Table S. 1. Reagents, antibodies and primers

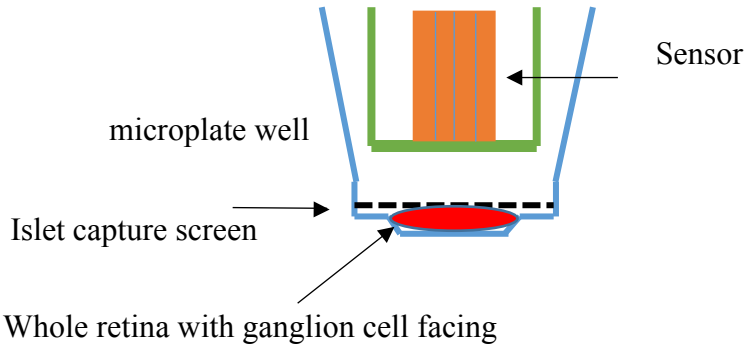
	Manufacturer Catalog Number
C57BL6J Mice	Jackson Labs# 000664
Streptozotocin (STZ)	Sigma# S0130
Glucometer	CVS True Metrix# RE4007-01
Hba1c Kit	PTS Diagnostics# 3021
Glucose Estimation Assay Kit	Cell Biolabs, Inc# STA-680
Trizol	Ambion# 15596
cDNA Synthesis Kit	Biorad# 1708841
4% PFA	EMS# 50-980-487
OCT Compound	EMS# 62550-01
H& E Kit	EMS# 26754-1A, 26762-01
Periodic Acid Schiff Reagents	Sigma# 395B-1KT
Elastase Solution	Calbiochem# 324682
Lipopolysacchrides (LPS)- E. Coli	Sigma# L2880
Seahorse XF DMEM Medium	AT# 103575-100
Seahorse XF24 Islet Capture Microplate	AT# 101122-100
Seahorse Microplate Insert Tool	AT# 101135-100
Isolectin1B-4	Vector laboratories # FL-1201
Anti- Vimentin (IHC)	Cell Signaling# 5741S
Anti- TRIB3 Antibody (IHC)	Abcam# 73547
Anti- TRIB3 Antibody (WB)	Santa Cruz# (B-2): sc-390242
Recombinant Human TRIB3 protein	Abcam# 186077
Anti- VEGF Antibody (WB-RETINA)	Santa Cruz # 7269 HRP
Anti- VEGF Antibody (WB-MULLER CELL)	Invitrogen JH121# MA5-13182
Anti- GFAP Antibody (IHC &WB)	Sigma# C9205
Anti- B-Actin Antibody (WB)	Sigma# A2066
Anti- CD 31/PECAM-1 (IHC)	Novus Biologicals# NB600-562
Anti- IBA-1 Antibody (IHC)	Wako Chemicals# 019-19741
Anti- BRN-3A Antibody (IHC)	Santa Cruz # 8429 AF488
Anti- Glut-1 Antibody (WB& IHC)	Cell Signaling# Rabbit mAb12939
Anti- HIF-1A Antibody (WB)	Cell Signaling# Rabbit mAb14179
Anti- EGFR Antibody (WB)	Santa Cruz # sc-03-G
Peroxidase AffiniPure Goat Anti-Mouse IgG, Fcγ subclass 2a specific (WB)	JacksonImmunoresearch#115-035-206
HRP Goat Anti-Mouse (WB)	LICOR# 926-80010
IR Goat Anti-Mouse (WB)	LICOR#926-32210
Donkey anti-Rabbit IgG Secondary Antibody, Alexa Fluor 555 (IHC)	Invitrogen Catalog # A32794

Donkey anti-Rabbit IgG Secondary Antibody, Alexa Fluor 488 (IHC)	Invitrogen Catalog # A-21206
VECTASHIELD® Antifade Mounting Medium with DAPI	Vector laboratories# H-1200-10
Taqman Primer- TRIB3	Mm00454879_m1
Taqman Primer- AIF-1	Mm00479862_g1
Taqman Primer- COX-2	Mm03294838_g1
Taqman Primer- ICAM-1	Mm00516023_m1
Taqman Primer- HIF-1A	Mm00468869_m1
Tagman Primer- GLUT1	Mm00441480_m1
Taqman Primer- NFKB-1	Mm00476361_m1
Taqman Primer- RC3HI	Mm01284492_m1
Taqman Primer- VEGF-A	Mm00437304_m1
Taqman Primer- ZC3HI2A	Mm00462533_m1
Taqman Primer- GAPDH	Mm99999915_g1
Taqman Primer- GUSB	Mm00446953_m1
Trib3 (BC012955) Mouse Tagged ORF Clone	Origene- Product number MR204806
MIO-M1 cells- Müller glia cell line	XIP (Prof Limb)
AGE-BSA	Abcam# ab51995
Oxoid™ AnaeroGen™ W-Zip Compact Gas Generator System	Thermo Scientific™ AN0010W
MTT Solution	Sigma-Aldrich# M5655
HIF-1A siRNA	Thermofisher Scientific Assay ID# 42840
EGFR siRNA	Thermofisher Scientific Assay ID# 646
Neg. Control siRNA	Thermofisher Scientific# 4390844
2-NBDG Glucose Uptake Assay Kit	BioVision# K68250

