Proximity Labeling (PL): Optimizing and Applications

Journal Club

Yancheng Wu 2021.05.18

Molecular interactions

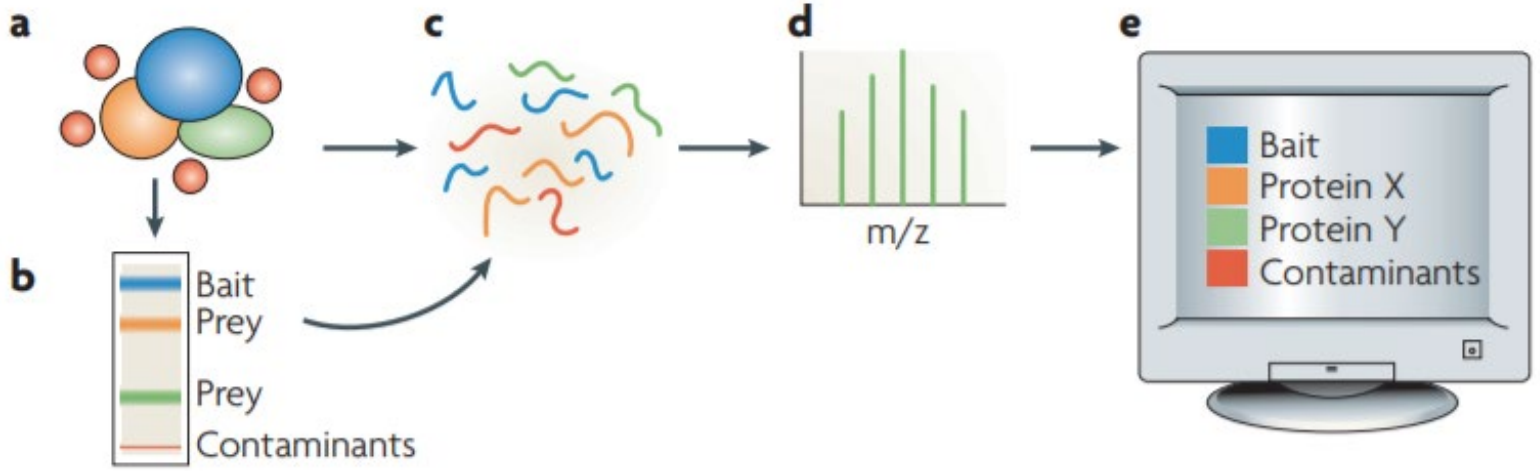
Protein–protein interactions (PPIs)
Signal transduction network (MAPK, Hippo, adrenergic GPCR), enzyme-substrate interaction (E3 ubiquitin ligases, kinase)

Protein–RNA interactions
Transcription and translation of cellular functions and stress response

Protein–DNA interactions
Regulation of gene expression, genome integrity and chromatin organization

Strategies for study molecular interactions

Affinity purification and mass spectrometry (AP-MS)

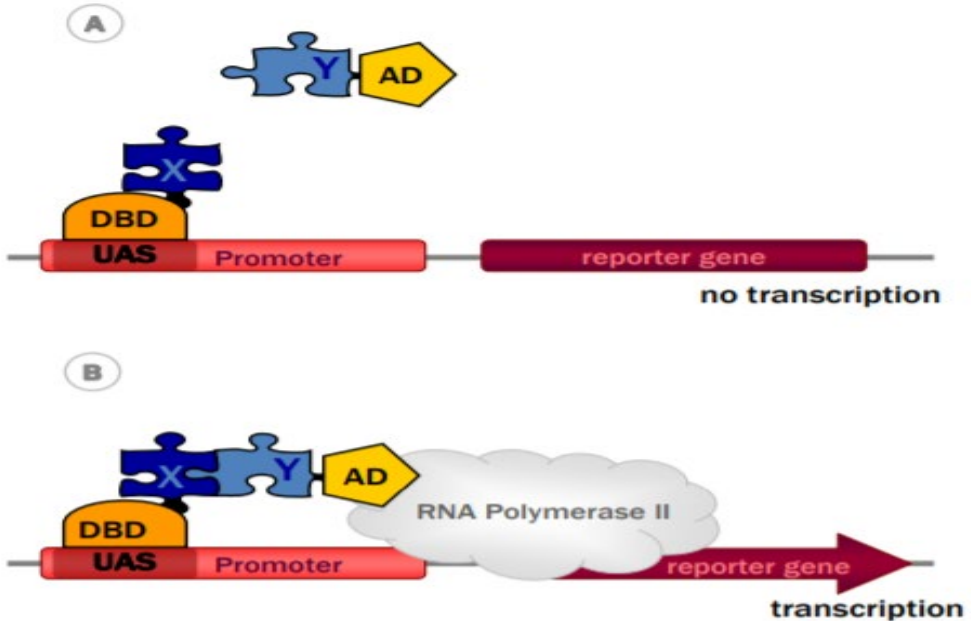


Combine sequencing

- Chromatin immunoprecipitation sequencing (ChIP-seq)
- RNA immunoprecipitation sequencing (RIP-seq)

Anne-Claude Gingras. et al. 2007.

Yeast two-hybrid



Anne Brückner. et al. 2009.

Limitations

Affinity purification

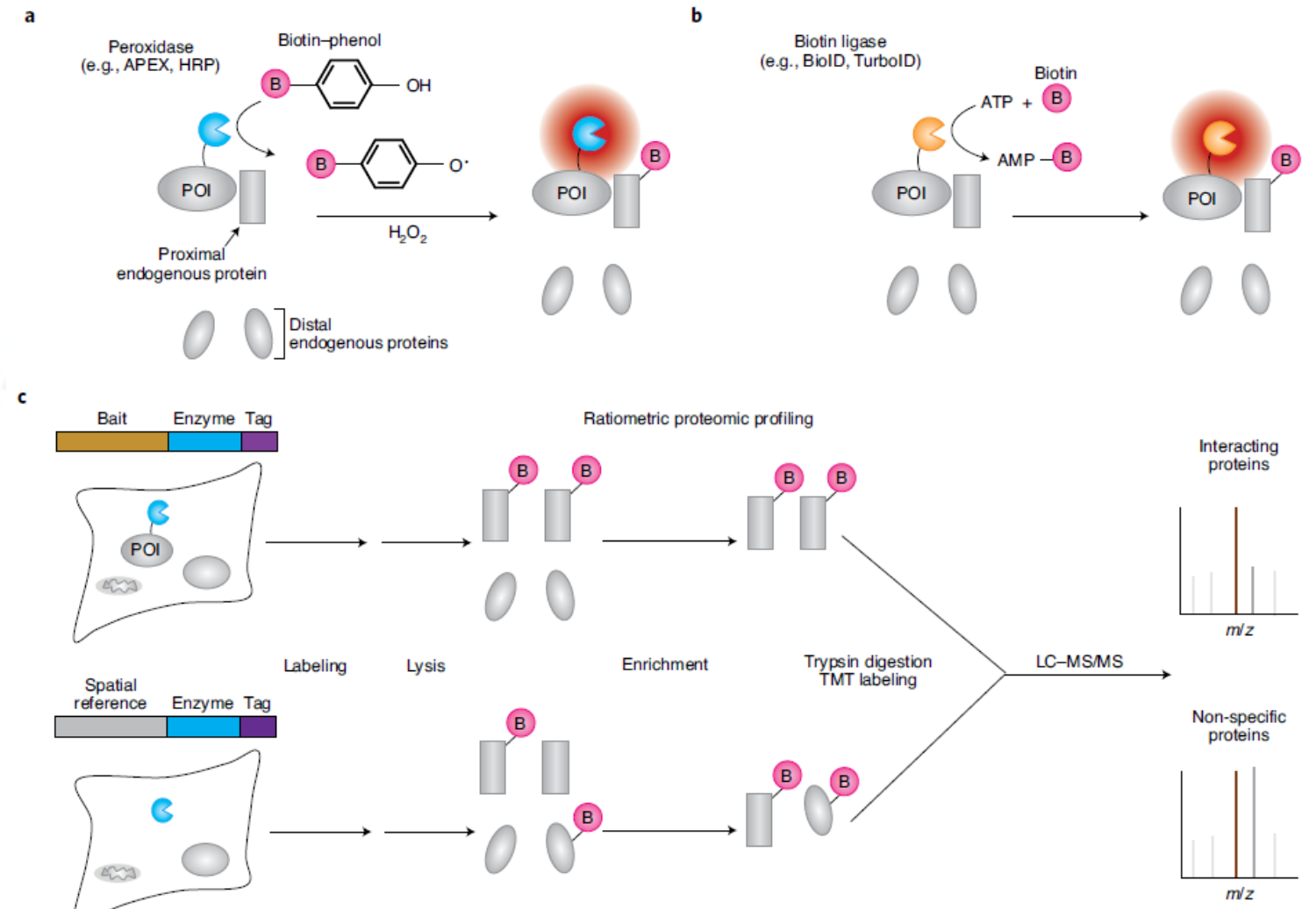
Cell lysis /washing steps lost Weak or transient interactions.
Combined with crosslinking, increases the rate of false positives.
x Insoluble targets or protein baits lacking high-affinity antibodies.

Yeast two-hybrid

Assays (prey protein) have cell type and organelle type restrictions
False positives from overexpression and tagging of both bait and prey
False negatives from steric interference by or geometric constraints of the required tags

PL as a complementary approach

Proximity labeling--PPI



Wei Qin. et al. 2021.

APEX: ascorbate peroxidase
HRP: horseradish peroxidase
TMT: tandem mass tag
LC-MS/MS: liquid chromatography and tandem mass spectrometry

Peroxidase- and biotin ligase-based proximity labeling methods for PPI mapping

Proximity labeling

Table 1 | Overview of PL enzymes

Enzyme	Type	Size (kDa)	Labeling time	Modification sites	Advantages	Limitations
APEX	Peroxidase	28	1 min	Tyr, Trp, Cys, His	High temporal resolution; versatility for both protein and RNA labeling	Limited application in vivo because of the toxicity of H_2O_2
APEX2	Peroxidase	28	1 min	Tyr, Trp, Cys, His	High temporal resolution; versatility for both protein and RNA labeling	Limited application in vivo because of the toxicity of H_2O_2
HRP	Peroxidase	44	1 min	Tyr, Trp, Cys, His	High temporal resolution; versatility for both protein and RNA labeling	Limited application in vivo because of the toxicity of H_2O_2; limited to secretory pathway and extracellular applications
BioID	Biotin ligase	35	18 h	Lys	Non-toxic for in vivo applications	Poor temporal resolution as a result of low catalytic activity
BioID2	Biotin ligase	27	18 h	Lys	Non-toxic for in vivo applications	Poor temporal resolution as a result of low catalytic activity
BASU	Biotin ligase	29	18 h	Lys	Non-toxic for in vivo applications	Poor temporal resolution as a result of low catalytic activity
TurboID	Biotin ligase	35	10 min	Lys	Highest activity biotin ligase; non-toxic for in vivo applications	Potentially less control of labeling window as a result of high biotin affinity
miniTurbo	Biotin ligase	28	10 min	Lys	High activity; non-toxic for in vivo applications; smaller than TurboID	Lower catalytic activity and stability as compared to TurboID

Wei Qin. et al. 2021.

