# Supplemental Data

**Ciliary gene *RPGRIP1L* is required for hypothalamic arcuate neuron development.**

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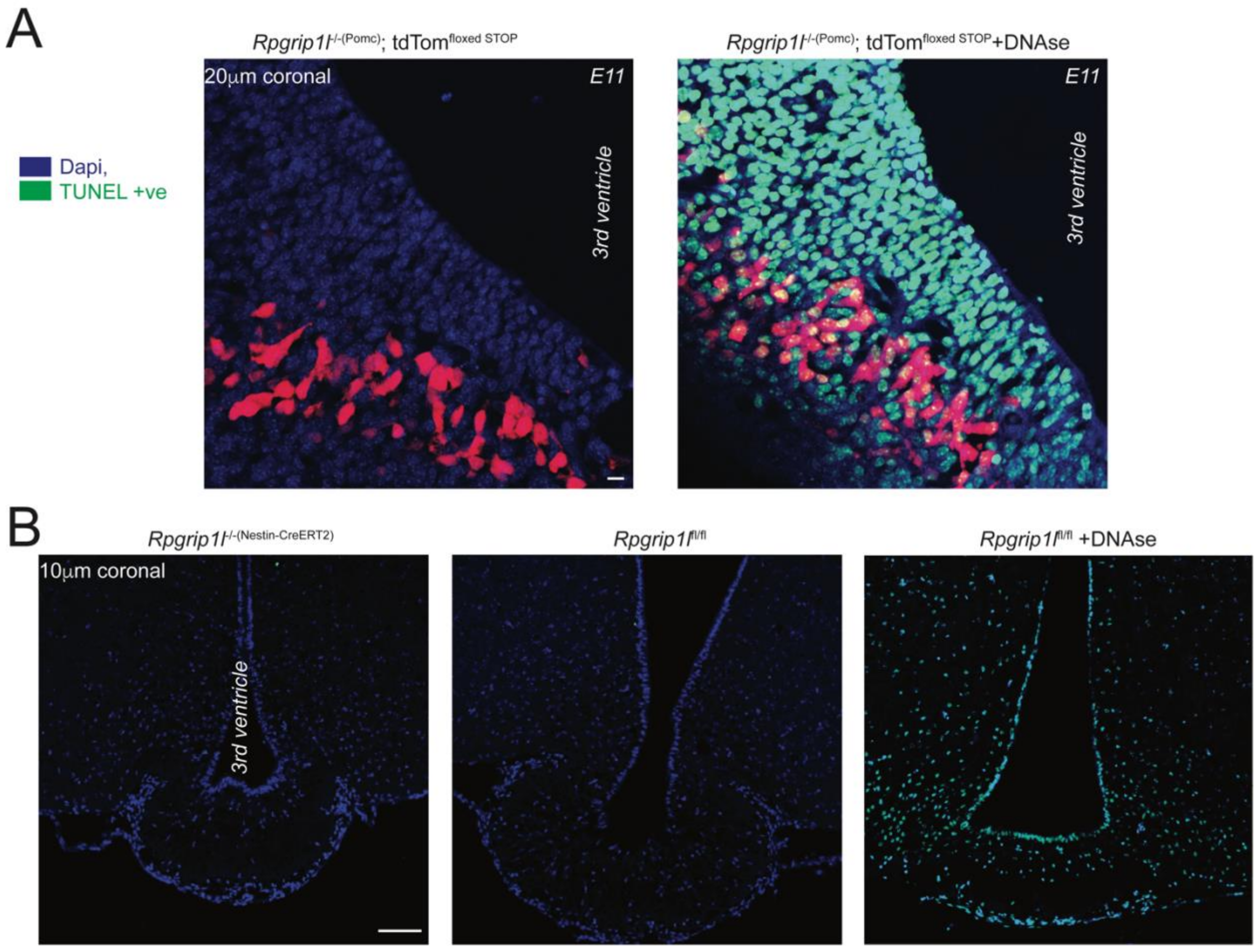


