Supplemental Figures

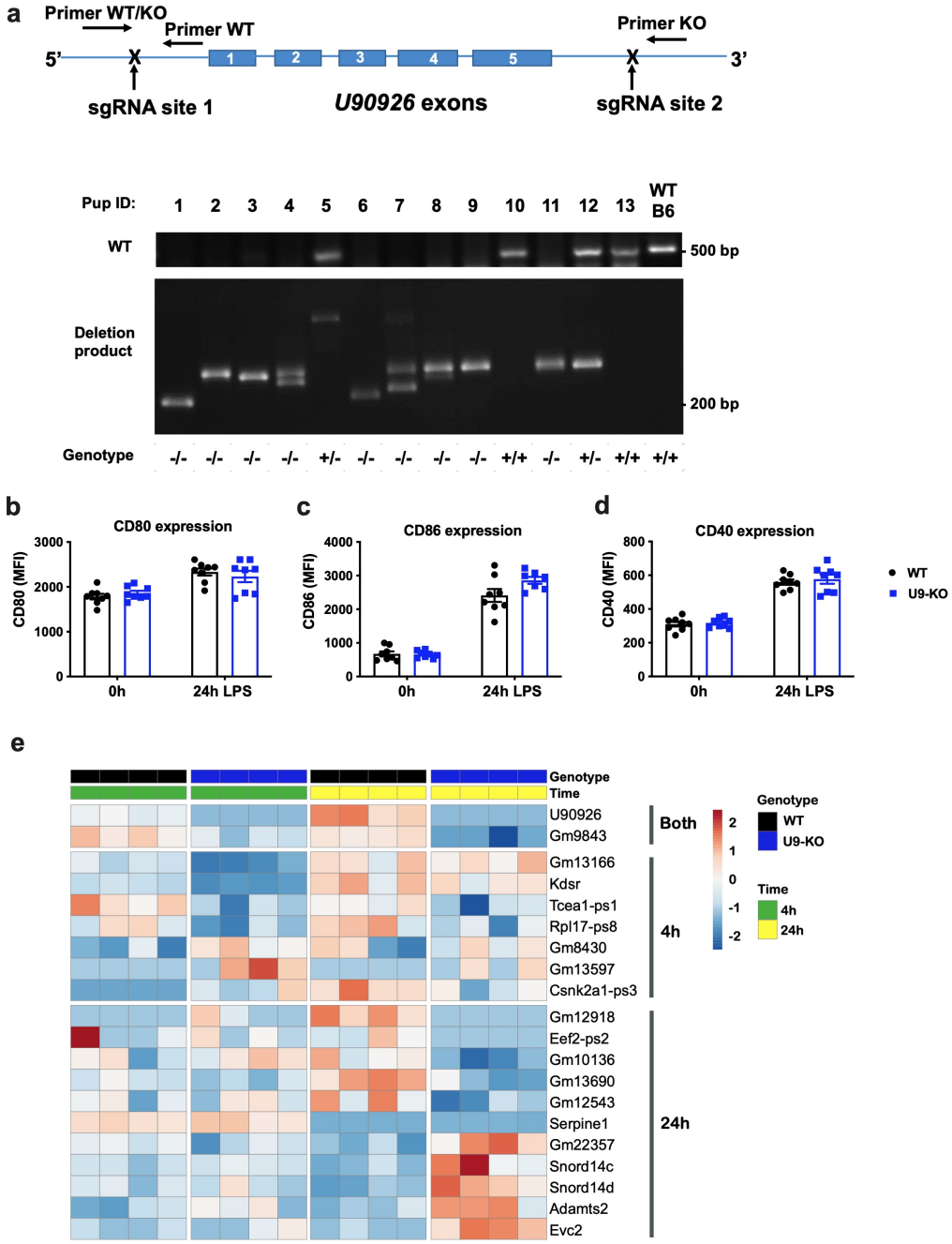
