Inhibition of protein geranylgeranylation and farnesylation protects against graft-*versus*-host disease via effects on CD4 effector T cells

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Online Supplementary Design and Methods

Mice

C57BL/6 (H2[b], Thy-1.2), FVB/N (H2[q], Thy-1.1) and BALB/c (H2[d], Thy-1.2) mice were purchased either from Charles River Laboratory (Sulzburg, Germany) or from the local stock of the animal facility at Freiburg University. The luciferase (luc+) transgenic C57BL/6 mice have been previously described.[1] The animal protocols (G-08/102, X-10/13H) were approved by the Committee on the Use of Laboratory Animals at Albert-Ludwigs University Freiburg, Freiburg, Germany.

Murine cytomegalovirus model

At day 28 after infection the mice were sacrificed. The spleen was removed and used for flow cytometry-based analysis for murine cytomegalovirus (MCMV)-specific T cells. MHC class I tetramers presenting the MCMV epitopes pp89 (H-2L[d]) and M164 (H-2D[d]) were used for staining.[2] The tetramers were generated in the Pircher laboratory as described elsewhere.[3] The livers and salivary glands were homogenized by sonication. The viral load was determined by plaque assay on mouse embryonic fibroblasts.

Flow cytometry

The following antibodies were used for flow cytometric analysis: CD4 (GK 1.5/RM4-5), CD8 (53-6.7), CD25 (PC61), CD19 (6D5), Thy-1.1 (H1S51), H-K[b] (AF6-88.5), H-K[d] (GF1 1.1), CD45 (30F11), UEA-1 (polyclonal), PDGFR1 (CD140b) (APB5), MHCII (M5/114.15.2), CD62L (MEL-14), CD27 (LG.3A10), CD127 (A7R34), KLRG1 (2F1), Alexa Fluor 488-Phalloidin (Invitrogen), Mouse Vβ TCR Screening Panel (BD Biosciences).

References

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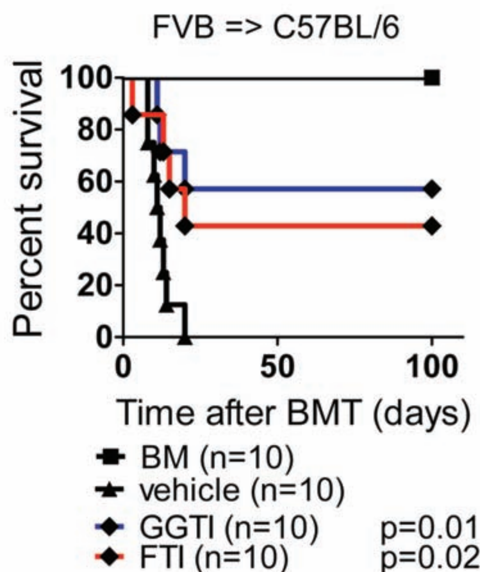
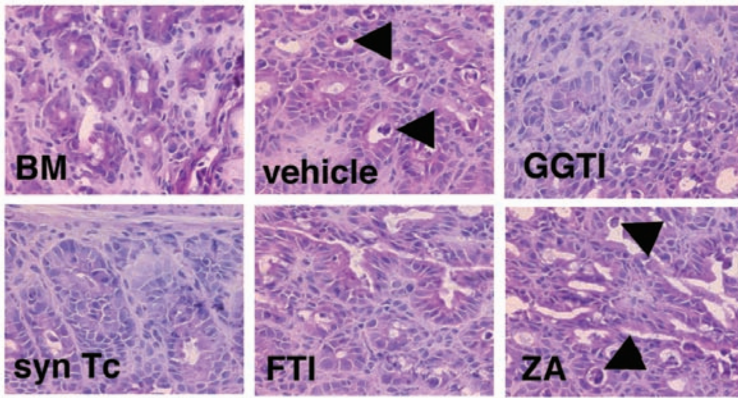
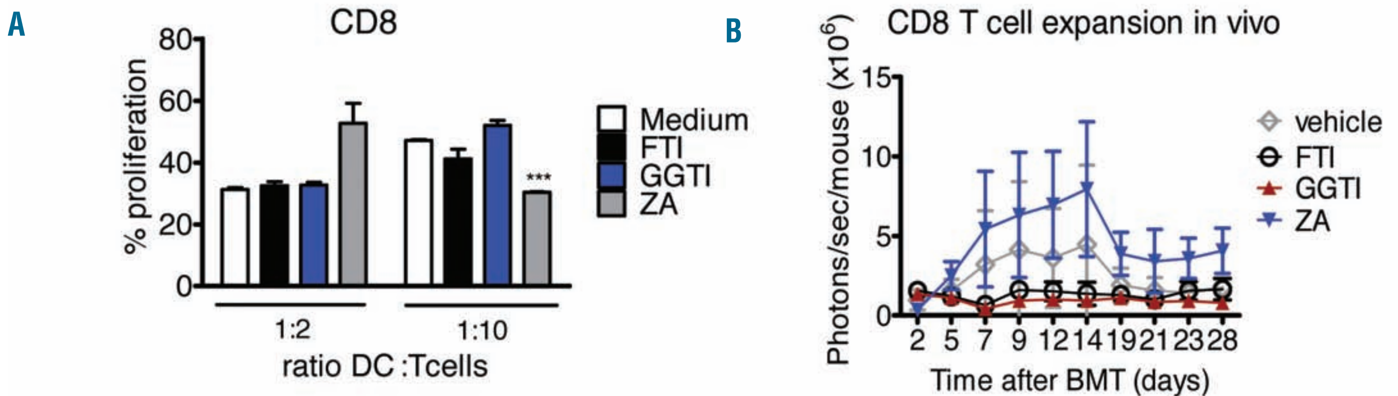
3. Altman JD, Davis MM. MHC-peptide tetramers to visualize antigen-specific T cells. Curr Protoc Immunol. 2003;Chapter 17:Unit 17.3.



