Neuromodulation of Behavioral Specialization: Tachykinin Signaling Inhibits Task-specific Behavioral Responsiveness in Honeybee Workers

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Abstract

Behavioral specialization is key to the success of social insects and often compartmentalized among colony members leading to division of labor. Response thresholds to task-specific stimuli proximally regulate behavioral specialization but their neurobiological regulation is not understood. Here, we show that response thresholds to task-relevant stimuli correspond to the specialization of three behavioral phenotypes of honeybee workers. Quantitative neuropeptidome comparisons suggest two tachykinin-related peptides (TRP2 and TRP3) as candidates for the modification of these response thresholds. Based on our characterization of their receptor binding and downstream signaling, we then confirm the functional role of tachykinins: TRP2 injection and RNAi cause consistent, opposite effects on responsiveness to task-specific stimuli of each behaviorally specialized phenotype but not to stimuli that are unrelated to their tasks. Thus, our study demonstrates that TRP-signaling regulates the degree of task-specific responsiveness of specialized honeybee workers and may control the context-specificity of behavior in animals more generally.

1. Introduction

Behavioral responses of animals to external and internal stimuli have evolved to optimize survival and reproduction under average circumstances [1]. However, environmental and inter-individual variability commonly cause deviations from the average, resulting in selection for context-specific and condition-dependent behavior [2-4]. Evolutionary constraint [5] of behavior occurs in form of behavioral syndromes, differences among individuals that manifest across different contexts [6]. Advantages of behavioral plasticity and specificity have been documented in many systems and some neuroendocrine mechanisms have been identified [7, 8]. However, general neural mechanisms that allow the sophistication of behavioral repertoires by increasing context-specificity of behavioral responses remain insufficiently understood.

Behavioral modulation is particularly important in social species in which social interactions provide a high diversity of behavioral context [9, 10]. However, social evolution also allows individuals to restrict their behavioral repertoires through temporal or permanent behavioral specialization [11]. This specialization and the resulting division of labor are believed to be major contributors to the successful colony life of many social insects despite its potential costs [12]. Advanced social evolution thus allows inter-individual plasticity to replace individual behavioral plasticity and decoupling of behavioral responses may be more efficient across different individuals than within solitary individuals. Nevertheless, the principal problem of behavioral plasticity across different contexts remains the same, and social insects can be constrained in their behavioral evolution by correlated selection responses across different behaviors or castes [13, 14].

Behavior often occurs in response to a specific stimulus exceeding an individual's specific response threshold [15, 16]. Response thresholds depend on internal physiological states [17], particularly the concentration of neurotransmitters and neuromodulators in the central nervous system [18, 19]. Response thresholds translate the value of perceived stimuli into probabilities of behavioral responses and vary among individuals [20]. In social insects, individual variation in response thresholds is linked to division of labor [21-23] and numerous studies have characterized this link across multiple levels of biological organization [20, 24, 25]. Many aspects of the division of labor in the social model *Apis mellifera* are driven by a life-long behavioral ontogeny, leading to age-polyethism [26]. Young bees perform numerous inside tasks, most prominently brood care in form of alloparental nursing behavior, before transitioning to a mix of other in-hive tasks [27]. Similar to the highly-specialized nursing stage, the final behavioral state of older bees as outside foragers is almost exclusive of other tasks [26]. Moreover, foragers often specialize on collecting only one of the principal food sources, pollen or nectar [28]. These behavioral specialists (nurses, nectar foragers, and pollen foragers) exhibit pronounced differences in their responsiveness to task-related stimuli. Responsiveness to brood pheromones peaks at typical nursing age [29]. In contrast, foragers have a lower response threshold to sugars and light than nurses [30, 31]. Among foragers, pollen specialists exhibit higher responsiveness to sucrose and pollen stimuli than nectar foragers [32, 33]. Response thresholds can be quantified based on the honeybees' reflexive extension of their proboscis in response to stimuli, such as sucrose [20]. The spontaneous proboscis extension reflex (PER) to sucrose has been expanded to other stimuli that bees spontaneously respond to [34, 35] and conditioned stimuli to which no spontaneous responses

occur [36].

Response thresholds can be modified by biogenic amines, and dopamine, 5-hydroxy-tryptamine, octopamine, and tyramine have been implicated in the regulation of different behaviors of worker bees [37]. However, neuropeptides have not been studied although they are a diverse group of neurotransmitters that can also act as neurohormones on distal targets to coordinate a wide range of internal states and behavioral processes [38]. Neuropeptides are intimately involved in food perception and social interaction of insects [39], two processes that are central to division of labor in social insects [40]. Neuropeptides mediate pheromonal effects on physiology [41, 42] and usually exhibit a high degree of specificity [43, 44]. Therefore, neuropeptides are prime candidates for mediating the independent adjustment of socially relevant response thresholds that mediate honeybee workers specialization and division of labor.

More than 100 mature neuropeptides derived from 22 protein precursors have been identified in the Western honeybee, *Apis mellifera* [45, 46]. Several neuropeptides, including allatostatin and tachykinin-related peptides (TRPs), may be involved in the control of social behavior of honeybees, such as aggression [47], foraging [48], brood care [45], and possibly a wide array of other behaviors [49]. However, these results are based on correlations between behavior and neuropeptide expression and more detailed studies are needed to understand the causal roles of neuropeptides in the behavioral specialization among honeybee workers. Here, we report the results of a comprehensive study to test the hypothesis that neuropeptides regulate the division of labor in honeybees. We initially compared response thresholds to task-relevant stimuli among behaviorally-defined worker groups of two honeybee species.

These response thresholds were correlated with neuropeptide expression levels, especially TRPs, suggesting a role of TRPs in worker specialization. Based on these results, we characterized the TRP signaling pathway molecularly. Finally, we demonstrated in a series of TRP injections and RNAi-mediated knockdown of the *TRP* and its receptor *TRPR* a causal role of this pathway in modulating different response thresholds in a task-specific manner.

2. Results

2.1 The task-specific responsiveness of worker bees shows significant variations between behavioral phenotypes and the two honeybee species

In our comparisons of the PER of worker bees to task-specific stimuli, including sucrose solution, pollen, and larva, significant differences were found between behavioral phenotypes and the two honeybee species (Fig. 1A, Table S1 and S2).

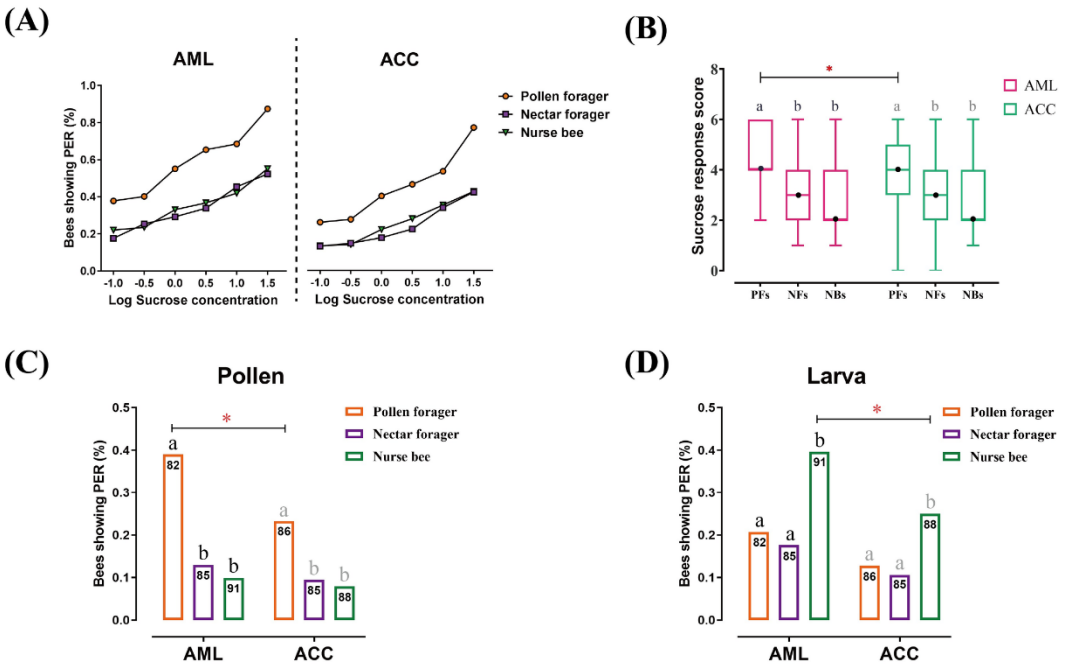

Fig. 1: Responses to sucrose solution, pollen, and larva stimulations are significant different among behavioral phenotypes and between honeybee species. (A) The proportion of pollen foragers (PFs), nectar foragers (NFs), and nurse bees (NBs) showing a proboscis extension reflex (PER)

increased with increasing concentrations of sucrose solutions. Left: *Apis mellifera ligustica* (AML), right: *Apis cerana cerana* (ACC). Details of the statistical results of our comparisons of sucrose responsiveness between behavioral phenotypes and bee species are listed in Table S2. (B) Median sucrose response scores (SRS; intermediate lines) and quartiles (upper and lower lines) of PFs, NFs, and NBs. Kruskal-Wallis tests with Bonferroni correction were used to compare the SRSs of the three behavioral phenotypes in the same species and significant differences are denoted by letters at $p < 0.05$. Pairwise Mann-Whitney U tests were used for comparing the same phenotype between two honeybee species (* denotes $p < 0.05$). (C) Proportion of PFs, NFs, and NBs showing PER to pollen stimulation of their antennae. (D) Proportion of PFs, NFs, and NBs showing PER to antennal stimulation with larvae. Numbers in bars represent the number of individuals sampled in each group. Independent Chi-square tests were used to compare the responsiveness to pollen or larvae between species (* denotes $p < 0.05$) and among behavioral phenotypes within species (letters indicate significant difference at $p < 0.05$).

The percentage of bees showing a PER increased with sucrose concentration across all experimental groups (Fig. 1A). In both, AML and ACC, the sucrose response scores (SRSs) of PFs were higher than the SRSs of NFs (AML: $Z = 7.0$, $p = <0.001$; ACC: $Z = 6.1$, $p < 0.001$) and NBs (AML: $Z = 5.9$, $p < 0.001$; ACC: $Z = 5.2$, $p < 0.001$), while no significant difference between NFs and NBs was observed in either species. PFs were more responsive than NFs and NBs to all sucrose concentrations. The species comparison between AML and ACC showed significant higher sucrose responsiveness in PFs of AML than in PFs of ACC ($Z = 2.361$, $p = 0.018$), specifically at sucrose concentrations of 0.3% ($\chi^2 = 4.1$, $p = 0.042$), 1.0% ($\chi^2 = 5.2$, $p = 0.001$), 3.0% ($\chi^2 = 8.4$, $p = 0.023$), and 10.0% ($\chi^2 = 5.3$, $p = 0.021$). Nectar foragers of AML and ACC showed no significant difference in overall SRS, but NFs of AML were more responsive than NFs of ACC at sucrose concentrations of 0.3% ($\chi^2 = 4.5$, $p = 0.035$), 1.0% ($\chi^2 = 4.5$, $p = 0.033$), and 3.0% ($\chi^2 = 4.0$, $p = 0.046$). There was no significant difference between NBs of AML and ACC in sucrose responsiveness.

In AML, PFs were more responsive to pollen stimulation than NFs ($\chi^2 = 14.9$, $p = 0.002$) and NBs ($\chi^2 = 20.2$, $p < 0.001$), while there were no significantly statistical differences

between NFs and NBs. Likewise, PFs of ACC were more sensitive than NFs ($\chi^2 = 6.0$, $p = 0.015$) and NBs ($\chi^2 = 7.8$, $p = 0.001$) without a statistically significant difference between NFs and NBs. Pollen foragers of AML showed a significant higher pollen responsiveness than of ACC ($\chi^2 = 4.9$, $p = 0.031$), with no significant species differences in NFs and NBs (Fig. 1B).

In larva responsiveness assay, NBs of AML showed increased responsiveness to larva stimulation compared to PFs ($\chi^2 = 7.2$, $p = 0.006$) and NFs ($\chi^2 = 10.3$, $p = 0.001$). Likewise, NBs of ACC were more sensitive than PFs ($\chi^2 = 4.2$, $p = 0.013$) and NFs ($\chi^2 = 6.1$, $p = 0.002$). Nurse bees of AML were significantly more sensitive to larvae ($\chi^2 = 4.3$, $p = 0.027$) than NBs of ACC, with no significant species differences in PFs and NFs. (Fig. 1C).

2.2 Quantitative peptidomics reveal brain neuropeptide signatures of behavior

Our LC-MS/MS-based comparisons of the brain neuropeptidomes of NBs, PFs, and NFs of AML and ACC revealed numerous differences among experimental groups but only two tachykinins showed consistent patterns relating to the task-specific responsiveness of the experimental groups. Overall, 132 unique neuropeptides derived from 23 neuropeptide families were identified in the brain of AML worker bees (Table S3). In the brain of ACC worker bees, for the first time, 116 unique neuropeptides derived from 22 neuropeptide families were identified (Table S4).

Quantitative comparison among the three behavioral phenotypes of AML showed that 40 neuropeptides derived from 16 neuropeptide families were differentially expressed the brain (Fig. 2, Table S5). Among 19 differential expressed neuropeptides between PFs and NFs, 9 neuropeptides were upregulated in PFs and 10 were upregulated in NFs. Among 24 differential expressed neuropeptides between PFs and NBs, 18 were upregulated in PFs and 6

were upregulated in NBs. Moreover, 21 differential expressed neuropeptides were found between NFs and NBs, with 14 upregulated in PFs and 7 upregulated in NBs.

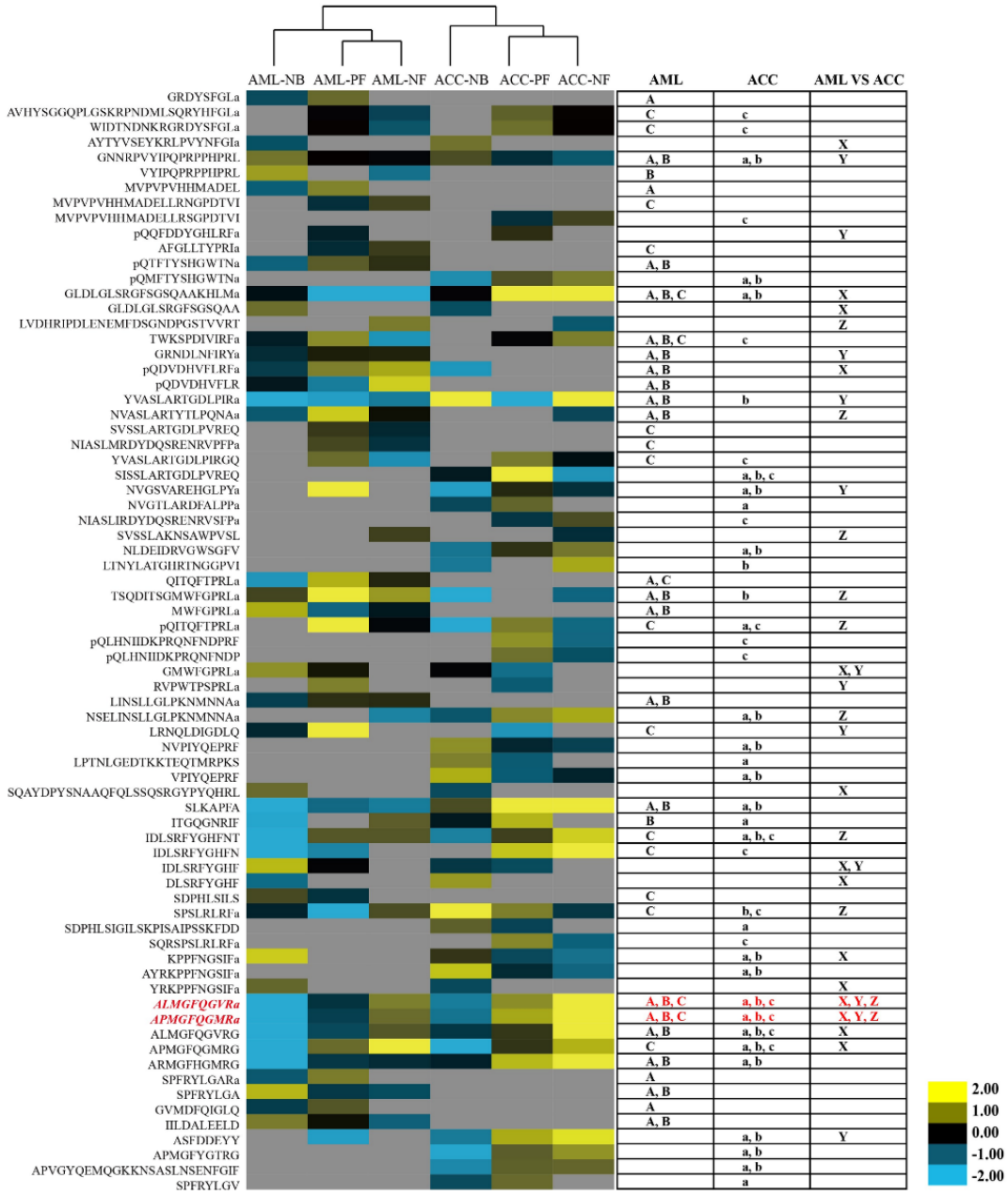

Fig. 2: Quantitative comparison of the brain neuropeptides. The brain neuropeptides were quantitatively compared between nurse bees (NBs), pollen foragers (PFs), and nectar foragers (NFs) of *Apis* mellifera *ligustica* (AML) and *Apis cerana cerana* (ACC). The up- and down-regulated peptides are indicated by yellow and blue colors, respectively. Color intensity indicates the relative expressional level, as noted in the key. Letters A, B, and C on the right represent significant differences between NBs and PFs, NBs and NFs, and PFs and NFs in AML, respectively; a, b, and c represent significant differences between NBs and PFs, NBs and NFs, and PFs and NFs in ACC, respectively; X, Y, and Z

represent significant differences of NBs, PFs, and NFs between AML and ACC, respectively. For detailed quantification data, see Table S5 S6, and S7.

In ACC 18 neuropeptides were differentially expressed between PFs and NFs, with 9 upregulated in each group. Between PFs and NBs, 27 neuropeptides showed different expression levels: 20 were upregulated in PFs and 7 were upregulated in NBs (Table S6). Twenty-five neuropeptides were differentially expressed between NFs and NBs, with 19 upregulated in NFs and 6 in NBs. The species comparison between AML and ACC, the number of differentially expressed neuropeptides in NBs, PFs and NFs was 13, 10, and 11, of which 7, 6, and 6 were upregulated in AML respectively (Table S7).

2.3 TRP/TRPR signaling couples to $G_{\alpha q}$ and $G_{\alpha s}$ pathways and triggers the ERK cascade

A series of cellular and molecular experiments confirmed that honeybee TRPR was expressed in the cell membrane and specifically activated by TRP, triggering intracellular cAMP accumulation, Ca^{2+} mobilization, and ERK phosphorylation by dually coupling $G_{\alpha s}$ and $G_{\alpha q}$ signaling pathways.

The honeybee *TRPR* gene was successfully cloned and expressed in the human embryonic kidney cells (HEK293) and the insect *Spodoptera frugiperda* pupal ovary cells (Sf21). Significant cell surface expression was observed by fluorescence microscopy (Fig. 3A and 3B), revealing that the honeybee TRPR was exclusively localized in the cell membrane in HEK293 and Sf21 cells.

Competitive binding assays with labeled TRP2 and TRP3 confirmed high affinity of the TRPR for both. The observed IC_{50} values for TRP2 and TRP3 were 2.34 nM and 6.29 nM in HEK293 cells and 8.76 nM and 34.88 nM in Sf21 cells, respectively (Fig. 3C and 3D). These

competition binding analyses strongly suggested a direct binding of TRP to TRPR, and also indicated that TRP2 displayed a higher affinity than TRP3 to TRPR.

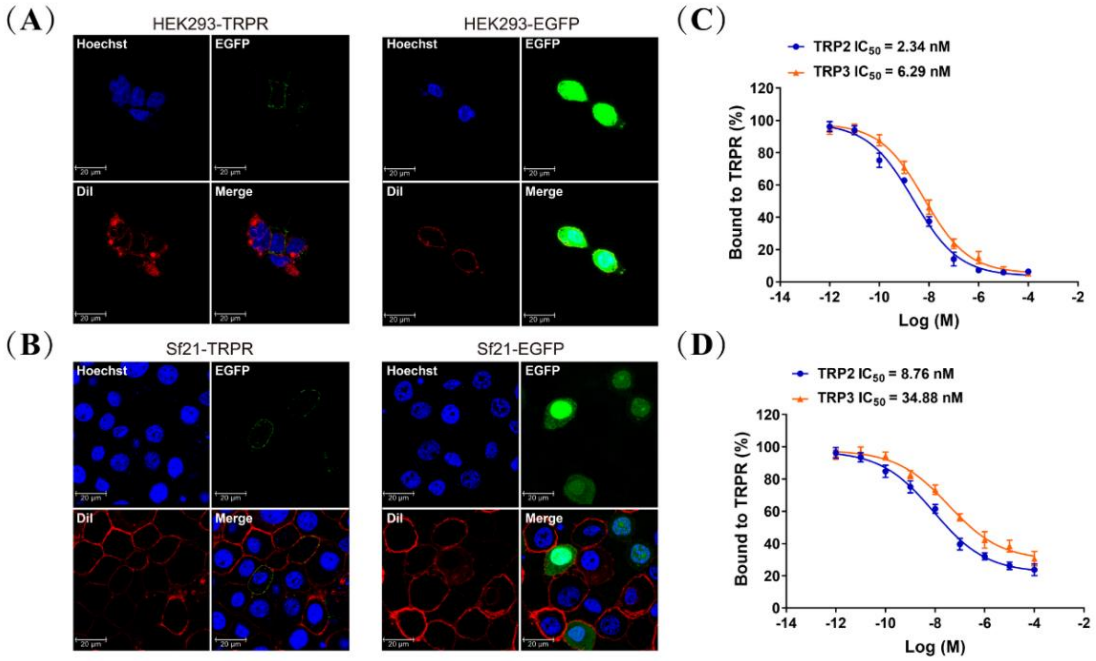

Fig. 3: Expression of TRPR and direct interaction of TRPs with TRPR in cell culture. (A) and (B) HEK293 and Sf21 cells expressing TRPR-EGFP and EGFP (green) were stained with a membrane plasma probe DiI (red) and a nuclei probe Hoechst (blue), and assessed by confocal microscopy. (C) and (D) Competitive inhibition of TAMRA-TRP2 and TAMRA-TRP3 binding to TRPR in HEK293 and Sf21 cells, and all data are presented as mean ± s.e.m. from three independent experiments.

The detected accumulation of intracellular cAMP concentration only in HEK293 cells transformed with TRPR (Fig. 4A) confirmed that TRP2 and TRP3 can activate TRPR and trigger cAMP signaling. This effect was confirmed in a second experiment and compared to other neuropeptides, including short neuropeptide F (NPF), pigment spreading hormone (PSH), and corazonin (CRZ), which did not induce any detectable responses in both HEK293 cells (Fig. 4B) and Sf21 cells (Fig. 4C).

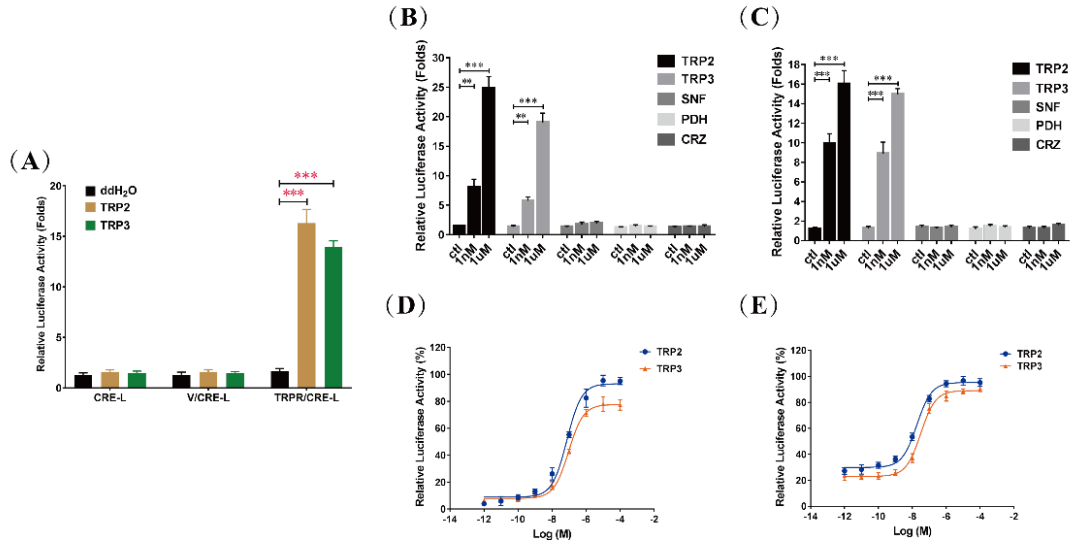

Fig. 4: TRP/TRPR-mediated cAMP accumulation in cells. (**A**), Luciferase activity of HEK293 cells transfected with the reporter gene pCRE-Luc (CRE-L), and co-transfected with pFLAG-TRPR (TRPR) or vehicle vector (V) were determined in response to ddH_2O and TRP (TRP2 or TRP3, 1 μM) treatment. TRP-dependent TRPR activation increases cAMP levels more than 10-fold. Luciferase activity of HEK293 cells (**B**) and Sf21 cells (**C**) co-transfected with TRPR and CRE-L were determined in response to different neuropeptides (TRP2, TRP3, short neuropeptide F (SNF), pigment-dispersing hormone (PDH), and corazonin (CRZ)) at different concentrations (1 nM or 1 μM). Increase of cAMP was specific to TRP2 and TRP3. Dose-dependent changes of luciferase activities, indicating cAMP increases, in HEK293 cells (**D**) and Sf21 cells (**E**) co-transfected with TRPR and CRE-L revealed typical kinetics in response to TRP2 and TRP3. All data are presented as mean ± s.e.m. from three independent experiments. Student's t-tests were used for pairwise comparisons (**p<0.01, ***p<0.001).

Additional dose-depended assays of TPR2 and TPR3 on cAMP accumulation in both HEK293 cells (Fig. 4D) and Sf21 cells (Fig. 4E) confirmed the direct correlation between TRP stimulation and cAMP signaling, and indicated that TRPR was more sensitive to TRP2 than to TRP3. Further analysis showed that pretreatment with PTX (an inhibitor of $G_{\alpha i}$ subunit) had no effect on cAMP accumulation, whereas stimulation with CTX (an activator of $G_{\alpha s}$ subunit) elicited a dramatically increase in abundance of cAMP (Fig. 5A), suggesting that $G_{\alpha s}$ was involved in TRPR-mediated cAMP signaling. In addition, TRP-induced cAMP generation was significantly inhibited by $G_{\alpha q}$ inhibitor YM-254890, and PKA inhibitor H89

(Fig. 5B). Collectively, these results established that both $G_{\alpha s}$ and $G_{\alpha q}$ are involved in TRP/TRPR-mediated cAMP signaling.

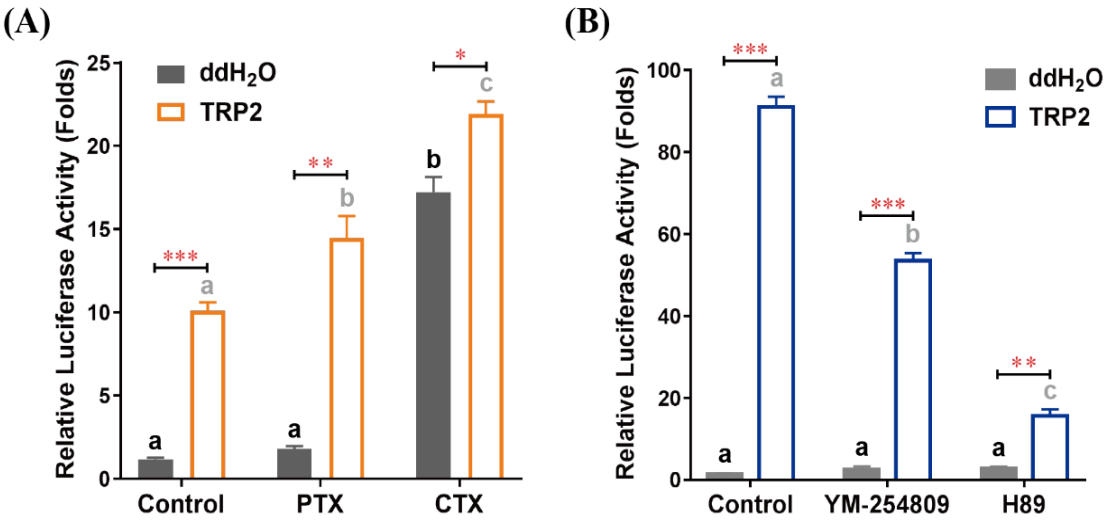

Fig. 5: TRP/TRPR signaling induces cAMP accumulation via $G_{\alpha q}$ and $G_{\alpha s}$ pathways. (**A**), Effects of $G_{\alpha i}$ inhibitor pertussis toxin (PTX) and $G_{\alpha s}$ activator cholera toxin (CTX) on TRP2-mediated stimulation of cAMP accumulation. HEK293 cells expressing TRPR were pretreated with PTX (100 ng/ml) or CTX (300 ng/ml) overnight prior to treatment with TRP2 (1 μM). (**B**), Effects of $G_{\alpha q}$ inhibitor YM-254890 and PKA inhibitor H89 on TRP2-mediated stimulation of cAMP accumulation. HEK293 cells expressing TRPR were pretreated with YM-254890 (1 μM) or H89 (10 μM) for 2 hours prior to treatment with TRP2 (1 μM). All data are presented as mean ± s.e.m. from three independent experiments. Student's t-tests were used for pairwise comparisons between water and TRP2 treatments (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$). One-way ANOVAs followed by Tukey's post-hoc tests were used for comparisons among control, PTX, and CTX groups within water or TRP2 treatments, and significant differences ($p < 0.05$) are denoted by letters.

