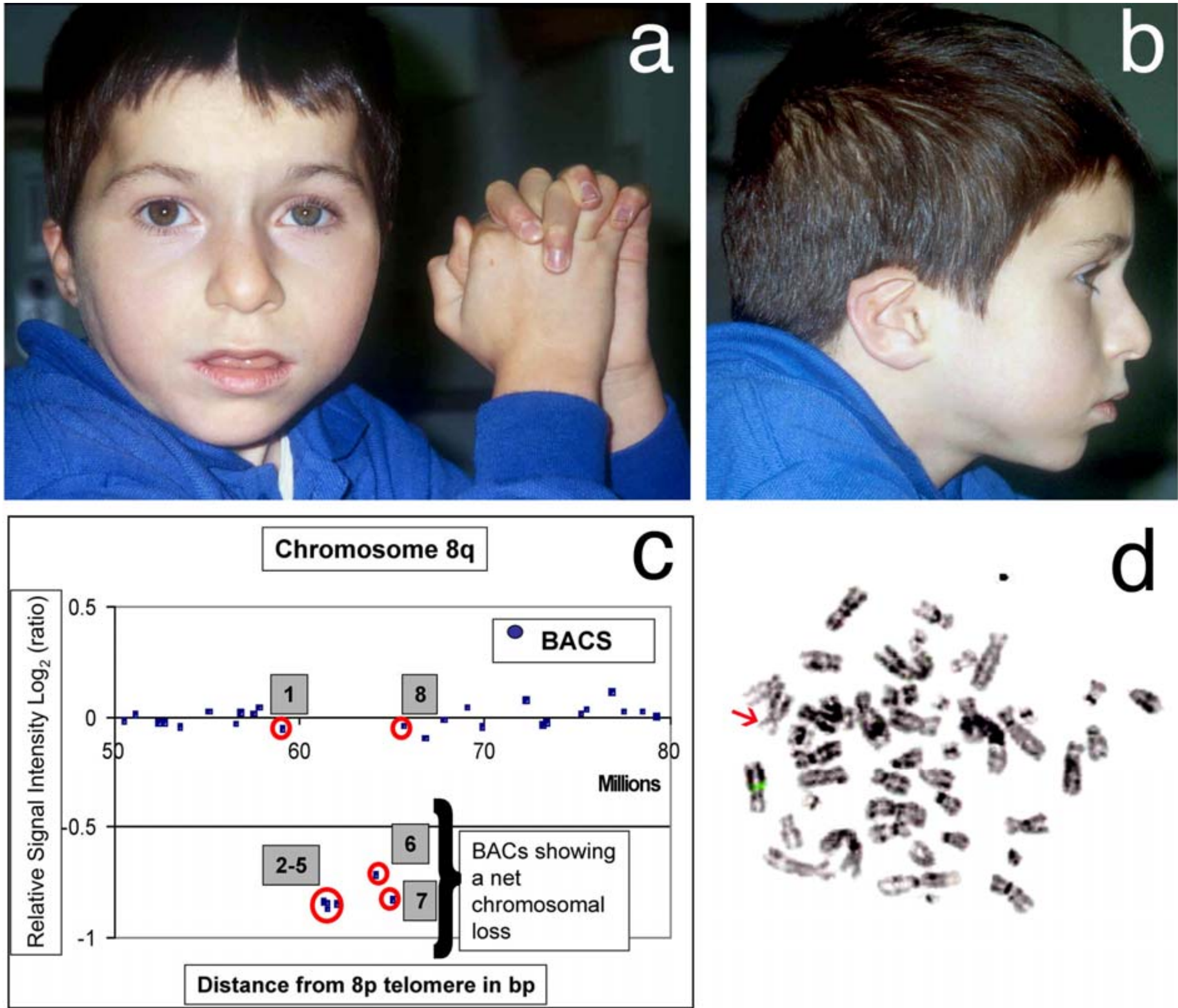
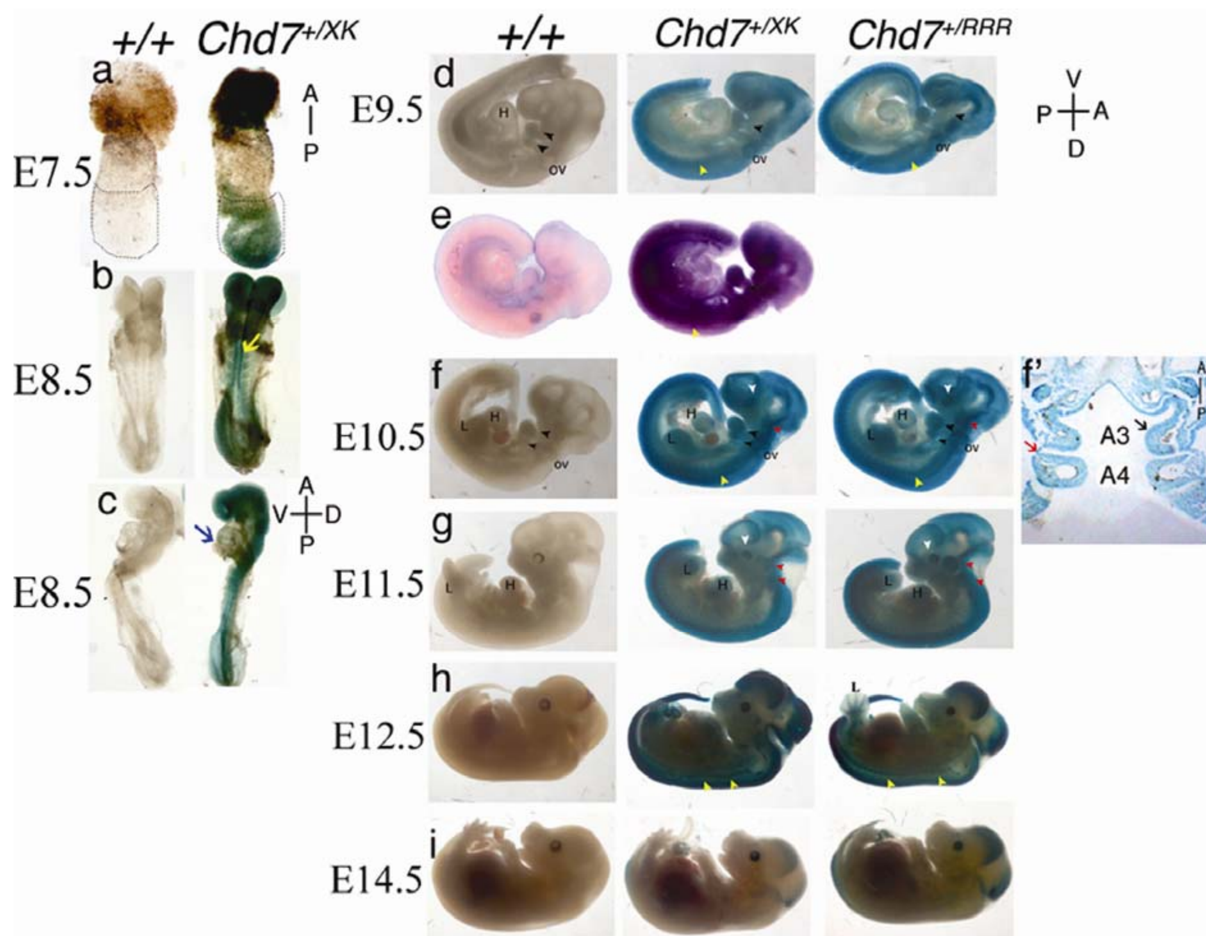
Supplementary Figure 1: **CHD7 Deletion in Patient PS15**

