

Expanded View Figures

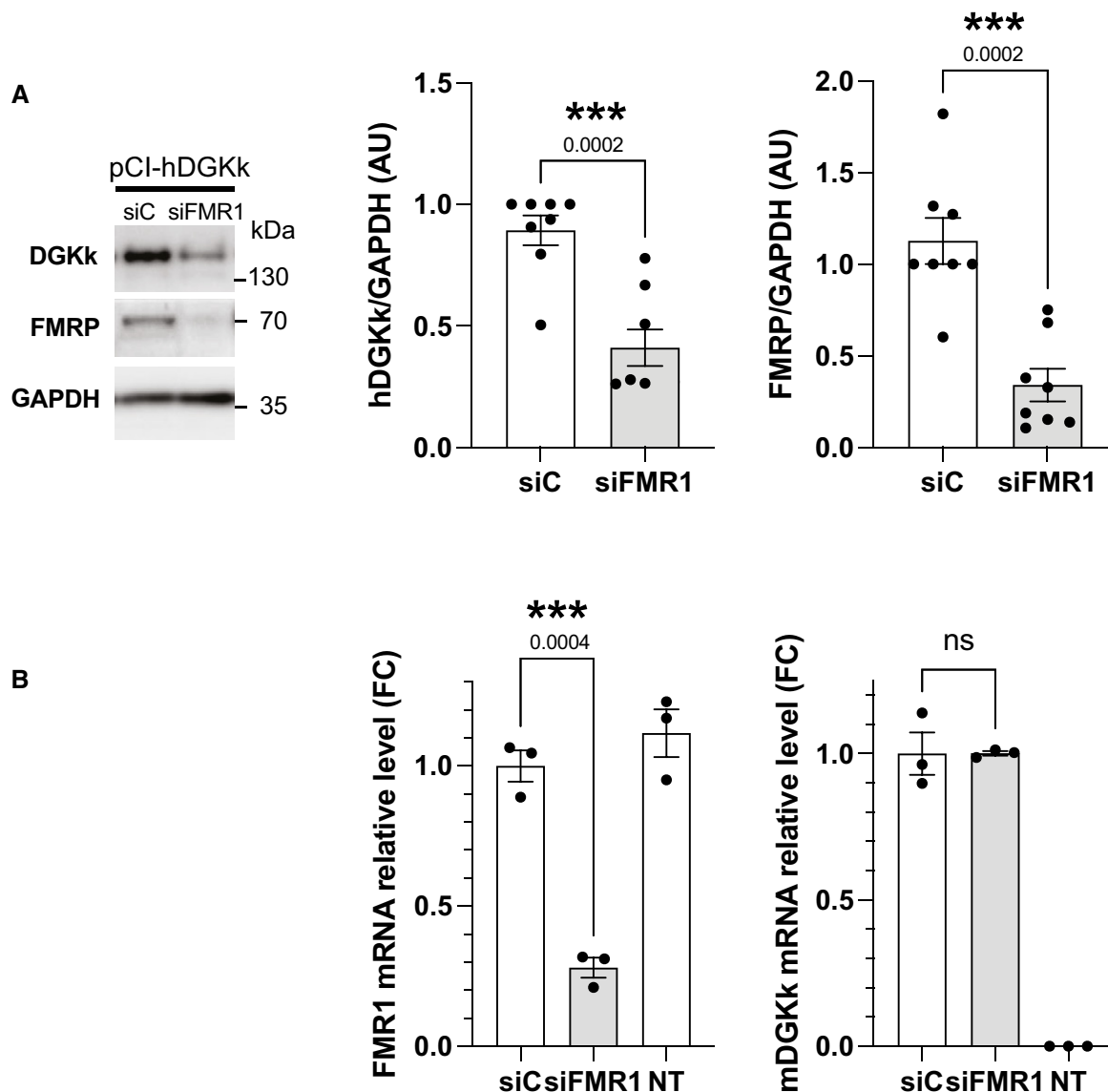


Figure EV1. Influence of FMRP protein on human DGKk protein expression and on mouse DGKk mRNA level.

A Immunoblots and quantification of lysates from HeLa cells transfected with plasmid pCI-hDGKk encoding human DGKk and pre-transfected 24 h before with siRNA control (siC) or against FMRP (siFMR1). GAPDH was used as a loading control. For quantification, DGKk and FMRP signals were normalized against GAPDH signal and presented relative to the signal for siC-treated cells. Each point represents data from an individual culture, and all values are shown as mean \pm SEM. *** $P < 0.001$, calculated by unpaired Student *t*-test.

