bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239343; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

The human PDZome 2.0: characterization of a new resource to test for PDZ interactions by Yeast Two-Hybrid

Monica Castro-Cruz [1,2], Frédérique Lembo [2], Jean-Paul Borg[3], Gilles Trave [4], Renaud Vincentelli [5], Pascale Zimmermann [1,2 *]

[1] Department of Human Genetics, KU Leuven, B-3000 Leuven, Belgium

[2] Équipe Labellisée Ligue 2018, Centre de Recherche en Cancérologie de Marseille (CRCM), Aix-Marseille Université, Marseille F-13284, France

[3] Marseille Proteomics Platform, CRCM, Institute Paoli-Calmettes, Aix-Marseille Université, Inserm, CNRS, 13009 Marseille, France

[4] Équipe Labellisée Ligue 2015, Department of Integrated Structural Biology, Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), INSERM U1258/CNRS UMR 7104/Université de Strasbourg, Illkirch, France

[5] Architecture et Fonction des Macromolécules Biologiques (AFMB), Unité Mixte de Recherche (UMR) 7257, Centre National de la Recherche Scientifique (CNRS) Aix-Marseille Université, Marseille cedex 9, France

[*]Correspondence should be addressed to Pascale Zimmermann pascale.zimmermann@kuleuven.be

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239343; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Keywords

PDZ, PDZome, protein-protein interaction, HPV16-E6, Yeast two-hybrid.

Abbreviations

HPV16, human papilloma virus-16

PDZ, postsynaptic density-95, disc large-1, zona occludens-1.

PBM, PDZ binding motif

PDZome, PDZ proteome

Y2H, Yeast two-hybrid

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239343; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Abstract

PSD95-disc large-zonula occludens (PDZ) domains are globular modules of 80-90 amino acids that co-evolved with multicellularity. They commonly bind to carboxy-terminal sequences of a plethora of membrane-associated proteins and influence their trafficking and signaling. We previously built a PDZ resource (PDZome) allowing to unveil human PDZ interactions by Yeast two-hybrid. Yet, this resource is partial according to the current knowledge on the human PDZ proteome. Here we built the PDZome 2.0 library for Yeast two-hybrid, based in a PDZ library manually curated from online resources. The PDZome2.0 contains 305 individual clones (266 PDZ domains in isolation and 39 tandems), for which all boundaries have been designed based on available PDZ structures. Using as bait the E6 oncoprotein from HPV16, a known promiscuous PDZ interactor, we show that PDZome 2.0 outperforms the previous resource.

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239343; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Introduction

PDZ scaffold proteins are involved in a wide range of cellular processes including the establishment and maintenance of polarity, protein trafficking, signaling and the coordination of synaptic events (1–3). They contain one or more PDZ domain that is an abundant and promiscuous protein interaction module. PDZ domains were first identified in the proteins **P**SD-95 (postsynaptic density-95), **D**lg-1 (disc large-1), and **Z**O-1 (zona occludens-1) (4–8). PDZ domains generally recognize short linear motifs of minimum 4 amino acids (PDZ binding motifs or PBM) located at the C-terminal region of receptors, co-receptors, or adhesion molecules (9). Additionally, PDZ domains can interact with internal protein motifs, lipids and other PDZ domains (10–12). PDZ interactions can be tuned in various ways. Changes in salt content and pH (13), auto-inhibition (14), allosteric regulation (15) and phosphorylation (16) are some of the features that modulate PDZ interactions (for reviews see (17,18)).

PDZ domains are composed by 80- 90 amino acid residues which fold in six β-strands (A-F) and two α-helices (A-B), forming a partially opened antiparallel B barrel structure (1,19). The PBM binds in a groove formed by the α-helix B and the β-sheet B (19). The PDZ binding groove is connected by a loop which often contains the GLGF motif. The GLGF motif, also described as R/K-X-X-X-G-φ-G- motif where X is any and φ is an hydrophobic residue, can vary significantly and contributes to the affinity of the interactions with the PBM (19,20). Structural and functional studies suggest that PDZ domains prefer specific residues in a PBM. One can currently identify three main PBM classes that can occur in 16 specificity sub-classes (20). Yet, approaches like e.g. phage display suggest that PBM specifities go beyond such classification (21,22).

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239343; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

PDZ domains are rare in non-metazoans. For example, bacteria and yeast display no more than 2 and 4 PDZ-domain containing proteins, respectively (23,24). In contrast, PDZ proteins are abundant in metazoans, suggesting they co-evolved with multicellularity (24). Several studies based on sequence analysis using SMART (www.smart.embl-heidelberg.de), Interpro (https://www.ebi.ac.uk/interpro/), and PFAM (https://pfam.xfam.org/) suggest that the number of PDZ domains in the human proteome ranges from 234 to 450 (25–27). Based on these strictly *in silico* studies, a first collection of human PDZ domains was built (PDZome) to test for PDZ interactions by Yeast-two-hybrid (Y2H) (27). This resource contains 246 PDZ domains. Yet according to more refined study including a 3D-structure based approach and careful manual annotation, this resource contains PDZ domains truncated at their N- and C-termini, by 5 to 16 amino-acids (28). Such truncation might compromise proper folding and binding activities (28–31). In this refined study, we identified 266 PDZ domains embedded in 150 proteins (omitting spliced forms) in the human proteome.

Noteworthy, it became clear that some PDZ domains occurring in tandem (separated by a short conserved linker region) can function as supramodules (32,33). The binding properties of these supramodules are different from those of PDZ domains taken in isolation. Generally, PDZ tandems display higher affinity for their target and in some case the tandem might be necessary for proper folding of individual domains (33,34).

Because the original Y2H PDZome resource (27) misses some PDZ domains, does not contain tandems and also because of the presence of suboptimal boundaries, we prepared a new resource that we called PDZome 2.0. The PDZome 2.0, is more comprehensive including the 266 manually annotated sequences of single PDZ domains (28). Additionally, it contains 39 PDZ domains in tandem. To test for the performance of PDZome 2.0, we used the E6 oncoprotein present in the human papilloma virus-16 (HPV16). The PDZome 2.0 detected a total of 54 E6-PDZ interactions. Twenty-nine are common with the 36 previously

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239343; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

identified by the PDZome and 25 are newly identified. We therefore propose the PDZome 2.0 as a more performant resource to comprehensively map human PDZ interactions by Y2H approach.

