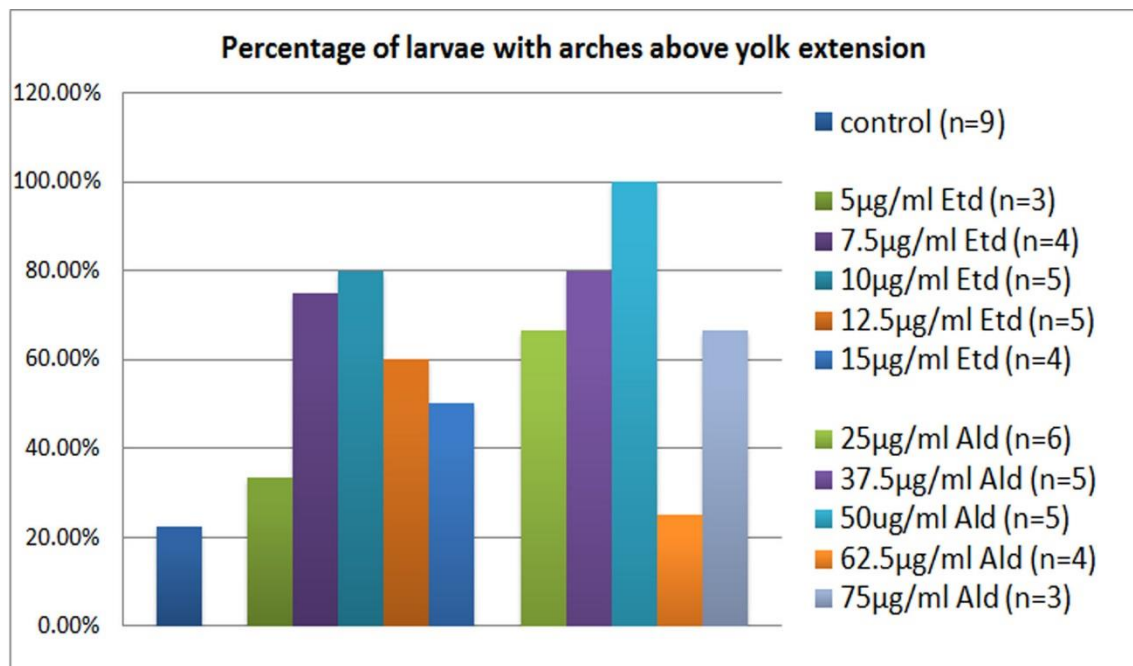
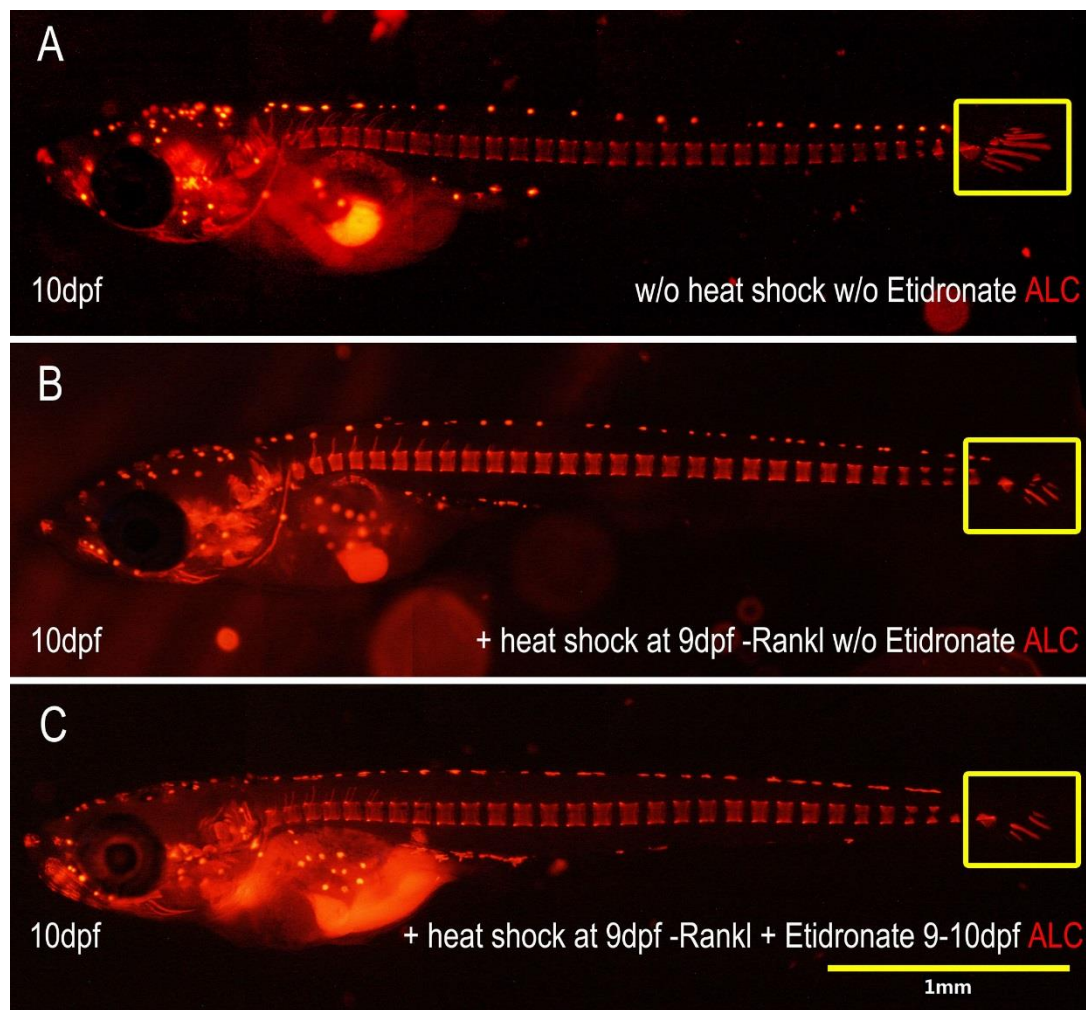


