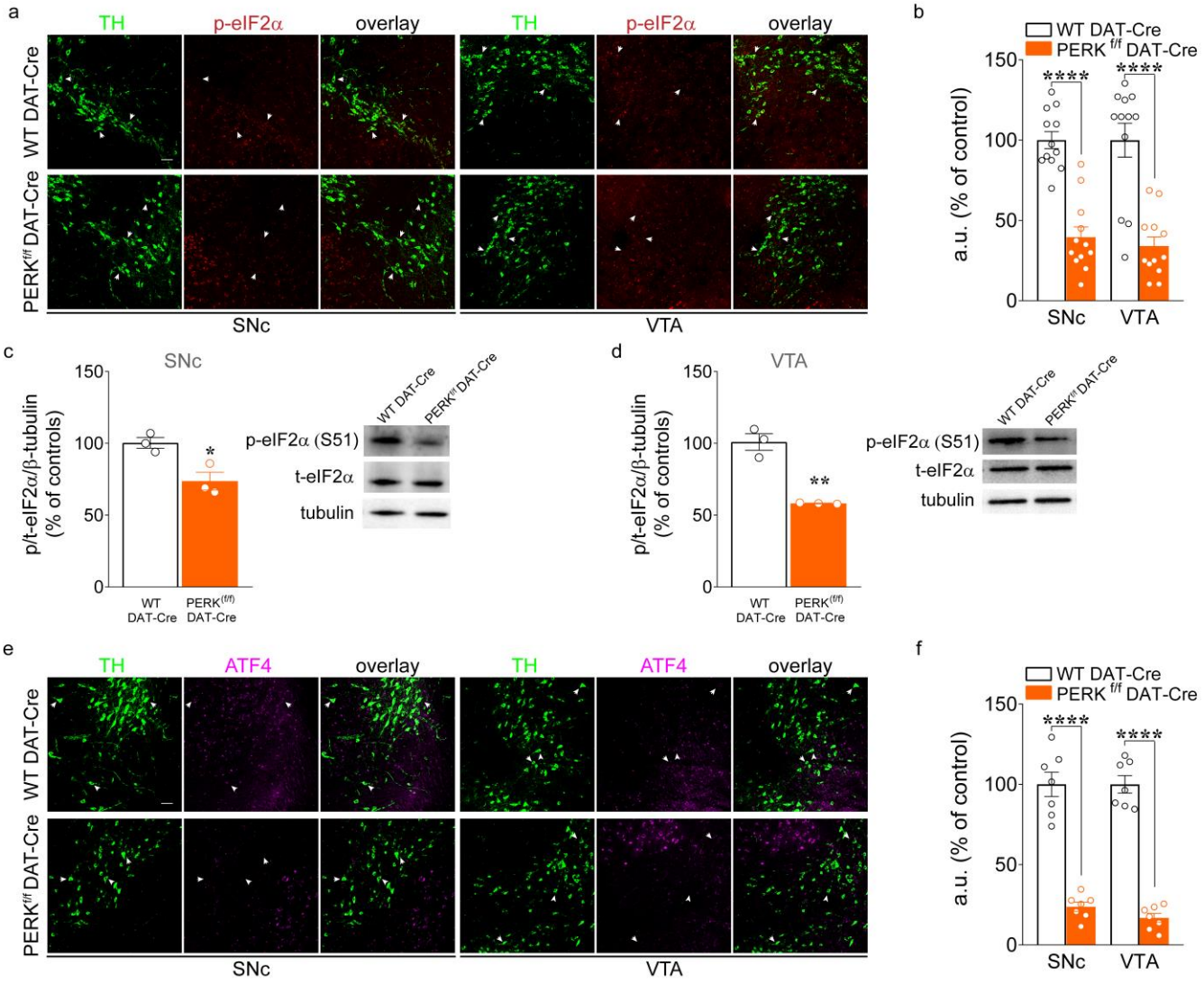