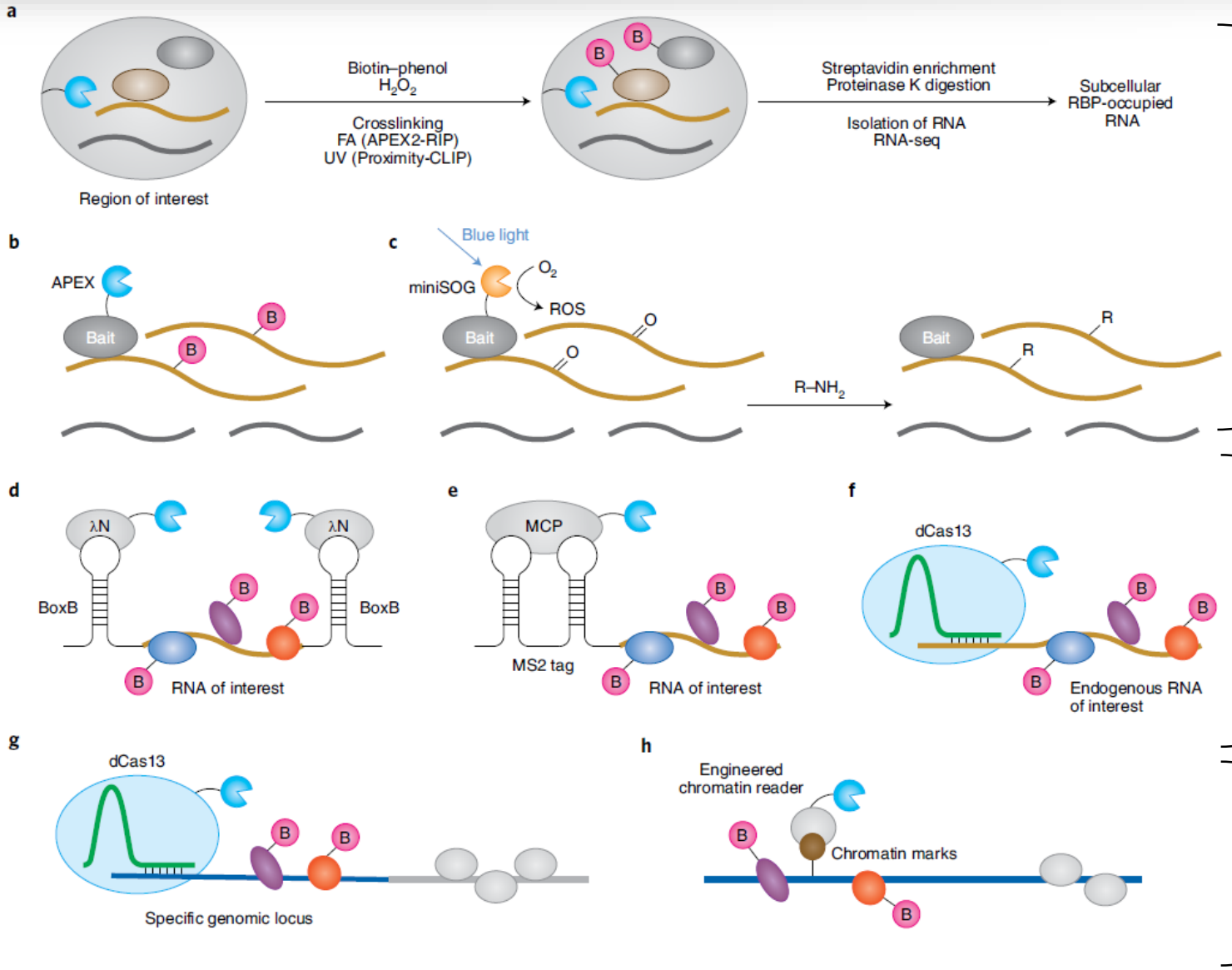
Proximity labeling

Table 2 | Examples of proximity labeling for mapping PPIs

PPI category	Notes	Enzyme	Baits	Refs.
Protein aggregates	Insoluble complexes by definition	BioID	TDP43 aggregates	61
Nuclear membrane and nuclear structures	Low-solubility complexes as a result of membrane function and/or complex size	BioID	Lamin A	11
		BioID	Lamin B1	59
		BioID	Various nuclear transport receptors	60
		BioID2	Lamin A, Sun2	58
Enzyme–substrate interactions	Low-affinity or transient interactions as a result of enzyme turnover	BioID	Hippo pathway (including Mst1/Mst2 kinases)	36
		BioID	p190/p210 BCR-ABL kinases	42
		APEX2	p38 MAPK	34
		BioID2	p38 MAPK	35
		BioID	SCF E3 ligases	40
		APEX2	KREP, Kelch E3 ligase adaptors	41
		BioID	ClpP protease	124
Other signaling pathways	Low-affinity or transient interactions	BioID2	TLR9, MYD88 (NF-κB pathway)	62
		BioID2	KRas4B	63
		APEX2	$Ca_v1.2$ GPCR (adrenergic pathway)	37
Intracellular sorting	Transient interactions, low-affinity interactors for trafficking machinery	BioID	Golgin-97, Golgin-245	125
		APEX2	LAMP1	45
		BioID2	Golgi glycosyltransferases	126
Dynamic processes	APEX for minute-scale interactome capture	APEX2	DOR (GPCR)	39
		APEX2	AT1R, β2AR (GPCRs)	38
		APEX2	Fzd9b (GPCR)	70
		APEX2	Gal8, Gal3, Gal9	127
		APEX2	TssA (bacteria)	47
In vivo PL in plants	PL in plant systems	BioID	HopF2	52
		BioID	AvrPto	77
		TurboID	N NLR	78
		TurboID	FAMA	53
In vivo PL in other organisms	Biotin ligase–based in vivo PL	BioID	Sun1 (*Dictyostelium*)	79
		BioID	CDK5RAP2 (*Dictyostelium*)	80
		BioID	ISP3 (*Toxoplasma gondii*)	82
		BioID	Cyst wall proteins (*T. gondii*)	85

Wei Qin. et al. 2021.

Proximity labeling—Protein nucleic acid interaction



Protein centric

RNA centric

DNA centric

FA: formaldehyde
UV: ultraviolet
RBP: RNA binding protein

Wei Qin. et al. 2021.

Proximity labeling-Paper 1

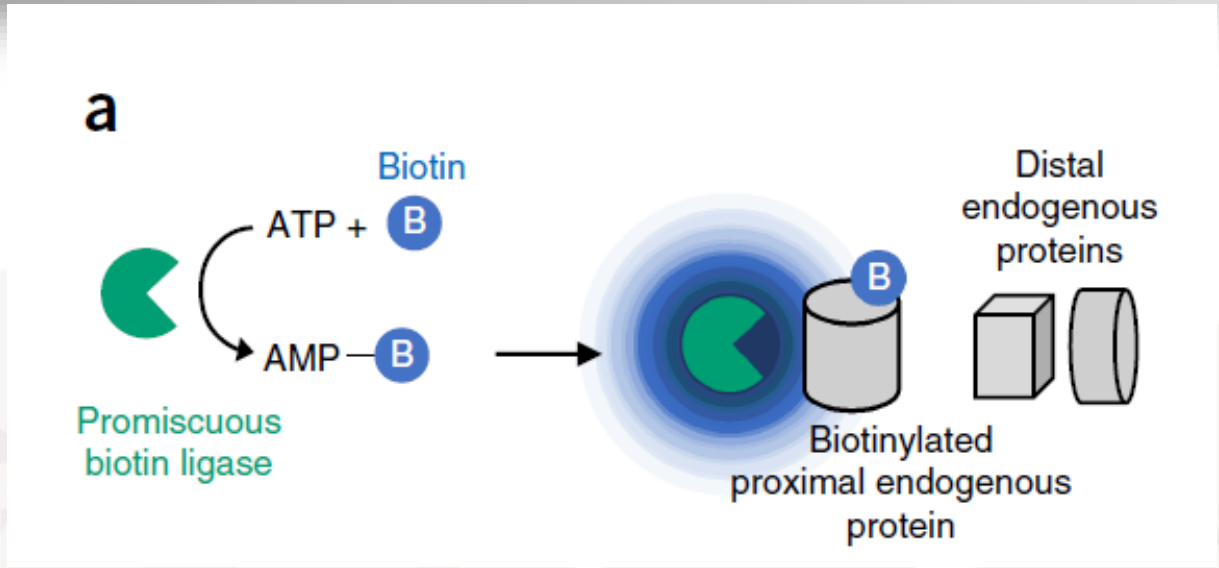
LETTERS

nature biotechnology

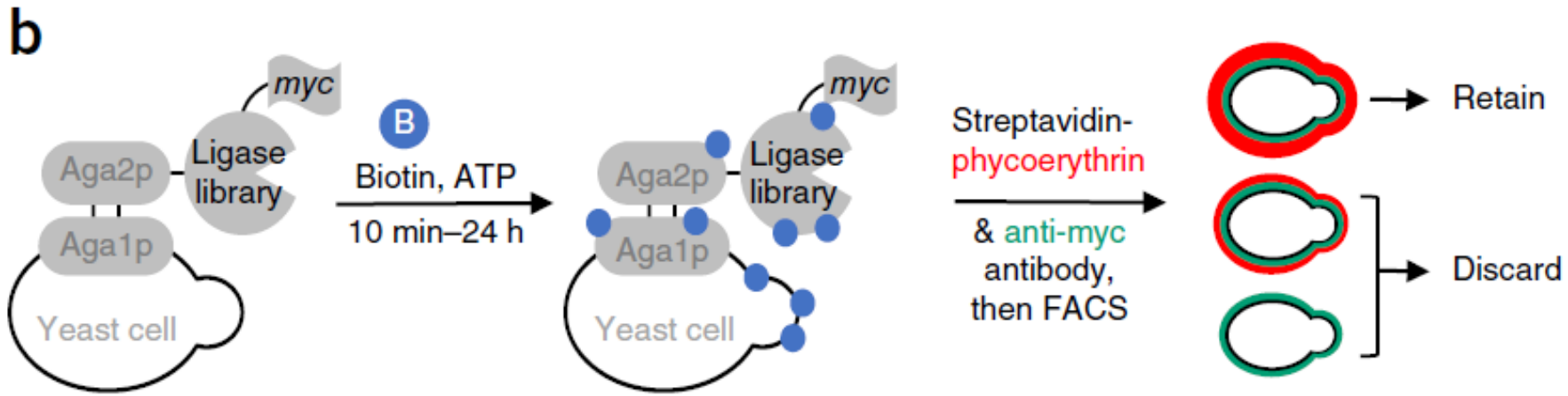
Efficient proximity labeling in living cells and organisms with TurboID

Tess C Branon[1–4], Justin A Bosch[5], Ariana D Sanchez[4], Namrata D Udeshi[6], Tanya Svinkina[6], Steven A Carr[6], Jessica L Feldman[4], Norbert Perrimon[5,7] & Alice Y Ting[1–4,8]

