Supplementary figures

Supplemental Figure 1. Nmi knock out strategy and validation

(A) Schematic of strategy for knocking out Nmi gene: Gene targeting via homologous recombination in 129SV embryonic stem cells was used to insert loxp sites flanking exon 8 and 9, including the polyadenylation sequence. Cre-mediated recombination destabilizes the mRNA transcript leading to nonsense mediated decay. (B) Western blot analysis shows Nmi knockout at multiple stages of mammary development. Note that Nmi protein expression is less in heterozygous ($Nmi^{+/-}$) compared to control ($Nmi^{fl/fl}$).

Supplemental Figure 2. Nmi ko causes precocious alveologenesis during mid-pregnancy. N=4 knock outs and 3 controls.

(A-B) Alveoli were counted in H&E sections, three fields per mouse were quantified and averaged before comparing the mean number of alveoli/field across phenotypes scale bar = 500 microns. Alveoli were defined as enclosed epithelial structures with envidence of lumen formation. Scale bar= 100 microns

Supplemental Figure 3. Preneoplastic regions in Nmi KO mammary glands exhibit invasive features.

(A) Whole mounted mammary glands of carcinogen treated Nmi knock out and control mice were examined for pre-neoplastic lesions in the mammary ductal tree after staining with carmine aluminum scale bar= 1000 microns inset = 100 microns. (B) The morphological characteristics of the border of each lesion was visualized with a higher magnification. Glands were also scanned for the presence of invasive preneoplastic lesions histlogically scale bar= 100 microns

Supplemental Figure 4. Nmi knock out inhibits luminal differentiation and activates Wnt Signaling

Schematic displaying the consequences of Nmi loss on luminal differentiation in MMTV-Neu and carcinogen-induced models. The mammary epithelial hierarchy is depicted with potential tumor initiating cells highlighted in either purple (MMTV-Neu) or blue (DMBA/MPA) boxes. Markers of initiating populations of the MMTV Neu model (CD49f+CD61+) and the DMBA/MPA model (CD29hiCD24+) are included.

Supplemental Figure 5. Effect of Conditional Nmi knock out on mammary development and tumor progression.

Within the mammary ductal tree of Nmi knock out mice the presence of luminal progenitor cells is increased resulting in a precocious alveolar development phenotype. These cells are marked by the markers CD61 and CD49f in MMTV-Neu nonparous mice. Luminal progenitors within the Nmi knock out mice exhibit extensive nuclear beta-catenin indicative of active Wnt signaling. Tumors formed from these hyperproliferative mammary epithelial cells are less differentiated and therefore more invasive leading to pulmonary metastasis in two spontaneous models breast cancer.