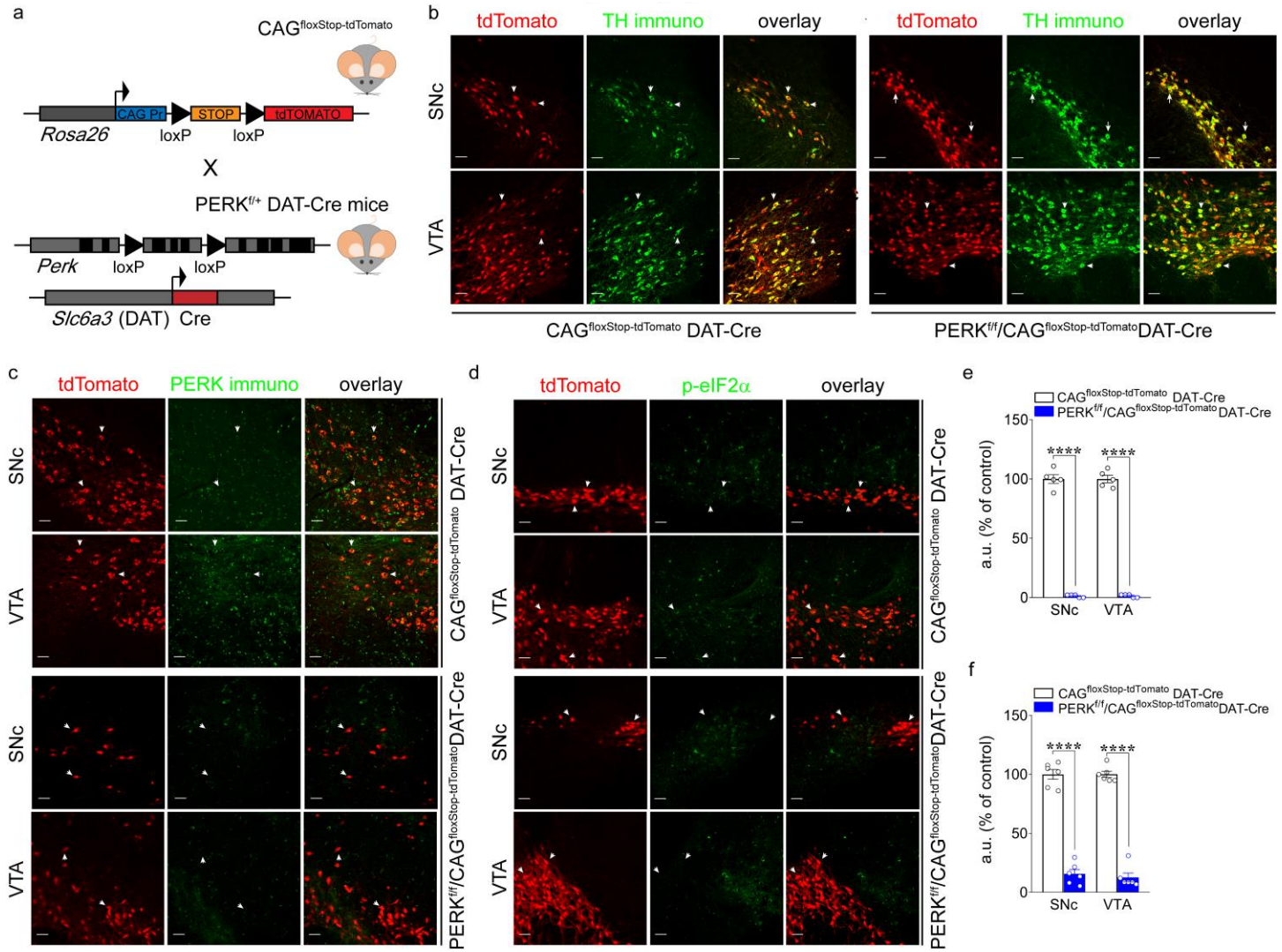
Supplementary Figures

