Additional file

Interplay between CCR7 and Notch1 axes promotes stemness in MMTV-PyMT mammary cancer cells.

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Figure S1. CCR7 does not regulate expression of Notch ligands

(a) Representative gating strategy for the delineation of the stem cell-enriched $CD24^{+}CD29^{hi}$ population as used in this project. Within each experiment, the $CD24^{+}CD29^{hi}$ population was either analyzed further by flow cytometric analysis, or sorted by FACS from the bulk tumor to analyze by Western or qPCR. **(b-c)** PyMT-*Ccr7*^{WT} and *Ccr7*$^{-/-}$ mammary tumors were analyzed by multi-color flow cytometry for expression of Notch ligands within the $CD24^{+}CD29^{hi}$ stem cell-enriched population. Top=representative flow cytometry histograms, bottom=quantification. **(b)** Cell surface expression of Notch ligand DLL1 in the PyMT-*Ccr7*^{WT} and *Ccr7*$^{-/-}$ mammary stem cell-like population. **(c)** Cell surface expression of Notch ligand Jagged1 in the PyMT-*Ccr7*^{WT} and *Ccr7*$^{-/-}$ mammary stem cell-like population. **(b-c)** n=4 mice/genotype. FMO=fluorescence minus one, MFI=mean fluorescence intensity.

Figure S2. Notch does not regulate expression of CCR7 or its ligands in mammary cancer stem cell-enriched culture

(a) Relative mRNA levels of Hes1 in PyMT-*Ccr7*^{WT} secondary mammospheres cultured with or without γ-secretase inhibitor RO4929097 to block Notch activation. **(b)** Relative mRNA levels of CCR7 in secondary mammospheres derived from PyMT-*Ccr7*^{WT} mammary tumors, cultured with or without RO4929097. **(c)** Cell surface expression of CCR7 on secondary mammospheres derived from PyMT-*Ccr7*^{WT} mammary tumors, cultured with or without RO4929097, as assessed by flow cytometry of mammosphere cells. Top=representative flow cytometry histograms, bottom=quantification. FMO=fluorescence minus one, MFI=mean fluorescence intensity. **(d)** Relative mRNA levels of CCR7 ligands CCL19 (left) and CCL21 (right) in secondary mammospheres derived from PyMT-*Ccr7*^{WT} mammary tumors, cultured with or without RO4929097. **(a-d)** n=6 mice/experiment.

