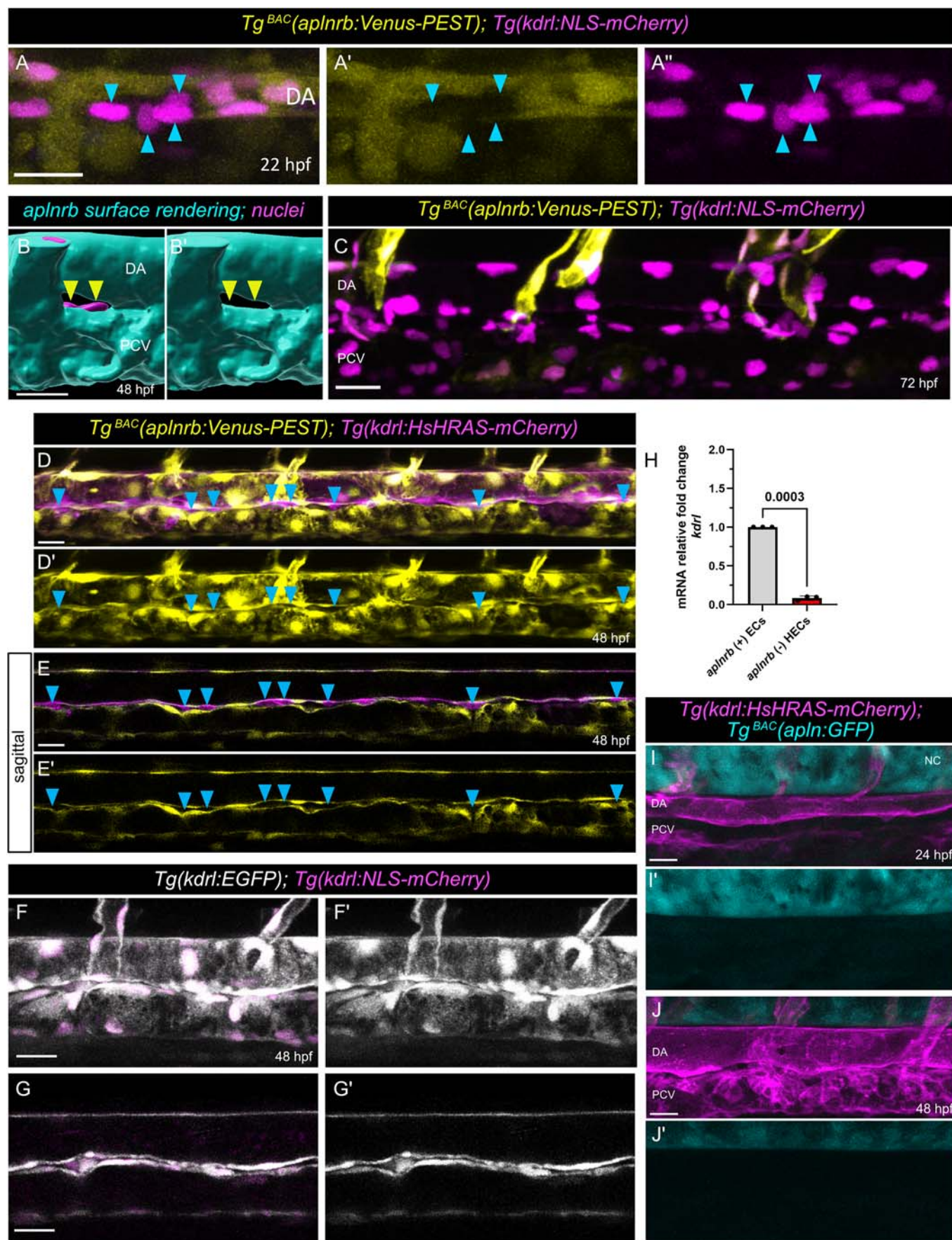


Expanded View Figures

Figure EV1. *aplnrb* negative cells are restricted to the VDA.

