

**Syndecan-4 modulates the proliferation of neural cells and the formation of CaP axons
during zebrafish embryonic neurogenesis**

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Supplementary figure legend

Figure S1. *Syn4* is required for the migration of neural crest.

(A, B): Knockdown efficiency of *syn4* MO at 9 hpf. (A): Zebrafish embryos were injected with 30 pg of pEGFP-N1-*syn4* recombinant plasmid containing the MO-binding site. (B): Green fluorescence was totally abolished by co-injection with 6 ng *syn4* MO. (C-D''): WISH assays of *crestin* (C-C''), *sox10* (D-D'') at 18 somites stage. Arrows indicate the expression of *crestin/sox10* in the morphants are inhibited. (E, F): Quantification of phenotypes produced by *syn4* morphants at 18 somites stage, with or without *syn4* mRNA. Lateral views, dorsal to the top and anterior to the left in C-D''.

Figure S2. Knockdown *syn4* disrupt the muscle patterning of zebrafish.

In situ hybridization for indicated genes. (A-D'') WISH assays of *myod* at 18 somites stage, white bars marks vertical. The morphants and *syn4*-C2 mRNA injected fishes appeared abnormal curvature. (E): Quantification of phenotypes at 18 somites stage. (F-K): Expression pattern of *islet1/islet2a* in different embryos at 18 somites stage.

Figure S3. Knockdown *syn4* disrupt the muscle patterning of zaebrfish.

(A-C'') Fast and slow muscle structures in Tg(Hb9:GFP line) at 18s. Fast and slow muscles in the trunk are stained with phalloidin, which recognizes actin fibers of both muscle types, PMNs in green fluorescence. Phalloidin labeling revealed an impaired muscle structure in morphants. (D-F'') muscle structures in Tg(Hb9:GFP line) at 26 dpf. Phalloidin labeling revealed the fibers are abnormally thick and wavy in morphants. The box regions were magnified.

Figure S4. The C2 domain of *syn4* is crucial for the proliferation of neural cells and the formation of CaP axons

(A): Schematic representation of Syndecan 4 constructs. (B-G''): Expression pattern of *nestin* in different embryos at 24 hpf (B-D) and 48 hpf (E-G''). (H): Embryos at 26 hpf showing PMNs in green fluorescence. The box regions were magnified. Arrows indicate shortened CaP axons and abnormal branches. (I): Statistical results of relative ratio of *nestin* expression level in (E-G''). (J): Quantification of CaP axon branching in (H), the number of axonal branches was significantly higher in *syn4* morphants and *syn4*- Δ C2 mRNA injected embryos than in controls. For each group 84 axons from 14 embryos were scored (mean \pm s.e.m, n=3, ***P<0.001, **P<0.01, *P<0.05, ns= not significant, t-test). Hb: hindbrain, sc: spinal cord.