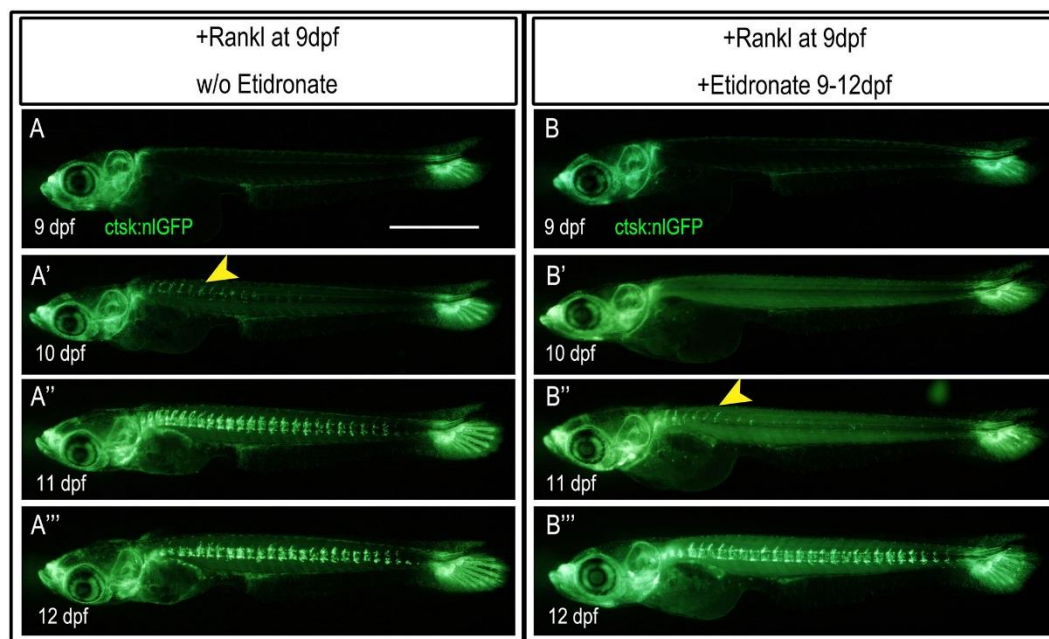
## SUPPLEMENTAL FIGURES



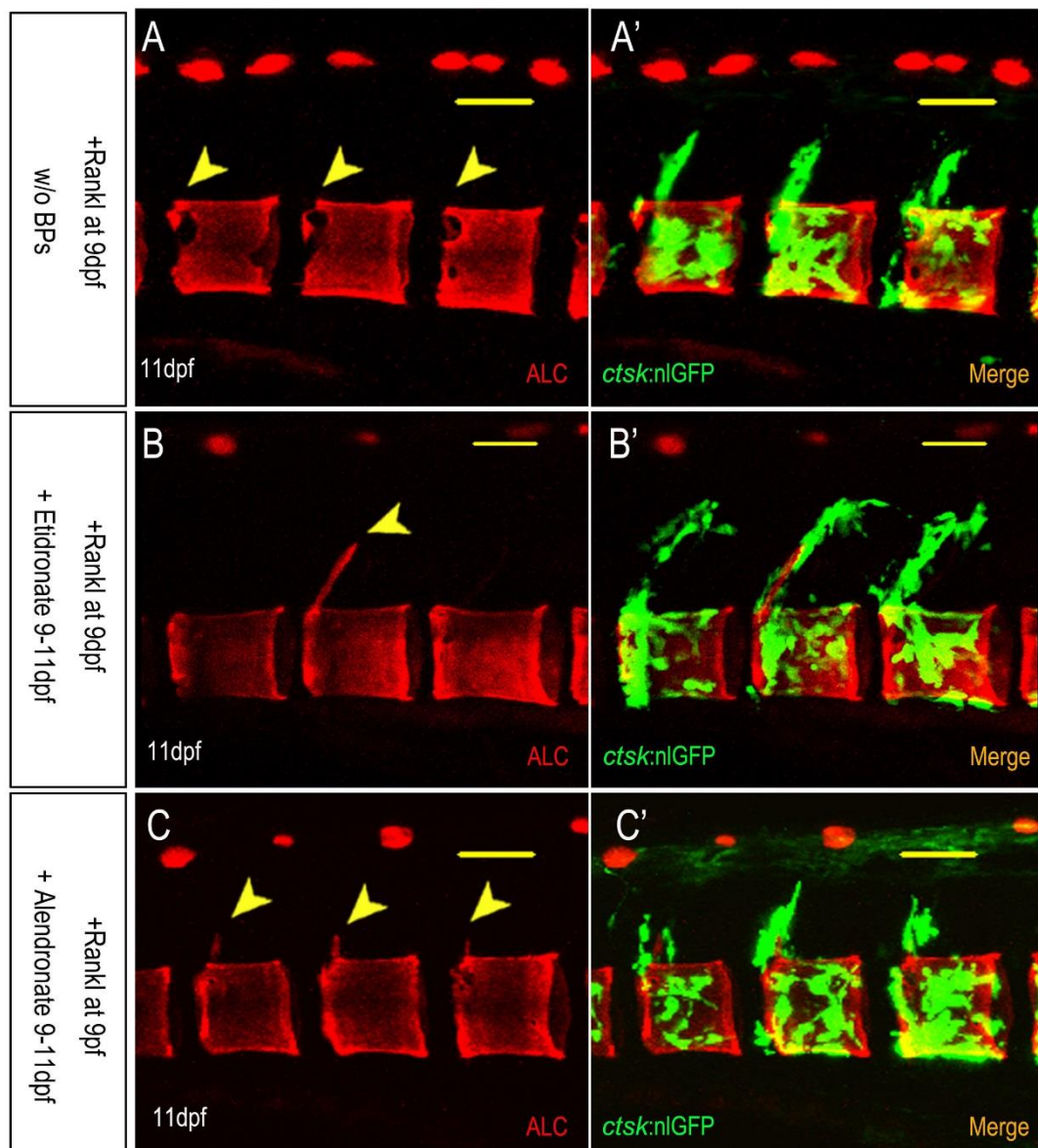
**Supplemental Figure S1: Dose-dependent effect of bisphosphonates on neural arch mineralization.** Percentage of larvae with re-mineralized neural arches after heat shock-induced Rankl expression and addition of BPs at different concentrations (for details see Fig. 4). Presence of arches was recorded in the area above the yolk extension. Number of analyzed fish is indicated.



**Supplemental Figure S2: Effect of heat shock on larval development.** (A-C) ALC staining of mineralized matrix in larvae at 10 dpf without heat shock and BP treatment (A), after heat shock, without both Rankl induction and BP treatment (B), and after heat shock, without Rankl induction but with Etidronate treatment (C). Note slightly delayed development as evident by reduced number of mineralized caudal fin rays (yellow box) in heat shock-treated larvae (B,C). Importantly, the developmental stage is similar in non-BP treated and BP treated larvae (B,C). Scale bar, 1 mm.

