**Supplementary Information** 

#### SCF<sup>FBW7</sup>-mediated Degradation of Brg1 Suppresses Gastric Cancer Metastasis

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#### Supplementary Figure 1: FBW7 negatively regulates Brg1.

- (a) Immunoblot (IB) analysis of whole cell lysates (WCL) derived from wild-type  $(FBW7^{+/+})$  and  $FBW7^{-/-}$  HCT116 cells.
- (b) Real-time PCR analysis of Brg1 gene expression in WT and *FBW7*<sup>-/-</sup> DLD1 and HCT116 cells.
- (c) IB analysis of WCL derived from different gastric cancer cell lines. GES1 is a normal gastric epithelial cell line as a negative control.
- (d) IB analysis of WCLs derived from AGS cells transfected with the indicated *FBW*7 shRNA lentivirus.
- (e) Real-time PCR analysis of Brg1 gene expression in MKN45 and AGS cells transfected with the indicated shRNA.
- (f) IB analysis of WCLs derived from AGS cells transfected with the indicated *Cullin1* shRNA lentiviruses.
- (g) Co-IP experiments in AGS cells were performed using anti-Brg1 antibody (sc-17796, Santa Cruz). Mouse IgG was used as a control.



# Supplementary Figure 2: FBW7α is the dominant isoform of FBW7 expressed in gastric cancer cells that regulates Brg1.

- (a) Real-time PCR analysis of three FBW7 isoforms ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) gene expression in MKN45, and AGS gastric cancer cells. Specific Primers are designed: FBW7 $\alpha$  Forward: 5-CGGAGTCTCCCAAACCTGAC-3, Reverse: 5-GGCGCGGTACTCCTCTTTT-3; FBW7 $\beta$  Forward: 5-AGTCTGCATTGCTGAATCCTG-3, Reverse: 5-ACTATGCCC TGTCAAAGCCT; FBW7 $\gamma$  Forward: 5-GGTCAGGACATTTGGTAGGG-3, Reverse: 5-GACAAAAAGGGAGGCCTTGG-3.
- (b) IB analysis of WCLs and IPs derived from MKN45 cells transfected with HA-Brg1 together with three Flag-FBW7 isoforms ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) plasmids.
- (c) IB analysis of WCLs derived from  $FBW7^{-/-}$  DLD1 cells transfected with three Flag-FBW7 isoforms ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) plasmids.



# Supplementary Figure 3: $CK1\delta$ is the crucial upstream kinase of Brg1 and promotes the FBW7-mediated Brg1 ubiquitination.

- (a) IB analysis of WCL derived from 293T cells transfected with the Flag-Brg1 and HA-FBW7 plasmids in the presence of indicated constructs. A plasmid encoding GFP was used as a negative control for transfection efficiency.
- (**b**) IB analysis of 293T cells transfected with the Flag-Brg1 and HA-FBW7 plasmids in the presence of the indicated constructs.
- (c) IB analysis of WCLs and IPs derived from 293T cells transfected with Flag-Brg1 together with the indicated Myc-tagged CK1 family vectors.
- (**d**) IB analysis of WCLs derived from AGS cells infected with the indicated lentiviral shRNA-CK1δ constructs.
- (e) IB analysis of WCLs and His tag pull-down of 293 cells transfected with indicated Flag-Brg1(1-104aa) constructs together with the HA-FBW7, Myc–CK1δ and His-Ub plasmids.



# Supplementary Figure 4: Brg1 degradation and ubiquitination mediated by FBW7/CK1δ is triggered under DNA damage conditions.

- (a) MKN45 cells were treated with 25 uM etoposide or 100 uM Cisplatin. At the indicated time points, WCLs were prepared and immunoblots were probed with the indicated antibodies.
- (**b**) IB analysis of MKN45 cells treated by serum starvation overnight and followed by serum readdition for the indicated times.
- (c) IB analysis of MKN45 cells synchronized by growth in nocodazole and then released for the indicated periods of time.
- (d) IB analysis of WCLs derived from MKN45 cells treated with 25 uM etoposide.
- (e) IB analysis of WCLs derived from MKN45 cells treated with 100 uM Cisplatin.
- (f) Endogenous co-IPs using FBW7 antibody and IB analysis of WCLs derived from MKN45 cells treated with 25 uM etoposide, or 100 uM Cisplatin for 1.5h.
- (g) Endogenous co-IPs using Brg1 antibody and IB analysis of WCLs derived from MKN45 cells treated with 25 uM etoposide, or 100 uM Cisplatin for 1.5h.



