Supporting Information

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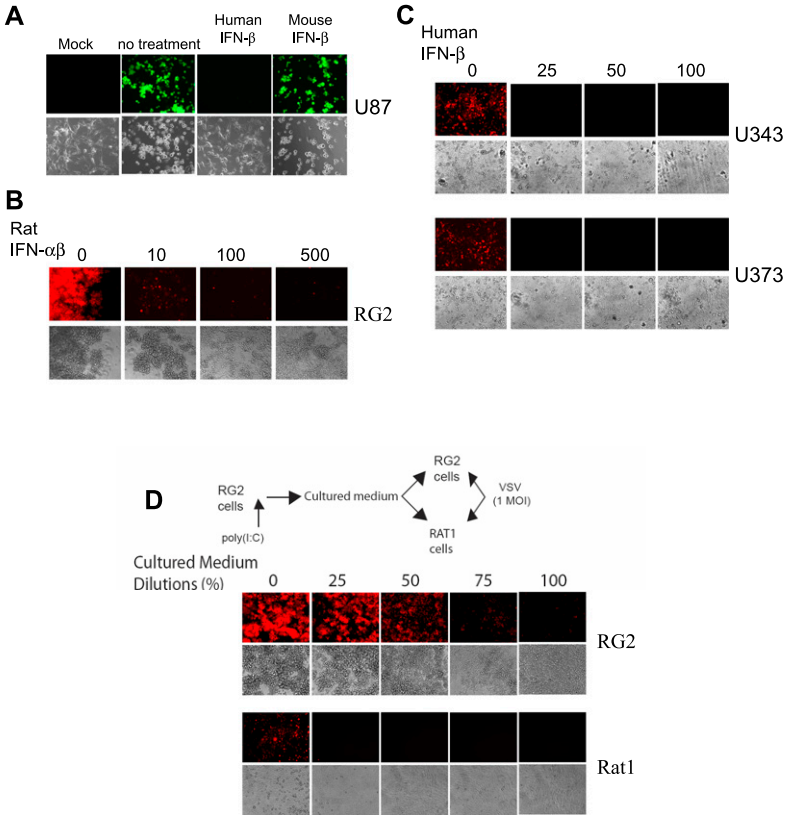

Fig. S1. Human and rat glioma cell lines are protected from VSV oncolysis when pretreated with type I IFN. (*A*) The human glioma cell line U87 was treated with 50 U of either human IFN-β or mouse IFN-β for 6 h before being infected with VSVΔM51-GFP at an MOI of 1. GFP fluorescence and CPE were analyzed at 24 h after infection by phase-contrast and fluorescent microscopy. (*B*) The rat glioma cell line RG2 was treated with increasing units of rat IFN-α/β for 6 h before being infected with $VSV^{\Delta M51}$-RFP at an MOI of 0.1. (*C*) The human glioma cell lines U343 and U373 were treated with increasing units of human IFN-β for 6 h before being infected with $VSV^{\Delta M51}$-RFP at an MOI of 1. RFP fluorescence and CPE were analyzed as in *A*. (*D*) RG2 cells were transfected with poly(I:C) (1 μg/mL) for 6 h. RG2 and Rat1 cells were then incubated with increasing amount of conditioned medium for 12 h and subsequently infected with $VSV^{\Delta M51}$-RFP (MOI of 1) for 24 h.

