Cell Reports, Volume *42*

Supplemental information

A macrophage subpopulation promotes airineme-mediated intercellular communication in a matrix metalloproteinase-9 dependent manner

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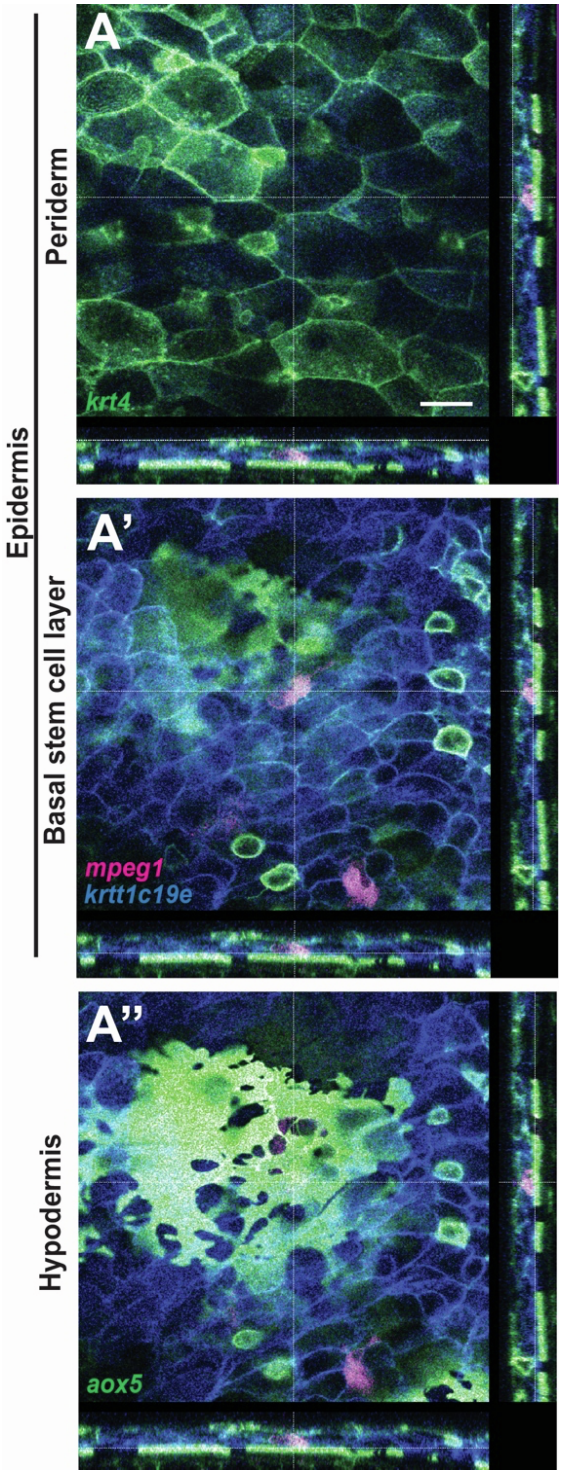


Figure S1. Metamorphic zebrafish skin layers, Related to Figure 1
(A) Visualization of the peridermal layer using *Tg(krt4:palmEGFP).* (A') Basal stem cell layer and intermediate layer between the periderm, indicated by *Tg(krtt1c19e:lyn-tdtomato)*, peudo-colored in blue. (A'') Hypodermis labeled with the injected xanthophore-lineage marker *aox5:palmEGFP*. Amoeboid *mpeg1+* macrophages (magenta) observed on top of xanthoblasts in the hypodermal layer. Scale bar: 20μm.

