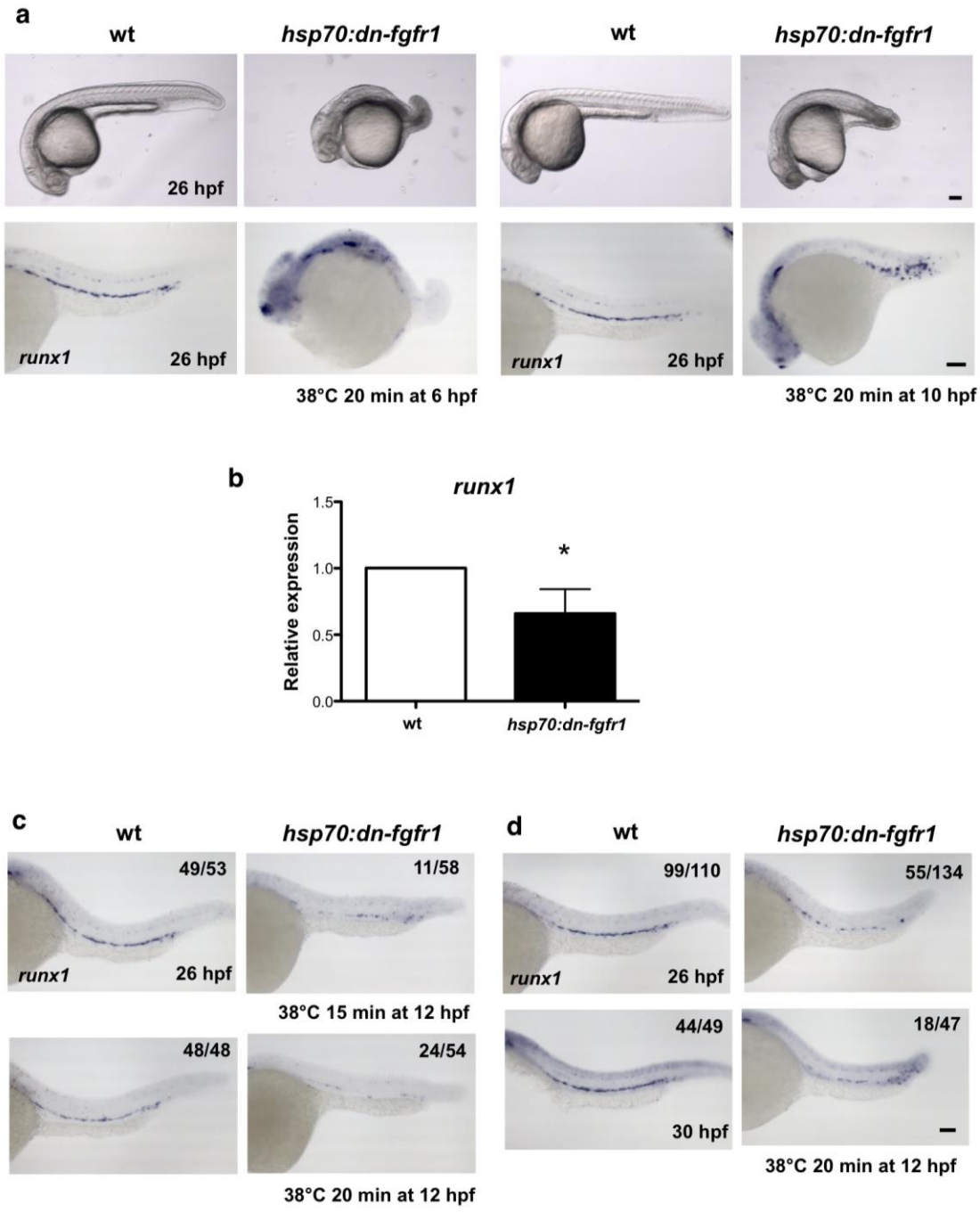