B Quantification of mouse DGKk mRNA by qRT-PCR in RNA extracts of Cos-1 cells transfected with plasmid pCI-mDGKk-HA and pre-transfected with siRNA control (siC) or siRNA against FMRP (siFMR1) or mock transfected (NT). Each point represents data from an individual culture, and all values are means of fold change \pm SEM, determined using $\Delta\Delta C_t$ method with Actb as normalizer, $n = 3$ biological replicates. *P* values were calculated using one-way ANOVA with Tukey's multiple comparison test. *** $P < 0.001$; ns, $P > 0.05$.

Source data are available online for this figure.

Figure EV2. Δ N-DGKk expression modulates mTOR and EIF4E signaling without significant toxicity.

- A Immunoblots and quantification of lysates from Cos-1 cells transfected with plasmid pCI control or pCI-HA- Δ N-DGKk, with the indicated amount of plasmid (μ g) and after 24 h incubated with puromycin (20 μ g/ml for 30 min) to measure basal rates of protein synthesis. GAPDH was used as a loading control. Densitogram of puromycin incorporation is presented as change relative to pCI (0.2 μ g) condition.
- B Cells transfected with 0.5 μ g pCI, pCI-HA-FL-mDGKk, or pCI-HA- Δ N-mDGKk plasmids were serum starved for 24 h and then incubated with 10% FCS for the indicated time period. Cells were then collected and immunoblotted with HA (DGKk), p-mTOR, mTOR, p-EIF4E, or EIF4E antibodies. Blots were normalized for GAPDH and then phospho/non-phospho ratio was calculated. Upper panel: mDGKk expression. Middle panel: m-TOR phosphorylation at Ser-2448. Bottom panel: p-EIF4E phosphorylation at Ser-209. Significance was determined by 2-way ANOVA compared to pCI control ($n = 3$).
- C–F Quantification of caspase 3/7 activity (C), release of lactate dehydrogenase (LDH) (D), percentage of NeunN (E), and GFAP positive cells (F), in WT and *Fmr1*-KO cortical neurons untreated (NT) or transduced at 8 DIV for 8 days with indicated titers of AAV (viral genome copies) AAVrh10- Δ N-DGKk or AAVrh10-FMRP by immunofluorescence high-throughput cell imaging. Positive control wells were treated with apoptotic inducer staurosporine (STS) at 0.1 and 1 μ M for 6 h.

Data information: Data are mean \pm SEM of individual cultures and analyzed using one-way ANOVA and Tukey's multiple comparisons test. * $P < 0.05$, **** $P < 0.0001$. Source data are available online for this figure.

