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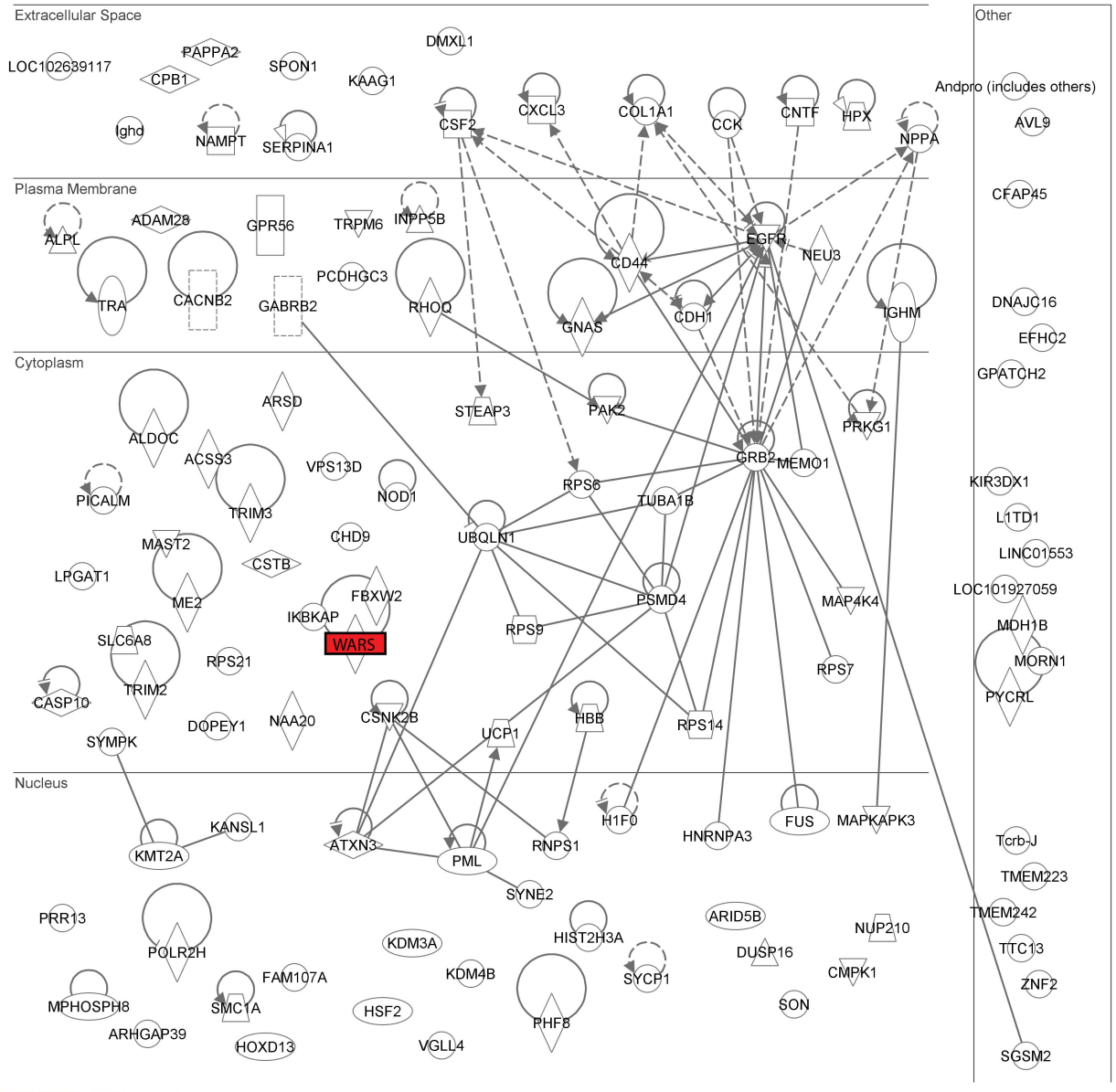

