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Supplemental information

Phospho-Ser^{784}-VCP Is Required for DNA Damage Response and Is Associated with Poor Prognosis of Chemotherapy-Treated Breast Cancer

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Supplementary Figures

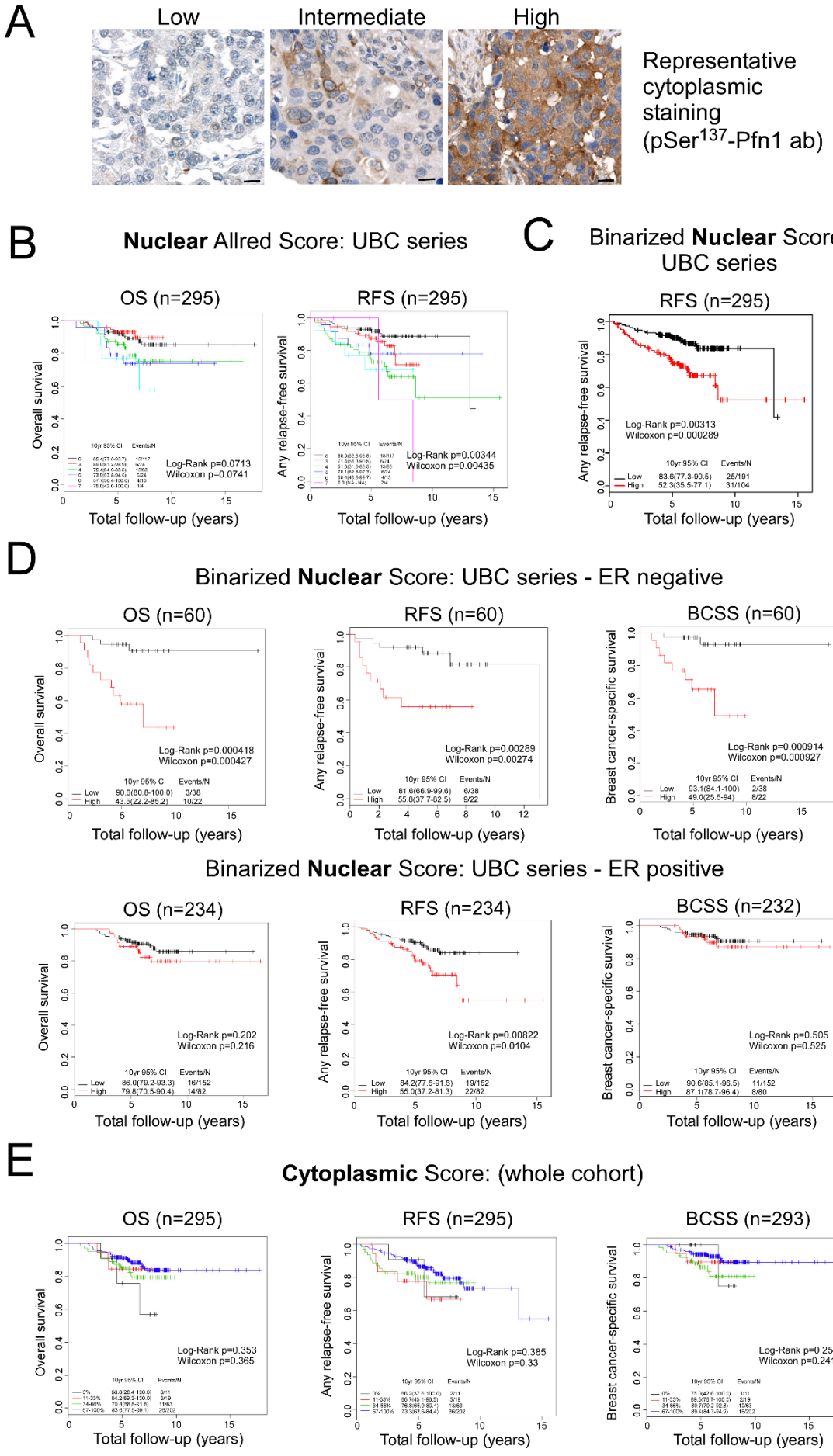

Fig. S1 Nuclear antigen recognized by the pSer^{137}-Pfn1 antibody inversely correlates with breast cancer survival in the UBC series, related to Fig.1C and 1D. (A) Representative IHC images of cytoplasmic staining of breast tumors by the pSer^{137}-Pfn1 antibody. (**B**) Individual nuclear Allred scores of the UBC TMA series were used for Kaplan-Meier analysis for association with the overall (OS) and relapse-free survival (RFS). (**C**) Nuclear Allred scores from (**B**) were binarized into low vs. high groups and subjected to Kaplan-Meier analysis for association with RFS as in (**B**). (**D**) Samples in the UBC series were divided into estrogen receptor negative (ER negative) vs. positive (ER positive) sub-groups and subjected to the same survival analysis as in (**C**) using the binarized nuclear Allred scores. (**E**) Cytoplasmic staining of the UBC TMA by the pSer^{137}-Pfn1 antibody was scored based on the percentage of positive cells (intensity was similar across tissues) into four groups (0-10%, 11-33%, 34-66%, 67-100%). The scores were used in univariate Kaplan-Meier analysis for association with overall (OS), relapse-free (RFS), and breast cancer-specific survival (BCSS). Log-rank and Wilcoxon tests were used to generate the p values which were considered statistically significant when being less than 0.05. Scale bars, 10μm.

