**SUPPLEMENTAL MATERIALS AND METHODS**

**Primers for genotyping of transgenic mice**

 Genotyping was conducted on genomic DNA isolated from tail biopsies using the KapaBiosystems genotyping kit and the following primers. SNAIL1-CBR: p1: caaatgaagttgtccgtggtgccacac; p2: gagtgcttgcggagagcac; p3: gccttcttgacgagttcttc; p4: tctggggacctaaagctccagg. SNAIL1*fl/fl*: (for genotyping) Fw: ctgccaggtgggaaggact; Rev: caaggacatgcgggagaaggt; p5: tacaaatatggacccccag; p6: acaaagcaccttcccaag; p7: ggaatttgctgctgctagg; p8: cacacacgcgtcctgcag. NeuNT: Fw: ccccgggagtatgtgagtga; Rv: tgagctgttttgaggctgaca. MMTV-PyMT: Fw: ggaagcaagtacttcacaaggg; Rv: ggaaagtcactaggagcaggg. MTA: Fw: tgccgccattattacgacaagc; Rv: accgtactcgtcaattccaaggg.

**ES cell differentiation assay**

 Targeted ES cells were cultured in the absence of LIF on non-culture-coated plates to induce differentiation and cells harvested at indicated times and processed for immunoblotting for indicated proteins and for RT-PCR for indicated mRNA.

**Transgenic mice used and *in vivo* experimental design**

 MMTV-NeuNT mice express an activated form of the rat oncogene Her2Neu or ErbB2 specifically in the mammary epithelium. Approximately 50% of these mice develop multifocal breast tumors with a latency period ranging from 5-8 months and 25-30% of tumor-bearing mice progress to develop lung and liver metastases with a metastatic latency of 2-3 months [[1](#_ENREF_1)].

 MTA; TAN mice (MMTV-rtTA; TetO-neuNT) are a tet-inducible activated rat Her2Neu model. Mice were fed drinking water containing 1 mg/ml dox starting at 6 weeks of age. By 12 weeks of age (6 weeks of dox treatment) >90% of mice had developed primary breast tumors of approximately 0.5 cmin diameterwithout evidence of lung metastasis [[2](#_ENREF_2)]. The first appearance of lung metastases appeared in a fraction of mice at age 15 weeks (or 9 weeks of dox treatment). If dox treatment was continued, essentially all mice developed detectable lung metastases by 18 weeks of age.

 MMTV-PyMT mice express the oncogene polyoma middle T antigen specifically in mammary epithelia. Tumor penetrance is nearly 100%, tumor latency is 3-5 weeks from puberty (i.e. 7-9 weeks of age), and nearly 100% mice develop lung metastases with a latency of approximately 4 weeks from first palpable tumors. Breast tumor development in MMTV-PyMT mice closely recapitulates human breast cancer development demonstrating a transition from dysplasia to adenoma to invasive carcinoma and the eventual loss of expression of estrogen and progesterone receptors coincident with the upregulation of Her2Neu in metastatic disease [[3](#_ENREF_3)].

 ROSA26-LSL-tdTomato Red mice contain a transgene encoding an enhanced tandem dimer tomato red fluorescent protein (tdTomato Red) in the ROSA26 locus with a lox-transcriptional stop-lox cassette (LSL) inserted proximal to the transcriptional start site [[4](#_ENREF_4)]. Expression of the tdTomato Red protein is suppressed unless the LSL is excised by CRE. To confirm the tissue specificity of CRE expression, tissue samples from breast, lung, liver and salivary gland were collected from 8-week-old ROSA26-LSL-tdTomato Red; MMTV-Cre mice and sections analyzed for tdTomato Red expression.

Tumor burden was assessed by physical measurements of all excised tumors at the time of euthanasia,

 For SNAIL1-CBR imaging experiment, mice were imaged biweekly starting at 16 weeks of age and continued until at least 16 weeks after first palpable tumors (approximately 0.5 cm in diameter that corresponded to between approximately 20 to 30 weeks of age) or until primary tumors exceeded 2 cm in diameter, whichever came first. Mice were then euthanized and the status of lung metastasis assessed. In mice with visible lung metastases, the number of visible superficial lung metastases was limited, ranging from 1 to 20 in most. The absence of internal lung metastases in lungs free of visible superficial lung metastases was confirmed by histologic examination of at least 3 separate sections. Due to the modest lung metastatic rate (e.g. ~25%) and low numbers of lung metastases in the MMTV-neuNT model, we scored the incidence of lung metastasis instead of quantifying the number of lung metastases.

 For SNAIL1 KO experiments, aged and genetic background matched SNAIL1 KO and control MMTV-PyMT mice were monitored for breast tumor development and euthanized between 17-18 weeks of age and the number of BM DTCs and lung metastases determined.

**Western Analysis**

 Cells were lysed in cell lysis buffer containing 1% Triton X-100, 1mM EDTA, 1mM EGTA, 10mM dithiothreitol, 1mM Na3VO4 and complete protease inhibitor mixture. Cellular lysates were separated by electrophoresis on 4-20% polyacrylamide gradient gel and transferred to PVDF membranes. The membranes were then probed with primary antibodies specific for SNAIL1 (Cell Signaling) or FLAG M2 (Sigma). The signal was detected using HRP-conjugated secondary antibody (Jackson ImmunoResearch) and reagents for enhanced chemiluminescence (Amersham).

**References for supplemental methods:**

1. Fantozzi A and Christofori G, Mouse models of breast cancer metastasis*.* Breast Cancer Res 2006; 8:212.

2. Moody SE, Sarkisian CJ, Hahn KT, Gunther EJ, Pickup S, Dugan KD, et al., Conditional activation of Neu in the mammary epithelium of transgenic mice results in reversible pulmonary metastasis*.* Cancer Cell 2002; 2:451-61.

3. Lin EY, Jones JG, Li P, Zhu L, Whitney KD, Muller WJ, et al., Progression to Malignancy in the Polyoma Middle T Oncoprotein Mouse Breast Cancer Model Provides a Reliable Model for Human Diseases*.* Am J Pathol 2003; 163:2113-26.

4. Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, et al., A robust and high-throughput Cre reporting and characterization system for the whole mouse brain*.* Nat Neurosci 2010; 13:133-40.