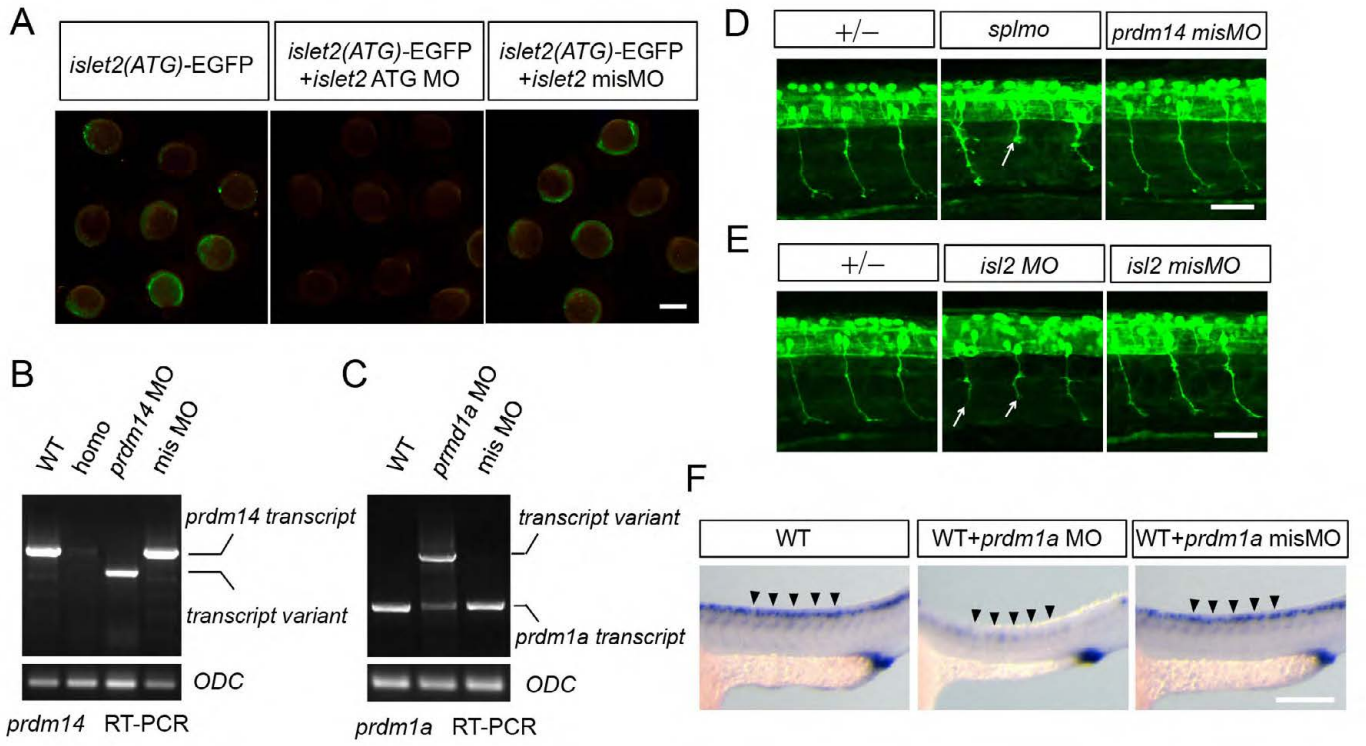


Fig. S1. Morpholinos used in this study are efficient and specific. (**A**) *islet2* MO is sufficient to suppress translation of *EGFP* mRNA containing the MO binding site (*islet2* ATG). *islet2* misMO shows no effect on the EGFP reporter translation. (**B**,**C**) *prdm14* and *prdm1a* splicing MO injections result in reduction of their respective transcripts and appearance of aberrant transcript variants. The control mismatched MOs have no effect. (**D**,**E**) *prdm14* and *islet2* misMO injections have no effects on CaP axon outgrowth. (**F**) *prdm1a* misMO injection affects *islet2* expression in RB. Arrows (D,E) indicate shortened CaP axons; arrowheads (F) indicate RB neurons. Scale bars: 500 μm in A; 50 μm in D,E; 200 μm in F.

