Deubiquitinase USP20 promotes breast cancer metastasis by stabilizing SNAI2

Wenyang Li, Minhong Shen, Yi-Zhou Jiang, Ruina Zhang, Hanqiu Zheng, Yong Wei, Zhi-Ming Shao, Yibin Kang

SUPPLEMENTAL INFORMATION

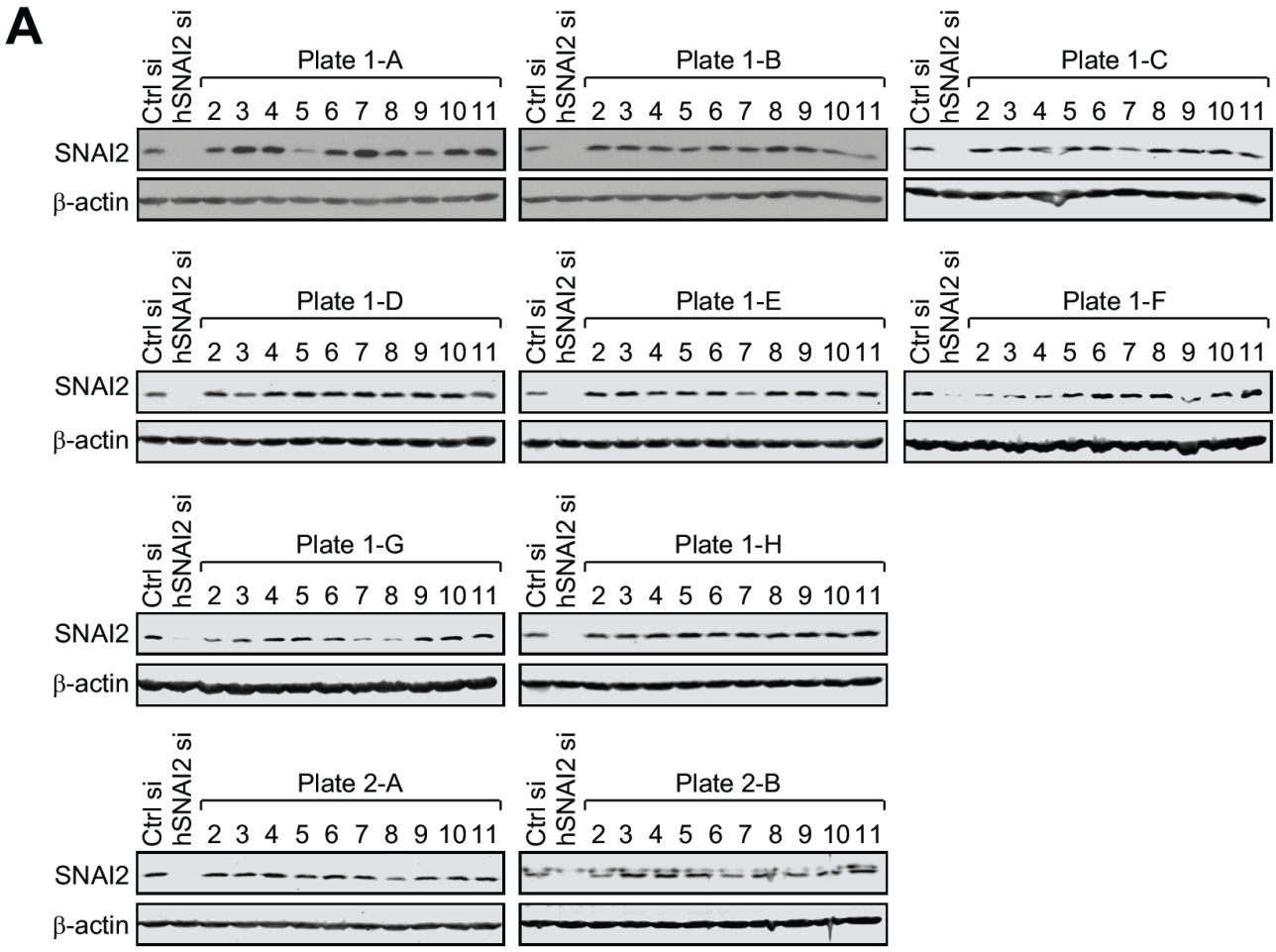
- Supplemental materials and methods
- Four supplemental figures with legends
- Two supplemental tables

