Expanded View Figures

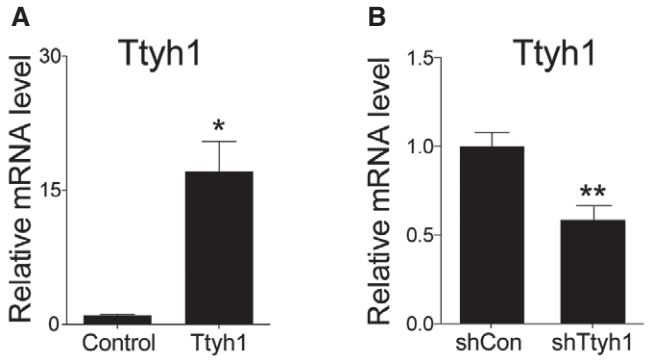

Figure EV1. mRNA levels of Ttyh1 after gene manipulation in primary mouse neural progenitor cells *in vitro*.

A, B Two days after E14.5 primary neural progenitor cells were transduced with retroviral vectors expressing (A) Ttyh1 or (B) shTtyh1, mRNA expression levels of Ttyh1 were measured by qPCR. Error bars represent SD. Student's *t*-test was used to determine statistical significance. $^{*}P < 0.05$, $^{**}P < 0.01$, $n = 3$ for (A), $n = 4$ for (B).

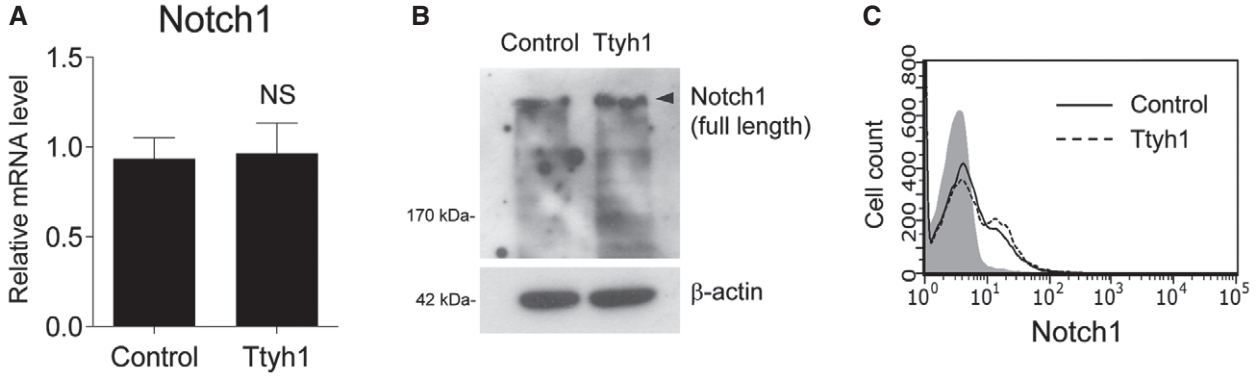

Figure EV2. Notch1 receptor expression was not changed by Ttyh1 overexpression.

A, B Two days after E14.5 primary neural progenitor cells were transduced with retroviral vectors expressing Ttyh1, Notch1 mRNA (A) and protein (B) levels were assessed by qPCR. Error bars represent SD. Student's *t*-test was used to determine statistical significance. NS, not significant. $n = 3$ for (A).

C E14.5 neural progenitor cells were transduced with Ttyh1 retroviruses. At 2 days posttransduction, a representative FACS histogram showing cell surface levels of Notch1 was obtained using an anti-Notch1 antibody (Invitrogen, MA5-11961). The lightly shaded curve represents the secondary antibody-only control.

Source data are available online for this figure.

