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Supplemental information

Transcription factor induction

of vascular blood stem cell niches *in vivo*

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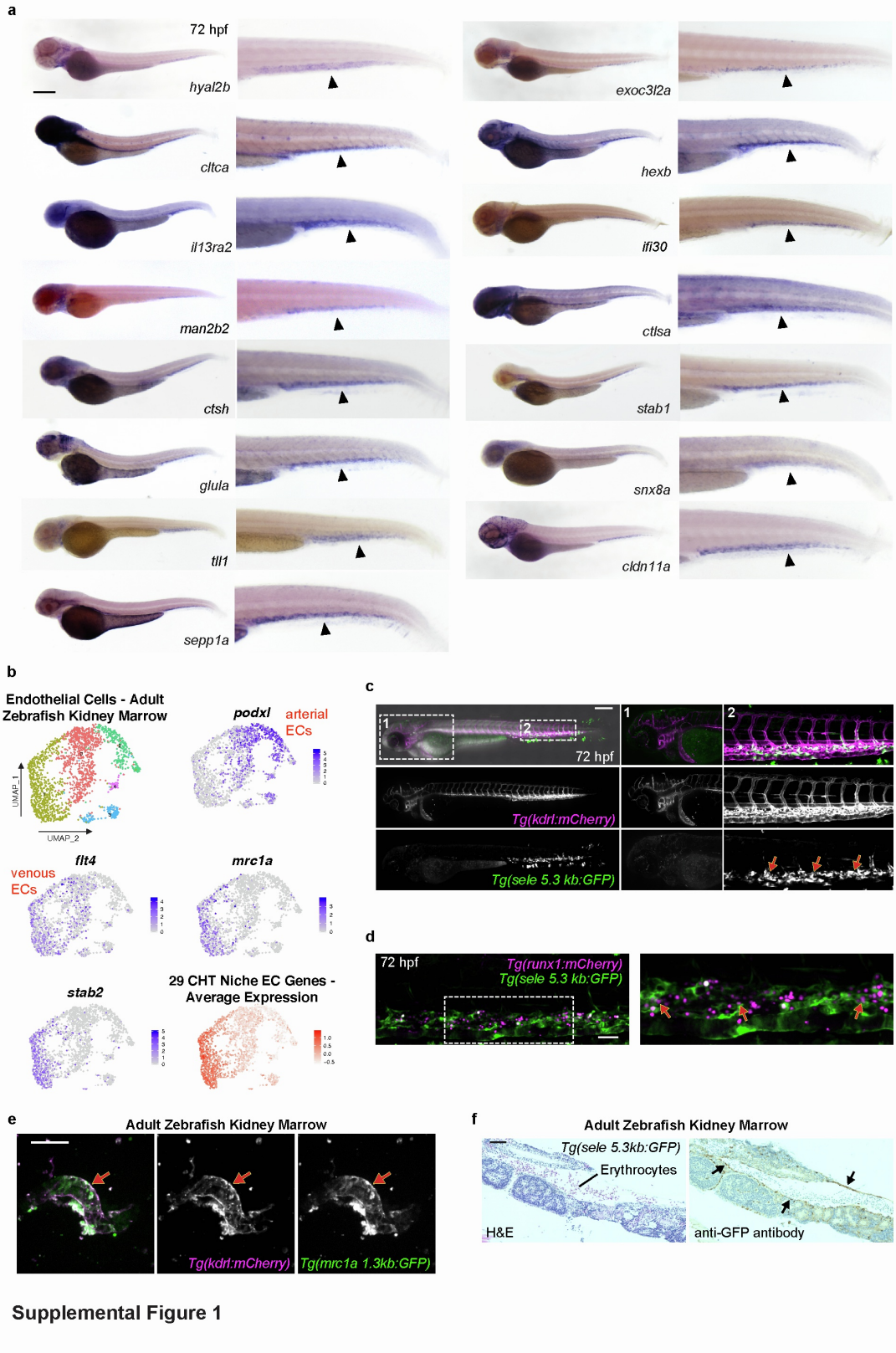
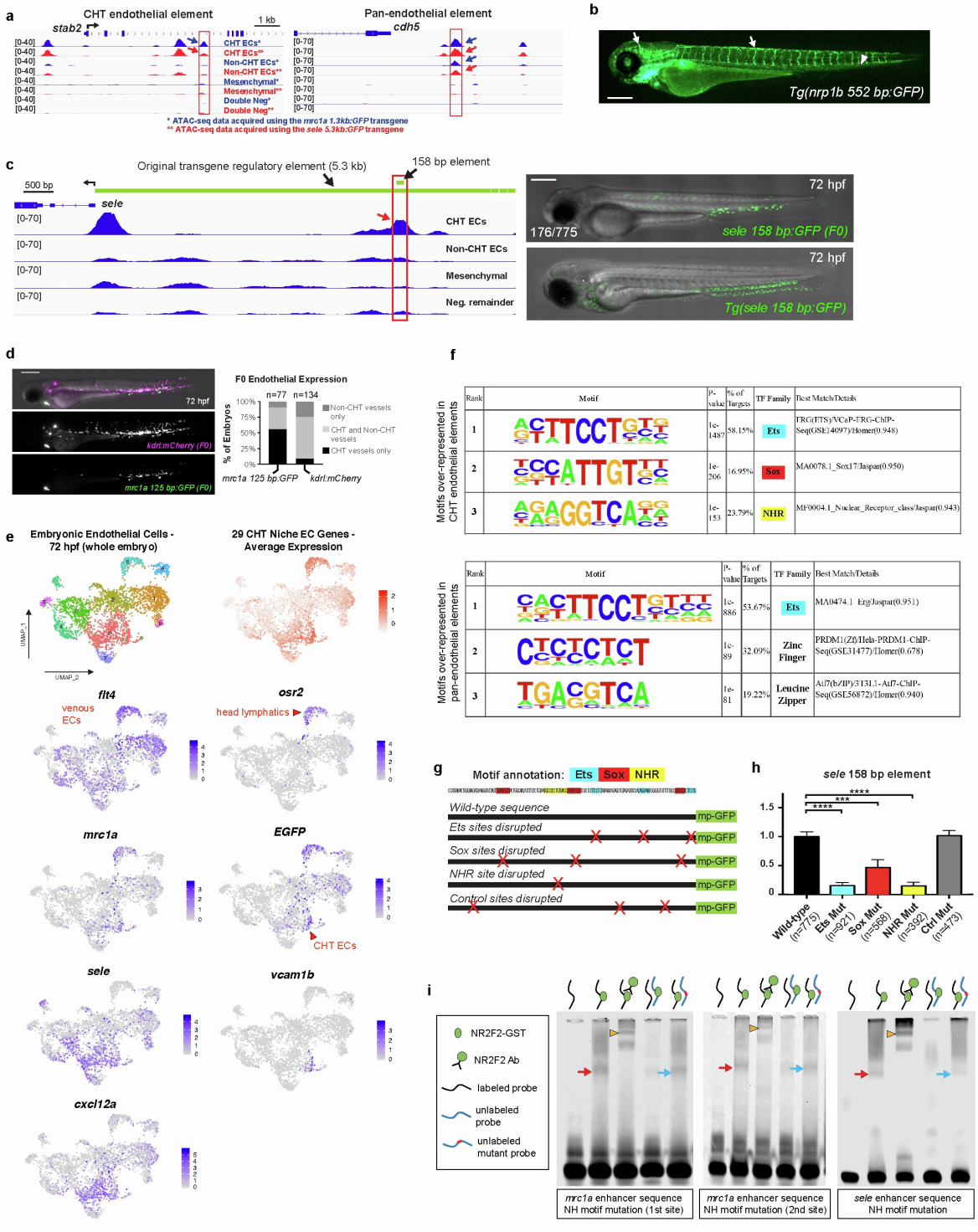


