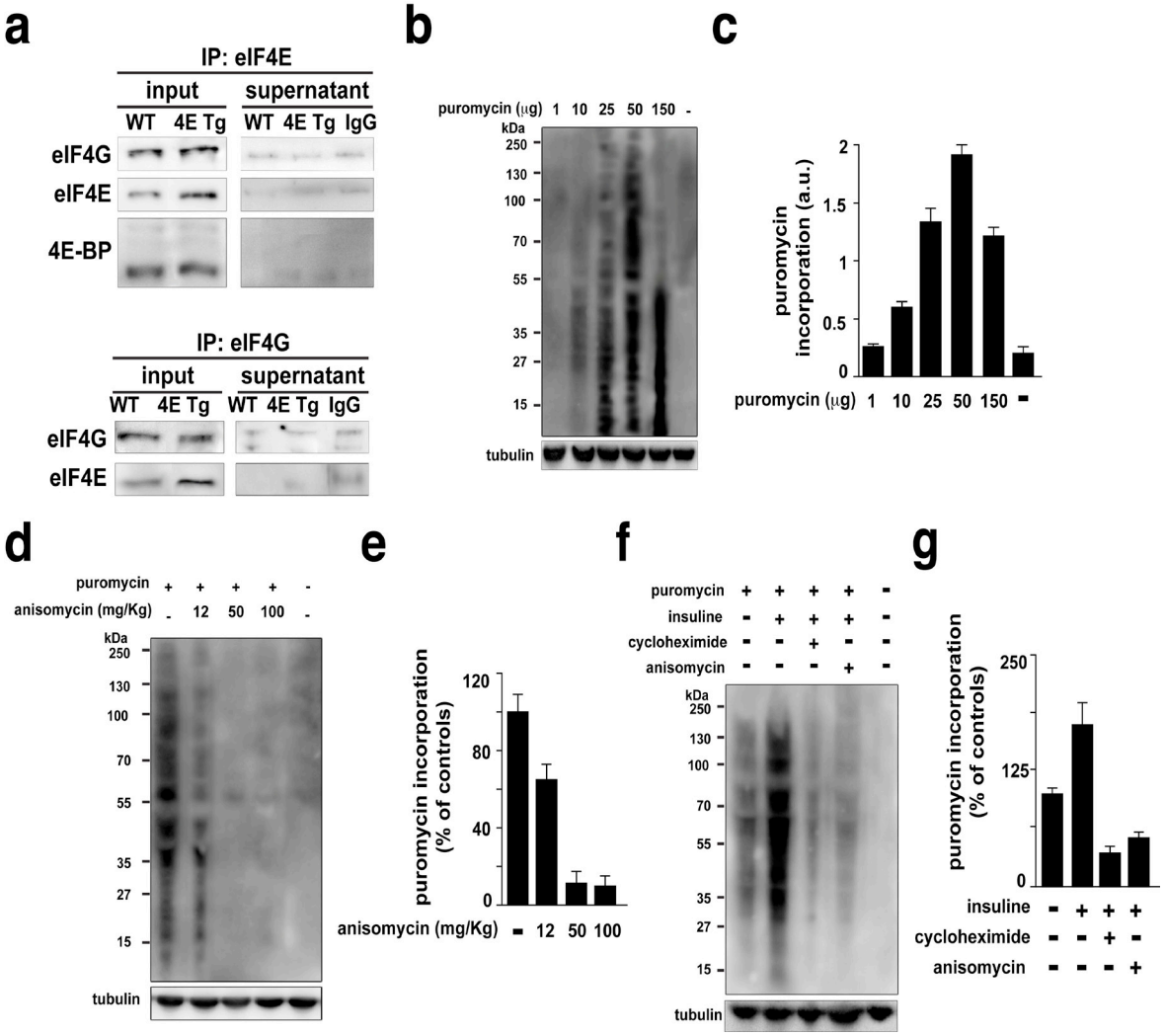
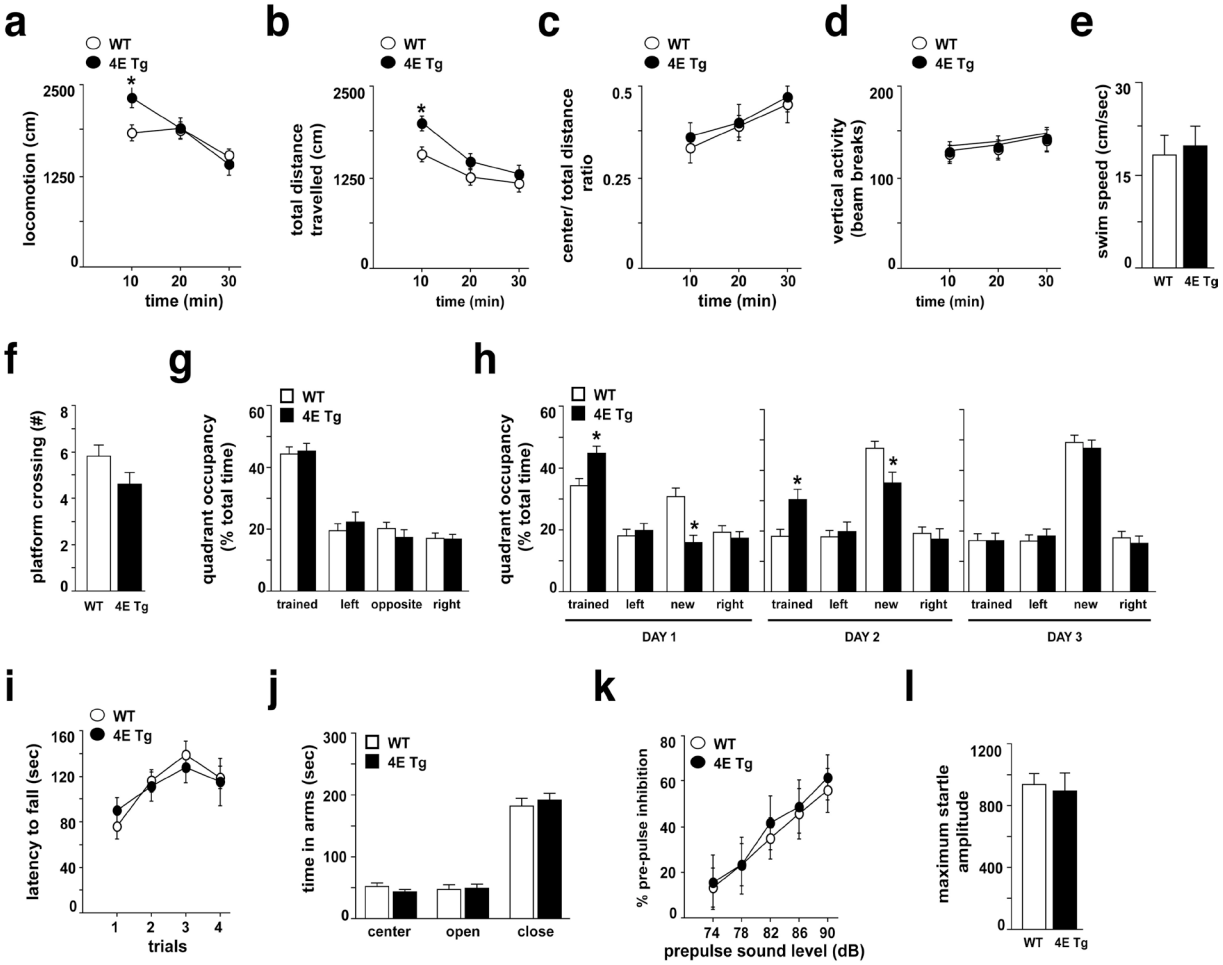
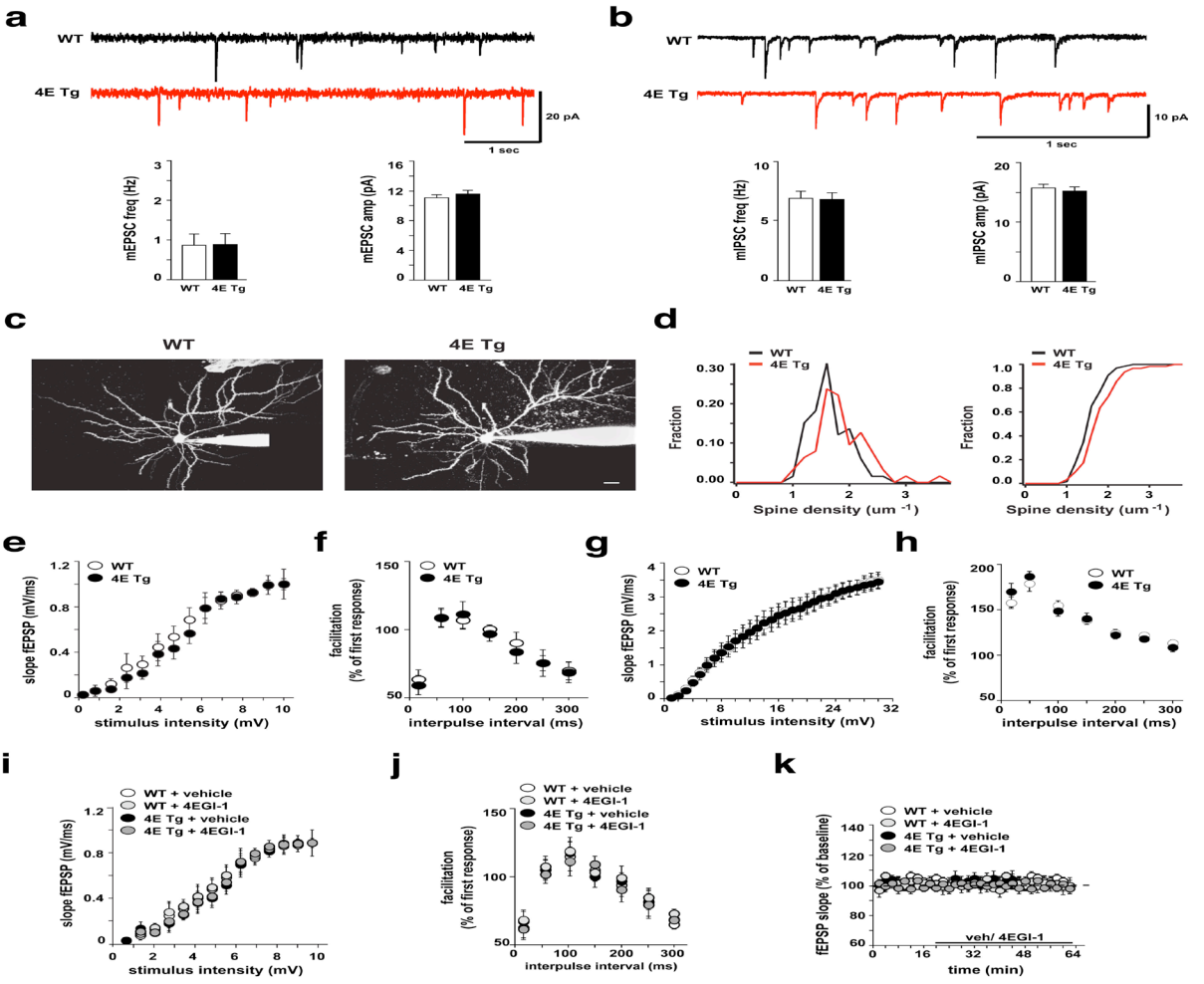
doi:10.1038/nature11782



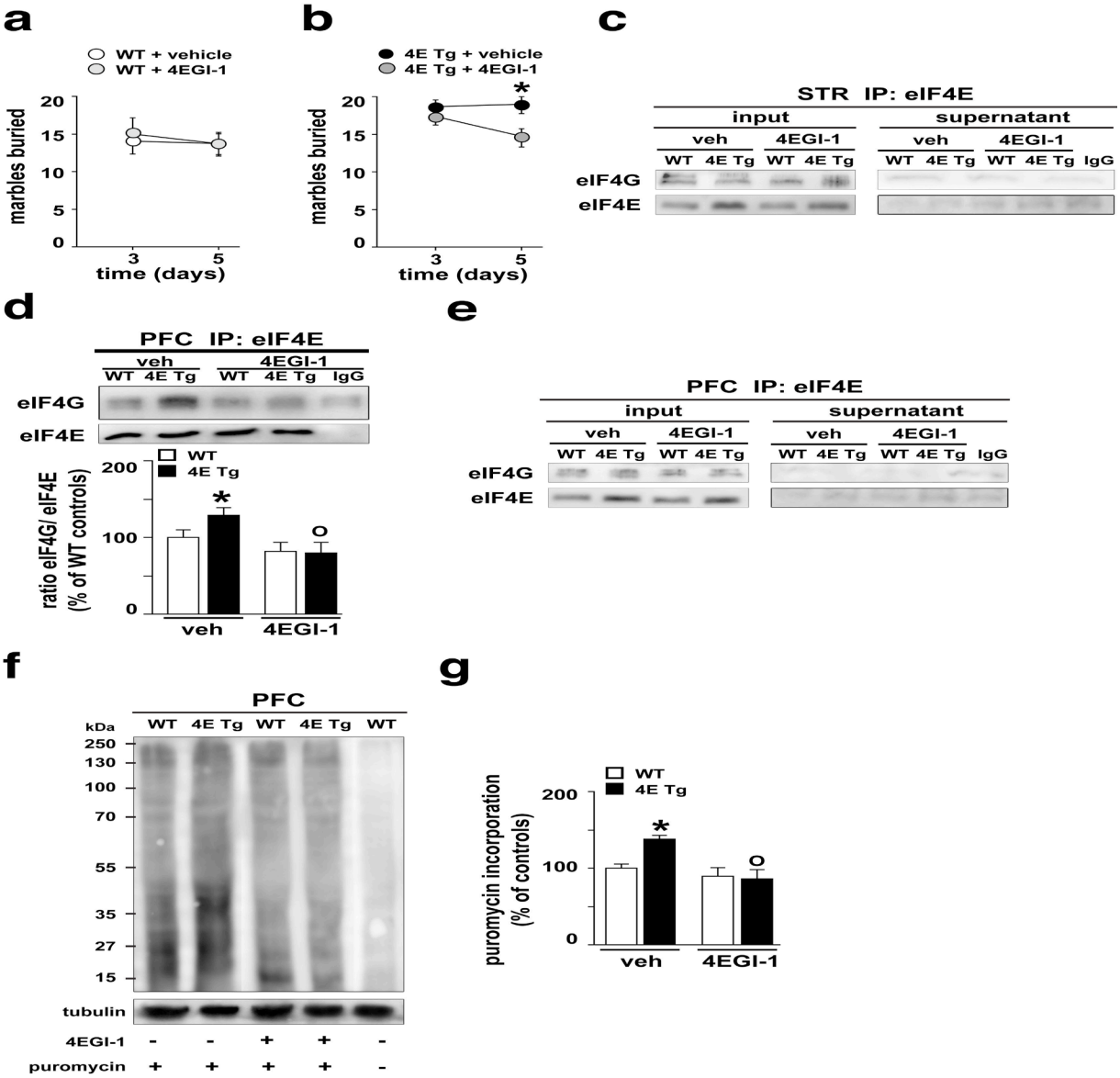
Supplementary Figure 1. (**a**) Representative Western blots showing brain levels of eIF4E, eIF4G and 4E-BP (input) and the supernatant after immunoprecipitation with eIF4E and eIF4G. All data are shown as mean ± SEM. (**b, c**) Dose response of puromycin infused ICV. Representative Western blots (**b**) and quantification (**c**) of newly synthesized brain proteins labeled with increasing doses of puromycin using the SUnSET method (see Supplementary Methods). n=3 mice/dose. One-way ANOVA [treatment