

Supplementary information for article:

Sea lamprey enlightens the origin of the coupling of retinoic acid signaling to vertebrate hindbrain segmentation

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Vertebrate Cyp26A1

Pm 1 **MA**LLQLAITVTITIFTPLLLLATWKLWEKFLRDPSCLPLPPGSMGLPI**GET**LHYVLGRGSF**ME**KKRQRYG**PV**YR**TH**LG**FTPTVR**VI**GS**EN**VR**RL**LL**GE**HL**VA
Rt 1 MVLVTLCATFLCTFVLPLALFVA**AV**KLWEVY**CI**SGR**PT**CEK**PL**PPGSMGL**P**LG**ET**LHLVLQRYK**FL**MMKVRKYGYIY**K**THLFG**NP**TVR**IM**GAEN**V**KQ**IL**GE**HK**LVS**V**
Lc 1 MVLHSLFATVLCITFVLPLSLFLAAV**KL**WEIY**CV**SGR**DP**SCDR**PL**PPG**TM**GF**PL**GETLQ**ML**QRR**K**FLQ**MK**RRKYGYIY**K**THLFG**RP**TVR**VM**GAEN**R**Q**IL**GE**HL**VA
Mm 1 **MG**LPA**L**LSALCTFVLPLSLFLAA**L**KLDLY**CV**SSRDR**SC**AL**PL**PPG**TM**GF**FF**GETLQ**ML**QRR**K**FLQ**MK**RRKYGYIY**K**THLFG**RP**TVR**VM**GAD**N**VR**RL**ILGE**HL**LVS**V**

Pm 111 **Q**W**P**TSV**S**RL**I**GA**G**CLSS**F**EGA**HR**MK**K**V**L**KA**S**REALQ**NY**VP**T**IVE**VS**AM**L**AD**CS**RS**-**RPV**LV**Y**PE**V**K**LV**M**F**R**AM**R**L**L**GI**D**VS**G**MA**R**G**-**E**I**HA**L**V**G**V**F**E**E**M**I**R**N**
Rt 111 **Q**W**P**ASV**R**T**L**GA**CS**LN**L**HSE**HK**RR**K**V**L**KA**S**REALQ**NY**VP**M**G**E**E**I**RAG**V**RR**LD**SG**-**CV**LV**Y**PE**M**K**RL**M**F**G**IAM**R**IL**L**GF**E**PTH**S****-**P**Q**TQ**Q**EL**I**Q**V**F**E**E**M**I**R**N
Lc 111 **Q**W**P**ASV**R**T**L**GS**G**CL**N**LHDS**Q**HK**RR**K**V**IM**K**AF**S**Q**AL**EN**I**VP**I**VE**VS**AV**C**Q**LN**SSGS**-**CV**LV**Y**PE**V**K**RL**M**F**R**IA**M**RI**L**LGF**E**PQ**T**K**-**P**E**N**E**LQ**L**VE**A**F**E**E**M**I**R**N
Mm 111 **H**W**P**AL**S**RL**T**GA**G**CL**N**LHDS**SH**K**RR**K**V**IM**Q**AF**S**REALQ**CV**Y**P**IA**E**EV**SS**CL**Q**N**L**SC**G**ER**GL**LV**Y**PE**V**K**R**L**M**F**R**IA**M**RI**L**L**G**CE**P**G**A**GG**E**DE**Q**L**VE**A**F**E**E**M**TR**N

Pm 219 **L**FS**L**PI**E**VP**F**GG**LY**R**GL**KAR**N**VI**HS**K**IE**ES**I**KK**L**SS**P**----PTD**G**CK**D**ALQ**ML**IE**HS**RE**NG**NC**L**S**L**Q**L**K**E**SA**T**ELL**FG**GH**ET**TAS**A**AT**S**LV**M**HL**A**I**HP**H**V**RS**R**L**R**SE**L**
Rt 219 **L**FS**L**PI**D**VP**F**SG**LY**R**GL**KAR**N**VI**HA**K**IE**EN**I**R**K**K**V**ARR**---**EST**G**Q**F**K**D**ALQ**L**L**IE**Q**S**EQ**LP**RL**Q**EL**K**ESA**T**ELL**FG**GH**ET**TAS**A**T**S**LV**T**FL**A**L**H**RE**V**LQ**K**V**R**RE**L**
Lc 219 **L**FS**L**PI**D**VP**F**SG**LY**R**GL**KAR**N**VI**HE**K**IE**EN**I**K**A**L**T**K**P**----NT**E**TQ**H**Q**D**VLQ**L**L**IE**Q**E**Y**K**ENG**E**Q**LN**MQ**EL**K**E**SA**T**ELL**FG**GH**ET**TAS**A**AT**S**L**I**T**FL**GL**H**HE**V**LE**K**V**R**KE**L**
Mm 221 **L**FS**L**PI**D**VP**F**SG**LY**R**GV**KAR**N**L**I**HA**R**IE**N**IR**A**K**IR**RLQ**A**TE**P**DG**G**CK**D**ALQ**L**L**IE**HS**W**ER**GE**RL**D**MQ**AL**K**Q**SS**T**ELL**FG**GH**ET**TAS**A**AT**S**L**I**T**LY**GL**Y**PH**V**LQ**K**V**R**E**E**L

Pm 325 KQ**Q**G**F**TA**E**RT**---**ISS**L**AE**L**E**L**K**F**VA**S**AV**K**ET**LR**V**SP**PA**G**GF**R**VA**L**K**T**FE**IN**G**Y**Q**IP**K**G**W**N**VI**YS**I**K**ETH**N**MA**EN**F**P**K**R**ND**FD**PD**R**W**MT**G**D**AG**G**NG**D**A**SS**R**F**T**Y**
Rt 326 QE**Q**GL**L**SSD**G**TE**I**R**H**IT**M**EL**EQ**L**K**Y**T**GS**VI**K**ET**LR**L**SP**P**GG**F**RV**A**L**K**TF**VI**NG**Y**Q**IP**K**G**W**N**VI**YS**I**C**D**TH**X**AT**N**F**SK**K**DE**F**N**P**DR**F**ME**G**CS**-----**END**S**CR**F**S**Y**
Lc 326 K**T**KE**L**CE**H**HK**D**KN**Y**L**D**ME**V**LE**Q**L**K**Y**T**GS**II**K**ET**LR**L**SP**P**GG**F**RV**A**L**K**TF**EL**SG**Y**Q**IP**K**G**W**N**VI**YS**I**C**D**TH**Q**AD**IT**SN**K**Q**E**F**N**P**DR**F**MS**S**Y**-----**ED**S**R**F**S**F**
Mm 331 K**S**GL**L**CK**S**ND**Q**N**K**-L**D**ME**T**LE**Q**L**K**Y**T**GC**VI**K**ET**LR**L**NP**P**GG**F**RV**A**L**K**TF**EL**NG**Y**Q**IP**K**G**W**N**VI**YS**I**C**D**TH**Q**AD**IT**FN**KE**E**F**N**DR**F**I**V**PH**P**-----ED**A**S**R**F**S**F

Pm 431 **I**PF**GG**GS**R**SC**V**G**K**E**L**AR**L**IL**RI**V**V**VEL**CR**CE**ML**P**NG**P**PT**LV**T**AP**S**LV**PD**N**L**PAR**FS**A**S**ADD**WR**
Rt 430 **I**PF**GG**GS**R**SC**V**G**K**E**F**AK**IL**L**K**IFT**VEL**VR**AC**D**W**ELL**NG**P**PT**M**K**NT**NP**Y**VP**DN**L**PT**K**TF**P**Y**NI**--
Lc 429 **I**PF**GG**GS**R**SC**V**G**K**E**F**AK**IL**L**K**IFT**VEL**AS**SC**D**W**QL**NG**P**PT**M**K**T**G**TV**Y**PD**N**LPT**K**TF**TH**FN**CK**T--
Mm 433 **I**PF**GG**GL**R**SC**V**G**K**E**F**AK**IL**L**K**IFT**VEL**AR**H**CD**W**QL**NG**P**PT**M**K**T**SP**TV**Y**PD**N**L**PAR**TF**TH**Q**DI**--