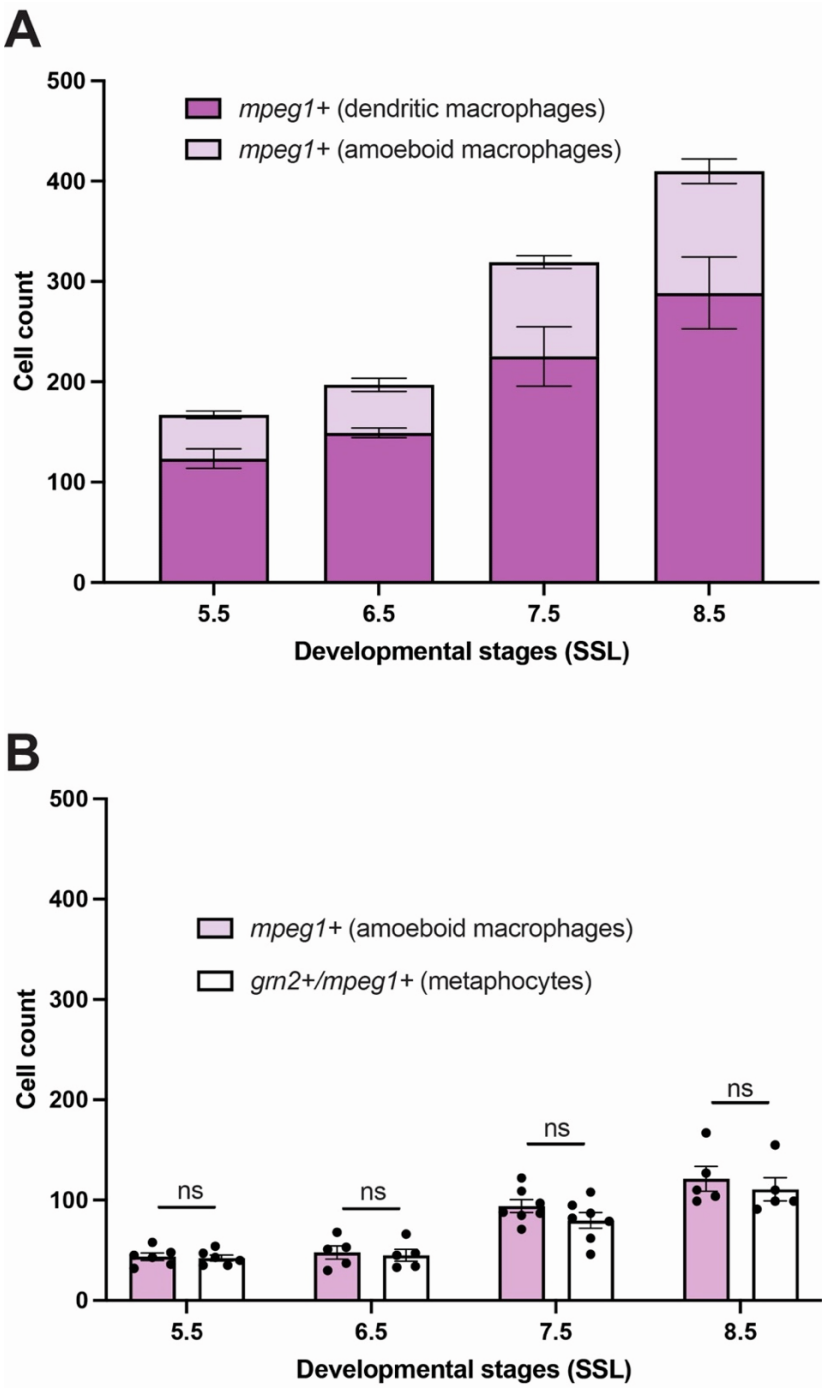


Figure S2. Skin-resident macrophage populations in various developmental stages in zebrafish, Related to Figure 1 and 2

(A) Developmental stage-dependent cell counts in both dendritic and amoeboid macrophage subpopulation. The proportion of amoeboid macrophages gradually increases as fish develops (~24% at SSL5.5 to ~29% at SSL8.5), but no dramatic expansion at SSL 7.5 (n=8 trunks at SSL5.5, 5 at SSL 6.5, 7 at SSL 7.5, 6 at SSL8.5). (B) Numbers of amoeboid macrophages (*mpeg1+)* and metaphocytes (*mpeg1+/grn2+)* are not differ in various developmental stages in *Tg(mpeg1:tdtomato), Tg(mpeg1:tdtomato; grn2:EGFP)*; n=5 trunks at SSL 5.5, n=5 at SSL 6.5, n=7 at SSL 7.5, n=5 at SSL 8.5.

