

Figure S1

**Fig. S1. Knockdown of *jag2b* in zebrafish embryos**

(A) Schematic of genomic loci (upper panel) and mRNAs (lower panel) of *jag2b*. Four different gRNA sequences were designed in the exons shown in red in the upper panel. The two different primer sets shown in blue arrows in the lower panel were used for qRT-PCR analysis in sgRNA-injected embryos. (B) Relative expression of *jag2b* in wild type and *jag2b*<sup>sgRNA</sup> embryos. Two different primer sets were used. Error bars, s.d. (C) Schematic of *jag2b* mRNA. The recognition site of the *jag2b* MO is shown as a red bar. (D) Expression of *runx1* in the DA of wild type or *jag2b* morphants. Black arrowheads denote *runx1* expression in the DA. Right panel shows the phenotype distribution of embryos exhibiting "high", "middle", or "low" *runx1* expression in each type. Bars, 100 µm (D).

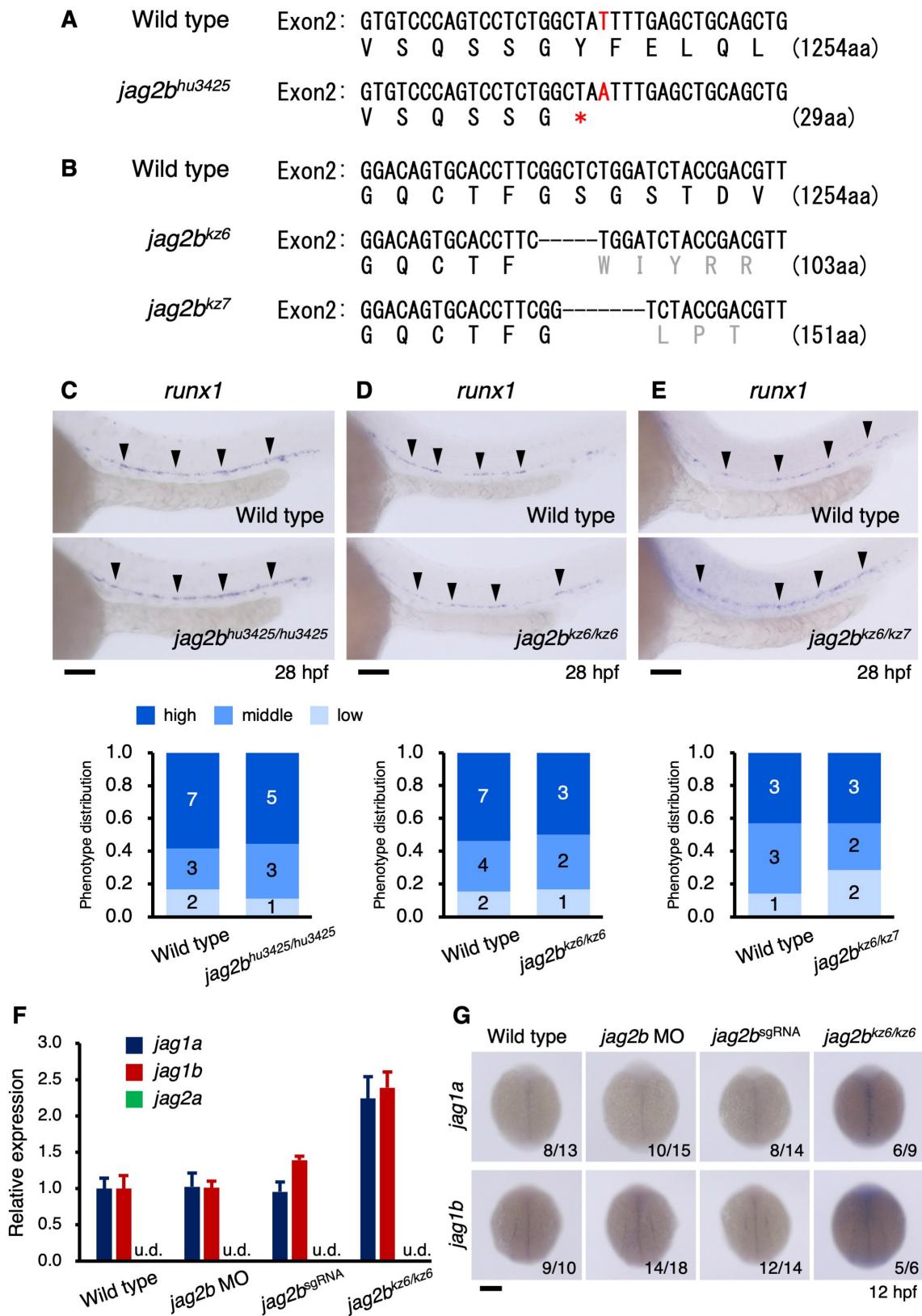


Figure S2

