Supplementary Information for

Members of the KCTD family are major regulators of cAMP signaling

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Materials and Methods

Animals

All studies were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institute of Health. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) protocol (#16-032) at The Scripps Research Institute. Every effort was made to minimize the number of animals used in the following experiments. Animal studies are reported in compliance with the ARRIVE 2.0 guidelines.

Kctd5 heterozygous (Kctd5\(^{+/-}\)) mice were generated by the Knockout Mouse Phenotyping Program (KOMP) and obtained from The Jackson Laboratory (C57BL/6NJ-Kctd5\(^{em1(IMPC)J}\)/Mmjax; MMRRC Stock No: 51084-JAX). A colony of Kctd5 wild-type (Kctd5\(^{+/+}\)) and Kctd5 heterozygous (Kctd5\(^{+/-}\)) was obtained by heterozygous breeding at The Scripps Research Institute and all mice evaluated were littermates. After weaning, female and male mice were separately group-housed (3-5 per cage) by genotype in standard housing cages with ad libitum access to water and food. Male and female mice of each genotype were used in all behavioral tasks. Mice were maintained on a 12:12 h light/dark cycle with lights on from 6 a.m. to 6 p.m. and had ad libitum access to food and water. For zinc chelation treatment, TPEN (N, N, N', N'-Tetrakis (2-pyridylmethyl) ethylenediamine (Sigma-Aldrich) was freshly prepared in 10% ethanol in normal physiological saline (0.9% NaCl, Merek, Darmstadt, Germany) and administered under the nape skin of the animals. Mouse were treated for seven successive days at doses of 5 mg/kg body weight. As a control, an equivalent volume of 10% ethanol was administered daily for 7 days. The injection was performed 1 h before behavior assays.

Behavioral assessment

Mice were 8 weeks old at the beginning of the experiments. The design of the behavior test battery was adapted from previous reports (1-3), and tests were ordered from least to most stressful paradigms. Inter-test intervals were selected to allow mice to recover between tests fully. A 3-day interval was chosen between tests of the same category. All behavioural tests were performed during the light cycle between 8 am and 2 pm. Animals were housed in a dedicated sound-proof behavioural facility. In all the experiments, mice were habituated for one hour to the experimental room where the test was later performed. During this time, the handling was done by the same experimenter who performed the tests. Group sizes were equal and no animal was excluded from the study. After each test, the equipment was cleaned with 70% ethanol to prevent bias based on olfactory cues. Behavioral tests were performed according to the test order described below.

Open-Field Test (OFT)

The OFT was used as an additional measure of anxious-like behaviour, as well as to evaluate locomotor performance and exploratory activity, as described previously(4). The multiple-unit open field maze consisted of four activity chambers. Each chamber was made from high density and non-porous plastic and measured 50 x 50 x 38 cm\(^3\). The mouse was allowed to explore the apparatus for 80 min with 10 min bins. The time spent in the center and the total distance traveled were recorded using a digital video system and EthoVision XT16 software (Noldus, Netherlands) considering two previously defined areas: a central
and an outer arena. Distance traveled in each of the zones were recorded and analyzed. The arena was cleaned between each test using alcohol 70% to avoid interfering with the smell of the previously tested animal.

**Horizontal Pole test**
The fine motor balance of mice was assessed by measuring the ability of the mice to traverse a graded series of narrow beams to reach an enclosed safety platform, as described previously (5). Animals were successively tested on 3 different circular beams with a diameter of 15.8 (L), 11 (M), and 8 (S) mm. The beam was located 100 cm above a padded floor with 50 cm away from an enclosed box (20 cm square) into which the mouse could escape (6). During training, mice were placed at the start of the beam and trained over 3 days (3 trials per day) to traverse the beam to the enclosed box. Mice were placed on the beam and the time taken to transverse 50 cm of the beam, as predefined on the beam, was recorded. The time taken to traverse the beam and the number of times the hind feet slipped off on each beam were recorded for each trial. For each measurement, the mean scores of the three trials for each beam width were used in the analysis.

**Hindlimb clasping test**
Hindlimb clasping test was performed as described previously (7-9). Briefly, mouse was lifted in the air and grasped with tail near its base and observed for both limbs position for 30 s. The behavior was scored as follows: score 0 - if the hindlimbs were consistently splayed outward and away from the abdomen, score 1 – if one hindlimb was retracted towards the abdomen for more than 50% of the time suspended, score 2 – if both hindlimbs were partially retracted towards the abdomen for more than 50% of the time, and score 3 – if the hindlimbs were entirely retracted and touching the abdomen for more than 50% of the time suspended. Mouse was scored once a day for three days.

