Materials and methods

Additional methods for qPCR. The oligonucleotide sequences of primers are listed in the supplementary data file. All data values were normalized using the geomean of three different housekeeping genes (HPRT, TBP, and Ywhaz; selected from a set of seven housekeeping genes using geNorm software (www.genequantification.com), and relative gene expression data were determined using the $2^{-\Delta\Delta Ct}$ method (1); $\Delta Ct_{(sample)} = Ct_{(sample)} - Ct_{(geomean\ of\ housekeeping\ genes)}$, $\Delta\Delta Ct_{(sample)} = \Delta Ct_{(sample)} - \Delta Ct_{(non\text{-}transgenic\ control\ sample)}$. The relative fold induction was calculated by dividing each value by average value of non-transgenic control samples.

Primer sets for qPCR assay

Gene name		Sequence
TATA box binding protein	mhTBP-F mhTBP-R	5'-accttatgctcagggcttg-3' 5'-tggtgttctgaataggctgtg-3'
Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	mhYwhaz-F mhYwhaz-R	5'-aagacagcacgctaataatgc-3' 5'-ttggaaggccggttaattttc-3'
Hypoxanthine guanine phosphoribosyl transferase 1	mhHPRT-F mhHPRT-R	5'-cctcatggactgattatggacag-3' 5'-aatccagcaggtcagcaaag-3'
Conductin/Axin2	mAxin2-F mAxin2-R	5'-gtctggcagtggatgttagag-3' 5'-gcacaggcagactccaat-3'
Lymphoid enhancer binding factor 1	mLef1-F mLef1-R	5'-gacggaggcctgtacaacaa-3' 5'-acatgtacgggtcgctgttc-3'
Wnt1-induced secreted protein 1	Wisp1-F Wisp1-R	5'-tgatgatgacgcaaggagac-3' 5'-gttctcataccgttgctccac-3'

All primers showed average 96±5% of PCR efficiency as measured by DART-PCR (2).

Supplemental Table 1

C**omparison of the kinetics of tumor development.** 40% of MMTV-Wnt1 transgenic mice develop tumors more quickly than in MMTV-ΔNβcat mice, starting over 11 weeks (82 days) earlier (onset defined (arbitrarily) as 10% of the total population). Thus, after 20 weeks, only 8% of *MMTV-ΔNβcat* mice were tumor bearing, compared to 40% of *MMTV-Wnt1* mice (Log Rank test, p==0.0046). Others show a substantially delayed pattern of progression (typically associated with *Ras* activation, data not shown), and together these two opposing effects lead to a complex tumor survival curve (Fig 1C).

Genotype	No. mice	Tumor onset time (10% of total cohort; days)	No. tumor-bearing mice at 20 weeks	Time for 80% mice to develop tumors (days)
MMTV-Wnt1	*48*	44	19	320
MMTV-ΔNβcatenin	*25*	126	2 *p*=0.0046	102

Supplementary Figure 1. Morphology of tumors. H&E stained tissue sections from Group 1 and 2 Wnt1-induced tumors. 60% of Group 2 Wnt1-induced tumors were associated with hemorrhagic cysts (*), whereas only 11% of Group 1 Wnt1-induced tumors displayed the blood lakes.

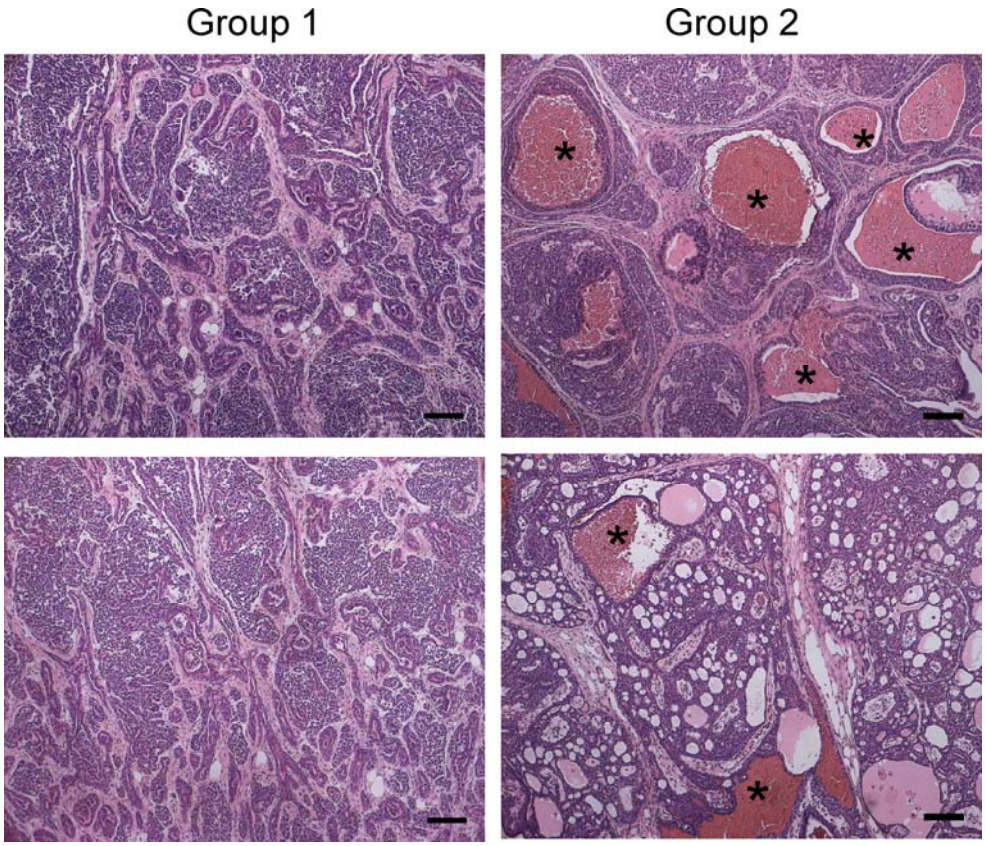
Supplementary Figure 2. Growth curves of NMFs. $2x10^4$ NMFs were cultured in either Wnt3a conditioned medium (CM) or control L cell CM. In this low serum (<2% FBS) condition, NMFs survived better in Wnt3a CM. Conditioned media were prepared as described previously (3).

