Supplementary Figure Legends

Figure S1: Calpain expression in SKBR3 cells correlates with sensitivity to HSP90 inhibitor 17AAG. (A) Immunoblot analysis of CAPN1, CAPN2 and CAPNS1 in SKBR3 cells infected with control lentivirus (control) or lentivirus expressing one of two shRNA constructs targeting *CAPNS1* (shRNA-*capns1-1*, shRNA-*capns1-2*). Blotting for RasGAP served as a loading control. **(B)** Cell death analysis of 17AAG treated SKBR3 cells described in (A). Data is mean ± SD. LD50 values for Control: 38.4μM; shRNA-Capns1-1: 0.66μM; shRNA-Capns1-2: 0.35μM. Student's t-test were performed. * indicates p-value ≤ 0.01 relative to Control. One-way Anova was used to detect significant differences in LD50 values.

Figure S2: Calpain knockdown enhances 17AAG-mediated suppression of migration and invasion *in vitro,* and metastasis *in vivo.* Representative images of MDA-MB-231 cells transduced with control or lentivirus expressing shRNA-*capns1* at end points of transwell migration **(A)** or matrigel invasion **(B)** assays in the presence of vehicle or the indicated concentrations of 17AAG for 8 or 24 hrs, respectively. These data were presented in quantitative form in Figure 3A,B.

(C) Representative images of biophotonically imaged lungs from orthotopic xenografts of control or calpain knockdown MDA-MB-231 cells with and without 17AAG treatment. Metastatic lesions are scored as independent GFP fluorescent signals using Image Pro. Data are presented in quantitative from in Figure 3D.

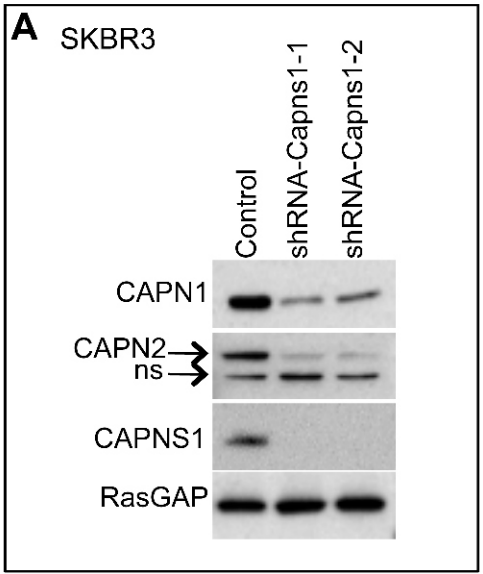
Figure S3: Loss of calpain enhances the rate of decline of Hsp90 clients. Densitometry of AKT **(A)**, Cyclin D1 **(B)**, and MRP2 **(C)** in control and calpain knockdown MDA-MB-231 cells

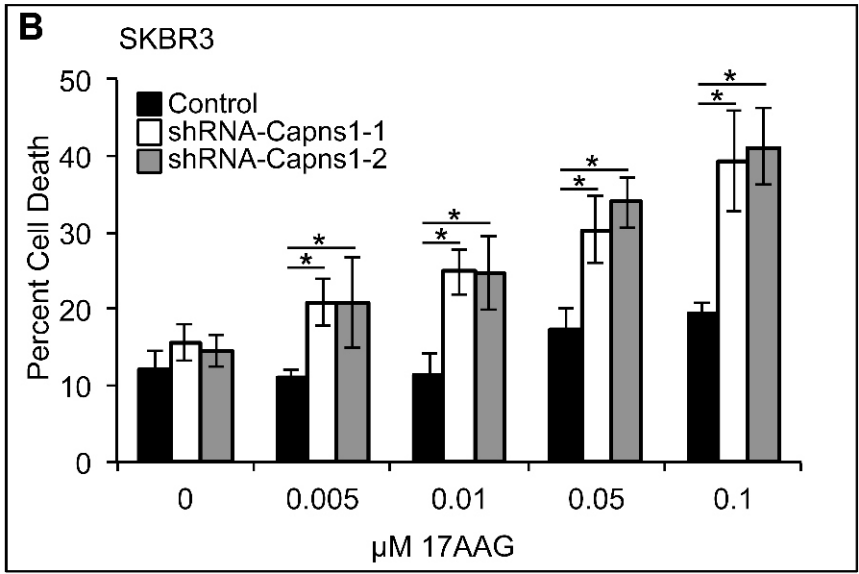
treated with increasing concentrations of 17AAG. Expression of proteins was normalized to tubulin (AKT, Cyclin D1) or membrane fraction Na^+K^+ ATPase (MRP2). Values are presented relative to untreated control within each cell line. Data is from two independent experiments. Nonlinear regressions were performed to assess differences in the rate of decline (k). * indicates p value ≤ 0.05 compared to Control.