**Grip strength**
Grip Strength Meter (Ugo Basile, Italy) was used to evaluate mouse muscle force and limb strength in vivo (10). Mouse was suspended by its tail and its tendency to grasp a horizontal metal bar was recorded. The bar was attached to a force transducer, and the force produced during the pull on the bar was repeatedly.

**Ledge test**
Mouse gait was measured for the motor coordination and muscle function based on the method described previously (7). Briefly, mouse was placed individually on the lip of mouse home cage (Allentown Inc., Allentown, NJ, USA) and away from the investigator. Mouse behavior was observed from behind as it walked and the following scored were assigned: score 0 – if the mouse moves normally, with its body weight support all limbs, and both hindlimbs participating evenly, score 1 – if it appears to limp while walking, score 2 – if it shows severe limp, or the feet point away from the body during locomotion, score 3 – if the mouse has difficulty moving forward and drags its abdomen along the ground. The test was performed once a day for 2 days.
**Vertical pole test**
The vertical pole test was performed to assess motor dysfunction as described previously (11, 12) with slight modification. Mice were placed on the top of a 60 cm vertical pole with a diameter of 11 mm. The mouse was placed grasping the rod with four paws and the head pointing upwards. The pole was mounted on the home cage base stand and placed in the home cage with bedding to motivate mouse to descend to the floor of the cage. The behavior was recorded when mice began to turn completely downward (Tturn) and total time to descend the floor was recorded. Each mouse had to perform three consecutive trials with an inter-trial interval of 30 s. The ability of a mouse to turn 180° and climb down with the head pointing downwards was evaluated. The behavior was scored as follows: score 0 – mice turned around completely and climbed down the pole, score 1 - mice turns with difficulty, score 2 – mice slides down the pole, score 3 – mice fell off from the pole.

**Accelerating Rotarod test**
The accelerating rotarod test was performed using a five-station rotarod treadmill (IITC Life Sciences, USA) and used to access forelimb and hindlimb motor coordination as well as motor learning behavior with slight modifications (13). Each mouse was placed on a rotating drum with 3 cm diameter accelerating rotarod and latency to fall off from the rotarod was recorded. The speed of the rotarod accelerated from 4 to 40 r.p.m. over a 300-s time period. Training to the apparatus consisted of 10 trials per day with 300 s between intertrial interval for 5 consecutive days, by which time a steady baseline level of performance was attained. On the test day, the experiment was repeated, and the latency to fall off the rotarod was recorded. The trial was ended when the mouse fell off the rod of completed one full revolution or after 300 s elapsed. Learning rate was calculated as follows (mean latency to fall trials 9 and 10) – (mean latency to fall trials 1 and 2)/9 (number of intertrial intervals)(14).

**Reverse Rotarod test**
Motor capabilities in the mouse was evaluated by placing the mouse on the accelerated rotarod (IITC Life Science Inc., Woodland Hills, CA, USA) on 4-40 r.p.m. for 5 min. The mouse was made to walk backward and rotarod was fitted to ensure that mice were not able to turn around and walk forward (15, 16). Mice were tested thrice a day with 10 min inter trial interval for 3 consecutive days. For each day, the average time spent on the rotarod was calculated. For the TPEN administration experiments, mice were tested on three trials with 10 min inter trial interval on a single day.

**Plasmids/Cloning**
Gβ2 (GNB0200000), Gγ7 (GNG0700000), and GαoA (GNA0OA0000) were acquired from the cDNA Resource Center. pmCherry-N1 was acquired from Takara Bio (#632523). pAAV-hSyn-DIO-mCherry was acquired from Addgene (#50459). Grk3ct(17) and AC5(18) constructs have been previously reported. The following were obtained from Addgene: Slc39a14-GFP (104380), ubiquitin (18712), Cul3 (19893), and NEDD8 (159322). The Zip14 coding sequence was cloned into pcDNA 3.1 (EcoRI/XbaI sites) utilizing the In-Fusion HD Cloning Kit (Clontech, Mountain View, CA) as previously reported (19). KCTD5-flag was purchased from Origene (RC200180).
**Cell Culture**

HEK293T/17 cells were grown at 37°C in a humidified incubator containing 5% CO2 in DMEM supplemented with 10% FBS, minimum Eagle’s medium non-essential amino acids, 1 mM sodium pyruvate, 100 units/ml penicillin, and 100 µg/ml streptomycin. Cells were transfected with Lipofectamine PLUS and LTX reagents in Opti-MEM, as previously described (19). Where indicated, cells were incubated with bortezomib (100 nM), NaF (10 mM), MgCl2 (9 mM), and AlCl3 (30 µM) for 2 hours.