(A–A'') Confocal projection images of the trunk region of *Tg^{BAC}(aplnrb:Venus-PEST); Tg(kdrl:NLS-mCherry)* double transgenic zebrafish embryos at 22 hpf. *aplnrb:Venus-PEST* negative ECs can be detected in the ventral DA (cyan arrowheads). (B, B'') Surface rendering of a confocal projection image of a double transgenic *Tg^{BAC}(aplnrb:Venus-PEST); Tg(kdrl:NLS-mCherry)* embryos at 48 hpf highlighting the absence of *aplnrb:Venus-PEST* expression (cyan) from putative HECs (yellow arrowheads) in the ventral DA (nuclei in magenta). (C) Confocal projection image of a double transgenic *Tg^{BAC}(aplnrb:Venus-PEST); Tg(kdrl:NLS-mCherry)* larva at 72 hpf. Expression of *aplnrb:Venus-PEST* is absent from the DA and PCV and is restricted to the intersegmental vessels. (D, D') Confocal projection images of a *Tg^{BAC}(aplnrb:Venus-PEST); Tg(kdrl:HsHRAS-mCherry)* double transgenic zebrafish embryo at 48 hpf. *aplnrb:Venus-PEST* negative ECs can be detected in the ventral DA (cyan arrowheads). (E, E') Sagittal slice of the images in (D, D'). (F, F') Confocal projection images of the trunk region of a *Tg(kdrl:GFP); Tg(kdrl:NLS-mCherry)* double transgenic embryo at 48 hpf. All ECs in the DA are marked with *kdrl:GFP* and *kdrl:NLS-mCherry* expression. (G, G') Sagittal slice of the images in (F, F'). (H) Quantitative RT-PCR results for *kdrl* on FACS sorted *aplnrb:Venus-PEST* expressing and non-expressing ECs, referring to Fig. 1E–G ($n = 3$, paired t test, $P < 0.0003$). Quantifications are displayed as mean $\pm$ SD. (I–J') Confocal projection images of double transgenic *Tg(kdrl:HsHRAS-mCherry); Tg^{BAC}(apln:GFP)* zebrafish embryo at 24 hpf (I, I') and embryo at 48 hpf (J, J'). At both timepoints displayed, no expression of the reporter for *apln* can be detected in the DA and the PCV. The notochord shows *apln* expression that is decreasing over time. DA dorsal aorta, PCV posterior cardinal vein, HECs hemogenic endothelia cells, ECs endothelial cells, NC notochord. Scale bars: 20 μ m.



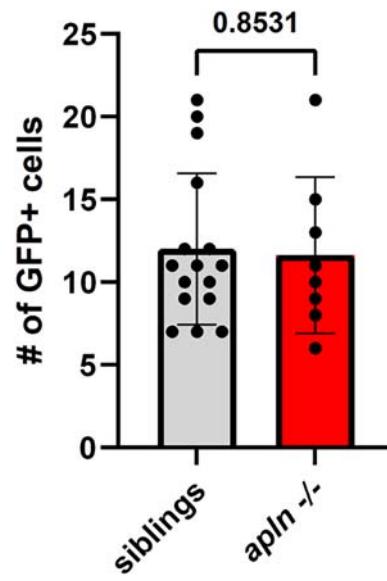


Figure EV2. No detectable change in HSPC numbers in zygotic *apl n* mutant embryos.

Quantification of *itga2b*:GFP expressing cells in the DA at 52 hpf ($n = 32$, unpaired t test, $P = 0.0044$); Quantifications are displayed as mean $\pm$ SD.

