Figure legends for Supplemental Figures

Figure S1. Changes in ganglioside compositions after transfection of GD3 synthase cDNA into SK-MEL-28 N1 cell. TLC of gangliosides extracted from SK-MEL-28 (lane 1: parent line), B78 (lane 2: murine melanoma line), V9 (lane 3: a control line of SK-MEL-28 N1), and G5 (lane 4: a GD3 synthase cDNA transfectant line). BBG is bovine brain ganglioside mixture used as a control. TLC was developed by a solvent system of C:M:DW (55:45:10). Resorcinol was used for detection of bands. These data are essentially same as reported in Ref. 7.

Figure S2. Expression of Src family kinases in melanoma cells. Expression of Src family kinase Lck in melanoma cells. Total cell lysates from two GD3+ cells were immunoblotted with anti-Lck antibody. No bands were detected. Jurkat is a T lympho-blastic leukemia cell line used for a positive control.

Figure S3. Implication of Src family kinases in the phenotypes of melanoma cells. (A) Knock down of Src was performed as that of Fyn was done. As shown in knockdown of Fyn, knockdown of Src did not affect levels of p130Cas, FAK, paxillin and their phosphorylated forms. (B and C) Effects of Src knockdown on cell proliferation (B) and invasion activity (C). BrdU uptake and cell invasion with Boyden chamber assays were performed as described for Fyn. No effects could be detected in both assay systems.

Figure S4. Mesurement of kinase activity of Yes *in vitro*. Yes was immuno-precipitated from lysates of G5 (GD3+), then in vitro kinase assay using enolase (2.62, 6.99 μg) and ATP (1, 10 μM) was done to optimize assay conditions. Autoradiogram of the products from individual conditions was shown. Three main bands were detected. In addition to enolase added as a substrate, Yes itself and p125, probably FAK were also phosphorylated. Intensities of bands indicating kinase activity of Yes were stronger when 2.62 μg enolase and 1μM ATP were used.

Figure S5. Uniform protein levels of Yes in G5 (GD3+) and V9 (GD3-) during FCS stimulation. G5 (GD3+) and V9 (GD3-) were treated with FCS after serum starvation

for 12 hrs, and their total cell lysates at time points indicated were immunoblotted with an anti-Yes antibody. (A) samples used in Fig. 5A were served for immunoblotting with anti-Yes antibody. (B) samples used in Fig. 5B was applied as in A.

Figure S6. Fractionation of Triton X-100 extracts by sucrose density gradient centrifugations. To examine the floating patterns of Yes in the extracts from G5 (GD3+) and V9 (GD3-), Triton X-100-extracts were fractionated by sucrose density gradient ultracentrifugation at 0 and 30 min after FCS treatment. Each fraction was used for immunoblotting by an anti-flotillin-1 antibody. (A) Results for G5 (GD3+) and V9 (GD3-) before FCS treatment. (B) Results for G5 (GD3+) and V9 (GD3-) at 30 min after FCS treatment. Stable localization of flotillin-1 in GEM/raft fractions (fractions 2-4) was demonstrated in both types of cells at time 0 and 30 min after FCS treatment.

Figure S7. Intracellular localization of Yes as prepared using a non-detergent extraction method. (A) The GEM/raft fractions were isolated using a non-detergent method, each fraction was used for immunoblotting by an anti-Yes antibody, then the relative intensities of bands in GEM/raft fractions (fractions 2-4) against those in GEM/raft fractions and non-GEM/raft fractions (fractions 6-10) were plotted. (B) This is essentially same data as those in Figure 6C. The GEM/raft fractions were isolated using Triton X-100, each fraction was used for immunoblotting by an anti-Yes antibody, then the relative intensities in GEM/raft fractions (fractions 2-4) against those in GEM/raft fractions and non-GEM/raft fractions (fractions 6-10) were plotted. The results show very similar distribution patterns.

