SUPPLEMENTARY INFORMATION FOR

In vivo mapping of a GPCR interactome using knock-in mice

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Supplementary Materials and Methods

Reagents and drugs

GDP, GTPγS, DPCPX, Poly-L-lysine, mouse monoclonal anti-FLAG M2 antibody (uncoupled or immobilized on magnetic beads), rabbit polyclonal anti-FLAG antibody, the anti-mouse IgG alkaline-phosphatase antibody and the alkaline phosphatase substrate kit were purchased from Sigma-Aldrich (Oakville, ON). Mouse monoclonal anti-Rab10 (clone 4E2) and rabbit polyclonal anti-VPS41 (ABC928) were from EMD Millipore and chicken anti-GFP antibody was from Molecular Probes. ProLong Gold antifade reagent, Lipofectamine 2000, Alexa Fluor 488 goat anti-chicken IgY and Alexa Fluor 647 goat anti-mouse IgG antibodies were purchased from Invitrogen. Alexa Fluor 594 goat anti-rabbit IgG antibody was purchased from ThermoFisher Scientific. Anti-rabbit-HRP IgG and anti-mouse-HRP IgG were purchased from GE Healthcare (Mississauga, ON). The protein G-agarose beads and the rabbit polyclonal anti-GAPDH (FL-335) antibody were obtained from Santa Cruz Biotechnology (Dallas, TX). The rat monoclonal anti-HA-Peroxidase (3F10) antibody was from Roche. The anti-GST polyclonal antibody was from Bethyl Laboratories. [D-Ala2] Deltorphin II (product #28-9-20, lot W01124T1) and [D-Ala2] Deltorphin I (cat.28-9-10, lot U04147T1) were purchased from American peptide or synthesized by the Plateforme de Synthèse de Peptides et de Sondes d'imagerie de l'Université de Sherbrooke. DAMGO was purchased from Tocris (cat.1171, lots 29A and 30G) or synthesized in-house. SNC80 was synthesized and generously provided by Dr Kenner C. Rice (National Institute on Drug Abuse, National Institutes of Health, Bethesda, MD, USA) The iodination of Deltorphin I and DAMGO was carried out using the iodogen method previously described (1).

Mice generation and genotyping

All animals were maintained on a 12 h light/dark cycle (06:00 - 18:00 h) in a temperature-controlled environment (22°C) with free access to water and food. All procedures were approved by the animal care committee of the Université de Sherbrooke (protocol # 315-17; 242-18) and were in accordance with the ethical guidelines of the Canadian Council on animal care and the International Association for the Study of Pain (IASP).

The FLAG- tagged DOPr sequence, the loxP sites and the STOP cassette were cloned into pKO Scrambler DT vector, which allows for both positive (G418) and negative (ganciclovir) selection of ES cells. The construction was confirmed by sequencing and linearized endotoxin-free DNA was purified and electroporated into 129X1/SvJ ES cells. ES cell clones showing homologous recombination by Southern Blot analysis were injected into mouse blastocysts, implanted into pseudopregnant females, and chimeric pups scored for germline transmission of the targeted allele. Homozygous FLAG-DOPr-KO (KO) mice were obtained from HET(KO) x HET(KO) crosses. FLAG-DOPr-KI mice (KI) were obtained by crossing HET(KO) males with Zp3-cre (JAX stock number 003651; http://jaxmice.jax.org/strain/003651.html) females expressing a recombinase allowing the excision of the STOP cassette, and thereby enabling the expression of FLAG-DOPr in all tissues. Homozygous KI mice were obtained by breeding HET(KI) x HET(KI). Both congenic background lines were established by backcrossing KO or KI males with females C567BL/6J mice (Charles River, Qc, Canada) through at least N = 5, or about 15 months.

For genotyping, mice ear clips were collected and incubated at 57ºC O/N in 100 µl of the PCR Buffer with Non-ionic Detergents solution (PBND: 50 mM KCl, 10 mM Tris-HCl pH 8.0, 2.5 mM $MgCl_2 \cdot H_2O$, 0.1 mg/ml gelatin, 0.45% v/v Nonidet P-40 and 0.45% v/v Tween-20) supplemented with 1:25 of a stock solution of 20 mg/ml of Proteinase K previously prepared in 50 mM Tris and 1 mM $CaCl_2$, pH 8.0. Samples were then incubated for 10 min at 95ºC to inactivate Proteinase K and touchdown PCR amplifications (from T_m+10ºC to T_m-5ºC with a gradual reduction of the annealing temperature of 1ºC every cycle) were performed on 2 µl of the supernatants. Sequences of the primers used in PCR amplifications are listed in Table S5. Genomic DNA from ears of KO and KI mice was also sequenced by the RNomics Platform at the Université de Sherbrooke (Qc, Canada).

Saturation binding assays

The protocol for preparation of brain membrane extracts was adapted from the previously described method (2). Briefly, forebrains from WT, KO and KI mice were harvested and homogenized with ice-cold 50 mM Tris buffer pH 7.4 supplemented with EDTA-free cOmplete™ protease inhibitor cocktail tablets (Buffer A) using a Polytron

device (3 x 15 s), then a Dounce homogenizer (3 x 5 strokes). The homogenates were centrifuged for 15 min at 2 000 *g* at 4ºC and the supernatants were centrifuged again for 15 min at 26 000 *g* at 4ºC. The pellets containing the membrane preparation were resuspended in 1 ml of buffer A and proteins were quantified using the *DC™ Protein Assay* kit (Bio-Rad Laboratories Canada, St-Laurent, Qc). Binding was performed on 60-75 µg of proteins and incubated for 1 h at room temperature with a range of concentration of 0.01-16 nM of isotopically diluted [^{125}I]-Deltorphin I (specific activity ~2200 Ci/mmol) in 50 mM Tris buffer pH 7.4 supplemented with 0.1% bovine serum albumin (BSA). The reaction was then stopped by rapid vacuum filtration with ice-cold 50 mM Tris-HCl pH 7.4 on 96-well filter plates. Filters were then placed in 5 ml tubes, and the radioactivity was determined using a Wizard2 Automatic Gamma Counter (PerkinElmer, Woodbridge, ON, Canada). Data were analyzed using a one site non-linear fitting analysis and the *Kd* and *Bmax* values were calculated using GraphPad Prism 7.01 (GraphPad Software, San Diego, CA).

Fresh tissue preparation

After mice were decapitated, brain and spinal cord tissues were removed and flash-freeze in isopentane maintained at -50°C on dry ice. Frozen tissues were then cut in 20 µm slices using a Leica cryostat (Leica CM1860) and mounted on gelatin-coated slides. Frozen sections were kept at -80°C until use.

Autoradiography

Frozen sections (20 µm) mounted on gelatin-coated slides were thawed and pre-incubated for 30 min at room temperature with a Tris buffer solution (50 mM, pH 7.4) containing 0.5% BSA and EDTA-free cOmplete™ protease inhibitor cocktail tablets. Sections were then incubated for 1h with [^{125}I]-Deltorphin I (50 pM) or [^{125}I]-DAMGO (50 pM) and washed 3 times with Tris 50 mM, pH 7.4 and once with cold water. For non-specific binding, sections were co-incubated with 1 µM Deltorphin II or DAMGO. Sections were then air-dried and exposed for one to two weeks with Carestream Kodak biomax MR Film.

Functional GTPγS assays

Coronal sections of fresh frozen olfactory bulbs (20 µm) mounted on gelatin-coated slides were preincubated for 20 min with buffer A (50 mM HEPES, 100 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, cOmplete^{TM} protease inhibitor cocktail tablets). Sections were then incubated for 1 h with Buffer B (1 mM GDP and 1 µM 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX; selective adenosine A1 receptor antagonist) diluted in buffer A) and stimulated for 1.5 h with a 10 µM Deltorphin II solution containing 50 pM [^{35}S]-GTPγS (PerkinElmer, Walthan, MA) and 1 mM DTT diluted in buffer B. For non-specific binding, 10 µM of GTPγS was added. Sections were rinsed 3 times with buffer A and one time with cold water before being air-dried and exposed for 3-6 days with Carestream Kodak biomax MR Film (Sigma-Aldrich). Protocol was adapted from (2). Films were then scanned and quantification was carried out using Image J software. For each genotype, the mean density of 4-8 olfactory bulb sections per animal were acquired for each of the following conditions: control and Deltorphin II-stimulated. An equivalent area was used as the background signal.

Behavioural tests

Locomotor activity. Animals were placed separately in locomotor activity cages (transparent homecage surrounded with infrared beams; Kinder Scientific, Poway, CA, USA) and kept for 30 min for habituation (basal activity). Mice were then injected subcutaneously with a solution containing 10 mg/kg of SNC80 in saline containing 1 *eq*. HCl or vehicle and the distance traveled was recorded for the next 3 h using the Motor monitor software (Kinder Scientific). Movements were detected by a set of infrared beams and data were acquired over a period of 3.5 h and analyzed in 5 min bins.

