Supplemental figures

Supplemental Figure 1: Fluorescence recovery for FRAP experiments depicted in Figure 1. Percent of original fluorescence was plotted as a function of time following photobleaching for ADFP-YFP and LSDP5-YFP. Data are means ± SEM from 7 or 8 experiments.

Supplemental Figure 2: PAT proteins at the lipid droplet surface recover similarly from 50% photobleaching. FRAP analysis of perilipin-GFP, ADFP-GFP and LDSP5-YFP in CHO-K1 cells incubated overnight with 400 μM oleic acid. Live cells were examined with a confocal microscope using a 40x oil immersion objective. Red boxed regions were bleached at time 0; fluorescence within these regions was monitored at 15 s intervals. Bar: 1 μm.

Supplemental Figure 3: Negative and positive controls used for AFRET experiments. (A) Confocal imaging of CHO-K1 cells co-transfected with perilipin-YFP and LSDP5-CFP (top), and CGI-58-CFP and perilipin-YFP (bottom). (B) Measurement of AFRET between perilipin-YFP and LSDP5-CFP, and CGI-58-CFP and perilipin-YFP in basal conditions. Data are means ± SEM from 10 or 12 experiments. (C) IP of perilipin-YFP and LSDP5-YFP from CHO-K1 cell extracts. Lysates were immunoprecipitated without antibody (None), or with anti-GFP poly-clonal antibody, anti-perilipin antibody, or anti-LSDP5 antibody; immunoblots were probed with GFP monoclonal antibody. Experiment is representative of 7 experiments. (D) Co-IP of perilipin A (untagged) with CGI-58-CFP from CHO-K1 cell extracts in basal (B) conditions. IPs were performed with control IgG or anti-CGI-58 antibody. Western blot was first incubated with anti-perilipin antibody and subsequently with anti-CGI-58 antibody. Experiment is representative of 9 experiments.

Supplemental Figure 4: Untagged HSL interacts with untagged perilipin A. CHO cells expressing ectopic Perilipin A (Peri A) cells were transduced with HSL adenovirus. Cells were incubated in basal conditions (B) or stimulated conditions (S). IPs were performed using a rabbit anti-HSL IgG or pre-immune rabbit IgG (IgG). Immunoprecipitates were analyzed by Western blot probed with an anti-perilipin antibody or an anti-HSL antibody. Experiment is representative of 2 experiments.

Supplemental Figure 5: Overexpression of the PAT-1 domain of perilipin prevents binding of phosphorylated HSL to the surfaces of perilipin-coated lipid droplets. (A) CHO cells stably overexpressing untagged perilipin A (Peri A cells) were co-transfected with either 1) HSL-CFP and a construct driving expression of YFP or 2) CFP-HSL and perilipin (1-121)-YFP. Cells were incubated overnight with 400 μM oleic acid. The following day, the cells were incubated with 5 μM triacsin C with no further additions (basal), or stimulated for 15 min with 10 μM forskolin, 1 mM IBMX and 5 μM triacsin C; live cells were examined with confocal microscopy as in Fig 1. Bar, 10 μm. (B) Quantitative analysis for HSL binding to the lipid droplet under PKA-stimulated conditions. Data are means ± SEM from 8 to 13 experiments (*, $p < 0.05$).

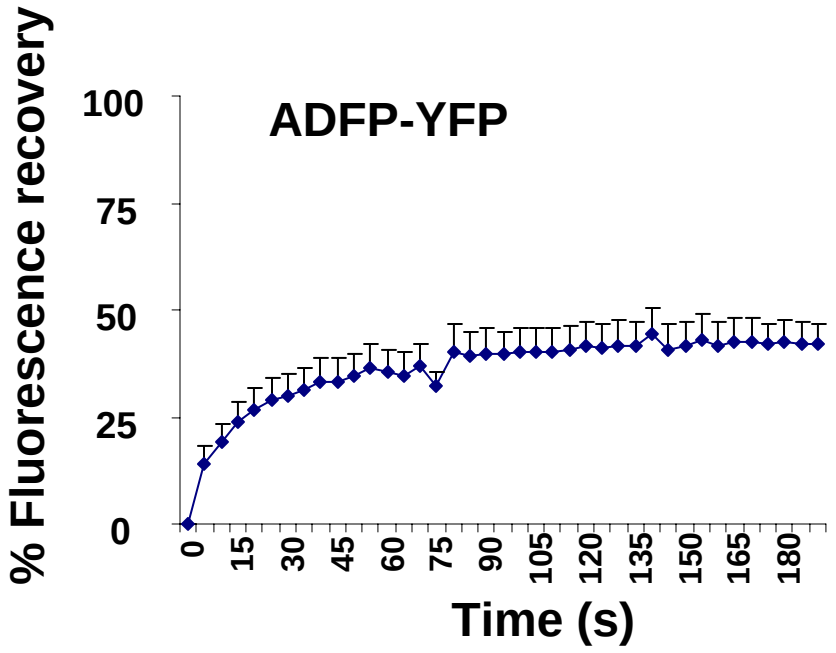
Supplemental Figure 6: The PAT-1 domains of both LSDP5 and perilipin interact with HSL. (A)Western blot of IP of HSL with the PAT-1 domain of perilipin (left; Peri-YFP 1-121)