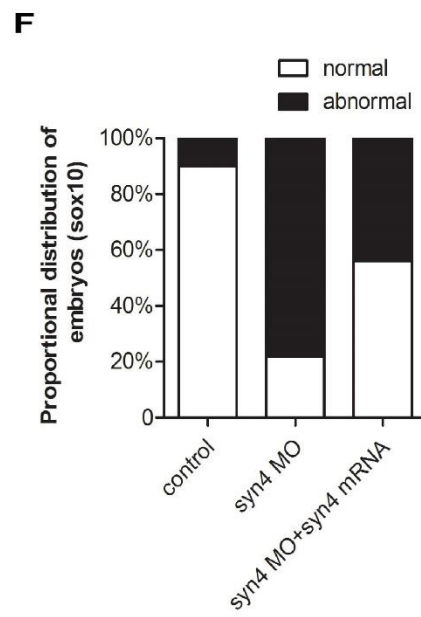
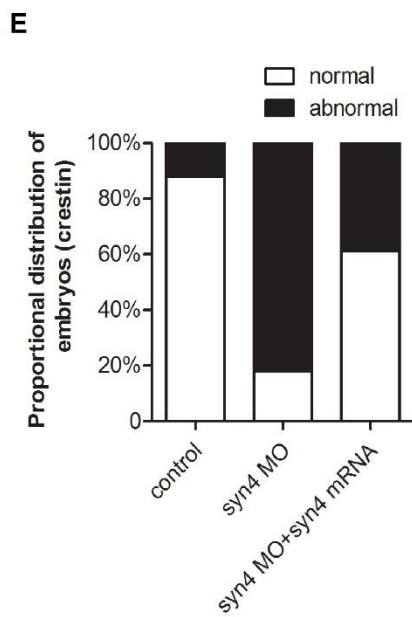
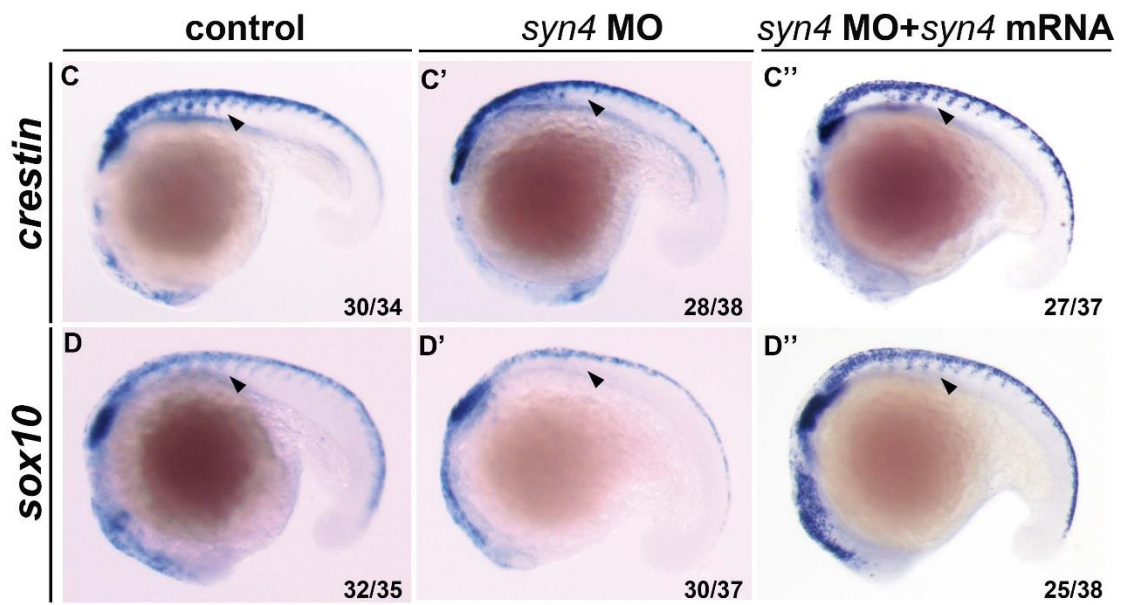
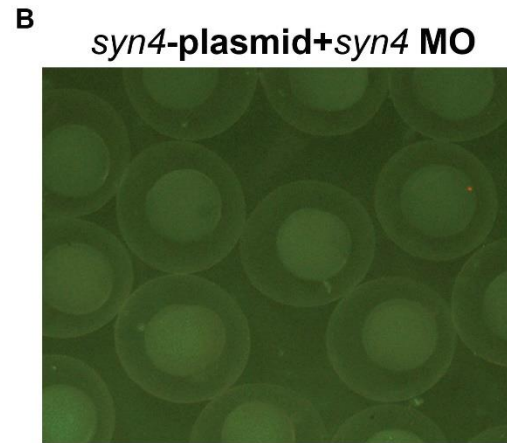
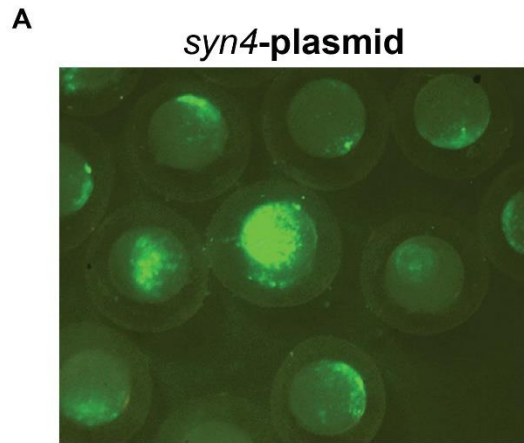
Figure S5. Overexpression of *syn2* is unable to rescue the phenotypes caused by knockdown of *Syn4* functions