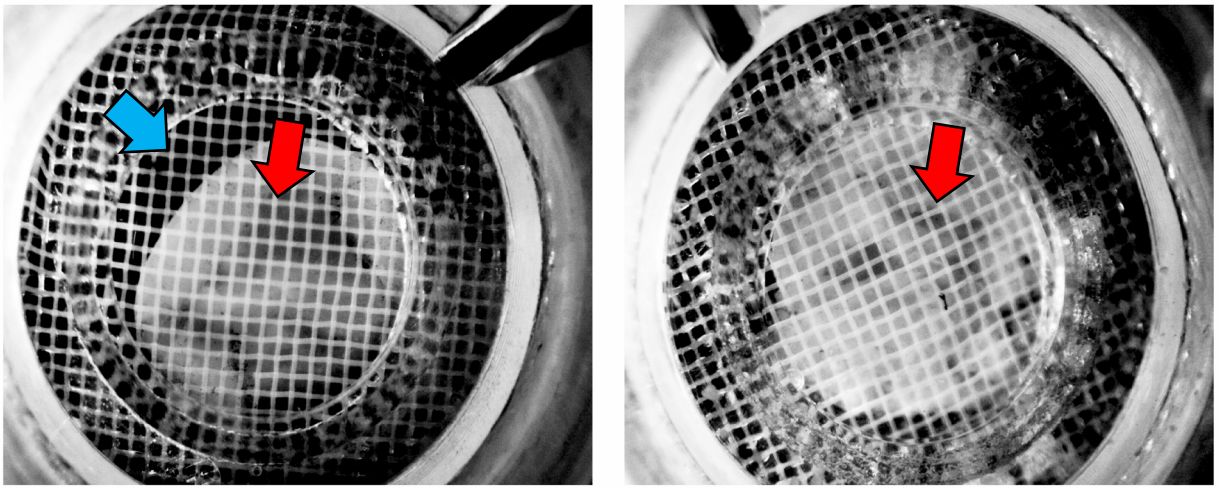
Table S. 2. Glucose levels in blood and retina

Experimental groups of mice	**50mg /kg STZ- 5 days dosage**			
	Mean HbA1c levels (%HbA1c; mmol/mol)			
	15-weeks	P value	30-weeks	P value
C57BL6 (n=9)	4.7; 28		4.7; 28	
C57BL6 STZ (n=9)	10.8; 95	<0.0001	11.7; 104	<0.0001
TRIB3KO (n=10)	5.3; 34		5.1; 32	
TRIB3KO STZ (n=13)	10.0; 86	<0.0001	10.2; 88	<0.0001
Experimental groups of mice	**150mg /kg STZ- single dosage**			
	Mean Blood glucose levels (mg/dl)			
	4-weeks	P value	6-weeks	P value
C57BL6 (n=8)	147.5		139.5	
C57BL6 STZ (n=12)	285	<0.0001	291.8	0.0006
TRIB3KO (n=9)	184		138.5	
TRIB3KO STZ (n=12)	391.5	0.0205	351.0	0.0133
Experimental groups of mice	**150mg /kg STZ- single dosage**			
	Mean Blood glucose levels (mg/dl)		**Mean Retinal glucose levels (mg/dl)**	
	4-weeks	P value	4-weeks	P value
C57BL6 (n=9)	139.9		0.3	
C57BL6 STZ (n=9)	470.6	<0.0001	4.4	<0.0001
TRIB3KO (n=11)	152.2		0.3	
TRIB3KO STZ (n=5)	434.4	NS	1.3	0.0009

A Schematic representation of the Aligent Islet



B Placement of retina in the islet

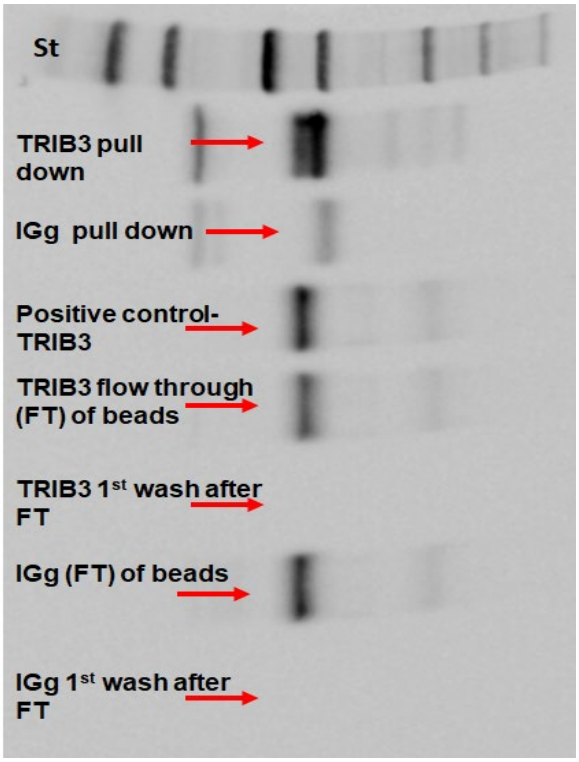


Islet capture screen

Whole retina with ganglion cell facing down

Supplementary Figure 1. Images of the retina placed in the islet microplate and prepared for the Seahorse analysis. **A.** Schematic representation of the well with the retina in the microplate. **B.** Retina placed under the islet capture screen mesh.

A.


