Supplementary Tables

ef1a F	GAGAAGTTCGAGAAGGAAGC
ef1a R	CGTAGTATTTGCTGGTCTCG
hapln1b F	ACAACTTTGAGGAGGCGTACA
hapln1b R	TGTGCTGTGTCGACTGAGAGT
kitlgb F	ATGGCATGAACTCTGCGGTT
kitlgb R	CGCATTCTTGCTCCACAACG

Table S1. QPCR primers

hapln1b ISH probe F	CAGAGCTGACAACTTTGAGG
hapln1b ISH probe R	ATCCCATAGTTCCTGACTCC
hapln1b full length F	AAAGAATTCAAGATGACCTTCCTGCTCCT
hapln1b full length R	AAACTCGAGGCGAACTCAGTTGTTGCTCT
Hapln1b R (STOP at the endo of exon 3)	AAACTCGAGTCAACCTTGTAGGTCAAGAGAAAC
Hapln1b full length R- HA tag	AAACTCGAGAACTCAAGCGTAATCTGGAACATCGTATGGGTAGTTG TTGCTCTTG
Hapln1b R (STOP at the endo of exon 3)- HA tag	AAACTCGAGTCAAGCGTAATCTGGAACATCGTATGGGTAACCTTGTA GGTCAAGAGAAAC

Table S2. Cloning primers

Std Ctl	CCTCCTACCTCAGTTACAATTTATA
hapln1b	ATCCTAAACATCCTCTCACCTGAGA

Table S3. Morpholinos

Supplementary Movies

Movie S1-S2

CHT region of ctl morphant (Movie S1) or *hapln1b* morphant (Movie S2) at 30hpf in *globin:GFP* embryos.

Movies S3-6

Time lapse imaging of AGM region from 32hpf to 42hpf in non-injected (Movie S3-4) and *hapln1b* mRNA injected (Movies S5-6) in *cmyb:GFP/flk1:mCherry* embryos.

Movie S7-8

AGM region at 28hpf of non-injected (Movie S7) or *hapln1b* mRNA injected (Movie S8).

Supplementary Figures

Figure S1

A

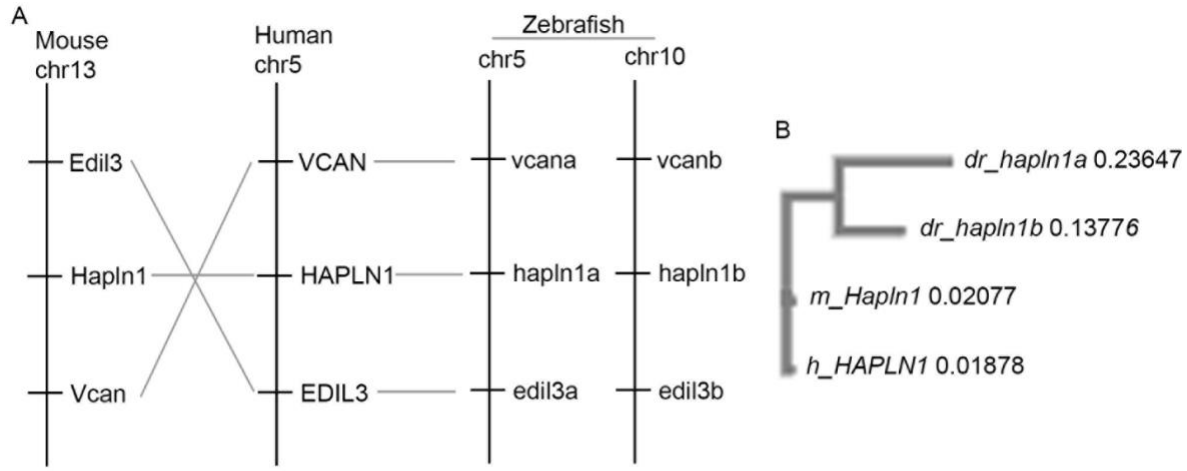
Gene	Expression pattern	Reference
hapln1a	Multiple cranial structures and pectoral fins	Kange et al., Zoological science, 2008
	Heart	Derrick et al., Cardiovascular Res, 2021
hapln1b	Endothelial cells, HSCs, EMPs and cranial cartilage	This study
hapln2	Otic vesicle	ZFIN
hapln3	Median fin fold, weak in cadinal vein and post. ISVs	ZFIN
hapln4	Unknown	–

