Volk, Supplementary Figure 1

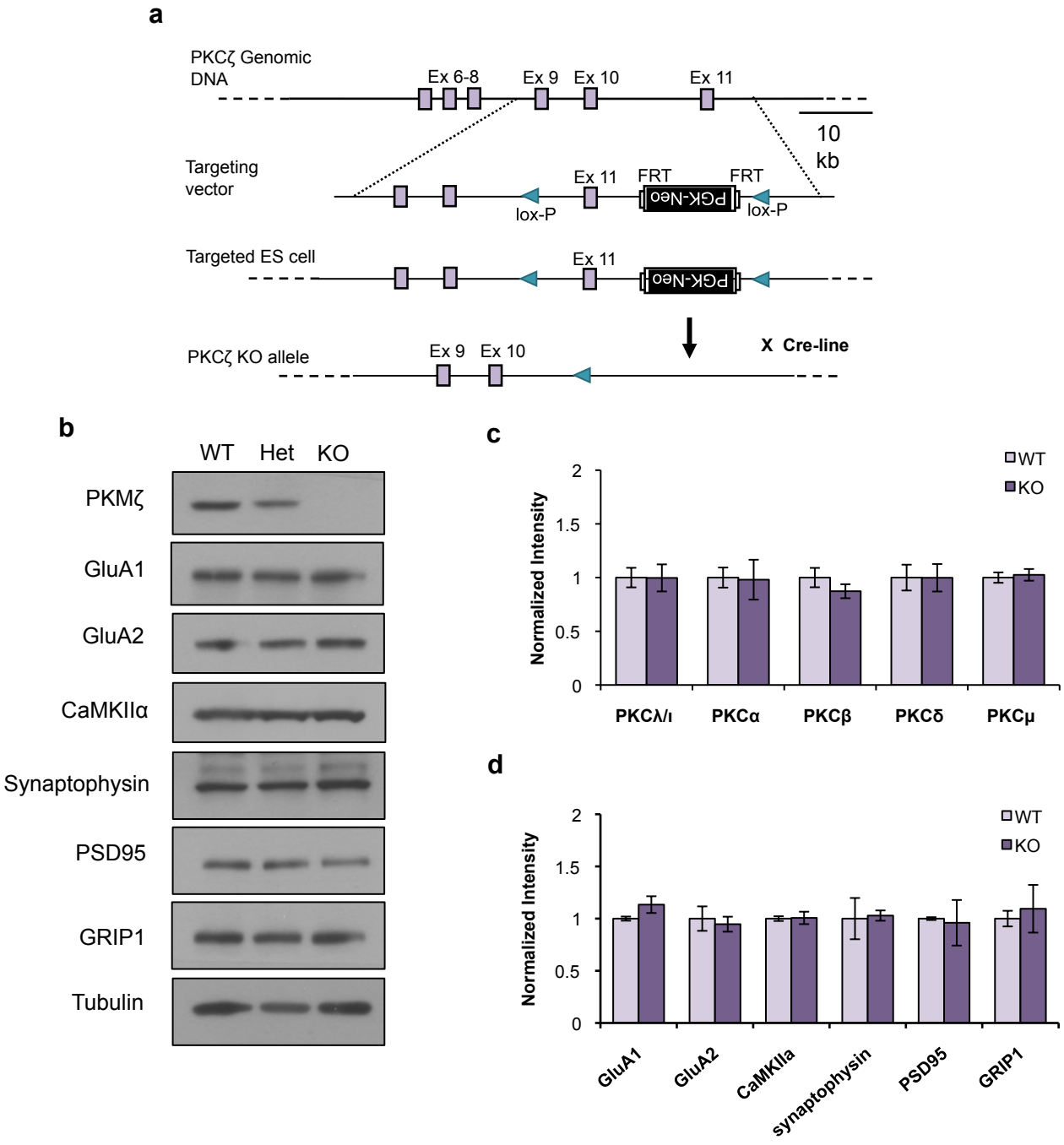


Figure S1: Characterization of PKC/Mζ KO mice. a, Schematic for PKC/Mζ KO targeting strategy in which exon 11 is floxed and deleted by Cre recombinase to create a frameshift mutation in *Prkcζ*. **b,** Western blots of synaptic proteins from whole brain lysate in wild-type, heterozygous and homozygous PKC/Mζ mice. **c,d,** Quantification of western blots shown in Fig. 1c and Fig. S1, respectively (n = 6-9). Values are normalized to tubulin as a loading control and then to WT values and represent mean ± s.e.m.