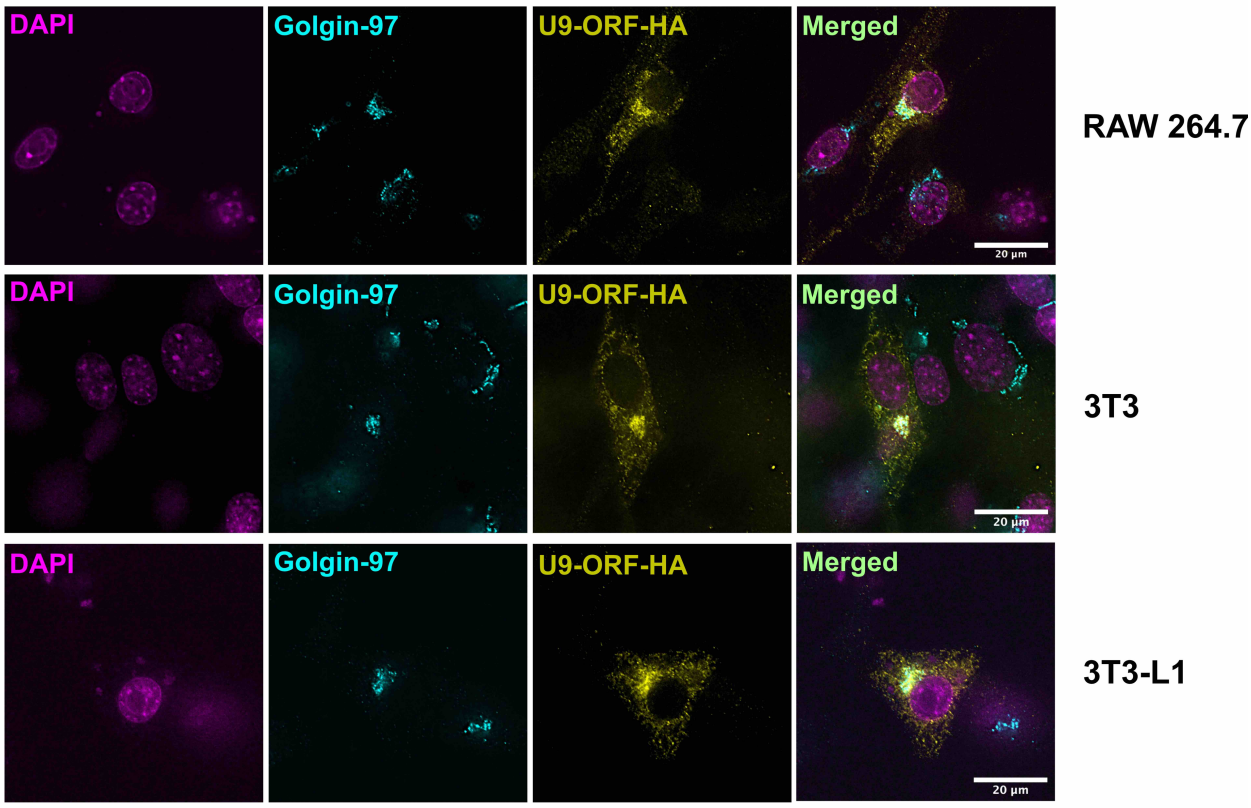