**Fig. S1.** (Supplemental data for **Fig. 4**) Cell death was not detected upon *Rpgrip1l* hypomorphism. (**A**) No TUNEL staining was detected in the developing hypothalamus of *Rpgrip1l*$^{-/-(Pomc)}$ E11 embryos (*n*=3) segregating for the Rosa26 floxed STOP tdTomato (tdTom$^{floxed\ stop}$). At E11, Pomc-positive progenitors appear. Scale bar - 10μm. (**B**) Lack of TUNEL staining in mice deleted for *Rpgrip1l* in the adult CNS (*Rpgrip1l*$^{hyp(Nestin)}$; *n*=3) 1 day after tamoxifen injection. Similar results were obtained after 2 and 3 days after Tamoxifen injection. Scale bar - 100μm.

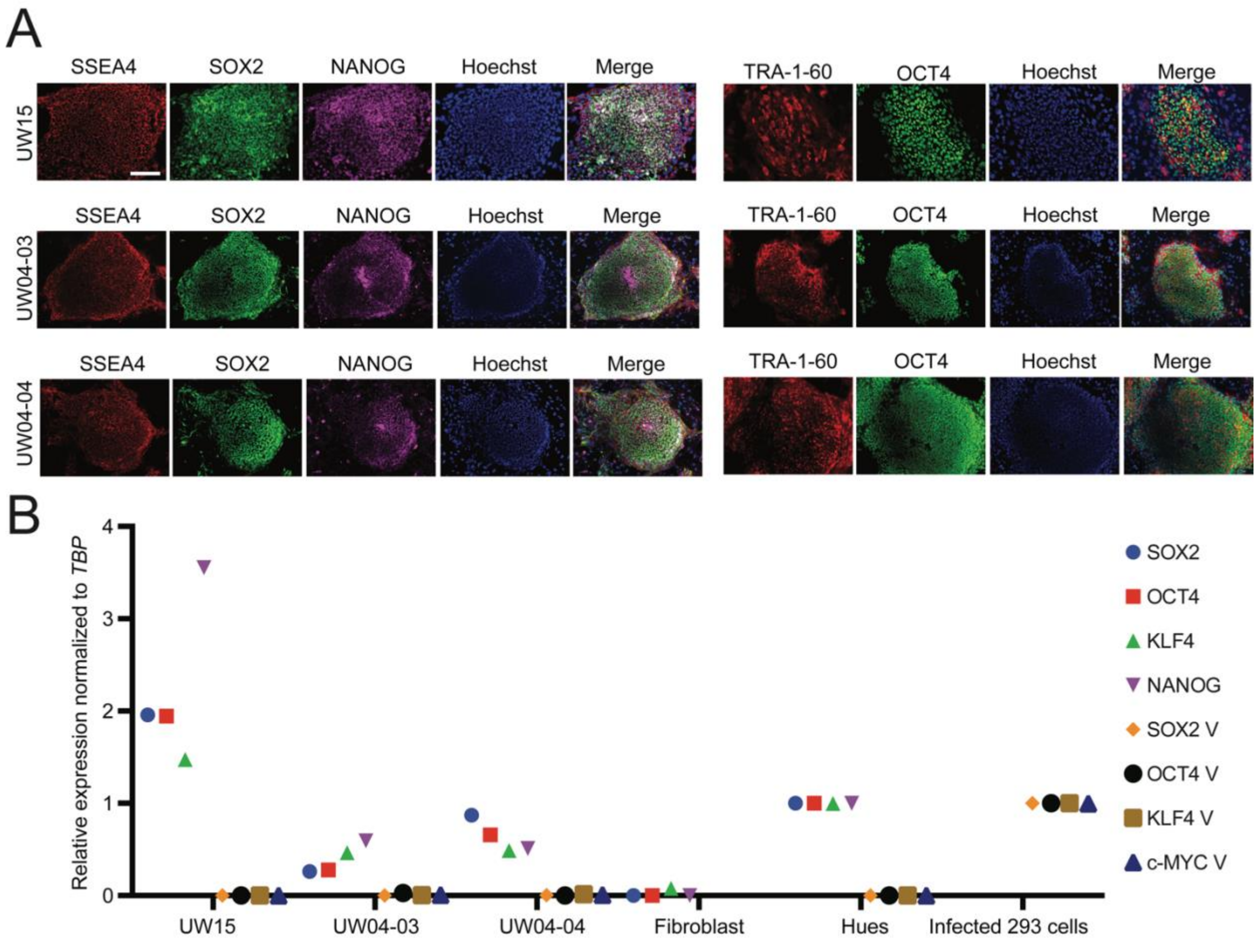


**Fig. S2.** (Supplemental data for **Fig. 6**) Proof of pluripotency of JBST iPSCs. (**A**) Immunopositive JBST iPSC lines UW15, UW04-03 and UW04-04 for pluripotency markers SSEA4, SOX2, NANOG, TRA-1-60 and OCT4. Scale bar - 200µm. (**B**) JBST iPSCs express pluripotency markers SOX2, OCT4, KLF4 and NANOG. Virus-packaged genes used to generate iPSCs from JBST fibroblasts (SOX2 V, OCT4 V, KLF4 V and c-MYC V) are silenced in JBST iPSCs. Untransduced fibroblasts were used as a negative control. Hues and viro-transduced 293 cells were used as a positive control for pluripotent gene and viral gene expression respectively. Expression was normalized to the TATA box binding protein (*TBP*).

