



Abstract

Cell surface proteins mediate essential signaling, trafficking, and cell-cell interactions, representing key biomarker and drug target classes. Yet, comprehensive characterization of human cells surfaceome remains challenging due to the limited spatial precision and labeling specificity of existing proteomic methods. We present an integrated workflow using SynCell Microscope® platform that employs two-photon photo-biotinylation to covalently tag proteins within user-defined microscopy regions of interest (ROI). In combination with Synlight-Rich and Synlight-Pure reagents, it enables nanometer-scale, unbiased proteome discovery with exceptionally high specificity and high spatial control. While Synlight-Rich achieves ~350 nm lateral resolution, Synlight-Pure introduces antibody-mediated proximity photolabeling, restricting biotinylation to within ~25–50 nm of the target structure. Labeled proteins are recovered via the Synpull kit and analyzed by LC-MS/MS, enabling deep interrogation of whole cell, subcellular domains, or cell surface proteomes. Here, SynCell Microscope platform's dual-precision approach—image-guided and chemistry-confined—enabled selective, high-specificity enrichment of membrane-associated and interaction-proximal proteins. In HeLa cells, Microscope with Synlight-Rich identified >3,500 proteins, including >1,600 known plasma-membrane proteins, with >40% showing ≥1.5-fold enrichment ($p < 0.05$) over unlabeled controls. Gene Ontology analysis revealed that ~76% of the top 200 enriched proteins localized to plasma-membrane compartments. Integration of Synlight-Pure increased membrane-specific identifications to ~90% within the same enrichment group, highlighting substantial improvement in labeling specificity. Both chemistries enabled the discovery of novel proteins that show potential associations with plasma membrane proteins, as indicated by protein interaction analysis using STRING, thereby motivating future validation. By extending spatial proteomics from the micrometer to the nanometer scale, SynCell's Microscope® Mint platform - paired with Synlight-Rich or Synlight-Pure - combines unbiased proteome discovery with targeted molecular precision to deliver comprehensive cell-surfaceome. Notably, the enhanced specificity of Synlight-Pure is ready to accelerate biomarker discovery and therapeutic target identification by unlocking interaction-proximity surfaceome across oncology, neurodegeneration, and immunology.

Microscope®: Microscopy-guided photo-biotinylation platform

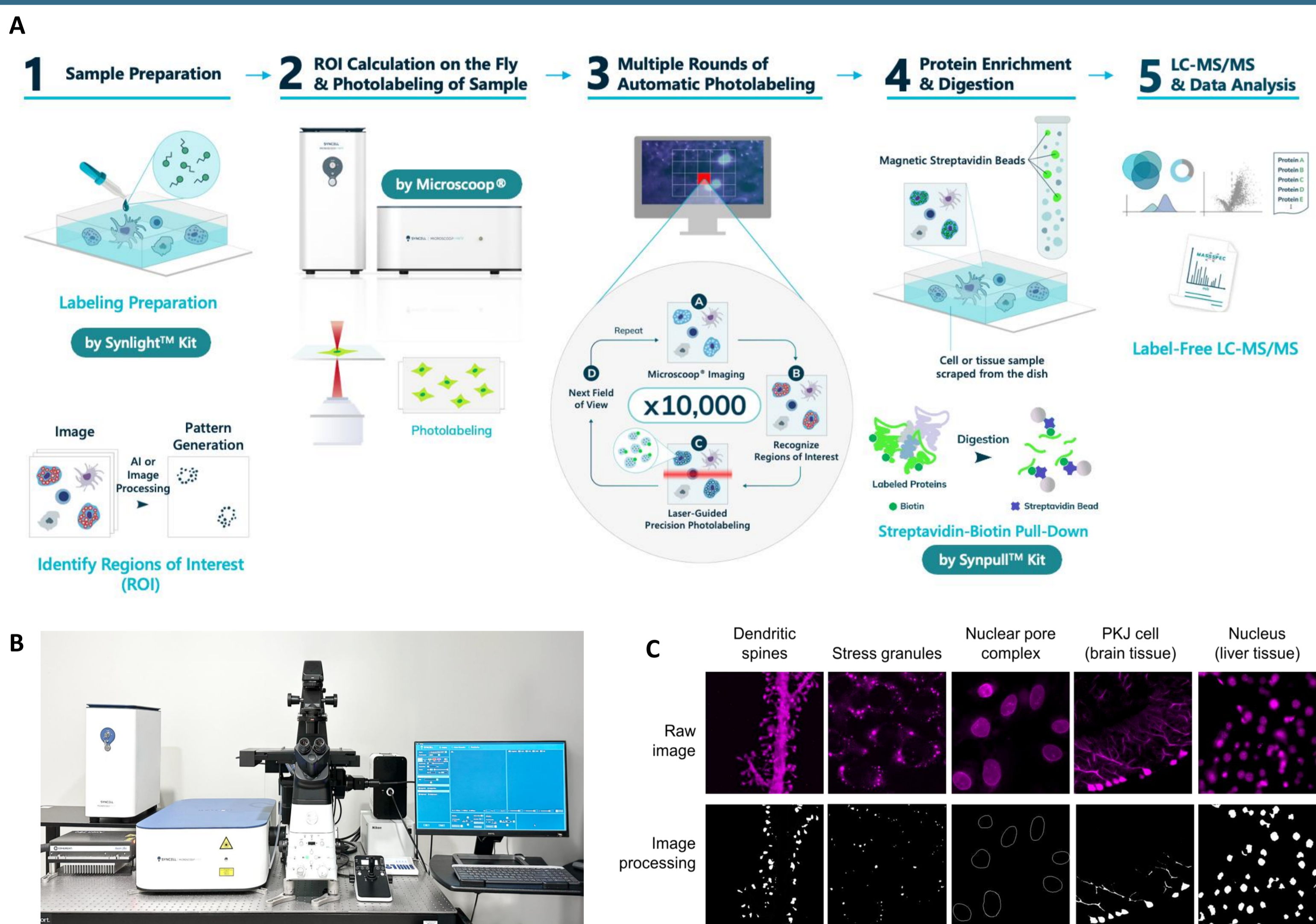


Fig. 1 | A. SynCell Microscope® workflow. The platform integrates image acquisition, photochemistry, microscopy, optics, and FPGA-based mechatronics to enable high-content photolabeling followed by protein purification for mass spectrometry analysis. **B.** Microscope Mint includes the controller, optical engine, microscope, LED illuminator, and Autoscoop software. **C.** Image mask created for ROI across cell and tissue types.

