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Supplemental material:

Supplemental Fig. S1. Comparison of early activation of retinoic acid signaling and anterior *Hox* gene expressions. (A-O) Lateral view of embryos at E7.25, E7.5, E7.75 and E8.5 stages. Embryos were analyzed by whole-mount RNA *in situ* hybridization (ISH) for *Raldh2* (A-C), *Hoxb1* (G-I), *Hoxa1* (J-L), *Hoxa3* (M-O) and by X-gal staining from *RARE-lacZ* transgenic embryos (D-F). (A-C) Whole-mount ISH with *Raldh2* probe showing expansion in the anterior lateral mesoderm. (D-F) β-galactosidase activity of *RARE-lacZ* transgenic embryo is detected in *Raldh2* domain at stage E7.5. (G-O) Whole-mount ISH showing expansion of the expression of *Hoxb1*, *Hoxa1*, *Hoxa3* at E7.25 (G,J,M), E7.5 (H,K,N) and E8.5 (I,L,O). em, extra-embryonic mesoderm; hf, head folds ; A, anterior ; P, posterior ; ps, primitive streak.

Supplemental Fig. S2. Hoxb1 and Hoxa1 are expressed in the second heart field. (A,B) Lateral view of double *in situ* hybridization of *Hoxb1* and *Hoxa1* mRNA with *Isl1*, which marks the second heart field (SHF), as shown in panel C. (A',B') Transverse sections of embryos depicted in A and B. Dotted lines indicate planes of sections. (A') Section showing co-expression of *Hoxb1* and *Isl1* in the splanchnic mesoderm and the anterior foregut endoderm (arrowheads). (B') Transverse section showing expression of *Hoxa1* in the *Isl1*+ splanchnic mesoderm (arrowheads). (C') Transverse section showing expression of *Isl1* in the endoderm and the SHF (arrowheads). en, endoderm; ht, heart tube; r4, rhombomere 4; sm, splanchnic mesoderm.

Supplemental Fig. S3. Hoxb1 co-localizes with Isl1 in the second heart field. (A-F) Expression of Hoxb1 was followed by an anti-Hoxb1 on wild-type (A), by an anti-β−galactosidase on *Hoxb1*$^{IRES\text{-}Cre}$; *R26R-lacZ* (D). (B,E) Expression of Isl1 protein is detected in the anterior foregut endoderm and the splanchnic mesoderm called the second heart field (SHF). (A,D) Immunostaining showing expression of Hoxb1, β−galactosidase in the splanchnic mesoderm (white arrowhead) and the anterior foregut endoderm. (C,F) Merges of Hoxb1, β−galactosidase and Isl1 immunofluorescence illustrating expression Hoxb1 in a sub-domain of the SHF. en, endoderm; ht, heart tube ; me, mesoderm; r4, rhombomere 4; VP, venous pole.

Supplemental Fig. S4. *Hoxb1* expression and genetic lineage analysis in early embryos. (A-E) Lateral view of X-gal stained embryos from *Hoxb1*$^{IRES\text{-}Cre}$; *R26R-lacZ* mice at E7.25, E7.5, E7.75 and E8.5. (F-J) Lateral view of whole- mount *in situ* hybridization (ISH) with *Hoxb1*