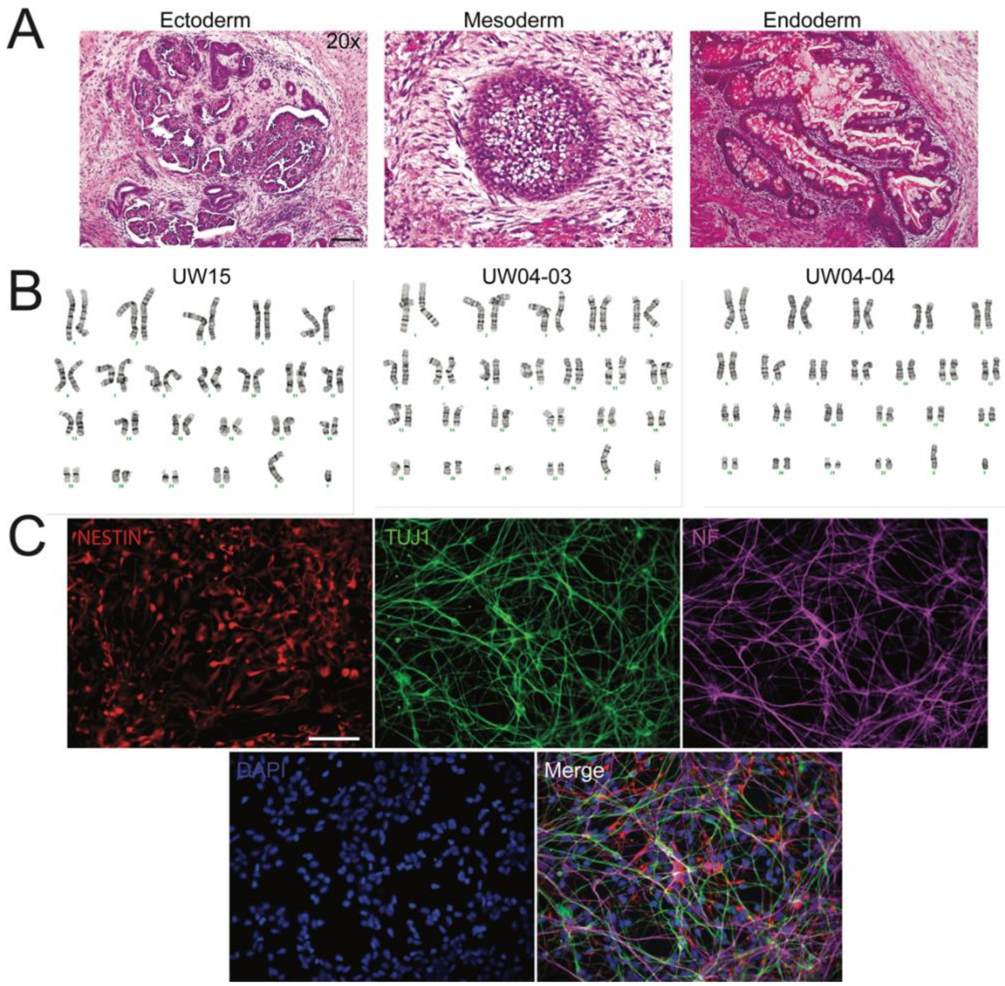


**Fig. S3.** (Supplemental data for **Figs. 6, S2**) Neurodifferentiation of JBST iPSCs. (**A**) HE staining of teratoma sections from JBST iPSCs demonstrating all three germ layers. Scale bar - 100μm. (**B**) Normal karyotype for all 3 JBST iPSCs. (**C**) Immunostaining of JBST iPSC-derived neurons for neuron markers NESTIN, TUJ1 and Neurofilament (NF). Scale bar - 100μm.

