Supplementary Information

Title: CHD7 promotes neural progenitor differentiation in embryonic stem cells via altered chromatin accessibility and nascent gene expression

Hui Yao[1,§], Douglas F. Hannum[2,§], Yiwen Zhai[1,3], Sophie F. Hill[4], Ricardo D'Oliveira Albanus[5], Wenjia Lou[1], Jennifer M. Skidmore[1], Gilson Sanchez[1], Alina Saiakhova[6], Stephanie L. Bielas[7], Peter Scacheri[6], Mats Ljungman[8], Stephen C. J. Parker[5,7] and *Donna M. Martin[1,7]

§Co-first authors

Department of Pediatrics[1], Department of Biostatistics[2], College of Literature, Science, and the Arts[4], Department of Computational Medicine and Bioinformatics[5], Department of Human Genetics[7], Department of Radiation Oncology[8], University of Michigan, Ann Arbor, MI; Center of Genetic and Prenatal Diagnosis[3], The First Affiliated Hospital of Zhenzhou University; Department of Genetics and Genome Sciences[6], Case Western Reserve University, Cleveland, OH

Running title: CHD7 promotes ESC neuronal differentiation

Keywords: CHD7, neuronal differentiation, ATAC-seq

*Correspondence to:

Donna M. Martin, M.D., Ph.D.

