

Supplemental table legends

Supplemental Table S1. GLP-1R interactors revealed by AP-MS in CHO cells.

Supplemental Table S2. GLP-1R interactors revealed by AP-MS in MIN6 cells.

Supplemental Table S3. Primer sequences for qPCR.

Supplemental Table S4. Protein abundance estimation of 4 potential GLP-1R interactors in CHO and MIN6 cells in liganded and unliganded states by using emPAI score normalized to bait protein (GLP-1R). All values represent the emPAI score of interactors normalized to GLP-1R identified in the same run.

Supplemental Table S5. Proteins and peptides identification by mass spectrometry (Separate EXCEL file).

Supplemental Table S6. emPAI score of all potential GLP-1R interactors identified by AP-MS in CHO and MIN6 cells (Separate EXCEL file).

Supplemental Table S1.

NCBI accession number	Protein name	Gene Name	Unliganded GLP-1R interactors	Liganded GLP-1R interactors	Subcellular location	Functions
354503280	PREDICTED: annexin A2-like, partial [Cricetulus griseus]	ANXA2		✓	Secreted, extracellular	Calcium binding.
354498588	PREDICTED: dnaJ homolog subfamily A member 2-like [Cricetulus griseus]	DNAJA2	✓	✓	Membrane	Cell proliferation and protein folding.
354497358	PREDICTED: peptidyl-prolyl cis-trans isomerase FKBP11-like [Cricetulus griseus]	FKBP11	✓		Membrane	Protein folding.
354488169	PREDICTED: ras-related protein Rab-5B-like [Cricetulus griseus]	RAB5B	✓	✓	Cell membrane, endosome	Protein transport.
354471953	PREDICTED: vesicular integral-membrane protein VIP36-like [Cricetulus griseus]	LMAN2	✓		Golgi apparatus membrane, ER membrane	Protein transport.
354477232	PREDICTED: caveolin-1-like [Cricetulus griseus]	CAV1	✓	✓	Golgi membrane, cell membrane	Endocytosis and protein localization.
354485034	PREDICTED: ras-related protein Rab-5C-like [Cricetulus griseus]	RAB5C	✓	✓	Cell membrane	Protein transport.
354467387	PREDICTED: ADP-ribosylation factor 4-like [Cricetulus griseus]	ARF4	✓	✓	Golgi apparatus	GTP-binding protein.
354494420	PREDICTED: protein RER1-like [Cricetulus griseus]	RER1		✓	Golgi apparatus	Retrograde vesicle-mediated transport.
354502651	PREDICTED: 14-3-3 protein theta-like [Cricetulus griseus]	YWHAQ	✓	✓	Cytoplasm	Adapter protein.
354478978	PREDICTED: protein S100-A6-like [Cricetulus griseus]	S100A6	✓	✓	Cytoplasm, nucleus, Plasma membrane	Calcium signaling.
354503516	PREDICTED: transmembrane protein 33-like [Cricetulus griseus]	TMEM33	✓	✓	Plasma membrane	Transport.
354498400	PREDICTED: ephrin type-A receptor 2-like [Cricetulus griseus]	EPHA2	✓	✓	Plasma membrane	Receptor tyrosine kinase.
346227187	guanine nucleotide-binding protein G(i) subunit alpha-2 [Cricetulus griseus]	GNAI2	✓	✓	Plasma membrane, Cytoplasm	Guanine nucleotide-binding protein.
354498129	PREDICTED: guanine nucleotide-binding protein G(l)/G(S)/G(O) subunit gamma-12-like [Cricetulus griseus]	GNG12	✓	✓	Plasma membrane, Cytoplasm	Guanine nucleotide-binding protein.
350539723	guanine nucleotide-binding protein G(l)/G(S)/G(T) subunit beta-1 [Cricetulus griseus]	GNB1	✓	✓	Plasma membrane, Cytoplasm	Guanine nucleotide-binding protein.
346227195	guanine nucleotide-binding protein G(k) subunit alpha [Cricetulus griseus]	GNAI3	✓	✓	Plasma membrane, Cytoplasm	Guanine nucleotide-binding protein.
350538021	matrix metalloproteinase-14 [Cricetulus griseus]	MMP14	✓	✓	Plasma membrane	Activate progelatinase A.
354475732	PREDICTED: membrane-associated	PGRMC1		✓	Plasma membrane	Receptor for