effect, $F_{(5,17)}$=78.07, p<0.001] (**d, e**) Anisomycin blocks the incorporation of puromycin into newly synthesized brain proteins in a dose-dependent manner. Puromycin (25 μg in 0.5 μl; IVC) was infused either with or without anisomycin (12, 50, 100 mg/Kg; i.p.). Representative Western blots (**d**) and quantification (**e**) of proteins labeled with puromycin using the SUnSET method (see Supplementary Methods). n=3 mice/treatments. One-way ANOVA [treatment effect, $F_{(3,12)}$=35.52, p<0.001]. (**f, g**) Protein labeling with puromycin using the SUnSET is sensitive to stimulation and inhibition of protein synthesis. Hippocampal slices incubated with puromycin (10 mg/ml) and stimulated with either insulin (1 μM) alone or in combination with either cycloheximide (300 μM) or anisomycin (40 μM). Representative Western blots (**f**) and quantification (**g**) of proteins labeled puromycin using SUnSET (see Supplementary Methods). n=3 slices from 3 mice/treatments. One-way ANOVA [treatment effect, $F_{(3,11)}$=16.62, p<0.001] .In all the SUnSET experiments (panels **b-g**): – represents a control sample without puromycin. Lower panel shows the levels of tubulin, which was used as loading control. All data are shown as mean ± SEM.



Supplementary Figure 2. (**a**) Novelty-induced locomotor activity shown in 10 min intervals. *p<0.05 vs WT, repeated measures ANOVA [genotype X