Materials and Methods

Sub-cloning of prey and baits

Prey entry clones were collected in the pZeo or the pDONOR201 Gateway ® vectors (NZYTech, Ltd.). All the entry clones were subcloned into the Y2H expression vector pACT2-AD using Gateway ® LR reactions (Invitrogen). After sequence validation, all pACT2-AD clones were transformed into the haploid Y187 yeast strain (MATα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, gal4Δ, met-, gal80Δ, MEL1, URA3::GAL1UAS -GAL1TATA-lacZ).

The two baits used here, correspond to a fragment of the HVP16 E6 oncoprotein wildtype (MSCCRSSRTRRETQL), and the same fragment without the PDZ binding motif or ΔTQL (MSCCRSSRTRRE). The E6 fragments were subcloned into the pGBT9-BD vector for expression in yeast, as reported previously (27). After sequence validation, E6 constructs were transformed into the haploid AH109 yeast strain (MATa, trp1-901, leu2-3, 112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1UAS -GAL1TATA -HIS3, GAL2UAS -GAL2TATA -ADE2, URA3::MEL1UAS -MEL1 TATA -lacZ).

Y2H assays.

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239343; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

PDZ interactions were tested screened by Y2H assay (36). Briefly, the Y2H was performed through mating of the two yeast strains Y187 (α) and AH109 (a). The yeasts were grown together (α + a) in liquid Yeast extract-Peptone-Dextrose (YPD) supplemented with 10% PEG for 5 - 6 h at 30 ºC under gentle agitation (140 rpm). After one wash in sterile water, the yeasts were spotted on solid medium. To test the mating efficiency, the yeasts were spotted on a solid permissive medium SC Agar -L -W. To test for interactions, the yeasts were spotted on a solid selective medium SC Agar -L -W -H. All SC-Agar plates were incubated at least 72 h and up to 1 week at 30 ºC or 2 weeks at room temperature. Images from the solid selective medium plates were captured and analyzed. Random positive clones were verified by PCR amplification and automated sequencing with the GAL-AD primer (Eurofins GATC).

Results

Construction of the human PDZ resource for Y2H assays

To build the human PDZome 2.0 resource allowing to test for PDZ interactions by Y2H, the 266 known human PDZ domain sequences (**Table S1**), bearing boundaries optimized based on available structural data (28), were introduced in the prey vector by Gateway ® approach (**Fig. 1A**). We also included 39 PDZ tandems (**Table S2**). The PDZ tandems were designed using the online UniProt resource (https://www.uniprot.org/). First, all PDZ proteins with more than one PDZ domain were included in the list (multi-PDZ proteins). Then, within these multi-PDZ proteins, those in which 2 PDZ domains were connected by a linker region of up to 36 amino acid residues acids were included. The final list of 39 tandems, belonging to 28 PDZ proteins, represent around 20% of the human PDZ proteome (**Table S2**).

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239343; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

All recombinant clones present in the prey pACT2-AD vector were transformed into the haploid Y187 (α) yeast strain. The final collection of individual clones was arrayed in four 96-well plates (**Fig. 1 A)**.

Fig 1 Construction of the PDZome 2.0 for yeast two-hybrid screens.

The PDZome 2.0 was built using the Gateway ® cloning system. (**A**) The entry clones corresponding to the open reading frames (ORF) of the 266 PDZ domains and 39 PDZ domains in tandem were subcloned from pZeo or pDONOR entry vectors. The ORFs were then introduced into the pACT2-AD vector using Gateway ® LR clonase. After validation by sequencing, pACT2-AD clones were transformed into the Y187 (type α) yeast strain. Ready for mating yeast containing the PDZome fused to the Gal4 activation domain were arranged in 4 plates of 96 wells (a, b, c correspond to single PDZ domains, whereas t corresponds to tandems). (**B**) Two peptides corresponding to the C-terminal part of the E6 protein from the HPV16 were used as baits. The wild type (MSCCRSSRTRRETQL) and the ΔTQL (or ΔPBM) were subcloned in the pGBT9-BD vector as described previously (27) and transformed into the AH109 (type a) yeast strain.

The PDZome 2.0 for Y2H screenings is validated using the HPV16 E6 oncoprotein

To characterize the performance of the PDZome 2.0 we used a fragment of the HPV16 E6 oncoprotein as bait in Y2H screenings. The HVP16 E6 oncoprotein is involved in the development of human cervical cancer by exploiting its class I PBM which has been previously described to bind at least 29 PDZ scaffold proteins (27,37–39).

Two E6 constructs were used to validate the new resource. The wild-type E6 (MSCCRSSRTRRETQL) and the mutant E6 ΔPBM, in which the PBM is disrupted by removing the last 3 amino acids (MSCCRSSRTRRE) (**Fig. 1 B**). Bait constructs were

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239343; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

178 subcloned in the pGBT9-BD expression vector and fusion proteins were expressed in the
179 AH109 yeast strain for Y2H (**Fig. 1 B**).

180 Y2H screens were carried out by mating the two recombinant yeast strains Y187 (α) and
181 AH109 (a), allowing the formation of diploid yeasts expressing the prey and the bait
182 constructs (**Fig. 2 A**). According to Y2H principles, in case the E6 bait interacts with a given
183 PDZ prey, a complex is formed and the activating domain (AD) is recruited near the reporter
184 gene, where it can stimulate its expression (**Fig. 2 B, C**). To control mating efficiency, we
185 cultured our mated yeasts in SC-Agar medium lacking leucine and tryptophan (-LW). The
186 growth of dense white colonies indicated an efficient mating (**Fig. 2 C upper panel**).
187 Simultaneously, to test for PDZ interactions, the mated yeasts were grown in SC-Agar
188 medium lacking leucine, tryptophan, and histidine (-LWH). The growth of dense white
189 colonies in the medium -LWH were indicative of E6-PDZ interactions (**Fig. 2 C middle**
190 **panel**). As expected, when the E6 PBM was disrupted (E6 ΔTQL), yeasts failed to grow in
191 the -LWH medium (**Fig. 2 C lower panel**) indicating that the PBM is essential.

192

193 **Fig 2 Y2H mating and selection process**.