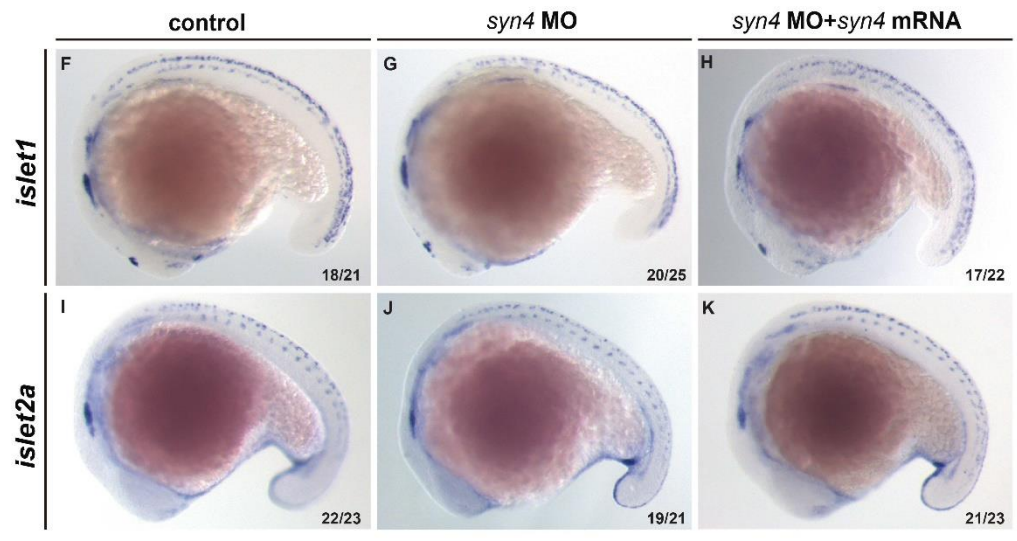
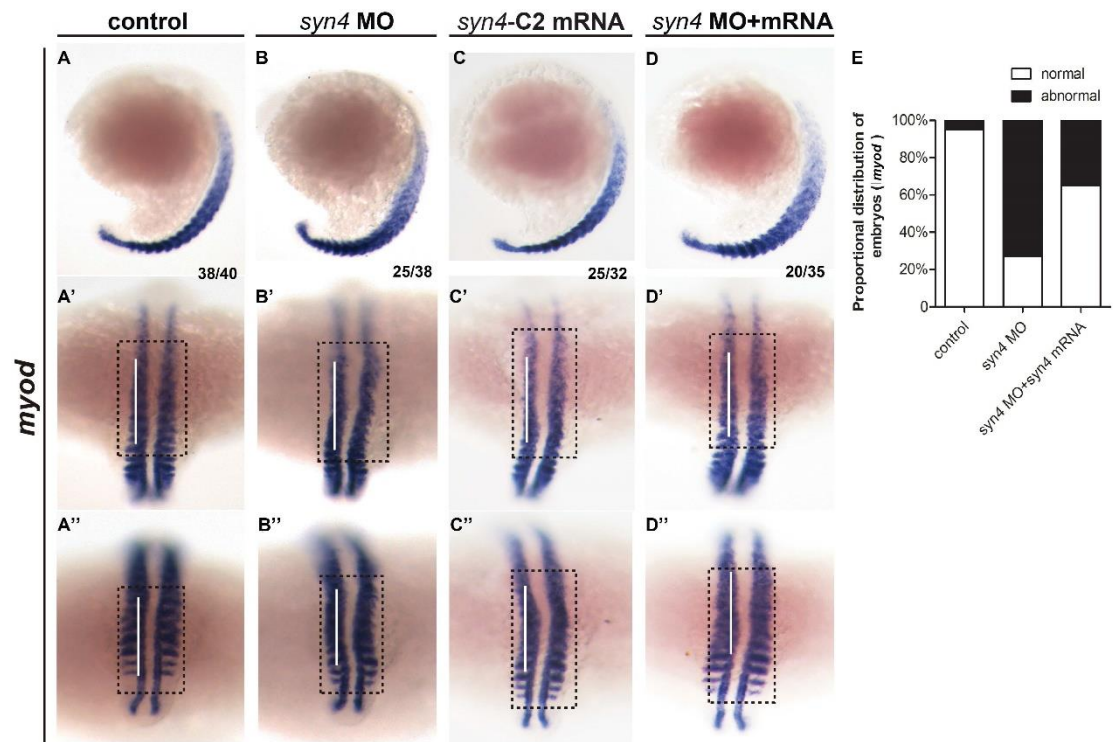
In situ hybridization for indicated genes. (A-F''): WISH assays of *gfap* and *elavl3* in different

