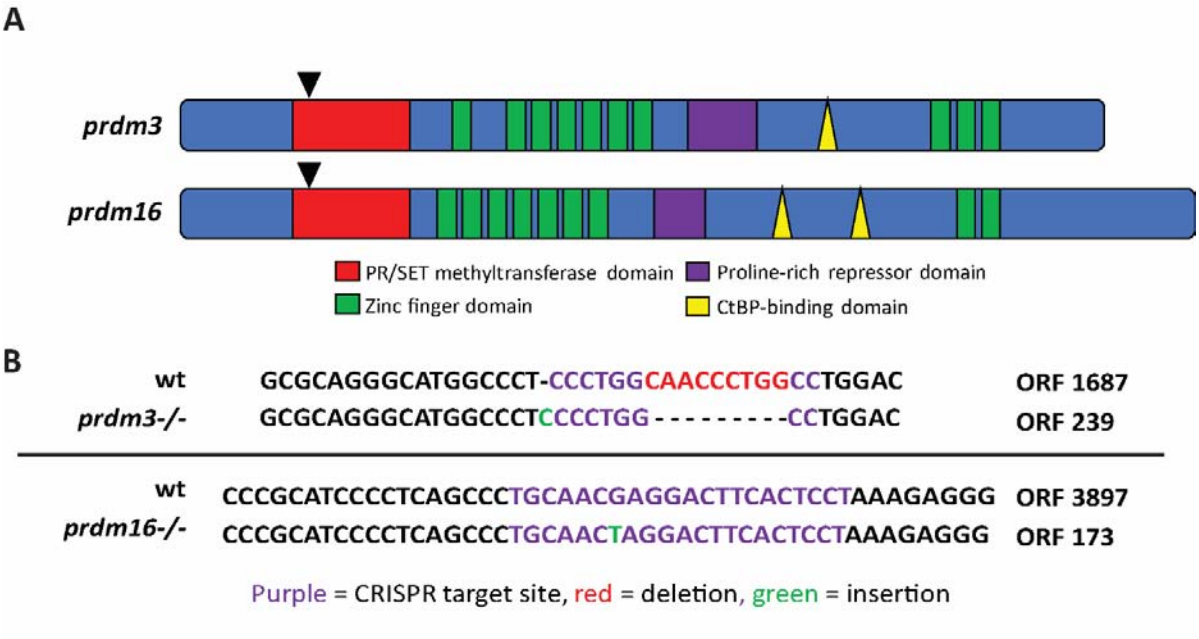
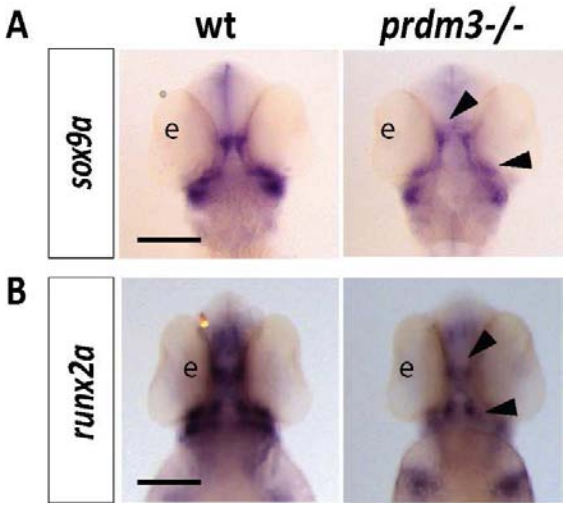
