SUPPLEMENTARY FIGURES

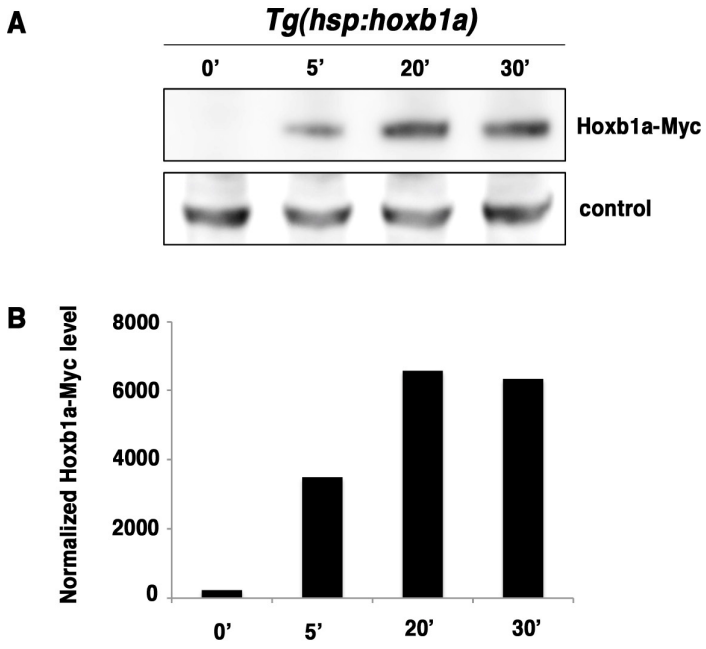

Figure S1. Dependence of the level of exogenous Hoxb1a on the length of the heat shock. (A) *Tg(hsp:hoxb1a-myc)* embryos were either not heat-shocked or heat-shocked for increasing lengths of time as indicated at 95% epiboly and collected one hour after heat shock. Pools of embryos were analysed by western blotting using an antibody directed against the Myc tag. A non-specific band corresponding to a protein not affected by the heat shock was used to normalize the amount of loaded material. (B) Quantification of the data presented in (A) using the ImageQuant software.

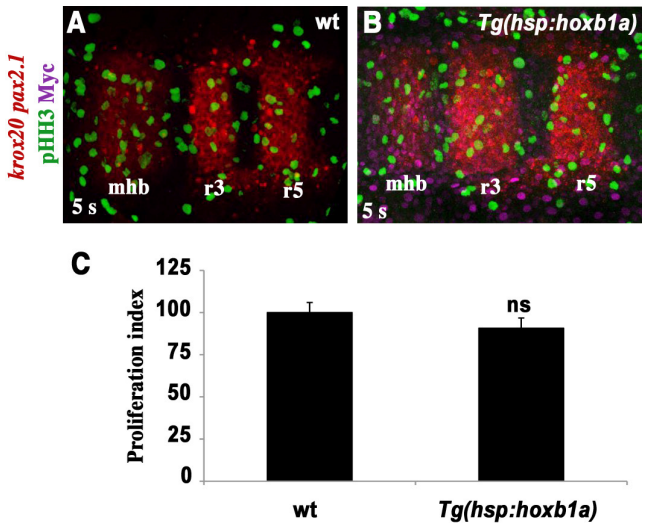

Figure S2. Cell proliferation in r3 is not affected by *hoxb1a* overexpression. (A,B) Wild-type (wt) and *Tg(hsp:hoxb1a-Myc)* embryos were heat-shocked for 5 min at 95% epiboly and collected at 5s. They were subjected to fluorescent in situ hybridization with a *krox20* probe (red), followed by double immunostaining using anti-phospho-Histone H3 (pHH3, green), a marker of mitosis, and anti-Myc-tag (magenta) antibodies. Embryos were flat-mounted with the anterior towards the left. Each figure shows merging of *z*-projections of confocal sections. (C) Normalized proliferation indexes corresponding to the mean ratio of the number of pHH3-positive cells within the rostral *krox20*-positive domain divided by the area of this domain, normalized by the mean ratio obtained with wild type embryos. No significant difference between wild type and *Tg(hsp:hoxb1a-Myc)* embryos was detected by *t*-test, $P>0.05$. Errors bars indicate s.e.m. mhb, mid-hindbrain boundary.

