

Supplemental Data

Supplementary Figures

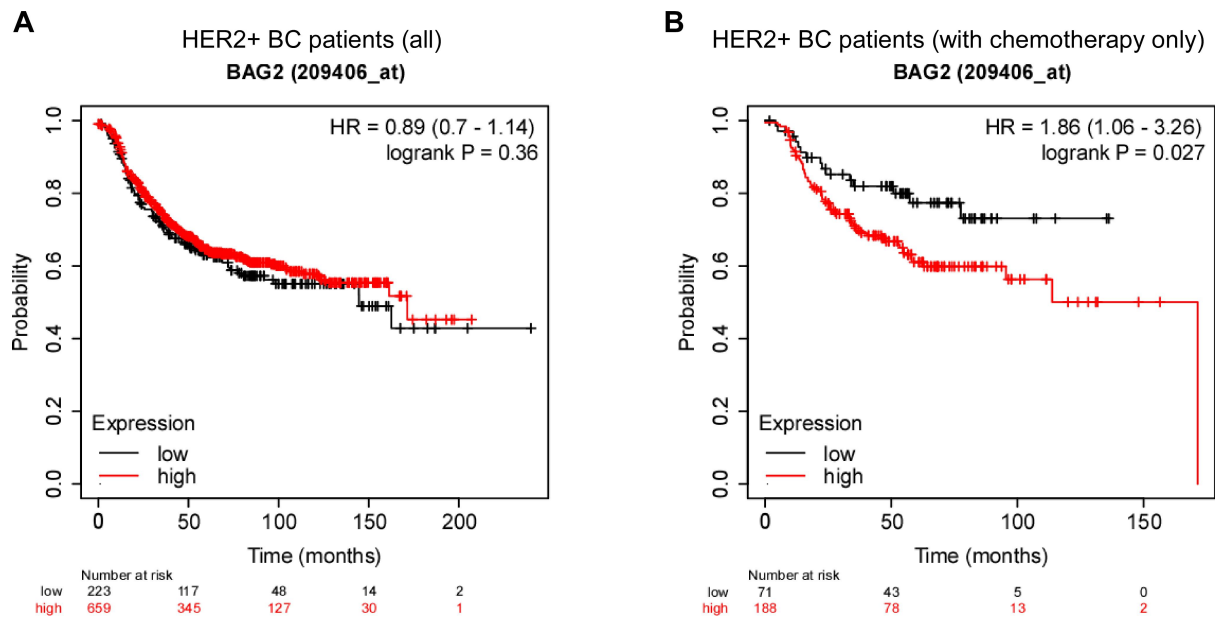


Figure S1. (A-B) Kaplan-Meier curves indicate the prognostic value of BAG2 expression in the RFS for HER2-positive breast cancer patients (A) or HER2-positive breast cancer patients treated with chemotherapy only (B) from online database (<http://kmplot.com/analysis>).