a, b: Frontal and side views of face of PS15. Note the lack of coloboma, but mild heterochromia of the iris. c: comparative genome hybridisation revealed diminished signal strength for BACS at chromosome 8, 62-65Mb. d: FISH using BAC RP11- 414L17 reveals a hemizygous deletion including *CHD7*. The signal is shown in green, the deleted chromosome 8 is indicated by the red arrow.



Supplementary Figure 2: ***Chd7* Expression From E7.5 to E14.5 Visualized Using X-Gal Staining and *In Situ* Hybridization.**

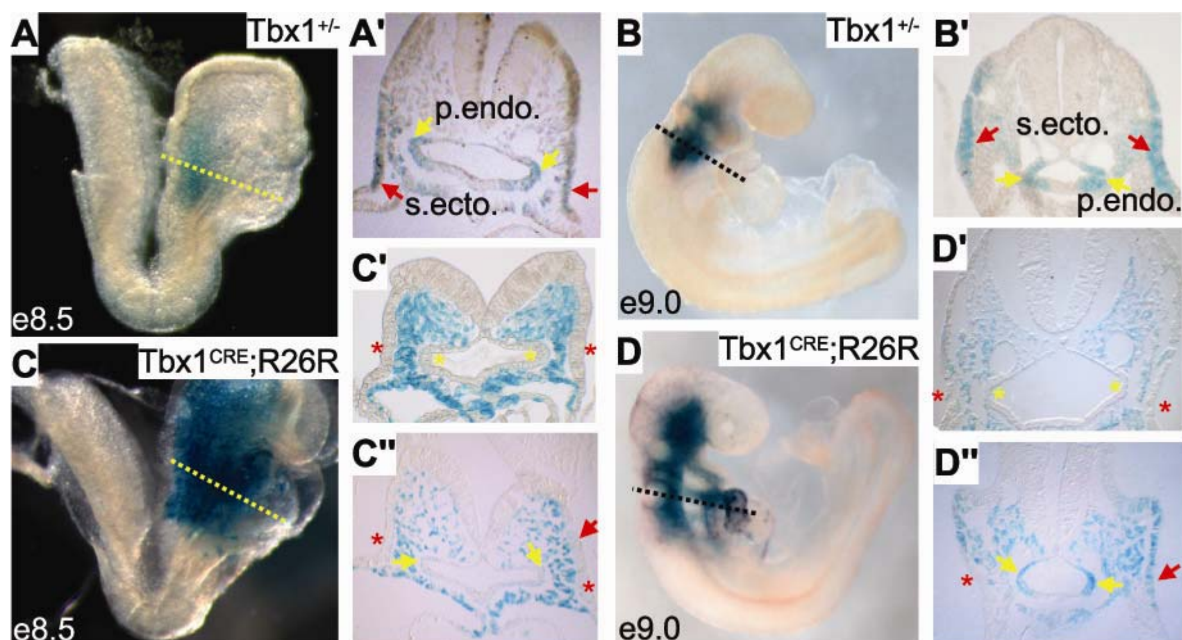


(a) At E7.5 *Chd7* was expressed at the embryonic region. Dotted line delineates the embryonic region at identical magnification to highlight the decreased size in the mutant. (b) E8.5 dorsal view. X-gal staining could clearly be seen in the somites and the neural tube, yellow arrow. (c) E8.5 lateral view. At E8.5 expression was detected throughout the embryo but appears weaker in the heart tube, blue arrow. (d) At E9.5 expression was seen throughout the embryo but was weaker in the heart tube. Expression was slightly higher than surrounding tissues in the pharyngeal arches (black arrowheads) and the somites (yellow arrowheads). (e) *Chd7* expression in wild type embryos analysed by *in situ* hybridisation. The sense probe did not produce non-specific staining (left). Anti-sense probe *Chd7* expression (right) could be seen throughout the embryo but was strongest in the pharyngeal arches (black arrowheads), somites (yellow arrowheads). (f) By E10.5 strong expression was seen in the first and second pharyngeal arches (black arrowheads), the otic vesicle (OV) and the developing fore limb (L). Expression was seen in the OFT and presumptive ventricles of the heart (H) and in the forming trigeminal ganglion (red arrowhead). There was also expression in the developing eye as indicated by white arrowheads. (f') Section through the pharyngeal arches at E10.5 show that the strongest staining was seen in the pharyngeal ectoderm (red arrow) and pharyngeal endoderm (black arrow). The darker staining within the pharyngeal arch arteries is India ink, which was injected to visualise these vessels. (g) At E11.5 expression was evident in the diencephalon and the floor and roof of the fourth ventricle. There was also expression in the trigeminal and vestibulo-cochlear ganglia as indicated by red arrowheads. (h) At E12.5 expression was pronounced throughout the central nervous system. LacZ expression could be

