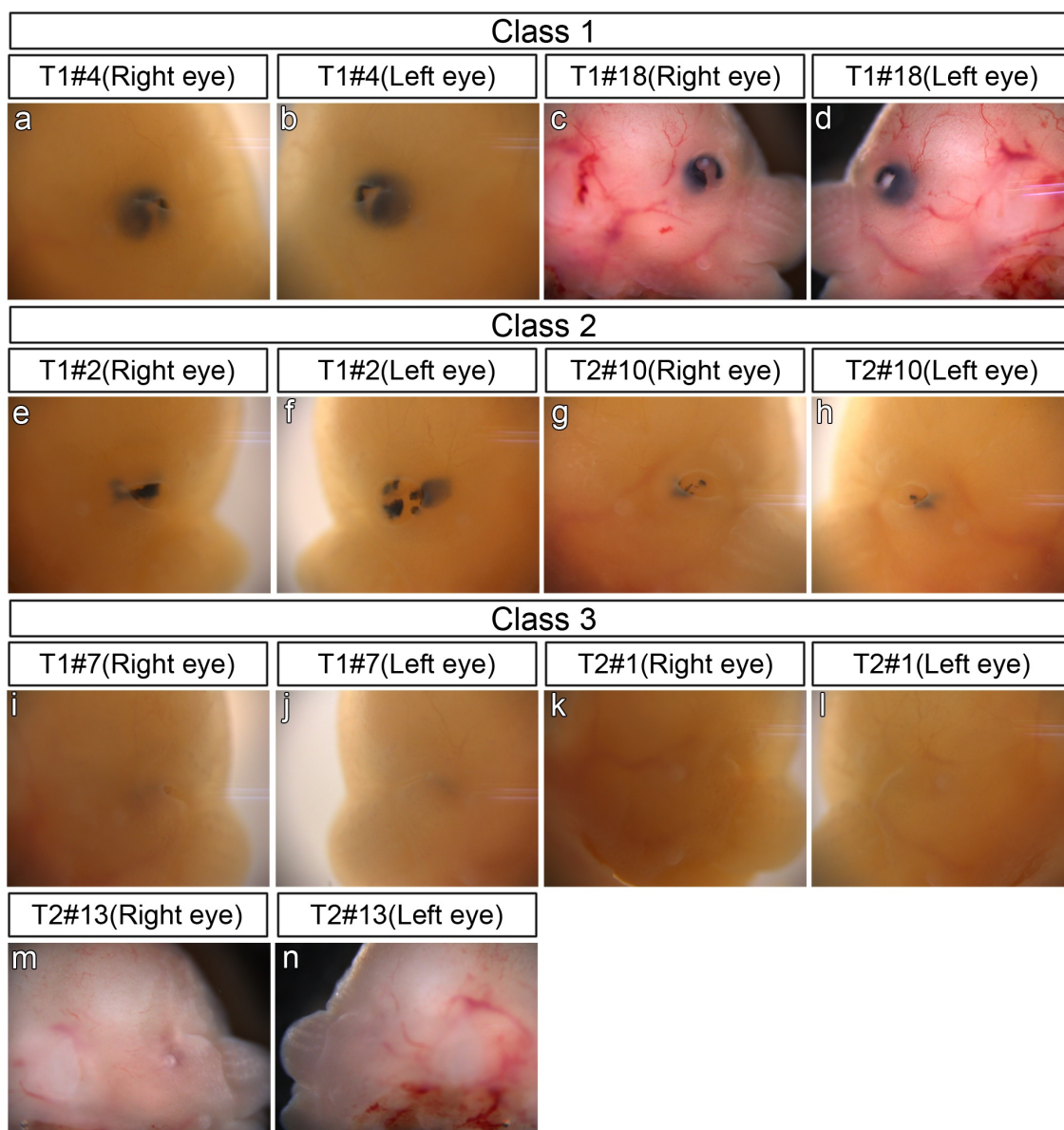


Supplementary Information 1**Relationship between somatic mosaicism of *Pax6* mutation and variable developmental eye abnormalities—an analysis of CRISPR genome-edited mouse embryos**

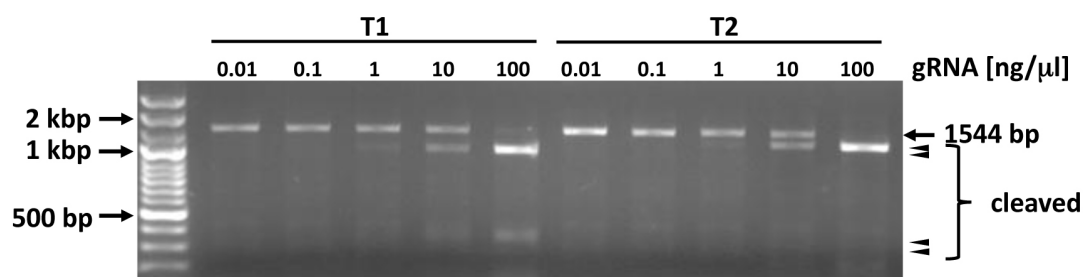
Akihiro Yasue^{1,*}, Hitomi Kono², Munenori Habuta², Tetsuya Bando², Keita Sato², Junji Inoue², Seiichi Oyadomari³, Sumihare Noji⁴, Eiji Tanaka¹ and Hideyo Ohuchi^{2,*}

