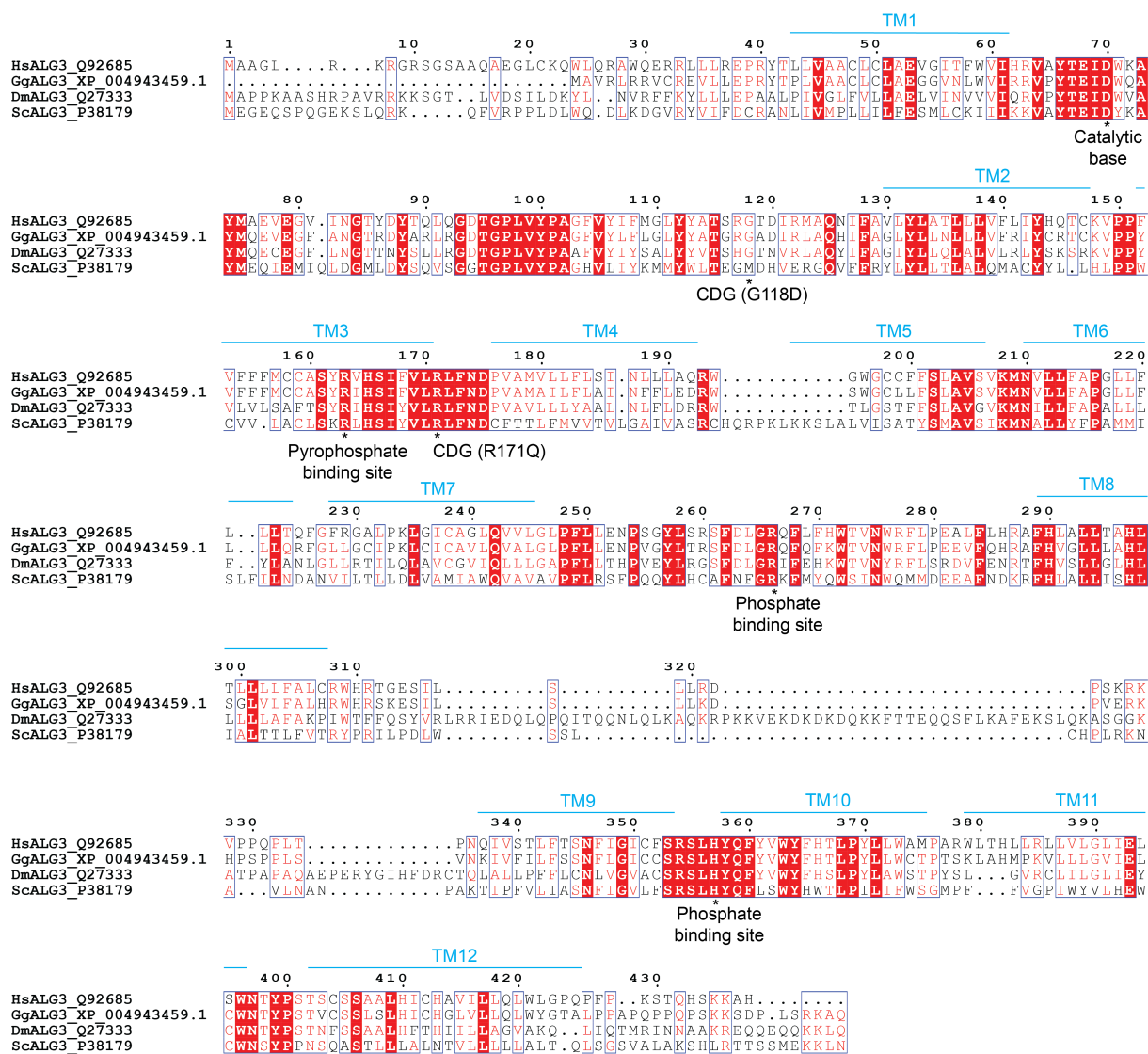
