Supplemental Material

Supplemental Methods

*Lentiviral vector production and titration*

SJ293TS cells was transfected with pCAG-kGP1-1R, pCAG-VSVG and pCAG4-RTR2 using PEIpro (Polyplus Transfection). The next day, transfected cells were diluted with media containing 12.5 U/mL Benzonase (Sigma). Vector supernatants were collected 48 hr post-transfection, clarified by centrifugation at 330 xg for 5 min and filtered. Lentiviral vector containing supernatants were adjusted to 300 mM NaCl, 50 mM Tris pH 8.0 and loaded onto an Acrodisc Mustang Q membrane (Pall Life Sciences) according to the manufacturer’s instructions using an Akta Avant chromatography system (GE Healthcare). After washing the column 300 mM NaCl, 50 mM Tris pH 8.0, viral particles were eluted from the column using 2 M NaCl, Tris pH 8.0 directly onto a PD10 desalting column (GE Healthcare) according to the manufacturer’s instructions. Vector containing flow-through was diluted with an equal volume of X-VIVO 10 media (Lonza) or phosphate buffered saline containing 0.5% (v/v) human serum albumin (Grifols Biologics) to achieve an approximate 50-fold concentration from the starting material, sterile filtered, aliquoted and stored at −80 °C.

Titration of lentiviral vectors was performed by transducing HOS cells with serially diluted vector preparations in the presence 5-8 mg/mL Polybrene (Sigma). Four days post-transduction, genomic DNA was isolated from transduced HOS cells using a Quick-DNA Miniprep kit (Zymo Research). Vector titers were determined by calculating the ratio between the copies of HIV psi and every two copies of RPP30 via QX200 digital droplet PCR system (Bio-Rad), multiplied by the number of cells transduced and if necessary, multiplied by the dilution factor.
Quantitative Proteomic / Mass Spectrometry

For adrenal gland, pituitary, liver, brain stem and WAT: the TMT labeled samples were analyzed on an Orbitrap Fusion Lumos mass spectrometer (ThermoFisher). Samples were injected directly onto a 25 cm, 100 µm ID column packed with BEH 1.7 µm C18 resin (Waters). Samples were separated at a flow rate of 400 nL/min on EASY-nLC (ThermoFisher). Buffer A and B were 0.1% (v/v) formic acid in water and acetonitrile, respectively. A gradient of 1–25% B over 180 min, an increase to 40% B over 40 min, an increase to 90% over 10 min and held at 90% B for 10 min was used for a 240 min total run time. The column was re-equilibrated with 20 µL of buffer A prior to the injection of the sample. Peptides were eluted directly from the tip of the column and nanospray directly into the mass spectrometer by application of 2.8 kV voltage at the back of the column. The Lumos was operated in a data-dependent mode. Full MS1 scans were collected in the Orbitrap at 120k resolution. The cycle time was set to 3 s, and within this 3 s the most abundant ions per scan were selected for CID MS/MS in the ion trap. MS3 analysis with multi-notch isolation (SPS3) was utilized for detection of TMT reporter ions at 60k resolution. Monoisotopic precursor selection was enabled and dynamic exclusion was used with an exclusion duration of 10 sec.

For hypothalamus: the TMT labeled samples were analyzed on an Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher). Samples were injected directly onto a 25 cm, 100 µm ID column packed with BEH 1.7 µm C18 resin (Waters). Samples were separated at a flow rate of 300 nL/min on EASY-nLC (ThermoFisher). Buffer A and B were 0.1% formic acid in water and acetonitrile, respectively. A gradient of 1–25% B over 200 min, an increase to 50% B over 120 min, an increase to 90% over 30 min and held at 90% B for 10 min was used for a 360 min total run time. The column was re-equilibrated with 20 µL of buffer A prior to the injection of the sample. Peptides were eluted directly from the tip of the column and nanospray directly into the
mass spectrometer by application of 2.8 kV voltage at the back of the column. The Fusion was operated in a data-dependent mode. Full MS1 scans were collected in the Orbitrap at 120k resolution. The cycle time was set to 3 s, and within this 3 s the most abundant ions per scan were selected for CID MS/MS in the ion trap. MS3 analysis with multi-notch isolation (SPS3) was utilized for detection of TMT reporter ions at 60k resolution. Monoisotopic precursor selection was enabled and dynamic exclusion was used with an exclusion duration of 10 sec.

