

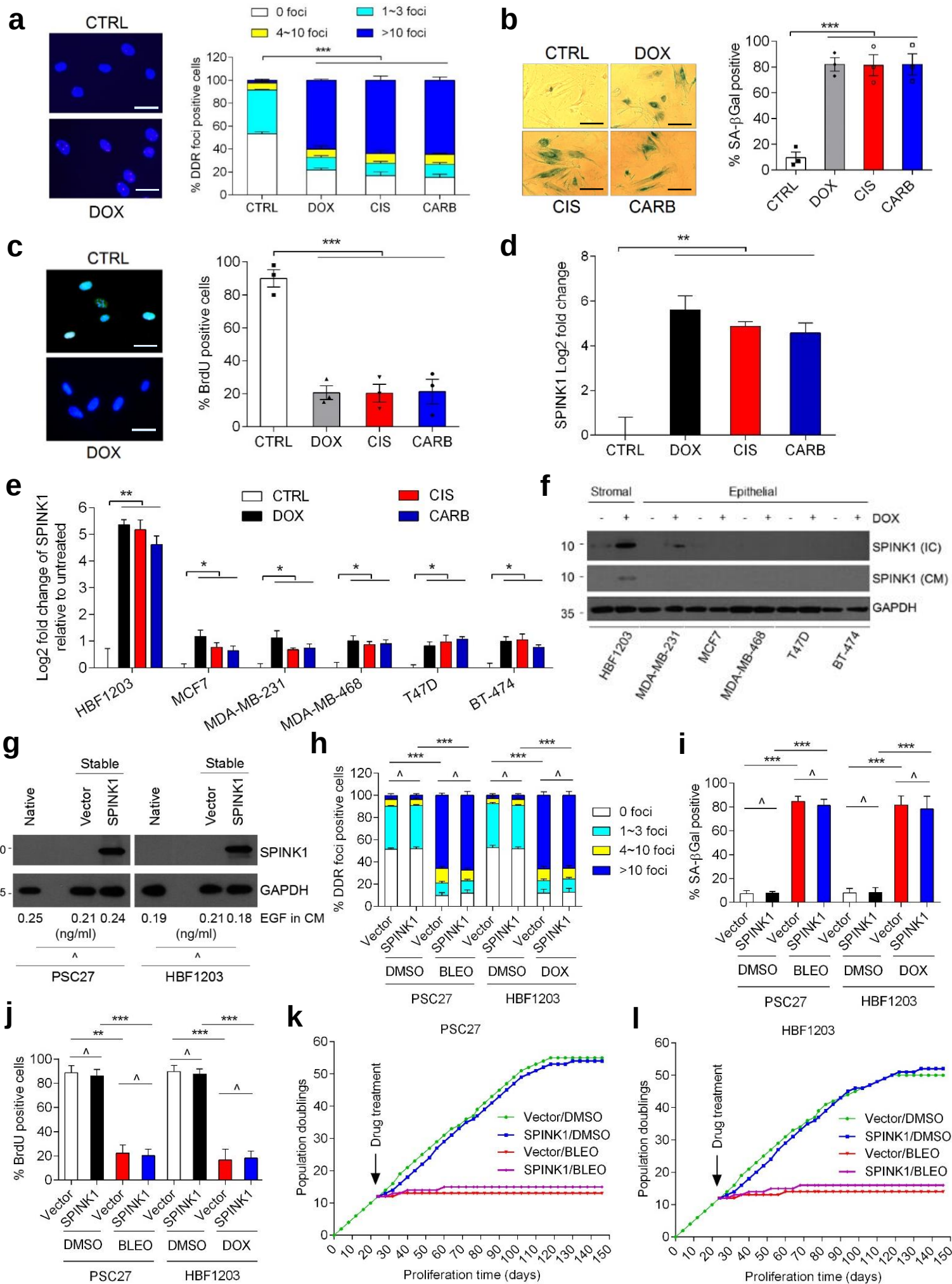
Targeting SPINK1 in the damaged tumour microenvironment alleviates
therapeutic resistance
Sun et al.

File Name: Supplementary Information

Description: Supplementary Figures and Supplementary Tables

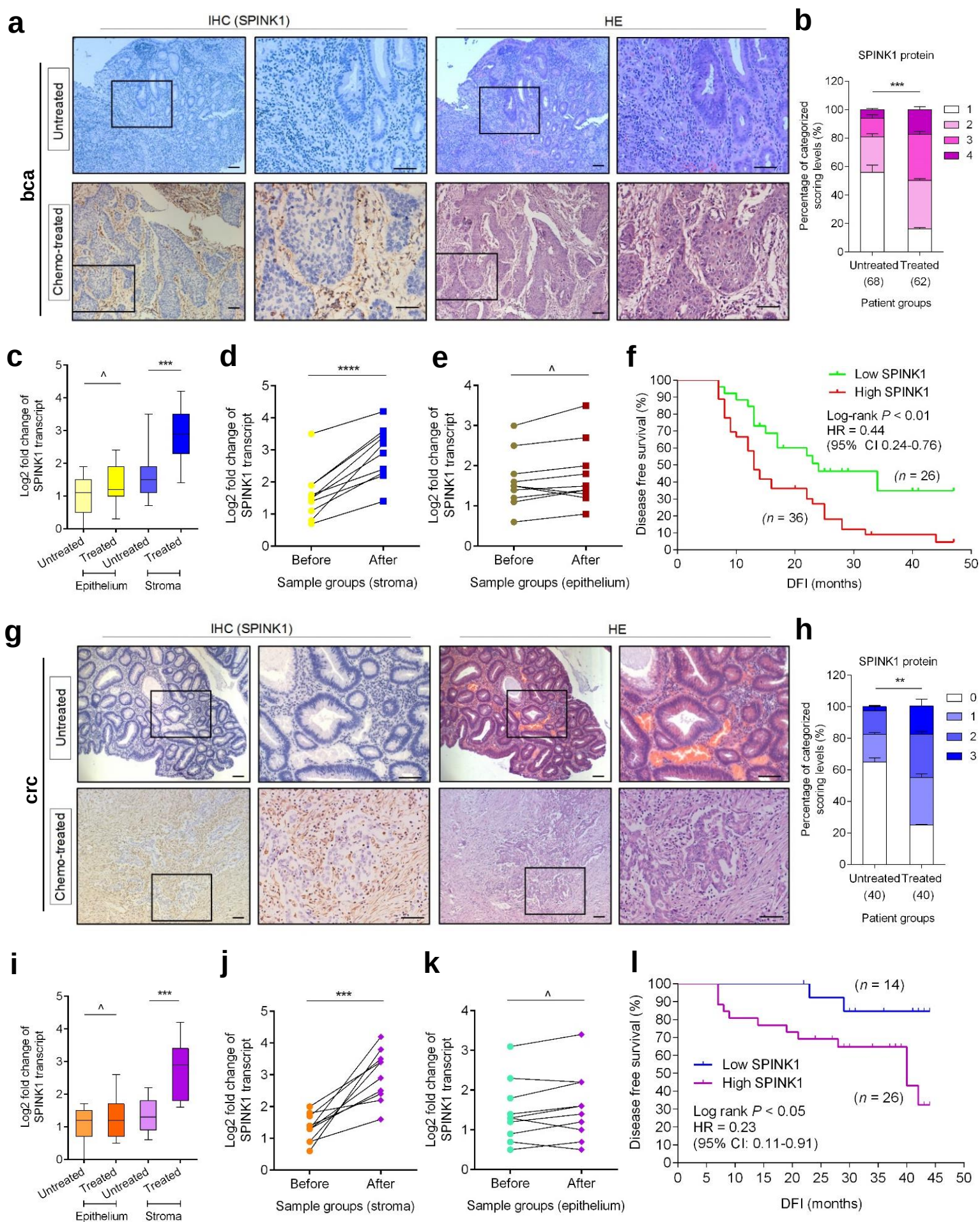
Supplementary Figures: Supplementary Figure 1-8

Supplementary Tables: Supplementary Table 1-5



Supplementary Figure 1. SPINK1 expression is upregulated in human breast stromal cells treated by genotoxic agents. (a) Representative immunofluorescence staining (IF) images (γ H2AX, left) and comparative statistics (right) of DNA damage foci (DDR) in human breast stromal cell line HBF1203. Cells were treated by DOX (doxorubicin), CIS (cisplatin) or CARB (carboplatin). DDR was profiled into 4 categories including 0 foci, 1–3 foci, 4–10 foci and > 10 foci per cell. CTRL, control. DOX, doxorubicin. CIS, cisplatin. CARB, carboplatin. DOX-treated cells were stained by IF for example. Scale bars, 15 μ m. (b) Senescence assessment of HBF1203 cells treated by various agents used in (a). Cells were stained for SA- β -Gal activity 7 d after treatments. Scale bars, 15 μ m. (c) DNA incorporation assay of HBF1203 by BrdU staining. Cells were treated by different agents as applied in (a) and (b). Scale bars, 20 μ m. (d) Transcript expression of SPINK1 in HBF1203 cells 7 d after treatment by several agents. Signal of each treatment condition was normalized to the CTRL group. (e) Comparative analysis of SPINK1 transcript expression in breast stromal (HBF1203) and epithelial cells (MCF7, MDA-MB-231, MDA-MB-468, T47D and BT-474) of human breast origin. Signals normalized to untreated sample (CTRL) per cell line. (f) Immunoblot of SPINK1 expression in breast stromal and epithelial cells after doxorubicin (DOX) treatment as performed in (e). IC, intracellular extracts. CM, conditioned media. GAPDH, loading control. (g) Immunoblot of SPINK1 expression in PSC27 and HBF1203 lines. Native, primary stromal cell line. Vector, stromal cells lentivirally infected with a control vector. SPINK1, stromal cells lentivirally infected with the SPINK1 construct. Concentration of EGF in the CM of cells was measured by ELISA to evaluate influence of SPINK1 expression on the production of other soluble factors. (h) Comparative statistics of DNA damage foci (DDR) by immunofluorescence (IF) staining against

γ H2AX in PSC27 and HBF1203 subline cells treated by genotoxic agents (PSC27 by BLEO, HBF1203 by DOX, to simulate the clinical conditions of PCa and BCa, respectively). DDR were classified into 4 sub-categories including 0 foci, 1~3 foci, 4~10 foci and > 10 foci per cell. (i) Statistics of SA- β -Gal staining results of PSC27 and HBF1203 cells treated by genotoxic agents as used in (h). Cells were stained 7 d after *in vitro* treatments. (j) Statistics of BrdU staining results of stromal cells treated by genotoxic agents as used in (h). (k) Population doubling assay of PSC27 cells grown under several different conditions. BLEO was applied to cell culture at day 22nd, and cell proliferation evaluated through day 150th. (l) Population doubling assessment of HBF1203 performed as described in (k), except that DOX was employed to damage breast stromal cells. Data are shown as mean $\pm$ SD and representative of 3 independent experiments, with 3 technical replicates run per cell-based assay. *P* values were calculated by Student's *t*-test (b, c, d, e, i, j) and two-way ANOVA (a, h) (^, *P* > 0.05; *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001).



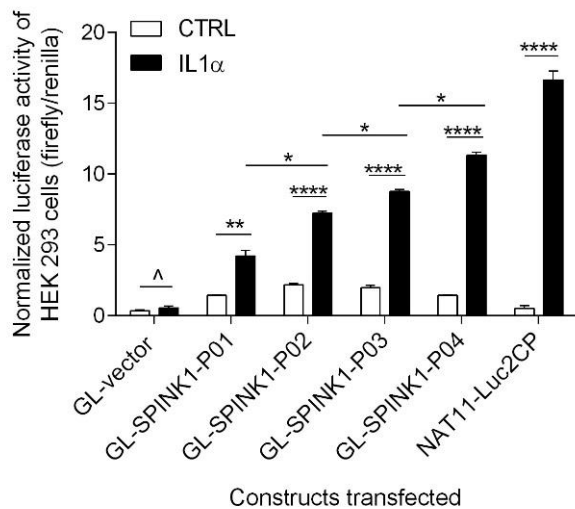
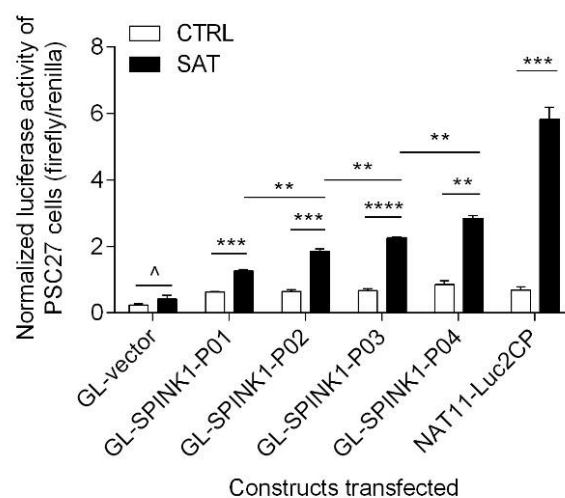
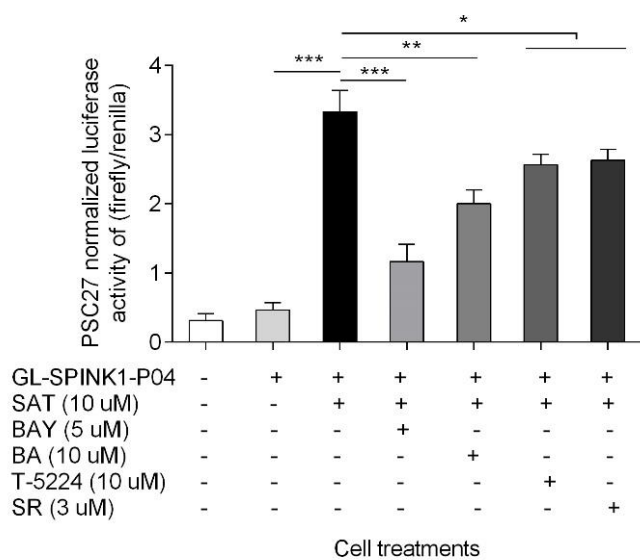
Supplementary Figure 2. SPINK1 is expressed in the tumor microenvironment (TME) of human breast cancer (BCa) and colorectal cancer (CRC) patients after chemotherapy. (a)

