Scientific Reports

A Split Biotin Ligase Approach to Revealing Proteins Associated with Oligomeric Alpha-Synuclein During Aggregation

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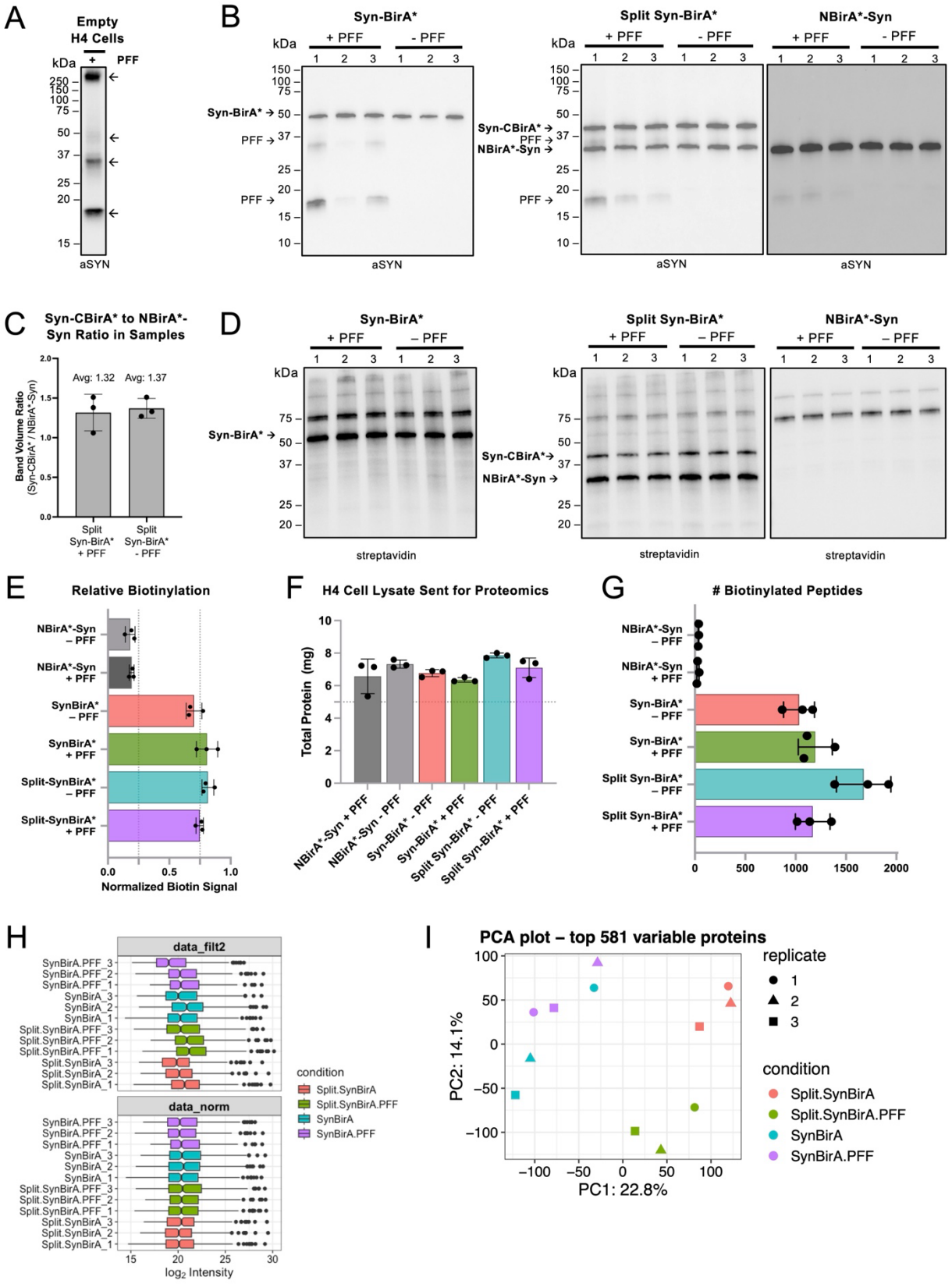
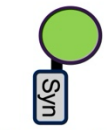