Measurements of a Ca^{2+}-sensitive fluorescent indicator suggested that intracellular Ca^{2+} signaling was also elicited by TRP/TRPR signaling. Both, TRP2 and TRP3, could induce a rapid intracellular Ca^{2+} accumulation in HEK293 cells (Fig. 6A) and Sf21 cells (Fig. 6B). The TRP/TRPR-mediated intracellular Ca^{2+} mobilization was decreased by $G_{\alpha q}$ inhibitor YM-254890 and phospholipase C (PLC) inhibitor U73122 (Fig. 6C), suggesting the $G_{\alpha q}$/PLC pathway was involved in TRP/TRPR-mediated Ca^{2+} signaling.

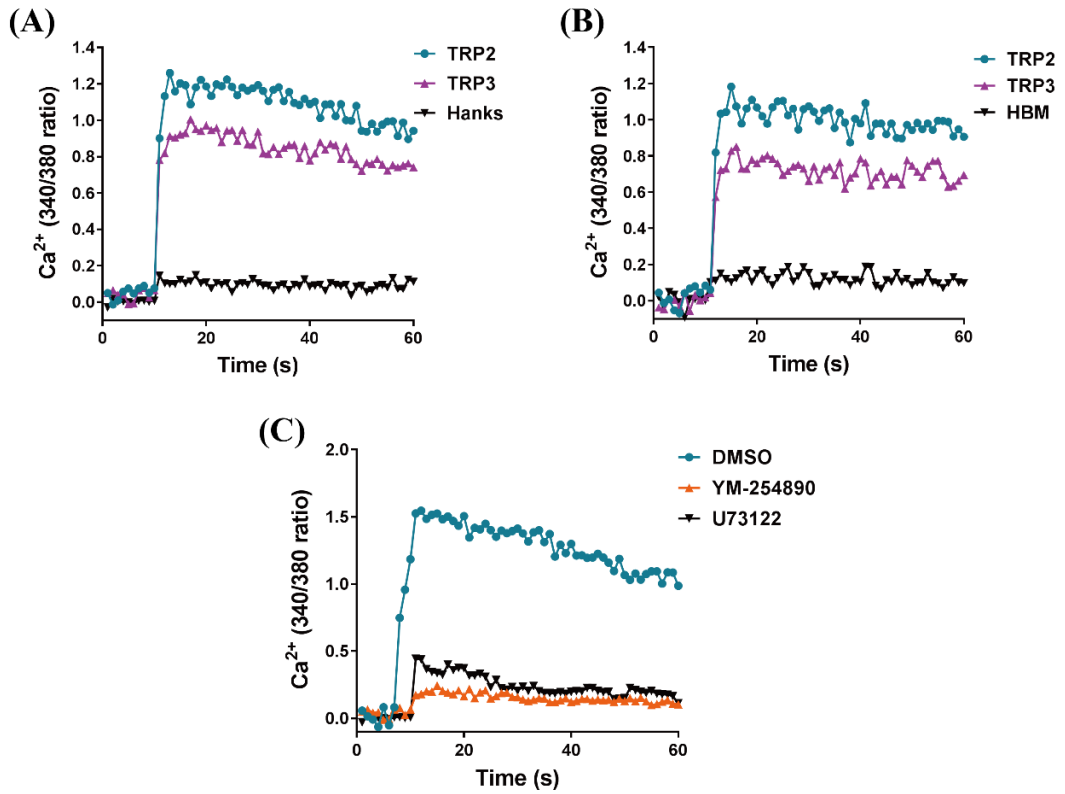

Fig. 6: TRP/TRPR-mediated intracellular Ca^{2+} influx via $G_{\alpha q}$/PLC pathways. HEK293 cells (**A**) and Sf21 cells (**B**) expressing TRPR were measured in response to TRP2 and TRP3 using the fluorescent Ca^{2+} indicator Fura-2 AM. Hanks solution (Hanks) and Hepes-buffered medium (HBM) were used as a control, respectively. (**C**), Effects of $G_{\alpha q}$ inhibitor YM-254890 and PLC inhibitor U73122 compared to vehicle control DMSO on TRP2-mediated intracellular Ca^{2+} influx. HEK293 cells expressing TRPR were pretreated with YM-254890 (1 μM) or U73122 (10 μM) for 2 hours then stimulated with TRP2 (1 μM). Each figure is representative of three independent repliates of each experiment.

Western blot analyses proved that phosphorylation of ERK was induced by TRP/TRPR signaling. Treatment with different concentrations of TRP2 induced a dose-dependent phosphorylation of ERK in both HEK293 (EC_{50}=68.04 nM) and Sf21 (EC_{50}=1.68 nM) cells (Fig. 7A and 7B). Further time-dependent analysis indicated that TRP2 elicited transient phosphorylation of ERK with maximal phosphorylation at 2 min and near basal levels by 90 min (Fig. 7C). Moreover, specific inhibitors were used to elucidate TRP/TRPR signaling-mediated ERK activation in both HEK293 and Sf21 cells. Treatment with MEK

inhibitor U0126, PKA inhibitor H89, and PKC inhibitor Go6983, respectively, led to a significant inhibition of TRP/TRPR-mediated ERK activation, whereas $G_{\alpha i}$ inhibitor PTX had no effect, demonstrating that honeybee TRP/TRPR signaling dually coupled to $G_{\alpha s}$ and $G_{\alpha q}$ proteins to activate the ERK signaling pathway (Fig. 7D).

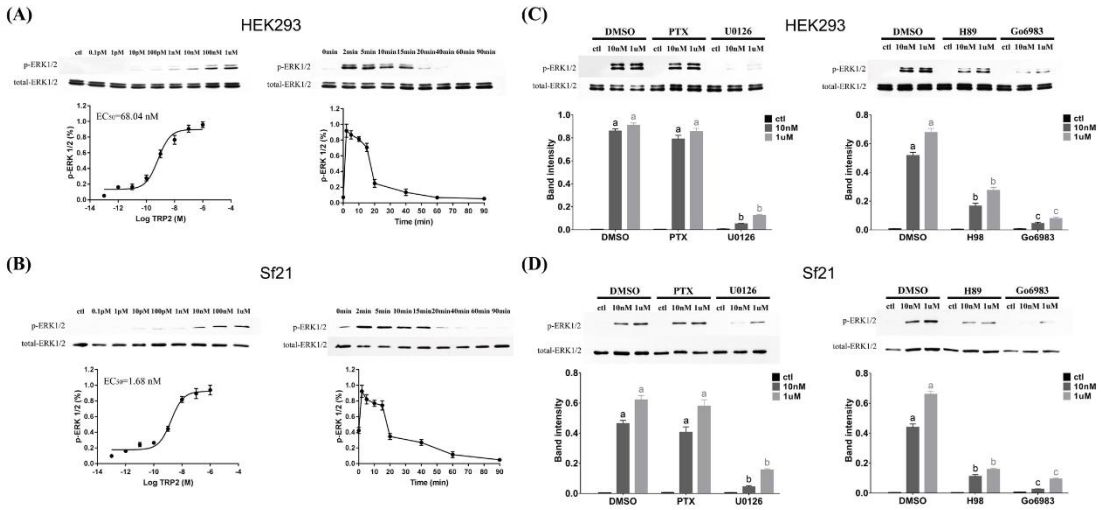

Fig. 7: $G_{\alpha q}$/PKC and $G_{\alpha s}$/PKA pathways involved in TRP/TRPR-induced ERK1/2 phosphorylation. Dose- and time-response analyses of TRP/TRPR-induced ERK1/2 phosphorylation in HEK293 cells (A) and Sf21 cells (B). Cells expressing TRPR were serum-starved then incubated either with an increasing dose of TRP2, (from 0.1 pM to 1 μM) for 10 min or with 100 nM TRP2 for different times (from 0 to 90 min), then harvested to quantify ERK1/2 phosphorylation. Effects of $G_{\alpha i}$ inhibitor pertussis toxin (PTX), MEK inhibitor U0126, PKA inhibitor H89, and PKC inhibitor Go6983 on TRP2-induced ERK1/2 phosphorylation in HEK293 cells (C) and Sf21 cells (D). The cells were pretreated with or without inhibitors for 2 hours then stimulated with ddH_2O (control) or TRP2 (10 nM or 1 μM) for 10 min. The phosphorylated ERK was normalized to a loading control (total ERK). All data are presented as mean ± s.e.m. from three independent replicates, and blots shown are representative of these experiments. One-way ANOVAs followed by Tukey's post-hoc tests were used for multi-group comparisons, and significant differences ($p < 0.05$) are denoted by letters.

2.4 TRP/TRPR signaling acts as negative regulator of task-specific responsiveness

2.4.1 TRP2 injection decreases task-specific responsiveness

Task-specific responsiveness of the different behavioral phenotypes (PFs, NFs, and NBs) was decreased by injection of TPR2 in a task-specific manner (Fig. 8, Table S8).

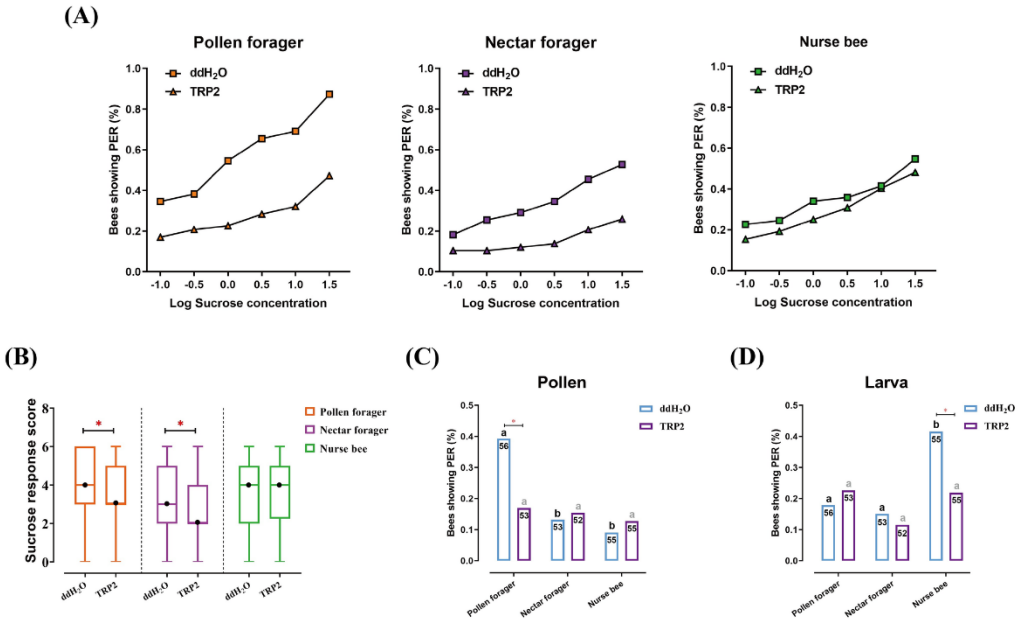

Fig. 8: Injection of TRP2 decreases task-specific responsiveness of worker bees. (A) The proportion of pollen foragers (PFs), nectar foragers (NFs), and nurse bees (NBs) exhibiting a positive proboscis extension reflex (PER) increases with increasing concentrations of sucrose solutions but is overall decreased in PFs and NFs after injection of TRP2 compared to ddH_2O injection. (B) Median sucrose response scores (SRS; intermediate lines) and quartiles (upper and lower lines) of ddH_2O injected and TRP2 injected groups of PFs, NFs, and NBs. Mann-Whitney U tests were used to compare the SRS (*: $p < 0.05$). The proportion of PFs, NFs, and NBs showing PER to pollen stimulation (C) and larva stimulation (D) after injection of TRP2 or ddH_2O. Numbers in bars are the number of individuals sampled in each group. Independent Chi-square tests were used to compare the responsiveness between different treatments (*: $p < 0.05$) and between different behavioral phenotypes within treatments (significant differences are denoted by letters, $p < 0.05$).

Injection of the TRP2 peptide significantly reduced the SRS of PFs ($Z = 2.2$, $p = 0.031$), significantly reducing PER responses to all sucrose concentrations used. Similarly, NFs injected with TRP2 displayed significantly lower SRS than control-injected NFs ($Z = 2.3$, $p = 0.019$), significantly reducing PER responses to all sucrose concentrations except 0.1% (Fig. 8A and 8B). In contrast, TRP2-injected NBs did not show significant responsiveness changes to sucrose relative to controls. For pollen stimulation, PFs showed significantly decreased responsiveness to pollen loads after TRP2 injection ($\chi^2 = 6.7$, $p = 0.017$), while no significant

306 effects were observed in PFs and NFs (Fig. 8C). In the larval responsiveness assay, injection
307 of TRP2 only significantly affected the responsiveness of NBs (χ^2 = 6.1, p = 0.001) but not
308 NFs or PFs (Fig. 8D).

309 2.4.2 Downregulation of *TRP* or *TRPR* increased task-specific responsiveness

310 The function of TPR/TRPR signaling on task-specific responsiveness was further confirmed
311 by RNAi-mediated downregulation of *TRP* or *TRPR* that complemented the results of the
312 TRP2 injection.

313 Knockdown efficiencies were close to 60% for *TRP* and *TRPR* mRNA levels at 24 hours
314 post-injection of the corresponding dsRNA (Fig. S1). Therefore, subsequent PER assays were
315 performed 24 hours after dsRNA injection. Relative to control injections, knockdown of
316 either *TRP* or *TRPR* significantly increased the SRS of NFs (ds*TRP*: Z = 2.4, p = 0.049;
317 ds*TRPR*: Z = 2.6, p = 0.025), specifically increasing the responses of NFs to sucrose at
318 concentrations of 0.1% (ds*TRP*: χ^2 = 3.9 p = 0.039; ds*TRPR*: χ^2 = 4.9, p = 0.023), 0.3%
319 (ds*TRP*: χ^2 = 5.3, p = 0.018; ds*TRPR*: χ^2 = 4.3, p = 0.030), 1.0% (ds*TRP*: χ^2 = 7.0, p = 0.007;
320 ds*TRPR*: χ^2 = 6.6, p = 0.009), and 3.0% (ds*TRP*: χ^2 = 6.0, p = 0.012; ds*TRPR*: χ^2 = 7.4, p =
321 0.006) (Fig. 9A and 9B, Table S9 and S10). Knockdown of *TRP* and *TRPR* didn't
322 significantly change the overall SRS of PFs and NBs, although it significantly increased the
323 responses of PFs to sucrose at concentrations of 0.1% (ds*TRP*: χ^2 = 4.4, p = 0.029; ds*TRPR*: χ^2
324 = 6.1, p = 0.011), 0.3% (ds*TRP*: χ^2 = 5.2, p = 0.018; ds*TRPR*: χ^2 = 6.0, p = 0.011), and 1.0%
325 (ds*TRP*: χ^2 = 5.0, p = 0.020; ds*TRPR*: χ^2 = 4.7, p = 0.025). Responses to pollen stimulation
326 after dsRNA injection indicated that knockdown of either *TRP* or *TRPR* specifically increased
327 the pollen responsiveness of PFs (ds*TRP*: χ^2 = 6.5, p = 0.018; ds*TRPR*: χ^2 = 6.4, p = 0.010),

whereas the effects on NFs and NBs were not significant (Fig. 9C). The responsiveness of NBs to larvae was significantly increased after gene knockdown of either *TRP* ($\chi^2 = 4.4$, $p = 0.029$) or *TRPR* ($\chi^2 = 4.8$, $p = 0.023$) but NFs and PFs were not affected (Fig. 9D).

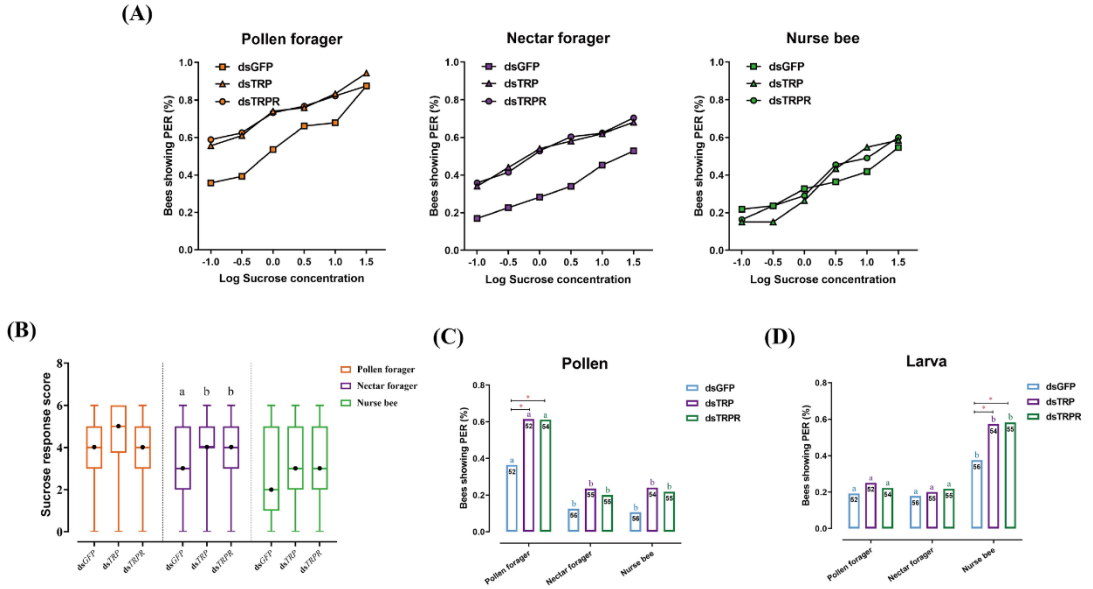

Fig. 9: RNAi-mediated knockdown of *TPR* and *TRPR* expression alter task-specific responses of worker bees. (A) Proportion of positive proboscis extension reflex (PER) responses of pollen foragers (PFs), nectar foragers (NFs), and nurse bees (NBs) increases with increasing concentrations of sucrose solutions but overall increases occur only in PFs and NFs after knockdown of *TPR* or *TRPR* transcripts compared to GFP control. Statistical details of these sucrose responsiveness comparisons are shown in Table S10. (B) Median sucrose response scores (SRS; intermediate lines) and quartiles (upper and lower lines) of ddH_2O injected and TRP2 injected PFs, NFs, and NBs. Kruskal-Wallis tests with Bonferroni correction were used to compare the SRSs of the three treatment groups of each behavioral phenotype and significant differences are denoted by letters ($p < 0.05$). The proportion of PFs, NFs, and NBs showing PER to pollen stimulation (C) and larvae stimulation (D) after *GFP*, *TPR*, or *TRPR* knockdown. Numbers in bars are the number of individuals sampled in each group. Independent Chi-square tests were used to compare the task-specific responsiveness between different treatments (*: $p < 0.05$, **: $p < 0.01$) within behavioral phenotypes and between different behavioral phenotypes within each treatment (significant differences are denoted by letters, $p < 0.05$).

2.5 TRP/TRPR signaling regulates ERK signaling *in-vivo*

To complement our finding that TRP/TRPR signaling activates ERK phosphorylation in cell culture, we used our *in-vivo* manipulations of TRP-signaling to confirm the link between

TRP- and ERK signaling in living honeybee workers. Western blot results confirmed that TRP/TRPR signaling triggers ERK signaling *in vivo*. The level of phosphorylated ERK significantly increased after injection of TRP2 peptide into NBs, PFs, and NFs (Fig. 10A) and decreased after knockdown of the *TRP* or *TRPR* transcripts (Fig. 10B).

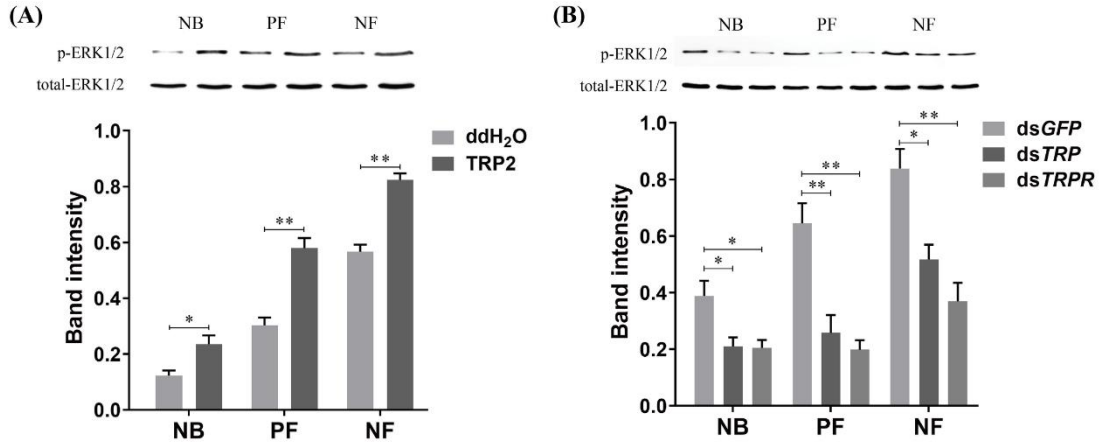

Fig. 10: Manipulations of TRP and TRPR levels change ERK phosphorylation states in the worker bee brains. (A) The ERK phosphorylation (p-ERK) levels after injection of TRP2 or ddH_2O into pollen foragers (PFs), nectar foragers (NFs), and nurse bees (NBs) of *Apis mellifera ligustica*. (B) The p-ERK levels after transcript knockdown of *GFP*, *TPR*, or *TRPR* in PFs, NFs, and NBs. The p-ERK was normalized to a loading control (total-ERK). The data shown are representative of three independent experiments, and blots shown are representative of these experiments. Student's t-tests were used for pairwise comparisons between control and treatment groups within each behavioral phenotype (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

3. Discussion

Behavioral plasticity plays a central role in animal adaptation and modulating behavioral responsiveness to different stimuli and contexts is key to individual fitness. The success of social insects is partly due to their efficient division of labor, a form of behavioral plasticity among instead of within individuals. In this study, we demonstrated that the responsiveness to task-relevant stimuli correlates with behavioral specialization in two different honeybee species. Through parallel characterization of the neuropeptidome, we identified two

369 tachykinin-related peptides (TRP2 and TRP3) as putative mechanism to adjust task-specific
370 response thresholds and thus proximally guide division of labor. Subsequently, we
371 characterized the molecular action of TRP2 and TRP3 in cell culture by verifying their
372 binding to their membrane-bound receptor and demonstrating activation of multiple
373 down-stream signaling mechanisms. Finally, we verified causal involvement of TRP
374 signaling in modulating task-specific behavioral response thresholds through complementary
375 outcomes of TRP2 injection and RNAi-mediated knockdown of *TRP* and its receptor *TRPR*:
376 while injection decreased task-specific responses, down-regulation of TRP or TRPR increased
377 the same specific responses. Thus, we present the first process that tunes the behavioral
378 responsiveness of animals to specific stimuli compared to others. We use behaviorally
379 specialized honeybee workers as models but hypothesize that this function of TRP signaling
380 could be more widely conserved to adjust the context-specificity of behavioral responses in
381 animals.

382 Among all the signaling molecules in the nervous system, neuropeptides represent the
383 largest and most diverse category and are crucial in orchestrating various biological processes
384 and behavioral actions [50, 51]. Thus, we quantitatively compared the entire neuropeptidome
385 among three behavioral worker phenotypes of *Apis mellifera ligustica* (AML) and *Apis*
386 *cerana cerana* (ACC) without an *a-priori* assumption. In addition to characterizing the ACC
387 neuropeptidome for the first time and discovering several new neuropeptides from the AML
388 brain, we identified TRP2 and TRP3 as candidates. TRPs have been associated with the
389 modulation of appetitive olfactory sensation [52-54], sex pheromone perception [41], and
390 aggression [55]. Particularly in honeybees, *TRP* is preferentially expressed in the mushroom

391 body and some neurons scattered in the antennal and optic lobes [56]. This expression is
392 consistent with our hypothesis that TRP-signaling may be a general modulator of behavioral
393 responsiveness. TRPs expression in the honeybee worker brain increases during the transition
394 from nursing to foraging, further implicating it in the regulation honeybee social behavior [49,
395 57].

396 In our study, only expression of TPR2 and TRP3 varied consistently among behavioral
397 phenotypes of AML and ACC. In both species, TRP2 and TRP3 were most abundant in the
398 brain of NFs, followed by PFs, and finally NBs. This is consistent with the very specific
399 responsiveness of NBs to brood stimuli observed in our PER experiments, while the
400 responsiveness of PFs and NFs was successively less specific: PFs responded specifically to
401 two stimuli, while NFs did not show specifically strong responses to any stimuli. Moreover,
402 the comparison between AML and ACC indicated higher TRP2 and TRP3 abundance in ACC
403 in each behavioral phenotype, commensurate with the less specific PER responsiveness in
404 ACC compared to AML. A few other neuropeptides, such as apidaecins, diuretic hormone,
405 and prohormone-3, showed somewhat similar expression patterns in both species, but none of
406 these was as tightly correlated to behavioral responsiveness and none has previously been
407 connected with behavioral regulation in insects or other animals. Therefore, the TRPs were
408 chosen as candidates of the control of honeybee division of labor for subsequent functional
409 tests and molecular characterization.

410 The action of most insect neuropeptides is mediated by binding to G-protein–coupled
411 receptors (GPCRs) and often involves cAMP and Ca^{2+} as second messengers [58]. The TRPR
412 is activated by TRPs triggering intracellular cAMP accumulation and Ca^{2+} mobilization in

fruit flies and silkworms (*Bombyx mori*) [59, 60], while no cAMP-responses were discovered in stable flies (*Stomoxys calcitrans*) [61]. The results of our peptide-based binding assays functionally confirmed that the honeybee TRPR is indeed the receptor for TRP2 and TRP3. The subsequent functional assays revealed that TRP signaling results in a dose-dependent increase in both intracellular cAMP and Ca^{2+}. Together, these results indicate that TRPs can activate TRPR and trigger second messengers to regulate downstream functions. TRP2 displayed a higher affinity to TRPR and induced higher cAMP and Ca^{2+} signaling than TRP3, leading us to focus on TRP2 in the later *in vivo* experiments. Moreover, TRP signaling is sensitive to $G_{\alpha s}$ activation and is significantly blocked by $G_{\alpha q}$ and PKA inhibitors, suggesting both $G_{\alpha s}$ and $G_{\alpha q}$ are involved in TRP signaling in honeybees. Many GPCRs are able to induce mitogen-activated protein kinase (MAPK) cascades via cooperation of $G_{\alpha s}$, $G_{\alpha q,}$ and $G_{\alpha i}$ signals, leading to the phosphorylation of ERK1/2, which plays critical roles in diverse biological processes [62]. Our results indicate that honeybee TRP signaling mediates phosphorylation of ERK1/2 in a dose- and time-dependent manner in both HEK293 and Sf21 cells. In addition, ERK1/2 activation was significantly inhibited by the PKA, PKC, and MEK inhibitors, which is in line with the observation of intracellular cAMP accumulation and Ca^{2+} mobilization. Thus, honeybees seem to be very similar to silkworms with regards to the involvement of the $G_{\alpha s}$/cAMP/PKA and $G_{\alpha q}$/Ca^{2+}/PKC signaling pathways in the regulation of TRP-induced ERK1/2 activation [60]. Taken together, our results demonstrate that the honeybee TRPR is specifically activated by TRPs, eliciting intracellular cAMP accumulation, Ca^{2+} mobilization, and ERK phosphorylation by dually coupling $G_{\alpha s}$ and $G_{\alpha q}$ signaling pathways.

435 Our *in vitro* and *in vivo* demonstrations that TRP signaling activates the ERK putatively
436 link TRP signaling also to the insulin/insulin-like signaling (IIS) pathway. IIS is controlled by
437 neuropeptides through ERK in *Drosophila* [63], and this connection in honeybees ties TRP
438 back to the age-based division of labor among workers: IIS signaling influences the timing of
439 the behavioral maturation of honeybee workers and brain *AmIlp1* is significantly higher
440 expressed in foragers than nurses [64], consistent with our finding that TRPs are higher in
441 foragers than nurses. Numerous other physiological changes accompany the transition from
442 in-hive nurse bees to foragers [37, 65-67] and our results integrate TRPs as the most
443 important neuropeptides into the regulation of the behavioral ontogeny of honeybee workers
444 and potential feedback loops to the modulation of behavioral response thresholds. The
445 specialization of nectar and pollen foragers has also been linked to IIS signaling [68, 69] and
446 explained by differences in sucrose response thresholds [70]. Our findings here may connect
447 the differences in response thresholds and IIS mechanistically through the TRP and ERK
448 signaling pathways.

449 The PER paradigm is well-suited to test behavioral response thresholds and has been
450 used for over 50 years in honeybees [36]. Consistent with previous studies, we found pollen
451 foragers to be more responsive to sucrose than nectar foragers and nurses in *Apis mellifera*
452 [32]. Moreover, we found corresponding differences between these behavioral groups in the
453 closely related *Apis cerana*. The pollen forager's responsiveness to low sucrose
454 concentrations might also make them more responsive to pollen, but the causation of the PER
455 to pollen is unclear [71] and other components of pollen may functionally distinguish pollen
456 from sucrose responsiveness [34]. Our results support the view that pollen and sucrose are

457 distinct stimuli: While our experimental manipulations of TRP signaling altered the
458 responsiveness of pollen foragers to pollen and sucrose, only responsiveness to sucrose was
459 affected in nectar foragers and only responsiveness to larvae was affected in nurses. The
460 functional significance of the PER in response to larvae is currently unclear, but we show that
461 it is specific to nurses and it has previously been linked to brood provisioning [35]. Thus, our
462 diverse PER results in two species comprehensively support the hypothesis that task-specific
463 response thresholds guide behavioral specialization, leading to division of labor among
464 honeybee workers [21-23].