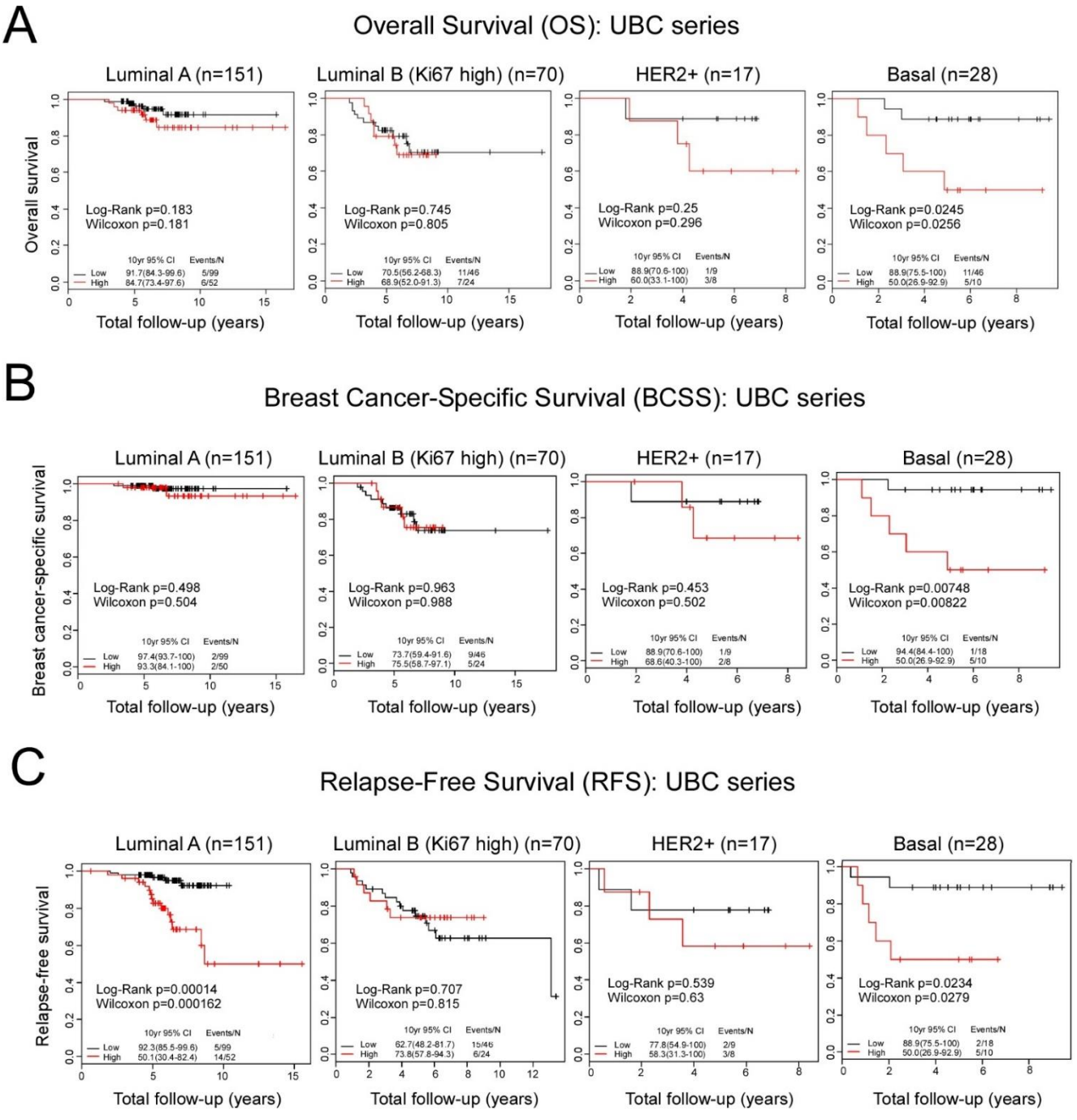

Fig. S2 The association between the nuclear antigen recognized by the pSer^{137}-Pfn1 antibody and breast cancer survival according to IHC subtype in the UBC series, related to Fig.1C and 1D. (**A-C**) The UBC series was divided into different breast cancer subtypes (luminal A, luminal B, HER2+, and basal) as previously described (Cheang et al., 2008; Goldhirsch et al., 2011). Luminal A: ER+ and/or PR+, HER2−, and ki67<14%; luminal B: ER+ and/or PR+, HER2−, and ki67≥14% or ER+ and/or PR+, any ki67 and HER2+; HER2+: ER-, PR-, HER2+; triple negative: ER-, PR-, HER2-; basal-like: ER-, PR, HER2- and [EGFR+ or CK5+]. Binarized nuclear Allred scores as described in Fig.S1C-S1D were subjected to univariate Kaplan-Meier analysis for association with overall survival (A), breast cancer-specific survival (B), and relapse-free survival (C). Log-rank and Wilcoxon tests were used to generate the p values which were considered statistically significant when being less than 0.05. Unadjusted P values for the outcome in the different IHC subtypes are displayed.

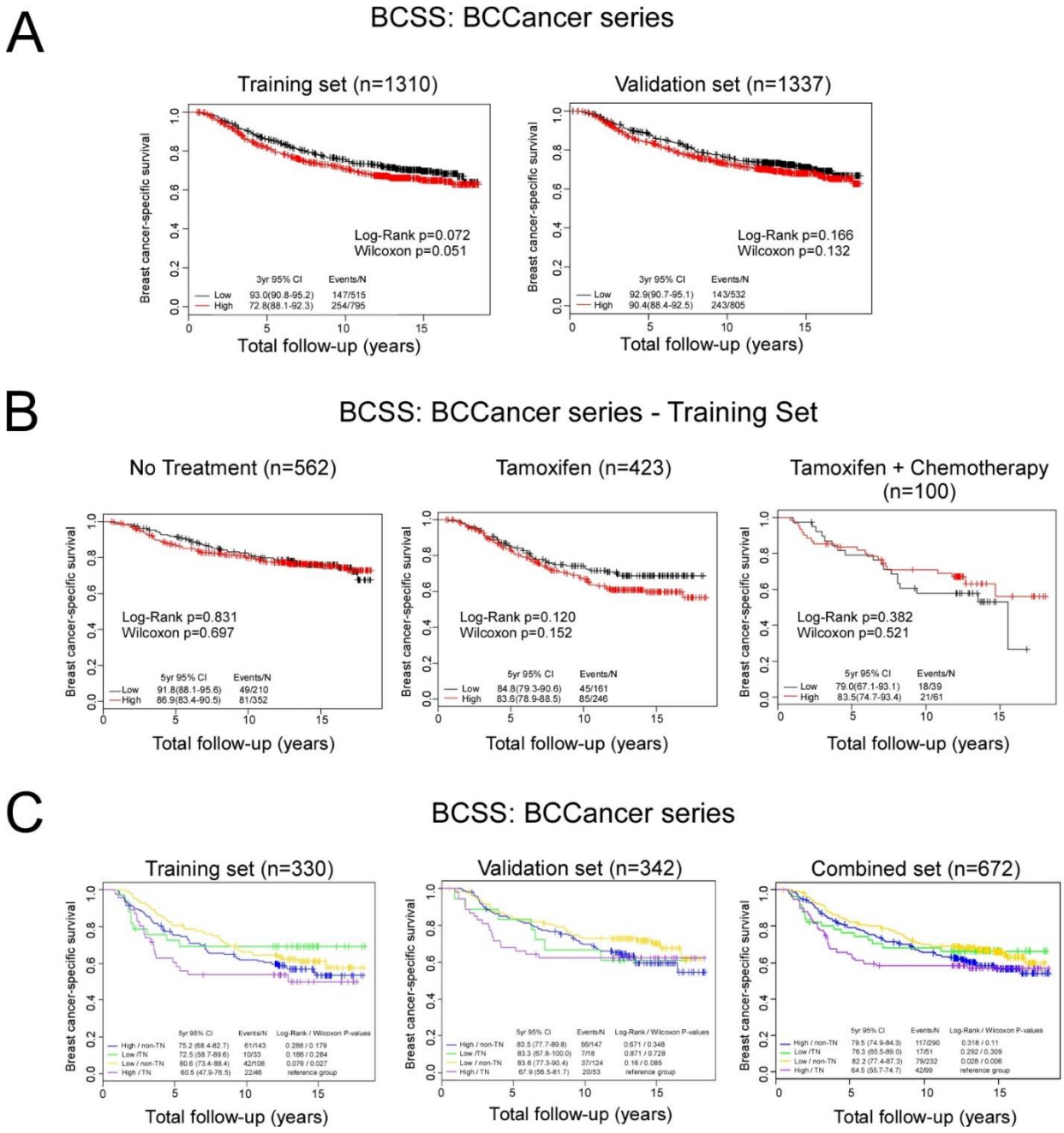

Fig. S3 Nuclear antigen recognized by the pSer^{137}-Pfn1 antibody associates with worse outcome of breast cancer treated with chemotherapy in the BCCancer series, related to Fig.1E. (**A**) Binarized nuclear Allred scores (low vs. high) were used in the univariate Kaplan-Meier analysis of the BCCancer cohort for breast

cancer-specific survival (BCSS), shown for the training (n=1311) and validation (n=1340) sets separately. (**B**) The training set of the BCCancer series (n=1311) was divided into subgroups based on systematic adjuvant treatments and subjected to univariate Kaplan-Meier analysis for BCSS. Shown here are the no treatment (n=562), tamoxifen (n=407) and chemotherapy plus tamoxifen (n=100) groups. No validation for these treatment groups was performed due to the lack of statistically significant association between nuclear scores and survival. (**C**) Chemotherapy-treated cases within the whole BCCancer series (n=672) were divided into triple negative vs. non-triple negative subgroups and subjected to the same Kaplan-Meier analysis for the association between binarized nuclear Allred scores and BCSS. Training, validation, and combined datasets are shown. Log-rank and Wilcoxon tests were used to generate the p values which were considered statistically significant when being less than 0.05. Unadjusted P values for the outcome in the different treatment groups of BCCancer series are displayed.