Synlight-Rich and Synlight-Pure

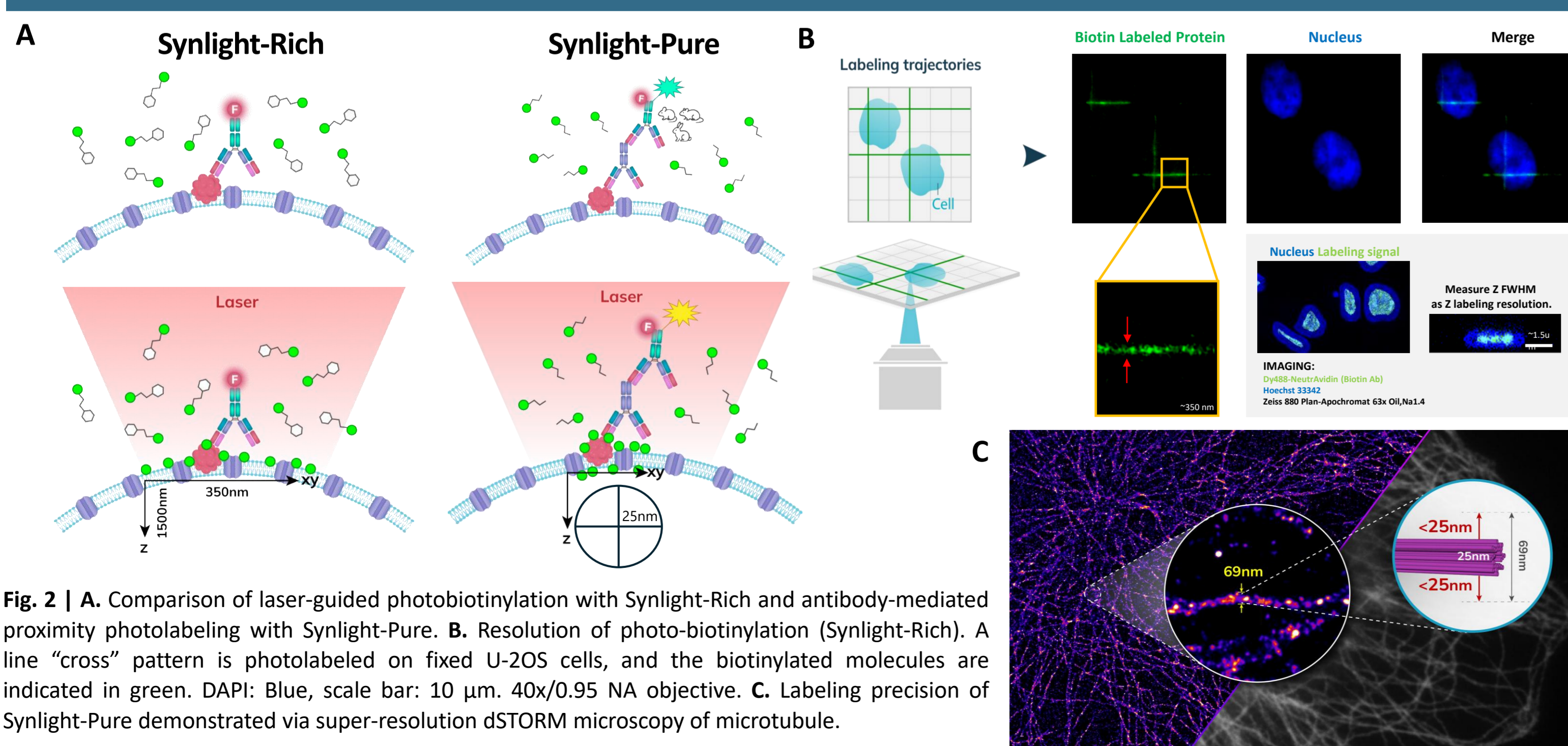
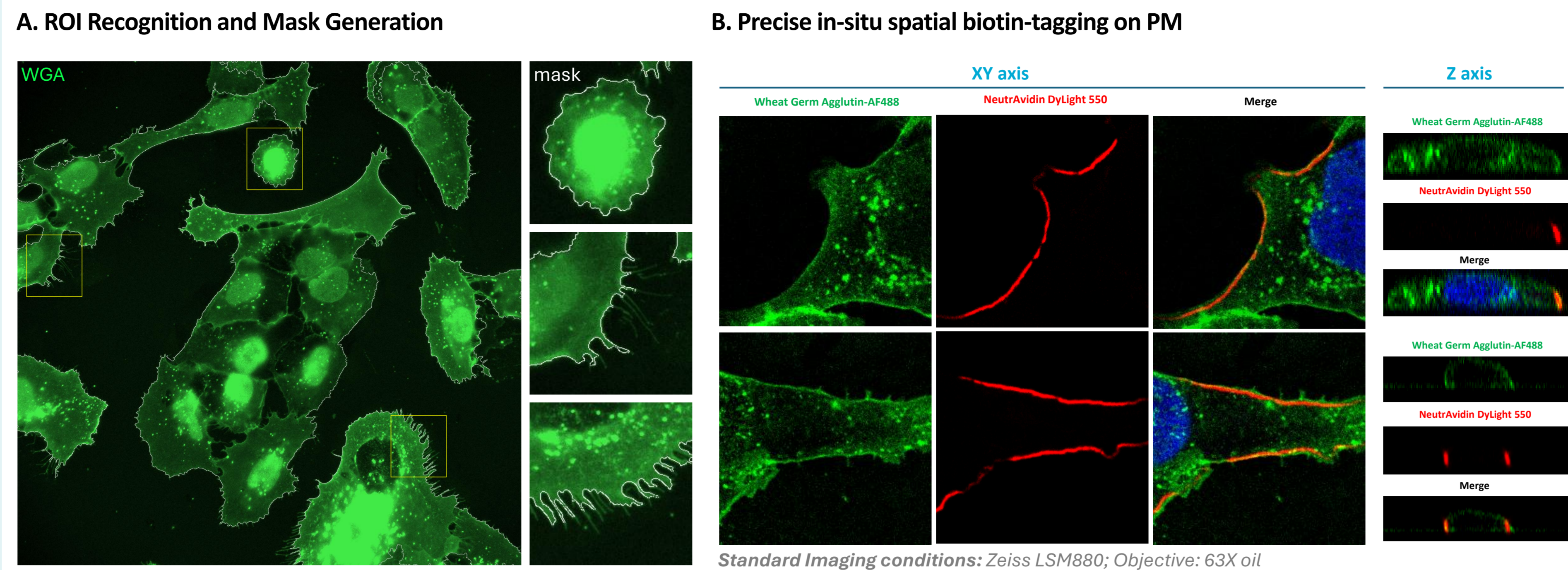
