Supplemental Data

Supplemental Figure Legends

Figure S1. Expression of *GIT1* in human brain and *GIT1* in mouse brain (related to Figure 1). (**A**) mRNA expression pattern of *GIT1* in human brain regions relative to a selection of non-neuronal tissue from the Genotype-Tissue Expression (GTEx) Project (http://www.gtexportal.org/home/). (**B**) mRNA expression pattern of *GIT1* in C57BL/6J mouse brain from Allen Brain Atlas. Note high expression of GIT1 in hippocampus and throughout the cortex**,** which is similar to previous observations using a gene trap β-galactosidase reporter mouse (13)**.**

Figure S2. Specific coupling of GIT1 to PAK3 over PAK1 (related to Figure 2). (**A**) GIT1 alone cannot activate MAPK kinases or AKT in HEK293 cells with normal culture conditions or with serum starvation as judged by phospho-specific antibodies. HEK293 cells were transfected with the indicated control or GIT1 expression vectors. One day after transfection, cells were harvested in 2X SDS sample buffer, and analyzed with immunoblot analysis using the indicated antibodies. For the serum starvation, 4hrs post transfection, culture media was replaced with serum free media. For quantification, band intensities were normalized to GAPDH intensities, and then to empty vector controls, in order to obtain fold changes in the presence (**B**) or absence (**C**) of serum. (**D-F**) Lack of effect of wild-type GIT1 and GIT1-R283W on PAK1 and MAPK activation in HEK293 cells. Cells were co-transfected with the indicated expression vectors and 24hrs later cells were harvested in 2X SDS sample buffer. (**D**) Protein samples were subjected to immunoblot analysis using the indicated antibodies. (**E**) For immunoblots other than those for the phospho-PAKs, band intensities were normalized to GAPDH band intensities, and then to values observed in wild-type GIT1-transfected cells in order to obtain

fold changes (data represent mean ± SEM; N = 3; ns = not significant; two-way ANOVA). For phospho-PAK immunoblots (**F**), band intensities were normalized to total Myc-PAK1 signals, and then to values for wild-type GIT1-transfected cells, in order to obtain fold changes (data represent mean ± SEM; N = 3; ns = not significant; two-way ANOVA).

Figure S3. Effects of lentivirus-mediated overexpression of GIT1 and GIT1-R283W on synaptic protein expression in primary hippocampal cultures (related to Figures 3 and 4). Primary cultured hippocampal neurons at DIV 14-16 were infected with lentiviruses expressing the indicated wild-type GIT1, GIT1-R283W, or empty vector control constructs. One week after infection, hippocampal neurons were harvested in 2X SDS sample buffer and analyzed by immunoblot using the indicated antibodies (GAPDH and GIT1-FLAG blots shown here are the same blots as shown in Figure 3) (**A**). (**B**) Synaptic protein immunoblot band intensities were normalized to GAPDH levels, and then to empty vector control lentivirus to obtain fold changes. Data represent mean ± SEM; N = 8; * = $p < 0.05$; ns = not significant; two-way ANOVA with *post hoc* across row comparisons using a Tukey correction.

Figure S4. Homology model of GIT1 ArfGAP variants. (**A**) Location of three SNVs within the ArfGAP domain of GIT1. (**B**) Structure-guided sequence alignment of GIT1 and related GAPs for which X-ray structures are known and in the Protein Data Bank (http://www.rcsb.org/pdb). Residues E33 (pink highlight and arrow) and V37 (pink highlight and arrow) map to a well-conserved α-helix and are flanked by regions of high sequence similarity. R39 is the conserved arginine finger catalytic residue (turquoise highlight and arrow). All proteins in alignment are human except PFL2140c (*Plasmodium falciparum*), cgd5_1040 (*Cryptosporium parvum*) and ASAP2 (*Mus musculus*). Consensus secondary structure prediction symbols: alpha-helix: h; beta-strand: e. (**C-E**) Homology model of the ArfGAP domain of human GIT1 based on the atomic coordinates of the ArfGAP domain from human ACAP1 (pdbid: 3JUE). Color-coding for

