Cell, volume 126

Supplemental Data

Resource

A Rapid, Reversible, and Tunable Method to Regulate Protein Function in Living Cells Using Synthetic Small Molecules

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Supplemental Figures

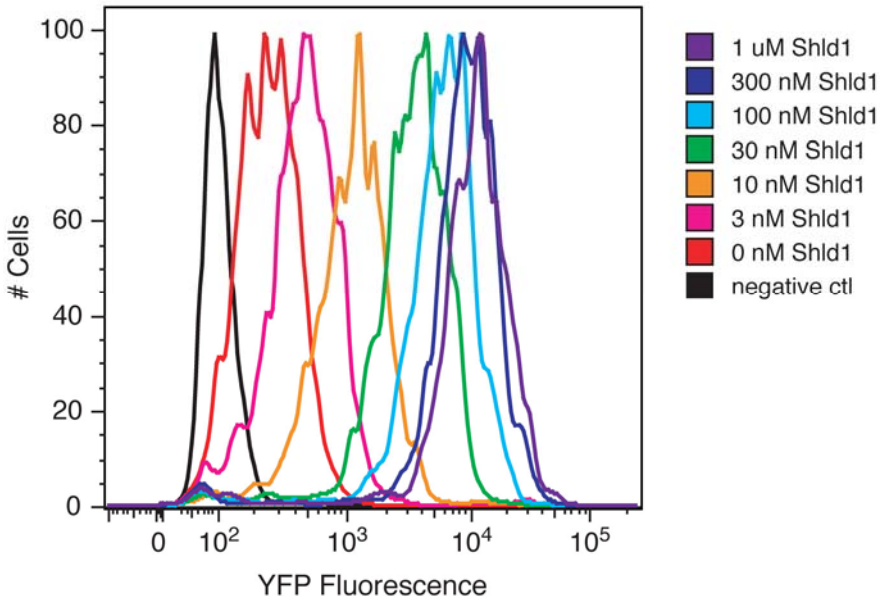

Figure S1. Response of the L106P-YFP fusion to varying concentrations of Shld1. NIH3T3 cells stably expressing the L106P-YFP fusion were titrated with three-fold dilutions of Shld1 (1 μM to 3 nM) and monitored by flow cytometry. Data shown are a representative example of an experiment performed in triplicate.

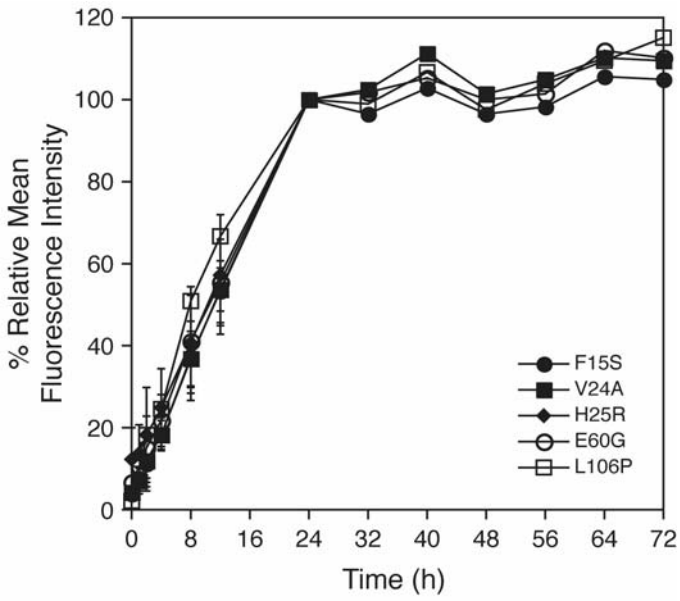

Figure S2. Kinetics of FKBP-YFP fluorescence growth upon addition of Shld1. NIH3T3 cells stably expressing the indicated FKBP-YFP fusions were treated with 1 μM Shld1, and increases in fluorescence were monitored over time using flow cytometry. Data are presented as the average mean fluorescence intensity (MFI) ± SEM relative to that of the maximum fluorescence intensity observed for the individual mutant. The experiment was performed in triplicate, and MFI was normalized to 100% at 24 hr.