For peptide identification, tandem mass spectra were searched against a database including the UniProt mouse database one entry per gene (21982 entries released 12/1/2019) with common contaminants and reversed sequences using ProLuCID (69). The search was set with 50 and 600 ppm for precursor and fragments mass tolerance, respectively. The precursor mass range was set from 600 to 6000 with half or fully tryptic status. The N-term static modification was considered as (+229.1629) for TMT labeling and the amino acid residue specific static modifications were (+57.02146) on cysteine for carbamidomethylation and (+229.1629) on lysine for TMT labeling. Data was filtered using DTASelect with the following parameters, -p 1 -y 2 --trypstat --pfp 0.01 --extra --pI -DM 10 --DB --dm -in -t 1 --brief --quiet.
Supplemental Figures

Figure S1. Western blot analysis of Magel2+/+ and Magel2Δ/m+ mouse hypothalami at 2, 4 and 20wks. A) Immunohistochemistry of 8wk old Magel2Δ/m+ mouse at the hypothalamus and amygdala using anti-Magel2 antibody. B-D) Western blot analysis confirmed reduced expression of Pcsk1, Pcsk2 and Chgb in B) 2wk; C) 4wk; and D) 20wk old Magel2Δ/m+ mouse hypothalami. Asterisks mark a non-specific band detected by Magel2 antibody. Gapdh served as loading control.
Figure S2. Quantitative proteomics of brain stem and adrenal gland in *Magel2*Δ/+ mice. Volcano plots showing proteins that are significantly changed in 8wk old *Magel2*Δ/+ mouse A) brain stem; and B) adrenal glands detected by TMT-M/S. The horizontal lines denoting p-value thresholds (p≤0.05; unpaired, analyzed by two-tailed student t-test) and vertical lines denoting log2 fold-change thresholds (>0.5 and <-0.5) n=5 per genotype.
Figure S3. Characterization of NGN2-generated human iN. A) Schematic of chromosome 15 PWS region and genomic editing. B) Representative images showing the time course of conversion of human iPSC into iN by GFP-NGN2. C) Representative images of immunofluorescence staining of control and PWS iN with various neuronal markers. Scale bars=50 µm.
**Figure S4. Endosomal protein recycling is not impaired in undifferentiated DPSC and DPSC-derived adipocytes.**

**A** There is no difference in proportion of cells with impaired M6PR trafficking between control and PWS deletion DPSC and DPSC-derived adipocytes. Each data point represents one individual, plotted as mean±SD; >75 cells/data point, and analyzed by t-test.

**B** Quantification of western deletion analysis showed no difference in the expression of alpha-5, alpha-V and beta-1 between control and PWS deletion DPSC. Each target protein is first normalized to GAPDH, and then PWS deletion DPSC is normalized to averaged control. Each data point represents one individual, plotted as mean±S.D. and analyzed by unpaired, two-tailed student t-test.