CFA pain model and Hargreaves tests. Animals were acclimatized to their new environment (Plexiglas boxes placed on a glass floor) for 30 min sessions during 3 consecutive days. On the third day, thermal thresholds of paw withdrawal latency were assessed by a light beam (~115mW/cm) directed to the plantar surface of each hind paw. For CFA inflammation, adult animals under isoflurane anesthesia were injected in the plantar surface of the

right hind paw with a 50 µl volume of an emulsified solution of complete Freund's adjuvant (CFA) (25 µg/50 µl *Mycobacterium butyricum*, Calbiochem). Hargreaves tests were carried out 72 h after CFA injection. Thermal thresholds were measured for every 15 min following intrathecal administration of Deltorphin II (1µg) on a 60 min period duration (cut-off was set at 20s).

AAV injections

Intrathecal injections of the recombinant adeno-associated AAV2/9-CBA-Cre-GFP was carried out as previously described (3). Briefly, 25 days after birth, non-anesthetized mice received intrathecal injections (5 µl) of a recombinant adeno-associated virus combining a serotype 2 replicase and serotype 9 capsid (AAV2/9) engineered to express a chimeric fluorescent Cre recombinase (Cre-GFP) under the chicken beta actin promoter (CBA) (AAV2/9-CBA-Cre-GFP; 1.25 - 1.6 x 10^{13} GC/ml, purchased from the Molecular Tools Platform, Université Laval, Québec, QC, Canada). Two months later, CFA was injected in a hindpaw and the intrathecal effect of Deltorphin II using the Hargreaves test, as described above. As we previously reported (3), the intrathecal administration of AAV2/9-CBA-Cre-GFP induced the expression of the recombinase in approximately 52% of L3-L5 DRG neurons and in less than 5% of the spinal cord neurons, suggesting that recombination is mainly present in primary afferents.

Immunoprecipitation of FLAG-DOPr from mice central nervous system

The forebrains from 5 mice/condition (WT, KI and KO) were harvested, snap-freezed in liquid nitrogen and homogenized with 15 ml of lysis buffer/condition (150 mM NaCl, 50 mM Tris pH 8.0, 0.5% sodium deoxycholate, 0.1% SDS, 10 mM $Na_4P_2O_7$, 1% IGEPAL and 5 mM ethylenediaminetetraacetic acid (EDTA)) supplemented with protease inhibitors (10 µM pepstatin, 10 µM antipain, 10 µM leupeptin and 10 µM chymostatin) using a Polytron device (5 x10s), then an electric Doppler (5 strokes). After 2.5 h of incubation in lysis buffer at 4ºC with rotation, the lysates were centrifuged for 5 min at 450 *g* at 4ºC. Supernatants were

transferred into new tubes and proteins were quantified using the *DC™ Protein Assay* kit. Concentrations of proteins were adjusted to be the same in each condition in a final volume of 10 ml with lysis buffer. 100 µl/condition of the M2 mouse monoclonal anti-FLAG antibody immobilized on magnetic beads were washed 4 times with 1 ml of equilibration buffer (50 mM Tris-HCl pH 7.4 and 150 mM NaCl) prior to be added to the lysates. After an overnight incubation at 4ºC with rotation, beads were washed twice with 2 ml of lysis buffer, transferred into new tubes and washed 3 times with 1 ml of lysis buffer. Immunoprecipitated proteins were eluted by the addition of 75 µl of SDS sample buffer, incubated at room temperature for 60 min, resolved by SDS-PAGE, transferred to nitrocellulose membranes and immunoblotted with a rabbit polyclonal anti-FLAG antibody.

Liquid chromatography tandem-mass spectrometry (LC-MS/MS) analysis

FLAG-DOPr were immunoprecipitated from KI and KO mice (forebrain) as described above with some modifications. After the overnight incubation, beads were washed twice with 2 ml of lysis buffer without protease inhibitors, transferred into new tubes, washed 3 times with 1 ml of lysis buffer without protease inhibitors and twice with 1 ml of sterile phosphate-buffered saline (PBS). Beads were then transferred again into a new tube and washed 5 times for 5 min with 1 ml of ammonium bicarbonate buffer (50 mM ammonium bicarbonate, 75 mM KCl, pH 8.0). Immunoprecipitation of FLAG-DOPr was confirmed in every experiment by SDS-PAGE and immunoblotting using a rabbit polyclonal anti-FLAG antibody. Preparation of samples for mass spectrometry analysis was carried out as we described previously (4) with some modifications. Trypsin was added to the beads and incubated at 37ºC for at least 5 h up to overnight and the digestion was stopped by acidifying the samples with a final concentration of 1% formic acid. The supernatant was transferred to a clean Eppendorf® Protein LoBind tube. Beads were then resuspended in 60% acetonitrile 0.1% formic acid for 5 min at room temperature and the supernatants were pooled. Samples were dried in a speed vac, resuspended in 20 µl sample buffer (0.1% TFA), desalted on a ZipTip, separated by HPLC (Ultimate 3000 Binary RSLCnano-Thermo Scientific) and injected into a mass spectrometer (Q Exactive™ Hybrid Quadrupole-Orbitrap™-Thermo Scientific) at the Plateforme de Protéomique of the Université de Sherbrooke (Qc, Canada). A protein was considered as a potential

DOPr-interacting partner when the ratio of peptide intensity displayed at least a 1.5-fold increase in the KI condition compared to KO. Candidate proteins were then sorted according to the number of experiments in which the interaction was considered positive within our criterion. Proteomic classification of the identified DOPr-interacting partners according to their cellular localization was performed using the PANTHER Classification System online tool (5, 6) and their reported molecular functions were classified using the Gene Ontology (GO) project terms provided by the UniProt Knowledgebase (UniProtKB) database (7).

Cell culture and transfection

Human embryonic kidney 293 (HEK293) cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Wisent) supplemented with 10% (v/v) fetal bovine serum (Gibco) at 37°C in a humidified atmosphere containing 5% CO_2. Transient transfections were performed at 50-70% confluence using *Trans*IT-LT1 reagent (Mirus Bio) according to the manufacturer's instructions. Empty pcDNA3 vector was added to keep the total DNA amount constant per plate. Dicer-substrate short interfering RNAs (DsiRNAs) targeting respectively the exons 6 and 3 of the human Rab10 gene, the exon 3 and 15 (isoform 1) of the human AS160 (TBC1D4) gene (sequences are listed in Table S5) and the negative control DsiRNA (DS NC1) were purchased from Integrated DNA Technologies. Transfections were carried out as we described before (8). HEK293 cells stably expressing the N-terminally FLAG-tagged mouse DOPr were obtained from Dr. Richard Howells (New Jersey Medical School, Newark, KJ, USA) and cultured in presence of 200 µg/ml of G418.

Plasmid constructs

The Rab10 cDNA (NCBI accession: NM_016131.4) was amplified from a human fetal brain MATCHMAKER cDNA library (#638804, Clontech). The HA-tagged Rab10 construct was generated by PCR using the Q5® High-Fidelity DNA Polymerase (New England Biolabs) and primers containing the HA epitope in frame with the N-terminal open reading frame. The human FLAG-DOPr construct (NCBI accession: EU883570.1) was generated from the previously described construct (9) using primers containing the FLAG epitope in frame with the N-

terminal open reading frame, and the C27F mutation was carried out by site-directed mutagenesis. The HA-Rab10 and FLAG-DOPr PCR fragments were both digested with BamHI and EcoRI and ligated into pcDNA3 or pRSET A vectors previously digested with the same restriction enzymes. HA-Rabaptin-5 was prepared by PCR from the cDNA template (Clone ID: 6046320) purchased from GE Dharmacon (Lafayette, Colorado, USA) and ARF6-HA from the cDNA template (Clone ID: ARF0600000) purchased from Guthrie cDNA Resource Center (Guthrie Research Institute, Sayre, Pennsylvania, USA). The GalT-YFP construct (UDP-galactose: β-D-*N*-acetylglucosaminide β-1,4-galactosyltransferase, EC 2.4.1.22) was a kind gift from Marilyn Farquhar (University of California, San Diego, La Jolla, California, USA). The cDNA fragment coding for the intracellular loops (ICL1: residues 76-85, ICL2: residues 145-163 and ICL3: residues 239-261) and the carboxy-terminal tail (CT: residues 322-372) of the human DOPr were introduced into the pGEX-4T1 vector between BamHI and EcoRI. A STOP codon was added at the end of each sequence. For the ICLs, the fragments were generated by annealing 10 µl of complementary oligonucleotides (100 µM) at 94°C for 3 min. The mix was incubated 1 h at room temperature before being ligated into pGEX-4T1. For the CT, the fragment was amplified by PCR using the Q5® High-Fidelity DNA Polymerase. Sequences are shown in Table S6.

The integrity of the coding sequences of all constructs was confirmed by sequencing at Génome Québec (McGill University, QC, Canada).

