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Supplemental information

Organotypic endothelial adhesion molecules are key for *Trypanosoma brucei* tropism and virulence

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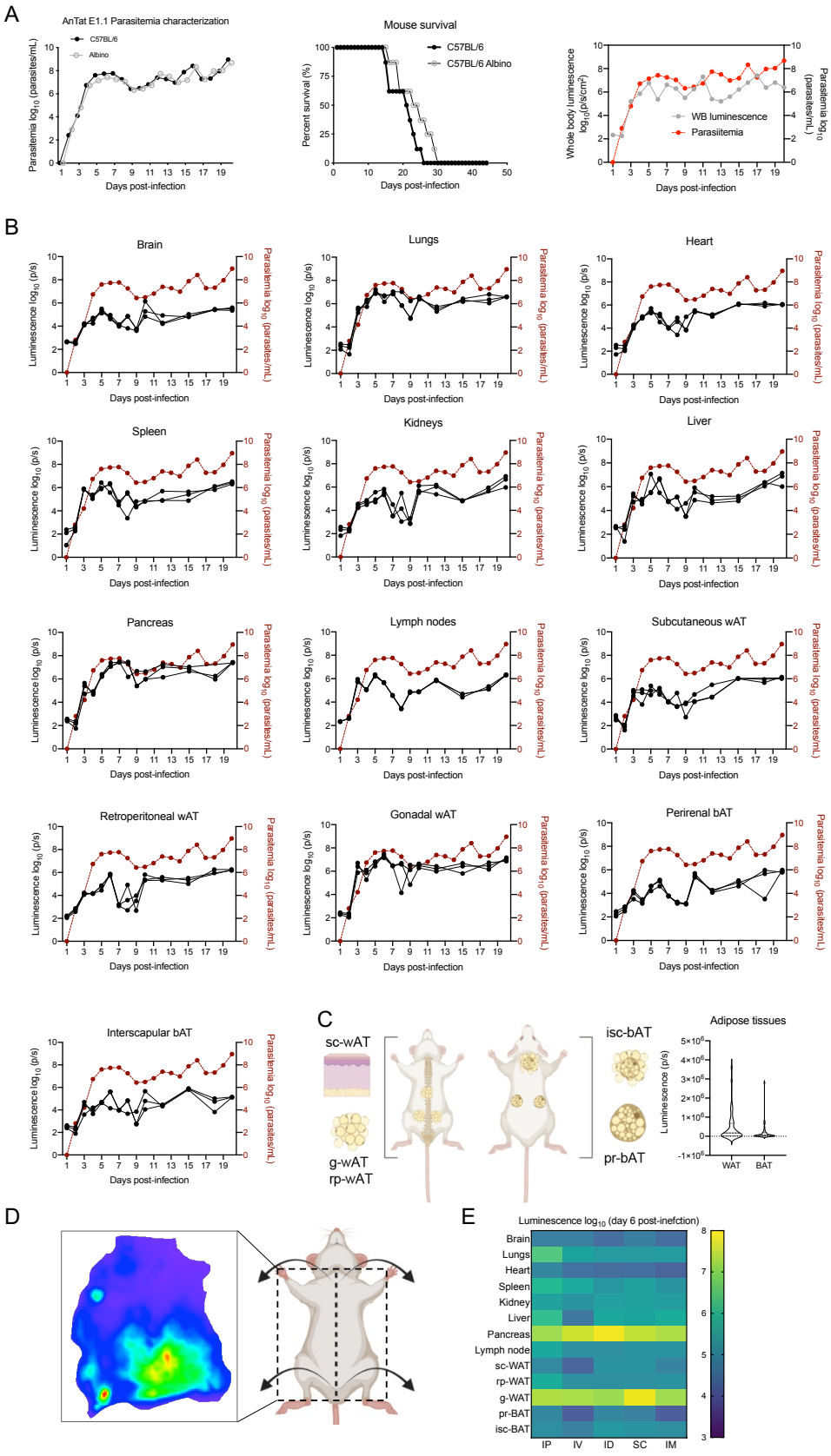


Figure S1. Characterization of the triple reporter *T. brucei* line, and parasite density measured by bioluminescence in mice individual organs. Related to Figure 1. A. C57BL/6 WT and Albino mice (males, 8 weeks old) were infected with a *T. brucei* reporter line expressing

TY1, TdTomato and Firefly luciferase, and their parasitemia (haemocytometer) (left panel), survival (middle panel) and whole body bioluminescence (left panel) were determined. **B.** Bioluminescence imaging of non-perfused organs was performed using an IVIS Lumina system, following injection with RediJect luciferin. While Figure 1 shows the organ mean of 3 separate experiments as a viridis map, Figure S1 shows the mean independent values of each organ separately, as well as the parasitemia curve (in red). Each black line is the log_{10} value of 3 independent experiments with 3 mice per experiment. **C.** Schematic showing the location of brown and white adipose tissue depots investigated in this study. Violin plots show the global comparison of parasite density (measured by bioluminescence) in WAT and BATs. **D.** Representative image of *T. brucei* distribution in the whole skin of a mouse at day 6 post-infection. **E.** Viridis map shows parasite density on day 6 post-infection when infection was initiated by different routes: intraperitoneal, intravenous, intradermal, sub-cutaneous and intramuscular. Values show the mean of 6 mice, which were perfused prior to scoring bioluminescence.

*All relevant data used to generate this figure is included in Data S1 (Tabs 1-5).