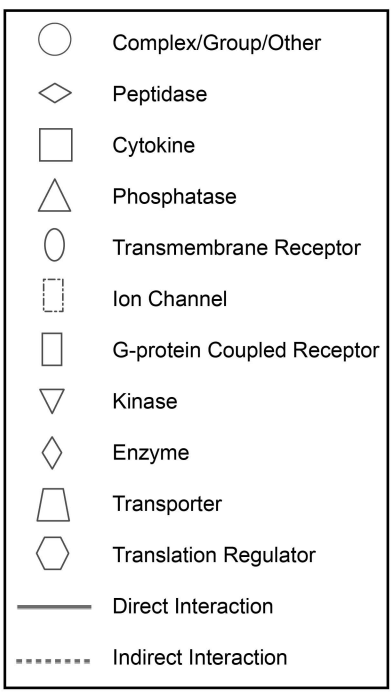

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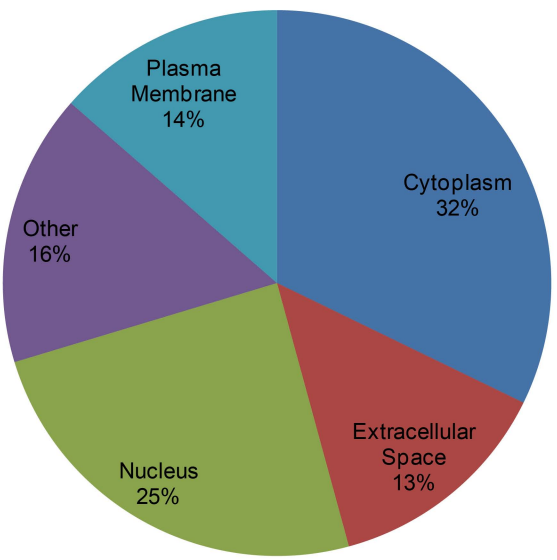
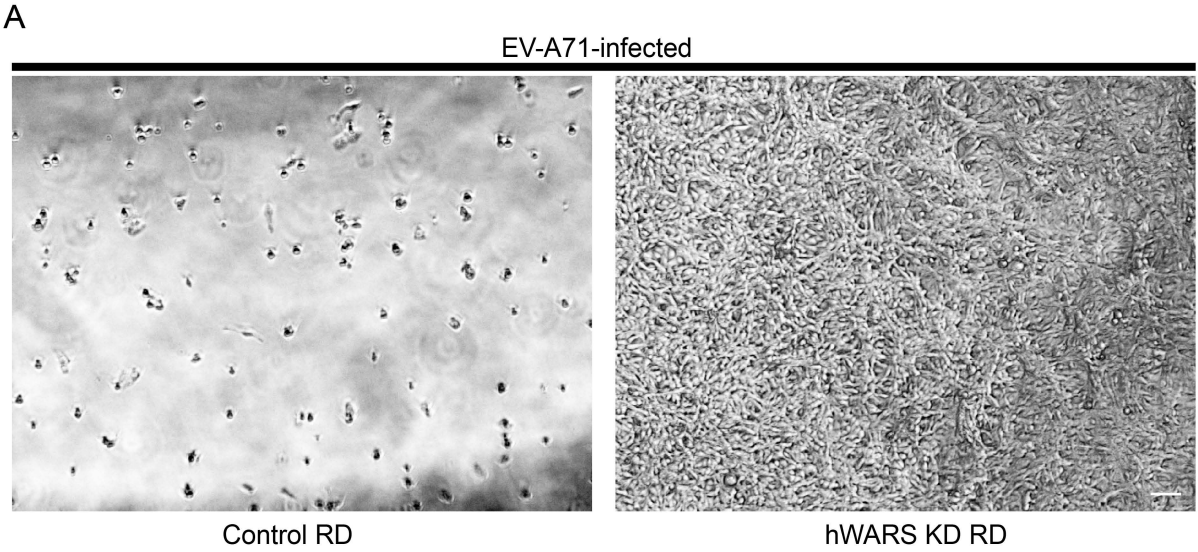
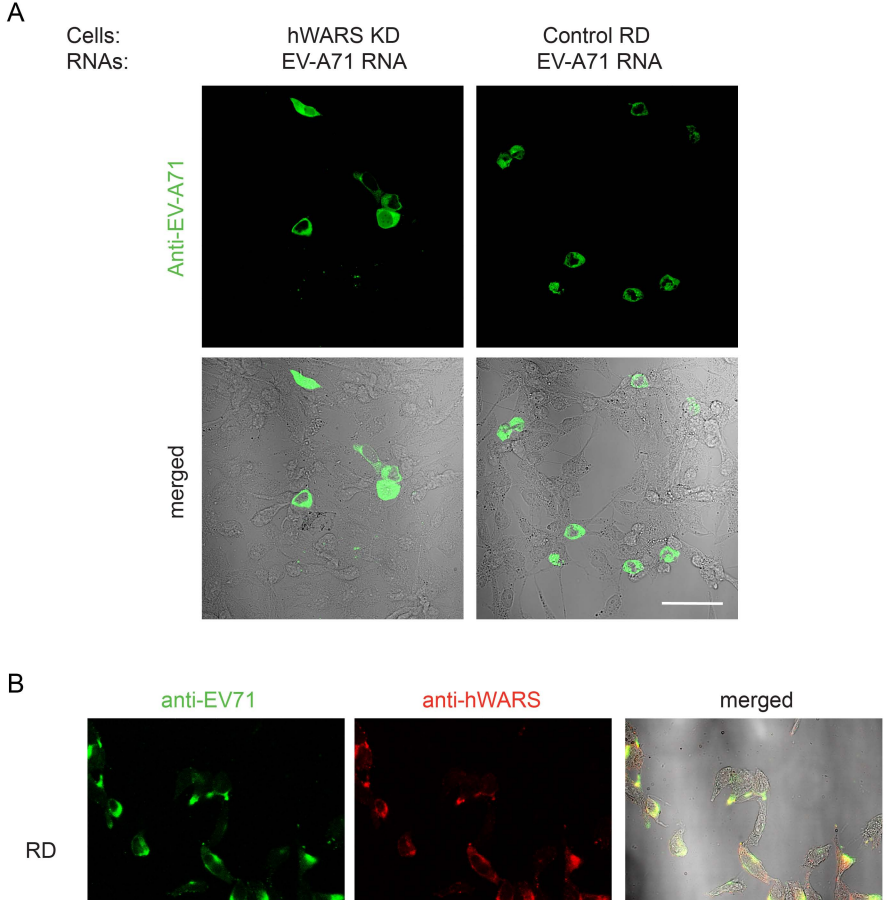


