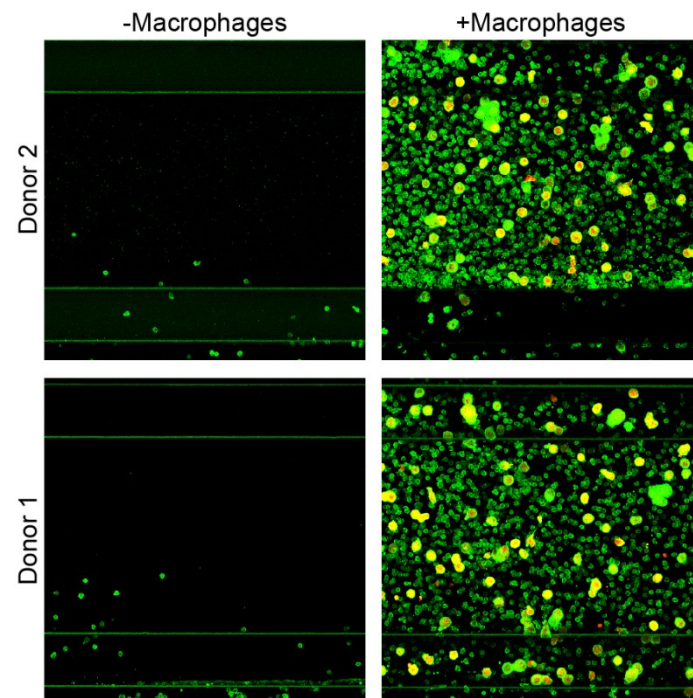
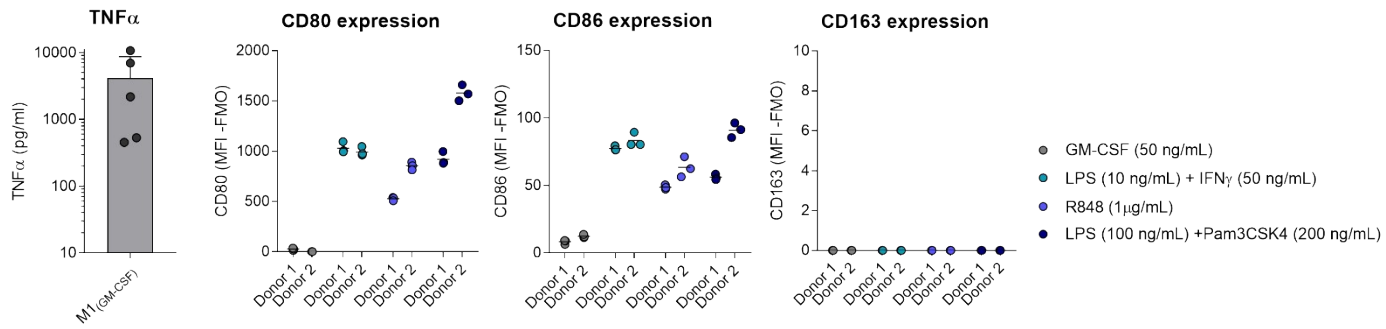


