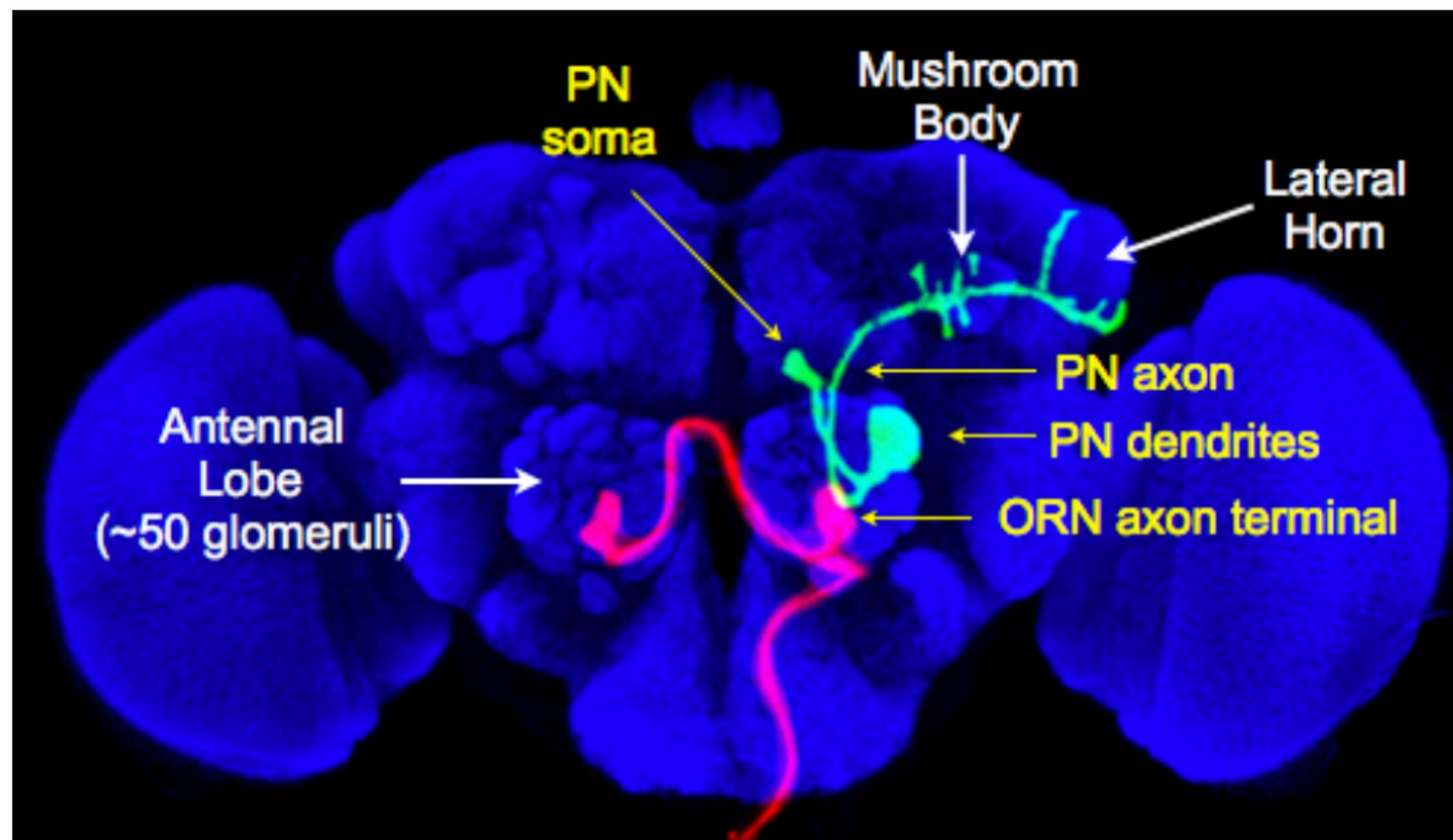


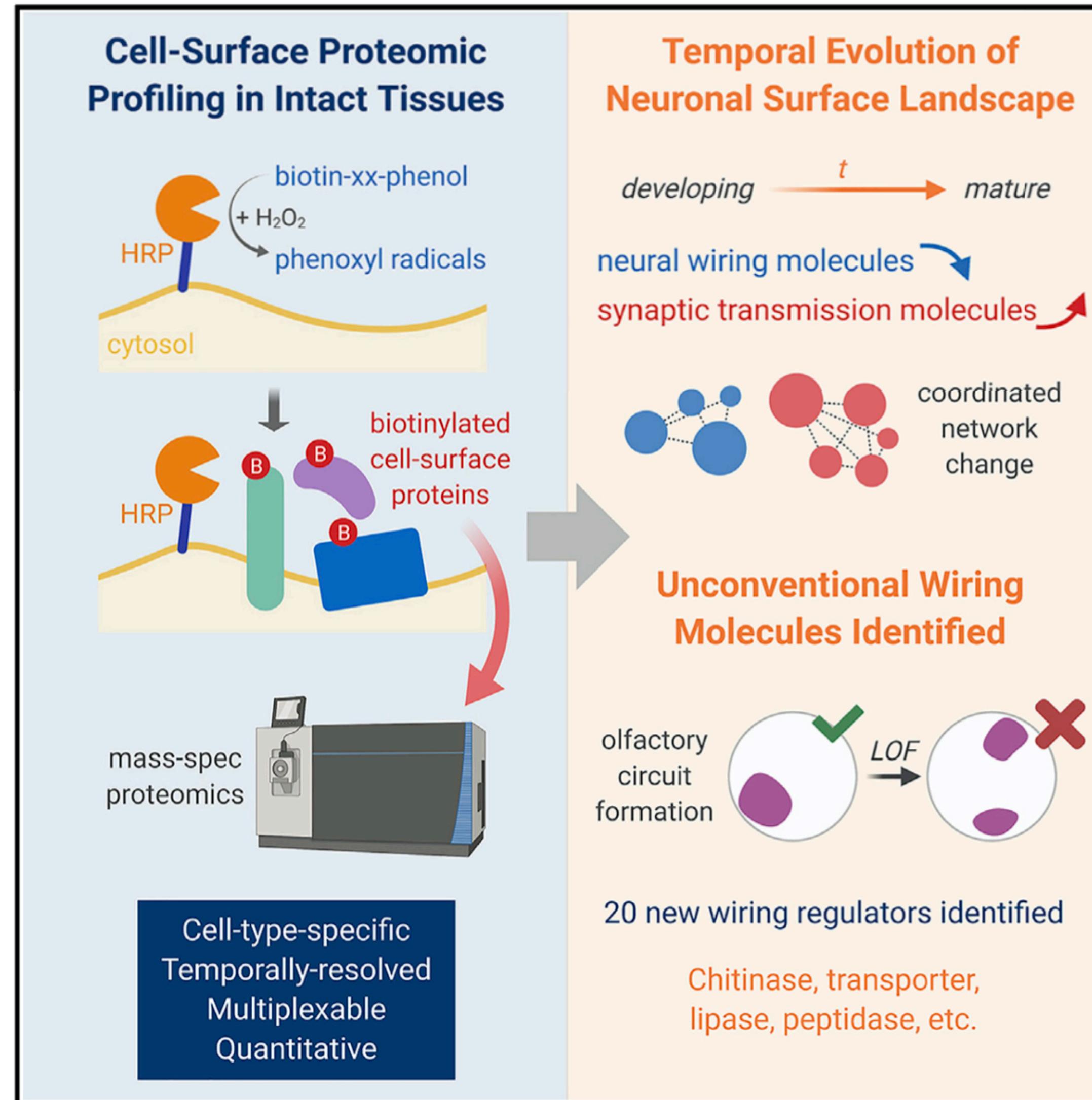
Cell-Surface Proteomic Profiling in the Fly Brain Uncovers Wiring Regulators

Jiefu Li, Shuo Han, ..., Alice Ting, Liqun Luo



- Graphic Abstract
- Background
 1. Olfactory System
 2. Membrane Protein
 3. Proximity Labeling
- Surface Labeling
- Temporal Evolution
Developing v.s. Mature
- Spatial Precision
ORN v.s. PN
- Regulation Pattern

