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Supplemental information

Gene-environment interactions mediate stress susceptibility and resilience through the CaMKIIβ/TARPγ-8/AMPAR pathway

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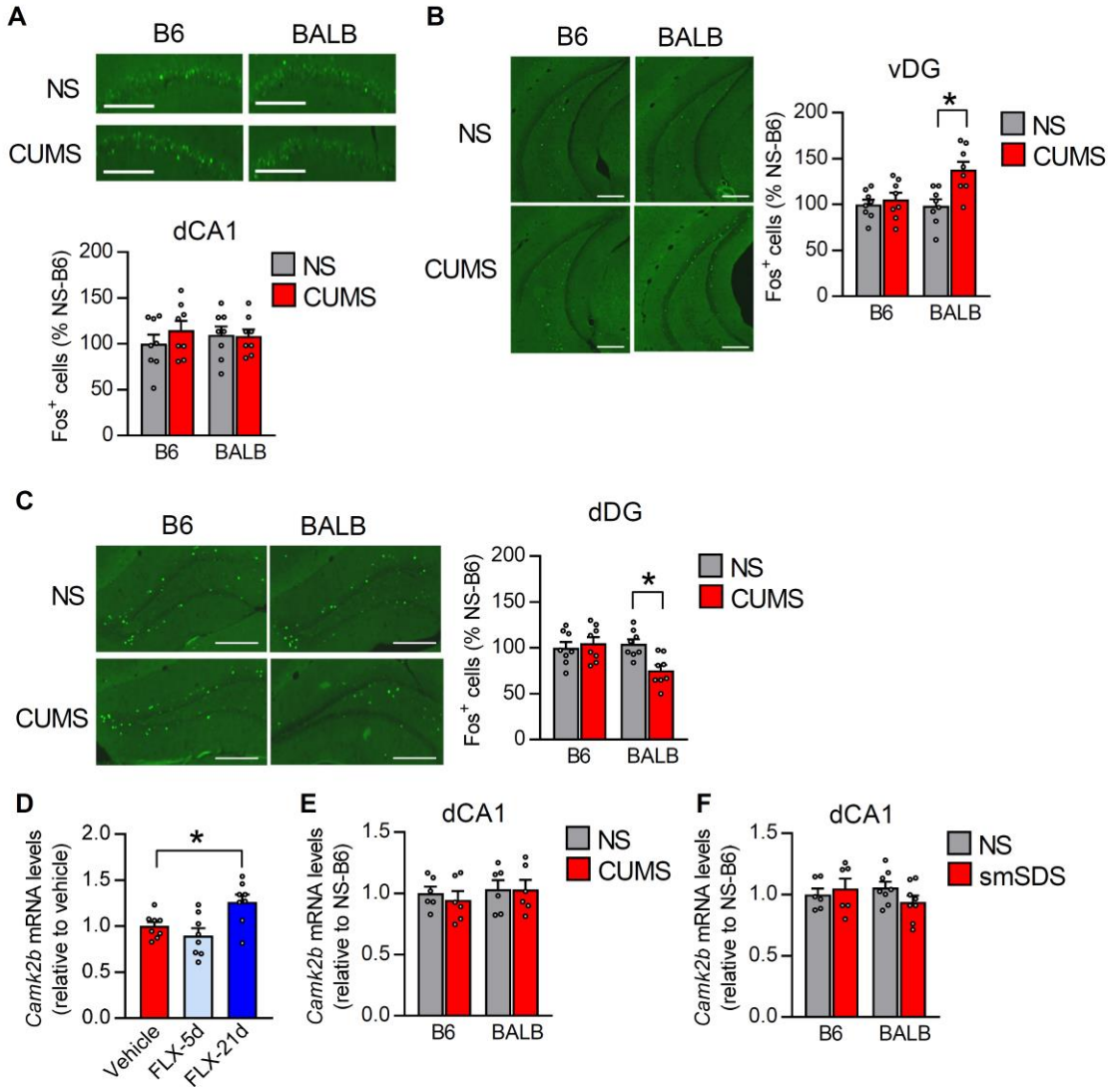

Figure. S1. Fos and *Camk2b* expression in BALB and B6 mice following stress exposure, related to Figure 1

A. Representative coronal slices showing Fos-positive cells in the dorsal CA1 (dCA1). Chronic ultra-mild stress (CUMS) exposure does not affect Fos expression in the dCA1 of BALB and B6 mice. *n* = 8 mice per group. Scale bar, 500 μm.