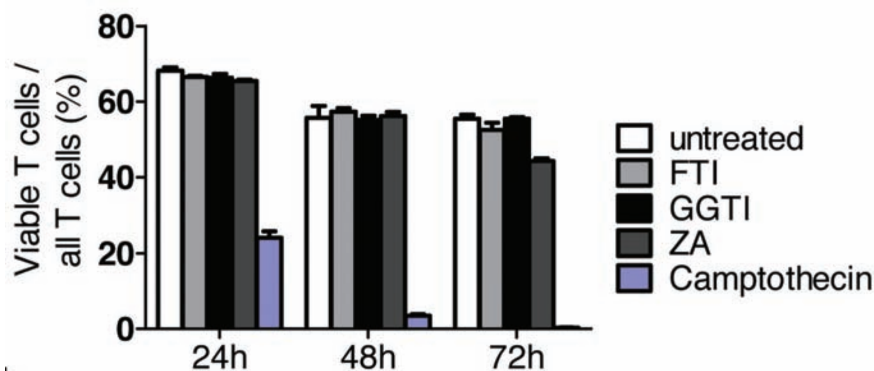
Online Supplementary Figure S1. Pretreatment with FTI/GGTI improves survival after major MHC mismatch bone marrow transplantation (BMT). Survival of mice receiving bone marrow alone (BM, black line, square), or with T cells and treatment with vehicle (black line, triangle), FTI (red line) or GGTI (blue line), in the FVB into C57BL/6 model. The following dosage was applied: GGTI-2133, 20 mg/kg day -1 to +10 and FTI-276, 20 mg/kg day -1 to +10. Percentage survival of BMT recipients is significantly higher as compared to the vehicle group when FTI or GGTI is given (number of animals and *P*-values for the comparison with the vehicle group are indicated next to each treatment group).