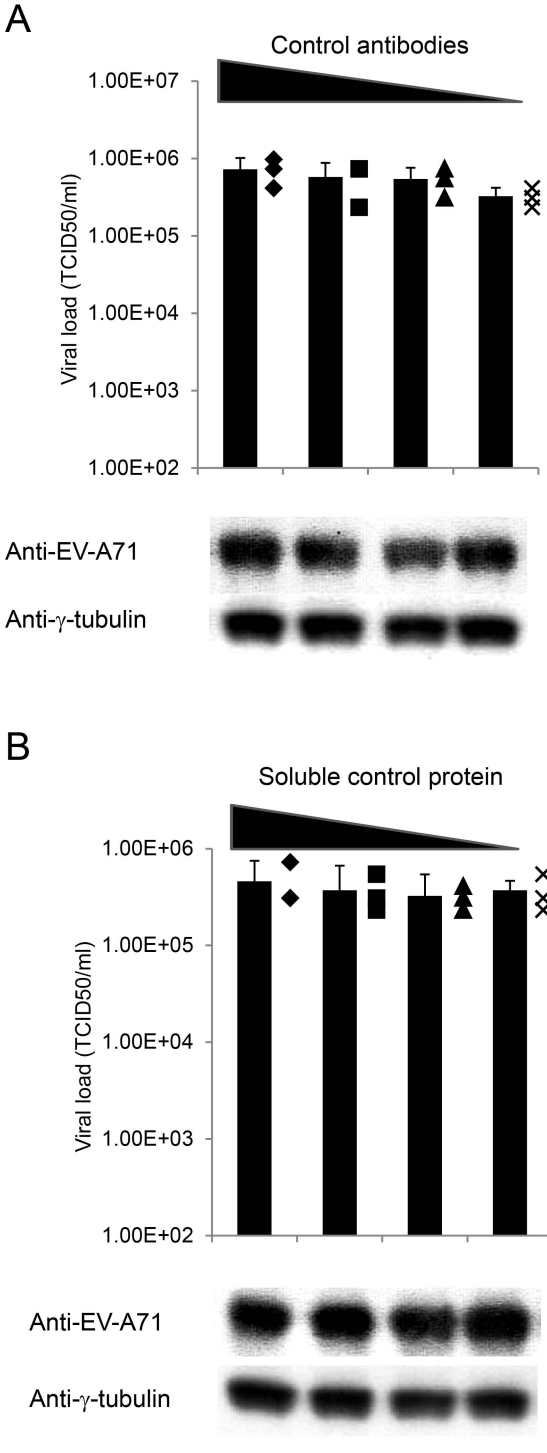
Supplemental Figure 1. Ingenuity pathway analysis of candidate genes required for EV-A71 infection. (A) Figurative representation of the molecular relationships between the identified candidates. The WARS gene is highlighted in red. (B) Pie-chart indicating their subcellular localization (Ingenuity Systems, www.ingenuity.com).