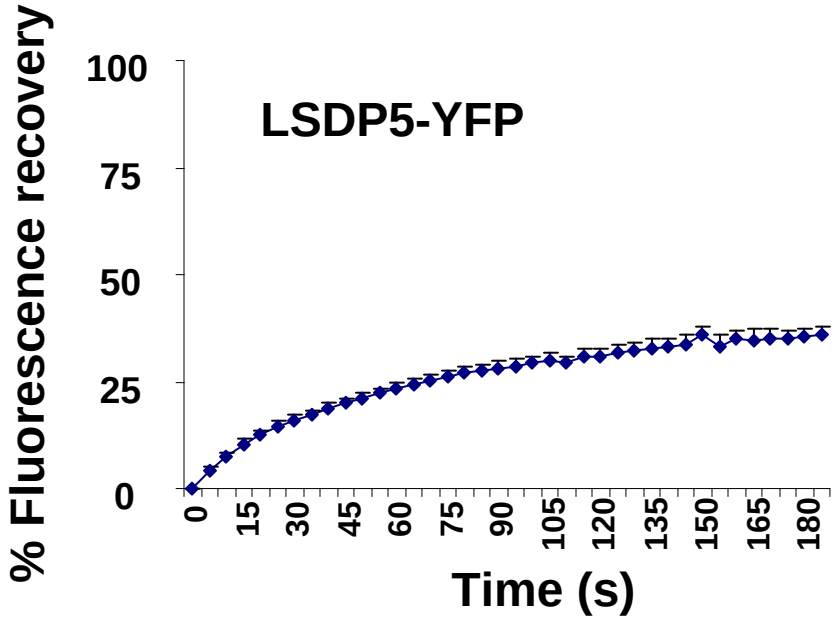
and the PAT-1 domain of LSDP5 (right; LSDP5-YFP 1-123) from CHO-K1 cell lysates. (B) Western blot of IP of HSL with the full length LSDP5 (top) and the 11-mer repeat sequence (PAT-2 domain) of LSDP5 (right; LSDP5-YFP 11-mer) from CHO-K1 cell lysates. Cells were incubated in basal conditions (B) or stimulated conditions (S). IPs were performed using a rabbit anti-HSL IgG or mouse anti-rabbit (control) IgG (IgG). Immunoprecipitates were analyzed by Western blot probed with a GFP antibody. Experiment is representative of 2 experiments.

Supplemental Figure 7: The phosphorylation of serine 81, 222, 276 of perilipin is required for recruitment of HSL to perilipin-coated lipid droplets. Complete set of representative confocal images for Figure 6, including images for the YFP channel, CFP channel and merged images. Cells were incubated overnight with 400 μM oleic acid. The following day, the cells were incubated with 5 μM triacsin C (basal) or stimulated for 10 min with 10 μM forskolin, 1 mM IBMX and 5 μM triacsin C; live cells were examined with a confocal microscope as in Fig 1. Bar, 10 μm.