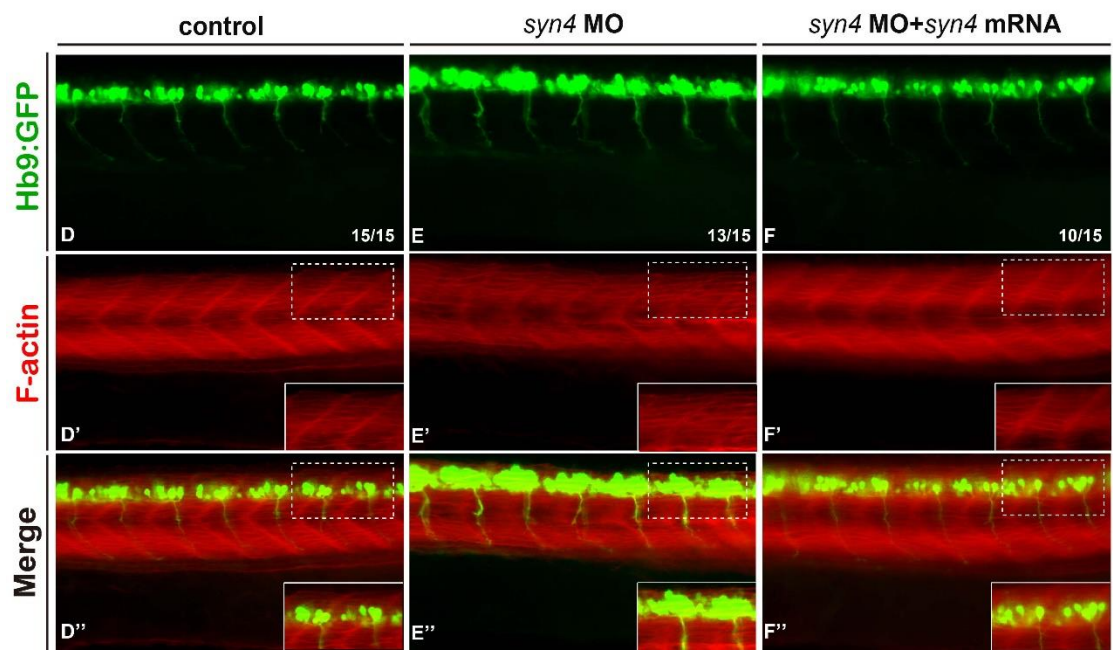
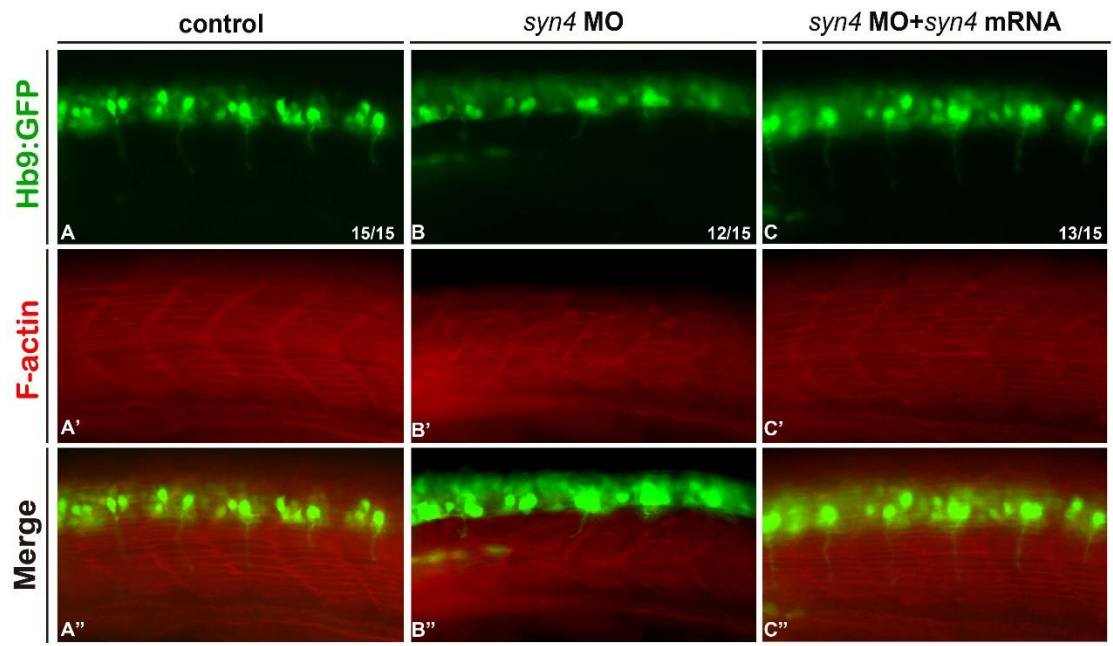
embryos at 48 hpf. The higher expression level of *gfap/elavl3* in morphants at 48 hpf couldn't be rescued by co-injection with *syn2* mRNA. FV: ventricular zone in the forebrain, Hb: hindbrain, Mb: midbrain.