	progesterone receptor component 1-like [Cricetulus griseus]					progesterone.
354507141	PREDICTED: protein NDRG1 [Cricetulus griseus]	NDRG1	✓	✓	Plasma membrane, Cytoplasm	Stress-responsive protein.
354473456	PREDICTED: ras-related protein Rab-18-like [Cricetulus griseus]	RAB18	✓	✓	Plasma membrane	Protein transport.
354503613	PREDICTED: ras-related protein Rab-34-like isoform 1 [Cricetulus griseus]	RAB34	✓	✓	Cytoplasm	GTP binding protein.
354476904	PREDICTED: sodium/potassium-transporting ATPase subunit alpha-1 [Cricetulus griseus]	ATP1A1	✓	✓	Plasma membrane	ATP biosynthetic process.
354481282	PREDICTED: transmembrane protein Tmp21-like [Cricetulus griseus]	TMED10	✓	✓	Plasma membrane, Cytoplasmic vesicle	Vesicular protein trafficking.
354472663	PREDICTED: voltage-dependent anion-selective channel protein 1-like, partial [Cricetulus griseus]	VDAC1	✓	✓	Plasma membrane, mitochondrial membrane	Cell apoptosis.
350537945	peroxiredoxin-1 [Cricetulus griseus]	PRDX1	✓	✓	Cytoplasm	Redox regulation.
354469527	PREDICTED: cullin-associated NEDD8-dissociated protein 1-like [Cricetulus griseus]	CAND1	✓	✓	Cytoplasm. Nucleus.	Cell differentiation.
354476229	PREDICTED: coatamer subunit alpha isoform 1 [Cricetulus griseus]	COPA	✓	✓	Cytoplasm, vesicle	Vesicle-mediated transport.
354466134	PREDICTED: coatamer subunit beta~ [Cricetulus griseus]	COPB2	✓	✓	Cytoplasm, vesicle	Vesicle-mediated transport.
354488599	PREDICTED: coatamer subunit beta [Cricetulus griseus]	COPB1	✓	✓	Cytoplasm, vesicle	Vesicle-mediated transport.

Supplemental Table S2.

NCBI accession number	Protein name	Gene Name	Unliganded GLP-1R interactors	Liganded GLP-1R interactors	Subcellular location	Functions
33468885	calcyclin binding protein	Cacybp	✓	✓	Cytoplasm	Cellular response to calcium ion.
6755963	voltage-dependent anion channel 1	Vdac1	✓	✓	Mitochondrion membrane. Cell membrane	Ion transport
226823359	coatamer protein complex subunit alpha	Copa	✓	✓	Cytoplasma, Golgi membrane, cytoplasmic vesicle	ER-Golgi transport, protein transport
6754012	guanine nucleotide binding protein, alpha O	Gnao1	✓	✓	Cytosol, cell membrane	Guanine nucleotide-binding protein.
28916687	Rab5b protein	Rab5b	✓	✓	Cell membrane, Endosome	Protein transport.
62241017	ATP-binding cassette, sub-family C (CFTR/MRP), member 8	Abcc8	✓	✓	Cell membrane	Regulation of insulin secretion.
29789080	coatamer protein complex, subunit beta 2 (beta prime)	Copb2	✓	✓	Cytoplasma, Golgi membrane, cytoplasmic vesicle	ER-Golgi transport
113866024	Rab5c protein	Rab5c	✓	✓	Membrane	Protein transport
33563256	guanine nucleotide binding protein (G protein), alpha inhibiting 3	Gnai3	✓	✓	Cytosol, cell membrane	Guanine nucleotide-binding protein.
41054806	guanine nucleotide binding protein (G protein), alpha inhibiting 2	Gnai2	✓	✓	Cytosol, cell membrane	Guanine nucleotide-binding protein.
117959930	Guanine nucleotide-binding protein G(s) subunit alpha isoforms XLas	Gnas	✓	✓	Cytosol, cell membrane	Guanine nucleotide-binding protein.
153792526	ADP-ribosylation factor 1	Arl1	✓	✓	Golgi apparatus membrane	GTP-binding protein.
18390323	Ras-related protein Rab-14	Rab14	✓	✓	Endosome	Protein transport.
21312062	transmembrane emp24-like trafficking protein 10	Tmed10	✓	✓	Membrane	Vesicular protein trafficking.
21703854	dehydrogenase/reductase (SDR family) member 7B	Dhrs7b	✓	✓	Peroxisome membrane	Putative oxidoreductase
22267448	transmembrane protein 33	Tmem33	✓	✓	Membrane	Unknown.
21704066	RAS-related protein-1a	Rap1a	✓	✓	Cell membrane	Rap protein signal transduction.
30841008	Ras-related protein Rab-18	Rab18	✓	✓	Cell membrane	Protein transport.
224994186	gamma-glutamyltransferase 7	Ggt7	✓	✓	Membrane	Cleaves glutathione conjugates.
226442772	progesterone receptor membrane component 2	Pgrmc2	✓	✓	Membrane	Receptor for steroids
6753138	ATPase, Na ⁺ /K ⁺ transporting, beta 1 polypeptide	Atp1b1	✓	✓	Cell membrane	Cell adhesion.
31560774	Ras-related protein Rab-34	Rab34	✓	✓	Cytoplasm, Golgi	Cell adhesion.
226693367	glucosidase, alpha, acid	Gaa	✓	✓	Lysosome	Glucose metabolic process.
13385434	Multifunctional protein ADE2	Paics	✓	✓	Cytoplasm	Purine biosynthesis.
20070422	PRA1 family protein 2	Praf2	✓	✓	Endosome	ER/Golgi transport and vesicular traffic.

