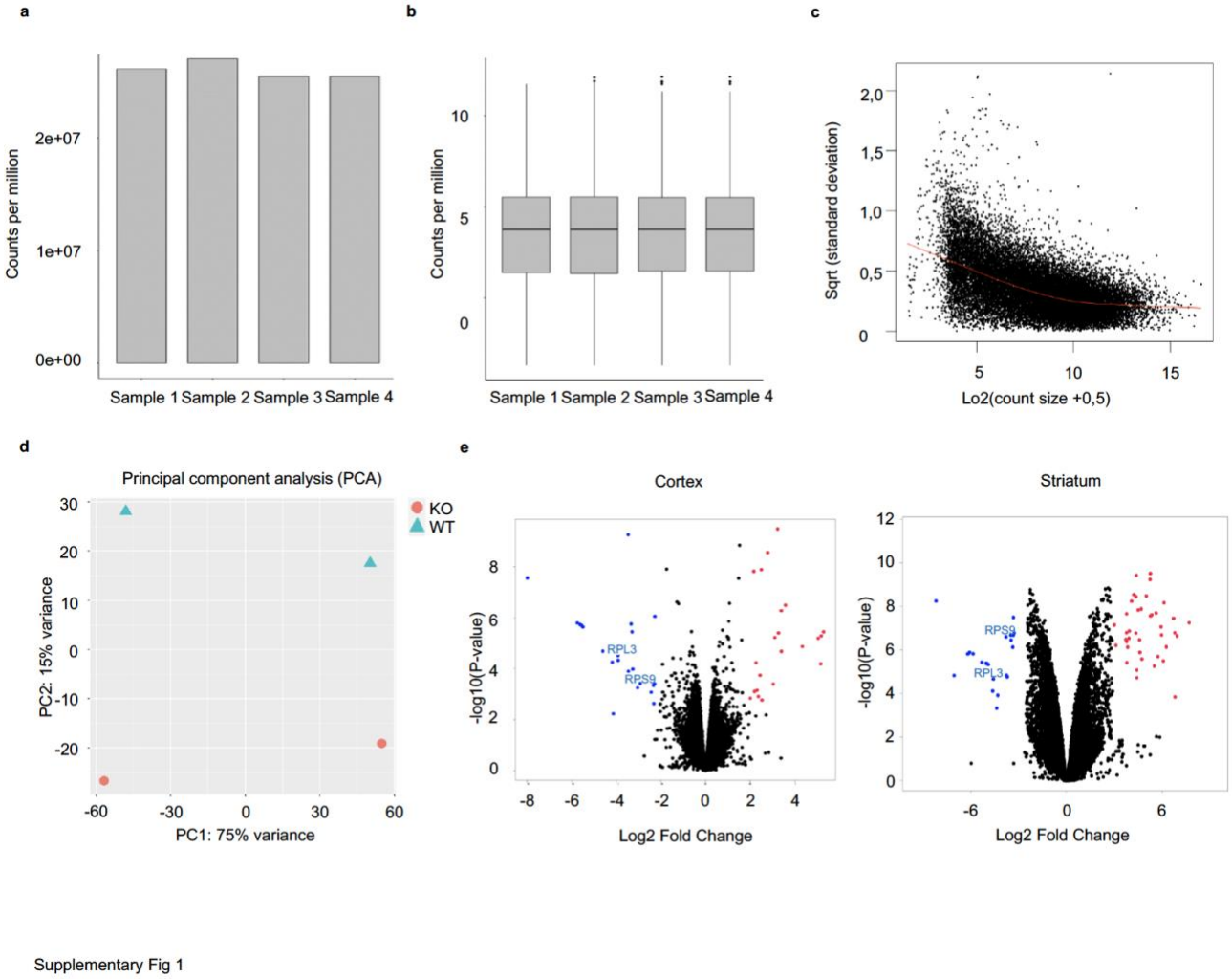