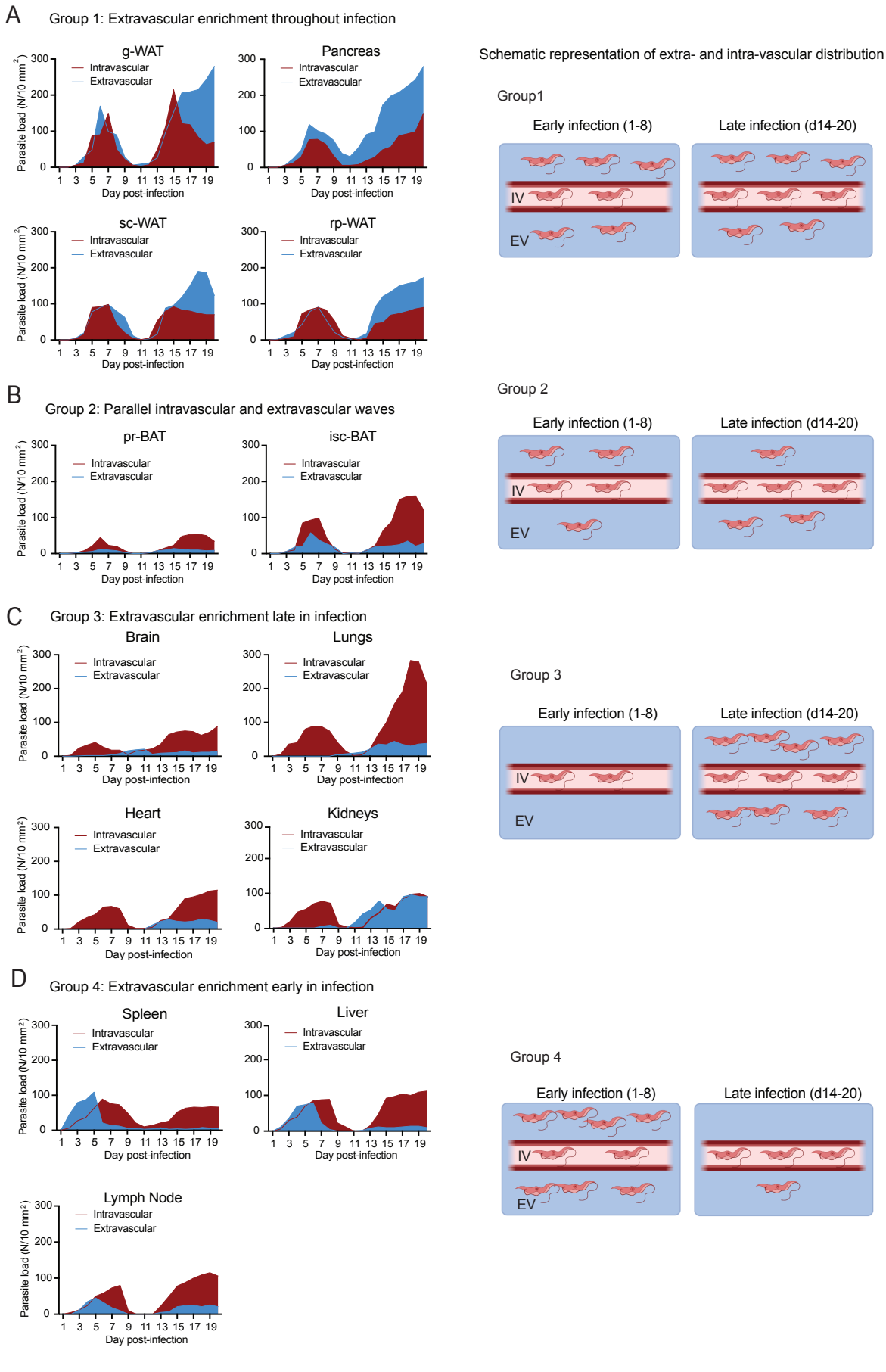

A
Group 1: Extravascular enrichment throughout infection
g-WAT
Pancreas
sc-WAT
rp-WAT
Intravascular
Extravascular
Parasite load (N/10 mm²)
Day post-infection
B
Group 2: Parallel intravascular and extravascular waves
pr-BAT
isc-BAT
C
Group 3: Extravascular enrichment late in infection
Brain
Lungs
Heart
Kidneys
D
Group 4: Extravascular enrichment early in infection
Spleen
Liver
Lymph Node
Schematic representation of extra- and intra-vascular distribution
Group1
Early infection (1-8)
Late infection (d14-20)
IV
EV
Group 2
Group 3
Group 4

Figure S2. Intravital imaging and *ex vivo* imaging of intravascular and extravascular parasites. A-D. Related to Figure 1. Number of parasites per vascular area (normalized to 10 mm^2) quantified by intravital and *ex vivo* imaging in each organ in the extravascular (blue) and intravascular (red) space throughout infection. Relative parasite distribution in these two compartments is represented in four groups of similar phenotype**.** Schematic representation of parasite distribution at early and late times post-infection are shown for each group. *All relevant data used to generate this figure is included in Data S1 (Tab 4).