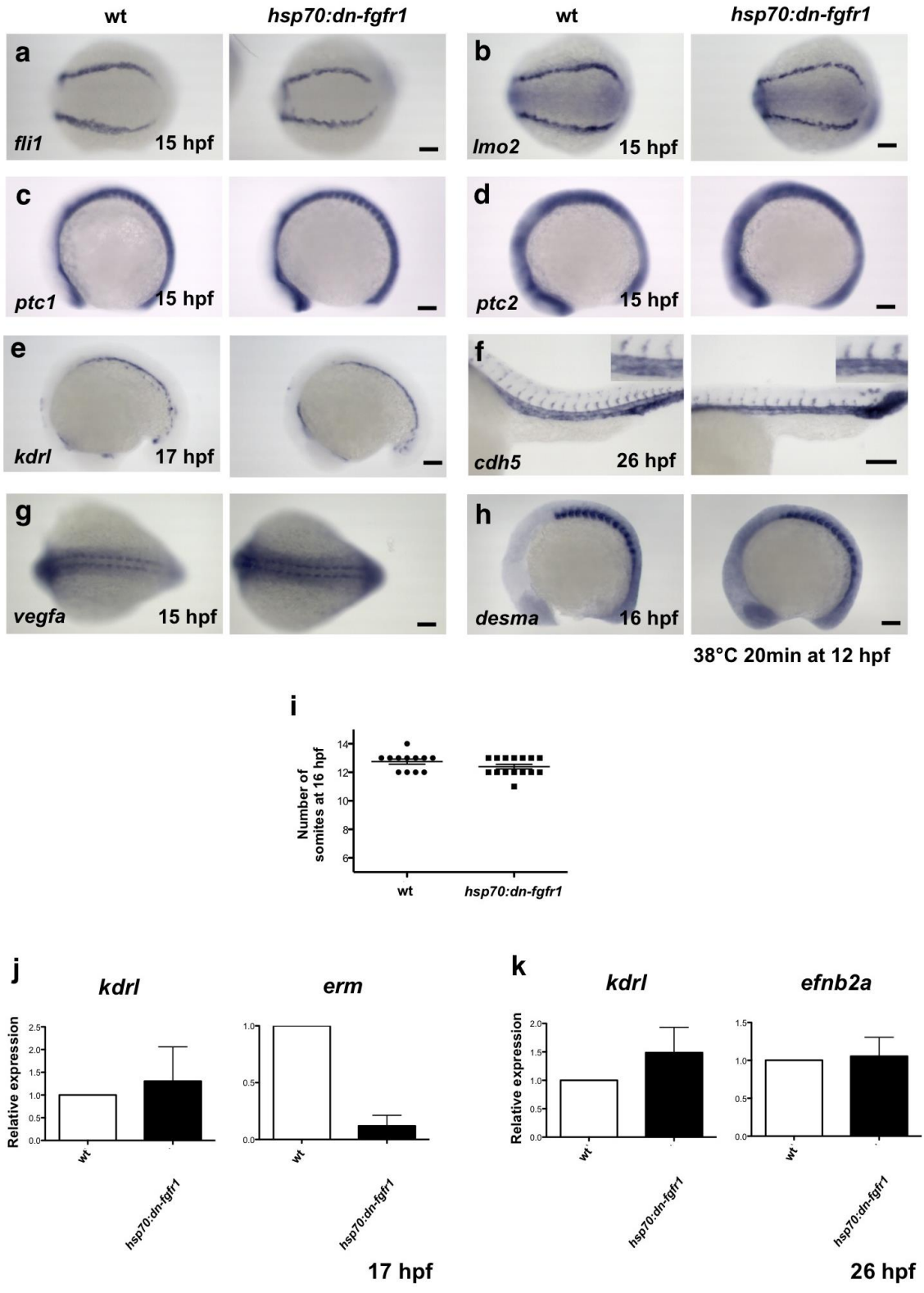
SUPPLEMENTARY INFORMATION



