SUPPLEMENTARY DATA

Sequences Prior to Conserved Catalytic Motifs of the Polysialyltransferase, ST8Sia IV, are Required for Substrate Recognition

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SUPPLEMENTAL EXPERIMENTAL METHODS

Transfection of COS-1 Cells with Wild-type or Mutant PST cDNAs—COS-1 cells maintained in Dulbecco's modified Eagle's medium (DMEM), 10% fetal bovine serum (FBS) were plated onto 12-mm glass coverslips and grown in a 37^{o}C, 5% CO_2 cell incubator until 50-70% confluent. Cells on each coverslip were then transected with 0.5 μg of PST-, sPST- or mutant PST-Myc DNA in 300 μL of OPTI-MEM I supplemented with 3 μL of Lipofectin transfection reagent. Cells were then incubated with the transfection mixture for 6 h at 37^{o}C, followed by the addition of 1 mL of DMEM, 10% FBS, and allowed to grow in the incubator for 18 h.

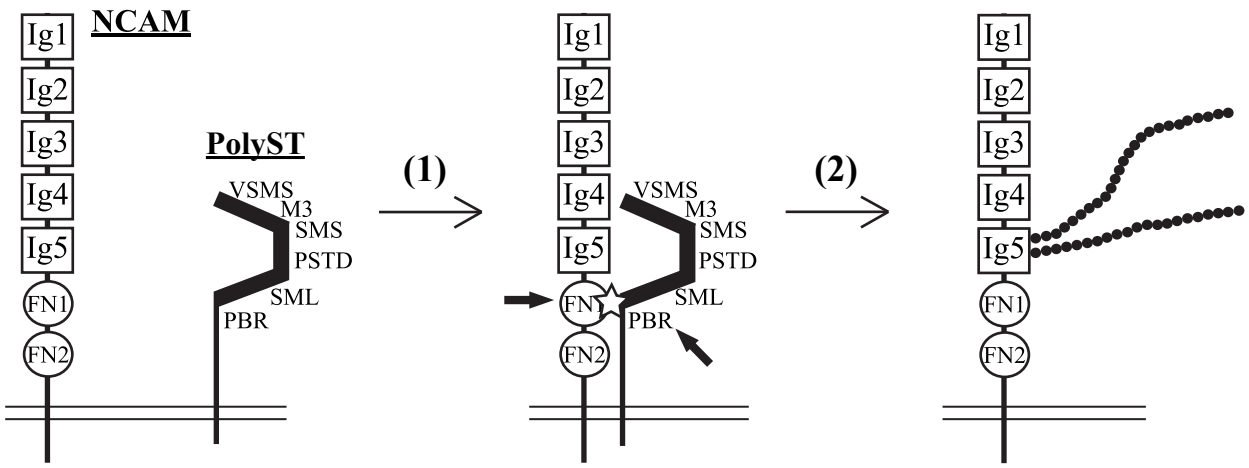
Immunofluorescence Analysis of PST and Mutant PST Enzyme Localization and Activity—COS-1 cells on glass coverslips were transfected with PST-, sPST- or a PST-Myc mutant cDNA as described above. Eighteen hours post-transfection, cells were washed with PBS then permeabilized with -20^{o}C methanol to view internal structures as well as the cell membrane. Cells were incubated with the following primary antibodies diluted in immunofluorescence blocking buffer (5% normal goat serum in PBS): anti-Myc tag antibody (1:250) to detect expression and localization of PST or a mutant PST, and OL.28 anti-polySia antibody (1:100) to determine PST catalytic activity by detecting protein autopolysialylation. Following incubation at room temperature with the primary antibodies for 2 h, the cells were incubated for one hour with the following secondary antibodies diluted in blocking buffer: FITC-conjugated goat anti-mouse IgG (1:100) to visualize protein localization, and TRITC-conjugated goat anti-mouse IgM (1:100) to visualize autopolysialylation. After washing, the cells were briefly treated with 300 nM 4', 6-diamidino-2-phenyl-indole, dihydrochloride (DAPI) in PBS, and the coverlips were then mounted on glass slides using 20 μL mounting medium (15% Vinol 205 polyvinyl alcohol (w:v), 33% glycerol (v:v), 0.1% azide, pH 8.5). Cells were visualized with a Zeiss Axiovert 200 M inverted confocal microscope using a 63X oil immersion objective.

