SUPPLEMENTAL MATERIAL

Material and methods

RNA Isolation of white blood cells from whole blood

Whole blood was kept on ice until further handling and centrifuged for 10 min at 14000g and 4˚C. Plasma was removed and red blood cells were lysed twice with 10 mL ice-cold NH_4Cl. Cells were pelleted by centrifugation and the pellet was suspended in Trizol (Invitrogen, Breda, The Netherlands). RNA was isolated according to standard protocols.

Supplemental Table

Supplemental Table I: CT values of *Gapdh*, *Ptprc*, and *Tek* in white blood cells of individual Tie2$^{+/+}$ and Tie2$^{+/-}$ mice as assessed with RT-qPCR.

Gene	**Tie2$^{+/+}$ (*n*=3)**			**Tie2$^{+/-}$ (*n*=4)**			
Gapdh	23.9	23.5	21.9	24.8	22.8	23.7	24.4
Ptprc	26.1	26.2	24.9	27.2	24.4	27.0	28.4
Tek	37.4	36.7	36.6	>40	36.8	36.3	>40

Gapdh: glyceraldehyde 3-phosphate dehydrogenase; Ptprc: protein tyrosine phosphatase, receptor type C, CD45; Tek: tyrosine kinase, Tie2

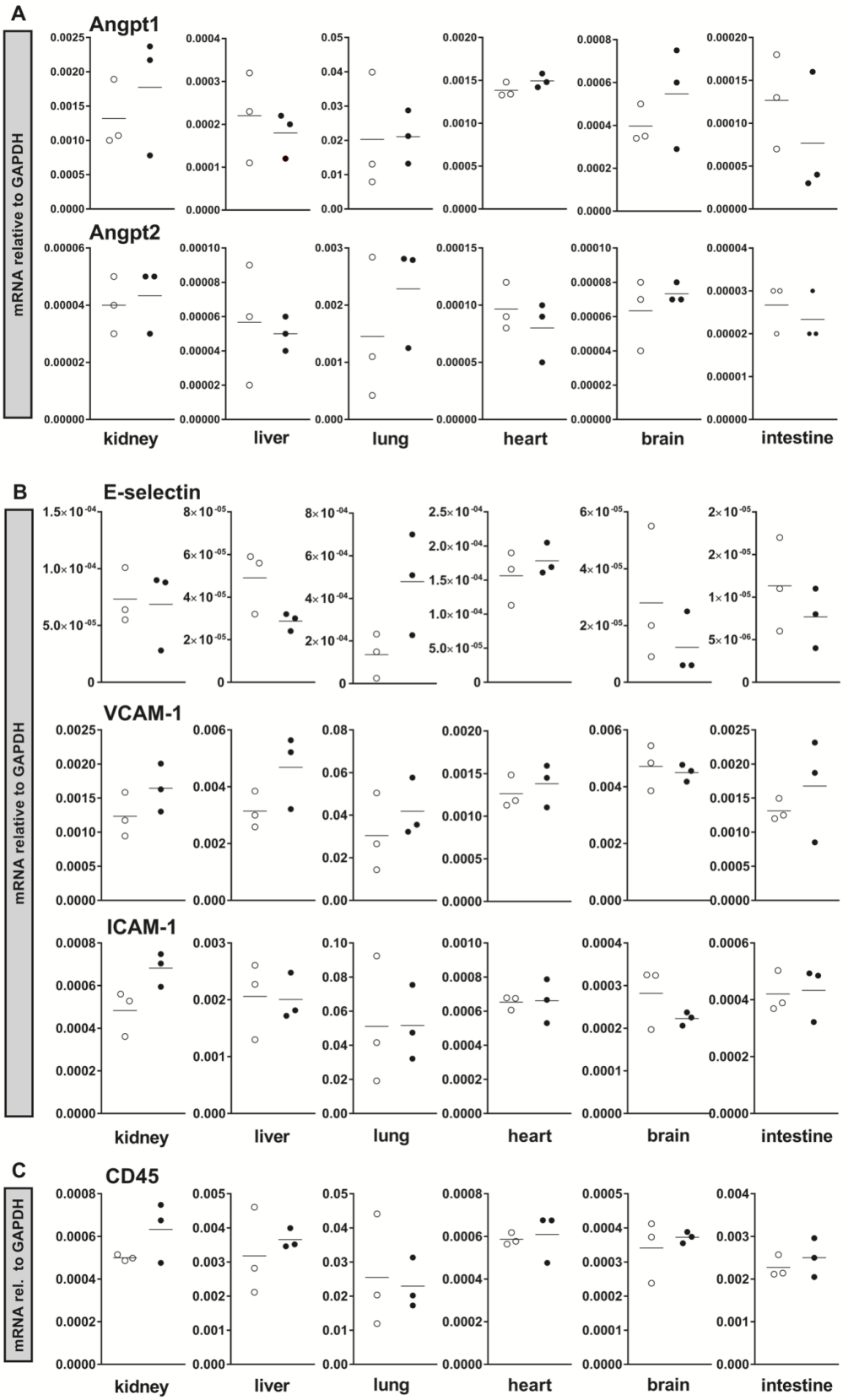
A
Angpt1
Angpt2
mRNA relative to GAPDH
kidney
liver
lung
heart
brain
intestine
B
E-selectin
VCAM-1
ICAM-1
mRNA relative to GAPDH
kidney
liver
lung
heart
brain
intestine
C
CD45
mRNA rel. to GAPDH
kidney
liver
lung
heart
brain
intestine