Supplementary Figure 1



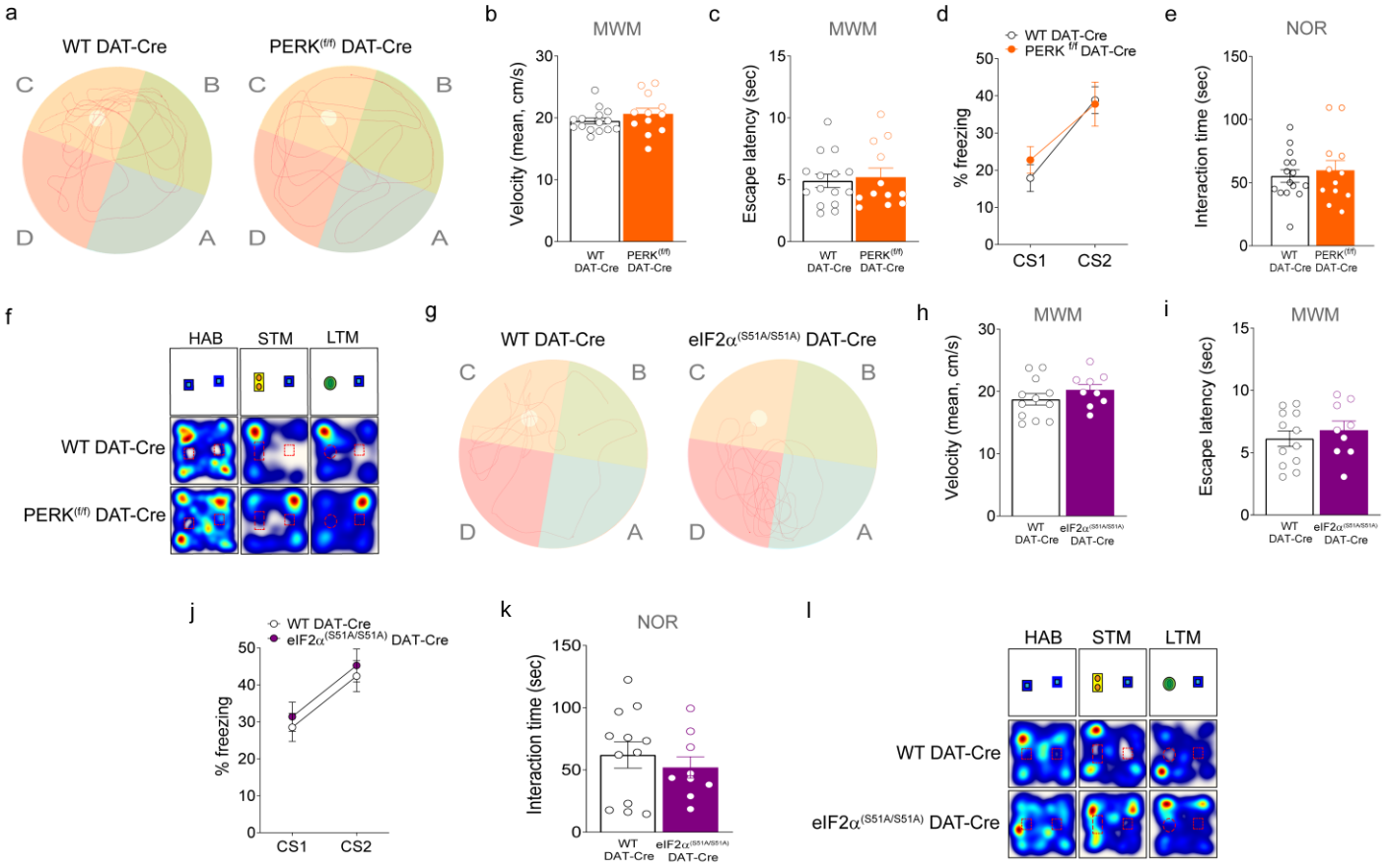
Supplementary Figure 1. Cell-specific deletion of PERK in VTA and SNc DA neurons results in reduced UPR after thapsigargin-induced ER-stress. (**a**) Immunofluorescent detection of TH$^+$ neuron (green) and phosphorylated eIF2α (red) in SNc and VTA DA neurons of PERK$^{f/f}$ DAT-Cre and WT DAT-Cre mice, in thapsigargin-treated brain slices (scale bars represent 50 µm). Arrows indicate dopaminergic neurons (green) and p-eIF2α (red) co-stain. (**b**) Summary plot showing reduced p-eIF2α signal after ER-stress induction, expressed as fluorescent arbitrary units (a.u.; % of control) in TH+ neurons from SNc and VTA of PERK$^{f/f}$ DAT-Cre vs. WT DAT-Cre mice. Cell soma intensity was measured in ImageJ. Statistical significance was determined by using Student's *t* test (PERK$^{f/f}$ DAT-Cre vs. WT DAT-Cre mice; unpaired t test; SNc, $t_{(22)}$ = 7.277, $P < 0.0001$; VTA, $t_{(22)}$ = 5.496, $P < 0.0001$). Data are shown as mean ± s.e.m. of n = 12 slices per group (average of n = 40 somas per slice, n = 2 slices per mouse, from three independent experiments) ****$P < 0.0001$. (**c,d**) Representative western blots (right panel) and quantification of phosphorylation of eIF2α in SNc (**c**) and VTA (**d**) of PERK$^{f/f}$ DAT-Cre and WT DAT-Cre mice. Summary plot showed a significant decrease in the phosphorylation of eIF2α in both SNc (**c**; unpaired t test, $t_{(4)}$ = 3.667, P = 0.023; n= 3 independent lysates from 3 mice per group) and VTA (**d**; unpaired t test, $t_{(4)}$ = 7.414, P = 0.002; n= 3 independent lysates from 3 mice per group) of PERK$^{f/f}$ DAT-Cre mice compared with controls after treatment with thapsigargin. (**e**) Immunofluorescent detection of TH$^+$ neuron (green) and ATF4 (magenta) in SNc and VTA DA neurons of PERK$^{f/f}$ DAT-Cre and WT DAT-Cre mice, in thapsigargin-treated brain slices (scale bars represent 50 µm). Arrows indicate dopaminergic neurons (green) and ATF4 (magenta) co-stain. (**f**) Summary plot showing reduced ATF4 signal after ER-stress induction, expressed as fluorescent arbitrary units (a.u.; % of control) in TH+ neurons from SNc and VTA of PERK$^{f/f}$ DAT-Cre *vs.* WT DAT-Cre mice. Cell soma intensity was measured in ImageJ. Statistical significance was determined by using Student's *t* test (PERK$^{f/f}$ DAT-Cre vs. WT DAT-Cre mice; unpaired t test; SNc, $t_{(12)}$ = 9.407, $P < 0.0001$; VTA, $t_{(12)}$ = 13.66, $P < 0.0001$). Data are shown as mean ± s.e.m. of n = 7 slices per group (average of n = 40 somas per slice, n = 2-3 slices per mouse, from three independent experiments) ****$P < 0.0001$.