**Fig. S2. *jag2b* mutant embryos did not recapitulate *jag2b*<sup>sgRNA</sup> embryos or *jag2b* morphants (A, B)** Schematic diagram of the genomic sequence of *jag2b* in wild type, *jag2b*<sup>hu3425</sup>, *jag2b*<sup>kz6</sup>, or *jag2b*<sup>kz7</sup>. A point mutation or small deletion in exon 2 of *jag2b* was observed in each mutant allele. **(C-E)** Expression of *runx1* in the DA of wild type, *jag2b*<sup>hu3425/hu3425</sup>, *jag2b*<sup>kz6/kz6</sup>, or *jag2b*<sup>kz6/kz7</sup> embryos. Black arrowheads denote *runx1* expression in the DA. Lower panels show the phenotype distribution of embryos exhibiting "high", "middle", or "low" *runx1* expression in each type. **(F)** Relative expression of *jag1a*, *jag1b*, and *jag2a* in wild type, *jag2b* MO, *jag2b*<sup>sgRNA</sup>, and *jag2b*<sup>kz6/kz6</sup> embryos at 24 hpf. Error bars, s.d.; u.d., undetected. **(G)** Expression of *jag1a* and *jag1b* in wild type, *jag2b* MO, *jag2b*<sup>sgRNA</sup>, and *jag2b*<sup>kz6/kz6</sup> embryos. Bars, 100 μm (C-E); 200 μm (G).