d



#### **Supplementary Figure 5: Brg1 overexpression correlates with gastric tumor progression.**

- (a) The immunofluorescence of FBW7 (ab109617, Abcam) in wild-type ( $FBW7^{+/+}$ ) and  $FBW7^{-/-}$  DLD1 cells. Scale Bar, 100  $\mu$ m.
- (b) The association of FBW7 expression with overall survival was examined by Kaplan-Meier analysis in gastric cancer patients from the Zhongshan cohort.
- (c) Representative images of Brg1 expression in tumor sections and adjacent non-tumor tissues according to TNM stage. Scale Bar, 100 μm.
- (d) High Brg1 expression positively correlates with distant metastasis in gastric cancer. Their correlation was analyzed by Spearman rank correlation test.





b

# Supplementary Figure 6: The Brg1 level is inversely correlated with the activation of CK1δ in gastric cancer samples.

- (a) Representative immunofluorescence (IF) images of Brg1(sc-17796, Santa Cruz) and CK1δ (14388-1-AP, Proteintech) in tumor sections. Scale Bar, 100 μm.
- (b) The Brg1 expression inversely correlates with CK1δ activation in gastric cancer. Their correlation was analyzed by Spearman rank correlation test.



Supplementary Figure 7: The FBW7-Brg1 axis regulates the mRNA expression of E-cadherin.

Real-time PCR analysis of E-cadherin gene expression in MKN45 cells transfected with the indicated shRNA.



# Supplementary Figure 8: Brg1 has no significant effect on the cell proliferation of gastric cancer cells.

- (a) *In vitro* scratch assays were performed with cell lines generated in Fig 4a. Cells were seeded on 60 mm dishes and scratched on the surface with a pipette tip. Representative photographs at time points 0, 24 and 48 hr were imaged after the scratch.
- (b) Cell growth curves of MKN45 cells treated with the indicated shRNA lentiviruses were plotted.
- (c) Colony formation assays of MKN45 cells treated with the indicated shRNA lentiviruses were performed. The quantification of the colonies was shown in the bottom. Data was presented as mean  $\pm$  SD.
- (d) In vitro scratch assay was performed with cell lines in Fig 4h.
- (e) Cell growth curves of AGS cells transfected with the indicated Flag-Brg1 vectors were plotted.
- (f) Colony formation assays of AGS cells transfected with the indicated Flag-Brg1 vectors. The quantification of the colonies was shown in the bottom. Data was presented as mean  $\pm$  SD.



# Supplementary Figure 9: The knockdown of *c-Jun* or *KLF2* does not attenuate the increased cell migration induced by *FBW7* knockdown in gastric cancer cell line MKN45.

- (a) IB analysis of WCLs derived from AGS cells infected with the indicated lentiviral shRNA constructs.
- (b-c)Representative images of migrated AGS cells infected with the indicated lentiviral shRNA constructs in a transwell assay (b) and quantification of migrated cells in (c). Data were shown as mean  $\pm$  SD of three independent experiments. \*p < 0.05, \*\*p < 0.01, Student's t test. Scale Bar, 100 µm.
- (d) IB analysis of WCLs derived from AGS cells infected with the indicated lentiviral shRNA constructs.
- (e-f) Representative images of migrated AGS cells infected with the indicated lentiviral shRNA constructs in a transwell assay (e) and quantification of migrated cells in (f). Data were shown as mean  $\pm$  SD of three independent experiments. \*p < 0.05, \*\*\*p < 0.001,Student's t test. Scale Bar, 100 µm.

#### а



#### Supplementary Figure 10: Brg1 expression correlates with EMT in gastric cancer.

- (a) Representative images of Brg1, Vimentin and E-cadherin expression in tumor sections. Scale Bar, 100 μm.
- (b) High Brg1 expression positively correlates with Vimentin expression in gastric cancer. Their correlation was analyzed by Spearman rank correlation test.