(D) and (E): carbons in GIT1 side chains are green for wild-type residues and pink for mutant residues; carbons in SMAP1 (pdbid: 2IQJ) side chains are grey; oxygen, red; nitrogen, blue; and sulfur, yellow. The Zn^{2+}-ion is shown as a blue-grey sphere. (**C**) Ribbon diagram showing the location of residues E33, V37, and A55 and the Zn^{2+} ion. C- and N-termini are indicated. Residues E33 and V37 are located on a helix that is central to the ArfGAP fold near the Zn^{2+}-finger. The GIT1-A55T variant replaces a small residue located in a poorly conserved loop at the protein surface and is not expected to disrupt the GIT1 structure. (**D**) The GIT1-E33K variant replaces a negatively charged residue with a positive charged residue at the surface of the protein near the Zn^{2+}-binding site. However, the ArfGAP fold can tolerate a variety of residues at this position, as shown in the sequence alignment in (**B**), including arginine (R50) in SMAP1 that is accommodated by a compensatory small residue V110 (K103 in GIT1). This suggests that GIT1-E33K may have complex effects on protein folding, stability, degradation and/or interactions with its partners. (**E**) The GIT1-V37M variant replaces a small hydrophobic residue with a large one and is expected to lead to steric clashes with I107 near the Zn^{2+}-binding site and destabilization of the protein leading to lower protein levels.

Supplemental Table Legends

Table S1, related to Figure 1. Summary of coding variants found in SCZ patients and controls by exome sequencing in the Swedish schizophrenia case-control study (10). Coordinates of each variant are given for genome build GRCh37/hg19.

Table S2, related to Figure 1. Summary of unique coding variants in GIT1, allele frequencies in the ExAC exome database (http://exac.broadinstitute.org/gene/ENSG00000108262), and predicted impact on function by PolyPhen. At the time of analysis, the ExAC database contained exome sequencing data from 60,706 unrelated individuals, including the Swedish SCZ cases and controls, as well as the parents but not probands from the Bulgarian trio studies.

Supplemental Tables

Table S1.

#	Chr	Pos	ID	Ref/Alt	Schizophrenia (A) : Control (U)
1	chr17	27901774	.	G/A	A=5;U=1
2	chr17	27901903	rs11548560	T/C	A=42;U=50
3	chr17	27902156	rs145991218	C/T	A=0;U=1
4	chr17	27902326	.	G/T	A=3;U=0
5	chr17	27902672	.	G/A	A=1;U=1
6	chr17	27902708	.	C/CG	A=0;U=1
7	chr17	27902709	.	G/A	A=0;U=1
8	chr17	27902831	.	G/A	A=2;U=1
9	chr17	27902849	.	T/A	A=1;U=0
10	chr17	27902866	.	C/T	A=1;U=2
11	chr17	27903117	.	G/A	A=1;U=0
12	chr17	27903301	rs144464404	C/G	A=2;U=2
13	chr17	27903304	rs148402352	G/A	A=0;U=2
14	chr17	27903359	rs147741808	C/G	A=1;U=0
15	chr17	27903418	.	C/T	A=1;U=0
16	chr17	27903544	.	C/T	A=2;U=1
17	chr17	27903555	rs145208703	C/G	A=2;U=2
18	chr17	27903666	.	G/A	A=1;U=0
19	chr17	27903908	rs141462052	G/A	A=1;U=0
20	chr17	27903993	.	C/T	A=0;U=1
21	chr17	27903998	rs144787773	A/C	A=2;U=1
22	chr17	27904711	rs11080105	G/A	A=1731;U=1706
23	chr17	27904723	.	T/A	A=1;U=2
24	chr17	27905359	.	T/C	A=0;U=1
25	chr17	27905380	.	G/A	A=4;U=0
26	chr17	27905809	.	G/A	A=1;U=0
27	chr17	27906001	.	C/G	A=1;U=0
28	chr17	27908989	.	A/T	A=1;U=0
29	chr17	27909004	.	C/T	A=0;U=1
30	chr17	27909707	rs182492370	C/T	A=19;U=19
31	chr17	27909978	.	G/A	A=0;U=1
32	chr17	27910032	.	G/A	A=0;U=1
33	chr17	27910510	rs141157150	C/T	A=0;U=1
34	chr17	27910524	rs187487955	C/T	A=0;U=1
35	chr17	27910537	rs147244911	G/A	A=3;U=3
36	chr17	27910578	.	C/T	A=1;U=0
37	chr17	27910590	.	C/T	A=1;U=0