Heme

Vertebrate Cyp26B1/C1

CcCyp26C1 1 ---M**F**LL**G**Y**V**Y**L**SALAT**A**L**T**SL**S**VL**L**LLAV**S**RQ**L**W**L**GN**V**TR**D**R-G**N**K**L**PL**P**K**G**SM**G**W**P**LL**G**ET**L**H**M**V**Q**GS**H**FAS**R**RE**K**Y**GN**V**F**K**TH**LL**GR**P**V**I**R**VT**GA**EN**I**R**K**IL
LcCyp26C1 1 ---M**F**LL**E**FS**V**Y**I**VD**I**IT**S**IM**S**LL**L**AV**S**RQ**L**W**L**IR**N**L**T**R**D**R-G**S**K**L**PL**P**K**G**SM**G**W**P**LG**ET**M**H**LV**Q**GS**N**F**H**CR**R**K**Y**GN**V**F**K**TH**LL**GR**P**V**I**R**VT**GA**EN**I**R**IL
MmCyp26C1 1 ---M**I**SW**G**LS**C**SV**L**GAAG**T**LL**C**AG**L**LL**G**LAQ**L**W**L**R**W**TS**L**R**D**W-AST**L**PL**P**K**G**SM**G**W**P**FF**GE**T**L**H**W**LV**Q**GS**R**F**H**SS**R**RE**R**Y**GT**V**F**K**TH**LL**GR**P**V**I**R**VS**GA**EN**V**R**IT**L
PmCyp26B1/C1b 1 MAM**E**LG**A**LV**A**SV**L**AS**L**AS**A**AG**L**GS**A**LL**L**LV**S**Q**L**W**A**M**R**WA**AT**R**D**-S**CK**L**PL**PS**G**SM**G**LP**L**IG**ES**L**H**W**V**QGS**R**F**H**SS**R**AR**H**GN**V**F**K**TH**LL**GR**P**V**I**R**VT**GA**Q**Y**V**R**IL**
EbCyp26_10538 1 ---ML**G**A-L**G**AL**S**V**L**ATA**V**AC**L**MS**L**V**LL**LA**V**AQ**L**W**L**W**R**TA**R**DR-**A**FQ**L**PL**P**K**G**SM**G**LP**L**IG**ET**L**H**M**V**Q**G**AN**F**HS**S**RR**E**K**F**GN**V**F**K**TH**LL**GR**P**V**I**R**VT**GA**E**Y**R**K**II**
EbCyp26_6009 1 -----ML**G**A---T**TA**ST**F**PC**R**AP**W**AF**P**W**E**K**S**IG**S**C**R**Q**VI**ST**L**-----L**A**VS**S**---A**M**Y**S**K**P**T**C**W**A**G**P**V**V**RV**T**GG**E**N**V**R**IL**
PmCyp26B1/C1a 1 ---M**L**LS**L**PS**L**DA**V**SA**A**SV**T**AC**L**AS**L**V**LL**TV**V**Q**L**W**L**RL**R**NS**A**DR**K**-S**S**AL**PL**PS**G**SM**G**FP**V**VG**ET**F**H**M**V**QGS**R**F**H**SS**R**RE**R**Y**GN**V**F**K**TH**LL**GR**P**V**I**R**VT**GA**EN**V**R**IL**
RtCyp26B1 1 ---M**F**W**E**GL**S**VA**S**AV**TT**AC**L**VS**LL**LL**L**TV**S**RQ**L**W**Q**F**R**TV**S**R**D**K-NC**N**L**P**IP**K**G**S**M**G**LP**L**IG**ET**F**H**W**VE**GS**R**F**H**SS**R**RE**K**Y**GN**V**F**K**TH**LL**GR**P**L**I**R**VT**G**SE**N**V**R**K**IL**
LcCyp26B1 1 -----M**S**L**T**LA**A**CL**S**V**IL**LL**L**AV**S**Q**L**W**L**R**WA**AT**R**D**K**-NC**K**L**P**IP**K**G**S**M**G**FP**I**IG**ET**F**H**W**L**Q**S**E**F**Q**S**SR**E**K**Y**GN**V**F**K**TH**LL**GR**P**L**I**R**VT**GAD**N**V**R**K**IL**
MmCyp26B1 1 ---M**L**FE**G**EL**S**VA**L**AT**LA**AC**L**VS**VT**LL**L**AV**S**Q**L**W**L**R**WA**AT**R**D**K**-S**CK**L**P**IP**K**G**S**M**G**FP**L**IG**ET**G**H**W**L**Q**S**G**F**Q**S**SR**E**K**Y**GN**V**F**K**TH**LL**GR**P**L**I**R**VT**GA**EN**V**R**K**IL**

CcCyp26C1 107 L**G**EH**S**LV**S**QA**W**PH**S**TR**IL**L**G**FN**T**LAN**S**I**G**DL**HR**Q**R**KK**L**MA**R**VF**S**HA**AL**DT**Y**PI**G**I**Q**LV**S**R**SV**RG**W**CRE**P**-AP**V**AV**Y**PA**T**K**AL**TF**CI**A**IR**ILL**GL**RA**AD**Q**L**DR**S**TS**F**E
LcCyp26C1 107 L**G**EH**S**LV**S**TQ**W**PH**S**TR**IL**L**G**PN**T**LV**NS**NG**L**HR**Q**R**K**IL**A**K**V**FS**HT**AL**E**IF**L**PI**R**IQ**L**VR**GE**I**Q**GW**CRE**Q-AA**I**AV**Y**PA**T**K**AL**TF**RI**A**VR**ILL**GL**S**ID**DR**Q**LQ**N**L**A**KT**F**E
MmCyp26C1 107 L**G**EH**R**L**V**RS**Q**W**PH**S**A**HL**L**GS**HT**L**G**AV**GE**PH**R**Q**R**K**L**VA**R**VF**S**RS**SL**EQ**F**VP**RL**Q**G**AL**R**RE**V**RS**W**CA**Q**-RP**V**AV**Y**QA**A**K**AL**TF**RM**A**AR**ILL**GL**Q**D**EA**R**CT**E**LA**HT**F**E**
PmCyp26B1/C1b 110 M**G**EH**A**LV**S**AC**W**PA**S**TR**ML**LG**S**N**L**INS**A**GE**A**HR**TR**R**K**VL**S**K**V**FS**RA**AL**ES**Y**A**GV**AR**V**V**RA**AV**RE**WR**AR**AG**AE**VR**Y**P**AC**Q**AL**M**F**R**AV**RV**L**L**GL**E**PS**PD**MT**F**S**AD**FT
EbCyp26_10538 106 M**G**EH**S**LV**S**QA**W**PK**S**VR**LL**L**G**DT**L**NS**V**GD**V**HR**H**K**R**L**L**FS**K**VS**R**T**AL**EN**Y**L**P**Q**L**Q**A**VA**R**AV**S**WR**AP**-DE**V**N**V**Y**PE**Q**AL**TF**H**VA**RV**L**L**GL**R**PS**E**AD**MT**R**I**GA**F**K**A**F
EbCyp26_6009 70 M**G**EH**Q**L**V**LS**Q**W**PH**S**TR**RL**L**GS**SA**F**I**NS**V**GD**I**HR**F**K**R**K**VM**TP**VL**S**HA**LES**Y**LP**S**IQ**H**V**R**DA**V**SA**W**SA**S**L-T**P**TT**V**Y**P**ACK**N**L**TF**RI**A**MR**V**LL**GF**R**PS**DA**E**LEN**S**N**V**E
PmCyp26B1/C1a 108 M**G**EH**A**LV**S**Q**W**PL**S**TR**LL**L**G**AN**S**L**G**MS**V**GD**A**HR**N**K**R**K**V**MA**Q**VF**S**HA**AL**E**G**Y**L**P**Q**V**Q**LV**M**VA**AV**SR**W**RD**CA**-SV**T**TV**Y**PE**CK**TL**TF**RV**AL**HW**V**SS**R**IA**P**HE**A**L**LA**DA**F**E
RtCyp26B1 107 M**G**EH**T**LV**S**Q**W**PR**S**TR**ML**LG**S**T**L**INS**I**GD**I**HR**Q**K**R**IF**A**K**V**FS**HY**AL**EC**Y**L**PK**I**Q**AI**Q**D**GR**D**W**SS**SG-EP**I**TV**Y**HE**AK**KL**TF**RI**A**VR**VL**LG**F**RV**S**ET**E**LN**L**SE**S**F**E**
LcCyp26B1 98 M**G**EH**T**LV**S**TE**W**PR**S**TR**ML**LG**PN**SL**V**NS**I**GD**I**HR**N**K**R**K**V**FS**K**LS**HE**AL**ES**Y**L**PK**I**Q**V**I**Q**DN**L**RE**W**SS**N**P-EP**I**N**V**Y**R**ES**Q**KL**TF**RI**A**MR**V**LL**GF**R**VS**EE**M**HN**L**FE**A**F**Q**
MmCyp26B1 107 L**G**EH**Q**L**V**ST**EW**PR**S**AR**VL**LG**PN**T**V**AN**S**I**GD**I**HR**N**K**R**K**V**F**SK**FS**HE**A**LES**Y**L**PK**I**Q**L**V**I**Q**DT**L**RA**W**SS**Q**-EA**I**N**V**Y**Q**E**A**Q**RL**TF**RM**AV**RV**L**L**GF**S**I**P**E**ED**L**G**HL**F**EV**V**Q