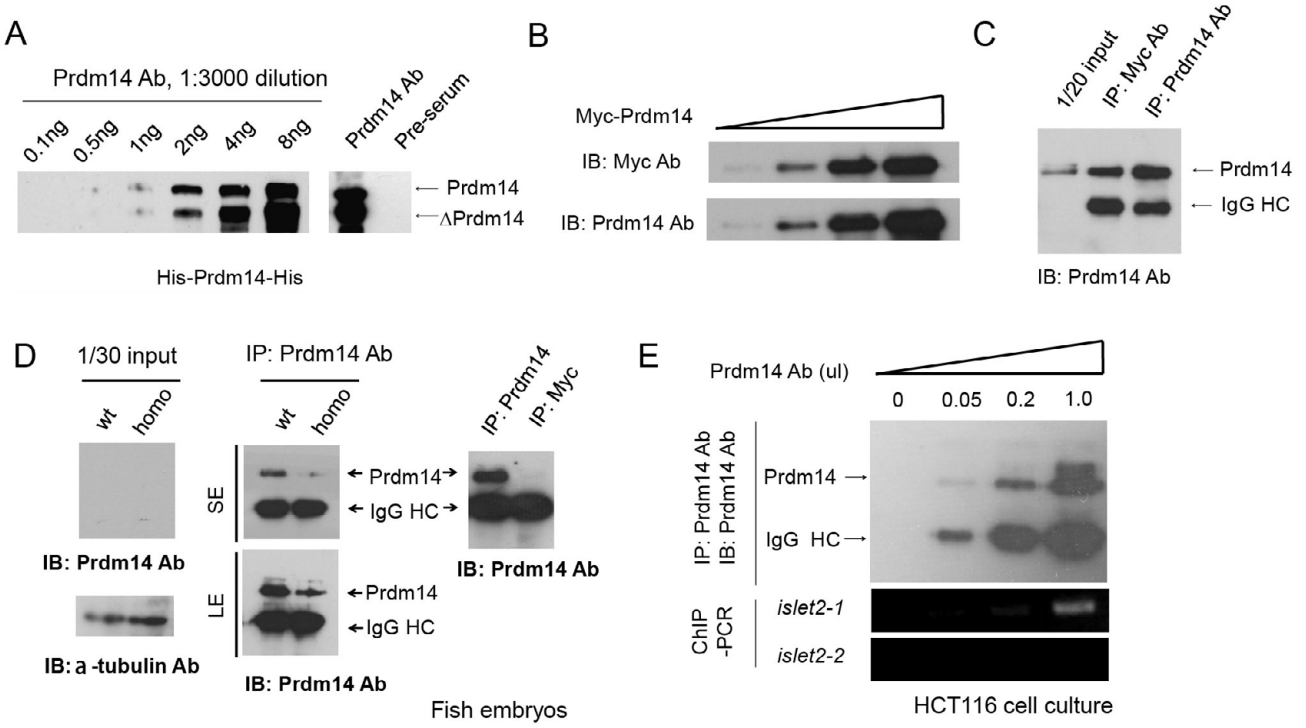


Fig. S2. Purified Prdm14 antibodies can pull down endogenous Prdm14. (**A**) Prdm14 antibodies (Ab) (diluted 1:3000) recognize His-Prdm14-His expressed from BL21 bacteria. (**B**) Prdm14 Ab recognize Myc-Prdm14 expressed from HEK293 cells. The detection efficiency is comparable to a commercialized Myc Ab (MBL, cat. 562). (**C**) Prdm14 Ab pull down Myc-Prdm14 with a comparable or even higher efficiency compared with Myc Ab. (**D**) Prdm14 Ab enrich and pull down endogenous Prdm14 protein from zebrafish embryo lysate (~200 embryos at 24 hpf). SE, short-time exposure; LE, long-time exposure. (**E**) Prdm14 Ab dose-dependently precipitate fragments containing pBS in the ChIP-PCR assay from HCT116 lysate.

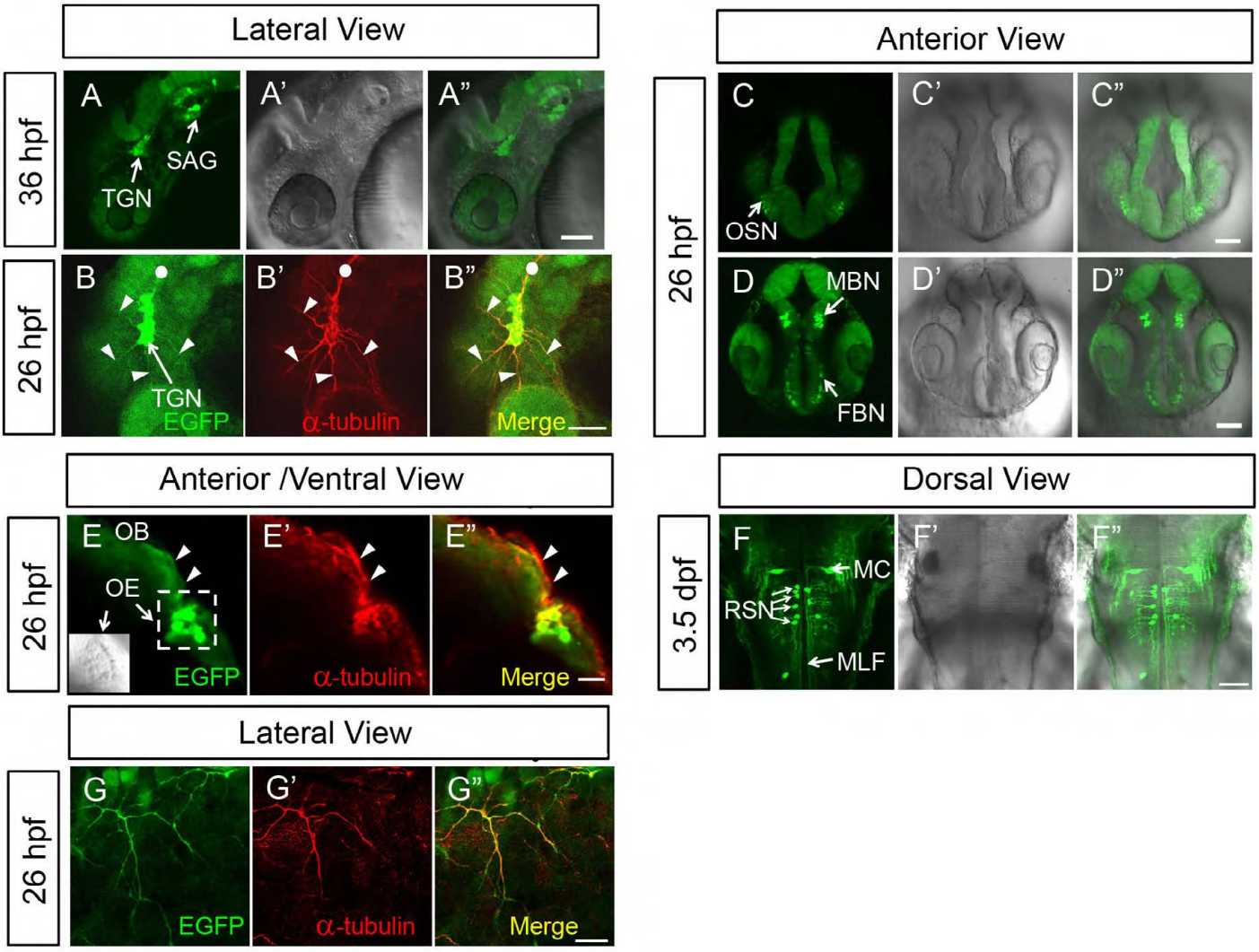


Fig. S3. Expression patterns of the trapped gene in other neurons. (**A-A″**) GFP expression in TGN and SAG in 36-hpf embryos. (**B-B″**) Acetylated α-tubulin expression in TGN in 26-hpf embryos (circle, central axon; arrowheads, peripheral axons). (**C-D″**) GFP expression in OSN, FBN and MBN in 26-hpf embryos. (**E-E″**) Acetylated α-tubulin in OSN in 26-hpf embryos (square, OE; arrowheads, axons projected from OSN to OE). (**F-F″**) GFP expression in MC, RSN and their afferents (MLF) in 3.5-dpf embryos. (**G-G″**) Acetylated α-tubulin in RB in 26-hpf embryos. FBN, forebrain neurons; MBN, midbrain neurons; MC, Mauthner cells; MLF, medial longitudinal fasciculus; OB, olfactory bulb; OE, olfactory epithelium; OSN, olfactory sensory neurons; RSN, reticulospinal neurons; SAG, statoacoustic ganglion neurons; TGN, trigeminal neurons. Scale bars: 50 μm in A-D″,F-F″; 20 μm in E-E″,G-G″.

