Supplementary Figure 1. Accumulation of macrophages in the ventral wall of the human aorta during the emergence of HSPCs, related to Figure 1.

(a-c’) Transverse sections through the DA of 26 (a-a’, n=3), 34 (b-b’, n=2) and 37-day human embryos (c-c’, n=3). Immunostaining by anti-CD68 reveals that the number of macrophages (arrowhead) in the mesoderm beneath the aorta increases with development. (a’, b’, c’) Higher magnification of black box in a, b, c. DA, dorsal aorta; Mn, mesonephros; NC, notochord. Scale bar: 100 µm (a-c); 50 µm (a’-c’).
Supplementary Figure 2. Macrophage depletion by L-clodronate has no toxic effect on zebrafish vasculature nor on neutrophil emergence and functions, related to Figure 2.

(a-b) L-clodronate injection at 32 hpf Tg(mpeg1:mCherry) specifically depletes macrophages (b, 52 hpf, high magnification, n=200) compared to injection with L-PBS (a, white arrow, high magnification, n=25) but has no effect on vasculature development (d vs c, n=15), Tg(kdrl:caax:mCherry), 52 hpf, high magnification) nor neutrophil survival (arrowhead) and function (arrow) as shown by the tail wound test (f vs e, Tg(mpx:eGFP), 72 hpf, high magnifications, n=35).

Scale bar: 250 µm (a). AGM, aorta gonad mesonephros; CHT, caudal haematopoietic tissue.

(g) Graph represents the evolution of macrophages (n=10) in the AGM and the CHT every hour during 18 hours after L-clodronate injection. Red columns stand for normal macrophages, grey for vacuolated or fragmented macrophages. Data shown as an average ± SEM.

(h) Graph represents the evolution of neutrophils in the AGM (n=10) and the CHT every hour during 18 hours after L-clodronate injection. Data shown as an average ± SEM.
Supplementary Figure 3. Re-appearance of macrophages in genetically depleted embryos directly rescues mobilisation of HSPCs/cd41:eGFP+ cells and haematopoietic organ colonisation. Related to Figure 3.

(a-b) Graphs represent the evolution of the number of macrophages/mpeg:mCherry+ (red line) and HSPCs/cd41:eGFP+ (columns) in the AGM (a) and CHT (b) over the time after metronidazole removal (at 60 hpf, then 20 and 40 hours after removal respectively at 80 hpf and 100 hpf, n=11). Data shown as relative ratio ± SEM compared to control embryos represented as 100% (n=9).
Supplementary Figure 4. Mmps expressed by macrophages affect colonisation of haematopoietic organs by HSPCs/cd41:eGFP+. Related to Figure 4. 
(a-c) Accumulation of HSPC/cd41:eGFP+ cells in the thymus (arrow) at 72 hpf. Control embryo (a), treated with GM6001 (b) or SB-3CT (c). Fluorescence is merged with transmitted light. 
(d) Quantification of the HSPC/cd41:eGFP+ number after treatment with Mmps inhibitors from 38 hpf compared to control. Graph shows HSPC/cd41:eGFP+ accumulation in the AGM at 60 hpf,
induced by GM6001 or SB-3CT (+26% and +51% respectively), decrease of colonisation in the

CHT at 60 hpf (-43% and -66% respectively) and in the thymus at 72 hpf (-44% and -75%
respectively). Data are shown as average ±SEM. * P < 0.05; *** P < 0.001 (Student’s t-test for
AGM and Wilcoxon test for CHT and thymus); n = number of embryos from minimum 3
independent experiments.

Confocal imaging of WISH for mmp-2 (e) combined with L-plastin immunofluorescence
(green) (e’) are merged in e’’. (f-g’) WISH for mmp-2 in the AGM in control embryos (f) and after
macrophage depletion by L-clodronate (g). Close-up of macrophage depleted embryo in g’. Arrow
indicates the presence of mmp-2 expressing cells. Scale bar: 50µm (a-c, f-g), 10 µm (e-e’’, g’).
Supplementary Figure 5. Gelatin degradation depends on macrophage-expressing mmps.

Related to Figure 5.
(a-c) Release of FITC signals from degraded gelatin-FITC in the AGM in Tg(mpeg1:mCherry) after macrophage passage (red cells) is disrupted by treatment with MMP inhibitors GM6001 (middle column) and SB-3CT (right column) (n=4).

(d-e) Degradation of gelatin-FITC in the AGM in Tg(kdrl:caax:mCherry) (d, arrow) is impaired after chemical L-clodronate mediated macrophage depletion (e, n=4).

(f) Graph represents the number of FITC+ foci appearing either after macrophage passage (macrophage induced, full line) or without macrophage interaction (independent, spaced line) every hour during over-night time-lapse (n=3).

Scale bar: 10µm (a-c), 50µm (d-e)