

Supplemental Table S1. Summary of GPR126 446-807 domain specific pulldown partners from sciatic nerve.

Peptide number	Gene name	Protein description	localization
18	HS90B	Heat shock protein HSP 90-beta	cytoplasm
10	IgG1	Ig heavy chain V region	
10	ENPL	Endoplasmin	Endoplasmic reticulum
8	KPYM	Pyruvate kinase PKM	Cytoplasm Nucleus
8	FUT8	Alpha-(1,6)-fucosyltransferase	Golgi
5	HS90A	Heat shock protein HSP 90-alpha	cytoplasm
5	IgG2a	Ig gamma-2A chain C region	
5	NID2	Nidogen-2	Basement membrane Extracellular matrix Secreted
5	Q9Z1A1	Evidence at transcript level	
4	GPR126	G protein coupled receptor 126	membrane
4	EF2	Elongation factor 2	nucleus
4	FINC	Fibronectin	Extracellular matrix Secreted
4	FBLN5	Fibulin-5	Secreted
4	SERA	D-3-phosphoglycerate dehydrogenase	
4	ACLY	Acly protein	mitochondrion
4	TCPA	T-complex protein 1 subunit alpha	Cytoplasm Cytoskeleton
3	ACTS	Actin, alpha skeletal muscle	Cytoplasm Cytoskeleton Basement membrane
3	LAMA2	Laminin subunit alpha-2	Extracellular matrix Secreted
3	CO6A1	Collagen alpha-1(VI) chain	Extracellular matrix Secreted
3	CN37	2',3'-cyclic-nucleotide 3'-phosphodiesterase	Membrane
3	PCNA	Proliferating cell nuclear antigen	Nucleus
3	ECHA	Trifunctional enzyme subunit alpha, mitochondrial	mitochondrial
3	BACH	Transcription regulator protein BACH1	Nucleus
3	NONO	Non-POU domain-containing octamer-binding protein	Nucleus

Protocol for sciatic nerve co-IP:

Step I: Cell Lysate Preparation:

1. collect the SN in an Eppendorf and with a plastic pestle (http://www.fishersci.com/ecom/servlet/fsproductdetail_10652_617322_-1_0) on liquid nitrogen to reduce them as a powder.
2. add 300ul of ice cold RAPI Buffer containing protease inhibitors, lyse cells with a cell disruptor for 10' and homogenization with syringe needle 26 G.
- 3.. Centrifuge the cell lysate at 10,000xg for 10minutes at 4°C.
3. Carefully collect the supernatant without disturbing the pellet and transfer to a clean tube. The Pellet can be discarded
- 4.The protein concentration can be determined by Bradford assay.

Step II: Cell Lysate Preclearing

1. preclearing cell lysate with 100ul protein G beads (from Invitrogen) equilibrated in the corresponding IP buffer/Lysis Buffer.

Step III: Immunoprecipitation

1. Incubation 250ug (in 300ul) of precleared cell lysate 3 hours with 2ug of Fc or Gpr126-hFc recombinant proteins followed by 1h with 40 ul of protein G sepharose beads (GE Healthcare 17-0618-01)
2. Centrifuge the tube at 2,500xg for 30 seconds at 4°C.
3. Carefully discard the supernatant completely. Wash the beads four times with 900μL of ice-cold Lysis Buffer, one time with TBS buffer. Centrifuge to pellet the beads in between each wash. In order to minimize background, remove the supernatant completely after each wash.
5. After the last wash, carefully aspirate the supernatant and add 50μL 1X Laemmli loading buffer to the bead pellet.
5. Vortex and heat at 90-100°C for 5 minutes.
6. Centrifuge at 10,000xg for 1 minutes to pellet the beads. Collect the supernatant carefully and load onto an SDS-PAGE gel.
7. Transfer to membrane, followed by western blot analysis.

Protocol for sciatic nerve lysing and GPR126-mFc-biotag pull- down:

Step I: Cell Lysate Preparation:

1. collect the SN in an Eppendorf and with a plastic pestle (http://www.fishersci.com/ecom/servlet/fsproductdetail_10652_617322_-1_0) on liquid nitrogen to reduce them as a powder.
2. add 300ul of ice cold RAPI Buffer containing protease inhibitors, lyse cells with a cell disruptor for 10' and homogenization with syringe needle 26 G.
- 3.. Centrifuge the cell lysate at 10,000xg for 10minutes at 4°C.
3. Carefully collect the supernatant without disturbing the pellet and transfer to a clean tube. The Pellet can be discarded
- 4.The protein concentration can be determined by Bradford assay.

Step II: Cell Lysate Preclearing

1. preclearing cell lysate with 300ul streptavidin beads, equilibrated in the corresponding IP buffer/Lysis Buffer.