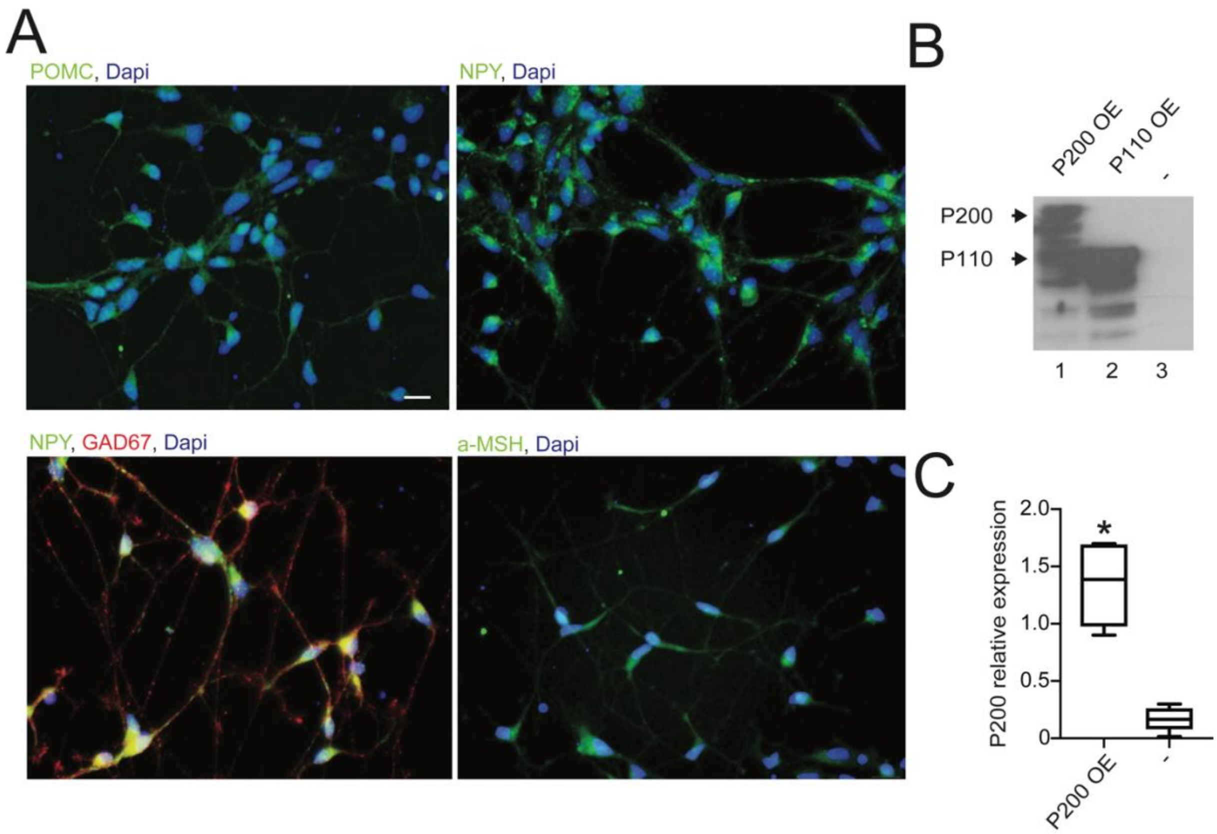


**Fig. S4.** (Supplemental data for **Fig. 8**) POMC staining of ESc-derived ARH neurons and ESc-derived P200 overexpression levels. (**A**) Staining of ESc-derived ARH neurons for POMC, NPY, alpha-Melanocyte-stimulating hormone (a-MSH), and the GABAergic neuron marker Glutamate Decarboxylase (GAD67). Scale bar - 20μm. (**B**) Western blot of whole protein extracts from ESc-derived ARH JBST neurons overexpressing full-length CUX1 (P200, lane 1), whole protein extracts from 293FT cells (Thermofisher Scientific) overexpressing CUX1 isoform P110 (lane 2), and whole protein extracts of ESc-derived ARH JBST neurons (lane 3). (**C**) P200 mRNA levels in ESc-derived ARH JBST neurons +/- P200 overexpression. Data in panel **C** are represented as box-and-whisker plots; boxes are the interquartile range, lines are the median value, and whiskers are minimum and maximum values. Each column represents the average of 6 isogenic cell lines. *$P < 0.01$, 2-tailed Student's $t$ test.