¹Department of Orthodontics Dentofacial Orthopedics, Institute of Biomedical Sciences, Tokushima University Graduate School, 3-18-15 Kuramoto-cho, Tokushima, 770-8504, Japan. ²Department of Cytology and Histology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Kita-ku, Okayama, 700-8558, Japan. ³Division of Molecular Biology, Institute for Advanced Enzyme Research, Tokushima University, 3-18-15 Kuramoto-cho, Tokushima, 770-8503, Japan. ⁴Tokushima University, 2-24 Shinkura-cho, Tokushima, 770-8501, Japan. *Correspondence and requests for materials should be addressed to A.Y. (yasuea@tokushima-u.ac.jp) or H.O. (hohuchi@okayama-u.ac.jp)

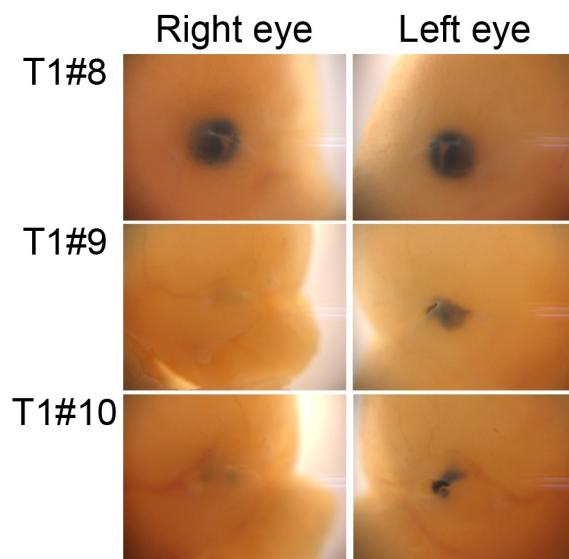
Supplementary Figure S1

Supplementary Figure S1. Lateral views of the eye in the *Pax6*-CRISPR founder mice at E16.5.

Class 1 (a-d), class 2 (e-h), and class 3 (i-n).

Supplementary Figure S2**Supplementary Figure S2. Analysis of cleavage products by T1 or T2 sgRNA.**

T1 and T2 sgRNAs were *in vitro* transcribed, respectively, and tested against its target sites in the mouse *Pax6* genomic region. A 1544 base pair-fragment of the *Pax6* genome was PCR-amplified using primers (5'-TTGTGGTCTTTTCTTCCTTCCT-3' and 5'-GGGCAAGGTAGACACACGAT-3'). The 1.5 kb fragment, T1 or T2 sgRNA, and recombinant Cas9 enzyme, using Guide-it sgRNA Screening Kit (Takara Bio Inc., Shiga, Japan), were combined in an *in vitro* cleavage reaction according to the manufacturer's protocol. Agarose gel analysis indicated that 100 ng of T1 or T2 sgRNA was each sufficient for 100% cleavage of the target fragment.

Supplementary Figure S3**Supplementary Figure S3. Lateral views of the eyes.**

In T1#8 embryo, both eyes exhibit class 1 phenotype. In T1#9 and T1#10 embryos, the right eyes exhibit a class 3 phenotype, while the left eyes exhibit a class 2 phenotype.