Volk, Supplementary Figure 2

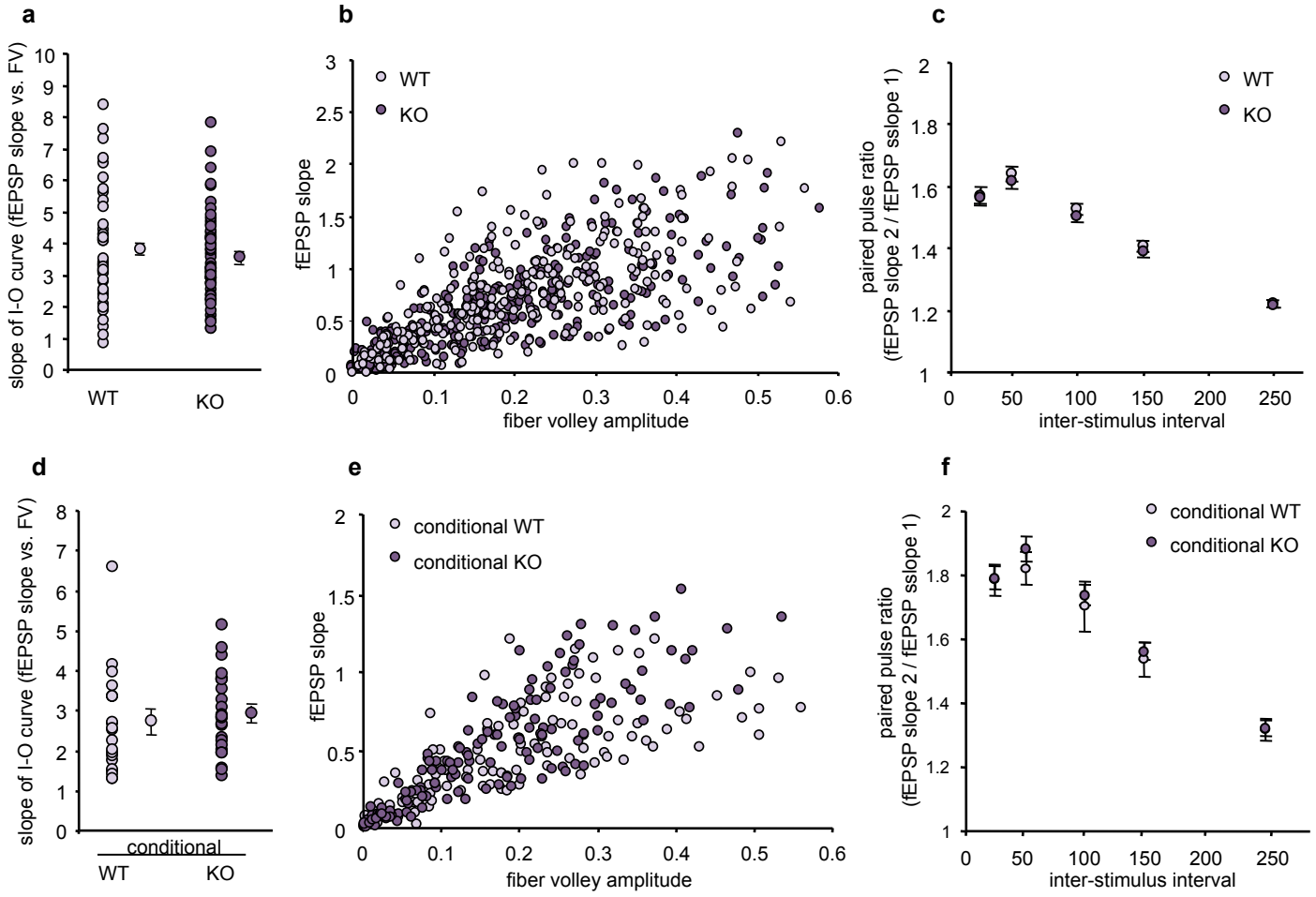


Figure S2: Basal synaptic transmission is unaffected in conventional and conditional PKC/Mζ KO mice. **a,** The slope of the input output curve is not altered in conventional PKC/Mζ KO mice. WT, n=45, 3.794 ± 0.13 ms-1; KO, n=54, 3.54 ± 0.19 ms-1; $p > 0.4$. **b,** Raw data from all input-output curves shown in a. **c,** Paired pulse facilitation is not altered in conventional PKC/Mζ KO mice. WT, n = 56, KO, n = 57. **d,** The slope of the input output curve is not altered in conditional PKC/Mζ KO mice. WT, n=21, 2.73 ± 0.31 ms-1; KO, n=25, 2.92 ± 0.2 ms-1; $p > 0.4$. **e,** Raw data from all input-output curves shown in d. **f,** Paired pulse facilitation is not altered in conditional PKC/Mζ KO mice. WT, n = 28, KO, n = 30. In all figures error bars represent ± s.e.m.

