Expanded View Figures

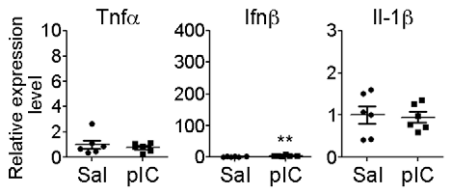

Figure EV1. *Trif*-mutant neurons lose the response to poly(I:C) stimulation.

At 5 DIV, *Trif*-mutant neurons were treated with poly(I:C) for 6 h, RNAs were extracted, and the expression levels of *Tnfa*, *Ifnb*, and *Il1b* were examined by using quantitative RT-PCR. The scales of *y*-axes are identical to those in Fig 4A for the responses of wild-type neurons to poly(I:C) and saline control. For *Ifnb*, the expression levels in response to poly(I:C) decreased dramatically from 100-fold to 4-fold relative to *Hprt* transcripts when comparing wild-type and *Trif*-mutant neurons; although, compared with saline control, poly(I:C) still increased *Ifnb* expression in *Trif*-mutant neurons. Data were analyzed by unpaired *t*-test. $N = 6$. Mean values ± SEM are shown. $^{**}P < 0.001$.

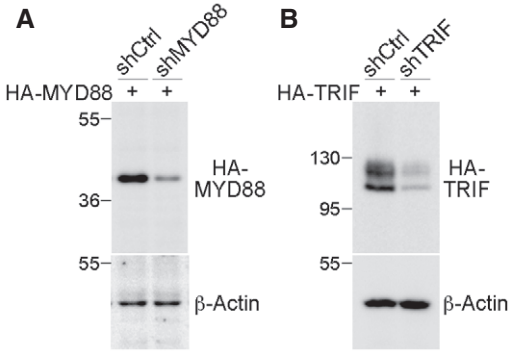

Figure EV2. The knockdown efficiency of MYD88- and TRIF-knockdown constructs.

A, B Non-silencing control shRNA (shCtrl), MYD88 shRNA (shMYD88), or TRIF shRNA (shTRIF) was cotransfected with HA-tagged MYD88 or HA-TRIF into HEK293T cells. Twenty-four hours later, total cell extract was collected and subjected to immunoblot analysis. The effects of (A) shMYD88 and (B) shTRIF are shown. Immunoblotting was performed with HA tag and β-actin antibodies. β-Actin was used as a loading control. Molecular mass standards (kD) are indicated next to the gel blots.

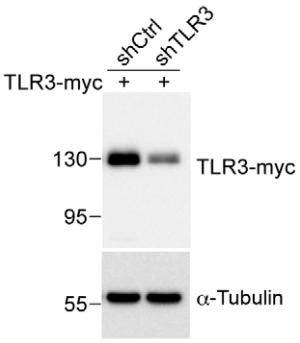

Figure EV3. The effect of the TLR3-knockdown construct on TLR3 expression.

Control shRNA (shCtrl) or TLR3 shRNA (shTLR3) was cotransfected with Myc-tagged TLR3 into HEK293T cells. Total cell extract was collected 24 h later and subjected to immunoblot analysis. Immunoblotting was performed with Myc tag and α-tubulin antibodies. α-Tubulin was used as a loading control.

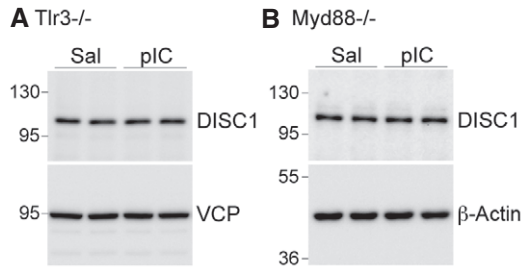

Figure EV4. Poly(I:C) does not alter DISC1 protein levels in *Tlr3*$^{-/-}$ neurons or *Myd88*$^{-/-}$ neurons.

A, B At 5 DIV, *Tlr3*$^{-/-}$ (A) and *Myd88*$^{-/-}$ (B) neurons were treated with poly(I:C) for 24 h. The total neuronal lysates were subjected to immunoblotting with the indicated antibodies. VCP and β-actin were used as loading controls.

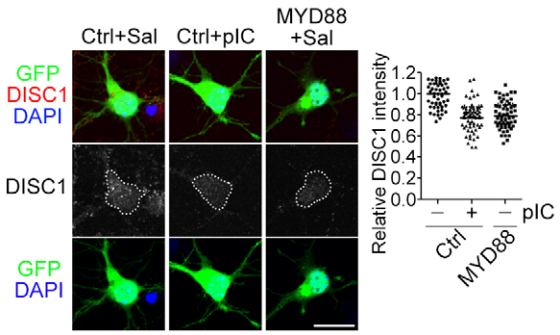

Figure EV5. Poly(I:C) treatment and MYD88 overexpression reduce the DISC1 protein levels in neurons.

Neurons were transfected with control vector (Ctrl) or HA-MYD88 (MYD88) with a GFP construct at 4 DIV. One day later, neurons were treated with saline or 10 μg/ml poly(I:C) for 24 h. Neurons were fixed and immunostained with DISC1 antibody at 6 DIV. Counterstaining with DAPI was performed to label the nuclei. DISC1 signal within the soma region (white dashed line as indicated) was quantified using ImageJ. The data were reproduced in two independent experiments. Only one set of representative data is shown. The dots indicate the relative DISC1 intensities of individual cells. The bars indicate mean of each group. Data were analyzed by one-way ANOVA followed by Bonferroni's multiple comparison test. Ctrl − pIC versus Ctrl + pIC, $P < 0.0001$; Ctrl − pIC versus MYD88 − pIC, $P < 0.0001$. Scale bar, 20 μm.