Supplementary Data accompanying:

Regulated proteolysis of NOTCH2 and NOTCH3 receptors by A-Disintegrin-And-Metalloproteinase (ADAM) 10 and Presenilins

Figure Legends, Table T1 and Figures 1-3

Supplementary Figure S1 Relative Dll4 mRNA expression in TSt-4/Dll4 cells, DSL ligand-dependent activation of endogenous NOTCH2 in HEK293 cells and NOTCH3 activation by Dll4-Fc

(A) Relative Dll4 mRNA expression in TSt-4 and Dll4 overexpressing cells. Experiments were performed in triplicate. Error bars represent mean + standard deviation. **(B)** Co-culture of HEK293 cells with DSL ligand overexpressing cells in the presence or absence of GSI. Lysates were immunoblotted and probed for endogenous NOTCH2. In response to ligand stimulation, NOTCH2 TMIC levels were diminished and accumulation of N2EXT could be observed in the presence of GSI. Ligand-dependent endogenous NOTCH2 activation and proteolytic processing is identical to that of exogenous NOTCH2 in Figure 1. **(C)** U2OS NOTCH3-HA cells cultured on top of coated Dll4-Fc molecules in the absence or presence of GSI. Upper panel: HA immunoblotting showed both the full length (FL) and the S1 processed TMIC form of NOTCH3. Dll4-FC stimulation led to diminished TMIC levels and N3EXT accumulation in the presence of GSI. Lower panel: Lamin A/C serves as a loading control (Ctrl). Molecular are weights indicated in kDa.

Supplementary Figure S2 Functional characterization of Adam10 and Adam17 reconstituted Adam10/17dKO mEF

(A) Relative Adam10 and Adam17 mRNA expression levels in Adam10/17dKO cells, reconstituted with either Adam10 (A10) or Adam17 (A17). Increased mRNA expression of Adam10 was detected in cells reconstituted with Adam10, whereas mRNA expression of Adam17 was detected in cells reconstituted with Adam17. Experiments were performed in triplicate. Error bars represent mean + standard deviation. **(B)** Relative Hey1 mRNA expression in reconstituted Adam10/17dKO-JAGGED2 cells, complementary to Figure 4B. Hey1 mRNA expression was increased only in cells expressing Adam10 and not in Adam17 expressing cells. GSI treatment completely blocked induced Hey1 mRNA expression. Experiments were performed in triplicate. Error bars represent mean + standard deviation. **(C)** Control immunoblots of monotypic cell cultures that complement Figure 4A. Endogenous Notch2 and Notch1 receptors were not cleaved in these cultures, indicated by the absence of GSI dependent accumulation of N2EXT and total absence of S3 cleaved Notch1 (N1ICD at Val1744). **(D)** Immunoblottin against Notch2 on lysates from Adam10/17dKO cells, reconstituted with either Adam10 or Adam17 cultured with recombinant Dll4-Fc molecules. N2EXT could only be accumulated in the presence of Adam10 and GSI. **(D)** Immunoblot for endogenous Notch2 on lysates from Adam10/17dKO cells, reconstituted with either Adam10 or Adam17 cultured with recombinant Dll4-Fc molecules. N2EXT could only be accumulated in the presence of Adam10 and GSI. Molecular weights are indicated in kDa.

Supplementary Figure S3 Depletion of ADAM10 and ADAM17 in U2OS cells does not influence ligand-dependent NOTCH3 turnover but prevents downstream signaling through N3EXT