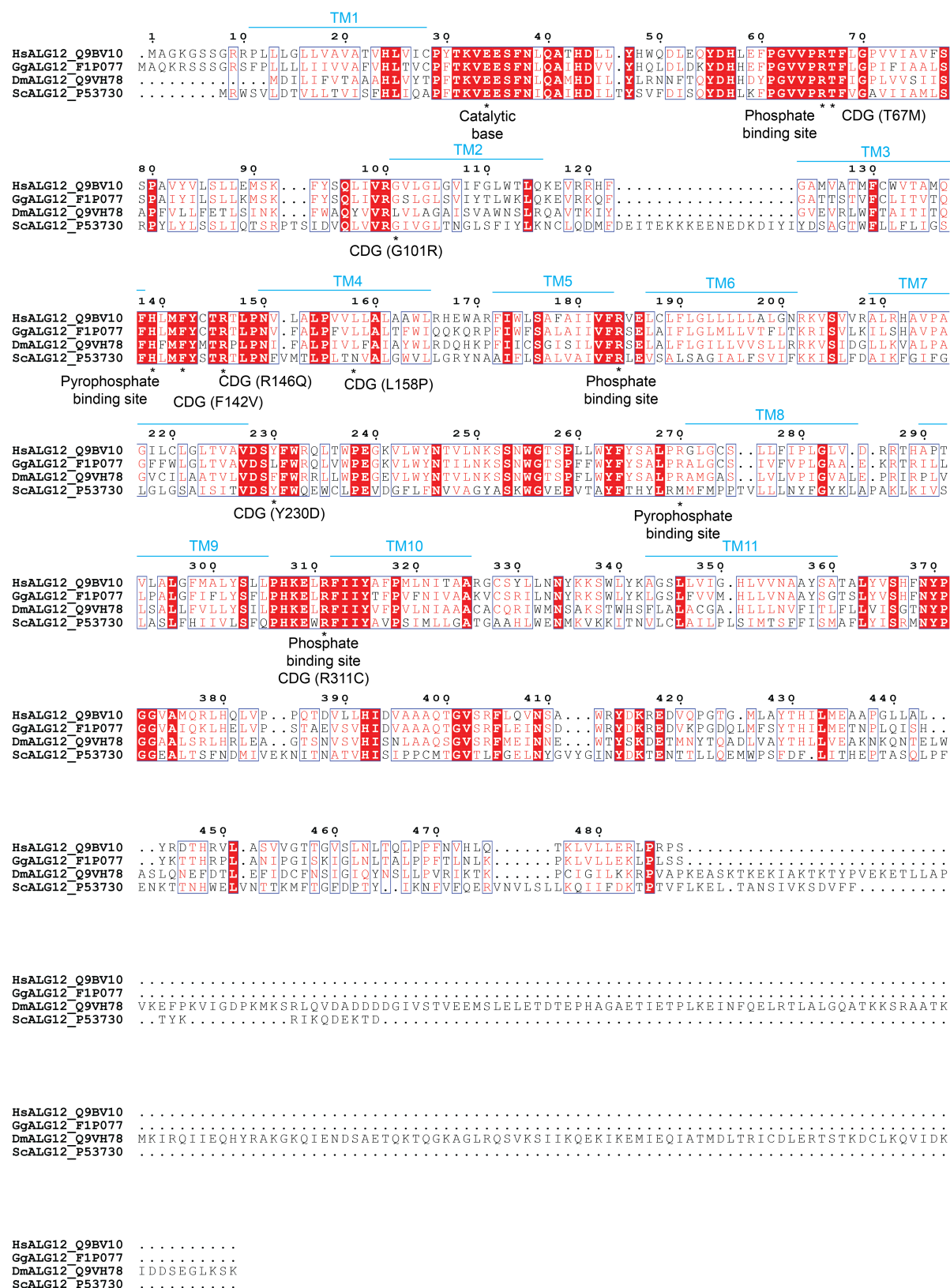