1150 W. Medical Center Dr.

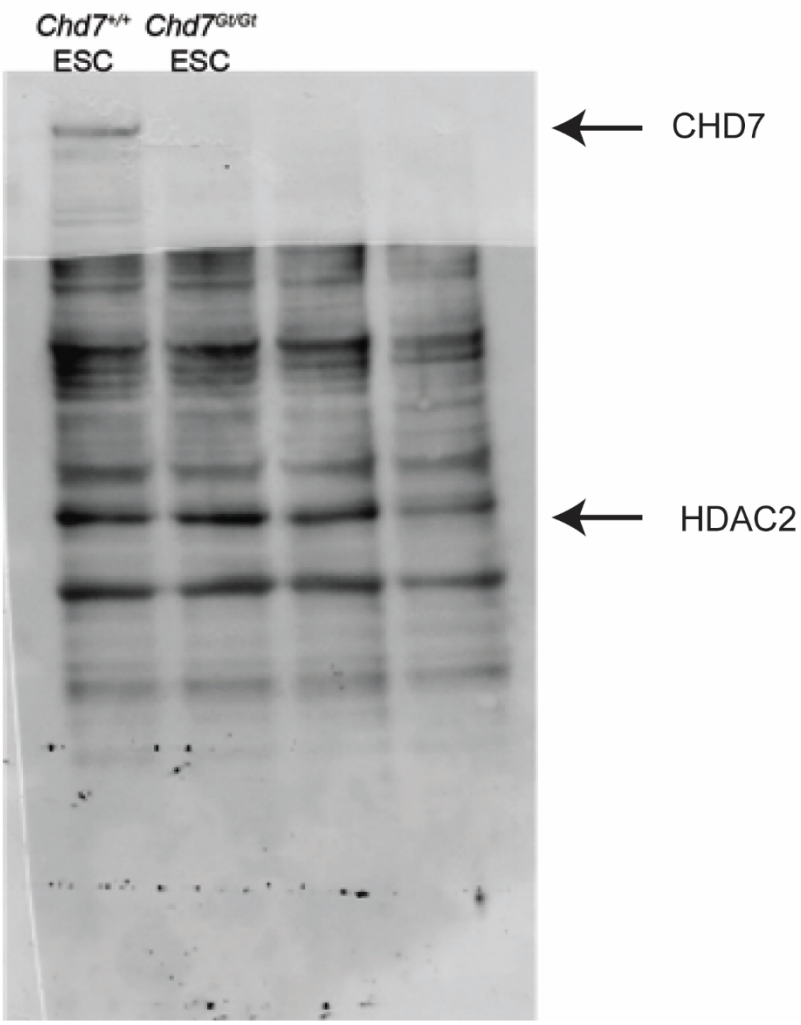
8220C MSRB III

Ann Arbor, MI 48109-5652

donnamm@umich.edu

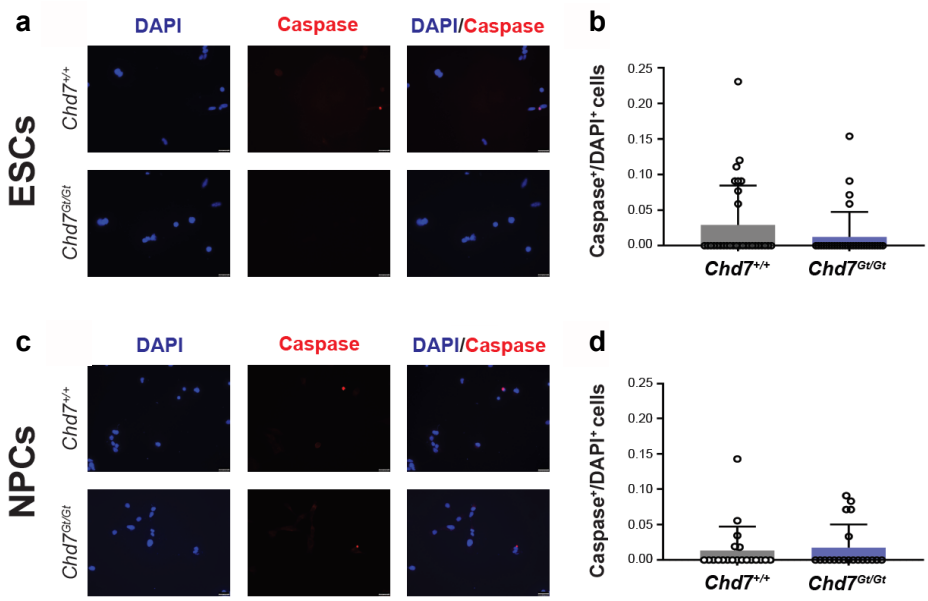
TEL: (734) 647-4859

FAX: (734) 763-9512



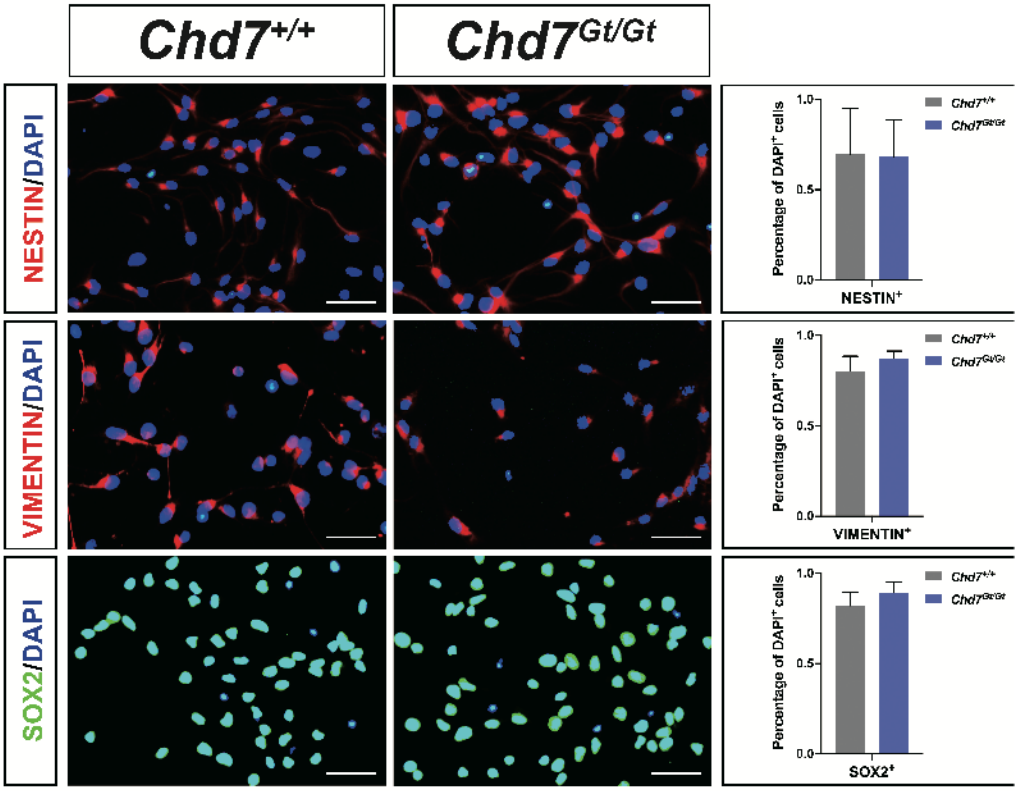
Supplementary Figure 1. Western Blot for CHD7

Western blot of the *Chd7*$^{+/+}$ and *Chd7*$^{Gt/Gt}$ ESCs confirming lack of CHD7 in the *Chd7*$^{Gt/Gt}$ cell line, using HDAC2 as a control.