194 (**A**) Scheme illustrating the mating of the two yeasts strains. The 'a' type yeasts hosting the E6-
195 pGBT9-BD baits and the 'α' type yeasts hosting the PDZome 2.0-pACT2-AD were allowed to mate.
196 Diploid yeasts containing both the PDZ and the E6 constructs were selected in synthetic agar medium.
197 (**B**) Scheme illustrating the detection of protein interaction by Y2H. In case the E6-bait coupled to the
198 Gal4 binding domain (BD) interacts with the given PDZ-prey coupled to the Gal4 activation domain
199 (AD), the *HIS3* reporter gene is expressed, allowing growth of the diploid yeasts in a synthetic
200 medium without histidine. In case there is no interaction between bait and prey, the AD is not
201 recruited and the *HIS3* reporter gene is not expressed. (**C**) Photographs exemplifying the growth of
202 diploid yeasts containing both the PDZ and the E6 constructs. Diploid yeasts are selected in

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239343; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

permissive culture medium without leucine and tryptophan (-LW). White dense colonies in the -LW medium, suggest an effective mating (upper panel). Simultaneously, the phenotypic test for interactions is performed in selective culture medium without leucine, tryptophan, and histidine (-LWH). White and dense colonies in the -LWH medium correspond to interaction pairs (middle panel). Disruption of the PBM effectively impairs the appearance of white dense colonies in the -LWH medium, confirming a PBM-mediated mode of interaction (lower panel).

We identified 53 PDZ domains interacting with the PBM of the E6 protein from the HPV16. These interactions were confirmed with the tandem constructs. In addition, the tandems identified four interactions not detected when PDZ domains are taken in isolation (**Fig. 3, Fig. S1**). Globally, the PDZome 2.0 outperforms the previous PDZome version that solely identified 36 PDZ domain interacting with E6 (27) (**Fig. 3**). Nevertheless, 8 interactions observed with the PDZome were not detected with the PDZome 2.0. In total, the PDZome 2.0 identified 43 PDZ proteins and 57 PDZ domains able to interact with the E6 protein of the HPV16. The previous version of the PDZome detected 28 PDZ proteins and 36 PDZ domains.

Fig 3 Mapping of E6-PDZ interactions using PDZome 2.0 as compared to the previous resource. Venn diagram representing the positive interactions identified by Y2H screens using the first PDZome (yellow) and PDZome 2.0 (blue). Common interactions detected using both resources are shown in the intersection region (green). Interactions revealed using PDZ tandems are highlighted in red. Note that USH1C interaction was detected using the PDZ 2 domain taken in isolation as present in the first PDZome and using the tandem (USH1C_1-2) from PDZome 2.0.

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239343; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Discussion and conclusion

In this study, we built and validated a new and most comprehensive resource to test for human PDZ interactions by Y2H. Compared to the previous version (27), this resource contains 20 additional PDZ domains (266 instead of 246). Moreover, PDZome 2.0 contains 39 PDZ domains in tandem. Finally, PDZ domains are flanked by extended boundaries meant to support proper folding (28) and avoid false negative results.

Consistently, the PDZome 2.0 revealed 25 interactions that were not detected previously for the viral oncoprotein E6. Among those 25 interactions, 9 were previously detected using the chromatographic holdup approach (HU) (38). Curiously, the PDZome 2.0 failed to detect 7 interactions that were detected with the previous version of the PDZome. The reasons are unclear. One trivial reason could be that some constructs were erroneously annotated in the PDZome compared to PDZome 2.0 (27,35,38). Another possible explanation could be that the extended sequences in the PDZ domains restrain particular interactions or contribute to the auto-inhibition of the PDZ domain (14,28). Finally, these interactions might correspond to false positives (40).

The presence of tandem structures in a protein (i.e. co-folding domains) can enhance the affinity for a particular ligand (32). Consistently, the PDZ tandem constructs not solely validated interactions observed with PDZ in isolation but also revealed additional interactions. Three of these extra interactions were not described previously in papers reporting the HPV16-E6 -PDZ interactomes (27,38,39,41–44). Obviously, the PDZome 2.0 might still be prone to false negative. It is always recommended to verify interactomes using complementary biochemical or biophysical methods such as HU or surface plasmon resonance, before performing functional analyses (38,45,46).

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239343; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

In conclusion, PDZome 2.0 represents a valuable additional resource to test for PDZ interactions by Y2H and certainly an easy going first line choice when one aims to investigate in a comprehensive manner the PDZ interactome of a protein of interest.

Acknowledgements

This work was supported by grants from the French National Research Agency (ANR-18-CE13-0017, Project SynTEV), the Fund for Scientific Research–Flanders (Fonds Wetenschappelijk Onderzoek—Vlaanderen Grants G.0846.15 and G0C5718N), and the Institut National du Cancer (Projets Libres de Recherche "Biologie et Sciences du Cancer" INCa 9474). J.-P.B. is a scholar of Institut Universitaire de France. M.C. benefited of a CONACYT fellowship.

References

1. Harris BZ, Lim WA. Mechanism and role of PDZ domains in signaling complex assembly. J Cell Sci. 2001 Sep;114(Pt 18):3219–31.

2. Nourry C, Grant SGN, Borg J-P. PDZ Domain Proteins: Plug and Play! Sci Signal. 2003 Apr 22;2003(179):re7–re7.

3. Manjunath GP, Ramanujam PL, Galande S. Structure function relations in PDZ-domain-containing proteins: Implications for protein networks in cellular signalling. J Biosci. 2018 Mar;43(1):155–71.

4. Cho KO, Hunt CA, Kennedy MB. The rat brain postsynaptic density fraction contains a homolog of the Drosophila discs-large tumor suppressor protein. Neuron. 1992 Nov;9(5):929–42.

5. Itoh M, Nagafuchi A, Yonemura S, Kitani-Yasuda T, Tsukita S, Tsukita S. The 220-kD protein colocalizing with cadherins in non-epithelial cells is identical to ZO-1, a tight junction-associated protein in epithelial cells: cDNA cloning and immunoelectron microscopy. J Cell Biol. 1993 May;121(3):491–502.

6. Woods DF, Bryant PJ. ZO-1, DlgA and PSD-95/SAP90: homologous proteins in tight, septate and synaptic cell junctions. Mech Dev. 1993 Dec;44(2–3):85–9.

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239343; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

7. Woods DF, Bryant PJ. The discs-large tumor suppressor gene of Drosophila encodes a guanylate kinase homolog localized at septate junctions. Cell. 1991 Aug;66(3):451–64.

8. Kennedy M. Origin of PDZ (DHR, GLGF) domains. Trends Biochem Sci. 1995 Sep;20(9):350.

9. Songyang Z. Recognition of Unique Carboxyl-Terminal Motifs by Distinct PDZ Domains. Science. 1997 Jan 3;275(5296):73–7.