Figure S4: Calpain affects doxorubicin localization. (A) MEFs or **(B)** MDA-MB-231 cells of the indicated genotypes (described in Figure 7) were plated on coverslips and treated with 3.5 μM doxorubicin for 1hr, fixed and stained with DAPI. Representative images of DAPI, Doxorubicin and merged fluorescence are shown with total intracellular doxorubicin fluorescence intensity quantified below.

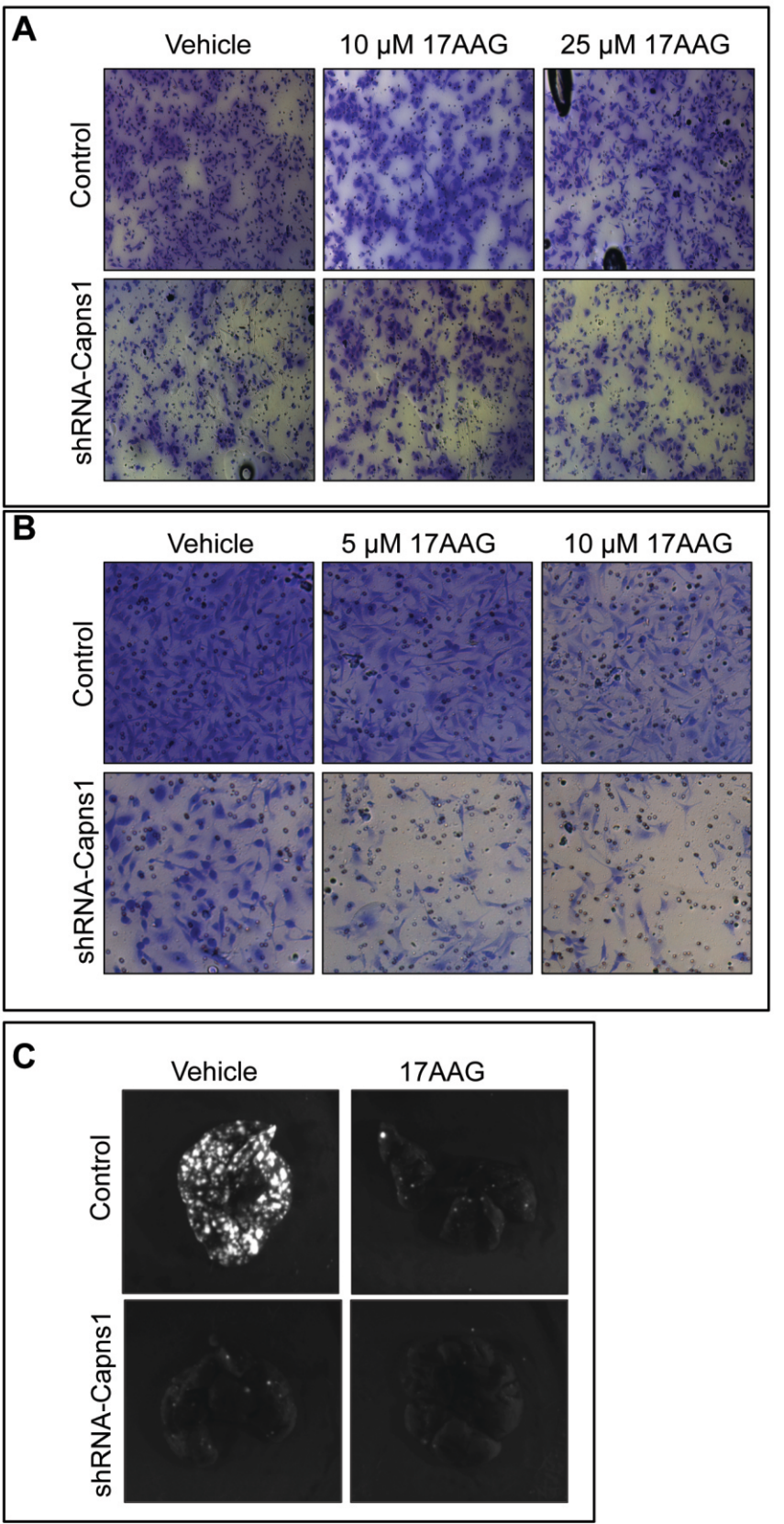
Figure S5: Model for cooperative role of calpain and HSP90 in regulating sensitivity to ABC transporter substrates. Calpain and HSP90 each promote functions and stability of mitogenic and survival signaling proteins, as well as selected ABC transporters. 17AAG is a substrate of specific ABC transporters, but can also inhibit some through inhibiting HSP90. The combined effect of inhibiting HSP90 and calpain is predicted to render cancer cells hypersensitive to 17AAG, as well as other chemotherapeutics that are efflux substrates of the ABC transporters acted upon by calpain and HSP90.

