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Supplementary Information for

**Cxcl9l and Cxcr3.2 regulate recruitment of osteoclast progenitors to bone matrix in a medaka osteoporosis model**

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A

| | baseMean | | Fold change RNAseq | | Fold change qPCR | |
|---|---|---|---|---|---|---|
| | *col10a1* 10 dpf | *ctsk* 12 dpf | *col10a1* 10 dpf | *ctsk* 12 dpf | *col10a1* 10 dpf | *ctsk* 12 dpf |
| ***cxcl9l*** | 1575 | 182 | 12.85 up | 1.4 down | 9.1 up * | n. s. |
| ***cxcr3.2*** | 30 | 1695 | 1.3 down | 1.4 up | 1.5 up * | 6.9 up ** |

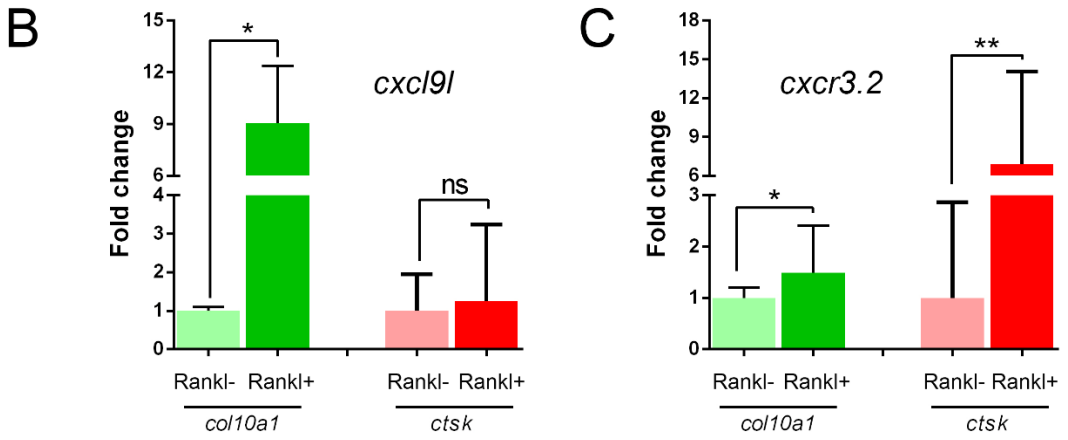



**Fig. S1. qPCR validation of *cxcl9l* and *cxcr3.2* regulation in osteoblast progenitors (*col10a1*) and osteoclasts (*ctsk*) after Rankl induction.** *(A)* Table summarizing baseMean and fold change values obtained by RNAseq and qPCR analysis, respectively. Samples used for RNAseq and qPCR were from independent experiments. The 'baseMean' value indicates average of transcript numbers in controls and Rankl-induced cell types for both cell types, respectively. *(B)* qPCR analysis of *cxcl9l* transcription in FAC-sorted *col10a1* (at 10 dpf) and *ctsk* cells (at 12 dpf) with and without Rankl induction. *(C)* qPCR analysis of *cxcr3.2* transcription in FAC-sorted *col10a1* (at 10 dpf) and *ctsk* cells (at 12 dpf) with and without Rankl induction. Three biological and three technical replicates each were analyzed. Error bars show mean value ± SD; * $p < 0.05$, ** $p < 0.01$; n.s. = no significant difference, Student's t-test.

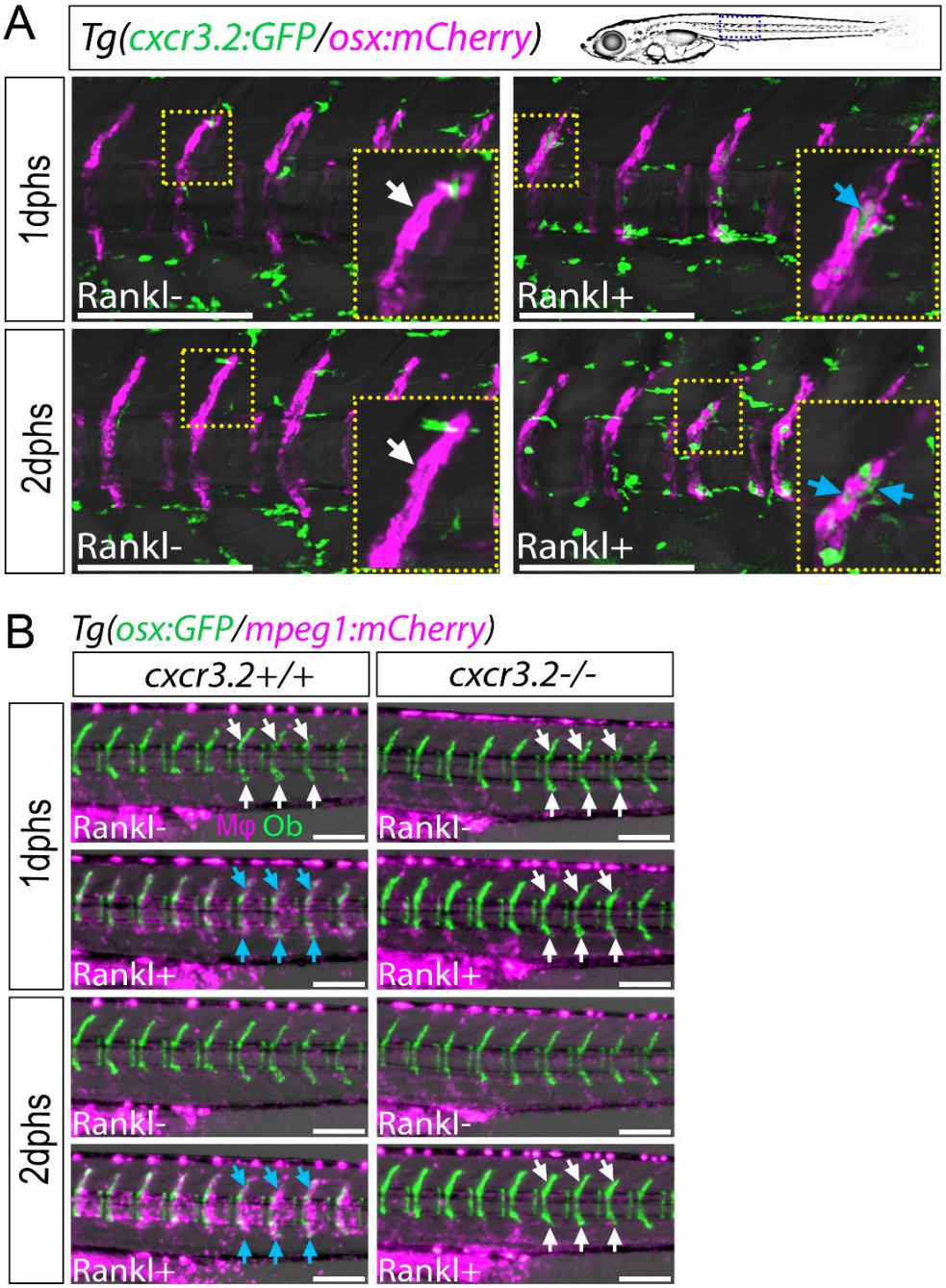



**Fig. S2. *Cxcr3.2* expression and function in osteoblasts.** *(A) cxcr3.2:GFP* does not overlap with *osx:mCherry* expression in osteoblasts without (left) or with Rankl induction (right). Upon Rankl induction, *cxcr3.2:GFP* cell numbers are increased in centra and neural arches at 1 and 2 dphs. Note tight interaction of *cxcr3.2:GFP* cells with *osx:mCherry* osteoblasts in arches (blue arrows). Inset shows higher magnification view of boxed area. *(B)* In the absence of ectopic Rankl, *osx:GFP* osteoblasts are similarly distributed in vertebrae of *cxcr3.2*+/+ and *cxcr3.2-/-* siblings at 1 and 2 dphs (white arrows). Upon Rankl induction, *osx:GFP* cells are redistributed in *cxcr3.2*+/+ siblings (blue arrows), as a consequence of ectopic osteoclast differentiation. In contrast, osteoblast distribution in *cxcr3.2-/-* mutants is not affected (right column, white arrows). Scale bars: 200 μm.

