

## SUPPLEMENTAL INFORMATION

### **Figure S1 refers to Figure 1 and STAR methods section. Lentiviral constructs that allow multiplexing.**

(A) Schematic shows the structure of the lentiviral vectors used in this study. See STAR methods for details on construction of this vector from its backbone. Basically, we have made multiple vectors with combinations of fluorescent protein tags and drug selection. The excitation, emission, and brightness of each fluorescent protein is shown.

### **Figure S2 refers to Figure 2. Co-IP of LMO2 and LDB1 and mutant proteins shows lysineless LMO2 is deficient in binding to LDB1.**

- (A) Clustal alignment of LIM domain Only paralogs, LMO1, LMO2, and LMO4. Arrows denote lysine residues throughout LMO2 protein.
- (B) Immunoblot with various antibodies of transduced Jurkat cell lysate. Expression control (GFP, mScarlet) and loading controls (VCP) are included. LDB1 WT and mutant proteins, LDB1 $\Delta$ LID, LDB1(I322A), LMO2 WT, and LMO2 K(0), a lysineless version of LMO2 with arginine substituted for each lysine.
- (C) Immunoprecipitation from same Jurkat cell lysate with anti-FLAG to pull down FLAG-LDB1. IgG capture is shown with blots for Fc and light chain.
- (D) Immunoprecipitation from same Jurkat cell lysate with anti-LMO2 antibody to pull down LMO2.

### **Figure S3 refers to Figure 4. SSBP2 and SSBP3 half-lives and immunoblots.**

- (A) Immunoblot of transduced Jurkat cells with various antibodies. SSBP2 and SSBP3 were N-terminally tagged with Halo and co-expressed with LDB1 WT or various mutant LDB1 proteins, LDB1 $\Delta$ LCCD is deficient in binding to SSBP proteins; K134R and K365R are arginine substitutions that can stabilize LDB1 from degradation. Expression control (EBFP2) and loading controls (tubulin and VCP) are included.
- (B) HaloLife assay was performed in Jurkat cells for Halo-SSBP2 with  $t_{1/2}$  shown.

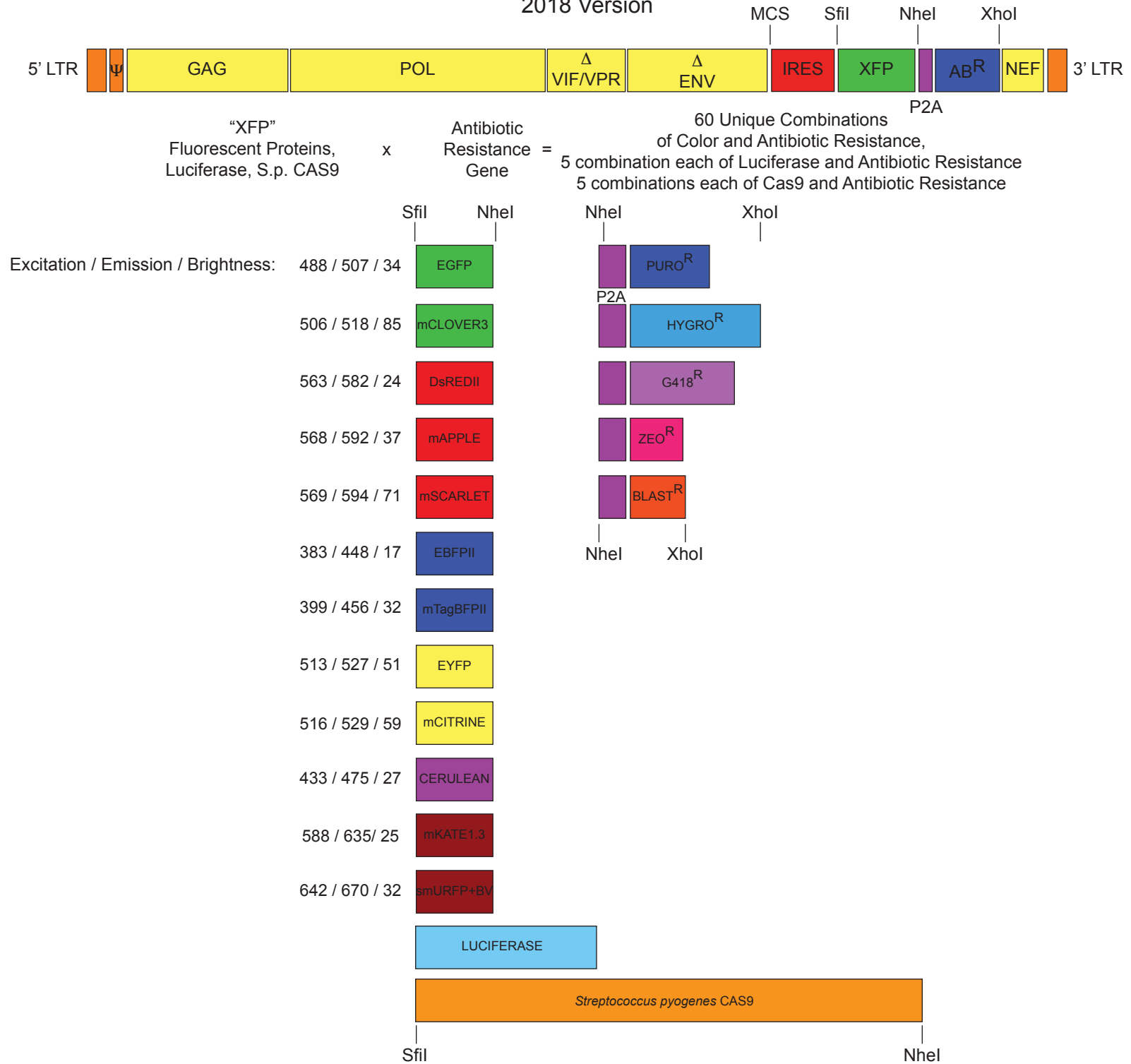
(C) HaloLife assay was performed in Jurkat cells for Halo-SSBP3 with  $t_{1/2}$  shown. Table in inset shows the lysines within SSBP proteins are located within the LUFS domain responsible for binding to LDB1 protein.

