

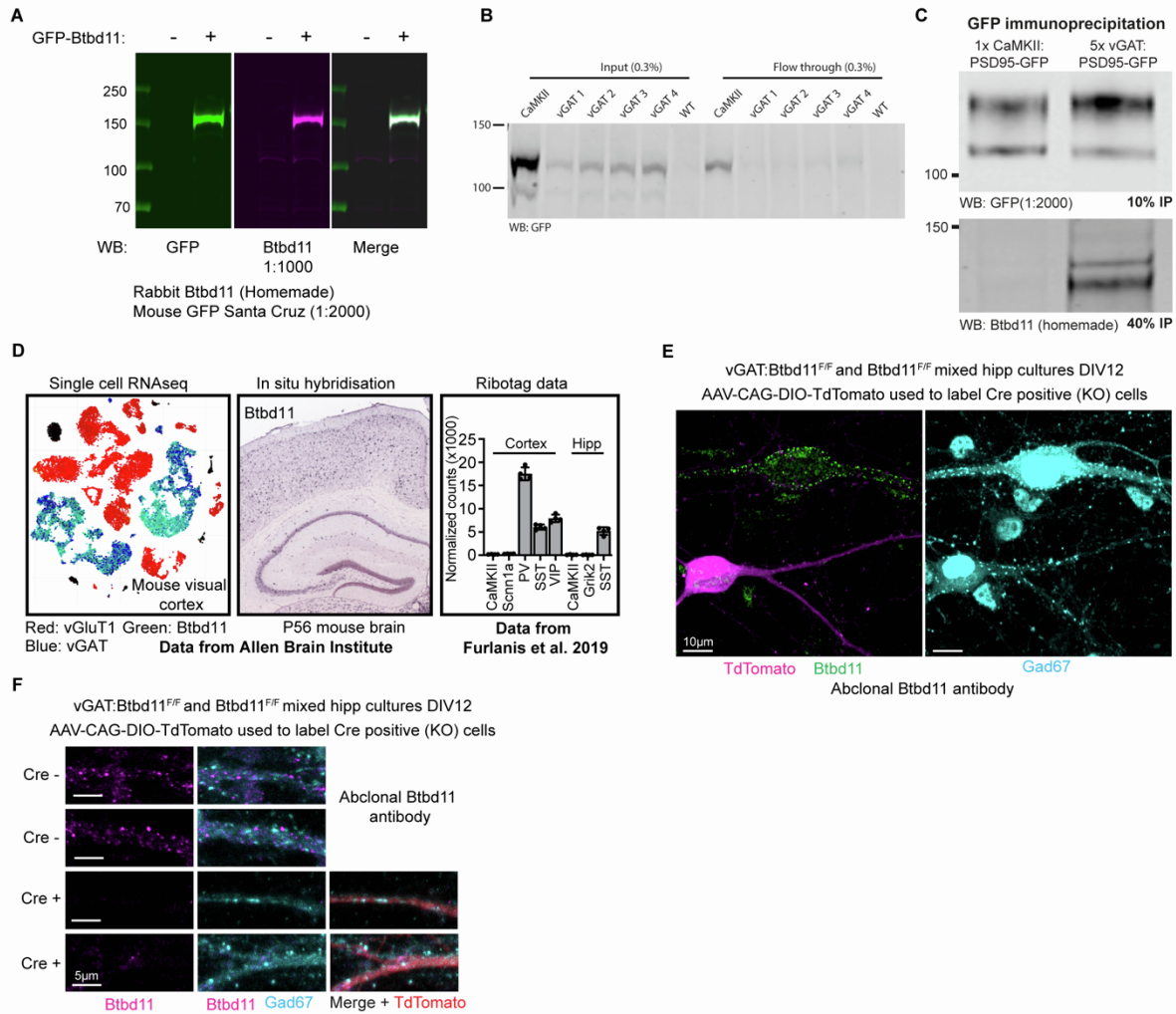
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**Supplemental information**

**Btbd11 supports cell-type-specific  
synaptic function**

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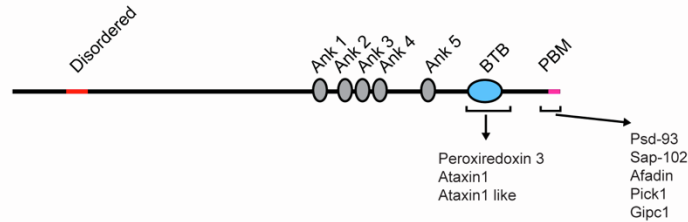
## SUPPLEMENTARY DATA



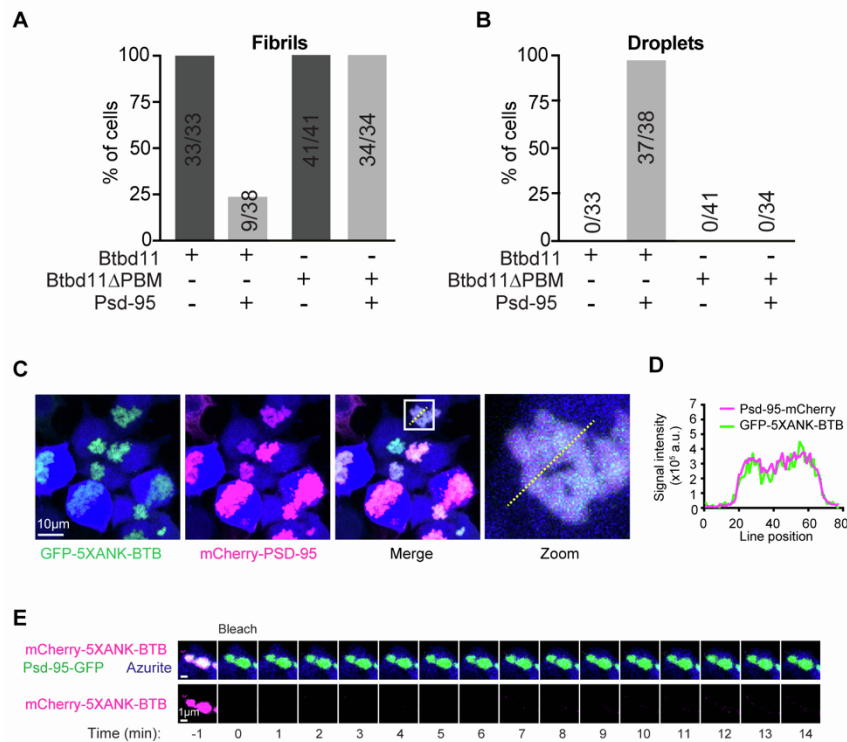
**Figure S1.** Validation of Btbd11 antibodies, GFP immunoprecipitation, and Btbd11 RNA expression profiles, related to Figure 1

