

Allosteric modulation of solute carrier transporter SLC39A8 potentiates manganese and cadmium uptake

Kelly L. Damm-Ganamet¹, Clara Moon², Alan D. Wickenden¹, Mark Tichenor¹, Yunhui Ge¹, Eduardo V. Mercado-Marin¹, Brian Chiou¹, Ayla Manughian-Peter², Taraneh Mirzadegan¹, Jennifer D. Venable¹, Ramnik J. Xavier^{3,4}, Jennifer E. Towne², Daniel B. Graham^{3,4,*}, Jacqueline Perrigoue^{5,*}

¹Therapeutics Discovery, Johnson & Johnson, 3210 Merryfield Row, San Diego, CA, 92121, USA

²Discovery Immunology, Johnson & Johnson, 3210 Merryfield Row, San Diego, CA, 92121, USA

³Broad Institute of MIT and Harvard, Cambridge, MA, 02142, USA

⁴Center for Computational and Integrative Biology, Department of Molecular Biology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, 02114, USA

⁵Discovery Immunology, Johnson & Johnson, 1400 McKean Rd, Spring House, PA, 19477, USA

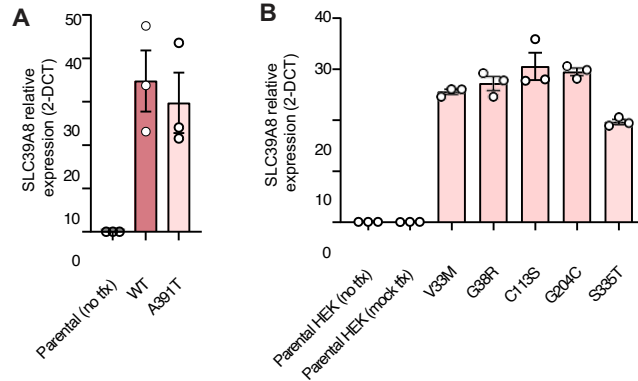
**Corresponding authors: Jacqueline Perrigoue (jperrigo@its.jnj.com) and Daniel B. Graham (dgraham@broadinstitute.org)*

Supplemental Information contains:

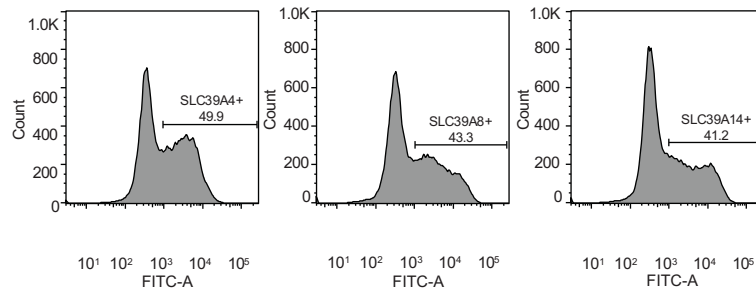
Supplemental Figures 1-9

Supplemental Table 1

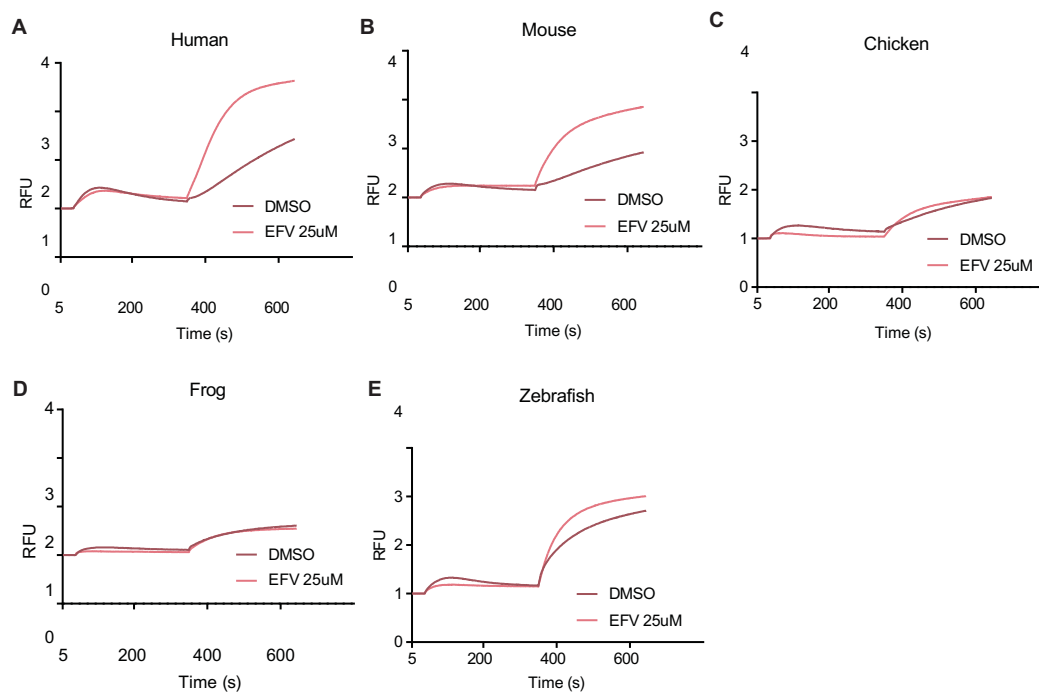
Supplemental materials and Methods including chemistry, plasmid constructs, and cell culture reagents



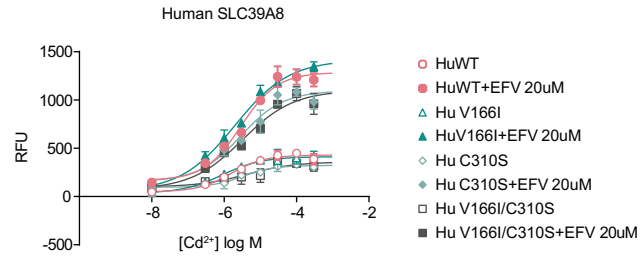
Supplemental Figure 1. *SLC39A8* gene expression in HEK293T cells after transient transfection. HEK293T cells were transiently transfected to express WT or CDG mutant *SLC39A8*. **A.** For parental HEK293T as well as WT and A391T transfected cells, *SLC39A8* mRNA expression was quantified using SYBR chemistry with custom designed primers as codon-optimized constructs were utilized. Data were normalized to the housekeeping gene *GAPDH*. **B.** *SLC39A8* mRNA expression was quantified using Taqman chemistry with commercially available probes for parental HEK293T cells as well as V33M, G38R, C113S, G204C, and S335T variant transfected cells. Data were normalized to *GAPDH*. No tfx = no transfection; mock tfx = mock transfection.



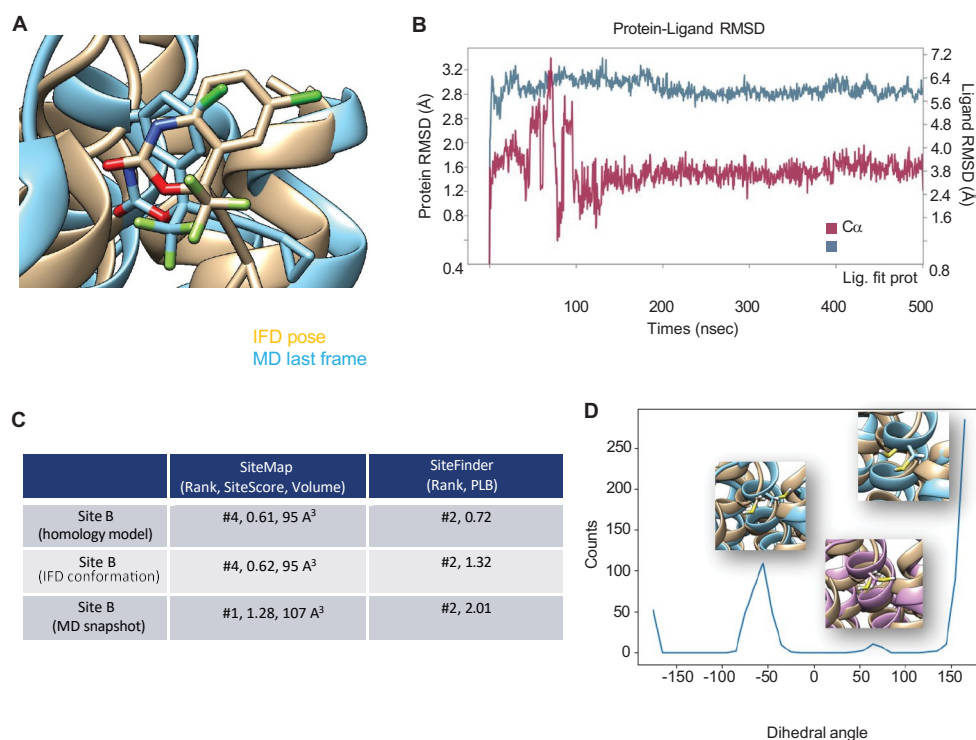
Supplemental Figure 2. Functional expression of SLC39A4, SLC39A8, and SLC39A14 by fluozin-3 uptake by flow cytometry. HEK293T cells were transiently transfected to express SLC39A4, SLC39A8, or SLC39A14. Intracellular zinc was measured using FluoZin-3 cell permeant Zn indicator dye to assess zinc uptake by these transporters.



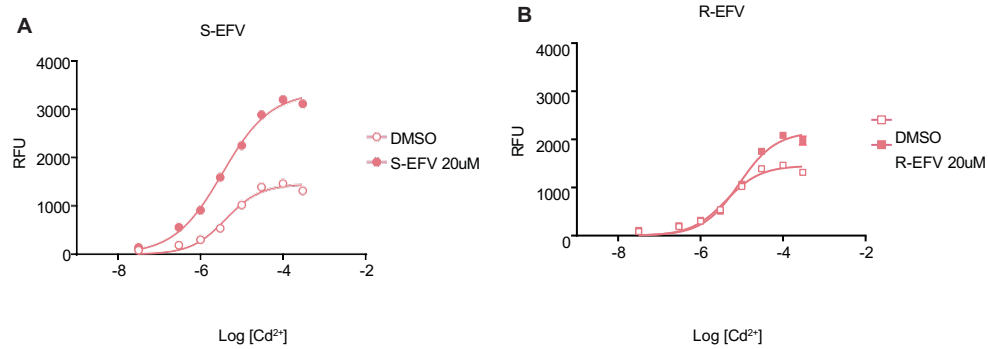
Supplemental Figure 3. Efavirenz potentiates human and mouse SLC39A8. A-E. Efavirenz potentiates human and mouse SLC39A8 (A-B), but not chicken (C), frog (D), or zebrafish (E).



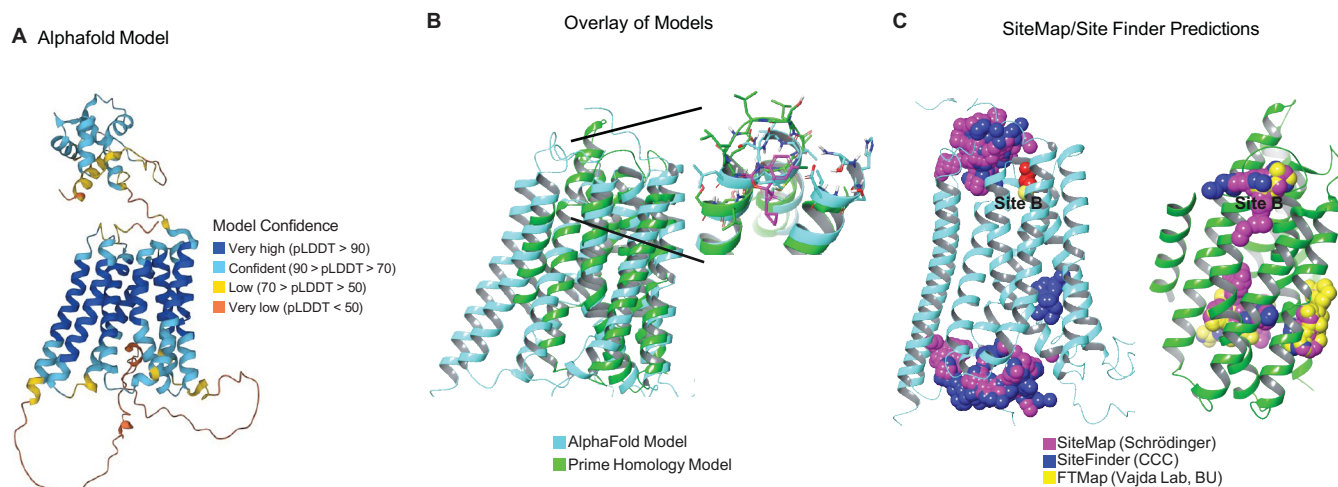
Supplemental Figure 5. Mutagenesis data reveals mutations in putative metal exit pathway does not alter EFV sensitivity. SLC39A8 mutations V166I, C301S, and V166I/C301S do not alter EFV sensitivity by cadmium uptake. Hu = human; EFV = efavirenz; WT = wild type; RFU = relative fluorescence units.



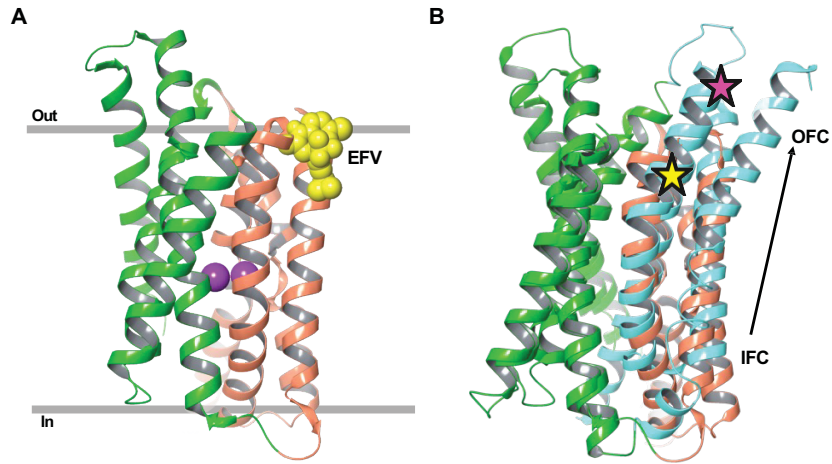
Supplemental Figure 6. Representative molecular dynamics stimulation of EFV in complex with hSLC39A8. Induced fit docking pose used as starting coordinates. **A.** Overlay of IFD pose with last frame of MD simulation; RMSD is 3.127 Ang. **B.** Protein and ligand RMSD across 500 ns MD trajectory. **C.** Pocket identification assessments from SiteMap, SiteFinder and FTMap for Sites A-C. SiteMap and SiteFinder ranking and scores improve after conformational sampling. **D.** Cys326 turns away from pocket in majority simulation.



Supplemental Figure 7. Potentiation of SLC39A8 by R-EFV and S-EFV. R-EFV is less effective at potentiating SLC39A8, showing a 70% reduction in Emax compared to S-EFV. EFV = efavirenz; RFU = relative fluorescence units.



Supplemental Figure 8. Comparison of Prime homology model to AlphaFold prediction. A. AlphaFold model. **B.** Overlay of Prime (green) and AlphaFold (cyan) models. RMSD = 3.31 (Å). Site B is shown zoomed in. **C.** SiteMap and SiteFinder predictions for Prime (green) and AlphaFold (cyan) models. Site B is highlighted on both models and Cys326 is shown in red spheres on the AlphaFold model to demonstrate where the pocket would be located had it been identified by SiteMap or SiteFinder. SiteMap spheres are shown in pink, SiteFinder in blue and FTMap probes in yellow.



Supplemental Figure 9. Mapping of putative EFV binding site onto the transport domain for both inward- facing and outward-facing conformations. **A.** ZIP proteins function with a two-domain structure, comprising a static scaffold domain (green) and a transport domain (pink), shown here in the inward-facing conformation (IFC). The putative EFV (shown in yellow sphere representation) binding site is located in the transport domain, facing away from the scaffold interface. Metal ions (shown in purple sphere representation) occupy the putative metal binding site. **B.** The scaffold domain remains static (green) between the inward-facing (IFC) and outward-facing (OFC) conformations whereas the transport domain (IFC pink; OFC cyan) moves vertically (highlighted with arrow) to facilitate the transfer of zinc ions (purple) across the membrane. The putative EFV binding pocket location is highlighted on the IFC (yellow star) and OFC (fuchsia star). The OFC was modeled using the ZIP13 AlphaFold prediction.

Supplemental Table 1: Summary of cadmium uptake experiments.