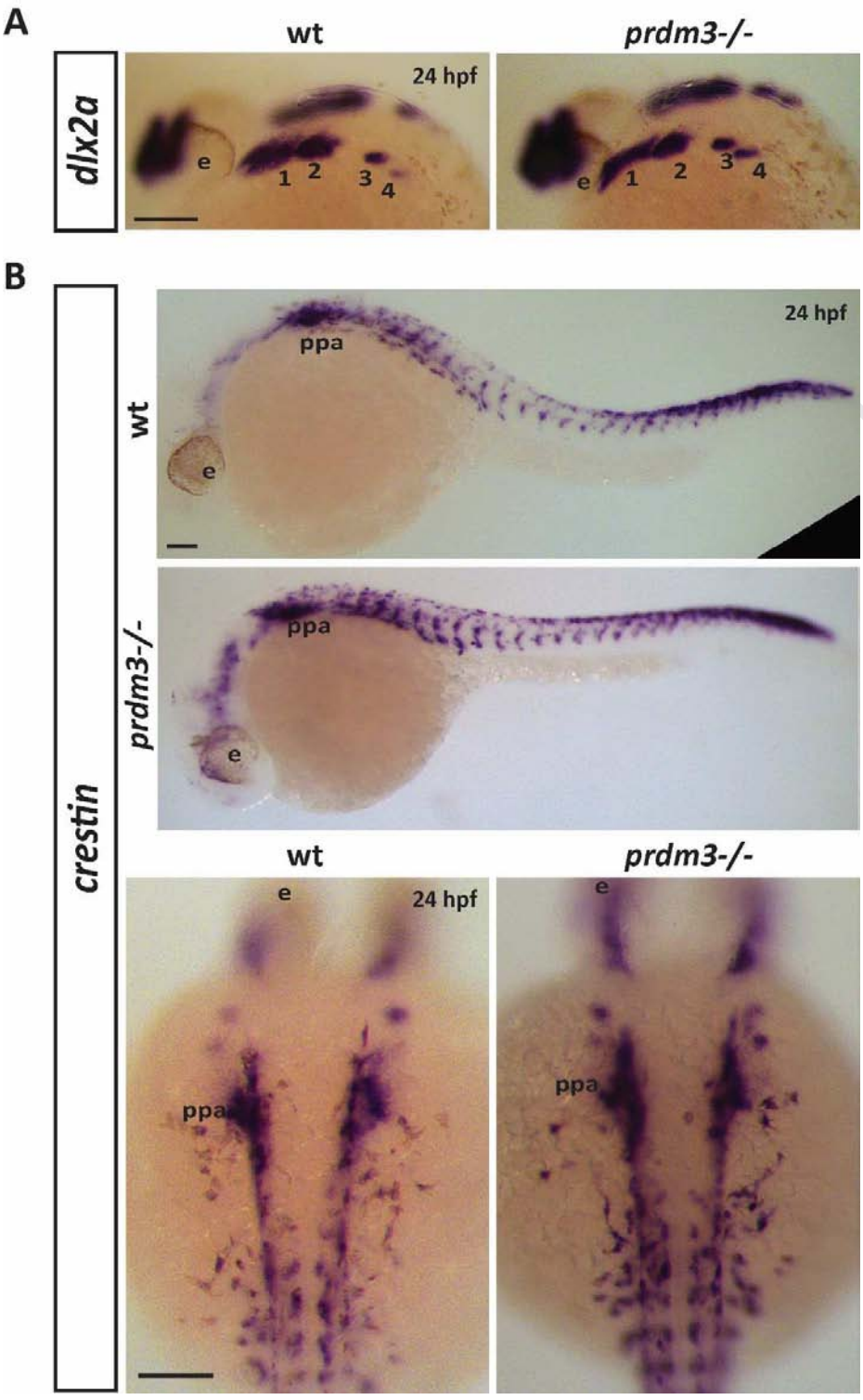