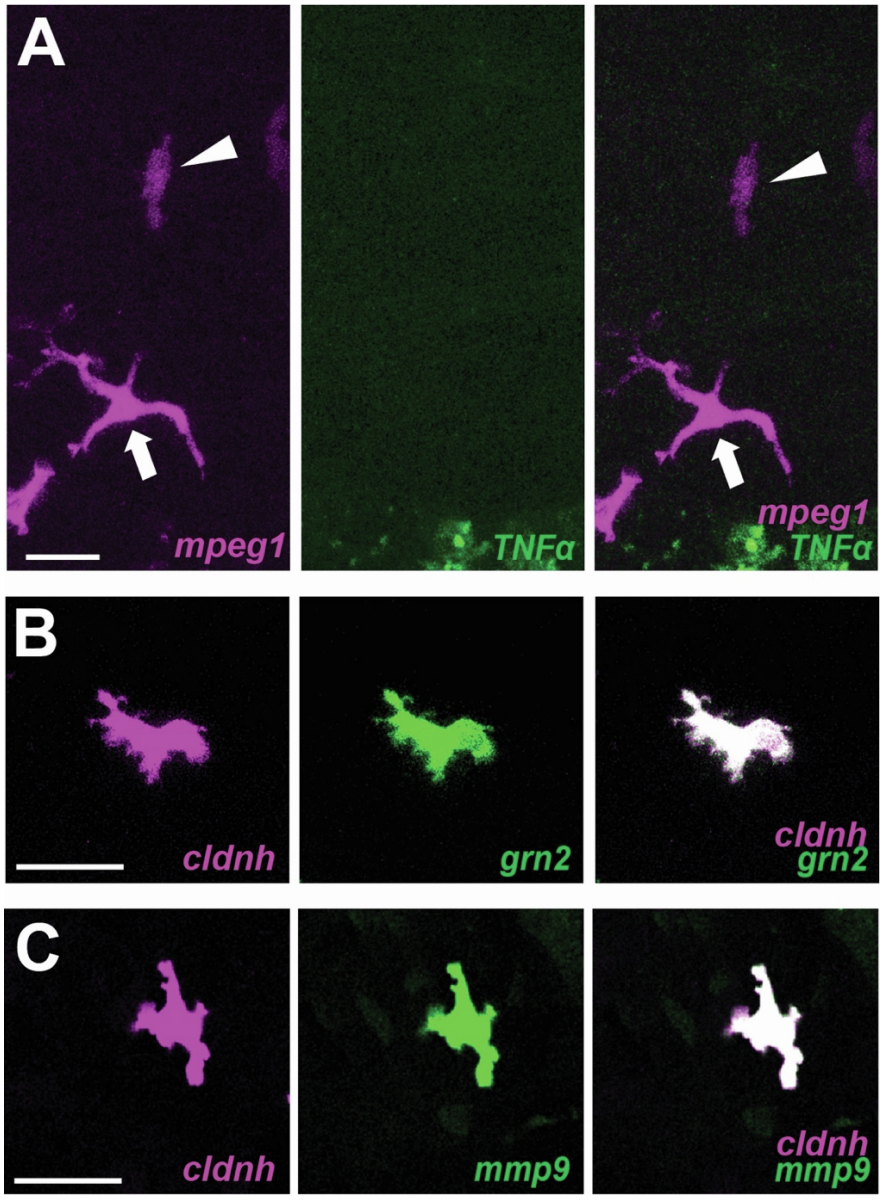


Figure S3. Amoeboid macrophage subpopulation, Related to Figure 2 and 4
(A) Neither amoeboid (arrowhead) and dendritic (arrow) macrophages are *TNFα* positive. *Tg(mpeg1:tdtomato; TNFα:EGFP)* were imaged. (B) Amoeboid macrophage subpopulation express both metaphocyte markers (*grn2* and *cldnh*). (C) Amoeboid macrophages express metaphocyte marker, *cldnh* and *mmp9*. *cldnh:mCherry* construct was injected into *Tg(grn2:EGFP)* or *Tg(mmp9:EGFP).* Scale bars: 20μm.