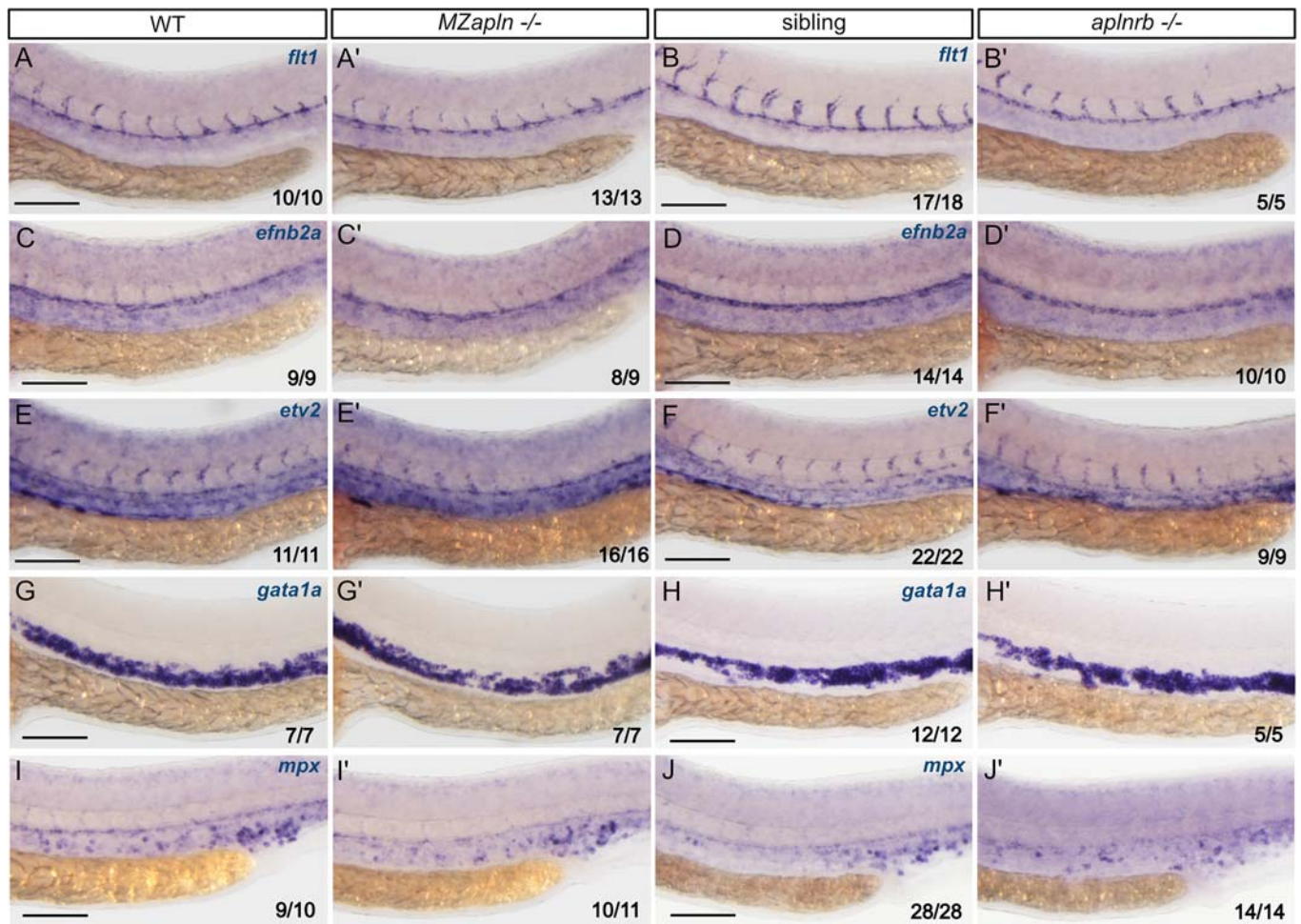


Figure EV3. Arterial differentiation and primitive hematopoiesis are unaltered upon loss of Apelin signaling.

(A–J') WISH on wild-type and *MZapln* ^{-/-} mutant (A, A', C, C', E, E', G, G', I, I') and siblings and *aplnr b* ^{-/-} mutant (B, B', D, D', F, F', H, H', J, J') zebrafish embryos at 24 hpf. (A–B') WISH for *ftl1* showing WT-like expression upon loss of Apelin signaling. (C–D') WISH for *efnb2a* showing WT-like expression upon loss of Apelin signaling. (E–F') WISH for *etv2* showing WT-like expression upon loss of Apelin signaling. (G–H') WISH for *gata1a* showing WT-like expression upon loss of Apelin signaling. (I–J') WISH for *mpx* showing WT-like expression upon loss of Apelin signaling. Scale bars: 100 μ m.