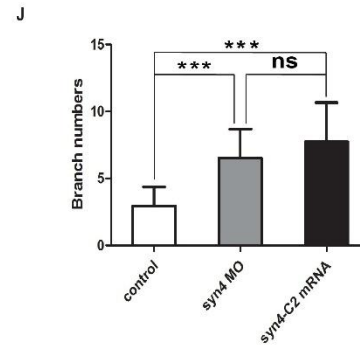
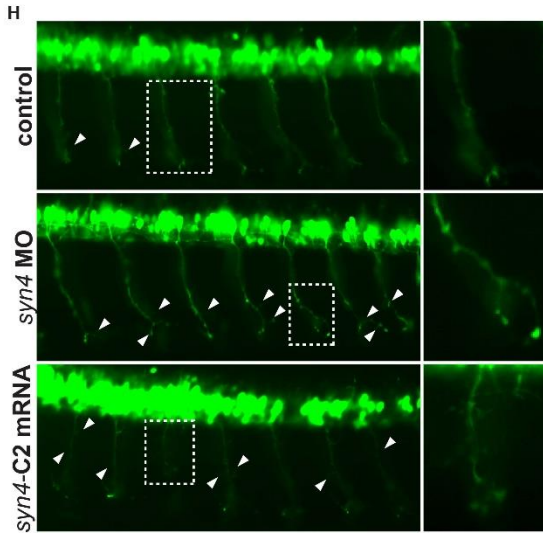
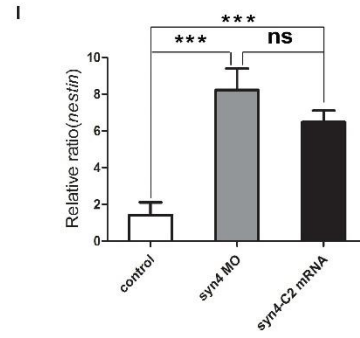
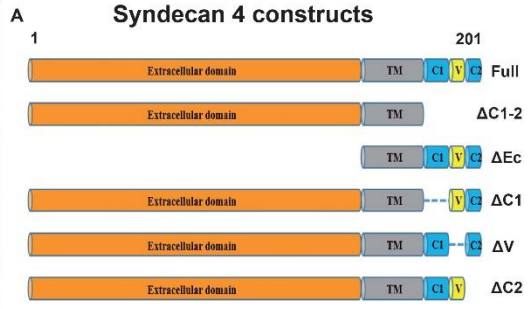
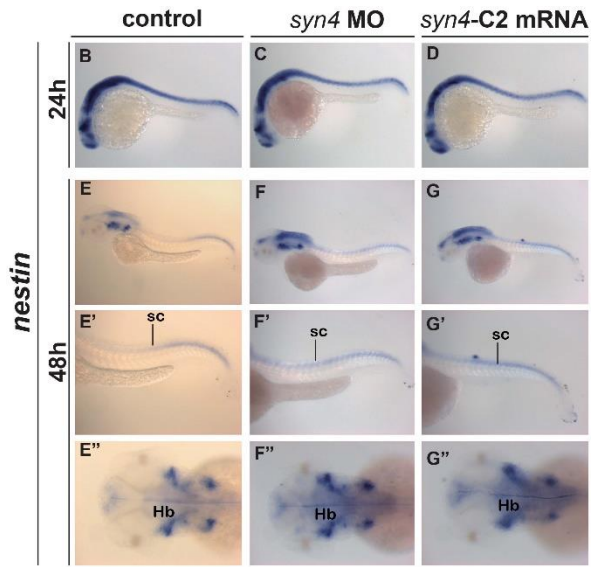
Figure S6. Immunofluorescence staining of H3S10 in zebrafish embryos