**C-D** There is no difference in the fluorescence intensity of F-actin (C) or ArpC5 (D) on VPS35-marked endosomes between control and PWS deletion DPSC, and DPSC-derived adipocytes. Each data point represents one individual, plotted as mean±SD; >75 cells/data point, and analyzed by unpaired, two-tailed student t-test.
Figure S5. SG protein transcript levels are not altered in Magel2<sub>pΔ/m+</sub> mice and PWS human iN. A-B) Transcript levels of Pcsk1, Pcsk2, Chgb and Cpe are unaltered between 2wk (A) and...
20wk (B) old Magel2pA/m+ and Magel2+/+ mouse hypothalami. Each data point represents one animal, plotted as mean±S.D. and analyzed by unpaired, two-tailed student t-test, ****p<0.0001. (n>3 per genotype) C) Transcript levels of PCSK1, PCSK2, CHGB and CPE are unaltered between control and PWS iN at 3d, 7d, 10d and 14d post-induction. Each data point represents one induction experiment (n=2), plotted as mean±S.D. and analyzed by ANOVA. D) Representative images of immunofluorescence staining with CHGB and LAMP1 in control and PWS iN. E) Transcript levels of PCSK1, PCSK2, CHGB and CPE are unaltered between control #3 and PWS #3 iN at 14d post-induction. Each data point represents one induction experiment (n=3), plotted as mean±S.D. and analyzed by t-test.
Figure S6. SG proteins colocalize with WASH and retromer components within the soma of human iN. A) Western blot analysis of Pcsk1, Pcsk2, Chgb and Magel2 in 2wk old Washc1 cKO mouse hypothalami. Gapdh served as loading control. Each data point represents one animal, n=4 per genotype, plotted as mean±S.D. and analyzed by unpaired, two-tailed student t-test. B) Representative images of immunofluorescence staining with CHGB and FAM21 at both soma and neurite in control iN. Scale bars=10 µm. C) Representative images of immunofluorescence staining with CHGB and VPS35 at both soma and neurite in control iN. Scale bars=10 µm. D) Representative images of immunofluorescence staining with CHGA and WASH at both soma and neurite in control iN. Scale bars represent 10 µm. E) Representative images of immunofluorescence staining with CHGA and VPS35 at both soma and neurite in control iN. Scale bars=10 µm.
Figure S7. Neuropeptide transcript levels are not altered in Magel2\textsuperscript{pΔ/m+} mice. Transcript levels of various neuropeptides between 2wk (A) and 20wk (B) old Magel2\textsuperscript{pΔ/m+} and Magel2\textsuperscript{+/+} mouse hypothalami. Each data point represents one animal, (n>3 per genotype), plotted as mean±S.D. and analyzed by unpaired, two-tailed student t-test. C) Transcript levels of various neuropeptides between Ctrl#3 and PWS#3 iN at 14d post-induction. Each data point represents one induction experiment (n=3), plotted as mean±S.D. and analyzed by t-test.
Supplemental Tables

Table S1. Protein expression differences between Magel2\(^{+/+}\) (WT) and Magel2\(^{p/-m+}\) (KO) (n=5 each) as determined by TMT proteomics for the indicated tissues.

*Attached .xls file*

Table S2. Gene ontology (GO) analysis of TMT proteomics data for the indicated tissues.

*Attached .xls file*

Table S3. Information on Prader-Willi and Schaaf-Yang syndrome patients and control subjects in which DPSCs were created from.