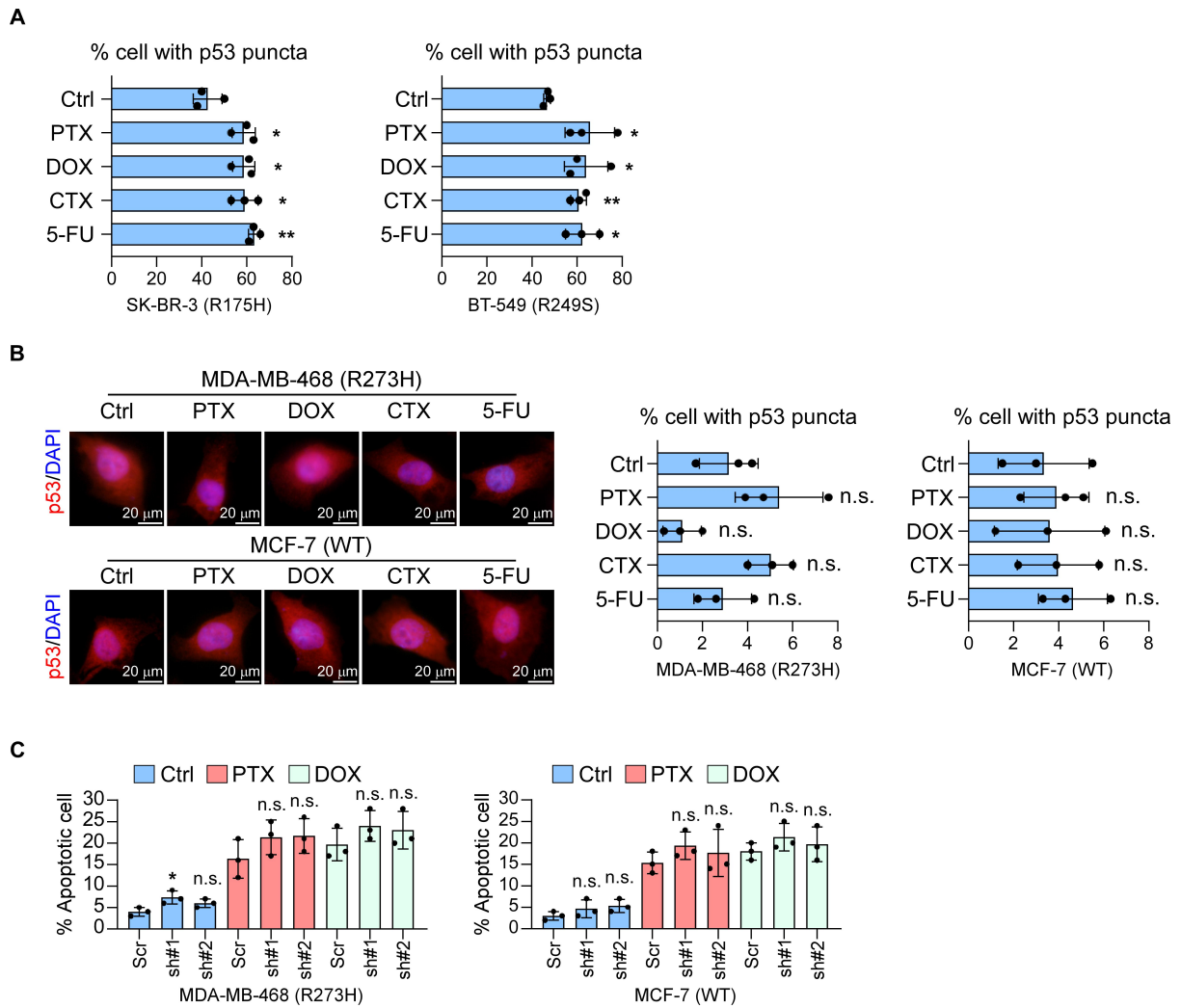


Figure S2. (A) Quantification of p53 aggregates in SK-BR-3 (R175H) and BT-549 (R249S) cells which harbor structural mutants under stationary, PTX, DOX, CTX, and 5-FU treatment. **(B)** IF staining of p53 (red) with anti-p53 DO-1. Shown are the representative images (left panel) and quantification (right panel) of endogenous aggregations of p53 proteins in MDA-MB-468 (R273H) cells, which harbor contact mutants and MCF-7 (WT) cells, under the indicated conditions. **(C)** Quantification of annexin V and PI staining for the MDA-MB-468 (R273H) and MCF-7 (WT) cells treated with PTX or DOX. Histograms show the portion of annexin V+ cells in each group. Each error bar represents the mean \pm SD of three biological replicates. Two-sided Student's t test was used for all panels. * $P < 0.05$, ** $P < 0.01$, and n.s.: not significant.