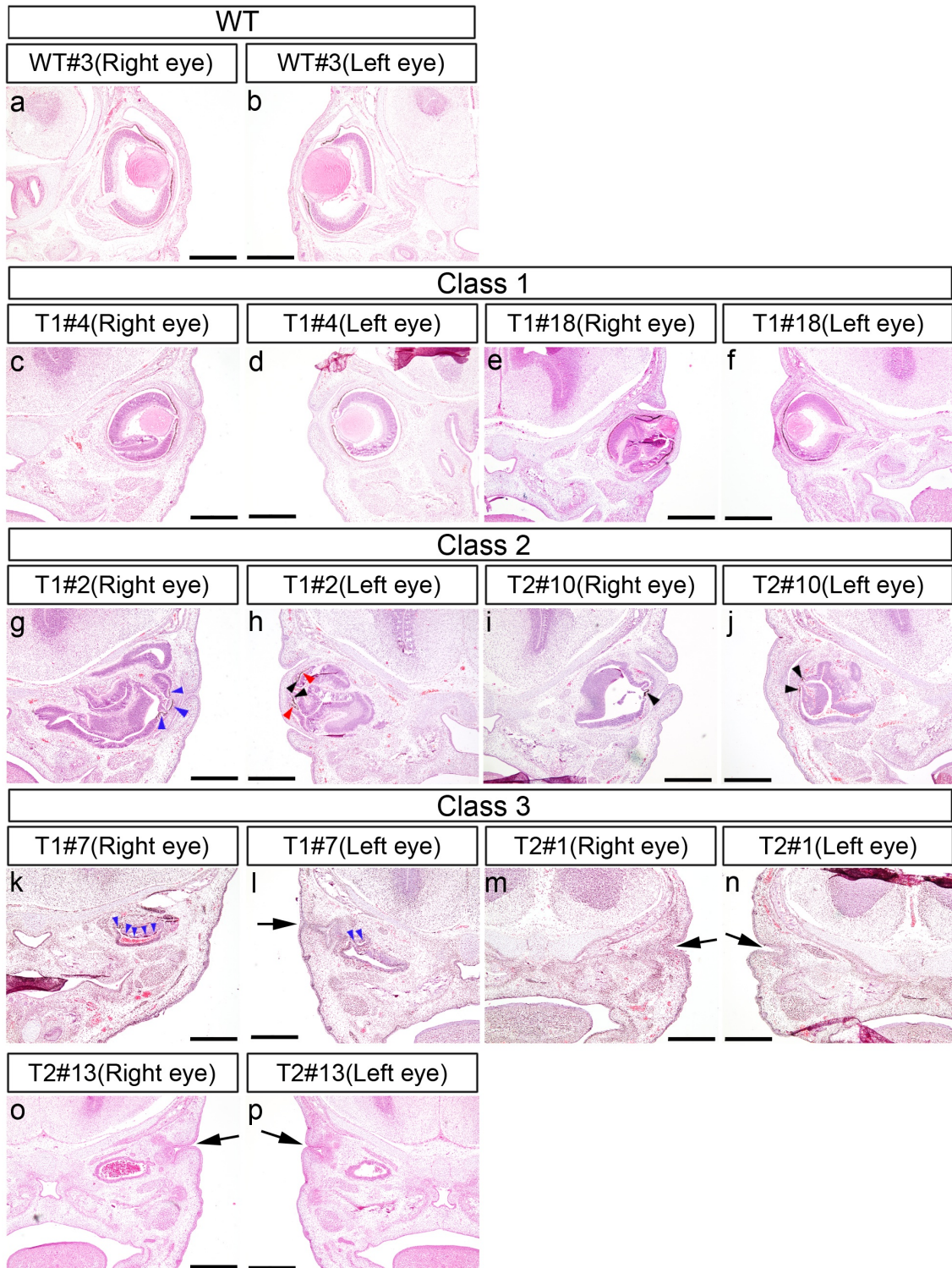
Supplementary Table S1. Test for association between genome-editing of the *Pax6* gene and occurrence of eye defects

Severity	Category	Observed	Expected	E11.5		E16.5		Statistical significance	
				T1	T2	T1	T2		
R=L	1	27	14.7	7	3	13	4	Chi-square	16.409
R>L	2	11	14.7	3	6	0	2	Degree of freedom	2
R<L	3	6	14.7	2	3	1	0	<i>P</i>	<i>P</i> <0.0001
Total									
	(n)	44		12	12	14	6		

Supplementary Table S2. Test for association between the parameters (right or left side) involved in occurrence of eye defects

Severity	Category	Observed	Expected	E11.5		E16.5		Statistical significance	
				T1	T2	T1	T2		
R>L	2	11	8.5	3	6	0	2	Chi-square	1.471
R<L	3	6	8.5	2	3	1	0	Degree of freedom	1
Total									
	(n)	17		9	6	14	6	<i>P</i>	<i>P</i> =0.225

Another set of experiment (T1#XH and T2#XH series) was performed to examine whether there is a trend towards more severe eye defects in right vs left eyes examined at E11.5 or E16.5 (n=44). R>L, right eyes exhibit more severe defects than left eyes; R<L, left eyes exhibit more severe defects than right eyes; R=L, similar eye defects on both sides, by macroscopic observation. Table S1 shows a highly significant deviation among frequencies of R>L, R=L, and R<L. Table S2 shows no significant deviation between frequencies of R>L and R<L in occurrence of eye defects. These results indicate that R=L defects occur at a significantly higher frequency than defects of R>L or R<L.

Supplementary Figure S4

Supplementary Figure S4. Histology of the head region from the wild-type (a, b) and *Pax6*-CRISPR embryos (c-p) shown in Fig. 3.