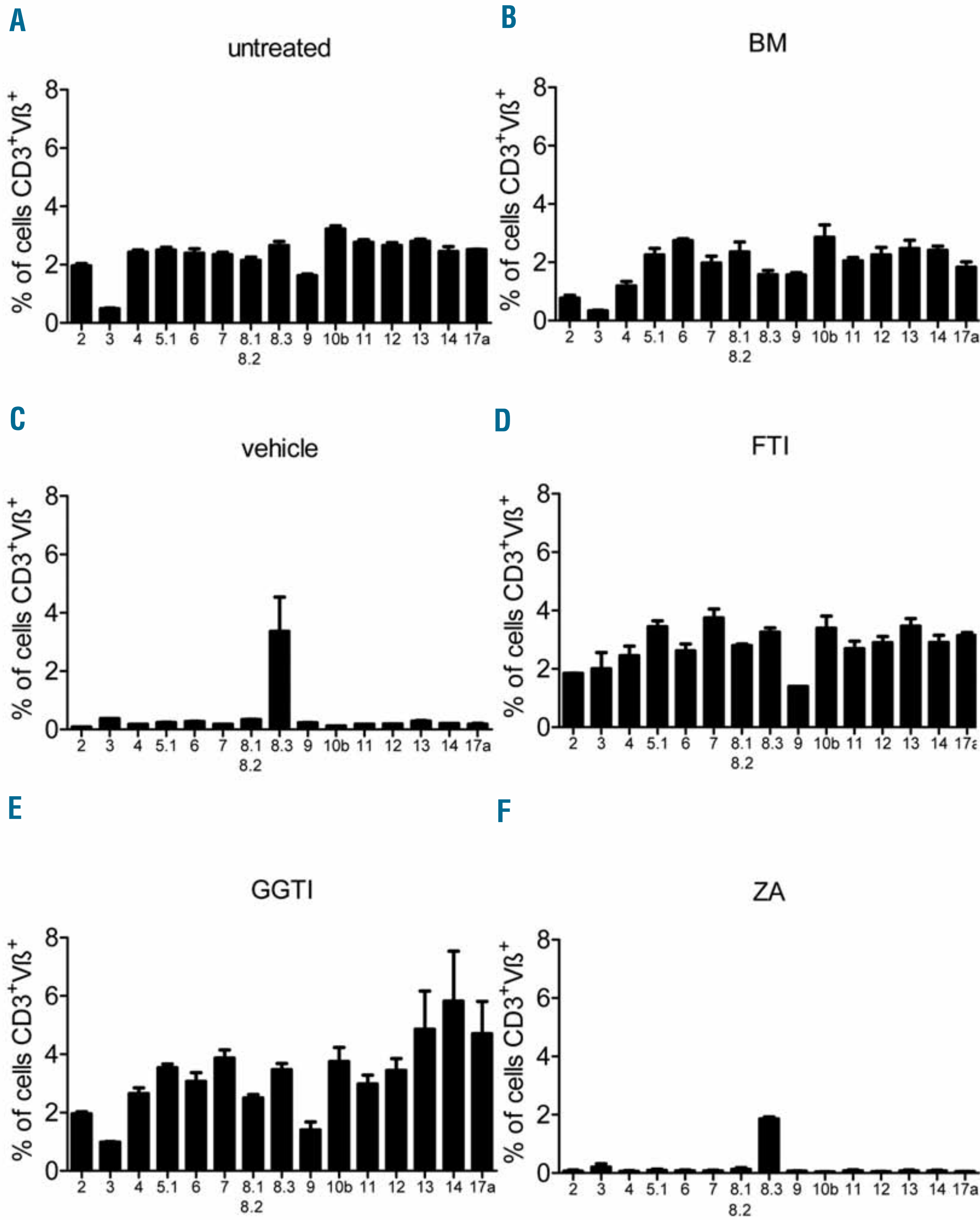
Online Supplementary Figure S2. FTI and GGTI treatment reduces GvHD severity. Ten days after transplantation, samples of small bowel, large bowel, the liver and skin were stained with hematoxylin/eosin. Representative colon samples from mice receiving bone marrow alone (BM), with syngeneic T cells (syn Tc), allogeneic T cells and treatment with vehicle (vehicle), FTI-276 (FTI), GGTI-2133 (GGTI) or zaragocic acid (ZA) are shown. Magnification x400. Arrows indicate crypt apoptosis.