10. Zimmermann P, Meerschaert K, Reekmans G, Leenaerts I, Small JV, Vandekerckhove J, et al. PIP(2)-PDZ domain binding controls the association of syntenin with the plasma membrane. Mol Cell. 2002 Jun;9(6):1215–25.

11. Brenman JE, Chao DS, Gee SH, McGee AW, Craven SE, Santillano DR, et al. Interaction of Nitric Oxide Synthase with the Postsynaptic Density Protein PSD-95 and α1-Syntrophin Mediated by PDZ Domains. Cell. 1996 Mar;84(5):757–67.

12. Harris BZ, Hillier BJ, Lim WA. Energetic Determinants of Internal Motif Recognition by PDZ Domains. Biochemistry. 2001 May;40(20):5921–30.

13. Liu W, Wen W, Wei Z, Yu J, Ye F, Liu C-H, et al. The INAD scaffold is a dynamic, redox-regulated modulator of signaling in the Drosophila eye. Cell. 2011 Jun 24;145(7):1088–101.

14. LaLonde DP, Bretscher A. The scaffold protein PDZK1 undergoes a head-to-tail intramolecular association that negatively regulates its interaction with EBP50. Biochemistry. 2009;48(10):2261–71.

15. van den Berk LCJ, Landi E, Walma T, Vuister GW, Dente L, Hendriks WJAJ. An allosteric intramolecular PDZ-PDZ interaction modulates PTP-BL PDZ2 binding specificity. Biochemistry. 2007 Nov 27;46(47):13629–37.

16. Raghuram V, Hormuth H, Foskett JK. A kinase-regulated mechanism controls CFTR channel gating by disrupting bivalent PDZ domain interactions. Proc Natl Acad Sci U S A. 2003 Aug 5;100(16):9620–5.

17. Ivarsson Y. Plasticity of PDZ domains in ligand recognition and signaling. FEBS Lett. 2012 Aug 14;586(17):2638–47.

18. Lee H-J, Zheng JJ. PDZ domains and their binding partners: structure, specificity, and modification. Cell Commun Signal CCS. 2010 May 28;8:8.

19. Doyle DA, Lee A, Lewis J, Kim E, Sheng M, MacKinnon R. Crystal structures of a complexed and peptide-free membrane protein-binding domain: molecular basis of peptide recognition by PDZ. Cell. 1996 Jun 28;85(7):1067–76.

20. Tonikian R, Zhang Y, Sazinsky SL, Currell B, Yeh J-H, Reva B, et al. A specificity map for the PDZ domain family. PLoS Biol. 2008 Sep 30;6(9):e239.

21. Ivarsson Y, Arnold R, McLaughlin M, Nim S, Joshi R, Ray D, et al. Large-scale interaction profiling of PDZ domains through proteomic peptide-phage display using human and viral phage peptidomes. Proc Natl Acad Sci. 2014 Feb 18;111(7):2542–7.

22. Garrido-Urbani S, Garg P, Ghossoub R, Arnold R, Lembo F, Sundell GN, et al. Proteomic peptide phage display uncovers novel interactions of the PDZ1-2 supramodule of syntenin. Valencia A, editor. FEBS Lett. 2016 Jan;590(1):3–12.

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239343; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

23. Ponting CP. Evidence for PDZ domains in bacteria, yeast, and plants. Protein Sci Publ Protein Soc. 1997 Feb;6(2):464–8.

24. Sakarya O, Conaco C, Egecioglu O, Solla SA, Oakley TH, Kosik KS. Evolutionary Expansion and Specialization of the PDZ Domains. Mol Biol Evol. 2010 May 1;27(5):1058–69.

25. Letunic I, Doerks T, Bork P. SMART 7: recent updates to the protein domain annotation resource. Nucleic Acids Res. 2012 Jan;40(Database issue):D302-305.

26. Spaller MR. Act globally, think locally: systems biology addresses the PDZ domain. ACS Chem Biol. 2006 May 23;1(4):207–10.

27. Belotti E, Polanowska J, Daulat AM, Audebert S, Thomé V, Lissitzky J-C, et al. The Human PDZome: A Gateway to PSD95-Disc Large-Zonula Occludens (PDZ)-mediated Functions. Mol Cell Proteomics. 2013 Sep;12(9):2587–603.

28. Luck K, Charbonnier S, Travé G. The emerging contribution of sequence context to the specificity of protein interactions mediated by PDZ domains. FEBS Lett. 2012 Aug 14;586(17):2648–61.

29. Wawrzyniak AM, Vermeiren E, Zimmermann P, Ivarsson Y. Extensions of PSD-95/discs large/ZO-1 (PDZ) domains influence lipid binding and membrane targeting of syntenin-1. FEBS Lett. 2012 May 21;586(10):1445–51.

30. Wang CK, Pan L, Chen J, Zhang M. Extensions of PDZ domains as important structural and functional elements. Protein Cell. 2010 Aug;1(8):737–51.

31. Zhang J, Petit CM, King DS, Lee AL. Phosphorylation of a PDZ domain extension modulates binding affinity and interdomain interactions in postsynaptic density-95 (PSD-95) protein, a membrane-associated guanylate kinase (MAGUK). J Biol Chem. 2011 Dec 2;286(48):41776–85.

32. Feng W, Zhang M. Organization and dynamics of PDZ-domain-related supramodules in the postsynaptic density. Vol. 10, Nature Reviews Neuroscience. 2009. p. 87–99.

33. Ye F, Zhang M. Structures and target recognition modes of PDZ domains: recurring themes and emerging pictures. Biochem J. 2013 Oct 1;455(1):1–14.

34. Grootjans JJ, Reekmans G, Ceulemans H, David G. Syntenin-syndecan binding requires syndecan-synteny and the co-operation of both PDZ domains of syntenin. J Biol Chem. 2000 Jun 30;275(26):19933–41.

35. Duhoo Y, Girault V, Turchetto J, Ramond L, Durbesson F, Fourquet P, et al. High-Throughput Production of a New Library of Human Single and Tandem PDZ Domains Allows Quantitative PDZ-Peptide Interaction Screening Through High-Throughput Holdup Assay. Methods Mol Biol Clifton NJ. 2019;2025:439–76.

36. Fields S, Song O. A novel genetic system to detect protein–protein interactions. Nature. 1989 Jul;340(6230):245–6.

37. Thomas M, Narayan N, Pim D, Tomaić V, Massimi P, Nagasaka K, et al. Human papillomaviruses, cervical cancer and cell polarity. Oncogene. 2008 Nov 24;27(55):7018–30.