(A) Western blot using lysate from HEK cells transfected with GFP-Btbd11 or non-transfected cells. Blots were probed with a homemade antibody against Btbd11 (magenta) or GFP (green). The merged image shows the overlap of the bands identified with each antibody. (B) Western blots showing the input and flow through fractions from the GFP immunoprecipitation experiment (pull down data shown in **Figure 1C**) probed with GFP antibody to detect Psd-95-GFP. (C) A repeat of the GFP immunoprecipitation as in **Figure 1C** except comparing 1x CaMKII:Psd-95-GFP animal with 5x vGAT:Psd-95-GFP (pooled) probed with a homemade Btbd11 antibody. (D) Btbd11 RNA expression data from the Allen Brain Institute mouse visual cortex RNAseq dataset (left panel) showing Btbd11 positive cells (green) also expressed vGAT (IN marker, blue), but not vGluT1 (EN marker, red). Data from the Allen Brain Institute *in situ* hybridization project (center panel) showing Btbd11 RNA expression. The sparse expression of Btbd11-positive cells is consistent with selective expression in inhibitory neurons but does not validate that the expression is in inhibitory neurons. (Right panel) Ribotag data, replotted using data from the SpliceCode resource<sup>24</sup> showing levels of Btbd11 being actively translated in ENs (CaMKII, Scnn1a, Grik2) and INs (PV, SST, VIP) neuron populations. Note the lack of Btbd11 RNA translation in excitatory neurons. (E) Validation of the specificity of the Abclonal Btbd11 antibody in mixed cultures of Btbd11<sup>F/F</sup> and vGAT:Btbd11<sup>F/F</sup> mice. Cultures made from mixed litters enable there to be INs in that same culture that were either Btbd11 KO

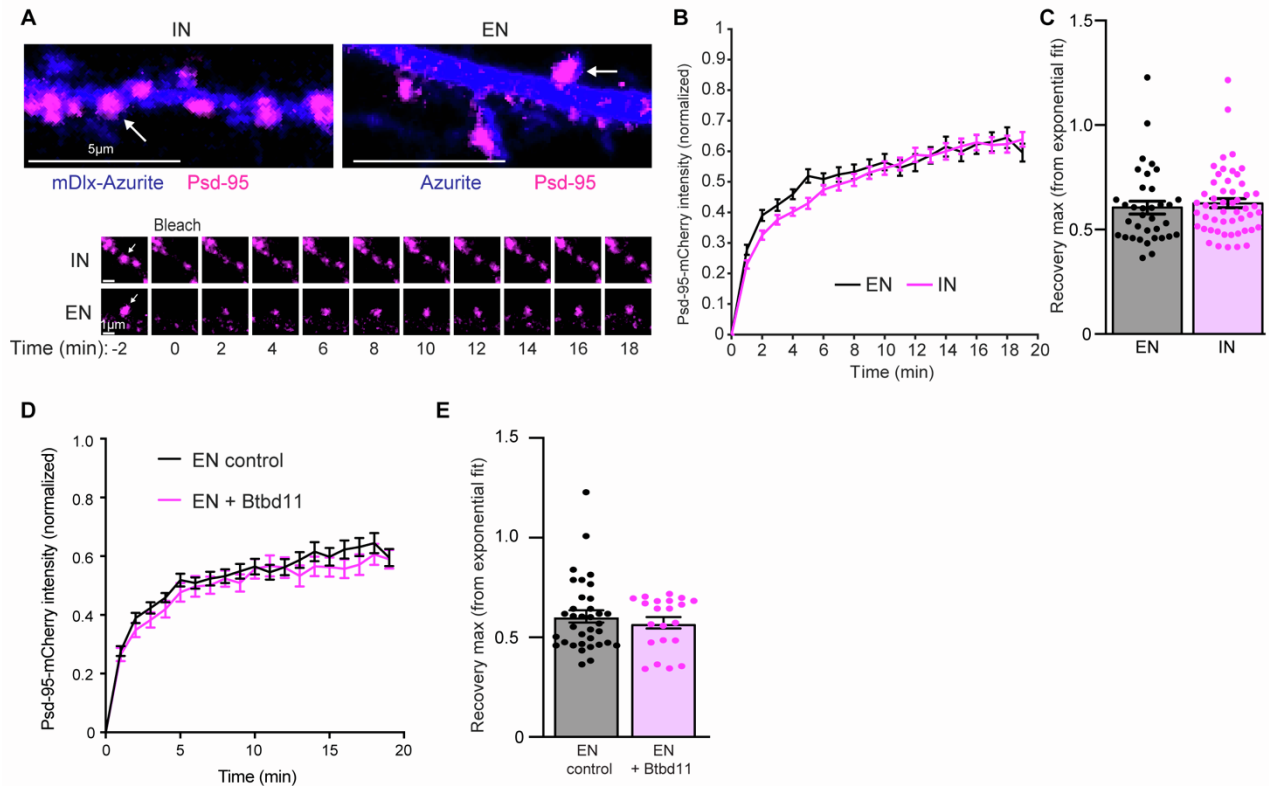
or control. There are 2 inhibitory neurons labeled in the field of view (Gad67 positive, cyan) where one of them is Cre-positive (tdTomato-positive, magenta). Note that the Cre positive cell is lacking the distinctive Btdb11 puncta seen in the other inhibitory neuron. Scale bar = 10 $\mu$ m. (F) Zoomed in dendritic regions of other Gad67-positive cells that were Cre negative (top two rows) or Cre positive (bottom two rows). Note that the Btdb11 puncta (magenta) are always absent in the Cre-positive Btdb11 KO cells. Scale bar = 5 $\mu$ m



**Figure S2.** Yeast 2-hybrid screen identifies putative Btdb11 interaction partners, *related to Figure 2*  
Schematic depiction of results from the yeast two-hybrid screen with the BTB domain or C-terminal portion of Btdb11 containing the PBM.