**CRISPR/Cas9 Lentivirus Production**

As previously described (20, 21), sgRNA sequences were selected utilizing Chopchop design tool (https://chopchop.cbu.uib.no/) (Supplementary Table 1). Oligos containing sgRNA targets (Integrated DNA Technologies, IA, USA) were phosphorylated with T4 Polynucleotide Kinase, annealed, and ligated with T4 DNA Ligase into the pSECC cloning vector (Addgene #60820). Three unique constructs were generated for each gene target and purified from Stbl3 E. coli. Lentiviral particles were generated from HEK293T/17 cells, which were seeded in 100 mm plates for transfection. All three sgRNA pSECC constructs per gene were transfected along with pCMV-VSV-G (Addgene #8454), pMDLg/pRRE (Addgene #12251), and pRSV-Rev (Addgene #12253). The supernatant containing the lentiviral particles was collected at 48 hr post-transfection. Viral titers of fresh supernatants ranged between 1x10⁷-1x10⁸ VP/ml as determined by QuickTiter Lentivirus Quantitation Kit (Cell Biolabs; VPK-112).

**Primary Culture**

As previously described (20, 21), P0 pups from CAMPER mice were sacrificed to remove the brain and isolate the striatum. Striata were dissected in cold HBSS (supplemented with 20% FBS, 4.2 mM NaHCO3, and 1 mM HEPES), washed in HBSS without FBS, and digested for 15 minutes at 37°C in pH 7.2 buffer containing (in mM): NaCl (137), KCl (5), Na₂HPO₄ (7), HEPES (25), and 0.3 mg/ml Papain (Worthington). The striata were then washed three times with HBSS (20% FBS), HBSS, and growth media (Neurobasal-A supplemented with 2 mM GlutaMAX, 2% B27 Supplement, 100 units/ml penicillin, and 100 µg/ml streptomycin). Tissue was then dissociated by mechanical pipette action through a standard P1000 pipette in growth media containing DNAse I (0.05 U/µl). Neurons were then plated on Poly-D-Lysine coated glass coverslips and cultures were maintained at 37°C/5% CO2 in a humidified incubator. At day in vitro 2 (DIV2), half of the media was replaced with fresh growth media containing pSECC lentiviral supernatant (50-100 µl). Half of the growth media was further replenished every three days for the duration of the culture. As previously described (22), Lipofectamine 2000 was used to co-transfect pmCherry-N1 or pAAV-hSyn-DIO-mCherry as a marker for studies utilizing overexpression of Gß2γ7, Grk3ct, and Zip14.

**Confocal Imaging**

Glass coverslips containing neuronal cultures were transferred to a recording chamber for live imaging and constantly perfused at 2 ml/min with a recording buffer, unless otherwise indicated, consisting of (in mM): CaCl2 (1.3), MgCl2 (0.5), MgSO4 (0.4), KH2PO4 (0.4), NaHCO3 (4.2), NaCl (138), Na₂HPO₄ (0.3), D-Glucose (5.6), and HEPES (20). All imaging experiments were recorded on a Leica TCS SP8 MP confocal microscope through
a 25x water-immersion objective lens. For cAMP imaging, the mTurquoise FRET donor was excited with a 442 nm diode laser paired with simultaneous emission collection from 465-505 nm (mTurquoise FRET donor) and 525-600 nm (Venus FRET acceptor). XYZT planes were acquired at 10 second intervals in order to quantify fluorescence intensity from neuronal cell bodies using ImageJ to calculate FRET from the inverse ratio of donor:acceptor. A cAMP standard curve from permeabilized CAMPER neurons was utilized to calculate absolute cAMP values (23). For zinc imaging, neurons were incubated with 1 µM Fluozin-3 AM (ThermoFisher #F24195) for 30 minutes before transfer to the recording chamber for imaging experiments. The Fluozin-3 sensor was excited by a 488 nm laser line paired with emission collected from 495-585 nm. XYZT planes were acquired at 15 second intervals in order to quantify fluorescence intensity change (ΔF) from neuronal cell bodies using ImageJ to calculate intensity (F) for reference to initial intensity (F0). Drugs were either applied in the bath solution or in a phasic puff utilizing a perfusion system pressure kit set to 1 PSI (Warner Instruments; VPP-6).