Figure S8. Exogenous GD3 enhanced kinase activity of Yes from GD3- cells. (A) In order to examine whether exogenous GD3 enhances Yes kinase activity of immunoprecipitated Yes, in vitro kinase assays using immunoprecipitated Yes from GD3- cells (V9) in the presence of GD3 (1, 10, 50, 100 μM) were performed. Autoradiogram of the products was shown. (B) Relative intensities of bands of phosphorylated enolase and p125 as well as phosphorylated Yes in (A) were measured using NIH image 1.61 and plotted after correction with that of Yes in graphs. The experiments were performed by triplicates, and average values ±SD were presented. The asterisks indicate $p < 0.05$ (*) and $p < 0.01$ (**). Consequently, intensities of

phosphorylation of enolase and p125 as well as autophosphorylation of Yes were increased. Namely, kinase activity of Yes was enhanced at concentrations of 1-50 μM of GD3, and phosphorylated p125 was significantly increased at 10-50 μM of GD3.

Figure S9. Liposome-embedded GM1 or GD1a did not alter kinase activity of Yes from GD3- cells. (A) In vitro kinase assays using immunoprecipitated Yes from GD3- cells were performed in the presence of GM1 (5, 10, 20 μM). (B) Relative intensities of phosphorylation bands of enolase and p125 as well as of Yes were measured using NIH image 1.61 and plotted in graphs after correction with that of Yes. (C) In vitro kinase assays using immunoprecipitated Yes from GD3- cells were performed in the presence of GD1a (5, 10, 20 μM). (D) Relative intensities of phosphorylation bands of enolase and p125 as well as of Yes were measured using NIH image 1.61 and plotted in graphs after correction with that of Yes. The experiments were performed by triplicates, and average values ±SD were presented. The asterisks indicate $p < 0.05$ (*).

