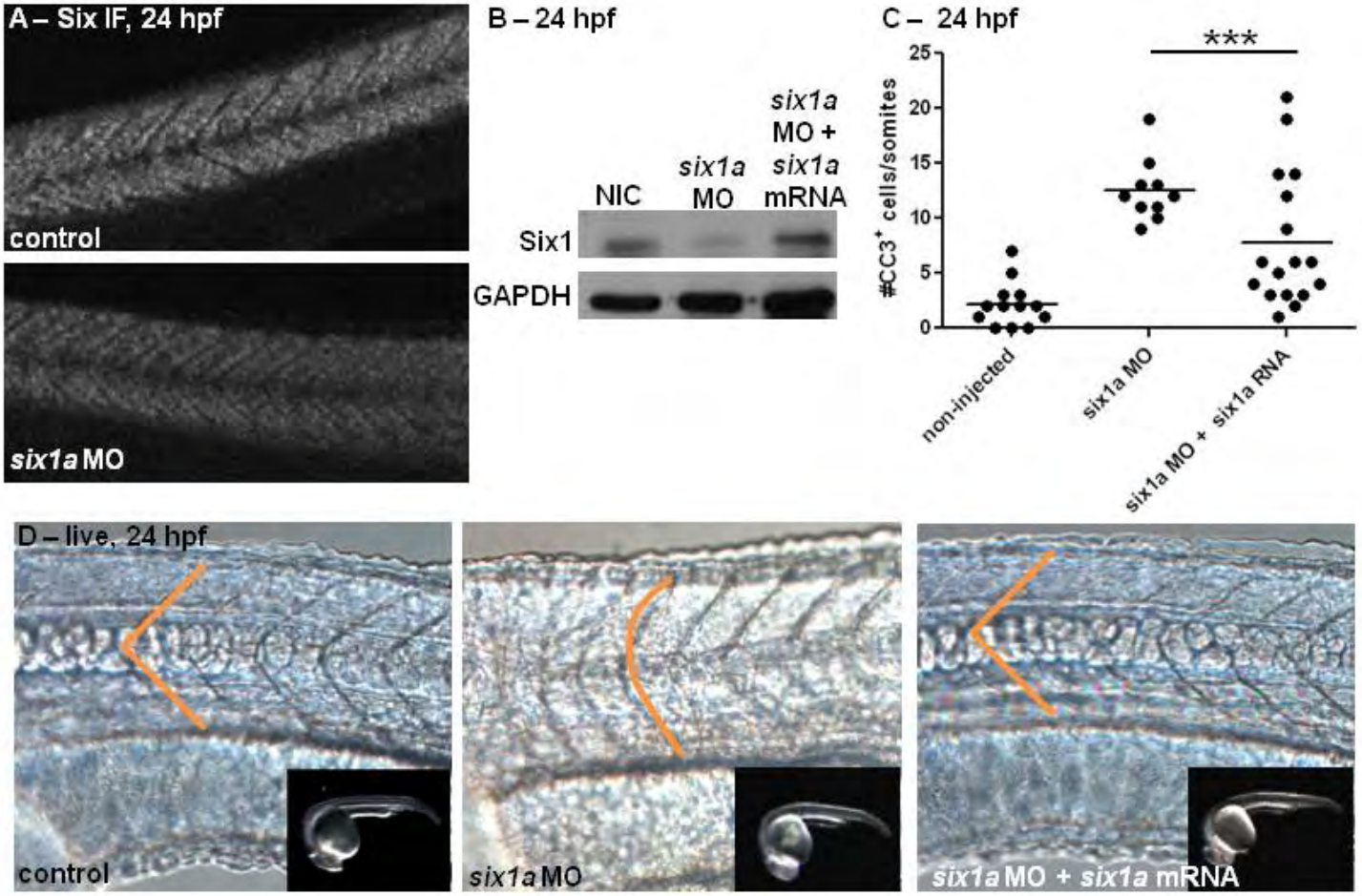

Fig. S1. Rescue of *six1a* morphant phenotypes is observed with overexpression of *six1a* mRNA. Immunofluorescent (IF) staining for multiple SIX family members at 24 hours post-fertilization (hpf) reveals a decrease in protein expression in the somites when *six1a* morpholino (MO) is injected in 1-4 cell embryos (**A**). Injection of *sixla* MO leads to downregulation of Six1 protein specifically as observed by Western blot analysis (**B**); with this protein expression rescued by co-injection of *six1a* mRNA with *six1a* MO. Quantification of cleaved-caspase-3 IF staining (**C**) at 24 hpf demonstrates that cell survival is also rescued with *six1a* mRNA overexpression in *six1a* morphants, ***p<0.0001, ANOVA with Bonferroni's post-hoc test. Live images (**D**) at 24 hpf, anterior to the left, show that the U-shaped somite morphology observed in *six1a* morphants is rescued to normal V-shape with *six1a* mRNA overexpression (control: n=13/13, 13 ng *six1a* MO: n=8/10, 13 ng *six1a* MO + 100pg *six1a* mRNA: n=12/17). Inset images confirm stage-matching across groups.

