**SUPPLEMENTAL TABLE AND FIGURE LEGENDS**

**Table S1.** **Spatiotemporal expression of SNAIL1 in MMTV-PyMT mice**

A table of SNAIL1-positive fraction in indicated tissue types is shown. Three 10-m sections each of primary tumor and lungs with metastases and BM DTC spreads from 5 animals were subjected to IHC for SNAIL1, E-cadherin and Vimentin (primary tumor and lung) or co-IF for panCK and SNAIL1 (BM DTCs) and the SNAIL1-positive percentage in each tissue type shown. **Related to Figure S4**.

**Figure S1, related to Figure 1: Generation and characterization of the SNAIL1-CBR mouse**

**A**, A diagram of the endogenous SNAIL1 locus is shown.

**B**, A diagram of the Snail-CBR targeting construct is shown. E1-3: SNAIL1 exons; CBR: clic beetle red luciferase; TRE-SNAIL1: tet-inducible SNAIL1 transgene; frt: FRT site; Lox: LoxP site; pA: polyA tail; 3’UTR: 3’ untranslated region; Stop: stop codon; Blue arrows labeled p1-5: PCR primers used to screen ES cells and mouse tails. Note p1 and p4 are in genomic DNA (denoted in red) that is outside the targeting construct. Neo/TK: the selection cassette.

(**C and D**) SNAIL1-3Flag transgene retains its EMT-inducing functions. MCFA10A cells were infected with a lentivirus containing either the pFLRu-SNAIL1-3Flag or the control pFLRu-3Flag. Infected cells were selected with puromycin. The ability of SNAIL1-3Flag to induce EMT was confirmed by both morphological changes (**C**) and molecularly as evidenced by downregulation of E-cadherin and upregulation of Vimentin (**D**).

(**E and F**) A survey of SNAIL1-CBR signal intensity in internal organs is shown.The baseline physiologic SNAIL1-CBR signal is low in mammary glands and skin (**E**) and most internal organs (**F**).

**Figure S2, related to Figure 2A-E:** **SNAIL1 expression in primary breast tumors strongly correlates with increased metastasis in MMTV-neuNT mice**

**A**, A histogram of relative difference in radiance (defined as the number of photons emitted per second per cm2) between SNAIL1-CBR positive and SNAIL1-CBR negative tumors in MMTV-NeuNT; SNAIL1-CBR mice as a function of lung metastasis is shown. Tumors with SNAIL1-CBR bioluminescence intensity of 107 photons/cm2 or greater (mean of 1.75x107 photons/cm2) were highly associated with the presence of lung metastasis (designated SNAIL1-CBR positive tumors), whereas those with SNAIL1-CBR intensity of 3x106 photons/cm2 or less (mean of 2.5x106 photons/cm2) were not (designated SNAIL1-CBR negative tumors). N=23 mice without lung metastasis and n=15 mice with lung metastasis. \*p=0.001.

(**B and C**) The number of BM DTCs correlates well with that of lung DTCs in MMTV-NeuNT mice, and therefore can be used as a marker of overall DTC burden.

**B**, A histogram demonstrating that SNAIL1 expression in primary breast tumors strongly correlated with the number of lung DTCs is shown. Lung DTCs were detected by IF for Her2Neu of total dissociated lung cells and quantified and expressed as number of lung DTCs per 106 total lung cells. N=4 for each group, \*p=0.002,

**C**, A histogram of the ratio of number of Her2Neu-positive BM DTCs per 106 total BM cells over that of Her2Neu-positive lung DTCs per 106 total lung cells is shown demonstrating that the ratio was comparable between mice bearing either SNAIL1-CBR positive and negative tumors. Therefore the number of BM DTCs can be used as a surrogate marker for total DTC burden. N=4 for each group.

**D**,A histogram of the fraction of Her2Neu-positive cells in SNAIL1-CBR positive and negative tumors is shown, demonstrating that they had comparable expression of Her2Neu. SNAIL1-CBR positive and negative primary tumors were isolated and dissociated with collagenase. Dissociated tumor cells were stained with an anti-Her2Neu monoclonal antibody and Alexaflor 488 conjugated secondary antibody and analyzed by FACS. The value for no stain control, which had secondary antiboby but not primary antibody, was subtracted from all values as background. 5 tumors in each group isolated from 3 different mice were used for the analyses.

**Figure S3, related to Figure 2F-J: SNAIL1 expression in primary breast tumors correlates with increased invasion**

**A**, Expression of endogenous SNAIL1 and SNAIL1-CBR in SNAIL1-CBR positive and negative tumors was confirmed by RT-PCR.

B, The same H&E stained sections as in Figure 2H are shown at higher magnification.

**Figure S4, related to Figure 3:**  **SNAIL1 expression in primary breast tumors precedes detection of DTCs, but SNAIL1 is not expressed by DTCs and lung metastases in MMTV-PyMT mice**

**(A to D)** SNAIL1 is upregulated in primary breast tumors prior to detection of BM DTCs

**A**, Representative images of serial bioluminescence imaging of MMTV-PyMT; SNAIL1-CBR mice are shown.

**B**, A histogram of SNAIL1-CBR bioluminescence as a function of tumor progression in the same mice is shown. N=12 mice. Note the milestones as marked by red arrows.

**C,** A histogram of relative SNAIL1 mRNA levels in primary breast tumors of mice at indicated age as determined by quantitative RT-PCR and expressed as relative to GAPDH mRNA levels is shown. N=3 mice for each age group, \*p=0.04.

**D**, A histogram of number of BM DTCs per 106 BM cells as quantified by panCK IF of whole BM cells is shown. N=3 mice for each age group, \*p=0.01.

**(E)** SNAIL1 is not expressed by BM DTCs and lung metastases.Representative images of the same H&E and IHC stained sections for indicated protein as in Table S1 are shown. M denotes lung metastatic focus; L denotes normal lung.

**Figure S5, related to Figure 4C-E:** **Primary breast tumors in Early SNAIL1 KO exhibit loss of EMT phenotype and metastatic potential**

(**A and B**) Representative images of sections of primary tumors and lungs with metastases from the same Control (**A**) and Early SNAIL1 KO (**B**) mice as in Figure 4C-E and stained with H&E or IHC for indicated proteins are shown. M denotes lung metastatic focus; L denotes normal lung.

**C.** The few lung metastases observed in early SNAIL1 KO mice arose from tumor cells that likely had escaped CRE-mediated deletion of floxed SNAIL1. Total genomic DNA isolated from paired primary tumor and individual lung metastases were subjected to PCR using primer pair p5 and p6 that specifically amplifies the CRE-deleted floxed SNAIL1 allele. Primer pair p7 and p8 was used as internal control for genomic DNA input.

**Figure S6, related to Figure 5D: SNAIL1 is not expressed by lung metastases in mice overexpressing SNAIL1 in primary breast tumors**

Representative images of sections of primary tumors and lung metastases from the same Control (**A**), Continuous SNAIL1 (**B**) and Transient SNAIL1 (**C**) as in Figure 5D and stained with H&E or IHC for indicated proteins are shown, again demonstrating that lung metastases had low expression of SNAIL1 even when SNAIL1 expression was widespread in primary tumors of continuously SNAIL1 overexpressing mice. Lung metastases also exhibited epithelial-like phenotype. Hematoxylin was used for nuclear counterstain.