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Supplemental Information

G3BPs tether the TSC complex to lysosomes and suppress mTORC1 signaling

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Table S1. siRNA, shRNA, and sgRNA targeting sequences mapped on the nucleotide sequence of G3BP1, related to Figure 1. The targeting sequences of the four different siRNAs from the G3BP1 siRNA pool (light green), two shRNA sequences against G3BP1 (dark green), and the two sgRNAs used for CRIPSR/Cas9 mediated knockout (orange) are highlighted.

5′UTR - **CCGGCGACCTTCCGGCGGGA**... (15689bp)
ATGGTGATGGAGAAGCCTAGTCCCCTGCTGGTCGGGCGGGAATTTGTGAGACAGTATTACACACTGCTGAACCAGGCCCCAGACA
TGCTGCATAGATTTTATGGAAAGAACTCTTCTTATGTCCATGGGGGATTGGATTCAAATGGA**AAGCCAGCAGATGCAGTCTA**CGG
ACAGAAAGAAATCCAC**AGGAAAGTGATGTCACAAA**ACTTCACCAACTGCCACACCAAGATTCGCCATGTTGATGCTCATGCCACG
CTAAATGATGGTGTGGTAGTCCAGGTGATGGGGCTTCTCTCTAACAACAACCAGGCTTTGAGGAGATTCATGCAAACGTTTGTCC
TTGCTCCTGAGGGGTCTGTTGCAAATAAATTCTATGTTCACAATGATATCTTCAGATACCAAGATGAGGTCTTTGGTGGGTTTGT
CACTGAGCCTCAGGAGGAGTCTGAAGAAGAAGTAGAGGAACCTGAAGAAAGACAGCAAACACCTGAGGTGGTACCTGATGATTCT
GGAACTTTCTATGATCAGGCAGTTGTCAGTAATGACATGGAAGAACATTTAGAGGAGCCTGTTGCTGAACCAGAGCCTGATCCTG
AACCAGAACCAGAACAAGAACCTGTATCTGAAATCCAAGAGGAAAAGCCTGAGCCAGTATTAGAAGAAACTGCCCCTGAGGATGC
TCAGAAGAGTTCTTCTCCAGCACCTGC**AGACATAGCTCAGACAGTA**CAGGAAGACTTGAGGACATTTTCTTGGGCATCTGTGACC
AGTAAGAATCTTCCACCCAGTGGAGCTGTTCCAGTTACTGGGATACCACCTCATGTTGTTAAAGTACCAGCTTCACAGCCCCGTC
CAGAGTCTAAGCCTGAATCTCAGATTCCACCACAAAGACCTCAGCGGGATCAAAGAGT**GCGAGAACAACGAATAAAT**ATTCCTCC
CCAAAGGGGACCCAGACCAATCCGTGAGGCTGGTGAGCAAGGTGACATTGAACCCCGAAGAATGGTGAGACACCCTGACAGTCAC
CAACTCTTCATTGGCAACCTGCCTCATGAAGTGGACAAATCAGAGCTTAAAGATTTCTTTCAAAGTTATGGAAAC**GTGGTGGAGT**
TGCGCATTAACAGTGGTGGGAAATTACCCAATTTTGGTTTTGTTGTGTTTGATGATTCTGAGCCTGTTCAGAAAGTCCTTAGCAA
CAGGCCCATCATGTTCAGAGGTGAGGTC**CGTCTGAATGTCGAAGAGA**AGAAGACTCGAGCTGCCAGG**GAAGGCGACCGACGAGAT**
AATCGCCTTCGGGGACCTGGAGGCCCTCGAGGTGGGCTGGGTGGTGGAATGAGAGGCCCTCCCCGTGGAGGCATGGTGCAGAAAC
CAGGATTTGGAGTGGGAAGGGGGCTTGCGCCACGGCAGTGA

Name	Target sequence
siG3BP1 (pool)	GTGGTGGAGTTGCGCATTA
	AGACATAGCTCAGACAGTA
	GAAGGCGACCGACGAGATA
	GCGAGAACAACGAATAAAT
shG3BP1 #1	CGTCTGAATGTCGAAGAGA
shG3BP1 #2	AGGAAAGTGATGTCACAAA
sgG3BP1 #1	AAGCCAGCAGATGCAGTCTA
sgG3BP1 #2	CCGGCGACCTTCCGGCGGGA

Table S2. Heterozygous variants in G3BP1 or G3BP2 strongly predicted to alter protein function, identified in the GEL and Epi4K cohorts, related to Figure 7. Rows are colored as indicated in Figure S3B according to protein domains of G3BP1 and G3BP2 in which the alterations occur.