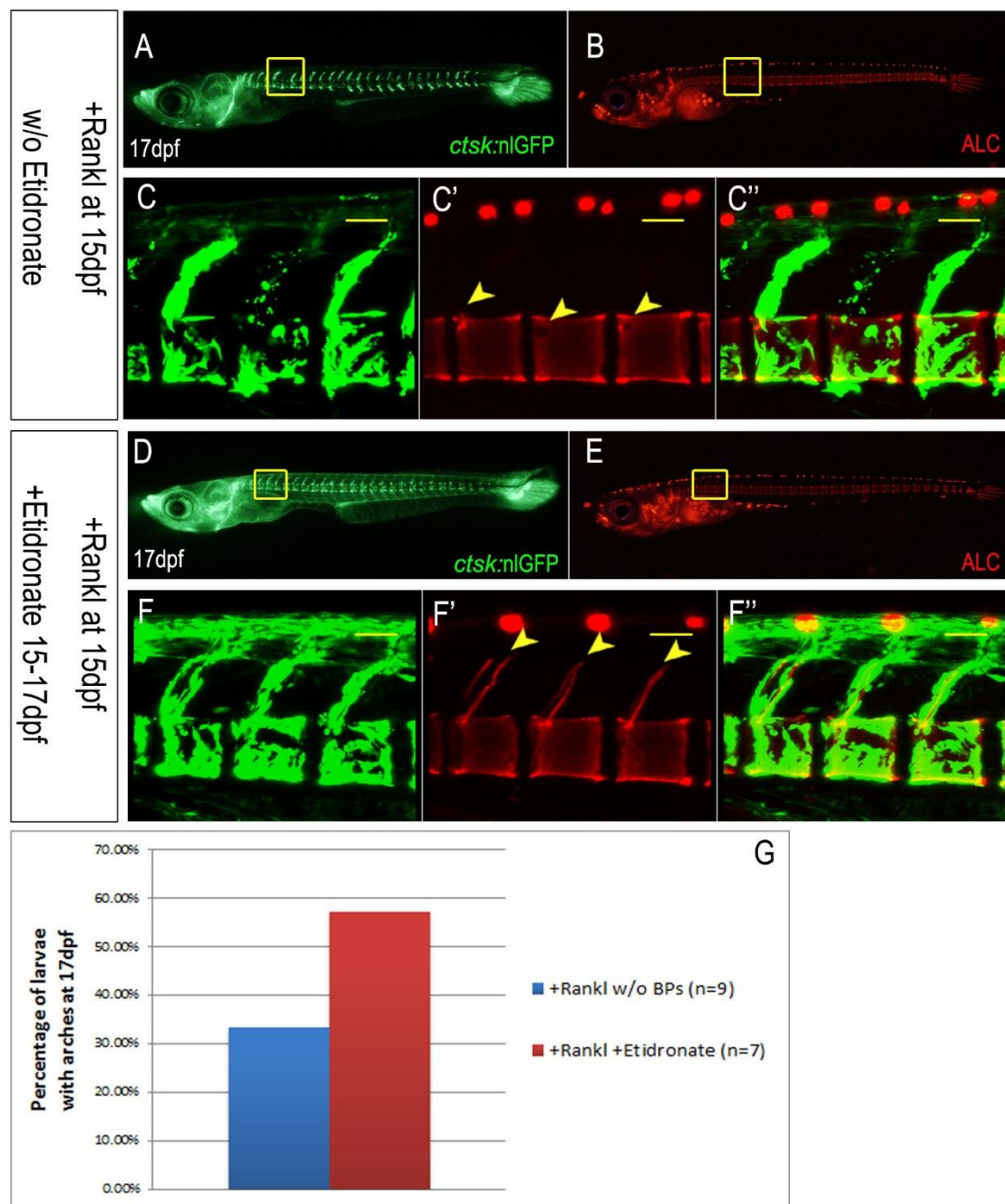


**Supplemental Figure S3: Effect of Etidronate treatment on osteoclast induction.** (A-A''') (A-A''') Ectopic osteoclast formation in larvae after Rankl induction without Etidronate treatment. *ctsk:nlGFP* positive osteoclasts can be seen in anterior regions of the vertebral column one day after Rankl induction at 10 dpf (arrowhead; A'). Increased osteoclast numbers can be observed in the same larvae at 11 and 12 dpf (A'-A''). (B-B''') Delayed formation of ectopic osteoclasts after Rankl induction and Etidronate treatment. No *ctsk:nlGFP* positive osteoclasts are detectable at 10 dpf (B) but appear in the same larvae one day later at 11 dpf (arrowhead; B''). At 12 dpf, the extent of Rankl-induced osteoclast in the Etidronate-treated larva (B''') is similar to non-treated larvae at 11 dpf (A''). Scale bar, 1mm.