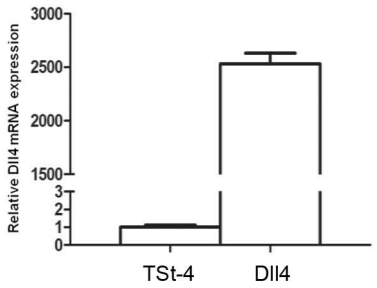
(A) Co-culture experiment of NOTCH3-HA expressing U2OS scrambled (SCR) or U2OS double knock-down for ADAM10 and ADAM17 (dKD) with Dll1 expressing cells compared to OP9 cells. Percentage of KD for both ADAMs is given compared to U2OS SCR cells. HA immunoblotting showed decreased TMIC levels in response to Dll1 and N3EXT accumulation was only observed in U2OS SCR cells, in the presence of GSI, but not in U2OS dKD cells. Molecular weights are indicated in kDa. **(B)** Firefly luciferase transcriptional Notch CSL reporter activity, corrected for Renilla luciferase, in U2OS SCR or dKD cells. Co-culture with OP9-Dll1 cells induces NOTCH3 dependent CSL activity which could be blocked by GSI in U2OS SCR cells. dKD cells showed strongly reduced ligand-dependent induced NOTCH3 CSL activity. Experiments were performed in triplicate and measurements are displayed relative light units (RLU). Error bars represent mean ± standard deviation.

Supplementary Table T1 q-PCR primers used in this study

hADAM10qRTfor	5`-TCCACAGCCCATTCAGCAA-3`
hADAM10qRTrev	5`-GCGTCTCATGTGTCCCATTTG-3`
hADAM17qRTfor	5`-GAAGTGCCAGGAGGCGATTA-3`
hADAM17qRTrev	5`-CGGGCACTCACTGCTATTACC-3`
mADAM10qRTfor	5`-AGCAACATCTGGGGACAAAC-3`
mADAM10qRTrev	5`-TGGCCAGATTCAACAAAACA-3`
mADAM17qRTfor	5`-GTACGTCGATGCAGAGCAAA-3`
mADAM17qRTrev	5`-AAACCAGAACAGACCCAACG-3`

Supplementary Figure S1

A



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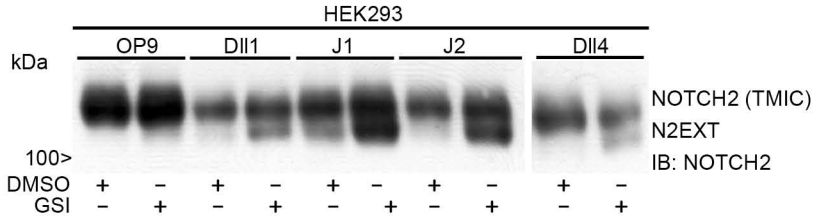