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239343; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

355 38. Vincentelli R, Luck K, Poirson J, Polanowska J, Abdat J, Blémont M, et al. Quantifying
356 domain-ligand affinities and specificities by high-throughput holdup assay. Nat Methods. 2015
357 Aug;12(8):787–93.

358 39. Ganti K, Broniarczyk J, Manoubi W, Massimi P, Mittal S, Pim D, et al. The Human
359 Papillomavirus E6 PDZ Binding Motif: From Life Cycle to Malignancy. Viruses. 2015 Jul
360 2;7(7):3530–51.

361 40. Brückner A, Polge C, Lentze N, Auerbach D, Schlattner U. Yeast Two-Hybrid, a Powerful Tool
362 for Systems Biology. Int J Mol Sci. 2009 Jun 18;10(6):2763–88.

363 41. Javier RT, Rice AP. Emerging theme: cellular PDZ proteins as common targets of pathogenic
364 viruses. J Virol. 2011 Nov;85(22):11544–56.

365 42. Neveu G, Cassonnet P, Vidalain P-O, Rolloy C, Mendoza J, Jones L, et al. Comparative analysis
366 of virus-host interactomes with a mammalian high-throughput protein complementation assay
367 based on Gaussia princeps luciferase. Methods San Diego Calif. 2012 Dec;58(4):349–59.

368 43. Pim D, Bergant M, Boon SS, Ganti K, Kranjec C, Massimi P, et al. Human papillomaviruses
369 and the specificity of PDZ domain targeting. FEBS J. 2012 Oct;279(19):3530–7.

370 44. Poirson J, Biquand E, Straub M-L, Cassonnet P, Nominé Y, Jones L, et al. Mapping the
371 interactome of HPV E6 and E7 oncoproteins with the ubiquitin-proteasome system. FEBS J.
372 2017;284(19):3171–201.

373 45. Grootjans JJ, Zimmermann P, Reekmans G, Smets A, Degeest G, Durr J, et al. Syntenin, a PDZ
374 protein that binds syndecan cytoplasmic domains. Proc Natl Acad Sci. 1997 Dec
375 9;94(25):13683–8.

376 46. Baietti MF, Zhang Z, Mortier E, Melchior A, Degeest G, Geeraerts A, et al. Syndecan–
377 syntenin–ALIX regulates the biogenesis of exosomes. Nat Cell Biol. 2012 Jul;14(7):677–85.

378

379 Supporting information

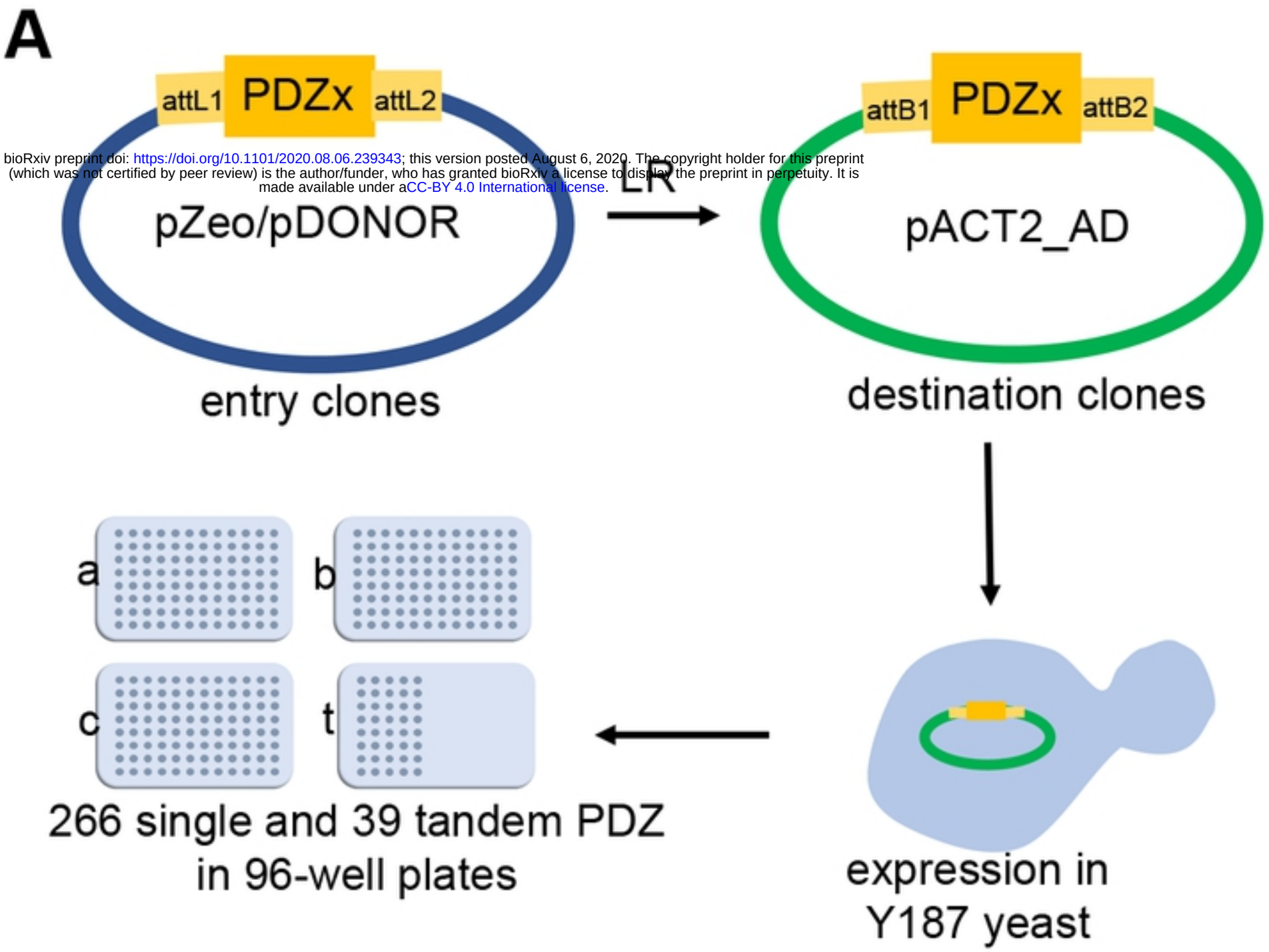
380 **Fig. S1. Summary of the Yeast-Two-Hybrid raw data.** Summary of the PDZ interactions
381 detected by Yeast-Two-Hybrid using the E6 protein C-terminal region of HPV16 wild type as
382 bait. (**A**) Comparison of the interactions detected using the PDZome and the single PDZ
383 collection of the PDZome 2.0 (as indicated). (**B**) Comparison of the interactions detected
384 using the collection of tandems of the PDZome 2.0 and the correspondent PDZ taken in
385 isolation in PDZome and PDZome 2.0 (as indicated). No interaction detected is indicated in
386 red, positive interaction detected is indicated in blue. Numbers correspond to positive
387 colonies / detected from *n* independent experiments.