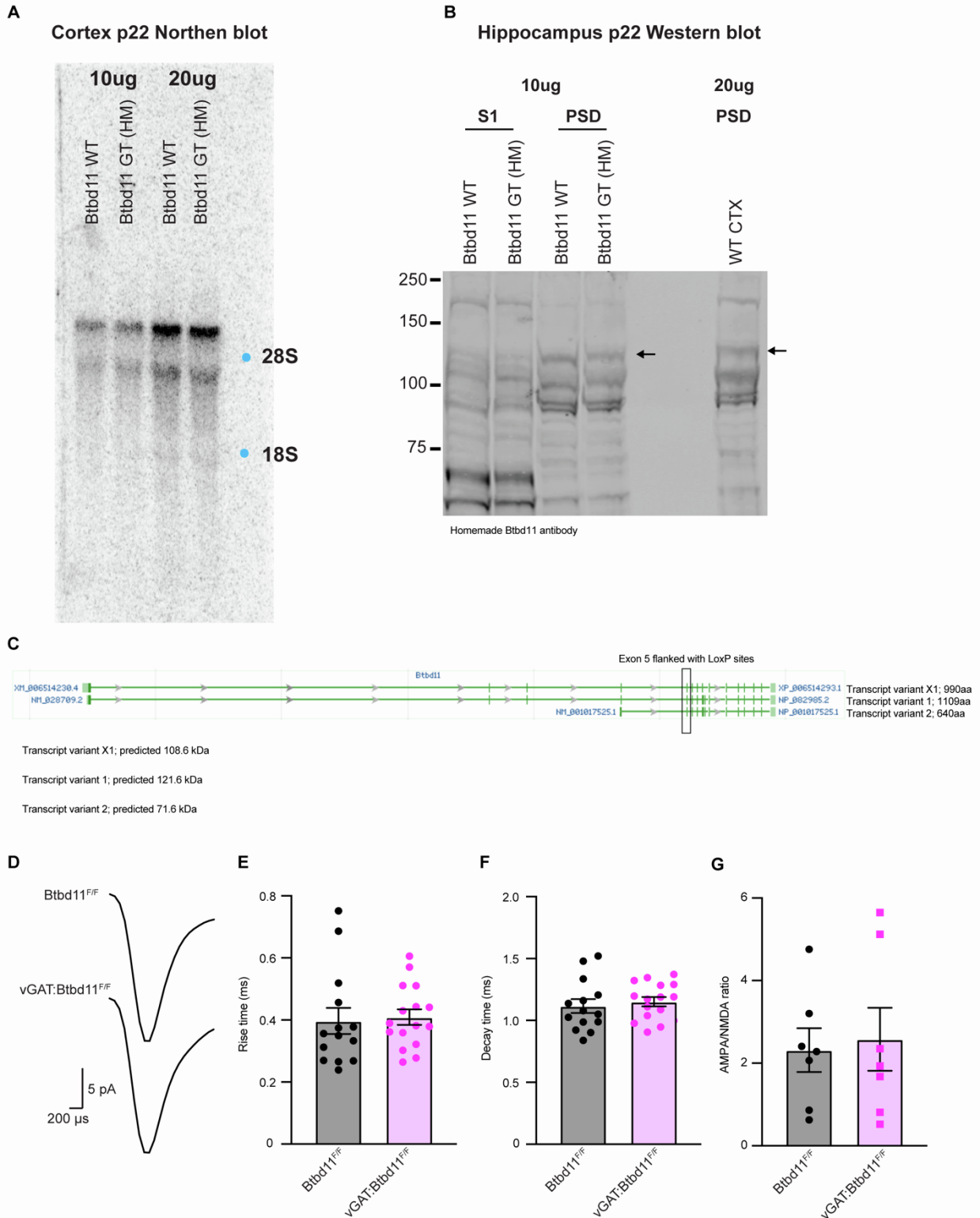


**Figure S3.** Liquid-liquid phase separation of Btdb11, *related to Figure 3*  
(A and B) Proportion of HEK cells which contain fibril-like assemblies or droplets, respectively, when GFP-Btdb11 or GFP-Btdb11 $\Delta$ PBM is expressed with or without Psd-95-mCherry. (C) Confocal image of HEK cells expressing GFP-5XANK-BTB (green), Psd-95-mCherry (magenta) and azurite (blue). Scale bar indicates 10 $\mu$ m. The white boxed area is enlarged on the right. The yellow dotted line indicates where a line-scan was evaluated. (D) Line scan showing signal intensity of GFP-5XANK-BTB (green) and Psd-95-mCherry (magenta). (E) FRAP of a mCherry-5XANK-BTB (magenta) and Psd-95-GFP (green) puncta with a composite image shown in the upper panels and just mCherry-5XANK-BTB shown on the bottom. Scale bar = 1 $\mu$ m. Note the lack of mCherry-5XANK-BTB signal recovery.