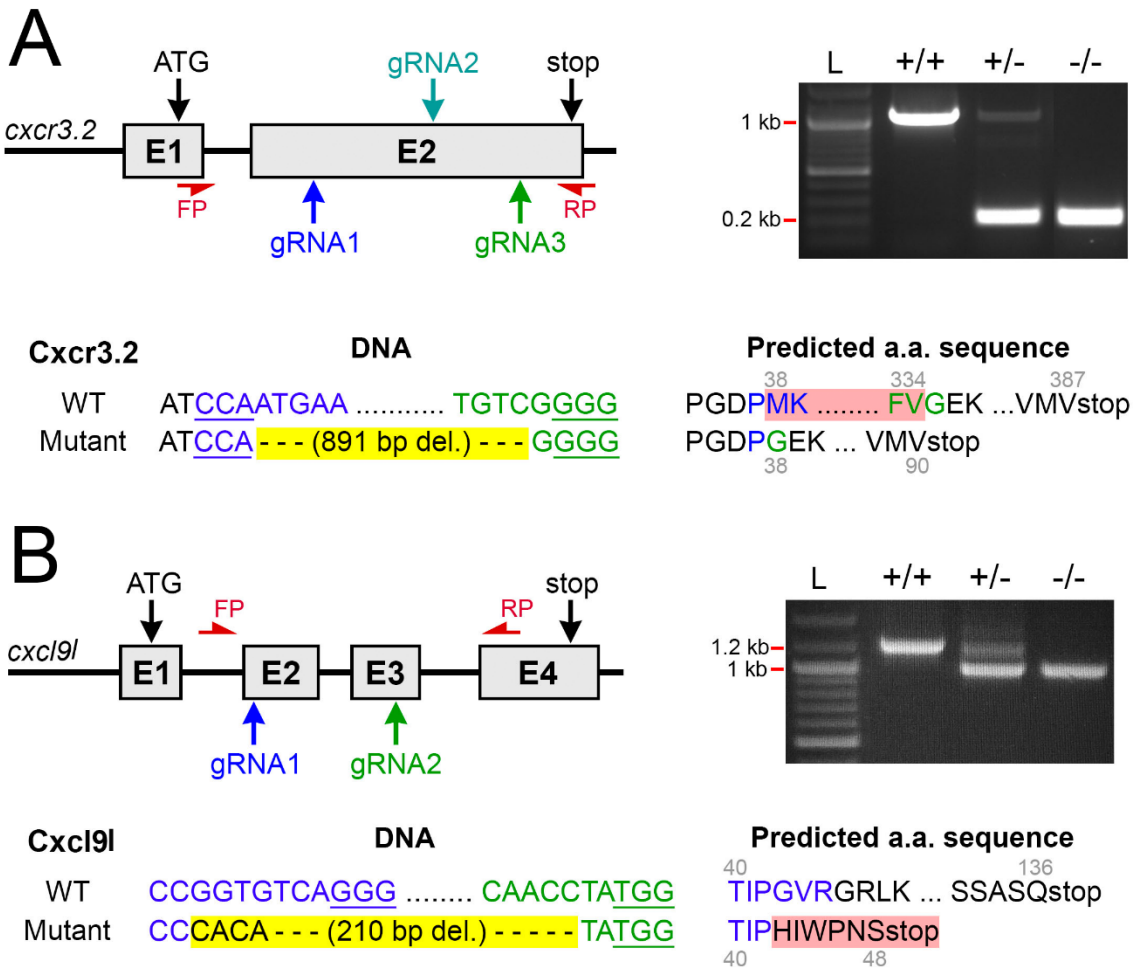



**Fig. S3. Generation of medaka *cxcl9l* and *cxcr3.2* mutants.** *(A)* Generation of *cxcr3.2* CRISPR/Cas9 mutants using three guide RNAs (gRNA1-3) targeting exon 2 (E2). Red arrows indicate position of forward (FP) and reverse (RP) primers for PCR genotyping (WT (+/+): approx. 1.1 kb; homozygous mutants (-/-): approx. 0.2 kb; heterozygous carriers (+/-): 1.1 and 0.2 kb). Mutants have a 891 bp deletion (yellow) between gRNA1 and gRNA3 target sites, leading to a predicted a.a. sequence missing aa 38 to 334. *(B)* Generation of *cxcl9l* mutants. Two guide RNAs (blue, green) targeting *cxcl9l* exon 2 (E2) and exon 3 (E3) were injected. PCR genotyping identified WT (+/+; approx. 1.15 kb), homozygous mutants (-/-; approx. 0.95 kb) and heterozygous siblings (+/-; 1.15 and 0.95 kb). Mutants have a 214 bp indel, leading to a predicted prematurely truncated aa sequence (red). PAM sequences are underlined. Sequences of guide RNAs and primers are listed in Table S5.

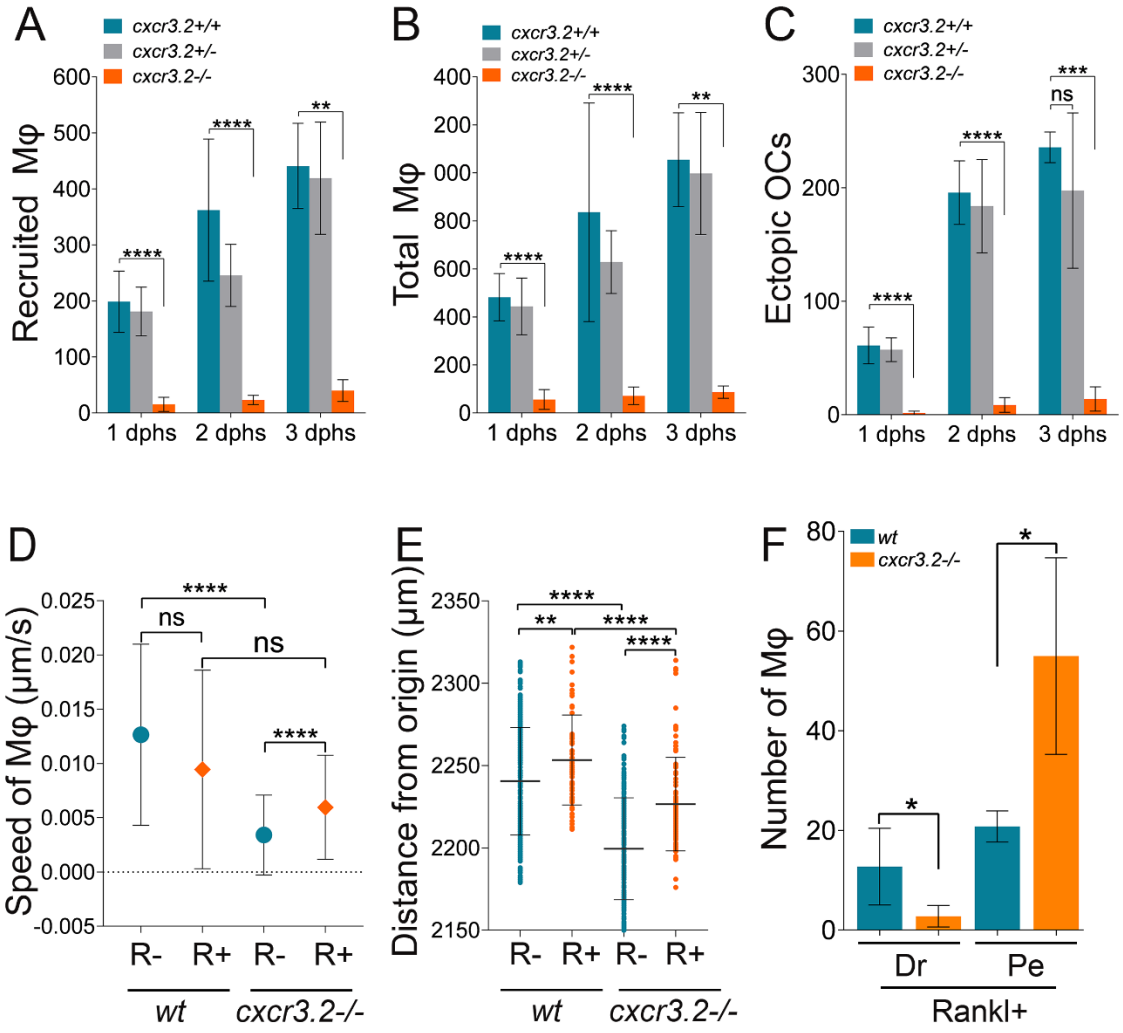



**Fig. S4. Quantification of macrophage dynamics and differentiation in *cxcr3.2* mutants.** *(A-C)* Sibling embryos obtained from *cxcr3.2* heterozygous incrosses were induced for Rankl, imaged for cell counting and genotyped. Numbers of recruited macrophages *(A)*, total macrophages *(B)* and ectopic osteoclasts *(C)* are shown. Error bars show mean number of cells ± SD, Mann-Whitney test, **p<0.01, ***p<0.001, ****p<0.0001, ns: non-significant, $7 \leq N_{cxcr3.2+/+larvae}$, $8 \leq N_{cxcr3.2+/-larvae} \leq 13$, $5 \leq N_{cxcr3.2-/-larvae} \leq 20$, from three independent experiments. *(D-E)* Speed and distance from origin of macrophages were extracted from time-lapse movies at six hours after the start of the movies (i.e. 10 hphs). Although speed and traveling distance of *cxcr3.2-/-* macrophages increased upon Rankl induction, both parameters were lower compared to that of wide type siblings. *(F)* Number of macrophages moving from AGM towards vertebral column (10-14 hphs, measured in three vertebral bodies), and cells retaining in the AGM (at 14 hphs). More macrophages migrated directly from the AGM to the vertebral column in wild type siblings, while most *cxcr3.2-/-* mutant macrophages remained in the AGM. Error bars show mean values ± SD, Mann-Whitney test, *p<0.05, **p<0.01, ****p<0.0001, ns: non-significant; $2 \leq N_{movies} \leq 5$. R-: Rankl negative; R+: Rankl positive; Dr: Directed recruitment towards vertebral column; Pe: Persistence in the AGM; Mφ: macrophage; OCs: osteoclasts.