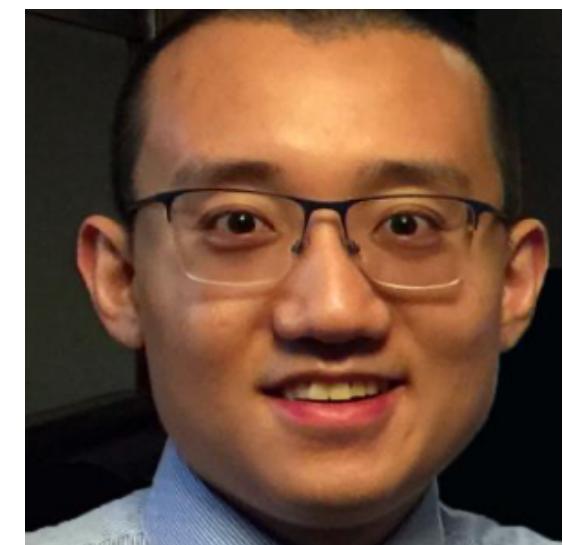
Graphical Abstract



Alice Y. Ting
APEX/APEX2
proximity biotinylation

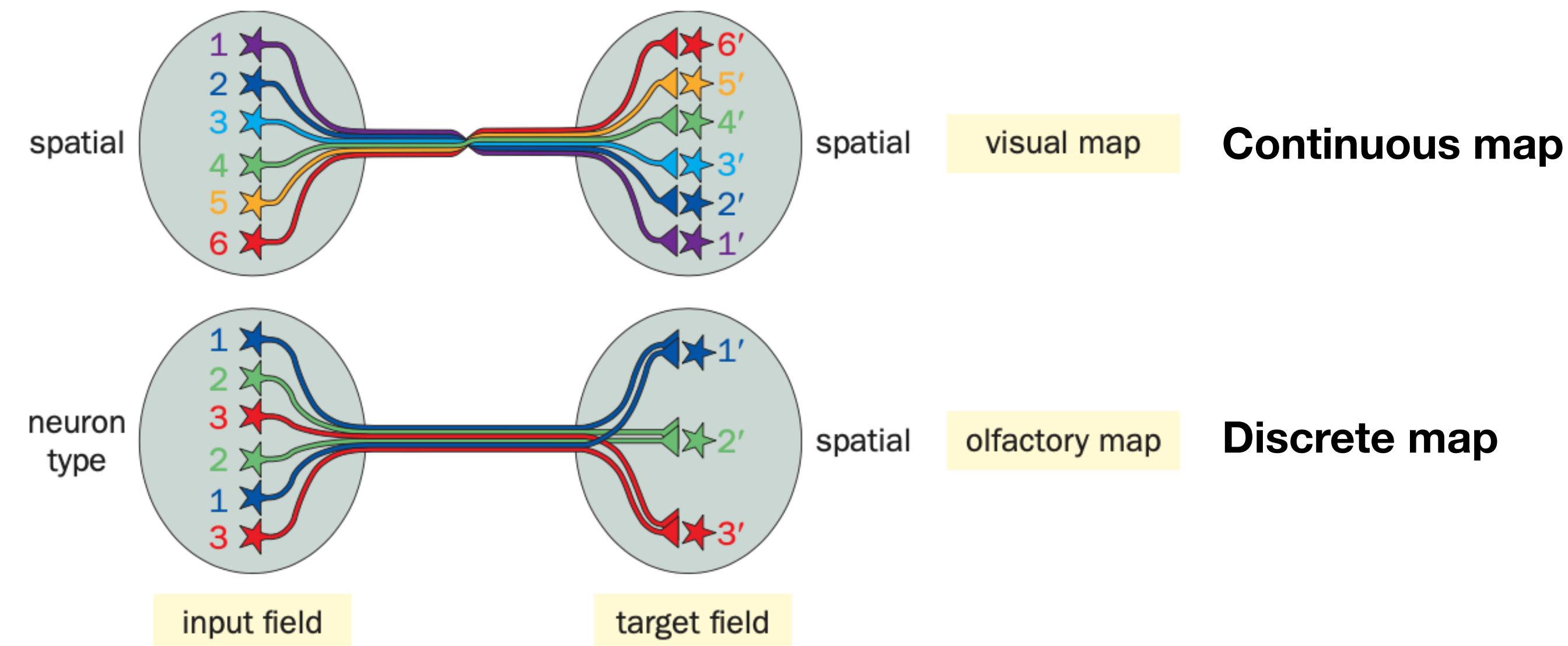


Liqun Luo
Fly olfactory assembly wiring specificity

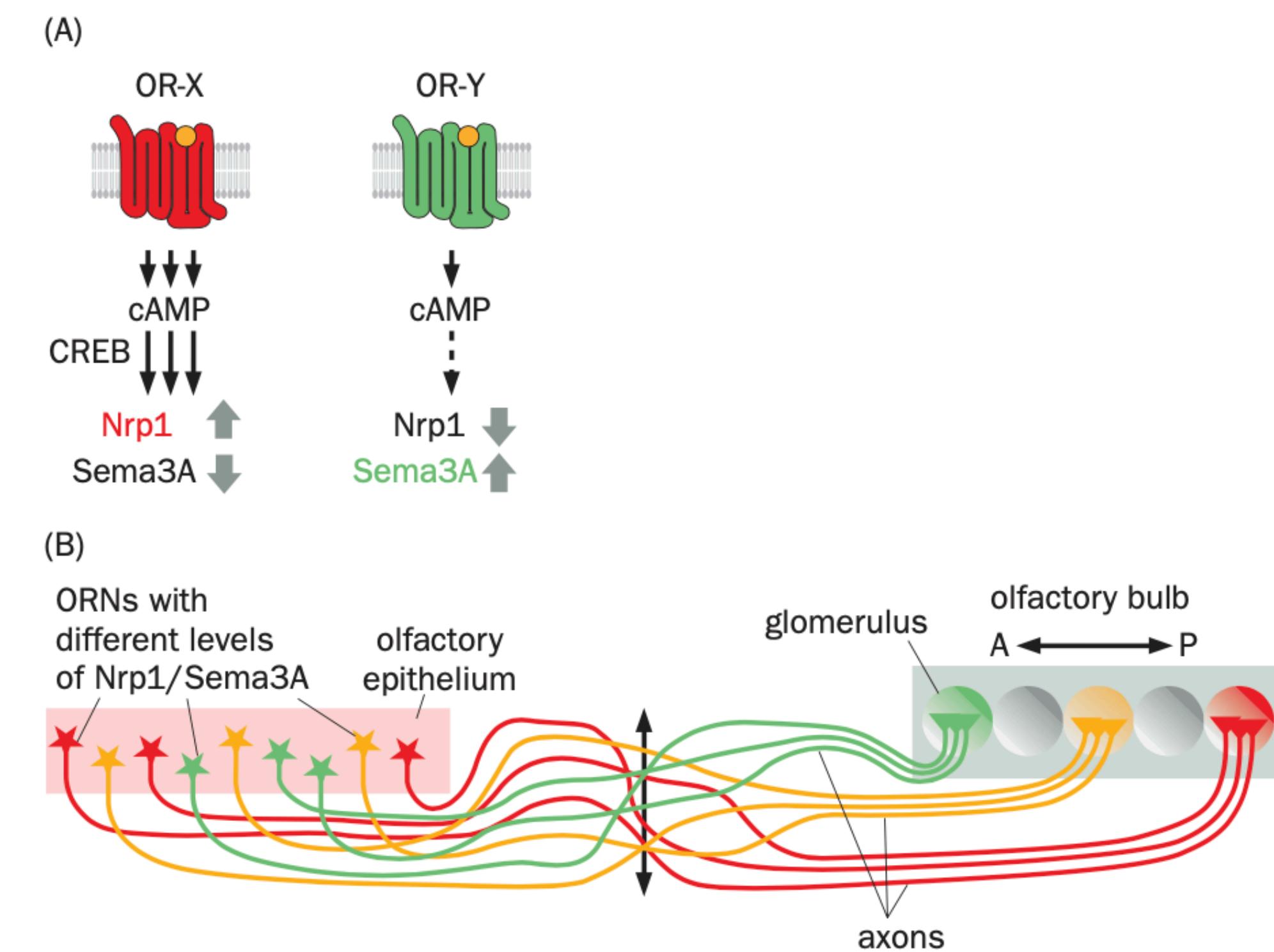
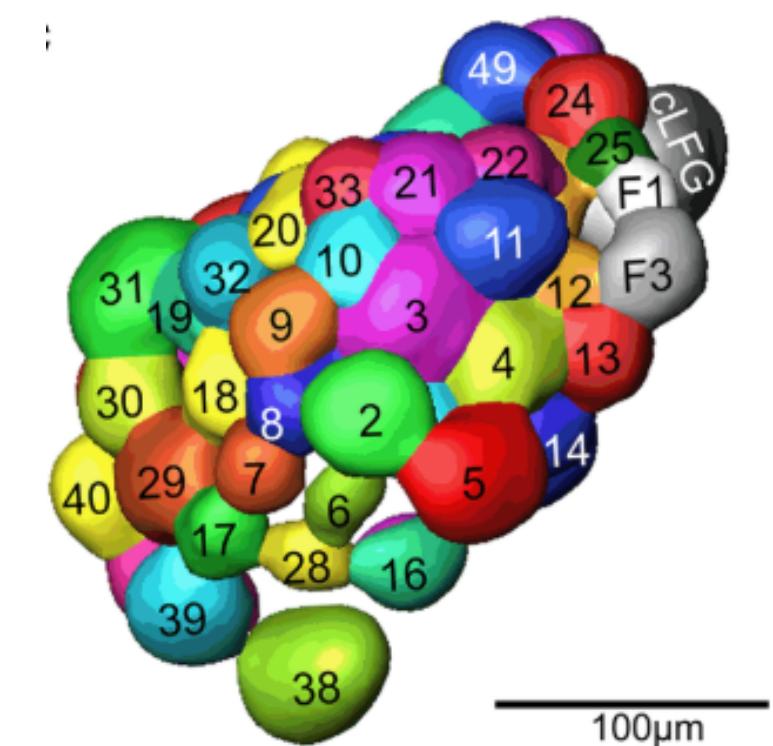


Jiefu Li
SJTU '14
iGEM - patent
Jan Lab - JN paper
Luo&Ting co-advise

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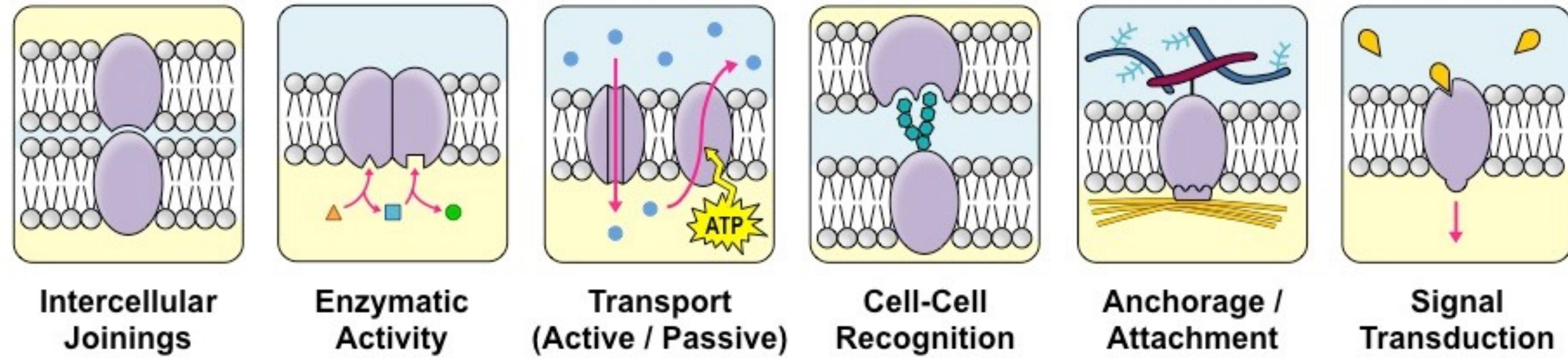


