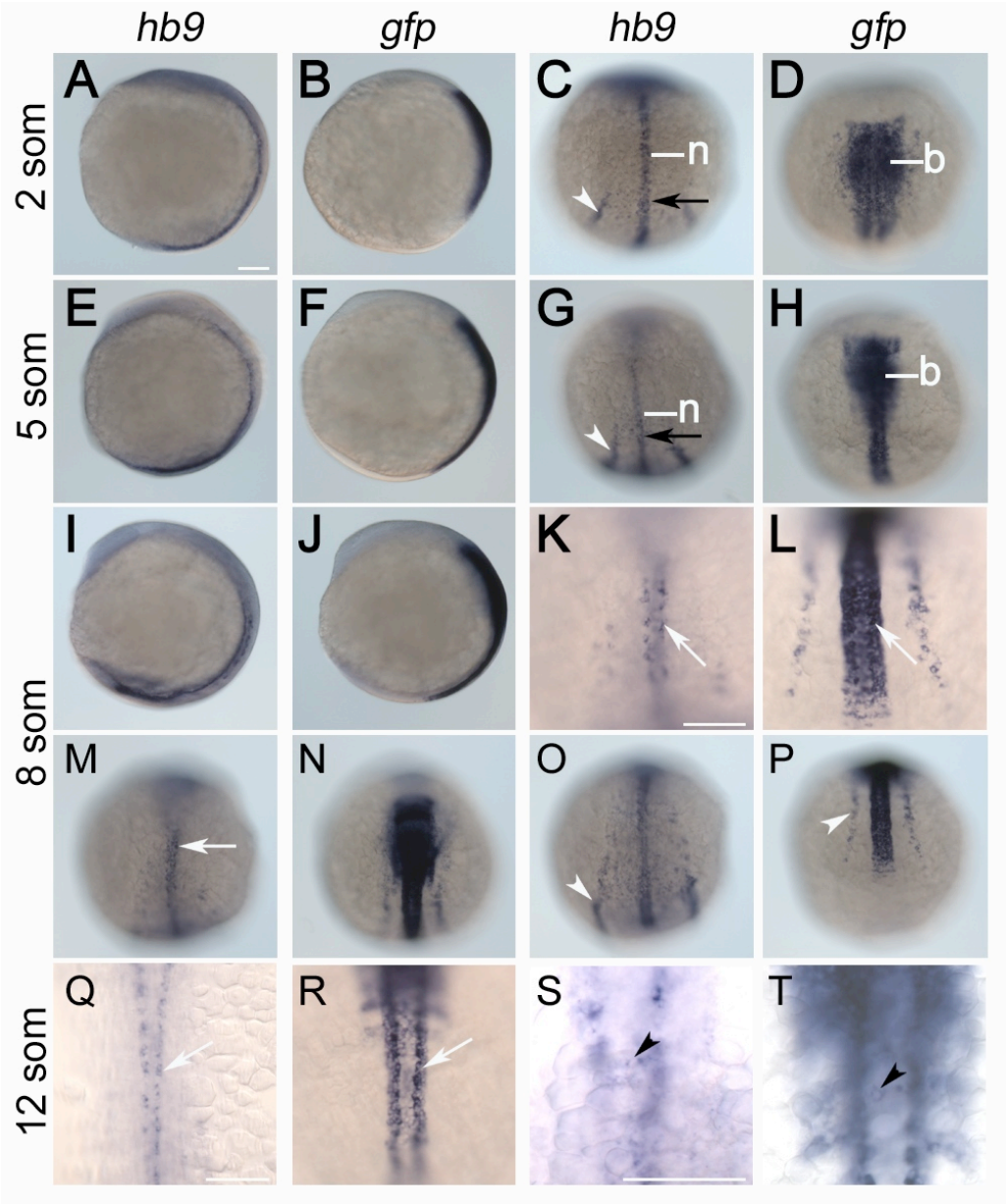
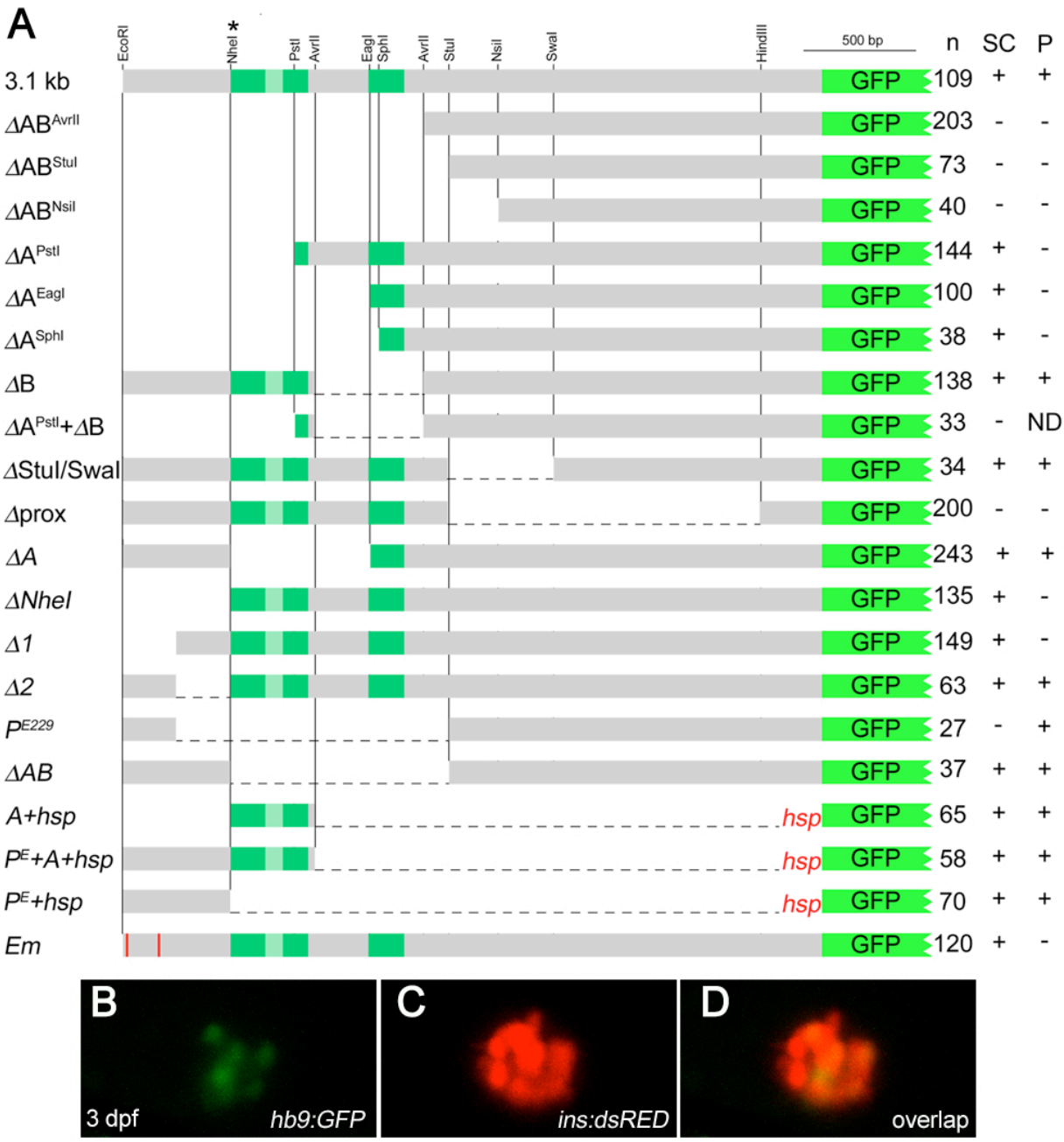