Immunoprecipitation and Western blot

HEK293 cells were seeded at a density of 1.5 x 10^6 cells in 60-mm Petri dishes, transiently transfected with the indicated constructs and maintained as described above for 48 h. Cells were then washed with ice-cold PBS and harvested in 300 µl of lysis buffer (150 mM NaCl, 50 mM Tris pH 8.0, 0.5% sodium deoxycholate, 0.1% SDS, 10 mM $Na_4P_2O_7$, 1% IGEPAL and 5 mM EDTA) supplemented with protease inhibitors (10 µM pepstatin, 10 µM antipain, 10 µM leupeptin and 10 µM chymostatin). After incubation in lysis buffer for 60 min at 4°C, lysates were centrifuged for 20 min at 17 000 x *g* at 4°C. The protein concentrations in the supernatants were determined using the *DC™ Protein Assay* kit and adjusted to be the same in each condition in a final volume of 500 µl with

lysis buffer. Lysates were then incubated with 1µg of the monoclonal anti-FLAG M2 antibody for 1.5 h at 4°C with rotation and 35 µl of 50% protein G-agarose beads were added before an overnight incubation at 4°C with rotation. Samples were then centrifuged, washed four times with 1 ml of lysis buffer and immunoprecipitated proteins were eluted by the addition of 50 µl of SDS sample buffer. After an incubation at 37°C for 60 min, immunoprecipitated proteins were resolved by SDS-PAGE, transferred to nitrocellulose membranes and immunoblotted with the indicated antibodies.

Recombinant protein production and GST-pulldown assays

To produce the hexa-histidine-tagged Rab10, the HA-Rab10 PCR fragment (described above in the "Plasmid constructs" section) was inserted into the pRSETA expression vector (Invitrogen) between BamHI and EcoRI. This construct was then used to produce fusion protein in OverExpressTM C41 (DE3) *Escherichia coli* strain (Avidis, Roubais, France) according to the manufacturer's instructions. Recombinant proteins were purified using nickel-nitrilotriacetic acid-agarose resin (Qiagen, Toronto, Canada) and eluted as detailed by the manufacturer. The cDNA fragments coding for the intracellular loops and the C-terminal tail of the human DOPr described above were introduced in the pGEX-4T1 vector (Amersham Biosciences, Baie d'Urfé, Canada). GST fusion proteins were produced using the same *E. coli* strain mentioned above and purified using glutathione-Sepharose 4B beads (Amersham Biosciences) according to the manufacturer's instructions. Purified recombinant proteins were analyzed by SDS-PAGE followed by Coomassie brilliant blue R-250 staining. Purified recombinant GST-tagged ICLs and CT of DOPr were incubated with purified recombinant His_6-tagged HA-Rab10 for 1 h at 4°C in 500 µl of buffer A (150 mM NaCl, 10 mM Tris-HCl pH 7.4, 1 mM EDTA pH 8.0, 10% glycerol, 0.5% IGEPAL, 2 mM dithiothreitol) supplemented with protease inhibitors (10 µM pepstatin, 10 µM antipain, 10 µM leupeptin and 10 µM chymostatin). Binding reactions were then washed 4 times with buffer A and 35 µl of SDS sample buffer was added. Samples were boiled for 5 min and pulldown reactions were analyzed by Western blotting using the indicated specific antibodies.

Immunofluorescence staining and confocal microscopy

For co-localization experiments, HEK293 cells were seeded directly onto coverslips previously coated with 0.1 mg/ml of poly-L-lysine at a density of 1 x 10^5 cells/well in 6-well plates and transiently transfected with the indicated constructs using *Trans*IT-LT1 reagent. After 48 h, cells were fixed with 4% paraformaldehyde (PFA) in PBS, washed with PBS, permeabilized with 0.1% Triton X-100 in PBS and blocked in a solution of PBS containing 0.1% Triton X-100 and 2% BSA. After a 60 min incubation at room temperature with primary antibodies diluted in blocking solution, cells were washed twice with 0.1% Triton X-100 in PBS and blocked again with blocking solution. They were then incubated with the indicated secondary antibodies diluted in the blocking solution for 60 min at room temperature, washed twice with 0.1% Triton X-100 in PBS, washed three times with PBS and the coverslips were mounted using ProLong Gold antifade reagent. Confocal microscopy was performed using a scanning confocal system (TCS SP8; Leica) coupled to an inverted microscope with an 60x oil-immersion objective (DMI8; Leica) and the images were processed using LAS X software (Leica).

Measurement of cell-surface receptor expression by ELISA

Quantification of receptor cell-surface expression was carried out as we described before (8). For Rab10 overexpression assays, 5x10^4 HEK293 cells were seeded in 24-well plates previously coated with 0.1 mg/ml poly-L-lysine, transiently transfected with the indicated constructs using *Trans*IT-LT1 reagent and maintained for 48 h. For DsiRNAs assays, 5x10^4 HEK293 cells stably expressing FLAG-DOPr were seeded in 24-well plates precoated with 0.1 mg/ml poly-L-lysine, transfected with 50 nM (DsiRNAs-Rab10) or 25 nM (DsiRNAs-AS160) using Lipofectamine 2000 and maintained for an additional 72 h. For internalization assays, cells were treated with SNC80 (1 µM) for the indicated times in stimulation buffer (serum-free DMEM containing 20 mM HEPES and 0.5% BSA). In all cases, cells were then fixed with 3.7% (v/v) formaldehyde in Tris-buffered saline (TBS: 20 mM Tris pH 8.0 and 150 mM NaCl) for 5 min at room temperature, washed twice with TBS and non-specific binding was blocked by incubation with TBS containing 1% BSA for 30 min. A rabbit polyclonal FLAG-specific antibody (1:2000) in TBS-BSA 1% was added and incubated for 60 min. Cells were washed twice with

TBS, blocked again with TBS-BSA 1% for 10 min and incubated with an alkaline phosphatase-conjugated goat anti-rabbit antibody (1:10 000) in TBS-BSA 1% for 60 min. Cells were then washed twice with TBS and 250 µl of a colorimetric alkaline phosphatase substrate was added according to the manufacturer's instructions. The plates were incubated at 37°C until a yellow color appeared, and the reaction was stopped by the addition of 250 µl of NaOH 0.4 M. Then, 200 µl of the colorimetric reaction was taken and the absorbance was measured at 405 nm using a NanoQuant Infinite M200 Microplate reader (TECAN, Switzerland). All conditions were done in triplicate for each experiment. After the assay, cells were harvested in 150 µl/triplicate of sample buffer (62.5 mM Tris pH 7.0, 2% SDS, 10% glycerol, 50 mM dithiothreitol, 0.01% bromophenol blue), sonicated, incubated at 37°C for 60 min, analyzed by SDS-PAGE, transferred to nitrocellulose membranes and immunoblotted with the indicated antibodies. Total expression of receptors and downregulation of Rab10 and AS160 by DsiRNAs were assessed following Western blot or RT-PCR analyses, respectively. Quantification was performed by densitometry analysis using NIH ImageJ 1.8.0. In both cases, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as control.

Statistical analysis

Statistical analysis was performed using Prism version 7.01 (GraphPad Software, La Jolla, CA, USA) using one-way or two-way analysis of variance (ANOVA) followed by Dunnett's or Tukey's multiple comparisons test, one sample Wilcoxon test or unpaired two-tailed Student's *t* test as described in the figure legends. All data are presented as mean ± S.E.M and exact *P* values are indicated in the figure legends.