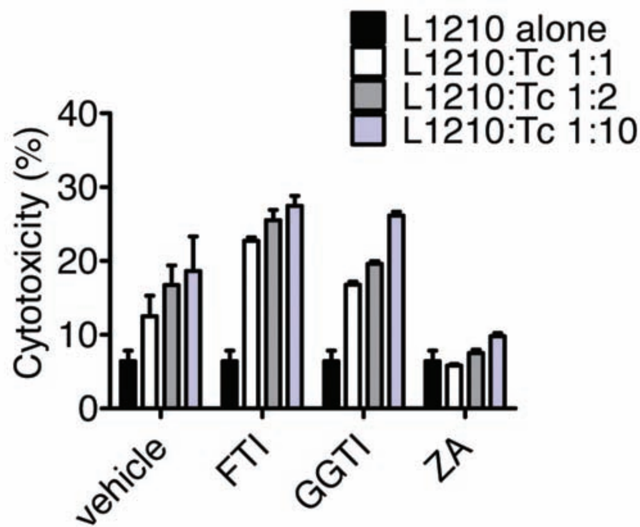
Online Supplementary Figure S3. Impact of FTI and GGTI on CD8 T-cell (Tc) expansion *in vitro* and *in vivo*. (A) *In vitro* expansion of CFSE-stained CD8 T cells ($H2^b$) stimulated with dendritic cells (DC) ($H2^d$). Comparison of the absolute values when FTI-276, GGTI-2133 or ZA were included as compared to alone as indicated. Proliferation was assessed as reduced CFSE intensity by flow cytometry. Ratios represent DC : Tc. Data from one of three independent experiments with comparable results are shown (***P*<0.01; ****P*<0.001). (B) Allogeneic HCT was performed as described in the *Design and Methods* section with only CD8 T cells being transferred, with at least three mice in each group. *In vivo* expansion of luc transgenic CD8 T cells ($H2^b$) in allogeneic recipients ($H2^d$) was monitored at serial time points. There was no significant difference between FTI/GGTI/ZA as compared to vehicle treatment with respect to expanding CD8 T cells, except for day 2 for ZA (*P*=0.045).



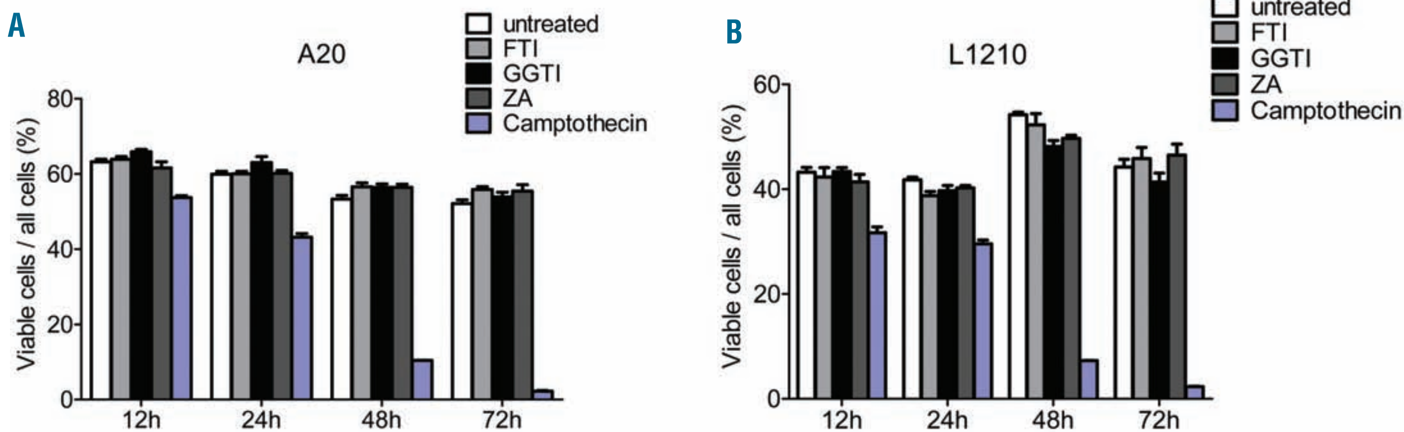
Online Supplementary Figure S4. FTI, GGTI or ZA had no effect on viability of T cells. T cells exposed to FTI, GGTI or ZA (10 μM) for different periods. One representative bar diagram of three independent experiments is shown.