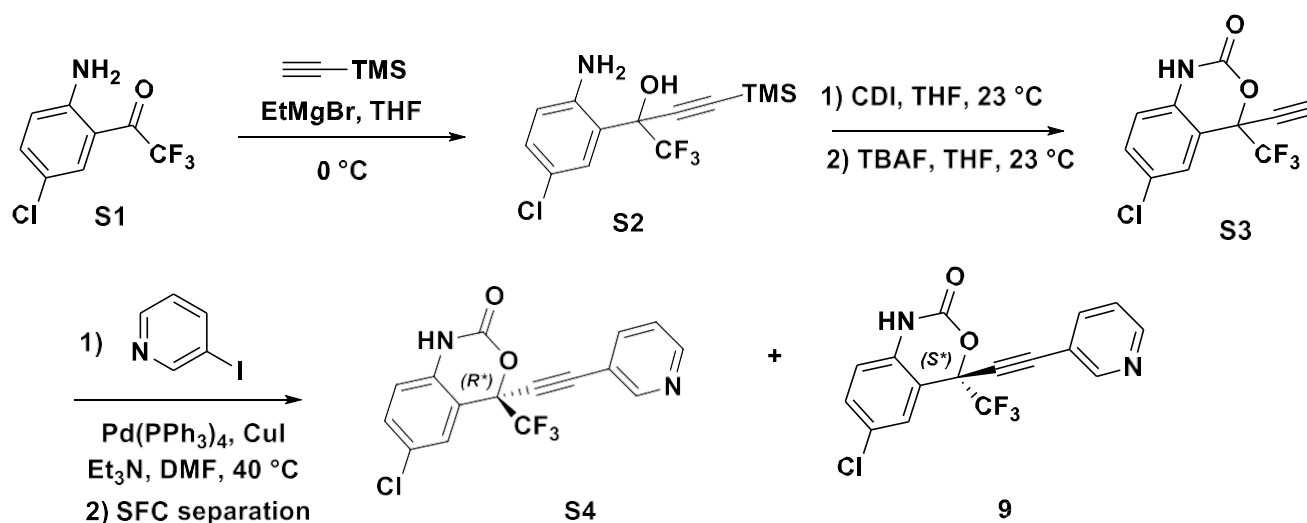
Cadmium uptake experiments

n=28	Control	Efavirenz (20uM)	P-value
Cd EC50 (uM)	3.4 +/- 0.29	4.5 +/- 0.39	0.0014
Slope	1.2 +/- 0.06	0.99 +/- 0.04	0.0012
E _{max}	607 +/- 71	1710 +/- 187	<0.0001

Supplementary Information: Chemistry Methods

General materials and methods for chemical synthesis

All solvents and chemicals were used as purchased without further purification. Nuclear magnetic resonance spectra were obtained on Bruker model DRX spectrometers. Chemical shifts (δ) are expressed in parts per million, relative to internal standard tetramethylsilane; coupling constants (J) are in Hz. The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), m (multiplet). HPLC–MS chromatograms and spectra were obtained using one of the following methods: (1) Agilent 1200 HPLC and G6100 system on X-Bridge ShieldRP18 (50×2.1 mm, $5 \mu\text{m}$) and a gradient system of 0.05% NH_4OH in $\text{H}_2\text{O}/\text{CH}_3\text{CN}$, 100:0 to 5:95 over 7.5 min, then 100:0 for 2.5 min at a temperature of 40°C ; (2) Agilent 1200 HPLC and G6100 system on Phenomenex Luna-C18 (50×2 mm, $5 \mu\text{m}$) and a gradient system of 0.1% TFA in $\text{H}_2\text{O}/0.05\%$ TFA in CH_3CN , 100:0 to 15:85 over 7.5 min, then 100:0 for 2.5 min at a temperature of 50°C ; or (3) Agilent 1100 HPLC and G1367A system on X-Bridge C18 (100×3 mm, $3.5 \mu\text{m}$) and a gradient system of 20 mM NH_4OH in $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ 90:10 over 2 min, then 0:100 for 1 min at a flow rate of $2.4 \text{ mL}/\text{min}$ at a temperature of 45°C . All compounds tested were a minimum of 95% purity as determined by HPLC.



S2: 2-(2-amino-5-chlorophenyl)-1,1,1-trifluoro-4-(trimethylsilyl)but-3-yn-2-ol

To a solution of trimethylsilylacetylene (4400 mg, 44.8 mmol) in THF (45 mL) was cooled to 0 °C and then ethylmagnesium bromide (3 M in diethylether, 15 mL, 44.8 mmol) was added slowly and the resulting solution was stirred at 0 °C for 1 hour and then warmed to 23 °C for 1 hour. The mixture was then cooled to 0 °C and 1-(2-amino-5-chlorophenyl)-2,2,2-trifluoroethanone (5000 mg, 23.4 mmol, **S1**) was added portion wise over 5 minutes. The reaction was stirred at 0 °C for 1 hour and then warmed to 23 °C and stirred for 2 hours. The reaction was then quenched by the addition of sat. aq. NH₄Cl (40 mL). The layers were then separated and the aqueous was extracted with EtOAc (3 x 30 mL). The combined organics were dried with MgSO₄, filtered, and concentrated in vacuo. The crude material was purified by silica gel chromatography using 0-100% EtOAc in hexanes to give the title compound (6.16 g, 85% yield) as a white solid. ¹H NMR (600 MHz, DMSO) δ 8.22 (s, 1H), 7.39 (d, J = 2.5 Hz, 1H), 7.11 (dd, J = 8.7, 2.6 Hz, 1H), 6.68 (d, J = 8.7 Hz, 1H), 5.64 (s, 2H), 0.24 (s, 9H). LCMS (ESI) mass calcd for C₁₃H₁₅ClF₃NOSi, 321.06; m/z found; 322.1 [M+H]⁺.

S3: 6-chloro-4-ethynyl-4-(trifluoromethyl)-1,4-dihydro-2H-benzo[d][1,3]oxazin-2-one

To a solution of 2-(2-amino-5-chlorophenyl)-1,1,1-trifluoro-4-(trimethylsilyl)but-3-yn-2-ol (**S2**) (6.00 g, 18.6 mmol) in THF (150 mL) was added CDI (7.56 g, 46.6 mmol) and the resulting mixture was then heated to 55 °C for 16 hours. The mixture was concentrated in vacuo then diluted with water (150 mL), sat. aq. NaHCO₃ (100 mL) and EtOAc (100 mL). The layers were separated and the aqueous extracted with EtOAc (2 x 100 mL). The combined organics were washed with 0.4 N HCl (2 x 50 mL), followed by 10 wt% brine (1 x 50 mL) then dried with MgSO₄, filtered

and to the organic layer was concentrated in vacuo. The crude material was diluted in THF (150 mL) and cooled to 0 °C and then TBAF (1.0 M in THF, 18.4 mL, 18.4 mmol) was added. After 1 hour, the mixture was warmed to 23 °C and then concentrated in vacuo. The crude material was dissolved in EtOAc (150 mL) and washed with 13 wt% NaCl (2 x 50 mL), then the organics were dried with MgSO₄, filtered, and concentrated in vacuo. The crude material was purified by silica gel chromatography using 0-60% EtOAc in hexanes to give the title compound (4.20 g, 83 % yield) as a white powder. ¹H NMR (600 MHz, DMSO) δ 11.18 (s, 1H), 7.60 (dd, J = 8.6, 2.3 Hz, 1H), 7.48 (d, J = 2.3 Hz, 1H), 7.03 (d, J = 8.6 Hz, 1H), 4.61 (s, 1H). LCMS (ESI) mass calcd for C₁₁H₅ClF₃NO₂, 274.9; m/z found; 276.0 [M+H]⁺.

Synthesis of compound S4 and 9: (R)-6-chloro-4-(pyridin-3-ylethynyl)-4-(trifluoromethyl)-1,4-dihydro-2H-benzo[d][1,3]oxazin-2-one and (S)-6-chloro-4-(pyridin-3-ylethynyl)-4-(trifluoromethyl)-1,4-dihydro-2H-benzo[d][1,3]oxazin-2-one.

To a solution of 6-chloro-4-ethynyl-4-(trifluoromethyl)-1,4-dihydro-2H-benzo[d][1,3]oxazin-2-one (**S3**) (3.80 g, 13.8 mmol), 3-iodopyridine (3.10 g, 15.2 mmol), triethylamine (5.3 mL, 38.4 mmol), Pd(PPh₃)₄ (796 mg, 0.69 mmol) and CuI (131 mg, 0.69 mmol) in DMF (40 mL) was evacuated and backfilled with inert N₂ (3x) and then the mixture was stirred at 40 °C for 4 hours. The mixture was cooled to 23 °C and diluted with water (120 mL) and EtOAc (40 mL). The layers were separated and the aqueous extracted with EtOAc (2 x 40 mL). The combined organics were dried with MgSO₄, filtered, and concentrated in vacuo. The crude material was purified by silica gel chromatography using 0-50% EtOAc in hexanes to give racemate (3.56 g, 73% yield) as a pale-yellow solid. The racemate was purified by chiral SFC (Stationary phase: Chiralpak AD-H 5µm

250 x 30 mm, Mobile phase: 15% methanol: isopropanol (1:1) with 0.2% isopropylamine, 85% CO₂) to give compound **S4** (1.15 g) and compound **9** (1.30 g) as the first and second eluting peaks, respectively. The stereochemistry was tentatively assigned S for the active enantiomer, corresponding to the active S-enantiomer of EFV. ¹H NMR and LCMS were identical for both compounds: ¹H NMR (600 MHz, DMSO) δ 11.21 (s, 1H), 8.87 (s, 1H), 8.70 (d, J = 4.3 Hz, 1H), 8.13 (dt, J = 7.9, 1.9 Hz, 1H), 7.73 (d, J = 2.3 Hz, 1H), 7.61 (dd, J = 8.6, 2.3 Hz, 1H), 7.52 (ddd, J = 7.9, 4.9, 0.8 Hz, 1H), 7.06 (d, J = 8.6 Hz, 1H). LCMS (ESI) mass calcd for C₁₆H₈ClF₃N₂O₂, 352.0; m/z found; 353.0 [M+H]⁺.

Abbreviations: TMS – trimethylsilyl, CDI – 1,1'-carbonyldiimidazole, TBAF – tetrabutylammonium fluoride, THF – tetrahydrofuran, DMF - *N,N*-dimethylformamide

Supplementary Information: qPCR methods for supplemental Figure S1

RNA Extraction

Frozen cell pellets were placed on ice and lysed in Qiagen RLT Plus Lysis Buffer (Qiagen 1053393) containing 0.01% β -mercaptoethanol (Sigma-Aldrich 444203). Cell pellets were homogenized using a p1000 pipet. The RNA extraction was performed according to the manufacturer's instructions using the RNeasy Miniprep Plus Kit (Qiagen 95134). Samples were eluted in 35 μ l nuclease free water, and concentrations were measured by Nanodrop.

qRT-PCR: Taqman Chemistry

cDNA synthesis and qPCR were performed in a single reaction using the qScript XLT One-Step RT-qPCR ToughMix (QuantaBio 95134) and Taqman primer-probe sets (ThermoFisher 431182), using 20 ng RNA for each reaction. The reaction was performed in a total volume of 20 μ l as follows: step 1) 15 min at 50°C; step 2) 1 min 95°C; step 3) 15 sec at 95°C; step 4) 1 min at 60°C; repeat steps 3-4 for 40 cycles. The resulting data was analyzed using the Applied Biosystems Expression Suite Software. Data were normalized to housekeeping GAPDH. Taqman probe information can be found in **Supplemental Table S2**.

qRT-PCR: SYBR Chemistry

100 ng of RNA was used for each sample to perform cDNA synthesis using the High-Capacity cDNA Reverse Transcription kit (Thermo 4374966). This reaction was performed in a total volume of 20 μ l. The cDNA synthesis PCR cycle was as follows: step 1) 10 min at 25°C; step 2) 120 min at 37°C; step 3) 5 min at 85°C; step 4) hold at 4°C.

The resulting cDNA was used for qPCR. The reaction was performed using SsoAdvanced Universal SYBR Green Supermix (BioRad 1725272) and custom primers from Integrated DNA Technologies as follows: step 1) 30 sec at 95°C; step 2) 15 sec at 95°C; step 3) 30 sec at 60°C; repeat steps 2-3 for 40 cycles; step 4) 15 sec at 95°C, followed by continuous melt curve from 60°C -95°C, 0.05°C/s. The resulting data was analyzed using the Applied Biosystems Expression Suite Software. Data were normalized to housekeeping GAPDH. Primer sequence information can be found in **Supplemental Table 2**.

Supplemental Table 2. SLC39A8 detection reagents

Gene name	Taqman Probe ID	Forward Primer (SYBR)	Reverse primer (SYBR)
SLC39A8	Hs00223357_m1	CAACACCTGCTGGAACAGA	GAACTTGCTGCTGGTGATCT
GAPDH	Hs99999905_m1	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG

Supplementary Information: Plasmid Construct

1. SLC39A2 (Human)

Gene name: SLC39A2, Length: 945 bp

Sequence:

```
GGTACCACCATGGAGCAGCTGCTGGGCATCAAGCTGGGCTGCCTGTTTGA  
CTGCTGGCCCTGACCCTGGGATGC  
GGACTGACACCTATCTGTTTTAAGTGGTTCCAGATCGACGCCGCCAGAG  
GCCACCACAGACTGGTGCTGAGGCTG  
CTGGGCTGTATCAGCGCCGGCGTGTCTGGGAGCAGGCTTCATGCACATG  
ACCGCAGAGGCCCTGGAGGAGATC  
GAGTCCCAGATCCAGAAGTTCATGGTGCAGAACCGCTCCGCCTCTGAGCG  
GAATAGCTCCGGCGACGCAGATAGC  
GCCCACATGGAGTACCCTTATGGCGAGCTGATCATCTCTCTGGGCTTCTT  
TTTCGTGTTTTCTGGAGAGCCTGGC  
CCTGCAGTGTCTCAGGCGCAGCAGGCGGCTCCACAGTGCAGGATGAGGAG  
TGGGGAGGCGCCACATCTTTG  
AGCTGCACTCTACGGCCACCTGCCAAGCCCTTCCAAGGGACCACTGCGG  
GCCCTGGTGCTGCTGTCTCTGAG  
CTTTCACAGCGTGTTCGAGGGCCTGGCAGTGGGACTGCAGCCTACCGTG  
GCAGCAACAGTGCAGCTGTGCCTGGC  
CGTGCTGGCACACAAGGGACTGGTGGTGTGGCGTGGGCATGAGACTGGT  
GCACCTGGGCACCTCTAGCAGGT  
GGGCCGTGTTCTCCATCCTGCTGCTGGCCCTGATGTCTCCACTGGGCCT  
GGCCGTGGCCGTGGCCGTGACAGGCG  
GCGACTCCGAGGGCGGCAGAGGACTGGCACAGGCCGTGCTGGAGGGCGT  
GGCCGCCGGCACCTTCTGTACGTG  
ACATTCTGGAGATCCTGCCAGAGAGCTGGCAAGCCAGAGGCACCACTGG  
CAAAGTGGTCTGCGTGGCCGCC  
GGCTTGCCTTCATGCGCTTCATCGCCCTGTGGGCCTAATCTAGA
```

1. SLC39A4 (Human)

Gene name: SLC39A4, Length: 1959 bp

Sequence:

```
GGTACCACCATGGCCTCTCTGGTGAGCCTGGAGCTGGGACTGCTGCTGGCC  
GTGCTGGTGGTGACCGCAACAGCA  
AGCCACCTGCAGGACTGCTGTCTGCTGACAAGCGGACAGGGCGCCCTGG  
ATCAGGAGGCCCTGGGAGGACT  
GCTGAACACCCTGGCAGACAGGGTGCCTGCGCAAATGGACCCTGCGGCA  
AGTGTCTGTCTGTGGAGGATGCCCT  
GGGCCTGGGCGAGCCAGAGGGCAGCGGACTGCCACCCGGCCCTGTGCTG  
GAGGCAAGATACGTGGCAAGGCTG  
AGCGCCGCCCGTGTCTGATCTGAGCAACCCTGAGGGCACCTGTGAGGAT  
GCCAGAGCCGGACTGTGGGCATC  
CCACGCAGACCACCTGCTGGCCCTGCTGGAGTCCCCAAAGGCCCTGAC  
ACCCGGCCTGTCTTGGCTGCTGCAGAG  
GATGCAGGCCAGAGCCGCAGGACAGACCCCAAAGATGGCATGCGTGGA  
CATCCCCAGCTGCTGGAGGAGGCCG  
TGGGAGCAGGCGCCCCAGGCAGCGCCGGAGGCGTGTGGCCGCCCTGCT  
GGATCACGTGCGGTCTGGCAGCTGT  
TTCCACGCCCTGCCATCCCCCAGTACTTTGTGGACTTCGTGTTTCAGC  
AGCACAGCTCCGAGGTGCCATGACCCT  
GGCAGAGCTGAGCGCCCTGATGCAGAGGCTGGGAGTGGGAAGGGAGGC  
ACACTCCGATCACTCTCACAGGCACA  
GGGGAGCCTTAGCCGGGACCCTGTGCCACTGATCTCCTTAGCAATTCT  
CTAGCGTGTGGGATACAGTGTGCCT  
GTCCGCCAGAGACGTGATGGCAGCCTATGGCCTGTCTGAGCAGGCAGG  
AGTGACCCCTGAGGCCTGGGCCAGC  
TGAGCCAGCCCTGCTGCAGCAGCAGCTGTCCGGAGCATGTACATCCCA  
GTCTAGGCCTCCAGTGCAGGATCAGC  
TGAGCCAGTCCGAGAGATACCTGTATGGCTCTCTGGCCACCCTGCTG  
ATCTGCCTGTGCGCCGTGTTGCGACTGCT  
GCTGCTGACCTGCACAGGCTGTAGAGGCGTGACCCACTACATCCTGC  
AGACATTTCTGTCCCTGGCAGTGGGAGC  
AGTGACCGGCGACGCCGTGCTGCACCTGACACCCAAGGTGCTGGGACT  
GCACACCCACAGCGAGGAGGGACTGT  
CCCCACAGCCTACATGGAGGCTGCTGGCAATGCTGGCAGGACTGTATG  
CCTTCTTTCTGTTGAGAACCTGTTTAA  
TCTGCTGCTGCCCCGCGACCCTGAGGATCTGGAGGACGGCCCTGCGG  
ACACTCCTCTCACTCCACGGCGGACAC  
TCCCACGGCGTGTCTCTGCAGCTGGCACCTTCTGAGCTGAGGCAGCC  
AAAGCCACCTCACGAGGGCAGCCGGGCC  
GATCTGGTGGCCGAGGAGAGCCAGAGCTGCTGAACCCAGAGCCACGG  
AGACTGAGCCAGAGCTGAGGCTGCT  
GCCTTACATGATCACCTGGGCGATGCCGTGCACAATTTGCGAGACGG  
ACTGGCAGTGGGAGCAGCCTTTGCCAG  
CTCCTGGAAGACCGGACTGGCCACATCCCTGGCCGTGTTCTGCCACG  
AGCTGCCACACGAGCTGGGCGACTTTGC  
CGCCCTGCTGCACGCAGGACTGAGCGTGCGCCAGGCCCTGCTGCTG  
AACCTGGCATCCGCCCTGACCGCCTTCGCC  
GGCCTGTACGTGGCCCTGGCCGTGGGCGTGAGCGAGGAGTCCGAGG  
CCTGGATTCTGGCCGTGGCCACAGGCCT  
GTTCTGTATGTGGCCCTGTGCGATATGCTGCCTGCCATGCTGAAGGT  
GCGGGACCCTAGACCCTGGCTGCTGTTT
```