Supplemental Figure 1

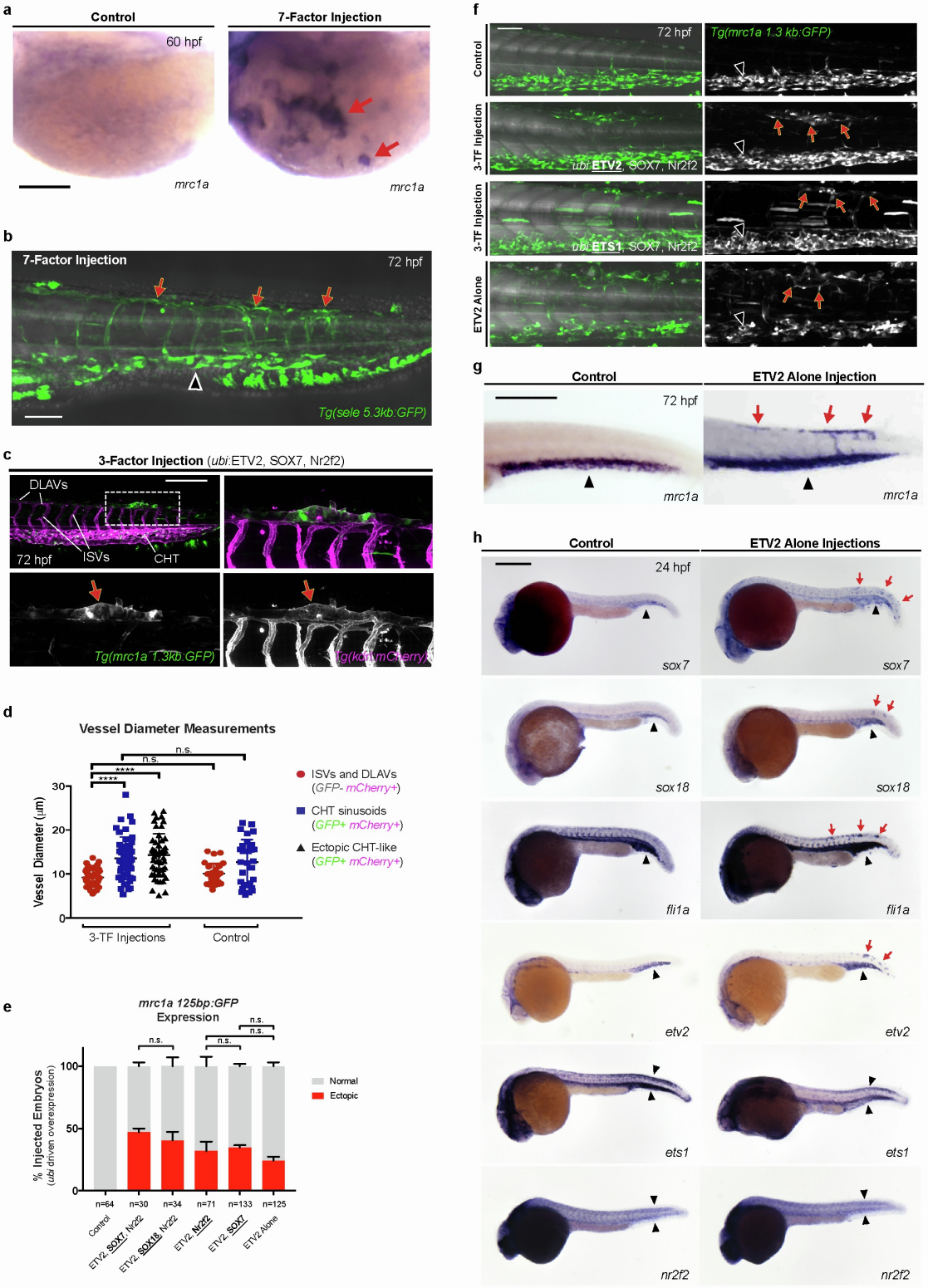
Supplemental Figure 1 | Niche-specific endothelial gene expression (related to Figures 1 and 2). **a**, WISH validates the CHT-enriched expression (arrowheads) of CHT EC genes identified using a combination of tomo-seq and tissue-specific RNA-seq. Scale bars represent 250 μm in this and all subsequent Supplemental Figures unless noted otherwise. **b**, Uniform Manifold Approximation and Projection (UMAP) plots show cell clustering and gene expression from a single cell RNA-seq analysis of *kdrl:mCherry*$^{+}$ endothelial cells isolated from adult zebrafish kidney marrow. Marker genes are shown for arterial (*podxl*) and venous (*flt4*) endothelial cells, as well as CHT ECs (*mrc1a* and *stab2*). The average expression of the 29 CHT niche EC genes is shown in the bottom right plot. Spectral scales report z-scores. **c**, Images show a double transgenic embryo carrying the pan-endothelial marker *kdrl:mCherry* (magenta) and the *sele 5.3kb:GFP* transgene (green). Magnifications of boxed areas are shown on the right. The highest levels of vascular GFP expression are observed in CHT ECs (red arrows); while lower levels of expression are observed in the anterior head region, although some of these cells do not express the *kdrl:mCherry* transgene. **d**, Images show *runx1:mCherry*$^{+}$ HSPCs (magenta) directly interacting with *sele 5.3kb:GFP*$^{+}$ ECs within the CHT niche (red arrows). Panel on right shows magnification of boxed area. **e**, Images show a segment of vasculature (red arrows) dissected from the kidney of a *mrc1a 1.3kb:GFP; kdrl:mCherry* double transgenic adult zebrafish. **f**, Images show sequential sections through an adult kidney isolated from a *sele 5.3kb:GFP* transgenic fish. Sections were stained with H&E (left) and with an antibody against GFP (right). Black arrows point to GFP^{+} vascular endothelial cells. Scale bars represent 50 μm in **d-f**.



