

Fig. S1 (Satou et al.)

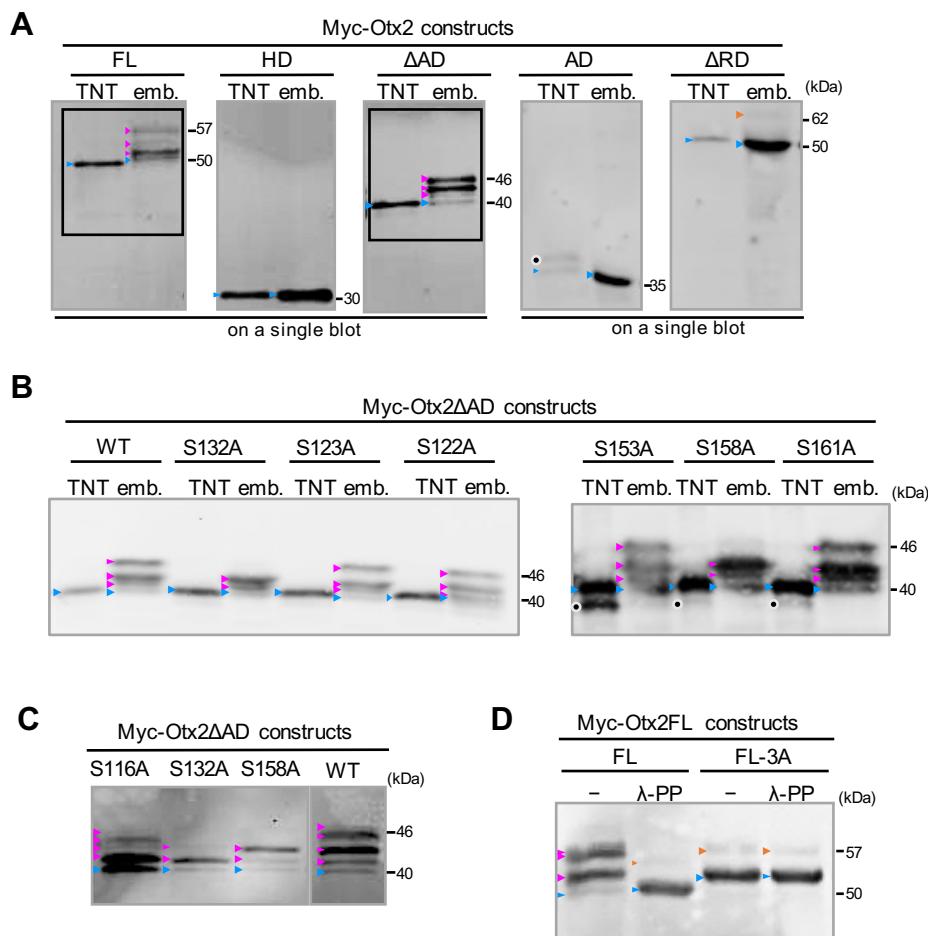


Fig. S1. Post-translational modifications of Otx2 around the repression domain. Western blot analysis of Myc-Otx2 constructs expressed in *X. laevis* embryos. (A) Myc-Otx2 (FL) and its deletion constructs (HD, ΔAD, AD and ΔRD). Boxes indicate the data presented in Fig. 1B. ΔRD appeared to have a modified band (orange arrowhead). (B) WT and alanine mutants at S132 (S132A), S123 (S123A), S122 (S122A), S153 (S153A), S158 (S158A) and S161 (S161A) in Myc-Otx2ΔAD constructs. (C) WT and alanine mutants at S116 (S116A), S132 (S132A), S158 (S158A) in Myc-Otx2ΔAD constructs. (D) λ-PP treatment removed most modified bands of WT and 3A constructs of Myc-Otx2FL. Note that the different electrophoretic mobility of nascent bands (blue arrowheads) between FL and FL-3A may be due to substitutions of serine with alanine, though Myc-Otx2-ΔAD and ΔAD-3A migrated similarly (see Fig. 1D). Calculated and apparent molecular masses of constructs (kDa) were 43 and 50 for Myc-Otx2FL, 22 and 30 for Myc-Otx2-HD, 32 and 40 for Myc-Otx2ΔAD, 23 and 35 for Myc-Otx2-AD, and 34 and 50 for Myc-Otx2ΔRD, respectively. Black circles, undesired products or degradation products; orange arrowheads, bands resistant to λ-PP, which may correspond to an upper band of ΔRD in panel A.

Fig. S2 (Satou et al.)