Fig. S4. Prdm14 protein contains a highly conserved PR domain and a ZF domain. Alignment of zebrafish, mouse and human Prdm14 proteins. The blue box indicates the PR domain sequences and the red box indicates the ZF domain.

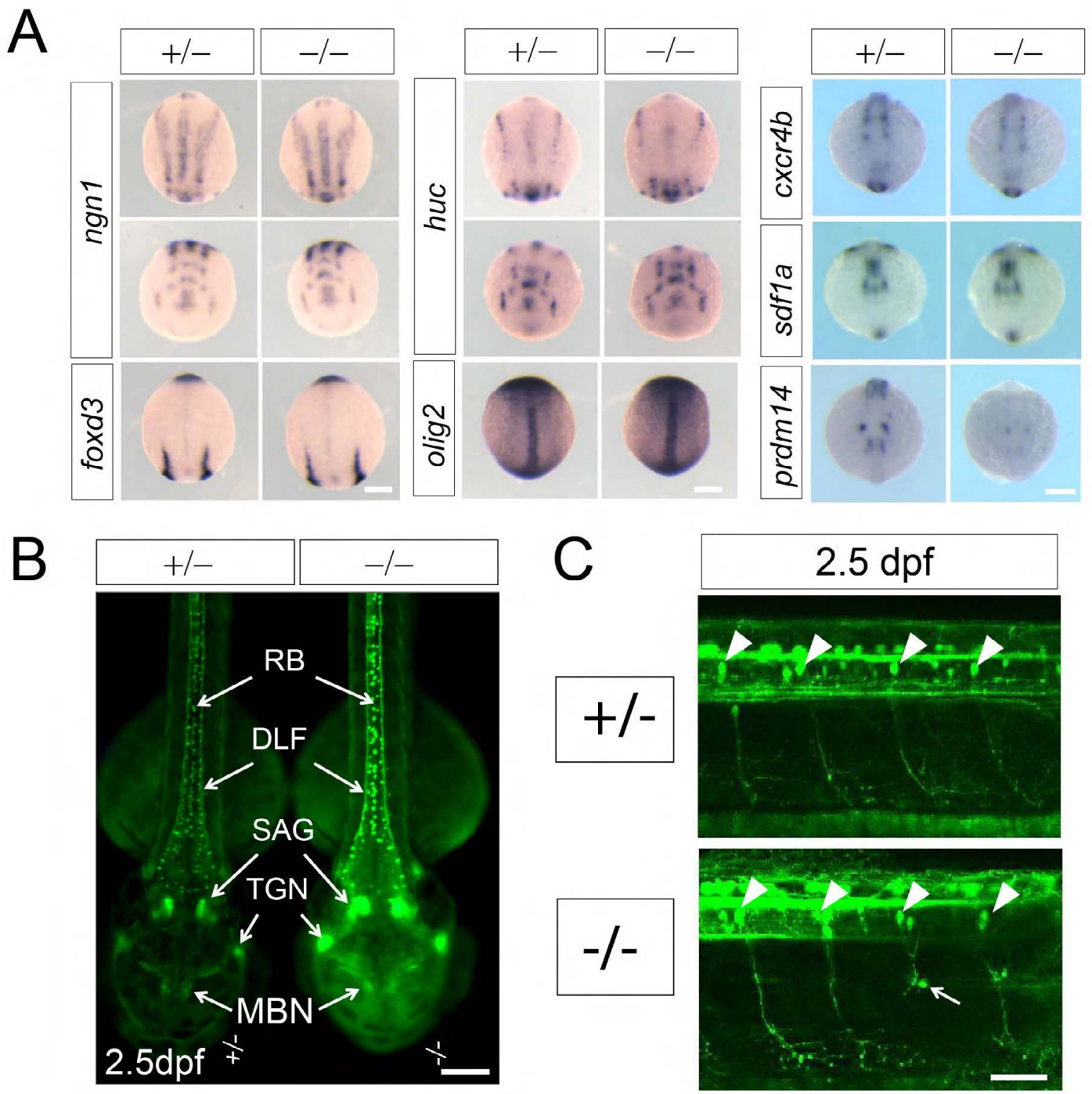


Fig. S5. Reduction of Prdm14 expression may not affect neuron specification. (**A**) Expression of the early neuron markers *ngn1*, *foxd3*, *huc*, *olig2* and *cxcr4b* is not substantially changed in *slg* mutant embryos at the 1- to 3-somite stage. *sdf1a* is used as a control. (**B**) At 2.5 dpf, neuron positions and cell numbers of MBN, TGN, SAG and RB are not greatly affected in *slg* mutant embryos. (**C**) At 2.5 dpf, CaP neurons (arrowheads) are present and the majority of the CaP neurons extend their axons to the ventral targets and form interactions in *slg* mutant embryos; a few shortened CaP axons are indicated by an arrow. Scale bars: 200 μm in A; 50 μm in B,C.