SUPPLEMENTAL TABLE 1

PCR Primers used for site-directed mutagenesis

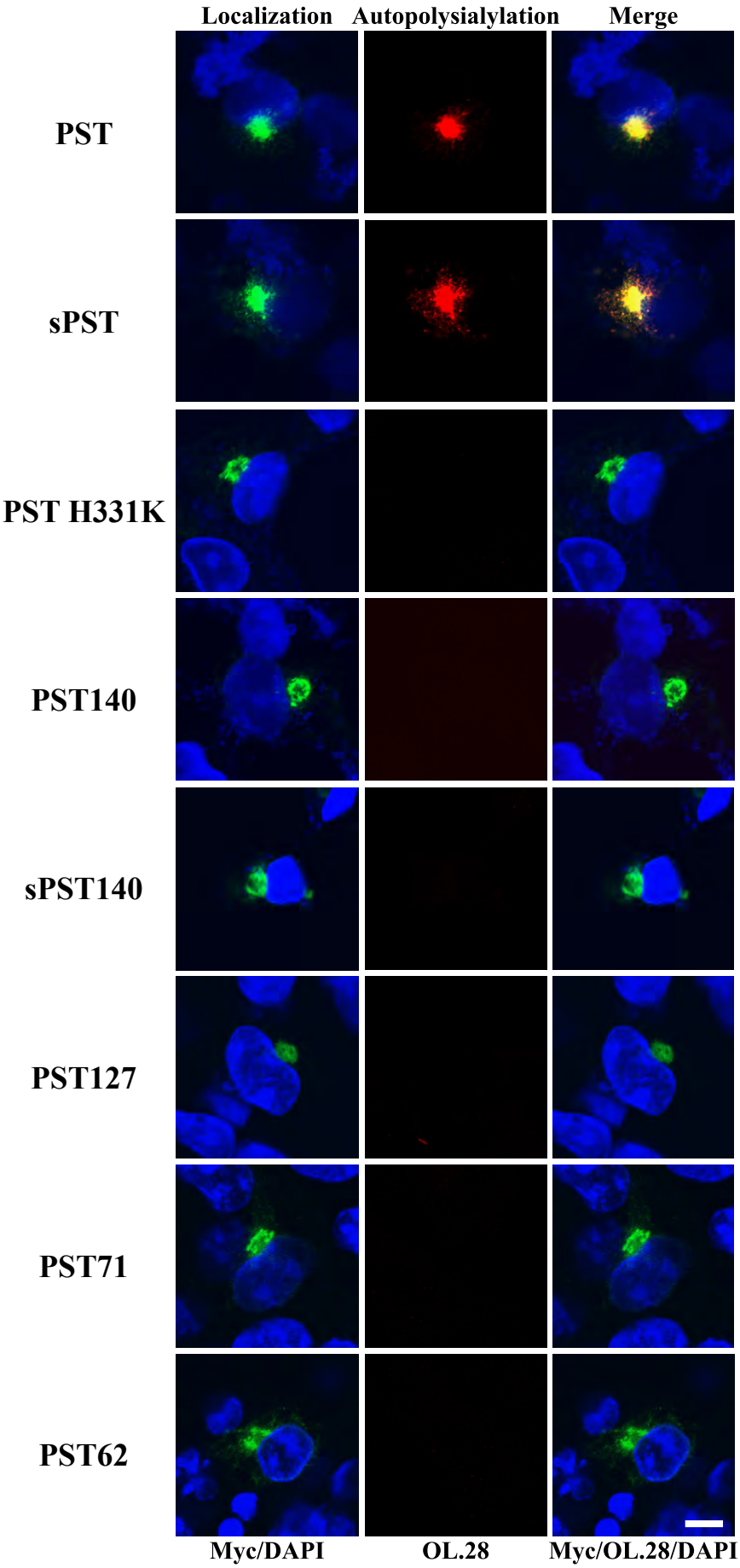
PST H331K	5’-CCAATGCAAGCCCTAAGAGAATGCCATTAG-3’ 5’-CTAATGGCATTCTCTTAGGGCTTGCATTGG-3’
PST140 XbaI site	5’-CGCAGGTTTAAGACTCTAGACTGTGCAGTTG-3’ 5’-CAACTGCACAGTCTAGAGTCTTAAACCTGCG-3’
PST127 XbaI site	5’-CATGATCTACATAGCCTCCTTCTAGAAGTTTCACCAATG-3’ 5’-CATTGGTGAAACTTCTAGAAGGAGGCTATGTAGATCATG-3’
PST105 XbaI site	5’-AGTTTTAAGCCTGGTGATCTAGAACACTATGTGCTT-3’ 5’-AAGCACATAGTGTTCTAGATCACCAGGCTTAAAACT-3’
PST71 XbaI site	5’-GTAGAAGGTTGGAATCTAGATTCCTCTTTG-3’ 5’-CAAAGAGGAATCTAGATTCCAACCTTCTAC-3’
PST62 XbaI site	5’-GCTGGCTCTTCAATTCTAGAGCACAATGTAGAA-3’ 5’-TTCTACATTGTGCTCTAGAATTGAAGAGCCAGC-3’
sPST #1,2	5’-AAAAAAAAGCTTGCTAGCTTGCTTGTTCTTTTTGCAG-3’ 5’-AAAAAAGATATCAGAGTCAACGAAGGCTGCGGTG-3’
sPST #3,4	5’-AAAAAAGATATCTCAATCTTCCAGCACAATGTAGAAGG-3’ 5’-AAAAAATCTAGACCTTGCTTTACACACTTTCCTGTTGTC-3’
PST / PSTH331K R82A	5’-GTCCTAGAGATAGCGAAGAACATACTT-3’ 5’-AAGTATGTTCTTCGCTATCTCTAGGAC-3’
PST / PST H331K R93A	5’-TTAGATGCAGAAGCGGATGTGTCAGTG-3’ 5’-CACTGACACATCCGCTTCTGCATCTAA-3’