Supplementary Fig. 1 Characterization of Samples Analyzed by Proteomics

A. Western blot of cell lysate from construct-free H4 neuroglioma cells treated with human wildtype alpha-Synuclein (aSYN) recombinant pre-formed fibrils (PFF). Probed for aSYN (MJFR1). **B.** Construct expression remained equal across replicates sent for proteomics. Western blot of cell lysate from triplicates of the 6 groups sent for proteomics. Probed for aSYN (MJFR1). Left: seeded (+ PFF) and non-seeded (– PFF) Syn-BirA*. Center: seeded (+ PFF) and non-seeded (– PFF) Split Syn-BirA*. Right: seeded (+ PFF) and non-seeded (– PFF) NBirA*-Syn. All blots demonstrate self-biotinylation of the construct expressed: self-biotinylated Syn-BirA* at 51kDa (left), self-biotinylated Syn-CBirA* at 40kDa and NBirA*-Syn at 27kDa (center) or self-biotinylated NBirA*-Syn only at 27kDa (right). PFF-specific bands are visible in groups treated with PFFs on all blots. **C.** Quantification of bands from panel B demonstrates ratios of Syn-CBirA* and NBirA*-Syn were similar across Split Syn-BirA* samples. **D-E.** Syn-BirA* and Split Syn-BirA* cell lines expressing a functioning biotin ligase enzyme demonstrate more overall biotinylation than the negative control cell line, NBirA*-Syn, expressing an incomplete enzyme fragment. (D) Western blot of cell lysate from triplicates of the 6 groups sent for proteomics. Probed for biotin (neutravidin). All blots demonstrate self-biotinylation of the construct expressed. Bands present in NBirA*-Syn cell lysate correspond with sizes of known endogenously biotinylated proteins: pyruvate carboxylase (130kDa), 3-methylcrotonyl-CoA carboxylase (75kDa), and propionyl-CoA carboxylase (72kDa). (E) Quantification of biotinylation signal from each sample in panel D. **F.** For each replicate, a cell pellet containing > 5mg of total protein were sent for BioSITe and LC-MS/MS. **G.** The number of unique biotinylated peptides reported in the raw proteomic data, by group. **H.** Box plots of the proteomic data, by sample, before ('data_filt2') and after ('data_norm') normalization. Proteins present in negative control NBirA*-Syn groups or identified in < 2 of 3 replicates across all groups were excluded prior to normalization. **I.** Principal component analysis (PCA) plot demonstrating clustering among similar samples for the post-processed dataset of 581 proteins.

A



Syn-BirA*
N = 342

Gene Names of Identified Proteins in the Non-Seeded Syn-BirA* Interactome. Organized by Encoding Protein Class.

Calcium-Binding Protein

ANXA2	*EFHD2*	*S100A11*	*S100A6*

Cell Junction Protein

TJP1	*TJP2*

Chaperone

AHSA1	*BAG2*	*BAG3*	*CCT8*	*DNAJB1*	*FKBP3*	*HSP90AA1*	*HSP90AB1*	*HSPA1B*	*HSPA8*	*HSPH1*	*NPM1*
TBCB											

Chromatin/Chr.-Binding/Regulatory Protein

BAZ2A	*H2AZ1*	*H2BC18*	*H2BC21*	*H2BC26*	*H2BC5*	*H3-7*	*NAP1L4*	*SET*	*SIN3B*

H3C1, H3C2, H3C3, H3C4, H3C6, H3C7, H3C8, H3C10, H3C11, H3C12 (Accession ID: P68431)

H4C1, H4C2, H4C3, H4C4, H4C5, H4C6, H4C7, H4C8, H4C9, H4C10, H4C11, H4C12, H4C13, H4C14, H4C15, H4C16 (Accession ID: P62805)

Cytoskeletal Protein

ACTB	*ACTC1*	*ANLN*	*ARPC1B*	*CALD1*	*CAP1*	*CAPG*	*CFL1*	*CKAP5*	*CORO1B*	*CORO1C*	*CSRP1*
EPB41	*EPB41L2*	*EPB41L3*	*EZR*	*GSN*	*JPT2*	*LIMA1*	*LIMCH1*	*MAP4*	*MSN*	*MYH13*	*MYH9*
MYO1B	*NUDC*	*NUDCD3*	*NUMA1*	*PDLIM1*	*PDLIM4*	*PDLIM5*	*PDLIM7*	*PFN1*	*PLS3*	*SEPTIN9*	*STMN1*
SYNPO2L	*TPM3*	*TPM4*	*TPX2*	*TWF1*	*VIM*	*ZYX*					

Defense / Immunity Protein

ANKRD17

DNA Metabolism Protein

BCLAF1	*RPA1*	*SMARCA5*	*THRAP3*	*TRIR*	*YLPM1*

Extracellular Matrix Protein

MFAP1	*SART1*

Gene-Specific Transcriptional Regulator

AFF4	*GABPA*	*GATAD2B*	*HDGFL2*	*HMGA1*	*HMGB3*	*IFI16*	*LRRFIP1*	*MTA1*	*RLF*	*SAP30BP*	*STAT1*
STAT3	*STAT6*	*TEAD1*	*TFE3*	*THOC2*	*TOX4*	*ZBTB33*	*ZC3H4*	*ZNF280C*			

Intercellular Signal Molecule

TMPO (Accession ID: P42166)

Membrane Traffic Protein

ARCN1	*BET1*	*CHMP5*	*CLINT1*	*COPG2*	*GAK*	*REPS1*	*SEC61B*	*VAMP1*	*VAPA*	*VAPB*

Metabolite Interconversion Enzyme

DPYSL4	*G6PD*	*GAPDH*	*HINT1*	*LBR*	*NAA10*	*NME2*	*NUDT5*	*PFKP*	*PGAM1*	*PIP4K2C*	*PKM*
PLA2G4A	*PRDX1*	*TXNRD1*	*WWOX*								