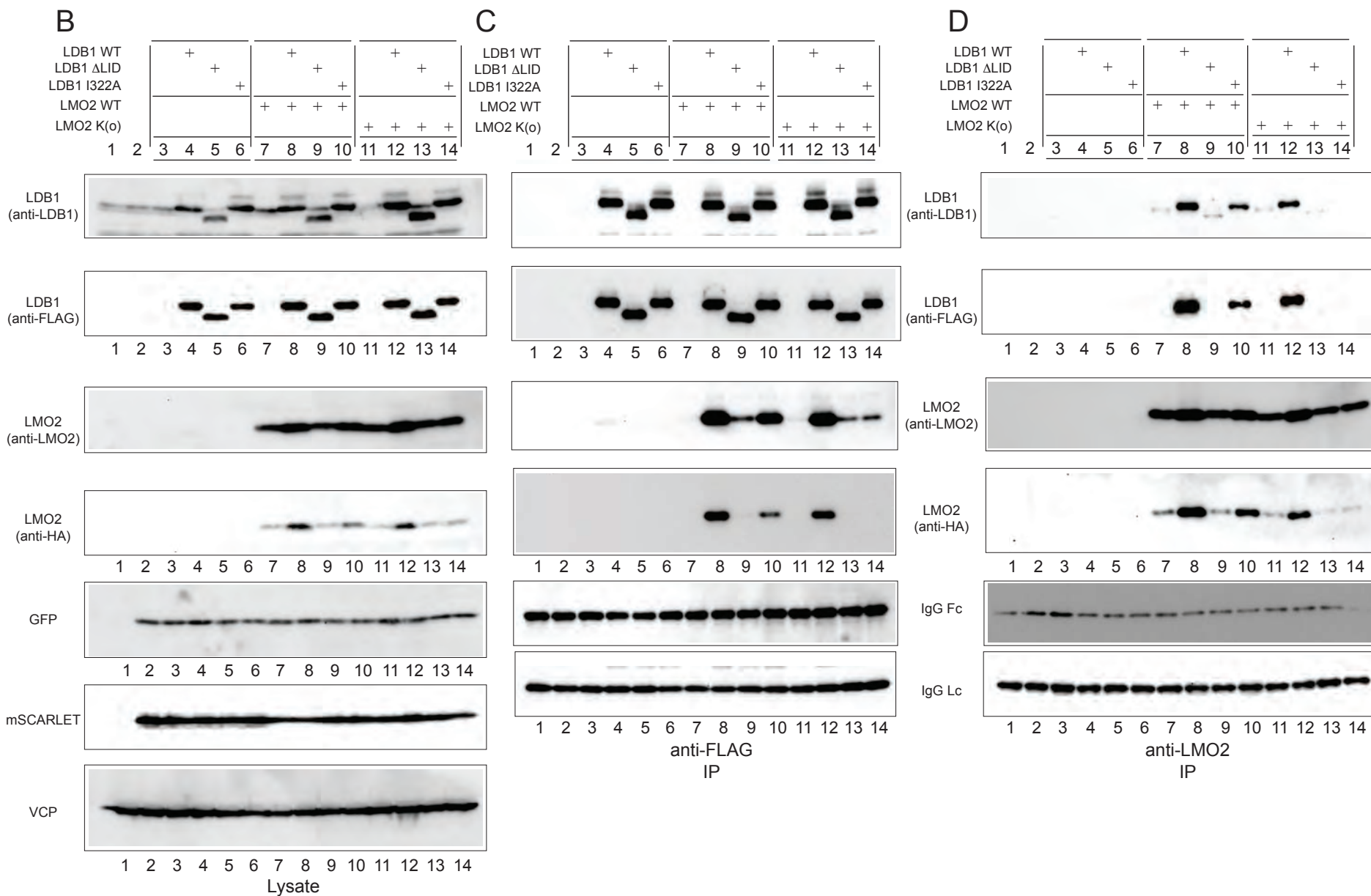
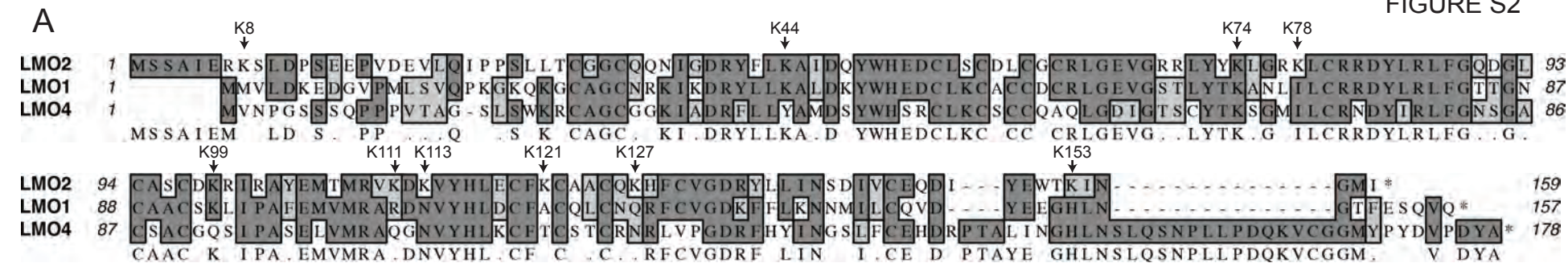
**Figure S4 refers to Figure 7. Full table of DUB screen using HaloLife assay.** Top left panel shows schematic of experiment for screening DUBs in the HaloLife assay. The effect of DUB shRNA knockdown was analyzed for each Halo-tagged subunit protein. In the following tables, DUBs are grouped in families. First column shows whether they are expressed in Jurkat cells followed by their effect upon the stability of Halo-tagged proteins. Yellow denotes 2 out of 3 criteria met. Red shows 3 out of 3 criteria met.