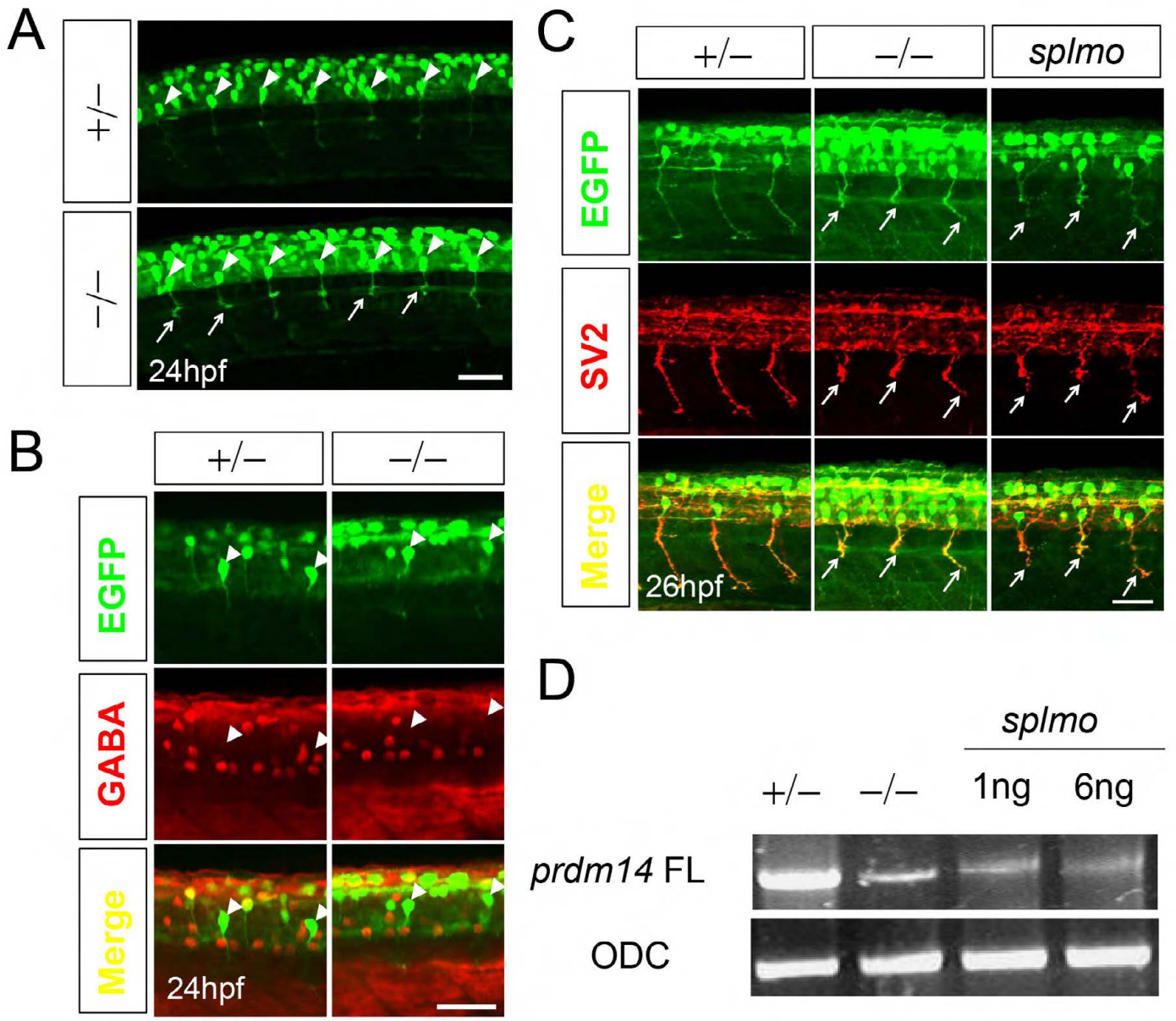


Fig. S6. Reduction of Prdm14 expression may not affect CaP specification. (**A**) Cell body position and cell number of CaP are not significantly changed in homozygous *slg* mutant embryos at 24 hpf, as compared with heterozygous embryos. Arrowheads indicate CaP neurons, arrows indicate shortened CaP axons. (**B**) No ectopic GABA expression in GFP-positive CaP neurons (arrowheads) in *slg* mutant embryos at 24 hpf. (**C**) At 26 hpf, CaP axons stained with the axon-specific marker SV2 show a shortened axon outgrowth phenotype in *slg* mutant and *splmo* morphant embryos. (**D**) In *slg* mutant, full-length *prdm14* is greatly reduced. In *splmo* morphant, *prdm14* reduction is dose dependent and is even more severe than that seen in the *slg* mutant. Scale bars: 50 μm.

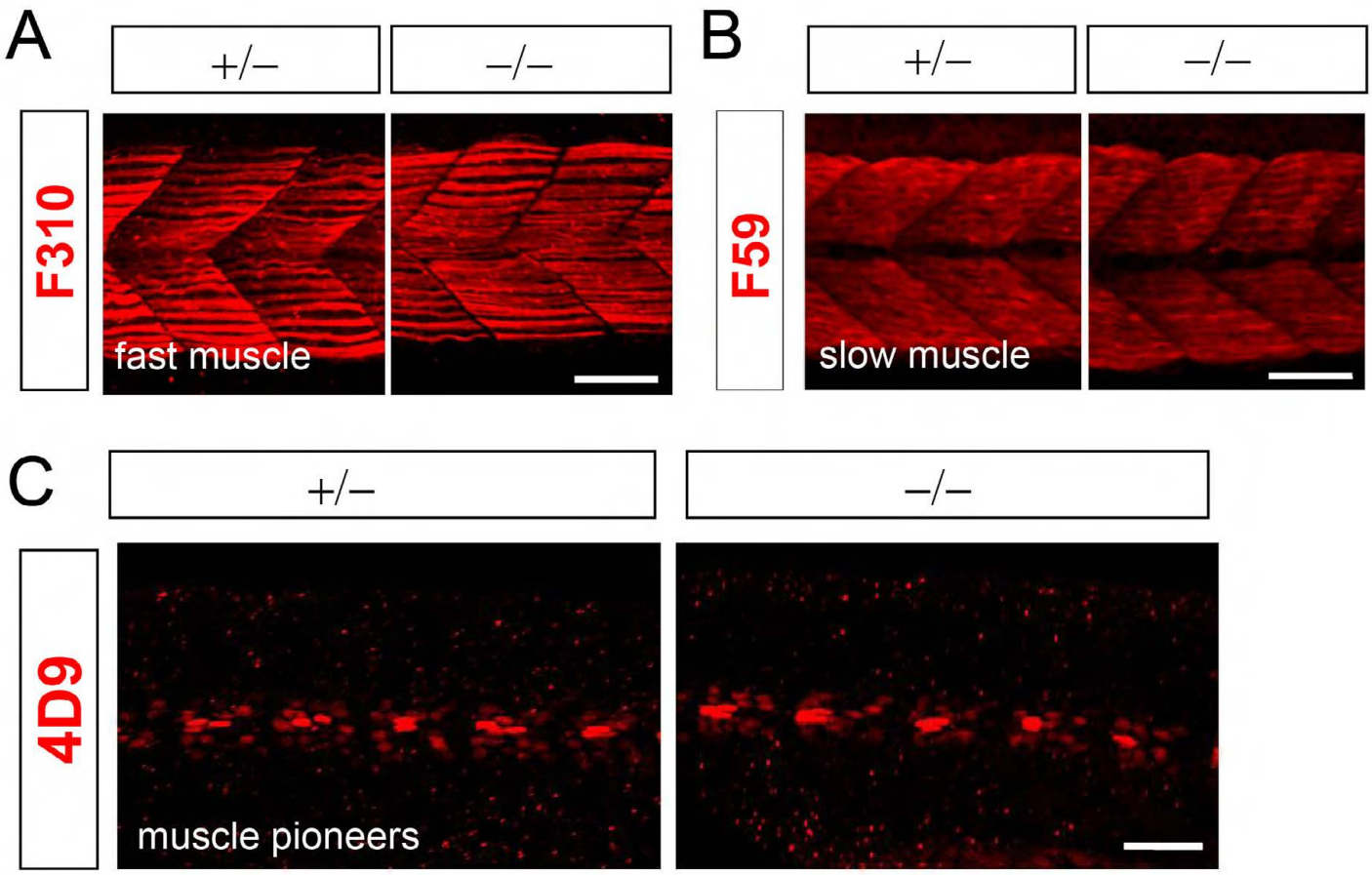


Fig. S7. Reduction of Prdm14 may not affect muscle development. Fast muscle (**A**), slow muscle (**B**) and muscle pioneer (**C**) cells are stained with F310, F59 and 4D9 antibodies, respectively. No obvious difference in muscle development is observed between wild-type and *slg* mutant embryos. Scale bars: 50 μm.