Column header	Comment
Gene	G3BP1 or G3BP2
Protein Domain	Protein domains of G3BP1 and G3BP2 are indicated according to Figure S4B
Cohort	**GEL** = The National Genomics Research and Healthcare Knowledgebase v5, Genomics England. doi:10.6084/m9.figshare.4530893.v5. 2019; **Epi4K** = Epi4K data from the Duke Center for Human Genome Variation (now Institute for Genomic Medicine, Columbia University). PMID: 25262651.
Location (GRCh 38, Hg38)	Genome Reference Consortium Human Build 38 (2013)
Protein	Amino acid or exon modification of the variant
MAF (gnomAD v.2 1.1*) and number of alleles	Minor allele frequencies assessed by GnomAD
Segregation	Information on variant occurence in the parents
Phenotype	Reported disease phenotypes
Similarity TSC, TBC1D7, mTORopathy	Overlap with phenotypes known for variants in TSC1, TSC2, TBC1D7 or genes related to mTORopathies
Neurological phenotype	Indicates whether the reported phenotypes relate to neurological disturbances

Gene	Protein domain	Cohort	Location (GRCh 38, Hg 38)	Protein	MAF (gnomAD v.2 1.1*) and number of alleles	Segregation	Phenotype	Similarity TSC, TBC1D7, mTORopathy	Neurological phenotype
G3BP1	delta acidic+ proline-rich+RRM+RGG (frameshift in NTF2L domain)	GEL	151786716	Exon 2 c.95+1	0	*De novo*	Macrocephaly Intellectual disability Skeletal dysplasia Gait disturbance	Macrocephaly is characteristic of mTORopathies and TBC1D7 disease	yes
	NTF2L	GEL	151786673	Q18R	0	Parents not available	Functional abnormality of the bladder Abnormality of eye movement Ataxia Dysarthria Hypercholesterolemia Peripheral neuropathy Vertigo Dysphagia		yes
	Acidic	GEL	151797322	E212G	0.00001194 (3/251328)	Mother unaffected and does not carry the variant	Heterotopia Subependymal nodules	Heterotopia and subependymal nodules are neuronal migration abnormalities	yes
	RRM	GEL	151803906	V406F	0.000003985 (1/250960)	Parents not available	Hypertension Gout Stage 5 chronic kidney disease		no
	delta RRM+RGG (stop in proline rich domain)	Epi4K	151179525	R307X	0.000003186 (1/31390)	*De novo*	Epileptic encephalopathy Infantile spasms	Infantile spasms and epilepsy frequent in TSC and mTORopathies	yes
	delta RRM+RGG (stop in proline rich domain)	GEL	151800235	Q325X	0	*De novo*	Upper limb spasticity		yes
	RGG	GEL	151803987	G433S	0	Parents not available	Dysarthria Tremor Weight loss Lower limb hyperreflexia Bowel incontinence Fatigue		yes
	RGG	GEL	151804021	G444D	0	Parents not available	Visual impairment Retinal dystrophy		yes
G3BP2	Acidic	GEL	75655786	Y176C	0.000007959 (2/251290)	Parents not available	Parkinsonism Abnormal rapid eye movement sleep		yes
	RGG	GEL	75645539	R447H	0	*De novo*	Ataxia Cognitive impairment		yes

* location of variant converted to GRCh 37, Hg19

Table S3. Antibody dilutions, related to STAR Methods.