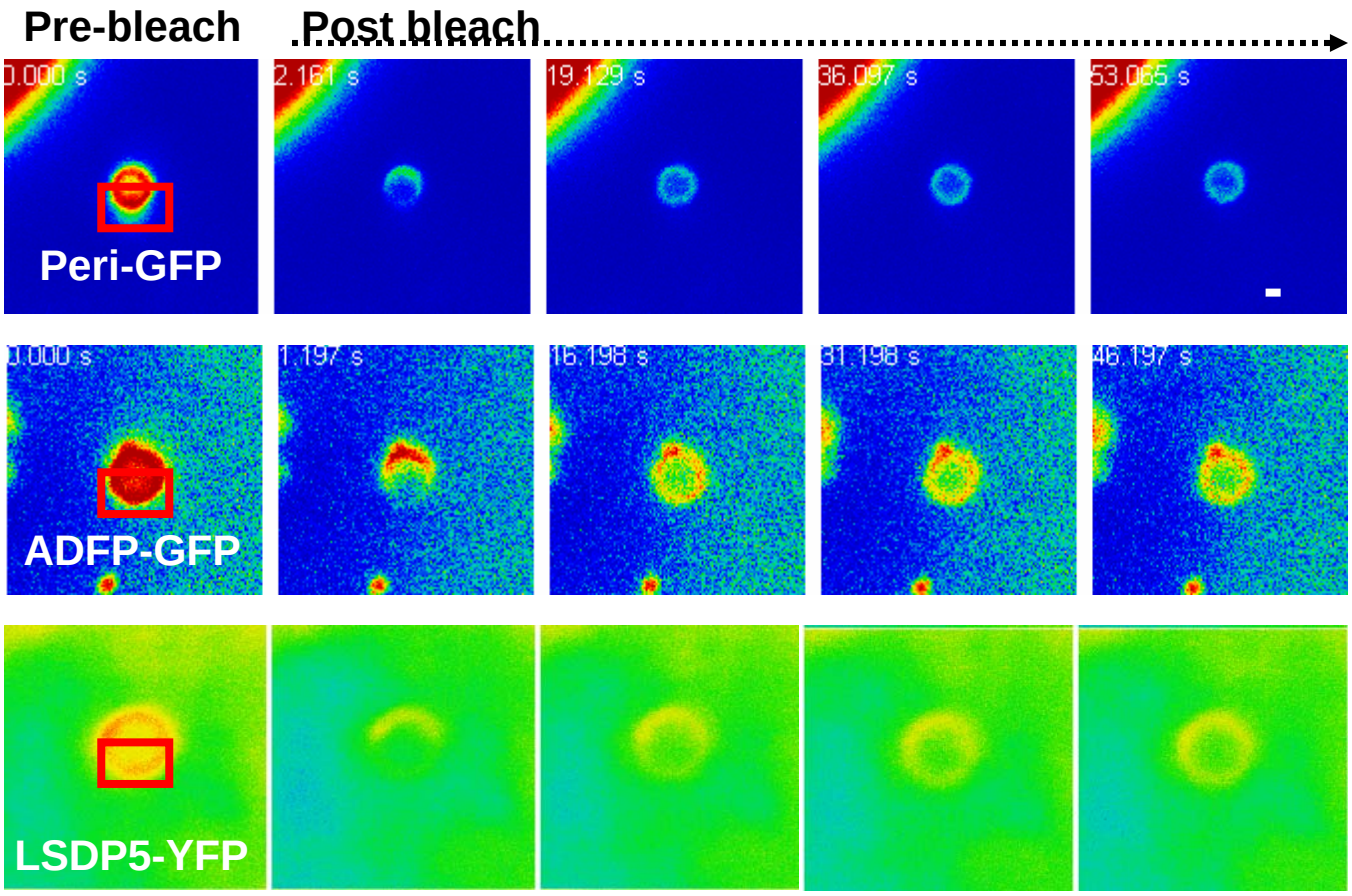
Supplemental Figure 8: HSL binding to perilipin requires the phosphorylation of serine 81, 222 and 276 of perilipin A. (A) Representative Western blot of IPs of HSL with mutated forms of perilipin. Plasmids for S81A Perilipin-YFP, S222A perilipin-YFP, S276A perilipin-YFP, perilipin STriA-YFP, and perilipin SQuadA-YFP were transfected into CHO-K1 cells stably expressing HSL-GFP. Cells were incubated in basal (B) and stimulated (S) conditions. Cell extracts were used for IP with control rabbit pre-immune IgG or rabbit anti-mouse HSL IgG, and Western blots were probed using a GFP antibody. Experiment depicted is representative of three experiments.