465 TRPs may adjust specific sensory neural circuits, potentially acting in concert with other
466 neuromodulators [72, 73]. However, we have currently no evidence to support the hypothesis
467 of different molecular TRP actions in different stimulus-response pathways and our consistent
468 results from two very different cell cultures indicate that TRP signaling may be relatively
469 robust to the cellular environment. Thus, we favor the more parsimonious explanation is that
470 TRP signaling acts generally through the identified mechanisms to decreases task-specific
471 response thresholds of behavioral specialists: It decreases pollen and sucrose responsiveness
472 in pollen foragers, sucrose responsiveness in nectar foragers, and responsiveness to larvae in
473 nurses. TRP signaling may thus be a general regulator of how task-specific stimuli are
474 weighted relative to others and consequently how specialized behavioral specialists are. This
475 effect translates to different degrees of division of labor in social insect colonies and may
476 control the context-specificity of behavioral responses in animals more generally [74].

477 Although AML and ACC are close relatives with similar basic biology, some behavioral
478 differences have evolved since their speciation [75]. AML and ACC share the age-based

division of labor, with younger bees specializing on nursing before maturing to foraging activities [76] and ACC foragers also specialize in nectar or pollen collection [77] similar to AML [28]. Accordingly, we found the main differences of stimulus responsiveness and TRPs expression among worker phenotypes conserved. However, ACC exhibited less responses to the task-specific stimuli than AML. Consistent PER differences in AML and ACC between nectar and pollen foragers and a generally lower responsiveness of ACC have been identified before [78], but the biological interpretation has remained unclear. It is possible that the species differences arise due to methodological bias, favoring AML performance in PER assays. However, our study offers the alternative explanation that ACC workers are less specialized than AML workers due to higher TRP signaling. Lower innate specialization may accompany better learning of ACC [79], facilitating its more opportunistic worker task allocation and resource exploitation than AML [80]. These alternative life history strategies are plausible, given the typical differences in colony size and habitat [73, 74, 81]. All three worker phenotypes of ACC exhibited higher levels of TRPs than their AML counterparts but functional verification at the level of colony phenotypes will be required to unambiguously link TRP signaling to such interspecific differences in life history.

4. Materials and Methods

4.1. Honeybee sources and sampling

Two honeybee species, *Apis mellifera ligustica* (AML) and *Apis cerana cerana* (ACC), were maintained in the apiary of the Institute of Apicultural Research at the Chinese Academy of Agricultural Sciences in Beijing. Three colonies of each species with mated queens of identical age were selected as experimental colonies, and before experiments the colonies

were equalized in terms of adult bee population, brood combs, and food storage. Frames containing old pupae (1-2 days before emergence) were put into an incubator (34°C and 80% relative humidity) for eclosion. Newly emerged worker bees were paint marked on their thoraxes and placed back into their parent colonies. Ten days later, marked bees that had their head and thorax in open brood cells while contracting their abdomen for more than 10 seconds were collected as nurse bees (NBs). Twenty day-old, marked bees were collected during early morning (between 8:00 am and 10:00 am) in good weather conditions during the blooming period of black locusts (*Robinia pseudoacacia* L.) as forager bees. The entrance to the hives were blocked to facilitate collecting. Bees flying into the hive with pollen loads were collected as pollen foragers (PFs), returning foragers without pollen loads were collected as nectar foragers (NFs). The experimental design of six groups (three behavioral phenotypes in two species) was used to compare responsiveness to task-specific stimuli (section 4.2) and to relate these phenotypes to differences in the brain neuropeptidome (section 4.3).

4.2. Comparative Proboscis Extension Reflex (PER) experiments

To investigate the responsiveness of different worker bee behavioral phenotypes (NBs, PFs, and NFs of AML and ACC) to different stimulus modalities (sucrose solution, pollen, and larva), series of PER experiments were performed. One hundred bees of each behavioral phenotype were collected from each experimental colony in the morning, transferred to the laboratory and narcotized on ice, then harnessed using a previously described protocol [82]. All harnessed bees were fed to satiation with 50% sucrose solution and placed in a dark incubator (20°C and 65% relative humidity) overnight. After 24 hours, all surviving bees were assayed for their PER following the methodology of Page et al. [32]. Each stimulus was

523 assessed independently with a new set of bees.

524 To investigate the sucrose responsiveness, bees were assayed using an ascending order of
525 sucrose concentrations: 0.1, 0.3, 1, 3, 10, and 30% (weight/weight). A small droplet of each
526 solution was touched to the bees' antennae for 3 seconds and the extension of the proboscis
527 was monitored during this time. The interval between each sucrose solution trial was 5 min to
528 exclude sensitization or habituation effects. The total number of PER responses after
529 stimulation with the six different sucrose concentrations were combined into a sucrose
530 response score (SRS) of a bee [83-85]. The SRSs of the three behavioral phenotypes in the
531 same species were compared using Kruskal-Wallis tests with Bonferroni correction. Pairwise
532 Mann-Whitney U tests were used for comparing the same phenotype from two honeybee
533 species. The sucrose responsiveness for specific sucrose concentrations was further compared
534 between different groups with independent Chi-square tests.

535 To test pollen stimulation, fresh pollen loads that had been removed from the leg of
536 randomly selected pollen foragers of the test group were used: AML were tested with pollen
537 collected by AML foragers and ACC with pollen collected by ACC foragers. These loads
538 contained a mixture of different pollen, predominated by black locust (*Robinia pseudoacacia*).
539 As a control for mechanical stimulation, each bee had both antennae first touched with a piece
540 of filter paper, and spontaneous responders were excluded. Subsequently both antennae of
541 each bee were gently touched with a pollen load and PER responses were recorded. The
542 pollen responsiveness was compared with independent Chi-square tests between different
543 groups.

544 To test responsiveness to larva, one-day-old larvae from each honeybee species were

545 collected, briefly rinsed in distilled water to remove royal jelly residue and dried on a filter
546 paper. As before, both antennae of bees were touched with a piece of filter paper first, and
547 spontaneous responders were excluded, then PERs in response to a larva touching the
548 antennae were recorded. The responsiveness to larvae was compared with independent
549 Chi-square tests between different groups. Statistical analyses were conducted using SPSS
550 20.0 (IBM, USA).

551 **4.3. Quantitative comparisons of brain neuropeptidomes**

552 To explore brain neuropeptide functions in behavioral regulation, a label-free quantitative
553 strategy was employed to compare neuropeptidomic variations between behavioral
554 phenotypes and the two honeybee species. Three independent biological replicate samples
555 (120 bees/sample) of NBs, PFs, and NFs of both AML and ACC (18 samples total) were
556 collected and immediately frozen in liquid nitrogen. Individual brains were carefully
557 dissected from the head capsule while remaining chilled on ice, and the dissected brains were
558 frozen at -80°C until neuropeptide extraction.

559 The brains were homogenized at 4°C by using a 90:9:1 solution of methanol, H_2O, and
560 acetic acid. The homogenates were centrifuged at 12000g for 10 min at 4°C. The resulting
561 supernatant containing the neuropeptides was collected and dried. The extracted neuropeptide
562 samples were dissolved in 0.1% formic acid in distilled water, and the peptide concentration
563 was quantified using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA).
564 LC-MS/MS analysis was performed on an Easy-nLC 1200 (Thermo Fisher Scientific)
565 coupled Q-Exactive HF mass spectrometer (Thermo Fisher Scientific). Buffer A (0.1% formic
566 acid in water) and buffer B (0.1% formic acid in acetonitrile) were used as mobile phase

buffers. Neuropeptides were separated using the following gradients: from 3 to 8% buffer B in 5 min, from 8 to 20 % buffer B in 80 min, from 20 to 30% buffer B in 20 min, from 30 to 90% buffer B in 5 min, and remaining at 90% buffer B for 10 min. The eluted neuropeptides were injected into the mass spectrometer via a nano-ESI source (Thermo Fisher Scientific). Ion signals were collected in a data-dependent mode and run with the following settings: full scan resolution at 70,000, automatic gain control (AGC) target: 3×10^6, maximum inject time (MIT): 20 ms, scan range: m/z 300-1,800; MS/MS scans resolution at 17,500, AGC target: 1×10^5, MIT: 60 ms, isolation window: 2 m/z, normalized collision energy: 27, loop count 10, and dynamic exclusion: charge exclusion: unassigned, 1, 8, >8; peptide match: preferred; exclude isotopes: on; dynamic exclusion: 30 s. Raw data were retrieved using Xcalibur 3.0 software (Thermo Fisher Scientific).

The extracted MS/MS spectra were searched against a composite database of *Apis mellifera* (23,491 protein sequences, downloaded from NCBI on July, 2018) or *Apis cerana* (20,934 protein sequences, downloaded from NCBI on July, 2018) using in-house PEAKS 8.5 software (Bioinformatics Solutions, Canada). Amidation (A, -0.98) and pyro-glu from Q (P, -17.03) were selected as variable modifications. The other parameters used were: parent ion mass tolerance, 20.0 ppm; fragment ion mass tolerance, 0.05 Da; enzyme, trypsin; allowing a nonspecific cleavage at both ends of the peptide; maximum missed cleavages per peptide, 2; maximum allowed variable PTM per peptide, 2. A fusion target-decoy approach was used for the estimation of the false discovery rate (FDR) and controlled at $\leq 1.0\%$ ($-10 \log P \geq 20.0$) both at protein and peptide levels. Neuropeptide identifications were only used if ≥ 2 spectra were identified in at least two of the three replicates of each sample type.

589 Quantitative comparison of brain neuropeptidomes was performed by the label-free approach
590 in PEAKS Q module. Feature detection was performed separately on each sample by using
591 the expectation-maximization algorithm. The features of the same peptide from different
592 samples were reliably aligned together using a high-performance retention time alignment
593 algorithm [86]. Peptide features were considered significantly different between experimental
594 groups if pairwise $p < 0.01$ and fold change ≥ 1.5. A heat map of differentially expressed
595 proteins was created by Gene cluster 3.0 using the unsupervised hierarchical clustering, and
596 the result was visualized using Java Tree view software. The LC−MS/MS data and search
597 results are deposited in ProteomeXchange Consortium
598 (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository with the
599 dataset identifier PXD018713.

600 **4.4. Characterization of honeybee tachykinin related peptide (TRP) signaling pathway**

601 To characterize honeybee TRP signaling pathway, the TRP receptor (TRPR) gene was first
602 cloned and expressed in human and insect cell lines to identify its cellular location and verify
603 its binding to TRPs. Additionally, these cells were used to test whether TRP/TRPR signaling
604 triggers intracellular cAMP accumulation, Ca^{2+} mobilization, and ERK phosphorylation.

605 2.4.1. TRPR gene clone and expression

606 To amplify the full-length sequence encoding *TRPR* of *Apis mellifera*, primers were designed
607 using Primer Premier 5.0 software (PREMIER Biosoft, USA) based on the sequence from
608 GenBank™ KT232312. The coding sequence of TRPR was amplified and cloned into
609 FLAG-tag expression vectors (pCMV-FLAG and pBmIE1-FLAG) and EGFP-tag expression
610 vectors (pEGFP-N1 and pBmIE1-EGFP). The primers used are documented in Table S11. All

611 constructs were sequenced to verify the correct sequence, orientation, and reading frame of
612 the inserts.

613 The human embryonic kidney cell line HEK293 and the insect *Spodoptera frugiperda*
614 pupal ovary cell line Sf21 were used for honeybee TRPR expression. HEK293 cells were
615 cultured in DMEM medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS).
616 Sf21 cells were cultured in TC100 medium (Gibco) supplemented with heat-inactivated 10%
617 FBS. Transfection of HEK293 cells was performed using Lipo6000™ transfection reagent
618 (Beyotime, China), while transfection of Sf21 cells was performed using LipoInsect™
619 transfection reagent (Beyotime), according to the manufacturer's instructions.

620 2.4.2. Cellular location of TRPR

621 To confirm the location of the honeybee TRPR, receptor surface expression assays were
622 performed. HEK293 or Sf21 cells expressing TRPR-EGFP were seeded onto poly-L-lysine
623 coated glass coverslips and allowed to attach overnight under normal growth conditions. After
624 24 hours, cells were incubated with the membrane probe DiI (Beyotime) and the nucleic acid
625 probe Hoechst 33342 (Beyotime) at 37°C for 10 min, then fixed with 4% paraformaldehyde
626 for 15 min. Cells transfected with empty EGFP-tag expression vectors were used as a control.
627 The cells were imaged using a Leica SP8 (Leica Microsystems, Germany) confocal
628 microscope equipped with an HC PL APO CS2 63×/1.40 oil objective. Images were acquired
629 with the sequence program in the Leica LAS X software.

630 2.4.3. Binding of TRPs to TRPR

631 To confirm the direct binding of the honeybee TRPs to TRPR, competitive binding
632 experiments were performed using synthesized TAMRA-TRP2 (TAMRA-ALMGFQGVRa)

and TAMRA-TRP3 (TAMRA-APMGFQGMRa), with TAMRA labeled at the N-terminus. The neuropeptides used as ligands in here and in later sections were commercially synthesized by SynPeptide Co, Ltd (China). All peptides were purified by reverse-phase high performance liquid chromatography with a purity > 98%, lyophilized, and diluted to the desired concentrations for subsequent experiments. The peptide sequences were verified by us using a Q-Exactive HF mass spectrometer (Thermo Fisher Scientific).

HEK293 and Sf21 cells expressing FLAG-TRPR were first seeded onto poly-L-lysine-coated 96-well plates and cultured overnight. On the next day, cells were washed once with phosphate-buffered saline (PBS), then incubated with 25 mL TAMRA-TRP2 or TAMRA-TRP3 (10 nM) in the presence of increasing concentrations of unlabeled TRP2 and TRP3 in a final volume of 100 mL of binding buffer (PBS containing 0.2% bovine serum albumin). Cells were incubated at room temperature for 2 hours. Fluorescence intensity was measured with a fluorescence spectrometer microplate reader (Tecan Infinite 200 PRO, Tecan, Switzerland) after washing twice with binding buffer. The cells transfected with empty FLAG-tag expression vectors were used as a control. The binding displacement curves were analyzed by GraphPad Prism 8.0 (GraphPad Software, USA) using the non-linear logistic regression method.

2.4.4. TRP/TRPR signaling targets: cAMP, Ca^{2+}, and ERK

To test whether TRP/TRPR signaling affects cAMP accumulation, intracellular cAMP was measured after incubation of HEK293 and Sf21 cells expressing FLAG-TRPR and pCRE-Luc with TRP2 and TRP3. After seeding in a 96-well plate overnight, HEK293 or Sf21 cells co-transfected with pFLAG-TRPR and pCRE-Luc were grown to about 90% confluence.

After washing once with PBS, cells were incubated with the neuropeptides TRP2, TRP3, short neuropeptide F (SNF), pigment-dispersing hormone (PDH), and corazonin (CRZ) in serum-free medium for 4 hours at 37°C for HEK293 cells, and at 28°C for Sf21 cells. Cells transfected with empty EGFP-tag expression vectors were used as a control. Luciferase activity was detected by a luciferase assay system (Promega, USA). Fluorescence intensity was measured with a Tecan fluorescence spectrometer. When characterizing the TRP-mediated cAMP accumulation, cells were pretreated with $G_{\alpha i}$ inhibitor pertussis toxin (PTX), $G_{\alpha s}$ activator cholera toxin (CTX), $G_{\alpha q}$ inhibitor YM-254890, and PKA inhibitor H89before stimulation with TRP2.

To test whether TRP signaling also affects intracellular Ca^{2+} concentrations, intracellular Ca^{2+} was measured after incubation of HEK293 and Sf21 cells expressing FLAG-TRPR with TRP2 or TRP3. Cells were detached by a non-enzymatic cell dissociation solution (Sigma-Aldrich, USA), washed twice with PBS, and resuspended at a density of 5×10^6 cells/ml in HEPES buffered saline (Macklin, China). Cells were then incubated with 3 μM Fura-2 AM (MedChemExpress, USA) for 30 min at 37°C for HEK293 cells, and at 28°C for Sf21 cells. Intracellular Ca^{2+} flux was measured using excitation wavelengths alternating at 340 and 380 nm with emission measured at 510 nm in a Tecan fluorescence spectrometer. When characterizing the detailed TRP-mediated intracellular Ca^{2+} mobilization, cells were pretreated with $G_{\alpha q}$ inhibitor YM-254890 and PLC inhibitor U73122 before stimulation with TRP2.

To assess whether TRP signaling mediates ERK1/2 signaling, ERK1/2 phosphorylation was measured by Western blot analysis after incubation of HEK293 and Sf21 cells expressing

677 FLAG-TRPR with TRP2. Cells were seeded in 24-well plates and starved for 4 hours in
678 serum-free medium to reduce background ERK1/2 activation and eliminate the effects of the
679 change of medium. After incubation with TRP2, cells were lysed by RIPA buffer (Beyotime)
680 at 4°C for 30 min. Protein concentration was determined according to the Bradford method
681 using BSA as the standard and the absorption was measured at 595 nm (spectrophotometer
682 DU800, Beckman Coulter, Los Angeles, CA), then all the samples were kept in -80°C for
683 further use. For Western blot, equal amounts of total cell lysate (20 μg/lane) were fractionated
684 by SDS-PAGE (10%) and transferred to a PVDF membrane (Millipore, USA) using an iBlot
685 dry blotting system (Invitrogen, USA). The membranes were blocked for 2 hours at room
686 temperature and then incubated with rabbit monoclonal anti-pERK1/2 antibody (Cell
687 Signaling Technology, USA) and anti-rabbit horseradish peroxidase-conjugated secondary
688 antibody (Cell Signaling Technology) according to the manufacturers' protocols. Antibody
689 reactive bands were visualized using Pierce™ ECL western blotting substrate (Thermo Fisher
690 Scientific, USA) followed by photographic film exposure. Total ERK1/2 was assessed as a
691 loading control after p-ERK1/2 chemiluminescence detection. Quantification analyses were
692 performed using Gel-Pro Analyzer 4.0 software (Media Cybernetics, USA).

693 To explore the detailed TRP-mediated ERK1/2 signaling, cells were pretreated with $G_{\alpha i}$
694 inhibitor pertussis toxin (PTX), MEK inhibitor U0126, PKA inhibitor H89, and PKC inhibitor
695 Go6983 before stimulation with TRP2.

696 **4.5. Effects of TRP2 injection on task-specific responsiveness**

697 To confirm the function of TPR on task-specific responsiveness, NBs, PFs, and NFs of AML
698 were injected with TRP2 and tested for their PER response to sucrose solution, pollen, and

699 larva. About 150 bees of each behavioral phenotype were collected in the morning, then
700 harnessed, fed and placed in a dark incubator as described in section 4.2. After 24 hours, all
701 surviving bees were evenly divided into two groups and injected with 1 μl TRP2 solution (1
702 μg/μl, synthesized TRP2 dissolved in ddH_2O) or 1 μl of ddH_2O into the head of honeybees via
703 the central ocellus using a glass capillary needle coupled to a microinjector. Bees injected
704 with ddH_2O were used as control. All injected bees were put back to the dark incubator and 1
705 hour after injection all surviving bees were assayed for their PER to stimulations of sucrose
706 solution, pollen, and larva as described in section 4.2. Each experiment was performed with a
707 new set of bees containing about 55 individuals per experimental and control group.

708 The average sucrose response scores of the TRP2 injection group and the ddH_2O
709 injection group were compared separately for each of the three behavioral phenotypes (NBs,
710 PFs, and NFs) using pairwise Mann-Whitney U tests. The sucrose responsiveness was further
711 compared between different groups at each specific sucrose concentration with independent
712 Chi-square tests. The responsiveness to pollen and larvae was compared between TRP2
713 injection group and ddH_2O injection group with independent Chi-square tests for each
714 behavioral phenotype separately. All statistical analyses were performed with SPSS Statistics
715 20.0 (IBM).

716 **4.6. Effects of RNAi-mediated downregulation of *TRP* or *TRPR* on responsiveness**

717 To further confirm the hypothesized effects of TPR/TRPR signaling on task-specific
718 responsiveness, RNAi-mediated downregulation of *TRP* and *TRPR* were performed on NBs,
719 PFs, and NFs of AML and then their PER to sucrose solution, pollen, and larva were
720 compared to controls.

721 Before evaluating the behavioral effects of transcript knockdown of *TRP* or *TRPR*,
722 preliminary experiments were performed to test the dsRNA-mediated knockdown efficiencies
723 of *TRP* and *TRPR*. The dsRNAs of the *TRP* and *TRPR* genes were prepared using the T7
724 RiboMAX Express RNAi system (Promega). The primers used are listed in Table S11. Sixty
725 bees were randomly collected from each of the three AML colonies. Bees were harnessed, fed
726 with sucrose and put into the dark incubator (20°C and 65% relative humidity) to acclimatize
727 to the experimental conditions. After 30 min, dsRNA (200 ng/bee for *TRP*, 2 μg/bee for *TRPR*)
728 was microinjected into the head of honeybees via the central ocellus using a glass capillary
729 needle coupled microinjector. dsRNA of green fluorescent protein gene (ds*GFP*, 2 μg/bee)
730 was used as control in all RNAi experiments. All harnessed bees were fed with 50% sucrose
731 solution every 12 hours. At 0, 12, 24, and 48 hours after injection, a group of 6 individual
732 bees were collected from each injection group (ds*TRP*, ds*TRPR*, and ds*GFP*) for comparing
733 *TRP* and *TRPR* expression. Individual brains were carefully dissected and frozen at -80°C
734 until RNA extraction. Three independent replicate groups per condition were collected and
735 qRT-PCR was performed to calculate the RNAi efficiency. Total RNA was isolated using
736 TRIzol reagent (Takara, Japan). Total RNA quantification was performed by NanoDrop 2000
737 spectrophotometer (Thermo Fisher Scientific), and the quality of RNA was evaluated by 1.0%
738 denaturing agarose gel electrophoresis. Reverse transcription was performed using a
739 PrimeScript^{TM} RT reagent kit (Takara), according to the manufacturer's instructions.
740 Gene-specific mRNA levels were assessed by qPCR using TB Green Fast qPCR Mix (Takara)
741 on a LightCycler 480II instrument (Roche, Switzerland). The *β-actin* gene was used as a
742 reference gene. After verifying amplification efficiency of the selected genes and β-actin

743 (from 96.8% to 100.5%), the differences in gene expression levels were calculated using the
744 $2^{-\Delta\Delta Ct}$ method. Pairwise differences in gene expression were considered significant at $p < 0.05$,
745 using one-way ANOVA (SPSS Statistics 20.0). The primers used for qPCR are shown in
746 Table S11.

747 After determination of knockdown efficiencies (see results), 24 hours post-injection was
748 chosen as the timepoint to study the PER effects of dsRNA-mediated knockdown of *TRP* and
749 *TRPR*. About 200 bees of each behavioral phenotype (NBs, PFs, and NFs of AML) were
750 collected in the morning, harnessed, and remained in a dark incubator to acclimatize. After 30
751 min, all surviving bees of each behavioral phenotype were evenly divided into three groups,
752 injected with ds*TRP*, ds*TRPR*, and ds*GFP* and kept as described above. After 24 hours, all
753 surviving bees were assayed for their PER to stimulations of sucrose solution, pollen, or
754 larvae as described in section 4.2. Each stimulus was assessed with a new set of bees
755 containing about 55 individuals for each treatment group (ds*TRP*, ds*TRPR*, and ds*GFP*). The
756 SRSs of the *TPR*-knockdown, *TRPR*-knockdown, and control groups were compared using
757 Kruskal-Wallis tests with Bonferroni correction for each behavioral phenotype separately.
758 The sucrose responsiveness was further compared between the different groups at the same
759 sucrose concentration with independent Chi-square tests. The responsiveness to pollen and
760 larvae was compared between the *TPR*-knockdown, *TRPR*-knockdown, and control groups
761 using independent Chi-square tests for each behavioral phenotype separately. All statistical
762 analyses were performed with SPSS Statistics 20.0 (IBM).

4.7. Effects of TRP2 injection and RNAi-mediated downregulation of *TRP* and *TRPR* on ERK signaling in honeybee workers

To test whether manipulating TRP/TRPR signaling has effect on honeybee ERK signaling a group of 10 individual worker bees were collected from each injection group (ddH_2O, TRP2, ds*TRP*, ds*TRPR*, and ds*GFP*) to compare ERK phosphorylation levels. Three independent replicate groups per condition were collected and Western blot analyses were performed: Honeybeebrains were carefully dissected and frozen at -80°C until protein extraction. Brain protein extractions were carried out according to our previously described method with some modifications. Briefly, the larvae were homogenized with lysis buffer (LB, 8 M urea, 2 M thiourea, 4% CHAPS, 20 mM Tris-base, 30 mM dithiothreitol). The mixture was homogenized for 30 min on ice and sonicated 20 s per 5 min during this time, then centrifuged at 12 000g and 4 °C for 10 min. Ice-cold acetone were added to the collected supernatants, and then the mixture was kept on ice for 30 min for protein precipitation. Subsequently, the mixture was centrifuged at 12 000g and 4 °C for 10 min. The supernatant was discarded and the pellets were resolved in LB and kept at -20°C for further use. Western blot analyses were performed as described in section 4.4.4.

Data Availability

Original data have been deposited to ProteomeXchange Consortium with the dataset identifier PXD018713 under http://proteomecentral.proteomexchange.org. Other data not provided in the supplementary materials and materials are available from the first author upon request.

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Competing Interests

None of the authors have any competing interests.

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Supplementary Materials

Fig. S1. Efficiencies of dsRNA-mediated knockdown of *TRP* and *TRPR*.

Table S1. The proboscis extension response of different behavioral phenotypes to different sucrose solutions.

Table S2. Statistical differences in sucrose responsiveness of different behavioral phenotypes.

Table S3. Neuropeptides identified in the brain of *Apis mellifera ligustica* workers.

Table S4. Neuropeptides identified in the brain of *Apis cerana cerana* workers.

Table S5. Quantitative neuropeptide comparison of different behavioral phenotypes of *Apis*

1024 *mellifera ligustica* workers.

1025 Table S6. Quantitative neuropeptide comparison of different behavioral phenotypes of *Apis*
1026 *cerana cerana* workers.

1027 Table S7. Quantitative neuropeptide comparison between *Apis cerana cerana* and *Apis mellifera*
1028 *ligustica*.

1029 Table S8. The proboscis extension response of workers after injection of ddH_2O and TRP2.

1030 Table S9. The proboscis extension response of workers after injection of ds*GFP*, ds*TRP*, and
1031 ds*TRPR*.

1032 Table S10. Statistical differences in sucrose responsiveness after injection of ds*GFP*, ds*TRP*, and
1033 ds*TRPR*.

1034 Table S11. Sequence information of primers used in this study.

Fig S1

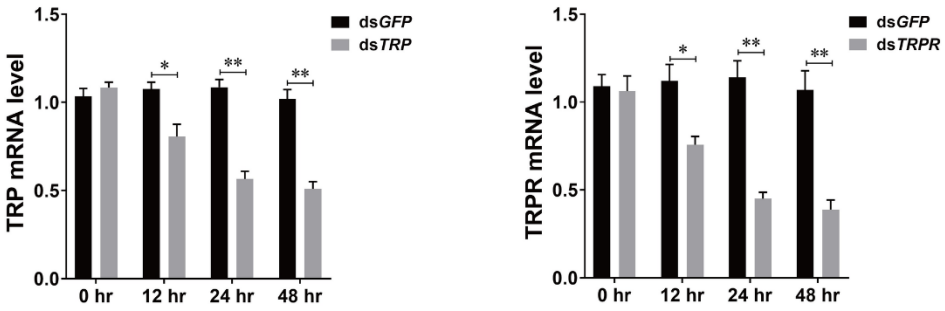

Fig S1. Efficiencies of dsRNA-mediated knockdown of *TRP* and *TRPR*. dsRNA (200 ng/bee for *TRP*, 2 μg/bee for *TRPR*) was microinjected into the head of honeybees via the central ocellus using a microinjector. dsRNA of green fluorescent protein gene (ds*GFP*, 2 μg/bee) was used as control. At 0, 12, 24, and 48 hours after injection, a group of 6 individual bees were collected from each injection group. Three independent replicate groups per condition were collected and qRT-PCR was performed to calculate the RNAi efficiency. Student's t-tests were used for pairwise comparisons (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

Table S1. The proboscis extension response of different behavioral phenotypes to different sucrose solutions. The proboscis extension response of *Apis mellifera ligustica* (AML) and *Apis cerana cerana* (ACC) worker bees to different sucrose solutions.

AML pollen foragers

Concentration	Show PER	No PER	PER ratio
0.1%	48	79	37.80%
0.3%	51	76	40.16%
1.0%	70	57	55.12%
3.0%	83	44	65.35%
10.0%	87	40	68.50%
30.0%	111	16	87.40%
Pollen	32	50	39.02%
Larva	17	65	20.73%

ACC pollen foragers

Concentration	Show PER	No PER	PER ratio
0.1%	33	92	26.40%
0.3%	35	90	28.00%
1.0%	51	74	40.80%
3.0%	59	66	47.20%
10.0%	68	57	54.40%
30.0%	98	27	78.40%
Pollen	20	66	23.26%
Larva	11	75	12.79%

AML nectar foragers

Concentration	Show PER	No PER	PER ratio
0.1%	23	107	17.69%
0.3%	33	97	25.38%
1.0%	38	92	29.23%
3.0%	44	86	33.85%
10.0%	59	71	45.38%
30.0%	68	62	52.31%
Pollen	11	74	12.94%
Larva	15	70	17.65%

ACC nectar foragers

Concentration	Show PER	No PER	PER ratio
0.1%	17	111	13.28%
0.3%	19	109	14.84%
1.0%	23	105	17.97%
3.0%	29	99	22.66%
10.0%	44	84	34.38%
30.0%	55	73	42.97%
Pollen	8	77	9.41%
Larva	9	76	10.59%

AML nurse bees

Concentration	Show PER	No PER	PER ratio
0.1%	30	106	22.06%
0.3%	32	104	23.53%
1.0%	45	91	33.09%
3.0%	50	86	36.76%
10.0%	57	79	41.91%
30.0%	75	61	55.15%
Pollen	9	82	9.89%
Larva	36	55	39.56%

ACC nurse bees

Concentration	Show PER	No PER	PER ratio
0.1%	18	113	13.74%
0.3%	19	112	14.50%
1.0%	30	101	22.90%
3.0%	38	93	29.01%
10.0%	48	83	36.64%
30.0%	58	73	44.27%
Pollen	7	81	7.95%
Larva	22	66	25.00%

Table S2. Statistical differences in sucrose responsiveness of different behavioral phenotypes.