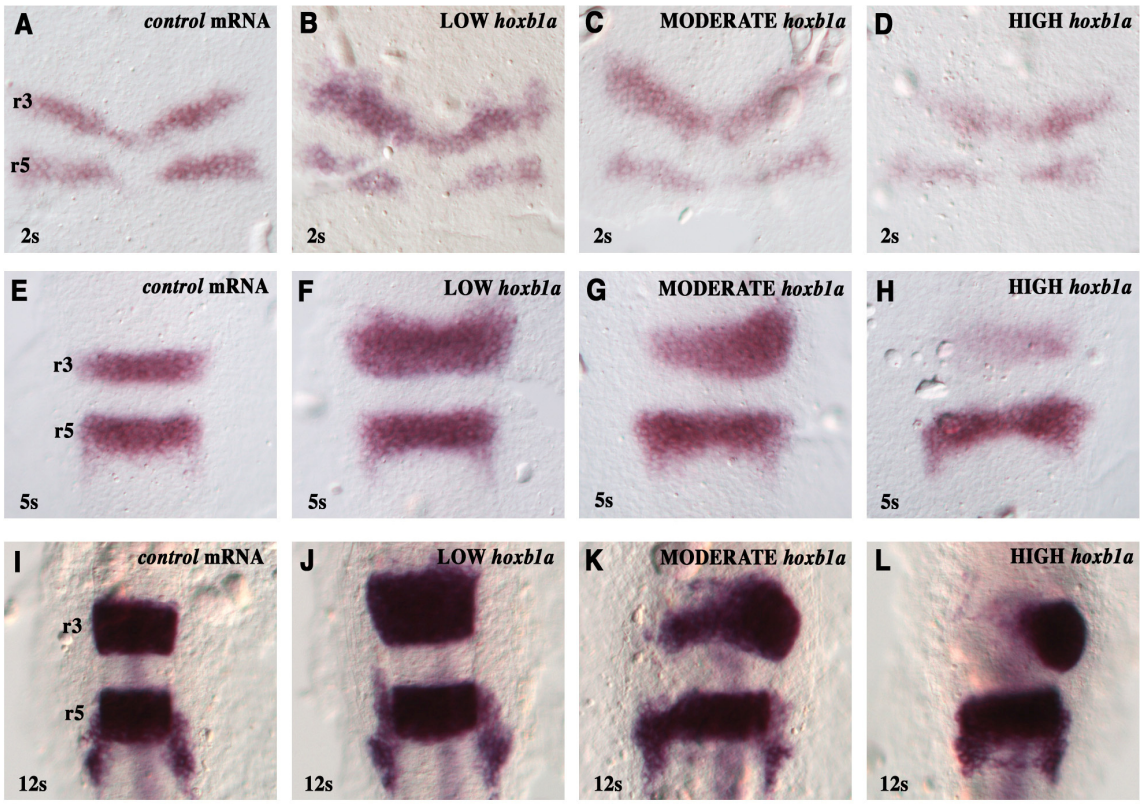

Figure S3. Dose-dependent effect of Hoxb1a on *krox20* expression. (A-L) Wild-type embryos were injected with control mRNA (*cherry-h2b)* or increasing amounts of *hoxb1a* mRNA (5, 20 and 50 ng/µl, respectively) as indicated. Embryos were collected at 2s, 5s or 12s as indicated and subjected to in situ hybridization with a *krox20* probe. The non-uniform effects of *hoxb1a* mRNA injection on *krox20* expression are due to unequal distribution of the RNA, in particular across the midline, as shown by lineage tracing analysis (Labalette, unpublished).