~50 glomeruli in antennal lobe



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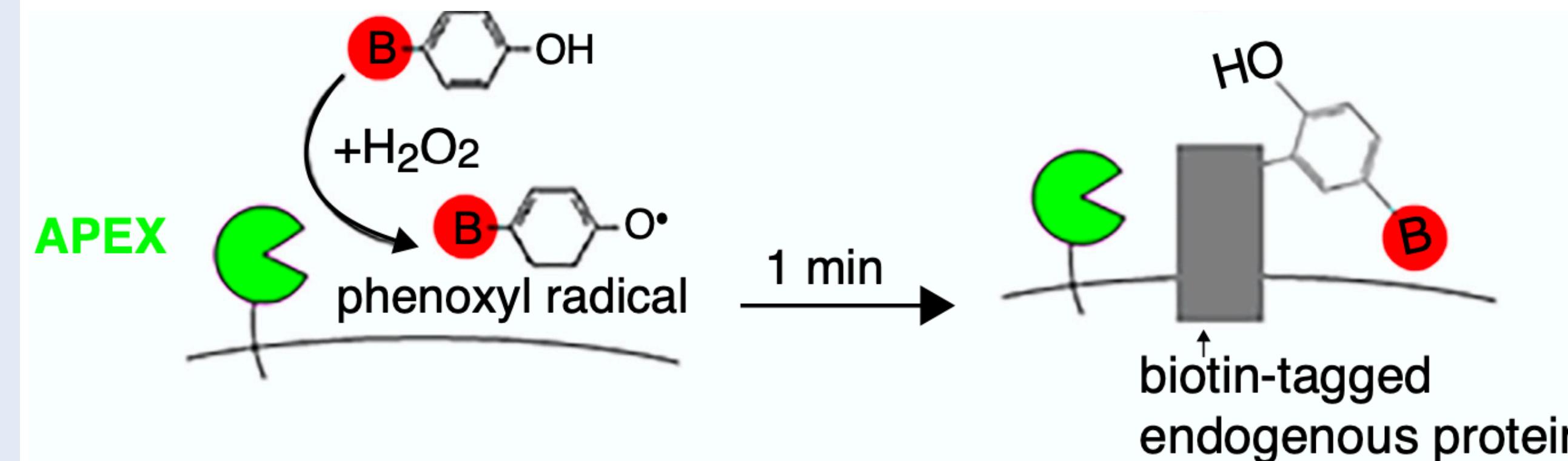
Membrane Proteins



- Specific functional area, e.g. synaptic cleft, PSD, growth cone
- Neuron-glia interaction
- Wiring regulators
- Orphan GPCR gene

PL=Proximity Labeling Use engineered enzyme to covalently tag neighboring proteins in living cells

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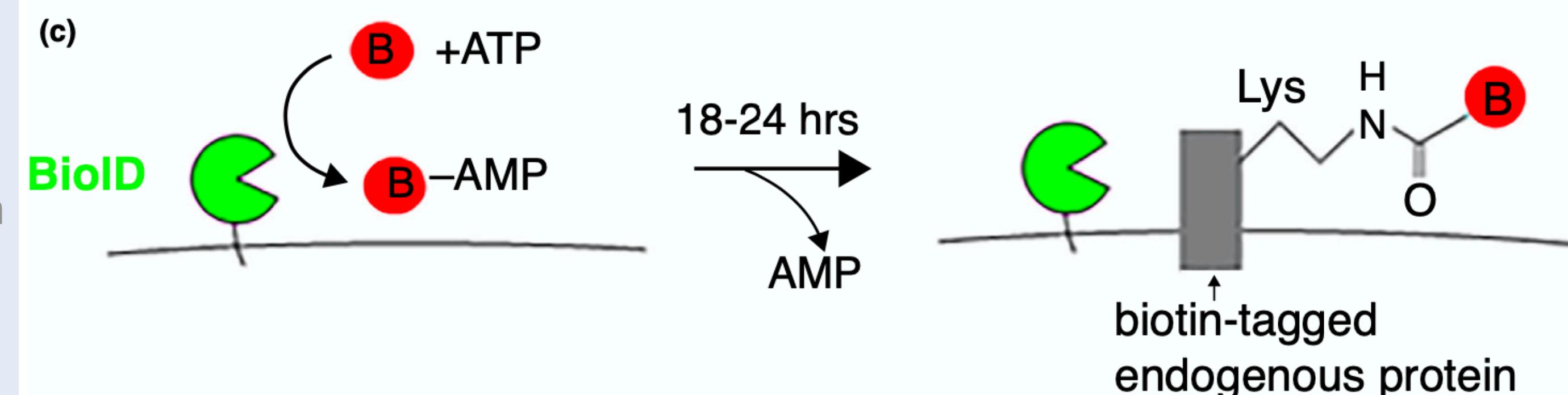


Peroxidase-based PL 过氧化物酶

Ascorbate peroxidase (APEX)
Horseradish peroxidase (HRP)
+
Biotin phenol (BP) or BxxP
=

Phenoxy Radical \rightarrow electron

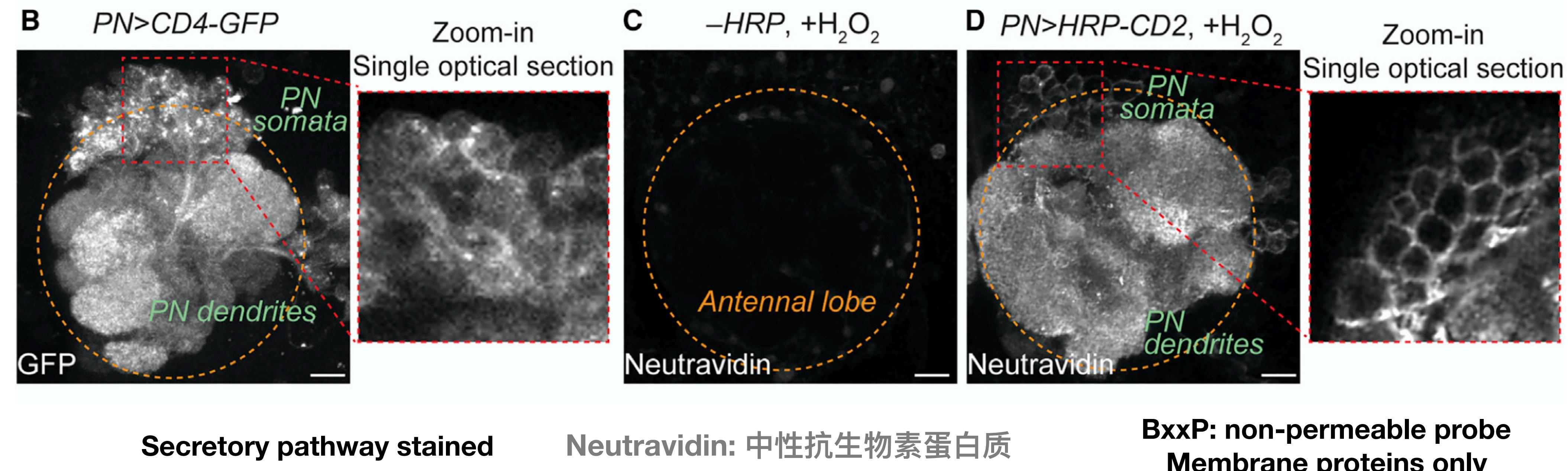
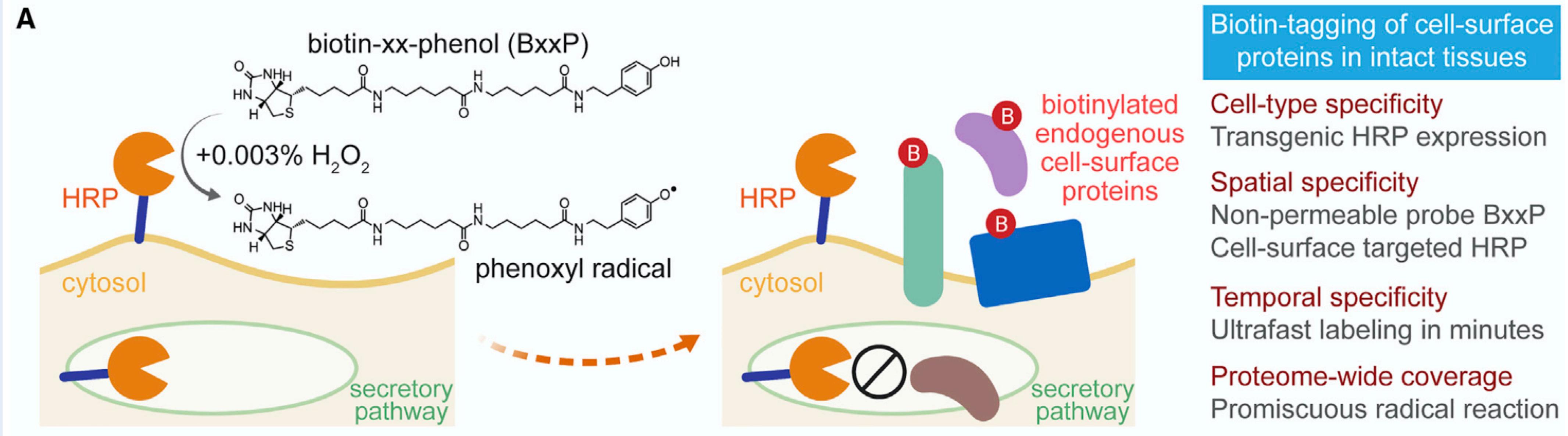
HRP is only active in secretory pathway or extracellular environment // yet more active than APEX