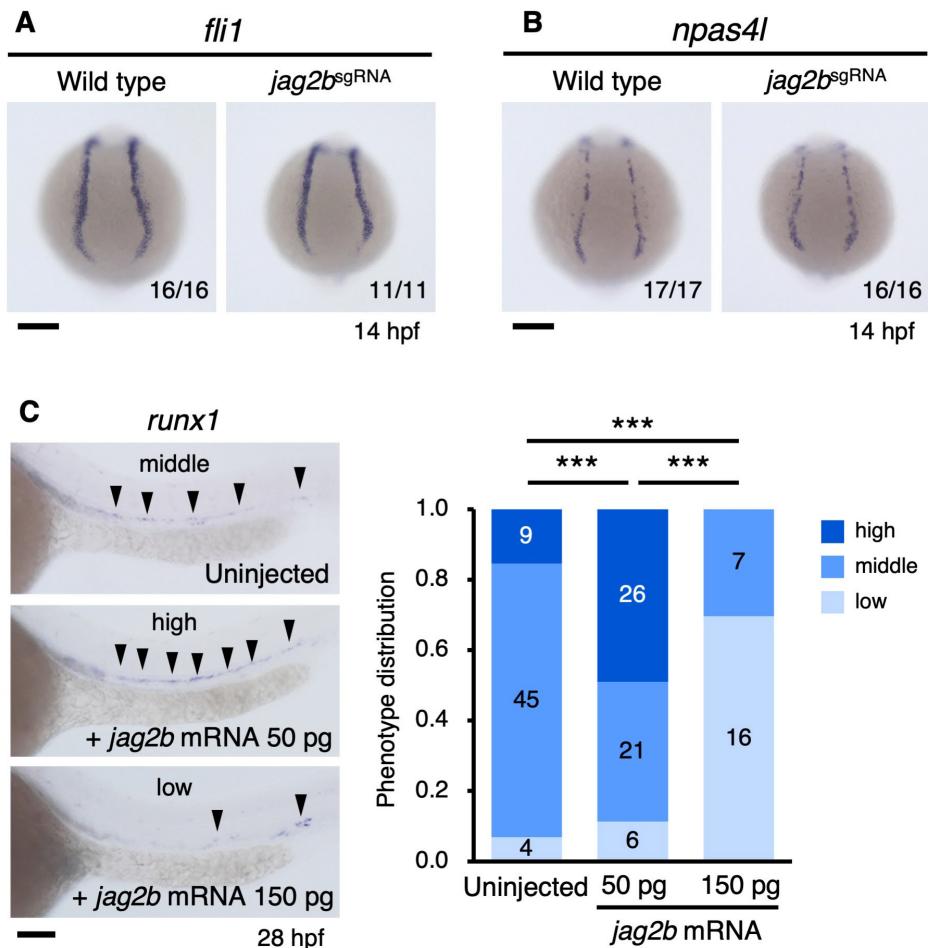


Figure S3

**Fig. S3. Injection of *jag2b* mRNA altered *runx1* expression**

(A, B) Expression of *fli1* and *npas4l* in the PLPM of wild type or *jag2b*<sup>sgRNA</sup> embryos. (C) Expression of *runx1* in the DA of embryos uninjected or injected with 50 pg or 150 pg of *jag2b* mRNA. Arrowheads in left panels denote the expression domain of *runx1*. Black arrowheads denote *runx1* expression in the DA. Right panel shows the phenotype distribution of embryos exhibiting "high", "middle", or "low" *runx1* expression in each type. \*\*\* $p < 0.001$ ; Bar, 200  $\mu\text{m}$  (A, B); 100  $\mu\text{m}$  (C).