Supplemental Figure 1. CRISPR/Cas9 mediated genome editing at the prdm3^{CO1005} and prdm16^{CO1006} loci creates frameshift mutations resulting in truncated proteins. (A) Schematic of the structures of *prdm3* and *prdm16*, highlighting the specific domains, including the PR/SET methyltransferase domain (red), zinc finger domains (green). Black arrowheads indicate the location of CRISPR/Cas9 mediated mutation for each gene. (B) CRISPR/Cas9 directed genome editing created an indel (green) and a 9 base pair deletion (red) in the CRISPR target site (purple) in *prdm3* and an indel (green) in the *prmd16* CRISPR target site, both resulting in frameshifts and truncated proteins.

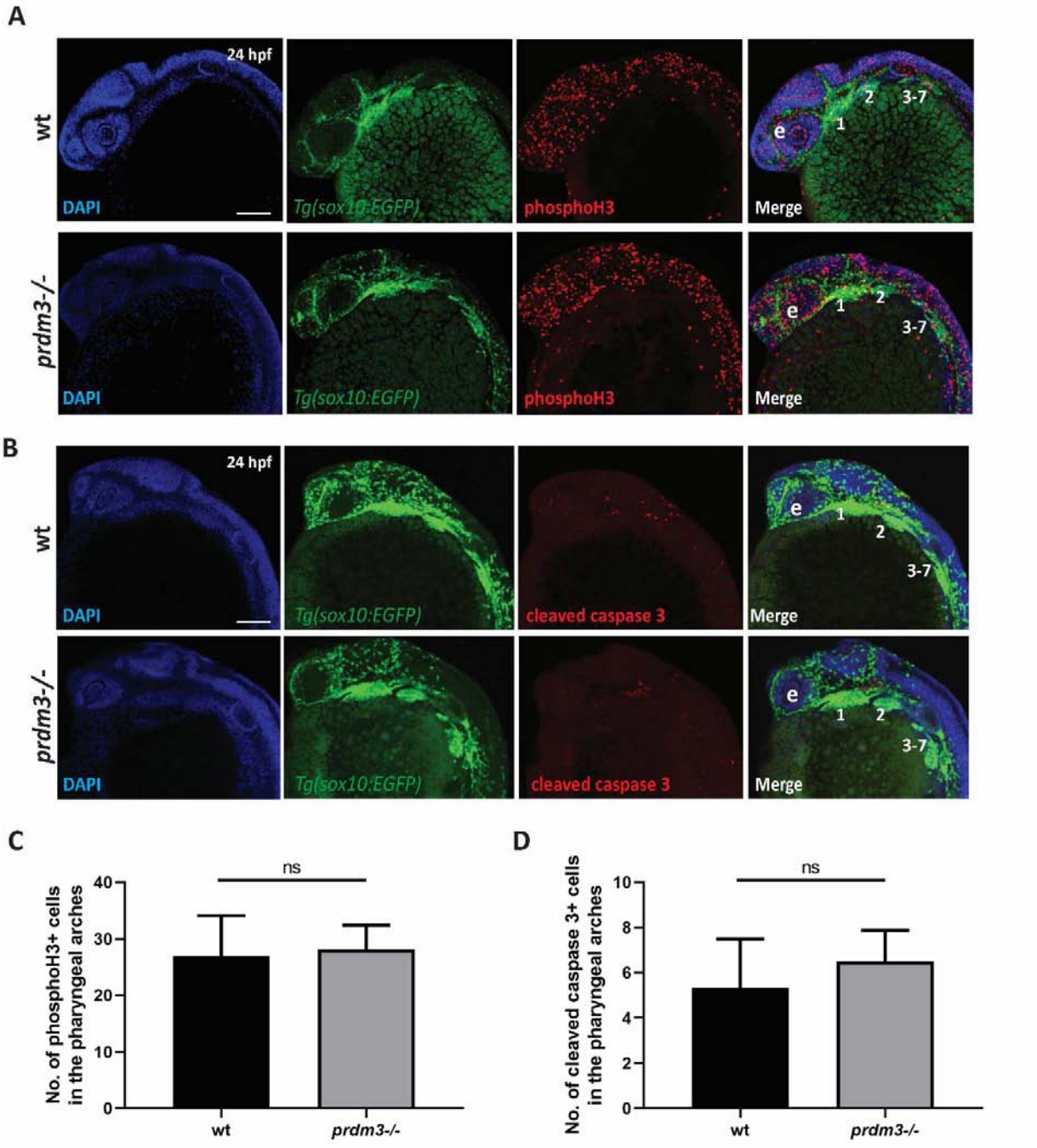


Supplemental Figure 2. Cartilage and bone markers, *sox9a* and *runx2a*, are reduced in the pharyngeal arches with loss of *prdm3* in zebrafish. (A-B) Wildtype or *prdm3-/-* mutant embryos were collected and *in situ hybridization* was performed for *sox9a* (A) or *runx2a* (B) at 48 hpf. Shown are ventral views for each transcript. Black arrow heads indicate areas of decreased expression of these markers in the developing viscerocranium and neurocranium. Scale bars, 250 µm. Abbreviations: eye (e).