Protein Modifying Enzyme

BAZ1B	*CACYBP*	*CUL4B*	*HERC4*	*PAK2*	*PPME1*	*PSMD1*	*PSMD8*	*PTPN1*	*TRIM25*	*UBE2M*	*USP15*
USP7	*UTRN*	*VCPIP1*									

Protein-Binding Activity Modulator

ARHGAP35	*CAST*	*CCNK*	*CCNT1*	*DOCK7*	*GAPVD1*	*NSFL1C*	*PPP1R37*	*RAB11FIP5*	*RABL3*	*RANGAP1*	*SIPA1L2*

RNA Metabolism Protein

ATXN2	*ATXN2L*	*CSTF2T*	*DDX42*	*DDX46*	*DHX9*	*GTF2F2*	*HDLBP*	*HNRNPA1*	*HNRNPC*	*KHSRP*	*LARP1*
NCL	*NONO*	*NOP58*	*PABPC1*	*PCBP2*	*PRRC2A*	*PRRC2C*	*PSPC1*	*SERBP1*	*SFPQ*	*SNRPD2*	*SUB1*
SURF6	*TAF1*	*TAF7*	*TCOF1*	*ZC3H7A*	*ZFR*	*ZMAT2*					

Scaffold/Adaptor Protein

ANKS1A	*CACTIN*	*CD2AP*	*CEP152*	*CEP170*	*CLASRP*	*DBNL*	*DLG1*	*DLGAP4*	*EHBP1L1*	*ENAH*	*HNRNPF*
LRBA	*MAGED1*	*MVP*	*NCK2*	*NUDT21*	*NUP50*	*PLIN3*	*PUF60*	*RANBP2*	*RANBP3*	*RBM17*	*RBM39*
SF3A1	*SF3B2*	*SH3GL1*	*SH3PXD2B*	*SHC1*	*SNRPA1*	*SNX2*	*SUGP1*	*TANC1*	*XAB2*	*YWHAB*	*ZRANB2*

Transfer / Carrier Protein

OSBPL8

Translational Protein

ABCF2	*EEF1A1*	*EEF1A2*	*EEF1B2*	*EEF1D*	*EEF2*	*EIF3G*	*EIF4B*	*EIF4E*	*EIF4G1*	*EIF4G2*	*EIF4G3*
EIF4H	*EIF5*	*EPRS1*	*GSPT2*	*RARS1*	*RPL19*	*RPL24*	*RPL4*	*RPL6*	*RPS2*	*RPS20*	*RPS3A*

Transmembrane Signal Receptor

ATP6AP2	*PGRMC2*

Transporter

AP3D1	*PPP4R3A*	*TPR*

Unclassified

AAK1	*ACIN1*	*ADNP*	*AHNAK*	*AHNAK2*	*ARGLU1*	*ARPC2*	*BOD1L1*	*C1orf198*	*CAMSAP1*	*CBX3*	*CCDC124*
CCDC6	*CDV3*	*CKAP4*	*CSDE1*	*CWC15*	*DAP*	*DNMBP*	*EBAG9*	*EMD*	*ESF1*	*FAM184B*	*FASN*
FHOD1	*FLNB*	*GEMIN5*	*GNL2*	*GSDME*	*HCFC1*	*HGS*	*HNRNPU*	*HNRNPUL2*	*LASP1*	*LMO7*	*LUZP1*
MTUS1	*NCAPG*	*NHLRC2*	*NOL11*	*NOLC1*	*NUCKS1*	*NUFIP2*	*PAICS*	*PCNP*	*PDAP1*	*PLEKHA1*	*PNISR*
PNN	*PPP1R18*	*PTMA*	*PUS7*	*RAI14*	*RBM10*	*RCC2*	*RNF213*	*RP9*	*RUFY1*	*RUVBL1*	*SAFB*
SAMD9	*SLTM*	*SMAP*	*SMC4*	*SND1*	*SP100*	*SRPK1*	*STBD1*	*STEEP1*	*STIP1*	*TAGLN2*	*TLN1*
TMA7	*TMEM109*	*UBXN4*	*UNC45A*	*UVSSA*	*VMA12*	*ZC3H18*	*ZC3HAV1*	*ZC3HC1*	*ZFAND3*	*ZMYND8*	*ZNF609*

Not Found

PALM2AKAP2	*TMPO (Accession ID: P42167)*

Supplementary Fig. 2 Proximal Proteome of Unseeded Monomeric and Multimeric aSYN

A. Gene names of the 342 identified proteins proximal to non-seeded Syn-BirA*, organized by encoding protein class. Proteins included were identified in ≥ 2 of 3 replicates. Protein classes were queried through PANTHER Database v19.

A

Split
Syn-BirA*
N = 487

Gene Names of Identified Proteins in the Non-Seeded Split Syn-BirA* Interactome. Organized by Encoding Protein Class.

Calcium-Binding Protein

ANXA1	ANXA2	EFHD2	S100A11	S100A6

Cell Junction Protein

TJP1	TJP2

Cell Adhesion Molecule

DSG2	ITGB1

Chaperone

AHSA1	BAG3	CCT8	CLIP1	DNAJB1
DNAJC13	DNAJC8	FKBP3	HSP90AA1	HSP90AB1
HSPA1B	HSPA8	NASP	NPM1	TBCB

Chromatin/Chr.-Binding/Regulatory Protein

BAZ2A	CHD7	CHD8	H2AZ1	H2BC18	H2BC21
H2BC26	H2BC5	H3-7	H4C1	JMJD1C	KMT2B
NAP1L4	NCOR2	SAP130	SCML2	SIN3B	
H4C1, H4C2, H4C3, H4C4, H4C5, H4C6, H4C7, H4C8, H4C9, H4C10, H4C11, H4C12, H4C13, H4C14, H4C15, H4C16 (Accession ID: P62805)					