St
TRIB3 pull down
IGg pull down
Positive control-TRIB3
TRIB3 flow through (FT) of beads
TRIB3 1st wash after FT
IGg (FT) of beads
IGg 1st wash after FT

B

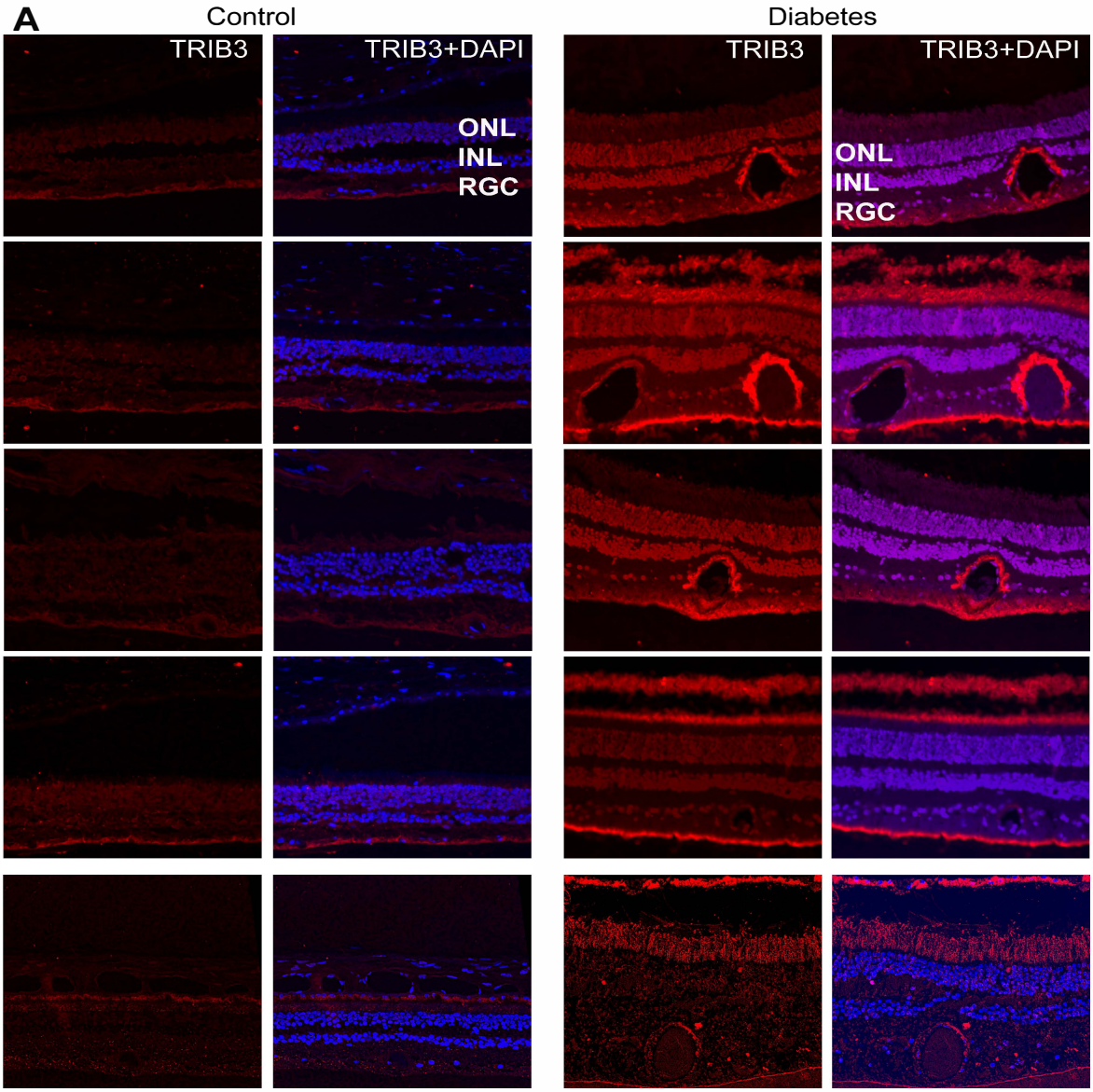
Protein Grouping Strategy: Experiment-wide grouping with binary peptide-protein weights Peptide Thresholds: 95.0% minimum; Protein Thresholds: 99.0% minimum and 2 peptides minimum

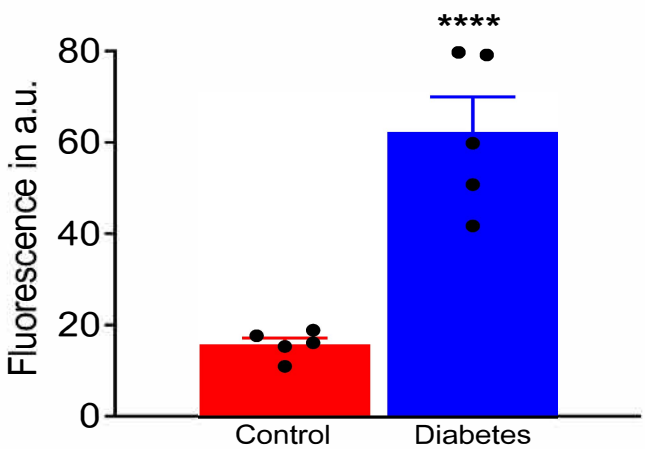
Peptide FDR: 0.9% (Prophet) Protein FDR: 0.0% (Prophet)