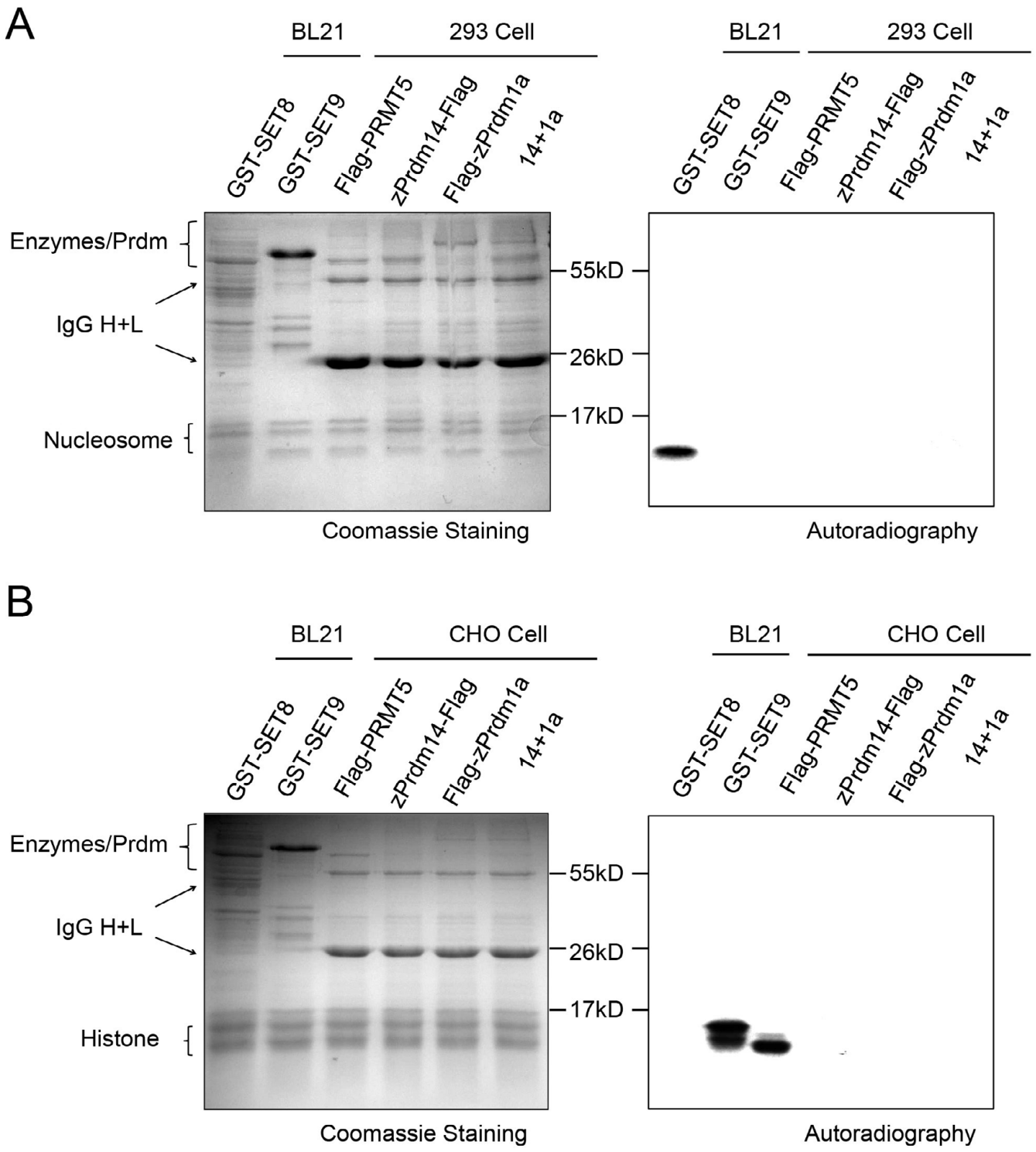


Fig. S8. Prdm14 protein may not possess methyltransferase activity. (**A**) Prdm14 expressed in HEK293T cells shows no methyltransferase activity with recombinant nucleosomes as substrate. (**B**) Prdm14 expressed in CHO cells shows no methyltransferase activity with histone octamers as substrate. As controls, SET8 methylates H3 in nucleosomes, SET9 methylates H3 and PRMT5 methylates H4 in the histone octamer.

