Supporting Information

Wilson *et al*. 10.1073/pnas.0711846105

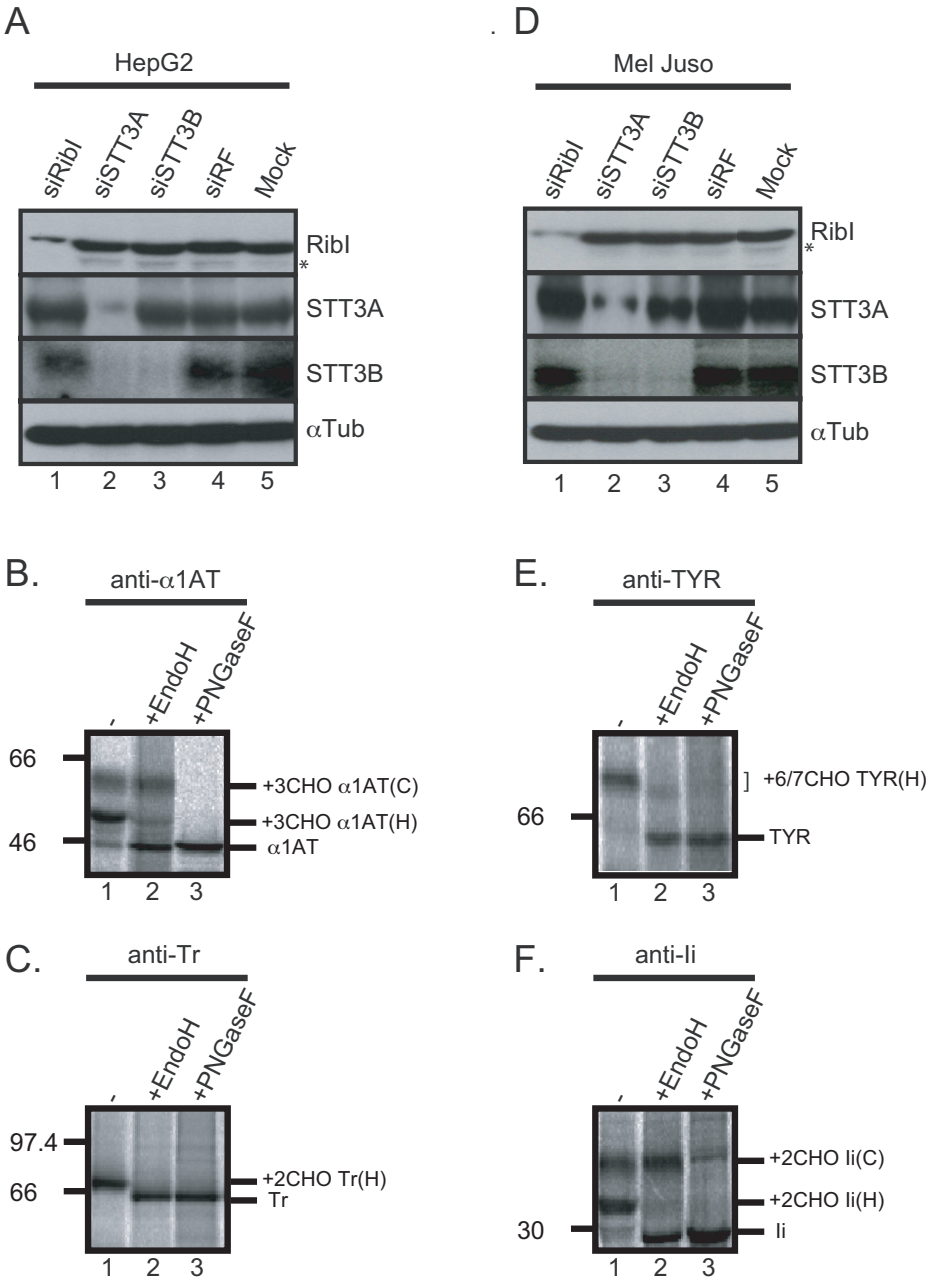

Fig. S1. siRNA mediated knockdown of ribophorin I, STT3A and STT3B. (*A*) HepG$_2$ cell lysates 48 h after transfection with ribophorin I, STT3A and STT3B siRNA duplexes (lanes 1–3), a non-functional siRisc-free control (siRF) (lane 4), or mock-transfected cells (lane 5) were probed with antibodies specific for ribophorin I (RibI), STT3A, STT3B or α-tubulin (αTub). An unglycosylated form of ribophorin I is indicated by an asterisk, and the loss of STT3B upon siRNA mediated knockdown of STT3A has been described in HeLa cells (1). HepG$_2$ cells were pulse labeled with [^{35}S]methionine/cysteine for 45 min, solubilized in Triton X-100 IP buffer and specific products recovered by immunoprecipitating with antisera recognizing α1-antitrypsin (*B*) or transferrin (*C*). The immunoprecipitated products were treated with either endoglycosidase H (Endo-H), Peptide: N-Glycosidase F (PNGase F) or mock treated (–) before resolving on SDS/PAGE. (*D*) Cell lysates from Mel Juso cells were prepared and immunoblotted as for *A*. Mel Juso cells were pulse labeled with [^{35}S]methionine/cysteine for 30 min, solubilized in Triton X-100 IP buffer and specific products recovered by immunoprecipitation using antisera recognizing tyrosinase (*E*) or the invariant chain (*F*). The immunoprecipitated products were either Endo-H, PNGase F or mock treated before resolving on SDS/PAGE.

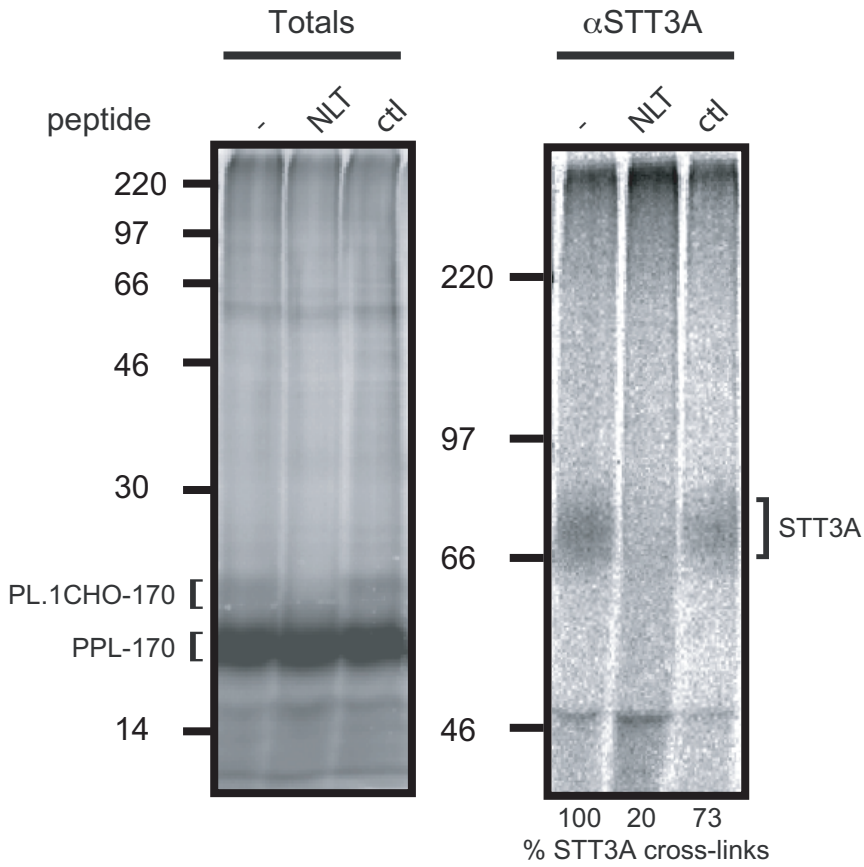

Fig. S2. An acceptor tripeptide blocks nascent chain cross-linking to STT3A. A 170-residue PPLNKT translocation intermediate was synthesized and cross-linked as described in the main text with no additions or in the presence of a NLT tripeptide or a control. Samples were divided in two and one half analyzed as total products, and the other half immunoprecipitated with an antiserum against STT3A.

