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Supplementary Materials for

Intercellular transmission of the unfolded protein response promotes survival and drug resistance in cancer cells

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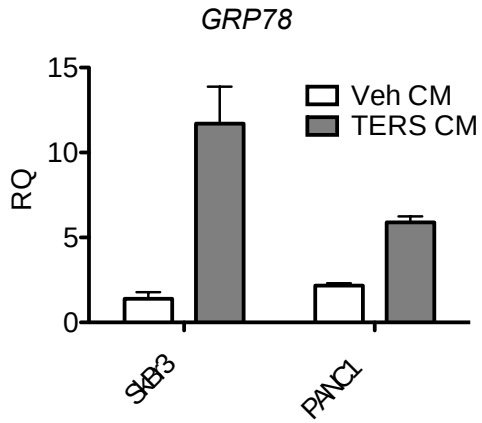

Fig. S1. TERS transmission and reception occur among various human cancer cell lines. (**A**) Expression of *GRP78* by RT-qPCR in human breast cancer cell line SkBr3 and human pancreatic cancer cell line PANC1 cultured in homologous vehicle- or TERS-conditioned medium (CM) for 24 hours. Expression was normalized to Veh CM SkBr3 cells. Data are means ± SEM (n=2), representative of three independent experiments. RQ, relative quantification.

