Supporting Information

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SI Text

Endocytosis and Recycling Assays. To assay endocytosis, OP9 cells stably expressing one of the three Dll1 derivates were labeled on ice for 40 min with the reversible reagent NHS-SS-biotin (0.5 mg/ml in PBS). After a 20 min incubation at 37°C, biotin was stripped by three 25-min incubations with 50 mM MesNa (sodium 2-mercapto-ethanesulfonate) in TNEB buffer (20 mM Tris, pH 8.3; 150 mM NaCl; 1 mM EDTA; 0.2% BSA) on ice. Cells were then lysed in RIPA buffer (50 mM Tris, pH 7.5; 150 mM NaCl; 1% Triton X-100; 0.1% SDS; 0.5% DOC), and biotinylated species were purified on streptavidin-agarose and analyzed by immunoblotting with anti-Dll1CT or anti-Dll3ic antibody. To assay recycling, OP9 cells stably expressing a Dll1 derivate were labeled on ice with NHS-SS-biotin and incubated for 20 min at 37°C. Biotin was stripped with MesNa as described above. Cells were incubated for 10 min on ice with 5 mg/ml iodoacetamide in TNEB. After a second incubation at 37°C for 10, 20, or 30 min, cells were treated or not with MesNa and lysed in RIPA buffer, and biotinylated species were purified on streptavidin-agarose and analyzed by immunoblotting with anti-Dll1CT or anti-Dll3ic antibody. Immunoreactivity was quantified using Quantity One software (Bio-Rad Laboratories). For each time point, the amount of recycled ligand was estimated by subtracting the amount measured in MesNa-treated cells from that in non-treated cells and expressed as a percentage of endocytosed ligand.

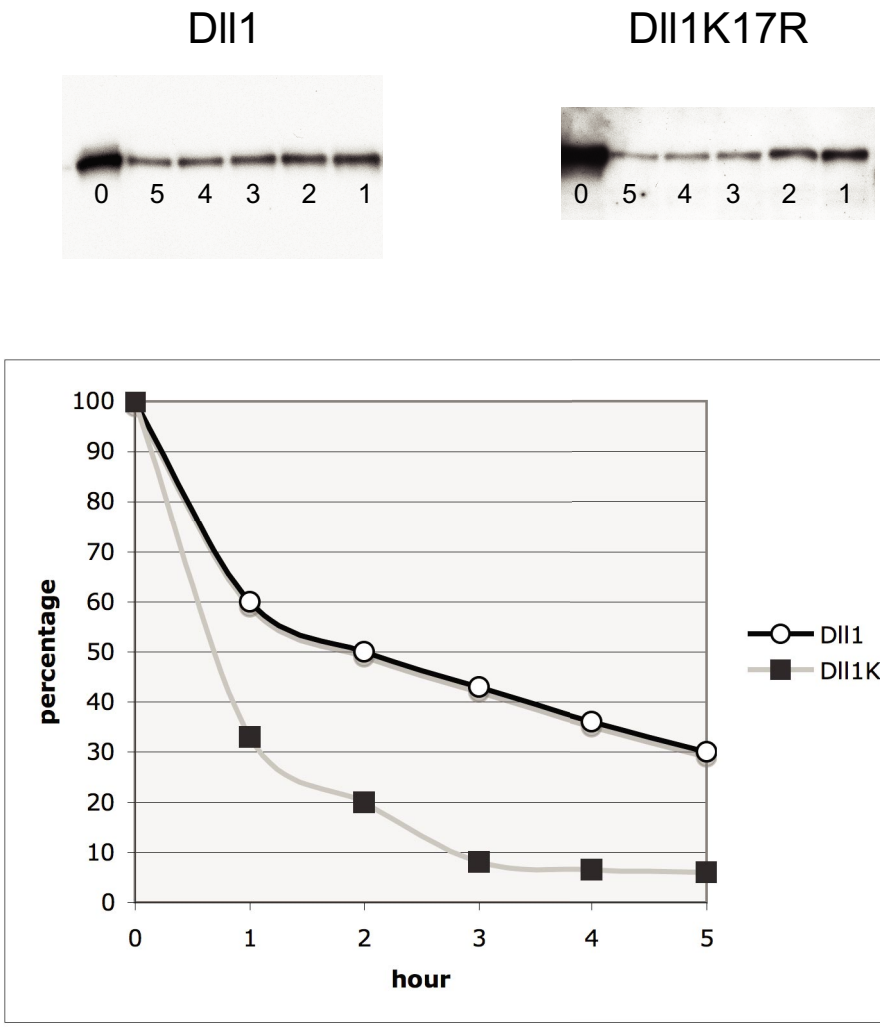

Fig. S1. Measurement of half-life of surface biotinylated Dll1 and Dll1K17R. OP9 cells expressing Dll1 or Dll1K17R were biotinylated at 4°C and shifted to 37°C at 1, 2, 3, 4, or 5 h as indicated. Whole-cell extracts were incubated with streptavidin-agarose, and biotinylated species were analyzed by immunoblotting with anti-Dll1CT antibody. Quantitation was performed after scanning of the film using Quantity One program. The percentage of biotinylated ligands at a given time point vs. that at time 0 is shown.

