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## Supplemental information

# Multi-omic profiling reveals the ataxia protein sacsin is required for integrin trafficking and synaptic organization

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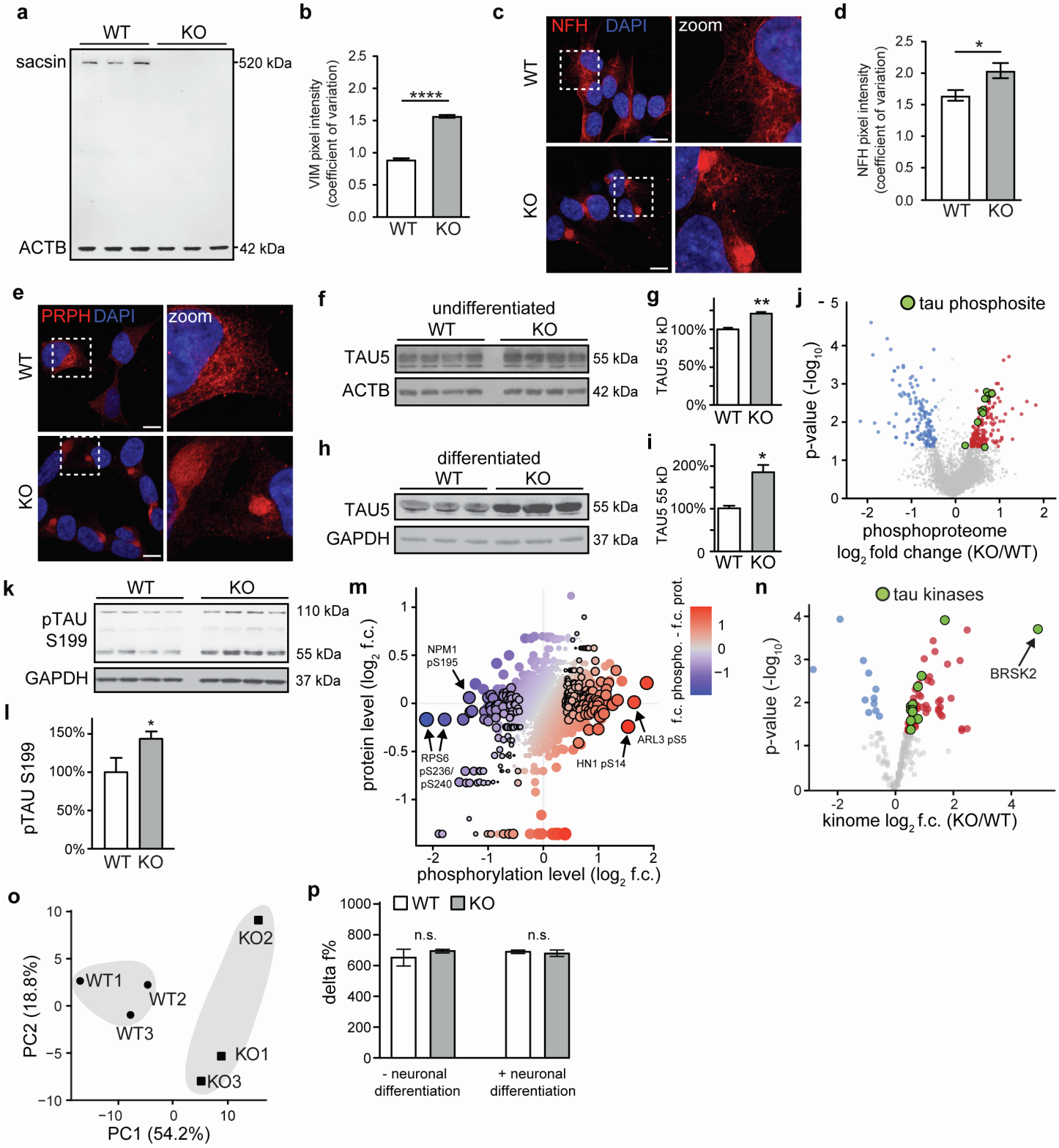



**Extended Data Figure 1 – sacsin KO SH-SY5Y cells recapitulate cellular phenotypes consistent with known deficits**

a. Western blot for sacsin and ACTB demonstrating the loss of sacsin in SH-SY5Y KO cells.

b. Coefficient of variation of vimentin pixel intensity values across the cell, with lower values indicating uniform distribution and higher values indicating polarized distribution. n = 3, S.E.M., Student's t-test, ****p<0.0001.

c. Representative confocal images of WT and sacsin KO cells immunostained for the neurofilament heavy chain.

d. Coefficient of variation of NFH pixel intensity. n = 3, S.E.M., Student's t-test, *p<0.05.

e. Representative confocal images of WT and sacsin KO cells immunostained for peripherin, an intermediate filament protein found in neurons in the peripheral nervous system.

f-i. Western blot analysis quantification of pan-tau (Tau5) in sacsin KO and WT cells in undifferentiated (f,g) and neuronally differentiated (h,i) SH-SY5Y cells. n = 3-4, S.E.M., Student's t-test, *p<0.05, **p<0.01.

j. Phosphoproteomic analysis of sacsin KO cells. Green circles mark specific phosphorylated residues on tau.