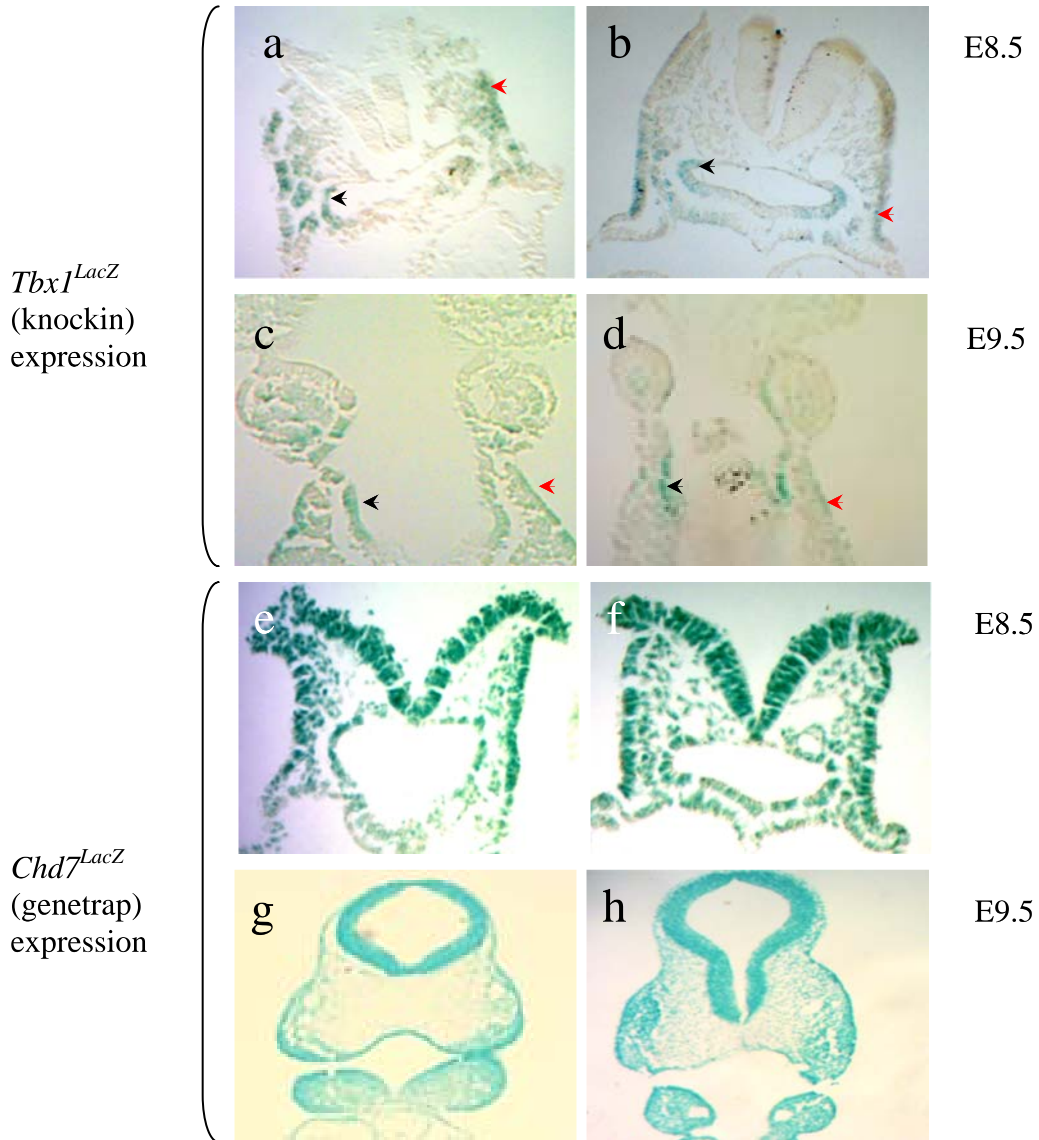
seen in somite derived tissues as indicated by yellow arrowheads and staining was evident in the skeletal components of the limb. (i) At E14.5 expression was restricted to regions of the forebrain, mid-hindbrain, eye and the caudal neural tube.

