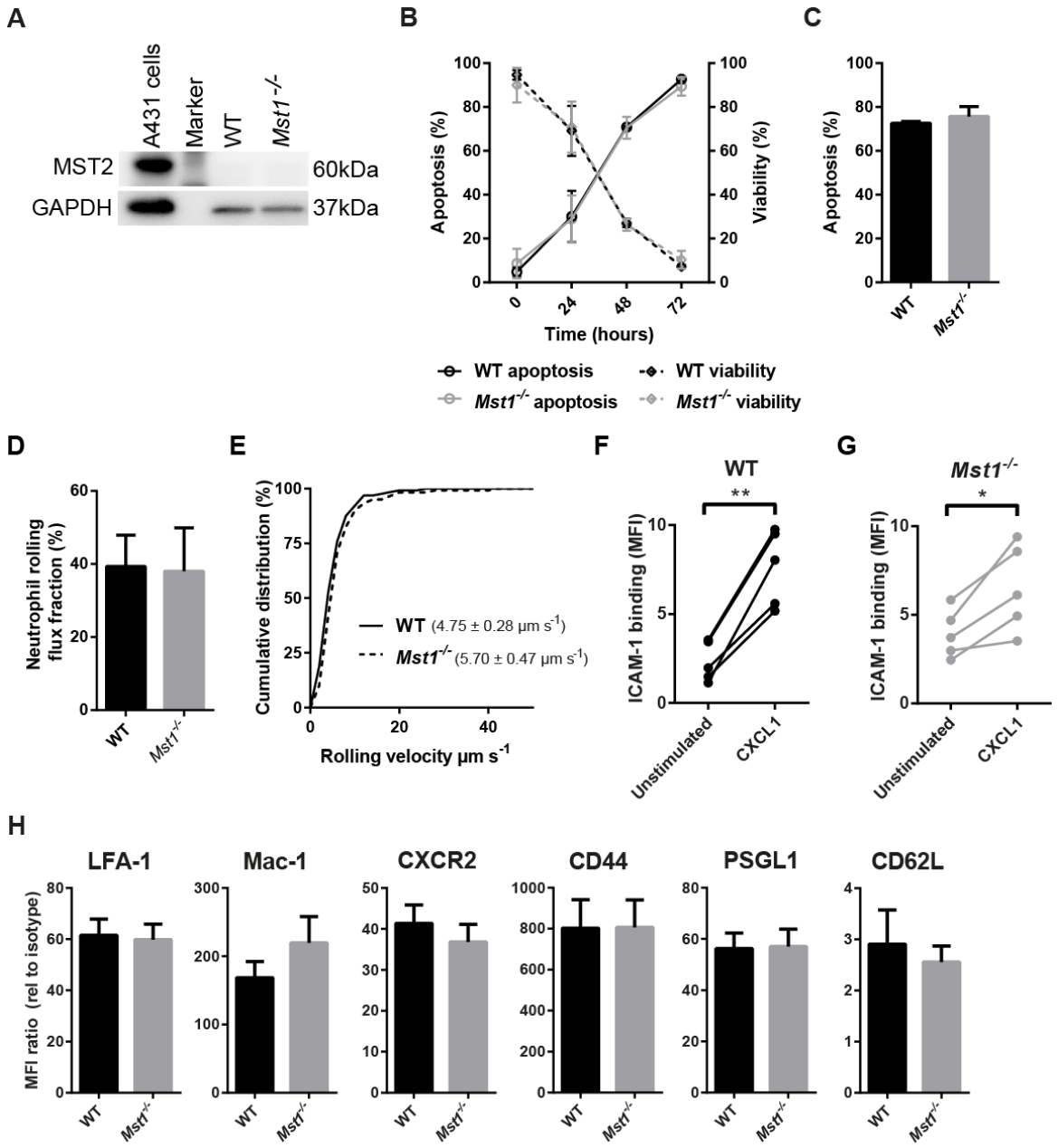
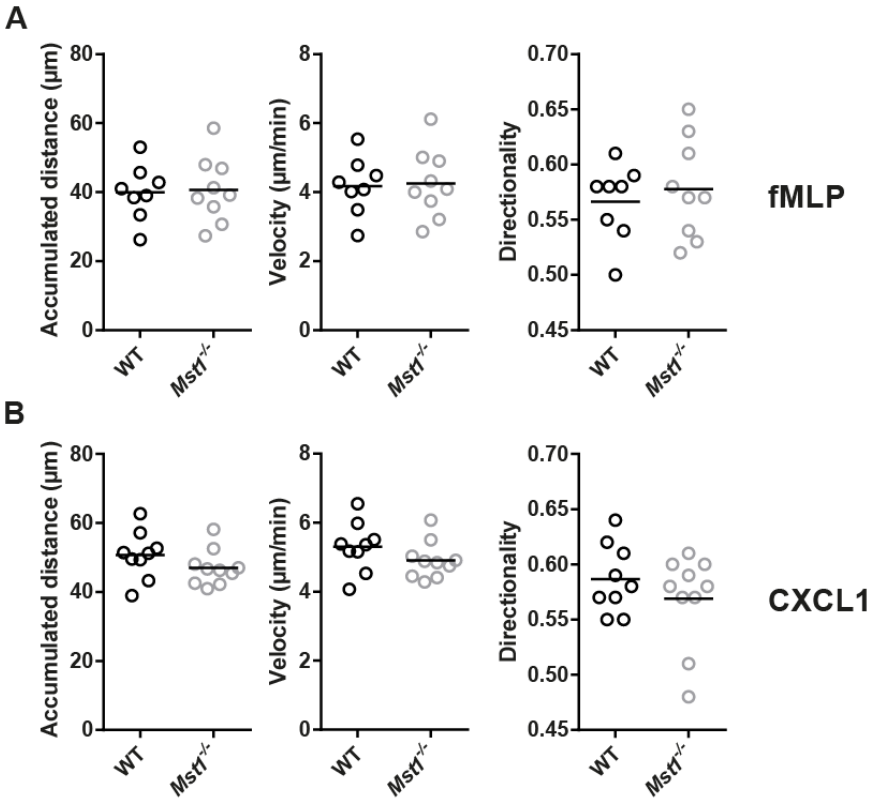
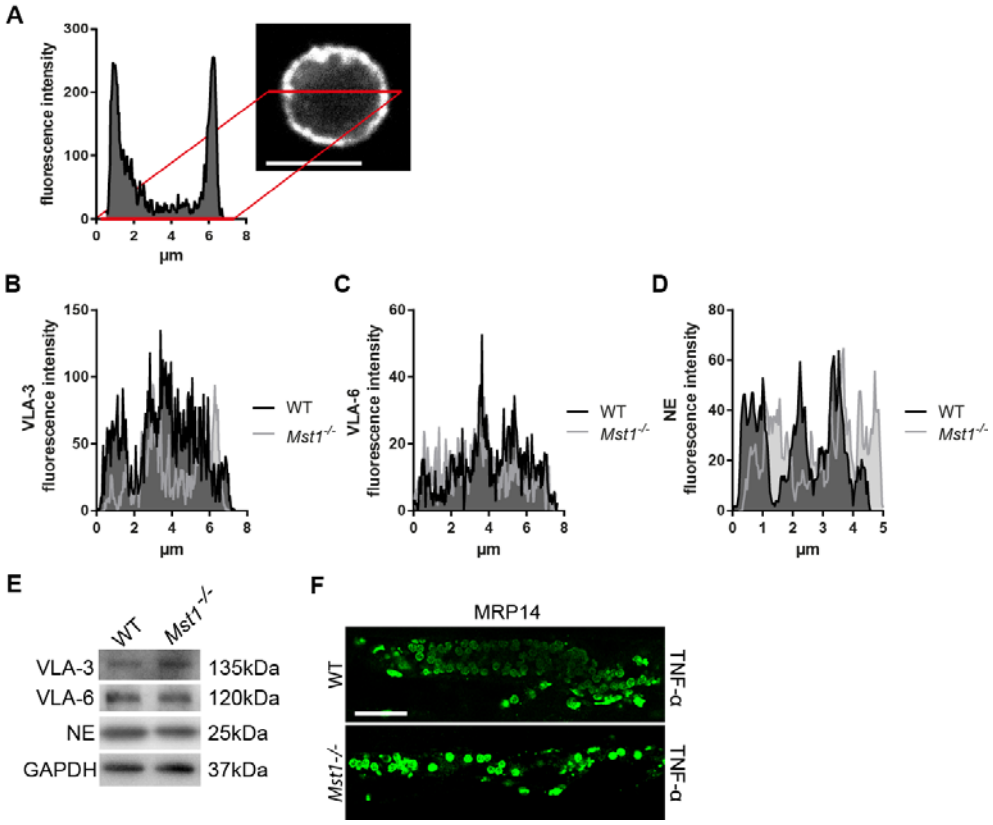


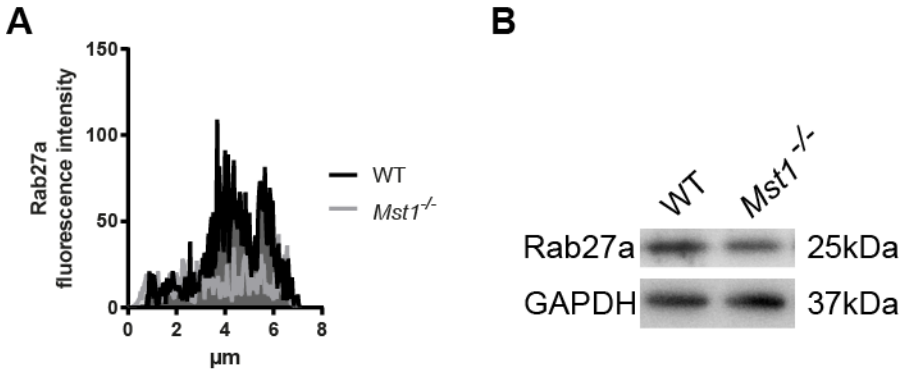
Supplemental Figure 1 **Expression, viability and adhesion properties of WT and *Mst1*$^{-/-}$ neutrophils. (A)** MST2 protein levels of EGF-stimulated A431 cells, used as positive control, and of WT and *Mst1*$^{-/-}$ neutrophils. GAPDH served as loading control (n=3). **(B)** FACS analysis of viable (TO-PRO3^{-} T-MRE^{+}) and apoptotic (TO-PRO3^{+} T-MRE^{-}) WT (back circles) and *Mst1*$^{-/-}$ neutrophils (grey circles) after cultivation for indicated times (n=3 mice per group, mean ± SEM, n.s., 2way ANOVA, Sidak's multiple comparisions test) **(C)** Apoptosis rate (TO-PRO3^{+} T-MRE^{-}) of neutrophils isolated from cremaster muscles of WT and *Mst1*$^{-/-}$ mice 2 h after i.s. injection of TNF-α (n=3 mice per group, n.s. unpaired t-test). **(D)** Rolling flux fraction and **(E)** cumulative distribution of neutrophil rolling velocity in cremaster muscle venules of WT and *Mst1*$^{-/-}$ mice 2 h after i.s. injection of TNF-α (n ≤ 5, mean ± SEM, n.s., unpaired t-test). **(F,G)** FACS analysis of ICAM-1 binding to **(F)** WT or **(G)** *Mst1*$^{-/-}$ neutrophils with or without CXCL1 stimulation (n=5 * $p < 0.05$, ** $p<0.01$ paired t-test). **(H)** FACS analysis of WT and *Mst1*$^{-/-}$ neutrophils for surface expression of LFA-1, Mac-1, CXCR2, CD44, PSGL-1, CD62L and corresponding isotype controls. The mean fluorescent intensity (MFI) was evaluated relative to the isotype control on Ly6G^{+} cells (neutrophils) (n= 3 mice, mean ± SEM, n.s., unpaired t-test).