B. Representative coronal slices showing Fos-positive cells in the ventral dentate gyrus (vDG). CUMS exposure increases Fos expression in the vDG of BALB, but not in that of B6 mice. *n* = 8 mice per group. Scale bar, 500 μm.

C. Representative coronal slices showing Fos-positive cells in the dorsal DG (dDG). CUMS exposure

reduces Fos expression in the dDG of BALB, but not B6 mice. *n* = 8 mice per group. Scale bar, 500 μm.

D. Increased *Camk2b* expression following chronic treatment with fluoxetine (FLX). *n* = 8 mice per group.

E. Normal *Camk2b* expression in the dCA1 of BALB and B6 mice following CUMS exposure. *n* = 6 mice per group.

F. Normal *Camk2b* expression in the dCA1 of BALB and B6 mice following smSDS exposure. *n* = 6-8 mice per group.

Two-way ANOVA followed by a Tukey's post hoc test (in A-C, E, and F) and one-way ANOVA followed by a Tukey's post hoc test (in D) were used for statistical analyses. $*p < 0.05$. Bar graphs show mean ± standard error of mean. Complete statistical summaries of the data are provided in Table S2.

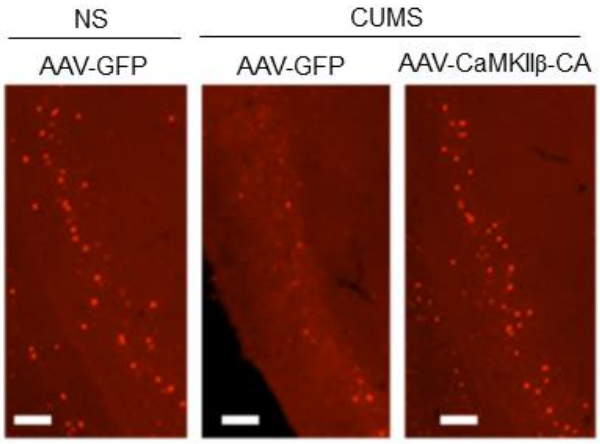

Figure S2. Representative images of immunofluorescence staining of Fos in non-stressed (NS) and stressed (CUMS) BALB mice injected with AAV-GFP or AAV-CaMKIIβ-CA, related to Figure 2.

Scale bar, 100 μm.