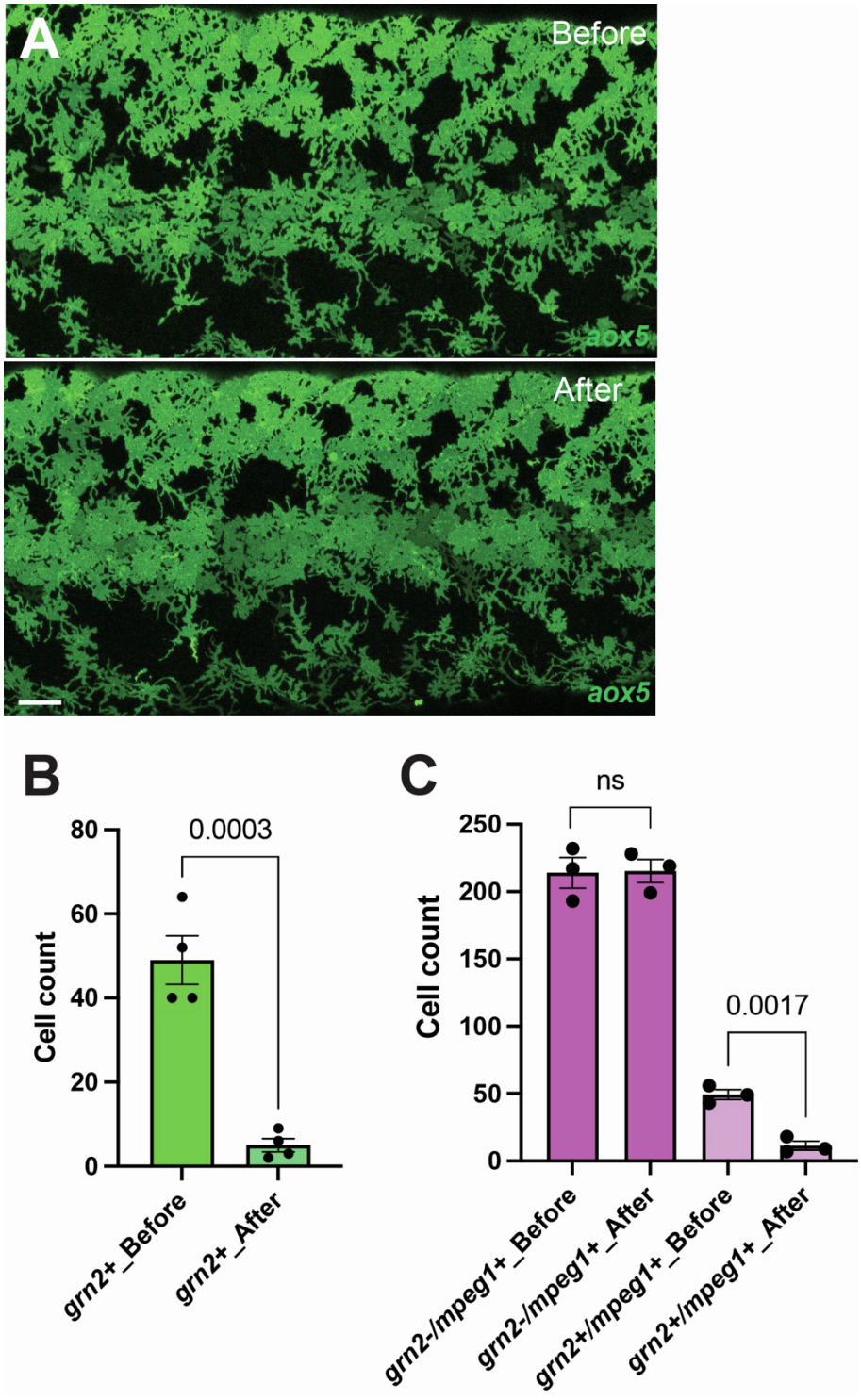


Figure S4. Metaphocyte-specific ablation, Related to Figure 2
(A) Xanthophore-lineage cells remained unaffected by metaphocyte depletion. (B) Effective ablation of metaphocytes was observed in *Tg(grn2:nfsB)* fish treated with mtz (n=4, 4 trunks). (C) Metaphocyte depletion was specific, with no significant effect on the dendritic population (n=3 trunks each). Scale bar: 20μm (A).