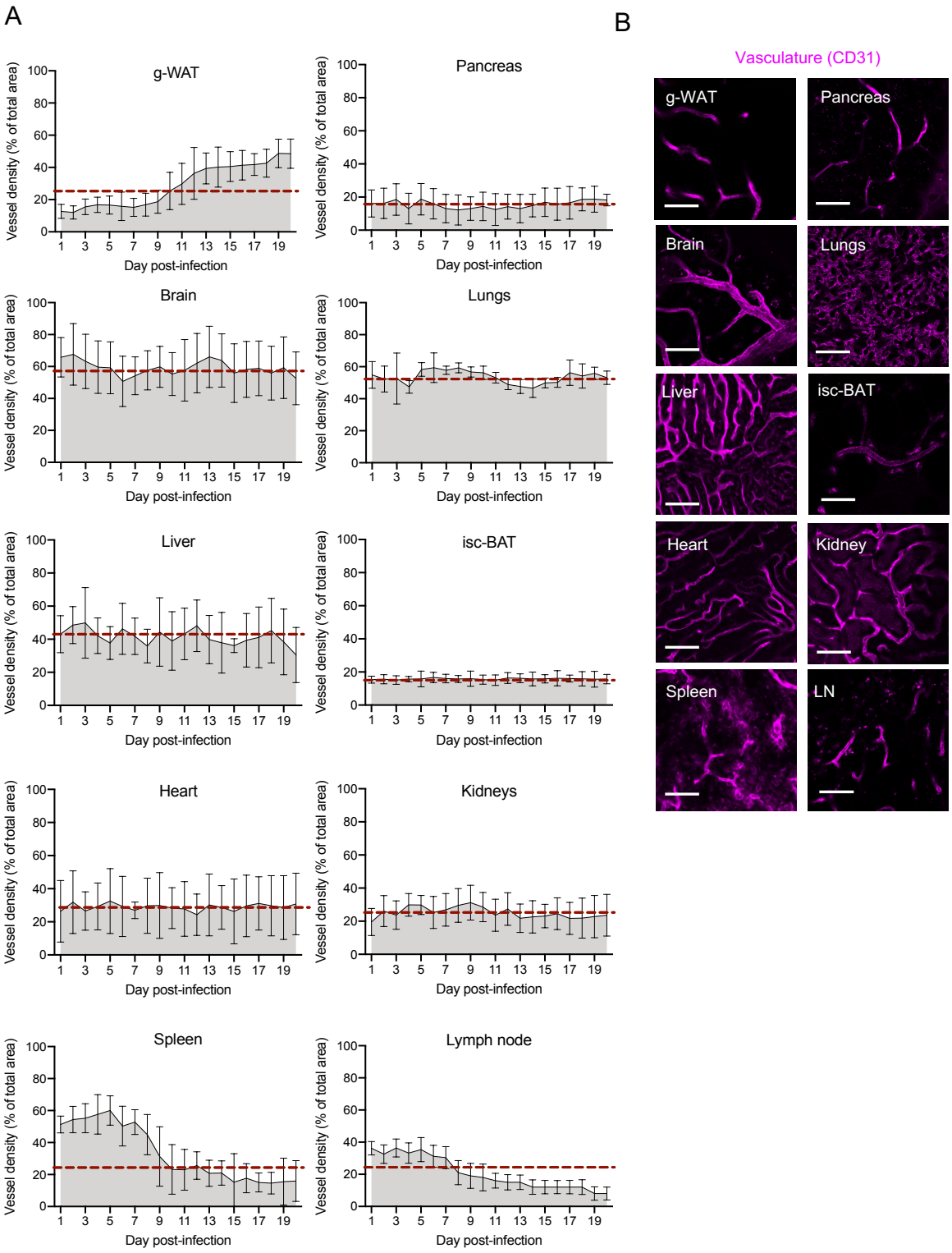


Figure S3. Changes in vascular density per organ throughout infection. Related to Figure 2. A. Vascular density is calculated as the area occupied by the vessel divided by the total area of the field of view were performed following vascular segmentation based on the marker CD31, as

shown in Figure 2. This figure shows the individual measurements, with SD for each organ. The red dotted line represents the organ average vascular density throughout infection. **B.** Representative figures for each organ showing CD31 labeling are included. Scale bar = 50 μm. *All relevant data used to generate this figure is included in Data S1 (Tab 6).

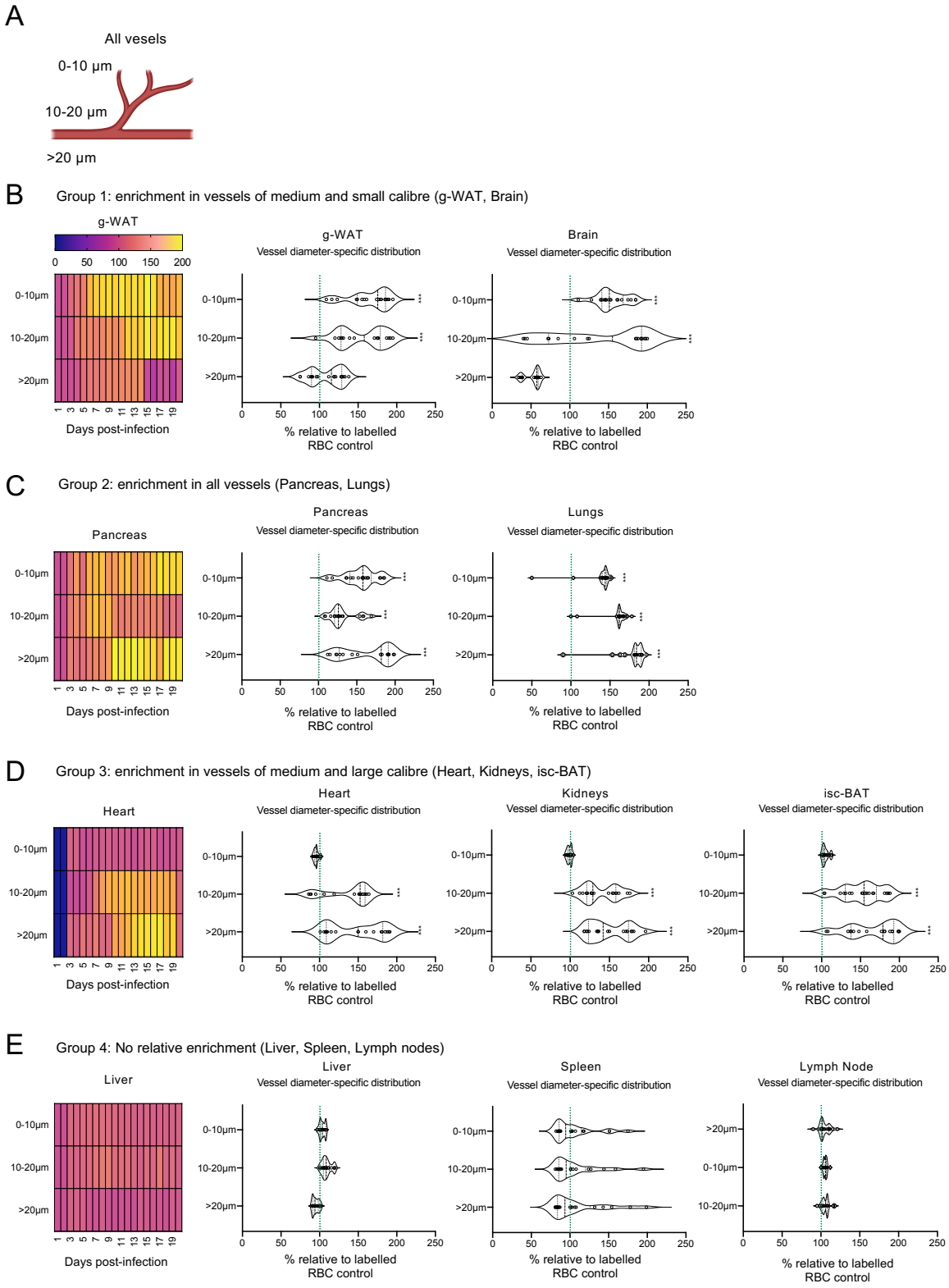


Figure S4. Distribution of *T. brucei* across vasculature of different diameters is organ-specific. Related to Figure 3. A. Schematic of vascular branches and respective diameters. **B-E**

left panels. Heat-maps of parasite distribution in vasculature, relative to labeled RBCs. Organs were grouped by similar parasite distribution. **B-E violin plots.** Violin plots showing quantifications of parasite enrichment relative to labeled RBCs (marked as a green dotted line) divided by vessel diameter. For all graphs, significance relative to RBC normalizers is shown as $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*). *All relevant data used to generate this figure is included in Data S1 (Tab 8).