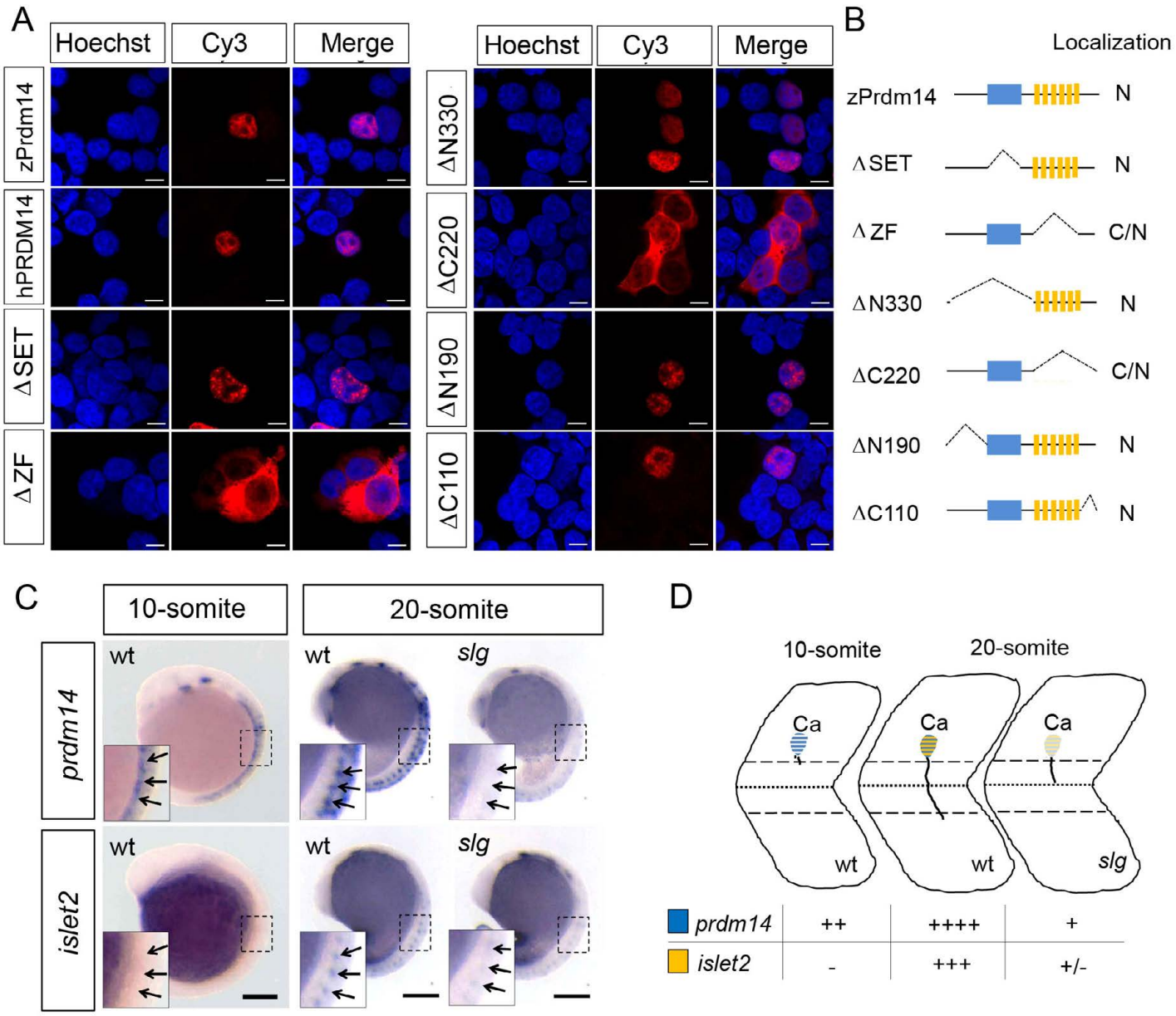


Fig. S9. Prdm14 is a nuclear protein and *prdm14* is expressed before *islet2* in zebrafish embryos. (**A**) Zebrafish Prdm14 is localized to the nucleus, similar to human PRDM14. The localization is ZF domain dependent. (**B**) The localization of different zebrafish Prdm14 isoforms is summarized. N, nucleus localized; C, cytoplasm localized. (**C**) *prdm14* is expressed earlier than *islet2* in CaP (arrows) at the 10-somite stage. Prdm14 downregulation causes decreased *islet2* expression at the 20-somite stage. (**D**) Diagram illustrating the results in C. Scale bars: 10 μm in A; 200 μm in C.

