# Supplementary information

## Type I interferon shapes brain distribution and tropism of tick-borne flavivirus

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**Other supplementary materials for this manuscript include the following:**

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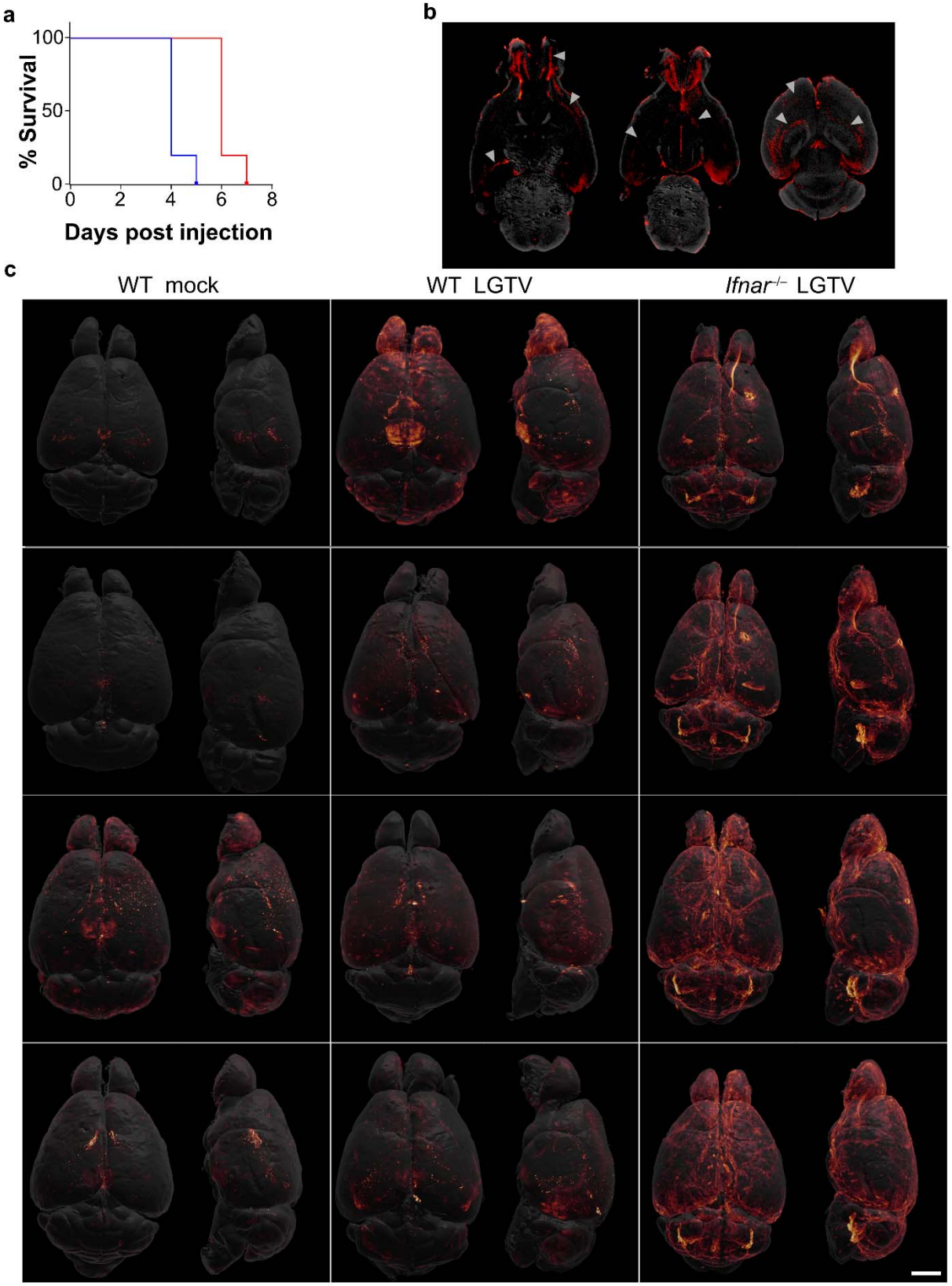
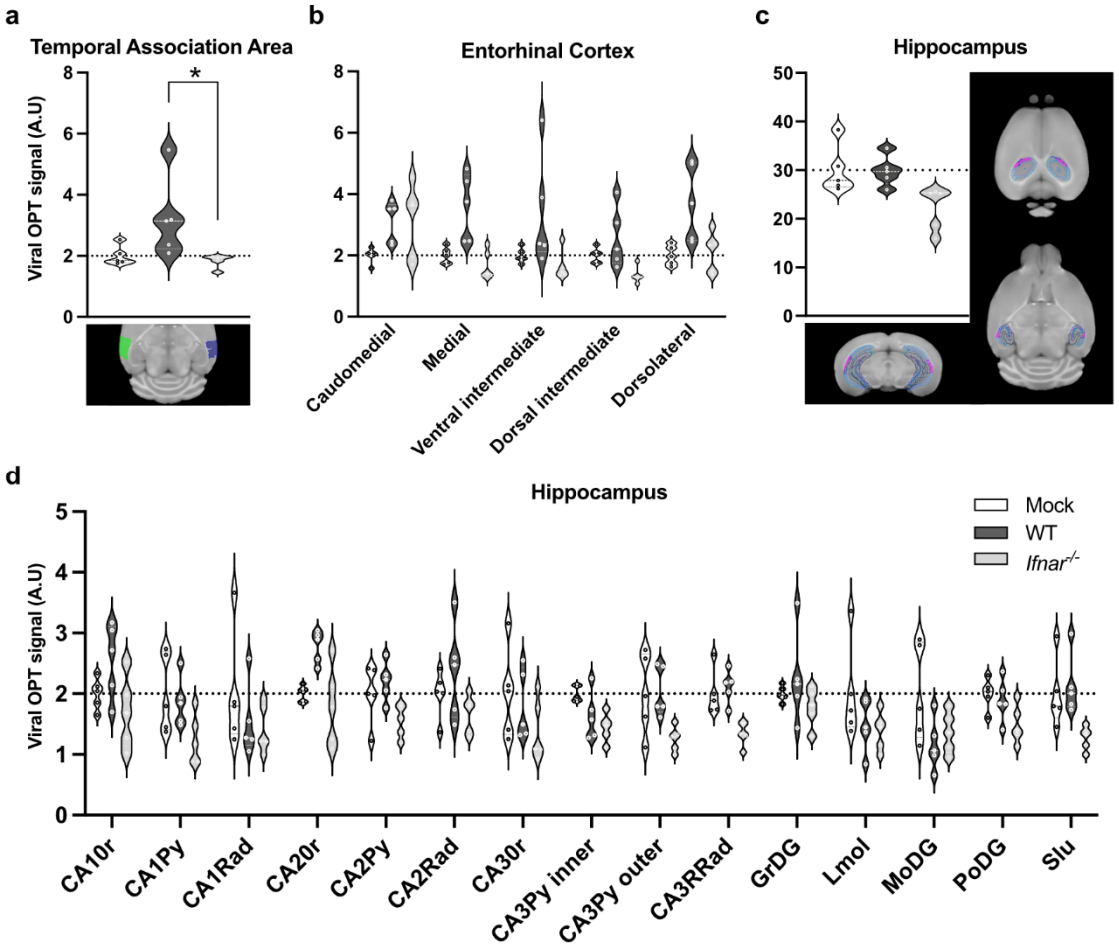
Supplementary Data 1-2