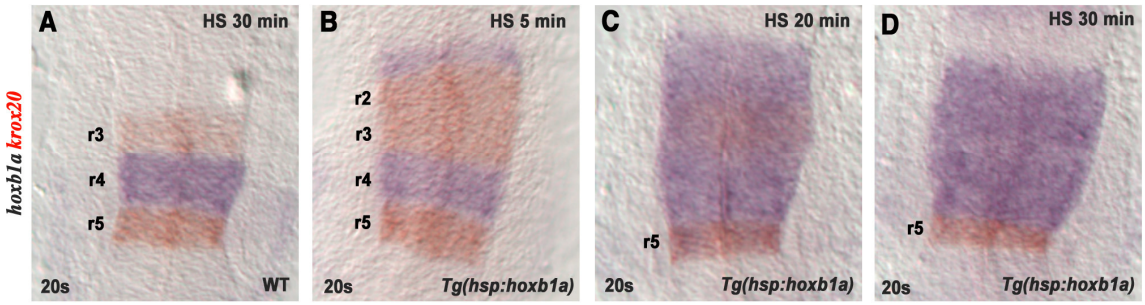

Figure S4. Ectopic Hoxb1a affects endogenous *hoxb1a* expression. Wild type (WT) and *Tg(hsp:hoxb1a)* embryos were heat-shocked for 5 (B), 20 (C) or 30 minutes (A,D) at 100% epiboly, collected at 20s and subjected to in situ hybridization for *krox20* (orange) and endogenous *hoxb1a* (purple, the probe specifically recognizes the endogenous mRNA). Embryos were flat-mounted with the anterior towards the top. At low doses of exogenous Hoxb1a, endogenous *hoxb1a* is activated anteriorly, presumably in a part of r1 (probably due to autoregulation). At higher levels, endogenous *hoxb1a* is activated over the entire r1-r4 region, reflecting loss of *krox20* expression and subsequent release of *hoxb1a* repression by Krox20. These results indicate that modifications in gene expression are not limited to *krox20*, but affect genes normally activated by Hoxb1a and repressed by Krox20.

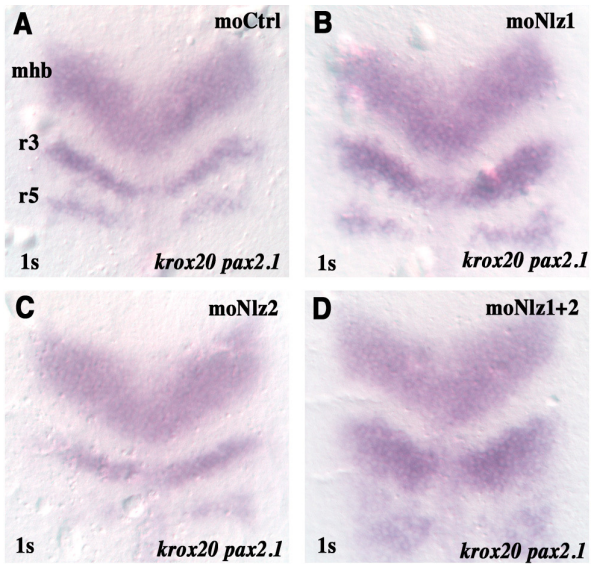

Figure S5. Nlz1 is the major actor of *krox20* repression. Wild-type embryos were injected with a control morpholino (moCtrl) or with morpholinos for *nlz1* (moNlz1), *nlz2* (moNlz2) or both (moNlz1+2), collected at 1 s and subjected to in situ hybridization with *krox20* and *pax2.1* probes (three independent experiments).