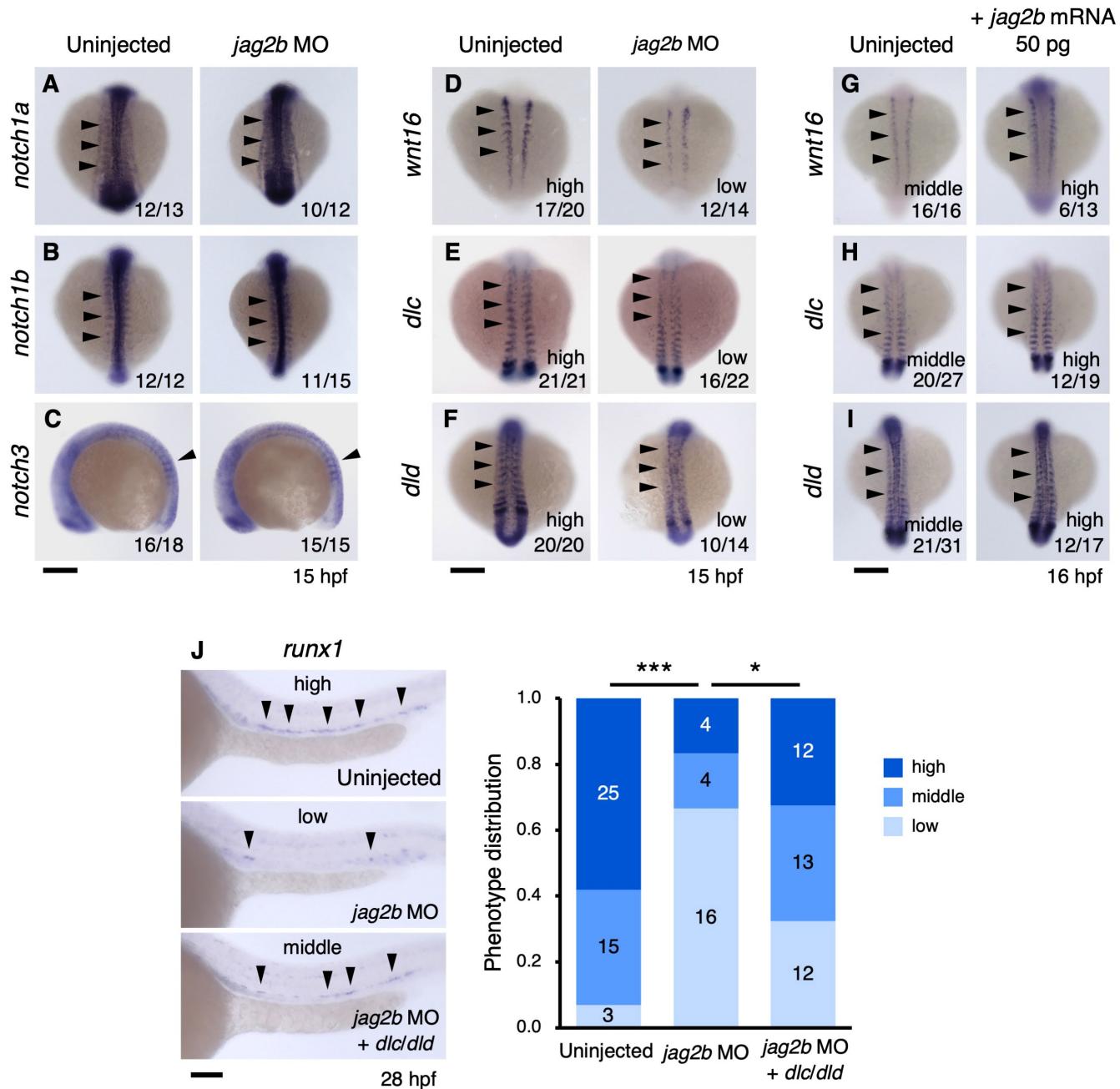


Figure S4

**Fig. S4. Jag2b regulates the Wnt16 – Dlc/Dld signaling axis**

(A-F) Expression of *notch1a*, *1b*, *3*, *wnt16*, *dlc*, and *dld* in embryos uninjected or injected with *jag2b* MO. Black arrowheads denote expression of each gene in the somite. (G-I) Expression of *wnt16*, *dlc*, and *dld* in the somite of uninjected or injected with *jag2b* mRNA. (J) Expression of *runx1* in the DA of embryos uninjected or injected with *jag2b* MO with or without *dlc* and *dld* mRNA. Right panel shows the phenotype distribution of embryos exhibiting "high", "middle", or "low" *runx1* expression in each type. Black arrowheads denote the expression domain of each gene in the somite (A-I) or DA (J). \* $p < 0.05$ ; \*\*\* $p < 0.001$ ; Bars, 200  $\mu\text{m}$  (A-I); 100  $\mu\text{m}$  (J).