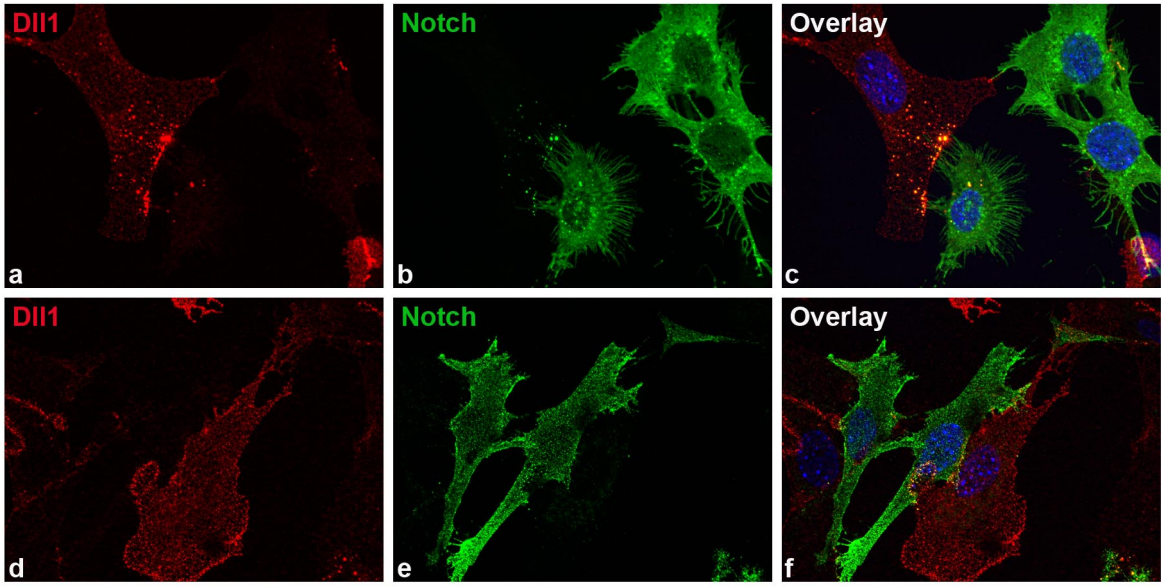

Fig. S2. Notch1 is taken up by Dll1-expressing cells. OP9 cells expressing VSV-tagged Dll1 were cocultured with MEFs expressing HA-tagged Notch1 for 24 h. Both tags are in the extracellular domain of the molecules. After fixation, cells were permeabilized with detergent (*A*–*C*) or not (*D*–*F*). Dll1 was detected using a Cy3-coupled anti-VSV antibody (red), and Notch1 was detected using an Alexa488-coupled anti-HA antibody (green). Hoechst staining is shown. Overlay: superposition of the two stainings.

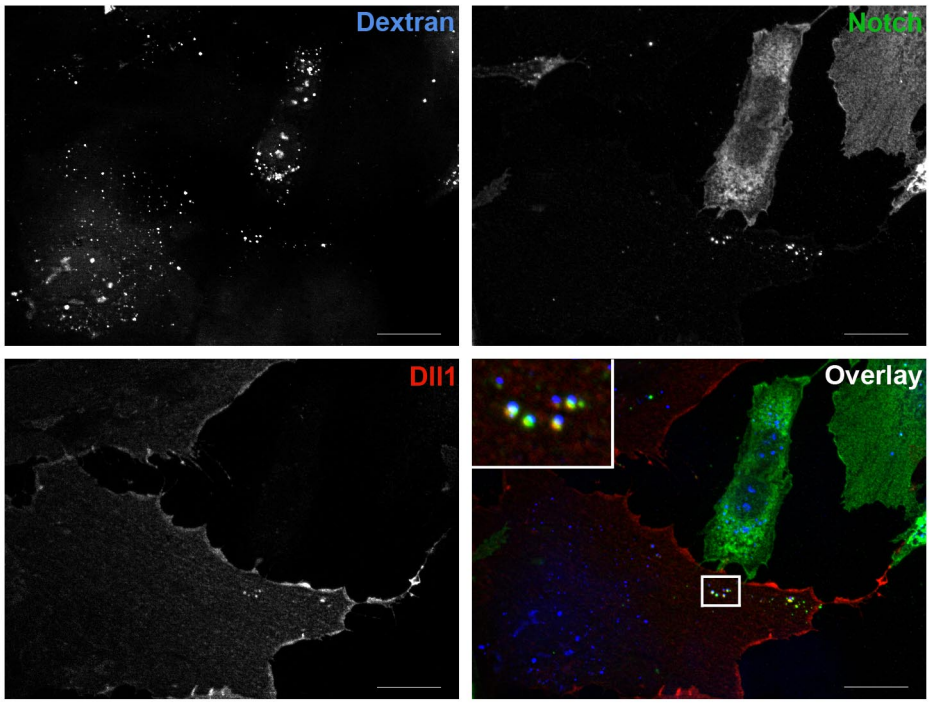

Fig. S3. Notch1 is found associated with endocytic vesicles in Dll1-expressing cells. OP9 cells expressing VSV-tagged Dll1 were cocultured with MEFs expressing HA-tagged Notch1 for 24 h. Cells were incubated for 4 h at 37°C in the presence of 4 mg/ml of Cascade Blue dextran (Molecular Probes) (blue in overlay panel). Then cells were washed, fixed, and permeabilized. Dll1 (red) was visualized with a rabbit anti-Dll1CT and an Alexa647-coupled secondary antibody (Molecular Probes). Notch (green) was detected with a mouse anti-HA antibody (HA-11, Covance) and a Cy3-coupled secondary antibody (Sigma). Overlay: superposition of the three stainings. Insert represents an enlarged view (12-fold) of the boxed region. (Scale bar, 20 μm.)