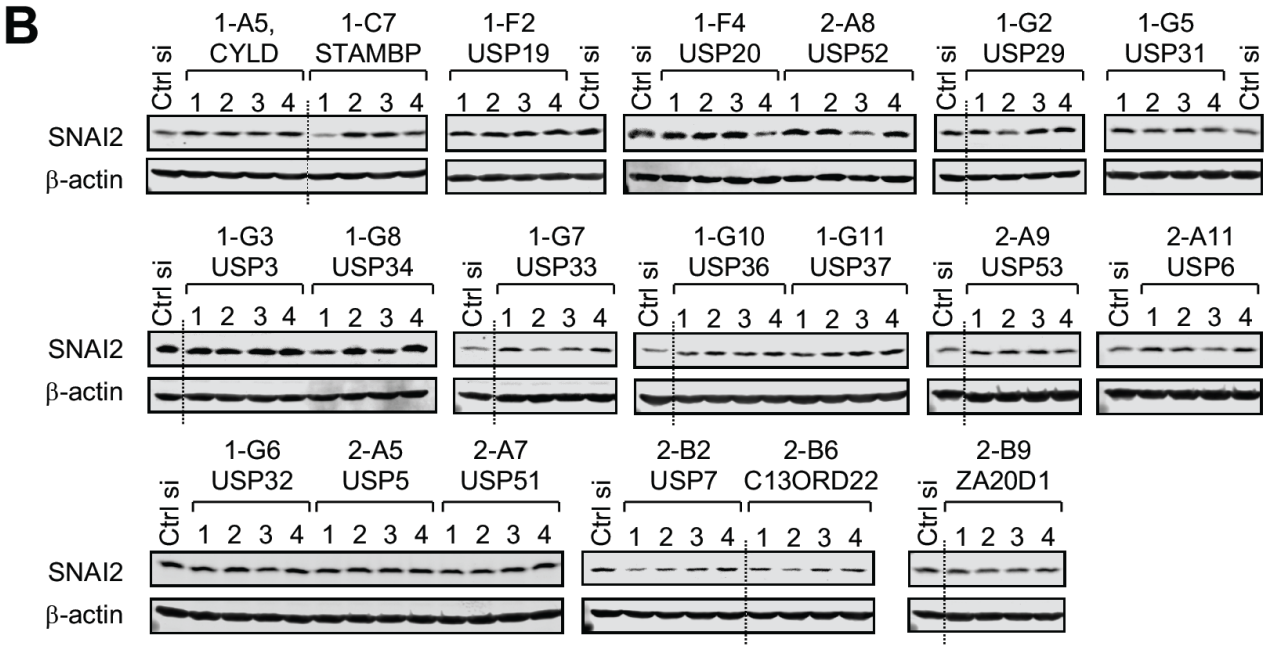
Supplemental Materials and Methods

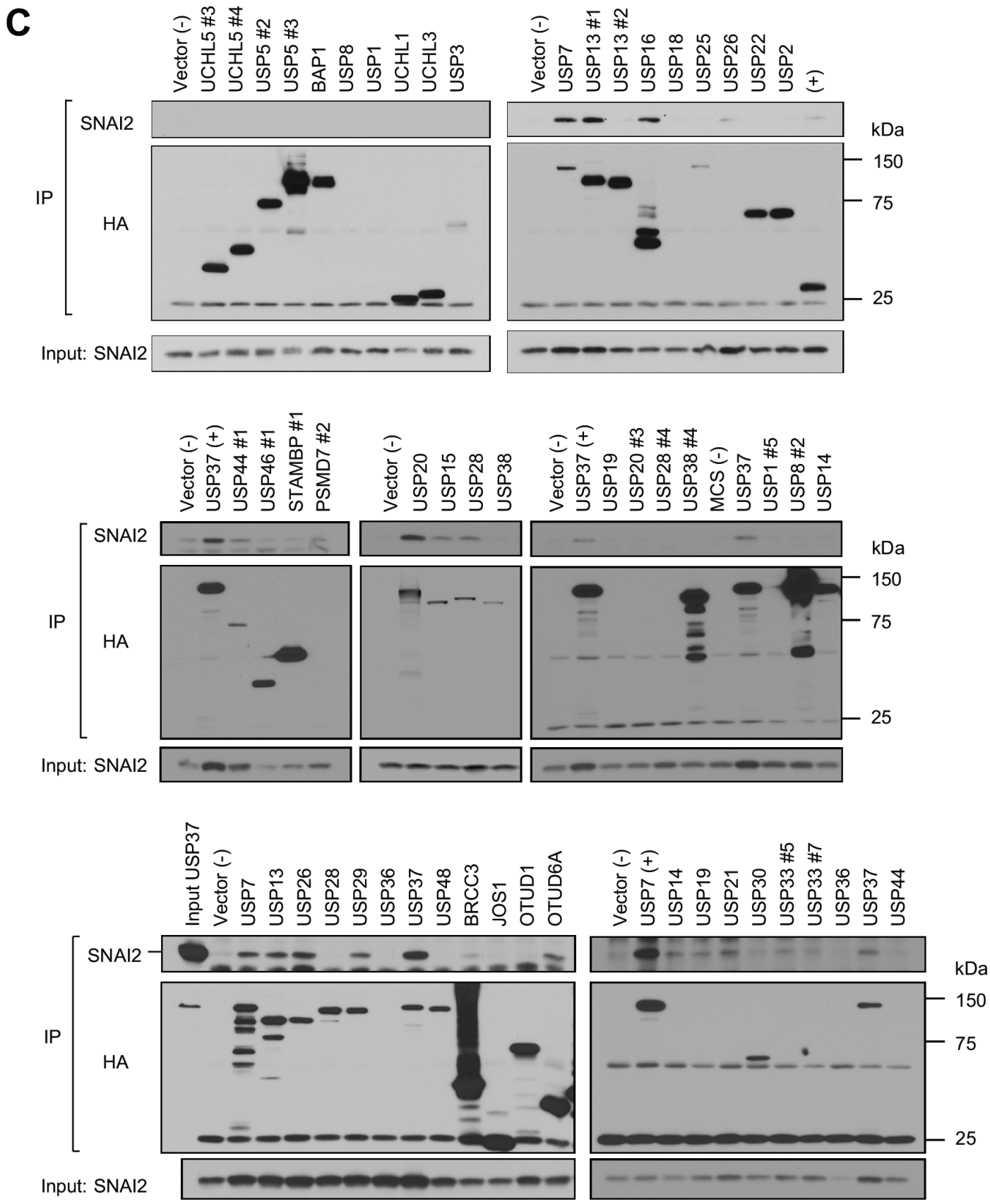
Sulforhodamine B (SRB) proliferation assay