Supplementary Figure 2



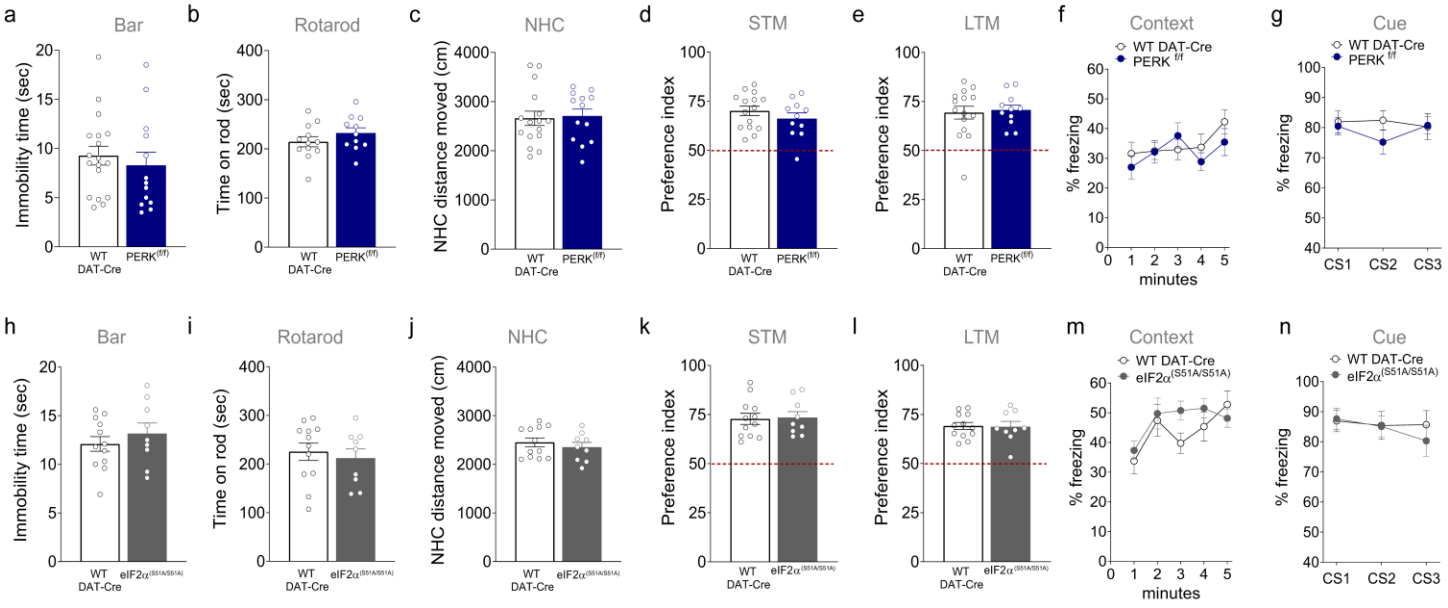
Supplementary Figure 2. Gt(ROSA)26Sor$^{tm14(CAG-tdTomato)Hze}$ Cre-dependent reporter mice crossed with PERK$^{f/+}$ DAT-Cre mice confirm positive targeting of DAT^{+} neurons for deletion of PERK and reduction of phosphorylated eIF2α. (**a**) Schematic representation of DAT^{+} neuron-specific expression of tdTomato in DAT-Cre mice crossed with Gt(ROSA)26Sor$^{tm14(CAG-tdTomato)Hze}$ Cre-dependent reporter mice. (**b**) Immunofluorescent detection of tdTomato (red) DAT^{+} neurons and TH-IR (green) in SNc and VTA DA neurons of PERK$^{f/f}$/CAG$^{floxStop-tdTomato}$DAT-Cre and WT/CAG$^{floxStop-tdTomato}$DAT-Cre (control) mice, confirming specificity of the Cre-system (scale bars represent 50 µm). Arrows indicate dopaminergic neurons (green) and tdTomato (red) co-stain. (**c**) Immunofluorescent detection of tdTomato (red) DAT^{+} neurons and PERK (green) expression in SNc and VTA DA neurons of PERK$^{f/f}$/CAG$^{floxStop-tdTomato}$DAT-Cre and WT/CAG$^{floxStop-tdTomato}$DAT-Cre mice, confirming positive targeting of DAT neurons for the deletion of PERK (scale bars represent 50 µm). Arrows indicate dopaminergic neurons (tdTomato; red) and PERK (green) co-stain. (**d**) Immunofluorescent detection of tdTomato (red) DAT^{+} neurons and phosphorylated eIF2α (green) expression in SNc and VTA DA neurons of PERK$^{f/f}$/CAG$^{floxStop-tdTomato}$DAT-Cre and WT/CAG$^{floxStop-tdTomato}$DAT-Cre mice, confirming reduction of phosphorylated eIF2α following deletion of PERK in DA neurons (scale bars represent 50 µm). Arrows indicate dopaminergic neurons (tdTomato; red) and p-eIF2α (green) co-stain. (**e,f**) Summary plot showing reduced PERK (**e**) and p-eIF2α (**f**) signal expressed as fluorescent arbitrary units (a.u.; % of control) in tdTomato^{+} (dopaminergic) neurons from SNc and VTA of PERK$^{f/f}$/CAG$^{floxStop-tdTomato}$DAT-Cre *vs.* WT/CAG$^{floxStop-tdTomato}$DAT-Cre mice. Cell soma intensity was measured in ImageJ. Statistical significance was determined by using Student's *t* test (PERK$^{f/f}$/CAG$^{floxStop-tdTomato}$DAT-Cre *vs.* WT/CAG$^{floxStop-tdTomato}$DAT-Cre mice; **e**, unpaired *t* test, SNc, $t_{(8)} = 27.04$, $P < 0.0001$; VTA, $t_{(8)} = 31.14$, $P < 0.0001$; **f**, unpaired *t* test, SNc, $t_{(10)} = 15.31$, $P < 0.0001$; VTA, $t_{(10)} = 19.00$, $P < 0.0001$) Data are shown as mean ± s.e.m. of n = 5-6 slices per group (average of n = 40 somas per slice, n = 2-3 slices per mouse, from three independent experiments) ****$P < 0.0001$.