SUPPLEMENTAL FIGURE S1



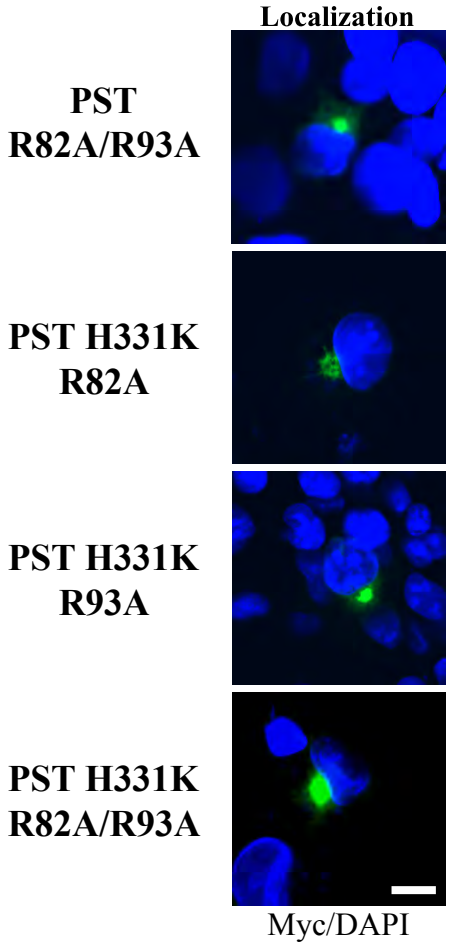
Supplemental Fig. S1. Working Model of NCAM Polysialylation by the polySTs.

SUPPLEMENTAL FIGURE S2



Supplemental Fig. S2. PST, sPST, PST H331K and all truncated PST mutants are predominantly localized to the Golgi apparatus in COS-1 cells. Myc-tagged PST, sPST, or a PST mutant were expressed in COS-1 cells as described in supplemental "Experimental Methods." Cells were then washed, fixed with methanol, and subjected to staining with anti-Myc antibody (*Localization, Myc, green*) to determine protein localization, and OL.28 anti-polySia antibody (*Autopolysialylation, OL.28, red*) to detect autopolysialylation and hence enzyme activty. DAPI staining was utilized to indicate the location of the nucleus (*Localization and Merge, DAPI, blue*). *Bar,* 10 µm.

SUPPLEMENTAL FIGURE S3



Supplemental Fig. S3. PST R82A/R93A, PST H331K R82A, PST H331K R93A, and PST H331K R82A/R93A are predominantly localized to the Golgi apparatus in COS-1 cells. The Myc-tagged PST R82A/R93A and PST H331K PBR mutants shown above were expressed in COS-1 cells as described in supplemental "Experimental Methods." Cells were then washed, fixed with methanol, and subjected to staining with anti-Myc antibody (*green*) to determine protein localization. Staining with DAPI was utilized to indicate the location of the nucleus (*blue*). Bar, 10 μm.