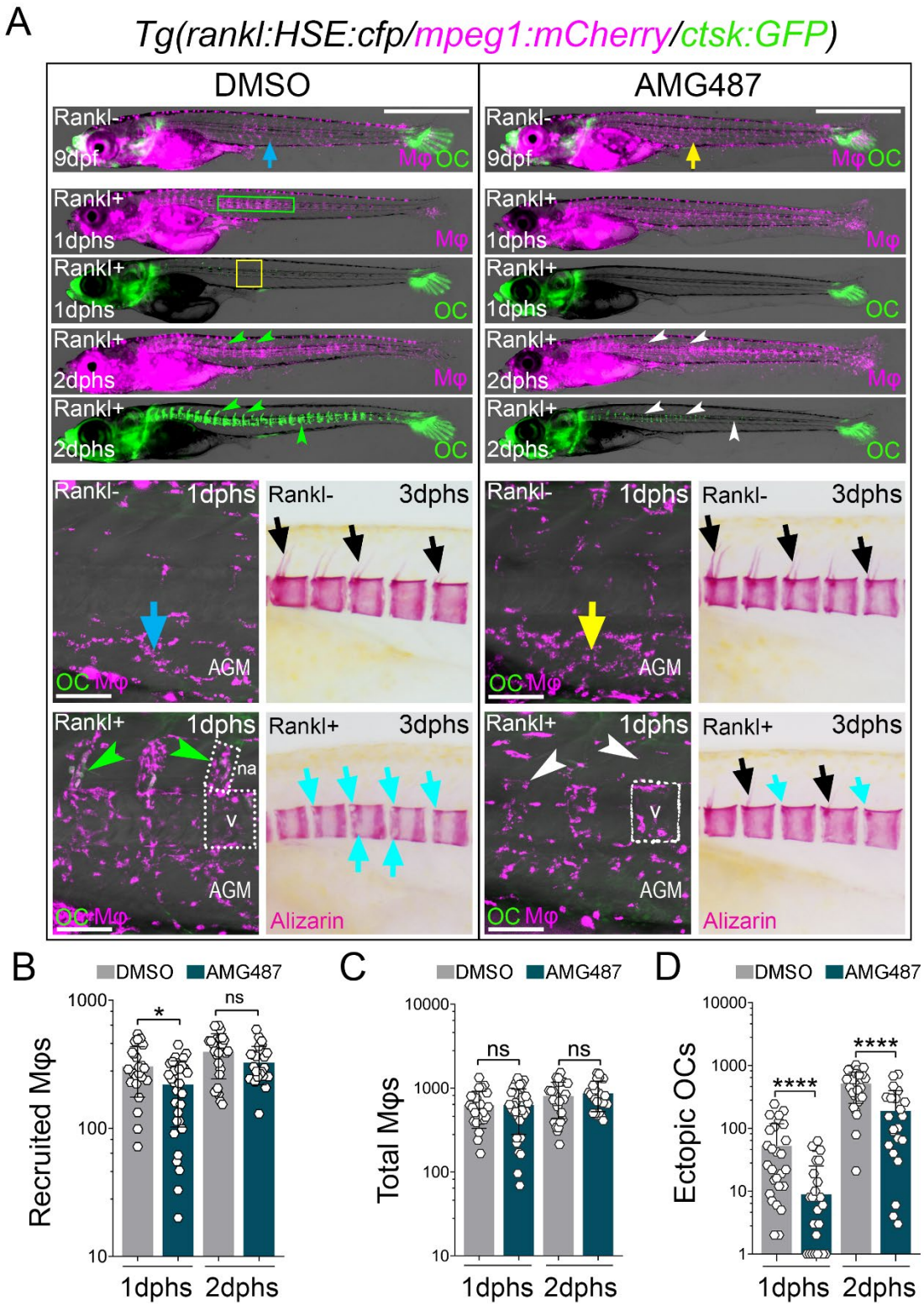



**Fig. S5. AMG487 treatment protects bone upon Rankl induction.** *(A)* 20 μM AMG487 was injected into the yolk of *rankl:HSE:cfp/mpeg1:mCherry/ctsk:GFP* transgenic embryos two hours prior to heat-shock for Rankl induction. In Rankl- embryos, AMG487 treatment does not affect

development but mildly alters macrophage distribution in the AGM (arrows). Upon Rankl induction, recruitment of macrophages and osteoclast formation is considerably reduced in AMG487 treated embryos (white arrowheads at 2 dphs) when compared to controls (green arrowheads). Alizarin Red staining of mineralized matrix shows severe bone lesions in control embryos (cyan arrows) and less lesions after AMG487 treatment (black arrows). Green box indicates region for cell quantification; yellow box shows region imaged with higher magnification. AGM: aorta-gonad-mesonephros, Mφ: macrophage; OC: osteoclast; na: neural arch, V: vertebra. Scale bar: 1 mm (whole embryo image), 100 μm (zoom-in image). *(B)* Number of macrophages recruited to vertebral column. *(C)* Total number of macrophages in trunk (including AGM). *(D)* Number of ectopic osteoclasts in vertebral column. Y axes show cell numbers and error bars indicate mean values ± SD, unpaired two-tailed Student's t-test *(B-C)*, Mann-Whitney test *(D)*, *P<0.05, ****P<0.0001, ns: non-significant, $N_{DMSO\ Larvae}$ = 23, $N_{AMG487\ Larvae}$ = 32, four independent experiments.