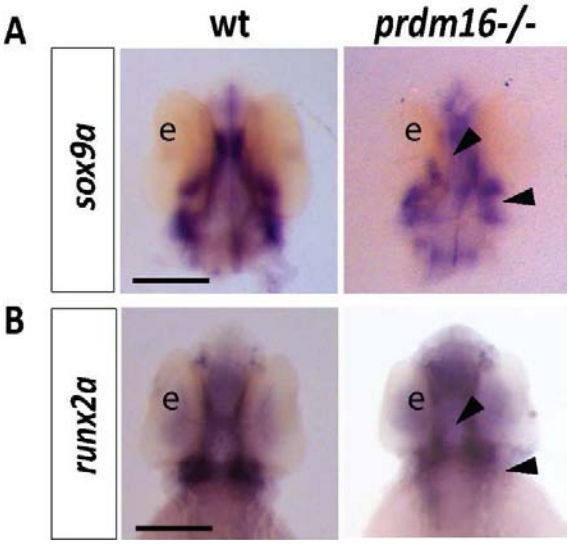
Supplemental Figure 3. Neural crest markers, *dlx2a* and *crestin*, are unchanged in the pharyngeal arches with loss of *prdm3* in zebrafish. (A-B) Wildtype or *prdm3-/-* mutant embryos were collected and *in situ hybridization* was performed for *dlx2a* (A) or *crestin* (B) at 24 hpf. Shown are lateral views of *dlx2a* expression in the pharyngeal arches (1-4) (A) and lateral views of *crestin* across the whole body to

show neural crest migration is unaffected in *prdm3* mutant zebrafish. Higher magnification, dorsal views of *crestin* expression is also shown in (B). Scale bars, 100 µm. Abbreviations: eye (e), 1-4 (pharyngeal arches 1-4), posterior pharyngeal arches (ppa).

