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Supplementary Materials for

Transitional premonocytes emerge in the periphery for host defense against bacterial infections

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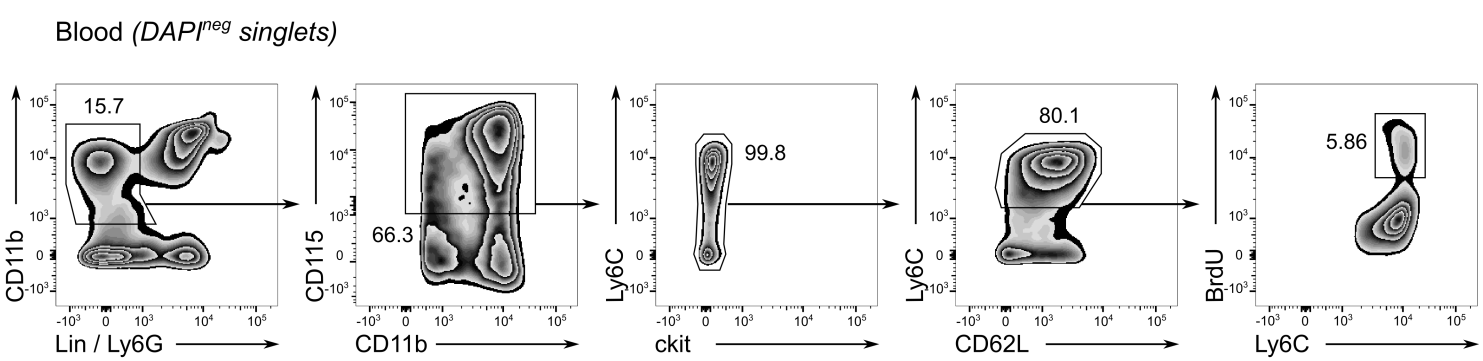
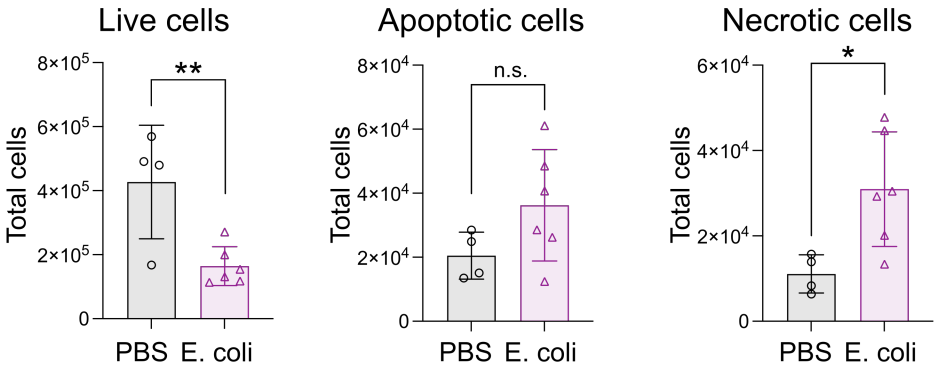
This PDF file includes:

Figure S1



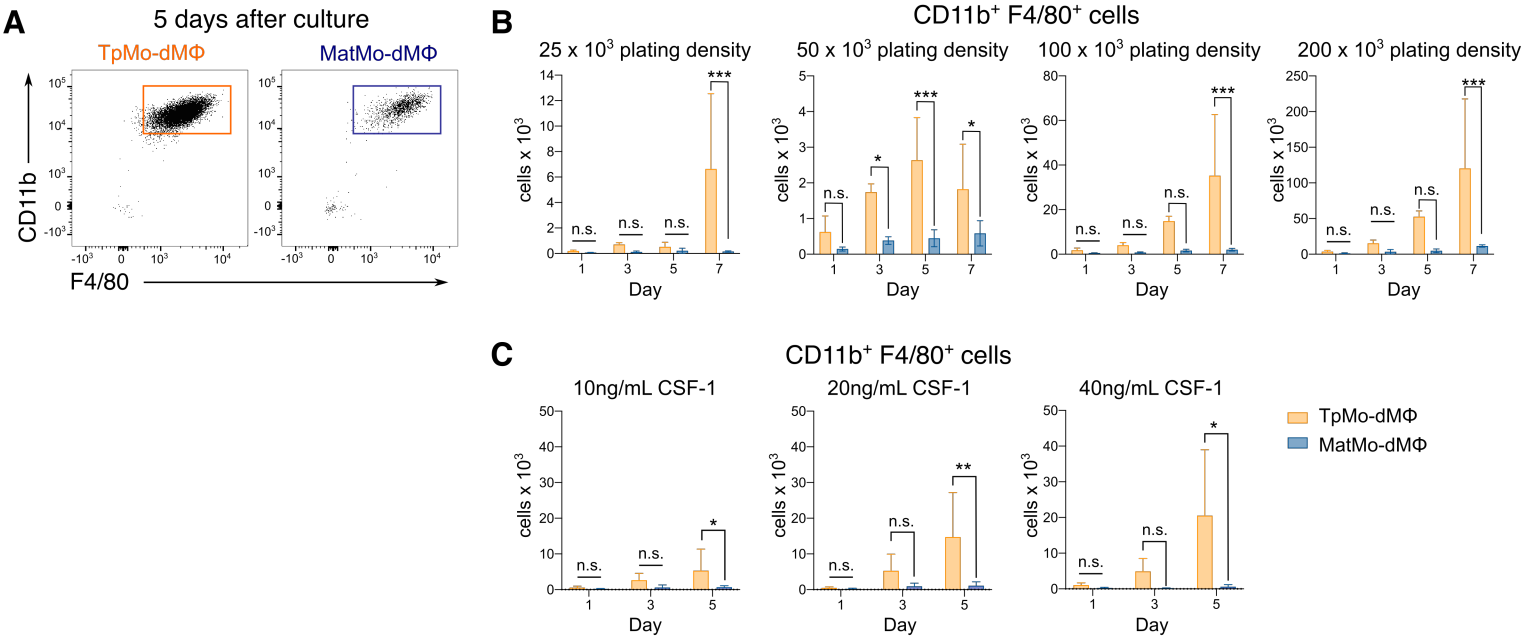
Supplementary Figure S1: Gating strategy of blood proliferating Ly6C^{hi} monocytes (pregated on DAPI^{neg}/ singlets). Blood proliferating Ly6C^{hi} monocytes are defined as Lin(B220/ CD90.2/ NK1.1)^{neg} Ly6G^{neg} CD11b^{pos} CD115^{pos} ckit^{neg} Ly6C^{hi} BrdU^{pos} or Fucci^{pos}. Flow cytometry plots are representative of one out of at least three independent experiments.