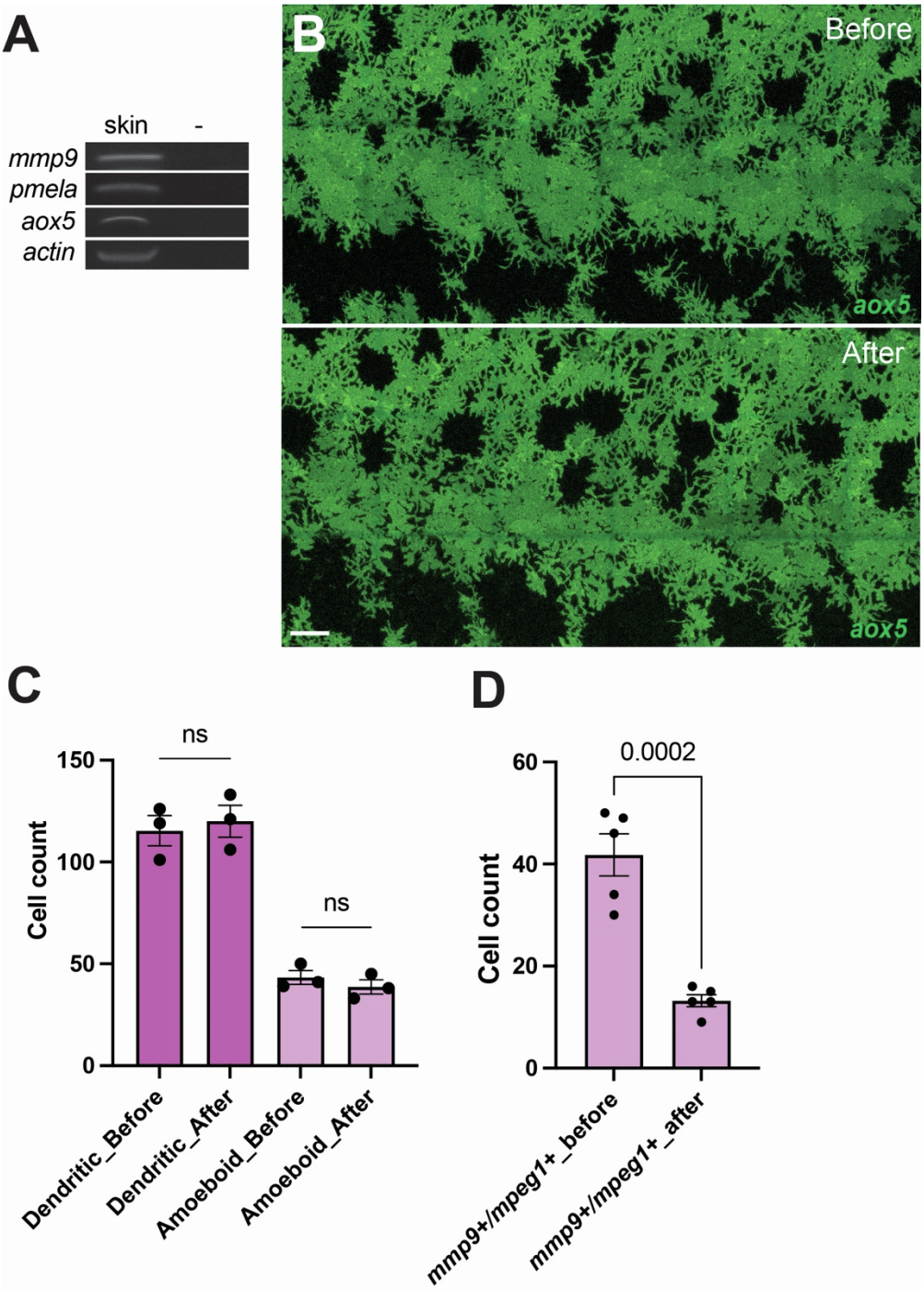


Figure S5. MMP9 inhibitor treatment does not affect the number of xanthophore-lineage cells and *mpeg1*+ macrophages, Related to Figure 4

(A) RT-PCR revealed endogenous mmp9 expression in metamorphic zebrafish skin. *aox5* marks xanthophore lineages, *pmela* for melanophores, and actin control. (B) Xanthophore-lineage cells are unaffected by MMP9 inhibitor treatment. (C) Neither dendritic nor amoeboid macrophage numbers were changed upon MMP9 inhibitor treatment (n=3 trunk each). (D) Effective ablation of *mmp9*+ cells was observed in *Tg(mmp9:nfsB)* fish treated with mtz (n=5 trunk each). Scale bar: 20μm (B).

