Supplementary Figure legends

Figure S1: Hepatocytes are susceptible to VACV infection *in vitro*. WT hepatocytes were isolated from liver and infected with VACV at MOI 0.1 and 1. 24 h post infection supernatant and cells were harvested and virus titers were determined by plaque assay (n=3 technical replicates).

Figure S2: Upon VACV infection, IFNAR-triggering of myeloid cells protects from hepatitis. C57BL/6, IFNAR$^{-/-}$, LysM-Cre$^{+/-}$IFNAR$^{fl/fl}$, and AlbCre$^{+/-}$IFNAR$^{fl/fl}$ mice were i.v. infected with 2 x 10^6 pfu VACV. (A) Hematoxylin-and-eosin staining and immunohistochemical analysis of caspase-3 (Casp-3) and iNOS of infected livers 5 dpi. Scalebars 100 µm. (B/C/D) Quantification of (A) (n = 3-5). Error bars indicate mean ± SEM; *p ≤ 0.05; one-tailed Mann-Whitney U test.

Figure S3: Infiltrating myeloid cells in the liver express the Kupffer cell marker CLEC4F. C57BL/6 mice were i.v. infected with 2 x 10^6 pfu VACV. Myeloid cells were isolated from liver at the indicated time points and CLEC4F expression was analyzed by flow-cytometry. FACS-plots are pre-gated on live CD45$^+$ Ly6G$^-$ Ly6C$^-$ CD11b$^+$ single cells.

Figure S4: The M1/M2 phenotype of myeloid cells is independent of direct IFNAR-triggering. (A) C57BL/6, IFNAR$^{-/-}$, and LysM-Cre$^{+/-}$IFNAR$^{fl/fl}$ mice were i.v. infected with 2 x 10^6 pfu VACV. KC were FACS-sorted 0 and 4 dpi. The expression of iNOS, YM-1, and Arg-1 was analyzed by qPCR (n ≥ 6). (B) WT and IFNAR$^{-/-}$ monocytes were isolated 0 and 2 dpi from mixed BM chimeric mice by FACS-sorting. The expression of iNOS, YM-1, and Arg-1 were analyzed by qPCR. Error bars indicate mean ± SEM; ***p ≤ 0.001, **p ≤ 0.01; one-tailed Mann-Whitney U test.

Figure S5: IFNAR-triggering of Kupffer cells controls early viral replication. C57BL/6, IFNAR$^{-/-}$, LysM-Cre$^{+/-}$IFNAR$^{fl/fl}$, and AlbCre$^{+/-}$IFNAR$^{fl/fl}$ mice were i.v. infected with 2 x 10^6 pfu VACV. Virus load in liver was determined 1, 2, and 4 days

post infection (dpi) by plaque assay (n = 3-10). Error bars indicate mean ± SEM; ***p ≤ 0.001, *p ≤ 0.05; one-tailed Mann-Whitney U test.

Figure S6: Replenished MoKC show enhanced Ly6C expression and morphological differences compared to Kupffer cells. (A) C57BL/6 and IFNAR$^{-/-}$ mice were i.v. infected with 2 x 10^6 pfu VACV. 5 dpi mice were sacrificed and paraffin sections of liver tissue were analyzed histologically for F4/80 expression; scalebars 10 µm. (B) C57BL/6 mice were i.v. infected with 2 x 10^6 pfu VACV. Myeloid cells were isolated from liver of WT mice 0 and 4 dpi and Ly6C expression of MoMF, DC, and KC was analyzed by flow cytometry. (C) Percentage of Ly6C-expressing myeloid cells in (B) (n ≥ 7).Error bars indicate mean ± SEM; **p ≤ 0.01; one-tailed Mann-Whitney U test.