Cytoskeletal Protein

ACTB	ACTC1	ACTN4	ADD2	AFDN	ANLN	ARPC3	CALD1	CAP1	CAPG	CFL1	CKAP5
CLASP1	CORO1C	CSRP1	CTNND1	EPB41	EPB41L2	EPB41L3	EVPL	EZR	GSN	JPT1	JPT2
KIF15	KIF20B	KIF5B	KRT18	LIMA1	LIMCH1	MACF1	MAP1A	MAP1B	MAP4	MAPRE2	MPRIP
MSN	MYH9	MYL12B	MYO1B	NUDC	NUMA1	PARVA	PDLIM1	PDLIM4	PDLIM5	PDLIM7	PFN1
PLS3	RDX	SEPTIN2	SEPTIN9	SPTAN1	SPTBN1	STMN1	SYNPO2L	TMSB10	TPM1	TPM3	TPM4
TPX2	TWF1	VIM	ZYX								

Defense / Immunity Protein

ANKRD17

DNA Metabolism Protein

BCLAF1	FEN1	MLH1	PRIM2	RPA1
SMARCA1	SMARCA5	THRAP3	TRIR	

Extracellular Matrix Protein

MFAP1	SART1

Gene-Specific Transcriptional Regulator

AFF4	AHR	ATF7IP	BTF3	EMSY	GATAD2B	HDGF	HMGA1
HMGB3	HSF1	IFI16	LRRFIP1	MTA1	RLF	SAP30BP	STAT1
STAT3	TEAD1	TFE3	TOX4	ZBTB33	ZC3H15	ZNF148	ZNF451
ZNF93							

Intercellular Signal Molecule

IK
TMPO (Accession ID: P42166)

Membrane Traffic Protein

ARCN1	ARFIP1	BCAP31	BET1	BET1L	CHMP4B	CHMP5	CLINT1	CLTA	COPG2	ERC1	ESYT1
ESYT2	GAK	IGF2R	REPS1	SDCBP	SEC61B	SNCB	VAMP1	VAPA	VAPB		

Metabolite Interconversion Enzyme

ADSL	ALDH3A2	ALG13	DPYSL4	G6PD	GAPDH	GFPT1	HINT1	HLCS	KYNU	NME2	NMT1
NUDT5	PFKP	PGAM1	PIP4K2C	PKM	PLA2G4A	PPIP5K2	PRDX1	TXNRD1	WWOX		

Phosphatase

INPP5F

Protein Modifying Enzyme

ATG3	BAZ1B	CACYBP	CUL4B	FER	HERC4	MAP4K4	NEDD4L	NRBP1	PA2G4	
PAK2	PAK4	PPME1	PPP1CB	PSMD1	PSMD12	PSMD2	PSMD8	PTPN1	PTPN11	
RFPL4A	RIPK2	SYVN1	TRIM25	TRIM56	UBE2M	UBE2O	USP7	UTRN	VCPIP1	

Protein-Binding Activity Modulator

ARF3	ARFGAP1	ARHGAP1	ARHGAP35	CAST	CCNK	CCNT1	DOCK7	GAPVD1	NSFL1C	PDCD5	RAB11FIP5
RAB1A	RAB3GAP1	RABL3	RAN	RANGAP1	SIPA1L2	TRIO					

RNA Metabolism Protein

ATXN2L	CSTF2	CSTF2T	DDX10	DDX3X	DDX42	DDX46	DHX36	DHX9	DIDO1	EIF4A1	FUS
G3BP1	GTF2F1	GTF2F2	GTF2I	GTF3C5	HNRNPA1	IGF2BP3	KHSRP	LARP1	LSM14A	NCL	NONO
NOP58	PCBP2	PES1	PRRC2A	PRRC2C	PSPC1	RBM26	SERBP1	SFPQ	SNRPD2	SPEN	SUB1
TAF1	TAF7	TARDBP	TCOF1	U2SURP	YTHDC2	ZC3H7A	ZFR	ZMAT2			

Scaffold/Adaptor Protein

ABI1	AIMP2	ANKS1A	CACTIN	CD2AP	CEP152	CEP170	CHORDC1	CLASRP	DBNL	DLG1	DLGAP4
EHBP1	EHBP1L1	ENAH	GCN1	HNRNPF	HP1BP3	LRBA	MAGED1	MAGED2	MVP	NCK2	NUP50
PLIN3	PRPF3	PUF60	RANBP1	RANBP2	RANBP3	RBM17	RBM39	RRBP1	SART3	SF3A1	SF3B2
SH3D19	SH3GL1	SH3PXD2B	SNRPA1	SNRPGP15	SNX1	SNX4	SON	SUGP1	TANC1	XAB2	YWHAB
ZRANB2											

Structural Protein

CRYBG1

Transfer / Carrier Protein

OSBPL11	OSBPL8

Translational Protein

ABCF2	EEF1A1	EEF1A2	EEF1B2	EEF1D	EEF2	EIF3G	EIF3H	EIF4B	EIF4E	EIF4G1	EIF4G2
EIF4G3	EIF4H	EIF5	EPRS1	GSPT2	RARS1	RPL19	RPL24	RPL6	RPS2	RPS20	RPS3A
TARS1											