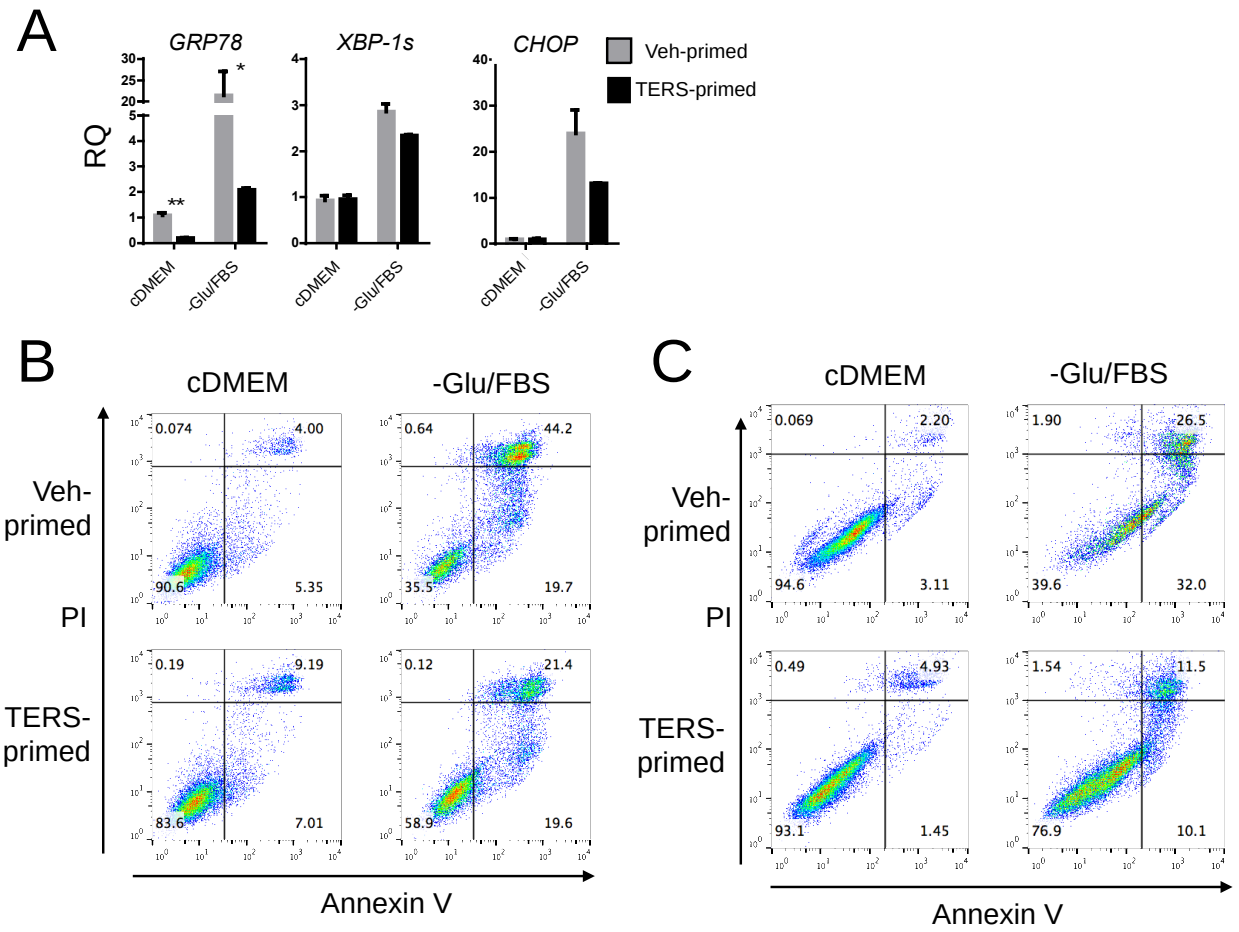

Fig. S2. Nutrient-starved TERS-primed cells have increased viability during nutrient deprivation. (**A**) Veh or TERS CM-primed PC3 cells were cultured in the presence (cDMEM) or absence of glucose and serum proteins (-Glu/FBS) for 48 hours and were analyzed by RT-qPCR for UPR status (*GRP78*, *XBP-1s*, *CHOP*). Gene expression was normalized to Veh-primed, cDMEM condition to determine relative quantification (RQ) of gene expression. Data are means ± SEM (n=2 per condition). * $p < 0.05$, ** $p < 0.01$, paired two-tailed Student's *t*-test. (**B**) Flow cytometry analysis for annexin V and propidium iodide (PI) staining in DU145 (B) or LNCaP (C) cells Veh-primed or TERS-primed and cultured in cDMEM or -Glu/FBS for 48 hours. Data are representative of two independent experiments.