Biological sample name	Protein name	Protein id
TRIB3-IP	Liprin-beta-1 n=3 Tax=Homo sapiens RepID=LIPB1_HUMAN	100.00%
TRIB3-IP	Potassium voltage-gated channel subfamily B member 1 n=1 Tax=Homo sapiens RepID=KCNB1_HUMAN	99.90%
TRIB3-IP	Endonuclease domain-containing 1 protein n=1 Tax=Homo sapiens RepID=ENDD1_HUMAN Metastasis-associated	97.00%
TRIB3-IP	protein MTA2 n=4 Tax=Homininae RepID=MTA2_HUMAN	100.00%
TRIB3-IP	FWP004 n=1 Tax=Homo sapiens RepID=Q5QTS3_HUMAN	98.60%
TRIB3-IP	HLA-Cw12 n=2 Tax=Homo sapiens RepID=A9X165_HUMAN	90.00%
TRIB3-IP	Tubulin alpha-4A chain n=17 Tax=Boreoeutheria RepID=TBA4A_HUMAN	97.00%
TRIB3-IP	Low-density lipoprotein receptor-related protein 1B n=10 Tax=Homo sapiens RepID=LRP1B_HUMAN 60S ribosomal	100.00%
TRIB3-IP	protein L6 n=7 Tax=Homininae RepID=RL6_HUMAN	99.60%
TRIB3-IP	Striatin-interacting protein 2 n=2 Tax=Homo sapiens RepID=STRP2_HUMAN	100.00%
TRIB3-IP	ATP-binding cassette sub-family A member 10 n=2 Tax=Homo sapiens RepID=ABCAA_HUMAN Integrin beta-1 n=2	99.70%
TRIB3-IP	Tax=Homininae RepID=ITB1_HUMAN	99.60%
TRIB3-IP	Arf-GAP with Rho-GAP domain, ANK repeat and PH domain-containing protein 3 n=7 Tax=Homo sapiens RepID	99.90%
TRIB3-IP	Vesicle-fusing ATPase n=3 Tax=Homo sapiens RepID=NSF_HUMAN	100.00%
TRIB3-IP	Piezo-type mechanosensitive ion channel component 1 n=1 Tax=Homo sapiens RepID=PIEZ1_HUMAN Heat shock 70	100.00%
TRIB3-IP	kDa protein 1A/1B n=9 Tax=cellular organisms RepID=HSP71_HUMAN	89.70%
TRIB3-IP	Sequestosome-1 n=3 Tax=Homininae RepID=SQSTM_HUMAN	99.00%
TRIB3-IP	Unconventional myosin-Ie n=2 Tax=Homo sapiens RepID=MYO1E_HUMAN	98.10%
TRIB3-IP	Histone-lysine N-methyltransferase 2B n=3 Tax=Homo sapiens RepID=KMT2B_HUMAN	100.00%
TRIB3-IP	Titin, isoform CRA_a n=1 Tax=Homo sapiens RepID=D3DPG0_HUMAN	100.00%
TRIB3-IP	Myosin-9 n=2 Tax=Homo sapiens RepID=MYH9_HUMAN	100.00%
TRIB3-IP	Tubulin beta chain n=53 Tax=Amniota RepID=TBB5_HUMAN	100.00%
TRIB3-IP	Syntaxin-12 n=5 Tax=Homininae RepID=STX12_HUMAN	98.70%
TRIB3-IP	Potassium voltage-gated channel subfamily C member 2 n=5 Tax=Catarrhini RepID=KCNC2_HUMAN Ubiquitin	100.00%
TRIB3-IP	carboxyl-terminal hydrolase 35 n=4 Tax=Homo sapiens RepID=UBP35_HUMAN	100.00%
TRIB3-IP	Sodium/nucleoside cotransporter 1 n=1 Tax=Homo sapiens RepID=S28A1_HUMAN	99.90%
TRIB3-IP	Annexin A2 n=10 Tax=Catarrhini RepID=ANXA2_HUMAN	100.00%
TRIB3-IP	Dedicator of cytokinesis protein 7 n=1 Tax=Homo sapiens RepID=DOCK7_HUMAN	96.90%
TRIB3-IP	Alpha-actinin-4 n=8 Tax=Catarrhini RepID=ACTN4_HUMAN	100.00%
TRIB3-IP	Reticulocalbin-1 n=4 Tax=Homininae RepID=RCN1_HUMAN	97.00%
TRIB3-IP	Ubiquitin-40S ribosomal protein S27a n=24 Tax=Tetrapoda RepID=RS27A_HUMAN	99.80%