Concentration	0.10%	0.30%	1.00%	3.00%	10.00%	30.00%
AML						
PF vs NF	***	*	***	***	***	***
PF vs NB	**	**	***	***	***	***
NF vs NB	ns	ns	ns	ns	ns	ns
ACC						
PF vs NF	**	**	***	***	**	***
PF vs NB	*	**	**	**	**	***
NF vs NB	ns	ns	ns	ns	ns	ns
AML vs ACC						
PF	ns	*	*	**	*	ns
NF	ns	*	*	*	ns	ns
NB	ns	ns	ns	ns	ns	ns

AML: *Apis mellifera ligustica*, ACC: *Apis cerana cerana*, PF: pollen forager, NF: nectar forager, NB: nurse bee. ns = $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Table S3. Neuropeptides identified in the brain of *Apis mellifera ligustica* workers. "NB" is nurse bee. "PF" is pollen forager. "NF" is nectar forager. "Protein Accession" is the unique number given to mark the entry of a protein in the database NCBInr. "Peptide" is the amino acid sequence of the peptide as determined in PEAKS Search. "-10lgP" is the score indicates the scoring significance of a peptide-spectrum match. "Mass" is monoisotopic mass of the peptide. "ppm" is precursor mass error, calculated as 10^6 × (precursor mass - peptide mass) / peptide mass. "m/z" is precursor mass-to-charge ratio. "z" is peptide charge. "RT" is retention time (elution time) of the spectrum as recorded in the data. "#Spec" is the number of scanned spectrums of the peptide. "PTM" is post translational modification types present in the peptide.

Sample	Protein Accession	Peptide	10lgP	Mass	ppm	m/z	z	RT	#Spec	PTM
NB	Q868G6.1	NSIINDVKNELFPEDIN	67.29	1972.974	-0.3	987.494	2	98.51	10	
NB	Q868G6.1	VLSMDGYQNILDKKDELLGEWE	61.58	2594.257	-7	1298.127	2	96.42	10	
NB	A8CL69.1	pQLHNIVDKPRQN	51.73	1443.758	0.7	482.2603	3	13.7	6	Pyro-glu from Q
NB	A8CL69.1	pQLHNIVDKPRQNFNDPRF	51.12	2220.119	0.3	556.0372	4	41.34	6	Pyro-glu from Q
NB	A8CL69.1	TSQDITSGMWFGPRLa	47.39	1693.825	0.1	847.9196	2	80.85	11	Amidation
NB	A8CL69.1	pQLHNIVDKP	45.99	1045.556	0.8	523.7855	2	23.83	4	Pyro-glu from Q
NB	A8CL69.1	GMWFGPRLa	33.41	961.4956	0	481.7551	2	68.13	9	Amidation
NB	A8CL69.1	RVPWTPSPRLa	30.85	1206.699	0.3	604.3567	2	25.21	6	Amidation
NB	A8CL69.1	pQITQFTPRL	27.13	1085.587	-0.1	543.8007	2	78.09	3	Pyro-glu from Q
NB	A8CL69.1	MWFGPRLa	26.77	904.4741	-0.5	453.2441	2	71.2	5	Amidation
NB	A8CL69.1	QITQFTPRLa	25.1	1101.63	0.5	551.8223	2	34.24	12	Amidation
NB	A8CL69.1	pQITQFTPRLa	37.99	1084.603	0	543.3087	2	69.95	21	Pyro-glu from Q; Amidation
NB	ACI90290.1	TWKSPDIVIRFa	50.93	1359.766	-0.3	454.2625	3	59.62	13	Amidation
NB	ACI90290.1	GRNDLNFIRYa	48.35	1265.663	-0.1	633.8386	2	33.11	11	Amidation
NB	NP_001161192.1	PEIFTSPEELRRYIDHVSDYYLLSGKARYa	43.49	3515.784	0.4	586.9714	6	95.9	5	Amidation
NB	P85527.1	QDVDHVFLRFa	55.21	1273.657	0	637.8356	2	50.66	9	Amidation

NB	P85527.1	pQDVDHVFLRFa	53.95	1256.63	-0.7	629.3219	2	74.39	15	Pyro-glu from Q; Amidation
NB	P85527.1	pQDVDHVFLRF	47.89	1257.614	0.8	629.8148	2	79.69	5	Pyro-glu from Q
NB	P85527.1	pQDVDHVFLR	47.86	1110.546	-0.5	556.2799	2	43.23	7	Pyro-glu from Q
NB	P85527.1	pQDVDHVFL	28.42	954.4447	1.8	478.2305	2	69.45	5	Pyro-glu from Q
NB	P85798.1	LRNQLDIGDLQ	50.23	1283.683	-0.5	642.8486	2	42.48	10	
NB	P85798.1	IPAADKERLLN	47.66	1238.698	0.9	620.3569	2	15.09	6	
NB	P85798.1	LRNQLDIGDL	38.2	1155.625	0	578.8196	2	51.53	5	
NB	P85799.1	SQAYDPYSNAAQFQLSSQSRGYPYQHRLVY	71.75	3523.655	-0.6	881.9204	4	60.92	51	
NB	P85799.1	SQAYDPYSNAAQFQLSSQSRGYPYQHRLV	70.34	3360.591	-0.4	841.1547	4	57.58	11	
NB	P85799.1	SQAYDPYSNAAQFQLSSQSRGYPYQHRL	64.21	3261.523	-1.2	816.387	4	53.22	8	
NB	P85799.1	LPTNLAEDTKKTEQTMRPKS	55.28	2287.184	0.1	572.8033	4	21.04	22	
NB	P85799.1	GYPYQHRLVY	49.18	1294.646	0.4	648.3304	2	25.22	15	
NB	P85799.1	NVPIYQEPRF	46	1261.646	-0.3	631.8298	2	46.32	11	
NB	P85799.1	VPIYQEPRF	43.34	1147.603	-0.1	574.8085	2	42.88	6	
NB	P85799.1	PIYQEPRF	28.55	1048.534	0.4	525.2746	2	46.03	7	
NB	P85828.1	ITGQGNRIF	46.68	1004.54	-0.7	503.2771	2	19.63	8	
NB	P85828.1	SLKAPFA	41.78	732.417	-0.6	367.2155	2	22.72	5	
NB	P85828.1	SLKAPF	36.02	661.3799	0.3	331.6973	2	23.77	4	
NB	P85829.1	MVPVPVHHMADELLRNGPDTVI	62.4	2439.24	0	1220.627	2	76.66	17	
NB	P85829.1	VHHMADELLRNGPDTVI	49.65	1915.957	0.1	639.6598	3	46.23	8	
NB	P85829.1	VPVPVHHMADELL	43.49	1455.754	1.3	728.8854	2	47.73	7	
NB	P85829.1	LLRNGPDTVI	35	1096.624	0.2	549.3194	2	28.64	5	
NB	P85829.1	LRNGPDTVI	22.27	983.54	0.5	492.7775	2	16.98	6	
NB	P85830.1	GLDLGLSRGFSGSQAAKHLMGLAAANYAGGPa	70.62	2985.524	-0.9	996.1811	3	84.68	9	Amidation

NB	P85830.1	GLDLGLSRGFSGSQAA	62.96	1534.774	1.2	768.3951	2	55.2	6	
NB	P85830.1	GLDLGLSRGFSGSQAAKH	53.33	1799.928	-0.5	600.9829	3	31.47	10	
NB	P85830.1	GLDLGLSRGFSGSQAAKHLMa	46.31	2043.068	1	682.0308	3	57.54	8	Amidation
NB	P85831.1	IDLSRFYGHFNT	60.52	1468.71	-0.3	735.362	2	64.92	29	
NB	P85831.1	IDLSRFYGHFN	56.71	1367.662	-0.1	684.8383	2	62.93	18	
NB	P85831.1	IDLSRFYGHF	52.75	1253.619	-0.6	627.8165	2	70.59	19	
NB	P85831.1	IDLSRFYGHFNTKR	48.89	1752.906	0	439.2337	4	43.28	23	
NB	P85831.1	FYGHFNT	44.93	884.3817	-0.2	443.1981	2	21.44	7	
NB	P85831.1	DLSRFYGHF	25.85	1140.535	0.2	571.275	2	70.16	3	
NB	P85831.1	DLSRFYGHFN	20.23	1254.578	0.4	628.2966	2	62.68	22	
NB	P85832.1	LTNYLATTGHGTNTGGPVLT	82.04	1987.001	-1.4	994.5065	2	47.57	22	
NB	P85832.1	LTNYLATTGHGTNTGGPVL	69.52	1885.953	-0.6	943.9834	2	52.3	4	
NB	P85832.1	NLDEIDRVGWSGFV	62.73	1605.779	0.3	803.8969	2	88.41	3	
NB	P85832.1	LTNYLATTGHGTNTGGPVLTRRFa	49.49	2445.288	-0.4	816.1028	3	39.57	13	Amidation
NB	P85832.1	NIDEIDRTAFDNFF	46.68	1715.779	-1.2	858.8958	2	96.46	9	
NB	P85832.1	LVDELSPVSERETLERFa	33.35	2017.048	0.3	673.3568	3	63.4	7	Amidation
NB	P85832.1	ELVDELSPVSERETLERFa	30.33	2146.091	0.6	716.3712	3	74.41	9	Amidation
NB	Q06601.1	GNNRPVYIPQPRPPHPRL	33.24	2107.155	0.3	422.4384	5	25.01	15	
NB	Q06601.1	VYIPQPRPPHPRL	23.32	1568.894	-0.2	393.2307	4	29.92	20	
NB	Q06601.1	AVHYSGGQPLGSKRPNDMLSQRYHFGLa	65.31	3013.509	-1.6	754.3834	4	38.67	9	Amidation
NB	Q06601.1	PNDMLSQRYHFGLa	66.71	1575.762	0.3	526.2613	3	56.93	13	Amidation
NB	Q06601.1	AYTYVSEYKRLPVYNFGIa	29.98	2181.126	-0.2	728.0491	3	72.4	8	Amidation
NB	Q06601.1	ADYPLRLNLD	48.67	1188.614	0	595.3142	2	56.85	11	
NB	Q06601.1	YPLRLNLD	43.48	1002.55	0.6	502.2825	2	49.18	8	
NB	Q06601.1	RQYSFGLa	31.09	868.4555	0	435.235	2	26.91	10	Amidation

NB	Q06601.1	GRQPYSFGLa	35.27	1022.53	-0.1	512.2721	2	32.39	6	Amidation
NB	Q06601.1	GRDYSFGLa	31.03	912.4453	0	457.2299	2	30.51	3	Amidation
NB	Q06601.1	WIDTNDNKRGRDYSFGLa	29.02	2054.992	0	686.0047	3	38.47	7	Amidation
NB	Q06601.1	LDYLPVDNPAFH	51.58	1399.677	0.6	700.8463	8	65.51	4	
NB	Q06602.1	EAEPEAEPGNNRPVYIPQPRPPHPRL	50.05	2959.505	-0.5	592.908	5	33.75	26	
NB	Q06602.1	GNNRPVYIPQPRPPHPRL	33.24	2107.155	0.3	422.4384	5	25.01	15	
NB	Q06602.1	VYIPQPRPPHPRL	23.32	1568.894	-0.2	393.2307	4	29.92	20	
NB	Q5DW47.1	STSLEELANR	39.7	1118.557	0.9	560.2861	2	24.18	4	
NB	Q5DW47.1	STSLEELANRN	38.16	1232.6	0.7	617.3075	2	23.07	5	
NB	Q5DW47.1	pQTFTYSHGWTNa	18.99	1322.568	-0.1	662.2912	2	51.14	10	Pyro-glu from Q; Amidation
NB	Q868G6.1	ASFDDEYYKRAPMGFQGMRa	55.4	2267.025	0.5	567.7639	4	45.63	9	Amidation
NB	Q868G6.1	APMGFQGMRG	50.96	1050.474	0	526.2442	2	22.9	6	
NB	Q868G6.1	GVMDFQIGLQ	50.62	1106.543	1	554.2793	2	85.81	6	
NB	Q868G6.1	APMGFQGMRa	49.03	992.4684	-1.1	497.241	2	18.87	16	Amidation
NB	Q868G6.1	VLSMDGYQNILD	47.52	1366.644	0.6	684.3296	2	80.98	15	
NB	Q868G6.1	NPRWEFRGKFVGVRa	47.04	1745.959	-0.2	437.4969	4	24.64	9	Amidation
NB	Q868G6.1	ARMGFHGMRa	46.29	1060.517	-0.4	354.5128	3	8.86	3	Amidation
NB	Q868G6.1	ALMGFQGVRG	46.07	1034.533	0.1	518.2739	2	35.2	6	
NB	Q868G6.1	SPFRYLGA	45.4	909.4708	0	455.7427	2	36.86	10	
NB	Q868G6.1	APMGFYGTRa	45.2	997.4803	-0.1	499.7474	2	16.94	3	Amidation
NB	Q868G6.1	APMGFYGTRG	45.18	1055.486	0.4	528.7504	2	20.63	7	
NB	Q868G6.1	ALMGFQGVRa	44.3	976.5276	-0.5	489.2709	2	29.63	13	Amidation
NB	Q868G6.1	SPFRYLGARG	44.18	1122.593	-0.4	375.2049	3	20.59	11	
NB	Q868G6.1	GVMDFQIGLQRKKD	44.03	1633.861	-0.2	817.9376	2	35.7	14	
NB	Q868G6.1	SPFRYLGARa	43.32	1064.588	0.2	355.87	3	16.59	8	Amidation

NB	Q868G6.1	NPRWEFRGKFVGV	42.84	1590.842	0.1	531.288	3	43.57	15	
NB	Q868G6.1	SPFRYLG	37.59	838.4337	0	420.2241	2	31.97	7	
NB	Q868G6.1	SLEEILDEIK	33.02	1187.629	0	594.8215	2	88.28	6	
NB	Q868G6.1	SLEEILDEI	29.37	1059.534	0.1	530.7741	2	108.02	4	
NB	Q868G6.1	ASFDDEYY	28.99	1008.371	0	505.1929	2	43.35	4	
NB	XP_006557714.1	pQQFDDYGHLRFa	47.97	1406.637	-2.3	704.324	2	68.3	4	Pyro-glu from Q; Amidation
NB	XP_006559359.1	NVASLARTYTLPQNAa	64.35	1616.863	-1.3	809.4379	2	43.18	6	Amidation
NB	XP_006559359.1	SVSSLAKNSAWPVSL	62.69	1544.82	-1.3	773.4162	2	68.52	8	
NB	XP_006559359.1	FLLLPATDNNYFHQKLPSSLRSKSL	56.55	2888.555	1	578.7188	5	71.13	15	
NB	XP_006559359.1	NVGSVAREHGLPYa	55.04	1396.721	-0.8	699.3672	2	21.03	15	Amidation
NB	XP_006559359.1	SVSSLARTGDLPVREQ	53.68	1713.901	0.5	572.3079	3	25.97	12	
NB	XP_006559359.1	YVASLARTGDLPIRGQ	51.94	1715.932	0.4	572.9847	3	35.62	12	
NB	XP_006559359.1	NIASLMRDYDQSRENRVPFPa	47.38	2406.186	0.1	803.0695	3	63.84	12	Amidation
NB	XP_006559359.1	HIGALARLGWLPSLRTA	42.32	1831.058	-0.2	611.3598	3	70.88	7	
NB	XP_006559359.1	HIGALARLGWLPSLRTARFS	42	2221.26	-0.4	556.322	4	71.36	9	
NB	XP_006559359.1	NVGTLARDFALPPa	40.53	1368.751	-0.1	685.3829	2	60.79	16	Amidation
NB	XP_006559359.1	YVASLARTGDLPIRa	40.29	1529.868	0.6	510.9635	3	34.02	8	Amidation
NB	XP_006559359.1	GIFLPGSVILR	37.83	1170.712	-0.1	586.3634	2	77.93	5	
NB	XP_006559359.1	LPGSVILRALS	36.35	1124.692	2.1	563.3543	2	72.8	8	
NB	XP_006559359.1	GIFLPGSVILRALSRQa	36.3	1725.041	-0.9	576.0205	3	95.14	10	Amidation
NB	XP_006559359.1	NVGTLARDFALPPGRRNIASLMRDYDQSRENRVPFPa	21.57	4127.135	0.2	688.8632	6	75.08	9	Amidation
NB	XP_006559865.1	AFGLLTYPRIa	40.74	1148.671	0.5	575.3428	2	70.98	6	Amidation
NB	XP_006559865.1	SNAPISNLNFN	35.48	1189.573	0.3	595.7938	2	48.7	4	
NB	XP_006559865.1	EKLKPNMRRAFGLLTYPRIa	28.33	2301.325	0.6	576.339	4	50.2	8	Amidation

NB	XP_006560385.1	AYRKPPFNGSIFa	42.26	1394.746	-0.5	698.3799	2	36.92	12	Amidation
NB	XP_006560385.1	KPPFNGSIFa	39.41	1004.544	0.2	503.2795	2	43.74	6	Amidation
NB	XP_006560385.1	RKPPFNGSIFa	32.35	1160.645	0	581.33	2	28.38	7	Amidation
NB	XP_006560385.1	YRKPPFNGSIFa	25.22	1323.709	0.5	662.862	2	36.42	6	Amidation
NB	XP_006562922.1	GFKPEYISTAYGFa	40.22	1477.724	0.2	739.8695	2	64.18	4	Amidation
NB	XP_006565207.1	SDPHLSILSKPMSAIPSYKFDD	81.44	2447.204	0.4	816.7423	3	71.96	17	
NB	XP_006565207.1	SPSLRLRFa	40.12	973.5821	0.2	487.7984	2	24.53	13	Amidation
NB	XP_006565207.1	SDPHLSILS	39.05	967.4974	0.4	484.7562	2	34.74	7	
NB	XP_006565207.1	SQRSPSLRLRFa	38.06	1344.774	0.4	449.2654	3	16.83	10	Amidation
NB	XP_006565207.1	SDPHLSILSKPMSAIP	32.57	1691.892	-1.1	846.9521	2	64.26	4	
NB	XP_006570344.1	NSELINSLLGLPKNMNNAa	65.94	1940.015	0.5	971.0152	2	87.45	11	Amidation
NB	XP_006570344.1	LINSLLGLPKNMNNAa	35.9	1609.897	1.1	805.9568	2	62.46	6	Amidation
NB	XP_016769998.1	LVDHRIPDLENEMFDSGNDPGSTVVRT	78.07	3012.425	0.1	754.1135	4	63.86	16	
NB	XP_016769998.1	HPISYNTYDERELSRDHPPLLL	30.5	2664.33	-1.6	667.0886	4	54.55	3	
NB	XP_016769998.1	IGSLSIVNSMDVLRQRVLLELARRKALQD QAQIDANRRLLETIa	27.71	4913.782	-0.3	819.9707	6	98.39	12	Amidation
PF	Q868G6.1	NSIINDVKNELFPEDIN	48.21	1972.974	-0.3	987.494	2	100.35	14	
PF	A8CL69.1	TSQDITSGMWFGPRLa	42.05	1693.825	0.5	847.92	2	79	22	Amidation
PF	A8CL69.1	pQLHNIVDKPRQN	37.86	1443.758	0.3	482.2602	3	14.35	3	Pyro-glu from Q
PF	A8CL69.1	pQLHNIVDKP	36.78	1045.556	0	523.7851	2	25.52	6	Pyro-glu from Q
PF	A8CL69.1	pQITQFTPRLa	29.72	1084.603	0.4	543.309	2	68.45	3	Pyro-glu from Q; Amidation
PF	A8CL69.1	RVPWTPSPRLa	25.44	1206.699	1.6	604.3575	2	28.86	5	Amidation
PF	A8CL69.1	pQLHNIVDKPRQNFNDPRF	23.63	2220.119	-0.9	556.0365	4	47.19	4	Pyro-glu from Q
PF	A8CL69.1	QITQFTPRLa	22.79	1101.63	-0.5	551.8218	2	37.4	7	Amidation
PF	A8CL69.1	MWFGPRLa	20.27	904.4741	-0.2	453.2443	2	75.27	10	Amidation

PF	A8CL69.1	GMWFGPRLa	17.98	961.4956	0.7	481.7554	2	72.51	12	Amidation
PF	ACI90290.1	TWKSPDIVIRFa	42	1359.766	0.1	454.2627	3	63.04	13	Amidation
PF	ACI90290.1	GRNDLNFIRYa	36.79	1265.663	0.2	633.8388	2	37.18	24	Amidation
PF	ACI90290.1	AGFKNLNREQ	35.46	1175.605	0	392.8755	3	10.1	6	
PF	ACI90290.1	SPDIVIRFa	28.69	944.5443	-1.1	473.2789	2	51.32	7	Amidation
PF	NP_001161192.1	PEIFTSPEELRRYIDHVSDYYLLSGKARYa	45.15	3515.784	0.4	586.9714	6	95.9	8	Amidation
PF	P85527.1	pQDVDHVFLRFa	43.1	1256.63	-0.6	629.322	2	76.1	19	Pyro-glu from Q; Amidation
PF	P85527.1	pQDVDHVFLR	40.39	1110.546	0.1	556.2802	2	44.76	7	Pyro-glu from Q
PF	P85527.1	QDVDHVFLRFa	40.1	1273.657	0.1	637.8357	2	54.09	9	Amidation
PF	P85527.1	pQDVDHVFLRF	38.93	1257.614	0	629.8143	2	82.73	8	Pyro-glu from Q
PF	P85527.1	pQDVDHVFL	27.57	954.4447	0.2	478.2297	2	70.88	3	Pyro-glu from Q
PF	P85798.1	LRNQLDIGDLQ	38.97	1283.683	-1	642.8483	2	44.49	12	
PF	P85798.1	IPAADKERLLN	33.42	1238.698	1.2	413.9072	3	15.96	6	
PF	P85798.1	LRNQLDIGDL	31.19	1155.625	0.3	578.8198	2	54.4	11	
PF	P85799.1	SQAYDPYSNAAQFQLSSQSRGYPYQHRLVY	51.05	3523.655	-0.1	881.9208	4	64.95	41	
PF	P85799.1	SQAYDPYSNAAQFQLSSQSRGYPYQHRLV	42.31	3360.591	0.4	841.1554	4	59.88	14	
PF	P85799.1	LPTNLAEDTKKTEQTMRPKS	37.87	2287.184	0.3	572.8035	4	24.72	28	
PF	P85799.1	SQAYDPYSNAAQFQLSSQSRGYPYQHRL	37.71	3261.523	-3.4	816.3852	4	56.14	10	
PF	P85799.1	GYPYQHRLVY	37.44	1294.646	0.3	648.3304	2	29.02	28	
PF	P85799.1	NVPIYQEPRF	37.08	1261.646	-1.1	631.8293	2	48.46	16	
PF	P85799.1	VPIYQEPRF	35.84	1147.603	-0.1	574.8085	2	45.61	13	
PF	P85799.1	PIYQEPRF	25.33	1048.534	0.1	525.2744	2	49.81	6	
PF	P85828.1	ITGQGNRIF	37.41	1004.54	-0.8	503.277	2	21.59	21	
PF	P85828.1	SLKAPFA	34.04	732.417	0.1	367.2158	2	24.36	11	

PF	P85828.1	SLKAPF	29.98	661.3799	0.1	331.6973	2	26.29	15	
PF	P85829.1	MVPVPVHHMADELLRNGPDTVI	36.48	2439.24	0.7	814.0879	3	81.53	22	
PF	P85829.1	VHHMADELLRNGPDTVI	33.81	1915.957	1.1	639.6605	3	51.24	10	
PF	P85829.1	LLRNGPDTVI	31.63	1096.624	0.4	549.3195	2	30.43	6	
PF	P85829.1	LRNGPDTVI	28.06	983.54	0.1	492.7773	2	17.56	6	
PF	P85829.1	VPVPVHHMADELL	20.81	1455.754	0.4	486.2589	3	51.21	6	
PF	P85830.1	GLDLGLSRGFSGSQAAKHLMGLAAANYAGGPa	46.77	2985.524	-0.3	747.3881	4	90.26	20	Amidation
PF	P85830.1	GLDLGLSRGFSGSQAA	43.99	1534.774	0.5	768.3947	2	57.69	5	
PF	P85830.1	HLMGLAAANYAGGPa	32.71	1340.666	-0.7	671.3398	2	41.33	7	Amidation
PF	P85830.1	GLDLGLSRGFSGSQAAKHLMa	31.39	2043.068	0.5	682.0304	3	61.51	6	Amidation
PF	P85830.1	GLDLGLSRGFSGSQAAKH	28.75	1799.928	0.1	600.9833	3	34.79	8	
PF	P85831.1	IDLSRFYGHFNT	45.95	1468.71	-0.5	735.3618	2	69.86	42	
PF	P85831.1	IDLSRFYGHFN	42.44	1367.662	-1.9	684.8371	2	65.48	27	
PF	P85831.1	IDLSRFYGHF	41.39	1253.619	-0.6	627.8165	2	74.6	23	
PF	P85831.1	FYGHFNT	36.1	884.3817	0.6	443.1984	2	23.94	9	
PF	P85831.1	DLSRFYGHFN	34.65	1254.578	0.1	628.2964	2	65.54	4	
PF	P85831.1	IDLSRFYGHFNTKR	32.23	1752.906	-0.1	439.2337	4	49.93	10	
PF	P85832.1	LTNYLATTGHGTNTGGPVLT	52.74	1987.001	0.2	994.5081	2	50.02	12	
PF	P85832.1	NLDEIDRVGWSGFV	47.92	1605.779	0	803.8966	2	93.23	14	
PF	P85832.1	LTNYLATTGHGTNTGGPVL	45.56	1885.953	0.3	943.9843	2	54.77	5	
PF	P85832.1	NIDEIDRTAFDNFF	43.44	1715.779	1.3	858.8979	2	98.86	7	
PF	P85832.1	LTNYLATTGHGTNTGGPVLTRRFa	37.72	2445.288	0.5	612.3295	4	44.18	11	Amidation
PF	P85832.1	ELVDELSPVSERETLERFa	32.49	2146.091	0.9	716.3715	3	77.64	9	Amidation
PF	P85832.1	LVDELSPVSERETLERFa	31.14	2017.048	-0.5	673.3563	3	66.54	10	Amidation
PF	Q06601.1	AVHYSGGQPLGSKRPNDMLSQRYHFGLa	60.6	3013.509	0.6	754.3851	4	40.53	8	Amidation

PF	Q06601.1	PNDMLSQRYHFGLa	65.73	1575.762	0.1	788.8882	2	47.77	9	Amidation
PF	Q06601.1	AYTYVSEYKRLPVYNFGIa	30.64	2181.126	0.3	728.0494	3	72.56	3	Amidation
PF	Q06601.1	ADYPLRLNLD	46.77	1188.614	0	595.3142	2	56.69	6	
PF	Q06601.1	YPLRLNLD	42.12	1002.55	0.3	502.2823	2	49.35	12	
PF	Q06601.1	RQYSFGLa	30.29	868.4555	-0.2	435.235	2	26.45	19	Amidation
PF	Q06601.1	GRQPYSFGLa	34.37	1022.53	0.3	512.2723	2	31.51	5	Amidation
PF	Q06601.1	GRDYSFGLa	28.95	912.4453	0.2	457.23	2	30.84	8	Amidation
PF	Q06601.1	WIDTNDNKRGRDYSFGLa	24.14	2054.992	0.4	686.0049	3	38.84	3	Amidation
PF	Q06601.1	LDYLPVDNPAFH	40.17	1399.677	-0.4	700.8456	2	67.71	7	
PF	Q06601.1	AVHYSGGQPLGS	39.1	1171.562	0.2	586.7885	2	14.58	6	
PF	Q06602.1	EAEPEAEPGNNRPVYIPQPRPPHPRL	23.07	2959.505	0.1	592.9084	5	39.74	5	
PF	Q5DW47.1	STSLEELANR	28.15	1118.557	0.3	560.2858	2	25.81	5	
PF	Q5DW47.1	STSLEELANRN	26.91	1232.6	-0.5	617.3068	2	24.93	3	
PF	Q5DW47.1	pQTFTYSHGWTNa	22.74	1322.568	0.6	662.2916	2	52.52	6	Pyro-glu from Q; Amidation
PF	Q868G6.1	ASFDDEYYKRAPMGFQGMRa	42.06	2267.025	0.6	567.7639	4	49.19	22	Amidation
PF	Q868G6.1	VLSMDGYQNILDKKDELLGEWE	41.48	2594.257	-0.4	865.7594	3	98.46	12	
PF	Q868G6.1	VLSMDGYQNILD	40.43	1366.644	-0.3	684.329	2	82.61	11	
PF	Q868G6.1	GVMDFQIGLQ	40.09	1106.543	-0.7	554.2784	2	87.57	16	
PF	Q868G6.1	APMGFQGMRG	38.9	1050.474	-0.5	526.244	2	24.95	13	
PF	Q868G6.1	APMGFQGMRa	38.73	992.4684	-0.7	497.2411	2	20.02	38	Amidation
PF	Q868G6.1	APMGFYGTRG	38.3	1055.486	-0.9	528.7497	2	21.9	13	
PF	Q868G6.1	ARMGFHGMRa	37.1	1060.517	0	531.2658	2	9	18	Amidation
PF	Q868G6.1	APMGFYGTRa	37.08	997.4803	-1.1	499.7469	2	18.43	23	Amidation
PF	Q868G6.1	ALMGFQGVRa	36.88	976.5276	-0.7	489.2708	2	31.91	23	Amidation
PF	Q868G6.1	ALMGFQGVRG	36.33	1034.533	0.2	518.2739	2	38.06	10	