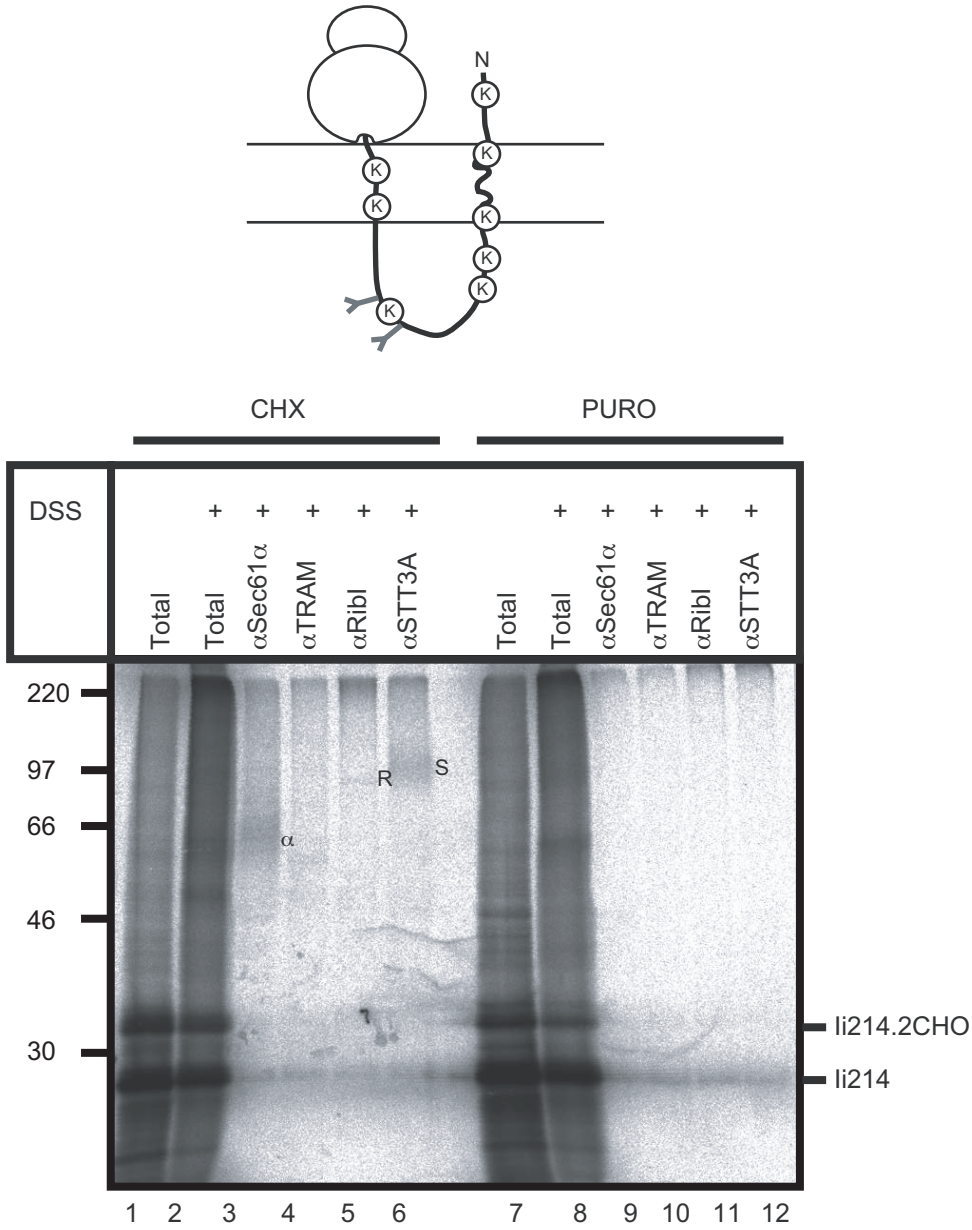

Fig. S3. Cross-linking analysis of an Invariant chain integration intermediate. A 214-residue fragment of the Invariant chain (Ii214) was synthesized from mRNA lacking a stop codon using a rabbit reticulocyte lysate system supplemented with semipermeabilized HeLa cells and incubated with either 2 mM cycloheximide (lanes 1–6) or 2 mM puromycin (lanes 7–12). Membrane-associated products were isolated and treated with 1 mM DSS or Me_2SO as indicated. A fraction of the total products was analyzed directly (lanes 1, 2, 7, and 8), whereas the remainder was subjected to immunoprecipitation with antisera specific for Sec61α (lanes 3 and 9), TRAM (4 and 10), ribophorin I (5 and 11), and STT3A (6 and 12). Discrete adducts with Sec61α (α), ribophorin I (R), and STT3A (S) are indicated.

1. Wilson CM, High S (2007) Ribophorin I acts as a substrate-specific facilitator of N-glycosylation. *J Cell Sci* 120:648–657.