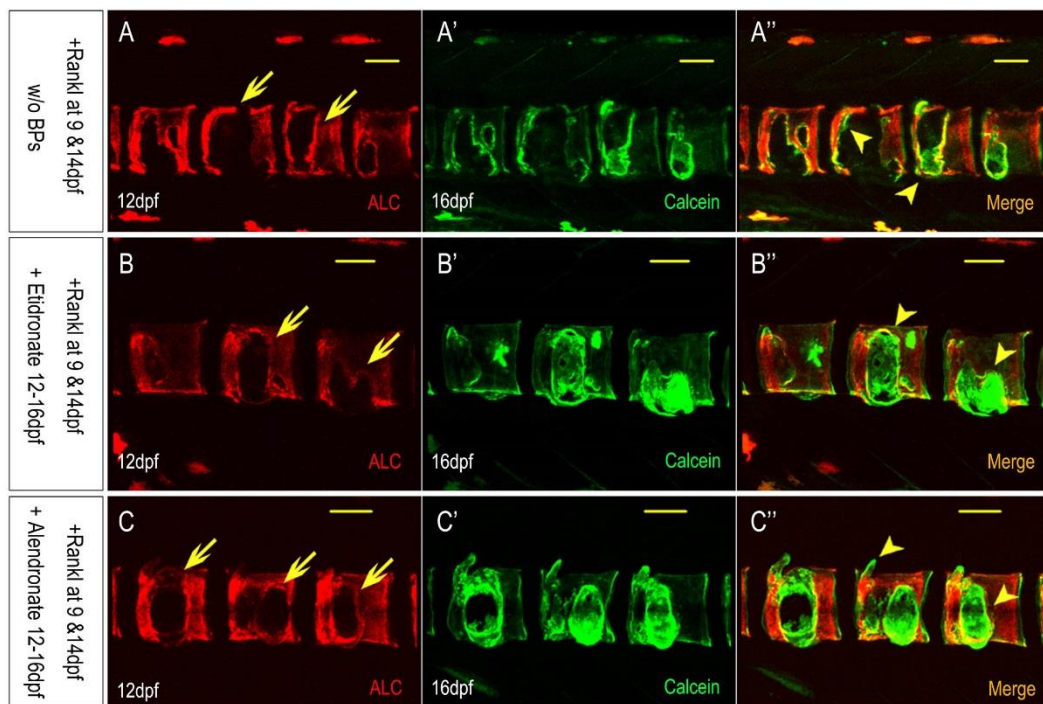
**Supplemental Figure S4: Mineralization defects in arches of BP-treated larvae are less severe than in non-BP treated larvae.** (A) More than 80% of non BP-treated Rankl expressing larvae exhibit a complete absence of mineralized neural arches (arrowheads) and cavities in the vertebral centra. (B-C) Significantly more BP-treated larvae show completely mineralized arches (see statistics in Fig. 2). The remaining BP-treated larvae show less severe defects than non BP-treated larvae in (A), with partial mineralization (arrowheads) after Etidronate (B) or Alendronate treatment (C). Scale bar, 50  $\mu$ m.