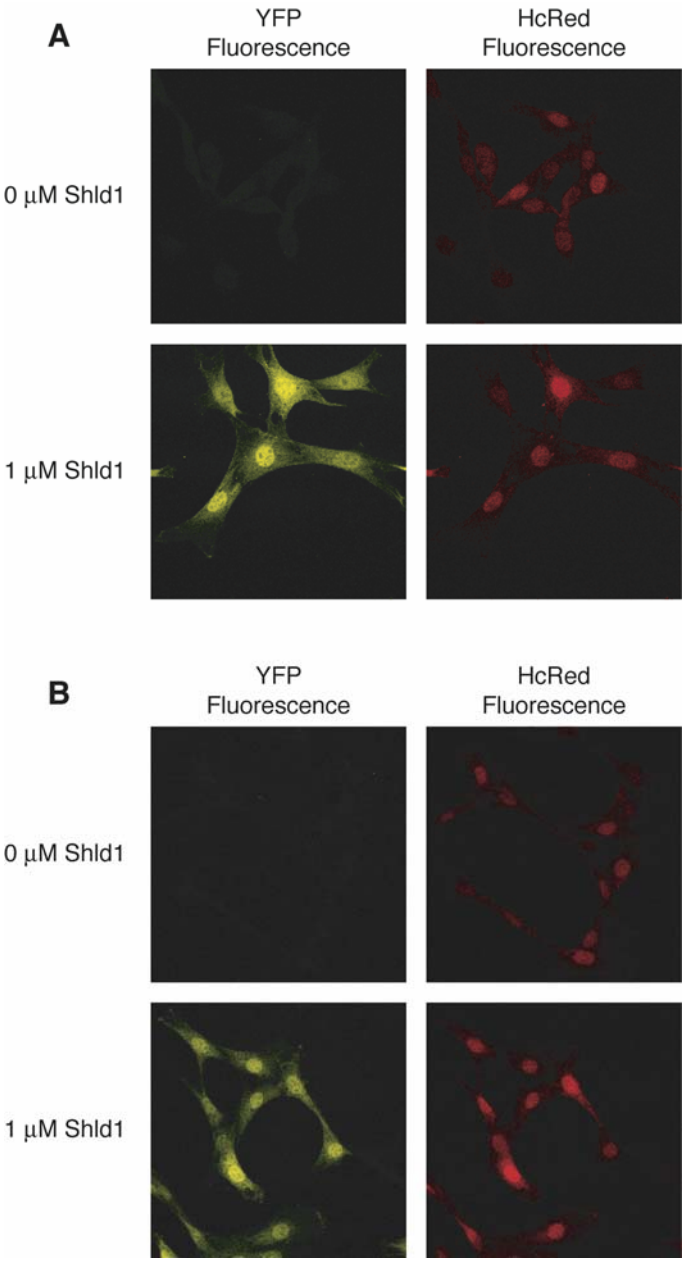

Figure S3. Fusion of an FKBP destabilizing domain to YFP results in Shld1-dependent fluorescence. **(A)** NIH3T3 cells stably expressing an FKBP F15S-YFP fusion were either mock-treated or treated with 1 μM Shld1 for 24 hrs. Cells were then fixed and visualized using fluorescence microscopy. HcRed fluorescence serves as a marker of infection. **(B)** NIH3T3 cells stably expressing an FKBP L106P-YFP fusion were treated as above.