Volk, Supplementary Figure 3

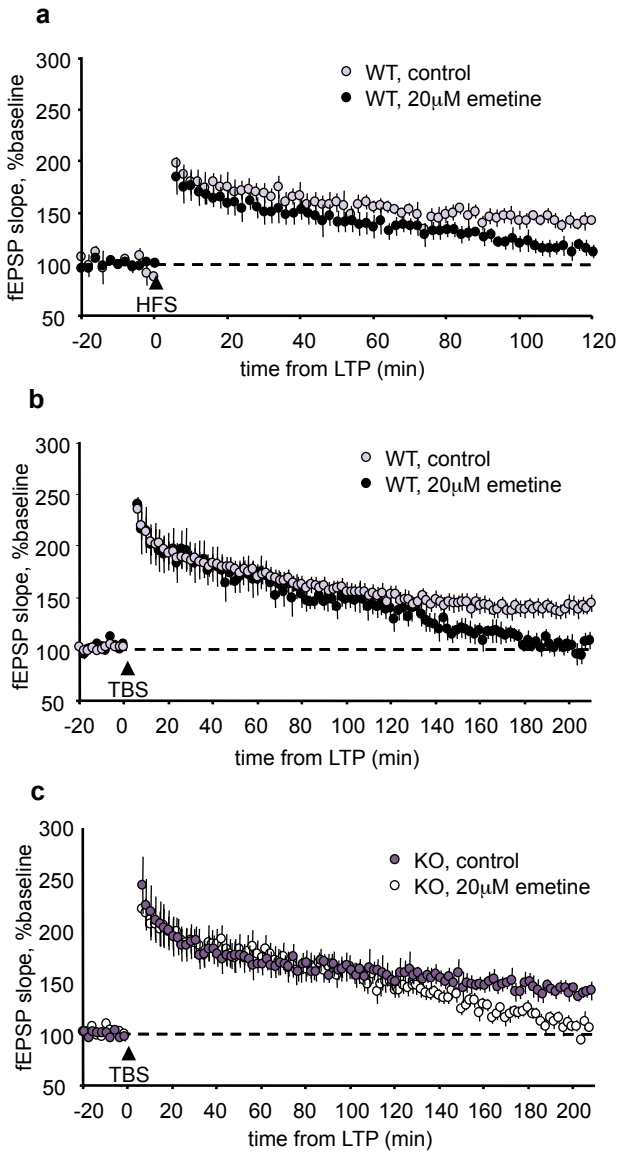
