

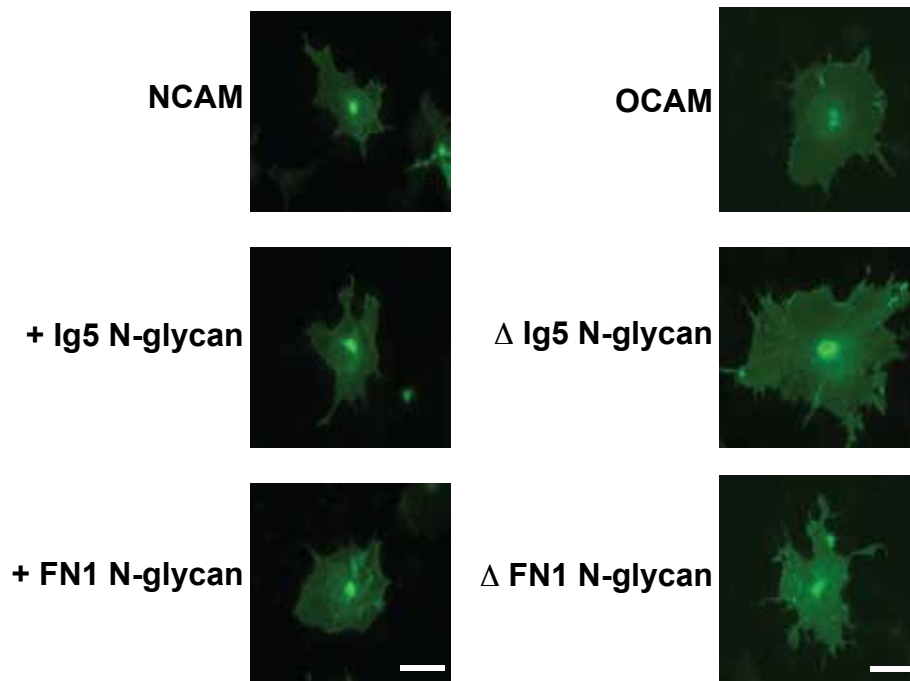
Supplementary Table 1. Primers used in construction of mutants and chimeras.

MUTANT	TEMPLATE	PRIMER SEQUENCE
NCAM G410N/P411Q/ V412T	NCAM	5'-GCCCCAAAGCTACAGAATCAGACAGCTGTGTACACTTGG-3' 5'-CCAAGTGTACACAGCTGTCTGATTCTGTAGCTTTGGGGC-3'
NCAM E569TT-NTT	NCAM	5'-GGCCTGAAGCCCCAACACAACGTACGCC-3' 5'-GGCGTACGTTGTGTTGGGCTTCAGGCC-3'
OCAM N406QT-QQT	OCAM	5'-CTAAGTTTGTTCACAGCAGACAATGTATTAC-3' 5'-GTAATACATTGTCTGCTGTGAAACAAACTTAG-3'
OCAM N562TT-QTT	OCAM	5'-GAGCAGTCTGGAACCACAGACGACTTACGAAATTAG-3' 5'-CTAATTCGTAAGTCGTCTGTGGTTCCAGACTGCTC-3'
NCAM NTPSASY- SVGRKMI	NCAM	5'- CAATATCAAGATCTACAGCGTCGGCCGTAAGATGATTCTGGAGG TGACCCC-3' 5'- GGGGTCACCTCCAGAATCATCTTACGGCCGACGCTGTAGATCTT GATATTG-3'
NCAM QESL-TRFQ	NCAM	5'-GAACCGCATTGGGACGAGGTTCCAGGAATTCATCCTTG-3' 5'-CAAGGATGAATTCCTGGAACCTCGTCCCAATGCGGTTC-3'
NCAM S448K/N476R	NCAM	5'-CAGCTGCTGCCAAGCAAGAATTACAGCAATATC-3' 5'-GATATTGCTGTAATTCTTGCTTGGCAGCAGCTG-3'
	NCAM S448K	5'-GAGAATGATTTTGGGAGATACAACTGTACTGC-3' 5'-GCAGTACAGTTGTATCTCCCAAATCATTCTC-3'
NCAM S448A/N476A	NCAM	5'-CTGCTGCCAAGCGCCAATTACAGC-3' 5'-GCTGTAATTGGCGCTTGGCAGCAG-3'
	NCAM S448A	5'-GAATGATTTTGGGGCCTACAACTGTAC-3' 5'-GTACAGTTGTAGGCCCCAAAATCATTTC-3'
OCAM NQT-NQA	OCAM	5'-GTTTGTTCAAATCAGGCCATGTATTACTCTTGGG-3' 5'-CCCAAGAGTAATACATGGCCTGATTTGAAACAAAC-3'
OCAM NQA/K444S	OCAM NQA	5'-CTTACCAGCTAGCAATACGACTC-3' 5'-GAGTCGATTGCTAGCTGGTAAG-3'
OCAM NQA/R472N	OCAM NQA	5'-GACAATGACTTTGGAACTATAACTGCACAGC-3' 5'-GCTGTGCAGTTATAGTTTCCAAAGTCATTGTC-3'

