
Article:
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Supplemental Data

Supplemental Methods

Zebrafish husbandry and experimentation.

Zebrafish were kept at 28.5˚C as described in the zebrafish book[1]. Genetically altered zebrafish are listed in Table S1. Embryos older than 30 hpf that were meant to be used in WISH experiments were treated with N-phenylthiourea from about 20 hpf (see Table S2 for chemicals). All procedures on animals were performed by licensed staff under the authority of the UK Home Office project licenses 40/3457, 40/3708, 30/3356 and 30/3378.

Table S1: Zebrafish lines used in this study.

Name of line	Abbreviation	Reference
gfi1aa^{qmc551Gt}	*qmc551*	[2]
gfi1aa^{sa11633}	*sa11633*	[3]
gfi1b^{sh339}	*sh339*	this manuscript
gfi1b^{qmc554}	*qmc554*	this manuscript

Table S2: Information on enzymes and selected chemicals

Chemical/Inhibitor	Company	Cat. No.	Instructions
Dulbecco's phosphate buffered saline (DPBS)	Invitrogen (GIBCO)	14200-067	Dilute 10x stock to 1x working solution
Hanks' buffered salt solution (HBSS)	Invitrogen (GIBCO)	14065	Dilute 10x stock to 1x working solution
Liberase Blendzyme	Sigma-Aldrich (Roche)	05 401 020 001	Dilute 50 mg in 1.86 mL distilled H_2O. Make 160 µL aliquots of stock solution. Mix one aliquot with 2 mL HBSS to generate working solution
MS222	Sigma-Aldrich	A5040	Stock at 4 g/l H_2O, pH7.5; use at 130 µg/ml
N-Phenylthiourea	Sigma-Aldrich	P7629	Stock at 0.3g/l H_2O, use at 0.03 g/l
Giemsa	BDH	350864X	Use 1:20 diluted

DPX Mountant for Histology	Fluka BioChemika	44581	Apply directly to dried slide and put cover slip on top

RNA isolation from FAC-sorted embryonic cells

Heterozygous and homozygous *qmc551* embryos were collected from crosses of *qmc551* homozygous females to *wt* males and to homozygous *qmc551* males, respectively. Half of the embryos of each batch were injected with 0.5 ng of two *gfi1b* morpholinos and 0.5 ng of *p53* morpholino as described previously[2]. Morpholino sequences are provided in Table S3. All embryos were grown to the 20 hpf. Embryos were dechorionated using a pronase solution (2.5 mg/mL). Embryos were washed first in E3 buffer and then in 1x Hanks' Balanced Salt Solution (10x HBSS buffer; Gibco, 14065 distributed by Invitrogen). Two hundred embryos were dissociated in 500 µL of a Liberase Blendzyme solution at 33°C. Embryos were pipetted up and down with a P200 pipette every 10 min for a total of about 40 to 60 min until all yolk cells were detached and the embryos were dissociated. The cell suspensions were moved onto ice. Cells were filtered through a 40 µm pore nylon filter (Falcon, 352340, distributed by VWR) into 50 mL conical centrifuge tubes. Cells were pelleted at 1,500 rpm (~400g) at 4 °C for 5 min in a Heraeus Multifuge 3 S-R. The supernatant was removed and then replaced with 10 mL sort buffer (1x Dulbecco's phosphate buffered saline, 2% foetal calf serum, 25 mM Hepes and 5 mM EDTA). Cells were filtered and pelleted again (as above), before being resuspended in 200 µL of sort buffer. Cells were analyzed on a Beckman Coulter MoFlo Astrios with a 488 nm laser. Fluorescence was detected through a 529/28 nm band pass filter. For gating purposes, wildtype cells were isolated from embryos that were derived from an outcross of a heterozygous *qmc551* female to a wt male. Wildtype embryos in the batch showed maternal GFP expression similar to the experimental embryos. Gating these cells allowed the exclusion of cells with only maternal GFP fluorescence. Cells from all samples were sorted three consecutive times, with the second sort always reaching a purity of at least 80%. Three batches of between 1,700 and 4,000 cells were collected in 100 µL of sort buffer for each experiment. Cells were pelleted and the supernatant decanted. Cells were lysed in 300 µL RLT Plus buffer with **β**-mercaptoethanol (10 µL per 1 mL of RLT Plus buffer

provided with the RNeasy Plus Micro Kit (Qiagen, 74034)). Genomic DNA was removed on gDNA eliminator columns and the RNA was cleaned up on RNeasy minElute spin columns following the manufacturer's instructions. RNA was eluted with 14 µL of RNase-free H_2O. RNA QC was done using Agilent RNA 6000 Pico kit (Agilent Biotechnologies, 5067-1513). Between 2.7 and 10.5 ng of total RNA was obtained from 1,700 to 4,000 cells. All RNAs achieved RIN numbers of 8 and higher.

Table S3: Morpholinos.

Experiment	Oligo Name	Sequence Reference
gfi1b exon 4 splice morpholino 1	MO1	5' –TCTGATGGAGGTATGATGGAAACAT-3' [2]
gfi1b exon 4 splice morpholino 2	MO2	5'-GCTGTGTTCACTATCTGACCTTGTC-3' [2]
p53 ATG morpholino	p53 MO	5'-GCGCCATTGCTTTGCAAGAATTG-3' [4]
etv2 5'UTR morpholino	*etv2* MO	5'-CACTGAGTCCTTATTTCACTATATC-3' [5,6]

RNA sequencing and Bioinformatics

1.9 ng of total RNA was used to generate and amplify full length cDNA of polyadenylated transcripts for sequencing using SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing (Takara, 634889). Sequencing libraries were prepared from full length cDNA using Nextera XT library preparation kit (Illumina, FC-131-1096, FC-131-1002). Library QC was performed using Agilent DNA High Sensitivity kit (Agilent biotechnologies, 5067-4626). Sequencing library concentrations were determined through qPCR (Kapa Biosystems, KK4824). Sequencing was done on an equimolar library pool using Illumina NextSeq500 sequencing platform using 2x75bp V2 chemistry according to manufacturers' guidelines (Illumina, FC-404-2002).

Raw reads were trimmed to remove adaptor and low quality sequences using Scythe (https://github.com/vsbuffalo/scythe) and Sickle (https://github.com/najoshi/sickle), respectively. The trimmed reads are available from ENA under accession number PRJEB25583. These reads were filtered to remove tRNA and rRNA sequences. Filtered reads were subsequently mapped onto

the zv9 reference genome using the TopHat mapping tool (https://ccb.jhu.edu/software/tophat/index.shtml). Information on the number of trimmed, filtered and mapped sequence reads for each sample is provided in Table S4. The read alignments were recorded in BAM formatted alignment files. Companion BAM index files were generated. Bam files were visualized with the Integrative Genomics Viewer (IGV, (http://software.broadinstitute.org/software/igv/) and with SeqMonk (https://www.bioinformatics.babraham.ac.uk/projects/seqmonk/). Read counts for each gene were calculated using 'htseq-count' (http://htseq.readthedocs.io/en/release_0.9.0/). Only uniquely and correctly aligned reads were used to generate counts per gene. Read counts were normalized against the length of every gene's exon model and against the total number of mapped reads. Normalized values are provided as RPKM (reads per kilobase of exon model per million reads)[7]. GraphPad Prism was used for standard statistics applications. Advanced count-based statistics was performed using the DE-Seq method[8] to produce the MA plots shown in Fig. 2D and 2M. Endothelial and erythroid genes highlighted in the MA plot shown in Fig. 2M are listed in Table S5.

Table S4: Information on the number of trimmed, filtered and mapped sequence reads. Sequence reads were trimmed and filtered using Scythe and Sickle software. Filtered reads were mapped using TopHat software. The table summarizes the number of trimmed, filtered and mapped sequence reads derived from the DNA libraries generated on full-length cDNA reverse transcribed on polyadenylated RNA isolated from 20 somite stage GFP+ cells isolated from heterozygous *qmc551* embryos, *gfi1b* morpholino-injected heterozygous *qmc551* embryos, homozygous *qmc551* embryos and *gfi1b*-morpholino-injected homozygous *qmc551* embryos.

