

# Social Behavior Atlas: A computational framework for tracking and mapping 3D close interactions of free-moving animals

**Yaning Han**<sup>1, 2, 3, 4, #</sup>, **Ke Chen**<sup>1, 2, 3, 4, #</sup>, **Yunke Wang**<sup>1, 3, 4, #</sup>, **Wenhao Liu**<sup>1, 3, 4, 5</sup>, **Xiaojing Wang**<sup>1, 3, 4, 6</sup>, **Jiahui Liao**<sup>1, 3, 4, 7</sup>, **Yiting Huang**<sup>1, 3, 4</sup>, **Chuanliang Han**<sup>1, 3, 4</sup>, **Kang Huang**<sup>1, 2, 3, 4</sup>, **Jiajia Zhang**<sup>1, 3, 4</sup>, **Shengyuan Cai**<sup>1, 3, 4</sup>, **Zhouwei Wang**<sup>1, 2, 3, 4</sup>, **Yongji Wu**<sup>1, 3, 4</sup>, **Gao Gao**<sup>1, 3, 4, 8</sup>, **Nan Wang**<sup>1, 2, 3, 4</sup>, **Jinxiu Li**<sup>9</sup>, **Yangwangzi Song**<sup>9</sup>, **Jing Li**<sup>10</sup>, **Guodong Wang**<sup>9</sup>, **Liping Wang**<sup>1, 3, 4</sup>, **Yaping Zhang**<sup>9</sup>, and **Pengfei Wei**<sup>1, 3, 4, \*</sup>

<sup>1</sup>Shenzhen Key Laboratory of Neuropsychiatric Modulation and Collaborative Innovation Center for Brain Science, Shenzhen-Hong Kong Institute of Brain Science, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China

<sup>2</sup>University of Chinese Academy of Sciences, Beijing 100049, China

<sup>3</sup>CAS Key Laboratory of Brain Connectome and Manipulation, the Brain Cognition and Brain Disease Institute, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China

<sup>4</sup>Guangdong Provincial Key Laboratory of Brain Connectome and Behavior, the Brain Cognition and Brain Disease Institute, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China

<sup>5</sup>Department of Neuroscience, City University of Hong Kong, Kowloon Tong, Hong Kong, China

<sup>6</sup>Department of Physical Education, China University of Geosciences, Beijing 100083, China

<sup>7</sup>School of Biomedical Engineering, Southern Medical University, Guangzhou 510515, China

<sup>8</sup>Honam University, Gwangju 62399, South Korea

<sup>9</sup>State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese

25 Academy of Sciences, Kunming 650223, China

26 <sup>10</sup>Kunming Police Dog Base of the Chinese Ministry of Public Security, Kunming 650204, China

27 <sup>#</sup>The authors contributed equally to this paper.

28

29 **Correspondence to:**

30 \*Pengfei Wei, Brain Cognition and Brain Disease Institute, Shenzhen Institutes of Advanced  
31 Technology, 1068 Xueyuan Avenue, Shenzhen University Town, Shenzhen, 518055, P.R.China. E-mail:

32 pf.wei@siat.ac.cn

33

34 **Abstract**

35 The study of social behaviors in animals is essential for understanding their survival and  
36 reproductive strategies. However, accurately tracking and analyzing the social interactions of free-  
37 moving animals has remained a challenge. Existing multi-animal pose estimation techniques suffer  
38 from drawbacks such as the need for extensive manual annotation and difficulty in discriminating  
39 between similar-looking animals in close social interactions. In this paper, we present the Social  
40 Behavior Atlas (SBeA), a novel computational framework that solves these challenges by employing  
41 a deep learning-based video instance segmentation model, 3D pose reconstruction, and unsupervised  
42 dynamic behavioral clustering. SBeA framework also involves a multi-camera setup to prevent  
43 occlusion, and a novel approach to identify individual animals in close social interactions. We  
44 demonstrate the effectiveness of SBeA in tracking and mapping the 3D close interactions of free-  
45 moving animals using the example of genetic mutant mice, birds, and dogs. Our results show that  
46 SBeA is capable of identifying subtle social interaction abnormalities, and the models and frameworks  
47 developed can be applied to a wide range of animal species. SBeA is a powerful tool for researchers  
48 in the fields of neuroscience and ecology to study animal social behaviors with a high degree of  
49 accuracy and reliability.

50

51 **Introduction**

52 Close social interactions are critical for the survival and reproduction of animals<sup>1</sup>. However, the  
53 study of social behaviors has traditionally relied on rudimentary measures, such as the duration of time  
54 spent in specific areas during experiments like three-chamber tests<sup>2</sup>. To address this limitation, deep  
55 learning-based quantitative measurements have emerged as a potential solution<sup>3</sup>. In particular, there  
56 has been a surge of interest in developing multi-animal pose estimation and behavioral mapping  
57 techniques across various disciplines, including neuroscience and ecology<sup>4</sup>. Although single-animal  
58 pose estimation has been achieved with high accuracy through deep learning, accurately tracking and  
59 mapping the social behaviors of multiple animals remains a challenging task<sup>5</sup>.

60 Advanced multi-animal pose estimation toolboxes, such as Multi-animal DeepLabCut (maDLC)  
61 and Social LEAP Estimate Animal Poses (SLEAP), have enabled markerless and precise tracking of  
62 body parts for different species based on videography<sup>6-8</sup>. However, these techniques suffer from  
63 several limitations. Firstly, the high level of tracking precision necessitates a significant amount of  
64 manual annotation, which becomes increasingly laborious as the number of animals in the study  
65 increases. Secondly, occlusion can occur when multiple animals are present in the same video frame,  
66 resulting in poor inference about the behavior of each animal. Thirdly, in close social interactions  
67 between similar-looking animals<sup>9</sup>, it becomes challenging to distinguish between individual identities,  
68 particularly over extended periods of time<sup>10</sup>.

69 The Social Behavior Atlas (SBeA) offers a solution to the challenges proposed by existing multi-  
70 animal pose estimation techniques. Firstly, the number of manual annotations can be reduced by  
71 comprising two processes. The first is the acquisition of each animal's contour. As fewer as 400  
72 annotations are enough to separate adjacent animals. These data generate millions of labeled frames to

73 train the deep learning-based video instance segmentation (VIS) model. Secondly, to address the issue  
74 of occlusion, SBeA employs multiple cameras to capture video streams, which are used to reconstruct  
75 3D poses and prevent complete occlusion<sup>11-13</sup>. Thirdly, SBeA resolves the multi-animal identification  
76 problem by merging the contour of each animal with the characteristic identity of multiple view angles,  
77 which achieves over 90% identification precision without human data annotation. Furthermore, after  
78 solving those problems, inspired by the natural structures of social behavior, the unsupervised dynamic  
79 behavioral metric learning is finally designed. The behavioral metric is composed of a time-series low-  
80 dimensional representation of the behavior module. The behavioral mapping generates the social  
81 behavior atlas, and one-third of the cluster purity reaches over 95%. Using SBeA, we found the subtle  
82 social interaction abnormalities of Shank3B KO mice, which verifies the availability of SBeA. The  
83 models and frameworks developed for SBeA can be also applied to birds and dogs, showcasing its  
84 strong generalization abilities suitable for various application scenarios.

85 **Results**

86 **SBeA: from multi-animal markerless 3D pose tracking to unsupervised social behavior mapping.**

87 The focus of SBeA is on quantifying the behavior of freely social animals comprehensively. It  
88 presents two significant challenges: pose tracking and behavior mapping. The pose tracking involves  
89 identifying the key body parts of each animal as well as their identities, which is particularly  
90 challenging when dealing with animals that look similar<sup>10</sup>. To address this issue, a novel free social  
91 behavior test paradigm has been developed that involves a multi-view camera array (Fig. 1a). This  
92 approach captures the animals covering more view angles and helps to overcome the challenge of  
93 frequent occlusion<sup>11-14</sup>. The camera array is used to capture images of a checkerboard for camera  
94 calibration, followed by videos of two free-moving animals for the social behavior test (Video capture  
95 phase 1, Fig. 1a). Finally, the array captures videos of single free-moving animals to facilitate animal  
96 identification without the need for human intervention (Video capture phase 2, Fig. 1a).

97 After the video acquisition, the multi-animal contour of video capture phase 1 and the single-  
98 animal pose of video capture phase 2 are manually annotated for the training of AI to output the 3D  
99 poses with identities of animals (Fig. 1b and c). The design of this AI model was separated into four  
100 stages for function integration. Through these multistage networks, the task of multi-animal video  
101 instance segmentation, pose estimation, and identity recognition was achieved with a relatively small  
102 number of manual annotations (~400 frames), as shown in Fig. 1d (left). By incorporating camera  
103 parameters, the above results from various camera angles were combined and matched based on  
104 geometric constraints to reconstruct 3D pose trajectories with identities for each animal (Fig. 1d, center  
105 and right).

106 After conducting pose tracking, the process of behavior mapping involves breaking down the

107 trajectories of animals into distinct behavior modules, and then, using appropriate metrics to obtain a  
108 low-dimensional representation of these modules<sup>12</sup>. In the context of SBeA, the framework for  
109 decomposing behavior is extended from a single animal to multiple animals<sup>12</sup>, with their 3D  
110 trajectories being separately decomposed into locomotion, non-locomotor movement, and body  
111 distance components (Fig. 1e top and middle). These parallel components are then divided into  
112 segments and subsequently merged into social behavioral modules using the dynamic behavior metric  
113 (Fig. 1e bottom). Overall, this process utilizes a nature-inspired structure for behavior decomposition  
114 and provides a dynamic approach to understanding social behaviors in groups of animals.

115 To gain insight into the distribution of features within social behavioral modules, it is necessary  
116 to convert them into low-dimensional representations (Fig. 1f). These representations incorporate both  
117 spatial and temporal aspects, with the spatial aspect being captured by low-dimensional embeddings  
118 of distance features in the SBeA framework (Fig. 1f left). The temporal aspect is represented by the  
119 social ethogram (Fig. 1f right). In SBeA, social behavioral modules are first clustered based on their  
120 spatial characteristics and then expanded into the temporal dimension to construct the social ethogram.  
121 This approach allows for a more comprehensive understanding of the distribution of features within  
122 social behavioral modules.

123

#### 124 **Fewer manual data annotations for multi-animal 3D pose tracking of SBeA.**

125 The use of deep learning for social pose estimation has been beneficial in enhancing the  
126 acquisition of data for multiple body parts in animals, as previously demonstrated in literature<sup>7,8</sup>.  
127 However, due to the flexible social interactions among animals, creating a comprehensive training  
128 dataset for deep learning-based social pose estimation is a challenge. Inadequately trained deep neural

129 networks tend to produce higher tracking errors, particularly in frames with close animal interactions  
130 <sup>10</sup>. To address this issue, we introduce a novel animal tracking method using continuously occluded  
131 copy-paste data augmentation (Fig. 2a) in our SBeA framework. There are pieces of evidence that  
132 simple copy-paste can increase the precision of instance segmentation<sup>15</sup>. Additionally, the continuous  
133 copy-paste further increases the performance of multi-object tracking and segmentation<sup>16</sup>. Here for  
134 multi-animal tracking, we extend the above methods to continuously occluded copy-paste, which  
135 generates the virtual scenario with instance occlusion. By capturing a short video of multiple animals  
136 behaving freely in an open field, SBeA obtains sufficient elements (background and animal instances)  
137 to generate the virtual dataset. These elements synthesize the complex interactive relationships  
138 between animals without the need for manual annotations, resulting in a sufficiently large virtual  
139 dataset to train deep neural networks.

140 During free behavior, it is common for animals to overlap, leading to loss of tracking in single-  
141 view cameras. To address this, SBeA employs a multi-view camera array to capture video streams,  
142 enabling compensation for the visual field of the cameras and facilitating continuous tracking (Fig. 2b)  
143 <sup>11-13</sup>. Background and trajectories can be extracted through background subtraction algorithms applied  
144 to the raw video streams (Fig. 2c left top and left middle). In addition, frames with close social  
145 interactions can be extracted for manual contour annotations (Fig. 2c left bottom). A lightweight  
146 instance segmentation deep neural network, YOLACT++, is trained with self-training using  
147 approximately 400-800 annotated contour frames (Fig. 2c center bottom), which enhances its  
148 performance while ensuring time-efficiency<sup>17,18</sup>. The well-trained self-training YOLACT++ predicts  
149 animal masks of video streams, and animal instances can be cropped based on these masks. As some  
150 trajectories of two animals may overlap in the same spatial position across different periods, merged

151 animal instances, backgrounds, trajectories, and masks can generate virtual scenarios with various  
152 occlusion relationships and mask labels (Fig. 2c center top and center middle). The continuously  
153 occluded copy-paste data augmentation increases the scale of the training dataset without additional  
154 manual annotations, producing a VIS dataset with successive frames of behaving animals and  
155 annotations. To capture the spatial-temporal patterns of occluded animals, the video instance  
156 segmentation with transformers (VisTR) method is modified and applied to the virtual VIS dataset as  
157 it can segment instances at the sequence level as a whole (Fig. 2c right top)<sup>19</sup>. Well-trained VisTR can  
158 patch the raw video streams to display only one animal in each video (Fig. 2d left top and left middle).  
159 Thus, pose estimation models trained for single animals, such as DeepLabCut, can be used to predict  
160 single animal poses on these patched videos after fine-tuning using patched frames (Fig. 2c right  
161 bottom, and 2d left bottom). Finally, the single-animal poses of each patched frame are merged into  
162 multi-animal poses (Fig. 2d left top, left middle, and left bottom).

163 The subsequent step in SBeA, following the acquisition of multi-animal poses from video frames,  
164 is the 3D reconstruction (Fig. 2e). Firstly, the MouseVenue3D automatic camera calibration system is  
165 employed to acquire the camera parameters of the camera array (Fig. 2e left top)<sup>11,13</sup>. Then, based on  
166 the epipolar constraint of camera parameters, the combination of each animal instance in each camera  
167 view is optimized to achieve minimum reprojection error (Fig. 2e left bottom). The optimized 3D  
168 skeletons of the single frame in Fig. 2d right bottom are presented in Fig. 2e right top and bottom. In  
169 the 3D skeleton, the close contact between two animals, such as anogenital sniffing, can be quantified  
170 (Fig. 2e right top and bottom).

171 Compared with the square increasing of routine multi-animal pose estimation methods such as  
172 maDLC, the pose annotation strategy in SBeA is linearly increasing with body points and the number

173 of animals (Fig. 2f)<sup>8</sup>. Considering the diversiform social interaction of animals, routine multi-animal  
174 pose estimation methods need to annotate more data on frames with various social interactions to get  
175 higher precision. Here, we create a well-annotated dataset Social Black Mice for Video Instance  
176 Segmentation (SBM-VIS) to quantify the performance of SBeA. According to the distance distribution  
177 of the test dataset, the clustering algorithm is used to separate close interaction (Fig. 2g, the left orange  
178 stem) and other conditions. The pixel root-mean-square error (RMSE) of all data is significantly lower  
179 than the close interaction of about 2 pixels of different body parts (Fig. 2h). But compared with maDLC,  
180 SBeA still has significantly lower RMSE of animal close interaction, with 800 pose-labeled frames are  
181 used to train maDLC and 400 pose- and 400 mask-labeled frames are used to train SBeA (Extended  
182 Data Fig. 1). For all of the test data, SBeA has significantly lower RMSE than maDLC in the Nose,  
183 Left ear, Right ear, Root tail, Mid tail, and Tip tail while maDLC has significantly lower pixel RMSE  
184 in the Back, Right front paw and Left front paw (Extended Data Fig. 1a). For the close contact part of  
185 the test data, SBeA has significantly lower RMSE in Nose, Left ear, Right ear, Left front limb, Right  
186 front limb, Left hind limb, Left hind paw, Root tail, Mid tail, and Tip tail (Extended Data Fig. 1b).  
187 These results show that SBeA can get higher precision with fewer manual annotations than routine  
188 multi-animal pose estimation methods such as maDLC. To get a similar precision of maDLC, SBeA  
189 only needs about a quarter of pose annotation points.

190  
191 **SBeA needs no data annotations for multi-animal identification.**

192 Accurately distinguishing the identities of free-moving animals is crucial for social behavior tests,  
193 particularly in studying treatment-induced behaviors in transgenic animal models<sup>12,20,21</sup>. However,  
194 frequent occlusion of these animals can lead to imprecise identification even with physical markers.

195 Moreover, the animals are the same breed to reduce the influence of irrelevant experiment variables  
196 with indistinguishable appearances for human annotators. That causes difficulties in creating the  
197 animal identification dataset to train deep neural networks such as SIPEC<sup>22</sup>. To address these  
198 challenges, we propose a solution in SBeA, which involves combining a camera array with  
199 bidirectional transfer learning in animal identification (Fig. 3a). Transfer learning allows artificial  
200 neural networks to use previous knowledge in new tasks<sup>23</sup>. For animal segmentation and identification  
201 tasks, the knowledge between them can be shared bidirectionally with each other. So, the segmentation  
202 model trained for multi-animals can be transferred to single-animal segmentation, and the  
203 identification model trained for single-animals can be transferred to multi-animal identification. The  
204 bidirectional transfer learning of them avoids unnecessary manual data annotations.

205 Well-trained VisTR in Fig. 2 can be used to segment single-animal instances from multiple view  
206 angles (Fig. 3b). These instances are then cropped, cascaded, and resized to generate training data for  
207 an identification model based on EfficientNet architecture (Fig. 3c, left and center)<sup>24</sup>. After that,  
208 LayerCAM is used to evaluate the patterns for identification recognition (Fig. 3c right)<sup>25</sup>. Before using  
209 the identification model in multi-animal instances, the cascaded and resized image frames were  
210 prepared (Fig. 3d, right). By using the best geometric constraint of 3D poses, instances from each  
211 frame view angle of each animal were matched to construct input frames of the identification model  
212 (Fig. 3d, left). Finally, the well-trained model outputted the top prediction probabilities to append the  
213 identities of instances and 3D poses. LayerCAM was also employed to verify the recognition patterns  
214 for identification (Fig. 3e).