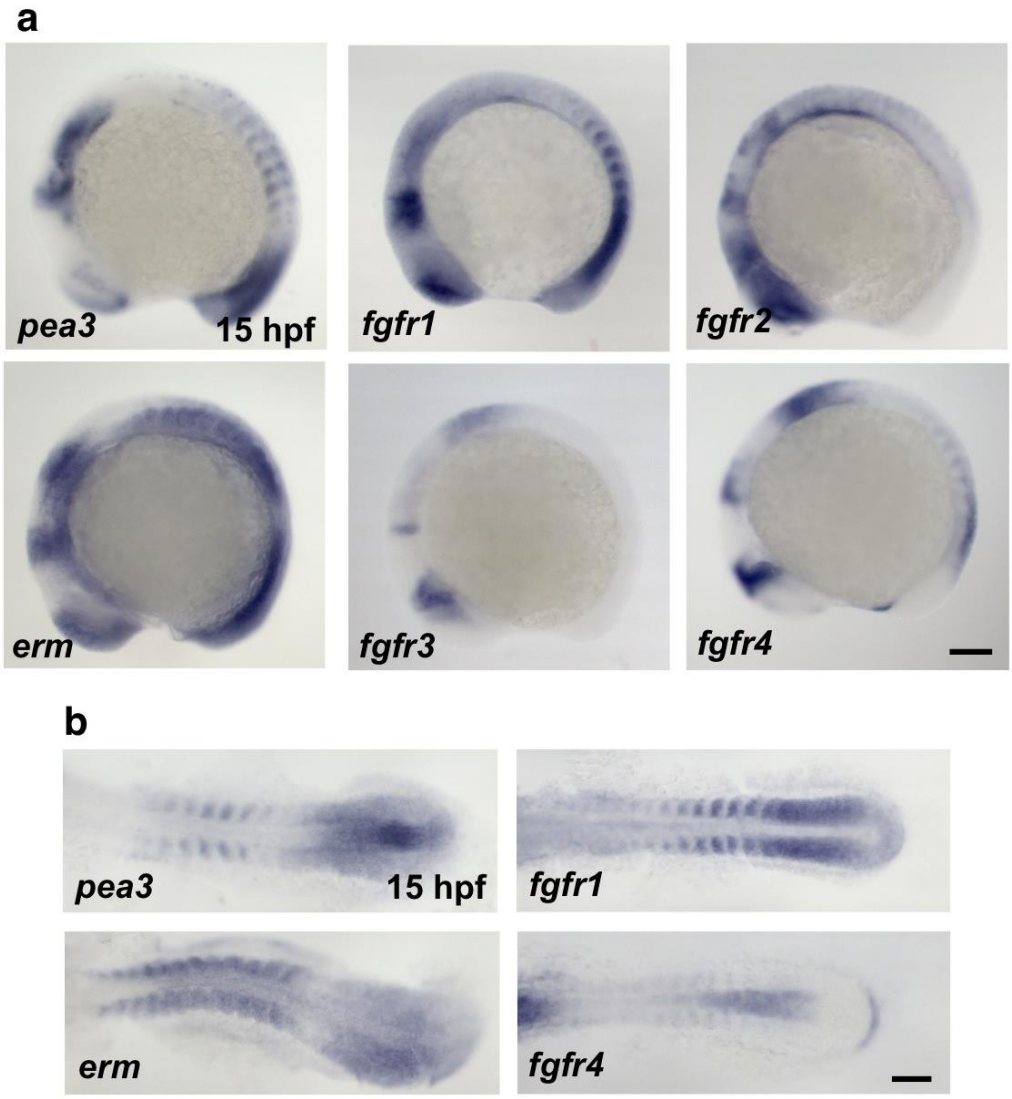
Supplementary Figure 1. Knock-down of FGF signaling with *hsp70*:*dn-fgfr1* induced at 12 hpf blocks formation of HSCs. (**a**) Zebrafish embryos of 26 hpf wt and *hsp70:dn-fgfr1* heat-shocked at shield stage (6 hpf) and bud stage (10