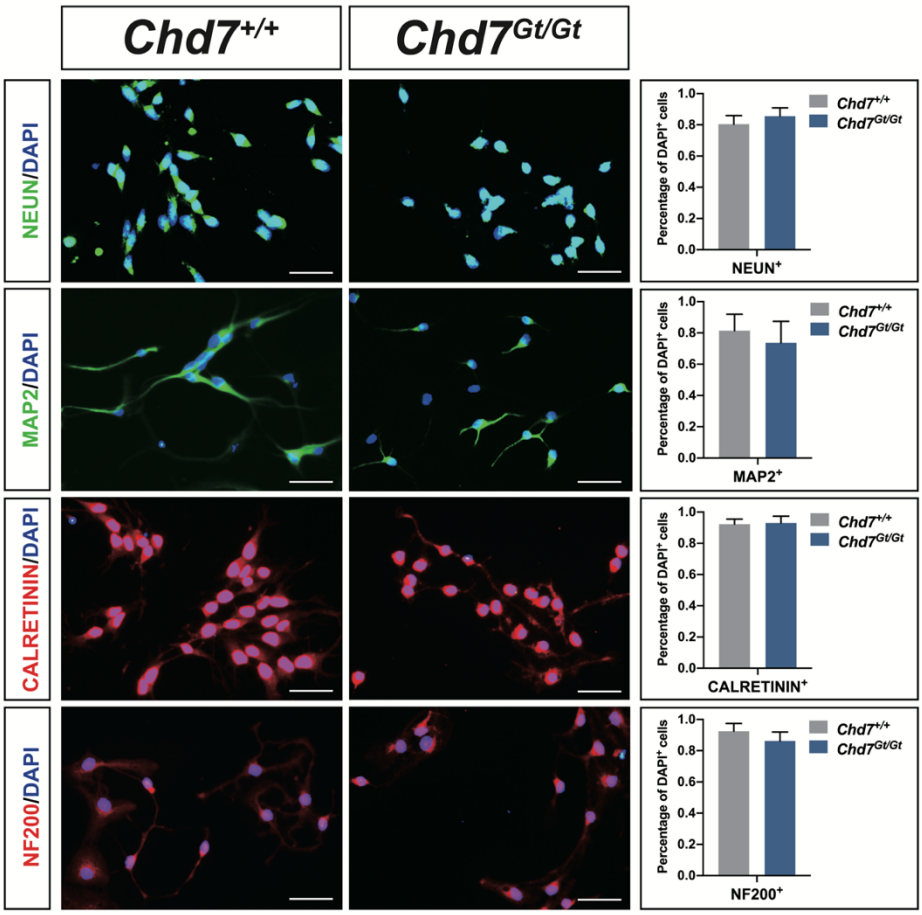
Supplementary Figure 2. Apoptosis is unchanged with loss of *Chd7*

(a, c) Representative images of cleaved Caspase 3 immunostained *Chd7*$^{+/+}$ and *Chd7*$^{Gt/Gt}$ embryonic stem cells (ESCs) (N = 30) and neural progenitor cells (NPCs) (N = 20), with quantification (b, d) of images.



