SUPPLEMENTAL

Supplementary Table 1

BAC Clone List

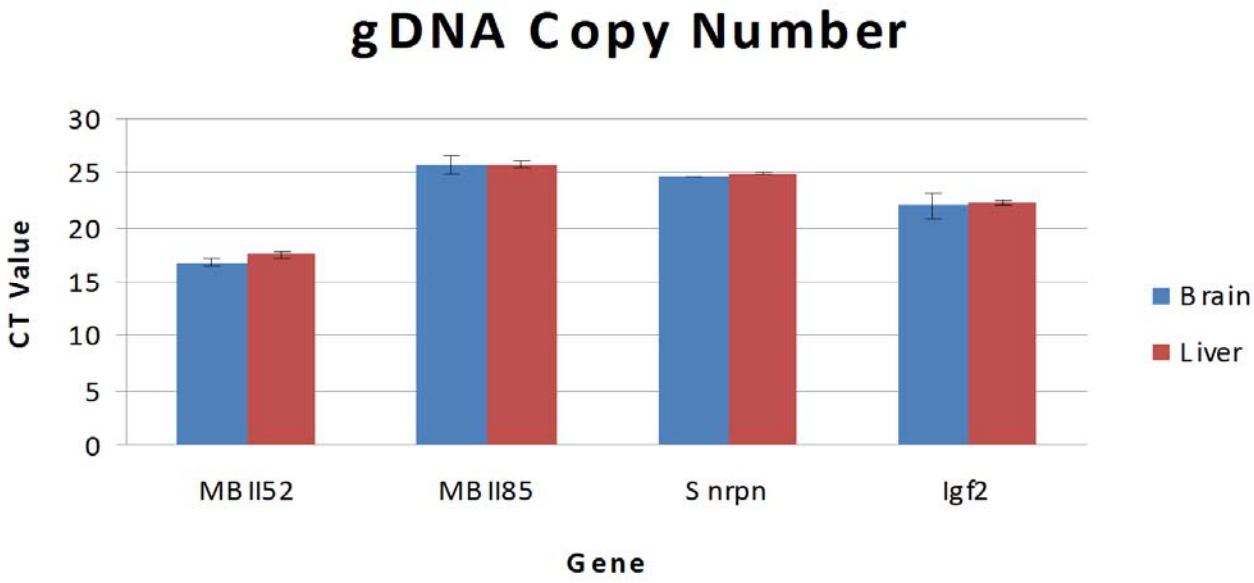
Human Clones

Clone #	Genes	Chr. Location	Size (Kb)	Notes
RP11-373J1	*NDN, MAGEL2*	15q11.2	199	
RP11-125E1	*SNRPN* (ICR, upstream)	15q11.2	175	*SNRPN-UBE3A probe*
RP11-186C7	*SNRPN, IPW & HBII-85* cluster	15q11.2	170	*SNRPN-UBE3A probe*
RP11-171C8	*HBII-52* cluster	15q11.2	161	*SNRPN-UBE3A probe*
RP11-1081A4	*UBE3A* (entire gene & downstream)	15q11.2 - 15q12	171	*SNRPN-UBE3A probe*
RP11-339C21	*ATP10A* (downstream)	15q12	168	