Sample	TrimmedReads	FilteredReads	MappedReads		Uniquely and Correctly Mapped Reads	
			Count	Percentage	Count	Percentage
Het1	88,466,580	85,422,004	74,118,229	86.77%	63,089,028	73.86%
Het2	79,519,376	76,739,726	66,777,069	87.02%	56,862,346	74.10%
Het3	84,780,446	82,037,832	71,404,026	87.04%	60,918,890	74.26%
HetMO1	82,671,702	80,214,178	70,009,095	87.28%	60,507,330	75.43%
HetMO2	79,036,454	76,463,384	66,820,002	87.39%	57,619,748	75.36%
HetMO3	83,182,998	80,506,118	70,104,145	87.08%	60,556,414	75.22%
Hom1	89,726,254	86,187,382	74,900,243	86.90%	65,229,184	75.68%
Hom2	77,274,014	74,249,960	64,468,007	86.83%	56,124,066	75.59%
Hom3	90,140,318	86,328,686	74,598,523	86.41%	64,662,084	74.90%
HomMO1	86,129,542	83,492,948	72,639,859	87.00%	63,169,918	75.66%
HomMO2	77,721,362	75,455,238	65,523,086	86.84%	57,007,298	75.55%
HomMO3	76,777,196	74,078,006	64,541,722	87.13%	56,302,842	76.00%

Table S5: List of endothelial (green) and erythroid (red) genes highlighted in the MA plot shown in Fig. 2M of the main manuscript

Endothelial Genes			
Probe	Chr	Start	End
smox-001 Chr1:42236840-42248137 FOR (11.2 kbp)	1	42236840	42248137
ap1s3b-201 Chr2:47710480-47724793 REV (14.3 kbp)	2	47710480	47724793
ramp2-201 Chr3:36832716-36841242 REV (8.5 kbp)	3	36832716	36841242
notch3-201 Chr3:54168014-54197194 REV (29.1 kbp)	3	54168014	54197194
stab2-001 Chr4:9786418-9827747 FOR (41.3 kbp)	4	9786418	9827747
tek-201 Chr5:840269-863799 FOR (23.5 kbp)	5	840269	863799
agtr2-001 Chr5:24916909-24917991 FOR (1 kbp)	5	24916909	24917991
dab2-001 Chr5:33963456-33972240 REV (8.7 kbp)	5	33963456	33972240
notch1b-001 Chr5:68668507-68771307 REV (102.8 kbp)	5	68668507	68771307
ephb4a-201 Chr5:70801978-70856493 FOR (54.5 kbp)	5	70801978	70856493
tie1-001 Chr6:33682045-33706271 REV (24.2 kbp)	6	33682045	33706271

cdh5-202 Chr7:45444316-45455076 FOR (10.7 kbp)	7	45444316	45455076
mrc1a-201 Chr7:65408686-65468212 REV (59.5 kbp)	7	65408686	65468212
plk2b-001 Chr8:17598123-17605185 FOR (7 kbp)	8	17598123	17605185
efnb2a-001 Chr9:27137159-27157051 FOR (19.8 kbp)	9	27137159	27157051
erg-201 Chr10:117107-122303 FOR (5.1 kbp)	10	117107	122303
tmem88a-001 Chr10:22976133-22979486 FOR (3.3 kbp)	10	22976133	22979486
cldn5b-001 Chr10:45496701-45497363 FOR (663 bp)	10	45496701	45497363
egfl7-201 Chr11:38486348-38507545 FOR (21.1 kbp)	11	38486348	38507545
aqp8a.1-001 Chr12:21718815-21725518 REV (6.7 kbp)	12	21718815	21725518
flt4-001 Chr14:19717288-19856559 REV (139.2 kbp)	14	19717288	19856559
kdrl-201 Chr14:33840326-33899405 REV (59 kbp)	14	33840326	33899405
ldb2a-001 Chr14:49620323-49768406 REV (148 kbp)	14	49620323	49768406
mcamb-001 Chr15:22627036-22696183 REV (69.1 kbp)	15	22627036	22696183
sesn3-201 Chr15:34339411-34386820 FOR (47.4 kbp)	15	34339411	34386820
RASIP1-201 Chr16:14272315-14310785 FOR (38.4 kbp)	16	14272315	14310785
she-001 Chr16:24768468-24783484 FOR (15 kbp)	16	24768468	24783484
etv2-001 Chr16:44778571-44783094 REV (4.5 kbp)	16	44778571	44783094
fli1b-002 Chr16:44787606-44815039 FOR (27.4 kbp)	16	44787606	44815039
clec14a-001 Chr17:10357902-10359008 REV (1.1 kbp)	17	10357902	10359008
CD93-201 Chr17:45726703-45728361 REV (1.6 kbp)	17	45726703	45728361
lyve1b-001 Chr18:16403529-16406850 FOR (3.3 kbp)	18	16403529	16406850
fli1a-002 Chr18:46987627-47050017 FOR (62.3 kbp)	18	46987627	47050017
sox7-001 Chr20:19147468-19149714 FOR (2.2 kbp)	20	19147468	19149714
kdr-001 Chr20:22293376-22348625 FOR (55.2 kbp)	20	22293376	22348625
dll4-001 Chr20:28275194-28282936 FOR (7.7 kbp)	20	28275194	28282936
sele-001 Chr20:33996012-34001480 REV (5.4 kbp)	20	33996012	34001480
hey2-001 Chr20:39615275-39621875 REV (6.6 kbp)	20	39615275	39621875
notch1a-204 Chr21:5028934-5036108 REV (7.1 kbp)	21	5028934	5036108
notch1a-201 Chr21:5057239-5083787 FOR (26.5 kbp)	21	5057239	5083787
notch1a-002 Chr21:5088100-5088163 REV (64 bp)	21	5088100	5088163
dusp5-001 Chr22:32601322-32612453 REV (11.1 kbp)	22	32601322	32612453
mafba-201 Chr23:3422313-3423383 REV (1 kbp)	23	3422313	3423383
sox18-001 Chr23:8904266-8906769 FOR (2.5 kbp)	23	8904266	8906769
krt18-201 Chr23:10454077-10457614 FOR (3.5 kbp)	23	10454077	10457614
her9-001 Chr23:23693973-23695173 REV (1.2 kbp)	23	23693973	23695173

gpr182-001 Chr23:36700336-36701412 FOR (1 kbp)	23	36700336	36701412
ephb4b-201 Chr23:44476466-44527722 FOR (51.2 kbp)	23	44476466	44527722
flt1-001 Chr24:22358980-22416554 REV (57.5 kbp)	24	22358980	22416554
sh3gl3b-001 Chr25:20013171-20025548 FOR (12.3 kbp)	25	20013171	20025548
Erythroid Genes			
Probe	Chr	Start	End
clgn-001 Chr1:40966162-41000205 REV (34 kbp)	1	40966162	41000205
prdx2-001 Chr1:52217037-52223503 FOR (6.4 kbp)	1	52217037	52223503
tfr1a-001 Chr2:4738513-4756725 REV (18.2 kbp)	2	4738513	4756725
urod-001 Chr2:27704376-27723829 REV (19.4 kbp)	2	27704376	27723829
cahz-001 Chr2:29192910-29199877 REV (6.9 kbp)	2	29192910	29199877
klf4b-001 Chr2:33044469-33046240 FOR (1.7 kbp)	2	33044469	33046240
slc4a1a-001 Chr3:19955787-19977887 REV (22.1 kbp)	3	19955787	19977887
pigq-001 Chr3:26212433-26224514 FOR (12 kbp)	3	26212433	26224514
hbbe1.1-202 Chr3:55965826-55966464 FOR (639 bp)	3	55965826	55966464
hbae1-202 Chr3:55972112-55972706 REV (595 bp)	3	55972112	55972706
hbbe1.1-201 Chr3:55983018-55983643 FOR (626 bp)	3	55983018	55983643
hbae1-201 Chr3:55990173-55990764 REV (592 bp)	3	55990173	55990764
hbae3-202 Chr3:55998358-55999373 REV (1 kbp)	3	55998358	55999373
add2-001 Chr5:14991725-15028531 REV (36.8 kbp)	5	14991725	15028531
hmbsa-002 Chr5:32157627-32172574 FOR (14.9 kbp)	5	32157627	32172574
alad-001 Chr5:62952095-62964449 FOR (12.3 kbp)	5	62952095	62964449
ank1-003 Chr5:71805570-71930274 REV (124.7 kbp)	5	71805570	71930274
klfd-001 Chr6:9656485-9666473 REV (9.9 kbp)	6	9656485	9666473
tspo-201 Chr6:40509383-40512240 REV (2.8 kbp)	6	40509383	40512240
fth1a-201 Chr7:13755565-13760561 FOR (4.9 kbp)	7	13755565	13760561
txn-001 Chr7:25524574-25531535 REV (6.9 kbp)	7	25524574	25531535
cpox-201 Chr8:19121660-19137899 REV (16.2 kbp)	8	19121660	19137899
alas2-001 Chr8:21894049-21906355 FOR (12.3 kbp)	8	21894049	21906355
slc38a5a-201 Chr8:26468724-26476425 FOR (7.7 kbp)	8	26468724	26476425
slc25a37-001 Chr8:52578850-52604409 REV (25.5 kbp)	8	52578850	52604409
steap3-001 Chr9:879590-885678 FOR (6 kbp)	9	879590	885678
nt5c2l1-001 Chr10:17491066-17514776 REV (23.7 kbp)	10	17491066	17514776
dmtn-001 Chr10:20162200-20180477 FOR (18.2 kbp)	10	20162200	20180477

slc11a2-201 Chr11:268489-273356 REV (4.8 kbp)	11	268489	273356
gata1a-001 Chr11:26345782-26350835 REV (5 kbp)	11	26345782	26350835
nmt1b-001 Chr12:16513733-16522908 FOR (9.1 kbp)	12	16513733	16522908
hbbe3-001 Chr12:21691975-21693024 REV (1 kbp)	12	21691975	21693024
hbbe2-001 Chr12:21702428-21703046 REV (619 bp)	12	21702428	21703046
hbz-001 Chr12:21704974-21705956 FOR (983 bp)	12	21704974	21705956
uros-202 Chr12:50189064-50195981 REV (6.9 kbp)	12	50189064	50195981
abcb10-201 Chr13:24487030-24499769 FOR (12.7 kbp)	13	24487030	24499769
rhd-001 Chr13:46117582-46143172 REV (25.5 kbp)	13	46117582	46143172
hmbsb-001 Chr15:22866441-22883493 REV (17 kbp)	15	22866441	22883493
epb41b-001 Chr16:28700637-28738636 FOR (38 kbp)	16	28700637	28738636
ppox-202 Chr16:45060563-45074680 REV (14.1 kbp)	16	45060563	45074680
sptb-001 Chr17:38741022-38766260 FOR (25.2 kbp)	17	38741022	38766260
ampd3b-001 Chr18:16422256-16454142 REV (31.8 kbp)	18	16422256	16454142
blvrb-001 Chr18:44560126-44573884 FOR (13.7 kbp)	18	44560126	44573884
slc10a4-001 Chr20:23419226-23428468 REV (9.2 kbp)	20	23419226	23428468
snx3-001 Chr20:32444979-32471034 FOR (26 kbp)	20	32444979	32471034
fech-001 Chr21:1970835-1992908 FOR (22 kbp)	21	1970835	1992908
rfesd-001 Chr21:10254213-10256989 REV (2.7 kbp)	21	10254213	10256989
gpd1b-001 Chr22:6943109-6953790 REV (10.6 kbp)	22	6943109	6953790
tal1-001 Chr22:16759536-16765797 FOR (6.2 kbp)	22	16759536	16765797
nfe2-001 Chr23:36363157-36367611 REV (4.4 kbp)	23	36363157	36367611
tmem14c-201 Chr24:8536514-8541305 FOR (4.7 kbp)	24	8536514	8541305