**Figure S4.** FRAP of Psd-95 in excitatory neurons, *related to Figure 4*

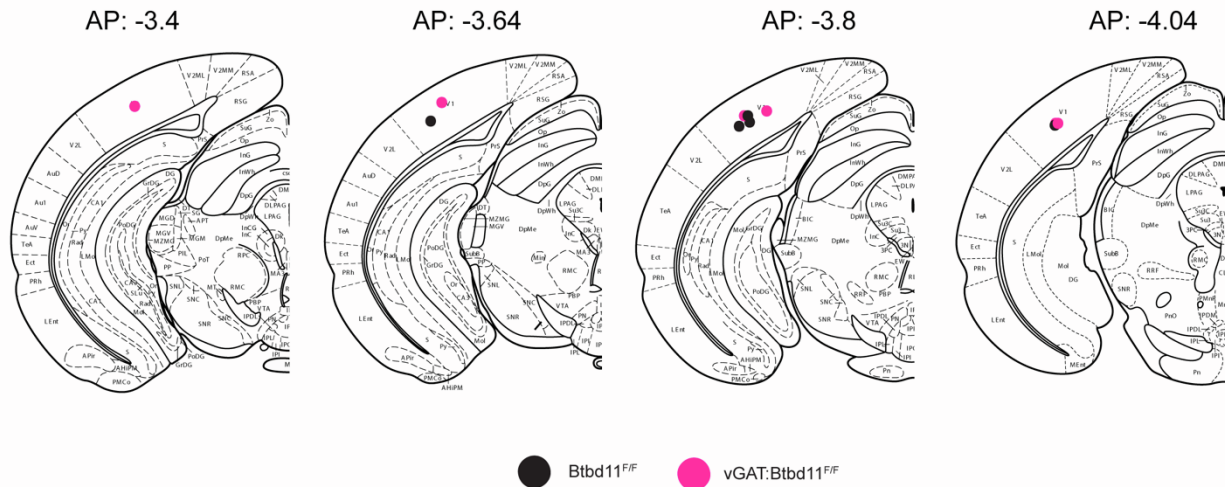
(A) Live-cell confocal imaging and FRAP of Psd-95-mCherry (magenta) in a putative interneuron (IN, identified with mDlx-Azurite) or excitatory neuron (EN, identified by the presence of dendritic spines with an Azurite cell-fill). The lower panels show FRAP of the individual puncta labeled in the upper panels with a white arrow. Respective scale bars are indicated at the bottom left of each image. (B) Quantification of Psd-95-mCherry FRAP in ENs (black) or INs (magenta). Lines display the mean and error bars display S.E.M. (C) Quantification of the estimated recovery maximum from an exponential fit of the FRAP data for each bleached punctum. EN data is shown in black and IN data is in magenta. Bars display mean and error bars show S.E.M. (D) Quantification of Psd-95-mCherry FRAP in ENs alone (black) or with overexpression of Btbd11 (magenta). Lines display the mean and error bars display S.E.M. (E) Quantification of the estimated recovery maximum from an exponential fit of the FRAP data for each bleached punctum. EN control data is shown in black and EN data with Btbd11 overexpression is in magenta. Bars display mean and error bars show S.E.M. Note the EN data without Btbd11 in panel (B) is the same as the control data presented in panel (D). See **Table S2** for full statistical information.



**Figure S5.** Characterization of Btbd11 Gene Trap mice and electrophysiology assessment of IN-specific Btbd11 KO animals, related to Figure 5

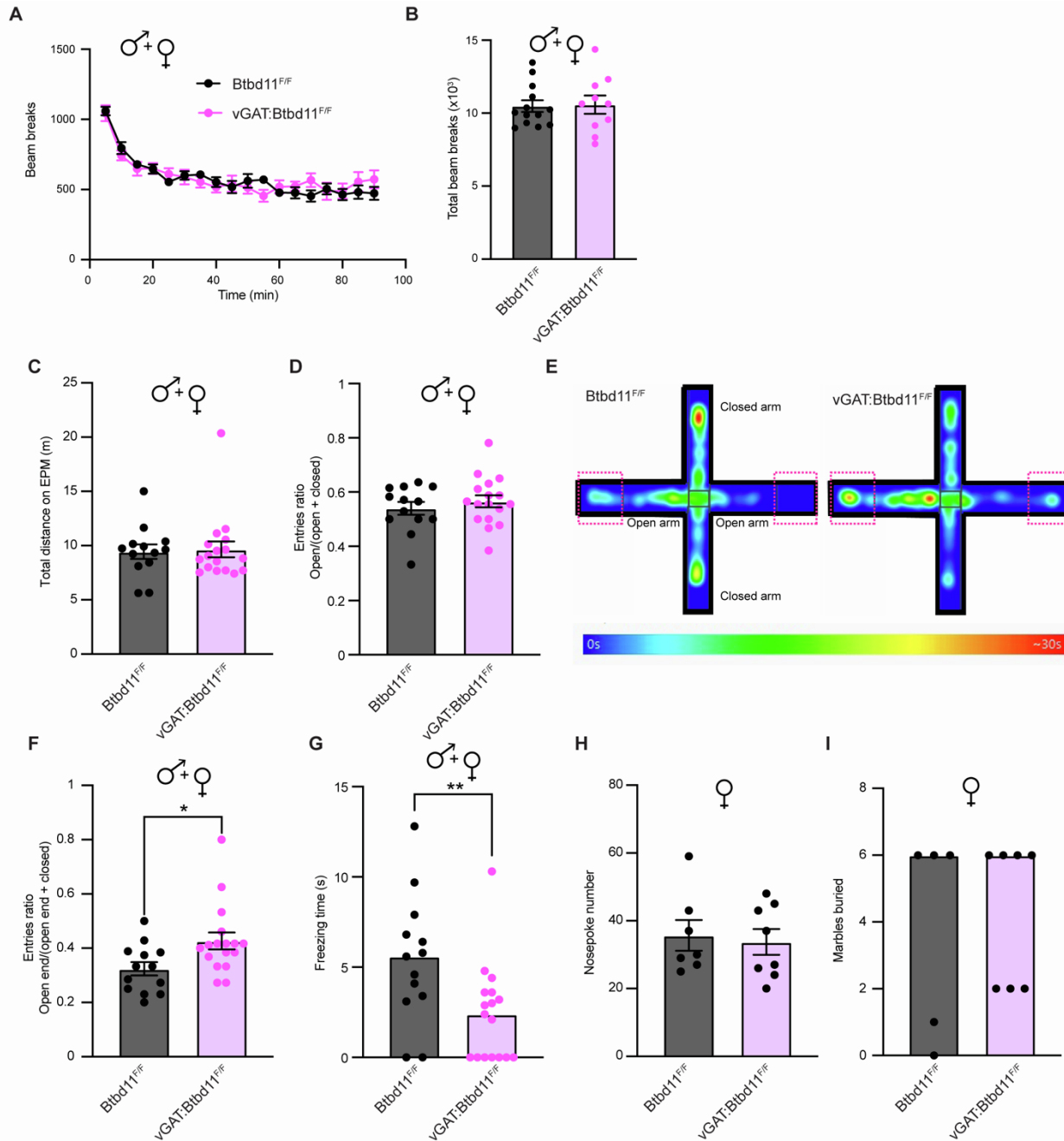
(A) Northern blots to assess the levels of Btbd11 mRNA in the cortex of postnatal day 22 Btbd11 wildtype (WT) or homozygous Btbd11 Gene Trap (GT) animals. Either 10μg or 20μg of mRNA was loaded. A blue