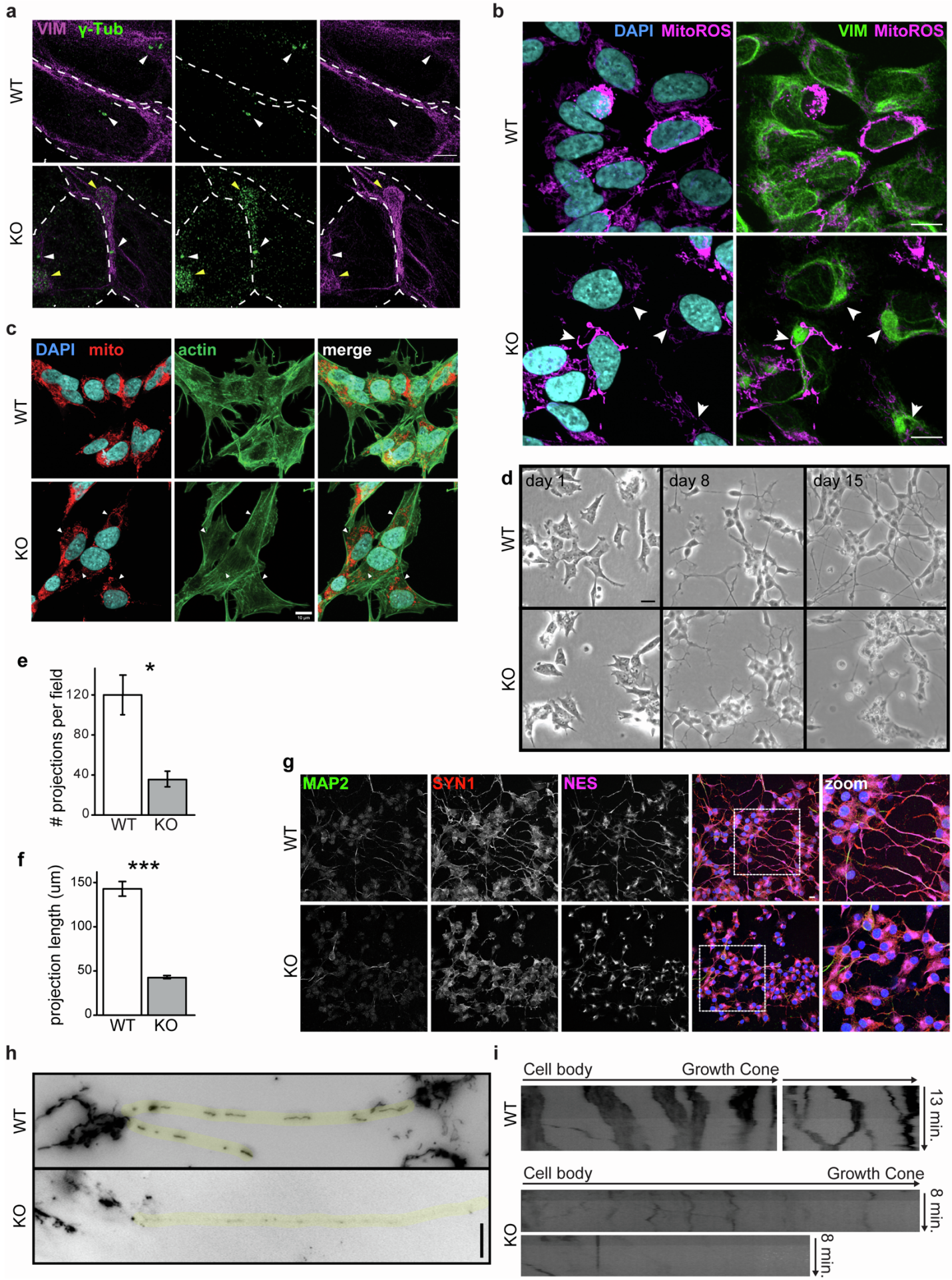
k,l. Western blot and quantification of phosphorylated tau at serine 199. n = 3, S.E.M., Student's t-test, *p<0.05.

m. Phosphopeptide levels compared to changes in total protein levels. Color scale reflects the difference in $\log_2$ f.c. between each dataset. Black outline marks phosphosites with p<0.05 and $\log_2$ f.c. -/+0.4.

n. Kinome profiling of sacsin KO cells. Green circles mark kinases which are known to directly phosphorylate tau.

o. Principle component analysis of all kinases identified in kinome profiling data (Supp. Table 1). Unsupervised hierarchical clustering separated WT and KO cells (grey shading), suggesting widespread changes in the kinome of sacsin KO cells.

p. Biochemical analysis of tau aggregation using homogeneous time resolved fluorescence (HTRF) and anti-Tau antibodies conjugated with either Tb (donor) or d2 (acceptor) fluorophores. Graph represents the HTRF ratio, or Delta f%, of the two emission signals comparing WT/KO SH-SHY5Y lysates. n = 3, S.E.M., Student's t-test, n.s. = not significant.


a
VIM γ-Tub
WT
KO
b
DAPI MitoROS
VIM MitoROS
WT
KO
c
DAPI mito
actin
merge
WT
KO
d
day 1
day 8
day 15
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KO
e
# projections per field
120
80
40
0
*
WT KO
f
projection length (um)
150
100
50
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WT KO
g
MAP2
SYN1
NES
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WT
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h
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i
Cell body
Growth Cone
13 min.
WT
Cell body
Growth Cone
8 min.
KO
8 min.