Mouse Clones

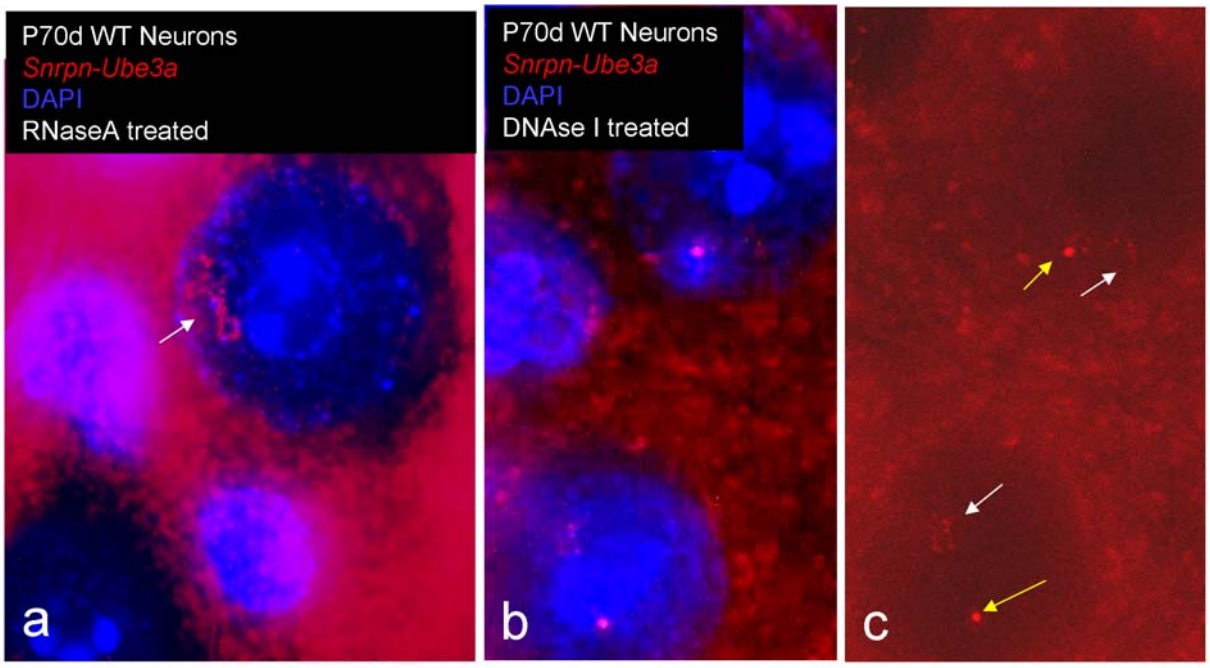
Clone #	Genes	Chr. Location	Size (Kb)	*Notes*
RP24-232N8	*Ndn, Mage12, Mkrn3 & Peg12*	7qC	169	
RP23-351F6	Upstream of *Snrpn*	7qC	207	flanking probe
RP23-380J10	*Snrpn* (tg380a BAC)	7qC	161	
RP24-275J20	*Snrpn*	7qC	140	*Snrpn-Ube3a probe*
RP23-358G20	*Ipw*	7qC	127	*Snrpn-Ube3a probe*
RP23-410L2	snoRNAs	7qC	181	*Snrpn-Ube3a probe*
RP24-147O20	*Ube3a*	7qC	168	*Snrpn-Ube3a probe*
RP23-141P24	Between *Ube3a & Atp10a*	7qC	191	*Snrpn-Ube3a probe*
RP23-318C6	*Atp10a*	7qC	206	flanking probe
RP23-50N22	*Igf2/H19*	7qF5	244	
RP23-60E10	*Gtl2*	12qF1	244	
RP24-241I15	*Dlk1,* upstream *Gtl2*	12qF1	165	
RP23-218J8	downstream *of Gtl2*	12qF1	214	
RP23-386G18	*Dlx 5/6*	6qA1	203	
RP23-443I23	*Htr2c*	XqF2	190	
RP23-422O20	*Igf2r*	17qA1	204	
RP23-309B17	*Tsix*	XqD	224	
RP23-39F15	*Dmd*	XqB - XqC1	245	
RP23-3D16	*Stmn4*	14qD1	202	