**Immunoprecipitation**

HEK293T/17 cells were transfected with AC5 and/or Zip14-GFP followed by sonication in lysis buffer (PBS supplemented with 150 mM NaCl, 0.5% n-dodecanoylsucrose, cOmplete protease inhibitor, and Sigma phosphatase inhibitor cocktail 1). Lysates were centrifuged at 14,000 x g for 15 minutes, supernatants were rotated for 1 hour at 4°C with 2 µg of antibody (anti-AC5 or anti-GFP) and 20 µl of Dynabeads Protein G. Samples were then washed three times in lysis buffer followed by elution in 2X SDS sample buffer and heated at 37°C for 15 minutes for Western blot analysis.

**Western Blot**

For tissue samples, three hours after the final TPEN injection dorsal striatal brain punches were harvested, snap frozen in liquid nitrogen, and stored at -80°C. Samples were homogenized in lysis PBS supplemented with 300 mM NaCl, 1% Triton X-100, cOmplete protease inhibitor. Primary culture and cell culture samples were resuspended in PBS supplemented with 150 mm NaCl, 1% Triton X-100, cOmplete protease inhibitor, and Sigma phosphatase inhibitor cocktail 1. Samples were then sonicated, centrifuged at 14,000 x g for 15 minutes, and supernatant protein concentration determined by Pierce 660 nm protein assay reagent (ThermoFisher). Samples were diluted to the same concentration, incubated at 37°C in SDS buffer for 15 minutes. Samples were resolved by SDS polyacrylamide gel electrophoresis, transferred to PVDF membranes, and incubated at room temperature for 1 hour in PBS containing 0.1% Tween-20 (PBST) with 5% dry non-fat milk. Membranes were then incubated with the following primary antibodies: rabbit anti-KCTD2/5/17 (ProteinTech #15553-1-AP), rabbit anti-KCTD8/16 (Novus Biologicals #NB1-86327), rabbit anti-KCTD12 (Novus Biologicals #NB1-80568) rabbit anti-Zip14 (Novus Biologicals #NB1-76510), rabbit anti-Gβ1(24), mouse anti-Gβ (H-1) (Santa Cruz sc-166123), mouse anti-Gαs/olf (A-5) (Santa Cruz sc-55545), mouse anti-AC5(25), mouse anti-GAPDH (Millipore Sigma #MAB374), mouse anti-GFP clones 7.1 and 13.1 (Millipore Sigma #11814460001), and rabbit anti-Flag (D6W5B) (Cell Signaling Technology #14793). Membranes were then washed in PBST, incubated with secondary antibodies conjugated to horseradish peroxidase in PBST (containing 1% milk), and
visualized bands with Kwik Quant Imager (Kindle Biosciences). ImageJ was then used to quantify band intensity.

**Data Analysis**
Statistical analysis was performed using GraphPad Prism 9. All data are represented as mean ± SEM. Student t-test and one-way ANOVA followed by indicated posttest were utilized as well as number of biological replicates are described in appropriate figure legends for each experimental comparison. The use of asterisks indicates statistical significance (* = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001). For behavioral experiments, a D’Agostino-Pearson test and Shapiro-Wilk normality tests were applied to evaluate data normality and homogeneity. Parametric statistics for normally distributed variables included one-way ANOVA and two-way repeated measures ANOVA for data followed over time. In addition, group differences using two factors or independent variables were evaluated by two-way ANOVA. Sidak’s *post-hoc* for multiple comparisons was applied when the main effects of factor were significant in the ANOVA analysis. A non-parametric test (Spearman rank, $R$) was used to check correlations when one of the variables was not normally distributed. Kruskal-Wallis non-parametric test followed by Dunn’s multiple comparison test was applied for the data that was not normally distributed. An alpha level of 0.05 was used to determine the statistical significance for all tests.
Supplemental Figures

Fig. S1. KCTD proteins regulate stimulatory GPCR inputs to cAMP signaling in striatal neurons.