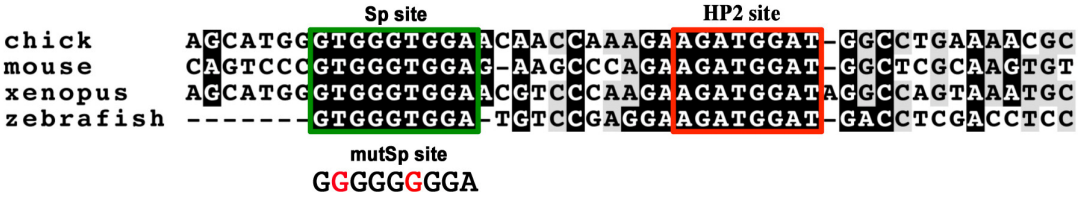

Figure S6. Alignment of vertebrate element C nucleotide sequences showing the conserved putative Sp site (green box) in the vicinity of a Hox/Pbx binding site (HP2, red box). The nucleotides modified in the mutant version of the Sp site (mutSp) are indicated in red underneath.

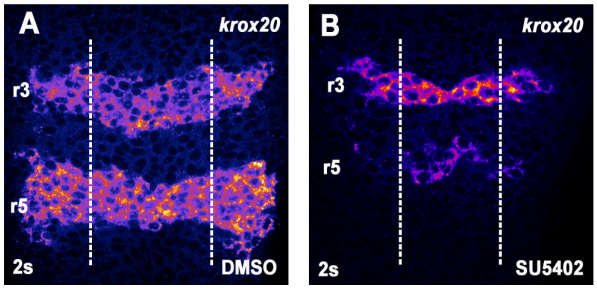

Figure S7. Analysis of *krox20* mRNA distribution using semi-quantitative, fluorescent *in situ* hybridization. (A,B) The analysis was performed on 2 s wild-type embryos, treated with DMSO or SU5402, as indicated. Embryos were flat-mounted with anterior towards the top. The dashed lines in the middle of each embryo side indicate the two AP lines along which fluorescence was measured in each cell.