hpf). Images of WISH for *runx1* of 26 hpf wt and *hsp70:dn-fgfr1* embryos heat-shocked at shield stage and bud stage. (**b**) qRT-PCR expression of *runx1* in the trunk of 26 hpf wt and *hsp70:dn-fgfr1* heat-shocked at 12 hpf (38°C 20 min) (wt, n=4; *hsp70:dn-fgfr1*, n=4, **P*-value < 0.05, significantly different from wt, Student's *t*-test). Expression data were normalized to ***ef1α*** levels. Error bar indicates *mean ± s. e. m*. (**c**) *runx1* WISH of 26 hpf *hsp70:dn-fgfr1* heat-shocked at 12 hpf with 38°C 15 min (upper panels) and 38°C 20 min (lower panels) compared to wt. (**d**) *runx1* WISH of 26 hpf (upper panels) and 30 hpf (lower panels) *hsp70:dn-fgfr1* heat-shocked at 12 hpf with 38°C 20 min. Scale bars=100μm

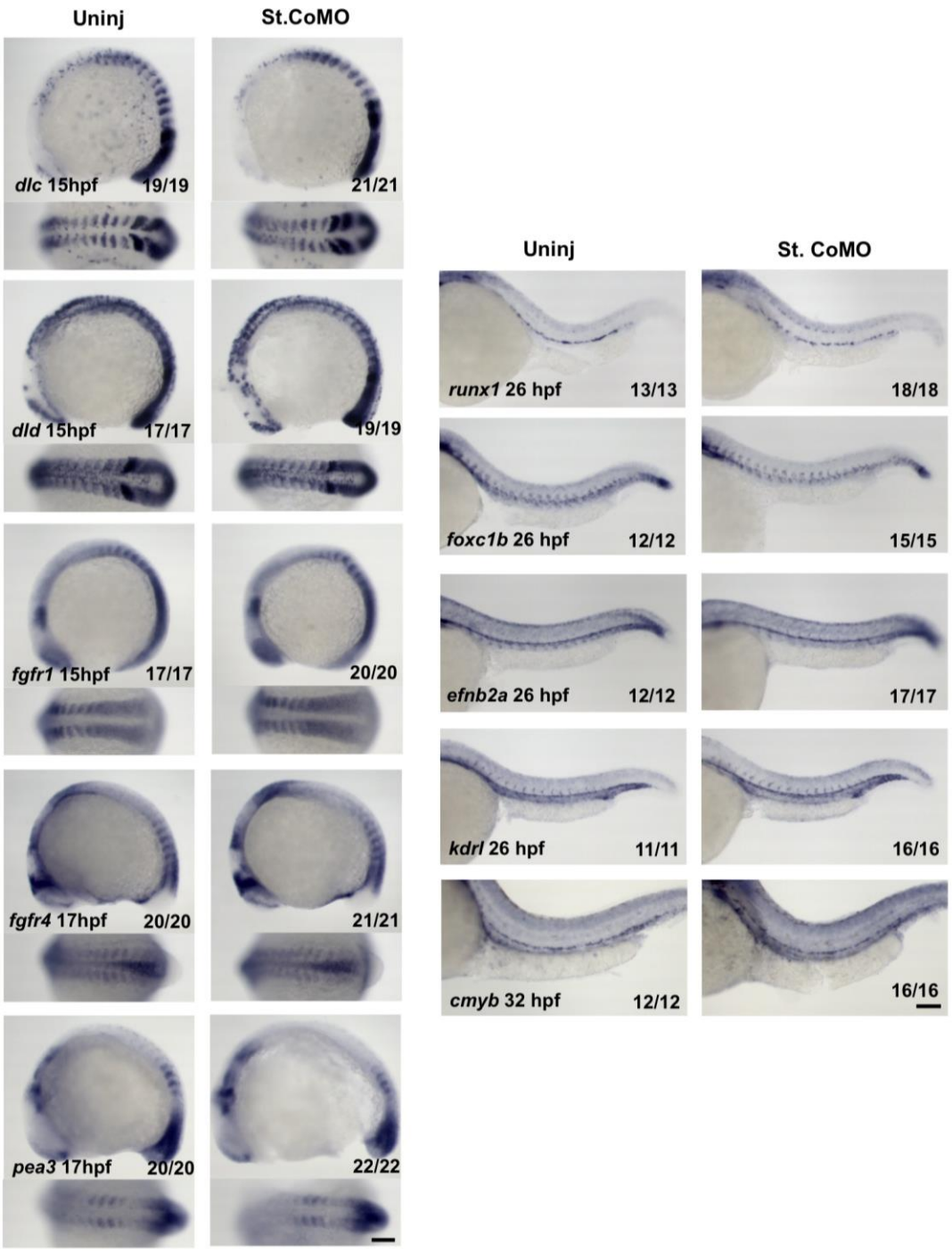


Supplementary Figure 2. Blocking FGF signaling specifically inhibits HSC formation. (**a-h**) WISH of wt and *hsp70:dn-fgfr1* embryos heat-shocked at 12 hpf

with the PLM markers *fli1* (**a**) and *lmo2* (**b**) at 15 hpf, Shh target genes *ptc1* (**c**) and *ptc2* (**d**) at 15 hpf, the endothelial markers *kdrl* (**e**) at 17 hpf and *cdh5* (**f**) at 26 hpf, the somitic markers *vegfa* (**g**) at 15 hpf and *desma* at 16 hpf (**h**). (**i**) Quantitation of number of *desma*-expressing somites at 16 hpf of wt and *hsp:dn-fgfr1* embryos heat-shocked at 12 hpf. Black lines indicate *mean ± s. e. m*. (**j, k**) qRT-PCR expression of *kdrl* and *erm* in the 17 hpf wt (n=3) and *hsp70:dn-fgfr1* (n=3) embryos (**j**) and *kdrl* and *efnb2a* in the 26 hpf wt (n=2) and *hsp70:dn-fgfr1* (n=2) embryos heat-shocked at 12 hpf (k) (38°C 20 min). Expression data were normalized to *ef1α* levels. Error bars indicate *mean ± s. e. m.* Scale bars=100μm.