13384618	guanine nucleotide binding protein (G protein), gamma 12	Gng12	✓	✓	Cytosol, cell membrane	Guanine nucleotide-binding protein.
23956096	USO1 homolog, vesicle docking protein	Uso1	✓	✓	Cytoplasm, Golgi	ER-Golgi transport.
15277331	FK506 binding protein 11	Fkbp11	✓	✓	Membrane	Protein synthesis.
112817607	thioredoxin-related transmembrane protein 4	Tmx4	✓	✓	Membrane	Redox-active center
13384696	keratinocyte associated protein 2	Krtcap2	✓	✓	membrane	Protein N-linked glycosylation.
145966911	transmembrane emp24 protein transport domain containing 9	Tmed9	✓	✓	Membrane	Vesicular protein trafficking.
15426055	coatomer protein complex, subunit beta 1	Copb1	✓	✓	Cytoplasm, Golgi membrane, cytoplasm vesicle	ER-Golgi transport
148747410	Coatomer subunit delta	Arcn1	✓	✓	Cytoplasm	ER-Golgi transport
115511018	ubiquitin specific peptidase 9, X chromosome	Usp9x	✓	✓	Cytoplasm	Protein deubiquitination.
13385392	N-ethylmaleimide sensitive fusion protein attachment protein alpha	Napa	✓	✓	Membrane	Vesicular transport.
6680045	guanine nucleotide binding protein (G protein), beta 1	Gnb1	✓	✓	Cytosol, cell membrane	Guanine nucleotide-binding protein.
6755588	synaptosomal-associated protein 25	Snap25	✓	✓	Cytoplasm, cell membrane	Neurotransmitter secretion
127139140	tripartite motif-containing 21; similar to Tripartite motif protein 21	Trim21	✓	✓	Cytoplasm. Nucleus.	Protein ubiquitination.
226958349	triosephosphate isomerase 1	Tpi1	✓	✓	Cytoplasm	Gluconeogenesis and glycolysis.
62234487	ATPase, Ca ⁺⁺ transporting, plasma membrane 1	Atp2b1	✓	✓	Cell membrane	Calcium transport.
11596855	transferrin receptor	Tfrc	✓	✓	Cell membrane	Cellular uptake of iron.
189409138	cullin associated and neddylation disassociated 1	Cand1	✓	✓	Cytoplasm. Nucleus.	Cell differentiation.
8567338	coatomer protein complex, subunit gamma	Copg	✓	✓	Cytoplasm, Golgi membrane, cytoplasm vesicle	ER-Golgi transport.
6680716	ADP-ribosylation factor 1	Arf1	✓	✓	Cytoplasm, Golgi	GTP-binding protein.
30794386	translocating chain-associating membrane protein 1	Tram1	✓	✓	Cytoplasm, Nucleus	Transcription regulation.
6756039	14-3-3 protein theta	YWHAQ	✓	✓	Cytoplasm	Adapter protein.
19526878	pyrroline-5-carboxylate reductase family, member 2	Pycr2	✓	✓	Membrane	Proline biosynthesis.
21617845	adipocyte-related X-chromosome expressed sequence 1	Arxes1	✓	✓	ER membrane	Unknown.
31543940	vesicle-associated membrane protein, associated protein B and C	Vapb	✓	✓	Cytoplasm	ER unfolded protein response.
51093867	carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase	Cad	✓	✓	Cytoplasm	Pyrimidine biosynthesis.
34538601	Cytochrome c oxidase subunit 2	COX2	✓		Cytoplasm, Golgi	Fatty acid biosynthesis.