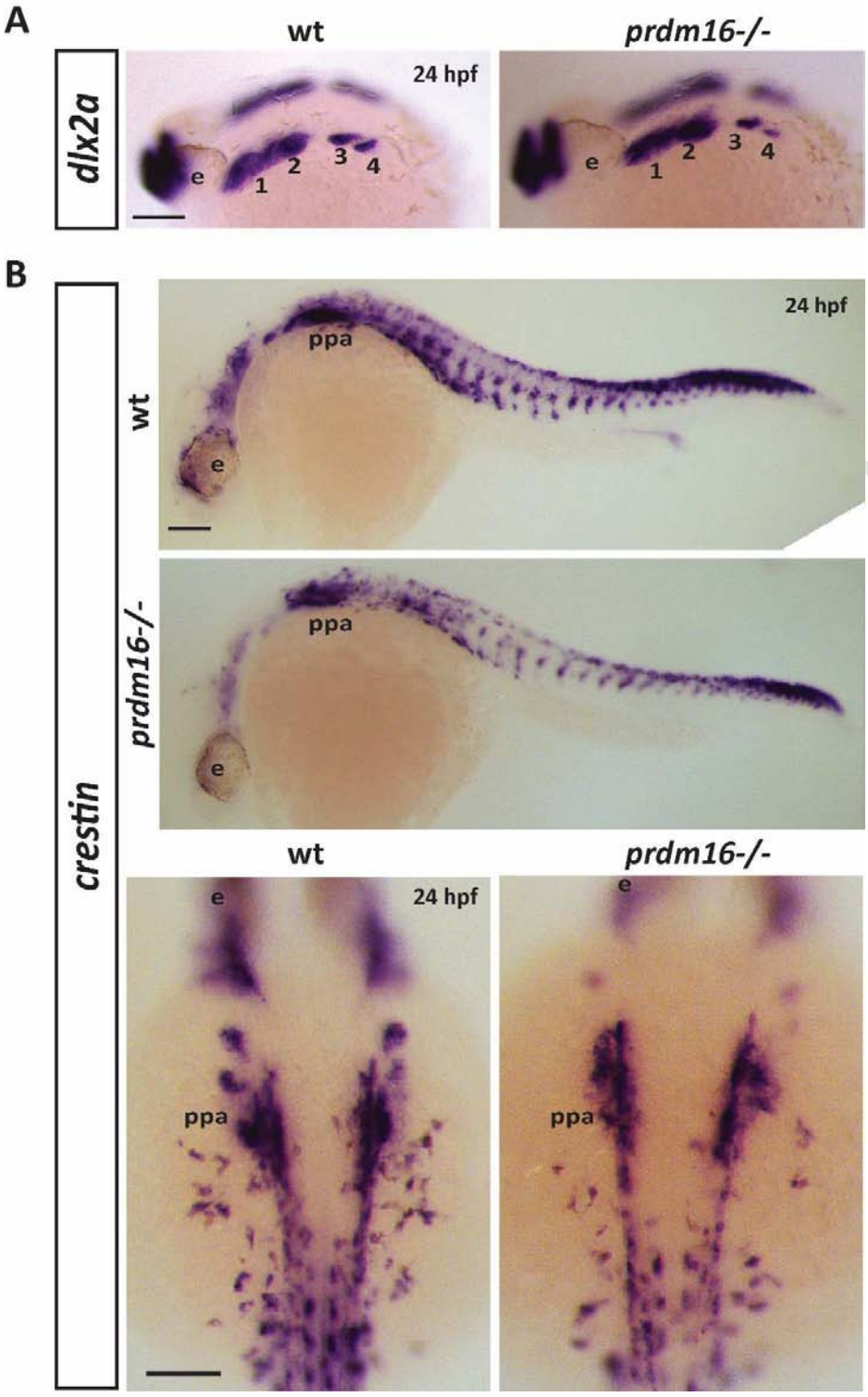


Supplemental Figure 4. Loss of *prdm3* does not lead to changes in pharyngeal arch cell proliferation or cell death. (A-D) Wildtype or *prdm3-/-* zebrafish embryos crossed into the *Tg(sox10:EGFP)* transgenic line were collected at 24 hpf and whole mount immunofluorescence was performed for phosphorylated histone H3 (A) or cleaved caspase 3 (B) to assess cell proliferation or apoptosis in the pharyngeal arches, respectively. Shown are lateral max projection images of the pharyngeal arches 1-7. (C-D) Quantification of the number of phosphorylated histone H3 positive cells (yellow) (C) or cleaved caspase 3 positive cells (yellow) (D) within the pharyngeal arches (n = 6 embryos

for each group). Abbreviations: eye (e), pharyngeal arches (1-7). Scale bar, 100 µm. ns, not significant, Student's *t* test.

