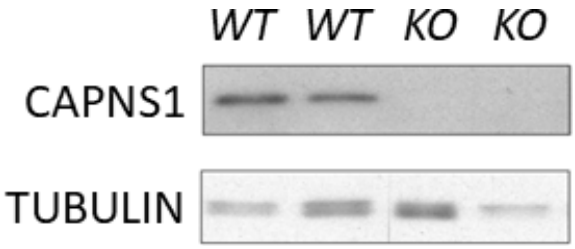
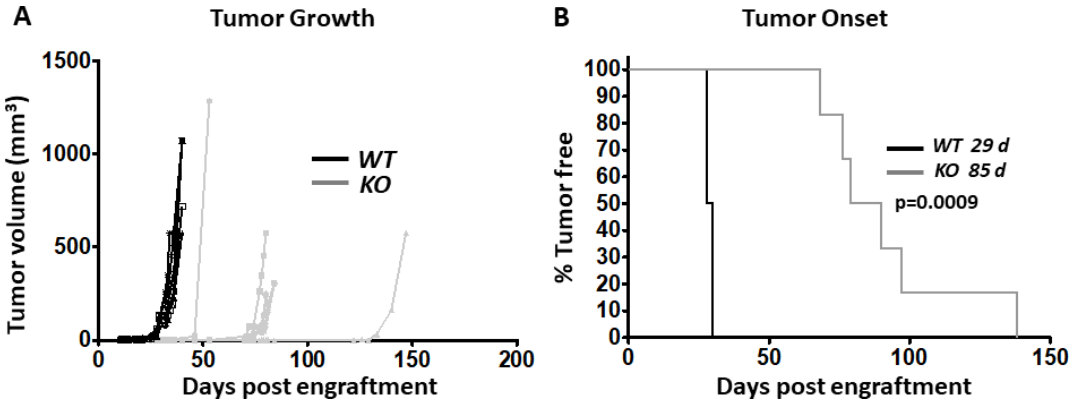
# Genetic disruption of calpain-1 and calpain-2 attenuates tumorigenesis in mouse models of HER2+ breast cancer and sensitizes cancer cells to doxorubicin and lapatinib

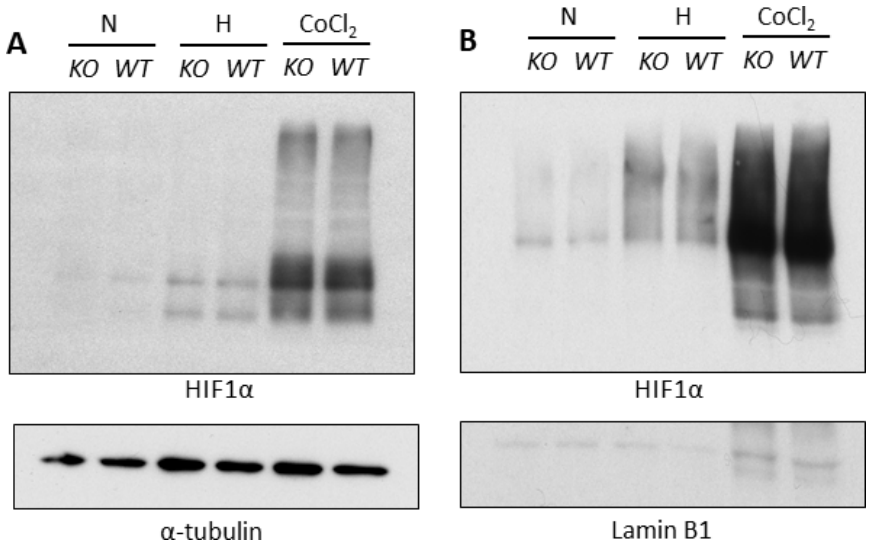
## SUPPLEMENTARY MATERIALS



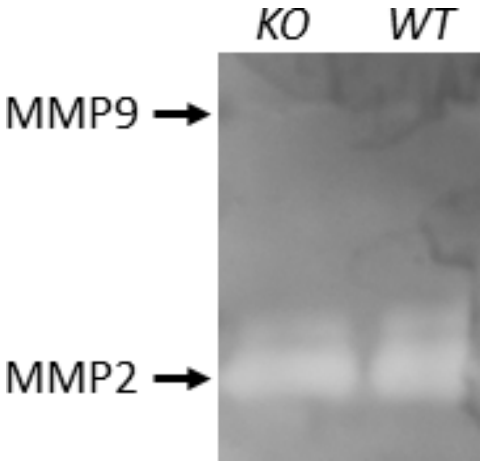

**Supplementary Figure 1: CAPNS1 expression is ablated in mammary tumors from NIC *capns1*$^{flox/flox}$ mice.** Tumor lysates from NIC *capns1*$^{flox/flox}$ (*KO*) or NIC *capns1*$^{+/+}$ (*WT*) female mice were assessed for CAPNS1 or tubulin expression by immunoblotting.




**Supplementary Figure 2: *capns1 KO* is associated with reduced tumorigenic potential.** One × 10$^6$ capns1 *KO* or *WT* MTECs were engrafted into the number four mammary glands of female *Rag2*$^{-/-}$ I*L-2Rγc*$^{-/-}$ mice ($n = 6$ for each cohort). (**A**) Caliper measurements of tumor volumes. (**B**) Median time to onset of palpable tumors at the engraftment site were 85 or 29 days for *KO* and *WT*, respectively ($p = 0.0009$, Log-rank (Mantel-Cox) test). All tumors arising in the KO group displayed expression of *capns1* (data not shown).




**Supplementary Figure 3: *capns1* knockout does not affect hypoxia-induced stabilization of HIF1α.** One and a half × $10^6$ *capns1* KO or *WT* MTECs were seeded for 24 h under conditions of hypoxia (H) (0.1% $O_2$), normoxia (N) (21% $O_2$) or challenge with $CoCl_2$ (100 μM). Lysates were collected and levels of HIF1α were assessed by immunoblotting. Tubulin and nuclear Lamin B1 immunoblotting was performed to assess sample loading. (**A**) Whole cell lysate immunoblotting for HIF1α (**B**) Nuclear enriched immunoblotting for HIF1α.




**Supplementary Figure 4: *capns1 KO* does not affect secretion or activation of matrix metalloproteinases 2 and 9.** Seven × $10^5$ *capns1 KO* or *WT* MTECs were seeded and cultured overnight, then changed to serum-free medium the following day. Conditioned media was collected after 24 hours and gelatin zymography performed to assess MMP activity.