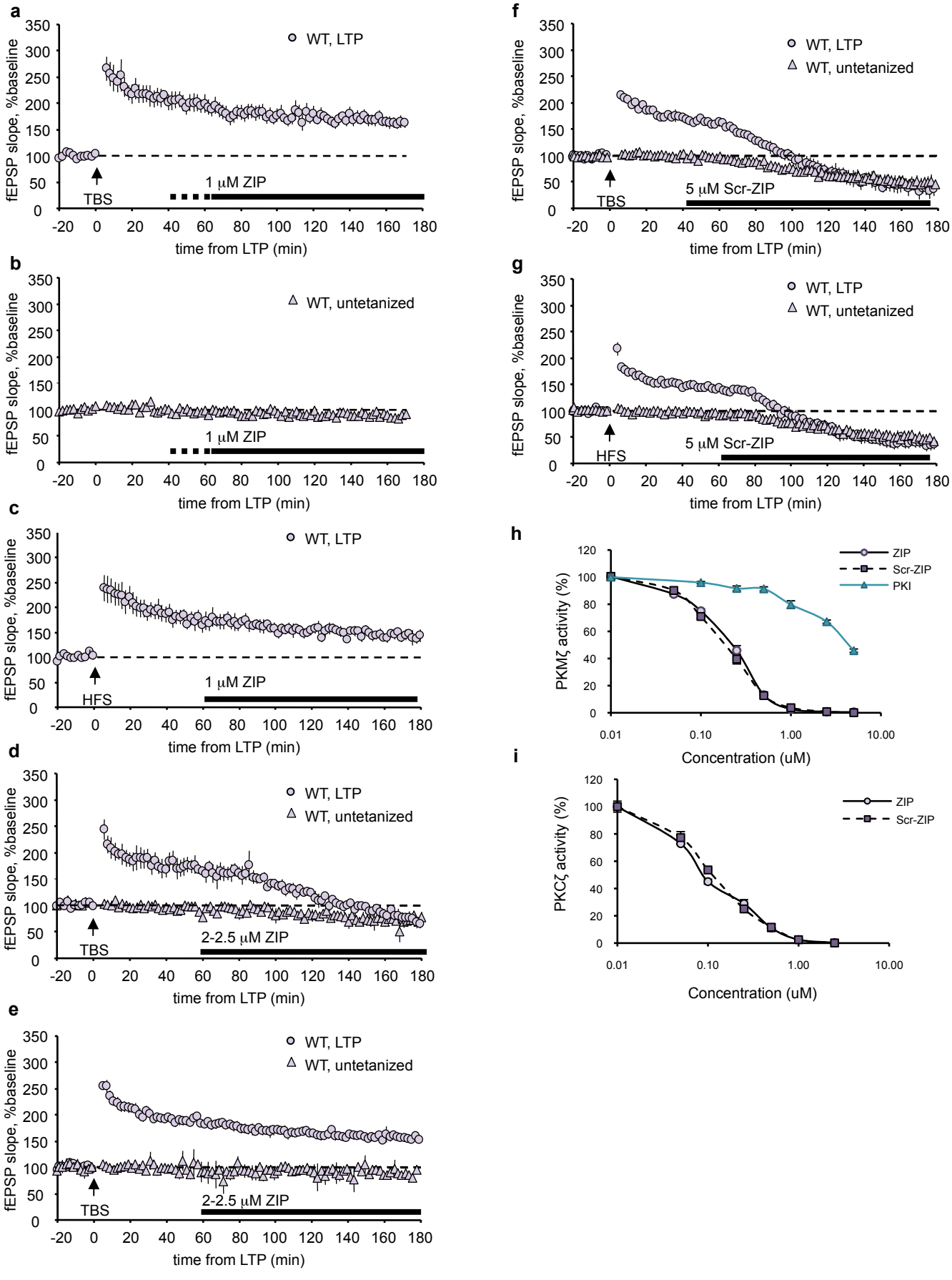