Supplemental Figure 1

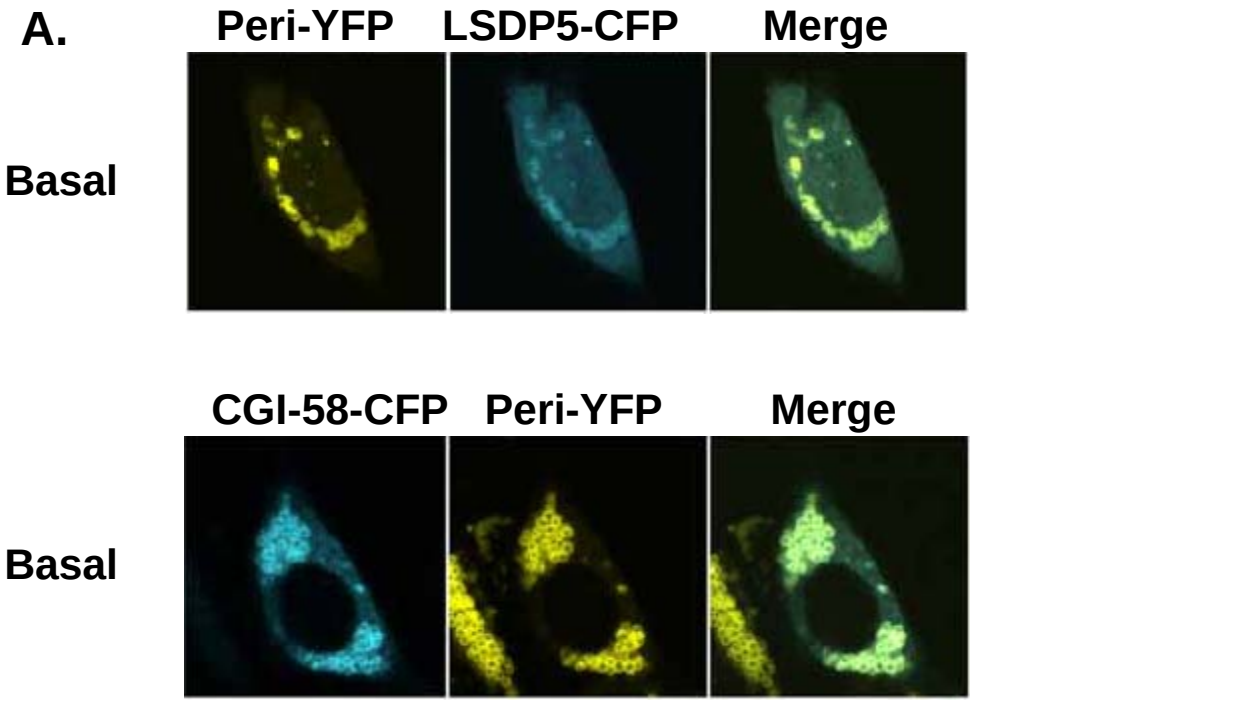




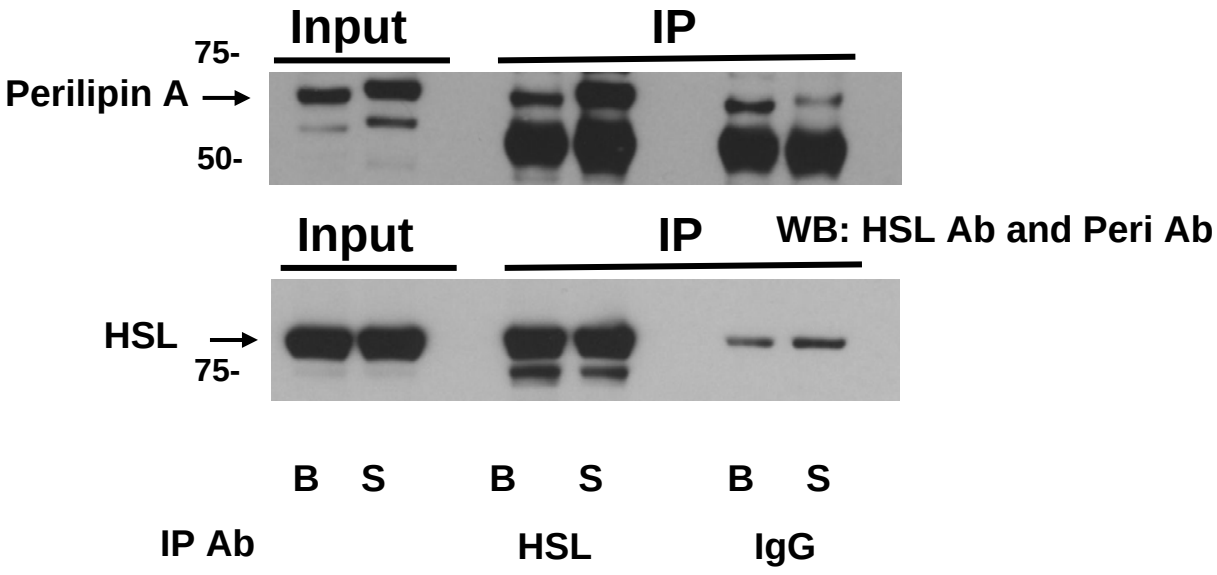
Supplemental Figure 2



Supplemental Figure 3

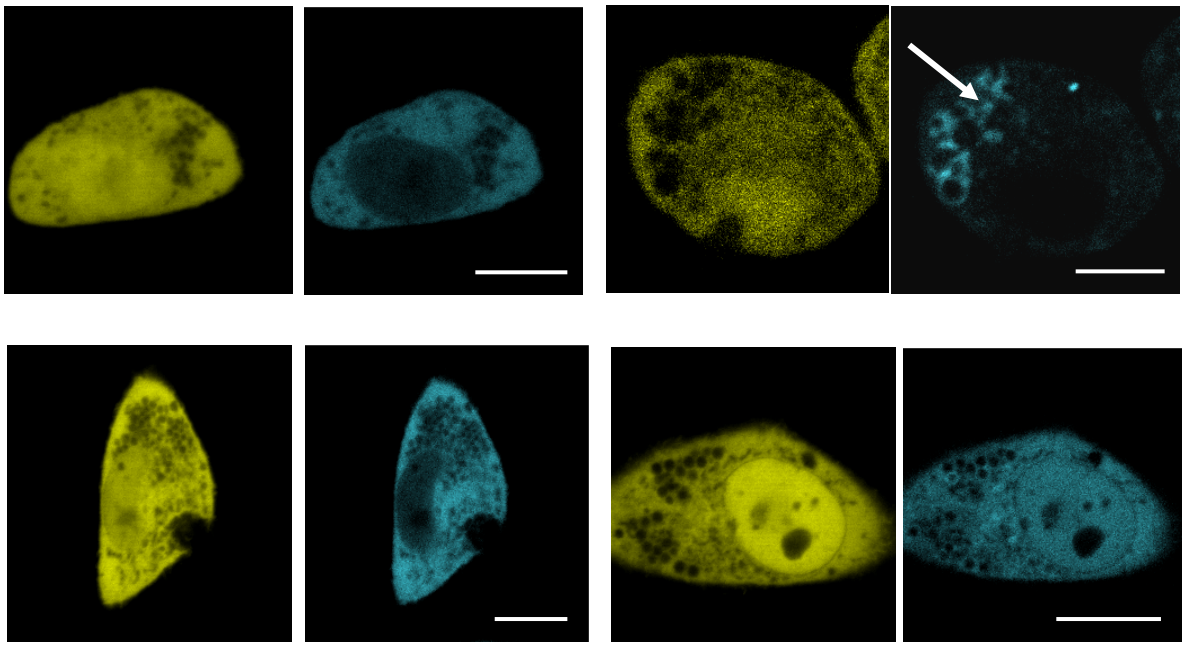


Supplemental Figure 4



Supplemental Figure 5

A.