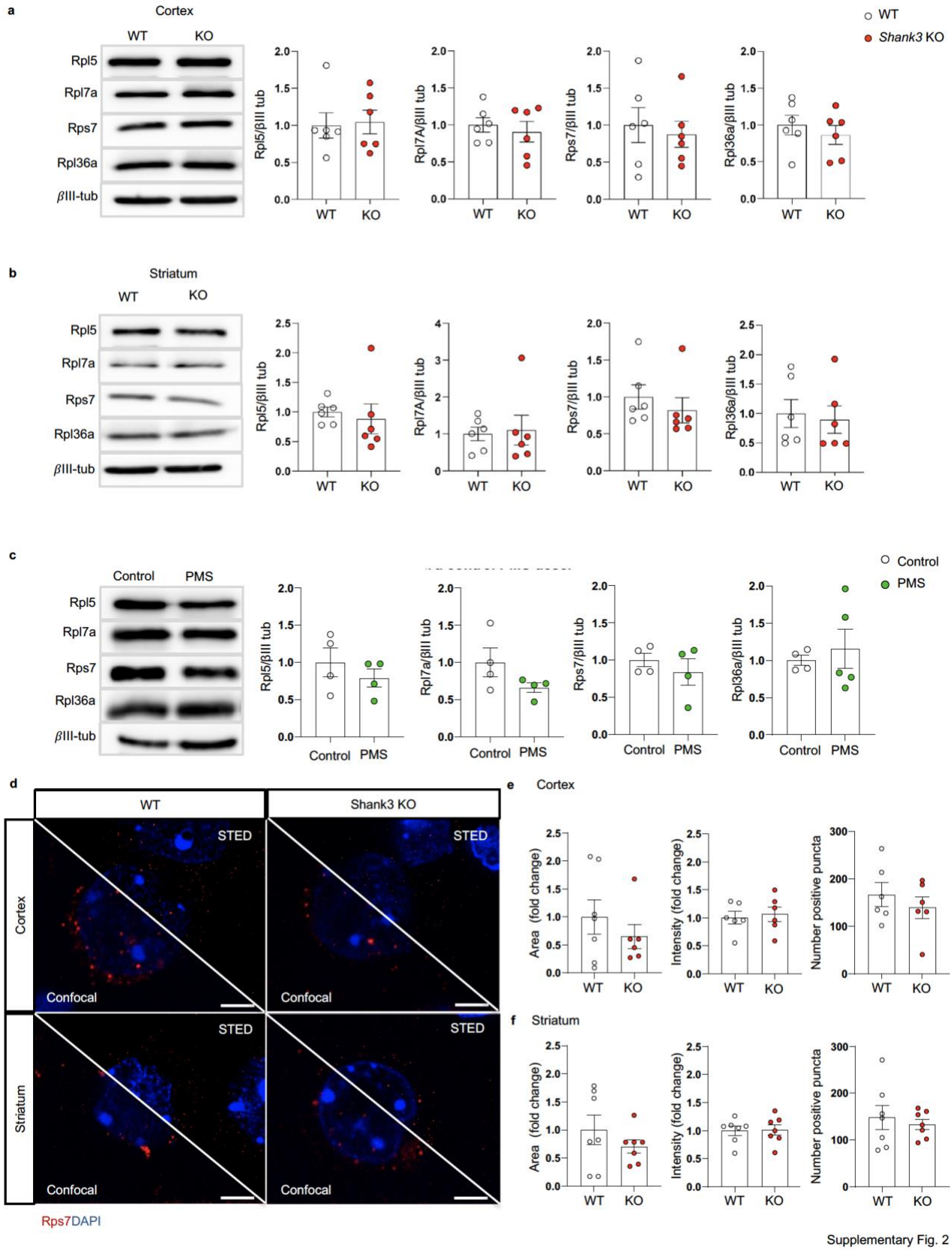
Supplementary Figures