**Figure S5 refers to discussion section. Confocal imaging of Halo-tagged proteins.** Confocal microscopy images of Jurkat cells showing localization of Halo-tagged proteins. Cells were transduced with our multiplexed lentiviral expression vectors to express HALO-tagged LMO2, LDB1, Tal1, Lyl1, SSBP2, and SSBP3, respectively. Non-transduced Jurkat cells were imaged as a negative control, shown in first row. Cellular localization of Halo-tagged proteins was determined by calculating the ratio of mean HaloTag R110 signal intensities within the nucleus versus the cytosol. Nuclear and cytosolic regions were established from SYTO Red 17 and EBFPII signals, respectively.

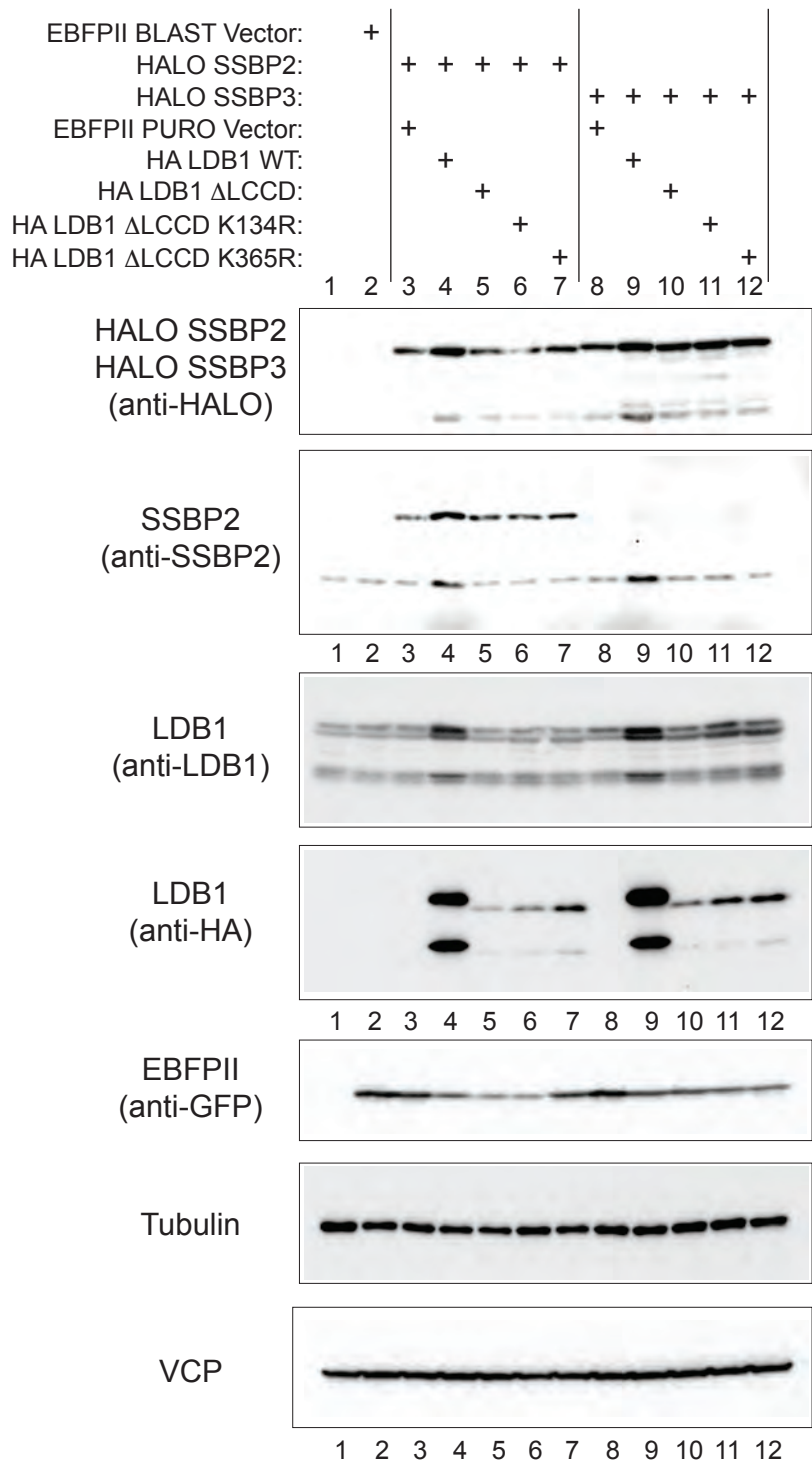
FIGURE S1

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 Multiplex Lentiviral Expression Vector System  
 2018 Version