Figure S1

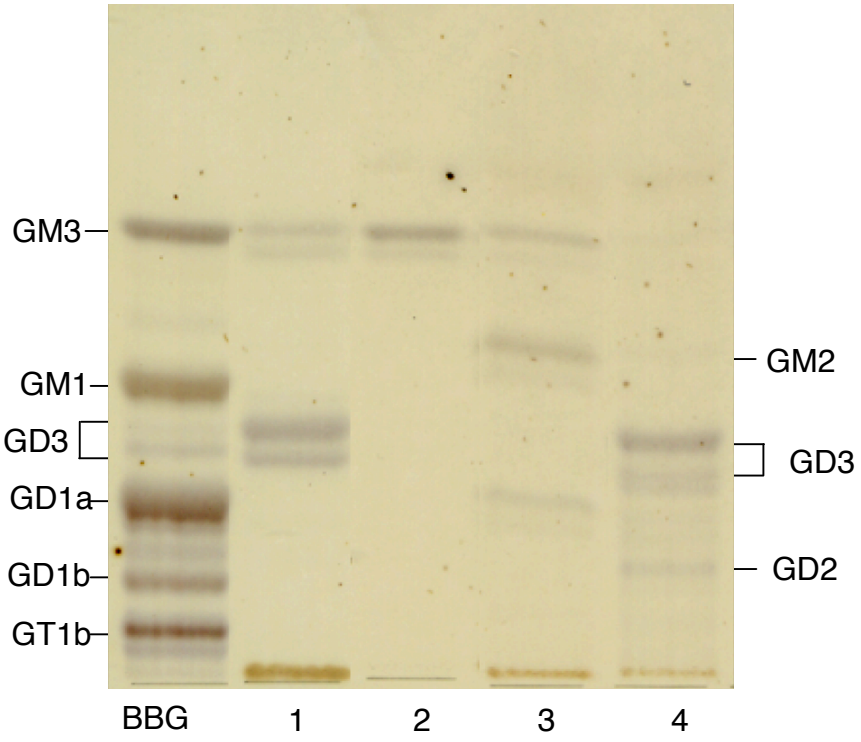


Figure S2

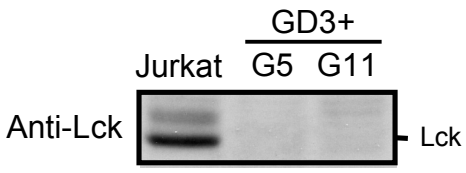


Figure S3

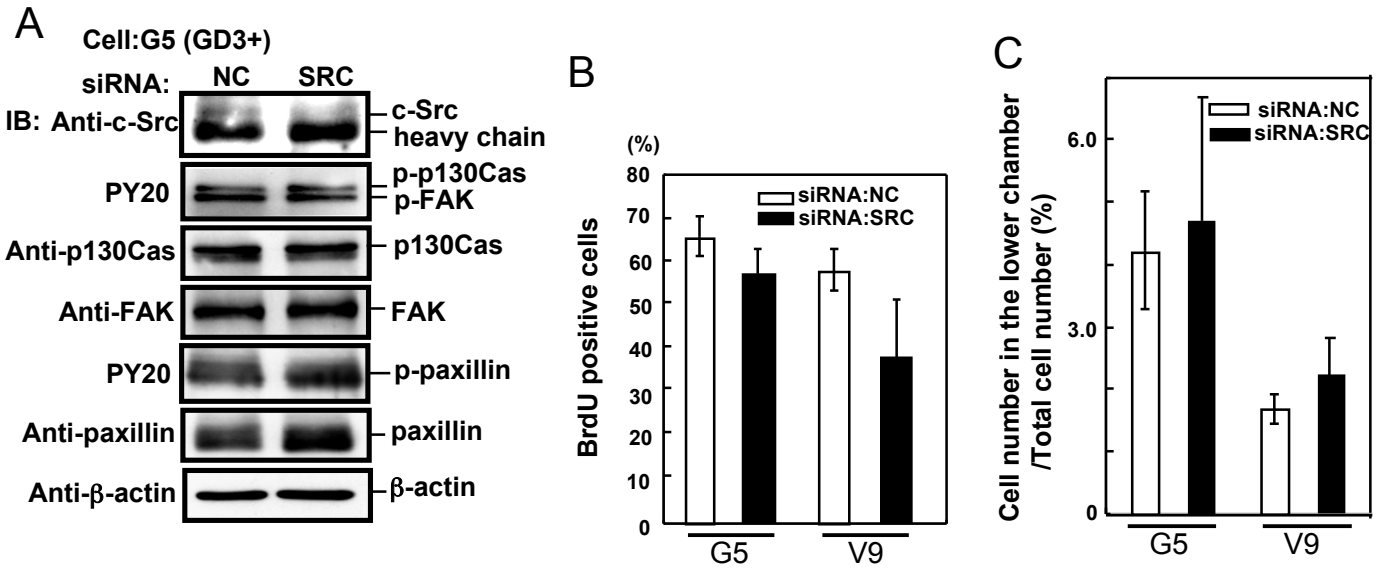

A
Cell:G5 (GD3+)
siRNA:
NC
SRC
IB: Anti-c-Src
c-Src
heavy chain
PY20
p-p130Cas
p-FAK
Anti-p130Cas
p130Cas
Anti-FAK
FAK
PY20
p-paxillin
Anti-paxillin
paxillin
Anti-β-actin
β-actin
B
(%)
BrdU positive cells
80
70
60
50
40
30
20
10
0
siRNA:NC
siRNA:SRC
G5
V9
C
Cell number in the lower chamber /Total cell number (%)
6.0
3.0
0
siRNA:NC
siRNA:SRC
G5
V9

Figure S4

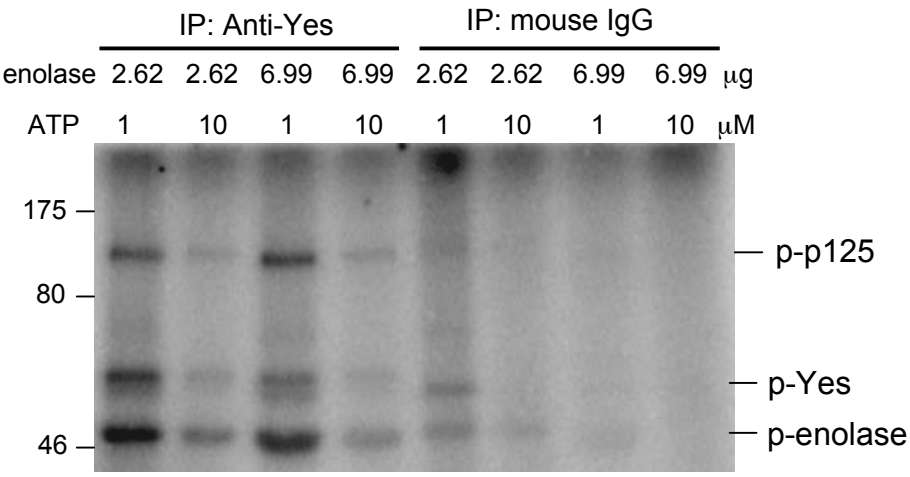

IP: Anti-Yes
IP: mouse IgG
enolase 2.62 2.62 6.99 6.99 2.62 2.62 6.99 6.99 μg
ATP 1 10 1 10 1 10 1 10 μM
175
80
46
p-p125
p-Yes
p-enolase