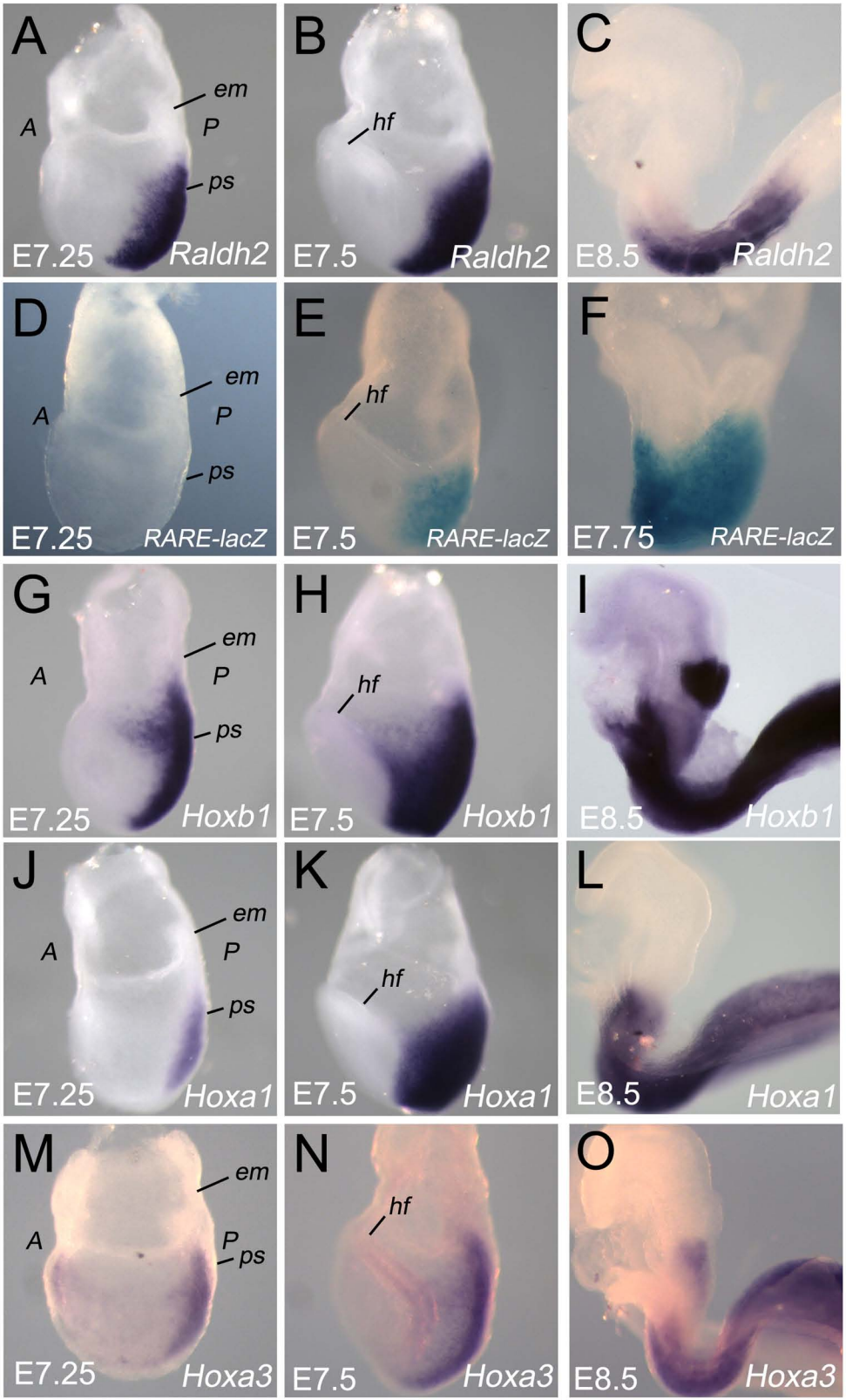
probe on embryos at E7.25, E7.5, E7.75 and E8.5. (A-C,F-H) X-gal staining showing early anterior expansion of β-galactosidase activity similar as those of Hoxb1 transcript. (D,E,I,J) At E8.5, X-gal-positive cells are detected in the venous pole of the heart, whereas Hoxb1 mRNA is not detected in the heart. Right side view showing X-gal labeled cells in the SHF of *Hoxb1*$^{IRES\text{-}Cre}$; *R26R-lacZ* embryo (arrowhead). Note that anterior expansion of *Hoxb1* in the SHF is discordant to those of X-gal staining. CC, cardiac crescent; ht, heart tube; ps, primitive streak; r4, rhombomere 4; vp, venous pole.