Gene targeting in zebrafish

A published protocol was followed to target the *gfi1b* locus using transcription activator-like effector nucleases (TALENs)[9]. The sequence 5' TCCCCCGAGCGGCCT 3' targeted by the TALENs was located at the beginning of exon 3. The TALEN nuclease was assembled in pCAG-T7-TALEN(Sangamo)-Destination vector using the Golden Gate TALEN Kit with repeat-variable diresidues (RVDs) NN NN NI NI HD HD NI NI NI NI NN NI NN NG NN NG NI NI NI and HD NI HD NG NN NN NI NG HD NG NI HD HD NG NN. The mutation in *sh339* allele

destroys a *BslI* restriction site. In a second approach, the *gfi1b* gene was also targeted using CRISPR/Cas9 technology[10]. Suitable target sites within *gfi1b* exon sequences were identified with the help of the CRISPRscan UCSC track[11]. The two sequences targeted to generate the *qmc554* allele were 5' GGAGGAAACTCTGCCAGCTG 3' and 5' GGTGATGCAGTGGTATGTGT 3'. These are located on the template strand at the beginning and at the end of *gfi1b* exon 4, respectively. They had score values of 58 and 45. Guide RNAs targeting these sites were predicted not to also target any off-target sites. Complementary oligos representing the target site sequences (see Table S6: oligos DB567 and DB568, as well as DB584 and DB585) were designed, hybridized and cloned into the *BsaI*-digested guide RNA expression vector pDR274[10]. Successful cloning of the correct sequences was confirmed by Sanger sequencing (at Source Biosciences, Nottingham). Plasmids carrying the correct target sequences were linearized with *DraI* and run-off transcripts were generated in vitro using T7 RNA polymerase (Thermo Fisher, EP0111). After a 2 hour incubation at 37°C, samples were treated with *DNase*I (Ambion) for 15 min. RNA products were verified by agarose gel electrophoresis and measured on the NanoDrop spectrophotometer. Guide RNAs were mixed with in vitro generated mRNA that encoded Cas9 mRNA codon-optimized for zebrafish. The mix was injected into 1 cell-stage zebrafish embryos that were heterozygous for the *qmc551Gt* allele of *gfi1aa*. Ten of the injected embryos were collected the following day for genotyping (see method below). PCR on genomic DNA with oligos DB569 and DB570 indicated the presence of a genomic deletion between the two target sites. As this indicated successful targeting of the *gfi1b* locus in these embryos, their siblings were raised. Once sexually mature, the fish were crossed to *wild-type* fish, and their progeny were analyzed for the presence of a deletion allele. This way the *qmc554* allele was identified that carried a 197 bp deletion and a 1 bp insertion. Siblings of the genotyped embryos were grown up to establish the *qmc554* line.

Table S6: Oligonucleotides used for target site cloning into pDR274

Name	Sequence	Purpose
DB567	5' taGGAGGAAACTCTGCCAGCTG 3'	gRNA1-forward
DB568	5' aaacCAGCTGGCAGAGTTTCCTCC	gRNA1-reverse
DB584	5' taGGTGATGCAGTGGTATGTGT 3'	gRNA2-forward
DB585	5' aaacACACATACCACTGCATCACC	gRNA2-reverse

Whole-mount in situ hybridisation (WISH)

WISH experiments followed a published protocol[12]. Probes used are listed in Table S7. After staining embryos were photographed in 80% glycerol in PBS (phosphate-buffered saline) plus 0.1% Tween 20. For genotyping, individual embryos were washed back into PBS/Tween via 50% and 30% glycerol. Following 3 washes in PBS/Tween, embryos were incubated first in 100 µL 300 mM NaCl at 65 °C for 4 hours[13] and then in 25 µL 1x Base solution (5x Base solution: 0.5g NaOH, 2 mL 0.5M EDTA (pH8), filled up with H_2O to 100 mL) at 95 °C for 20 min. The sample was cooled for a minute at room temperature (RT) before 25 µL 1x Neutralisation solution (5x Neutralisation solution: 3.15g Tris-HCl in 100 mL of H_2O) was added. The sample was vortexed and then centrifuged at 3,000 rpm for 5 min in a Microfuge. 2 µL of the supernatant was used for PCR.

Table S7: Information on WISH probes used in the study

Gene	Reference
cahz	14
cdh5	15
clec14a	15
cmyb	16
epor	17
etv2/etsrp	5
flk1/kdrl	18–20
gfi1aa	2,21,22
gfi1ab	2,23
gfi1b	2,22
klf1	24
rag1	25
slc4a1a	26
sox7	27–29
sptlc2a	This publication, ENSDARG00000018976
myoD1	30
pax2a	31
nkx2.5	32
npas4l/cloche	33

Genomic DNA isolation

Adult fish were anaesthetized with MS222 for caudal fin clipping. Fin clips and fresh embryos used for genotyping were incubated in proteinase K-containing lysis buffer at 55°C overnight. The volumes were 50 µL for a fin clip, 10 µL per embryo if batches were analysed, and 20 µL for a single embryo. Following the overnight incubation, proteinase K was heat-inactivated for 20 min at 99°C. Water was added to 100 µL. 1-10 µL were used for PCR amplification.

PCR amplification, DNA purification and sequencing

Standard PCRs on cDNA and genomic DNA were performed using Taq polymerase (New England Biolabs, M0273). PCR fragments were cleaned up by agarose gel electrophoresis. DNA was eluted from agarose gels using the GenElute Gel extraction kit (Sigma, NA1111). Some PCR fragments were sent for Sanger sequencing (Source Bioscience Nottingham). Others were cloned into pGEM-Teasy (Promega, A1360) and then sequenced.

Genotyping of the mutant and wildtype zebrafish lines

The oligos used for genotyping are listed in Table S7. Genotyping of the mutant *gfi1b* allele *sh339* involves the use of a nested PCR using oligos DB913 and DB914 in the first and oligos DB915 and DB916 in the second step. The expected PCR products are 954 and 687 bp fragments, respectively. Oligo DB826 is used to sequence the gel-extracted nested product to detect the single base deletion present in the mutant allele. Genotyping of the *qmc554* allele is done by PCR using oligos DB569 and DB570. The PCR on the wild-type allele gives a 305 bp fragment, while the PCR on the mutant allele generates a 109 bp fragment. Genotyping of the *qmc551* and the two alternative wild-type alleles of *gfi1aa* also relies purely on PCR. Oligos DB278 and DB306 are used to amplify the *qmc551* allele. The resulting PCR fragment has a size of 491 bp. The wild-type allele with the larger intron 1 sequence is amplified using oligo pairs DB1019 and DB986, giving a fragment of 1330 bp. Oligos DB1019 and DB239 are used to detect the smaller wild-type allele, producing a PCR product of 477 bp. Genotyping of the *sa11633* allele of *gfi1aa* involved a PCR

with oligos DB819 and DB820. The resulting 233 bp fragment is sequenced with DB820 to detect the C to A substitution in exon 4 of *gfi1aa*. Oligo sequences are provided in Table S8.

Table S8: Oligonucleotides used for PCR amplification and DNA Sequencing

Name	Sequence	Purpose
DB913	5' ACACCTTGTAATAAACTGCCACT	*sh339* first PCR
DB914	5' TGAACCTGGAAACCTGTAACC 3'	*sh339* first PCR
DB915	5' TCCAAAGTCGACAGAGCAGA 3'	*sh339* nested PCR
DB916	5' TTGGAACCACTTGAGGGAGA 3'	*sh339* nested PCR
DB826	5' TGTAAAAGGGACGTCTGGTG 3'	*sh339* sequencing
DB569	5' TGTGGCATGTGTAGGGTAGA 3'	*qmc554* PCR
DB570	5' GACCTTGTCGCAGGTGATG 3'	*qmc554* PCR
DB278	5' TCCACTTTGCCTTTCTCTCC 3'	*qmc551* PCR
DB306	5' CGATGCCCTTCAGCTCGAT 3'	*qmc551* PCR
DB1019	5' TATGGCCTGCTCGGAGTTTG 3'	*gfi1aa wt1* and *wt2* PCR
DB986	5' GGTTATCTCTATGCTGATCACGC 3'	*gfi1aa* wt1 PCR
DB239	5' GCGCCCGTTTGCTCTTCACC 3'	*gfi1aa* wt2 PCR
DB819	5' TCAGCCTTTCGACATGTCCT 3'	*sa11633* PCR
DB820	5' AGCCTCGATTATCCAGCAGG 3'	*sa11633* PCR + seq.