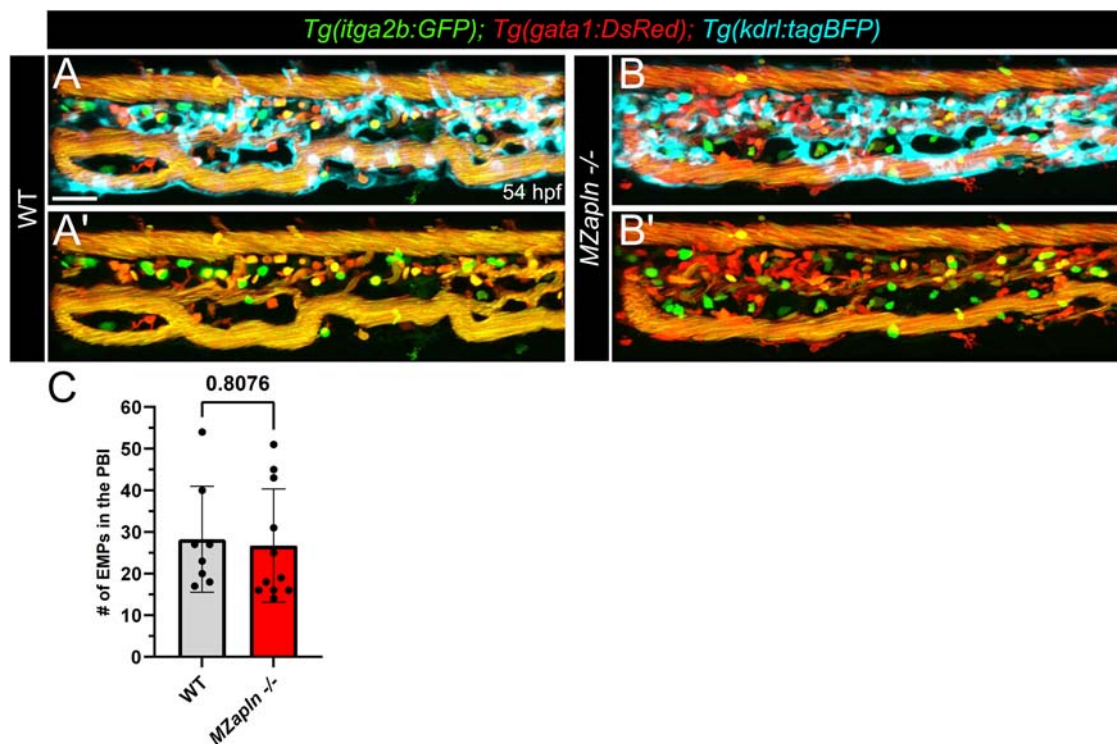


Figure EV4. EMP development is independent of Apelin signaling.

(A–B') Confocal projection images of the CHT region of *Tg(itga2b:GFP); Tg(gata1:DsRed); Tg(kdrl:tagBFP)* triple transgenic zebrafish larvae at 54 and 72 hpf. (A–B') Exemplary images of wild-type and *MZapln*^{-/-} mutant zebrafish larvae at 54 hpf. (C) Quantification corresponding to (A–B'). *itga2b:GFP* + / *gata1a:DsRed* + EMPs were counted in the CHT ($n = 19$, unpaired t test, $P = 0.8076$). EMP erythro-myeloid progenitors, CHT caudal hematopoietic tissue. Quantifications are displayed as mean $\pm$ SD. Scale bars: 40 μ m.