Supplemental Figure 2 Chemotactic migration of WT and *Mst1*$^{-/-}$ neutrophils. (A,B) Chemotactic migration of WT and *Mst1*$^{-/-}$ neutrophils seeded in 3D collagen gels towards a gradient of **(A)** fMLP or **(B)** CXCL1 for 30 min. Neutrophils were tracked using Fiji software (62). Accumulated distance, velocity and directionality of cells were evaluated (n= 3, scatter blots with mean, n.s., unpaired t-test).



Supplemental Figure 3VLA-3, VLA-6 and NE – protein levels, distribution and NE activity. Fluorescence intensity profiles along a line cut through the center of the cell as exemplified in **(A)** for **(B)** VLA-3, **(C)** VLA-6 and **(D)** NE of a representative WT (black line) and *Mst1*$^{-/-}$ neutrophil (grey line) seeded on BSA coated wells. Scale bar: 5 µm. **(E)** Total protein levels of VLA-3, VLA-6 and NE from neutrophils of WT and *Mst1*$^{-/-}$ mice. GAPDH served as loading control (n=3 mice). **(F)** Immunostaining of MRP14 (green) in TNF-α stimulated cremaster muscle whole mounts from WT and *Mst1*$^{-/-}$ mice (n=3 per group). Scale bar: 50 µm.



Supplemental Figure 4 **Protein levels and distribution under unstimulated conditions of Rab27a. (A)** Fluorescence intensity profile along a line cut through the center of the cell for Rab27a of a representative WT (black line) and *Mst1*$^{-/-}$ neutrophil (grey line) seeded on BSA coated wells. **(B)** Total protein levels of Rab27a from neutrophils of WT and *Mst1*$^{-/-}$ mice. GAPDH served as loading control (n=3 mice).

924 **Supplemental Movie 1 LFA-1 clustering in WT and *Mst1*$^{-/-}$ neutrophils.** Whole blood from **(A)** WT and
925 **(B)** *Mst1*$^{-/-}$ mice was incubated with a non-blocking LFA-1 antibody and perfused through flow chambers
926 coated with E-selectin, ICAM-1 and CXCL1. Using confocal microscopy, interacting cells were recorded
927 under flow conditions. Images were recorded for 195 s (one stack every 13 s). The movies were
928 generated and converted using Fiji software.
929
930 **Supplemental Movie 2 Intravital multi photon laser scanning microscopy of transmigrating**
931 **neutrophils.** Extravasation of neutrophils (green) was recorded from postcapillary venules (red) in a TNF-
932 α stimulated mouse cremaster muscle of **(A)** Lyz2^{GFP} and **(B)** *Mst1*$^{-/-}$ x Lyz2^{GFP}mice. Z-stacks of
933 postcapillary venules were obtained every 30 seconds over a time period of 240 min. Sequences of z-
934 stacks were processed and converted using Imaris 7 and Fiji software.
935
936 **Supplemental Movie 3 Intravital multi photon laser scanning microscopy of laser injury.** Four hours
937 after intrascrotal injection of TNF- α in **(A)** Lyz2^{GFP} and **(B)** *Mst1*$^{-/-}$ x Lyz2^{GFP}mice, a laser injury was
938 induced near postcapillary cremaster muscle venules (red). Swarming of neutrophils (green) was then
939 recorded to the sites of laser injury. Z-stacks of postcapillary venules (red) were obtained every 30
940 seconds over a time period of 30 min. Sequences of z-stacks were processed and converted using Imaris
941 7 and Fiji software.