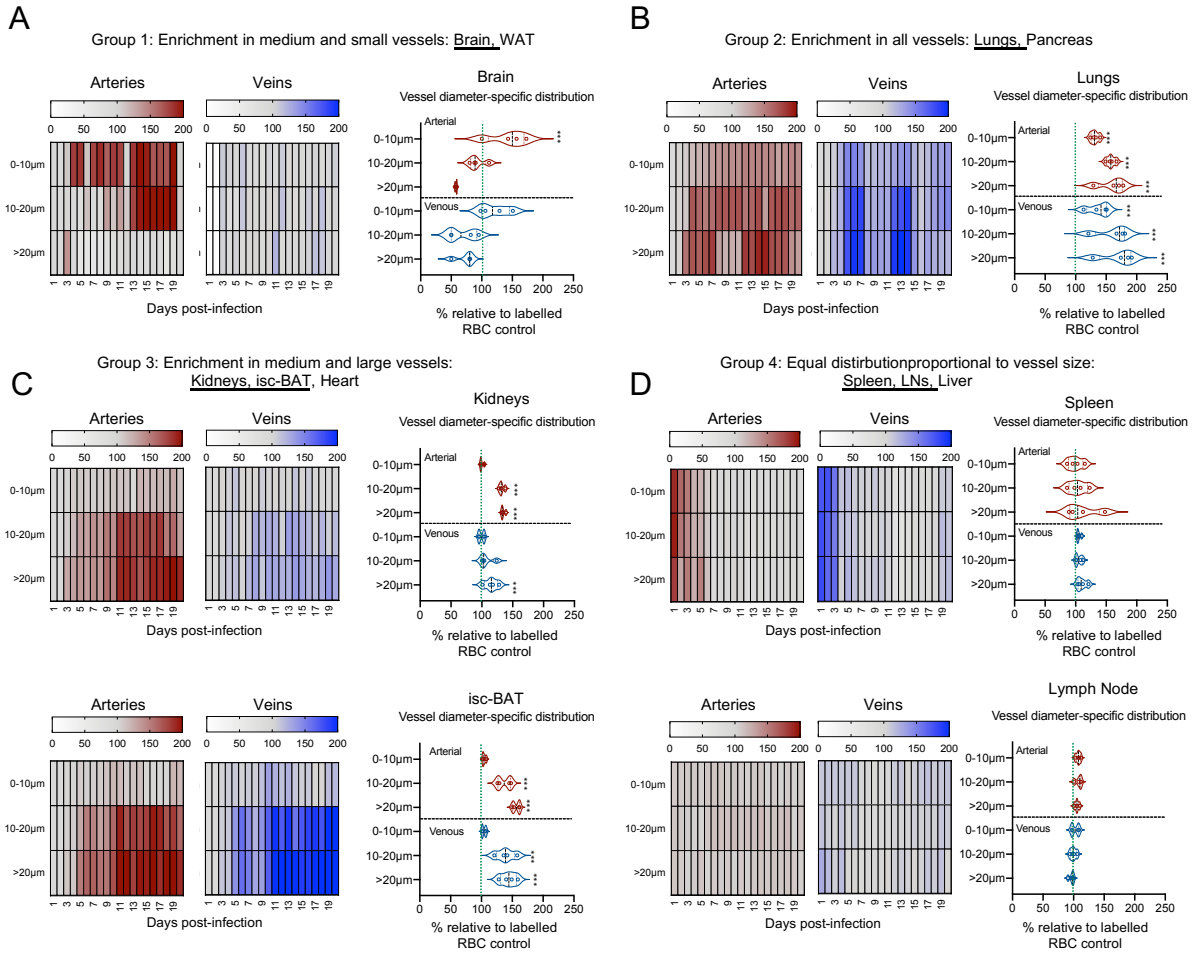
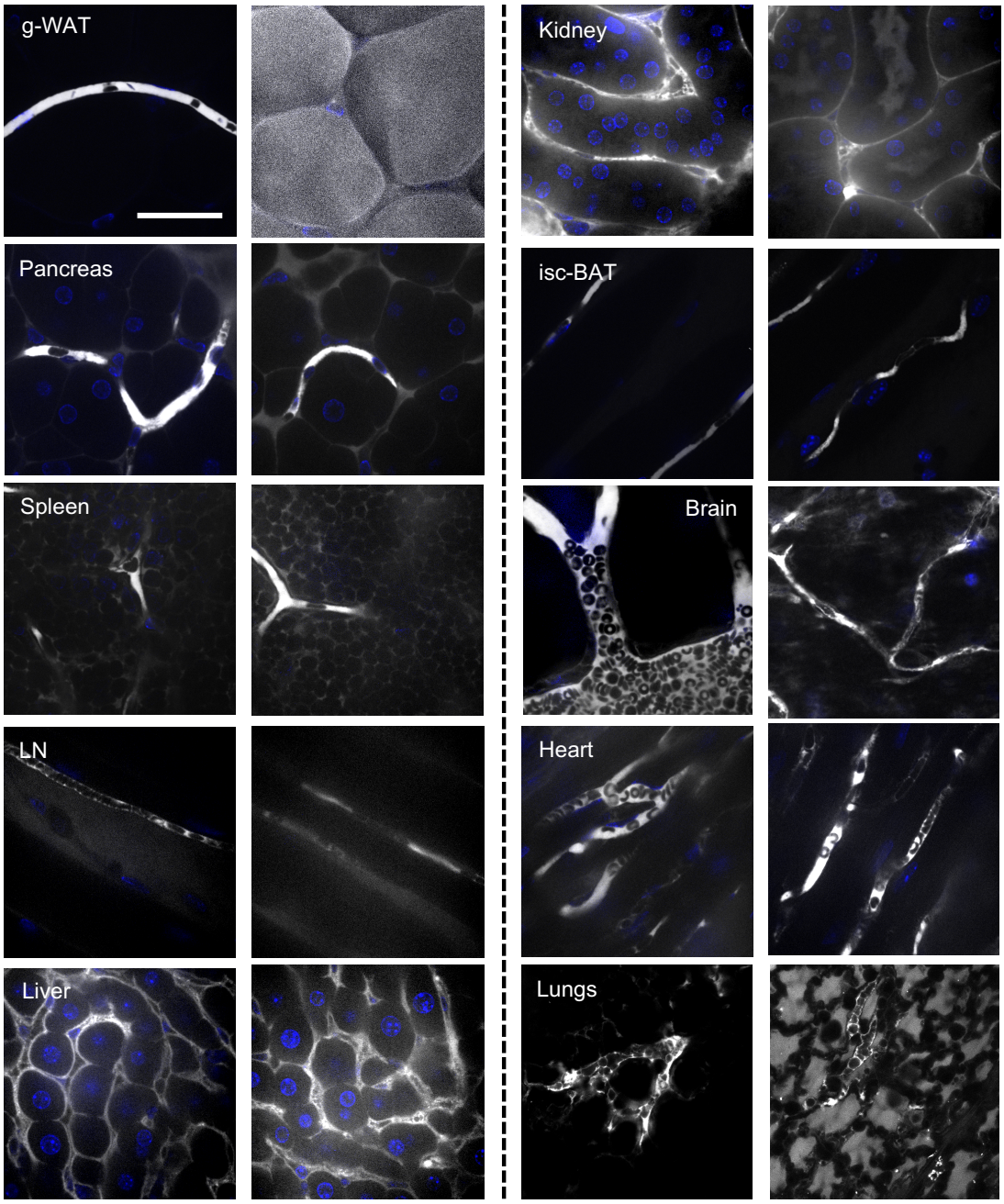
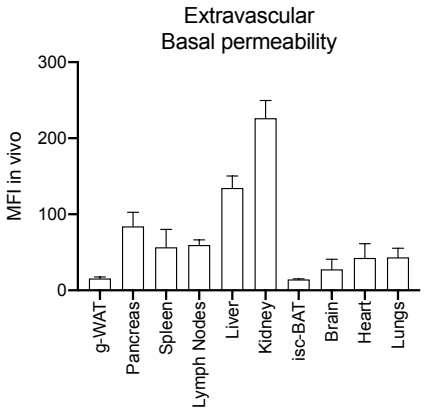