dot indicates the location of 18S and 28S ribosomal RNA. (B) Western blots to assess the level of Btbd11 in the hippocampus of Btbd11 wildtype (WT) or homozygous Btbd11 Gene Trap (GT) animals. 10 $\mu$ g of the PSD fraction was run, and membranes were probed with an antibody for Btbd11 (homemade antibody). A black arrow indicates the band corresponding to Btbd11. On the right 20 $\mu$ g of WT cortical PSD fraction was run to ensure that endogenous Btbd11 could be detected. (C) An illustration of the genomic region of Btbd11 (From NCBI, <https://www.ncbi.nlm.nih.gov/>). The black box indicates exon 5 that is flanked by loxP sites in the Btbd11<sup>F/F</sup> mice. Note that this variant is common to the three predicted splice variants of Btbd11. Transcript variant 1, with a predicted Mw of 121.6 kDa, is considered the major isoform and is what we have used for all Btbd11 overexpression studies. (D) The average mEPSC trace from representative cells from PV-INs from Btbd11<sup>F/F</sup> and vGAT:Btbd11<sup>F/F</sup> animals shown in **Figure 5F**. (E,F) Average mEPSC half-maximal rise time and half-maximal decay time, respectively, recorded from PV-INs in Btbd11<sup>F/F</sup> (Black) and vGAT:Btbd11<sup>F/F</sup> (magenta) animals. Bars display the mean and error bars show S.E.M. (G) AMPA/NMDA ratio in PV cells from Btbd11<sup>F/F</sup> (Black) and vGAT:Btbd11<sup>F/F</sup> (magenta) animals. Bars display the mean and error bars show S.E.M. See **Table S2** for full statistical information.



**Figure S6.** Electrode placement reconstructions, *related to Figure 6*  
Reconstructions of estimated electrode positioning based on electrolytic lesions. Black dots = Btbd11<sup>F/F</sup> and magenta dots = vGAT:Btbd11<sup>F/F</sup> animals, respectively. AP = anterior/posterior location relative to bregma. Note: there are two vGAT:Btbd11<sup>F/F</sup> with overlapping estimated lesion sites in the AP: -4.04 image.





**Figure S7. Behavioral experiments in IN-specific Btbd11 KO mice, related to Figure 7**

(A) Infrared beam breaks as a proxy for locomotor activity in an open field arena in the dark. Btbd11<sup>F/F</sup> (black) and vGAT:Btbd11<sup>F/F</sup> (magenta) animals explored the novel environment for 90 mins. Lines display the mean in 5 min time bins. Error bars show S.E.M. (B) Total beam breaks in the 90 min exploration period for Btbd11<sup>F/F</sup> (black) and vGAT:Btbd11<sup>F/F</sup> (magenta) animals. Bars display the mean and error bars show S.E.M. (C) The total distance traveled, and (D) ratio of entries to the open arm vs the closed arm of Btbd11<sup>F/F</sup> (black) and vGAT:Btbd11<sup>F/F</sup> (magenta) animals during 5 min exploration of the elevated plus maze. Bars display the mean, and error bars show S.E.M. (E) Representative heat maps of Btbd11<sup>F/F</sup> and vGAT:Btbd11<sup>F/F</sup> mice showing the time spent in different portions of the maze. (F) The ratio of entries into the end of the open arm (magenta box in panel E) compared to the closed arm in Btbd11<sup>F/F</sup> (black) and vGAT:Btbd11<sup>F/F</sup> (magenta) animals. Bars display the mean, and error bars show the S.E.M. \*  $p < 0.05$

0.05. (G) Time that  $Btbd11^{F/F}$  (black) and  $vGAT:Btbd11^{F/F}$  (magenta) animals spent freezing on the elevated plus maze apparatus during the 5 min test. Bars display the median. \*\*  $p < 0.01$ . (H) Number of nose pokes made by female  $Btbd11^{F/F}$  (black) and  $vGAT:Btbd11^{F/F}$  (magenta) animals on the hole board task. Bars display the mean, and error bars show the S.E.M. (I) Number of marbles buried by female  $Btbd11^{F/F}$  (black) and  $vGAT:Btbd11^{F/F}$  (magenta) animals. Bars display the median. See **Table S2** for full statistical information.