Figure S2



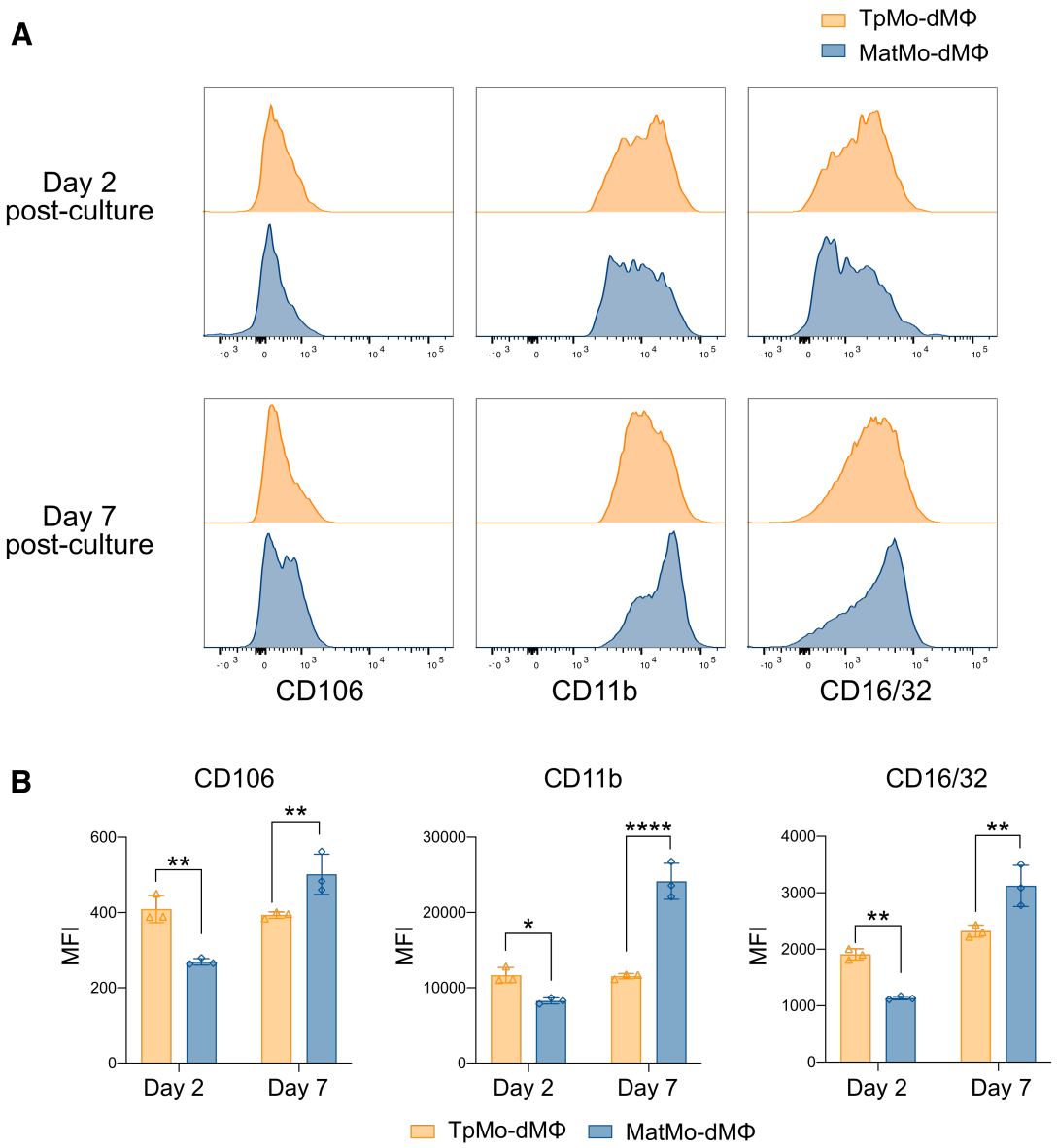
Supplementary Figure S2: Cellular numbers of live, apoptotic, and necrotic peritoneal macrophages after *E.coli* infection. The number of live, apoptotic, and necrotic peritoneal macrophages were quantified after 18 h of *E. coli* infection. Results are quantified as triplicates and representative of one out of three independent experiments. *n.s. not significant, *P<0.05, **P<0.01* (Student's t-test).

