Supplemental Figure Legends

Figure S1. Ligand binding properties at hS1P1 and mS1P4.
Ligand binding properties at hS1P1 and mS1P4 are examined with radiorabeled ligand binding assay. CHO cells stably transfected with (A) empty vector, (B) FLAG-S1P1-HA or (C) FLAG-S1P4-HA were incubated with indicated concentrations of [32P]S1P, [32P] DHS1P or [32P] PHS1P in the absence or presence of 100 times-excess unlabeled competitor for 30min at 4°C. Cells were lysed and bound radioligand was quantified by liquid scintillation counting. Data show specific binding, which is binding in the absence of unlabeled competitor minus binding in the presence of unlabeled competitor. These data represent the mean + s.e. from three independent experiments.

Figure S2. Electrostatic Surfaces of TM3-5 in S1P Complexes.
Gauss Connelly surfaces colored by atomic partial charge (red indicates negative, white indicates neutral and blue indicates positive partial charge) for atoms within 4.5 Å of S1P in TM3-5 were computed with the MOE program. The bound position of S1P is shown as a stick model. A. hS1P1 surface with docked position of S1P. B. mS1P4 surface with docked position of S1P.

Figure S3. [32P]S1P binding to hS1P1 and hS1P1-W4.64 mutant.
CHO cells transiently transfected with empty vector, hS1P1-HA or hS1P1-HA W4.64 mutant were incubated with 60 nM [32P]S1P in the absence (total binding) or presence (non-specific binding) of 6μM unlabeled competitor for 30min at 4°C. Cells were lysed and bound radioligand was quantified by liquid scintillation counting. These data represent the average of three independent experiments with error bars indicating the s.e. Statistical significance: *$P < 0.05$ vs. vector control.

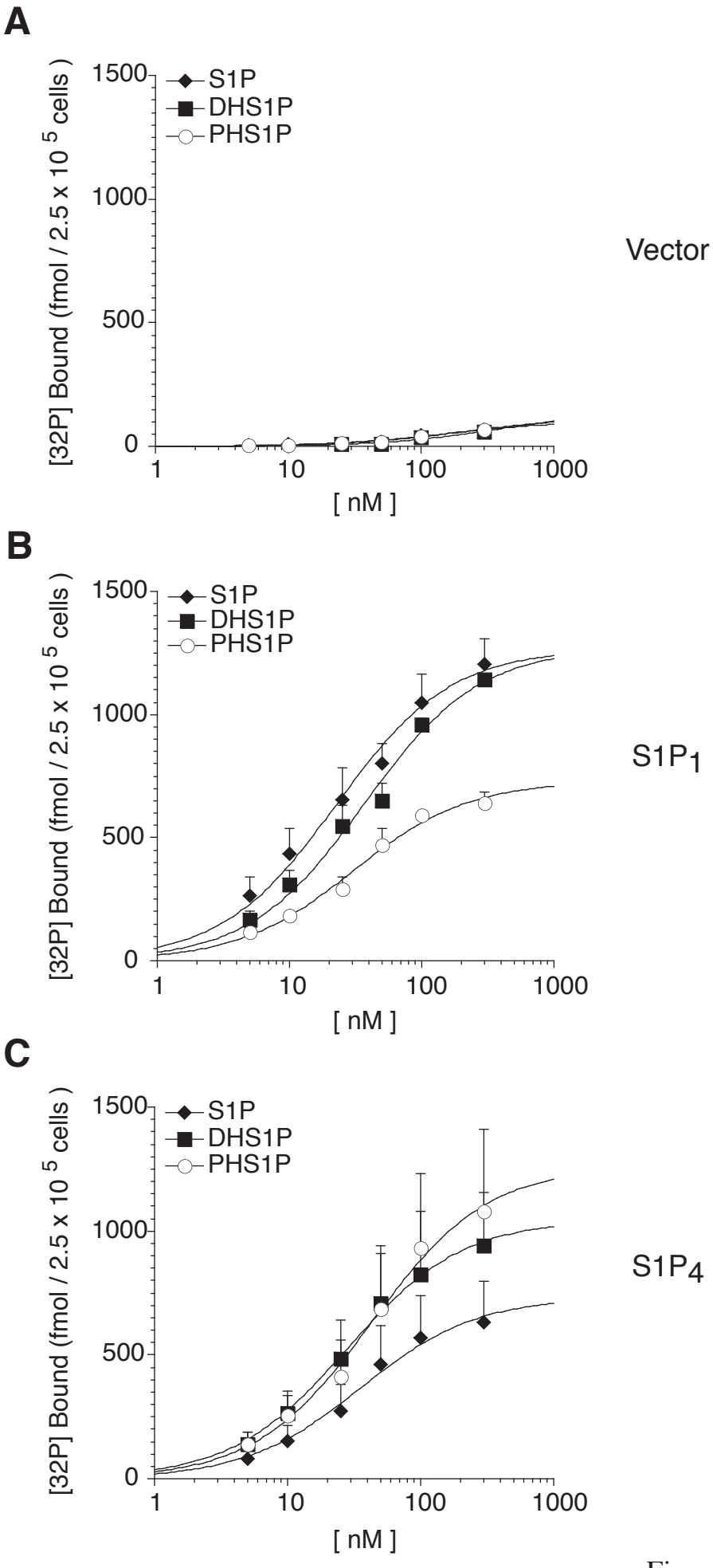
A
B
C
S1P
DHS1P
PHS1P
[32P] Bound (fmol / 2.5 x 10 5 cells)
[nM]
1500
1000
500
0
1
10
100
1000
Vector
S1P1
S1P4