PF	Q868G6.1	SPFRYLGA	35.81	909.4708	-0.3	455.7426	2	40.27	14	
PF	Q868G6.1	GVMDFQIGLQRKKD	34.42	1633.861	0.6	545.6279	3	39.49	8	
PF	Q868G6.1	SPFRYLGARG	34.3	1122.593	-0.5	375.2049	3	23.99	7	
PF	Q868G6.1	SPFRYLGARa	33.85	1064.588	0	533.3012	2	19.83	7	Amidation
PF	Q868G6.1	SLEEILDEIK	33.49	1187.629	0.1	594.8216	2	93.96	13	
PF	Q868G6.1	SPFRYLG	30.94	838.4337	0.3	420.2242	2	36.16	10	
PF	Q868G6.1	NPRWEFRGKFVGVRa	30.44	1745.959	0.5	437.4972	4	32.34	10	Amidation
PF	Q868G6.1	NPRWEFRGKFVGV	30.3	1590.842	0.3	531.2881	3	49.83	3	
PF	Q868G6.1	ASFDDEYY	28.5	1008.371	0.1	505.1929	2	44.7	5	
PF	Q868G6.1	SLEEILDEI	25.89	1059.534	0.4	530.7743	2	108.72	6	
PF	Q868G6.1	IILDALEELD	25.61	1142.607	-0.2	572.3107	2	100.26	3	
PF	XP_006557714.1	pQQFDDYGHLRFa	41.67	1406.637	0.5	704.326	2	69.79	13	Pyro-glu from Q; Amidation
PF	XP_006559359.1	SVSSLAKNSAWPVSL	46.58	1544.82	-0.3	773.417	2	71.29	11	
PF	XP_006559359.1	NVASLARTYTLPQNAa	44.1	1616.863	-0.5	809.4386	2	46.96	8	Amidation
PF	XP_006559359.1	FLLLPATDNNYFHQKLPSSLRSKSL	42.63	2888.555	0.4	578.7184	5	77.31	22	
PF	XP_006559359.1	NVGSVAREHGLPYa	41.93	1396.721	-0.5	699.3674	2	24.98	21	Amidation
PF	XP_006559359.1	YVASLARTGDLPIRGQ	40.96	1715.932	0	572.9846	3	37.62	10	
PF	XP_006559359.1	HIGALARLGWLPSLRTA	40.77	1831.058	-0.1	611.3599	3	78.84	14	
PF	XP_006559359.1	NIASLMRDYDQSRENRVPFPa	39.13	2406.186	0.9	803.0701	3	69.67	13	Amidation
PF	XP_006559359.1	SVSSLARTGDLPVREQ	38.63	1713.901	-0.4	572.3073	3	27.58	8	
PF	XP_006559359.1	NVGTLARDFALPPa	38.03	1368.751	-0.6	685.3825	2	63.59	18	Amidation
PF	XP_006559359.1	GIFLPGSVILRALSRQa	37.1	1725.041	0	576.0211	3	98.8	12	Amidation
PF	XP_006559359.1	YVASLARTGDLPIRa	33.92	1529.868	0	510.9632	3	36.19	7	Amidation
PF	XP_006559359.1	LPGSVILRALS	28.72	1124.692	0.4	563.3533	2	76.05	10	
PF	XP_006559359.1	GIFLPGSVILR	27.16	1170.712	1.5	586.3644	2	80.54	14	

PF	XP_006559359.1	HIGALARLGWLPSLRTARFS	25.34	2221.26	0.4	556.3224	4	81.47	4	
PF	XP_006559865.1	AFGLLTYPRIa	34.68	1148.671	-0.1	575.3425	2	73.38	13	Amidation
PF	XP_006560385.1	AYRKPPFNGSIFa	37.99	1394.746	-0.3	698.38	2	40.92	20	Amidation
PF	XP_006560385.1	YRKPPFNGSIFa	26.07	1323.709	0.1	662.8617	2	41.73	5	Amidation
PF	XP_006560385.1	RKPPFNGSIFa	24.15	1160.645	0.4	581.3302	2	33.49	11	Amidation
PF	XP_006562922.1	GFKPEYISTAYGFa	40.49	1477.724	0	739.8693	2	66.76	9	Amidation
PF	XP_006565207.1	SDPHLSILSKPMSAIPSYKFDD	45.28	2447.204	-0.6	816.7415	3	75.81	11	
PF	XP_006565207.1	SPSLRLRFa	30.48	973.5821	-0.3	487.7982	2	28.72	19	Amidation
PF	XP_006565207.1	SDPHLSILS	27.48	967.4974	0	484.756	2	36.83	8	
PF	XP_006565207.1	SQRSPSLRLRFa	24.3	1344.774	0.2	337.2008	4	20.45	5	Amidation
PF	XP_006570344.1	NSELINSLLGLPKNMNNAa	46.64	1940.015	0.2	971.015	2	91.06	12	Amidation
PF	XP_006570344.1	LINSLLGLPKNMNNAa	39.96	1609.897	-0.2	805.9557	2	65.66	10	Amidation
PF	XP_016769998.1	LVDHRIPDLENEMFDSGNDPGSTVVRT	45.09	3012.425	-0.7	754.1129	4	66.6	23	
NF	Q868G6.1	NSIINDVKNELFPEDIN	71.58	1972.974	-0.7	987.4937	2	96.19	11	
NF	Q868G6.1	VLSMDGYQNILDKKDELLGEWE	58.61	2594.257	5.3	1298.143	2	95.2	4	
NF	A8CL69.1	TSQDITSGMWFGPRLa	41.46	1693.825	1.1	847.9205	2	75.51	4	Amidation
NF	A8CL69.1	pQLHNIVDKPRQNFNDPRF	38.15	2220.119	-1.1	556.0364	4	40.26	9	Pyro-glu from Q
NF	A8CL69.1	pQITQFTPRLa	18.4	1084.603	2	543.3098	2	65.6	8	Pyro-glu from Q; Amidation
NF	A8CL69.1	GMWFGPRLa	36.12	961.4956	1.3	481.7557	2	50.46	11	Amidation
NF	A8CL69.1	MWFGPRLa	26.19	904.4741	0.9	453.2448	2	53.31	9	Amidation
NF	A8CL69.1	pQLHNIVDKPRQN	50.72	1443.758	0.9	482.2604	3	14.04	8	Pyro-glu from Q
NF	A8CL69.1	pQLHNIVDKP	45.19	1045.556	0.8	523.7855	2	23.94	9	Pyro-glu from Q
NF	A8CL69.1	RVPWTPSPRLa	41.57	1206.699	-0.9	604.356	2	23.89	5	Amidation
NF	ACI90290.1	TWKSPDIVIRFa	51.85	1359.766	-0.7	454.2624	3	56.58	5	Amidation
NF	ACI90290.1	GRNDLNFIRYa	36.79	1265.663	0.2	633.8388	2	30.39	9	Amidation

NF	ACI90290.1	QITQFTPRLa	33.64	1101.63	-0.4	551.8218	2	32.4	10	Amidation
NF	ACI90290.1	AGFKNLNREQ	36.45	1175.605	-0.1	588.8096	2	10.17	12	
NF	ACI90290.1	SPDIVIRFa	33.7	944.5443	-0.9	473.279	2	45.58	8	Amidation
NF	NP_001161192.1	PEIFTSPEELRRYIDHVSDYYLLSGKARYa	46.23	3515.784	0.6	586.9714	6	94.48	7	Amidation
NF	P85527.1	pQDVDHVFLRFa	52.69	1256.63	0	629.3223	2	72.23	6	Pyro-glu from Q; Amidation
NF	P85527.1	QDVDHVFLRFa	48.09	1273.657	0.3	425.5596	3	48.21	5	Amidation
NF	P85527.1	pQDVDHVFLRF	49.46	1257.614	0.2	629.8145	2	74.75	6	Pyro-glu from Q
NF	P85527.1	pQDVDHVFLR	43.61	1110.546	2.4	556.2815	2	30.9	8	Pyro-glu from Q
NF	P85527.1	pQDVDHVFL	24.41	954.4447	0.5	478.2299	2	70.18	6	Pyro-glu from Q
NF	P85798.1	LRNQLDIGDLQ	49.85	1283.683	0	642.8489	2	41.44	6	
NF	P85798.1	LRNQLDIGDL	46.57	1155.625	0.7	578.8201	2	35.03	3	
NF	P85798.1	IPAADKERLLN	48.73	1238.698	0.9	620.3569	2	15.09	7	
NF	P85799.1	SQAYDPYSNAAQFQLSSQSRGYPYQHRLVY	76.59	3523.655	-0.9	881.9201	4	60.11	31	
NF	P85799.1	SQAYDPYSNAAQFQLSSQSRGYPYQHRL	66.47	3261.523	-1.1	816.3871	4	51.7	6	
NF	P85799.1	SQAYDPYSNAAQFQLSSQSRGYPYQHRLV	62.02	3360.591	-2	841.1534	4	55.98	5	
NF	P85799.1	LPTNLAEDTKKTEQTMRPKS	60.38	2287.184	-0.6	572.803	4	20.72	19	
NF	P85799.1	NVPIYQEPRF	46.81	1261.646	-0.8	631.8295	2	45.2	8	
NF	P85799.1	VPIYQEPRF	45.86	1147.603	-0.2	574.8084	2	42.06	7	
NF	P85799.1	GYPYQHRLVY	39.06	1294.646	-0.3	648.33	2	23.28	7	
NF	P85799.1	PIYQEPRF	25.88	1048.534	0.2	525.2745	2	45.14	3	
NF	P85828.1	ITGQGNRIF	48.34	1004.54	0.4	503.2776	2	19.51	3	
NF	P85828.1	SLKAPFA	40.92	732.417	0.1	367.2158	2	29.4	5	
NF	P85828.1	SLKAPF	35.22	661.3799	-0.1	331.6972	2	23.98	5	
NF	P85829.1	MVPVPVHHMADELLRNGPDTVI	49.4	2439.24	0.1	814.0874	3	74.93	6	

NF	P85829.1	VHHMADELLRNGPDTVI	42.7	1915.957	-0.5	639.6594	3	44.62	5	
NF	P85829.1	VPVPVHHMADELL	49.99	1455.754	0.2	728.8846	2	33.13	7	
NF	P85829.1	LLRNGPDTVI	35.24	1096.624	-0.1	549.3192	2	28.76	11	
NF	P85829.1	LRNGPDTVI	28.81	983.54	0.5	492.7775	2	16.64	12	
NF	P85830.1	GLDLGLSRGFSGSQAAKHLMGLAAANYAGGPa	75.12	2985.524	0.3	996.1823	3	83.37	8	Amidation
NF	P85830.1	GLDLGLSRGFSGSQAAKH	47.59	1799.928	-0.2	600.9831	3	30.26	7	
NF	P85830.1	GLDLGLSRGFSGSQAAKHLMa	34.44	2043.068	1.2	682.0309	3	54.93	8	Amidation
NF	P85830.1	GLDLGLSRGFSGSQAA	70.04	1534.774	0.5	768.3947	2	43.37	5	
NF	P85830.1	HLMGLAAANYAGGPa	47.07	1340.666	-0.8	671.3397	2	35.45	8	Amidation
NF	P85831.1	IDLSRFYGHFNT	66.33	1468.71	-0.8	735.3616	2	62.1	15	
NF	P85831.1	IDLSRFYGHFN	59.58	1367.662	0	684.8384	2	59.48	12	
NF	P85831.1	IDLSRFYGHF	54.45	1253.619	0.1	627.817	2	66.68	7	
NF	P85831.1	IDLSRFYGHFNTKR	45.5	1752.906	0.5	585.3095	3	39.93	6	
NF	P85831.1	DLSRFYGHFN	33.5	1254.578	-0.6	628.8198	2	65.09	14	
NF	P85831.1	FYGHFNT	44.01	884.3817	-0.2	443.1981	2	20.92	13	
NF	P85832.1	LTNYLATTGHGTNTGGPVLT	85.24	1987.001	-0.6	994.5072	2	47.09	8	
NF	P85832.1	NLDEIDRVGWSGFV	56.68	1605.779	-0.9	803.8959	2	86.63	4	
NF	P85832.1	NIDEIDRTAFDNFF	54.74	1715.779	-0.7	858.8962	2	94.63	7	
NF	P85832.1	LTNYLATTGHGTNTGGPVLTRRFa	52.84	2445.288	0	612.3292	4	38.23	12	Amidation
NF	P85832.1	ELVDELSPVSERETLERFa	29.85	2146.091	-1.2	716.3699	3	72.71	4	Amidation
NF	P85832.1	LVDELSPVSERETLERFa	26.21	2017.048	-1.1	673.3558	3	61.74	3	Amidation
NF	P85832.1	LTNYLATTGHGTNTGGPVL	83.65	1885.953	3.4	943.9872	2	37.57	3	
NF	Q06601.1	PNDMLSQRYHFGLa	69.72	1575.762	-0.4	526.2609	3	47.76	6	Amidation
NF	Q06601.1	AVHYSGGQPLGSKRPNDMLSQRYHFGLa	66.38	3013.509	0.1	754.3846	3	41.05	10	Amidation
NF	Q06601.1	AYTYVSEYKRLPVYNFGIa	69.78	2181.126	-1.2	1091.569	2	73.7	8	Amidation

NF	Q06601.1	ADYPLRLNLD	50.74	1188.614	0.6	595.3146	2	57.13	15	
NF	Q06601.1	YPLRLNLD	44.94	1002.55	0.3	502.2823	2	49.7	21	
NF	Q06601.1	LDYLPVDNPAFH	54.33	1399.677	1.6	700.8469	2	61.58	5	
NF	Q06601.1	RQYSFGLa	34.76	868.4555	0.1	435.2351	2	23.68	7	Amidation
NF	Q06601.1	GRQPYSFGLa	38.89	1022.53	0.2	512.2722	2	29.28	7	Amidation
NF	Q06601.1	GRDYSFGLa	30.15	912.4453	0	457.2299	2	29.2	10	Amidation
NF	Q06601.1	WIDTNDNKRGRDYSFGLa	36.47	2054.992	1	686.0054	3	34.51	7	Amidation
NF	Q06601.1	AVHYSGGQPLGS	39.03	1171.562	0.3	586.7885	2	13.57	11	
NF	Q06602.1	EAEPEAEPGNNRPVYIPQPRPPHPRL	66.9	2959.505	3.2	592.9102	5	21.55	23	
NF	Q5DW47.1	STSLEELANR	36.25	1118.557	0.9	560.2861	2	24.18	7	
NF	Q5DW47.1	STSLEELANRN	39.72	1232.6	0.7	617.3075	2	23.07	9	
NF	Q5DW47.1	pQTFTYSHGWTNa	51.7	1322.568	-0.6	662.2909	2	50.66	19	Pyro-glu from Q; Amidation
NF	Q868G6.1	ASFDDEYYKRAPMGFQGMRa	58.13	2267.025	-0.1	567.7635	4	43.53	9	Amidation
NF	Q868G6.1	ARMGFHGMRa	54.74	1060.517	-0.5	531.2656	2	8.87	7	Amidation
NF	Q868G6.1	APMGFQGMRa	49.97	992.4684	0	497.2415	2	19.16	6	Amidation
NF	Q868G6.1	SLEEILDEIK	47.34	1187.629	0.3	594.8217	2	84.58	9	
NF	Q868G6.1	NPRWEFRGKFVGV	45.98	1590.842	0.3	531.2881	3	40.1	9	
NF	Q868G6.1	SPFRYLGARa	42.47	1064.588	0.1	533.3013	2	15.98	4	Amidation
NF	Q868G6.1	ALMGFQGVRa	42.22	976.5276	-0.3	489.2709	2	29.18	6	Amidation
NF	Q868G6.1	NPRWEFRGKFVGVRa	40.99	1745.959	-0.2	437.4969	4	21.41	7	Amidation
NF	Q868G6.1	SPFRYLGA	40.83	909.4708	0	455.7427	2	35.07	7	
NF	Q868G6.1	GVMDFQIGLQRKKD	40.37	1633.861	0.1	545.6276	3	34.17	7	
NF	Q868G6.1	SPFRYLGARG	39.19	1122.593	-0.4	375.2049	3	19.38	4	
NF	Q868G6.1	ALMGFQGVRG	38.17	1034.533	-0.1	518.2737	2	34.43	13	
NF	Q868G6.1	GVMDFQIGLQ	37.68	1106.543	0.3	554.2789	2	84.25	6	

NF	Q868G6.1	VLSMDGYQNILD	36.56	1366.644	0.1	684.3292	2	79.28	3	
NF	Q868G6.1	APMGFYGTRa	36.12	997.4803	1.1	499.748	2	16.97	3	Amidation
NF	Q868G6.1	ASFDDEYY	30.67	1008.371	0.1	505.1929	2	40.98	4	
NF	Q868G6.1	SLEEILDEI	23.62	1059.534	-1.5	530.7733	2	104.74	3	
NF	Q868G6.1	IILDALEELD	28	1142.607	0.6	572.3112	2	102.74	5	
NF	Q868G6.1	APMGFQGMRG	50.06	1050.474	-0.3	526.2441	2	23.14	5	
NF	Q868G6.1	APMGFYGTRG	43.78	1055.486	-0.3	528.7501	2	20.72	3	
NF	XP_006557714.1	pQQFDDYGHLRFa	59.46	1406.637	0.4	704.3259	2	41.18	4	Pyro-glu from Q; Amidation
NF	XP_006559359.1	SVSSLAKNSAWPVSL	70.04	1544.82	0	773.4172	2	66.98	6	
NF	XP_006559359.1	NVASLARTYTLPQNAa	66.96	1616.863	-0.3	809.4387	2	42.45	6	Amidation
NF	XP_006559359.1	NVGSVAREHGLPYa	62.66	1396.721	-0.5	699.3675	2	20.47	11	Amidation
NF	XP_006559359.1	YVASLARTGDLPIRGQ	57.46	1715.932	0.2	572.9846	3	35.02	9	
NF	XP_006559359.1	FLLLPATDNNYFHQKLPSSLRSKSL	51.46	2888.555	1.1	578.7189	5	69.14	13	
NF	XP_006559359.1	SVSSLARTGDLPVREQ	47.87	1713.901	0	572.3076	3	24.99	6	
NF	XP_006559359.1	NIASLMRDYDQSRENRVPFPa	45.43	2406.186	-0.5	803.069	3	60.51	5	Amidation
NF	XP_006559359.1	YVASLARTGDLPIRa	35.3	1529.868	0.8	510.9636	3	31.89	4	Amidation
NF	XP_006559359.1	LPGSVILRALS	34.9	1124.692	0.5	563.3534	2	70.13	8	
NF	XP_006559359.1	NVGTLARDFALPPa	33.49	1368.751	0.1	685.383	2	59.01	11	Amidation
NF	XP_006559359.1	GIFLPGSVILR	32.7	1170.712	0.2	586.3636	2	76.91	5	
NF	XP_006559359.1	GIFLPGSVILRALSRQa	31.65	1725.041	-1	576.0204	3	94.65	8	Amidation
NF	XP_006559359.1	HIGALARLGWLPSLRTARFS	29.69	2221.26	-0.6	556.3218	4	68.04	3	
NF	XP_006559359.1	HIGALARLGWLPSLRTA	24.36	1831.058	0.4	611.3602	3	67.47	5	
NF	XP_006559359.1	NVGTLARDFALPPGRRNIASLMRDYDQSRENRVPFPa	19.42	4127.135	0.3	688.8633	6	72.55	6	Amidation
NF	XP_006559865.1	AFGLLTYPRIa	34	1148.671	-0.2	575.3424	2	68.55	5	Amidation

NF	XP_006559865.1	EKLKPNMRRAFGLLTYPRIa	21.02	2301.325	0.5	576.3389	4	45.4	4	Amidation
NF	XP_006560385.1	AYRKPPFNGSIFa	41.64	1394.746	-0.1	698.3801	2	35.3	6	Amidation
NF	XP_006560385.1	KPPFNGSIFa	36.34	1004.544	0	503.2794	2	42.18	7	Amidation
NF	XP_006560385.1	YRKPPFNGSIFa	47.47	1323.709	0.3	662.8619	2	32.59	7	Amidation
NF	XP_006560385.1	RKPPFNGSIFa	40.45	1160.645	-0.2	581.3298	2	24.42	9	Amidation
NF	XP_006562922.1	GFKPEYISTAYGFa	52.25	1477.724	0.6	739.8698	2	70.58	29	Amidation
NF	XP_006565207.1	SDPHLSILSKPMSAIPSYKFDD	72.01	2447.204	-0.3	816.7418	3	70.27	6	
NF	XP_006565207.1	SQRSPSLRLRFa	32.44	1344.774	-0.2	449.2651	3	15.82	6	Amidation
NF	XP_006565207.1	SPSLRLRFa	28.98	973.5821	0.5	487.7986	2	22.89	5	Amidation
NF	XP_006565207.1	SDPHLSILS	38.23	967.4974	0	484.756	2	33.67	7	
NF	XP_006570344.1	NSELINSLLGLPKNMNNAa	64.9	1940.015	3.8	971.0184	2	85.93	7	Amidation
NF	XP_006570344.1	LINSLLGLPKNMNNAa	54.36	1609.897	-0.3	805.9557	2	61.69	6	Amidation
NF	XP_016769998.1	LVDHRIPDLENEMFDSGNDPGSTVVRT	65.72	3012.425	-0.4	1005.148	3	63.14	13	
NF	XP_016769998.1	IGSLSIVNSMDVLRQRVLLELARRKALQD QAQIDANRRLLETIa	34.17	4913.782	-0.5	819.9706	6	96.85	12	Amidation
NF	XP_016769998.1	HPISYNTYDERELSRDHPPLLL	33.16	2664.33	0.3	667.0898	4	52.44	6	

Table S4. Neuropeptides identified in the brain of *Apis cerana cerana* workers. "NB" is nurse bee. "PF" is pollen forager. "NF" is nectar forager. "Protein Accession" is the unique number given to mark the entry of a protein in the database NCBInr. "Peptide" is the amino acid sequence of the peptide as determined in PEAKS Search. "-10lgP" is the score indicates the scoring significance of a peptide-spectrum match. "Mass" is monoisotopic mass of the peptide. "ppm" is precursor mass error, calculated as $10^6 \times$ (precursor mass - peptide mass) / peptide mass. "m/z" is precursor mass-to-charge ratio. "z" is peptide charge. "RT" is retention time (elution time) of the spectrums as recorded in the data. "#Spec" is the number of scanned spectrums of the peptide. "PTM" is post translational modification types present in the peptide.

Sample	Protein Accession	Peptide	-10lgP	Mass	ppm	m/z	z	RT	#Spec	PTM
NB	PBC25365.1	pQQFDDYGHLRFa	26.97	1406.637	-2	704.3242	2	56.29	6	Pyro-glu from Q; Amidation
NB	PBC27532.1	LVDHRIPDLENEMF	48.92	1726.835	1.8	864.4263	2	49.73	8	
NB	PBC27532.1	ISYDTYDERELSRDHPPLLL	47.44	2431.202	2	811.4095	3	51.01	9	
NB	PBC27532.1	HPISYDTYDERELSRDHPPLLL	45.5	2665.314	0.7	889.4457	3	41.65	14	
NB	PBC27532.1	SLPLYGGNMSKTGDSRLKSE	45.37	2139.063	1	535.7736	4	19.63	8	
NB	PBC27532.1	SLPLYGGNMSKTGDSRLKSEFE	43.99	2415.174	1.1	806.0662	3	30.88	7	
NB	PBC27532.1	IGSLSIVNSMDVLRQRVLLELARRKALQD QAQIDANRRLLETIa	41.71	4913.782	0.6	983.7643	5	87.07	14	Amidation
NB	PBC27532.1	ARRKALQDQAQIDANRRLLETIa	37.21	2577.458	0.4	516.499	5	22.74	4	Amidation
NB	PBC27532.1	LVDHRIPDLENEMFDSGNDPGSTVVRT	58.45	3012.425	0.6	1005.149	3	50.38	18	
NB	PBC27982.1	ITGQGNRIF	39.25	1004.54	0.5	503.2777	2	18.96	8	
NB	PBC27982.1	SLKAPFA	34.7	732.417	-0.5	367.2156	2	20.05	5	
NB	PBC27985.1	YLLSGKARYa	31.25	1068.608	0.7	535.3116	2	11.7	5	Amidation
NB	PBC28057.1	GNNRPVYIPQPRPPHP	45.28	1837.97	0.8	613.6644	3	17.05	10	
NB	PBC28057.1	GNNRPVYIPQPRPPHPRL	38.71	2107.155	1.9	703.3936	3	19.33	10	
NB	PBC28057.1	PVYIPQPRPPHP	36.58	1396.762	0	466.5944	3	22.15	3	
NB	PBC28214.1	GLDLGLSRGFSGSQAAKHLMGLAAANYA GGPa	55.91	2985.524	2.3	996.1843	3	69.28	15	Amidation

NB	PBC28214.1	GLDLGLSRGFSGSQAAKH	43.4	1799.928	0.6	900.9717	2	23.14	9	
NB	PBC28214.1	GLDLGLSRGFSGSQAA	51.79	1534.774	1.7	768.3955	2	42.16	6	
NB	PBC28214.1	GLDLGLSRGFSGSQAAKHLMa	37.61	2043.068	0.8	682.0306	3	41.21	3	Amidation
NB	PBC30406.1	SDPHLSIGILSKPISAIPSSKFDD	54.85	2523.322	1.1	842.1155	3	58.84	15	
NB	PBC30406.1	SPSLRLRFa	33.83	973.5821	0.1	487.7984	2	18.81	4	Amidation
NB	PBC30406.1	SDPHLSIGILSKPISAIP	32.67	1844.041	1.8	615.6886	3	64.64	5	
NB	PBC30406.1	SQRSPSLRLRFa	30.49	1344.774	-0.2	449.2651	3	14.25	3	Amidation
NB	PBC30406.1	SDPHLSIGILSKP	47.29	1362.751	1.1	682.3834	2	31.95	8	
NB	PBC31004.1	pQMFTYSHGWTNa	36.09	1352.561	1.4	677.2886	2	54.66	3	Pyro-glu from Q; Amidation
NB	PBC31004.1	STSLEELVNR	32.15	1146.588	0.5	574.3016	2	27.94	3	
NB	PBC31251.1	YRKPPFNGSIFa	37.89	1323.709	0.8	662.8622	2	24.67	4	Amidation
NB	PBC31251.1	AYRKPPFNGSIFa	36.33	1394.746	1.3	698.3811	2	25.09	13	Amidation
NB	PBC31251.1	KPPFNGSIFa	27.98	1004.544	0.7	503.2798	2	30.98	5	Amidation
NB	PBC31251.1	RKPPFNGSIFa	20.29	1160.645	1	581.3306	2	21.64	12	Amidation
NB	PBC31431.1	APVGYQEMQGKKNSASLNSENFGIF	54.25	2715.296	2.7	906.1084	3	48.51	8	
NB	PBC31431.1	NSIINDVKNELFPEDIN	51.1	1972.974	0.9	987.4952	2	83.93	25	
NB	PBC31431.1	STDFQDVESGSESFKRARMGFHGMRa	45.17	2860.313	0.4	573.0701	5	25.77	7	Amidation
NB	PBC31431.1	ARMGFHGMRa	43.34	1060.517	1	531.2664	2	7.55	19	Amidation
NB	PBC31431.1	APMGFQGMRG	41.6	1050.474	1	526.2448	2	19.99	4	
NB	PBC31431.1	SPFRYLGV	41.02	937.5021	0.2	469.7584	2	36.47	9	
NB	PBC31431.1	APMGFYGTRG	40.33	1055.486	0.8	528.7506	2	18.81	3	
NB	PBC31431.1	APMGFQGMRa	40.14	992.4684	0.5	497.2418	2	17.14	9	Amidation
NB	PBC31431.1	ALMGFQGVRG	38.56	1034.533	1.2	518.2744	2	26.54	4	
NB	PBC31431.1	ALMGFQGVRa	38.13	976.5276	0.8	489.2715	2	23.4	5	Amidation
NB	PBC31431.1	APMGFYGTRa	37.78	997.4803	0.3	499.7476	2	15.26	6	Amidation