Supplemental Fig. S5. Genetic lineage analysis of *Hoxa1-enhIII-Cre* and *Hoxa3*$^{IRES\text{-}Cre}$ embryos at early stages. (A-D) *Hoxa1*-lineage and *Hoxa3*-lineage visualized by X-gal staining of *Hoxa1-enhIII-Cre; R26R-lacZ* and *Hoxa3*$^{IRES\text{-}Cre}$*; R26R-lacZ* embryos at E8.5 and E9.5. (A,C) β-galactosidase activity visualized by X-gal staining shows the same anterior border as *Hoxa1* and *Hoxa3* expression at E8.5. (B,D) X-gal staining of later embryos highlights all regions of the embryo that are derived from the *Hoxa1* and *Hoxa3*-expression domains including the pharyngeal region (asterisk). Ba1, branchial arch 1; g, gut epithelium; ht, heart tube; oft, outflow tract; rv, right ventricle.

Supplemental Fig. S6. Expression of *Hoxb1*, *Hoxa1* and *Hoxa3* genes is sensitive to retinoic acid signaling. (A-I) Lateral view of whole-mount *in situ* hybridization with *Hoxb1*, *Hoxa1* or *Hoxa3* probes on wild-type (*WT*), *Raldh2*$^{-/-}$ mutant and RA-treated embryos at E8.5. Insets present transverse sections of embryos in A-D,F,G,I. Dotted lines indicated the planes of sections. Treated embryos were subjected to a 70mg/kg dose of all-trans RA at E7.75 and then analyzed 18 hours later at E8.5. (A-C) Whole-mount ISH showing that expression of *Hoxb1* in the splanchnic mesoderm situated posterior and adjacent to the cardiac tube is reduced in *Raldh2*$^{-/-}$ mutant embryos, whereas it is shifted anteriorly in RA-treated embryos. Transverse section displayed in insets, indicates that expression of *Hoxb1* is largely activated in the pharyngeal mesoderm under RA-treatment. (D-F) Whole-mount ISH showing reduction of *Hoxa1* expression in *Raldh2*$^{-/-}$ mutant, whereas it is increased in RA-treated embryos. Transverse section indicates that expression of *Hoxa1* is largely activated in ectoderm, mesoderm and endoderm under RA-treatment (G-I) Whole-mount ISH showing posterior shifting of the anterior border of *Hoxa3* expression in *Raldh2*$^{-/-}$ mutant, whereas it is anteriorly expanded in RA-treated embryos. Inset shows transverse sections of embryos in G and I. Sections display that activation of *Hoxa3* is mainly observed in the ectoderm in RA-treated embryo. ht, heart tube.

Supplemental Fig. S7. Retinoic acid signaling has restricted effect on Hox3-lineage. (A,B) Lateral view of X-gal stained (A) *Hoxa3*$^{IRES\text{-}Cre}$; *R26R-lacZ* wild-type (*WT*) and (B) RA-treated

embryos at E9.5. X-gal staining reveals β-galactosidase activity in the second heart field (the splanchnic mesoderm) contiguous to the outflow tract (arrow). (A',B') Sagittal sections (same embryo as in A and B) show a small number of X-gal positive cells in region anterior of the otic vesicle in embryo which received a single all-trans RA injection (85mg/Kg). oft, outflow tract; ov, otic vesicle; rv; right ventricle.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em
A
P
ps
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E
hf
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F
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G
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Figure S1

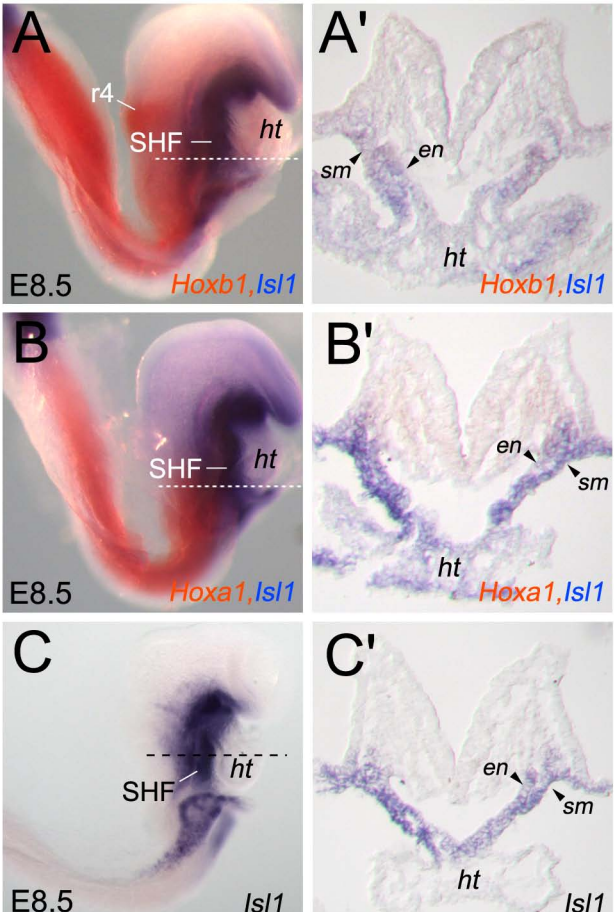
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Figure S2