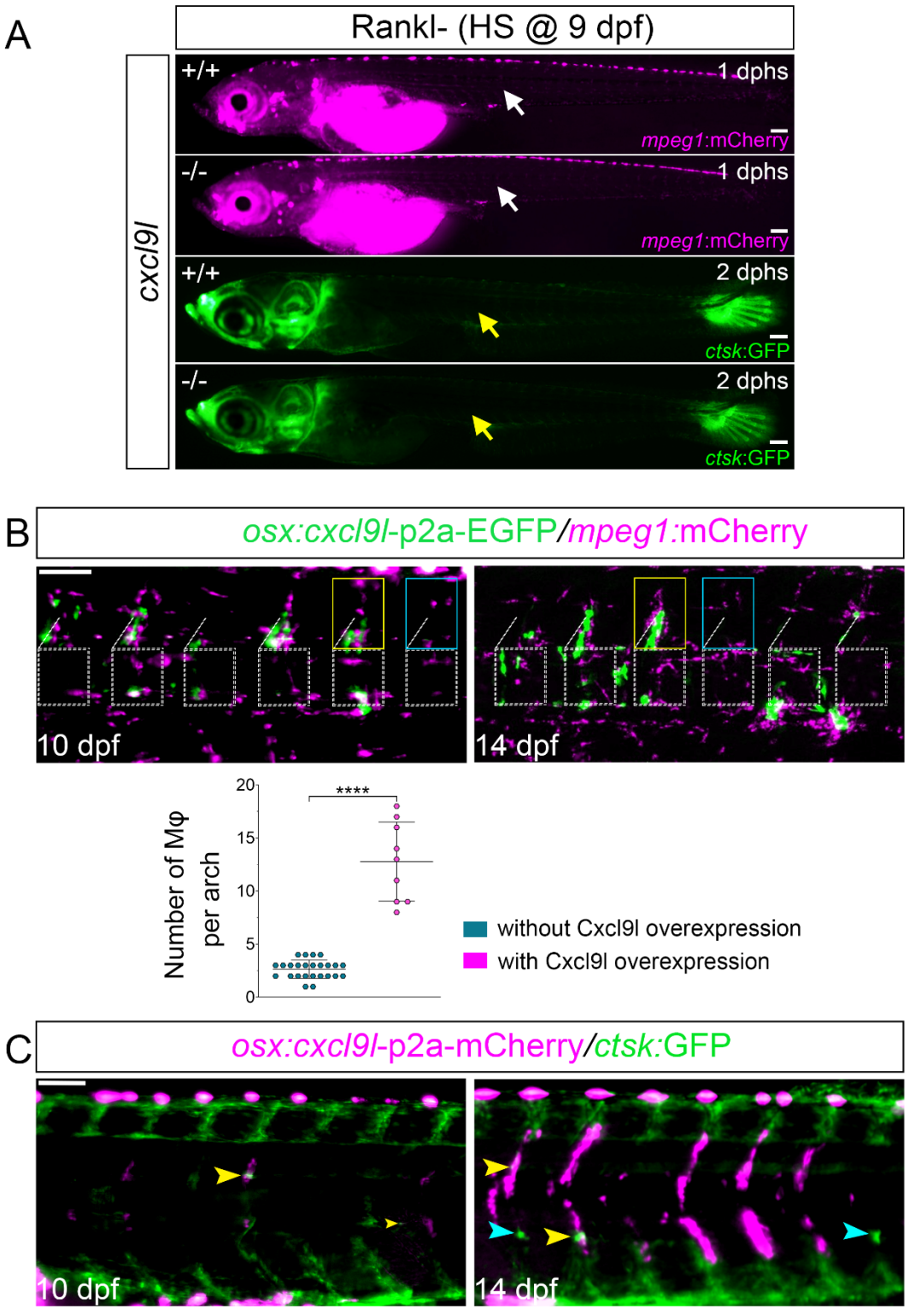
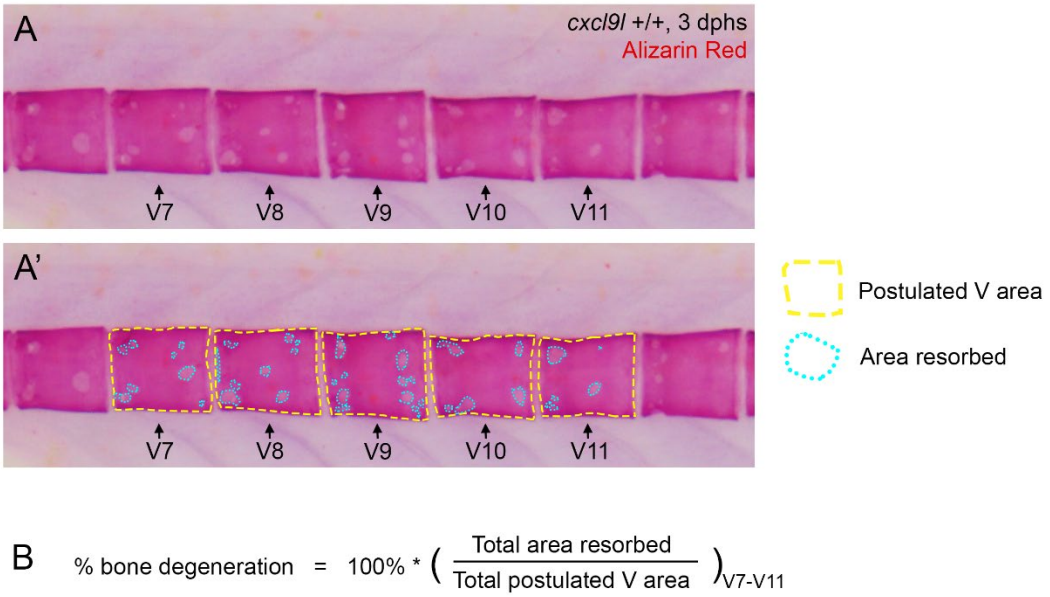



**Fig. S6. Macrophage and osteoclast distribution in *cxcl9l-/-* mutants without Rankl induction and after ectopic Cxcl9l overexpression.** *(A)* In the absence of Rankl induction, *cxcl9l-/-* mutants develop normally and show similar distribution of *mpeg1*:mCherry and *ctsk*:GFP cells as *cxcl9l*+/+ siblings. Scale bars: 100 μm. *(B)* Distribution of *cxcl9l-EGFP* expressing cells (green) and

*mpeg1*:mCherry macrophages (magenta) in vertebral bodies of *osx*:*cxcl9*-p2a-GFP injected larva at 10 and 14 dpf. Yellow boxes demarcate neural arches with Cxcl9l overexpression, cyan boxes indicate neural arch regions without Cxcl9l overexpression, white boxes indicate centra of vertebral bodies. Quantification of macrophages in neural arches without and with Cxcl9l overexpression. *(C)*. Distribution of *cxcl9l-mCherry* expressing cells (magenta) and *ctsk*:GFP positive osteoclasts (green) in vertebral bodies of *osx*:*cxcl9*-p2a-mCherry injected larva at 10 and 14 dpf. Yellow arrowheads indicate osteoclasts in vicinity of Cxcl9l producing cells, cyan arrowheads label osteoclasts away from Cxcl9l producing cells. Scale bars: 100 µm in *(B)* and *(C)*.




B $\%\ \text{bone degeneration} = 100\% * \left( \frac{\text{Total area resorbed}}{\text{Total postulated V area}} \right)_{\text{V7-V11}}$

**Fig. S7. Quantification of bone degeneration.** *(A)* Example of Alizarin Red stained vertebral column used for quantification of bone degeneration. V7-V11, 7$^{th}$ to 11$^{th}$ vertebral bodies. *(A')* To quantify bone degeneration, the postulated V area (enclosed by yellow dotted lines) and area resorbed (enclosed by cyan dotted lines) for V7-V11 were determined using ImageJ. *(B)* Equation for calculation of percentage (%) bone degeneration using values obtained from the analysis indicated in *(A')*.

