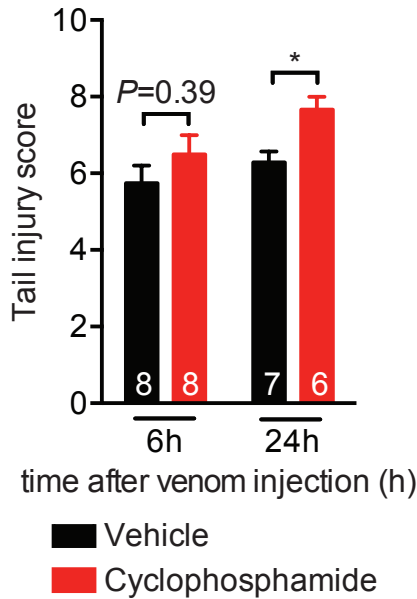
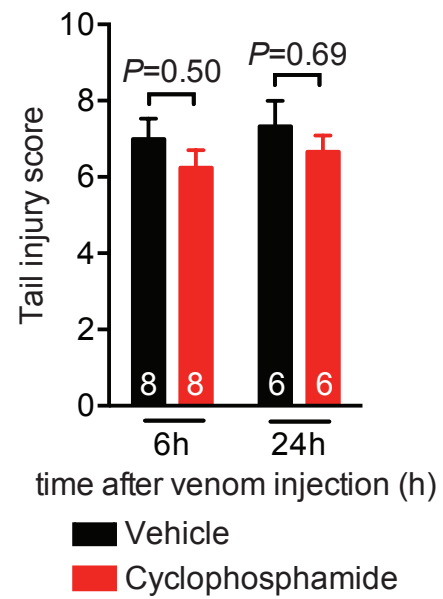
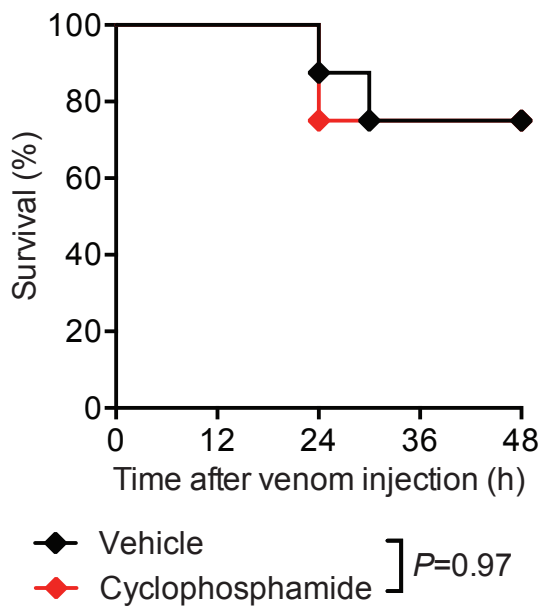
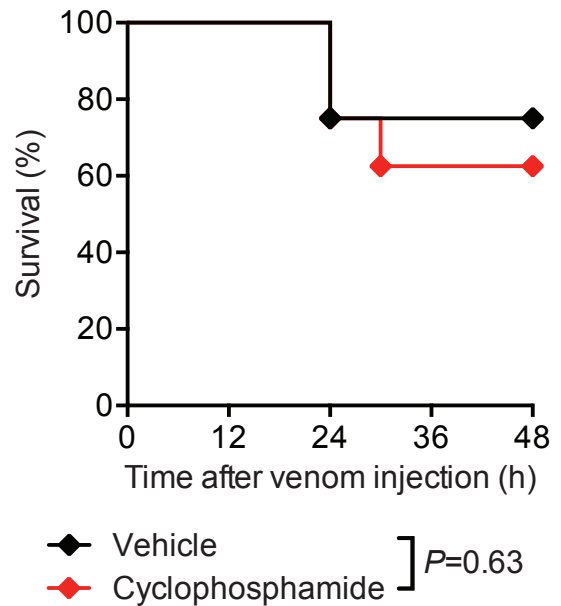


## **Supplementary Information**

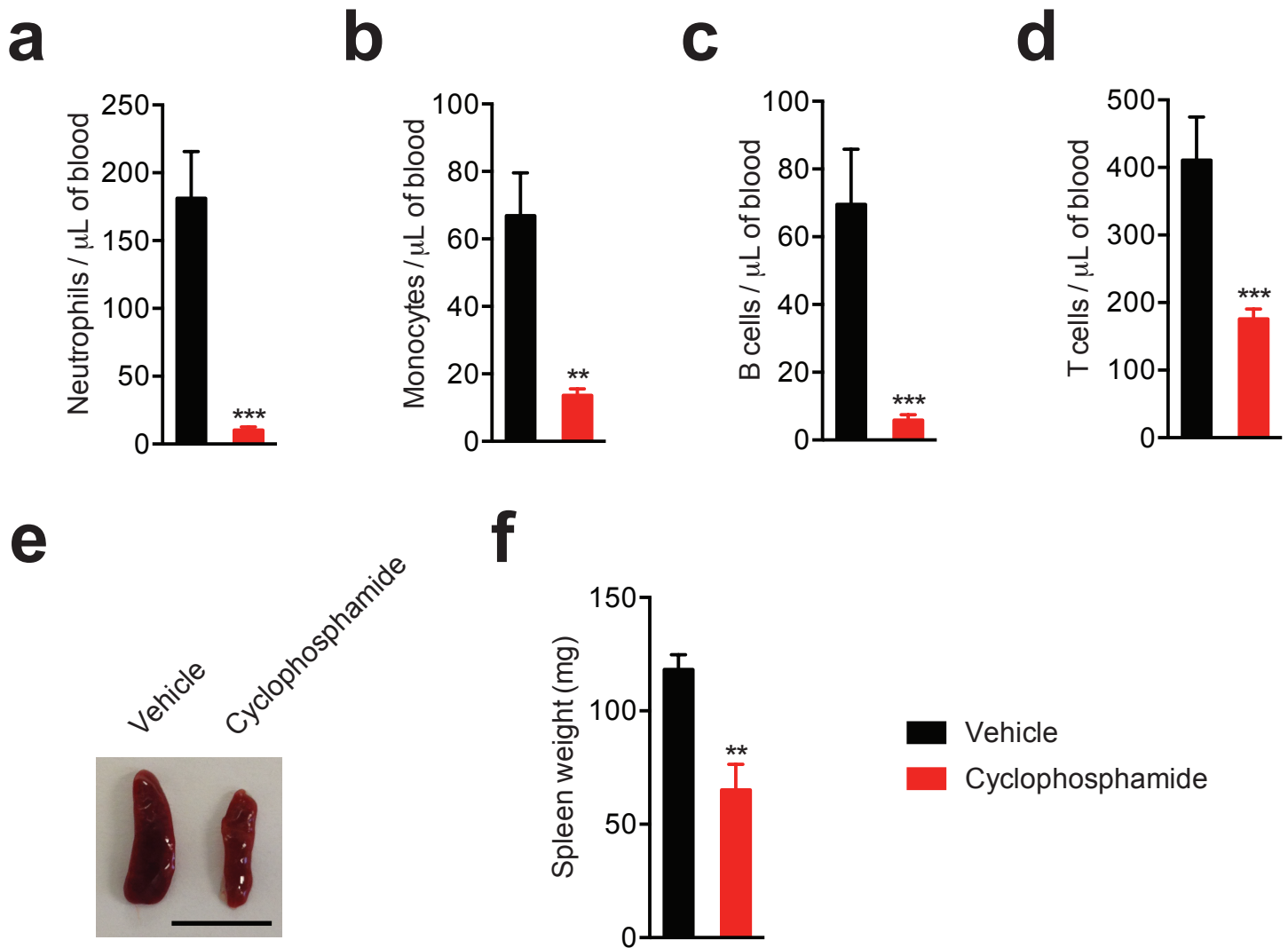
**‘Correspondence: Evidence that neutrophils do not promote**

***Echis carinatus* venom-induced tissue destruction’**

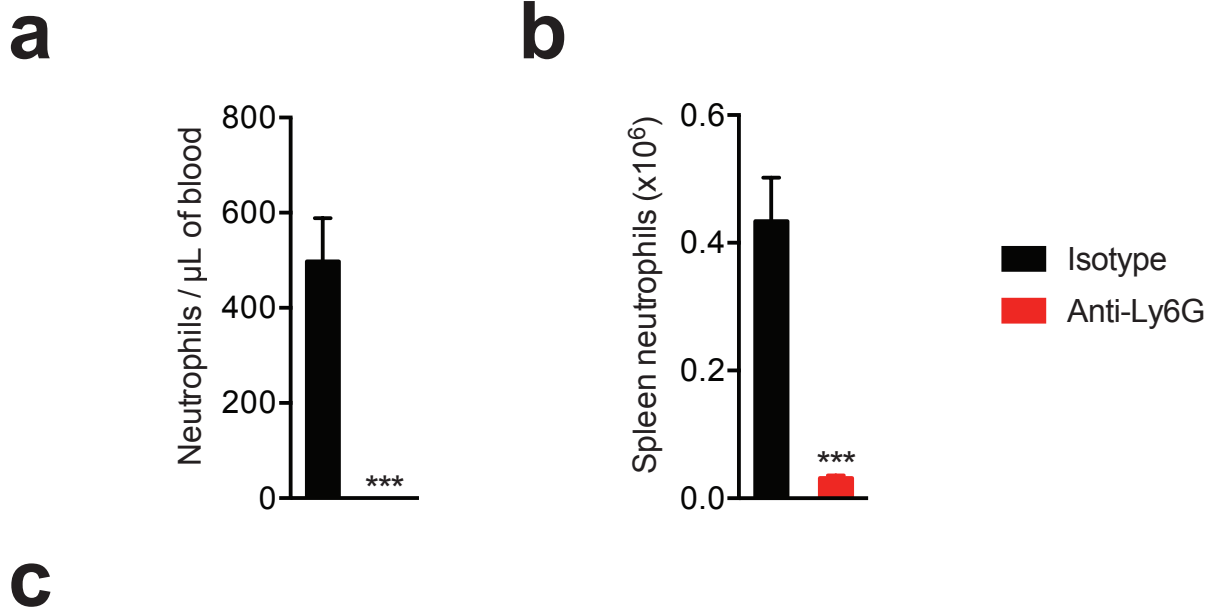
**Stackowicz et al.**

**a*****Echis carinatus multisquamatus*****b*****Echis carinatus pyramidum*****c*****Echis carinatus multisquamatus*****d*****Echis carinatus pyramidum***

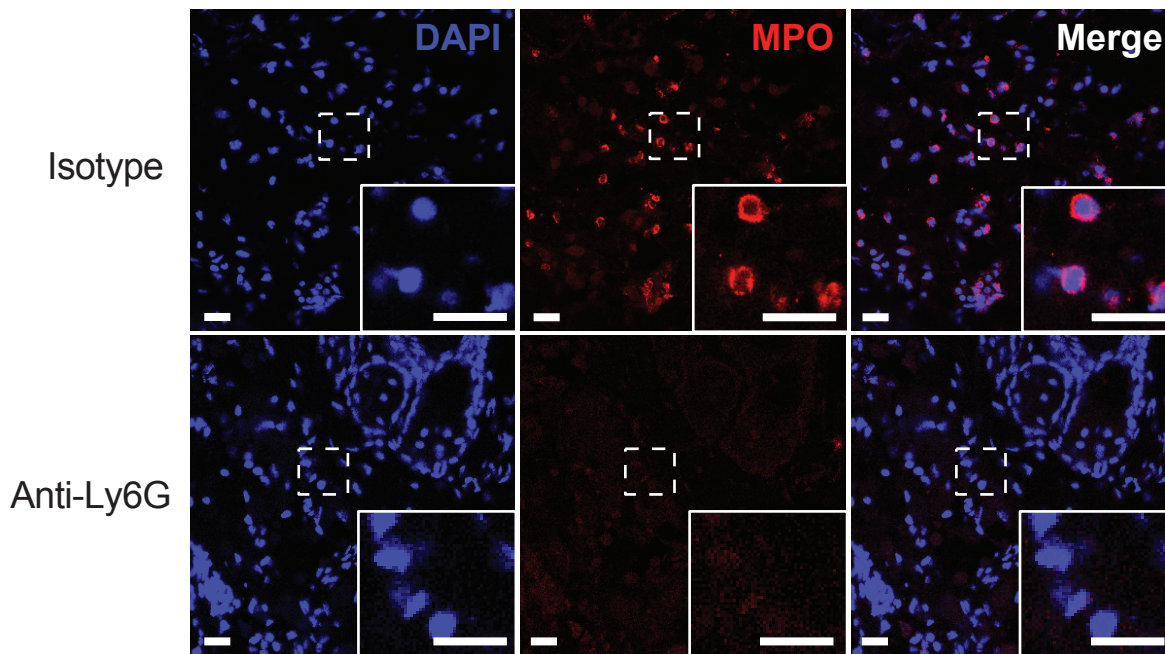