Figure S5. Distribution of *T. brucei* across vasculature is organ-specific. Related to Figure 3. Organs were grouped by similar parasite distribution. **A-D.** Heat-maps of parasite distribution in vasculature, relative to labeled RBCs. Panels are divided as arterial vasculature (left) and venous vasculature (right). Organs were grouped by similar parasite distribution. **A-D.** Violin plots showing quantifications of enrichment relative to loaded RBCs (marked as a vertical green dotted line). Plots show distribution of parasites by vessel diameter (0-10, 10-20 and >20 μm) and vessel type (arterial or venous). Data for other organs is shown in Figure 3. Significance between vasculature of different diameters per vessel type is defined as follows: $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*). *All relevant data used to generate this figure is included in Data S1 (Tab 9).

A


g-WAT
Kidney
Pancreas
isc-BAT
Spleen
Brain
LN
Heart
Liver
Lungs

B


Extravascular
Basal permeability
MFI in vivo
300
200
100
0
g-WAT
Pancreas
Spleen
Lymph Nodes
Liver
Kidney
isc-BAT
Brain
Heart
Lungs

C

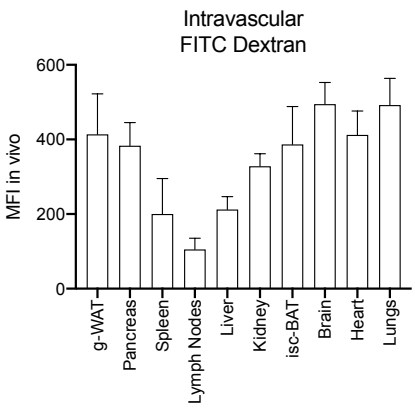

Intravascular
FITC Dextran
MFI in vivo
600
400
200
0
g-WAT
Pancreas
Spleen
Lymph Nodes
Liver
Kidney
isc-BAT
Brain
Heart
Lungs

Figure S6. Basal vascular permeability in uninfected mice. Related to Figure 4. Control mice were injected with 70kDa FITC-Dextran (shown in grey values) and Hoechst (blue). **(A)** Intravital images were acquired from 10 organs. Two representative images per organ are included. Scale bar, 50 μm. **(B)** MFI values of the extravascular **(C)** and intravascular niche are included. Error bars show SEM values. Quantifications were obtained from at least 100 fields of view in at least 3 separate animals. These values were taken as baseline for all quantifications relative to Figure 4. *All relevant data used to generate this figure is included in Data S1 (Tab 10).