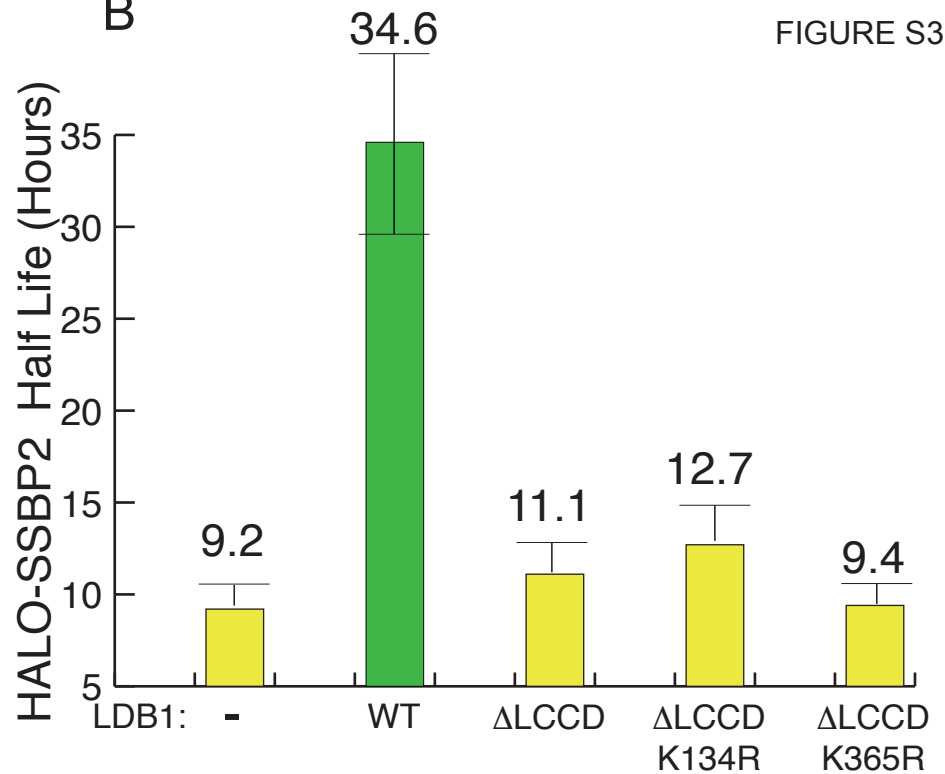




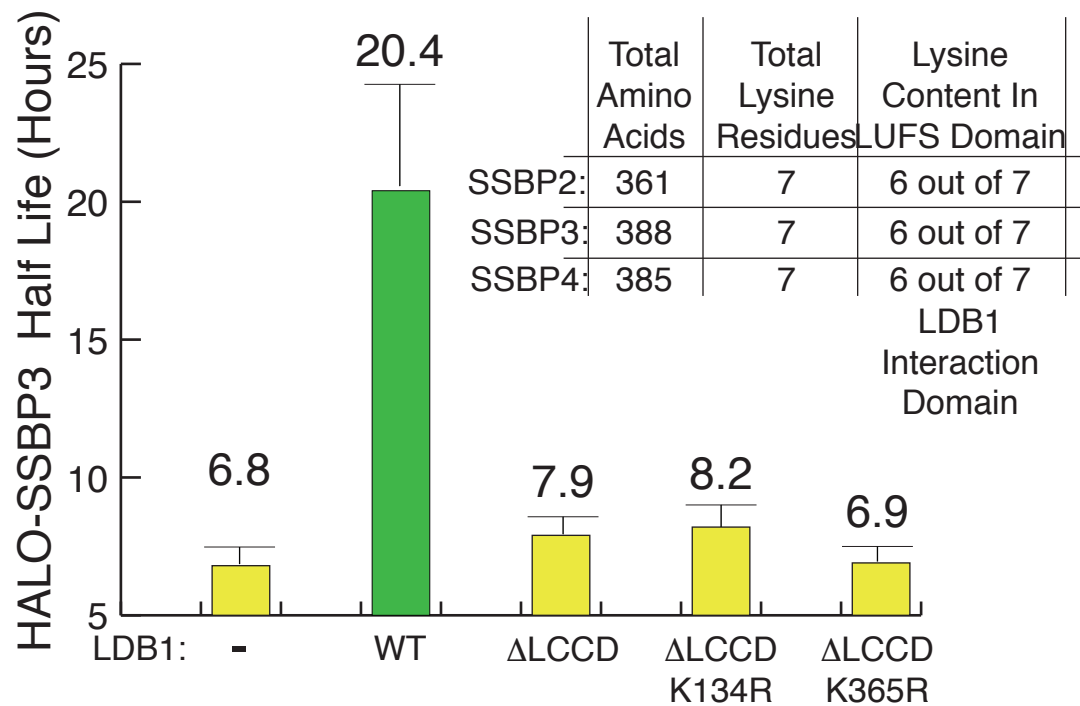
A



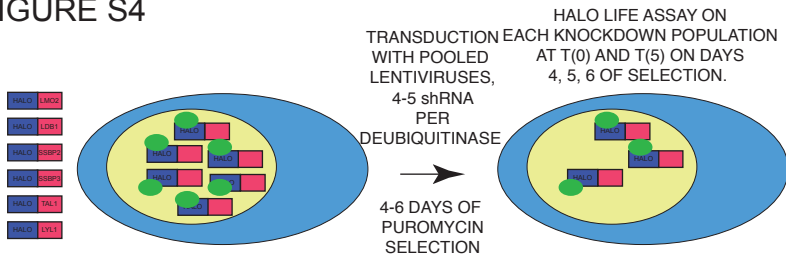
B



C



**FIGURE S4**



POSITIVE HITS SCORED BY:

- I. REDUCED % OF T(0) DUB shRNA TO T(0) SCRAMBLED shRNA
- II. REDUCED T(0) HALO SIGNAL
- III. REDUCED % OF T(0) at T(5)

FULFILLS AT LEAST 2 CRITERIA

FULFILLS ALL 3 CRITERIA

MISSING shRNA (14):

- USP19
- USP24
- USP27
- USP35
- USP45
- FAM63A
- OTULIN
- OTU1
- OTUD1
- OTUD3
- OTUD4
- AT7L3
- JOSD2
- ZUFSP

**OTU FAMILY (11 Tested)**

	Present in JURKAT?	$\Sigma\Delta\Omega$ TPOQTH?	HALO LMO2	HALO LDB1	HALO SSBP2	HALO SSBP3	HALO TAL1	HALO LYL1
OTUB1	NO	NO						
OTUB2	YES	NO		YES				
OTUD5	YES	NO						
OTUD6A	?	NO				YES		
OTUD6B	YES	YES					YES	
OTUD7A	YES	YES						
OTUD7B	YES	NO	YES	YES	YES	YES	YES	YES
ALG13	YES	SEVERE	YES	YES	YES	YES	YES	YES
TNFAIP3	?	SEVERE						
VCIPI1	YES	NO						YES
ZRANB1	?	NO		YES				YES

**MINDY FAMILY (5 Tested)**

	Present in JURKAT?	$\Sigma\Delta\Omega$ TPOQTH?	HALO LMO2	HALO LDB1	HALO SSBP2	HALO SSBP3	HALO TAL1	HALO LYL1
FAM63B	YES	YES					YES	YES
FAM105A	?	NO						
FAM105B	?	NO			YES	YES		YES
FAM188A	YES	YES					YES	
FAM188B	YES	YES						YES

**UCLH FAMILY (3 Tested)**

	Present in JURKAT?	$\Sigma\Delta\Omega$ TPOQTH?	HALO LMO2	HALO LDB1	HALO SSBP2	HALO SSBP3	HALO TAL1	HALO LYL1
UCLH1	NO	YES		YES	YES	YES		
UCLH3	YES	NO			YES			
UCLH5	YES	NO						
BAP1	YES	N.D.						

**MJD FAMILY (1 Tested)**

	Present in JURKAT?	$\Sigma\Delta\Omega$ TPOQTH?	HALO LMO2	HALO LDB1	HALO SSBP2	HALO SSBP3	HALO TAL1	HALO LYL1
ATXN3	YES	N.D.						
JOSD1	YES	NO						

**USP FAMILY (35 Tested)**

	Present in JURKAT?	$\Sigma\Delta\Omega$ TPOQTH?	HALO LMO2	HALO LDB1	HALO SSBP2	HALO SSBP3	HALO TAL1	HALO LYL1
USP1	?	SEVERE			YES		YES	
USP2	NO	NO						
USP3	YES	NO	YES	YES	YES	YES		YES
USP4	YES	NO	YES	YES	YES	YES		
USP5	YES	SEVERE						
USP6	YES	N.D.						
USP7	YES	YES						
USP8	YES	N.D.						
USP9X	YES	N.D.						
USP9Y	?	N.D.						
USP10	YES	N.D.						
USP11	YES	N.D.						
USP12	?	NO						
USP13	YES	NO						
USP14	YES	N.D.						
USP15	YES	YES						YES
USP16	YES	NO						
USP17L	?	N.D.						
USP18	?	NO						
USP20	YES	SEVERE						
USP21	?	NO						
USP22	YES	NO						
USP25	YES	NO						
USP26	?	NO						YES
USP28	YES	SEVERE						
USP29	?	NO						
USP30	YES	NO						
USP31	NO	YES						
USP32	YES	SEVERE						
USP33	YES	NO						
USP34	YES	N.D.						
USP36	YES	NO						
USP37	YES	NO						
USP38	YES	N.D.						
USP39	?	NO						
USP40	YES	NO			YES			YES
USP42	YES	NO						
USP44	?	NO						
USP46	NO	NO						
USP47	YES	NO						
USP48	YES	NO		YES	YES	YES		YES
USP49	?	NO						YES
USP50	?	N.D.						
USP51	?	N.D.						
USP53	NO	N.D.						
USP54	NO	N.D.						
USPL1	?	NO						
CYLD	YES	NO						
PAN2	?	YES						

FIGURE S5