B

Figure S1. Comparison of hapln family member protein structures in zebrafish

(A) Summary of known expression pattern of other members of the *hapln* family in zebrafish. ZFIN: https://zfin.org/. (B) Amino acid sequence alignment (using uniprot) with highlighted signal peptide and disulphide bonds of zebrafish hapln family and percentage identity compared to hapln1b as calculated by clustal 2.1. Q1LXE1= *hapln1a*, E7FBW8= *hapln1b*, A2CF10=*hapln2*, Q6NV41=*hapln3*, Q1LV25=*hapln4*.

Figure S2



C

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HPLN1_HUMAN          1   MKSLLLLVLISICWADHL-SDNYT-LDHDRAIHIQAENGPHLLVEAEQAKVFSHRGGNVT      58
HPLN1_MOUSE          1   MRSLLLLVLISVCWADHHLSDSYTPPDQDRVIHIQAENGPRLLVEAEQAKVFSHRGGNVT      60
Q1LXE1_DANRE         1   MIALFSVALMV--FA-------------SSLHV------EAQDSESRVKVYANRGDNIT      38
E7FBW8_DANRE         1   MTFLLLFLLVLSESRADNFEEAYSDLETYRTIY-ISENGPRLSVDTAQPKVISQRGGNAT      59
                         *  *: . *:                      ::       .   .  : ** ::**.* *

HPLN1_HUMAN         59   LPCKFYRDPTAFGSGIHKIRIKWTKLTSDYLKEVDVFVSMGYHKKTYGGYQGRVFLKGGS      118
HPLN1_MOUSE         61   LPCKFYRDPTAFGSGIHKIRIKWTKLTSDYLREVDVFVSMGYHKKTYGGYQGRVFLKGGS      120
Q1LXE1_DANRE        39   LPCRLDGQSTLFGN-----RIKWTKIEDD-STETDVLISMGFHKKAYGSYQDRAYLLEVD       92
E7FBW8_DANRE        60   LPCKFNRDSSLP--PNPKLRIKWTKLTSDYLKEIDVFVAMGFHKKSYGRFHGRVHLQAAS      117
                         ***::  : :         ******: .*   * **:::**:***:** ::.*..*   .

HPLN1_HUMAN         119  DSDASLVITDLTLEDYGRYKCEVIEGLEDDTVVVALD--------LQGVVFPYFPRLGRY      170
HPLN1_MOUSE         121  DNDASLVITDLTLEDYGRYKCEVIEGLEDDTAVVALE--------LQGVVFPYFPRLGRY      172
Q1LXE1_DANRE         93  DSDATLVITDLDVNDYGTYRCEFIDGMNDMTLEVDLEVQGKDSLSFTGVVFPYSPRLGRY      152
E7FBW8_DANRE        118  ESDATLVITEITLEDYGKYKCEVIDGLEDDTAVVSLD--------LQGIVFPYFPRLGRY      169
                         :.**:****:: ::*** *:**.*:*::* *  * *:        : *:**** ******

HPLN1_HUMAN         171  NLNFHEAQQACLDQDAVIASFDQLYDAWRGGLDWCNAGWLSDGSVQYPITKPREPCGGQN      230
HPLN1_MOUSE         173  NLNFHEARQACLDQDAVIASFDQLYDAWRGGLDWCNAGWLSDGSVQYPITKPREPCGGQN      232
Q1LXE1_DANRE        153  NLNFQNAVAACQEQDAVVASFEQLYGEWKSGLDWCNAGWLSDGTVQYPITKPREPCGGAR      212
E7FBW8_DANRE        170  NLNFLEAEKACRDQDAIVASFEQLYDAWRDGLDWCNAGWLSDGSVQYPITKPREPCGGKN      229
                         **** :*  ** :***::***:***. *:.************:************** .