Biotin ligase-based PL 生物素连接酶

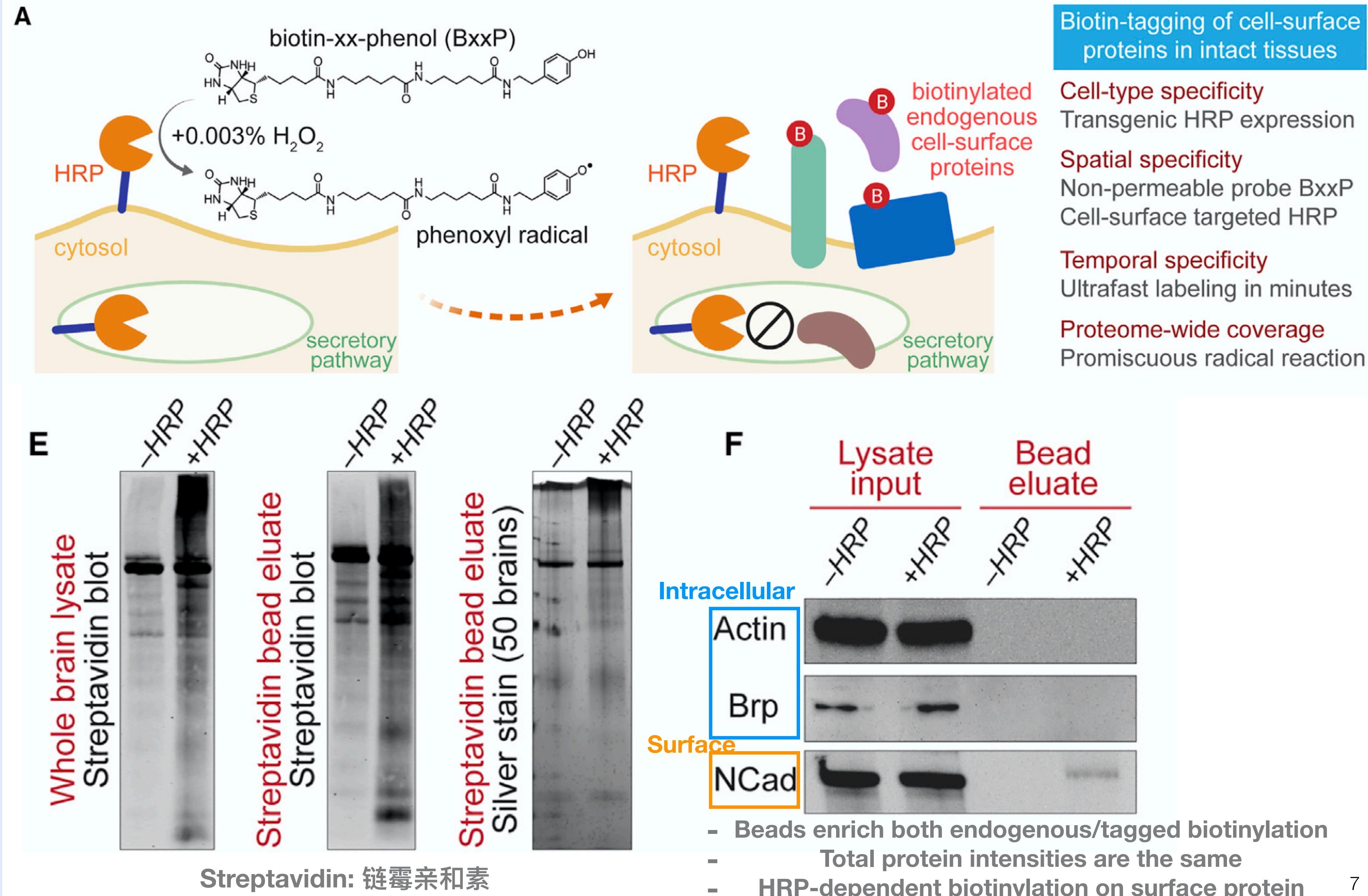
BioID: R118G mutant of BirA
Reduced affinity to Biotin-AMP
Biotin-AMP \rightarrow lysine residue

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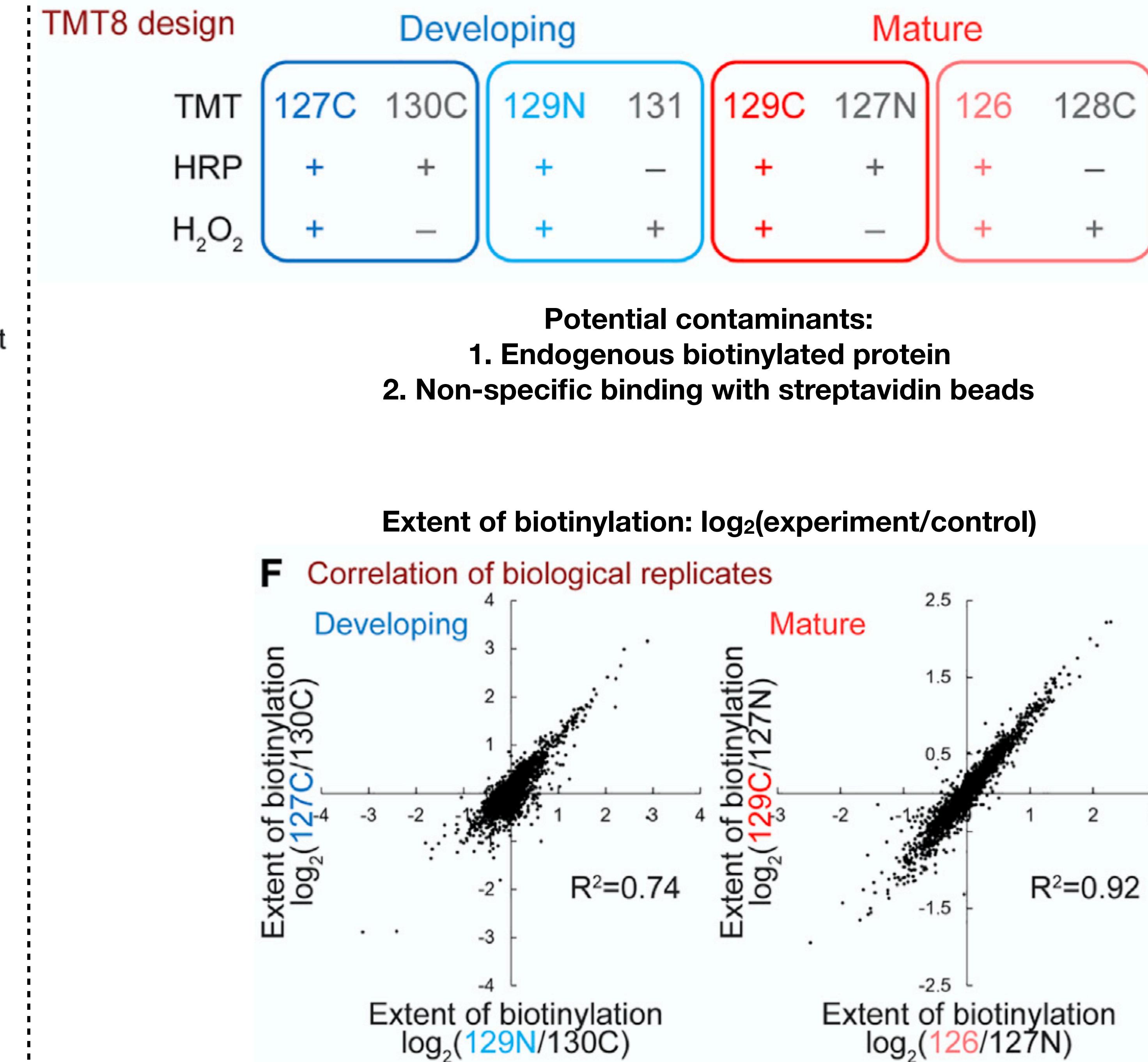
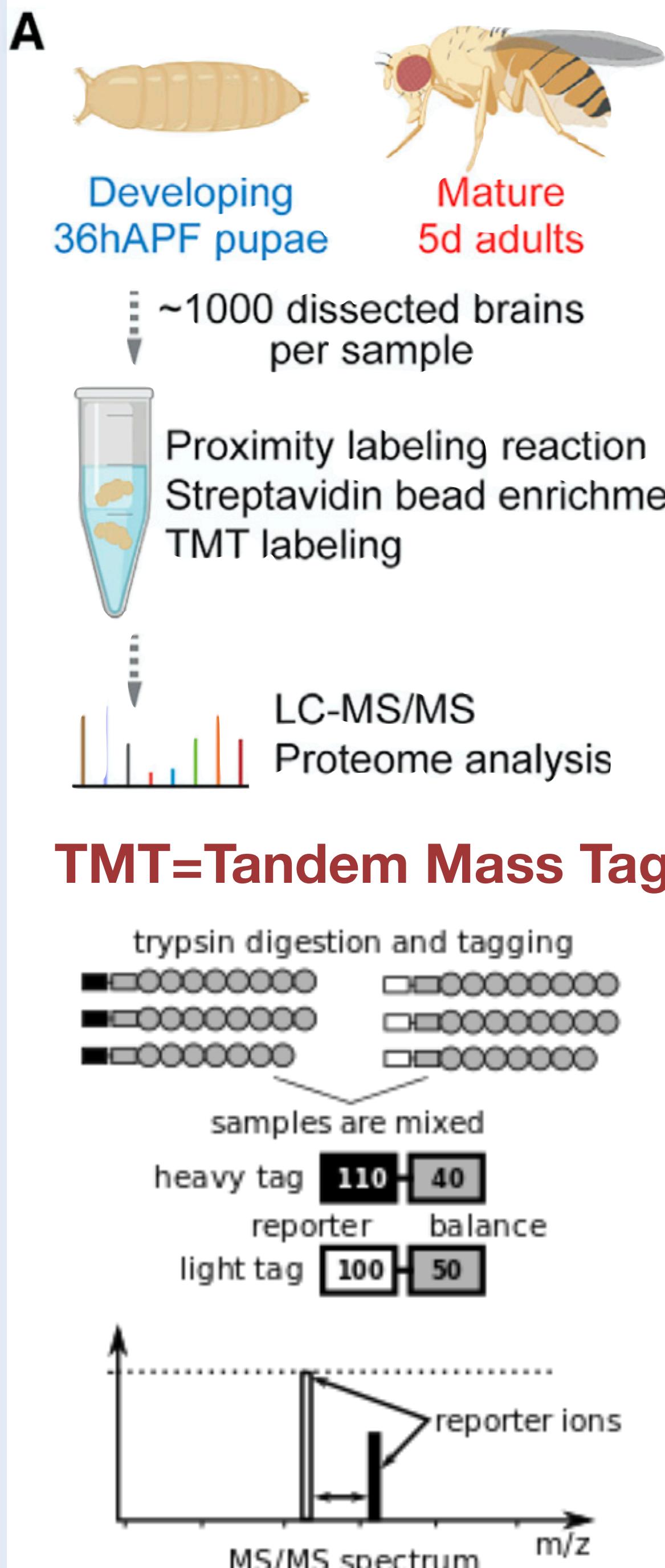