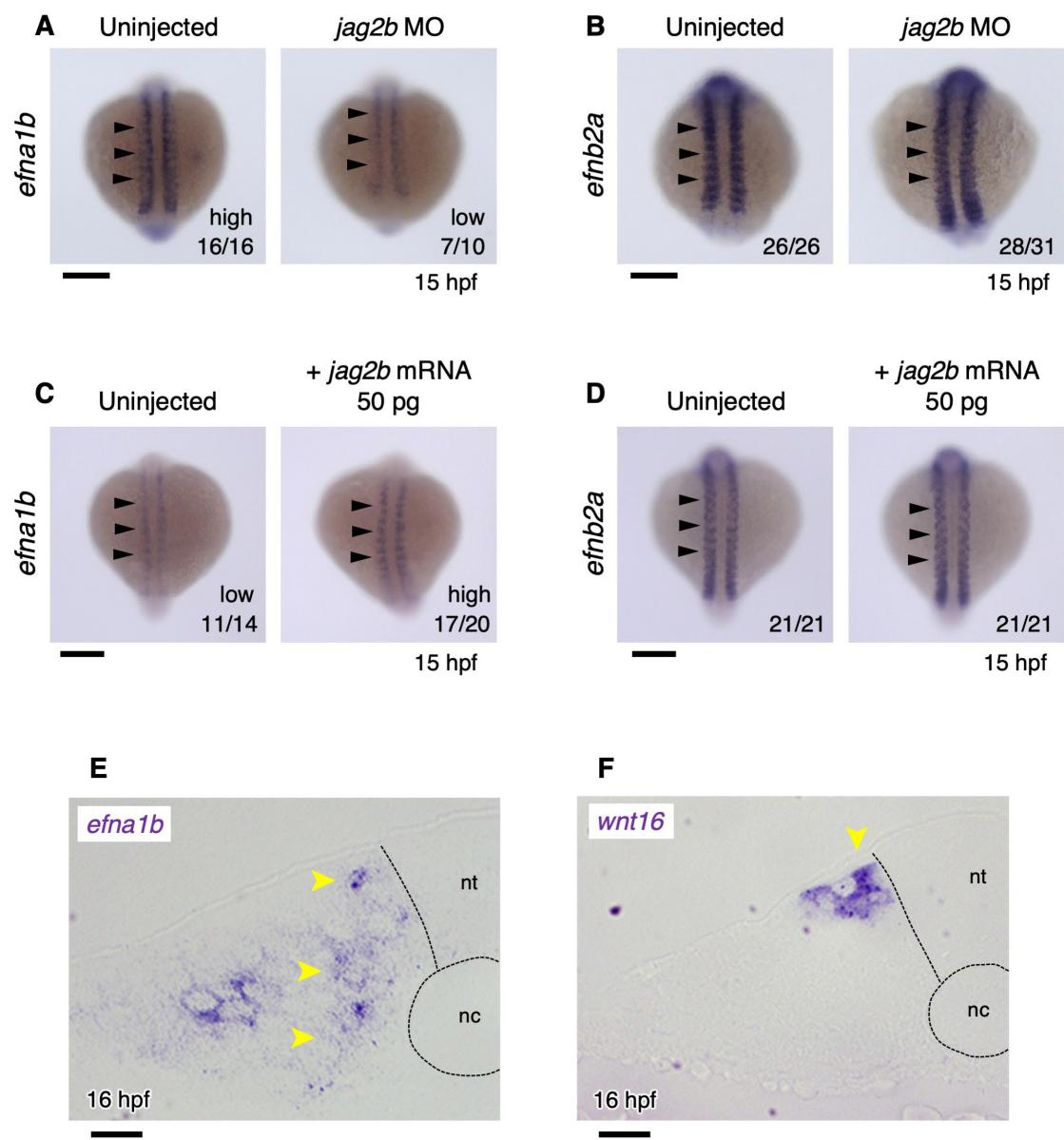


Figure S5

**Fig. S5. Loss and gain of *jag2b* altered *efna1b* expression**

(A, B) Expression of *efna1b* and *efnb2a* in embryos uninjected or injected with *jag2b* MO. Black arrowheads denote the expression domain of each gene. (C, D) Expression of *efna1b* and *efnb2a* in embryos uninjected or injected with *jag2b* mRNA. (E, F) Expression of *efna1b* and *wnt16* in the somite at 16 hpf. Yellow arrowheads denote the expression domain in the adaxial region of the somite. Bars, 200  $\mu$ m (A-D); 10  $\mu$ m (E, F).

