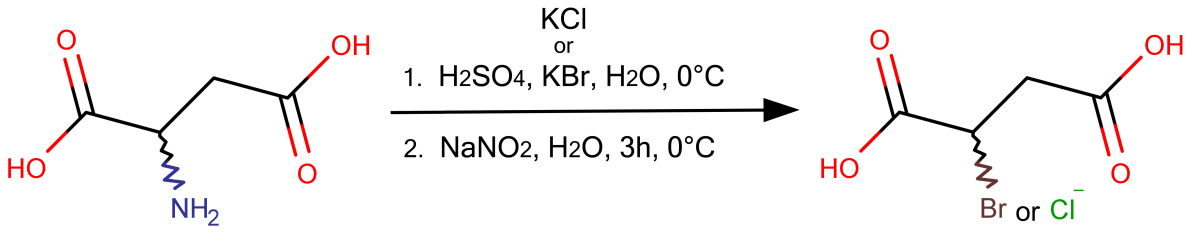
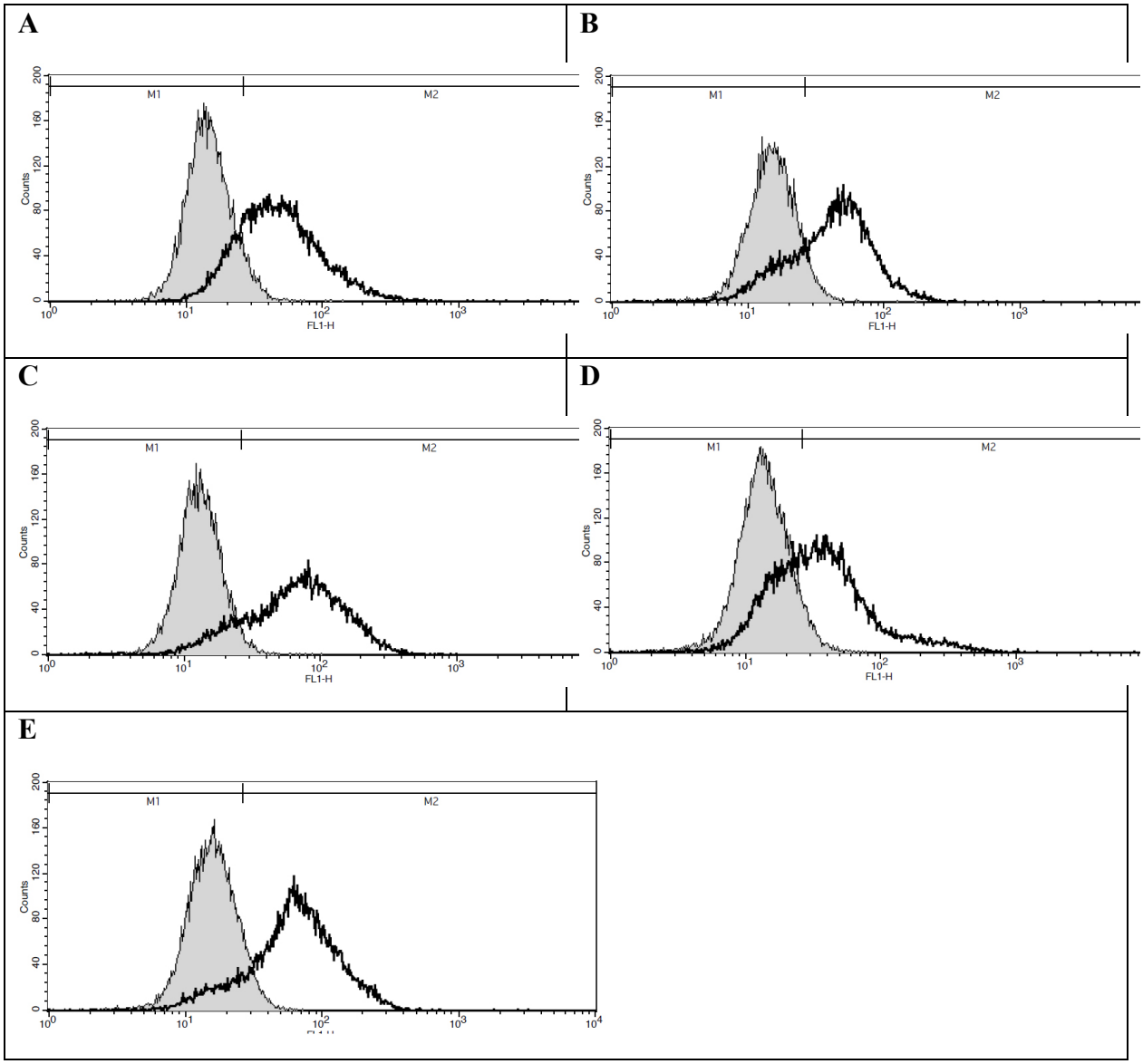
Supplementary information