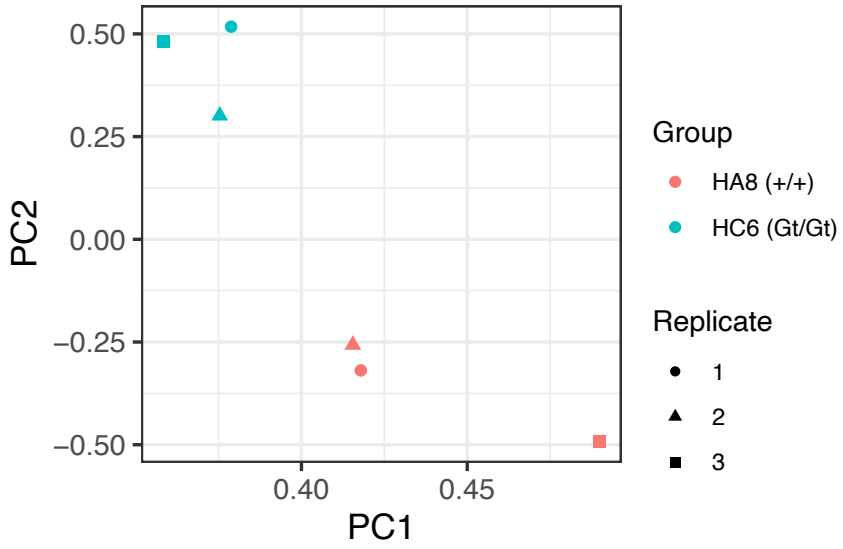
Supplementary Figure 3. Immunostaining of neuronal progenitor markers.

Immunostaining for the neuronal progenitor markers NESTIN, VEMENTIN and SOX2 in cells derived from *Chd7*$^{+/+}$ and *Chd7*$^{Gt/Gt}$ ESCs (left). Quantification of immunostaining (right) shows no significant differences between *Chd7*$^{+/+}$ and *Chd7*$^{Gt/Gt}$ in the proportion of NESTIN^{+} cells, VIMENTIN^{+} cells, and SOX2^{+} cells. Data from three independent experiments are represented as mean ± SEM. Unpaired student's *t*-tests were used for statistical analysis. Scale bars = 50 µm.



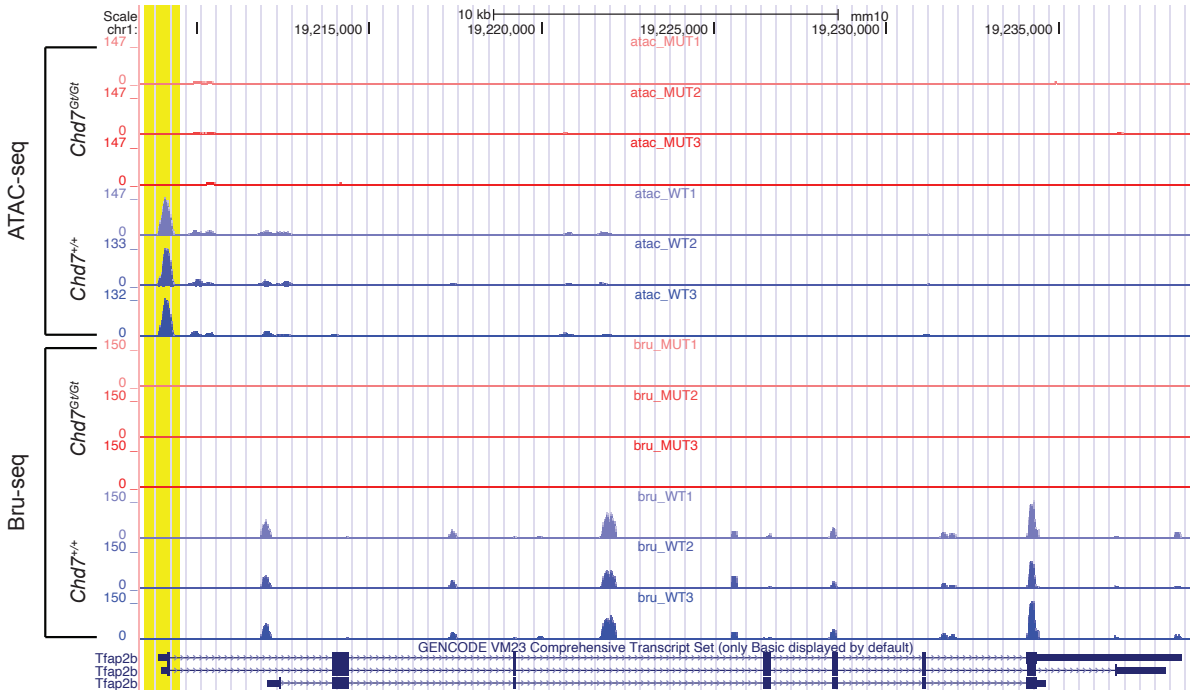
Supplementary Figure 4. Immunostaining of neuronal markers.

