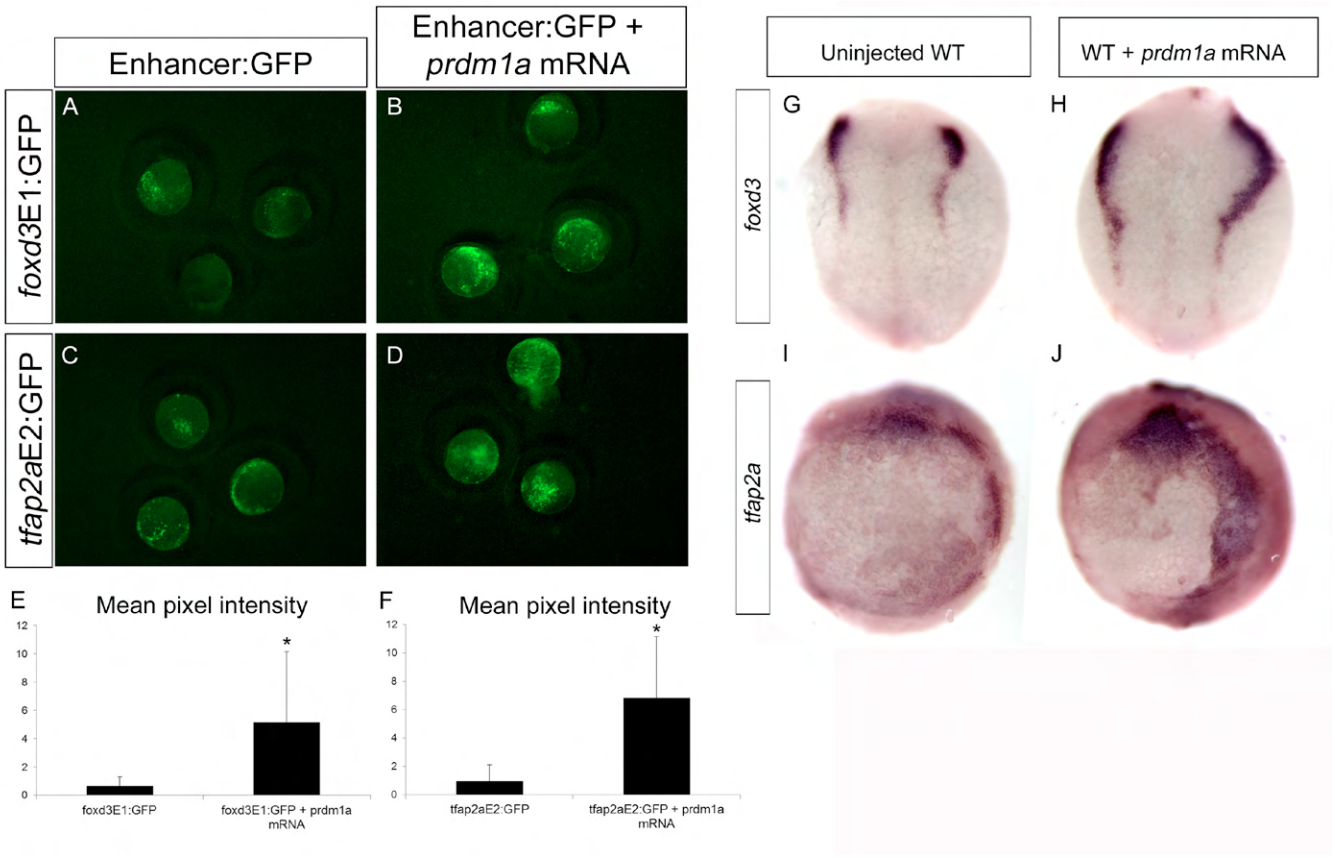

Fig. S1. *prdm1a* overexpression leads to expansion of *foxd3* and *tfap2a* enhancer reporters and endogenous expression. (**A-F**) *prdm1a* mRNA co-injected at the single-cell stage with *foxd3*E1:GFP (B,E; *n*=11) and *tfap2a*E2:GFP (D,F; *n*=25) and imaged at 2-somites produces increased GFP expression when compared with enhancer:GFP constructs alone (A,C). (**G-J**) *prdm1a* mRNA overexpression also increases the expression of endogenous *foxd3* along the NPB (G,H; dorsal views; *n*=15) and expansion of the *tfap2a* expression domain at 2-somites (I,J; lateral views; *n*=10).