CcCyp26C1 216 EL**M**EN**I**FS**L**PL**VD**PF**S**GL**R**K**G**IK**A**R**N**VL**H**EY**L**E**K**A**IT**E**K**L**Q**RT**S**---RG**A**Y**P**D**AL**D**V**ML**NS**ARE**NG**KE**PN**LQ**EL**KE**V**AV**E**L**I**FA**A**F**ST**T**A**SA**S**TS**L**V**LL**LL**Q**HS**Q**ARE**K**V**R**
LcCyp26C1 216 Q**L**ID**N**L**FS**L**PL**DI**PF**CG**L**R**K**G**K**ARD**T**L**H**EY**M**E**K**A**IT**E**K**L**Q**KK**H**---SE**G**Y**H**D**AL**DF**MM**SS**A**K**E**L**G**VEL**SM**Q**EL**KE**S**AV**E**L**I**FA**A**F**ST**T**A**SA**S**TS**L**IL**Q**LL**K**HP**F**AVE**K**V**R**
MmCyp26C1 216 Q**L**VEN**L**FS**L**PL**VD**PF**S**GL**R**K**G**IR**AR**D**Q**L**Y**E**H**LDE**A**VA**E**L**K**Q**E**K---T**A**EP**G**D**ALL**I**NS**ARE**L**GH**EP**S**V**Q**EL**KE**L**AV**ELL**FA**A**F**FT**T**A**SA**S**TS**L**LL**L**Q**HP**AA**IT**K**I**Q
PmCyp26B1/C1b 220 Q**V**DN**I**FS**L**PL**VD**PF**S**GL**R**K**G**LR**AR**D**S**MM**H**K**F**LE**ST**L**SE**L**AS**R**H**AG**GR**T**H**AD**A**DI**Y**ME**S**ARE**Q**E**I**LS**A**KE**I**KE**A**AV**E**L**I**FA**A**Y**ST**T**A**SA**S**CT**S**LV**LL**LL**L**HP**E**AL**A**K**L**R
EbCyp26_10538 215 Q**V**AN**I**FS**L**PL**VD**PF**S**GL**R**K**G**LR**Q**AR**E**L**H**NY**L**ET**AL**RE**L**KA**AR**D**S**ES**G**ED**AM**D**Y**LES**A**RE**Q**GIS**I**S**I**KE**L**KE**A**AV**E**L**I**FA**A**Y**ST**T**S**SA**S**CT**S**LV**LQ**LL**L**HP**V**LE**K**L**R**
EbCyp26_6009 179 Q**V**EN**L**FS**L**PL**VD**PF**S**GL**R**K**G**IN**AR**N**K**L**HL**FL**E**K**F**IL**E**K**LE**SE---T**K**D**Y**D**AL**D**IL**IS**SA**N**H**Q**D**K**S**MS**M**EL**EL**KE**A**AV**E**L**I**FA**A**Y**ST**T**A**SA**S**TS**L**LQ**L**L**K**HP**AV**L**T**K**L**Q
PmCyp26B1/C1a 217 Q**V**EN**F**FS**L**PL**VD**PF**S**GL**R**K**G**L**K**AR**K**VL**H**GY**L**DK**L**RD**K**LER**PE**---F**K**ES**Q**D**AM**D**IL**IS**SA**KE**H**G**K**EMS**M**EL**EL**KE**A**AV**E**L**I**FA**A**H**AT**T**A**SA**S**TS**L**V**LQ**ML**K**HP**D**OT**L**AR**L**
RtCyp26B1 216 Q**L**AN**I**FS**L**PL**VD**PF**S**GY**R**K**G**MR**AR**DN**L**HY**K**LE**K**A**IT**E**K**L**Q**Y**KE**---E**K**D**Y**S**D**AM**D**IL**IS**ARE**Q**G**K**EL**T**MQ**EL**KE**ST**VEL**I**FA**A**F**AT**T**S**AT**S**LV**YL**Q**L**HP**L**VE**K**L**R**
LcCyp26B1 207 Q**F**EN**I**FS**L**PL**VD**PF**S**GY**R**R**G**I**C**AR**S**LE**K**GLE**K**A**IT**E**K**L**Q**SN**Q**---G**K**D**Y**AD**AL**D**IL**IES**SK**ENS**RE**LS**MQ**EL**K**D**AT**IEL**I**FA**A**F**AT**T**A**SA**S**TS**L**MQ**L**L**K**HP**V**VL**E**K**L**H
MmCyp26B1 216 Q**V**EN**V**FS**L**PL**VD**PF**S**GY**R**R**G**I**Q**AR**Q**IL**Q**K**L**E**K**A**IT**E**K**L**Q**CT**Q**---G**K**D**Y**S**D**AL**D**IL**IES**KE**H**G**K**EM**T**MQ**EL**K**D**GT**EL**I**FA**Y**A**Y**AT**T**A**SA**S**TS**L**MQ**L**L**K**HP**AV**LE**K**L**R**

CcCyp26C1 324 Q**E**LE**Q**HS**L**IR**N**Y**GE**F-----
LcCyp26C1 324 R**E**LV**S**H**G**IL**N**CH**CL**-----
MmCyp26C1 324 Q**E**LS**A**Q**L**GR**A**CT**CT**-----P**R**A-----
PmCyp26B1/C1b 330 A**E**LRA**H**GL**P**E**A**PC**CG**CA**HR**PD**A**AD**A**AA**S**R**PH**A**E**GA**G**IS**T**SPA**E**E**A**GD**ASA**--V**R**LE**G**HR**RA**E**G**EP**G**-----G**E**TE**G**K**RED**G---R**R**T**A**SG**A**RE**W**Q**N**-----
EbCyp26_10538 325 A**E**LRA**H**AV**P**PL**G**C---T**C**RP**AD**ME**I**-----
EbCyp26_6009 287 E**E**LVD**A**GL**S**PP**PT**D-----
PmCyp26B1/C1a 325 R**E**LA**D**AG**L**GG**G**PA**AAA**-----
RtCyp26B1 324 E**E**LR**S**NG**I**L**H**N-G-----
LcCyp26B1 315 E**E**LR**S**NG**I**L**H**N-G-----
MmCyp26B1 324 E**E**LA**R**Q**L**L**H**GG**G**-----

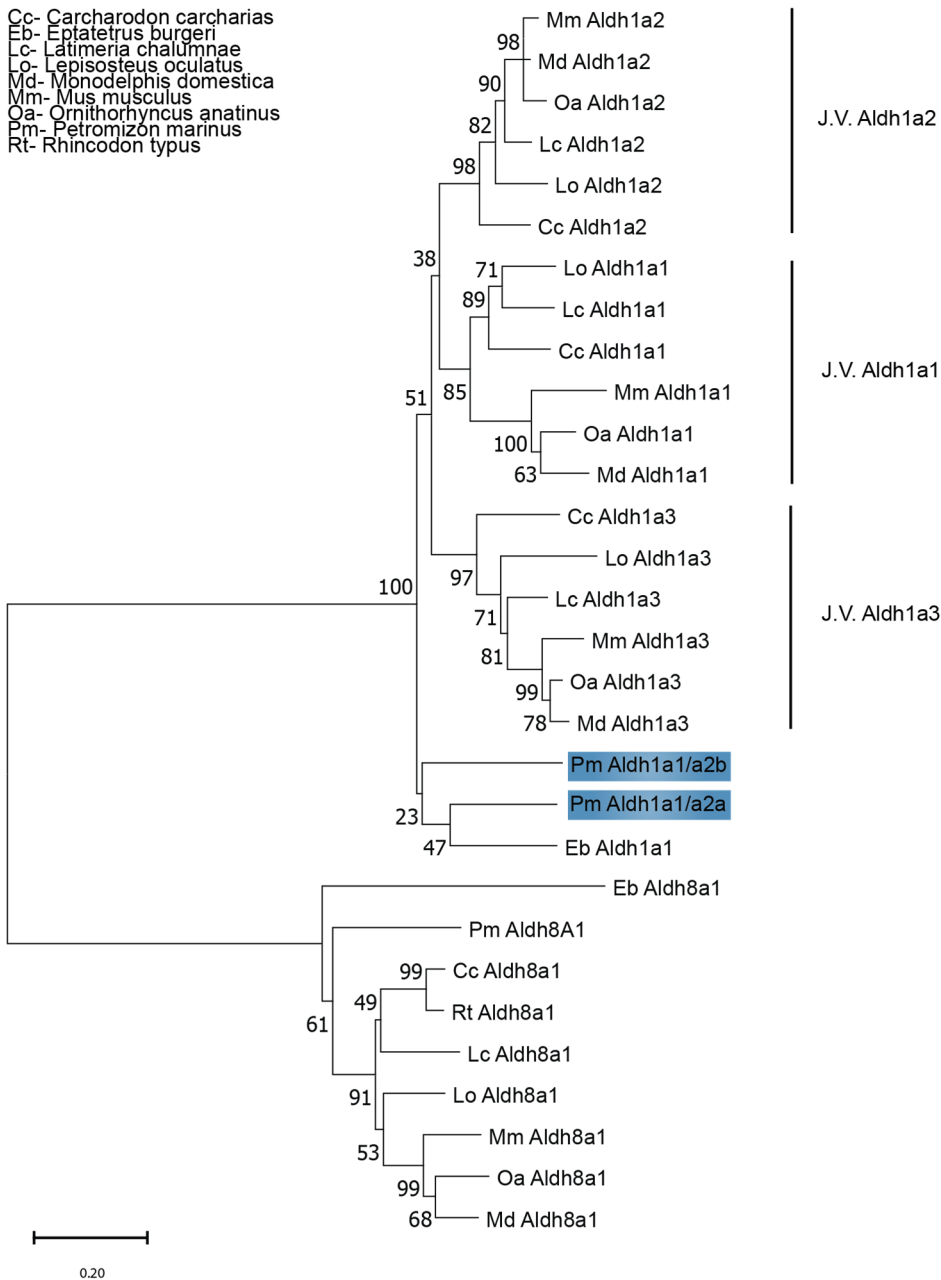
CcCyp26C1 338 ---PG-----AA**V**I**Q**SE**T**A**Q**MS**R**NC-DC**Q**H**I**N**L**DT**L**SR**L**RY**L**DC**V**VE**V**L**R**LL**PP**VS**GG**Y**R**T**AL**Q**T**FE**L**NG**C**Q**IP**K**G**W**N**II**YS**I**R**D**T**Q**E**TA**A**V**Y**LN-P**D**T
LcCyp26C1 430 DES**C**PQ**S**VI**T**Q**N**K**MS**HS**C**NS**AD**FT**NN**L**V**K**I**T**N**VC-EC**Q**PH**L**N**D**K**L**SR**L**C**Y**LG**CV**VE**V**L**R**LL**PP**VS**GG**Y**R**T**VL**Q**T**FE**L**E**G**Y**Q**IP**K**G**S**W**Y**MS**I**R**D**T**H**ET**S**SV**Y**Q**N**-P**D**L
MmCyp26C1 341 ---SG**S**PP**D**C-G**E**PD**L**S**L**AM**L**GR**L**RY**Y**DC**V**VE**V**L**R**LL**PP**VS**GG**Y**R**T**AL**R**T**FE**L**D**G**Y**Q**IP**K**G**S**W**Y**MS**I**R**D**T**H**ET**A**AV**Y**RS**P**PE**G**
PmCyp26B1/C1b 419 PA**E**E**G**N**L**VER**M**K**V**-----A**A**GE**A**E**E**GD**A**RE**D**G**CR**KE**PT**L**GP**ER**L**SR**L**RY**L**DC**V**VE**V**L**R**ML**PP**VS**GG**Y**R**T**AL**R**T**FE**L**D**G**F**Q**IP**K**G**S**W**Y**MS**I**R**D**T**H**D**T**AP**V**FP**D**-P**E**H
EbCyp26_10538 353 R-----D**E**RP**EC**MG**CG**AR**L**SL**E**Q**L**GL**S**Y**L**DC**V**VE**V**L**R**LL**PP**VS**GG**Y**R**T**AL**Q**T**FE**I**D**G**Y**Q**IP**K**G**S**W**Y**MS**I**R**D**T**H**D**T**AP**Q**FA**R**-P**E**R
Eb

Supplementary Figure 1: Vertebrate Cyp26 protein alignments. Protein alignments of vertebrate Cyp26A1 and vertebrate Cyp26B1/C1. The yellow highlighting represents the identity between sequences with a 95% threshold for sequence identity. Conserved K, I helices and Heme domains are indicated. Cutting sites of the gRNAs used for the CRISPR/Cas9 experiments are indicated with a teal arrow. Cc, *Carcharodon carcharias*; Eb, *Eptatretus burgeri*; Lc, *Latimeria chalumnae*; Mm, *Mus musculus*; Pm, *Petromyzon marinus*; Rt, *Rhincodon typus*.