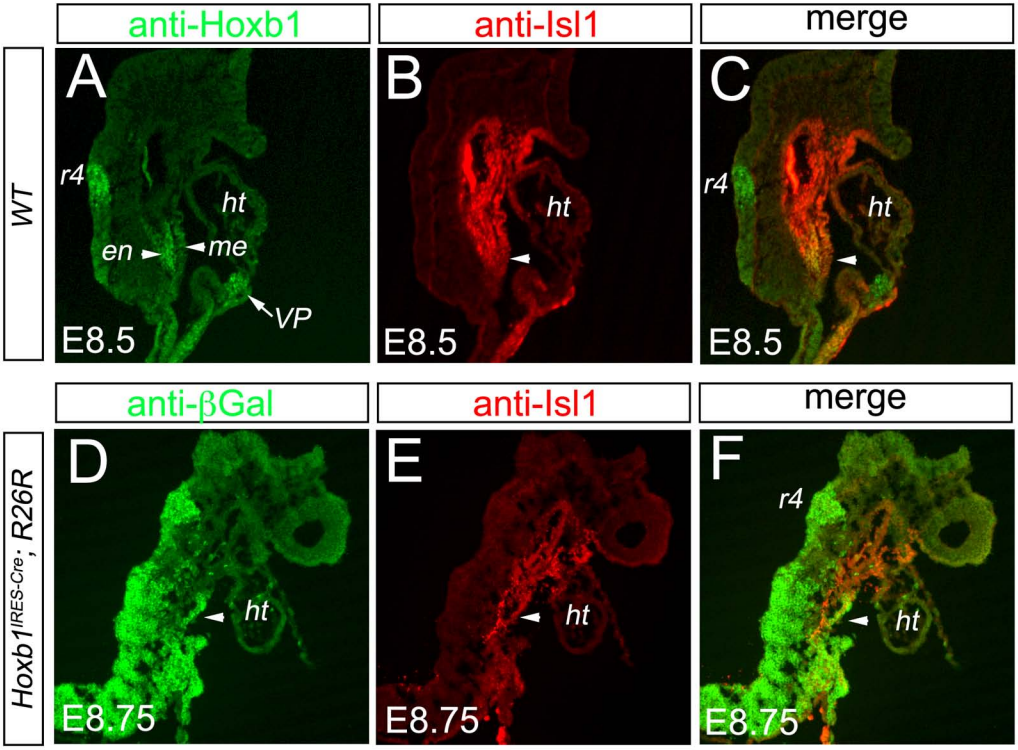
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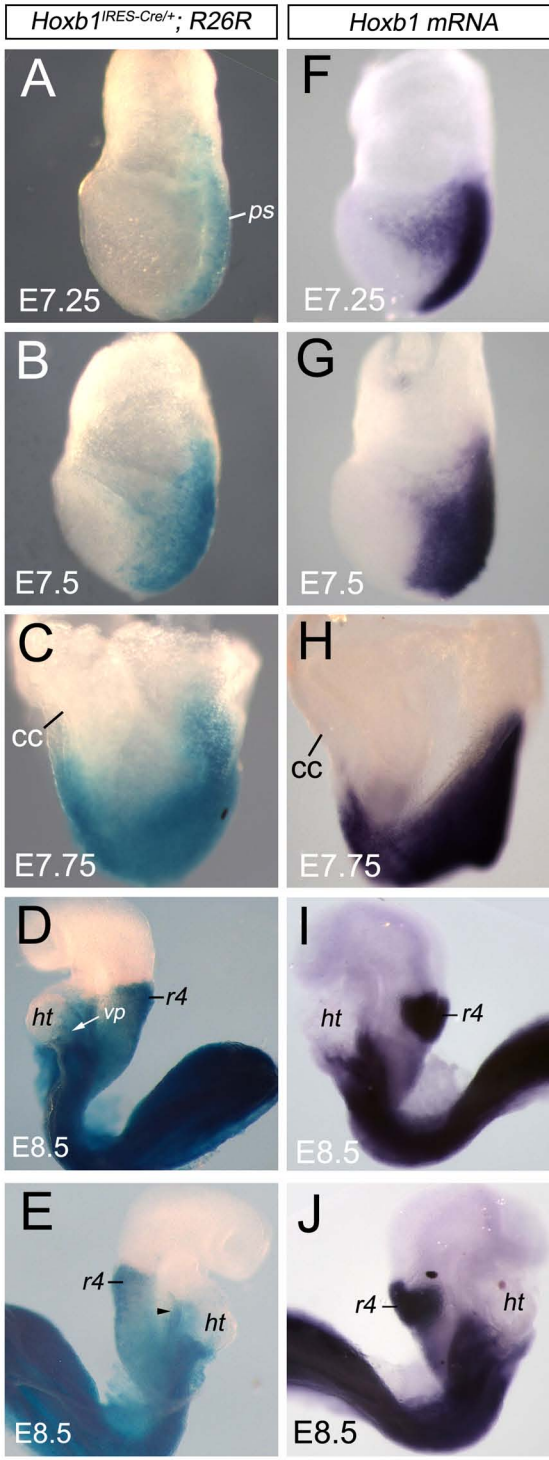
Figure S3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D
ht
vp
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Figure S4