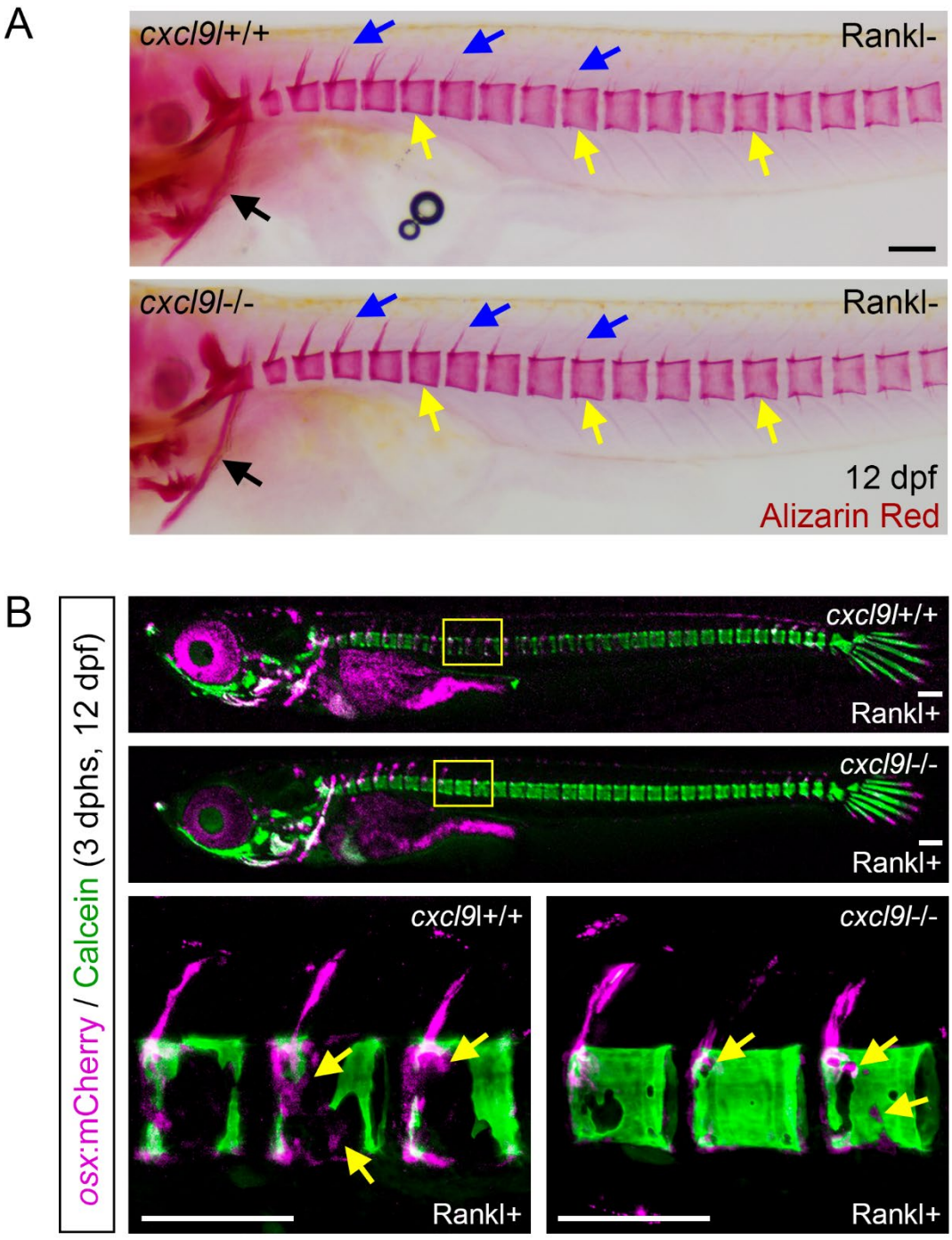



**Fig. S8. Bone development in *cxcl9l* mutants.** *(A)* In the absence of Rankl induction, Alizarin Red staining revealed normal bone mineralization in the cleithrum (black arrows), neural arches (blue arrows) and vertebral bodies (yellow arrows) of *cxcl9l-/-* mutants as compared to *cxcl9l+/+* siblings at 12 dpf. *(B)* After Rankl induction, calcein staining revealed less severe bone lesions in *cxcl9l-/-* mutants due to reduced osteoclast formation. Overview and magnified confocal images showing *osx*:mCherry-expressing osteoblasts accumulating at bone lesion sites (arrows) in both *cxcl9l+/+* and *cxcl9l-/-* siblings (3 dphs, 12 dpf). Yellow boxes indicate regions shown in the magnified views. Scale bars: 100 µm.

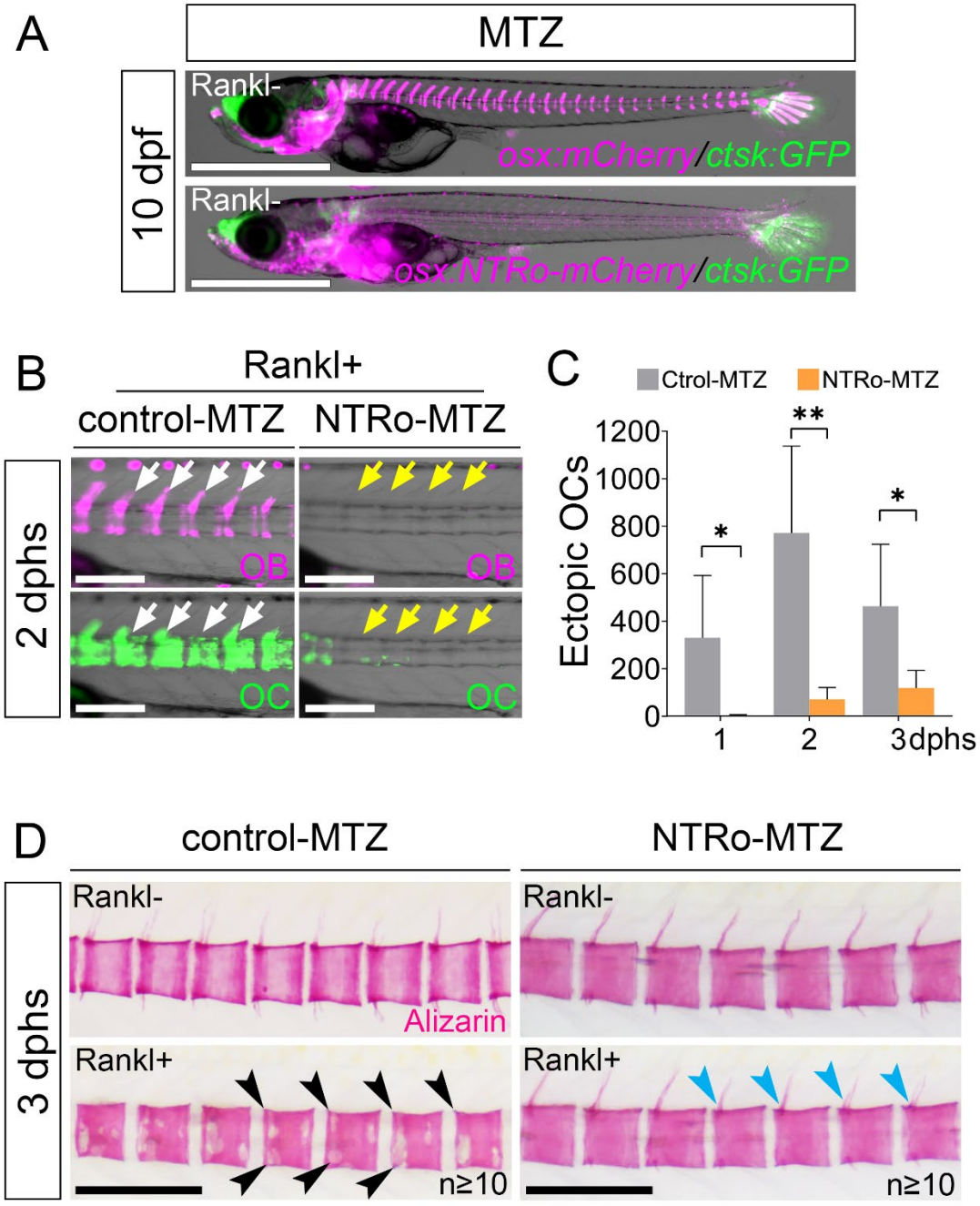



**Fig. S9. Osteoclast formation depends on the presence of osteoblasts.** *(A)* Metronidazole (MTZ) treatment results in efficient ablation of osteoblasts in *osx:mCherry-NTRo/ctsk:GFP* embryos when compared to *osx:mCherry/ctsk:GFP* control embryos. *(B)* After ablation of osteoblasts, Rankl fails to stimulate the formation of ectopic osteoclasts (yellow arrows) compared to massive osteoclast formation in control embryos (white arrows). *(C)* Quantification of osteoclast numbers showing mean number ± SD, Brown-Forsythe ANOVA with Games Howell's test, *p<0.05, **p<0.01, 9≤N≤11, from two independent experiments. OB: osteoblast; OC: osteoclast. *(D)* MTZ treatment does not affect normal bone development (control-MTZ; Rankl-). Ablation of osteoblasts protects bone from lesions by Rankl induction (Rankl+; arrowheads). Scale bars: 1 mm *(A)*, 200 µm *(B-D)*.

**Table S1. Genes exclusively up-regulated in *col10a1 cells* or *osx* cells at 10 dpf, or commonly up-regulated in both cell types.**

| **Up-regulated exclusively in "col10 10dpf" (n=32)** | | **Up-regulated in "col10 10dpf" and "osx 10dpf" (n=13)** | | **Up-regulated exclusively in "osx 10dpf" (n=15)** | |
|---|---|---|---|---|---|
| symbol | description | symbol | description | symbol | description |
| cd74 | HLA class II histocompatibility antigen gamma chain | itgb8 | integrin beta-8 | LOC101160009 | calcium/calmodulin-dependent protein kinase type II subunit beta isoform X1 |
| psmb9-like | proteasome subunit%2C beta type 9-like | c6 | complement component C6 isoform X1 | LOC101160009 | calcium/calmodulin-dependent protein kinase type II subunit beta isoform X2 |
| orla-uaa | major histocompatibility complex class I-related gene protein-like precursor | c6 | complement component C6 isoform X2 | LOC101160009 | calcium/calmodulin-dependent protein kinase type II subunit beta isoform X3 |
| orla-uba | major histocompatibility complex class I-related gene protein-like isoform X1 | LOC101156669 | collagenase 3 | LOC101160009 | calcium/calmodulin-dependent protein kinase type II subunit beta isoform X4 |
| orla-uba | major histocompatibility complex class I-related gene protein-like precursor | LOC101168980 | C-X-C motif chemokine 2 | LOC101160009 | calcium/calmodulin-dependent protein kinase type II subunit beta isoform X5 |
| LOC101172593 | tumor necrosis factor-like | LOC110016535 | no description | LOC101156421 | neurofilament light polypeptide |
| str-3 | stromelysin-3 precursor | LOC101159531 | tumor necrosis factor-like | relt | tumor necrosis factor receptor superfamily member 19L |
| LOC101169056 | C5a anaphylatoxin chemotactic receptor 1-like | LOC101157923 | insulin-like growth factor-binding protein 5 | cutc | copper homeostasis protein cutC homolog |
| LOC101163430 | interferon regulatory factor 1 isoform X1 | LOC101162522 | bactericidal permeability-increasing protein | LOC101166002 | uncharacterized protein |
| LOC101163430 | interferon regulatory factor 1 isoform X2 | cdk5rap1 | CDK5 regulatory subunit-associated protein 1 isoform X1 | LOC111949221 | no description |
| LOC101171022 | transcription regulator protein BACH1 | cdk5rap1 | CDK5 regulatory subunit-associated protein 1 isoform X2 | otog | otogelin isoform X1 |
| LOC101163059 | heme-binding protein 2 | mmp9 | matrix metalloproteinase-9 precursor | otog | otogelin isoform X2 |
| LOC101166736 | disintegrin and metalloproteinase domain-containing protein 8 | nfe2 | transcription factor NF-E2 45 kDa subunit | LOC101162414 | adenylate cyclase type 8-like |
| steap4 | metalloreductase STEAP4 | | | LOC101160286 | heme-binding protein 1 |
| mmp14 | matrix metalloproteinase-14 precursor | | | LOC111947687 | no description |
| LOC101163459 | mamu class II histocompatibility antigen DR alpha chain | | | | |