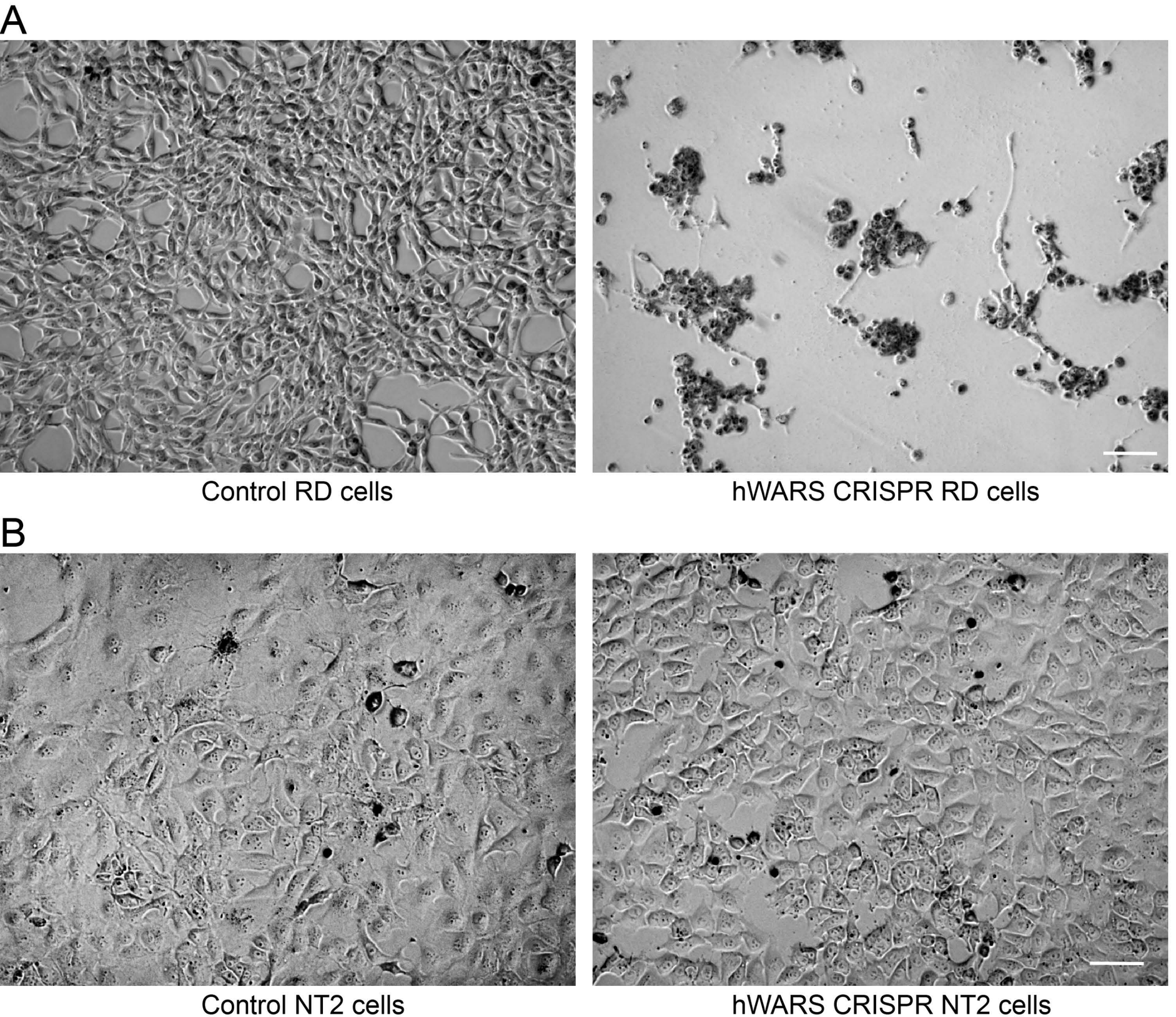
Supplemental Figure 2. Rhabdomyosarcoma (RD) and hWARS knockdown RD (hWARS KD RD) cells infected with EV-A71. (A) Microscopic examination of the control (left) and hWARS KD RD (right) cells 2 days post-EV-A71 infection. (B) Modified MTT assays of the hWARS KD RD (grey) and control RD (dark) cells post-challenged with EV-A71. Mock infection of control RD (dark grey) cells was included. The error bars represent the mean ±s.d. of three independent experiments. The images are representative of three independent experiments. The scale bar indicates 500 μm.