Evolution of TurboID

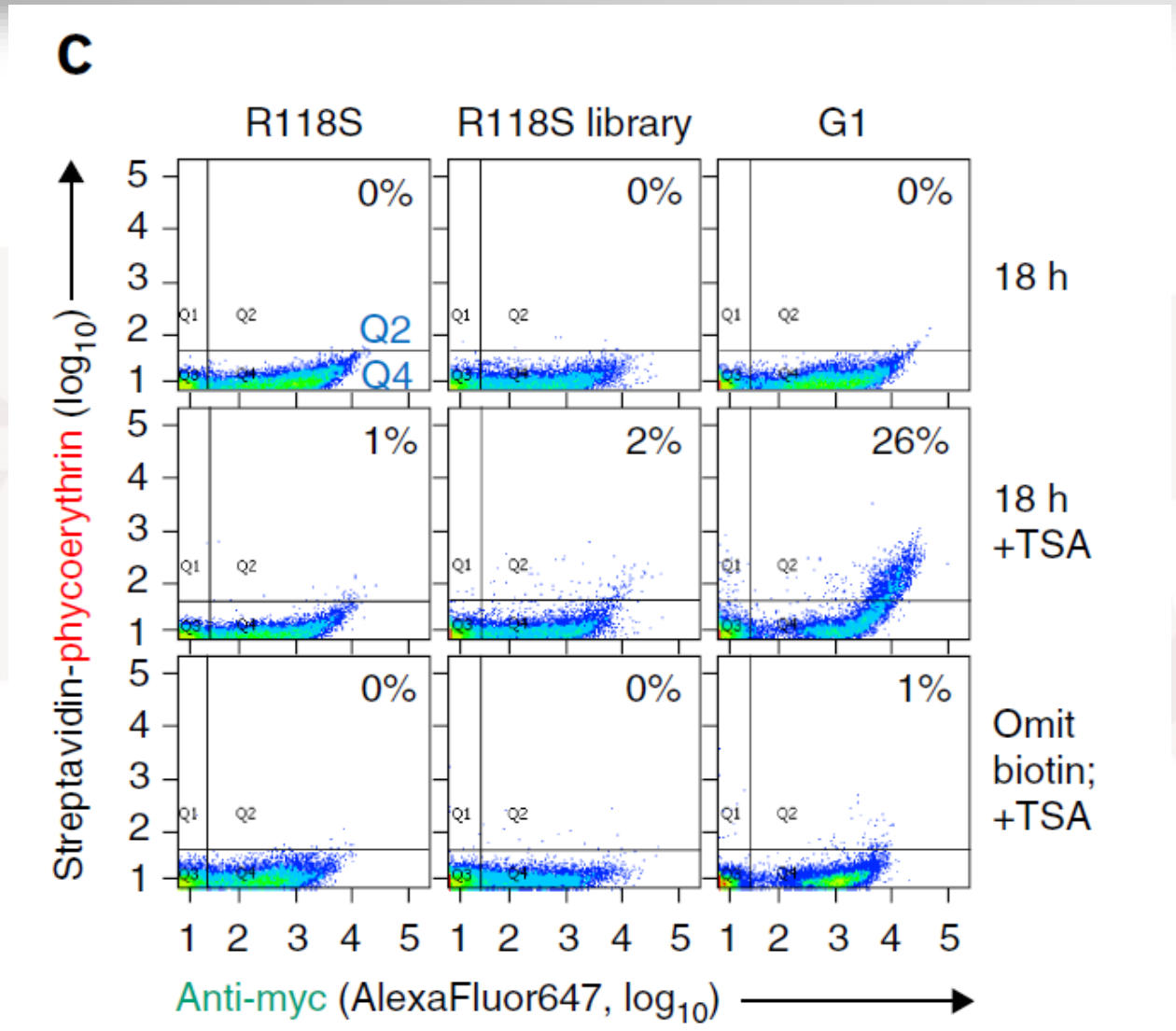


Proximity-dependent biotinylation catalyzed by promiscuous biotin ligases



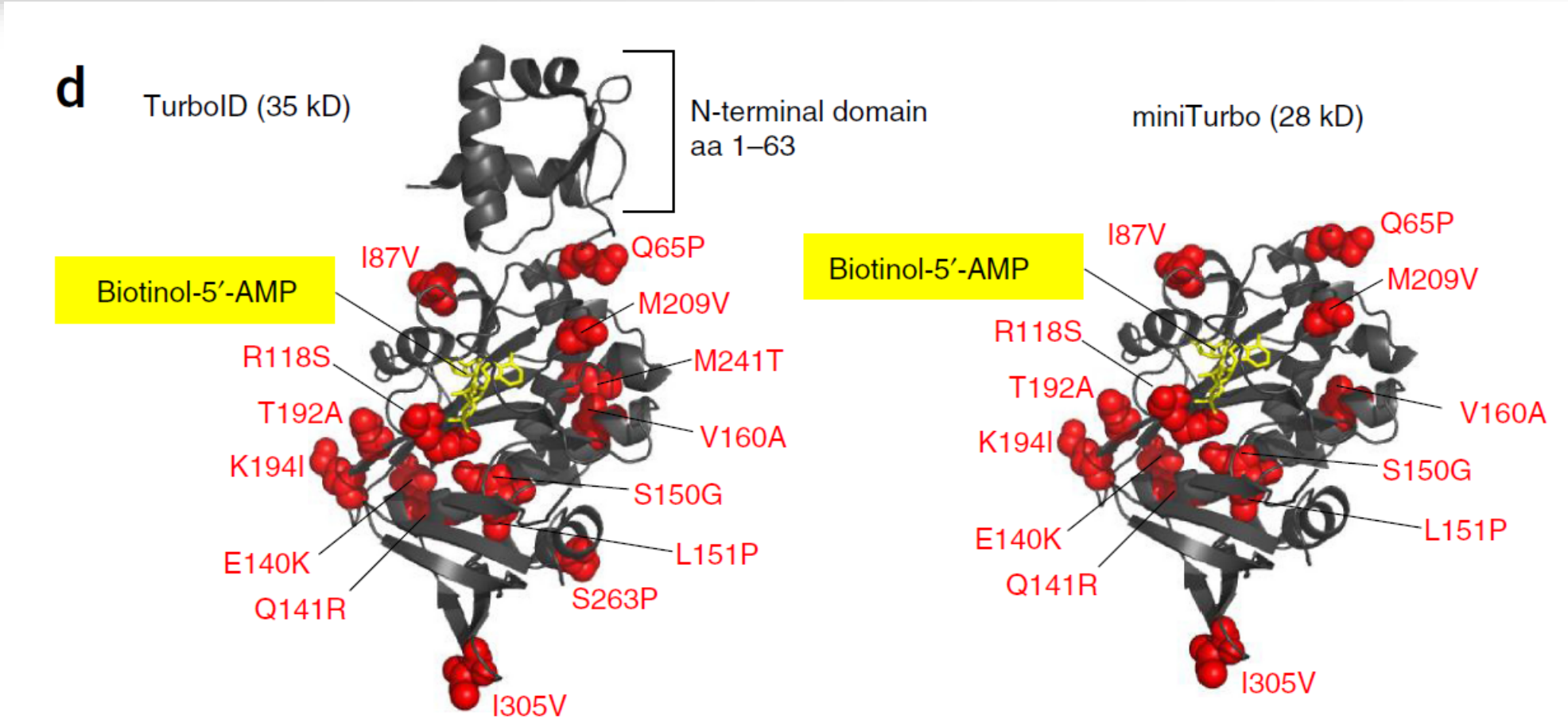
Yeast-display-based selection scheme

Evolution of TurboID

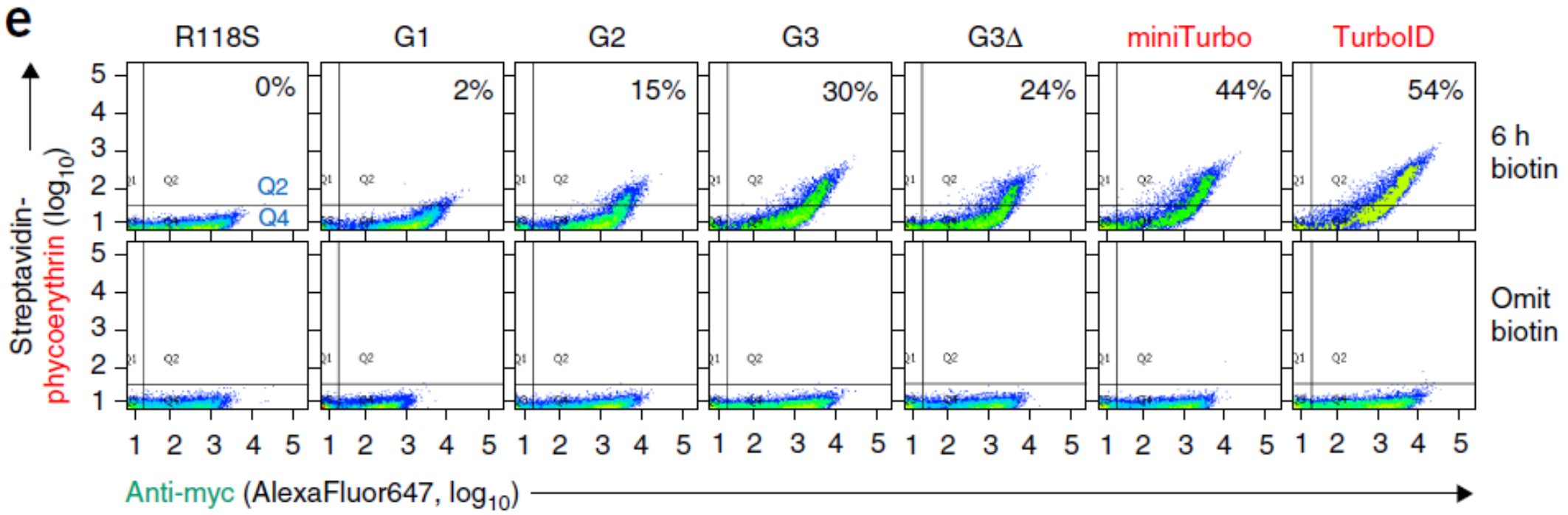


G1: winning clone from the first mutant generation TSA: tyramide signal amplification