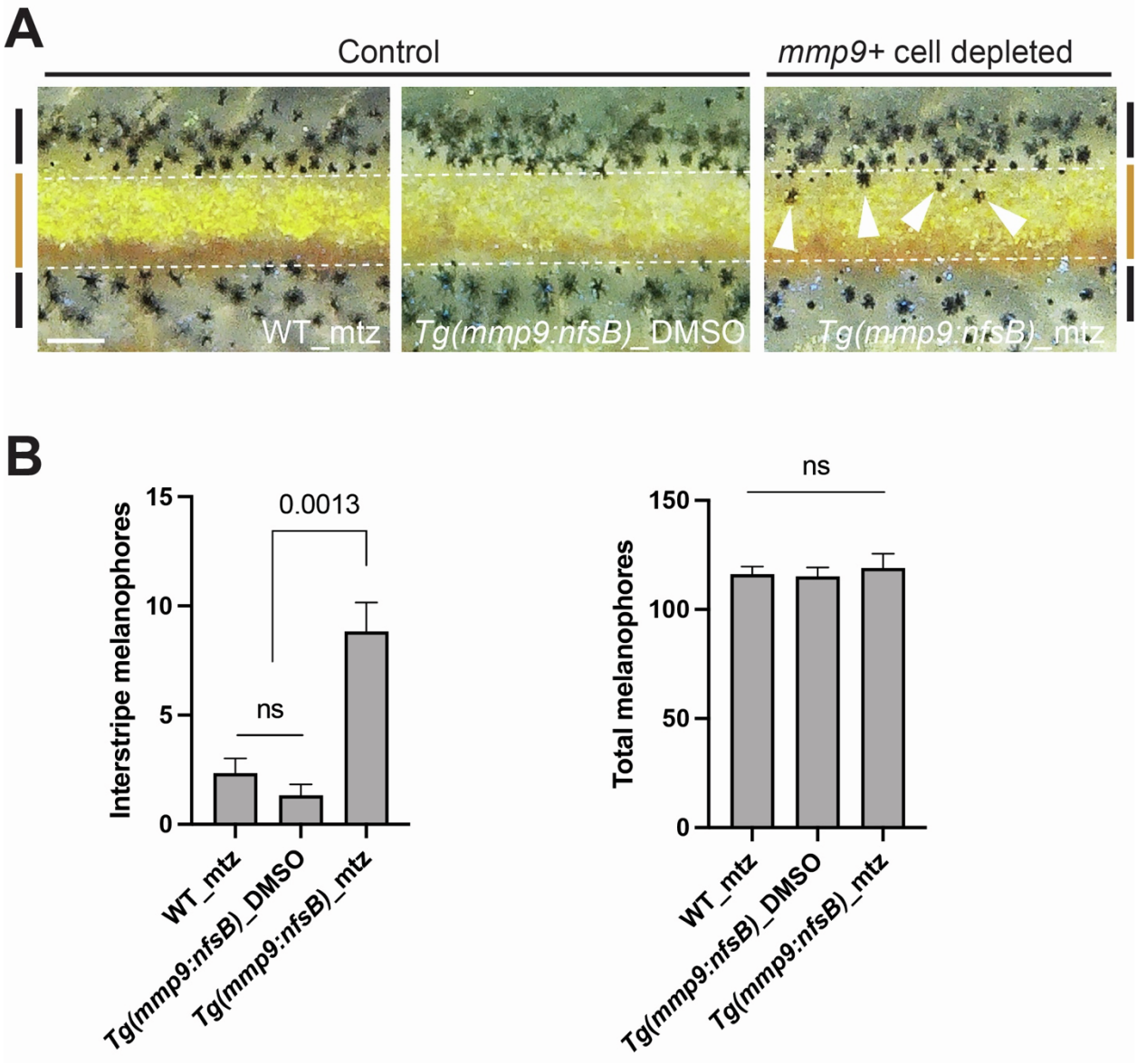