| inPSD hits with vGAT/CaMKII ratio with value > mean + 2x standard deviation |  |                              |
|---|--|------------------------------|
| Accession   | Description  | log norm ratio (vGAT/CaMKII) |
| NP_082985.2   | ankyrin repeat and BTB/POZ domain-containing protein BTBD11 isoform 1 [Mus musculus] | 3.37                         |
| NP_058017.1   | stress-induced-phosphoprotein 1 [Mus musculus]                                       | 2.41                         |
| XP_006534552.1  | PREDICTED: myosin-10 isoform X1 [Mus musculus]                                       | 2.23                         |
| NP_076288.1   | protein O-GlcNAcase [Mus musculus]   | 2.08                         |
| XP_006515861.1  | PREDICTED: TNF receptor-associated factor 3 isoform X1 [Mus musculus]                | 2.05                         |
| XP_006498025.1  | PREDICTED: E3 ubiquitin-protein ligase LRSAM1 isoform X1 [Mus musculus]              | 2.02                         |
| XP_006538016.1  | PREDICTED: F-box/LRR-repeat protein 4 isoform X1 [Mus musculus]                      | 2.00                         |
| XP_017175463.1  | PREDICTED: fatty-acid amide hydrolase 1 isoform X1 [Mus musculus]                    | 1.93                         |
| XP_006527892.1  | PREDICTED: glycerol kinase isoform X1 [Mus musculus]                                 | 1.92                         |
| NP_659136.1   | palmitoyltransferase ZDHHC5 [Mus musculus]   | 1.89                         |
| XP_017171979.1  | PREDICTED: oxidation resistance protein 1 isoform X1 [Mus musculus]                  | 1.88                         |
| XP_006511788.1  | PREDICTED: E3 ubiquitin-protein ligase ARIH2 isoform X1 [Mus musculus]               | 1.87                         |
| NP_085041.1   | E3 ubiquitin-protein ligase RNF34 [Mus musculus]                                     | 1.86                         |
| XP_006519125.1  | PREDICTED: E3 ubiquitin-protein ligase RNF31 isoform X1 [Mus musculus]               | 1.83                         |
| XP_006537872.1  | PREDICTED: PHD finger protein 24 isoform X1 [Mus musculus]                           | 1.75                         |
| NP_033750.1   | a disintegrin and metallopeptidase domain 4 precursor [Mus musculus]                 | 1.72                         |
| XP_011249102.1  | PREDICTED: glutamate receptor ionotropic, NMDA 2D isoform X1 [Mus musculus]          | 1.68                         |
| XP_006533949.1  | PREDICTED: mitochondrial Rho GTPase 1 isoform X1 [Mus musculus]                      | 1.65                         |
| NP_035234.1   | protein kinase C epsilon type [Mus musculus]   | 1.64                         |
| XP_006526184.1  | PREDICTED: E3 ubiquitin-protein ligase RNF14 isoform X1 [Mus musculus]               | 1.62                         |
| XP_006516266.1  | PREDICTED: WD repeat-containing protein 20 isoform X1 [Mus musculus]                 | 1.62                         |
| NP_033534.2   | vesicular inhibitory amino acid transporter [Mus musculus]                           | 1.60                         |

**Table S1.** Mass spectrometry results, *related to Figure 1*

Table of inPSD candidates identified in the cell type-specific Psd-95-GFP immunoprecipitation experiment. Proteins were quantified with label-free quantification and then normalized to levels of Psd-95. Then the vGAT/CaMKII ratio was calculated to identify putative proteins enriched at the PSD of inhibitory interneurons.