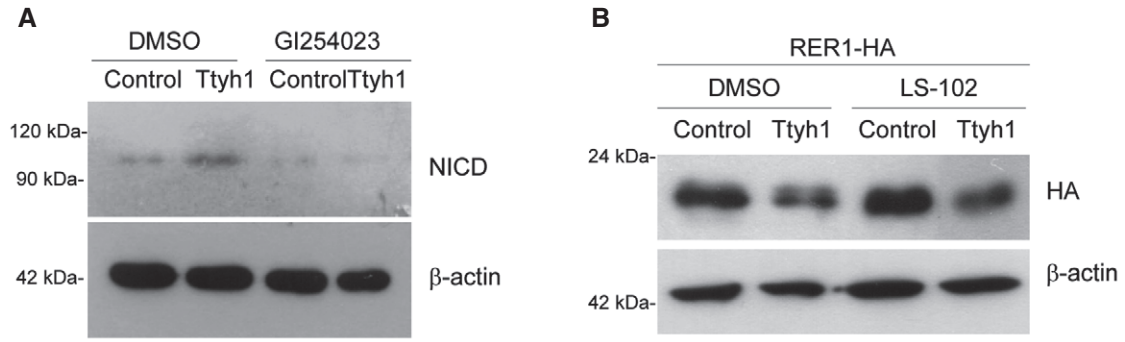

Figure EV3. Effects of ADAM10 inhibition on Ttyh1-enhanced NICD production and effects of synoviolin (Syvn) inhibition on Ttyh1-induced Rer1 reduction.

A Ttyh1-transduced E14.5 neural progenitors were incubated with or without an ADAM10 inhibitor, GI254023, for 2 days, then cells were harvested for Western blot analysis using anti-NICD antibody.

B HEK293T cells were transfected with Ttyh1-Myc and Rer1-HA expression plasmids and treated with a Syvn inhibitor LS102. At 2 days posttransfection, cells were harvested and analyzed by Western blotting using anti-HA antibody.

Source data are available online for this figure.

A

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Human              LPRAWALFPPSDDYDDTDDDDPFNPQ-ESKRFVQWQSSI
Horse              LPRAWALFPPSDDYDDTDDDDPFNPQ-ESKRFVQWQSSI
Cattle             LPRAWALFPPSDDYEDTDDDDPFNPQ-ESKRFVQWQSSI
Dog                LPRAWALFPPSDDYDDTDDDDPFNPQ-ESKRFVQWQSSI
Rat                LPRAWALFPPSDDYDDTDDDDPFNPQ-ESKRFVQWQSSI
Mouse              LPRAWALFPPSDDYDDTDDDDPFNPQ-ESKRFVQWQSSI
Chimpanzee         LPRAWALFPPSDDYDDTDDDDPFNPQQESKRFVQWQSSI
Horse              LPRAWALFPPSDDYDDTDDDDPFNPQQESKRFVQWQSSI
                   **************:********** ************
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B

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mTtyh1          LPRAWALFPPSD-DYDDTDDDDPFNPQES------------KRFVQWQSSI---------
mTtyh2          GPRAWKYFINRDRDYDDIDDDDPFNPQARRIAAHNPTRGQLHSFCSYSSGLGSQCSLQPP
mTtyh3          IPHTWQQKRGPDDDGEEETAPGPRQAHDSLYRVHMP------SLYSCGSSYGSEASIPAA
                 *::*      * * ::    .* :.:                : .  *.

mTtyh1          ------------------------------------------------------------
mTtyh2          SQTISNAPVSEYMNQAILFGGNPRYENVPLIGRGSPPPTYSPSMRPTYMSVADEHLRHYE
mTtyh3          AHTVSNAPVTEYITPPA-----------------------------------------

mTtyh1          ---
mTtyh2          FPS
mTtyh3          ---
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Figure EV4. Protein sequence comparison of mammalian Ttyh C-terminal tail regions.

A Alignment of Ttyh1 C-terminal tail regions from cattle (GenBank accession number NM_001077015), chimpanzee (XM_512893), dog (XM_857514), horse (XM_023650408), human (NM_001005367), mouse (NM_001109765), and rat (NM_001106225).

B Protein sequence analysis of C-terminal tail regions of mouse Ttyh1 (NM_001109765), Ttyh2 (NM_053273), and Ttyh3 (NM_175274).