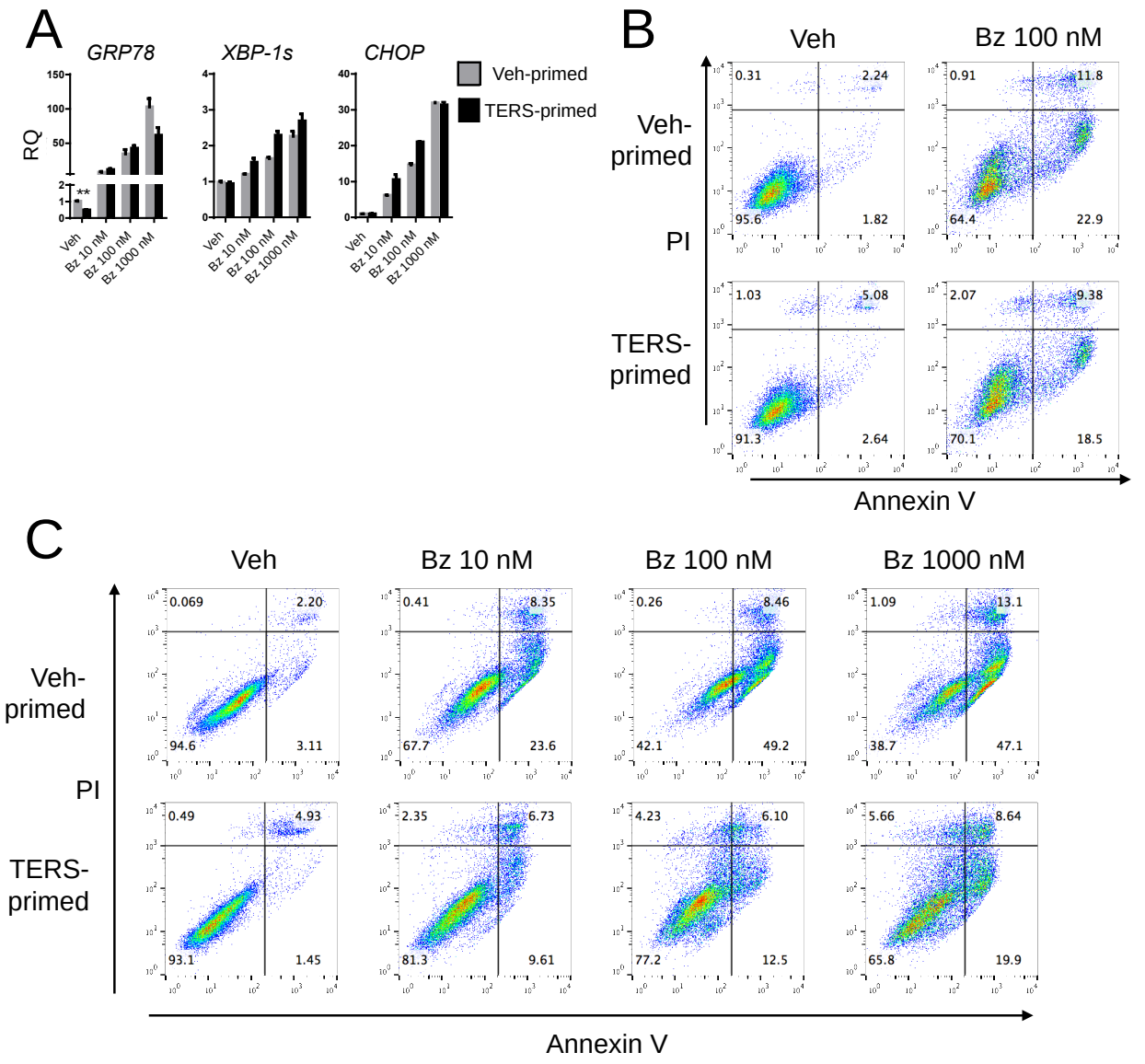

Fig. S3. Bortezomib does not affect UPR transcription between vehicle- and TERS-primed cells but is less cytotoxic to TERS-primed cells. (**A**) RT-qPCR analysis of *GRP78*, *XBP-1S*, and *CHOP* after 24 hours treatment with bortezomib or vehicle control (Veh) in vehicle- or TERS-primed PC3 cells. Relative quantification (RQ) was established by normalizing gene expression to Veh treated cells of Veh-primed PC3 cells. Data are means ± SEM of three independent experiments (n=2 per condition).** $p < 0.01$, paired two-tailed Student's *t*-test. (**B and C**) Flow cytometric analysis for annexin V and propidium iodide (PI) staining in DU145 (B) or LNCaP (C) cells Veh-primed or TERS-primed and cultured in bortezomib (Bz) or Veh control for 24 hours. Data are representative of two (B) and five independent experiments (C), respectively.