**Supplementary Figure 1. Effect of cyclophosphamide in mice injected with *Echis carinatus multisquamatus* venom (ECMV), or *Echis carinatus pyramidum* venom (ECPV).** RjOrl:SWISS mice were treated with cyclophosphamide as described by Katkar *et al.*<sup>3</sup>. Mice were injected i.p. with cyclophosphamide twice (150 mg/kg on day one and 100 mg/kg on day four, in 500  $\mu$ L PBS). Control mice were injected with vehicle only (PBS). On day 5, mice were injected in the tail with 3 mg/kg ECMV or ECPV, as indicated. (a-d) Tail injury scores (a & b) and survival (percentage of live animals) (c & d) after injection of venom (3 mg/kg) in mice pre-treated with cyclophosphamide or vehicle (PBS) ( $n=8$  per group). Numbers of live animals per group in a & b are indicated in white. Data are pooled from two independent experiments. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$  by two-tailed Mann–Whitney  $U$  test (a & b) or Mantel–Cox log-rank test (c & d).



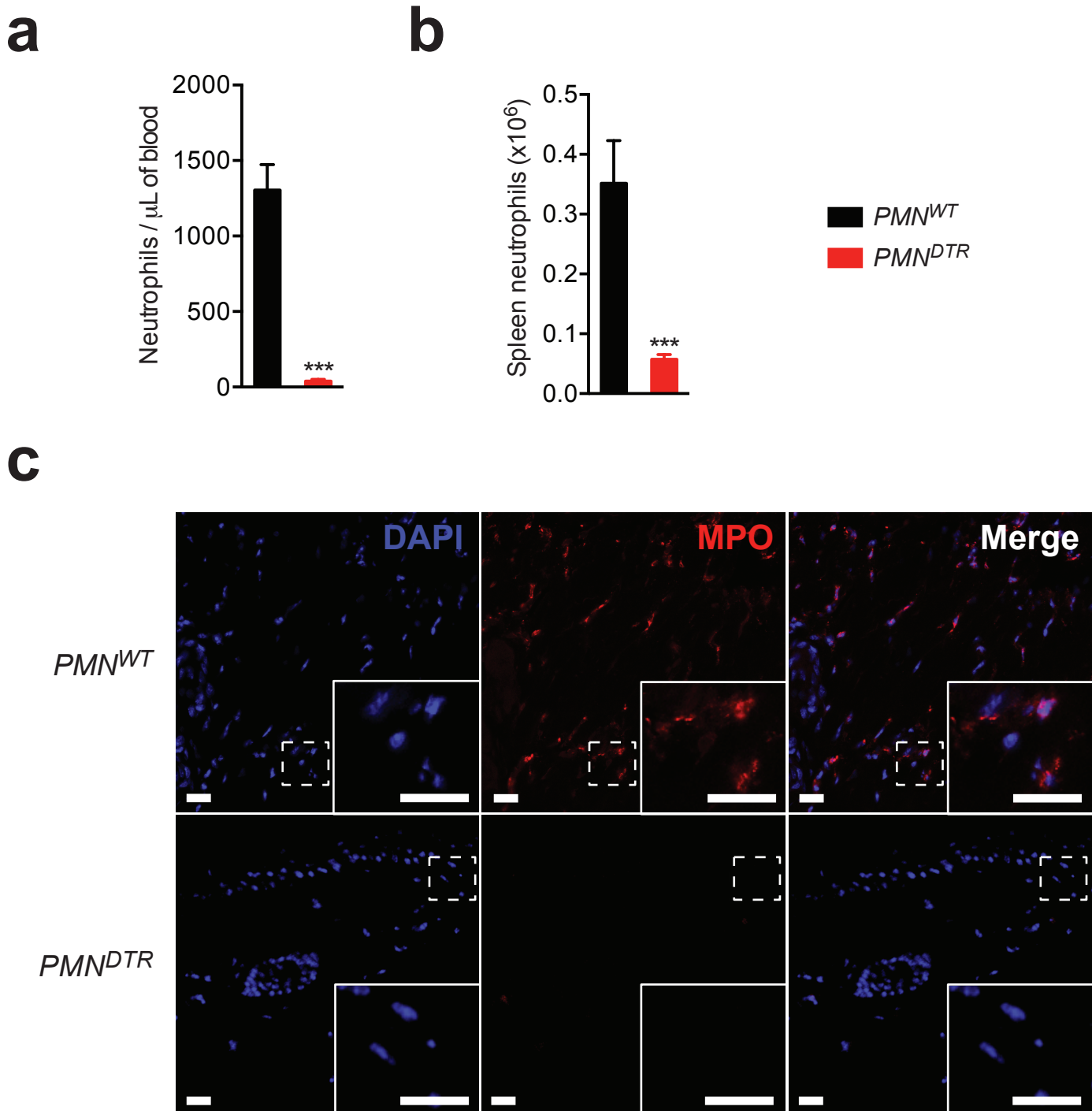