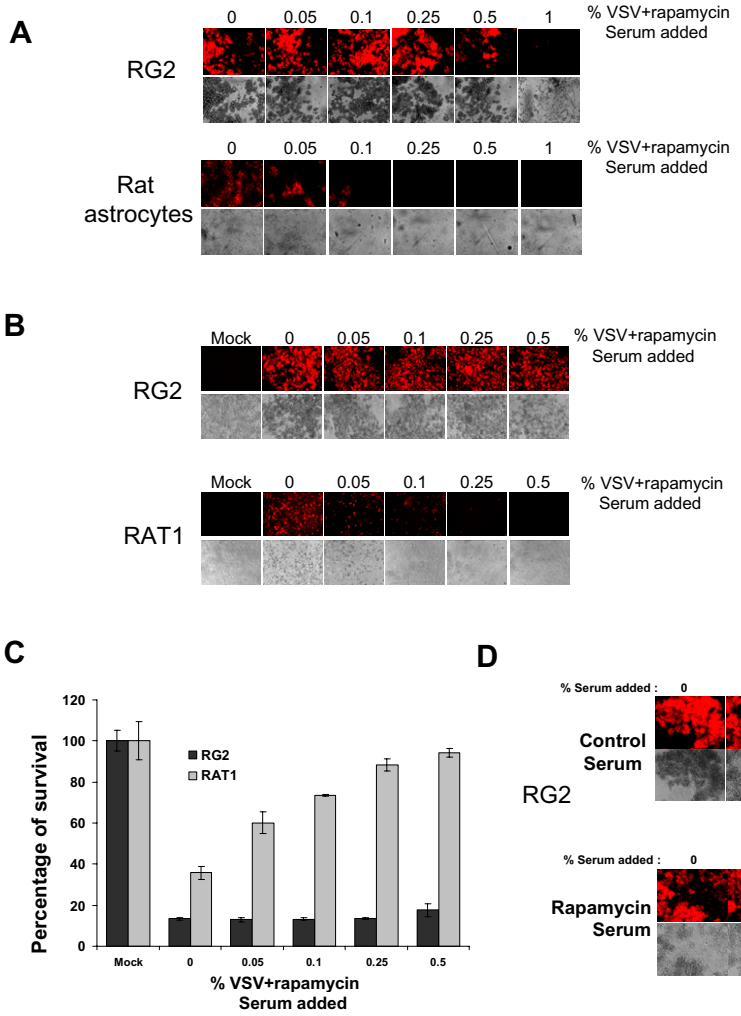

Fig. S2. Low concentration of sera from rapamycin and VSV-treated rats protected normal rat astrocytes and fibroblasts but not rat glioma cells. Sera from rapamycin plus VSV$^{\Delta M51}$-GFP–treated rats were collected at 48 h after VSV$^{\Delta M51}$ infection. Increasing concentrations of the sera were added to either RG2, Rat astrocytes (*A*), or Rat1 cells (*B*) for 1 h before infection with VSV$^{\Delta M51}$-RFP at an MOI of 1. RFP fluorescence and CPE were analyzed as in Fig. S1*A*. (*C*) Viability from RG2 and Rat1 cells was measured by MTT assay. (Error bars correspond to the mean ± SD of experiments done in triplicate.) (*D*) Sera from control and rapamycin-treated rats were added to RG2 cells 1 h before VSV$^{\Delta M51}$-RFP infection at an MOI of 0.1.