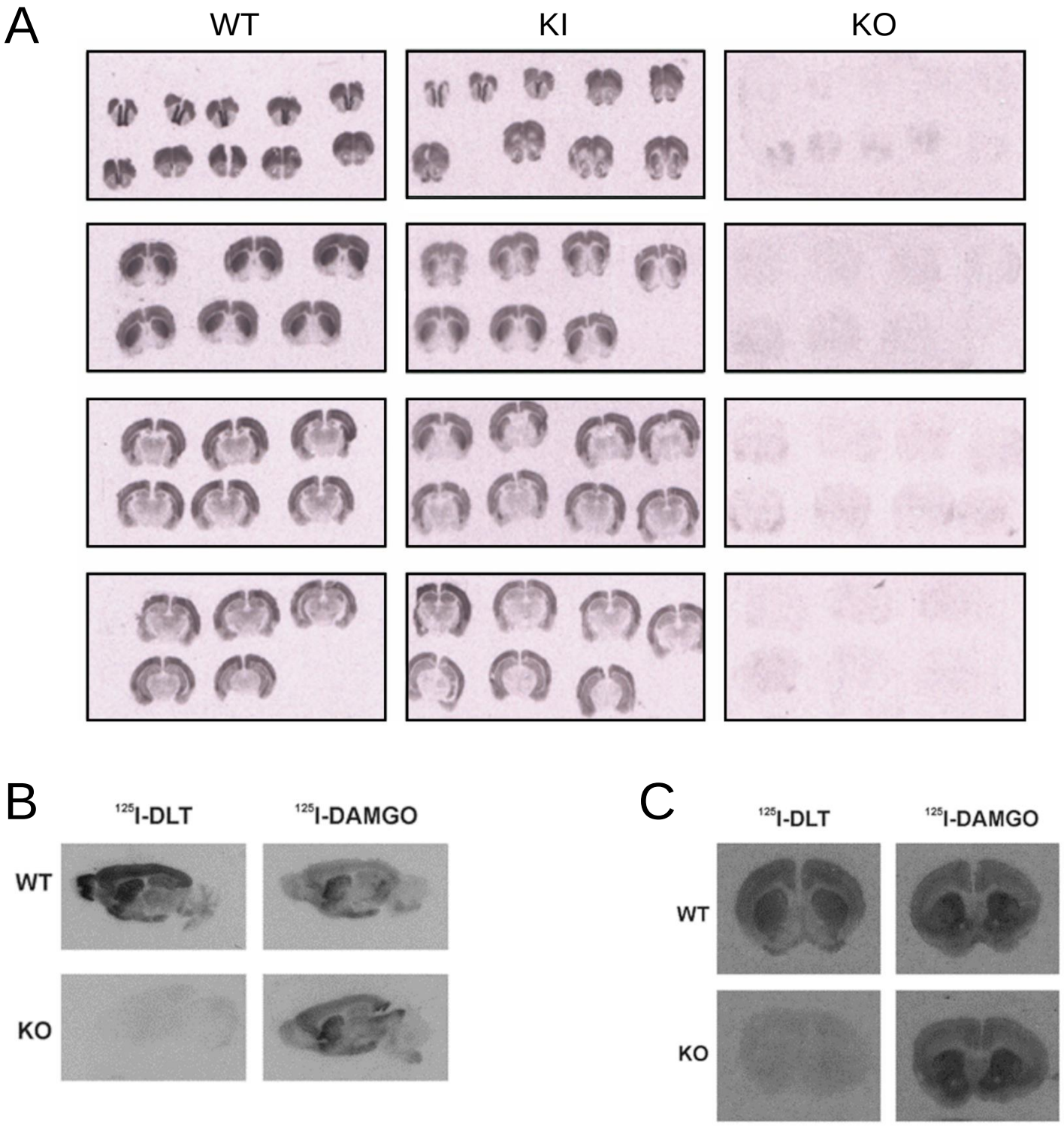

Figure S1. Expression and distribution of opioid receptors in the brain of WT, KI and KO mice. Autoradiography of coronal **(A, C)** and sagittal **(B)** sections using [^{125}I]-DLT **(A-C)** or [^{125}I]-DAMGO **(B, C)**, a selective MOPr agonist (representative of 3 independent experiments).

TABLE S1: Novel DOPr-interacting partners identified by LC-MS/MS analysis involved in intracellular trafficking

GENE SYMBOL	PROTEIN IDENTITY	N/2	FOLD INTENSITY KI/KO	NUMBER OF UNIQUE PEPTIDE	COVERAGE (%)
Proteins appearing in both experiments with an intensity ratio of at least 1.5-Fold					
ARF6	ADP-ribosylation factor 6	2	2.3 – 2.0	2 – 4	18.3 – 33.1
EEA1	Early endosome antigen 1	2	N/A – N/A	2 – 2	2.1 – 1.7
EHD3	EH domain-containing protein 3	2	1.8 – 2.2	7 – 5	26.5 – 27.1
EXOC3	Exocyst complex component 3	2	1.8 – 1.5	10 – 9	17.6 – 15.4
GAK	Cyclin-G-associated kinase	2	1.5 – N/A	1 – 1	4.4 – 4.4
HTT	Huntingtin	2	2.5 – 2.2	14 – 19	6.3 – 8.7
MON2	Protein MON2 homolog	2	N/A – 1.6	2 – 1	1.4 – 0.9
RAB10	Ras-related protein Rab-10	2	51.0 – 1.5	2 – 2	17.0 – 23.0
RAB30	Ras-related protein Rab-30	2	N/A – 2.5	1 – 2	6.3 – 12.6
RALGAPB	Ral GTPase-activating protein subunit beta	2	1.5 – 1.6	2 – 4	3.2 – 5.5
RPH3A	Rabphilin-3A	2	4.6 – 2.7	3 – 4	8.8 – 12.2
SCFD1	Sec1 family domain-containing protein 1	2	3.4 – 2.0	3 – 5	11.1 – 16.1
SEC31B	Protein transport protein Sec31B	2	N/A – 1.5	2 – 1	3.3 – 0.7
UBE2O	(E3-independent) E2 ubiquitin-conjugating enzyme UBE2O	2	1.6 – 7.6	6 – 7	7.0 – 8.1
USO1	General vesicular transport factor p115	2	1.7 – 2.6	7 – 8	10.7 – 12.4
Proteins appearing in KI group only in 1 out of 2 experiments					
AP3M1	AP-3 complex subunit mu-1	1	N/A	2	12.2
ARAP1	Arf-GAP with Rho-GAP domain, ANK repeat and PH domain-containing protein 1	1	N/A	1	2.3
COPZ1	Coatomer subunit zeta-1	1	N/A	1	8.6
CUL3	Cullin-3	1	N/A	2	4.0
DLG2	Disks large homolog 2	1	N/A	2	6.4
DNM1	Dynamin-1	1	N/A	1	46.5
EHD1	EH domain-containing protein 1	1	N/A	2	22.7
EPS15	Epidermal growth factor receptor substrate 15	1	N/A	1	1.4
ERC1	ELKS/Rab6-interacting/CAST family member 1	1	N/A	1	2.9
ESYT2	Extended synaptotagmin-2	1	N/A	3	5.7
EXOC8	Exocyst complex component 8	1	N/A	1	2.8
GAPVD1	GTPase-activating protein and VPS9 domain-containing protein 1	1	N/A	3	2.4
LLGL1	Lethal(2) giant larvae protein homolog 1	1	N/A	1	1.4
NECAP1	Adaptin ear-binding coat-associated protein 1	1	N/A	1	3.3
OSBPL1A	Oxysterol-binding protein-related protein 1	1	N/A	1	9.2
PICK1	PRKCA-binding protein	1	N/A	1	2.9
PIK3C3	Phosphatidylinositol 3-kinase catalytic subunit type 3	1	N/A	1	1.4
PIP5K1C	Phosphatidylinositol 4-phosphate 5-kinase type-1 gamma	1	N/A	1	61.6
RABEP1	Rab GTPase-binding effector protein 1	1	N/A	1	6.6
RAB6B	Ras-related protein Rab-6B	1	N/A	2	38.0

TABLE S1 (continued)

GENE SYMBOL	PROTEIN IDENTITY	N/2	FOLD INTENSITY KI/KO	NUMBER OF UNIQUE PEPTIDE	COVERAGE (%)
RAB9B	**Ras-related protein Rab-9B**	1	N/A	1	8.0
RAB11FIP5	**Rab11 family-interacting protein 5**	1	N/A	1	3.1
RAB31	**Ras-related protein Rab-31**	1	N/A	1	7.2
SCAMP4	**Secretory carrier-associated membrane protein 4**	1	N/A	1	4.8
SEC61A1	**Protein transport protein Sec61 subunit alpha isoform 1**	1	N/A	1	4.2
SNX2	**Sorting nexin-2**	1	N/A	1	5.2
SNX4	**Sorting nexin-4**	1	N/A	1	3.7
SNX6	**Sorting nexin-6**	1	N/A	1	2.7
SNX27	**Sorting nexin-27**	1	N/A	2	5.5
SORBS1	**Sorbin and SH3 domain-containing protein 1**	1	N/A	2	2.6
STX1A	**Syntaxin-1A**	1	N/A	1	7.0
STX5	**Syntaxin-5**	1	N/A	1	40.4
STX12	**Syntaxin-12**	1	N/A	1	6.2
STXBP3	**Syntaxin-binding protein 3**	1	N/A	2	4.7
STXBP5L	**Syntaxin-binding protein 5-like**	1	N/A	1	1.4
TRAPPC12	**Trafficking protein particle complex subunit 12**	1	N/A	1	1.7
VPS26A	**Vacuolar protein sorting-associated protein 26A**	1	N/A	1	13.0
VPS33A	**Vacuolar protein sorting-associated protein 33A**	1	N/A	1	2.7
VPS41	**Vacuolar protein sorting-associated protein 41 homolog**	1	N/A	1	1.8
VTA1	**Vacuolar protein sorting-associated protein VTA1 homolog**	1	N/A	2	7.3
Proteins appearing in 1 out of 2 experiments with an intensity ratio of at least 1.5-Fold					
ANKFY1	**Rabankyrin-5**	1	1.8	34	39.9
AP2A1	**AP-2 complex subunit alpha-1**	1	1.7	23	41.0
AP2M1	**AP-2 complex subunit mu**	1	1.5	17	46.7
AP2S1	**AP-2 complex subunit sigma**	1	1.9	6	44.4
AP3B2	**AP-3 complex subunit beta-2**	1	1.5	56	51.2
AP3D1	**AP-3 complex subunit delta-1**	1	1.5	29	29.6
AP3M2	**AP-3 complex subunit mu-2**	1	1.8	7	31.8
AP3S1	**AP-3 complex subunit sigma-1**	1	1.6	3	18.7
ARF5	**ADP-ribosylation factor 5**	1	2.0	5	56.7
ARFGEF2	**Brefeldin A-inhibited guanine nucleotide-exchange protein 2**	1	3.1	2	2.3
ARHGAP33	**Rho GTPase-activating protein 33**	1	2.4	1	2.0
BIN1	**Myc box-dependent-interacting protein 1**	1	2.3	11	32.5
CNIH4	**Protein cornichon homolog 4**	1	1.7	1	14.4
CTTN	**Src substrate cortactin**	1	3.0	7	14.1
DBNL	**Drebrin-like protein**	1	2.8	4	13.9
DLG4	**Disks large homolog 4**	1	2.2	5	15.1
DNAJC13	**DnaJ homolog subfamily C member 13**	1	2.9	4	4.4