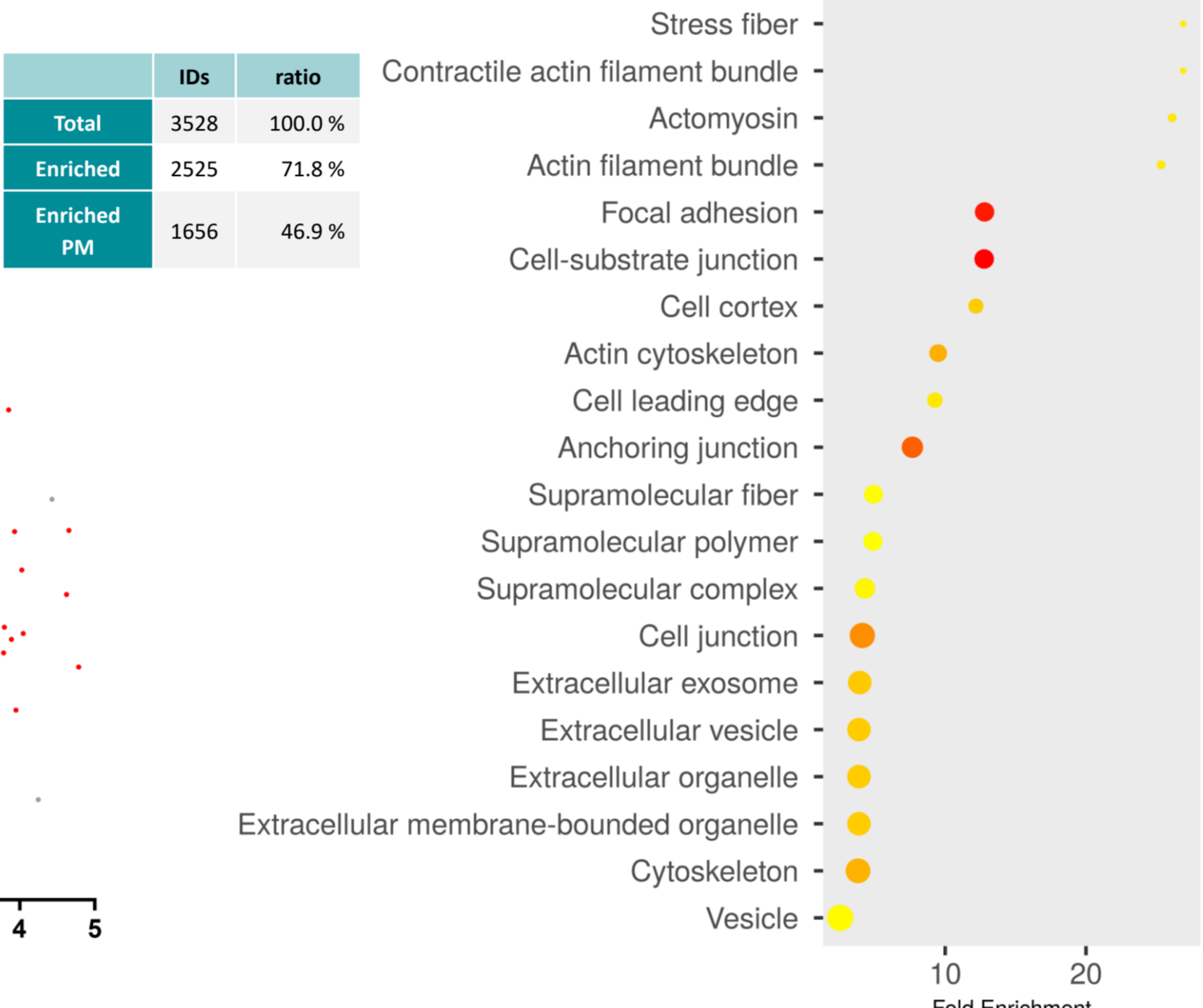
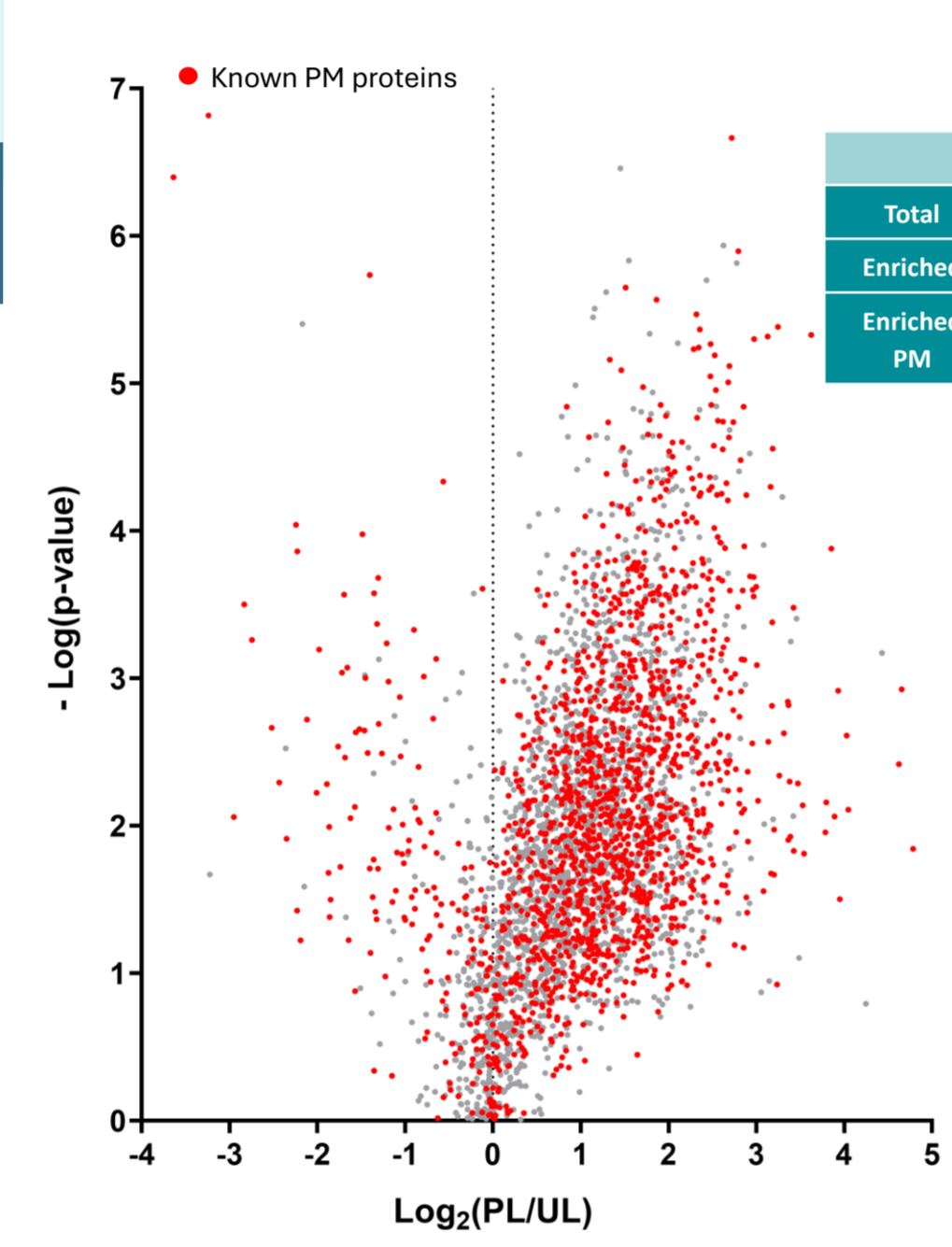