**Extended Data Figure 2 – Microtubule and mitochondria deficits in sacsin KO cells**

a. Super resolution structural illumination microscopy images showing accumulation of gamma-tubulin within perinuclear vimentin bundles of sacsin KO cells. White arrows point to centrioles, yellow arrowheads highlight the presence of gamma-tubulin within vimentin bundles in KO cells. Dashed white lines denote boundaries between adjacent cells. Scale bar = 1um.

b. Representative confocal images of WT and sacsin KO cells stained for the mitochondria membrane potential dependent dye CMXRos, vimentin, and nuclei (DAPI). Arrowheads highlight the exclusion of mitochondria from vimentin bundles.

c. Representative confocal images of WT and sacsin KO cells immunostained for mitotracker, actin, and nuclei (DAPI). Arrowheads highlight the exclusion of mitochondria from vimentin bundles.

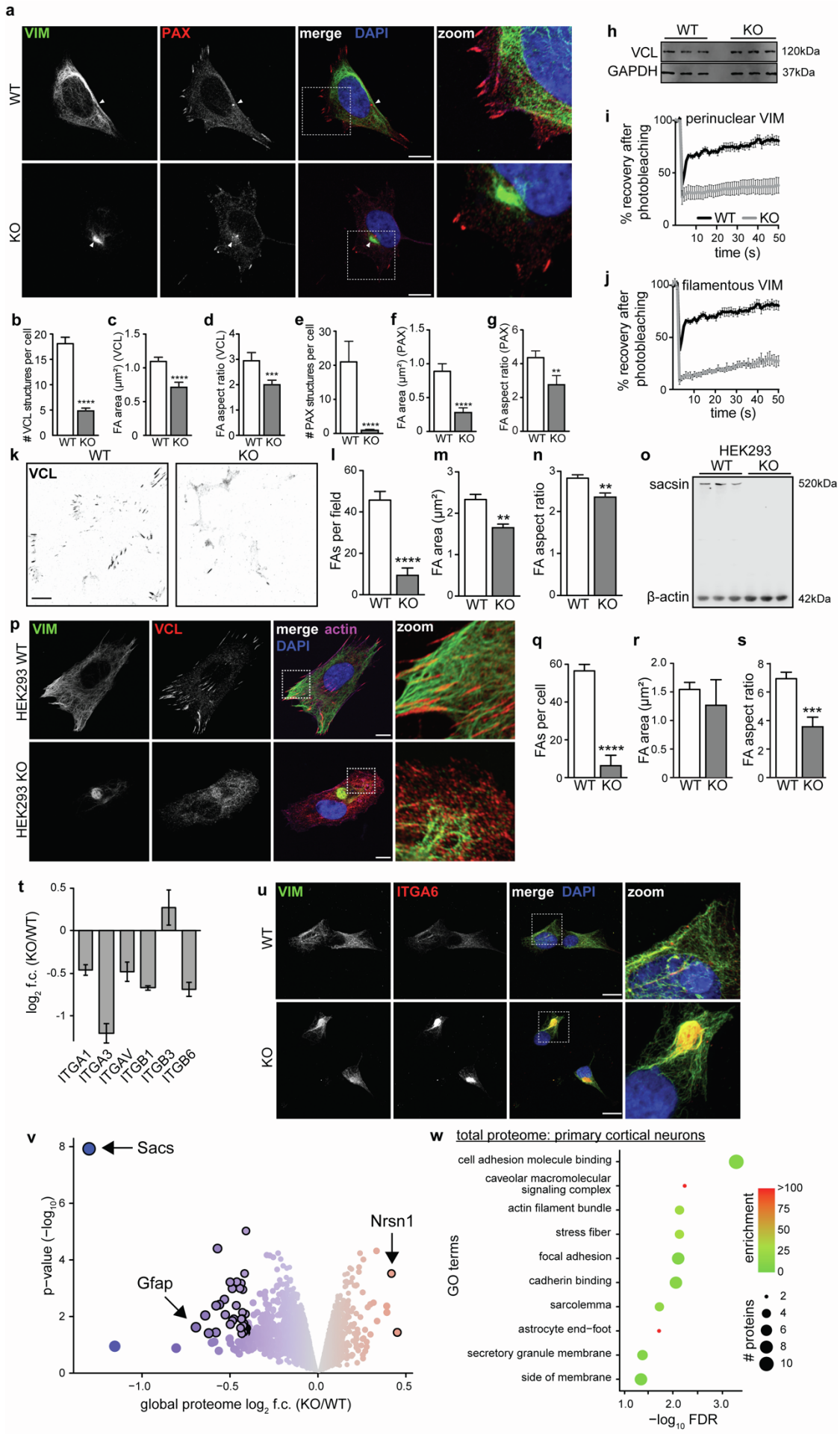
d. Representative phase contrast brightfield images of WT and sacsin KO cells across 15 days of neuronal differentiation.