| | |
|---|---|
| adamts5 | A disintegrin and metalloproteinase with thrombospondin motifs 5 |
| map3k8 | mitogen-activated protein kinase kinase kinase 8 |
| tank | TRAF family member-associated NF-kappa-B activator |
| orla-b2m | b2-microglobulin isoform X1 |
| orla-b2m | b2-microglobulin precursor |
| LOC101156150 | HLA class II histocompatibility antigen%2C DP alpha 1 chain |
| prdm1b | uncharacterized protein LOC101173518 |
| prdm1b | uncharacterized protein LOC101173518 isoform X1 |
| tnfrsf21 | tumor necrosis factor receptor superfamily member 21 |
| LOC110017367 | no description |
| cd276 | CD276 antigen |
| LOC101166708 | podocin |
| alpl | alkaline phosphatase%2C tissue-nonspecific isozyme |
| LOC101170656 | olfactomedin-4 |
| il34 | interleukin-34 |
| mafb | transcription factor MafB |

**Table S2. Top up-regulated genes in *col10a1* cells at 10 dpf (log2 FC >1; baseMean >= 10; adj p-value < 0.05).**

| geneID | baseMean | log2 FC | p adj | symbol | description |
|---|---|---|---|---|---|
| gene11555 | 2593.35 | 4.11 | 0.00 | cd74 | HLA class II histocompatibility antigen gamma chain |
| gene12485 | 1019.15 | 3.53 | 0.00 | itgb8 | integrin beta-8 |
| gene12651 | 16.64 | 2.97 | 0.03 | psmb9-like | proteasome subunit%2C beta type 9-like |
| gene12654 | 399.86 | 1.63 | 0.04 | orla-uaa | major histocompatibility complex class I-related gene protein-like precursor |
| gene12655 | 453.16 | 1.85 | 0.00 | orla-uba | major histocompatibility complex class I-related gene protein-like isoform X1 |
| gene12655 | 453.16 | 1.85 | 0.00 | orla-uba | major histocompatibility complex class I-related gene protein-like precursor |
| gene12867 | 44.57 | 4.71 | 0.00 | LOC101172593 | tumor necrosis factor-like |
| gene13945 | 179.45 | 1.57 | 0.00 | str-3 | stromelysin-3 precursor |
| gene14071 | 2666.33 | 4.77 | 0.00 | c6 | complement component C6 isoform X1 |
| gene14071 | 2666.33 | 4.77 | 0.00 | c6 | complement component C6 isoform X2 |
| gene15152 | 131.20 | 3.28 | 0.01 | LOC101169056 | C5a anaphylatoxin chemotactic receptor 1-like |
| gene15223 | 1052.85 | 5.91 | 0.00 | LOC101156669 | collagenase 3 |
| gene15559 | 306.69 | 1.32 | 0.00 | LOC101163430 | interferon regulatory factor 1 isoform X1 |
| gene15559 | 306.69 | 1.32 | 0.00 | LOC101163430 | interferon regulatory factor 1 isoform X2 |
| gene15803 | 205.87 | 2.06 | 0.00 | LOC101171022 | transcription regulator protein BACH1 |
| gene16569 | 516.26 | 2.47 | 0.03 | LOC101163059 | heme-binding protein 2 |
| gene17282 | 1574.72 | 3.68 | 0.00 | LOC101168980 | C-X-C motif chemokine 2 |
| gene17283 | 822.76 | 2.15 | 0.02 | LOC110016535 | no description |
| gene17293 | 197.66 | 3.02 | 0.03 | LOC101166736 | disintegrin and metalloproteinase domain-containing protein 8 |
| gene18053 | 3924.33 | 1.45 | 0.00 | steap4 | metalloreductase STEAP4 |
| gene18583 | 884.44 | 4.54 | 0.00 | LOC101159531 | tumor necrosis factor-like |
| gene19596 | 4639.77 | 1.81 | 0.02 | mmp14 | matrix metalloproteinase-14 precursor |
| gene2037 | 1005.57 | 4.64 | 0.00 | LOC101157923 | insulin-like growth factor-binding protein 5 |
| gene21042 | 162.58 | 3.41 | 0.03 | LOC101163459 | mamu class II histocompatibility antigen%2C DR alpha chain |
| gene2216 | 903.66 | 1.65 | 0.00 | adamts5 | A disintegrin and metalloproteinase with thrombospondin motifs 5 |
| gene22512 | 498.72 | 2.23 | 0.00 | map3k8 | mitogen-activated protein kinase kinase kinase 8 |
| gene23741 | 289.11 | 1.72 | 0.01 | tank | TRAF family member-associated NF-kappa-B activator |
| gene25463 | 821.34 | 1.85 | 0.00 | orla-b2m | b2-microglobulin isoform X1 |
| gene25463 | 821.34 | 1.85 | 0.00 | orla-b2m | b2-microglobulin precursor |
| gene2562 | 431.02 | 3.75 | 0.00 | LOC101156150 | HLA class II histocompatibility antigen%2C DP alpha 1 chain |
| gene26177 | 35.32 | 2.79 | 0.04 | prdm1b | uncharacterized protein LOC101173518 |
| gene26177 | 35.32 | 2.79 | 0.04 | prdm1b | uncharacterized protein LOC101173518 isoform X1 |
| gene26348 | 832.01 | 2.12 | 0.01 | tnfrsf21 | tumor necrosis factor receptor superfamily member 21 |

| | | | | | |
|---|---|---|---|---|---|
| gene2926 | 19.73 | 3.19 | 0.01 | LOC110017367 | no description |
| gene3342 | 2116.96 | 1.27 | 0.00 | cd276 | CD276 antigen |
| gene4117 | 75.73 | 2.86 | 0.00 | LOC101166708 | podocin |
| gene5496 | 4243.16 | 4.38 | 0.00 | LOC101162522 | bactericidal permeability-increasing protein |
| gene5497 | 737.83 | 3.33 | 0.00 | cdk5rap1 | CDK5 regulatory subunit-associated protein 1 isoform X1 |
| gene5497 | 737.83 | 3.33 | 0.00 | cdk5rap1 | CDK5 regulatory subunit-associated protein 1 isoform X2 |
| gene5832 | 1363.09 | 1.75 | 0.04 | alpl | alkaline phosphatase%2C tissue-nonspecific isozyme |
| gene6029 | 110.24 | 4.09 | 0.01 | LOC101170656 | olfactomedin-4 |
| gene6853 | 246.58 | 3.14 | 0.00 | il34 | interleukin-34 |
| gene7554 | 14765.11 | 5.17 | 0.00 | mmp9 | matrix metalloproteinase-9 precursor |
| gene7663 | 2198.70 | 2.65 | 0.00 | nfe2 | transcription factor NF-E2 45 kDa subunit |
| gene7750 | 1791.19 | 1.48 | 0.01 | mafb | transcription factor MafB |