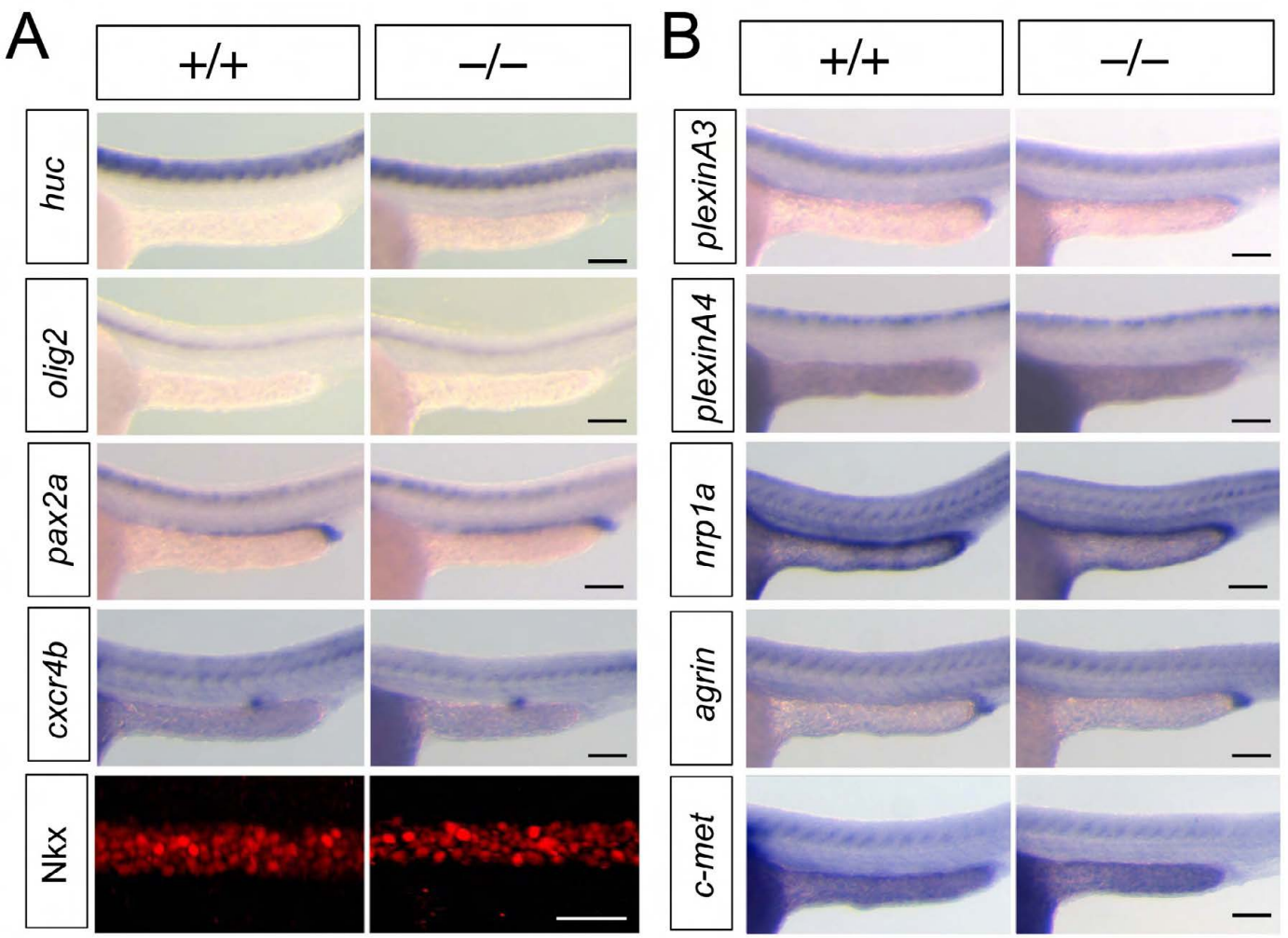
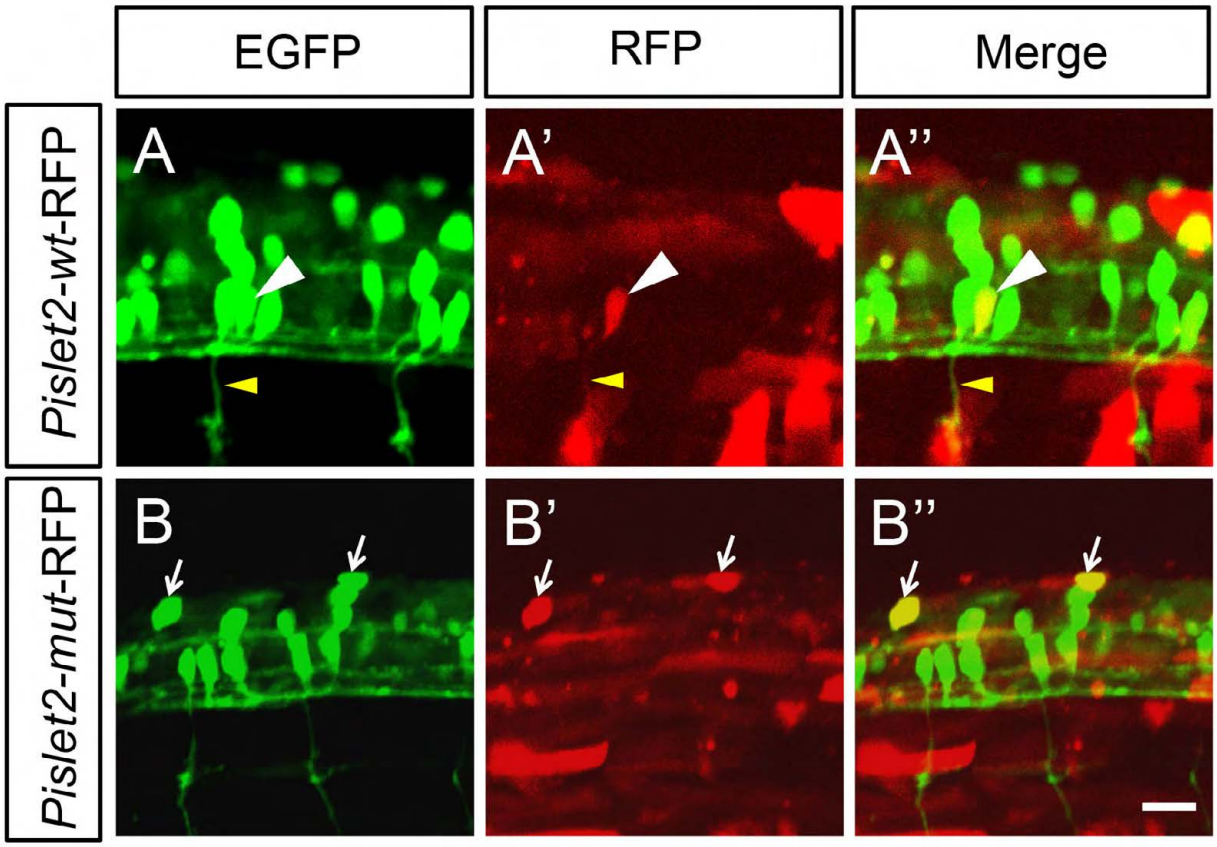


Fig. S10. Neuronal transcription factors and effectors are not substantially affected in the *slg* mutant. (**A**) Transcription factors involved in neuron development, including *huc, olig2*, *pax2a* and Nkx6.1, are not greatly changed in *slg* mutant embryos. (**B**) Effectors that might function downstream of Prdm14 in CaP axon outgrowth, including *cxcr4b*, *nrp1a*, *plexin 3A*, *plexin 4*, *agrin* and *c-met*, are not greatly changed in *slg* mutant embryos. However, *c-met* may be slightly downregulated in the mutant. Scale bars: 100 μm.



C

	Transient RFP Expression Pattern	
	RB positive	CaP positive
Pislet2-wt-RFP (N=27)	n=31, N=12	n=4, N=4
Pislet2-mut-RFP (N=36)	n=50, N=18	N=0

Fig. S11. The Prdm14 binding site (BS) may be required for *islet2* expression in CaP. (**A-A″**) The *islet2* promoter with wild-type BS drives RFP expression in CaP (white arrowheads, cell body; yellow arrowheads, axon). (**B-B″**) A promoter with a mutated BS does not affect RFP transgene expression in RB (arrow), but no longer drives RFP expression in CaP. (**C**) Summary of A and B. N, number of embryos; n, RFP-positive RB or CaP neurons. Scale bar: 20 μm.

Table S1. Sequences of *bcl2* splicing acceptor and bovine growth hormone poly(A) and pause site