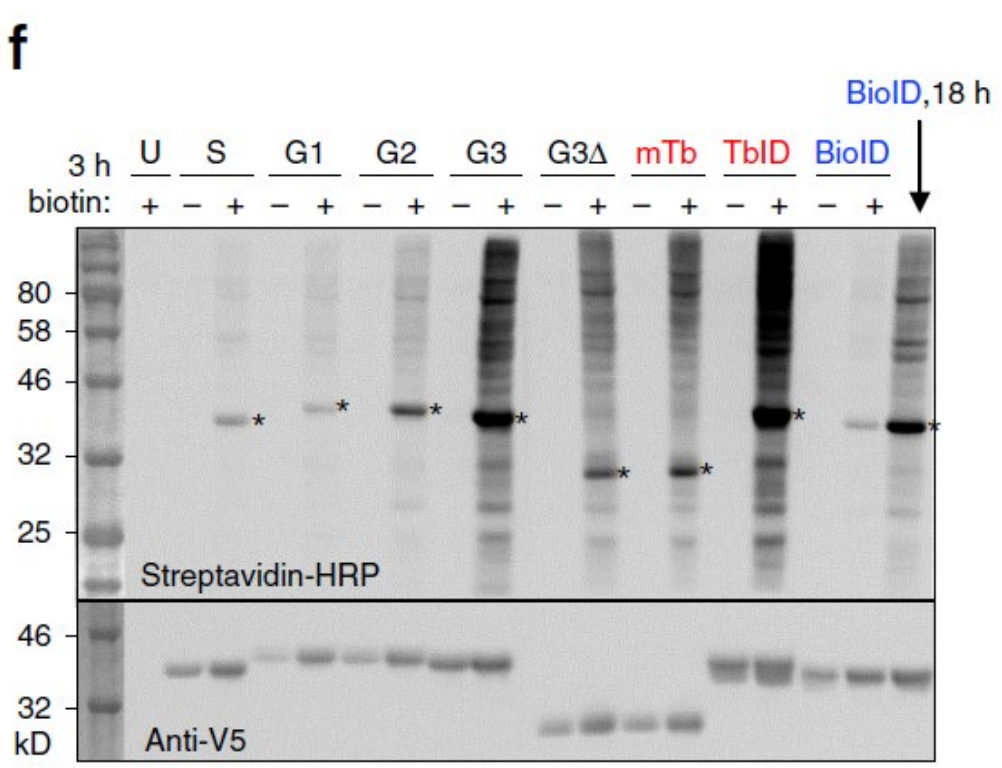
Evolution of TurboID

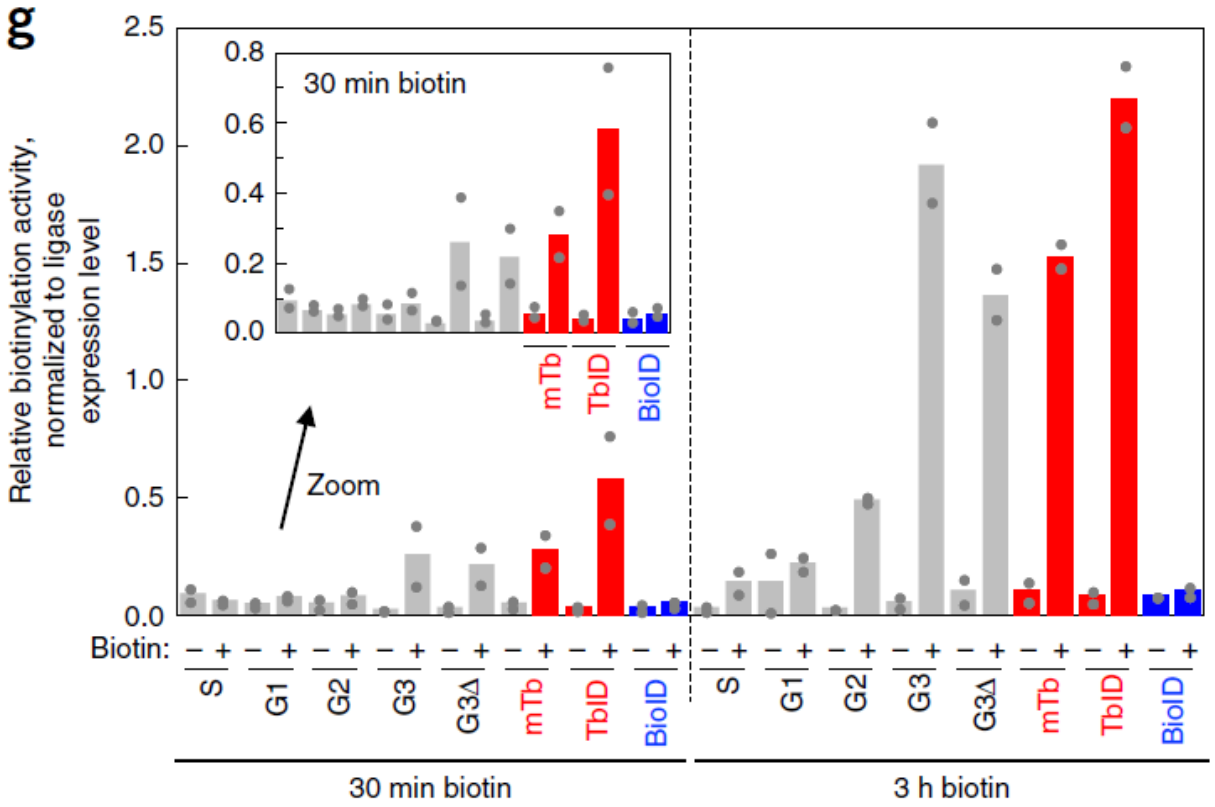


Evolution of TurboID



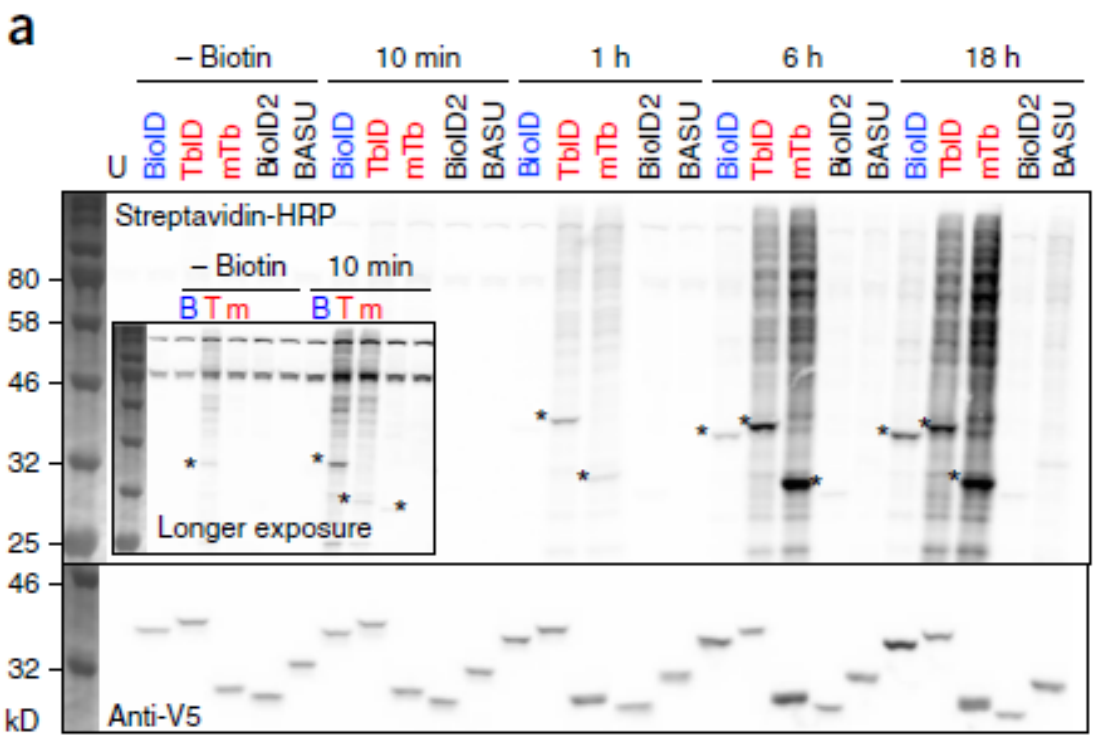
Evolution of TurboID

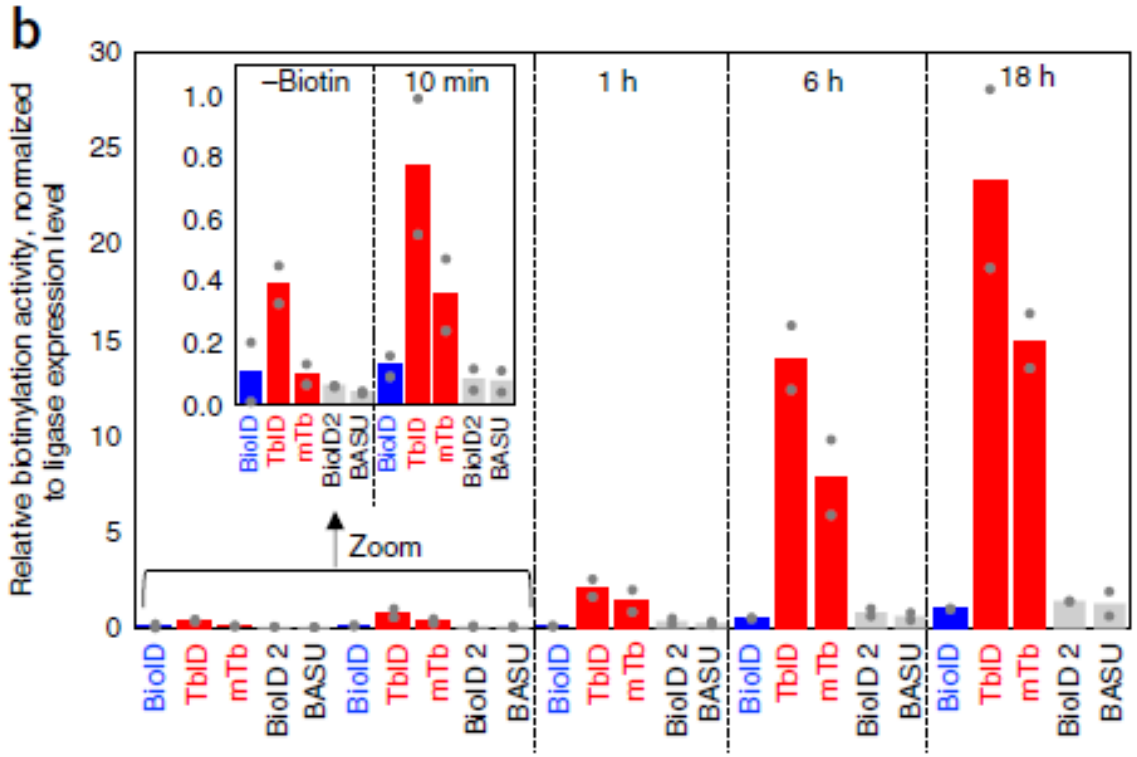




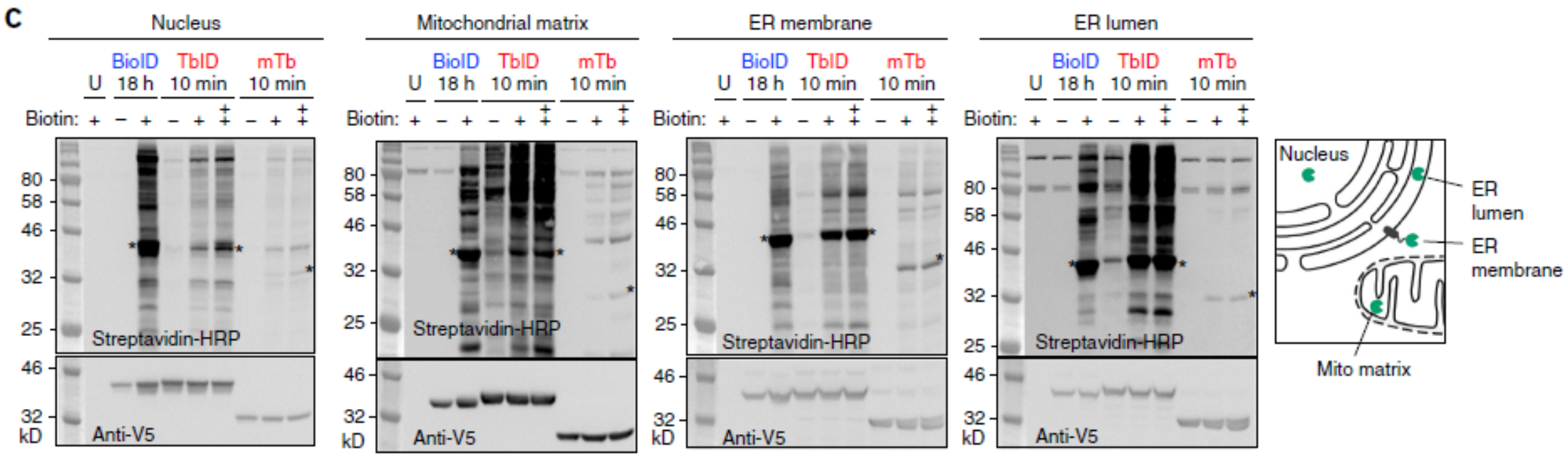
U: untransfected S: BirA-R118S

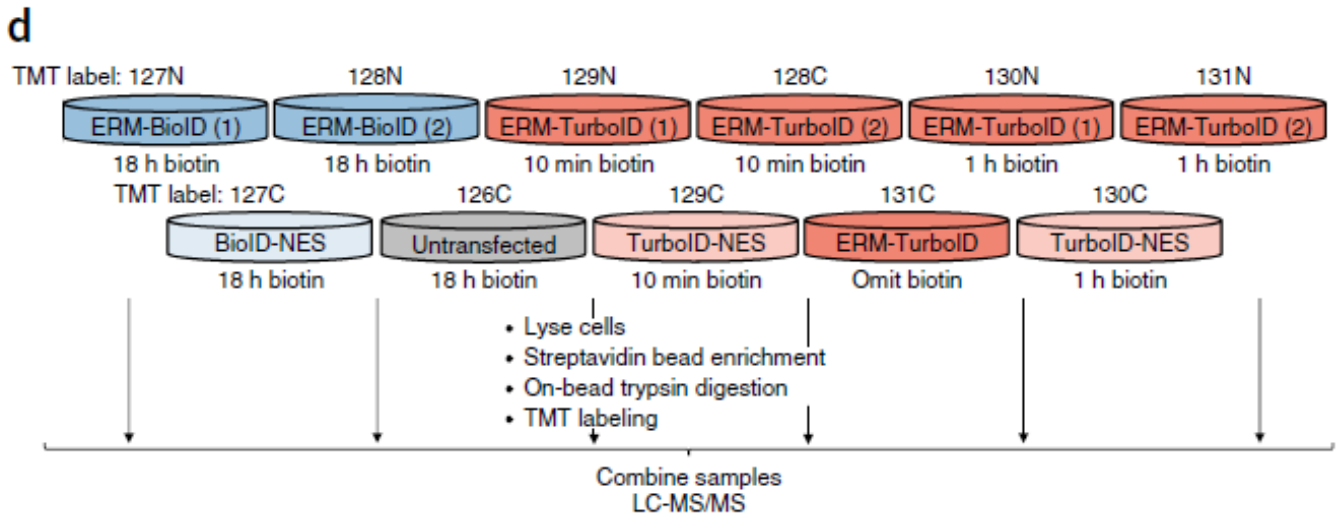
Characterization of TurboID and miniTurbo in mammalian cells

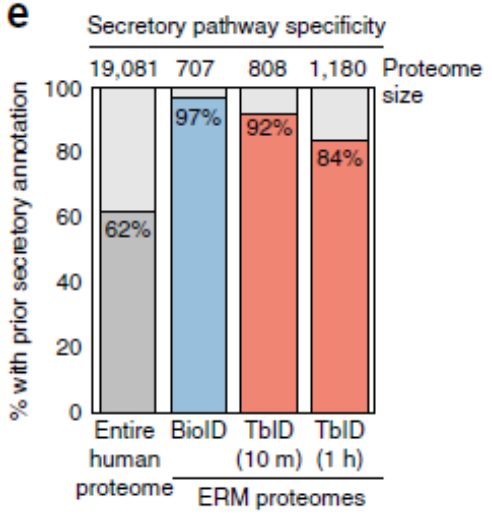




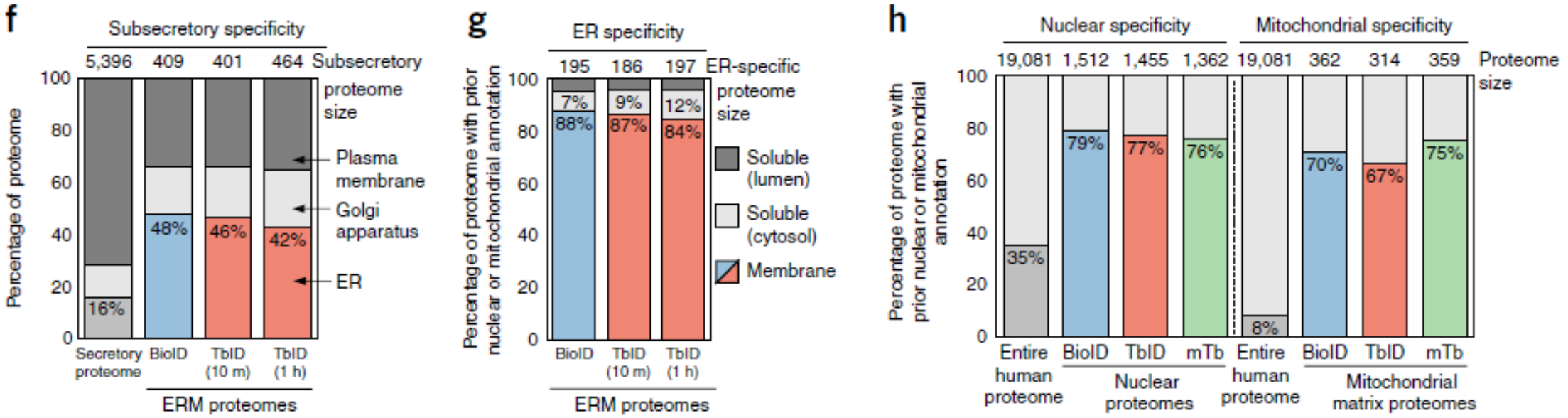
Characterization of TurboID and miniTurbo in mammalian cells