Figure S1. Genotype screening of CRISPR/Cas9 edited pups and *U90926*-deficiency has none to minimal effect in BMDCs and BMDMs. (a) Two sgRNAs and recombinant Cas9 were microinjected into mouse single-cell embryos. PCR genotyping results from the resulting offspring are shown. Two primer sets were used: one set targeting the WT region, (absent in KO), and a second set flanking the entire region, which only yields product if the deletion is present (200-500 bp, depending on size of deletion). Here, U9-KO = -/-, WT B6 = +/+, and heterozygous = +/-. **(b-d)** BMDCs were generated from WT and U9-KO mice and stimulated with 100ng/ml LPS for 24h. Cell surface expression of co-stimulatory markers, CD80, CD86, and CD40, was measured by flow cytometry and expressed as mean fluorescence intensity (MFI). Data are represented as the mean ± SEM. Significance was determined by student's t test. **(e)** RNAseq data shown in **Fig. 3k** and **3l** were reanalyzed using a relaxed cutoff of |Log2 Fold Change|=0.6, Padj <0.2. A heatmap demonstrating the row-normalized gene expression of the top 20 (by log2 fold change) differentially expressed genes at the indicated time points in WT and U9-KO BMDM.

a) Cells transfected with U9-ORF-HA



b) HeLa cells transfected with U9-ORF-HA and treated with Brefeldin A

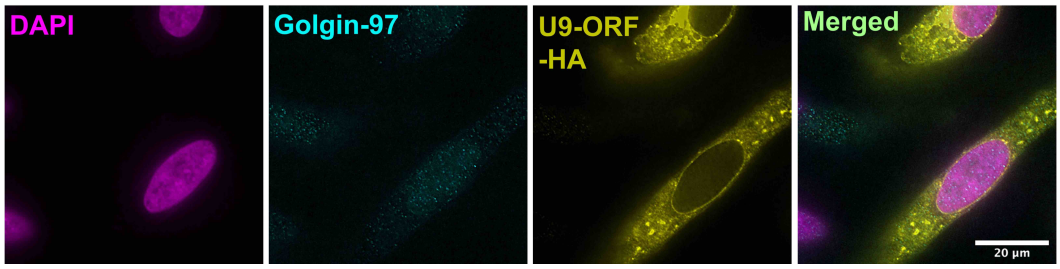


Figure S2. U9-ORF protein localizes to the Golgi apparatus. (**a**) RAW 264.7 mouse macrophages, mouse 3T3 fibroblasts, and mouse 3T3-L1 preadipocytes were transfected with U9-ORF-HA plasmid, followed by immunostaining. (**b**) HeLa cells were transfected with U9-ORF-HA plasmid and treated with Brefeldin A, followed by immunostaining. Stained markers are as follows, Golgin-97 (cyan; Golgi marker), HA (yellow: U9-ORF peptide), and DAPI nuclear staining (magenta).

