

SUPPLEMENTAL INFORMATION

Sculpting therapeutic monoclonal antibody *N*-glycans using endoglycosidases

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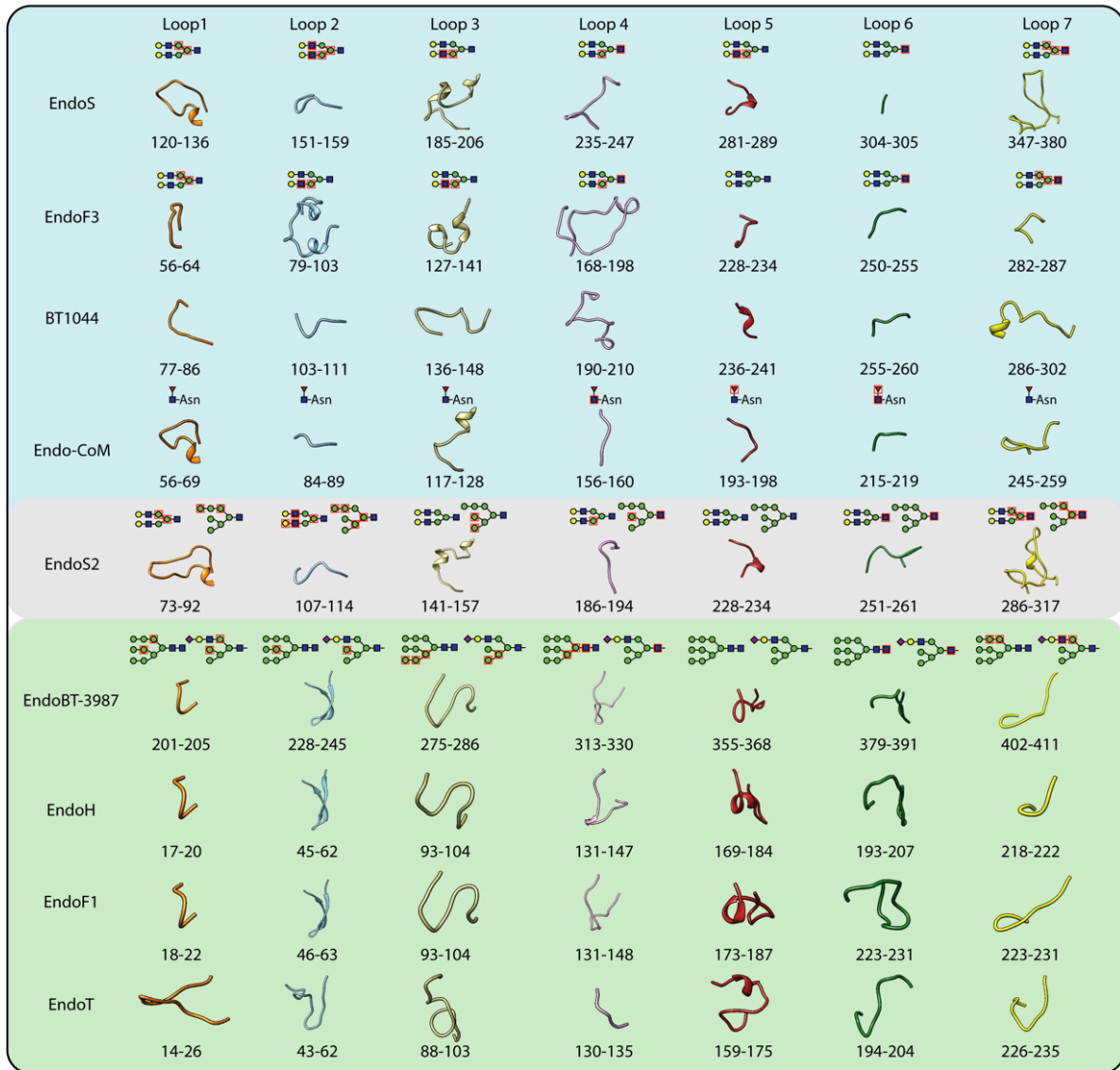
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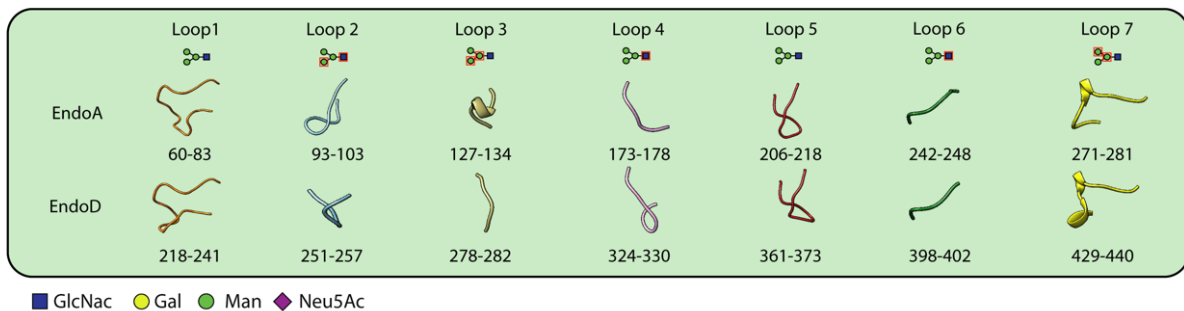
Supplementary Figure 1: Structural basis of ENGases specificity for *N*-glycans.