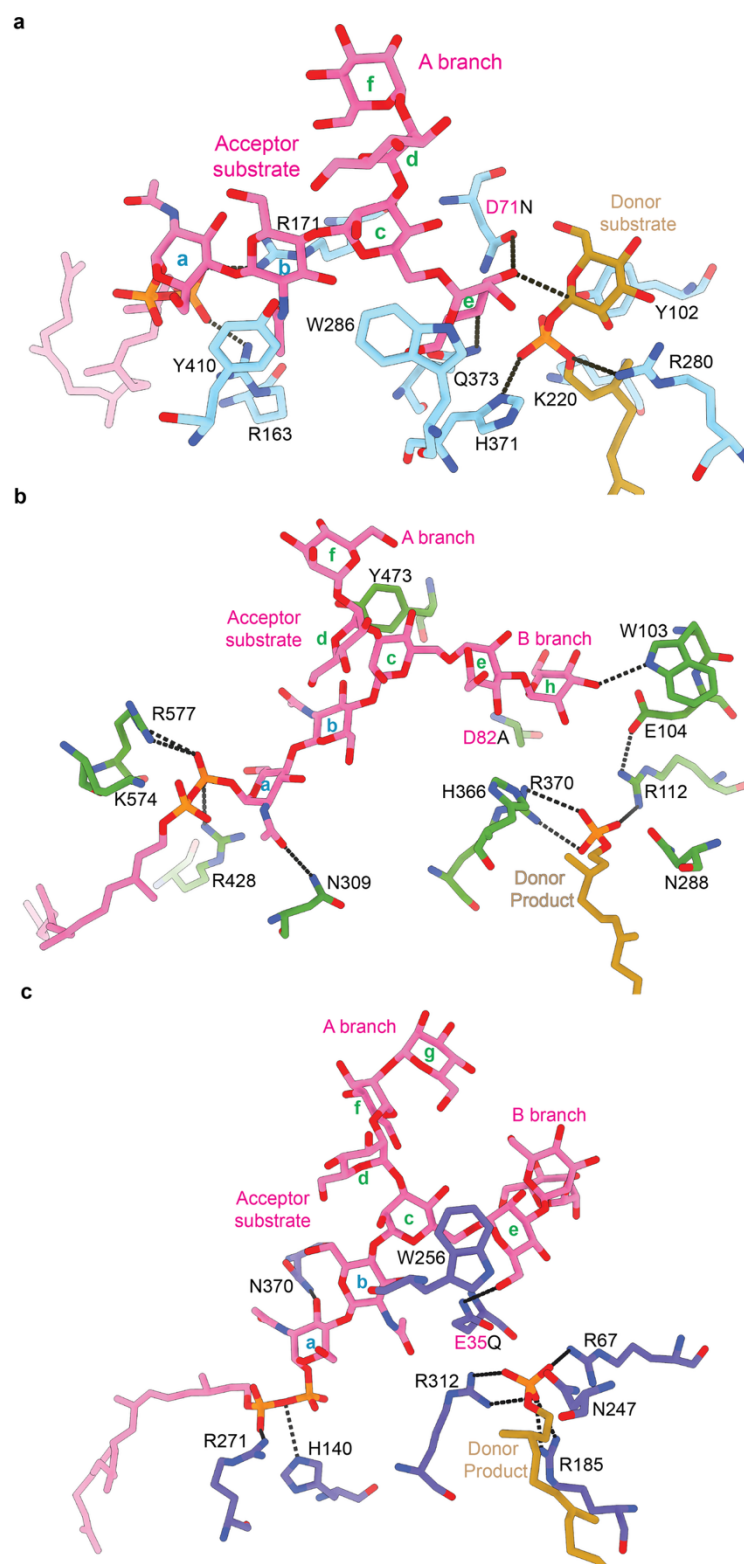
Structures of ALG3/9/12 reveal the assembly logic of the *N*-glycan oligomannose core