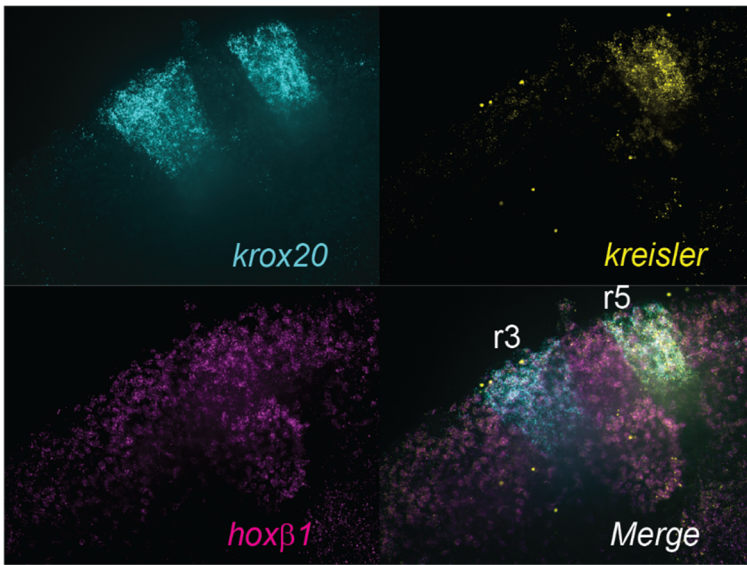
Vertebrate Aldh1a1 and Aldh1a2

PmAldh1a1/a2b	1	MA-----QRGD-----GVGRVGEAGGGPIPTVPADPMQMYTQLFINNEWQEAASGRKFDVTNPSTGQLLCSVAEADEEDVDRAVRAARAARLGLSPWRRMDPSARGRLLA
EbAldh1a1	1	MSEVASRTDGVL-----SNAFDGLSISPPSPVMDLQIKYTKIFINNSWHDLSNGDTFTPTVDPNSGCKLCDVQEGREVDVAVKAAREAFRPGSPWRRLDASERGRLLN
PmAldh1a1/a2a	1	-MSGGAEPVGGDAAQAGLAGLLASLPGVPPSPVRDLARYTKIFIGNEWDRDISGRFTPTFDPASGEKLCDVQEGDHEDVNVAVVAAREAFVLGLSPWRRMDASDRGVLLS
RtAldh1a2	1	MTSSEIEIPSEVKT--DPAA-LIASLQLLPSPTPNLEIQHTKLFINNEWHHSVSCKTFSTYNPSTGEKICDVQEAADKADVDKAVQAARIAFSPGVSWRRMDASERGRLLD
LoAldh1a2	1	MTSSKIELPGEVKT--DPAA-LVASLHLVPSVPNLEIKHTKIFINNEWQNSVSGKTFPTYNPSNGEKICDVQEAADKADVDKAVQAARLAFSLGSVWRIMDASERGRLLD
LcAldh1a2	1	MTSSKIELPGEVKT--DPAA-LMASLHLLPSPTPNLEIKHTKIFINNEWQNSVSGKTFPVYNPSNGEKICDVQEAADKADVDKAVQAARLAFSLGSVWRIMDASERGRLLN
MmAldh1a2	1	MTSSEIAMPGEVKA--DPAA-LMASLQLLPSPTPNLEIKYTKIFINNEWQNSVSGRVFPVCNPATGEQVCEVQEAADKVDIDKAVQAARLAFSLGSVWRIMDASERGRLLD
MmAldh1a1	1	-----M-SSPAQPAVPAPLADLKIQTHTKIFINNEWHNSVSCKTFPVLPNATPEEIVCHVEGDKADVDKAVKAARQAQFQIGSPWRRMDASERGRLLN
RtAldh1a1	1	MS----A----PAA--DDES-PAPAPRYVPAPLTELQIKYTKIFINNEWHNSVSCKFTTYPNATGEKICDIEEGDKVEVDKAVKAAREAFQIGSTWRRMDASERGRLLH
LcAldh1a1	1	MS----ATPCESQN--SDLK-PAVVPGLPVVPSNLEIKYTKIFINNDWHDSSNGKRFPTYPNATGEKICEVEGDKVEDVDKAVKAAREAFQLGSLWRITMDASERGRLLN
PmAldh1a1/a2b	101	RLADLVERDRALLSTLECLDAGKPFLLTTYFVDLDGVIRTLRYAGWADKVGQRTIPVDGDFISYTRHEPIGVCGQIIPWNFPLLMFAWKIPALCCGNTVVVKPAEQTPL
EbAldh1a1	105	RLAELVERDVTLSTLESLSGKPFLLHTFFVDMGIVKTLRYAGWCDKVQGRITIPVDGEYFTYTRHEPIGVCGQIIPWNFPLLMFVWIKIPALCCGNTIVIKPAEQTPL
PmAldh1a1/a2a	110	RLADLVERERTTLATLESMDSGKPFLLPAFFVDVTVGAVKTLRYFAGWCDKIQGRITIPVDGEYFTYTRHEPIGVCGQIIPWNFPLLMFIWKIPALCCGNTVVVKPAEQTPL
RtAldh1a2	108	KLADLVERDRALATLESLSGKPFLLQAYYVDLQGVIKTLRYFAGWTDKIHGKTIPTVDGDFYFTTRHEPIGVCGQIIPWNFPLLMFAWKIPALCCGNTVVIKPAEQTPL
LoAldh1a2	108	KLANLVERDRVLLATLESLSGKPFLLQAYYVDLQGTIKTLRYFAGWADKIHGMITIPMDGDFYFTTRNEPIGVCGQIIPWNFPLLMFAWKIPALCCGNTVVVKPAEQTPL
LcAldh1a2	108	KLADLVERDSTLLATMESLNGGKPFLLQAFYVDLQGVIKTLRYFAGWADKIHGMITIPVDGDFYFTTRHEPIGVCGQIIPWNFPLLMFAWKIPALCCGNTVVVKPAEQTPL
MmAldh1a2	108	KLADLVERDRATLATMESLNGGKPFLLQAFYIDLQGVIKTLRYAGWADKIHGMITIPVDGDFYFTTRHEPIGVCGQIIPWNFPLLMFTWKIPALCCGNTVVVKPAEQTPL
MmAldh1a1	91	KLADLVERDRLLLATMEALNGGKVFANAYLSDLGGSIKALKYKAGWADKIHGQITPSDGDIFTYTRHEPIGVCGQIIPWNFPLLMFTWKIPALCCGNTVVVKPAEQTPL
RtAldh1a1	100	KLADLVERDRVILSTIESIDSGKFLHAYFVDLDGSIKTLRYAGWADKIQGRITIPVDGDFYFTTRHEPIGVCGQIIPWNFPLLMFTWKIPALCCGNTVVVKPAEQTPL
LcAldh1a1	104	KLADLVERDQMLSTIESVDSGKPFLLSAYTIDLTAITKTLRYAGWTDKIQGRITIPVDGDFYFTTRHEPIGVCGQIIPWNFPLLMFTWKIPALCCGNTVVVKPAEQTPL
PmAldh1a1/a2b	211	SALHVCSLILEAGFPPGVVNIIPGFGPTAGAAIARHVDVDKVAFTGSTEVGKLIQQAASASNLKRVTLLEGGKSPNIIIFADADLDLAVEQAHQGSFNNQGCCCTAGSRVF
EbAldh1a1	215	TALHMGALIKEAGFPAGVNIIPGVGPTAGAAIARHPDINKVSFTGSTEVGKLIQEAAGKSNLKRVTLELGGKSPNIIIFVPDADLDVAVKETHEAVFFNQGCCCTAGSRVF
PmAldh1a1/a2a	220	TALHVGALIAEAGFPPGVVNIIPGFGPTAGAAIVQHPDIDKIAFTGSTEVGKLIQEAAGKSNLKRVTLELGGKSPNIIIFADADLDAAVEQAHQGVFFNQGCCCTAGSRVF
RtAldh1a2	218	TALYMGALITEAGFPPGVVNIIPGFGPTAGAAIASHMDIDKIAFTGSTEVGKLIQEAAGKSNLKRVTLELGGKSPNIIIFADADLDYAVEQAHQGVFFNQGCCCTAGSRVF
LoAldh1a2	218	SCLYMGALIKEAGFPPGVVNIIPGFGPTAGAAIANHMGIDKIAFTGSTEVGKLIQEAAGKSNLKRVTLELGGKSPNIIIFADADLDYAVEQAHQGVFFNQGCCCTAGSRVF
LcAldh1a2	218	SALYMGALIKEAGFPPGVVNIIPGVGPTAGATAIAAHMGIDKIAFTGSTEVGKLIQEAAGKSNLKRVTLELGGKSPNIIIFADADLDYAVEQAHQGVFFNQGCCCTAGSRVF
MmAldh1a2	218	SALYMGALIKEAGFPPGVVNIIPGVGPTAGAAIASHMDIDKIAFTGSTEVGKLIQEAAGKSNLKRVTLELGGKSPNIIIFADADLDYAVEQAHQGVFFNQGCCCTAGSRVF
MmAldh1a1	201	TALHLSALIKEAGFPPGVVNIIPGVGPTAGAAISSHMDVDKVAFTGSTQVGLKIEAAGKSNLKRVTLELGGKSPNIIIFADADLDYAVEFAHHGVFYHQGCCVAAASRIF
RtAldh1a1	210	TALYMGALVKEAGFPPGVVNIIPGVGPTAGATITSHMDIDKIAFTGSTEVGKLIQEAAGKSNLKRVTLELGGKSPNIIIFADADLDYAVEQAHHALFFHQGCCCLAGSRIF
LcAldh1a1	214	TALYLGAALIKEAGFPPGVVNIIPGFGPTAGAAISQHMDIDKIAFTGSTEVGKLIQQAAGKSNLKRVTLELGGKSPNIIIFADADLDYAVEQAHHALFFHQGCCCLAGSRIF
PmAldh1a1/a2b	321	VQAPVYDEFVRRTERARRRALGDPLRPGTDQGPQVDQTFQDKVLELVESGKTEGAHVACGGGADTVAGALFIQPTVFTDVQDHMRITVEEIFGPVQIMILKFDVTVEEVL
EbAldh1a1	325	VHESIYRDFVRRSVECARRRRLGNPLHPSTQQGPQIDETQRKRIELIESGVRREGARLECGGCTWGD-QGYFLEPTVFSVDVDDMRIACEEIFGPVQQIMSFTSSEVLA
PmAldh1a1/a2a	330	VEDPVYDEFVRRSAQRARRRRVGHFPCPSTEHGPDIDEKQSKLIELVQSALEEGAHLECGGVACEG-RGYFVQPTVFSVDVPSDARIITREEIFGPVQILMRFDLSLEDDVA
RtAldh1a2	328	VEEPIYDEFVRKSTERAQRRVTGNPFDPAITEQGPDIDKQCKVLLEVLVSGIAEGAKLECGGKSLGK-KGFFIEPTVFSNVTDMDRIAKEEIFGPVQIILKFKTMEEV
LoAldh1a2	328	VEEPVYEEFVRKSVERAKRRVGSFPDPTTEQGPDISKEQSQSILELIQSGISEGAKLECGGKAIGR-KGYFIEPTVFSNVDRDEMIRIAKEEIFGPVQIIMKFKSIDEVIE
LcAldh1a2	328	VEEPIYEEFVRRSIERAKRRIVGSPFDPTTEQGPDINKEQYNNKILGLIQSGISEGAKLECGGKALGQ-KGFFIEPTVFSNVTDMDRIAKEEIFGPVQIIMKFKSTEEVIE
MmAldh1a2	328	VEESIYEEFVRKSVERAKRRIVGSPFDPTTEQGPDIDKQYNNKVLIELIQSGVAEGAKLECGGKGLGR-KGFFIEPTVFSNVTDMDRIAKEEIFGPVQIILKFKTMEEVIE
MmAldh1a1	311	VEESVYDEFVRKSVERAKKYVLGNPLTPGINQGPQIDKEQHKDILDLIESGKKEGAKLECGGRWGN-KGFFVQPTVFSNVTDMDRIAKEEIFGPVQIIMKFKSVDDVIK
RtAldh1a1	320	VEEPVYEFVCKSISLAQKHVIGNPLHEAVTHGPDIDKEQYDKILNLIESGKKEGAKLECGGLPWGD-KGFFIQPTVFSVTDMDRIAKEEIFGPVQIIMKFKTMEDEVIE
LcAldh1a1	324	VEESIYEEFVHKSVEQAQRRLGNPLIPGVDQGPQINQYHKDILDLIESGKKEGAKLECGGTSWGD-KGFFIQPTVFSVDVDDMDRIAKEEIFGPVQIIMKFKTIDEVIE
PmAldh1a1/a2b	431	RANNTRYGLAAAVFTRDLDTALTAAAGLQAGTVVWNCYNVIAQATFAGGFKMSGNGREMGEYGLQYETVKTVTIRVPKKS
EbAldh1a1	434	RANDSYGLAAGVFSKDLDTVLHVAALQAGTVVWNCYNVAVNCQSPFGGFKMSGNGREMGEYGLQYETVKTVTIRLPNKML
PmAldh1a1/a2a	439	RANASPYGLVAVFTRDLERALAVSAAMQAGTVWNCYNVAMNAQSPFGGFKMSGNGREMGEYGLQYETVKTVTIKISQKNS
RtAldh1a2	437	RANKSQFGLVAAVFTKDIDKALTVAAMQAGTVWNCYNVAVNCQSPFGGFKMSGNGREMGEYGLREYETIKTIVTRIQKNS
LoAldh1a2	437	RANNSEYGLTAGVFTRDINKAMTVSTAMQAGTVWNCYNFALSAQCPFGGFKMSGNGREMGEYGLREYSEVKTTITIKVPQKNS
LcAldh1a2	437	RANNPDFGLVAAVFTNDLNKALTVAAMQAGTVWNCYNVAVNCQSPFGGFKMSGNGREMGEYGLREYSEVKTTITIKVPQKNS
MmAldh1a2	437	RANNPDFGLVAAVFTNDINKALMVSSAMQAGTVWNCYNVAVNCQSPFGGFKMSGNGREMGEYGLREYSEVKTTITIKVPQKNS
MmAldh1a1	420	RANNTTYGLAAGLFTKDLKAITVSSALQAGVWVWNCYMLSAQCPFGGFKMSGNGRELGHEGLYETELKTVAMKISQKNS
RtAldh1a1	429	RANNTHYGLVAAVFTKDLINKAFTVASALQAGTVVWNCYNAMHVQSPFGGFKMSGNGREMGEYGLQYETIKTITIKIPQKNS
LcAldh1a1	433	RANNTHYGLVAGVFTKDLNKAMTIASSLQGTGVWNCYNAMTPQCPFGGFKMSGNGREMGEYGLQYETIKTITIKISQKNS