Representative images of SPINK1 expression in the primary foci of human BCa patients. Left, immunohistochemical (IHC) staining. Right, hematoxylin and eosin (HE) staining. In each staining set, top tissues are from untreated patients; bottom tissues from treated. Rectangular region in the left image per staining is zoomed into the right image. Scale bars, 100 μ m. **(b)** Pathological assessment of stromal SPINK1 expression in BCa patients (untreated, 68; treated, 62). Patients were pathologically assigned into 4 categories per SPINK1 staining intensity in the stroma. 1, negative; 2, weak; 3, moderate; 4, strong. Statistical comparison of untreated and treated groups is displayed. $P < 0.001$ by ANOVA. **(c)** Comparative analysis of SPINK1 expression at transcript level between different cell lineages after chemotherapy. Briefly, epithelial and stromal cells were separately acquired by laser capture microdissection (LCM) from the primary tumors of BCa patients, with RNA subsequently extracted for quantitative assessment. **(d)** Statistical appraisal of SPINK1 induction in the stroma of 10 randomly selected BCa patients. Stromal cells from both before and after treatment per same patient were isolated by LCM and subject to transcript examination. **(e)** Statistical appraisal of SPINK1 induction in the epithelium of the tumor foci of 10 randomly selected BCa patients. Cells from the same individual patients were isolated by LCM and subject to transcript examination. **(f)** Kaplan-Meier analysis of BCa patients. Disease free survival (DFS) stratified according to SPINK1 expression (low, average score < 2 , green line, $n = 26$; high, average score ≥ 2 , red line, $n = 36$). DFS represents the length (months) of period calculated from the date of BCa diagnosis to the point of first time disease relapse. Survival curves were plotted according to the Kaplan–Meier

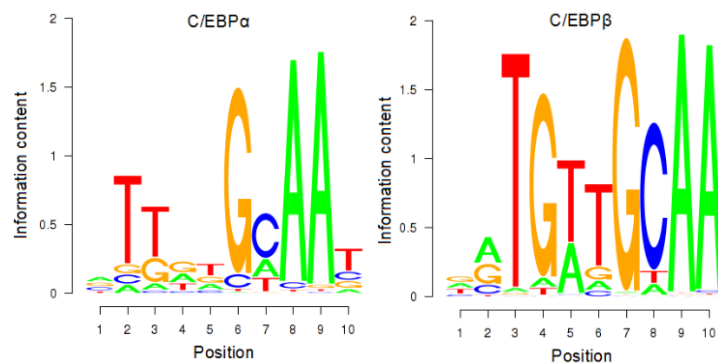
method, while *P* value was calculated using a log-rank (Mantel-Cox) test. **(g)** Representative images of SPINK1 expression in the primary foci of human colorectal cancer (CRC) patients. Left, IHC staining. Right, HE staining. In each staining set, top tissues are from untreated patients; bottom tissues from treated. Rectangular region in the left image per staining is amplified into the right image. Scale bars, 100 μ m. **(h)** Pathological assessment of stromal SPINK1 expression in BCa patients (untreated, 40; treated, 40). Patients were pathologically assigned into 4 categories per SPINK1 staining intensity in the stroma. 1, negative; 2, weak; 3, moderate; 4, strong. Statistical comparison of untreated and treated groups is displayed. *P* < 0.01 by ANOVA. **(i)** Comparative analysis of SPINK1 expression at transcript level between different cell lineages after chemotherapy. Stromal cells were separately acquired by LCM from the primary tumors of CRC patients, with transcripts subsequently prepared for quantitative assessment. **(j)** Induction appraisal of SPINK1 in the stroma of 10 randomly selected CRC patients. Stromal cells from both before and after treatment per patient were isolated by LCM and subject to transcript examination. **(k)** Induction appraisal of SPINK1 in the epithelium of 10 randomly selected CRC patients. Epithelial cells from both before and after treatment per patient were isolated by LCM and subject to transcript examination. **(l)** Kaplan-Meier analysis of CRC patients. Disease free survival (DFS) stratified according to SPINK1 expression (low, average score < 2, blue line, n = 14; high, average score $\geq$ 2, pink line, n = 26). DFS represents the length (months) of period calculated from the date of CRC diagnosis to the point of first time disease relapse. Survival curves generated according to the Kaplan–Meier method, with *P* value calculated using a log-rank (Mantel-Cox) test. Data are shown as mean $\pm$ SD and representative of 3 independent experiments. *P* values were calculated by Student's *t*-test **(c,**

d, e, i, j, k), one-way ANOVA (**b, h**) and log-rank test (**f, l**) ($^{\wedge}$, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$;

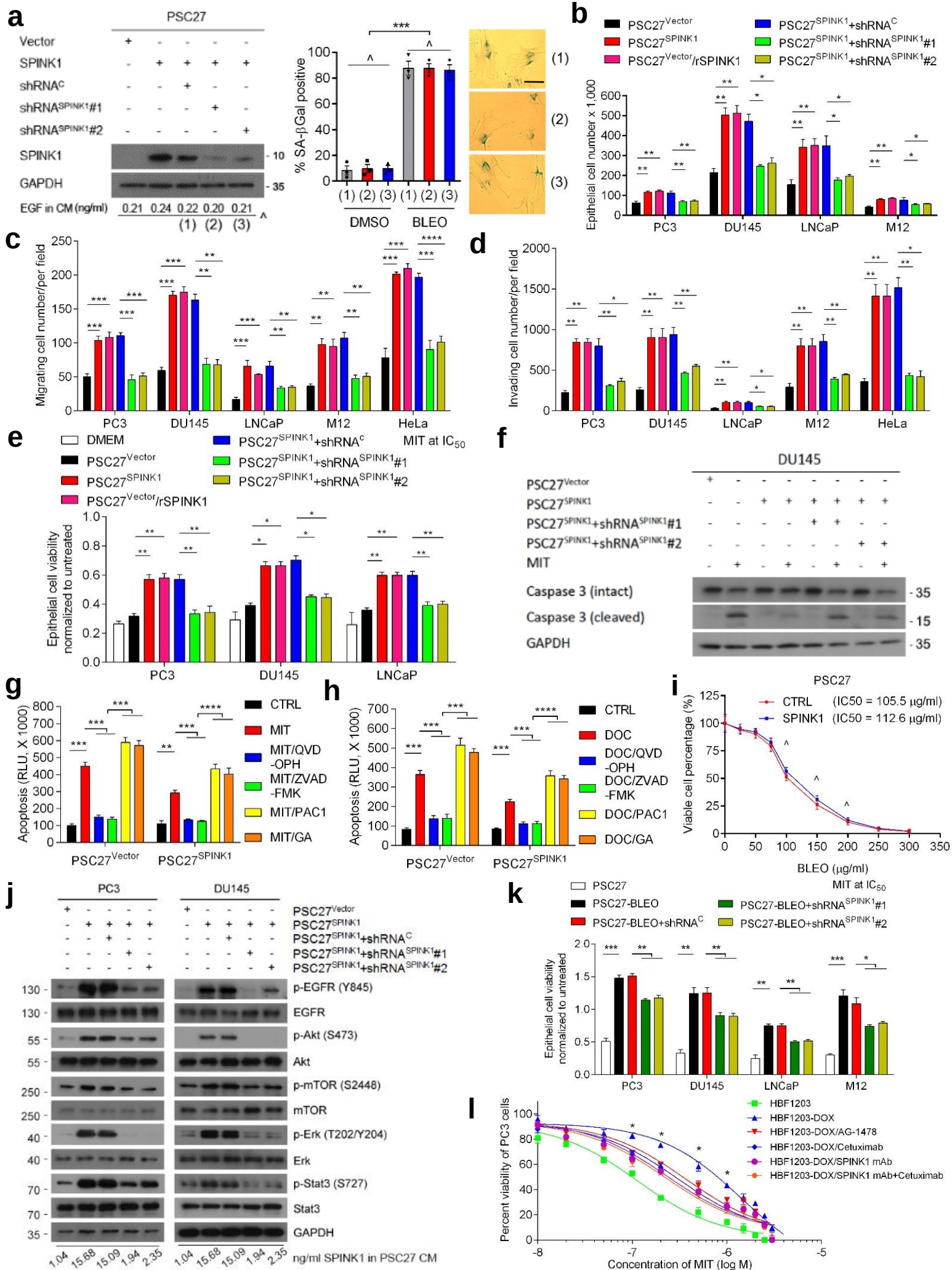
, $P < 0.001$; *, $P < 0.0001$). HR, hazard ratio.

a**b****c****d**

Transcription factor	Putative binding sites
NF- κ B	4
C/EBP	9
AP-1	7



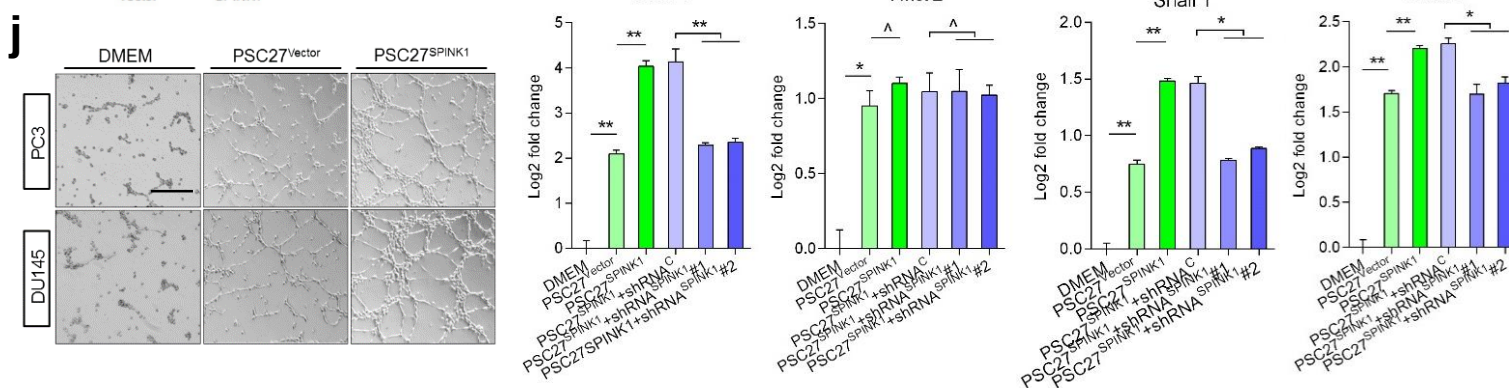
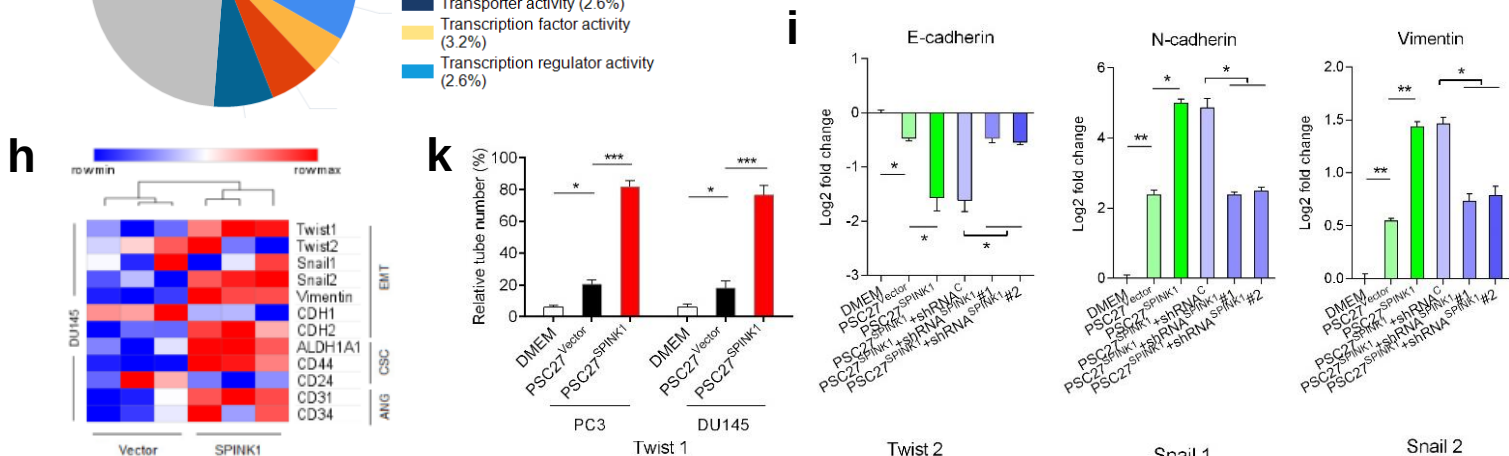
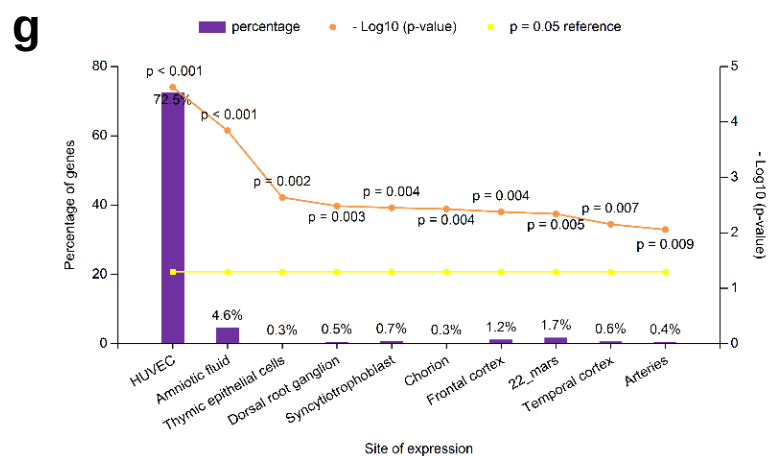
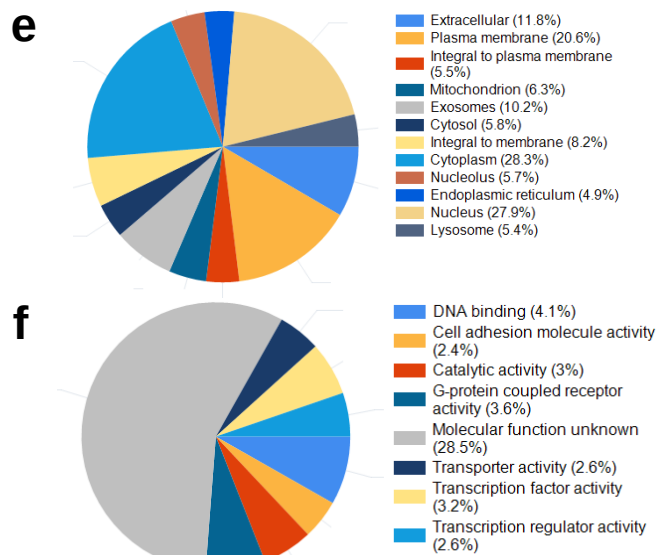
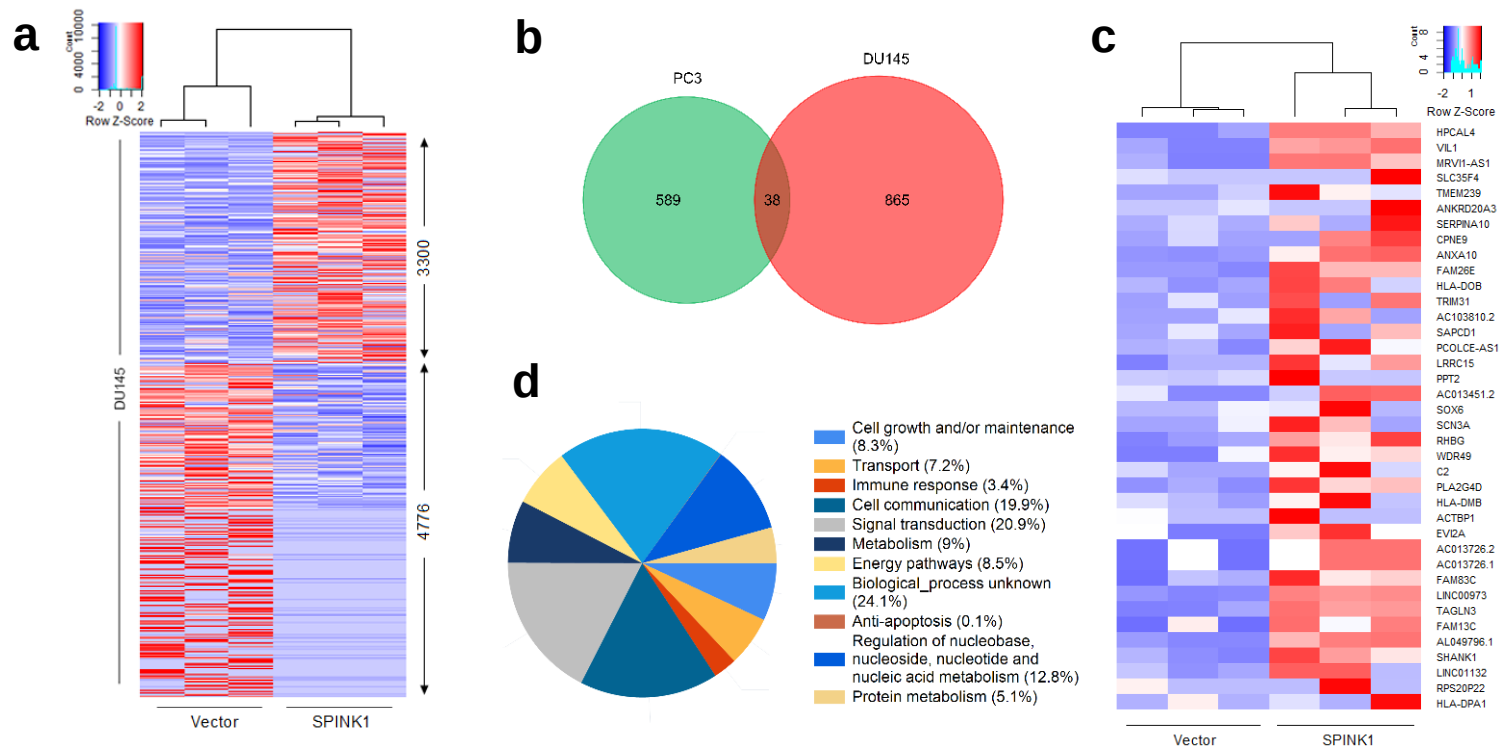
Supplementary Figure 3. SPINK1 expression is mainly mediated by the NF- κ B complex in response to cytokine stimulation or genotoxic stress. (a) Luciferase activity assay with lysates of 293T cells pre-transfected with each of GL-vector, GL-SPINK1-P01, GL-SPINK1-P02, GL-SPINK1-P03 and GL-SPINK1-P04 and subsequently treated by 10 ng/ml IL- α in culture. Data are presented as relative ratios of firefly/renilla luciferase signals. (b) PSC27 cells transiently transfected with the constructs used in (a) were treated by SAT (satraplatin, 10 μ M), with cell lysates collected for luciferase assay. The NAT11-Luc2CP construct which encodes multiple NF- κ B binding sites was used as a positive control. (c) The reporter construct pGL-SPINK1-P04 encoding the longest approximal SPINK1 promoter (4000 bp upstream of TSS) was transiently transfected into PSC27 cells before treated by SAT. BAY (Bay 11-7982, 5 μ M), BA (betulinic acid, 10 μ M) and T-5224 (10 μ M) were applied simultaneously with SAT as small molecule inhibitors against NF- κ B, C/EBP family and AP-1, respectively. SR (SR 11302, 3 μ M) was used as an extra control for AP-1 inhibition. Cells were lysed 7 d after treatment and subject to luciferase activity assay. Data were calculated as ratios of firefly/renilla luciferase signals. (d) Statistical summary of binding sites for transcription factors functionally associated with SPINK1 expression in stroma cells. Upper, putative counts provided for binding sites of NF- κ B, C/EBP and AP-1 on human SPINK1 approximal promoter, with data derived from bioinformatics evaluation. Lower, representative binding motif of C/EBP α and C/EBP β . Data are shown as mean $\pm$ SD and representative of 3 independent experiments. *P* values were calculated by Student's *t*-test (a, b, c) (^, *P* > 0.05; *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001; ****, *P* < 0.0001).