Supplementary Figure 3



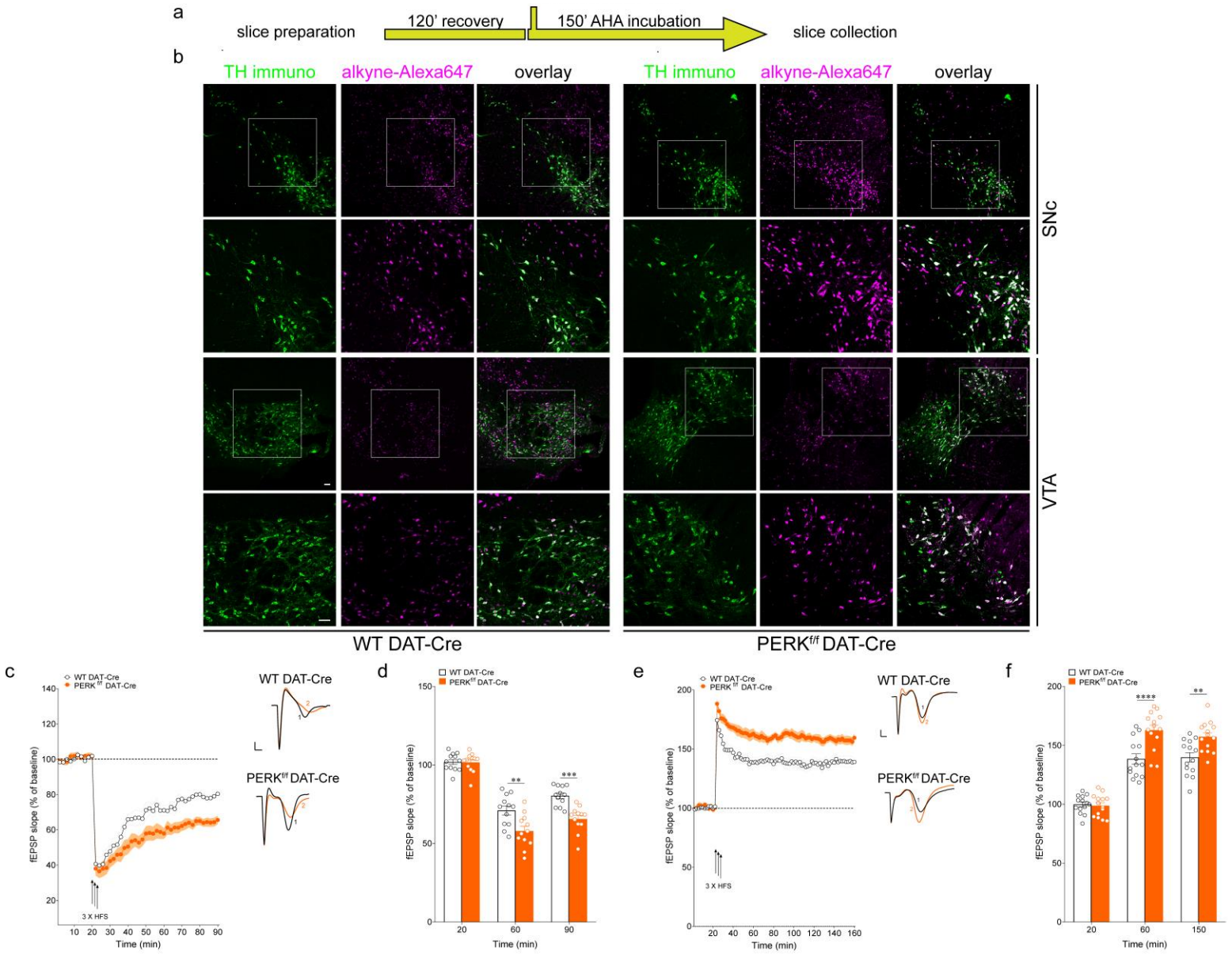
Supplementary Figure 3. PERK$^{f/f}$ DAT-Cre and eIF2α$^{(S51A/S51A)}$ DAT-Cre mice exhibit normal vision during MWM test and, normal freezing behavior and object interaction during training in FC and NOR tests, respectively.
(**a**) Representative swim paths in the MWM test. (**b-e**) Summary plot of (**b**) average velocity during the MWM test (unpaired *t* test; $t_{(25)}$= 1.15, *P* = 0.26); (**c**) escape latency during visible platform test (unpaired *t* test; $t_{(25)}$= 0.33, *P* = 0.74); (**d**) average percentage of freezing during training in the associative threat memory test (two-way RM ANOVA, followed by Bonferroni's multiple comparisons test, time x genotype, $F_{(1,25)}$ = 0.76, *P* = 0.39), and (**e**) interaction time with familiar objects during habituation in the novel object recognition test (unpaired *t* test; $t_{(25)}$ = 0.513, *P* = 0.61) in 3-month old PERK$^{f/f}$ DAT-Cre *versus* WT DAT-Cre mice. (**f**) Representative heat maps of the interaction with the objects for each genotype in the novel object recognition test. (**g**) Representative swim paths in the MWM test. (**h-k**) Summary plot of (**h**) average velocity during the MWM test (unpaired *t* test; $t_{(19)}$= 1.15, *P* = 0.27); (**i**) escape latency during visible platform test (unpaired *t* test; $t_{(19)}$= 0.71, *P* = 0.49); (**j**) average percentage of freezing during training in the associative threat memory test (two-way RM ANOVA, followed by Bonferroni's multiple comparisons test, time, $F_{(1,19)}$ = 78.34, *P* < 0.001), and (**k**) interaction time with familiar objects during habituation in the novel object recognition test (unpaired *t* test; $t_{(19)}$= 0.702, *P* = 0.49) in 3-month old eIF2α$^{(S51A/S51A)}$ DAT-Cre *versus* WT DAT-Cre mice. (**l**) Representative heat maps of the interaction with the objects for each genotype in the novel object recognition test.

