Supplementary Figures

Fig. S1 Chemical synthesis of (Bdp-So and (*S*)-FTY720 regioisomer. (A) Synthetic schemes used to prepare the (*S*)-FTY720 regioisomer. (B) Synthetic scheme for the synthesis of Bdp-So.

Fig. S2 Inhibitor-kinetic analysis for stably expressed SK1 in HEK 293 cells. Dixon, S/V *versus* S and 1/V *versus* 1/S plots for Ki determinations for (A) FTY720, (B) (*S*)-FTY720 vinylphosphonate, (C) SKi. Results are representative of three independent experiments.

Supplementary Information

To prepare the (*S*)-FTY720 regioisomer, the positions of the amino group and one of the prochiral hydroxymethyl groups of FTY720 were interchanged as follows. Epoxide (*R*)-**2** was prepared by Sharpless asymmetric epoxidation of allyl alcohol **1** as previously described (1). Regioselective opening of 2,3-epoxy alcohol (*R*)-**2** with sodium azide in the presence of ammonium chloride in aqueous MeOH at reflux gave azido diol (*S*)-**3** as the only product in 93% yield. The azido group was reduced to an amino group with Pearlman's catalyst to give the desired (*S*)-FTY720 regioisomer in 92% yield (Suppl. Fig. 1A). The intermediates and products were characterized by ^{1}H- and ^{13}C-NMR spectroscopy and high-resolution mass spectrometry with electrospray ionization.

The preparation of Bdp-So is outlined in Suppl Fig. 1B. An *E*-selective olefin cross-metathesis reaction of (*S*)-Garner allylic alcohol **5**, which was prepared by *p*-toluenesulfonic acid mediated opening of **4**, with intermediate **6** (2) provided *N*-Boc-Bodipy-sphingosine derivative **7**. Deprotection of **7** using $BF_3{\cdot}OEt_2$ in the presence of 4 Å molecular sieves afforded Bdp-So (3, 4).

1. Lu, X., Sun, C., Valentine, W.J., E.S. Liu, J., Tigyi, G. & Bittman, R. (2009) *J. Org. Chem.* **74**:3192-3195.

2. Peters, C., Billich, A., Ghobrial, M., Högenauer, K., Ullrich, T. & Nussbaumer, P. (2007) *J. Org. Chem.* **72**:1842-1845.

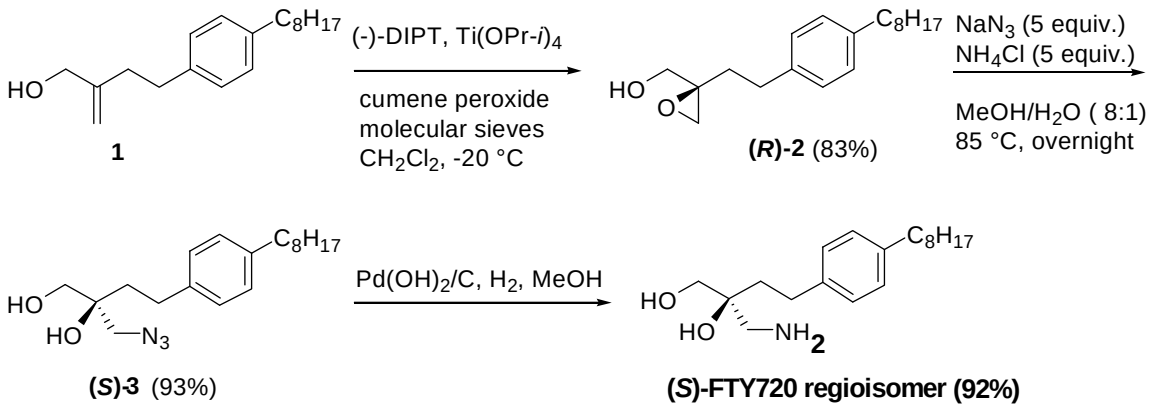
3. Li, Z. & Bittman, R. (2007) *J. Org. Chem.* **72**:8376-8382.

4. Bode, C., Sensken, S.-C., Peest, U., Beutel, G., Thol, F., Levkau, B., Li, Z., Bittman, R., Huang, T., Tölle, M., van der Giet, M. & Gräler, M.H. (2010) *J. Cell. Biochem.* **109**:1232-1243.

