

## Supplementary Information

### Supplementary Figure Legends

**Figure S1.** (A) The indicated TM region deletion constructs of NgBR were transfected into COS-7 cells. 24h post-transfection, cell lysates were treated with EndoH. Deletion of TM1 or TM3 precludes C-terminal glycosylation events. (B) The indicated HA-tagged NgBR constructs were co-transfected with NPC2 into COS-7 cells. 24h post-transfection, cells were lysed and NgBR was immunoprecipitated with anti-HA agarose. NPC2 only co-immunoprecipitates with NgBR when the C-terminus of NgBR is present in a luminal orientation (i.e., with WT and  $\Delta$ TM2 constructs).

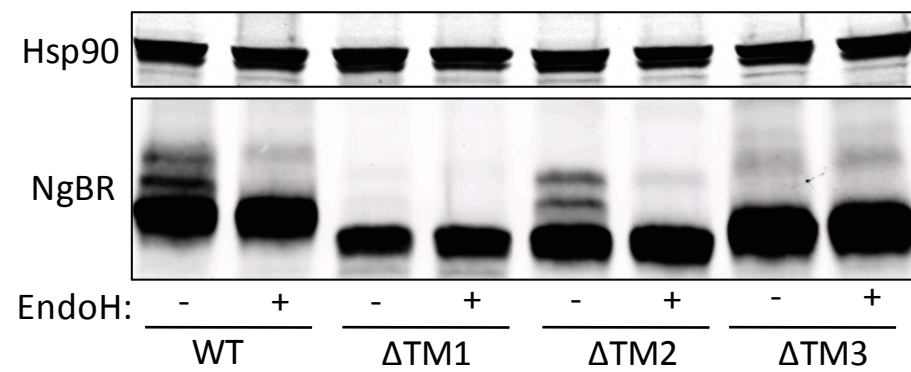
**Figure S2.** (A) SDS-PAGE of lysates from cells treated with Ctrl RNAi (lane 1), NgBR RNAi (lane 2) or simultaneous treatment with NgBR RNAi and Ad-NgBR-FLAG (lane 3). (B) FACE analysis of LLOs from these cells. Cells were treated with RNAi for 72h to allow for sufficient expression of the adenovirus. (C-D) Free Asn-linked glycans were measured by FACE analysis (loading was normalized to protein). Total protein precipitates were treated with N-glycanase and neutral and acidic glycans were separated by ion exchange chromatography, followed by FACE analysis of the free glycans. (E) Quantification of results from (C-D) (n=6, \*p<0.001).

**Figure S3.** SDS-PAGE analysis of post-nuclear supernatant (W), cytosolic fraction (C) and membrane (M)-associated NgBR. After centrifugation at 1000 x g, post-nuclear supernatants were centrifuged at 100,000 x g to separate cytosol from crude membranes in cells expressing either endogenous (NgBR) or ectopic (NgBR-HA) NgBR.

**Figure S4.** Sequence alignment of cis-IPTases. Alignment was performed with ClustalW2. Residues important for catalysis are shaded in red, residues critical for IPP binding are shaded in blue, and residues important for allylic-PP binding are shaded in purple. NgBR does not contain many of the residues deemed important for catalytic activity and substrate binding in UPPS.

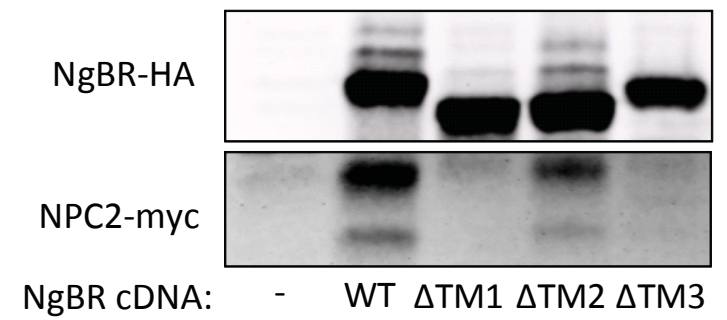
Figure S1

A



B

IP: HA



WCL:

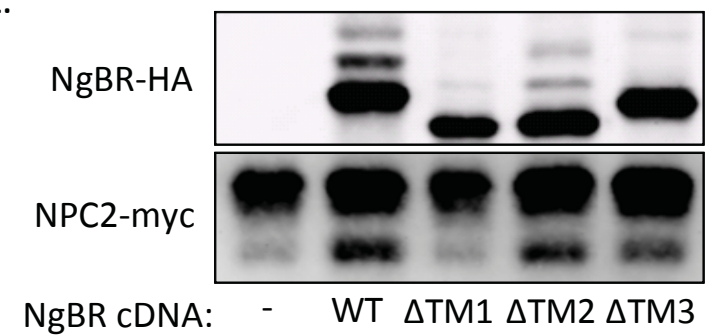
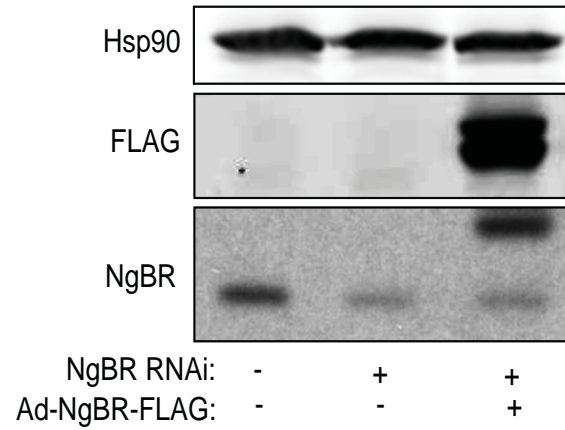
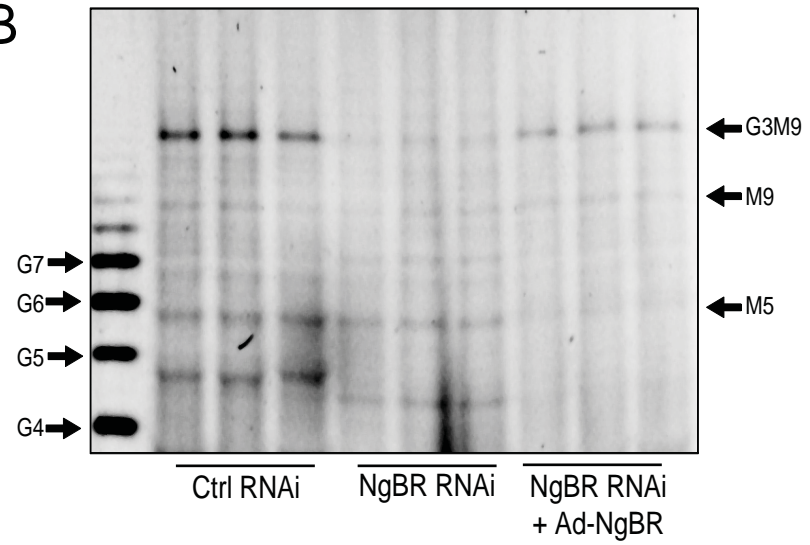