A) Western blot quantification of KCTD level from primary neurons subject to CRISPR/Cas9 sgRNA. n=4 cultures/group; One-way ANOVA Dunnett comparison test to control sgRNA; * p<0.05, ** p<0.01.

B) Adenosine → A2AR dose-response curve. n≥10 neurons/dose.
C) Max A2AR response amplitude from 100 µM adenosine. n≥10 neurons/group; One-way ANOVA; *** p<0.001.
D) A2AR response EC50 from adenosine. n≥10 neurons/dose; One-way ANOVA; * p<0.05.
E) Averaged D1R-mediated cAMP responses to a phasic puff of 100 µM dopamine. n≥14 neurons/group.
F) Dopamine → D1R dose-response curve. n≥14 neurons/dose.
G) Max D1R response amplitude from 100 µM dopamine. n≥14 neurons/group; One-way ANOVA; **** p<0.0001.
H) D1R response EC50 from dopamine. n≥14 neurons/dose; One-way ANOVA; **** p<0.0001.
I) Averaged A2AR-mediated cAMP responses to a phasic puff of 100 µM adenosine. n≥12 neurons/group.
J) Adenosine → A2AR dose-response curve. n≥12 neurons/dose.
K) Max A2AR response amplitude from 100 µM adenosine. n≥12 neurons/group; One-way ANOVA; **** p<0.0001.
L) A2AR response EC50 from adenosine. n≥12 neurons/dose; One-way ANOVA; **** p<0.0001.
M) Western blot quantification of KCTD5 level from primary neurons subject to CRISPR/Cas9 with individual sgRNA sequences. n=3 cultures/group; One-way ANOVA Dunnett comparison test to control sgRNA; *** p=0.0001.
N) Baseline cAMP values in D1R+ striatal neurons classified by directionality of response to dopamine. n≥6 neurons/group; One-way ANOVA; **** p<0.0001.
O) Max cAMP response amplitude to bath application of 100 µM forskolin in striatal neurons. n≥13 neurons/group; One-way ANOVA; **** p<0.0001.
P) Representative D1R-mediated cAMP responses to a phasic puff of 100 µM dopamine.
Q) Max D1R response amplitude from 100 µM dopamine. n≥6 neurons/group; One-way ANOVA; **** p<0.0001.

All Data represented as mean ± SEM.
Fig. S2. KCTD8, KCTD12, and KCTD16 collectively regulate inhibitory GPCR inputs to cAMP signaling in striatal neurons.

A) D2R antagonist raclopride blocks D2R dopamine-mediated cAMP signaling. Representative trace from three independent experiments.

B) D2R antagonist raclopride did not influence D1R dopamine-mediated cAMP signaling. Representative trace from three independent experiments.

C) Adenosine → A1R dose-response curve. n≥9 neurons/dose.

D) Max A1R response amplitude from 100 µM adenosine. n≥9 neurons/group.

E) A1R response EC50 from adenosine. n≥9 neurons/dose.

F) Averaged D2R-mediated cAMP responses to a phasic puff of 100 µM dopamine. n≥16 neurons/group.

G) Dopamine → D2R dose-response curve. n≥16 neurons/dose.

H) Max D2R response amplitude from 100 µM dopamine. n≥16 neurons/group; One-way ANOVA, **** p<0.0001.

I) D2R response EC50 from dopamine. n≥16 neurons/dose.

J) Averaged A1R-mediated cAMP responses to a phasic puff of 100 µM adenosine. n≥11 neurons/group.

K) Adenosine → A1R dose-response curve. n≥11 neurons/dose.

L) Max A1R response amplitude from 100 µM adenosine. n≥11 neurons/group; One-way ANOVA, **** p<0.0001.
M) A1R response EC50 from adenosine. n≥11 neurons/dose. All Data represented as mean ± SEM.
Fig. S3. KCTD5 sensitizes A2AR cAMP signaling in striatal neurons through Gβγ regulation.
A) Western blot quantification of Gβ level from primary striatal neurons subject to CRISPR/Cas9 sgRNA. n=4 cultures/group; One-way ANOVA Dunnett comparison test to control sgRNA; * p<0.05, ** p<0.01, *** p<0.001.
B) Representative A2AR-mediated cAMP responses to a phasic puff of 100 µM adenosine (left), with Grk3ct overexpression (Gβγ block; middle) or Gβγ overexpression (+Gβγ; right). n≥9 neurons/group.
C) Max A2AR response amplitude from 100 µM adenosine. n≥9 neurons/group; non-parametric Mann-Whitney test; * p<0.05, ** p<0.01, **** p<0.001.
D) Adenosine → A2AR dose-response curve with Grk3ct overexpression (Gβγ block; left) or Gβγ overexpression (+Gβγ; right). n≥9 neurons/dose.
E) A2AR response EC50 from adenosine. n≥9 neurons/dose; non-parametric Mann-Whitney test; *** p<0.001, **** p<0.0001.
All Data represented as mean ± SEM.
Fig. S4. Baseline motor behavior comparison between *Kctd5*<sup>+/+</sup> and *Kctd5*<sup>-/-</sup> mice.