Flow cytometric analysis of kidney marrow cells

Blood cells were collected from adult kidneys and analysed on the flow cytometer following a published protocol[34]. Forward scatter, side scatter and GFP fluorescence characteristics of KM cells were analyzed on a Beckman Coulter MoFlo Astrios cell sorter using the Kaluza software. Sytox was used to exclude dead cells.

Microscopy and Imaging

Embryos were examined on a Nikon SMZ-1500 microscope with epifluorescence attachment. GFP was detected with the help of a FITC filter set. Sections were analyzed on a Nikon Eclipse i80. Images were taken with a Nikon DS-5Mc/DS-U1 camera setup operated by the Nikon ACT-2U software. Images were collated in

Photoshop CS6. Diagrams were drawn with Apple Graphic Software.

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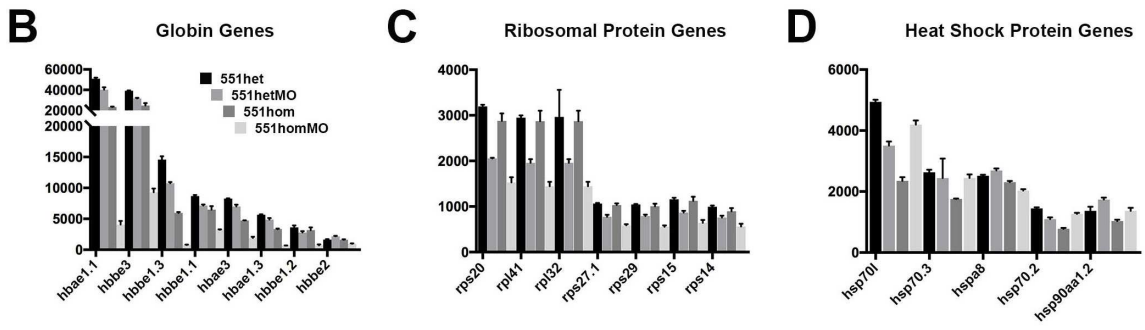
Supplemental Figures

A Genes with the highest expression (RPKM) in *qmc551 het* prRBCs at 20 hpf

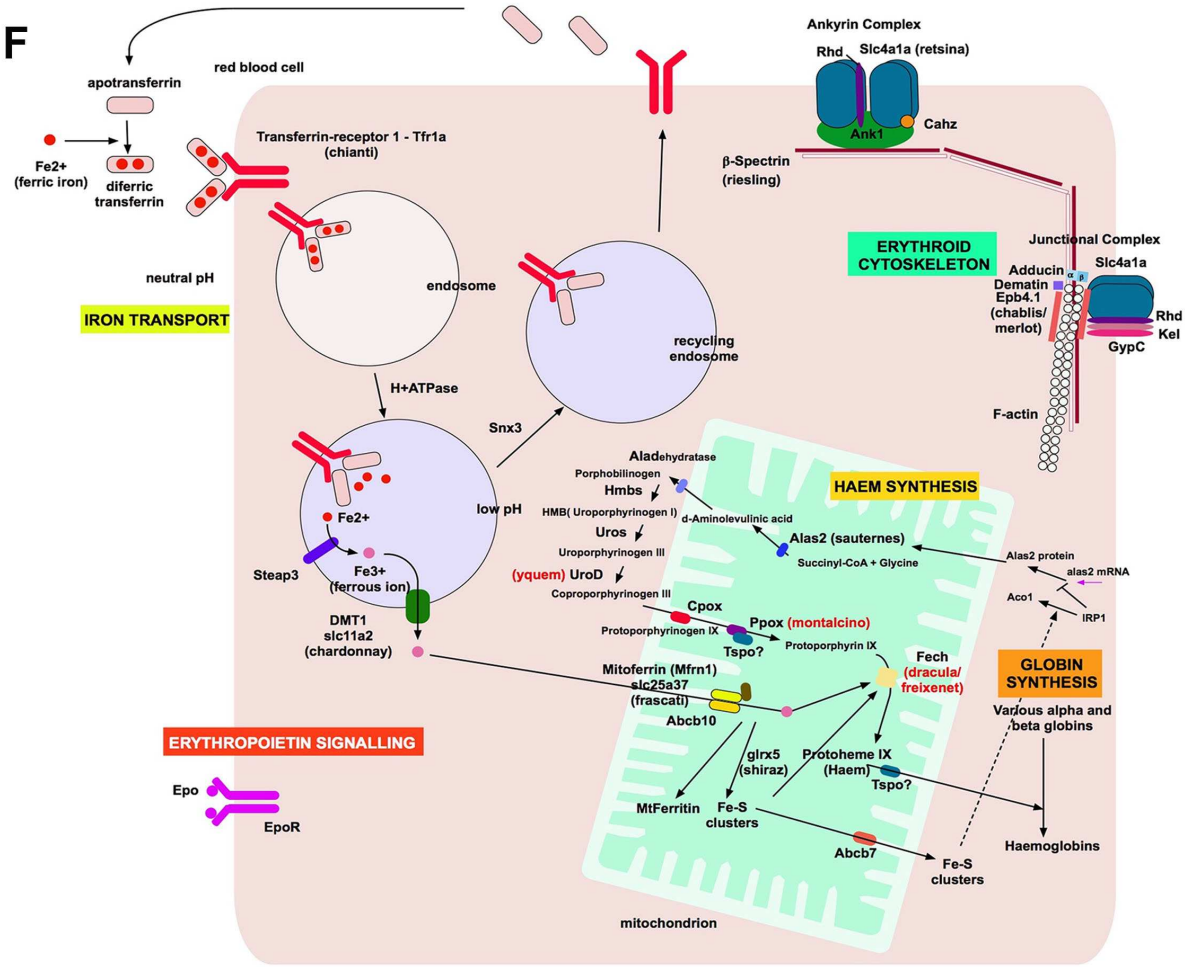
GeneID	GeneName	Het	HomMO	log2(HomMO/Het)
ENSDARG00000089475	hbae1	50932.95	4069.97	-3.65
ENSDARG00000038147	hbbe3	39256.99	9295.60	-2.08
ENSDARG00000087390	hbbe1.3	14579.52	777.46	-4.23
ENSDARG00000089963	hbbe1.1	8677.93	3236.74	-1.42
ENSDARG00000079305	hbae3	8265.50	1916.24	-2.11
ENSDARG00000089124	hbae1.3	5669.17	686.12	-3.05
ENSDARG00000055723	hsp70l	4941.06	4198.98	-0.23
ENSDARG00000090689	hbbe1.2	3618.63	725.86	-2.32
ENSDARG00000036044	rps20	3195.65	1529.80	-1.06
ENSDARG00000092807	rpl41	2947.73	1445.86	-1.03
ENSDARG00000054818	rpl32	2631.49	1904.14	-0.47
ENSDARG00000021924	hsp70.3	2630.99	2445.10	-0.11
ENSDARG00000068992	hspa8	2515.63	2032.45	-0.31
ENSDARG00000006818	urod	2496.66	475.96	-2.39
ENSDARG00000076532	wu:fb18c02-Prothymosin A	2489.90	3665.67	0.56
ENSDARG00000015551	fth1a	2435.46	875.31	-1.48
ENSDARG00000045143	hbbe2	1653.67	953.95	-0.79
ENSDARG00000092362	hsp70.2	1446.34	1268.82	-0.19
ENSDARG00000054155	pcna	1377.28	759.89	-0.86
ENSDARG00000024746	hsp90aa1.2	1369.11	1366.00	0.00



E Genes with the highest expression (RPKM) in *qmc551 hom* and *gfi1b* morphant prRBCs at 20 hpf

GeneID	GeneName	Het	HomMO	log2(HomMO/Het)
ENSDARG00000038147	hbbe3	39256.99	9295.60	-2.08
ENSDARG00000055723	hsp70l	4941.06	4198.98	-0.23
ENSDARG00000089475	hbae1	50932.95	4069.97	-3.65
ENSDARG00000076532	wu:fb18c02 - Prothymosin A	2489.90	3665.67	0.56
ENSDARG00000089963	hbbe1.1	8677.93	3236.74	-1.42
ENSDARG00000090186	rps27.2	423.43	3124.38	2.88
ENSDARG00000021924	hsp70.3	2630.99	2445.10	-0.11
ENSDARG00000077777	tmsb4x	700.73	2284.29	1.70
ENSDARG00000017624	krt4	239.75	2067.37	3.11
ENSDARG00000076667	ccng1	422.25	2051.02	2.28
ENSDARG00000068992	hspa8	2515.63	2032.45	-0.31
ENSDARG00000079305	hbae3	8265.50	1916.24	-2.11
ENSDARG00000054818	rpl32	2631.49	1904.14	-0.47
ENSDARG00000004665	hspa5	855.45	1625.66	0.93
ENSDARG00000036044	rps20	3195.65	1529.80	-1.06
ENSDARG00000092807	si:dkey-151g10.6 - rpl41	2947.73	1445.86	-1.03
ENSDARG00000037746	actb1	880.73	1437.02	0.71
ENSDARG00000024746	hsp90aa1.2	1369.11	1366.00	0.00
ENSDARG00000035423	zgc:123194 - tubb2b	1285.68	1359.98	0.08
ENSDARG00000092362	hsp70.2	1446.34	1268.82	-0.19