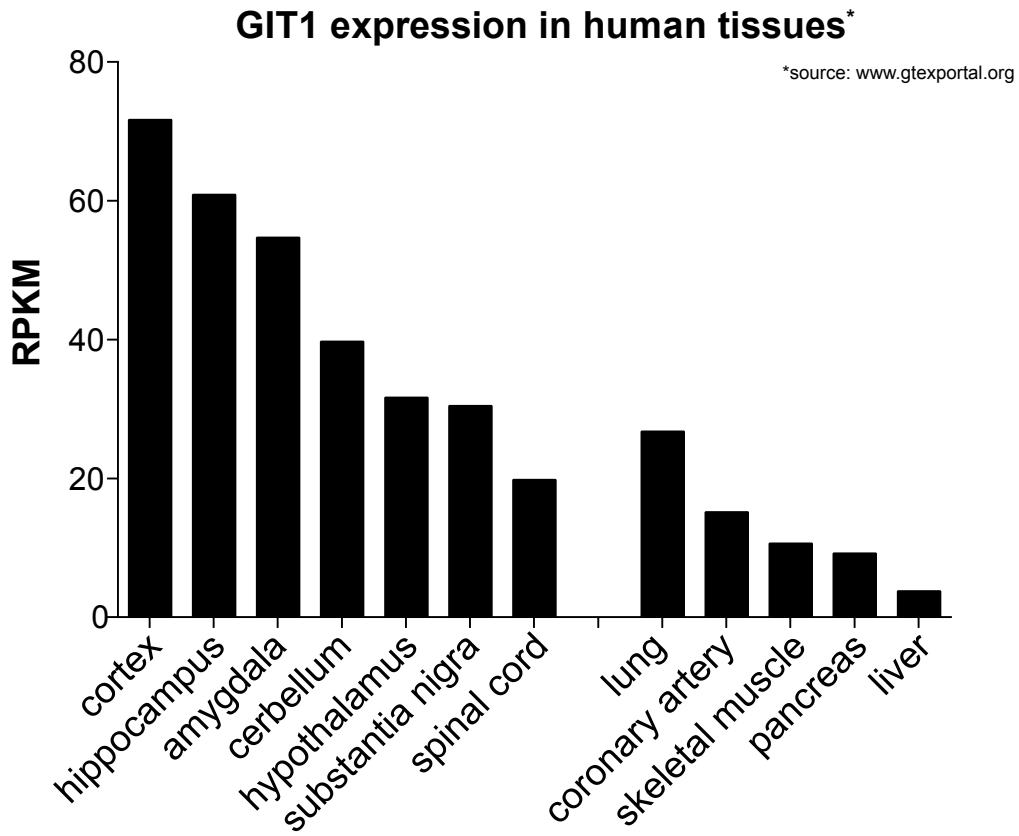
Table S2. Summary of GIT1 variant allele frequency data and PolyPhen2 predictions scores.

Swed./Bulg. Studies* SNV	Hg19 Genomic Position (Chr17)	Amino Acid Variant	dbSNP Variant	ExAC Allele Count*	PolyPhen2 Prediction
Case Unique	27910590	E33K	no	1	0.997
Case Unique	27910578	V37M	no	10	0.997
Case Unique	27906001	R256P	no	1	0.158
Case Unique	27905380	R283W [4:0]	no	6 [5 known to be from SCZ cases]	0.999
Case Unique	27903359	G506A	rs147741808	9	0
Case Unique	27902849	Q587L	no	1	0.005
De Novo	27902699	S601N	no	N/A	0.935
Control Unique	27910524	A55T	rs187487955	27	0.051
Control Unique	27905359	M290V	no	2	0.763
Control Unique	27903993	R381Q	no	1	0.075
Control Unique	27902709	R598C	no	1	0.994
Control Unique	27902156	V681M	rs145991218	3	0.517

*(8, 10)

