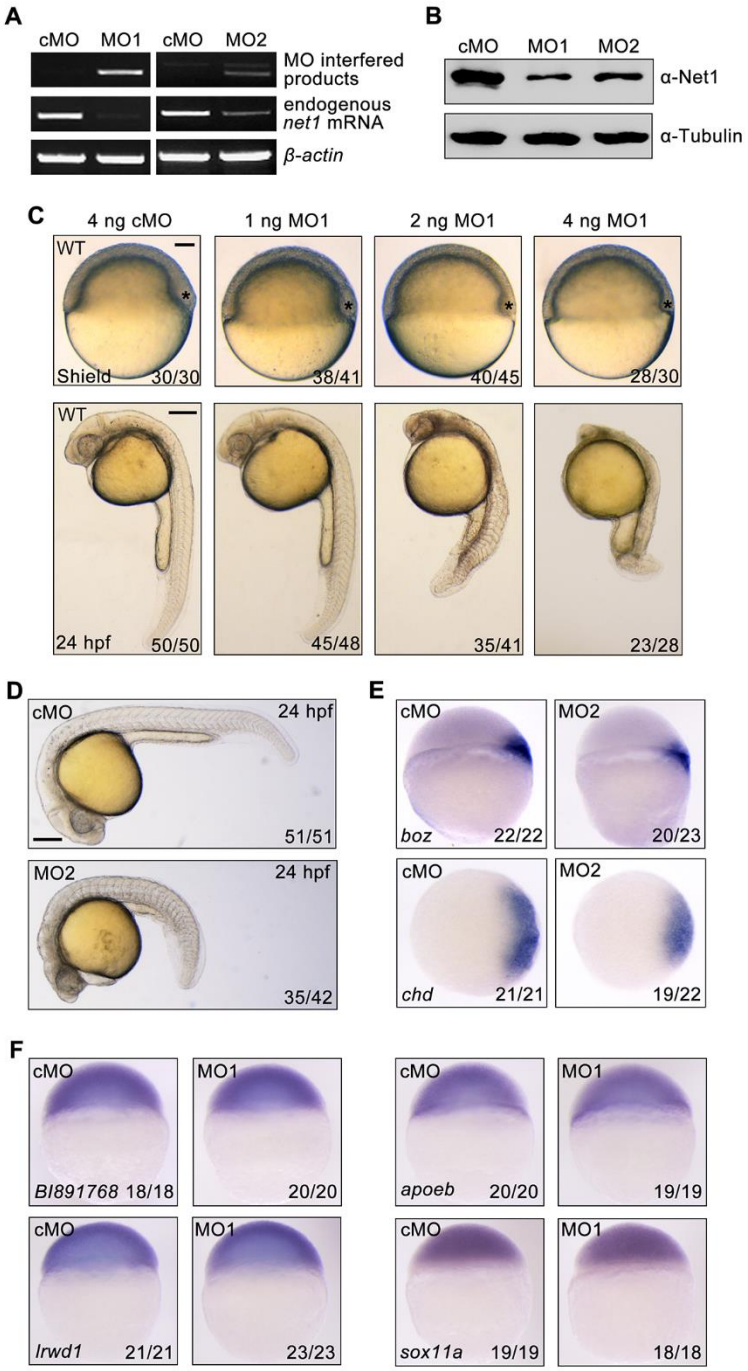

Figure S2 Knockdown of *net1* by MO injection blocks zebrafish dorsal axis formation. (A and B) Efficiency of *net1* MO1 and MO2 in knocking down endogenous net1 expression. Embryos were injected with 4 ng net1 cMO, 4 ng *net1* MO1 (targeting the splicing region between exon2 and intron2), 4 ng *net1* MO2 (targeting the splicing region between exon3 and intron3), respectively. All embryos were harvested at 30%-epiboly stage for semi-quantitative

RT-PCR (A) or western blot analysis (B). Note that the specific splice interference MO mRNA products are only detected in morphants. (C) Morphological defects in *net1* MO1 injected embryos at shield stage (upper panels: lateral views with dorsal to the right; scale bar, 100 μm) and 24 hpf (lower panels: lateral views with anterior to the top; scale bar, 200 μm). Note that the dorsal organizer becomes smaller in *net1* MO1 injected embryos (black star). The ratios of affected embryos are indicated. (D) Morphological defects in *net1* MO2-injected embryos. Images of embryos injected with 4 ng *net1* cMO or MO2 were acquired at 24 hpf. All embryos are shown in lateral views with the anterior side at the left. Scale bar, 200 μm. (E) Expression of dorsal marker genes in *net1* morphants. Embryos were injected with 4 ng *net1* cMO or MO2 at one cell stage and harvested for *in situ* hybridization at sphere stage. (F) The expression of indicated early zygotic genes at sphere stage in the embryos injected with 4 ng cMO or 4 ng *net1* MO1.