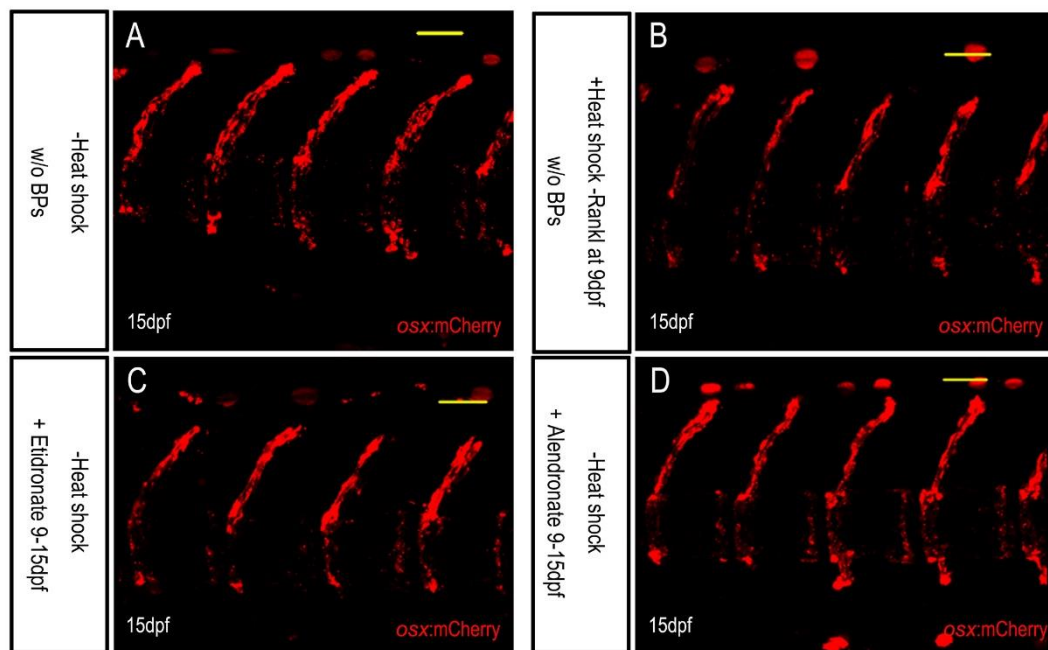


**Supplemental Figure S5: Etidronate treatment blocks osteoclast function and bone resorption after late Rankl induction.** (A-B) Ectopic osteoclast formation at 17 dpf, two days after Rankl induction at 15 dpf in *rankl:HSE:CFP/ctsk:nlGFP* larvae (A), and ALC stained mineralization (B). (C-C'') Confocal stack of area boxed in (A-B), showing absence of mineralized neural arches and cavities in vertebral centra (arrowheads; C'), where active osteoclasts cover the vertebral bodies (overlay in C''). (D-E) Expression of *ctsk:nlGFP* in ectopic osteoclasts after addition of Etidronate at the same day as Rankl induction at 15 dpf.

(F-F'') Confocal imaging shows vertebral bodies with almost intact neural arches (arrowheads; F'), in the presence of abundant *ctsk:nlGFP* positive osteoclasts (F''). (G) Statistical analysis of larvae with intact neural arches at 17 dpf, after Rankl induction (blue), and after Rankl induction with Etidronate (red). Scale bar, 50  $\mu$ m.