Supplementary Figure 3. Quantification of microvessel density. (A) Measurement of $CD31^+$ area ($pixel^2$). CD31 stained sections were examined with 20x objective lens of confocal microscope, and pictures were taken with Lasersharp 2000 software (a). The images were processed by ImageJ software (1.37v) and converted to 8-bit binary black & white using process > binary > make binary tool (b). To determine the total area of marker-positive cells, the binary images were then analyzed for particles (size ($pixel^2$): 10-infinity, circularity: 0.00-1.00) (c). (B) Determination of the total number of nuclei. Nuclei were stained with TO-PRO3, and the images were converted to 8-bit black & white (a). The total number of white nuclei was counted using ImageJ plug-in software, ITCN (Center for Bio-Image Informatics at UC-Santa Barbara), with following settings; 10 pixel width, 5 pixel minimum distance, and 1.0 threshold. The counted nuclei were marked with red dot (b). Since the size of nuclei was not uniform in these tumors and some of nuclei had weak intensity because all cells are not on the same plane, some nuclei were counted twice while some were not counted (c; the magnified view of white box in

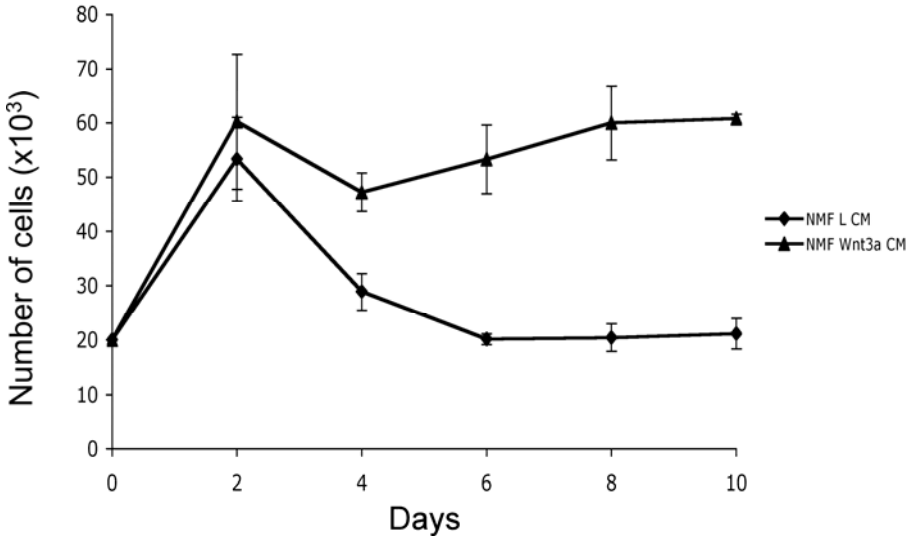
b). However, because that offsets each other, error range of the automatic counting was less than 10% compared to the manual counting (data not shown).

Supplementary Figure 4. Specificity of anti-WISP1 immunohistochemical localization. A representative sample of a tumor from a *MMTV-Wnt1* gland stained with anti-WISP1 is shown, together with a section from a *MMTV-ΔNβcat*-induced tumor (negative) and a control section from a Wnt1-induced tumor not incubated in primary antibody (biotinylated sheep anti-WISP1; see Materials and Methods).