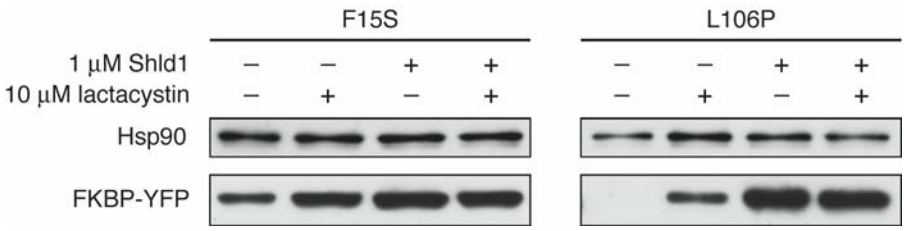

Figure S4. Degradation of FKBP-YFP fusions is mediated by the proteasome. NIH3T3 cells stably expressing F15S-YFP and L106P-YFP were treated with 1 μM Shld1 for 24 hrs. Cells were then washed and treated with 10 μM lactacystin in the presence and absence of 1 μM Shld1 for 4 hrs. Immunoblotting was performed with an anti-YFP antibody.

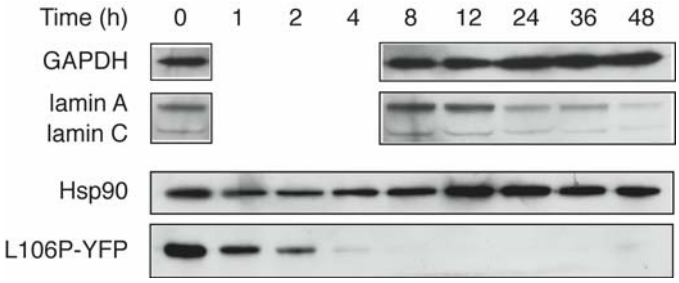

Figure S5. DD-mediated degradation shows faster kinetics than RNAi-mediated silencing. HeLa cells were transfected with 30 nM siRNA against lamin A/C and monitored over time. Time required for knockdown of lamin A/C is compared against time required for degradation of L106P-YFP upon removal of Shld1 from NIH3T3 cells stably expressing the fusion.

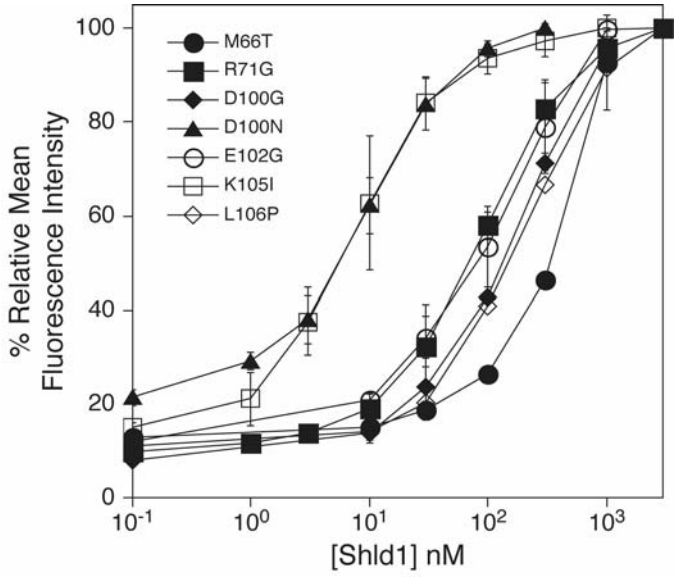

Figure S6. Response of YFP-FKBP fusions to varying concentrations of Shld1. NIH3T3 cells stably expressing YFP-FKBP fusions were titrated with three-fold dilutions of Shld1 (3 μM to 0.1 nM) and monitored by flow cytometry. Data are presented as the average mean fluorescence intensity ± SEM relative to that of the maximum fluorescence intensity observed for the individual mutant. Experiment was performed in triplicate.