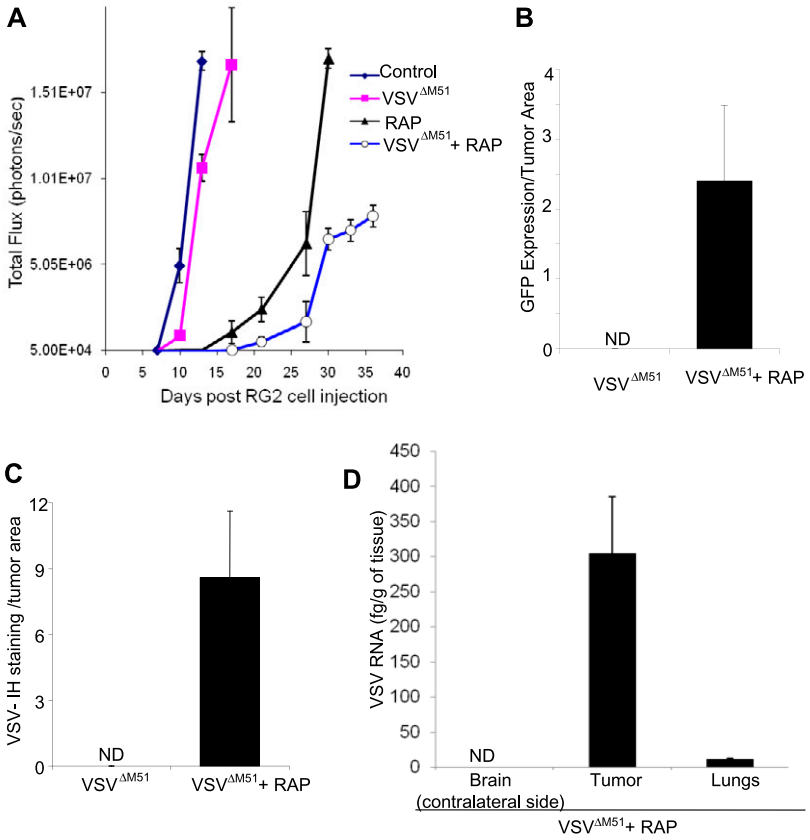

Fig. S3. The combination of rapamycin and VSV$^{\Delta M51}$ reduces tumor size and specifically increases VSV$^{\Delta M51}$ replication within the tumor. (*A*) Quantification of the bioluminescence imaging signal corresponding to real-time monitoring of tumor size. RG2-expressing luciferase cells were injected intracranially and treated with PBS solution (*Control*), VSV$^{\Delta M51}$, rapamycin (*RAP*), and VSV$^{\Delta M51}$ plus RAP. The total photon flux emission (photons/sec) was recorded during the course of the experiment. (RAP vs. VSV$^{\Delta M51}$ plus RAP: ANOVA, $P < 0.05$.) (*B*) The brains of VSV$^{\Delta M51}$ and VSV$^{\Delta M51}$ plus RAP–treated animals were imaged in situ 72 h after infection using a stereotactic microscope with a GFP filter. Two dimensional measurement of GFP expression was quantified as a percentage of the entire tumor area using ImageJ software. (*C*) Brains of VSV$^{\Delta M51}$ and VSV$^{\Delta M51}$ plus RAP–treated animals were sectioned for immunohistochemistry staining against VSV. The VSV immunohistochemistry stained area was quantified as a percentage of the tumor area using ImageJ. (*D*) The lungs and brains of VSV$^{\Delta M51}$ plus RAP-treated animals 24 h after infection were cut in half and tumors were excised from the half that contained them. RNA was extracted and amplified by quantitative real-time PCR. Viral load (in fg/g of tissue) was determined using a standard curve generated with the full-length VSV-N RNA.