Fig. 2 | A. Comparison of laser-guided photobiotinylation with Synlight-Rich and antibody-mediated proximity photolabeling with Synlight-Pure. **B.** Resolution of photo-biotinylation (Synlight-Rich). A line "cross" pattern is photolabeled on fixed U-2OS cells, and the biotinylated molecules are indicated in green. DAPI: Blue, scale bar: 10 μ m. 40x/0.95 NA objective. **C.** Labeling precision of Synlight-Pure demonstrated via super-resolution dSTORM microscopy of microtubule.

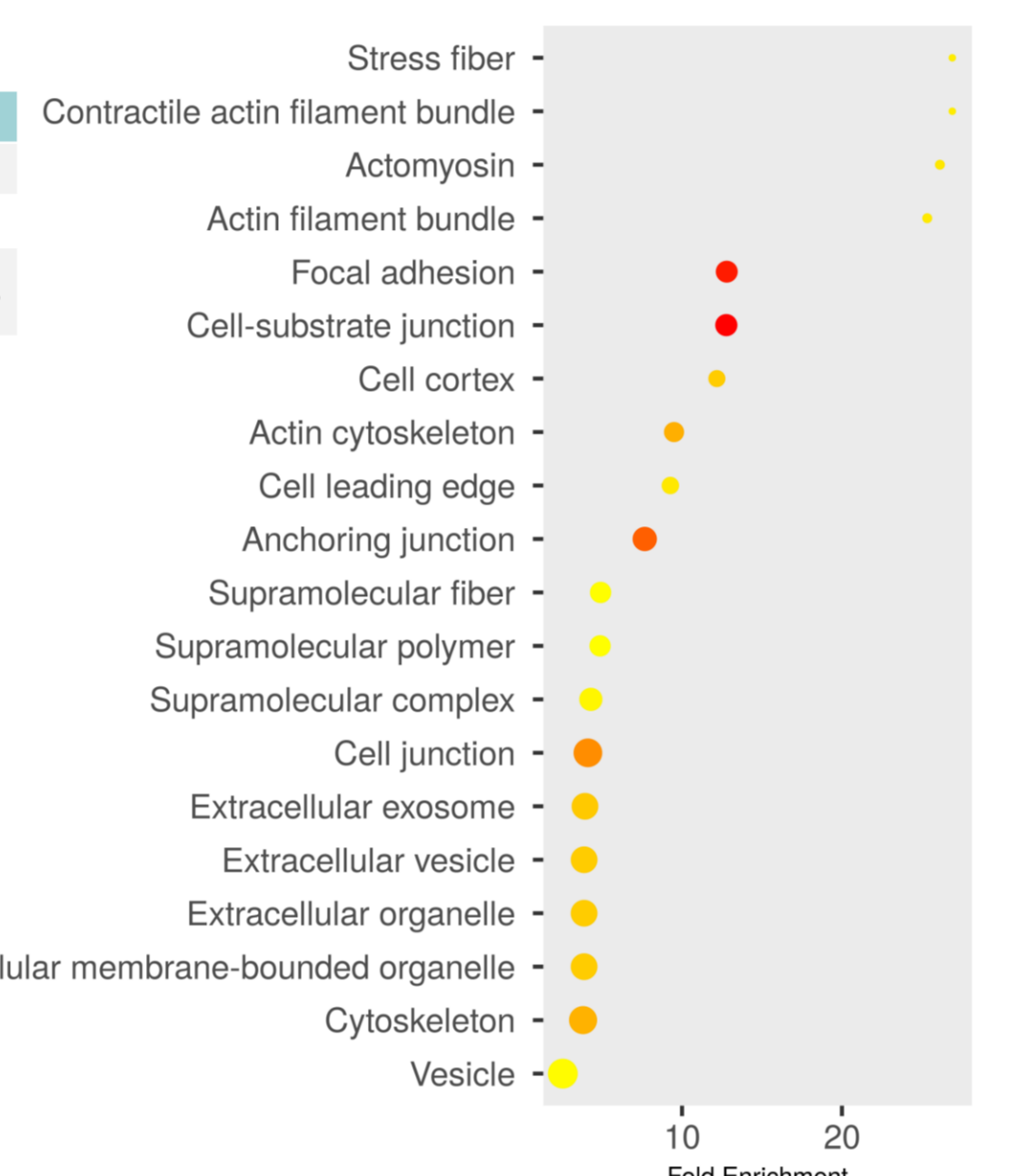
Spatially-resolved proteomic mapping of the plasma membrane (PM) using Synlight-Rich