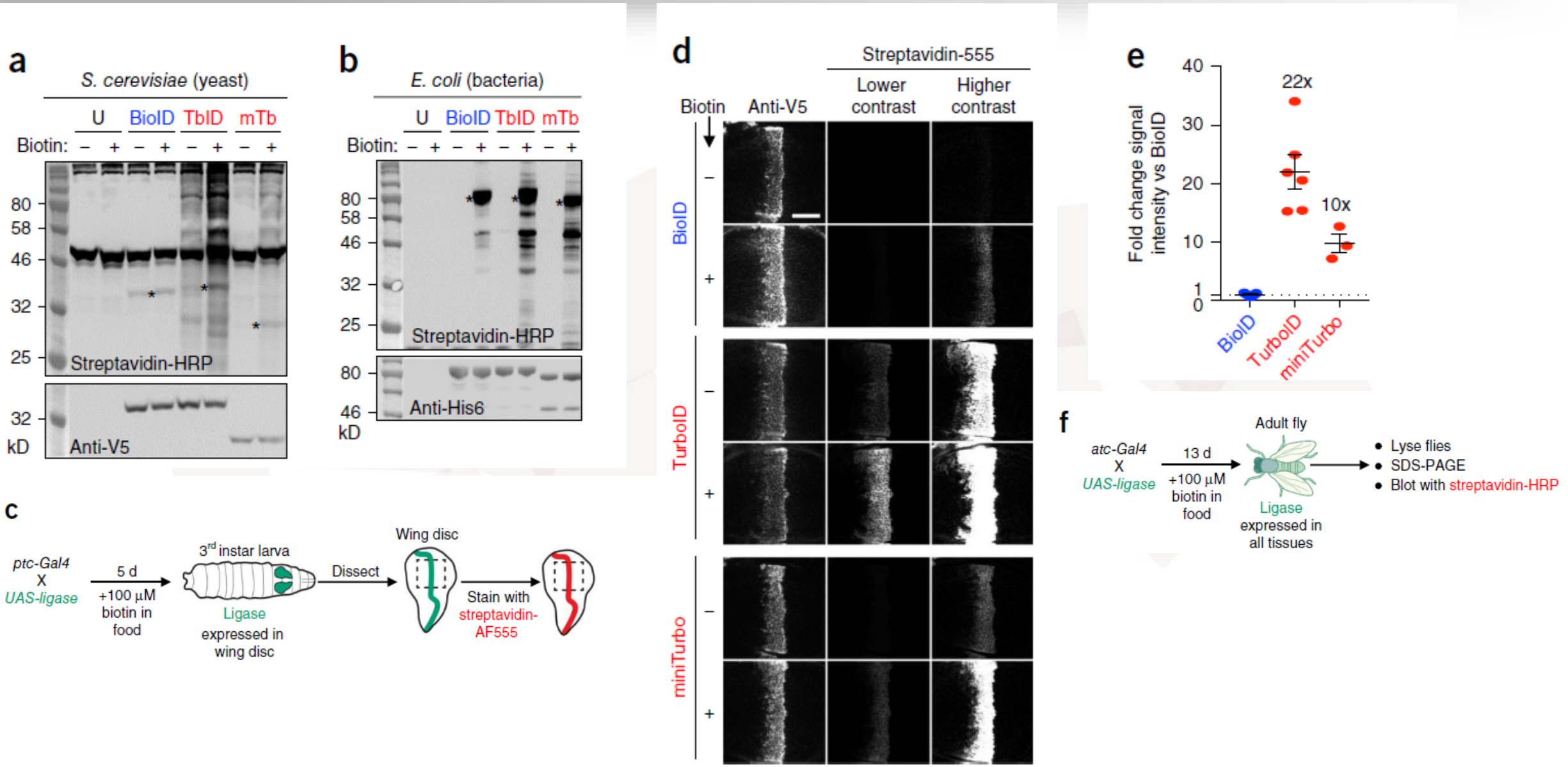




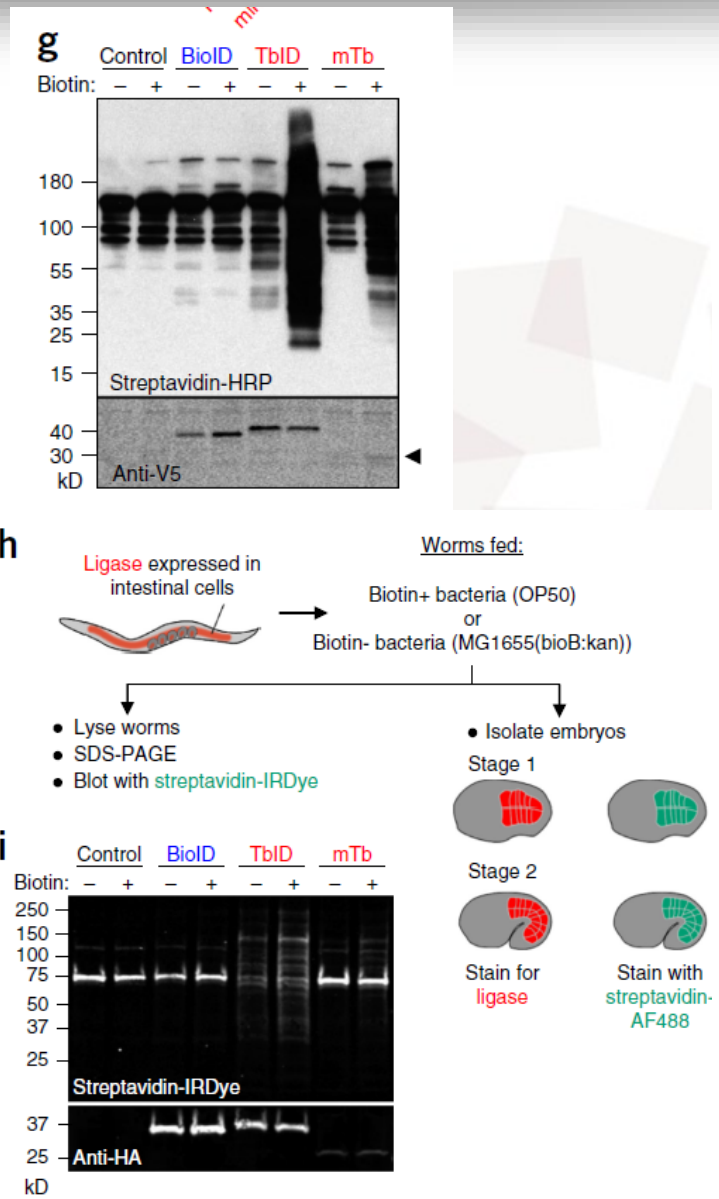
Characterization of TurboID and miniTurbo in mammalian cells



TurboID and miniTurbo in flies, worms and other species



TurboID and miniTurbo in flies, worms and other species



Summary

1. Yeast-display-based directed evolution, generated two new ligases for PL applications: TurboID and miniTurbo.

2. TurboID is the most active, and should be used when the priority is to maximize biotinylation yield and sensitivity and/or recovery.

