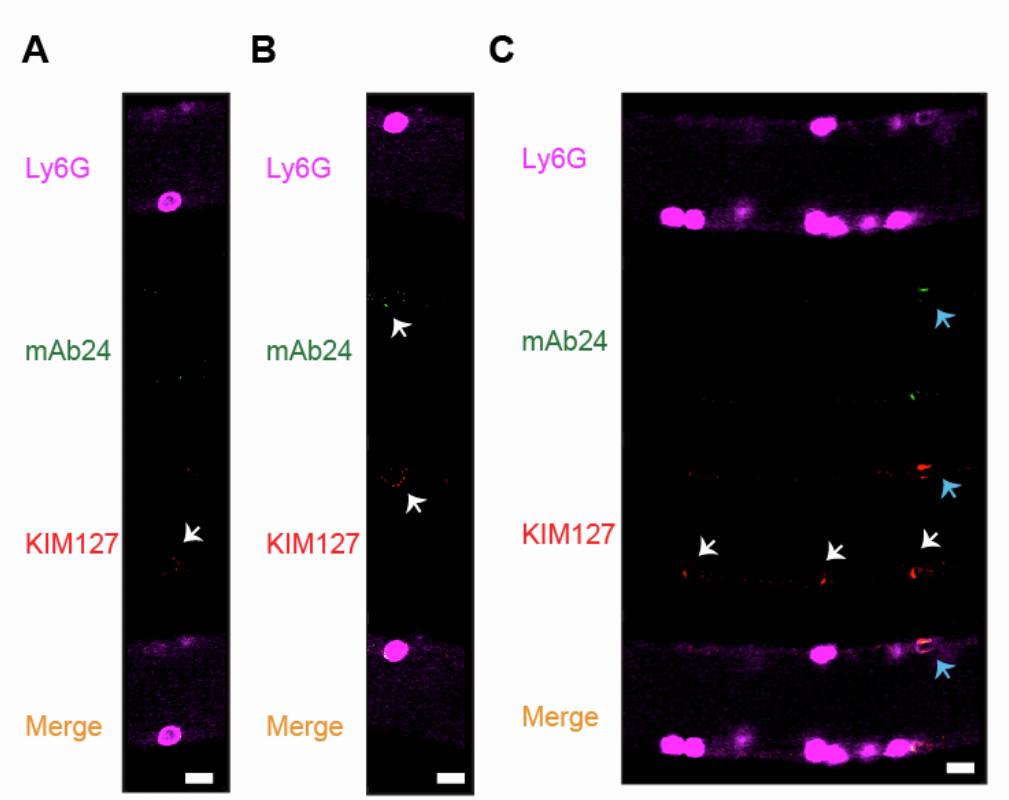
Cell Reports, Volume *39*

Supplemental information

A humanized β_2 integrin knockin mouse reveals localized intra- and extravascular neutrophil integrin activation *in vivo*

Lai Wen, Alex Marki, Zhihao Wang, Marco Orecchioni, Jeffrey Makings, Monica Billitti, Erpei Wang, Sujit S.A. Suthahar, Kenneth Kim, William B. Kiosses, Zbigniew Mikulski, and Klaus Ley

Supplemental Figures



D

Integrin	Ly6G+	Percentage
E-H-	94	92.15%
E+H-	7	6.86%
E-H+	0	0
E+H+	1	0.98%

Figure S1. Rare mAb24 and KIM127 binding in rolling neutrophils before KC application. Related to Figure 4. A. A rolling neutrophil display $E^{+}H^{-}$ integrins. **B**. A rolling neutrophil displays $E^{+}H^{+}$ integrins. **C**. A spontaneous adhering neutrophil with $E^{+}H^{+}$ integrins (Blue arrow). **D**. Summary of Ly6G+ rollers with different status of activated integrins during an observation period of 5 minutes. Scale bars, 10 μm.