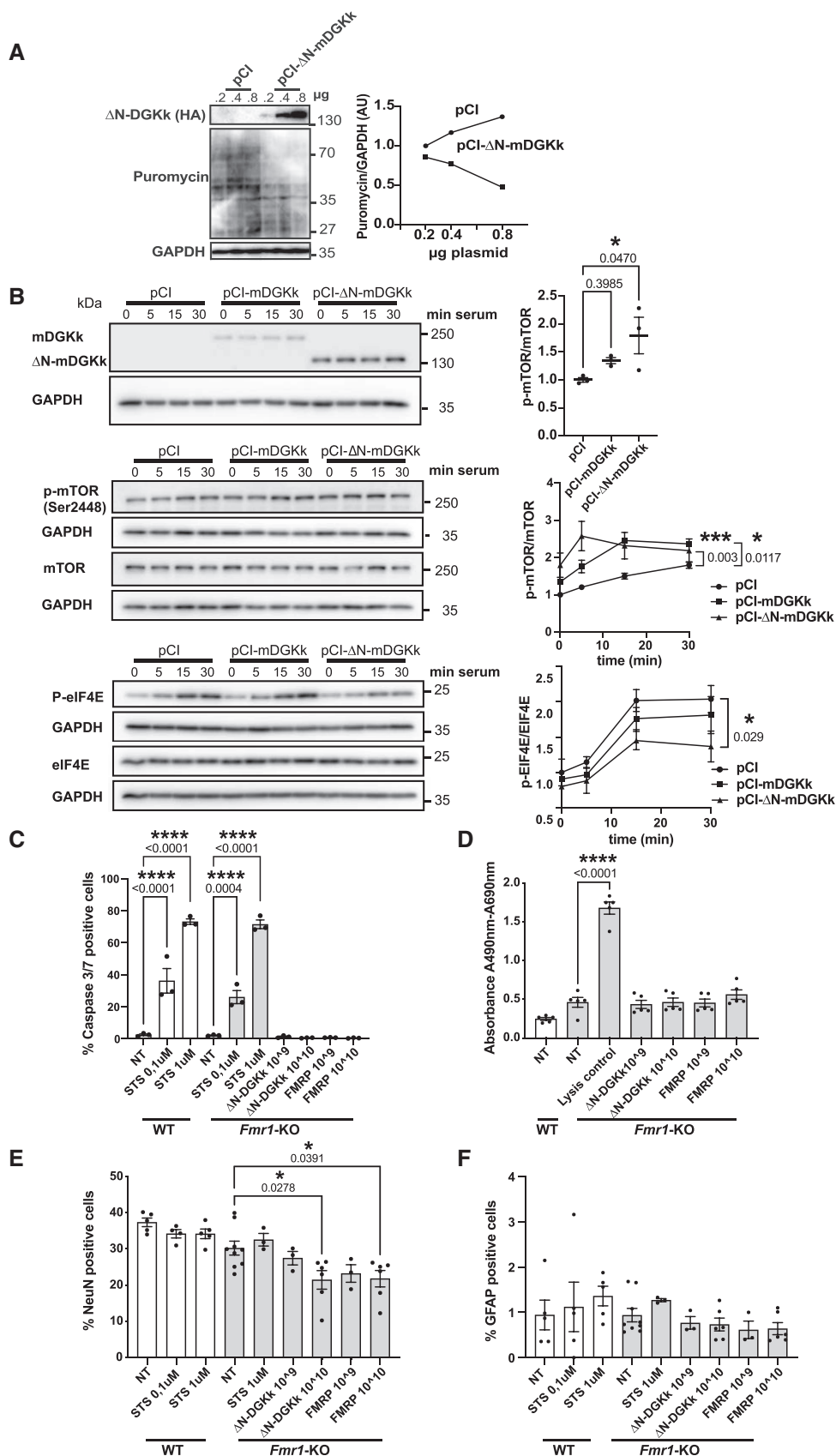


Figure EV2.

Figure EV3. Δ N-DGKk expression in brain with AAV vectors is stable over time and normalizes abnormal phosphatidic acid level in *Fmr1*-KO cortex.

- A Representative coronal brain sections processed for detection of Δ N-DGKk (HA) at 8 and 12 weeks post-injections using immunohistochemistry on *Fmr1*-KO mice treated with indicated treatment, counterstained with eosin hematoxylin. Three regions, a, b, c, are shown with their corresponding position on brain sagittal map. Scale bar 2 mm. Image of brain 8W b is a reuse of Fig 3D Rh10.
- B Immunoblots and quantification of Δ N-DGKk protein in *Fmr1*-WT brain lysates from cortex (c), hippocampus (h), and rest (r) areas as in Fig 3C. GAPDH was used as a loading control. AU, arbitrary units.
- C Measure of total phosphatidic acid (PA) level by mass spectrometry in hippocampus and rest of brain of WT mice treated with saline solution (WT) and *Fmr1*-KO mice treated with saline (*Fmr1*-S), AAVPHP.eB- Δ N-DGKk (*Fmr1*-PHP.eB), AAVRh10- Δ N-DGKk (*Fmr1*-Rh10) 8 weeks after injections. Data are expressed as mol % of total lipids and analyzed using one-way ANOVA and Tukey's multiple comparisons test, $n = 8$ individual animals, except for WT and *Fmr1*-S $n = 11$ and represented as median with interquartile range with minimum and maximum values.
- D Total diacylglycerol (DAG) level in cortex measured as in C).

Source data are available online for this figure.