Supplemental Figure 2

Supplemental Figure 2 | A *cis*-regulatory landscape for HSPC niche- and pan-endothelial gene expression (related to Figures 3 and 4). **a**, Gene tracks show regions of chromatin that were uniquely open in the mCherry^{+}; GFP^{+} CHT EC fraction (left; red box and arrows) or a region of chromatin open in both the mCherry^{+}GFP^{+} (CHT EC) and mCherry^{+}GFP^{-} (non-CHT EC) populations (right; red box and arrows). The blue tracks show ATAC-seq data obtained using the *mrc1a 1.3kb:GFP* transgene, while the red tracks show ATAC-seq data obtained using the *sele 5.3kb:GFP* transgene. **b**, Image shows reporter expression for the stable *nrp1b:552bp:GFP* enhancer transgene. Arrows point to GFP expression in non-CHT ECs and arrowhead points to expression in CHT ECs. **c**, Gene tracks show a region of chromatin upstream of *sele* that was uniquely open in the double positive CHT EC fraction but not the other three cell populations (red box and arrow). Green bars denote the position of the 158 bp enhancer sequence and the 5.3 kb sequence used to generate the *sele:GFP* reporter transgenes. Images show transient F0 (upper right) and stably integrated (lower right) transgene expression of the *sele 158 bp:GFP* construct. **d**, Images show an F0 embryo injected with *mrc1a 125 bp:GFP* and *kdrl:mCherry* plasmids. Graph reports the anatomical location of endothelial expression in F0 embryos that were injected with each construct. **e**, Uniform Manifold Approximation and Projection (UMAP) plots show cell clustering and gene expression from a single cell RNA-seq analysis of endothelial cells (whole embryo) isolated from *mrc1a 125bp:GFP*; *kdrl:mCherry* double positive embryos at 72 hpf. *osr2* expression is shown as a marker of the head lymphatic EC population. Spectral scales report z-scores. **f**, Tables show the transcription factor binding motifs most enriched in CHT EC regions (top) or pan-endothelial regions (bottom). **g**, Wild-type sequence of the 158 bp *sele*

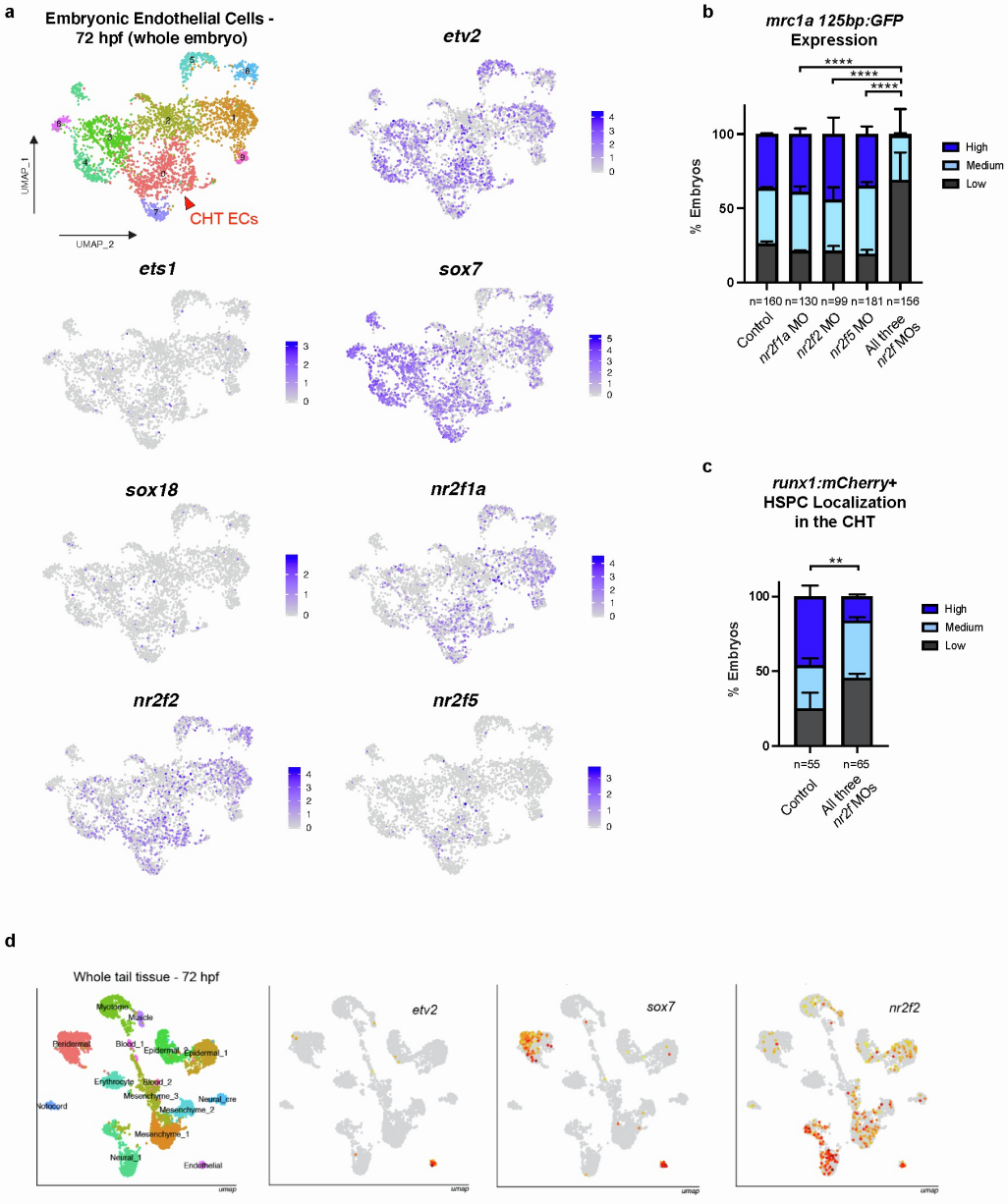
enhancer is shown, annotated with colors highlighting the Ets, Sox and NHR binding motifs (top). Schematic depicts sequence variants in which each class of motif or control regions were targeted by mutation. Red X's denote the location of targeted sites. mp-GFP: mouse *Beta-globin* minimal promoter fused to GFP. **h**, Graph reports the frequency of embryos with GFP expression in CHT ECs after injection with wild-type sequences or mutated variants of the *sele* 158 bp enhancer. Data is normalized to the wild-type control (23% GFP^+ CHT ECs (176/775)). Mean +/- s.e.m., One-way ANOVA with Dunnett's multiple comparisons test; ***P<0.001, ****P<0.0001. **i**, Images show electrophoretic mobility shift assays with recombinant Nr2f2-GST that was incubated with DNA sequences spanning the NHR motifs present in the 125 bp *mrc1a* (left two gels) or 158 bp *sele* (right gel) enhancer sequences. Red arrows point to DNA:protein binding while orange arrowheads point to super-shifted DNA:protein complexes. Labeled DNA:protein complexes were outcompeted by unlabeled wild-type probe (lane 4) but not by unlabeled probe in which the NHR motif was disrupted by mutation (blue arrows). All experiments were performed at least three times, with independent clutches.