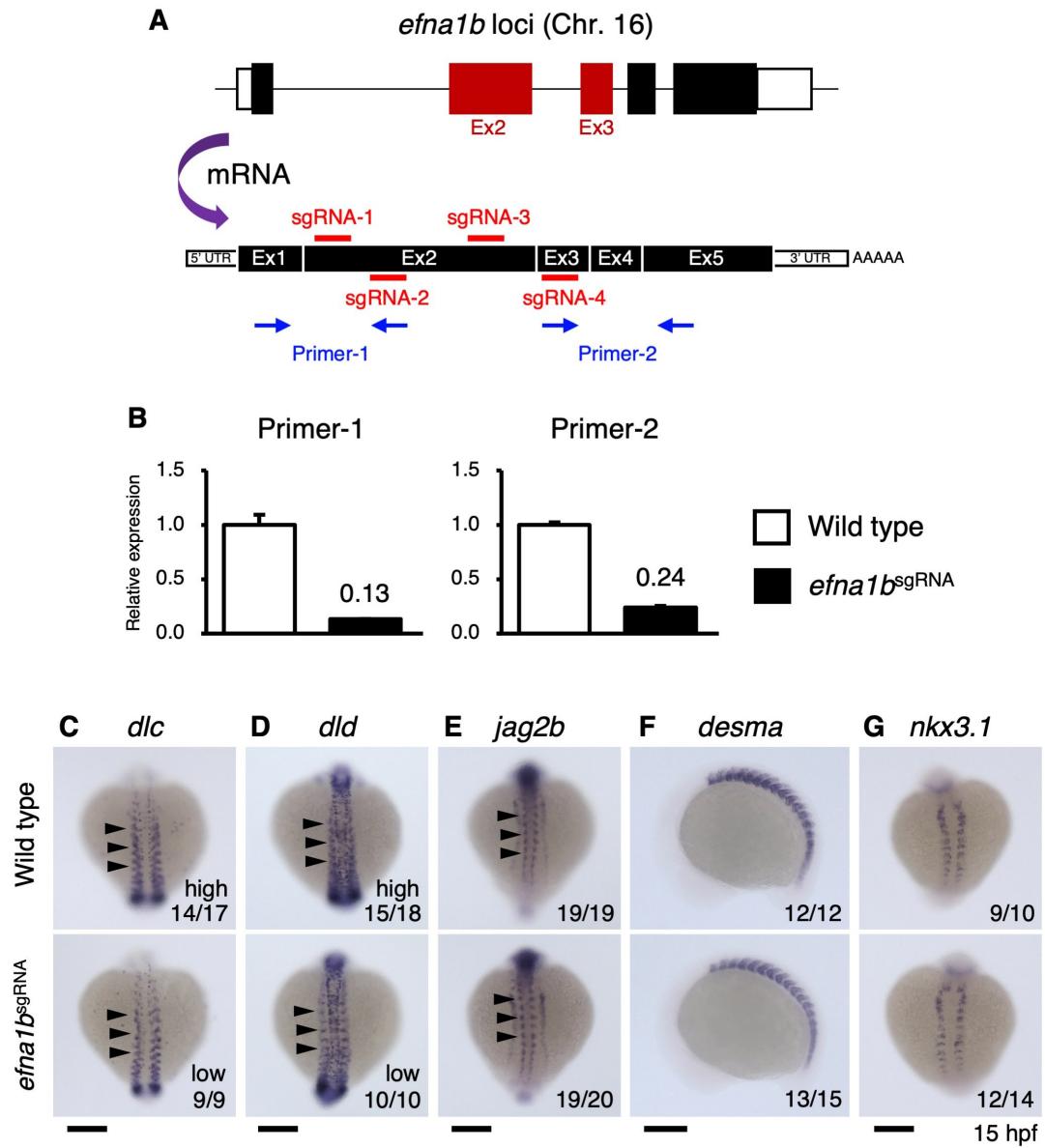


Figure S6

**Fig. S6. Phenotypic effects in *efna1b*<sup>sgRNA</sup> embryos**

(A) Schematic of genomic loci (upper panel) and mRNAs (lower panel) of *efna1b*. Four different gRNA sequences were designed in the exons shown in red in the upper panel. The two different primer sets shown in blue arrows in the lower panel were used for qRT-PCR analysis in sgRNA-injected embryos. (B) Relative expression of *efna1b* in wild type and *efna1b*<sup>sgRNA</sup> embryos. Two different primer sets were used. Error bars, s.d. (C-G) Expression of *dlc*, *dld*, *jag2b*, *desma*, and *nkx3.1* in wild type or *efna1b*<sup>sgRNA</sup> embryos. Black arrowheads denote the expression domain of each gene in the somite. Bars, 200 μm.