HPLN1_HUMAN         231  TVPGVRNYGFWDKDKSRYDVFCFTSNFNGRFYYLIHPTKLTYDEAVQACLNDGAQIAKVG      290
HPLN1_MOUSE         233  TVPGVRNYGFWDKDKSRYDVFCFTSNFNGRFYYLIHPTKLTYDEAVQACLNDGAQIAKVG      292
Q1LXE1_DANRE        213  ATAGLRNYGQRDKSNSRYDVFCFTAGLKGRFYYMIQPKKLNFDEAVDACKGDGAEIAKVG      272
E7FBW8_DANRE        230  TIPGVRNYGIRDKQKEQYDVFCFTSNYKGRFYYLIHSSKLTYDEAVQACQKDGAQIAKVG      289
                         :  *:****  **.:.:*******:. :*****:*: .**.:****:**  ***:*****

HPLN1_HUMAN         291  QIFAAWKILGYDRCDAGWLADGSVRYPISRPRRRCSPTEAAVRFVGFPDKKHKLYGVYCF      350
HPLN1_MOUSE         293  QIFAAWKLLGYDRCDAGWLADGSVRYPISRPRRRCSPTEAAVRFVGFPDKKHKLYGVYCF      352
Q1LXE1_DANRE        273  HMFAAWKLQGYDRCDAGWLADGSVRYPISRPRKNCSPTEAAVRFVGFPDKKQKLYGVYCY      332
E7FBW8_DANRE        290  QMYAAWKLLGYDRCDAGWLADGSVRYPISKPRRRCSPTEAAVRFSGFPDKKHKLYGVYCY      349
                         :::****: ********************:**:.********** ******:*******:

HPLN1_HUMAN         351  RAYN 73.79%
HPLN1_MOUSE         353  RAYN 73.09%
Q1LXE1_DANRE        333  KAQQ 62.58%
E7FBW8_DANRE        350  KSNN
                         :: :
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percentage identity (clustal2.1), compared to hapln1b

☑ Disulfide bond
☑ Propeptide
☑ Signal peptide
☑ Glycosylation

Figure S2. *hapln1b* and *hapln1a* arise from duplication of the mammalian orthologue

(A) Synteny analysis, showing duplication of the mammalian *hapln1* to give *hapln1a* and *hapln1b* in zebrafish. (B) phylogenetic analysis of *hapln1b* and its comparison to *hapln1a* in zebrafish (dr) and hapln1b in mice (m) and humans (h) as calculated by clustal 2.1. (C) Amino acid sequence alignment of zebrafish *hapln1b, and hapln1a, Hapln1 (mouse) and HAPLN1 (human)* with highlighted disulphide bond, propeptide, signal peptide and glycosylation site (analysed using uniprot). Percentage identity is compared to *hapln1b* as calculated by clustal 2.1. Q1LXE1= hapln1a, E7FBW8= hapln1b.