Figure S7: Upon VACV infection, liver infiltrating monocytes are directly triggered by IFN-I. Mixed BM chimeras were i.v. infected with 2 x 10^6 pfu VACV. (A) Virus load in livers was determined 4 dpi by plaque assay (n = 5). (B) Mx2-luc mice were i.v. infected with 2 x 10^6 pfu VACV. At the indicated time points luciferin was injected i.v. and luciferase activity was determined by *in vivo* imaging. Light signals detected in the regions of interest (ROI) equivalent to liver were quantified (n = 3). (C) MxCre$^{+/-}$Rosa26YFP$^{ST/ST}$ mice were i.v. infected with 2 x 10^6 pfu VACV. Myeloid cells were isolated from liver 4 dpi and analyzed by flow-cytometry. MoMF = monocyte-derived marcrophages (CD11b$^+$ CD11c$^+$I-A/I-E$^-$), DC = dendritic cells (CD11b$^+$CD11c$^+$I-A/I-E$^+$), KC = Kupffer cells (F4/80$^+$CLEC4F$^+$). (D) Quantification of C. Percentages of YFP$^+$ cells are shown (n = 5).

Figure S8: MCMV infection induces KC loss. C57BL/6 mice were i.v. infected with 5 x 10^5 pfu MCMV. (A) Myeloid cells were isolated from liver 0 and 3 dpi and analyzed by flow-cytometry. (B) Quantification of KC in the liver of (A) (n ≥ 5). Error bars indicate mean ± SEM; **p ≤ 0.01; one-tailed Mann-Whitney U test.

Supplementary Material and Methods:

Mouse strains

IFNAR$^{-/-}$: Ubiquitous deletion of the type I interferon receptor 1

LysM-Cre$^{+/-}$IFNAR$^{fl/fl}$: Cell-type specific deletion of IFNAR on myeloid cells, including KC

Alb-Cre$^{+/-}$IFNAR$^{fl/fl}$: Cell-type specific deletion of IFNAR on hepatocytes

Cx3cr1$^{+/gfp}$: GFP-reporter expression under the control of the Cx3CR1 promotor. Blood monocytes are Cx3Cr1 and thus GFP positive, facilitating to follow such cells upon infiltration into organs. The half-life time of GFP is approximately 24 h, thus organ resident macrophages, such as KC, do not express GFP under homeostatic conditions.

Mx-Cre$^{+/-}$Rosa26eYFP$^{ST/ST}$: YFP-reporter expression under the control of the Mx promotor (Interferon stimulated gene (ISG)). Upon IFNAR-triggering Cre activity is induced that removes the STOP cassette. Thus YFP expression is permanently induced and therefore can be used to trace interferon induced cells.

Mx2-luc: Luciferase-reporter expression under the control of the Mx2 promotor (ISG). Upon luciferin administration IFNAR-triggering can be visualized in *in vivo* imaging system. Because luciferase expression is reversible in this system the kinetics of IFNAR-triggering can be determined.

IFN-β$^{WT/\Delta\beta\text{-luc}}$: Luciferase-reporter expression under the control of the IFN-β promoter. Upon luciferin administration IFN-β expression can be visualized in *in vivo* imaging system. Because luciferase expression is reversible in this system the kinetics of IFN-β expression can be determined.

Isolation of primary murine hepatocytes

Murine hepatocytes were isolated from C57BL/6 mice using a 2-step liberase perfusion as described previously (*1*) and cultured in Primaria 6-well plates (BD Biosciences, Franklin Lakes, USA) at a density of 1×10^6 cells per well in 2 ml of HBM Basal Medium supplemented with HCM™ SingleQuots® (Lonza, Basel, Switzerland). Hepatocytes were infected with VACV at MOIs 0.1 and 1 and incubated for 2h. Medium was changed and the cells were incubated for another 22 h at 37°C.

RNA isolation and quantitative real-time PCR (qPCR)

RNA was isolated using the Arcturus PicoPure kit (Applied Biosystems). RNA was reverse-transcribed to cDNA using PrimeScript™ kit (Takara Clonetech). qPCR was carried out using Power SYBR Green (Bioline) and was run on a Lightcycler 480 II Real-Time PCR system (Roche). Expression values were calculated in relation to *Actb* as an endogenous control gene.

References

1. M. Rothe, I. Rittelmeyer, M. Iken, U. Rudrich, A. Schambach, S. Glage, M. P. Manns, C. Baum, M. Bock, M. Ott, U. Modlich, Epidermal growth factor improves lentivirus vector gene transfer into primary mouse hepatocytes. *Gene Ther* **19**, 425-434 (2012).