HE-stained coronal sections. In (l-p), from the surface ectoderm, there was an invagination (arrow) that did not make contact with the pseudo-optic cup and no lens develops. Scale bars: 500 μ m.

Supplementary Figure S5

		encoding truncated Pax6 proteins		
a	WT	ProCysAspIleSerArgIleLeuGln CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc		
	T1#2	CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc	(Total: 66) WT (x14) -1+31 (x6) +1 (C) (x4) -8+7 (x2) -8 (x11) -12 (x8) -13 (x8) -24+1 (x13)	
	T1#4	CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc	(Total: 52) WT (x18) -1 (T) (x34)	
	T1#5H	CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc	(Total: 79) WT (x5) -4+4 (x4) +1 (T) (x13) -5+1 (x52) -22 (x5)	
	T1#6	CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc	(Total: 52) WT (x40) -3 (x11) -22 (x1)	
	T1#7	CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc	(Total: 54) -6 (x23) -19 (x31)	
	T1#11	CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc	(Total: 38) +9 (x16) -1 (T) (x22)	
	T1#12	CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc	(Total: 47) WT (x21) +1 (T) (x1) -1 (T) (x19) -25 (x6)	
	T1#18	CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc CCGTGCGACATTTCCGAAATCTGCAGgtgatcctccatggtgcccactactgcccgtcc	(Total: 49) WT (x14) +1 (T) (x2) -1 (T) (x2) -9 (x20) -22 (x11)	
	WT	ValSerAsnGlyCysValSerLysIleLeuGlyArgTyrTyrGluThrGlySer aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC		
	T2#1	aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC	(Total: 30) WT (x1) +1 (T) (x29)	
	T2#1H	aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC	(Total: 89) +1 (T) (x10) -1 (A) (x37) -1 (T) (x38) -12 (x4)	
	T2#3	aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC	(Total: 32) +1 (A) (x3) +1 (T) (x3) -2+1 (x14) -1 (T) (x12)	
	T2#5H	aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC	(Total: 46) +1 (T) (x3) -21 (x4) -25 (x39)	
	T2#10	aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC	(Total: 40) WT (x13) -3+7 (x5) +2 (x16) -1+1 (x6)	
	T2#13	aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTTGTGTGAGTAAAATCTGGGCAGGTATTACGAGACTGGCTCC	(Total: 31) -2 (x23) -12 (x3) -22 (x5)	
				Phenotype Class 2 Class 1 Class 2 Class 1 Class 3 Class 3 Class 2 Class 1 Class 3 Class 3 Class 3 Class 3 Class 2 Class 3

Supplementary Figure S5 (continued)

