Expanded View Figures

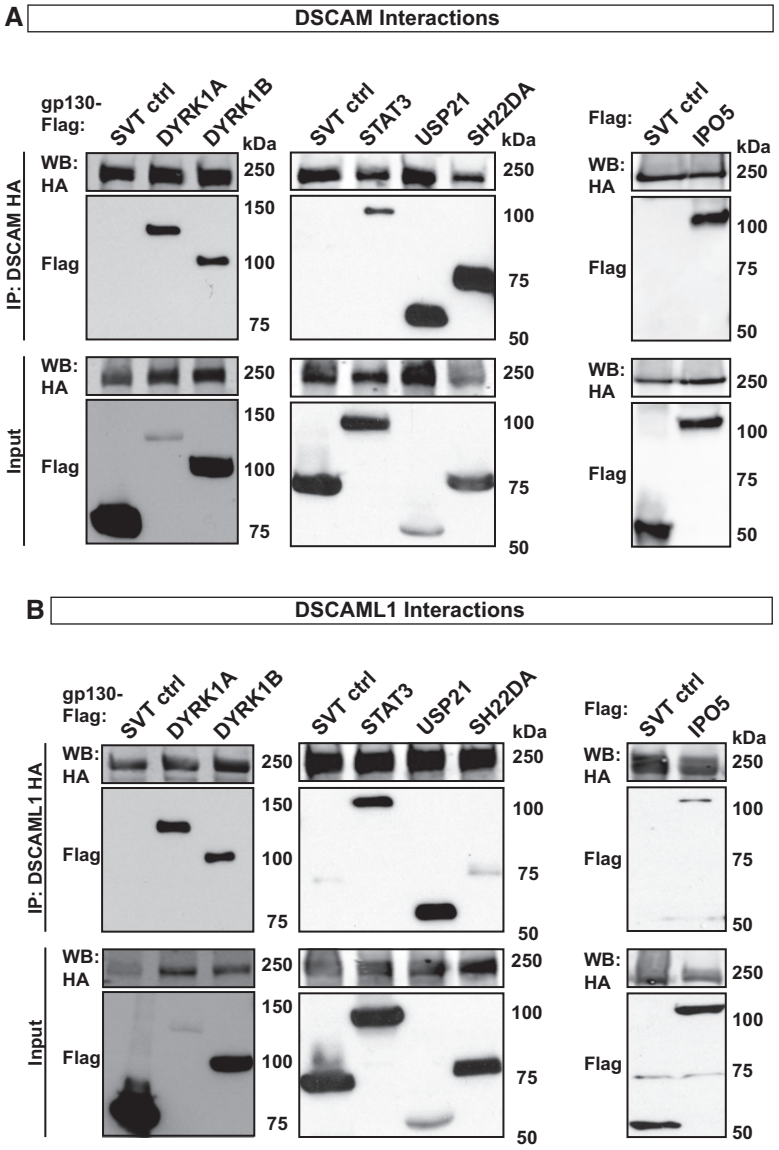

Figure EV1. Validation of MAPPIT Interactions by co-immunoprecipitation (related to Fig 1).

A, B HEK293T cells or SH-SY5Y cells were co-transfected with either DSCAM-HA or DSCAML1-HA together with gp130-Flag-tagged SVT (SV40 large T antigen, unrelated negative control), DYRK1A, DYRK1B, STAT3, USP21, SH2D2A, or Flag-tagged IPO5. HA-tagged DSCAM/L1 proteins were precipitated using anti-HA magnetic beads and co-precipitated protein complexes were analyzed by Western blot using HA- and Flag-tag specific antibodies. (A) DSCAM-HA co-precipitates with gp130-Flag-tagged DYRK1A, DYRK1B, STAT3, SH2D2A and USP21 in HEK293T cells or Flag-tagged IPO5 in SH-SY5Y cells. (B) DSCAML1-HA co-precipitates with gp130-Flag-tagged DYRK1A, DYRK1B, STAT3, USP21, SH2D2A, or Flag-tagged IPO5 in HEK293T cells.

Source data are available online for this figure.

