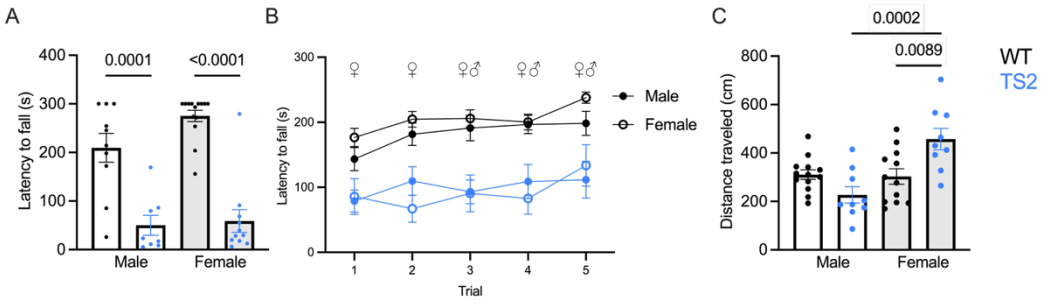
SUPPLEMENTAL MATERIAL FOR

Dystonia in a Timothy syndrome mouse model uncovers an interaction between Ca^{2+} and metabolism

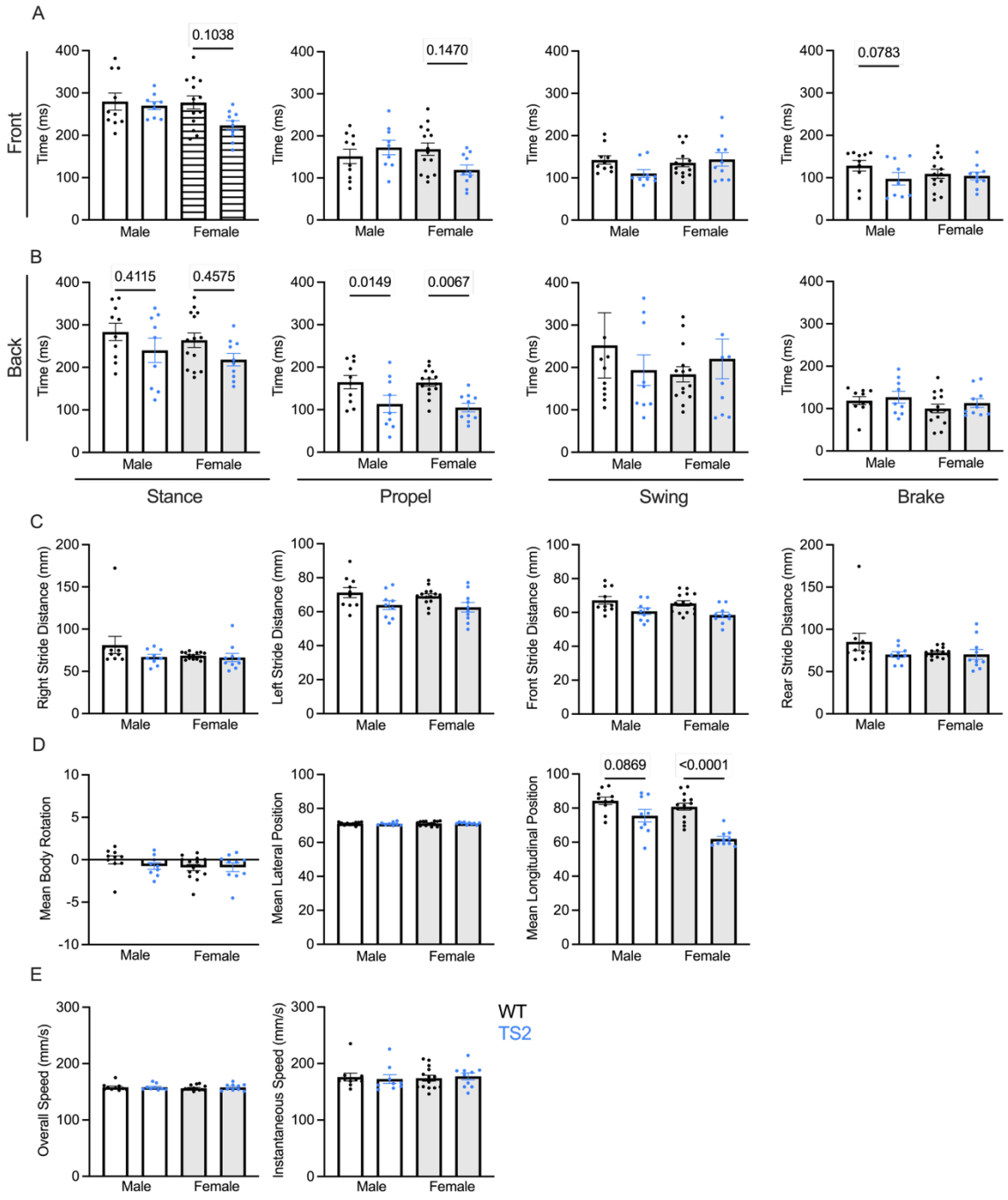
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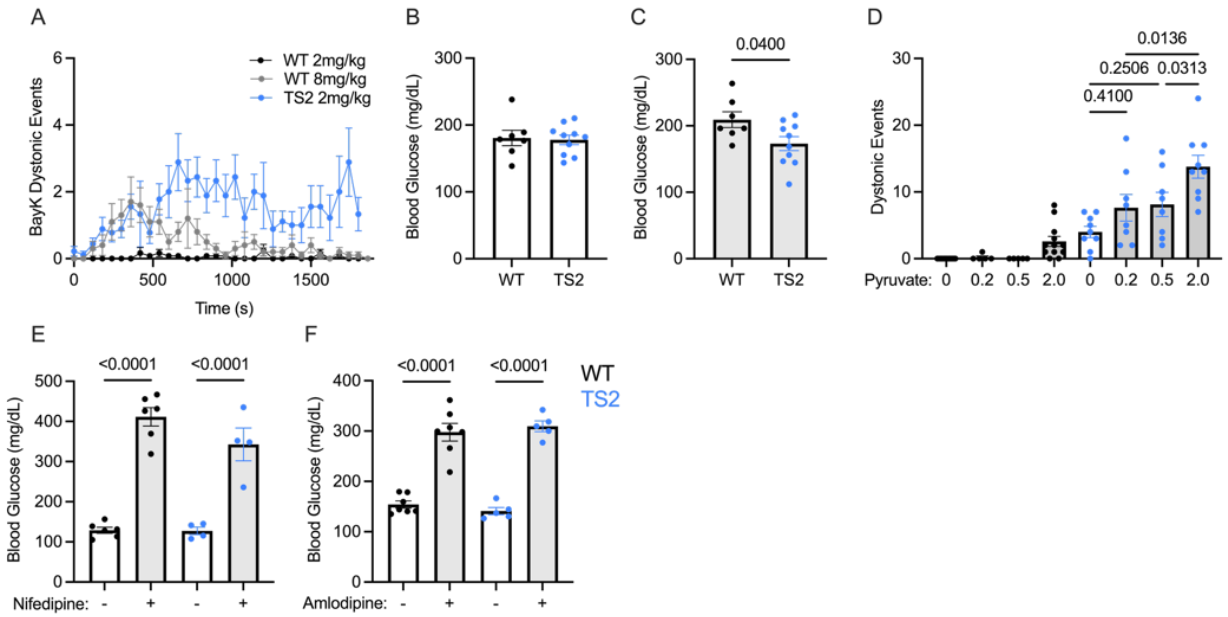