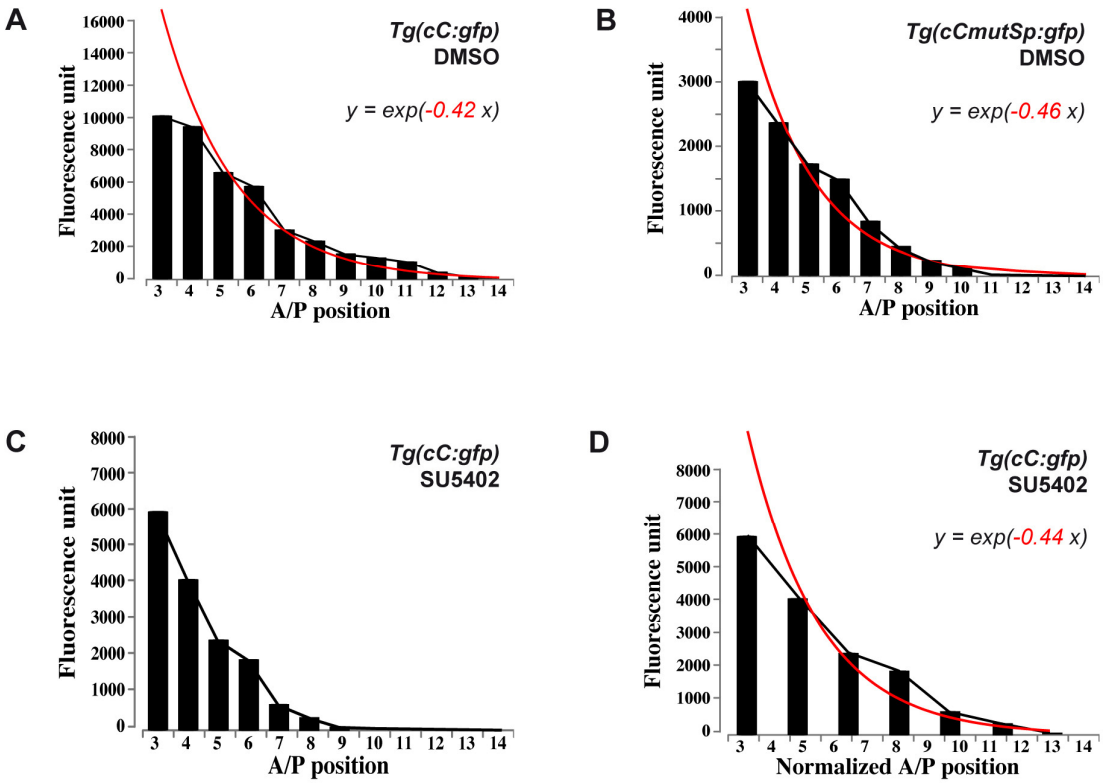

Figure S8. Quantitative analysis of element C activity profile upon variations in FGF signalling. (A-C) The profiles shown in Fig. 7B-E (black bars) are represented after normalization along the y axis, omitting the first point (point 2 in Fig. 7). (A,B) The profiles were fitted with decreasing exponentials (red curves) using the Matlab software and the equation of the best-fitting curve is shown. (D) The profile shown in C was first normalized along the *x*-axis to take into account the reduction in size of the hindbrain following SU5402 treatment (38% reduction in the length of the MHB-r6 region, data not shown) and then fitted with a decreasing exponential (red curve). Close exponential factors are found for the different curves, indicating that the normalized profiles are quantitatively similar.