Step III: in vitro biotinylation

- 1 part Biotin: 1ul
- 1 part Biomix A: 1ul
- 1 part Biomix B: 1ul
- 6 parts substrate solution: 6ul (1ug/ul)
- BirA enzyme 1 ul (1ug/ul)
- Incubate at RM for overnight

BirA	Biotin-Protein	Ligase	Cat. BIRA-500	MSPB-BIRA500
Standard Reaction Kit				

Delivery information: Standard Reaction Kit includes: 2 vials of 20 µl each 1mg/ml BirA biotin-protein ligase (40 µg total); 2 vials of 1.5 ml each BiomixA (0.5 M bicine buffer solution, pH 8.3); 2 vials of 1.5 ml each BiomixB (100 mM ATP, 100 mM Mg(OAc)₂, 500 µM d-biotin); 2 vials of 1.5 ml each additional BIO-200 (500 µM d-biotin).

Step IV: Immunoprecipitation

1. Incubation precleared cell lysate 3 hours with 4ug of in vitro biotinylated **GPR56-mFc-biotag protein, or mFc-biotag as negative control**, followed by 1h with 100 ul of Streptavidin beads (Sigma)
- 2.Centrifuge the tube at 2,500xg for 30 seconds at 4°C.
- 3.Carefully remove the supernatant completely. Wash the beads four times with 900µL of ice-cold Lysis Buffer, one time (final wash) with TBS buffer. Centrifuge to pellet the beads in between each wash. In order to minimize background, care should be given to remove the supernatant completely after each wash.
- 5.After the last wash, carefully aspirate the supernatant and add 60µL of 1X Laemmli sample buffer to the bead pellet.
5. Vortex and heat at 90-100°C for 5 minutes.

6. Centrifuge at 10,000xg for 1 minute to pellet the beads. Collect the supernatant carefully and load onto an SDS-PAGE gel. The whole lane was excised and submitted to Taplin Biological Mass Spectrometry Facility, Harvard Medical School.

Step V: Protein Sequence Analysis by LC-MS/MS

At Taplin Biological Mass Spectrometry Facility, Harvard Medical School

The gels were cut into approximately 1 mm³ pieces. Gel pieces were then subjected to a modified in-gel trypsin digestion procedure (1). Gel pieces were washed and dehydrated with acetonitrile for 10 min. followed by removal of acetonitrile. Pieces were then completely dried in a speed-vac. Rehydration of the gel pieces was with 50 mM ammonium bicarbonate solution containing 12.5 ng/μl modified sequencing-grade trypsin (Promega, Madison, WI) at 4°C. After 45 min., the excess trypsin solution was removed and replaced with 50 mM ammonium bicarbonate solution to just cover the gel pieces. Samples were then placed in a 37°C room overnight. Peptides were later extracted by removing the ammonium bicarbonate solution, followed by one wash with a solution containing 50% acetonitrile and 1% formic acid. The extracts were then dried in a speed-vac (~1 hr). The samples were then stored at 4°C until analysis.

On the day of analysis the samples were reconstituted in 5 - 10 μl of HPLC solvent A (2.5% acetonitrile, 0.1% formic acid). A nano-scale reverse-phase HPLC capillary column was created by packing 2.6 μm C18 spherical silica beads into a fused silica capillary (100 μm inner diameter x ~30 cm length) with a flame-drawn tip (2). After equilibrating the column each sample was loaded via a Famos auto sampler (LC Packings, San Francisco CA) onto the column. A gradient was formed and peptides were eluted with increasing concentrations of solvent B (97.5% acetonitrile, 0.1% formic acid).

As peptides eluted they were subjected to electrospray ionization and then entered into an LTQ Orbitrap Velos Pro ion-trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA). Peptides were detected, isolated, and fragmented to produce a tandem mass spectrum of specific fragment ions for each peptide. Peptide sequences (and hence protein identity) were determined by matching protein databases with the acquired fragmentation pattern by the software program, Sequest (Thermo Fisher Scientific, Waltham, MA) (3). All databases include a reversed version of all the sequences.

1. Shevchenko, A.; Wilm, M.; Vorm, O.; Mann, M. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. *Anal Chem.* 1996, 68, 850–858. [https://doi: 10.1021/ac950914h](https://doi.org/10.1021/ac950914h).
2. Peng, J.; Gygi, S.P. Proteomics: The move to mixtures. *J. Mass Spec.* 2001, 36, 1083–1091. [https://doi: 10.1002/jms.229](https://doi.org/10.1002/jms.229).
3. Eng, J.K.; McCormack, A.L.; Yates, J.R. An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. *J Am Soc Mass Spectrom.* 1994, 5, 976–989. [https://doi: 10.1016/1044-0305\(94\)80016-2](https://doi.org/10.1016/1044-0305(94)80016-2).

260 —
160 —
110 —
80 —
60 —
50 —
40 —

20 —

20 —



IB:hFC
5/10/16

GPR126 31-348

GPR126 446-807

hFC vector

260

160

110

80

60

50

40

30

20

IB: Collagen VI