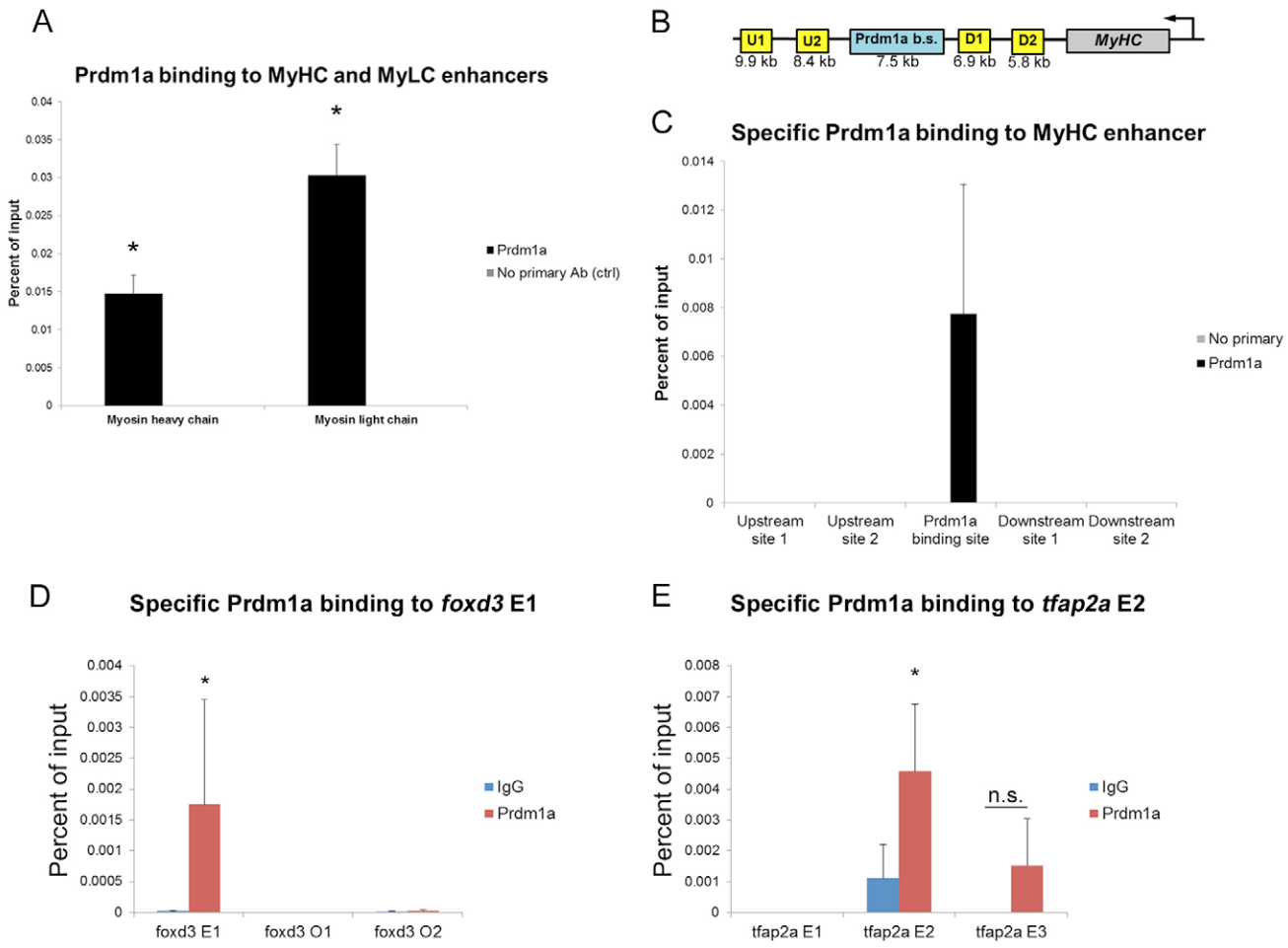

Fig. S2. ChIP controls demonstrate the specificity of the Prdm1a antibody. (**A-C**) Prdm1a pulls down previously identified enhancers for MyHC (A,C) and MyLC (A) at 24 hpf and does not pull down the genomic regions Upstream 1 and 2 and Downstream 1 and 2 (distance from the *MyHC* transcription start site is indicated) flanking the Prdm1a binding site in the MyHC enhancer (B,C). (**D**,**E**) Prdm1a specifically pulls down the *foxd3* E1 enhancer and not the *foxd3* off-target regions O1 or O2 (D) and pulls down *tfap2a* E2 and not the other identified enhancers with Prdm1a binding sequences E1 or E3 (E) at 2-somites.