TABLE S1 (continued)

GENE SYMBOL	PROTEIN IDENTITY	N/2	FOLD INTENSITY KI/KO	NUMBER OF UNIQUE PEPTIDE	COVERAGE (%)
DNM2	Dynamin-2	1	1.6	1	13.5
DNM3	Dynamin-3	1	1.5	15	37.2
EFR3B	Protein EFR3 homolog B	1	1.9	8	14.0
EHBP1	EH domain-binding protein 1	1	1.6	11	12.9
EHD4	EH domain-containing protein 4	1	2.2	2	3.5
EPN1	Epsin-1	1	3.2	1	3.8
EPS15L1	Epidermal growth factor receptor substrate 15-like 1	1	1.9	13	22.8
ERGIC1	Endoplasmic reticulum-Golgi intermediate compartment protein 1	1	1.5	3	16.6
EXOC2	Exocyst complex component 2	1	2.1	8	12.4
EXOC4	Exocyst complex component 4	1	1.7	19	24.8
EXOC5	Exocyst complex component 5	1	1.9	6	10.9
GET4	Golgi to ER traffic protein 4 homolog	1	1.6	1	7.6
HOMER1	Homer protein homolog 1	1	2.0	10	35.0
ITSN1	Intersectin-1	1	3.3	6	5.6
NAPA	Alpha-soluble NSF attachment protein	1	1.9	4	31.5
NAPB	Beta-soluble NSF attachment protein	1	1.5	11	54.7
NBEA	Neurobeachin	1	2.5	32	15.4
ODR4	Protein odr-4 homolog	1	1.5	12	29.3
PACS1	Phosphofurin acidic cluster sorting protein 1	1	1.7	17	22.1
PDCD6	Programmed cell death protein 6	1	2.3	5	35.6
PEF1	Peflin	1	5.5	2	15.6
PRAF2	PRA1 family protein 2	1	1.8	2	12.4
RAB2A	Ras-related protein Rab-2A	1	1.7	3	50.9
RAB3D	Ras-related protein Rab-3D	1	1.5	1	35.8
RAB5B	Ras-related protein Rab-5B	1	1.7	2	23.3
RAB5C	Ras-related protein Rab-5C	1	2.4	4	37.5
RAB14	Ras-related protein Rab-14	1	1.5	8	51.2
RAB15	Ras-related protein Rab-15	1	7.7	1	17.5
RAB21	Ras-related protein Rab-21	1	3.4	5	29.3
RAB33B	Ras-related protein Rab-33B	1	3.1	2	12.9
RAB35	Ras-related protein Rab-35	1	3.2	2	11.9
RAB39B	Ras-related protein Rab-39B	1	6.4	3	23.9
RTN3	Reticulon-3	1	1.6	13	20.7
SEC13	Protein SEC13 homolog	1	12.4	3	14.3
SEC22B	Vesicle-trafficking protein SEC22B	1	2.5	4	21.4
SEC23A	Protein transport protein Sec23A	1	2.1	3	8.2
SEC61A2	Protein transport protein Sec61 subunit alpha isoform 2	1	3.1	1	6.6

TABLE S1 (continued)

GENE SYMBOL	PROTEIN IDENTITY	N/2	FOLD INTENSITY KI/KO	NUMBER OF UNIQUE PEPTIDE	COVERAGE (%)
SGIP1	**SH3-containing GRB2-like protein 3-interacting protein 1**	1	4.4	4	6.4
SMAP2	**Stromal membrane-associated protein 2**	1	4.8	3	13.1
SNAP25	**Synaptosomal-associated protein 25**	1	1.7	1	55.8
SNAP91	**Clathrin coat assembly protein AP180**	1	2.3	12	19.2
SNTA1	**Alpha-1-syntrophin**	1	2.3	2	8.0
TBC1D10B	**TBC1 domain family member 10B**	1	2.4	11	17.0
TOM1L2	**TOM1-like protein 2**	1	6.9	6	25.5
TRAPPC6B	**Trafficking protein particle complex subunit 6B**	1	1.5	1	12.0
TRAPPC9	**Trafficking protein particle complex subunit 9**	1	2.0	9	10.9
TRAPPC10	**Trafficking protein particle complex subunit 10**	1	3.8	4	5.7
TRAPPC11	**Trafficking protein particle complex subunit 11**	1	1.6	1	1.5
TRIM3	**Tripartite motif-containing protein 3**	1	3.9	7	13.7
VPS13A	**Vacuolar protein sorting-associated protein 13A**	1	2.5	4	2.5
VPS16	**Vacuolar protein sorting-associated protein 16 homolog**	1	2.9	5	10.7
VPS18	**Vacuolar protein sorting-associated protein 18 homolog**	1	1.9	1	1.8
VPS26B	**Vacuolar protein sorting-associated protein 26B**	1	2.3	7	29.5
VPS29	**Vacuolar protein sorting-associated protein 29**	1	1.8	5	47.9
VPS35	**Vacuolar protein sorting-associated protein 35**	1	1.6	18	27.8
VPS45	**Vacuolar protein sorting-associated protein 45**	1	1.5	1	2.5
VPS51	**Vacuolar protein sorting-associated protein 51 homolog**	1	2.6	2	4.2
VPS52	**Vacuolar protein sorting-associated protein 52 homolog**	1	8.0	5	8.9
YWHAH	**14-3-3 protein eta**	1	1.5	7	37.4
YWHAZ	**14-3-3 protein zeta/delta**	1	1.6	9	44.9

TABLE S1-S4 LEGEND: A protein was considered as a positive hit when the ratio of peptide intensity KI/KO displayed at least a 1.5-fold increase. The number of experiments in which a protein was considered positive (N/2) is shown and results from both experiments are separated by a hyphen when applicable. *N/A* indicates that peptides were detected only in KI condition

TABLE S2: Novel DOPr-interacting partners identified by LC-MS/MS analysis involved in protein folding

GENE SYMBOL	PROTEIN IDENTITY	N/2	FOLD INTENSITY KI/KO	NUMBER OF UNIQUE PEPTIDE	COVERAGE (%)
Proteins appearing in both experiments with an intensity ratio of at least 1.5-Fold					
CCT6A	**T-complex protein 1 subunit zeta**	2	1.5 – 2.3	12 – 17	28.8 – 38.2
DNAJA2	**DnaJ homolog subfamily A member 2**	2	1.9 – 4.0	4 – 8	13.3 – 28.6
HYOU1	**Hypoxia up-regulated protein 1**	2	1.9 – 3.1	10 – 10	16.4 – 15.2
TMX3	**Protein disulfide-isomerase TMX3**	2	2.4 – N/A	1 – 2	4.2 – 4.8
Proteins appearing in KI group only in 1 out of 2 experiments					
DNAJB6	**DnaJ homolog subfamily B member 6**	1	N/A	1	9.5
PFDN5	**Prefoldin subunit 5**	1	N/A	1	24.7
PPP2R4	**Serine/threonine-protein phosphatase 2A activator**	1	N/A	1	21.3
Proteins appearing in 1 out of 2 experiments with an intensity ratio of at least 1.5-Fold					
AHSA1	**Activator of 90 kDa heat shock protein ATPase homolog 1**	1	1.9	11	44.1
BAG6	**Large proline-rich protein BAG6**	1	1.6	3	3.9
CCT2	**T-complex protein 1 subunit beta**	1	1.9	16	41.1
CCT3	**T-complex protein 1 subunit gamma**	1	1.7	15	36.0
CCT4	**T-complex protein 1 subunit delta**	1	1.9	13	26.0
CCT5	**T-complex protein 1 subunit epsilon**	1	1.8	16	33.5
CCT8	**T-complex protein 1 subunit theta**	1	1.6	20	40.5
CLU	**Clusterin**	1	3.1	7	16.3
DNAJB2	**DnaJ homolog subfamily B member 2**	1	1.5	1	6.8
CRYAB	**Alpha-crystallin B chain**	1	2.2	4	24.6
FKBP1A	**Peptidyl-prolyl cis-trans isomerase FKBP1A**	1	2.9	2	25.0
HSP90B1	**Endoplasmin**	1	4.1	2	5.6
HSPA2	**Heat shock-related 70 kDa protein 2**	1	1.9	7	26.7
HSPA4	**Heat shock 70 kDa protein 4**	1	1.6	14	22.1
HSPA4L	**Heat shock 70 kDa protein 4L**	1	1.9	12	19.1
HSPA8	**Heat shock cognate 71 kDa protein**	1	1.5	15	47.7
HSPH1	**Heat shock protein 105 kDa**	1	1.7	17	29.5
PDIA6	**Protein disulfide-isomerase A6**	1	2.0	6	18.0
PPIG	**Peptidyl-prolyl cis-trans isomerase G**	1	1.5	5	19.4
TCP1	**T-complex protein 1 subunit alpha**	1	1.7	20	49.6

