



Figure S13 *net1* null alleles exhibit no obvious developmental defects. (A) Generation of *net1* mutants using Cas9/gRNA system. Two deletion mutants named *net1* Δ 20 and *net1* Δ 53 were identified from the F1 embryos of founder fish. (B and C) The expression of *net1* transcripts

and proteins were examined by *in situ* hybridization (B) and western blot analysis (C) in sphere stage wild-type embryos and *net1* homozygous mutants. (D) Phenotypes at 24 hpf of wild-type embryos and *net1* homozygous mutants. (E) The expression pattern of *boz* and *chd* in sphere-stage wild-type embryos and homozygous *net1Δ53* mutants. Note that compared to wild-type control, *net1Δ20* and *net1Δ53* homozygotes exhibited no obvious morphological defects and showed normal expression patterns of dorsal marker genes. (F and G) The expression pattern of *boz* and *chd* in sphere-stage wild-type and homozygous *net1Δ53* mutant embryos injected with 4 ng MO1 (F) or 1 ng siRNA1 (G). (H) Compactin treatment disrupts the dorsal development of *net1Δ53* mutants. *net1Δ53* mutant embryos were treated with DMSO or 10 μM compactin from 16-cell stage and harvested for *in situ* hybridization at sphere stage. (I) The expression pattern of *boz* and *chd* in sphere-stage *net1Δ53* mutant embryos injected with or without 225 pg *dnpak1* mRNA.