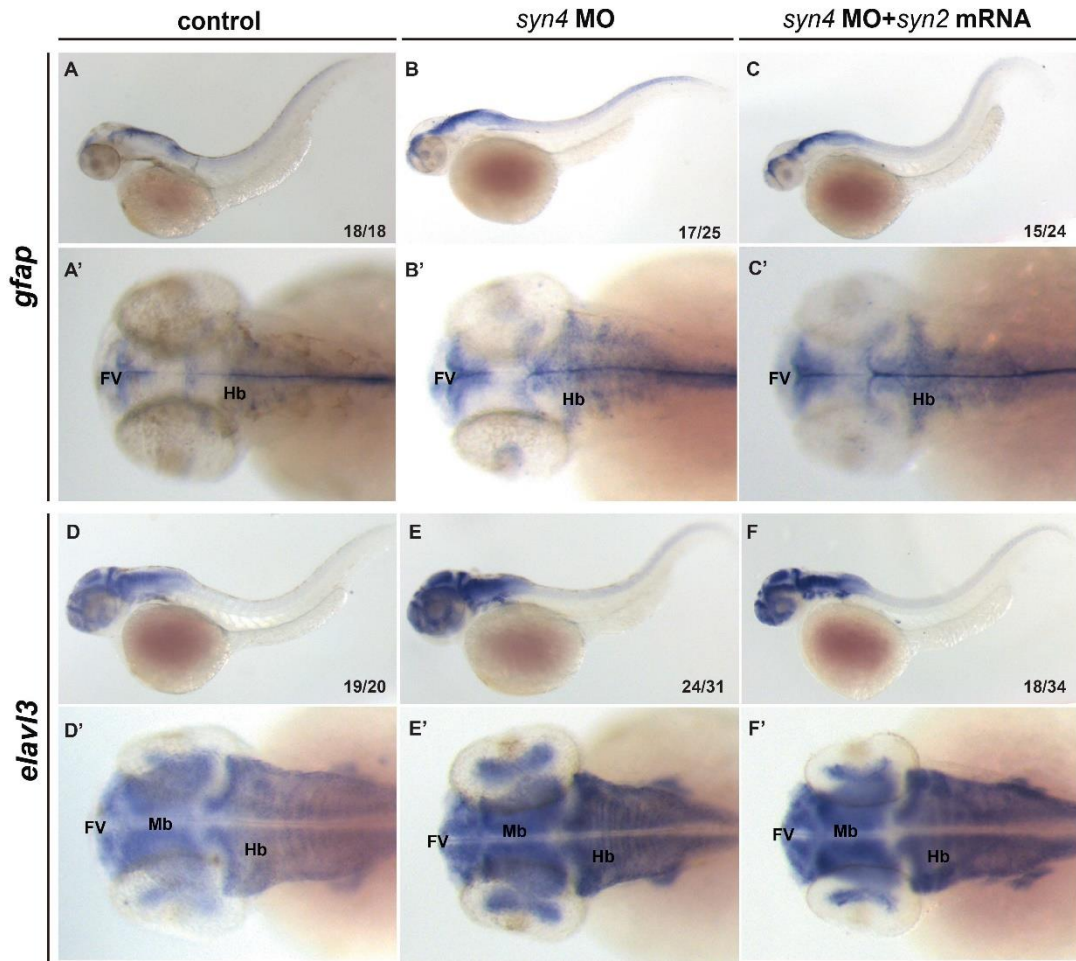
(A-C'): Immunostaining of H3S10 in 48 hpf embryos. (D-F''): Transverse sections of forebrain (Top), midbrain (middle) and hindbrain (below) of different embryos immunostained for H3S10 (green). G: Quantification of H3S10 -positive cells. For each group, 6 embryos are scored (mean \pm s.e.m, n=3, ***P<0.001, **P<0.01, *P<0.05, ns= not significant, Student's unpaired t-test). e: eye, Fb: forebrain, Hb: hindbrain, Mb: midbrain, t: tectum. The dotted boxes mark the brain regions.