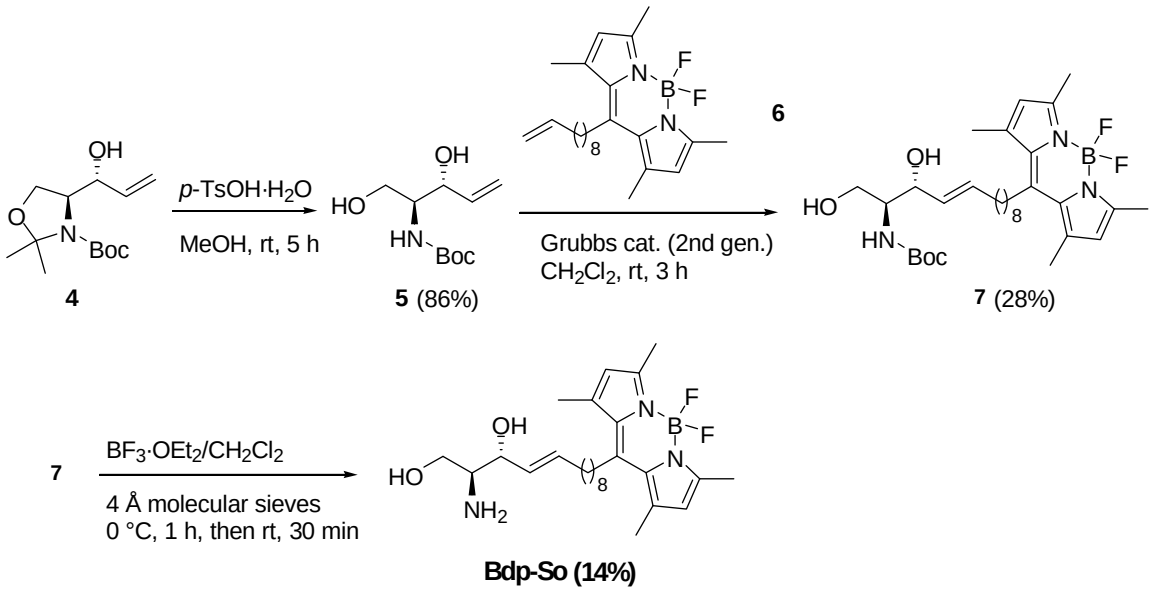
(2*S*)-2-(Aminomethyl)-4-(4'-*n*-octylphenyl)butane-1,2-diol [(*S*)-FTY720 regioisomer]. $[\alpha]^{25}_{D}$ +1.0° (*c* 1.84, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.26-1.38 (m, 10H), 1.58 (m, 2H), 1.70 (m, 2H), 2.55 (t, 2H, *J* = 7.6 Hz), 2.64 (m, 2H), 2.96 (s, 2H), 3.64 (m, 2H), 3.71 (br s, 4H), 7.09 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 29.0, 29.3, 29.4, 29.5, 31.6, 31.9, 35.6, 38.3, 48.0, 68.5, 72.3, 128.1, 128.4, 139.1, 140.5. HRMS ($M+Na^+$) *m/z* calcd for $C_{19}H_{33}NO_2Na^+$ 330.2403, found 330.2407.

Bdp-So. ESI-HRMS $(M+H)^+$ *m/z* calculated for $C_{26}H_{41}BF_2N_3O_2^+$ 476.3254, found 476.3250.

