SUPPLEMENTAL MATERIAL

Kang et al., https://doi.org/10.1084/jem.20160974

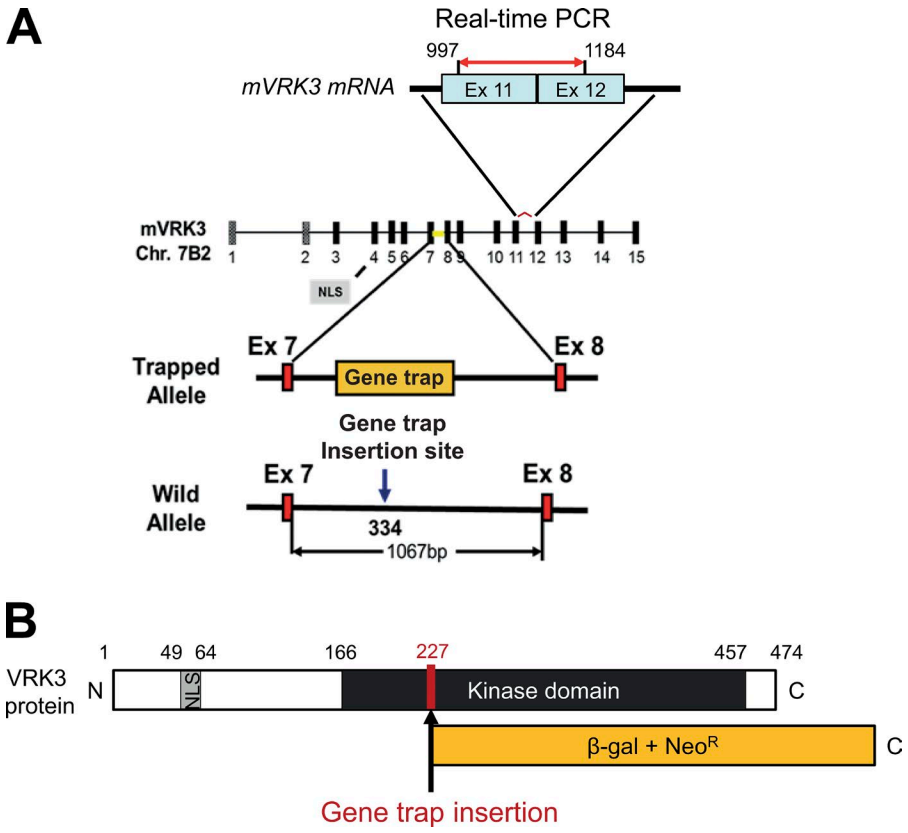

Figure S1. **Gene trap insertion site and effect on VRK3 protein.** (A) Schematic representation of WT and mutant gene locus of *VRK3* in mice. For detection and quantification, the positions of the primers used in quantitative RT-PCR. Ex, exon. (B) *VRK3* protein consists of 474 amino acids. It has two domains. One is a nuclear localized signal (NLS) at the N terminus, and the other is a kinase domain, which is 292 amino acids long. Because exon 7 encodes until amino acid 226, the β-galactosidase (β-gal) and neomycin resistance (Neo^R) fusion protein, which is the coding region of the gene trap vector, is translated subsequently.