CTGCTGCACAATGTGGGACTGCTGGGAGGATGGACAGTGCTGCTGCTGCTGAGCCTGTATGAGGACGACATCACC
TTCTAATCTAGA

2. SLC39A14 (Human)

Gene name: SLC39A14, Length: 1494 bp

Sequence:

GGTACCACCATGAAGCTGCTGCTGCTGCACCCTGCCTTTTCAGAGCTGTCTGCTGCTGACCCTGCTGGGACTGTGGA
GGACCACACCAGAGGCACACGCCAGCTCCCTGGGCGCCCCTGCCATCAGCGCCGCCTCCTCCTGCAGGACCTGAT
CCACAGATACGGCGAGGGCGATAGCCTGACCCTGCAGCAGCTGAAGGCCCTGCTGAATCACCTGGACGTGGGAG
TGGGAAGGGGAAACGTGACACAGCACGTGCAGGGCCACAGAAATCTGTCCACCTGCTTTTCTAGCGGCGATCTGT
TTACAGCCCACAACCTTCTCTGAGCAGAGCAGGATCGGCTCCTCTGAGCTGCAGGAGTTCTGTCCAACCATCCTGCA
GCAGCTGGACTCCAGGGCCTGCACATCTGAGAACCAGGAGAATGAGGAGAACGAGCAGACCGAGGAGGGCCGC
CCATCTGCCGTGGAAGTGTGGGGATACGGACTGCTGTGCGTGACAGTGATCTCCCTGTGCTCTCTGCTGGGAGCA
TCCGTGGTGCCCTTTATGAAGAAGACCTTCTACAAGCGCCTGCTGCTGTACTTCATCGCCCTGGCCATCGGCACACT
GTATTCCAACGCCCTGTTCCAGCTGATCCCCGAGGCCTTTGGCTTCAATCCTCTGGAGGACTACTACGTGAGCAAG
AGCGCCGTGGTGTGGCGGCTTCTACCTGTTCTTTTTCACAGAGAAGATCCTGAAGATCCTGCTGAAGCAGAAGA
ACGAGCACCACCACGGCCACAGCCACTATGCCTCCGAGTCTCTGCCCTCCAAGAAGGATCAGGAGGAGGGCGTGA
TGGAGAAGCTGCAGAAATGGCGACCTGGATCACATGATCCCTCAGCACTGTAGCTCCGAGCTGGACGGCAAGGCC
CAATGGTGGATGAGAAAGTGATCGTGGGCAGCCTGTCCGTGCAGGACCTGCAGGCCTCTCAGAGCGCCTGCTACT
GGCTGAAGGGCGTGCGGTATTCCGACATCGGCACCCTGGCCTGGATGATCACACTGTCTGACGGCCTGCACAACT
TCATCGATGGCCTGGCCATCGGCGCCTCTTTTACCGTGAGCGTGTTCAGGGCATCTCCACATCTGTGGCCATCCTG
TGCGAGGAGTTTCTCACGAGCTGGGCGATTCGTGATCCTGCTGAACGCCGGCATGTCTATCCAGCAGGCCCTGT
TTTTCAATTTTCTGAGCGCCTGCTGTTGCTACCTGGGACTGGCCTTCGGCATCCTGGCAGGCAGCCACTTTTCCGCC
AATTGGATCTTCGCCCTGGCCGGCGGCATGTTTCTGTATATCAGCCTGGCCGACATGTTCCCCGAGATGAACGAGG
TGTGCCAGGAGGATGAGCGGAAGGGCTCCATCCTGATCCCTTTATCATCCAGAATCTGGGCCTGCTGACCGGCTT
CACAATCATGGTGGTGCTGACCATGTATAGCGCCAGATCCAGATCGGCTAATCTAG

3. SLC39A8 SPECIES

**tr|F1NBL5|F1NBL5_CHICK Uncharacterized protein OS=Gallus gallus GN=SLC39A8
PE=4 SV=2**

>CHICKEN

MLSGVAVRGS PAATTFAEDVLRVFGSNRSLSAGQLSALLSQLGAAPALSAVLPLPHLHHN
QCLTGEEIFSLHGIPNNSHISNSDFSIIICPAVLQQLIFHPCDHQENSVDASRPHSLQVWG
FGFLAVTIINLASLLGFILTPLLKSYFPKILTYFVGLAVGTLFSNAIFQLIPEAFGFDS
HVDNYIEKAVAVFGGFYILFFVERILKVILKIYNQPGHNDVENGEENQSKDKTSPSKPPS
SSNGVTCYANSAIVESNGNFGFDSISVVS AQEEAVQGS LCKCLGGRPLSKIGTIAWMVTL
SDAVHNFLDGLAIGASF TSLFQGLSTSIAILCEEFPHELGD FVILLNAGMSIRQALFFN
FLSACSCYIGMALGILVGNNFAPTII FAVAGGMFLYISLADMFPEMNDMLREKVTGRKMD
LTFFLIQNAGLLTGFAAILLITLYEGNIQL

**>tr|F1R5W1|F1R5W1_DANRE Solute carrier family 39 (zinc transporter), member 8
OS=Danio rerio GN=slc39a8 PE=4 SV=2**

>ZF

MEESFLQNVLGFYGEESLSVGNLELFLQLITSRRAAAVEDEGNPLKSAECLSLSELLSA
FGLSNASVSVSNLEMMCPAILNQVLIPACPYTSSNLNASASISDHKVGWGYGLAVTVIN
LAALLGLFLVPFTKKKYFPKVL MYFIGLAIGTLFSNAVLQLIPEALGLDPKDDNYVLNVV
GIFGGFYILFVTERILKMVLKTDTELGHSHFPPLQSSDVTISTISNDVVISNISGDIITN

NTNHEQNSISEKSNNPSESPAVEQNACALFACRWLKGSAmsNIKTVAWMITVSDALHNFI
DGLAIGASFTLSLLSGFSTSIaIFCEEFPHELGDfVILLNSGMSVgQAaFFNLLSAMCCY
LGLALGILLGSNFAPNaIFaIAGGMFLYISLADMFPEMNSIMASHTKDYQERVVFFLIQn
AGLLTGFTIILLITLFAgDINLQ

>tr|A0A5G3H9Q7|A0A5G3H9Q7_XENTR Solute carrier family 39 member 8 OS=Xenopus tropicalis OX=8364 GN=slc39a8 PE=4 SV=1

MSFPGYRFLFLALYGAVFVILVGANDAGDMFTQDILQLYGHdGRlSSSNVSRlMNGCDARD
ELHNLEHLHYNRCLSTEDIFSIYNIKNDQITNVTFETFCPaILHQIFFHPCtFESQDEST
QRPSAQVWGfSFLSVTIINLTSLGLFITPLIKKPYfPKILTYfVGLaIGTLfSNaIFQ
LIPEAFGfDPKVDNYVPKAVAIFGGFYILLFVERLLKLILNIYGEgVHThLEIDHVPHQE
LHIESPANKIPNGNIiYSNPavaEINGVNHLdNIKVSSKDAVEEVYCKVLKWRPLKSiG
TLAWMITLSdALHNfIDGLaIGASFTLSVLQGLSTSIaILCEEFPHEFGDFaILINAGMS
IPQALTfNfLSACSCYIGLVFGILVGNNfEPsIIFaIAGGMFLYIGLADMFPELNEMLKD
KIKGRRSDLIYFSIQNaGLLSGFaIILLITLYSKEIKLn

>RAT ZIP8

MAPGRAVAGLLLLAATGLGRPSEGPELaFTEDVLRVFGANQSLSaAQlGRLLERlGAAPQ
QGALElGQLHfNQCLSaEDIfSLHGfSNVTQITSSNfTaICPaILQQLNfHPCEDPQKHS
VKPSfSEVWGyGFLSVTIINLaSLGLILtPLIKKSYfPKILTYfVGLaIGTLfSNaIFQ
LIPEAFGfNPkIDNYVEKAVAVFGGFYMLFFVERTLKMLLKTYGQNDHThFRNDdFGSKE
KAHQPKTLPLPPVNGVTCYANPAVTEPNGHIHfDTVSVVSLQDGKAESSSCTCLKGPKLS
EIGTIaWMITLcDALHNfIDGLaIGASyTSLlQGLSTSIaILCEEFPHELGDfVILLNa
GMSTRQALLfNfLSACSCYVGLAFGILVGNNfAPNIIFaLAGGMFLYISLADMFPEMNDM
LREKVTGRQTDfTFFMIQNaGMLTGfTaILLITLYAGDIeLQ

>PIG ZIP8

MAPGRAVAGLLLLAAAGLGGETAGPELaFSedVLSVFGANRSLSaAQlRRLLeQLGSGPV
EGaPElGQLHfNQCLSaEEIfSLHGfSNATQITSSNfSVICPaVLQQLNfHPCkDRPKHK
TKPSLSEVWGyGFLSVTVINLaSLGLILtPLIKKSYfPKILTYfVGLaIGTLfSNaIFQ
LIPEAFGfNPkVDNYVEKAVAVFGGFYIFFFVERMLKMLLKTYGQNGHThFGNDdFGPSQ
EKThQPKTLPaINGVTCYANPAVTEPNGHIHfDNVSVVSLQDGKKESSCCSCLKGPKLSE
IGTIaWMITLSdALHNfIDGLaIGASFTLSLLQGLSTSIaILCEEFPHELGDfVILLNaG
MSTRQALLfNfLSACSCYVGLALGILVGNNfAPNIIFaLAGGMFLYISLADMFPEMNDML
REKVTGRKTDfTFFMIQNaGMLTGfTaILLITLYAGEIELE

>HUMAN SLC39A8

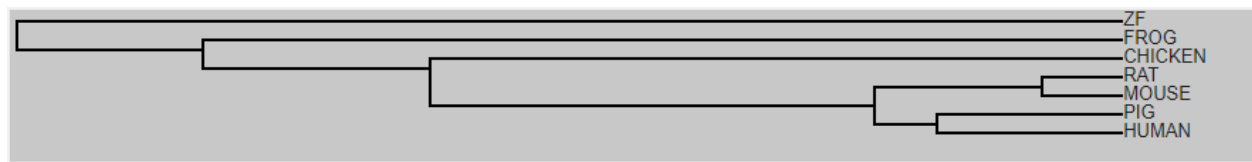
MAPGRAVAGLLLLAAAGLGgVAEGPGLAFSEdVLSVFGANLSLSaAQlQHLLeQMGAASR
VGvPEPGQLHfNQCLTaEEIfSLHGfSNATQITSSKfSVICPaVLQQLNfHPCEDRPKHK
TRPSHSEVW GYGfLSVTIINLaSLGLILtPLIKKSYfPKILtFFVGLaIGTLfSNaIFQ
LIPEAFGfDPKVDsYVEKAVAVFGGFYLLFFFERMLK MLLKTYGQNGHThFGNDNFGPQe
KThQPKaLPaINGVTCYANPAVTEANGHIHfDNVSVVSLQDGKKEPSSCTCLKGPKLSEI
GTIaWMITLcDALHNfIDGLaIGASCTLSLLQGLSTSIaILCEEFPHELGDfVILLNaG
STRQALLfNfLSACSCYVGLAFGILVGNNfAPNIIFaLAGGMFLYISLADMFPEMNDMLR
EKVTGRKTDfTFFMIQNaGMLTGfTaILLITLYAGEIELE

>sp|Q91W10|S39A8_MOUSE Zinc transporter ZIP8 OS=Mus musculus OX=10090 GN=Slc39a8 PE=2 SV=1

>MOUSE

MAPGRAVAGLLLLAATSLGHPSEGPELaFSedVLSVFGANRSLSaAQlGRLLERlGAASQ
QGALDLGQLHfNQCLSaEDIfSLHGfSNVTQITSSNfSaICPaILQQLNfHPCEDLRKHn
AKPSLSEVWGyGFLSVTIINLaSLGLILtPLIKKSYfPKILTYfVGLaIGTLfSNaIFQ
LIPEAFGfNPkIDNYVEKAVAVFGGFYMLFFVERTLKMLLKTYGQNDHThFRNDdFGSKE
KThQPKTLPLPAVNGVTCYANPAVTEPNGHIHfDTVSVVSLQDGKTEPSSCTCLKGPKLS
EIGTIaWMITLcDALHNfIDGLaIGASCTLSLLQGLSTSIaILCEEFPHELGDfVILLNa

GMSTRQALLFNFLSACSCYVGLAFGLVGNFAPNIIIFALAGGMFLYISLADMFPENNDM
LREKVTGRQTDFTFFMIQNAGMLTGFTAILLITLYAGDIELQ



4. Chimera

Gene name: _HHF_SLC39A8-C-terminal, Length: 726 bp, Sequence:

CTGCTGAAGACCTACGGTCAGAACGGCCACACCCACTTCGGCAACGACAACCTTTGGTCCGCAGGAGAAGACCCAC
CAACCGAAGGCGCTGCCGGCGATCAACGGTGTGACCTGCTATGCGAACCCGGCGGTTACCGAAGCGAACGGTCA
CATTCACTTCGACAACGTGAGCGTGGTTAGCCTGCAAGATGGCAAGAAAGAGCCGAGCAGCTGCACCTGCCTGAA
GGGTCCGAAACTGAGCGAAATCGGCACCATTCGCTGGATGATCACCTGTGCGACGCGCTGCACAACCTTTATCGA
TGGTCTGGCGATCGGTGCGAGCTGCACCCTGAGCCTGCTGCAGGGCCTGAGCACCAGCATCGCGATTCTGTGCGA
GGAGTTCCCGCACGAGCTGGGTGACTTTGTGATCCTGCTGAACAGCGGTATGAGCGTTGGCCAAGCGGCGTTCTT
TAACCTGCTGAGCGCGATGTGCTGCTACCTGGGTCTGGCGCTGGGCATTCTGCTGGGTAGCAACTTCGCGCCGAA
CGCGATCTTTGCGATTGCGGGTGGCATGTTCTGTACATCAGCCTGGCGGACATGTTTCCGGAGATGAACAGCATT
ATGGCGAGCCACACCAAAGATTATCAGGAACGTGTGGTTTTCTTTCTGATCCAAAACGCGGGCCTGCTGACCGGTT
TCACCATCATTCTGCTGATCACCTGTTTGGGGTGACATCAACCTGCAG

Gene name: _FHH_SLC39A8-c-terminal, Length: 309 bp, Sequence:

GCGGGTATGAGCACCCGTCAGGCGCTGCTGTTCAACTTTCTGAGCGCGTGACGCTGCTACGTGGGTCTGGCGTTT
GGTATCCTGGTTGGTAACAACCTTCGCGCCGAACATCATTTTTGCGCTGGCGGGTGGCATGTTCTGTATATTAGCC
TGGCGGACATGTTTCCGGAAATGAACGATATGCTGCGTGAGAAGGTGACCGGCCGTAACACCGACTTCACCTTCT
TTATGATCCAAAACGCGGGCATGCTGACCGGTTTTACCGCGATCCTGCTGATTACCCTGTACGCGGGCGAGATTGA
ACTGGAG

Gene name: NF_Human SLC39A8-middle, Length: 264 bp, Sequence:

GGCTACGGCTTTCTGAGCGTGACCATCATCAACCTGGCCTCCCTGCTGGGCCTGATCCTGACACCACTGATCAAGA
AGTCTTATTTCCCAAGATCCTGACCTTTTGTGGGCCTGGCCATCGGCACACTGTTTAGCAATGCCATCTTCCAG
CTGATCCCCGAGGCCTTTGGCTTCGACCCTAAGGTGGATAGCTACGTGGAGAAGGCCGTGGCCGTGTTTGGCGGC
TTCTATCTGCTGTTCTTTTTCGAGCGGATGCTGAAG

Gene name: NF_HHF SLC39A8-C-terminal, Length: 309 bp, Sequence:

GCAGGAATGTCTACCAGGCAGGCCCTGCTGTTCAACTTTCTGAGCGCCTGCTCCTGTTACGTGGGACTGGCCTTCG
GCATCCTGGTGGGCAACAATTTGCCCCAATATCATCTTTGCCCTGGCCGGCGGCATGTTCTGTATATCAGCCTG
GCCGACATGTTTCTGAGATGAACGATATGCTGCGGGAGAAGGTGACCGGCAGAAAGACCGACTTCACATTCTTT
ATGATCCAGAATGCCGGCATGCTGACCGGCTTTACAGCCATCCTGCTGATCACACTGTACGCCGGCGAGATCGAG
CTGGAG

Gene name: NH_FHH SLC39A8-middle, Length: 264 bp, Sequence:

GGCTACGGCTTCTGGCCGTGACCGTGATCAACCTGGCCGCCCTGCTGGGACTGTTCTGGTGCCCTTCACCAAGA
AGAAGTACTTCCCAAGGTGCTGATGTACTTCATCGGCCTGGCCATCGGCACCCTGTTAGCAATGCCGTGCTGCA

GCTGATCCCAGAGGCCCTGGGCCTGGACCCTAAGGACGATAACTACGTGCTGAATGTGGTGGGCATCTTCGGCGG
CTTTTATATCCTGTTTGTGACAGAGAGAATCCTGAAG

Gene name: NH_Fish SLC39A8-middle, Length: 645 bp, Sequence:

GGCTACGGCTTCCTGGCCGTGACCGTGATCAACCTGGCCGCCCTGCTGGGACTGTTCTTGGTGCCCTTCACCAAGA
AGAAGTACTTCCCTAAGGTGCTGATGTACTTCATCGGCCTGGCCATCGGCACCCTGTTTAGCAATGCCGTGCTGCA
GCTGATCCCTGAGGCCCTGGGCCTGGACCCCAAGGACGATAACTACGTGCTGAATGTGGTGGGCATCTTCGGCGG
CTTTTATATCCTGTTCTGTGACAGAGAGAATCCTGAAGATGGTGTGAAGACCGACACAGAGCTGGGCCACAGCCA
CTTTCCCTCTGACAGAGCAGCGACGTGACCATCTCTACAATCAGCAACGACGTGGTCATCAGCAATATCTCTGGC
GACATCATCACCACAATAACAAACCACGAGCAGAACAGCATCAGCGAGAAGTCCAACAATCCATCCGAGTCTCCA
GCAGTGGAGCAGAACGCATGCGCACTGTTGCGCTGTAGATGGCTGAAGGGCAGCGCCATGTCCAACATCAAGAC
CGTGGCCTGGATGATCACAGTGTCCGACGCCCTGCACAATTTTCATCGATGGCCTGGCCATCGGCGCCTCTTTTACC
CTGTCTCTGCTGAGCGGCTTCAGCACATCCATCGCCATCTTT

5. TM1/4/5 and additional mutants

Gene name : HUMAN_Fish_TM1_SLC39A8_C326F

- Variant name: HUMAN_Fish_TM1_SLC39A8
- Variant name: HUMAN_SLC39A8_C326F

Gene name : _FISH_Human_TM1_SLC39A8_F309C

- Variant name: FISH Human TM1 SLC39A8
- Variant name: Fish SLC39A8 F309C
- Variant name: FISH Human TM1 SLC39A8 F309C_S315Q
- Variant name: FISH Human TM1 SLC39A8 F309C_F317L
- Variant name: FISH Human TM1 SLC39A8 F309C_S315Q_F317L

Gene name: SLC39A8, Sequence:

- Variant name: HUMAN_SLC39A8_C326A
- Variant name: HUMAN_SLC39A8_C326W
- Variant name: HUMAN_SLC39A8_C326Y
- Variant name: HUMAN_SLC39A8_Y131A
- Variant name: HUMAN_SLC39A8_S135A
- Variant name: HUMAN_SLC39A8_Q332S
- Variant name: HUMAN_SLC39A8_Q332A
- Variant name: HUMAN_SLC39A8_L334F

Gene name : HUMAN_Fish_TM1_SLC39A8_C326F, Sequence:

GGTACCACCATGGCCCCTGGCCGGGCCGTGGCCGGCCTGCTGCTGCTGGCTGCCGCCGGACTGGGAGGAGTGGC
AGAGGGCCCAGGCCTGGCCTTTTCCGAGGACGTGCTGAGCGTGTTCGGAGCAAACCTGTCCCTGTCTGCCGCACA
GCTGCAGCACCTGCTGGAGCAGATGGGAGCAGCCAGCCGGGTGGGCGTGCCCGAGCCTGGCCAGCTGCACTTCA
ACCAAGTGCCTGACCGCCGAGGAGATCTTTAGCCTGCACGGCTTCTCCAATGCCACCCAGATCACAAGCTCCAAGTT
TAGCGTGATCTGCCAGCCGTGCTGCAGCAGCTGAACTTCCACCCTTGTGAGGATCGGCCAAAGCACAAAGACCAG

ACCTAGCCACTCCGAAGTGTGGGGCTACGGCTTTCTGGCCGTGACAGTGATCAATCTGGCCGCCCTGCTGGGACT
GTTTCTGGTGCCATTACCAAGAAGTCTTATTTCCCCAAGATCCTGACCTTCTTTGTGGGCCTGGCCATCGGCACAC
TGTTTAGCAACGCCATCTTCCAGCTGATCCCCGAGGCCTTTGGCTTCGACCCTAAGGTGGATTCTACGTGGAGAA
GGCCGTGGCCGTGTTTGGCGGCTTCTATCTGCTGTTCTTTTCGAGAGGATGCTGAAGATGCTGCTGAAGACCTAC
GGCCAGAATGGCCACACACACTTTGGCAACGACAATTCGGCCCCCAGGAGAAGACACACCAGCCAAAGGCCCTG
CCAGCCATCAACGGAGTGACCTGTTATGCCAATCCTGCCGTGACAGAGGCCAACGGCCACATCCACTTTGACAACG
TGAGCGTGGTGTCTCTGCAGGATGGCAAGAAGGAGCCTTCTAGCTGCACCTGTCTGAAGGGCCCCAAAGCTGTCCG
AGATCGGCACCATCGCCTGGATGATCAGCTGTGCGACGCCCTGCACAACTTTATCGATGGCCTGGCCATCGGCGC
CTCTTTCACCCTGAGCCTGCTGCAGGGCCTGTCTACAAGCATCGCCATCCTGTGCGAGGAGTTTCCCCACGAGCTG
GGCGACTTCGTGATCCTGCTGAACGCCGGCATGTCCACCAGACAGGCCCTGCTGTTAATTTCTGAGCGCCTGCT
CCTGTTACGTGGGACTGGCCTTCGGCATCCTGGTGGGCAACAATTTTGGCCCAAATATCATCTTCGCCCTGGCCGG
CGGCATGTTTCTGTATATCAGCCTGGCCGACATGTTCCCCGAGATGAACGATATGCTGAGGGAGAAGGTGACAGG
CCGCAAGACCGACTTCACCTTCTCATGATCCAGAATGCCGGCATGCTGACCGGCTTCACAGCCATCCTGCTGATCA
CCCTGTACGCCGGCGAGATCGAGCTGGAGTAATCTAGA

Template Name: HUMAN_Fish_TM1_SLC39A8_C326F, Mutagenesis instruction: Variant name:

HUMAN_Fish_TM1_SLC39A8, Variant sequence:

GGTACCACCATGGCCCCTGGCCGGGCCGTGGCCGGCCTGCTGCTGCTGGCTGCCGCCGGACTGGGAGGAGTGGC
AGAGGGCCCAGGCCTGGCCTTTTCCGAGGACGTGCTGAGCGTGTTTCGGAGCAAACCTGTCCCTGTCTGCCGCACA
GCTGCAGCACCTGCTGGAGCAGATGGGAGCAGCCAGCCGGGTGGGCGTGCCCCGAGCCTGGCCAGCTGCACTTCA
ACCAAGTGCCTGACCGCCGAGGAGATCTTTAGCCTGCACGGCTTCTCCAATGCCACCCAGATCACAAGCTCCAAGTT
TAGCGTGATCTGCCAGCCGTGCTGCAGCAGCTGAACTTCCACCCTTGTGAGGATCGGCCAAAGCACAAGACCAG
ACCTAGCCACTCCGAAGTGTGGGGCTACGGCTTTCTGGCCGTGACAGTGATCAATCTGGCCGCCCTGCTGGGACT
GTTTCTGGTGCCATTACCAAGAAGTCTTATTTCCCCAAGATCCTGACCTTCTTTGTGGGCCTGGCCATCGGCACAC
TGTTTAGCAACGCCATCTTCCAGCTGATCCCCGAGGCCTTTGGCTTCGACCCTAAGGTGGATTCTACGTGGAGAA
GGCCGTGGCCGTGTTTGGCGGCTTCTATCTGCTGTTCTTTTCGAGAGGATGCTGAAGATGCTGCTGAAGACCTAC
GGCCAGAATGGCCACACACACTTTGGCAACGACAATTCGGCCCCCAGGAGAAGACACACCAGCCAAAGGCCCTG
CCAGCCATCAACGGAGTGACCTGTTATGCCAATCCTGCCGTGACAGAGGCCAACGGCCACATCCACTTTGACAACG
TGAGCGTGGTGTCTCTGCAGGATGGCAAGAAGGAGCCTTCTAGCTGCACCTGTCTGAAGGGCCCCAAAGCTGTCCG
AGATCGGCACCATCGCCTGGATGATCAGCTGTGCGACGCCCTGCACAACTTTATCGATGGCCTGGCCATCGGCGC
CTCTTGACCCTGAGCCTGCTGCAGGGCCTGTCTACAAGCATCGCCATCCTGTGCGAGGAGTTTCCCCACGAGCTG
GGCGACTTCGTGATCCTGCTGAACGCCGGCATGTCCACCAGACAGGCCCTGCTGTTAATTTCTGAGCGCCTGCT
CCTGTTACGTGGGACTGGCCTTCGGCATCCTGGTGGGCAACAATTTTGGCCCAAATATCATCTTCGCCCTGGCCGG
CGGCATGTTTCTGTATATCAGCCTGGCCGACATGTTCCCCGAGATGAACGATATGCTGAGGGAGAAGGTGACAGG
CCGCAAGACCGACTTCACCTTCTCATGATCCAGAATGCCGGCATGCTGACCGGCTTCACAGCCATCCTGCTGATCA
CCCTGTACGCCGGCGAGATCGAGCTGGAGTAATCTAGA

Template Name: HUMAN_Fish_TM1_SLC39A8_C326F, Mutagenesis instruction: Variant name:

HUMAN_SLC39A8_C326F, Variant sequence:

GGTACCACCATGGCCCCTGGCCGGGCCGTGGCCGGCCTGCTGCTGCTGGCTGCCGCCGGACTGGGAGGAGTGGC
AGAGGGCCCAGGCCTGGCCTTTTCCGAGGACGTGCTGAGCGTGTTTCGGAGCAAACCTGTCCCTGTCTGCCGCACA
GCTGCAGCACCTGCTGGAGCAGATGGGAGCAGCCAGCCGGGTGGGCGTGCCCCGAGCCTGGCCAGCTGCACTTCA
ACCAAGTGCCTGACCGCCGAGGAGATCTTTAGCCTGCACGGCTTCTCCAATGCCACCCAGATCACAAGCTCCAAGTT
TAGCGTGATCTGCCAGCCGTGCTGCAGCAGCTGAACTTCCACCCTTGTGAGGATCGGCCAAAGCACAAGACCAG

ACCTAGCCACTCCGAAGTGTGGGGCTACGGCTTTCTGAGCGTGACAATCATCAATCTGGCCAGCCTGCTGGGACT
GATCCTGACCCCACTGATCAAGAAGTCTTATTTCCCAAGATCCTGACCTTCTTTGTGGGCCTGGCCATCGGCACAC
TGTTTAGCAACGCCATCTTCCAGCTGATCCCCGAGGCCTTTGGCTTCGACCCTAAGGTGGATTCTACGTGGAGAA
GGCCGTGGCCGTGTTTGGCGGCTTCTATCTGCTGTTCTTTTCGAGAGGATGCTGAAGATGCTGCTGAAGACCTAC
GGCCAGAATGGCCACACACACTTTGGCAACGACAATTCGGCCCCCAGGAGAAGACACACCAGCCAAAGGCCCTG
CCAGCCATCAACGGAGTGACCTGTTATGCCAATCCTGCCGTGACAGAGGCCAACGGCCACATCCACTTTGACAACG
TGAGCGTGGTGTCTCTGCAGGATGGCAAGAAGGAGCCTTCTAGCTGCACCTGTCTGAAGGGCCCAAAGCTGTCCG
AGATCGGCACCATCGCCTGGATGATCACACTGTGCGACGCCCTGCACAACTTTATCGATGGCCTGGCCATCGGCGC
CTCTTTCACCCTGAGCCTGCTGCAGGGCCTGTCTACAAGCATCGCCATCCTGTGCGAGGAGTTTCCCCACGAGCTG
GGCGACTTCGTGATCCTGCTGAACGCCGGCATGTCCACCAGACAGGCCCTGCTGTTTAATTTCTGAGCGCCTGCT
CCTGTTACGTGGGACTGGCCTTCGGCATCCTGGTGGGCAACAATTTGCCCAAATATCATCTTCGCCCTGGCCGG
CGGCATGTTTCTGTATATCAGCCTGGCCGACATGTTCCCCGAGATGAACGATATGCTGAGGGAGAAGGTGACAGG
CCGCAAGACCGACTTCACCTTCTCATGATCCAGAATGCCGGCATGCTGACCGGCTTCACAGCCATCCTGCTGATCA
CCCTGTACGCCGGCGAGATCGAGCTGGAGTAATCTAGA

Gene name: FISH_Human_TM1_SLC39A8_F309C, Sequence:

GGTACCACCATGGAGGAGTCCTTCCTGCAGAACGTGCTGGGCTTTTACGGCGAGGAGAGCAGCCTGTCCGTGGGC
AATCTGGAGCTGTTTCTGCAGCTGATCACCTCTCGGAGAGCTGCCGCCGTGGAGGATGAGGGAAATCCCCTGAAG
TCTGCCGAGTGCCTGAGCCTGTCCGAGCTGCTGAGCGCCTTCGGCCTGTCCAACGCCTCTGTGGTGTCTGTGAGCA
ATCTGGAGATGATGTGCCCTGCCATCCTGAACCAGGTGCTGATCCCAGCCTGTCCCTACACCTCTAGCAACCTGAA
TGCCTCCGCCTCTATCAGCGACCACAAAGTGTGGGGCTATGGCTTTCTGAGCGTGACCATCATCAATCTGGCCTCC
CTGCTGGGCCTGATCCTGACACCACTGATCAAGAAGAAGTACTTCCCCAAGGTGCTGATGTACTTCATCGGCCTGG
CCATCGGCACCCTGTTTCAGCAACGCCGTGCTGCAGCTGATCCCAGAGGCCCTGGGCCTGGACCCCAAGGACGATA
ACTACGTGCTGAATGTGGTGGGCATCTTCGGCGGCTTTTATATCCTGTTTGTGACAGAGAGAATCCTGAAGATGGT
GCTGAAGACCGACACAGAGCTGGGCCACTCCACTTCCCCCTCTGCAGTCCTCTGACGTGACCATCAGCACAATC
TCCAACGACGTGGTCATCAGCAATATCAGCGGCGACATCATCACCACAATACAAACCACGAGCAGAACAGCATC
AGCGAGAAGAGCAACAATCCTAGCGAGTCCCCAGCAGTGAGCAGAACGCATGCGCACTGTTTCGCATGTAGGTG
GCTGAAGGGCTCCGCCATGTCTAACATCAAGACCGTGGCCTGGATGATCACAGTGAGCGACGCCCTGCACAATTT
CATCGATGGACTGGCAATCGGAGCATCTGCACCCTGAGCCTGCTGTCCGGCTTTTCTACAAGCATCGCCATCTTCT
GTGAGGAGTTTCCCCACGAGCTGGGCGATTTCTGTATCCTGCTGAACTCTGGCATGAGCGTGGGCCAGGCCGCCT
TCTTTAATCTGCTGTCTGCCATGTGCTGTTATCTGGGACTGGCCCTGGGCATCCTGCTGGGCAGCAACTTCGCCCT
AATGCCATCTTGGCATCGCCGGCGGCATGTTCTGTACATCTCCCTGGCCGATATGTTTCAGAGATGAACTCCAT
CATGGCCTCTCACACCAAGGACTATCAGGAGCGGGTGGTGTCTTTCTGATCCAGAATGCCGGCCTGCTGACCGGC
TTCACAATCATCCTGCTGATCACACTGTTTGCCGGCGACATCAACCTGCAGTAATCTAGA

**Template Name: FISH_Human_TM1_SLC39A8_F309C, Mutagenesis instruction: Variant name: FISH
Human TM1 SLC39A8, Variant sequence:**

GGTACCACCATGGAGGAGTCCTTCCTGCAGAACGTGCTGGGCTTTTACGGCGAGGAGAGCAGCCTGTCCGTGGGC
AATCTGGAGCTGTTTCTGCAGCTGATCACCTCTCGGAGAGCTGCCGCCGTGGAGGATGAGGGAAATCCCCTGAAG
TCTGCCGAGTGCCTGAGCCTGTCCGAGCTGCTGAGCGCCTTCGGCCTGTCCAACGCCTCTGTGGTGTCTGTGAGCA
ATCTGGAGATGATGTGCCCTGCCATCCTGAACCAGGTGCTGATCCCAGCCTGTCCCTACACCTCTAGCAACCTGAA
TGCCTCCGCCTCTATCAGCGACCACAAAGTGTGGGGCTATGGCTTTCTGAGCGTGACCATCATCAATCTGGCCTCC
CTGCTGGGCCTGATCCTGACACCACTGATCAAGAAGAAGTACTTCCCCAAGGTGCTGATGTACTTCATCGGCCTGG

CCATCGGCACCCTGTTTCAGCAACGCCGTGCTGCAGCTGATCCCAGAGGCCCTGGGCCTGGACCCCAAGGACGATA
ACTACGTGCTGAATGTGGTGGGCATCTTCGGCGGCTTTTATATCCTGTTTGTGACAGAGAGAATCCTGAAGATGGT
GCTGAAGACCGACACAGAGCTGGGCCACTCCCCTCTGAGTCCTCTGACGTGACCATCAGCACAATC
TCCAACGACGTGGTCATCAGCAATATCAGCGGCGACATCATCACCAACAATACAAACCACGAGCAGAACAGCATC
AGCGAGAAGAGCAACAATCCTAGCGAGTCCCCAGCAGTGGAGCAGAACGCATGCGCACTGTTTCGCATGTAGGTG
GCTGAAGGGCTCCGCCATGTCTAACATCAAGACCGTGGCCTGGATGATCACAGTGAGCGACGCCCTGCACAATTT
CATCGATGGACTGGCAATCGGAGCATCCTTCACCCTGAGCCTGCTGTCCGGCTTTTCTACAAGCATCGCCATCTTCT
GTGAGGAGTTTCCCCACGAGCTGGGCGATTTCGTGATCCTGCTGAACTCTGGCATGAGCGTGGGCCAGGCCGCCT
TCTTTAATCTGCTGTCTGCCATGTGCTGTTATCTGGGACTGGCCCTGGGCATCCTGCTGGGCAGCAACTTCGCCCT
AATGCCATCTTTGCCATCGCCGGCGGCATGTTCTGTACATCTCCCTGGCCGATATGTTTCCAGAGATGAACTCCAT
CATGGCCTCTCACACCAAGGACTATCAGGAGCGGGTGGTGTCTTTCTGATCCAGAATGCCGGCCTGCTGACCGGC
TTCACAATCATCCTGCTGATCACACTGTTTGCCGGCGACATCAACCTGCAGTAATCTAGA