Biotin-tagging of cell-surface proteins in intact tissues

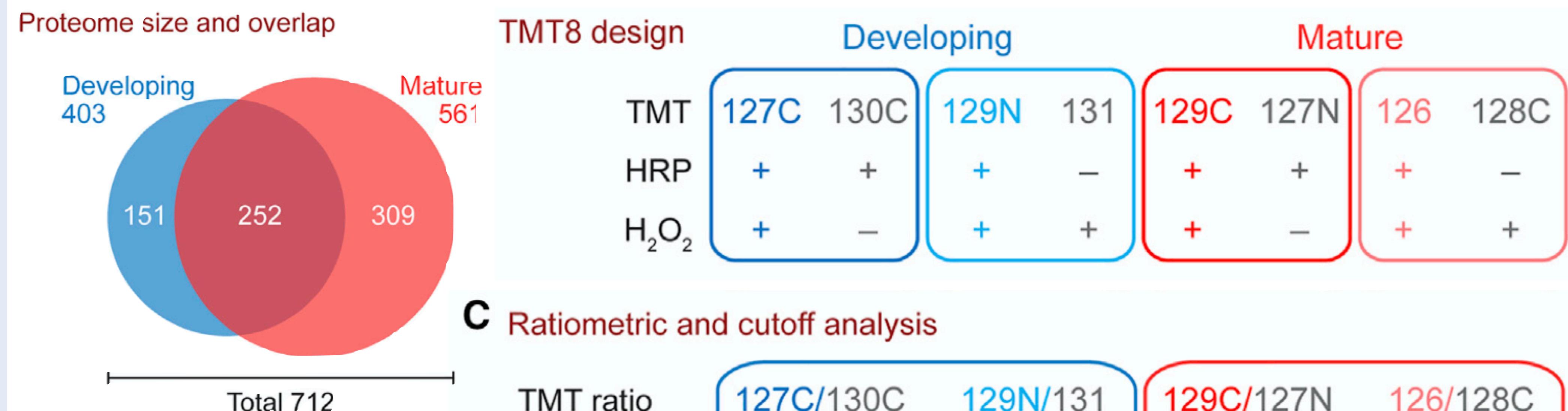
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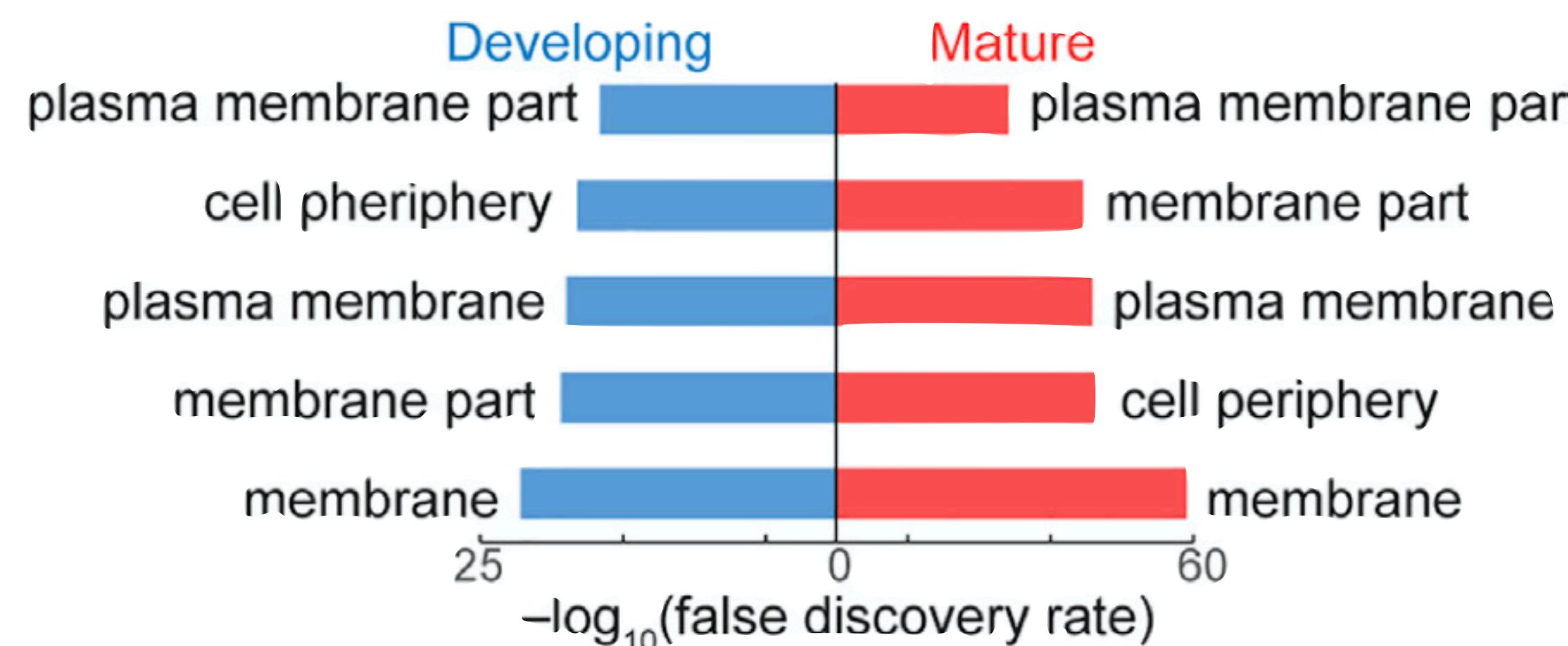


C Ratiometric and cutoff analysis

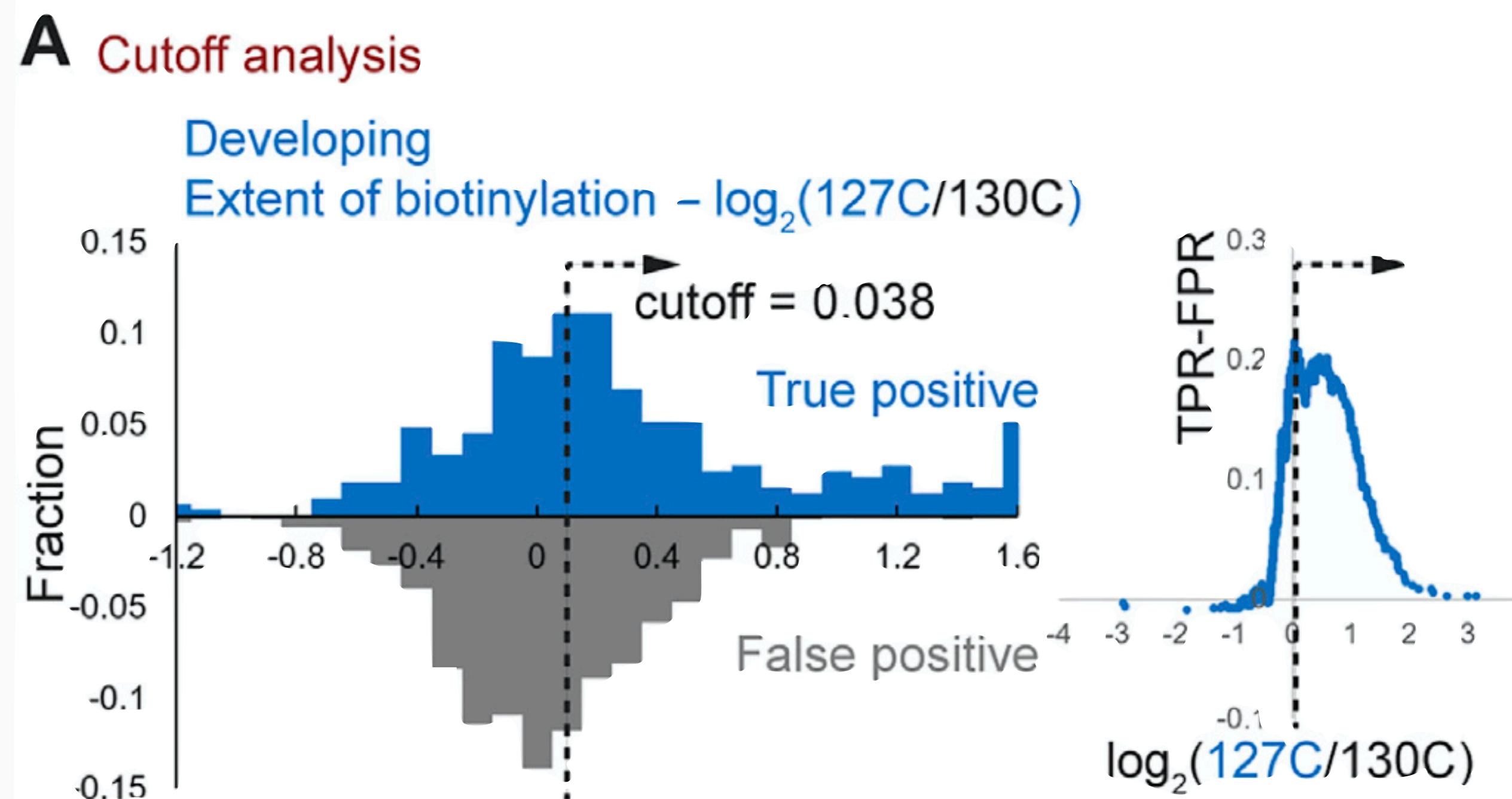
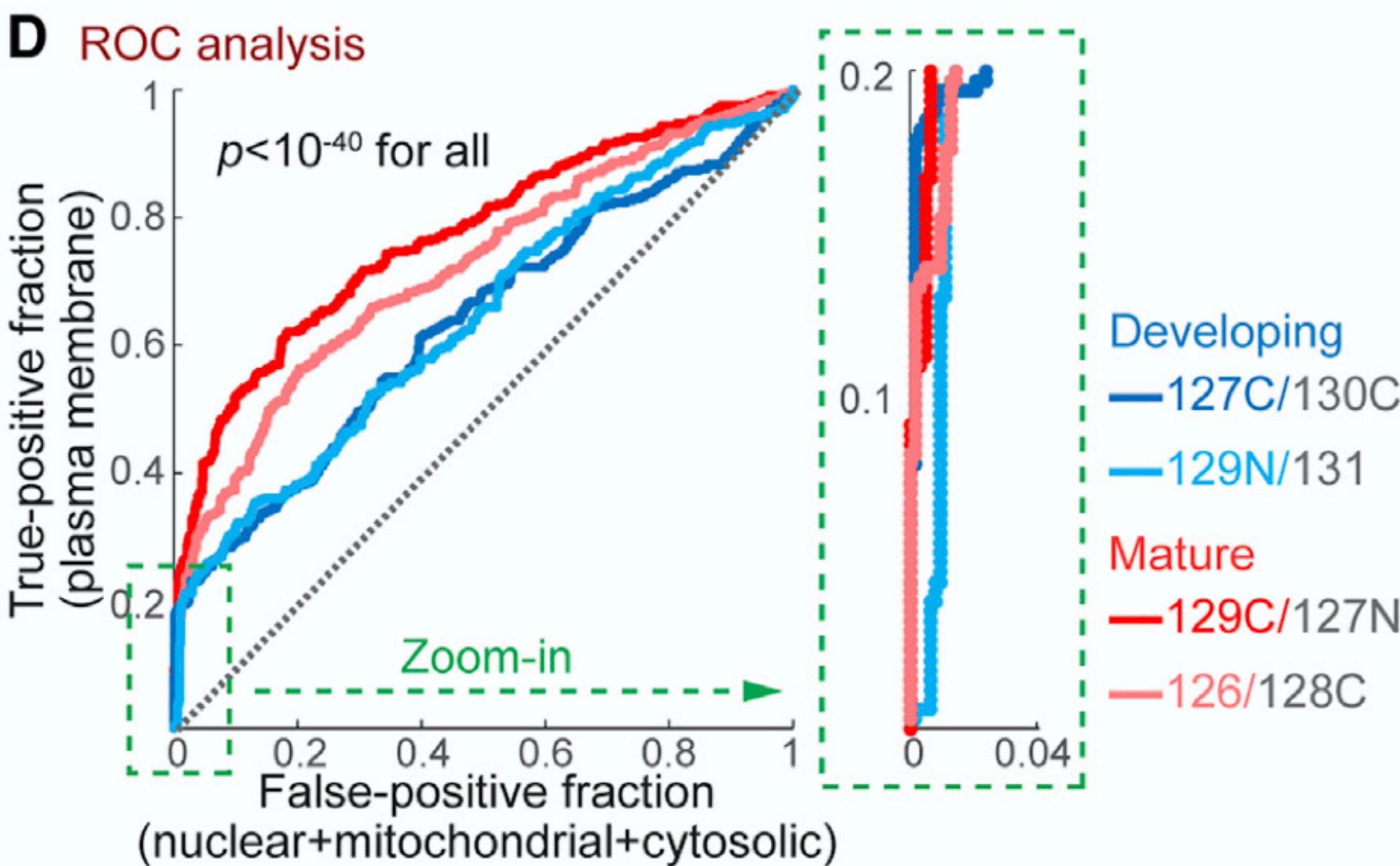
	TMT ratio	127C/130C	129N/131	129C/127N	126/128C
Proteins detected			2020 (with 2 or more unique peptides)		
Surface enriched		928	461	741	713
Intersection		403 (FDR 17.4%)		561 (FDR 13.4%)	