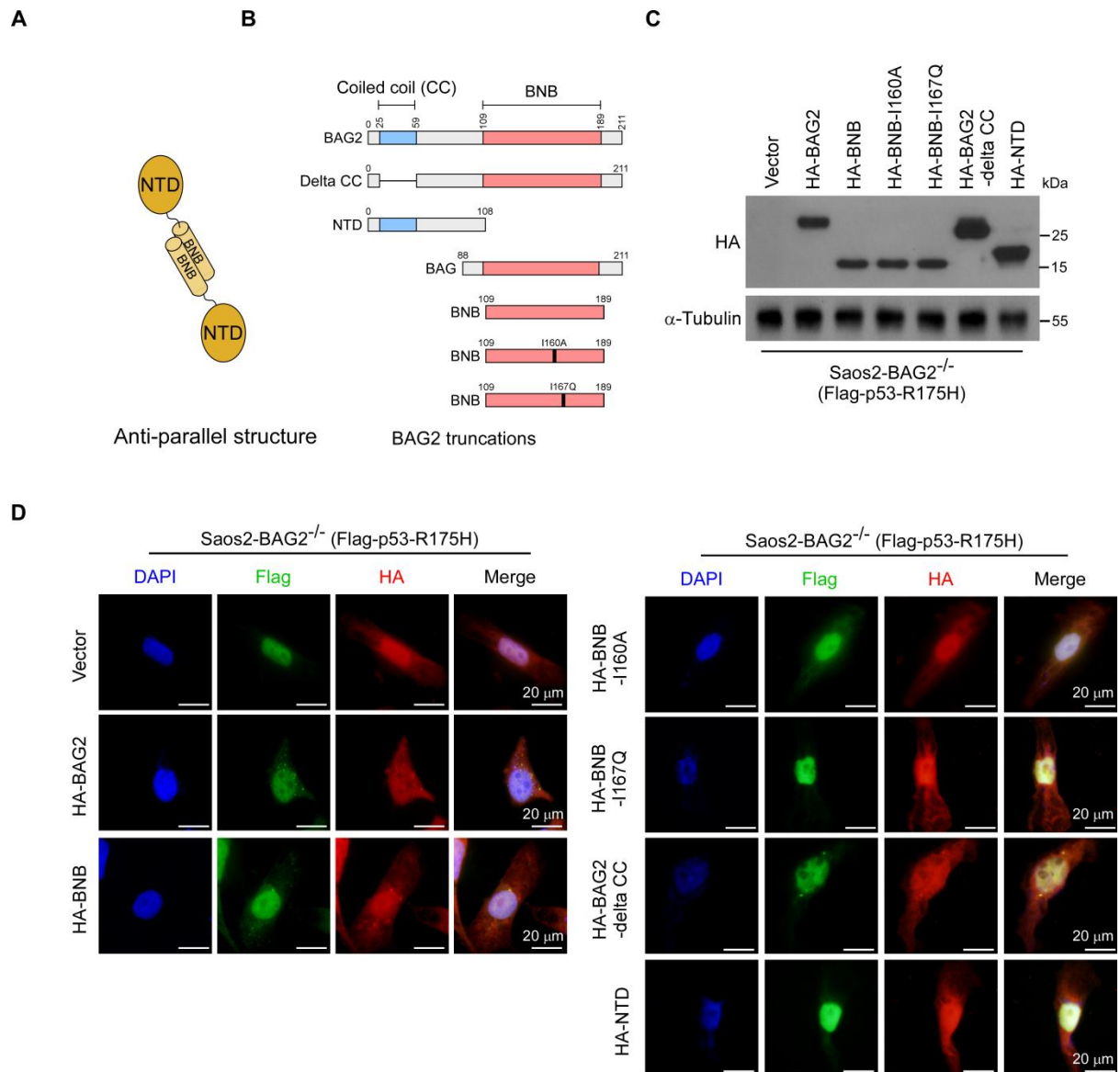


Figure S3. (A) Schematic illustration of the BNB and NTD structures and the antiparallel dimer structure of the BAG2 protein. (B-C) Schematic illustration and confirmation of the BAG2 truncation constructs. (D) Representative images of immunofluorescence (IF) staining of Flag and HA co-aggregations in Saos2-BAG2^{-/-} cells transfected with the indicated truncations of BAG2.