NB	PBC31431.1	ARMGFHGMRG	36.45	1118.523	-0.5	373.848	3	9.58	3	
NB	PBC31431.1	SPFRYLGVRa	35.49	1092.619	0.3	547.3171	2	17.93	10	Amidation
NB	PBC31431.1	ASFDDEYY	22.86	1008.371	0.6	505.1932	2	33.37	7	
NB	PBC31431.1	ASFDDEYYKRAPMGFQGMRa	50.81	2267.025	1.1	567.7642	4	31.82	6	Amidation
NB	PBC31431.1	STDFQDVESGSESF	45.52	1533.611	0.9	767.8133	2	45.31	8	
NB	PBC32274.1	pQLHNIIDKPRQN	42.12	1457.774	1.2	729.8951	2	16.05	6	Pyro-glu from Q
NB	PBC32274.1	RVPWTPSPRLa	36.39	1206.699	1.2	604.3572	2	19.93	5	Amidation
NB	PBC32274.1	pQLHNIIDKPRQNFNDPRF	36.19	2234.135	0.3	559.5411	4	32.1	7	Pyro-glu from Q
NB	PBC32274.1	pQITQFTPRLa	33.03	1084.603	0.4	543.309	2	53.9	4	Pyro-glu from Q; Amidation
NB	PBC32274.1	VPWTPSPRLa	32.1	1050.597	0.2	526.3061	2	25.14	3	Amidation
NB	PBC32274.1	pQLHNIIDKPRQNFNDP	28.81	1930.965	1.6	966.4913	2	26.9	4	Pyro-glu from Q
NB	PBC32274.1	SGMWFGPRLa	27.1	1048.528	1.5	525.2719	2	49.17	3	Amidation
NB	PBC32274.1	TSQDITSGMWFGPRLa	42.69	1693.825	1.1	847.9205	2	63.45	10	Amidation
NB	PBC32274.1	DITSGMWFGPRLa	33.24	1377.686	1.6	689.8515	2	77.83	3	Amidation
NB	PBC32274.1	pQLHNIIDKP	32.19	1059.571	-0.1	530.7928	2	24.84	5	Pyro-glu from Q
NB	PBC32274.1	SQDITSGMWFGPRLa	31.19	1592.777	2.4	797.3976	2	67.49	3	Amidation
NB	PBC32274.1	GMWFGPRLa	31.02	961.4956	0.7	481.7554	2	50.43	5	Amidation
NB	PBC32496.1	IPAADKERLLN	41.66	1238.698	0.7	620.3568	2	14.79	5	
NB	PBC32496.1	LRNQLDIGDLQ	40.78	1283.683	2.1	642.8503	2	31.12	6	
NB	PBC32496.1	SYWKQCAFNAVSCFa	39.16	1651.728	1.1	826.8719	2	69.48	5	Amidation
NB	PBC32545.1	NSELINSLLGLPKNMNNAa	46.62	1940.015	1.7	971.0164	2	72.76	8	Amidation
NB	PBC32608.1	IDLSRFYGHFNTKR	47.18	1752.906	1.2	585.3099	3	28.6	11	
NB	PBC32608.1	IDLSRFYGHFNT	45.98	1468.71	1.8	735.3635	2	49.16	9	
NB	PBC32608.1	IDLSRFYGHF	43.61	1253.619	0.9	627.8174	2	53.8	6	
NB	PBC32608.1	DLSRFYGHF	26.84	1140.535	0.4	571.2751	2	36.46	7	

NB	PBC32608.1	IDLSRFYGHFNTK	29.22	1596.805	-0.1	533.2755	3	35.94	3	
NB	PBC32678.1	pQDVDHVFLRFa	40.98	1256.63	0.8	629.3228	2	61.33	6	Pyro-glu from Q; Amidation
NB	PBC32678.1	QDVDHVFLRFa	40.61	1273.657	0.9	637.8362	2	36.74	8	Amidation
NB	PBC32678.1	pQDVDHVFLR	39.04	1110.546	1.2	556.2808	2	33.73	4	Pyro-glu from Q
NB	PBC32727.1	LPTNLGEDTKKTEQTMRPKS	49.08	2273.169	0.9	569.2999	4	15.3	14	
NB	PBC32727.1	SQAYDPYSNAAQFQLSSQSRGYPYQHRL	48.8	3261.523	0.5	816.3884	4	39.12	7	
NB	PBC32727.1	NVPIYQEPRF	35.7	1261.646	0.1	631.8301	2	32.71	5	
NB	PBC32727.1	YPYQHRLIY	34.97	1251.64	1.2	626.8281	2	21.18	4	
NB	PBC32727.1	SQAYDPYSNAAQFQLSSQSRGYPYQHRLI Y	55.22	3537.67	1.8	885.4265	4	51.39	26	
NB	PBC32727.1	SQAYDPYSNAAQFQLSSQSRGYPYQHRLI	51.38	3374.607	1.7	844.6604	4	46.46	5	
NB	PBC32727.1	VPIYQEPRF	36.9	1147.603	0.8	574.809	2	30.77	3	
NB	PBC32727.1	GYPYQHRLIY	27.89	1308.662	1.5	655.339	2	22.03	5	
NB	PBC32914.1	SIATLAKNDDLPISLHDRMAENEDDEE	54.94	3040.393	0.8	1014.472	3	42.04	10	
NB	PBC32914.1	FLLLPATDNNYFHQKLPSSLRSKSL	47.88	2888.555	1.7	578.7192	5	54.83	13	
NB	PBC32914.1	YVASLARTGDLPIRGQ	44.56	1715.932	1	858.974	2	24.87	10	
NB	PBC32914.1	NVGSVAREHGLPYa	43.9	1396.721	1	699.3685	2	17.05	11	Amidation
NB	PBC32914.1	NIASLIRDYDQSRENRVSFPa	40.86	2378.209	0.9	793.7443	3	48.95	11	Amidation
NB	PBC32914.1	NVGTLARDFALPPa	39.83	1368.751	1.4	685.3839	2	44	19	Amidation
NB	PBC32914.1	SISSLARTGDLPVREQ	39.66	1727.917	1.5	576.9803	3	23.16	8	
NB	PBC32914.1	YVASLARTGDLPIRa	36.12	1529.868	0.5	510.9635	3	22.56	6	Amidation
NB	PBC32914.1	NVASLARTYTLPQNAa	34.95	1616.863	1.2	809.4399	2	30.41	7	Amidation
NB	PBC32914.1	GIFVPGSVILRALSRQa	42.55	1711.026	1.8	856.5216	2	70.58	13	Amidation
NB	PBC32914.1	SVSSLAKNSAWPVSL	38.37	1544.82	1.6	773.4184	2	53.19	5	
NB	PBC34787.1	AYTYVSEYKRLPVYNFGIa	51.99	2181.126	0.6	1091.571	2	58.6	7	Amidation

NB	PBC34787.1	PNDMLSQRYHFGLa	48.95	1575.762	0.4	788.8884	2	32.82	5	Amidation
NB	PBC34787.1	AVHYSGGQPLGSKRPNDMLSQRYHFGLa	47.29	3013.509	1.4	754.3856	4	26.02	14	Amidation
NB	PBC34787.1	AVHYSGGQPLGS	37.66	1171.562	0.7	586.7888	2	13.39	6	
NB	PBC34787.1	RQYSFGLa	31.08	868.4555	0.6	435.2353	2	21.52	3	Amidation
NB	PBC34787.1	WIDTNDNKRGRDYSFGLa	28.42	2054.992	1.5	686.0057	3	26.77	3	Amidation
NB	PBC34787.1	LDYLPVDNPAFH	42.16	1399.677	1.7	700.847	2	52.91	3	
NB	PBC34787.1	YPLRLNLD	32.63	1002.55	0.2	502.2823	2	35.29	3	
NB	PBC34787.1	GRDYSFGLa	30.69	912.4453	0.4	457.2301	2	24.05	3	Amidation
NB	PBC34787.1	GRQPYSFGLa	30.68	1022.53	0.4	512.2723	2	23.69	5	Amidation
NB	XP_016905690.1	LNSDSRNSQVNGYTPRLa	44.7	1918.961	1.8	640.662	3	15.42	3	Amidation
NB	XP_016905690.1	SNAPVSNLNFN	42.02	1175.557	1.4	588.7867	2	30.68	3	
NB	XP_016905690.1	NSDSRNSQVNGYTPRLa	40.74	1805.877	1.5	602.9671	3	14.5	3	Amidation
NB	XP_016905690.1	RASGLLSYPRIa	25.05	1230.72	0.3	411.2473	3	22.05	3	Amidation
NB	XP_016908608.1	LTNYLATGHRTNGGPVI	51.9	1782.938	1	892.477	2	24.82	11	
NB	XP_016908608.1	NLDEIDRVGWSGFV	49.71	1605.779	2.2	803.8984	2	74.07	6	
NB	XP_016908608.1	LTNYLATGHRTNGGPVIRRFa	35.11	2241.224	0.8	748.0826	3	18.66	14	Amidation
NB	XP_016908608.1	NIDEIDRTAFDNFF	48.19	1715.779	1	858.8976	2	83.39	6	
NB	XP_016908970.1	MVPVPVHHMADELLRSGPDTVI	54.41	2412.229	0.5	1207.123	2	60.48	21	
NB	XP_016908970.1	VHHMADELLRSGPDTVI	51.6	1888.947	0.8	945.4813	2	32.22	9	
NB	XP_016908970.1	MVPVPVHHMADEL	33.03	1473.711	1.3	737.8636	2	27.55	4	
NB	XP_016908970.1	LRSGPDTVI	25.3	956.5291	0.3	479.2719	2	16.69	3	
NB	XP_016908970.1	VPVPVHHMADELL	47.4	1455.754	0.2	728.8846	2	32.18	6	
NB	XP_016920932.1	TWKSPDIVIRFa	44.03	1359.766	0.2	454.2628	3	42.36	11	Amidation
NB	XP_016920932.1	GRNDLNFIRYa	42.19	1265.663	1.3	633.8395	2	23.39	5	Amidation
PF	PBC25365.1	pQQFDDYGHLRFa	36.55	1406.637	-1.5	704.3246	2	56.2	15	Pyro-glu from Q; Amidation

PF	PBC27532.1	LVDHRIPDLENEMF	57.77	1726.835	2	864.4264	2	49.65	10	
PF	PBC27532.1	ISYDTYDERELSRDHPPLLL	53.81	2431.202	1.3	811.4089	3	50.84	16	
PF	PBC27532.1	SLPLYGGNMSKTGDSRLKSEFE	52.9	2415.174	1.1	806.0662	3	30.78	16	
PF	PBC27532.1	SLPLYGGNMSKTGDSRLKSE	52.19	2139.063	1.2	1070.54	2	15.87	8	
PF	PBC27532.1	HPISYDTYDERELSRDHPPLLL	51.83	2665.314	1.1	889.4461	3	40.68	31	
PF	PBC27532.1	IGSLSIVNSMDVLRQRVLLELARRKALQD QAQIDANRRLLETIa	40.04	4913.782	2.3	983.766	5	87.46	19	Amidation
PF	PBC27532.1	ARRKALQDQAQIDANRRLLETIa	26.54	2577.458	0.2	516.4989	5	21.29	6	Amidation
PF	PBC27532.1	LVDHRIPDLENEMFDSGNDPGSTVVRT	72.85	3012.425	0.5	1005.149	3	50.35	25	
PF	PBC27982.1	ITGQGNRIF	44.58	1004.54	0.3	503.2776	2	15.7	12	
PF	PBC27982.1	SLKAPFA	33.45	732.417	-0.4	367.2156	2	18.98	3	
PF	PBC27985.1	YLLSGKARYa	30.64	1068.608	0.9	535.3118	2	10.92	4	Amidation
PF	PBC28057.1	GNNRPVYIPQPRPPHP	52.22	1837.97	0.5	919.9927	2	17.43	18	
PF	PBC28057.1	GNNRPVYIPQPRPPHPRL	49.35	2107.155	0.4	703.3926	3	17.9	21	
PF	PBC28057.1	PVYIPQPRPPHP	42.58	1396.762	0.4	466.5946	3	21.67	7	
PF	PBC28214.1	GLDLGLSRGFSGSQAAKHLMGLAAANYA GGPa	63.47	2985.524	2.3	747.39	4	69.13	14	Amidation
PF	PBC28214.1	GLDLGLSRGFSGSQAAKH	45.58	1799.928	1.1	900.9721	2	22.16	8	
PF	PBC28214.1	GLDLGLSRGFSGSQAA	58.47	1534.774	2.1	768.3959	2	42.52	5	
PF	PBC28214.1	GLDLGLSRGFSGSQAAKHLMa	25.64	2043.068	0.4	682.0303	3	41.15	4	Amidation
PF	PBC28214.1	HLMGLAAANYAGGPa	41.31	1340.666	1.6	671.3413	2	26.72	8	Amidation
PF	PBC30406.1	SDPHLSIGILSKPISAIPSSKFDD	66.06	2523.322	1.2	842.1157	3	58.92	17	
PF	PBC30406.1	SQRSPSLRLRFa	38.3	1344.774	-0.2	449.2651	3	14.04	4	Amidation
PF	PBC30406.1	SPSLRLRFa	37.71	973.5821	0.2	487.7984	2	16.5	4	Amidation
PF	PBC30406.1	SDPHLSIGILSKPISAIP	27.97	1844.041	1.2	923.0287	2	63.92	8	
PF	PBC30406.1	SDPHLSIGILSKP	37.51	1362.751	-0.6	455.2573	3	31.93	9	

PF	PBC31004.1	pQMFTYSHGWTNa	42.17	1352.561	1.5	677.2887	2	53.87	6	Pyro-glu from Q; Amidation
PF	PBC31004.1	SFSENMINDHRQPASTNNNY	56.41	2338.003	-0.2	1170.009	2	20.18	7	
PF	PBC31251.1	AYRKPPFNGSIFa	39.22	1394.746	1.1	698.381	2	23.96	9	Amidation
PF	PBC31251.1	RKPPFNGSIFa	27.47	1160.645	1.8	581.331	2	20.02	3	Amidation
PF	PBC31251.1	KPPFNGSIFa	25.66	1004.544	0.6	503.2798	2	29.89	3	Amidation
PF	PBC31431.1	APVGYQEMQGKKNSASLNSENFGIF	77.23	2715.296	0.2	1358.656	2	48.2	11	
PF	PBC31431.1	NSIINDVKNELFPEDIN	57.78	1972.974	1.6	987.4959	2	84.36	25	
PF	PBC31431.1	ARMGFHGMRa	51.41	1060.517	0.9	531.2663	2	6.72	21	Amidation
PF	PBC31431.1	APMGFQGMRG	46.15	1050.474	0.4	526.2444	2	19.43	3	
PF	PBC31431.1	STDFQDVESGSESFKRARMGFHGMRa	45.36	2860.313	1.5	716.0867	4	24.71	5	Amidation
PF	PBC31431.1	SPFRYLGV	45.28	937.5021	0.6	469.7586	2	35.51	7	
PF	PBC31431.1	APMGFQGMRa	44.07	992.4684	-0.1	497.2415	2	15.49	6	Amidation
PF	PBC31431.1	APMGFYGTRG	41.45	1055.486	0.8	528.7506	2	18.36	3	
PF	PBC31431.1	ARMGFHGMRG	40.87	1118.523	-0.6	373.8479	3	9.39	3	
PF	PBC31431.1	ALMGFQGVRG	39.1	1034.533	0.9	518.2743	2	25.78	3	
PF	PBC31431.1	APMGFYGTRa	39.01	997.4803	0.6	499.7478	2	15.72	4	Amidation
PF	PBC31431.1	ALMGFQGVRa	37.61	976.5276	0.6	489.2714	2	22.47	4	Amidation
PF	PBC31431.1	SPFRYLGVRa	34.87	1092.619	0.9	547.3174	2	16.65	10	Amidation
PF	PBC31431.1	ASFDDEYY	26.92	1008.371	0.4	505.1931	2	32.7	4	
PF	PBC31431.1	ASFDDEYYKRAPMGFQGMRa	54.61	2267.025	0.7	567.764	4	30.79	8	Amidation
PF	PBC31431.1	STDFQDVESGSESF	46.63	1533.611	1.5	767.8138	2	44.9	12	
PF	PBC32274.1	pQLHNIIDKPRQN	47.32	1457.774	1.2	729.8951	2	16.62	6	Pyro-glu from Q
PF	PBC32274.1	pQLHNIIDKPRQNFNDP	40.55	1930.965	1.2	966.4909	2	26.43	6	Pyro-glu from Q
PF	PBC32274.1	pQITQFTPRLa	39.64	1084.603	1	543.3093	2	54	4	Pyro-glu from Q; Amidation

PF	PBC32274.1	SGMWFGPRLa	39.39	1048.528	1.1	525.2717	2	47.79	4	Amidation
PF	PBC32274.1	RVPWTPSPRLa	39.12	1206.699	0.6	604.3569	2	19.13	7	Amidation
PF	PBC32274.1	VPWTPSPRLa	37.19	1050.597	0.4	526.3062	2	24.2	4	Amidation
PF	PBC32274.1	pQLHNIIDKPRQNFNDPRF	30.58	2234.135	1.6	559.5418	4	32.43	4	Pyro-glu from Q
PF	PBC32274.1	TSQDITSGMWFGPRLa	46.98	1693.825	1.5	847.9208	2	62.81	10	Amidation
PF	PBC32274.1	SQDITSGMWFGPRLa	39.1	1592.777	1.5	797.397	2	66.63	4	Amidation
PF	PBC32274.1	pQLHNIIDKP	37.74	1059.571	0.2	530.793	2	24.59	3	Pyro-glu from Q
PF	PBC32274.1	DITSGMWFGPRLa	36.73	1377.686	1.7	689.8516	2	76.96	4	Amidation
PF	PBC32274.1	GMWFGPRLa	25.78	961.4956	0.9	481.7555	2	49.4	6	Amidation
PF	PBC32496.1	IPAADKERLLN	44.95	1238.698	-0.1	620.3563	2	15.01	4	
PF	PBC32496.1	LRNQLDIGDLQ	43.76	1283.683	2.6	642.8506	2	30.89	5	
PF	PBC32496.1	SYWKQCAFNAVSCFa	41.11	1651.728	1.9	826.8726	2	69.18	7	Amidation
PF	PBC32545.1	NSELINSLLGLPKNMNNAa	50.59	1940.015	1.2	971.0159	2	72.01	11	Amidation
PF	PBC32608.1	IDLSRFYGHFNT	52.52	1468.71	1.3	735.3632	2	47.67	11	
PF	PBC32608.1	IDLSRFYGHF	45.01	1253.619	0.3	627.8171	2	52.78	9	
PF	PBC32608.1	IDLSRFYGHFNTKR	38.28	1752.906	0.4	439.2339	4	27.86	5	
PF	PBC32608.1	DLSRFYGHF	26.3	1140.535	0.7	571.2753	2	35.46	8	
PF	PBC32608.1	IDLSRFYGHFNTK	34.1	1596.805	-0.9	533.2751	3	35.46	10	
PF	PBC32678.1	pQDVDHVFLRFa	43.61	1256.63	1.4	629.3232	2	60.92	9	Pyro-glu from Q; Amidation
PF	PBC32678.1	pQDVDHVFLR	36.49	1110.546	-0.8	556.2797	2	32.82	3	Pyro-glu from Q
PF	PBC32678.1	QDVDHVFLRFa	30.34	1273.657	2.2	637.837	2	35.62	10	Amidation
PF	PBC32727.1	SQAYDPYSNAAQFQLSSQSRGYPYQHRL	62.05	3261.523	1.3	816.389	4	38.35	9	
PF	PBC32727.1	LPTNLGEDTKKTEQTMRPKS	55.51	2273.169	1.6	569.3003	4	15.34	14	
PF	PBC32727.1	YPYQHRLIY	44.17	1251.64	0.7	626.8277	2	19.76	5	
PF	PBC32727.1	NVPIYQEPRF	40.73	1261.646	0.6	631.8304	2	32.28	3	

PF	PBC32727.1	SQAYDPYSNAAQFQLSSQSRGYPYQHRLIY	67.17	3537.67	1.4	885.4261	4	48.14	22	
PF	PBC32727.1	SQAYDPYSNAAQFQLSSQSRGYPYQHRLI	42.19	3374.607	1.3	844.66	4	46.04	3	
PF	PBC32727.1	VPIYQEPRF	39.88	1147.603	0.8	574.809	2	30.27	4	
PF	PBC32727.1	GYPYQHRLIY	35.91	1308.662	1.2	655.3388	2	20.49	7	
PF	PBC32914.1	SIATLAKNDDLPISLHDRMAENEDDEE	66.67	3040.393	0.9	1014.473	3	41.42	13	
PF	PBC32914.1	FLLLPATDNNYFHQKLPSSLRSKSL	51.8	2888.555	0.9	963.8597	3	53.93	7	
PF	PBC32914.1	YVASLARTGDLPIRGQ	48.79	1715.932	0.7	858.9738	2	24.81	12	
PF	PBC32914.1	NVGSVAREHGLPYa	47.94	1396.721	1	699.3685	2	15.44	7	Amidation
PF	PBC32914.1	NVASLARTYTLPQNAa	47.62	1616.863	1.3	809.44	2	30.12	4	Amidation
PF	PBC32914.1	NIASLIRDYDQSRENRVSFPa	46.82	2378.209	0.5	793.744	3	48.39	10	Amidation
PF	PBC32914.1	SISSLARTGDLPVREQ	45.9	1727.917	1.2	576.9802	3	23.96	9	
PF	PBC32914.1	NVGTLARDFALPPa	45.12	1368.751	1.3	685.3839	2	44.83	20	Amidation
PF	PBC32914.1	YVASLARTGDLPIRa	37.25	1529.868	0.5	510.9634	3	22.77	6	Amidation
PF	PBC32914.1	SVSSLAKNSAWPVSL	47.07	1544.82	2.4	773.419	2	52.64	6	
PF	PBC32914.1	GIFVPGSVILRALSRQa	38.99	1711.026	0.9	571.3497	3	69.51	19	Amidation
PF	PBC34787.1	AYTYVSEYKRLPVYNFGIa	59.77	2181.126	0.7	1091.571	2	58.48	9	Amidation
PF	PBC34787.1	PNDMLSQRYHFGLa	59.21	1575.762	1	788.8889	2	32.45	8	Amidation
PF	PBC34787.1	AVHYSGGQPLGSKRPNDMLSQRYHFGLa	50.41	3013.509	0.8	1005.511	3	26.04	12	Amidation
PF	PBC34787.1	AVHYSGGQPLGS	38.4	1171.562	1	586.7889	2	13.02	5	
PF	PBC34787.1	RQYSFGLa	33.4	868.4555	-0.2	435.235	2	19.76	3	Amidation
PF	PBC34787.1	WIDTNDNKRGRDYSFGLa	30.34	2054.992	1.4	686.0056	3	26.1	4	Amidation
PF	PBC34787.1	LDYLPVDNPAFH	48.74	1399.677	1	700.8466	2	53.02	6	
PF	PBC34787.1	GRQPYSFGLa	33.85	1022.53	1.3	512.2728	2	23.75	5	Amidation
PF	PBC34787.1	GRDYSFGLa	29.14	912.4453	0	457.2299	2	23.11	7	Amidation
PF	PBC34787.1	YPLRLNLD	28.83	1002.55	0.5	502.2824	2	34.88	3	

PF	XP_016905690.1	LNSDSRNSQVNGYTPRLa	51.57	1918.961	1.2	640.6617	3	15.11	5	Amidation
PF	XP_016905690.1	NSDSRNSQVNGYTPRLa	49.71	1805.877	1.4	602.967	3	14.65	4	Amidation
PF	XP_016905690.1	RASGLLSYPRIa	37.06	1230.72	1.3	616.3679	2	20.79	4	Amidation
PF	XP_016908608.1	LTNYLATGHRTNGGPVI	61.58	1782.938	-0.6	892.4755	2	24.48	7	
PF	XP_016908608.1	NLDEIDRVGWSGFV	55.78	1605.779	1.8	803.8981	2	73.08	9	
PF	XP_016908608.1	LTNYLATGHRTNGGPVIRRFa	38.36	2241.224	0.2	449.2522	5	15.5	13	Amidation
PF	XP_016908608.1	NIDEIDRTAFDNFF	54.7	1715.779	0.8	858.8975	2	83.7	6	
PF	XP_016908970.1	MVPVPVHHMADELLRSGPDTVI	63.82	2412.229	1	1207.123	2	59.89	23	
PF	XP_016908970.1	VHHMADELLRSGPDTVI	59.42	1888.947	1	945.4814	2	31.3	12	
PF	XP_016908970.1	MVPVPVHHMADEL	38.66	1473.711	0.6	737.8632	2	26.32	4	
PF	XP_016908970.1	LRSGPDTVI	33.3	956.5291	0.5	479.2721	2	16.34	3	
PF	XP_016908970.1	VPVPVHHMADELL	33.21	1455.754	1.1	728.8853	2	31.73	13	
PF	XP_016920932.1	TWKSPDIVIRFa	42.29	1359.766	0.3	454.2628	3	41.77	11	Amidation
PF	XP_016920932.1	GRNDLNFIRYa	46.06	1265.663	0.5	633.839	2	23.5	8	Amidation
NF	PBC25365.1	pQQFDDYGHLRFa	57.86	1406.637	1.1	704.3264	2	57.22	3	Pyro-glu from Q; Amidation
NF	PBC27532.1	SLPLYGGNMSKTGDSRLKSEFE	72.83	2415.174	-1.7	1208.592	2	29.14	9	
NF	PBC27532.1	SLPLYGGNMSKTGDSRLKSE	70.09	2139.063	-1.1	1070.538	2	19.53	9	
NF	PBC27532.1	HPISYDTYDERELSRDHPPLLL	51.4	2665.314	1.7	889.4467	3	40.88	12	
NF	PBC27532.1	ARRKALQDQAQIDANRRLLETIa	50.38	2577.458	0.9	645.3723	4	21.77	9	Amidation
NF	PBC27532.1	LVDHRIPDLENEMF	49.87	1726.835	1.2	576.6196	3	48.67	5	
NF	PBC27532.1	ISYDTYDERELSRDHPPLLL	47.14	2431.202	-1.4	811.4067	3	49.77	5	
NF	PBC27532.1	IGSLSIVNSMDVLRQRVLLELARRKALQDQAQIDANRRLLETIa	32.59	4913.782	2.9	819.9734	6	88.02	6	Amidation
NF	PBC27532.1	LVDHRIPDLENEMFDSGNDPGSTVVRT	69.38	3012.425	2.7	1005.152	3	56.97	3	
NF	PBC27982.1	ITGQGNRIF	47.84	1004.54	1	503.2779	2	17.63	4	

NF	PBC27982.1	SLKAPFA	35.82	732.417	-1.3	367.2153	2	19.3	3	
NF	PBC27985.1	YLLSGKARYa	28.55	1068.608	0.4	535.3115	2	11.92	6	Amidation
NF	PBC28057.1	GNNRPVYIPQPRPPHP	61.04	1837.97	0.9	613.6645	3	13.46	17	
NF	PBC28057.1	GNNRPVYIPQPRPPHPRL	57.25	2107.155	1.7	703.3935	3	15.04	61	
NF	PBC28057.1	PVYIPQPRPPHP	42.53	1396.762	0	466.5945	3	18.93	7	
NF	PBC28214.1	GLDLGLSRGFSGSQAAKHLMGLAAANYAGGPa	48.64	2985.524	2.3	996.1843	3	69.28	8	Amidation
NF	PBC28214.1	GLDLGLSRGFSGSQAA	38.12	1534.774	1.7	768.3955	2	42.16	5	
NF	PBC28214.1	GLDLGLSRGFSGSQAAKH	35.47	1799.928	0.4	450.9894	4	18.94	4	
NF	PBC28214.1	HLMGLAAANYAGGPa	52.17	1340.666	0.2	671.3403	2	24.83	3	Amidation
NF	PBC28214.1	GLDLGLSRGFSGSQAAKHLMa	53.99	2985.524	-1.5	1493.767	2	69.37	19	Amidation
NF	PBC30406.1	SDPHLSIGILSKPISAIPSSKFDD	66.11	2523.322	-2.7	1262.665	2	57.71	9	
NF	PBC30406.1	SQRSPSLRLRFa	41.59	1344.774	0.2	449.2653	3	13.35	8	Amidation
NF	PBC30406.1	SPSLRLRFa	33.76	973.5821	0.3	487.7985	2	17.83	3	Amidation
NF	PBC30406.1	SDPHLSIGILSKPISAIP	33.69	1844.041	3.3	923.0306	2	63.22	6	
NF	PBC30406.1	SDPHLSIGILSKP	56.41	1362.751	0.6	682.3831	2	30.59	3	
NF	PBC31004.1	pQMFTYSHGWTNa	35.37	1352.561	2.1	677.2891	2	54.56	16	Pyro-glu from Q; Amidation
NF	PBC31251.1	AYRKPPFNGSIFa	56.07	1394.746	1.2	698.381	2	20.1	23	Amidation
NF	PBC31251.1	KPPFNGSIFa	39.77	1004.544	0.3	503.2796	2	26.3	8	Amidation
NF	PBC31251.1	RKPPFNGSIFa	28.99	1160.645	0.6	581.3303	2	20.94	6	Amidation
NF	PBC31431.1	APVGYQEMQGKKNSASLNSENFGIF	76.23	2715.296	0.9	1358.657	2	47.19	9	
NF	PBC31431.1	NSIINDVKNELFPEDIN	61.58	1972.974	1.8	987.4961	2	85.69	17	
NF	PBC31431.1	ARMGFHGMRa	56.1	1060.517	0.4	531.2661	2	8.49	4	Amidation
NF	PBC31431.1	STDFQDVESGSESFKRARMGFHGMRa	52.07	2860.313	-0.9	477.7257	6	24.04	8	Amidation
NF	PBC31431.1	SPFRYLGV	49.43	937.5021	0.4	469.7585	2	34.68	6	