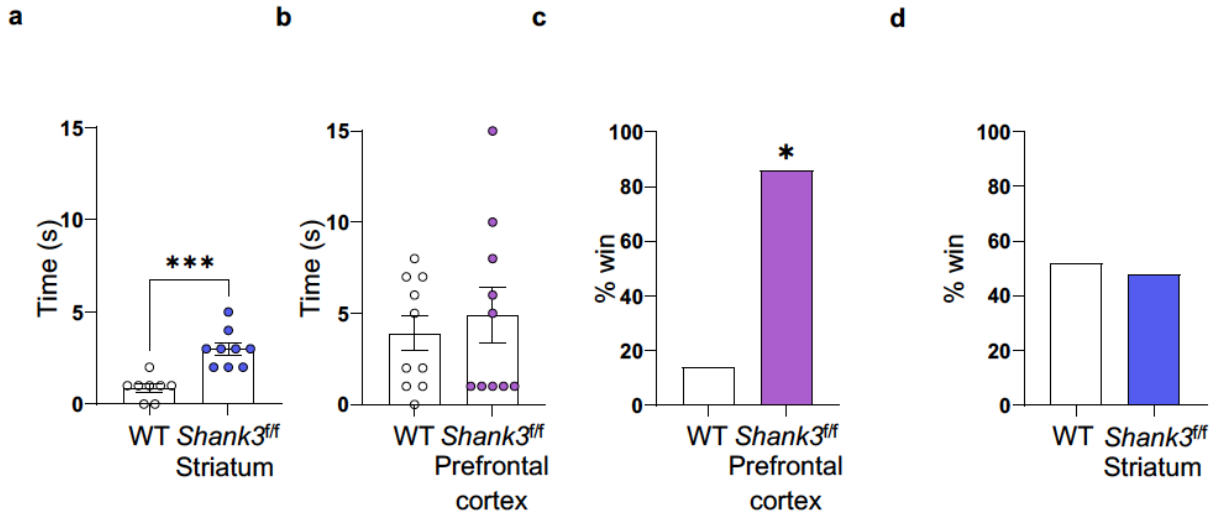
Supplementary Fig 1

Supplementary Fig.1: (**a**) Total read-counts per library and (**b**) a box plot of log transformed count data. (**c**) Model fitting data with the DSEQ2 algorithm (**d**) Principal component analysis of the genes represented in the two top-most components which account for a total of the 90% data variance. A clear separation between condition can be obtained by thresholding at zero the second component. **(e)** Volcano plot of log2 (fold change, FC) versus log10 (p-value) of RNASeq-identified transcripts in wild-type and *Shank3Δ11*$^{-/-}$ cortex and striatum. Transcripts significantly differentially expressed between replicates are highlighted. Red dots indicate significant transcripts with a FC ≤ 1.5; blue dots indicate transcripts with a FC ≥± 1.5 but with an FDR corrected p-value of ≥0.05. RPL3 and RPS9 are indicated. Sample1 is the KO cortex, Sample2 is the KO striatum, Sample3 is the WT striatum and the Sample4 is the WT cortex.