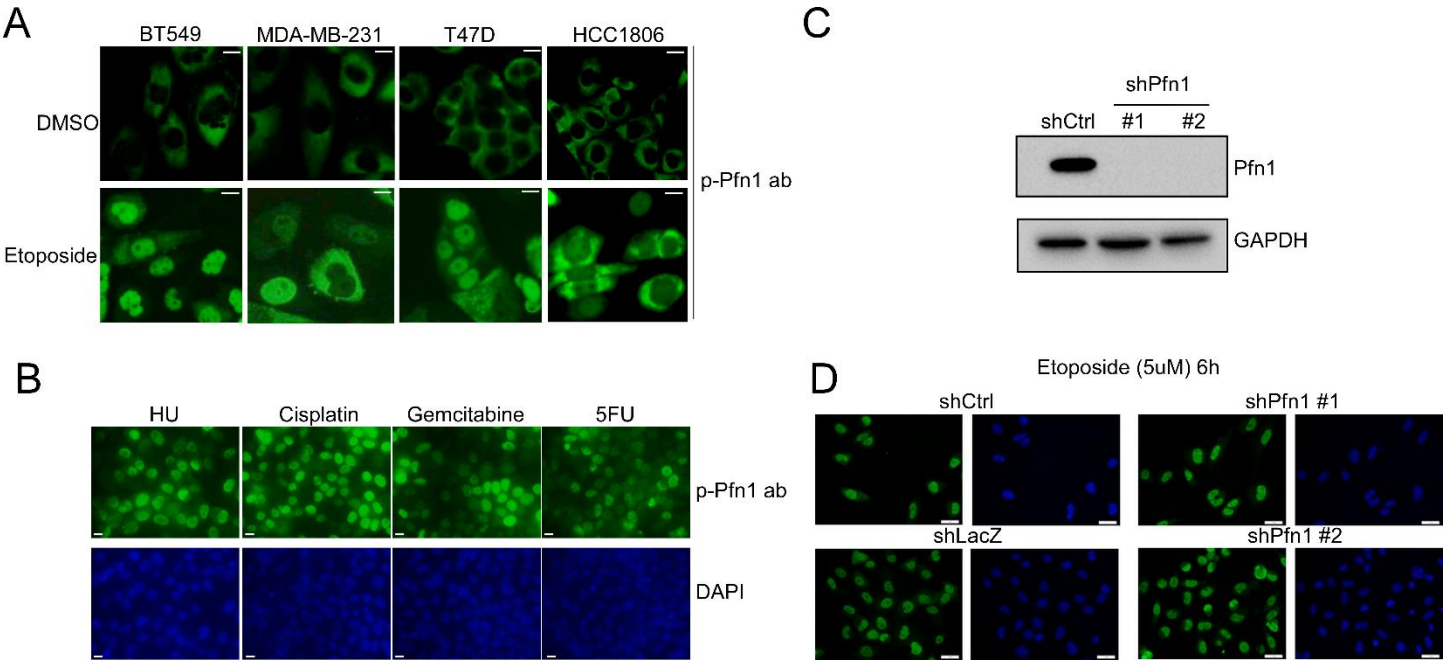

Fig. S4 Genotoxin-induced nuclear antigen of the pSer^{137}-Pfn1 antibody is not Pfn1, related to Fig.2A. (**A**) Different breast cancer cell lines were treated with DMSO or etoposide (5μM) for 6hr, followed by immunofluorescence staining using the pSer^{137}-Pfn1 antibody. (**B**) HeLa cells were treated with various genotoxic agents (1mM HU, 1μM cisplatin, 1μM gemcitabine, 50μM 5-fluorouracil) for 16hr, followed by immunofluorescence staining using the pSer^{137}-Pfn1 antibody and counterstaining by DAPI. Scale bars, 20μm. (**C**) HeLa cells were infected with a control shRNA or two shRNAs targeting human Pfn1. Western blot showing complete Pfn1 knockdown 4 days after infection. (**D**) HeLa cells infected with two control shRNAs (shCtrl and shLacZ) and the two Pfn1-specific shRNAs were treated with 5μM etoposide for 6h, followed by immunofluorescence staining using the pSer^{137}-Pfn1 antibody and counterstaining by DAPI. Scale bars, 40μm.