3. TurboID –PL: small degree of labeling before exogenous biotin is supplied.
   MiniTurboID-PL: If the priority is to precisely define the labeling time window.

4. miniTurbo is less stable than TurboID (likely due to removal of its N-terminal domain), resulting in lower expression levels

Proximity labeling—Paper2

Cell

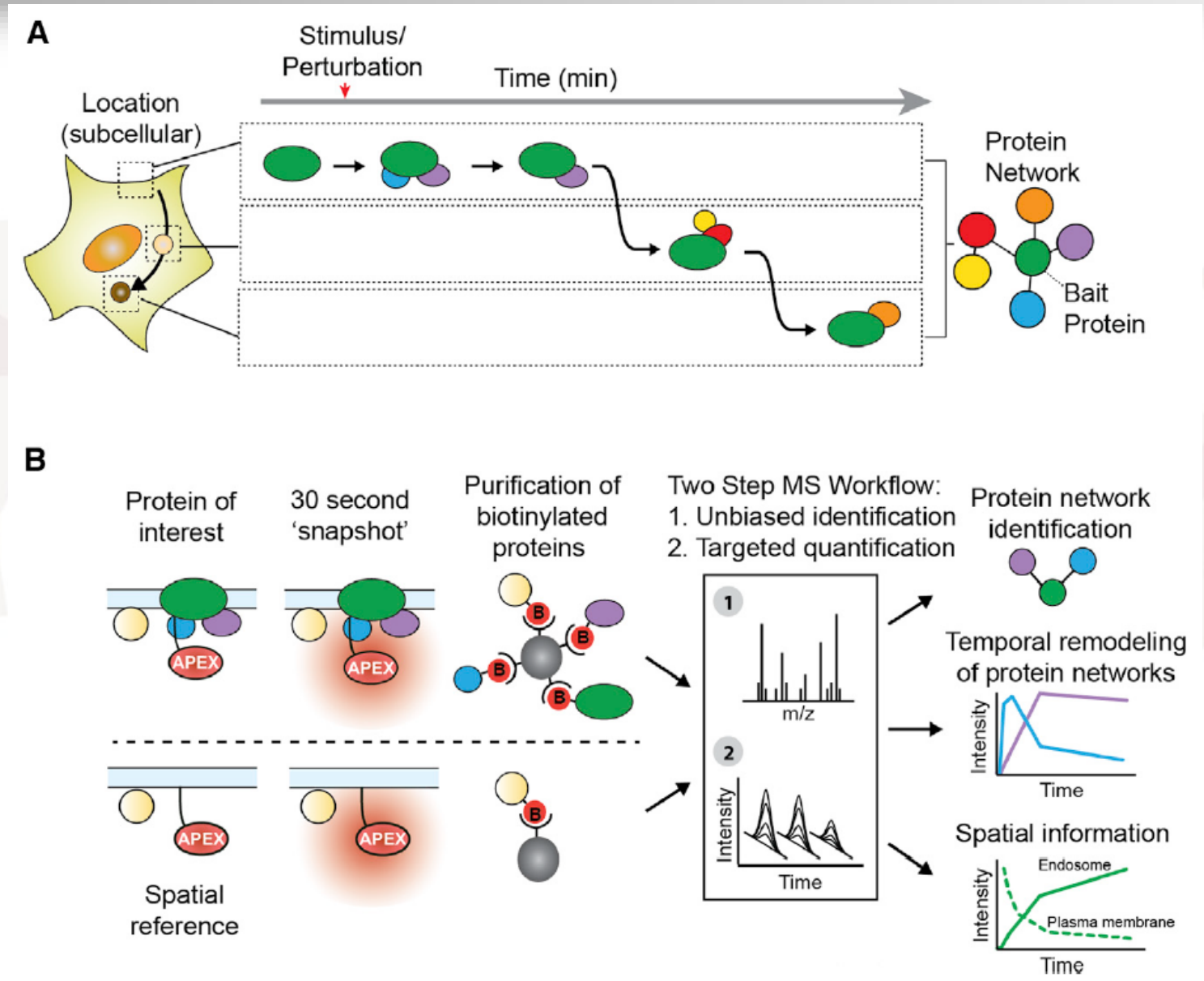
Volume 169, Issue 2, 6 April 2017, Pages 350-360.e12

Resource

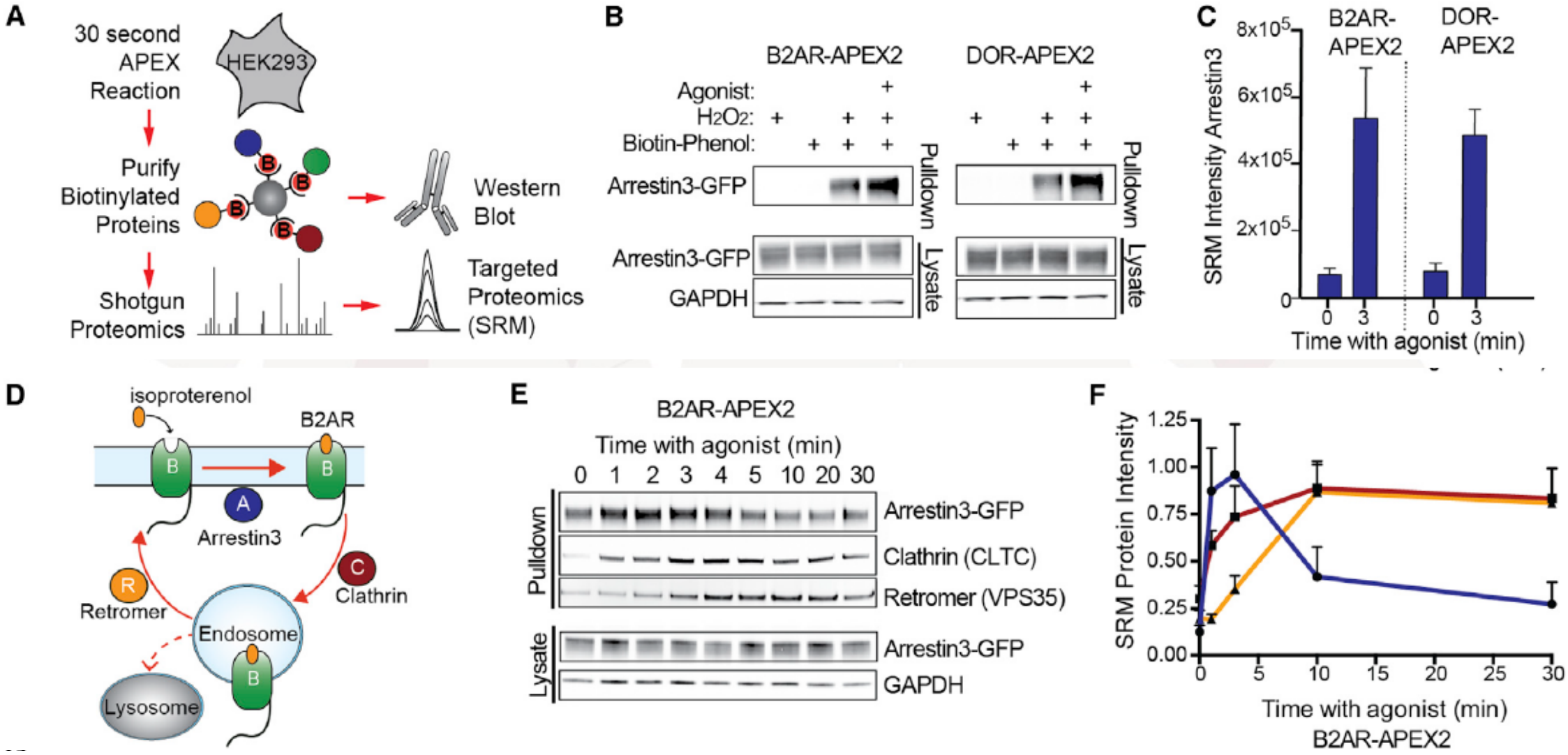
An Approach to Spatiotemporally Resolve Protein Interaction Networks in Living Cells

Braden T. Lobingier [1, 8], Ruth Hüttenhain [2, 3, 4, 8], Kelsie Eichel [5], Kenneth B. Miller [6], Alice Y. Ting [7], Mark von Zastrow [1, 2, 9] ✉, Nevan J. Krogan [2, 3, 4] ✉

