

Supplemental Information

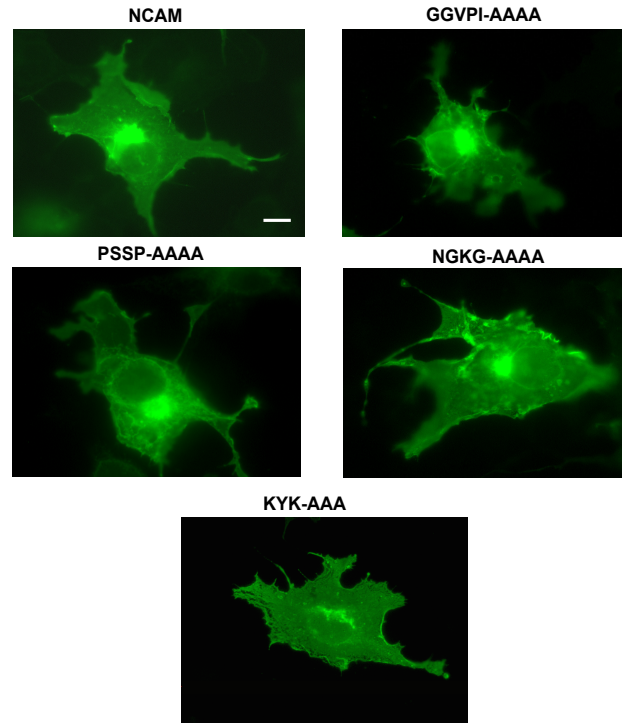
Supplemental Table 1

Mutant	Templates	5'-3' Primer	3'-5' Primer
GGVPI-AAAAA	NCAM or NCAM7	5'-GAGGCCACAGGTGCGG CGGCCGCCCTCAAATAC-3'	5'-GTATTTGAGGGCGGCCG CCGCACCTGTGGCCTC-3'
NGKG-AAAA	NCAM or NCAM7	5'-CTGGCGGCGCTCGC TGCCGCAGCGCTGGGT GAGATC-3'	5'-GATCTCACCCAGCGC TGCGGCAGCGAGCGCCG CCAG-3'
PSSP-AAAA	NCAM or NCAM7	5'-CAAGCAGACACCGC CGCTGCAGCATCCATC GACCAG-3'	5'-CTGGTCGATGGATG CTGCAGCGGCGGTGTC TGCTTG-3'
KYK-AAA	NCAM	5'-GTGCCCATCCTCGCAGC CGCAGCTGAGTGGAG-3'	5'-CTCCACTCAGCTGCGG CTGCGAGGATGGGCAC-3'
sPST-myc	sNCAM- pcDNA 3.1/V5- HisB ST8SiaIV/PST- pcDNA3.1/V5- HisB	Primer A 5'-AAAAAAAAGCTTGCTAG CTTGCTTGTCTTTTT GCAG-3' Primer C 5'-AAAAAAGATATCTCAAT CTTCAGCACAATGTAGAA GG-3'	Primer B 5'-AAAAAAGATATCAGAGTC AACGAAGGCTGCGGTG-3' Primer D 5'-AAAAAATCTAGACCTTGC TTTACACACTTTCCTGTTG TC-3'

Supplemental Experimental Methods

Analysis of NCAM and NCAM mutant protein localization-Cos-1 cells grown on 12-mm coverslips were transfected using 3 µl Lipofectin in 300 µl Opti-MEM 1, and 0.5 µg NCAM cDNA. After a 6h incubation, 1 ml DMEM, 10% FBS, was added to each well. Sixteen hours later, cell media was removed and cells were washed twice with 1 ml PBS. One milliliter of -20°C methanol was used to fix and permeabilize cells to visualize both internal and cell surface structures. The coverslips were then incubated with 1 ml immunofluorescence blocking buffer (5% normal goat serum in PBS). To evaluate localization, cells were incubated with anti-V5 tag antibody diluted 1:250 in blocking buffer, washed with PBS four times for 5 min, followed by incubation with FITC-conjugated goat anti-mouse IgG (Jackson ImmunoResearch) diluted 1:100 in blocking buffer. After further washing, coverslips were mounted on glass slides using mounting medium (15% (w/v) Vinol 205 polyvinyl alcohol, 33% (w/v) glycerol, 0.1% azide in PBS, pH 8.5). A Nikon Axiophot microscope equipped with epifluorescence illumination and a 60X oil immersion Plan Apochromat objective was used to visualize the cells and pictures taken with a SPOT RT color digital camera and processed with SPOT RT software version 3.5.1 (Diagnostic Instruments Inc, Sterling Heights, MI)

Supplemental Figure 1



Supplemental Fig. 1. **NCAM and NCAM mutants localize to the Golgi complex and cell surface.** COS-1 cells transiently expressing NCAM or NCAM mutants with sequence replacements in the FN1 domain (PSSP-AAAA, GGVPI-AAAA, NGKG-AAAA, KYK-AAA) were fixed with -20°C methanol and proteins were localized by indirect immunofluorescence microscopy using the anti-V5 epitope tag antibody and an FITC-anti-mouse IgG secondary antibody as described in “Supplemental Experimental Procedures.” *Bar*, 10 μ m.