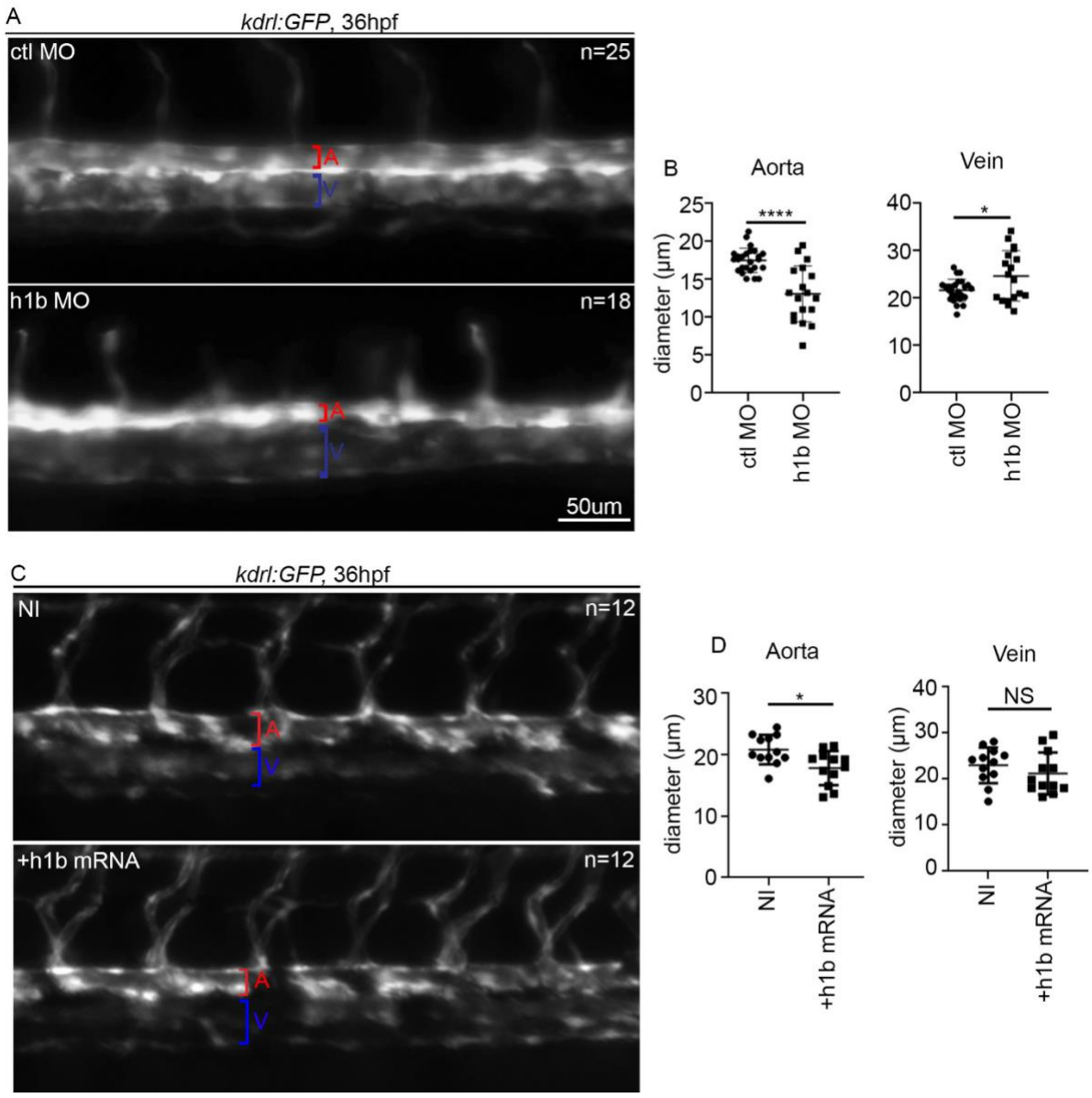

Figure S3. *Hapln1b* expression is required for correct artery and vein formation.

(A) Imaging of *kdrl:GFP* embryos after ctl MO or hapln1b MO injection at 36hpf. (B) analysis of aorta/vein diameter. For aorta, $p<0.0001$ (Student's unpaired t test). For vein, $p=0.0159$ (Student's unpaired t test). (C) Imaging of *kdrl:GFP* non-injected or *hapln1b* mRNA injected embryos at 36hpf. (D) analysis of aorta/vein diameter. For aorta, update after converting to um.