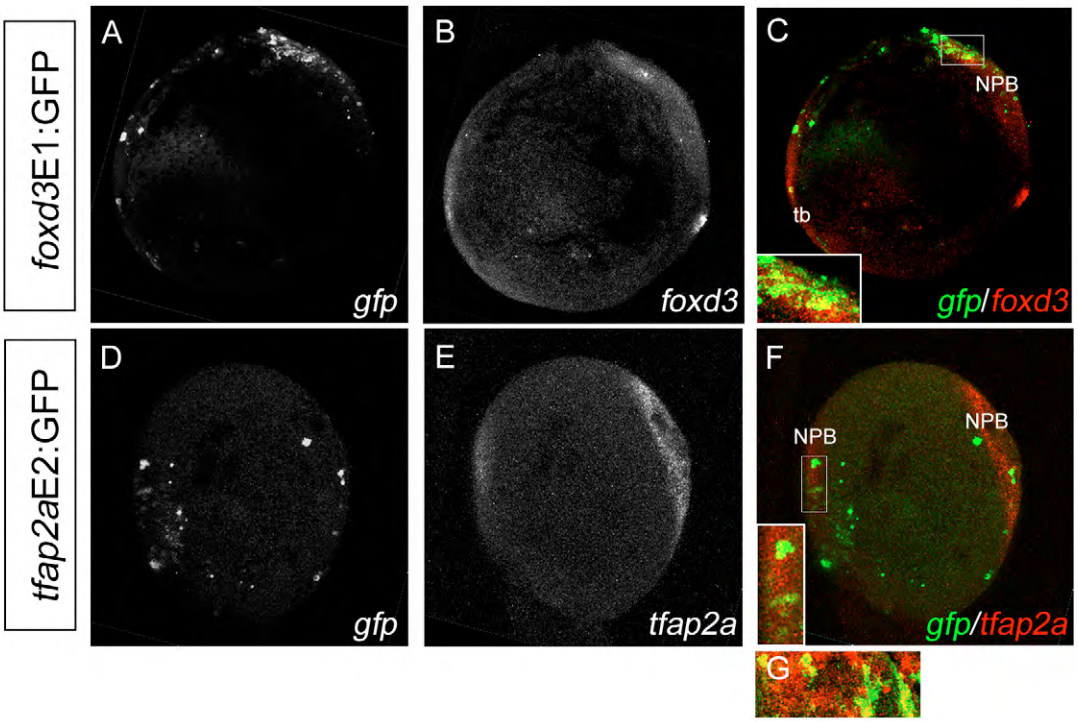

Fig. S3. Double fluorescent ISH shows mosaic colocalization of enhancer:GFP with endogenous *foxd3* and *tfap2a* mRNA expression. (**A-F**) Double fluorescent ISH for *gfp* (A) and *foxd3* (B) in *foxd3*E1:GFP-injected embryos (merge in C, lateral view) and for *gfp* (D) and *tfap2a* (E) in *tfap2a*E2:GFP-injected embryos (merge in F, dorsal view) at 2-somites demonstrate mosaic colocalization of enhancer:GFP with the endogenous gene along the NPB (insets in C,F). (**G**) *tfap2a* and *gfp* also colocalize in the most anterior NPB as shown in a lateral magnification from a separate WT embryo. NPB, neural plate border; tb, tailbud.