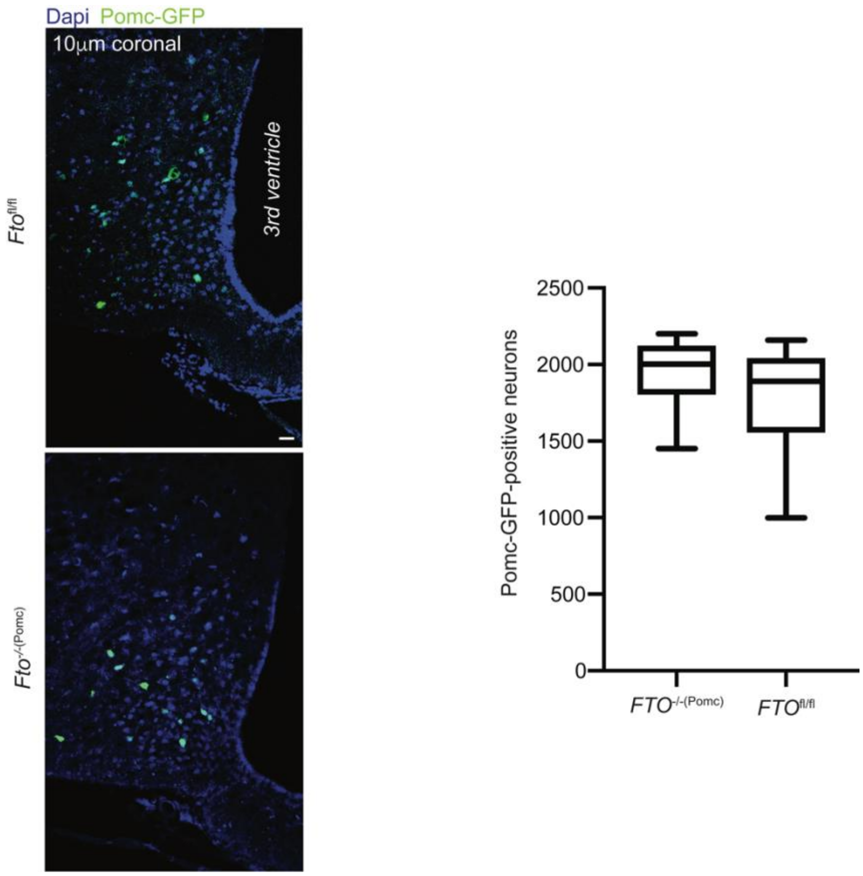


**Fig. S5.** (Supplemental data for **Fig. 4 & 8**) Congenital deletion of *Fto* in Pomc neurons has no effect on ARH Pomc neuron number. No difference in ARH Pomc neuron number between 6-week old $Fto^{fl/fl}$ mice segregating for the Pomc-Cre and Pomc-GFP alleles ($Fto^{-/-(Pomc)}$) and $Fto^{fl/fl}$ littermates. *n*=6 per column. Data are represented as box-and-whisker plots; boxes are the interquartile range, lines are the median value, and whiskers are minimum and maximum values. Scale bar – 40μm.

**Table 1. Oligonucleotides for quantitative PCR**

| Gene | Oligonucleotide pair |
|---|---|
| RPGRIP1L | 5’-CCATGGATCAAGCAATTCGACTTTATCGAG<br>5’-GGGACTGCAGGTGGTTGCAAC |
| FTO | 5-GAGCTTGAAGACACTTGGCTCCC<br>5-GCAGCCATGCTTGTGCAGTGTGA |
| CUX1 P200 | 5’-CGAACAGAGCCGGGAGTTCAAGAA<br>5’-GCTTCCTTGCTTCTTTTACTCAGTGCATC |