Supplementary Figure 4. Stromal cell-derived SPINK1 changes the phenotypes of cancer

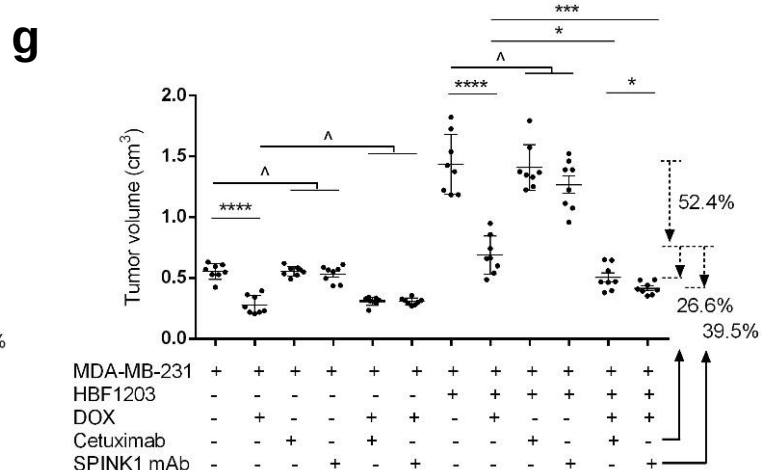
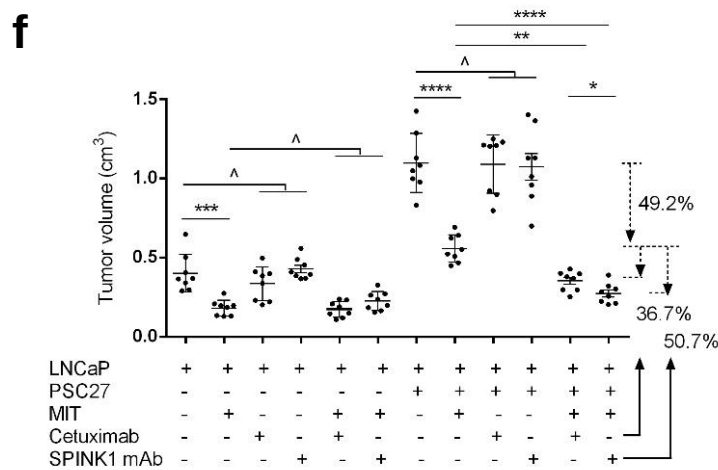
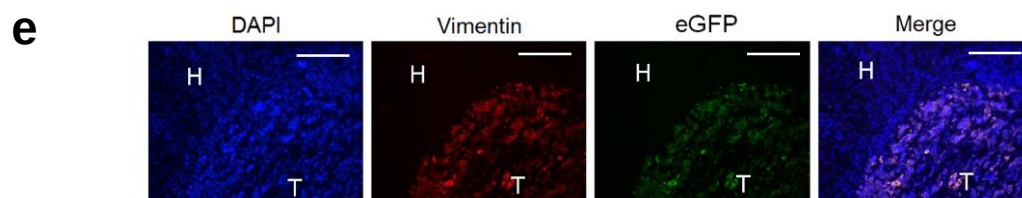
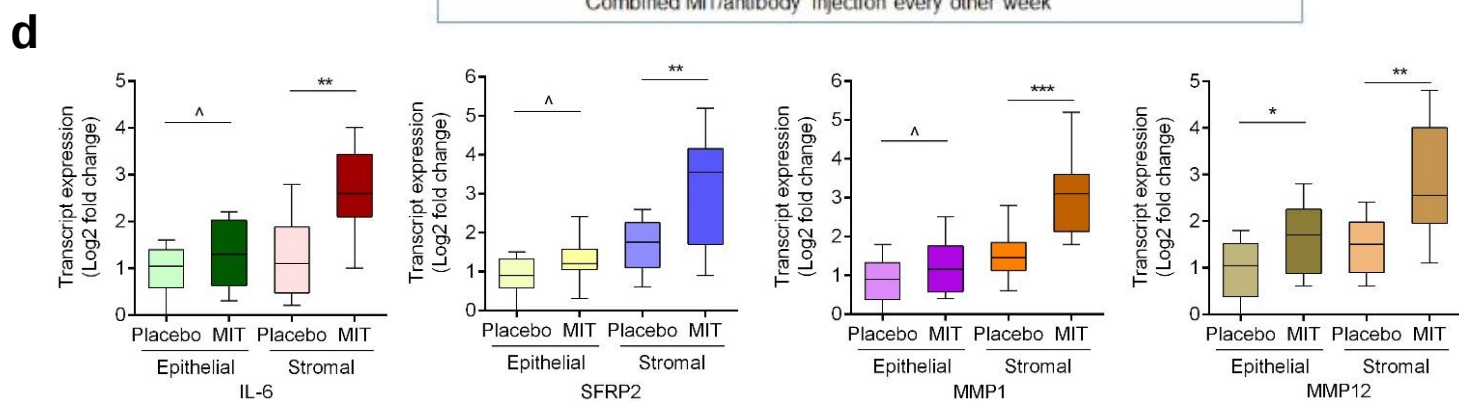
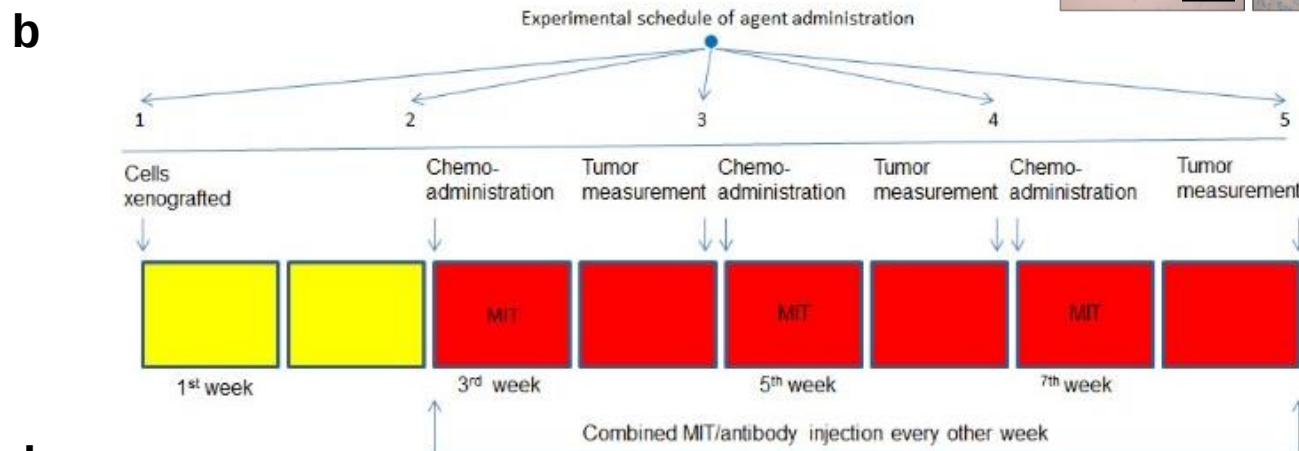
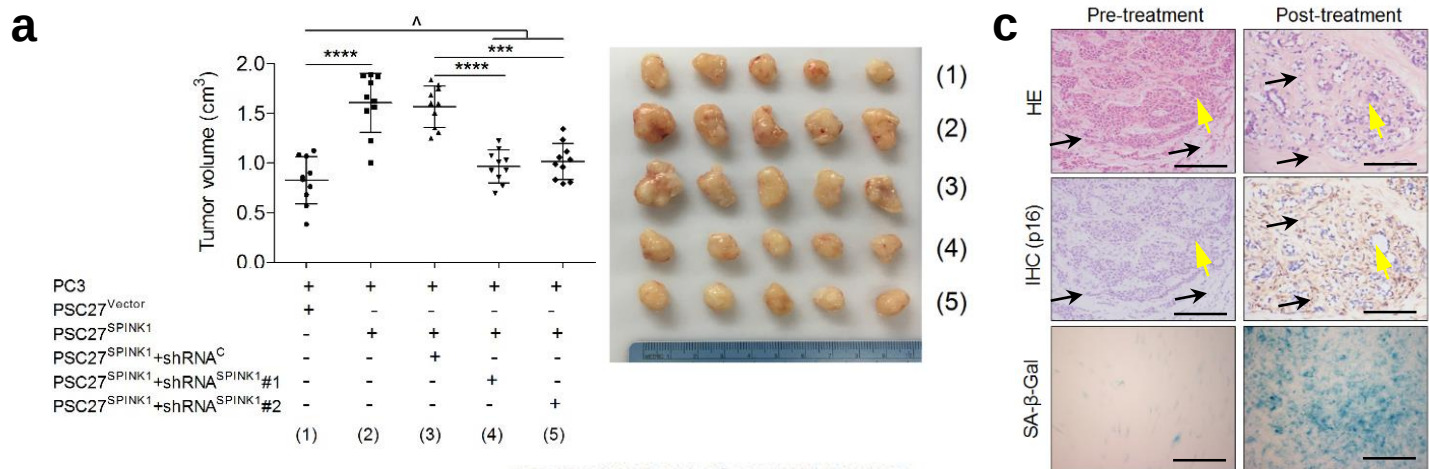
cells. (a) Immunoblot examination of SPINK1 expressed in PSC27 sublines established with different lentiviral constructs. Cells were infected with lentiviral particles encoding either the control vector or full length human SPINK1 sequence. Stable PSC27-SPINK1 cells were subsequently transduced with scramble or SPINK1-specific shRNAs. GAPDH, loading control for protein lysates. Bottom, EGF concentration in the CM was measured by ELISA to determine the influence of SPINK1 knockdown in stromal cells. Right, statistical profiling of SA- β -Gal staining results of PSC27 sublines after BLEO treatment. DMSO, vehicle control. **(b)** PCa cell lines including PC3, DU145, LNCaP and M12 were treated with the conditioned media (CM) from PSC27 cells for 3 days, and subject to cell proliferation assay. PSC27^{Vector} was used as control for cells exogenously expressing SPINK1 (PSC27^{SPINK1}). Scramble or SPINK-specific shRNAs were used to transduce PSC27^{SPINK1} cells to generate stable sublines (PSC27^{SPINK1}+shRNA^C, PSC27^{SPINK1}+PSC27^{SPINK1} #1 and PSC27^{SPINK1}+PSC27^{SPINK1}#2). **(c)** Migration assessment of PCa cells after cultured for 3 days in the CM from sublines of PSC27. HeLa cells were examined as a positive control. **(d)** Invasion assay of PCa cells across the basement membrane of transwells upon culture with the CM from sublines of PSC27 for 3 days. HeLa line served as a positive control. **(e)** Chemoresistance assay of PCa cells upon culture with the CM from PSC27 sublines. MIT (mitoxantrone) was applied at the concentration of IC₅₀ value pre-determined per cell line. **(f)** Immunoblot analysis of protein lysates prepared from DU145, cultured with the CM of PSC27 sublines for consecutive 3 days in the presence or absence of MIT. Intact and cleaved caspase 3 was probed. GAPDH, loading control. **(g)** Apoptotic assay for combined activities of caspase 3/7 determined 24 h after exposure of PC3