TABLE S3: Novel DOPr-interacting partners identified by LC-MS/MS analysis involved in signal transduction

GENE SYMBOL	PROTEIN IDENTITY	N/2	FOLD INTENSITY KI/KO	NUMBER OF UNIQUE PEPTIDE	COVERAGE (%)
Proteins appearing in both experiments with an intensity ratio of at least 1.5-Fold					
ADCY9	Adenylate cyclase type 9	2	3.3 – 1.6	1 – 1	1.3 – 1.3
AKT2	RAC-beta serine/threonine-protein kinase	2	1.5 – 1.5	1 – 3	4.5 – 11.9
ARHGAP26	Rho GTPase-activating protein 26	2	4.8 – 2.6	2 – 3	3.9 – 7.2
ARHGAP32	Rho GTPase-activating protein 32	2	2.1 – 1.5	7 – 4	6.5 – 3.3
ARHGAP44	Rho GTPase-activating protein 44	2	2.5 – 1.5	3 – 1	5.2 – 1.7
CASKIN1	Caskin-1	2	1.5 – 2.1	4 – 6	4.6 – 6.8
CDC42BPB	Serine/threonine-protein kinase MRCK beta	2	1.6 – 1.9	11 – 10	8.3 – 8.1
CRKL	Crk-like protein	2	2.2 – 2.4	3 – 3	16.8 – 9.1
DAAM1	Disheveled-associated activator of morphogenesis 1	2	3.3 – 1.5	5 – 5	5.9 – 6.4
DCLK2	Serine/threonine-protein kinase DCLK2	2	1.6 – 2.8	5 – 5	11.0 – 11.3
DGKQ	Diacylglycerol kinase theta	2	N/A – 1.6	1 – 1	3.1 – 1.5
GNA13	Guanine nucleotide-binding protein subunit alpha-13	2	2.4 – 3.6	3 – 3	10.3 – 10.3
GNG3	Guanine nucleotide-binding protein G(i)/G(s)/G(o) subunit gamma-3	2	1.6 – 1.8	2 – 2	22.7 – 22.7
GPRIN1	G protein-regulated inducer of neurite outgrowth 1	2	3.5 – 1.5	10 – 9	15.6 – 17.0
KNDC1	Kinase non-catalytic C-lobe domain-containing protein 1	2	3.4 – 2.5	3 – 4	7.3 – 4.2
MARK2	Serine/threonine-protein kinase MARK2	2	1.6 – 1.9	5 – 10	16.0 – 24.0
PDE4B	cAMP-specific 3',5'-cyclic phosphodiesterase 4B	2	1.5 – N/A	1 – 1	3.4 – 9.6
PDE4D	cAMP-specific 3',5'-cyclic phosphodiesterase 4D	2	2.1 – N/A	1 – 1	10.1 – 6.2
PPM1H	Protein phosphatase 1H	2	1.7 – 4.0	4 – 4	10.9 – 16.6
PPP1R1B	Protein phosphatase 1 regulatory subunit 1B	2	3.7 – 2.6	3 – 2	30.4 – 16.5
PPP3CA	Serine/threonine-protein phosphatase 2B catalytic subunit alpha isoform	2	2.6 – 1.9	6 – 6	27.1 – 28.8
PTK2	Focal adhesion kinase 1	2	2.4 – 1.9	1 – 3	0.9 – 3.8
PTK2B	Protein-tyrosine kinase 2-beta	2	1.5 – 1.9	10 – 17	14.5 – 19.2
PTPN11	Tyrosine-protein phosphatase non receptor type 11	2	N/A – 2.4	1 – 3	7.9 – 9.1
RASGRF2	Ras-specific guanine nucleotide-releasing factor 2	2	3.5 – 3.3	3 – 5	4.9 – 7.6
SIPA1L1	Signal-induced proliferation-associated 1-like protein 1	2	5.2 – 1.8	3 – 8	3.0 – 6.8
STRAP	Serine-threonine kinase receptor-associated protein	2	N/A – 3.1	3 – 2	8.4 – 8.6
YWHAG	14-3-3 protein gamma	2	N/A – 1.6	1 – 4	14.2 – 28.3
Proteins appearing in KI group only in 1 out of 2 experiments					
ADCY5	Adenylate cyclase type 5	1	N/A	3	4.6
AKAP12	A-kinase anchor protein 12	1	N/A	1	2.0
ARHGEF6	Rho guanine nucleotide exchange factor 6	1	N/A	1	2.9
ARHGEF9	Rho guanine nucleotide exchange factor 9	1	N/A	1	1.8
BRSK2	Serine/threonine-protein kinase BRSK2	1	N/A	2	4.6
CTNND1	Catenin delta-1	1	N/A	1	3.6
GNAL	Guanine nucleotide-binding protein G(olf) subunit alpha	1	N/A	2	11.0
IQGAP2	Ras GTPase-activating-like protein IQGAP2	1	N/A	1	0.8

TABLE S3 (continued)

GENE SYMBOL	PROTEIN IDENTITY	N/2	FOLD INTENSITY KI/KO	NUMBER OF UNIQUE PEPTIDE	COVERAGE (%)
MAP2K7	Dual specificity mitogen-activated protein kinase kinase 7	1	N/A	1	4.6
MAPK8IP3	C-Jun-amino-terminal kinase-interacting protein 3	1	N/A	1	4.1
MRAS	Ras-related protein M-Ras	1	N/A	1	8.5
PDE9A	High affinity cGMP-specific 3',5'-cyclic phosphodiesterase 9A	1	N/A	1	15.0
RPS6KA1	Ribosomal protein S6-kinase alpha-1	1	N/A	1	8.0
RPS6KA4	Ribosomal protein S6-kinase alpha-4	1	N/A	1	1.3
SIPA1L2	Signal-induced proliferation-associated 1-like protein 2	1	N/A	1	0.9
STAM	Signal transducing adapter molecule 1	1	N/A	3	8.4
STAT1	Signal transducer and activator of transcription 1	1	N/A	1	5.3
STK10	Serine/threonine-protein kinase 10	1	N/A	1	1.7
TAB1	TGF-beta-activated kinase 1 and MAP3K7-binding protein 1	1	N/A	2	6.6
WASF3	Wiskott-Aldrich syndrome protein family member 3	1	N/A	2	6.2
Proteins appearing in 1 out of 2 experiments with an intensity ratio of at least 1.5-Fold					
AGAP2	Arf-GAP with GTPase, ANK repeat and PH domain-containing protein 2	1	1.8	9	10.4
AKAP5	A-kinase anchor protein 5	1	1.6	9	28.5
AKT3	RAC-gamma serine/threonine-protein kinase	1	2.6	2	12.6
APPL1	DCC-interacting protein 13-alpha	1	4.8	6	11.5
ARHGEF7	Rho guanine nucleotide exchange factor 7	1	1.5	3	10.2
CAMK2G	Calcium/calmodulin-dependent protein kinase type II subunit gamma	1	1.7	6	27.8
CAMK4	Calcium/calmodulin-dependent protein kinase type IV	1	2.3	6	17.7
CCDC88A	Girdin	1	1.5	12	8.8
CDC42	Cell division control protein 42 homolog	1	1.5	1	16.8
CDC42BPA	Serine/threonine-protein kinase MRCK alpha	1	4.1	4	4.8
CDK18	Cyclin-dependent kinase 18	1	3.5	4	15.7
CNKSR2	Connector enhancer of kinase suppressor of ras 2	1	1.6	10	13.5
CPNE6	Copine-6	1	1.5	5	12.7
CTNNB1	Catenin beta-1	1	1.6	7	13.1
CTNND2	Catenin delta-2	1	2.7	5	6.6
DCLK1	Serine/threonine-protein kinase DCLK1	1	1.5	17	28.8
DOCK11	Dedicator of cytokinesis protein 11	1	2.9	12	11.2
DUSP3	Dual specificity protein phosphatase 3	1	2.4	4	39.3
FARP1	FERM, ARHGEF and pleckstrin domain-containing protein 1	1	2.2	8	12.3
FRRS1L	DOMON domain-containing protein FRRS1L	1	3.8	2	10.2
GNA11	Guanine nucleotide-binding protein subunit alpha-11	1	1.5	2	19.2
GNB5	Guanine nucleotide-binding protein subunit beta-5	1	2.1	5	16.4
GNG2	Guanine nucleotide-binding protein G(i)/G(s)/G(o) subunit gamma-2	1	2.1	2	39.4
GNG4	Guanine nucleotide-binding protein G(i)/G(s)/G(o) subunit gamma-4	1	2.1	1	21.3
GRB2	Growth factor receptor-bound protein 2	1	4.1	7	44.7