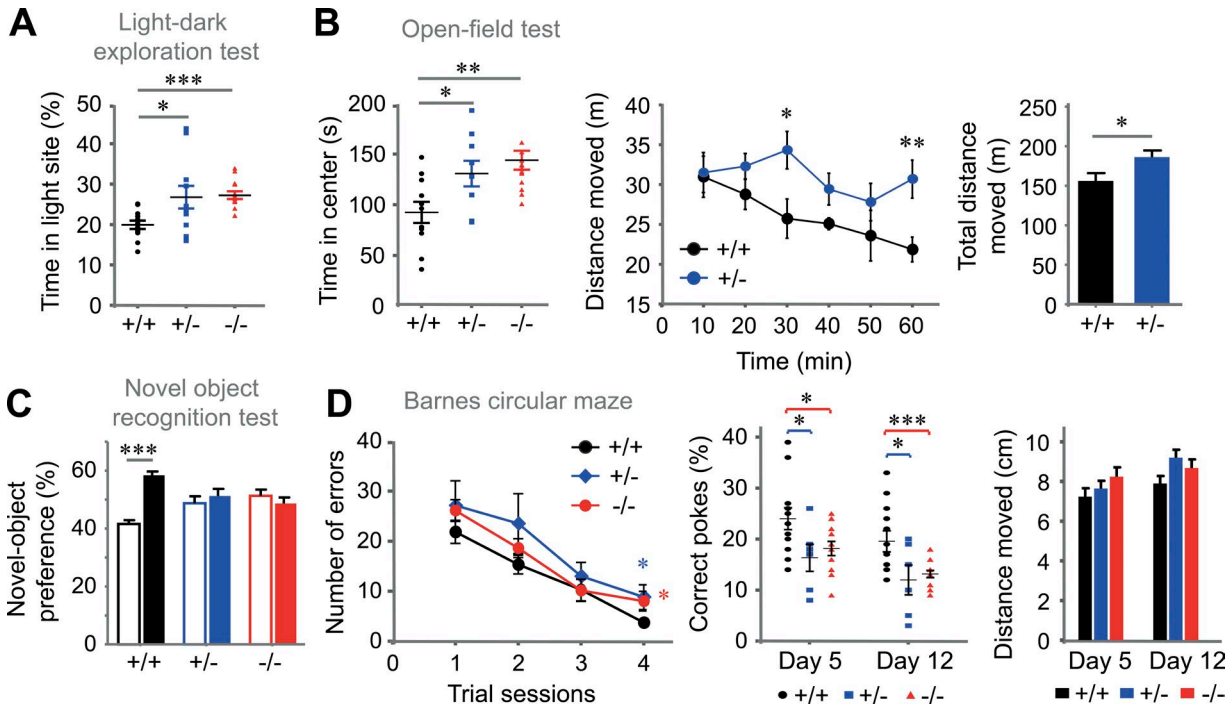

Figure S2. ***VRK3* mutant mice exhibit impaired learning and memory performance along with hyperactivity and anxiolytic behavior.** (A) Duration of time spent in the light compartment in the light–dark exploration test (n = 12, 11 and 14 for WT, *VRK3*-heterozygous [HT] and KO mice). (B) Total time spent in the center region of the open field (left; n = 11, 9 and 14 for WT (+/+), *VRK3* heterozygous (+/−), and KO (−/−) mice), and total locomotor activity and distance moved in the open field for 1 h (middle and right; n = 7, 7 for WT and *VRK3* HT mice). (C) Novel object recognition test 24 h after exposure to a familiar object (F) for WT (+/+), *VRK3*-heterozygous (+/−), and *VRK3*-KO (−/−) mice (n = 10, 11 and 14 for WT, *VRK3* heterozygous and KO mice). (D) Barnes circular maze with the number of errors across each day of training (left; n = 11, 6 and 12 for WT, *VRK3*-heterozygous, and *VRK3*-KO mice). Probe test on the fifth and 12th day (middle) and distance moved in the probe test (right) for WT, *VRK3*-heterozygous, and *VRK3*-KO mice (n = 11, 6 and 12 for WT, *VRK3*-heterozygous, and *VRK3*-KO mice). n.s., not significant. *, P < 0.05; **, P < 0.01; ***, P < 0.001; two-tailed t test. All values represent mean ± SEM.