Figure S1



Iron Transport and Metabolism			
Gene	**Protein**	**Het**	**HomMO**
tfr1a	Transferrin receptor 1a	133	45
steap3a	Endosomal Ferrireductase	16	1
slc11a2	solute-coupled divalent metal ion transporter (DMT1)	263	101
slc25a37	Mitochondrial ion transporter	47	2
abcb10	abc binding cassette b10	182	53
snx3	sorting nexin 3	95	33
frrs1b	ferric-chelate reductase 1b	48	7
glrx5	glutaredoxin 5	1260	847
abcb7	abc binding cassette b7	74	37
aco1	iron regul. pr. 1 / aconitase 1	62	47

Haem synthesis			
Gene	**Protein**	**Het**	**HomMO**
alas2	aminolaevulinate synthase 2	924	145
alad	aminolaevulinate dehydratase	459	87
hmbsa	hydroxymethyl-bilane synthase A	651	66
hmbsb	hydroxymethyl- bilane synthase B	702	50
uros	uroporphyrinogen III synthase	131	23
urod	uroporphyrinogen III decarboxylase	2497	476
cpox	coproporphyrinogen oxidase	1020	292
ppox	protoporphyrinogen oxidase	57	9
fech	Ferrochelatase	67	10
tspo	translocator protein	460	51

Erythroid cytoskeleton			
Gene	**Protein**	**Het**	**HomMO**
slc4a1a	Erythroid band 3	158	1
ebp41b	Erythroid Protein Band 4.1b	70	6
sptb	beta-Spectrin	99	18
rhd	Rh blood group, D antigen	138	11
add1	Adducin 1	17	14
add2	Adducin 2	18	1
dmtn	dematin actin binding protein	37	11
ank1	Ankyrin 1	8	2
cahz	Carbonic anhydrase	365	70
gypc	glycophorin C	22	71
kel	kell blood group	4	3

Figure S1

Figure S1: The late erythroid transcription programme is depressed in Gfi1aa/1b-deficient primitive red blood cells. Analysis of the transcriptomes of populations of prRBCs isolated from Gfi1aa and/or Gfi1b-deficient embryos. **(A)** List of genes that are most highly expressed in 20 hpf *qmc551* heterozygous prRBCs. **(B-D)** Expression levels of globin genes, ribosomal protein genes and heat shock protein genes in all samples. Of these highly expressed genes only the globin genes were reduced at a lower level in the absence of Gfi1aa and Gfi1b. **(E)** List of genes that are most highly expressed in *gfi1b* morphant *qmc551hom* embryos. **(F)** The red blood cell diagram depicts proteins involved in important aspects of RBC biology, like iron transport and metabolism, haem synthesis and the erythroid cytoskeleton. Mutations affecting some of these proteins are known to give erythroid phenotypes in zebrafish. The names of the hypochromic mutants are given in brackets. Mutants which display erythroporphyria are highlighted in red.

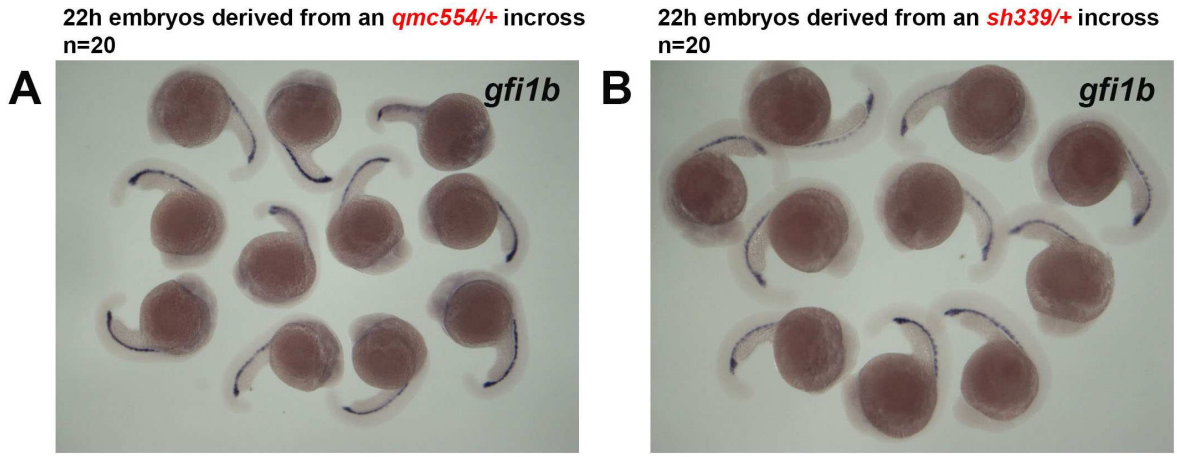


Figure S2

Figure S2: *Gfi1b*^{qmc554} and *gfi1b*^{sh339} mRNAs are stable. WISH experiments with *gfi1b* probe on 22 hpf embryos derived from incrosses of *gfi1b*^{qmc554} (A) and *gfi1b*^{sh339} (B) heterozygous fish. Genotyping identified 5 homozygous *gfi1b*^{qmc554} carriers in the cohort shown in (A). Siblings of the embryos shown in (B) were stained for *gfi1aa* expression (Fig. 4E). Some of these displayed elevated gfi1aa expression and turned out to be *gfi1b*^{sh339}*hom* mutants.

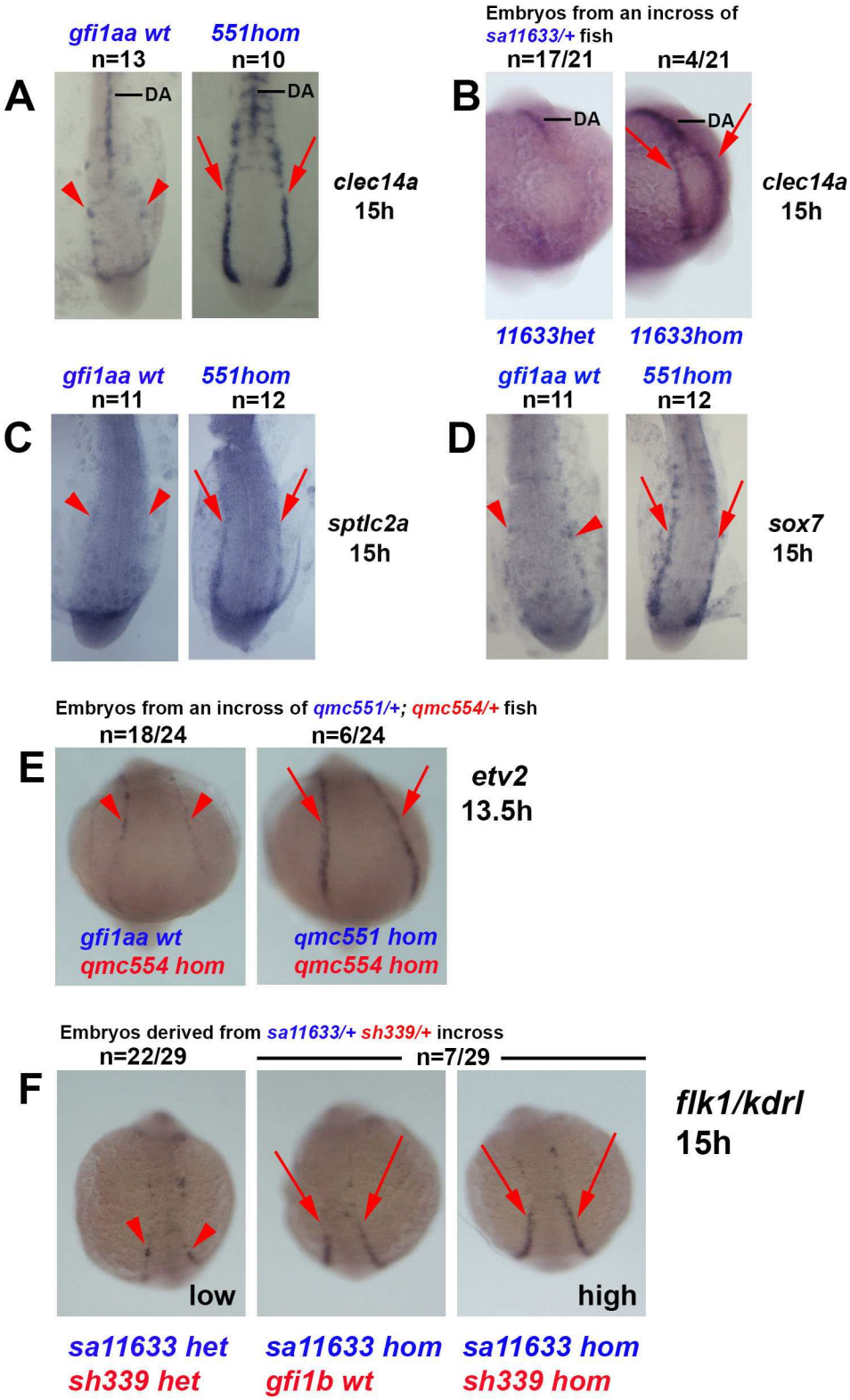


Figure S3

Figure S3: Endothelial genes display expanded expression patterns in the posterior lateral mesoderm of *gfi1aa*^{qmc551}*hom* and *gfi1aa*^{sa11633}*hom* embryos. Images show embryos after WISH experiments. Flat-mounts are shown in panels (A), (C) and (D). Posterior views of embryos are depicted in panels (B) and (E).