Supplementary Figure 1



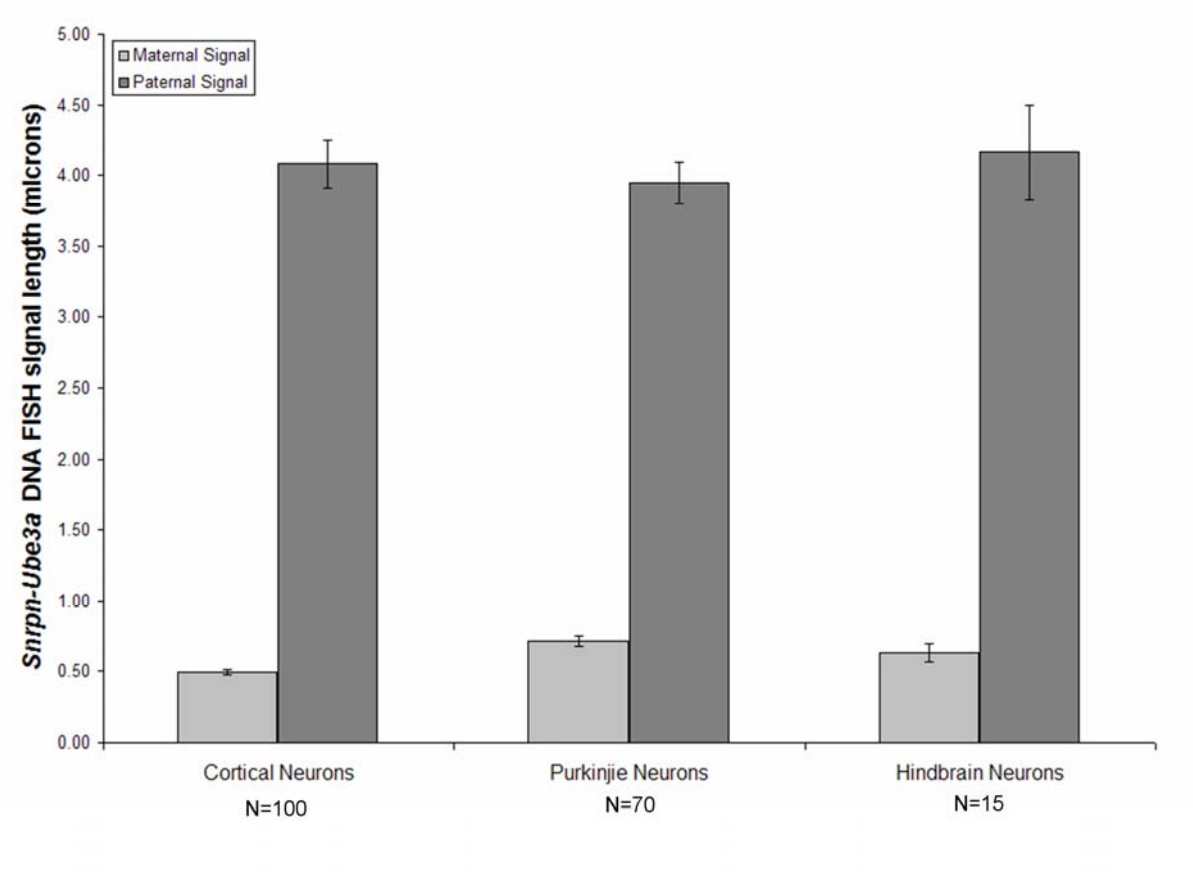
qPCR performed on gDNA from adult mouse brain and liver tissue for *Snrpn*, MBII-85, MBII-52 and *Igf2* (control) to test for DNA duplication in the *Snrpn-Ube3a* region in adult neurons reveals no evidence for DNA duplication in brain versus liver. Results represent the mean ± SEM of triplicate reactions.