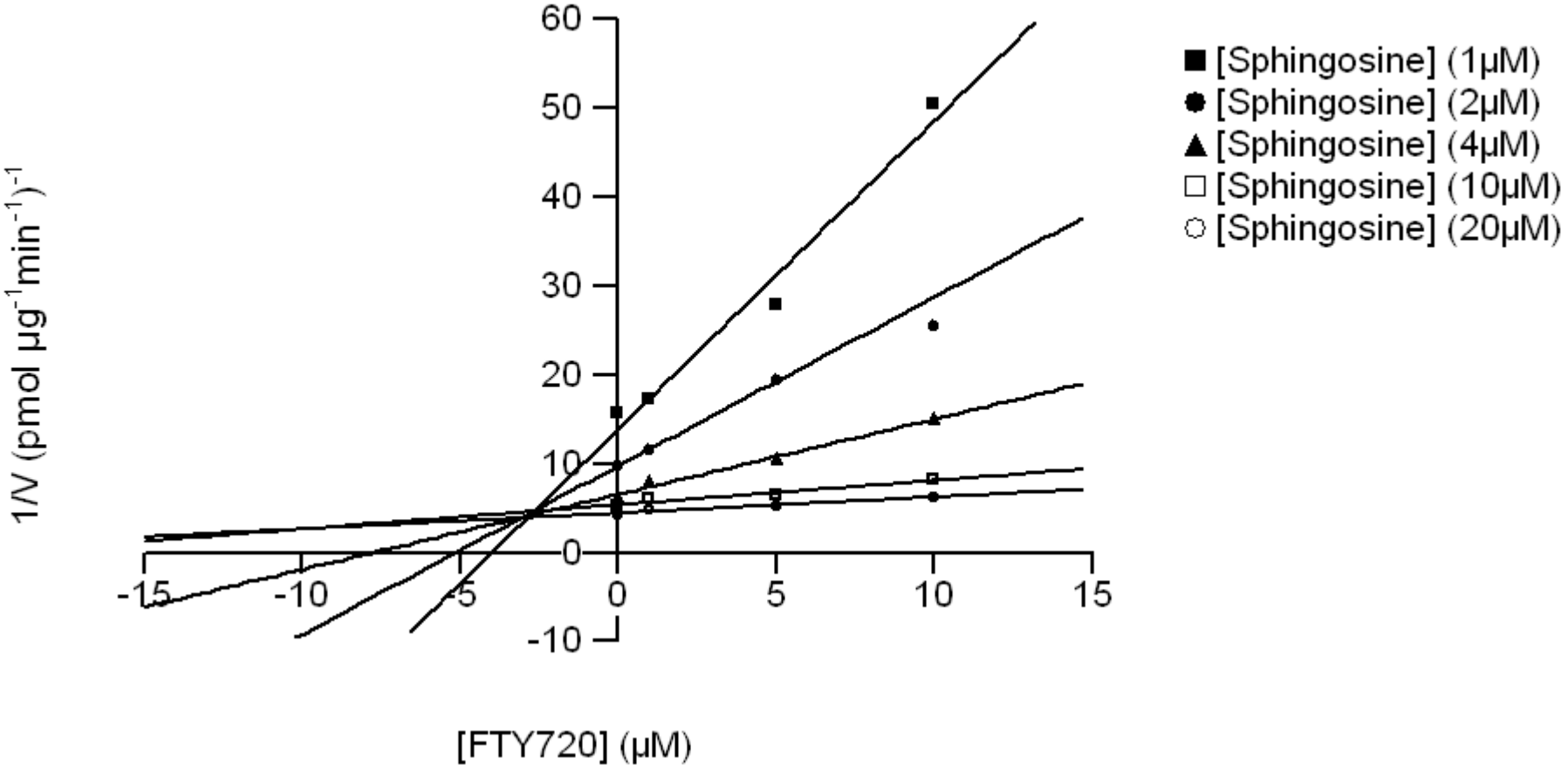
Suppl Fig. 1A

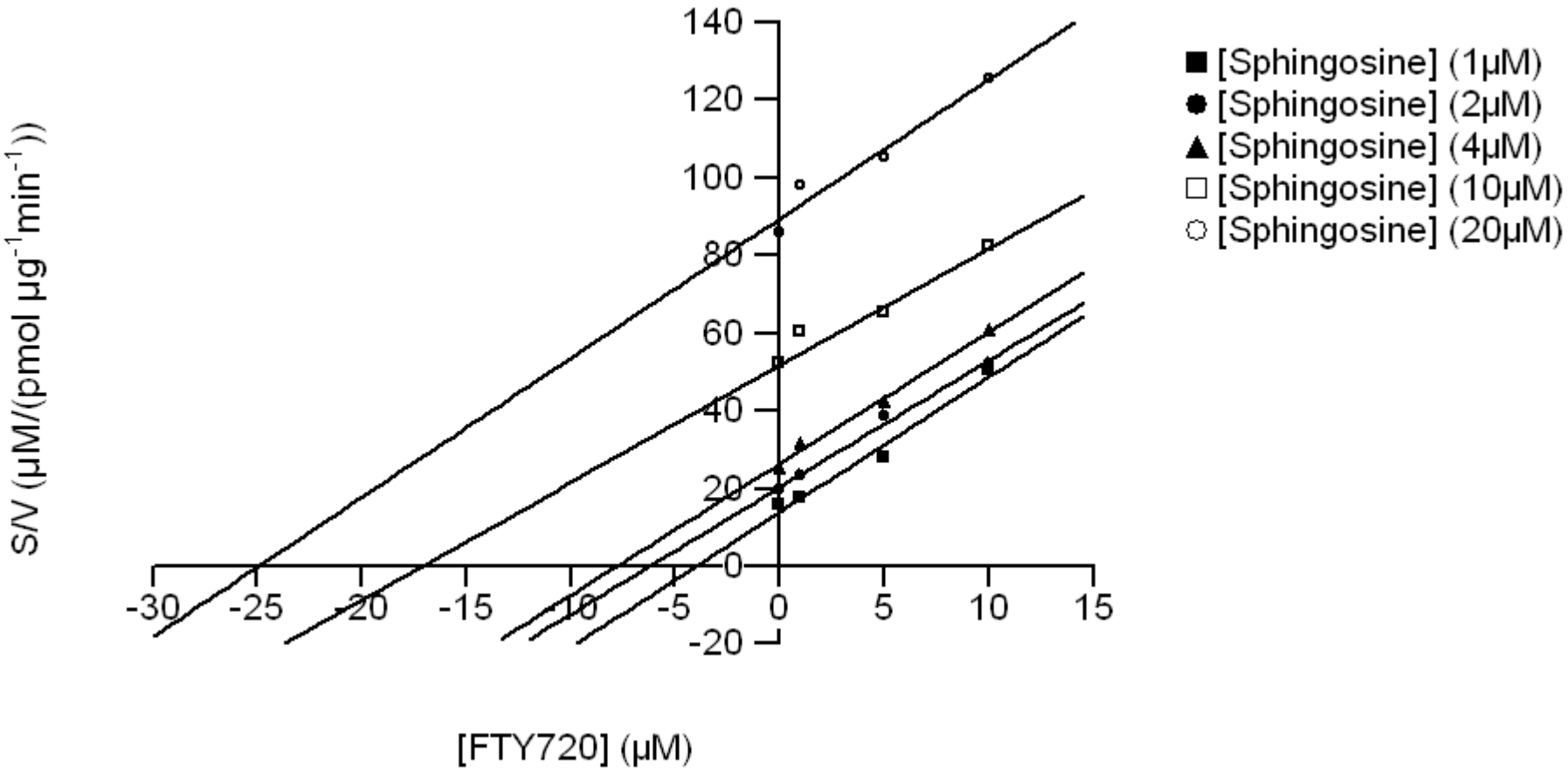

C8H17
HO
1
(-)-DIPT, Ti(OPr-i)4
cumene peroxide
molecular sieves
CH2Cl2, -20 °C
HO
O
C8H17
(R)-2 (83%)
NaN3 (5 equiv.)
NH4Cl (5 equiv.)
MeOH/H2O (8:1)
85 °C, overnight
HO
HO
N3
C8H17
(S)-3 (93%)
Pd(OH)2/C, H2, MeOH
HO
HO
NH2
C8H17
(S)-FTY720 regioisomer (92%)