Figure S3



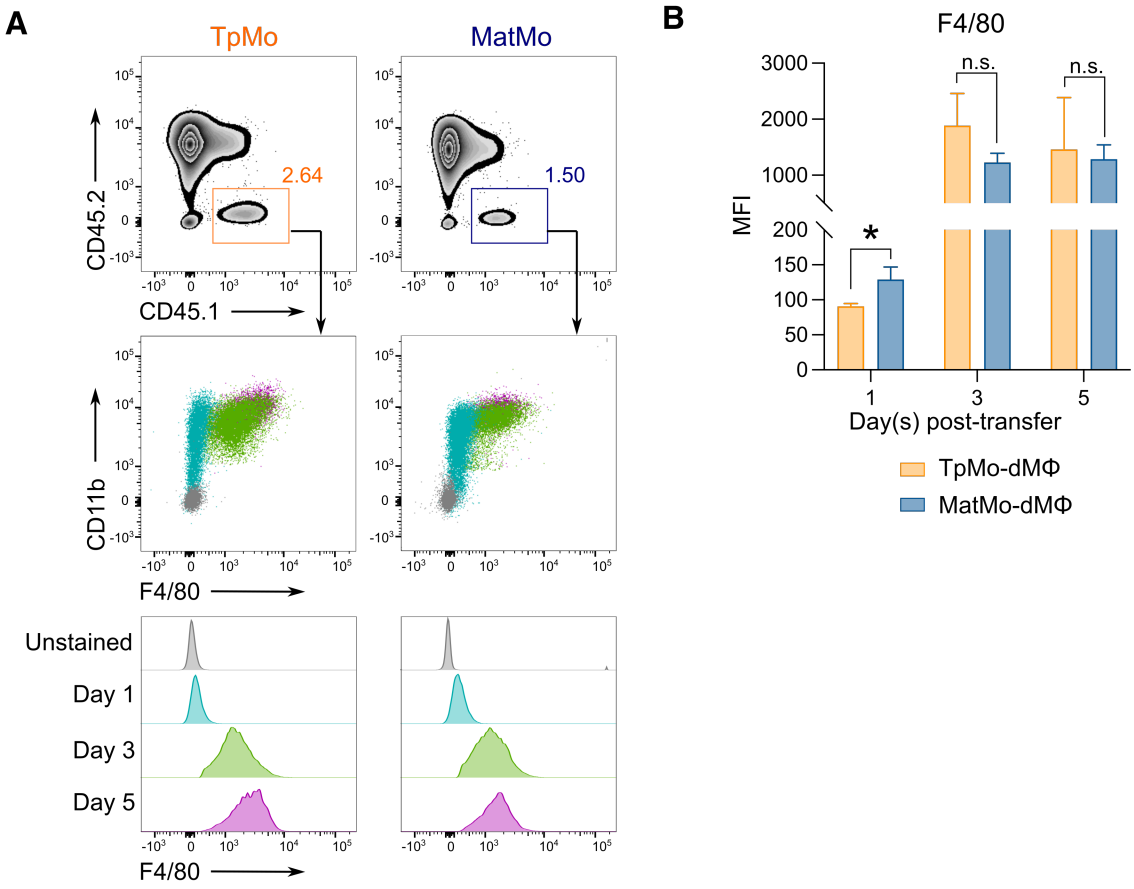
Supplementary Figure S3: TpMos generated more macrophages than MatMos at titrated doses of CSF-1. **(A-C)** Sorted TpMos or MatMos were cultured *in vitro* and macrophages were analyzed based on F4/80 and CD11b. **(A)** TpMo-derived macrophages (TpMo-dMΦs) and MatMo-derived macrophages (MatMo-dMΦs) harvested at indicated timepoints and plating densities **(B)** as well as doses of CSF-1 **(C)**. Results are quantified as triplicates and representative of one out of three independent experiments. *n.s. not significant, *P<0.05, **P<0.01, ***P<0.001* (Student's t-test).