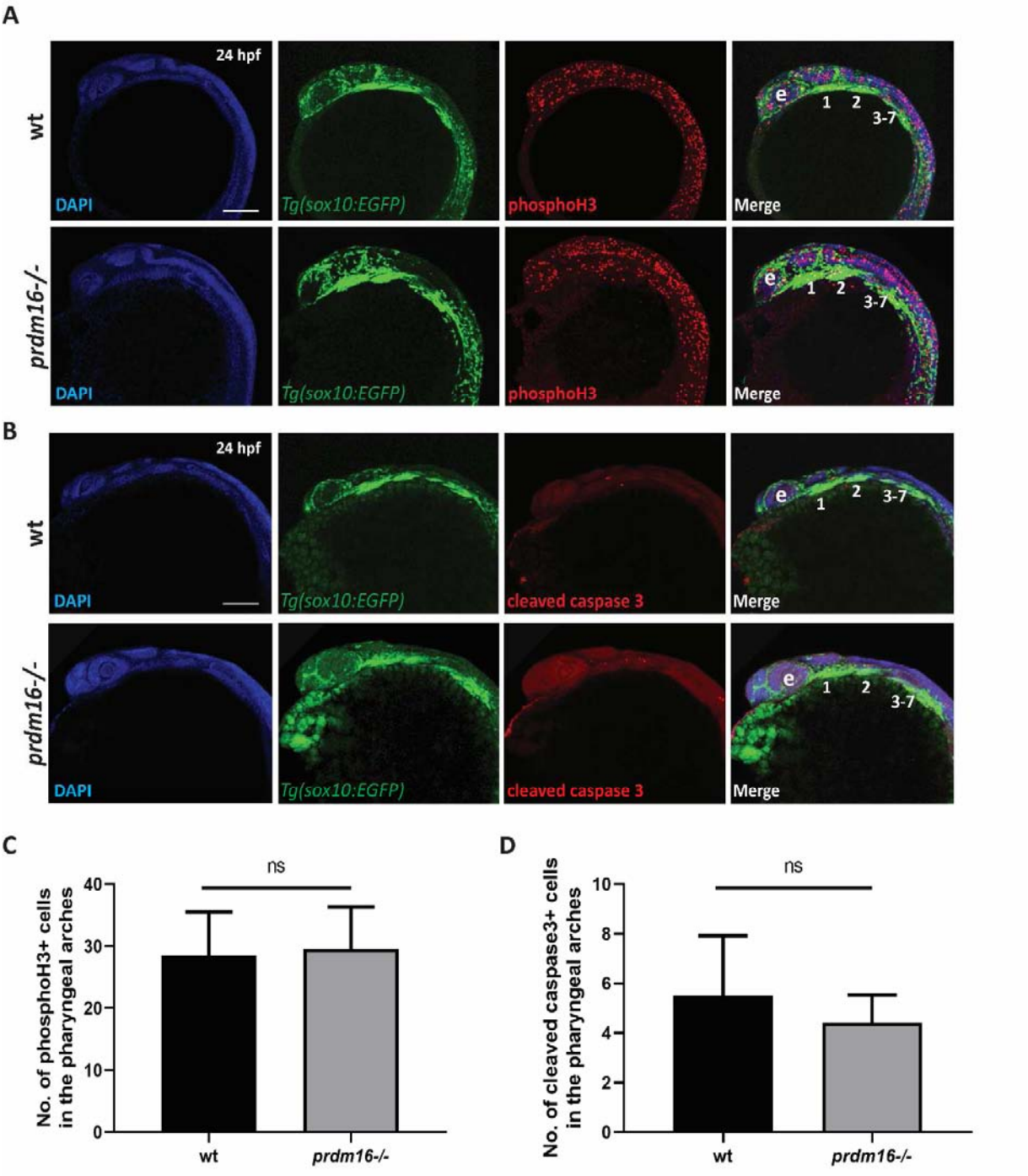


Supplemental Figure 5. Loss of *prdm16* decreases expression of cartilage and bone markers, *sox9a* and *runx2a*, in zebrafish. (A-B) Wildtype or *prdm16-/-* mutant embryos were collected and *in situ hybridization* was performed for *sox9a* (A) or *runx2a* (B) at 48 hpf. Shown are ventral views for each transcript. Black arrow heads indicate areas of reduced expression of these transcripts in the developing cartilage and bone structures of the craniofacial skeleton. Scale bars, 250 µm. Abbreviations: eye (e).