Figure S3: Protein synthesis-dependence of LTP in WT and PKC/Mζ KO mice. **a,** HSF LTP in WT mice is sensitive to protein synthesis inhibition by ~2 hours (at 120min. control, n=5, 140 ± 6%; 20μM emetine, n=6, 115 ± 7%; $p < 0.05$). **b,** TBS LTP in WT mice is sensitive to protein synthesis inhibition by ~3 hours (at 180min, control, n=9, 139 ± 8%; 20μM emetine, n=7, 109 ± 8%; $p < 0.05$). **c,** TBS LTP in PKC/Mζ KO mice is also sensitive to protein synthesis inhibition by ~3 hours (at 180min, control, n=6, 144 ± 7%; 20μM emetine, n=9, 119 ± 8%; $p < 0.05$). When used, emetine was present throughout the experiment.

Volk, Supplementary Figure 4



Volk, Supplementary Figure 4

Figure S4: 5 μM ZIP is the minimum effective concentration for reversing LTP, but the 5 μM Scr-ZIP also reverses LTP. a, 1 μM ZIP has no effect on TBS LTP in WT mice, n=6. ZIP was applied 40 or 60 min after TBS with the same result, so these data are combined. **b,** 1 μM ZIP has no effect on basal synaptic transmission, n=6. ZIP was applied to untetanized slices 40 or 60 min after LTP was induced in the paired slice. **c,** 1 μM ZIP has no effect on HFS LTP, n=5. **d,e,** 2-2.5 μM ZIP is close to the threshold for effective concentration in reversing LTP. 2-2.5 μM ZIP reduced tetanized and basal synaptic transmission in half of our experiments (d, tetanized, n=4; untetanized, n=2) and had no effect in half of our experiments (e, tetanized, n=4; untetanized, n=2). **f,g,** 5 μM myr-scrambled-ZIP ('control' peptide) reverses TBS and HFS LTP in addition to reducing basal transimission (f, TBS LTP, n=5; untetanized, n=7: g: HFS LTP, n=8; untetanized =7). **h,i** ZIP and Scr-ZIP are equally effective at inhibiting PKMζ (h) and PKCζ (i) biochemically *in vitro* (each point, n=6). Data represent mean ± s.e.m.