Transmembrane Signal Receptor

GPRC5A	PGRMC2	TMEM87A

Transporter

ATP11B	CCAR2	NUP214	NUP98	TPR	VCP	

Unclassified

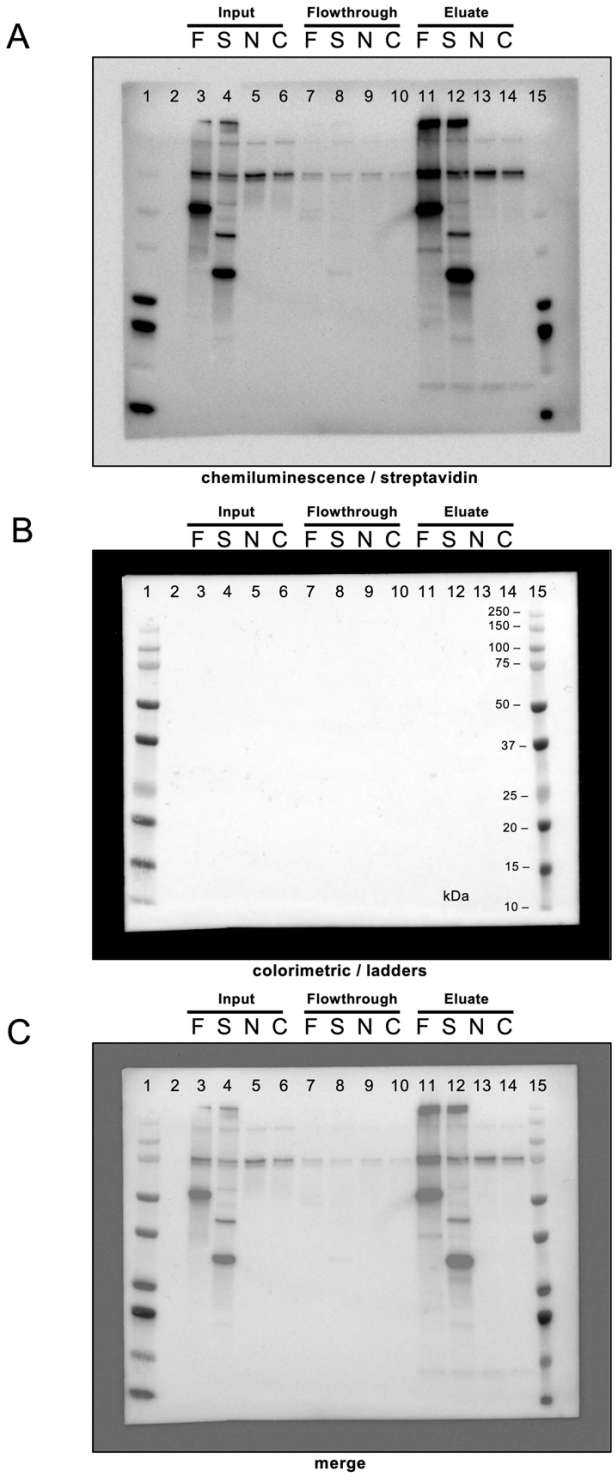
AAK1	ACIN1	ADNP	AGFG1	AHNAK	AHNAK2	ALMS1	AP1AR	ATIC	BOD1L1	C1orf198	C2CD2
CBX3	CCDC124	CCDC50	CCZ1B	CDV3	CISD2	CKAP4	CNN2	COMMD8	CSDE1	CWC15	DAP
DBN1	DNMBP	EGLN1	EMD	ENSA	ESF1	FAM107B	FASN	FIP1L1	FLNB	GEMIN5	GSDME
HCFC1	HGS	HIVEP1	HNRNPU	HNRNPUL1	HNRNPUL2	IWS1	KNL1	LASP1	LMO7	LSM12	LUZP1
MIA	MTDH	MTUS1	NBR1	NCAPG	NCAPH	NEDD8	NELFE	NHLRC2	NKAPD1	NOL11	NUCKS1
NUFIP2	PAICS	PCNP	PDAP1	PLEKHA1	PNISR	PNN	PPP1R10	PPP1R18	PTMA	PTPN12	PUS7
RAI14	RBM10	RBM33	RCC2	RIC8A	RP9	RUFY1	RUVBL1	SAFB	SAMD9	SKA3	SLAIN2
SLTM	SMAP	SMC4	SND1	SP100	SQSTM1	SRP72	SRRM2	STBD1	STEEP1	STIP1	SYMPK
SYNM	TACC3	TAGLN2	THOC7	TLN1	TMA7	TNKS1BP1	UBAP2L	UFD1	UNC45A	VCL	VMA12
WBP11	WDR36	YKT6	ZC3H18	ZC3HC1	ZDBF2	ZMYND8	ZNF318				

Not Found

PALM2AKAP2	TMPO (Accession ID: P42167)