Xl_Otx2.L	MMSYIJKQ-PPYAVNGLSSLTSGMDLLHPSVGVYPATPRK	QRRERTTFTTRAQLDILEALFAK	59
Xl_Otx5.L	MMSYIJKQ-PHYAVNGLTLAGTMGDLHSAVGYPNTPRK	QRRERTTFTTRAQLDILEALFAK	59
Xt_Otx2	MMSYIJKQ-PPYAVNGLSSLTSGMDLLHPSVGVYPATPRK	QRRERTTFTTRAQLDILEALFAK	59
Xt_Otx5	MMSYIJKQ-PHYAVNGLTLAGTMGDLHSAVGYPNTPRK	QRRERTTFTTRAQLDILEALFAK	59
Dr_Otx2	MMSYIJKQ-PPYAVNGLSSLTSGMDLLHPSVGVYPATPRK	QRRERTTFTTRAQLDILEALFAK	59
Dr_Otx5	MMSYIJKQ-PHYAVNGLTLAGTMGDLHSAVGYPNTPRK	QRRERTTFTTRAQLDILEALFAK	59
Gg_Otx2	MMSYIJKQ-PPYAVNGLSSLTSGMDLLHPSVGVYPATPRK	QRRERTTFTTRAQLDILEALFAK	59
Gg_Otx5	MMSYIJKQ-PHYAVNGLTLAGTMGDLHSAVGYPNTPRK	QRRERTTFTTRAQLDILEALFAK	59
Mm_Otx2	MMSYIJKQ-PPYAVNGLSSLTSGMDLLHPSVGVYPATPRK	QRRERTTFTTRAQLDILEALFAK	59
Mm_Crx	MMAYMNPAPHYSVNALASGPNVNDLHMQAVPYSSAPRK	QRRERTTFTRSQLEEEALFAK	60
Hs_Otx2	MMSYIJKQ-PPYAVNGLSSLTSGMDLLHPSVGVYPATPRK	QRRERTTFTRAQLDVEALFAK	59
Hs_Crx	MMAYMNPAPHYSVNALASGPNSVNDLHMQAVPYSSAPRK	QRRERTTFTRSQLEEEALFAK	60
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Xl_Otx2.L	TRYPDFMREEVALKINLPESRVQVWFKNRRAKCR Q-----QQQQQQNGQNKRPSKK	114	
Xl_Otx5.L	TRYPDFMREEVALKINLPESRVQVWFKNRRAKCRQ-----QQQQ-STGQAKPRPAKK	112	
Xt_Otx2	TRYPDFMREEVALKINLPESRVQVWFKNRRAKCRQ-----QQQQQQNGQNKRPSKK	114	
Xt_Otx5	TRYPDFMREEVALKINLPESRVQVWFKNRRAKCRQ-----QQQQ-STGQAKPRPAKK	112	
Dr_Otx2	TRYPDFMREEVALKINLPESRVQVWFKNRRAKCRQ-----QQQQQQNGQNKRPAKK	114	
Dr_Otx5	TRYPDFMREEVALKINLPESRVQVWFKNRRAKCRQ-----QQQQ-QTSGQTTPRPPKK	113	
Gg_Otx2	TRYPDFMREEVALKINLPESRVQVWFKNRRAKCRQ-----QQQQQQNGQNKRPAKK	114	
Gg_Otx5	TRYPDFMREEVALKINLPESRVQVWFKNRRAKCRQ-----QQQQ-SSCQPKARPAKK	112	
Mm_Otx2	TRYPDFMREEVALKINLPESRVQVWFKNRRAKCRQ-----QQQQQQNGQNKRPAKK	114	
Mm_Crx	TQYPDVYAREEVALKINLPESRVQVWFKNRRAKCRQRQQQQKQQQPPGAQTKPARKR	120	
Hs_Otx2	TRYPDFMREEVALKINLPESRVQVWFKNRRAKCRQ-----QQQQQQNGQNKRPAKK	114	
Hs_Crx	TQYPDVYAREEVALKINLPESRVQVWFKNRRAKCR QQRQQQQKQQQPPGGQAKARPAKR	120	
*:***: * *****:*****:*****:***: * * :***:*			
Xl_Otx2.L	T ----SPAREVSSE---SGTSGQFSPPCS---TSGPVISSTAPVSIWSPASISP LSDPL	164	
Xl_Otx5.L	T----SPARETNSE---ASTNGQYSPPPPG---TAVTPSSSASATVSIWSPASISP IPDPL	163	
Xt_Otx2	P----SPAREVSSE---SGTSGQFSPPCS---TSVPVISSTAPVSIWSPASISP LSDPL	164	
Xt_Otx5	T----SPARETNSE---ASTNGQYSPPPPG---TAVTPSSSASATVSIWSPASISP IPDPL	163	
Dr_Otx2	S----SPAREASSE---SGASGQFTPPSS---TSVPAISTTAPVSIWSPASISP LSDPL	164	
Dr_Otx5	S----SPARDSSASEPASSTASGSPYSPPPPPGTAITP---SSSSTATVSIWSPASISP LPDPL	168	
Gg_Otx2	N----SPAREVSSE---SGTSGQFTPPSS---TSVPTISSLAPVSIWSPASISP LSDPL	164	
Gg_Otx5	P----TPPREAPND---AGGAGPYSPPTQP---GPAGTPGSAPVSIWSPASISP VPDPD	161	
Mm_Otx2	S----SPAREVSSE---SGTSGQFSPPSS---TSVPTIASSSAPVSIWSPASISP LSDPL	164	
Mm_Crx	AGTSPRSTDVCTDP---LGISDYSYSPSLP --GPSGSPTTAVATVSIWSPASAEPLPEAQ	175	