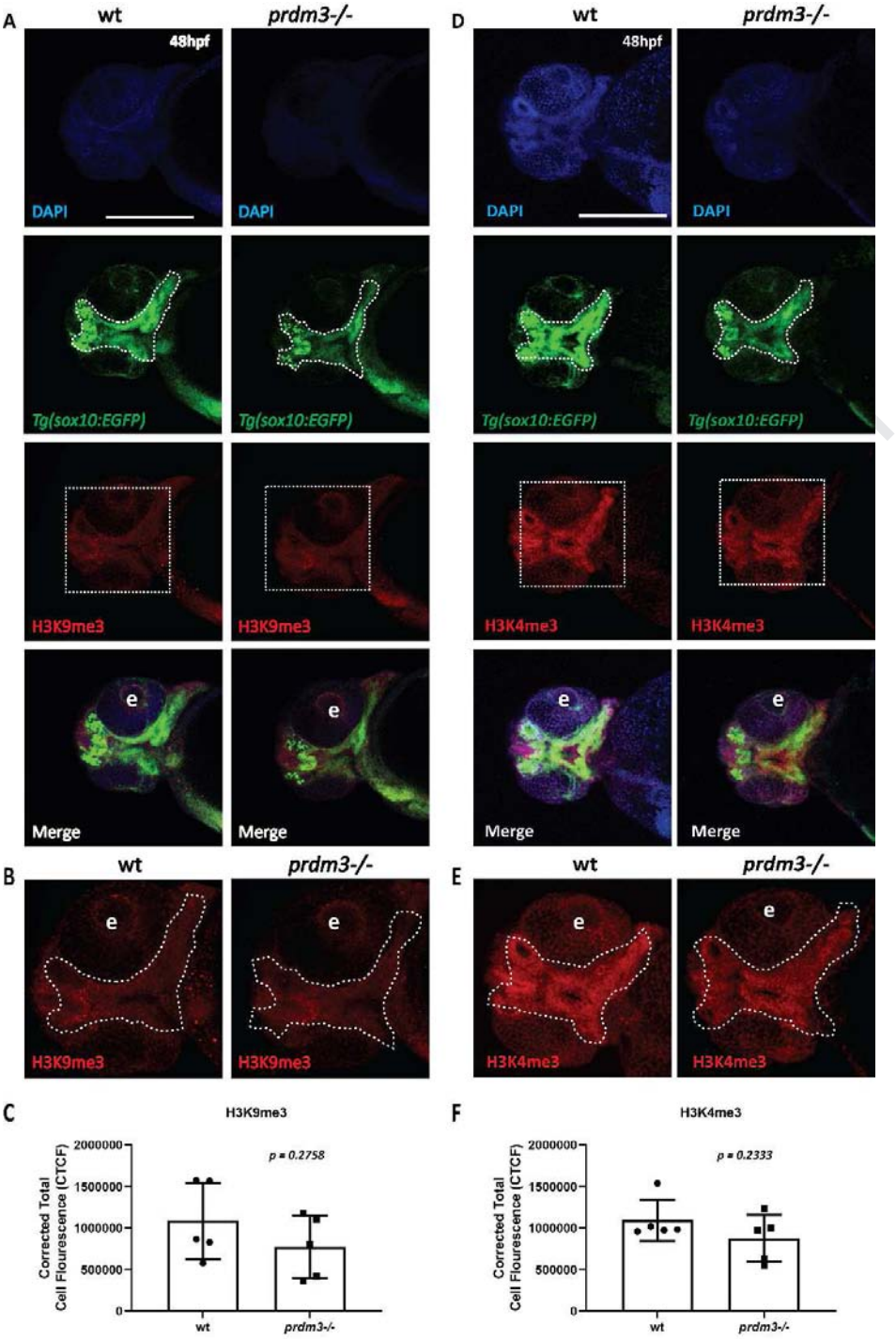
Supplemental Figure 6. Loss of *prdm16* causes a subtle decrease in expression of neural crest markers *dlx2a* or *crestin* in the pharyngeal arches. (A-B) Wildtype or *prdm16-/-* mutant embryos were collected and *in situ hybridization* for *dlx2a* (A) or *crestin* (B) was performed at 24 hpf. Shown are lateral views of *dlx2a* expression in the pharyngeal arches (1-4) (A) and lateral views of *crestin* across the whole body to show neural crest migration is unchanged in *prdm16* mutant zebrafish. Higher