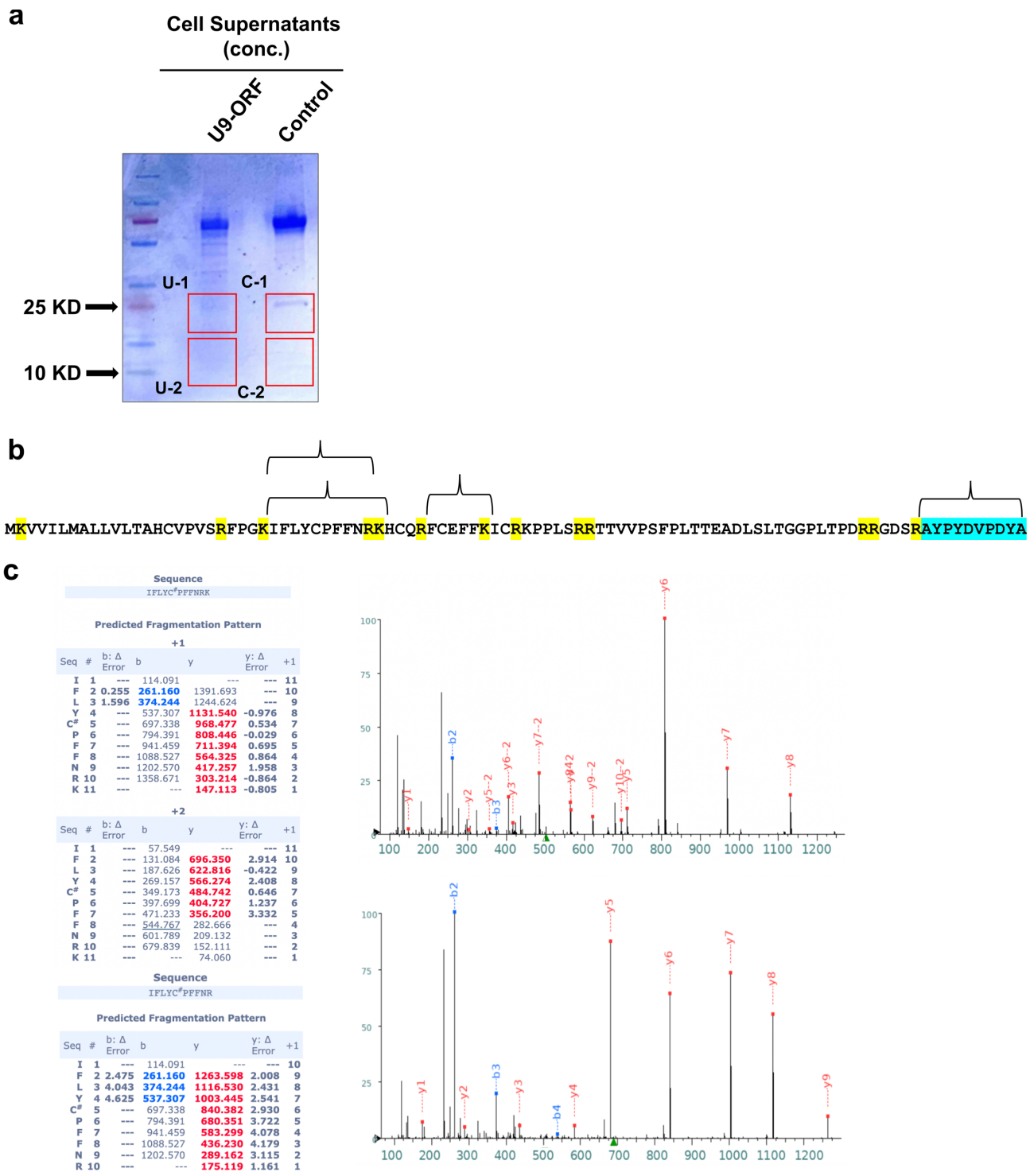


Figure S3: LC-MS/MS identification of U9-ORF protein. HeLa cells were mock-transfected (control) or transfected with a plasmid encoding U9-ORF-HA. Supernatant was collected and concentrated as described earlier and subjected to SDS-PAGE. The gel was stained with Coomassie blue (a). Gel regions shown in (a) were excised and subjected to in-gel tryptic digestion. Tryptic peptides were extracted and subjected to LC-MS/MS. Tryptic peptides for U9-ORF were identified only in the transfected sample and the high confidence identified peptides are listed in Table 1 and are graphically depicted in (b), with brackets indicating the identified tryptic fragments within the U9-ORF sequence. Note, yellow highlights indicate potential tryptic cleavage sites. Blue highlight indicates the HA-tag with an N-terminal alanine linker. (c) MS/MS spectra for two of the identified tryptic peptides showing with b- and y-type ions labeled. Mass tables are included for each spectrum denoting expected fragment ion masses and mass accuracy (Δ Error, in ppm). Note upper and lower spectra overlap and display strong y-type ion series as expected.