Figure S1. Basal mRNA expression levels of Tie2 ligands Angpt1 and Angpt2, endothelial inflammatory adhesion molecules, and surveilling leukocytes in organs of Tie2$^{+/+}$ and Tie2$^{+/-}$ mice. Organs of Tie2$^{+/+}$ and Tie2$^{+/-}$ mice were assessed for mRNA levels by RT-qPCR relative to GAPDH. **A.** Angpt1 and Angpt2 mRNA levels. **B.** E-selectin, VCAM-1, and ICAM-1 mRNA levels. **C**. CD45 mRNA levels. Dots represent individual Tie2$^{+/+}$ mice (○), Tie2$^{+/-}$ mice (●), horizontal lines indicate average values of 3 mice per group

Supplementary figure S2

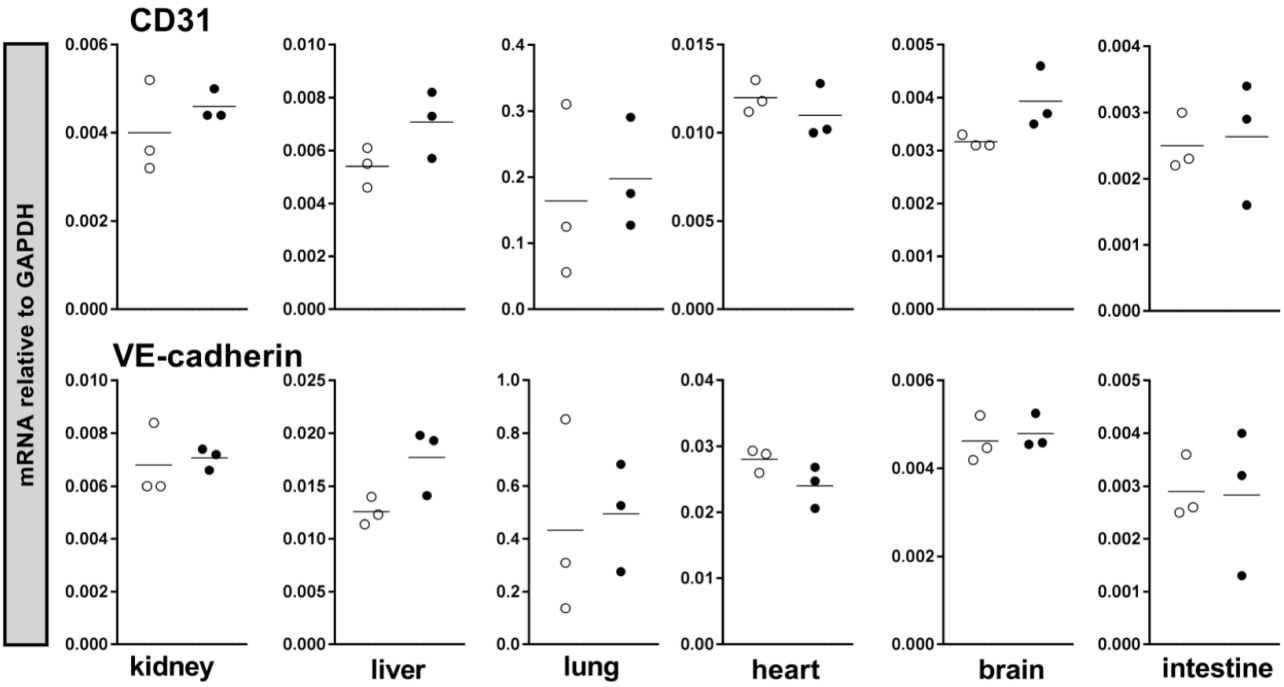

Figure S2. Basal expression levels of pan-endothelial genes CD31 and VEcadherin in organs of Tie2$^{+/+}$ and Tie2$^{+/-}$ mice. Organs of Tie2$^{+/+}$ and Tie2$^{+/-}$ mice were assessed for mRNA levels by RT-qPCR relative to GAPDH. **A.