

Supplementary Figure S1

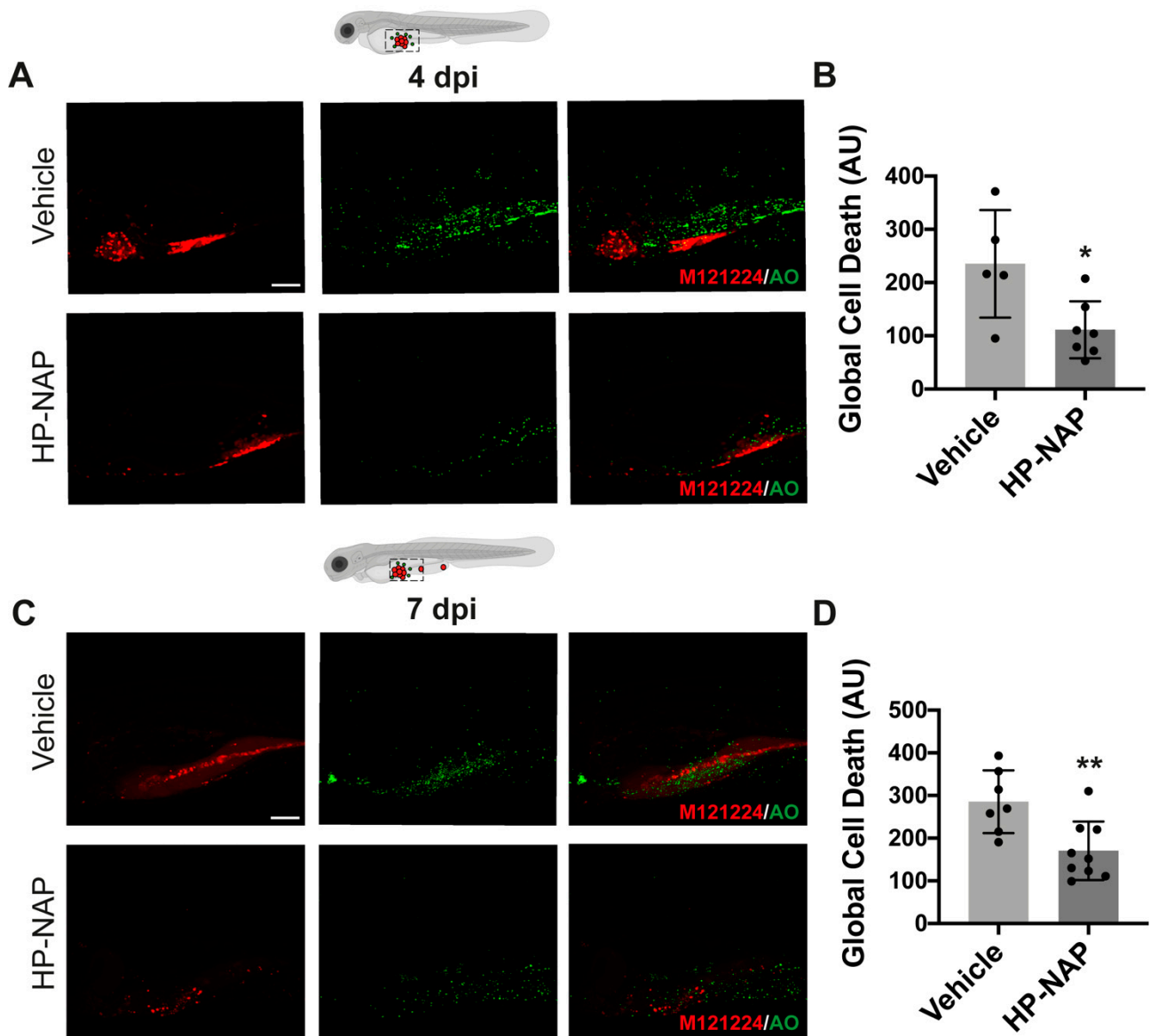


Figure S1. (A) Representative Z-projections of confocal images of the yolk-sac region, stained with Acridine Orange (AO, green dots), in 4 dpi larvae injected with M121224 human melanoma cells (red signals) and treated with vehicle or HP-NAP. Scale bar: 100 μ m. **B.** Scatter plots show the quantification (AU) of the cell death marker AO. Values are expressed as mean \pm SEM and analyzed by Student's *t* test; *, $p < 0.05$; vehicle ($n=5$) vs. HP-NAP ($n=7$). **C.** Representative Z-projections of confocal images of the yolk-sac region, stained with Acridine Orange (AO, green dots), in 7 dpi larvae injected with M121224 human melanoma cells (red signals) and treated with vehicle or HP-NAP. Scale bar: 100 μ m. **D.** Scatter plots show the quantification (AU) of the cell death marker AO. Values are shown as mean \pm SEM and analyzed by Student's *t* test; **, $p < 0.01$; HP-NAP ($n=9$) vs. vehicle ($n=7$).