Supplementary Figure 1

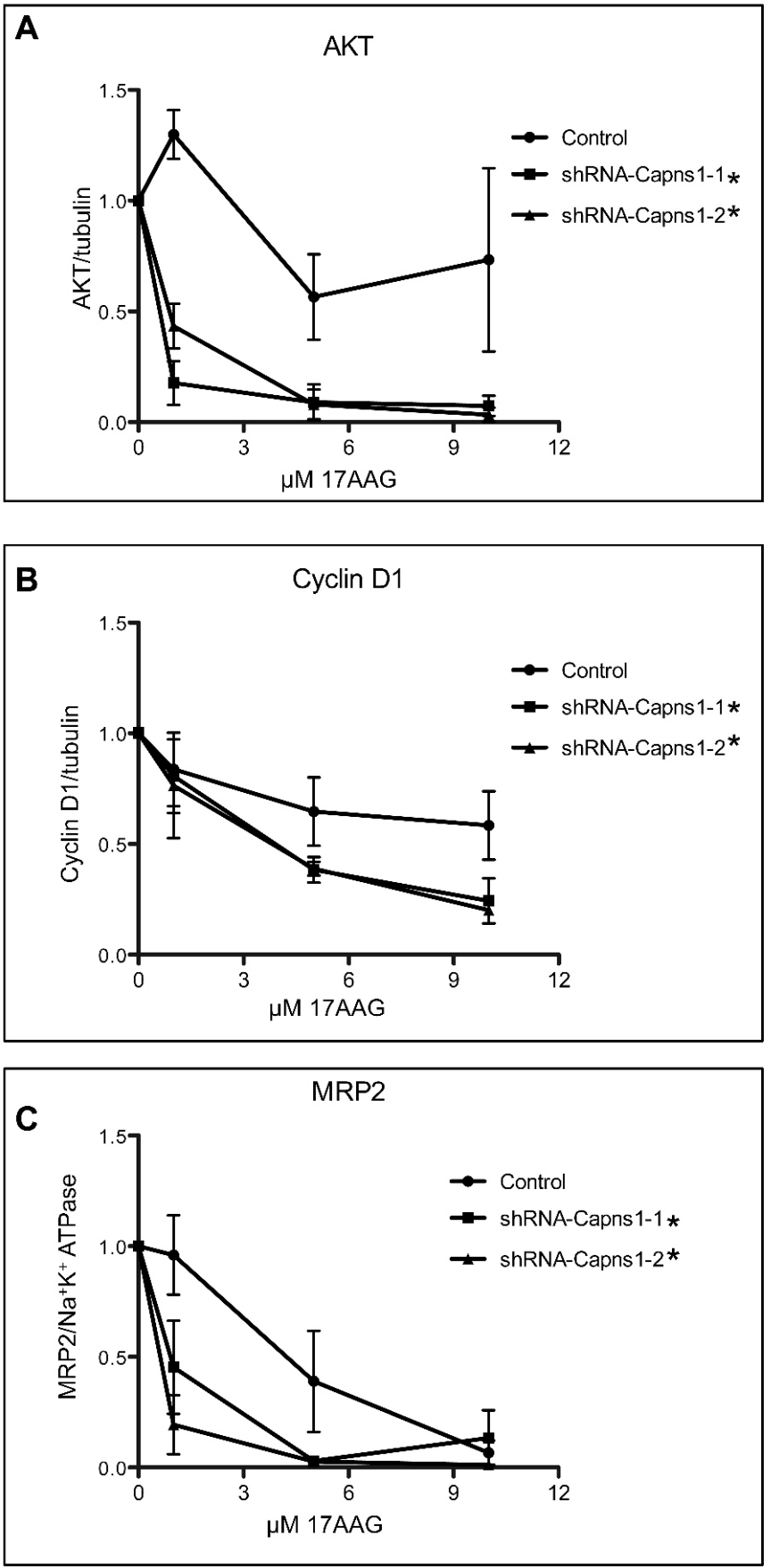