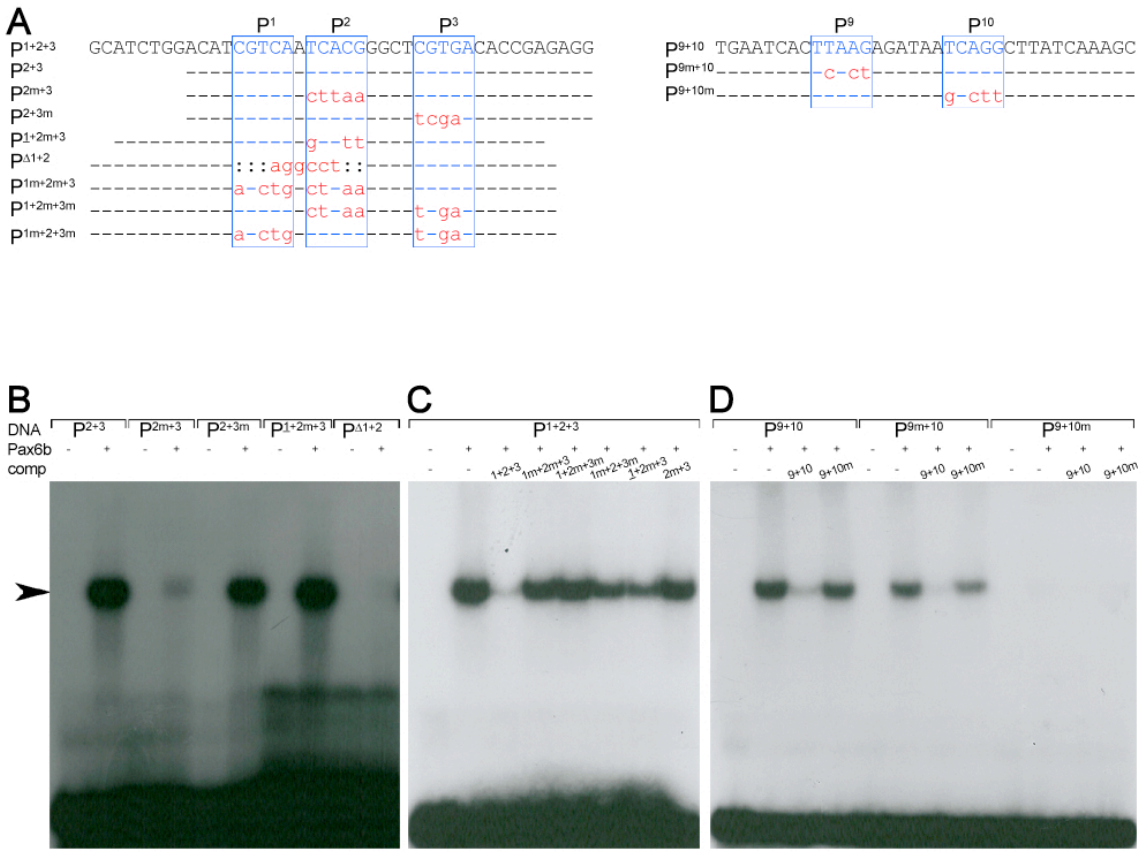