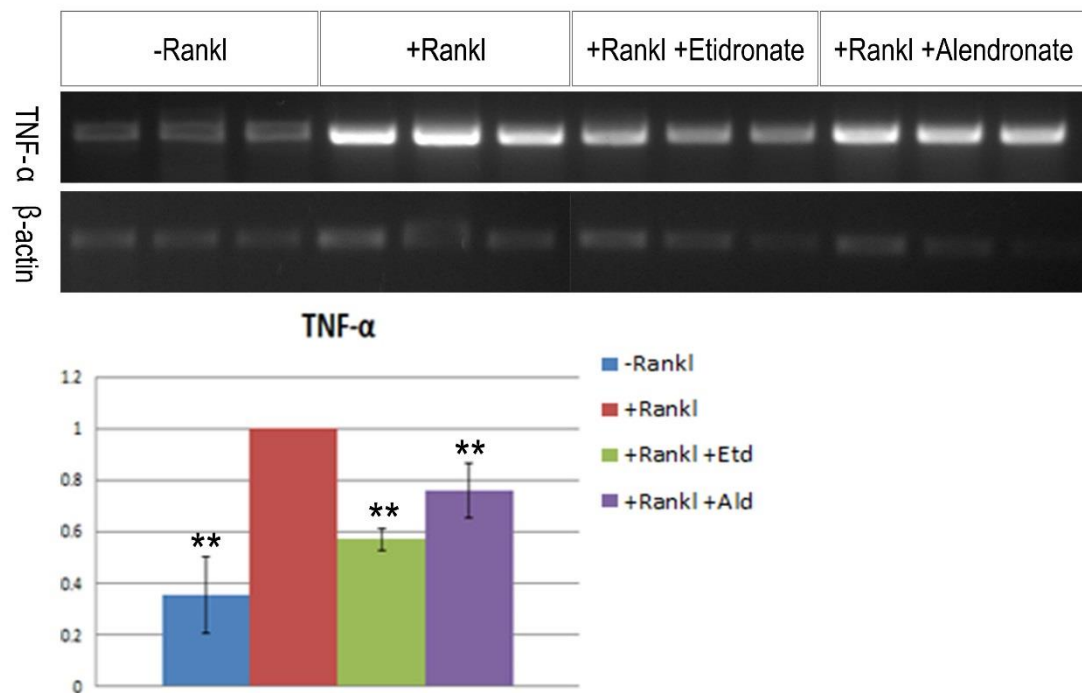


**Supplemental Figure S6: Etidronate and Alendronate accelerate bone recovery in *rankl*:HSE:CFP larvae.** (A-C) ALC stained bone matrix at 12 dpf, three days after heat shock-induced Rankl expression. A second heat shock was done at 14 dpf. The mineralized matrix was successively stained with Calcein at 16 dpf in the same larvae without (A') and with BP treatment (B', C'). Note significantly reduced mineralization in vertebral centra at 12 dpf (arrows; A-C). In the absence of BPs, re-mineralization occurred only around the edges of lesions in centra (arrowheads; A''). In contrast, centra lesions were almost completely re-mineralized four days after addition of Etidronate (arrowheads; B'') or Alendronate (arrowheads; C''). Scale bar, 50  $\mu$ m.

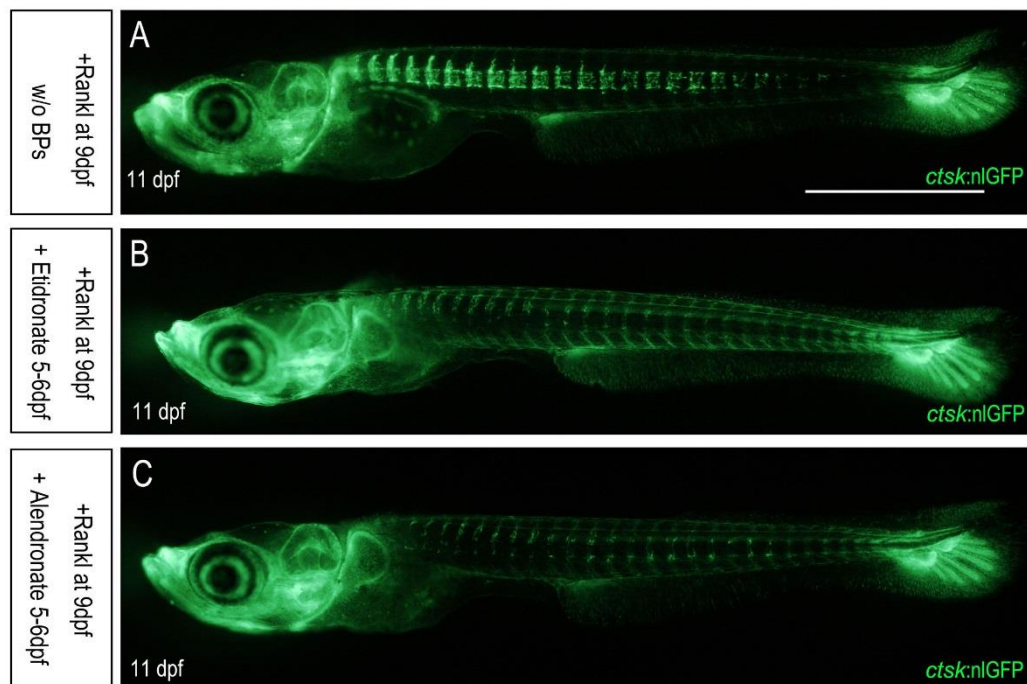


**Supplemental Figure S7: Heat shock or BP treatment alone does not affect osteoblast distribution in *osx:mCherry* larvae.** (A) *osx:mCherry* expressing osteoblasts at neural arches and vertebral centra in larvae without heat shock and BP treatment. (B) *osx:mCherry* cells after heat shock, without both Rankl induction and BPs treatment. (C-D) *osx:mCherry* cells after six day Etidronate (C) or Alendronate (D) treatment. Scale bar, 50 μm.