*case/control status of new allele counts not identified

A



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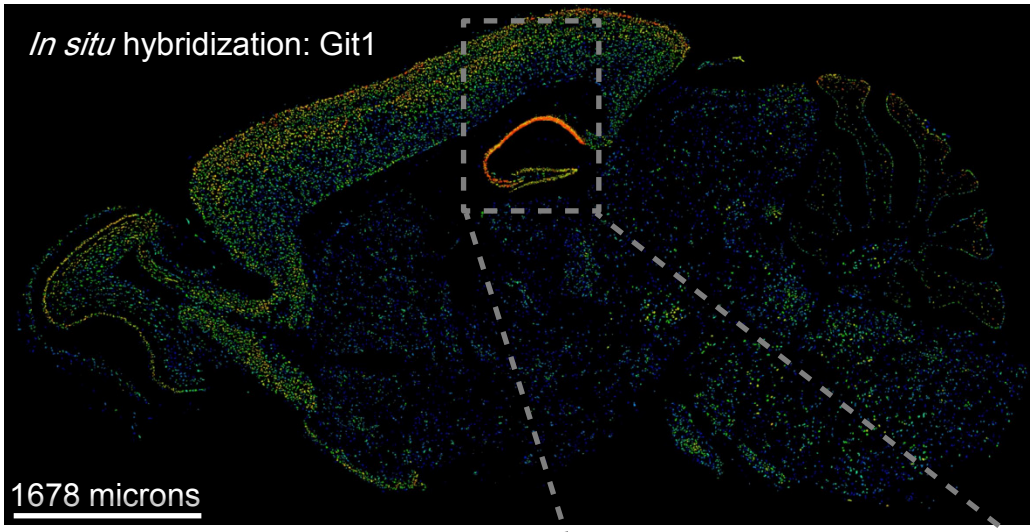
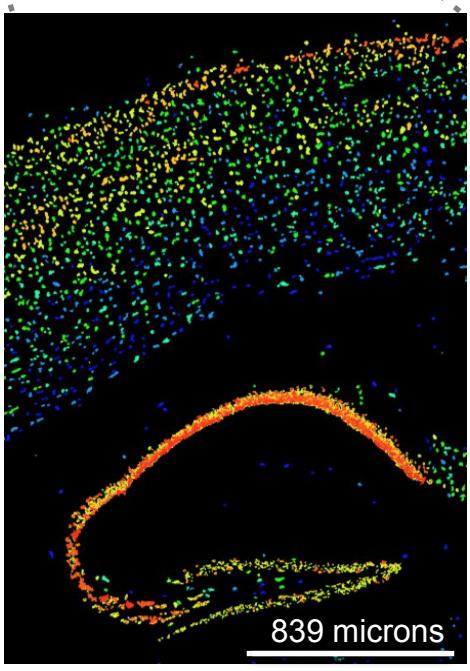
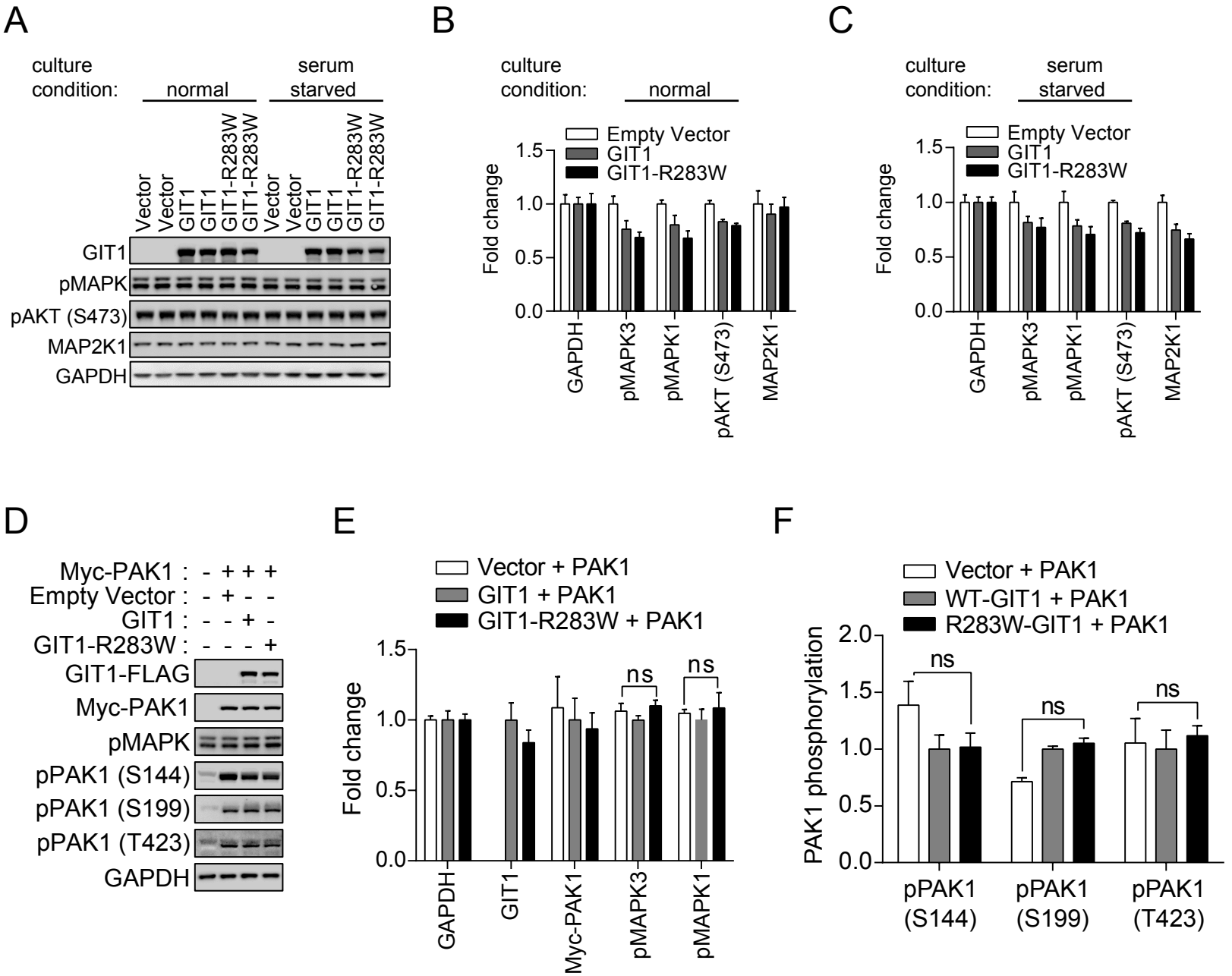