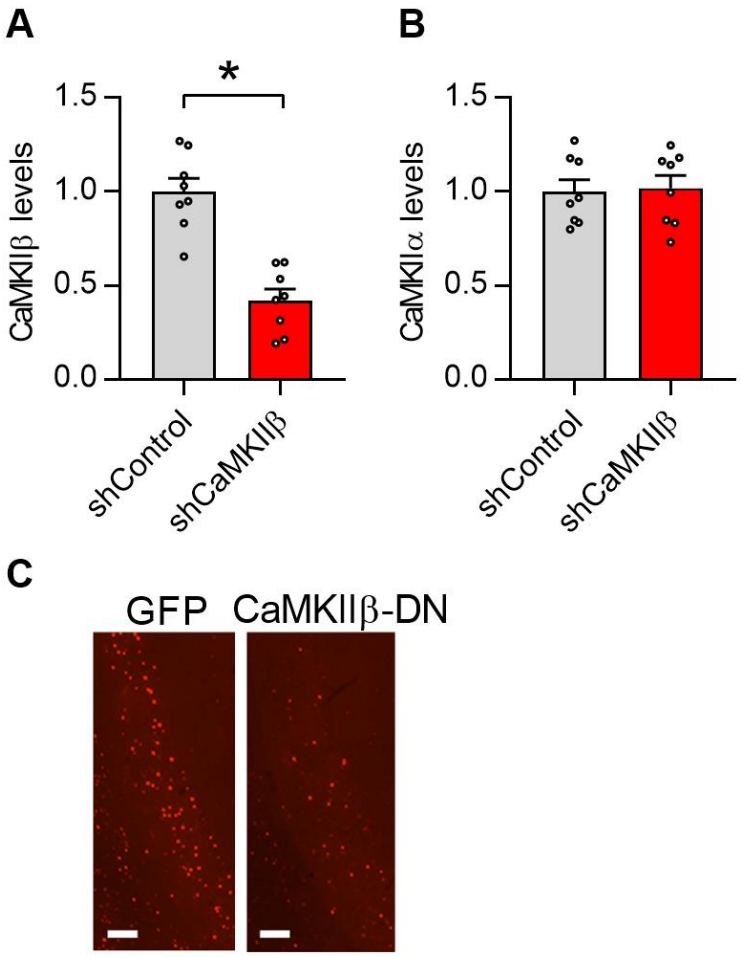

Figure S3. Effect of CaMKIIβ knockdown and representative images for Fos staining, related to Figure 3

A and B. Specific knockdown of Ca^{2+}/calmodulin-dependent protein kinase II β (CaMKIIβ) (**A**) but not CaMKIIα (**B**) by the CaMKIIβ knockdown vector (AAV-shCaMKIIβ).

C. Representative images of immunofluorescence staining of Fos in stressed (smSDS) B6 mice injected with AAV-GFP or AAV-CaMKIIβ-DN. Scale bar, 100 μm.

Two-tailed Student's t-test (in A and B) was used for statistical analyses. *$P < 0.05$. Bar graph shows the mean ± standard error of mean. Complete statistical summaries of the data are provided in Table S2.