Schematic and work flow of Spatiotemporal remodeling protein interaction network

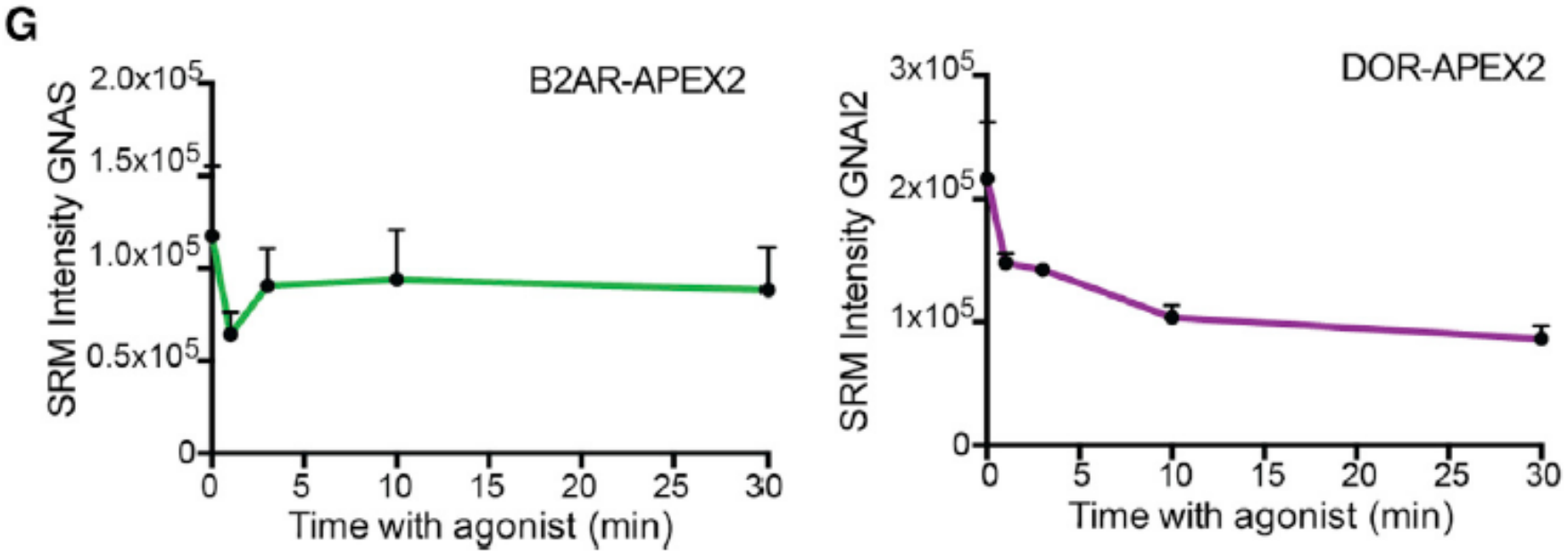


APEX Captures GPCR Protein Interaction Networks

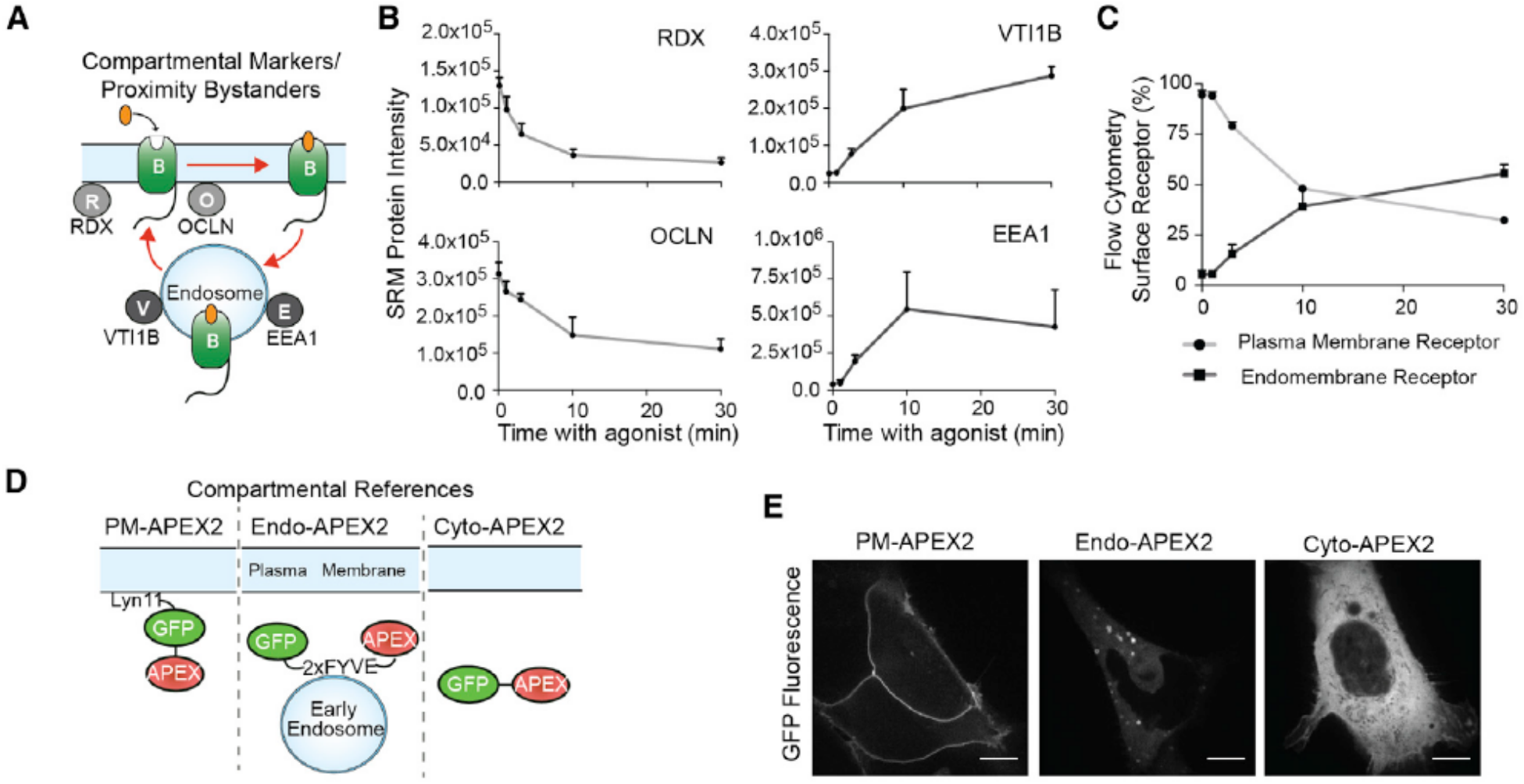


SRM: selected reaction monitoring B2AR: beta-2 adrenergic receptor DOR:delta opioid receptor

APEX Captures GPCR Protein Interaction Networks

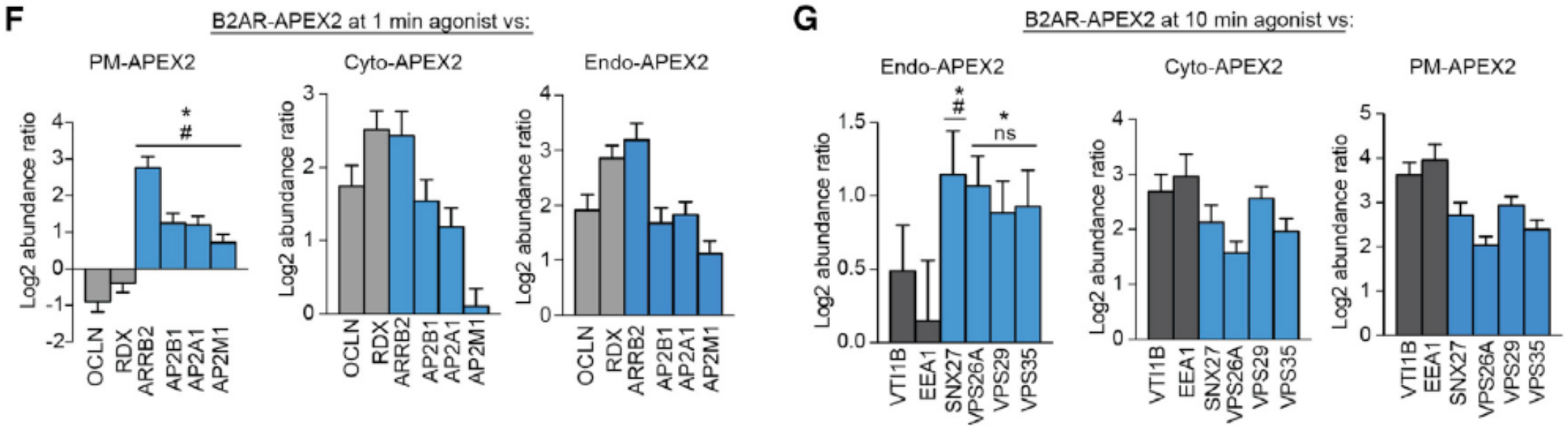


APEX Captures Information about Relative Spatial Location



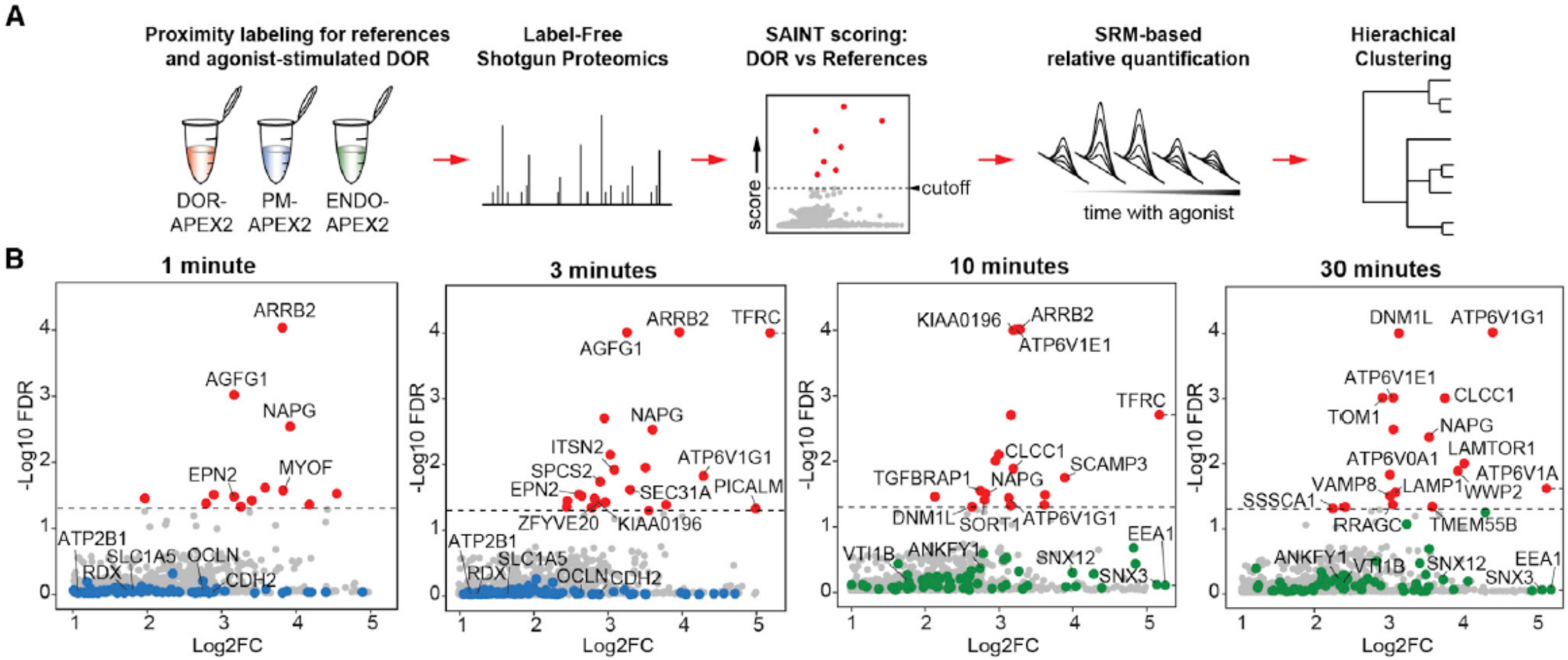
RDX: radixin OCLN: occluding EEA1: endosomal antigen 1 VTI1B: t-SNAREs homolog 1B