FDR=False Discovery Rate
UniProt Database

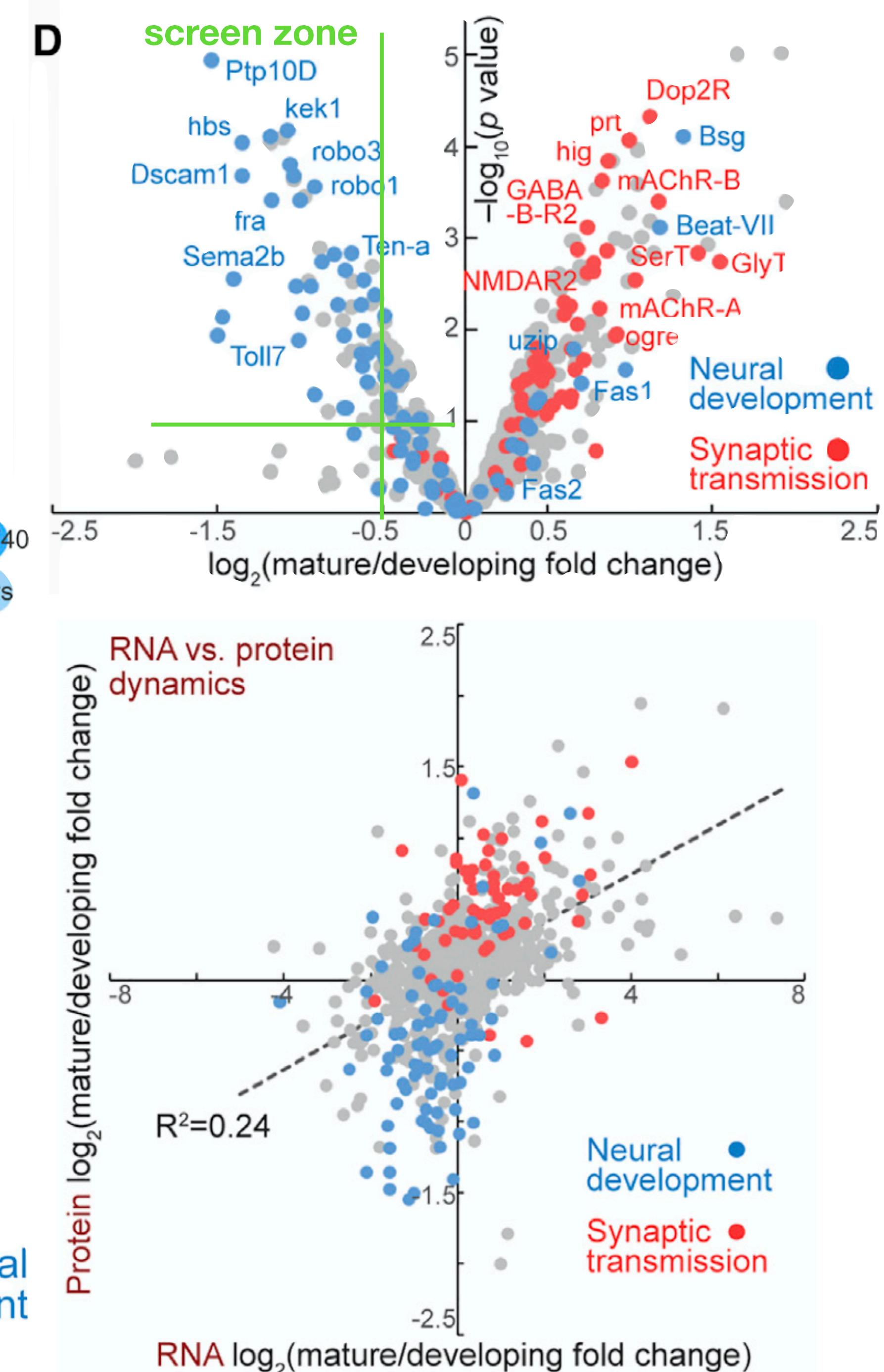
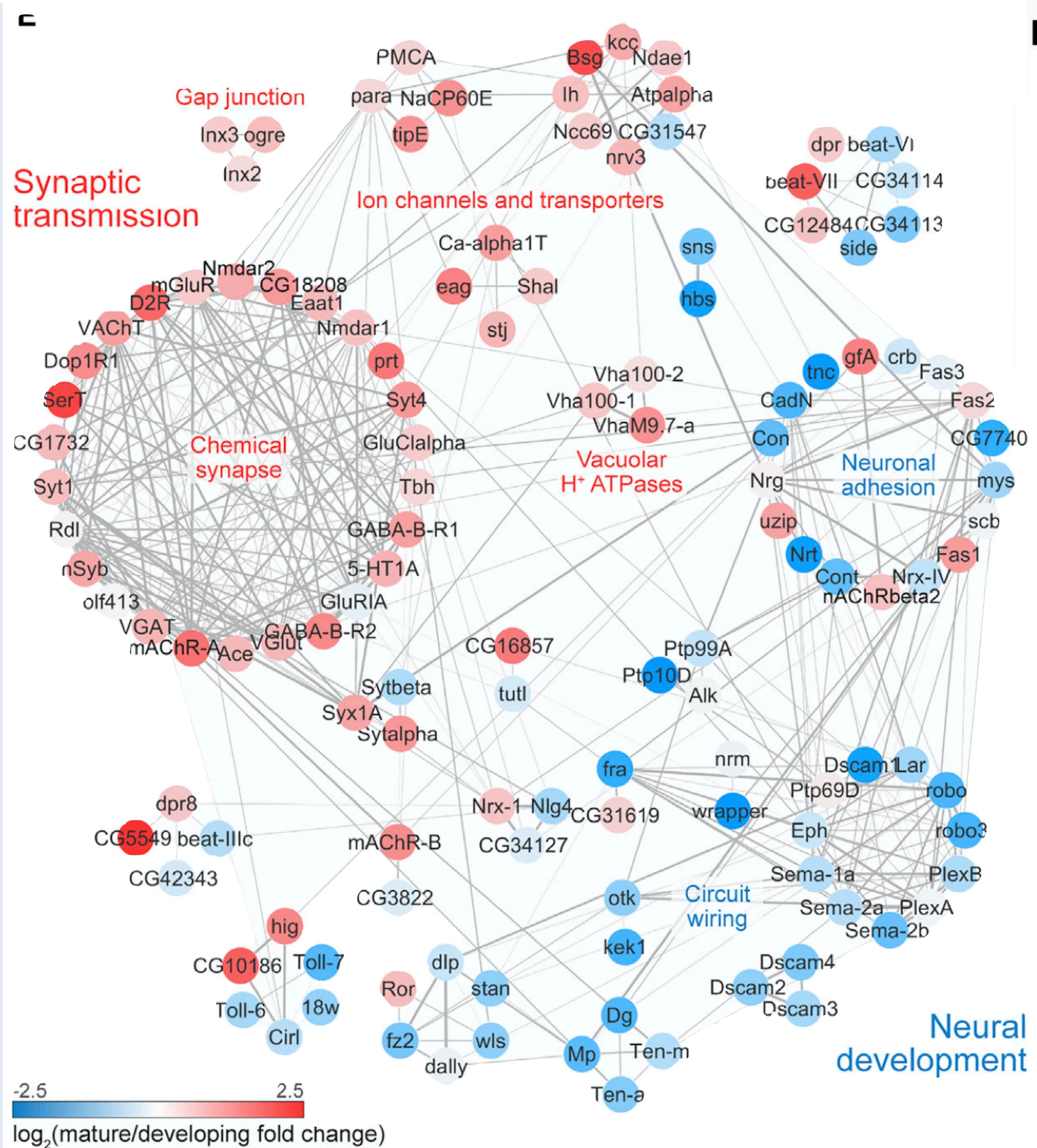
G Gene Ontology – Cellular Compartment