TRIB3-IP	Keratin 1 n=1 Tax=Homo sapiens RepID=H6VRG3_HUMAN	100.00%
TRIB3-IP	Tenascin-X n=4 Tax=Homo sapiens RepID=TENX_HUMAN	100.00%
TRIB3-IP	Putative ankyrin repeat domain-containing protein 31 n=1 Tax=Homo sapiens RepID=ANR31_HUMAN WW domain-	100.00%
TRIB3-IP	binding protein 11 n=2 Tax=Homo sapiens RepID=WBP11_HUMAN	100.00%
TRIB3-IP	Heat shock cognate 71 kDa protein n=44 Tax=Tetrapoda RepID=HSP7C_HUMAN	100.00%
TRIB3-IP	TBC1 domain family member 28 n=3 Tax=Homo sapiens RepID=TBC28_HUMAN	100.00%
TRIB3-IP	Relaxin-3 receptor 1 n=1 Tax=Homo sapiens RepID=RL3R1_HUMAN	85.90%
TRIB3-IP	Sodium-dependent neutral amino acid transporter B(0)AT3 n=2 Tax=Homo sapiens RepID=S6A18_HUMAN Filamin-A	99.90%
TRIB3-IP	n=5 Tax=Homo sapiens RepID=FLNA_HUMAN	100.00%
TRIB3-IP	Thioredoxin domain-containing protein 5 n=5 Tax=Homo sapiens RepID=TXND5_HUMAN	100.00%
TRIB3-IP	Sortilin-related receptor n=3 Tax=Homo sapiens RepID=SORL_HUMAN	98.20%
TRIB3-IP	182 kDa tankyrase-1-binding protein n=5 Tax=Homo sapiens RepID=TB182_HUMAN	100.00%
TRIB3-IP	78 kDa glucose-regulated protein n=6 Tax=Catarrhini RepID=GRP78_HUMAN	99.90%
TRIB3-IP	Serum albumin n=1 Tax=Bos taurus RepID=ALBU_BOVIN	100.00%
TRIB3-IP	Serum albumin n=1 Tax=Homo sapiens RepID=ALBU_HUMAN	100.00%
TRIB3-IP	T-cell immunoreceptor with Ig and ITIM domains n=3 Tax=Homo sapiens RepID=TIGIT_HUMAN	97.50%
TRIB3-IP	Low-density lipoprotein receptor-related protein 4 n=1 Tax=Homo sapiens RepID=LRP4_HUMAN	99.80%
TRIB3-IP	Low-density lipoprotein receptor-related protein 4 n=1 Tax=Homo sapiens RepID=LRP4_HUMAN	100.00%
TRIB3-IP	Keratin, type II cytoskeletal 6B n=2 Tax=Euarchontoglires RepID=K2C6B_HUMAN	100.00%
TRIB3-IP	Heat shock protein HSP 90-beta n=3 Tax=Homo sapiens RepID=HS90B_HUMAN	100.00%
TRIB3-IP	Laminin subunit beta-1 n=1 Tax=Homo sapiens RepID=LAMB1_HUMAN	100.00%
TRIB3-IP	Granulins n=8 Tax=Homo sapiens RepID=GRN_HUMAN	89.20%
TRIB3-IP	40S ribosomal protein S7 n=29 Tax=Tetrapoda RepID=RS7_HUMAN	100.00%
TRIB3-IP	Atrial natriuretic peptide-converting enzyme n=4 Tax=Homo sapiens RepID=CORIN_HUMAN	98.70%
TRIB3-IP	Prolow-density lipoprotein receptor-related protein 1 n=1 Tax=Homo sapiens RepID=LRP1_HUMAN	98.50%
TRIB3-IP	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 n=4 Tax=Homo sapiens RepID=RPN1_	90.40%
TRIB3-IP	ALK tyrosine kinase receptor n=2 Tax=Homo sapiens RepID=ALK_HUMAN	100.00%
TRIB3-IP	Keratin, type I cytoskeletal 9 n=2 Tax=Homo sapiens RepID=K1C9_HUMAN	100.00%
TRIB3-IP	POTE ankyrin domain family member E n=2 Tax=Homo sapiens RepID=POTEE_HUMAN Keratin,	89.30%
TRIB3-IP	type II cytoskeletal 2 epidermal n=1 Tax=Homo sapiens RepID=K22E_HUMAN Glycine--tRNA	100.00%
TRIB3-IP	ligase n=3 Tax=Homo sapiens RepID=SYG_HUMAN	99.90%