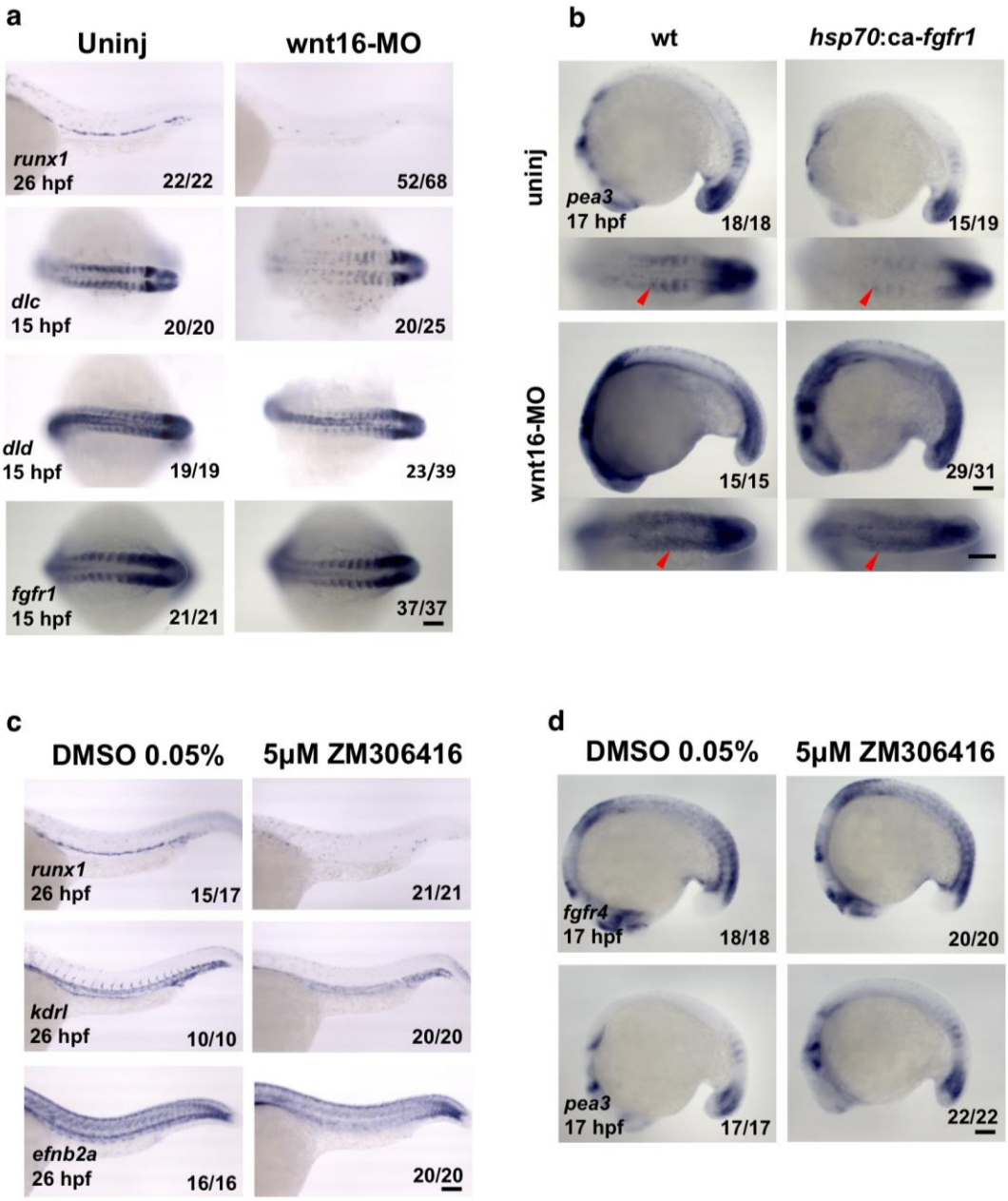


Supplementary Figure 3. Expression of FGF targets and Fgfrs during mid-somitogenesis. WISH analysis of whole-mount (**a**) and flat-mount (**b**) 15 hpf zebrafish embryos for Fgf targets *pea3* and *erm*, and *fgfrs* (*fgfr1*, *fgfr2*, *fgfr3*, and *fgfr4*). Scale bars=100μm.