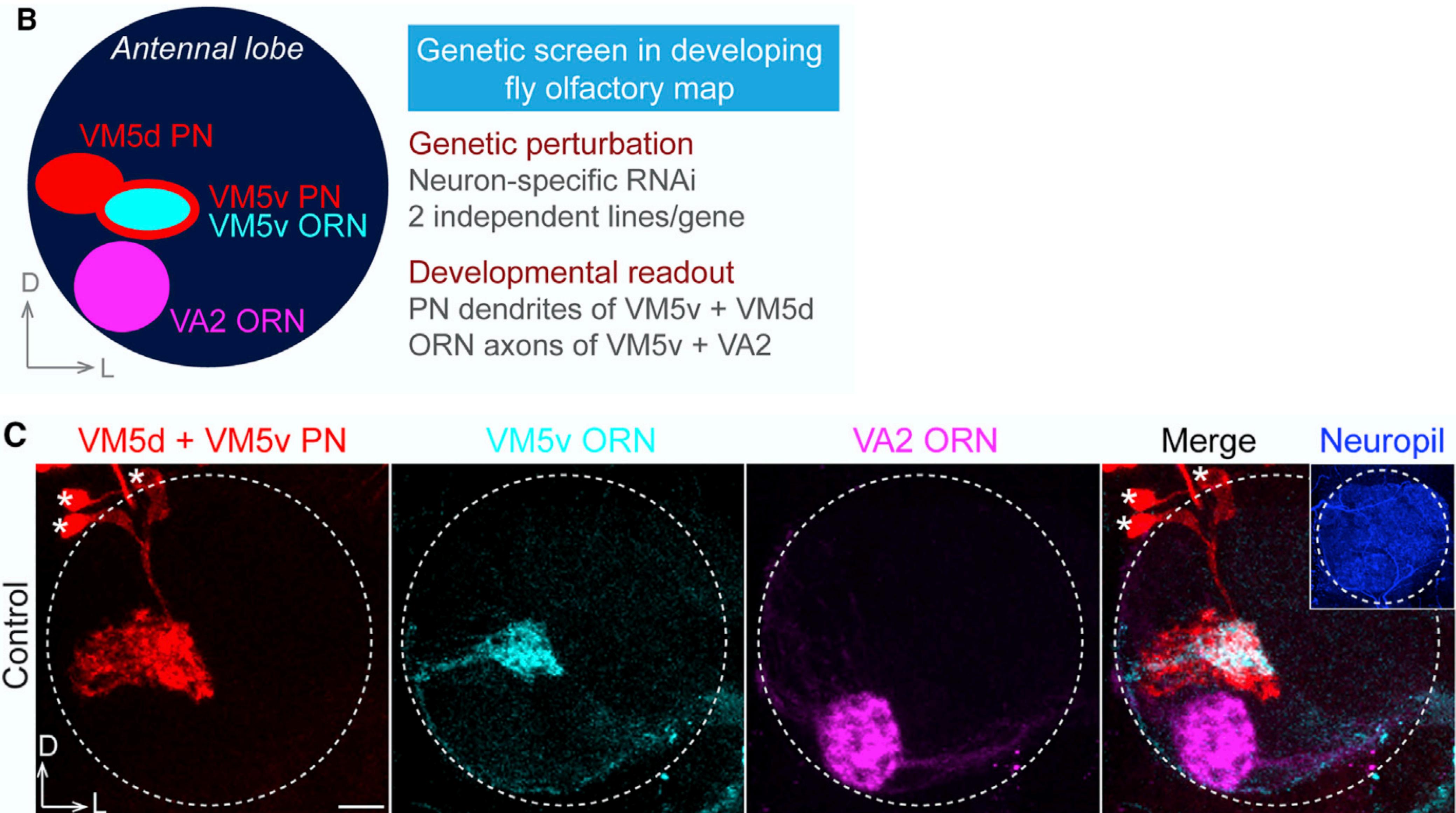
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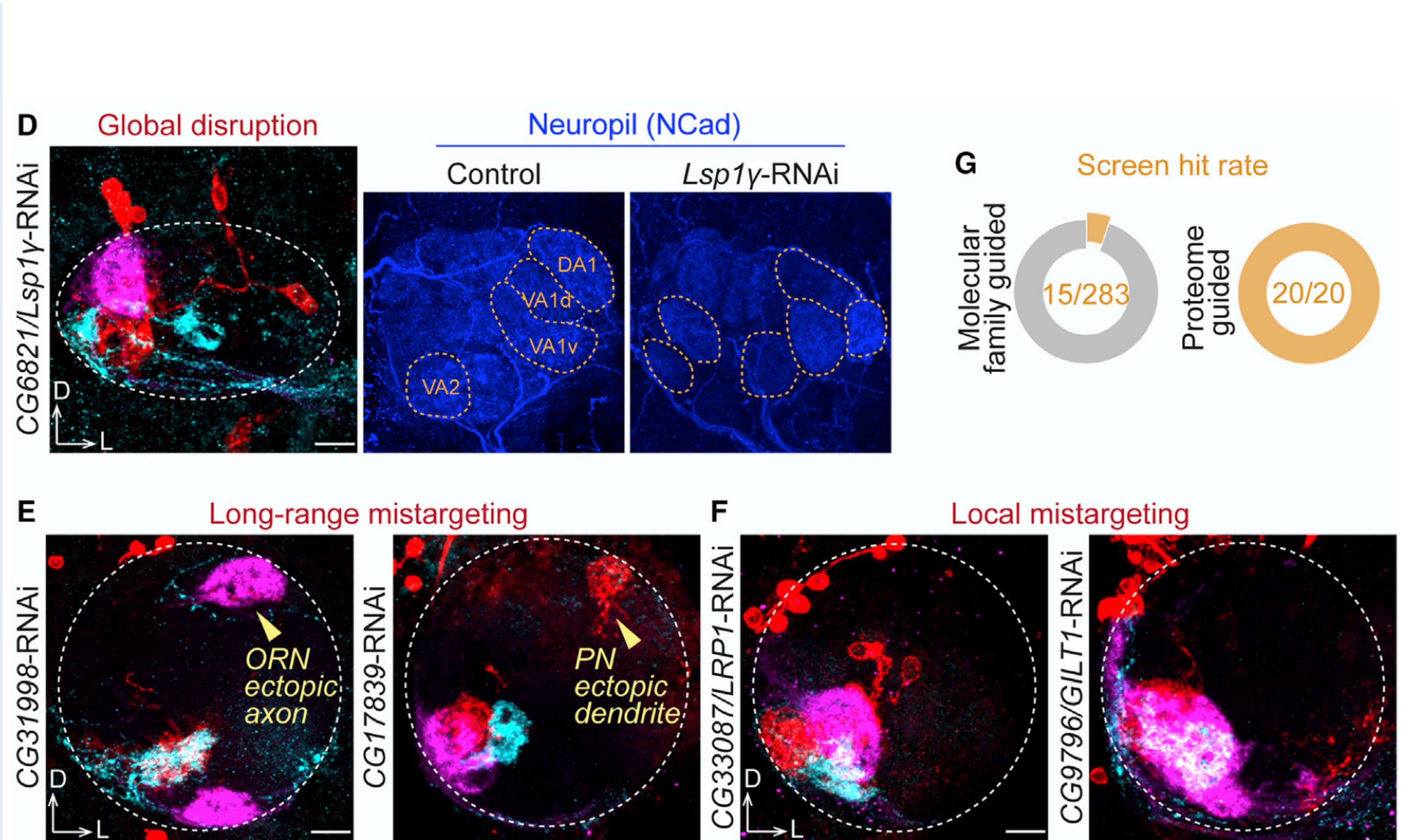
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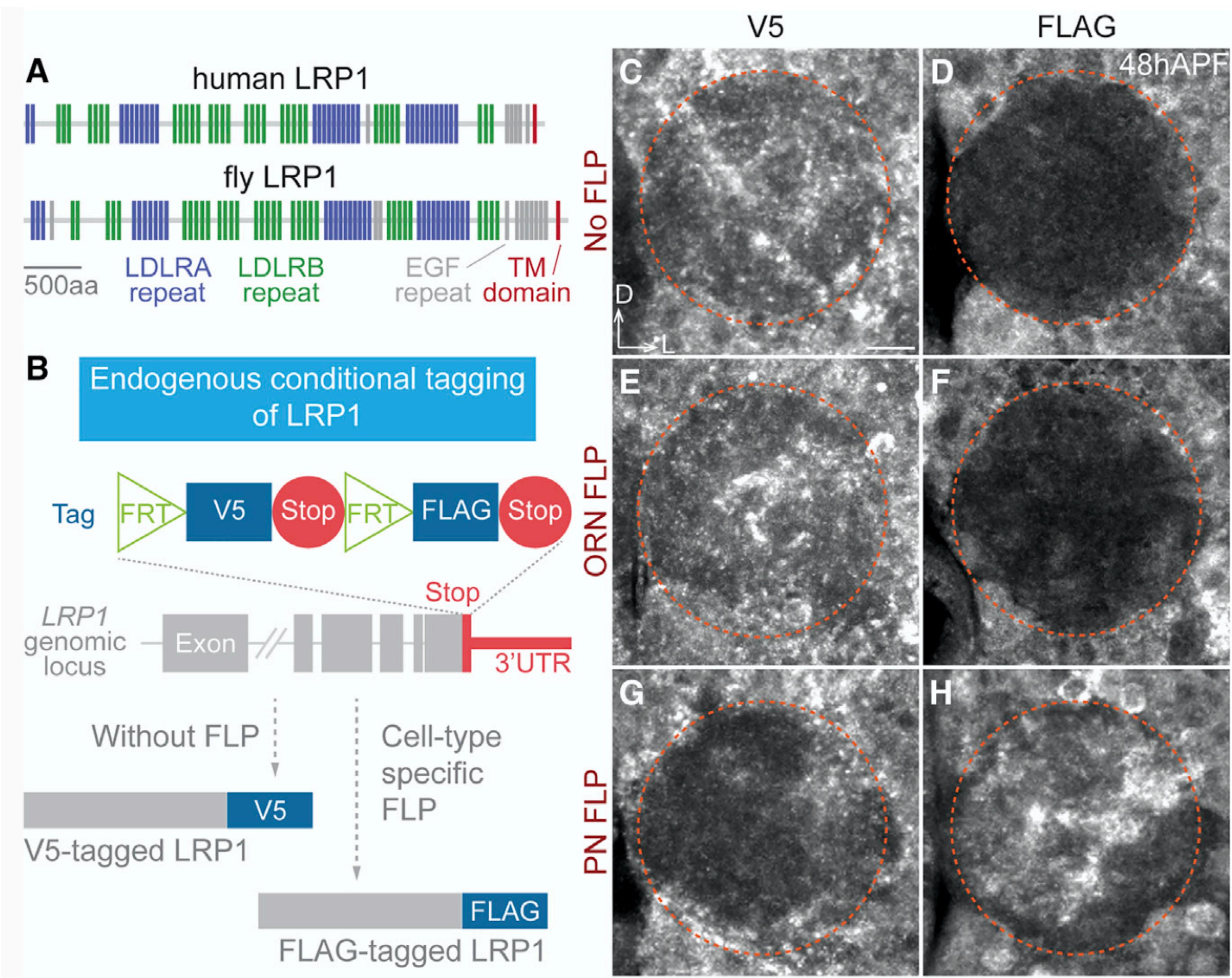
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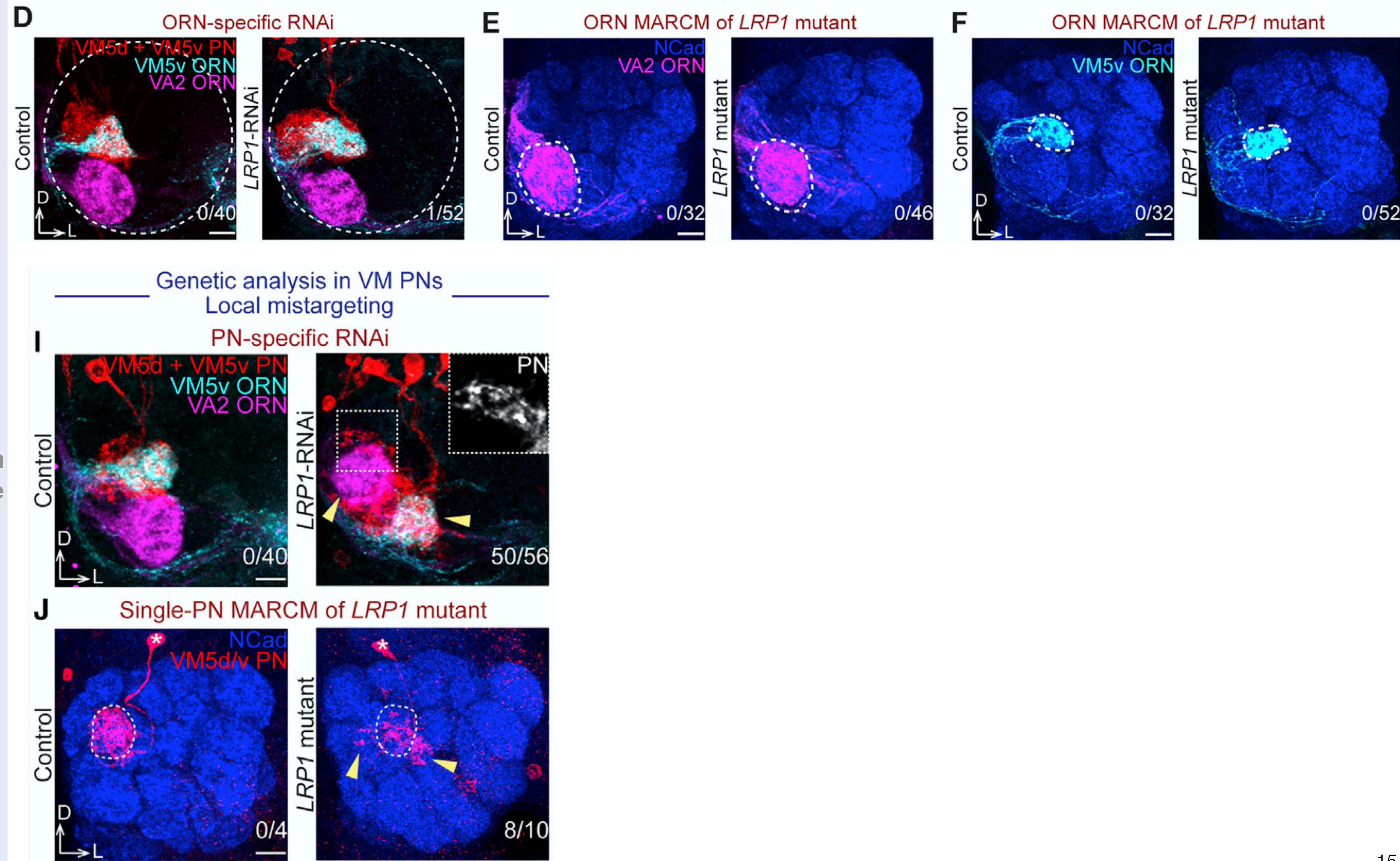