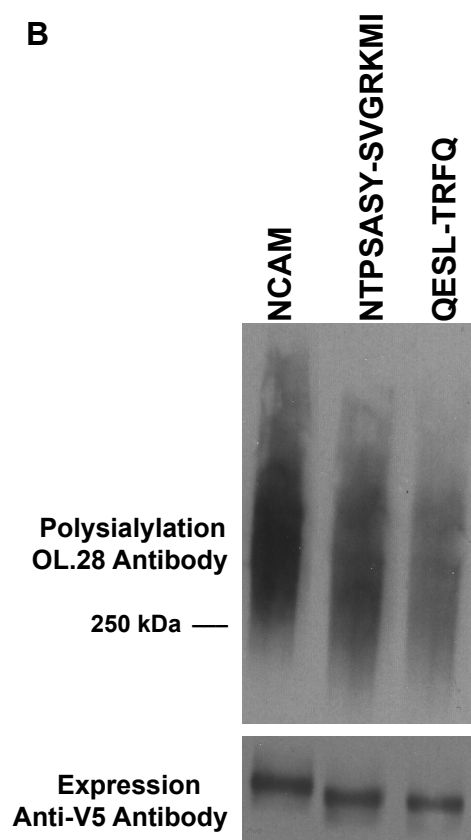
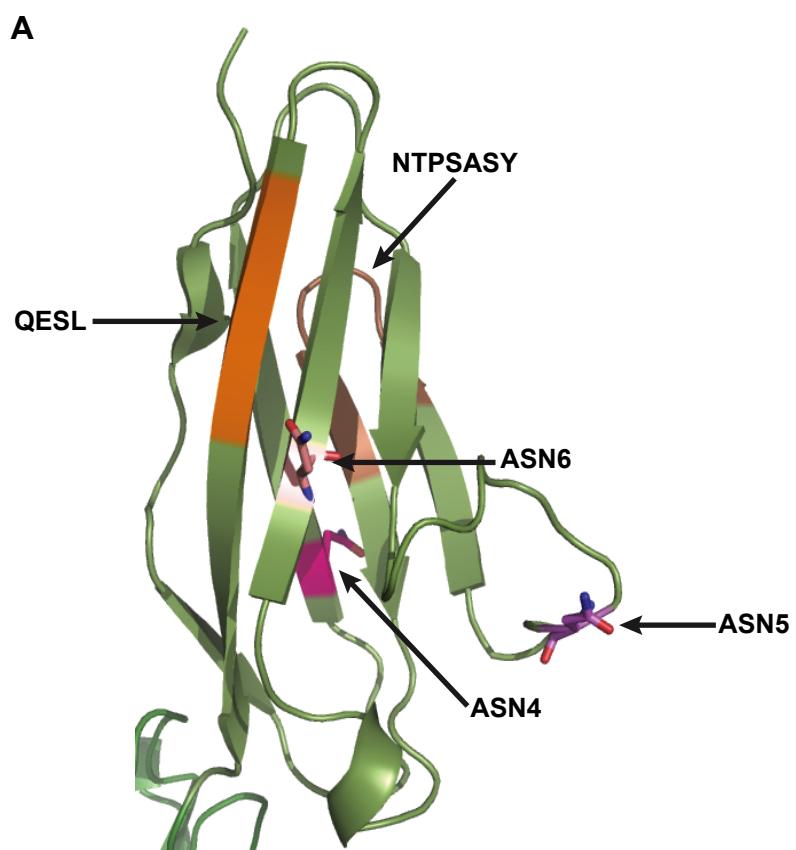
A