Dorsal views are shown in (F). The genotype of the embryos shown in (A), (C) and (D) was known as the embryos were derived from incrosses of *wt* and *gfi1aa*^{qmc551}*hom* fish. Embryos in (B), (E) and (F) had to be genotyped after the WISH experiment. In (B), all four embryos with increased *clec14a* expression were *gfi1aa*^{sa11633} homozygous mutants. In (E), the 6 embryos with an expanded *etv2* expression pattern were also genotyped and found to be homozygous *gfi1aa*^{qmc551} mutants. In (F), two of the seven embryos with increased *flk1/kdrl* expression were genotyped and revealed to be *gfi1aa*^{sa11633} homozygous. None of the control embryos we genotyped in (B), (E) and (F) were homozygous *gfi1aa* mutants. Some of them in (E) and (F) were *gfi1b* mutants, confirming that loss of Gfi1b expression does not cause EC gene expression to be expanded. The figure panels show representative examples. Red arrowheads point at normal EC gene expression in small clusters of EC progenitors. Red arrows highlight expanded expression of ECs in the posterior lateral mesoderm.

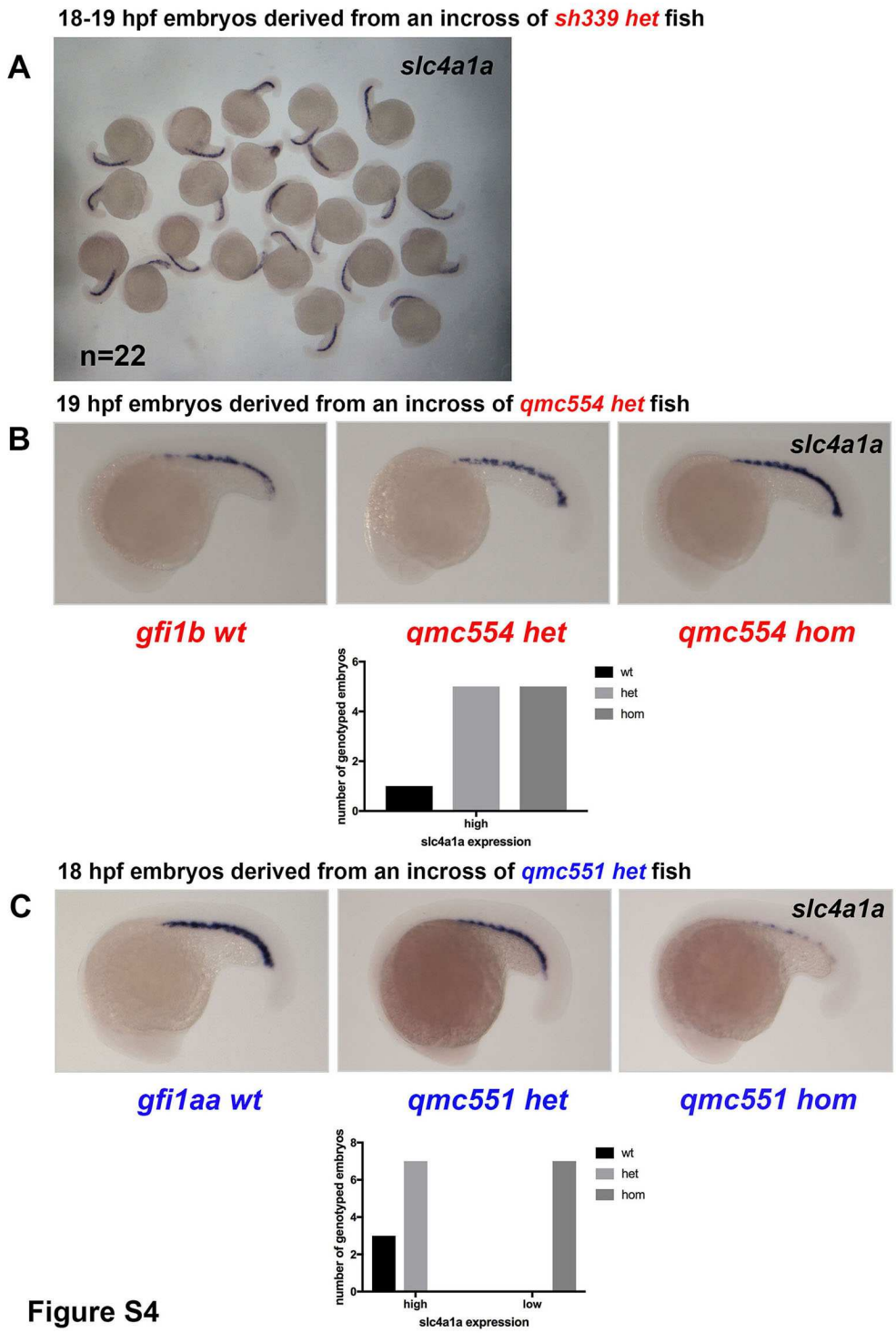


Figure S4

Figure S4: The timely onset of *slc4a1a* expression in red blood cells of 18-20 hpf embryos requires the presence of Gfi1aa, but not the expression of Gfi1b. Embryos were stained by whole-mount in situ hybridization. Lateral views of embryos are presented in all panels. A subset of embryos was genotyped. The genotyping results are presented in bar charts below the photographs.

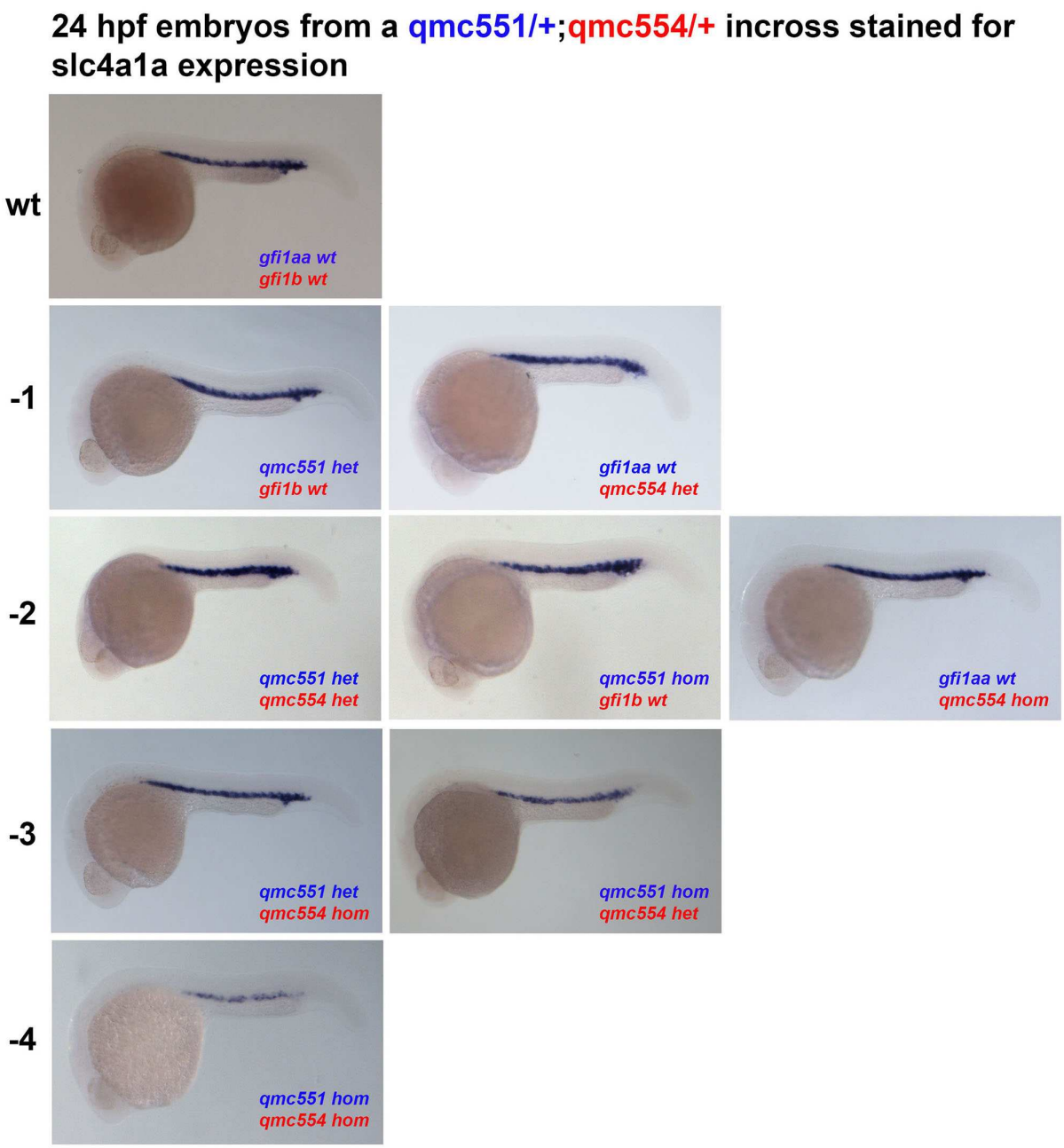


Figure S5

Figure S5: At 24 hpf, erythroid *slc4a1a* expression is reduced most strongly in *gfi1aa/gfi1b* double mutant embryos. 7 out of 89 embryos derived from an incross of double heterozygous *gfi1aa*^{qmc551} and *gfi1b*^{qmc554} parents displayed strongly reduced *slc4a1a* expression in a whole-mount in situ hybridization experiment. All 7 turned out to be double homozygous embryos in the subsequent PCR-based genotyping experiment. A further 40 embryos were photographed and genotyped. Representative embryos for each genotype are shown. The images show lateral views of the stained embryos. They are arranged in the shape of a pyramid based on the combined number of *wt* copies of *gfi1aa* and *gfi1b* lost in the genotyped embryos.