ANTIBODIES	DILUTION	SOURCE	IDENTIFIER
CALR	dilution in IB 1:1000	Cell Signaling	Cat# 12238; RRID: AB_2688013
CANX	dilution in IB 1:1000	Cell Signaling	Cat# 2679; RRID: AB_2228381
CTSD	dilution in IB 1:1000	Cell Signaling	Cat# 2284; RRID: AB_10694258
EEA1	dilution in IB 1:1000	Cell Signaling	Cat# 3288; RRID: AB_2096811
EIF2S1	dilution in IB 1:1000	Cell Signaling	Cat# 9722; RRID: AB_2230924
EIF2S1-pS51	dilution in IB 1:1000	Cell Signaling	Cat# 9721; RRID: AB_330951
EIF3A	dilution in IB 1:10000 dilution in IF 1:1000	Cell Signaling	Cat# 3411; RRID: AB_2096523
FLAG	used in IP at 1ug/mL	Sigma-Aldrich	Cat# F3165; RRID: AB_259529
G3BP1	dilution in IB 1:1000	Santa Cruz	Cat# sc-365338; RRID: AB_10846950
G3BP1	dilution in IB 1:1000 dilution in IF 1:200 dilution in PLA 1:2000	Santa Cruz	Cat# sc-81940; RRID: AB_1123055
G3BP2	dilution in IB 1:1000	Bethyl	Cat# A302-040A; RRID: AB_1576545
GAPDH	dilution in IB 1:10000	Abcam	Cat# ab37187; RRID: AB_732651
GAPDH (zebrafish)	dilution in IB 1:1000	Sigma-Aldrich	Cat# SAB2701826
GFP	dilution in IB 1:1000 used in IP at 1 µg/mL	Roche	Cat# 11814460001; RRID: AB_390913
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	dilution in IF 1:500	Invitrogen	Cat# A-11001; RRID: AB_2534069
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 568	dilution in IF 1:500	Invitrogen	Cat# A-11011; RRID: AB_143157
Goat anti-Mouse IgG (H+L) cross-adsorbed secondary, Alexa Fluor 555	dilution in IF 1:1000	Thermo Fisher Scientific	Cat# A-21422; RRID: AB_2535844
Goat anti-Rabbit IgG (H+L) cross-adsorbed secondary, Alexa Fluor 488	dilution in IF 1:1000	Thermo Fisher Scientific	Cat# A-11008; RRID: AB_143165
Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP-coupled	dilution in IB 1:4000	Thermo Fisher Scientific	Cat# 31430; RRID: AB_228307
Goat anti-Rabbit IgG (H+L) Secondary Antibody, HRP-coupled	dilution in IB 1:4000	Thermo Fisher Scientific	Cat# 31460; RRID: AB_228341
Goat anti-Rabbit IgG (H+L) Secondary Antibody, Dylight 800 (zebrafish)	dilution in IB 1:10000	Thermo Fisher Scientific	Cat# SA5-35571; RRID: AB_2556775
Goat anti-Rat IgG (H+L) Secondary Antibody, HRP-coupled	dilution in IB 1:4000	Thermo Fisher Scientific	Cat# 31470; RRID: AB_228356
GOLGA1	dilution in IB 1:1000	Cell Signaling	Cat# 13192; RRID: AB_2798144

GOLGA2	dilution in IB 1:1000	Cell Signaling	Cat# 12480; RRID: AB_2797933
HA	dilution in IB 1:1000	Roche	Cat# 11867423001; RRID: AB_390918
Histone H3 (H3C1)	dilution in IB 1:1000	Bethyl	Cat# A300-822A; RRID: AB_597872
HSP60 (HSPD1)	dilution in IB 1:10000	Cell Signaling	Cat# 12165; RRID: AB_2636980
HSP90 (CDC37)	dilution in IB 1:10000	Cell Signaling	Cat# 4877; RRID: AB_2233307
LMNA A/C	dilution in IB 1:10000	Cell Signaling	Cat# 2032; RRID: AB_2136278
LAMP1	dilution in IB 1:1000;	Cell Signaling	Cat# 9091; RRID: AB_2687579
LAMP1	dilution in IF 1:1000	Developmental Studies Hybridoma Bank	Cat# H4A3; RRID: AB_2296838
LAMP2	dilution in IB 1:10000	Cell Signaling	Cat# 49067; RRID: AB_2799349
LAMP2	dilution in IB 1:1000;	Santa Cruz	Cat# sc-18822; RRID: AB_626858
LAMP2	dilution in PLA 1:200	Developmental Studies Hybridoma Bank	Cat# H4B4; RRID: AB_2134755
MTOR	dilution in IB 1:1000 dilution in IF 1:200	Cell Signaling	Cat# 2983; RRID: AB_2105622
MTOR epitope maps to residues 221 and 261 of human mTOR	used in IP at 7.5 µg/mL	Monoclonal Antibody Core Unit. Helmholtz Zentrum München, Germany	TQREP-3G6
Mock antibody mouse	used in IP at 7.5 µg/mL	Santa Cruz	Cat# sc-2025; RRID: AB_737182
Mock antibody rabbit	used in IP at 7.5 µg/mL	Bethyl	Cat# P120-101; RRID: AB_479829
	used in IP from rat brains at 4 µg/mL	Sigma-Aldrich	Cat# I5006; RRID: AB_1163659
Mock antibody rat	used in IP at 7.5 µg/mL	Monoclonal Antibody Core Unit. Helmholtz Zentrum München, Germany	RmC3-7H8
Mouse IgG HRP Linked Whole Ab	used in IB of lysosomal isolation 1:5000	Merck	Cat# GENA931
MYC-tag	dilution in IB 1:1000	Cell Signaling	Cat# 2276; RRID: AB_331783
Rabbit IgG HRP Linked Whole Ab	used in IB of lysosomal isolation 1:5000	Merck	Cat# GENA934; RRID: AB_2722659
RPS6KB1	dilution in IB 1:1000	Cell Signaling	Cat# 2708; RRID: AB_390722
RPS6KB1-pT389	dilution in IB 1:1000	Cell Signaling	Cat# 9206; RRID: AB_2285392
RPS6KB1-pT389	dilution in IB 1:1000	Cell Signaling	Cat# 9205; RRID: AB_330944
RAB5A	dilution in IB 1:1000	Cell Signaling	Cat# 3547; RRID: AB_2300649
RAB7A	dilution in IB 1:1000	Cell Signaling	Cat# 9367; RRID: AB_1904103
RPTOR	dilution in IB 1:1000	Cell Signaling	Cat# 2280; RRID: AB_561245
RPTOR #1 epitope maps to residues 686 and 704 of human Raptor	used in IP at 7.5 µg/mL	Monoclonal Antibody Core Unit. Helmholtz Zentrum München, Germany	RAP1-20C4
RPTOR #2	used in IP at 7.5 µg/mL	Bethyl	Cat# A300-553A; RRID: AB_2130793