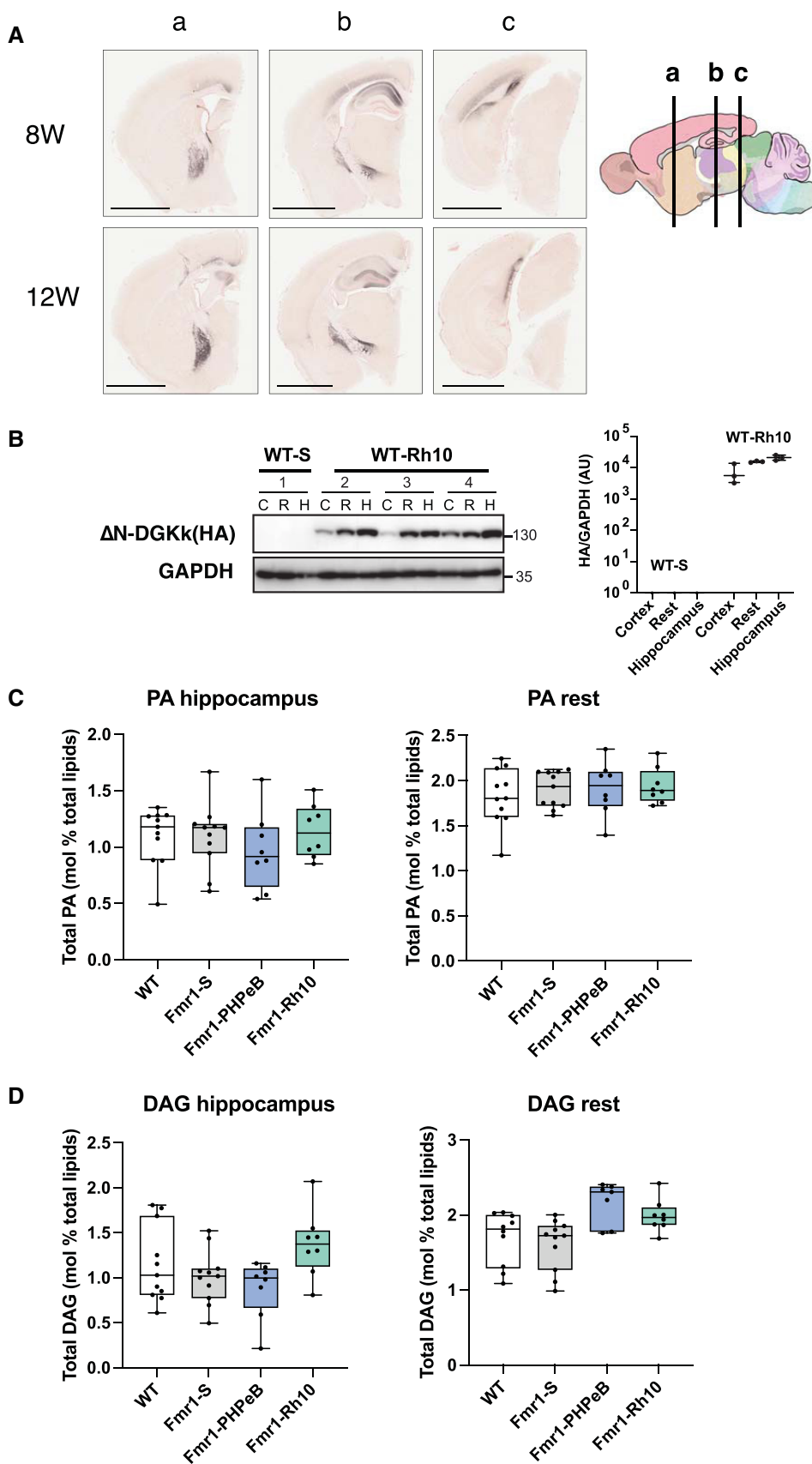


Figure EV3.

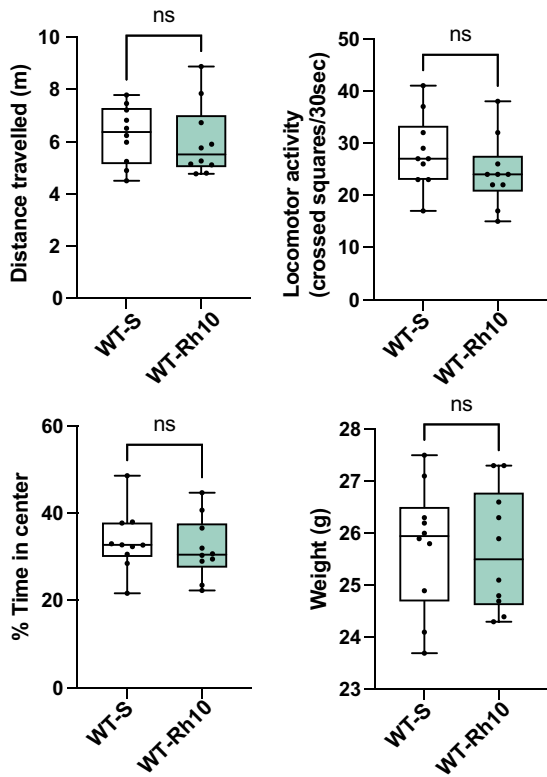


Figure EV4. AAVRh10-ΔN-DGKk does not lead to visible behavior effect in *Fmr1*-WT mice 4 weeks after its administration.

Locomotor activity (distance in m and crossed squares per 30 s) in the whole arena and percentage of time spent in the center during 15 min habituation, weight of vehicle-treated WT mice (WT-S) compared to AAVRh10-ΔN-DGKk-treated WT mice (WT-Rh10).

Data information: Data are expressed as median with interquartile range with minimum and maximum values. Statistical analysis: unpaired t-test ($n = 10$ mice per group), ns not significant.

Source data are available online for this figure.