Hs_Otx2	T----SPAREVSSE---SGTSGQFTPESS---TSVPTIASSSAPVSIWSPASISP LSDPL	164	
Hs_Crx	AGTSPRSTDVCPDP---LGISDYSYSPPLP --GPSGSPTTAVATVSIWSPASISP LSDPL	175	
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Xl_Otx2.L	S---TSSS-CMQRS---YPMTYTQASGYSQG---YASSTS YFGGMDCGSYLTPMHHQLSG	214	
Xl_Otx5.L	S---AVTPCMQRST-GYPMTYSQAPAYTQS---YGGSSSYFTGLDCGSYLSMPHQLSA	216	
Xt_Otx2	S---TSSS-CMQRS---YPMTYTQASGYSQG---YAGSTS YFGGMDCGSYLTPMHHQLSG	214	
Xt_Otx5	S---AATTPCMQRSA-GYPMTYSQAPAYTQS---YGGSSSYFTGLDCGSYLSMPHQLSA	216	
Dr_Otx2	S---TSSS-CMQRS---YPMTYTQASGYSQG---YAGSTS YFGGMDCGSYLTPMHHQLTG	214	
Dr_Otx5	S---APSTACLQRSS---YPMTYTQASGYSQG---YAASSYYFTGLDCSSYLSMPHQLSA	220	
Gg_Otx2	S---TSSS-CMQRS---YPMTYTQASGYSQG---YAGSTS YFGGMDCGSYLTPMHHQLPG	214	
Gg_Otx5	A---AGSAPGLPRSAFPASA PYNQ TAPYQGOS---YGGSAAYFGLDCGAYLSPMPHPLGA	215	
Mm_Otx2	S---TSSS-CMQRS---YPMTYTQASGYSQG---YAGSTS YFGGMDCGSYLTPMHHQLPG	214	
Mm_Crx	RAGLVASGSPSLTASP---YAMTYAPASAFCSSPSSAYGSPSSYFSGLDP---YLSPMPVQLGG	231	
Hs_Otx2	S---TSSS-CMQRS---YPMTYTQASGYSQG---YAGSTS YFGGMDCGSYLTPMHHQLPG	214	
Hs_Crx	RAGLVASGSPSLTASP---YAMTYAPASAFCSSPSSAYGSPSSYFSGLDP---YLSPMPVQLGG	231	
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Xl_Otx2.L	PGATLSPMSTNAVTSHLNQSQALSSQAYGASSLGFNSTADCLDYKDQTASWKLFNDA-D	273	
Xl_Otx5.L	PGATLSPPIATPTMGSHLSQSPASLSAQGYGAASLGFTS-VDCLDYKDQTASWKLFNAD	275	
Xt_Otx2	AGATLSPMGTNAVTSHLNQSPALSSQAYGASSLGFNSTADCLDYKDQTASWKLFNDA-D	273	
Xt_Otx5	PGATLSPPIATPTMGSHLSQSPASLSAQGYGAASLGFTS-VDCLDYKDQTASWKLFNAD	275	
Dr_Otx2	PGSTLSPMSSNAV TSHLNQSPASLPTQCYGAGSLGFNSTADCLDYKDQASSWKLFNDA-D	273	
Dr_Otx5	SGGALSPMSG---ALSGSPASLSSQGYTAASLGFTG+VDCLDYKDQTASWKLFNAD	274	
Gg_Otx2	PGATLSPMGNANAV TSHLNQSPASLSTQGYGASSLGFNSTTDCLDYKDQTASWKLFNDA-D	273	
Gg_Otx5	PGAALSPLGAP-MGAHLTPSPASLGSQSFAG-LGFGA-VDCLEYKEQAGAWKLNFNAAD	272	
Mm_Otx2	PGATLSPMGTNAVTSHLNQSPASLSTQGYGASSLGFNSTTDCLDYKDQTASWKLFNDA-D	273	
Mm_Crx	P--ALSPLSGPSVGP SLAQSPSTLSGQSYSTYSP---VDSLEFKDPTGTWKFTYNPMD	284	
Hs_Otx2	PGATLSPMGTNAVTSHLNQSPASLSTQGYGASSLGFNSTTDCLDYKDQTASWKLFNDA-D	273	
Hs_Crx	P--ALSPLSGPSVGP SLAQSPSTLSGQSYGAYSP---VDSLEFKDPTGTWKFTYNPMD	284	
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Xl_Otx2.L	C LDYKDQTSSWKFQVL 289		
Xl_Otx5.L	C LDYKDQ-SSWKFQVL 290		
Xt_Otx2	C LDYKDQTSSWKFQVL 289		
Xt_Otx5	C LDYKDQ-SSWKFQVL 290		
Dr_Otx2	C LDYKDQTSSWKFQVL 289		
Dr_Otx5	C LDYKDQ-NSWKFQVL 289		
Gg_Otx2	C LDYKDQTSSWKFQVL 289		
Gg_Otx5	C LDYKEQ-SSWKFQVL 287		
Mm_Otx2	C LDYKDQTSSWKFQVL 289		
Mm_Crx	PLDYKDQ-SAWKFQIL 299		
Hs_Otx2	CLDYKDQTSSWKFQVL 289		
Hs_Crx	PLDYKDQ-SAWKFQIL 299		
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Fig. S2 (Satou et al.)