B

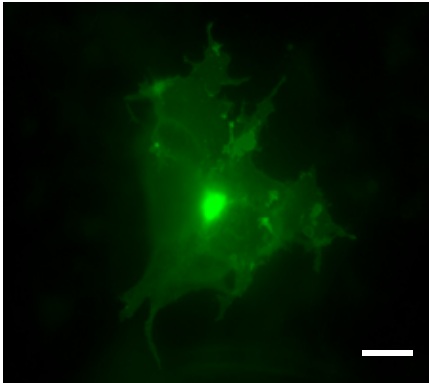


Supplementary Figure 1

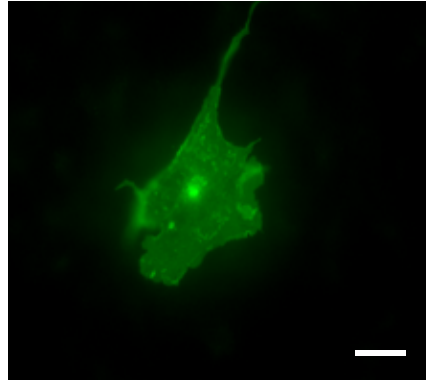


Supplementary Figure 2

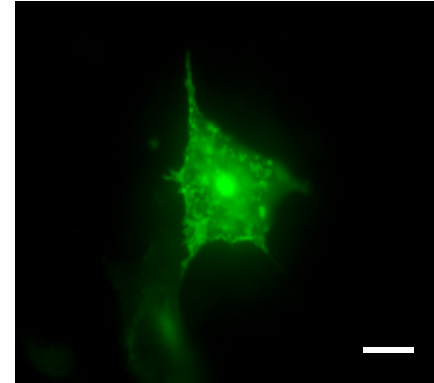
NCAM



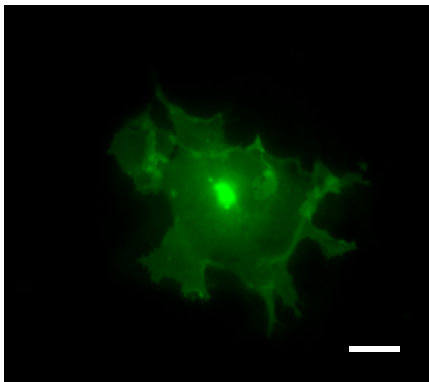
NCAM S448K



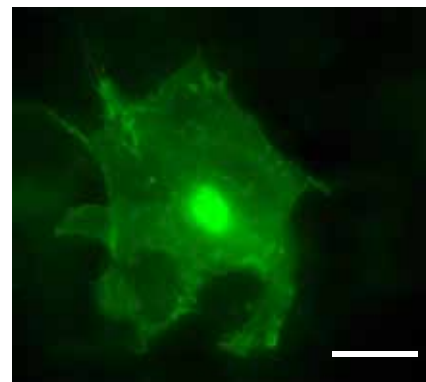
NCAM N476R



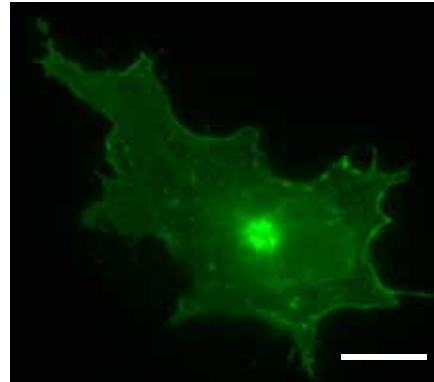
S448K N476R



S448E N476E



S448A N476A



Supplementary Figure 3

Supplementary Figure Legends

Supplementary Fig. 1. **Localization of NCAM and OCAM chimeric proteins and glycosylation mutants expressed in COS-1 cells.** The localization of NCAM, OCAM and O-N chimeric proteins, O-N FN1, O-N Ig5 and O-N Ig5-FN1 (A), or NCAM and OCAM glycosylation mutants (B), was determined by indirect immunofluorescence microscopy of methanol fixed and permeabilized COS-1 cells expressing these proteins. An anti-V5 epitope tag antibody and a FITC-conjugated goat anti-mouse IgG secondary antibody were used to detect proteins in cells, as described in *Experimental Procedures*. Bar, 10 μ m.

Supplementary Fig. 2. **Replacing nonconserved NCAM Ig5 sequences has minimal effect on polysialylation.** A, a schematic diagram of NCAM Ig5 highlighting two regions not conserved in OCAM Ig5. The sequence Asn⁴⁵⁷-Thr⁴⁵⁸-Pro⁴⁵⁹-Ser⁴⁶⁰-Ala⁴⁶¹-Ser⁴⁶²-Tyr⁴⁶³ (NTPSASY) is shown in brown and Gln⁴⁸⁷-Glu⁴⁸⁸-Ser⁴⁸⁹-Leu⁴⁹⁰ (QESL) is in orange. The three consensus N-linked glycosylation sites in NCAM Ig5 are also indicated. B, *upper panel*, wild type or mutated NCAM proteins were co-expressed with ST8SiaIV/PST in COS-1 cells. NCAM proteins were immunoprecipitated from cell lysates, and polysialylation determined by immunoblotting with the OL28 anti-polySia antibody. *Lower panel*, relative NCAM protein expression levels were measured by immunoblotting with the anti-V5 epitope tag antibody.

Supplementary Fig. 3. **Localization of NCAM Ser⁴⁴⁸ and Asn⁴⁷⁶ mutants expressed in COS-1 cells.** The localization of NCAM and mutant NCAM proteins with S448 or N476 replaced individually or together with analogous OCAM Ig5 residues, alanine or glutamic acid residues, was determined by indirect immunofluorescence microscopy of methanol fixed and permeabilized COS-1 cells expressing these proteins. An anti-V5 epitope tag antibody and a FITC-conjugated goat anti-mouse IgG secondary antibody were used to detect proteins in cells, as described in *Experimental Procedures*. Bar, 10 μ m.