TABLE S3 (continued)

GENE SYMBOL	PROTEIN IDENTITY	N/2	FOLD INTENSITY KI/KO	NUMBER OF UNIQUE PEPTIDE	COVERAGE (%)
GSK3B	Glycogen synthase kinase-3 beta	1	1.9	6	30.7
GUCY1A2	Guanylate cyclase soluble subunit alpha-2	1	4.0	3	9.0
GUCY1A3	Guanylate cyclase soluble subunit alpha-1	1	2.5	2	3.6
GUCY1B3	Guanylate cyclase soluble subunit beta-1	1	1.6	13	27.6
HPCA	Neuron-specific calcium-binding protein hippocalcin	1	1.6	3	31.0
ILK	Integrin-linked protein kinase	1	1.6	1	3.1
IQSEC1	IQ motif and SEC7 domain-containing protein 1	1	1.5	9	14.8
ITPKA	Inositol-trisphosphate 3-kinase A	1	1.6	5	13.9
MAPK3	Mitogen-activated protein kinase 3	1	1.5	5	23.9
MAP2K1	Dual specificity mitogen-activated protein kinase kinase 1	1	1.5	9	35.9
MAP2K2	Dual specificity mitogen-activated protein kinase kinase 2	1	2.0	2	16.8
MAP3K7	Mitogen-activated protein kinase kinase kinase 7	1	3.5	2	4.9
MAP3K19	Mitogen-activated protein kinase kinase kinase 19	1	1.5	1	0.5
MINK1	Misshapen-like kinase 1	1	2.3	2	4.6
MTOR	Serine/threonine-protein kinase mTOR	1	2.2	8	4.9
NECAB2	N-terminal EF-hand calcium-binding protein 2	1	3.5	2	9.9
NF1	Neurofibromin	1	2.8	14	7.8
PDE1B	Calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase 1B	1	1.9	7	18.6
PDE2A	cGMP-dependent 3',5'-cyclic phosphodiesterase	1	1.8	10	15.1
PDE3A	cGMP-inhibited 3',5'-cyclic phosphodiesterase A	1	1.9	1	4.4
PDE8B	High affinity cAMP-specific and IBMX-insensitive 3',5'-cyclic phosphodiesterase 8B	1	2.4	1	7.1
PDPK1	3-phosphoinositide-dependent protein kinase 1	1	2.1	1	3.7
PEBP1	Phosphatidylethanolamine-binding protein 1	1	3.0	9	52.4
PI4KA	Phosphatidylinositol 4-kinase alpha	1	1.7	53	33.6
PIK3R4	Phosphoinositide 3-kinase regulatory subunit 4	1	1.5	3	2.6
PIP5K1A	Phosphatidylinositol 4-phosphate 5-kinase type-1 alpha	1	1.5	4	18.5
PPP1CA	Serine/threonine-protein phosphatase PP1-alpha catalytic subunit	1	1.5	3	18.5
PPP1CC	Serine/threonine-protein phosphatase PP1-gamma catalytic subunit	1	1.5	2	17.0
PPP2R1A	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	1	1.5	15	31.2
PPP2R2A	Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform	1	1.5	8	36.9
PPP2R5C	Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit gamma isoform	1	3.8	1	6.2
PPP2R5E	Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit epsilon isoform	1	1.9	3	14.3
PPP5C	Serine/threonine-protein phosphatase 5	1	1.9	1	2.7
PRKAR1A	cAMP-dependent protein kinase type I-alpha regulatory subunit	1	1.9	2	19.5
PRKAR1B	cAMP-dependent protein kinase type I-beta regulatory subunit	1	1.5	1	14.8
PRKCG	Protein kinase C gamma type	1	1.8	20	35.7
PSD3	PH and SEC7 domain-containing protein 3	1	1.7	2	8.2

TABLE S3 (continued)

GENE SYMBOL	PROTEIN IDENTITY	N/2	FOLD INTENSITY KI/KO	NUMBER OF UNIQUE PEPTIDE	COVERAGE (%)
PTPN5	**Tyrosine-protein phosphatase non-receptor type 5**	1	3.7	2	3.8
RALGAPA1	**Ral GTPase-activating protein subunit alpha-1**	1	9.6	2	1.8
RAP2A	**Ras-related protein Rap-2a**	1	1.7	3	41.5
RAPGEF2	**Rap guanine nucleotide exchange factor 2**	1	2.3	6	6.7
RAPGEF4	**Rap guanine nucleotide exchange factor 4**	1	3.2	3	4.0
RASAL1	**RasGAP-activating-like protein 1**	1	1.7	18	33.3
ROCK1	**Rho-associated protein kinase 1**	1	3.3	1	5.7
ROCK2	**Rho-associated protein kinase 2**	1	2.1	25	24.6
RPTOR	**Regulatory-associated protein of mTOR**	1	3.0	4	5.2
SBF2	**Myotubularin-related protein 13**	1	1.6	23	18.2
SIK3	**Serine/threonine-protein kinase SIK3**	1	1.7	11	12.6
STAT3	**Signal transducer and activator of transcription 3**	1	1.6	16	28.7
STK38	**Serine/threonine-protein kinase 38**	1	1.7	21	61.7
STK39	**STE20/SPS1-related proline-alanine-rich protein kinase**	1	3.7	4	7.9
SYNGAP1	**Ras/Rap GTPase-activating protein SynGAP**	1	1.7	10	12.8
TOLLIP	**Toll-interacting protein**	1	3.1	2	12.3
UBR1	**E3 ubiquitin-protein ligase UBR1**	1	1.6	1	1.8
YWHAQ	**14-3-3 protein theta**	1	1.6	5	29.0

TABLE S4: Novel DOPr-interacting partners identified by LC-MS/MS analysis and classified as receptors, transporters or channels.

GENE SYMBOL	PROTEIN IDENTITY	N/2	FOLD INTENSITY KI/KO	NUMBER OF UNIQUE PEPTIDE	COVERAGE (%)
Proteins appearing in both experiments with an intensity ratio of at least 1.5-Fold					
ADGRB2	Adhesion G protein-coupled receptor B2	2	N/A – N/A	1 – 2	1.0 – 3.6
CACNG8	Voltage-dependent calcium channel gamma-8 subunit	2	2.5 – N/A	2 – 1	7.8 – 3.5
GABBR1	Gamma-aminobutyric acid type B receptor subunit 1	2	1.6 – 2.0	4 – 4	6.0 – 5.6
GABBR2	Gamma-aminobutyric acid type B receptor subunit 2	2	1.9 – 3.0	4 – 4	5.3 – 5.7
GABRG2	Gamma-aminobutyric acid receptor subunit gamma-2	2	1.9 – 2.0	3 – 4	10.2 – 15.9
ITPR1	Inositol 1,4,5-trisphosphate receptor type 1	2	1.6 – 2.4	13 – 10	7.2 – 5.4
PTPRZ1	Receptor-type tyrosine-protein phosphatase zeta	2	1.7 – 1.8	4 – 6	3.0 – 4.8
SCN1A	Sodium channel protein type 1 subunit alpha	2	N/A – 3.8	1 – 5	3.4 – 7.2
SCN1B	Sodium channel subunit beta-1	2	N/A – N/A	2 – 1	19.7 – 7.8
SLC2A1	Solute carrier family 2, facilitated glucose transporter member 1	2	1.8 – 2.1	4 – 5	8.5 – 10.6
SLC2A13	Proton myo-inositol cotransporter	2	2.9 – N/A	1 – 1	2.0 – 1.4
SLC6A9	Sodium- and chloride-dependent glycine transporter 1	2	1.5 – 1.8	3 – 3	8.4 – 8.4
SLC7A5	Large neutral amino acids transporter small subunit 1	2	1.5 – 2.0	2 – 2	5.9 – 5.9
SLC8A1	Sodium/calcium exchanger 1	2	1.9 – N/A	2 – 1	6.2 – 4.4
SLC24A2	Sodium/potassium/calcium exchanger 2	2	2.8 – 1.5	3 – 1	6.0 – 7.1
TTYH1	Protein tweety homolog 1	2	2.0 – 1.9	3 – 1	9.3 – 2.9
Proteins appearing in KI group only in 1 out of 2 experiments					
ABCA2	ATP-binding cassette sub-family A member 2	1	N/A	1	1.8
CACNA1E	Voltage-dependent R-type calcium channel subunit alpha-1E	1	N/A	3	2.3
FLT3	Receptor-type tyrosine-protein kinase FLT3	1	N/A	1	2.0
GABRA3	Gamma-aminobutyric acid receptor subunit alpha-3	1	N/A	1	7.5
ITGA3	Integrin alpha-3	1	N/A	3	4.3
KCND3	Potassium voltage-gated channel subfamily D member 3	1	N/A	1	6.6
KCNK10	Potassium channel subfamily K member 10	1	N/A	1	2.6
KCNN2	Small conductance calcium-activated potassium channel protein 2	1	N/A	1	4.6
KCNQ2	Potassium voltage-gated channel subfamily KQT member 2	1	N/A	1	4.4
OLFR138	Olfactory receptor	1	N/A	1	26.8
SLC1A6	Excitatory amino acid transporter 4	1	N/A	1	3.7
SLC4A3	Anion exchange protein 3	1	N/A	1	1.5
SLC9A6	Sodium/hydrogen exchanger 6	1	N/A	2	3.0
SLC10A1	Sodium/bile acid cotransporter	1	N/A	1	3.9
SLC16A10	Monocarboxylate transporter 10	1	N/A	1	8.1
SLC30A9	Zinc transporter 9	1	N/A	1	2.6
SLC32A1	Vesicular inhibitory amino acid transporter	1	N/A	1	2.1
SLC39A10	Zinc transporter ZIP10	1	N/A	1	1.4
TAS2R4	Taste receptor type 2 member 4	1	N/A	1	5.4