215 To evaluate the identification model's performance in SBeA, we conducted experiments with ten  
216 C57BL/6J mice having tail markers, where we recorded their free behaviors for 5 minutes. The tail

217 markers are convenient for experimenters to distinguish the identities of each mouse. The first 4  
218 minutes of data were used for training the identification model, and the last 1 minute was used for  
219 validation. The confusion matrix of the validation data demonstrated that the EfficientNet model can  
220 identify most of the mice (Fig. 3f). The t-SNE algorithm was used to create a 2D feature representation  
221 of the identified mice (Fig. 3j). However, the features of mice with ID M4 and M5 were found to be  
222 mixed with other classes, as quantified by the silhouette coefficient Fig. 3h). The sorted validation  
223 precision of the identified mice showed that the mouse with ID M4 had the lowest precision of  
224 approximately 0.4 (Fig. 3i). Even though the features of M5 were mixed with other classes, its  
225 precision was found to be around 0.8 (Fig. 3i).

226 To assess the identification model's performance in multi-animal data, we recorded the free social  
227 behaviors of 5 paired C57BL/6J mice identified by SBeA for 15 minutes. We manually verified the  
228 identities of mask reprojection images and 3D poses frame by frame (Fig. 3j). The results indicated  
229 that although some of the single mouse identity precisions were lower (Fig. 3i), the overall precision  
230 in identifying pairs of mice could be higher than 0.85, as seen in the case of the pairs of M3&M4 and  
231 M5&M6. Additionally, the validation precision in single-animal identification was found to be  
232 positively correlated with precision in multi-animal identification, as evidenced by the other pairs (Fig.  
233 3j).

234 We also investigate if the number of animals would influence the identification recognition  
235 precision. Previous research suggests that the identification precision may decrease with an increasing  
236 number of animals involved in the study<sup>26,27</sup>. To counteract this trend, we increased the amount of  
237 training data to balance the precision decrease. Our results indicate that for a group of 22 mice, a 15-  
238 minute video recording can achieve similar precision to that of 10 mice with a 5-minute recording (Fig.

239 3k). These findings have important implications for optimizing study design and ensuring accurate  
240 identification of individual animals in social behavior experiments.

241 Our research has revealed that the precision of animal identification can be influenced by the  
242 experiment apparatus used in social behavior tests (Fig. 3l). Specifically, we found that open fields  
243 with different diameters - 50cm and - 20cm can impact the precision of animal identification conducted  
244 on the same ten mice. Our results indicate that the precision of identification in the 20cm open field is  
245 significantly higher than that in the 50cm field (Fig. 3l). This difference may be due to the higher dots  
246 per inch (DPI) of mice.

247 Further, we tested the stability of identification patterns. Animals would groom themselves, which  
248 could change the patterns of identities<sup>9</sup>. We compared the identification precision of two separate  
249 groups of mice. One group underwent both identity video recording and social behavior tests on the  
250 same day, while the other group underwent social behavior tests one week after their identity videos  
251 were recorded (Fig. 3m). We manually verified the identities of mask reprojection images and 3D  
252 poses frame-by-frame. Our analysis revealed that while there was no significant difference in the  
253 precision of mask reprojection images between the two groups, the precision of 3D poses in the group  
254 that underwent social behavior tests one week after the recording of their identity videos was  
255 significantly lower than that of the group that underwent both on the same day (Fig. 3m). As the  
256 precision of 3D poses is equivalent to the identification precision of cascade and resize images, the  
257 observed decrease in precision of 3D poses indicates a decline in identification precision. Shorter  
258 intervals between the recording of identity videos and social behavior tests could potentially enhance  
259 the accuracy of identification recognition.

260 We evaluated the feature intensity of the identification model used to distinguish different animals

261 at last in this chapter. To this end, we designed open fields with diameters of 50 and 20 cm, respectively,  
262 in which the same ten mice were allowed to freely engage in social behavior 2 mice per trial. The pose  
263 tracking point "Root tail" with tail markers was used as a control against other body parts (Fig. 3n).  
264 We calculated LayerCAM values to quantify the feature intensity of each body point. The results  
265 showed that the Root tail in the 50 cm group had more significant feature intensities than in the 20 cm  
266 group. This finding suggests that a higher DPI can enable the identification model to capture more  
267 available fur pattern features and thereby overcome errors resulting from marker occlusion.  
268 Additionally, we found that identification using low animal DPI requires the use of stronger markers  
269 to maintain sufficient recognition precision.

270

271 **SBeA reveals the social behavioral structure in the atlas by unsupervised machine learning.**

272 Following pose tracking, it is necessary to map the trajectories with animal identities to a low-  
273 dimensional space to gain insights into behavior (Fig. 4a). Recent research has indicated that the body  
274 language of social animals can be represented through sequential behavioral motifs or modules<sup>28</sup>. Thus,  
275 we expand our prior work on the animal behavior mapping framework to encompass multiple animals,  
276 Behavior Atlas (BeA), which was initially developed for a single animal. The concepts of parallel and  
277 dynamic behavior decomposition from BeA have been adopted in our new framework SBeA (Fig. 4b  
278 and c). In the social process, the distance between animals is an essential component, as noted in  
279 previous studies<sup>29</sup>. In addition to using non-locomotor movement to assess body movement and  
280 locomotion to evaluate body displacement, body distance is also utilized to evaluate the relationships  
281 of body position (Fig. 4b). After parallel decomposition, each component is decomposed further using  
282 dynamic time alignment kernel (DTAK) to retain the natural dynamic structures of behavior (Fig. 4c).

283 To distinguish subtle structures of social behavior, the temporal points of decomposition for each  
284 component are merged through logical addition (Fig. 4d). The aforementioned steps enable the metric  
285 of social behavior, resulting in the transformation of continuous pose trajectories into discrete social  
286 behavior modules.

287 Then, the social behavior modules are embedded in a low-dimensional space for behavior  
288 representation (Fig. 4e and f). All of the social behavior modules from different experimental trials  
289 need to be represented in a common feature space. That induces two questions, the first is what feature  
290 is reasonable to represent social behavior in a low-dimensional space, and the second is how to create  
291 a common feature space under the big behavioral data<sup>30,31</sup>. For the first question, the distance  
292 component is chosen for the feature representation of social behavior modules (Fig. 4e left). The  
293 dimensionally reduced distance component by uniform manifold approximation and projection  
294 (UMAP) is beneficial to improve the separation of behavior atlas verified by our previous studies<sup>11-</sup>  
295 <sup>14,32</sup>. But with the increase of data scale, the computational consumption of UMAP would be  
296 unacceptable because of limited memory space, which is the second question. To solve the second  
297 question, the residual multilayer perceptron (ResMLP) is combined with UMAP for feature  
298 representation (Fig. 4e right)<sup>33</sup>. A part of the social behavior feature frames is extracted randomly to  
299 build up the feature representation of distance dynamics by the UMAP. Then, the mapping from  
300 extracted social behavior feature frames to distance dynamics is trained by ResMLP for the feature  
301 encoding. Further, the rest of the social behavior feature frames are decoded by ResMLP to distance  
302 dynamics. The distance dynamics are embedded by DTAK and UMAP to construct the social behavior  
303 atlas (Fig. 4f). To reveal the distributions of different social behavior modules, based on density  
304 clustering, we modified the watershed algorithm to automatically determine the best cluster density

305 with upper and lower boundaries. At last, the social behavior modules of the same clusters are manually  
306 identified and defined (Fig. 4g).

307 In constructing the social behavior atlas, the inclusion of the distance component is crucial. By  
308 using the distance component, the social behavior atlas can maintain the overall body distance  
309 structures of social behavior modules (Fig. 4h left), while the absence of the distance component leads  
310 to a lack of observable patterns in the distribution of distance (Fig. 4h right). To compare the  
311 effectiveness of the distance representations in the atlases, the map/body distance metric is utilized,  
312 with higher values indicating better performance in distance representation (Fig. 4i). Results show that  
313 the distance component is essential in achieving a high map/body distance, indicating the importance  
314 of including this component in constructing the social behavior atlas. Additionally, the  $0.45 \pm 0.32$ s  
315 temporal duration of merged behavioral modules reveals that the SBeA framework can effectively  
316 decompose social behavior into dynamic sub-second motifs (Fig. 4j)<sup>12,34</sup>. The ResMLP can address  
317 issues related to the memory cost of large behavioral data, while also reducing computational time  
318 consumption compared to using UMAP alone. More than 5000 frames can get time benefits from  
319 ResMLP, and the time benefits will increase with the number of frames (Fig. 4k). Then, the time  
320 proportion of identified behavioral modules is quantified to evaluate their temporal precision (Fig. 4l).  
321 The time proportion of the typical social behavior such as allogrooming conforms to previous studies  
322 on social behavior<sup>35</sup>. Further, the feature correlations between the intra- and inter-clusters of each social  
323 behavior class are compared for the evaluation of clustering consistency (Fig. 4m). The intra-feature  
324 correlations of each social behavior class are significantly higher than inter-feature correlations, and  
325 the intra-feature correlations distribute consistently near to 1, in turn, the inter-feature correlations  
326 distribute in the weak negative correlation. These unsupervised validation measures demonstrate the

327 effectiveness of the SBeA framework in accurately mapping social behavior.

328 In addition to unsupervised validation, we conducted supervised validation of SBeA using the  
329 PAIR-R24M dataset (Fig. 4n)<sup>36</sup>. The dataset provides 3D poses, social behavior labels, and subject  
330 behavior labels of rats in free behavior. We used SBeA to construct the social behavior atlas for the  
331 dataset, and appended the three social labels (close, chase, and explore) to each behavior module (Fig.  
332 4o). The distributions of the three social labels were separated and matched their similarity relationship.  
333 The 121 combinations of subject behavior labels also showed distribution patterns in the social  
334 behavior atlas (Fig. 4p). The social labels such as close and explore were consistent with the close  
335 distance distribution in the distance map, and the chase label was consistent with the distance transition  
336 zone of the distance map (Fig. 4q). To quantify the clustering performance, we used the cluster purity  
337 of social and subject behavior labels (Fig. 4r and s). For the upper boundary of clustering, 14 classes  
338 were clustered with a mean cluster purity of  $0.77 \pm 0.16$  (Fig. 4r). For the lower boundary of clustering,  
339 405 classes were clustered, and the probability of cluster purities greater than 0.95 was significantly  
340 higher than for other purities (Fig. 4s). These results provide further validation of the performance of  
341 SBeA in supervised contexts.

342

### 343 **SBeA identifies *Shank3B* knockout mice in free-social interactions by subtle behavior modules.**

344 Social behavior can serve as an indicator of the genetic variations that underlie neuropsychiatric  
345 disorders<sup>37</sup>. SBeA is well-suited for this purpose, as it allows for a detailed characterization of social  
346 behavior at an atlas-level. To test the ability of SBeA to detect genetic differences from social behavior,  
347 we utilized an animal model of autism spectrum disorder (ASD): Shank3B knockout mice<sup>12,20</sup>. While  
348 abnormal individual behaviors of these mice have been previously identified, the limitations of existing

349 techniques have made it difficult to fully understand their abnormal free social behaviors<sup>12,20</sup>.

350 To distinguish between Shank3B knockout (KO) mice and wild-type (WT) mice, a free-social  
351 behavioral paradigm was designed based on the framework of SBeA, which consists of three steps:  
352 identity recording, social behavior recording, and SBeA processing (Fig. 5a). First, the home-caged  
353 WT and KO mice were randomly shuffled and recorded for 5 minutes each using the MouseVenue3D  
354 system to obtain identity information. After identity recording, the mice were randomly grouped into  
355 three pairs (WT-WT, WT-KO, and KO-KO) for social behavior recording, with each pair of mice  
356 recorded for 15 minutes. The identity and social behavior data were then processed using SBeA for 3D  
357 pose tracking and behavior mapping. The experiment used a total of 10 WT and 10 KO mice, resulting  
358 in 45 unique pairs of mice, including 10 WT-WT, 10 KO-KO, and 25 WT-KO pairs. To ensure equal  
359 representation of each group, the number of WT-KO pairs was reduced from 25 to 10 through random  
360 sampling. Before behavior mapping, the raw trajectories were copied and switched to capture the  
361 direction of social behavior between WT and KO mice. This resulted in a total of 60 pairs of trajectories  
362 for behavior mapping using SBeA.

363 The social behavior atlas with distance map is shown in Fig. 5b. After the construction of the  
364 social behavior atlas, the density map is calculated to compare the social behavior distribution of each  
365 group by kernel density estimation (Fig. 5c). Density map shows obvious differences across the three  
366 groups. Combing with the distance map, the WT-WT group shows social behavior phenotypes with  
367 flexible distances from close to far, the KO-KO group shows more abnormal social behaviors than the  
368 WT-WT group, and WT-KO shows more close social interaction than the WT-WT group. From the  
369 global level, the social behaviors of KO mice show differences from WT mice.

370 The 260 social behavior modules identified in the social behavior atlas were clustered to reveal

371 their coincident patterns, which displayed distinct speckled patterns for each group, ranging from 1 to  
372 20 social behavior modules in the KO-KO group (Fig. 5d). To compare the differences in behavior  
373 components among the three groups, principal component analysis (PCA) was employed to determine  
374 the percent variability explained by each principal component (Fig. 5e). The results indicated that three  
375 components could account for 90% of the variance, while 11 components could account for 99% of  
376 the variance. To construct the phenotype space of the three groups, UMAP was used for dimensional  
377 reduction of the social behavior modules, with the dimension number set to 3 based on the 90%  
378 variance explanation, owing to the more robust feature representation of non-linear dimensional  
379 reduction (Fig. 5f). The distributions of the three groups in the phenotype space were found to be  
380 segregated, matching the distribution of the density map, and distinguishing KO mice from WT mice  
381 (Fig. 5c).

382 Further, SBeA was utilized to identify subtle social behavior modules that distinguish KO and  
383 WT mice. The two-way ANOVA was used to compare the behavior fractions between the three groups,  
384 and 24 social behavior modules were found to have significant differences (Fig. 5h). To reduce the  
385 redundancy of these results, angle spectrum clustering, which combines PCA and hierarchical  
386 clustering, was proposed (Fig. 5g). The social behavior modules were merged based on their angular  
387 separation of features, resulting in the identification of 9 social behaviors, as determined by human  
388 analysis (Fig. 5i). The color of mice represented the behavior cases with the highest mean fraction in  
389 Fig. 5g.

390 The 9 social behavior modules identified through SBeA highlighted significant differences among  
391 the three groups. The WT-WT group exhibited more allogrooming, a prosocial behavior, than the WT-  
392 KO and KO-KO groups<sup>38</sup>. Conversely, allogrooming was rare in unstressed partners and even rarer in

393 Shank3B KO mice, suggesting an antisocial behavioral phenotype<sup>35</sup>. The exploring behavior of the  
394 WT-WT group was significantly higher than that of the KO-KO group, which displayed reduced motor  
395 ability or social novelty<sup>12,20</sup>. In the WT-KO group, social behavior with significant differences were  
396 divided into two parts, namely, peer sniffing and independent grooming. Peer sniffing was observed  
397 more frequently in the WT mouse, particularly when the KO mouse was grooming or in locomotion,  
398 indicating a behavioral phenotype of curiosity. Furthermore, the KO mouse could induce higher  
399 interest in the WT mouse than vice versa. Independent grooming could be an imitation of the WT  
400 mouse by the KO mouse, and in the KO-KO groups, the higher incidence of independent grooming  
401 could be attributed to the increased individual grooming of each mouse. In addition to increased  
402 independent grooming, two abnormal behavior phenotypes, namely, synchronous behaviors and  
403 immobility, were observed. The synchronous behaviors displayed 5 subtypes, including grooming,  
404 hunching, rearing, sniffing, and micromovement, indicating greater behavior variability in free-social  
405 conditions compared to individual spontaneous behavior of KO mice<sup>12</sup>. Most instances of immobility  
406 occurred in only one pair of KO-KO mice, indicating that abnormal autistic-like behaviors vary even  
407 among mice with the same genetic background. These findings demonstrate that SBeA can  
408 differentiate genetic mutant animals based on social behavior and identify genetic mutant-related  
409 subtle social behavior modules.

410  
411 **SBeA is robust to be used in different environments across species.**

412 To assess the generalizability of SBeA to different animal species and experimental settings, the  
413 behaviors of birds and dogs were captured using the MouseVenue3D system with varying device  
414 configurations<sup>11</sup>. The animals were prepared to have as similar appearances as possible (Fig. 5a top

415 and 5e top), and it was difficult for human experimenters to separate two animals from the randomly  
416 selected frames. The resulting videos were manually annotated to train the AI of the pose tracking  
417 component of SBeA (Fig. 6a bottom and 6e bottom), using 19 body parts for birds and 17 body parts  
418 for dogs, based on previous studies<sup>39,40</sup>. The well-trained AI was then used to predict video instances,  
419 body poses, and identities (Fig. 6b and f), which were mapped to a social ethogram and behavior atlas  
420 using the behavior mapping component of SBeA (Fig. 6c and g). In total, 34 and 15 social behavior  
421 classes were identified for birds and dogs, respectively, and their typical cases were visualized in 3D  
422 (Fig. 6d and h). The 3D pose tracking of birds showed clear identification of their claw touching their  
423 rectrix, while the 3D pose tracking of dogs was robust to occlusion even in the lying posture.

424 To evaluate the performance of the SBeA algorithm in tracking birds and dogs, various metrics  
425 were employed, including tracking likelihood, 3D reprojection error, identity confusion matrix, and  
426 feature correlation (FC) (Fig. 6i-m). The results indicate that while dogs have a higher tracking  
427 likelihood than birds, both achieve a satisfactory level of tracking precision (Fig. 6i)<sup>12</sup>. But the 3D  
428 reprojection error is significantly higher for dogs due to incomplete camera coverage and annotation  
429 errors (Fig. 6j). In terms of identity recognition, both birds and dogs have higher precision than mice  
430 due to their distinct fur patterns (Fig. 6k). The results of FC show that all of the intra-FC of clusters  
431 are significantly higher than inter-FC (Fig. 6l and m). But from the distribution of FCs, the clustering  
432 performance of birds is better than dogs. The feature mix-up of intra- and inter-clusters is influenced  
433 by the 3D pose tracking precision. The error of 3D pose tracking such as target loss in dogs would  
434 degrade the performance of SBeA clustering. The LayerCAM analysis reveals no significant  
435 differences in feature values between birds and dogs, except for the Mid back and Nose of dogs, which  
436 may be attributed to the loss of nose detection in video captures (Fig. 6n and o). The identification

437 recognition network automatically reduces the feature weights on the body part with target loss or  
438 occlusion to keep the higher recognition precision of identities. These results demonstrate that SBeA  
439 is robust enough to be applied to different animal species in various experimental settings, making it a  
440 versatile tool for the study of social behavior in animals.