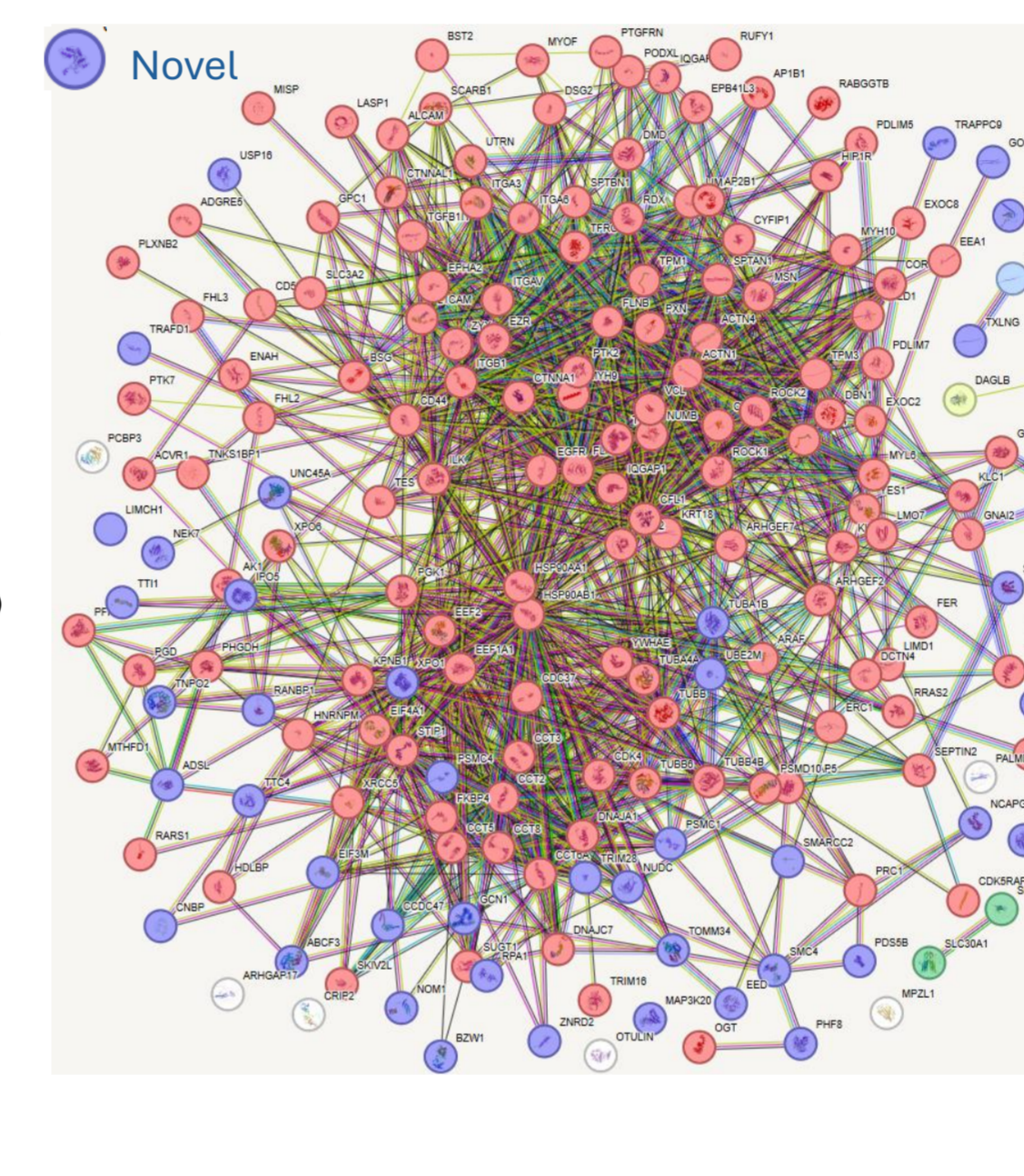
C. Protein enrichment by Synlight-Rich



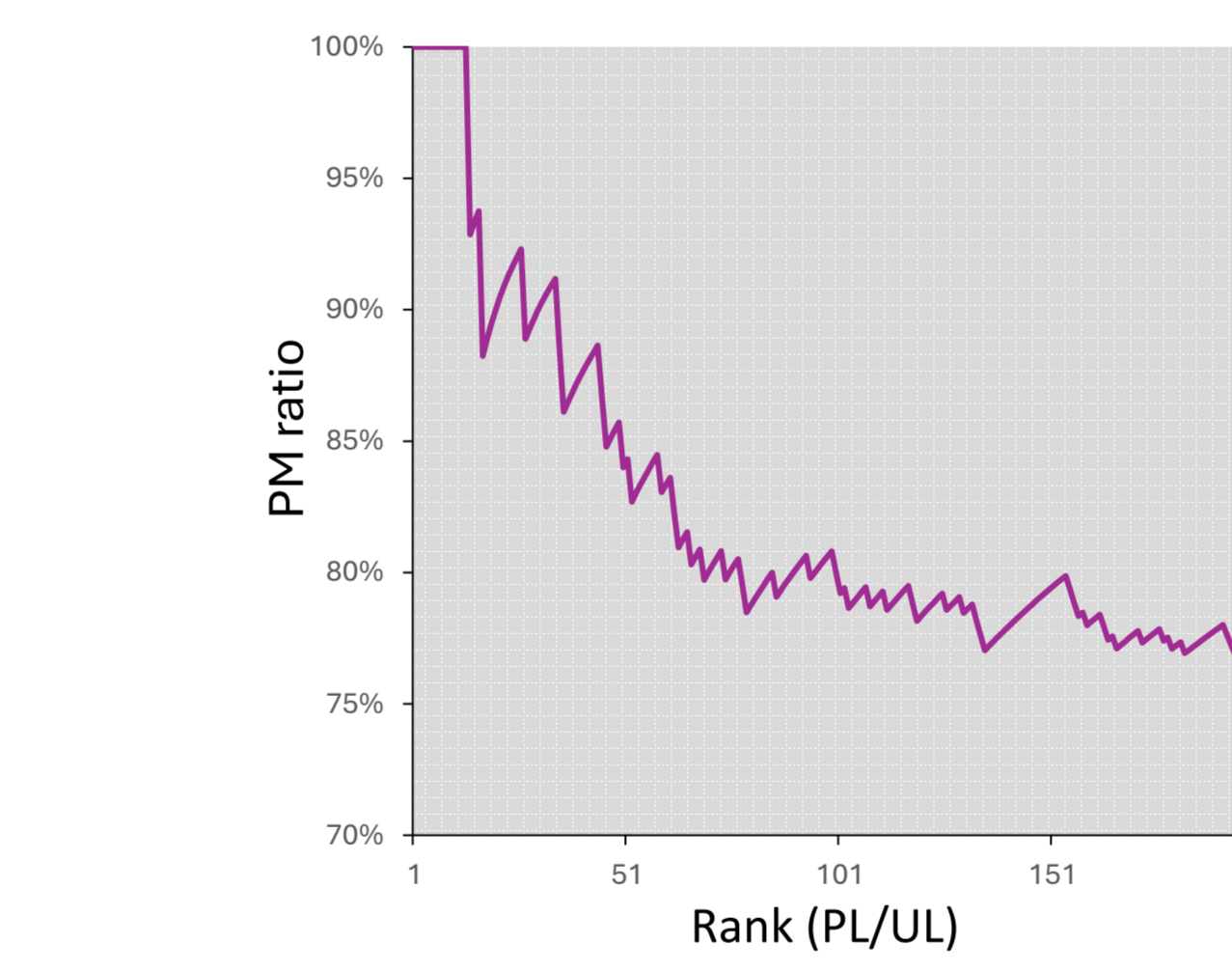
E. Cellular component of Top 200 enriched proteins