	AspIleSerArgIleLeuGln	ValSerAsnGlyCysValSerLysIleLeuGlyArgTyr	
WT	GACATTTCCCGAATTCGCAggtgatcctccca.....cagGTATCCAACGGTGTGTGAGTAAATTTCTGGGCAGGTAT		Class 2 (Total: 22)
T1+T2#6	GACATTTCCCGAATTCGCAggtgatcctccca.....cagGTATCCAACGGTGTGTGAGTAAATTTCTGGGCAGGTAT		T1:WT, T2:WT (x2)
	GACATTTCCCGAATTCGCAggtgatcctccca.....cagGTATCCAACGGTGTGTGAGTAAATTTCTGGGCAGGTAT		T1:WT, T2:+1 (T) (x2)
	GACA-----ggtgatcctccca.....cagGTATCCAACGGTGTGTGAGTAAATTTCTGGGCAGGTAT		T1:-7, T2:+1 (T) (x4)
			-1064 (x13)
b			
WT	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKILGRYYETGSIRPRAIGGSKPRVATPEVVSQIAQY KRECPISIFAWEIRDRLLESEVCNTNDNIPSSVINRVLRLNLAASEKQMGADGMYDKLRMLNGQTGSWGTRPGWYPTGSVPGQPTQDGCQQQ EGGGENTNSISSNGEDSDEAQMRLQLKRRKLRNRTSFTQEQIEALEKEFERTHYDVFARERLAAKIDLPEARIQVWFSNRRAKWRREEK LRNQRRQASNTPSHIPISSSFSTSVYQPIQPPTPVSSFTSGSMLGRDALTALNTYSALPPMPSFTMANNLPMQPPVPSQTSYSSCMCLPT SPSVNGRSYDITYTPPHMQTHMNSQPMGTSGETTGLISPGVSVFVQVPGSEPDMSQYWPRLQ*		
T1	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRITV*		
-1+31	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRQVTFLQVSNCGCVSKILGRYYETGSIRPRAIGGSKPRVATPE...RLQ*		
+9	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRISAGIQRICE*		
+1 (C/T)	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKILGRYYETGSIRPRAIGGSKPRVATPE...RLQ*		
-4+4	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKILGRYYETGSIRPRAIGGSKPRVATPE...RLQ*		
-1 (T)	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKILGRYYETGSIRPRAIGGSKPRVATPE...RLQ*		
-8+7	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKILGRYYETGSIRPRAIGGSKPRVATPE...RLQ*		
-3	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKILGRYYETGSIRPRAIGGSKPRVATPE...RLQ*		
-5+1	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKILGRYYETGSIRPRAIGGSKPRVATPE...RLQ*		
-6	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKILGRYYETGSIRPRAIGGSKPRVATPE...RLQ*		
-8	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKILGRYYETGSIRPRAIGGSKPRVATPE...RLQ*		
-9	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKILGRYYETGSIRPRAIGGSKPRVATPE...RLQ*		
-12	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKILGRYYETGSIRPRAIGGSKPRVATPE...RLQ*		
-13	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKILGRYYETGSIRPRAIGGSKPRVATPE...RLQ*		
-19	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKILGRYYETGSIRPRAIGGSKPRVATPE...RLQ*		
-22	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKILGRYYETGSIRPRAIGGSKPRVATPE...RLQ*		
-24+1	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKILGRYYETGSIRPRAIGGSKPRVATPE...RLQ*		
-25	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARP*		
T2	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKITVGVLRDHLHQTQGNRRE*		
-3+7	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKIWAGITRLAPSDPGQSEGVSQEWRLQKL*		
+2	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKISGQVLRDHLHQTQGNRRE*		
+1 (A/T)	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKITLGRYYETGSIRPRAIGGSKPRVATPE...RLQ*		
-1+1	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKITLGRYYETGSIRPRAIGGSKPRVATPE...RLQ*		
-1 (A)	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKIWAGITRLAPSDPGQSEGVSQEWRLQKL*		
-1 (T)	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKIWAGITRLAPSDPGQSEGVSQEWRLQKL*		
-2+1	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKIWAGITRLAPSDPGQSEGVSQEWRLQKL*		
-2	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKIWAGITRLAPSDPGQSEGVSQEWRLQKL*		
-12	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKIWAGITRLAPSDPGQSEGVSQEWRLQKL*		
-21	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKIWAGITRLAPSDPGQSEGVSQEWRLQKL*		
-22	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKIWAGITRLAPSDPGQSEGVSQEWRLQKL*		
-25	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKIWAGITRLAPSDPGQSEGVSQEWRLQKL*		
T1+T2	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKISGQVLRDHLHQTQGNRRE*		
T1:WT, T2:+1 (T)	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKISGQVLRDHLHQTQGNRRE*		
T1:-7, T2:+1 (T)	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKISGQVLRDHLHQTQGNRRE*		
-1064	MQNSHSGVNQLGGVFVNGRPLDPDSTRQKIVELAHSGARPDISRILQVSNCGCVSKISGQVLRDHLHQTQGNRRE*		

Sequencing primers for T1+T2 were 5'-TTGTGGTCTTTTCTTCCTTCCT-3' and 5'-GGGCAAGGTAGACACACGAT-3'. T1#5H, T2#1H, and T2#5H embryos were from another set of experiments, which is different from those shown in Tables 1 and 2.

Supplementary Table S3. Summary of genotypes for the HE-stained embryos (E16.5) shown in Fig. 3 and Supplementary Fig. S4

Phenotype Class (Microscopic)	1	1	2	2	3	3	3
Embryo No.	T1#4	T1#18	T1#2	T2#10	T1#7	T2#1	T2#13
Pax6 Mutation: Truncation	34 (65.4%)	15(30.6%)	44 (66.7%)	21(52.5%)	31(57.4%)	29(96.7%)	28(90.3%)
Pax6 Mutation: In-frame (In/Del/Sub)	0	20(40.8%;3aaDel)	8(12.1%;4aaDel)	6(15%;1aaSub)	23(42.6%;2aaDel)	0	3(9.7%;4aaDel)
Wild-type (%)	18 (34.6%)	14(28.6%)	14(21.2%)	13(32.5%)	0	1(3.3%)	0
Sequencing Clone No.	52	49	66	40	54	30	31
Mosaicism	2	5	8	4	2	2	3

1aaSub:1 amino acid substitution

Supplementary Table S4. Summary of genotypes for the immunostained embryos (E16.5) shown in Fig. 4

Phenotype Class (Microscopic)	1	2	3	3
Embryo No.	T1#6	T1#12	T1#11	T2#3
Pax6 Mutation: Truncation	1(1.9%)	26 (54.3%)	22 (57.9%)	32(100%)
Pax6 Mutation: In-frame (In/Del)	11(21.2%; 1aaDel)	0	16 (42.1%; 3aaIn)	0
Wild-type (%)	40 (76.9%)	21 (45.7%)	0	0
Sequencing Clone No.	52	47	38	32
Mosaicism	3	4	2	4

