Supplemental data

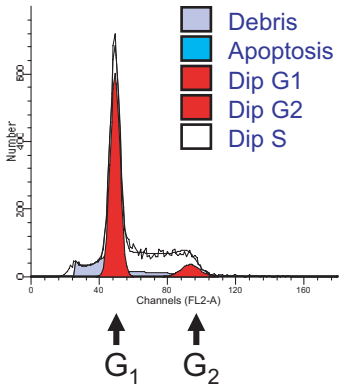
Fig. S1. Primary FACS data. MCF-7 cells were treated with 10 μM of P61-A6, P61-B6 or DMSO for 48 hours. Data shown here are representative of two independent experiments for each treatment.

Fig. S2. Inhibition of RabGGTase activity in cells. (A) P49-F6 treatment inhibits Rab5b geranylgeranylation in NIH3T3 cells. Whole cell lysates from cells treated with DMSO or P49-F6 for 48 hours were prepared and processed for immunoblot analysis using antibody against Rab5b (upper panel) or actin (lower panel). (B) Whole cell lysates from NIH3T3 cells treated with DMSO or P49-F6 for 48 hours were prepared and processed for immunoblot analysis using antibody against unprenylated form of Rap1 (upper panel), total-Rap1 (upper middle panel), H-Ras (lower middle panel) or actin (lower panel).

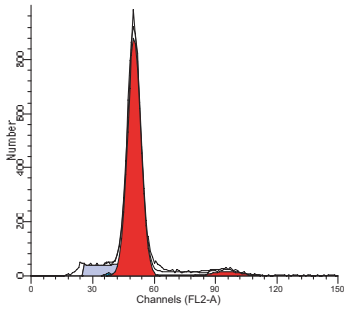
Fig. S3. Effects of RabGGTI on membrane association of Rab5b protein. Western blots of Rab5b in the soluble fractions or membrane fractions prepared from NIH3T3 cells treated for 48 hours as indicated in "Experimental Procedures". Rho GDI and Na^+/K^+ ATPase were used as marker proteins for the soluble and membrane fractions, respectively. Bars indicate intensity of protein bands after normalization using loading control.

Fig. S1

DMSO



P61-A6



P61-B6

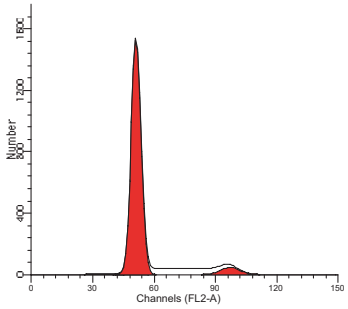


Fig. S2

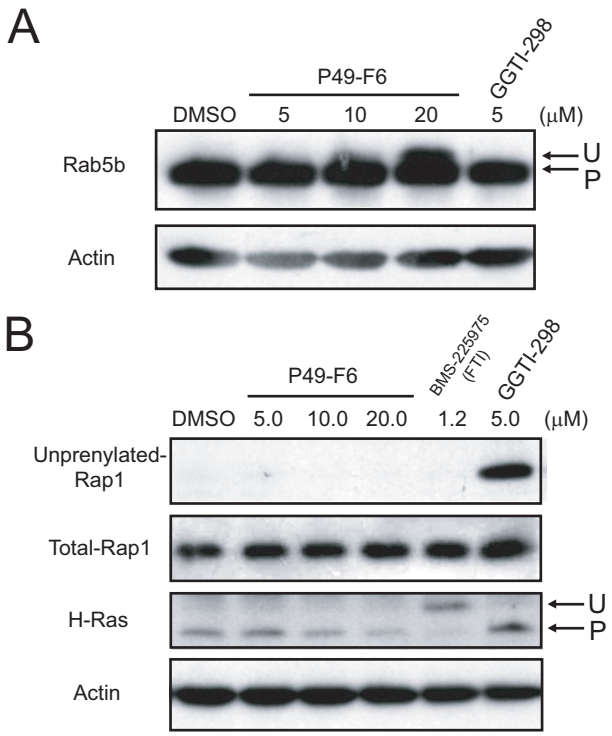

A
P49-F6
GGTI-298
DMSO
5
10
20
5
(μM)
Rab5b
U
P
Actin
B
P49-F6
BMS-225975 (FTI)
GGTI-298
DMSO
5.0
10.0
20.0
1.2
5.0
(μM)
Unprenylated-Rap1
Total-Rap1
H-Ras
U
P
Actin

