

**Supplementary Figure 1.** Effects of *Btbd3* knockout on additional behavioral measures. In the Go/No-Go paradigm (n = 12 WT, 11 HT, 16 KO mice, all male), there was no difference between genotypes for discrimination index *d'* overall (a, left) or across days (a, right). *Btbd3* genotype did not affect false alarm rate overall (b, left) or across days (b, right), but did affect premature responses, with an overall reduction in *Btbd3* HT and KO mice (c, left) that did not differ across days (c, right). *Btbd3* genotype did not affect winstay responses on the target port (d) or lose-shift responses on the non-target port (e) in the PLT (n = 13 WT, 16 HT, 17 KO mice, all male). No differences in prepulse inhibition (f) or acoustic startle amplitude (g) were identified between genotypes (n = 14 female/15 male WT, 15 female/14 male HT, 14 female/14 male KO mice). *Btbd3* genotype did not affect spatial *d* in the open field (n = 17 female/27 male WT, 34 female/27 male HT, and 11 female/20 male KO mice). Results are expressed as mean values ± SEM. \*p<.05 vs WT group as determined by ANOVA and post hoc tests. WT: wild-type HT: heterozygous KO: knockout PLT: probabilistic learning task.

## Olfactory Dishabituation & Memory а Trial p<.0001 16 \*[**‡**₩ 12 % Sniffs Sniffs ☐ Trial 1 ☐ Trial 2 ☐ Trial 3 8 Trial 1 Trial 2 Whisker Brushing Test С Right Whisker Response Left Whisker Response 8. .8 .6 .6 .4 .4 .2 .2 0 WT HT KO WT HT KO **Footprint Test** f е Forelimb Stride (cm) Hindlimb Stride (cm) 2 0 WT HT KO WT HT KO h g Hind Base Width (cm) 2.0 WT HT KO WT HT KO Bodyweight i j 30] Overlap (cm) Bodyweight (g) 20 10 0. WT HT KO WT HT KO

Supplementary Figure 2. Btbd3 knockout does not have robust effects on sensory or motor capabilities (n = 12/genotype/sex except for female WT, n = 11 mice). In the olfactory dis/habituation test (a), no effect of Btbd3 genotype was identified for habituation to repetition of an odor or dishabituation when a new odor was introduced. In the olfactory memory test (b), no effect of Btbd3 genotype on olfactory memory was found, but an overall increase in instances of sniffing was seen in Btbd3 KO mice. No effect of genotype on left (c) or right (d) whisker response was found (n = 15/genotype/sex). No effect of Btbd3 genotype was identified in the footprint test (n = 12/genotype/sex) for any measure (e-i). Young adult Btbd3 KO had significantly reduced bodyweight relative to WT mice (j). Results are expressed as mean values ± SEM, except for panels depicting categorical data, which does not have error. \*p<.05 vs WT group as determined by ANOVA and post hoc tests. WT: wild-type HT: heterozygous KO: knockout.

Supplementary Figure 3. Btbd3 knockout does not cause major alterations in limbic CSTC circuit morphology. dendritic Apical dendritic branching was unaffected by Btbd3 genotype in granule neurons of the dentate gyrus (n = 6 WT, 5 HT, 5 KO mice, all female) (a), CA1 pyramidal neurons (c), and spiny stellate neurons in mediodorsal thalamus (e). Spine density was unaffected by genotype in the hippocampus, in both the dentate gyrus (b) and CA1 (d). Layer II/III pyramidal neurons in ACC showed a mild effect of Btbd3 genotype on apical dendritic branching, but with no differences between groups (f). Spine density was elevated in Btbd3 KO ACC layer II/III pyramidal neurons relative to WT mice (g). Representative dendritic spine images from ACC layer II/III pyramidal neurons (h). Results are expressed as mean values ± SEM. \*p<.05 vs WT group determined by ANOVA and post hoc tests. WT: wild-type HT: heterozygous KO: knockout ACC: anterior cingulate cortex.

