SUPPLEMENTAL FIGURE LEGENDS

Figure S1, related to Figure 4. JAM-A is dispensable for reovirus neural infection. Primary murine cortical neuron cultures prepared from wildtype JAM-A$^{+/+}$ or JAM-A$^{-/-}$ embryos were absorbed with reovirus T3D at an MOI of 10 PFU/cell. Titers of virus in cell lysates at the indicated intervals were determined by plaque assay using L929 cells. Results are expressed as mean viral yields. Error bars indicate SD. (B) Primary murine embryonic fibroblasts or (C) cortical neurons derived from JAM-A$^{+/+}$ or JAM-A$^{-/-}$ embryos were pretreated with PBS, a CAR-specific antibody, or increasing concentrations of JAM-A-specific antibody prior to adsorption with reovirus T3D at an MOI of 100 or 1000 PFU/cell, respectively. Cells were fixed at 20 h and scored for reovirus antigen using indirect immunofluorescence. Results are expressed as the mean FFU/field for three fields of view in triplicate samples of confluent fibroblast monolayers. FFU/field was normalized to the total number of cells per three fields of view in triplicate samples of neurons. Error bars indicate SD. ***, $P < 0.0005$ (as determined by Student's *t* test in comparison to PBS-treated infected cells).

Figure S2, related to Figure 1. Identification of NgR1 as a cellular mediator of reovirus-induced cytotoxicity. HeLa S3 cells were transfected with nonspecific, control, or siRNAs specific for 18,055 human genes. After 48 h incubation, cells were infected with reovirus strain T3SA+ at an MOI of 1000 PFU/cell. After an additional 48 h incubation, cell viability was determined using an ATP-dependent luminescent assay. Luminescence was quantified, and robust Z scores (median absolute deviation) were calculated for individual samples. Shown is the average robust Z score for the top 25

cellular candidates of reovirus-induced cytotoxicity identified in three independent screening experiments. NgR1 is highlighted in red.

Figure S3, related to Figure 1. Binding to sialic acid enhances but is not required for NgR1-mediated infectivity. CHO cells were either mock transfected or transiently transfected with plasmid encoding NgR1 or JAM-A for 48 h. Cells were treated with either PBS or 40 mU/mL *A. ureafaciens* neuraminidase (NA) for 1 h prior to absorption with reovirus T3D or T3SA- at an MOI of 10 PFU/cell. Cells were fixed at 20 h and scored for reovirus antigen using indirect immunofluorescence. Results are expressed as the mean FFU/field for three fields of view in triplicate samples. Error bars indicate SD. **, $P < 0.005$; ***, $P < 0.0005$ (as determined by Student's *t* test in comparison to PBS-treated infected cells).

Figure S4, related to Figure 3. Cleaved NgR1 is detected in culture supernatants following PI-PLC treatment. CHO cells were mock transfected or transiently transfected with plasmid encoding NgR1 or JAM-A. After 48 h, GPI-anchored proteins were removed from the cell surface by incubating cells with various concentrations of PI-PLC at 37°C for 1 h. To detect soluble NgR1 cleaved from the cell surface following PI-PLC treatment, culture supernatants (15% of total volume) were resolved by SDS-PAGE and immunoblotted using an NgR1-specific antiserum (R&D Systems AF1208) and fluorescent secondary antibodies. Cell lysates from NgR1-expressing cells served as a positive control. Immunoblots were visualized using the Odyssey imaging system.

Figure S5, related to Figure 4. Primary murine cortical neurons express cell-surface NgR1. Primary murine cortical neurons were prepared from wildtype embryos and cultured in vitro. Cells were fixed, stained with NgR1-specific antibody, and visualized by indirect immunofluorescence microscopy. Representative images from (A) fluorescence and (B) bright field combined with fluorescence views are shown. NgR1 antigen is depicted in green. No antigen staining was detected with a CAR-specific control antibody or secondary antibody alone.

Figure S6, related to Figure 1. NgR1 mediates reovirus T1 and T3 infectivity in non-susceptible CHO cells. CHO cells were either mock transfected or transiently transfected with plasmid encoding NgR1 or JAM-A. After 48 h, cells were adsorbed with either reovirus T1L or T3SA- at an MOI of 10 PFU/cell. Cells were fixed at 20 h and scored for reovirus antigen using indirect immunofluorescence. Results are expressed as the mean FFU/field for three fields of view in triplicate samples. Error bars indicate SD.

Figure S7, related to Figure 5. Cultured primary cortical neurons recapitulate reovirus in vivo serotype-specific tropism. Primary murine cortical neuron cultures prepared from wildtype $NgR1^{+/+}$ or isogenic $NgR1^{-/-}$ embryos were adsorbed with reovirus T3D, T3SA+, T3SA-, or T1L at an MOI of 500 PFU/cell. Cells were fixed at 20 h and scored for reovirus antigen using indirect immunofluorescence. Results are expressed as the mean FFU/field for three fields of view in triplicate samples. Error bars

indicate SD. ***, $P < 0.0005$ (as determined by Student's *t* test in comparison to $NgR1^{+/+}$ neurons).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cells, viruses, and antibodies. Primary murine embryonic fibroblasts and HeLa S3 cells were maintained as described (Antar et al., 2009; Mainou et al., 2013). Reovirus strains T1L and T3D are laboratory stocks. Antiserum specific for human CAR was provided by Jeffrey Bergelson (University of Pennsylvania). NgR1-specific polyclonal antibody (Abcam ab26291) was used to stain cell-surface NgR1. Alexa Fluor-conjugated antibodies (Invitrogen) were used as secondary antibodies.

Whole genome siRNA screen. Lipofectamine® RNAiMAX (Life Technologies) was incubated in DMEM medium for 5 min and dispensed into 384-well plates containing either nonspecific, control (Luciferase, JAM-A, VHA-6 [Life Technologies] or AllStars [Qiagen]), or pooled siRNAs from the Dharmacon ON-TARGETplus® SMARTpool® Human siRNA Library (Thermo Scientific). After 15 min, HeLa S3 cells that had been passaged daily for a week were added to wells and incubated at 37ºC for 48 h. Reovirus T3SA+ was inoculated into wells at an MOI of 1000 PFU/cell and incubated at 37ºC for 48 h. Cell viability was assessed using an ATP-dependent luminescent assay (CellTiter-Glo®, Promega). Luminescence was quantified using the Synergy 2 Multi-Mode Microplate Reader (Biotek). All liquid handling steps were performed using a MultiFlo Microplate Dispenser (Biotek). Robust Z scores (median absolute deviation) from individual samples were calculated from the luminescence data.

SUPPLEMENTAL REFERENCES

Mainou, B., Zamora, P.F., Ashbrook, A.W., Dorset, D.C., Kim, K.S., and Dermody, T.S. (2013). Reovirus cell entry requires functional microtubules. mBio *4*, e00405-00413.