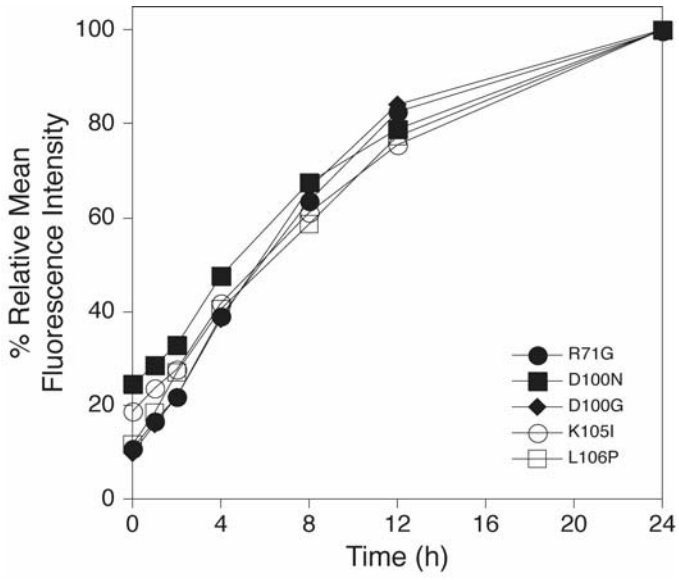

Figure S7. Kinetics of YFP-FKBP fluorescence growth upon addition of Shld1. NIH3T3 cells stably expressing YFP-FKBP fusions were treated with 1 μM Shld1, and increases in fluorescence were monitored over time using flow cytometry. Data are presented as the average mean fluorescence intensity (MFI) relative to that of the maximum fluorescence intensity observed for the individual mutant. MFI was normalized to 100% at 24 hr.

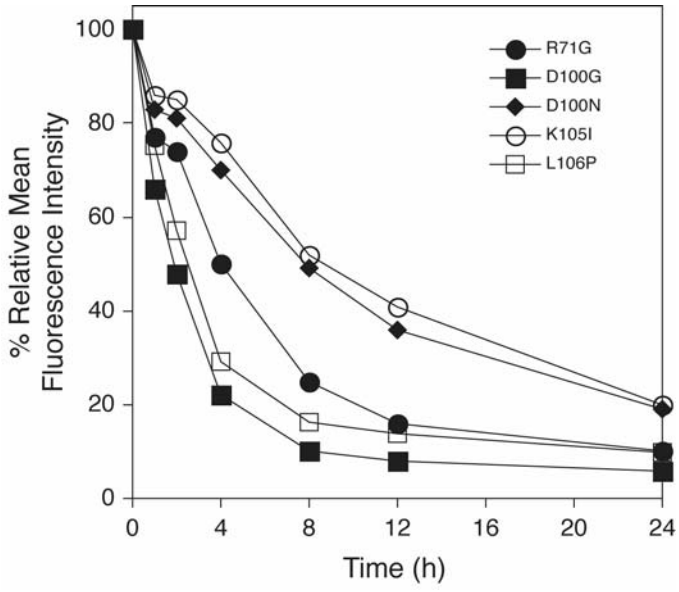

Figure S8. Kinetics of YFP-FKBP fluorescence decay upon removal of Shld1. NIH3T3 cells stably expressing YFP-FKBP fusions were treated with 1 μM Shld1 for 24 hours at which point the cells were washed with media to remove Shld1, and decreases in fluorescence were monitored using flow cytometry. Data are presented as the average mean fluorescence intensity relative to that of the maximum fluorescence intensity observed for the individual mutant.