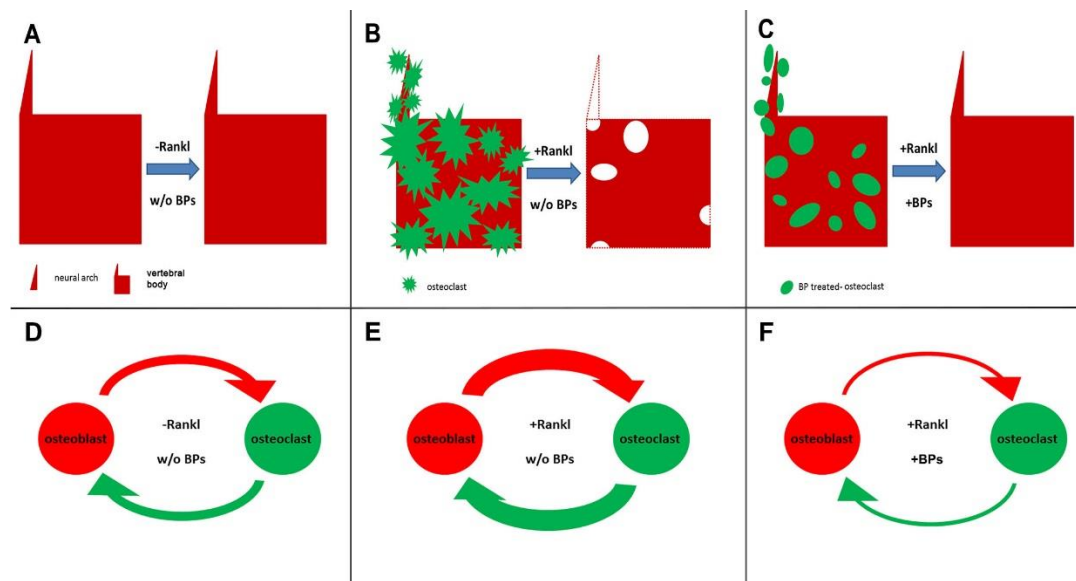




**Supplemental Figure S8: BP treatment reduces Rankl-induced TNF- $\alpha$  expression.** TNF- $\alpha$ , a common marker for systemic inflammation, is up-regulated at 11 dpf, two days after Rankl induction. After two days of Etidronate or Alendronate treatment, TNF- $\alpha$  levels are down-regulated. Bottom: Fold regulation values compared between groups without BP treatment with (red) and without (blue) Rankl induction, and groups with Rankl induction without BP (red) and with either Etidronate (green) or Alendronate (purple) treatment.  $\beta$ -actin loading control was used for normalization. Significance values are for comparison to +Rankl (\*\* =  $P < 0.01$ ).



**Supplemental Figure S9: Early BP treatment affects subsequent osteoclastogenesis.** (A) Formation of ectopic osteoclasts in *rankl:HSE:CFP/ctsk:nlGFP* larvae at 11 dpf, two days after Rankl induction at 9 dpf. (B-C) Pre-treatment of larvae with Etidronate (B) or Alendronate (C) from 5-6 dpf results in reduced numbers of ectopic osteoclasts at 11 dpf after Rankl was induced at 9 dpf. The same phenotype is observed at 11 dpf when BPs were added from 6-7 dpf or 7-8 dpf, i.e. one or two days before Rankl induction at 9 dpf (data not shown). Scale bar, 1mm.



**Supplemental Figure S10:** (A-C) Model of dynamic osteoclast behaviour in vertebral bodies without Rankl and BP treatment (A), with Rankl but without BP treatment (B), and with both Rankl and BP treatment (C). The change in osteoclast morphology after BP treatment (less extensions, smaller size) and the reduction of mineralized matrix in neural arches and centra after Rankl induction is indicated. (D-F) Model of osteoblast-osteoclast coupling in the absence of Rankl and BP treatment (D), with Rankl but without BP treatment (E), and with both Rankl and BP treatment (F). Width of arrows indicate hypothesized extent of cross talk between both cell types.