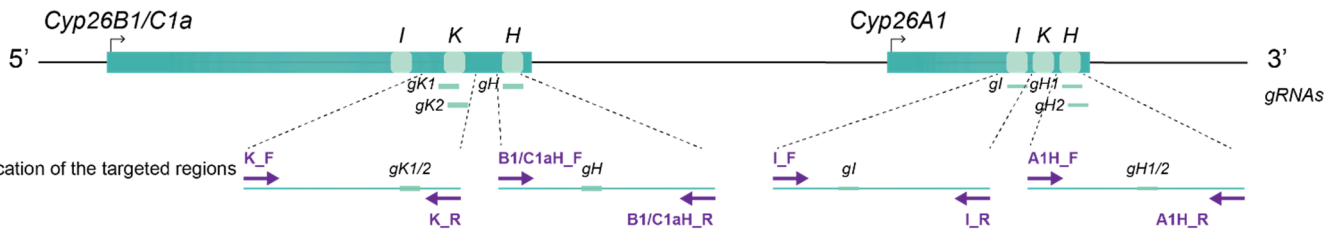
Supplementary Figure 2: Vertebrate Aldh1a protein alignments. Protein alignments of sea lamprey (Pm) Aldh1a1/a2a and Aldh1a1/a2b with Aldh1a1 and Aldh1a2 from various jawed vertebrates. The yellow highlighting represents the identity between sequences with a 95% identity threshold. Conserved Glutamic acid (Glu) and Cysteine (Cys) domains are indicated. Cutting sites of the gRNAs used for the CRISPR/Cas9 experiments are indicated with a blue arrow. Eb, *Eptatretus burgeri*; Lc, *Latimeria chalumnae*; Lo *Lepisosteus oculatus*; Mm, *Mus musculus*; Pm, *Petromyzon marinus*; Rt, *Rhincodon typus*.



Supplementary Figure 3: Vertebrate *Aldh1a* Phylogeny. Phylogenetic analysis of the vertebrate *Aldh1a* complement with *Aldh1a8* used as an outgroup. Jawed Vertebrate (J.V.) *Aldh1a1/Aldh1a2/Aldh1a3* clades are indicated with black lines. Trees were generated by Maximum Likelihood using the WAG model with 500 iterations for bootstrap testing, and the resulting supporting value for each node is shown as a percentage. A scale bar for the evolutionary distance is indicated below each tree. Species name abbreviations are indicated on the top left of the panel.



Supplementary Figure 4: HCRv3 development and validation in the sea lamprey. HCR panel representing a high-power (40X) image of *krox20* (cyan), *kreisler* (yellow) and *hoxβ1* (magenta) with their merged expression. This image was obtained after optimizing and adapting our HCRv3 protocol to be able to visualize hindbrain segments. We then used this protocol with this combination of genes to understand the changes in hindbrain segmentation in Talarazole vs. DMSO embryos (**Fig. 6a**).