**Template Name: _FISH_Human_TM1_SLC39A8_F309C, Mutagenesis instruction: Variant name: Fish
SLC39A8 F309C, Variant sequence:**

GGTACCACCATGGAGGAGTCCTTCCTGCAGAACGTGCTGGGCTTTTACGGCGAGGAGAGCAGCCTGTCCGTGGGC
AATCTGGAGCTGTTTCTGCAGCTGATCACCTCTCGGAGAGCTGCCGCCGTGGAGGATGAGGGAAATCCCCTGAAG
TCTGCCGAGTGCCTGAGCCTGTCCGAGCTGCTGAGCGCCTTCGGCCTGTCCAACGCCTCTGTGGTGTCTGTGAGCA
ATCTGGAGATGATGTGCCCTGCCATCCTGAACCAGGTGCTGATCCCAGCCTGTCCCTACACCTCTAGCAACCTGAA
TGCCTCCGCCTCTATCAGCGACCACAAAGTGTGGGGCTATGGCTTTCTGGCCGTGACCGTGATCAATCTGGCCGCC
CTGCTGGGCCTGTTCTGGTGCCATTACCAAGAAGAAGTACTTCCCCAAGGTGCTGATGTACTTCATCGGCCTGG
CCATCGGCACCCTGTTTCAGCAACGCCGTGCTGCAGCTGATCCCAGAGGCCCTGGGCCTGGACCCCAAGGACGATA
ACTACGTGCTGAATGTGGTGGGCATCTTCGGCGGCTTTTATATCCTGTTTGTGACAGAGAGAATCCTGAAGATGGT
GCTGAAGACCGACACAGAGCTGGGCCACTCCCCTCTGAGTCCTCTGACGTGACCATCAGCACAATC
TCCAACGACGTGGTCATCAGCAATATCAGCGGCGACATCATCACCAACAATACAAACCACGAGCAGAACAGCATC
AGCGAGAAGAGCAACAATCCTAGCGAGTCCCCAGCAGTGGAGCAGAACGCATGCGCACTGTTTCGCATGTAGGTG
GCTGAAGGGCTCCGCCATGTCTAACATCAAGACCGTGGCCTGGATGATCACAGTGAGCGACGCCCTGCACAATTT
CATCGATGGACTGGCAATCGGAGCATCCTGCACCCTGAGCCTGCTGTCCGGCTTTTCTACAAGCATCGCCATCTTCT
GTGAGGAGTTTCCCCACGAGCTGGGCGATTTCGTGATCCTGCTGAACTCTGGCATGAGCGTGGGCCAGGCCGCCT
TCTTTAATCTGCTGTCTGCCATGTGCTGTTATCTGGGACTGGCCCTGGGCATCCTGCTGGGCAGCAACTTCGCCCT
AATGCCATCTTTGCCATCGCCGGCGGCATGTTCTGTACATCTCCCTGGCCGATATGTTTCCAGAGATGAACTCCAT
CATGGCCTCTCACACCAAGGACTATCAGGAGCGGGTGGTGTCTTTCTGATCCAGAATGCCGGCCTGCTGACCGGC
TTCACAATCATCCTGCTGATCACACTGTTTGCCGGCGACATCAACCTGCAGTAATCTAGA

**Template name: _FISH_Human_TM1_SLC39A8_F309C, Mutagenesis instruction: Variant name: FISH
Human TM1 SLC39A8 F309C_S315Q, Variant sequence:**

GGTACCACCATGGAGGAGTCCTTCCTGCAGAACGTGCTGGGCTTTTACGGCGAGGAGAGCAGCCTGTCCGTGGGC
AATCTGGAGCTGTTTCTGCAGCTGATCACCTCTCGGAGAGCTGCCGCCGTGGAGGATGAGGGAAATCCCCTGAAG
TCTGCCGAGTGCCTGAGCCTGTCCGAGCTGCTGAGCGCCTTCGGCCTGTCCAACGCCTCTGTGGTGTCTGTGAGCA
ATCTGGAGATGATGTGCCCTGCCATCCTGAACCAGGTGCTGATCCCAGCCTGTCCCTACACCTCTAGCAACCTGAA
TGCCTCCGCCTCTATCAGCGACCACAAAGTGTGGGGCTATGGCTTTCTGAGCGTGACCATCATCAATCTGGCCTCC
CTGCTGGGCCTGATCCTGACACCACTGATCAAGAAGAAGTACTTCCCCAAGGTGCTGATGTACTTCATCGGCCTGG
CCATCGGCACCCTGTTTCAGCAACGCCGTGCTGCAGCTGATCCCAGAGGCCCTGGGCCTGGACCCCAAGGACGATA
ACTACGTGCTGAATGTGGTGGGCATCTTCGGCGGCTTTTATATCCTGTTTGTGACAGAGAGAATCCTGAAGATGGT

GCTGAAGACCGACACAGAGCTGGGCCACTCCACTTCCCCCTCTGCAGTCCTCTGACGTGACCATCAGCACAATC
TCCAACGACGTGGTCATCAGCAATATCAGCGGCGACATCATCACCAACAATACAAACCACGAGCAGAACAGCATC
AGCGAGAAGAGCAACAATCCTAGCGAGTCCCCAGCAGTGGAGCAGAACGCATGCGCACTGTTTCGCATGTAGGTG
GCTGAAGGGCTCCGCCATGTCTAACATCAAGACCGTGGCCTGGATGATCACAGTGAGCGACGCCCTGCACAATTT
CATCGATGGACTGGCAATCGGAGCATCCTGCACCCTGAGCCTGCTGCAGGGCTTTTCTACAAGCATCGCCATCTTC
TGTGAGGAGTTTCCCCACGAGCTGGGCGATTTTCGTGATCCTGCTGAACTCTGGCATGAGCGTGGGCCAGGCCGCC
TTCTTTAATCTGCTGTCTGCCATGTGCTGTTATCTGGGACTGGCCCTGGGCATCCTGCTGGGCAGCAACTTCGCCCC
TAATGCCATCTTTGCCATCGCCGGCGGCATGTTCTGTACATCTCCCTGGCCGATATGTTTCCAGAGATGAACTCCA
TCATGGCCTCTCACACCAAGGACTATCAGGAGCGGGTGGTGTCTTTCTGATCCAGAATGCCGGCCTGCTGACCGG
CTTCACAATCATCCTGCTGATCACACTGTTTGCCGGCGACATCAACCTGCAGTAATCTAGA

**Template name: _FISH_Human_TM1_SLC39A8_F309C, Mutagenesis instruction: Variant name: FISH
Human TM1 SLC39A8 F309C_F317L, Variant sequence:**

GGTACCACCATGGAGGAGTCCTTCCTGCAGAACGTGCTGGGCTTTTACGGCGAGGAGAGCAGCCTGTCCGTGGGC
AATCTGGAGCTGTTTCTGCAGCTGATCACCTCTCGGAGAGCTGCCGCCGTGGAGGATGAGGGAAATCCCCTGAAG
TCTGCCGAGTGCCTGAGCCTGTCCGAGCTGCTGAGCGCCTTCGGCCTGTCCAACGCCTCTGTGGTGTCTGTGAGCA
ATCTGGAGATGATGTGCCCTGCCATCCTGAACCAGGTGCTGATCCCAGCCTGTCCCTACACCTCTAGCAACCTGAA
TGCCTCCGCCTCTATCAGCGACCACAAAGTGTGGGGCTATGGCTTTCTGAGCGTGACCATCATCAATCTGGCCTCC
CTGCTGGGCCTGATCCTGACACCACTGATCAAGAAGAAGTACTTCCCCAAGGTGCTGATGTACTTCATCGGCCTGG
CCATCGGCACCCTGTTTCAGCAACGCCGTGCTGCAGCTGATCCCAGAGGCCCTGGGCCTGGACCCCAAGGACGATA
ACTACGTGCTGAATGTGGTGGGCATCTTCGGCGGCTTTTATATCCTGTTTGTGACAGAGAGAATCCTGAAGATGGT
GCTGAAGACCGACACAGAGCTGGGCCACTCCACTTCCCCCTCTGCAGTCCTCTGACGTGACCATCAGCACAATC
TCCAACGACGTGGTCATCAGCAATATCAGCGGCGACATCATCACCAACAATACAAACCACGAGCAGAACAGCATC
AGCGAGAAGAGCAACAATCCTAGCGAGTCCCCAGCAGTGGAGCAGAACGCATGCGCACTGTTTCGCATGTAGGTG
GCTGAAGGGCTCCGCCATGTCTAACATCAAGACCGTGGCCTGGATGATCACAGTGAGCGACGCCCTGCACAATTT
CATCGATGGACTGGCAATCGGAGCATCCTGCACCCTGAGCCTGCTGTCCGGCCTGTCTACAAGCATCGCCATCTTC
TGTGAGGAGTTTCCCCACGAGCTGGGCGATTTTCGTGATCCTGCTGAACTCTGGCATGAGCGTGGGCCAGGCCGCC
TTCTTTAATCTGCTGTCTGCCATGTGCTGTTATCTGGGACTGGCCCTGGGCATCCTGCTGGGCAGCAACTTCGCCCC
TAATGCCATCTTTGCCATCGCCGGCGGCATGTTCTGTACATCTCCCTGGCCGATATGTTTCCAGAGATGAACTCCA
TCATGGCCTCTCACACCAAGGACTATCAGGAGCGGGTGGTGTCTTTCTGATCCAGAATGCCGGCCTGCTGACCGG
CTTCACAATCATCCTGCTGATCACACTGTTTGCCGGCGACATCAACCTGCAGTAATCTAGA

**Template name: _FISH_Human_TM1_SLC39A8_F309C, Mutagenesis instruction: Variant name: FISH
Human TM1 SLC39A8 F309C_S315Q_F317L, Variant sequence:**

GGTACCACCATGGAGGAGTCCTTCCTGCAGAACGTGCTGGGCTTTTACGGCGAGGAGAGCAGCCTGTCCGTGGGC
AATCTGGAGCTGTTTCTGCAGCTGATCACCTCTCGGAGAGCTGCCGCCGTGGAGGATGAGGGAAATCCCCTGAAG
TCTGCCGAGTGCCTGAGCCTGTCCGAGCTGCTGAGCGCCTTCGGCCTGTCCAACGCCTCTGTGGTGTCTGTGAGCA
ATCTGGAGATGATGTGCCCTGCCATCCTGAACCAGGTGCTGATCCCAGCCTGTCCCTACACCTCTAGCAACCTGAA
TGCCTCCGCCTCTATCAGCGACCACAAAGTGTGGGGCTATGGCTTTCTGAGCGTGACCATCATCAATCTGGCCTCC
CTGCTGGGCCTGATCCTGACACCACTGATCAAGAAGAAGTACTTCCCCAAGGTGCTGATGTACTTCATCGGCCTGG
CCATCGGCACCCTGTTTCAGCAACGCCGTGCTGCAGCTGATCCCAGAGGCCCTGGGCCTGGACCCCAAGGACGATA
ACTACGTGCTGAATGTGGTGGGCATCTTCGGCGGCTTTTATATCCTGTTTGTGACAGAGAGAATCCTGAAGATGGT
GCTGAAGACCGACACAGAGCTGGGCCACTCCACTTCCCCCTCTGCAGTCCTCTGACGTGACCATCAGCACAATC
TCCAACGACGTGGTCATCAGCAATATCAGCGGCGACATCATCACCAACAATACAAACCACGAGCAGAACAGCATC

AGCGAGAAGAGCAACAATCCTAGCGAGTCCCCAGCAGTGGAGCAGAACGCATGCGCACTGTTGCGATGTAGGTG
GCTGAAGGGGCTCCGCCATGTCTAACATCAAGACCGTGGCCTGGATGATCACAGTGAGCGACGCCCTGCACAATTT
CATCGATGGACTGGCAATCGGAGCATCCTGCACCCTGAGCCTGCTGCAGGGCCTGTCTACAAGCATCGCCATCTTC
TGTGAGGAGTTTTCCCCACGAGCTGGGCGATTCGTGATCCTGCTGAACTCTGGCATGAGCGTGGGCCAGGCCGCC
TTCTTTAATCTGCTGTCTGCCATGTGCTGTTATCTGGGACTGGCCCTGGGCATCCTGCTGGGCAGCAACTTCGCCCC
TAATGCCATCTTTGCCATCGCCGGCGGCATGTTCTGTACATCTCCCTGGCCGATATGTTTCCAGAGATGAACTCCA
TCATGGCCTCTCACACCAAGGACTATCAGGAGCGGGTGGTGTCTTTCTGATCCAGAATGCCGGCCTGCTGACCGG
CTTCACAATCATCCTGCTGATCACACTGTTTGCCGGCGACATCAACCTGCAGTAATCTAGA

Gene name: SLC39A8, Sequence:

GGTACCACCATGGCCCCGGGTCGCGCGGTGGCCGGGCTCCTGTTGCTGGCGGCCGCCGGCCTCGGAGGAGTGGC
GGAGGGGGCCAGGGCTAGCCTTCAGCGAGGATGTGCTGAGCGTGTTCCGGCGCAATCTGAGCCTGTGGCGGGCGC
AGCTCCAGCACTTGCTGGAGCAGATGGGAGCCGCTCCCGCGTGGGCGTCCCGGAGCCTGGCCAGCTGCACTTCA
ACCAAGTGTTTAACTGCTGAAGAGATCTTTCCCTTCATGGCTTTTCAAATGCTACCCAAATAACCAGCTCCAAATTCT
CTGTCATCTGTCCAGCAGTCTTACAGCAATTGAACTTTACCCATGTGAGGATCGGCCCAAGCACAAAACAAGACC
AAGTCATTGAGAAGTTTGGGGATATGGATTCTGTGAGTGACGATTATTAATCTGGCATCTCTCCTCGGATTGATTT
TGACTCCACTGATAAAGAAATCTTATTTCCCAAAGATTTTGACCTTTTTGTGGGGCTGGCTATTGGGACTCTTTTT
CAAATGCAATTTTCCAACCTATTCCAGAGGCATTTGGATTTGATCCCAAAGTCGACAGTTATGTTGAGAAGGCAGT
TGCTGTGTTTGGTGGATTTTACCTACTTTTCTTTTTGAAAGAATGCTAAAGATGTTATTAAGACATATGGTCAGA
ATGGTCATACCCACTTTGGAAATGATAACTTTGGTCCTCAAGAAAAAACTCATCAACCTAAAGCATTACCTGCCATC
AATGGTGTGACATGCTATGCAAATCCTGCTGTACAGAAGCTAATGGACATATCCATTTTGATAATGTCAGTGTGG
TATCTCTACAGGATGGAAAAAAGAGCCAAGTTCATGTACCTGTTTGAAGGGGGCCAACTGTCAGAAATAGGGA
CGATTGCCTGGATGATAACGCTCTGCGATGCCCTCCACAATTTTCATCGATGGCCTGGCGATTGGGGCTTCTGCAC
CTTGCTCTCTCCTTCAGGGACTCAGTACTTCCATAGCAATCCTATGTGAGGAGTTTCCCCACGAGTTAGGAGACTTTG
TGATCCTACTCAATGCAGGGATGAGCACTCGACAAGCCTTGCTATTCAACTTCCTTTCTGCATGTTCTGCTATGTT
GGGCTAGCTTTTGGCATTTTGGTGGGCAACAATTTGCTCCAAATATTATTTGCACTTGCTGGAGGCATGTTCTCT
CTATATTTCTCTGGCAGATATGTTTCCAGAGATGAATGATATGCTGAGAGAAAAGGTAAGTGAAGAAAAACCGA
TTTACCTTCTTCATGATTGAGAATGCTGGAATGTTAACTGGATTACAGCCATTCTACTCATTACCTTGATGCAGG
AGAAATCGAATTGGAGTAATCTAGA

Template name: SLC39A8, Mutagenesis instruction: Variant name: HUMAN_SLC39A8_C326A, Variant sequence:

GGTACCACCATGGCCCCGGGTCGCGCGGTGGCCGGGCTCCTGTTGCTGGCGGCCGCCGGCCTCGGAGGAGTGGC
GGAGGGGGCCAGGGCTAGCCTTCAGCGAGGATGTGCTGAGCGTGTTCCGGCGCAATCTGAGCCTGTGGCGGGCGC
AGCTCCAGCACTTGCTGGAGCAGATGGGAGCCGCTCCCGCGTGGGCGTCCCGGAGCCTGGCCAGCTGCACTTCA
ACCAAGTGTTTAACTGCTGAAGAGATCTTTCCCTTCATGGCTTTTCAAATGCTACCCAAATAACCAGCTCCAAATTCT
CTGTCATCTGTCCAGCAGTCTTACAGCAATTGAACTTTACCCATGTGAGGATCGGCCCAAGCACAAAACAAGACC
AAGTCATTGAGAAGTTTGGGGATATGGATTCTGTGAGTGACGATTATTAATCTGGCATCTCTCCTCGGATTGATTT
TGACTCCACTGATAAAGAAATCTTATTTCCCAAAGATTTTGACCTTTTTGTGGGGCTGGCTATTGGGACTCTTTTT
CAAATGCAATTTTCCAACCTATTCCAGAGGCATTTGGATTTGATCCCAAAGTCGACAGTTATGTTGAGAAGGCAGT
TGCTGTGTTTGGTGGATTTTACCTACTTTTCTTTTTGAAAGAATGCTAAAGATGTTATTAAGACATATGGTCAGA
ATGGTCATACCCACTTTGGAAATGATAACTTTGGTCCTCAAGAAAAAACTCATCAACCTAAAGCATTACCTGCCATC
AATGGTGTGACATGCTATGCAAATCCTGCTGTACAGAAGCTAATGGACATATCCATTTTGATAATGTCAGTGTGG