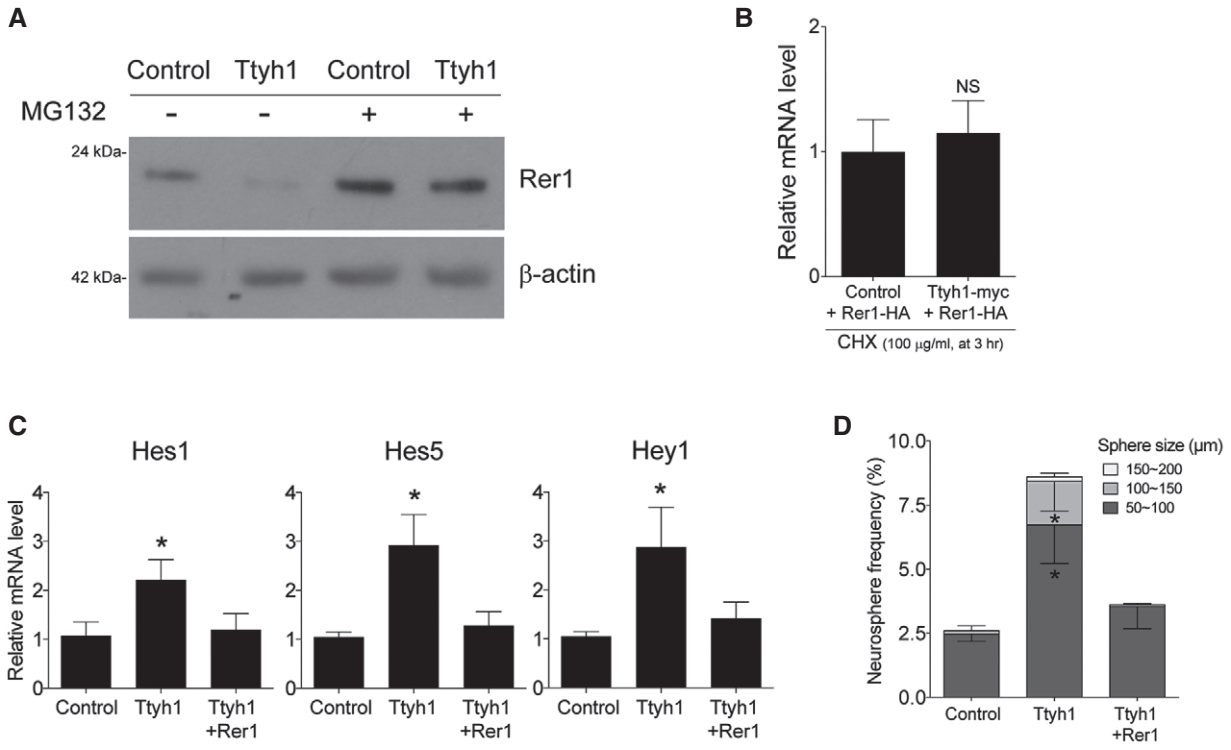

Figure EV5. Ttyh1 downregulates Rer1, and Rer1 expression abolishes Ttyh1-induced enhancement of the Notch signaling pathway.

A Cell extracts from primary neural progenitor cells transduced with Ttyh1 retroviruses in the presence of the DMSO vehicle or the proteasome inhibitor MG132 (5 μM) were immunoblotted with an antibody to Rer1.

B qPCR analysis for Rer1-HA mRNA was performed using samples corresponding to lane 4 and lane 8 in Fig 5I (n = 4).

C, D E14.5 neural progenitor cells transduced with the indicated retroviruses were used for (C) qPCR analysis of Notch target mRNAs at 2 days posttransduction or (D) cultured for 7 days for the neurosphere assay. n = 4 for (C) and n = 3 for (D)

Data information: Error bars represent SD. Student's t-test was used to determine statistical significance. *$P < 0.05$. NS, not significant.
Source data are available online for this figure.