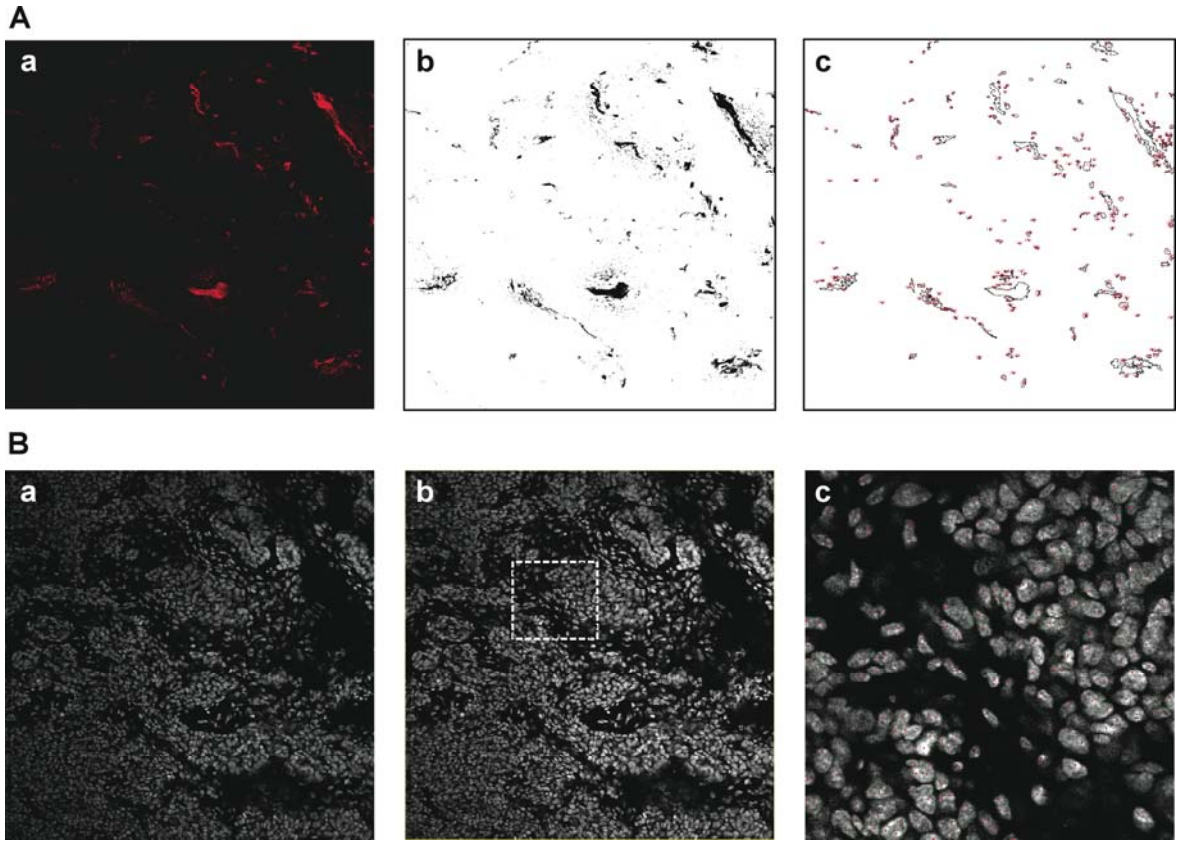
Kim et al. Supplemental Fig_1



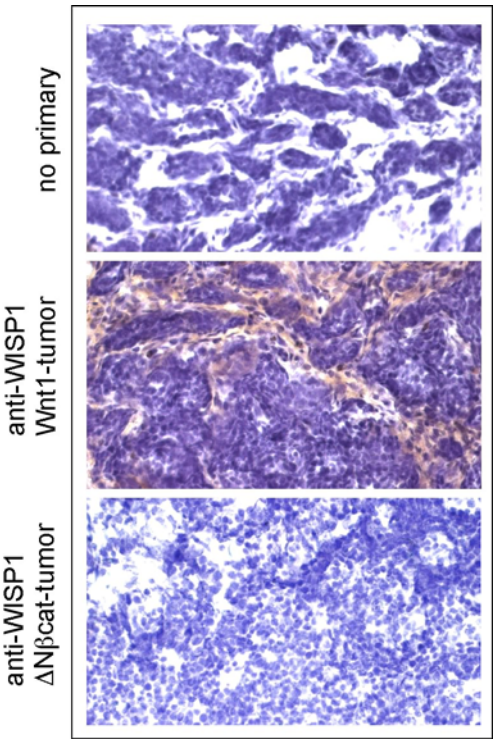
Kim et al. Supplemental Fig_2



Kim et al. Supplemental Fig_3



Kim et al. Supplemental Fig_4



References

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3. Liu BY, Kim YC, Leatherberry V, Cowin P, Alexander CM. Mammary gland development requires syndecan-1 to create a beta-catenin/TCF-responsive mammary epithelial subpopulation. Oncogene 2003; 22(58): 9243-53.