cells to CM of stromal cells while being treated by mitoxantrone (MIT) in the presence of absence of caspase inhibitors including QVD-OPH and ZVAD-FMK, or caspase activators including PAC1 and gambogic acid (GA). RLU, relative luciferase unit. **(h)** Apoptotic assay performed in the way similar as the one in **(g)**, except that PAC (paclitaxel) was applied instead of MIT. RLU, relative luciferase unit. **(i)** Survival curves of PSC27 sublines transduced with either empty vector (CTRL) or a SPINK1 construct (SPINK1). Cells were exposed to increasing concentrations of BLEO (25-300 $\mu\text{g/ml}$), and the number of surviving cells after 3-day consecutive culture was counted and normalized to the untreated control, with the IC50 value calculated per line. **(j)** Immunoblot analysis of EGFR-associated pathways in PC3 and DU145 cells treated by the CM from PSC27 sublines as examined in **(a)**. Antibodies of p-EGFR (Y845), p-Akt (S473), p-mTOR (S2448), p-Erk (T202/Y204) and p-Stat3 (S727) were used, with the total protein per molecule and GAPDH probed as loading control. **(k)** Chemoresistance assay of PCa cells cultured with the CM from PSC27 sublines analyzed in **(a)** after treatment by BLEO. MIT was applied at the concentration of IC50 value pre-determined per cell line. **(l)** Dose-response curves (non-linear regression/curve fit) plotted from drug-based survival assays of MDA-MB-231 cells cultured with the CM of HBF1203 native or damaged by doxorubicin (HBF1203-DOX), and concurrently treated by a wide range of concentrations MIT. AG-1478 (2 μM), cetuximab (50 $\mu\text{g/ml}$) or SPINK1 mAb (1 $\mu\text{g/ml}$) were applied with PSC27 CM. Data are shown as mean $\pm$ SD and representative of 3 independent experiments. *P* values were calculated by Student's *t*-test (**a, b, c, d, e, g, h, i, k**) ($^{\wedge}$, $P > 0.05$. *, $P < 0.05$. **, $P < 0.01$. ***, $P < 0.001$. ****, $P < 0.0001$).



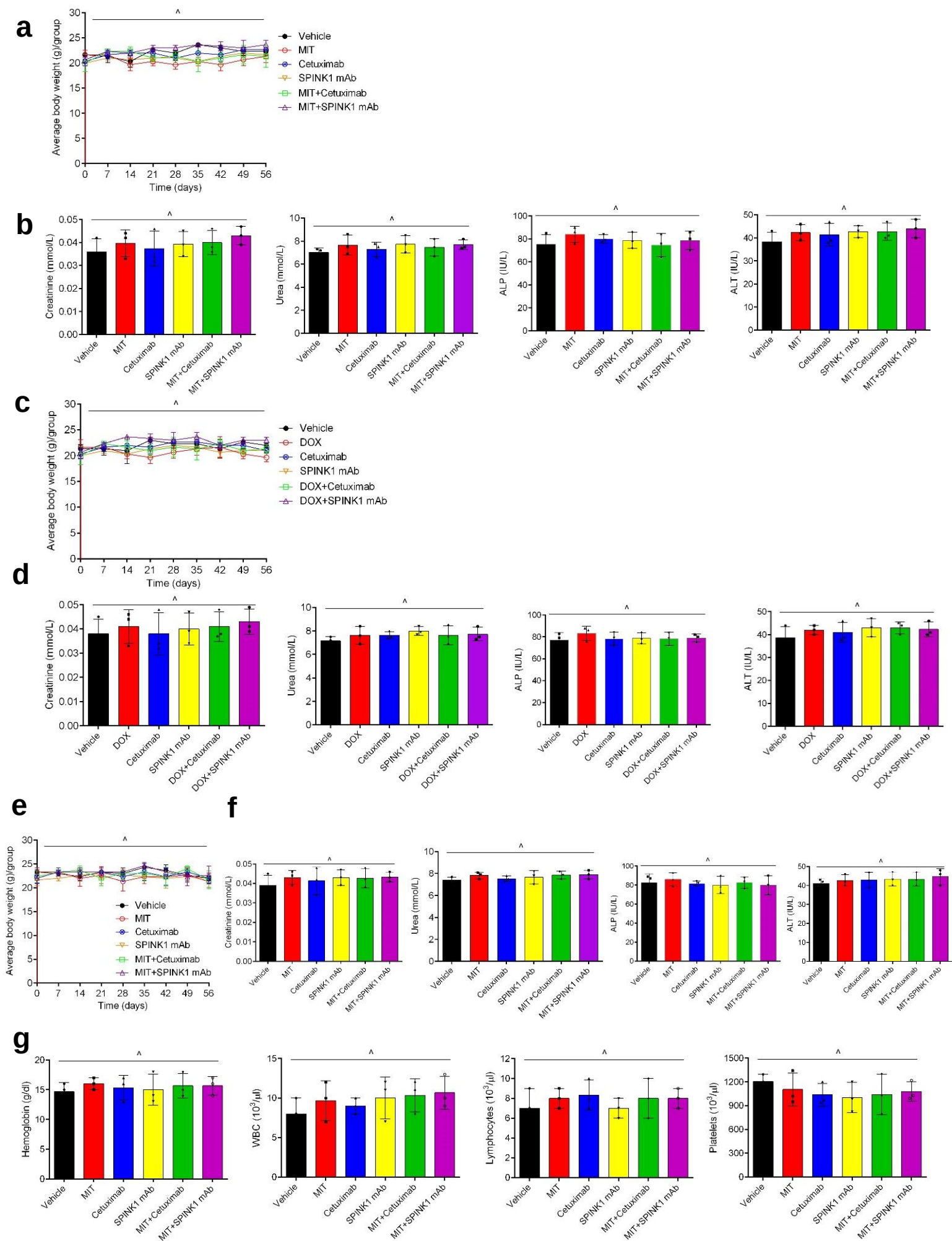
Supplementary Figure 5. Transcriptomic-wide expression of prostate cancer (PCa) cells is modified by stromal cell-derived SPINK1. (a) Heatmap showing differentially expressed human transcripts in DU145 cells after consecutive 3-day culture with SPINK1-containing CM collected from PSC27 stromal cells. In contrast to cancer cells cultured with control media (vector), there were 3300 and 4776 genes upregulated and downregulated, respectively, in those treated with conditioned media from SPINK1-expressing PSC27 cells (SPINK1). (b) Venn diagram presenting the overlap of 38 transcripts upregulated in PC3 and DU145 cells upon treatment with SPINK1-containing CM from stromal cells (627 and 903 genes were significantly upregulated with average fold change ≥ 4 after SPINK1-CM treatment of PC3 and DU145, respectively). (c) Heatmap depicting the top 38 co-upregulated transcripts by both PC3 and DU145 cells. Genes sorted according to their expression fold changes in DU145 cells. (d) GO-pie chart displaying the biological process of the 3300 transcripts upregulated in DU145 after SPINK1-CM treatment. (e) GO-pie chart manifesting the cellular component of the 3300 transcripts upregulated in DU145 after SPINK1 stimulation. (f) GO-pie chart showing the molecular function of the 3300 DU145 transcripts analyzed in (d) and (e). (g) Column chart depicting the sites of expression of 3300 transcripts upregulated in DU145 cells after SPINK1 stimulation, with percentage and log₁₀ (*P* value) per specific site indicated on the left and right Y axis, respectively. (h) Heatmap of gene expression signatures associated with phenotypic changes, namely EMT/CSC/ANG, after SPINK1 stimulation of DU145 cells *in vitro*. EMT, epithelial-to-mesenchymal transition; CSC, cancer stem cell; ANG, angiogenesis. Data derived from qRT-PCR assays. (i) Quantitative RT-PCR analysis of the transcript expression of EMT-specific markers E-cadherin, N-cadherin and vimentin, as well as EMT regulators Twist 1, Twist

2, Snail 1 and Snail 2 in DU145 cells treated with the CM of PSC27 sublines. Data per factor were normalized to DMEM-cultured cells. (j) *In vitro* tube formation assay to measure the angiogenesis-inducing capacity of stromal SPINK1. PCa cells (PC3 and DU145) were seeded on top of a basement membrane matrigel layer and incubated in the CM of PSC27 sublines for 10 h. Cells were stained with calcein-AM and photographed by phase contrast microscopy. Scale bars, 100 μ m. (k) Statistical assessment of the capacity of tube formation by PCa cells cultured on the top of polymerized matrigel in 12-well plates loaded with different CM of PSC27 cells. Tubular structures were quantified by manual counting of high power fields (HPFs). Data are shown as mean $\pm$ SD and representative of 3 independent experiments. *P* values were calculated by Student's *t*-test (i) ($^{\wedge}$, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$).



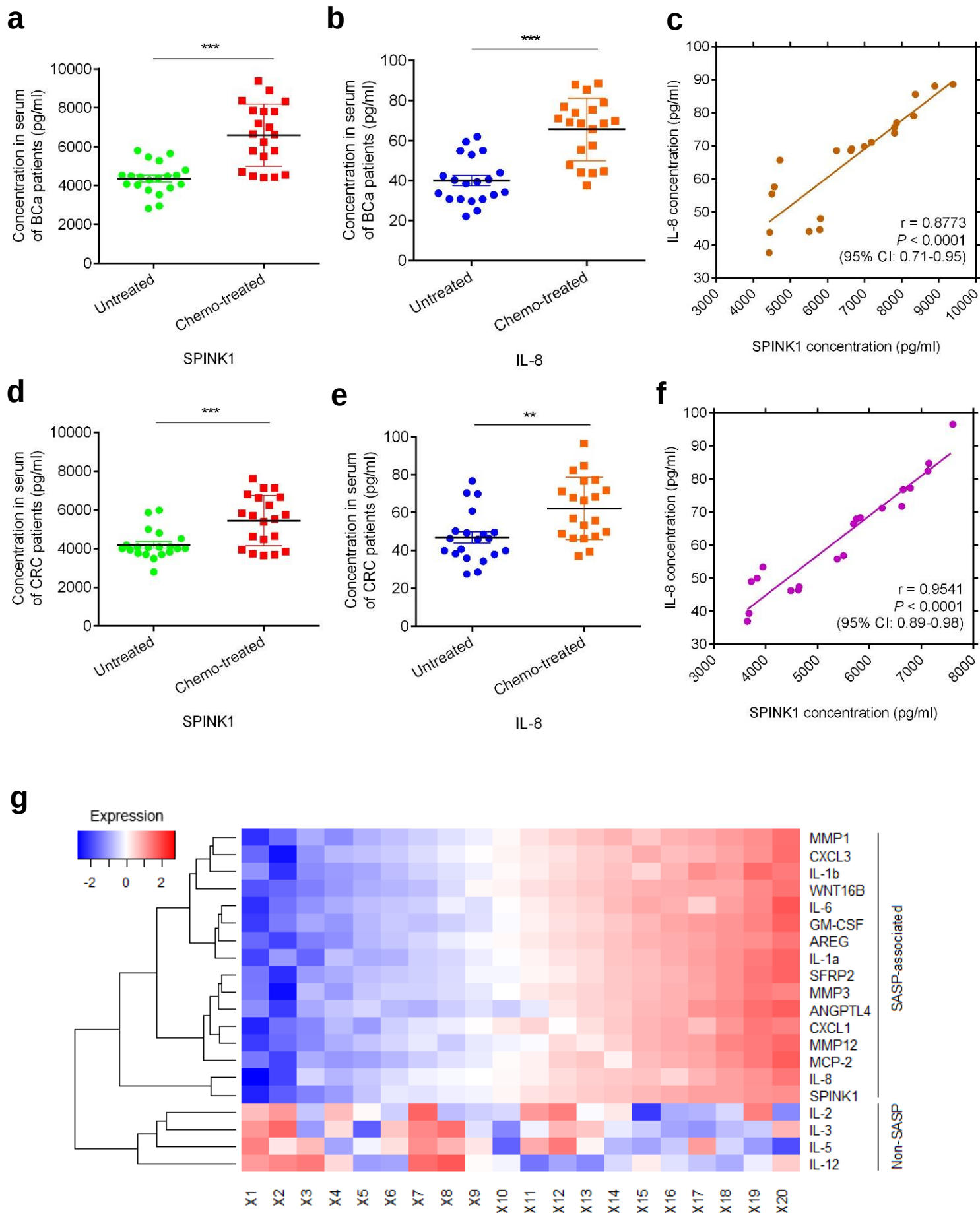
Supplementary Figure 6. Paracrine SPINK1 confers therapeutic resistance on solid tumors developed in experimental animals. (a) Statistics of tumor volume measured at the end of an 8-week growth period. PC3 cells were xenografted alone or together with PSC27 cells to the hind flank of SCID mice. Prior to implantation, PSC27 cells were transduced with the control vector or SPINK1 construct to make stable sublines (PSC27^{Vector}, and PSC27^{SPINK1}, respectively). PSC27^{SPINK1} cells were subsequently subject to lentiviral infection to make SPINK1-null sublines (PSC27^{SPINK1}+shRNA^{SPINK1}#1 and PSC27^{SPINK1}+shRNA^{SPINK1}#2), with scramble shRNA used as non-specific control (PSC27^{SPINK1}+shRNA^C). Right, representative images for tumors isolated from SCID mice, with numeric numbers corresponding to those provided in the left graph. (b) Strategic design of drug administration and tumor surveillance for preclinical trial. PC3 cells alone or combined with PSC27 cells were inoculated subcutaneously to SCID mice 2 weeks prior to the initiation of chemotherapy. The chemotherapeutic agent MIT was provided on the first day of each week starting from the 3rd week, then given every other week with a total number of 3 doses. Therapeutic antibodies (cetuximab or SPINK1 mAb) were given 12 h before each time of MIT delivery (totally 3 doses in the regimen). At the end of 8 weeks mice were sacrificed with tumor volume measured, and histologically analyzed. (c) Representative images of *in vivo* cellular senescence after MIT-mediated chemotherapy. PC3/PSC27 tumors were collected at the end of therapeutic regimen and subject to histological assessment. Images from staining of HE, IHC (anti-p16 as primary antibody) and SA- β -gal staining are acquired for comparative analysis. Black arrows, stromal cells. Yellow arrowheads, cancer cells. Scale bars, 150 μ m. (d) Transcript analysis of several canonical SASP factors including IL-6, SFRP2, MMP1 and MMP12 expressed in stromal cells isolated from the tumors

of SCID mice. Tissues from animals implanted with both stromal and cancer cells in tumor grafts were subject to LCM isolation, RNA preparation and qRT-PCR assays. (e) Immunofluorescence assessment of tumor xenografts. Tumor sections were subject to staining with an antibody against vimentin. Blue, DAPI; red, vimentin; green, signals from PSC27 cells pre-transduced with a marker vector encoding enhanced green fluorescent protein (eGFP). H, host; T, tumor. Scale bars, 150 μ m. (f) Statistical comparison of tumor growth in animals after treatment by different agents. Mice received LNCaP cells implanted alone or combined with PSC27 cells, before treated by the chemotherapeutic drug (MIT) alone or combined with cetuximab or SPINK1 mAb. Tumor volumes were measured at the end of an 8-week preclinical regimen. (g) A similar statistical comparison performed for tumors grown in mice as depicted in (f), except that animals received breast cancer cells MDA-MB-231 alone or combined with breast stromal cells HBF12037. Mice were treated by the chemotherapeutic drug DOX only or together with monoclonal antibodies (cetuximab or SPINK1 mAb). Tumor volumes were measured at the end of an 8-week preclinical regimen. Data are shown as mean $\pm$ SD and representative of 3 independent experiments. n = 10 per treatment arm. MIT, mitoxantrone. DOX, doxorubicin. *P* values were calculated by Student's *t*-test (a, d, f, g) ($^{\wedge}$, $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$).