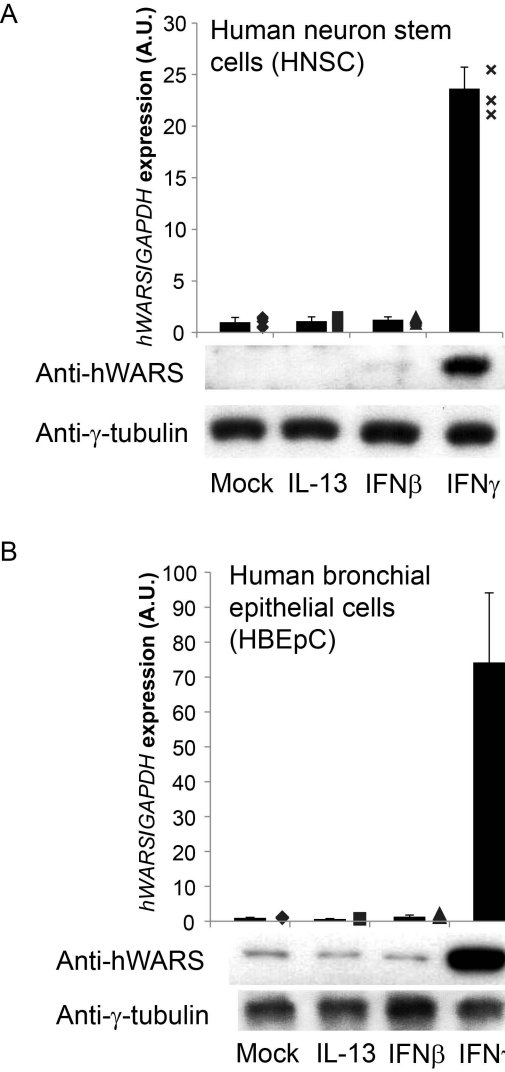
Supplemental Figure 3. Confocal microscopy analyses of EV-A71 protein and hWARS expressions. (A) hWARS KD (left) and control RD (right) cells transfected with EV-A71 RNA. The cytoplasmic expression of EV-A71 protein was detected by a specific antibody. (B) EV-A71 attachment on RD cells was confirmed by the co-immunostaining of EV-A71 protein (by Millipore; MAB979; green) and endogenous hWARS (by Abcam; ab31536; red). The images are representatives of three independent experiments. The scale bar indicates 100 μm.



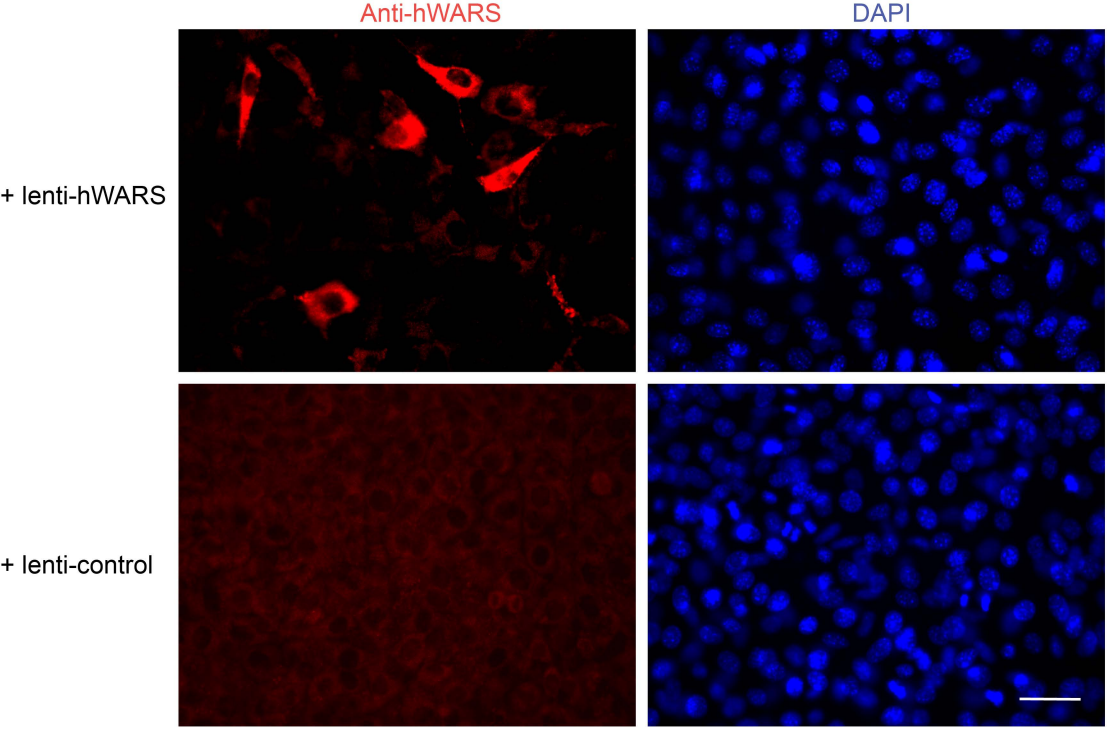
Supplemental Figure 4. Controls of antibody/antigen blocking experiments. The same amounts of control antibodies (A) and soluble control proteins (B) were added to the RD cells under the same experimental conditions as indicated in Figure 4D and E, respectively. Virus production in conditioned supernatants (upper) and viral protein expression in infected cell lysates (bottom) are shown. The error bars represent the mean ± s.d. of three independent experiments. The gel images are representatives of three independent experiments.