Figure S2

A

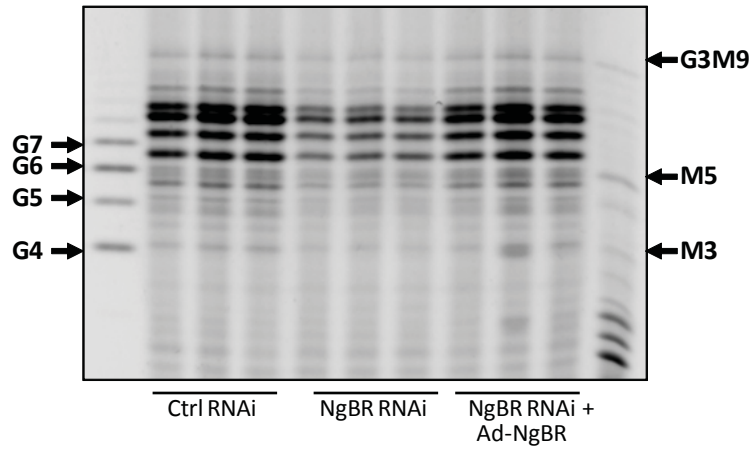


B



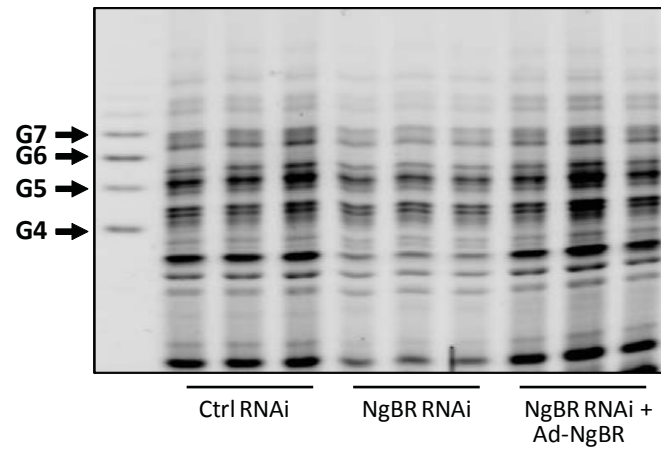
C

*Neutral N-glycans*



D

*Negatively-Charged N-glycans*



E

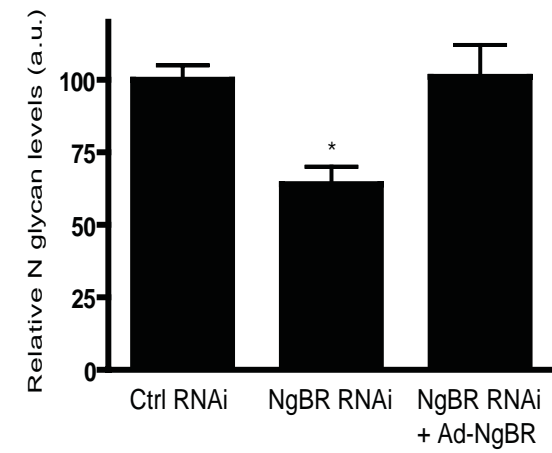