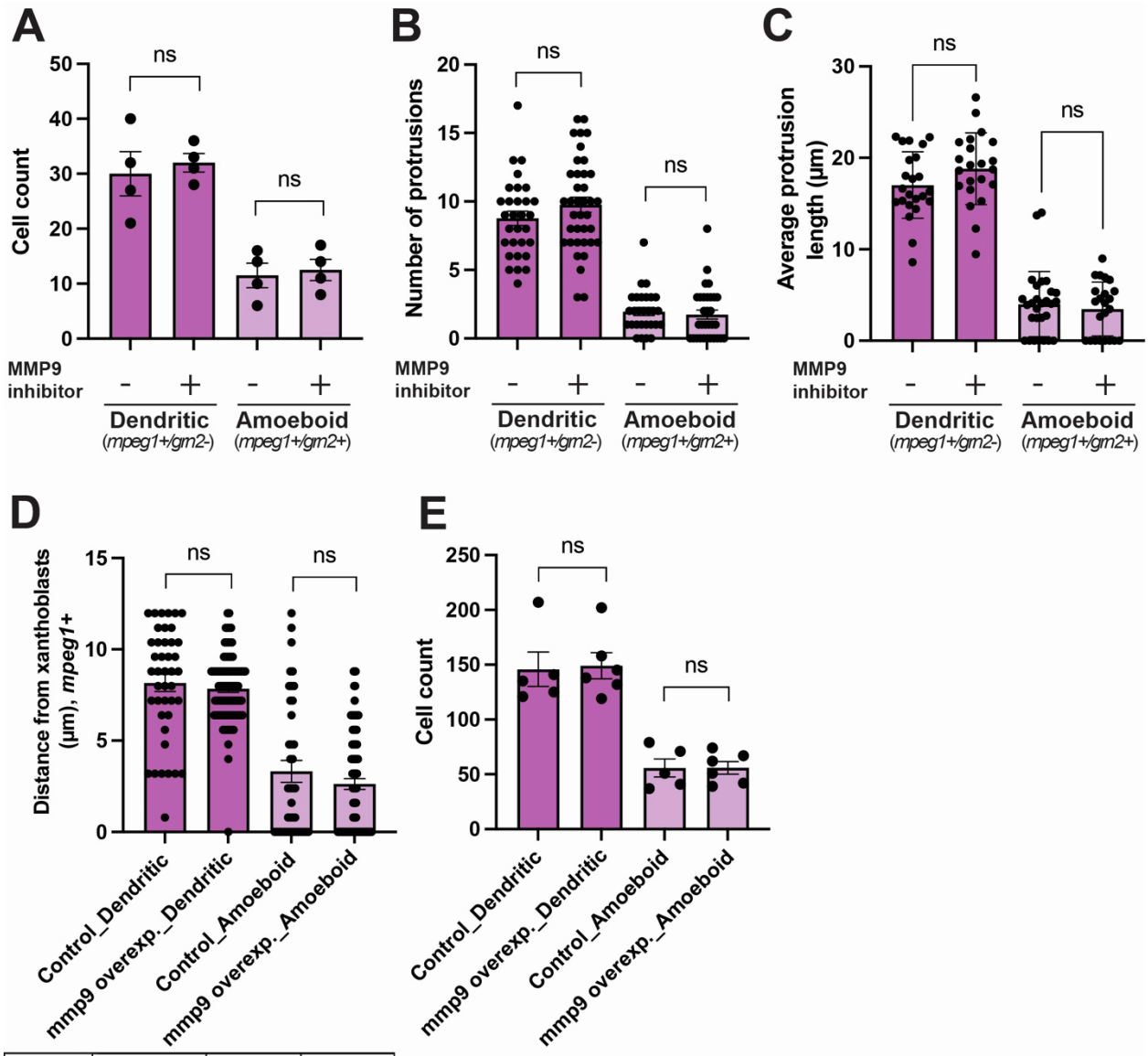