Supplemental Figure 5. Generation of hWARS CRISPR cells using the CRISPR/Cas9 system. The cell morphologies of the RD (A) and NT2 (B) cells are shown after their hWARS expressions were targeted by CRISPR/Cas9. Their parental cells (the control RD and control NT2 cells) are also pictured for comparison. The images are representatives of three independent experiments. The scale bar indicates 100 μm.

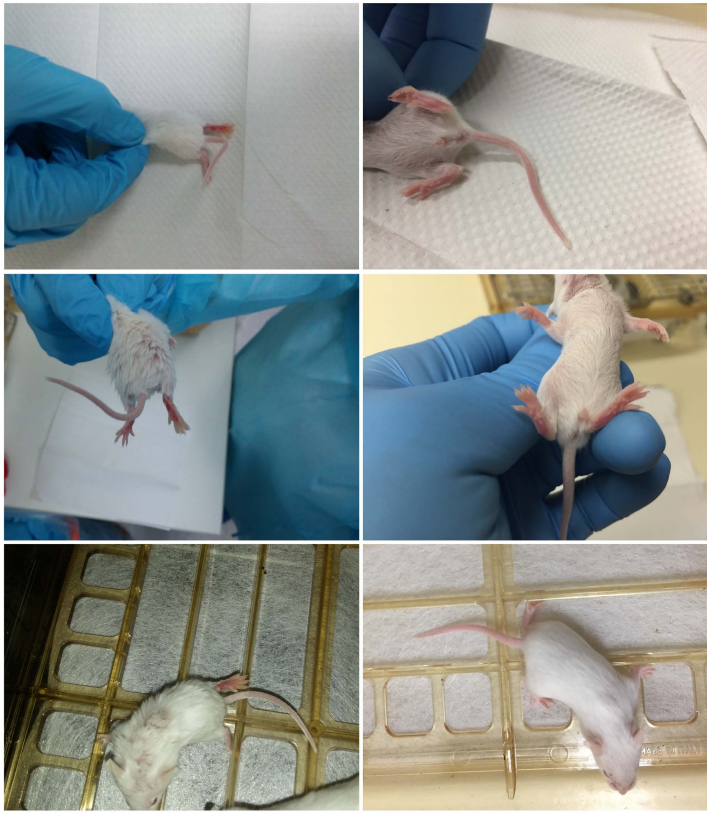


Supplemental Figure 6. Expression of hWARS in response to pro-inflammatory cytokine treatment in human neuron stem cells (HNSC) and human bronchial epithelial cells (HBEpC). Both the HNSC (A) and HBEpC (B) were treated with IFNγ (100 U/ml), IFNβ (100 U/ml) and IL-13 (10 ng/ml). At 48 hours post-treatment, the mRNAs and proteins of the treated cells were harvested for quantitative measurement using quantitative reverse transcription polymerase chain reaction (top) and Western blot (bottom) analyses, respectively. Endogenous glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and γ-tubulin were used as loading controls. The error bars represent the mean ± s.d. of four independent experiments. The images are representatives of four independent experiments. A.U. stands for arbitrary unit.