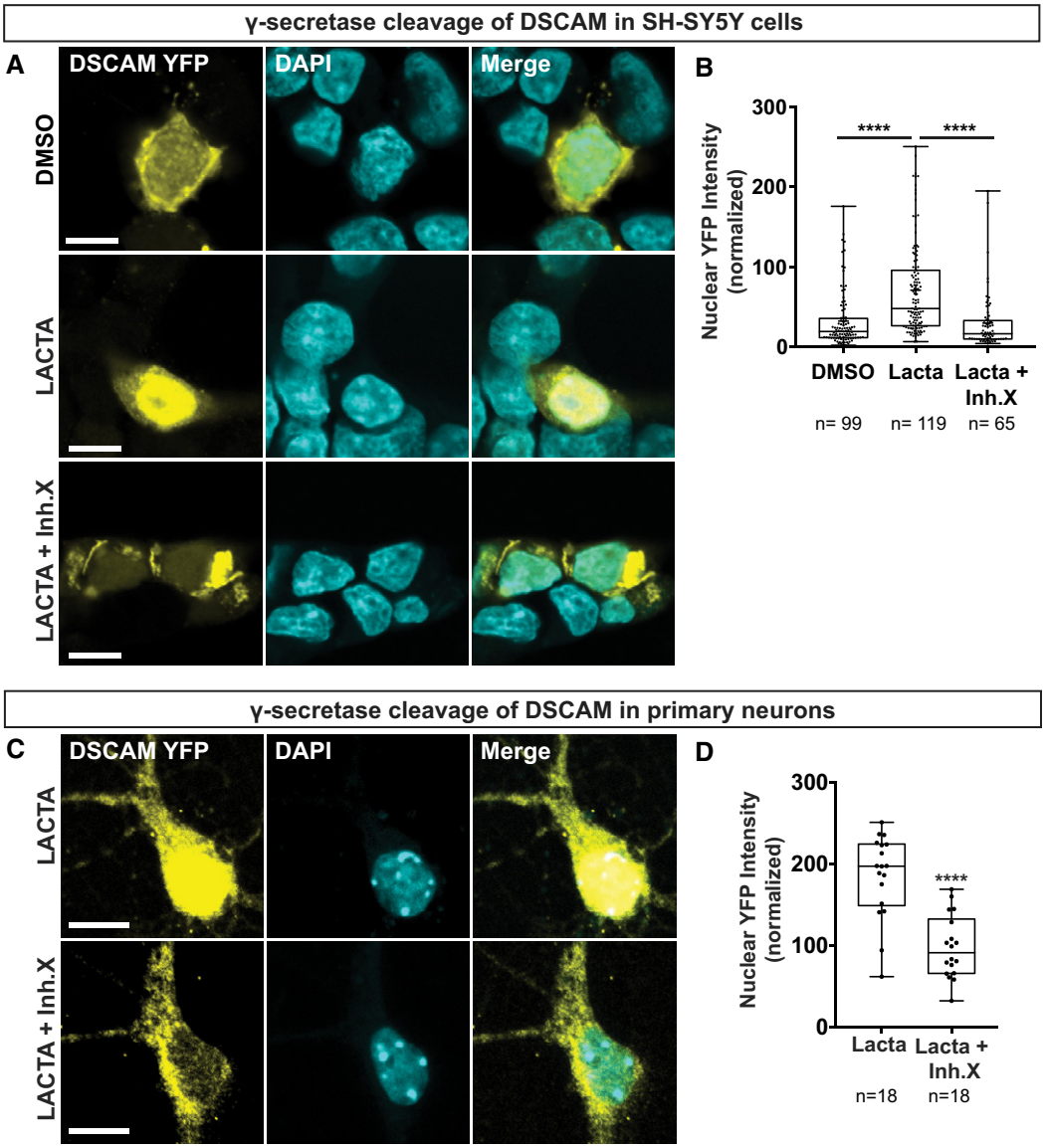

Figure EV2. γ-Secretase regulates nuclear enrichment of the DSCAM ICD (related to Fig 3).

DSCAM nuclear localization decreases significantly upon γ-secretase inhibitor treatment in SH-SY5Y cells and primary hippocampal neurons transfected with C-terminally YFP-tagged DSCAM.

A, B 24 h post-transfection SH-SY5Y cells were incubated over night with DMSO (control) or Lactacystin (10 μM) in the presence or absence of γ-secretase inhibitor X (10 μM). (A) Cells were immuno-stained for YFP and treated with DAPI. (B) Quantification of DSCAM nuclear localization, which is expressed as nuclear YFP intensity normalized to the nuclear area.

C, D Primary mouse hippocampal neurons were isolated at E18 and nucleofected at DIV0. At DIV9, neurons were incubated over night with Lactacystin (5 μM) in the presence or absence of inhibitor X (10 μM) and immuno-stained on DIV10 for YFP and DAPI (C). (D) Quantification of DSCAM nuclear localization, which is expressed as nuclear YFP intensity normalized to the nuclear area.