Supplemental Fig. 1. Comparison of early *hb9* and *gfp* expression in *Tg[hb9:GFP]*^{ml2} . Stains are shown at 2 **(A-D)**, 5 **(E-H)**, 8 **(I-P)** and 12 somite stage **(Q-T)**. Expression of *hb9* is restricted to notochord (n), posterior endoderm (black arrow) and posterior intermediate mesoderm (white arrowhead), motoneurons (white arrow) and at 12 stage also to beta-cell progenitors (black arrowhead). Differently, *gfp* mRNA in early *Tg[hb9:GFP]*^{ml2} embryos is expressed in the entire hindbrain (b), the anterior spinal cord and in intermediate mesoderm. At 12 somites stage *gfp* expression in the trunk marks anterior somites, beta-cells and ventral spinal neurons. Embryos are shown from lateral **(A, B, E, F, I, J)** or dorsal **(C, D, G, H, K-N, Q-T)** with anterior pole to the top or from posterior with the dorsal side up **(O, P)**. Scale bars correspond to 100 μm.

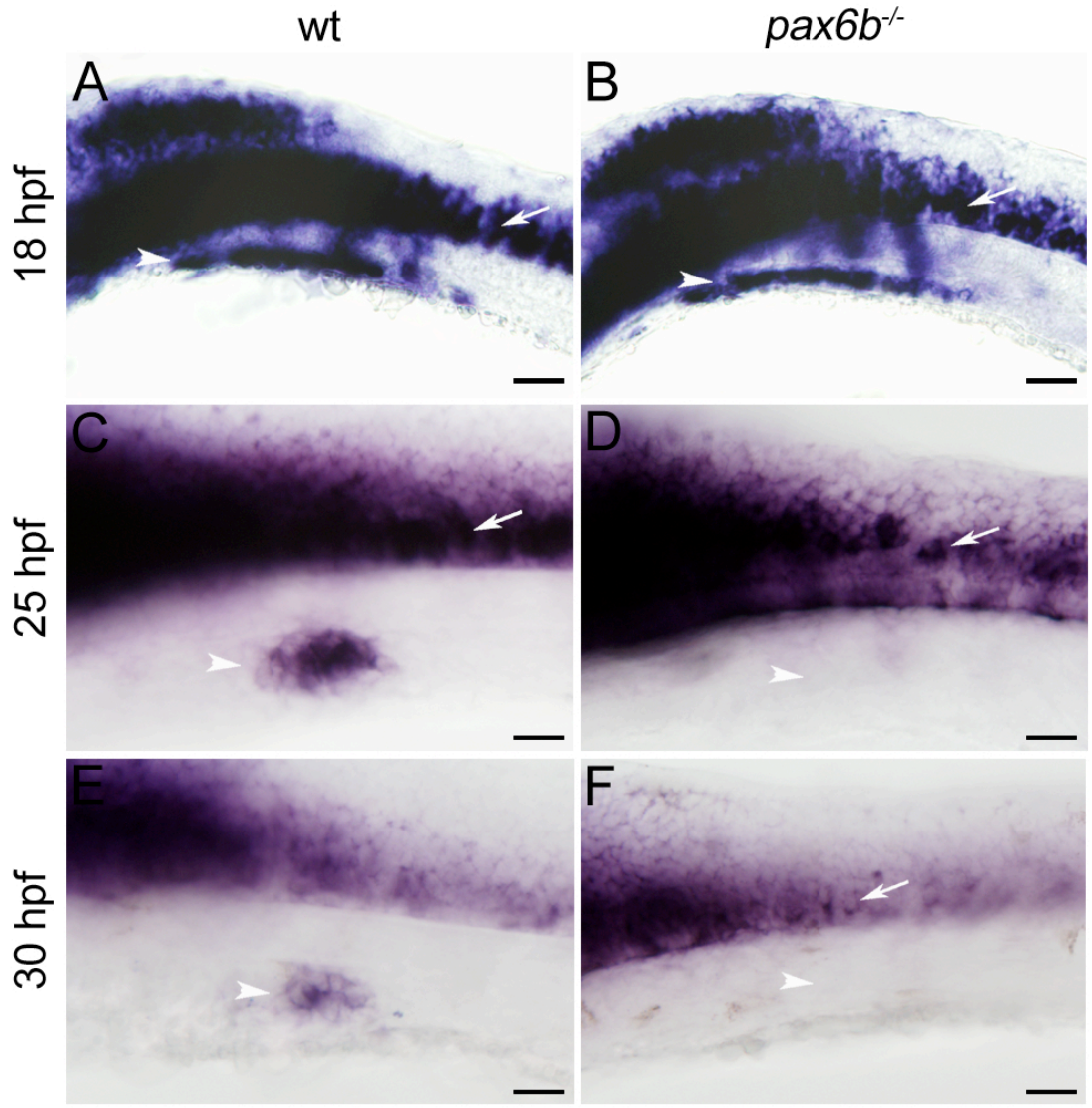


Supplemental Fig. 2. Characterization of 3.1 kb of upstream *hb9* promoter in zebrafish (A). Scheme of deletion constructs that were tested in transient and transgenic GFP reporter assays in 24 hpf embryos. Indicated are the number of GFP expressing embryos (n) and the presence (+) or absence (-) of GFP expression in spinal cord (SC) and pancreas (P). NheI* indicates an additional