A

*foxd3*E1 ...TCTTGTCAGCAAATGAAAGAGATCTGCTTGTCGCG...
*foxd3*mutE1 ...TCTTGTCAATCACAAACTGTCGCGATGTTGTCGCG...

B

*tfap2a*E2 ...TTCGTGTTTGAAGTGAATGTGTGTAGTTTTAGCCC...
*tfap2a*MutE2 ...TTCGTGTTTTAACCAGATCAGCTGTCGTTTAGCCC...

Fig. S4. Mutation of the Prdm1a binding site in enhancer-GFP constructs. (**A**) Sequence encompassing the Prdm1a binding site in *foxd3* E1 (core recognition site in red) and sequence of mutated *foxd3* E1 (*foxd3*mutE1, recognition and flanking sequence mutated as shown in gray). (**B**) Sequence of *tfap2a* E2 containing the Prdm1a binding site (core in red) and mutated sequence (gray).

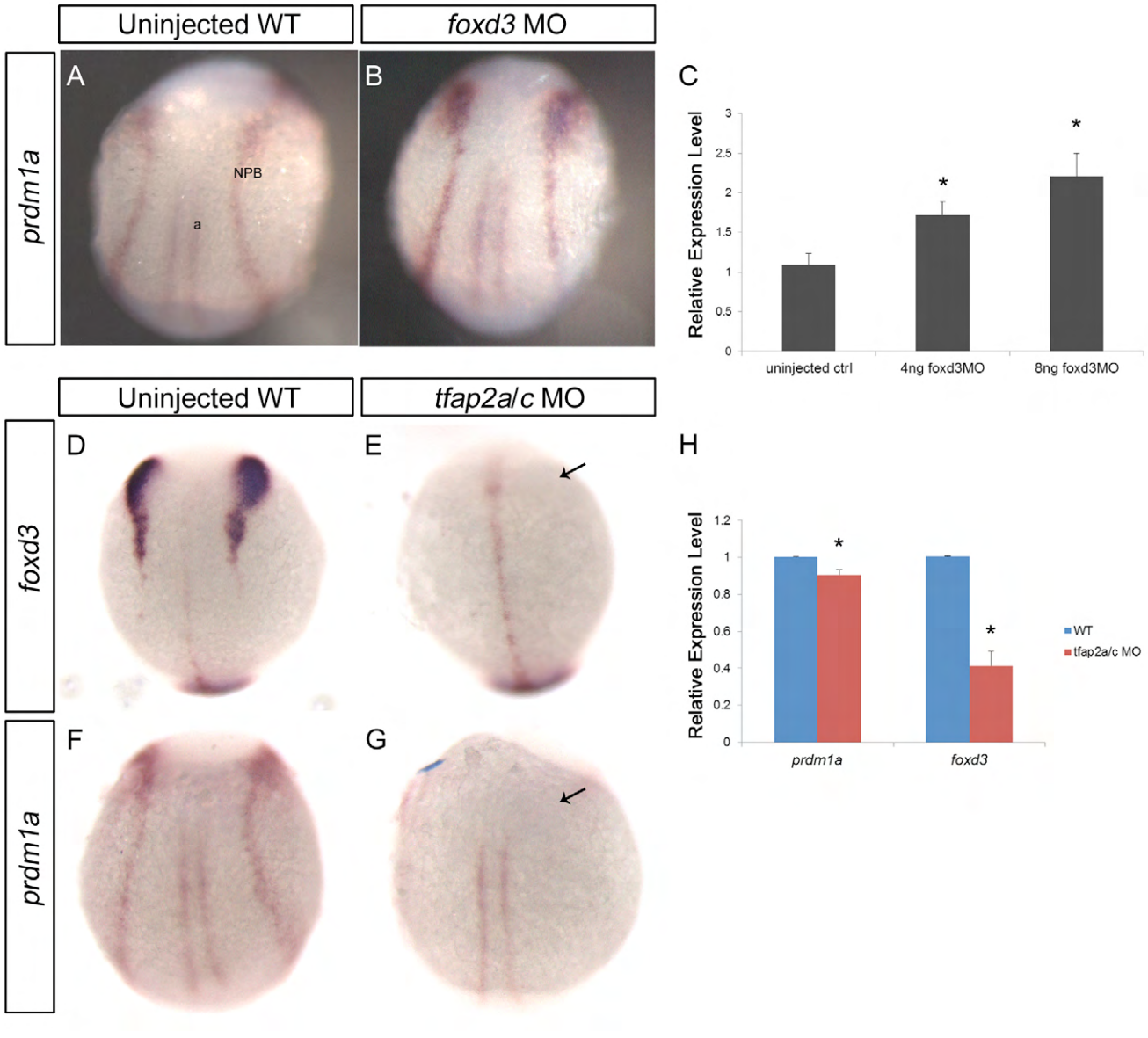

Fig. S5. *tfap2a* expression is reduced by *prdm1a*-MO at the NPB. ISH was performed on uninjected WT embryos (**A**) and embryos injected with *prdm1a*-MO (**B**) for *tfap2a* at the 2-somite stage. Lateral view shows decreased *tfap2a* expression at the NPB in *prdm1a* morphants.