6 hours after transfected with non-targeting control, USP20 or SNAI2 siRNA, the cells were seeded to 96-well plates at 1×10^4 cells per well. After culturing for different days as indicated, the cells were fixed with 10% trichloroacetic acid for 1 hour at 4°C, washed with ddH_2O, and air dried overnight. SRB (Sigma, cat# 230162, 0.4% in 1% acetic acid) was used to stain cells for 20-30 min before washing with 1% acetic acid to remove unincorporated dye. Plates were air dried overnight, and then 10 mM Tris buffer was used to dissolve the dye. Absorbance at a wavelength of 540 nm was measured to indicate the total biomass after subtracting the background absorbance at 690 nm.

Supplemental Figures



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USP7
USP13 #1
USP13 #2
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USP18
USP25
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USP22
USP2
(+)
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kDa
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75
25
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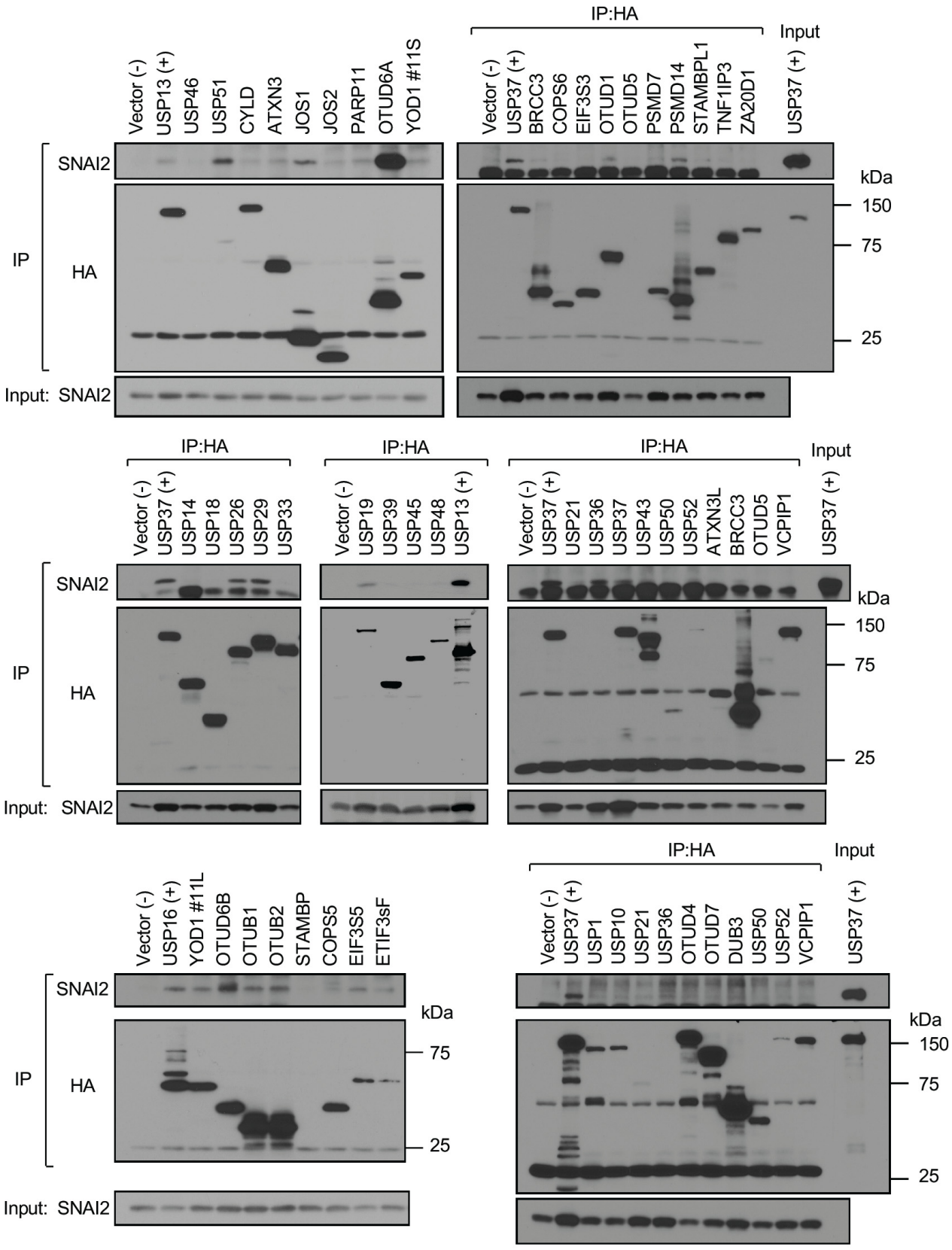
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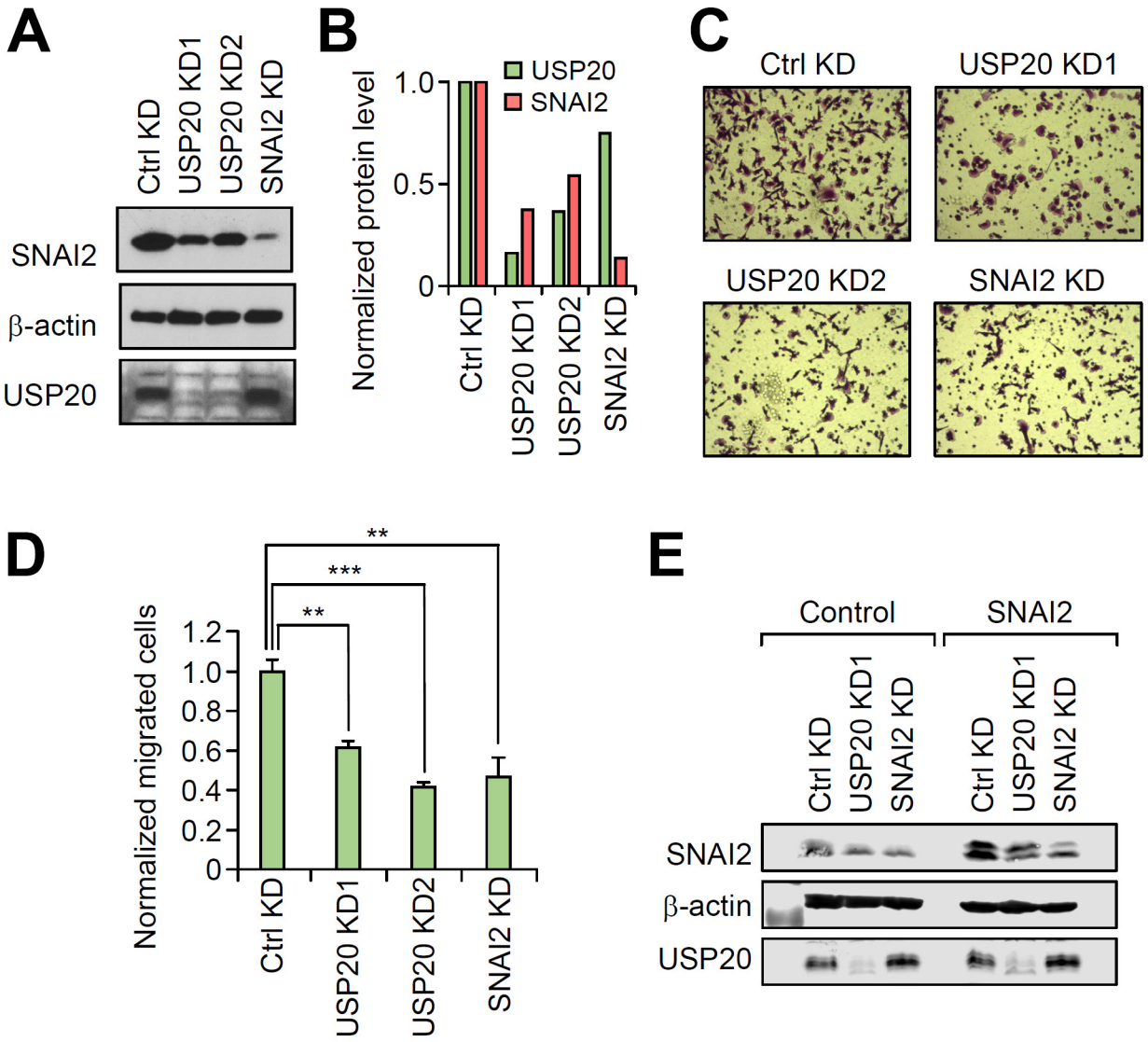
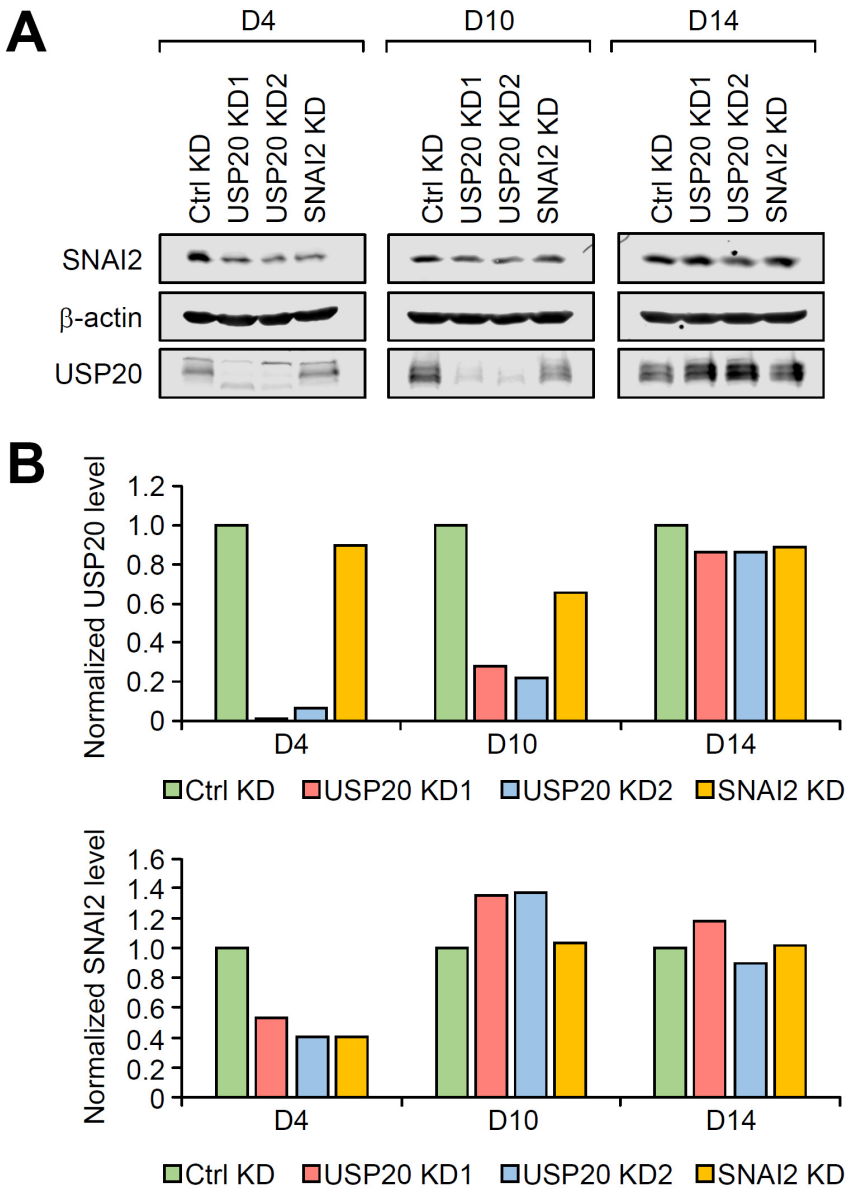
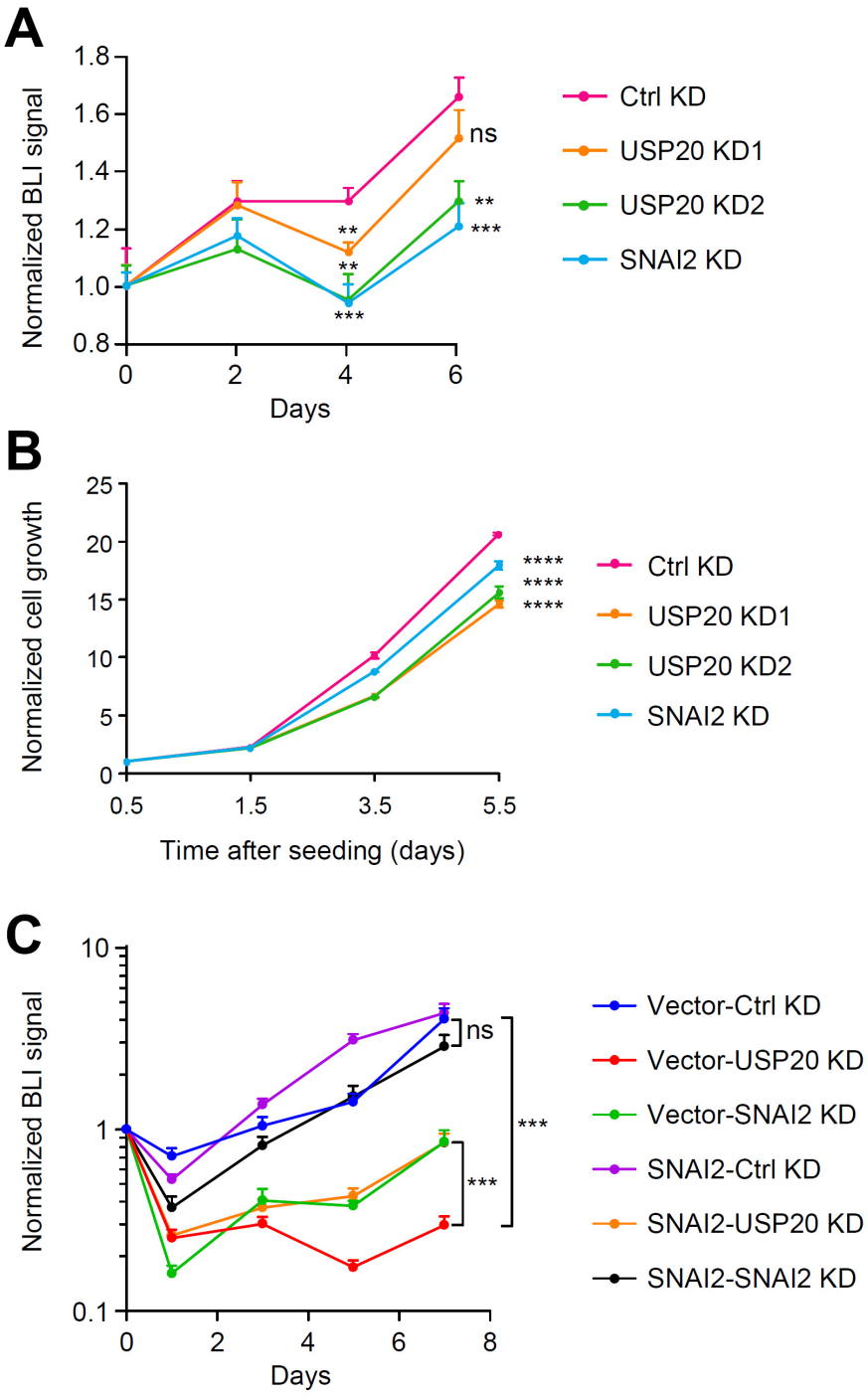
Supplemental Figure S1. DUB siRNA library and cDNA library screening. ***(A)*** Western blot results of the siRNA library screening. Each of the DUBs was knocked down in MDA-MB-231 cells by pooled siRNAs, and SNAI2 level was determined by western blot at 48 hours after transfection. **(*B)*** Western blot results of the second-round siRNA library validation. Each of the DUBs was knocked down in MDA-MB-231 cells by four individual siRNAs, and SNAI2 level was determined by western blot at 48 hours after transfection. ***(C)*** Western blot results of the cDNA library co-IP screening. SNAI2 and different HA tagged DUB expression constructs were co-overexpressed in HEK293T cells. DUBs were pulled down from cell lysates using HA antibody. Co-IPed SNAI2 was detected by SNAI2 antibody.