Supplementary Figure 3: **Comparison of the *Tbx1enCre* Line Efficiency in a R26R Background With Endogenous *Tbx1* Gene Expression.**

Whole mount LacZ staining of *Tbx1*$^{+/-}$ (A-B) and *Tbx1Cre;R26R* (C-D) embryos at the indicated developmental stages and their respective transversal sections through the pharyngeal region (A', B' and B''), (C', D' and D''). Dashed lines indicate section planes. Red and yellow arrows indicate regions where *Tbx1* expression is present in the pharyngeal surface ectoderm (s.ecto.) and pharyngeal endoderm (p.endo.) respectively. Red and yellow asterisks indicate regions where *Tbx1* expression is missing in the s.ecto and the p.endo. respectively. At E8.5, LacZ expression indicates that *Tbx1*^{CRE} is strongly active in the head mesoderm (h.meso.) and the splanchnic mesoderm (sp.meso.) whereas it is chimeric in the s.ecto. and the p.endo. (A', C', C''). Similarly, analyses of *Tbx1Cre;R26R* E9.0 embryos indicate a high degree of chimerism in the pharyngeal epithelium (s.ecto. and p.endo.) when compared to wt (B, D, B', D', D''). The poor ectodermal recombination explains the lack of apparent rescue of the *Chd7*$^{+/xk}$ phenotype in the sample we analysed.



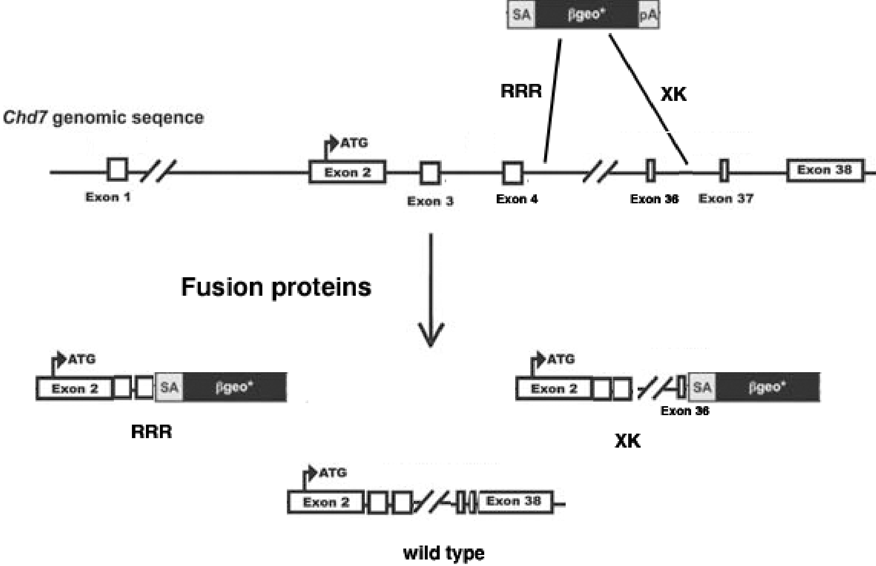
Supplementary Figure 4. **Ectodermal Expression of *Tbx1* and *Chd7* is Maintained in *Chd7* and T*bx1* Heterozygotes, Respectively**