e,f. Quantitation of the number of projections per field (e) and length of projection (f) of WT/KO cells demonstrating significantly reduced number and length of projections in sacsin KO cells. n = 3, S.E.M., Student's t-test, *$p<0.05$, ***$p<0.001$.

g. Confocal images of WT/KO cells after 15 days in differentiation conditions, stained for neuronal markers microtubule associated protein 2 (MAP2) and synapsin1 (SYN1), and the intermediate filament protein nestin (NES), a marker of immature neurons. Scale bar = 10 μm.

h. Mitochondria labeled with mitoTracker GreenFM in neurites (highlighted in yellow) of 15 day differentiated WT/KO cells demonstrating the lack of elongated mitochondria in sacsin KO neurites. Images were snapshots from live-cell time-lapse imaging.

i. Kymograph illustrating mitochondrial transport along neurites of differentiated WT/KO cells. Note that mitochondrial undergo both retrograde and anterograde movement in control but are relatively static in sacsin KO cells. Scale bar = 10 μm.


a
VIM
PAX
merge DAPI
zoom
WT
KO
h
WT
KO
VCL
GAPDH
120kDa
37kDa
i
perinuclear VIM
% recovery after photobleaching
WT
KO
time (s)
j
filamentous VIM
% recovery after photobleaching
time (s)
b
# VCL structures per cell
c
FA area (µm²) (VCL)
d
FA aspect ratio (VCL)
e
# PAX structures per cell
f
FA area (µm²) (PAX)
g
FA aspect ratio (PAX)
k
VCL
l
FAs per field
m
FA area (µm²)
n
FA aspect ratio
o
HEK293
sacsin
520kDa
β-actin
42kDa
p
HEK293 WT
HEK293 KO
VIM
VCL
merge actin DAPI
zoom
q
FAs per cell
r
FA area (µm²)
s
FA aspect ratio
t
log2 f.c. (KO/WT)
ITGA1
ITGA3
ITGAV
ITGB1
ITGB3
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merge DAPI
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Nrsn1
Gfap
p−value (−log10)
global proteome log2 f.c. (KO/WT)
w
total proteome: primary cortical neurons
cell adhesion molecule binding
caveolar macromolecular signaling complex
actin filament bundle
stress fiber
focal adhesion
cadherin binding
sarcolemma
astrocyte end−foot
secretory granule membrane
side of membrane
GO terms
enrichment
# proteins
−log10 FDR

**Extended Data Figure 3 – FAs are disrupted in sacsin KO cells**

a. Representative confocal image of WT/KO cells labelled with vimentin and paxillin. Arrowhead marks the PAX positive MTOC, which is sequestered in the vimentin bundle in *SACS* KO cells. Scale bar = 10 μm.

b-g. Quantification of images from Fig. 3c (b-d), and Extended Data Figure 3a (e-g). Aspect ratio = width:height ratio. n = 3 independent cultures, S.E.M., Student's t-test, **p<0.01, ***p<0.001, ****p<0.0001.

h. Western blot for vinculin, showing that levels of the FA protein are unaltered in KO cells.

i,j. FRAP analysis of perinuclear vimentin (i), and filamentous vimentin on the periphery of the cell away from vimentin bundle (j). Cells were transfected with EGFP-VIM expression vector and defined 2×2 μm regions of interest were bleached with a 488-nm laser. Recovery was monitored over 50 cycles of imaging with a 1-s interval. n=10 cells from each of three independent experiments.

f. Representative image of cover slips treated with hypotonic shock to remove cell bodies, leaving FAs retained through ECM interaction. Staining for the FA protein vinculin. Scale bar = 10 μm.

l-n. Quantification of the incidence, area, and aspect ratio of paxillin positive FAs in WT/KO cells treated with hypotonic shock. n = 3 independent cultures, S.E.M., Student's t-test, **p<0.01, ****p<0.0001.

o. Western blot for sacsin and ACTB demonstrating the loss of sacsin in HEK293 KO cells.

p. Confocal images of HEK293 cells immunolabeled for vimentin, vinculin, and actin. Scale bar = 10 μm.