| Cell Line | Genotype       | Sex | Age (Years) | Autism Status |
|-----------|----------------|-----|-------------|---------------|
| 48        | Control        | M   | 7           | Possible ASD  |
| 50        | Control        | M   | unknown     | unknown       |
| 195       | Control        | M   | 4.4         | Normal        |
| 238       | Control        | M   | 8.3         | Normal        |
| 302       | Control        | M   | 9.9         | Normal        |
| 38        | Control        | F   | 10.3        | unknown       |
| 76        | Control        | F   | 4.3         | unknown       |
| 182       | Control        | F   | 12.8        | Normal        |
| 188       | Control        | F   | 5.2         | Normal        |
| 189       | Control        | F   | 8.4         | unknown       |
| 233       | Control        | F   | 11.9        | Normal        |
| 312       | Control        | F   | 8           | Normal        |
| 148       | PWS, deletion  | M   | 5.8         | Normal        |
| 152       | PWS, deletion  | M   | 5.4         | Normal        |
| 198       | PWS, deletion  | M   | 8           | Normal        |
| 225       | PWS, deletion  | M   | 11.6        | Normal        |
| 297       | PWS, deletion  | M   | 5.6         | Normal        |
| 192       | PWS, deletion  | F   | 13.3        | Normal        |
| 258       | PWS, deletion  | F   | 5.5         | Normal        |
| 235       | PWS, UPD       | M   | 10.1        | Normal        |
| 94        | PWS, UPD       | F   | 5.1         | Possible ASD  |
| 162       | PWS, UPD       | F   | 4.6         | Normal        |
| 249       | PWS, UPD       | F   | 8.1         | Normal        |
| 268       | PWS, UPD       | F   | 6.6         | Possible ASD  |
| 270       | PWS, UPD       | F   | 4.6         | Normal        |
| 277       | PWS, UPD       | F   | 16.2        | Possible ASD  |
| 306       | Schaaf-Yang    | F   | 7           | Possible ASD  |
| 274       | Schaaf-Yang    | F   | 7.2         | Possible ASD  |
| Sample ID | Genotype          | Familial status | Gender | Age (YRS) |
|-----------|------------------|-----------------|--------|-----------|
| 0030 - CU | Unaffected Control | Parent          | Female | 34.0      |
| 0031 - CU | Unaffected Control | Parent          | Male   | 34.3      |
| 0034 - CU | Unaffected Control | Parent          | Female | 30.6      |
| 0035 - CU | Unaffected Control | Parent          | Male   | 32.7      |
| 0037 - CU | Unaffected Control | Sibling         | Female | 1.7       |
| 0038 - CU | Unaffected Control | Parent          | Female | 38.0      |
| 0039 - CU | Unaffected Control | Parent          | Male   | 40.3      |
| 0041 - CU | Unaffected Control | Sibling         | Female | 3.7       |
| 0042 - CU | Unaffected Control | Parent          | Female | 35.3      |
| 0043 - CU | Unaffected Control | Parent          | Male   | 35.3      |
| 0045 - CU | Unaffected Control | Sibling         | Female | 9.2       |
| 0046 - CU | Unaffected Control | Parent          | Female | 39.3      |
| 0047 - CU | Unaffected Control | Parent          | Male   | 43.4      |
| 0049 - CU | Unaffected Control | Parent          | Female | 30.3      |
| 0050 - CU | Unaffected Control | Parent          | Male   | 33.4      |
| 0052 - CU | Unaffected Control | Sibling         | Male   | 4.5       |
| 0053 - CU | Unaffected Control | Sibling         | Female | 1.6       |
| 0054 - CU | Unaffected Control | Parent          | Female | 39.4      |
| 0055 - CU | Unaffected Control | Parent          | Male   | 40.8      |
| 0056 - CU | Unaffected Control | Sibling         | Male   | 1.5       |
| 0058 - CU | Unaffected Control | Parent          | Male   | 48.7      |
| 0060 - CU | Unaffected Control | Parent          | Female | 47.0      |
| 0061 - CU | Unaffected Control | Parent          | Male   | 48.2      |
| 0063 - CU | Unaffected Control | Sibling         | Female | 11.5      |
| 0064 - CU | Unaffected Control | Sibling         | Female | 6.4       |
| 0065 - CU | Unaffected Control | Parent          | Male   | 38.2      |
| 0067 - CU | Unaffected Control | Parent          | Female | 44.2      |
| 0068 - CU | Unaffected Control | Parent          | Male   | 54.7      |
| 0070 - CU | Unaffected Control | Sibling         | Male   | 5.1       |
| 0032 - CU | PWS Deletion      | Proband         | Female | 5.6       |
| 0036 - CU | PWS Deletion      | Proband         | Male   | 3.1       |
| 0040 - CU | PWS Deletion      | Proband         | Female | 1.6       |
| 0044 - CU | PWS UPD           | Proband         | Female | 6.5       |
| 0048 - CU | PWS Deletion      | Proband         | Male   | 5.2       |
| 0051 - CU | PWS Deletion      | Proband         | Male   | 6.1       |
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| 0057 - CU | PWS UPD | Proband | Female | 3.2 |   |
| 0059 - CU | PWS | Proband | Female | 7.7 |   |
| 0062 - CU | PWS UPD | Proband | Male | 8.4 |   |
| 0066 - CU | PWS UPD | Proband | Male | 6.5 |   |
| 0069 - CU | PWS UPD | Proband | Male | 2.3 |   |

