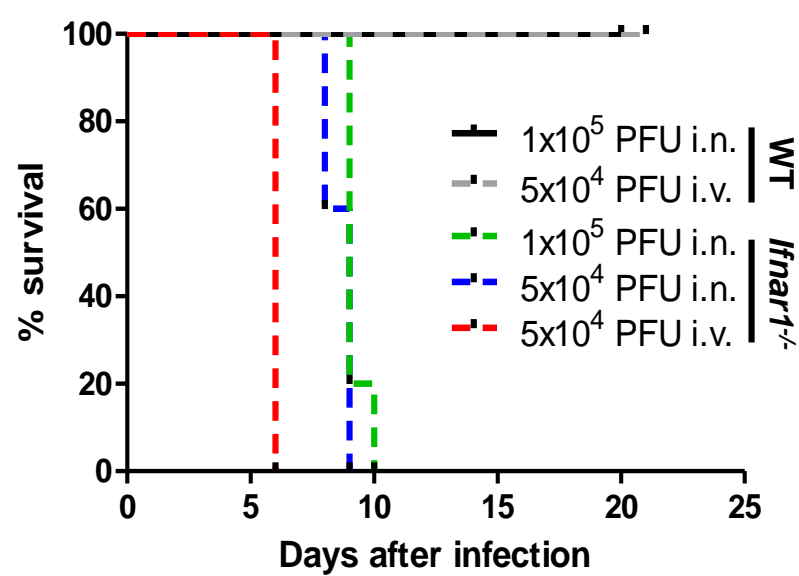


# **Type I Interferon Signaling Controls Gammaherpesvirus Latency *in vivo***

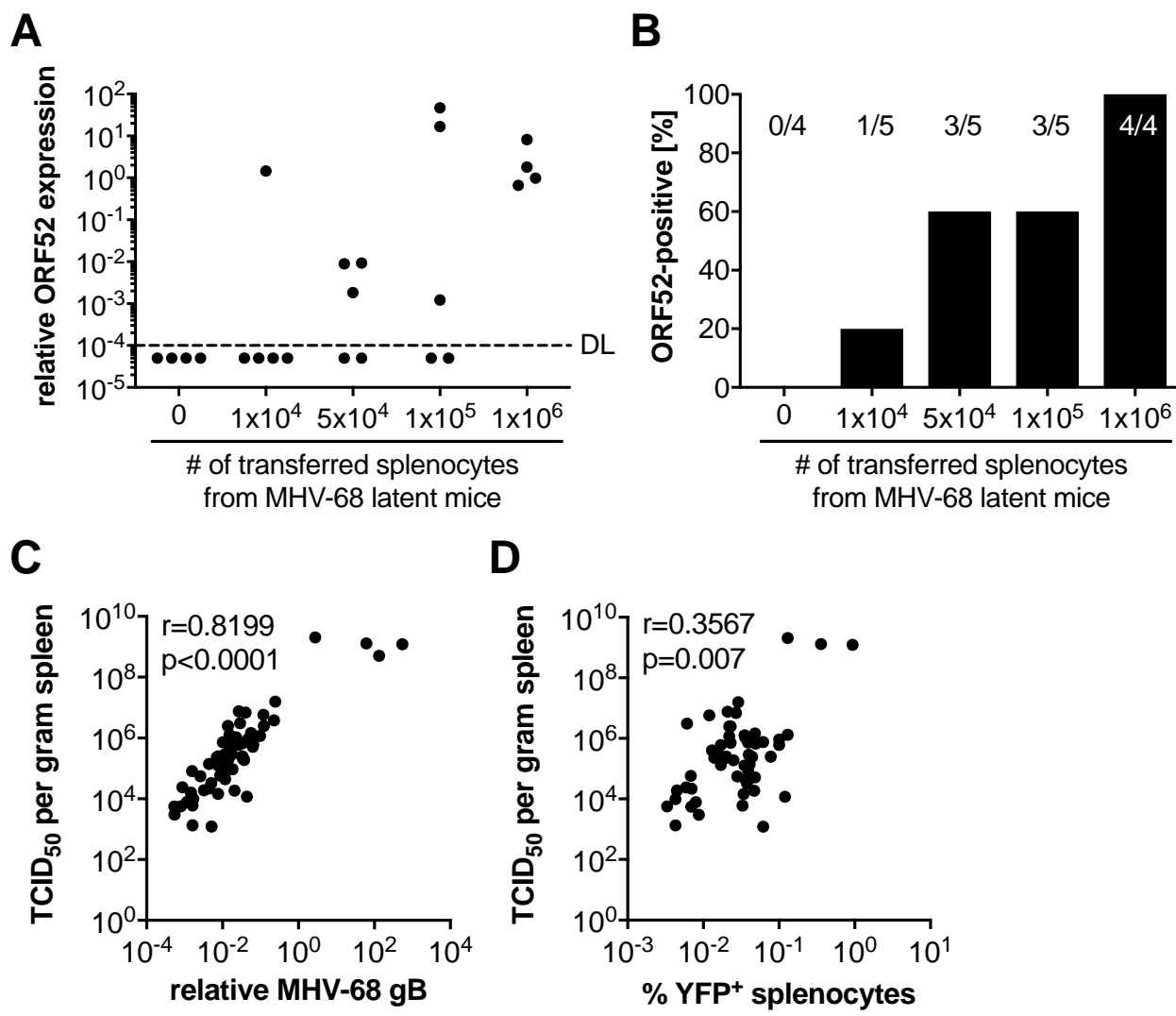
Johannes Schwerk, Lucas Kemper, Kendra A. Bussey, Stefan Lienenklaus, Siegfried Weiss, Luka Čičin-Šain, Andrea Kröger, Ulrich Kalinke, Christopher M. Collins, Samuel H. Speck, Martin Messerle, Dagmar Wirth, Melanie M. Brinkmann, Hansjörg Hauser and Mario Köster