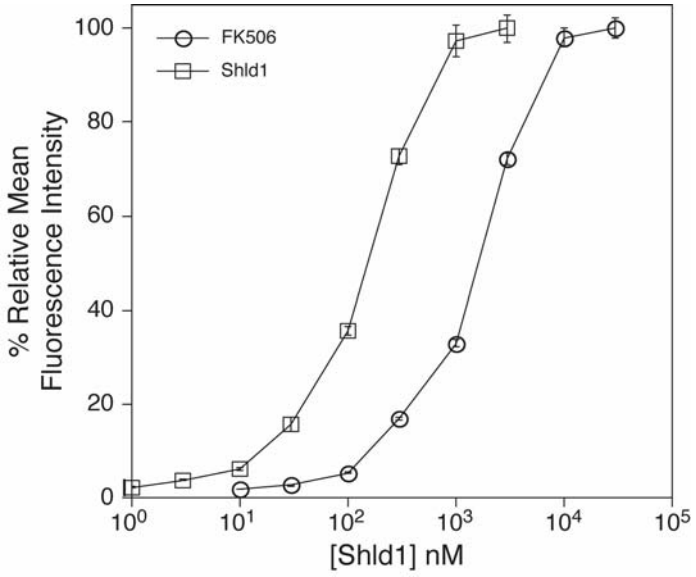

Figure S9. FKBP-YFP fusions are stabilized by multiple FKBP ligands. NIH3T3 cells stably expressing the L106P-YFP fusion were treated with three-fold dilutions of FK506 (30 μM to 10 nM) or Shld1 (3 μM to 1 nM) and monitored by flow cytometry. Data are presented as the average mean fluorescence intensity ± SEM relative to that of the maximum fluorescence intensity observed for the L106P-YFP mutant. Experiments were performed in triplicate.

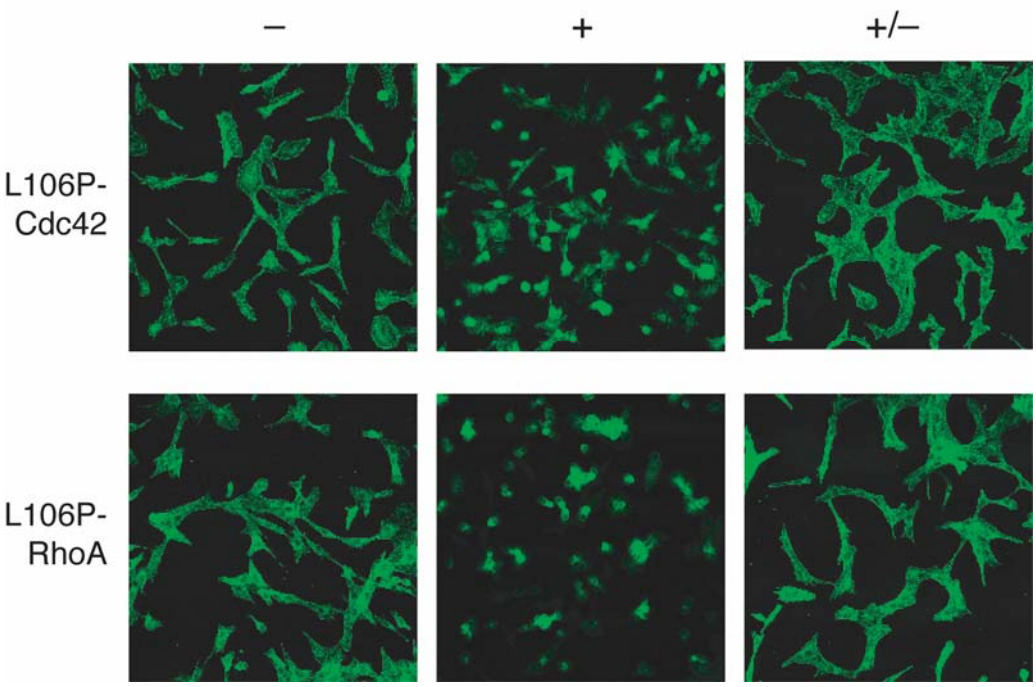

Figure S10. Stabilization of specific proteins with Shld1 results in predictable changes in cellular morphologies. NIH3T3 cells stably expressing fusions of a constitutively active small GTPase to the L106P destabilization domain were split into three pools. The first population (–) was mock-treated and the second population (+) was treated with 1 μM Shld1 for 24 hrs. The third population (+/–) was treated with 1 μM Shld1 for 24 hrs, then washed with media and cultured in the absence of Shld1 for 24 hrs (RhoA Q63L) or 48 hrs (Cdc42 Q61L). Cells were serum-starved for 12 hrs, fixed, stained with Alexa Fluor 488-conjugated phalloidin, and visualized with a 20× objective using confocal microscopy.