Immunostaining for the neuronal markers NEUN, MAP2, CALR and NF200 in cells derived from *Chd7*$^{+/+}$ and *Chd7*$^{Gt/Gt}$ NPCs (left). Quantification of immunostaining (right) shows no difference between *Chd7*$^{+/+}$ and *Chd7*$^{Gt/Gt}$ in the proportion of NEUN^{+} cells, MAP2^{+} cells, CALR^{+} cells, and NF200^{+} cells. Data from three independent experiments are represented as mean ±SD. Unpaired student's *t*-tests were used for statistical analysis. Scale bars = 50 μm.



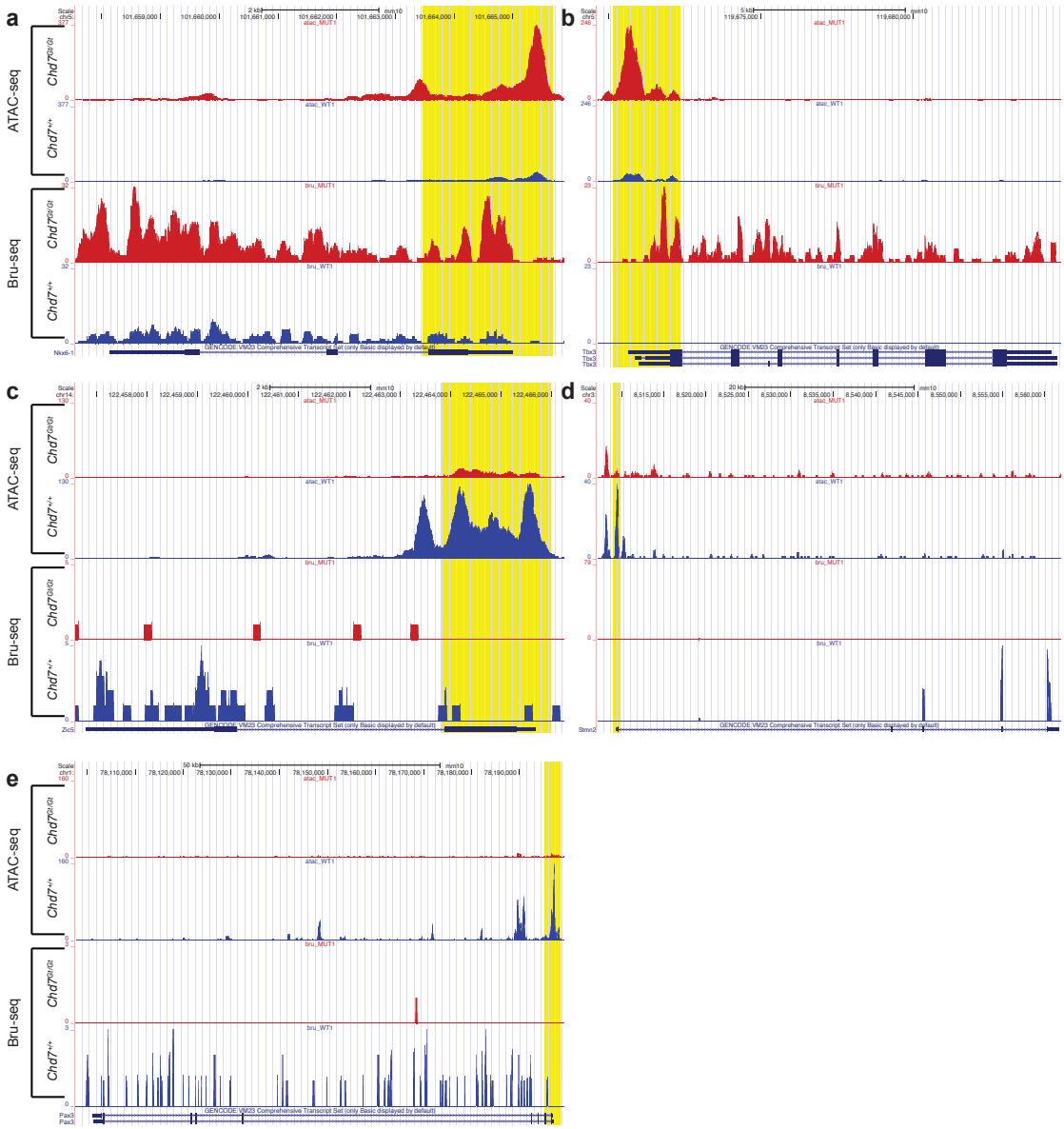
Supplementary Figure 5. Principle component analysis of Bru-Seq data