Figure S4



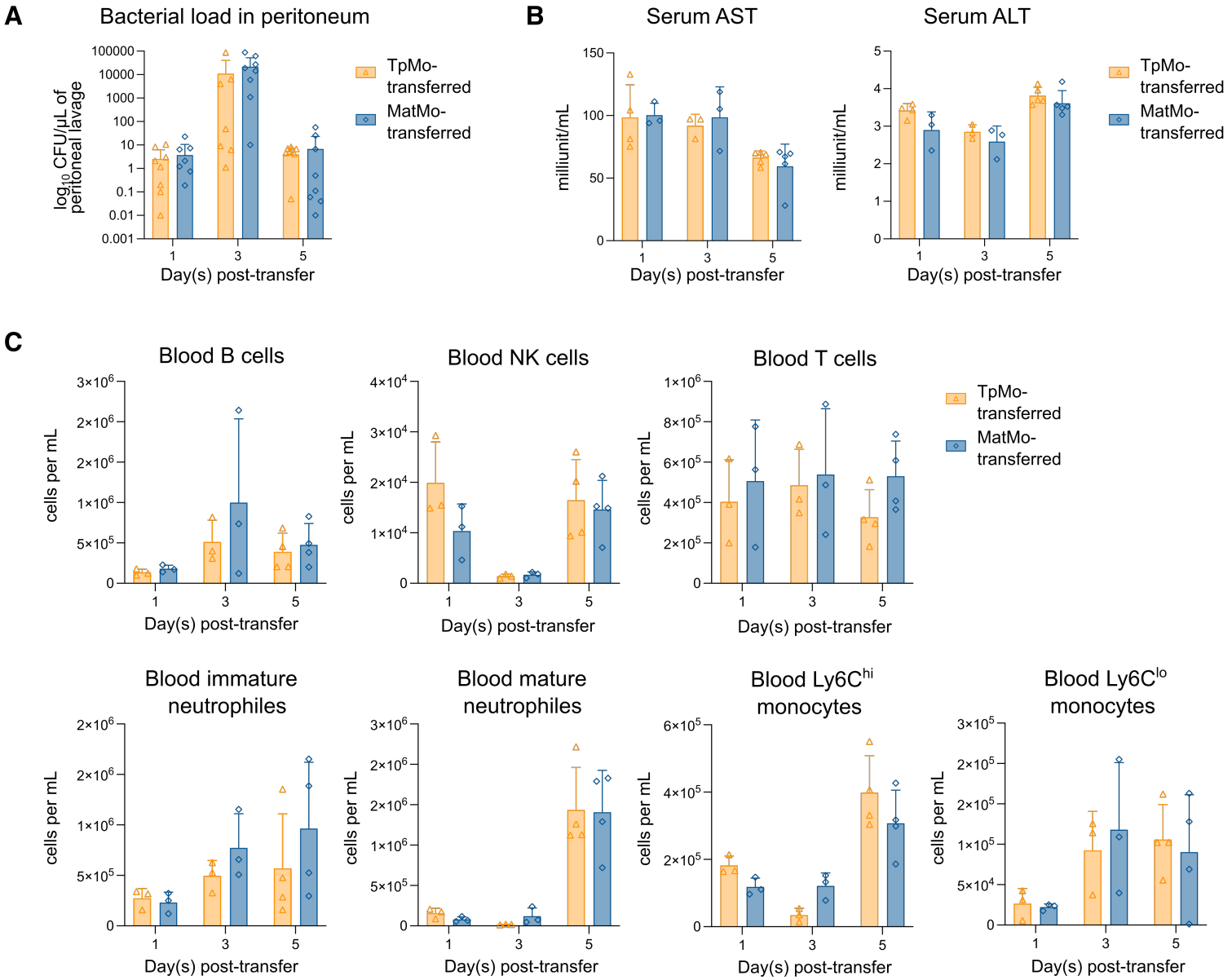
Supplementary Figure S4: Phenotypic characteristics of TpMo-derived macrophages (TpMo-dMΦs) and MatMo-derived macrophages (MatMo-dMΦs). (A-B) TpMos and MatMos were cultured with 20ng/ml of CSF-1 and analyzed at 2- or 7-days post-culture for surface markers **(A)** with median fluorescence intensity quantified **(B)**. Results are expressed as mean ± SD and representative of one out of three experiments. **P<0.05, **P<0.01, ****P<0.0001* (Student's t-test).