In the format provided by the
authors and unedited

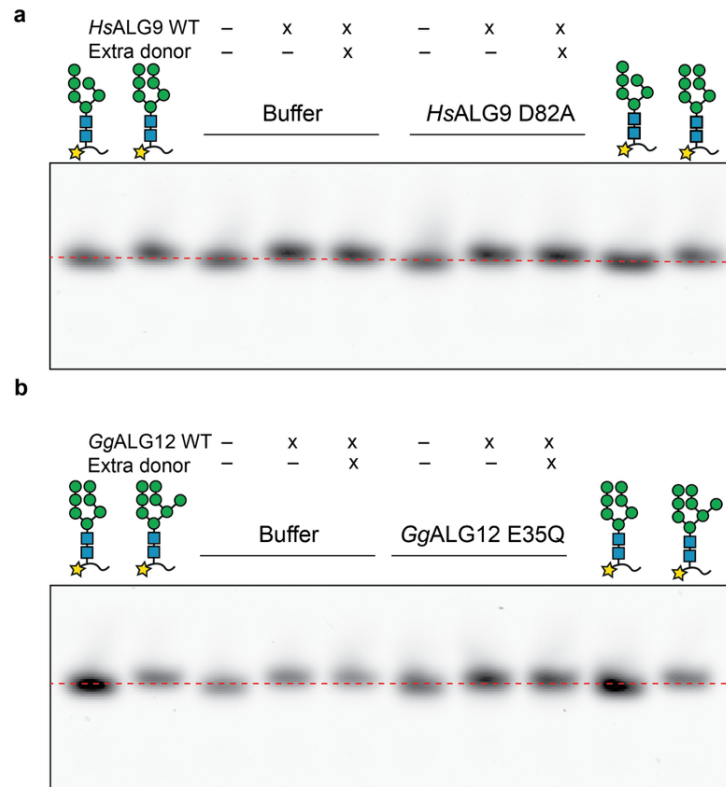


Supplementary Figure 1 | Homologue protein sequence alignments of ALG3 from *Homo sapiens*, *Gallus gallus*, *Drosophila melanogaster* and *Saccharomyces cerevisiae*. The catalytic base, substrate pyrophosphate and phosphate binding residues are indicated along with residues reported to be involved in CDGs. See Extended Data Table 2 for references for the variants associated with CDGs. The protein names are followed by the accession number.