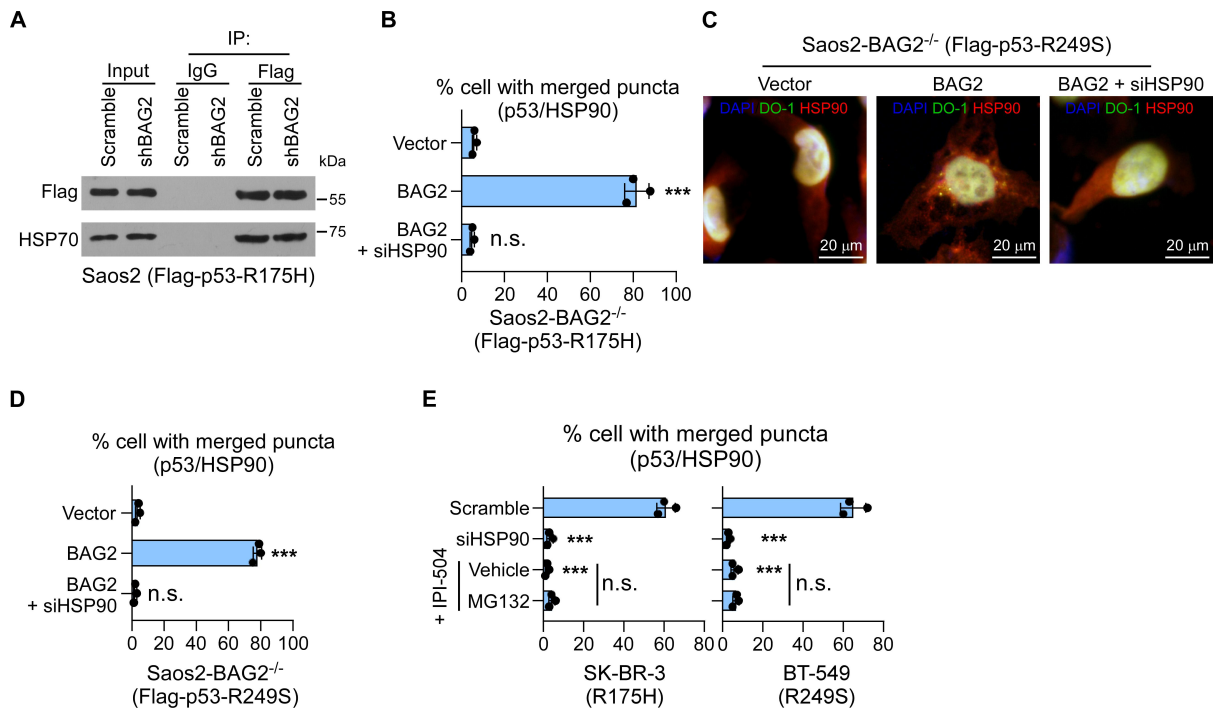


Figure S4. (A) Saos2-p53-R175H cells with silencing HSP90 were followed by IP assays and analyzed by western blotting. (B) Immunofluorescence (IF) staining quantification of p53-R175H and HSP90 aggregations in Saos2-BAG2^{-/-} (Flag-p53-R175H) cells transfected with vector, BAG2, or BAG2 and HSP90 siRNA. (C and D) Immunofluorescence (IF) staining (C) and quantification (D) of p53-R249S and HSP90 aggregations in Saos2-BAG2^{-/-} cells transfected with vector, BAG2, or BAG2 and HSP90 siRNA. (E) Quantification of p53/HSP90 aggregations in SK-BR-3 and BT-549 cells treating with or without IPI-504 and MG-132 (10 μ M for 6 hours). Each error bar in B, D and E represents the mean \pm SD of three biological replicates. Two-sided Student's t test was used for all panels. *** $P < 0.001$, n.s.: not significant.