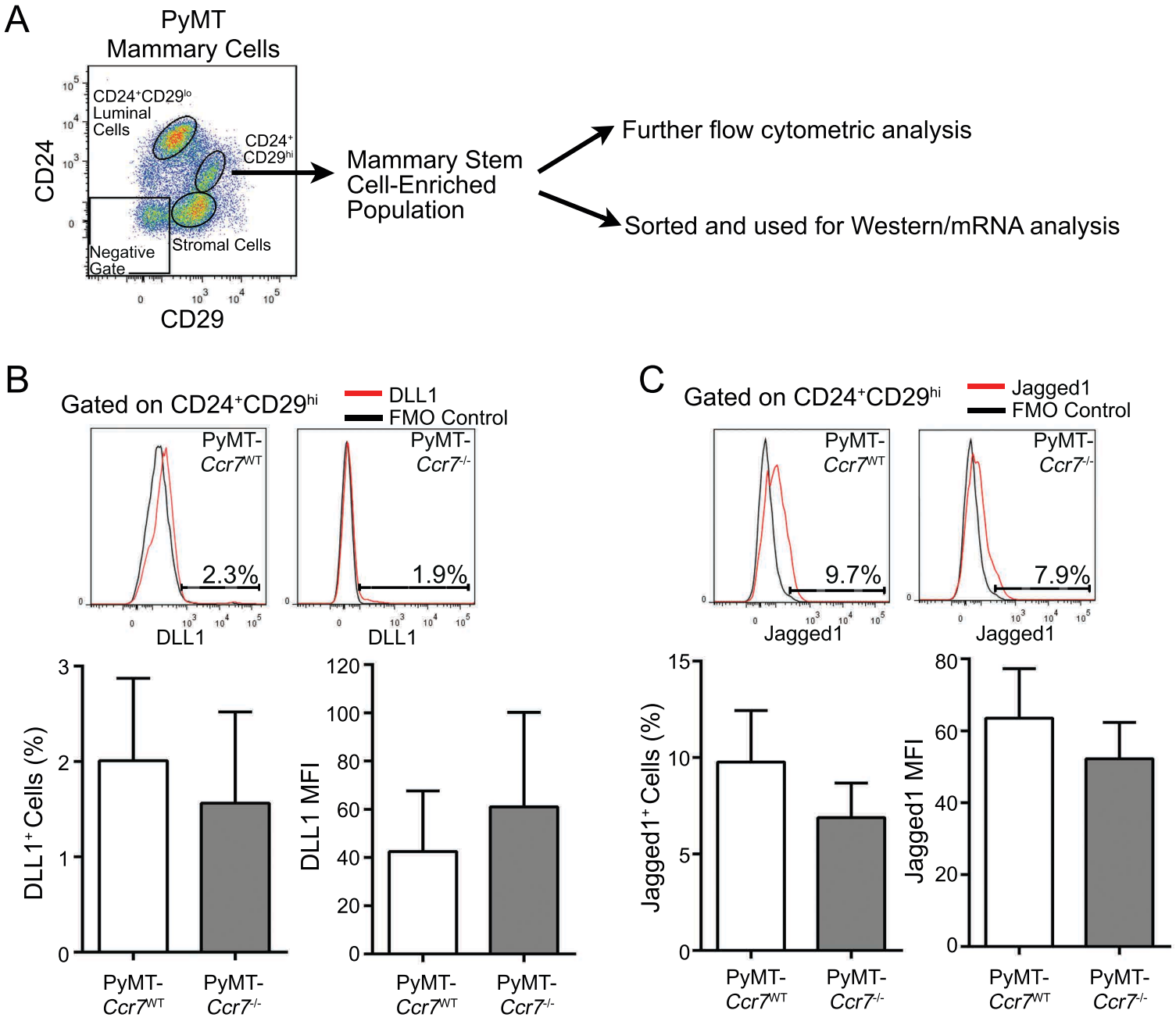
Figure S3. Wnt signaling inhibition dos not affect the ability of CCR7 to stimulate mammosphere formation.

Mammosphere-forming efficiency (number of spheres/cells seeded) of PyMT-*Ccr7*^{WT} primary mammary tumor cells cultured with and without chemokine CCL21 and Wnt inhibitor XAV-939. n=6 mice/experiment.

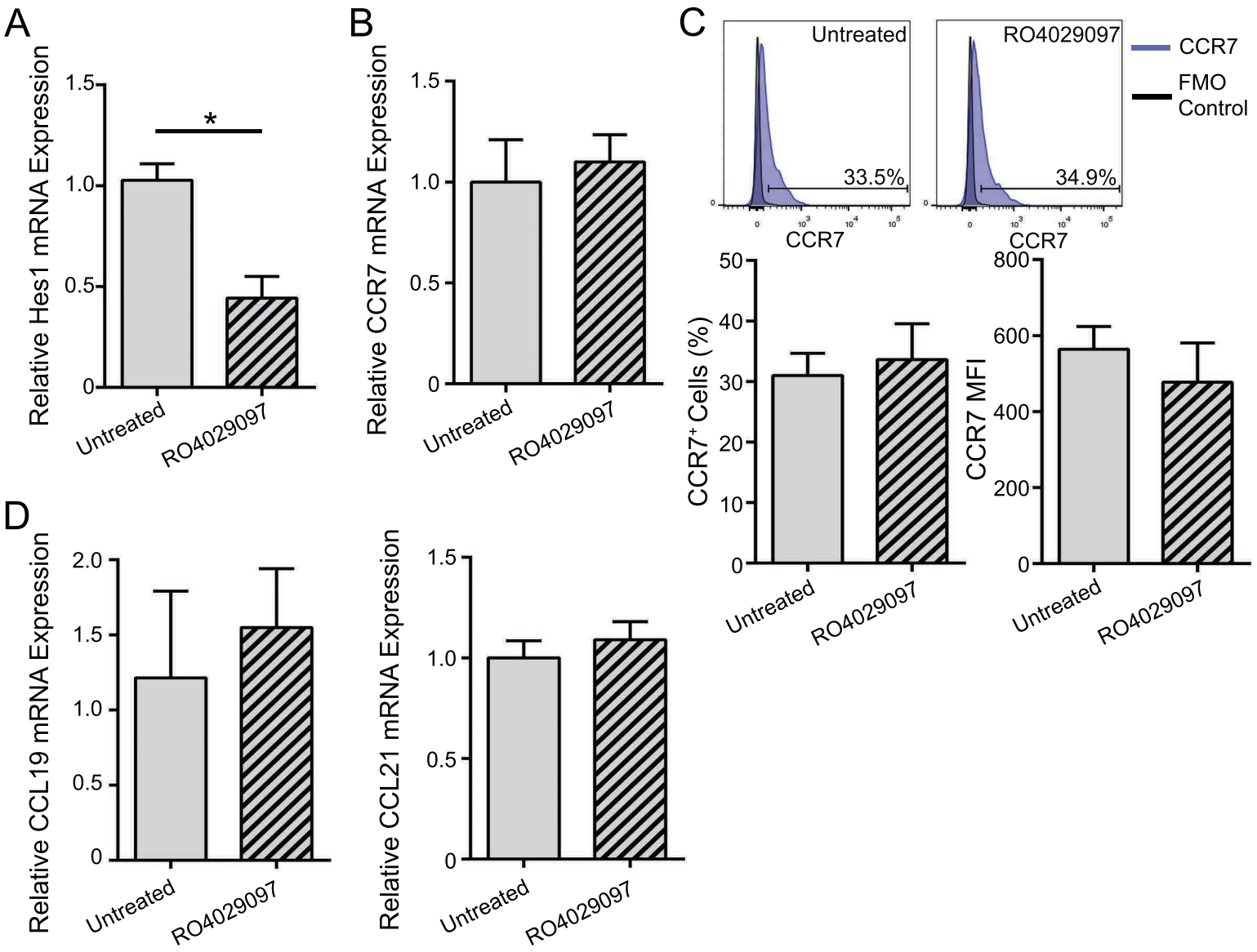
Figure S4. Correlation of CCR7 and Notch1 expression in primary human breast cancer.