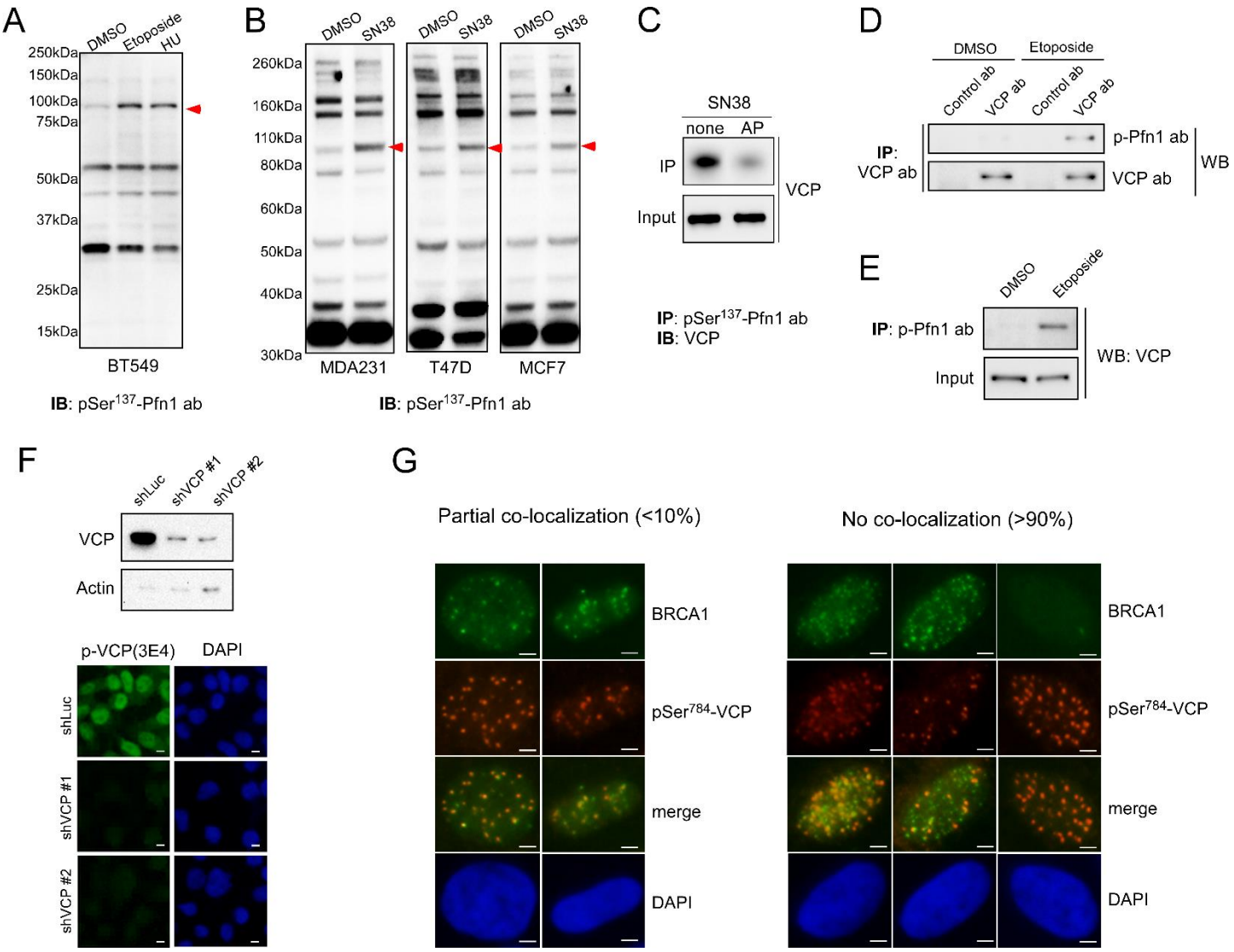

Fig. S5 Identification and characterization of pSer^{784}-VCP, related to Fig.2B-2D and Fig.3. (**A**) BT549 cells were treated with DMSO, 5μM etoposide, and 1mM HU for 12hr, followed by Western blot analysis using the pSer^{137}-Pfn1 antibody. (**B**) MDA-MB-231, T47D, and MCF-7 cells were treated with DMSO or 200nM SN38 for 16hr, followed by Western blot analysis using the pSer^{137}-Pfn1 antibody. Red arrowheads indicate the DNA damage-induced 100kDa protein. (**C**) HeLa cells were treated with 200nM SN38 for 16hr, lysed in SDS-free RIPA, and treated with or without alkaline phosphatase (AP) at 37°C for 1hr. Lysates were immunoprecipitated by the pSer^{137}-Pfn1 antibody followed by Western blot analysis of proteins bound to the antibody and present in the inputs using a VCP-specific antibody. (**D-E**) HeLa cells were treated with DMSO or 50μM etoposide for 24 hours, lysed in denaturing buffer (1% SDS, 20mM Tris-HCl, pH 7.4, 150mM NaCl), and heated at 95°C for 10min. Samples were diluted 20-fold with ice cold buffer containing 20mM Tris-HCl, pH 7.4, 150mM NaCl, protease and phosphatase inhibitors, and clarified at >16,000g for 10min. Supernatants were subjected to immunoprecipitation by control IgG or VCP pan antibody (**D**) or the pSer^{137}-Pfn1 antibody (**E**). IP samples from (**D**) were analyzed by Western blot using the pSer^{137}-Pfn1 or VCP pan antibodies. IP and input samples from (**E**) were analyzed by Western blot using the VCP pan antibody. (**F**) HeLa cells were infected with shLuc or two distinct shRNAs targeting human VCP. Western blot showing effective VCP knockdown after 4 days. Cells were treated with 5μM etoposide for 6hr followed by immunofluorescence staining by the pSer^{784}-VCP antibody and counterstaining by DAPI. Scale bars, 10μm. (**G**) U2OS cells were treated with 50μM etoposide for 1hr, recovered for 90min, detergent-extracted, fixed, and subjected to double immunofluorescence labeling using the pSer^{137}-Pfn1 (rabbit, detecting pSer^{784}-VCP) antibody and a BRCA1-specific (mouse) antibody. DAPI was used for counterstaining. Representative images showing partial co-

localization of pSer^{784}-VCP and BRCA1 in DNA damage foci of <10% of the cells and no co-localization in >90% of the cells. More than 100 cells per condition have been analyzed. Scale bars, 4µm.

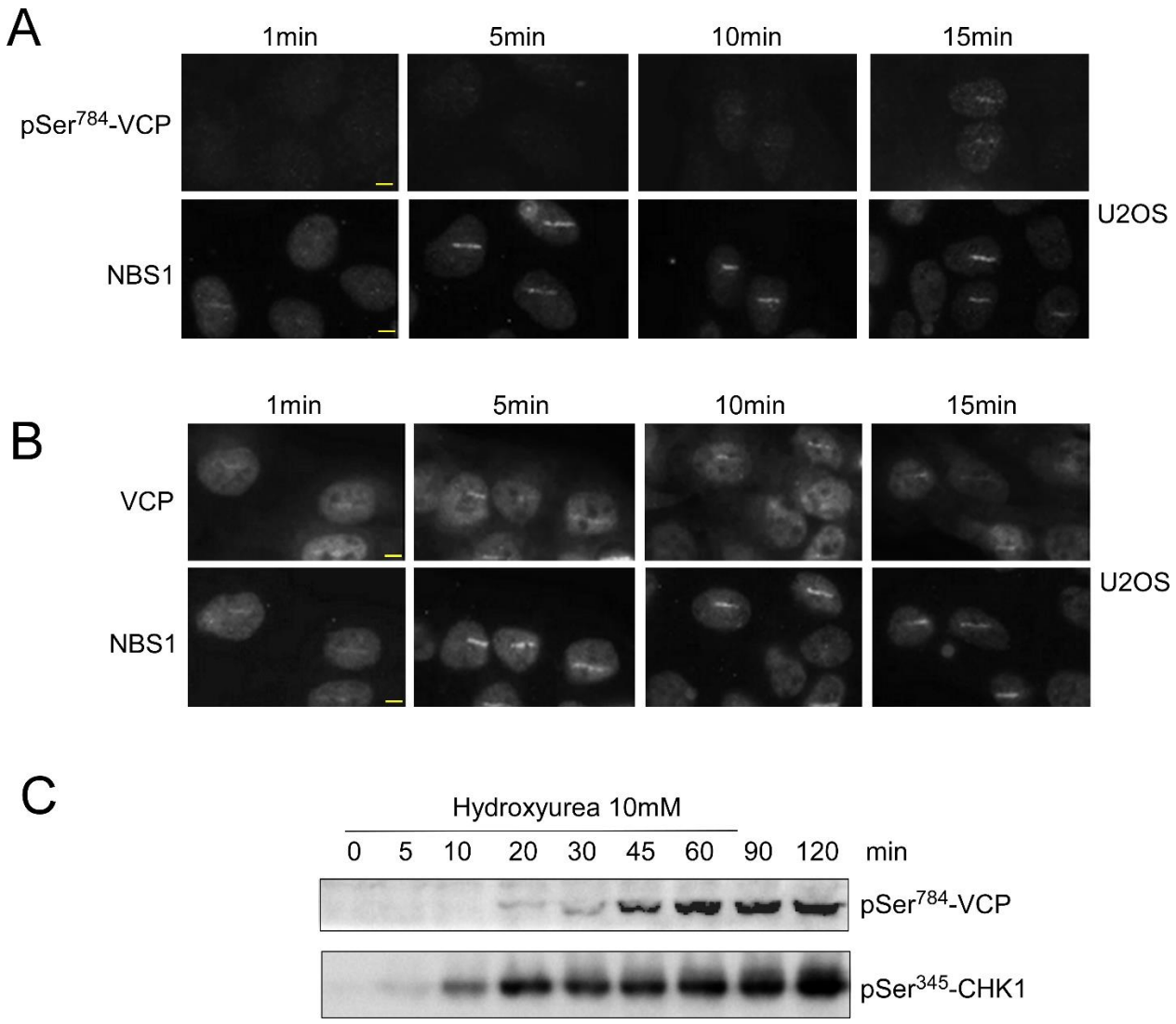

Fig. S6 pSer^{784}-VCP is a late DDR event, related to Fig.4. (**A-B**) U2OS cells were laser micro-irradiated, fixed at different time points, and subjected to double immunofluorescence staining using the pSer^{784}-VCP/NBS1 (**A**) or VCP/NBS1 (**B**) antibody pairs. Scale bars, 10µm. (**C**) HeLa cells were treated with 10mM hydroxyurea for the indicated amounts of time and analyzed by Western blot for pSer^{784}-VCP and pSer^{345}-Chk1.