time, $F_{(5,100)}$=3.69, p<0.01] followed by Bonferroni-Dunn test. (**b, c, d**) Open field test. Total distance (**b**), ratio center/total distance (**c**) and vertical activity (**d**) shown in 10 min intervals. *p<0.05 vs WT, repeated measures ANOVA [genotype X time, $F_{(5,100)}$=3.82, p<0.01] followed by Bonferroni-Dunn test. (**e**) Swim speed (cm/sec) in the MWM. n.s., Student's t-test. (**f, g**) Number of platform crossings (**f**) and quadrant occupancy (**g**) during the probe test of the MWM. . n.s., repeated measure ANOVA. (**h**) Quadrant occupancy for each day of the reversal learning phase of the MWM. *p<0.05 vs WT, repeated measures ANOVA [DAY1: genotype X time, $F_{(3,92)}$=6.12, p<0.001; DAY2: genotype X time, $F_{(3,92)}$=5.93, p<0.01; DAY3: n.s.] followed by Bonferroni-Dunn test. (**i**) Accelerating rotarod test. Latency to fall from the accelerating rod (sec) is shown for four test trials. n.s., repeated measure ANOVA. (**j**) Elevated plus maze test. Time spent in the arms and in the center of the maze. n.s., two-way ANOVA. (**k, l**) Prepulse inhibition (PPI) of the acoustic startle response is represented as % of PPI of the startle response (**k**). n.s., repeated measures ANOVA. Acoustic startle response is expressed as maximum startle amplitude to the 120-dB stimulus (**l**). n.s., Student's t-test. In all the experiments n=12-13 mice/genotype. All data are shown as mean ± SEM.