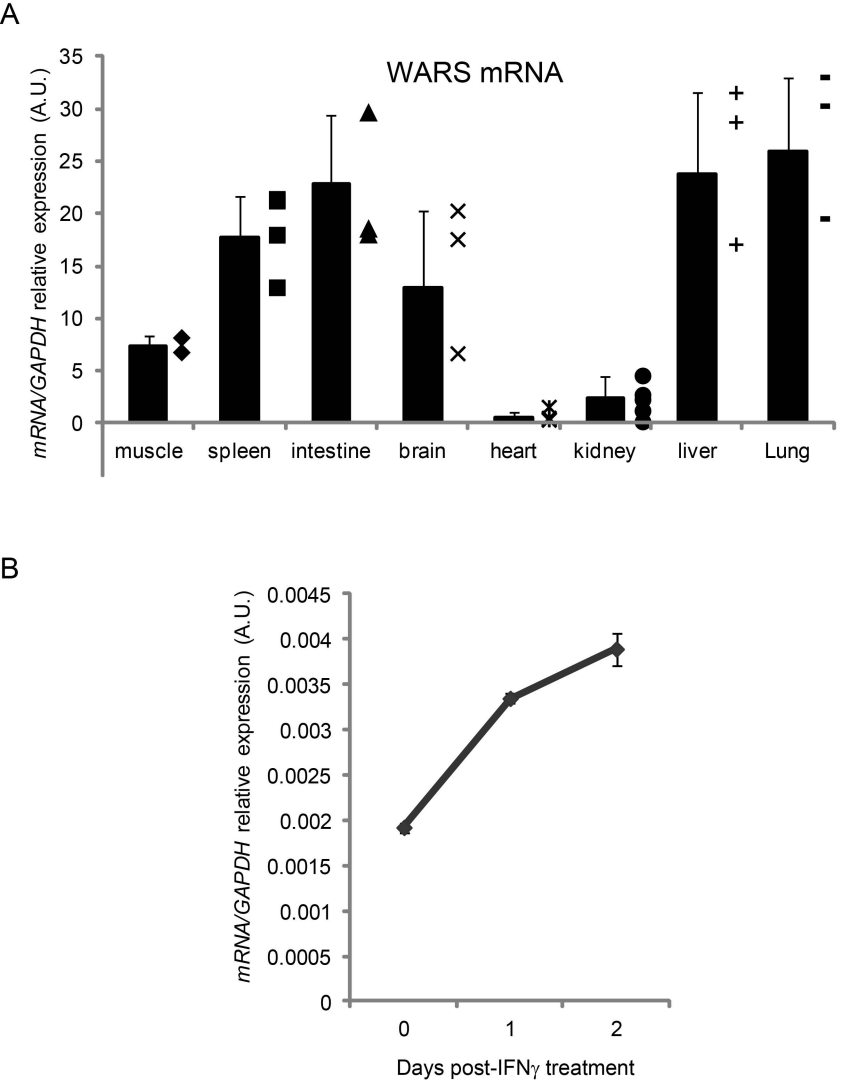


Supplemental Figure 7. Overexpression of hWARS in mouse cells. L929 mouse cells were transduced with lentiviral vector expressing hWARS (top). The cells expressing hWARS were stained with anti-hWARS antibodies (red). The control cells transduced with empty lentiviral vector were also stained (bottom). DAPI staining of the nuclei of the cells is indicated on the right. The images are representative of three independent experiments. The scale bar indicates 50 μm.