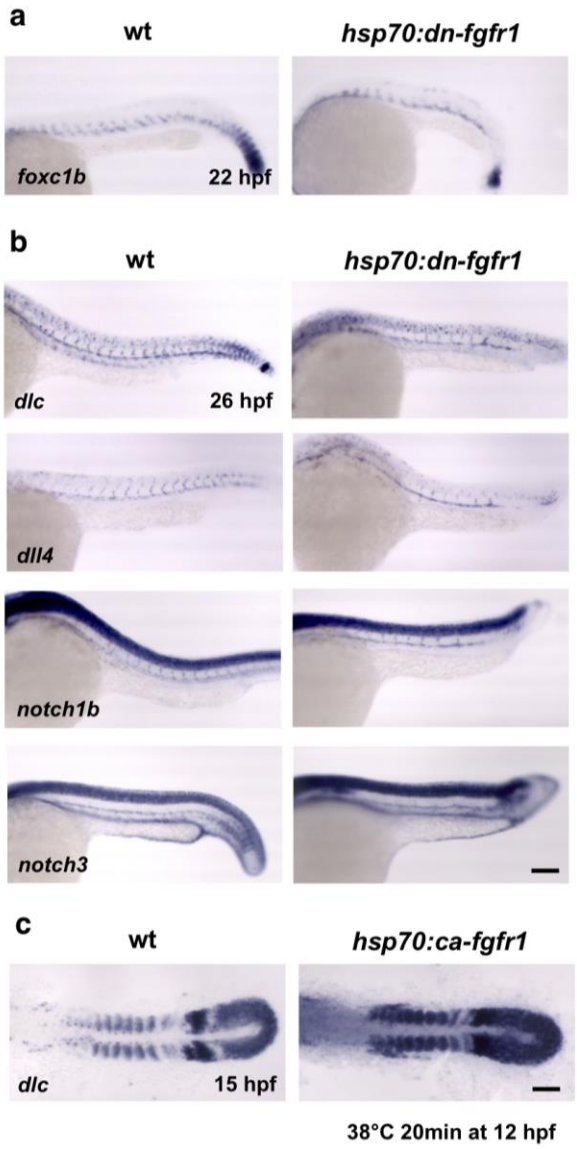
Supplementary Figure 4. No off-target effects of St. CoMO injected embryos. Embryos injected with 5 ng standard control MO (St. Co MO) at 1-2

cell stage led to no alteration of expression of following genes: *dlc*, *dld*, *fgfr1*, *fgfr4*, *pea3, runx1, foxc1b, efnb2a, kdrl, cmyb.* Scale bars=100μm.