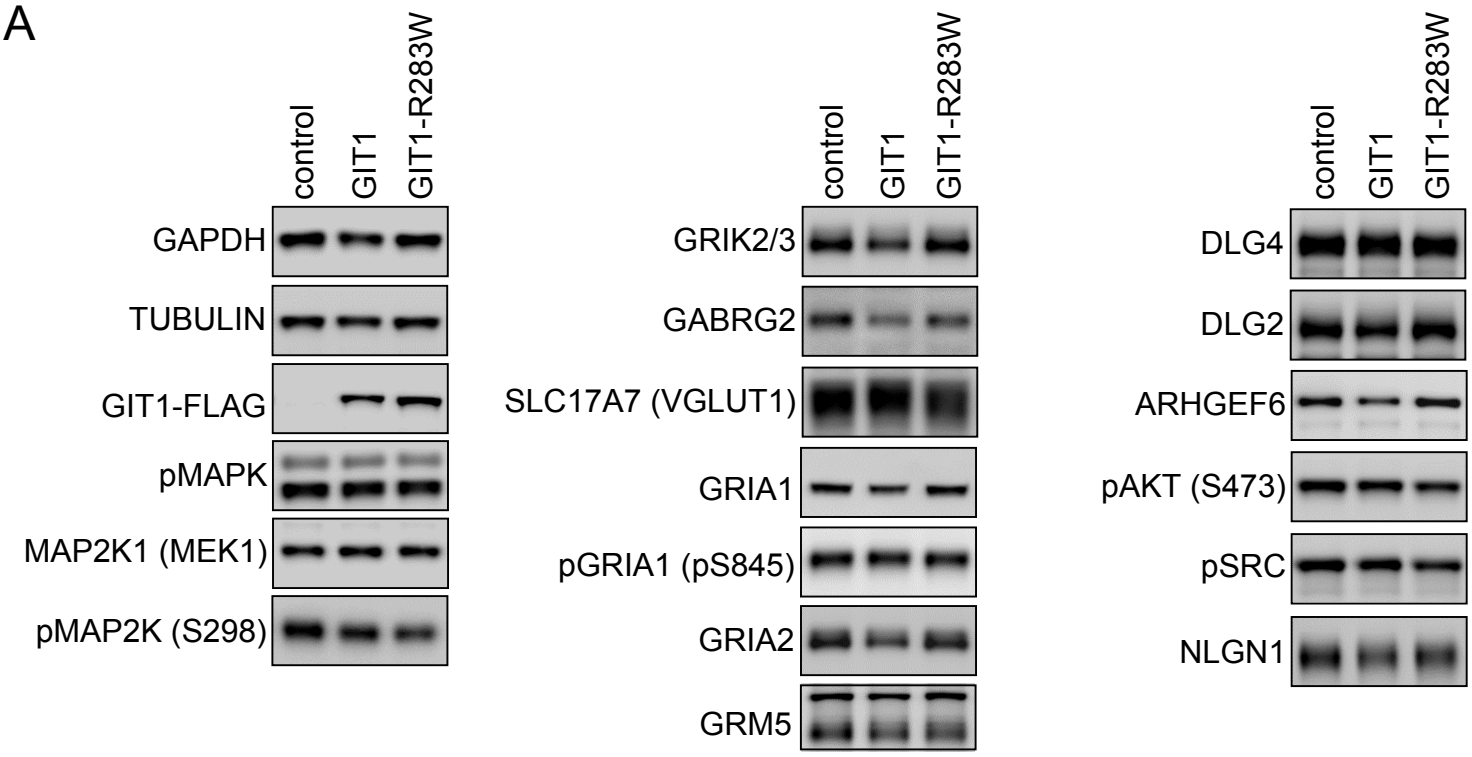


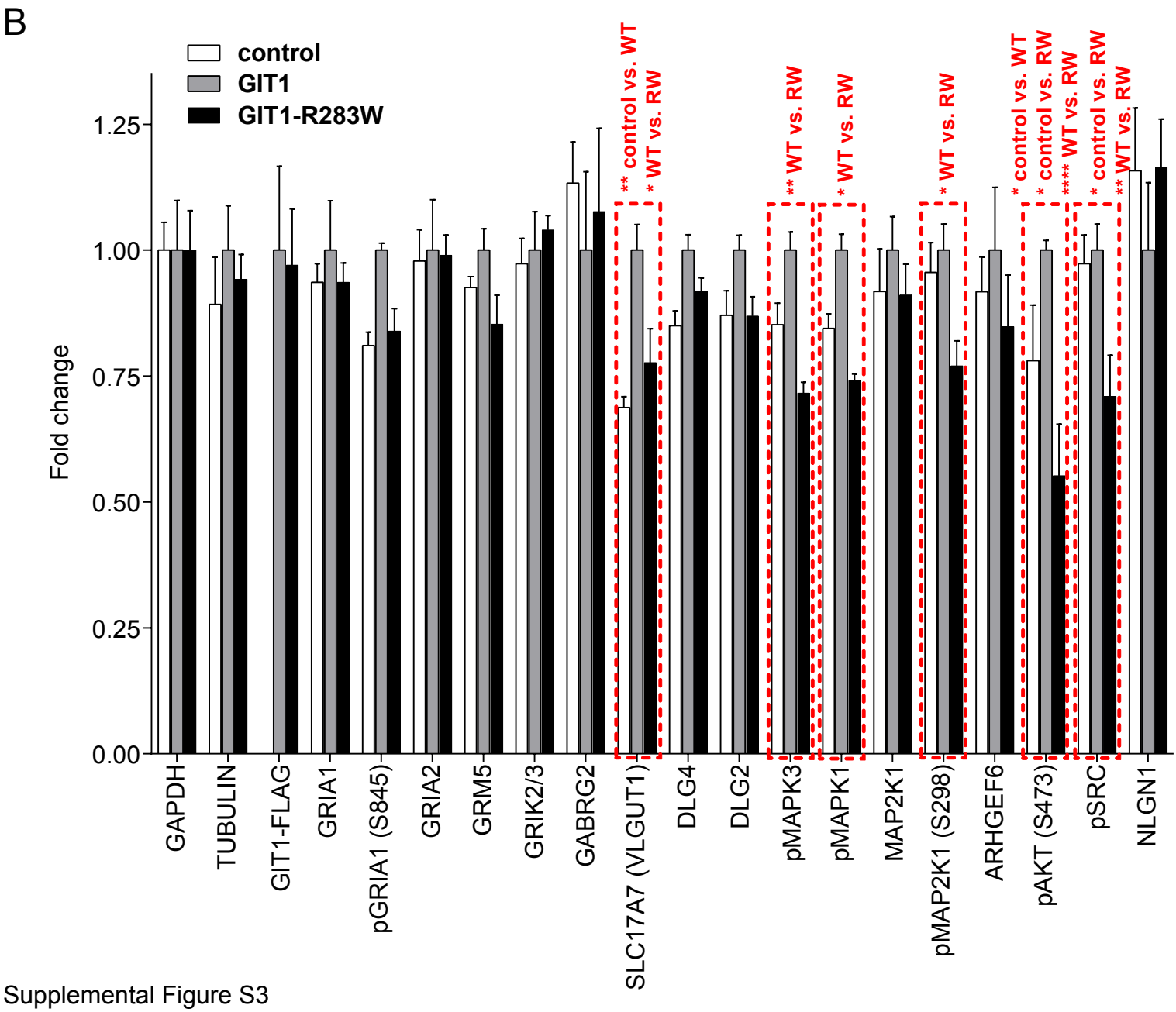
C57BL/6J
male
post-natal day 56
Git1-RP_050428_04_F12
sagittal