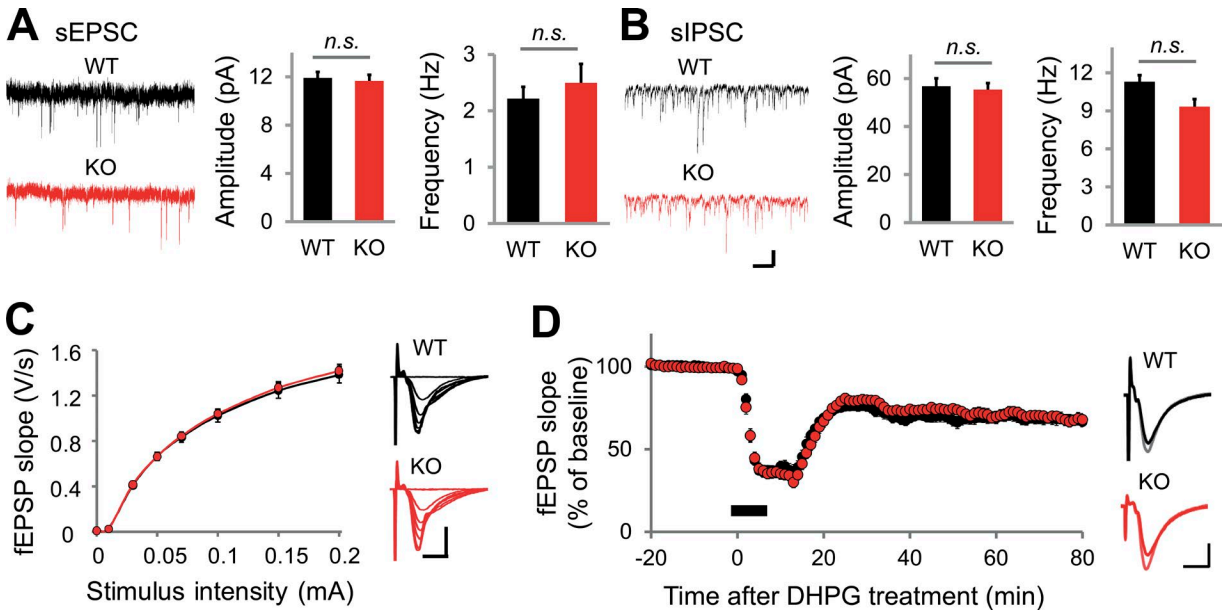

Figure S3. **Normal basal synaptic transmission and metabotropic glutamate receptor–induced LTD in *VRK3*-KO mice.** (A and B) Representative traces and mean amplitude and frequency of sEPSCs (A; n = 15, 17 neurons from 3, 3 mice; P > 0.05, t test) and sIPSCs (B; n = 16, 17 neurons from 3, 3 mice; P > 0.05, t test) from 3–4-wk-old WT and *VRK3* knockout (*VRK3*-KO) mice. Bars, 10 pA, 1 s. (C) Summary graphs and representative traces of the input–output test at SC-CA1 synapses from 3–4-wk-old WT and *VRK3*-KO mice (n = 31, 41 slices from 4, 6 mice; P > 0.05, t test). Bars, 0.5 mV, 10 ms. (D) Group I metabotropic glutamate receptor-induced LTD induced by 10-min bath application of 100 µM (R,S)-3,5-DHPG (n = 18, 18 slices from 6, 7 mice; P > 0.05, t test) in hippocampal SC-CA1 synapses from 3–4-wk-old WT and *VRK3*-KO mice. Field excitatory postsynaptic potential (fEPSP) slope is shown as a percentage of baseline. Traces were taken 1 min before DHPG treatment (WT, gray; *VRK3* KO, light red) and at the end of the recording period (WT, black; *VRK3* KO, red). Bars, 0.5 mV, 10 ms. All values represent mean ± SEM.