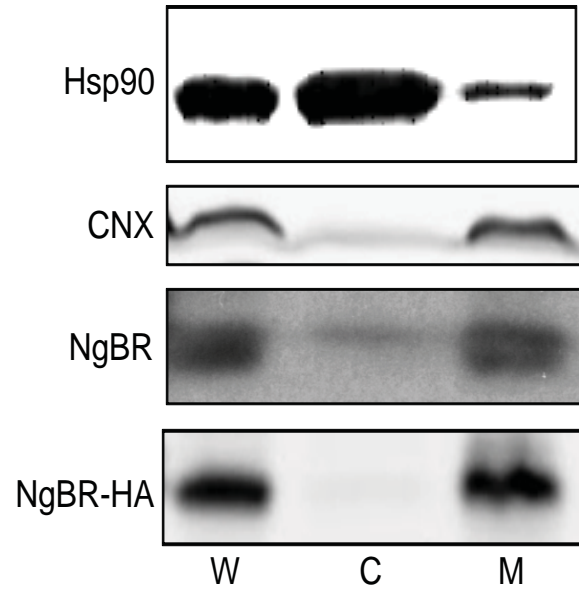
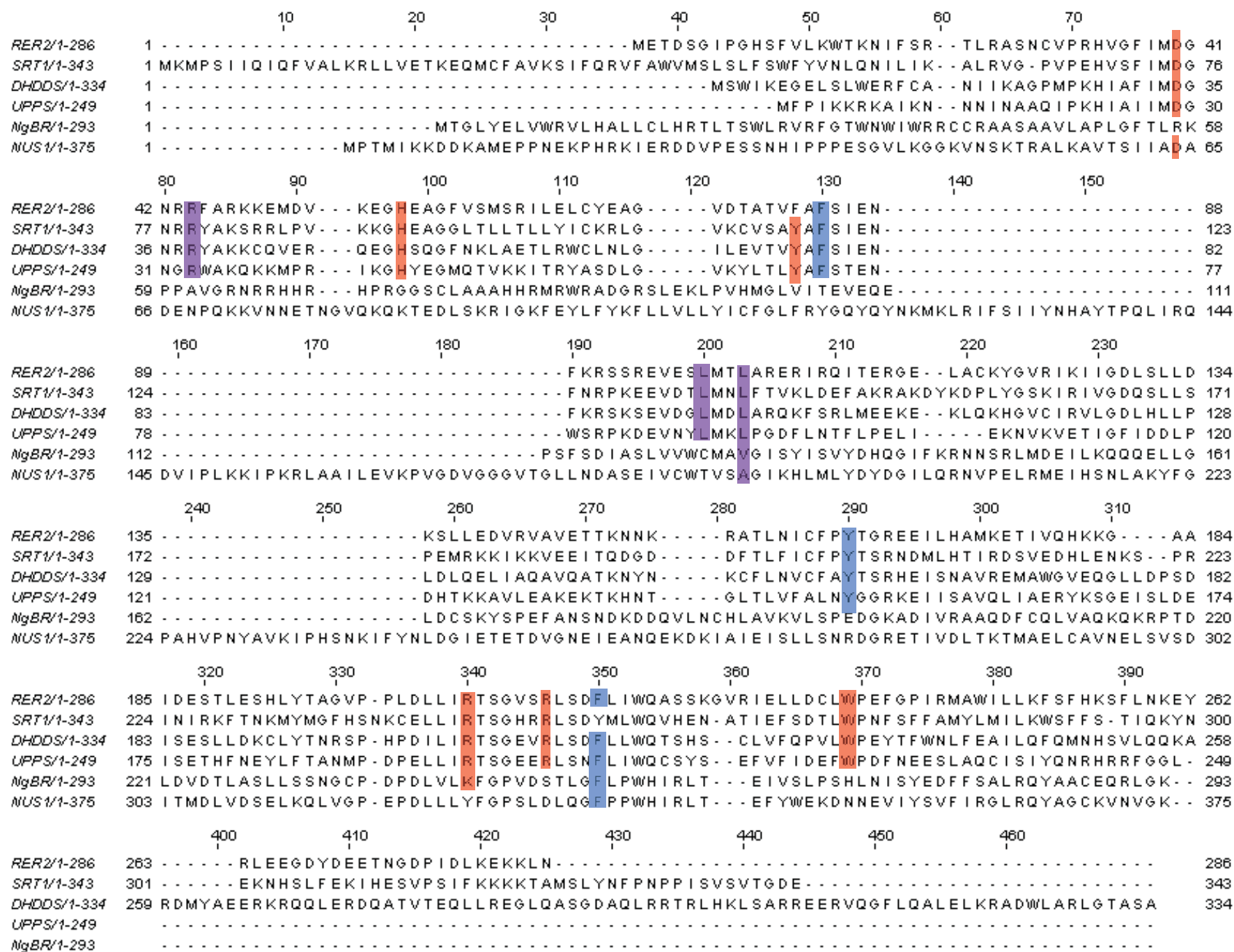


Figure S3



# Figure S4



█ Catalytic site  
█ IPP binding site  
█ allylic-PP binding site