Supplemental Figure S2. USP20 knockdown inhibits cell migration and invasion. ***(A-B)*** USP20 and SNAI2 were knocked down by siRNA in SUM159-M1a cells. 48 hours after transfection, cells were lysed for western blot. ***(C-D)*** USP20 and SNAI2 were knocked down by siRNA in SCP28 cells. 48 hours after transfection, cells were seeded for transwell migration assay. ***(C)*** Representative microscope images. ***(D)*** Quantified results. Data represent mean ± SEM. **p < 0.01, ***p < 0.001 by one-tailed Student's t test. ***(E)*** USP20 and SNAI2 were knocked down by siRNA in control or SNAI2-overexpressing LM2 cells. 48 hours after transfection, cells were lysed for western blot.



Supplemental Figure S3. USP20 siRNA knockdown lasts for at least 10 days. ***(A)*** Western blots of LM2 cells at 4, 10 or 14 days after transfection with USP20 or SNAI2 siRNAs. ***(B)*** Quantified results of the western blots.



Supplemental Figure S4. SNAI2 and USP20 knockdown reduce metastatic seeding in the lung. ***(A)*** The seeding of LM2 cells in the lung was monitored by bioluminescence imaging (BLI) every other day and was normalized to day 0. Data represent mean ± SEM. ns: non-significant, **p < 0.01, ***p < 0.001 by one-tailed Student's t test. ***(B)*** Proliferation assay of LM2 cells after siRNA-mediated knockdown

of USP20 or SNAI2. LM2 cells were transfected with non-targeting control siRNA or siRNAs targeting USP20 or SNAI2 and plated on 96 well plates 6 hours later at the same density. The cells were fixed at different time points as indicated in the figure, stained with sulphorhodamine B (SRB) to measure cell growth. Data represent mean ± SEM. ****p < 0.0001 by two-tailed Student's t test. ***(C)*** Control LM2 cells and LM2 cells stably over-expressing SNAI2 were transfected with control siRNA, USP20 siRNA or SNAI2 siRNA. 48 hours after transfection, cells were collected, and 30,000 cells were injected into female NSG mice. BLI was performed and was normalized to day 0. Data represent mean ± SEM. ns: non-significant, *p < 0.05, **p < 0.01 by two-tailed Student's t test.

Supplemental Tables

Supplemental Table S1. Summary of the quantified DUB siRNA library screening results.

Supplemental Table S2. Summary of the DUB cDNA library co-immunoprecipitation screening results.

Supplemental Table S1. Summary of the quantified DUB siRNA library screening results

1st Round siRNA library screening

Plate	Well	Number	Gene Name	Normalized SNAI2
1-A	2	1	*BAP1*	1.27
	3	2	*COPS5*	2.58
	4	3	*CXORF53*	2.61
	5	4	*CYLD*	0.60
	6	5	*DUB1A*	2.11
	7	6	*DUB3*	3.40
	8	7	*FBXO7*	2.37
	9	8	*FBXO8*	0.92
	10	9	*FLJ14981*	2.41
	11	10	*JOSD1*	2.16
1-B	2	11	*MJD*	1.84
	3	12	*MYSM1*	1.89
	4	13	*OTUB1*	1.51
	5	14	*OTUB2*	1.31
	6	15	*OTUD1*	1.57
	7	16	*OTUD4*	1.25
	8	17	*OTUD5*	1.68
	9	18	*OTUD6B*	1.45
	10	19	*OTUD7*	1.01
	11	20	*ZA20D1*	0.67
1-C	2	21	*PARP11*	1.38
	3	22	*PRPF8*	2.10
	4	23	*PSMD14*	1.12
	5	24	*SBBI54*	1.35
	6	25	*SENP2*	1.47
	7	26	*STAMBP*	0.72
	8	27	*STAMBPL1*	1.61
	9	28	*TNFAIP3*	1.40
	10	29	*UBL3*	1.81
	11	30	*UBL4*	1.06
1-D	2	31	*UBL5*	1.33
	3	32	*UBR1*	0.98
	4	33	*UBTD1*	1.48
	5	34	*DC-UBP*	1.82
	6	35	*UCHL1*	1.49
	7	36	*UCHL3*	1.99
	8	37	*UCHL5*	1.36
	9	38	*UMPK*	1.63
	10	39	*UEVLD*	1.70
	11	40	*UFD1L*	0.95
1-E	2	41	*USP1*	1.86
	3	42	*USP10*	2.06
	4	43	*USP11*	1.34
	5	44	*USP12*	1.67
	6	45	*USP13*	1.62
	7	46	*USP14*	1.07
	8	47	*USP15*	2.00
	9	48	*USP16*	2.34
	10	49	*USP17*	2.23
	11	50	*USP18*	1.61