Supplementary Figure 4



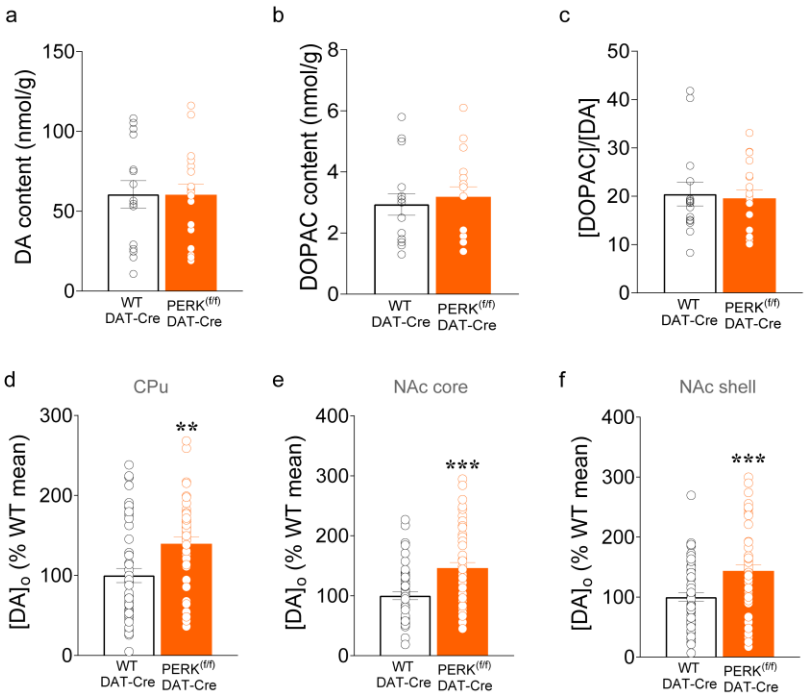
Supplementary Figure 4. Either mice harboring floxed PERK gene (PERK$^{f/f}$) or eIF2α$^{(S51A/\ S51A)}$ mice show no differences in motor and cognitive phenotype compared to their WT-Cre littermates. PERK$^{f/f}$, eIF2α$^{(S51A/\ S51A)}$ and their respective WT DAT-Cre littermates mice were subjected to a set of tests, including the bar (**a,h**), rotarod (**b,i**) and novel home (**c,j**) tests to investigate locomotor activity, and to NOR (**d,e; k,l**) and FC (**f,g; m,n**) tests to investigate memory. (**a,h**) Summary plot of immobility time (sec) during bar test in (**a**) PERK$^{f/f}$ *versus* WT DAT-Cre mice (unpaired *t* test, $t_{(29)}$= 0.62, *P* =0.54) and (**h**) eIF2α$^{(S51A/\ S51A)}$ *versus* WT DAT-Cre mice (unpaired *t* test, $t_{(19)}$= 0.83, *P* =0.42). (**b,i**) Summary plot of latency to fall from the rotating rod measured as average of two days (4 trials/day) test in (**b**) PERK$^{f/f}$ *versus* WT DAT-Cre mice (unpaired *t* test, $t_{(21)}$= 1.19, *P* =0.25) and (**i**) eIF2α$^{(S51A/\ S51A)}$ *versus* WT DAT-Cre mice (unpaired *t* test, $t_{(19)}$= 0.50, *P* =0.62). (**c,j**) Summary plot of the novelty-induced locomotor activity expressed as a novel home cage (NHC) distance moved (cm) in the first 10 minutes interval of a 60 minutes test during novel home cage test in (**c**) PERK$^{f/f}$ *versus* WT DAT-Cre mice (unpaired *t* test, $t_{(27)}$= 0.23, *P* =0.82) and (**j**) eIF2α$^{(S51A/\ S51A)}$ *versus* WT DAT-Cre mice (unpaired *t* test, $t_{(19)}$= 0.74, *P* =0.47). (**d,e**) Summary plots of preference indices of mice towards a novel object introduced in the novel object recognition test in PERK$^{f/f}$ *versus* WT DAT-Cre mice (unpaired *t* test; **d**, $t_{(24)}$ = 1.06, *P* =0.30; **e**, $t_{(24)}$ = 0.30, *P* =0.76). (**k,l**) Summary plots of preference indices of mice towards a novel object introduced in the novel object recognition test in eIF2α$^{(S51A/\ S51A)}$ *versus* WT DAT-Cre mice (unpaired *t* test; **k**, $t_{(19)}$ = 0.16, *P* =0.88; **l**, $t_{(19)}$ = 0.12, *P* =0.90). (**f,g**) Summary plots of average percentage of freezing during (**f**) exposure to the context 24 hours after training, and (**g**) exposure to 3 CS presentations in a novel context in the associative threat memory test PERK$^{f/f}$ *versus* WT DAT-Cre mice (**f**, two-way RM ANOVA, followed by Bonferroni's multiple comparisons test, genotype, $F_{(1, 28)}$ = 0.38, *P* = 0.54; **g**, two-way RM ANOVA, followed by Bonferroni's multiple comparisons test, genotype, $F_{(1, 28)}$ = 0.53, *P* = 0.47). (**m,n**) Summary plots of average percentage of freezing during (**m**) exposure to the context 24 hours after training, and (**n**) exposure to 3 CS presentations in a novel context in the associative threat memory test eIF2α$^{(S51A/\ S51A)}$ *versus* WT DAT-Cre mice (**m**, two-way RM ANOVA, followed by Bonferroni's multiple comparisons test, genotype, $F_{(1, 22)}$ = 0.97, *P* = 0.34; **n**, two-way RM ANOVA, followed by Bonferroni's multiple comparisons test, genotype, $F_{(1, 22)}$ = 0.10 *P* = 0.75). 3-month old mice were analyzed using Student's *t* test or two-way RM ANOVA, followed by Bonferroni's test for multiple comparisons. All data are shown as mean ± s.e.m. of *n* = 13-18 (a), *n* = 11-12 (b), *n* = 13-16 (c), *n* = 9-12 (h-l), *n* = 11-15 (d,e), *n* = 15 (f,g), and *n* = 12 (m,n) mice/genotype.