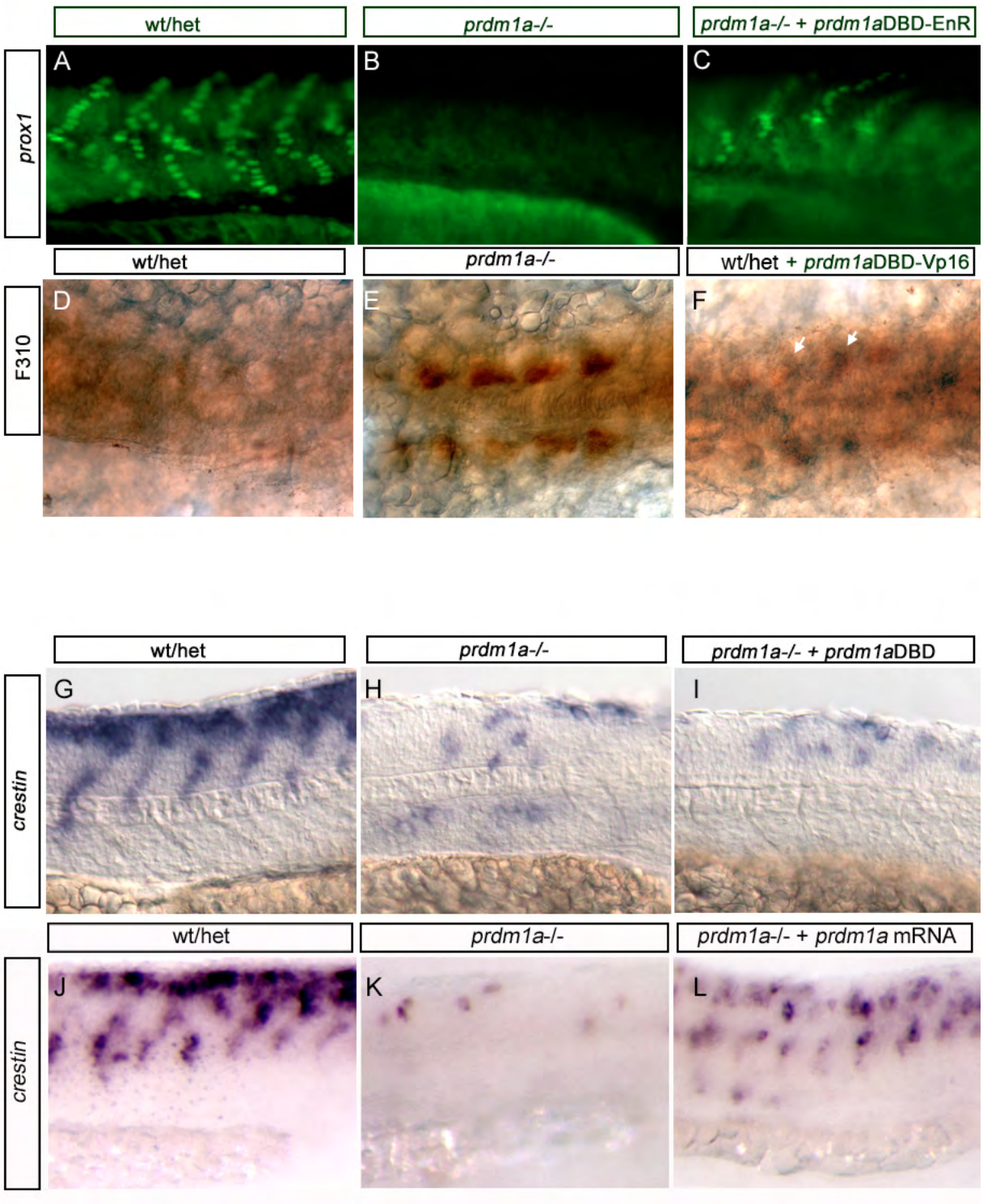

Fig. S6. *foxd3* and *tfap2a* interact reciprocally with *prdm1a* at the NPB. (**A**,**B**) WT embryos (A) and embryos injected with 8 ng *foxd3*-MO (B) were fixed at 2-somites and ISH was performed for *prdm1a*. Dorsal view of embryos shows increased *prdm1a* expression in *foxd3* morphants. (**C**) Embryos at 2-somites were also analyzed for *prdm1a* expression by qRT-PCR, showing a dose-dependent increase in *prdm1a* expression in response to *foxd3*-MO. (**D-G**) ISH for *foxd3* (D,E) and *prdm1a* (F,G) was performed on uninjected WT (D,F) and *tfap2a*/*tfap2c* double-morphant embryos (E,G) at 2-somites. Dorsal views show the absence of *foxd3* and *prdm1a* expression at the NPB in *tfap2a*/*tfap2c* morphants (arrows, E,G). (**H**) qRT-PCR for *prdm1a* and *foxd3* was also performed on WT and *tfap2a*/*c* morphants and showed decreased expression of both genes in the morphants. NPB, neural plate border; a, adaxial cells. *P<0.05.