TRIB3-IP	B-cell receptor-associated protein 31 n=2 Tax=Homo sapiens RepID=BAP31_HUMAN	96.80%
TRIB3-IP	TRIB3_Mouse_Myc_DDK	100.00%
TRIB3-IP	Translational activator GCN1 n=2 Tax=Homo sapiens RepID=GCN1L_HUMAN	99.40%
TRIB3-IP	Vesicle-associated membrane protein 4 n=8 Tax=Catarrhini RepID=VAMP4_HUMAN	100.00%
TRIB3-IP	Keratin, type I cytoskeletal 10 n=1 Tax=Homo sapiens RepID=K1C10_HUMAN	100.00%
TRIB3-IP	Receptor-type tyrosine-protein phosphatase zeta n=1 Tax=Homo sapiens RepID=PTPRZ_HUMAN Hydrocephalus-	91.40%
TRIB3-IP	inducing protein homolog n=4 Tax=Homo sapiens RepID=HYDIN_HUMAN	100.00%
TRIB3-IP	POTE ankyrin domain family member J n=1 Tax=Homo sapiens RepID=POTEJ_HUMAN	23.80%
TRIB3-IP	Heat shock protein HSP 90-alpha n=19 Tax=Tetrapoda RepID=HS90A_HUMAN	96.00%
TRIB3-IP	Histone-lysine N-methyltransferase ASH1L n=1 Tax=Homo sapiens RepID=ASH1L_HUMAN	95.80%
TRIB3-IP	Eukaryotic initiation factor 4A-I n=22 Tax=Boreoeutheria RepID=IF4A1_HUMAN	99.30%
TRIB3-IP	60 kDa heat shock protein, mitochondrial n=2 Tax=Homo sapiens RepID=CH60_HUMAN	100.00%
TRIB3-IP	Vimentin n=4 Tax=Homininae RepID=VIME_HUMAN	100.00%
TRIB3-IP	Pyruvate kinase PKM n=5 Tax=Homininae RepID=KPYM_HUMAN	100.00%
TRIB3-IP	Pyruvate kinase PKM n=5 Tax=Homininae RepID=KPYM_HUMAN	100.00%
TRIB3-IP	Pyruvate kinase PKM n=5 Tax=Homininae RepID=KPYM_HUMAN	100.00%
TRIB3-IP	Epidermal growth factor receptor n=4 Tax=Homo sapiens RepID=EGFR_HUMAN	98.10%
TRIB3-IP	Keratin, type I cytoskeletal 14 n=1 Tax=Homo sapiens RepID=K1C14_HUMAN	100.00%
TRIB3-IP	KN motif and ankyrin repeat domain-containing protein 2 n=1 Tax=Homo sapiens RepID=KANK2_HUMAN	100.00%
TRIB3-IP	Stress-70 protein, mitochondrial n=1 Tax=Homo sapiens RepID=GRP75_HUMAN	100.00%
TRIB3-IP	Actin, cytoplasmic 1 n=68 Tax=Amniota RepID=ACTB_HUMAN	100.00%
TRIB3-IP	Heterogeneous nuclear ribonucleoprotein K n=10 Tax=Boreoeutheria RepID=HNRPK_HUMAN	94.30%
TRIB3-IP	Calnexin n=7 Tax=Homo sapiens RepID=CALX_HUMAN	99.60%
TRIB3-IP	Elongation factor 1-alpha 1 n=73 Tax=cellular organisms RepID=EF1A1_HUMAN	100.00%
TRIB3-IP	Keratin, type II cytoskeletal 5 n=2 Tax=Homo sapiens RepID=K2C5_HUMAN	99.90%
TRIB3-IP	Tubulin alpha-1A chain n=19 Tax=Tetrapoda RepID=TBA1A_HUMAN	100.00%
TRIB3-IP	Class IVb beta tubulin n=1 Tax=Homo sapiens RepID=Q8IWP6_HUMAN	100.00%
TRIB3-IP	Tubulin beta-2A chain n=12 Tax=Mammalia RepID=TBB2A_HUMAN	100.00%
TRIB3-IP	Ras suppressor protein 1 n=1 Tax=Homo sapiens RepID=RSU1_HUMAN	97.80%
TRIB3-IP	Alpha-enolase n=6 Tax=Hominidae RepID=ENOA_HUMAN	100.00%
TRIB3-IP	ADP-sugar pyrophosphatase n=1 Tax=Homo sapiens RepID=NUDT5_HUMAN	92.70%
TRIB3-IP	Annexin A1 n=4 Tax=Homininae RepID=ANXA1_HUMAN	100.00%
TRIB3-IP	Glyceraldehyde-3-phosphate dehydrogenase n=11 Tax=Catarrhini RepID=G3P_HUMAN Glyceraldehyde-3-	100.00%
TRIB3-IP	phosphate dehydrogenase n=11 Tax=Catarrhini RepID=G3P_HUMAN	100.00%
TRIB3-IP	T-complex protein 1 subunit beta n=12 Tax=Catarrhini RepID=TCPB_HUMAN	98.50%
TRIB3-IP	60S acidic ribosomal protein P0 n=3 Tax=Hominidae RepID=RLA0_HUMAN	99.30%
TRIB3-IP	Nuclease-sensitive element-binding protein 1 n=5 Tax=Boreoeutheria RepID=YBOX1_HUMAN	100.00%
TRIB3-IP	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochond	95.80%
TRIB3-IP	HLA class I histocompatibility antigen, B-54 alpha chain n=13 Tax=Homo sapiens RepID=1B54_HUMAN ATP	91.20%
TRIB3-IP	synthase subunit alpha, mitochondrial n=7 Tax=Homininae RepID=ATPA_HUMAN	100.00%
TRIB3-IP	Elongation factor Tu, mitochondrial n=2 Tax=Homininae RepID=EFTU_HUMAN	99.00%
TRIB3-IP	Poly(rC)-binding protein 2 n=3 Tax=Theria RepID=PCBP2_HUMAN	86.90%
TRIB3-IP	14-3-3 protein theta n=24 Tax=Theria RepID=1433T_HUMAN	98.80%
TRIB3-IP	Myosin regulatory light chain 12B n=21 Tax=Theria RepID=ML12B_HUMAN	92.10%
TRIB3-IP	Peptidyl-prolyl cis-trans isomerase A n=19 Tax=Catarrhini RepID=PPIA_HUMAN	90.00%