Ctrl_C1H	GGAGCGTCCTCTACAGCATCCGCGACACGCACGAGACGGCGCCGGCCTTCAGCTCGCCGC	60
Cr_C1H_1	GGAGCGTCCTCTACAGCATCCGCGACACGCACGAGACGGCGCCGGCCTTCAGCTCGCCGC	60
Cr_C1H_2	GGAGCGTCCTGTACAGCATCCGCGACACGCACGAGACGGCGCCGGCCTTCAGCTCGCCGC	60
Cr_C1H_3	GGAGCGTCCTATACAGCATCCGCGACACGCACGAGACGGCGCCGGCCTTCAGCTCGCCGC	60
Cr_C1H_4	GGAGCGTCCTCTACAGCATCCGCGACACGCACGAGACGGCGCCGGCCTTCAGCTCGCCGC	60
Cr_C1H_5	GGAGCGTCCTCTACAGCATCCGCGACACGCACGAGACGGCGCCGGCCTTCAGCTCGCCGC	60

Ctrl_C1H	TTGACTTCGACCCCGACCGCTTCGACGCGACGCGCGCCGAGGACTCGAAGGAGCGCTTCA	120
Cr_C1H_1	TTGACTTCGACCCCGACCGCTTAGACGCGACGCGCGCCGAGGACTCGAAGGAGCGCTTCA	120
Cr_C1H_2	TTGACTTCGACCCCGACCGCTTCGACGCGACGCGCGCCGAGGACTCGAAGGAGCGCTTCA	120
Cr_C1H_3	TTGACTTCGACCCCGACCGCTTCGACGCGACGCGCGCCGAGGACTCGAAGGAGCGCTTCA	120
Cr_C1H_4	TTGACTTCGACCCCGACCGCTTCGACGCGACGCGCGCCGAGGACTCGAAGGAGCGCTTCA	120
Cr_C1H_5	TTGACTTCGACCCCGACCGCTTCG-----	69

Ctrl_C1H	GCTACCTGCCGTTTCGGCGGCGGTGCGCAGCTGCCTGGGCAAGGAGCTCGCCAAGCTCA	180
Cr_C1H_1	GCTACCTGCC----GGCGGCGGTGCGCAGCTGCCTGGGCAAGGAGCTCGCCAAGCTCA	176
Cr_C1H_2	GCTACCTGCC----CGGCGGCGGTGCGCAGCTGCCTGGGCAAGGAGCTCGCCAAGCTCA	176
Cr_C1H_3	GCTACCTGCC----CGGCGGCGGTGCGCAGCTGCCTGGGCAAGGAGCTCGCCAAGCTCA	176
Cr_C1H_4	GCTACCTGCC----GGCGGCGGTGCGCAGCTGCCTGGGCAAGGAGCTCGCCAAGCTCA	176
Cr_C1H_5	-----CGGCGGCGGTGCGCAGCTGCCTGGGCAAGGAGCTCGCCAAGCTCA	129

Ctrl_A1H	CCCGACCGCTGGATGACCGGCGGCGACGCCGGCGGCGGCAACGAGACGCGGCATCGTCG	120
Cr_A1H_1	CCCGACCGCTGGATGACCGGCGGCGACGCCGGCGGCGGCAACGAGACGCGGCATCGTC-	119
Cr_A1H_2	CCCGACCGCTGGATGACCGGCGGCGACGCCGGCGGCGGCAACGAGACGCGGCATCGTCG	120
Cr_A1H_3	CCCGCCCGCGGGATGACCGGCGGCGGCGCTGCGGCGGCATCGGAGACGTGGCATTGTCTG	120
Cr_A1H_4	CCCGACCGCTGGATGACCGGCGGCGACGCCGGCGGCGGCAACGAGACGCGGCATCGTC-	119
*** **		
Ctrl_A1H	CGCTTCACCTACATCCCGGGGGCGGCTCGCGCAGCTGCGTGGGCAAGGAGCTGGCGCGAC	180
Cr_A1H_1	-----GCGCAGCTGCGTGGGCAAGGAGCTGGCGCGAC	151
Cr_A1H_2	CGCTTCACCTCG-----CGCTGCGTGGGCAAGGAGCTGGCGCGAC	160
Cr_A1H_3	CGCGTCTC-----CTCCGCTGCGTGGGCAAGGAGCTGGCGCGAC	160
Cr_A1H_4	-----GCGCAGCTGCGTGGGCAAGGAGCTGGCGCGAC	151

Ctrl_A1H	TCATCCTGAGGATCGTGGTGGTGGAGCTGTGCCGCGCTGCGAGTGGGAGCTGCCCAACG	240
Cr_A1H_1	TCATCCTGAGGATCGTGGTGGTGGAGCTGTGCCGCGCTGCGAGTGGGAGCTGCCCAACG	211
Cr_A1H_2	TCATCCTGAGGATCGTGGTGGTGGAGCTGTGCCGCGCTGCGAGTGGGAGCTGCCCAACG	220
Cr_A1H_3	TCATCCTGAGGATCGTGGTGGTGGAGCTGTGCCGCGCTGCGAGTGGGAGCTGCCCAACG	220
Cr_A1H_4	TCATCCTGAGGATCGTGGTGGTGGAGCTGTGCCGCGCTGCGAGTGGGAGCTGCCCAACG	211

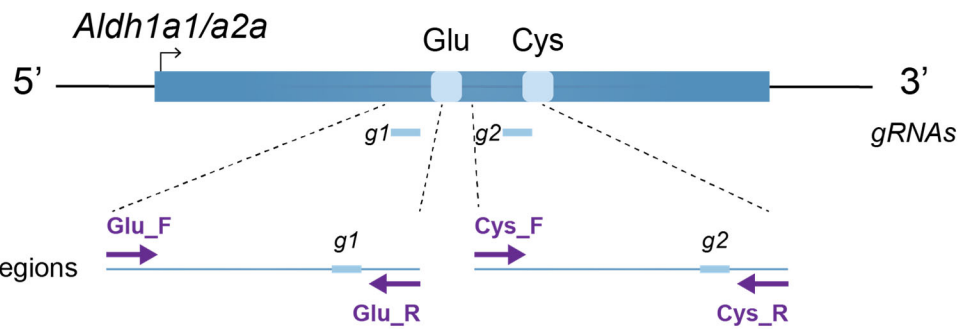
Ctrl_A1I	CCGCTCACCCTCCCGCCCCCTCCAGGACCTGAAGGAGTCGCCACGGAGCTCCTGTTCG	420
Cr_A1I	CCGCTCACCCTCCCGCCCCCTCCAGGACCTGAAGGAGTCGCCACGGAGCTCCTGT--G	419

Ctrl_A1I	GGGACACGAGACGACGCCAGCGCCGCCACGTCACTCGTCATGCACCTGGCAATCCACC	480
Cr_A1I	GGGACACGAGACGACGCCAGCGCCGCCACGTCACTCGTCATGCACCTGGCAATCCACC	478

	Cyp26A1_H	Cyp26A1_I	Cyp26B1/C1a_H
Mutant CRISPR loci	4/7	1/12	5/6
Control CRISPR loci	5/5	6/6	3/3

Supplementary Figure 5: Genotyping strategy and sequencing results for *Cyp26* CRISPR embryos