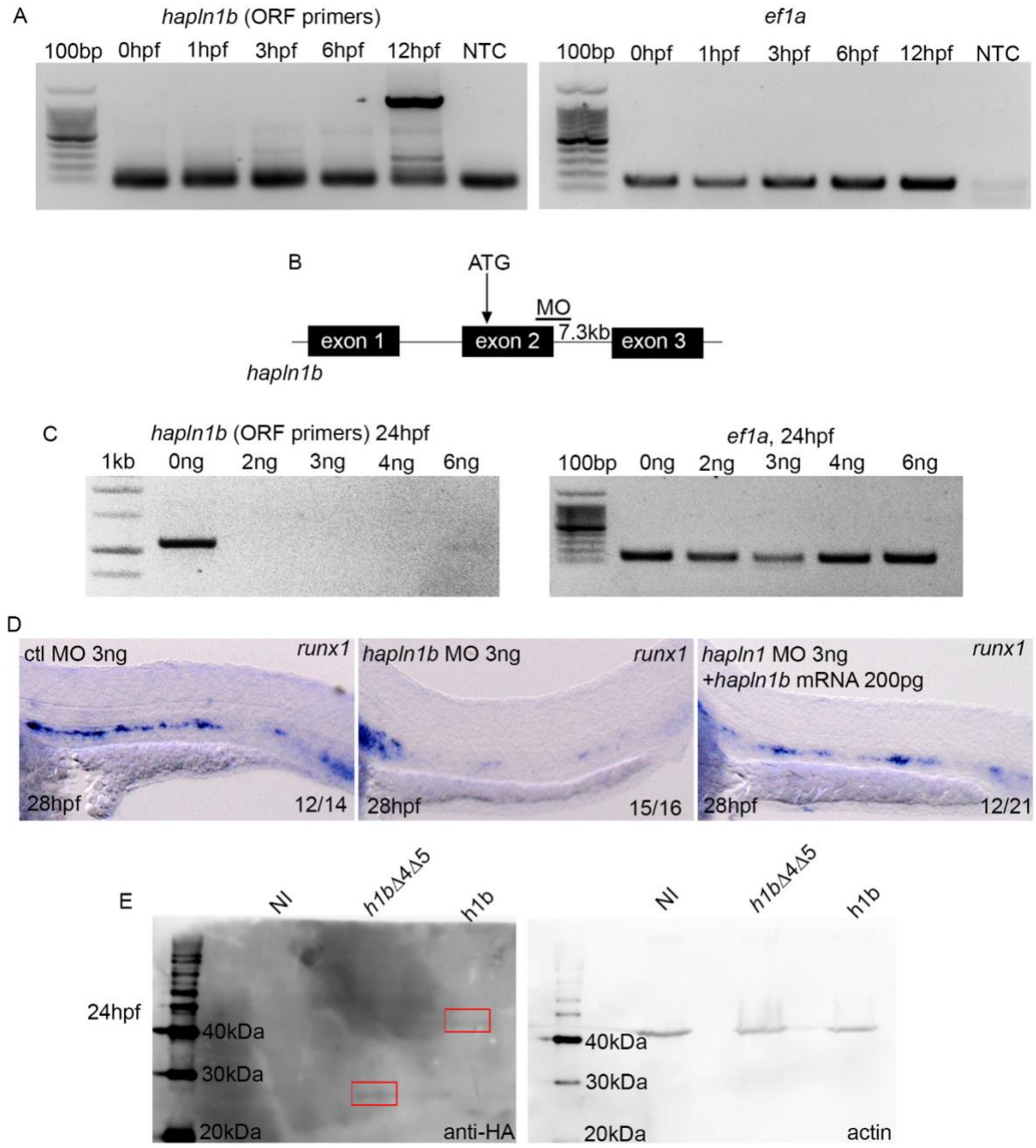

Figure S4. Validation of the *hapln1b* MO and mRNA injections

(A) *hapln1b* expression by PCR from pools of 8-10 embryos extracted at the indicated timepoints. Primers for cloning full length hapln1b were used. *Ef1a* was used as a housekeeping control. NTC, no template control. (B) Schematic indicating MO target site, schematic is not to scale. (C) PCR after injection of the hapln1b MO at the indicated timepoints. RNA was extracted from pools of 8-10 embryos at 24hpf. *Hapln1b* full length

primers used (ORF: open reading frame). *Ef1a* was used as a housekeeping control. (D) ISH expression of *runx1* in control morphants, *hapln1b* morphants and *hapln1b* morphants injected with *hapln1b* mRNA. (E) Western blot of non-injected (NI), HA tagged truncated *hapln1b* (h1bΔ4Δ5) injected or *hapln1b* (h1b) injected embryos at 24hpf.