Source Data file

## Supplementary figures



**Supplementary Fig. 1. IFN-I response influences host survival and distribution of LGTV infection. a** Survival analysis of WT and *Ifnar*$^{-/-}$ mice, intracranially injected with 1000 pfu of LGTV (n = 5). Survival differences between groups were significant (log-rank test, p = 0.0031). **b** OPT cross sections of a representative *Ifnar*$^{-/-}$ brain showing antibody penetration in the deeper areas of the brain (arrows). **c** OPT-scanned immunolabeled brain reveals the distribution of LGTV infection in the adult mouse brain. Volumetric 3D render of supplementary OPT scans of the brain from mock and LGTV infected mice immunolabeled with antibodies against viral NS5. The signal intensity was normalized within an individual brain and adjusted to identical minimum and maximum. The viral signal was overlaid onto the anatomical outlines created from iso-surface rendering of the autofluorescence signal of each brain. For each image pair, the top and lateral views of the same specimen are shown. Scale bar = 2000 µm. Source data are provided as a Source Data file.

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60 **Supplementary Fig. 2. Image and quantification of viral infection in cerebral cortex regions.**
61 Quantification and statistical analysis (two-tailed unpaired t-test (a and c) with Holm-Sidak multiple
62 comparison correction (c and d) of viral OPT signal in **a** temporal association area (*p=0.047), **b** individual
63 VOIs in the composite entorhinal cortical region, **c** composite hippocampal region, and **d** individual VOIs in
64 the hippocampal region. Source data are provided as a Source Data file.

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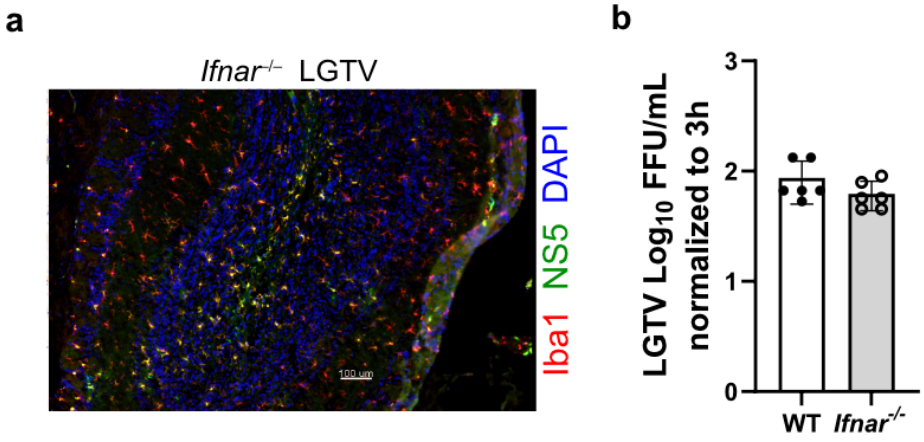
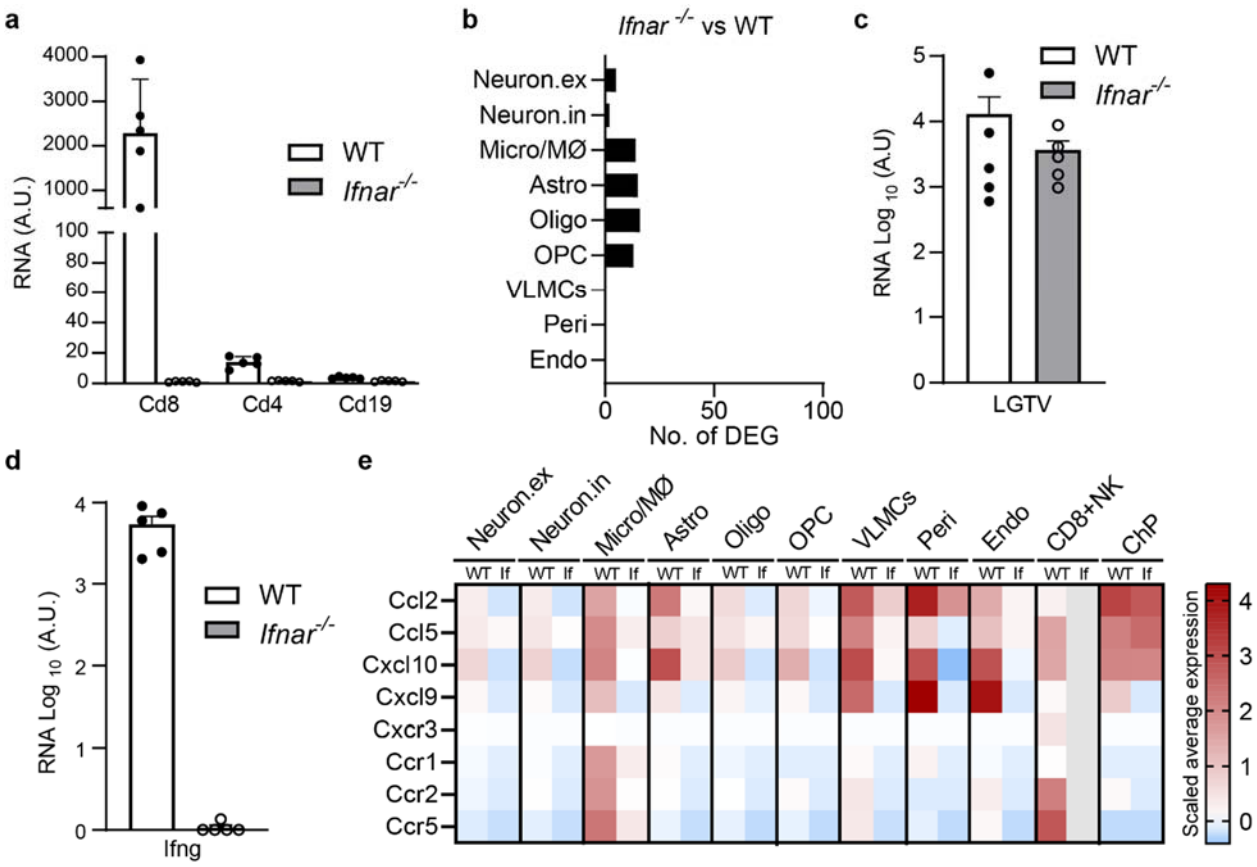
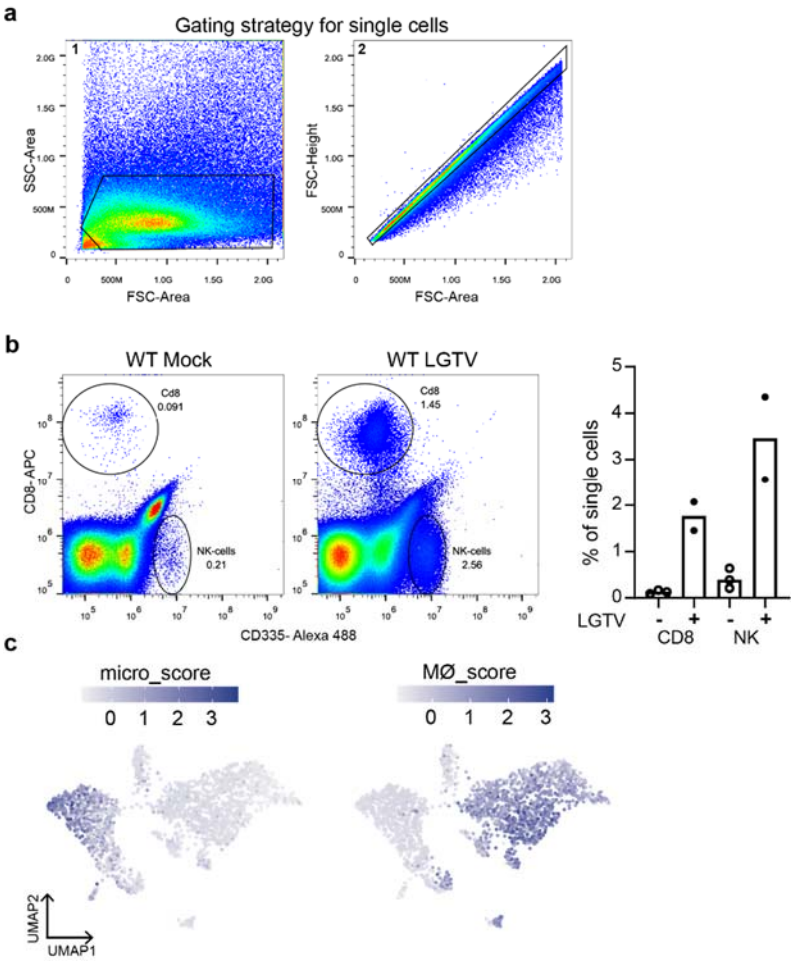