Data information: (A, C) Single planes of representative images from each condition are shown. Scale bars 10 μm. (A–D) Lacta, Lactacystin; Inh.X, inhibitor X. Box plots in (B, D) show whiskers spanning the minimum to maximum of normalized YFP intensities and individual values for each analyzed cell (*n*) as dots. In (B), ****$P < 0.0001$ (Kruskal–Wallis and Dunn's multiple comparisons test). In (D), ****$P < 0.0001$ (Welchs *t* test, two-tailed).

Figure EV3. Enriched Biological Functions in DSCAM and DSCAML1 RNA-seq datasets (related to Fig 4).

A, B Volcano plots showing all profiled up- and down-regulated genes as log2 fold change versus −log10 of the false discovery rate (FDR) corrected *P*-value in the DSCAM (A) and DSCAML1 (B) data sets. Statistically relevant DEGs are separated from statistically non-relevant DEGs by a horizontal striped line by FDR ≤ 0.0005 cutoff. Vertical striped lines indicate the log ratio (LR) cutoff (LR ≥ 0.58, ≤ −0.58; fold change ≥ 1.5, ≤ 1.5). For simplicity, genes differentially expressed in nuclear versus cytoplasmic YFP control cell lines (FDR ≥ 0.1) are not shown. (A) Volcano plot showing all profiled genes in DSCAM ICD versus nuclear YFP expressing cell lines. Note that DSCAM is highly up-regulated (log2 fold change, 6.0345; −log10 of *P*-value, 124.678) due to experimental overexpression of the DSCAM ICD. (B) Volcano plot showing all profiled genes in DSCAML1 ICD versus nuclear YFP expressing cell lines. DSCAML1 is highly up-regulated (log2 fold change, 7.553) due to experimental overexpression of the DSCAML1 ICD. The arrow indicates that due to a FDR *P*-value of 0, this data point cannot be plotted on a −log10 scale.

C, D Results of Ingenuity Pathway Core Analysis (FDR ≤ 0.0005; LR ≥ 0.58, ≤ −0.58). Enriched biological functions related to physiological system development and function and canonical pathways. *X*-axis shows likelihood of association between a set of genes in our dataset and a biological function, expressed as −log (*P*-value). Enriched biological functions and pathways were scored according to their *P*-value, calculated with the Fischer's exact test. The *y*-axis shows the annotations of the enriched biological functions or canonical pathways of differentially expressed genes. (C) Functional classification of DEGs in DSCAM ICD relative to YFP-NLS-expressing cells (*P*-value ≤ 0.05). (D) Functional classification of DEGs in DSCAML1 ICD relative to YFP-NLS-expressing cells (*P*-value ≤ 0.05).

E Validation of DEGs of the DSCAM Dataset in Neuro2A cells. Mouse Neuro 2A cells were transfected with the YFP-tagged DSCAM ICD (DSCAM ICD), a nuclear YFP control (YFP Ctrl), or left non-transfected (NT Ctrl). 48 h post-transfection total mRNA was isolated and reverse transcribed into cDNA. mRNA transcription levels were quantified by semi-quantitative real-time PCR (qRT–PCR) using gene specific primers. mRNA levels between different samples were normalized using GAPDH and actin as reference genes.

Data information: Bar graphs in (E) show the mean from three experiments ± SEM. **P* ≤ 0.05 and ***P* ≤ 0.01 (ordinary one-way ANOVA with Dunnett's multiple comparisons test).