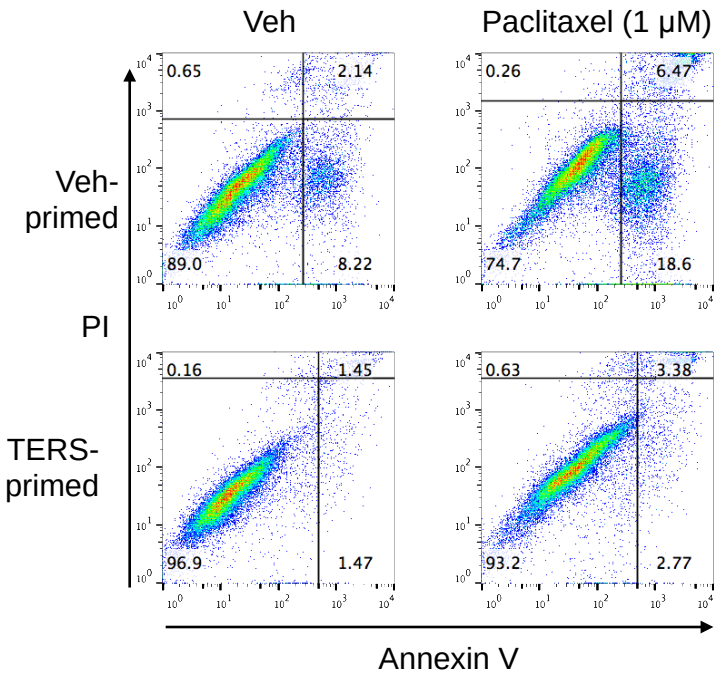

Fig. S4. TERS-primed LNCaP cells are protected from paclitaxel cytotoxicity. Flow cytometry analysis of annexin V and PI staining in vehicle- or TERS-primed LNCaP cells 24 hours after addition of paclitaxel compared to Veh control cultures. Data are representative of three independent experiments.

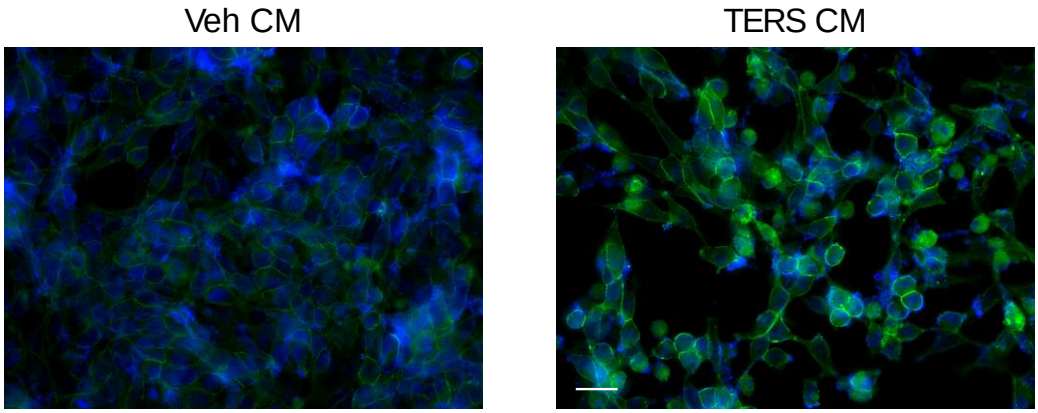

Fig. S5. TERS CM promotes abundance in β-catenin. Immunofluorescence staining for β-catenin (green) in LNCaP cells cultured in vehicle (Veh) CM or TERS CM for 48 hours. Nuclei are counterstained with DAPI (blue). Scale bars, 25 μm. Microscopy images are representative of one field from three images of two independent experiments.