441 **Discussion**

442 Here we have presented SBeA, a framework for 3D pose tracking and behavior mapping of multiple  
443 free-social animals. SBeA builds upon the BeA framework, extending it to enable multi-animal pose  
444 estimation and social behavior clustering<sup>11-14</sup>. The method reduces the labor required for annotation  
445 by up to fifty percent compared to traditional approaches for pose estimation. By utilizing four  
446 cameras, SBeA overcomes the issue of occlusion and reconstructs 3D behaviors accurately. Notably,  
447 SBeA resolves the challenge of animal identification over extended time frames, facilitating the study  
448 of close social interactions. The technique is highly versatile and has been successfully applied to  
449 various animal species, including *Shank3B* knockout mice, where it revealed synchronous behaviors  
450 and reduced social interest. SBeA's cross-species application has been verified in birds and dogs. In  
451 summary, SBeA represents a breakthrough in deep learning-based pose estimation and identification,  
452 offering numerous potential applications in animal behavior research.

453 Both maDLC and SLEAP are versatile tools that can be applied to a variety of animal models, from  
454 fish to humans.<sup>7,8</sup> However, a major drawback of these tools is the lack of a framework for maintaining  
455 animal identities during long-term experiments, which can be fatal to the accuracy of results<sup>10</sup>. SBeA  
456 incorporates the identity recognition approach of idTracker.ai and TRex, utilizing deep neural networks  
457 to directly learn the appearance features of animals<sup>26,41</sup>. This results in a lower error rate than maDLC  
458 or SLEAP and allows for frames with low accuracy to be filtered without affecting the entire video.  
459 Additionally, SBeA provides an extension of 2D tracking tools to 3D movement tracking, which is  
460 critical for making accurate inferences about animal behavior.

461 One potential area for future research to improve SBeA is the development of an end-to-end model  
462 that can reduce storage consumption. To accomplish this, the process of data generation could be

463 incorporated into a video instance segmentation model. Additionally, the identity videos available in  
464 this context may contain sufficient information to train a deep learning model for tasks such as multi-  
465 animal segmentation, identification, and pose estimation. Furthermore, the behavior atlas of a single  
466 animal could be combined with a social behavior atlas of multiple animals through an algorithmic  
467 bridge from BeA to SBeA that facilitates not only social behavior analysis but also other forms of  
468 analysis within the field.

469

470 **Online content**

471 The online version of SBeA will be released after the peer review of this work. Anyone interested in  
472 our work can contact us for the further corporation.

473

474

475

476 **Methods**

477 **Experiments of mice, birds, and dogs.** There are four experiments in this study.

478 The first is the free-social behavior test of two wild-type mice for the program design of SBeA.

479 32 adult male C57BL/6 mice (7–12 weeks old) are used for the free-social behavior test. The mice

480 were housed at 4-5 mice per cage under a 12-h light–dark cycle at 22–25 °C with 40–70% humidity,

481 and were allowed to access water and food ad libitum (Shenzhen Institutes of Advanced Technology,

482 Shenzhen, China). Before the social behavior test, the mice are added tail tags using black mark pen.

483 The tail tags are constructed by horizontal and vertical lines. The horizontal line represents one, and

484 the vertical line represents five. Using the combination of horizontal and vertical lines, the mice are

485 marked according to the sequence of the experiment. After that, the mice are put into a circular open

486 field made of a transparent acrylic wall and white plastic ground, with a base diameter of 50 cm or 20

487 cm and a height of 50 cm for 5 min or 15 min identity recording one by one using MouseVenue3D.

488 Then, the mice are paired and put into the same circular open field for the free-social behavior test.

489 The second is the free-social behavior test of mice with different genotypes. 5 adult (8 weeks old)

490 Shank3B knockout (KO; *Shank3B*<sup>−/−</sup>) mice on C57BL/6J genetic background and 5 adults (8 weeks

491 old) male C57BL/6 mice, were used in the behavioral experiments. *Shank3B*<sup>−/−</sup> mice were obtained

492 from the Jackson Laboratory (Jax No. 017688) and were described previously<sup>20</sup>. The mice were housed

493 at 4-5 mice per cage under a 12-h light–dark cycle at 22–25 °C with 40–70% humidity, and were

494 allowed to access water and food ad libitum (Shenzhen Institutes of Advanced Technology, Shenzhen,

495 China). The mice have added the tail tag introduced above. After that, the mice are put into a circular

496 open field with a base diameter of 20 cm introduced before for 5 min identity recording. Then the mice

497 are paired to WT-WT, WT-KO, and KO-KO groups and put into the same circular open field for the

498 free-social behavior test. The combinations of groups and the sequence of experiments are random  
499 generated by customized MATLAB code.

500 The third is the free-social behavior test of two birds. One male and one female *Melopsittacus*  
501 *Undulatus* (about 26 weeks old) are used in this experiment. They are housed in a conventional  
502 environment with feed regularly (Shenzhen Institutes of Advanced Technology, Shenzhen, China). The  
503 birds are first put into a circular open field with a base diameter of 20 cm introduced before for 5 min  
504 identity recording one by one, and then put in it together for 15 min free-social behavior test and  
505 recording.

506 The fourth is the free-social behavior test of two dogs. Two female Belgian Malinois (13 weeks  
507 old) are used in this experiment. They are housed in Kunming Police Dog Base of the Chinese Ministry  
508 of Public Security, Kunming, 650204, China, and their behavior test of them is finished in the State  
509 Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy  
510 of Sciences, Kunming, 650223, China. The dogs are first put into a  $2 \times 2 \text{ m}^2$  open field made by  
511 fences one by one for the identity recording. Restricted by the locomotion of dogs, there are only 6  
512 min and 11 min identity frames captured by MouseVenue3D and both of them are used for  
513 identification. Then, they are both put into the open field for 15 min free-social behavior test.

514 All husbandry and experimental procedures of mice and birds in this study were approved by  
515 Animal Care and Use Committees at the Shenzhen Institute of Advanced Technology, Chinese  
516 Academy of Sciences. And all husbandry and experimental procedures of dogs in this study were  
517 approved by Animal Care and Use Committees at the Kunming Institute of Zoology, Chinese Academy  
518 of Sciences.

519

520 **MouseVenue3D subtle behavior capture system.** There are three versions of MouseVenue3D  
521 systems used in this study.

522 The first version is used for the data capture of the SBM-VIS dataset. Four Intel RealSense D435  
523 cameras are mounted orthogonally on four supporting pillars made of stainless steel. The distance  
524 between the nearest cameras is 90 cm. The cameras are adjusted to 75 cm off the ground to capture the  
525 whole view of the animal activities in the open field. Images were simultaneously recorded at 30  
526 frames in  $640 \times 480$  sizes per second by a PCI-E USB-3.0 data acquisition card and the pyrealsense2  
527 Python camera interface package. The cameras are connected to a high-performance computer (i7-  
528 9700K, 16G RAM) equipped with a 1-terabyte SSD and 12-terabyte HDD as an image acquisition  
529 platform. The computer also controls the camera calibration module.

530 The second version is used for the behavioral capturing of mice and birds. Four Point Grey FLIR  
531 Chameleon3 CM3-U3-13S2 cameras with adaptive zoom lenses are mounted orthogonally on four  
532 supporting pillars made of stainless steel. The distance between the nearest cameras is 85 cm. The  
533 cameras are adjusted to 45 cm off the ground to capture the whole view of the animal activities in the  
534 open field. To adapt to the size of the open field, the focal length and the pitch angle of cameras are  
535 flexibly adjusted before each experiment. Images were simultaneously recorded at 30 frames in  $1288$   
536  $\times 964$  sizes in grayscale per second by a PCI-E USB-3.0 data acquisition card and the Spinnaker  
537 Python camera interface package. The cameras are connected to a high-performance computer (i9-  
538 10900K, 128G RAM) equipped with a 512-gigabyte SSD and two 16-terabyte HDDs as an image  
539 acquisition platform. The computer also controls the camera calibration module.

540 The third version is used for the behavioral capturing of dogs. Four Intel RealSense D435 cameras  
541 are mounted orthogonally on walls. The distance between the nearest cameras is 210 cm. The cameras

542 are adjusted to 150 cm off the ground to capture the whole view of the dog activities in the open field.  
543 Images were simultaneously recorded at 30 frames in  $640 \times 360$  sizes per second by a PCI-E USB-3.0  
544 data acquisition card and the pyrealsense2 Python camera interface package. The cameras are  
545 connected to a high-performance computer (i7-9700K, 16G RAM) equipped with a 1-terabyte SSD  
546 and 12-terabyte HDD as an image acquisition platform. The computer also controls the camera  
547 calibration module.

548

549 **SBM-VIS Dataset.** The free-social behavior of two C57BL/6 mice introduced above is captured by  
550 the first version of MouseVenue3D. The first 1 min frames of four cameras are annotated as the SBM-  
551 VIS dataset, which is 7200 frames in total. To accelerate the data annotation, we take deep learning for  
552 assistance. 30% of the contours are manually labeled, and the rest are firstly labeled by YOLACT++  
553 trained by the manually labeled 30% contours then checked by humans. Then, the single animal  
554 DeepLabCut is used to predict the poses of masked frames with the human check. Per 18 frames are  
555 grouped for a video instance and saved as YouTubeVIS format<sup>42</sup>. And the poses are saved as a .csv file.  
556 The identities across different cameras are corrected by human annotators.

557

558 **New scenario generation for video instance segmentation.** The new scenario generation for video  
559 instance segmentation is divided into several steps: contour extraction, trajectory extraction, dataset  
560 labeling, background calculation, model self-training, and video dataset generation. After that, it can  
561 be input into the instance segmentation model for large-scale training. Suppose the number of animals  
562 in the video is n. Conda virtual environment configuration includes OpenCV 4.5.5.62, Python 3.8.12,

563 Pytorch 1.10.1, The computer was configured with Intel(R) Xeon(R) Silver 4210R CPU @ 2.40GHz  
564 and NVIDIA RTX3090 GPU.

565 In the animal contour step, image thresholding is first done, and then the contour in the image is  
566 extracted, and the following formula is used to determine whether the frame is social or not, where  $i$   
567 stands for a frame,  $R_i$  stands for the judgment result of this frame and  $num_i$  stands for the number of  
568 contours in this frame:

$$569 R_i = \begin{cases} social, num_i < n \cap num_i > 0 \\ nonsocial, num_i = n \\ error, others \end{cases} \quad (1)$$

570 When extracting the animal trajectory, due to the influence of noise, all the contour center points  
571 are recorded as the candidates of the animal frame center point, and the closest point to each animal in  
572 the previous frame is selected from multiple center points as the true center point of this frame, and  
573 then the Hungarian matching idea is used to remove the matching points successfully, to optimize the  
574 animal trajectory.

575 For dataset annotation, different manually annotated datasets were used for different animals. We  
576 manually annotated 272 images in the 50 cm mice open field experiment, 805 images in the 20 cm  
577 mice open field experiment, 600 images in the birds experiment, and 800 images in the dogs  
578 experiment.

579 For background calculation, the non-mask position (the background) of each image is extracted  
580 and fused into the final background image using the labeled data set. The above operation is repeated  
581 for all data sets to obtain a clean background image.

582 The labeled data set is used for YOLACT++ round training, and the trained model is used to  
583 predict video frames. The predicted high-quality frames will be added to the original data set for the

584 next round of training. Among them, the selection method of high-quality frames is as follows:  $i$   
585 represents a certain frame,  $f_i$  is the segmentation result of the frame  $i$ ,  $f_{i-1}$  is the segmentation result  
586 of the frame  $i-1$ ,  $F$  is the calculation process of scoring matrix of all segmentation results in two  
587 frames, the calculation idea refers to the Hungarian matching idea, and the calculation result is  $G_i$ :

588 
$$G_i = F(f_i, f_{i-1}) \quad (2)$$

589 Then, all  $G_i$  are merged and clustered, and the class with the higher overall matrix score is  
590 selected as the high-quality frame class and added to the training data set. YOLACT++ selects the  
591 ResNet50 model as the pre-training model, and the maximum number of iterations is 150,000  
592 generations. The training process takes about 5 hours. After YOLACT++ finishes training, its final  
593 model is used to predict the results for all frames.

594 The video dataset required for instance segmentation training is subsequently generated. The data  
595 set is divided into three parts, which are real data set, social area data set, and randomly generated data  
596 set. The real data set is the continuous high-quality frames predicted and filtered by YOLACT++,  
597 which are written into the video data set after data enhancement, where the data enhancement is  
598 performed by flipping the image left and right. Since there are many occlusions during social  
599 interaction and the performance of the model decreases, it is necessary to generate multiple datasets in  
600 the social area. Here, consecutive frames of animals in the social area are selected and augmented to  
601 generate the social area dataset, where  $N$  forms of enhancement are generated by data augmentation,  
602 as shown below, where  $C$  represents combination (that is, the combination of different masks is  
603 selected for flipping in each frame).  $A$  stands for alignment (that is, all masks are aligned to occlusion):

604 
$$N = \left( \sum_n^{i=0} C_n^i \right) * A_n^n \quad (3)$$

605 Since the number of real data and social area data sets may be far from enough to complete the  
606 model training task, some data sets in the animal activity area are randomly generated after this step.  
607 In this part, the real animal trajectory in the video, the obtained animal mask, and the background  
608 calculated in the previous step are used for data collection, and the video data set is written after data  
609 enhancement. 14940 video datasets were generated for the 50cm mice open field experiment, 15130  
610 for the 20cm mice open field experiment, 5970 for the bird experiment, and 41,755 for the dog  
611 experiment.

612

613 **The training and validation of video instance segmentation model.** Here, the video instance  
614 segmentation model adopts the Transformer-based VisTR model, which regards the video instance  
615 segmentation task as a parallel sequence encoding and decoding problem. The pre-training model was  
616 the ResNet101 model trained on the COCO dataset, the learning rate was set to 0.0001, the dropout  
617 parameter was set to 0.1, the training epochs is 30, the frame length was set to 9, the sequence length  
618 was set to 19, the number of encoding layers was 6, the number of decoding layers was 6, and Adam  
619 was used for the optimizer. The model training takes about 1.5 days. The trained model is evaluated  
620 on one minute of standard data, and the model accuracy for video instance segmentation is as follows:  
621 IST (Identity swap times) is  $5.500 \pm 3.640$ , ISTP (Identity swap times percentage) is  $0.003 \pm 0.002$ ,  
622 IOU<sub>NID</sub> (The Intersection of the union without identity) is  $0.746 \pm 0.017$ , mAP50<sub>NID</sub> (Mean of average  
623 precision without identity, the threshold value is greater than 0.5) is  $0.985 \pm 0.013$ , mAP50<sub>ID</sub> (Mean of  
624 average precision with identity, the threshold value is greater than 0.5) is  $0.605 \pm 0.319$ , similarly,  
625 mAP70<sub>NID</sub> is  $0.805 \pm 0.068$ , mAP70<sub>ID</sub> is  $0.497 \pm 0.271$ .

626

627 **Single animal pose estimation.** Single animal pose estimation was performed using DeepLabCut  
628 2.2.0.4 with a Conda virtual environment with Python 3.8.12. Four different animals were used in the  
629 manual labeling of the dataset, with 709 images labeled for mice in a 50cm open field, 1421 images  
630 labeled for mice in a 20cm open field, 1035 images labeled for birds, and 819 images labeled for dogs.  
631 The number of body posture points varied for each animal, with 16 for each mouse(nose, left ear, right  
632 ear, neck, left front limb, right front limb, left hind limb, right hind limb, left front claw, right front  
633 claw, left hind claw, right hind claw, back, root tail, mid tail, tip tail), 19 for each bird(beak, calvaria,  
634 left eye, right eye, neck, left wing root, left wing mid, left wing tip, right wing root, right wing mid,  
635 right wing tip, left leg root, left leg tip, right leg root, right leg tip, back, belly, tail root, tail tip), and  
636 17 for each dog(nose, left ear, right ear, neck, left front limb, left front paw, right front limb, right front  
637 paw, left hind limb, left hind paw, right hind limb, right hind paw, front back, mid back, hind back, tail  
638 root, tail tip). ResNet50 was used as the pre-trained model. The model was trained for a maximum of  
639 103 million iterations with a batch size of 8 and took approximately 10 hours to train on an NVIDIA  
640 RTX3090 GPU using Python. The prediction results were saved in a CSV file.

641

642 **3D pose reconstruction of multi-animals.** Here, we use the multi-view geometry method in computer  
643 vision for the 3D reconstruction of multiple animals. The basic projection formula between 2D points  
644 and 3D space points is as follows.

$$645 s \begin{bmatrix} x \\ y \\ 1 \end{bmatrix} = K \begin{bmatrix} R & t \\ 0 & 1 \end{bmatrix} \begin{bmatrix} X \\ Y \\ Z \\ 1 \end{bmatrix} \quad (4)$$

646 Here,  $s$  represents the scaling factor,  $x$  and  $y$  are the points in the image,  $K$  is the camera  
647 internal reference,  $R$  is the rotation matrix,  $t$  is the translation matrix, and  $X$ ,  $Y$ , and  $Z$  represent  
648 the coordinates of the 3D points. Specifically, firstly, all two-dimensional skeleton information of  
649 multi-animal and multi-view was read, and the points in the two-dimensional file with too low a  
650 confidence rate were directly set to NaN. Then, the relative position parameters between multiple  
651 cameras are read and the triangulation algorithm is used for the 3D reconstruction of a single animal.