**Supplementary fig. 3 Microglia staining of OB and primary microglia infected with LGTV *in vitro***. a) Maximum-intensity projection of confocal z-stack. The images were taken from sagittal brain sections (10 µm) using confocal microscope, representative picture of OB is shown. Scale bars = 100 µm. The sections were immunolabeled using anti-NS5 (green), anti-Iba1 (red) for microglia, and DAPI for nucleus (blue). b) Primary microglia were isolated from WT and *Ifnar*$^{-/-}$ brains. The cells were infected with LGTV (MOI 1) for 72 h and viral growth was measured by focus forming assay, bars show mean ± SD (n = 6). Source data are provided as a Source Data file.




**Supplementary Fig. 4. Transcriptional differences and viral load in WT and *Ifnar*$^{-/-}$ mice. a** Gene expression of *Cd8*, *Cd4* and *Cd19* in cortex of infected WT (n=5) and *Ifnar*$^{-/-}$ (n=5) mice. Quantified by qPCR and normalized to housekeeping gene *Gapdh,* bars show mean ±SD. **b** Number of DEGs (log2FC>1, padj < 0.05) between *Ifnar*$^{-/-}$ and WT mice in uninfected samples. **c** Viral load in cerebral cortex at the humane endpoint in WT (n=5) and *Ifnar*$^{-/-}$ mice (n=5) infected with 10,000 PFU intracranially. Quantified by qPCR and normalized to housekeeping gene *Gapdh*. Bars represent mean ±SEM. **d** Gene expression of *Cd8*, *Cd4* and *Cd19* in cortex of infected WT (n=5) and *Ifnar*$^{-/-}$ (n=5) mice, bars show mean ±SEM. Quantified by qPCR and normalized to housekeeping gene *Gapdh*. **e** Heatmap showing expression of T cell chemoattractants and corresponding receptors upon infection in WT and *Ifnar*$^{-/-}$ (If) mice. Source data are provided as a Source Data file.




**Supplementary Fig. 5. Infiltration of immune cells in WT and *Ifnar*$^{-/-}$ mice. a** Representative scatterplots showing gating strategy based on forward scatter (FSC) and side scatter (SSC) for obtaining single cells used in downstream analysis of brain leucocytes. **b** Flow cytometry of brain leukocytes from the cerebral cortex of untreated and infected WT mice. Representative scatterplots and percentage CD8+ T cells and NK cells (CD335$^{+}$). Boxes represent means of untreated (n=3) and LGTV infected (n=2). **c** Subclustering of 2,363 nuclei of micro/MØ cell subset belonging to all datasets, colored by gene signatures for microglia (*Cx3cr1, P2ry12, Slco2b1, Tmem119*) or MØ (*Slfn4, Ms4a8a, Clec4e, Itga4*) (PMID: 31325960). Source data are provided as a Source Data file.

**Supplementary Table 1. Key Antibody Table**

| Antibodies | Origin | Clonality | Clone name | Working dilution | Use in the current study | Company (Cat#) |
|---|---|---|---|---|---|---|
| **Anti-chicken Alexa Fluor 488** | Goat | Polyclonal | - | 1:400 | IHC | Thermo Fisher Scientific (A11039) |
| **Anti-chicken Alexa Fluor 555** | Goat | Polyclonal | - | 1:500 | IHC | Thermo Fisher Scientific (A21437) |
| **Anti-chicken Alexa Fluor 680** | Goat | Polyclonal | - | 1:500 | OPT, IHC | Abcam (ab175779) |
| **Anti-mouse Alexa Fluor 647** | Goat | Polyclonal | - | 1:500 | IHC | Abcam (ab150115) |
| **Anti-rabbit Alexa Fluor 488** | Donkey | Polyclonal | - | 1:500 | IHC | Thermo Fisher Scientific (A21206) |
| **Anti-rabbit Alexa Fluor 594 (pre-absorbed)** | Donkey | Polyclonal | - | 1:500 | OPT | Abcam (ab150064) |
| **Anti-mouse CD45 Alexa 488** | Rat | Monoclonal | 30F-11 | 1:100 | IHC | Thermo Fisher Scientific (53-0451-82) |
| **AQPI** | Mouse | Monoclonal | sc-32737 | 1:50 | IHC | Santa Cruz Biotechnology (sc-32737) |
| **CD8** | Rat | Monoclonal | Clone 53-6.7 (RUO) | 1:200 | IHC | BD Pharmigen (561093) |
| **CD335** | Rat | Monoclonal | 29A1.4 | 1:500 | IHC | Thermo Fisher Scientific (AB_1724164 |
| **Doublecortin** | Rabbit | Polyclonal | - | 1:800 | IHC | Cell Signaling (4604) |
| **Iba1** | Rabbit | Polyclonal | - | 1:400 | IHC | Histolab (CP290) |
| **NS5*** | Chicken | Polyclonal | - | 1:1000 | OPT, IHC | Agrisera AB |
| **TMEM119** | Rabbit | Monoclonal | 28-3 | 0.5µg/ml | IHC | Abcam (ab209064) |
| **TUBB3** | Rabbit | Polyclonal | - | 1:3000 | IHC | BioLegend (PRB-435P) |

*Affinity-purified NS5 antibody was produced in chicken, according to the manufacturer's protocol, using the following peptide sequence of NS5 from tick-borne encephalitis virus strain Torö (GenBank: DQ401140): (carboxylated)-CMDRHDLHWELRLESS-(amidated).