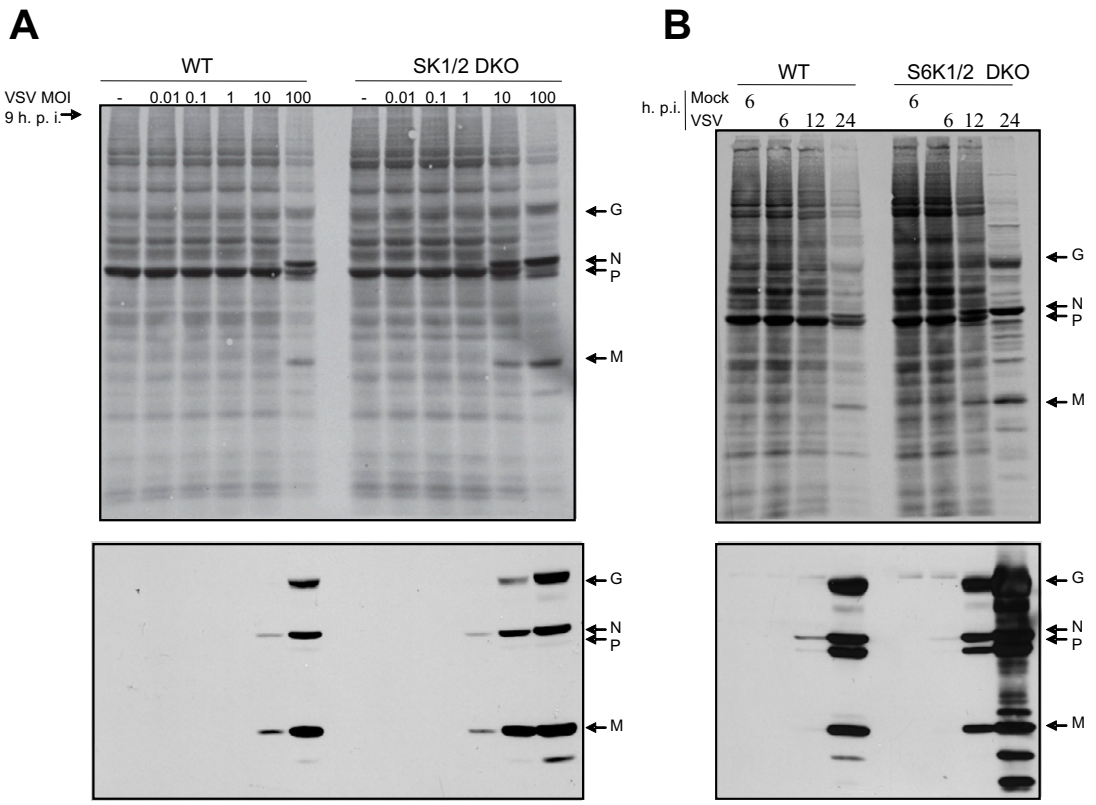

Fig. S4. S6K1/2 DKO MEFs are more susceptible to VSV infection compared with WT MEFs. (*A*) WT and S6K1/2 DKO MEFs were infected with increasing MOIs of VSV. At 8 h after infection, MEFs were incubated with [^{35}S]methionine for 1 h and subsequently lysed. Proteins were subjected to SDS-PAGE (10%) and transferred to a nitrocellulose membrane. An autoradiogram and Western blotting analysis against VSV proteins are shown. (*B*) WT and S6K1/2 DKO MEFs were infected with VSV (MOI of 1) and viral infection was followed up to 24 h as in *A*. Viral proteins are indicated on the right. *G*, glycoprotein; *M*, matrix protein; *N*/*P*, nucleocapsid protein/phosphoprotein.