652 The basic principle is as follows:

$$\begin{aligned} \alpha_1 &= K_1[R_1 t_1]P \\ \alpha_2 &= K_2[R_2 t_2]P \\ &\dots \\ \alpha_n &= K_n[R_n t_n]P \end{aligned} \tag{5}$$

653 Here,  $\alpha_1$  to  $\alpha_n$  represent the two-dimensional points with the same content in different cameras,  
654  $K_1$  to  $K_n$  represent the internal parameter matrix of different cameras,  $R_1$  to  $R_n$  represent the rotation  
655 matrix of different cameras,  $t_1$  to  $t_n$  represent the translation matrix of different cameras, and the three-  
656 dimensional point  $P$  can be solved by combining these equations, and we use the SVD decomposition  
657 to solve the least squares regression problem.

658 Next, since the appearance of animals in different views is very similar, the identities of instance  
659 segmentation may be swapped, and the wrong 3D point coordinates may be calculated. Therefore, we  
660 first obtain the full permutation index list of all 2D points of multiple animals in each view angle, and  
661 then obtain the 3D point coordinates in all cases. Eventually, the point with the smallest error is selected  
662 as the final multi-animal 3D skeleton point.

664

665 **The training and validation of animal identification model.** In multi-animal experiments, because  
666 the animal hair is too similar, its identity is likely to be wrongly assigned. Here, we use the deep  
667 learning model to identify two-dimensional animals under four perspectives, to determine the identity,  
668 and to ensure that the animal identity of the whole video can be corrected back.  
669 The data set of identity recognition needs to record the individual activity videos of all experimental  
670 animals in the same scene, and then obtain two-dimensional pictures of animals from multiple  
671 perspectives. The trained video instance segmentation model is used to predict the mask of the whole  
672 body of a single animal (the effect of manually selecting some body parts of animals for identity  
673 recognition is not good). Then, the four obtained prediction images are processed by image stretching,  
674 stitching, thresholding, and so on, and finally, a complete image is obtained as the training data. The  
675 labels are the animal numbers, so there is no need to manually annotate the data. The size of the dataset  
676 depends on the duration of recording individual activity videos of animals. In the mouse experiment  
677 with a 50 cm open field, the data set size was 594,000, in the mouse experiment with a 20 cm open  
678 field, the data set size was 180,000, in the bird social experiment, and the dog social experiment, the  
679 data set size was 54161.  
680 The deep learning model uses the Efficientnet-b4 model, the maximum number of iterations is set to  
681 120, the initial value of the learning rate is 0.005, and the batch size is set to 32. It is trained on NVIDIA  
682 RTX3090 GPU, and each round of training takes about 40 minutes.  
683 In the mouse experiment with a 50 cm open field, the accuracy of the identification network in the  
684 training set was 0.993, and the accuracy of the validation set was 0.922. In the mouse experiment with  
685 a 20 cm open field, the accuracy of the training set was 0.999, and the accuracy of the validation set

686 was 0.911. In the dog social experiment, the training set accuracy is 0.999, and the validation set  
687 accuracy is 0.999.

688

689 **The pattern visualization of animal identification by LayerCAM.** LayerCAM can generate the  
690 class activation maps (CAM) of each layer of CNN-based models<sup>25</sup>. The LayerCAM of each layer of  
691 the EfficientNet-based identity recognition network is averaged to output a global visualization pattern  
692 of animal identities. To further compare the feature weights of different body parts of animals, the 2D  
693 poses are used for the body part location of identity frames. From the 2D poses to identity frames,  
694 there is a coordinate transformation. The transformed 2D poses on identity frames  $P_t$  can be calculated  
695 as:

$$696 \quad P_t = K_r [K_b^1(P_1 + B_b^1), K_b^2(P_2 + B_b^2), \dots, K_b^{cam}(P_{cam} + B_b^{cam})] \quad (6)$$

697 where  $K_r$  is the resized matrix of cascade frames,  $K_b$  is the scale matrix of the bounding box of  
698 single camera view,  $P$  is the raw 2D poses,  $B_b$  is the bias matrix of the bounding box of single camera  
699 view, and the index  $cam$  is the camera number. The  $K_b$  is decided by the size of frames and the  
700 bounding box size of the cropped animal instance. To reduce the disturbance of 2D pose estimation, a  
701 box centered on  $P_t$  of each transformed 2D pose crops the LayerCAM value. And the mean value of  
702 them represents the CAM weights of each body part.

703

704 **The mask reprojection from 3D poses to video instances.** The 3D poses of each animal connect the  
705 geometric relationships of the video instances in different camera views. In the step of 3D  
706 reconstruction of multi-animals, the 2D poses of each camera view angle have been re-grouped by  
707 optimization. Because the 2D poses of multiple animals are constructed by the single animal after

708 video instance segmentation, the masks of instances are matched to the 2D poses. Therefore, the 3D  
709 poses of each animal are corresponding to the masks of video instances frame by frame. A table saves  
710 the corresponding indexes from 3D poses to video instances and is checked frame by frame for mask  
711 reprojection.

712

713 **Parallel decomposition of trajectories.** The parallel decomposition of trajectories includes three parts.

714 The first part is the decomposition of non-locomotor movement. Let  $X_{ij}^m$  is the behavior  
715 trajectories of animals  $m$  with  $i$  frames and  $j$  dimensions, the non-locomotor movement component  
716  $Y_{NM}$  can be calculated as follows:

$$717 Y_{NM} = \{X^m - J \frac{1}{N} \sum_{n=1}^N X_{nj}^m\} \quad (7),$$

718 where  $J$  is all one vector, and  $N$  is the number of frames. After this step, the center of the body  
719 of the animals can be aligned together.

720 The second part is the decomposition of locomotion. The locomotion component  $Y_L$  can be  
721 calculated as follows:

$$722 Y_L = \left\{ \frac{\partial X^m}{\partial i} \right\} \quad (8).$$

723 The third part is the decomposition of distance. The distance component  $Y_D$  can be calculated as  
724 follows:

$$725 Y_D = \sqrt{(X^1 - X^2)^2} \quad (9).$$

726

727 **Feature representation of distance dynamics.** The distance dynamics  $Y_{DD}$  can be calculated as  
728 follows:

729

$$\begin{cases} Y_{DD} = f_{UMAP}(Y_D), i < I_{thres} \\ Y_{DD} = f_{ResMLP}(Y_D), i \geq I_{thres} \end{cases} \quad (10),$$

730 where  $f_{UMAP}(\cdot)$  is the UMAP mapping including the parameters n\_neighbors set to 199, and

731  $I_{thres}$  is the threshold of frames set to 200000, and  $f_{ResMLP}(\cdot)$  is the feature

732 representation including ResMLP. For  $f_{ResMLP}(\cdot)$ , firstly, the  $Y_D$  is randomly sampled to  $Y_{Ds}$

733 according to  $I_{thres}$ . And the rest of  $Y_D$  is  $Y_{Dr}$ . Then,  $Y_{Ds}$  and  $Y_{DDs} = f_{UMAP}(Y_{Ds})$ , the UMAP of  $Y_{Ds}$ , is

734 used to train ResMLP for feature encoding. After the training, the ResMLP predicts the  $Y_{DDr}$  from  $Y_{Dr}$ ,

735 and the  $Y_{DD}$  can be recombined by  $Y_{DDs}$  and  $Y_{DDr}$  according to the sample point.

736 The ResMLP is based on the residual module and multi-layer perceptron<sup>43,44</sup>. The residual block

737 is constructed by multi-layer perceptron with two layers. Each layer has 64 neurons, and two residual

738 blocks are stacked to construct the residual part. The head of ResMLP is one 1d convolution layer and

739 one global max pooling layer for the feature encoding of distance dynamics<sup>45</sup>. The output part of

740 ResMLP is constructed by one fully connected layer with one sigmoid layer for the continuous value

741 representation<sup>46</sup>. The activation function of ResMLP uses ReLU layers<sup>46</sup>. The optimizer of ResMLP is

742 adam, the initial learning rate is set to 0.001, the mini batch size is set to 2000, and the epoch number

743 is set to 100<sup>47</sup>. The final RMSE of validation is 0.02~0.06, and the training time of ResMLP is about 4

744 min on NVIDIA GeForce RTX 3090 GPU.

745

746 **The time consumption comparison of ResMLP.** After the manually time consumption test of UMAP,

747 the quadratic function is used for the estimation time comparison. The coefficient of quadratic function

748 is 0.00002. The time consumption of ResMLP is estimated as a linear function with slope set to

749 0.000008 and intercept set to 240 based on the training and prediction time of ResMLP. The functions  
750 of the time consumption are as follows:

751 
$$T_{UMAP} = k_{UMAP} y_D^2 \quad (11),$$

752 
$$T_{ResMLP} = k_{ResMLP} y_D^2 + b_{ResMLP} \quad (12),$$

753 where  $T_{UMAP}$  is the time consumption of UMAP,  $k_{UMAP}$  is the coefficient of quadratic function,  $y_D$   
754 is the number of distance components,  $T_{ResMLP}$  is the time consumption of ResMLP,  $k_{ResMLP}$  is the  
755 slope of ResMLP, and  $b_{ResMLP}$  is the intercept.

756

757 **The distance map.** Let  $Y_E$  is the low-dimensional embedding of the social behavior atlas, and  $Y_{DM}$  is  
758 the distance of  $Y_E$ . The  $Y_{DM}$  can be calculated as follows:

759 
$$Y_{DM}^j = \frac{1}{q^j - p^j + 1} \sum_{i=p^j}^{q^j} y_D^i \quad (13),$$

760 where  $j$  is one of the point in  $Y_{DM}$ ,  $p$  is the start time point of  $Y_{DM}^j$ , and  $q$  is the end time point  
761 of  $Y_{DM}^j$ .

762

763 **The map/body distance.** The body distance is equivalent to  $Y_{DM}$ . The map distance  $Y_{EM}$  can be  
764 calculated as follows:

765 
$$Y_{EM}^j = \arg \min (Jy_E^j - Y_E) \quad (14),$$

766 where  $y_E$  is one point of  $Y_E$ . And the map/body distance  $Y_{MB}$  can be calculated as follows:

767 
$$Y_{MB}^j = \frac{Y_{EM}^j}{Y_{DM}^j} \quad (15).$$

768

769 **The adaptive watershed clustering.** The variable of watershed clustering on 2D embeddings is the  
 770 kernel bandwidth  $k_b$ , which decides the density  $d$ . The adaptive watershed clustering is designed to  
 771 automatically choose the best  $d$ . The best  $d$  is determined by the stable number of clusters  $c_{st}$ . To  
 772 get  $c_{st}$ , the clusters under certain  $k_b$  are firstly calculated as:

773 
$$c_n^i = f_{WC}(Y_E, k_b^i) \quad (16),$$

774 where  $f_{WC}(\cdot)$  is the watershed clustering,  $c_n$  is the number of clusters. Then, the  $c_{st}$  is calculated  
 775 as:

776 
$$c_{st} = c_n^{f_{Mode}\left(\left|\frac{dc_n^i}{di}\right|\right)} \quad (17),$$

777 where  $f_{Mode}(\cdot)$  is the mode function. The  $c_s$  is the lower bound of watershed clustering with  
 778 larger kernel bandwidth. To improve the sensitivity of watershed clustering for the subtle differences  
 779 of social behavior, a threshold  $u_{thres}$  is set to 0.9 to restrict  $k_b$  in more fine grain. So, the number of  
 780 sensitivity clusters  $c_{se}$  can be calculated as:

781 
$$c_{se} = \arg \max \left( \left| \frac{dc_n^i}{di} \right| - u_{thres} \times \left( f_{Max} \left( \left| \frac{dc_n^i}{di} \right| \right) - f_{Min} \left( \left| \frac{dc_n^i}{di} \right| \right) \right) \right) \quad (18),$$

782 where  $f_{Max}(\cdot)$  is the maximum function, and  $f_{Min}(\cdot)$  is the minimum function. The  $c_{st}$  and  $c_{se}$   
 783 together determine the lower and upper bound of watershed clustering.

784

785 **Behavior mapping of the PAIR-R24M dataset.** The 3D trajectories of PAIR-R24M dataset are  
 786 captured by high-performance cameras with high frame rate. To reduce the processing time and keep  
 787 the global features of different mice, the frame rate is downsampled from 120 Hz to 30 Hz. The  
 788 classification of the behavioral interactions of the animals includes 4 categories especially close, chase,

789 explore and NaN value. The NaN value in social behavior atlas is defined as others. Because the  
790 interaction classes are imbalance in quantity, four coefficients are used to balance the visual effect of  
791 data distribution in atlas.

792

793 **The cluster purity.** The cluster purity is an indicator to quantify the uniformity of a cluster. Let the  
794  $P = \{p_1, p_2, \dots, p_N\}$  is the ground truth indexes of all data, the  $Q = \{q_1, q_2, \dots, q_N\}$  is the cluster indexes  
795 of all data, and  $N$  is the number of clusters, the cluster purity  $C_p$  can be calculated as:

$$796 C_p^i = \frac{\sum p_i \cap q_i}{\sum p_i \cup q_i} \quad (19).$$

797

798 **The cluster gram of grouped mice.** To reveal the inherent patterns of behavior fractions of each group,  
799 the cluster gram is firstly stacked group by group. Then, all of the behavior fractions are normalized  
800 according to the dimension of subject and sorted by hierarchical clustering according to the dimension  
801 of social behavior module. The clustering tree is normalized for better visualization. Further, the  
802 behavior fractions of each group are sorted according to Euclidean distance for the similarity metric.  
803 The initial row of each group for sorting is chose by the maximum change rate  $R_m$ . The  $R_m$  can be  
804 calculated as:

$$805 R_m = \sum \left| \frac{ds_m^i}{di} \right| \quad (20),$$

806 where  $s_m$  is the sorted social behavior fractions by hierarchical clustering.

807

808 **The angle spectrum clustering.** The angle spectrum clustering is used to merge the similar sub-  
809 clusters of behavior in feature vector space. Let  $V$  is the feature vector matrix of social behavior  
810 modules in PCA space, the angle spectrum  $A_s$  can be calculated as:

$$a_s^{ij} = \arccos\left(\frac{\mathbf{v}_i \cdot \mathbf{v}_j}{|\mathbf{v}_i| \times |\mathbf{v}_j|}\right) \quad (21),$$

812 where  $\mathbf{v}$  is one of the feature vector in  $V$ . Then, the  $A_s$  is clustered by hierarchical clustering  
813 according to the 11 components of 99% variance explanation.

814

815 **Computational software and hardware.** The development of 3D tracking part of SBeA is based on  
816 the Python 3.8.12 in Conda environment on Ubuntu 20.04. The development of behavior mapping part  
817 and figure plot uses MATLAB R2021b. All of the statistics are finished by Prism 8.0 (GraphPad  
818 Software). The development of SBeA is on a high-performance workstation with two Intel Xeon Silver  
819 4210R, eight NVIDIA GeForce RTX 3090, 2 Tera Byte RAM and a 140 Tera Byte Network Attached  
820 Storage. SBeA has been verified to be able to applied in a workstation with one Intel i9-12900K CPU,  
821 at least one NVIDIA GeForce RTX 3090 GPU and 128 Giga Byte RAM.

822

823 **Statistics.** Before hypothesis testing, data were first tested for normality by the Shapiro–Wilk  
824 normality test and for homoscedasticity by the F test. For normally distributed data with homogeneous  
825 variances, parametric tests were used; otherwise, non-parametric tests were used. All of the ANOVA  
826 analysis are corrected by the recommended options of Prism 8.0. No data in this work are removed.  
827 All related data are included in analysis.

828



830 **References**

- 831 1. Chen, P. & Hong, W. Neural Circuit Mechanisms of Social Behavior. *Neuron* **98**, 16–30 (2018).
- 832 2. Barbera, G. *et al.* An open-source capacitive touch sensing device for three chamber social behavior test. *MethodsX* **7**, 101024 (2020).
- 833 3. Sturman, O. *et al.* Deep learning-based behavioral analysis reaches human accuracy and is capable of outperforming commercial solutions. *Neuropsychopharmacology* **45**, 1942–1952 (2020).
- 834 4. Schweihoff, J. F., Hsu, A. I., Schwarz, M. K. & Yttri, E. A. A-SOiD, an active learning platform for expert-guided, data efficient discovery of behavior. *bioRxiv* 2022.11.04.515138 (2022) doi:10.1101/2022.11.04.515138.
- 835 5. Mathis, M. W. & Mathis, A. Deep learning tools for the measurement of animal behavior in neuroscience. *Curr Opin Neurobiol* **60**, 1–11 (2020).
- 836 6. Chen, Z. *et al.* AlphaTracker: A multi-animal tracking and behavioral analysis tool. *bioRxiv* Preprint at <https://doi.org/10.1101/2020.12.04.405159> (2020).
- 837 7. Pereira, T. D. *et al.* SLEAP: A deep learning system for multi-animal pose tracking. *Nature Methods* **2022** *19*:4 **19**, 486–495 (2022).
- 838 8. Lauer, J. *et al.* Multi-animal pose estimation, identification and tracking with DeepLabCut. *Nature Methods* **2022** *19*:4 **19**, 496–504 (2022).
- 839 9. Vidal, M., Wolf, N., Rosenberg, B., Harris, B. P. & Mathis, A. Perspectives on Individual Animal Identification from Biology and Computer Vision. *Integr Comp Biol* **61**, 900–916 (2021).
- 840 10. Agezo, S. & Berman, G. J. Tracking together: estimating social poses. *Nature Methods* **2022** *19*:4 **19**, 410–411 (2022).
- 841 11. Han, Y. *et al.* MouseVenue3D: A Markerless Three-Dimension Behavioral Tracking System for Matching Two-Photon Brain Imaging in Free-Moving Mice. *Neurosci Bull* **38**, 303–317 (2022).
- 842 12. Huang, K. *et al.* A hierarchical 3D-motion learning framework for animal spontaneous behavior mapping. *Nat Commun* **12**, (2021).
- 843 13. Han, Y., Huang, K., Chen, K., Wang, L. & Wei, P. An automatic three dimensional markerless behavioral tracking system of free-moving mice. *2021 IEEE 11th Annual International Conference on CYBER Technology in Automation, Control, and Intelligent Systems, CYBER 2021* 306–310 (2021) doi:10.1109/CYBER53097.2021.9588299.
- 844 14. Liu, N. *et al.* Objective and comprehensive re-evaluation of anxiety-like behaviors in mice using the Behavior Atlas. *Biochem Biophys Res Commun* **559**, 1–7 (2021).
- 845 15. Ghiasi, G. *et al.* Simple Copy-Paste Is a Strong Data Augmentation Method for Instance Segmentation. 2918–2928 Preprint at <https://cocodataset.org/> (2021).
- 846 16. Xu, Z. *et al.* Continuous Copy-Paste for One-Stage Multi-Object Tracking and Segmentation. 15323–15332 Preprint at <http://www.cvlabs.net/> (2021).
- 847 17. Bolya, D., Zhou, C., Xiao, F. & Lee, Y. J. YOLACT++ Better Real-Time Instance Segmentation. *IEEE Trans Pattern Anal Mach Intell* **44**, 1108–1121 (2022).
- 848 18. Bolya, D., Fanyi, C. Z., Yong, X. & Lee, J. *YOLACT Real-time Instance Segmentation*. *openaccess.thecvf.com* <https://github.com/dbolya/yolact>. (2019).
- 849 19. Wang, Y. *et al.* End-to-End Video Instance Segmentation With Transformers. 8741–8750 Preprint at <https://git.io/VisTR> (2021).
- 850 20. Peça, J. *et al.* Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. *Nature* **472**, 437–442 (2011).