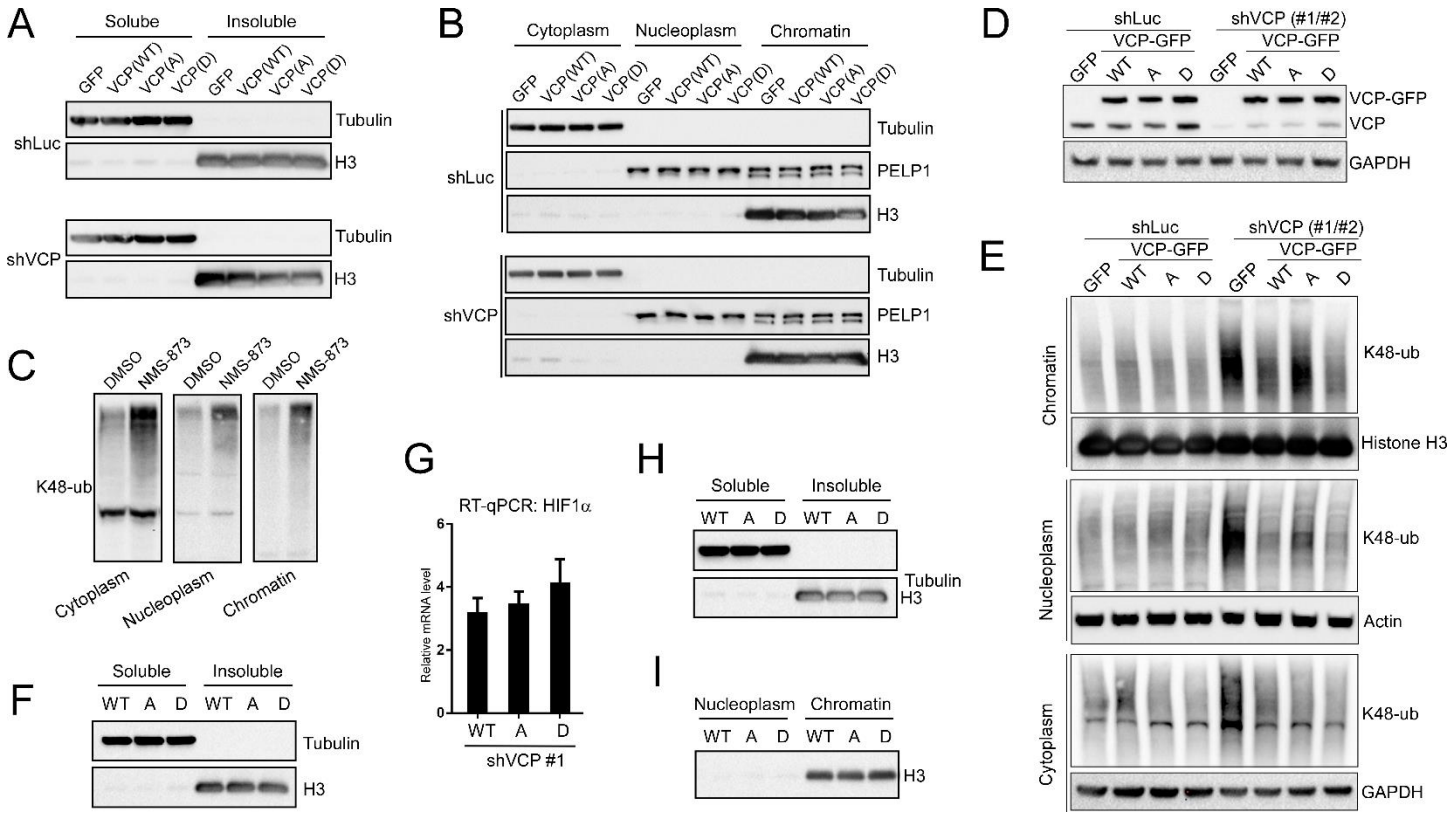

Fig.S7 Effects of Ser^{784} phosphorylation on VCP substrates, related to Fig.5 and Fig.6. (**A**) RIPA lysis of HeLa cells as described in Fig.5B. Samples were blotted for tubulin (soluble marker) and histone H3 (insoluble marker). (**B**) HeLa samples from Fig.5C were blotted for tubulin (cytosolic marker), PELP1 (nuclear marker), and histone H3 (chromatin marker). **(C)** Equal numbers of HeLa cells were treated with 5μM NMS-873 for 1hr and subjected to subcellular fractionation. Cytoplasm, nucleoplasm, and chromatin fractions were analyzed by Western blot using an antibody specific to K48-linked polyubiquitin. (**D**) U2OS cells stably expressing GFP or RNAi-resistant VCP-GFP (WT or mutants) were infected with shLuc or shVCP#1 and #2 combined. Cells were analyzed 4 days later by Western blot using antibodies against VCP (detecting both endogenous VCP and exogenous VCP-GFP) or actin. (**E**) Cells in (G) were treated with 50μM etoposide for 30min, recovered for 1hr, and subjected to subcellular fractionation followed by Western blot analysis of the cytoplasmic, nucleoplasmic, and chromatin fractions using the K48-ubiquitin antibody controlled by GAPDH, actin, and histone H3. (**F**) RIPA lysed HeLa samples from Fig.5E were blotted for tubulin (soluble marker) and histone H3 (insoluble marker). **(G)** HeLa cells expressing RNAi-resistant wild type or mutant VCP were infected with shVCP #1, treated with 50μM etoposide for 30min, recovered for 2h in the presence of 20μM MG-132, and subjected to RT-qPCR for HIF1α using GAPDH for normalization. Shown are mean ± SD of three technical replicates of one biological replicate. (**H**) RIPA lysed HeLa samples from Fig.6C were blotted for tubulin (soluble marker) and histone H3 (insoluble marker). (**I**) Nucleoplasmic and chromatin fractions of HeLa cells from Fig.6D were blotted for histone H3. Note that for (A, B, F, H, I) same samples from the experiments described in the main figures were rerun and reanalyzed here. Same results were confirmed by three biologically independent experiments.

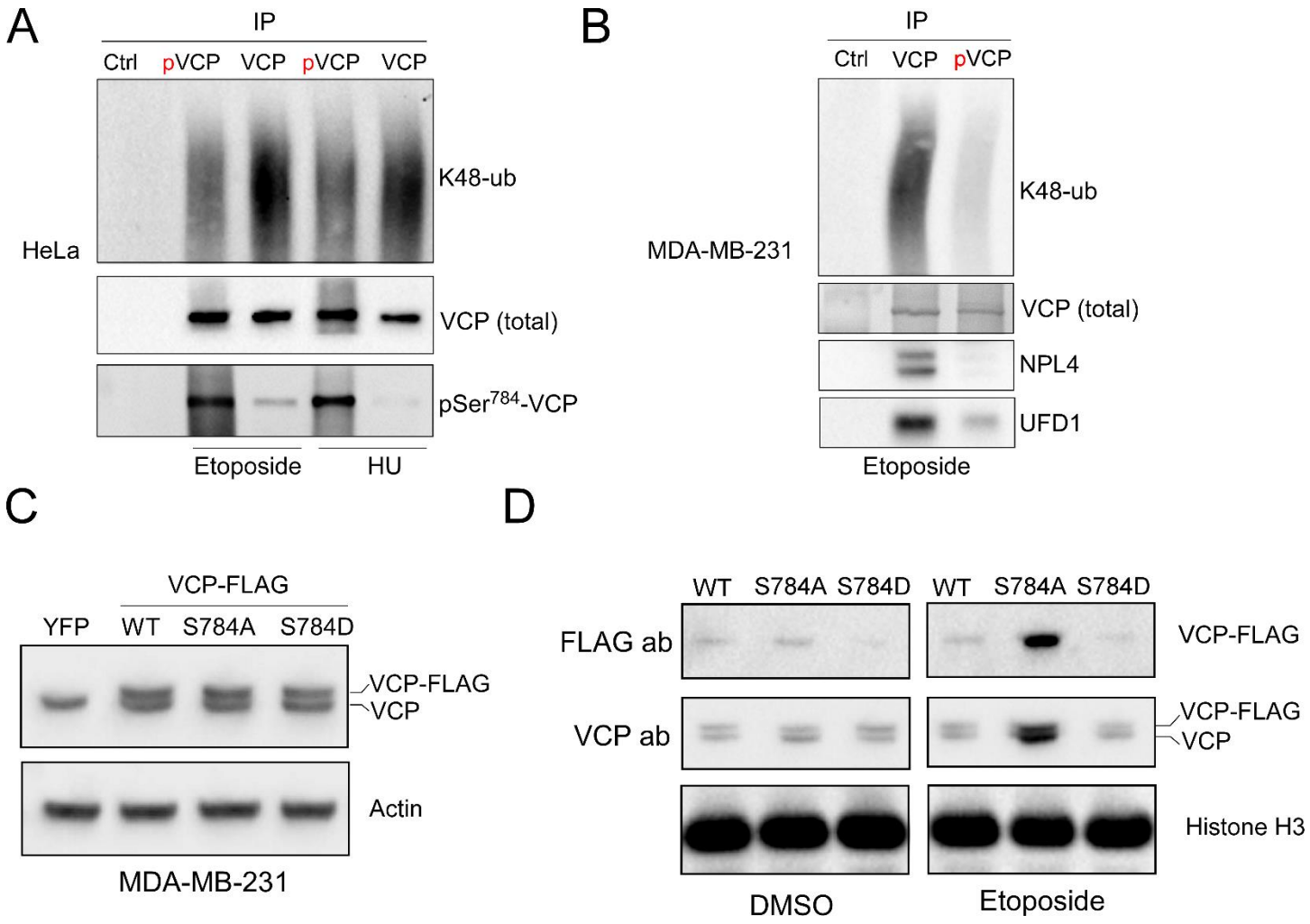

Fig. S8 Ser^{784} phosphorylation reduces VCP interaction with cofactors NPL4/UFD1 and K48-polyubiquinated proteins, related to Fig.6. (**A**) HeLa cells were treated with 5μM etoposide or 1mM HU for 20hr, lysed with SDS-free RIPA buffer, and immunoprecipitated by either the pSer^{784}-VCP antibody or a pan VCP antibody. Antibody-bound proteins were analyzed by Western blot for K48-ubiquitin, total VCP and pSer^{784}-VCP. Same amount of control IgG was used to bind the etoposide-treated HeLa samples to control for non-specific binding. (**B**) MDA-MB-231 cells were treated with 5μM etoposide for 22hr, lysed, and immunoprecipitated by the VCP or pSer^{784}-VCP antibodies, controlled by non-specific IgG as in (A). Samples were analyzed by Western blot for K48-ubiquitin, NPL4, and UFD1. Total VCP bound by the antibodies were visualized by silver staining. (**C**) Western blot showing similar expression levels of VCP-FLAG (WT and mutants) relative to endogenous VCP in the stable MDA-MB-231 cells. YFP-FLAG was expressed as a control. (**D**) MDA-MB-231 stable cells from (C) were treated with DMSO or 5μM etoposide for 12hr, subjected to chromatin fractionation, and analyzed by Western blot using anti-VCP or FLAG antibodies, with histone H3 as the loading control.