Supplementary Figure 5



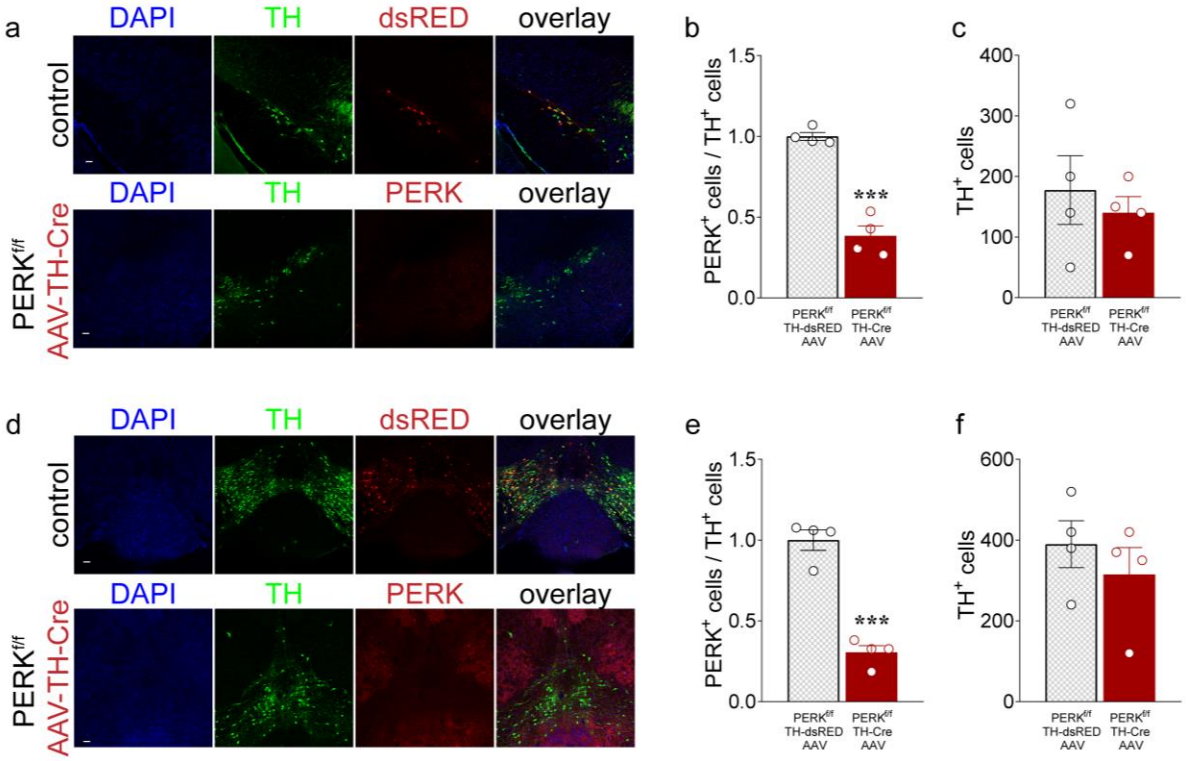
Supplementary Figure 5. **Deletion of PERK in DA neurons causes dysregulated *de novo* translation in mice and alters both striatal and hippocampal synaptic plasticity.** (**a**) Schematic for FUNCAT experiments. (**b**) Representative immunofluorescence images of TH-IR (green) and incorporation of AHA (magenta) detected by FUNCAT with alkyne-Alexa 647 in SNc and VTA DA neurons from midbrain coronal slices of 3-month old PERK$^{f/f}$ DAT-Cre mice and their WT DAT-Cre littermates. Insets in the first and third rows are magnified in the second and fourth rows, respectively (scale bars represent 50 µm). (**c,d**) Striatal long-term depression (LTD) in 3-month old PERK$^{f/f}$ DAT-Cre *vs*. WT DAT-Cre mice. (**c**) Plot showing normalized fEPSP mean slope (±s.e.m. displayed every 2 min) recorded from striatal slices. (**d**) Mean fEPSPs at baseline (20 min), at 60 (40 min after tetanus) and at 90 min (70 min after tetanus). LTD evoked by 3 trains of high frequency stimulation (HFS) was significantly enhanced in PERK$^{f/f}$ DAT-Cre striatal slices at both 60 min and 90 min (two-way RM ANOVA, followed by Bonferroni's multiple comparisons test, time x genotype, $F_{(2,44)} = 6.71$, $P = 0.003$; time, $F_{(2,44)} = 161.60$, P <0,0001; genotype $F_{(1,22)} = 19.85$, $P = 0,0002$; n=12 slices from 7 mice/genotype). Data are shown as mean ± s.e.m. $^{**}P < 0.01$ and $^{***}P < 0.001$ PERK$^{f/f}$ DAT-Cre *versus* WT DAT-Cre mice. (**e,f**) Hippocampal late-phase long-term potentiation (L-LTP) in 3-month old PERK$^{f/f}$ DAT-Cre *vs*. WT DAT-Cre mice. (**e**) Plot showing normalized fEPSP mean slope (±s.e.m. displayed every 2 min) recorded from hippocampal slices. (**f**) Mean fEPSPs at baseline (20 min), at 60 (40 min after tetanus) and at 150 min (130 min after tetanus). L-LTP evoked by 3 trains of high frequency stimulation (HFS) was significantly increased in PERK$^{f/f}$ DAT-Cre hippocampal slices at both 60 min and 150 min (two-way RM ANOVA, followed by Bonferroni's multiple comparisons test, time x genotype, $F_{(2,52)} = 8.36$, $P = 0.0007$; time, $F_{(2,52)} = 162$, P <0,0001; genotype $F_{(1,26)} = 162$, $P = 0,0004$; n=14 slices from 8 mice/genotype). Representative traces (**c,e** left panels) are superimposed fEPSPs (scale bars represent 1 mv/msec) recorded during baseline (1) and 60 min after HFS train (2). Arrows indicate delivery of HFS.