Supplementary Figure 7. Chemotherapeutic and/or targeting agents generate negligible effects on body weight, biochemistry, and blood counts of experimental mice. (a) Mouse body weights were determined once a week until the end of the therapeutic regimen. Chemotherapeutic agent MIT (0.2 mg/kg) was administered alone or with the targeting antibody (cetuximab or SPINK1 mAb, each 10.0 mg/kg) on 1st day of week 3, 5 and 7 after tumor implantation (PC3/PSC27) to SCID mice. (b) Terminal bleeds were taken via cardiac punctures on day 56. Serum levels of creatinine, urea, alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were analyzed for toxicity appraisal with SCID mice developing prostate tumors. (c) Mouse body weights were determined as described in a strategy similar to (a). Chemotherapeutic agent DOX (1.0 mg/kg) was administered alone or with the targeting antibody (cetuximab or SPINK1 mAb, each 10.0 mg/kg) on 1st day of week 3, 5 and 7 after tumor implantation (MDA-MB-231/HBF1203) to SCID mice. (d) Terminal bleeds were taken via cardiac punctures on the last day of the 8-week regimen. Serum levels of creatinine, urea, alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were analyzed for toxicity appraisal with SCID mice developing breast tumors. (e) Mouse body weights were determined once a week until the end of a therapeutic regimen applied to immunocompetent animals. Chemotherapeutic agent MIT (0.2 mg/kg) was administered alone or with the targeting antibody (cetuximab or SPINK1 mAb, each 10.0 mg/kg) on 1st day of week 3, 5 and 7 to wild type C57BL/6 mice. (f) Terminal bleeds were taken via cardiac punctures on the last day of the regime. Serum levels of creatinine, urea, alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were analyzed for potential toxicity to C57BL/6 mice. (g) Blood counts were measured to evaluate potential effect of therapeutic agents on the immune system and

tissue homeostasis of C57BL/6. WBC, white blood count. Data are shown as mean $\pm$ SD and representative of 3 independent experiments. $n = 3$ per treatment arm. MIT. P values were calculated by Student's t -test (**a-g**) ($^{\wedge}$, $P > 0.05$).



Supplementary Figure 8. SPINK1 is a novel biomarker that indicates the SASP development *in vivo* and predicts adverse outcome in the post-treatment stage of cancer patients. (a) Abundance of SPINK1 protein in the serum of untreated and chemo-treated BCa patients. Data were derived from ELISA measurement, n = 20. (b) Abundance of IL-8 protein in patient serum analyzed in (a), data from ELISA assays, n = 20. (c) Scatterplot showing correlation between SPINK1 and IL-8 in the serum of individual BCa patients studied in (a) and (b). Pearson's correlation coefficient, *P* value and confidence interval are indicated in the plot, n = 20. (d) Abundance of SPINK1 protein in the serum of untreated and chemo-treated CRC patients. Data were derived from ELISA measurement, n = 20. (e) Abundance of IL-8 protein in patient serum analyzed in (d), data from ELISA assays, n = 20. (f) Scatterplot showing correlation between SPINK1 and IL-8 in the serum of individual CRC patients studied in (d) and (e). Pearson's correlation coefficient, *P* value and confidence interval are indicated in the plot, n = 20. (g) Heatmap showing the relative expression of a panel of SASP signature factors in the tumor stroma of PCa patients, and the correlation of SPINK1/IL-8 with these factors (n = 20). Stromal cells in the tumor tissues were isolated via LCM and expression of each target factor was measured by qRT-PCR, with signals per factor group normalized to that of the sample showing the lowest expression value. A subset of inflammatory factors typically not considered as SASP components was examined as random control, including IL-2/3/5/12. Correlations of these factors are presented as dendrogram organized by hierarchical clustering. Data are shown as mean $\pm$ SD and representative of 3 independent experiments. *P* values were calculated by Student's *t*-test (a, b, d, e) and Pearson analysis (c, f) (***P* < 0.01; ****P* < 0.001).

Fig. 1f

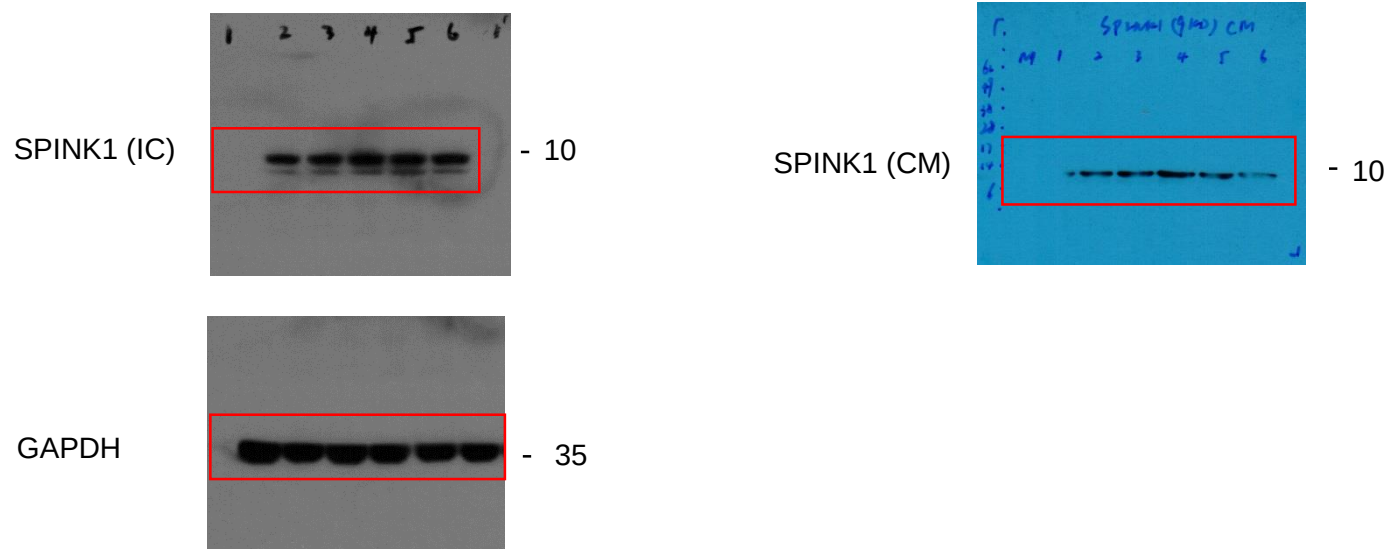
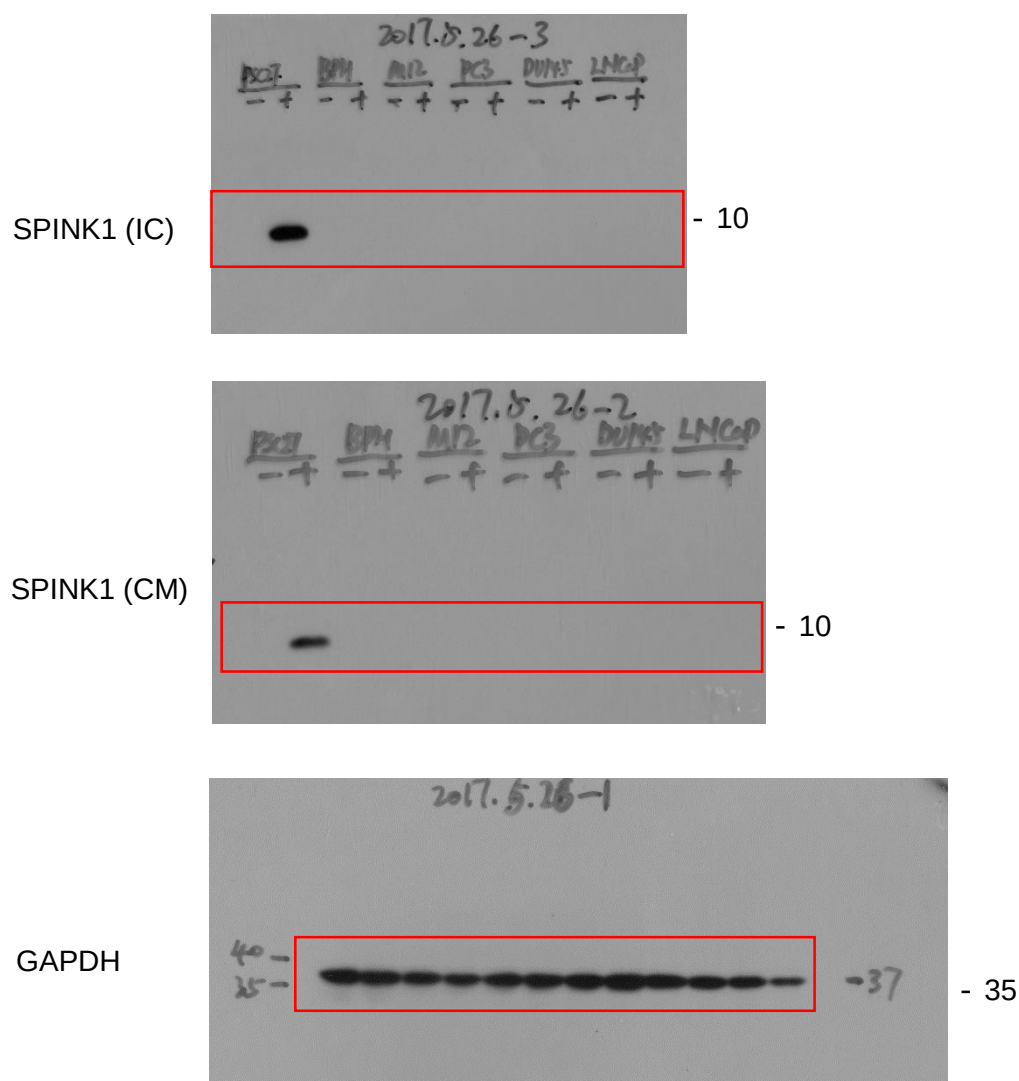
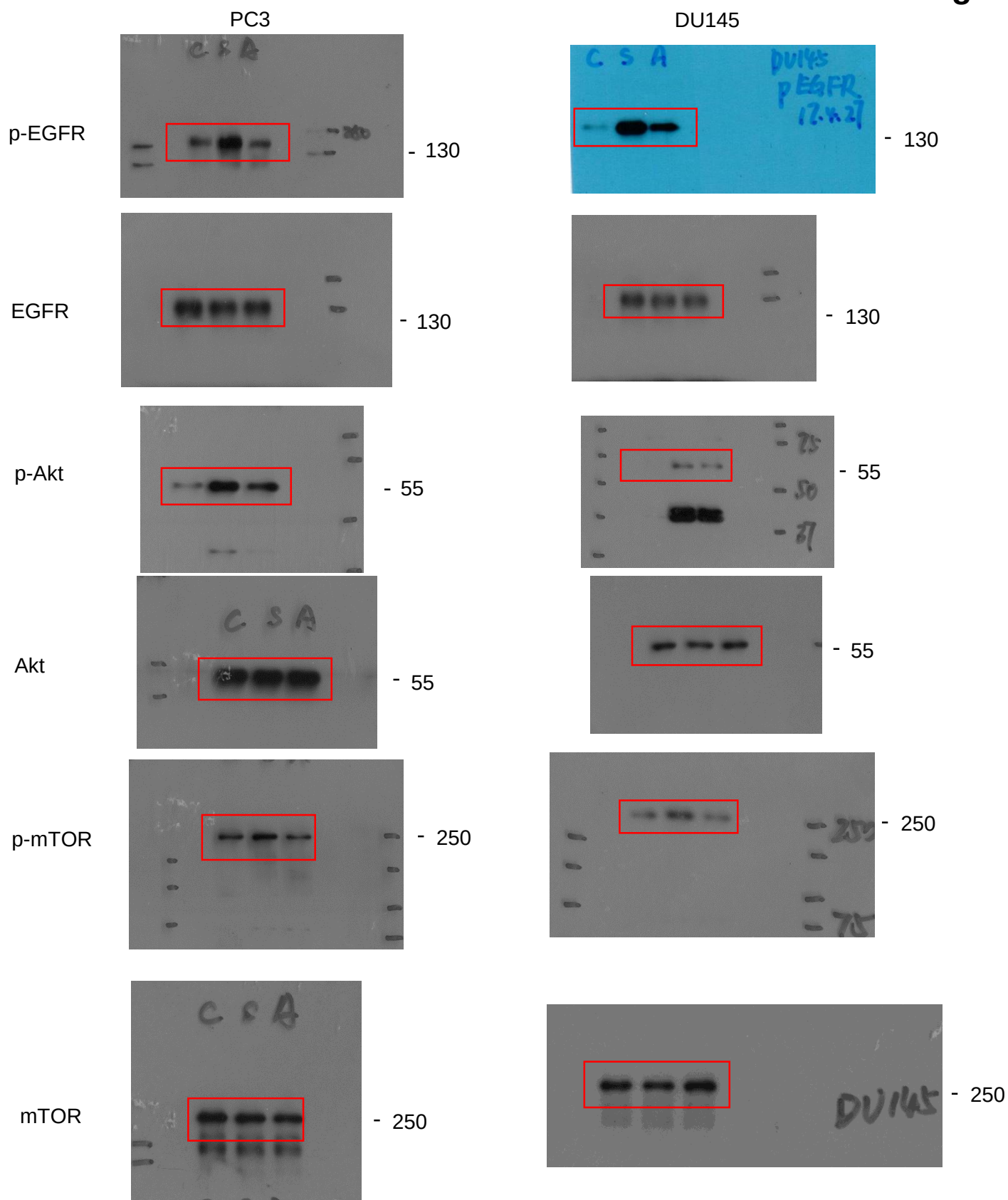