** CD31 mRNA levels. **B**. VEcadherin mRNA levels. Dots represent individual Tie2$^{+/+}$ mice (○), Tie2$^{+/-}$ mice (●), horizontal lines indicate average values of 3 mice per group.

Supplementary figure S3

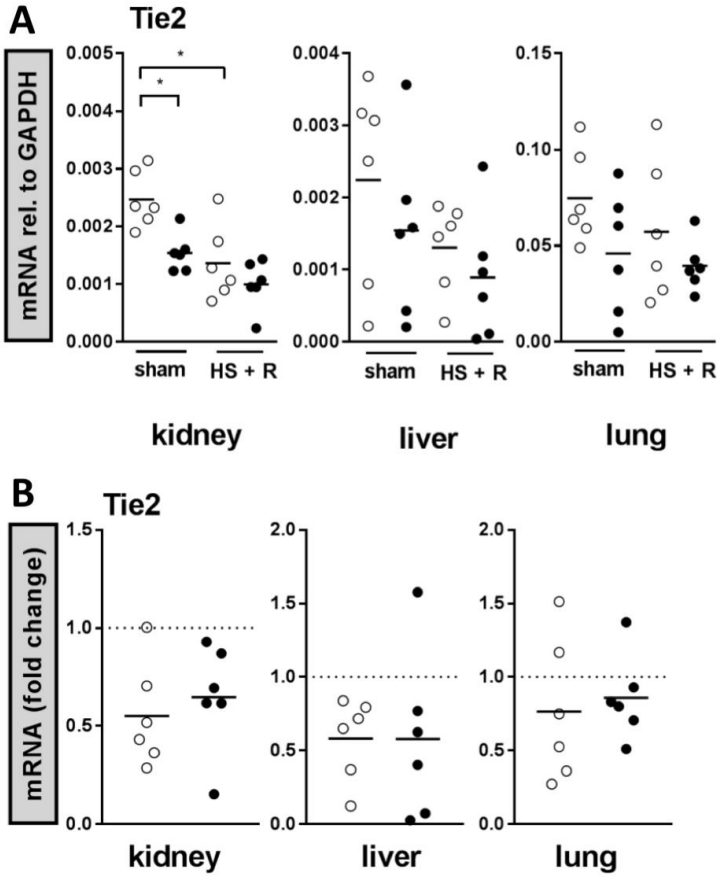

Figure S3. Effects of hemorrhagic shock and resuscitation on Tie2 mRNA expression in kidney, liver, and lung in Tie2$^{+/+}$ and Tie2$^{+/-}$ mice. Tie2$^{+/+}$ and Tie2$^{+/-}$ mice were subjected to hemorrhagic shock and resuscitation and sacrificed 1h after resuscitation (HS + R). Kidney, liver, and lungs were assessed for Tie2 mRNA levels relative to GAPDH (**A**) and as fold change (**B**) between HS + R and sham (set at 1, ----). Dots represent individual Tie2$^{+/+}$ mice (○), Tie2$^{+/-}$ mice (●), horizontal lines indicate average values of 6 mice per group, * $P<0.05$.