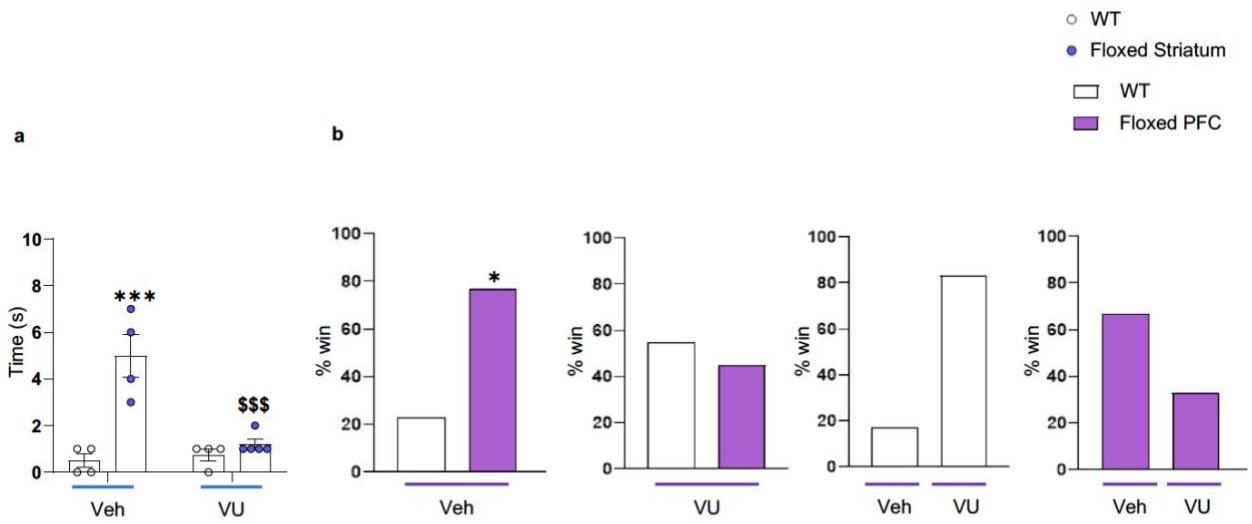
Supplementary Fig.2: (a and b) Protein levels of Rpl5, Rpl7a, Rpl36a, and Rps7 were analyzed using Western blot in total lysate obtained from tissue in cortex (**a**) and striatum (**b**) of WT and *Shank3* KO mice. Cortex and striatum n = 6 per group. (**c**) Protein levels of Rpl5, Rpl7a, Rpl36a, and

Rps7 were analyzed using Western blot in total lysates from cortical neurons derived from iPSCs of Phelan-McDermid syndrome patient and control**.** Rpl5: n = 4 per group; Rpl7a: n = 4 per group; Rps7: n = 4 per group; Rpl36a: Control n = 4 and PMS n = 5. Protein levels were normalized to the respective β-III-tubulin and ratios were compared between genotypes. The images indicate representative blots, and the results are shown as bar diagrams. (**d**) Comparison of Rps7 signal imaged by both confocal and STED microscopy, scale bar 3µm. (**e** and **f**) Rps7 signal resolved with STED microscopy show no difference between WT and KO in both cortex (**e**) and striatum (**f**) in area covered by rps7 signal, intensity, and number of puncta. Cortex: WT n = 6-7 per group *Shank3* KO n = 6 per group; striatum WT and *Shank3* KO n = 7 per group. All data are presented as mean ± SEM; all p-values were derived using Unpaired t-test.