At E8.5 (a,b) and E9.5 (c,d) T*bx1*^{LacZ} expression can be clearly seen in the ectoderm (red arrowhead) and endoderm (black arrowhead) of double (*Chd7*$^{+/whi}$;*Tbx1*^{LacZ}; a,c) and single (*Tbx1*^{LacZ}; b,d) heterozygotes. Thus *Tbx1* expression is not qualitatively altered by heterozygous loss of *Chd7*. See Supplementary table 2 for quantitative data.

At E8.5 (e,f) and E9.5 (g, h) *Chd7*^{LacZ} expression (from the *Chd7*^{xk} allele) is widespread throughout double (*Chd7*^{xk};*Tbx1*^{MCM}; e,g) and single (*Chd7*^{xk} (f,h) heterozygous embryos, including pharyngeal surface ectoderm. No qualitative alteration of *Chd7* expression can be observed as a consequence of heterozygous loss of *Tbx1*. See Supplementary table 2 for quantitative data.

Supplementary Figure 5: **Gene Trap Constructs.**



Two gene trap ES cell lines were acquired. The line designated RRR has a gene trap insertion site within intron 4. The RRR allele produces a fusion protein containing the first 3 exons and βgeo. The XK line has a more 3' insertion site between exons 36 and 37, and produces a protein containing all exons, except 37 and 38, fused to the gene trap cassette.

Supplementary Table 1: Lethality in *Chd7*$^{+/xk}$ and *Chd7*$^{+/xk}$; *Tbx1*$^{+/LacZ}$ Mice

Genotype	**n (mice)**	**Percentage**
Chd7$^{+/xk}$	11[a]	14.3
Tbx1$^{+/LacZ}$	23	29.9
Whi/+; *Tbx1*$^{+/LacZ}$	2 [b]	2.6
WT	41	53.2
	Total 77	

The expected ratios of genotypes in this *Chd7*$^{+/xk}$ x *Tbx1*$^{+/LacZ}$ cross are 25% for each genotype. Just 2 of 77 mice at P10 were *Chd7*$^{+/xk}$; *Tbx1*$^{+/LacZ}$ genotype.

[a] $P = 0.009$ and [b]$P = 1.8 \times 10^{-6}$ (Fisher's exact test).

Supplementary Table 2

A] Quantitation of genes dysregulated in *Tbx1* mutant embryos in *Chd7* heterozygotes

Gene	Mean Fold Change	Standard Deviation
Chd7	0.52	0.305
Tbx1	0.98	0.153
Fgf8	0.95	0.251
Etv4	1.00	0.315
Fgf10	0.92	0.279
Hes1	1.06	0.257
Gbx2	0.89	0.260
Smad7	0.97	0.189
Sema3c	0.98	0.214

Table A.1: RTQPCR results for *Chd7*$^{+/xk}$ E10.5. Individual E10.5 heterozygote embryos were compared to littermate wild type embryos and analysed by RTQPCR for gene expression changes. *Chd7* levels were reduced in heterozygote embryos by approximately 50%. No other expression changes were detected in the listed genes.

Gene	Mean Fold Change	Standard Deviation
Chd7	0.45	0.274
Tbx1	1.04	0.087
Fgf8	0.78	0.212
Etv4	1.11	0.263
Fgf10	0.87	0.147
Hes1	0.86	0.211
Gbx2	0.83	0.162

Table A.2: RTQPCR results for *Chd7*$^{+/xk}$ E9.5. Individual E9.5 heterozygote embryos were compared to littermate wild type embryos and analysed by RTQPCR for gene expression changes. *Chd7* levels were reduced in heterozygote embryos by approximately 50%. No other expression changes were detected in the listed genes.