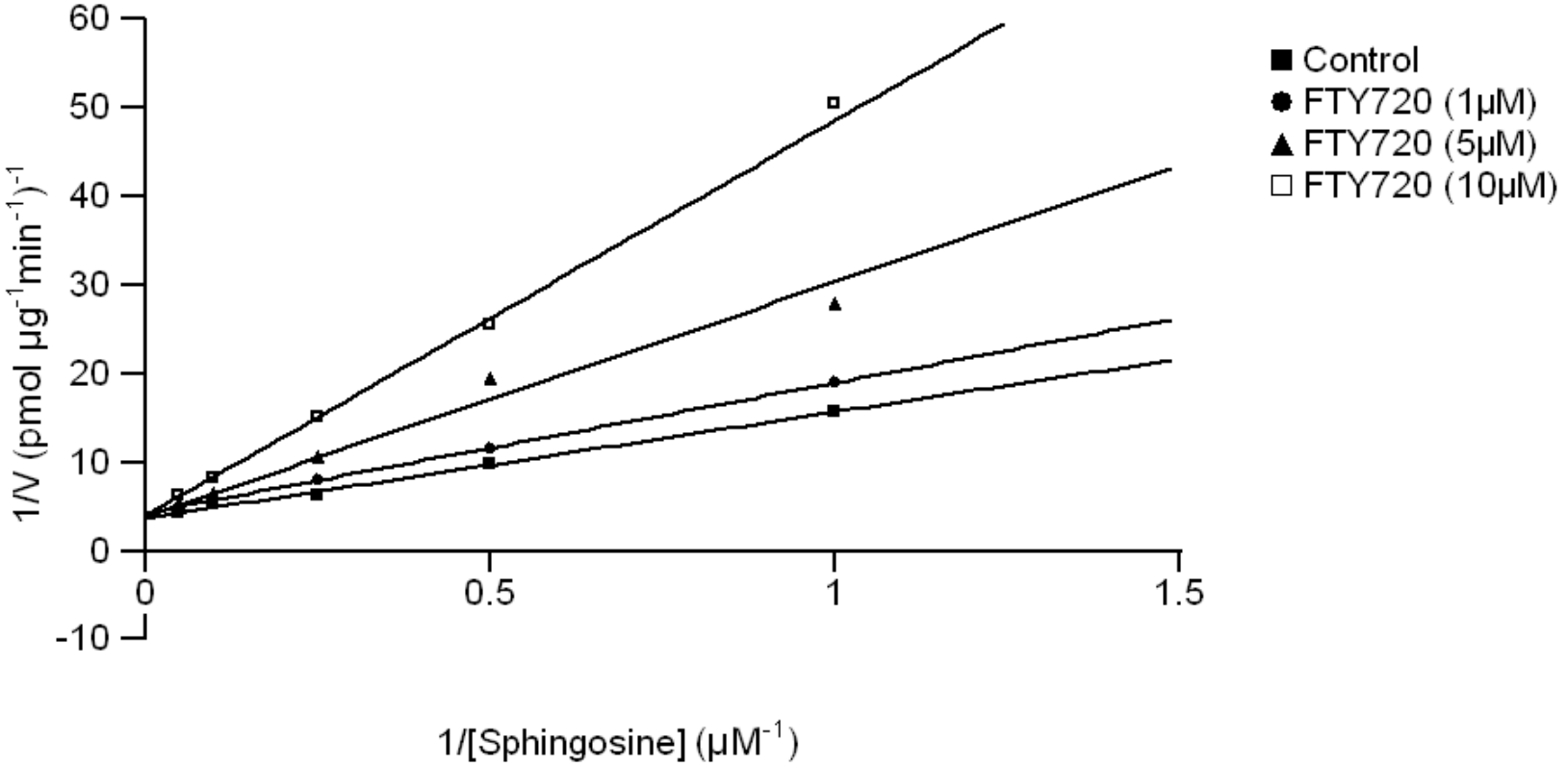
Suppl Fig. 1B


OH
O
N
Boc
4
p-TsOH·H2O
MeOH, rt, 5 h
OH
HO
HN
Boc
5 (86%)
6
F
B
F
N
8
Grubbs cat. (2nd gen.)
CH2Cl2, rt, 3 h
OH
HO
HN
Boc
7 (28%)
7
BF3·OEt2/CH2Cl2
4 Å molecular sieves
0 °C, 1 h, then rt, 30 min
OH
HO
NH2
Bdp-So (14%)

