Supplementary Information:

Delayed maturation of GABAergic signaling in the Scn1a and Scn1b mouse models of Dravet Syndrome

Yukun Yuan1*, Heather A. O'Malley1*, Melissa A. Smaldino1#, Alexandra A. Bouza1, Jacob M. Hull2, and Lori L. Isom1,2**

1Department of Pharmacology and 2Neuroscience Graduate Program, University of Michigan, Ann Arbor, MI 48109-5632

*These authors contributed equally to the work.

#Present address: Department of Biology, Ball State University, Muncie, IN 47306

**Corresponding Author: lisom@umich.edu, 734-936-3050
Supplementary Figures

Fig. S1: P17 Scn1b⁻/⁻ brain shows bicuculline-sensitive spontaneous inward currents. **A**: Representative spontaneous current events recorded with low [Cl⁻] in a CA1 pyramidal cell of a hippocampal slice from a P17 WT mouse in the absence (top) or presence (middle) of 10 µM bicuculline, and following washout with ACSF (bottom). The upward events were bicuculline-sensitive. Similar responses were also observed in cortical layer II/III neurons (data not shown). **B**: Representative spontaneous inward currents recorded with low [Cl⁻] in a layer II/III pyramidal cell of a cortical slice from a P16 Scn1b⁻/⁻ mouse in the absence (top) and presence of 10 µM bicuculline (middle). Washout of bicuculline completely recovered spontaneous inward currents (bottom), demonstrating that the disappearance of these bicuculline-sensitive inward currents was not due to current rundown or other artifacts. Data are representative of 9 cells from 5 WT and 16 cells from 12 Scn1b⁻/⁻ mice.
Fig. S2: mRNA transcript levels and RNAseq results from Scn1b brain. A-C: Relative mRNA transcript levels of A. Slc12a5 (KCC2), B. Slc12a2 (NKCC1), or C. Scn1a (Na\textsubscript{v} 1.1) are not different between Scn1b WT and -/- brains at P17. Data are shown relative to WT. RQ: Relative quantity. D: Volcano plot showing differentially expressed genes in P10 Scn1b WT or -/- cortical layer VI. Red: Downregulated genes, Blue: upregulated genes (log2FC $\geq 1.5$ and false discovery rate $\leq 0.05$). E: Table showing genes differentially expressed between Scn1b WT and -/- cortical layer VI. In addition, there were no differences in expression between genotypes for the genes encoding NKCC1, KCC2, WNK1, or STK39.
Fig. S3: Protein expression of KCC2 or β1 and mRNA expression of Slc12a5, Slc12a2, or Scn1b in Scn1a WT or +/- mouse brain. A-C: Protein expression levels of KCC2 and β1 are not different between Scn1a WT and +/- mouse brains. A: KCC2 protein expression at P16. Top: Quantification of KCC2 expression in Scn1a WT vs +/- brain,
normalized to α-tubulin expression and shown relative to WT. Bottom: Representative Western blot image. B: β1 protein expression at P16-17. Top: Quantification of β1 expression in Scn1a WT vs +/- brain, normalized to α-tubulin levels and shown relative to WT. Bottom: Representative Western blot image. C: β1 protein expression at P21-24. Top: Quantification of β1 expression in Scn1a WT vs +/- brain, normalized to α-tubulin levels and shown relative to WT. Bottom: Representative Western blot image. β1 null brain membranes were used as a negative control in B and C. D-I: Relative mRNA transcript levels of Slc12a5 (KCC2), Slc12a2 (NKCC1), and Scn1b (β1) are not different between Scn1a WT and +/- mice at P16 or P22. Data are shown relative to WT. D: Relative transcript levels of Slc12a5 at P16. E: Relative transcript levels of Slc12a2 at P16. F: Relative transcript levels of Scn1b at P16. G: Relative transcript levels of Slc12a5 at P22. H: Relative transcript levels of Slc12a2 at P22. I: Relative transcript levels of Scn1b at P22. RQ: Relative quantity.
Fig. S4: GABA induces excitatory responses in Scn1a<sup>+/−</sup> neurons. A, B:

Representative traces of GABA-evoked responses at different membrane holding potentials after establishing gramicidin perforated patch recording in two representative Scn1a<sup>+/−</sup> neurons. At membrane potentials more hyperpolarized than -60 mV (-70 and -80 mV are shown), puff application of GABA induced AP firing (arrows in B-III and B-IV). Each trace is representative of results from 6 individual mice.
Supplementary Tables:

<table>
<thead>
<tr>
<th>Patient</th>
<th>Variant</th>
<th>Growth Parameters</th>
<th>Age</th>
<th>Weight (kg)</th>
<th>Percentile</th>
<th>Height (cm)</th>
<th>Percentile</th>
<th>Head (cm)</th>
<th>Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>c.449-2A&gt;G</td>
<td></td>
<td>6 months</td>
<td>7.6</td>
<td>50-75</td>
<td>63</td>
<td>10-25</td>
<td>42.5</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7 months</td>
<td>7.6</td>
<td>50</td>
<td>67.7</td>
<td>50</td>
<td>43</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9 months</td>
<td>8.5</td>
<td>50</td>
<td>66</td>
<td>50</td>
<td>44.5</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 months</td>
<td>10.7</td>
<td>&lt;5</td>
<td>81</td>
<td>&lt;5</td>
<td>47</td>
<td>&lt;5</td>
</tr>
<tr>
<td>2</td>
<td>c.449-2A&gt;G</td>
<td></td>
<td>3 months</td>
<td>5.4</td>
<td>50</td>
<td>57</td>
<td>10</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8 months</td>
<td>8</td>
<td>25-50</td>
<td>68</td>
<td>25</td>
<td>43</td>
<td>50-75</td>
</tr>
<tr>
<td>3</td>
<td>c.355T&gt;G: p.Y119D</td>
<td></td>
<td>10 years</td>
<td>11.52</td>
<td>&lt;1</td>
<td>90</td>
<td>&lt;1</td>
<td>43.5</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

**Table S1:** Growth parameters of SCN1B-linked DS patients at multiple developmental milestones. Head: head circumference.
Original full-length images of Western blots are provided below:
Fig. 5A - loading controls
Fig. 5B, KCC2 and loading controls
Fig. 5C - loading control, lane 1 omitted
Fig. 5E, IP lanes and control
Fig. 5F - IP lanes
Fig. 5G - controls
Supp. Fig. 4A - KCC2
Supp. Fig. 4B - β1, lanes 3-9 shown in figure
Supp. Fig. 4B - loading controls, lanes 3-9 shown
Supp. Fig. 4C - $\beta_1$ - lane 1 not shown
Supp. Fig. 4C - loading controls, lane 1 not shown