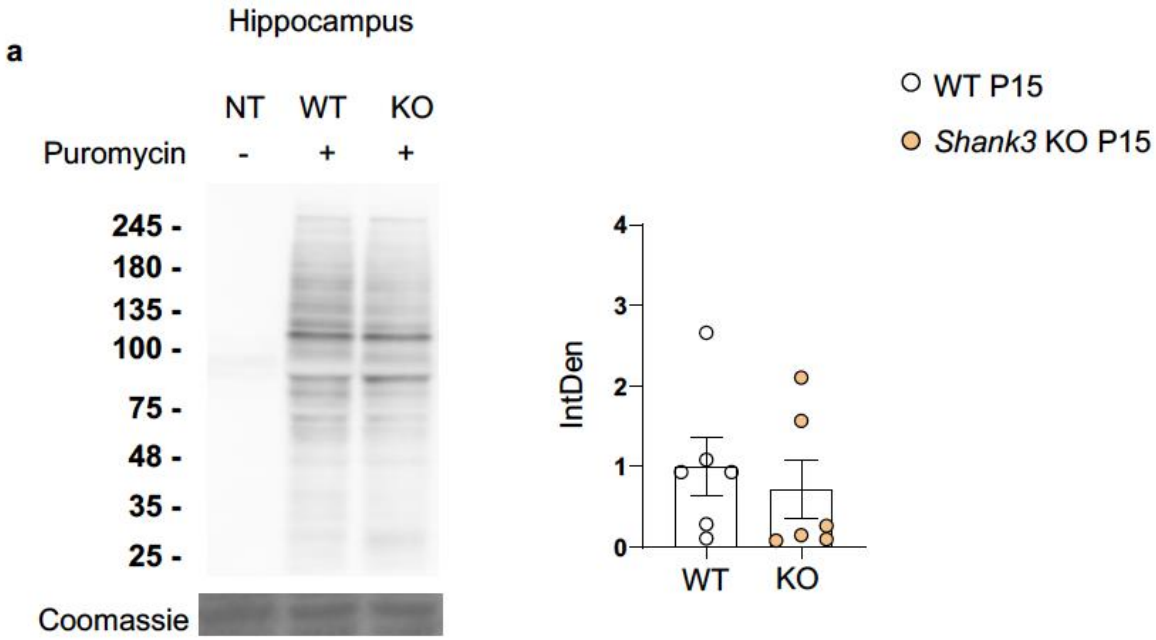
Supplementary Fig. 3

Supplementary Fig.3: *Shank3Δ11*$^{LoxP/LoxP}$ mice injected in the striatum or prefrontal cortex with AAV9-hsyn-Hi-eGFP-cre.WPRE.SV40. (**a and b**) Self-grooming behavior assessed as time spent grooming in the (**a**) striatum and (**b**) prefrontal cortex. Striatum WT n = 8 and *Shank3* KO n = 9; Cortex n = 10 per group. (**c and d**) Social dominance evaluated as percentage of matches won in the tube test in (**c**) prefrontal cortex and (**d**) striatum. Cortex n = 25 per group; Striatum n = 7 per group. Data are shown as the mean ± SEM. p-values were derived using Mann-Whitney (**a**-**b**), Fisher's Fisher's exact test (**c**-**d**) *P<0.05; *** P<0.001