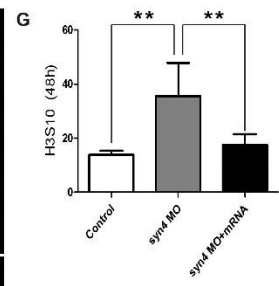
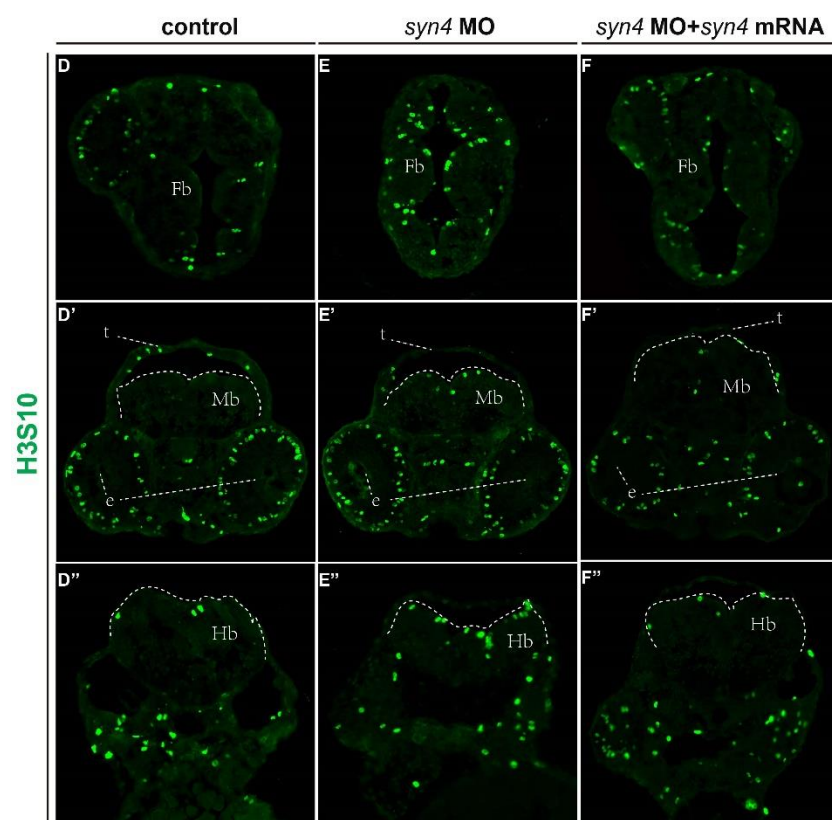
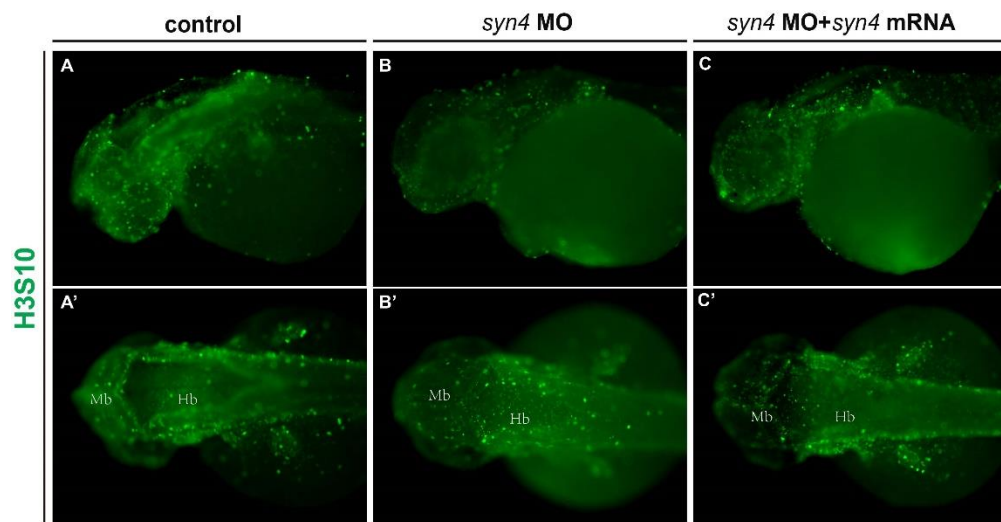