Table S5. Human (Hu) and mouse (Mo) qRT-PCR primers used in this study.

| Primer Name | Sequence |
|-------------|----------|
| Hu MAP2 F   | CCACCTGAGATTAAGGATCA |
| Hu MAP2 R   | GGCTTACTTTGCTTCTCTGA |
| Hu DCX F    | TATGCGCCGAAGCAAGTCTCCA |
| Hu DCX R    | CATCCAAGGACAGAGGCAAGGTA |
| Hu SST F    | CCAGACTCCGTACATGCTTGCA |
| Hu SST R    | TTCCAGGGCATTCTCCCGTC |
| Hu TRH F    | GGCTTACTCCGTACATGCTTGCA |
| Hu TRH R    | TGTGCTGGGTACATCGACT |
| Hu VGF F    | ACAAGGTGTTTTTCGTCCGT |
| Hu VGF R    | TGGCTTTCGCTTCCATCG |
| Hu PENK F   | GCTTTTCAATTCGCTTGTC |
| Hu PENK R   | ATTCAGTTACGCCAGCCAGGA |
| Hu POMC F   | TACGGCCGTTTACATCGC |
| Hu POMC R   | GCCTTCTTGATGATGCGTT |
| Hu OXT F    | GGTGGTGAACAGAGCTCCACC |
| Hu OXT R    | GCCAGTTCTGGATGACAGG |
| Hu AGRP F   | ACAAGGTGAGGCAACAGGCAAGA |
| Hu AGRP R   | TGCAGGGTCGCTACCTCCGAA |
| Hu PCSK1 F  | TGATCCCAACAAACGAGGAA |
| Hu PCSK1 R  | ACCAGGTGCTGCATTCTCG |
| Hu PCSK2 F  | AGAGAGGGCCATGCAATTC |
| Hu PCSK2 R  | AGGTCTCTCCATCCTCCCTCA |
| Hu CHGB F   | CAGCAACGCTTCTTCGAC |
| Hu CHGB R   | CAGCGTACATCCATTTCCTCA |
| Hu CPE F    | TAAATTCAGGCTACCAGGC |
| Hu CPE R    | CCACTACAGGATTTACAG |
| Hu MAGEL2 F | AAATCGCTTGCTGAGTTC |
| Hu MAGEL2 R | CCAGCTTATGTTGGCAAGG |
| Hu GAPDH F  | GTCTCCCTCTGAACATCAGC |
| Hu GAPDH R  | ACCACCACTGTGGCTGAGCCA |
| Mo Sst F    | ACCCCAGACTCCGTAGTT |
| Mo Sst R    | TACCTGCGCAGTTCTCTTCC |
| Mo Trh F    | GTGTGCTGCTTATGGAGTTC |
| Mo Trh R    | TTGTCTGGTGGCAGTGC |
| Mo Vgf F    | AGTACAHCCATGACCTCC |
| Mo Vgf R    | GCTTAGCATGGCTCCGACTG |
| Gene       | Forward Primer  | Reverse Primer   |
|------------|----------------|-----------------|
| Mo Penk F  | GCGACATCAATTTTCCCTGGCGTG |                 |
| Mo Penk R  | CCTTGCAGGTTCTCCCAGATTT  |                 |
| Mo Pome F  | GCTTGCAAAACTCGACCTCTC  |                 |
| Mo Pome R  | TTTCCGGGGTTTTTGAGTCAG  |                 |
| Mo Oxt F   | GAGGAACACTACCTGCCCTTCG |                 |
| Mo Oxt R   | GTATTCCCAAGAAGTGGGCT  |                 |
| Mo Agrp F  | CATCTTCCACCTTTGCAGGATT |                 |
| Mo Agrp R  | AGCATGGCCCTTTTGGCTTGGCTG |                |
| Mo Pesk1 F | TGGAGTTTGCATATAATTCCAAAGTT |               |
| Mo Pesk1 R | AGGCTCAATGGCATCAGTAC  |                 |
| Mo Pesk2 F | GGCCTGTTTCATGCTTTTCTT |                 |
| Mo Pesk2 R | GCACAGTCAGATGGTGATGT  |                 |
| Mo Chgb F  | CACTCTCAAATGCCCTATCCA |                 |
| Mo Chgb R  | CACCTTGGACCTCTTTTTCCACT |              |
| Mo Cpe F   | AACCTTACAGCCCTCCCGTC |                 |
| Mo Cpe R   | CAAGCTCAAAATGCCCTCC |                 |
| Mo Magel2 F| TGGAGGATTTTCTAGAGGGCTA |               |
| Mo Magel2 R| TGCCGGAGTTGAGAGGGATT |                 |
| Mo Gapdh F | GTCTCCTCTTCTCAGACAGACGC |              |
| Mo Gapdh R | ACCACCCCTGTTGCTTAGCCAA |               |
Figure 1