Supplementary Figure 2



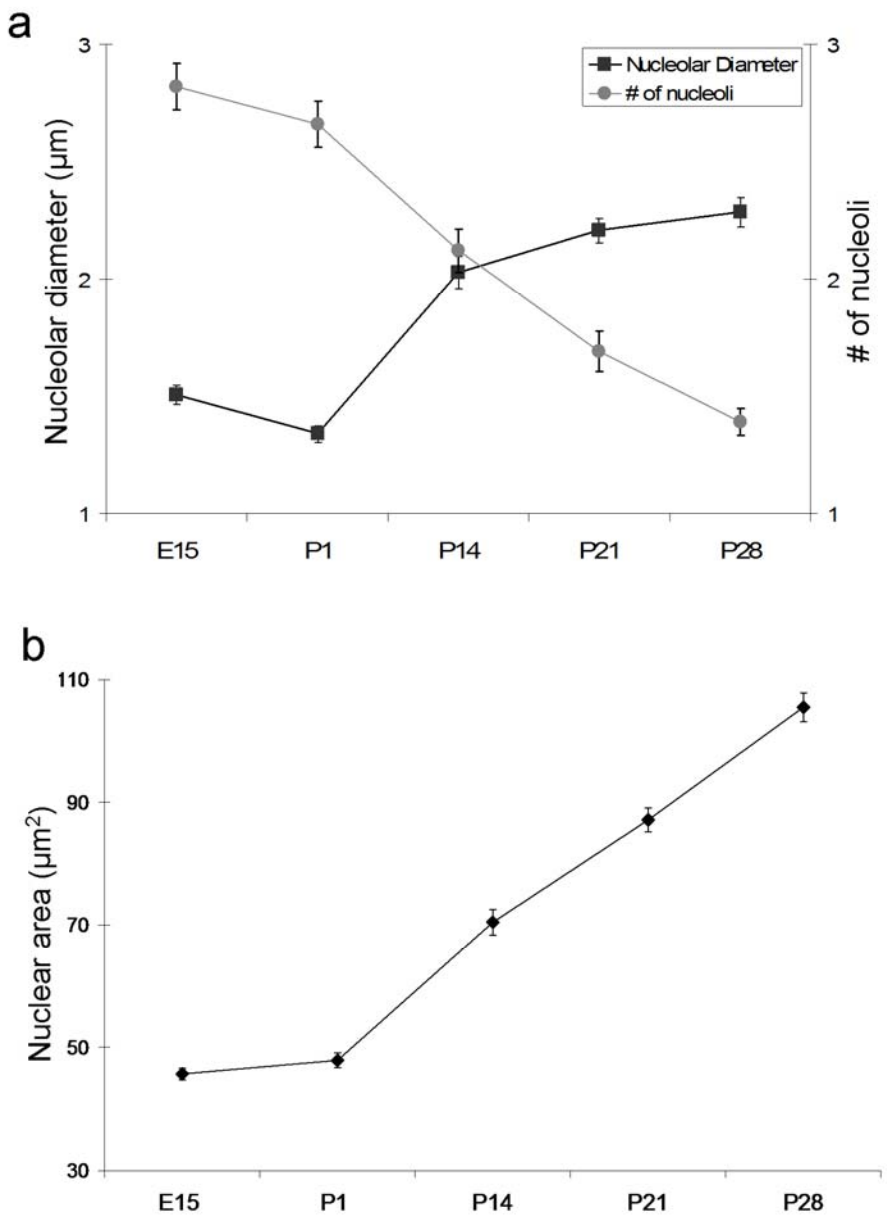
a. RNase A treatment pre- and post-hybridization does not decrease the length of the paternal *Snrpn-Ube3a* signals. Shown is a representative image of neuronal nuclei treated with 250 µg/mL of RNase A pre-hybridization showing an extended paternal signal (white arrow). **b-c.** DNase I treatment of adult mouse brain sections reveals a greater level of digestion of paternal *Snrpn-Ube3a* signals (white arrows) then maternal *Snrpn-Ube3a* signals (yellow arrows) indicating a decreased level of nucleosome coverage on the paternal allele compared to the maternal allele.

Supplementary Figure 3



Large neuronal nuclei in the cortex, hindbrain and Purkinje neurons exhibited the largest paternal *Snrpn-Ube3a* signals in adult wildtype mouse brain sections. Results represent the mean ± SEM of number (N) of nuclei indicated below each set of bars.