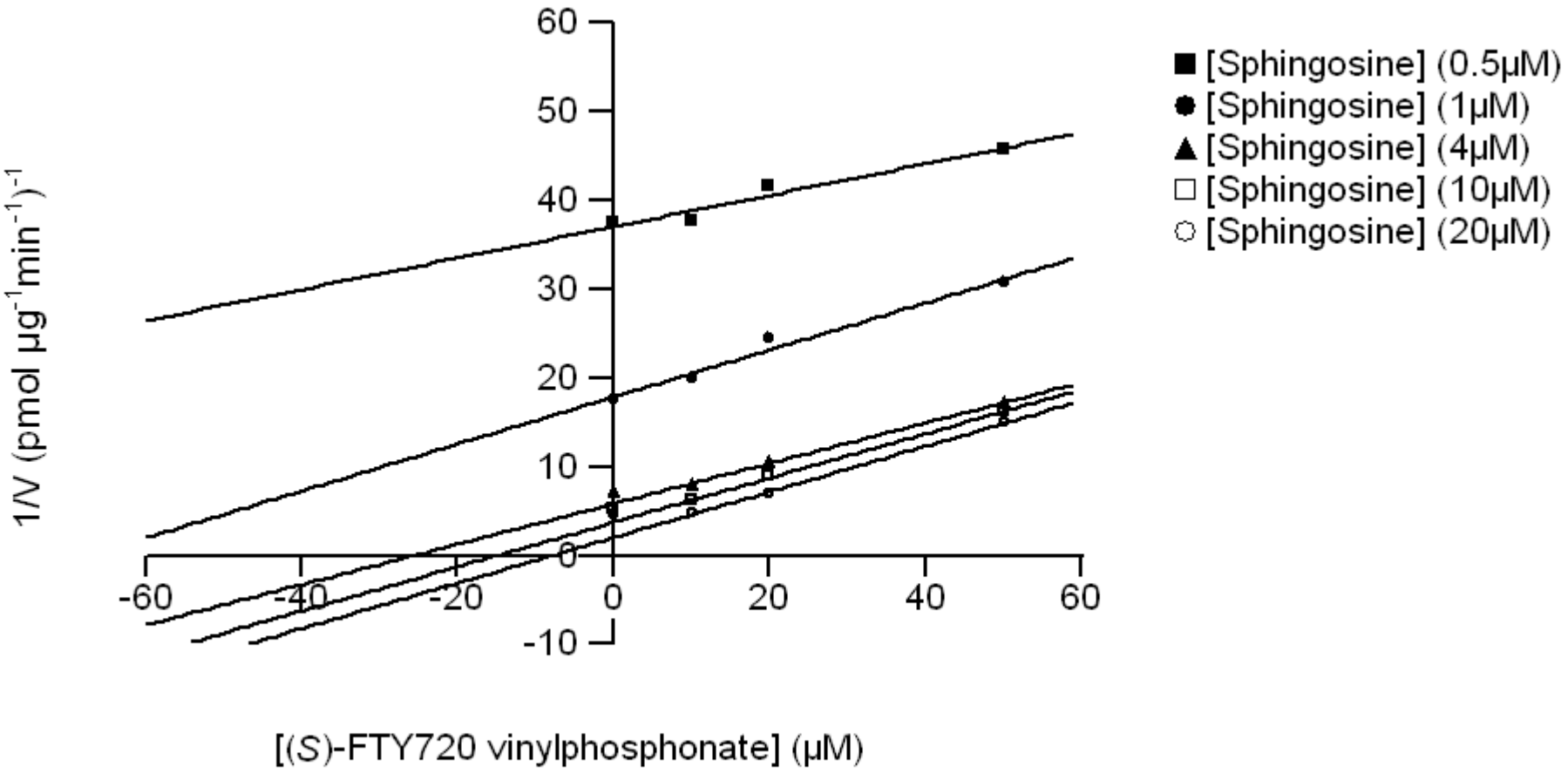
Suppl Fig. 2A

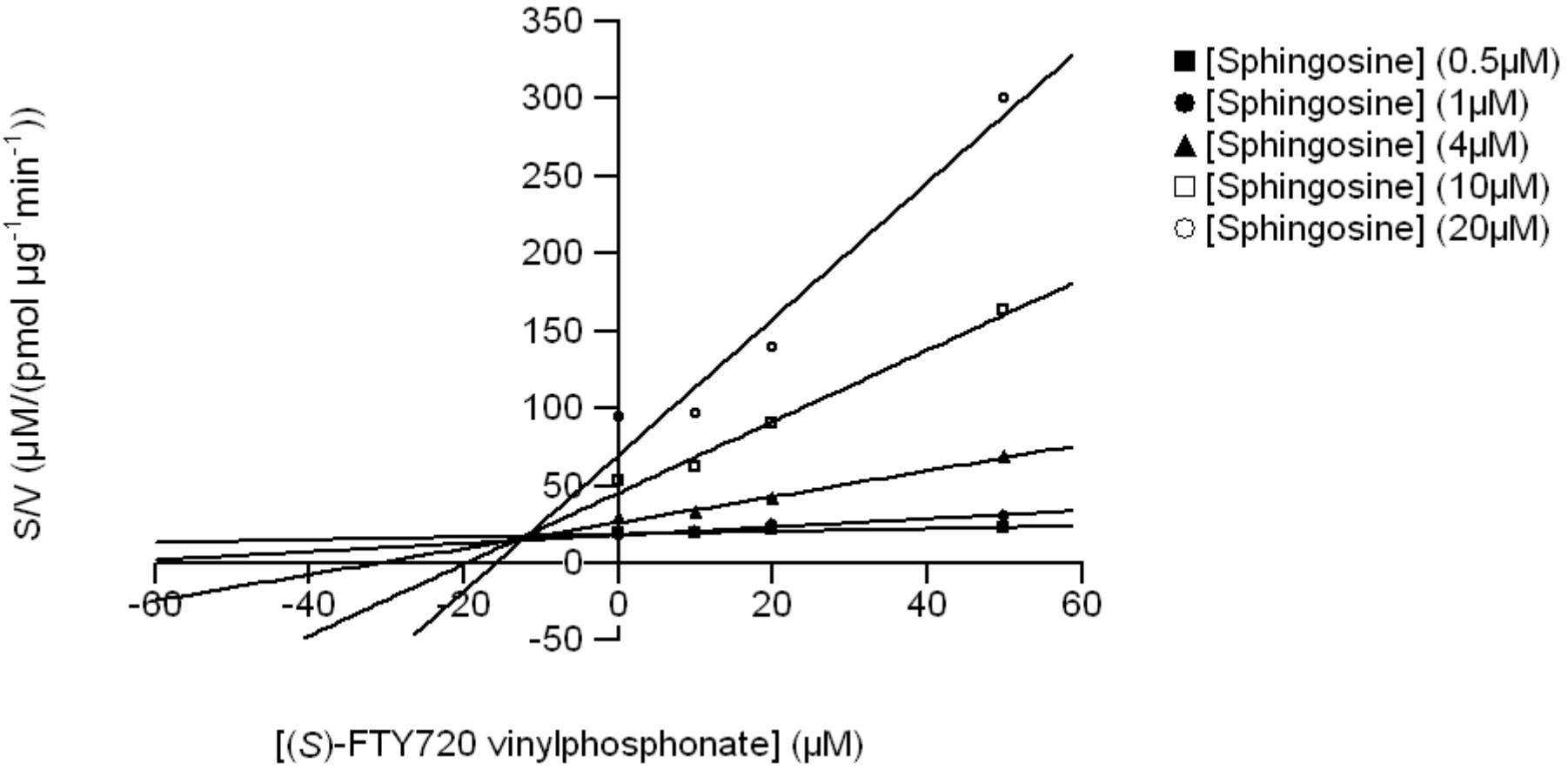

60
50
40
30
20
10
0
-10
-15
-10
-5
0
5
10
15
1/V (pmol µg-1min-1)-1
[FTY720] (µM)
[Sphingosine] (1µM)
[Sphingosine] (2µM)
[Sphingosine] (4µM)
[Sphingosine] (10µM)
[Sphingosine] (20µM)