Fig. 1i



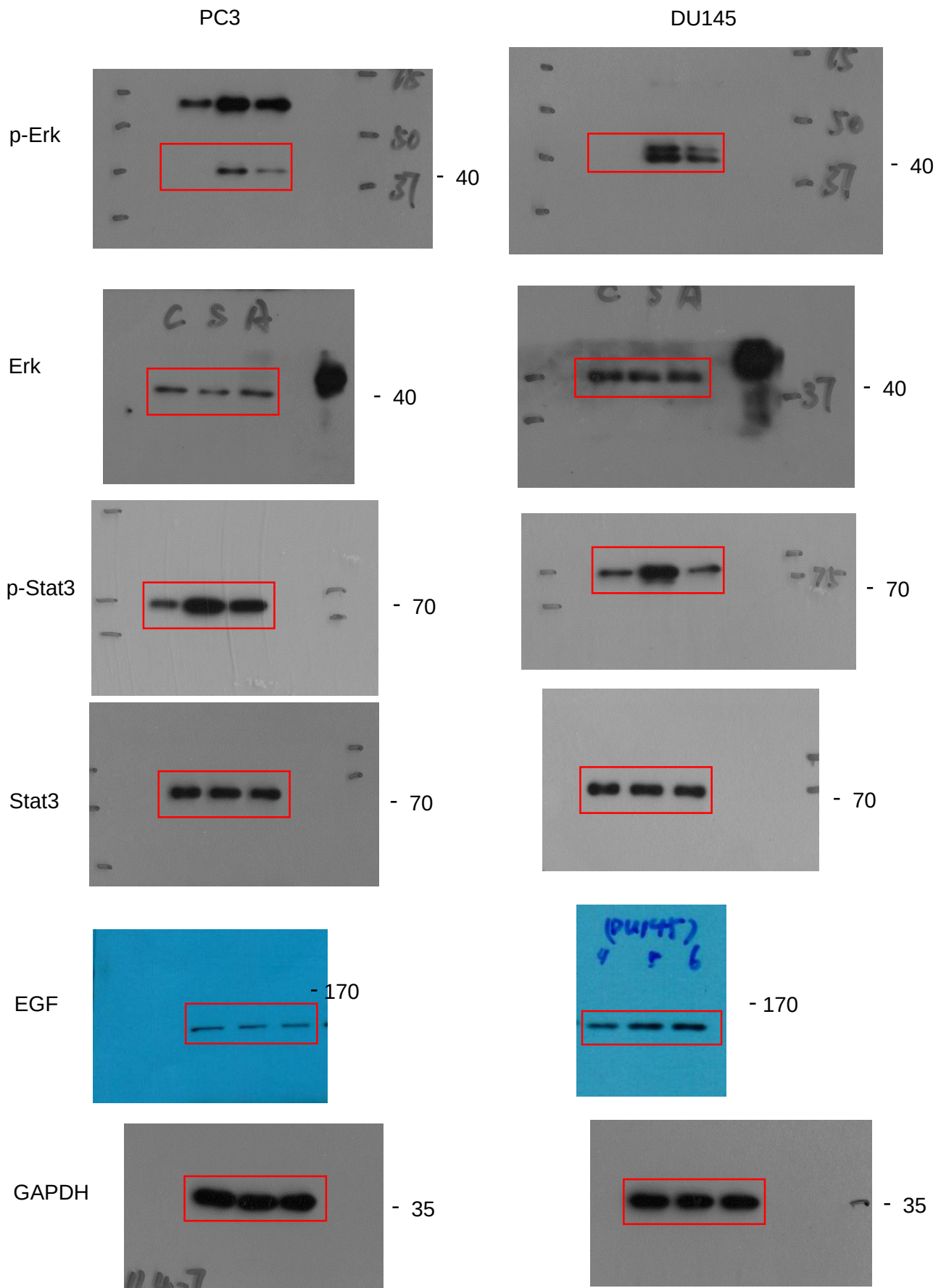
Supplementary Figure 9. The uncropped scans of immunoblots

Fig. 4a



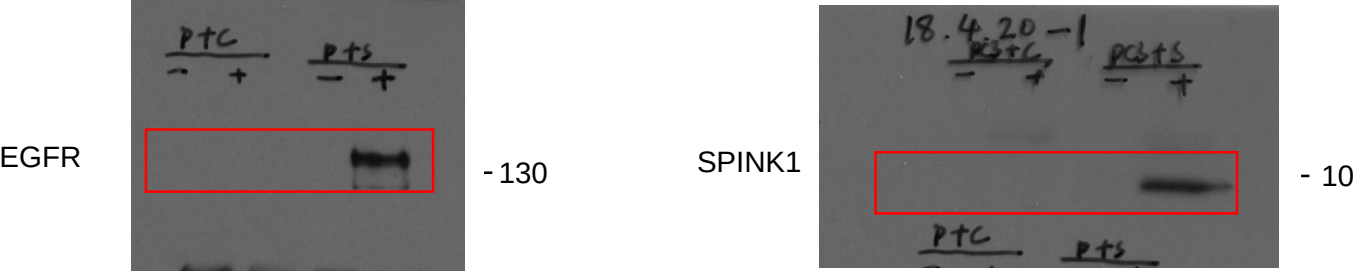
Supplementary Figure 9. The uncropped scans of immunoblots (continued)

**Fig. 4a
(continued)**

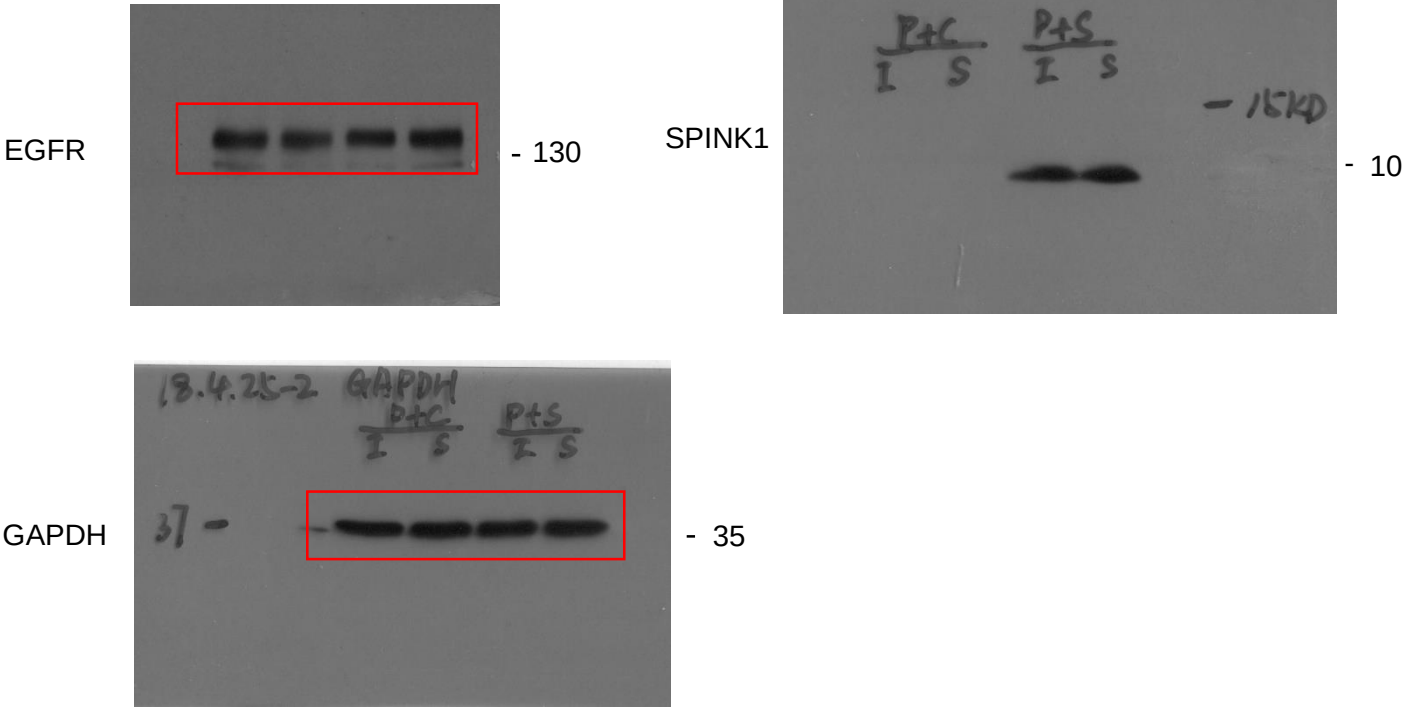


Supplementary Figure 9. The uncropped scans of immunoblots (continued)

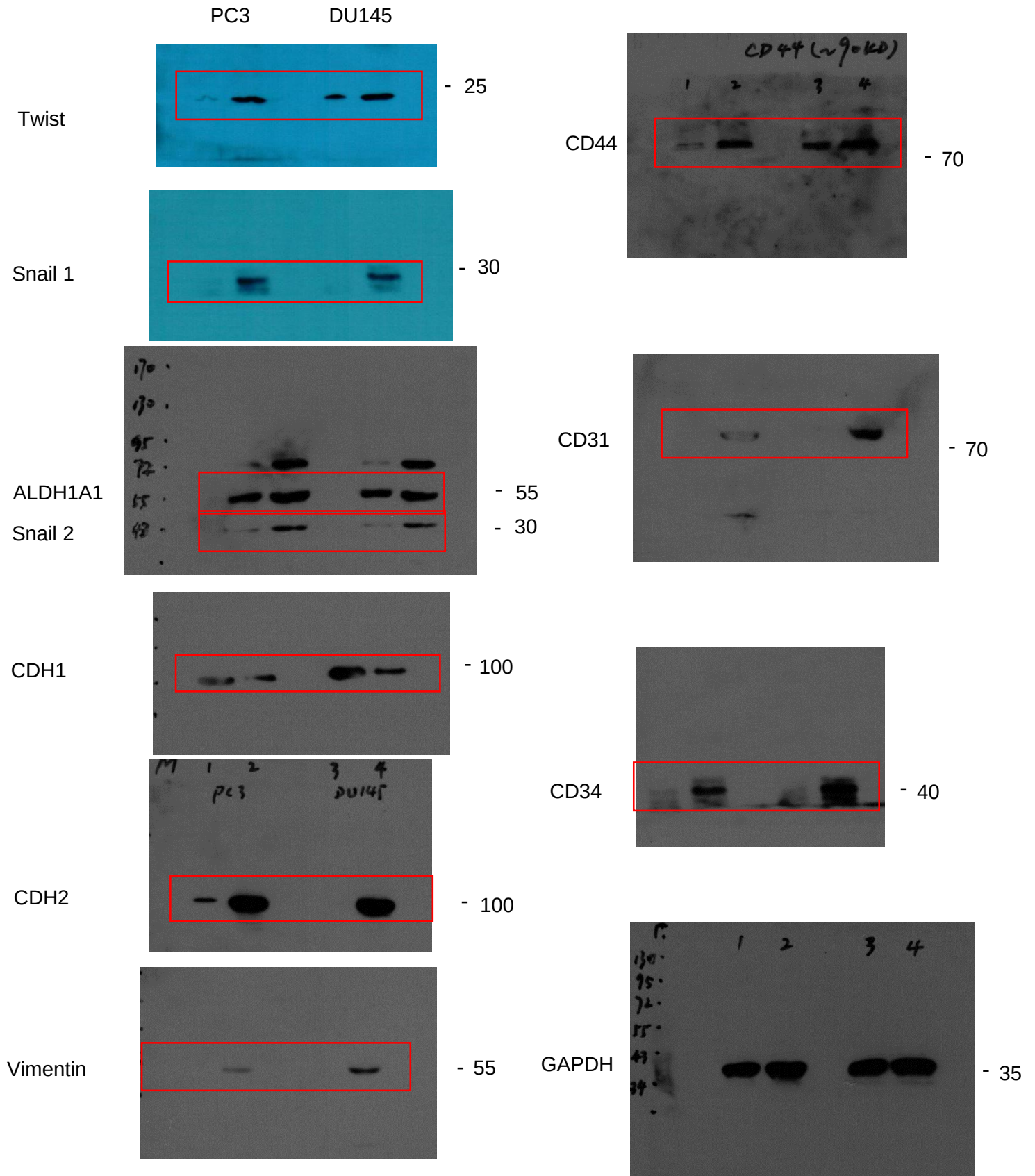
Pull down



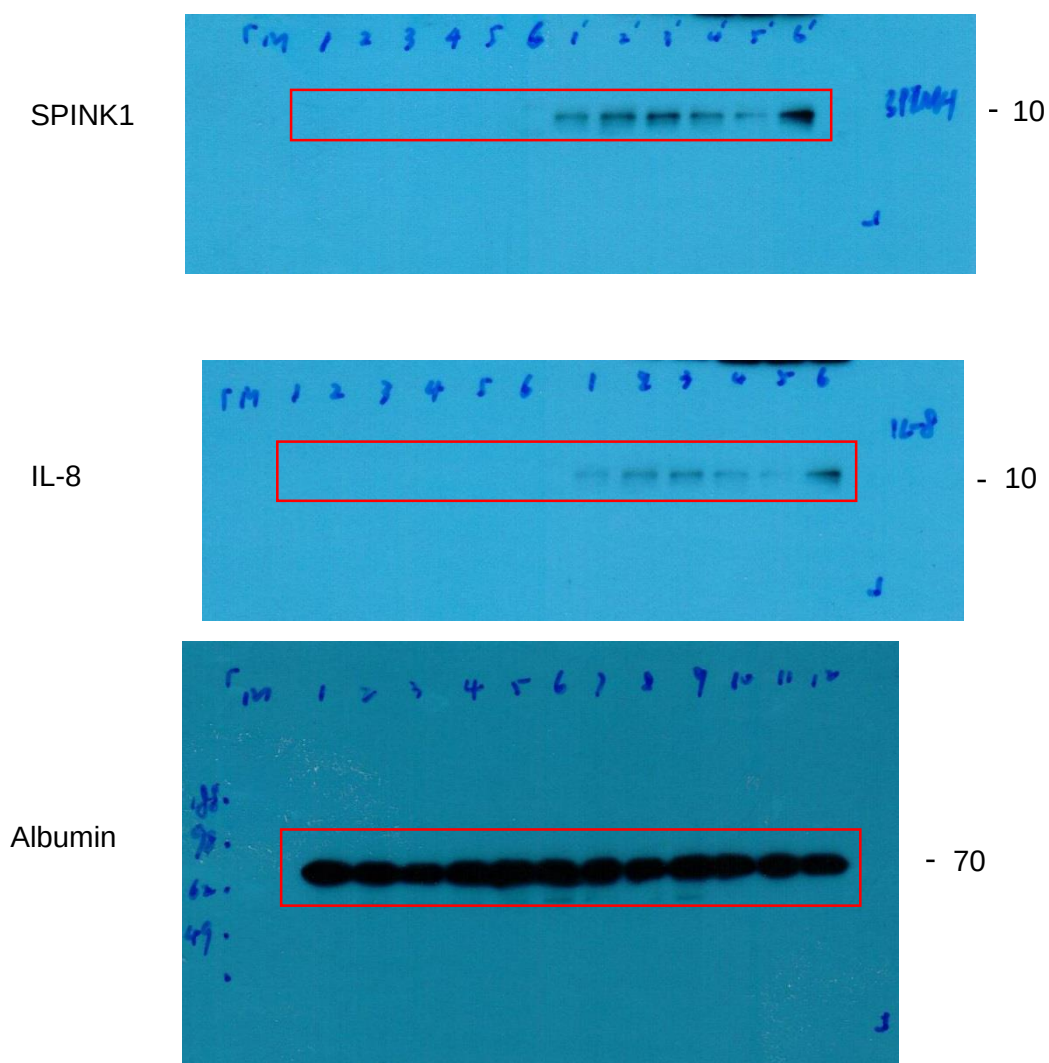
Input



Supplementary Figure 9. The uncropped scans of immunoblots (continued)