Figure S1

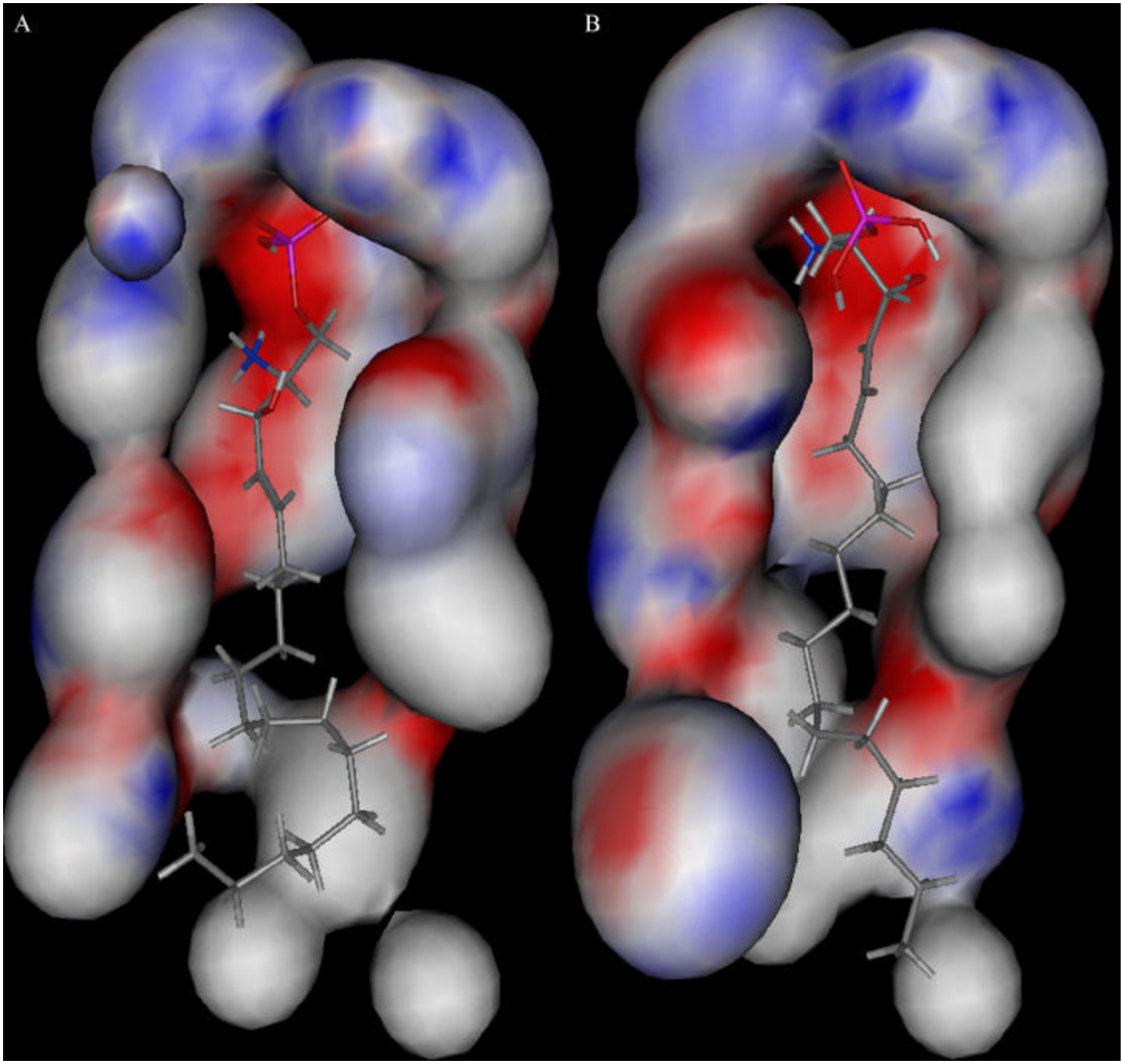

Figure S2

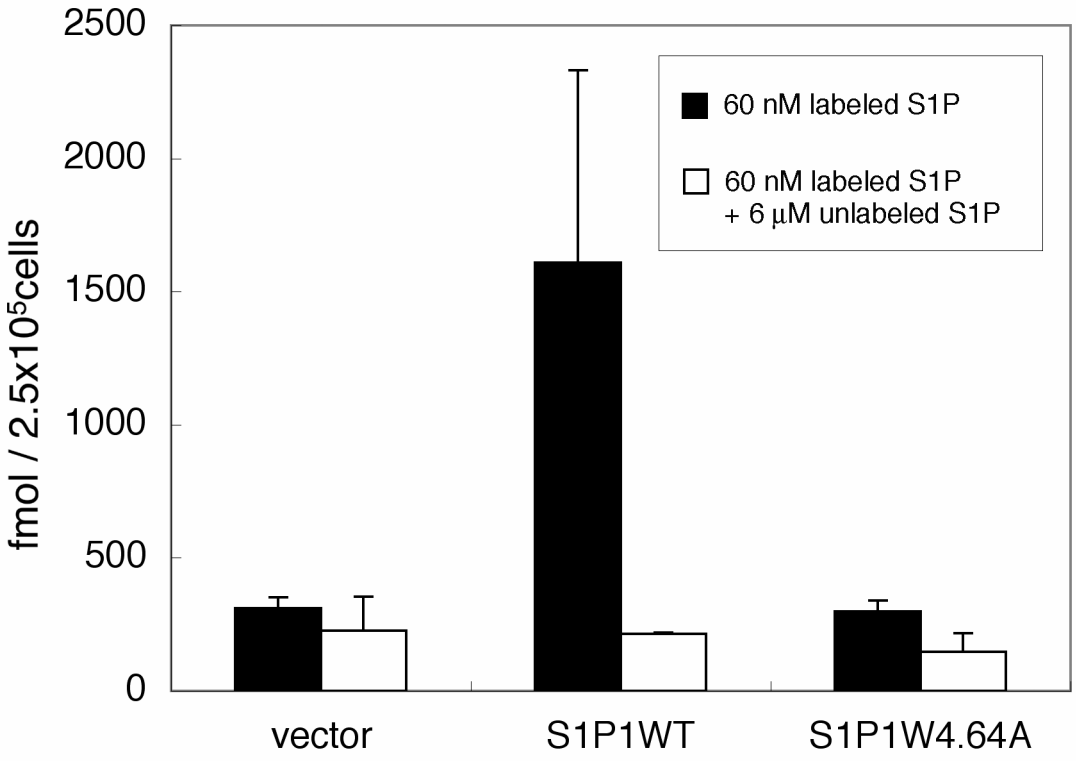
2500
2000
1500
1000
500
0
fmol / 2.5x10^5cells
60 nM labeled S1P
60 nM labeled S1P
+ 6 μM unlabeled S1P
vector
S1P1WT
S1P1W4.64A

Figure S3