Figure S5

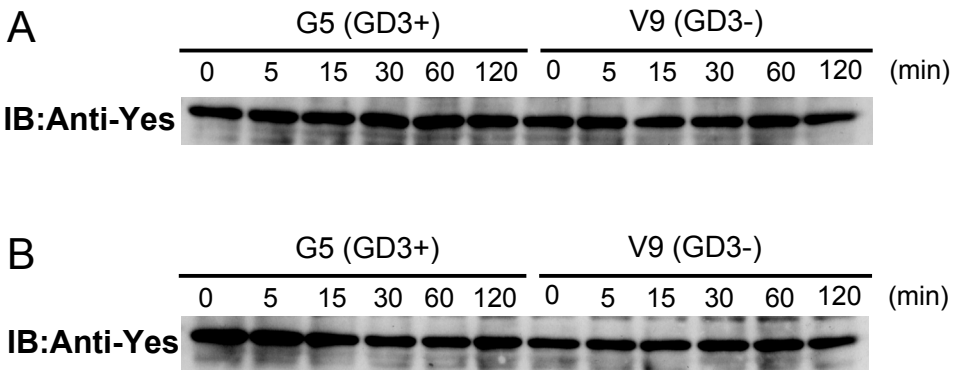

A
G5 (GD3+)
V9 (GD3-)
0 5 15 30 60 120 0 5 15 30 60 120 (min)
IB:Anti-Yes
B
G5 (GD3+)
V9 (GD3-)
0 5 15 30 60 120 0 5 15 30 60 120 (min)
IB:Anti-Yes