Supplementary Figure 9. The uncropped scans of immunoblots (continued)



Supplementary Figure 9. The uncropped scans of immunoblots (continued)

Fig. S1f

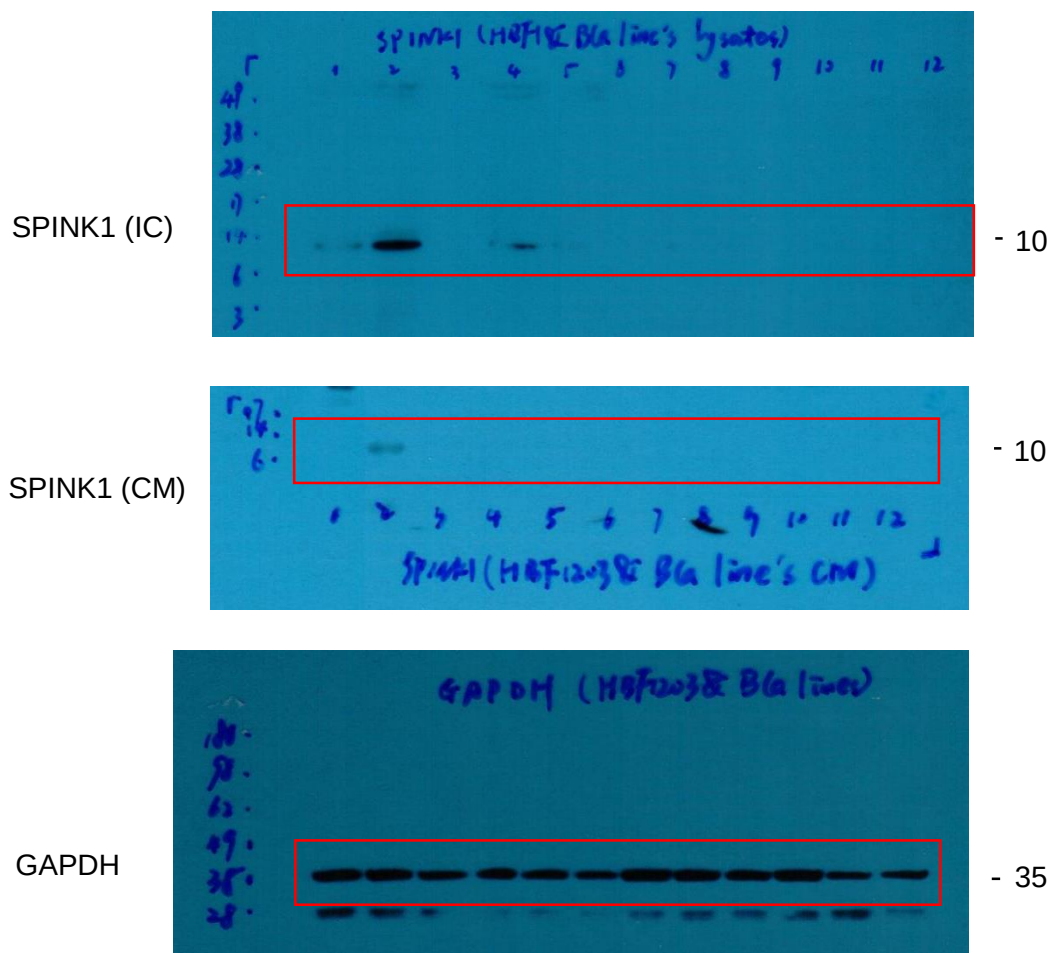


Fig. S1g

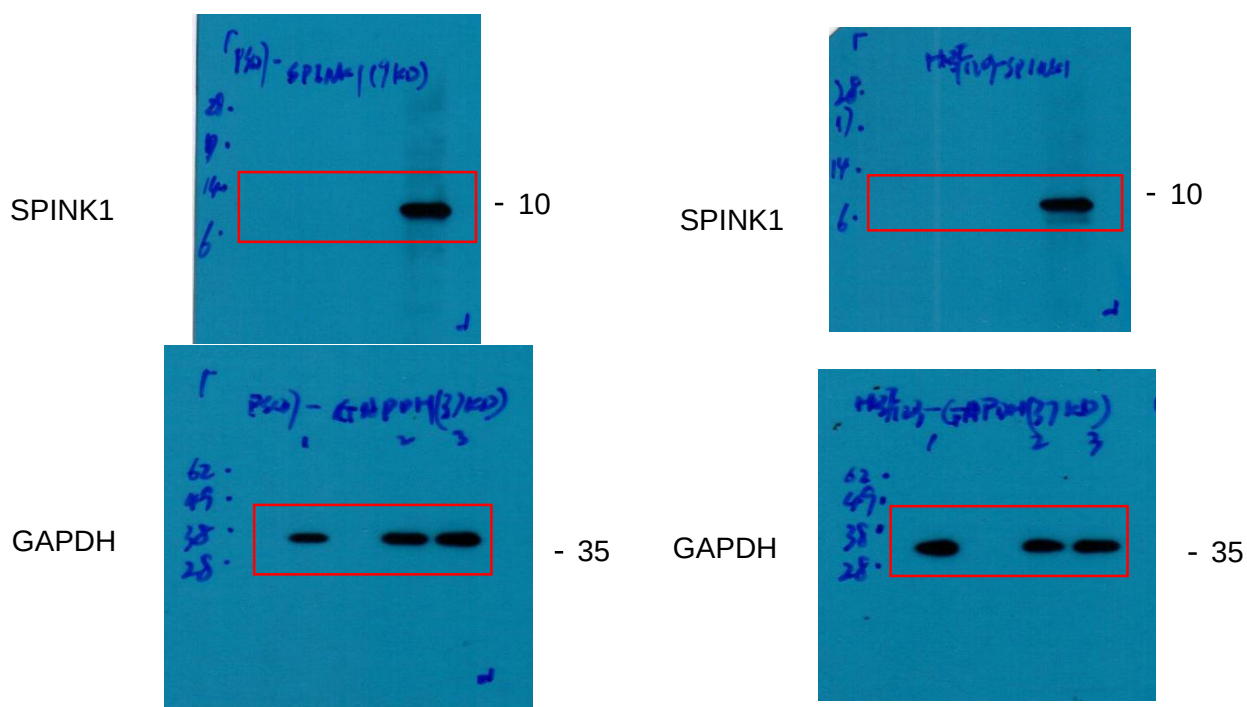


Fig. S4a

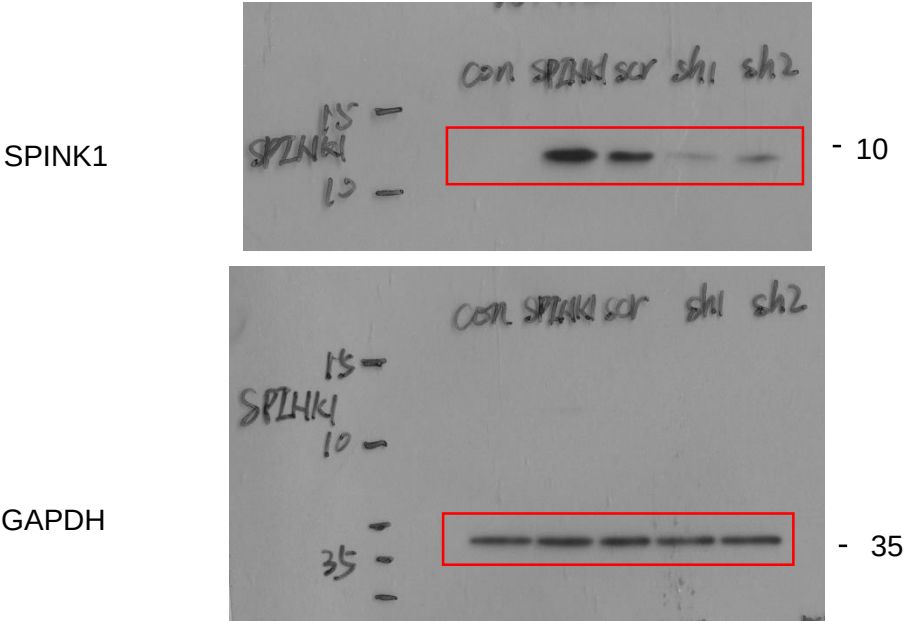
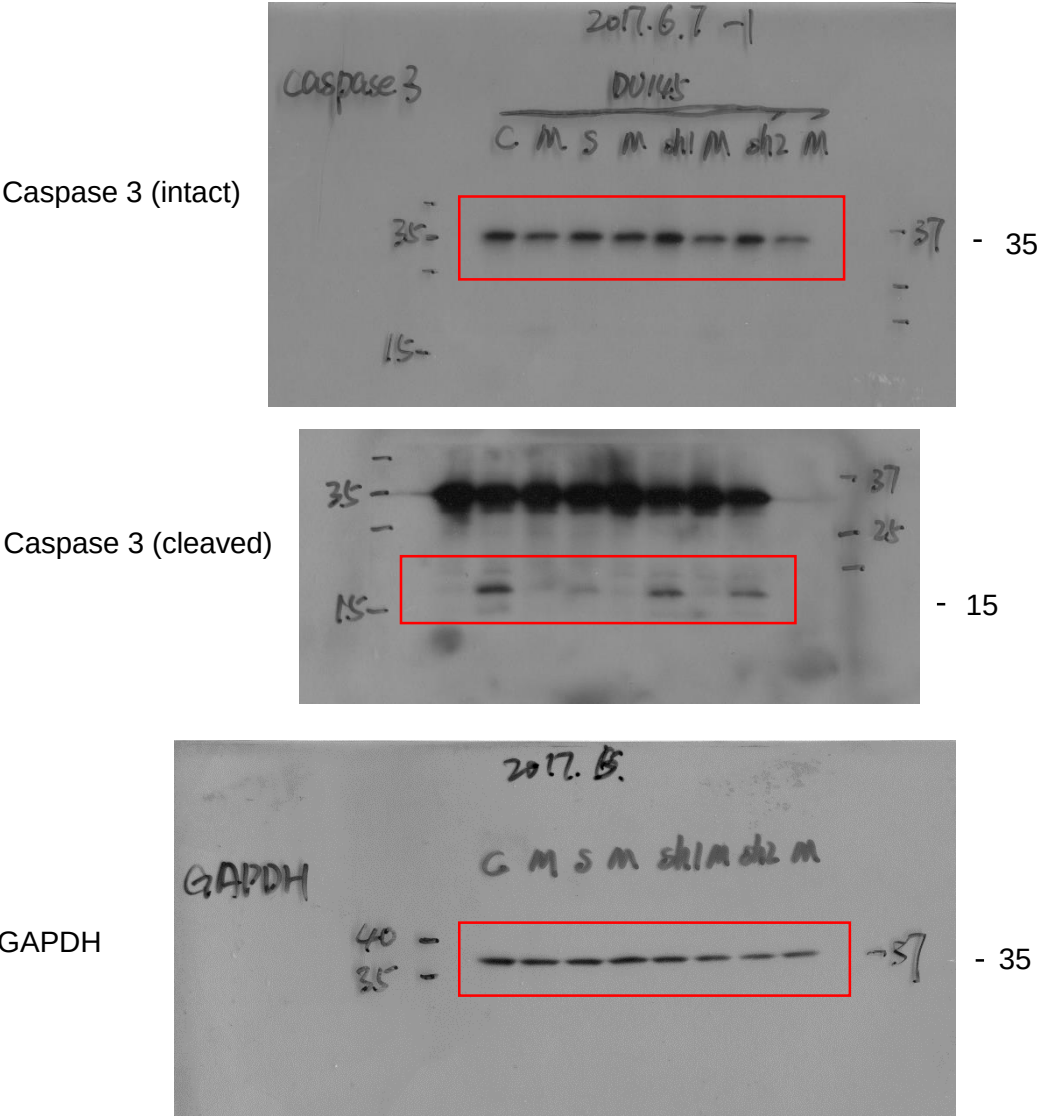
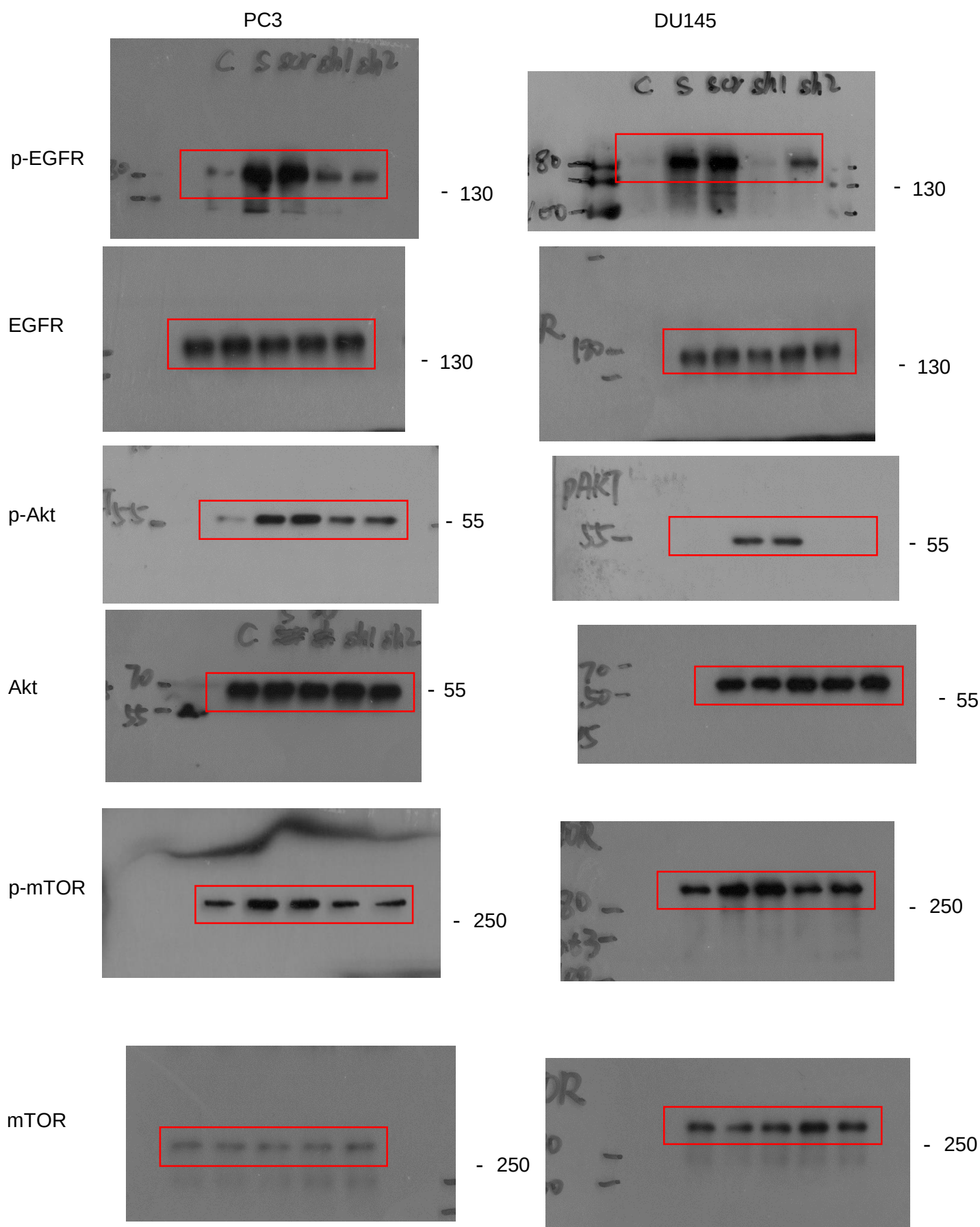