| Main Figures | Test  | Test statistic(s)                          | P-value  | N number information   |
|--------------|---|--|----------|--|
| Figure 1K    | Unpaired T-test (TOS)   | t=5.489, df=12                             | <0.0001  | Dendritic regions from 6 cells for Psd95, and 8 cells for gephyrin.  |
| Figure 1L    | Kolmogorov-Smirnov test (Puncta-to-puncta distance)           | Kolmogorov-Smirnov D = 0.7170              | <0.0001  | 528 Psd-95 puncta from 7 cells and 365 gephyrin puncta from 8 cells.   |
| Figure 2G    | One-way ANOVA (GST pull-down)                                 | F (2,6) = 514.0                            | <0.0001  | N = 3 per group  |
|              | Dunnett's multiple comparisons                                | WT vs. Btdb11DPBM; Mean Diff. = 90.05      | <0.0001  |  |
|              | Dunnett's multiple comparisons                                | WT vs. GST; Mean Diff. = 99.91             | <0.0001  |  |
| Figure 2I    | One-way ANOVA (GST pull-down)                                 | F (4,10) = 44.63                           | <0.0001  | N = 3 per group  |
|              | Dunnett's multiple comparisons                                | WT vs. dPDZ1,2; Mean Diff. = 75.17         | 0.0001   |  |
|              | Dunnett's multiple comparisons                                | WT vs. dPDZ3; Mean Diff. = -10.35          | 0.7386   |  |
|              | Dunnett's multiple comparisons                                | WT vs. dPDZ1,2,3; Mean Diff. = 80.50       | <0.0001  |  |
|              | Dunnett's multiple comparisons                                | WT vs. GST (no Btdb11); Mean Diff. = 99.90 | <0.0001  |  |
| Figure 4I    | Repeated measures ANOVA (FRAP)                                |  |          | N=52 puncta for control from 10 cells and N = 50 puncta with Btdb11 from 11 cells.                                 |
|              | Time vs. Condition (+/- Btdb11)                               | F (4,400) = 0.3815                         | 0.8219   |  |
|              | Time  | F (4,400) = 2.693                          | 0.0307   |  |
|              | Condition (+/- Btdb11)  | F (1,100) = 5.755                          | 0.0183   |  |
| Figure 4J    | Unpaired T-test (FRAP vMAX)                                   | t=2.417, df=100                            | 0.0175   | N=52 puncta for control from 10 cells and N = 50 puncta with Btdb11 from 11 cells.                                 |
| Figure 5G    | Unpaired T-test (mEPSC amplitude)                             | t=0.6264, df=28                            | 0.5361   | N = 14 cells from control (1 female, 2 male) and 16 cells from vGAT:Btdb11 KO (2 female and 1 male).               |
| Figure 5H    | Unpaired T-test (mEPSC frequency)                             | t=6.741, df=28                             | <0.0001  | N = 14 cells from control (1 female, 2 male) and 16 cells from vGAT:Btdb11 KO (2 female and 1 male).               |
| Figure 5K    | Unpaired T-test (PPR)   | t=0.7390, df=14                            | 0.4721   | N = 8 cells from 2 mice per group (all male).  |
| Figure 5M    | Unpaired T-test (Psd-95 puncta density)                       | t=0.4752, df=38                            | 0.6374   | N = 20 dendrites (one per cell) for each group. 4 mice per group aged 6 weeks (Con, 4 male; KO, 2 male, 2 female). |
| Figure 5N    | Unpaired T-test (Psd-95 puncta size)                          | t=2.191, df=38                             | 0.0347   | N = 20 dendrites (one per cell) for each group. 4 mice per group aged 6 weeks (Con, 4 male; KO, 2 male, 2 female). |
| Figure 6C    | Unpaired T-test (Spontaneous Ca <sup>2+</sup> transients)     | t=5.763, df=51                             | <0.0001  | 26 fields of view for control (GFP) and 27 fields of view for KO (GFPcre) from 3 batches of cultures.              |
| Figure 6E    | Unpaired T-test (PV immunofluorescence)                       | t=7.388, df=114                            | <0.0001  | N = 59 control cells (GFP) and 57 KO cells (GFPcre) from 4 batches of cultures.                                    |
| Figure 6G    | Unpaired T-test (PV western blot)                             | t=4.617, df=12                             | 0.0006   | N = 7 pair of samples (GFP or GFPcre) from 4 culture batches.  |
| Figure 6K    | Unpaired T-test (Ratio of mean 55-65 Hz)                      | t=2.797, df=9                              | 0.0208   | N = 5 for control and N = 6 for vGAT:Btdb11 (all male 2-5 months)  |
| Figure 6L    | Unpaired T-test (Ratio of peak 55-65 Hz)                      | t=2.969, df=9                              | 0.0157   | N = 5 for control and N = 6 for vGAT:Btdb11 (all male 2-5 months)  |
| Figure 7A    | Repeated measures ANOVA (Locomotor activity in light 30 mins) |  |          | N = 13 control (6 male, 7 female) and N = 17 vGAT:Btdb11 KO (9 male and 8 female) aged 2.5 - 7 months.             |
|              | Time vs. Genotype   | F (5, 140) = 0.7804                        | P=0.5654 |  |
|              | Time  | F (3.294, 92.23) = 49.98                   | P<0.0001 |  |
|              | Genotype  | F (1, 28) = 1.950                          | P=0.1735 |  |

|   |  |                                    |                |   |
|---|--|------------------------------------|----------------|---|
| Figure 7B   | Unpaired T-test (open field rearing events)                                  | t=3.766, df=28                     | 0.0008         | N = 13 control (6 male, 7 female) and N = 17 vGAT: Btbd11 KO (9 male and 8 female) aged 2.5 - 7 months.   |
| Figure 7C   | Unpaired T-test (open field center beam breaks in 5 min)                     | t=2.372, df=28                     | 0.0248         | N = 13 control (6 male, 7 female) and N = 17 vGAT: Btbd11 KO (9 male and 8 female) aged 2.5 - 7 months.   |
| Figure 7D   | Unpaired T-test (nose poke male only)  | t=2.935, df=13                     | 0.0116         | N = 13 control (6 male, 7 female) and N = 17 vGAT: Btbd11 KO (9 male and 8 female) aged 2.5 - 7 months.   |
| Figure 7F   | Mann Whitney test (marbles buried male only)                                 | Mann-Whitney U (21)                | 0.0187         | N = 13 control (male 5) and 8 vGAT: Btbd11 (male) aged 2.5 - 4 months.  |
| Figure 7G   | Unpaired T-test (Y maze spontaneous alternation)                             | t=1.469, df=21                     | 0.1567         | N = 13 control (9 female and 4 male) and 10 vGAT: Btbd11 (6 female and 4 male) animals aged 5-6 months.   |
| Figure 7H   | Unpaired T-test (T maze spontaneous alternation)                             | t=0.8051, df=28                    | 0.4276         | N = 13 control (6 male, 7 female) and N = 17 vGAT: Btbd11 KO (9 male and 8 female) aged 2.5 - 7 months.   |
| Figure 7I   | Unpaired T-test (contextual fear conditioning)                               | t=0.4983, df=25                    | 0.6226         | N = 17 control (13 male 4 female) and 10 vGAT: Btbd11 (3 female 7 male) aged 2.5 - 4 months.  |
| Figure 7K   | Two-way ANOVA (MK-801 induced locomotor activity)                            |                                    |                | N = 9 control (5 female 4 male) and 7 knockout (3 female and 4 male) aged 5-6 months.   |
|   | Drug vs. Genotype  | F (1, 14) = 4.875                  | 0.0444         |   |
|   | Drug   | F (1, 14) = 42.84                  | <0.0001        |   |
|   | Genotype   | F (1, 14) = 7.815                  | 0.0143         |   |
|   | Šídák's multiple comparisons test (Con vs. knockout under vehicle condition) | Predicted (LS) mean diff. = -168.3 | 0.9697         |   |
| Šídák's multiple comparisons test (Con vs. knockout under MK-801 condition) | Predicted (LS) mean diff. = -2663  | 0.0031                             |                |   |
| <b>Supplementary Figures</b>  | <b>Test</b>  | <b>Test statistic(s)</b>           | <b>P-value</b> | <b>N number information</b>   |
| Figure S4B  | Repeated measures ANOVA (FRAP INs vs. ENs)                                   |                                    |                | N=52 puncta from 10 inhibitory cells; N = 35 puncta from 7 excitatory neurons   |
|   | Time vs. Condition (EN vs. IN)   | F (4, 340) = 2.825                 | 0.0249         |   |
|   | Time   | F (4, 340) = 1.832                 | 0.1222         |   |
|   | Condition (EN vs. IN)  | F (1, 85) = 0.04326                | 0.8357         |   |
| Figure S4C  | Unpaired T-test (FRAP vMAX INs vs. ENs)                                      | t=0.5684, df=85                    | 0.5713         | N=52 puncta from 10 inhibitory cells; N = 35 puncta from 7 excitatory neurons   |
| Figure S4D  | Repeated measures ANOVA (FRAP in ENs)  |                                    |                | N = 35 puncta from 7 excitatory neurons for control; N=21 puncta from 4 excitatory neurons with Btbd11  |
|   | Time vs. Condition (+/- Btbd11)  | F (4, 216) = 1.572                 | 0.1829         |   |
|   | Time   | F (4, 216) = 3.272                 | 0.0125         |   |
|   | Condition (+/- Btbd11)   | F (1, 54) = 0.8301                 | 0.3663         |   |
| Figure S4E  | Unpaired T-test (FRAP vMAX +/- Btbd11 within ENs)                            | t=0.7092, df=54                    | 0.4813         | N = 35 puncta from 7 excitatory neurons for control; N=21 puncta from 4 excitatory neurons with Btbd11  |
| Figure S5B  | Unpaired T-test (mEPSC rise time)  | t=0.2549, df=28                    | 0.8007         | N = 14 cells from control (1 female, 2 male) and 16 cells from vGAT: Btbd11 KO (2 female and 1 male).   |
| Figure S5C  | Unpaired T-test (mEPSC decay time)   | t=0.5366, df=28                    | 0.5958         | N = 14 cells from control (1 female, 2 male) and 16 cells from vGAT: Btbd11 KO (2 female and 1 male).   |
| Figure SD   | Unpaired T-test (AMPA/NMDA ratio)  | t=0.2855, df=12                    | 0.7801         | N = 8 cells from 2 mice per group (all male). Note, one cell was removed per group as there were deemed outliers (value greater than mean +2SD) |
| Figure S7A  | Repeated measures ANOVA  |                                    |                |   |