NF	PBC31431.1	APMGFQGMRG	46.35	1050.474	1	526.2448	2	19.72	4	
NF	PBC31431.1	ARMGFHGMRG	45.29	1118.523	-0.7	373.8479	3	9.3	3	
NF	PBC31431.1	APMGFYGTRa	45.15	997.4803	0.5	499.7477	2	15.19	4	Amidation
NF	PBC31431.1	APMGFYGTRG	44.23	1055.486	0.1	528.7502	2	17.78	3	
NF	PBC31431.1	APMGFQGMRa	43.83	992.4684	-0.1	497.2414	2	16.14	3	Amidation
NF	PBC31431.1	ALMGFQGVRG	42.24	1034.533	0	518.2738	2	25.21	3	
NF	PBC31431.1	ALMGFQGVRa	39.26	976.5276	1.2	489.2717	2	22.01	3	Amidation
NF	PBC31431.1	SPFRYLGVRa	36.21	1092.619	-0.1	365.2137	3	17.16	3	Amidation
NF	PBC31431.1	ASFDDEYY	24.23	1008.371	0.2	505.193	2	32.63	3	
NF	PBC31431.1	ASFDDEYYKRAPMGFQGMRa	64.73	2267.025	1.2	567.7642	4	26.98	10	Amidation
NF	PBC31431.1	STDFQDVESGSESF	43.46	1533.611	1.2	767.8135	2	45.7	8	
NF	PBC32274.1	RVPWTPSPRLa	42.45	1206.699	1.2	604.3572	2	18.98	4	Amidation
NF	PBC32274.1	pQLHNIIDKPRQN	41.7	1457.774	3.2	729.8966	2	15.91	4	Pyro-glu from Q
NF	PBC32274.1	pQLHNIIDKPRQNFNDPRF	33.79	2234.135	1.6	745.72	3	31.38	4	Pyro-glu from Q
NF	PBC32274.1	pQITQFTPRLa	30.83	1084.603	1	543.3093	2	53.25	9	Pyro-glu from Q; Amidation
NF	PBC32274.1	pQLHNIIDKPRQNFNDP	28.77	1930.965	-2.1	966.4877	2	25.76	5	Pyro-glu from Q
NF	PBC32274.1	SGMWFGPRLa	28	1048.528	-0.7	525.2707	2	47.79	12	Amidation
NF	PBC32274.1	VPWTPSPRLa	27.27	1050.597	1.5	526.3068	2	23.67	3	Amidation
NF	PBC32274.1	DITSGMWFGPRLa	43.5	1377.686	1.1	689.8512	2	91.87	4	Amidation
NF	PBC32274.1	GMWFGPRLa	26.94	961.4956	0.8	481.7555	2	47.5	7	Amidation
NF	PBC32274.1	pQLHNIIDKP	33.03	1059.571	0.7	530.7933	2	20.67	12	Pyro-glu from Q
NF	PBC32274.1	SQDITSGMWFGPRLa	48.18	1592.777	1	797.3965	2	75.27	4	Amidation
NF	PBC32274.1	TSQDITSGMWFGPRLa	54.49	1693.825	1.1	847.9205	2	72.22	5	Amidation
NF	PBC32496.1	LRNQLDIGDLQ	48.94	1283.683	1.5	642.8499	2	30.84	5	
NF	PBC32496.1	IPAADKERLLN	46.28	1238.698	0.4	620.3566	2	13.93	5	

NF	PBC32496.1	SYWKQCAFNAVSCFa	38.98	1651.728	1.1	826.8719	2	70.16	9	Amidation
NF	PBC32545.1	NSELINSLLGLPKNMNNAa	37.17	1940.015	2.1	971.0167	2	71.55	3	Amidation
NF	PBC32608.1	IDLSRFYGHF	50.48	1253.619	1.4	627.8178	2	51.77	4	
NF	PBC32608.1	IDLSRFYGHFNT	44.72	1468.71	1	735.3629	2	47.98	3	
NF	PBC32608.1	IDLSRFYGHFNTKR	37.96	1752.906	-0.2	585.3091	3	26.76	4	
NF	PBC32608.1	DLSRFYGHF	24.76	1140.535	1.2	571.2755	2	34.83	3	
NF	PBC32608.1	IDLSRFYGHFNTK	28.71	1596.805	1.3	799.4107	2	36.19	9	
NF	PBC32678.1	pQDVDHVFLR	49.86	1110.546	0.4	556.2804	2	30.38	5	Pyro-glu from Q
NF	PBC32678.1	pQDVDHVFLRFa	54.14	1256.63	1	629.3229	2	67.87	16	Pyro-glu from Q; Amidation
NF	PBC32678.1	QDVDHVFLRFa	54.26	1273.657	0.8	637.8362	2	32.44	3	Amidation
NF	PBC32727.1	LPTNLGEDTKKTEQTMRPKS	61.45	2273.169	-0.7	1137.591	2	14.32	14	
NF	PBC32727.1	SQAYDPYSNAAQFQLSSQSRGYPYQHRL	55.41	3261.523	0.5	1088.182	3	37.25	3	
NF	PBC32727.1	NVPIYQEPRF	46.81	1261.646	0.4	631.8303	2	31.89	4	
NF	PBC32727.1	YPYQHRLIY	20.47	1251.64	1.1	418.2211	3	20.5	3	
NF	PBC32727.1	GYPYQHRLIY	20.84	1308.662	0.3	437.2279	3	15.38	11	
NF	PBC32727.1	SQAYDPYSNAAQFQLSSQSRGYPYQHRLI	35.98	3374.607	-0.2	1125.876	3	48.52	20	
NF	PBC32727.1	SQAYDPYSNAAQFQLSSQSRGYPYQHRLI Y	74.39	3537.67	1	885.4257	4	55.74	10	
NF	PBC32727.1	VPIYQEPRF	46.09	1147.603	1.1	574.8092	2	27.03	10	
NF	PBC32914.1	SIATLAKNDDLPISLHDRMAENEDDEE	79.73	3040.393	-0.6	1014.471	3	41.3	7	
NF	PBC32914.1	NVASLARTYTLPQNAa	58.15	1616.863	1.6	809.4402	2	28.93	3	Amidation
NF	PBC32914.1	NVGSVAREHGLPYa	56.28	1396.721	0.6	699.3682	2	16.87	6	Amidation
NF	PBC32914.1	YVASLARTGDLPIRGQ	53.24	1715.932	1.1	572.9852	3	25.03	9	
NF	PBC32914.1	FLLLPATDNNYFHQKLPSSLRSKSL	51.67	2888.555	2.3	723.1476	4	52.88	4	
NF	PBC32914.1	SISSLARTGDLPVREQ	49.75	1727.917	2.1	576.9807	3	22.94	6	

NF	PBC32914.1	NIASLIRDYDQSRENRVSFPa	47.5	2378.209	0.8	793.7443	3	47.24	6	Amidation
NF	PBC32914.1	YVASLARTGDLPIRa	26.48	1529.868	1.1	765.942	2	22.24	3	Amidation
NF	PBC32914.1	NVGTLARDFALPPa	19.92	1368.751	0.2	685.3831	2	43.82	4	Amidation
NF	PBC32914.1	GIFVPGSVILRALSRQa	48.99	1711.026	0.9	571.3497	3	83.9	14	Amidation
NF	PBC32914.1	SVSSLAKNSAWPVSL	48.47	1544.82	1.4	773.4183	2	57.72	4	
NF	PBC34787.1	PNDMLSQRYHFGLa	66.58	1575.762	0.1	788.8882	2	31.07	4	Amidation
NF	PBC34787.1	AVHYSGGQPLGSKRPNDMLSQRYHFGLa	53.14	3013.509	1.1	754.3854	4	24.91	11	Amidation
NF	PBC34787.1	AYTYVSEYKRLPVYNFGIa	49.81	2181.126	-0.5	1091.57	2	57.15	4	Amidation
NF	PBC34787.1	AVHYSGGQPLGS	38.77	1171.562	0.6	586.7887	2	13.17	3	
NF	PBC34787.1	WIDTNDNKRGRDYSFGLa	37	2054.992	0.6	686.0051	3	24.87	6	Amidation
NF	PBC34787.1	RQYSFGLa	32.4	868.4555	-0.3	435.2349	2	19.73	3	Amidation
NF	PBC34787.1	GRQPYSFGLa	32.58	1022.53	0.5	512.2724	2	18.09	3	Amidation
NF	PBC34787.1	YPLRLNLD	34.51	1002.55	0.3	502.2823	2	32.93	8	
NF	PBC34787.1	LDYLPVDNPAFH	40.13	1399.677	2.2	700.8474	2	53.16	14	
NF	PBC34787.1	GRDYSFGLa	20.29	912.4453	0	457.2299	2	22.83	13	Amidation
NF	XP_016905690.1	RASGLLSYPRIa	36.91	1230.72	1.6	616.368	2	17.49	5	Amidation
NF	XP_016905690.1	LNSDSRNSQVNGYTPRLa	43.12	1918.961	0.8	640.6614	3	15.95	22	Amidation
NF	XP_016905690.1	NSDSRNSQVNGYTPRLa	30.46	1805.877	2.7	602.9678	3	14.09	18	Amidation
NF	XP_016908608.1	LTNYLATGHRTNGGPVI	68.44	1782.938	1.1	892.4771	2	23.49	5	
NF	XP_016908608.1	NLDEIDRVGWSGFV	65.24	1605.779	1.8	803.8981	2	73.95	5	
NF	XP_016908608.1	LTNYLATGHRTNGGPVIRRFa	38.87	2241.224	0.5	449.2524	5	17.43	9	Amidation
NF	XP_016908608.1	NIDEIDRTAFDNFF	41.27	1715.779	2.6	858.899	2	96.53	8	
NF	XP_016908970.1	MVPVPVHHMADELLRSGPDTVI	64.98	2412.229	-0.6	1207.121	2	60.24	11	
NF	XP_016908970.1	VHHMADELLRSGPDTVI	57.42	1888.947	-0.9	945.4797	2	30.16	4	
NF	XP_016908970.1	MVPVPVHHMADEL	38.96	1473.711	1.1	737.8635	2	26.34	6	

NF	XP_016908970.1	LRSGPDTVI	33.48	956.5291	0.2	479.2719	2	15.76	3	
NF	XP_016908970.1	VPVPVHHMADELL	31.46	1455.754	0.5	486.259	3	33.15	4	
NF	XP_016920932.1	TWKSPDIVIRFa	49.3	1359.766	0.3	454.2628	3	40.95	9	Amidation
NF	XP_016920932.1	GRNDLNFIRYa	47.92	1265.663	-0.1	422.8949	3	17.89	3	Amidation

Table S5. Quantitative neuropeptide comparison of different behavioral phenotypes of *Apis mellifera ligustica* workers. "Protein Accession" is the unique number given to mark the entry of a protein in the database NCBInr. "Peptide" is the amino acid sequence of the peptide. "Significance (-10lgP)" is the peptide confidence score. "NB" is nurse bee. "PF" is pollen forager. "NF" is nectar forager. "Group Profile (Ratio)" is the relative abundance ratio to the base group. "PTM" is post translational modification types present in the peptide.

Protein	Protein Accession	Peptide	Significance	NB 1	NB 2	NB 3	PF 1	PF 2	PF 3	NB	PF	Group Profile (Ratio)	PTM
PBAN-type neuropeptides (PBAN)	**A8CL69.1**	QITQFTPRLa	60	6.51E+06	6.29E+06	6.44E+06	4.23E+07	4.34E+07	4.42E+07	6.41E+06	4.33E+07	1.00 : 6.76	Amidation
		TSQDITSGMWFGPRLa	60	6.88E+08	6.55E+08	6.65E+08	1.88E+09	1.85E+09	1.95E+09	6.69E+08	1.89E+09	1.00 : 2.83	Amidation
		MWFGPRLa	27.89	2.04E+06	2.04E+06	2.15E+06	4.40E+05	4.64E+05	4.47E+05	2.08E+06	4.50E+05	1.00 : 0.22	Amidation
FMRFamide	**ACI90290.1**	TWKSPDIVIRFa	60	1.81E+07	1.85E+07	1.69E+07	4.27E+07	4.30E+07	4.18E+07	1.78E+07	4.25E+07	1.00 : 2.38	Amidation
		GRNDLNFIRYa	42.6	2.94E+06	3.09E+06	3.11E+06	4.77E+06	4.84E+06	4.44E+06	3.05E+06	4.68E+06	1.00 : 1.54	Amidation
Myosuppressin	**P85527.1**	pQDVDHVFLRFa	30.88	1.33E+08	1.21E+08	1.28E+08	3.48E+08	3.65E+08	3.39E+08	1.27E+08	3.51E+08	1.00 : 2.75	Pyro-glu from Q; Amidation
		pQDVDHVFLR	60	1.23E+07	1.33E+07	1.39E+07	6.40E+06	6.49E+06	6.72E+06	1.32E+07	6.54E+06	1.00 : 0.5	Pyro-glu from Q
Prohormone-3	**P85828.1**	SLKAPFA	60	9.06E+06	9.13E+06	8.90E+06	1.88E+07	2.00E+07	2.06E+07	9.03E+06	1.98E+07	1.00 : 2.19	
Brian peptide	**P85829.1**	MVPVPVHHMADEL	60	6.94E+05	7.00E+05	6.87E+05	2.39E+06	2.46E+06	2.57E+06	6.94E+05	2.47E+06	1.00 : 3.57	
Diuretic hormone (DH)	**P85830.1**	GLDLGLSRGFSGSQAAKHLMa	24.42	4.01E+08	4.26E+08	4.21E+08	8.49E+07	8.43E+07	8.29E+07	4.16E+08	8.40E+07	1.00 : 0.2	Amidation
Allatostatin (AST)	**Q06601.1**	GRDYSFGLa	53.26	8.32E+07	8.35E+07	8.56E+07	2.19E+08	2.46E+08	2.26E+08	8.41E+07	2.30E+08	1.00 : 2.74	Amidation
Apidaecins	**Q06602.1**	GNNRPVYIPQPRPPHPRL	35.66	6.18E+09	6.04E+09	5.92E+09	3.37E+09	3.60E+09	3.47E+09	6.05E+09	3.48E+09	1.00 : 0.58	
Corazonin (CRZ)	**Q5DW47.1**	pQTFTYSHGWTNa	33.25	2.52E+06	2.65E+06	2.75E+06	7.79E+06	7.96E+06	8.01E+06	2.64E+06	7.92E+06	1.00 : 3	Pyro-glu from Q; Amidation
Tachykinins (TK)	**Q868G6.1**	ALMGFQGVRa	60	3.37E+08	3.66E+08	3.58E+08	1.47E+09	1.34E+09	1.32E+09	3.54E+08	1.38E+09	1.00 : 3.89	Amidation
		APMGFQGMRa	60	3.60E+08	3.69E+08	3.80E+08	1.32E+09	1.24E+09	1.43E+09	3.70E+08	1.33E+09	1.00 : 3.6	Amidation
		SPFRYLGARa	60	3.50E+07	3.75E+07	3.67E+07	1.21E+08	1.24E+08	1.15E+08	3.64E+07	1.20E+08	1.00 : 3.3	Amidation

		ARMGFHGMRG	60	5.23E+06	5.02E+06	5.11E+06	1.19E+07	1.23E+07	1.11E+07	5.12E+06	1.18E+07	1.00 : 2.3	
		GVMDFQIGLQ	60	5.17E+07	5.23E+07	5.19E+07	1.11E+08	1.28E+08	1.12E+08	5.20E+07	1.17E+08	1.00 : 2.25	
		ALMGFQGVRG	26.66	8.62E+05	8.72E+05	8.56E+05	1.87E+06	1.75E+06	1.69E+06	8.63E+05	1.77E+06	1.00 : 2.05	
		IILDALEELD	60	7.62E+06	7.96E+06	7.36E+06	4.66E+06	4.48E+06	4.39E+06	7.65E+06	4.51E+06	1.00 : 0.59	
		SPFRYLGA	60	3.01E+07	3.26E+07	3.10E+07	8.63E+06	8.72E+06	8.88E+06	3.12E+07	8.74E+06	1.00 : 0.28	
Neuropeptide like-1 (NPL1)	**XP_006559359.1**	YVASLARTGDLPIRa	30.62	2.05E+07	2.18E+07	2.15E+07	3.39E+07	3.47E+07	3.55E+07	2.13E+07	3.47E+07	1.00 : 1.63	Amidation
		NVASLARTYTLPQNAa	60	4.25E+07	4.23E+07	4.26E+07	2.11E+08	2.09E+08	2.12E+08	4.25E+07	2.11E+08	1.00 : 4.96	Amidation
Pigment-dispersing hormone (PDH)	**XP_006570344.1**	LINSLLGLPKNMNNAa	60	1.35E+07	1.49E+07	1.58E+07	2.85E+07	2.66E+07	2.98E+07	1.47E+07	2.83E+07	1.00 : 1.92	Amidation

Protein	Protein Accession	Peptide	Significance	NB 1	NB 2	NB 3	NF 1	NF 2	NF 3	NB	NF	Group Profile (Ratio)	PTM
Apidaecins	**Q06602.1**	GNNRPVYIPQPRPPHPRL	32.1	6.18E+09	6.04E+09	5.92E+09	3.15E+09	3.36E+09	3.31E+09	6.05E+09	3.27E+09	1.00 : 0.54	
		VYIPQPRPPHPRL	60	1.33E+09	1.30E+09	1.23E+09	2.84E+08	2.70E+08	2.74E+08	1.29E+09	2.76E+08	1.00 : 0.21	
Corazonin (CRZ)	**Q5DW47.1**	pQTFTYSHGWTNa	53.03	2.52E+06	2.65E+06	2.75E+06	6.36E+06	6.55E+06	6.30E+06	2.64E+06	6.40E+06	1.00 : 2.43	Pyro-glu from Q; Amidation
Diuretic hormone (DH)	**P85830.1**	GLDLGLSRGFSGSQAAKHLMa	60	4.01E+08	4.26E+08	4.21E+08	1.41E+08	1.25E+08	1.39E+08	4.16E+08	1.35E+08	1.00 : 0.33	Amidation
FMRFamide	**ACI90290.1**	GRNDLNFIRYa	43.64	2.94E+06	3.09E+06	3.11E+06	4.86E+06	4.79E+06	4.65E+06	3.05E+06	4.68E+06	1.00 : 1.56	Amidation
		TWKSPDIVIRFa	60	1.81E+07	1.85E+07	1.69E+07	7.71E+06	7.80E+06	7.68E+06	1.78E+07	7.73E+06	1.00 : 0.43	Amidation
Myosuppressin	**P85527.1**	pQDVDHVFLRFa	36.72	1.33E+08	1.21E+08	1.28E+08	4.23E+08	4.37E+08	4.36E+08	1.27E+08	4.32E+08	1.00 : 3.39	Pyro-glu from Q; Amidation
		pQDVDHVFLR	60	1.23E+07	1.33E+07	1.39E+07	4.15E+07	4.38E+07	4.21E+07	1.32E+07	4.25E+07	1.00 : 3.23	Pyro-glu from Q
Neuropeptide like-1 (NPL1)	**XP_006559359.1**	YVASLARTGDLPIRa	60	2.05E+07	2.18E+07	2.15E+07	4.49E+07	4.57E+07	4.39E+07	2.13E+07	4.48E+07	1.00 : 2.11	Amidation
		NVASLARTYTLPQNAa	60	4.25E+07	4.23E+07	4.26E+07	8.16E+07	8.31E+07	8.37E+07	4.25E+07	8.28E+07	1.00 : 1.95	Amidation
PBAN-type neuropeptides	**A8CL69.1**	TSQDITSGMWFGPRLa	30.19	6.88E+08	6.55E+08	6.65E+08	1.04E+09	1.02E+09	9.98E+08	6.69E+08	1.02E+09	1.00 : 1.52	Amidation

(PBAN)													
		MWFGPRLa	60	2.04E+06	2.04E+06	2.15E+06	7.56E+05	7.59E+05	7.49E+05	2.08E+06	7.55E+05	1.00 : 0.36	Amidation
Pigment-dispersing hormone (PDH)	**XP_006570344.1**	LINSLLGLPKNMNNAa	60	1.35E+07	1.49E+07	1.58E+07	2.78E+07	2.85E+07	2.66E+07	1.47E+07	2.76E+07	1.00 : 1.88	Amidation
Prohormone-3	**P85828.1**	ITGQGNRIF	60	8.78E+06	8.66E+06	8.67E+06	4.36E+07	4.27E+07	4.25E+07	8.70E+06	4.29E+07	1.00 : 4.93	
		SLKAPFA	38.97	9.06E+06	9.13E+06	8.90E+06	1.71E+07	1.68E+07	1.75E+07	9.03E+06	1.71E+07	1.00 : 1.9	
Tachykinins (TK)	**Q868G6.1**	ALMGFQGVRG	60	8.62E+05	8.72E+05	8.56E+05	4.27E+06	4.33E+06	4.47E+06	8.63E+05	4.36E+06	1.00 : 5.05	
		ALMGFQGVRa	30.19	3.37E+08	3.66E+08	3.58E+08	3.57E+09	3.63E+09	3.39E+09	3.54E+08	3.53E+09	1.00 : 9.98	Amidation
		APMGFQGMRa	60	3.60E+08	3.69E+08	3.80E+08	3.38E+09	3.27E+09	3.59E+09	3.70E+08	3.41E+09	1.00 : 9.23	Amidation
		ARMGFHGMRG	60	5.23E+06	5.02E+06	5.11E+06	1.26E+07	1.33E+07	1.21E+07	5.12E+06	1.28E+07	1.00 : 2.49	
		IILDALEELD	41.85	7.62E+06	7.96E+06	7.36E+06	2.13E+06	2.22E+06	2.30E+06	7.65E+06	2.22E+06	1.00 : 0.29	
		SPFRYLGA	31.06	3.01E+07	3.26E+07	3.10E+07	7.52E+06	7.72E+06	7.69E+06	3.12E+07	7.64E+06	1.00 : 0.24	

Protein	Protein Accession	Peptide	Significance	PF 1	PF 2	PF 3	NF 1	NF 2	NF 3	PF	NF	Group Profile (Ratio)	PTM
Allatostatin (AST)	**Q06601.1**	AVHYSGGQPLGSKRPNDMLSQRYHFGLa	30.34	4.90E+08	4.69E+08	4.98E+08	3.18E+08	3.25E+08	3.17E+08	4.86E+08	3.20E+08	1.00 : 0.66	Amidation
		WIDTNDNKRGRDYSFGLa	60	4.38E+07	4.15E+07	4.29E+07	2.24E+07	2.52E+07	2.38E+07	4.27E+07	2.38E+07	1.00 : 0.56	Amidation
Brian peptide	**P85829.1**	MVPVPVHHMADELLRNGPDTVI	60	9.95E+08	9.90E+08	1.04E+09	1.89E+09	1.98E+09	1.77E+09	1.01E+09	1.88E+09	1.00 : 1.86	
CAPA peptides-like	**XP_006559865.1**	AFGLLTYPRIa	60	2.88E+07	2.78E+07	2.63E+07	4.99E+07	4.66E+07	4.94E+07	2.76E+07	4.86E+07	1.00 : 1.76	Amidation
Diuretic hormone (DH)	**P85830.1**	GLDLGLSRGFSGSQAAKHLMa	60	8.49E+07	8.43E+07	8.29E+07	1.41E+08	1.25E+08	1.39E+08	8.41E+07	1.35E+08	1.00 : 1.61	Amidation
FMRFamide	**ACI90290.1**	TWKSPDIVIRFa	60	4.27E+07	4.30E+07	4.18E+07	7.71E+06	7.80E+06	7.68E+06	4.25E+07	7.73E+06	1.00 : 0.18	Amidation
Neuropeptide like-1 (NPL1)	**XP_006559359.1**	SVSSLARTGDLPVREQ	35.02	4.01E+07	4.21E+07	4.11E+07	2.52E+07	2.33E+07	2.38E+07	4.11E+07	2.41E+07	1.00 : 0.59	
		NIASLMRDYDQSRENRVPFPa	60	3.00E+08	2.86E+08	2.98E+08	1.42E+08	1.47E+08	1.64E+08	2.95E+08	1.51E+08	1.00 : 0.51	Amidation
		YVASLARTGDL	27	2.75E+08	2.92E+08	2.87E+08	6.32E+07	6.44E+07	6.27E+07	2.85E+08	6.34E+07	1.00 : 0.22	

		PIRGQ											
PBAN-type neuropeptides (PBAN)	**A8CL69.1**	QITQFTPRLa	60	4.23E+07	4.34E+07	4.42E+07	2.05E+07	2.24E+07	2.19E+07	4.33E+07	2.16E+07	1.00 : 0.5	Amidation
		pQITQFTPRLa	33.65	3.50E+08	3.36E+08	3.38E+08	8.50E+07	8.36E+07	8.38E+07	3.41E+08	8.41E+07	1.00 : 0.25	Pyro-glu from Q; Amidation
Prohormone-1	**P85798.1**	LRNQLDIGDLQ	42.97	9.56E+08	9.48E+08	9.34E+08	4.52E+09	4.41E+09	4.45E+09	9.46E+08	4.46E+09	1.00 : 4.71	
Prohormone-4	**P85831.1**	IDLSRFYGHFNT	60	6.46E+08	6.50E+08	6.36E+08	3.46E+09	3.50E+09	3.36E+09	6.44E+08	3.44E+09	1.00 : 5.34	
		IDLSRFYGHFN	34.77	1.76E+08	1.64E+08	1.54E+08	3.77E+08	3.96E+08	3.66E+08	1.65E+08	3.80E+08	1.00 : 2.31	
Short neuropeptide F (sNPF)	**XP_006565207.1**	SDPHLSILS	33.58	1.93E+06	1.84E+06	1.93E+06	9.67E+05	9.50E+05	9.57E+05	1.90E+06	9.58E+05	1.00 : 0.5	
		SPSLRLRFa	42.51	6.44E+06	6.16E+06	6.37E+06	1.11E+06	1.12E+06	1.33E+06	6.32E+06	1.19E+06	1.00 : 0.19	Amidation
Tachykinins (TK)	**Q868G6.1**	APMGFQGMRG	60	5.44E+07	5.60E+07	5.56E+07	2.38E+08	2.47E+08	2.43E+08	5.53E+07	2.43E+08	1.00 : 4.39	
		APMGFQGMRa	59.71	1.32E+09	1.24E+09	1.43E+09	3.38E+09	3.27E+09	3.59E+09	1.33E+09	3.41E+09	1.00 : 2.57	Amidation
		ALMGFQGVRa	60	1.47E+09	1.34E+09	1.32E+09	3.57E+09	3.63E+09	3.39E+09	1.38E+09	3.53E+09	1.00 : 2.56	Amidation

Table S6. Quantitative neuropeptide comparison of different behavioral phenotypes of *Apis cerana cerana* workers. "Protein Accession" is the unique number given to mark the entry of a protein in the database NCBInr. "Peptide" is the amino acid sequence of the peptide. "Significance (-10lgP)" is the peptide confidence score. "NB" is nurse bee. "PF" is pollen forager. "NF" is nectar forager. "Group Profile (Ratio)" is the relative abundance ratio to the base group. "PTM" is post translational modification types present in the peptide.