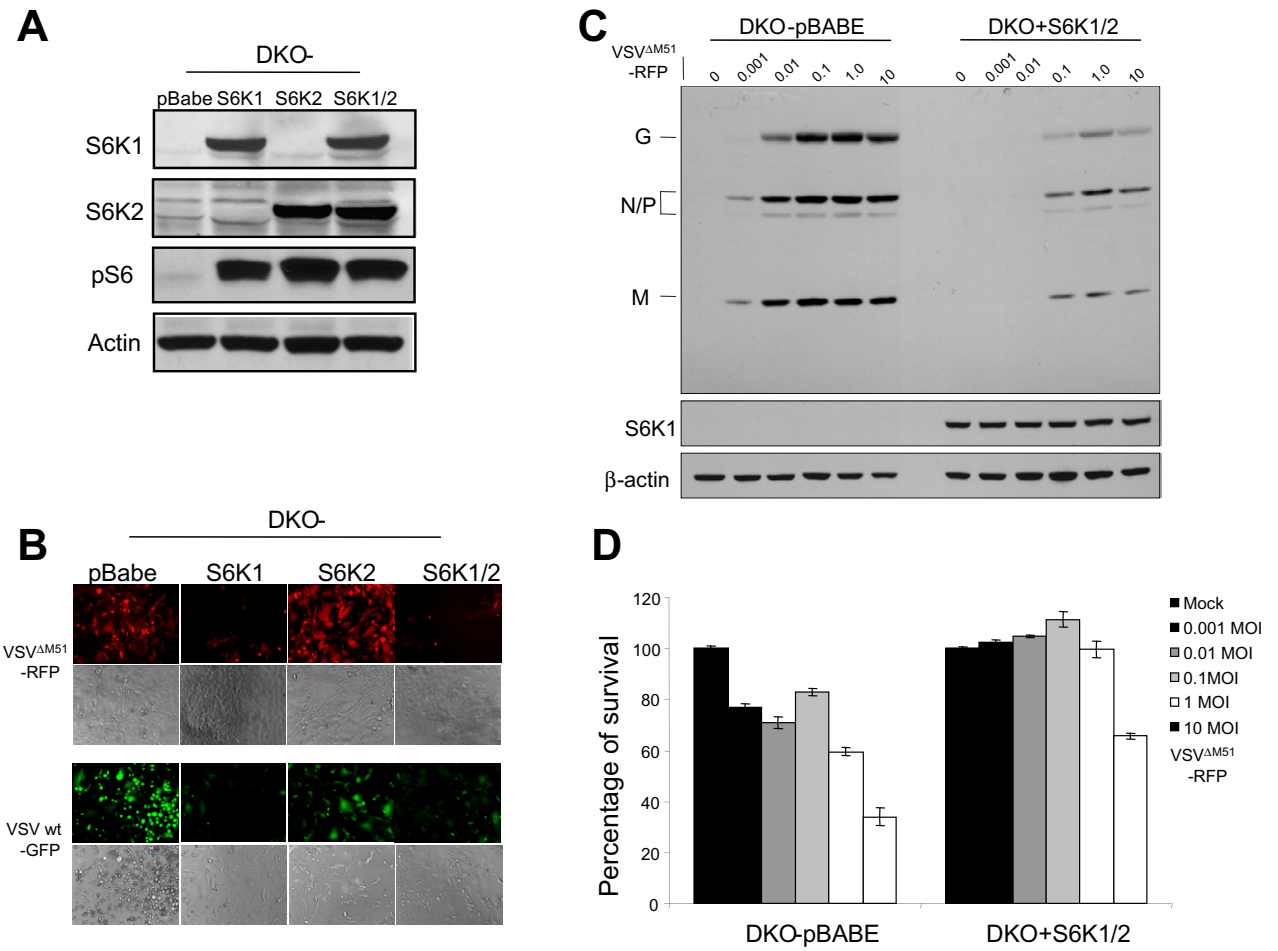

Fig. S5. Rescue of the VSV-sensitive phenotype in S6K1/2 DKO MEFs by ectopic reexpression of S6K1 and 2. Retroviruses carrying S6K1, S6K2, or empty vector (*pBabe*), were used to transfect DKO MEFs. (*A*) Western blot analysis to detect the expression of S6K1, S6K2, phosphorylated $S6^{240-244}$ (*pS6*), and β-actin in transfected S6K1/2 DKO MEFs. (*B*) Stably transfected MEFs expressing S6K1, S6K2, both S6K1 and 2, or empty vector (*pBabe*) were infected with $VSV^{\Delta M51}$-RFP or VSV^{WT}-GFP for 12 h at an MOI of 1 and resulting infection was assessed by RFP or GFP fluorescence and CPE. (*C*) Stably transfected cells expressing both S6K1/2 or empty vector were infected with $VSV^{\Delta M51}$-RFP at different MOIs for 20 h. Infected MEFs were harvested and protein lysates were subjected to Western blot analysis against VSV proteins, S6K1, and β-actin (*D*). The remaining viability of the cells from the same experiment as in *C* was measured by MTT assay. (Error bars correspond to the mean ± SD of experiments done in triplicate.)