Cartoon illustrating the design of primers for amplifying each site targeted by the *gRNAs* in the *Cyp26* loci. The primer binding sites are indicated in bold purple. For each site, we show an alignment of mutant loci (Cr_) sequences with the sequence of a control locus (Ctrl), the site targeted by the gRNA is indicated in bold and the PAM in red. A table indicating the proportion of mutant CRISPR or control CRISPR loci for each site is shown.



```

Ctrl_Glu      CCCTTACGTAACGCAGGTGGGGAAAGTTGATCCAGGAGGAGGCCGGCAAGAGTAACCTGAA 179
Cr_Glu_1      CCTTTACGTAACGCAGGTGGGGAAAGTTGATCCAGGA-----GTAACGTGAA 73
Cr_Glu_2      CCCTTACGTAACGCAGGTGGGGAAAGTTGATCCAGGA-----GTAACCTGAA 165
Cr_Glu_3      CCCTTACGTAACGCAGGTGGGGAAAGTTGATCCAGGA-----GTAACCTGAA 166
Cr_Glu_4      CCCTTACGTAACGCAGGTGGGGAAAGTTGATCCAGGAGTAACCTGAAGC----- 165
Cr_Glu_5      CCCTTACGTAACGCAGGTGGGGAAAGTTGATCCAGGA-----GTAACCTGAA 165
** *****

Ctrl_Glu      GCGCGTGACGCTGGAGCTTGGCGGGAAGAGCCCCATCATCGTCTTCGCCGACGCCGACCG 239
Cr_Glu_1      GCGAGTGACGCTGAAGCTTGGCGGGAAGAGCCCCATCATCGTATTTCGCCGAA-CCGACCG 132
Cr_Glu_2      GCGCGTGACGCTGGAGCTTGGCGGGAAGAGCCCCATCATCGTCTTCGCCGACGCCGACCG 225
Cr_Glu_3      GCGCGTGACGCTGGAGCTTGGCGGGAAGAGCCCCATCATCGTCTTCGCCGACGCCGACCG 226
Cr_Glu_4      ---GCGGACGCTGGAGCGTGGCGGGAAGAGCCCCATCATCGTTTTGACCGACGCCGACCG 222
Cr_Glu_5      GCGCGTGACGCTGGAGCTTGGCGGGAAGAGCCCCATCATCGTCTTCGCCGACGCCGACCG 225
***** ** *****

Ctrl_Cys      CCCCTGCTGTGACACTGCTGACTCGGCGTGGTTCCACCCCTCCACCCCCCCTCCAC 240
Cr_Cys_1      CCCCTGCTGTGACACTGCTGACTCGGCGTGGTTCCACCCCTCCACCCCCCCTCCAC 240
Cr_Cys_2      CCCCTGCTGTGACACTGCTGACTCGGCGTGGTTCCACCCCTCCACCCCCCCTCCAC 240
Cr_Cys_3      CCCCTGCTGTGACACTGCTGACTCGGCGTGGTTCCACCCCTCCACCCCCCCTCCAC 240
Cr_Cys_4      CCCCTGCTGTGACACTGCTGACTCGGCGTGGTTCCACCCCTCCACCCCCCCTCCAC 238
Cr_Cys_5      CCCCTGCTGTGACACTGCTGACTCGGCGTGGTTCCACCCCTCCACCC----- 238
Cr_Cys_6      CCCCTGCTGTGACACTGCTGACTCGGCGTGGTTCCACCCCTCCACCC----- 238
Cr_Cys_7      CCCCTGCTGTGACACTGCTGACTCGGCGTGGTTCCACCCCTCCACCC----- 240
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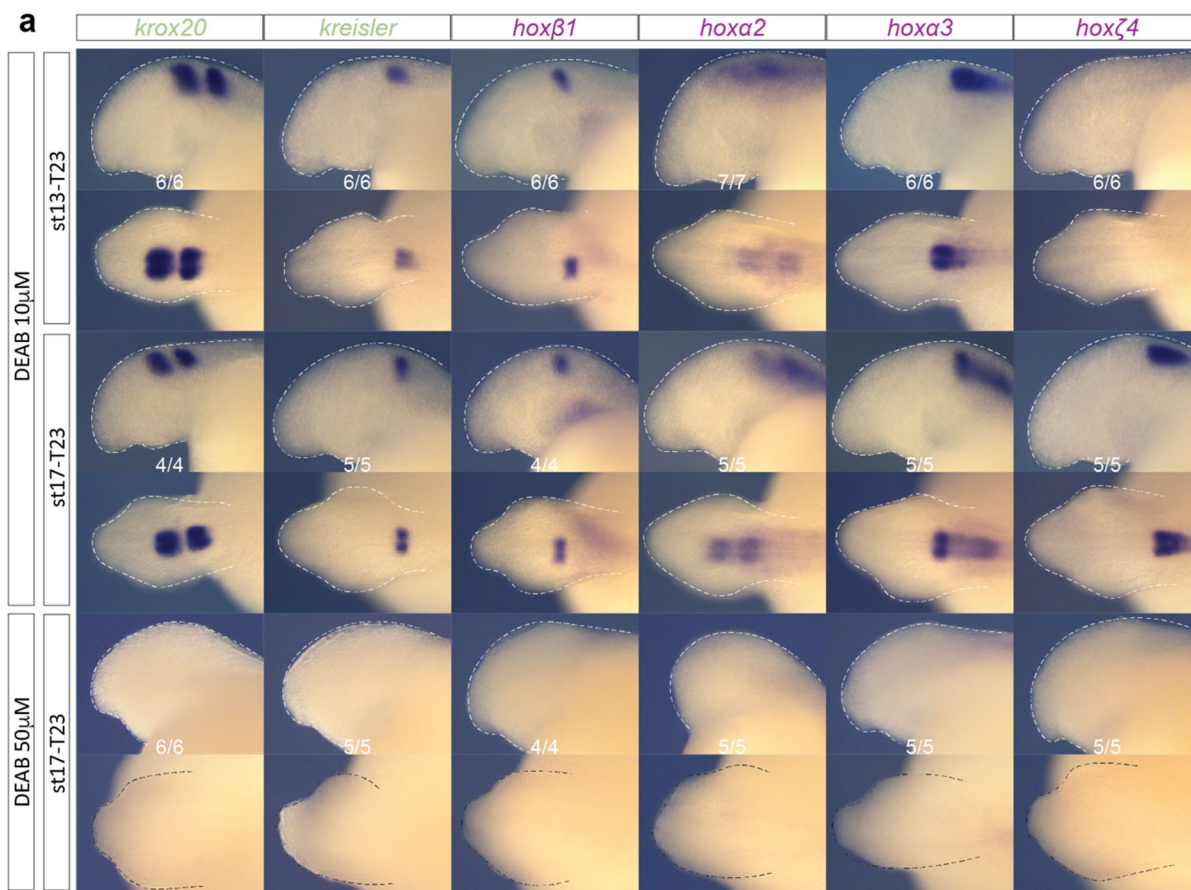
Ctrl_Cys      TACGAGCAGTGGACGCGCGGTGGAGCAGGCGCACCAGGGGGTGTCTTGGAACCAGGGCC 300
Cr_Cys_1      -----GGGCC 258
Cr_Cys_2      -----GGGCC 258
Cr_Cys_3      -----GGGCC 249
Cr_Cys_4      -----GGGCC 260
Cr_Cys_5      -----AGGGCC 250
Cr_Cys_6      -----AGGGCC 250
Cr_Cys_7      -----AGGGCC 244
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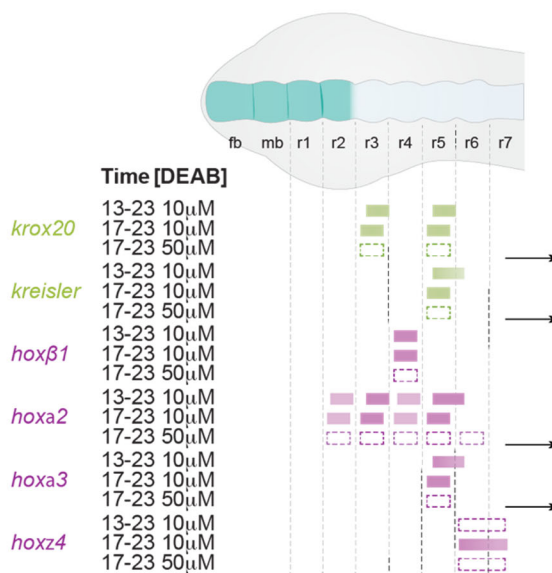
	Aldh1a1/a2a_Glu	Aldh1a1/a2a_Cys
Mutant CRISPR loci	5/5	7/11
Control CRISPR loci	5/5	3/3

Supplementary Figure 6: Genotyping strategy and sequencing results for *Aldh1a1/a2a* CRISPR embryos.

Cartoon illustrating the design of primers for amplifying each site targeted by the *gRNAs* in the *Aldh1a1/a2a* gene loci. Primer binding sites are indicated in bold purple. For each site, we show an alignment of mutant loci (Cr_) sequences with the sequence of a control locus (Ctrl), the site targeted by the *gRNA* is indicated in bold and the PAM in red. A table indicating the proportion of mutant CRISPR or control CRISPR loci for each site is shown.



b Summary of gene expression



Supplementary Figure 7: Effect of DEAB concentration and timing of treatment on hindbrain patterning. (a) cISH of key patterning hindbrain markers in embryos treated with 10 μM of DEAB at gastrulation (st13), 10 μM of DEAB at early neurulation (st17) or 50 μM of DEAB at early neurulation (st17). For each gene, the most representative phenotype is shown, and numbers of experimental replicates are indicated. These molecular phenotypes obtained for embryos treated with 10 μM of DEAB at gastrulation (st13) are less severe than the ones obtained when treating the embryos with 50 μM DEAB (**Fig. 5d**) and are reminiscent of the phenotypes obtained in the *Cr Aldh1a1/a2a* mutants (**Fig. 8c**). **(b)** Cartoon summarizing the effects of different concentrations and timing of treatments on hindbrain patterning.

Mouse (*Mus musculus*): Aldh1a1: NP_001348432.1; Aldh1a2: NP_033048.2; Aldh8a1: NP_848828.1; Cyp26A1: XP_017173542.1; Cyp26B1: NP_001171184.1; Cyp26C1: XP_017173748.1; Cyp51: NP_064394.2

Spotted gar (*Lepisosteus oculatus*): Aldh1a1: XP_015222024.1; Aldh1a2: XP_006628784.1; Aldh8a1: XP_006625886.1; Cyp26A1-LG5: XP_015202021.1; Cyp26B1-LG5: XP_015202022.1; Cyp26B1-LG4: XP_006629157.1; Cyp51: XP_006636075.1

West indian coelacanth (*Latimeria chalumnae*): Aldh1a1: XP_006001725.2; Aldh1a2: XP_005998797.2; Aldh8a1: XP_014352052.1; Cyp26A1: XP_005991616.1; Cyp26B1: XP_005997719.1; Cyp26C1: XP_014341348.1; Cyp51: XP_014350652.1

Whale shark (*Rhincodon typus*): Aldh1a1-like: XP_015222024.1; Aldh1a2: XP_020369553.1; Aldh8a1: XP_048452951.1; Cyp26A1: XP_020382659.1; Cyp26B1: XP_020378695.1; Cyp26C1: XP_020367038.1; Cyp51: XP_020371022.1

Inshore hagfish (*Eptatretus burgeri*): Aldh1a1: ENSEBUG00000010361; Aldh8a1: ENSEBUG00000011145; Cyp26C1 (10538): ENSEBUG00000010538; Cyp26C1 (06909): ENSEBUG00000006909; Cyp51: ENSEBUG00000006843

Sea lamprey (*Petromyzon marinus*): Aldh1a1/a2a (Chr1): XP_032805864.1; Aldh1a1/a2b (Ch40): XP_032824682.1; Aldh8a1: XP_032810842.1; Cyp26A1: XP_032808019.1-2; Cyp26B1/C1a (Chr11): XP_032808017.1; Cyp26B1/C1b (Chr9): XP_032806163.1; Cyp51: XP_032816590.1

Thorny Skate (*Amblyraja radiata*): Aldh1a1-like: XP_032873441.1; Aldh1a2: XP_032906516.1; Cyp26A1: XP_032889480.1; Cyp26B1: XP_032885953.1; Cyp26C1: XP_032889482.1;

Chicken (*Gallus gallus*): Aldh1a1: NP_989908.2; Aldh1a2: NP_001384737.1; Cyp26A1: NP_001001129.2; Cyp26B1: XP_015141554.1; Cyp26C1: XP_421678.6;

Chinese soft-shelled turtle (*Pelodiscus sinensis*): Aldh1a1: XP_006137885.1; Aldh1a2: XP_006112531.1; Cyp26A1: XP_006113245.1; Cyp26B1: XP_025042348.1; Cyp26C1: XP_025035575.1;

West African lungfish (*Protopterus annectens*): Aldh1a1: XP_043916765.1; Aldh1a2: XP_043934839.1; Cyp26A1: XP_043912621.1; Cyp26B1: XP_043919907.1; Cyp26C1: XP_043912622.1;