Fig. S3

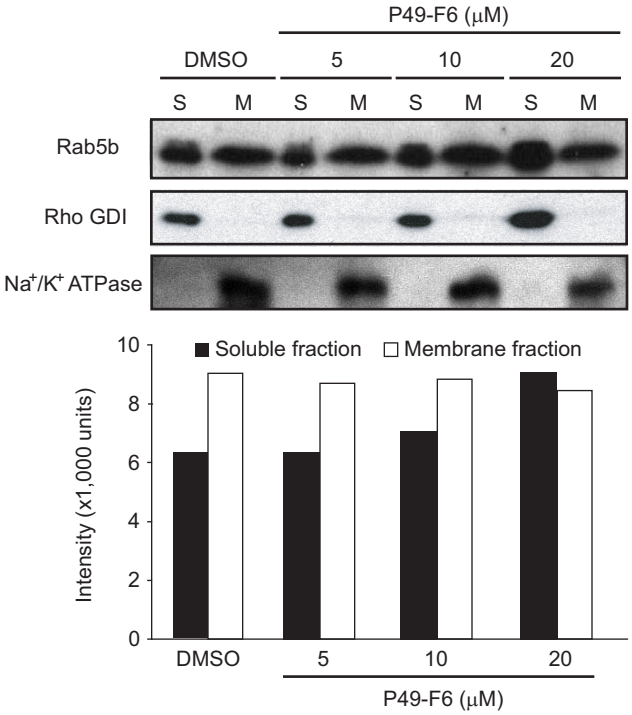

P49-F6 (μM)
DMSO
5
10
20
S
M
S
M
S
M
S
M
Rab5b
Rho GDI
Na+/K+ ATPase
Soluble fraction
Membrane fraction
Intensity (x1,000 units)
10
8
6
4
2
0
DMSO
5
10
20
P49-F6 (μM)

Table S1. Potencies of GGTIs toward human cancer cell lines

		P61-A6	P61-B6
Jurkat	Blood	2.9	1.3
SE-Mk2	Blood	6.4	14.1
PANC-1	Pancreas	5.2	12.6
MiaPaCa2	Pancreas	4.7	13.9
AsPc-1	Pancreas	11.7	> 20.0
Capan-2	Pancreas	11.5	11.1
CFpac-1	Pancreas	6.3	8.1
HPAC	Pancreas	3.9	11.7
MDA-MB-231	Breast	8.7	12.5
BT474	Breast	8.5	11.6
MCF-7	Breast	4.5	6.4

Cancer cells were treated with the indicated GGTIs for 3 days (Jurkat, PANC-1, MiaPaCa2, Capan-2, CFpac-1, HPAC, MDA-MB-231 and BT474) or 6 days (SE-MK2, AsPc-1 and MCF-7), and cell number was counted using CCK-8 and compared with vehicle (DMSO) treated cells. Values are the IC_{50} (μM) for at least two separate experiments.

Table S2. Effects of GGTIs on cell cycle phase distribution in cancer cells

Cell line	Tissue	Percent G_0/G_1			Percent G_2/M	
		DMSO	P61-A6	*P* value	DMSO	P61-A6
Jurkat	Blood	46.92	57.87	< 0.05	11.80	11.60
PANC-1	Pancreas	42.08	54.13	< 0.005	24.21	19.81
MiaPaCa2	Pancreas	77.85	87.98	< 0.05	14.12	10.81
MDA-MB-231	Breast	73.78	85.16	< 0.05	7.74	5.15

NOTE: Cancer cells were treated with the P61-A6 (10 μM) for 48 hours, and cell cycle distribution was determined by flow cytometry as described under "Experimental Procedures". Data are representative of at least two independent experiments.