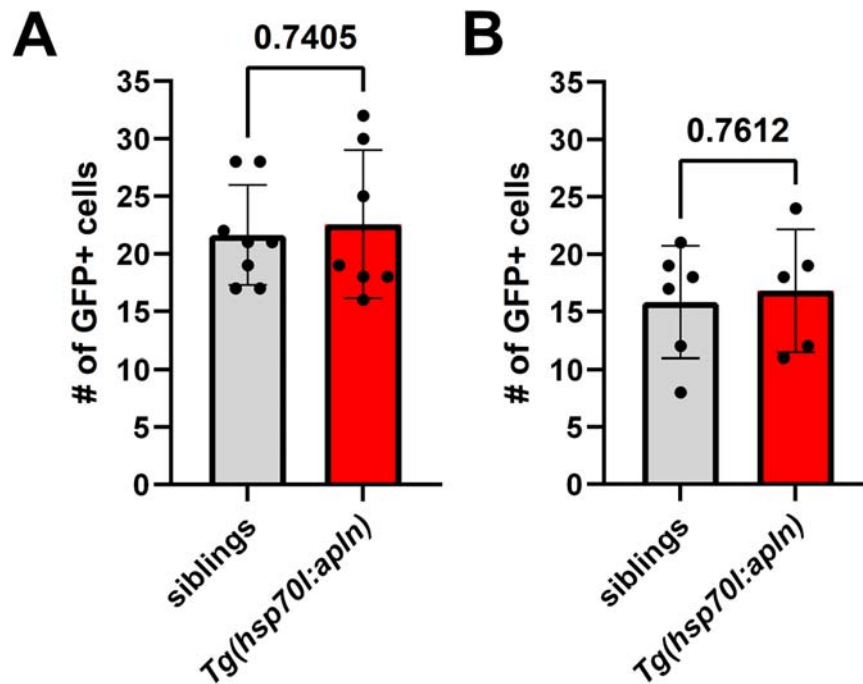
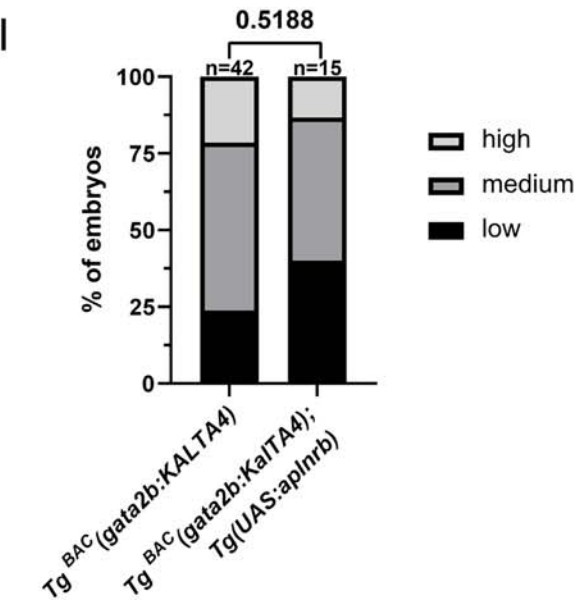
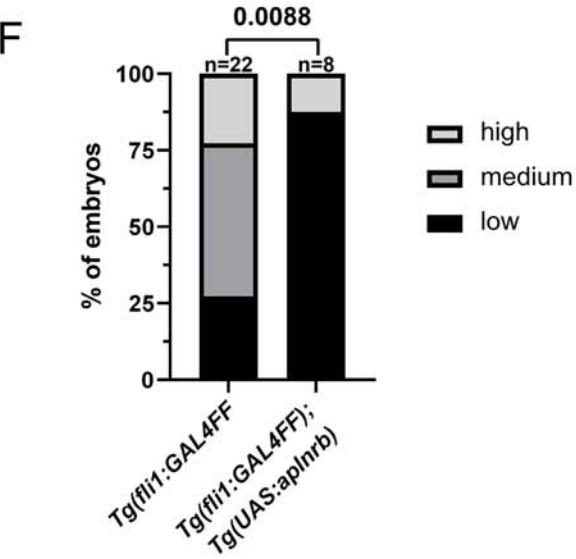
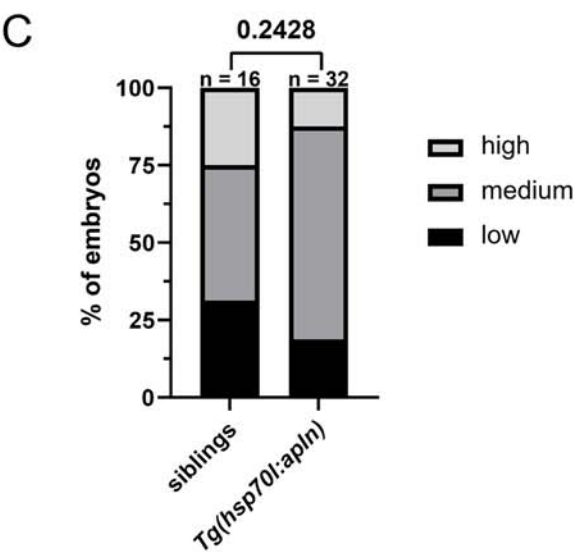
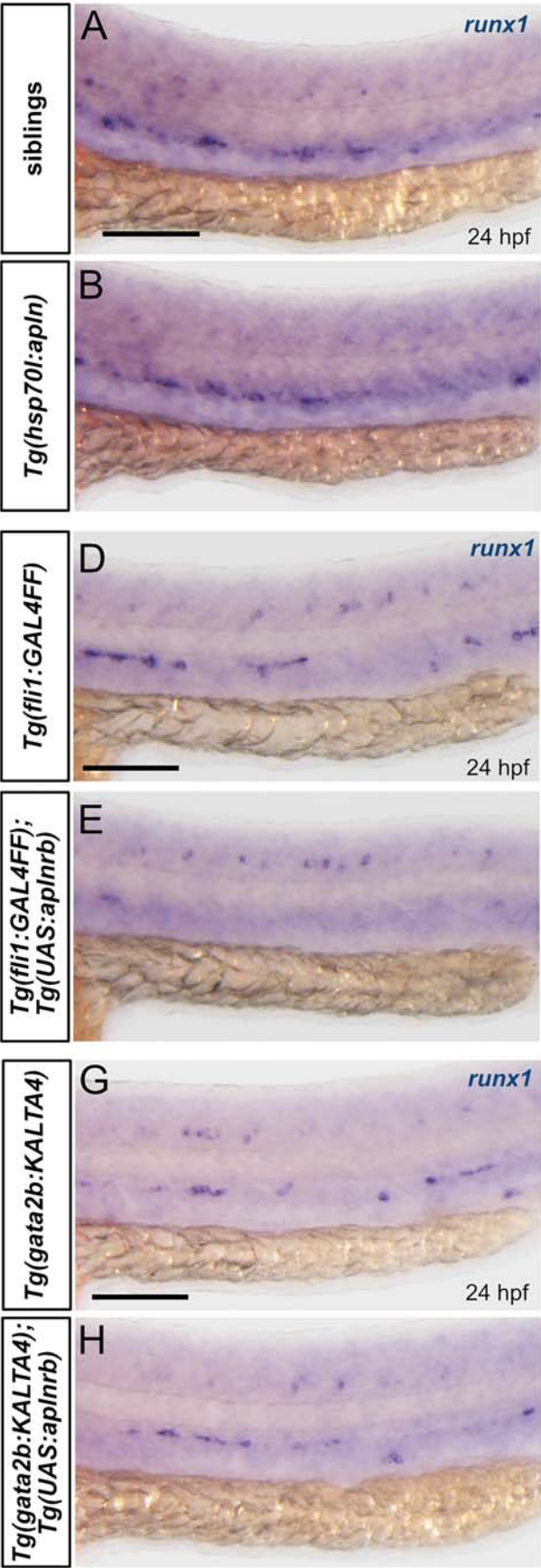