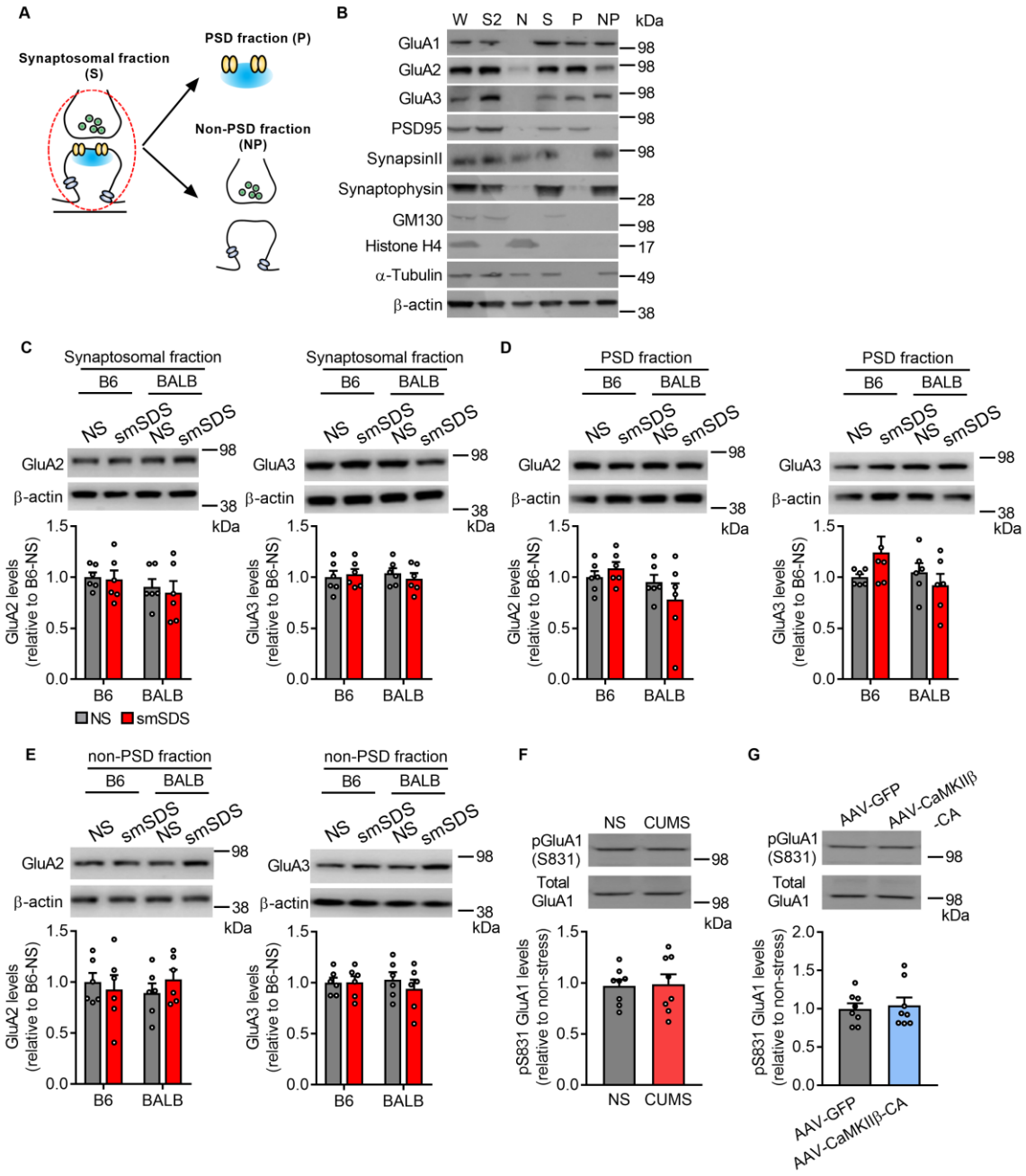

Figure S4. The levels of GluA2, GluA3, and phosphorylation of GluA1 following stress exposure, Related to Figure 4

A. Synaptosomes (S) were treated with Triton-X to yield insoluble postsynaptic membrane (PSD)-enriched (P) and soluble non-PSD-enriched (NP) fractions, corresponding to synaptic versus peri-/extra-synaptic and presynaptic membranes.

B. Distribution of the protein markers associated with the synaptosomes, PSD, and non-PSD sub-fractions. The integrity of the synaptosomal fractions was verified by immunoblotting with antibodies for GM130 (Golgi marker), Histone H4 (nuclear protein), synapsin II (presynaptic protein), synaptophysin (presynaptic protein), PSD95 (PSD protein), GluA1 (postsynaptic proteins), GluA2 (postsynaptic proteins), GluA3 (postsynaptic proteins), α-tubulin, and β-actin. W, whole cell extract; S2, cytosol and microsomes; N, nuclear fraction.

C-E. GluA2 and GluA3 expression in synaptosomal (**C**), PSD (**D**), and non-PSD (**E**) fractions in B6 and BALB mice subjected to smSDS or non-stressed (NS). *n* = 6-8 mice per group.

F. Normal pGluA1 (S831) expression in the vCA1 of BALB mice following CUMS exposure. *n* = 8 mice per group.

G. Normal pGluA1 (S831) expression in the vCA1 of BALB mice overexpressing CaMKIIβ-CA. *n* = 8 mice per group. AAV-GFP: adeno-associated virus [AAV]-green fluorescent protein [GFP]; AAV-CaMKIIβ-CA, AAV-constitutively active CaMKIIβ.

Two-way ANOVA (in C-E) and two-tailed Student's t-test (in F and G) were used for statistical analyses. $*P < 0.05$. Bar graph shows the mean ± standard error of mean. Complete statistical summaries of the data are provided in Table S2.