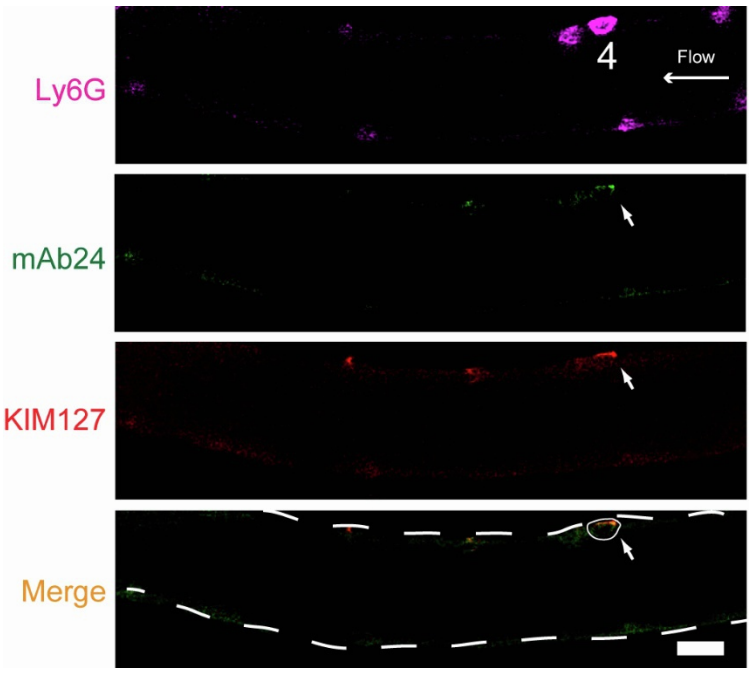


Figure S2. Arrested neutrophil #4 has activated integrins (E^+H^+) when observed in another focal plane shown here, which is not seen in Figure 4. Related to Figure 4.