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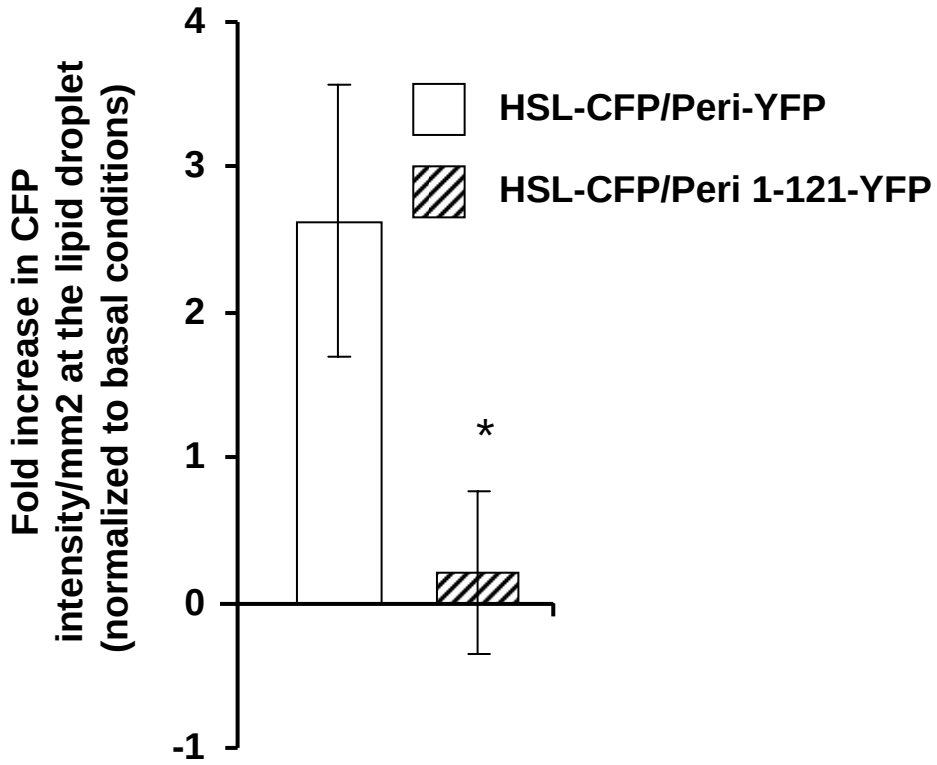
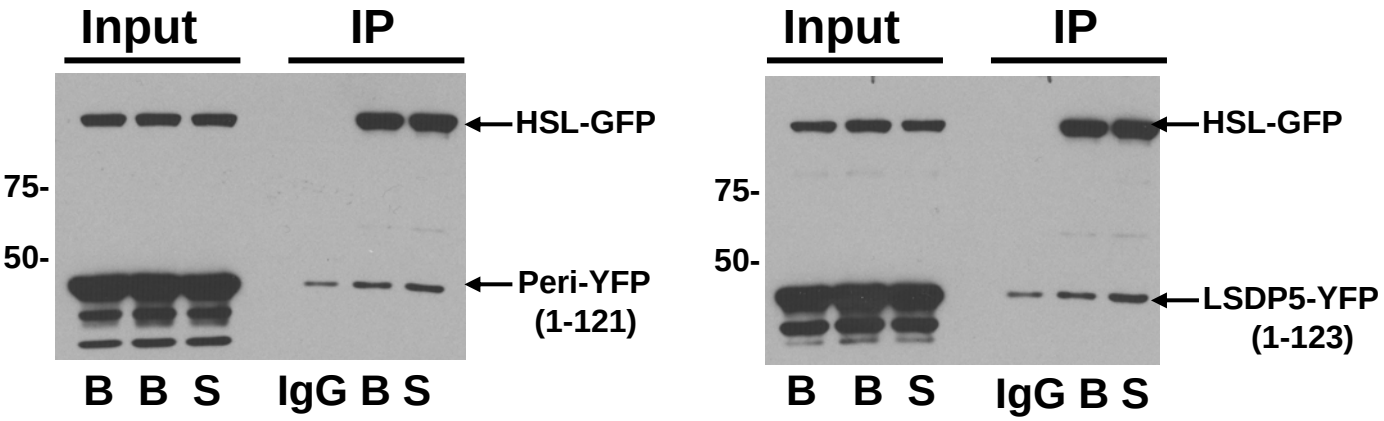


Supplement Figure 6

A.