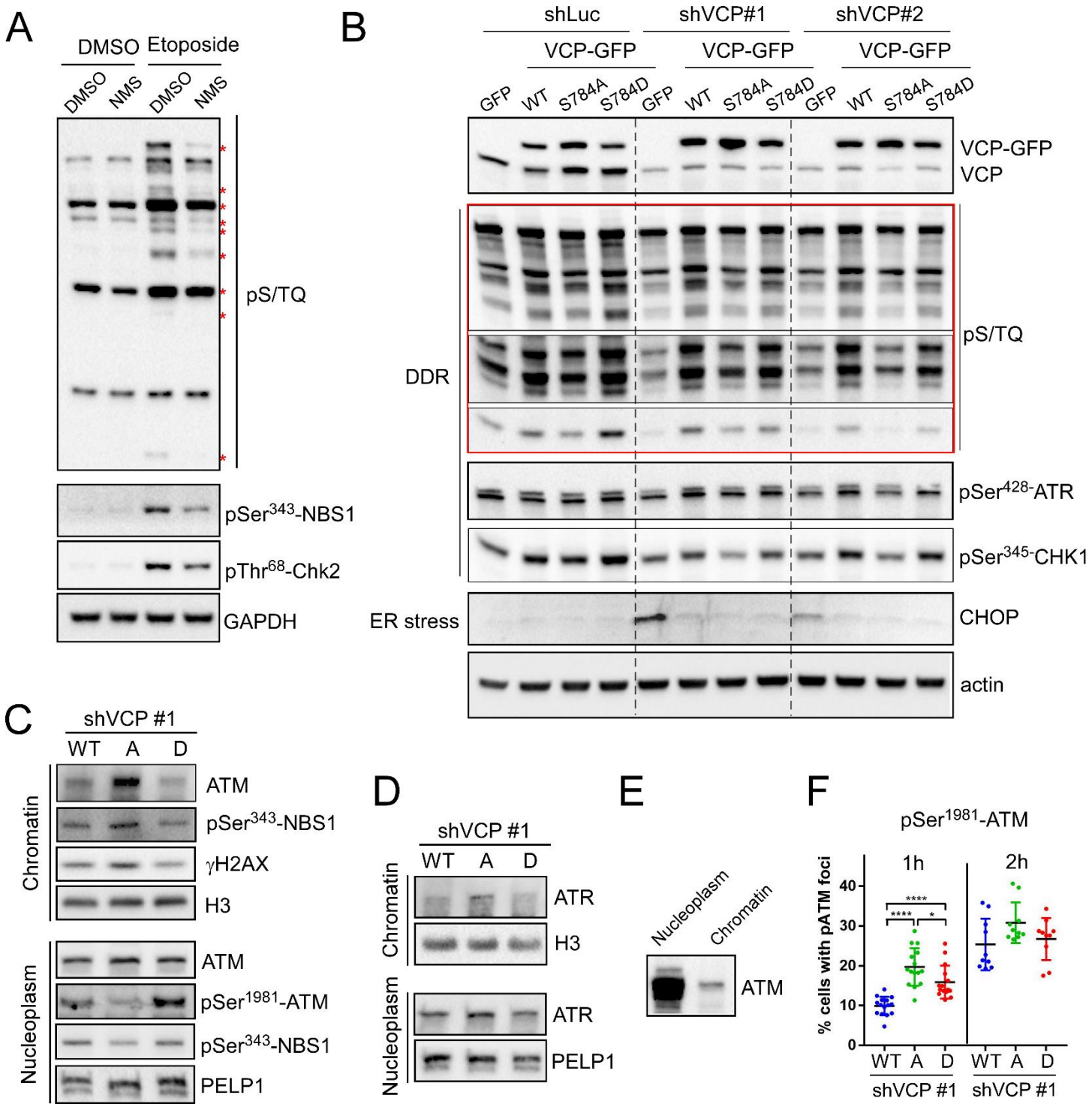

Fig. S9 Effects of Ser^{784} phosphorylation of VCP on PIKK signaling, related to Fig.7A. (A) MDA-MB-231 cells were pre-treated with NMS-873 for 30min followed by 20min treatment with 25μM etoposide. Cells were lysed by SDS-free RIPA buffer, and soluble fractions were analyzed by Western blot for the indicated proteins. Red asterisks indicate pS/TQ motif-containing proteins whose levels are reduced by NMS-873. (**B**) HeLa cells stably expressing GFP or VCP-GFP (WT and mutants) were infected with shLuc, shVCP #1, or shVCP #2 individually. Four days later, they were treated with 1mM HU for 4hr, lysed by SDS-free RIPA buffer, followed by Western blot analysis of the soluble proteins with the indicated antibodies. **(C)** VCP knockdown and rescue HeLa cells were treated with 50μM etoposide for 30min, recovered for 1hr, and subjected to cellular fractionation. Nucleoplasm and chromatin fractions were analyzed by Western blot using the indicated antibodies. PELP1 and histone H3 were blotted as loading controls. (**D**) VCP knockdown and rescue HeLa cells

were treated with 5mM HU for 4hr and subjected to cellular fractionation and Western blot for ATR similarly to (**C**). **(E)** Nucleoplasm and chromatin fractions from (C) were loaded proportionally to show the relative abundance of total ATM in either compartment. (**F**) VCP knockdown and rescue HeLa cells were treated for 15min with 50μM etoposide, recovered for 1hr or 2hr, detergent extracted, fixed with para-formaldehyde, and immunostained for pSer^{1981}-ATM. Shown are single biological replicates containing ~2000 cells per condition. Error bars represent SD. Similar results were confirmed by three biologically independent experiments. P values were based on 2-tailed unpaired t-test. *, p<0.05; ****, p<0.0001.