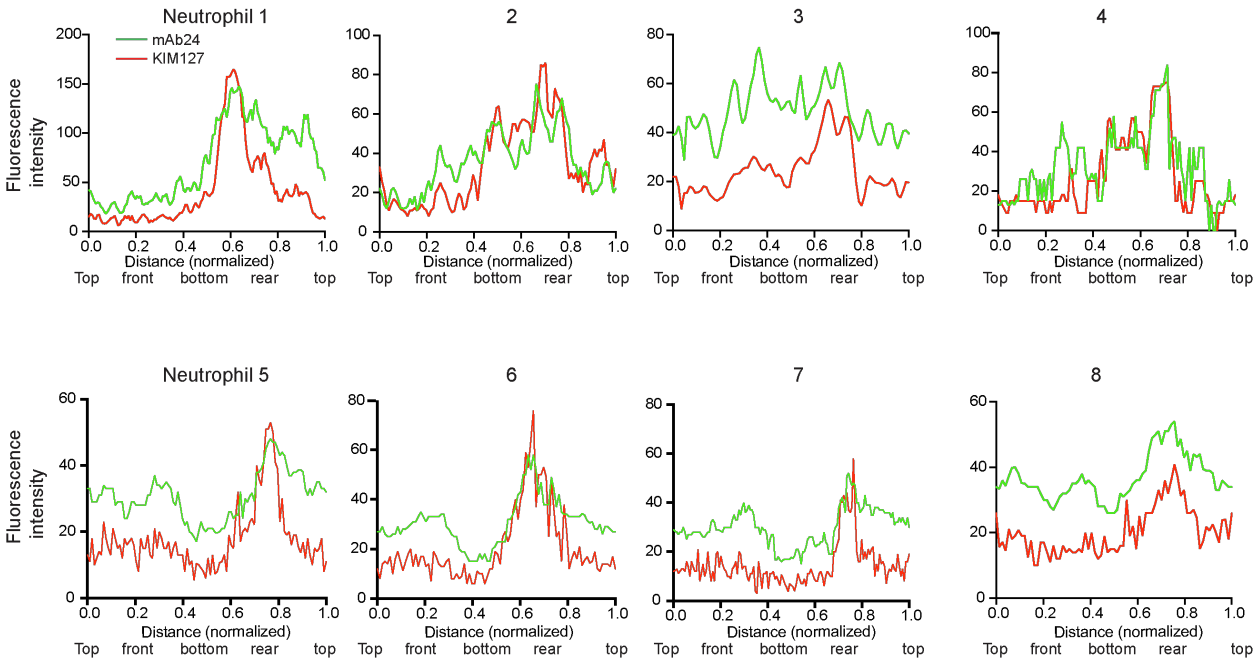


Figure S3. Fluorescence intensity profile of all 8 neutrophils in Figure 4. Related to Figure 4. A line (1 pixel wide using the plot profile function in ImageJ software) was drawn along the cell top, front, bottom and rear of each neutrophil based on Ly6G labelling. Profiles of mAb24 and KIM127 fluorescence intensities were measured as a function of location. Distance normalized to the circumference of each cell (0 to 1).