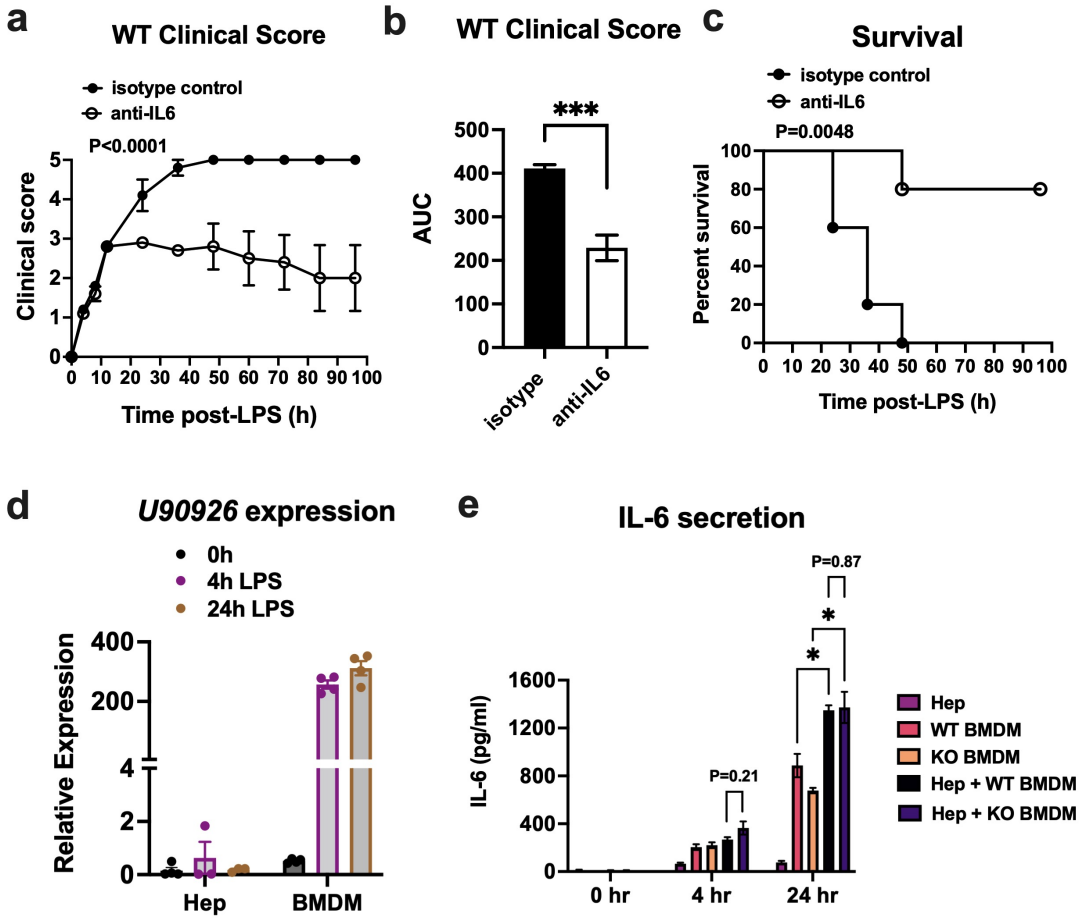


Figure S4. IL-6 Neutralization is protective during LPS endotoxemia in WT mice challenged with a higher LPS dose, and U9-ORF regulation in heaptocytes. WT B6 mice (n=10) were challenged with high dose of LPS (60 mg/kg) and simultaneously administered isotype control (anti-trinitrophenol) or anti-IL-6 antibodies (100 μg/mouse) by i.p. injection, followed by evaluation of sickness behavior **(a** and **b)** and survival **(c).** Area under the curve (AUC) is shown in **(b),** which was calculated from kinetic data in (**a**), to calculate a cumulative measurement of the disease severity. Clinical score evaluation measurement is described in the Methods section. **(d)** A mouse hepatocyte cell line, AML12, or BMDMs generated from WT B6 mice (n=4) and were stimulated with LPS (100 ng/ml) at different time points indicated above. RNA was extracted, followed by RT-qPCR to measure *U90926* expression. RT-qPCR data are expressed relative to the housekeeping gene, Beta-2-microglobulin (*B2m*), and multiplied by a factor of 10,000 for ease of visualization. Data are represented as the mean ± SEM. **(e)** *In vitro* co-culture experiments were set up using mouse hepatocyte, AML12 and WT and U9-KO BMDMs at a 4:1 ratio and their control groups containing either AML12 or BMDM alone. Cells were stimulated with LPS (100ng/ml) for 4 and 24 hours and supernatants were analyzed by ELISA to measure IL-6 secretion. Significance of differences was assessed using 2-way ANOVA (panel a and e), Welch's T test (panel b) and Mantel-Cox test (panel c). P values are represented as follows: ns= >0.05, *= ≤0.05, **=≤0.01, ***=≤0.001.