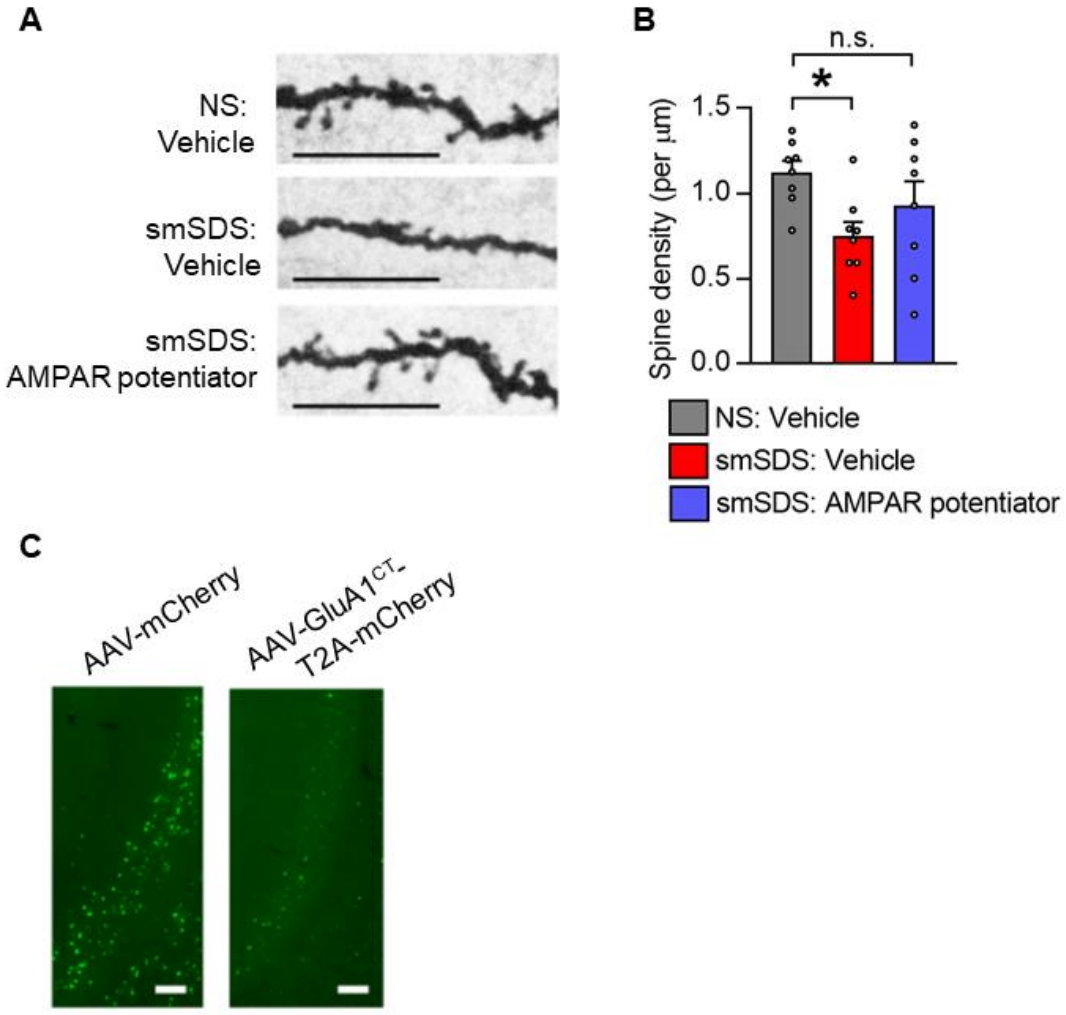

Figure S5. Effects of AMPAR activation on vCA1 spine density and Fos expression, related to Figure 5.

A and **B.** Golgi staining. smsSDS exposure reduces the dendritic spine density in the vCA1 neurons of BALB mice, whereas this reduction is blocked by treatment with AMPAR potentiator. Scale bar, 10 µm. *n* = 8 mice per group.

C. Representative images of immunofluorescence staining of Fos in stressed (smSDS) B6 mice injected with AAV-mCherry or AAV-GluA1^{CT}-T2A-mCherry. Scale bar, 100 µm.

One-way ANOVA followed by a Tukey's post hoc test (in B) was used for statistical analyses. *P < 0.05. Bar graph shows the mean ± standard error of mean. Complete statistical summaries of the data are provided in Table S2.