TABLE S4 (continued)

GENE SYMBOL	PROTEIN IDENTITY	N/2	FOLD INTENSITY KI/KO	NUMBER OF UNIQUE PEPTIDE	COVERAGE (%)
TLR13	Toll-like receptor 13	1	N/A	1	1.3
TMCO1	Calcium load-activated calcium channel	1	N/A	1	7.5
Proteins appearing in 1 out of 2 experiments with an intensity ratio of at least 1.5-Fold					
ADCYAP1R1	Pituitary adenylate cyclase-activating polypeptide type I receptor	1	2.3	2	3.6
ADGRB3	Adhesion G-protein coupled receptor B3	1	1.5	2	11.0
CACNA1B	Voltage-dependent N-type calcium channel subunit alpha-1B	1	1.9	3	1.9
CACNA1C	Voltage-dependent L-type calcium channel subunit alpha-1C	1	1.5	6	6.9
CCR1	C-C chemokine receptor type 1	1	1.5	2	5.6
EPHA4	Ephrin type-A receptor 4	1	1.8	2	4.0
GABRB2	Gamma-aminobutyric acid receptor subunit beta-2	1	1.6	2	11.2
GABRB3	Gamma-aminobutyric acid receptor subunit beta-3	1	6.1	1	8.7
GPR37	Prosaposin receptor GPR37	1	1.6	1	3.0
GPR158	Probable G-protein coupled receptor 158	1	6.3	4	5.2
GPRC5B	G-protein coupled receptor family C group 5 member B	1	1.7	2	9.2
GRIA2	Glutamate receptor 2	1	2.0	8	12.1
GRIA3	Glutamate receptor 3	1	1.8	3	6.6
GRIN1	Glutamate receptor ionotropic NMDA 1	1	2.2	6	9.4
GRIN2A	Glutamate receptor ionotropic NMDA 2A	1	2.6	2	2.3
GRIN2B	Glutamate receptor ionotropic NMDA 2B	1	1.7	2	1.3
GRM3	Metabotropic glutamate receptor 3	1	1.7	9	16.5
GRM5	Metabotropic glutamate receptor 5	1	3.4	7	6.8
HCN1	Brain cyclic nucleotide-gated channel 1	1	1.6	3	9.9
HCN2	Brain cyclic nucleotide-gated channel 2	1	2.5	11	24.0
HCN4	Brain cyclic nucleotide-gated channel 3	1	1.6	1	5.4
KCNA2	Potassium voltage-gated channel subfamily A member 2	1	1.7	4	22.0
KCNAB2	Voltage-gated potassium channel subunit beta-2	1	2.2	8	33.0
LRRC8A	Volume-regulated anion channel subunit LRRC8A	1	2.0	2	2.6
OLFR1018	Olfactory receptor	1	8.1	1	10.3
PKD1L3	Polycystic kidney disease protein 1-like 3	1	2.2	1	0.9
RYR2	Ryanodine receptor 2	1	3.9	17	6.4
SCN2A1	Sodium channel protein type 2 subunit alpha	1	1.8	9	10.9
SCN11A	Sodium channel protein 11 subunit alpha	1	3.4	1	2.0
SLC1A3	Excitatory amino acid transporter 1	1	1.7	6	17.9
SLC4A4	Electrogenic sodium bicarbonate cotransporter 1	1	1.6	16	22.8
SLC6A7	Sodium-dependent proline transporter	1	1.5	2	4.1
SLC6A11	Sodium- and chloride-dependent GABA transporter 3	1	2.1	7	12.1
SLC6A17	Sodium-dependent neutral amino acid transporter SLC6A17	1	2.9	5	10.2

GENE SYMBOL	PROTEIN IDENTITY	N/2	FOLD INTENSITY KI/KO	NUMBER OF UNIQUE PEPTIDE	COVERAGE (%)
SLC8A3	**Sodium/calcium exchanger 3**	1	1.5	24	32.8
SLC16A1	**Monocarboxylate transporter 1**	1	2.1	3	11.2
SLC17A6	**Vesicular glutamate transporter 2**	1	1.5	10	20.4
SLC24A4	**Sodium/potassium/calcium exchanger 4**	1	1.5	1	1.6
SLC30A3	**Zinc transporter 3**	1	1.7	6	15.2
TTYH3	**Protein tweety homolog 3**	1	1.5	1	2.9

TABLE S5: Primers and DsiRNAs sequences

Oligonucleotide names	Sequences
pDOPr-01	5’ – TGGCCTCCGTTTTCCGCGC – 3’
pDOPr-02	5’ – CTGGGAAAGGCGTCCGAGAGGT – 3’
pDOPr-03	5’ – GCCATGGATTACAAAGATGAC – 3’
pDOPr-04	5’ – GTCTGGATCTGACATGGTAAG – 3’
pDOPr-05	5’ – TGGGTATCCTGGTCTACAAAG – 3’
pAS160-For	5'- ATGGAGCCGCCCAGCTGCATTCAGGATGAGCCGTTCCCG C -3'
pAS160-Rev	5'- TTATGGCTTATTTCCTATCTTGGCTTTGTTGTTAGGGTTGCAGTTTAG GTCTCTCAGC -3'
pGAPDH-For	5'- GTGAAGGTCGGTGTGAACGG – 3’
pGAPDH-Rev	5'- CCAAAGTTGTCATGGATGAC – 3’
DsiRNA-Rab10-13.2 (exon 6)	5’ – rArArCrArGrUrGrArArArArUrGrUrArGrArUrArUrCrArGCA – 3’ 5’ – rUrGrCrUrGrArUrArUrCrUrArCrArUrUrUrUrCrArCrUrGrUrUrGrG – 3’
DsiRNA-Rab10-13.3 (exon 3)	5’ – rUrArUrGrArCrArUrCrArCrCrArArUrGrGrUrArArArArGTT – 3’ 5’ – rArArCrUrUrUrUrArCrCrArUrUrGrGrUrGrArUrGrUrCrArUrArUrA – 3’
DsiRNA-AS160-13.1 (exon 3)	5’ – rArCrUrArArArArUrCrArGrUrUrGrUrGrCrUrArGrArArArAGA – 3’ 5’ – rUrCrUrUrUrUrCrUrArGrCrArCrArArCrUrGrArUrUrUrArGrUrGrU – 3’
DsiRNA-AS160-13.2 (exon 15)	5’ – rArGrCrUrArArArArUrCrArGrArUrGrUrGrArUrArUrGrGAA – 3’ 5’ – rUrUrCrCrArUrArUrCrArCrArUrCrUrGrArUrUrUrUrArGrCrUrCrU – 3’

TABLE S6: Amino acid sequences of the recombinant GST-fused protein constructs

Constructs	Amino acid sequences
GST-hDOPr-ICL1	**GST**-RYTKMKTATN-**STOP**
GST-hDOPr-ICL2	**GST**- DRYIAVCHPVKALDFRTPA-**STOP**
GST-hDOPr-ICL3	**GST**- RLRSVRLLSGSKEKDRSLRRITR-**STOP**
GST-hDOPr-CT	**GST**- DENFKRCFRQLCRKPCGRPDPSSFSRAREATARERVTACTPSDGPGGGAAA-**STOP**

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