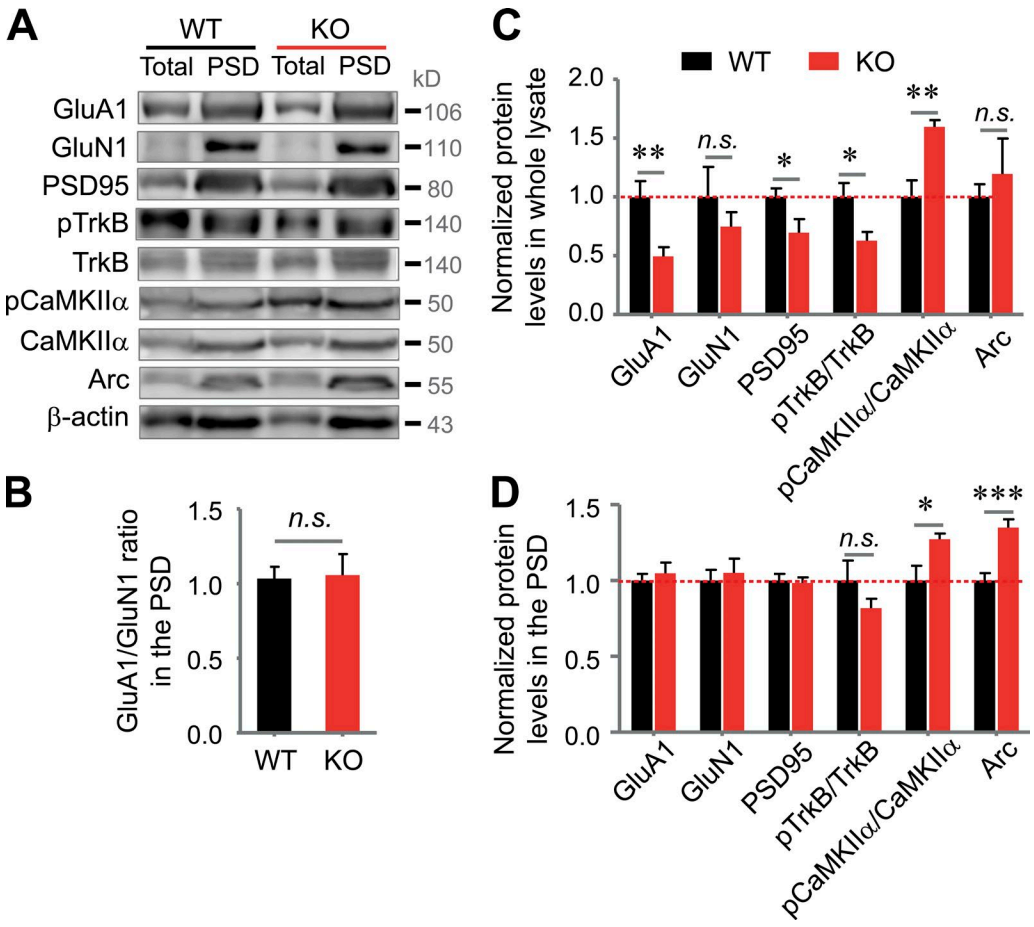

Figure S4. **Biochemical changes in the hippocampal PSD fraction and whole lysates of young *VRK3*-KO mice.** (A) Immunoblot analyses of PSD fractions and whole lysates from 4–5-wk old WT and *VRK3*-KO mice for the indicated proteins. GluA1, GluN1, and CaMKIIα are also known as GluR1, NR1, and CAMK2A, respectively. (B) Protein level of the ratio between GluA1 and GluN1 glutamate receptor subunits was not different between *VRK3*-KO and WT mice. (C and D) Protein levels of GluA1, GluN1, PSD-95, phosphorylated TrkB, phosphorylated CaMKIIα and Arc in hippocampal whole lysates (C) and PSD fractions (D) from *VRK3*-KO mice ($n = 6$ mice per genotype; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; *t* test). n.s., not significant. All values represent mean ± SEM.

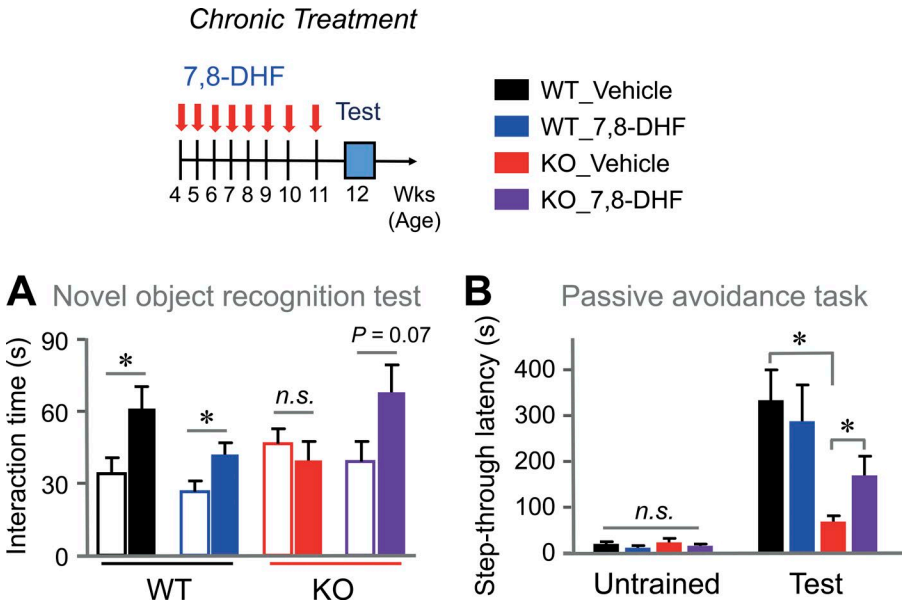

Figure S5. **Rescue effects of 7,8-DHF on the deficits in learning and memory in *VRK3*-KO mice.** (A) In a novel object recognition test, *VRK3*-KO mice exhibited an increased ability to discriminate between familiar and novel objects after chronic treatment with 7,8-DHF. (B) 7,8-DHF partially rescued impairment of learning and/or memory in a passive avoidance task in *VRK3*-KO mice. P = 0.07; *, P < 0.05; **, P < 0.01, two-tailed *t* test. All values represent mean ± SEM from 5–7 mice per genotype.