Supplemental Figure S1



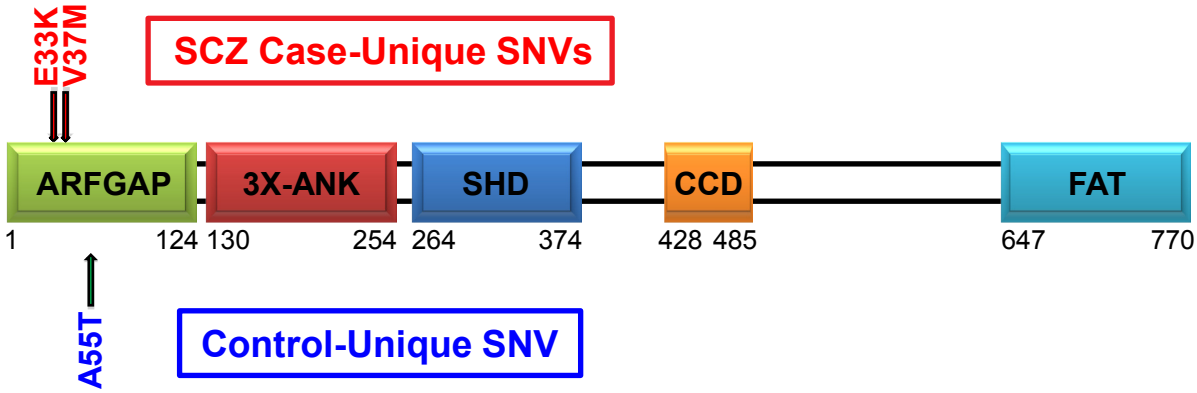
Supplemental Figure S2





Supplemental Figure S3

A



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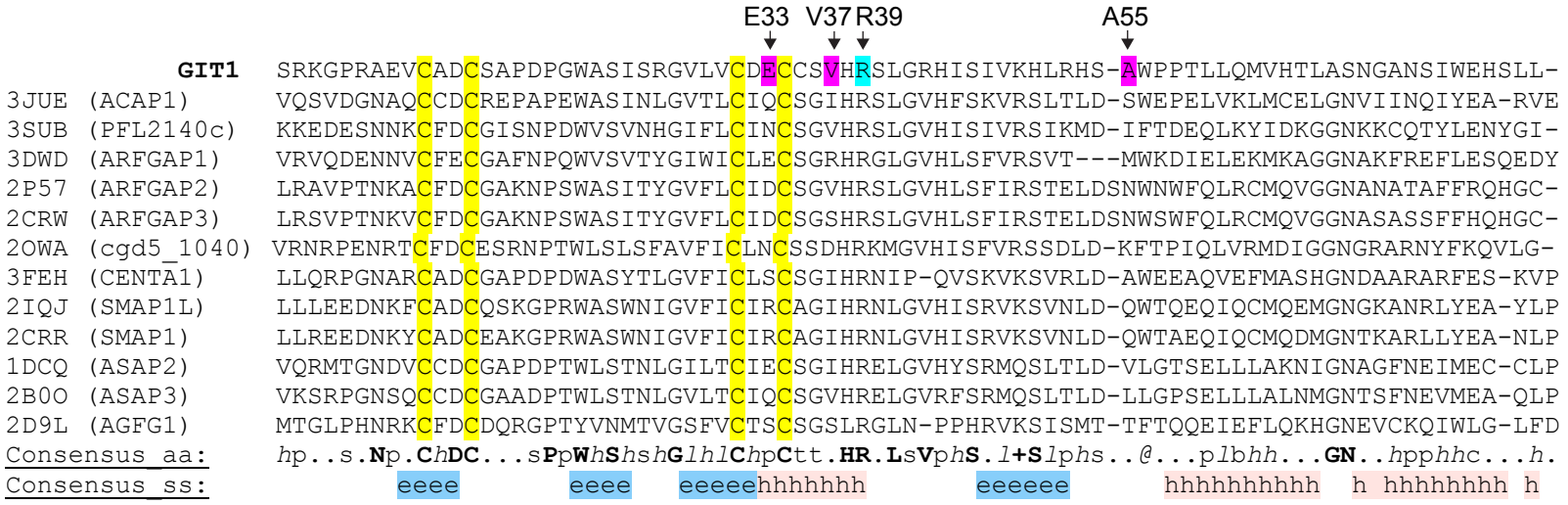

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