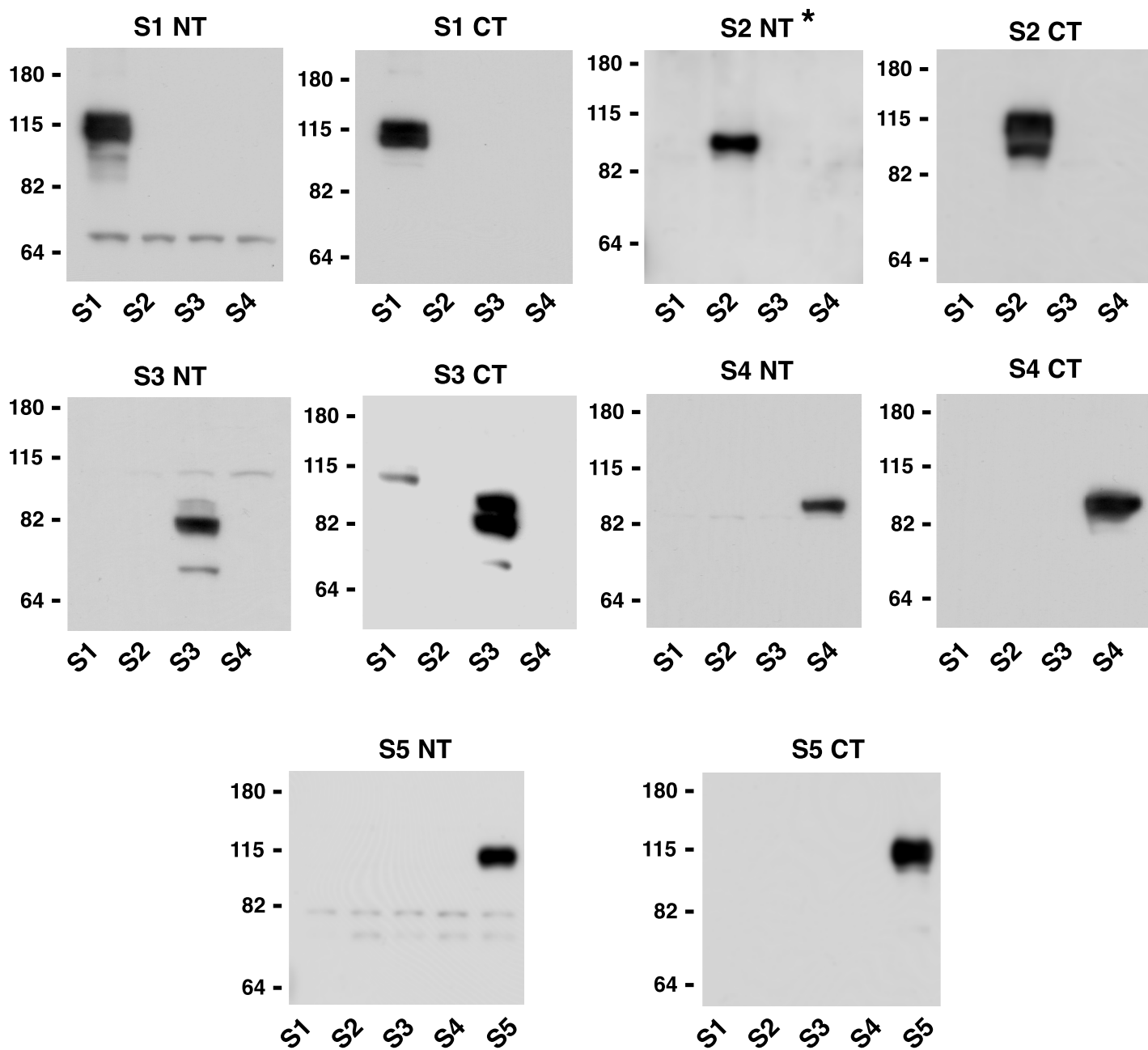


SUPPLEMENTAL FIGURE 1. SALM antibody characterization. Lysate from HEK293 cells singly transfected with SALMs 1, 2, 3, 4, or 5 was analyzed by SDS-PAGE to determine antibody specificity. Blots were probed with antibodies directed against the N-terminus (NT) or C-terminus (CT) of the SALMs. All of the antibodies are specific to the target SALM, except for the SALM3 CT antibody, which weakly recognizes SALM1 as well as SALM3. For the S2NT panel, the HEK293 lysate was treated with N-glycosidase, to deglycosylate the SALMs, prior to loading the protein samples on to the gel (asterisk).

SUPPLEMENTAL METHODS

EndoH Digestion and Deglycosylation-HEK293 cell pellets, expressing the SALM family members, were resuspended in denaturing buffer (10 mM NaH₂PO₄, pH 6, 0.5% SDS, and 2% glycerol), incubated for 3 min at 95°C, and diluted with 1% Nonidet P-40 in 10 mM NaH₂PO₄, pH 6, with protease inhibitors. Lysate was incubated with peptide N-glycosidase F (PNGase F) (3 U) (Prozyme) for 6 hrs at 37°C before being diluted with 5X sample buffer and loaded onto a 10% SDS-PAGE gel.



Suppl. Fig. 1