Supplemental Figure 3

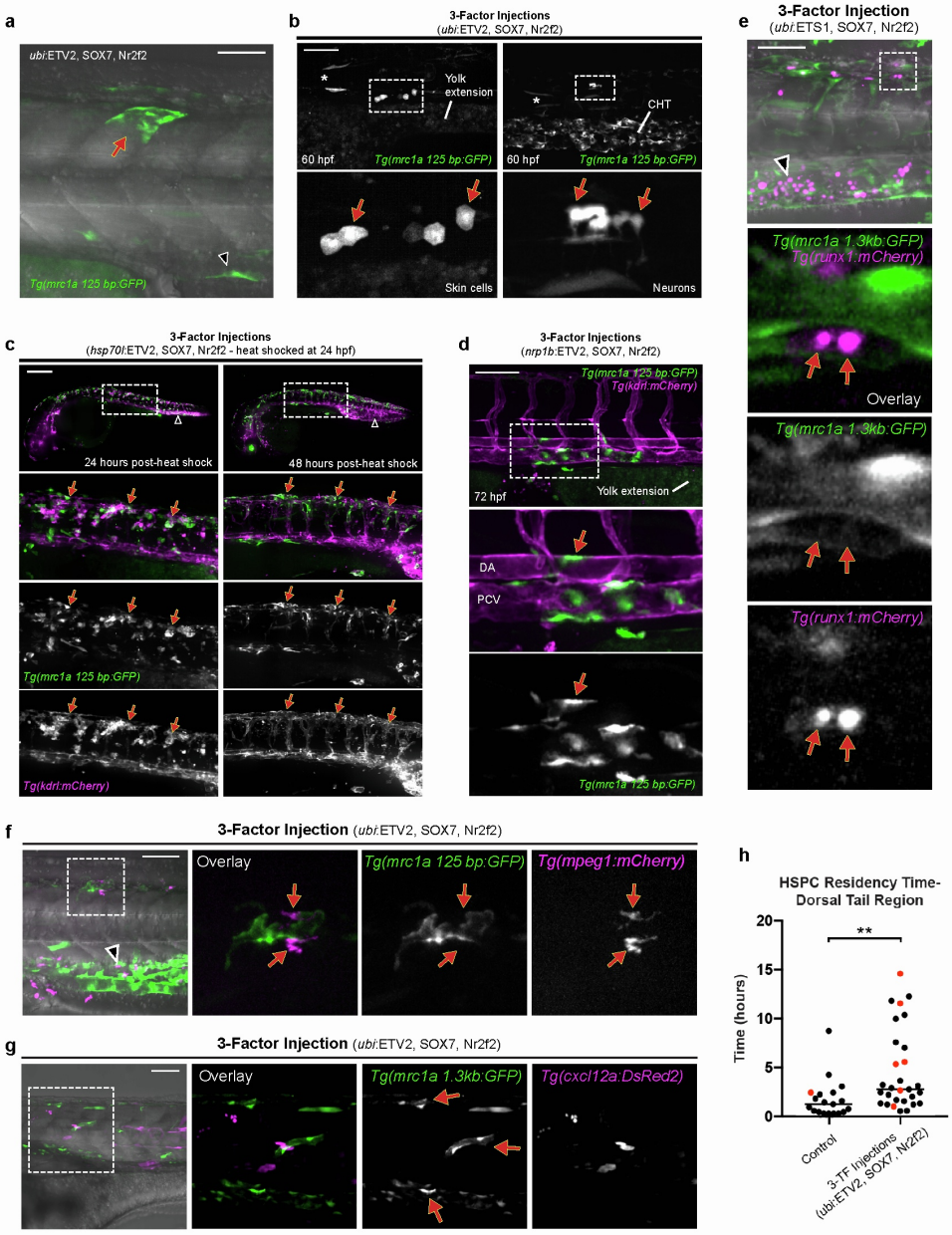
Supplemental Figure 3 | Transcription factor induction of niche EC gene expression (related to Figure 5 and 6). a, Images show WISH staining for *mrc1a* over the yolk ball in a control (left) and 7-factor injected embryo (right). **b**, Image shows a *sele 5.3kb:GFP* transgenic embryo that was injected with the 7-factor pool. **c**, Images show a large vessel in the dorsal tail region (red arrow) ectopically expressing *mrc1a 125bp:GFP* (green) and *kdrl:mCherry* (magenta). Magnification of white dotted box is shown on the right. Dorsal longitudinal anastomotic vessels (DLAVs); Intersegmental vessels (ISVs). **d**, Graph reports quantitative measurements of vessel diameter for intersegmental vessels (ISVs) and dorsal longitudinal anastomotic vessels (DLAVs), and CHT sinusoidal vessels in *ubi*:ETV2, SOX7 and N2f2 and control injected embryos. One-way ANOVA with Tukey's test for multiple comparisons; ****P<0.0001; n.s. = not significant. **e**, Graph reports the percentage of transcription factor-injected embryos that showed ectopic expression of *mrc1a 125bp:GFP.* Fisher's exact test for pairwise comparisons was used; n.s. = not significant. **e**, Graph reports the percentage of transcription factor-injected embryos that showed ectopic expression of *mrc1a 125bp:GFP.* Fisher's exact test for pairwise comparisons was used; n.s. = not significant. **f**, Images show *mrc1a 1.3kb:GFP* transgenic embryos that were injected with different combinations of transcription factors at the one-cell stage. Grayscale images of the GFP signal are shown on the right. Red arrows denote regions of ectopic expression and black arrowheads point to normal domains of expression in all panels of this figure. **g-h**, Injection of human ETV2 alone induces ectopic expression of *mrc1a* (**g**) and zebrafish transcription factors (**h**), including *sox7*, *sox18*, *fli1a* and *etv2*. Embryos shown range from 24-36 hpf. All experiments were