Fig. S2. Alignment of amino acid sequences of Otx2 and Otx5/Crx among vertebrates.

Abbreviations of species are *Xenopus laevis* (Xl), *Xenopus tropicalis* (Xt), *Danio rerio* (Dr), *Gallus gallus* (Gg), *Mus musculus* (Mm) and *Homo sapiens* (Hs). Protein sequences were obtained from the NCBI database: Xl_Otx2.L (NP_001084955), Xl_Otx5.L (NP_001081916), Xt_Otx2 (NP_001016177), Xt_Otx5 (NP_001016021), Dr_Otx2 (NP_571326), Dr_Otx5 (NP_851848), Gg_Otx2 (NP_989851), Gg_Otx5 (NP_001288716), Mm_Otx2 (NP_001273412), Mm_Crx (NP_031796), Hs_Otx2 (NP_001257453), Hs_Crx (AAH53672). Boxes coloured in green, homeodomain; blue, SIWSPAS motif; yellow, repeated Otx tail motif; red, Otx2 mutation sites (P133 and P134) that were reportedly associated with human ocular malformation. Blue or magenta letters, consensus motifs for Akt and Cdks. Bold cases, putative phosphorylation sites of Otx2 as shown in this study.

Fig. S3 (Satou et al.)

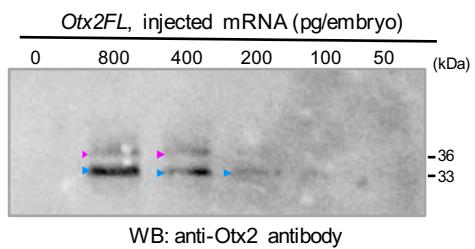
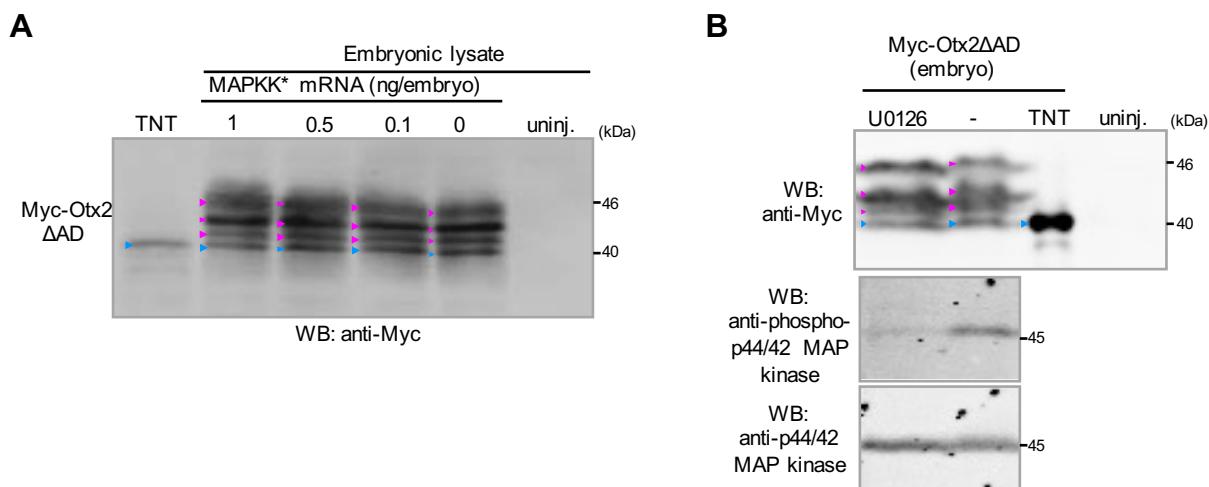


Fig. S3. The detection limit of Otx2FL by western blotting.

Embryos were injected with *Otx2FL* mRNA at various doses as indicated. Modified bands of *Otx2FL* was clearly detected when *Otx2FL* mRNAs were injected at higher than 200 pg per embryo. Embryonic lysate of 0.42 equivalent embryo was loaded per lane. *Otx2FL* was detected by western blotting with anti-*Otx2* antibodies. Blue arrowheads, nascent protein; magenta arrowheads, modified proteins.

Fig. S4 (Satou et al.)