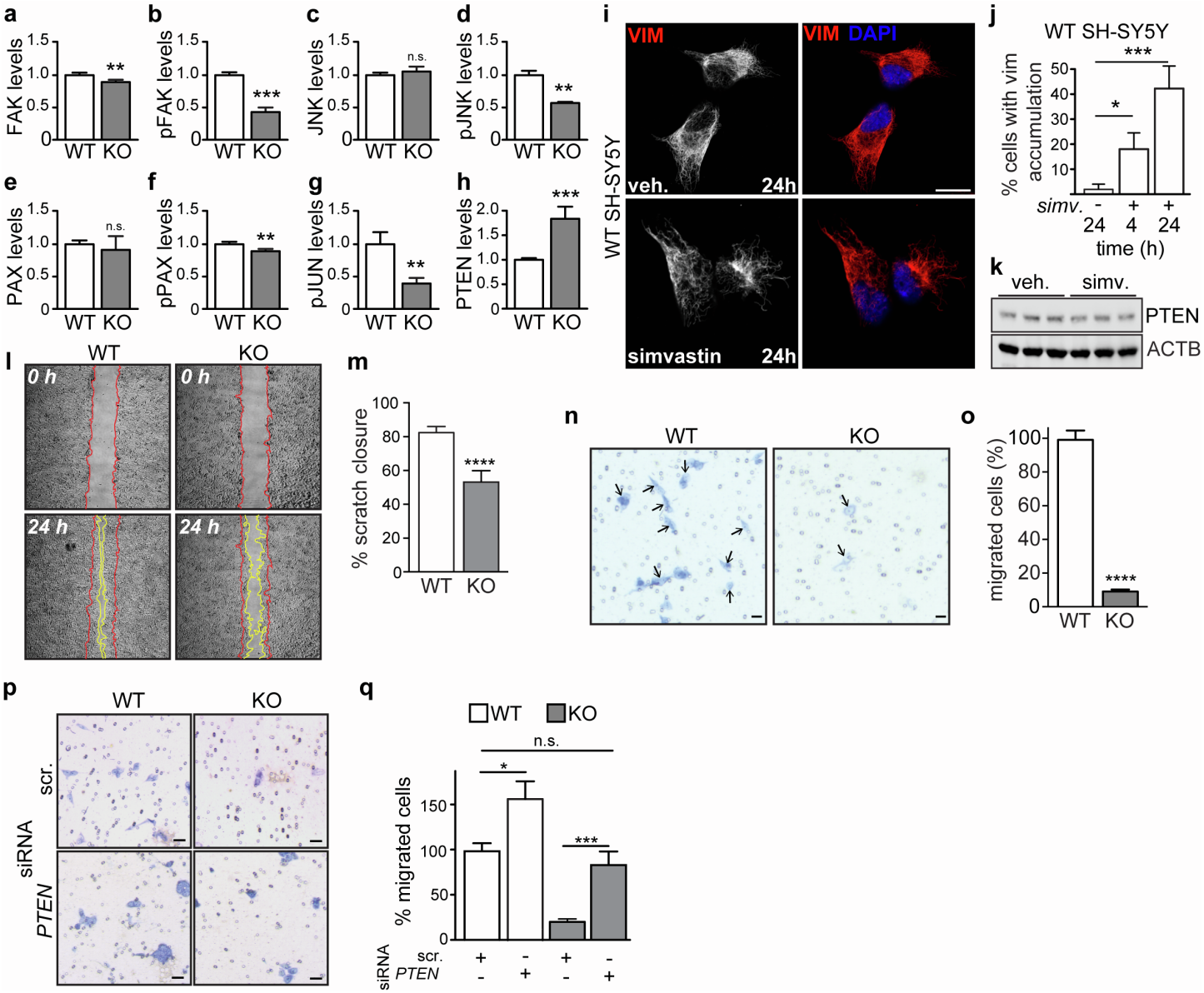
q-s. Quantification of images from Supp. Fig. 3p, suggesting FA deficits are consistent with SH-SY5Y cells. n = 3 independent cultures, S.E.M., Student's t-test, ***p<0.001, ****p<0.0001.

t. Changes in levels of integrin proteins quantified by mass-spectrometry (data from Supplementary Table 1, Fig. 1b). n = 3, S.E.M.

u. Representative confocal images of cells immunolabeled for ITGA6. Scale bar = 10 μm.

v. Global proteomic profiling of primary cortical cultures derived from E15.5 *Sacs*$^{(-/-)}$ mice. Cutoffs for significance were p<0.05 and $\log_2$ fold change (f.c.) -/+0.4, denoted by black outline. n=3 litters, replicate defined as litter mate control cultures from *Sacs*$^{(+/+)}$ and *Sacs*$^{(-/-)}$ mice.

w. GO term analysis of differentially expressed proteins in primary cortical cultures (p<0.05, $\log_2$ f.c. cutoff -/+0.4).




## Extended Data Figure 4 – Modulating *PTEN* rescues cellular phenotypes in sacsin KO cells

a-h. Quantification of immunoblots from Fig. 4c. Intensity normalized to ACTB. n=3 biological replicates, S.E.M., Student's t-test, **p<0.01, ***p<0.001.

i. Representative confocal images of the induction of vimentin bundling by simvastin. Scale bars = 10 µm.

j. Quantification of vimentin bundling phenotype induced by simvastin over time.

k. Western blot of PTEN levels in 24-hour simvastin treated WT cells, suggesting that vimentin bundling does not affect PTEN levels.