Figure S5



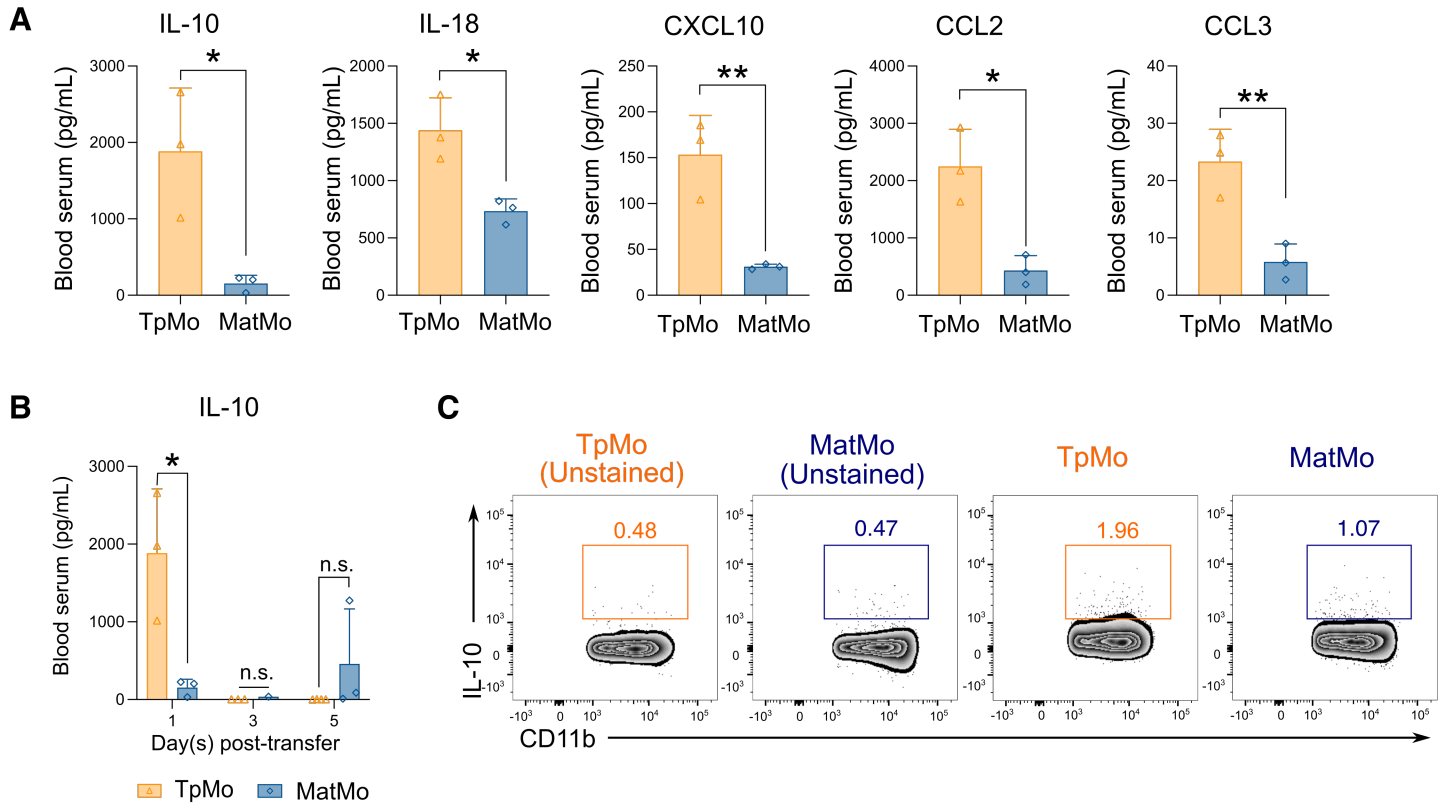
Supplementary Figure S5: Expression of F4/80 on TpMo-derived macrophages (TpMo-dMΦs) and MatMo-derived macrophages (MatMo-dMΦs) *in vivo*. (A-B) TpMos and MatMos from CD45.1 mice were sorted and adoptively transferred i.p. into CLP-induced CD45.2 mice. Analysis of peritoneal lavage of recipient mice for transferred cells were performed on Day 1, 3, and 5 after CLP for F4/80 expression **(A)** with median fluorescence intensity of F4/80 **(B)** quantified. Results are quantified as triplicates and representative of one out of three independent experiments. *n.s. not significant, *P<0.05* (Student's t-test).

Figure S6



Supplementary Figure S6: Assessment of sepsis parameters of mice that received TpMos and MatMos. **(A-C)** TpMos and MatMos were sorted and adoptively transferred i.p. into CLP-induced septic mice. Recipient mice were harvested on Day 1, 3, and 5 after CLP to check for bacterial load in the peritoneum **(A)**, serum AST/ALT levels **(B)**, and blood leukocyte numbers **(C)**.

Figure S7



Supplementary Figure S7: Serum cytokine and chemokine profile of septic mice after adoptive transfer of TpMos versus MatMos. (A-B) TpMos and MatMos from CD45.1 mice were sorted and adoptively transferred i.p. into CLP-induced CD45.2 mice. LUMINEX analysis of blood serum of recipient mice were performed on Day 1, 3, and 5 after the adoptive transfer for cytokine and chemokine expressions. Results are quantified as triplicates and representative of one out of three independent experiments. *n.s. not significant, *P<0.05, **P<0.01* (Student's t-test). **(C)** Sorted TpMos and MatMos were analyzed for the expression of IL-10 via intracellular cytokine staining. Flow cytometry plots are representative of one out of at least three independent experiments.