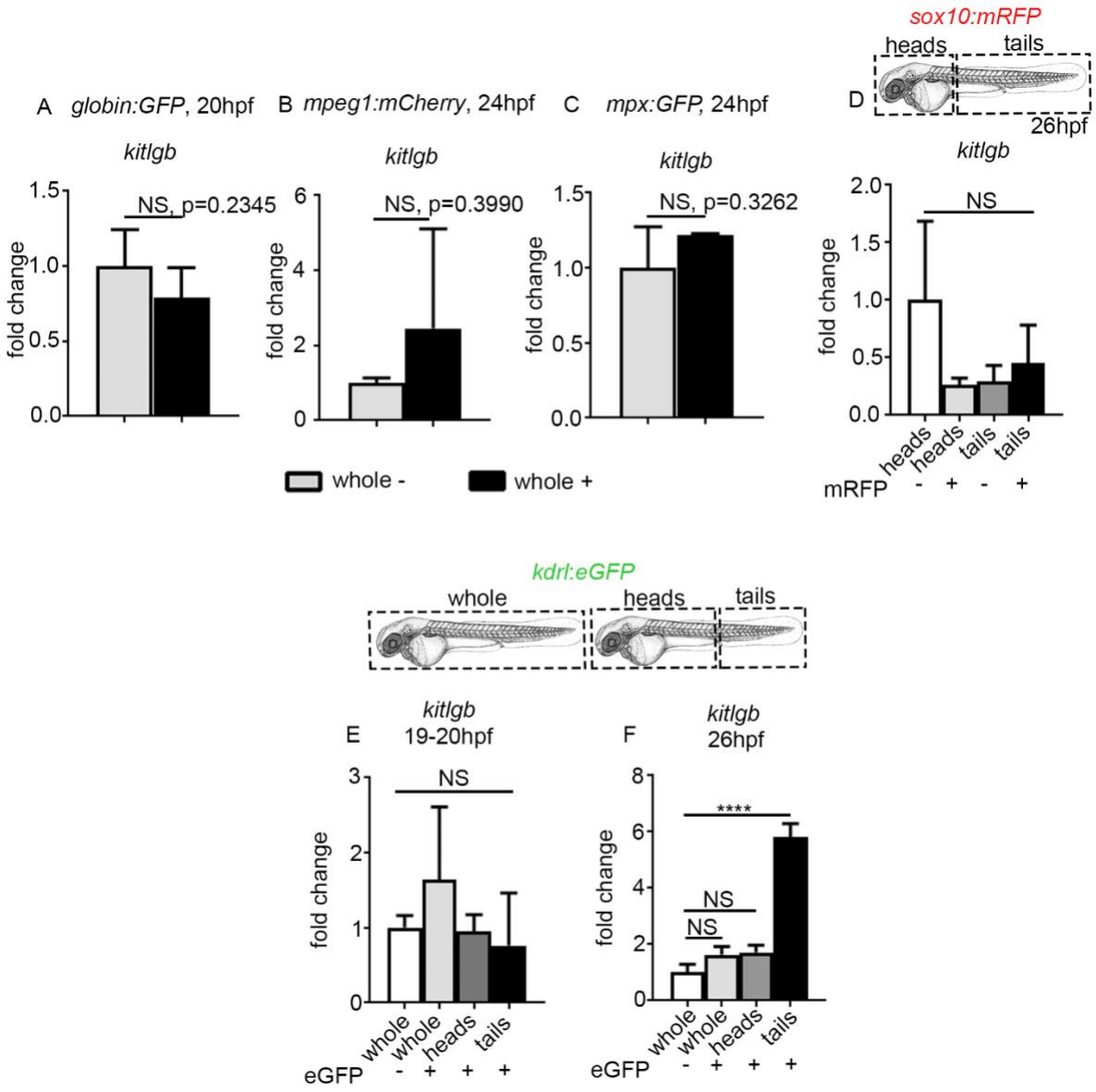

Figure S5. *kitlgb* is specifically expressed by caudal endothelial cells

(A-D) qPCR expression of *kitlgb* in either negative cells or positive cells from the indicated transgenic lines. In A-C, data is analysed using a two-tailed Student's t-test, A: p=0.2345, B: p=0.3990, C: p=0.3262. In D, cells were sorted from dissection of heads or tails as indicated in the schematic, data was analysed using one-way anova. Heads- vs. heads+: p=0.1137, heads- vs. tails -: p=0.1294, heads- vs. tails+: p=0.2708. (E,F) Experimental outline and qPCR expression of *kitlgb* from FACS sorted endothelial cells from different dissections at either 19-20hpf or 26hpf. Data was analysed using one way anova. In E,

whole- vs. whole+: p=0.4839, whole- vs. head +: p=0.9994, whole- vs. tail+: p=0.9338. In F, whole- vs. whole+: p=0.1300, whole- vs. head +: p=0.0866, whole- vs. tail+: p<0.0001.