A) Daily performance of *Kctd5*<sup>+/+</sup> (n=10) and *Kctd5*<sup>-/-</sup> (n=10) mice on the accelerating rotarod over 5 days (10 trials/day).

B) Grip strength for *Kctd5*<sup>+/+</sup> (n=10) and *Kctd5*<sup>-/-</sup> (n=10) mice. Unpaired t-test; parametric; p=0.1169.

C) Distance in open field test in 80 minutes for *Kctd5*<sup>+/+</sup> (n=10) and *Kctd5*<sup>-/-</sup> (n=10) mice. Unpaired t-test; parametric; ns p>0.05.
D) Sex differences in total distance traveled in 80 minutes. Two-way ANOVA to analyze sex difference; Sidak multiple comparison test; ns p>0.05.

E) Velocity in open field test over 80 minutes for $Kctd5^{+/+}$ (n=10) and $Kctd5^{+/−}$ (n=10) mice. Unpaired t-test; parametric; ns p>0.05.

F) Sex differences in average velocity over 80 minutes. Two-way ANOVA to analyze sex difference; Sidak multiple comparison test; ns p>0.05.

G) Time in center over 80 minutes for $Kctd5^{+/+}$ (n=10) and $Kctd5^{+/−}$ (n=10) mice. Unpaired t-test; parametric; ns p>0.05.

H) Sex differences in time in center over 80 minutes for $Kctd5^{+/+}$ and $Kctd5^{+/−}$ mice. Two-way ANOVA to analyze sex difference; Sidak multiple comparison test; ns p>0.05.

I) Time in corner over 80 minutes for $Kctd5^{+/+}$ (n=10) and $Kctd5^{+/−}$ (n=10) mice. Unpaired t-test; parametric; ns p>0.05.

J) Sex differences in time in corner over 80 minutes for $Kctd5^{+/+}$ and $Kctd5^{+/−}$ mice. Two-way ANOVA to analyze sex difference; Sidak multiple comparison test; ns p>0.05

All Data represented as mean ± SEM.
Table S1. CRISPR/Cas9 targeting sequences.

| Target  | sgRNA Target Sequence                  |
|---------|----------------------------------------|
| Kctd2   | CGTCATAGAGCGAGCGAAA                    |
| Kctd2   | ATCCTAAACTACCTCCGCCA                   |
| Kctd2   | GCCCGTGGAAGCAGCGTGTAC                  |
| Kctd5   | TATTAACAAAGACCTGCAG                    |
| Kctd5   | GGGATCTCTGTCGATTAAAT                   |
| Kctd5   | ACACGGGTACACATGCTTCAC                  |
| Kctd8   | GCGGACACCATCTCGCTGAT                   |
| Kctd8   | CGCTTCTTCATCGACCGCGA                   |
| Kctd8   | AGCGACGCACTGCTGCTGCG                   |
| Kctd12  | CGAGTCGCGCAGACGACACCA                  |
| Kctd12  | AGCGTCGCGCGCGATGGTGT                   |
| Kctd12  | GGAGCTCGTGTCGCCTCG                     |
| Kctd16  | GCTTCTTACGACCGCAAGT                    |
| Kctd16  | GATTCTGCTGTCGCTTTCC                    |
| Kctd16  | CCGGAGAGTCCCGCCGATTT                   |
| Kctd17  | AGACAACGGCGGCCGCGATG                   |
| Kctd17  | GCGAAGAGCCTCAATCGGAC                   |
| Kctd17  | ACTCGGTATACATGCTTTAGG                  |
| Slc39a14| TCAAGTTGTGCGCCGCAAAG                   |
| Slc39a14| ATGACCTTCTCGTGTCGGCC                   |
| Slc39a14| GGGGTCCGCTACTGATAT                    |
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