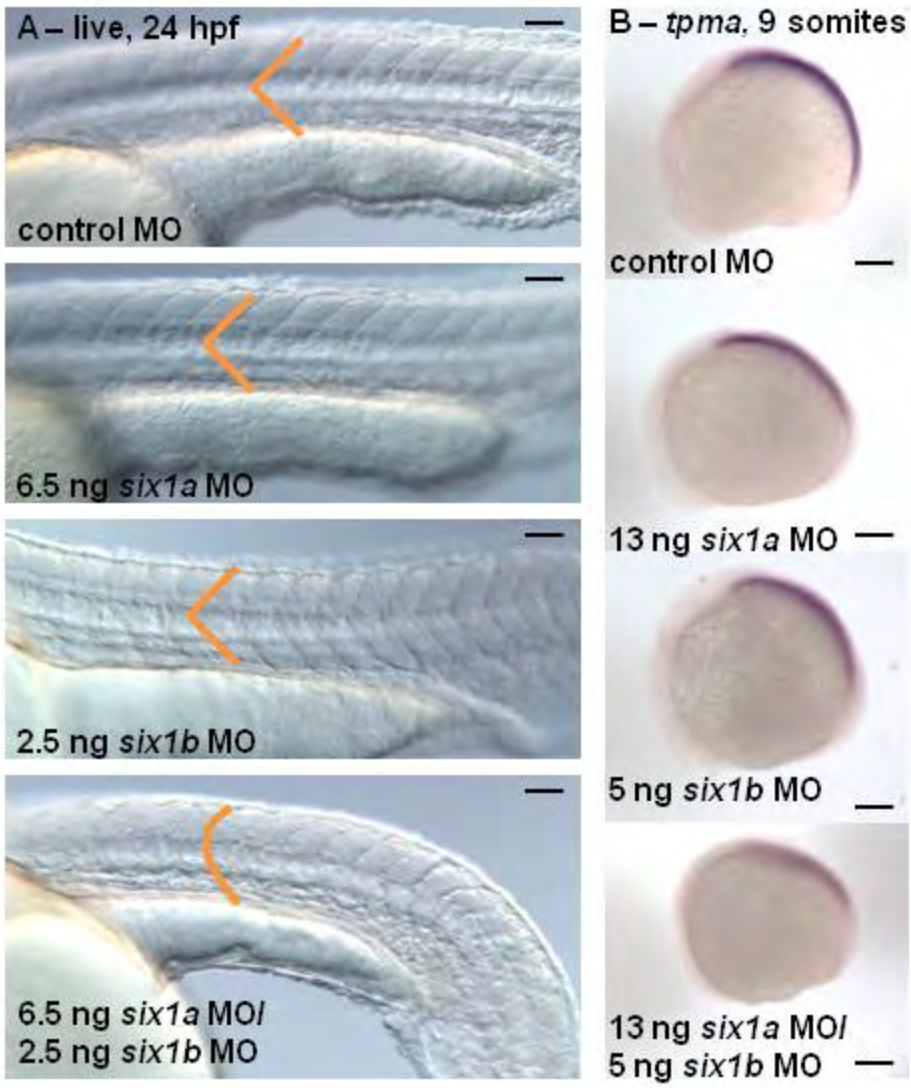

Fig. S2. Morpholino studies reveal that *six1a* and *six1b* function together during zebrafish myogenesis. Live images (**A**) at 24 hours post fertilization (hpf), anterior to the left, show that lower doses of *six1a* MO and *six1b* MO injected alone do not disrupt normal somite morphology individually, only when injected together are U-shaped somites observed (control: n=75/77, 6.5 ng *six1a* MO: n=61/64, 2.5 ng *six1b* MO: n=50/56, *six1a/b* MO: n=100/154). Lateral views of *in situ* hybridization (**B**) at 9 somites shows an early delay of *tropomyosin-α(tpma)* (control: n=25/27, *six1a* MO: n=21/25, *six1b* MO: n=20/25, *six1a/b* MO: n=10/14), which is strongest in the double *six1a/b* knockdown. Scalebar = 100 μm.

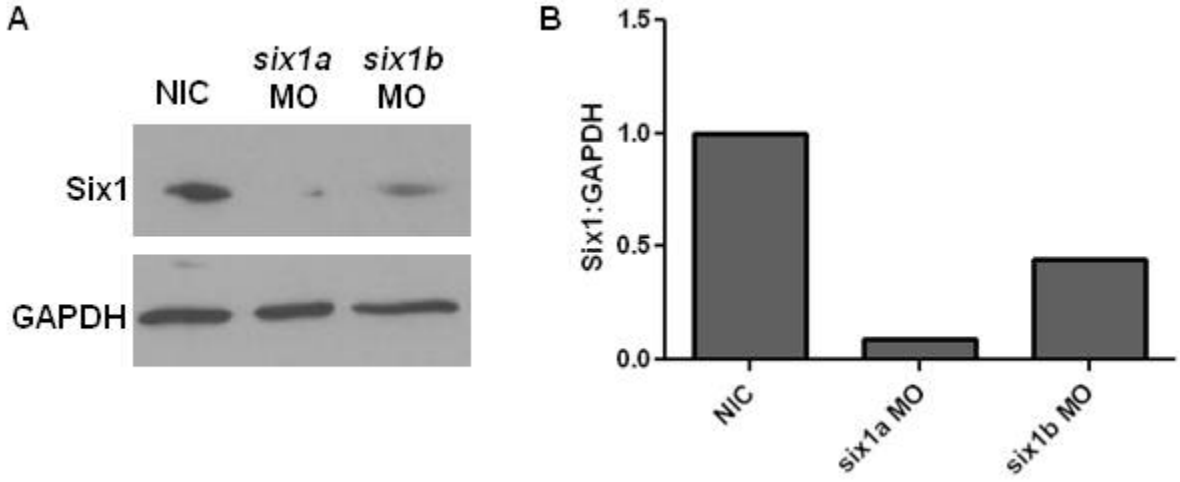

Fig. S3. Six1 antibody recognizes both Six1a and Six1b by Western blot. Western blot analysis (**A**) quantified at 24 hours post-fertilization following normalization to GAPDH (**B**) demonstrates that Six1 protein expression decreases following independent injection of *six1a*-targeted morpholino (MO) or *six1b*-targeted MO into 1-4 cell stage embryos, compared to non-injected controls (NIC).

