Supplemental figure S1. Jagged1 promotes EMT and stem cell phenotype through downregulation of miR-205. (A) miR-205/mRNA expression and (D) the percentage of CD24-CD44+ population of primary human mammary epithelial cells (HMEC), MCF12A and human primary breast tumor cells (PT) under treatment of 40µM control peptide or Jagged1 peptide for 1 day along with expression of the control vector or miR-205. (B) Protein (left) and miR-205/ZEB1 mRNA expression (right) of MCF12A cells expressing sh-Vec and sh-JAG1 (n=3, asterisk indicates P<0.05). (C) Correlation between the percentage of CD24-CD44+ population and the endogenous miR-205 expression level of HMEC, MCF12A and PT cells (n=3, asterisk indicates P<0.05). (E) The percentage of cells in each cell cycle phase of MCF12A cells expressing sh-Vec and sh-miR-205. Error bars denote ±SD.
Supplemental figure S2. Validation of NOTCH2 as an miR-205 target. (A) mRNA expression of putative miR-205 target genes identified by in silico analysis in MCF12A cells expressing sh-Vec and sh-miR-205 (n=3, asterisk indicates P<0.05). Error bars denote ±SD. (B) Protein expression of NOTCH2 (full length and NICD) and NOTCH4 in MCF12A cells that stably expressed sh-Vec or sh-miR-205. (C) Protein expression of NOTCH2 (full length and NICD) and NOTCH4 in breast cancer cells MDA-MB-231 and BT-549 that stably expressed the control vector or miR-205.
Supplemental figure S3. Activation of miR-205 signaling suppresses EMT and stemness phenotype in breast cancer cells. The number of primary spheres per 1000 cells from the control vector- and miR-205-expressed (A) MDA-MB-231 cells, (B) BT549 cells, and (C) human primary breast tumor cells (PT). (D) Expression of miR-205/mRNAs in MDA-MB-231, BT549, and PT cells that stably expressed miR-205. Expression of miR-205/mRNAs in (E) BT549 cells expressing si-NOTCH2 or (F) BT549 cells treated with 5 µM GSI for 1 day (n=3, asterisk indicates P<0.05). Insets showing full length NOTCH2 and NICD-NOTCH2 protein expression levels in (E) 1: BT549-sc, 2: BT549-siNOTCH2; (F) 1: BT549-DMSO, 2: BT549-GSI. Error bars denote ±SD.
Supplemental figure S4. Expression of miR-205 suppresses symmetric division of human primary breast tumor stem cells. (A) Confocal fluorescence images showing CD44 (red) and Numb (green) intracellular distribution during first cell division of the CD24^−CD44^high population that was isolated from human primary breast tumor cells cultured in suspension with the blebbistatin treatment (scale bar: 20 µm). (B) Pie chart showing the percentage of the symmetric vs. asymmetric cell division patterns of the cells expressing control vector and miR-205 (n=3, 70-110 cells were counted per sample, asterisk indicates P<0.05). (C) Pie chart (left) showing the percentage of the symmetric vs. asymmetric cell division, (D) NOTCH2/ZEB1 protein expression (right), and (E) mRNA expression levels of stemness-related or polarity genes of the cells expressing control vector or miR-205 co-expressing NICD-NOTCH2 or ZEB1 (n=3, asterisk indicates P<0.05).
Supplemental figure S5. ZEB1 transcriptionally represses polarity genes Llgl1 and Llgl2, leading to suppression of asymmetric division and promotion of symmetric division of mammary stem cells. (A) Diagram showing the promoter regions of Llgl1 and Llgl2 with the putative ZEB1 response elements (ZEB1-RE: A1, B1 in Llgl1; A2, B2 in Llgl2). (B) Chart showing the ChIP-PCR result with the percentage of the bound chromatin/input chromatin using ZEB1 antibody targeting ZEB1 binding elements (A1, B1, A2, B2) in MCF12A cells. IgG was used as a negative control. (n=3, asterisk indicates P<0.05). (C) Fold change in luciferase activity driven by the wild-type or mutant Llgl1 and Llgl2 reporters (with mutated A1 or B2, respectively) under expression of ZEB1 in MCF12A cells (n=3, asterisk indicates P<0.05). (D) The percentage of the symmetric vs. asymmetric cell division (upper) and protein expression of Llgl1 and Llgl2 (lower) of the CD24-CD44high-MCF12A cells expressing sh-Vec or sh-Llgl1+sh-Llgl2 (n=3, 70-110 cells counted per sample, asterisk indicates P<0.05).
Figure S6. In vivo GSI treatment suppresses the development of mouse mammary premalignant and malignant lesions caused by downregulation of miR-205. (A) Representative miR-205/NOTCH2 staining with H&E of the cropped regions of the control and miR-205 knock-down mammary tissues, and (B) the percentage of lin-CD24+CD29high population treated with DMSO or GSI for 2 weeks at three weeks after virus introduction (black scale bar: 100µm, blue scale bar: 30µm, n=5/group). (C) Representative NOTCH2 staining (scale bar: 50 µm), (D) the percentage of observed mammary carcinoma, and (E) the number of tumor spheres per 1000 cells isolated from miR-205 knock-down mammary tumors treated with DMSO or GSI for 2 weeks at three months after virus introduction (n=5/group, asterisk indicates P<0.05). Error bars denote ±SD.