NheI site that was introduced by site-directed mutagenesis. **(B-D)** Confocal image projection of a *Tg[ins:dsRED]* embryo showing mosaic GFP expression in beta-cells following injection of *hb9:GFP* DNA.



Supplemental Fig. 3. Pax6b binding properties of the sequence motives P^{1-3} and P^{9-10}

(A) Sequence of oligonucleotides used in the EMSA studies (top row). Indicated are the Pax6 core consensus sequences P^1, P^2, P^3, P^9 and P^{10} (blue box, letters in upper case) and nucleotides that were unchanged (line), mutated (red letters in lower case) or deleted (colon) in comparison to the genomic sequence. **(B-D)** Radiograms of EMSA studies with corresponding ^{32}P-labeled oligonucleotides (DNA, as indicated), Pax6b protein and non-labelled competitor DNA (comp, as indicated). **(B)** Weak or missing signals (arrow) for P^{2m+3}, $P^{\Delta 1+2}$ and strong signals for P^{2+3}, P^{2+3m} and P^{1+2m+3} suggest that Pax6b binds with high affinity to P^1 and P^2 but not to P^3. **(C)** The inefficient competition of all mutated oligonucleotides in comparison to P^{1+2+3} shows that highest affinity binding requires the presence of both sites, P^1 and P^2. **(D)** Missing signals for and inefficient competition by P^{9+10m} show that Pax6b binds with high affinity to P^{10} but not to P^9.