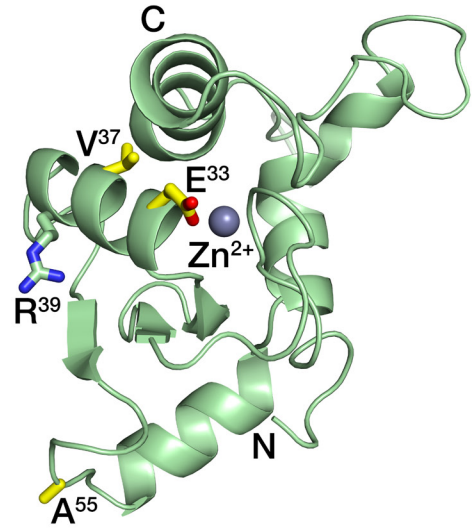

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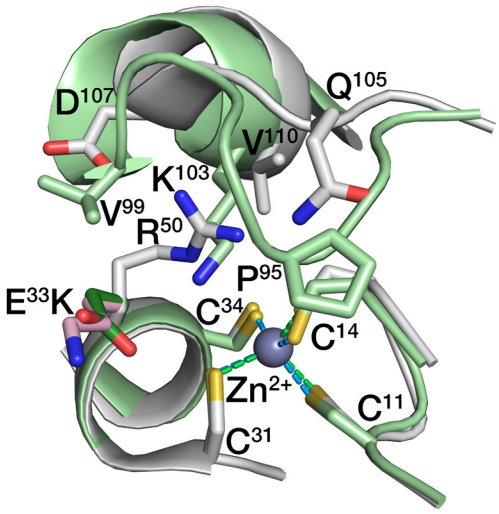

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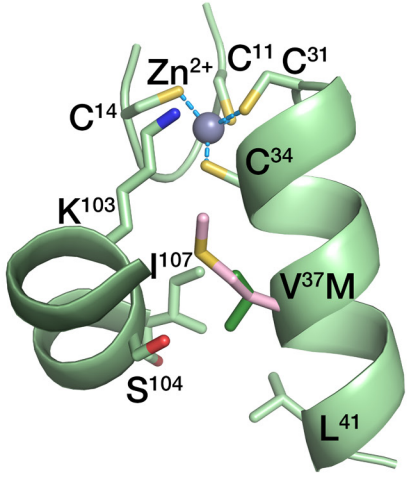

Supplemental Figure S4