Supplementary Fig. 3 Proximal Proteome of Unseeded Multimeric Only aSYN

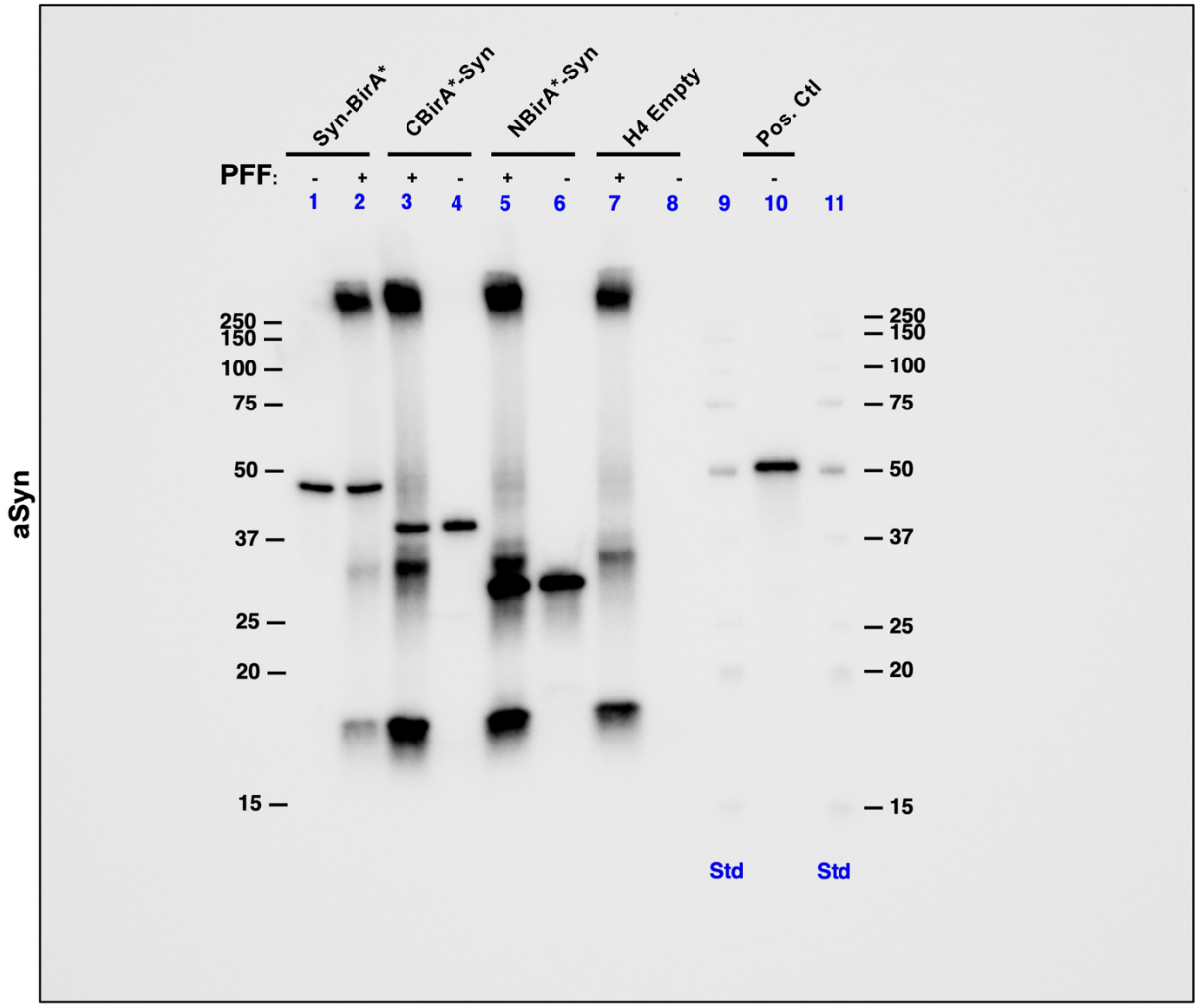
A. Gene names of the 487 identified proteins proximal to non-seeded Split Syn-BirA*, organized by encoding protein class. Proteins included were identified in ≥ 2 of 3 replicates. Protein classes were queried through PANTHER Database v19. These proteins represent potential interactors of physiologically healthy multimeric aSYN.



Supplementary Fig. 4 Uncropped Blot from Figure 1D Demonstrating Enrichment of Biotinylated Proteins