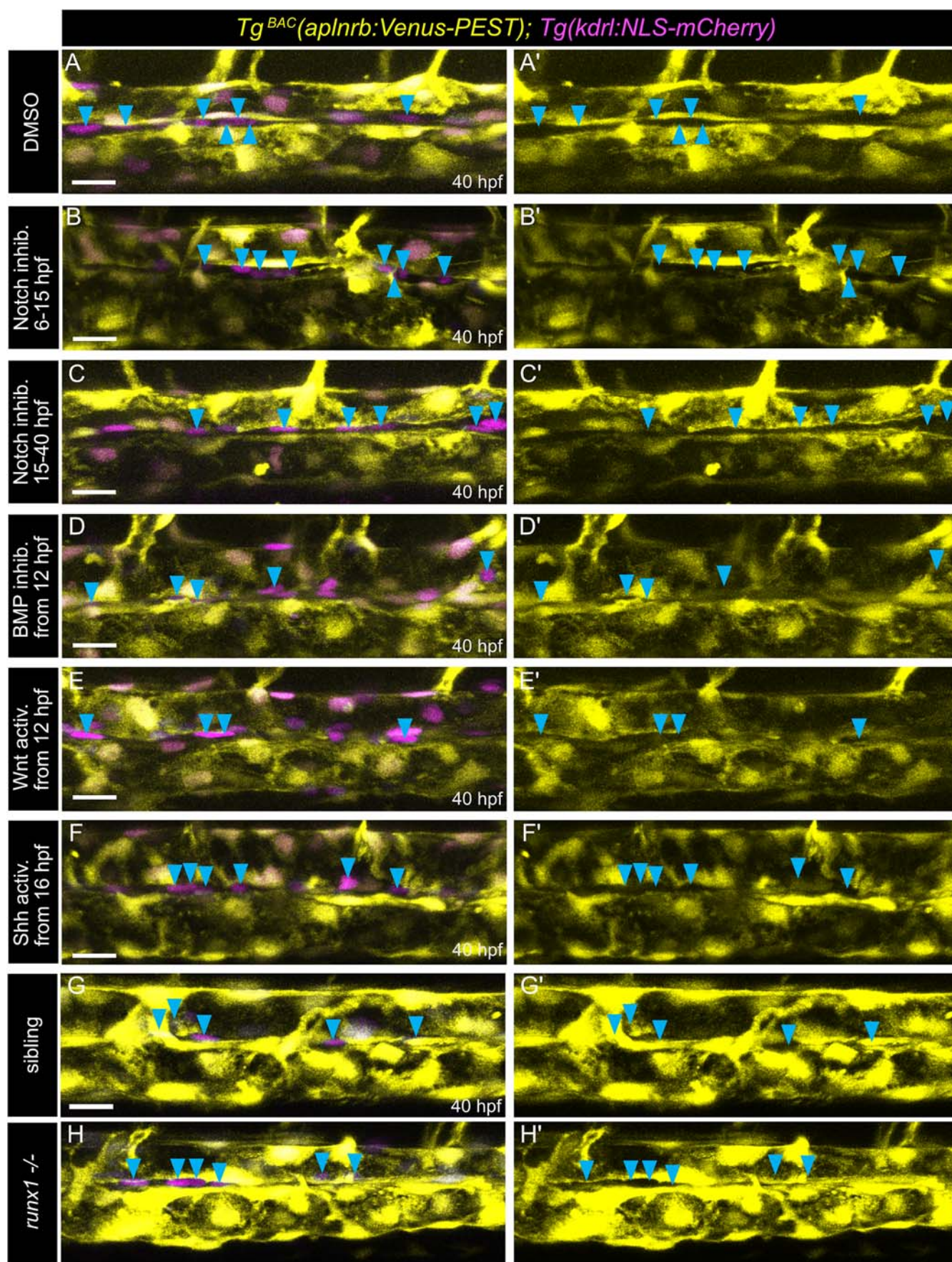
Figure EV5. Overexpression of *apln* does not influence HSPC specification and maintenance.