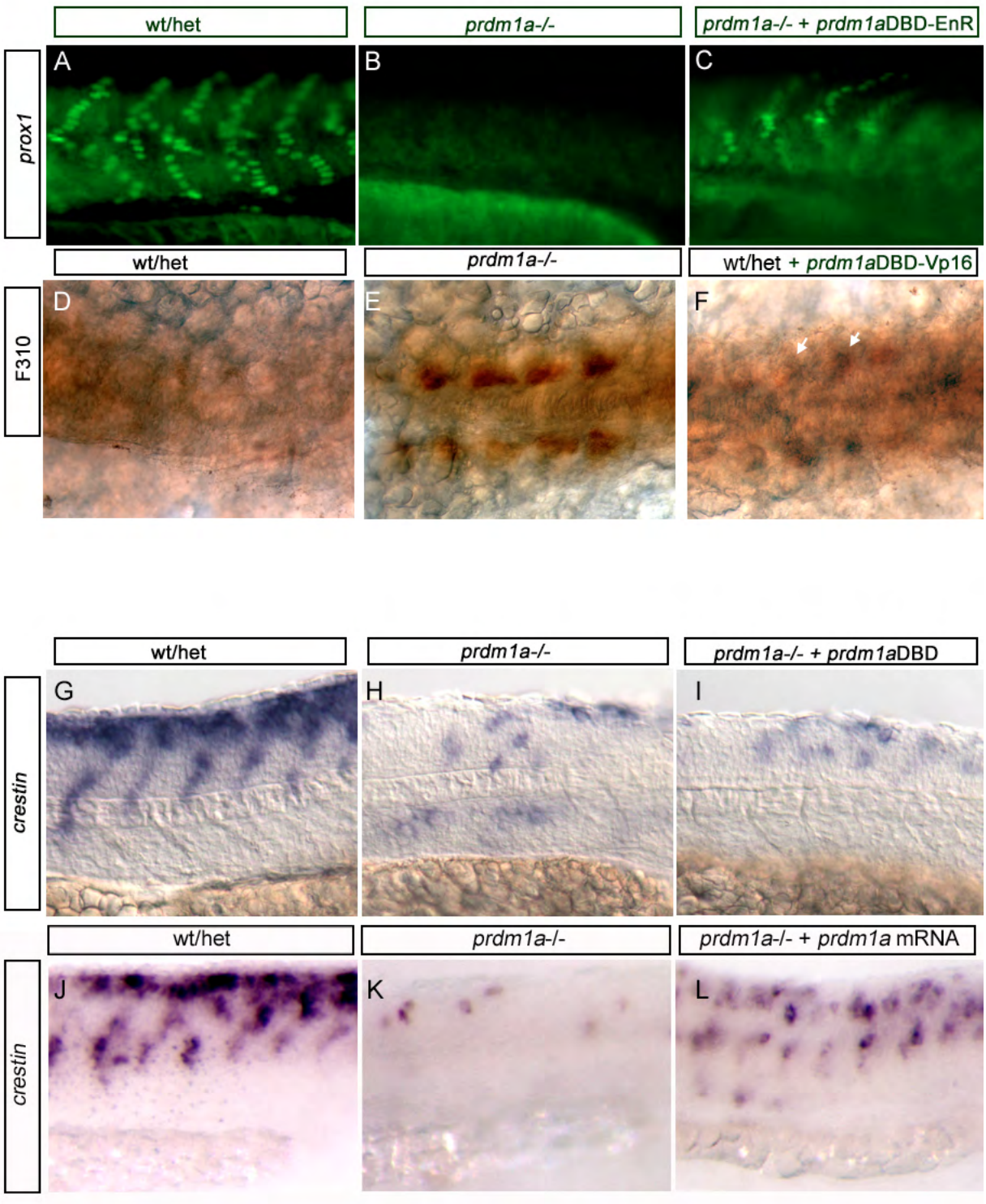

Fig. S7. *prdm1a*DBD-VP16 activating and *prdm1a*DBD-EnR repressing constructs are functional. (**A-C**) Injection of *prdm1a*DBD-EnR construct into *prdm1a*$^{-/-}$ embryos rescues *prox1* expression in slow-twitch muscle (10% rescued in C, *n*=4/20) as shown previously (von Hofsten et al., 2008). (**D-F**) Injection of *prdm1a*DBD-VP16 construct into WT embryos shows precocious F310 immunoreactivity (arrows in F), similar to what is observed in *prdm1a*$^{-/-}$ embryos (57% exhibit F310 immunoreactivity shown in F, *n*=47/82). (**G-I**) Injection of *prdm1a*DBD alone into *prdm1a*$^{-/-}$ embryos does not rescue *crestin* expression in *prdm1a* mutant embryos (100% of *prdm1a* mutants injected with *prdm1a*DBD alone exhibit reduced neural crest as in I, *n*=10/10). (**J-L**) *crestin* trunk expression in 24-hpf WT/het embryos (J), *prdm1a*$^{-/-}$ embryos (K) and *prdm1a*$^{-/-}$ embryos rescued with full- length *prdm1a* mRNA (L).