#### **Supplementary Figure 11: Brg1 drives EMT transcriptional program.**

- (a) IB analysis of WCLs derived from WT ( $FBW7^{+/+}$ ) and  $FBW7^{-/-}$  DLD1 cells.
- (b) IB analysis of WCLs derived from WT and *FBW7*<sup>-/-</sup> DLD1 cells infected with the indicated shRNA lentiviruses.
- (c) IB analysis of WCLs derived from WT DLD1 cells transfected with the indicated shRNA lentivirus and Flag-Brg1 constructs.



#### Supplementary Figure 12: Brg1 drives EMT in part through regulating Snail.

- (a) IB analysis of WCL derived from AGS cells transfected with indicated plasmid or lentiviral shRNA in the presence of indicated constructs.
- (b-c)Representative images of migrated AGS cells infected with indicated plasmid or lentiviral shRNA constructs in a transwell assay (b) and quantification of migrated cells in (c). Data were shown as mean ± SD of three independent experiments. \*\*p < 0.01, \*\*\*p < 0.001, Student's t test.</p>



**Supplementary Figure 13: FBW7-Brg1 regulates histone modification.** 

- (a) ChIP assays with anti-Brg1 antibody were performed in WT ( $FBW7^{+/+}$ ) and  $FBW7^{-/-}$  DLD1 cells.
- (b) ChIP assays with anti-FBW7 antibody were performed in WT ( $FBW7^{+/+}$ ) and  $FBW7^{-/-}$  DLD1 cells.
- (c) IB analysis of WCLs derived from WT ( $FBW7^{+/+}$ ) and  $FBW7^{-/-}$  DLD1 cells.

Factor	Patients		FBW7 expression			
	No.	%	Low	High	P-value	
All patients	400	100	222	178		
Age (vears)					0.062	
≤ <u></u> ≤65	248	62	147	101		
>65	152	38	75	77		
Gender					0.937	
Female	134	34	74	60		
Male	266	66	148	118		
Location					0.479	
Proximal	58	15	30	28		
Middle	87	21	53	34		
Distal	255	64	139	116		
Size					0.629	
≦2.5cm	115	29	66	49		
> 2.5cm	285	71	156	129		
Differentiation					0.363	
Well	75	19	47	28		
Moderately	114	29	60	54		
Poorly	211	52	115	96		
Lauren classification					0.001	
Intestinal type	245	61	120	125		
Diffuse type	134	34	85	49		
Mixed type	21	5	17	4		
T classification					0.537	
T1	61	15	29	32		
T2	36	10	19	17		
T3	120	30	68	52		
T4	183	45	106	77		
N classification					0.026	
NO	111	28	50	61		
N1	68	17	41	27		
N2	75	19	39	36		
N3	146	36	92	54		
Distant metastasis					0.013	
No	385	96	209	176		
Yes	15	4	13	2		
TNM stage					0.034	
Ι	67	16	31	36		
II	96	24	51	45		
III	222	56	127	95		
IV	15	4	13	2		
Vascular invasion					0.583	
No	282	71	159	123		
Yes	118	29	63	55		

# Supplementary Table 1. Relation between intratumoral FBW7 expression and clinical characteristics of gastric cancer, related to Figure 3

Abbreviation: TNM=tumour node metastasis.

P-value  $\leq 0.05$  marked in bold font shows statistical significant.

Factor	Patients		Brg1 expression		
	No.	%	Low	High	P-value
All patients	400	100	232	168	
Age (years)					0.199
≤65	248	62	150	98	
>65	152	38	82	70	
Gender					0.481
Female	134	34	81	53	
Male	266	66	151	115	
Location					0.971
Proximal	58	15	33	25	
Middle	87	21	50	37	
Distal	255	64	149	106	
Size					0.003
≦2.5cm	115	29	80	35	
> 2.5cm	285	71	152	133	
Differentiation					0.506
Well	75	19	47	28	
Moderately	114	29	68	46	
Poorly	211	52	117	94	
Lauren classification					0.384
Intestinal type	245	61	137	108	
Diffuse type	134	34	84	50	
Mixed type	21	5	11	10	
<b>F</b> classification					< 0.001
T1	61	15	55	6	
T2	36	10	21	15	
Т3	120	30	69	51	
T4	183	45	87	96	
N classification					< 0.001
NO	111	28	85	26	
N1	68	17	42	26	
N2	75	19	44	31	
N3	146	36	61	85	
Distant metastasis					< 0.001
No	385	96	230	155	
Yes	15	4	2	13	
TNM stage					< 0.001
Ι	67	16	57	10	
II	96	24	64	32	
III	222	56	109	113	
IV	15	4	2	13	
Vascular invasion					0.001
No	282	71	178	104	
Yes	118	29	54	64	

# Supplementary Table 2. Relation between intratumoral Brg1 expression and clinical characteristics of gastric cancer, related to Figure 3

Abbreviation: TNM=tumour node metastasis.