magnification, dorsal views of *crestin* expression is also shown in (B). Scale bars, 100 µm. Abbreviations: eye (e), 1-4 (pharyngeal arches 1-4), posterior pharyngeal arches (ppa).



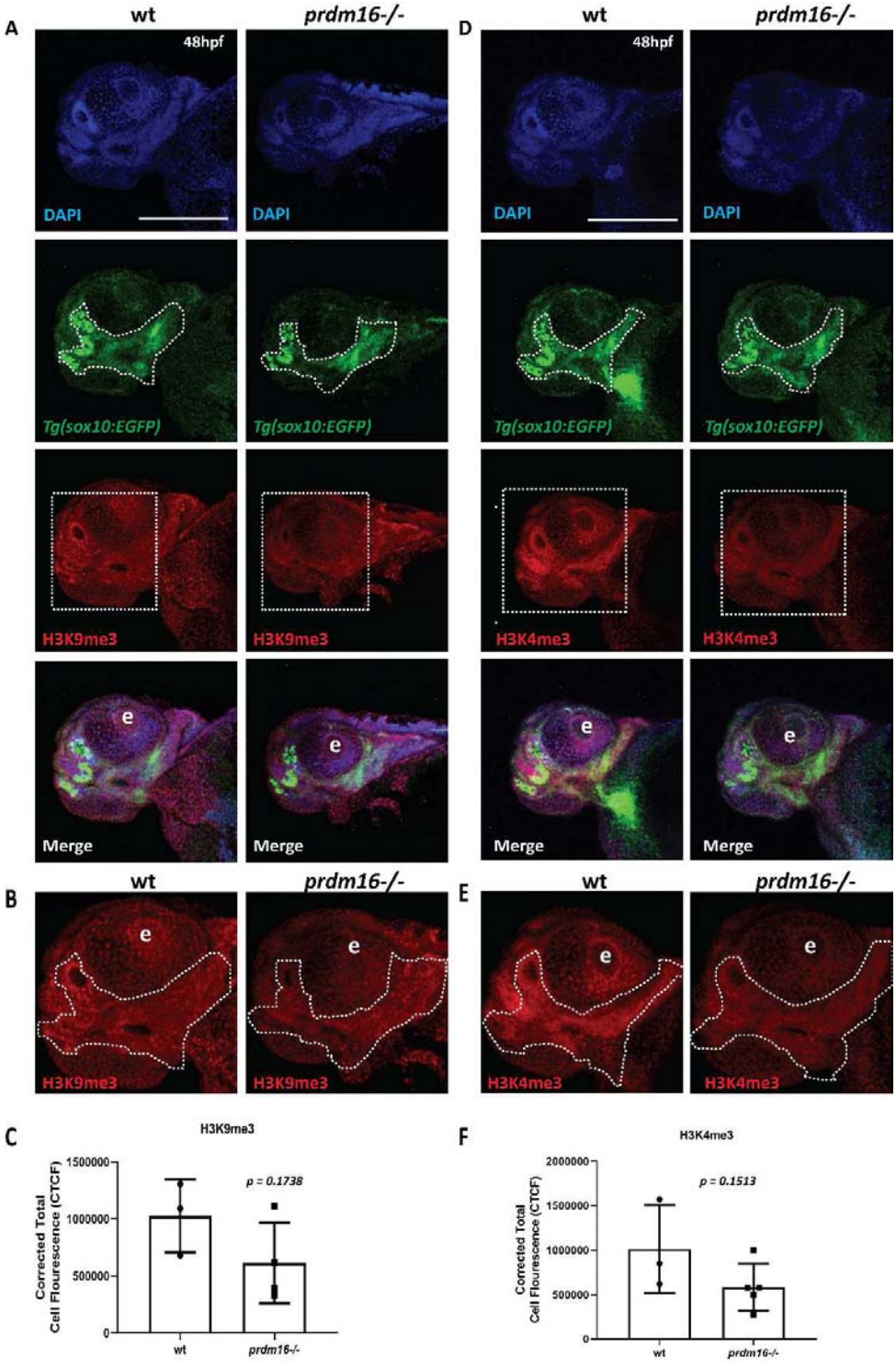
Supplemental Figure 7. Cell proliferation and cell death are not affected in the pharyngeal arches with loss of *prdm16*. (A-D) Wildtype or *prdm16-/-* zebrafish embryos crossed into the *Tg(sox10:EGFP)* transgenic line were collected at 24 hpf and whole mount immunofluorescence was performed for phosphorylated histone H3 (A) or cleaved caspase 3 (B) to assess cell proliferation or apoptosis in the pharyngeal arches, respectively. Shown are lateral max projection images of the pharyngeal arches 1-7. (C-D) Quantification of the number of phosphorylated histone H3 positive cells (yellow) (C) or cleaved

caspase 3 positive cells (yellow) (D) within the pharyngeal arches (n = 6 embryos for each group). Abbreviations: eye (e), pharyngeal arches (1-7). Scale bar 100 µm. ns, not significant, Student's *t* test.



Supplemental Figure 8. Loss of *prdm3* decreases trimethylation of histone H3 substrates in zebrafish cranial pharyngeal arch derivatives. (A-F) Wildtype or *prdm3-/-* zebrafish embryos crossed into the *Tg(sox10:EGFP)* transgenic line were collected at 48 hpf and whole mount immunofluorescence was performed for trimethylated H3 lysine 9 (A-C) or trimethylated H3 lysine 4 (D-F). Shown are ventral confocal max projection images of the head. (B, E) Zoomed images of H3K9me3

or H3K4me3 signal. (C, F) Quantification of the corrected total mean fluorescence intensity of H3K9me3 (C) or H3K4me3 (F) in the pharyngeal arch-derived craniofacial structures outlined with dotted line in (B) and (E) from 5 different embryos for each genotype and each mark. Abbreviation: eye (e). Scale bar 150 μm. p values shown above, Student's *t* test.



Supplemental Figure 9. Loss of *prdm16* reduces trimethylation of histone H3 substrates in zebrafish pharyngeal arch derived craniofacial structures. (A-F) Wildtype or *prdm16-/-* zebrafish embryos crossed into the *Tg(sox10:EGFP)* transgenic line were collected at 48 hpf and whole mount immunofluorescence was performed for trimethylated H3 lysine 9 (A-C) or trimethylated H3 lysine 4 (D-

F). Shown are ventral confocal max projection images of the head. (B, E) Zoomed images of H3K9me3 or H3K4me3. (C, F) Quantification of the corrected total mean fluorescence intensity of H3K9me3 (C) or H3K4me3 (F) in the pharyngeal arch-derived craniofacial structures outlined with dotted line in (B) and (E) from 3-5 different embryos for each genotype and each mark. Abbreviation: eye (e). Scale bar 150 μm. p values shown above, Student's *t* test.