performed at least three times, with independent clutches. Scale bar represents 100 μm in **a**-**c** and **f**.



Supplemental Figure 4

Supplemental Figure 4 | Redundancy in the transcription factor regulation of niche EC gene expression (related to Figure 6). **a**, Uniform Manifold Approximation and Projection (UMAP) plots show cell clustering and gene expression from a single cell RNA-seq analysis of endothelial cells isolated from embryos at 72 hpf. Spectral scales report z-scores. **b-c,** Bar graphs report the quantification of *mrc1a 125bp:GFP* expression (**b**) and HSPC localization (**c**) in animals injected with the *nr2f1a, nr2f2, nr2f5* or control morpholinos. Chi squared test; **P<0.01,****P<0.0001. **d**, UMAP plots show single cell RNA-seq data on whole tail tissue dissected from 72 hpf embryos. The plot on the left includes labels for the distinct cell populations. The remaining three plots show the expression for *etv2*, *sox7* and *nr2f2*. All experiments were performed at least three times, with independent clutches.



Supplemental Figure 5

Supplemental Figure 5 | Tissue-specific induction of niche EC gene expression and ectopic recruitment of HSPCs (related to Figures 6 and 7). a, Images show a cluster of GFP$^+$ muscle-shaped cells ectopically expressing the *mrc1a 125 bp:GFP* transgene in a *ubi*:ETV2, SOX7, Nr2f2 injected embryo. Image corresponds to Supplementary Video 4. **b**, Images show GFP$^+$ skin cells (left) and neurons (right) ectopically expressing the *mrc1a 125 bp:GFP* transgene in a *ubi*:ETV2, SOX7, Nr2f2 injected embryo. Asterisks denote expression in muscle cells. **c**, Images show ectopic expression in *mrc1a 125bp:GFP; kdrl:mCherry* double positive embryos that were injected with *hsp70l:*ETV2, SOX7, Nr2f2 plasmids at the one-cell stage and then heat shocked at 24 hpf. Magnification of boxed regions is shown at bottom. **d**, Images show ectopic expression in a *mrc1a 125bp:GFP; kdrl:mCherry* double positive embryo that was injected with endothelial-specific *nrp1b:*ETV2, SOX7, Nr2f2. Red arrow points to GFP expression in an arterial EC. Dorsal aorta (DA); posterior cardinal vein (PCV). **e**, Image shows *runx1:mCherry*$^+$ HSPCs localized outside the CHT within a dorsal ectopic region of *mrc1a 1.3kb:GFP* expression in an embryo injected with a pool of *ubi:*ETS1, SOX7 and Nr2f2. Magnifications of boxed region are shown. Red arrows point to ectopic expression or localization while black arrowheads point to normal expression or localization in this and other panels in this figure. Scale bars represent 100 μm. **f**, Images show ECs ectopically expressing *mrc1a 125bp:GFP* (boxed region) that are associated with *mpeg1:mCherry*$^+$ macrophages (red arrows), similar to ECs in the CHT (arrowhead). This data corresponds to Supplementary Video 7. **g**, Images show ECs (red arrows) in an anterior region of an embryo ectopically expressing *mrc1a 125bp:GFP* that are not associated with *cxcl12a:DsRed2*$^+$ stromal cells. **h**, Graph reports lifetime

measurements of HSPC residency outside of the CHT in control and 3-factor injected embryos. Red dots correspond to cells that divided. Mann-Whitney test; **P<0.01. Scale bars represent 100 μm in **a-b, d-g**. All experiments were performed at least three times, with independent clutches.