Name	Sequence (5′-3′)	Other information
***bcl2* splicing acceptor (*bcl2* SA)**	GGGCCCTGACCCTTATCCGCTCAATCTGTA ACAATGCAGATAAAAGAAAATGAGTAACT GTATAACTAAATACTGTATAATTAGATGCT GTCAGTACACCAGTATTTTACATTTGAGTG TCTATGCAAACATAAATAGGCTAAATCAT AGAACCATACTTTATTTAAAAAAATACTTT ATTTATATATAATTATTTAAATATTTGTGA GAATTATCACAGTAATGTTGAGGCAAATC AAATCTAATCAAAGATGCTGTAAGACTGT AATAACAACCCATTTTACCTTAATTCATTT AGCAGTTTCATGCACCATAGACCGCAGGG GCAAGCAAAGGGTATAAAATAGATACATA CATAGGAAATTTGCTGCAAGTTTGGTGGTC ATTCCACAAACAAACAAACATTTAATTTG ATTTATTTGGCATTTATTATCCATGCTTTGC TATTTTCACTAGTGCAATAATGTGATTTCT AATTGTCTGCTCCTAATACCCTTCTGTTTCT CTTTCAGGATGCCTTCGTGGAGATGTACGG TCAGCAGAGAGACTCTGTGTTCCACCCGTT TTCCATGG	Zebrafish *bcl2* splicing acceptor was cloned into T2AL200R150 with *Apa*I/*Nco*I to replace *EF1a* promoter and second intron of rabbit β-globin
Bovine growth hormone poly(A) and pause site (bGHpA.PS)	ATCGATCGACTGTGCCTTCTAGTTGCCAGC CATCTGTTGTTTGCCCCTCCCCCGTGCCTT CCTTGACCCTGGAAGGTGCCACTCCCACTG TCCTTTCCTAATAAAATGAGGAAATTGCAT CGCATTGTCTGAGTAGGTGTCATTCTATTC TGGGGGGTGGGGTGGGGCAGGACAGCAA GGGGGAGGATTGGGAAGACAATAGCAGG CATGCTGGGGATGCGGTGGGCTCTATGGC TTCTGAGAATTCAACATACGCTCTCCATCA AAACAAAACGAAACAAAACAAACTAGCA AAATAGGCTGTCCCCAGTGCAAGTGCAGG TGCCAGAACATTTCTCTATCGAT	Bovine growth hormone poly(A) and pause site were cloned upstream of original SV40 poly(A) with *Cla*I/*Cla*I

Table S2. Mismatched MOs and primers

Mismatched MO	**Sequence (mutations underlined)**
prdm14 misMO	TCATTGTTGGAGCAACGTGTGGTGG
islet2 misMO	GATTATGCACGATAGAGGACGGTTA
prdm1a misMO	TGCTGTGATACGTCTTTCGAGTGTG

Primers	**Sequence (5′-3′)**
Tail-PCR	
LAD1-1	ACGATGGACTCCAGAGGC(G/C/A)N(G/C/A)NNNGGAA
LAD1-2	ACGATGGACTCCAGAGGC(G/C/T)N(G/C/T)NNNGGTT
LAD1-3	ACGATGGACTCCAGAGGC(G/C/A)(G/C/A)N(G/C/A)NNNCC AA
LAD1-4	ACGATGGACTCCAGAGGC(G/C/T)(G/A/T)N(G/C/T)NNNCG GT
AC1	ACGATGGACTCCAGAG
T3-1	CTCTAGATCAGATCTAATACTCAAGTACAA
T3-2	ACGATGGACTCCAGTCCGGCCACTCAAGTAAGATTCTAG CCAGATACTT
T3-3	CCTAAGTACTTGTACTTTCACTTGAGTAA
T5-1	GACTGTAAATAAAATTGTAAGGAGTAAAAAGTACT
T5-2	ACGATGGACTCCAGTCCGGCCGTACTCAAGTAAAGTAAA AATCCCCAAAAA
T5-3	CAAGTAAAATTACTCAAGTACTTTACACCT
***prdm14* genotyping**	
*prdm14*G T up	GAGGCTTCATTAATGGTGACC
*prdm14*G T down	CAACAATATGCAGGTCAGACAC
*mutant*G T down	CGGTCTATGGTGCATGAAACT
P1, P2, P3 (see Fig. 3)	
P1-up	ACACCAGACCTCTTTTCATC

P1-down	ATGTGTGTGCGAAGGATGCT
P2-up	ATGTCGGTTTCTCTCTCCAG
P2-down	TTAGTTCCAGGGTCTGTACTC
P3-up	ACACCAGACCTCTTTTCATC
P3-down	GCCTGCTATTGTCTTCCCAA
Cloning mRFP, EGFP, *islet2*, *prdm14* into DEST394 destination vector (containing *mnx1-3_125bp* promoter) by GATEWAY system	
mRFP attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTGGATCCATG GCCTCCTCC
mRFP attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTTTAGGCGCCG GTGGAGTG
EGFP attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTGCCACCATG GTGAGCAAG
EGFP attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTTTATCTAGAT CCGGTGGA
Prdm14 attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTGCAGGATCC ACCATGGCT
Prdm14 attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTTTAGTTCCAG GGTCTGTACTC
Iset2 attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTGCAGGATCC ACCATGGCT
Iset2 attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTTCACGTCTCC ACGGGACTG
Antisense probes (SP6 promoter sequence is in bold)	
olig2 up	GAGTGAACTGGATAGCCTTA
olig2 down	**GATTTAGGTGACACTATAG**TGGTGGCTTCTCAAAGTTCT
pax2a up	CGACCTCAGTCGATTATCTT
pax2a down	**GATTTAGGTGACACTATAG**AATCCCTCTGACCATTCAGA
huc up	ATGGAAACTCAGGTGTCCAA
huc down	**GATTTAGGTGACACTATAG**GATGACCTTGACGTTTGTGA
c-met up	GCTTCCATCCTAATCATCCT
c-met down	**GATTTAGGTGACACTATAG**TCGCTCAGAGTAAATGCACT

mnx up	CGAGGCGTTAATCTGTTTGT
mnx down	**GATTTAGGTGACACTATAG**TCGCTCAGAGTAAATGCACT
agrin up	TACCTGAAAGGCAAGACCAT
agrin down	**GATTTAGGTGACACTATAG**TGGCGTTGAACTTACAACCA
plexinA3 up	AAGGTCTGGAATCATGAGGT
plexinA3 down	**GATTTAGGTGACACTATAG**TCAGTGTCATTGGAGCACAT
plexinA4 up	AAGGTCTGGAATCATGAGGT
plexinA4 down	**GATTTAGGTGACACTATAG**TACTCATCTCCATCCCATCA
Testing splicing MO	
prdm14 mo-up	ACACCAGACCTCTTTTCATC
prdm14 mo-down	ATGTGTGTGCGAAGGATGCT
prdm1a mo-up	GTCACTTACCATCTGGACTA
prdm1a mo-down	GGTTCTTGCAGCACATCTTT
Cloning *islet2* ATG to upstream of EGFP	
islet2 ATG up	GATCTCTGTAGCCTTCTGCCTTAACCCTCCTGTATGGTGG ATATTCTACC
islet2 ATG	CATGGGTAGAATATCCACCATACAGGAGGGTTAAGGCAG AAGGCTACAGA