**Table S3: Top up-regulated genes in *osx* cells at 10 dpf (log2 FC >1; baseMean >= 10; adj p-value < 0.05).**

| geneID | baseMean | logFC | p adj | symbol | description |
|---|---|---|---|---|---|
| gene12485 | 523.76 | 3.00 | 0.00 | itgb8 | integrin beta-8 |
| gene13252 | 123.30 | 1.78 | 0.01 | LOC101160009 | calcium/calmodulin-dependent protein kinase type II subunit beta isoform X1 |
| gene13252 | 123.30 | 1.78 | 0.01 | LOC101160009 | calcium/calmodulin-dependent protein kinase type II subunit beta isoform X2 |
| gene13252 | 123.30 | 1.78 | 0.01 | LOC101160009 | calcium/calmodulin-dependent protein kinase type II subunit beta isoform X3 |
| gene13252 | 123.30 | 1.78 | 0.01 | LOC101160009 | calcium/calmodulin-dependent protein kinase type II subunit beta isoform X4 |
| gene13252 | 123.30 | 1.78 | 0.01 | LOC101160009 | calcium/calmodulin-dependent protein kinase type II subunit beta isoform X5 |
| gene13829 | 10.25 | 4.03 | 0.00 | LOC101156421 | neurofilament light polypeptide |
| gene14071 | 1334.32 | 2.02 | 0.00 | c6 | complement component C6 isoform X1 |
| gene14071 | 1334.32 | 2.02 | 0.00 | c6 | complement component C6 isoform X2 |
| gene15005 | 14.41 | 3.68 | 0.01 | relt | tumor necrosis factor receptor superfamily member 19L |
| gene15223 | 772.16 | 2.84 | 0.04 | LOC101156669 | collagenase 3 |
| gene17248 | 61.84 | 3.05 | 0.03 | cutc | copper homeostasis protein cutC homolog |
| gene17282 | 1396.00 | 3.85 | 0.00 | LOC101168980 | C-X-C motif chemokine 2 |
| gene17283 | 763.32 | 1.97 | 0.01 | LOC110016535 | no description |
| gene18056 | 299.45 | 1.80 | 0.00 | LOC101166002 | uncharacterized protein LOC101166002 |
| gene18583 | 1181.39 | 3.73 | 0.00 | LOC101159531 | tumor necrosis factor-like |
| gene2037 | 451.82 | 4.46 | 0.00 | LOC101157923 | insulin-like growth factor-binding protein 5 |
| gene20621 | 29.80 | 3.87 | 0.01 | LOC111949221 | no description |
| gene2435 | 68.68 | 3.63 | 0.00 | otog | otogelin isoform X1 |
| gene2435 | 68.68 | 3.63 | 0.00 | otog | otogelin isoform X2 |
| gene26204 | 21.14 | 2.94 | 0.04 | LOC101162414 | adenylate cyclase type 8-like |
| gene5496 | 3946.75 | 1.95 | 0.02 | LOC101162522 | bactericidal permeability-increasing protein |
| gene5497 | 718.80 | 1.68 | 0.00 | cdk5rap1 | CDK5 regulatory subunit-associated protein 1 isoform X1 |
| gene5497 | 718.80 | 1.68 | 0.00 | cdk5rap1 | CDK5 regulatory subunit-associated protein 1 isoform X2 |
| gene7554 | 9157.18 | 3.00 | 0.01 | mmp9 | matrix metalloproteinase-9 precursor |
| gene7663 | 1796.27 | 1.68 | 0.00 | nfe2 | transcription factor NF-E2 45 kDa subunit |
| gene8 | 148.46 | 1.44 | 0.02 | LOC101160286 | heme-binding protein 1 |
| gene8360 | 13.82 | 4.49 | 0.00 | LOC111947687 | no description |

**Table S4: Top regulated genes in *ctsk* cells at 12 dpf (log2 FC <1/>1; baseMean >= 10; adj p-value < 0.05).**

| Down-regulated | | | | | | Up-regulated | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| geneID | baseMean | logFC | p adj | symbol | description | geneID | baseMean | logFC | p adj | symbol | description |
| gene10078 | 297.84 | -1.21 | 0.04 | LOC101161893 | no description | gene12655 | 154.29 | 1.56 | 0.00 | orla-uba | major histocompatibility complex class I-related gene protein-like precursor |
| gene11327 | 241.08 | -1.38 | 0.00 | gdpd2 | glycerophosphoinositol inositolphosphodiesterase GDPD2 | gene151 | 111.54 | 1.06 | 0.04 | LOC111947943 | histone H1-like |
| gene11841 | 79.20 | -1.41 | 0.04 | LOC101159027 | N-acetyllactosaminide beta-acetylglucosaminyltransferase 2 | gene2304 | 98.30 | 2.42 | 0.01 | cfap47 | cilia- and flagella-associated protein 47 isoform X1 |
| gene1186 | 180844.67 | -1.08 | 0.01 | LOC101159648 | keratin%2C type I cytoskeletal 13 | gene4068 | 1080.94 | 1.05 | 0.03 | comp | cartilage oligomeric matrix protein isoform X1 |
| gene13325 | 552.45 | -1.29 | 0.04 | LOC101174360 | proteinase-activated receptor 2 | gene4068 | 1080.94 | 1.05 | 0.03 | comp | cartilage oligomeric matrix protein isoform X2 |
| gene14417 | 378.81 | -1.02 | 0.01 | LOC101167722 | suppressor of tumorigenicity 14 protein homolog | gene6944 | 137.50 | 1.45 | 0.00 | b3gnt9 | UDP-GlcNAc:betaGal beta-acetylglucosaminyltransferase 9 |
| gene14893 | 30.76 | -2.71 | 0.00 | LOC101175669 | thymus-specific serine protease-like | gene7162 | 2397.26 | 1.23 | 0.01 | ddost | dolichyldiphosphooligosaccharide—protein glycosyl-transferase 48 kDa subunit |
| gene15169 | 33.74 | -2.14 | 0.03 | LOC101171510 | CMP-N-acetylneuraminate-beta-galactosamide-alpha-sialyltransferase 4-like | | | | | | |
| gene15879 | 75.86 | -1.94 | 0.04 | zpld1 | zona pellucida-like domain-containing protein 1 isoform X1 | | | | | | |
| gene15879 | 75.86 | -1.94 | 0.04 | zpld1 | zona pellucida-like domain-containing protein 1 isoform X2 | | | | | | |
| gene15949 | 8465.15 | -1.25 | 0.00 | LOC101165663 | lipocalin | | | | | | |
| gene16700 | 139.57 | -1.11 | 0.02 | LOC101156521 | hydroxycarboxylic acid receptor 2 isoform X1 | | | | | | |
| gene16700 | 139.57 | -1.11 | 0.02 | LOC101156521 | hydroxycarboxylic acid receptor 3 isoform X2 | | | | | | |
| gene16751 | 63.96 | -1.52 | 0.01 | LOC105358576 | B-cadherin-like | | | | | | |
| gene18443 | 78.71 | -1.52 | 0.01 | LOC101168736 | cannabinoid receptor 2 | | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| gene18722 | 231.12 | -1.85 | 0.03 | LOC105357484 | apoptosis-associated speck-like protein containing a CARD |
| gene18735 | 526.23 | -1.12 | 0.04 | LOC101162953 | uncharacterized protein LOC101162953 isoform X1 |
| gene18735 | 526.23 | -1.12 | 0.04 | LOC101162953 | uncharacterized protein LOC101162953 isoform X2 |
| gene18735 | 526.23 | -1.12 | 0.04 | LOC101162953 | uncharacterized protein LOC101162953 isoform X3 |
| gene18735 | 526.23 | -1.12 | 0.04 | LOC101162953 | uncharacterized protein LOC101162953 isoform X4 |
| gene18735 | 526.23 | -1.12 | 0.04 | LOC101162953 | uncharacterized protein LOC101162953 isoform X5 |
| gene20384 | 27.73 | -2.04 | 0.03 | LOC101156610 | uncharacterized protein LOC101156610 isoform X1 |
| gene20384 | 27.73 | -2.04 | 0.03 | LOC101156610 | uncharacterized protein LOC101156610 isoform X2 |
| gene20677 | 45.34 | -1.41 | 0.04 | LOC101161585 | ephexin-1 |
| gene21132 | 337.74 | -1.20 | 0.02 | LOC101174954 | nectin-4 isoform X1 |
| gene21132 | 337.74 | -1.20 | 0.02 | LOC101174954 | nectin-4 isoform X2 |
| gene21239 | 85.81 | -2.26 | 0.04 | LOC105358224 | no description |
| gene21278 | 195.27 | -1.07 | 0.02 | LOC111946396 | no description |
| gene21279 | 105.51 | -1.29 | 0.01 | LOC111946395 | no description |
| gene22836 | 26.24 | -3.16 | 0.00 | LOC101165601 | uncharacterized protein LOC101165601 |
| gene24481 | 205.32 | -1.31 | 0.01 | LOC105356917 | interleukin-31 receptor subunit alpha-like |
| gene26296 | 43.82 | -2.02 | 0.03 | LOC101170320 | sodium-dependent glucose transporter 1-like |
| gene2654 | 123.55 | -1.10 | 0.03 | ano1 | anoctamin-1 isoform X1 |
| gene2654 | 123.55 | -1.10 | 0.03 | ano1 | anoctamin-1 isoform X2 |
| gene2654 | 123.55 | -1.10 | 0.03 | ano1 | anoctamin-1 isoform X3 |
| gene26752 | 300.20 | -1.30 | 0.02 | rhov | rho-related GTP-binding protein RhoV |