Supplemental Fig. 4. *gfp* expression in *Tg[hb9:GFP]*^{ml2} is initiated but not maintained in *Pax6b* deficient embryos. (**A-F**) *gfp* mRNA expression in *Tg[hb9:GFP]*^{ml2} control embryos **(A, C, E)** and *pax6b*^{sa0086} mutant embryos **(B, D, F)** at 18 hpf **(A,B),** 25 hpf **(C,D)** and 30 hpf **(E,F)**. Pancreatic *gfp* expression (white arrowhead) is present at 18 hpf but missing in older embryos sinitin (white arrowhead). Expression in the spinal cord (white arrow) is not affected by the pax6b mutation. Embryos are shown in lateral view with anterior to the left. Scale bars in all figures correspond to 50 μm.

phenotype / genotype	pancreas	SC	kidney	muscle	Additional sites of expression
Tg[hb9:GFP]_1	+	+	+, post	+	
Tg[hb9:GFP]_2	+	+	+, post	+	
Tg[hb9$^{\Delta A}$:GFP]_1	+	+	++	++	brain
Tg[hb9$^{\Delta A}$:GFP]_2	-	+	++	++	brain
Tg[hb9$^{\Delta A}$:GFP]_3	+	+	++	++	brain
Tg[hb9$^{\Delta A}$:GFP]_4	-	+	++	++	brain, vessels
Tg[hb9$^{\Delta A}$:GFP]_5	+	+	++	++	brain, vessels
Tg[hb9$^{\Delta A}$:GFP]_6	+	+	++	+	brain
Tg[hb9$^{\Delta A}$:GFP]_7	+	+	+	+	brain
Tg[hb9$^{\Delta A}$:GFP]_8	+	+	-	-	
Tg[hb9$^{\Delta A}$:GFP]_9	+	+	+, post	+	brain
Tg[hb9$^{\Delta A}$:GFP]_10	-	+	++	+	brain, vessels
Tg[hb9$^{\Delta A}$:GFP]_11	+	+	++	+	brain, endoderm
Tg[hb9$^{\Delta A}$:GFP]_12	+	+	+	+	brain
Tg[hb9$^{\Delta NheI}$:GFP]_1	-	+	-	-	eyes
Tg[hb9$^{\Delta 1}$:GFP]_1	-	+	+, post	+	brain
Tg[hb9$^{\Delta 1}$:GFP]_2	-	+	-	-	brain
Tg[hb9$^{\Delta 1}$:GFP]_3	+	+	+	+	brain, fins
Tg[hb9$^{\Delta 1}$:GFP]_4	-	+	+, post	+	
Tg[hb9$^{\Delta 1}$:GFP]_5	-	+	+	+	brain, eyes
Tg[hb9$^{\Delta 1}$:GFP]_6	-	+	+	+	
Tg[hb9$^{\Delta 1}$:GFP]_7	-	+	+	-	eyes
Tg[hb9$^{\Delta 2}$:GFP]_1	+	+	+	++	brain
Tg[hb9$^{\Delta 2}$:GFP]_2	+	+	+	+	brain
Tg[hb9$^{\Delta 2}$:GFP]_3	-	+	+	+	brain
Tg[hb9$^{\Delta 2}$:GFP]_4	+	+	+	+	brain
Tg[hb9$^{\Delta 2}$:GFP]_5	+	+	-	+	brain
Tg[hb9$^{\Delta 2}$:GFP]_6	-	+	-	-	brain
Tg[hb9$^{\Delta 2}$:GFP]_7	-	+	+, post	+	brain
Tg[hb9$^{\Delta 2}$:GFP]_8	+	+	-	-	brain
Tg[hb9$^{\Delta 2}$:GFP]_9	+	+	++	+	brain, vessels
Tg[hb9$^{\Delta 2}$:GFP]_10	+	+	+, post	+	brain

Supplemental Table 1. GFP expression in *hb9:GFP* transgenic reporter lines corresponding to Fig. 2.