1aaDel: 3 amino acid deletion

3aaIn: 3 amino acid insertion

Supplementary Table S5. Data for Fig. 4v

Embryo No.	Class	X axis	Y axis
		% Pax6 truncated mutations	% Pax6 (+) area
WT#4		0	59.5
WT#5		0	53.2
T1#6	1	1.9	86.4
T1+T2 #6*	2	27.3	45.3
T1#12	2	54.3	75.1
T1#11**	3	57.9	77.6
T1#5H	2	88.6	44.6
T2#5H	3	91.3	36
T2#1H	3	95.5	46.3
T2#3	3	100	16.1

*also had an in-frame mutation of 17 amino acid deletion in the PD (59.0%)

**also had an in-frame mutation of 3 amino acid insertion in the PD (42.1%)

Supplementary Table S6. Data for Fig. 4w

Embryo No.	Class	X axis	Y axis
		Pax6 (+) area (μm^2)	Tuj1 (+) area (μm^2)
T2#3	3	6143	1578
T2#1H	3	10629	345
T2#5H	3	23409	10446
T1#11*	3	61027	24776
WT#5		115027	144856
T1#5H	2	116751	90795
T1+T2 #6**	2	142972	134391
WT#4		156576	193865
T1#6	1	219048	57744
T1#12	2	547239	231312

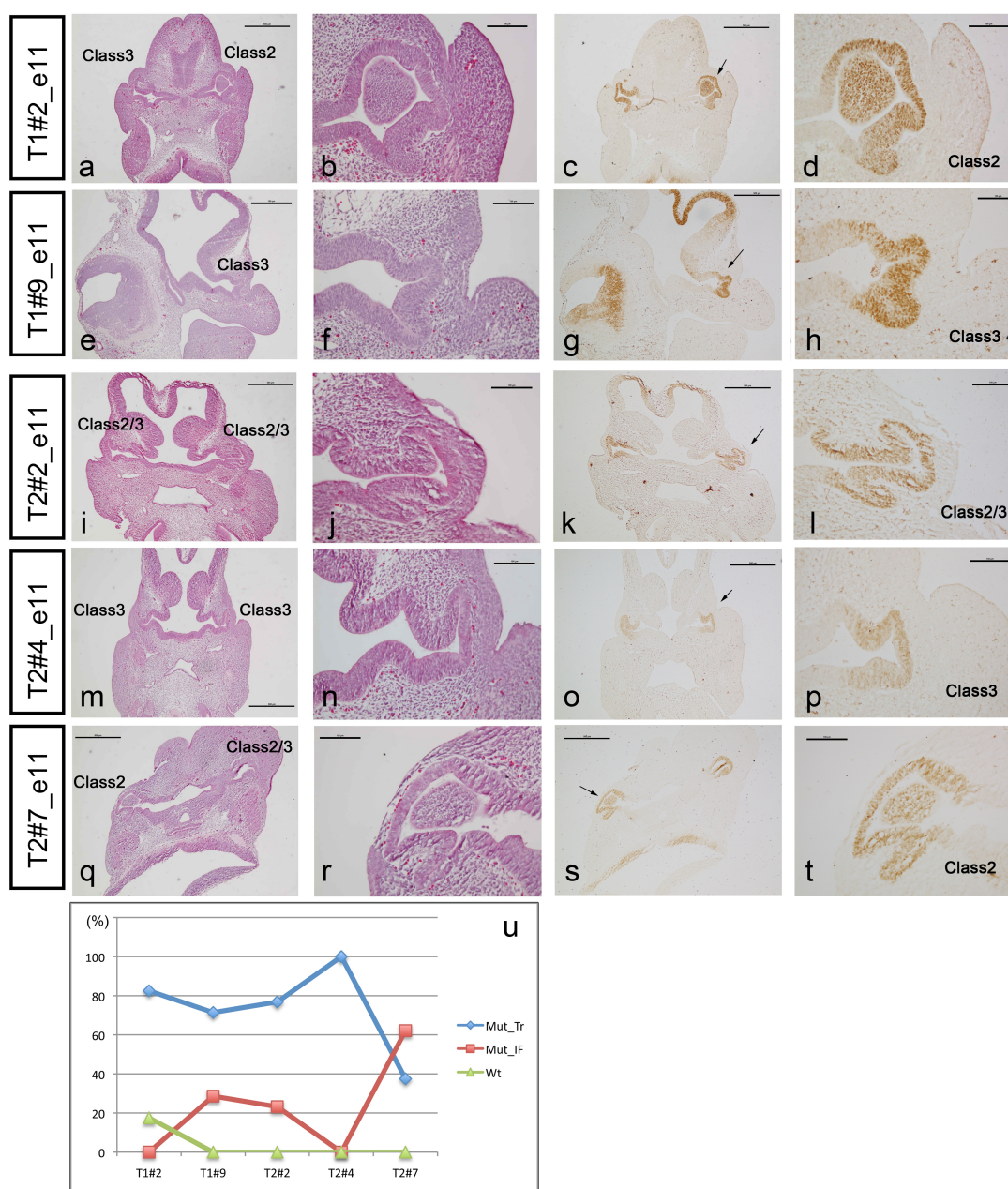
*also had an in-frame mutation of 3 amino acid insertion in the PD domain (42.1%)