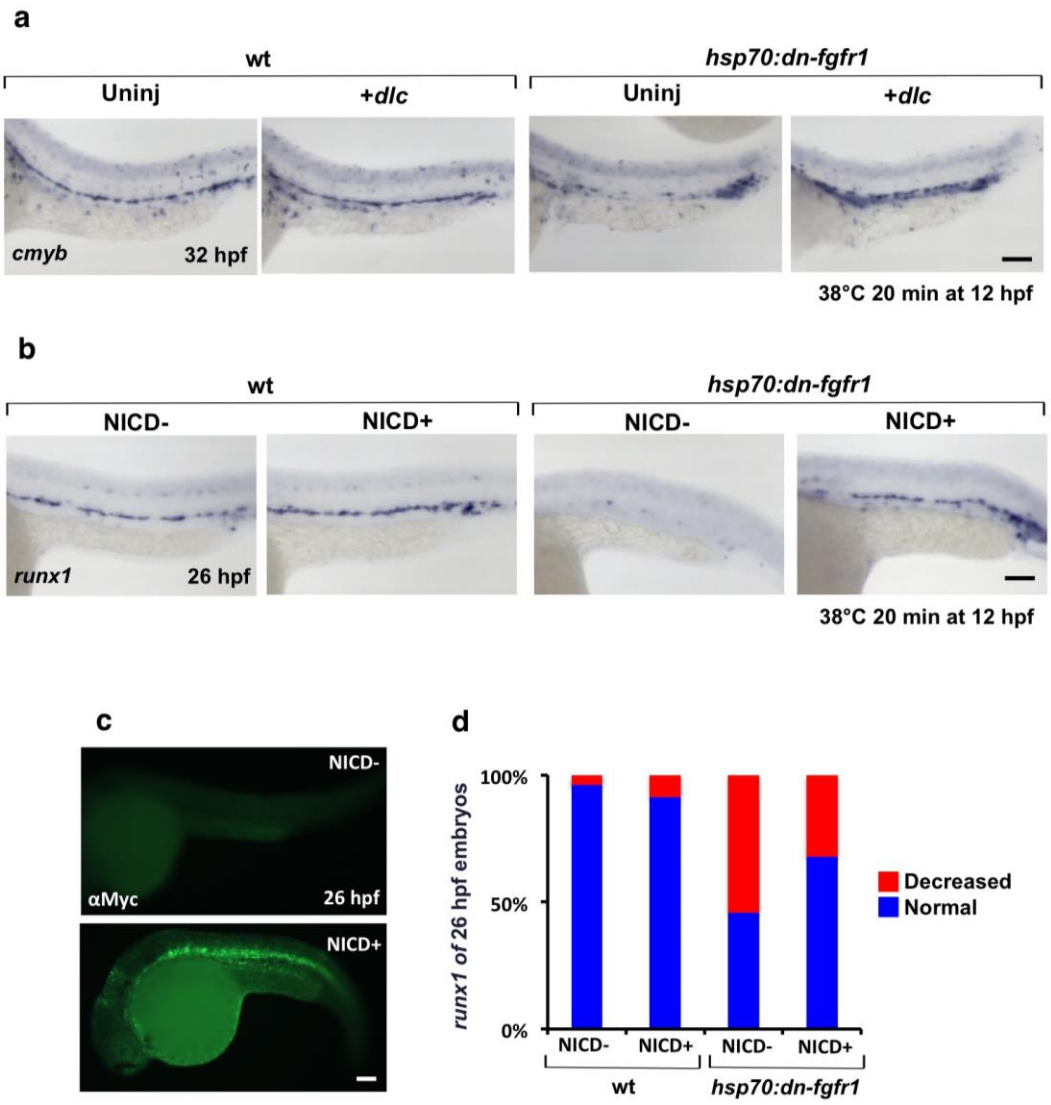
Supplementary Figure 5. FGF signaling is downstream of Wnt16 to specify HSCs. (**a**) WISH for *runx1* (26 hpf), *dlc* (15 hpf), *dld* (15 hpf), and *fgfr1* (15 hpf) in uninjected controls (left column) and *wnt16* morphants (right column). (**b**) *pea3* WISH at 17 hpf with ectopic activation of FGF signaling using *hsp70:ca-fgfr1* induced at 12 hpf (38°C 20 min) in *wnt16* morphants. Red arrowheads indicate

pea3 expression in the somites. (**c**, **d**) Expression of *runx1*, *kdrl* and *efnb2a* at 26 hpf (**c**) and *pea3* and *fgfr4* at 17 hpf (**d**) wt embryos incubated with the Vegfr-antagonist ZM306416 from 10 hpf compared to DMSO-treated controls. Scale bars=100µm