Supplementary Figure 6



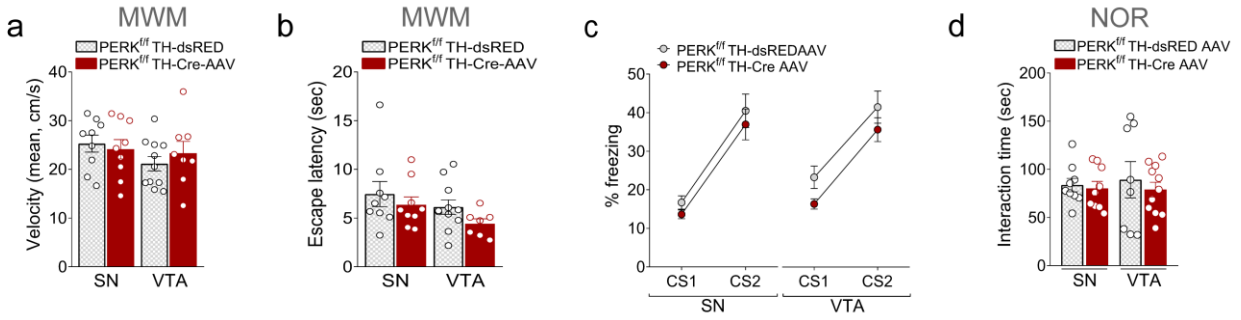
Supplementary Figure 6. Deletion of PERK from DA neurons does not affect DA content and the effect on DA release does not involve ACh and nAChRs. Tissue content (nmoles/g tissue wet weight) of DA (**a**), its metabolite DOPAC (**b**), was performed using HPLC with electrochemical detection in experimental striatal slices. The analysis of the metabolite/DA ratio ([DOPAC/DA]; **c**) is also reported. (**a-b**) Summary plot of average DA (**a**) and DOPAC (**b**) content in both dorsal (DS) and ventral (VS) striatum of WT DAT-Cre *vs.* PERK$^{f/f}$ DAT-Cre mice (unpaired *t*-test, n.s.). (**c**) Summary plot of average [DOPAC/DA] ratio (unpaired *t*-test, n.s). All data are shown as mean ± s.e.m. of $n = 15$-18 mice per genotype (**a-c**). (**d-f**) Average single-pulse-evoked $[DA]_o$ in dStr (**d**, unpaired *t*-test: $t_{(91)} = 3.160$, $P < 0.01$), NAc core (**e**, unpaired *t*-test: $t_{(96)} = 4.045$, $P < 0.001$), and NAc shell (**f,** unpaired *t*-test: $t_{(96)} = 4.491$, $P < 0.001$) in the presence of dihydro-β-erythroidine (DhβE; 1 μM), a selective antagonist for β2 subunit-containing (β2*) nAChRs that are enriched on DA axons. Data are are means ± s.e.m. normalized to 100% mean WT control for each region. $^{**}P < 0.01$; $^{***}P < 0.001$ for WT DAT-Cre *vs.* PERK$^{f/f}$ DAT-Cre mice, where *n* denotes the number of recording sites in each region sampled from 3 to 5 mice per genotype.

Supplementary Figure 7



Supplementary Figure 7. Selective deletion of PERK in DA neurons of the SNc and the VTA. (**a**) Immunofluorescence detection of TH-IR (green) and either PERK or dsREd (red) in the SNc of PERK$^{f/f}$ TH-Cre-AAV and PERK$^{f/f}$ TH-dsRED AAV control mice (scale bars represent 50 µm). (**b**) Summary data showing the ratio of TH$^+$ cells in the SNc that co-labeled for PERK in PERK$^{f/f}$ TH-Cre-AAV *vs*. PERK$^{f/f}$ TH-dsRED AAV control mice (n = 4 mice each, unpaired t test, $t_{(6)} = 9.373$, $P < 0.001$). (**c**) Summary data showing the overall number of TH$^+$ cells in the SNc that co-labeled for PERK in PERK$^{f/f}$ TH-Cre-AAV *vs*. PERK$^{f/f}$ TH-dsRED AAV control mice (n = 4 mice each, unpaired t test, $t_{(6)} = 0.599$, $P = 0.571$). (**d**) Immunofluorescence detection of TH-IR (green) and PERK or dsRED (red) in the VTA of PERK$^{f/f}$ TH-Cre-AAV and PERK$^{f/f}$ TH-dsRED AAV control mice (scale bars represent 50 µm). (**e**) Summary data showing the ratio of TH$^+$ cells in the VTA that co-labeled for PERK in PERK$^{f/f}$ TH-Cre-AAV *vs*. PERK$^{f/f}$ TH-dsRED AAV control mice (n = 4 mice each, unpaired t test, $t_{(6)} = 9.120$, $P < 0.001$). (**f**) Summary data showing the overall number of TH$^+$ cells in the VTA that co-labeled for PERK in PERK$^{f/f}$ TH-Cre-AAV *vs*. PERK$^{f/f}$ TH-dsRED AAV control mice (n = 4 mice each, unpaired t test, $t_{(6)} = 0.849$, $P = 0.429$).

Supplementary Figure 8



Supplementary Figure 8. Selective PERK deletion in DA neurons of the SN or the VTA does not alter freezing behavior and object interaction during training. (**a-d**) Summary plot of (**a**) average velocity during the MWM test (unpaired t test; d, n.s. in both SN and VTA); (**b**) escape latency during visible platform test (unpaired t test; d, n.s. in both SN and VTA); (**c**) average percentage of freezing during training in the associative threat memory test (two-way RM ANOVA, followed by Bonferroni's multiple comparisons test, n.s. in both SN and VTA), and (**d**) interaction time with familiar objects during habituation in the novel object recognition test (unpaired t test; d, n.s. in both SN and VTA) in 3-month old PERK$^{f/f}$ TH-Cre-AAV *versus* PERK$^{f/f}$ TH-dsRED AAV control mice.