RPS6	dilution in IB 1:1000	Cell Signaling	Cat# 2317; RRID: AB_2238583
RPS6-pS235/236	dilution in IB 1:1000	Cell Signaling	Cat# 4856; RRID: AB_2181037
RPS6-pS235/236 (zebrafish)	dilution in IB 1:1000	Cell Signaling	Cat# 2211; RRID: AB_331679
RPS6-pS235/236 (zebrafish)	dilution in IF 1:100	Cell Signaling	Cat# 4858; RRID: AB_916156
TSC1	dilution in IB 1:1000	Cell Signaling	Cat# 4906; RRID: AB_2209790
TSC1 #1	used in IP at 7.5 µg / mL	Gift from Michael N. Hall, Basel, Switzerland (Molle, 2006). Generated according to van Slegtenhorst et al. (1998).	N/A
TSC1 #2	used in IP at 7.5 µg / mL	Thermo Fisher Scientific (Invitrogen)	Cat# 37-0400; RRID: AB_2533292
TSC1 #3	dilution in IB 1:1000 used in IP at 4 µg/mL	Cell Signaling	Cat# 6935; RRID: AB_10860420
TSC2	dilution in IB 1:1000; dilution in IF 1:800; dilution in PLA 1:1600	Cell Signaling	Cat# 4308; RRID: AB_10547134
TSC2 #1	used in IP at 7.5 µg / mL	Thermo Fisher Scientific (Invitrogen)	Cat# 37-0500; RRID: AB_2533293
TSC2 #2 epitope maps to residues 1535 and 1784 of human TSC2	used in IP at 7.5 µg / mL	Gift from Michael N. Hall, Basel, Switzerland (Molle, 2006). Generated according to van Slegtenhorst et al. (1998).	N/A
TSC2 #3	used in IP at 7.5 µg / mL	Abcam	Cat# ab52936; RRID: AB_883283
TUBA1B	dilution in IB 1:10000	Abcam	Cat# ab108629; RRID: AB_10866252
VDAC	dilution in IB 1:1000	Cell Signaling	Cat# 4661; RRID: AB_10557420

Table S4. Primers, related to STAR Methods.