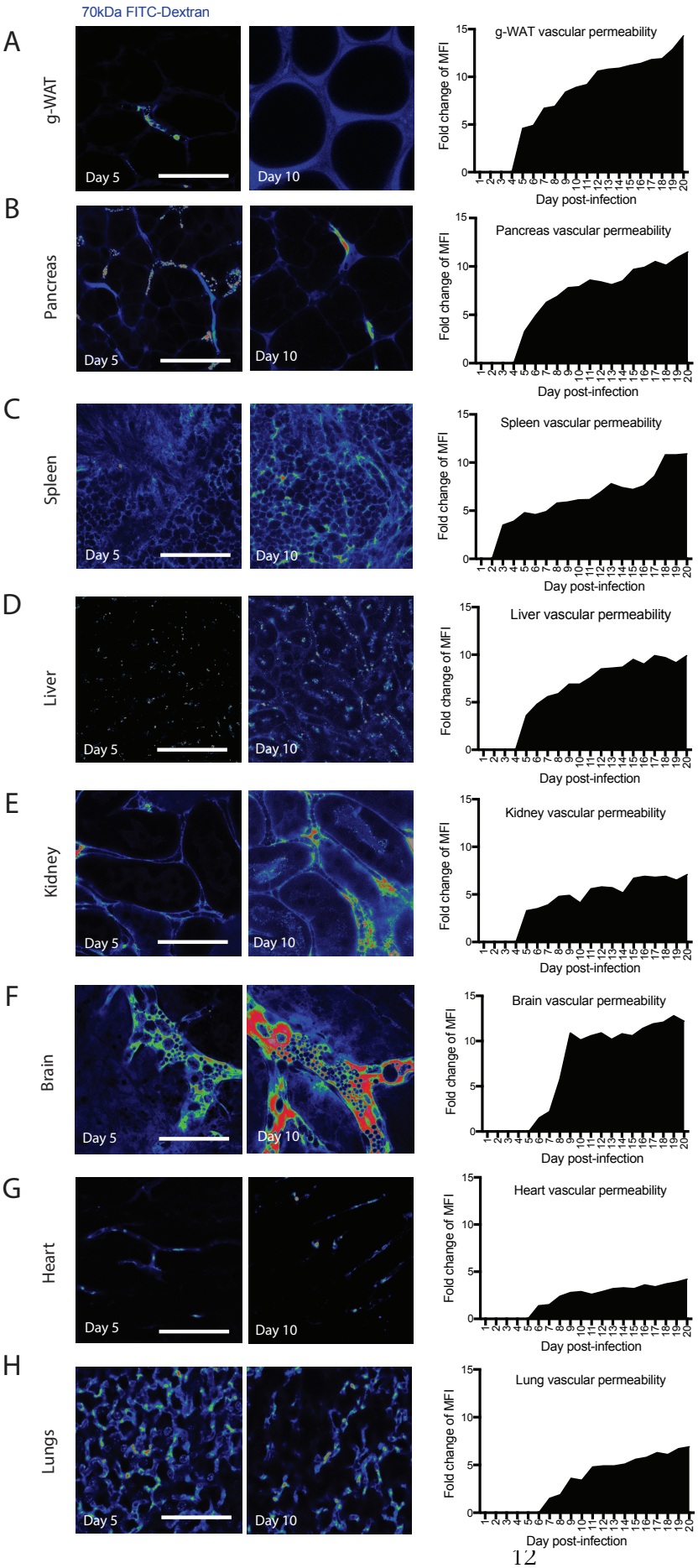
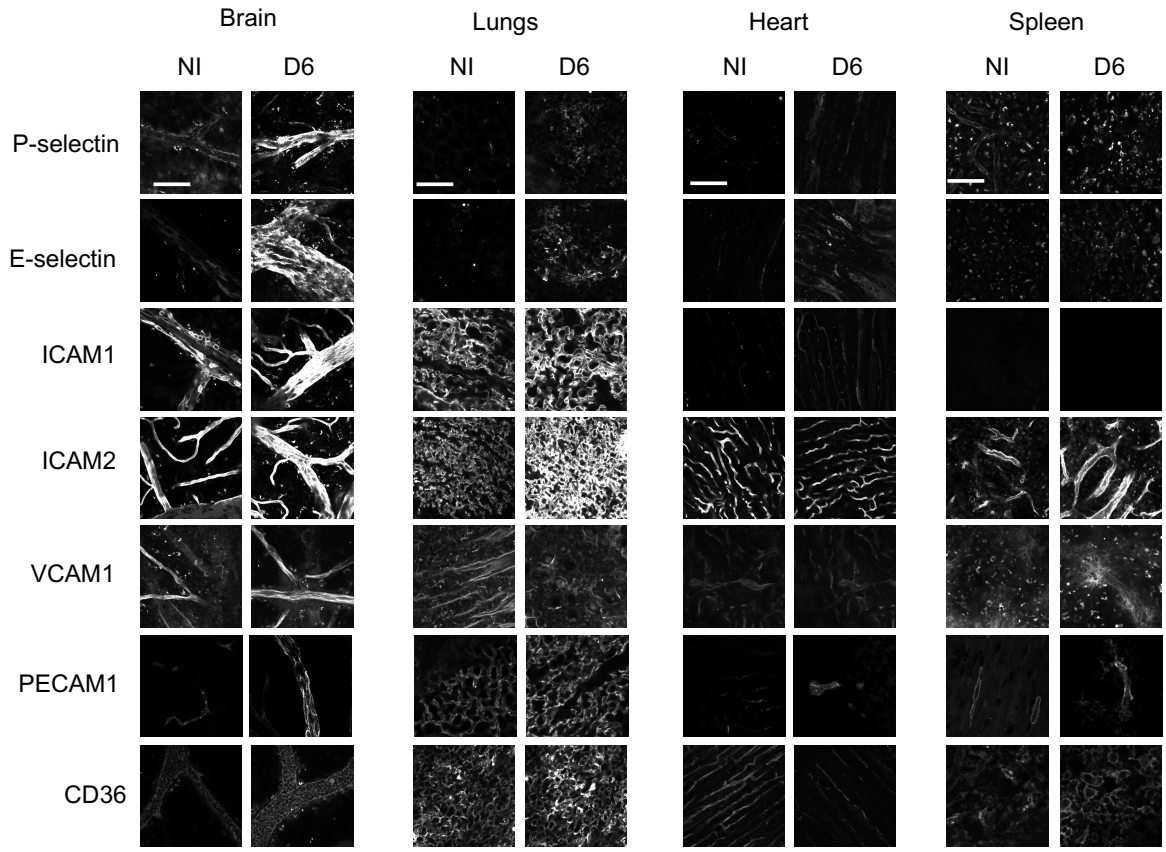

70kDa FITC-Dextran
A
g-WAT
Day 5
Day 10
g-WAT vascular permeability
Fold change of MFI
Day post-infection
B
Pancreas
Day 5
Day 10
Pancreas vascular permeability
Fold change of MFI
Day post-infection
C
Spleen
Day 5
Day 10
Spleen vascular permeability
Fold change of MFI
Day post-infection
D
Liver
Day 5
Day 10
Liver vascular permeability
Fold change of MFI
Day post-infection
E
Kidney
Day 5
Day 10
Kidney vascular permeability
Fold change of MFI
Day post-infection
F
Brain
Day 5
Day 10
Brain vascular permeability
Fold change of MFI
Day post-infection
G
Heart
Day 5
Day 10
Heart vascular permeability
Fold change of MFI
Day post-infection
H
Lungs
Day 5
Day 10
Lung vascular permeability
Fold change of MFI
Day post-infection

Figure S7. Vascular permeability changes upon *T. brucei* infections. Related to Figure 4. (**A-H**) Vascular permeability was measured in every organ as an increase in the mean fluorescence intensity (MFI) emitted by the 70kDa FITC-Dextran in the extravascular space. Representative microscopy images of each organ at days 6 and 10 post-infection are shown in the left panels. The rainbow color code reflects fluorescence intensity, with blue being the lowest values and red the highest. The graphics show the vascular permeability of each organ separately through time. Scale bar, 50 μm. *All relevant data used to generate this figure is included in Data S1 (Tab 11).


Brain
Lungs
Heart
Spleen
NI
D6
P-selectin
E-selectin
ICAM1
ICAM2
VCAM1
PECAM1
CD36