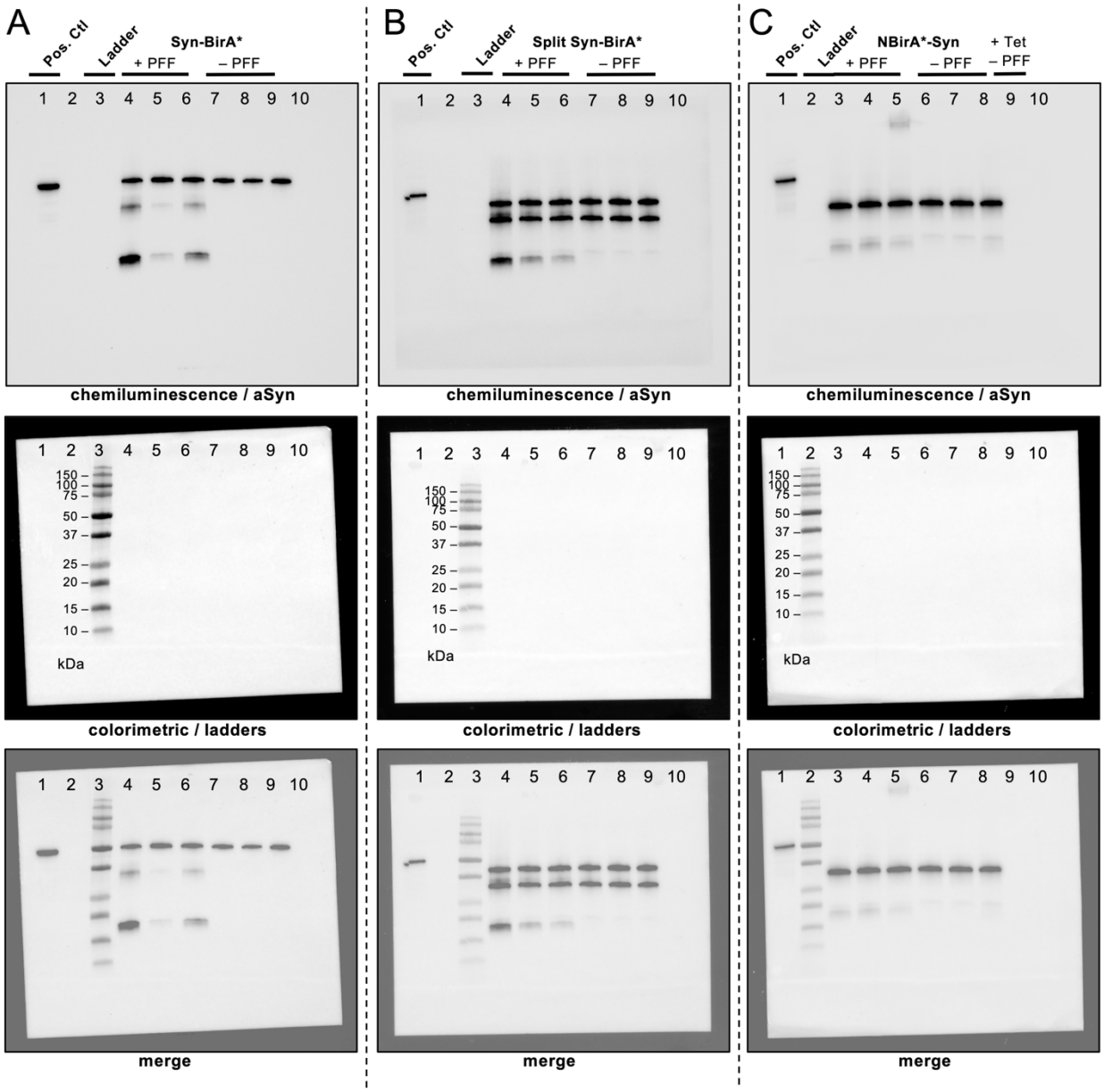
Lanes 11-14 were shown in the main text to highlight the biotinylated proteins found in lysates after streptavidin enrichment. Lanes 1 & 15 are ladders. Lane 2 is blank (no sample). Lanes 3-6 represent starting H4 stable cell lysate for pulldown (aka "input") for Syn-BirA* ("F"), Split Syn-BirA* ("S"), NBirA*-Syn ("N") and Syn-CBirA* ("C").

Lanes 7-10 represent the flowthrough from the streptavidin pulldown; these samples are expected to show reduced levels of biotinylated proteins. Lanes 11-14 represent final enriched biotinylated proteins (aka “eluate). **A.** Chemiluminescence only imaging showing anti-streptavidin HRP signal. **B.** Colormetric only imaging to visualize the ladder. **C.** Merge of blots from (A) and (B). Blot was not moved between colorimetric and chemiluminescent imaging.