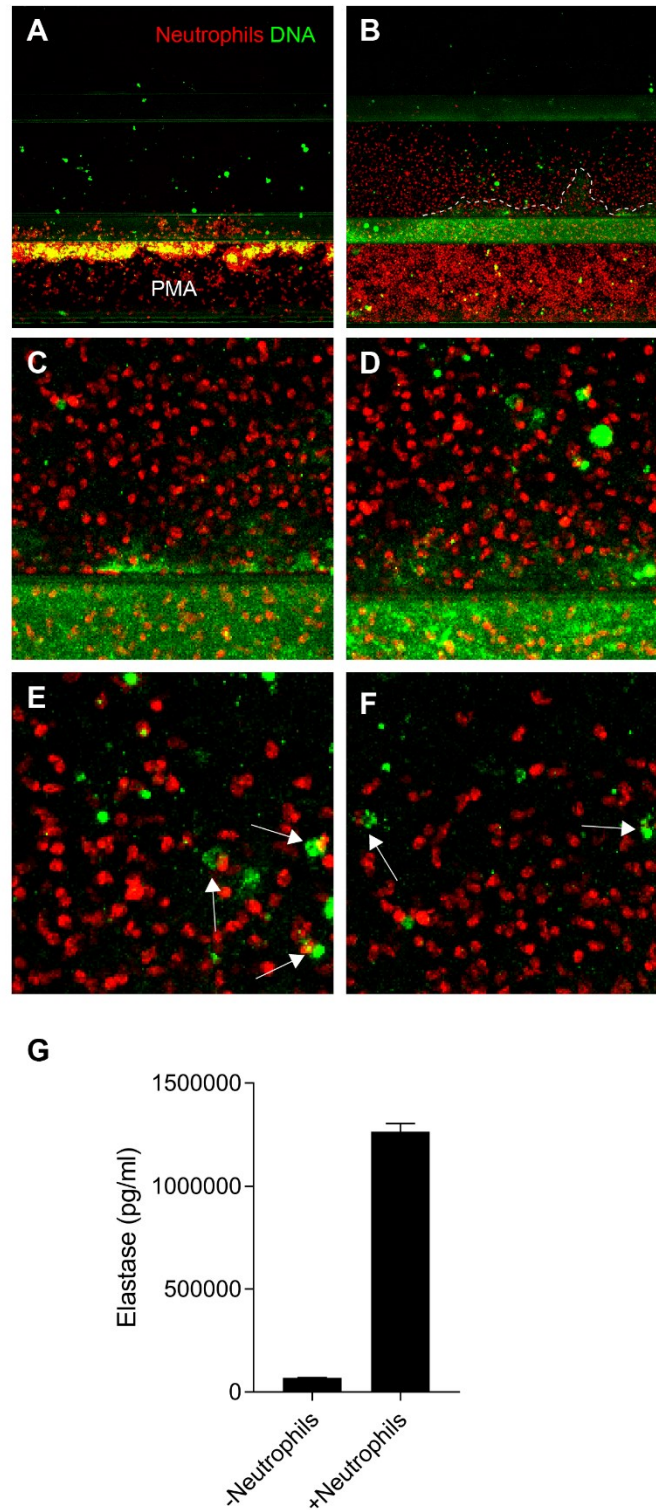
Supplementary Figure 1. Activation of NF κ B pathway in ECM-residing THP1 cells. THP1-blue cells (NF κ B reporter line) were seeded in the extracellular matrix (ECM) and co-cultivated with Caco-2 barrier as in Figure 1. PMA and TNF α were added to the basal channel (channel B) and secretion of SEAP was measured at the indicated time point **a**. Supernatant from the luminal and basal side was collected and analyzed. SEAP: secreted embryonic alkaline phosphatase. Data are shown as mean with s.d. of 3 microfluidic chambers.



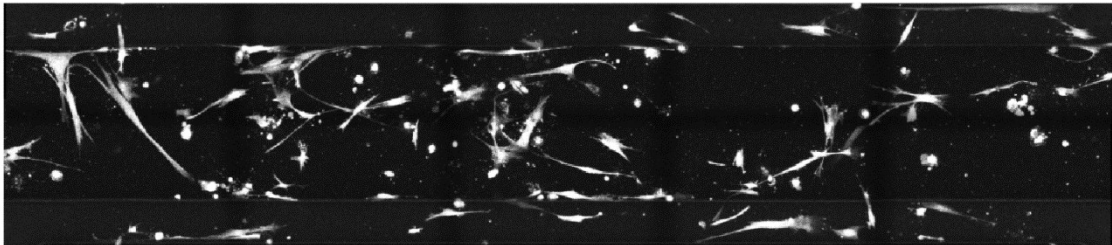
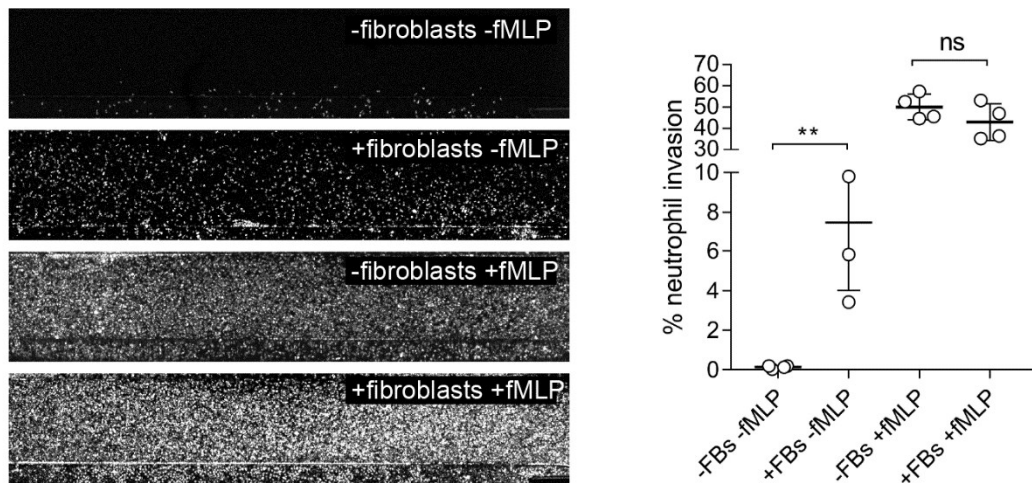
Supplementary Figure 2. Neutrophil recruitment by primary macrophages. Macrophages obtained by differentiating primary human monocytes in the presence of GM-CSF for one week promoted robust neutrophil infiltration into 3D collagen matrices. In this figure, green (F-actin) labels both neutrophils and macrophages and macrophages are uniquely labeled in red.