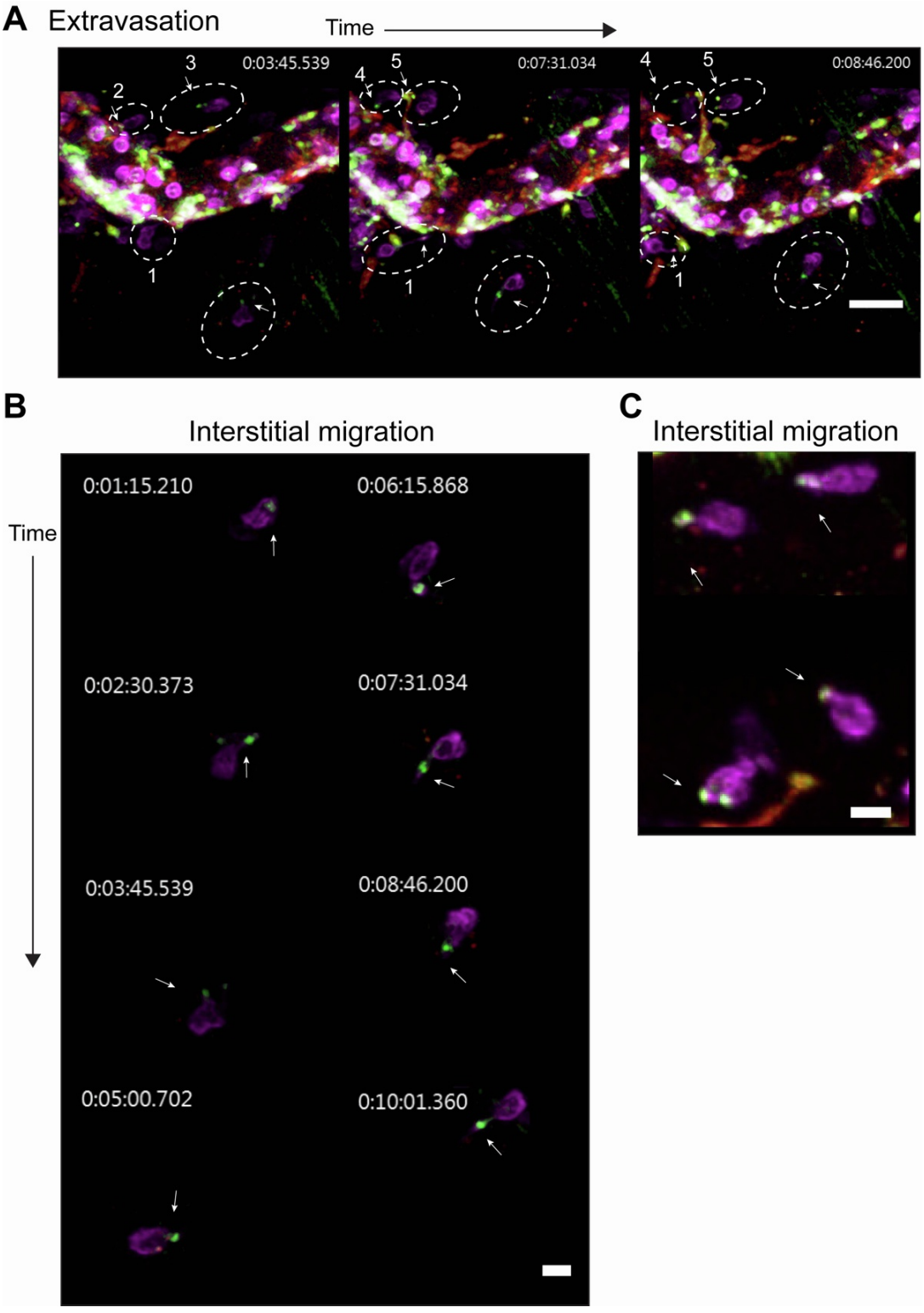


Figure S4. **Integrin activation during neutrophil transendothelial migration**. **Related to Figure 4. A**. Neutrophil extraversion. Open circle 1-5 indicates position of 5 neutrophils transmigrating across the vessel and migrating over time. The white arrows indicate activated integrins at the uropod of neutrophils. Scale bar, 30 μm. **B**. An interstitial migrating neutrophil with

high affinity integrins at the uropod. **C**, Neutrophils show $E^{+}H^{+}$ integrins at the rear during interstitial migration (n=9 neutrophils). Magenta, Ly6G; Green, mAb24; Red, KIM127; Yellow, overlay of mAb24 and KIM127. Scale bars, 10 μm.