(A, B) Quantification of *itga2b*:GFP expressing HSPCs in the DA at 48 hpf upon heatshock induced global *apln* overexpression. (A) heatshock at 16 hpf, imaging at 48 hpf ($n = 15$, unpaired t test, $P = 0.7405$). (B) heatshock at 32 hpf, imaging at 48 hpf ($n = 11$, unpaired t test, $P = 0.7612$). HSPC hematopoietic stem and progenitor cell, DA dorsal aorta. Quantifications are displayed as mean $\pm$ SD.



◀ **Figure EV6. Forced expression of *aplnrb* in the vasculature disturbs HE specification.**

(A, B, D, E, G, H) WISH for *runx1* at 24 hpf upon *apln/aplnrb* overexpression. Images of the trunk region above the yolk extension. (A, B) WISH for *runx1* in siblings or *hsp70l:apln* (heatshock inducible global overexpression of *apln*) zebrafish embryos. The heatshock was induced at 16 hpf. Embryos with global *apln* overexpression display no differences in *runx1* expression compared with control siblings. (C) Phenotypic distribution plot of embryos in (A, B) scored with low, medium and high *runx1* expression ($n = 48$, Chi-square test, $P = 0.2428$). (D, E) WISH for *runx1* in *fli1:Gal4FF* (control) or *fli1:Gal4FF/UAS:aplnrb* (pan-vascular *aplnrb* overexpression) zebrafish embryos. Embryos with vascular *aplnrb* overexpression display a drastic reduction in *runx1* expression compared with control siblings. (F) Phenotypic distribution plot of embryos in (D, E) scored with low, medium and high *runx1* expression ($n = 30$, Chi-square test, $P = 0.0088$). (G, H) WISH for *runx1* in *gata2b:KaltA4* (control) or *gata2b:KaltA4/UAS:aplnrb* (hemogenic endothelium specific overexpression of *aplnrb*) zebrafish embryos. Embryos with HE specific *aplnrb* overexpression display no differences in *runx1* expression compared with control siblings. (I) Phenotypic distribution plot of embryos in (G, H) scored with low, medium and high *runx1* expression ($n = 57$, Chi-square test, $P = 0.5188$). HE hemogenic endothelium. Quantifications are displayed as mean $\pm$ SD. Scale bars: 100 μ m.



◀ **Figure EV7. *aplnrb* repression in HEC is independent of major regulators of embryonic hematopoiesis.**