Figure S6

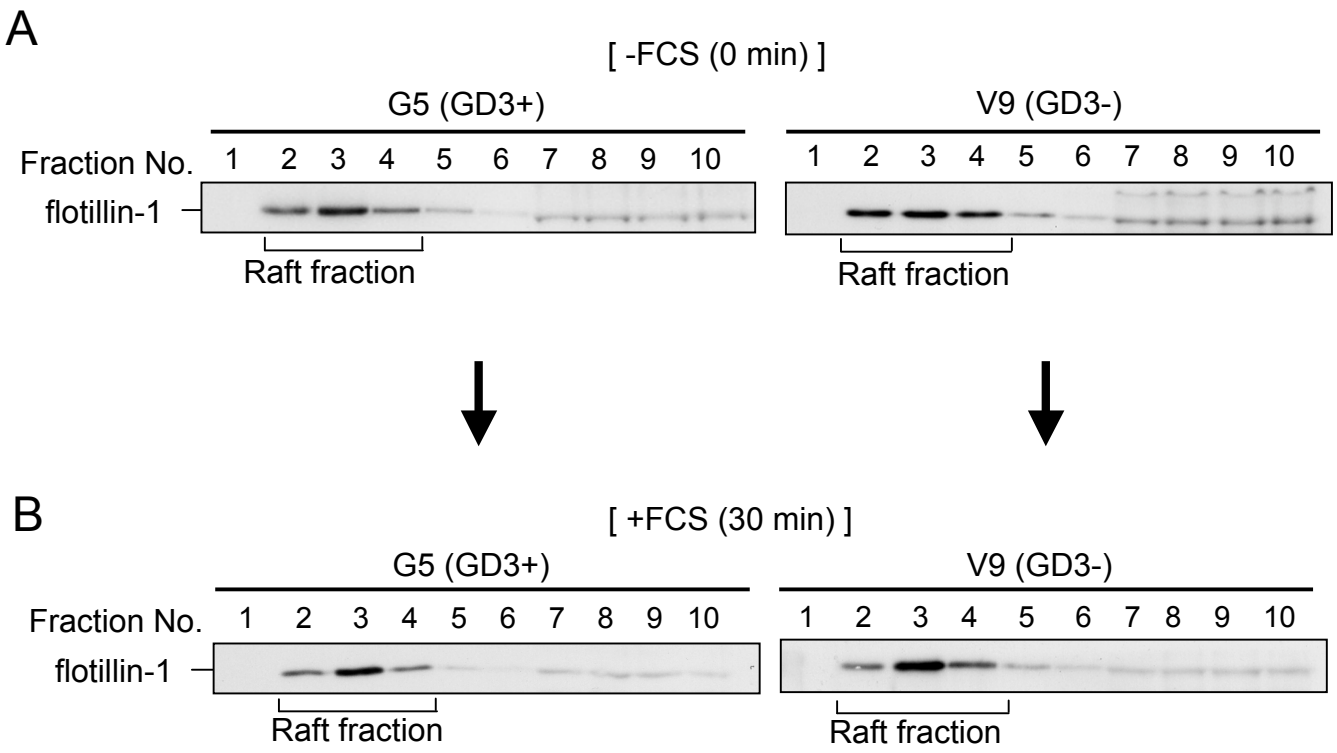

A
[-FCS (0 min)]
G5 (GD3+)
V9 (GD3-)
Fraction No.
1 2 3 4 5 6 7 8 9 10
flotillin-1
Raft fraction
Raft fraction
B
[+FCS (30 min)]
G5 (GD3+)
V9 (GD3-)
Fraction No.
1 2 3 4 5 6 7 8 9 10
flotillin-1
Raft fraction
Raft fraction

Figure S7

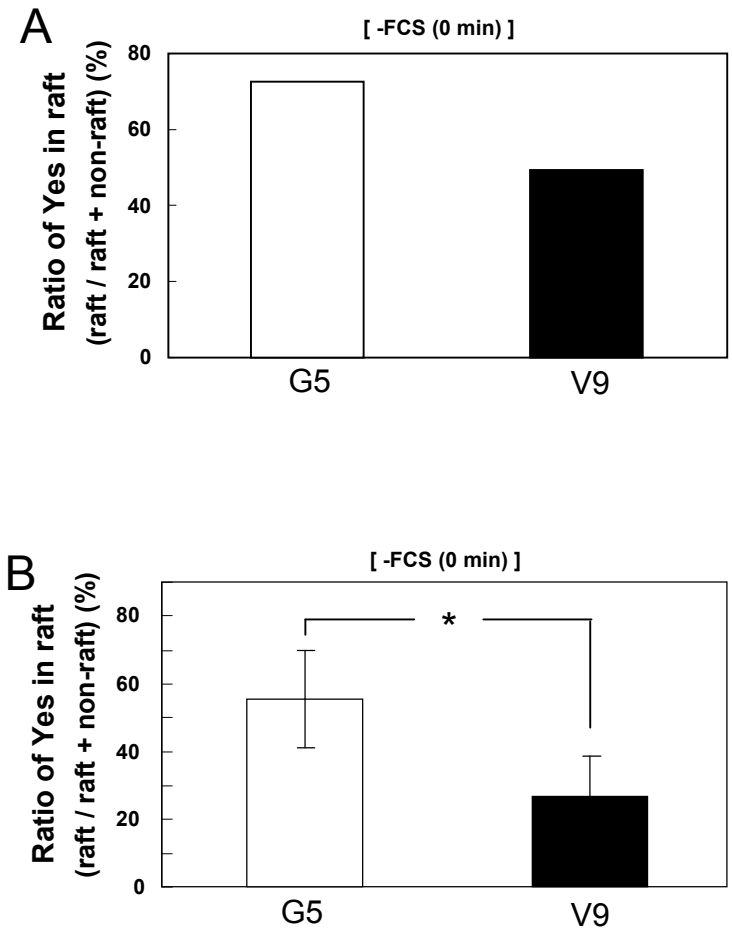

A
[-FCS (0 min)]
Ratio of Yes in raft
(raft / raft + non-raft) (%)
80
60
40
20
0
G5
V9
B
[-FCS (0 min)]
Ratio of Yes in raft
(raft / raft + non-raft) (%)
*
80
60
40
20
0
G5
V9