Figure S6. Melanophore retention in the interstripe of *mmp9*+ cell depleted fish, Related to Figure 4

(A) In two controls, melanophores reside in the stripe region (denoted by black bars at far left and right, and white dotted lines) and border the interstripe (orange bar). In contrast, mmp9+ cell depletion results in melanophores retention in the interstripe (arrowheads). (B) Numbers of melanophores in the interstripe. mmp9+ cell depleted fish (*Tg(mmp9:nfsB)*_mtz) had significantly more melanophores in the interstripe than controls (WT_mtz and *Tg(mmp9:nfsB)*_DMSO). However, there were no significant differences in total melanophore numbers; data are means ± SEM. At stage 12 standardized standard length (SSL) (n=6, WT_mtz, n=6, *Tg(mmp9:nfsB)*_DMSO, n=6, *Tg(mmp9:nfsB)_mtz* trunks). Statistical significances were assessed by Student's t-test. Scale bar: 200μm (A).



		% in epi.	% in hypo.
Dendritic	Control	100	0
	mmp9 overexp.	98.9	1.07
Amoeboid	Control	72.72	27.27
	mmp9 overexp.	70.86	29.13

Figure S7. MMP9 manipulations do not affect the proportion of macrophage subpopulation and their morphology, Related to Figure 4

(A) The proportion of both dendritic and amoeboid metaphocyte populations remained unchanged in MMP9 inhibitor treated fish (n=4 fish each). (B) The number of protrusions in both macrophage subpopulations showed no significant differences in MMP9 inhibitor treated fish (n=30, 38, 28, 31 cells, 3 trunk each). (C) The length of protrusions in both macrophage populations remained unaltered in MMP9 inhibitor treated fish (n=23, 22, 28, 23 cells, 3 trunk each). (D) Localization of both macrophage populations remained unchanged in *mmp9* overexpressed fish (n=42, 92, 40, 90 cells, 3, 3, 4, 4 trunks). (E) The proportion of dendritic and amoeboid populations remained unchanged in *mmp9* overexpressed fish (n=5, 6, 5, 6 fish). Statistical significances were assessed by Student's t-test.