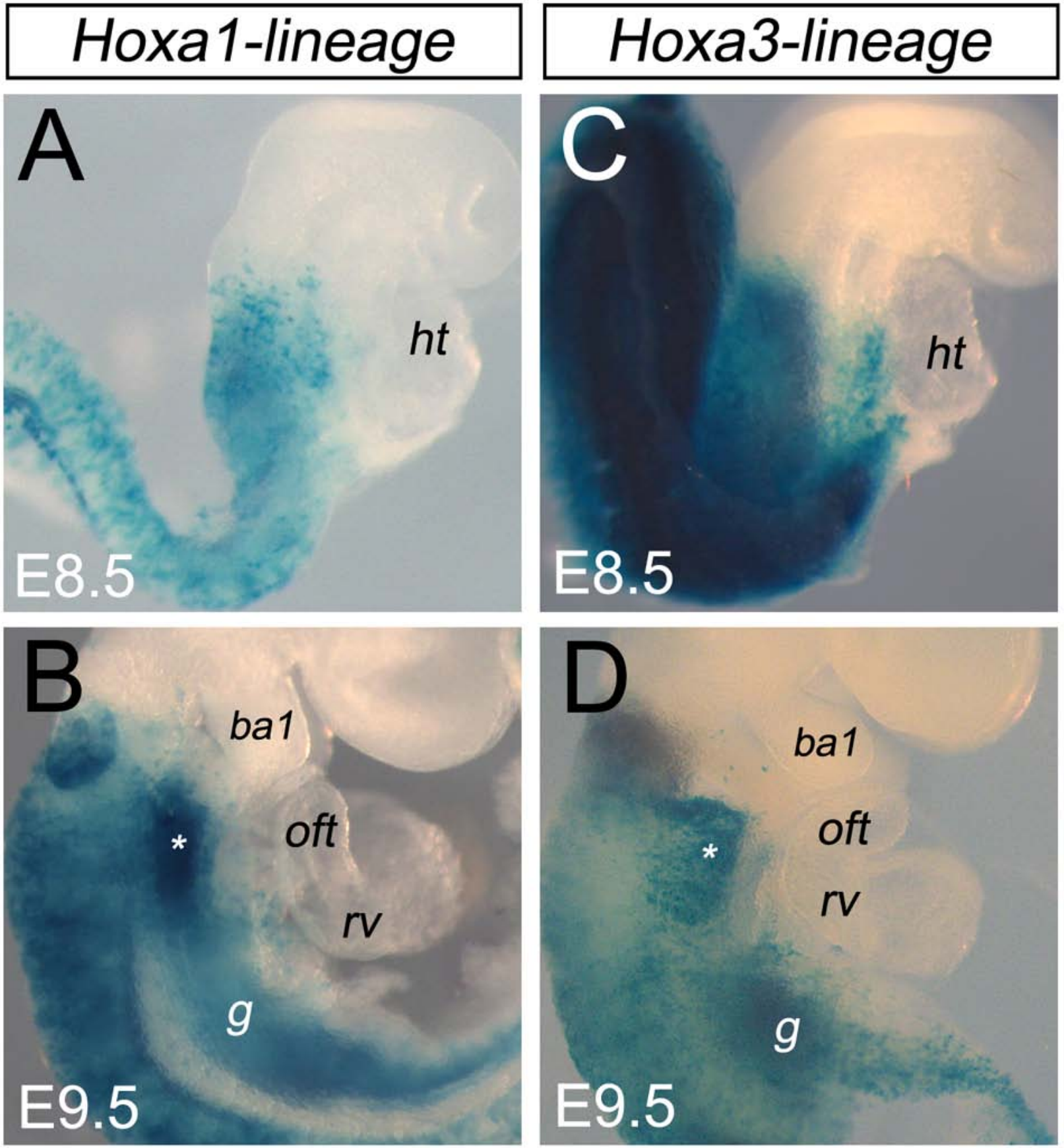
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Figure S5