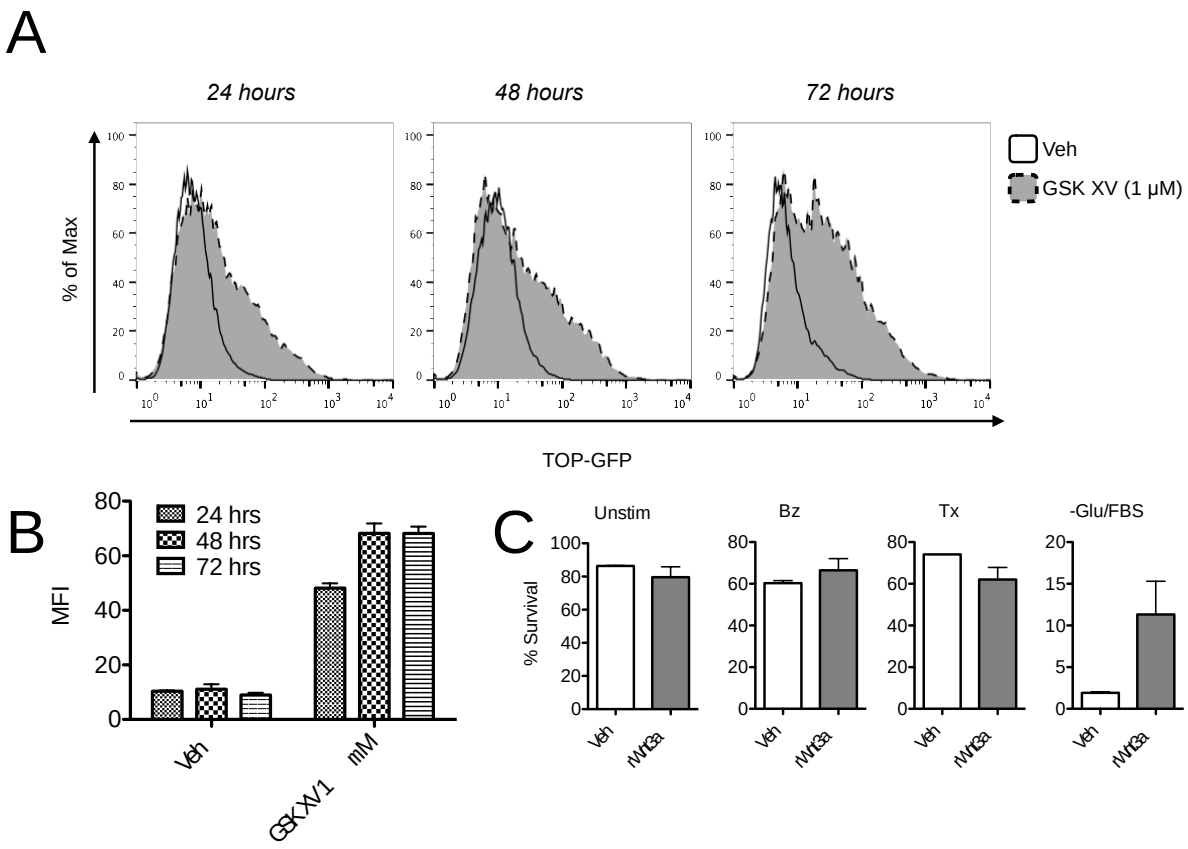

Fig. S6. Validation of PC3.TOP reporter system. (**A and B**) Flow cytometry analysis of PC3.TOP reporter cells treated with the GSK-3 inhibitor, GSK-XV, on days 1, 2, and 3. Representative histogram (A) and mean fluorescent intensity (MFI; B) (n=2) of treated or Veh control cells. (**C**) 7AAD-based analysis of viability of LNCaP cells cultured with vehicle (Veh) or recombinant WNT3a (rWNT3a; 20 ng/ml for 2 days) and treated as indicated for an additional 48 hours. Bz, bortezomib (100 nM); Tx, paclitaxel (1 μM); -Glu/FBS, nutrient-deprived medium. Viable cells were 7AAD-negative. Control cells were vehicle-treated (Veh). Data in (B and C) are MFI ± SEM from 10,000 events per sample. Flow cytometry data are representative of three independent experiments.