Protein	Protein Accession	Peptide	Significance	NB 1	NB 2	NB 3	PF 1	PF 2	PF 3	NB	PF	Group Profile (Ratio)	PTM
Prohormone-3	PBC27982.1	SLKAPFA	60	5.93E+07	5.68E+07	5.76E+07	1.40E+08	1.46E+08	1.44E+08	5.79E+07	1.43E+08	1.00 : 2.48	
		ITGQGNRIF	60	2.20E+07	2.39E+07	2.38E+07	6.70E+07	6.54E+07	6.88E+07	2.32E+07	6.71E+07	1.00 : 2.89	
Apidaecins	PBC28057.1	GNNRPVYIPQPR PPHPRL	60	4.98E+09	4.86E+09	5.07E+09	2.50E+09	2.59E+09	2.58E+09	4.97E+09	2.56E+09	1.00 : 0.51	
Diuretic hormone (DH)	PBC28214.1	GLDLGLSRGFSG SQAAKHLMa	39.87	4.60E+08	4.57E+08	4.22E+08	1.79E+09	1.91E+09	1.90E+09	4.46E+08	1.87E+09	1.00 : 4.18	Amidation
Short neuropeptide F (sNPF)	PBC30406.1	SDPHLSIGILSKPI SAIPSSKFDD	60	3.73E+08	3.94E+08	3.79E+08	1.46E+08	1.60E+08	1.57E+08	3.82E+08	1.54E+08	1.00 : 0.4	
Corazonin (CRZ)	PBC31004.1	pQMFTYSHGWT Na	28.91	9.94E+07	9.45E+07	9.63E+07	3.79E+08	3.73E+08	3.71E+08	9.67E+07	3.74E+08	1.00 : 3.87	Pyro-glu from Q; Amidation
SIFamide	PBC31251.1	KPPFNGSIFa	60	1.97E+08	1.84E+08	1.81E+08	8.11E+07	9.40E+07	9.48E+07	1.87E+08	9.00E+07	1.00 : 0.48	Amidation
		AYRKPPFNGSIFa	60	1.68E+09	1.55E+09	1.64E+09	4.56E+08	4.76E+08	4.75E+08	1.62E+09	4.69E+08	1.00 : 0.29	Amidation
Tachykinins (TK)	PBC31431.1	ASFDDEYY	56.6	6.50E+06	6.08E+06	6.11E+06	3.49E+07	3.10E+07	3.38E+07	6.23E+06	3.32E+07	1.00 : 5.33	
		APMGFQGMRa	60	9.27E+08	9.24E+08	9.17E+08	4.58E+09	4.73E+09	4.55E+09	9.23E+08	4.62E+09	1.00 : 5.01	Amidation
		APMGFYGTRG	60	7.36E+06	7.35E+06	7.19E+06	3.19E+07	3.83E+07	3.85E+07	7.30E+06	3.62E+07	1.00 : 4.96	
		APMGFQGMRG	40.07	9.10E+06	9.29E+06	9.11E+06	4.22E+07	4.19E+07	4.36E+07	9.17E+06	4.26E+07	1.00 : 4.64	
		ALMGFQGVRa	60	8.14E+08	8.19E+08	8.28E+08	3.87E+09	3.66E+09	3.89E+09	8.20E+08	3.81E+09	1.00 : 4.64	Amidation
		APVGYQEMQGK KNSASLNSENFG IF	55.82	4.61E+07	4.43E+07	4.48E+07	1.85E+08	1.83E+08	1.82E+08	4.51E+07	1.83E+08	1.00 : 4.07	
		ARMGFHGMRG	41.94	1.29E+07	1.40E+07	1.42E+07	4.16E+07	4.24E+07	4.17E+07	1.37E+07	4.19E+07	1.00 : 3.06	
		SPFRYLGV	60	5.53E+07	5.86E+07	5.75E+07	1.45E+08	1.64E+08	1.61E+08	5.71E+07	1.57E+08	1.00 : 2.74	
		ALMGFQGVRG	37.82	1.98E+06	2.09E+06	1.92E+06	3.64E+06	3.64E+06	3.91E+06	2.00E+06	3.73E+06	1.00 : 1.87	

Prohormone-2	PBC32727.1	NVPIYQEPRF	46.37	9.22E+08	9.28E+08	9.43E+08	3.25E+08	3.72E+08	3.88E+08	9.31E+08	3.62E+08	1.00 : 0.39	
		LPTNLGEDTKKTEQTMRPKS	60	5.12E+08	5.16E+08	5.02E+08	1.44E+08	1.56E+08	1.47E+08	5.10E+08	1.49E+08	1.00 : 0.29	
		VPIYQEPRF	33.21	9.74E+07	9.61E+07	9.48E+07	2.38E+07	2.16E+07	2.06E+07	9.61E+07	2.20E+07	1.00 : 0.23	
Neuropeptide like-1 (NPL1)	PBC32914.1	SISSLARTGDLPVREQ	30.75	3.69E+08	3.39E+08	3.46E+08	1.38E+09	1.33E+09	1.46E+09	3.51E+08	1.39E+09	1.00 : 3.96	
		NVGSVAREHGLPYa	60	6.26E+08	6.78E+08	6.89E+08	2.21E+09	2.73E+09	2.22E+09	6.64E+08	2.39E+09	1.00 : 3.59	Amidation
		NVGTLARDFALPPa	36.07	5.22E+07	5.05E+07	5.14E+07	1.31E+08	1.52E+08	1.21E+08	5.14E+07	1.35E+08	1.00 : 2.62	Amidation
Pigment-dispersing hormone (PDH)	PBC32545.1	NSELINSLLGLPKNMNNAa	23.88	7.62E+07	7.93E+07	7.72E+07	2.76E+08	2.75E+08	2.46E+08	7.76E+07	2.66E+08	1.00 : 3.43	Amidation
PBAN-type neuropeptides (PBAN)	PBC32274.1	pQITQFTPRLa	33.45	2.79E+07	2.85E+07	2.58E+07	1.59E+08	1.71E+08	1.55E+08	2.74E+07	1.62E+08	1.00 : 5.9	Pyro-glu from Q; Amidation
Orcokinin (ORC)	XP_016908608.1	NLDEIDRVGWSGFV	42.33	2.22E+08	2.48E+08	2.52E+08	6.87E+08	6.87E+08	6.53E+08	2.41E+08	6.76E+08	1.00 : 2.81	
Prohormone-4	PBC32608.1	IDLSRFYGHFNT	30.72	9.52E+08	9.58E+08	9.13E+08	3.06E+09	3.12E+09	3.03E+09	9.41E+08	3.07E+09	1.00 : 3.26	
Protein	**Protein Accession**	**Peptide**	**Significance**	**NB 1**	**NB 2**	**NB 3**	**NF 1**	**NF 2**	**NF 3**	**NB**	**NF**	**Group Profile (Ratio)**	**PTM**
Prohormone-3	PBC27982.1	SLKAPFA	60	5.93E+07	5.68E+07	5.76E+07	1.75E+08	1.62E+08	1.77E+08	5.79E+07	1.71E+08	1.00 : 2.96	
Apidaecins	PBC28057.1	GNNRPVYIPQPRPPHPRL	35.67	4.98E+09	4.86E+09	5.07E+09	1.89E+09	1.92E+09	1.98E+09	4.97E+09	1.93E+09	1.00 : 0.39	
Diuretic hormone (DH)	PBC28214.1	GLDLGLSRGFSGSQAAKHLMa	60	4.60E+08	4.57E+08	4.22E+08	2.39E+09	2.40E+09	2.51E+09	4.46E+08	2.43E+09	1.00 : 5.45	Amidation
Short neuropeptide F (sNPF)	PBC30406.1	SPSLRLRFa	60	3.86E+07	3.68E+07	3.63E+07	5.84E+06	5.43E+06	5.37E+06	3.72E+07	5.55E+06	1.00 : 0.15	Amidation
Corazonin (CRZ)	PBC31004.1	pQMFTYSHGWTNa	48.07	9.94E+07	9.45E+07	9.63E+07	4.49E+08	4.58E+08	4.78E+08	9.67E+07	4.62E+08	1.00 : 4.77	Amidation
SIFamide	PBC31251.1	KPPFNGSIFa	60	1.97E+08	1.84E+08	1.81E+08	6.87E+07	6.79E+07	6.70E+07	1.87E+08	6.79E+07	1.00 : 0.36	Amidation
		AYRKPPFNGSIFa	60	1.68E+09	1.55E+09	1.64E+09	3.26E+08	3.14E+08	3.06E+08	1.62E+09	3.15E+08	1.00 : 0.19	Amidation
Tachykinins (TK)	PBC31431.1	ALMGFQGVRa	60	8.14E+08	8.19E+08	8.28E+08	9.80E+09	9.58E+09	9.57E+09	8.20E+08	9.65E+09	1.00 : 11.76	Amidation
		APMGFQGMRa	60	9.27E+08	9.24E+08	9.17E+08	9.93E+09	1.02E+10	9.93E+09	9.23E+08	1.00E+10	1.00 : 10.86	Amidation

		APMGFQGMRG	56.21	9.10E+06	9.29E+06	9.11E+06	8.10E+07	7.91E+07	7.86E+07	9.17E+06	7.96E+07	1.00 : 8.68	
		ASFDDEYY	39.72	6.50E+06	6.08E+06	6.11E+06	4.46E+07	4.32E+07	4.21E+07	6.23E+06	4.33E+07	1.00 : 6.95	
		APMGFYGTRG	60	7.36E+06	7.35E+06	7.19E+06	4.62E+07	4.58E+07	4.99E+07	7.30E+06	4.73E+07	1.00 : 6.48	
		ALMGFQGVRG	60	1.98E+06	2.09E+06	1.92E+06	1.07E+07	1.21E+07	1.23E+07	2.00E+06	1.17E+07	1.00 : 5.86	
		APVGYQEMQGK KNSASLNSENFG IF	35.68	4.61E+07	4.43E+07	4.48E+07	1.86E+08	1.82E+08	1.92E+08	4.51E+07	1.87E+08	1.00 : 4.14	
		ARMGFHGMRG	28.15	1.29E+07	1.40E+07	1.42E+07	5.47E+07	5.44E+07	5.44E+07	1.37E+07	5.45E+07	1.00 : 3.98	
Prohormone-2	PBC32727.1	VPIYQEPRF	25.35	9.74E+07	9.51E+07	9.48E+07	3.12E+07	3.29E+07	3.30E+07	9.58E+07	3.24E+07	1.00 : 0.34	
		NVPIYQEPRF	60	9.22E+08	9.28E+08	9.43E+08	2.88E+08	3.15E+08	3.00E+08	9.31E+08	3.01E+08	1.00 : 0.32	
Neuropeptide like-1 (NPL1)	PBC32914.1	YVASLARTGDL PIRa	60	6.26E+08	6.78E+08	6.89E+08	3.17E+09	3.41E+09	3.20E+09	6.64E+08	3.26E+09	1.00 : 4.91	Amidation
		NVGSVAREHGL PYa	36.43	3.69E+08	3.39E+08	3.46E+08	1.45E+09	1.40E+09	1.50E+09	3.51E+08	1.45E+09	1.00 : 4.13	Amidation
		SISSLARTGDLP VREQ	60	5.22E+07	5.05E+07	5.14E+07	1.53E+08	1.55E+08	1.42E+08	5.14E+07	1.50E+08	1.00 : 2.92	
Pigment-dispersing hormone (PDH)	PBC32545.1	NSELINSLLGLP KNMNNAa	60	7.62E+07	7.93E+07	7.72E+07	3.02E+08	3.19E+08	3.18E+08	7.76E+07	3.13E+08	1.00 : 4.04	Amidation
PBAN-type neuropeptides (PBAN)	PBC32274.1	TSQDITSGMWF GPRLa	36.94	8.63E+07	8.83E+07	8.45E+07	2.38E+08	2.50E+08	2.47E+08	8.64E+07	2.45E+08	1.00 : 2.84	Amidation
Orcokinin (ORC)	XP_016908608.1	LTNYLATGHRT NGGPVI	43.31	4.29E+08	4.11E+08	4.24E+08	2.17E+09	2.13E+09	2.19E+09	4.21E+08	2.16E+09	1.00 : 5.13	
		NLDEIDRVGWS GFV	60	2.22E+08	2.48E+08	2.52E+08	9.30E+08	9.49E+08	9.42E+08	2.41E+08	9.40E+08	1.00 : 3.91	
Prohormone-4	PBC32608.1	IDLSRFYGHFNT	25.79	9.52E+08	9.58E+08	9.13E+08	6.23E+09	6.19E+09	6.34E+09	9.41E+08	6.25E+09	1.00 : 6.65	
Protein	**Protein Accession**	**Peptide**	**Significance**	**PF 1**	**PF 2**	**PF 3**	**NF 1**	**NF 2**	**NF 3**	**PF**	**NF**	**Group Profile (Ratio)**	**PTM**
Short neuropeptide F (sNPF)	PBC30406.1	SPSLRLRFa	60	1.37E+07	1.59E+07	1.46E+07	5.84E+06	5.43E+06	5.37E+06	1.47E+07	5.55E+06	1.00 : 0.38	Amidation
		SQRSPSLRLRFa	43.9	4.30E+07	4.03E+07	4.33E+07	1.12E+07	1.11E+07	1.20E+07	4.22E+07	1.14E+07	1.00 : 0.27	Amidation

Tachykinins (TK)	PBC31431.1	ALMGFQGVRG	51.11	3.64E+06	3.64E+06	3.91E+06	1.07E+07	1.21E+07	1.23E+07	3.73E+06	1.17E+07	1.00 : 3.14	
		ALMGFQGVRa	60	3.87E+09	3.66E+09	3.89E+09	9.80E+09	9.58E+09	9.57E+09	3.81E+09	9.65E+09	1.00 : 2.54	Amidation
		APMGFQGMRa	60	4.58E+09	4.63E+09	4.55E+09	9.93E+09	1.02E+10	9.93E+09	4.59E+09	1.00E+10	1.00 : 2.18	Amidation
		APMGFQGMRG	47.56	4.22E+07	4.19E+07	4.36E+07	8.10E+07	7.91E+07	7.86E+07	4.26E+07	7.96E+07	1.00 : 1.87	
PBAN-type neuropeptides (PBAN)	PBC32274.1	pQLHNIIDKPRQ NFNDPRF	60	6.45E+07	6.61E+07	6.72E+07	1.56E+07	1.61E+07	1.74E+07	6.59E+07	1.64E+07	1.00 : 0.25	Pyro-glu from Q
		pQITQFTPRLa	26.86	1.59E+08	1.71E+08	1.55E+08	4.20E+07	4.55E+07	4.41E+07	1.62E+08	4.39E+07	1.00 : 0.27	Pyro-glu from Q; Amidation
		pQLHNIIDKPRQ NFNDP	34.07	6.24E+06	6.13E+06	6.39E+06	2.29E+06	2.13E+06	2.01E+06	6.25E+06	2.14E+06	1.00 : 0.34	Pyro-glu from Q
Prohormone-4	PBC32608.1	IDLSRFYGHFN	49.52	2.50E+09	2.51E+09	2.49E+09	5.28E+09	5.29E+09	5.21E+09	2.50E+09	5.26E+09	1.00 : 2.1	
		IDLSRFYGHFNT	60	3.06E+09	3.12E+09	3.03E+09	6.23E+09	6.19E+09	6.34E+09	3.07E+09	6.25E+09	1.00 : 2.04	
Neuropeptide like-1 (NPL1)	PBC32914.1	SISSLARTGDLP VREQ	56.01	1.38E+09	1.33E+09	1.46E+09	1.53E+08	1.55E+08	1.42E+08	1.39E+09	1.50E+08	1.00 : 0.11	
		NIASLIRDYDQS RENRVSFPa	39.6	1.40E+08	1.59E+08	1.34E+08	2.99E+08	2.86E+08	3.03E+08	1.44E+08	2.96E+08	1.00 : 2.05	Amidation
		YVASLARTGDL PIRGQ	30.32	3.13E+08	3.00E+08	3.05E+08	1.50E+08	1.56E+08	1.58E+08	3.06E+08	1.55E+08	1.00 : 0.5	
Allatostatin (AST)	PBC34787.1	AVHYSGGQPLG SKRPNDMLSQR YHFGLa	60	8.06E+08	7.84E+08	7.90E+08	5.10E+08	5.17E+08	5.00E+08	7.93E+08	5.09E+08	1.00 : 0.64	Amidation
		WIDTNDNKRGR DYSFGLa	28.34	7.12E+07	7.11E+07	7.06E+07	4.35E+07	4.19E+07	4.22E+07	7.10E+07	4.25E+07	1.00 : 0.6	Amidation
Brain peptide	XP_016908970	MVPVPVHHMA DELLRSGPDTVI	60	5.20E+08	4.91E+08	4.94E+08	9.09E+08	9.84E+08	9.14E+08	5.02E+08	9.36E+08	1.00 : 1.87	
FMRFamide	XP_016920932.1	TWKSPDIVIRFa	60	1.94E+07	2.20E+07	2.10E+07	3.86E+07	3.92E+07	4.09E+07	2.08E+07	3.96E+07	1.00 : 1.9	Amidation

Table S7. Quantitative neuropeptide comparison between *Apis cerana cerana* and *Apis mellifera ligustica*. "NB" is nurse bee. "PF" is pollen forager. "NF" is nectar forager. "Peptide" is the amino acid sequence of the peptide. "Significance (-10lgP)" is the peptide confidence score. "Group Profile (Ratio)" is the relative abundance ratio to the base group. "PTM" is post translational modification types present in the peptide.

Protein	Peptide	Significance	ACC-NB 1	ACC-NB 2	ACC-NB 3	AML-NB 1	AML-NB 2	AML-NB 3	ACC-NB	AML-NB	Group Profile (Ratio)	PTM
Allatostatin (AST)	AYTYVSEYKRLPVYNFGIa	60	3.41E+08	3.10E+08	3.21E+08	1.09E+08	1.07E+08	1.11E+08	3.24E+08	1.09E+08	1.00 : 0.34	Amidation
Diuretic hormone (DH)	GLDLGLSRGFSGSQAA	36.51	1.22E+06	1.11E+06	1.08E+06	3.24E+06	3.37E+06	3.08E+06	1.14E+06	3.23E+06	1.00 : 2.84	
	GLDLGLSRGFSGSQAAKHLMa	60	2.39E+08	2.20E+08	2.27E+08	4.01E+08	4.26E+08	4.21E+08	2.29E+08	4.16E+08	1.00 : 1.82	Amidation
SIFamide	YRKPPFNGSIFa	60	4.54E+07	4.35E+07	4.46E+07	1.22E+08	1.17E+08	1.15E+08	4.45E+07	1.18E+08	1.00 : 2.65	Amidation
	KPPFNGSIFa	35.18	1.97E+08	1.84E+08	1.81E+08	3.97E+08	4.10E+08	3.83E+08	1.87E+08	3.97E+08	1.00 : 2.12	Amidation
Myosuppressin	pQDVDHVFLRFa	49.38	6.80E+07	6.50E+07	6.67E+07	1.33E+08	1.21E+08	1.28E+08	6.66E+07	1.27E+08	1.00 : 1.91	Pyro-glu from Q; Amidation
PBAN-type neuropeptide (PBAN)	GMWFGPRLa	60	7.02E+06	6.96E+06	6.87E+06	1.53E+07	1.37E+07	1.43E+07	6.95E+06	1.44E+07	1.00 : 2.08	Amidation
Prohormone-2	SQAYDPYSNAAQFQLSSQSRGYPYQHRL	60	4.18E+07	4.36E+07	4.23E+07	1.24E+08	1.19E+08	1.08E+08	4.26E+07	1.17E+08	1.00 : 2.75	
Prohormone-4	IDLSRFYGHF	41.13	2.19E+08	2.21E+08	2.01E+08	7.73E+08	7.77E+08	7.53E+08	2.14E+08	7.68E+08	1.00 : 3.59	
	DLSRFYGHF	52.14	1.52E+07	1.57E+07	1.43E+07	3.30E+06	3.76E+06	3.32E+06	1.51E+07	3.46E+06	1.00 : 0.23	
Tachykinins (TK)	ALMGFQGVRG	33.23	1.98E+06	2.09E+06	1.92E+06	8.62E+05	8.72E+05	8.56E+05	2.00E+06	8.63E+05	1.00 : 0.43	
	ALMGFQGVRa	60	8.14E+08	8.19E+08	8.28E+08	3.37E+08	3.66E+08	3.58E+08	8.20E+08	3.54E+08	1.00 : 0.43	Amidation
	APMGFQGMRa	60	9.27E+08	9.24E+08	9.17E+08	3.60E+08	3.69E+08	3.80E+08	9.23E+08	3.70E+08	1.00 : 0.4	Amidation
	APMGFQGMRG	60	9.10E+06	9.29E+06	9.11E+06	3.36E+06	3.19E+06	3.23E+06	9.17E+06	3.26E+06	1.00 : 0.36	
Protein	**Peptide**	**Significance**	**ACC-PF1**	**ACC-PF2**	**ACC-PF3**	**AML-PF1**	**AML-PF2**	**AML-PF3**	**ACC-PF**	**AML-PF**	**Group Profile (Ratio)**	**PTM**
Apidaecins	GNNRPVYIPQPRPPHPRL	60	1.38E+09	1.50E+09	1.46E+09	3.37E+09	3.60E+09	3.47E+09	1.447E+09	3.48E+09	1.00 : 2.41	

Callisulfakinin	pQQFDDYGHLRFa	60	6.46E+06	6.42E+06	6.39E+06	4.16E+06	4.32E+06	4.14E+06	6423333.3	4206667	1.00 : 0.65	Pyro-glu from Q; Amidation
FMRFamide-related peptides-like	GRNDLNFIRYa	60	1.66E+07	1.45E+07	1.48E+07	4.77E+06	4.84E+06	4.44E+06	15300000	4683333	1.00 : 0.31	Amidation
Neuropeptide like precursor 1 (NPLP1)	NVGSVAREHGLPYa	60	2.21E+09	2.73E+09	2.22E+09	6.84E+09	6.84E+09	6.87E+09	2.39E+09	6.85E+09	1.00 : 2.87	Amidation
	YVASLARTGDLPIRa	60	1.73E+07	1.77E+07	1.81E+07	3.39E+07	3.47E+07	3.55E+07	1.77E+07	47500000	1.00 : 1.96	Amidation
PBAN-type neuropeptide (PBAN)	RVPWTPSPRLa	60	7.45E+06	7.17E+06	7.21E+06	2.62E+07	2.50E+07	2.37E+07	7276666.7	24967000	1.00 : 3.43	Amidation
	GMWFGPRLa	31.51	3.25E+06	3.45E+06	3.52E+06	7.93E+06	7.83E+06	8.00E+06	3406666.7	7920333	1.00 : 2.32	
Prohormone-1	LRNQLDIGDLQ	60	4.46E+08	4.18E+08	4.51E+08	9.56E+08	9.48E+08	9.34E+08	438333333	9.46E+08	1.00 : 2.16	
Prohormone-4	IDLSRFYGHF	43.86	1.95E+08	1.88E+08	1.72E+08	2.94E+08	3.07E+08	2.88E+08	185000000	2.96E+08	1.00 : 1.6	
Tachykinins (TK)	ALMGFQGVRa	60	3.87E+09	3.66E+09	3.89E+09	1.47E+09	1.34E+09	1.32E+09	3.807E+09	1.38E+09	1.00 : 0.36	Amidation
	APMGFQGMRa	60	4.58E+09	4.63E+09	4.55E+09	1.32E+09	1.24E+09	1.43E+09	4.587E+09	1.33E+09	1.00 : 0.29	Amidation
	ASFDDEYY	60	3.49E+07	3.10E+07	3.38E+07	4.73E+06	4.93E+06	4.80E+06	3.32E+07	4.82E+06	1.00 : 0.14	
Protein	**Peptide**	**Significance**	**ACC-NF1**	**ACC-NF2**	**ACC-NF3**	**AML-NF1**	**AML-NF2**	**AML-NF3**	**ACC-NF**	**AML-NF**	**Group Profile (Ratio)**	**PTM**
Diuretic hormone (DH)	LVDHRIPDLENEMFDSGNDPGSTVVRT	31.21	2.54E+06	2.31E+06	2.51E+06	7.87E+06	7.99E+06	8.03E+06	2.45E+06	7.96E+06	1.00 : 3.25	
Neuropeptide like precursor 1 (NPLP1)	SVSSLAKNSAWPVSL	60	1.53E+08	1.55E+08	1.42E+08	2.62E+08	2.76E+08	2.89E+08	1.50E+08	2.76E+08	1.00 : 1.84	
	NVASLARTYTLPQNAa	27.49	4.77E+07	4.79E+07	4.99E+07	8.16E+07	8.31E+07	8.37E+07	4.85E+07	8.28E+07	1.00 : 1.71	Amidation
PBAN-type neuropeptide (PBAN)	TSQDITSGMWFGPRLa	60	2.38E+08	2.50E+08	2.47E+08	1.04E+09	1.02E+09	9.98E+08	2.45E+08	1.02E+09	1.00 : 4.16	Amidation
	pQITQFTPRLa	46.66	4.20E+07	4.55E+07	4.41E+07	8.50E+07	8.36E+07	8.38E+07	4.39E+07	8.41E+07	1.00 : 1.92	Pyro-glu from Q; Amidation
Pigment-dispersing hormone (PDH)	NSELINSLLGLPKNMNNAa	60	3.02E+08	3.19E+08	3.18E+08	5.49E+07	5.67E+07	5.49E+07	3.13E+08	5.55E+07	1.00 : 0.18	Amidation

Prohormone-4	IDLSRFYGHFNT	39.91	6.23E+09	6.19E+09	6.34E+09	3.46E+09	3.50E+09	3.36E+09	6.25E+09	3.44E+09	1.00 : 0.55	
Short neuropeptide F (sNPF)	SPSLRLRFa	28.41	5.84E+06	5.43E+06	5.37E+06	1.11E+07	1.12E+07	1.23E+07	5.55E+06	1.15E+07	1.00 : 2.08	Amidation
Tachykinins (TK)	ALMGFQGVRa	60	9.80E+09	9.58E+09	9.57E+09	3.57E+09	3.63E+09	3.39E+09	9.65E+09	3.53E+09	1.00 : 0.37	Amidation
	APMGFQGMRa	55.7	9.93E+09	1.02E+10	9.93E+09	3.38E+09	3.27E+09	3.59E+09	1.00E+10	3.41E+09	1.00 : 0.34	Amidation

Table S8. The proboscis extension response of workers after injection of ddH_2O and TRP2.

		ddH_2O			TRP2		
	Concentration	Show PER	No PER	PER ratio	Show PER	No PER	PER ratio
Pollen foragers	0.1%	19	36	34.55%	9	44	16.98%
	0.3%	21	34	38.18%	11	42	20.75%
	1.0%	30	25	54.55%	12	41	22.64%
	3.0%	36	19	65.45%	15	38	28.30%
	10.0%	38	17	69.09%	17	36	32.08%
	30.0%	48	7	87.27%	25	28	47.17%
	Pollen	22	34	39.29%	9	44	16.98%
	Larva	11	45	19.64%	12	41	22.64%

		ddH_2O			TRP2		
	Concentration	Show PER	No PER	PER ratio	Show PER	No PER	PER ratio
Nectar foragers	0.1%	10	45	18.18%	6	52	10.34%
	0.3%	14	41	25.45%	6	52	10.34%
	1.0%	16	39	29.09%	7	51	12.07%
	3.0%	19	36	34.55%	8	50	13.79%
	10.0%	25	30	45.45%	12	46	20.69%
	30.0%	29	26	52.73%	15	43	25.86%
	Pollen	7	46	13.21%	8	44	15.38%
	Larva	9	44	16.98%	6	46	11.54%

		ddH_2O			TRP2		
	Concentration	Show PER	No PER	PER ratio	Show PER	No PER	PER ratio
Nurse bees	0.1%	12	41	22.64%	8	44	15.38%
	0.3%	13	40	24.53%	10	42	19.23%
	1.0%	18	35	33.96%	13	39	25.00%
	3.0%	19	34	35.85%	16	36	30.77%
	10.0%	22	31	41.51%	21	31	40.38%
	30.0%	29	24	54.72%	25	27	48.08%
	Pollen	5	50	9.09%	7	48	12.73%
	Larva	21	32	39.62%	10	45	18.18%

Table S9. The proboscis extension response of workers after injection of ds*GFP*, ds*TRP*, and ds*TRPR*.

		ds*GFP*			ds*TRP*			ds*TRPR*		
Pollen foragers	Concentration	Show PER	No PER	PER ratio	Show PER	No PER	PER ratio	Show PER	No PER	PER ratio
	0.1%	20	36	35.71%	30	24	55.56%	33	23	58.93%
	0.3%	22	34	39.29%	33	21	61.11%	35	21	62.50%
	1.0%	30	26	53.57%	40	14	74.07%	41	15	73.21%
	3.0%	37	19	66.07%	41	13	75.93%	43	13	76.79%
	10.0%	38	18	67.86%	45	9	83.33%	46	10	82.14%
	30.0%	49	7	87.50%	51	3	94.44%	49	7	87.50%
	Pollen	19	33	36.54%	32	20	61.54%	33	21	61.11%
	Larva	10	42	19.23%	13	39	25.00%	12	42	22.22%

		ds*GFP*			ds*TRP*			ds*TRPR*		
Nectar foragers	Concentration	Show PER	No PER	PER ratio	Show PER	No PER	PER ratio	Show PER	No PER	PER ratio
	0.1%	9	44	16.98%	17	33	34.00%	19	34	35.85%
	0.3%	12	41	22.64%	22	28	44.00%	22	31	41.51%
	1.0%	15	38	28.30%	27	23	54.00%	28	25	52.83%
	3.0%	18	35	33.96%	29	21	58.00%	32	21	60.38%
	10.0%	24	29	45.28%	31	19	62.00%	33	20	62.26%
	30.0%	28	25	52.83%	34	16	68.00%	38	16	70.37%
	Pollen	7	49	12.50%	13	42	23.64%	11	44	20.00%
	Larva	10	46	17.86%	11	44	20.00%	12	43	21.82%

		ds*GFP*			ds*TRP*			ds*TRPR*		
	Concentration	Show PER	No PER	PER ratio	Show PER	No PER	PER ratio	Show PER	No PER	PER ratio
Nurse bees	0.1%	12	43	21.82%	8	45	15.09%	9	46	16.36%
	0.3%	13	42	23.64%	8	45	15.09%	13	42	23.64%
	1.0%	18	37	32.73%	14	39	26.42%	16	39	29.09%
	3.0%	20	35	36.36%	23	30	43.40%	25	30	45.45%
	10.0%	23	32	41.82%	29	24	54.72%	27	28	49.09%
	30.0%	30	25	54.55%	31	22	58.49%	33	22	60.00%
	Pollen	6	50	10.71%	13	41	24.07%	12	43	21.82%
	Larva	21	35	37.50%	31	23	57.41%	32	23	58.18%

Table S10. Statistical differences in sucrose responsiveness after injection of ds*GFP*, ds*TRP*, and ds*TRPR*.

Concentration	0.10%	0.30%	1.00%	3.00%	10.00%	30.00%
Pollen foragers						
ds*TRP* vs ds*GFP*	*	*	*	ns	ns	ns
ds*TRPR* vs ds*GFP*	*	*	*	ns	ns	ns
ds*TRP* vs ds*TRPR*	ns	ns	ns	ns	ns	ns
Nectar foragers						
ds*TRP* vs ds*GFP*	*	*	**	*	ns	ns
ds*TRPR* vs ds*GFP*	*	*	*	**	ns	ns
ds*TRP* vs ds*TRPR*	ns	ns	ns	ns	ns	ns
Nurse bees						
ds*TRP* vs ds*GFP*	ns	ns	ns	ns	ns	ns
ds*TRPR* vs ds*GFP*	ns	ns	ns	ns	ns	ns
ds*TRP* vs ds*TRPR*	ns	ns	ns	ns	ns	ns

ns = P > 0.05, *P < 0.05, **P < 0.01

Table S11. Sequence information of primers used in this study.

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')
***TRPR* clone (for FLAG-tag expression vectors)**	AAGCTTAAGCTTATGCAGACCGTAGAAGTTTTTCTAAAC	GGATCCTCAAGACACGTGACCCGTAGTTTGCGA
***TRPR* clone (for EGFP-tag expression vectors)**	AAGCTTGCCACCATGCAGACCGTAGAAGTTTTTCTA	GGATCCAGACACGTGACCCGTAGTTTGC
***TRPR* RNAi**	TAATACGACTCACTATAGGGGAGCAAACGAAGGGTGGTAA	TAATACGACTCACTATAGGGCGCGTCGAAATCTGGAGT
***TRPR* qPCR**	GAGCAAACGAAGGGTGGTAA	ACTCCAGATTTCGACGCG
***TRP* RNAi**	TAATACGACTCACTATAGGGGGTGTGCGTGGAAAGAAAAA	TAATACGACTCACTATAGGGTTTGATATCCATCCATCGACAA
***TRP* qPCR**	GTTATCAAGATATGAGGAAT	ATGGATTAGAAGACAGTT
***GFP* RNAi**	TAATACGACTCACTATAGGGAGTGGAGAGGGTGAAGGTGA	TAATACGACTCACTATAGGGGGTAAAAGGACAGGGCCATC

Red font indicates T7 promoter sequence.