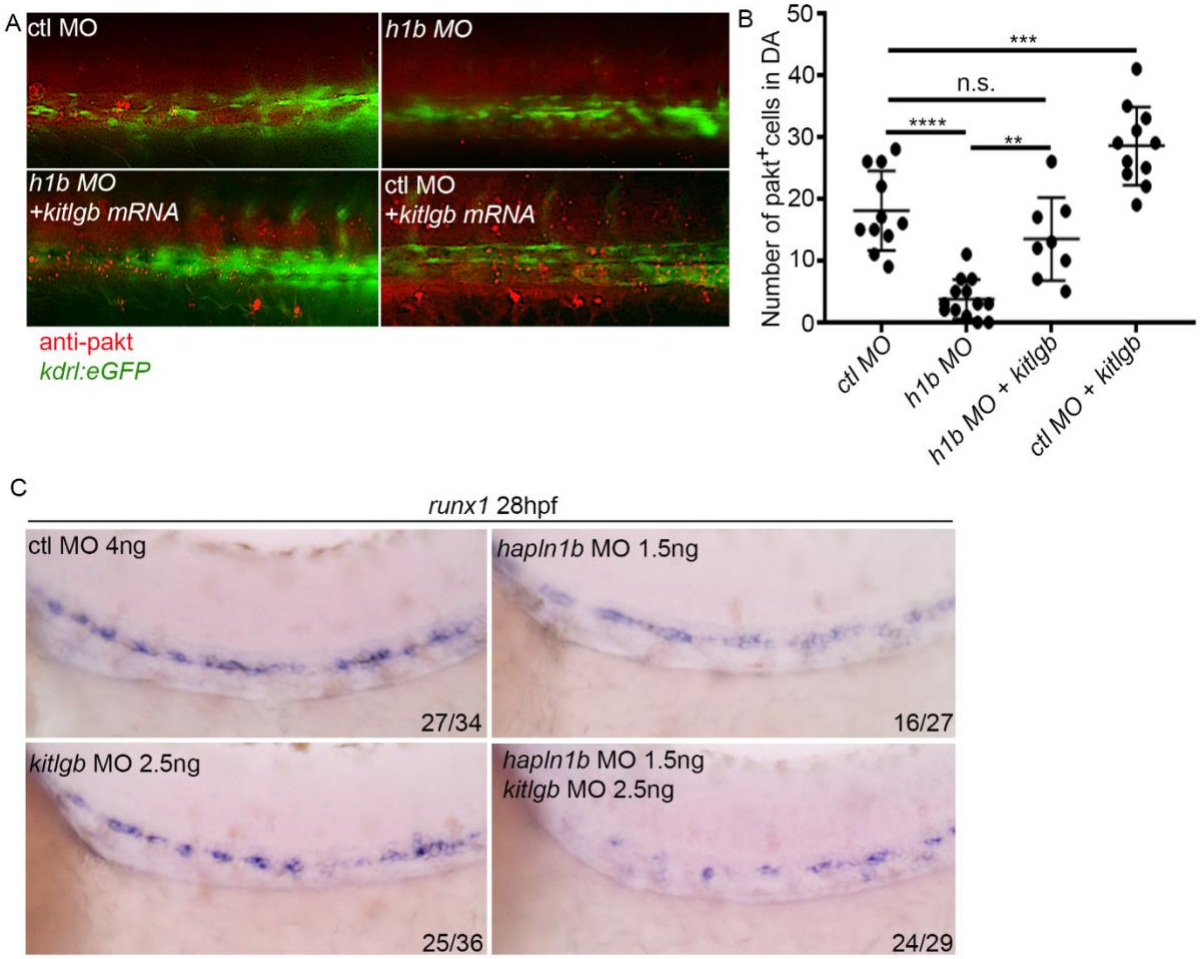

Figure S6. *Hapln1b* is required for *kitlgb* signalling in dorsal aorta cells

(A) p-akt staining by immunofluorescence to quantify the number of positive cells in *kdrl:GFP* embryos, at 26hpf, in the DA region after control MO injection (ctl MO), hapln1b MO injection (h1b MO), hapln1b MO and *kitlgb* mRNA injection (h1b MO + *kitlgb* mRNA) and control MO and *kitlgb* mRNA injection (ctl MO + *kitlgb* mRNA). (B) Quantification of phosphor-akt (pakt) positive cells in the dorsal aorta. Data was analysed using one way anova, ctl MO vs h1b MO $p<0.0001$, ctl MO vs h1b MO+*kitlgb* $p=0.314$, h1b MO vs h1b MO+*kitlgb* $p=0.0025$, ctl MO vs ctl MO +*kitlgb* $p=0.0006$. (C) *runx1* expression in embryos at 28hpf injected with control MO (ctl MO), hapln1b MO (injected at half dose), kitlgb MO

(injected at half dose) and both hapln1b MO (injected at half dose) and kitlgb MO (injected at half dose).