Embryos at 28 hpf were analysed for presence (+) or absence (-) of GFP expression in the endocrine islet (pancreas) and spinal cord (SC motoneurons and interneurons) and for intensity of GFP signals in the kidney and somatic muscle (+: weak signal, ++: strong signal, post: posterior expression). Additional sites of expression were more variable and include the brain, eyes, blood vessel (vessels), endoderm and fin epidermis (fins).

phenotype / genotype	pancreas	SC	kidney	muscle	additional sites of expression
Tg[hb9^{PE229}:GFP]_1	-	-	+	++	brain, vessels, fins
Tg[hb9^{PE229}:GFP]_2	-	-	++	+	brain, vessels
Tg[hb9^{PE229}:GFP]_3	(+)	-	+	++	brain, vessels, fins
Tg[hb9^{PE229}:GFP]_4	-	-	++	-	vessels
Tg[hb9^{PE229}:GFP]_5	(+)	+	++	+	brain , vessels
Tg[hb9^{PE229}:GFP]_6	(+)	+	-	+	brain vessels
Tg[hb9^{PE229}:GFP]_7	-	-	++	+	vessels
Tg[hb9^{PE229}:GFP]_8	-	-	++	+	brain, vessels, fins
Tg[hb9^{PE229}:GFP]_9	+	-	+	++	brain, fins
Tg[hb9$^{\Delta AB}$:GFP]_1	+	+	+	++	brain, vessels, lens
Tg[hb9$^{\Delta AB}$:GFP]_2	+	-	+	++	brain, eyes, floorplate
Tg[hb9$^{\Delta AB}$:GFP]_3	+	+	+	+	brain, vessels, floorplate
Tg[hb9$^{\Delta AB}$:GFP]_4	+	+	+	+	brain, vessels, fins, eyes
Tg[hb9$^{\Delta AB}$:GFP]_5	+	-	+	+	brain
Tg[hb9$^{\Delta AB}$:GFP]_6	+	+	+	+	brain, vessels
Tg[hb9$^{\Delta AB}$:GFP]_7	+	+	-	+	brain, vessels
Tg[hb9$^{\Delta AB}$:GFP]_8	+	+	-	++	brain, vessels, notochord
Tg[hb9$^{\Delta AB}$:GFP]_9	+	+	+	++	brain, HG, notochord, OP
Tg[P^{E}+hsp:GFP]_1	-	+	+	++	brain, eyes, OP, HG
Tg[P^{E}+hsp:GFP]_2	+	+	-	-	brain, endothelium,
Tg[P^{E}+hsp:GFP]_3	+	+	-	+	brain,
Tg[P^{E}+hsp:GFP]_4	+	+	-	-	brain, notochord
Tg[P^{E}+hsp:GFP]_5	+	+	-	-	brain, notochord,
Tg[A+hsp:GFP]_1	-	-	+	++	brain
Tg[A+hsp:GFP]_2	-	+	+	++	brain
Tg[A+hsp:GFP]_3	-	+	-	++	brain, fins
Tg[P^{E}+A+hsp:GFP]_1	+	+	-	+	brain, vessels, OP, floorpalte,
Tg[P^{E}+A+hsp:GFP]_2	+	+	+	+	brain, fins
Tg[hb9^{Em}:GFP]_1	-	+	-	-	eyes
Tg[hb9^{Em}:GFP]_2	-	+	-	-	eyes

Supplemental Table 2. GFP expression in *hb9:GFP* transgenic reporter lines corresponding to Fig. 3.

Embryos at 28 hpf were analysed for presence (+) or absence (-) of GFP expression in the endocrine islet (pancreas), spinal cord (SC – mainly interneurons) and for intensity of GFP signals in kidney and somatic muscle (+: weak signal, ++: strong signal, (+) – few number of positive cells, post: posterior expression). Additional sites of expression were variable and include diverse domains in the brain (brain), eyes, lens, blood vessel (vessels), otic placodes (OP), floorplate, notochord, hatching glands (HG), fin epidermis (fins).

	embryos with ectopic *gfp* mRNA expression	embryos with ectopic *hb9* mRNAexpression
non-injected	0/20	0/15
cas mRNA	0/17	0/14
cas/pax6b mRNA	13/16	7/11
cas/neuroD mRNA	0/15	0/13

Supplemental Table 3. Quantification of mRNA injection experiments.
Indicated are numbers of *Tg[hb9:GFP]*^{ml2} embryos with ectopic *gfp* or *hb9* expression at 9hpf following injected of indicated mRNAs.