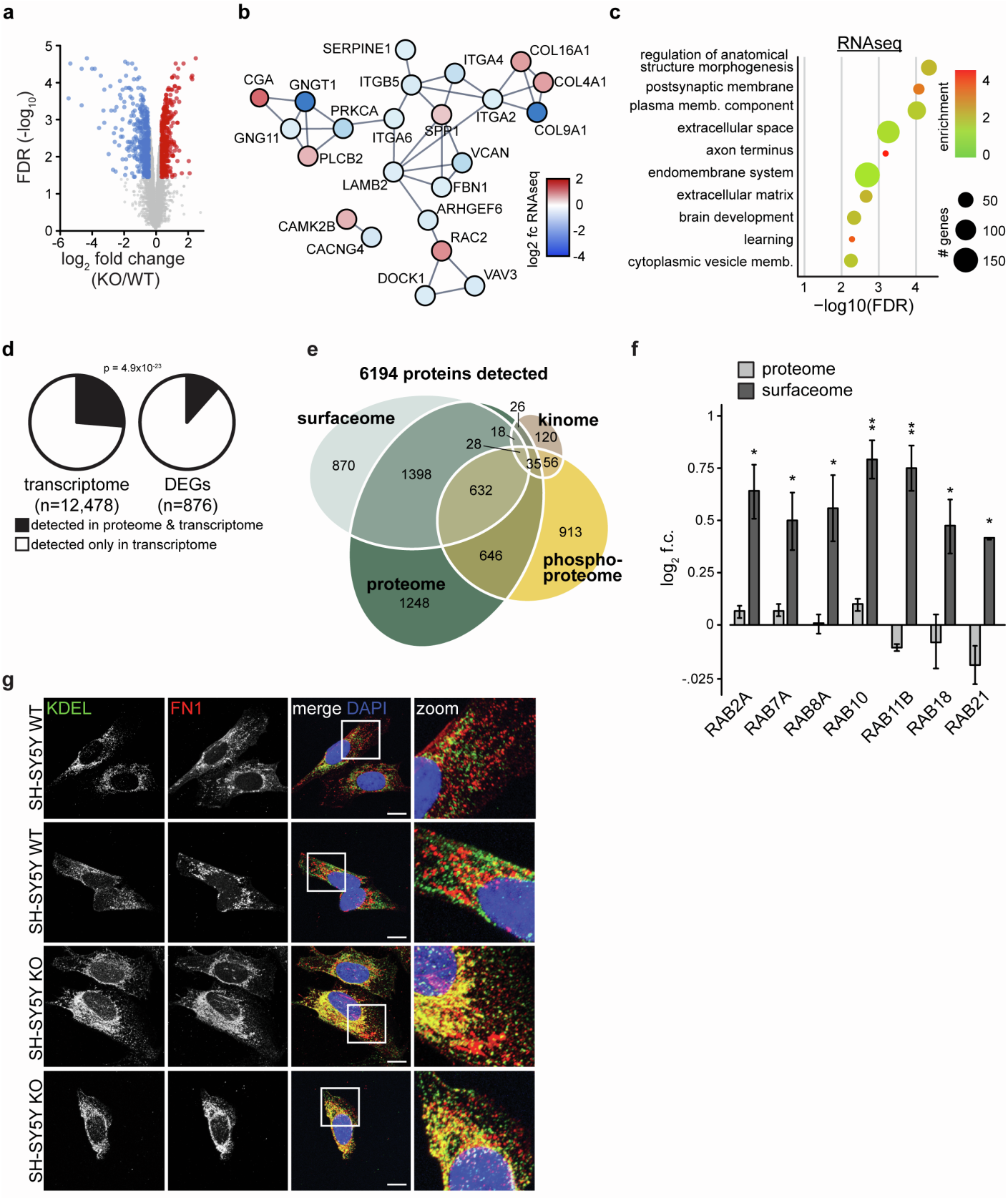
l,m. Representative bright field images of a scratch assay of WT/KO SH-SY5Y cells. Red and yellow lines mark the edge of the wound after 0 and 24 hours of recovery, respectively (l). Quantification of scratch closure in WT/KO 24 hours after the scratch was made. n = 3 independent cultures, S.E.M., Student's t-test, ****p<0.0001.

h. Representative images of WT/KO SH-SY5Y cells in Transwell chambers with 8 µm pores 24 hours after plating, fixed and stained with Giemsa blue. Arrows mark cell bodies, scale bar = 20 µm.

i. Quantification of the number of migrated cells after 24 hours, normalized to WT. n = 3 biological replicates, S.E.M., Student's t-test, ****p<0.0001.

j. Representative images of WT/KO SH-SY5Y cells transfected with the indicated siRNAs, and plated in Transwell chambers. Scale bar = 20 µm.

k. Quantification of Transwell assay 24 hours after plating. n = 5 per cell line/condition, S.E.M., Student's t-test, *p<0.05, ***p<0.001.