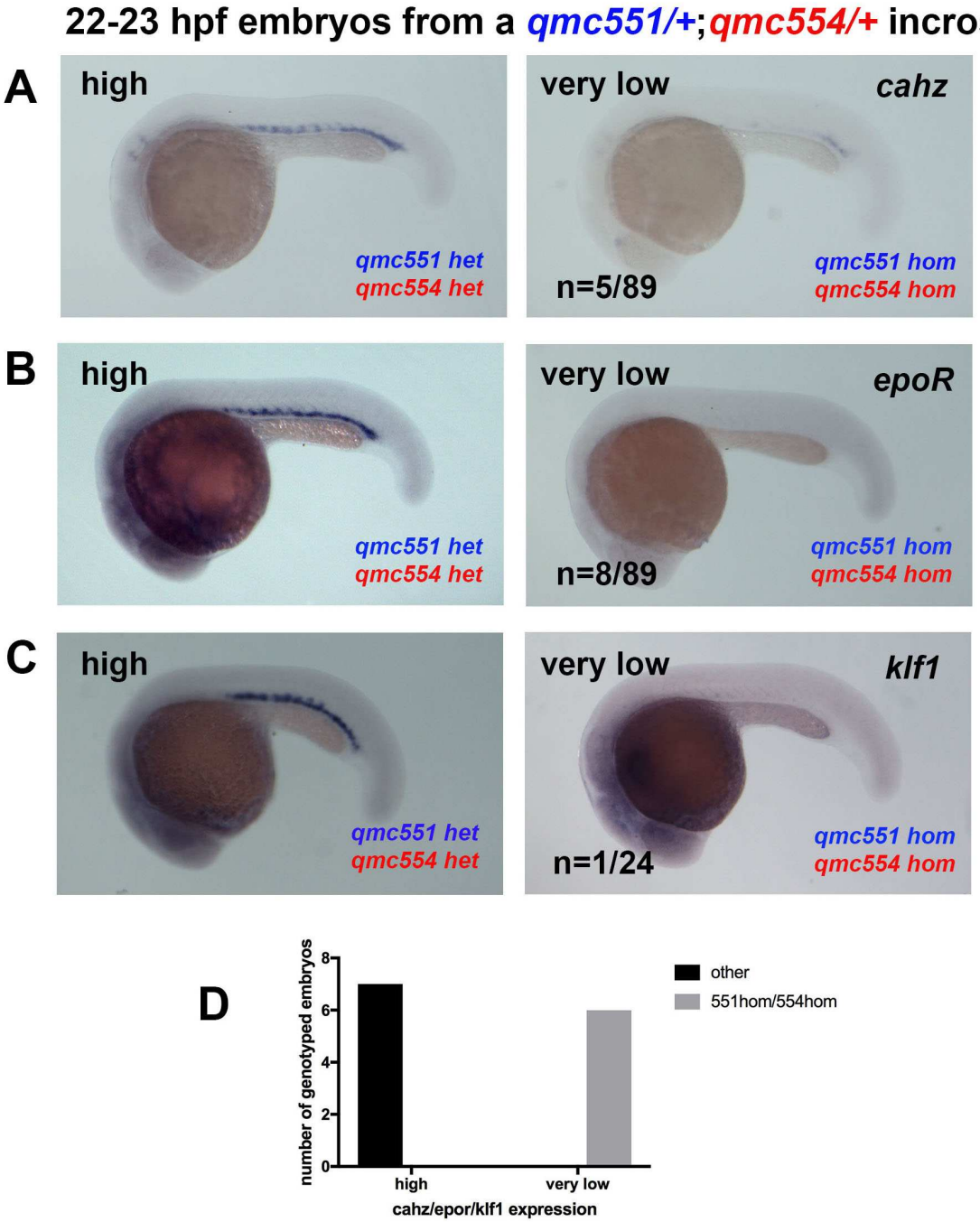


Figure S6

Figure S6: Gfi1b rescues the expression of the erythroid genes *cahz*, *epor* and *klf1* in Gfi1aa-depleted embryos. The panels show lateral views of representative genotyped embryos. Six of the embryos that showed very low expression of the 3 erythroid genes were genotyped and turned out to be double homozygous *gfi1aa*^{qmc551}/*gfi1b*^{qmc554} carriers. None of the well stained embryos displayed this genotype.

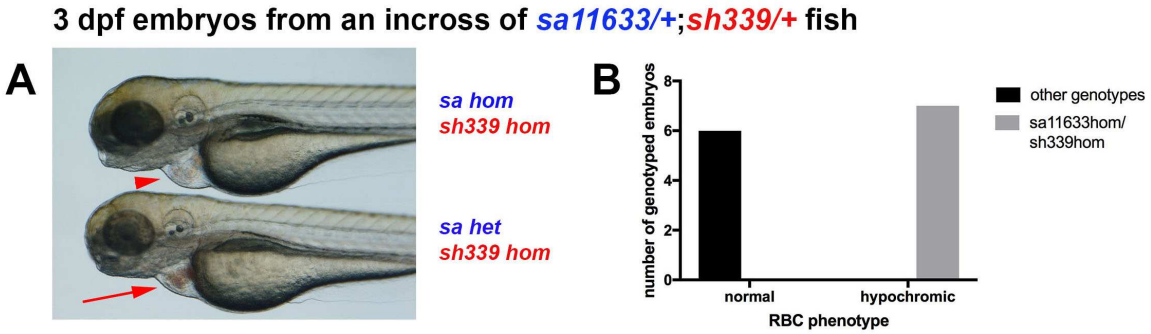


Figure S7

Figure S7: Double homozygous gfi1aa^{sa11633}/*gfi1b*^{sh339} embryos carry hypochromic blood cells. **(A)** Lateral view of 3 dpf representative embryos are presented. The red arrow highlights the normal red colour of blood cells in the heart. The red arrowhead points at hypochromic blood cells. **(B)** Embryos with normal or hypochromic blood were genotyped. Bar charts summarize the results of these experiments.