APEX Captures Information about Relative Spatial Location



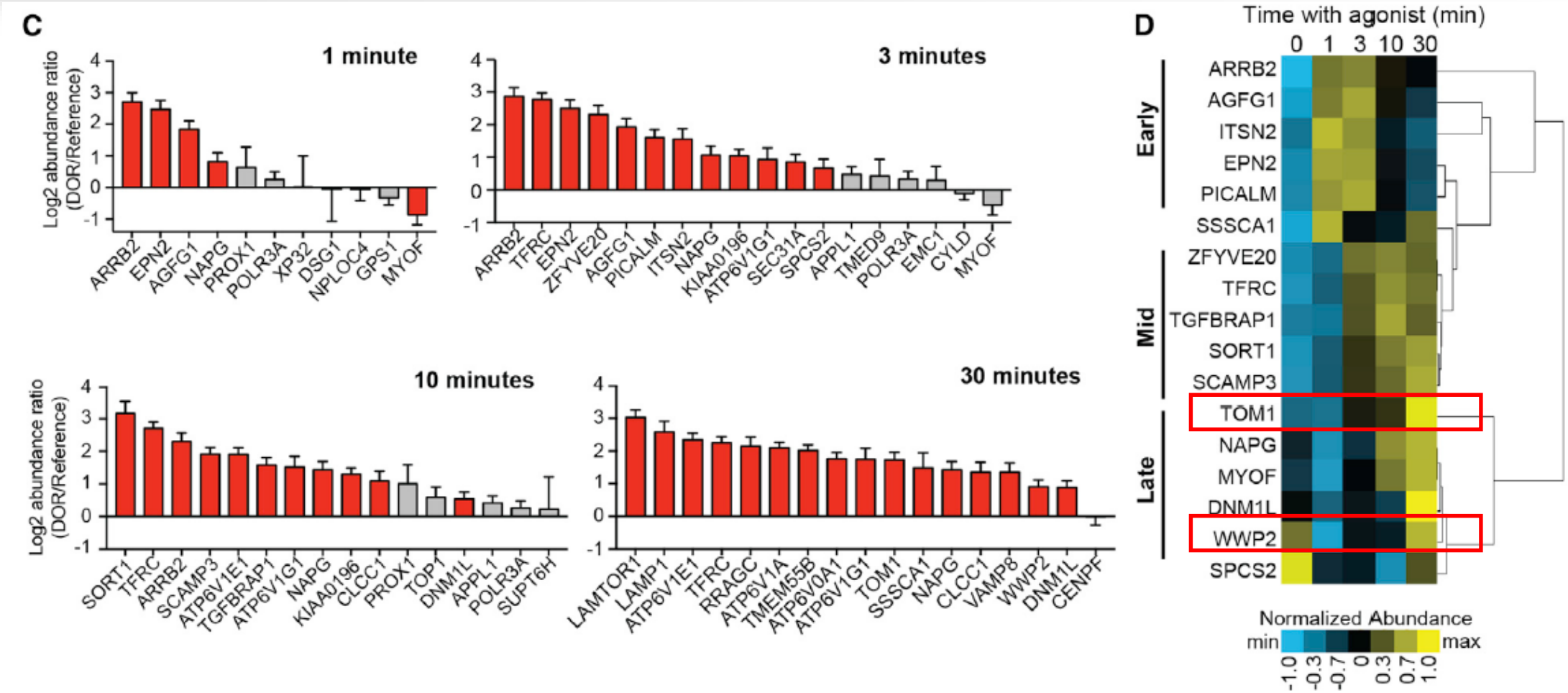
ARRB2: arrestin3 AP2B1/AP2A1/AP2M1 (AP2 complex)
SNX27: sorting nexin 27 VAS26A/VPS29/VPS35 (Retromer complex)

PL in Identification of Interacting Partners for the Delta Opioid Receptor



SAINT : Significance Analysis of INTeractome FDR: false discovery rate FC: fold change

PL in Identification of Interacting Partners for the Delta Opioid Receptor



WWP2 and TOM1

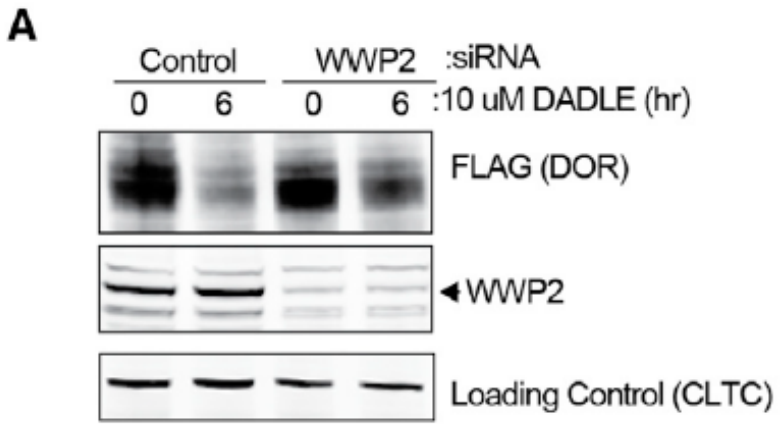
Two ubiquitin-linked proteins

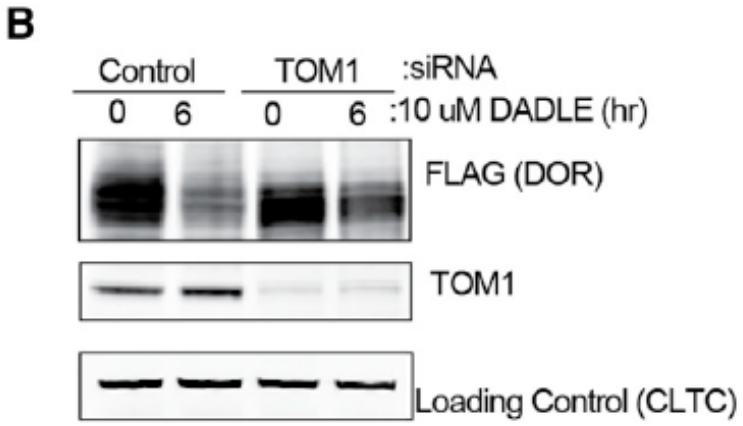
WWP2: a HECT family E3 ligase and has been linked to the degradation of two GPCRs: PAR1 and S1P1.

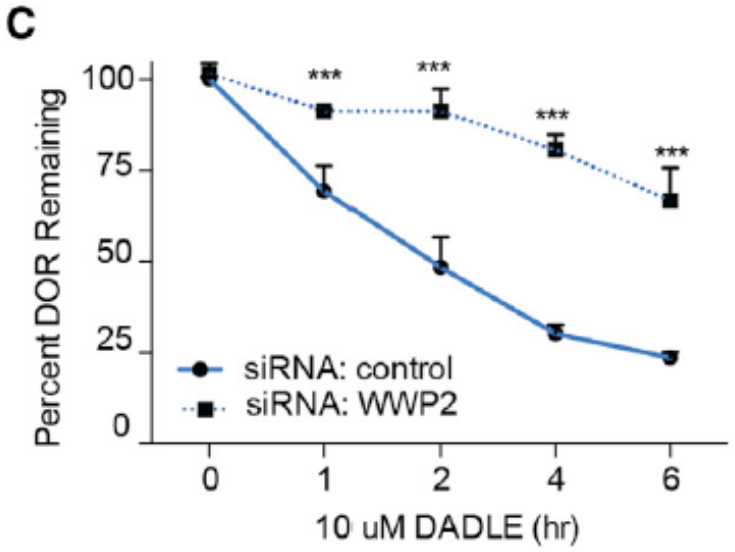
TOM1: has two ubiquitin interaction domains (VHS and GAT) that bind ubiquitin in vitro and it has been shown to localize to endosomes

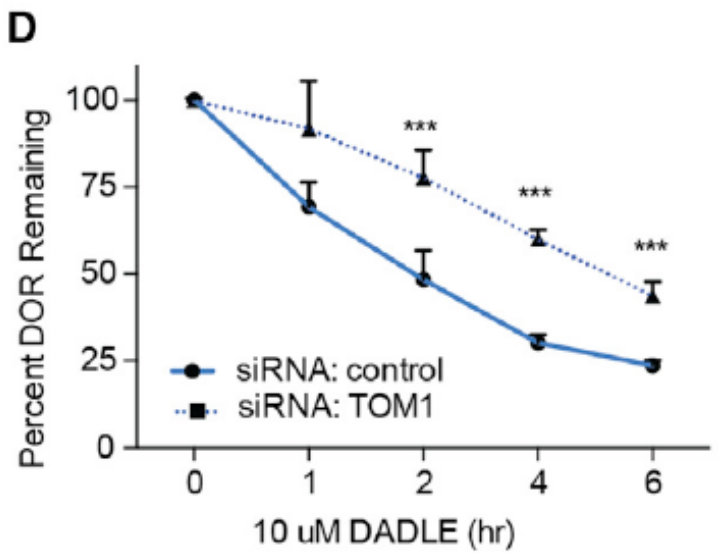
Knockdown of TOM1 or WWP2 → lead to
- endosomal mis-sorting
- reduction in lysosomal degradation

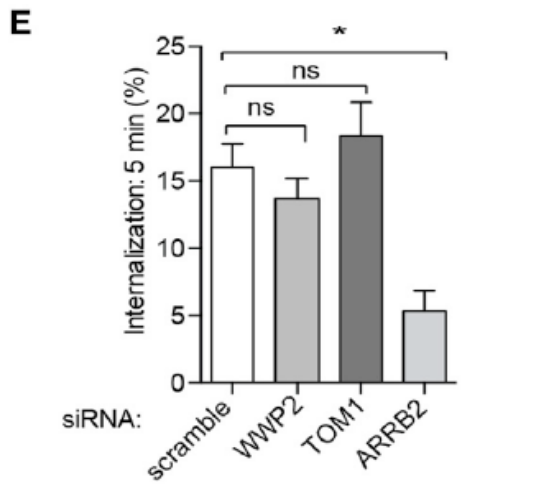
WWP2 or TOM1 as Ubiquitin Network Components Required for DOR Trafficking to Lysosomes

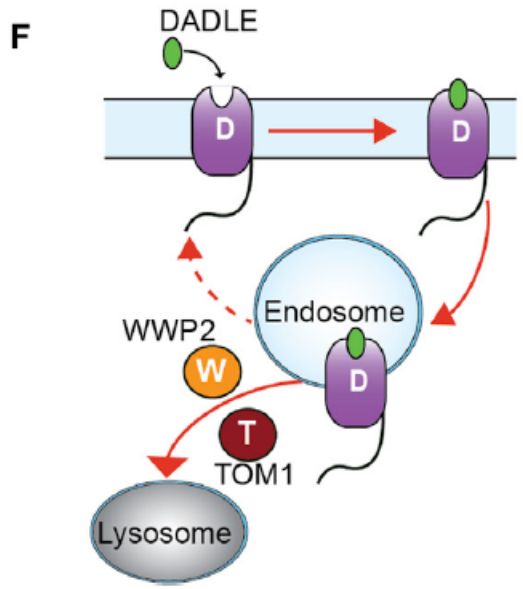












Summary

1. Presenting an approach based on APEX proximity labeling, spatial references, and quantitative MS that allows protein interaction networks to be resolved according to both location and timing.

2. Utility of this method by applying it to GPCRs, which are traditionally difficult targets due to their movement within cells and ligand-induced remodeling of the protein interaction networks that they engage.

3. Validated capture of known receptor binding partners, including those with transient or low-affinity interactions, and demonstrated that our pipeline can be used to discover components of protein interaction networks..

Conclusion

1. PL have enabled biological investigations previously difficult to access.

2. BioID and TurboID have been successfully used in many organisms for in vivo proteomic mapping.
   optimization such as the use of non-biotin probes to avoid background from endogenously biotinylated proteins may improve compatibility for PL in vivo.

3. Time resolved PL combined spatial specific references may provide comprehensive interactome maps and improve spatiotemporal specificity in a greater diversity of model systems.

4. Improving the efficiency of RNA or DNA labeling by PL enzymes will boost sensitivity and analysis of transcriptomes and genomes in distinct cell populations.

Continuing development of increasingly sophisticated PL technology may vastly expand the range of PL-based discoveries and address more challenging questions

THANKS for your attention!