**Extended Data Figure 5 – Altered transcription of synaptic adhesion and vesicular proteins**

a. RNA-seq of 15 day neuronally differentiated SH-SY5Y cells.

b. Interaction network of cell adhesion proteins that are differentially expressed.

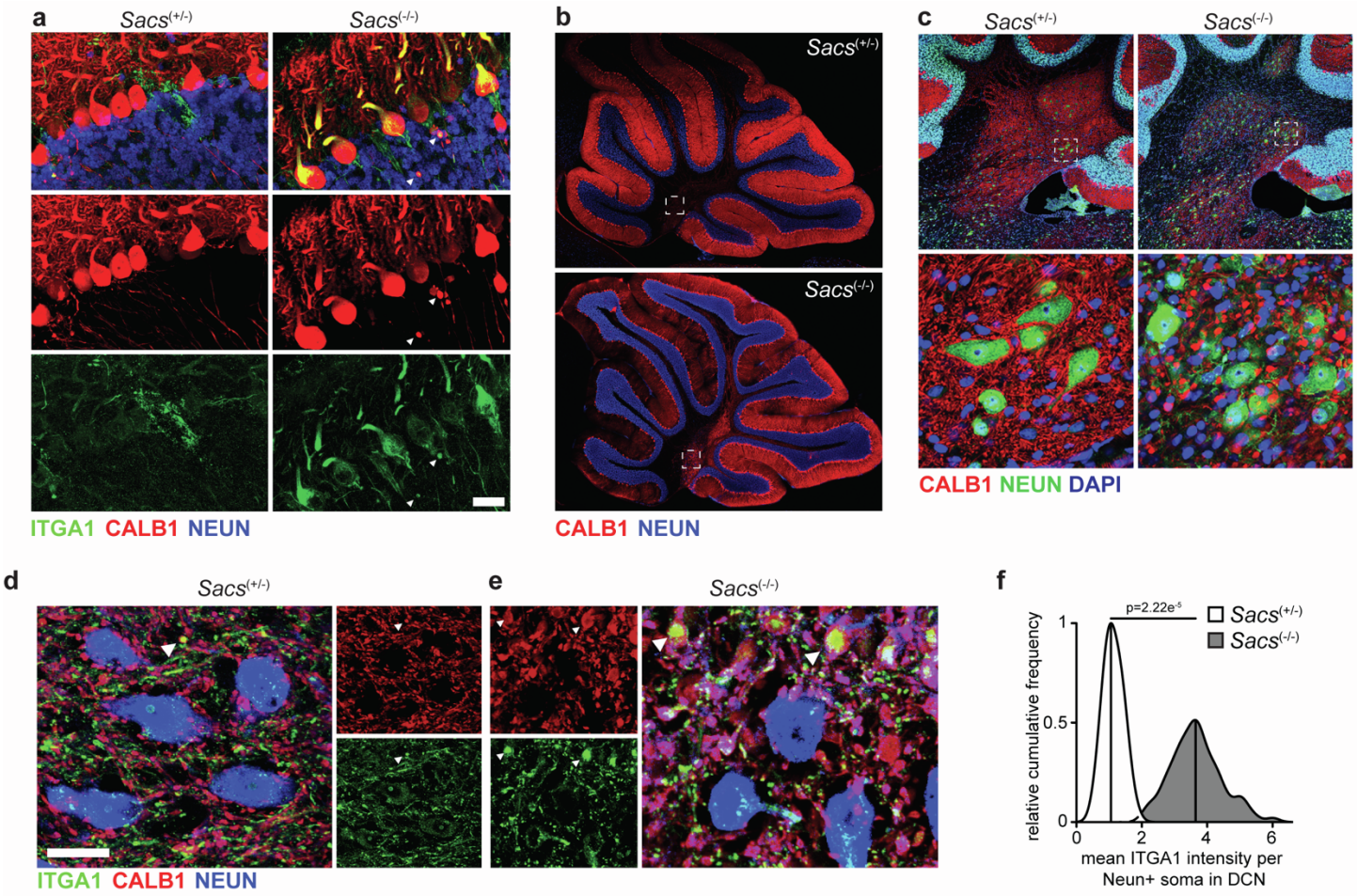
c. GO term analysis of differentially expressed genes suggests that synaptic and vesicular transport genes are altered in neurons ($p<0.05$, $\log_2$ f.c. -/+ 0.5).

d. Overlapping gene/protein identification from RNAseq and proteomics, showing that DEGs were not detected as readily in proteomics, as proteins that were not differentially expressed at the RNA level. Statistics: Hypergeometric test.

e. Euler diagram of protein identification across all mass-spec datasets.

f. $Log_2$ f.c. of Rab proteins in proteome and surfaceome datasets. Asterisks refer to statistical significance in each dataset. No Rabs were significantly affected in the proteome. n = 3, S.E.M., Student's t-test, *p<0.05, **p<0.01.

g. Representative confocal images of cells immunolabelled for fibronectin and KDEL in in WT/KO SH-SY5Y cells. Scale bar =10 μm.