**also had an in-frame mutation of 17 amino acid deletion in the PD domain (59.0%)

Supplementary Figure S6

		encoding truncated Pax6 proteins		
a	WT	ProCysAspIleSerArgIleLeuGln CCGTGCGACATTTCCCGAATTCTGCAGgtgatcctcc		
	T1#2_e11	CCGTGCGACATTTCCCGAATTCTGCAGgtgatcctccatggtgccctactgcccgtcc CCGTGCGACATTTCCCGAATTCTGCAGgtgatcctccatggtgccctactgcccgtcc CCGTGCGACATTTCCCGAATTCTGCAGgtgatcctccatggtgccctactgcccgtcc CCGTGCGACATTTCCCGAATG-----Ggtgatcctccatggtgccctactgcccgtcc CCGTGCGACATTTCC-----TGCAGgtgatcctccatggtgccctactgcccgtcc	(Total: 57) WT (x10) +2 (x12) +1 (T) (x4) -6+1 (x12) -8 (x19)	
	T1#9_e11	CCGTGCGACATTTCCC--AT-----gtgatcctccatggtgccctactgcccgtcc CCGTGCGACATTTCC-----ccatggtgccctactgcccgtcc	(Total: 28) -9 (x8) -22 (x20)	
	WT	ValSerAsnGlyCysValSerLysIleLeuGlyArgTyrTyrGluThrGlySer aatgcaagGTATCCAACGGTGTGTGAGTAAATCTGGGCAGGTATTACGAGACTGGCTCC		
	T2#2_e11	aatgcaagGTATCCAACGGTGTGTGAGTAAATCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTGTGTGAGTAA--CCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGT-----AATACTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTGTGTG-----GGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAAC-----GGTATTACGAGACTGGCTCC	(Total: 56) +1 (T) (x5) -4+1 (x13) -12+1 (x15) -14 (x8) -25 (x15)	
	T2#4_e11	aatgcaagGTATCCAACGGTGTGTGAGTAAATCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTGTGTGAGTAAAT-CTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTGTGTGAGTAAA-TTCTGGGCAGGTATTACGAGACTGGCTCC	(Total: 47) +1 (T) (x19) -1 (T) (x10) -1 (A) (x18)	
	T2#7_e11	aatgcaagGTATCCAACGGTGTGTGAGTAAATCTGGGCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTGTGTGAG-----GCAGGTATTACGAGACTGGCTCC aatgcaagGTATCCAACGGTGTGTG-----GGCAGGTATTACGAGACTGGCTCC	(Total: 24) +1 (G) (x6) -11 (x3) -12 (x15)	
	b	WT	MQNSHSGVNQLGGVFVNGRPLDPSTRQKIVELAHSGARPCDISRILQVSNCGVSKILGRYYETGSIRPRAIGGSKPRVATPEVVSQIAQY KRECPISIFAWEIRDRLLESEGVCTNDNIPVSSINRVLRLNLAESKQMGADGMYDKLRMLNGQTSWGTRPGWYPGTSVPGQPTQDGCQQQ EGGGENTNSISSNGEDSDEAQMRLQLKRLQRNRTSFTQEQIEALEKEFERTHYPDVFAFERLAAKIDLPEARIQWFNSNRRAKWRREEK LRNQRRQASNTPSHIPISSSFSTSVYQPIPOPTTPVSSFTSGSMLGRDPTALNTNYSALPMPSPFTMANNLPMQPPVPSQTSYSCLMPT SPSVNGRSYDITYTPPHMQTHMNSQPMGTSSTGLISPGVSVFVQVPGSEPDMSQYWRPRLQ*	
		T1	+2 MQNSHSGVNQLGGVFVNGRPLDPSTRQKIVELAHSGARPCDISRIFCR* +1 (T) MQNSHSGVNQLGGVFVNGRPLDPSTRQKIVELAHSGARPCDISRISAGIQRLCE* -6+1 MQNSHSGVNQLGGVFVNGRPLDPSTRQKIVELAHSGARPCDISRMGIQRLCE* -8 MQNSHSGVNQLGGVFVNGRPLDPSTRQKIVELAHSGARPCDISAGIQRLCE* -9 MQNSHSGVNQLGGVFVNGRPLDPSTRQKIVELAHSGARPCDISH---VSNCGVSKILGRYYETGSIRPRAIGGSKPRVATPE.....RLQ* -22 MQNSHSGVNQLGGVFVNGRPLDPSTRQKIVELAHSGARPCDISHGCPTRRSLEPTTPPPRPAALHSHKRLFLISYCKRY*	
		T2	+1 (G) MQNSHSGVNQLGGVFVNGRPLDPSTRQKIVELAHSGARPCDISRILQVSNCGVSKMSGQVLRDHLHQTQGNRRE* +1 (T) MQNSHSGVNQLGGVFVNGRPLDPSTRQKIVELAHSGARPCDISRILQVSNCGVSKISGQVLRDHLHQTQGNRRE* -1 (A) MQNSHSGVNQLGGVFVNGRPLDPSTRQKIVELAHSGARPCDISRILQVSNCGVSKIFWAGITRLAPSDPGQSEGVSQEWRLQKL* -1 (T) MQNSHSGVNQLGGVFVNGRPLDPSTRQKIVELAHSGARPCDISRILQVSNCGVSKIWAGITRLAPSDPGQSEGVSQEWRLQKL* -4+1 MQNSHSGVNQLGGVFVNGRPLDPSTRQKIVELAHSGARPCDISRILQVSNCGVSKI-GRYYETGSIRPRAIGGSKPRVATPE.....RLQ* -11 MQNSHSGVNQLGGVFVNGRPLDPSTRQKIVELAHSGARPCDISRILQVSNCGVSRQVLRDHLHQTQGNRRE* -12+1 MQNSHSGVNQLGGVFVNGRPLDPSTRQKIVELAHSGARPCDISRILQVSNCGTGGVLRDHLHQTQGNRRE* -12 MQNSHSGVNQLGGVFVNGRPLDPSTRQKIVELAHSGARPCDISRILQVSNCGV---GRYYETGSIRPRAIGGSKPRVATPE.....RLQ* -14 MQNSHSGVNQLGGVFVNGRPLDPSTRQKIVELAHSGARPCDISRILQVSNCGQVLRDHLHQTQGNRRE* -25 MQNSHSGVNQLGGVFVNGRPLDPSTRQKIVELAHSGARPCDISRILQVSNGITRLAPSDPGQSEGVSQEWRLQKL*	