Supplementary Fig. 1. Synthesis of halogeno succinic acid.



Supplementary figure 2. FACS Analysis of HEK293 stably transfected with several SUCNR1 mutants. A. WT; B. R252A; C. R281A; D. R99A; E. H103A

Supplementary Methods

Elemental analyses (C, H, N, S) were determined on a Thermo Scientific Flash EA 1112 elemental analyzer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and were within ±0.4% of the theoretical values. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 500 MHz instrument (Bruker, Billerica, Massachusetts, USA) using deuterated dimethyl sulfoxide (d6-DMSO) as the solvent and tetramethylsilane (TMS) as an internal standard; chemical shifts are reported in δ values (ppm) relative to that of internal TMS. The abbreviations s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, and br = broad are used throughout.

Synthesis

The synthesis of halogeno derivatives is slightly modified from Zurwerra *et al* (Zurwerra *et al.*, 2012). To a solution of R or S-Aspartic (1.00 g, 1 equiv) in 20 mL of H_2SO_4 2M (6.60 equiv) at 0°C (NaCl/ice) was added KBr (4.00 g, 4.5 equiv) or KCl (2.52 g, 4.5 equiv) followed by a drop addition of a $NaNO_2$ solution (0.92 g, 1.80 equiv) in H_2O (2 mL). After 3h at 0°C, the halogenosuccinate was extracted with EtOAc (15mL x 3) and dried over $MgSO_4$. The final compound was concentred under reduced pressure as a white to yellowish compound.

R-Bromosuccinic acid

(2R)-2-Bromosuccinic acid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.98 (br. s, 2H), 4.52 (dd, J = 8.5, 6.3 Hz, 1H), 3.08 (dd, J = 17.1, 8.6 Hz, 1H), 2.89 (dd, J = 17.1, 6.3 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.99, 170.11, 40.51, 39.49. Anal. ($C_4H_5O_4Br$) theoretical: C 24.39; H 2.56. Found: C 24.99; H 2.61

R-Chlorosuccinic acid

(2R)-2-Chlorosuccinic acid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.91 (br s, 2H), 4.63 (t, J = 7.0 Hz, 1H), 2.99 (dd, J = 17.0, 7.2 Hz, 1H), 2.84 (dd, J = 16.9, 6.7 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.60, 169.61, 52.61, 39.26. Anal. ($C_4H_5O_4Cl$) theoretical: C 31.50; H 3.30. Found: C 31.51; H 3.37

S-Chlorosuccinic acid

(2S)-2-Chlorosuccinic acid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.91 (br s, 2H), 4.63 (t, J = 7.0 Hz, 1H), 2.99 (dd, J = 17.0, 7.2 Hz, 1H), 2.84 (dd, J = 16.9, 6.7 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.60, 169.61, 52.61, 39.26. Anal. ($C_4H_5O_4Cl$) theoretical: C 31.50; H 3.30. Found: C 31.42; H 3.37

Flow cytometry analysis

The following commercially available antibodies was used for FACS analysis: monoclonal anti-FLAG clone M2 (F3165) from Sigma-Aldrich (St Louis, Missouri, USA), anti-Mouse (Alexa Fluor® 488 Conjugate, 4408) from Cell Signaling Technology (Danvers, Massachusetts, USA)). Cells (2 .10^5 cells per tube) were incubated with monoclonal ANTI-FLAG M2 for 45 min at 4°C. After wash, cells were incubated with anti-Mouse IgG (H+L), $F(ab')_2$ Fragment (1:1000, Alexa Fluor® 488 Conjugate) for 45 min at 4°C in the dark. Data were acquired on BD FACSCalibur 2 lasers (Becton Dickinson) and analysed with Cellquest pro. The gate on living cells was made using the SSC/FSC dot plot.