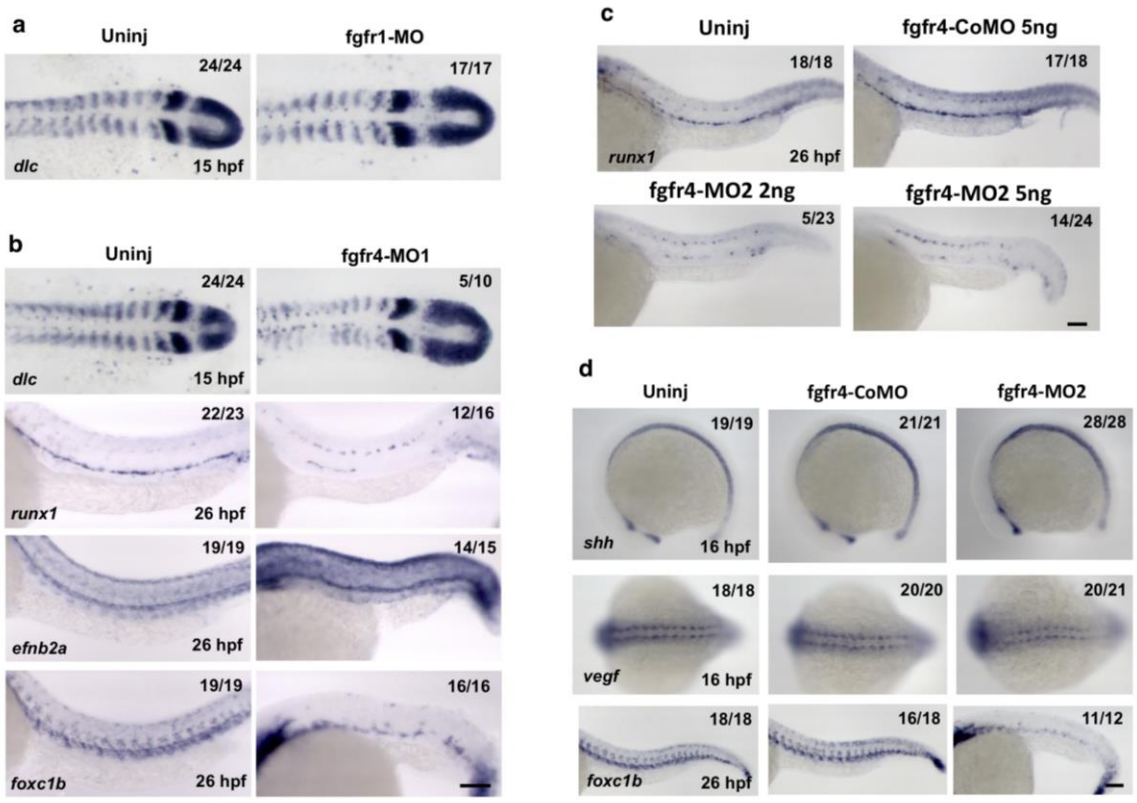
Supplementary Figure 6. Expression of Notch ligands and receptors in the absence or presence of FGF signaling. (**a**, **b**) WISH of wt (left column) and *hsp70:dn-fgfr1* (right column) heat-shocked at 12 hpf. (**a**) Sclerotomal marker *foxc1b* expression at 22 hpf. (**b**) Expression of Notch ligands *dlc*, *delta-like4* (*dll4*), and Notch receptors *notch1b* and *notch3* in the endothelium at 26 hpf. (**c**)

dlc expression at 15 hpf in wt (left) and *hsp70:ca-fgfr1* (right) embryos heat-shocked at 12 hpf. Scale bars=100μm.



Supplementary Figure 7. HSC formation attenuated by FGF signaling blockade can be recovered by Notch activation. (**a**) *cmyb* expression at 32 hpf in wt and *hsp70:dn-fgfr1* embryos injected with 100pg of *dlc* mRNA and uninjected controls. Embryos were given heat-shock at 12 hpf. (**b**) *runx1* expression at 26 hpf in *hsp70:gal4; UAS:NICD-myc; hsp70:dn-fgfr1* heat-shocked at12 hpf. (**c**) NICD+ embryos were identified by anti-myc antibody staining after WISH performed. (**d**) Percentage of embryos with a *runx1* phenotype at 26 hpf in wt or FGF-blocked, with or without enforced NICD

expression from (**b**) (NICD- wt, n=50; NICD+ wt, n=23; NICD- *hsp70:dn-fgfr1*, n=48; NICD+ *hsp70:dn-fgfr1*, n=25). Scale bars=100μm.



Supplementary Figure 8. Fgfr4 is required for somitic *dlc* and the sclerotomal *foxc1b* expression. (**a**) Expression of *dlc* at 15 hpf in fgfr1-MO injected embryos (right column) and uninjected controls (left column). (**b**) Expression of somitic *dlc* at 15 hpf, *runx1,* aortic *efnb2a* and sclerotomal marker *foxc1b* at 26 hpf in the fgfr4-MO1 injected embryos (right column), compared to uninjected sibling controls (left column). (**c**) *runx1* expression of 26 hpf wt embryos injected with 2 ng fgfr4-MO2 and 5 ng fgfr4-MO2 compared to controls. (**d**) Expression of *shh* and *vegf* at 16 hpf and *foxc1b* at 26 hpf in fgfr4 morpahnts compared to uninjected and fgfr4-CoMO injected control embryos. Scale bars=100μm