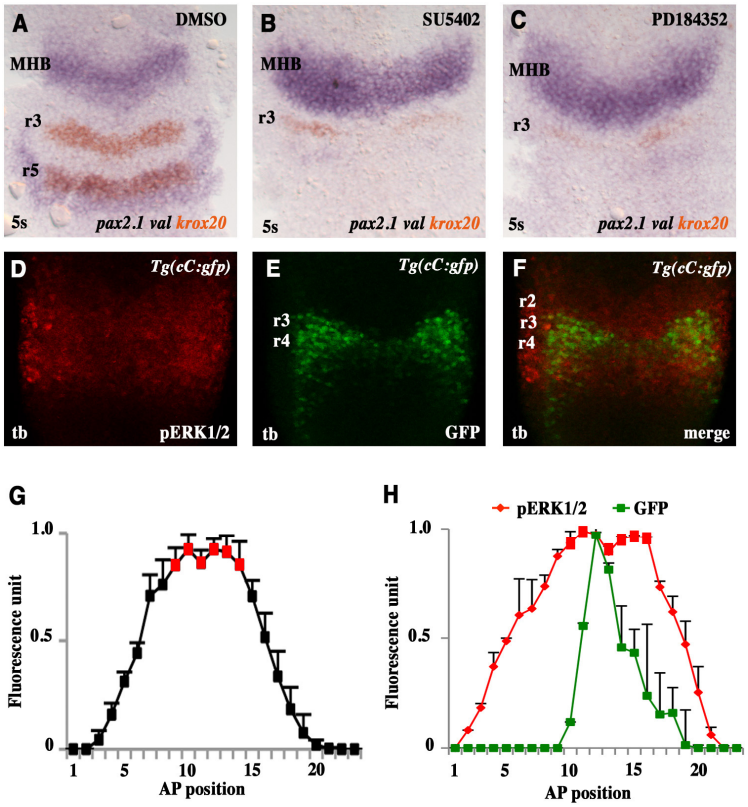

Figure S9. FGF signalling is homogeneous over a region including r3. (A-C) Embryos were treated as indicated with DMSO, SU5402 or the MEK inhibitor, PD184352, from shield to 90% epiboly, allowed to develop to 5s and subjected to *pax2.1* (purple), *krox20* (orange) and *val* (purple) in situ hybridization. (D-F) Tailbud *Tg(cC:gfp)* embryo analysed by double immunohistochemistry for pERK1/2 (red, D) and GFP (green, E). The merge is presented in (F). The positions of prospective rhombomeres are deduced from the GFP pattern. (G) Quantification of the levels of the phosphorylated forms of ERK1/2 (pERK1/2), estimated by fluorescent immunohistochemistry at 100% epiboly, along the AP axis. Measures represent the average of seven embryos. (H) Quantifications of the levels of pERK1/2 (red) and GFP (green) fluorescence at tail bud stage along the AP axis in the embryo shown in D-F. Measures represent the average of both sides of the embryos. In G,H, the red squares indicate the positions where the pERK1/2 signal differs by less than 5% from the mean of the plateau. Errors bars indicate s.e.m.

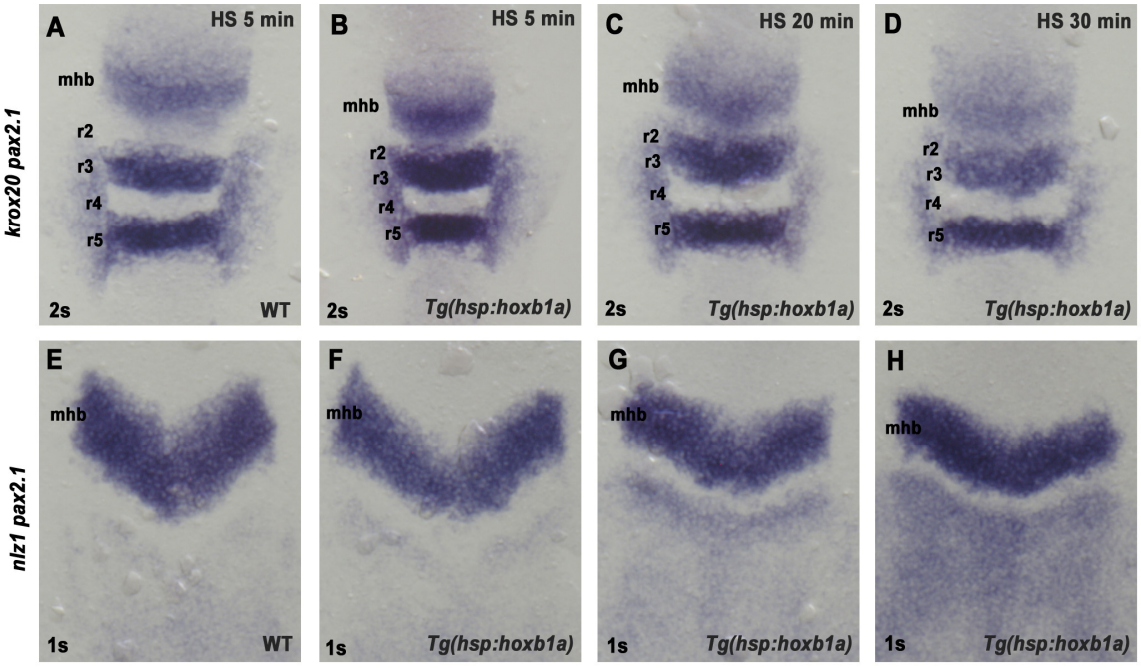

Figure S10. Different thresholds of activation by Hoxb1a for *krox20* and *nlz1*. Wild-type (WT) and *Tg(hsp:hoxb1a)* embryos were heat-shocked for 5 (A,B,E,F), 20 (C,G) or 30 min (D,H) at 100% epiboly, collected at 1-2 s and subjected to *in situ* hybridization for *krox20/pax2.1* (A-D) or *nlz/pax2.1* (E-H). Embryos were flat-mounted with the anterior towards the top. A 5 min heat shock results in full activation of *krox20* in r2, whereas *nlz1* expression is only marginally affected. Strong activation of *nlz1* in the r2-r4 region is only observed after 20-30 min heat shock, in parallel to the repression of *krox20* in r2-r3.