**Supplementary Figure 2. Effects of cyclophosphamide on various blood immune cell types and spleen weight.** RjOrl:SWISS mice were treated with cyclophosphamide as described by Katkar *et al.*<sup>3</sup>. Mice were injected i.p. with cyclophosphamide twice (150 mg/kg on day one and 100 mg/kg on day four, in 500  $\mu\text{L}$  PBS). Control mice were injected with vehicle only (PBS). Mice were sacrificed on day 5 for analysis of blood immune cell populations and spleen weights. **(a-d)** Numbers of blood CD11b<sup>+</sup> Ly6G<sup>+</sup> neutrophils **(a)**, CD11b<sup>+</sup> Ly6G<sup>-</sup> monocytes **(b)**, B220<sup>+</sup> CD3 $\epsilon$ <sup>-</sup> B cells **(c)** and B220<sup>-</sup> CD3 $\epsilon$ <sup>+</sup> T cells **(d)** in mice treated with cyclophosphamide or vehicle (PBS). **(e & f)** Representative picture of spleens **(e)** and quantification of spleen weight **(e)** in mice treated with cyclophosphamide or vehicle (PBS). Data are presented as mean + SEM, and pooled from two independent experiments with a total of 6 **(f)** or 8 **(a-d)** mice per group. \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$  by two-tailed Mann-Whitney  $U$  test. Scale bar in **e**: 1 cm.