873 21. Mei, Y. *et al.* Adult restoration of Shank3 expression rescues selective autistic-like phenotypes. *Nature* **530**,  
874 481–484 (2016).

875 22. Marks, M. *et al.* Deep-learning-based identification, tracking, pose estimation and behaviour classification of  
876 interacting primates and mice in complex environments. *Nature Machine Intelligence* **2022** *4*:4, 331–340  
877 (2022).

878 23. Zhuang, F. *et al.* A Comprehensive Survey on Transfer Learning. *Proceedings of the IEEE* vol. 109 Preprint  
879 at <https://doi.org/10.1109/JPROC.2020.3004555> (2021).

880 24. Tan, M. & Le, Q. v. EfficientNet: Rethinking Model Scaling for Convolutional Neural Networks. 6105–6114  
881 Preprint at <https://proceedings.mlr.press/v97/tan19a.html> (2019).

882 25. Jiang, P. T., Zhang, C. bin, Hou, Q., Cheng, M. M. & Wei, Y. LayerCAM: Exploring hierarchical class  
883 activation maps for localization. *IEEE Transactions on Image Processing* **30**, 5875–5888 (2021).

884 26. Romero-Ferrero, F., Bergomi, M. G., Hinz, R. C., Heras, F. J. H. & de Polavieja, G. G. idtracker.ai: tracking  
885 all individuals in small or large collectives of unmarked animals. *Nature Methods* **2019** *16*:2 **16**, 179–182  
886 (2019).

887 27. Pérez-Escudero, A., Vicente-Page, J., Hinz, R. C., Arganda, S. & de Polavieja, G. G. IdTracker: Tracking  
888 individuals in a group by automatic identification of unmarked animals. *Nat Methods* **11**, 743–748 (2014).

889 28. Ebbesen, C. L. & Froemke, R. C. Body language signals for rodent social communication. *Curr Opin  
890 Neurobiol* **68**, 91–106 (2021).

891 29. Bzdok, D. & Dunbar, R. I. M. The Neurobiology of Social Distance. *Trends Cogn Sci* **24**, 717–733 (2020).

892 30. von Ziegler, L., Sturman, O. & Bohacek, J. Big behavior: challenges and opportunities in a new era of deep  
893 behavior profiling. *Neuropsychopharmacology* 1–12 (2020) doi:10.1038/s41386-020-0751-7.

894 31. Gomez-Marin, A., Paton, J. J., Kampff, A. R., Costa, R. M. & Mainen, Z. F. Big behavioral data: Psychology,  
895 ethology and the foundations of neuroscience. *Nature Neuroscience* Preprint at  
896 <https://doi.org/10.1038/nn.3812> (2014).

897 32. McInnes, L., Healy, J. & Melville, J. UMAP: Uniform Manifold Approximation and Projection for Dimension  
898 Reduction. (2018).

899 33. Shi, S., Wang, Y., Dong, H., Gui, G. & Ohtsuki, T. Smartphone-Aided Human Activity Recognition Method  
900 using Residual Multi-Layer Perceptron. *INFOCOM WKSHPS 2022 - IEEE Conference on Computer  
901 Communications Workshops* (2022) doi:10.1109/INFOCOMWKSHPS54753.2022.9798274.

902 34. Wiltschko, A. B. *et al.* Mapping Sub-Second Structure in Mouse Behavior. *Neuron* **88**, 1121–1135 (2015).

903 35. Wu, Y. E. *et al.* Neural control of affiliative touch in prosocial interaction. *Nature* **2021** *599*:7884 **599**, 262–  
904 267 (2021).

905 36. Marshall, J. D. *et al.* The PAIR-R24M Dataset for Multi-animal 3D Pose Estimation. *bioRxiv*  
906 2021.11.23.469743 (2021) doi:10.1101/2021.11.23.469743.

907 37. Day, F. R., Ong, K. K. & Perry, J. R. B. Elucidating the genetic basis of social interaction and isolation. *Nat  
908 Commun* **9**, (2018).

909 38. Wu, Y. E. & Hong, W. Neural basis of prosocial behavior. *Trends Neurosci* (2022)  
910 doi:10.1016/J.TINS.2022.06.008.

911 39. Dunn, T. W. *et al.* Geometric deep learning enables 3D kinematic profiling across species and environments.  
912 *Nature Methods* **2021** *18*:5 **18**, 564–573 (2021).

913 40. Mathis, A. *et al.* Pretraining boosts out-of-domain robustness for pose estimation. in *Proceedings - 2021 IEEE  
914 Winter Conference on Applications of Computer Vision, WACV 2021* (2021).  
915 doi:10.1109/WACV48630.2021.00190.

916 41. Walter, T. & Couzin, I. D. Trex, a fast multi-animal tracking system with markerless identification, and 2d

917 estimation of posture and visual elds. *Elife* **10**, 1–73 (2021).

918 42. Yang, L., Fan, Y. & Xu, N. Video instance segmentation. in *Proceedings of the IEEE International Conference*  
919 *on Computer Vision* vols 2019–October (2019).

920 43. He, K., Zhang, X., Ren, S. & Sun, J. Deep residual learning for image recognition. in *Proceedings of the*  
921 *{IEEE} conference on computer vision and pattern recognition* 770–778 (2016). doi:10.1109/CVPR.2016.90.

922 44. Kruse, R., Mostaghim, S., Borgelt, C., Braune, C. & Steinbrecher, M. Multi-layer Perceptrons. 53–124 (2022)  
923 doi:10.1007/978-3-030-42227-1\_5.

924 45. Kiranyaz, S. *et al.* 1D convolutional neural networks and applications: A survey. *Mech Syst Signal Process*  
925 **151**, (2021).

926 46. Lecun, Y., Bengio, Y. & Hinton, G. Deep learning. *Nature* Preprint at <https://doi.org/10.1038/nature14539>  
927 (2015).

928 47. Zhang, Z. Improved Adam Optimizer for Deep Neural Networks. in *2018 IEEE/ACM 26th International*  
929 *Symposium on Quality of Service, IWQoS 2018* (2019). doi:10.1109/IWQoS.2018.8624183.

930 48. Kort, R. *et al.* Shaping the oral microbiota through intimate kissing. *Microbiome* **2**, (2014).

931 49. Clucas, B. Patterns of Behavior: Konrad Lorenz, Niko Tinbergen, and the Founding of Ethology. *J Mammal*  
932 **87**, (2006).

933 50. Kaminski, J. & Marshall-Pescini, S. *The Social Dog: Behavior and Cognition. The Social Dog: Behavior and*  
934 *Cognition* (2014). doi:10.1016/C2012-0-06593-3.

935 51. de Chaumont, F. *et al.* Real-time analysis of the behaviour of groups of mice via a depth-sensing camera and  
936 machine learning. *Nature Biomedical Engineering* **2019 3:11** **3**, 930–942 (2019).

937

938

939 **Acknowledgements**

940 This work was supported in part by STI2030-Major Projects(2021ZD0203900), National Natural  
941 Science Foundation of China (32222036), the Youth Innovation Promotion Association of the Chinese  
942 Academy of Sciences (Y2021100), the National Key R&D Program of China (2018YFA0701403),  
943 CAS Key Laboratory of Brain Connectome and Manipulation (2019DP173024), and Guangdong  
944 Provincial Key Laboratory of Brain Connectome and Behavior (2017B030301017). We thank  
945 ChatGPT for the English language editing of this paper.

946 **Author contributions**

947 Conceptualization was done by YN. H., K. C. and PF. W. Code was done by YN. H., K. C., JH. L. and  
948 ZW. W. Algorithm design was done by YN. H. and K. C. Mouse data were gathered by YN. H., WH.  
949 L., XJ. W., YT. H. Bird data were gathered by YN. H., CL. H. and YT. H. Dog data were gathered by  
950 JX. L., YWZ. S., N. W., J. L., GD. W., YP. Z., YN. H., YT. H., XJ. W. and JH. L. Hardware was set  
951 up by YN. H. and K. H. Data analysis was done by YN. H. Preliminary experiments were assisted by  
952 K. H., JJ. Z., SY. C., YJ. W., G. G. and LP. W. The article was written by YN. H., K. C., YK. W. and  
953 PF. W. with input from all authors. PF. W. supervised the project.

954

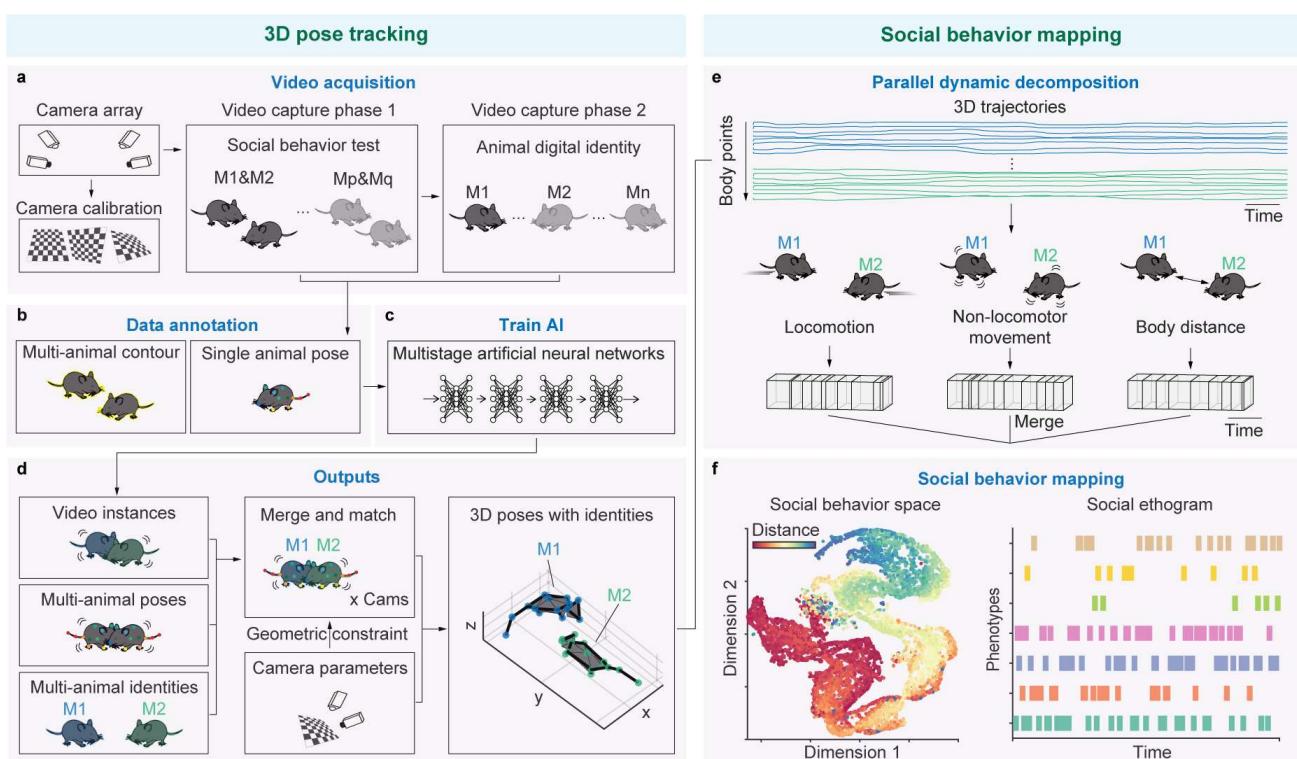
955 **Competing interests**

956 The authors declare no competing interests.

957

958

## Figures and legends

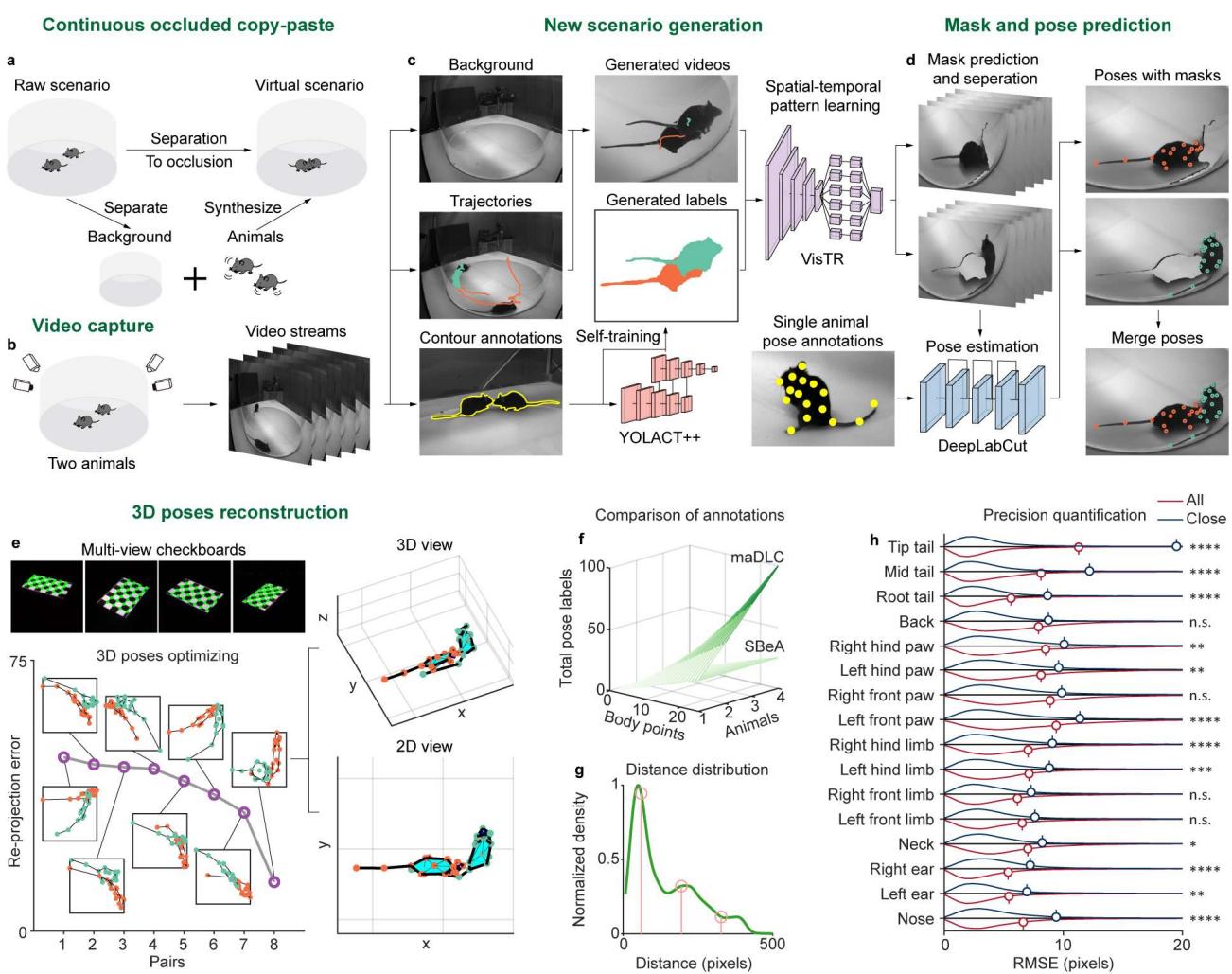


959

**Fig. 1| The architecture of Social Behavior Atlas.** **a**, Video acquisition for free social behavior test. The camera array is used for behavioral capturing, and it is calibrated by checkboard images. There are two phase for behavioral video capturing including social behavior test and animal digital identity. The phase 1 is to capture the videos of free-social interactions of two mice. The phase 2 is to capture the identities of each mice in phase 1. **b**, Data annotation for AI training. Social Behavior Atlas need the annotations of multi-animal contour and single animal pose. **c**, The multistage artificial neural networks for 3D pose tracking. **d**, The outputs of 3D pose tracking. Left: The outputs of AI including video instances, multi-animal poses, and multi-animal identities. Center: Combining video instances, multi-animal poses, and multi-animal identities with camera calibration parameters for 3D reconstruction with identities. Right: The visualization of 3D poses with identities. **e**, Parallel dynamic decompostion of body trajectories. Raw 3D trajectories of two animals can be decomposed into locomotion, non-locomotor movement and body distance. After dynamical temporal deomsition, these three parts are merged together as social behavior motifs for behavioral mapping. **f**, Social behavior metric. Social behavior motifs are clustered and pheonotyped according to the distribution in social behavior space.