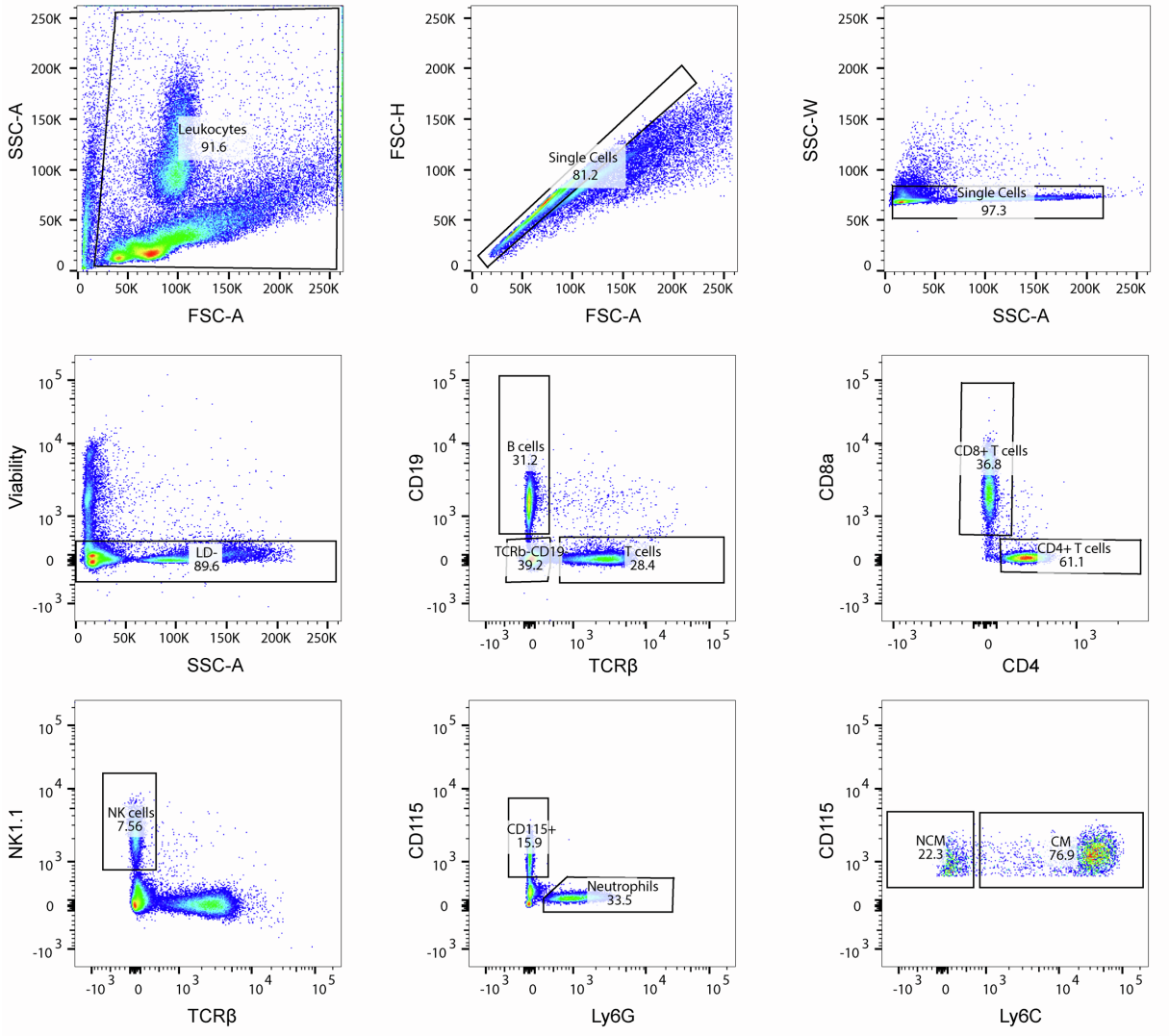


Figure S5. Gating strategy. Related to Figure 5. The gating scheme of blood flow cytometry assay. Singlets and live cells were gated first. Neutrophils: $Ly6G^{+}$; B cells: $CD19^{+}$; $CD4^{+}$ T cells: $TCR\beta^{+}$ $CD4^{+}$; $CD8^{+}$ T cells: $TCR\beta^{+}$ $CD8^{+}$; NK cells: $NK1.1^{+}$; Classical Monocytes: $CD115^{+}Ly6C^{hi}$; Non-classical monocytes: $CD115^{+}Ly6C^{lo}$.

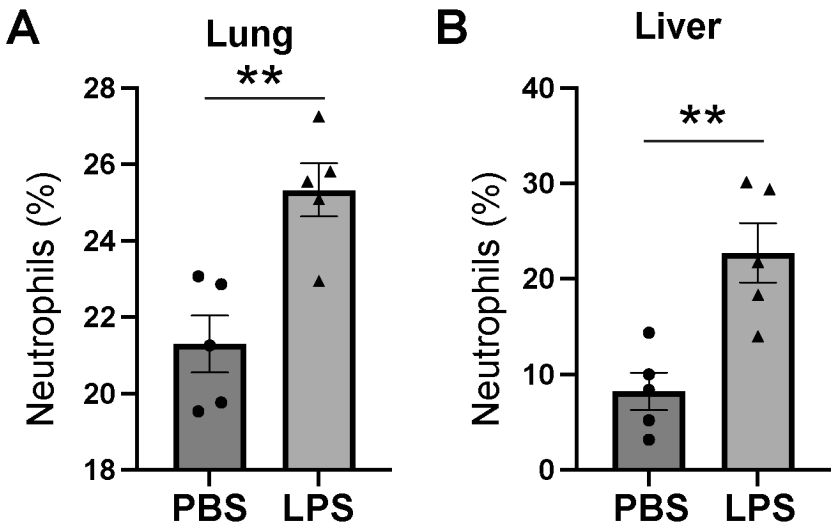


Figure S6. Percentage of neutrophils out of total dissociated cell suspensions from lung (A) and liver (B) were measured by Hemavet. Related to Figure 6.