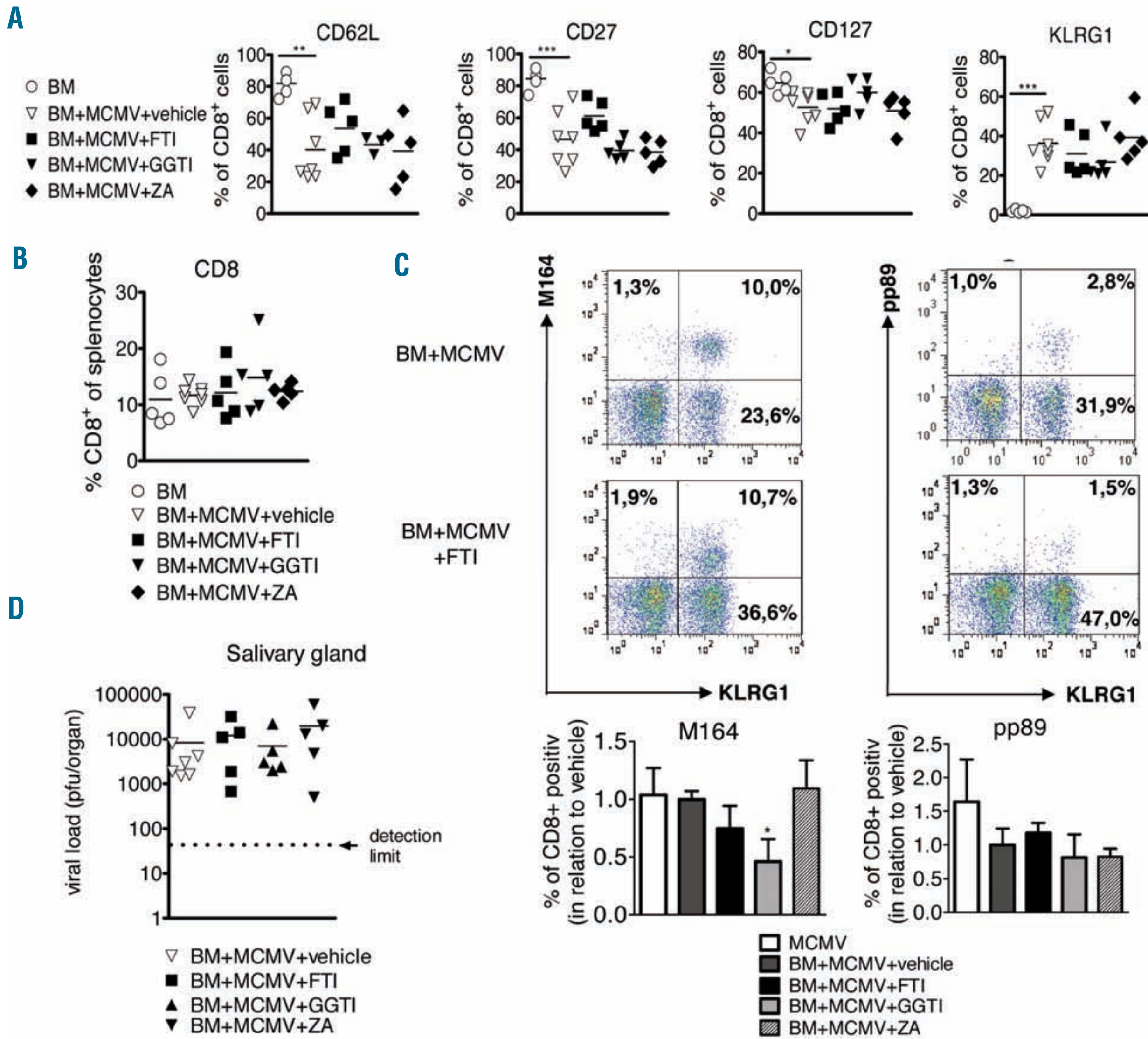
Online Supplementary Figure S5. FTI and GGTI favor a polyclonal TCR repertoire (A)-(F): TCR Vβ analysis by flow cytometry of splenic T cells (Tc) on day 20 after transplantation in BALB/c recipients is shown for the respective groups (untreated, BM only, +Tc/vehicle, +Tc/FTI, +Tc/GGTI and +Tc/ZA). Three mice in each group were analyzed. Analysis of T-cell Vβ expression was performed on day 20 after allogeneic HCT with the Mouse V TCR Screening Panel (BD, Germany) which recognizes Vβ 2, 3, 4, 5.1/5.2, 6, 7, 8.1/8.2, 8.3, 9, 10b, 11, 12, 13, 14, and 17a T-cell receptors by flow cytometry.