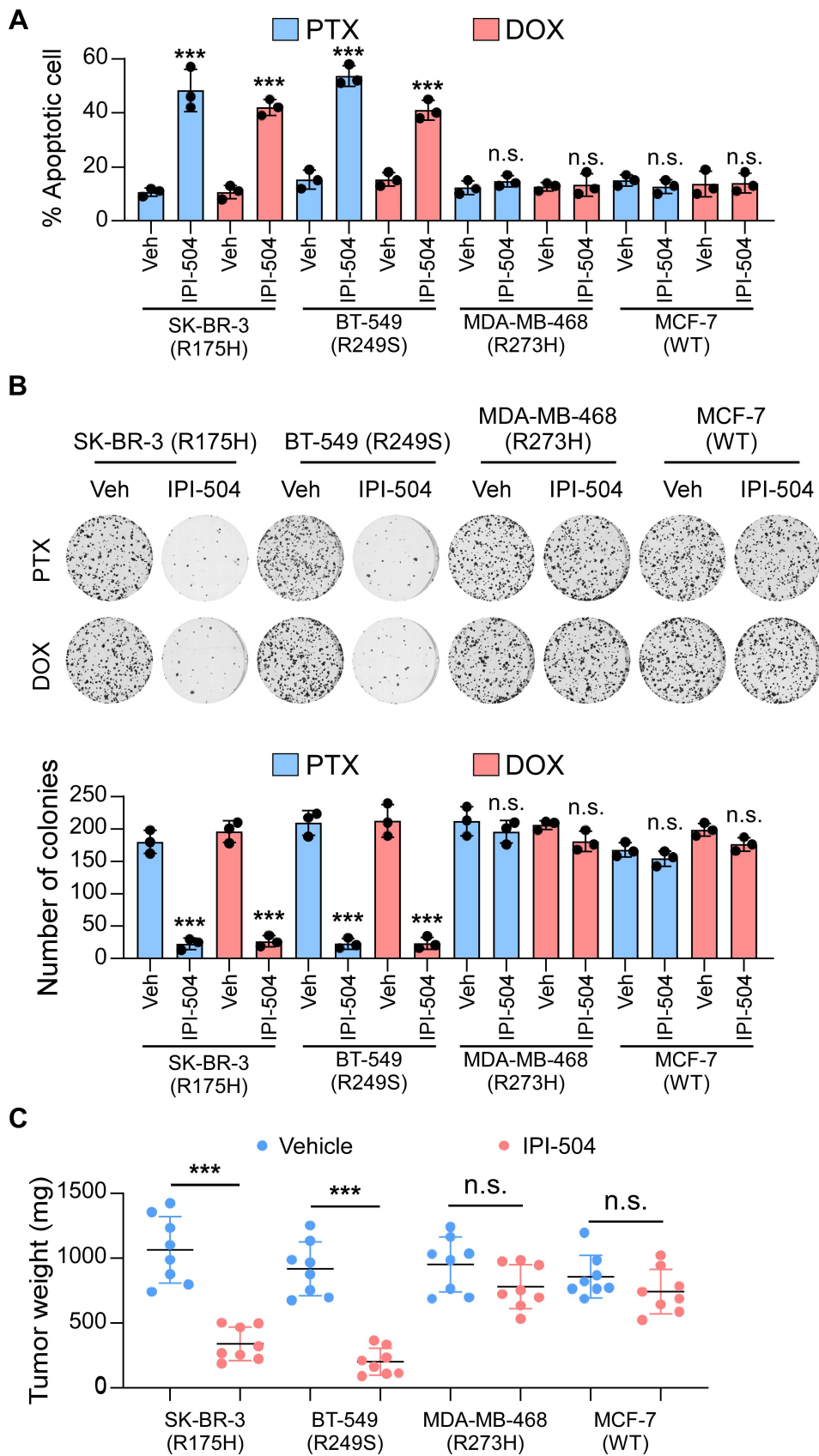


Figure S5. (A and B) The number of surviving colonies and apoptotic percentage of the SK-BR-3, BT-549, MDA-MB-468 and MCF-7 cells with or without treatment of IPI-504. **(C)** The tumor

weights of the indicated cells with or without treatment of IPI-504. Each error bar in A and B represents the mean \pm SD of three biological replicates. Error bar in C represents the mean \pm SD derived from tumor mouse models (n = 8 mice/group). Two-sided Student's t test was used for all panels. *** $P < 0.01$, n.s.: not significant.