Supplementary Figure 2. Muller Mio-M1 cells were transfected with plasmid expressing the TRIB3-DDK (Tag) cDNA**. A**: IP reaction was performed using TRIB3 antibody (Table S. 1). TRIB3 detection is shown on western blot membrane after transferring cellular protein extract. **B**: List of the TRIB3 binding partners


A
Control
Diabetes
TRIB3
TRIB3+DAPI
ONL
INL
RGC
TRIB3
TRIB3+DAPI
ONL
INL
RGC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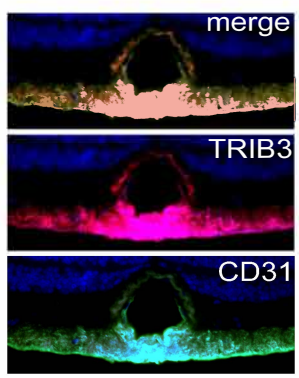
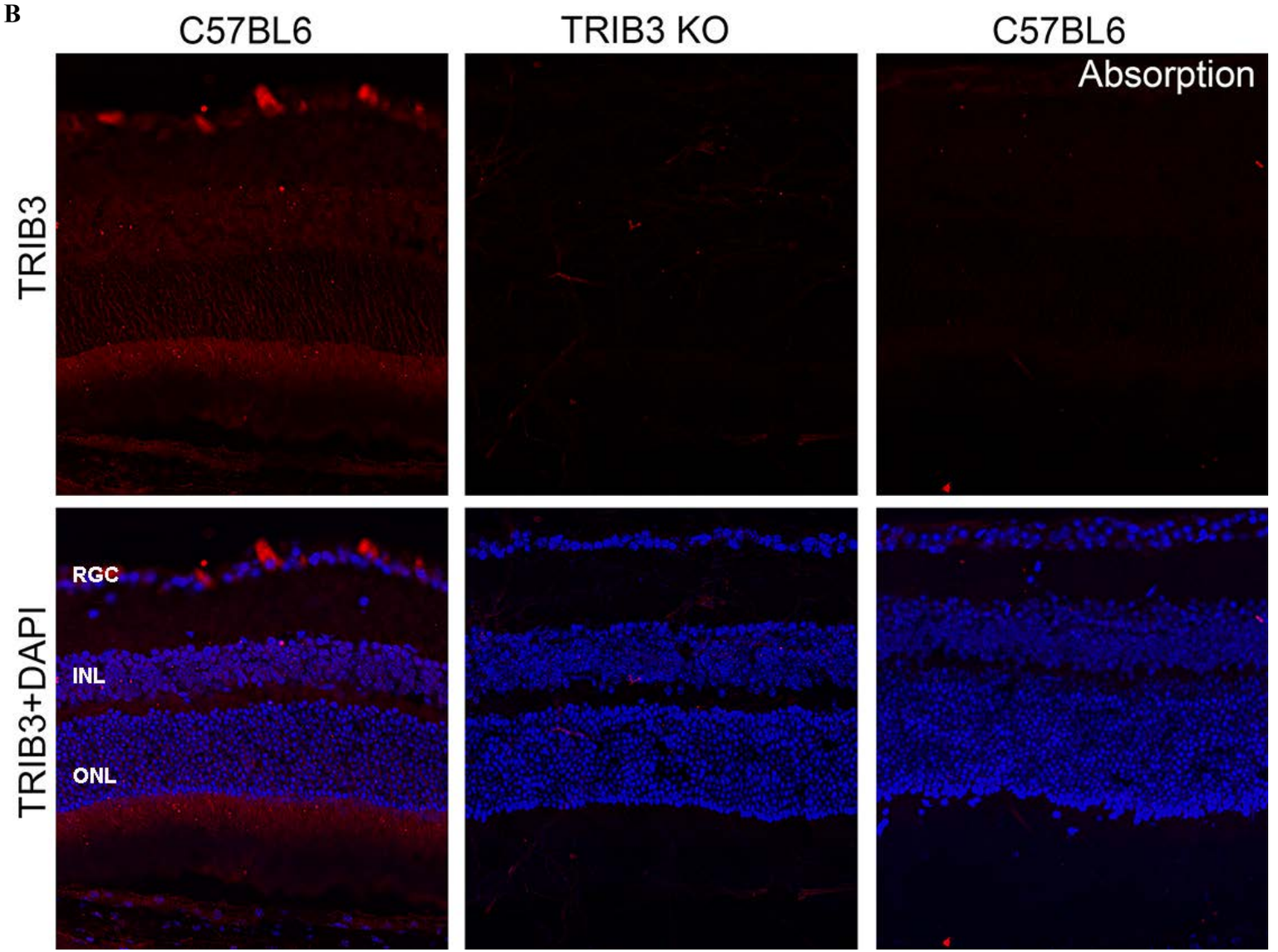
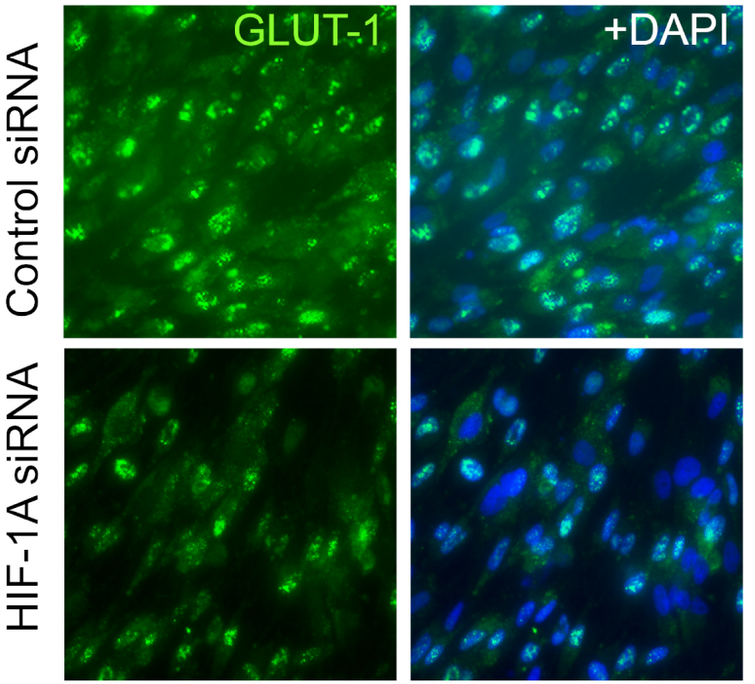
Fluorescence in a.u.
80
60
40
20
0
Control
Diabetes

