

Supplementary information

Supplementary figure legends

Figure S1. *In vitro* eIF2 α kinase activity of GCN2-immune complexes from wild type and GCN2^{-/-} MEFs. MEFs derived from GCN2^{-/-} (lanes 1 and 2) and control (lanes 3 and 4) animals were lysed and cell lysates were subjected to immunoprecipitation with anti-GCN2 antibodies. Immune complexes were assayed for their ability to phosphorylate eIF2 α in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of SV RNA as described under Materials and methods. Proteins were resolved into 10% SDS-PAGE and transferred to an immobilon-P membrane. The membrane was exposed to autoradiography to visualize GCN2 and eIF2 α phosphorylation (Autoradiography, upper panels) and then probed with different antisera to detect eIF2 α phosphorylated at serine 51, total eIF2 α , and phosphorylated and total GCN2 (Western blot, lower panels) as indicated.

Figure S2. MEFs derived from GCN2^{-/-} and control animals were not infected (hpi=0, lanes 1 and 4), or else infected with Sindbis virus at an MOI of 50 (lanes 2, 3, 5 and 6). Cell lysates were prepared at the indicated hpi and subjected to immunoprecipitation with anti-PKR antibody (D-20; Santa Cruz). Immune complexes were assayed for their ability to phosphorylate eIF2 α . Phosphoproteins were analyzed by SDS-PAGE and autoradiography as described (see Materials and methods) (top panel). Migration of phosphorylated eIF2 α is indicated on the left. The amount of radioactive phosphate incorporated in eIF2 α was quantified in a Phosphorimager BAS-1500 (Fujifilm). Shown is a representative experiment out of three that yielded similar results. In order to follow the course of the infection we analyzed the presence of viral proteins and phosphorylated eIF2 α by immunoblot using specific antisera for detection of SV E1 protein, eIF2 α phosphorylated at serine 51 and total eIF2 α as indicated.

Figure S3. Viral protein synthesis in infected MEFs derived from GCN2^{-/-} and control animals. Cells were not infected (Mock) or infected with the indicated viruses at an MOI of 25 and metabolically labeled with [³⁵S]-Met-Cys for 1 hr at the indicated hpi. Cells were lysed in a sample buffer and equivalent amounts of total protein were subjected to 12% SDS-PAGE, transferred to nitrocellulose membranes and subjected to autoradiography. The position of main viral protein bands of each virus are indicated. VV, vaccinia virus; FLU, influenza virus.

Figure S4. Viral protein synthesis in infected MEFs derived from GCN2^{-/-} and control animals. Cells were mock-infected or infected with Sindbis virus (SV) at an MOI of 10 and, at 4 hpi, metabolically labeled with [³⁵S]-Met-Cys (lanes 1-4) or just kept in normal growth medium (lanes 5-8), for 30 min. Cells were lysed in a sample buffer and equivalent amounts of total protein were subjected to 12% SDS-PAGE, transferred to nitrocellulose membranes and subjected to autoradiography (bottom panels). The membranes were then probed with specific antisera for detection of SV E1 protein, eIF2 α phosphorylated at serine 51 and total eIF2 α (upper panels) as indicated. The position of the main viral protein bands as well as that of the actin band is indicated.

Figure S5. A1-transduced BHK cells show an attenuated eIF2 α phosphorylation in response to different cellular stresses. Immunoblot to detect eIF2 α phosphorylated at serine 51 (eIF2 α -P) or total eIF2 α , in lysates from control and A1-transduced cells untreated (UT) or after exposure to dithiothreitol (DTT, 1.5 mM for 1 h), tunicamycin (Tm, 2 μ g/ml for 2 h) or thapsigargin (Tg, 200 nM for 1 h).