F. Protein interaction of Top 200 enriched proteins



D. PM protein ratio of Top 200 enriched proteins

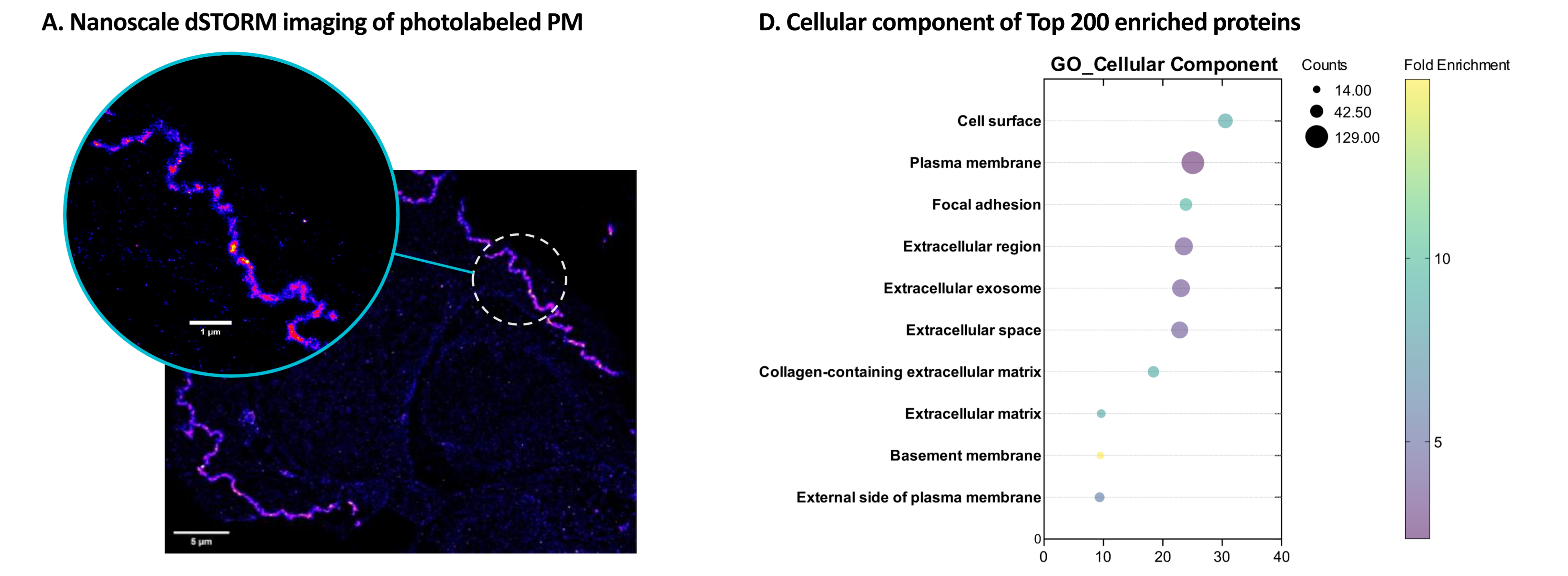


G. List of Top 200 enriched proteins

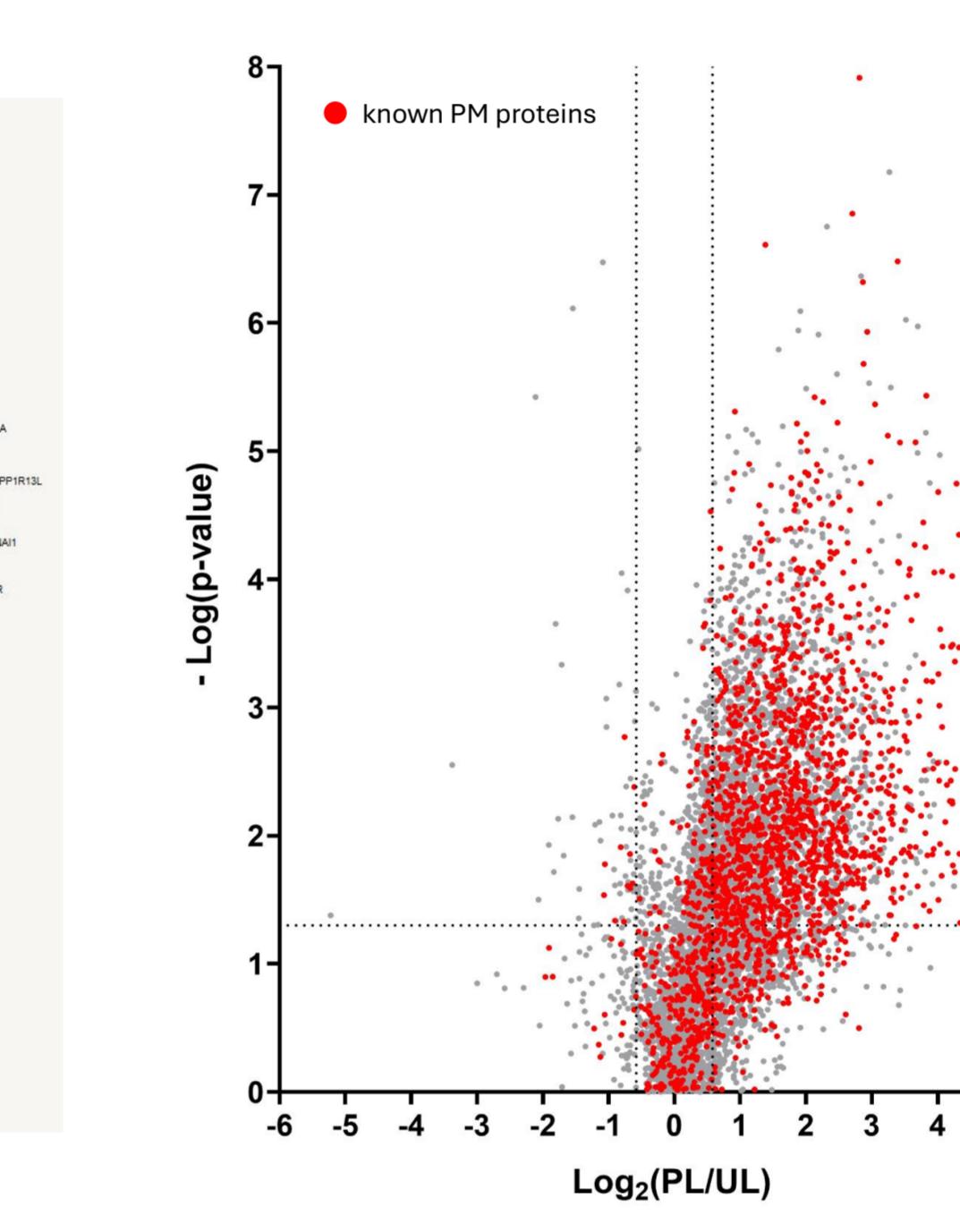
Known PM Proteins										Putative Novel		
ACTN1	CDC37	EGFR	HSP90AA1	MTHFD1	PTK2	TGFBI1	ABCF3	PSMC1	UNC45A	ADAM10	ADAM11	ADAM12
ACTN4	CDK4	E1F4A1	HSP90AB1	MYH10	PTK7	TLN1	ABR	PSMC4	USP16	ADAM13	ADAM14	ADAM15
ACVR1	CDKSRAP2	ENAH	ILK	MYH9	PKN	TNKS1BP1	ACTB	RAB11A/B	XPO1	ADAM16	ADAM17	ADAM18
ADGRFS	CFL1	EPH41L3	IQGAP1	MYL6	RABGGTB	TPM1	ADSL	RAB31	ZNRD2	ADAM19	ADAM20	ADAM21
AK1	CKAP5	EPHA2	IQGAP2	MYO6	RARS1	TPM3	AGPS	RANBP1		ADAM22	ADAM23	ADAM24
ALCAM	CNN2	ERL1	ITGA3	NUMB	RASA3	TPM4	BZV1	RPA1		ADAM25	ADAM26	ADAM27
AP1B1	CORO1C	EXOC2	ITGA6	OGT	RDX	TRIM16	CCDC47	SEPTIN9		ADAM28	ADAM29	ADAM30
AP2B1	CRIP2	EXOC8	ITGAV	OTULIN	ROCK1	TUBA4A	CNBP	SH2D4A		ADAM31	ADAM32	ADAM33
ARAF	CTNNA1	EZR	ITGB1	PALMD	ROCK2	TUBB	EED	SKIC2		ADAM34	ADAM35	ADAM36
ARHGAP17	CTNNA11	FER	KIF5B	PCBP3	RASA2	TUBB4B	EIF3M	SMARCC2		ADAM37	ADAM38	ADAM39
ARHGFE2	CTTN	FHL2	KLC1	PDLM5	RUFY1	TUBB6	GCN1	SMC4		ADAM40	ADAM41	ADAM42
ARHGFE7	CYFIP1	FHL3	KLC2	PDLM7	SCARB1	TXLNA	GOLGA1	TNPO2		ADAM43	ADAM44	ADAM45
BSG	DAGLB	FKBP4	KPNB1	PFKM	SEPTIN2	UTRN	IPOS	TOMM34		ADAM46	ADAM47	ADAM48
BST2	DBN1	FLNA	KRT18	PGD	SLC30A1	VASN	LUMCH1	TRABD		ADAM49	ADAM50	ADAM51
CALD1	DCTN4	FLNB	LICAM	PGK1	SLC39A6	VCL	MAP3K20	TRAFD1		ADAM52	ADAM53	ADAM54
CCT2	DMD	GNAI1	LASP1	PHGDH	SLC3A2	XPO6	NAP1L4	TRAPP9		ADAM55	ADAM56	ADAM57
CCT3	DNAJA1	GNAI2	LIMA1	PLXNB2	SPTAN1	XRCC5	NCAPG	TRIM28		ADAM58	ADAM59	ADAM60
CCTS	DNAIC7	GNAI3	LIMD1	PODXL	SPTBN1	YES1	NEK7	TTIC4		ADAM61	ADAM62	ADAM63
CCT6A	DSG2	GPC1	LMO7	PPP1R3L	STIP1	YWHAE	NUM1	TTI1		ADAM64	ADAM65	ADAM66
CCT8	EEA1	HDLBP	MISP	PRC1	SUGT1	ZYX	NUDC	TUBA1B		ADAM67	ADAM68	ADAM69
CD44	EEF1A1	HIP1R	MP2L1	PSMD10	TES		NUDC	TUBA1B		ADAM70	ADAM71	ADAM72
CD55	EEF2	HNRNPM	MSLN	PTGFRN	TFRC		PDS5B	TOMM34		ADAM73	ADAM74	ADAM75