Supplementary figure S4

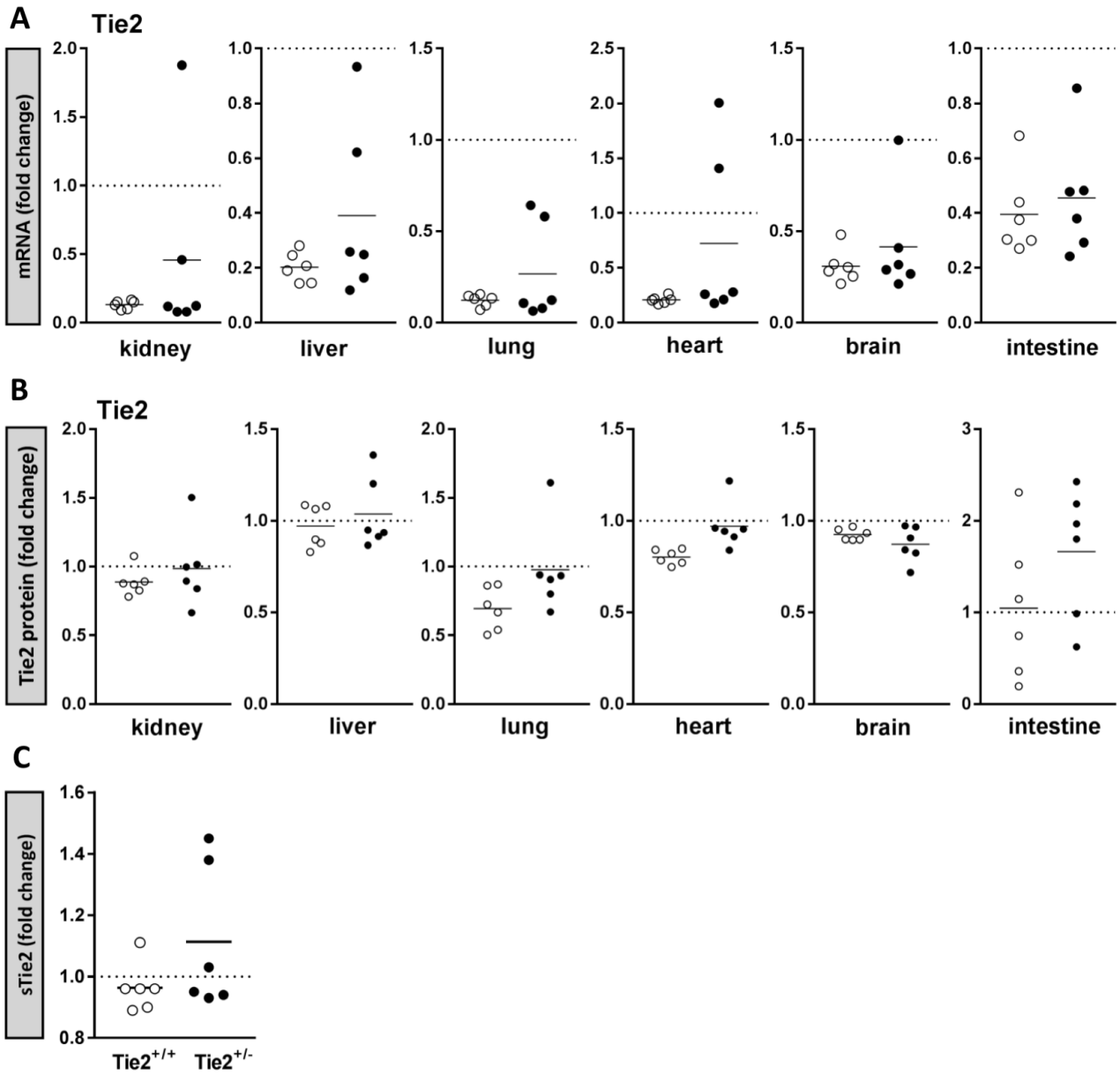

Figure S4. Effects of LPS challenge on Tie2 expression in organs of Tie2$^{+/+}$ and Tie2$^{+/-}$ mice. Tie2$^{+/+}$ and Tie2$^{+/-}$ mice were challenged with LPS *i.p*. (1 µg/g) and sacrificed 4h later. **A.** Tie2 mRNA levels in organs. **B.** Tie2 protein expression in organs. **C.** soluble Tie2 (sTie2) levels in plasma. Data are presented as fold change between LPS treated mice and vehicle control (set at 1, ----). Dots represent individual Tie2$^{+/+}$ mice (○), Tie2$^{+/-}$ mice (●), horizontal lines indicate average values of 6 mice per group.