Gene	Mean Fold Change	Standard Deviation
Chd7	0.50	0.157
Tbx1	0.99	0.081
Fgf8	0.84	0.131
Etv4	0.84	0.143
Fgf10	0.96	0.226
Hes1	0.95	0.201

Table A.3: RTQPCR results for *Chd7*$^{+/xk}$ E8.5. E8.5 heterozygote embryos were compared to littermate wild type embryos and analysed by RTQPCR for gene expression changes. *Chd7* levels were reduced in heterozygote embryos by approximately 50%. No other expression changes were detected in the listed genes.

B] *Chd7* is not dysregulated in *Tbx1*$^{+/-}$ embryos

Gene	Mean Fold Change	Standard Deviation
Chd7	0.95	0.262
Fgf8	0.78	0.244
Etv4	0.92	0.245
Fgf10	0.90	0.210

Table B.1: RTQPCR results for *Tbx1*$^{+/-}$ E10.5. Individual E10.5 heterozygote embryos were compared to littermate wild type embryos and analysed by RTQPCR for gene expression changes. No expression changes were detected in the listed genes.

Gene	Mean Fold Change	Standard Deviation
Chd7	0.99	0.266
Fgf8	0.81	0.312
Etv4	0.83	0.251
Fgf10	1.17	0.183

Table B.2: RTQPCR results for *Tbx1*$^{+/-}$ E9.5. Individual E9.5 heterozygote embryos were compared to littermate wild type embryos and analysed by RTQPCR for gene expression changes. No expression changes were detected in the listed genes.

Gene	Mean Fold Change	Standard Deviation
Chd7	0.89	0.161
Fgf8	0.96	0.146
Etv4	1.03	0.158
Fgf10	1.04	0.142
Hes1	0.83	0.143
Gbx2	0.95	0.143

Table B.3: RTQPCR results for *Tbx1*$^{+/-}$ E8.5. E8.5 heterozygote embryos were compared to littermate wild type embryos and analysed by RTQPCR for gene expression changes. No expression changes were detected in the listed genes.

Supplementary Table 3: No Effect of *Fgf8* on *Chd7* Haploinsufficiency Phenotype at E10.5.

The cross was undertaken on a mixed (CD1/C57Bl6) and inbred (C57Bl6) background. The first table gives the results separately, the second combined. No genetic interaction was observed.

Genotype	*n*	*n* Abnormal	*n* with 4th paa defects	*n* with 6th paa defects
$Fgf8^{\Delta/+}$ (CD1)	18	1 (5.5%)[a]	1	0
$Chd7^{+/xk}$ (CD1)	27	9 (33.3%)[b]	9	1
$Fgf8^{\Delta/+}$ (Bl6)	23	0 (0%)[c]	0	0
$Chd7^{+/xk}$ (Bl6)	11	5 (45.5%)[d]	5	1
$Chd7^{+/xk}$: $Fgf8^{\Delta/+}$ (CD1)	18	4 (22.2%)[e]	4	0
$Chd7^{+/xk}$: $Fgf8^{\Delta/+}$ (Bl6)	9	3 (33.3%)[f]	3	0

[a] P-value 0.17, [b] P-value 0.32, [c] P-value 0.017, [d] P-value 0.46, [e]P-value 0.6, [f]P-value 0.2 Fisher's exact test. No defects were observed in any wild type embryo.

Genotype	*n*	*n* Abnormal	*n* with 4th paa defects	*n* with 6th paa defects
$Fgf8^{\Delta/+}$	41	1 (2.4%)[a]	1	0
$Chd7^{+/xk}$	38	14 (36.8%)[b]	14	2
$Chd7^{+/xk}$: $Fgf8^{\Delta/+}$	27	7 (25.9%)[c]	7	0

[a] P-value 0.005, [b] P-value 0.26, [c] P-value 0.3 Fisher's exact test. No defects were observed in any wild type embryo.