Supplementary Figure S2

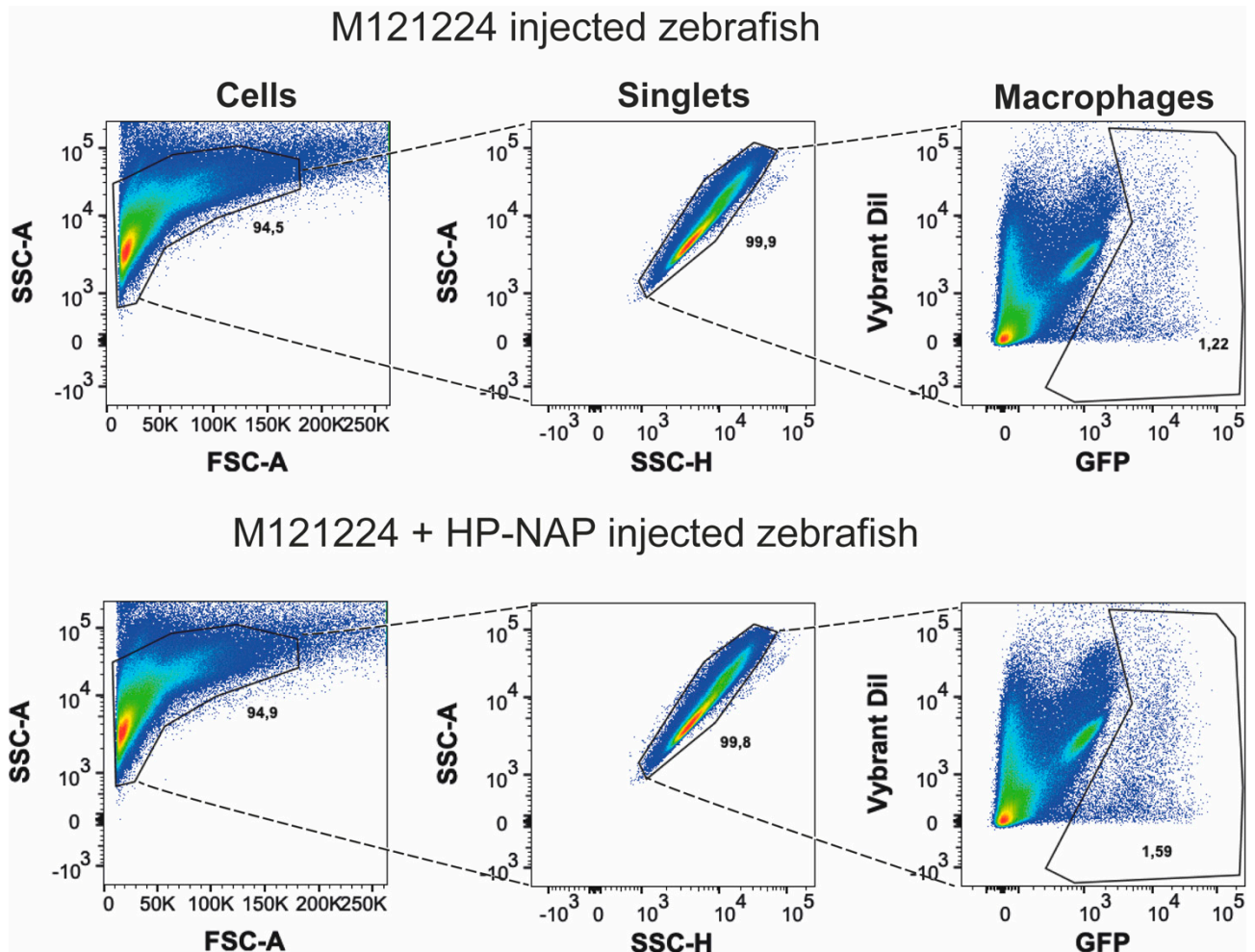


Figure S2. Representative gating strategy of macrophages sorting. Zebrafish *Tg(mpeg1:eGFP)gl22* larvae, treated with HP-NAP or untreated, were dissociated at 3 dpi, digested to obtain single cell suspension and GFP⁺ macrophages were sorted using FACS Aria III sorter. Cells from M121224-injected zebrafish treated or not with HP-NAP were first gated, based on the FCS-A and SSC-A, then single cells were gated based on SSC-A and SSC-H. Macrophages were gated based on GFP expression.

