Figure legends for Supplemental data

Supplemental Figure 1. CD9 does not affect the association of caveolin-1 and PKC-α with EGFR or EGFR tyrosine phosphorylation. CD9 expression was knocked down by 75% *(top row, lane 3)* by transfecting cells with CD9 siRNA as described in *"Materials and Methods"*. *Scrambled,* cells transfected with CD9 scrambled siRNA control. Both caveolin-1 (Cav-1) *(4th row)* and PKC-α (*5th row*) co-immunoprecipitate with EGFR, and this association is not impacted by significantly decreasing CD9 expression *(lane 3)*. Similarly, knockdown of CD9 did not reduce EGFR tyrosine phosphorylation *(bottom row)*.

Supplemental Figure 2. Go6976 prevents GM3-induced PKC-α phosphorylation at its threonine 638 site. SCC12 cells were treated with PPPP to deplete GM3 or antisense oligomers directed against GM2/GD2 synthase and GD3 synthase to increase GM3 as described in "*Materials and Methods*". After starvation of serum and growth factor overnight, cells were treated with 500 nM Go6976 for 30 mins before 10 nM EGF was added. Cells were lysed in boiling lysis buffer after stimulation with EGF for 10 mins as described above. Thirty μg of total protein from the whole cell lysate were applied onto a 10% SDS-PAGE mini-gel, transferred onto nitrocellulose membrane, and probed with anti-phosphothreonine-638 PKC-α antibody. Equal loading was confirmed by probing the same membrane with anti-actin antibody. *SCC12,* untreated cell control; *vehicle,* DMSO-treated cells, control for PPPP; *PPPP*, GM3-depleted cells; *sense,* cells treated with sense oligomers to both GM2/GD2 synthase and GD3 synthase, control for antisense treated cells; *antisense*, GM3-overexpressing cells. Treatment with Go6976 significantly inhibited PKC-α phosphorylation at its threonine 638 site in control cells and in cells with increased GM3, but not in GM3-depleted cells.

Supplemental Figure 3. Calcium chelation with BAPTA/AM prevents the inhibitory effect of GM3 on EGF-induced EGFR tyrosine phosphorylation and cell proliferation. SCC12 cells were untreated *(A)* or treated with sense oligomers *(B)* or antisense oligomers (to increase GM3) *(C)*. Cells were starved of both serum and growth factors overnight, then pretreated with BAPTA/AM for 30 mins before 10 nM EGF was added. Ten mins later, EGF stimulation was terminated by washing cells with cold PBS. Cells were lysed with boiling lysis buffer (10 mM Tris-HCl, pH 7.4, 1% SDS, 1 mM Na_3VO_4) and the whole cell lysate was collected. Ten μg of total protein from the whole cell lysate was applied onto a 10% SDS-PAGE gel. After electrophoresis, separated protein was transferred onto nitrocellulose membrane and immunoblotting was performed using antibody directed against phosphothreonine PKC-α at its 638 site or phosphotyrosine EGFR. Equal loading was confirmed by blotting the same membrane with anti-actin antibody. BAPTA/AM inhibited GM3-induced PKC-α activation in a dose-dependent manner. 50 μM BAPTA/AM was able to eliminate GM3-induced PKC-α threonine phosphorylation at its 638 site and maintained EGFR tyrosine phosphorylation at the same level as that of SCC12 cells and sense oligomer-treated controls *(last lane)*. Consistently, BAPTA/AM treatment reversed the inhibitory effect of GM3 on cell growth as demonstrated by the BrdU incorporation assay *(D)*.

Supplemental Figure 4. CD9 is not detectable in the complex that includes EGFR and PKC-α. CD9 does not co-immunoprecipitate with EGFR *(A)*, even in the presence of increased GM3 *(lane 5)*. Similarly, EGFR does not co-immunoprecipitate with CD9 *(B)*, even when GM3 is increased *(lane 5)*. PKC-α weakly associates with CD9, but this association is not altered by increased content of GM3 *(B)*. *SCC12*, untreated cell control; *vehicle*, DMSO-treated cells, control for PPPP; *PPPP*, GM3-depleted cells; *sense*, cells treated with sense oligomers to both GM2/GD2 synthase and GD3 synthase, control for antisense treated cells; *antisense*, GM3-overexpressing cells.