PRIMER	SEQUENCE	SOURCE
AttB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCACC	Stefan Pusch/Eurofins
AttB2	GGGGACCACTTTGTACAAGAAAGCTGGGTT	Stefan Pusch/Eurofins
G3BP1_B1	CAAAAAAGCAGGCTCCACCATGGTGATGGAGAAGCCTAGTC	Stefan Pusch/Eurofins
G3BP1_182_B2o	CAAGAAAGCTGGGTTGTCATTACTGACAACTGCCTGATC	Stefan Pusch/Eurofins
G3BP1_183_B1	CAAAAAAGCAGGCTCCACCATGGAAGAACATTTAGAGGAGCCTG	Stefan Pusch/Eurofins
G3BP1_332_B2o	CAAGAAAGCTGGGTTTCTTCGGGGTTCAATGTCAC	Stefan Pusch/Eurofins
G3BP1_333_B1	CAAAAAAGCAGGCTCCACCATGGTGAGACACCCTGACAG	Stefan Pusch/Eurofins
G3BP1_B2o	CAAGAAAGCTGGGTTCTGCCGTGGCGCAAG	Stefan Pusch/Eurofins
G3BP1_Fw_AsiSI	gaggcgatcgccatggtgatggagaagcctagt	Eurofins
G3BP1_Rev_MluI	gcgACGCGTctgccgtggcgcaagc	Eurofins
hG3BP1ΔRGG_F	gaatgtcgaagagaagaagtaagcggccgcact	Eurofins
hG3BP1ΔRGG_R	agtgcggccgcttacttcttctcttcgacattc	Eurofins
h-G3BP2-AsiSI-F	GAATTCGCGATCGCCATGGTTATGGAGAAGCCCAG	Eurofins
h -G3BP2-MluI –R	CG ACGCGT TTA GCGACGCTGTCCTGTG	Eurofins
LAMP1_B1	CAAAAAAGCAGGCTCCACCATGGCGGCCCCCGGCAG	Stefan Pusch/Eurofins
LAMP1_382_B2o	CAAGAAAGCTGGGTTCATGCTGTTCTCGTCCAGCAG	Stefan Pusch/Eurofins
LAMP1_383_BglII_f	ATGATCAGATCTATGCTGATCCCCATCGCTGTG	Stefan Pusch/Eurofins
LAMP1_383_SalI_ro	TACGATGTCGACGATAGTCTGGTAGCCTGCGTG	Stefan Pusch/Eurofins
Myc-DDK-G3BP1_ d70-483_F (ΔNTF2L)	gctcagtgacaaaccccatcgcgatcgcctta	Eurofins
Myc-DDK-G3BP1_ d70-483_R (ΔNTF2L)	taaggcgatcgcgatggggtttgtcactgagc	Eurofins
Myc-DDK-G3BP1_d484-1470_F (NTF2L)	cgagtgcggccgcttaaccaaagacctcatct	Eurofins
Myc-DDK-G3BP1_d484-1470_R (NTF2L)	agatgaggtctttggttaagcggccgcactcg	Eurofins
sgTSC2-Exon 2-F	5'- CACCGACGGAGTTTATCATCACCG	Invitrogen
sgTSC2-Exon 2-R	5'- AAACCGGTGATGATAAACTCCGTC	Invitrogen
sgG3BP1-Exon 3-F	5'- CACCGAAGCCAGCAGATGCAGTCTA	Invitrogen
sgG3BP1-Exon 3-R	5'- AAACTAGACTGCATCTGCTGGCTTC	Invitrogen
sgG3BP1#2-Exon 1-F	5'- CACCGTCCCGCCGGAAGGTCGCCGG	Invitrogen
sgG3BP1#2-Exon 1-R	5'- AAACCCGGCGACCTTCCGGCGGGAC	Invitrogen

Table S5. siRNAs and shRNAs, related to STAR Methods.

RNA	SOURCE	IDENTIFIER
siControl (ON-TARGET plus Non-targeting Pool)	Dharmacon	Cat# D-001810-10-05
siG3BP1 pool (ON-TARGET plus Human G3BP1 siRNA – SMART pool)	Dharmacon	Cat# L-012099-00-0020
siG3BP2 pool (ON-TARGET plus Human G3BP2 siRNA – SMART pool)	Dharmacon	Cat# L-015329-01-0020
siG3BP1 (siGENOME) (used for PLA experiments in **Figure 4A**)	Dharmacon	Cat# M-012099-02-0005
anti-Luc siRNA (used as Control in **Figure 4A**)	Dharmacon	Cat# D-002050-01-20
siTSC2 pool (ON-TARGET plus Human TSC2 siRNA – SMART pool)	Dharmacon	Cat# L-003029-00-0005
TRIPZ Inducible Lentiviral Human G3BP1 shRNA (F6 = shG3BP1 #1)	Dharmacon	Cat# RHS4696-200750396; CloneId: V3THS_329105
TRIPZ Inducible Lentiviral Human G3BP1 shRNA (H11 = shG3BP1 #2)	Dharmacon	Cat# RHS4696-200753099; CloneId: V3THS_329104
TRIPZ Inducible Lentiviral Non-silencing shRNA Control	Dharmacon	Cat# RHS4743