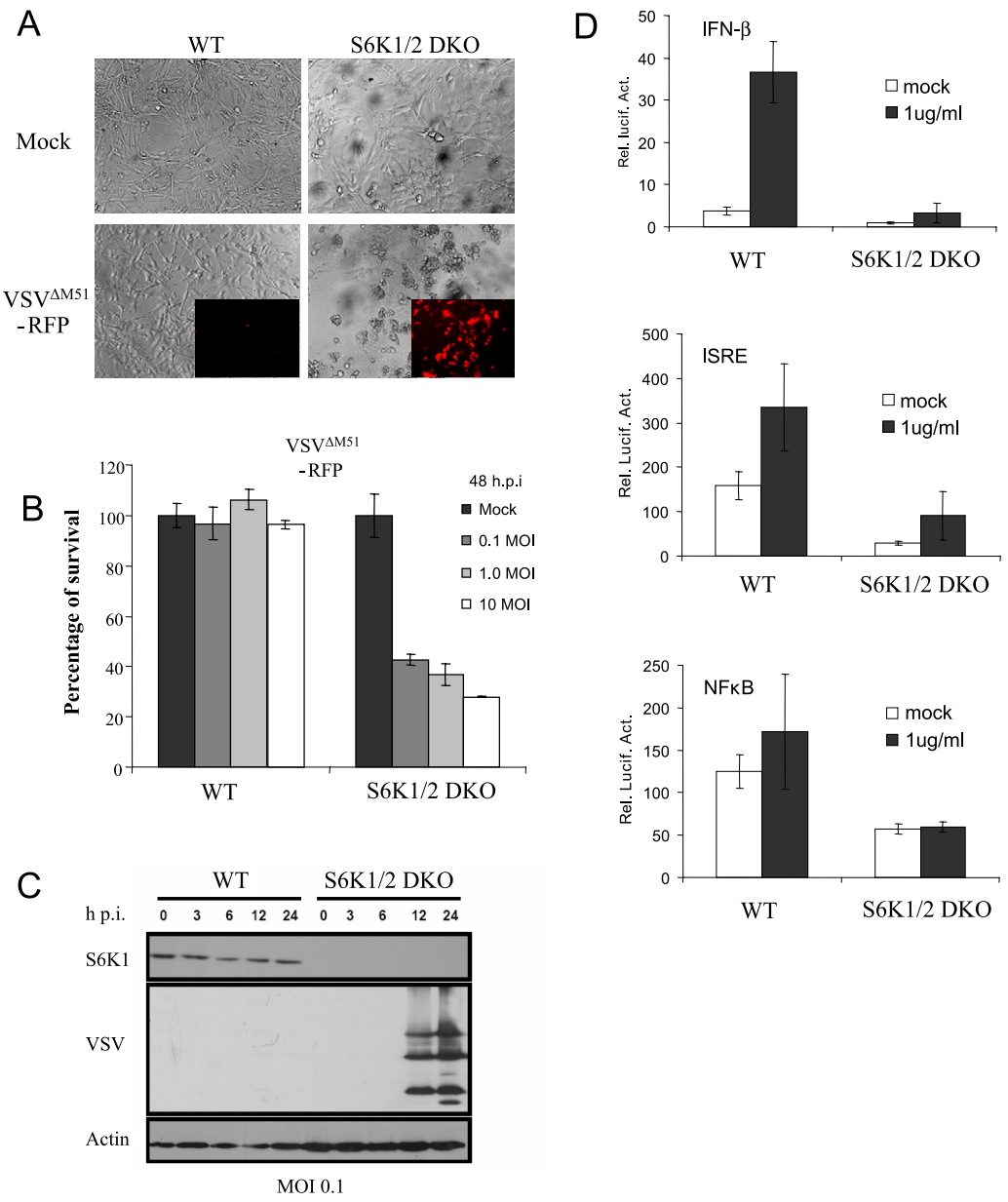

Fig. S6. VSV$^{\Delta M51}$ selectively infects MEFs lacking S6K1/2. (*A*) WT and S6K1/2 DKO MEFs were infected with the IFN-sensitive VSV mutant (VSV$^{\Delta M51}$-RFP) at an MOI of 1. Forty-eight hours after infection, RFP fluorescence and CPE were analyzed by phase-contrast and fluorescent microscopy. (*B*) Viability was measured by MTT assay on MEFs infected with VSV$^{\Delta M51}$-RFP at MOIs of 0.1, 1, and 10. (*C*) WT and S6K1/2 DKO MEFs were infected with VSV$^{\Delta M51}$-RFP at an MOI of 0.1 and viral proteins were analyzed by Western blotting. (*D*) WT and SK61/2 DKO MEFs were transfected with luciferase reporter containing the IFN-β, ISRE, or NFκB promoter, in conjunction with a TK-promoter renilla reporter, which was used as transfection control. Forty-eight hours after transfection, triplicate cultures were left untreated (mock) or treated with 1 