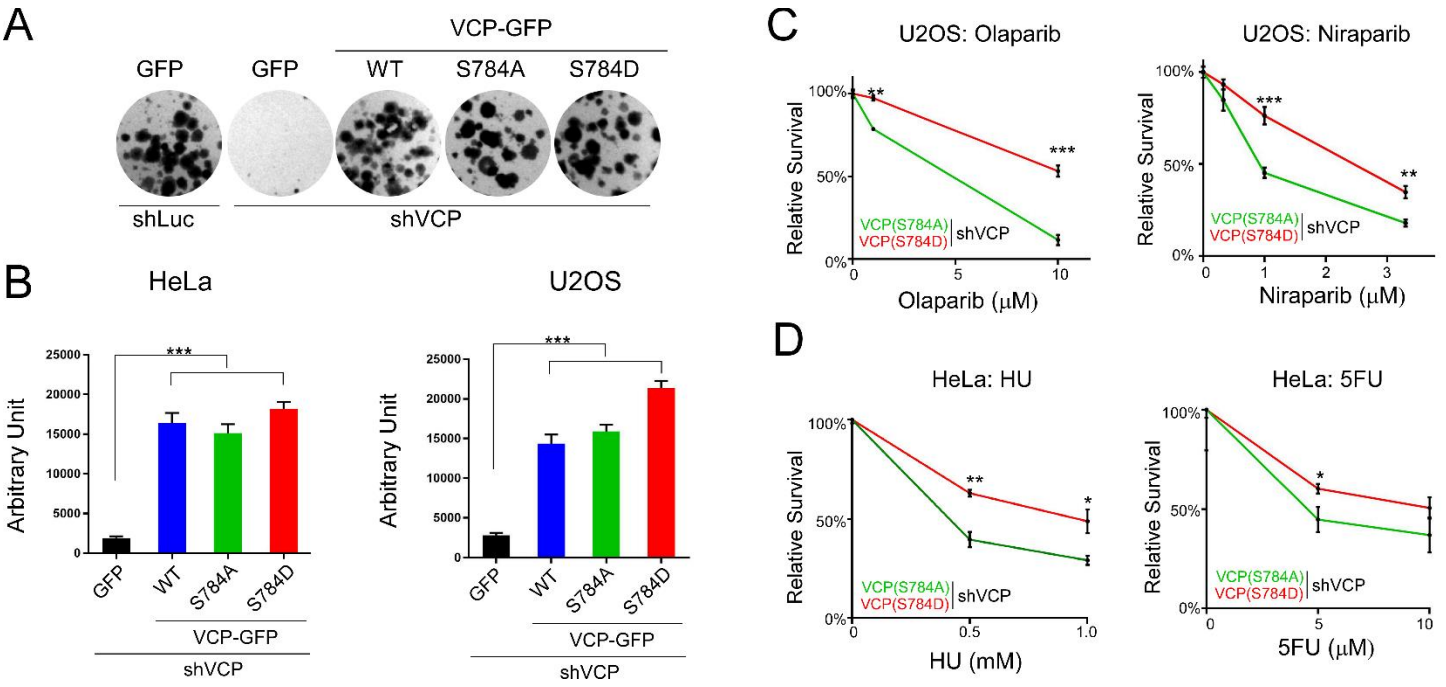

Fig. S10 Ser^{784} phosphorylation is important for VCP function specifically in the presence of genotoxic stress, related to Fig.7B. (**A**) HeLa cells stably expressing GFP or RNAi-resistant VCP-GFP (WT or mutants) were infected with shLuc or shVCP#1 and #2 combined. Same number of cells were plated and grown without treatment for 10 days in colony formation assays. (**B**) HeLa and U2OS stable cells expressing GFP or RNAi-resistant VCP-GFP (WT and mutants) were infected with shVCP #1 and #2 combined and grown in colony formation assays for 10 days as in (A). Viable cells were quantified by Alamar blue. (**C**) Stable U2OS cells expressing RNAi-resistant VCP-GFP (S784A vs. S784D) were infected with shVCP#2 for 4 days, treated with vehicle or PARP inhibtiors olaparib or niraparib at the indicated concentrations for 16hr, and allowed to grow in colony formation assays for 10-14 days. Colonies were stained and quantified, and relative survival was calculated by normalizing drug-treated values over vehicle controls. (**D**) Stable HeLa cells expressing RNAi-resistant VCP-GFP (S784A vs. S784D) were infected with combined shVCP#1 and shVCP#2, treated with HU and 5FU at the indicated concentrations for 16hr, and subjected to colony formation for 7-10 days and quantification as in (**C**). Values in (**B-D**) represent mean ± SEM of three technical replicates of single experiments. Results were confirmed by three biologically independent experiments. P values were based on unpaired student's t-test at the indicated drug concentrations. *<0.05, **<0.01, ***, p<0.001.

Supplemental Tables

Allred score	**Nuclear Allred scores of the antigen of the pSer^{137}-Pfn1 antibody**
Allred 0	117 (39.67%)
Allred 2	0 (0%)
Allred 3	74 (25.08%)
Allred 4	63 (21.35%)
Allred 5	24 (8.14%)
Allred 6	13 (4.41%)
Allred 7	4 (1.35%)
Allred 8	0 (0%)
Total	295

Table S1: Distributions of the level of the nuclear antigen of pSer^{137}-Pfn1 antibody in the UBC series, related to Fig.1C and 1D.

Clinicopathologic characteristic	**Allred score (0-3) (n=191)**	**Allred score (4-8) (n=104)**	**Total (n=295)**	**P-value**
Age, years				0.80
<50	19 (15%)	14 (13%)	33 (14%)	
≥50	111 (85%)	90 (87%)	201 (86%)	
Tumor Size, cm				0.55
≤2	19 (11%)	14 (13%)	33 (12%)	
>2	153 (89%)	90 (87%)	243 (88%)	
Involved lymph nodes				
Negative	128 (67%)	61 (59%)	189 (64%)	0.14
Positive	62 (33%)	43 (41%)	105 (36%)	
Tumor grade				0.02
1	44 (24%)	11 (11%)	55 (19%)	
2	70 (37%)	42 (40%)	112 (38%)	
3	74 (39%)	51 (49%)	125 (43%)	
Lympho-vascular Invasion				0.16
Negative	144 (79%)	72 (71%)	216 (76%)	
Positive	39 (21%)	29 (29%)	68 (24%)	
ER Status				0.81
Negative	38 (20%)	22 (21%)	60 (31%)	
Positive	152 (80%)	82 (79%)	134 (69%)	
PR Status				0.20
Negative	57 (30%)	39 (38%)	96 (33%)	
Positive	132 (70%)	65 (62%)	197 (67%)	
HER2 Status				0.28
Negative	176 (95%)	91 (92%)	267 (94%)	
positive	9 (5%)	8 (8%)	17 (6%)	

Ki67				0.66
<14%	111 (59%)	59 (57%)	170 (58%)	
≥14%	76 (41%)	45 (43%)	121 (42%)	
IHC Subtype				
Luminal A	99 (55%)	52 (53%)	151 (54%)	(Luminal A vs. others) = 0.82
Luminal B (high Ki67, HER2-)	46 (25%)	24 (24%)	70 (25%)	(Luminal B vs. others) = 0.94
Luminal B (HER2+)	4 (2%)	2 (2%)	6 (2%)	
Her2-Enriched	5 (3%)	6 (6%)	11 (4%)	(Her2-Enriched vs. others) = 0.20
Triple Negative	27 (15%)	15 (15%)	42 (15%)	(Triple Negative vs. others) = 0.90
Basal-like	18 (10%)	10 (10%)	28 (10%)	(Basal-like vs. others) = 0.87

Table S2: Clinicopathologic characteristics of the nuclear antigen of the pSer^{137}-Pfn1 antibody in the UBC series, related to Fig.1C and 1D.

	Univariate analysis for OS in the UBC series			
	Hazard ratio	**Lower (95% CI)**	**Upper (95%CI)**	**P-value**
pSer^{137}-Pfn1 (nuclear)	2.4	1.3	4.4	< 0.01
	Multivariate analysis for OS in the UBC series			
	Hazard ratio	**Lower (95% CI)**	**Upper (95%CI)**	**P-value**
pSer^{137}-Pfn1 (nuclear)	2.51	1.31	4.83	< 0.01
Age	1.06	1.03	1.09	< 0.01
Tumor grade	1.29	0.60	2.80	0.05
Nodal status	1.15	0.54	2.45	0.07
Tumor size	1.46	1.19	1.80	< 0.01
Lympho-vascular invasion	2.82	1.37	5.82	< 0.01
ER status	0.92	0.33	2.57	0.09
PR status	0.61	0.27	1.42	0.03
HER2 status	1.57	0.49	5.08	0.05

Table S3: Univariate and multivariate analysis of overall survival (OS) for the level of the nuclear antigen of pSer^{137}-Pfn1 antibody in the UBC series, related to Fig.1C and 1D.

	Univariate analysis for BCSS in the UBC series			
	Hazard ratio	**Lower (95% CI)**	**Upper (95%CI)**	**P-value**
pSer^{137}-Pfn1 (nuclear)	2.39	1.15	4.97	< 0.01
	Multivariate analysis for BCSS in the UBC series			
	Hazard ratio	**Lower (95% CI)**	**Upper (95%CI)**	**P-value**
pSer^{137}-Pfn1 (nuclear)	2.30	1.03	5.10	< 0.01
Age	1.02	0.99	1.06	0.02
Tumor grade	1.70	0.68	4.25	0.03
Nodal status	1.56	0.60	4.07	0.04
Tumor size	1.58	1.27	1.98	< 0.01
Lympho-vascular invasion	2.93	1.20	7.15	< 0.01
ER status	1.04	0.32	3.32	0.09
PR status	0.58	0.21	1.59	0.03
HER2 status	1.46	0.38	5.60	0.06

Table S4: Univariate and multivariate analysis of breast cancer specific survival (BCSS) for the level of the nuclear antigen of pSer^{137}-Pfn1 antibody in the UBC series, related to Fig.1C and 1D.