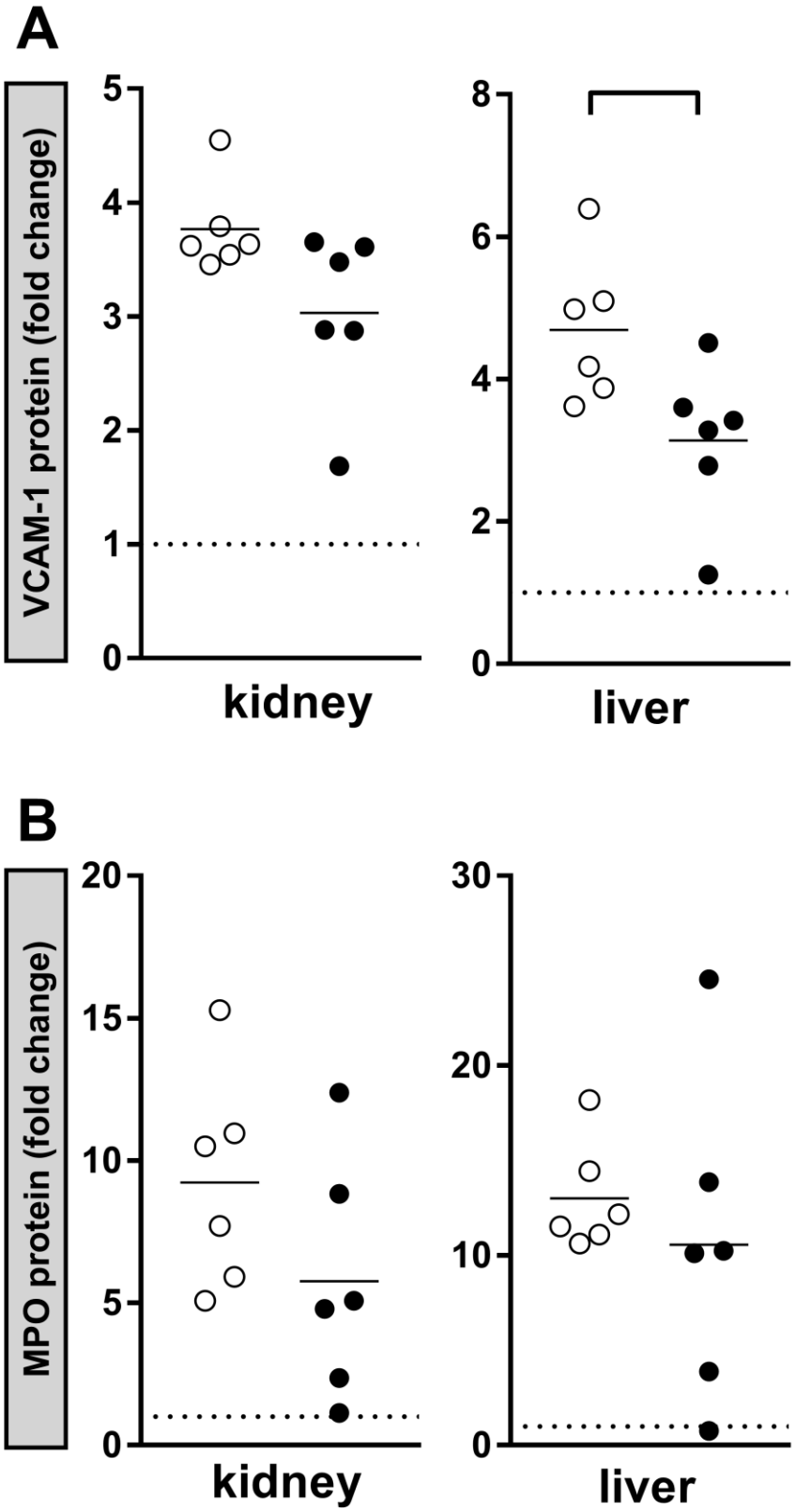
A
VCAM-1 protein (fold change)
5
4
3
2
1
0
kidney
8
6
4
2
0
liver
B
MPO protein (fold change)
20
15
10
5
0
kidney
30
20
10
0
liver

Figure S5: Effects of LPS challenge on VCAM-1 and MPO protein in kidney and liver of Tie2$^{+/+}$ and Tie2$^{+/-}$ mice. Tie2$^{+/+}$ and Tie2$^{+/-}$ mice were challenged with LPS *i.p.* (1µg/g) and sacrificed 4 hours later. Protein levels were determined by ELISA on whole tissue homogenates. **A.** VCAM-1 protein levels in kidney and liver. **B.** MPO protein levels in kidney and liver. Data are presented as fold change between LPS treated mice and vehicle control (set at 1, ----). Dots represent individual Tie2$^{+/+}$ mice (○), Tie2$^{+/-}$ mice (●), horizontal lines indicate average values of 6 mice per group.