(A–H') Confocal projection images of the trunk region of *Tg^{BAC}(aplnrb:Venus-PEST); Tg(kdrl:NLS-mCherry)* double transgenic zebrafish embryos. *aplnrb:Venus-PEST* negative HECs can be detected in the ventral DA (cyan arrowheads). (A, A') Trunk region of DMSO treated control embryos corresponding to (B–F'), imaging at 40 hpf. (B, B') Trunk region of Notch inhibitor (1 μ M RO4929097) treated embryos, treatment from 6–15 hpf, imaging at 40 hpf. WT-like *aplnrb:Venus-PEST* expression in the ventral DA can be observed. (C, C') Trunk region of Notch inhibitor (1 μ M RO4929097) treated embryos, treatment from 15–40 hpf, imaging at 40 hpf. WT-like *aplnrb:Venus-PEST* expression in the ventral DA can be observed. (D, D') Trunk region of BMP inhibitor (10 μ M DMH1) treated embryos, treatment from 12–40 hpf, imaging at 40 hpf. WT-like *aplnrb:Venus-PEST* expression in the ventral DA can be observed. (E, E') Trunk region of Wnt activator (0.15 M LiCl) treated embryos, treatment from 12–40 hpf, imaging at 40 hpf. WT-like *aplnrb:Venus-PEST* expression in the ventral DA can be observed. (F–F') Trunk region of Shh activator (20 μ M Purmorphamine) treated embryos, treatment from 16–40 hpf, imaging at 40 hpf. WT-like *aplnrb:Venus-PEST* expression in the ventral DA can be observed. (G–H') Trunk region of siblings and *runx1* $-/-$ mutant zebrafish embryos at 40 hpf. WT-like *aplnrb:Venus-PEST* expression can be observed in the ventral DA of *runx1* $-/-$ mutant embryos. HE hemogenic endothelium, EC endothelial cell, DA dorsal aorta. Scale bars: 20 μ m.

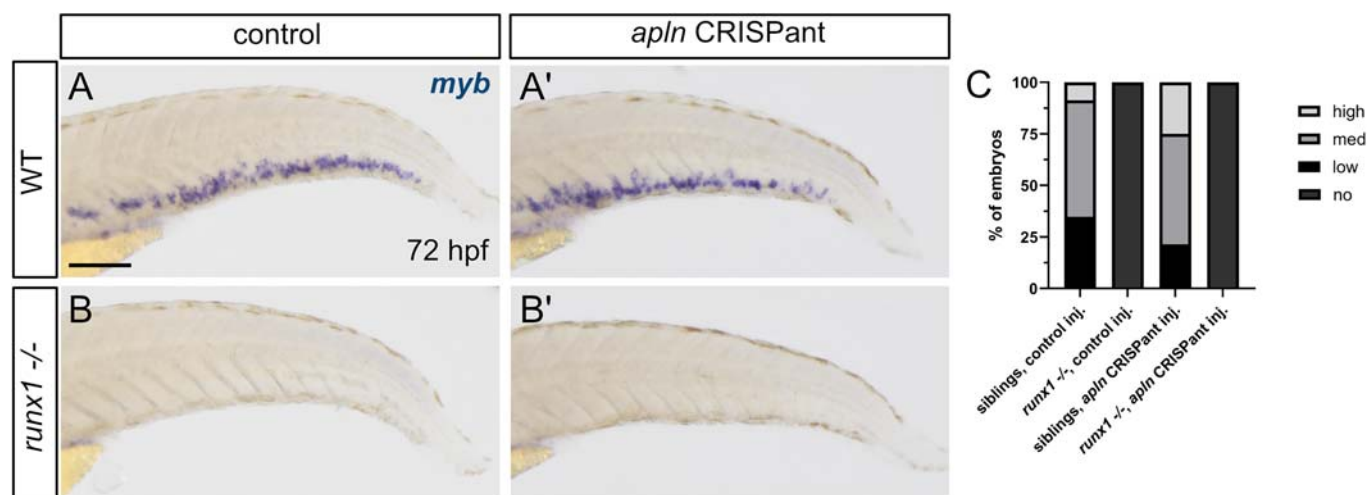


Figure EV8. Elevated HSPC numbers upon loss of Apelin signaling are dependent on Runx1 function.

(A, B) WISH for *myb* at 72 hpf in *runx1*^{-/-} mutant larvae and siblings. Images of the CHT region. (A, A') WISH for *myb* in control injected and *apl*n CRISPR injected wild-type siblings. Loss of Apelin signaling results in increased *myb* expression in the CHT compared with control injected siblings. (B, B') WISH for *myb* in control injected and *apl*n CRISPR injected *runx1*^{-/-} mutant larvae. Loss of Apelin signaling does not rescue *myb* expression in the CHT. (C) Phenotypic distribution plot of embryos in (A, B) scored for no, low, medium and high *myb* expression ($n = 71$). CHT caudal hematopoietic tissue. Scale bars: 200 μ m.