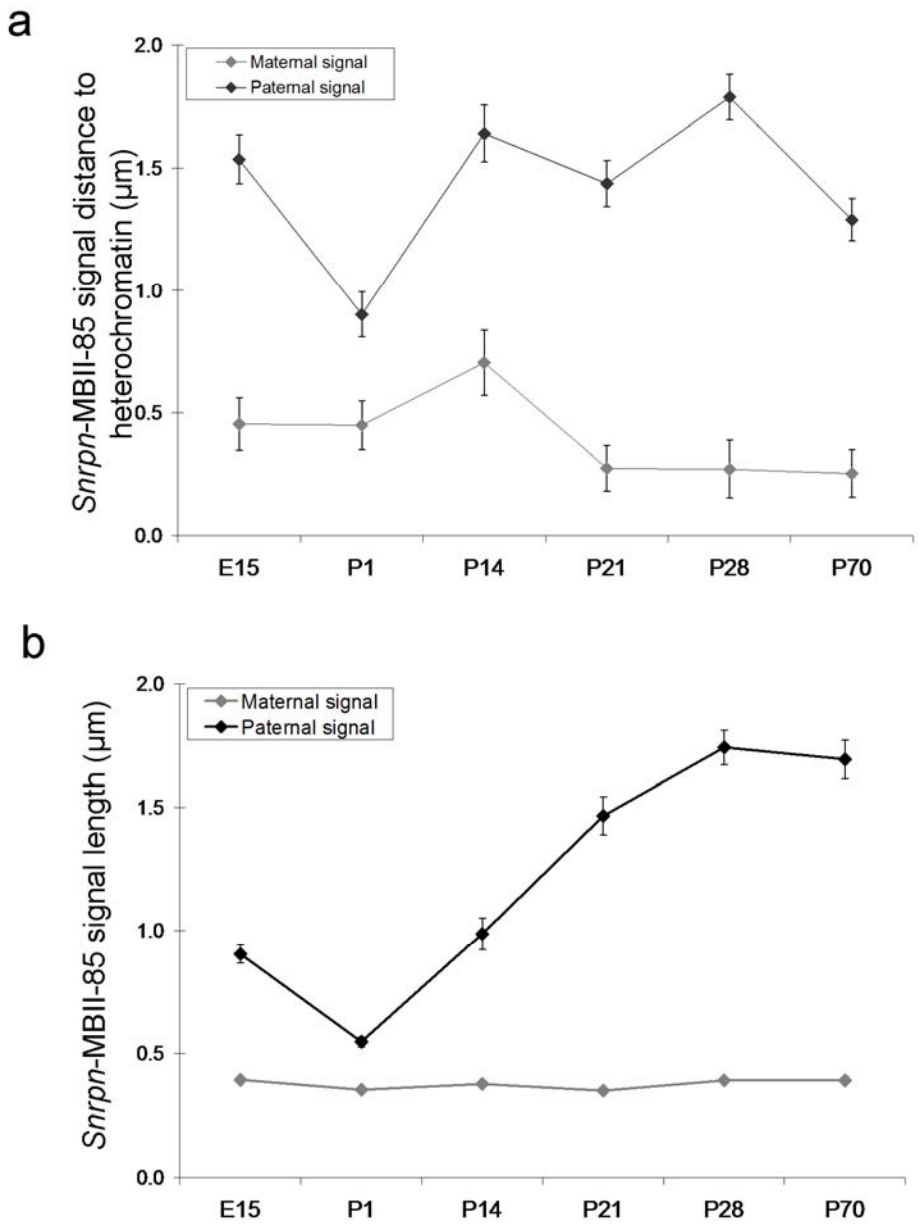
Supplementary Figure 4



a. Changes in nucleolar number (right axis) and diameter (left axis) during normal neurodevelopment with the number of nucleoli decreased with age while the diameter of largest nucleolus increased. **b.** Neuronal nuclear diameter increased with

neurodevelopment and in correlation with chromatin decondensation of the paternal *Snrpn-Ube3a* signals.