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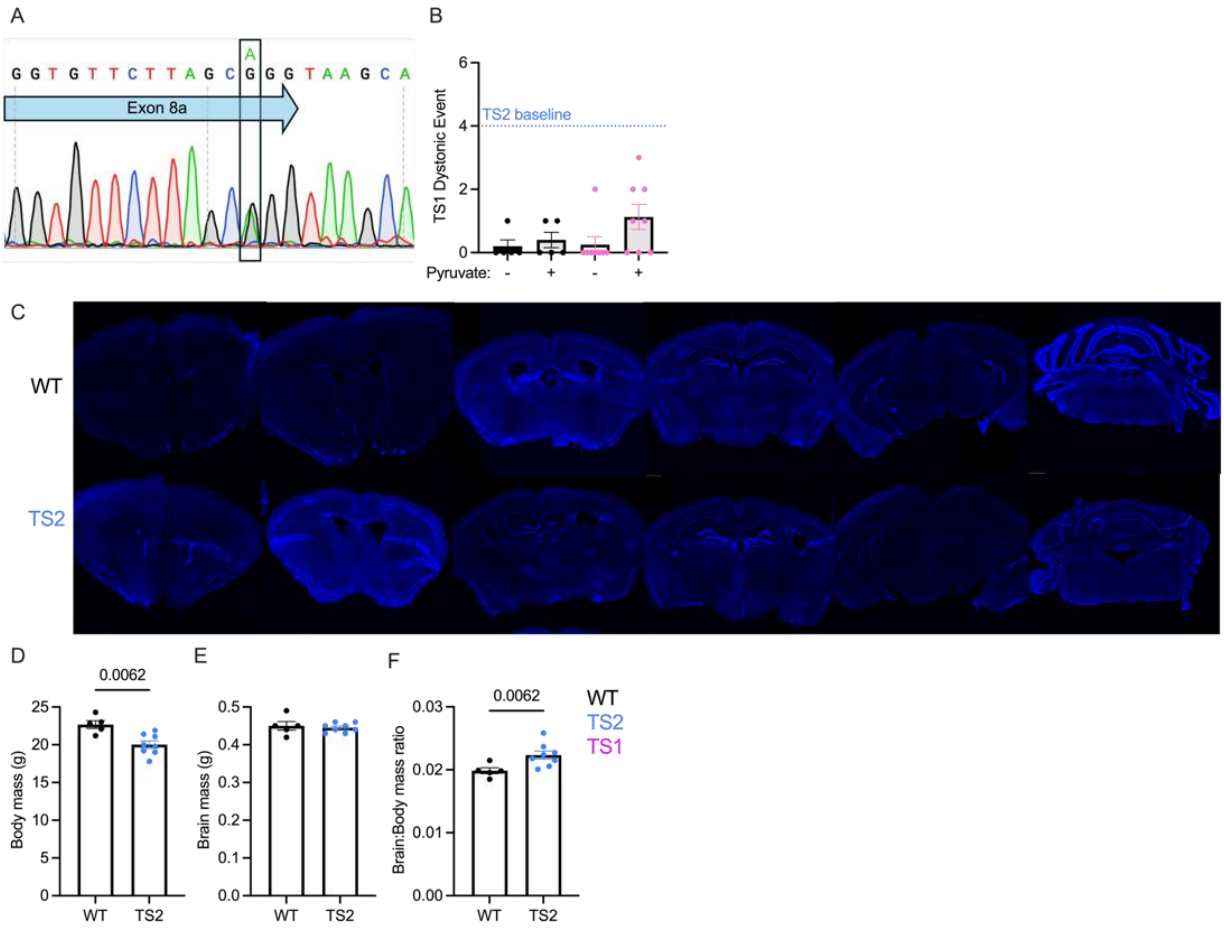
Supplemental Figure 1. Mild motor dysfunction in TS2 male and female mice
(**A,B**) Latency to fall on the steady-state (A) and accelerating (B) rotarod (n = 10 M and 14 F WT, 8 M and 11 F TS2). (**C**) Distance traveled in the open field per 5 minutes (n = 13 M and 12 F WT, 9 M and 9 F TS2). (**A-C** One-way ANOVA adjusted for multiple comparisons. **B** Male and female symbols indicate significance between WT and TS2 in each sex).