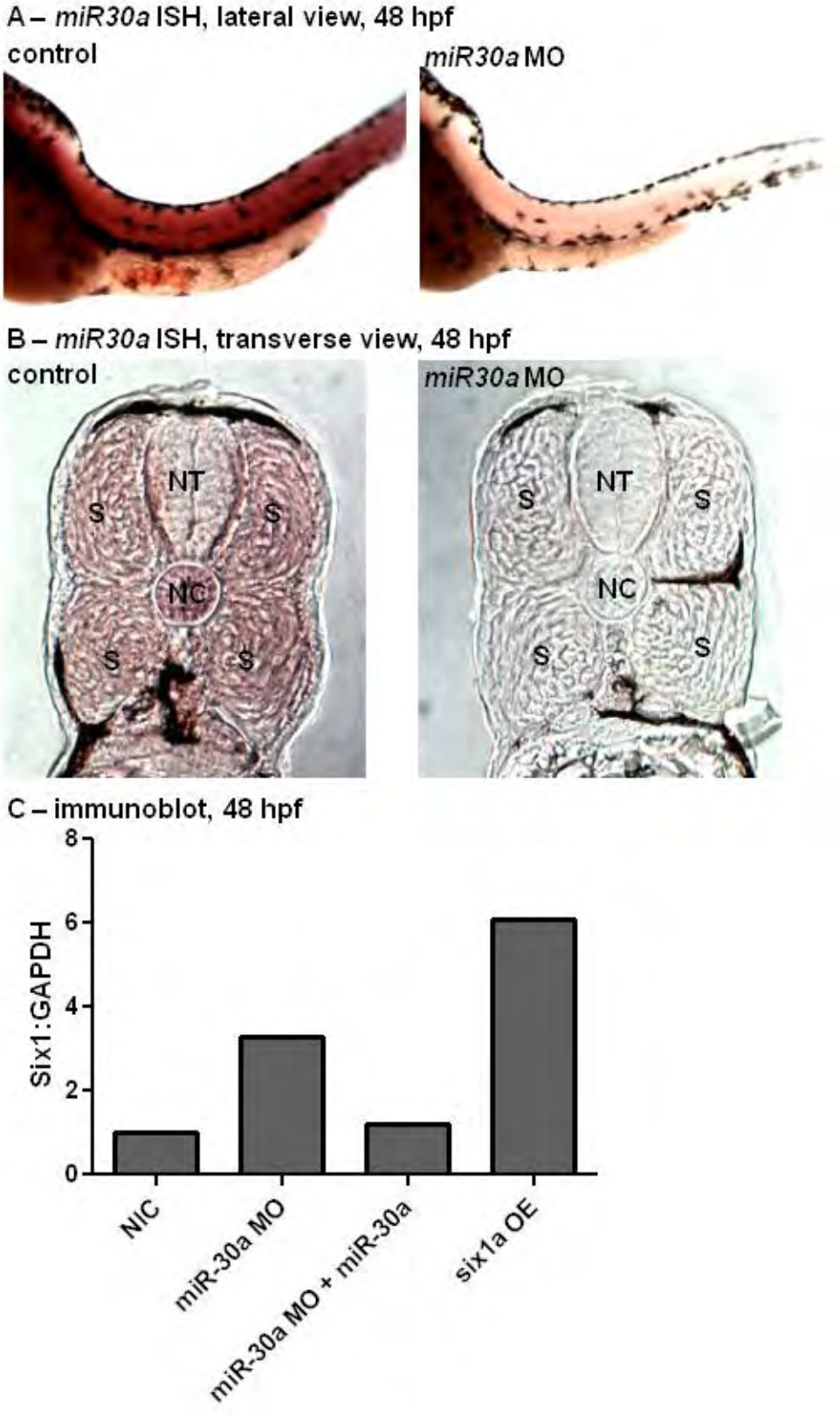

Fig. S4. microRNA-30a targeting morpholino specificity confirmed by in situ hybridization and immunoblot analyses. *In situ hybridization* (ISH) analysis (**A**, control: n=17/17 total embryos, miR30a MO: n=14/18) for microRNA-30a (miR30a) at 48 hours post fertilization (hpf) reveals a decreased expression of miR30a upon injection of miR30a-targeting morpholino (MO). Transverse cross-sections at 18 μm of 48 hpf embryos stained by ISH for miR30a (**B**) confirm miR30a is expressed in the somites (S), (NT=neural tube, NC=notochord). Western blot analysis (**C**) quantified at 48 hpf following normalization to GAPDH demonstrates that miR30a-targeted MO injection into 1-4 cell stage embryos leads to an increase in Six1 protein expression, which is lost if miR30a is co-injected with miR30a MO. Further, *six1a* mRNA injection does result in increased levels of Six1 protein expression.