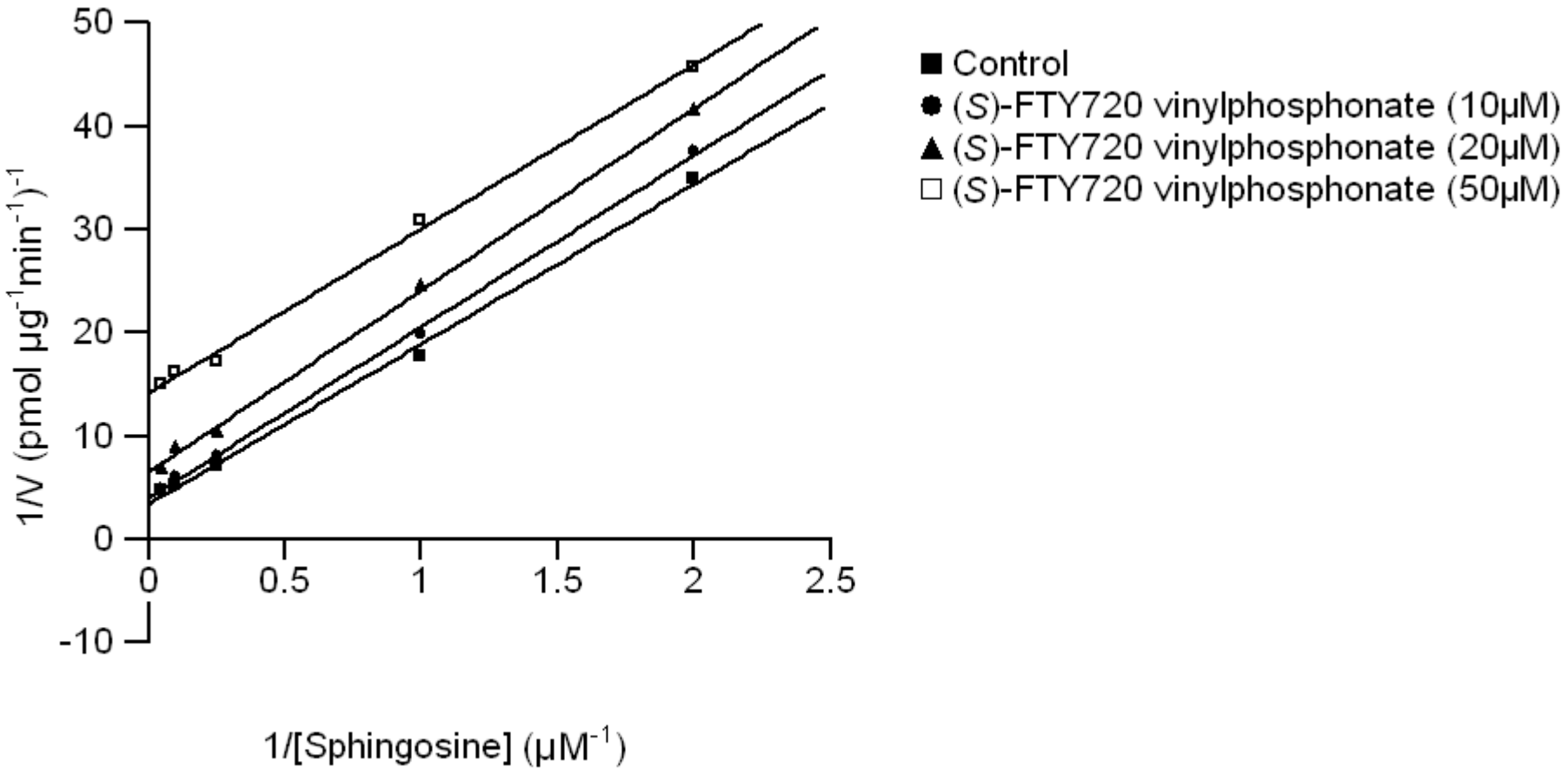

S/V (μM/(pmol μg-1min-1))
140
120
100
80
60
40
20
0
-20
-30
-25
-20
-15
-10
-5
0
5
10
15
[FTY720] (μM)
[Sphingosine] (1μM)
[Sphingosine] (2μM)
[Sphingosine] (4μM)
[Sphingosine] (10μM)
[Sphingosine] (20μM)


60
50
40
30
20
10
0
-10
0
0.5
1
1.5
1/V (pmol μg-1min-1)-1
1/[Sphingosine] (μM-1)
Control
FTY720 (1μM)
FTY720 (5μM)
FTY720 (10μM)