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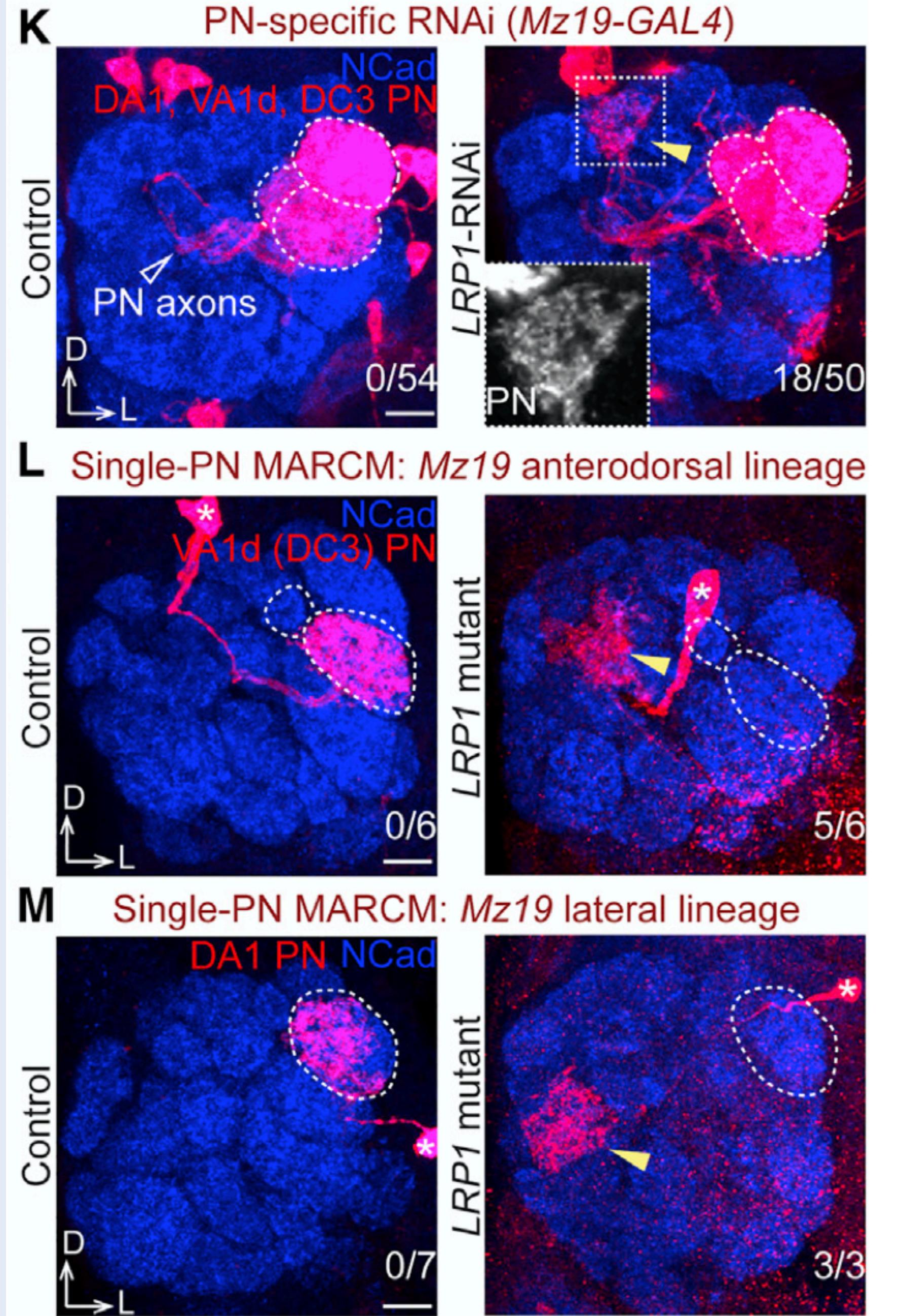
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Genetic analysis in ORNs
No wiring defects



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Genetic analysis in DL PNs
Long-range medial mistargeting



[Rhee H, Zou P, Udeshi ND, Martell JD, Mootha VK, Carr SA, Ting AY: Proteomic mapping of mitochondria in living cells via spatially restricted enzymatic tagging. Science 2013](#)

This paper establishes the use of an engineered ascorbate peroxidase, APEX, and its biotin-phenol substrate, for proteomic mapping of membrane-bound organelles. APEX is targeted to the mitochondria in HEK 293T cells to identify 495 proteins in the mitochondrial matrix with high specificity (>94%) and high coverage (>85%).

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[Loh KH, Stawski PS, Draycott AS, Udeshi ND, Lehrman EK, Wilton DK, Svinkina T, Deerinck TJ, Ellisman MH, Stevens B et al.: Proteomic analysis of unbounded cellular compartments: synaptic clefts. Cell 2016](#)

This paper used HRP to probe the composition of both the inhibitory and excitatory synaptic clefts in living neurons. The two proteomes discover dozens of novel synaptic candidates, which are validated by either fluorescence microscopy or western blotting. Many known synaptic proteins are also assigned to a specific cleft type in the dataset.