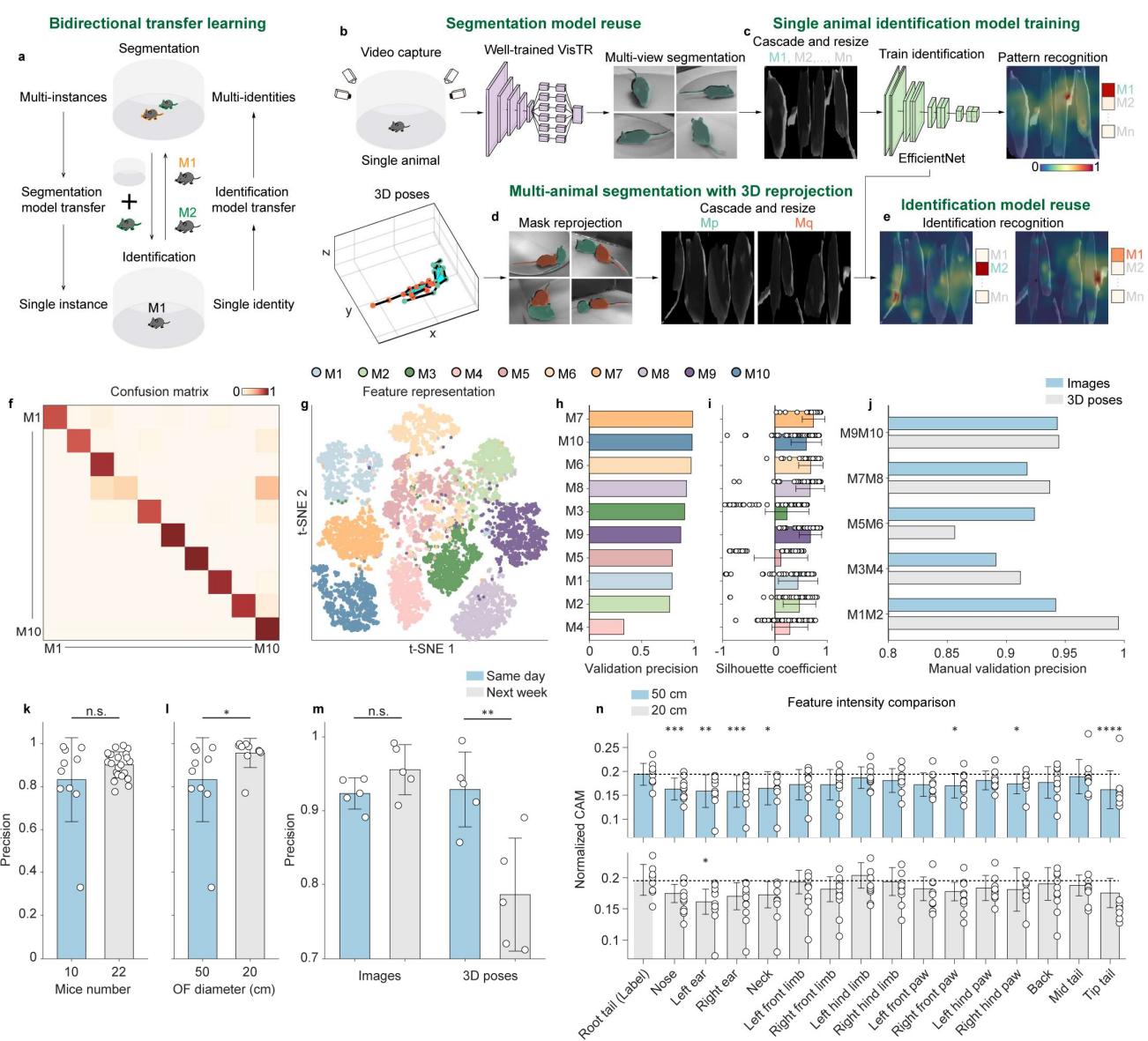
975

976



**Fig. 2| Continuously occluded copy-paste data augmentation-based multi-animal tracking.** **a**, Concept diagram of continuously occluded copy-paste data augmentation. From raw scenario, the instances of background and animals can be synthesized with occlusion in new combination. That achieves generating big data from small data. **b**, Video capture of two free-moving animals. Two animals are put in transparent circular open field and the video streams of behavior are captured by camera array. **c**, New scenario generation according to a little manually labeled data. Behavioral video streams are separated to backgrounds (top left), trajectories (medium left) and manually labeled masks (bottom left). Self-training YOLACT++ is used to predict more unlabeled masks from manually labeled masks. They then combined with backgrounds and trajectories to generate new scenarios of two free-moving mice. **d**, Mask and pose prediction. VisTR is used for the spatial-temporal learning of new scenarios and predict the masks of real mouse instances. Single animal pose estimation model such as DeepLabCut is used for each animal and further the 2D pose of them are merged together. **e**, 3D poses reconstruction. The camera array are calibrated by checkboard images using Zhang's calibration. And reprojection errors of all combination pairs of 2D poses of each animals are optimized for 3D reconstruction. Top right: 3D view of 3D poses of two mice in this case. Bottom right: 2D view of 3D poses of two mice in this case. **f**, Comparison of the number of manually labeled points of SBeA and maDLC. **g**, Distance distribution of two free-moving mice. Pink stems are distance boundaries clustered by k-means (close: 60.69, interim: 195.03, far: 327.47). **h**, Prediction error comparison of all validation data. The differences between all and close data are about  $\pm 2$  pixels (two-way ANOVA).

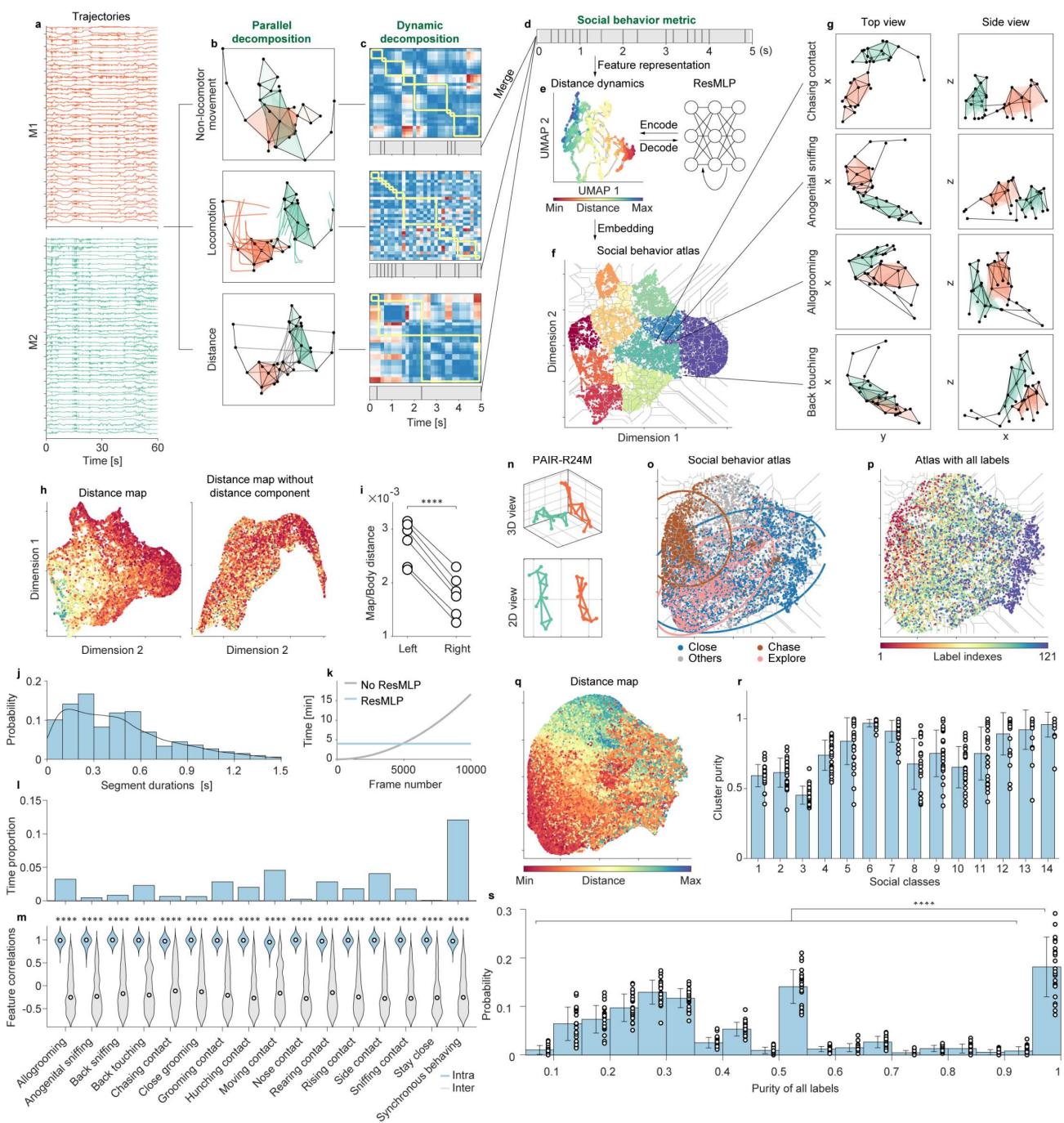
997 followed by Sidak multiple comparisons test). RMSE: root-mean squared error, n.s.: no significant  
998 difference, \*:  $P<0.05$ , \*\*:  $P<0.01$ , \*\*\*:  $P<0.001$ , \*\*\*\*:  $P<0.0001$ .  
999  
1000



1001

1002 **Fig. 3| Bidirectional transfer learning-based animal identification.** **a**, Concept diagram of  
1003 bidirectional transfer learning-based animal identification. Well trained segmentation model on multi-  
1004 animals can be transferred to the single animal, and well trained identity recognition model on the  
1005 single animal can also be transferred to multi-animals. The transfer learning of two models reduces  
1006 unnecessary manual annotations of animal identities. **b**, Segmentation model reuse. Left: an animal is  
1007 put in transparent circular open field and the video streams are captured by camera array. Center: The  
1008 well-trained VisTR is reused for the single animal. Right: The output of well-trained VisTR on the  
1009 single animal. **c**, Single animal identification model training. Left: the single animal instances of multi-  
1010 view are cropped, cascaded and resized to an image. Center: using EfficientNet as the backbone to  
1011 train multi-animal classifier. Right: The identity recognition pattern visualization by LayerCAM. **d**,  
1012 Multi-animal segmentation with 3D reprojection. Left: mask reprojection of each camera view. Right:  
1013 crop, cascade and resize of two animal instances from matched camera view angles. **e**, Identification  
1014 model reuse. The well-trained identification model on the single animal can be reused in multi-animal  
1015 identification. **f**, Confusion matrix of single animal identification. **g**, Feature representation of single  
1016 animal identification using t-SNE. **h**, The sorted validation precision of **f**. **i**, The sorted silhouette

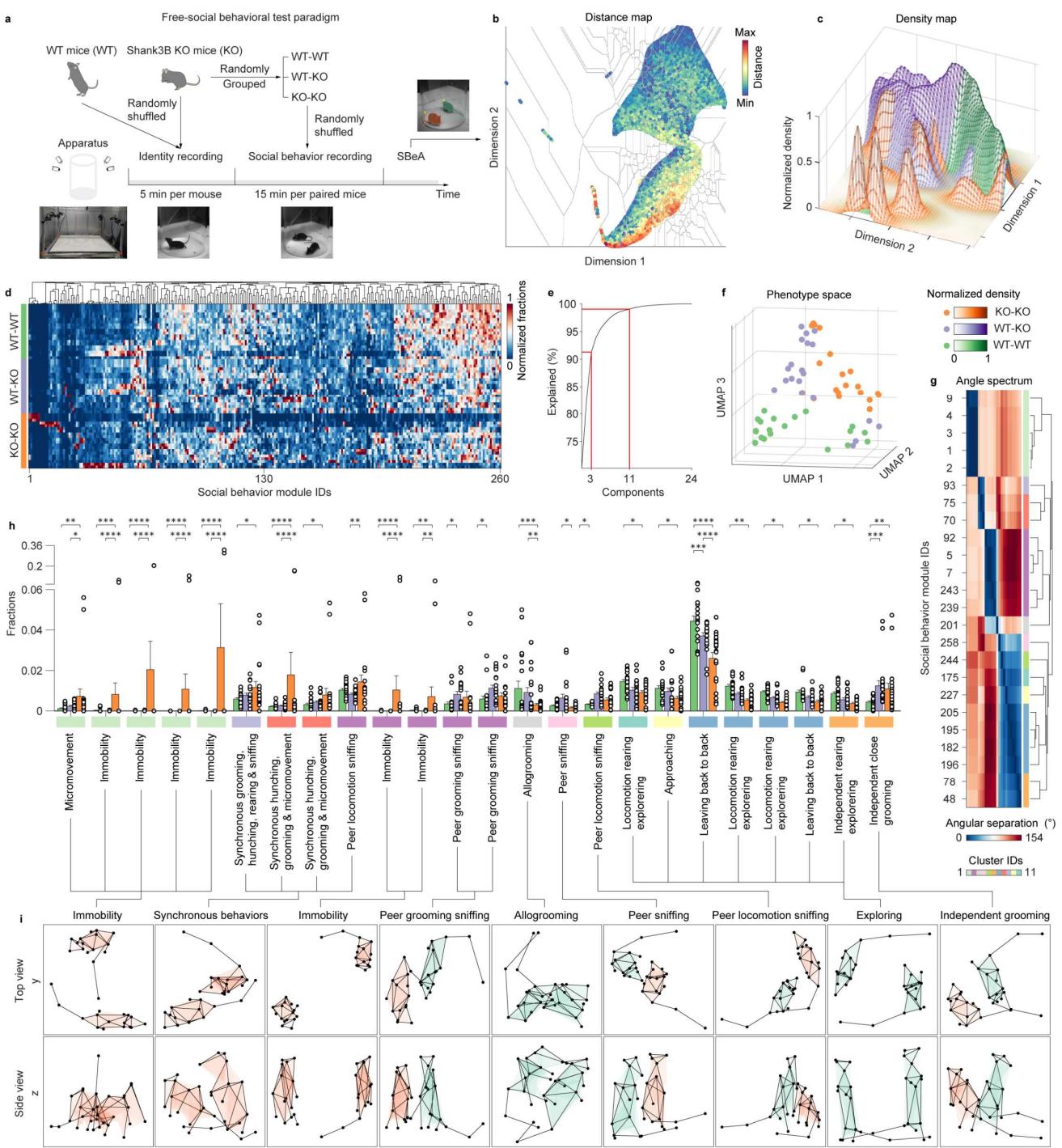
1017 coefficient of **g**. **j**, The manual validation precision of multi-animal identification. **k**, The identification  
1018 precision under different mice number. The identification of 10 mice uses 7200 frames for training and  
1019 1800 for validation, and 22 mice uses 21600 frames for training and 5400 frames for validation. With  
1020 the increase of animal number, the add of training frames can keep higher identification precision (two-  
1021 sided Mann–Whitney test). **l**, The identification precision under different open field (OF) diameter of  
1022 behavioral test(two-sided Mann–Whitney test). **m**, The identification precision in different interval  
1023 time between social behavior test and identify recording(two-sided unpaired T-test). **n**, The feature  
1024 intensity of the tracking body parts under different OF diameter of behavioral test. The root tail of mice  
1025 is labeled by different black line markers for the easy distinguish of human(one-way ANOVA followed  
1026 by Dunnett multiple comparisons test). n.s.: no significant difference, \*:  $P<0.05$ , \*\*:  $P<0.01$ , \*\*\*:  
1027  $P<0.001$ , \*\*\*\*:  $P<0.0001$ .  
1028  
1029  
1030



1043 performance for the representation of social behavior with different distances. **j**, The probability of  
1044 segment durations. **k**, The comparison of computational time consumption of feature representation  
1045 with or without ResMLP. **l**, The time proportion of different behavior. **m**, The feature correlations intra  
1046 and inter behavioral classes (two-sided Mann–Whitney test). **n-s**, The performance quantification of  
1047 SBeA on the PAIR-R24M dataset. **n**, The visualization of two mice in the PAIR-R24M dataset. **o**, The  
1048 social behavior atlas of PAIR-R24M dataset. The social classes of the PAIR-R24M dataset are  
1049 separated in social behavior atlas. The ellipse is the Gaussian model fitting of the three classes. **p**, The  
1050 social behavior atlas of all the class labels of PAIR-R24M dataset. The 11 classes of each mouse are  
1051 combined to 121 classes, and the 121 classes are distributed with patterns. **q**, The distance map of  
1052 social behavior atlas. The distance distribution of distance map is coincident with labels in **o**. **r**, The  
1053 cluster purity of social classes in **o**. **s**, The cluster purity probability of all labels in **p**. The cluster  
1054 purities greater than 0.95 are significant higher than others (one-way ANOVA followed by Tukey  
1055 multiple comparisons test). n.s.: no significant difference, \*:  $P<0.05$ , \*\*:  $P<0.01$ , \*\*\*:  $P<0.001$ , \*\*\*\*:  
1056  $P<0.0001$ .