Supplementary Table 4 | *In vivo* screening of predicted enhancer elements (related to Figure 3)

Type of Element	Gene Name	Genomic Coordinates of ATAC-seq Element[a]	Relative to TSS (kb)	Amplicon Size (bp)	Showed Predicted GFP Expression Pattern[b]	Element Contains Ets, SoxF and NHR Motifs
CHT EC Element	*ap1b1*	chr5:26463217-26463695	17	750	Yes	Yes
	cltca	chr10:29,047,274-29,047,619	2.8	404	Yes	Yes
	dab2	chr5:33,980,000-33,980,306	-3.5	394	Yes	Yes
	exoc3l2a	chr5:38359097-38359903	5.9	901	Yes	Yes
	glula	chr2:19,458,704-19,459,047	4.8	446	No	Yes
	gpr182	chr23:36701205-36701682	-4.9	481	Yes	Yes
	gpr182	chr23:36694073-36694476	-2.8	398	Yes	Yes
	gpr182	chr23:36696363-36696656	1.6	577	Yes	Yes
	lgmn	chr13:36,448,465-36,448,818	2.9	414	Yes	Yes
	prcp	chr15:10,400,588-10,400,868	23	334	No	No[c]
	sele	chr20:34,010,027-34,010,326	-9.7	398	Yes	Yes
	sele	chr20:34,011,251-34,011,563	-8.5	360	Yes	Yes
	snx8a	chr3:42,090,805-42,091,062	5.5	395	No	Yes
	stab1	chr22:10467346-10467937	-2.8	874	Yes	Yes
	stab2	chr4:9790795-9791116	4.3	422	Yes	Yes
Pan-EC Element	*cdh5*	chr7:45457842-45458791	13	823	Yes	Yes
	clec14a	chr17:10362325-10362844	-3.1	455	Yes	Yes
	dll4	chr20:28219013-28219619	-55	452	Yes	No[c]
	fli1a	chr18:47039842-47040466	47	800	Yes	No[c]
	lmo2	chr18:36722030-36722527	-3.6	367	Yes	No[c]
	nrp1b	chr2:43535098-43535801	-34	552	Yes	Yes

Table shows CHT EC-specific and pan-EC ATAC-seq elements that were fused to a minimal promoter and GFP and injected into one cell-stage zebrafish embryos. [a]Coordinates of MACS2 peak. [b]Expressed in CHT ECs for CHT EC elements and in vessels throughout the embryo for pan-EC elements. [c]Lacks NHR motif.

Supplementary Table 6 | Transcription factor expression in CHT ECs (related to Figure 5)

Transcription Factor	Family	FPKM	Associated with CHT EC ATAC-seq Element Containing Ets, Sox and NHR Sites*	Genomic Coordinates of Representative Element
fli1a	Ets	480.4	Yes	chr18:46966409-46966698
etv2	Ets	192.3	Yes	chr16:44782409-44782895
ets1	Ets	183	Yes	chr18:46883643-46884100
sox18	SoxF	206.4	Yes	chr23:8886011-8886744
sox7	SoxF	125.1	Yes	chr20:19158376-19158663
nr2f2	NHR	84.6	Yes	chr18:23728906-23729747
rxraa	NHR	45.9	Yes	chr21:16411020-16411531

Table shows FPKM expression values in CHT ECs for highly expressed members of the Ets, Sox and NHR transcription factor families.
*Within 100 kb of TSS; some genes are associated with multiple elements.

Supplementary Table 7 | Transcription factor expression in mouse hematopoietic niche (related to Figure 5)

Transcription Factor	Family	Mouse E14-E15 Liver EC FPKM	Mouse E16-E17 Liver EC FPKM	Mouse Adult Bone Marrow EC FPKM
Ets1	Ets	218.4666	251.9493	153.2657
Erg	Ets	46.64156	78.53131	45.14673
Elk4	Ets	9.369453	11.4226	22.83457
Elk1	Ets	7.003965	9.08418	6.8779
Etv1	Ets	2.203135	3.10327	1.488542
Etv2	Ets	0.235977	0	0
Sox18	SoxF	127.1509	262.44	130.1783
Sox7	SoxF	49.94503	46.9365	19.80563
Sox17	SoxF	33.01219	68.37438	90.24645
Sox11	SoxF	12.01665	11.1584	0.67509
Sox12	SoxF	11.81507	21.5267	0.556478
Sox6	SoxF	1.741399	1.158524	0.51182
Sox5	SoxF	0.193041	0.289841	0.437005
Sox9	SoxF	0.119527	0.072563	0
Nr2f2	NHR	58.97832	103.5558	63.17458
Rxra	NHR	23.98264	33.0942	22.08392
Rara	NHR	19.29841	27.37294	13.93433
Nr4a2	NHR	10.13413	3.130986	30.30394
Esrrb	NHR	6.219864	7.884516	0.586381
Rora	NHR	1.219604	1.086872	5.922048

Supplementary Table 9 | Primers used to clone promoter and enhancer elements (related to Figure 3)