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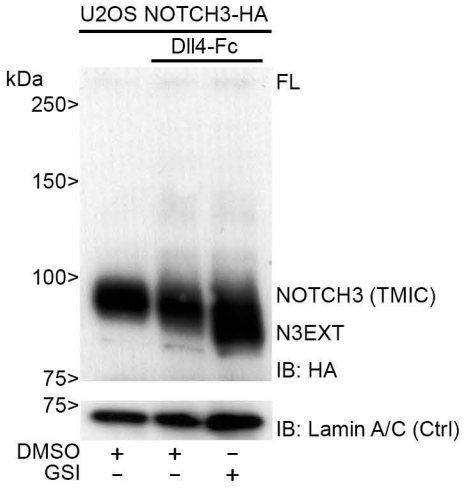
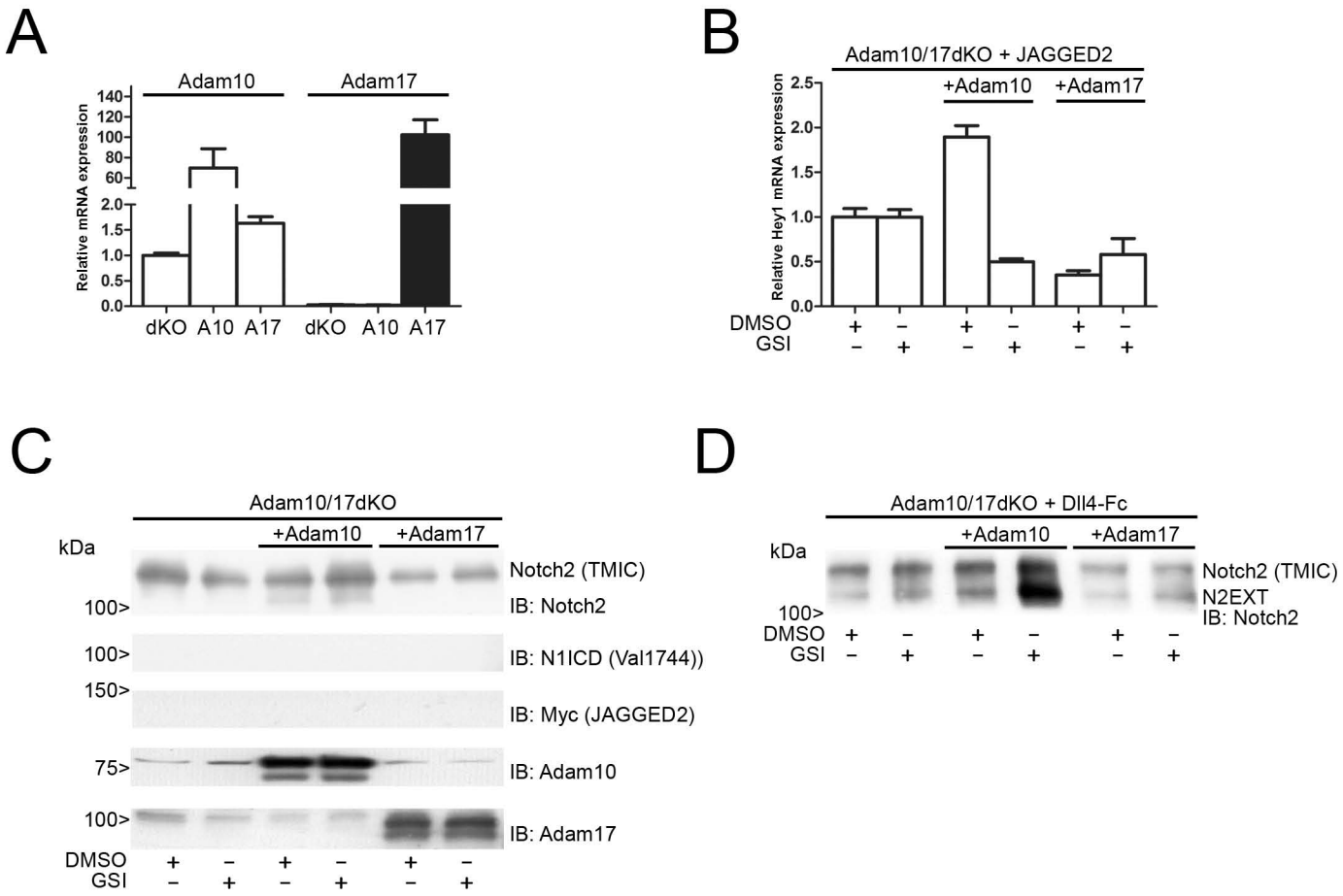
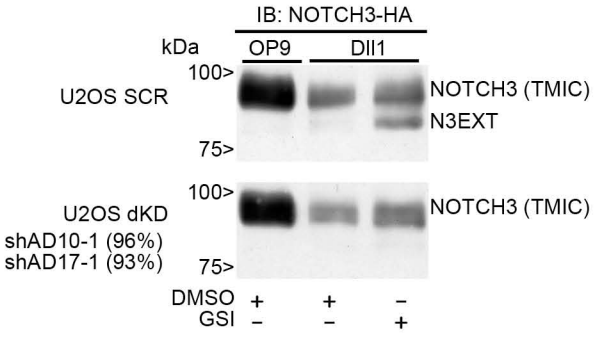


Supplementary Figure S2



Supplemetary Figure S3

A



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