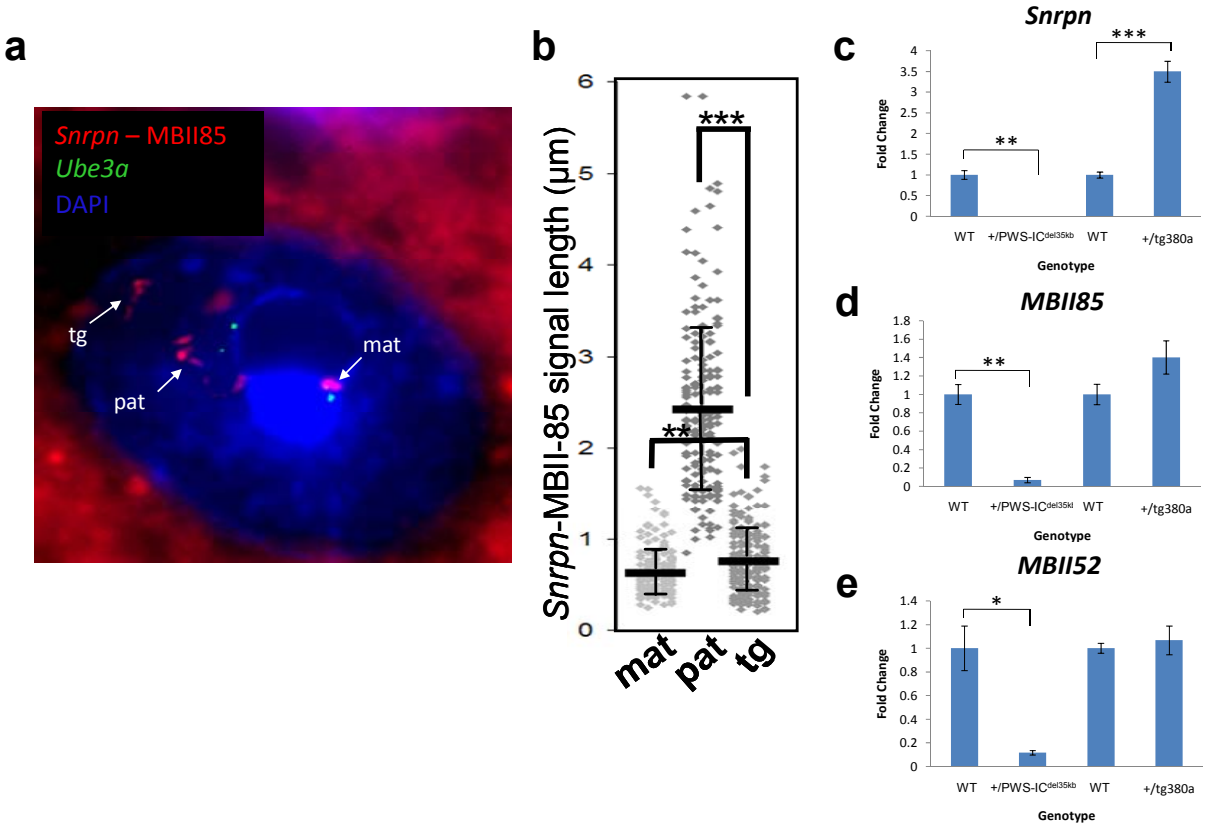
Supplementary Figure 5



a. Measurements fromin*Snrpn*-MBII-85 DNA FISH signals to closest heterochromatic foci (DAPI) reveal that the paternal allele was significantly further from heterochromatin then the maternal allele throughout neurodevelopment. **b.** *Snrpn*-MBII-85 maternal and

paternal signals follow a similar trend as *Snrpn-Ube3a* signals (Figure 3a) throughout neurodevelopment. Results represent the mean ± SEM for 100 nuclei per timepoint.