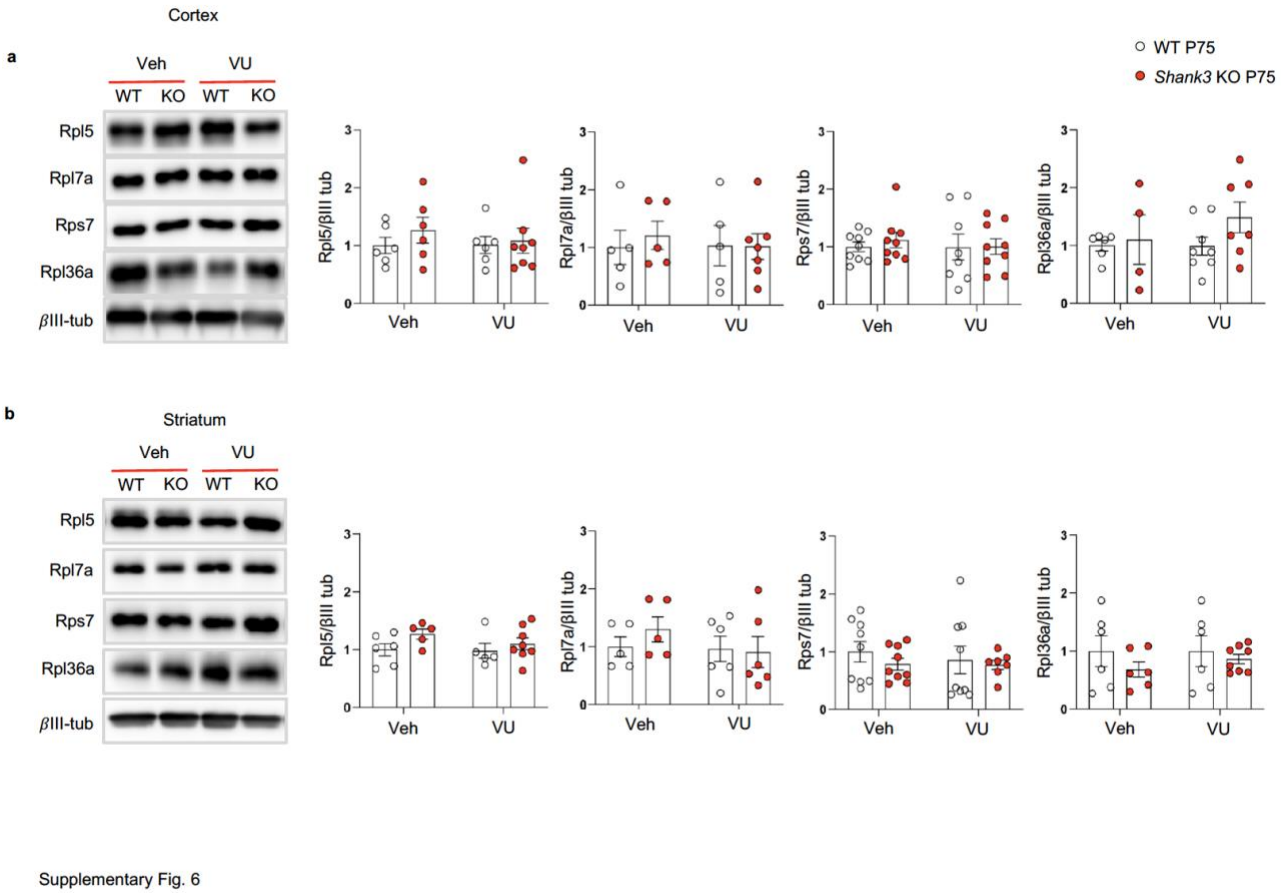
Supplementary Fig. 4

Supplementary Fig.4: (**a**) After striatal injection of AAV9-hsyn-Hi-eGFP-cre.WPRE.SV40, WT and *Shank3Δ11*$^{LoxP/LoxP}$ mice were treated with VU0409551 (5mg/kg) or vehicle Self-grooming behaviour assessed as time spent grooming. WT n = 4 and *Shank3* KO n = 4-5. All data are presented as mean SEM, and all p-values were calculated using the two-way ANOVA with the Bonferroni post hoc test. *** P<0.001 versus WT veh mice; $$ P<0.01; $$$ P<0.001 versus KO veh mice. (**b**) After being injected in the prefrontal cortex with AAV9-hsyn-Hi-eGFP-cre.WPRE.SV40 mice were treated with VU0409551 (5mg/kg) or vehicle. The graphs showed the percentage of won matches. Analyses are based on a sample size of n = 6–13 animals for each group. All data are presented as mean ± SEM; all p-values were derived using Fisher's exact test. *P<0.05