**Fig. S4. Effect of MAPK on modifications of Otx2.**

(A) No effect of a constitutively active mutant of MAPKK (MAPKK*) on modifications of Otx2. Overexpression of MAPKK* did not apparently affect the modified bands of Myc-Otx2 Δ AD even with the high-dose injection of MAPKK*. The amounts of injected MAPKK** mRNA were as indicated. mRNAs were injected into the animal pole region of both blastomeres at the 2-cell stage. (B) No effect of the chemical inhibitor for MEK (MAPKK), U0126, on modifications of Otx2. Myc-Otx2 Δ AD expressing embryos were injected with U0126 into the blastocoel at the early blastula (stage 6), and subjected at the gastrula (stage 10.5) to western blotting with anti-Myc, anti-p44/42 MAP kinase and anti-phospho-p44/42 MAP kinase antibodies as indicated. Blastocoel injection of U0126, which reduced the activation form of MAPK, phospho-p44/42 MAP kinase (Fig. S4B, middle and bottom panels), did not affect the modified bands of Myc-Otx2 Δ AD (upper panel). Blue arrowheads, nascent proteins; magenta arrowheads, modified proteins.

Fig. S5 (Satou et al.)

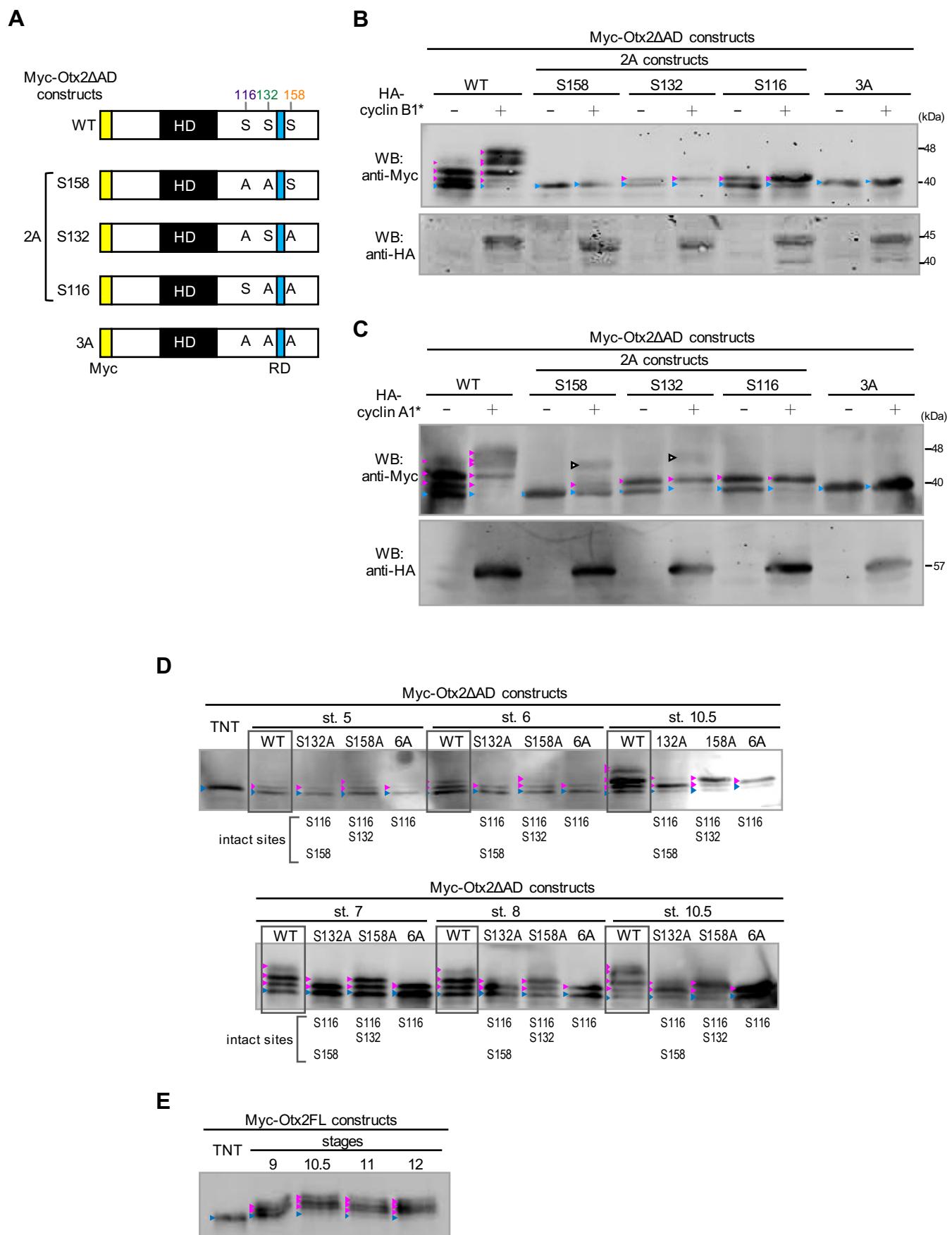
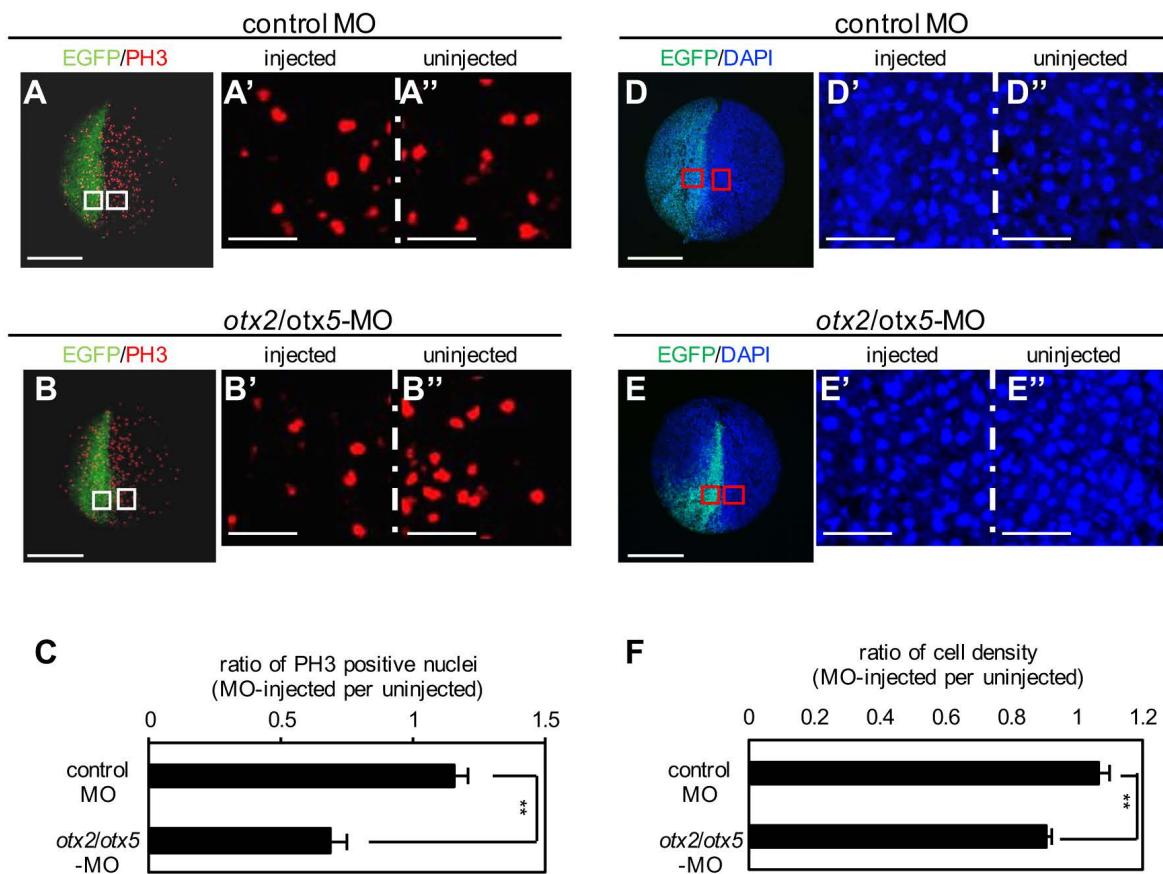