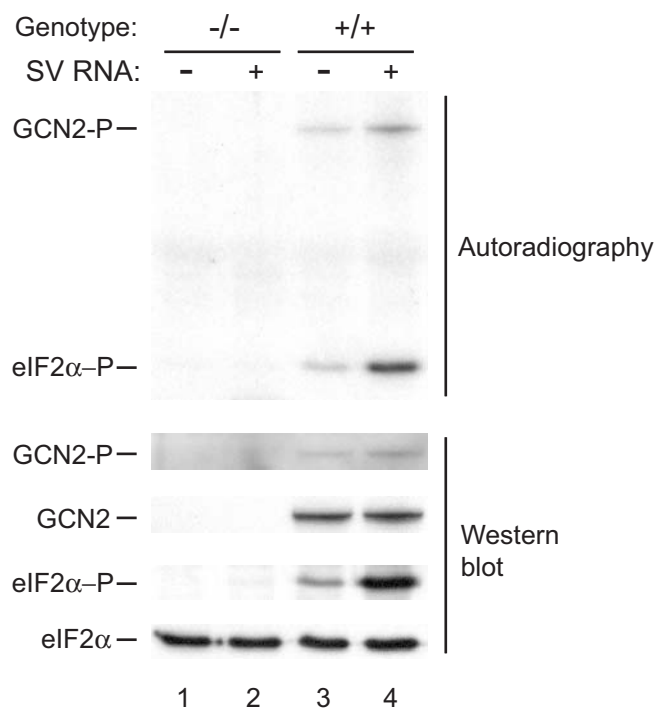


Figure S1

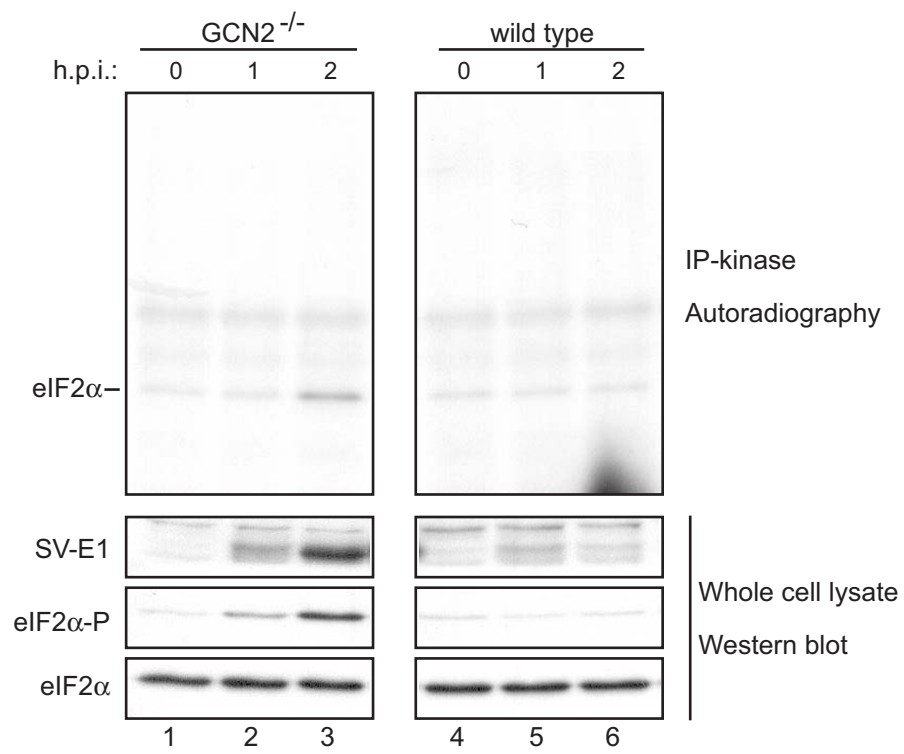


Figure S2

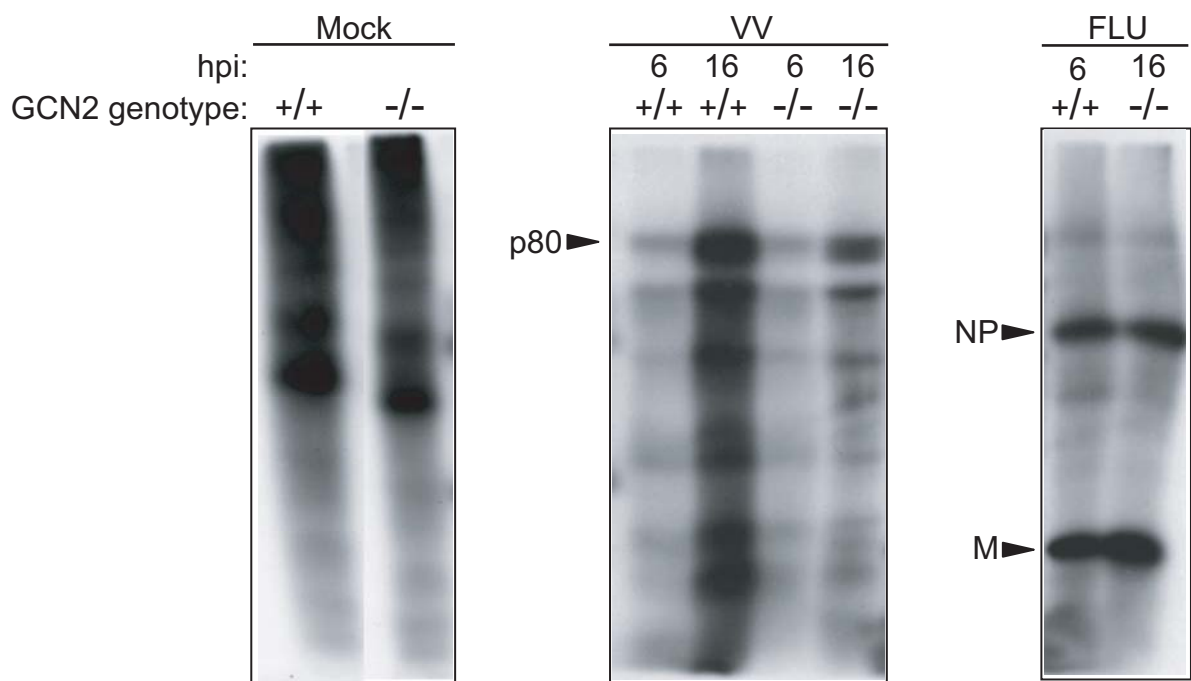