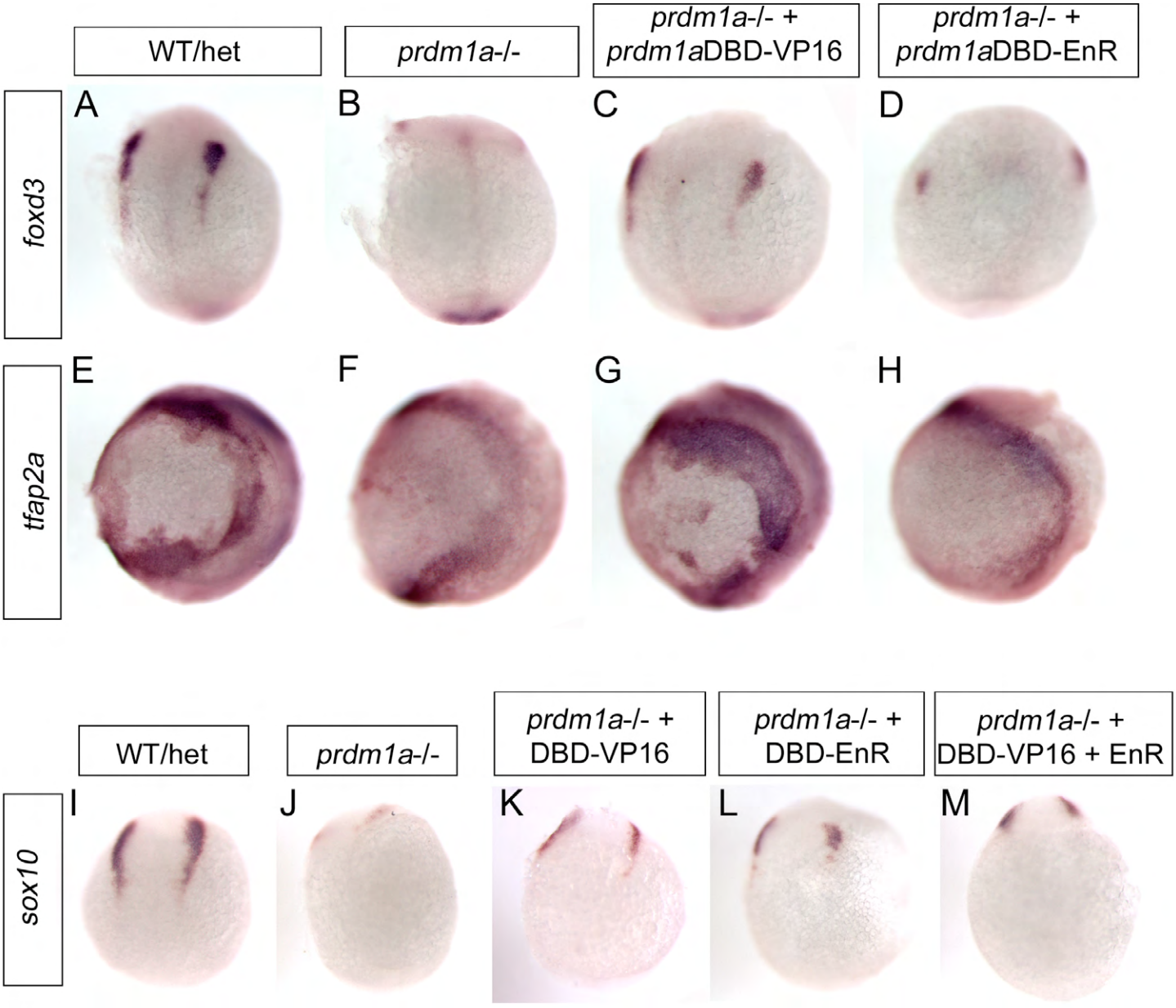

Fig. S8. *prdm1a* dominant activator rescues *foxd3* and *tfap2a* in *prdm1a* mutant embryos, and both activator and repressor forms rescue *sox10*. (**A-H**) Whole-mount ISH was performed on *prdm1a*$^{-/-}$ embryos injected with *prdm1a*DBD-VP16 activator or *prdm1a* DBD-EnR repressor at 2-somites for *foxd3* (A-D, dorsal views) and *tfap2a* (E-H, lateral views). *foxd3* and *tfap2a* are both decreased in *prdm1a*$^{-/-}$ embryos (B,F) compared with WT/het embryos (A,E). Injection of *prdm1a*DBD-VP16 partially rescues *foxd3* (C) and expands *tfap2a* (G) at the NPB in *prdm1a* mutants, whereas *prdm1a*DBD-EnR does not rescue either *foxd3* or *tfap2a* mRNA expression. (**I-M**) ISH for *sox10* was performed on *prdm1a*$^{-/-}$ embryos injected with *prdm1a*DBD-VP16, *prdm1a*DBD-EnR or both combined at 4-somites. *prdm1a*DBD-VP16 (K), *prdm1a*DBD-EnR (L) and both combined (M) partially rescued *sox10* expression in the NPB. Dorsal views.