Supplemental Figure 2. Mild gait and posture difference in TS2 male and female mice (**A,B**) Duration of each component of gait, (A) forepaw: stance, propel, swing, and brake, (B) hindpaw: stance, propel, swing, and brake. **(C)** Distance of right, left, front, and rear stride length. (**D**) Body rotation during gait; mean, mean lateral, and mean longitudinal body position during gait. **(E)** Overall and instantaneous run speed. (**A-E** n = 10 male and 14 female WT, 9 male and 10 female TS2). (**A-E** One-way ANOVA adjusted for multiple comparisons).

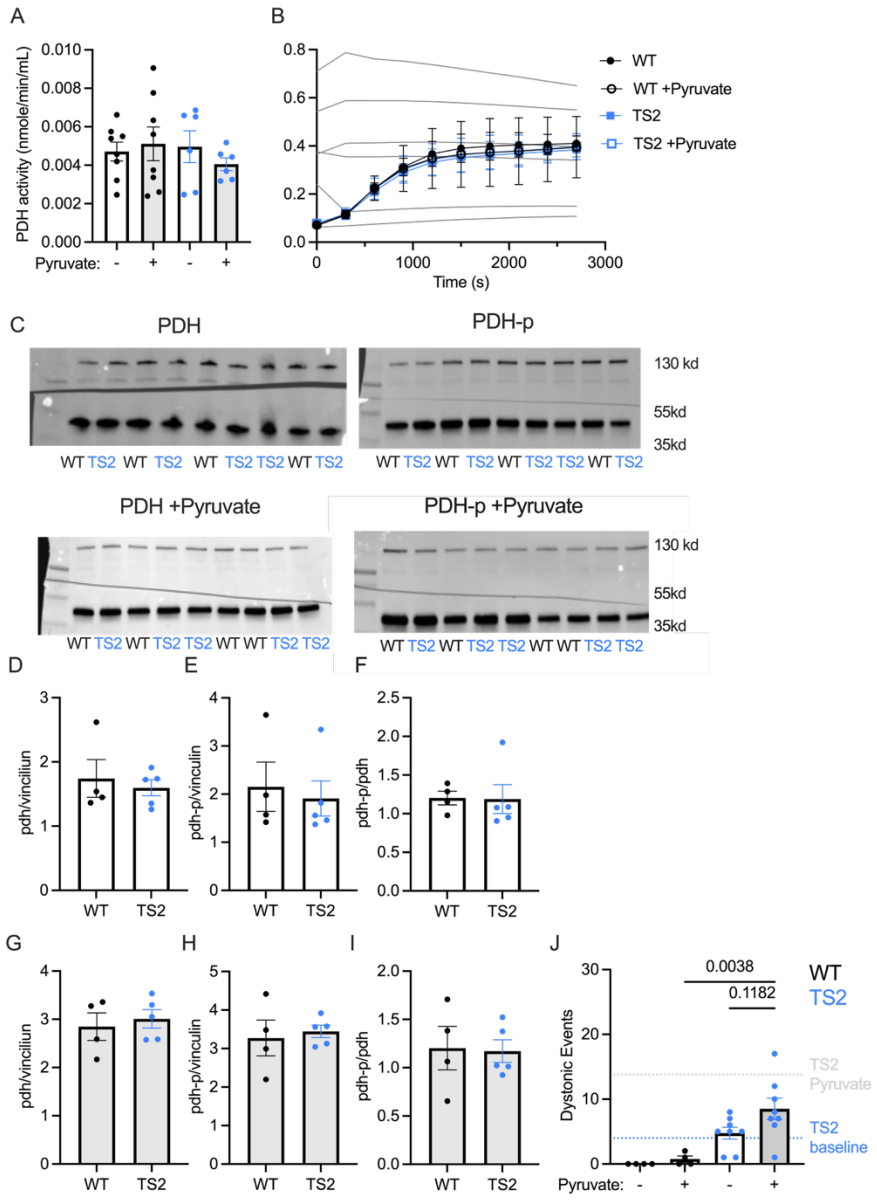


Supplemental Figure 3. TS2 mice have normal response to CCBs
(**A**) Time course of dystonic events post treatment with 2 or 8 mg/kg of Bay K 8644 (n = 12 WT 2 mg, 10 WT 8 mg, 9 TS2). (**B,C**) Blood glucose levels of WT and TS2 mice at baseline (B, Welch's t test p = 0.8363) and 1 minute after pyruvate administration (C, Welch's t test p = 0.04) (n = 7 WT, 10 TS2). (**D**) Dystonic events observed at baseline and after 0.2, 0.5, or 2.0 g/kg of pyruvate administration (n = 5-12 WT, 8-9 TS2 One-way ANOVA adjusted for multiple comparisons). (**E,F**) Blood glucose measurements at baseline and after nifedipine (E) or amlodipine (F) treatment. (n = 6 WT, 4 TS2 nifedipine and n = 7 WT, 5 TS amlodipine, One-way ANOVA adjusted for multiple comparisons).