Graphical representation of the first and second principal components for the six Bru-Seq samples. Colors indicate the two different genotypes: HA8 (+/+) is *Chd7*$^{+/+}$ and HC6 (Gt/Gt) is *Chd7*$^{Gt/Gt}$. Shapes represent the different replicates for each genotype. Groups cluster separately from each other.



Supplementary Figure 6. UCSC Genome Browser Tracks for *Tfap2b*

Image showing all tracks for the 3 replicates for Bru-Seq and ATAC-seq data at the *Tfap2b* locus. Yellow highlight indicates the promoter region showing differential accessibility between genotypes.



Supplementary Figure 7. Representative UCSC Genome Browser Images

(a-e) Images showing reads for the ATAC- and Bru-Seq experiments for (a) *Nkx6*-1, (b) *Tbx3*, (c) *Zic5*, (d) *Stmn2* and (e) *Pax3* as displayed in the UCSC Genome Browser. Promoter regions are highlighted in yellow. The ATAC-seq reads show a visible difference between *Chd7*$^{+/+}$ and *Chd7*$^{Gt/Gt}$, with subtler changes in expression by Bru-Seq analysis.

Supplementary Table 1. Primers Used for RT-qPCR

Gene/locus	Direction	Primer sequence (5' to 3')
Oct4	Forward	TCT TTC CC CAG GCC CCC GGC TC
	Reverse	TGC GGG CGG ACA TGG GGA GAT CC
Sox2	Forward	TTC GAG GAA AGG GTT CTT GCT G
	Reverse	TCC TTC CTT GTT TGT AAC GGT CCT
Nanog	Forward	AGG GTC TGC TAC TGA GAT GCT CTG
	Reverse	CAA CCA CTG GTT TTT CTG CCA CCG
C-Myc	Forward	TGA CCT AAC TCG AGG AGG AGC TGG
	Reverse	AAG TTT GAG GCA GTT AAA ATT ATG GCT GAA GC
Brn2	Forward	AGA GCC CAA GGC AGA AAA GT
	Reverse	GGC GCT CTG GTT AAA GGA G
Gpm6a	Forward	CTT GGA TCT GCG TCA GTT TG
	Reverse	GGA AGT TCT CAG AGG CAG TAC AA
Ncan	Forward	GGA CAG ACA ACA CAG GAC TGC
	Reverse	CCC ACC TGC GAA GAA ATT AT
Olig1	Forward	AGG GTT TCC GAG CTG GAT GTT
	Reverse	AGG AAC CTC TCC ACT TCG CAT C
Olig2	Forward	AGA CCG AGC CAA CAC CAG
	Reverse	AAG CTC TCG AAT GAT CCT TCT TT
Pax6	Forward	GTT CCC TGT CCT GTG GAC TC
	Reverse	ACC GCC CTT GGT TAA AGT CT
Sox1	Forward	ATA CCC CCA AAA TGC ATC AA
	Reverse	GGA AAC GGG CTT TTC TCT CT
Sox11	Forward	CAC CAC AGC CAC AAA GCG CAA
	Reverse	CAC ATG GGC ACA TCC AGG TT
Gapdh	Forward	TGT TCC TAC CCC CAA TGT GT
	Reverse	TGT GAG GGA GAT GCT CAG TG

Supplementary Table 2. Mapped reads in Bru-Seq experiments

Library	Cell Line	Number of Reads	Number of Unique Mapped Reads
HA8mNPC0h5a	HA-8	72,860,158	44,967,039
HA8mNPC0h6a	HA-8	62,109,913	41,434,129
HA8mNPC0h7a	HA-8	51,801,745	32,347,723
HC6mNPC0h5a	HC-6	62,130,545	56,056,777
HC6mNPC0h6a	HC-6	64,740,891	58,451,291
HC6mNPC0h7a	HC-6	55,515,348	49,884,359

* HA-8 refers to *Chd7*$^{+/+}$
HC-6 refers to *Chd7*$^{Gt/Gt}$