2nd round siRNA library screening

Plate	Well	Number	Gene Name	Normalized SNAI2
1-A	5	1	*CYLD*	1.10
1-C	7	3	*STAMBP*	0.57
1-F	2	4	*USP19*	0.91
1-F	4	5	*USP20*	0.42
1-G	2	6	*USP29*	0.53
1-G	3	7	*USP3*	0.68
1-G	5	8	*USP31*	1.25
1-G	6	9	*USP32*	0.77
1-G	7	10	*USP33*	0.59
1-G	8	11	*USP34*	0.54
1-G	10	12	*USP36*	1.11
1-G	11	13	*USP37*	1.07
2-A	5	14	*USP5*	1.02
2-A	7	15	*USP51*	0.83
2-A	8	16	*USP52*	0.47
2-A	9	17	*USP53*	1.15
2-A	11	18	*USP6*	0.88
2-B	2	19	*USP7*	0.56
2-B	6	20	*C13ORF22*	0.55
1-B or 2-B	11 or 9	2	*ZA20D1*	0.99

1-F	2	51	*USP19*	0.81
	3	52	*USP2*	0.88
	4	53	*USP20*	0.73
	5	54	*USP21*	1.00
	6	55	*USP22*	1.46
	7	56	*USP24*	1.16
	8	57	*USP25*	1.26
	9	58	*USP26*	1.02
	10	59	*USP27X*	1.16
	11	60	*USP28*	1.31
1-G	2	61	*USP29*	0.74
	3	62	*USP3*	0.73
	4	63	*USP30*	0.89
	5	64	*USP31*	0.83
	6	65	*USP32*	0.74
	7	66	*USP33*	0.66
	8	67	*USP34*	0.56
	9	68	*USP35*	0.92
	10	69	*USP36*	0.82
	11	70	*USP37*	0.81
1-H	2	71	*USP38*	1.05
	3	72	*USP39*	1.31
	4	73	*USP4*	1.32
	5	74	*USP40*	1.79
	6	75	*USP41*	1.35
	7	76	*USP42*	1.59
	8	77	*USP43*	1.37
	9	78	*USP44*	1.71
	10	79	*USP45*	1.62
	11	80	*USP46*	1.91
2-A	2	81	*USP47*	1.15
	3	82	*USP48*	1.05
	4	83	*USP49*	1.28
	5	84	*USP5*	0.81
	6	85	*USP50*	1.11
	7	86	*USP51*	0.74
	8	87	*USP52*	0.49
	9	88	*USP53*	0.82
	10	89	*USP54*	0.87
	11	90	*USP6*	0.83
2-B	2	91	*USP7*	0.74
	3	92	*USP8*	1.93
	4	93	*USP9X*	1.61
	5	94	*USP9Y*	1.33
	6	95	*C13ORF22*	0.68
	7	96	*VCPIP1*	1.23
	8	97	*YOD1*	0.95
	9	98	*ZA20D1*	0.92
	10	99	ZRANB1	1.34

Supplemental Table S2. Summary of the DUB cDNA library co-immunoprecipitation screening results.

	Protein Name	Interaction with SNAI2
1	ATXN3	No
2	ATXN3L	No
3	BAP1	No
4	BRCC3	No
5	COPS5	No
6	COPS6	No
7	CYLD	No
8	DUB3	No
9	EIF3S3	No
10	EIF3S5	Yes
11	ETIF3sF	Yes
12	JOS1	Yes
13	JOS2	No
14	OTUB1	Yes
15	OTUB2	Yes
16	OTUD1	No
17	OTUD4	No
18	OTUD5	No
19	OTUD6A	Yes
20	OTUD6B	Yes
21	OTUD7B	No
22	PSMD14	Yes
23	PSMD7	No
24	STAMBP	No
25	STAMBPL1	No
26	TNFAIP3	No
27	UBP33	No
28	UBP38	No
29	UBP46	No
30	UCHL1	No
31	UCHL3	No
32	UCHL5	No
33	USP1	No
34	USP10	No
35	USP13	Yes
36	USP14	No
37	USP15	Yes
38	USP16	Yes
39	USP18	No
40	USP19	No
41	USP2	No
42	USP20	Yes
43	USP21	No
44	USP22	No
45	USP25	No

46	USP26	Yes
47	USP28	Yes
48	USP29	Yes
49	USP3	No
50	USP30	No
51	USP37	Yes
52	USP39	No
53	USP43	No
54	USP44	Yes
55	USP45	No
56	USP48	Yes
57	USP5	No
58	USP50	No
59	USP51	No
60	USP52	No
61	USP7	Yes
62	USP8	No
63	VCPIP	No
64	YOD1	Yes
65	ZA20D1	No