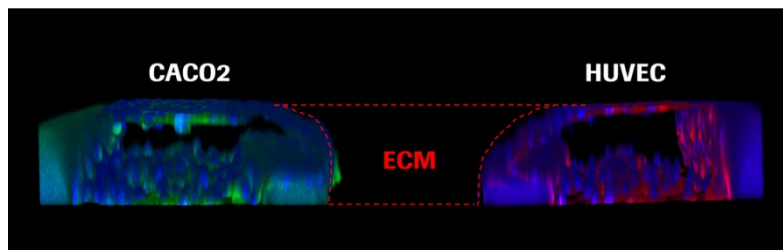
Supplementary Figure 3. Phenotyping of primary macrophages. Macrophages obtained by differentiating primary human monocytes in presence of GM-CSF exhibited high pro-inflammatory TNF α cytokine secretion in supernatants harvested after one week of culture (each dot corresponds to one different blood donor). These macrophages were prone to react to inflammatory signals as illustrated by CD80 and CD86 activation markers upregulation detected by surface staining flow cytometry assessment after one day of stimulation by Lipopolysaccharide LPS (10ng/mL) + IFN γ (50ng/mL), or Toll-like receptor 7/8 agonist Resiquimod R848 (1 μ g/mL) or LPS (100ng/mL) + Toll-like receptor 1/2 agonist Pam3CSK4 (200 ng/mL). On the contrary, the M2-like resident macrophage marker CD163 was undetectable by flow cytometry neither at basal nor stimulated state. MFI refers to Mean Fluorescence Intensity and FMO refers to Fluorescence Minus One).



Supplementary Figure 5. Neutrophil netosis and elastase production on-chip. (A) Formation of neutrophil extracellular traps (NETs) on-chip was monitored by fluorescent labeling of extracellular DNA. Introduction of PMA (as a positive control) into the basal channel resulted in massive netosis. Netosis was also observed in the absence of PMA, during neutrophil invasion of macrophage-containing collagen gels. Diffuse extracellular DNA was detected at the interface between the vascular and stromal channels of the chip. **Dashed line in B indicates region of diffuse DNA signal (B-D).** In addition, individual neutrophils showed strong localized pericellular DNA signal (**white arrows**), indicative of netosis (**E-F**). **Neutrophils also produce elastase in the Organoplate, as indicated by ELISA analysis of supernatants of chips with and without neutrophils (G).**

A**B**

Supplementary Figure 6. Human intestinal fibroblasts influence neutrophil infiltration in the gut-on-a-chip device. (A) Primary human intestinal fibroblasts are successfully cultured in the matrix compartment of the device, where they adopt a characteristic 3D morphology. (B) In the absence of a strong inflammatory signal, fibroblasts themselves can induce neutrophil infiltration into a 3D collagen-rich ECM. Data are shown as individual points, with mean and s.d. ****** $P < 0.01$.



Supplementary Figure 7. Vascular tube constructed within the Gut-on-a-chip device. Formation of Caco2 and HUVEC monolayers lining a collagen-based extracellular matrix is performed as described in main Figure 1 and corresponding methods for the Caco2 mono-culture, except that HUVEC medium is used instead of Caco2 medium at the time of seeding, during intestinal and vascular tube formation and in-chip maintenance.