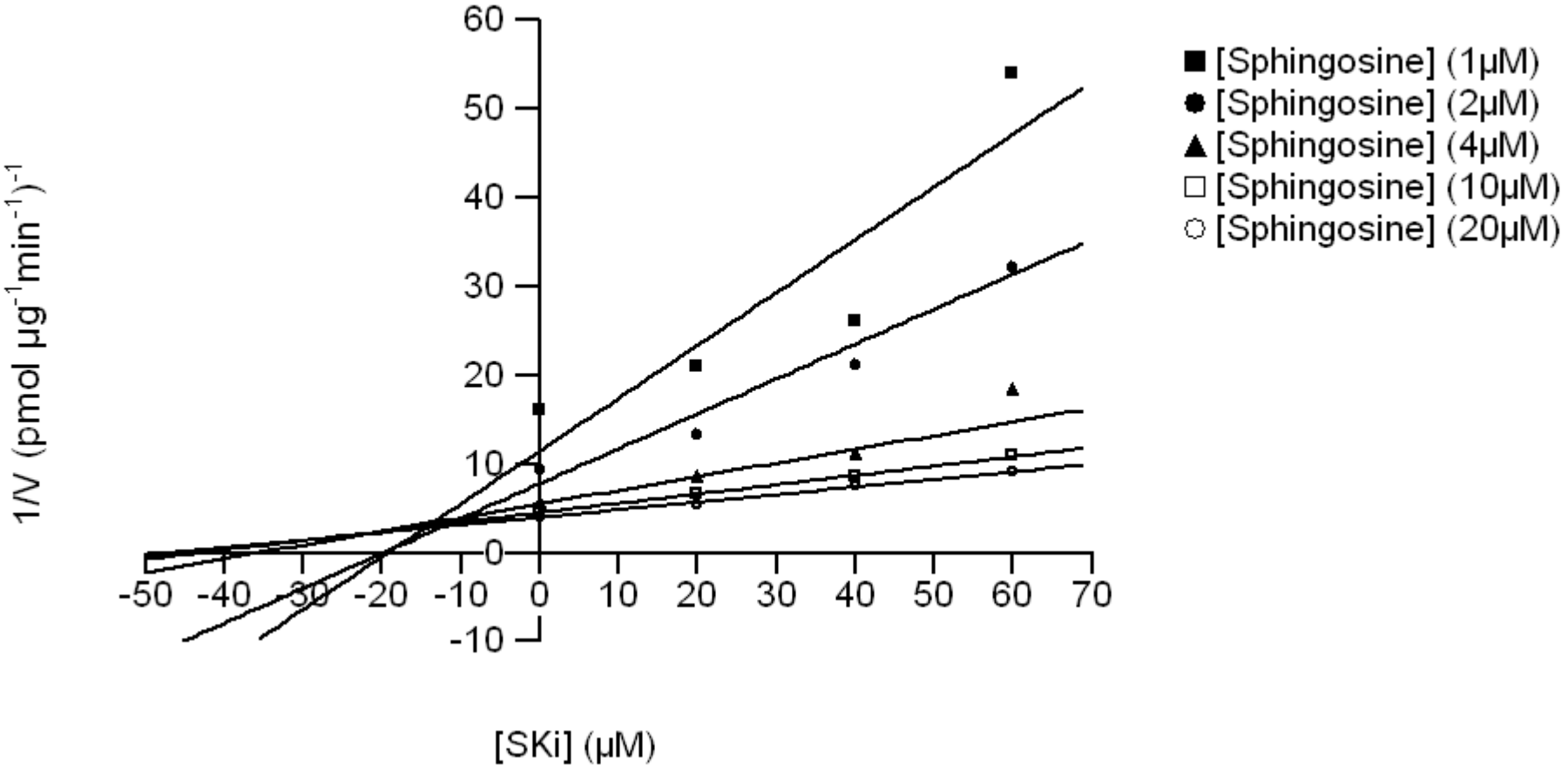
Suppl Fig. 2B

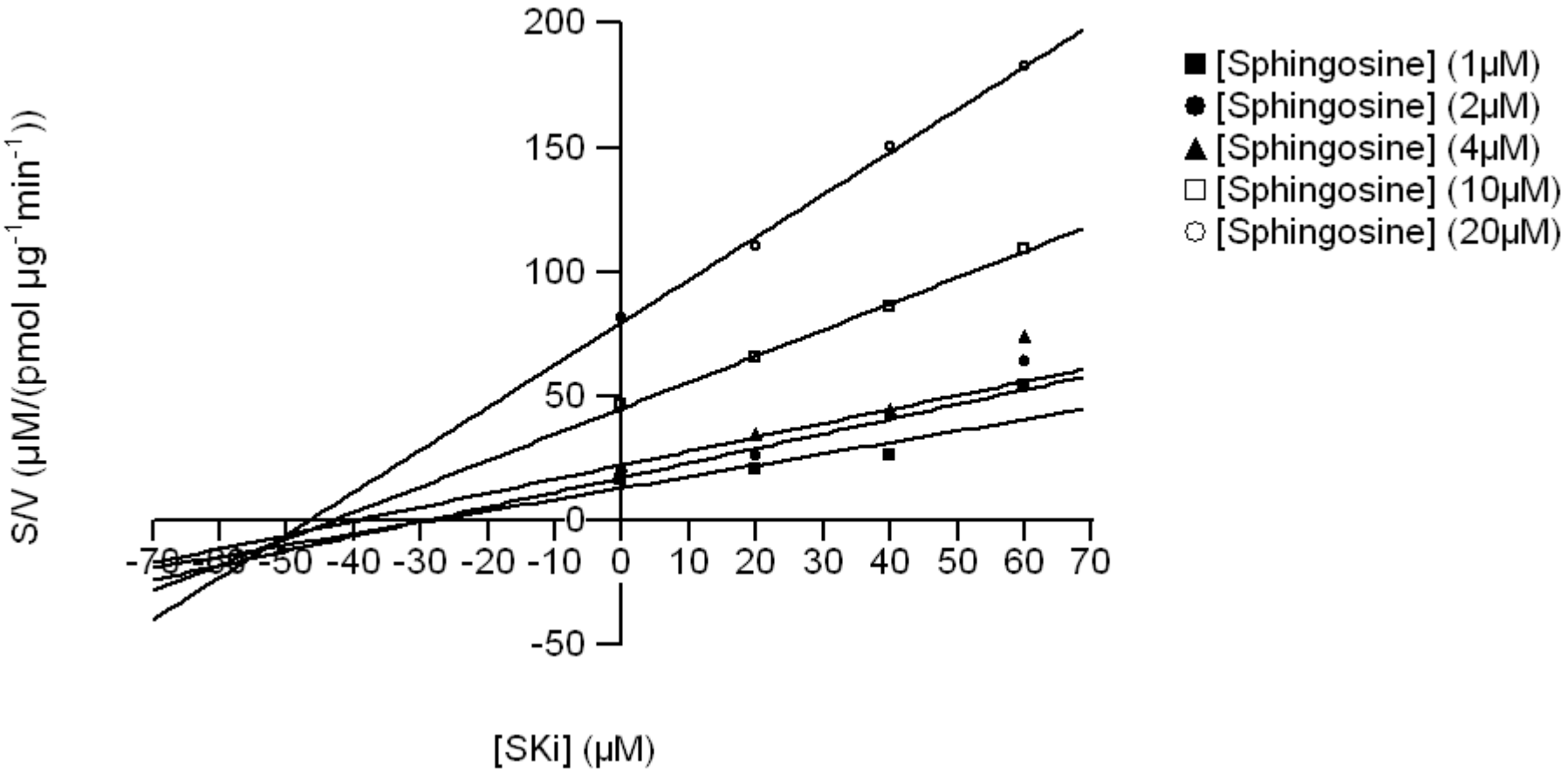

60
50
40
30
20
10
0
-10
-60
-40
-20
0
20
40
60
1/V (pmol µg-1min-1)-1
[(S)-FTY720 vinylphosphonate] (µM)
[Sphingosine] (0.5µM)
[Sphingosine] (1µM)
[Sphingosine] (4µM)
[Sphingosine] (10µM)
[Sphingosine] (20µM)