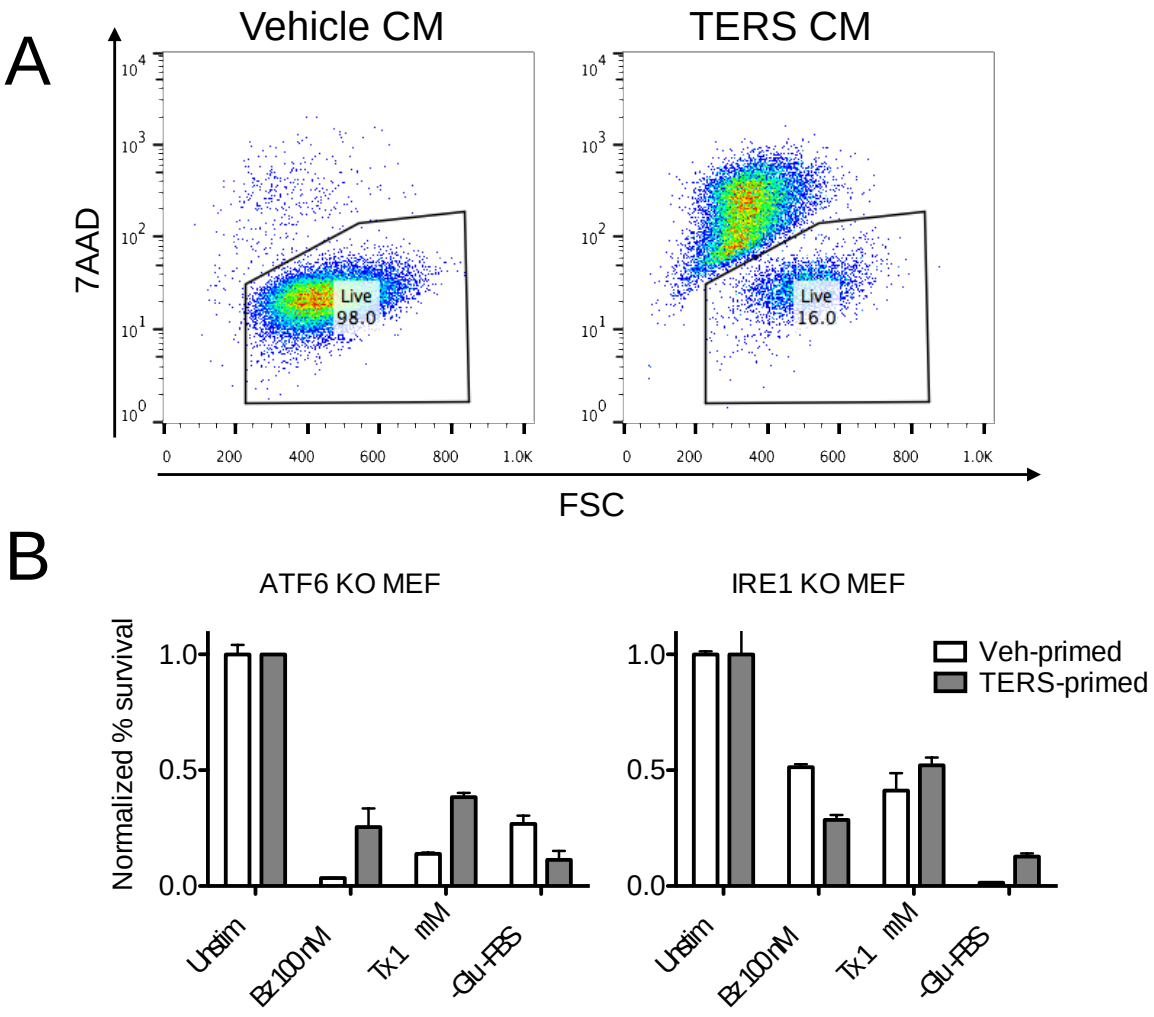

Fig. S7. MEF KO cells have selective sensitivity to TERS. (**A**) Flow cytometry analysis of viability of *PERK* KO MEFs cultured in TC1-derived Veh CM or TERS CM after two days of treatment as determined by 7AAD staining. Data were collected as 10,000 events/sample, and are representative of three independent experiments. FSC: forward scattered. (**B**) Flow cytometry analysis of 7AAD exclusion (viability) in *ATF6* and *IRE1* KO MEFs cultured in TC1 Veh or TERS CM and challenged for 48 hours as indicated. Bz, bortezomib; Tx, paclitaxel; -Glu/FBS, nutrient-deprived medium. Data were normalized to that of the corresponding untreated condition (unstim). Data are means ± SEM (n=2) from three independent experiments.