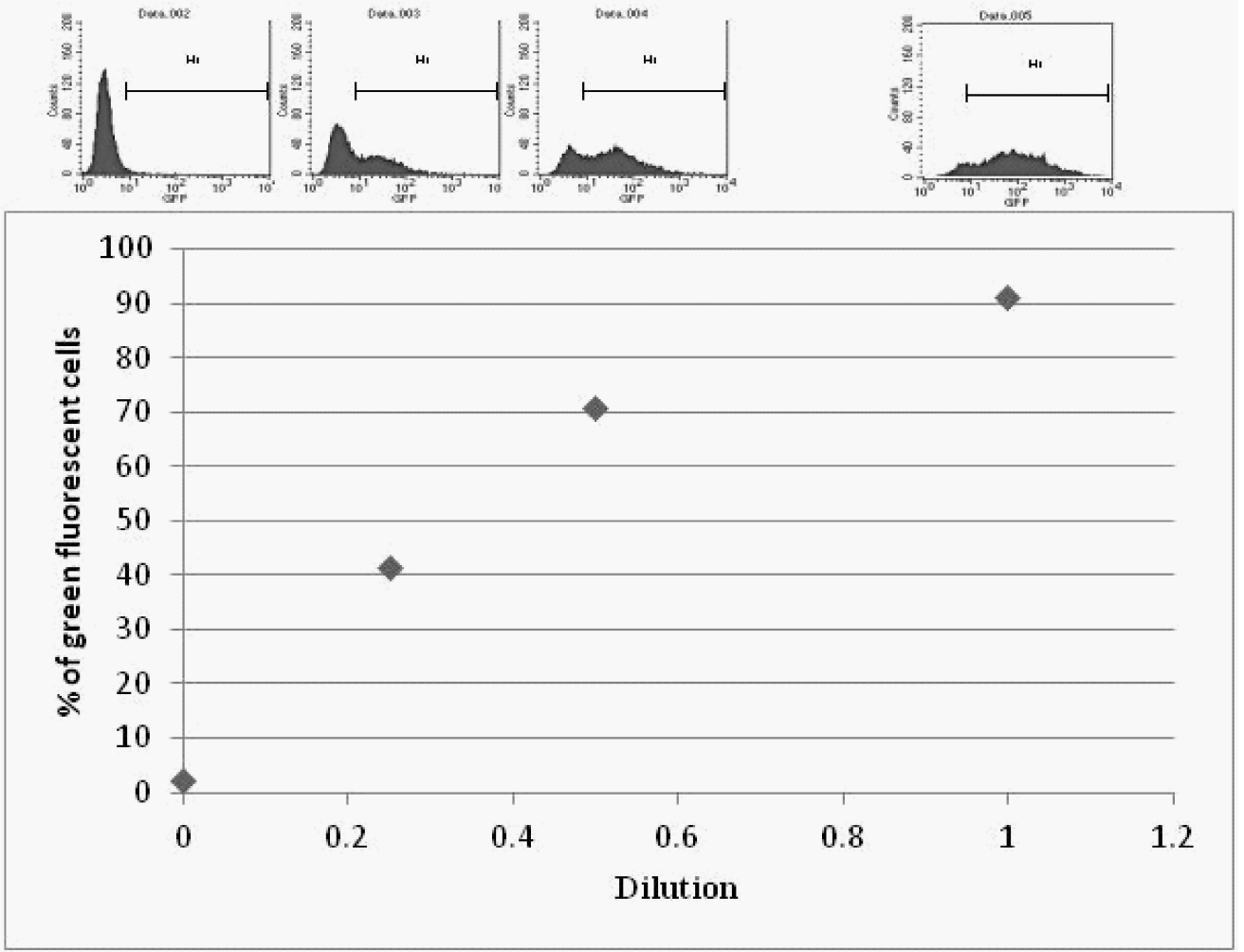
Mouse:	lenti-hWARS-transduced	lentiviral vector-transduced
Virus:	EV-A71	EV-A71



Supplemental Figure 8. Neurological manifestations of EV-A71-inoculated lentivirus-transduced mice on day 5 post-challenge. A lenti-hWARS-transduced mouse was challenged with EV-A71. A mouse with rear limb paralysis is shown (left). The control images taken from a lentiviral vector-transduced mouse challenged with EV-A71 are shown (right). The images are representatives of three independent experiments.



Supplemental Figure 9. Examination of endogenous WARS expression in mouse cells. (A). mRNA expression levels of WARS in various organs of BALB/c mice were quantified by quantitative reverse transcription polymerase chain reaction (qRT-PCR). Endogenous glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was detected as a normalization control. (B) IFNγ stimulated the expression of WARS in mouse L929 cells. Following 100 U/ml IFNγ-treatment, the expression levels of WARS in mouse L929 cells were monitored by qRT-PCR at the indicated time-points. The expression data were normalized with GAPDH. The error bars represent the mean ± s.d. of three independent experiments.



Supplemental Figure 10. Optimization of VSV-G-pseudotyped lentivirus transduction for shRNA library knockdown screening. The infectivity of 500 ml of the HEK293T supernatant containing VSV-G-pseudotyped GFP-expressing lentivirus on the RD cells is proportional to the virus input. The data were collected from the fluorescence-activated cell sorting of the RD cells 3 days post-transduction. The top panel shows gated areas of positive signal. The images are representatives of three independent experiments.