| IFT20 | 5’-GGTAACCTCTGGGTCCAACA<br>5’-CCTGTCTACATCAGCCTGGG |
| IFT88 | 5’-GGTCCAAGACATCTCTGGCATCATCA<br>5’-AAATGCAGAGCCTCTCAAAGCTGC |
| ADCY3 | 5’-CGCACAGGTAGAGGAAGACG<br>5’-ATCATCTCCGTGGTCTCCTG |
| PATCHED1 | 5’-TTCTTGGTTGTGGGCCTCCTCATA<br>5’-CTCTTCTCCAATCTTCTGGCGAGT |
| GLI1 | 5’-ACAGTCCTTCTGTCCCCACA<br>5’-CCAGCGCCCAGACAGAG |
| SOX2 | 5’-TTACCTCTTCCTCCCACTCCAG<br>5’-GGGTTTTCTCCATGCTGTTTCT |
| OCT4 | 5’-ATGCACAACGAGAGGATTTTGA<br>5’-CTTTGTGTTCCCAATTCCTTCC |
| KLF4 | 5’-ACCCACACAGGTGAGAAACCTT<br>5’-GTTGGGAACTTGACCATGATTG |
| NANOG | 5’-ACAACTGGCCGAAGAATAGCA<br>5’-GGTTCCCAGTCGGGTTCAC |
| SOX2 V | 5’-TTACCTCTTCCTCCCACTCCAG<br>5’-AACCTACAGGTGGGGTCTTTCA |
| OCT4 V | 5’-ATGCACAACGAGAGGATTTTGA<br>5’-AACCTACAGGTGGGGTCTTTCA |
| KLF4 V | 5’-ACCCACACAGGTGAGAAACCTT<br>5’-AACCTACAGGTGGGGTCTTTCA |
| c-MYC V | 5’-AGCAGAGGAGCAAAAGCTCATT<br>5’-CCAAAGTCCAATTTGAGGCAGT |