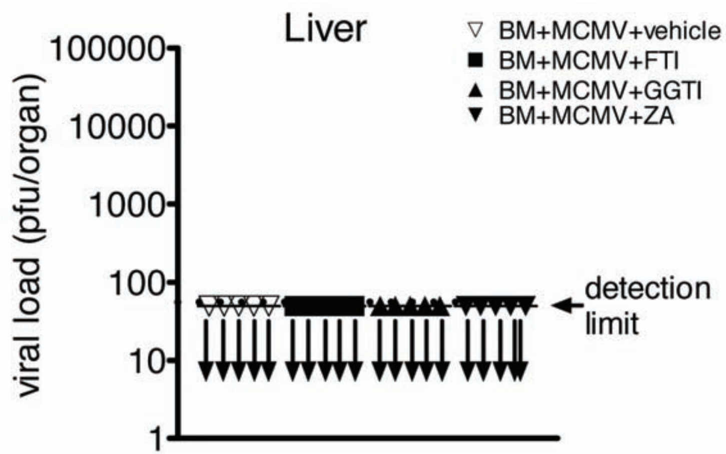
Online Supplementary Figure S6. FTI and GGTI do not influence anti tumour-activity *in vitro*. Allogeneic HCT was performed as described in the *Design and Methods* section with $5x10^5$ CD4/CD8 T cells. Mice were treated with: GGTI-2133, 20 mg/kg from day -1 to +10 and FTI-276, 20 mg/kg from day -1 to +10 or ZA, 10mg/kg from day -1 to +10. On day 12 CD4/CD8 T cells were isolated and were used in killing assays against L1210 cells. Data from one representative experiment of two with four mice in each group are shown.



Online Supplementary Figure S7. FTI and GGTI have no toxic effects on tumor cell lines. A20 (A) or L1210 (B) tumor cell lines were incubated with 10 μM of FTI, GGTI or ZA. Viability was measured by flow cytometric analysis of annexin/propidium iodide. One representative bar diagram of three independent experiments is shown.



Online Supplementary Figure S8. FTI does not interfere with anti-viral immune responses. Bone marrow (BM) transplantation was performed as described in the *Design and Methods* section without T cells. For all scatter dot plot diagrams each data point represents an individual animal. The following dosages were applied: GGTI-2133, 20 mg/kg day -1 to +10, FTI-276, 20 mg/kg day -1 to +10, and ZA, 10 mg/kg day -1 to +10. One group which did not receive a BMT was infected with MCMV. (A) Splenocytes were isolated on day 58 after BMT and stained against CD8 in combination with CD62L, CD27, CD127 and KLRG1. Percentages of cells positive for CD8 and the respective marker are displayed (n=5 per group). (*P<0.05,**P<0.01, ***P<0.001). (B) Percentage of CD8 positive cells in the spleen for the indicated groups are shown (n=5 per group). (C) Upper panel: representative flow cytometry analysis for the infected groups after gating on CD8 (n=5 per group). Lower panel: percentage of tetramer-positive cells within the CD8-positive splenocyte fraction (M164: vehicle *vs.* FTI P=0.28; vehicle *vs.* ZA P=0.72 pp89: vehicle *vs.* FTI: P=0.54; vehicle *vs.* GGTI P= 0.67; vehicle *vs.* ZA P=0.53). (D) Viral load in the salivary glands determined by the plaque assay is given for the individual groups (n=5 per group).



Online Supplementary Figure S9. **FTI does not interfere with anti-viral immune responses.** Bone marrow transplantation (BMT) was performed as described in the *Design and Methods* section without T cells. Viral load in the liver on day 58 after BMT, determined by a plaque assay, is shown for the individual groups (n=5 per group).