Table S1. Primers for PCR cloning and sqRT-PCR

Sequence	sense-primer	antisense-primer	Vector
<i>syn4</i> coding region (with 5'-UTR)	GGAATTCATTCACCTC GGGCTACTTG	CGGATCCCATGCGTAGATTTCTGT GGTTGG	pEGFP-N1
<i>syn4</i> coding region (without 5'-UTR)	CCGGAATTCATGTTG GTTTTGTCG	CCGCTCGAGTCATGCGTAGATTTTC	pcDNA 3.1+
<i>syn2</i> coding region	CCGGAATTCATGAG GAACTTTTGGAT	TGCTCTAGATTATGCGTAAAACCTC CTT	pcDNA 3.1+
<i>syn4-ΔC1</i>	CGCGGATCCATGTTG GTTTTGTCGGCGGCT	TCATTTTTTGTAGATGGGCGTCTTT CCGAGAACGAAGTGAACGAG	pGEM-T easy
	CGCGGATCCATGTTG GTTTTGTCGGCGGCT	TCATGCGTAGATTTCTGTGGTTGG AGCTTTTTTGTAGATGGGCGT	pcDNA 3.1+
<i>syn4-ΔV</i>	CGCGGATCCATGTTG GTTTTGTCGGCGGCT	CCGGAATTCTCATGCGTAGATTTTC TGTGGTTGGAGCCAGGTCGTAAC TCCTTC	pcDNA 3.1+
<i>syn4-ΔC2</i>	CGCGGATCCATGTTG GTTTTGTCGGCGGCT	CCGGAATTCTCATTTTTTGTAGATG GGCGT	pcDNA 3.1+
<i>HuC</i> sqRT-PCR	AGCGGAACCAGCCT GCCTAA	TGAGCGTGTGATAGCCTTGTCG	
<i>gfap</i> sqRT-PCR	TTCGCCAGCTACATC GAGAAGGT	TTGAGAGTGCCGAGGTCTGAGG	
<i>islet1</i> sqRT-PCR	GCAGCAGCAACCCA ACGACAA	TTGAGCCTGGACCACCTTCAGAA	
<i>islet2a</i> sqRT-PCR	CACGGGTCATACGA GTGTGGTTC	TGGTCCAGGTCGCTCTGTAAGG	
<i>olig2</i> sqRT-PCR	GCAACTCGCTGGAG GAGATGAAG	AAGAGATGCTGCGGTGGAGACT	
<i>slc1a3a</i> sqRT-PCR	ATTATCGCTGTTGAT TGGT	ATTTCTTGGTCTCGTTCTC	
<i>Nestin</i> sqRT-PCR	TGGACTGGAGGTGG CAACATACA	AGGCAGGAAGCAGCAGTGGTT	
<i>gapdh</i> sqRT-PCR	AGTTGTAAGCAATG CCTCCTG	CTGGGATGATGTTCTGACTGG	