Supplementary Figure 3. (**a**) mEPSC traces obtained from layer 5 mPFC pyramidal neurons (upper panel). n=22-25 neurons/genotype. n.s., Student's *t*-test. (**b**) mIPSC traces obtained from layer 5 mPFC pyramidal neurons (upper panel). n=34-35 neurons/genotype. n.s., Student's *t*-test. (**c**) Two-photon images showing layer 2/3 mPFC pyramidal neuron filled with Alexa Fluor-594 through the whole-cell recording pipettes. Scale bar= 20 µm. (**d**) Histograms (left) and cumulative histograms (right) of spine density in WT (black) and Tg (red) mice, showing a significant difference between the two populations. (**e**) Input versus output plot representing the slope of the striatal fEPSP in response to increasing stimulus intensity. n=10 slices from 8 mice/genotype. n.s., repeated measures ANOVA. (**f**) Percent facilitation in the striatum, determined by the ratio of the second fEPSP to the first fEPSP shown at interpulse intervals from 10 to 300 ms (right panel). n=13 slices from 8 mice/genotype. n.s., repeated measures ANOVA. (**g**) Input versus output plot representing the slope of the hippocampal fEPSP in response to increasing stimulus intensity (left panel). n=12 slices from 9 mice/genotype. n.s., repeated measures ANOVA. (**h**) Percent facilitation in the hippocampus, determined by the ratio of the second fEPSP to the first fEPSP is shown at interpulse intervals from 10 to 300 ms (right panel). n=13 slices from 8 mice/genotype. n.s., repeated measures ANOVA. (**i**) Input versus output plot representing the slope of the striatal fEPSP in response to increasing stimulus intensity. n=15 slices from 9 mice/genotype/treatment. n.s., repeated measures ANOVA. (**j**) Percent facilitation in the striatum, determined by the ratio of the second fEPSP to the first fEPSP is shown at interpulse intervals from 10 to 300 ms (right panel). n=13 slices from 9 mice/genotype/treatment. n.s., repeated measures ANOVA. 4EGI-1 (100 µM) was bath applied for 45 min prior and during the duration of the experiments. (**k**) 4EGI-1 does not alter basal synaptic transmission in striatal slices. Baseline fEPSP was recorded for 20 min prior and during the application of 4EGI-1 (100 µM). n=13 slices from 9 mice/genotype/treatment. n.s., repeated measures ANOVA. All data are shown as mean ± SEM.



Supplementary Figure 4. (**a, b**) Marble-burying behavior of WT (**a**) and eIF4E transgenic mice (**b**) tested five hours after the infusion of 4EGI-1. Data are shown as mean ± SEM (n=6 mice/genotype/treatment). *p<0.05 vs vehicle-treated 4E Tg mice, two-way repeated measures ANOVA [genotype X treatment, $F_{(1,20)}$=6.16, p<0.05] followed by Bonferroni-Dunn test. (**c**) Representative Western blots showing the levels of eIF4E and eIF4G in the striatum (**c**, STR, input,) and in the supernatant after immunoprecipitation with eIF4E also is shown (**c,** STR, supernatant). (**d**) Representative Western blots and quantification of proteins recovered after immunoprecipitation (IP) of eIF4E in the prefrontal cortex (PFC). n=4 mice/genotype. *p<0.05 and °p<0.05 vs vehicle-treated WT and 4E Tg, respectively, two-way ANOVA, followed by Bonferroni-Dunn test. (**e**) Representative Western blots showing the levels of eIF4E and eIF4G in the striatum (**e**, STR, input,) and in the supernatant after immunoprecipitation with eIF4E also is shown (**e,** STR, supernatant). (**f, g**) Representative Western blots and quantification of newly synthesized proteins labeled with puromycin using the SUnSET method in WT and 4E Tg mice infused with either vehicle or 4EGI-1 and puromycin in prefrontal cortex (PFC). The last sample represents a control without puromycin. Lower panel shows the levels of tubulin, used as loading control. *p<0.05 and °p<0.05 vs vehicle-treated WT and 4E Tg, respectively, two-way ANOVA, followed by Bonferroni-Dunn test. All data are shown as mean ± SEM.