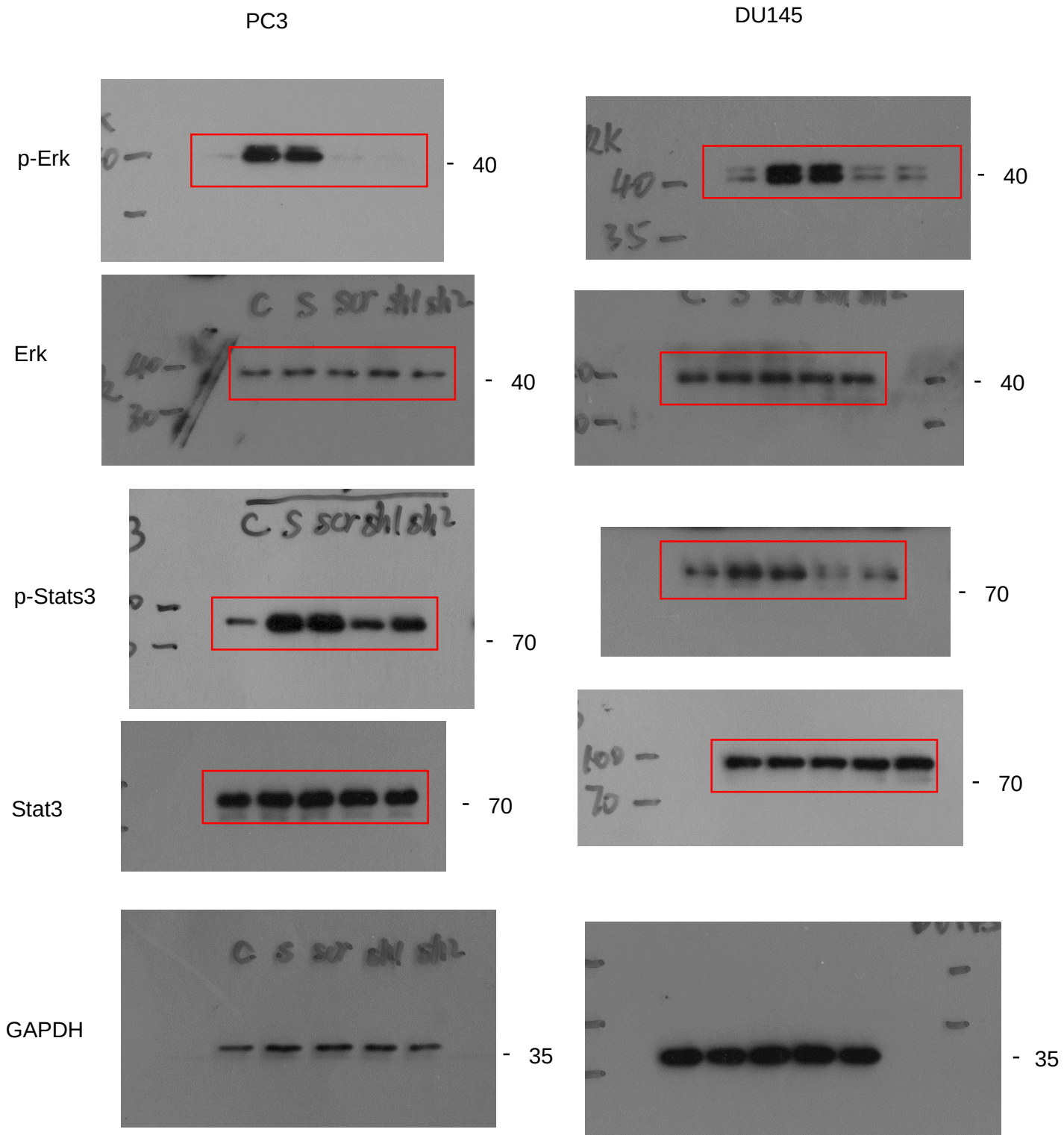
Fig. S4f



Supplementary Figure 9. The uncropped scans of immunoblots (continued)



Supplementary Figure 9. The uncropped scans of immunoblots (continued)



Supplementary Figure 9. The uncropped scans of immunoblots (continued)

Supplementary Table 1. Univariate and multivariate Cox proportional hazards model analysis of prognostic factors for PFS of PCa patients.

Variable	Univariate			Multivariate		
	HR	95% CI	<i>P</i>	HR	95% CI	<i>P</i>
Age: < 60 vs. ≥ 60	0.47	0.19–1.21	0.120	0.44	0.21–0.92	0.036
Tumor stage: IV vs. IIc-III	1.27	0.38–4.29	0.696	1.34	0.48–3.83	0.582
Tumor size: ≥ 3 vs. < 3 cm	1.52	0.67–3.44	0.042	2.07	1.08–4.27	0.038
SPINK1: low vs. high	0.74	0.33–1.65	0.016	0.34	0.17–1.68	0.004
Caspase 3 (cleaved): low vs. high	0.49	0.14–1.67	0.265	1.22	0.38–4.02	0.093
AR low vs. high	1.08	0.59–1.82	0.054	1.28	0.67–3.59	0.065

PFS, progression-free survival. PCa, prostate cancer. HR, hazard ratio. CI, confidence interval. AR, androgen receptor. $P < 0.05$ is statistically significant, with significant *P*-values highlighted in bold.

Supplementary Table 2. Univariate and multivariate Cox proportional hazards model analysis of prognostic factors for PFS of BCa patients.

Variable	Univariate			Multivariate		
	HR	95% CI	<i>P</i>	HR	95% CI	<i>P</i>
Age: < 60 vs. ≥ 60	0.39	0.15–1.04	0.096	0.42	0.19–0.82	0.048
Tumor stage: IV vs. Ia-III	1.39	0.32–4.02	0.508	1.30	0.38–3.65	0.498
Tumor size: ≥ 3 vs. < 3 cm	1.78	0.45–3.21	0.046	1.96	1.02–3.17	0.065
SPINK1: low vs. high	0.65	0.31–1.72	0.009	0.44	0.24–0.76	0.004
Caspase 3 (cleaved): low vs. high	0.67	0.25–1.89	0.228	1.56	0.39–3.22	0.106
HER2 high vs. low	2.45	1.76–3.12	0.015	1.85	1.23–2.79	0.032
PR high vs. low	2.90	0.92–3.87	0.072	1.54	0.93–2.75	0.098

PFS, progression-free survival. BCa, breast cancer. HR, hazard ratio. CI, confidence interval. HER2, human epidermal growth factor receptor 2. PR, Human progesterone *receptor*. *P* < 0.05 is statistically significant, with significant *P*-values highlighted in bold.

Supplementary Table 3. Univariate and multivariate Cox proportional hazards model analysis of prognostic factors for PFS of CRC patients.

Variable	Univariate			Multivariate		
	HR	95% CI	<i>P</i>	HR	95% CI	<i>P</i>
Age: < 60 vs. ≥ 60	1.78	0.45–3.21	0.246	1.96	1.02–3.17	0.065
Tumor stage: IV vs. II-III	1.45	0.31–4.57	0.386	1.56	0.39–2.15	0.389
Tumor size: < 3 vs. ≥ 3 cm	0.42	0.16–1.15	0.072	0.48	0.26–0.98	0.031
SPINK1: low vs. high	0.44	0.29–1.24	0.048	0.23	0.11–0.91	0.025
Caspase 3 (cleaved): low vs. high	0.39	0.16–1.03	0.346	1.21	0.33–2.42	0.235
EGFR low vs. high	0.737	0.324–1.602	0.189	0.760	0.382–1.491	0.167
MET low vs. high	0.821	0.319–2.059	0.231	0.840	0.390–1.863	0.175

PFS, progression-free survival. CRC, colon and rectal cancer. HR, hazard ratio. CI, confidence interval. EGFR, epidermal growth factor receptor. MET, c-MET proto-oncogene, receptor tyrosine kinase. *P* < 0.05 is statistically significant, with significant *P*-values highlighted in bold.

Supplementary Table 4. List of quantitative RT-PCR primers.

Target name	Forward (5'-3')	Reverse (5'-3')
<i>SPINK1</i>	CCTTGGCCCTGTTGAGTCTA	GCCCAGATTTTTGAATGAGG
<i>IL-6</i>	TACCCCCAGGAGAAGATTCC	TTTTCTGCCAGTGCCTCTTT
<i>IL-8</i>	GTGCAGTTTTGCCAAGGAGT	CTCTGCACCCAGTTTTCTT
<i>WNT16B</i>	GCTCCTGTGCTGTGAAAACA	TGCATTCTCTGCCTTGTGTC
<i>SFRP2</i>	GCCTCGATGACCTAGACGAG	GATGCAAAGGTCGTTGTCCT
<i>MMP1</i>	GGTCTCTGAGGGTCAAGCAG	AGTTCATGAGCTGCAACACG
<i>MMP3</i>	GCAGTTTGCTCAGCCTATCC	GAGTGTCGGAGTCCAGCTTC
<i>MMP12</i>	ACACATTTTCGCCTCTCTGCT	CCTTCAGCCAGAAGAACCTG
<i>AREG</i>	TGGATTGGACCTCAATGACA	AGCCAGGTATTTGTGGTTCG
<i>GM-CSF</i>	CCCCAGTCACCTGCTGTTAT	TGGAATCCTGAACCCACTTC
<i>ANGPTL4</i>	GCCTATAGCCTGCAGCTCAC	AGTACTGGCCGTTGAGGTTG
<i>IL-1a</i>	AATGACGCCCTCAATCAAAG	TGGGTATCTCAGGCATCTCC
<i>IL-1b</i>	GGGCCTCAAGGAAAAGAATC	TTCTGCTTGAGAGGTGCTGA
<i>CXCL1</i>	AGGGAATTCACCCCAAGAAC	TGGATTTGTCACTGTTCAAGCA
<i>CXCL3</i>	GCAGGGAATTCACCTCAAGA	GGTGCTCCCCTTGTTCAAGTA
<i>MCP-2</i>	TCACCTGCTGCTTTAACGTG	ATCCCTGACCCATCTCTCCT
<i>IL-2</i>	TGCAACTCCTGTCTTGCAAT	GCCTTCTTGGGCATGTAAAA
<i>IL-3</i>	CTTTGCCTTTGCTGGACTTC	CCGTCCTTGATATGGATTGG
<i>IL-5</i>	GAGACCTTGGCACTGCTTTC	CAGTACCCCCTTGACACAGTT
<i>IL-12</i>	GATGGCCCTGTGCCTTAGTA	TCAAGGGAGGATTTTTGTGG
<i>Twist 1</i>	GTCCGCAGTCTTACGAGGAG	CCAGCTTGAGGGTCTGAATC
<i>Twist 2</i>	AGCAAGAAGTCGAGCGAAGA	CAGCTTGAGCGTCTGGATCT
<i>Snail 1</i>	TTTACCTTCCAGCAGCCCTA	CCTCATCTGACAGGGAGGTC
<i>Snail 2</i>	CTTTTTCTTGCCCTCACTGC	ACAGCAGCCAGATTCCTCAT
<i>E-cadherin</i>	TGCCCAGAAAATGAAAAAGG	GTGTATGTGGCAATGCGTTC
<i>N-cadherin</i>	GACAATGCCCTCAAGTGTT	CCATTAAGCCGAGTGATGGT
<i>Vimentin</i>	GAGAACTTTGCCGTTGAAGC	TCCAGCAGCTTCCTGTAGGT
<i>ALDH1A1</i>	TGTTAGCTGATGCCGACTTG	TTCTTAGCCCGCTCAACACT
<i>CD44</i>	AGCAACCAAGAGGCAAGAAA	GTGTGGTTGAAATGGTGCTG
<i>CD24</i>	ACCCACGCAGATTTATTCCA	ACCACGAAGAGACTGGCTGT
<i>CD31</i>	GCAAAATGGGAAGAACCTGA	CACTCCTTCCACCAACACCT
<i>CD34</i>	CACCCTGTGTCTCAACATGG	GGCTTCAAGGTTGTCTCTGG
<i>RPL13A</i>	GTACGCTGTGAAGGCATCAA	CGCTTTTCTTGTCGTAGGG

Supplementary Table 5. Antibodies used for Western blot (WB), immunofluorescence staining (IF) and immunohistochemistry (IHC) staining.

<i>Antigen name</i>	<i>Commercial source</i>	<i>Catalog number (clone number)</i>	<i>Application</i>	<i>Dilution time</i>
SPINK1	Abnova	H00006690-M01 (clone 4D4)	WB, IHC	1:500; 1:200
IL-8	Proteintech	60141-2-Ig	WB	1:500
γH2AX	Cell signaling	9718	IF	1:250
BrdU	Cell signaling	5292	IF	1:500
EGFR	Proteintech	18986-1-AP	WB	1:1000
p-EGFR	Cell signaling	2231	WB	1:1000
mTOR	Proteintech	20657-1-AP	WB	1:500
p-mTOR	Abcam	ab1093	WB	1:200
AKT1	Proteintech	10176-2-AP	WB	1:500
p-AKT1	Abcam	ab8932	WB	1:300
Erk1/2	Proteintech	16443-1-AP	WB	1:1000
p-Erk1/2	Cell signaling	4377	WB	1:500
STAT3	Proteintech	10253-2-AP	WB	1:1000
p-STAT3	Abcam	ab32143	WB	1:500
E-cadherin	Proteintech	20874-1-AP	WB, IF	1:500
N-cadherin	Proteintech	22018-1-AP	WB	1:200
Vimentin	Proteintech	60330-1-Ig	WB	1:1000
Vimentin	GeneTex	GTX100619	IF	1:500
GAPDH	Vazyme	Ab103	WB	1:2000
Caspase 3 (cleaved)	Cell signaling	9661	WB, IHC	1:500
Caspase 3 (intact)	Cell signaling	9662	WB	1:1000