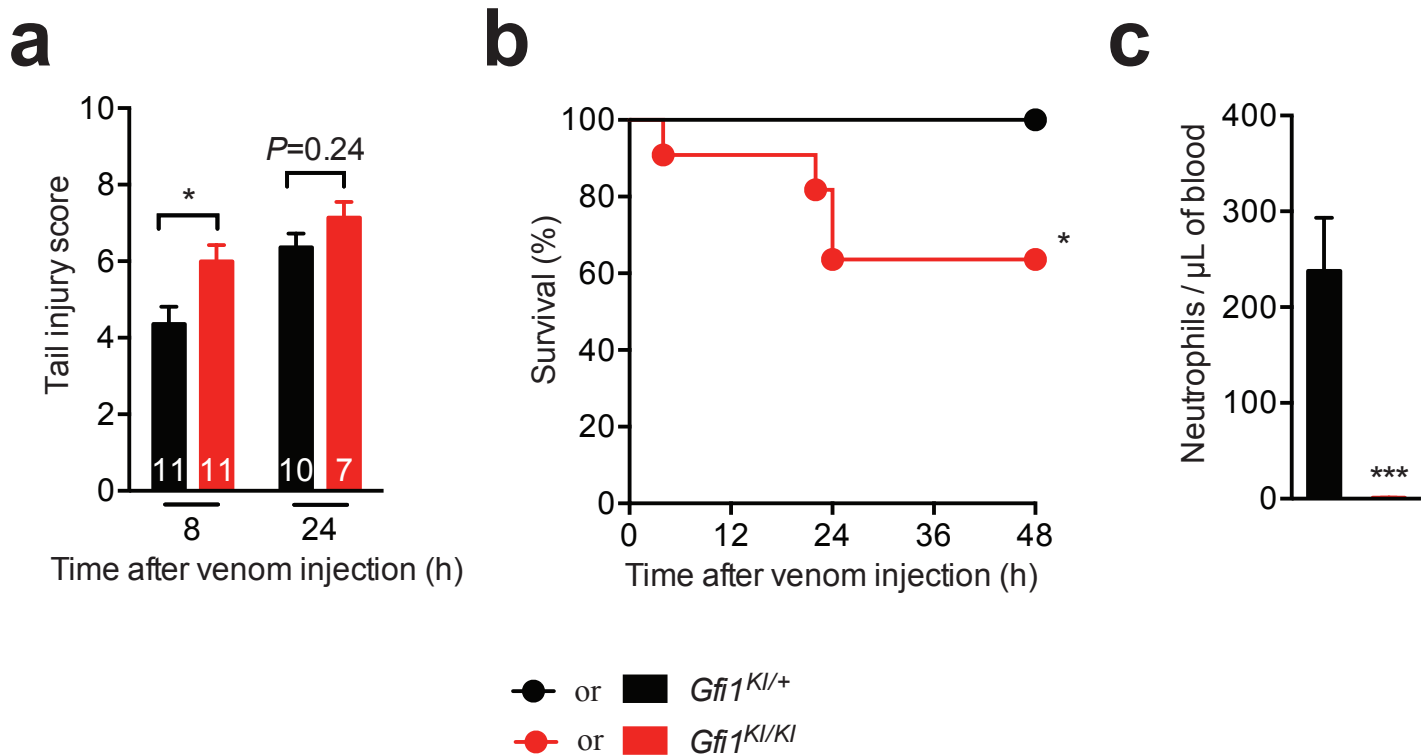
**c**



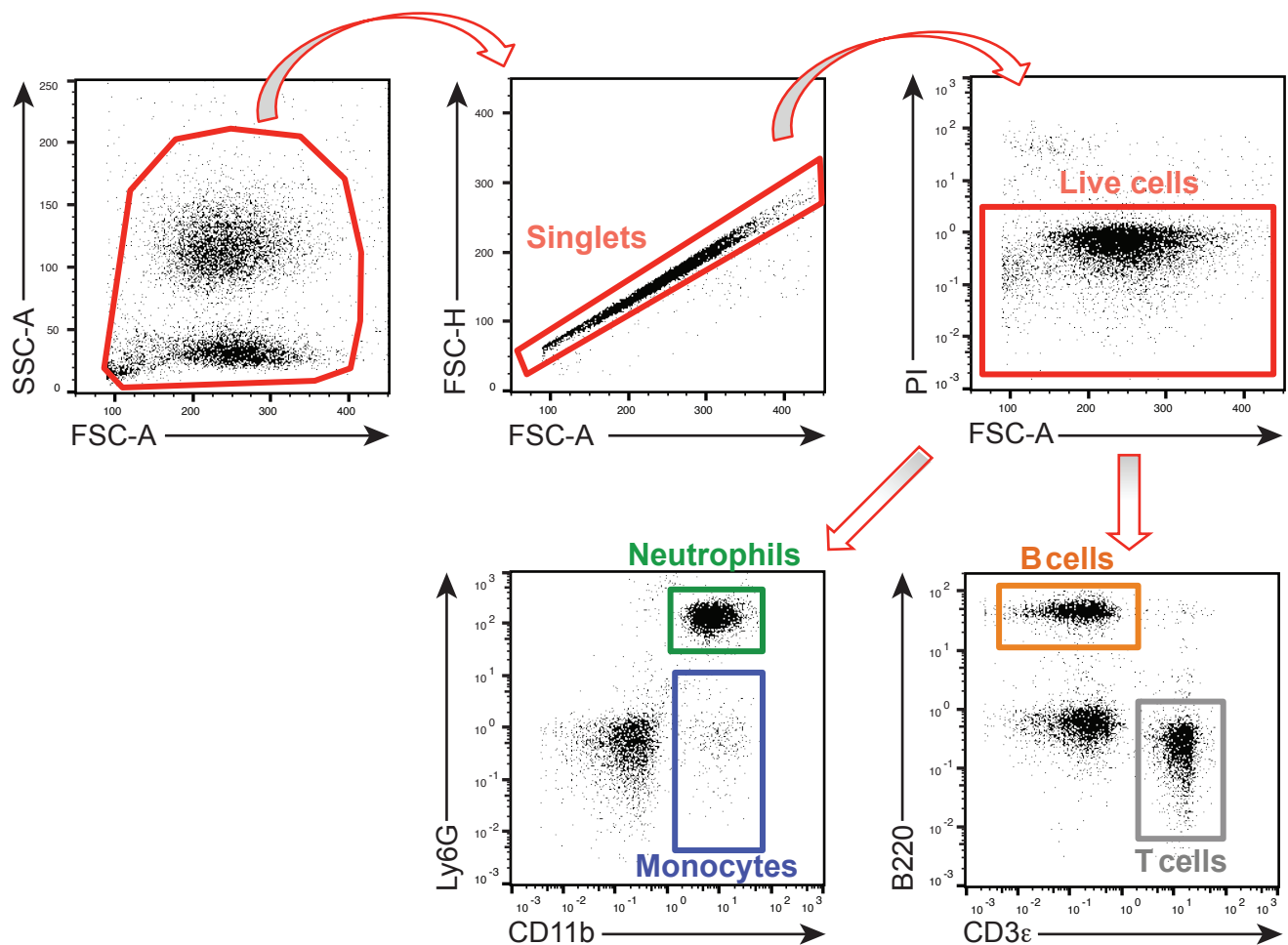
**Supplementary Figure 3. Levels of neutrophils 5 hours after injection of *Echis carinatus* venom in mice pre-treated with neutrophil-depleting anti-Ly6G antibodies or isotype control antibodies.** (a-c) RjOrl:SWISS mice were treated i.p. with anti-Ly6G antibodies (clone NIMP-R14) or isotype control antibodies (each at 300  $\mu\text{g}$  in 200  $\mu\text{L}$  PBS) 24 h and 2 h before injection of 3 mg/kg *Echis carinatus sochukeri* venom. Mice were sacrificed 5 h after venom injection, and levels of Ly6G<sup>+</sup> CD11b<sup>+</sup> neutrophils were quantified by flow cytometry in the blood (a) and spleen (b). Data are presented as mean + SEM, and pooled from two independent experiments with a total of 7 or 8 mice per group. \*\*\*,  $P < 0.001$  by two-tailed Mann–Whitney  $U$  test. (c) Confocal microscopy analyses of tail tissues collected after 5 h at the site of venom injection. Representative DNA staining (DAPI), together with myeloperoxidase (MPO) staining. Insets show enlargements of boxed areas. Scale bars, 20  $\mu\text{m}$ .