| | | | | | |
|---|---|---|---|---|---|
| gene3536 | 35.81 | -1.67 | 0.03 | LOC101163707 | aerolysin-like protein |
| gene3539 | 168.60 | -1.25 | 0.01 | LOC110013331 | aerolysin-like protein |
| gene3541 | 37.85 | -1.67 | 0.04 | LOC101160881 | aerolysin-like protein |
| gene4324 | 1311.78 | -1.34 | 0.00 | LOC101165224 | epiplakin isoform X1 |
| gene4324 | 1311.78 | -1.34 | 0.00 | LOC101165224 | epiplakin isoform X2 |
| gene4467 | 4415.93 | -1.41 | 0.00 | LOC101173929 | dimethylaniline monooxygenase [N-oxide-forming] 5 |
| gene6482 | 613.99 | -1.16 | 0.00 | LOC101165389 | plakophilin-3 isoform X1 |
| gene6482 | 613.99 | -1.16 | 0.00 | LOC101165389 | plakophilin-3 isoform X2 |
| gene6958 | 285.25 | -1.46 | 0.03 | LOC101175077 | carbohydrate sulfotransferase 6-like |
| gene7240 | 175297.40 | -1.06 | 0.00 | LOC101167707 | intermediate filament protein ON3 |
| gene7832 | 130.39 | -1.44 | 0.02 | il17rc | interleukin-17 receptor C |
| gene8486 | 375.87 | -1.42 | 0.04 | LOC101156896 | tryptase |

**Table S5: Used oligos.**

| Name of oligo | Sequence 5' to 3' | Application |
|---|---|---|
| mpeg1.2F | ATAGCGAGCGGATCCAGAACAGTTTCTGGCAGAAGTTAGTC | Promoter cloning |
| mpeg1.2R | AATATGCGGCCGCTGAATTCATCCATGTTCCGCAGGTTGTC | Promoter cloning |
| cxcl9lUP | TGATCCGCTGCTGGGATAGA | qPCR |
| cxcl9lDOWN | TTAGTCCCAAGGGCTTGTCC | qPCR |
| cxcr3.2UP | CTAATATGGGTTCACACACATCCACCAC | qPCR |
| cxcr3.2Down | CTCAGAACAAGGGGCTCCAATC | qPCR |
| β-actinUP | GCCAACAGGGAGAAGATGAC | qPCR |
| β-actinDOWN | CATCACCAGAGTCCATGACG | qPCR |
| cxcl9lF | GTTTAGCTTGTCTCCCGGGCAGGCCACC | Full length cDNA |
| cxcl9lR | TTGTGAGGCTGAGGAGGA | Full length cDNA |
| cxcl9l crRNA1 | TCAGACAATACCCGGTGTCAGGG | CRISPR mutant |
| cxcl9l crRNA2 | TTTGAATCCAGAGCAACCTATGG | CRISPR mutant |
| cxcr3.2 crRNA1 | CCAATGAAGATTGGAGCCCCTTG | CRISPR mutant |
| cxcr3.2 crRNA2 | CCTCGGTTTAAGCGGAGTGGACA | CRISPR mutant |
| cxcr3.2 crRNA3 | TCTGCTGTACGGATTTGTCGGGG | CRISPR mutant |
| cxcl9lgenoF | TCTGTATAGTGAGGGAAACGGACGG | genotyping |
| cxcl9lgenoR | CCTGGGCTTTCTTTTTGAACAATGC | genotyping |
| cxcr3.2genoF | TGTTTGTGCAGAAAATAGGCATTCC | genotyping |
| cxcr3.2genoR | TCGGAGCCTCTTTCCTTTCC | genotyping |

**Movie S1. Dynamics of *cxcr3.2:GFP* positive cells in medaka embryo without Rankl induction.** A *cxcr3.2:GFP/mpeg1:mCherry* transgenic embryo was heat-shocked at 9 dpf (2 hours, 39°C). Time-lapse started at 2 hphs. The majority of macrophages remained in the AGM while single cells were positioned in neural arches and centra. Most *mpeg1:mCherry* macrophages express *cxcr3.2* but at different levels. Box: vertebral body; VC: vertebral column; AGM: aorta-gonad-mesonephros; time: hh-mm-ss. Scale bar: 50 µm.

**Movie S2. Dynamics of *cxcr3.2:GFP* positive cells in medaka embryo after Rankl induction.** A *rankl:HSE:cfp/cxcr3.2:GFP/mpeg1:mCherry* transgenic embryo was heat-shocked at 9 dpf (2 hours, 39°C). Time-lapse started at 2 hphs. Upon Rankl induction, *cxcr3.2*:GFP cells migrate towards the mineralized matrix of the vertebral column, where they differentiate into osteoclasts (see Movie S3). Scale bar: 50 µm.

**Movie S3. Differentiation of *cxcr3.2:GFP* cells into *ctsk:mCherry* osteoclasts.** A *rankl:HSE:cfp/cxcr3.2:GFP/ctsk:mCherry* transgenic embryo was heat-shocked at 9 dpf (2 hours, 39°C). Time-lapse started at 24 hphs. The recruited *cxcr3.2* positive cells gradually express *ctsk*:mCherry during osteclast maturation (yellow arrowheads). Time: hh-mm-ss. Scale bar: 50 µm.

**Movie S4. Downregulation of *cxcr3.2:GFP* in maturing osteoclasts.** A *rankl:HSE:cfp/cxcr3.2:GFP/ctsk:mCherry* transgenic embryo was heat-shocked at 9 dpf (2 hours, 39°C). Time-lapse started at 1 dphs. *cxcr3.2:GFP* expression in *ctsk:mCherry* positive cells becomes weaker with time (white arrow). Note cellular protrusions characteristic for maturing osteoclast. Time: hh-mm-ss. Scale bar: 50 µm.

**Movie S5. Macrophage (*cxcr3.2*+/+) dynamics in Rankl- embryo.** A *mpeg1:mCherry* transgenic embryo was heat-shocked at 9 dpf (2 hours, 39°C). Time-lapse was started at 5 hphs. Most macrophages reside in the AGM, while only few cells are located in neural arches and centra. Scale bar: 50 µm.

**Movie S6. Macrophage (*cxcr3.2*+/+) dynamics in Rankl+ embryo.** A *rankl:HSE:cfp/mpeg1:mCherry* transgenic embryo was heat-shocked at 9 dpf (2 hours, 39°C). Time-lapse started at 5 hphs. Upon Rankl induction, increasing numbers of macrophages are present in vertebral bodies. Scale bar: 50 µm.

**Movie S7. Mutant macrophage (*cxcr3.2*-/-) dynamics in Rankl- embryo.** A *cxcr3.2*-/- mutant embryo with *mpeg1:mCherry* transgenic background was heat-shocked at 9 dpf (2 hours, 39°C). Time-lapse started at 5 hphs. Most macrophages reside in the AGM with only few cells located in neural arches and centra. Compared to wild-type macrophages, *cxcr3.2*-/- macrophages appear less dynamic, especially in the AGM. Scale bar: 50 µm.

**Movie S8. Mutant macrophage (*cxcr3.2*-/-) migration in Rankl+ embryo.** A *cxcr3.2*-/- mutant embryo with a *rankl:HSE:cfp/mpeg1:mCherry* transgenic background was heat-shocked at 9 dpf (2 hours, 39°C). Time-lapse was started at 5 hphs. Upon Rankl induction, *cxcr3.2*-/- macrophages become more motile but do not move towards vertebral column. Most cells remain in the AGM. Scale bar: 50 µm.

**Movie S9. Macrophages (*cxcr3.2*+/+) clear *E. coli* cells.** A *cxcr3.2*+/+ wild-type sibling with *mpeg1:mCherry* transgenic background was injected into muscle with non-pathogenic *E.coli K12*

expressing GFP. Time-lapse was started at 20 minutes post infection (mpi). White arrows indicate macrophages engulfing and clearing bacteria. Time: hh-mm-ss.

**Movie S10. Macrophages (cxcr3.2+/-) clear E. coli cells.** Same set-up as in Movie S9 but with a cxcr3.2+/- embryo. Time: hh-mm-ss.

**Movie S11. Mutant macrophages (*cxcr3.2-/-*) clear *E. coli* cells.** Same set-up as in Movies S9 and S10 but with a *cxcr3.2-/-* embryo. Time: hh-mm-ss.

**Movie S12. Macrophages (*cxcl9+/-*) clear *E. coli* cells.** Same set-up as in Movies S9-S11 but with a heterozygous *cxcl9l+/-* sibling with *mpeg1:mCherry* transgenic background. Time: hh-mm-ss.

**Movie S13. Mutant macrophages (*cxcl9-/-*) clear *E. coli* cells.** Same set-up as in Movies S9-S12 but with a *cxcl9l-/-* sibling with *mpeg1:mCherry* transgenic background. Time: hh-mm-ss.