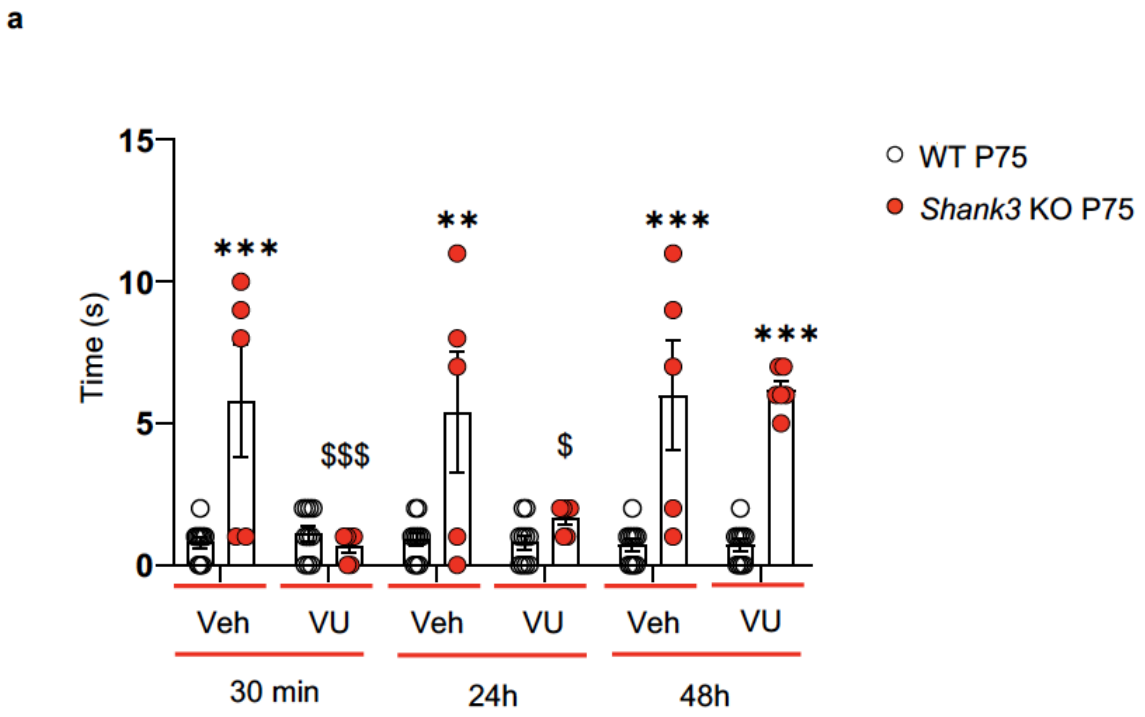
Supplementary Fig. 5

Supplementary Fig.5: (a) Level of protein synthesis in young (post-natal-day 15) WT and *Shank3* KO mice in the hippocampus. The puromycin signals were normalized to the respective Coomassie staining signals and the results are shown as bar diagrams. N = 6 per group. Data are presented as mean ± SEM; all p-values were derived using the unpaired t test.



Supplementary Fig. 6

Supplementary Fig.6: Protein levels of Rpl5, Rpl7a, Rpl36a, and Rps7 were analyzed using Western blot in total lysate obtained from tissue in the cortex (**a**) and striatum (**b**) wild type and *Shank3* KO mice after acute administration of VU0409551 (5mg/kg) or vehicle. Protein levels were normalized to the respective β-III-tubulin and ratios were compared between genotypes. The images indicate representative blots, and the results are shown as bar diagrams. Rpl5: Cortex WT n = 6, *Shank3* KO n = 6-8 per group, and striatum WT n = 6-5 and *Shank3* KO n = 5-8; Rpl7a: Cortex WT n = 5, *Shank3* KO n = 5-7 per group, and striatum WT n = 5-6 and *Shank3* KO n = 5-6; Rps7: Cortex WT n = 9-8, *Shank3* KO n = 9 per group, and striatum WT n = 9 and *Shank3* KO n=9-7; Rpl36a: Cortex WT n = 6-8, *Shank3* KO n = 4-7 per group, and striatum WT n = 6 and *Shank3* KO n = 6-8. All data is presented as the mean ± SEM; all p-values were derived using the Mann-Whitney test.



Supplementary Fig. 7

Supplementary Fig.7: (a) Duration of the VU0409551 effect Graphs indicate time spent grooming 30 min, 24 h, and 48 h after the drug administration. WT n = 10; *Shank3* KO n = 3-6 per condition. The number of animals is indicated in the figure. All data are presented as the mean SEM; all p-values were calculated using the Two-way ANOVA with the Bonferroni post hoc test. * <p 0.05; ** p <0.01; *** p <0.001 compared to the WT vehicle p< 0.05; $$P<0.01; $$$ p<0.001 when compared to WT VU0409551.