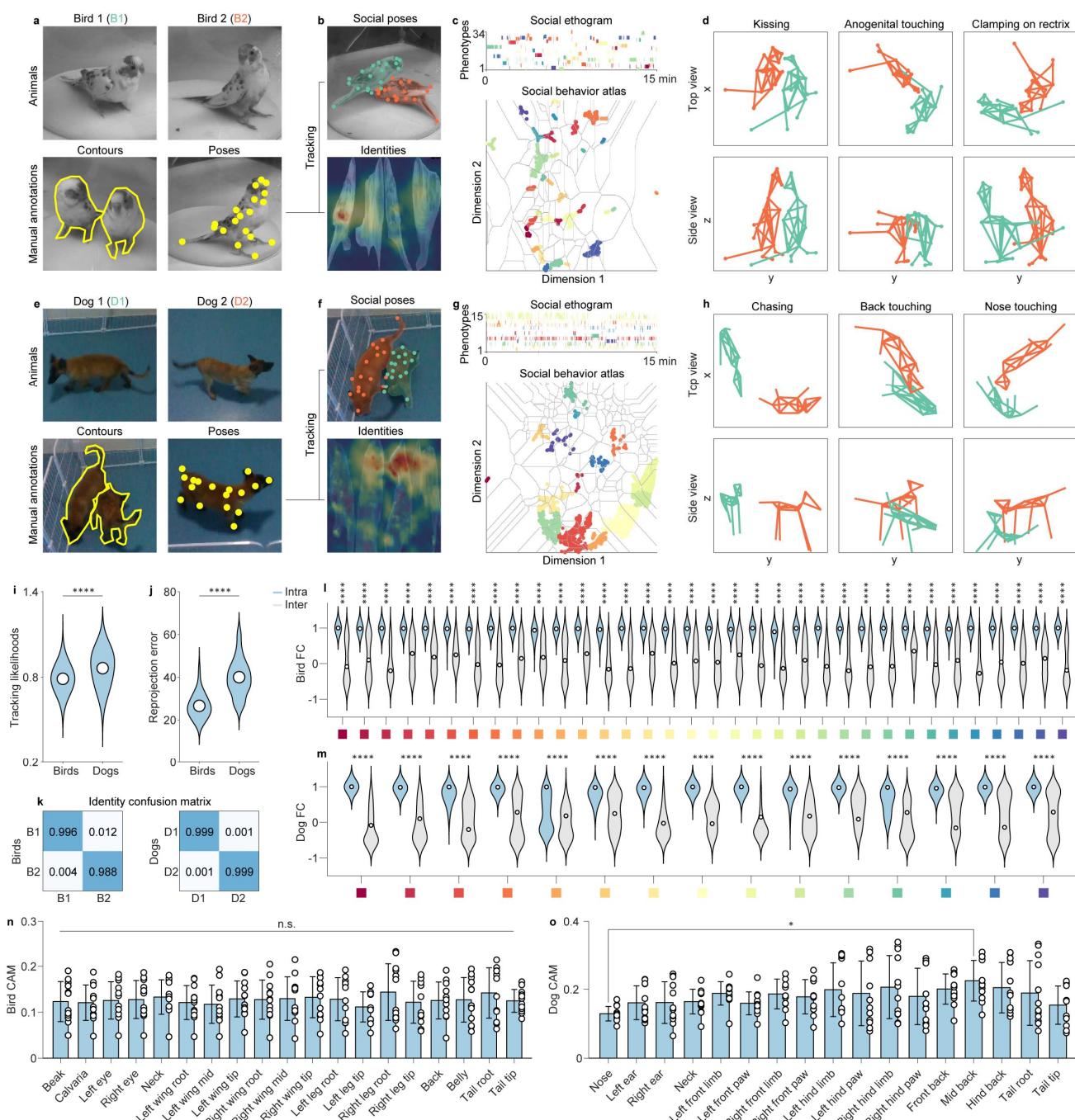
1057

1058



**Fig. 5| The identifying of abnormal social behavior modules in *Shank3B* knockout mice.** **a**, The paradigm of free-social behavioral test. WT: Wild type mice, KO: *Shank3B* knockout mice. **b**, The social behavior atlas with distance map of 3 grouped mice. **c**, The distribution of social behavioral modules of three social groups. A total of 260 social behavior modules are identified. **d**, The fractions of social behavioral modules of three social groups. The fractions of each group are normalized, and they are clustered and resorted according to the dimension of social behavior modules. **e**, Dimensional reduction of behavior fractions using principal component analysis (PCA) after hypothesis testing (two-way ANOVA followed by Tukey multiple comparisons test). 24 social behavior modules are significant differences in three groups. 3 components can explain more than 90% variances, and 11

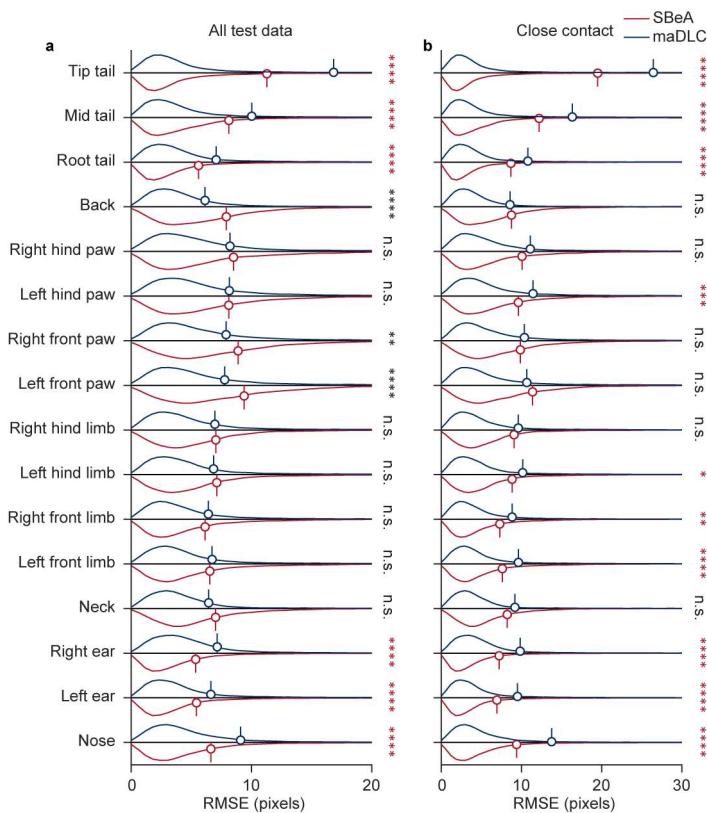
1069 components can explain more than 99% variances. **f**, The construction of phenotype space. UMAP is  
1070 used to reduce the 260 dimensions of social behavior modules to 3 dimensions according to **e**. Different  
1071 colors of dots represent different social groups. The phenotypes of 3 social groups can be separated in  
1072 phenotype space. **g**, The merging of social behavior modules according to behavioral feature angles  
1073 and **e**. 24 social behavior modules with significant differences are firstly mapped to PCA feature space  
1074 and then the angular separation are calculated to construct angle spectrum. Further, hierarchical  
1075 clustering is used to cluster angle spectrum to 11 clusters according to **e**. **h**, The comparison of  
1076 behavioral fractions of 3 social groups. 24 social behavior modules with significant differences are  
1077 manually identified. **i**, The visualization of merged social behavior modules. With the assistance of **g**,  
1078 9 social behavior modules are merged and identified from 24 social behavior modules. Orange 3D  
1079 mice represent KO mice, and green 3D mice represent WT mice. n.s.: no significant difference, \*:  
1080 P<0.05, \*\*: P<0.01, \*\*\*: P<0.001, \*\*\*\*: P<0.0001.  
1081  
1082  
1083



1084  
1085 **Fig. 6| SBeA for the applications across species such as birds and dogs. a-d**, SBeA is used for birds.  
1086 **a**, The preparation of birds. Two parrots with inconspicuous appearance difference are used for social  
1087 behavior test. After video recording of identity and free-social behavior by camera array, the contours  
1088 and poses are manually annotated. 19 body parts are defined for 3D pose tracking. **b**, The social poses  
1089 and identities outputs of SBeA. **c**, The social ethogram and social behavior atlas of birds. **d**, The 3D  
1090 social behavior cases of birds. **e-h**, SBeA is used for dogs. **e**, The preparation of dogs. Two Belgian  
1091 Malinois with inconspicuous appearance difference are used for the social behavior test. After video  
1092 recording of identity and free-social behavior by camera array, the contours and poses are manually  
1093 annotated. 17 body parts are defined for 3D pose tracking. **f**, The social poses and identities outputs  
1094 of SBeA. **g**, The social ethogram and social behavior atlas of dogs. **h**, The 3D social behavior cases  
1095 of dogs. **i-o**, The performance quantification of SBeA in birds and dogs. **i**, The tracking likelihoods of

1096 birds and dogs are significant different (two-sided Mann–Whitney test). **j**, The 3D reprojection error  
1097 of birds and dogs are significant different (two-sided Mann–Whitney test). **k**, The identity recognition  
1098 confusion matrix of birds and dogs. **l**, The feature correlations (FC) intra and inter behavioral classes  
1099 of birds (two-way ANOVA followed by Sidak multiple comparisons test). **m**, The FC intra and inter  
1100 behavioral classes of dogs (two-way ANOVA followed by Sidak multiple comparisons test). **n**, The  
1101 feature intensity of the tracking body parts of birds (one-way ANOVA followed by Tukey multiple  
1102 comparisons test). The feature intensities do not show significant differences. **o**, The feature intensity  
1103 of the tracking body parts of dogs (one-way ANOVA followed by Dunnett multiple comparisons  
1104 test). The feature intensities between nose and mid back show significant differences. n.s.: no  
1105 significant difference, \*:  $P<0.05$ , \*\*:  $P<0.01$ , \*\*\*:  $P<0.001$ , \*\*\*\*:  $P<0.0001$ .  
1106

1107 **Supplementary materials**



1108

1109 **Extended Data Fig. 1| Performance comparison of SBeA and maDLC.** **a**, Prediction error  
1110 compasion of all test data. The RMSE of most of the body parts of SBeA is significantly lower than  
1111 maDLC (two-way ANOVA followed by Sidak multiple comparisons test). **b**, Prediction error  
1112 compasion of close contact. The RMSE of all of the body parts of SBeA is significantly lower than  
1113 maDLC or even with maDLC (two-way ANOVA followed by Sidak multiple comparisons test). RMSE:  
1114 root-mean squared error, n.s.: no significant difference, \*: P<0.05, \*\*: P<0.01, \*\*\*: P<0.001, \*\*\*\*:  
1115 P<0.0001.

1116

Social behavior	Definition	Species
Approaching	One individual approaching another individual with locomotion.	Mouse

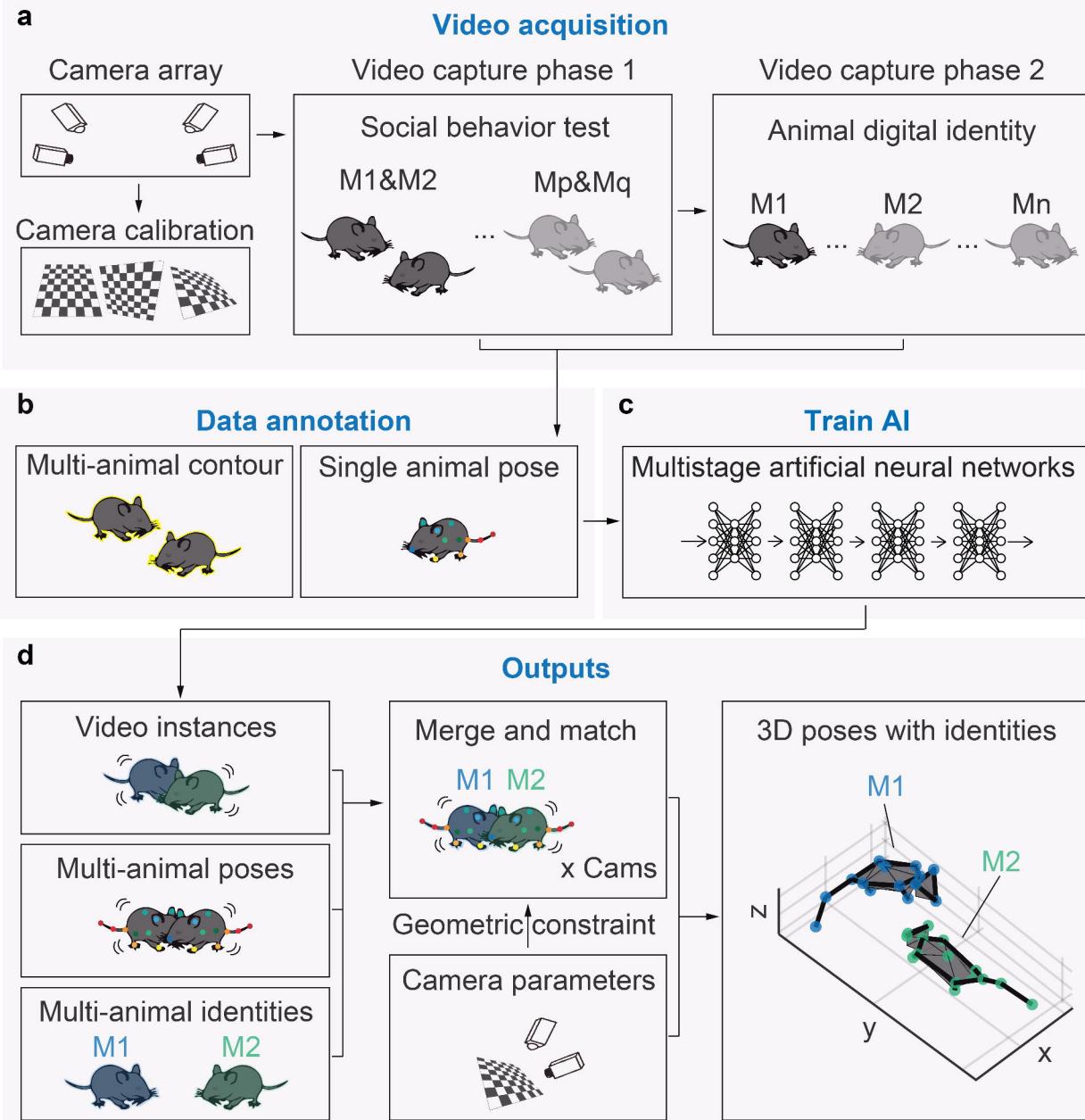
Allogrooming	Grooming behavior directed towards another individual.	Mouse
Anogenital sniffing	Sniffing and exploring the anogenital (perianal and genital) region of another individual.	Mouse
Back touching	One individual touching the back of another individual by nose or fore limbs.	Mouse
Chasing contact	One individual running after another individual with discrete contact.	Mouse
Immobility	All of the individuals are motionless.	Mouse
Independent close grooming	All of the individuals are self-grooming without the influence of another individual.	Mouse
Independent rearing exploring	All of the individuals are rearing towards the outside without the influence of another individual.	Mouse
Locomotion rearing exploring	All of the individuals are rearing towards the outside with locomotion.	Mouse
Leaving back to back	All of the individuals towards back to each other and in locomotion.	Mouse
Micromovement	Small, subtle movements of individuals.	Mouse
Peer sniffing	Sniffing behavior directed towards another individual.	Mouse
Peer locomotion sniffing	Sniffing behavior directed towards another locomotion individual.	Mouse
Peer grooming sniffing	Sniffing behavior directed towards another grooming individual.	Mouse

Synchronous behavior	The coordinated movement or activity of multiple individuals at the same time.	Mouse
Anogenital touching	Contacting the genital or anal region of another individual.	Bird
Clamping on rectrix	One individual holding onto the tail feathers of another individual by feet.	Bird
Kissing	Two individuals touching beaks.	Bird
Back touching	One individual touching the back of another individual by nose or fore limbs.	Dog
Chasing	One individual pursuing or running after another individual.	Dog
Nose touching	Two individual touching the noses of each other.	Dog

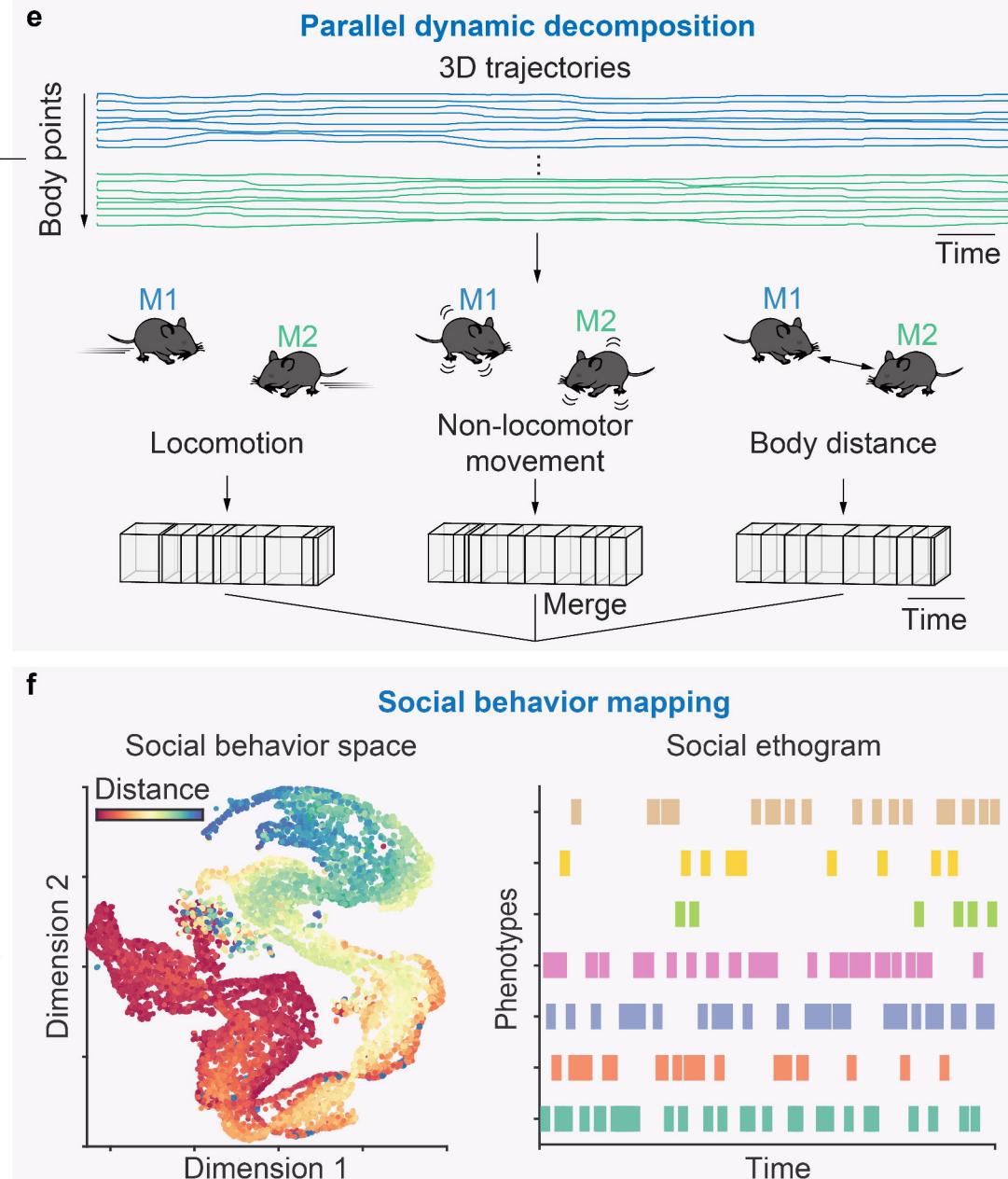
---

1117 **Extended Data Tab. 1| Social behavior definitions for manual labeling.** The definition of social  
1118 behavior of mouse, bird and dog refers to Mouse Ethogram database ([www.mousebehavior.org](http://www.mousebehavior.org)),  
1119 ref.<sup>35,48–51</sup>.  
1120