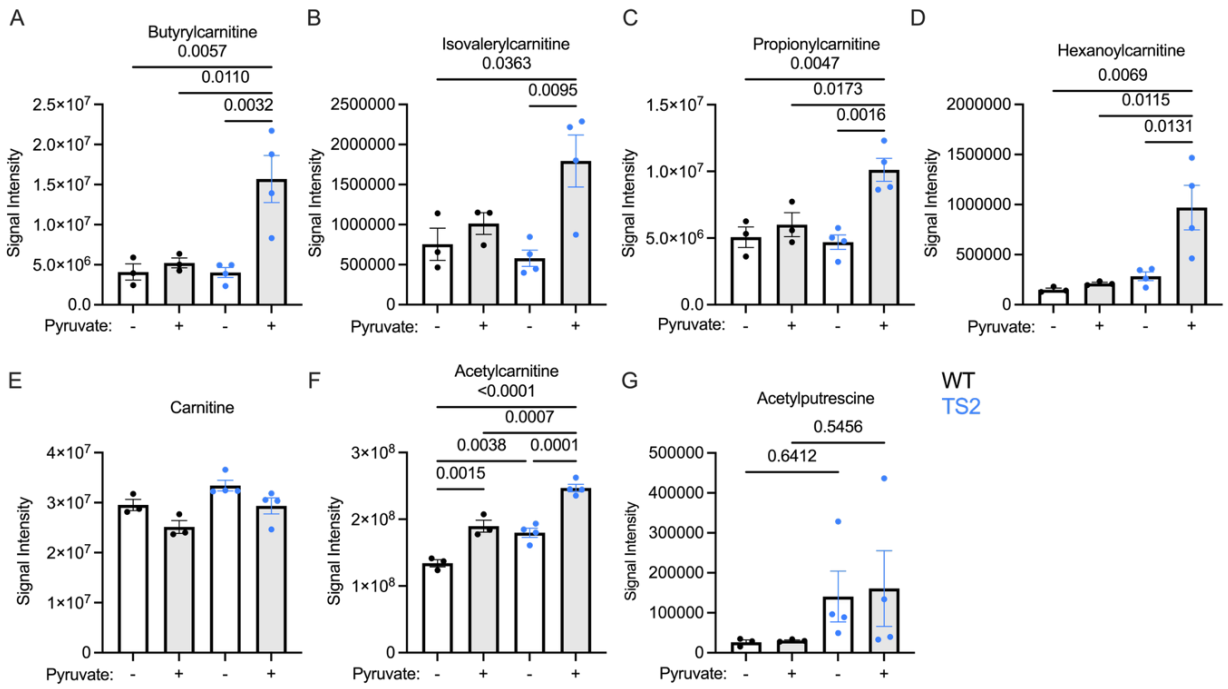


Supplemental Figure 4. TS2 mice have normal gross brain development

(**A**) Genomic sequencing from a TS1 mouse showing the single point mutation for the G406R mutation. (**B**) Dystonic events observed in TS1 mice at baseline and after pyruvate administration (n = 5 WT, 8 TS1, One-way ANOVA adjusted for multiple comparisons). (**C**) IHC of coronal brain sections stained with DAPI. (**D-F**) WT and TS body (D, Mann-Whitney test p = 0.0062) and brain (E, Mann-Whitney test p = 0.9308) weights and their ratio (F, Mann-Whitney test p = 0.0062) (n = 5 WT, 8 TS2). In B, dotted line represents basal mean TS2 dystonic events (y = 4, blue) from Figure 2C.