|            |  |                          |         |  |
|------------|--|--------------------------|---------|--|
|            | (Locomotor activity in dark 90 mins)             |                          |         | N = 13 control (9 female and 4 male) and 10 vGAT:Btbd11 (6 female and 4 male) animals aged 5-6 months. |
|            | Time vs. Genotype                                | F (17, 357) = 1.622      | 0.0565  |  |
|            | Time   | F (7.365, 154.7) = 35.12 | <0.0001 |  |
|            | Genotype   | F (1, 21) = 0.02106      | 0.8860  |  |
| Figure S7B | Unpaired T-test (total beam breaks)              | t=0.1451, df=21          | 0.8860  | N = 13 control (9 female and 4 male) and 10 vGAT:Btbd11 (6 female and 4 male) animals aged 5-6 months. |
| Figure S7C | Unpaired T-test (EPM distance)                   | t=0.1954, df=28          | 0.8465  | N = 13 control (6 male, 7 female) and N = 17 vGAT:Btbd11 KO (9 male and 8 female) aged 2.5 - 7 months. |
| Figure S7D | Unpaired T-test (EPM entries ratio)              | t=0.7875, df=28          | 0.4376  | N = 13 control (6 male, 7 female) and N = 17 vGAT:Btbd11 KO (9 male and 8 female) aged 2.5 - 7 months. |
| Figure S7F | Unpaired T-test (EPM entries at arm end ratio)   | t=2.449, df=28           | 0.0208  | N = 13 control (6 male, 7 female) and N = 17 vGAT:Btbd11 KO (9 male and 8 female) aged 2.5 - 7 months. |
| Figure S7G | Mann-Whitney U Test (EPM freezing time)          | Mann-Whitney U (49)      | 0.008   | N = 13 control (6 male, 7 female) and N = 17 vGAT:Btbd11 KO (9 male and 8 female) aged 2.5 - 7 months. |
| Figure S7H | Unpaired T-test (nose poke female only)          | t=0.3338, df=13          | 0.7439  | N = 7 control ( female) and N = 8 vGAT:Btbd11 KO (female) aged 2.5 - 7 months.                         |
| Figure S7I | Mann-Whitney U Test (marbles buried female only) | Mann-Whitney U (15)      | 0.7348  | N = 5 control (female) and 7 vGAT:Btbd11 (female) aged 2.5 - 4 months.                                 |

**Table S2.** Details of statistical tests

Table with statistical details for all tests performed and information about the N for each experiment.

## VIDEO TITLES

### Video S1

Alpha Fold prediction of Btbd11 structure. Based on UniProt annotations and the recognition of a putative PDZ binding motif (PBM) portions of the protein are shaded as follows: red = disordered, gray = ankyrin repeats (Ank), blue = BTB domain (BTB), magenta = PDM.

### Video S2

Time-lapse of two GFP-Btbd11 (green) and Psd-95-mCherry (magenta) puncta fusing over time. The cell also contains an azurite cell-fill (blue). This is the same data presented in **Figure 3K**.

### Video S3

Longitudinal live-cell imaging of a hippocampal neuron expressing an Azurite cell-fill (Blue) and GFP-Btbd11 (Green). A portion of a GFP-Btbd11 fibril is bleached. Note the lack of fluorescence recovery.

### Video S4

Live cell imaging of jRGECO1a in control cultures (Btbd11<sup>F/F</sup> with AAV-GFP). Note the synchronous Ca<sup>2+</sup> transients.

### Video S5

Live cell imaging of jRGECO1a in Btbd11 knockout cultures (Btbd11<sup>F/F</sup> with AAV-GFP-Cre). Note that the synchronous Ca<sup>2+</sup> transients appear more frequent than in control conditions (**Video S4**).