Fig. 3 | Microscopy-guided nanoscale photolabeling and proteomic profiling of plasma membrane using Synlight-Rich. **A.** Representative microscopy images of HeLa cells showing ROI identification via wheat germ agglutinin (WGA) staining and the corresponding image masks generated for automated photolabeling. **B.** Orthogonal projections (xy and z-axis) illustrating the spatial distribution of photolabeled subcellular compartments. **C.** Volcano plot showing differential protein enrichment (Log2 fold-change) of Synlight-Rich photolabeled (PL) versus unlabeled (UL) controls. **D.** Proportion of plasma membrane (PM)-associated proteins within the top 200 enriched candidates (thresholds: $FC \geq 1.5$, $p \leq 0.05$, unique peptides ≥ 2). **E.** Gene Ontology (GO) cellular component analysis of the top 200 enriched proteins. **F.** Protein-protein interaction network of the top 200 candidates analyzed via the STRING database. **G.** List of the top 200 enriched proteins identified by LC-MS/MS. PM reference dataset source: Nat Chem Biol. 2025 Dec;21(12):1895-1905.

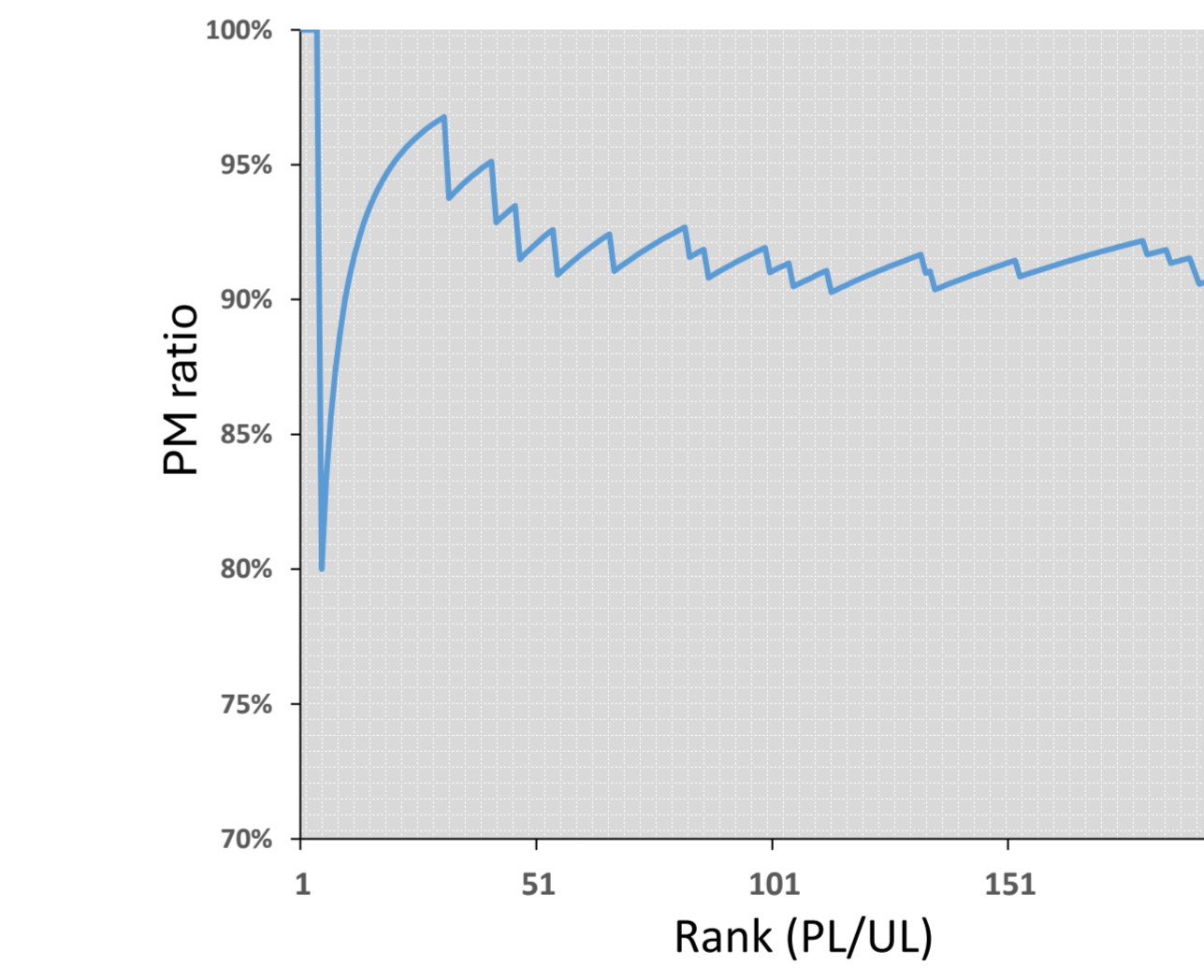
Spatially-resolved proteomic mapping of the plasma membrane (PM) using Synlight-Pure