Supplementary Fig. 5 Uncropped Blot Demonstrating PFF-associated Bands

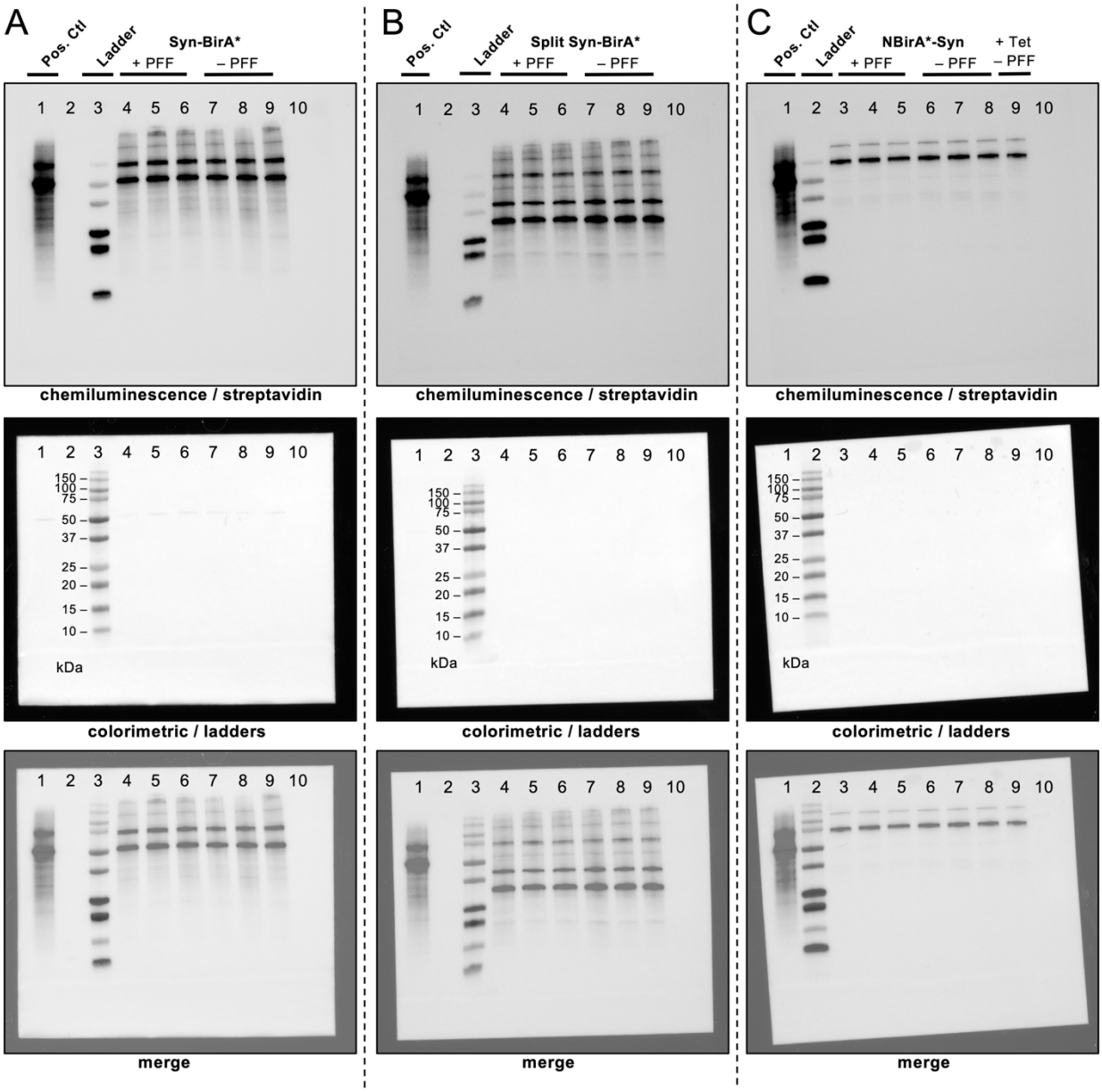
Uncropped blots used for Supp. Figure 1A. Lane 7 was chosen for Supplementary Figure 1A to demonstrate bands associated with the addition of pre-formed fibrils (PFFs) in H4 cells lacking the expression of a BirA* construct. Lanes 9 & 11 are ladders. Lanes 1, 4, 6 and 8 represent H4 cell lysates no fibrils added. Lanes 2, 3, 5 and 7 represent H4 cell lysates with the addition of PFFs. H4 Empty Cells had no construct integration or expression. Lane 10 represents a western blot positive control using Syn-BirA* cell lysate generated during a prior experiment. This control ensured the western blot procedure worked as expected and was not relevant to subsequent analysis of the blot. Chemiluminescence imaging was used.



Supplementary Fig. 6 Uncropped anti-aSyn Blots of Samples Analyzed by Proteomics

Uncropped blots used for Supp. Figure 1B. Three blots (panels A-C) were visualized via chemiluminescent imaging for the probe of interest (top), colorimetric imaging to visualize ladders (middle), and the merged images were used for analysis (bottom). In all blots, Lane 1 represents a western blot positive control using cell lysate generated during a prior experiment. This control ensured the western blot procedure worked as expected and was not relevant to subsequent analysis of the blot. Lane 10 was blank in all blots. Lysate from Syn-BirA* (**A**), Split Syn-BirA* (**B**) and NBirA*-Syn (**C**) samples analyzed by proteomics were run on western blot and probed for aSyn via

chemiluminescent imaging. Blots were not moved between colorimetric and chemiluminescent imaging. **C.** An additional cell lysate – H4 NBirA*-Syn cells + tetracycline was run on western blot (Panel C, Lane 9) demonstrating that no construct was expressed in the presence of tetracycline.



Supplementary Fig. 7 Uncropped Biotinylated Protein Blots of Samples Analyzed by Proteomics

Uncropped blots used for Supp. Figure 1D. Three blots (panels A-C) were visualized via chemiluminescent imaging for the probe of interest (top), colorimetric imaging to visualize ladders (middle), and the merged images were used for analysis (bottom). In all blots, Lane 1 represents a western blot positive control using cell lysate generated during a prior experiment. This control ensured the western blot procedure worked as expected and was not relevant to subsequent analysis of the blot. Lane 10 was blank in all blots. Lysate from Syn-BirA* (**A**), Split Syn-BirA* (**B**) and NBirA*-Syn (**C**) samples analyzed by proteomics were run on western blot and probed for biotinylated proteins via

chemiluminescent imaging using streptavidin-HRP. Blots were not moved between colorimetric and chemiluminescent imaging. **C.** An additional cell lysate – H4 NBirA*-Syn cells + tetracycline was run on western blot (Panel C, Lane 9) demonstrating that endogenous biotinylation detected was not noticeably different with or without NBirA*-Syn expression.