	Univariate analysis for RFS in the UBC series			
	Hazard ratio	**Lower (95% CI)**	**Upper (95%CI)**	**P-value**
pSer^{137}-Pfn1 (nuclear)	2.55	1.51	4.33	< 0.01
	Multivariate analysis for RFS in the UBC series			
	Hazard ratio	**Lower (95% CI)**	**Upper (95%CI)**	**P-value**
pSer^{137}-Pfn1 (nuclear)	2.30	1.31	4.04	< 0.01
Age	1.00	0.98	1.03	0.08
Tumor grade	1.13	0.61	2.10	0.07
Nodal status	1.19	0.62	2.29	0.06
Tumor size	1.29	1.09	1.52	< 0.01
Lympho-vascular invasion	2.10	1.09	4.06	< 0.01
ER status	0.95	0.38	2.33	0.09
PR status	0.73	0.34	1.56	0.04
HER2 status	1.49	0.54	4.12	0.04

Table S5: Univariate and multivariate analysis of relapse free survival (RFS) for the level of the nuclear antigen of pSer^{137}-Pfn1 antibody in the UBC series, related to Fig.1C and 1D.

Nuclear Allred scores of the antigen of the pSer^{137}-Pfn1 antibody			
Allred score	**Training set**	**Validation set**	**Combined set**
Allred 0	195 (14.9%)	183 (13.7%)	378 (14.3%)
Allred 2	16 (1.2%)	19 (1.4%)	35 (1.3%)
Allred 3	111 (8.5%)	138 (10.3%)	249 (9.4%)
Allred 4	193 (14.7%)	193 (14.4%)	386 (14.6%)
Allred 5	254 (19.4%)	246 (18.4%)	500 (18.9%)
Allred 6	321 (24.5%)	314 (23.4%)	635 (24%)
Allred 7	221 (16.9%)	247 (18.4%)	468 (17.7%)
Allred 8	0 (0%)	0 (0%)	0 (0%)
Total	1311	1340	2651

Table S6: Distributions of the levels of the nuclear antigen of pSer^{137}-Pfn1 antibody in the BCCancer series, related to Fig.1E.

Clinicopathologic characteristic	**Nuclear p-PFN1 Allred score (0-4) (n=515)**	**Nuclear p-PFN1 Allred score (5-8) (n=796)**	**Total (n=1311)**	**P-value**
Age, years				0.66
<50	147 (29%)	218 (27%)	365 (28%)	
≥50	368 (71%)	578 (73%)	946 (72%)	
Tumor Size, cm				0.04
≤2	281 (55%)	387 (49%)	668 (51%)	
>2	232 (45%)	406 (51%)	638 (49%)	
Involved lymph nodes				0.43
Negative	267 (54%)	433 (57%)	700 (56%)	
1-3	142 (29%)	218 (29%)	360 (29%)	
4-9	66 (13%)	79 (11%)	145 (12%)	
≥10	17 (4%)	25 (3%)	42 (3%)	
Stage				0.36
I	186 (36%)	264 (33%)	450 (34%)	
II	295 (58%)	473 (60%)	768 (59%)	
III	30 (6%)	58 (7%)	88 (7%)	
Tumor grade				0.002
1-2	256 (52%)	331 (43%)	578 (46%)	
3	233 (48%)	435 (57%)	668 (54%)	
Lympho-vascular Invasion				1.00
Negative	273 (55%)	421 (55%)	556 (44%)	
Positive	219 (45%)	337 (45%)	694 (56%)	
ER Status				0.02
Negative	121 (24%)	237 (30%)	358 (27%)	
Positive	394 (76%)	557 (70%)	951 (73%)	
HER2 Status				0.001
Positive	48 (10%)	125 (16%)	173 (13%)	
Negative	454 (90%)	657 (84%)	1111 (87%)	

Ki67				0.18
<14%	264 (56%)	391 (52%)	655 (54%)	
≥14%	205 (44%)	358 (48%)	563 (46%)	
Subtype				(Luminal vs. non-luminal) = 0.004
Luminal A	225 (44%)	306 (38%)	531 (41%)	
Luminal B	151 (29%)	242 (30%)	393 (30%)	
Her2-Enriched	22 (4%)	65 (8%)	87 (7%)	
Triple Negative	70 (14%)	138 (17%)	208 (16%)	(Triple negative vs. non-triple negative) = 0.07
Basal-like	34 (7%)	90 (11%)	124 (9%)	

Table S7: Clinicopathologic characteristics of the nuclear antigen of the pSer^{137}-Pfn1 antibody in the training set of the BCCancer series, related to Fig.1E.

Clinicopathologic characteristic	**Allred score (0-4) (n=533)**	**Allred score (5-8) (n=807)**	**Total (n=1340)**	**P-value**
Age, years				0.007
<50	179 (34%)	215 (27%)	394 (29%)	
≥50	354 (66%)	592 (73%)	946 (71%)	
Tumor Size, cm				0.29
≤2	285 (54%)	406 (51%)	691 (52%)	
>2	245 (46%)	395 (49%)	640 (48%)	
Involved lymph nodes				0.34
Negative	285 (56%)	426 (55%)	711 (56%)	
1-3	158 (31%)	222 (29%)	380 (30%)	
4-9	48 (10%)	91 (12%)	139 (11%)	
≥10	15 (3%)	32 (4%)	47 (4%)	
Stage				0.22
I	192 (36%)	258 (32%)	450 (34%)	
II	291 (55%)	478 (60%)	769 (58%)	
III	47 (9%)	65 (8%)	112 (8%)	
Tumor grade				0.001
1-2	250 (49%)	312 (40%)	562 (43%)	
3	259 (51%)	474 (60%)	733 (57%)	
Lympho-vascular Invasion				0.65
Negative	272 (53%)	404 (52%)	676 (52%)	
Positive	240 (47%)	378 (48%)	618 (48%)	
ER Status				<0.001
Negative	102 (19%)	267 (33%)	369 (28%)	
Positive	429 (81%)	539 (67%)	968 (72%)	
HER2 Status				<0.001
Positive	472 (90%)	653 (83%)	1125 (86%)	
Negative	51 (10%)	133 (17%)	184 (14%)	

KI67				<0.001
<14%	292 (60%)	377 (4%)	669 (53%)	
≥14%	197 (40%)	384 (51%)	581 (47%)	
Subtype				(Luminal vs. non-luminal) < 0.001
Luminal A	251 (51%)	295 (39%)	546 (40%)	
Luminal B	160 (33%)	230 (31%)	390 (28%)	
Her2-Enriched	22 (5%)	76 (10%)	98 (7%)	
Triple Negative	56 (11%)	153 (20%)	209 (15%)	(Triple negative vs. non-triple negative) < 0.001
Basal-like	24 (5%)	105 (21%)	129(10%)	

Table S8: Clinicopathologic characteristics of the nuclear antigen of the pSer^{137}-Pfn1 antibody in the validation set of the BCCancer series, related to Fig.1E.

Clinicopathologic characteristic	**Allred score (0-4) (n=1048)**	**Allred score (5-8) (n=1603)**	**Total (n=2651)**	**P-value**
Tumor grade				<0.001
1-2	506 (51%)	643 (41%)	1149 (45%)	
3	492 (49%)	909 (59%)	1401 (55%)	
ER Status				<0.001
Negative	223 (21%)	504 (31%)	727 (27%)	
Positive	823 (79%)	1096 (69%)	1919 (73%)	
HER2 Status				<0.001
Positive	926 (90%)	1310 (83%)	2236 (86%)	
Negative	99 (10%)	258 (17%)	357 (14%)	
Subtype				(Luminal vs. non-luminal) <0.001
Luminal A	476 (50%)	601(40%)	1077(41.3%)	
Luminal B	311 (32%)	472(31%)	783(30.1%)	
Her2-Enriched	44 (5%)	141(10%)	185(7.1%)	
Triple Negative	126 (13%)	291 (19%)	417 (16%)	(Triple negative vs. non-triple negative) < 0.001
Basal-like	58 (6%)	195(13%)	253(9.7%)	

Table S9: Clinicopathologic characteristics of the nuclear antigen of the pSer^{137}-Pfn1 antibody in the whole BCCancer series (only significant results on both training + validation sets are presented), related to Fig.1E.