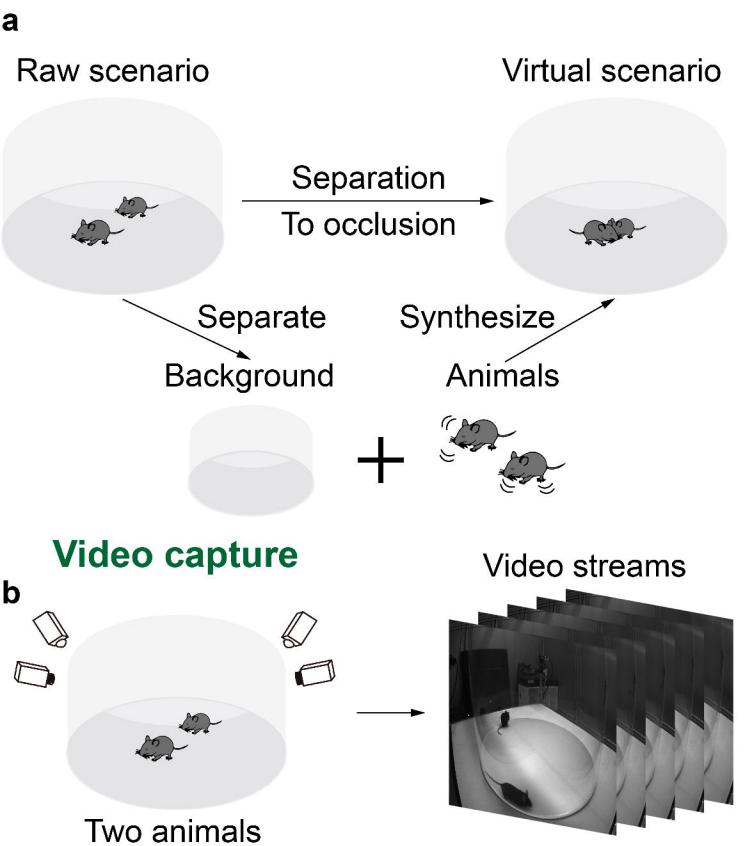
## 3D pose tracking



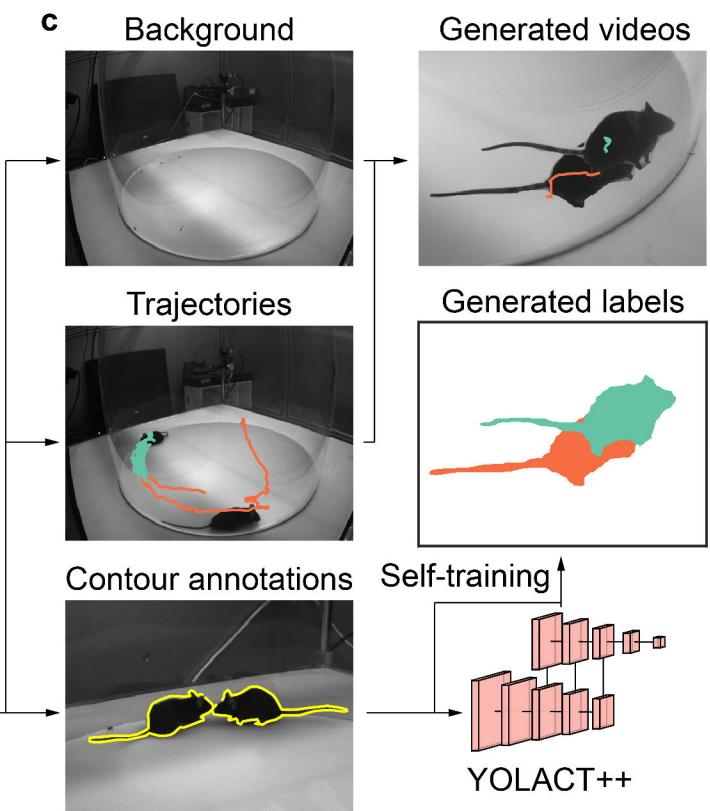
## Social behavior mapping



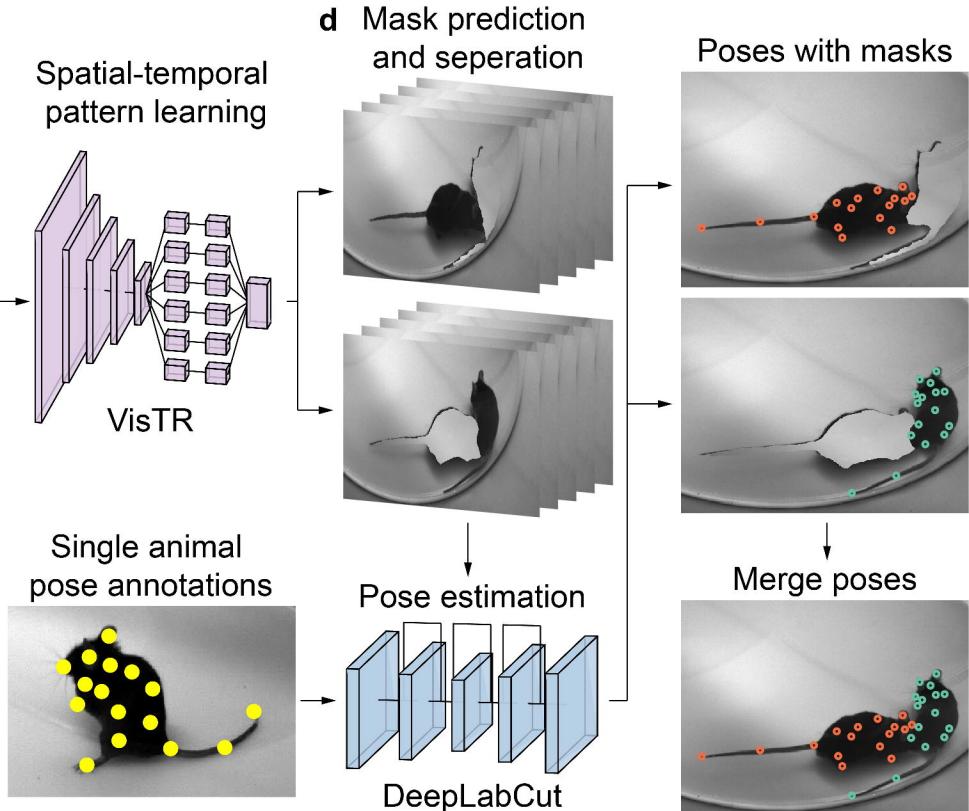
## Continuous occluded copy-paste



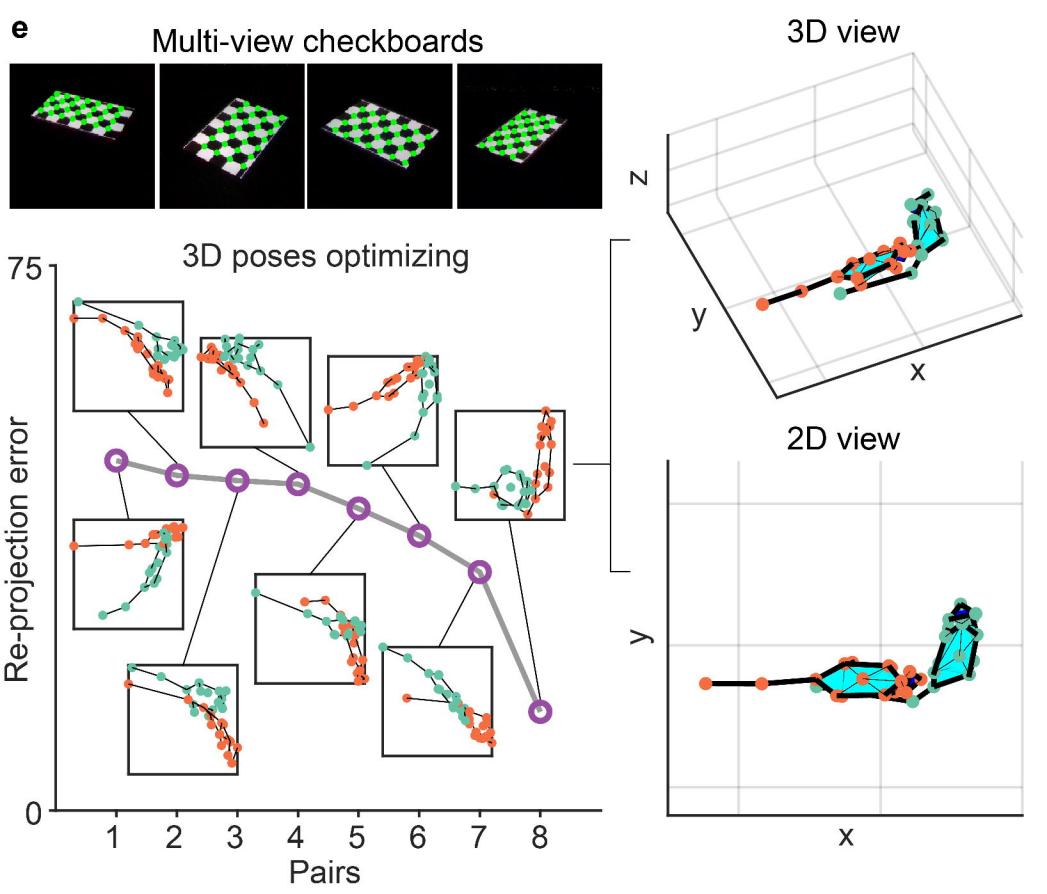
## New scenario generation



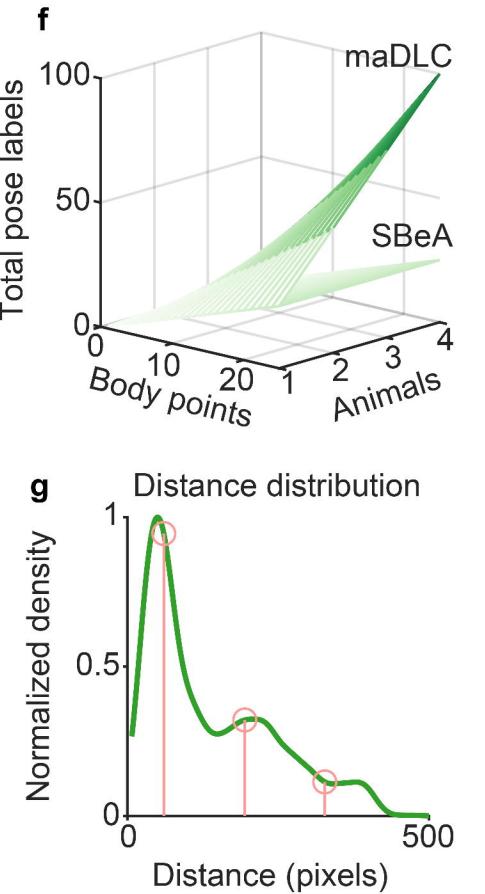
## Mask and pose prediction



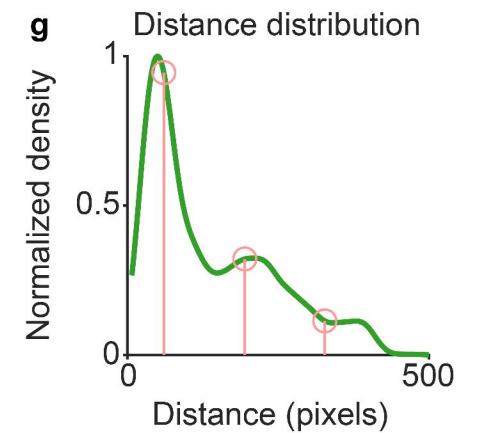
## 3D poses reconstruction



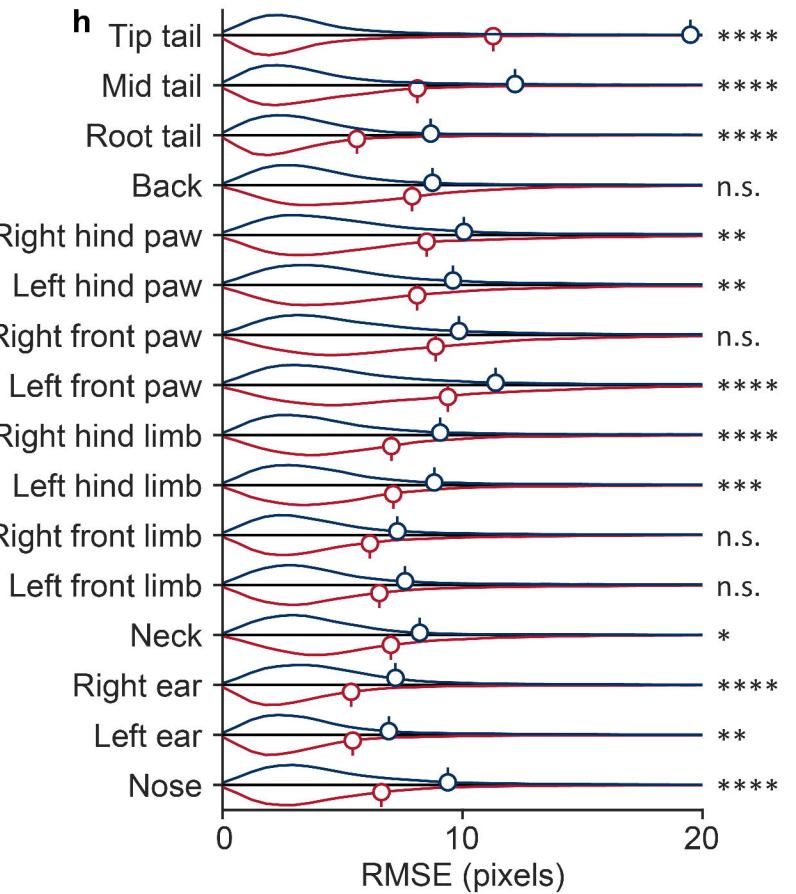
## Comparison of annotations



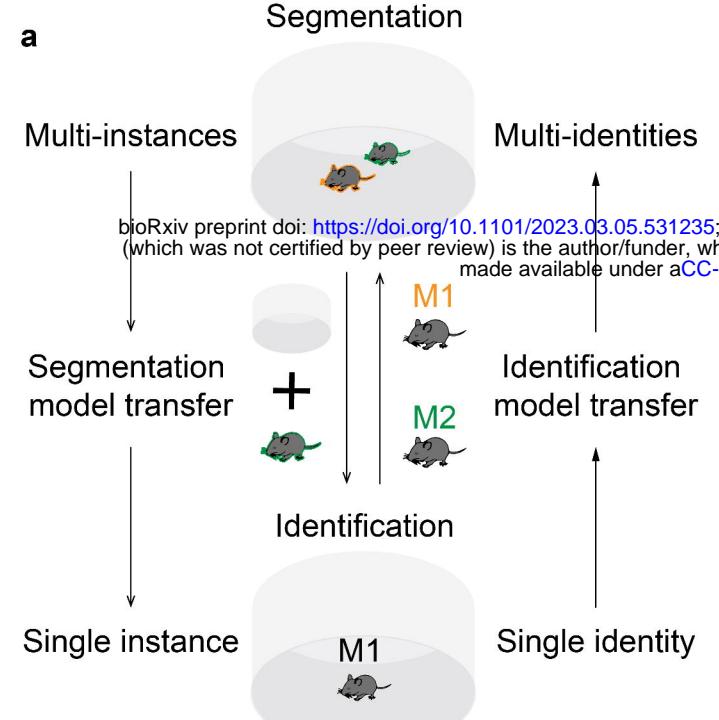
## Distance distribution



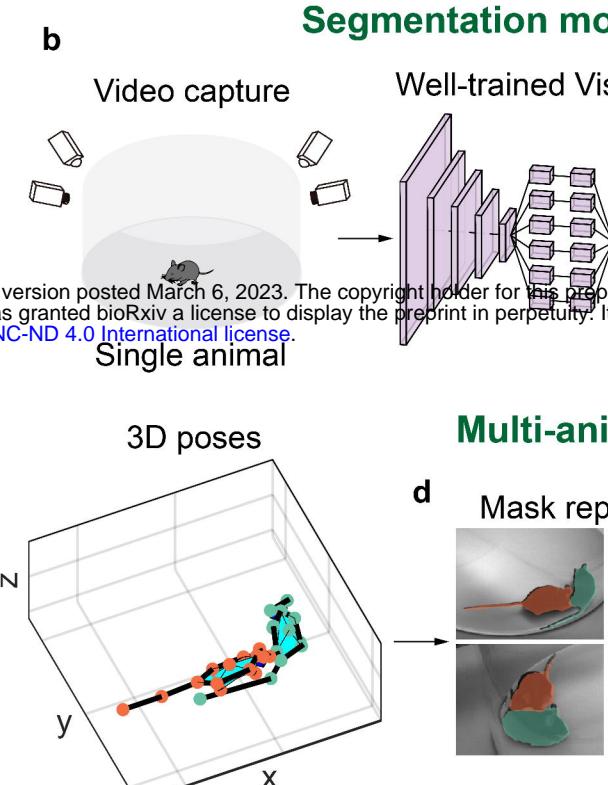
## Precision quantification



## Bidirectional transfer learning



9



## c Single animal identification model training