Figure S1



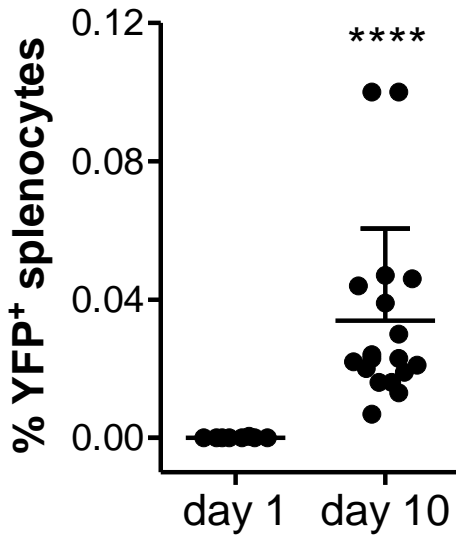
**Figure S1. Type I IFN signaling is essential for survival of MHV-68 infection.** Survival of WT and *Ifnar1*<sup>-/-</sup> mice upon intranasal (i.n.) and intravenous (i.v.) infection with indicated doses of MHV-68 WUMS (n=4-5).

Figure S2



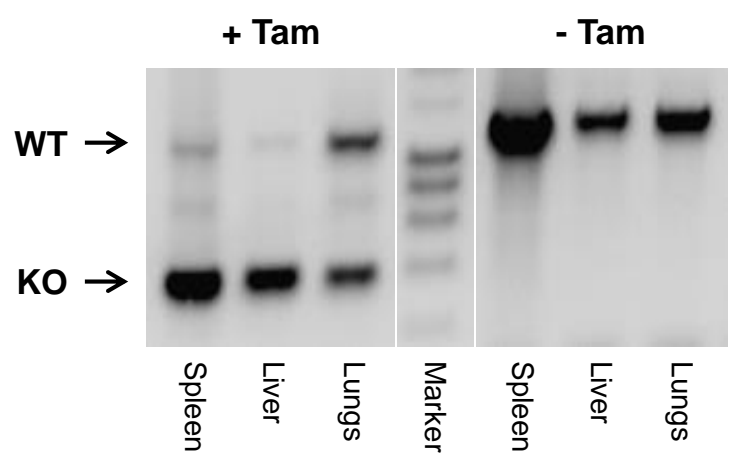
**Figure S2. *In vivo* reactivation efficiency and validation of MHV-68 gB DNA and YFP<sup>+</sup> cells as readout for recoverable infectious virus.** (A) Indicated amounts of splenocytes from latently infected donor mice, supplemented with uninfected splenocytes to a total number of  $1 \times 10^7$  cells, were adoptively transferred into *Ifnar1*<sup>-/-</sup> recipient mice. Spleens were isolated from recipient mice 12 days after transfer and analysed for viral ORF52 expression by qPCR (n=4-5). (B) Ratio of reactivation-positive mice relative to the amount of adoptively transferred splenocytes from latently infected donor mice. (C, D) MHV-68 genome load (gB) and frequency of YFP<sup>+</sup> cells in spleens was correlated to recoverable infectious virus. The Spearman correlation coefficient (r) was determined by pooling data of several experiments (n=56-63).

**Figure S3**



**Figure S3. Increase of MHV-68 infected cells between day 1 and day 10 after adoptive transfer of latently infected splenocytes.** Splenocytes from MHV-68 H2bYFP latently infected tamoxifen-inducible *Ifnar1*<sup>-/-</sup> mice (R26CreERT2 x *Ifnar1*<sup>fl/fl</sup>) were isolated and adoptively transferred into conventional *Ifnar1*<sup>-/-</sup> recipient mice (10<sup>7</sup> cells/mouse). The frequency of YFP<sup>+</sup> splenocytes was determined by flow cytometry at days 1 and 10 after adoptive transfer. The graph compares the frequency of YFP<sup>+</sup> cells in spleens of recipient mice at day 1 and day 10 (see Figure 3F) after adoptive transfer without tamoxifen treatment of donor cells (day 1, n=8; day 10, n=18; mean ± SD). *P* value was calculated by Mann-Whitney's U test (\*\*\*\**P* < 0.0001).

Figure S4



**Figure S4. Tamoxifen-inducible knockout of *Ifnar1*.** Novel R26CreERT2 x *Ifnar1<sup>fl/fl</sup>* mice were treated by oral administration of a single dose of 2 mg tamoxifen (+Tam). Mice were sacrificed 2 days later and genomic DNA was isolated from different tissue and subjected to PCR in order to verify efficiency and control of Cre-mediated recombination of *Ifnar1*. Untreated mice (-Tam) served as controls.