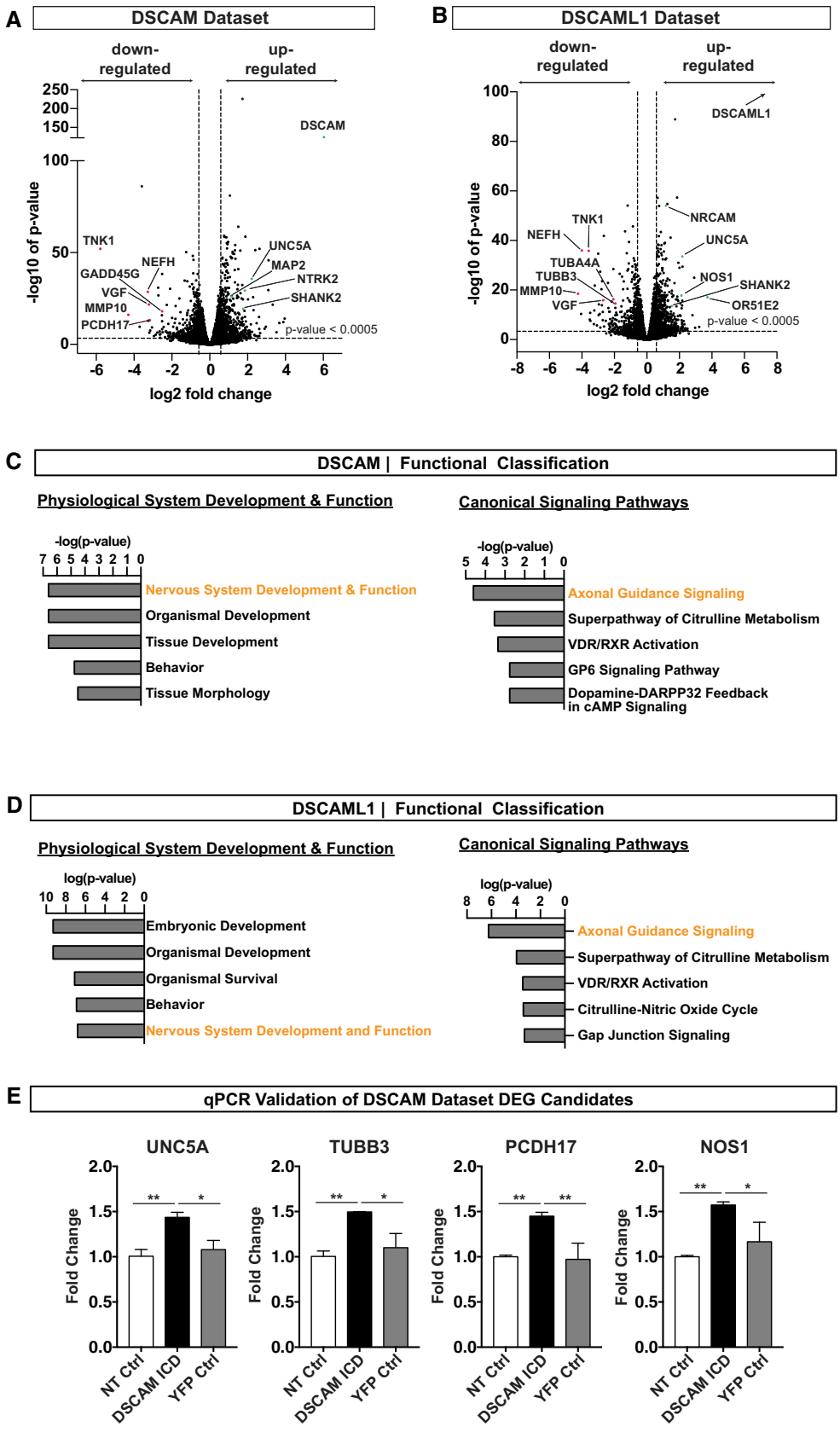
A
DSCAM Dataset
down-regulated
up-regulated
DSCAM
TNK1
NEFH
GADD45G
VGF
MMP10
PCDH17
UNC5A
MAP2
NTRK2
SHANK2
p-value < 0.0005
-log10 of p-value
log2 fold change
B
DSCAML1 Dataset
down-regulated
up-regulated
DSCAML1
TNK1
NEFH
TUBA4A
TUBB3
MMP10
VGF
NRCAM
UNC5A
NOS1
SHANK2
OR51E2
p-value < 0.0005
-log10 of p-value
log2 fold change
C
DSCAM | Functional Classification
Physiological System Development & Function
-log(p-value)
Nervous System Development & Function
Organismal Development
Tissue Development
Behavior
Tissue Morphology
Canonical Signaling Pathways
-log(p-value)
Axonal Guidance Signaling
Superpathway of Citrulline Metabolism
VDR/RXR Activation
GP6 Signaling Pathway
Dopamine-DARPP32 Feedback in cAMP Signaling
D
DSCAML1 | Functional Classification
Physiological System Development & Function
log(p-value)
Embryonic Development
Organismal Development
Organismal Survival
Behavior
Nervous System Development and Function
Canonical Signaling Pathways
log(p-value)
Axonal Guidance Signaling
Superpathway of Citrulline Metabolism
VDR/RXR Activation
Citrulline-Nitric Oxide Cycle
Gap Junction Signaling
E
qPCR Validation of DSCAM Dataset DEG Candidates
UNC5A
TUBB3
PCDH17
NOS1
Fold Change
NT Ctrl
DSCAM ICD
YFP Ctrl

Figure EV3.