TATCTCTACAGGATGGAAAAAAGAGCCAAGTTCATGTACCTGTTTGAAGGGGCCCAAAGTGTGAGAAATAGGGA
CGATTGCCTGGATGATAACGCTCTGCGATGCCCTCCACAATTTTCATCGATGGCCTGGCGATTGGGGCTTCCGCCAC
CTTGTCTCTCCTTCAGGGACTCAGTACTTCCATAGCAATCCTATGTGAGGAGTTTCCCCACGAGTTAGGAGACTTTG
TGATCCTACTCAATGCAGGGATGAGCACTCGACAAGCCTTGCTATTCAACTTCCTTTCTGCATGTTCTGCTATGTT
GGGCTAGCTTTTGGCATTTTGGTGGGCAACAATTTGCTCCAAATATTATATTGCACTTGCTGGAGGCATGTTCTC
CTATATTTCTCTGGCAGATATGTTTCCAGAGATGAATGATATGCTGAGAGAAAAGGTAAGTGAAGAAAAACCGA
TTTACCTTCTTCATGATTGAGAATGCTGGAATGTTAACTGGATTACAGCCATTCTACTCATTACCTTGATGCAGG
AGAAATCGAATTGGAGTAATCTAGA

Template name: SLC39A8, Mutagenesis instruction: Variant name: HUMAN_SLC39A8_C326W, Variant sequence:

GGTACCACCATGGCCCCGGGTCGCGCGGTGGCCGGGCTCCTGTTGCTGGCGGCCGCCGGCCTCGGAGGAGTGGC
GGAGGGGCCAGGGCTAGCCTTCAGCGAGGATGTGCTGAGCGTGTTCCGGCGCAATCTGAGCCTGTGGCGGGCGC
AGCTCCAGCACTTGCTGGAGCAGATGGGAGCCGCTCCCGCGTGGGCGTCCCGGAGCCTGGCCAGCTGCACTTCA
ACCAAGTGTTTAACTGCTGAAGAGATCTTTTCCCTTCATGGCTTTTCAAATGCTACCCAAATAACCAGCTCCAAATTCT
CTGTCATCTGTCCAGCAGTCTTACAGCAATTGAACTTTACCCATGTGAGGATCGGCCCCAAGCACAAAACAAGACC
AAGTCATTGAGAAGTTTGGGGATATGGATTCTGTGCTGAGTACGATTATTAATCTGGCATCTCTCCTCGGATTGATTT
TGACTCCACTGATAAAGAAATCTTATTTCCCAAAGATTTTGACCTTTTTTGTTGGGGCTGGCTATTGGGACTCTTTTT
CAAATGCAATTTTCCAACCTATTCCAGAGGCATTTGGATTTGATCCCAAAGTCGACAGTTATGTTGAGAAGGCAGT
TGCTGTGTTTGGTGGATTTTACCTACTTTTCTTTTTGAAAGAATGCTAAAGATGTTATTAAGACATATGGTCAGA
ATGGTCATACCCACTTTGGAAATGATAACTTTGGTCCTCAAGAAAAAACTCATCAACCTAAAGCATTACCTGCCATC
AATGGTGTGACATGCTATGCAAATCCTGCTGTACAGAAGCTAATGGACATATCCATTTTGATAATGTCAGTGTGG
TATCTCTACAGGATGGAAAAAAGAGCCAAGTTCATGTACCTGTTTGAAGGGGCCCAAAGTGTGAGAAATAGGGA
CGATTGCCTGGATGATAACGCTCTGCGATGCCCTCCACAATTTTCATCGATGGCCTGGCGATTGGGGCTTCTGGAC
CTTGTCTCTCCTTCAGGGACTCAGTACTTCCATAGCAATCCTATGTGAGGAGTTTCCCCACGAGTTAGGAGACTTTG
TGATCCTACTCAATGCAGGGATGAGCACTCGACAAGCCTTGCTATTCAACTTCCTTTCTGCATGTTCTGCTATGTT
GGGCTAGCTTTTGGCATTTTGGTGGGCAACAATTTGCTCCAAATATTATATTGCACTTGCTGGAGGCATGTTCTC
CTATATTTCTCTGGCAGATATGTTTCCAGAGATGAATGATATGCTGAGAGAAAAGGTAAGTGAAGAAAAACCGA
TTTACCTTCTTCATGATTGAGAATGCTGGAATGTTAACTGGATTACAGCCATTCTACTCATTACCTTGATGCAGG
AGAAATCGAATTGGAGTAATCTAGA

Template name: SLC39A8, Mutagenesis instruction: Variant name: HUMAN_SLC39A8_C326Y, Variant sequence:

GGTACCACCATGGCCCCGGGTCGCGCGGTGGCCGGGCTCCTGTTGCTGGCGGCCGCCGGCCTCGGAGGAGTGGC
GGAGGGGCCAGGGCTAGCCTTCAGCGAGGATGTGCTGAGCGTGTTCCGGCGCAATCTGAGCCTGTGGCGGGCGC
AGCTCCAGCACTTGCTGGAGCAGATGGGAGCCGCTCCCGCGTGGGCGTCCCGGAGCCTGGCCAGCTGCACTTCA
ACCAAGTGTTTAACTGCTGAAGAGATCTTTTCCCTTCATGGCTTTTCAAATGCTACCCAAATAACCAGCTCCAAATTCT
CTGTCATCTGTCCAGCAGTCTTACAGCAATTGAACTTTACCCATGTGAGGATCGGCCCCAAGCACAAAACAAGACC
AAGTCATTGAGAAGTTTGGGGATATGGATTCTGTGCTGAGTACGATTATTAATCTGGCATCTCTCCTCGGATTGATTT
TGACTCCACTGATAAAGAAATCTTATTTCCCAAAGATTTTGACCTTTTTTGTTGGGGCTGGCTATTGGGACTCTTTTT
CAAATGCAATTTTCCAACCTATTCCAGAGGCATTTGGATTTGATCCCAAAGTCGACAGTTATGTTGAGAAGGCAGT
TGCTGTGTTTGGTGGATTTTACCTACTTTTCTTTTTGAAAGAATGCTAAAGATGTTATTAAGACATATGGTCAGA
ATGGTCATACCCACTTTGGAAATGATAACTTTGGTCCTCAAGAAAAAACTCATCAACCTAAAGCATTACCTGCCATC
AATGGTGTGACATGCTATGCAAATCCTGCTGTACAGAAGCTAATGGACATATCCATTTTGATAATGTCAGTGTGG

TATCTCTACAGGATGGAAAAAAGAGCCAAGTTCATGTACCTGTTTGAAGGGGCCCAAAGTGTGAGAAATAGGGA
CGATTGCCTGGATGATAACGCTCTGCGATGCCCTCCACAATTTTCATCGATGGCCTGGCGATTGGGGCTTCCTACAC
CTTGTCTCTCCTTCAGGGACTCAGTACTTCCATAGCAATCCTATGTGAGGAGTTTCCCCACGAGTTAGGAGACTTTG
TGATCCTACTCAATGCAGGGATGAGCACTCGACAAGCCTTGCTATTCAACTTCCTTTCTGCATGTTCTGCTATGTT
GGGCTAGCTTTTGGCATTITGGTGGGCAACAATTTGCTCCAAATATTATATTGCACTTGCTGGAGGCATGTTCT
CTATATTTCTCTGGCAGATATGTTTCCAGAGATGAATGATATGCTGAGAGAAAAGGTAAGTGAAGAAAAACCGA
TTTACCTTCTTCATGATTGAGAATGCTGGAATGTTAACTGGATTACAGCCATTCTACTCATTACCTTGATGCAGG
AGAAATCGAATTGGAGTAATCTAGA

Template name: SLC39A8, Mutagenesis instruction: Variant name: HUMAN_SLC39A8_Y131A, Variant sequence:

GGTACCACCATGGCCCCGGGTCGCGCGGTGGCCGGGCTCCTGTTGCTGGCGGCCGCCGGCCTCGGAGGAGTGGC
GGAGGGGCCAGGGCTAGCCTTCAGCGAGGATGTGCTGAGCGTGTTCCGGCGCAATCTGAGCCTGTCGGCGGCGC
AGCTCCAGCACTTGCTGGAGCAGATGGGAGCCGCTCCCGCGTGGGCGTCCCGGAGCCTGGCCAGCTGCACTTCA
ACCAAGTGTTTAACTGCTGAAGAGATCTTTTCCCTTCATGGCTTTTCAAATGCTACCCAAATAACCAGCTCCAAATTCT
CTGTCATCTGTCCAGCAGTCTTACAGCAATTGAACTTTACCCATGTGAGGATCGGCCCCAAGCACAAAACAAGACC
AAGTCATTGAGAAGTTTGGGGAGCCGGATTCTGTGCTGACGATTATTAATCTGGCATCTCTCCTCGGATTGATTT
TGACTCCACTGATAAAGAAATCTTATTTCCCAAAGATTTTGACCTTTTTTGTTGGGGCTGGCTATTGGGACTCTTTTT
CAAATGCAATTTTCCAACCTATTCCAGAGGCATTTGGATTTGATCCCAAAGTCGACAGTTATGTTGAGAAGGCAGT
TGCTGTGTTTGGTGGATTTTACCTACTTTTCTTTTTGAAAGAATGCTAAAGATGTTATTAAGACATATGGTCAGA
ATGGTCATACCCACTTTGGAAATGATAACTTTGGTCCTCAAGAAAAAACTCATCAACCTAAAGCATTACCTGCCATC
AATGGTGTGACATGCTATGCAAATCCTGCTGTACAGAAGCTAATGGACATATCCATTTTGATAATGTCAGTGTGG
TATCTCTACAGGATGGAAAAAAGAGCCAAGTTCATGTACCTGTTTGAAGGGGCCCAAAGTGTGAGAAATAGGGA
CGATTGCCTGGATGATAACGCTCTGCGATGCCCTCCACAATTTTCATCGATGGCCTGGCGATTGGGGCTTCCTGCAC
CTTGTCTCTCCTTCAGGGACTCAGTACTTCCATAGCAATCCTATGTGAGGAGTTTCCCCACGAGTTAGGAGACTTTG
TGATCCTACTCAATGCAGGGATGAGCACTCGACAAGCCTTGCTATTCAACTTCCTTTCTGCATGTTCTGCTATGTT
GGGCTAGCTTTTGGCATTITGGTGGGCAACAATTTGCTCCAAATATTATATTGCACTTGCTGGAGGCATGTTCT
CTATATTTCTCTGGCAGATATGTTTCCAGAGATGAATGATATGCTGAGAGAAAAGGTAAGTGAAGAAAAACCGA
TTTACCTTCTTCATGATTGAGAATGCTGGAATGTTAACTGGATTACAGCCATTCTACTCATTACCTTGATGCAGG
AGAAATCGAATTGGAGTAATCTAGA

Template name: SLC39A8, Mutagenesis instruction: Variant name: HUMAN_SLC39A8_S135A, Variant sequence:

GGTACCACCATGGCCCCGGGTCGCGCGGTGGCCGGGCTCCTGTTGCTGGCGGCCGCCGGCCTCGGAGGAGTGGC
GGAGGGGCCAGGGCTAGCCTTCAGCGAGGATGTGCTGAGCGTGTTCCGGCGCAATCTGAGCCTGTCGGCGGCGC
AGCTCCAGCACTTGCTGGAGCAGATGGGAGCCGCTCCCGCGTGGGCGTCCCGGAGCCTGGCCAGCTGCACTTCA
ACCAAGTGTTTAACTGCTGAAGAGATCTTTTCCCTTCATGGCTTTTCAAATGCTACCCAAATAACCAGCTCCAAATTCT
CTGTCATCTGTCCAGCAGTCTTACAGCAATTGAACTTTACCCATGTGAGGATCGGCCCCAAGCACAAAACAAGACC
AAGTCATTGAGAAGTTTGGGGATATGGATTCTGGCCGTGACGATTATTAATCTGGCATCTCTCCTCGGATTGATTT
TGACTCCACTGATAAAGAAATCTTATTTCCCAAAGATTTTGACCTTTTTTGTTGGGGCTGGCTATTGGGACTCTTTTT
CAAATGCAATTTTCCAACCTATTCCAGAGGCATTTGGATTTGATCCCAAAGTCGACAGTTATGTTGAGAAGGCAGT
TGCTGTGTTTGGTGGATTTTACCTACTTTTCTTTTTGAAAGAATGCTAAAGATGTTATTAAGACATATGGTCAGA
ATGGTCATACCCACTTTGGAAATGATAACTTTGGTCCTCAAGAAAAAACTCATCAACCTAAAGCATTACCTGCCATC
AATGGTGTGACATGCTATGCAAATCCTGCTGTACAGAAGCTAATGGACATATCCATTTTGATAATGTCAGTGTGG

TATCTCTACAGGATGGAAAAAAGAGCCAAGTTCATGTACCTGTTTGAAGGGGCCCAAAGTGTGAGAAATAGGGA
CGATTGCCTGGATGATAACGCTCTGCGATGCCCTCCACAATTTTCATCGATGGCCTGGCGATTGGGGCTTCCTGCAC
CTTGTCTCTCCTTCAGGGACTCAGTACTTCCATAGCAATCCTATGTGAGGAGTTTCCCCACGAGTTAGGAGACTTTG
TGATCCTACTCAATGCAGGGATGAGCACTCGACAAGCCTTGCTATTCAACTTCCTTTCTGCATGTTCTGCTATGTT
GGGCTAGCTTTTGGCATTTTGGTGGGCAACAATTTGCTCCAAATATTATATTGCACTTGCTGGAGGCATGTTCTCT
CTATATTTCTCTGGCAGATATGTTTCCAGAGATGAATGATATGCTGAGAGAAAAGGTAAGTGAAGAAAAACCGA
TTTACCTTCTTCATGATTGAGAATGCTGGAATGTTAACTGGATTACAGCCATTCTACTCATTACCTTGATGCAGG
AGAAATCGAATTGGAGTAATCTAGA

Template name: SLC39A8, Mutagenesis instruction: Variant name: HUMAN_SLC39A8_Q332S, Variant sequence:

GGTACCACCATGGCCCCGGGTCGCGCGGTGGCCGGGCTCCTGTTGCTGGCGGCCGCCGGCCTCGGAGGAGTGGC
GGAGGGGCCAGGGCTAGCCTTCAGCGAGGATGTGCTGAGCGTGTTCCGGCGCAATCTGAGCCTGTGGCGGGCGC
AGCTCCAGCACTTGCTGGAGCAGATGGGAGCCGCTCCCGCGTGGGCGTCCCGGAGCCTGGCCAGCTGCACTTCA
ACCAAGTGTTTAACTGCTGAAGAGATCTTTTCCCTTCATGGCTTTTCAAATGCTACCCAAATAACCAGCTCCAAATTCT
CTGTCTCTGTCCAGCAGTCTTACAGCAATTGAACTTTACCCATGTGAGGATCGGCCCCAAGCACAAAACAAGACC
AAGTCATTGAGAAGTTTGGGGATATGGATTCTGTGCTGAGTACGATTATTAATCTGGCATCTCTCCTCGGATTGATTT
TGACTCCACTGATAAAGAAATCTTATTTCCCAAAGATTTTGACCTTTTTTGTTGGGGCTGGCTATTGGGACTCTTTTT
CAAATGCAATTTTCCAACCTATTCCAGAGGCATTTGGATTTGATCCCAAAGTCGACAGTTATGTTGAGAAGGCAGT
TGCTGTGTTTGGTGGATTTTACCTACTTTTCTTTTTTGAAAGAATGCTAAAGATGTTATTAAGACATATGGTCAGA
ATGGTCATACCCACTTTGGAAATGATAACTTTGGTCCTCAAGAAAAAACTCATCAACCTAAAGCATTACCTGCCATC
AATGGTGTGACATGCTATGCAAATCCTGCTGTACAGAAGCTAATGGACATATCCATTTTGATAATGTCAGTGTGG
TATCTCTACAGGATGGAAAAAAGAGCCAAGTTCATGTACCTGTTTGAAGGGGCCCAAAGTGTGAGAAATAGGGA
CGATTGCCTGGATGATAACGCTCTGCGATGCCCTCCACAATTTTCATCGATGGCCTGGCGATTGGGGCTTCCTGCAC
CTTGTCTCTCCTTAGCGGACTCAGTACTTCCATAGCAATCCTATGTGAGGAGTTTCCCCACGAGTTAGGAGACTTTG
TGATCCTACTCAATGCAGGGATGAGCACTCGACAAGCCTTGCTATTCAACTTCCTTTCTGCATGTTCTGCTATGTT
GGGCTAGCTTTTGGCATTTTGGTGGGCAACAATTTGCTCCAAATATTATATTGCACTTGCTGGAGGCATGTTCTCT
CTATATTTCTCTGGCAGATATGTTTCCAGAGATGAATGATATGCTGAGAGAAAAGGTAAGTGAAGAAAAACCGA
TTTACCTTCTTCATGATTGAGAATGCTGGAATGTTAACTGGATTACAGCCATTCTACTCATTACCTTGATGCAGG
AGAAATCGAATTGGAGTAATCTAGA

Template name: SLC39A8, Mutagenesis instruction: Variant name: HUMAN_SLC39A8_Q332A, Variant sequence:

GGTACCACCATGGCCCCGGGTCGCGCGGTGGCCGGGCTCCTGTTGCTGGCGGCCGCCGGCCTCGGAGGAGTGGC
GGAGGGGCCAGGGCTAGCCTTCAGCGAGGATGTGCTGAGCGTGTTCCGGCGCAATCTGAGCCTGTGGCGGGCGC
AGCTCCAGCACTTGCTGGAGCAGATGGGAGCCGCTCCCGCGTGGGCGTCCCGGAGCCTGGCCAGCTGCACTTCA
ACCAAGTGTTTAACTGCTGAAGAGATCTTTTCCCTTCATGGCTTTTCAAATGCTACCCAAATAACCAGCTCCAAATTCT
CTGTCTCTGTCCAGCAGTCTTACAGCAATTGAACTTTACCCATGTGAGGATCGGCCCCAAGCACAAAACAAGACC
AAGTCATTGAGAAGTTTGGGGATATGGATTCTGTGCTGAGTACGATTATTAATCTGGCATCTCTCCTCGGATTGATTT
TGACTCCACTGATAAAGAAATCTTATTTCCCAAAGATTTTGACCTTTTTTGTTGGGGCTGGCTATTGGGACTCTTTTT
CAAATGCAATTTTCCAACCTATTCCAGAGGCATTTGGATTTGATCCCAAAGTCGACAGTTATGTTGAGAAGGCAGT
TGCTGTGTTTGGTGGATTTTACCTACTTTTCTTTTTTGAAAGAATGCTAAAGATGTTATTAAGACATATGGTCAGA
ATGGTCATACCCACTTTGGAAATGATAACTTTGGTCCTCAAGAAAAAACTCATCAACCTAAAGCATTACCTGCCATC
AATGGTGTGACATGCTATGCAAATCCTGCTGTACAGAAGCTAATGGACATATCCATTTTGATAATGTCAGTGTGG

TATCTCTACAGGATGGAAAAAAGAGCCAAGTTCATGTACCTGTTTGAAGGGGCCCAAAGTGTGAGAAATAGGGA
CGATTGCCTGGATGATAACGCTCTGCGATGCCCTCCACAATTTTCATCGATGGCCTGGCGATTGGGGCTTCCTGCAC
CTTGTCTCTCCTTGCCGGACTCAGTACTTCCATAGCAATCCTATGTGAGGAGTTTCCCCACGAGTTAGGAGACTTTG
TGATCCTACTCAATGCAGGGATGAGCACTCGACAAGCCTTGCTATTCAACTTCCTTTCTGCATGTTCTGCTATGTT
GGGCTAGCTTTTGGCATTITGGTGGGCAACAATTTTCGCTCCAAATATTATATTGCACTTGCTGGAGGCATGTTCT
CTATATTTCTCTGGCAGATATGTTTCCAGAGATGAATGATATGCTGAGAGAAAAGGTAAGTGAAGAAAAACCGA
TTTACCTTCTTCATGATTGAGAATGCTGGAATGTTAACTGGATTACAGCCATTCTACTCATTACCTTGATGCAGG
AGAAATCGAATTGGAGTAATCTAGA

Template name: SLC39A8, Mutagenesis instruction: Variant name: HUMAN_SLC39A8_L334F, Variant sequence:

GGTACCACCATGGCCCCGGGTCGCGCGGTGGCCGGGCTCCTGTTGCTGGCGGCCGCCGGCCTCGGAGGAGTGGC
GGAGGGGCCAGGGCTAGCCTTCAGCGAGGATGTGCTGAGCGTGTTCCGGCGCAATCTGAGCCTGTGGCGGCGC
AGCTCCAGCACTTGCTGGAGCAGATGGGAGCCGCTCCCGCGTGGGCGTCCCGGAGCCTGGCCAGCTGCACTTCA
ACCAGTGTTTAACTGCTGAAGAGATCTTTTCCCTTCATGGCTTTTCAAATGCTACCCAAATAACCAGCTCCAAATTCT
CTGTCTCTGTCCAGCAGTCTTACAGCAATTGAACTTTACCCATGTGAGGATCGGCCCCAAGCACAAAACAAGACC
AAGTCATTCAGAAGTTTGGGGATATGGATTCTGTGCTGAGTGACGATTATTAATCTGGCATCTCTCCTCGGATTGATTT
TGACTCCACTGATAAAGAAATCTTATTTCCCAAAGATTTTGACCTTTTTTGTGGGGCTGGCTATTGGGACTCTTTTT
CAAATGCAATTTTCCAATTATTCCAGAGGCATTTGGATTTGATCCCAAAGTCGACAGTTATGTTGAGAAGGCAGT
TGCTGTGTTTGGTGGATTTTACCTACTTTTCTTTTTTGAAAGAATGCTAAAGATGTTATTAAAGACATATGGTCAGA
ATGGTCATACCCACTTTGGAAATGATAACTTTGGTCTCAAGAAAAAACTCATCAACCTAAAGCATTACCTGCCATC
AATGGTGTGACATGCTATGCAAATCCTGCTGTACAGAAGCTAATGGACATATCCATTTTGATAATGTCAGTGTGG
TATCTCTACAGGATGGAAAAAAGAGCCAAGTTCATGTACCTGTTTGAAGGGGCCCAAAGTGTGAGAAATAGGGA
CGATTGCCTGGATGATAACGCTCTGCGATGCCCTCCACAATTTTCATCGATGGCCTGGCGATTGGGGCTTCCTGCAC
CTTGTCTCTCCTTCAGGGATTCAGTACTTCCATAGCAATCCTATGTGAGGAGTTTCCCCACGAGTTAGGAGACTTTG
TGATCCTACTCAATGCAGGGATGAGCACTCGACAAGCCTTGCTATTCAACTTCCTTTCTGCATGTTCTGCTATGTT
GGGCTAGCTTTTGGCATTITGGTGGGCAACAATTTTCGCTCCAAATATTATATTGCACTTGCTGGAGGCATGTTCT
CTATATTTCTCTGGCAGATATGTTTCCAGAGATGAATGATATGCTGAGAGAAAAGGTAAGTGAAGAAAAACCGA
TTTACCTTCTTCATGATTGAGAATGCTGGAATGTTAACTGGATTACAGCCATTCTACTCATTACCTTGATGCAGG
AGAAATCGAATTGGAGTAATCTAGA

6. CDG Mutants

FLAG tag inserted after a putative signal peptide (aa 1-22) in the N-terminal region:

> hSLC39A8 Signal NFLAG_pcDNA3.1(+)

> hSLC39A8 A391T Signal NFLAG:

> hSLC39A8_V33M_pcDNA3.1+_Signal NFLAG pcDNA3.1(+)

> hSLC39A8_G38R_pcDNA3.1+_Signal NFLAG pcDNA3.1(+)

> hSLC39A8_C113S_pcDNA3.1+_Signal NFLAG pcDNA3.1(+)

> hSLC39A8_G204C_pcDNA3.1+_Signal NFLAG pcDNA3.1(+)

> hSLC39A8_S335T_pcDNA3.1+_Signal NFLAG pcDNA3.1(+)

> hSLC39A8_I340N_pcDNA3.1+_Signal NFLAG pcDNA3.1(+)

> hSLC39A8 Signal NFLAG_pcDNA3.1(+)

GGTACCACCATGGCCCCGGGTCGCGCGGTGGCCGGGCTCCTGTTGCTGGCGGCCGCCGGCCTCGGAGGAGTGGC
GGATTACAAGGATGACGACGATAAGGAGGGGCCAGGGCTAGCCTTCAGCGAGGATGTGCTGAGCGTGTTCGGC
GCGAATCTGAGCCTGTCGGCGGCGCAGCTCCAGCACTTGCTGGAGCAGATGGGAGCCGCCTCCCGCGTGGGCGT
CCCGGAGCCTGGCCAGCTGCACTTCAACCAAGTGTAACTGCTGAAGAGATCTTTCCCTTCATGGCTTTTCAAATG
CTACCCAAATAACCAGCTCCAAATTCTCTGTCATCTGTCCAGCAGTCTTACAGCAATTGAACTTTCACCCATGTGAG
GATCGGCCCCAAGCACAAAAACAAGACCAAGTCATTGAGAAGTTTGGGGATATGGATTCTGTGTCAGTGACGATTATT
AATCTGGCATCTCTCCTCGGATTGATTTTGACTCCACTGATAAAGAAATCTTATTTCCCAAAGATTTTGACCTTTTT
GTGGGGCTGGCTATTGGGACTCTTTTTCAAATGCAATTTCCAACCTATTCCAGAGGCATTTGGATTGATCCCAA
AGTCGACAGTTATGTTGAGAAGGCAGTTGCTGTGTTGGTGGATTTTACCTACTTTCTTTTTGAAAGAATGCTAA
AGATGTTATTAAAGACATATGGTCAGAATGGTCATACCCACTTTGGAAATGATAACTTTGGTCTCAAGAAAAAAC
TCATCAACCTAAAGCATTACCTGCCATCAATGGTGTGACATGCTATGCAAATCCTGCTGTCACAGAAGCTAATGGA
CATATCCATTTTGATAATGTCAGTGTGGTATCTCTACAGGATGGAAAAAAGAGCCAAGTTCATGTACCTGTTTGA
AGGGGCCCAAACCTGTCAGAAATAGGGACGATTGCCTGGATGATAACGCTCTGCGATGCCCTCCACAATTCATCG
ATGGCCTGGCGATTGGGGCTTCCTGCACCTTGCTCTCCTTCAGGGACTCAGTACTTCCATAGCAATCCTATGTGAG
GAGTTTCCCCACGAGTTAGGAGACTTTGTGATCCTACTCAATGCAGGGATGAGCACTCGACAAGCCTTGCTATTCA
ACTTCCTTTCTGCATGTTCTGCTATGTTGGGCTAGCTTTTGGCATTGTTGGTGGGCAACAATTCGCTCCAAATATTA
TATTTGCACTTGCTGGAGGCATGTTCTCTATATTTCTCTGGCAGATATGTTTCCAGAGATGAATGATATGCTGAGA
GAAAAGGTAAGTGAAGAAAAACCGATTTACCTTCTTCATGATTGAGAATGCTGGAATGTAACTGGATTACAG
CCATTCTACTCATTACCTTGTATGCAGGAGAAATCGAATTGGAGTAATCTAGA

> hSLC39A8 A391T Signal NFLAG:

GGTACCACCATGGCCCCGGGTCGCGCGGTGGCCGGGCTCCTGTTGCTGGCGGCCGCCGGCCTCGGAGGAGTGGC
GGATTACAAGGATGACGACGATAAGGAGGGGCCAGGGCTAGCCTTCAGCGAGGATGTGCTGAGCGTGTTCGGC
GCGAATCTGAGCCTGTCGGCGGCGCAGCTCCAGCACTTGCTGGAGCAGATGGGAGCCGCCTCCCGCGTGGGCGT
CCCGGAGCCTGGCCAGCTGCACTTCAACCAAGTGTAACTGCTGAAGAGATCTTTCCCTTCATGGCTTTTCAAATG
CTACCCAAATAACCAGCTCCAAATTCTCTGTCATCTGTCCAGCAGTCTTACAGCAATTGAACTTTCACCCATGTGAG
GATCGGCCCCAAGCACAAAAACAAGACCAAGTCATTGAGAAGTTTGGGGATATGGATTCTGTGTCAGTGACGATTATT
AATCTGGCATCTCTCCTCGGATTGATTTTGACTCCACTGATAAAGAAATCTTATTTCCCAAAGATTTTGACCTTTTT
GTGGGGCTGGCTATTGGGACTCTTTTTCAAATGCAATTTCCAACCTATTCCAGAGGCATTTGGATTGATCCCAA
AGTCGACAGTTATGTTGAGAAGGCAGTTGCTGTGTTGGTGGATTTTACCTACTTTCTTTTTGAAAGAATGCTAA
AGATGTTATTAAAGACATATGGTCAGAATGGTCATACCCACTTTGGAAATGATAACTTTGGTCTCAAGAAAAAAC
TCATCAACCTAAAGCATTACCTGCCATCAATGGTGTGACATGCTATGCAAATCCTGCTGTCACAGAAGCTAATGGA
CATATCCATTTTGATAATGTCAGTGTGGTATCTCTACAGGATGGAAAAAAGAGCCAAGTTCATGTACCTGTTTGA
AGGGGCCCAAACCTGTCAGAAATAGGGACGATTGCCTGGATGATAACGCTCTGCGATGCCCTCCACAATTCATCG
ATGGCCTGGCGATTGGGGCTTCCTGCACCTTGCTCTCCTTCAGGGACTCAGTACTTCCATAGCAATCCTATGTGAG
GAGTTTCCCCACGAGTTAGGAGACTTTGTGATCCTACTCAATGCAGGGATGAGCACTCGACAAGCCTTGCTATTCA
ACTTCCTTTCTGCATGTTCTGCTATGTTGGGCTAGCTTTTGGCATTGTTGGTGGGCAACAATTCACCCAAATATTA
TATTTGCACTTGCTGGAGGCATGTTCTCTATATTTCTCTGGCAGATATGTTTCCAGAGATGAATGATATGCTGAGA
GAAAAGGTAAGTGAAGAAAAACCGATTTACCTTCTTCATGATTGAGAATGCTGGAATGTAACTGGATTACAG
CCATTCTACTCATTACCTTGTATGCAGGAGAAATCGAATTGGAGTAATCTAGA

>hSLC39A8_V33M_pcDNA3.1+ Signal NFLAG pcDNA3.1(+)

GGTACCACCATGGCCCCGGGTCGCGCGGTGGCCGGGCTCCTGTTGCTGGCGGCCGCCGGCCTCGGAGGAGTGGC
GgattacaaggatgacgacgataagGAGGGGCCAGGGCTAGCCTTCAGCGAGGATATGCTGAGCGTGTTCGGCGCGAAT
CTGAGCCTGTCGGCGGCGCAGCTCCAGCACTTGCTGGAGCAGATGGGAGCCGCCTCCCGCGTGGGCGTCCCGGA
GCCTGGCCAGCTGCACTTCAACCAAGTGTAACTGCTGAAGAGATCTTTCCCTTCATGGCTTTTCAAATGCTACCC
AAATAACCAGCTCCAAATTCTCTGTCATCTGTCCAGCAGTCTTACAGCAATTGAACTTTCACCCATGTGAGGATCGG

CCCAAGCACAAAACAAGACCAAGTCATTCAGAAGTTTGGGGATATGGATTCCCTGTCAGTGACGATTATTAATCTGG
CATCTCTCCTCGGATTGATTTTGACTCCACTGATAAAGAAATCTTATTTCCCAAAGATTTTGACCTTTTTTGTTGGGGC
TGGCTATTGGGACTCTTTTTTCAAATGCAATTTTCCAACCTATTCCAGAGGCATTTGGATTGATCCCAAAGTCGAC
AGTTATGTTGAGAAGGCAGTTGCTGTGTTTGGTGGATTTTACCTACTTTTCTTTTTTGAAAGAATGCTAAAGATGTT
ATTAAGACATATGGTCAGAATGGTCATACCCACTTTGGAAATGATAACTTTGGTCCTCAAGAAAAAACTCATCAA
CCTAAAGCATTACCTGCCATCAATGGTGTGACATGCTATGCAAATCCTGCTGTCACAGAAGCTAATGGACATATCC
ATTTTGATAATGTCAGTGTGGTATCTCTACAGGATGGAAAAAAGAGCCAAGTTCATGTACCTGTTTGAAGGGGCC
CAAAGTGTGAGAAATAGGGACGATTGCCTGGATGATAACGCTCTGCGATGCCCTCCACAATTTTCATCGATGGCCTG
GCGATTGGGGCTTCTGACCTTGTCTCTCCTTCAGGGACTCAGTACTTCCATAGCAATCCTATGTGAGGAGTTTCC
CCACGAGTTAGGAGACTTTGTGATCCTACTCAATGCAGGGATGAGCACTCGACAAGCCTTGCTATTCAACTTCCTTT
CTGCATGTTTCTGCTATGTTGGGCTAGCTTTTGGCATTTTGGTGGGCAACAATTTTCGCTCCAAATATTATATTTGCA
CTTGCTGGAGGCATGTTCTCTATATTTCTCTGGCAGATATGTTTCCAGAGATGAATGATATGCTGAGAGAAAAGG
TAACTGGAAGAAAAACCGATTTACCTTCTTCATGATTGAGAATGCTGGAATGTTAACTGGATTACAGCCATTCTA
CTCATTACCTTGATGCAGGAGAAATCGAATTGGAGTAATCTAGA

>hSLC39A8_G38R_pcDNA3.1+_Signal NFLAG pcDNA3.1(+)