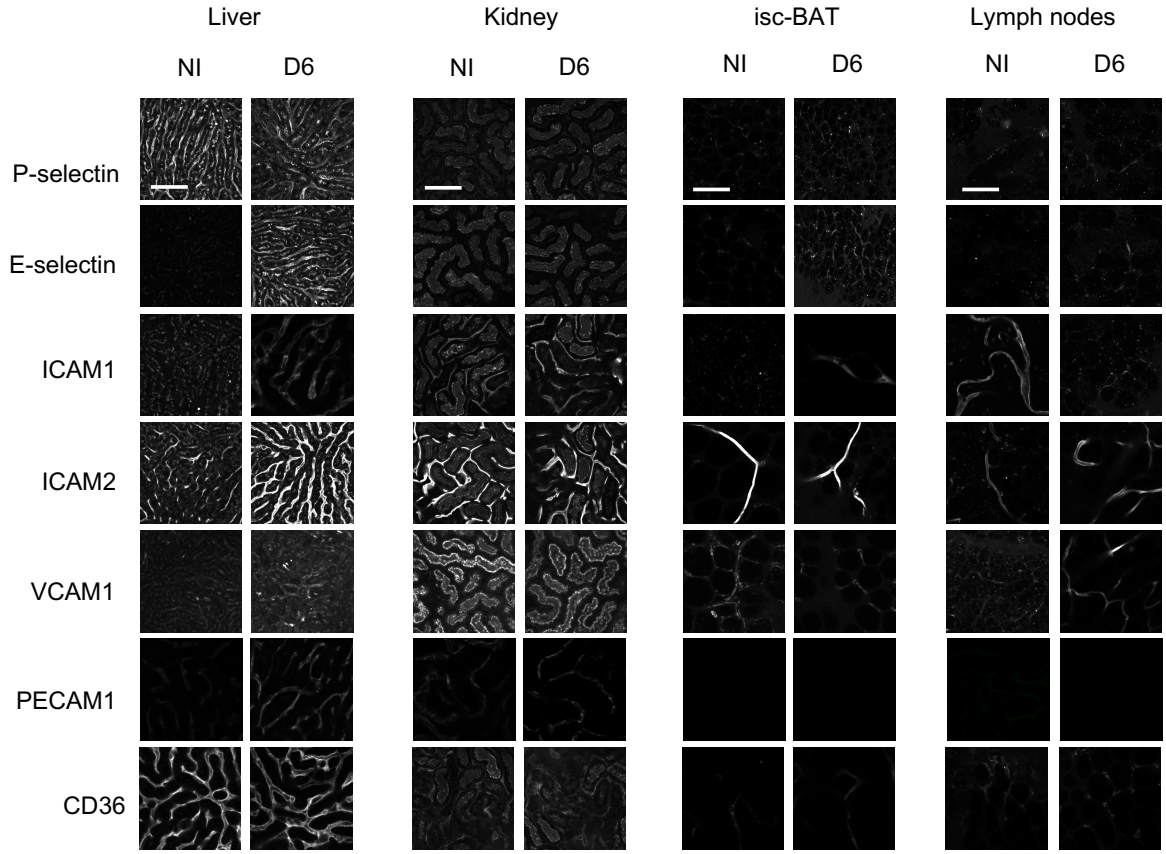

Liver
Kidney
isc-BAT
Lymph nodes
NI
D6
P-selectin
E-selectin
ICAM1
ICAM2
VCAM1
PECAM1
CD36

Figure S8. Representative images of vascular endothelial receptor expression across organs. Related to Figure 5. Each pair of columns represents uninfected and day 6-infected organs., while each row represents a vascular endothelial receptor (P-Selectin, E-selectin, ICAM1, ICAM2, VCAM1, PECAM1 and CD36). Scale bar = 50 μm.

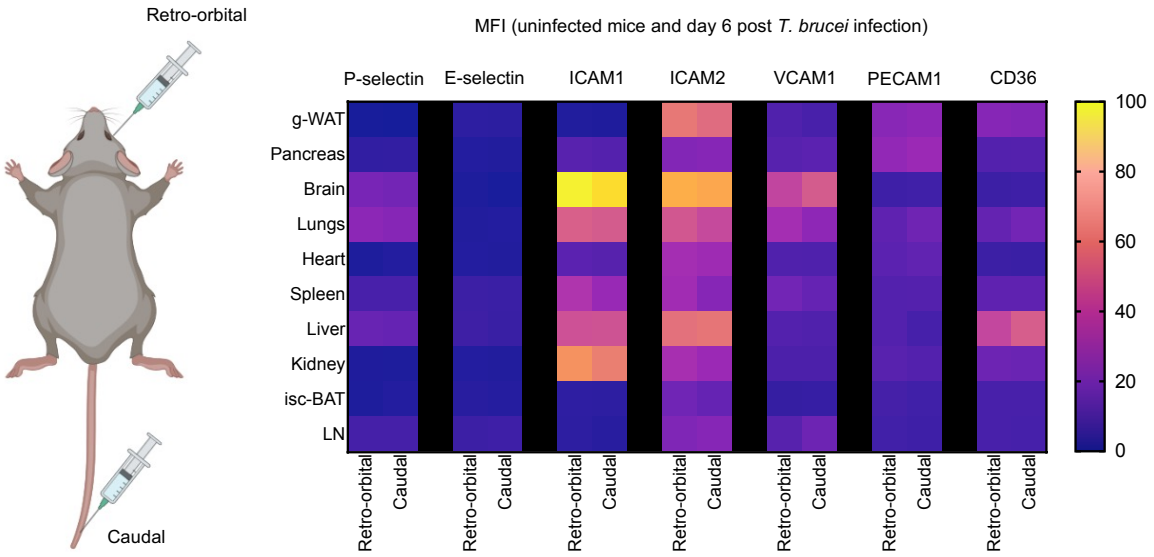


Figure S9. Comparison of vascular receptor expression upon retro-orbital and caudal intravenous routes in uninfected mice. Related to Figure 5. The viridis map shows the average of at least 100 MFI values obtained in vessels of mice intravenously injected with labeled antibodies via either the caudal or retro-orbital routes. *All relevant data used to generate this figure is included in Data S1 (Tab 16).