Type of Element	Gene	Genomic Coordinates of ATAC-seq Element	Amplicon Size (bp)	Forward	Reverse
5' upstream of TSS	*mrc1a* (1.3 kb)	chr7:65,468,213-65,469,565	1353	CTTTTGGCCATTACTGCCG	TTCTGTCTTTTAATCAGCAATCC
CHT EC element	*mrc1a* (125 bp)	chr7:65469086-65469210	125	GCTCTCAGTTCCTGGTATTTTTCT	TGAAGCTTGTACCTTTCATTTCC
5' upstream of TSS	*sele* (5.3 kb)	chr20:34,001,481-34.006.781	5301	TCGTTACTGCACTTGAAAGCGT	TATCAGTGATGTTCTGCAGTGGTC
CHT EC element	*sele* (158 bp)	chr20:34004805-34004962	158	CCATGAAACTGGGAAGATGAA	CAGGAAGAAATAATGGCAAAAA
CHT EC element	*ap1b1*	chr5:26463217-26463695	750	GAAGCTCTCCAGCAGCTCA	CATTTCCACCAGCTGTCTGAT
CHT EC element	*cltca*	chr10:29,047,274-29.047.619	404	GCTGTCAGCACATTCTTTTCC	CCCTGCTGATCACACATGAC
CHT EC element	*dab2*	chr5:33,980,000-33,980,306	394	ACTGCTCCTCACCAATCGTC	TGCACTAAATCTGTGCCAAGTC
CHT EC element	*exoc3l2a*	chr5:38359097-38359903	901	TTTATATAATCGGAAGGAACCTTTTT	TCCTGTCAGCTGTTTTCATCC
CHT EC element	*glula*	chr2:19,458,704-19,459,047	446	GGCAAAATGCTTAGATGCAGA	TGCGAGGAGGACATAAACAA
CHT EC element	*gpr182*	chr23:36701205-36701682	481	TAGCCTTGTGCAATGCTTGT	TGCTGAATTCAAAAGCCACTT
CHT EC element	*gpr182*	chr23:36694073-36694476	398	CACTTCTGGTACCAAATGATCAAC	GAGGGTTAAACGTGGCCTTA
CHT EC element	*gpr182*	chr23:36696363-36696656	577	GCGGCAAACTTTTTGAGTGT	GCCAGCCTCAAAGTTTGTTCT
CHT EC element	*lgmn*	chr13:36,448,465-36,448,818	414	CGCGTGATGAGGATCTGATT	GGTGTTGAAAGGTGATGCTG
CHT EC element	*prcp*	chr15:10,400,588-10,400,868	334	AAAATTAAGAGCGGGCAGACT	TGGAAACAACAACAGCCTGA
CHT EC element	*sele*	chr20:34,010,027-34,010,326	398	AAAGCACTTGATTGAGAATTGC	TGTTTGGTTCAGTTACACGTTTT
CHT EC element	*sele*	chr20:34,011,251-34,011,563	360	CAGTTTCCCAAGCTTCAAGG	TGTGATTACACATTCCCACACAT
CHT EC element	*snx8a*	chr3:42,090,805-42,091,062	395	AATGGTTGCAGCATTGTGTT	GCTTTTGTTTGGTGATGTGC
CHT EC element	*stab1*	chr22:10467346-10467937	874	GTTACCTGGCAACCACCAAC	TGGTCAGAATAAGCACGTTTCA
CHT EC element	*stab2*	chr4:9790795-9791116	422	ACGTTAACAAAGGCGATGTTTT	TCTAAACAATTTTTAAGGTAAACCAAA
Pan-EC element	*cdh5*	chr7:45457842-45458791	823	TGACAGGACTCATCAGCACG	AATAGTCTCTGGTCTGCTGTTAAA
Pan-EC element	*clec14a*	chr17:10362325-10362844	455	TGGGAAAAATACCAGGAAGCGT	AAGCAGCGAGCTCTCATAATAAA
Pan-EC element	*dll4*	chr20:28219013-28219619	452	AGATCAATGAGAGCGAGGCG	GGAGCAGATGAGGTTAAGTCCT
Pan-EC element	*fli1a*	chr18:47039842-47040466	800	CGGACAGTAATGTCTGGATGG	CCACAACTCCATACTGGGAAA
Pan-EC element	*lmo2*	chr18:36722030-36722527	367	TCATCATGGCCAACAGAATG	GTGCAGGAAATGAGCACAGA
Pan-EC element	*nrp1b*	chr2:43535098-43535801	552	TGACTCAACCAATCAATCAGCCT	TAGCAAAGCTCTCAGGCCC

Supplementary Table 10 | Sequences and primers for mutational variants of the 125 bp *mrc1a* and 158 bp *sele* enhancer elements (related to Figure 4)