P-value < 0.05 marked in bold font shows statistical significant.

## Uncropped blots related to Figure 1a



FBW7



## Uncropped blots related to Figure 1b and 1d

Fig1b-Brg-1



## Fig1b-Vinculin



Fig1d-Brg1



Fig1d-Vinculin



## Uncropped blots related to Figure 1e



c-Myc



cyclinE



Vinculin



FBW7



Fig1f-Brg-1





Fig1g-Myc (IP)







Fig1g-Myc (Lysates)



#### Fig1g-Flag (lysates)



## Uncropped blots related to Figure 1h

Brg1





c-Myc



Cul1

Vinculin





#### Uncropped blots related to Figure 1i and 1j





Fig1j-FBW7 (IP)



Fig1j-BAF155 (IP)



#### Uncropped blots related to Figure 1k

Brg1



BRM



cyclinE



Arid1a





## Vinculin





## Uncropped blots related to Figure 2b, 2c



Fig2b, HA (lysates)



Fig2b, Flag (IP)

Fig2b, Flag (lysates)













Fig 2e--Brg1









## Uncropped blots related to Figure 2f



Μус-СК1δ









## Uncropped blots related to Figure 2g

Flag-Brg1



HA-FBW7





#### Uncropped blots related to Figure 2i

Flag-Brg1





Μус-СК1δ



Vinculin



Flag (Ni-NTA)







HA (lysates)

Myc (lysates)





#### Uncropped blots related to Figure 4a, 4h



#### Uncropped blots related to Figure 5a



 $\beta$ -cateinin







#### Uncropped blots related to Figure 5d



Snail



TWIST-1







 $\beta$ -cateinin



Vinculin



## Uncropped blots related to Figure S1a





Arid1a





Vinculin

BRM





170 – 130 – 95 –

# Uncropped blots related to Figure S1c







## Uncropped blots related to Figure S1d



c-Myc









FBW7



#### Uncropped blots related to Figure S1f, S1g







FigS1g-Brg1 (IP)



FigS1g-FBW7 (IP)



#### Uncropped blots related to Figure S2b

Flag (IP)



HA (IP)



## HA (lysates)







Vinculin (lysates)



## Uncropped blots related to Figure S2c

Brg1



c-myc





Cyclin E

## Vinculin



Flag



## Uncropped blots related to Figure S3a



GFP





Vinculin



## Uncropped blots related to Figure S3b







Vinculin 130 – 95 –

#### Uncropped blots related to Figure S3c, S3d



43 -

34 -

170 -

130 -

130 —

95 — •

72 – 🎘

Flag (Ni-NTA)





Flag (lysates)



17 —

9





## Uncropped blots related to Figure S4a









Vinculin



## Uncropped blots related to Figure S4b

Brg-1







Vinculin



## Uncropped blots related to Figure S4c

Brg-1





Fbw7











#### Uncropped blots related to Figure S4d









## Uncropped blots related to Figure S4e

Brg-1













#### Uncropped blots related to Figure S4f, S4g

Fig S4f-Brg-1 (IP)



Fig S4f-Fbw7 (IP)



Fig S4g-Ub (IP)



Fig S4g-Brg-1 (Lysates)



## Fig S4g-Tubulin (Lysates)



#### Uncropped blots related to Figure S9a, S9d

Fig S9a-c-JUN



Fig S9a-FBW7



Fig S9a-Vinculin







Fig S9d-FBW7



Fig S9d-Vinculin



#### Uncropped blots related to Figure S11a







Snail





Vinculin





 $\beta$ -cateinin





#### Uncropped blots related to Figure S11b





35

## Uncropped blots related to Figure S11c



Snail

Vinculin



## Uncropped blots related to Figure S12a



#### Uncropped blots related to Figure S13c