**Extended Data Figure 6 – Cerebellar imaging in *SACS* KO mice**

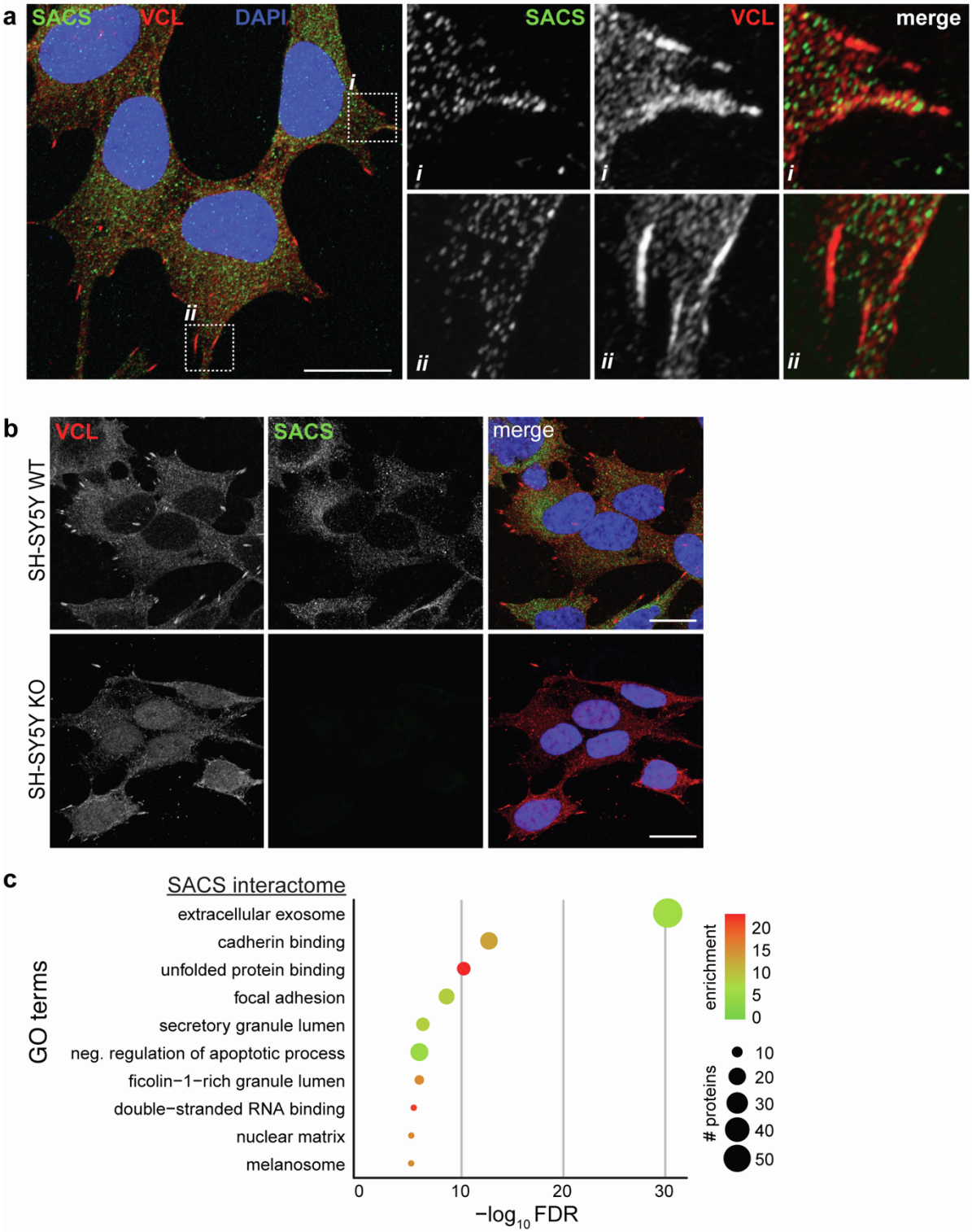
a. Purkinje cell layer in P120 mice. Arrowhead marks ITGA1 accumulation in axonal swellings. Scale bar = 20 µm.

b. Sagittal cerebellar section, marking the general DCN region analyzed in Figs. 6h-l, S6c-f.

c. DCN in P120 mice, demonstrating substantial disruption of Purkinje neuron termini on DCN neurons. Scale bar = 20 µm.

d,e. DCN in P60 mice. Arrowheads mark large CALB1+ structures, with accumulation of ITGA1. Scale bar = 20 µm.

a. Quantification of images in Fig. 6k. Large diameter DCN neuron soma defined as NEUN+/DAPI+ where diameter is between 20-25 µm. Replicates defined as in Fig. 6d, n=4, paired t-test.




**Extended Data Figure 7 – sacsin interactors**

a. Representative confocal image for sacsin and vinculin in WT SH-SY5Y cells demonstrating sacsin colocalizes with FAs.
b. Representative confocal image for sacsin KO cells processed in parallel to (a), demonstrating the specificity of sacsin staining.
c. GO term analysis of all proteins identified in the sacsin co-IP interactome (Supplementary Table 4).