μg/mL of poly(I:C) for 6 h. The data are presented as relative luciferase activity when normalized to a control vector (i.e., renilla luciferase).

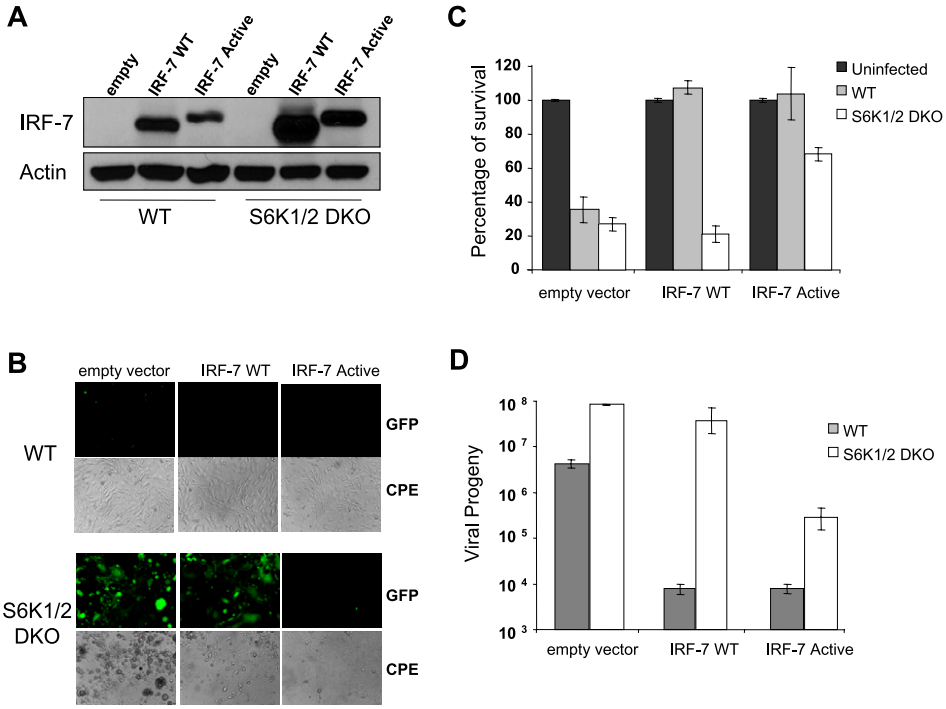

Fig. S7. S6K1/2 activity is important for IRF-7–mediated antiviral activity. WT and S6K1/2 DKO MEFs were transfected with constructs encoding an WT (IRF-7 WT) or a constitutively active form of IRF-7 (IRF-7 Active). (*A*) Western blot analysis for the expression of IRF-7 in transfected WT and S6K1/2 DKO MEFs. (*B*) WT and S6K1/2 DKO cells expressing either the WT or the constitutively active forms of IRF-7 were infected with VSV^{WT}-GFP for 20 h. Fluorescence and CPE were performed as shown in Fig. S1. (*C*) Remaining viability of WT and S6K1/2 DKO cells transfected with IRF-7 WT or IRF-7 Active and infected with VSV^{WT}-GFP at an MOI of 1 for 36 h. (*D*) Viral titers were measured by plaque assay at 36 h after infection.