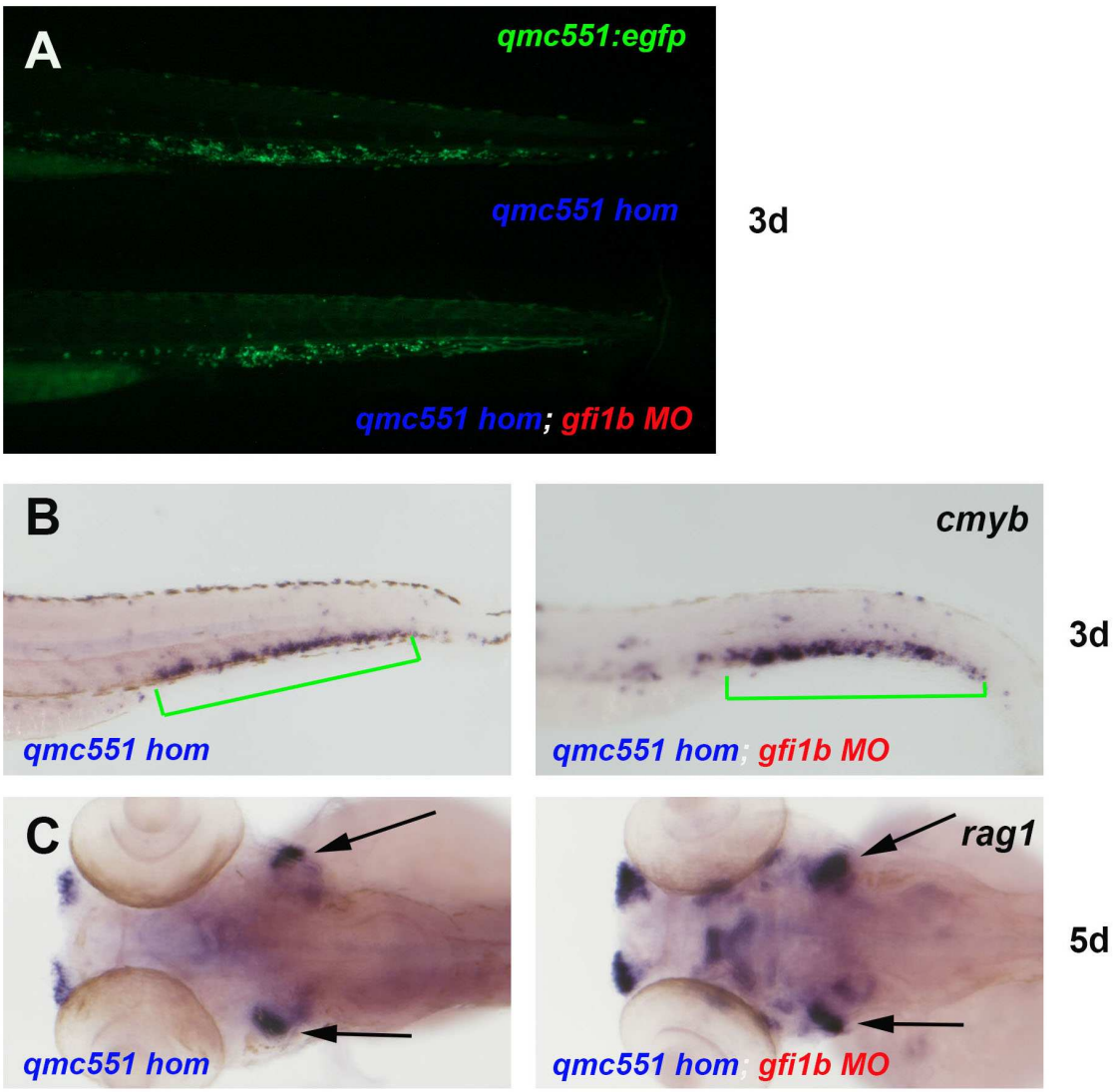


Figure S8

Figure S8: Gfi1b splice-morphant *gfi1aa*^{qmc551} homozygous mutant embryos do not display obvious defects in definitive haematopoiesis. (A) lateral view of the posterior trunk and tail region of *gfi1aa*^{qmc551} homozygous embryos at 3 dpf that were or were not injected with *gfi1b* exon 4 splice morpholinos (n=10 for each) (B-C) WISH experiments on *gfi1aa*^{qmc551} homozygous mutant embryos injected or not injected with *gfi1b* exon 4 splice morpholinos. The green brackets in (B) highlight *cmyb*-positive cells in the caudal haematopoietic tissue (n=12 for each). The black arrows point at *rag1* expression in the thymus (n=18 for each). Views are lateral in (B) and dorsal in (C). Anterior is towards the left.

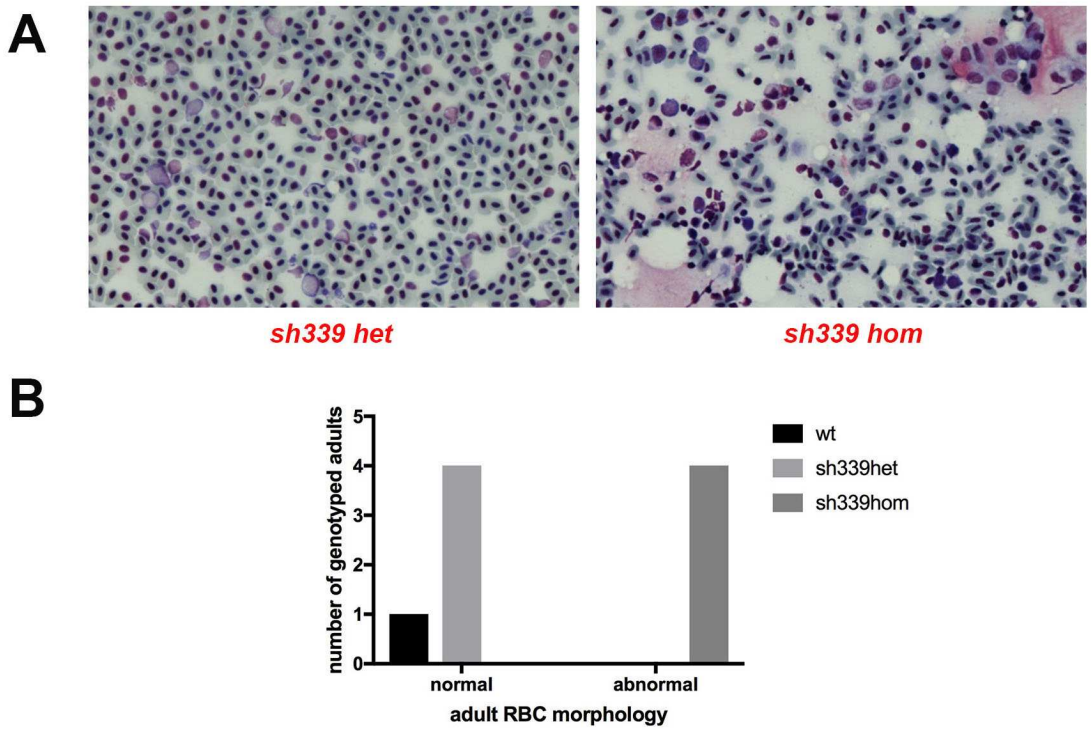


Figure S9

Figure S9: Adult homozygous *gfi1b*^{sh339} carriers carry red blood cells with abnormal morphology in their peripheral blood. **(A)** Blood smears were taken from adult progeny derived from a *gfi1b*^{sh339}*het* incross. Two examples are shown in (A). **(B)** Genotyping of tissue taken from 5 fish with normal and 4 fish with abnormal adult RBCs was performed. All abnormal blood smears were from *gfi1b*^{sh339}*hom* adult fish.

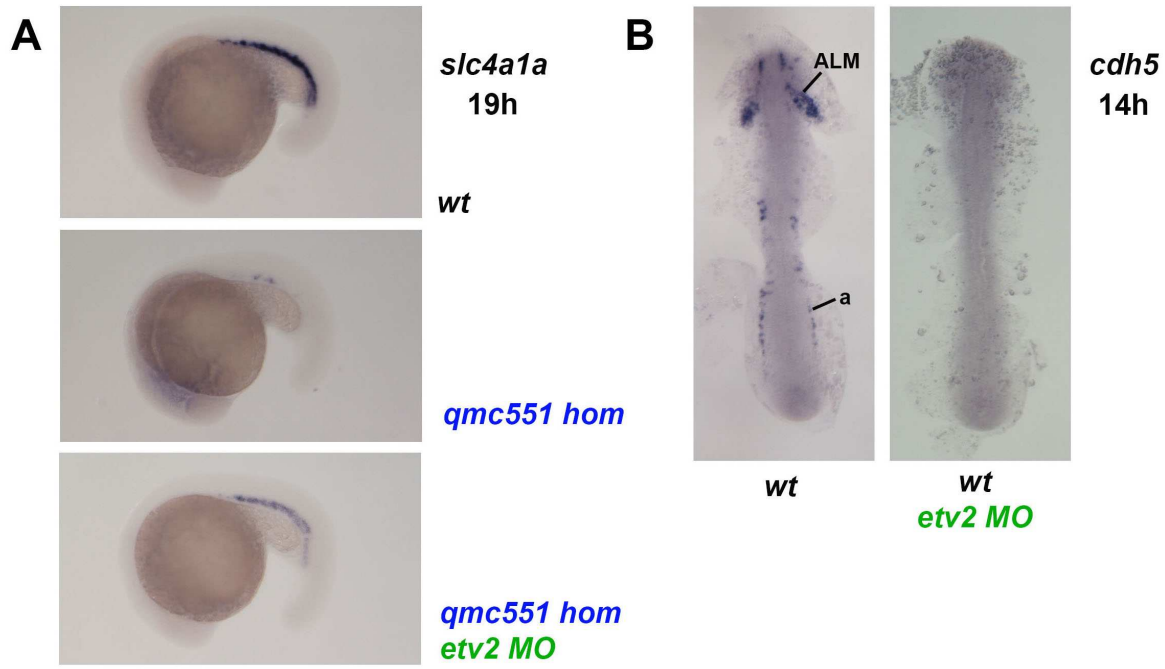


Figure S10

Figure S10: *Etv2* morpholino-mediated suppression of endothelial gene expression only partially rescues the level of *slc4a1a* expression in prRBCs of Gfi1aa-depleted embryos. 10 ng of an *etv2* 5'UTR MO were injected at the 2-4 cell stage embryos. Embryos in (A) were either *wt* or derived from an incross of *gfi1aa*^{qmc551} homozygous fish. Embryos in (B) were *wt*. **(A)** *slc4a1a* WISH experiment. 15 *wt*, 21 *gfi1aa* mutant and 20 *gfi1aa* mutant *etv2* morphant embryos were collected at 19 hpf and stained in a WISH experiment with an *slc4a1a* probe. Of the 14 *gfi1aa* mutant embryos that we could accurately stage-match with *gfi1aa* mutant *etv2* morphant embryos, 12 displayed weaker levels of *slc4a1a* expression than the corresponding *gfi1aa* mutant *etv2* morphants while 2 showed similar expression levels. **(B)** *cdh5* WISH experiment. 13 uninjected *wt* embryos and 20 *wt* embryos injected with the *etv2* morpholino were collected at 14 hpf and stained by WISH. While all of the *wt* embryos displayed *cdh5* expression in angioblasts derived from the anterior lateral mesoderm (ALM) and in arterial angioblasts in the posterior lateral mesoderm (a), no *cdh5* expression was detectable in any of the 20 *etv2*-morphant embryos. Embryos in (B) were flat-mounted. Embryos are shown with anterior up. The *cdh5* WISH experiments gives an indication of the efficiency of the morpholino injection. Please note that the *etv2* morpholino also caused a complete loss of *cdh5* expression in the 20 *qmc551* homozygous embryos shown in Figure 5E. In (A) and (B) representative embryos are shown.