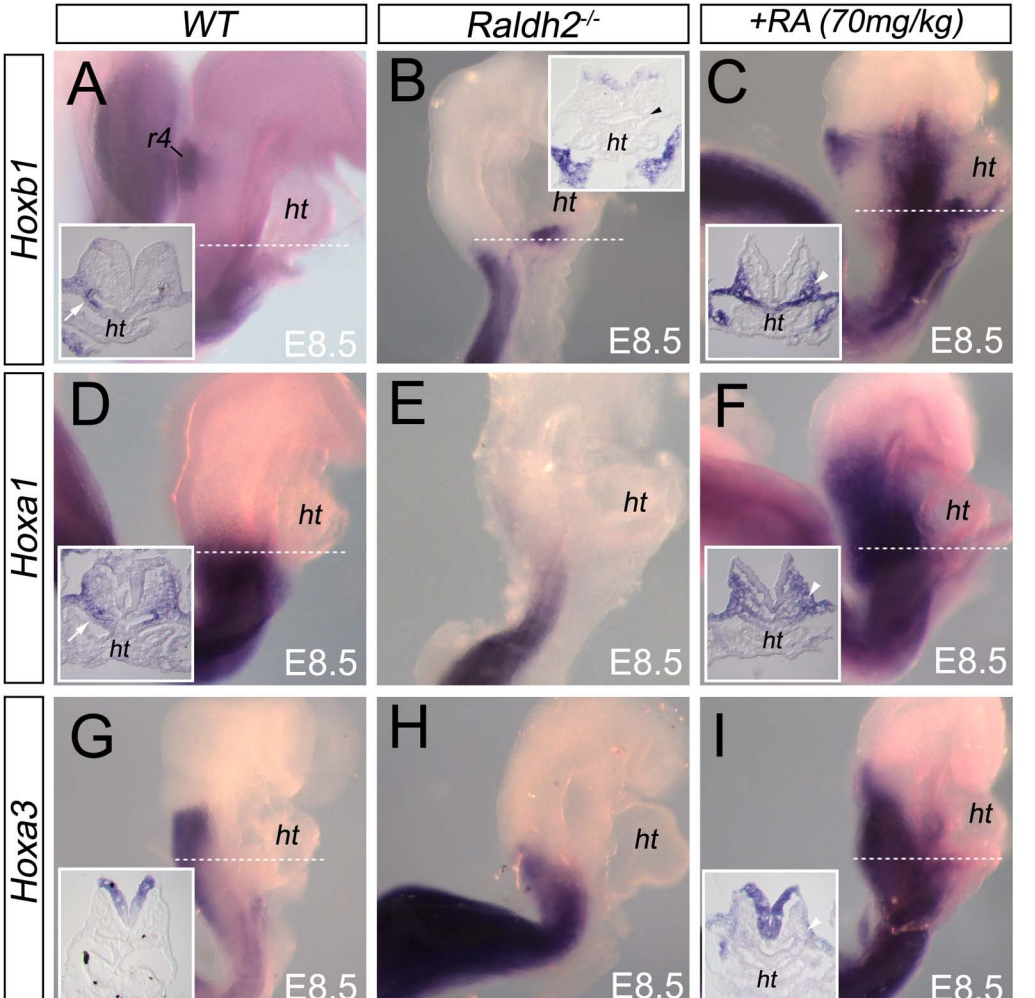

Figure S6

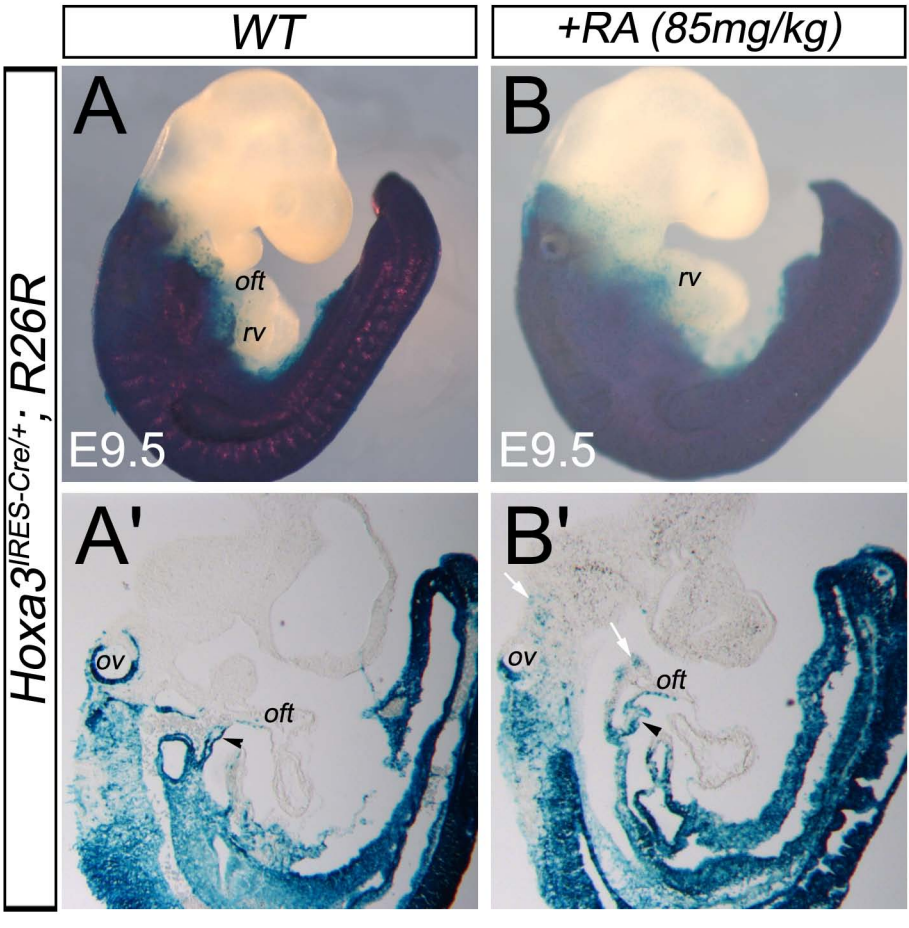
WT
+RA (85mg/kg)
Hoxa3IRES-Cre/+; R26R
A
oft
rv
E9.5
B
rv
E9.5
A'
ov
oft
B'
ov
oft

Figure S7