GGTACCACCATGGCCCCGGGTCGCGCGGTGGCCGGGCTCCTGTTGCTGGCGGCCGCCGGCCTCGGAGGAGTGGC
GgattacaaggatgacgacgataagGAGGGGCCAGGGCTAGCCTTCAGCGAGGATGTGCTGAGCGTGTTCGGGCGAAT
CTGAGCCTGTGCGCGGCGCAGCTCCAGCACTTGCTGGAGCAGATGGGAGCCGCCTCCCGCGTGGGCGTCCCGGA
GCCTGGCCAGCTGCACTTCAACCAGTGTTTAACTGCTGAAGAGATCTTTTCCCTTCATGGCTTTTCAAATGCTACCC
AAATAACCAGCTCCAAATTCTGTCTATCTGTCCAGCAGTCTTACAGCAATTGAATTTTACCCATGTGAGGATCGG
CCCAAGCACAAAACAAGACCAAGTCATTCAGAAGTTTGGGGATATGGATTCCCTGTCAGTGACGATTATTAATCTGG
CATCTCTCCTCGGATTGATTTTGACTCCACTGATAAAGAAATCTTATTTCCCAAAGATTTTGACCTTTTTTGTTGGGGC
TGGCTATTGGGACTCTTTTTTCAAATGCAATTTTCCAACCTATTCCAGAGGCATTTGGATTGATCCCAAAGTCGAC
AGTTATGTTGAGAAGGCAGTTGCTGTGTTTGGTGGATTTTACCTACTTTTCTTTTTTGAAAGAATGCTAAAGATGTT
ATTAAGACATATGGTCAGAATGGTCATACCCACTTTGGAAATGATAACTTTGGTCCTCAAGAAAAAACTCATCAA
CCTAAAGCATTACCTGCCATCAATGGTGTGACATGCTATGCAAATCCTGCTGTCACAGAAGCTAATGGACATATCC
ATTTTGATAATGTCAGTGTGGTATCTCTACAGGATGGAAAAAAGAGCCAAGTTCATGTACCTGTTTGAAGGGGCC
CAAAGTGTGAGAAATAGGGACGATTGCCTGGATGATAACGCTCTGCGATGCCCTCCACAATTTTCATCGATGGCCTG
GCGATTGGGGCTTCTGACCTTGTCTCTCCTTCAGGGACTCAGTACTTCCATAGCAATCCTATGTGAGGAGTTTCC
CCACGAGTTAGGAGACTTTGTGATCCTACTCAATGCAGGGATGAGCACTCGACAAGCCTTGCTATTCAACTTCCTTT
CTGCATGTTTCTGCTATGTTGGGCTAGCTTTTGGCATTTTGGTGGGCAACAATTTTCGCTCCAAATATTATATTTGCA
CTTGCTGGAGGCATGTTCTCTATATTTCTCTGGCAGATATGTTTCCAGAGATGAATGATATGCTGAGAGAAAAGG
TAACTGGAAGAAAAACCGATTTACCTTCTTCATGATTGAGAATGCTGGAATGTTAACTGGATTACAGCCATTCTA
CTCATTACCTTGATGCAGGAGAAATCGAATTGGAGTAATCTAGA

>hSLC39A8_C113S_pcDNA3.1+_Signal NFLAG pcDNA3.1(+)

GGTACCACCATGGCCCCGGGTCGCGCGGTGGCCGGGCTCCTGTTGCTGGCGGCCGCCGGCCTCGGAGGAGTGGC
GgattacaaggatgacgacgataagGAGGGGCCAGGGCTAGCCTTCAGCGAGGATGTGCTGAGCGTGTTCGGCGCGAAT
CTGAGCCTGTGCGCGGCGCAGCTCCAGCACTTGCTGGAGCAGATGGGAGCCGCCTCCCGCGTGGGCGTCCCGGA
GCCTGGCCAGCTGCACTTCAACCAGTGTTTAACTGCTGAAGAGATCTTTTCCCTTCATGGCTTTTCAAATGCTACCC
AAATAACCAGCTCCAAATTCTGTCTATCTGTCCAGCAGTCTTACAGCAATTGAATTTTACCCAAGCGAGGATCGG
CCCAAGCACAAAACAAGACCAAGTCATTCAGAAGTTTGGGGATATGGATTCCCTGTCAGTGACGATTATTAATCTGG
CATCTCTCCTCGGATTGATTTTGACTCCACTGATAAAGAAATCTTATTTCCCAAAGATTTTGACCTTTTTTGTTGGGGC
TGGCTATTGGGACTCTTTTTTCAAATGCAATTTTCCAACCTATTCCAGAGGCATTTGGATTGATCCCAAAGTCGAC
AGTTATGTTGAGAAGGCAGTTGCTGTGTTTGGTGGATTTTACCTACTTTTCTTTTTTGAAAGAATGCTAAAGATGTT
ATTAAGACATATGGTCAGAATGGTCATACCCACTTTGGAAATGATAACTTTGGTCCTCAAGAAAAAACTCATCAA
CCTAAAGCATTACCTGCCATCAATGGTGTGACATGCTATGCAAATCCTGCTGTCACAGAAGCTAATGGACATATCC

ATTTTGATAATGTCAGTGTGGTATCTCTACAGGATGGAAAAAAGAGCCAAGTTCATGTACCTGTTTGAAGGGGCC
CAAAGTGTGAGAAATAGGGACGATTGCCTGGATGATAACGCTCTGCGATGCCCTCCACAATTTTCATCGATGGCCTG
GCGATTGGGGCTTCCTGCACCTTGCTCTCCTTCAGGGAAGTCTCAGTACTTCCATAGCAATCCTATGTGAGGAGTTTCC
CCACGAGTTAGGAGACTTTGTGATCCTACTCAATGCAGGGATGAGCACTCGACAAGCCTTGCTATTCAACTTCCTTT
CTGCATGTTCTGCTATGTTGGGCTAGCTTTTGGCATTGTTGGTGGGCAACAATTCGCTCCAAATATTATATTTGCA
CTTGCTGGAGGCATGTTCTCTATATTTCTCTGGCAGATATGTTTCCAGAGATGAATGATATGCTGAGAGAAAAGG
TAACTGGAAGAAAAACCGATTTACCTTCTTCATGATTGAGAATGCTGGAATGTTAACTGGATTACAGCCATTCTA
CTCATTACCTTGATGCAGGAGAAATCGAATTGGAGTAATCTAGA

>hSLC39A8_G204C_pcDNA3.1+_Signal NFLAG pcDNA3.1(+)

GGTACCACCATGGCCCCGGGTCGCGCGGTGGCCGGGCTCCTGTTGCTGGCGGCCGCCGGCCTCGGAGGAGTGGC
GgattacaaggatgacgacgataagGAGGGGCCAGGGCTAGCCTTCAGCGAGGATGTGCTGAGCGTGTTCGGCGCGAAT
CTGAGCCTGTGCGCGGCGCAGCTCCAGCACTTGCTGGAGCAGATGGGAGCCGCCTCCGCGTGGGCGTCCCGGA
GCCTGGCCAGCTGCACTTCAACCAAGTGTAACTGCTGAAGAGATCTTTCCCTTCATGGCTTTTCAAATGCTACCC
AAATAACCAGCTCCAAATTCTGTCTCATCTGTCCAGCAGTCTTACAGCAATTGAACCTTTCACCCATGTGAGGATCGG
CCCAAGCACAAAACAAGACCAAGTCATTGAGAAGTTTGGGGATATGGATTCTGTGAGTGACGATTATTAATCTGG
CATCTCTCCTCGGATTGATTTTGACTCCACTGATAAAGAAATCTTATTTCCAAAGATTTTGACCTTTTTTGTTGGGGC
TGGCTATTGGGACTCTTTTTCAAATGCAATTTTCAACTTATTCAGAGGCATTTGGATTGATCCCAAAGTCGAC
AGTTATGTTGAGAAGGCAGTTGCTGTGTTTTGCGGATTTTACCTACTTTTCTTTTTGAAAGAATGCTAAAGATGTT
ATTAAAGACATATGGTCAGAATGGTCATACCCACTTTGGAAATGATAACTTTGGTCCTCAAGAAAAAACTCATCAA
CCTAAAGCATTACCTGCCATCAATGGTGTGACATGCTATGCAAATCCTGCTGTACAGAAGCTAATGGACATATCC
ATTTTGATAATGTCAGTGTGGTATCTCTACAGGATGGAAAAAAGAGCCAAGTTCATGTACCTGTTTGAAGGGGCC
CAAAGTGTGAGAAATAGGGACGATTGCCTGGATGATAACGCTCTGCGATGCCCTCCACAATTTTCATCGATGGCCTG
GCGATTGGGGCTTCCTGCACCTTGCTCTCCTTCAGGGAAGTCTCAGTACTTCCATAGCAATCCTATGTGAGGAGTTTCC
CCACGAGTTAGGAGACTTTGTGATCCTACTCAATGCAGGGATGAGCACTCGACAAGCCTTGCTATTCAACTTCCTTT
CTGCATGTTCTGCTATGTTGGGCTAGCTTTTGGCATTGTTGGTGGGCAACAATTCGCTCCAAATATTATATTTGCA
CTTGCTGGAGGCATGTTCTCTATATTTCTCTGGCAGATATGTTTCCAGAGATGAATGATATGCTGAGAGAAAAGG
TAACTGGAAGAAAAACCGATTTACCTTCTTCATGATTGAGAATGCTGGAATGTTAACTGGATTACAGCCATTCTA
CTCATTACCTTGATGCAGGAGAAATCGAATTGGAGTAATCTAGA

>hSLC39A8_S335T_pcDNA3.1+_Signal NFLAG pcDNA3.1(+)

GGTACCACCATGGCCCCGGGTCGCGCGGTGGCCGGGCTCCTGTTGCTGGCGGCCGCCGGCCTCGGAGGAGTGGC
GgattacaaggatgacgacgataagGAGGGGCCAGGGCTAGCCTTCAGCGAGGATGTGCTGAGCGTGTTCGGCGCGAAT
CTGAGCCTGTGCGCGGCGCAGCTCCAGCACTTGCTGGAGCAGATGGGAGCCGCCTCCGCGTGGGCGTCCCGGA
GCCTGGCCAGCTGCACTTCAACCAAGTGTAACTGCTGAAGAGATCTTTCCCTTCATGGCTTTTCAAATGCTACCC
AAATAACCAGCTCCAAATTCTGTCTCATCTGTCCAGCAGTCTTACAGCAATTGAACCTTTCACCCATGTGAGGATCGG
CCCAAGCACAAAACAAGACCAAGTCATTGAGAAGTTTGGGGATATGGATTCTGTGAGTGACGATTATTAATCTGG
CATCTCTCCTCGGATTGATTTTGACTCCACTGATAAAGAAATCTTATTTCCAAAGATTTTGACCTTTTTTGTTGGGGC
TGGCTATTGGGACTCTTTTTCAAATGCAATTTTCAACTTATTCAGAGGCATTTGGATTGATCCCAAAGTCGAC
AGTTATGTTGAGAAGGCAGTTGCTGTGTTTTGGTGGATTTTACCTACTTTTCTTTTTGAAAGAATGCTAAAGATGTT
ATTAAAGACATATGGTCAGAATGGTCATACCCACTTTGGAAATGATAACTTTGGTCCTCAAGAAAAAACTCATCAA
CCTAAAGCATTACCTGCCATCAATGGTGTGACATGCTATGCAAATCCTGCTGTACAGAAGCTAATGGACATATCC
ATTTTGATAATGTCAGTGTGGTATCTCTACAGGATGGAAAAAAGAGCCAAGTTCATGTACCTGTTTGAAGGGGCC
CAAAGTGTGAGAAATAGGGACGATTGCCTGGATGATAACGCTCTGCGATGCCCTCCACAATTTTCATCGATGGCCTG
GCGATTGGGGCTTCCTGCACCTTGCTCTCCTTCAGGGAAGTCTCAGTACTTCCATAGCAATCCTATGTGAGGAGTTTCC
CCACGAGTTAGGAGACTTTGTGATCCTACTCAATGCAGGGATGAGCACTCGACAAGCCTTGCTATTCAACTTCCTTT
CTGCATGTTCTGCTATGTTGGGCTAGCTTTTGGCATTGTTGGTGGGCAACAATTCGCTCCAAATATTATATTTGCA
CTTGCTGGAGGCATGTTCTCTATATTTCTCTGGCAGATATGTTTCCAGAGATGAATGATATGCTGAGAGAAAAGG

TAAGTGAAGAAAAACCGATTTACCTTCTTCATGATTCAGAATGCTGGAATGTAACTGGATTCACAGCCATTCTA
CTCATTACCTTGTATGCAGGAGAAATCGAATTGGAGTAATCTAGA

>hSLC39A8_I340N_pcDNA3.1+_Signal NFLAG pcDNA3.1(+)

GGTACCACCATGGCCCCGGGTCGCGCGGTGGCCGGGCTCCTGTTGCTGGCGGCCGCGGCCTCGGAGGAGTGGC
GgattacaaggatgacgacgataagGAGGGGCCAGGGCTAGCCTTCAGCGAGGATGTGCTGAGCGTGTTCCGGCGCAAT
CTGAGCCTGTGCGCGGCGCAGCTCCAGCACTTGCTGGAGCAGATGGGAGCCGCTCCCGCGTGGGCGTCCCGGA
GCCTGGCCAGCTGCACTTCAACCAGTGTAACTGCTGAAGAGATCTTTCCCTTCATGGCTTTTCAAATGCTACCC
AAATAACCAGCTCCAAATTCTGTCTATGTCCAGCAGTCTTACAGCAATTGAACTTTACCCATGTGAGGATCGG
CCCAAGCACAAAACAAGACCAAGTCATTGAGAAAGTTGGGGATATGGATTCTGTGAGTACGATTATTAATCTGG
CATCTCTCCTCGGATTGATTTTGACTCCACTGATAAAGAAATCTTATTTCCCAAAGATTTTGACCTTTTTTGTGGGGC
TGGCTATTGGGACTCTTTTTTCAAATGCAATTTTCAAATTTTCCAGAGGCATTTGGATTGATCCCAAAGTCGAC
AGTTATGTTGAGAAGGCAGTTGCTGTGTTTGGTGGATTTTACCTACTTTTCTTTTTGAAAGAATGCTAAAGATGTT
ATTAAGACATATGGTCAGAATGGTCATACCCACTTTGGAAATGATAACTTTGGTCTCAAGAAAAAACTCATCAA
CCTAAAGCATTACCTGCCATCAATGGTGTGACATGCTATGCAATCCTGCTGTCACAGAAGCTAATGGACATATCC
ATTTTGATAATGTGAGTGTGGTATCTCTACAGGATGGAAAAAAGAGCCAAAGTTCATGTACCTGTTTGAAGGGGCC
CAAATGTGAGAAATAGGGACGATTGCCTGGATGATAACGCTCTGCGATGCCCTCCACAATTTTCATCGATGGCCTG
GCGATTGGGGCTTCTGACCTTGTCTCTCCTTCAGGGACTCAGTACTTCCATAGCAAACCTATGTGAGGAGTTTCC
CCACGAGTTAGGAGACTTTGTGATCCTACTCAATGCAGGGATGAGCACTCGACAAGCCTTGCTATTCAACTTCCTTT
CTGCATGTTCTGCTATGTTGGGCTAGCTTTTGGCATTTTGGTGGGCAACAATTTGCTCCAAATATTATATTGCA
CTTGTGGAGGCATGTTCTCTATATTCTCTGGCAGATATGTTTCCAGAGATGAATGATATGCTGAGAGAAAAGG
TAAGTGAAGAAAAACCGATTTACCTTCTTCATGATTCAGAATGCTGGAATGTAACTGGATTCACAGCCATTCTA
CTCATTACCTTGTATGCAGGAGAAATCGAATTGGAGTAATCTAGA

Supplementary Information: Cell Culture Reagents

Critical reagents

Reagents

Reagent	Description	Catalog number
L-WRN Conditioned media	Conditioned media to enrich for stem cell growth and expansion	Analytical Biological Services C01-0770
Advanced DMEM/F12	Primary culture media	Gibco 12634
Pen/Strep	Primary culture media	Gibco 15140122
L-Glut	Primary culture media	Gibco 25030081
FBS	Primary culture media	Sigma F6178-500ml
Y-27632	Rho kinase inhibitor; used to improve cell viability after passage	Tocris 1254
DAPT	Gamma secretase inhibitor; for differentiation media	Millipore 565784
TPP 24-well plates	Cell culture plates	Sigma Z707791
Matrigel	Extracellular matrix	Corning 354234

PBS		Hyclone SH300028.02
0.5M EDTA	For passaging	Invitrogen 15575-020
70µm cell strainers	For passaging	Corning 08-771-2 (352350)
10X Trypsin solution	For passaging	Sigma T4549
DMEM/F12	Wash media for passaging	Sigma D6421-500ml
PC Transwell inserts	0.4 µm polycarbonate membrane; for submerged culture	Corning 3413
40µm cell strainers	For seeding TWs	Corning 05-771-1 (352340)
Attachment factor	For seeding TWs	Thermo S006100
Manganese		Sigma M4880
White clear bottom 96well plates	Assay plate for Apotox-Glo	Corning 3903
Qiazol	Lysis buffer for RNA extraction	Qiagen 79306
Direct-zol RNA mini prep kit	RNA extraction	Zymo research R2052
High-Capacity cDNA Reverse Transcription Kit	cDNA synthesis kit	Thermo 4374966
TaqMan universal PCR master mix	2X qPCR reaction mix for TLDA	Applied Biosystems 4304437
DMSO	Compound control	Sigma D2650
Collagenase type I	Establishment of spheroids	Invitrogen 17100-017
Gentamycin	Establishment of spheroids	Sigma G1397

Compounds

JNJ	BID	Notes
16535207	9351027, 48780907	Efavirenz R (inactive)
26892567	47806864, 43128694	Efavirenz S (active)
74701315	50444093	Efavirenz series analog, Cmpd 9

Media recipes

Primary culture media

Reagent	Volume
Advanced DMEM/F12	500 ml
100X L-Glutamine	6.25 ml
100X Pen/Strep	6.25 ml
FBS (heat inactivated)	125 ml

Washing media

Reagent	Volume
Advanced DMEM/F12 with HEPES	500 ml
100X L-Glutamine	5 ml
100X Pen/Strep	5 ml
FBS (heat inactivated)	50 ml

Collagenase solution

Reagent	Volume
Collagenase type I	20 mg
Gentamycin	10 μ l
Washing Media	10 ml

PBS-EDTA solution 0.5 mM – for passaging

Reagent	Volume
PBS	500 ml
0.5M EDTA	500 μ l

1X Trypsin-EDTA solution

Reagent	Volume
0.5 mM PBS-EDTA solution	9 ml
10X Trypsin	1 ml

50% L-WRN conditioned medium

Reagent	Volume
Primary Culture Media	40 ml
100% L-WRN media	40 ml

DAPT+LPS differentiation media

Reagent	Volume
Primary culture media	5 ml
25 mM DAPT	2 μ l (10 μ M final)
1 mg/ml LPS	5 μ l (1 μ g/ml final)