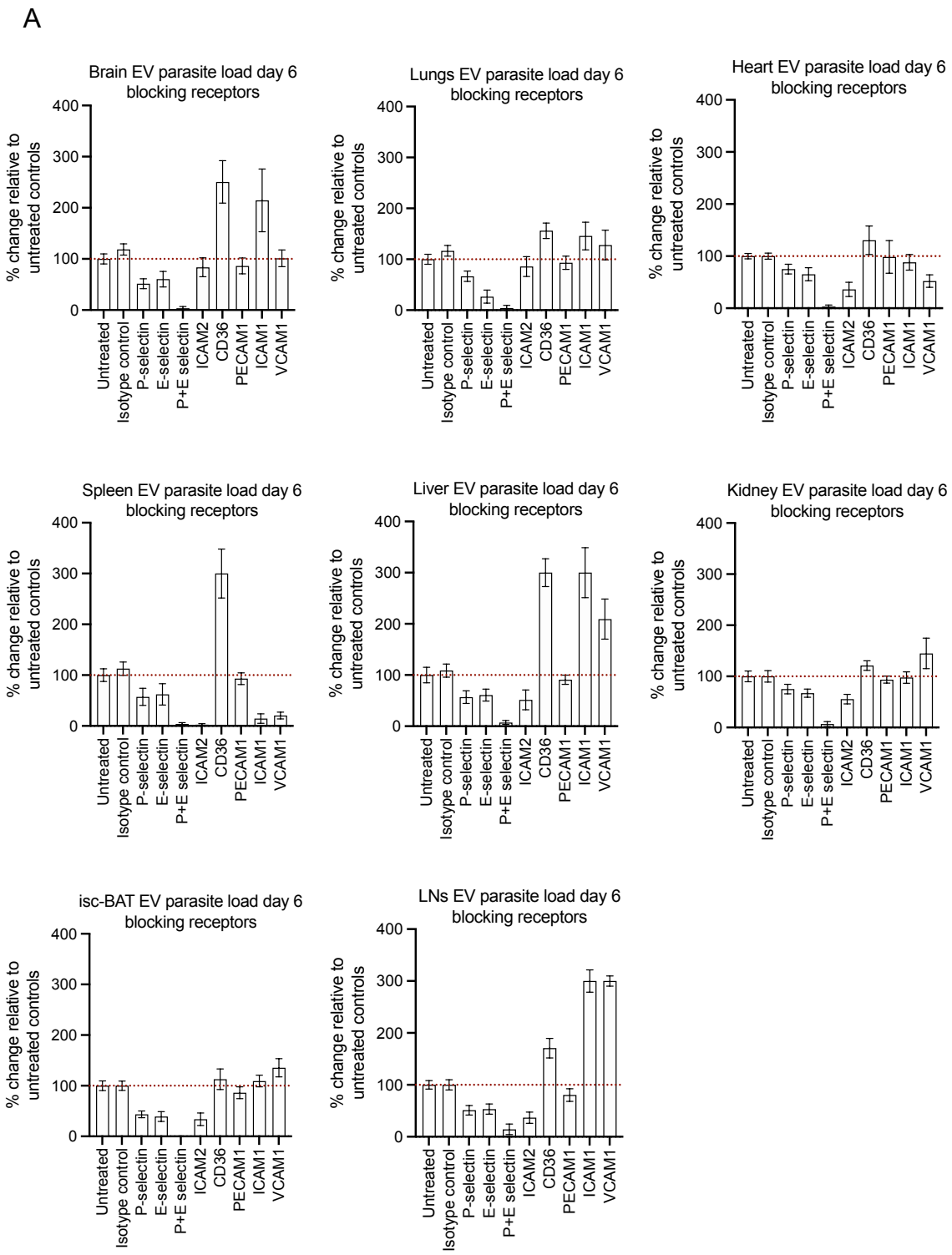


Figure S10. Parasite density in 8 organs following treatment with vascular receptor blocking antibodies. Related to Figure 6. Graphs show the respective quantitative values of parasite density measured by bioluminescence (p/s / area), in all organs except g-WAT and pancreas (included in Figure 6). Each column represents a condition (blocking antibody), while the red dotted line represents 100%. Error bars show SD. *All relevant data used to generate this figure is included in Data S1 (Tab 18).

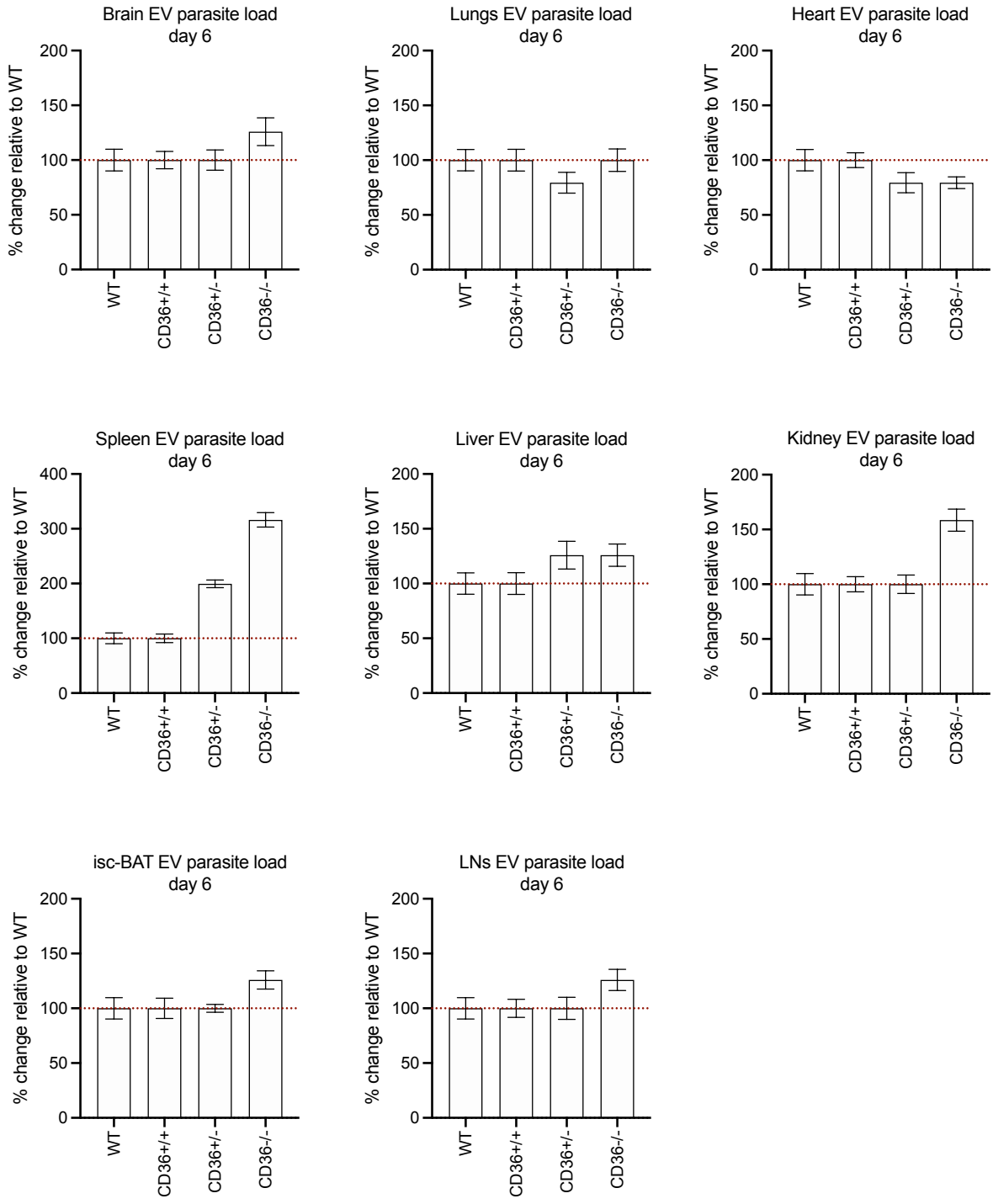


Figure S11. Parasite density in 8 organs of CD36 homozygous and heterozygous knock-out mice. Related to Figure 6. Graphs show the respective quantitative values of parasite density measured by bioluminescence (p/s / area), in all organs except g-WAT and pancreas (included in Figure 6). Each column represents a condition (mouse genotype), while the red dotted line represents 100%. Error bars show SD. *All relevant data used to generate this figure is included in Data S1 (Tab 20).