Supplementary Figure 2: Schematic representation of the binding site of GH18 ENGases.

A



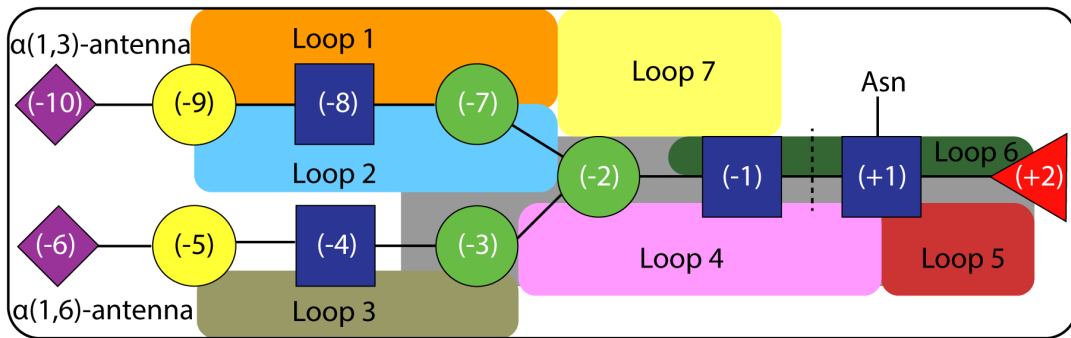
B



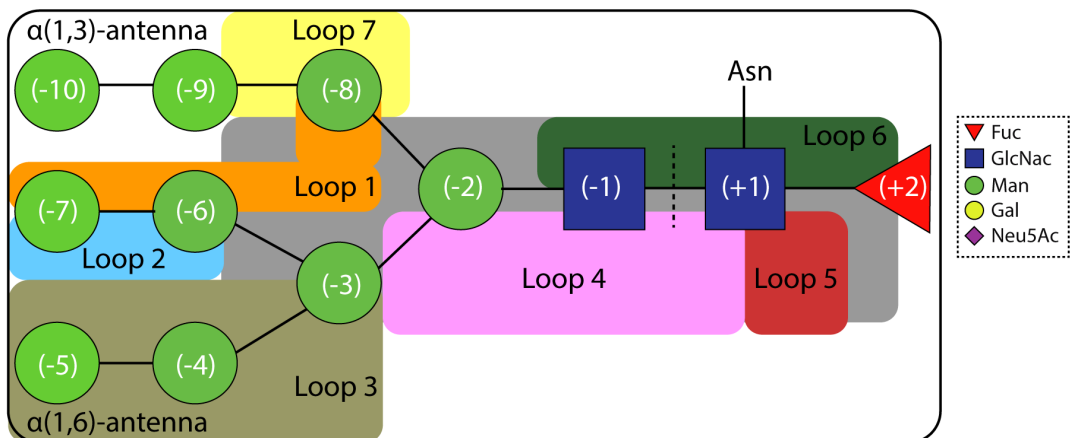
Supplementary Fig 1 | Structural basis of ENGases specificity for N-glycans. **A** Structural comparison of the loops surrounding the active site of GH18 family enzymes with ENGase activity and known X-ray crystal structure: EndoS (PDB code 6EN3), EndoF3 (1EOM), BT1044 (PDB code 6Q64), Endo-CoM (6KPN), EndoS2 (PDB codes 6MDS and 6MDV), EndoBT-3987 (PDB codes 6T8K and 7NWF), EndoH (PDB code 1C3F), EndoF1 (PDB code 2EBN) and EndoT (PDB code 4AC1). **B** Structural comparison of the loops surrounding the

active site of GH85 family enzymes with ENGase activity and known X-ray crystal structure: EndoA (PDB code 3FHA) and EndoD (PDB code 2W92). Enzymes that hydrolyze CT- and HM-type *N*-glycans are highlighted in blue and green, respectively and EndoS2 that is able to hydrolyze CT-, HM- and Hy-type *N*-glycans is highlighted in grey. Carbohydrate moieties that interact with each loop in the crystal structures are marked with red squares.

A



B



Supplementary Fig 2 | Schematic representation of the binding site of GH18 ENGases that hydrolyze CT-type (A) and HM-type (B) *N*-glycans. The loops and the β -barrel that interact with the glycan are highlighted in different colors and grey, respectively.