Figure S8

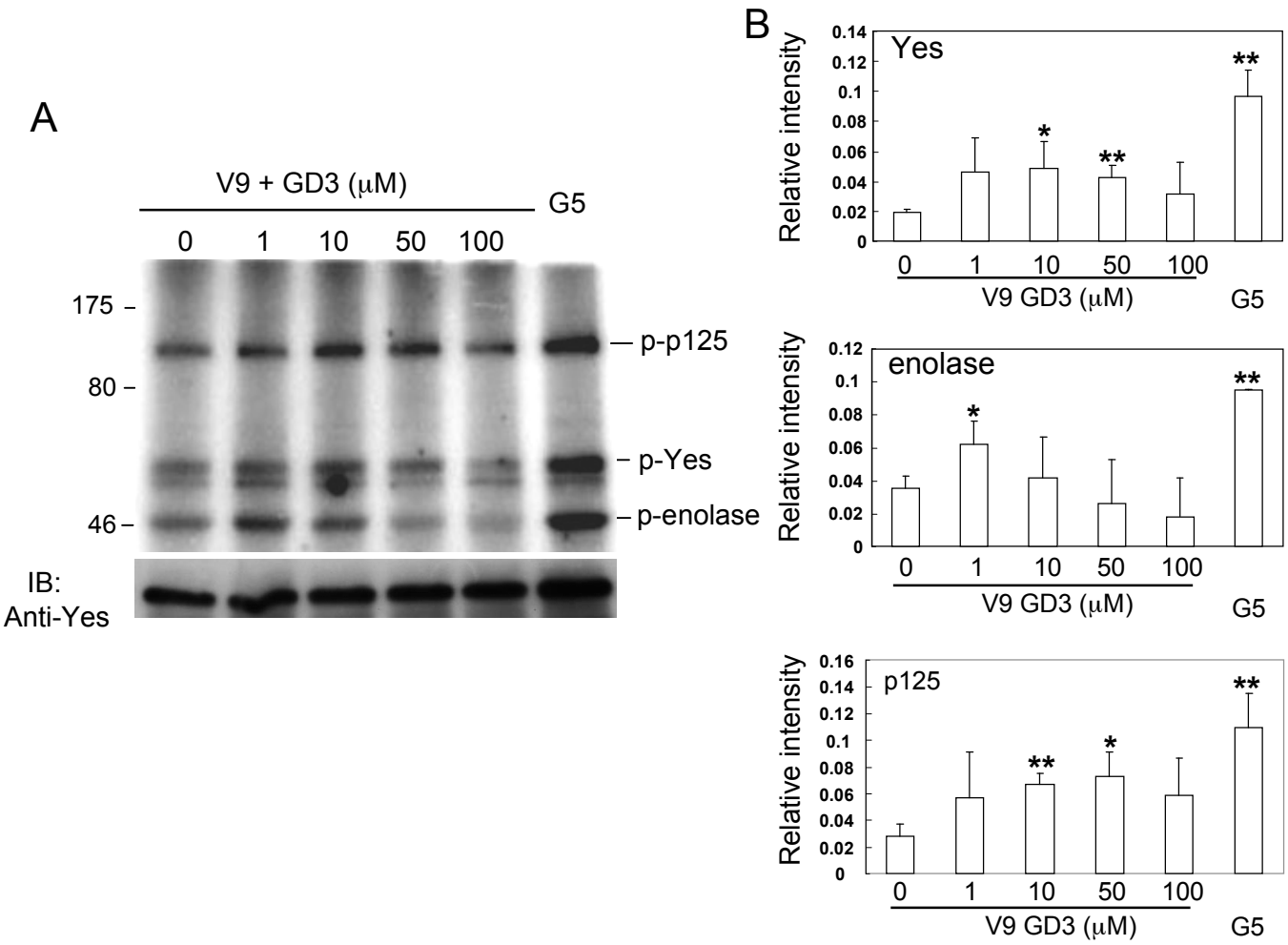
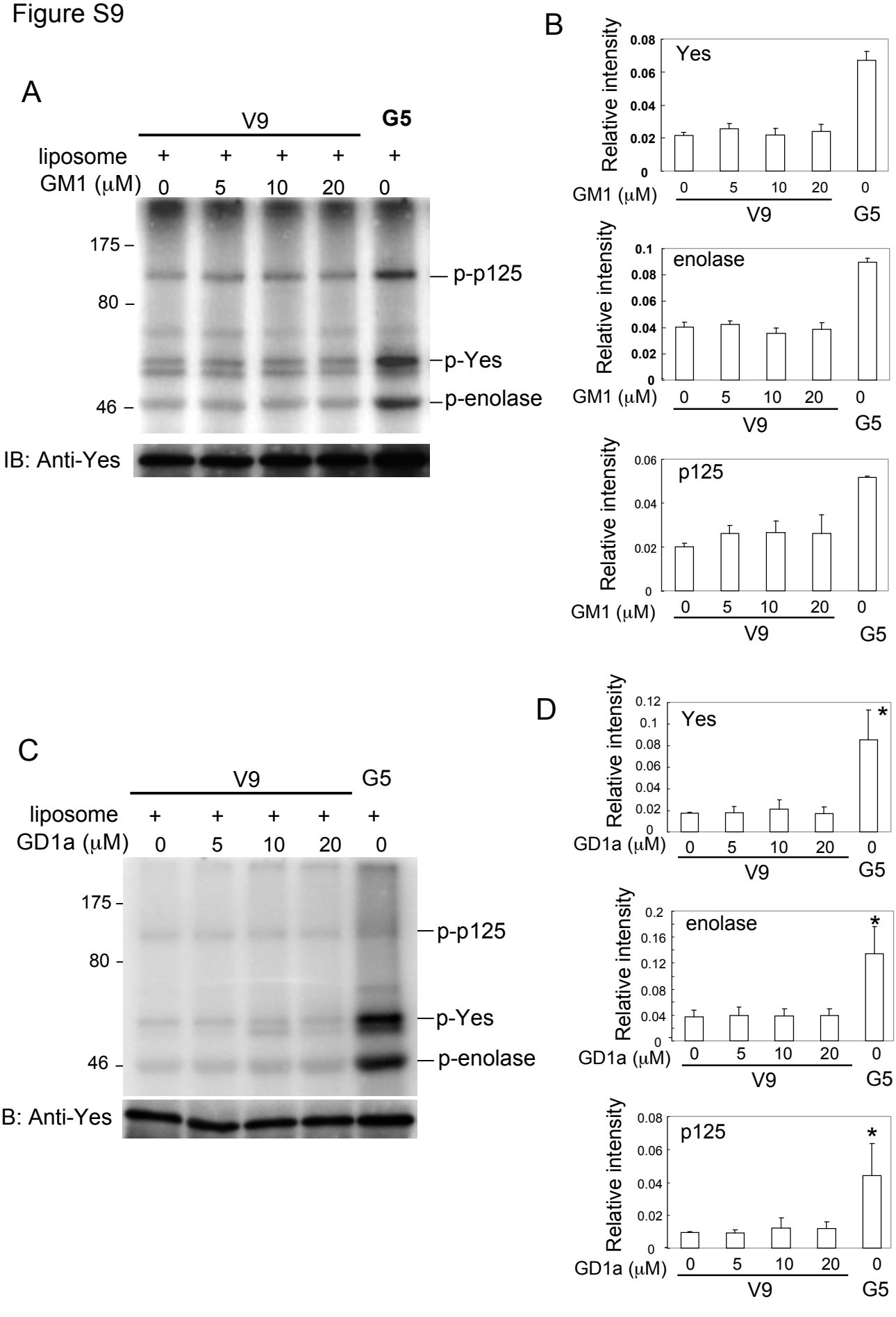

A
V9 + GD3 (μM)
G5
0
1
10
50
100
175
80
46
p-p125
p-Yes
p-enolase
IB:
Anti-Yes
B
Yes
enolase
p125
Relative intensity
V9 GD3 (μM)
G5

Figure S9


A
V9
G5
liposome + + + + +
GM1 (μM) 0 5 10 20 0
175 –
80 –
46 –
— p-p125
— p-Yes
— p-enolase
IB: Anti-Yes
B
Yes
enolase
p125
Relative intensity
GM1 (μM) 0 5 10 20 0
V9
G5
C
V9
G5
liposome + + + + +
GD1a (μM) 0 5 10 20 0
175 –
80 –
46 –
— p-p125
— p-Yes
— p-enolase
IB: Anti-Yes
D
Yes
enolase
p125
*
Relative intensity
GD1a (μM) 0 5 10 20 0
V9
G5