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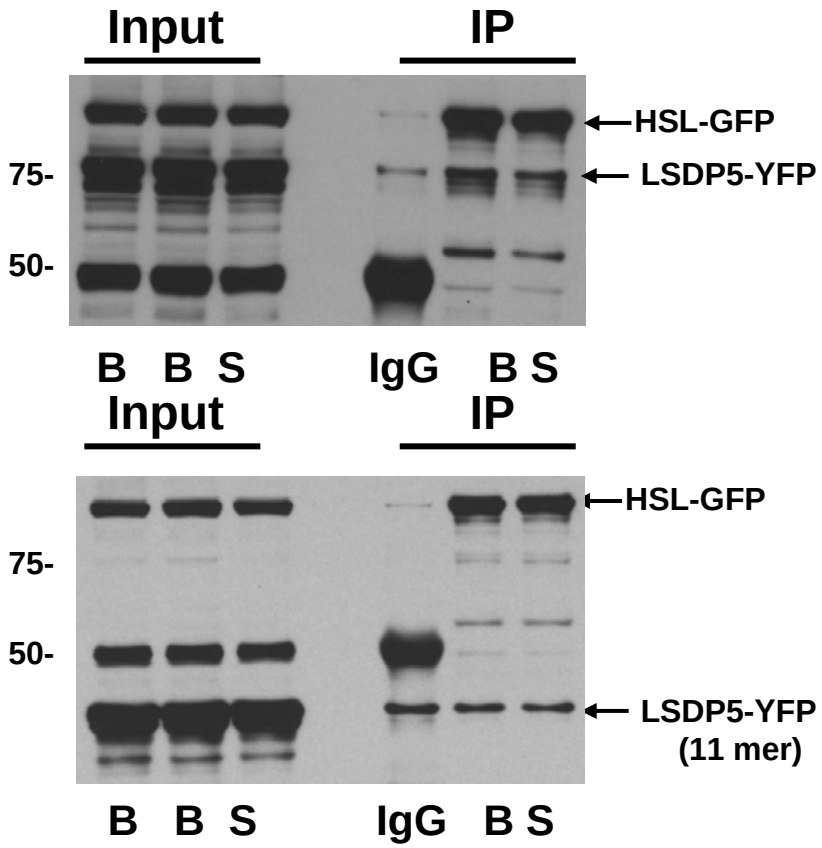
