**Supplemental Information**

Supplemental Information includes Materials and Methods, 7 figures and 2 tables.

**Supplemental Materials and Methods**

***PCR to detect β-catenin exon 3 recombination***

YFP+ cells and CAFs were FACS sorted according to described procedures. Subsequently, the genomic DNA was isolated from these cells and a PCR reaction was performed on the genomic DNA using primers flanking exon 3 of the β-catenin gene***.*** The PCR products were analyzed on an agarose gel. Forward primer sequence 5’→3’: GGTACCTGAAGCTCAGCGCACAGCTG, Reverse primer sequence 5’→3’: ACGTGTGGCAAGTTCCGCGTCATCC

**Supplemental Figure Legends**

***Figure S1. CAFs are generated in the stroma of Wnt-Met mammary gland tumors.*** A. Immunofluorescence of tissue sections from control mammary glands and Wnt-Met tumors at two weeks post-partum for the mesenchymal markers vimentin (VIM) and smooth muscle actin (SMA) and the epithelial markers keratin 5 (K5) and keratin 8 (K8). B. Immunofluorescence of tissue sections from Wnt-Met reporter mice at 1 and 2 weeks post-partum for YFP and -CTN. C. qRT-PCR analysis of RNA from YFP- and YFP+ cells sorted from Wnt-Met tumors for the genes Axin2, Hgf and Wap. D. Immunofluorescence of tissue sections from Wnt-Met tumors expressing the reporter gene YFP at 1 and 2 weeks post-partum for YFP and the epithelial marker keratin 14 (K14). E. Immunofluorescence of YFP+ cells sorted from Wnt-Met tumors at two weeks post-partum for FN and K14. Scale bars, 25 μm.

***Figure S2. CD140b+CD90+ cells isolated from Wnt-Met mammary gland tumors exhibit CAF features.*** A. qRT-PCR analysis of RNA samples from YFP+ cells, control fibroblasts (COFs) and CAFs for mesenchymal and epithelial cell surface markers. B. Flow cytometry analysis of COFs and CAFs for CD140b and CD90. C. Immunofluorescence of tissue sections from Wnt-Met tumors at two weeks post-partum for CD140b, K5 and VIM. Scale bar, 25 μm. D. Scheme of the β-catenin gene showing exon 3 flanked by two lox-p sites (left panel); exon 3 is lost in cells with activated Cre recombinase (right panel). E. Agarose gel electrophoresis of genomic DNA from YFP+ cells and CAFs for recombined β-catenin gene: a 700 bp PCR product is amplified in the sequence flanking exon 3 of the β-catenin gene, reflecting Cre-induced recombination in YFP+ cells; no PCR product is obtained in CAFs, indicating absence of recombination.

***Figure S3. CAFs promote mammosphere formation and enhance the capacity of CSCs to form invasive 3D structures in Wnt-Met mammary gland tumors.*** A. Scheme of the co-culture system used to generate mammospheres. B. Flow cytometry analysis of YFP+ cells for the cell surface markers CD24 and CD49f. C. Immunofluorescent staining of tumor sections showing co-localization of nuclear β-catenin (CSCs) and the proliferative marker Ki-67 and nuclear β-catenin (CSCs) stained cells in close proximity to Vimentin positive stroma (CAFs). D. Scheme of the co-culture system used to generate 3D structures. E. Representative brightfield (upper panel) and fluorescence (lower panel) pictures of 3D structures generated by CSCs grown alone, with COFs or CAFs over a two weeks culturing period. Scale bars, 100 μm. F. Bar chart of average sizes of 3D structures generated by CSCs grown alone, with COFs or CAFs.

***Figure S4. CAFs from Wnt-Met mammary gland tumors display activation of Hedgehog signaling.*** A. Gene ontology analysis for biological processes of genes upregulated in CAFs with respect to COFs with a minimum 2.5-fold change. B. qRT-PCR validation of the CAF gene signature for the genes *Cxcl12*, *Mmp9*, *Spp1*, and *Tnc*. C. Heat map and grouping of genes differentially expressed between CAFs and CSCs in Wnt-Met tumors. D. Gene ontology analysis for molecular pathways selectively activated in CAFs with respect to CSCs. E. Immunofluorescence of tissue sections from control mammary glands and Wnt-Met tumors at two weeks post-partum for SHH and PTCH1. Scale bar, 25 μm. F. Bar chart of the relative distance of CSCs (CD49f high) and cells in the bulk of the tumor (CD49f low) of the tumor to CAFs.

***Figure S5. Hedgehog signaling regulates CAFs in Wnt-Met mammary gland tumors.*** A. Bar chart of relative cell growth of CAFs stimulated with increasing concentrations of SHH for 72 hours. B. Bar chart of relative fluorescence intensity of CAFs stimulated with SHH and stained for SMA. C. Bar chart of relative fluorescence intensity of CAFs stimulated with SHH and stained for FN.

***Figure S6. Therapeutic treatment of Wnt-Met mice with a Hedgehog pathway inhibitor depletes tumor stroma and prevents CSC expansion.*** A. Body weight of Wnt-Met mice treated with increasing concentrations of vismodegib over 16 days. B. Bar chart showing number of Ki-67 positive cells/acinus. C. Bar chart showing number of Ki-67 positive stromal cells/field. D. Bar chart of relative cell growth of CAFs sorted from untreated and Vismodegib-treated Wnt-Met tumors, as measured by MTT assay; VISMO: Vismodegib. E. Bar chart of average percentages of YFP+ cells in untreated and vismodegib-treated Wnt-Met tumors. F. Bar chart of average numbers of mammospheres generated by single cell suspensions from untreated and vismodegib-treated Wnt-Met tumors. G. Bar chart of average sizes of 3D structures generated by CSCs sorted from untreated and vismodegib-treated Wnt-Met tumors. H. Bar chart of average sizes of mammosphere treated with DMSO or increasing concentrations of vismodegib over a two weeks culturing period. I. Number of mammospheres formed by CSCs treated with DMSO or increasing concentrations of Hedgehog inhibitor vismodegib after 2 weeks growth. Data points are mean±SEM. ns, not significant. \*\*, p<0.006.

***Figure S7. Hedgehog signaling regulates the production of CAF ligands involved in the regulation of CSCs in Wnt-Met mammary gland tumors.*** A. Heat map of gene the expression profiles of CSCs (CD24+CD49fhi) and CD24+CD49flo cells that form the bulk of the tumor mass. B. qRT-PCR validation of the CSC gene signature for *Erbb3*, *Hgf*, *Itga6* and *K14* expression. C. qRT-PCR analysis of CAF ligands in CAFs vs. YFP+ cells. D. Expression of identified CAF ligands in the stroma of breast cancer patients. E. Bar chart of numbers of primary mammospheres untreated or grown for 1 week in the presence of IGF-1 (100 nM), ACTIVIN A (10nM), NOV (50nM), and LIF (1nM).