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239343; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

S1 Table. Single PDZ domain constructs used to comprehensively map PDZ interactions.

S2 Table. Tandem PDZ constructs used as preys to comprehensively map PDZ interactions.

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239343; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.



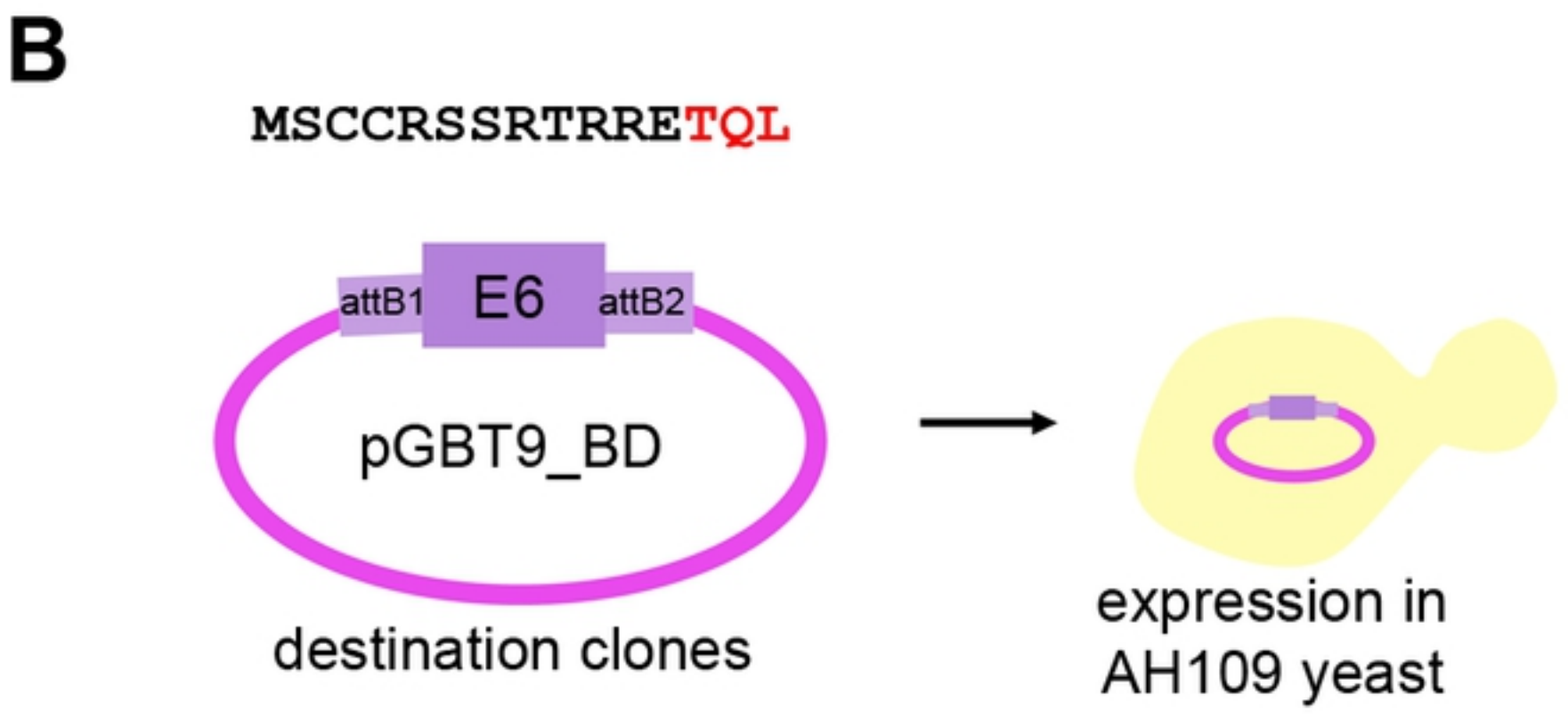
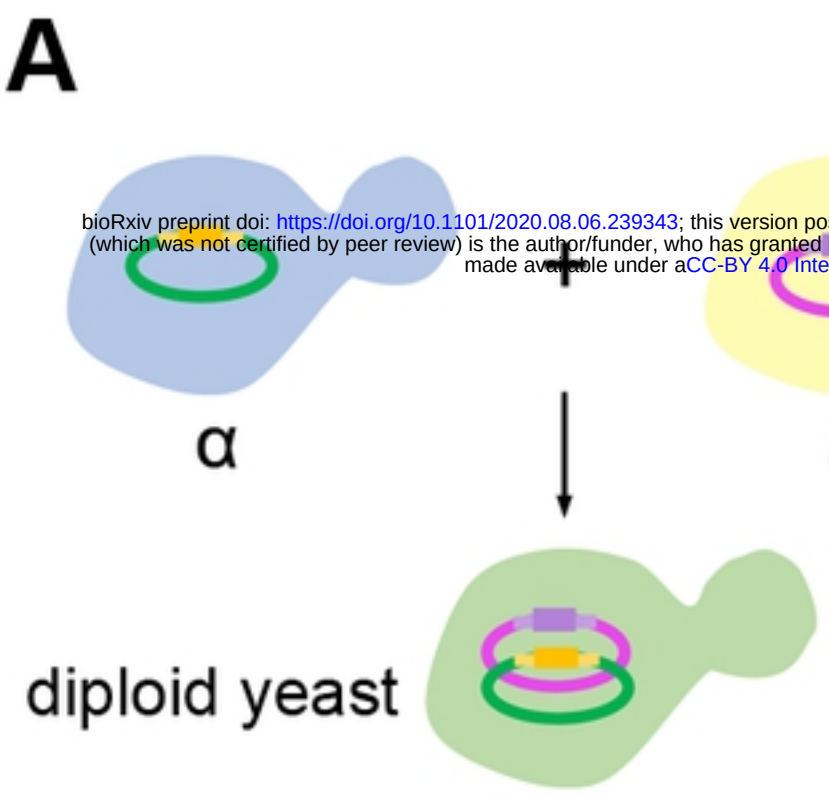
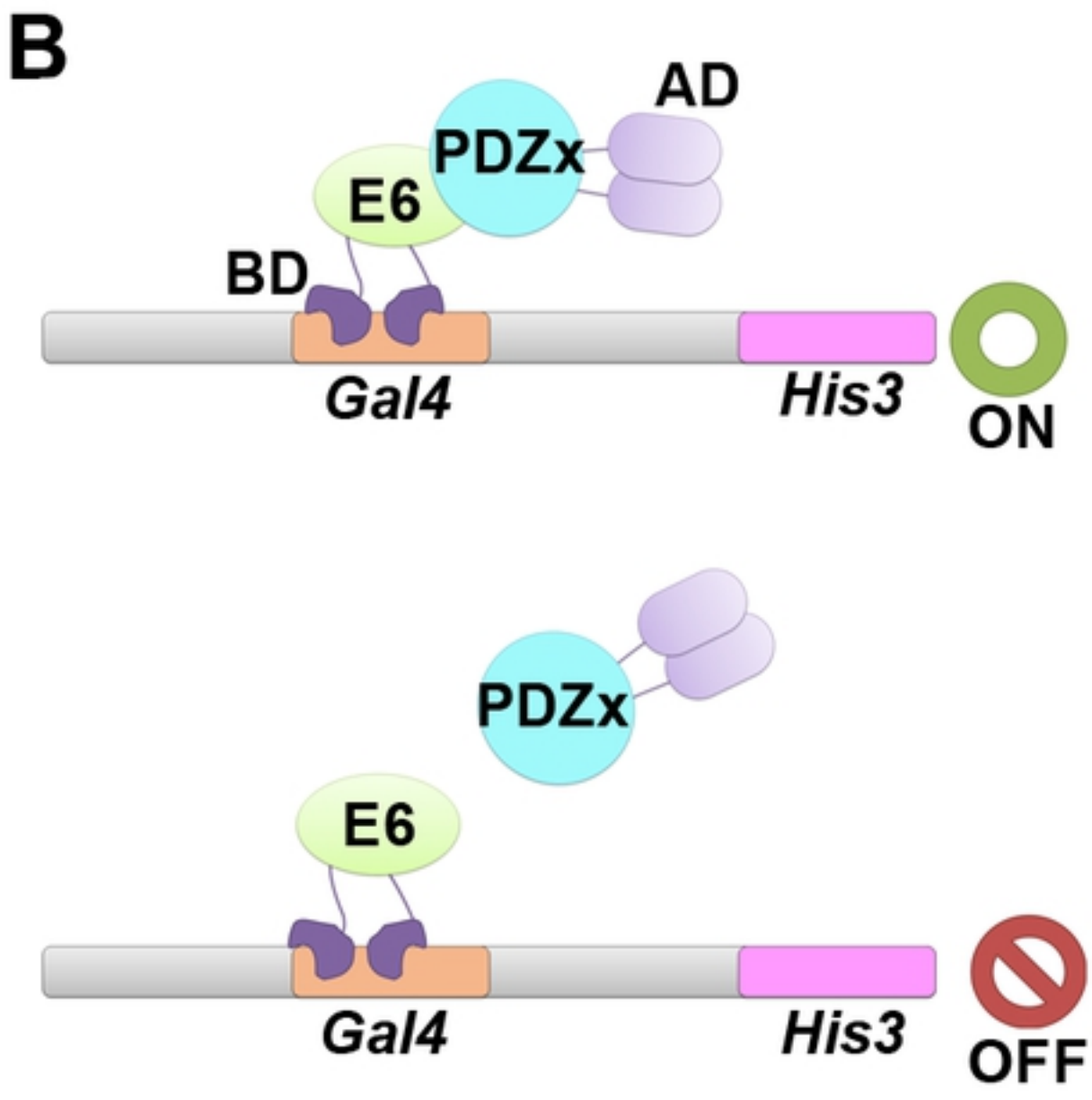