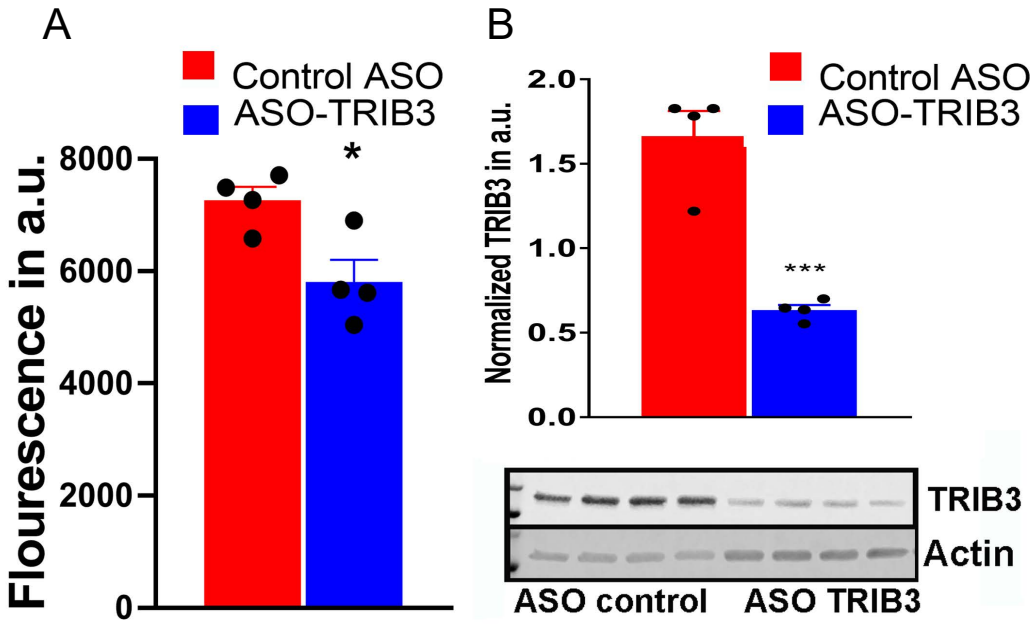

merge
TRIB3
CD31



Supplementary Figure 3. TRIB3 expression in human retinas. A: Human retinas from five diabetic patients and five control individuals were subjected to immunohistochemical analysis with anti-TRIB3 antibody. The diabetic group included the retinas of 62, 62, 82, 84, and 92-year-old patients, while the control group included the retinas of 66, 77, 77, 78, and 82-year-old individuals. Immunoreactivity was calculated using Image J program. Over 3-fold increase in TRIB3 signal was detected in diabetic group vs control retinas. Bottom: In human diabetic retinas, TRIB3 signal was co-localized with CD31 staining showing hypertrophic blood vessels. B: Absorption method was used to validate the antibody against TRIB3. Human recombinant TRIB3 protein was incubated with anti-TRIB3 antibody overnight. The mixture then was applied on mouse retinal cryosections. Secondary antibody was used to detect signal. TRIB3 immunoreactivity was detected in C57BL6 retinas.



Supplementary Figure 4. Representative images of MIO-M1 cells transfected with control and Hif1a siRNA. Detection of GLUT1 (in green) was achieved performing IHC analysis and confocal microscopy. Staining with DAPI (in blue) was used to detect nuclei.



Supplemental Figure 5. MIO-M1 cells overexpressing mouse TRIB3-DDK were treated with three micrograms of ASO-control and ASO-TRIB3 (mouse) synthesized by Sigma Aldrich[a]. Twenty-four hours after the co-transfection, Muller cells were exposed to hypoxia chamber for 48 hours. Glucose uptake was measured as described in the materials and method section. In addition, TRIB3 protein levels were measured in both groups. Here, we found that 21% reduction in 2-NBDG uptake (S. 5A; n=7) correlates with 60% knockdown of TRIB3 protein by ASO-TRIB3 (S.5B; n=4).

a. Weismann, D. et al. Knockdown of the gene encoding Drosophila tribbles homologue 3 (Trib3) improves insulin sensitivity through peroxisome proliferator-activated receptor-gamma (PPAR-gamma) activation in a rat model of insulin resistance. Diabetologia 54, 935-944, doi:10.1007/s00125-010-1984-5 (2011).

ASO-control [mC]*[mC]*[mT]*[mT]*[mC]*C*C*T*G*A*A*G*G*T*T*[mC]*[mC]*[mT]*[mC]*[mC]
ASO-TRIB3 [mG]*[mT]*[mC]*[mC]*[mA]*G*T*C*A*T*C*A*C*G*C*[mA]*[mG]*[mG]*[mC]*[mA]