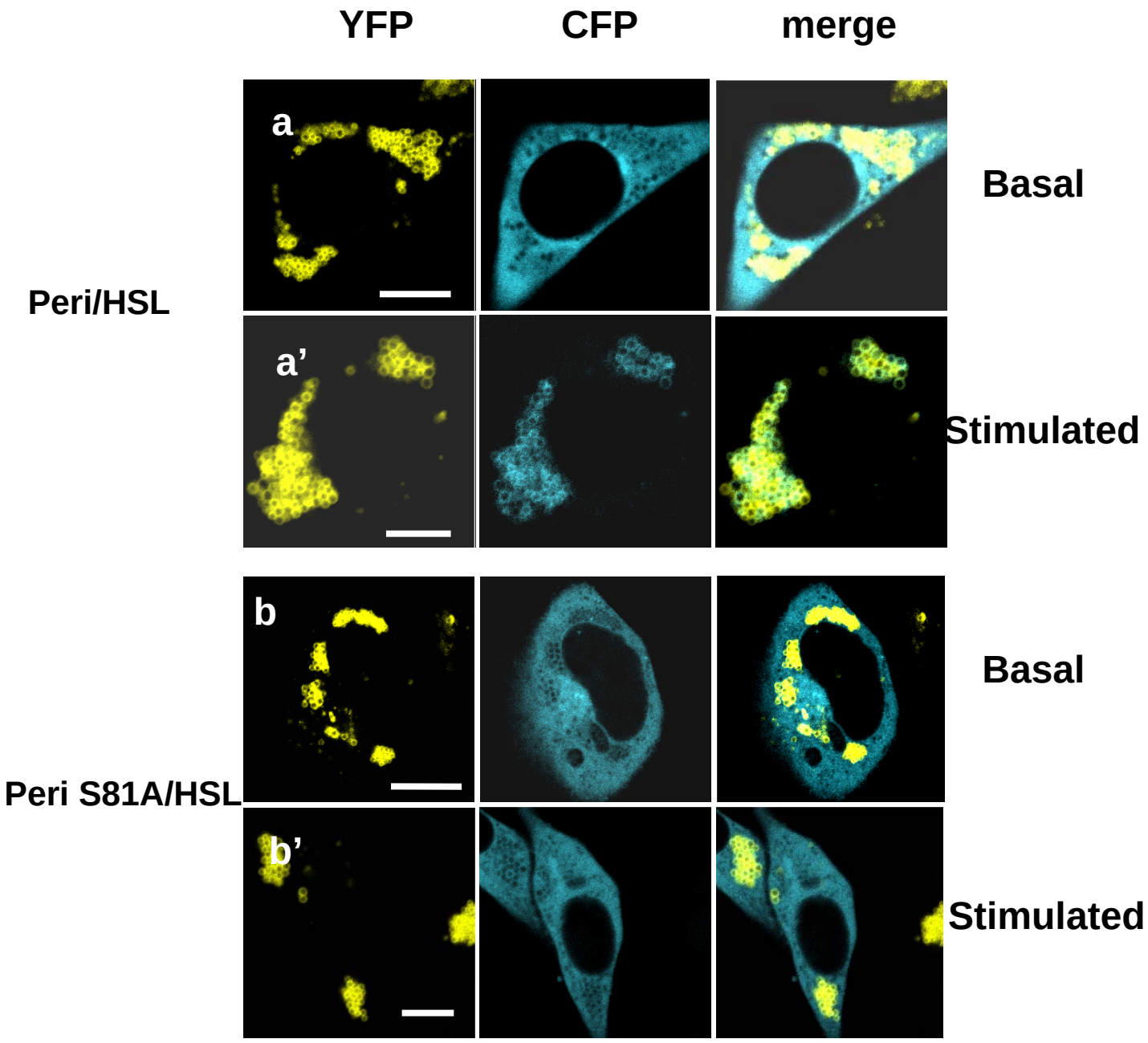
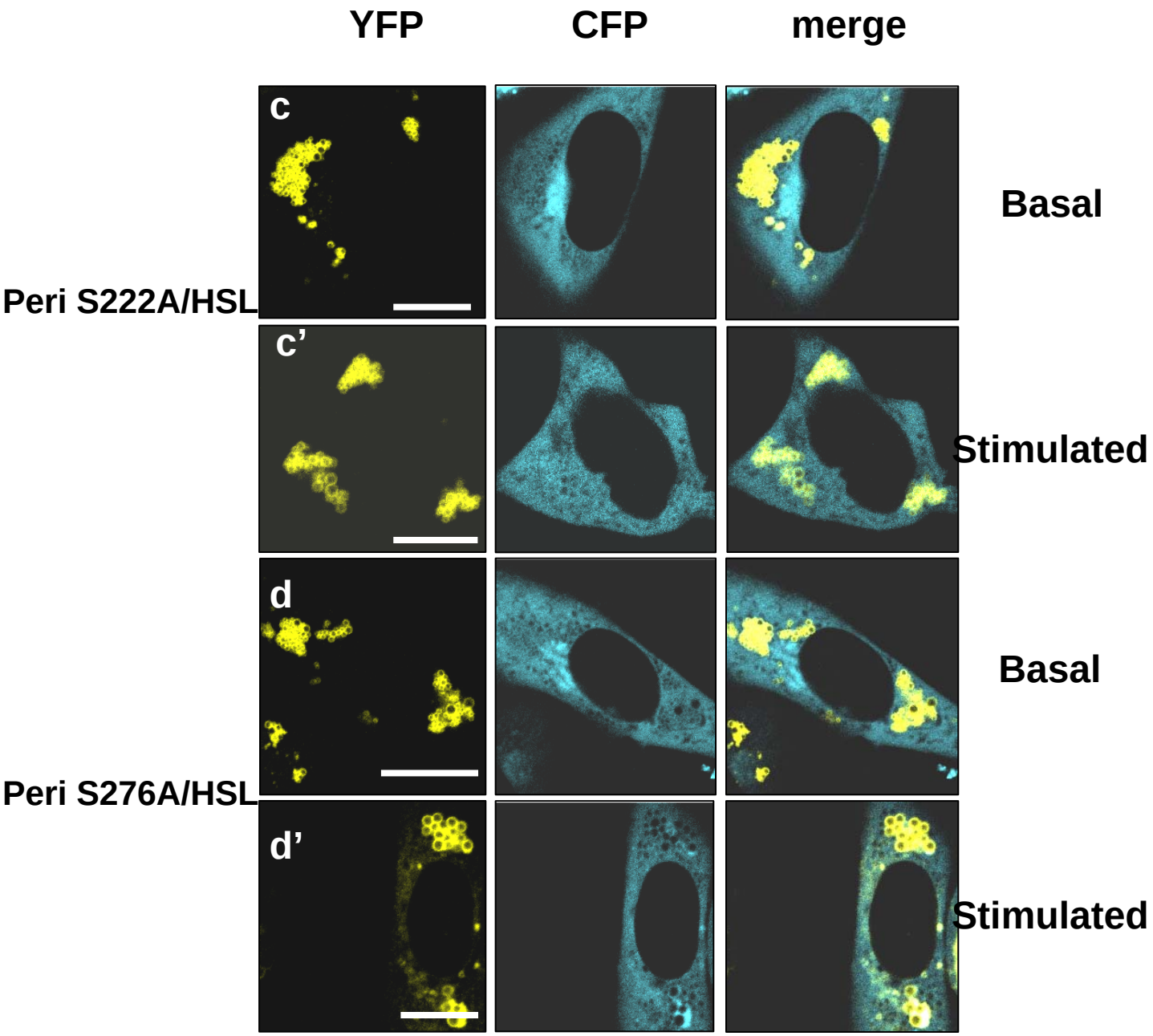