Figure S3

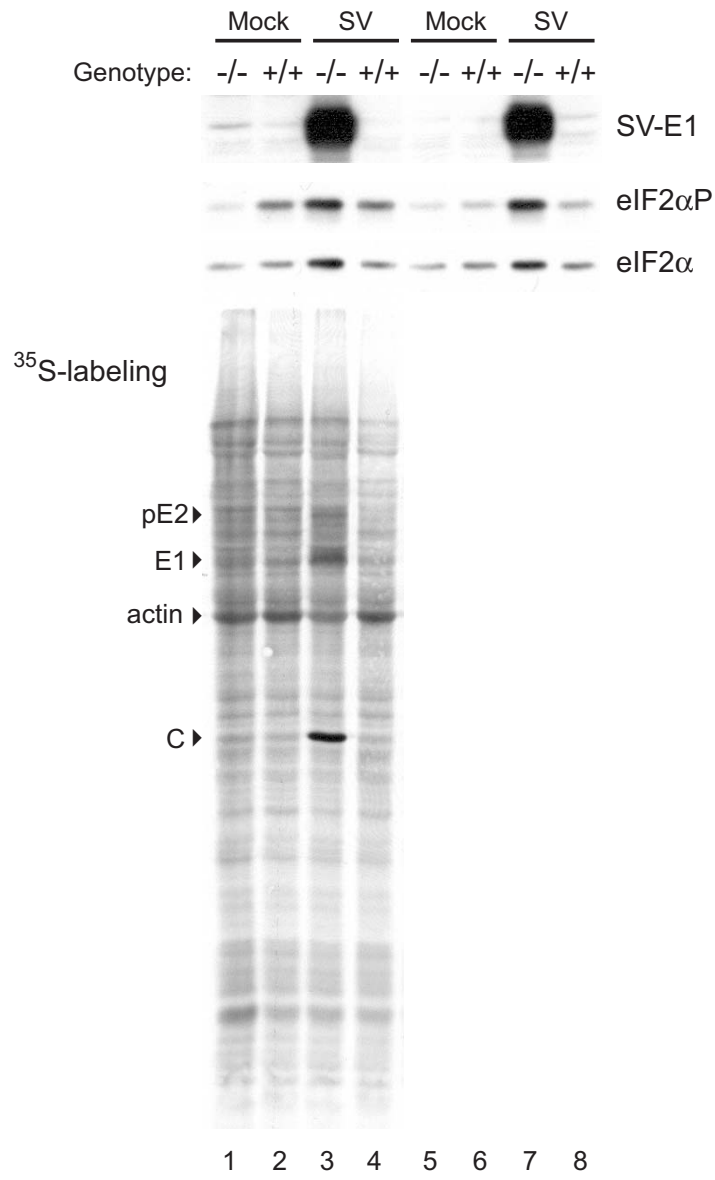


Figure S4

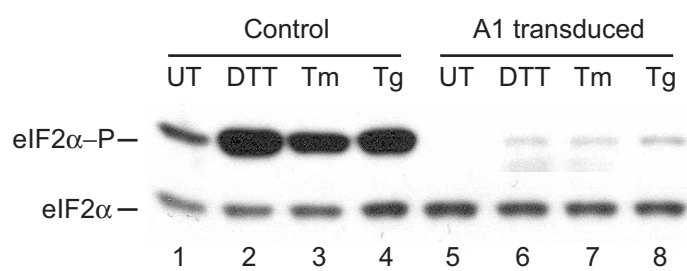


Figure S5