**Supplementary Figure 4. Levels of neutrophils 5 hours after injection of *Echis carinatus* venom in DT-treated  $PMN^{DTR}$  and  $PMN^{WT}$  mice.** (a-c)  $PMN^{DTR}$  and  $PMN^{WT}$  mice were treated i.p. with 500 ng diphtheria toxin (DT) 24 h and 2 h before injection of 3 mg/kg *Echis carinatus sochukeri* venom. Mice were sacrificed 5 h after venom injection, and levels of Ly6G<sup>+</sup> CD11b<sup>+</sup> neutrophils were quantified by flow cytometry in the blood (a) and spleen (b). Data are presented as mean + SEM, and pooled from two independent experiments with a total of 7 or 8 mice per group. \*\*\*,  $P < 0.001$  by two-tailed Mann–Whitney  $U$  test. (c) Confocal microscopy analyses of tail tissues collected after 5 h at the site of venom injection. Representative DNA staining (DAPI), together with myeloperoxidase (MPO) staining. Insets show enlargements of boxed areas. Scale bars, 20  $\mu$ m.



**Supplementary Figure 5. Tail injury score, mortality and neutrophil levels in neutrophil-deficient *Gfi1*<sup>KI/KI</sup> mice and neutrophil-sufficient *Gfi1*<sup>KI/+</sup> littermates after injection of 1 mg/kg *Echis carinatus* venom. (a & b) Tail injury scores (a) and survival (percentage of live animals) (b) after injection of 1 mg/kg *Echis carinatus sochukeri* venom in *Gfi1*<sup>KI/KI</sup> and *Gfi1*<sup>KI/+</sup> mice. (c) Levels of blood Ly6G<sup>+</sup> CD11b<sup>+</sup> neutrophils 24 h after injection of venom. Results in a & c are represented as means + SEM. Data in a-c are pooled from two independent experiments with a total of 7 (c) or 11 (a & b) mice per group. White numbers in a indicate numbers of live animals per group at each time-point. \*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$  by two-tailed Mann–Whitney  $U$  test (a & c) or Mantel–Cox log-rank test (b).**



**Supplementary Figure 6. Flow cytometry gating strategies.** Leukocytes were selected using a side scatter area (SSC-A) vs. forward scatter area (FSC-A) density plot. A FSC height (FSC-H) vs. FSC-A density plot was used to exclude doublets, and dead cells were excluded based on staining with propidium iodide (PI). Blood immune cell populations were identified among live cells as follows: neutrophils (CD11b<sup>+</sup> Ly6G<sup>+</sup>), monocytes (CD11b<sup>+</sup> Ly6G<sup>-</sup>), B cells (B220<sup>+</sup> CD3ε<sup>-</sup>) and T cells (B220<sup>-</sup> CD3ε<sup>+</sup>).