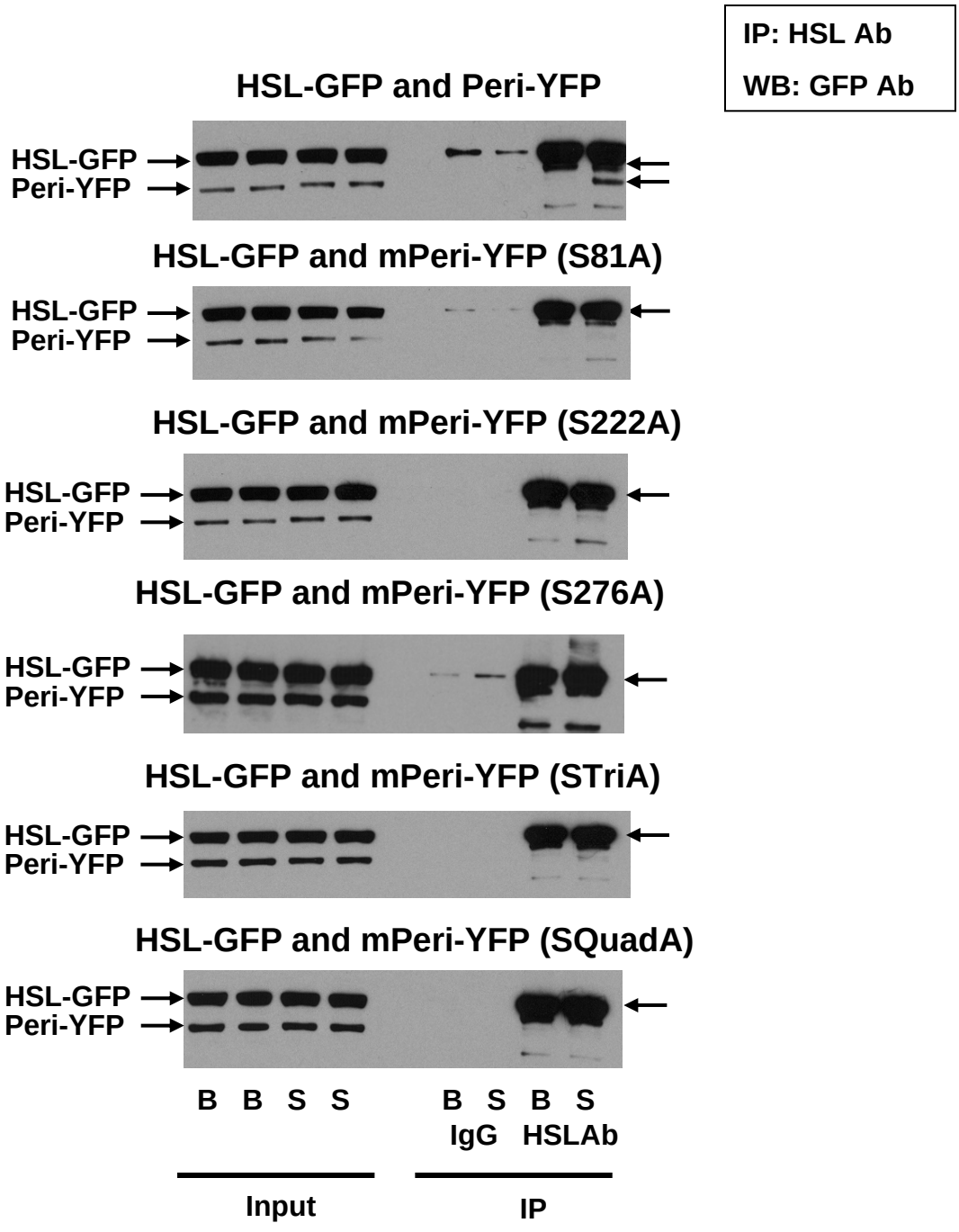
Supplement Figure 7A



Supplement Figure 7B



Supplemental Figure 8



Supplemental tables

Table 1 Primers used for constructs

Construct Name	PCR Primer Sequence	Digestion Sites
HSL-CFP-N1	Forward 5'-GGCAGATCTGATGGATTTACGCACAATGAC-3'	BglII
	Reverse 5'-ATTTAAAGCTTGGTCAGCGGTGCAGCAGG-3'	/HindIII
Perilipin-YFP-C1	Forward 5'-TAGAGCTCGGATGTCAATGAACAAGGGCC-3'	SacI
	Reverse 5'-TAGGTACCGCAGTCTGCTCAGCTCTTCTTGC-3'	/KpnI
LDSP5-YFP-C1	Forward 5'-TAGAGCTCAGGAAATGGACCAGAGAGGTGAAG-3′	SacI
	Reverse 5'-TAGGTACCCCTCGATAGTCAGAAGTCCAGCTC-3′	/KpnI
ADFP-YFP-C1	Forward 5'-TAGGTACCAAAATGGGAGCAGCAGTAGTGGAT-3′	KpnI
	Reverse 5'-TAGGTACCAGGAGGGGTTTACTGAGCTTTGAC-3′	/KpnI
Tip47-YFP-C1	Forward 5'-TAGAGCTCCCATGTCTAGCAATGGTACAGAT-3′	SacI
	Reverse 5'-TAGGTACCTCCCTACTTCCCTTCAGGGGTTT-3′	/KpnI
CGI-58-CFP-C1	Forward 5'-CAGTAAGCTTCGAAAGCGATGGCGGCGGAGGA-3'	HindIII
	Reverse 5'-CCGTCGGATCCTCAGTCTACTGTGTGGCAGATC-3'	/BamHI

Table 2
Cell types and antibodies used

Protein/protein Interaction	Cell line	Transfection	Immunoprecipitation antibody	Western Blot antibody
HSL/Perilipin	HSL-GFP	Perilipin-YFP	HSL	GFP
HSL/LDSP5	HSL-GFP	LDSP5-YFP	HSL	GFP
HSL/ADFP	HSL-GFP	ADFP-YFP	HSL	GFP
HSL/Tip47	HSL-GFP	Tip47-YFP	HSL	GFP
Perilipin/CGI-58	Perilipin A	CGI-58-C	CGI-58	Perilipin /CGI-58
Perilipin/LSPD-5	CHO-K1	Perilipin-YFP/ LDSP5-YFP	Perilipin/LDSP5	GFP