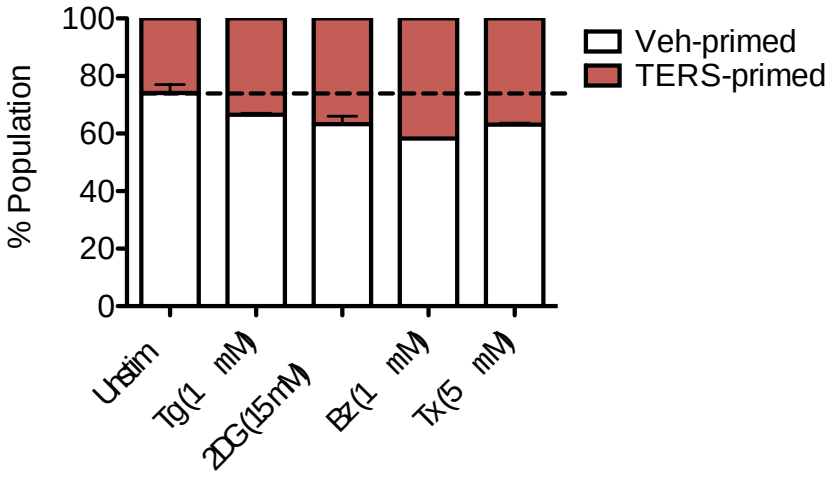

Fig. S8. Population fitness of TERS-primed cells. Percentage of TERS-primed TC1.RFP cells present in cocultures with Veh-primed TC1(untagged) cells after 24 hours treatment with thapsigargin (Tg), 2-deoxy glucose (2DG), bortezomib (Bz), or paclitaxel (Tx). Cell numbers were quantified by flow cytometry after exclusion of 7AAD-positive cells. Data are means ± SEM (n=2; 10,000 events/sample) from representative of two independent experiments.

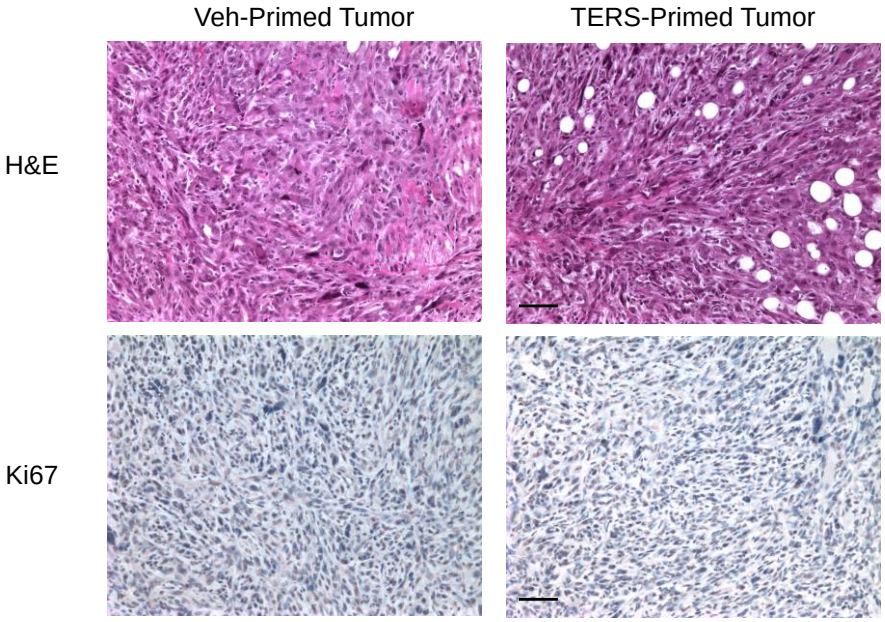

Fig. S9. Histology analysis of TC1 vehicle- and TERS-primed tumors. Haematoxylin and eosin (H&E) staining (for morphology; top) and Ki67 staining (for proliferation; bottom) in Veh-primed and TERS-primed TC1 tumors 30 days after implantation in C57BL/6 mice. Scale bar, 100 µm. Images are representative of three fields from three individual tumors per group.