A

Untreated
myc only
myc-Magel2
myc-MAGEL2

anti-MAGEL2

Untreated
myc only
myc-Magel2
myc-MAGEL2

anti-myc

GAPDH

B

Magel2

β-tub
Figure 2

D

| Magel2 | Gapdh | Chgb | Pcsk1 | Pcsk2 |
|--------|-------|------|-------|-------|
| +/+    | +/+   | +/+  | +/+   | +/+   |
| Magel2 | Magel2 | Magel2 | Magel2 | Magel2 |
| pΔ/m+  | pΔ/m+ | pΔ/m+ | pΔ/m+ | pΔ/m+ |

Note: Figure 2 shows the expression levels of Magel2, Gapdh, Chgb, Pcsk1, and Pcsk2 under different genotypes (+/+ and pΔ/m+) in the context of Magel2 knockout (Magel2−/−). The Western blot images indicate variations in protein expression across the conditions tested.
Figure 3

(C) 

| Ctrl #1 | Ctrl #2 | PWS #1 | PWS #2 |
|---------|---------|--------|--------|
| PCSK1   |         |        |        |
| MAGEL2  |         |        |        |
| GAPDH   |         |        |        |
| CHGB    |         |        |        |

(E) 

| Ctrl #1 | Days post NGN2 induction |
|---------|--------------------------|
| D0      | D3          | D7       | D10     | D14     |
| TUBB3   |             |          |         |         |
| PCSK1   |             |          |         |         |
| PCSK2   |             |          |         |         |
| CHGB    |             |          |         |         |
| CPE     |             |          |         |         |
| GAPDH   |             |          |         |         |
| MAP2    |             |          |         |         |
Figure 4

D

alpha-5

GAPDH

beta-1

alpha-v

GAPDH

H

|          | Ctrl #1 | Ctrl #2 | PWS #1 | PWS #2 |
|----------|---------|---------|--------|--------|
| M6PR     |         |         |        |        |
| alpha-5  |         |         |        |        |
| alpha-v  |         |         |        |        |
| beta-5   |         |         |        |        |
| GAPDH    |         |         |        |        |
Figure 5

A

Pcsk1

Pcsk2

ChgB

Gapdh

C

Pcsk1

Pcsk2

ChgB

Gapdh
Figure 6
Supplemental Figure 4

B

alpha-5

alpha-v

beta-1

GAPDH

GAPDH

GAPDH
Supplemental Figure 6

A

- **Wash**
- **Pcsk2**
- **Magel2**
- **Pcsk1**
- **Gapdh**
- **Chgb**

WT cKO