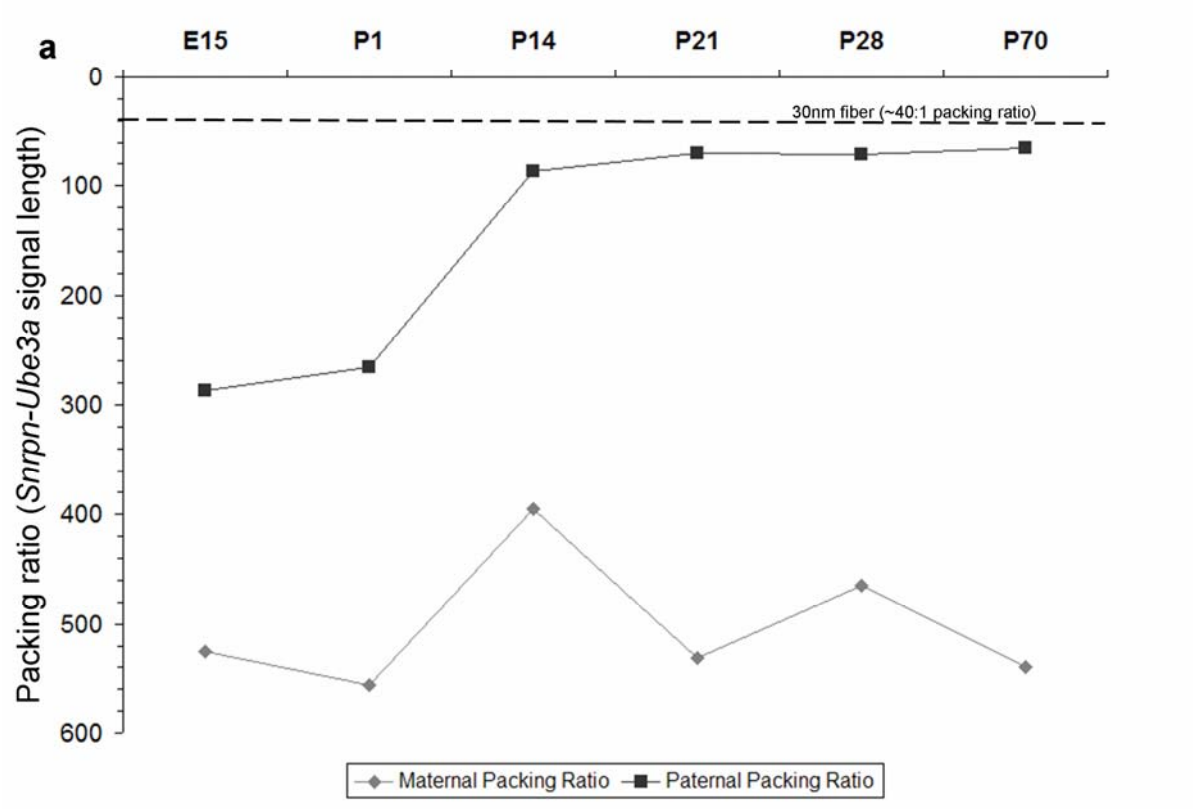
Supplementary Figure 6

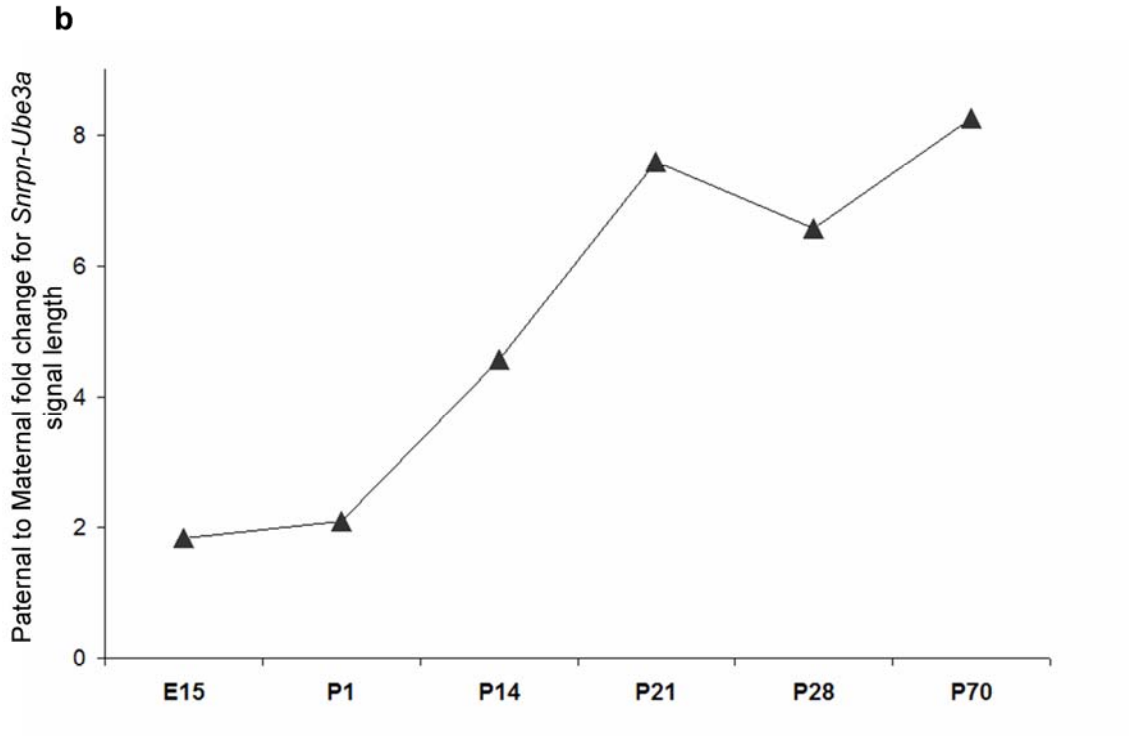


Analysis of a transgenic mouse line for chromatin decondensation and transcriptional activity. **a.** Adult brain sections of the tg380a transgene mouse line containing a tandem genomic BAC insertion (140 kb spanning PWS-IC through MBII-85) were hybridized with a DNA FISH probe from the inserted BAC (RP23-380J10, red) and a probe for *Ube3a* (RP23-147O20, green) to identify the endogenous paternal and maternal signals. Representative image of a +/tg380a neuron showing all three signals for *Snrpn-MBII-85* (red) with the tg380a allele identified by the lack of adjacent *Ube3a* (green). **b.** *Snrpn-MBII-85* lengths were measured for all three signals: maternal, paternal and transgene. While transgenic signals were significantly larger than the endogenous

maternal signals (19% larger than the maternal signals, **P<0.005), they were highly significantly smaller than the endogenous paternal signals (69% smaller than paternal signals, ***P<0.0001). Results represent the mean (black bars) ± S.D. for 200 nuclei from three transgenic adult mice. **c-e.** Analysis of quantitative RT-PCR for *Snrpn*, *MBII-85* and *MBII-52* expression in +/PWS-IC^{del35kb} whole brain compared to wt littermate, and +/tg380a whole brain compared to wt littermates. Error bars represent the S.E.M. for triplicate experiments, * p <0.05, ** p < 0.02, *** p < 0.0001.

Supplementary Figure 7





a. Chromatin packing ratios for *Snrpn-Ube3a* maternal and paternal alleles throughout murine neurodevelopment; the paternal allele decondenses almost to the level of a 30 nm fiber (indicated by the dotted line) while the maternal allele remains highly compact (~10x or more compact then a 30nm fiber). **b.** The paternal *Snrpn-Ube3a* allele decondenses, extending to over four times its original length and becoming over 8x longer then the maternal allele, represented by fold change. Results represent calculations of chromatin packing and fold changes using the mean values of 100 cortical neuronal nuclei per timepoint originally displayed in Fig 2A.