Fig. S5. Preference of cyclin B/Cdk and cyclin A/Cdk for C-sites of Otx2.

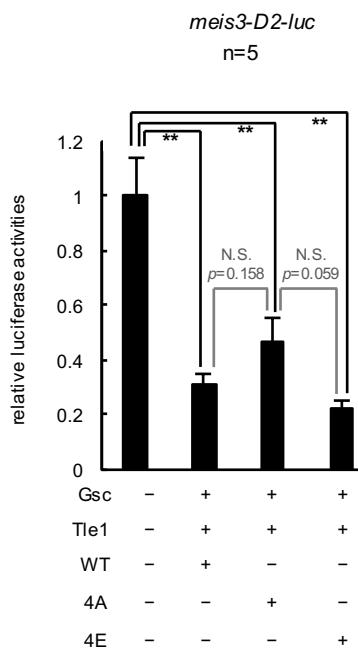
(A) Schematic presentation of Myc-Otx2 Δ AD with double (2A) and triple alanine (3A) mutations at S116, S132 and S158. To test a preference of cyclin B/Cdk and cyclin A/Cdks for Otx2 phosphorylation, double alanine mutants (2A) for S116, S132 and S158 in Δ AD were constructed, in which only one site is responsible for Otx2 phosphorylation. In the 2A constructs, for example, the S158 construct indicates mutations at S116 and S132. Yellow box, Myc-tag; black box, homeodomain (HD); blue box, repression domain (RD). S, serine phosphorylation sites (S116, S132, S158); A, alanine mutation. (B) Western blotting of Myc-Otx2 Δ AD constructs (2A or 3A) co-expressed with (+) or without (-) HA-cyclin B1*. The intensity of modified bands of S132 (S116A/S158A) and S116 (S132A/S158A) constructs, but not that of S158 (S116A/S132A), was increased by HA-cyclin B1* expression. (C) Western blotting of Myc-Otx2 Δ AD constructs (2A or 3A) co-expressed with (+) or without (-) HA-cyclin A1*. All modified bands were enhanced by HA-cyclin A1* expression, with an additional modified band (white arrowheads). These data suggest that S116 and S132 have a preference for both cyclin B/Cdk and cyclin A/Cdks, and that S158 has it for cyclin A/Cdks, if Otx2 is directly phosphorylated by cyclin/Cdks. (D,E) Developmental changes of phosphorylation of Otx2 as assayed by using Myc-Otx2 Δ AD (D) and Myc-Otx2FL (E) constructs. The wild type (WT) and alanine mutants of a single serine at S132 (S132A) and S158 (S158A), and of 6 serines at S122, S123, S132, S153, S158, and S161 (6A) in Myc-Otx2 Δ AD constructs were analyzed from stages 5 to 10.5 as indicated. Note that the 6A construct has S116, which is a phosphorylation site. Mutation at S132 (S132A and 6A constructs) reduced phosphorylation levels compared to S158 mutation, indicating that S132 and S116 are more efficiently phosphorylated than S158 during cleavage stages. Myc-Otx2FL was analyzed at stages 9 to 12 as indicated. TNT, *in vitro* translation products; blue arrowheads, nascent bands; magenta arrowheads, modified bands. Blue arrowheads, nascent proteins; magenta arrowheads, modified proteins; white arrowheads, additional modified bands at sites other than S116, S132 and S158.