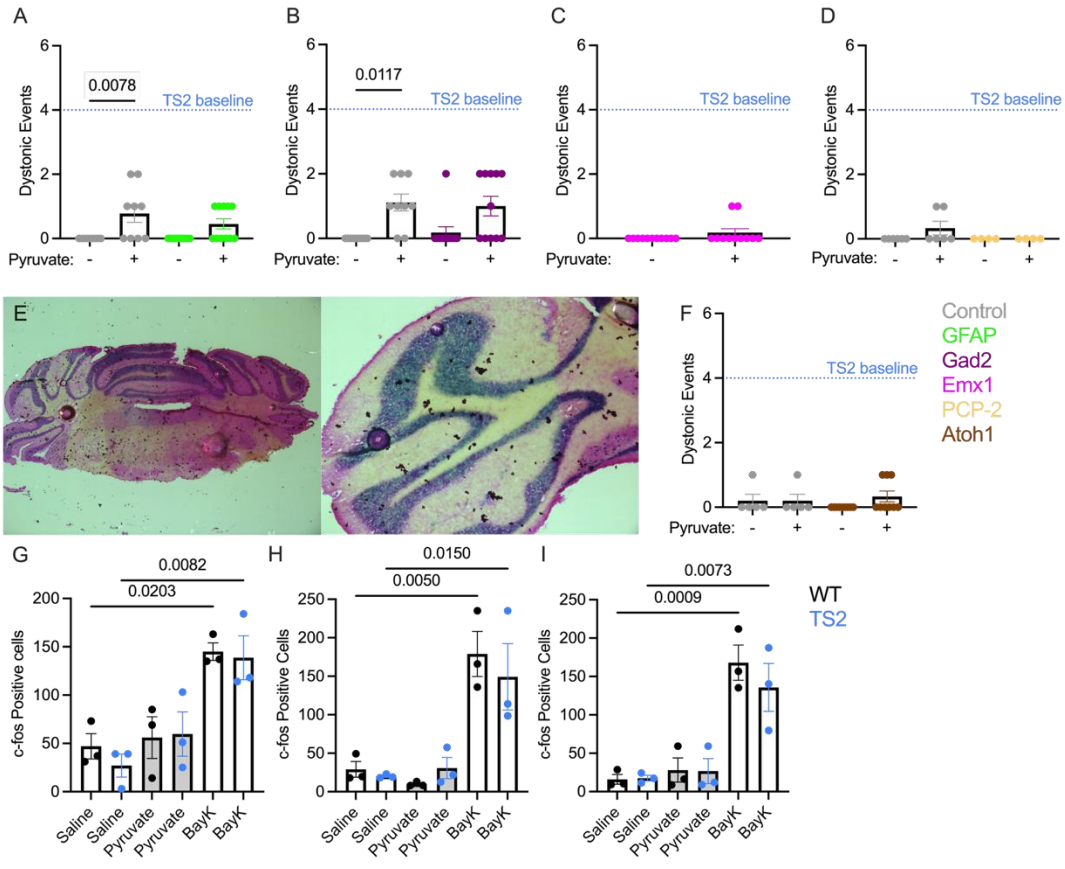


Supplemental Figure 5. Mutant Cav1.2 does not affect PDH level or activity
(**A,B**) PDH activity in WT and TS2 brain sample pretreated with either vehicle or pyruvate, measured by plate based colorimetric assay (n = 8 WT, 6 TS2, One-way ANOVA adjusted for multiple comparisons). (**C-I**) Western blots and quantification of whole brain lysates derived from WT and TS2 mice that were pretreated with either control or pyruvate administration before sample collection. (D-F) PDH (D, Mann-Whitney test p = 0.9048) and PDH-p (E, Mann-Whitney test p = 0.5556) levels at baseline and their ratio (F, Mann-Whitney test p = 0.4127). (G-I) PDH (G, Mann-Whitney test p = 0.9048) and PDH-p (H, Mann-Whitney test p > 0.9999) levels after pyruvate administration and their ratio (I, Mann-Whitney test p = 0.25) PDH (43 kd) and PDH-p (40 kd) were normalized to vinculin (117 kd) (n = 4 WT control, 3 WT pyruvate, 5 TS2 control, 5 TS2 pyruvate). (**J**) Dystonic events observed over 10 minutes following pretreatment with dichloroacetate (DCA 100 mg/kg) and administration of pyruvate (n = 4 WT and 8 TS2, One-way ANOVA adjusted for multiple comparisons). Dotted lines represent basal TS2 dystonic events (y = 4, blue) and TS2 pyruvate induced dystonic events (y = 13.78, silver) from Figure 2C.



Supplemental Figure 6. Serum metabolite levels affected by mutant $Ca_v1.2$ expression and pyruvate

(**A-G)** Relative levels of carnitine metabolites from serum profiling in WT and TS2 mice after pretreatment with either vehicle (saline) or pyruvate (n = 3 WT, 4 TS2). (**A-G** One-way ANOVA adjusted for multiple comparisons).



Supplemental Figure 7. Neuronal expression and effect of mutant $Ca_v1.2$ on response to pyruvate

(**A-D**) Dystonic events observed at baseline and following administration of pyruvate in cell-type specific expression of mutant $Ca_v1.2$ in astrocytes (A - GFAP), inhibitory neurons (B - Gad2), forebrain excitatory neurons (C - Emx1, Mann-Whitney test p = 0.4762), and cerebellar Purkinje cells (D - PCP-2). (**E**) LacZ reporter marking $Ca_v1.2$ expression in coronal brain sections of the cerebellum, with higher magnification demonstrating granule cell layer signal. (**F**) Dystonic events observed at baseline and following administration of pyruvate in cell-type specific expression of mutant $Ca_v1.2$ in cerebellar granule cells (Atoh1). (**G-I**) Quantification of IHC performed with c-fos antibody in coronal brain sections of TS2 and WT mice that were pretreated with saline, pyruvate, or Bay K 8644 before sample isolation. Cell counts were performed in the locus coeruleus (G) as well as medial (H) and lateral (I) fields of view in the cortex (n = 3, One-way ANOVA adjusted for multiple comparisons). In A-D and F, dotted line represents mean basal dystonic events (y = 4, blue) in TS2 mice from Figure 2C. (A,B,D,F n = 4-13 per *Cre* line, One-way ANOVA adjusted for multiple comparisons).