Volk, Supplementary Figure 5

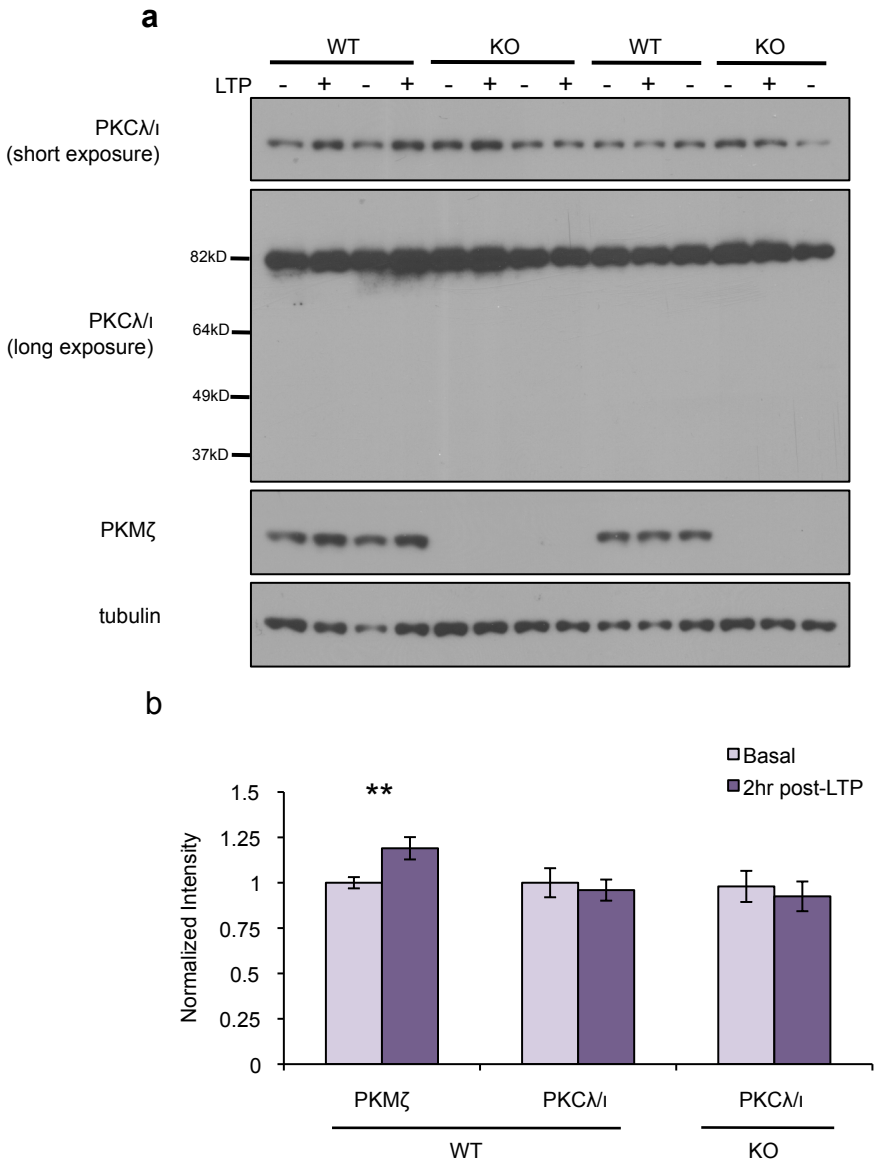


Figure S5: LTP in PKC/Mζ KO is not compensated by elevated or truncated PKCλ/ι products. a, Western blot analysis of microdissected CA1 regions 2hr after either basal stimulation (LTP -) or TBS (LTP +) in WT and PKC/Mζ KO mice. **b,** Quantification of PKMζ and PKCλ/ι expression (PKMζ: WT, basal n=14, 1.0 ± 0.03, LTP n=13, 1.19 ± 0.06, * $p < 0.01$ Student's *t*-test; PKCλ/ι: WT, basal n=12, 1.0 ± 0.08 LTP, n=12, 0.96 ± .06, $p > 0.6$; KO, basal n=12, 0.98 ± 0.09, LTP n=12, 0.93 ± 0.08, $p > 0.6$). Values are normalized to tubulin as a loading control and then to WT basal values and represent mean ± s.e.m.

Volk, Supplementary Figure 6

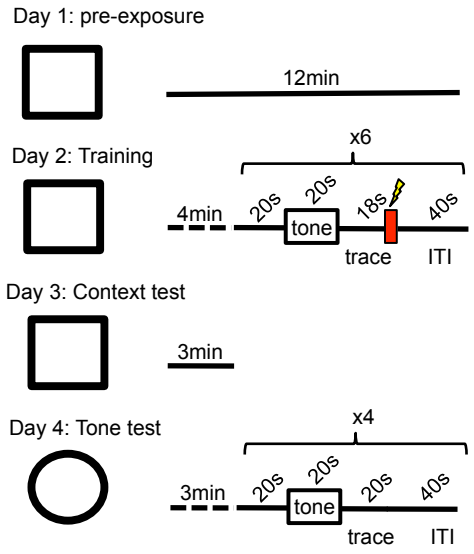


Figure S6: Trace fear conditioning protocol. Trace fear conditioning was conducted over the course of four days as shown.