Fig. S6 (Satou et al.)

**Fig. S6. Reduction of cell proliferation by *otx2/otx5* knockdown in *X. tropicalis* embryos.**

Knockdown experiments were performed using *X. tropicalis*. Previous study using MO-injection in *X. tropicalis* embryos has shown that Otx2 and its parologue Otx5, which are expressed in anterior neuroectoderm, have functional redundancy in head development (Yasuoka et al., 2014). *X. tropicalis* is a diploid species closely related to the tetraploid species *X. laevis* and suitable for MO knockdown experiments, and all three C-sites of Otx2 are conserved in Otx5 (Fig. S2). MOs together with FITC dextran as a tracer were injected into one blastomere at the 4-cell stage of *X. tropicalis* embryos; followed by PH3 immunostaining and DAPI staining at early neural stage. PH3 immunostaining, DAPI nuclear staining were the same as in Fig. 5. The numbers of stained nuclei were counted and compared between injected and uninjected areas. (A,B) Effects of *otx2/otx5*-MO injection on mitotically active cells. White boxes, enlarged area (A'-B''). (C) Quantitative analysis for the ratio of PH3-positive nuclei. PH3-positive nuclei in an injected or uninjected area (0.072 mm^2) in each embryo were counted (more than 60 nuclei in each area) with 9 embryos, and the ratio of the numbers of injected versus uninjected area was calculated for each embryo to obtain mean \pm s.e.m. from 9 embryos. The ratio of PH3-positive nuclei in control MO-injected embryos was 1.15 ± 0.055 (mean \pm s.e.m.), whereas that in *otx2/otx5*-MOs-injected embryos was 0.688 ± 0.063 , which significantly differed from the control ($p = 4.95 \times 10^{-5}$). (D,E) Effects of *otx2/otx5*-MO on cell density. Red boxes, enlarged area (D'-E''). (F) Quantitative analysis for the ratio of cell densities. More than 500 total nuclei in each area (0.072 mm^2) were counted in one embryo, and 9 embryos were analysed. The ratio of cell density in control MO-injected embryos was 1.063 ± 0.034 (mean \pm s.e.m.), whereas that in *otx2/otx5*-MOs-injected embryos was 0.906 ± 0.013 , which differed from the control ($p = 0.0015$). **P<0.01 (t-test); error bars, s.e.m. (C,F). Scale bar, 500 μm (A,B,D,E); 50 μm (A'-B'',D'-E''). The amount of injected MO (pmol/embryo):control MO, 1; *otx2/otx5*, 0.5 each.

Fig. S7 (Satou et al.)

**Fig. S7. Repression activity of Otx2-4A and -4E together with Gsc and Tle1.**

Experimental procedures were the same as Fig. 7L. Repression activity of Otx2-4A requires Gsc for *meis3-D2-luc* reporter. *meis3-D2-luc* reporter DNA was co-injected with mRNAs for Gsc (12.5 pg/embryo), Tle1 (12.5 pg/embryo), Otx2-WT, or -4A (25 pg/embryo), or 4E (25 pg/embryo) as indicated. **P<0.01 (t-test); error bars, s.e.m.; N.S., not significant; n, the number of samples.