B. Protein enrichment by Synlight-Pure



C. PM protein ratio of Top 200 enriched proteins



F. List of Top 200 enriched proteins

Known PM Proteins										Putative Novel			
ALPL	VASN	ITGB3	PLAUR	CALM1	SEMA3A	NUMA2	ALPL	VASN	ITGB3	PLAUR	CALM1	SEMA3A	NUMA2
THY1	KIRREL1	ICAM1	MST1R	PROCR	AB11	NUP54	THY1	KIRREL1	ICAM1	MST1R	PROCR	AB11	NUP54
CDS8	PDZD11	FZD2	THSD4	NHERF1	CD82	DYNT13	CDS8	PDZD11	FZD2	THSD4	NHERF1	CD82	DYNT13
PLP2	CSPG4	HABP2	MSLN	CRISP1D2	LTBP1	SULT1B1	PLP2	CSPG4	HABP2	MSLN	CRISP1D2	LTBP1	SULT1B1
SERPINE1	BCAM	TNC	EWSR1	FRZB	FNDCL1	C11orf68	SERPINE1	BCAM	TNC	EWSR1	FRZB	FNDCL1	C11orf68
CRISPLD1	MUC16	SEMA3B	SLC12A4	FN1	CORO1B	SEPTIN11	CRISPLD1	MUC16	SEMA3B	SLC12A4	FN1	CORO1B	SEPTIN11
ITGA1	ITGAV	CTNNA1	PIP4K2A	PLXNA2	SERPINE2	CASP6	ITGA1	ITGAV	CTNNA1	PIP4K2A	PLXNA2	SERPINE2	CASP6
FAT4	EPHA2	TGFB1	JAG1	LAMC1	ILIRAP	ABTB2	FAT4	EPHA2	TGFB1	JAG1	LAMC1	ILIRAP	ABTB2
CDH13	MCAM	LECT2	PPP1R9B	ADAMT53	NUP62		CDH13	MCAM	LECT2	PPP1R9B	ADAMT53	NUP62	
ITGA5	ALCAM	FERMT3	EZR	ACTN4	ARL6IP5	WDR54	ITGA5	ALCAM	FERMT3	EZR	ACTN4	ARL6IP5	WDR54
ENG	IGFBP5	ULBP3	PRK4	PTK7	ITPB8		ENG	IGFBP5	ULBP3	PRK4	PTK7	ITPB8	
HLA-A	NEGR1	TACSD2	GPC1	MUC1	WDR6	NCK2	HLA-A	NEGR1	TACSD2	GPC1	MUC1	WDR6	NCK2
LIN7C	LRI1	NHERF2	SEMA3C	EGFR	DLG3	SEPTIN9	LIN7C	LRI1	NHERF2	SEMA3C	EGFR	DLG3	SEPTIN9
CD55	CD109	SLC39A6	DDAH1	TIMP1	MET	NUP37	CD55	CD109	SLC39A6	DDAH1	TIMP1	MET	NUP37
SEPTIN2	ROBO2	RSPRY1	PLXND1	SRRP1	NUP43		SEPTIN2	ROBO2	RSPRY1	PLXND1	SRRP1	NUP43	
FOLR1	PTPRF	BLVRB	PSMD9	CDH2	NUP58		FOLR1	PTPRF	BLVRB	PSMD9	CDH2	NUP58	
ANOS1	MPZL1	ARPC5L	CA12	MPZL1	TGFB2	SPC24	ANOS1	MPZL1	ARPC5L	CA12	MPZL1	TGFB2	SPC24
MICA	CCN1	PTGFRN	OSQX1	IGFBP3	KIAA1522	RWDD4	MICA	CCN1	PTGFRN	OSQX1	IGFBP3	KIAA1522	RWDD4
CHAD	ITGA6	ADD3	NTSE	ANO10	ACTN1	RAE1	CHAD	ITGA6	ADD3	NTSE	ANO10	ACTN1	RAE1
CORO1A	TFPI	ADAMT51	EP58	TIMP3	FKBP3		CORO1A	TFPI	ADAMT51	EP58	TIMP3	FKBP3	
SLC3A2	ADGRFS	NRP3	AMIGO2	ALDH8A1	COL4A2	MYBPC3	SLC3A2	ADGRFS	NRP3	AMIGO2	ALDH8A1	COL4A2	MYBPC3
LICAM	CSF1	NTN4	SERPINC1	GM81	EPS15L1		LICAM	CSF1	NTN4	SERPINC1	GM81	EPS15L1	
ADGRG1	IGSF3	NECTIN2	ADAM22	CPM	STK10		ADGRG1	IGSF3	NECTIN2	ADAM22	CPM	STK10	
MAPKAP1	CCN2	CSAD	ITGA3	STOM	ITGB4		MAPKAP1	CCN2	CSAD	ITGA3	STOM	ITGB4	
ITGA2	TFPI2	RSP03	TRIP10	LPL	ITGB4		ITGA2	TFPI2	RSP03	TRIP10	LPL	ITGB4	
FGFR3P1	ITGB1	TPM1	TMEM132A	LRP1	GPNBP1		FGFR3P1	ITGB1	TPM1	TMEM132A	LRP1	GPNBP1	
SMOCL1	MRC2	CERS1	GREM1	FAM234A	KCN118		SMOCL1	MRC2	CERS1	GREM1	FAM234A	KCN118	
TRNRSF11B	IGSF8	EPH82	HLA-B	MOV10	CRLF1		TRNRSF11B	IGSF8	EPH82	HLA-B	MOV10	CRLF1	
HTRA3	PVR	COL3A1	CDH11	PPP1R16A	FBN1		HTRA3	PVR	COL3A1	CDH11	PPP1R16A	FBN1	
HTRA1	LAMAS	NOG	GPC5	RP519			HTRA1	LAMAS	NOG	GPC5	RP519		

Fig. 4 | Microscopy-guided nanoscale photolabeling and proteomic profiling of plasma membrane using Synlight-Pure. **A.** Super-resolution dSTORM imaging of photolabeled plasma membranes in HeLa cells. **B.** Volcano plot showing differential protein enrichment (Log2 fold-change) for Synlight-Pure photolabeled (PL) versus unlabeled (UL) controls; red data points denote annotated plasma membrane (PM) proteins. **C.** Proportion of plasma membrane (PM)-associated proteins within the top 200 enriched candidates (thresholds: $FC \geq 1.5$, $p \leq 0.05$, and unique peptides ≥ 2). **D.** Gene Ontology (GO) cellular component analysis of the top 200 enriched proteins. **E.** Protein-protein interaction network generated via STRING analysis for the top 200 candidates. **F.** List of the top 200 enriched proteins identified by LC-MS/MS. PM reference dataset source: Nat Chem Biol. 2025 Dec;21(12):1895-1905.

Summary

- SynCell Microscope enables Nanometer-Scale Spatial Proteomics**
Extends spatial resolution from micrometers to ~25–50 nm for high-sensitivity, high-specificity discovery of target structure proteomes.
- Image-Guided, ROI-Specific Protein Labeling**
Two-photon photo-biotinylation enables precise targeting of user-defined cellular regions.
- Dual-Precision Chemistry: Discovery or Proximity**
Synlight-Rich supports truly unbiased whole proteome discovery, while Synlight-Pure enables antibody-mediated proximity labeling for interaction-proximity proteins discovery.

- Deep, Unbiased Surfaceome Coverage**
Synlight-Rich and Synlight-Pure identify >3,500 proteins, including >1,600 known plasma-membrane components, as well as novel putative proteins.
- High Cell-Surface Specificity (~90% on Top 200 PM proteins)**
Synlight-Pure antibody-mediated photolabeling dramatically increases plasma-membrane proteins enrichment.
- Accelerated Biomarker & Target Discovery**
SynCell Microscope high specificity and spatial control enable disease-relevant surfaceome studies to accelerate biomarker discovery and therapeutic target identification across therapeutic areas.