Supplemental Tables

Table S1. Shld1-dependent N-terminal FKBP mutants isolated from library screen.

Clone	No. Mutations	Amino Acid Changes
1-37	0	None
38	0	Stop codon introduced
39	0	Dropped base
40	0	Mixed sequence
41	1	F15S
42	1	F15S
43	1	V24A
44	1	K34R
45	1	S38P
46	1	F46L
47	1	V63F
48	1	M66V
49	1	R71S
50	1	P78T
51	1	D79G
52	1	A81V
53	1	E102G
54	1	L106P
55	2	F15S, N43S
56	2	Y26H, Q53R
57	2	G28R, E31G
58	2	F48I, E60G
59	2	G51D, S77P
60	2	E54G, F99L
61	2	Q65R, L106P
62	3	V2A, L50A, L106A
63	3	T6A, V24A, I91A
64	3	Q3R, N43S, G69S
65	3	K44E, E60G, V63A
66	3	W59R, E60G, I76M
67	4	R13H, V24A, K35A, M49A
68	6	S8P, G28R, L30P, S39P, F99L, D100G
69	7	F15S, H25R, K47G, K73R, I76V, D79G, I90V
70	10	H25R, M29T, L30P, D32G, P45S, F48L, K52E, S67G, L104P, L106P
71	11	H25R, M29T, L30P, D32G, P45S, F48L, K52E, E54G, S67G, L104P, L106P
72	16	I7T, S8P, P9L, D11N, T14A, F15L, H25Y, L30P, D37N, D41N, A64T, I76M, G83D, T85A, I91V, F99V

Table S2. Shld1-dependent C-terminal FKBP mutants isolated from library screen.

Clone	No. Mutations	Amino Acid Changes
1-5	0	None
6	0	Stop codon introduced
7-8	0	Incomplete sequence
8-12	0	Mixed sequence
13	1	G1R
14	1	L30P
15	1	M66T
16	1	D100G
17	1	D100N
18	1	E102G
19	1	E102G
20	1	E107G
21	2	E5K, R71G
22	2	E5K, R71G
23	2	D11G, K73R
24	2	Q20L, T27A
25	2	T21A, H25R
26	2	C22F, M29T
27	2	M29T, D100G
28	2	E31G, E107G
29	2	K34Q, Q70R
30	2	S67G, Q70R
31	2	G89S, K105I
32	3	V4M, G33R, G58S
33	3	D11A, D32G, K44R
34	3	D11G, N43D, D79G
35	3	D11G, R13C, F48L
36	3	G19D, K35R, K105E
37	3	E31G, R71G, K105E
38	3	K35R, G69S, I76V
39	3	E61G, H94R, K105R
40	3	D79G, P93S, D100R
41	3	D79G, P93S, D100R
42	4	T6A, I7T, T14I, M66V
43	4	T21A, N43D, A72V, E107G
44	4	M29T, E31K, K52R, T75A
45	4	R42G, K52R, D79G, E107G
46	5	I7T, M29V, F48L, T85A, K105R
47	7	Q3R, F15S, T21A, K44E, K73E, P88T, K105R
48	8	T6S, P9S, M29V, K34R, R42G, Q53R, K73R, D79G

Table S3. Fluorescence of YFP-FKBP fusions in the absence of Shld1.

FKBP Mutant	% Residual YFP Fluorescence ± SEM*
M66T	13 ± 3.1
R71G	9.8 ± 0.6
D100G	8.0 ± 0.8
D100N	22 ± 1.5
E102G	12 ± 0.6
K105I	15 ± 4.7
L106P	11 ± 1.0

***** Data are presented as the average mean fluorescence intensity (MFI) ± SEM relative to that of the maximum fluorescence intensity observed for the individual mutant. Experiment was performed in triplicate.