Gene	Fragment Name	Total Fragment Sequence	Forward Primer	Reverse Primer
mrc1a	Wild-type	CCATGAAACTGGGAAGATGAAAGCATTAG TTGAATTGTTACTGGCAACATCTTCTCTGT AATGCCCCCTGTGACCCATATTGTCTCGCT CTTTCCTTTATAAACAGAGCTGTAGATATC CACAGGAAATGGGGGTGTTTTTGCCATTA TTTCTTCCTG	TGAAGCTTGTACCTTTCATTTCCTTTTTGC TGAGCTTTATTTTCTCTAGAATTGCCATTG TGTTTCCATTCTAG	GCTCTCAGTTCCTGGTATTTTTCTTTCAGC TGAAAAAAAAAATGCTGATTTGCTAGAATGG AAACACAATGGCAAT
mrc1a	Ets mutant	TGAAGCTTGTACCTTTCATTTaaaTTTTGCT GAGCTTTATTTTCTCTAGAATTGCCATTGT GTTTCCATTCTAGCAAATCAGCATTTTTTTT TCAGCTGAAAGAAAAATACCAtttACTGAGA GC	TGAAGCTTGTACCTTTCATTTaaaTTTTGCT GAGCTTTATTTTCTCTAGAATTGCCATTGT GTTTCCATTCTAG	GCTCTCAGTaaaTGGTATTTTTCTTTCAGCT GAAAAAAAAATGCTGATTTGCTAGAATGGA AACACAATGGCAAT
mrc1a	Sox mutant	TGAAGCTTGTACCTTTCATTTCCTTTTTGC TGAGCgggcggggaTCTAGAATTGCacggtgGTT TCCATTCTAGCAAATCAGCcgggggTTTCAG CTGAAAGAAAAATACCAGGAACTGAGAGC	TGAAGCTTGTACCTTTCATTTCCTTTTTGC TGAGCgggcggggaTCTAGAATTGCacggtgGTT TCCATTCTAG	GCTCTCAGTTCCTGGTATTTTTCTTTCAGC TGAAAccccccgGCTGATTTGCTAGAATGGA AACcaccgtGCAAT
mrc1a	NHR mutant	attAGCagatTtaaTTTCATTTCCTTTTTGCattaa TTTATTTTCTCTAGAATTGCCATTGTGTTT CCATTCTAGCAAATCAGCATTTTTTTTTCAG CTGAAAGAAAAATACCAGGAACTGAGAGC	attAGCagatTtaaTTTCATTTCCTTTTTGCattaa TTTATTTTCTCTAGAATTGCCATTGTGTTT CCATTCTA	GCTCTCAGTTCCTGGTATTTTTCTTTCAGC TGAAAAAAAAATGCTGATTTGCTAGAATGG AAACACAATGGCAAT
mrc1a	Control mutant	TGAAGCTTGTACCTTTCATTTCCTTTTTGC TGAGCTTTATTTTCTCTAGAATTGCCATTG TGTTTCCATTCcgGCAAATCAGCATTTTTTT TTCAGCTGAccGAAAAATACCAGGAACTGA GAGC	TGAAGCTTGTACCTTTCATTTCCTTTTTGC TGAGCTTTATTTTCTCTAGAATTGCCATTG TGTTTCCATTCTAG	GCTCTCAGTTCCTGGTATTTTTCggTCAGCT GAAAAAAAAATGCTGATTTGCcgGAATGGA AACACAATGGCAAT
sele	Wild-type	CCATGAAACTGGGAAGATGAAAGCATTAG TTGAATTGTTACTGGCAACATCTTCTCTGT AATGCCCCCTGTGACCCATATTGTCTCGCT CTTTCCTTTATAAACAGAGCTGTAGATATC CACAGGAAATGGGGGTGTTTTTGCCATTA TTTCTTCCTG	CCATGAAACTGGGAAGATGAAAGCATTAG TTGAATTGTTACTGGCAACATCTTCTCTGT AATGCCCCCTGTGACCCATATTGTCTCGCT CT	CAGGAAGAAATAATGGCAAAAACACCCCCA TTTCCTGTGGATATCTACAGCTCTGTTTAT AAAGGAAAGAGCGAGACAATATGGGTCAC AG
sele	Ets mutant	CCATGAAACTGGGAAGATGAAAGCATTAG TTGAATTGTTACTGGCAACATCTTCTCTGT AATGCCCCCTGTGACCCATATTGTCTCGCT CTTTaaaTTATAAACAGAGCTGTAGATATCC ACAtttAATGGGGGTGTTTTTGCCATTATTT CTaaaTG	CCATGAAACTGGGAAGATGAAAGCATTAG TTGAATTGTTACTGGCAACATCTTCTCTGT AATGCCCCCTGTGACCCATATTGTCTCGCT CT	CAtttAGAAATAATGGCAAAAACACCCCCATT aaaTGTGGATATCTACAGCTCTGTTTATAAtt tAAAGAGCGAGACAATATGGGTCACAG
sele	Sox mutant	CCATGAAACTGGGAAGATGAAAGCATTAG TTGAAggtggACTGGCAACATCTTCTCTGTAA TGCCCCCTGTGACCCATAggtgaTCGCTCTTT CCTTTATAAACAGAGCTGTAGATATCCACA GGAAATGGGGGTGTTTTTGCCATTATTTC TTCCTG	CCATGAAACTGGGAAGATGAAAGCATTAG TTGAAggtggACTGGCAACATCTTCTCTGTAA TGCCCCCTGTGACCCATAggtgaTCGCTCT	CAGGAAGtttattaGGCAAAAACACCCCCATTT CCTGTGGATATCTACAGCTCTGTTTATAAAA GGAAAGAGCGAtcaccTATGGGTCACAG
sele	NHR mutant	CCATGAAACTGGGAAGATGAAAGCATTAG TTGAATTGTTACTGGCAACATCTTCTCTGT AATGCCCCCTGattaaCATATTGTCTCGCTCT TTCCTTTATAAACAGAGCTGTAGATATCCA CAGGAAATGGGGGTGTTTTTGCCATTATT TCTTCCTG	CCATGAAACTGGGAAGATGAAAGCATTAG TTGAATTGTTACTGGCAACATCTTCTCTGT AATGCCCCCTGattaaCATATTGTCTCGCTCT	CAGGAAGAAATAATGGCAAAAACACCCCCA TTTCCTGTGGATATCTACAGCTCTGTTTAT AAAGGAAAGAGCGAGACAATATGttaatCAG
sele	Control mutant	CCATGAAACTGGGAAtcTGAAAGCATTAGT TGAATTGTTACTGGCAACATCTTCTCTGTA ATGCCCCCTGTGACCCATATTGTCTCGCTC TTTCCTTTATAAACAGAGagGTAGATATCCA CAGGAAATGGGGGacTTTTTGCCATTATTT CTTCCTG	CCATGAAACTGGGAAtcTGAAAGCATTAGT TGAATTGTTACTGGCAACATCTTCTCTGTA ATGCCCCCTGTGACCCATATTGTCTCGCTC T	CAGGAAGAAATAATGGCAAAAAgtCCCCCAT TTCCTGTGGATATCTAcctCTCTGTTTATAA AGGAAAGAGCGAGACAATATGGGTCACAG

Lowercase letters indicate base pair changes used to disrupt transcription factor binding motifs.

Supplementary Table 11 | Primers used for cloning and EMSA probe synthesis (related to Figure 4)

Category	Primer Name	Forward	Reverse	Comment
Cloning	*Nr2f2*	CGGGATCCatggca atggtagtca gcacg	CCGGAATTCCGGttgaattgccatatatggc	
Probe synthesis	*mrc1a* site 1 wild-type	tttaTGAAGCTTGTACCTTTCATTTCCTTTTTG	CAAAAAGGAAATGAAAGGTACAAGCTTCAtaaa	
Probe synthesis	*mrc1a* site 1 mutation	TTTAattAGCagatTtaaTTTCATTTCCTTTTTG	CAAAAAGGAAATGAAAttaAatctGCTaatTAAA	1st NHR site mutated like *in vivo* GFP reporter experiment
Probe synthesis	*mrc1a* site 2 wild-type	TTCATTTCCTTTTTGCTGAGCTTTATTTTC	GAAAATAAAGCTCAGCAAAAAGGAAATGAA	
Probe synthesis	*mrc1a* site 2 mutation	TTCATTTCCTTTTTGCattaaTTTATTTTC	GAAAATAAAttaatGCAAAAAGGAAATGAA	2nd NHR site mutated like *in vivo* GFP reporter experiment
Probe synthesis	*sele* wild-type	GTAATGCCCCCTGTGACCCATATTGTCTCGCTCTTTCCTTTATA	TATAAAGGAAAGAGCGAGACAATATGGGTCACAGGGGGCATTAC	
Probe synthesis	*sele* mutation	GTAATGCCCCCTGattaaCATATTGTCTCGCTCTTTCCTTTATA	TATAAAGGAAAGAGCGAGACAATATGttaatCAGGGGGCATTAC	NHR site mutated like *in vivo* GFP reporter experiment

Table shows primers used for cloning mouse *Nr2f2* into the pGEX2TK vector and DNA probes from the zebrafish *mrc1a* and *sele* enhancers.