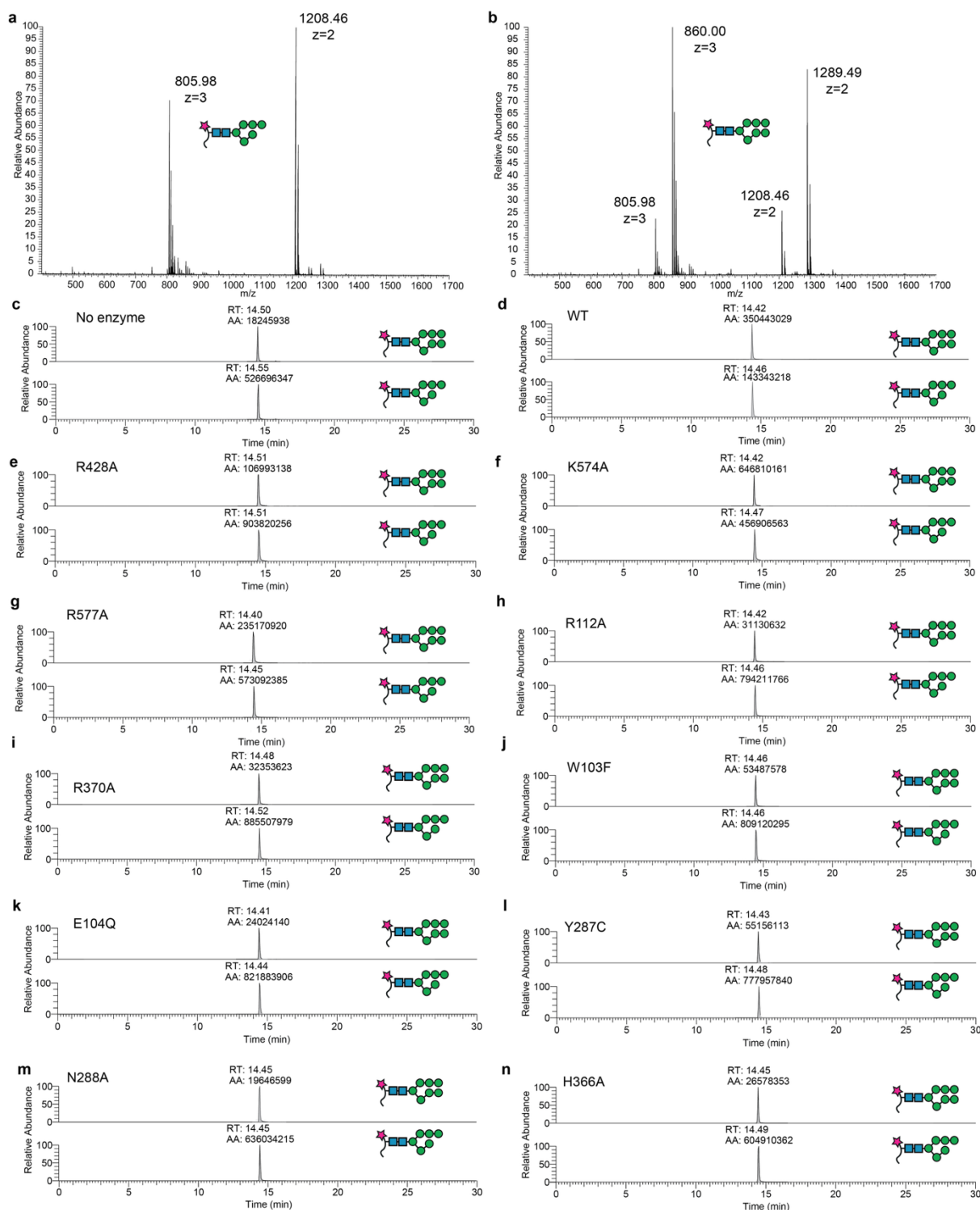




Supplementary Figure 4 | Close up of substrates and selected active site residues. a, *ScALG3*, b, *HsALG9* and c. *GgALG12* ternary complexes. Acceptor and donor substrates are depicted in pink and gold stick representation. Key residues are highlighted as sticks, and hydrogen bonding interactions are shown as dashed lines.



Supplementary Figure 5 | Assay to measure futile donor hydrolysis by *HsALG9* D82A or *GgALG12* E35Q. **a**, Assay with *HsALG9* D82A. **b**, Assay with *GgALG12* E35Q. After incubation of acceptor and donor substrates for 1 hour on ice as indicated above the gel, the samples were split into three and further processed with either buffer alone, WT enzyme or WT enzyme and fresh donor substrate as indicated above the gel. The reaction products were transferred to a fluorescent peptide (5-carboxyfluorescein-GSDAN^YTYTQ-NH₂) using *TbSTT3A* and separated by tricine gel electrophoresis. Lanes with glycopeptide cartoons over them serve as glycopeptide standards for the indicated glycan. Assays to measure donor hydrolysis were performed at least twice with similar results.



Supplementary Figure 6 | HRMS analysis of glycopeptides from the *HsALG9* activity assay. **a**, Representative LC-MS analysis of the GlcNac₂Man₆ glycopeptide. HRMS (m/z): [M+2H]²⁺ calcd. for C₁₀₃H₁₄₈N₁₂O₅₄²⁺, 1208.46; found, 1208.46. [M+3H]³⁺ calcd. for C₁₀₃H₁₄₉N₁₂O₅₄³⁺, 805.98; found, 805.98. **b**, Representative LC-MS analysis of the GlcNac₂Man₇ glycopeptide. HRMS (m/z): [M+2H]²⁺ calcd. for C₁₀₉H₁₅₈N₁₂O₅₉²⁺, 1289.49; found, 1289.49. [M+3H]³⁺ calcd. for C₁₀₉H₁₅₉N₁₂O₅₉³⁺, 859.99; found, 860.00. **c-n**, Extracted ion chromatograms for GlcNac₂Man₆ and GlcNac₂Man₇ glycopeptides from reactions with **c**, no enzyme, **d**, WT, **e**, R428A, **f**, K574A, **g**, R577A, **h**, R112A, **i**, R370A, **j**, W103A, **k**, E104Q, **l**, Y287C, **m**, N288A and **n**, H366A samples. The analyte areas for the GlcNac₂Man₆ (acceptor) and GlcNac₂Man₇ (product) glycopeptide are indicated on the spectra.

Supplementary Fig. 5a



Supplementary Fig. 5b



Supplementary Figure 7 | Unprocessed gels for Supplementary Figure 5.