224809376	nicastrin	Ncstn	✓		Membrane	Membrane protein proteolysis.
103472025	cleft lip and palate associated transmembrane protein 1	Clptm1	✓		Cytoplasm, membrane	T-cell development.
23956140	glycerophosphodiester phosphodiesterase domain containing 1	Gdpd1	✓		Golgi membrane, cytoplasmic vesicle	Glycerol metabolic process.
30424655	Ras-related protein Rab-6B	Rab6b	✓		Cytoplasmic vesicle	Protein transport.
6679229	proprotein convertase subtilisin/kexin type 2	Pcsk2	✓		Cell membrane	Protein autoprocessing.
9506541	diacylglycerol kinase, epsilon	Dgke	✓		Secreted	Intracellular signal transduction.
51317387	cyclin G associated kinase	Gak	✓		Cytoplasm	Cell cycle.
8394027	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), alpha isoform	Ppp2r1a	✓		Membrane	Chromosome segregation.
21361250	ATPase, H ⁺ transporting, lysosomal accessory protein 2	Atp6ap2	✓		Peroxisome membrane;Cytosol	Renin receptor.
60218877	ATP-binding cassette, sub-family D (ALD), member 3	Abcd3	✓		Membrane	Transport.
70778812	transmembrane protein 65	Tmem65	✓		Apical cell membrane;	Unknown.
238637277	solute carrier family 3 member 2	Slc3a2	✓		Membrane	Carbohydrate metabolic process
45597453	solute carrier family 27, member 4	Slc27a4	✓		Cytoplasm	Transmembrane transport.
85701756	DNA segment, Chr 10, Brigham & Women's Genetics 1379 expressed	D10Bwg1379e	✓		Membrane	Vesicle mediate transport
6754954	sequestosome 1	Sqstm1		✓	Cytoplasm, endosome	Apoptosis
29825825	hypothetical protein LOC100043986	C630004H02Rik		✓	Predicted	Protein transport
30520239	arylacetamide deacetylase-like 1	Nceh1		✓	Membrane	Lipid metabolism
161016826	receptor accessory protein 5	Reep5		✓	Membrane	Surface expression of receptors
94721328	vesicle-associated membrane protein, associated protein A	Vapa		✓	ER membrane	Vesicle trafficking.
10946644	glucose-6-phosphatase, catalytic, 2	G6pc2		✓	ER membrane	Glucose production.
70794816	enolase 1, alpha non-neuron	Eno1		✓	unknown	Unknown.
13128964	Ras-related protein Rab-27A	Rab27a		✓	Membrane, lysosome	Exocytosis.
27229185	transmembrane 9 superfamily member 1	Tm9sf1		✓	Lysosome membrane; cytoplasmic vesicle	Autophagy.
13385374	Ras-related protein Rab-5A	Rab5a		✓	Cell membrane, Early endosome membrane	Endocytosis.
31980806	progesterone receptor membrane component 1	PGRMC1		✓	Membrane	Receptor for progesterone.
162138894	phenylalanyl-tRNA synthetase, alpha subunit	Farsa		✓	Cytoplasm	Protein biosynthesis.
7305085	glutamine fructose-6-phosphate transaminase 1	Gfpt1		✓	Cytoplasm	Carbohydrate biosynthetic process.
34610207	alanyl-tRNA synthetase	Aars		✓	Cytoplasm	Protein biosynthesis.

158937258	Yip1 interacting factor homolog B	Yif1b	✓	Membrane	Vesicular trafficking.
149248877	Predicted gene 2890	Gm2890	✓	Unknown	Unknown.
31324569	Neurocalcin delta	Ncald	✓	Cytoplasm	Calcium-mediated signaling.
21312406	ADP-dependent glucokinase	Adpgk	✓	Secreted	Glycolysis.
33859498	Uridine monophosphate synthetase	Umps	✓	Cytoplasm, nucleus	Pyrimidine biosynthesis.
6753320	chaperonin containing Tcp1, subunit 3 (gamma)	Cct3	✓	Cytoplasm	Molecular chaperone.
31981588	ATPase, H ⁺ transporting, lysosomal V1 subunit H	Atp6v1h	✓	Cytosol, membrane	Transport.

Supplemental Table S3.

Gene name	Sequence
PGRMC1	Forward 5'- TTGGCCTCTGCATCTTCCTG-3' Reverse 5'-TCGTATCGCCTTAGTTCGGC-3'
Beta-Actin	Forward 5'-CACCCGCGAGTACAACCTTC-3' Reverse 5'-CCCATACCCACCATCACACC-3'
KCNJ11	Forward 5'- AGTGTGGCTGTGGCAAAGG-3' Reverse 5'-GGACCGCAACTCAGGACAA-3'

Supplemental Table S4.

Interactors	Min6			CHO			Min6			CHO		
	Unligand-run1	Unligand-run2	Unligand-run3	Unligand-run1	Unligand-run2	Unligand-run3	Ligand-run1	Ligand-run2	Ligand-run3	Ligand-run1	Ligand-run2	Ligand-run3
PGRMC1^A	0	0	0	0.952380952			2.571428571	2.714285714			3.333333333	3.333333333
Rab5b	2.545454545	4		0.380952381	2.833333333	1		4	4	9.333333333		4.666666667
Rab5c	2.590909091	4.071428571		0.833333333	1.333333333	1.029411765	8.142857143	5.857142857	5.857142857	5.833333333	2.916666667	2.916666667
Tmed10	1.5	3.785714286	7.571428571	0.357		0.441176471	4.714285714	2.357142857	2.357142857	2		1.25

A. Because no PGRMC1 protein was identified in Min6 unliganded runs, the missing values are set as 0.