Reedfish (*Erpetoichthys calabaricus*): Aldh1a1: XP_028658993.1; Aldh1a2: XP_028678887.1; Cyp26A1: XP_028651199.1; Cyp26B1: XP_028657295.1; Cyp26C1: XP_028651196.1

Amphioxus (*Branchiostoma floridae*): BfAldh1a1/a2a: XP_035683184.1; BfAldh1a1/a2b: XP_035666435.1; BfAldh1a1/a2c: XP_035683791.1; BfAldh1a1/a2d: XP_035683789.1; BfAldh1a1/a2e: XP_035659507.1; BfAldh1a1/a2f: XP_035659462.1; BfCyp26-1: LOC118413434; BfCyp26-2: XP_035672703.1; BfCyp26-3: XP_035672700.1;

Supplementary Figure 8: Protein accession numbers used for phylogeny analysis. Protein sequences corresponding to each gene were retrieved using both ENSEMBL and NCBI databases, focusing on the latest available version of the genome and/or the most complete available genome annotation. Amphioxus *Cyp26* and *Aldh1* gene families were used as outgroups for the analyses of *Cyp26* and *Aldh1a* complements.

Mouse (*Mus musculus*): *Aldh1a1*: NM_001361503.1; *Aldh1a2*: NM_009022.4;
Cyp26A1: XM_017318053.2; *Cyp26B1*: NM_001177713.1; *Cyp26C1*: XM_017318259.3

 Grey short-tailed opossum (*Monodelphis domestica*): *Aldh1a1*: XM_001373091.4;
Aldh1a2: XM_007479682.2; *Cyp26A1*: XM_001375255.4; *Cyp26C1*: XM_007478798.1

 Platypus (*Ornithorhynchus anatinus*): *Aldh1a1*: XM_007667019.3;
Aldh1a2: XM_029070050.1; *Cyp26B1*: XM_029046562.2; *Cyp26C1*: XM_029060242.1

 Chicken (*Gallus gallus*): *Aldh1a1*: NM_204577.5; *Aldh1a2*: NM_001397808.1;
Cyp26A1: XM_015288598.4; *Cyp26B1*: XM_015286068.4; *Cyp26C1*: XM_421678.8

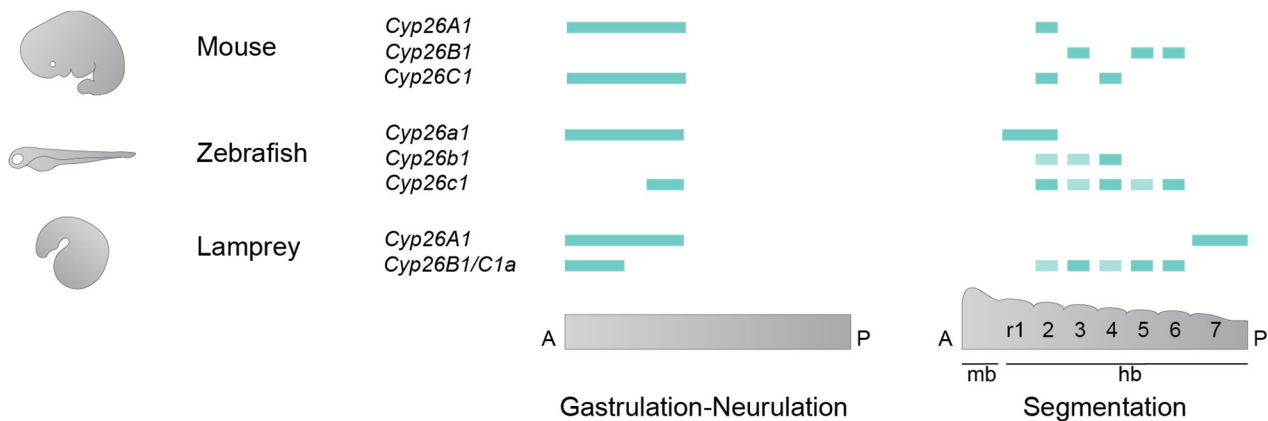
 Spotted gar (*Lepisosteus oculatus*): *Aldh1a1*: XM_015366538.1; *Aldh1a2*: XM_006628721.2;
*Cyp26A1*LG5: XM_015346535.1; *Cyp26B1*-LG5: XM_015346536.1; *Cyp26B1*-LG4: XM_006629094.2

 West indian coelacanth (*Latimeria chalumnae*): *Aldh1a1*: XM_006001663.2;
Aldh1a2: XM_005998735.2; *Cyp26A1*: XM_005991554.1; *Cyp26B1*: XM_005997657.2;
Cyp26C1: XM_014485862.1

 Whale shark (*Rhincodon typus*): *Aldh1a1*-like: XM_020527364.2; *Aldh1a2*: XM_020513964.2;
Cyp26A1: XM_048608684.1; *Cyp26B1*: XM_020523106.1; *Cyp26C1*: XM_020511449.1

 Sea lamprey (*Petromyzon marinus*): *Aldh1a1/a2a* (Chr1): XM_032949973.1;
Aldh1a1/a2b (Chr40): XM_032968791.1; *Cyp26A1*: XM_032952128.1;
Cyp26B1/C1a (Chr11): XM_032952126.1; *Cyp26B1/C1b* (Chr9): XM_032950272.1

Supplementary Figure 9: mRNA accession numbers used for synteny analysis. mRNA accession numbers of *Cyp26* and *Aldh1a* used to conduct the synteny analyses in different vertebrate models.



Supplementary Figure 10: comparison of *Cyp26* expression developmental dynamics in different vertebrates. Schematic representation of the dynamic changes in *Cyp26* expression during early processes of development (gastrulation-neurulation) and during hindbrain segmentation in mouse, zebrafish and lamprey, illustrating both similarities and differences between orthologues.

Species	Exons	AA
<i>Pm Cyp26A1</i>	7 exons	497 AA
<i>Pm Cyp26B1/C1a</i>	6 exons	529 AA
<i>Pm Cyp26B1/C1b</i>	6 exons	634 AA
<i>Eb Cyp26 06909</i>	6 exons	468 AA
<i>Eb Cyp26 10538</i>	6 exons	528 AA

Supplementary Table 1: Cyp26 gene structure and protein length in cyclostomes. *Pm*, *Petromyzon marinus* (sea lamprey); *Eb*, *Eptatretus burgeri* (inshore hagfish); AA. Amino-acid.

Species	<i>Cyp26A1</i>	<i>Cyp26A1</i>	<i>Cyp26B1</i>	<i>Cyp26B1</i>	<i>Cyp26C1</i>	<i>Cyp26C1</i>
Mm	7 exons	497 AA	6 exons	512 AA	6 exons	518 AA
Oa	NF		6 exons	511 AA	6 exons	571 AA
Lo	7 exons	492 AA	6 exons	511 AA	6 exons	554 AA
Lc	7 exons	493 AA	6 exons	511 AA	6 exons	634 AA
Rt	7 exons	494 AA	6 exons	511 AA	NF	
Cc					6exons	560 AA

Supplementary Table 2: Cyp26 gene structure and protein length in jawed vertebrates. *Mm*, *Mus musculus* (mouse); *Oa*, *Ornithorhynchus anatinus* (platypus); *Lo*, *Lepisosteus oculatus* (spotted gar); *Lc*, *Latimeria chalumnae* (coelacanth); *Rt*, *Rhincodon typus* (whale shark); *Cc*, *Carcharodon carcharias* (great white shark); NF: not found; AA. Amino-acid.

Species	Exons	AA
<i>Pm Aldh1a1/a2a</i>	13	520 AA
<i>Pm Aldh1a1/a2b</i>	13	512AA or 459 AA
<i>Eb Aldh1a1</i>	13	515 AA

Supplementary Table 3: Aldh1a gene structure and protein length in cyclostomes. *Pm*, *Petromyzon marinus* (sea lamprey); *Eb*, *Eptatretus burgeri* (inshore hagfish); AA. Amino-acid.

Species	<i>Aldh1a1</i>	<i>Aldh1a1</i>	<i>Aldh1a2</i>	<i>Aldh1a2</i>
Mm	13 exons	501 AA	13 exons	518 AA
Oa	13 exons	511 AA	13 exons	518 AA or 422 AA
Lo	13 exons	519 AA or 527 AA	13 exons	518 AA
Lc	13 exons	514	13 exons	518 AA or 422 AA
Rt	13 exons	510 or 537 AA	13 exons	518 AA

Supplementary Table 4: Aldh1a gene structure and protein length in jawed vertebrates. *Mm*, *Mus musculus* (mouse); *Oa*, *Ornithorhynchus anatinus* (platypus); *Lo*, *Lepisosteus oculatus* (spotted gar); *Lc*, *Latimeria chalumnae* (coelacanth); *Rt*, *Rhincodon typus* (whale shark); AA. Amino-acid.

Gene	Accession Number	Probe set size	Amplifier/AlexaFluor	Average Probe Concentration
<i>Aldh1a1/a2a</i>	LOC116940317	19	B3-488	6 nM
<i>Cyp26A1</i>	LOC116941264	23	B4-546	5 nM
<i>Cyp26B1/C1a</i>	LOC116941263	22	B1-647	5 nM
<i>krox20 (egr1)</i>	LOC116940673	18	B3-488	6 nM
<i>kreisler (mafB-like)</i>	LOC116954108	12	B4-546	11 nM
<i>hoxβ1</i>	LOC103091820	13	B1-647	8 nM

Supplementary Table 5: HCR FISH probe set design. Summary of the probe set design obtained for each gene targeted by FISH HCR; nM: nanomolar.

name	5'--> 3' sequence	description	product size
A1_H_F	TACAGCATCAAGGAGACGCA	Cyp26A1_H helix	405 bp
A1_H_R	CGTCTTGTGCTGGCATAAATG		
A1_I_F	GCTGAGACAAAGCGGGAAAG	Cyp26A1_I helix	568 bp
A1_I_R	CCAGGCGAGGGAGATAACAT		
name	5'--> 3' sequence	description	product size
C1_H_F	GGAGCGTCCTCTACAGCATC	Cyp26C1_H helix	480 bp
C1_H_R	CTCCTCCGTCTCTCTGTCGT		
name	5'--> 3' sequence	description	product size
Glu_F	TGGCGGTCGATTCTTCTCTT	Glutamate site genotyping	360 bp
Glu_R	TATTGGCTGCGCAATCTCAC		
Cys_F	ATATACAGCAGGCGTCAGGG	Cysteine site genotyping	355 bp
Cys_R	CGCACGAACTCGTCGTAGA		

Supplementary Table 6: Primers for CRISPR genotyping. Primers used for genotyping targeted regions in CRISPR embryo; F: Forward, R: Reverse.