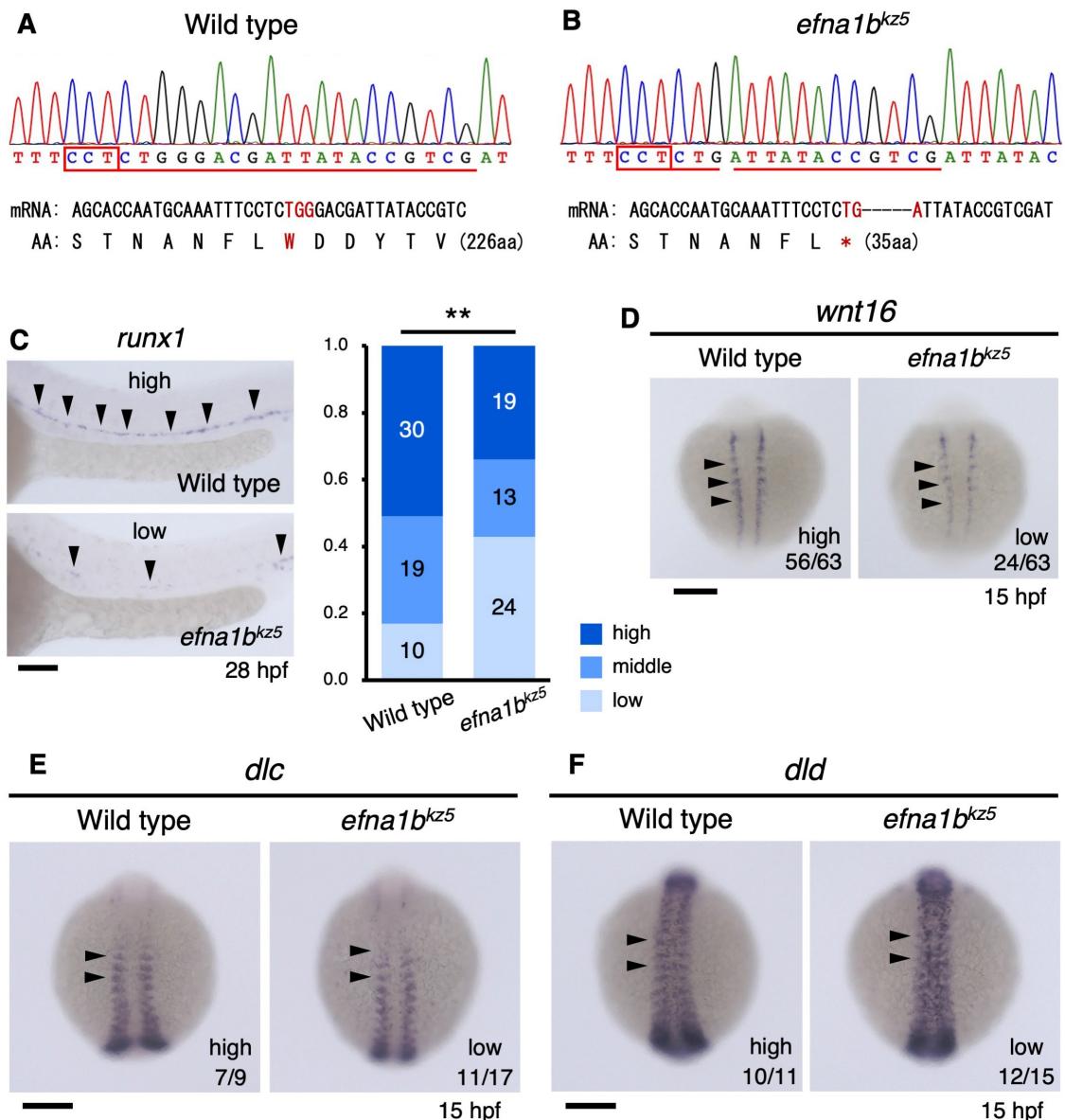


Figure S7

**Fig. S7. Mutation of *efna1b* reduced *runx1* and *wnt16* expression**

(A, B) Mutation of *efna1b* was verified by sequencing of genomic loci in the *efna1b<sup>kz5</sup>* line. A red box and red line indicate protospacer adjacent motif (PAM) and gRNA target sequence, respectively. The mutant line is predicted to have a premature stop codon in exon 2. (C-F) Expression of *runx1* in the DA and *wnt16*, *dlc*, and *dld* in the somite of wild type or *efna1b<sup>kz5</sup>* embryos. Black arrowheads denote the expression domain of each gene. Right panel in C show the phenotype distribution of embryos exhibiting "high", "middle", or "low" *runx1* expression in each type. \*\* $p < 0.01$ ; Bars, 100  $\mu\text{m}$  (C); 200  $\mu\text{m}$  (D-F).