Supplementary Figure S3

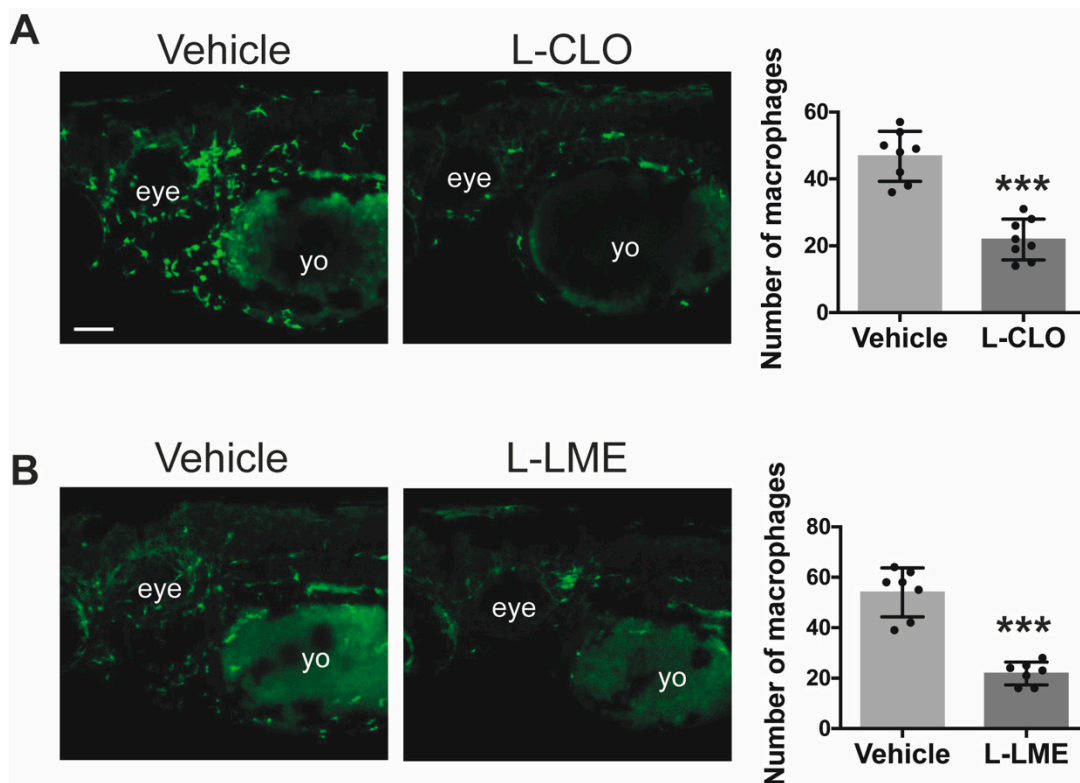


Figure S3. Depletion of macrophages with liposome-encapsulated clodronate (L-CLO) and L-Leucyl L-Leucine Methyl Ester (L-LME). **(A)** Representative Z-projections of confocal images of Tg(mpeg1:EGFP)gl22 in 3 dpf embryos (head, lateral view) transplanted with M121224 cells. Images show the decreased number of macrophages (green signals) 24 h after the administration of L-CLO compared to empty liposomes (vehicle). Scale bar: 200 μ m. Scatter plots show the number of macrophages in the selected area; ***, $p < 0.001$; $n = 8$ for each condition. **(B)** Representative Z-projections of confocal images of embryos at 3 dpf (head, lateral view). Images show the decreased number of macrophages (green signals) 24 h after the administration of L-LME compared to vehicle. Scale bar: 200 μ m. Scatter plots show the number of macrophages in the selected area; ***, $p < 0.001$; $n = 7$ for each condition. yo: yolk (diffused green fluorescence due to auto-fluorescence).

Supplementary Video S1: 3D reconstruction of macrophages and melanoma cells.

3D reconstruction of macrophages (green, *Tg(mpeg1:EGFP)gl22*) and melanoma cells (red, M121224) in vehicle- (A) and HP-NAP-treated (B) larvae at 4 dpi.