CCR7 and Notch1 expression in various grades of breast cancer, analyzed using a published dataset available in Oncomine, consisting of 2,000 primary breast cancers. Significant associations were identified using Pearson correlation coefficients.

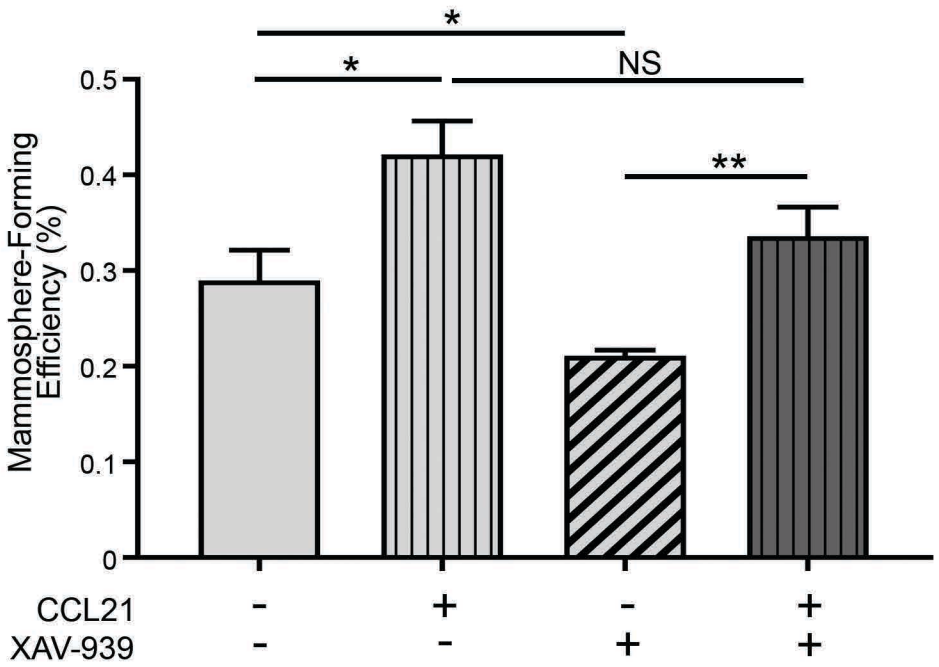
SUPPLEMENTARY FIGURE 1



SUPPLEMENTARY FIGURE 2



SUPPLEMENTARY FIGURE 3



SUPPLEMENTARY FIGURE 4