**Table S1. Sequences for primers and oligos**

Gene	Forward primer	Reverse primer	Description
<i>jag2b</i>	CCCTCAAATTAACATTAGCACA	CCCACAACTCACCGTTTACA	Genotyping for <i>jag2b</i> <sup>lu3425</sup>
<i>jag2b</i>	CCAGTCTGAAGTCACCACCA	TCCACCAAGAACGTGGTAG	Genotyping for <i>jag2b</i> <sup>kz7</sup> and <i>jag2b</i> <sup>kz8</sup>
<i>jag2b</i>	TCGAGAAGCCTCGTTTGT	GGAGGACTGGTAAGGGAAGG	In situ probe
<i>jag2b</i>	CACCTCGGCTCTGGATCTA	GTCCAAGCTCAAGGATGA	qRT-PCR primer-1
<i>jag2b</i>	TACCATTGCTGTGGCGAGTA	ATTCGACACGGATTGCTCA	qRT-PCR primer-2
<i>jag2b</i>	CAAGGATCCACCATGTGAAATTGTATCAGGATTAGG	CATCTCGAGTCATAACGAGTGTCTTGTGC	mRNA
<i>efna1b</i>	CCCTCTGGCTTTCTGTGT	AGTGAGGGCAGATGATGTCC	Genotyping for <i>efna1b</i> <sup>kz5</sup>
<i>efna1b</i>	ACAGAATTCCACCATGGATTTCTGTGGCTGCT	CAGCTCGAGTTGCAGGGTGTGTTCACATT	In situ probe
<i>efna1b</i>	CATCATGGCCAGGAGTGT	CCACCGGGATCATAGCAATA	qRT-PCR primer-1
<i>efna1b</i>	CCGAGCGACACAGTGT	ATATCGTTCTGCCTCCTGGG	qRT-PCR primer-2
<i>efna1b</i>	ACAGAATTCCACCATGGATTTCTGTGGCTGCT	TTACTCGAGCTATGAAGCTTGGTGGAGAAC	mRNA
<i>wnt16</i>	GCAGAATTCCGTTAGTCGGGACTTGTT	GCGCTCGAGTTACTTCAGGTGTGCATGTC	In situ probe
<i>wnt16</i>	TAAGAATTCCACCATGGATAATACCGGTTGTGGG	ATACTCGAGTTACTTCAGGTGTGCATGTC	mRNA
<i>jag1a</i>	ACGGAAGCGGATCTACTCCT	GTGTTTCAGGACCTGCCATT	qRT-PCR
<i>jag1b</i>	TGGTGAGCAAGCATAATGGA	GTGTTGCTGTGGGTGTTTG	qRT-PCR
<i>jag2a</i>	TTGTACGTACGGCACTGGAA	TCCAGGGTTCATCTCTCCAC	qRT-PCR
<i>ef1a</i>	ACCGGCCATCTGATCTACAA	CAATGGTATACCACGCTCA	qRT-PCR
CRISPR/Cas9			Description
	TAATACGACTCACTATAGGACAGTCACCTCGGCTCGTTAGAGCTAGAAATAGC		<i>jag2b</i> target-1
	TAATACGACTCACTATAGGCTTGCACACGGCTGGTTAGAGCTAGAAATAGC		<i>jag2b</i> target-2
	TAATACGACTCACTATAGGGAGCAATCCGTGCGAAAGTTAGAGCTAGAAATAGC		<i>jag2b</i> target-3
	TAATACGACTCACTATAGGCTTCTGCCAGGGACCCAGTTAGAGCTAGAAATAGC		<i>jag2b</i> target-4
	TAATACGACTCACTATAGGACGGTATAATCGTCCCAGGTTAGAGCTAGAAATAGC		<i>efna1b</i> target-1
	TAATACGACTCACTATAGGATGGCGAGATCGCTCCCGTTAGAGCTAGAAATAGC		<i>efna1b</i> target-2
	TAATACGACTCACTATAGGAAACTCTCGGGCGCGTGGTTAGAGCTAGAAATAGC		<i>efna1b</i> target-3
	TAATACGACTCACTATAGGAAACACTCCTGGCCATGAGTTAGAGCTAGAAATAGC		<i>efna1b</i> target-4
	AAAAGCACCGACTCGGTGCCACTTTCAAGTTGATAACGGACTAGCCTATTTAACTTGCTATTCTAGCTCTAAAAC		gRNA scaffold primer