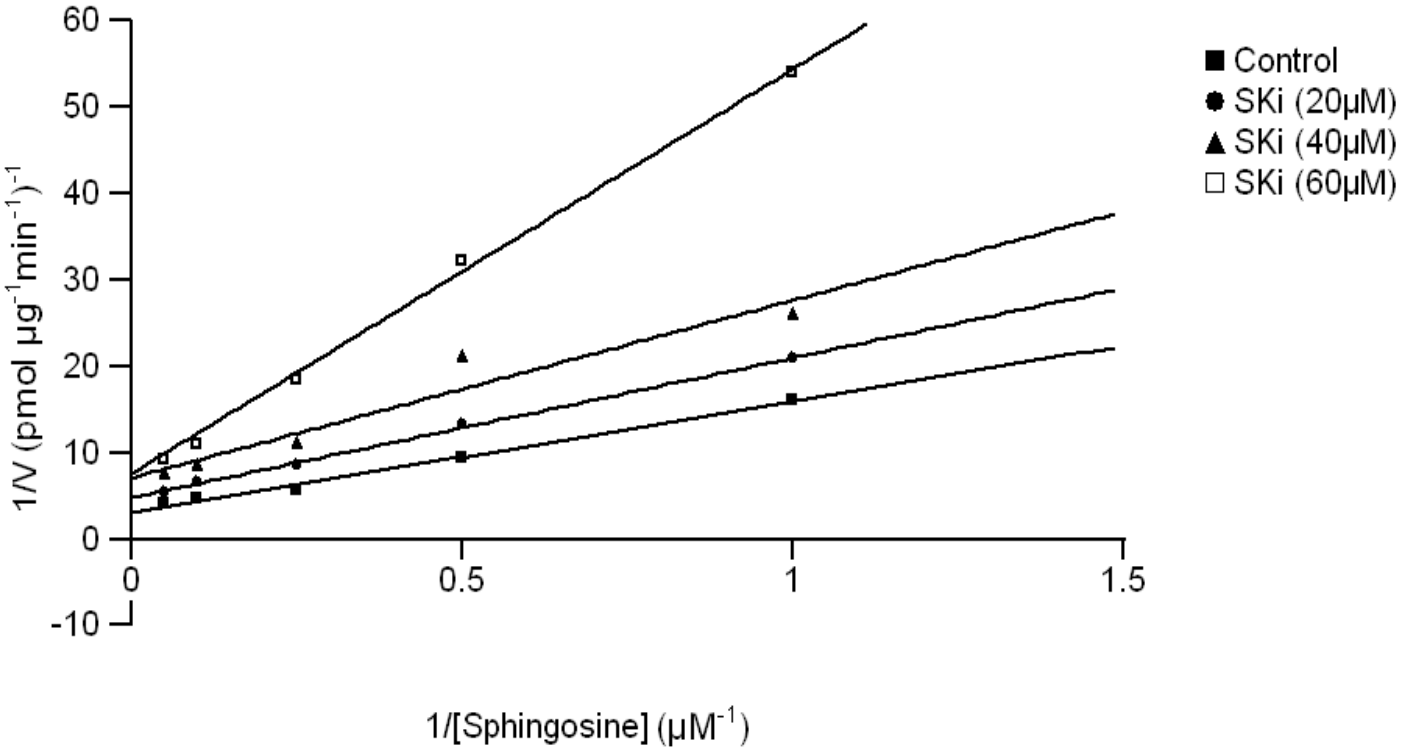

S/V (μM/(pmol μg-1min-1))
350
300
250
200
150
100
50
0
-50
-60
-40
-20
0
20
40
60
[(S)-FTY720 vinylphosphonate] (μM)
[Sphingosine] (0.5μM)
[Sphingosine] (1μM)
[Sphingosine] (4μM)
[Sphingosine] (10μM)
[Sphingosine] (20μM)


50
40
30
20
10
0
-10
0
0.5
1
1.5
2
2.5
1/V (pmol µg-1min-1)-1
1/[Sphingosine] (µM-1)
Control
(S)-FTY720 vinylphosphonate (10µM)
(S)-FTY720 vinylphosphonate (20µM)
(S)-FTY720 vinylphosphonate (50µM)

Suppl Fig. 2C


60
50
40
30
20
10
0
-10
-50 -40 -30 -20 -10 0 10 20 30 40 50 60 70
1/V (pmol µg-1min-1)-1
[SKi] (µM)
[Sphingosine] (1µM)
[Sphingosine] (2µM)
[Sphingosine] (4µM)
[Sphingosine] (10µM)
[Sphingosine] (20µM)


S/V (µM/(pmol µg-1min-1))
200
150
100
50
0
-50
-70 -60 -50 -40 -30 -20 -10 0 10 20 30 40 50 60 70
[SKi] (µM)
[Sphingosine] (1µM)
[Sphingosine] (2µM)
[Sphingosine] (4µM)
[Sphingosine] (10µM)
[Sphingosine] (20µM)


60
50
40
30
20
10
0
-10
0
0.5
1
1.5
1/V (pmol µg-1min-1)-1
1/[Sphingosine] (µM-1)
Control
SKi (20µM)
SKi (40µM)
SKi (60µM)