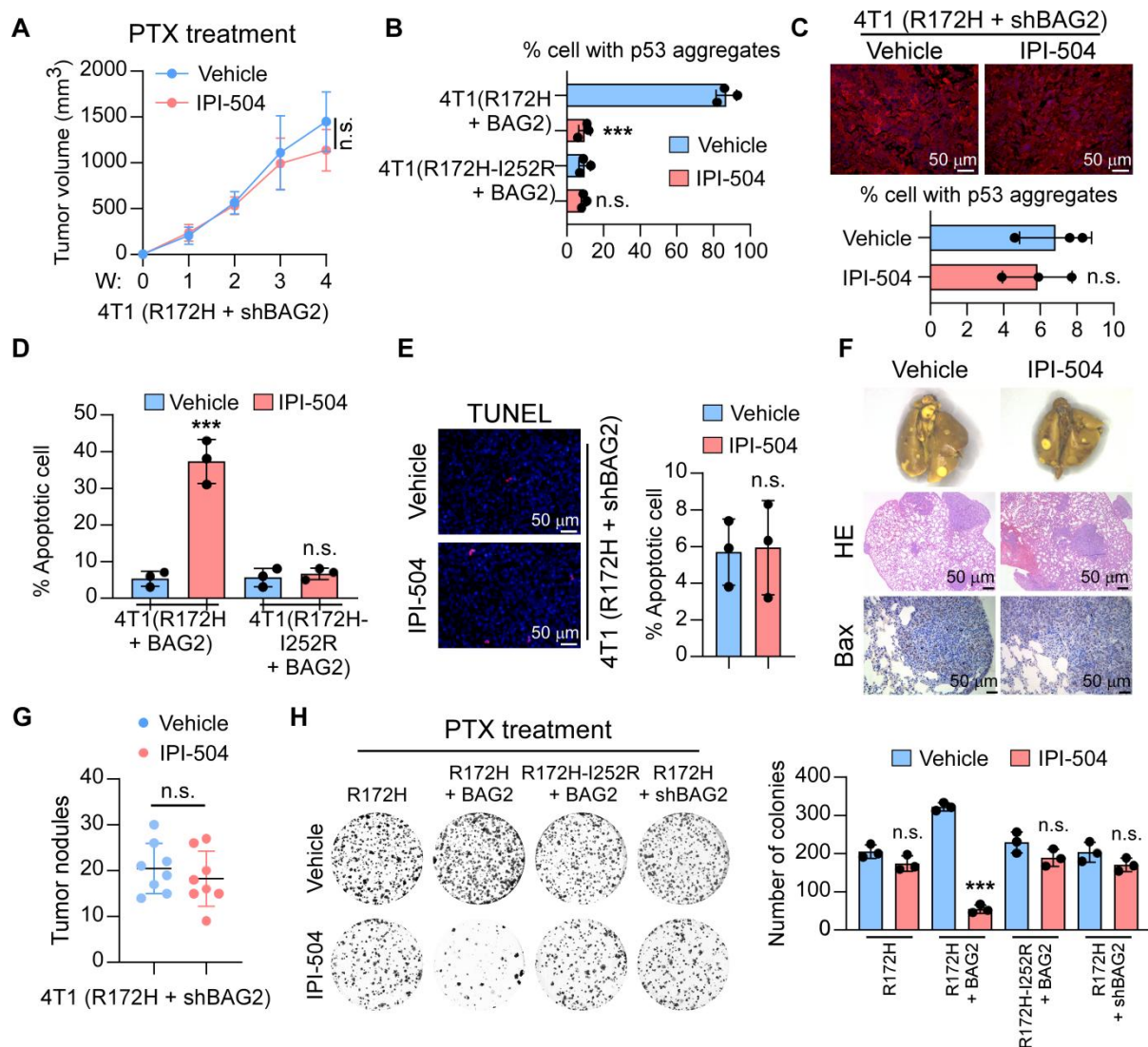


Figure S6. (A) The tumor volume of 4T1 (R172H + shBAG2) xenograft with or without IPI-504 treatment. (B) Quantification of aggregated p53 in 4T1 (R172H + BAG2) and 4T1 (R172H-I252R + BAG2) xenografts treating with or without IPI-504 were shown. (C) The representative images and quantification of aggregated p53 in the indicated sections xenografts were examined by IF staining. (D) Apoptosis index were calculated using the proportions of TUNEL positive cells in the indicated xenograft sections. (E) TUNEL staining and apoptosis index in the 4T1 (R172H + shBAG2) orthotopic tumors. (F) Images, HE staining and IHC of Bax staining of lung metastases. (G) Quantification of visible surface metastatic lesions in the indicated tumors. (H) Colony formation assays of the indicated cells with or without IPI-504 treatments. Surviving colonies were counted. Each error bar in A and G represents the mean \pm SD derived from tumor mouse models ($n = 8$ mice/group). Each error bar in B, C, D, E and H represents the mean \pm SD of three biological

replicates. Two-sided Student's t test (B, C, D, E, G, H), or One-way repeated-measures ANOVA test (A) was used for statistical analysis. *** $P < 0.001$, n.s.: not significant.