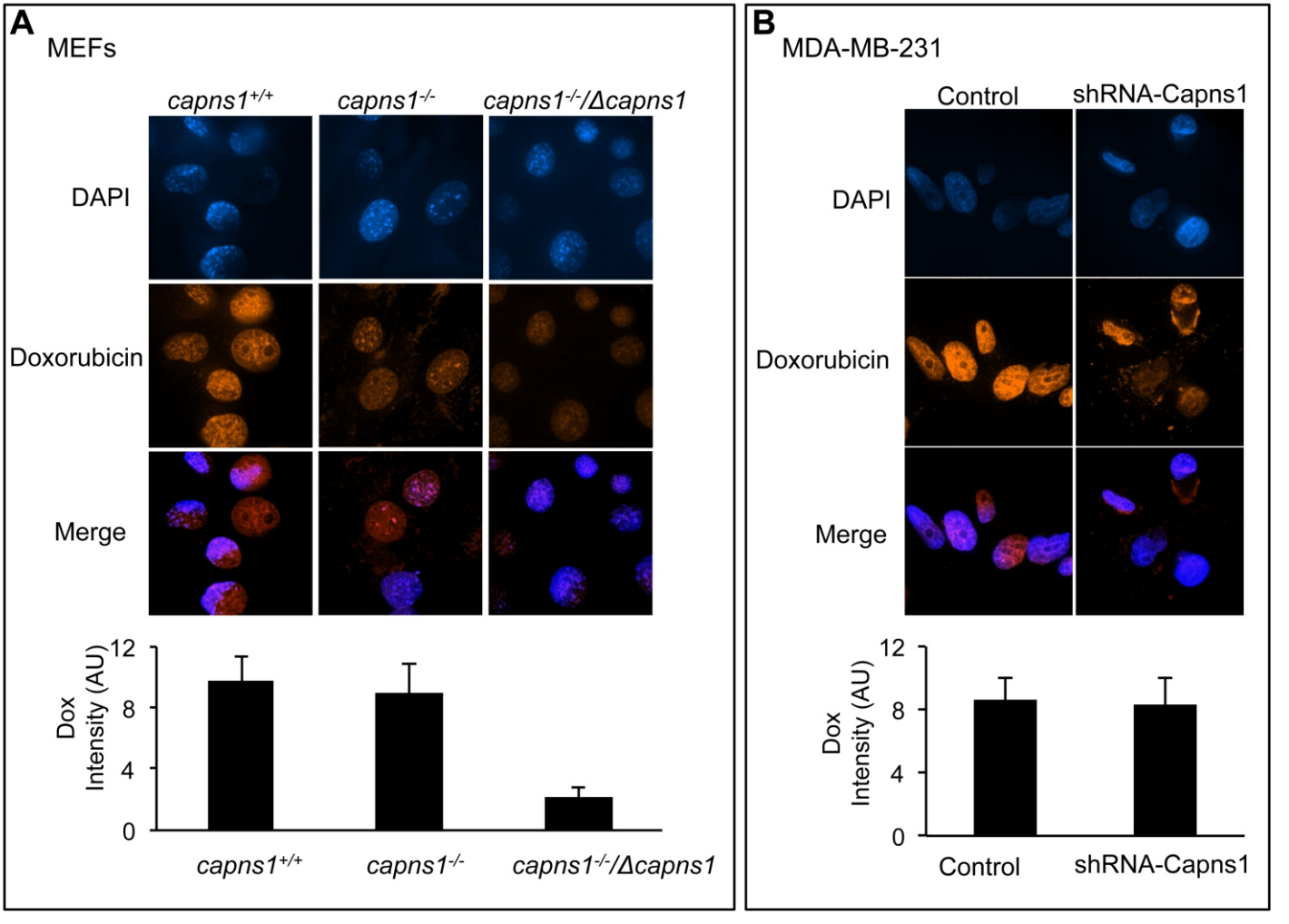
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5