Figure 1

bioRxiv preprint doi: https://doi.org/10.1101/2020.08.06.239343; this version posted August 6, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.





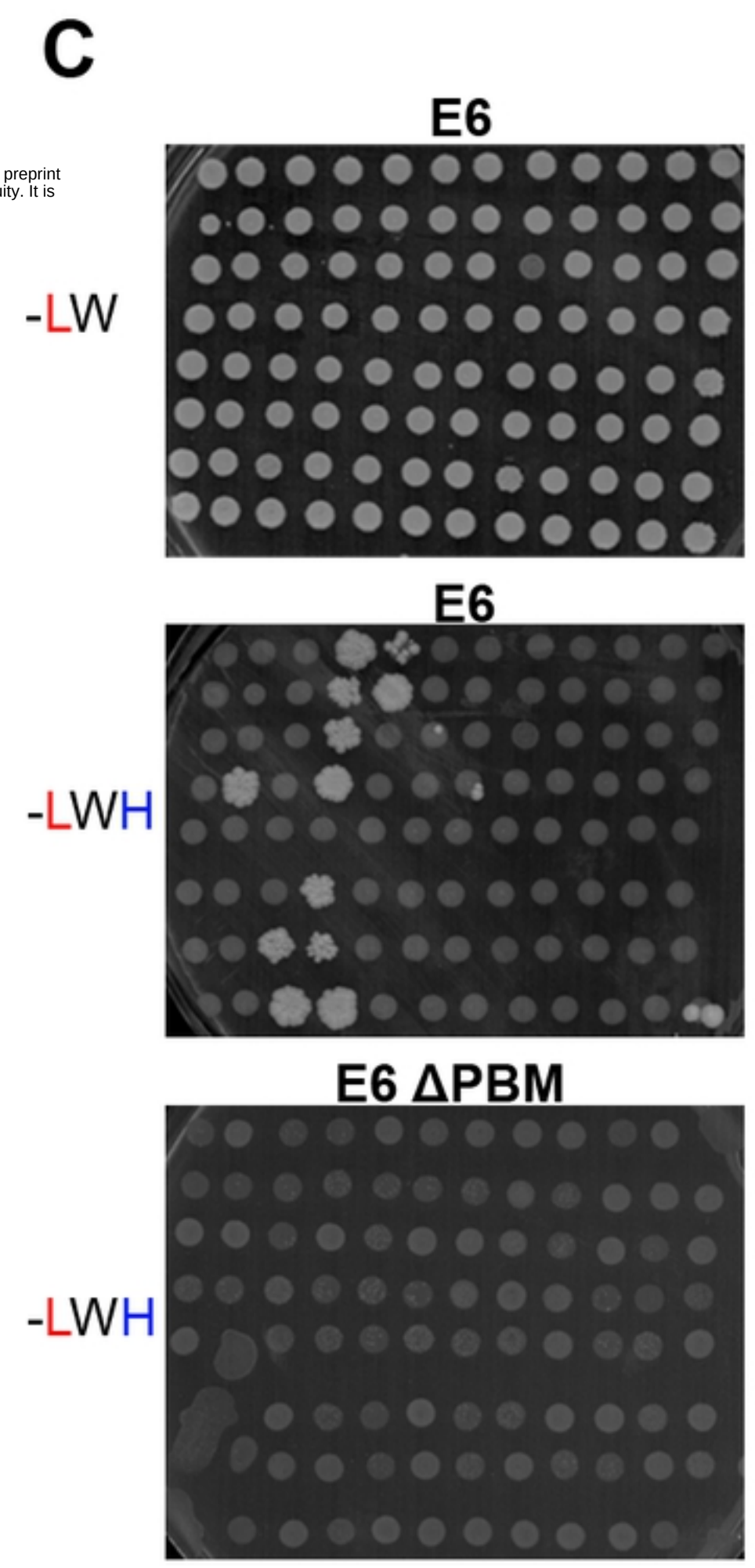


Figure 2

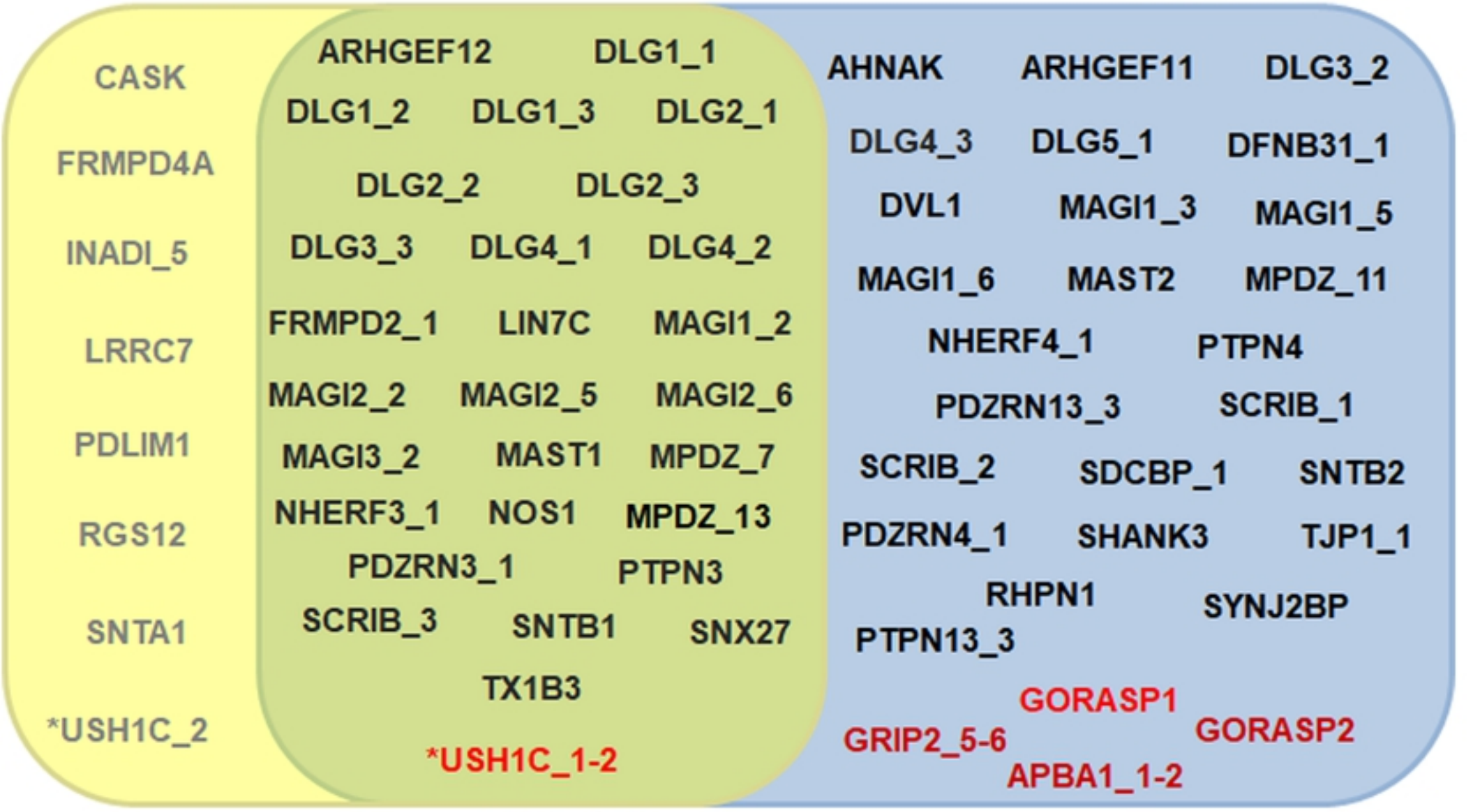

PDZome
PDZome 2.0
CASK
FRMPD4A
INADL_5
LRRC7
PDLIM1
RGS12
SNTA1
*USH1C_2
ARHGEF12
DLG1_1
DLG1_2
DLG1_3
DLG2_1
DLG2_2
DLG2_3
DLG3_3
DLG4_1
DLG4_2
FRMPD2_1
LIN7C
MAGI1_2
MAGI2_2
MAGI2_5
MAGI2_6
MAGI3_2
MAST1
MPDZ_7
NHERF3_1
NOS1
MPDZ_13
PDZRN3_1
PTPN3
SCRIB_3
SNTB1
SNX27
TX1B3
*USH1C_1-2
AHNAK
ARHGEF11
DLG3_2
DLG4_3
DLG5_1
DFNB31_1
DVL1
MAGI1_3
MAGI1_5
MAGI1_6
MAST2
MPDZ_11
NHERF4_1
PTPN4
PDZRN13_3
SCRIB_1
SCRIB_2
SDCBP_1
SNTB2
PDZRN4_1
SHANK3
TJP1_1
RHPN1
SYNJ2BP
PTPN13_3
GORASP1
GRIP2_5-6
GORASP2
APBA1_1-2
common

Figure 3