Supplementary Tables

Table S1, Clinicopathological characteristics of 236 breast cancer patients

Parameters	Number of cases (%)
Gender	
Female	236 (100)
Male	0 (0)
Age	
≤ 49	128 (54.2)
> 49	108 (45.8)
Clinical stage	
I-II	154 (65.3)
III-IV	82 (34.7)
T classification	
T1-2	203 (86.0)
T3-4	33 (14.0)
N classification	
N0	111 (47.0)
N1-3	125 (53.0)
Histologic grade	
G1-2	139 (58.9)
G3	97 (41.1)
Hormone receptor	
Negative	109 (46.2)
Positive	127 (53.8)
HER2 status	
Negative	154 (65.3)
Positive	82 (34.7)
Ki67	
≤ 15%	112 (47.5)
> 15%	124 (52.5)
TNBC	
No	165 (69.9)
Yes	71 (30.1)
Relapse	
No	160 (67.8)
Yes	76 (32.2)

BAG2 expression

Low 126 (53.4)

High 110 (46.6)

PAb240

Low 116 (49.2)

High 120 (50.8)

BAG2 combines with**PAb240**

High/high 67 (28.4)

High/low or low/high 96 (40.7)

Low/low 73 (30.9)

Table S2, Correlation between BAG2 and clinicopathological characteristics of 236 breast cancer patients

Characteristics	BAG2		<i>P</i> values
	low	high	
Age			
≤ 49	72	56	0.338
> 49	54	54	
Clinical stage			
I-II	88	69	0.248
III-IV	38	41	
T classification			
T1-2	113	90	0.082
T3-4	13	20	
N classification			
N0	64	47	0.216
N1-3	62	64	
Histologic grade			
G1-2	77	62	0.460
G3	49	48	
Hormone receptor			
Negative	56	53	0.566
Positive	70	57	
HER2 status			
Negative	86	68	0.300
Positive	40	42	
Ki67			
≤ 15	60	52	0.958
> 15	66	58	
TNBC			
No	89	76	0.796
Yes	37	34	
Relapse			
No	101	59	0.001
Yes	25	51	

Table S3, Correlation between BAG2 combined with PAb240 and clinicopathological characteristics of 236 breast cancer patients

Characteristics	BAG2/PAb240			P values
	-/-	+/-	+/+	
Age				
≤ 49	41	57	30	0.170
> 49	32	39	37	
Clinical stage				
I-II	53	64	37	0.091
III-IV	20	32	30	
T classification				
T1-2	67	82	54	0.159
T3-4	6	14	13	
N classification				
N0	43	43	25	0.032
N1-3	30	53	42	
Histologic grade				
G1-2	41	59	39	0.779
G3	32	37	28	
Hormone receptor				
Negative	30	49	30	0.422
Positive	43	47	37	
HER2 status				
Negative	49	63	42	0.855
Positive	24	33	25	
Ki67				
≤ 15	34	44	34	0.813
> 15	39	52	33	
TNBC				
No	53	64	48	0.661
Yes	20	32	19	
Relapse				
No	59	66	35	0.001
Yes	14	30	32	

Table S4, Univariate and multivariate analysis of factors associated with 5-year relapse-free survival in 236 breast cancer patients

Characteristics	Univariate analysis		Multivariate analysis	
	HR (95% CI)	<i>P</i> values	HR (95% CI)	<i>P</i> values
BAG2				
combines with PAb240 (high/high)	2.082 (1.415-3.063)	< 0.001	3.219 (1.413- 7.337)	0.005
T classification (T3-4)	2.622 (1.392-4.938)	0.003	1.518 (0.784- 2.938)	0.216
N classification (N1-3)	3.858 (1.974-7.539)	< 0.001	3.605 (1.771- 7.336)	< 0.001
HR status (negative)	0.844 (0.485-1.469)	0.549	0.660 (0.371- 1.174)	0.158
HER2 status (positive)	3.291 (1.867-5.799)	0.001	3.756 (2.088- 6.755)	< 0.001
Ki67 (> 15%)	1.069 (0.613-1.864)	0.814	0.834 (0.464- 1.499)	0.543
Histologic grade (G3)	1.317 (0.756-2.294)	0.331	1.035 (0.585- 1.832)	0.906

HR, hazard ratio; CI, confidence interval.