Supplementary Figure S7



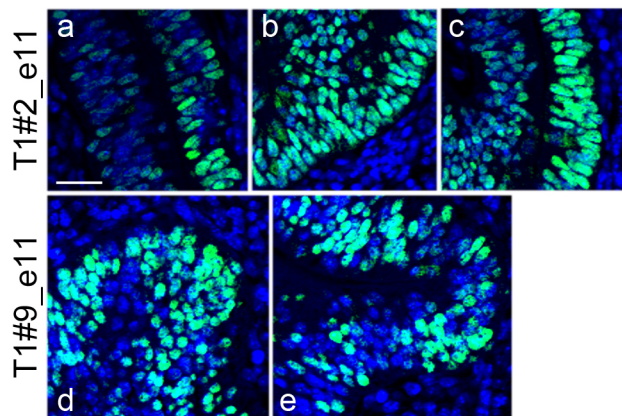
Supplementary Figure S7. HE-stained and immunostained head tissues from founder mice at E11.5.

Transverse sections are shown. Brown staining in (c, d, g, h, k, l, o, p, s, t) indicates localization of Pax6 proteins, wild-type and mutated. (u) Summary of genotypes for each embryo. Details are shown in Supplementary Fig. S6 and Table S5. Scale bars: 500 μ m in (a, c, e, g, i, k, m, o, q, s); 100 μ m in (b, d, f, h, j, l, n, p, r, t).

Supplementary Table S7. Summary of genotypes for the HE-stained embryos (E11.5) shown in Figure S6

Phenotype Class (Microscopic) R/L	Class3/Class2	n.d./Class3	Class2/3	Class3	Class2/Class2/3
Embryo No.	T1#2_e11	T1#9_e11	T2#2_e11	T2#4_e11	T2#7_e11
Pax6 Mutation: Truncation	47(82.5%)	20(71.4%)	43(76.8%)	47(100%)	9(37.5%)
Pax6 Mutation: In-frame (In/Del)	0	8(28.6%; 3aaDel)	13(23.2%; 1aaDel)	0	15(62.5%)
Wild-type (%)	10(17.5%)	0	0	0	0
Sequencing Clone No.	57	28	56	47	24
Mosaicism	5	2	5	3	3

n.d.: The optic primordium could not be determined on the sections.

Supplementary Figure S8**Supplementary Figure S8. Pax6 immunoreactivity in E11.5 *Pax6*-mosaic optic vesicle.**

High magnification images of optic vesicles shown in Fig. 5a (a-c) and Fig. 5c (d, e).

Scale bar: 25 μ m.