Table S1. The list of plasmid constructs made in this study

Plasmid names	Cloning sites	Vectors	Comments
pCSf107_MycOtx2FL_T	BamHI, XbaI	pCSf107_MTmT	aa 1-288 (Full length: FL) Otx2. S (<i>X. laevis</i>) NP_001084160
pCSf107_MycOtx2HD_T	BamHI, XbaI	pCSf107_MTmT	aa 1-96
pCSf107_MycOtx2ΔAD_T	BamHI, XbaI	pCSf107_MTmT	aa 1-184
pCSf107_MycOtx2AD_T	BamHI, XbaI	pCSf107_MTmT	aa 185-288
pCSf107_MycOtx2ΔRD_T	BamHI, XbaI	pCSf107_MTmT	aa 1-96/185-288
pCSf107_MycOtx2ΔAD-S116A_T	BamHI, XbaI	pCSf107_MTmT	
pCSf107_MycOtx2ΔAD-S122A_T	BamHI, XbaI	pCSf107_MTmT	
pCSf107_MycOtx2ΔAD-S123A_T	BamHI, XbaI	pCSf107_MTmT	
pCSf107_MycOtx2ΔAD-S132A_T	BamHI, XbaI	pCSf107_MTmT	
pCSf107_MycOtx2ΔAD-S153A_T	BamHI, XbaI	pCSf107_MTmT	
pCSf107_MycOtx2ΔAD-S158A_T	BamHI, XbaI	pCSf107_MTmT	
pCSf107_MycOtx2ΔAD-S161A_T	BamHI, XbaI	pCSf107_MTmT	
pCSf107_MycOtx2ΔAD-3A_T	BamHI, XbaI	pCSf107_MTmT	S116A, S132A, S158A
pCSf107_MycOtx2ΔAD-4A_T	BamHI, XbaI	pCSf107_MTmT	T115A, S116A, S132A, S158A
pCSf107_MycOtx2ΔAD-2A(S158)_T	BamHI, XbaI	pCSf107_MTmT	S116A, S132A
pCSf107_MycOtx2ΔAD-2A(S132)_T	BamHI, XbaI	pCSf107_MTmT	S116A, S158A
pCSf107_MycOtx2ΔAD-2A(S116)_T	BamHI, XbaI	pCSf107_MTmT	S132A, S158A
pCSf107_Otx2-WT_T	BamHI, XbaI	pCSf107_mT	Wild type: WT
pCSf107_Otx2-4E_T	BamHI, XbaI	pCSf107_mT	T115E, S116D, S132E, S158E
pCSf107_Otx2-T115A_T	BamHI, XbaI	pCSf107_mT	
pCSf107_Otx2-3A_T	BamHI, XbaI	pCSf107_mT	S116A, S132A, S158A
pCSf107_Otx2-4A_T	BamHI, XbaI	pCSf107_mT	T115A, S116A, S132A, S158A
pCSf107_Otx2-A2A(S116)_T	BamHI, XbaI	pCSf107_mT	T115A, S132A, S158A
pCSf107_Otx2-A2A(S132)_T	BamHI, XbaI	pCSf107_mT	T115A, S116A, S158A
pCSf107_Otx2-A2A(S158)_T	BamHI, XbaI	pCSf107_mT	T115A, S116A, S132A
pCSf107_Otx2ΔAD-4A_T	BamHI, XbaI	pCSf107_mT	
pCSf107_Otx2ΔAD-4E_T	BamHI, XbaI	pCSf107_mT	
pCSf107_HA-cyclin A1*_T	BamHI, XhoI	pCSf107_4HAMT	replaced Δcyclin A1 (from Dr. N. Furuno)
pCSf107_HA-cyclin B1*_T	BamHI, EcoRI	pCSf107_4HAMT	replaced GST-ΔN106cyclin B1 (Iwabuchi et al., 2002)
pCSf107_p27xic1_T	BamHI, XhoI	pCSf107_mT	
pCSf107_HATLE1_T	AgeI, XbaI	pCSf107_4HAMT	Replaced pCSf107-TLE1 (Yasuoka et al., 2014)
pCSf107_pax6_T	SfuI, NotI	pCSf107_mT	replaced pCS105-pax6

Note: the postfix “_T” indicates the presence of SP6/T7 terminators at the end of the transcribed region.

Table S2. The list of cutting sites and RNA polymerases for the in vitro transcription of anti-sense RNA probe

Plasmid names	Cutting sites	RNA polymerase	Comments
pCSf107_p27xic1_T	<i>BamH</i> I	T7	
pCSf107_Otx2-WT_T	<i>BamH</i> I	T7	
pBS SK(-)gbx2	<i>EcoR</i> I	T3	
pBSK_xcg1	<i>Not</i> I	T3	
PCS107BSX_rax	<i>Xho</i> I	SP6	
pCSf107_pax6_T	<i>Sal</i> I	T7	
BS4A 3(X pax2-2a)	<i>EcoR</i> I	T3	(Takada et al., 2005)

References

- Takada, H., Hattori, D., Kitayama, A., Ueno, N. and Taira, M.** (2005). Identification of target genes for the Xenopus Hes-related protein XHR1, a prepattern factor specifying the midbrain-hindbrain boundary. *Dev. Biol.* **283**, 253–267.