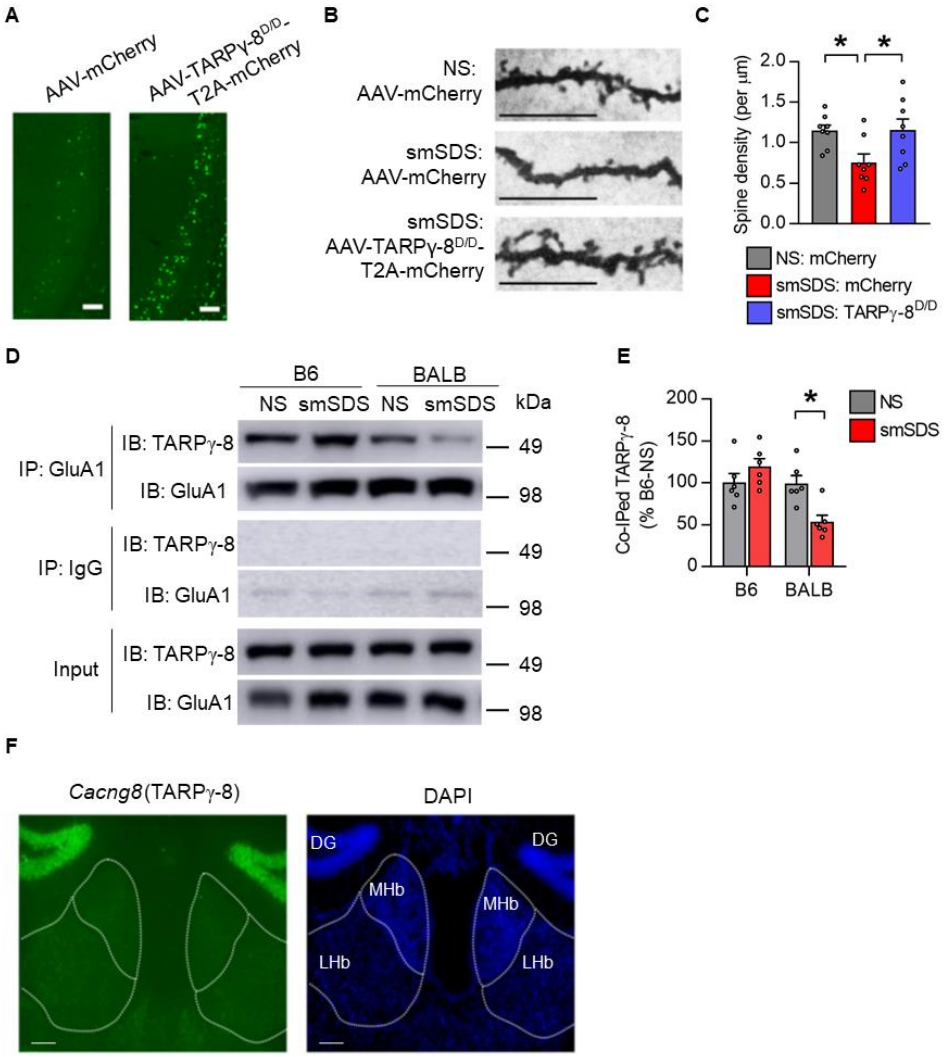

Figure S6. Effects of TARPγ-8 activation on Fos expression and dendritic spine density, and effect of stress on GluA1-TARPγ-8 interaction, related to Figure 6

A. Representative images of immunofluorescence staining of Fos in stressed (smSDS) BALB mice injected with AAV-mCherry or AAV-TARPγ-8$^{D/D}$-T2A-mCherry. Scale bar, 100 μm.

B and C. Golgi staining. smsSDS exposure reduces the dendritic spine density in the vCA1 neurons of BALB mice, whereas this reduction is blocked by TARPγ-8$^{D/D}$ overexpression. Scale bar, 10 μm. *n* = 8 mice per group.

D and E. Co-immunoprecipitation reveals decreased GluA1-TARPγ-8 complexes in the vHPC of stressed BALB but not B6 mice. *n* = 6 mice per group.

F. Expression of the *Cacng8* gene (encoding TARPγ-8) by RNAscope in a coronal section of the mouse brain. dCA1, dorsal part of cornu ammonis (CA1) of the hippocampus (HPC); LHb, lateral habenula. Scale bar, 500 μm.

One-way ANOVA followed by a Tukey's post hoc test (in C) and two-way ANOVA followed by a Tukey's post hoc test (in E) were used for statistical analyses. *$P < 0.05$. Bar graph shows the mean ± standard error of mean. Complete statistical summaries of the data are provided in Table S2.