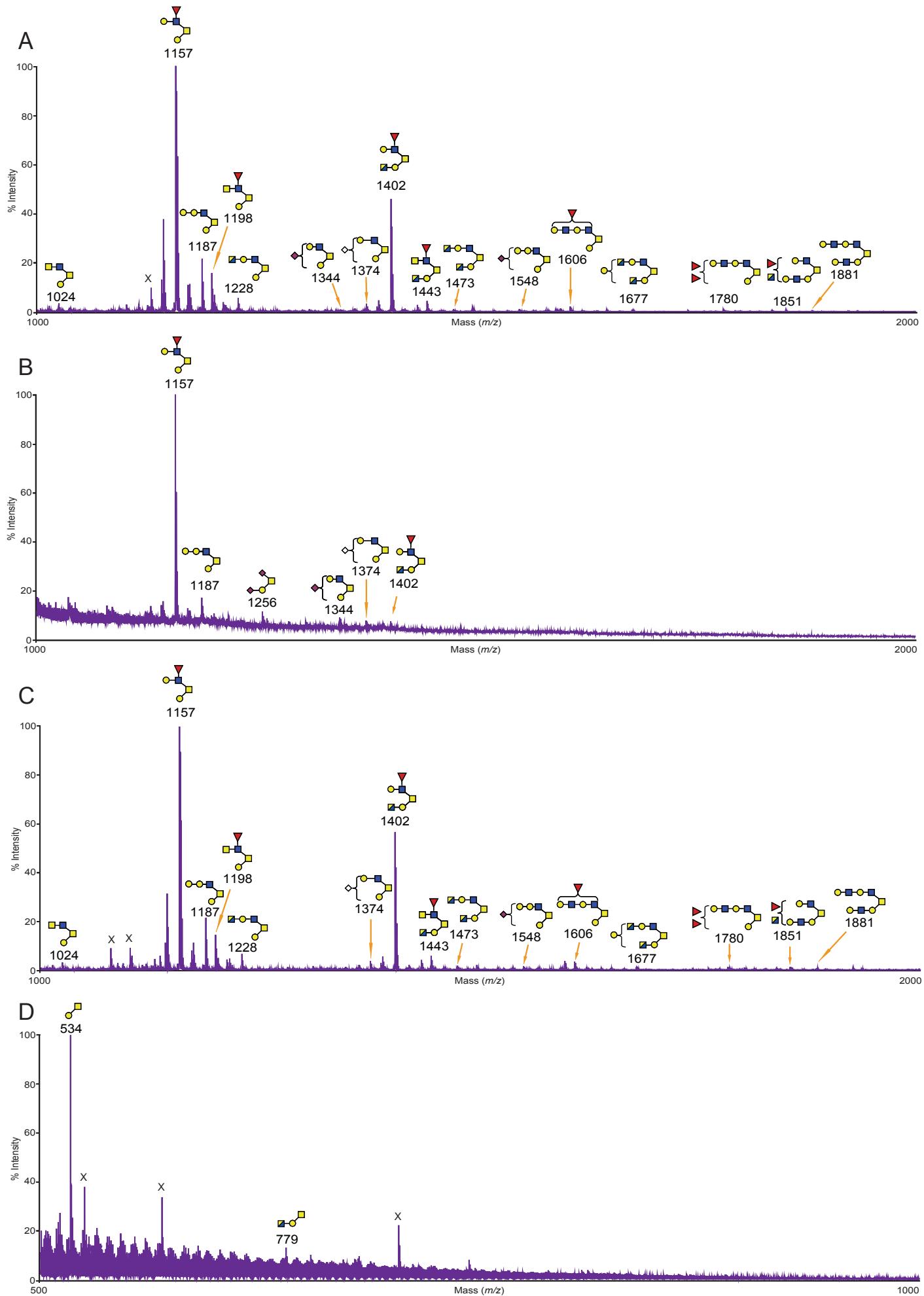


# **Supplementary Figures**

## **1 - 14**

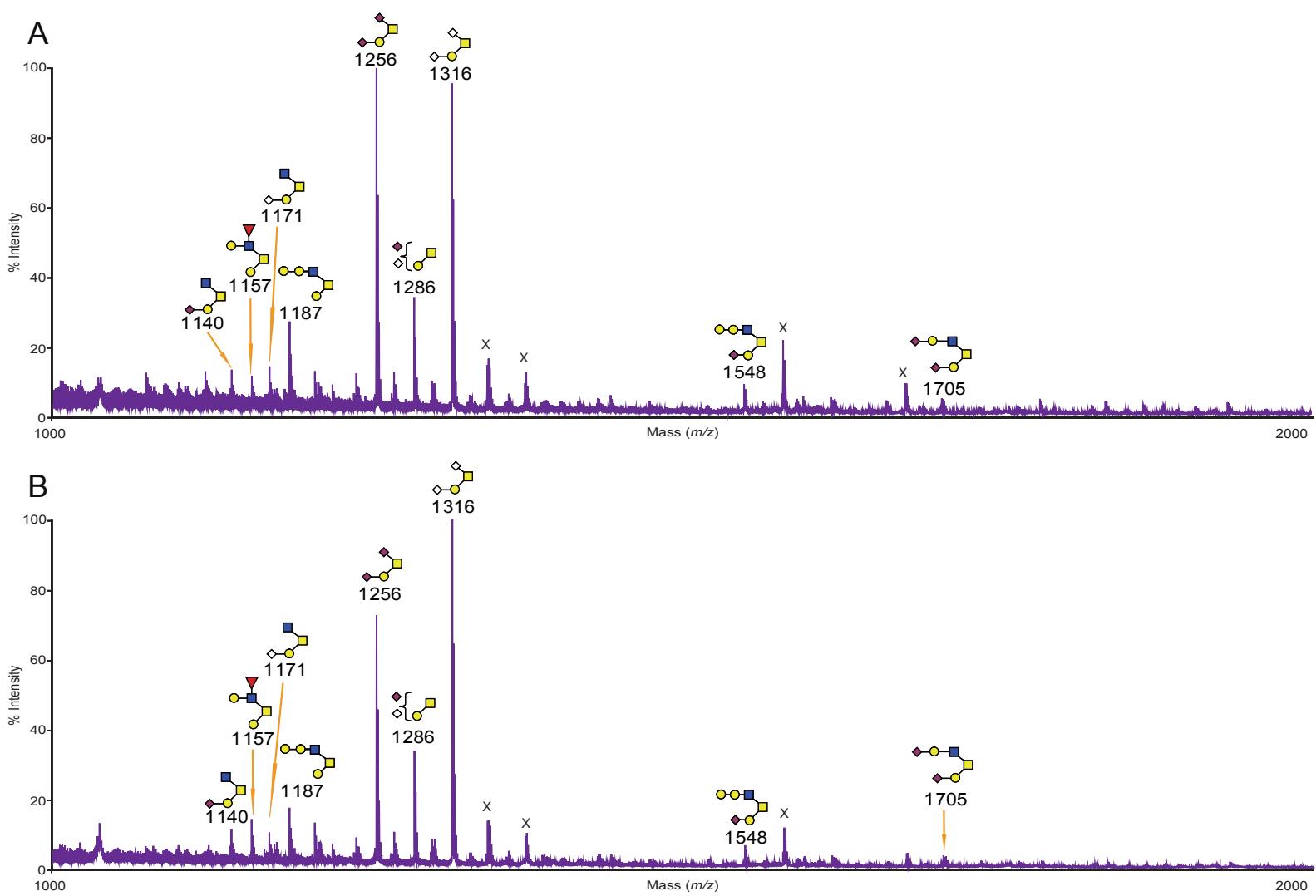
High Sensitivity O-glycomic Analysis of Mice Deficient  
in Core 2  $\beta$ 1,6-N-acetylglucosaminyltransferases

Ismail et al. 2010  
Glycobiology



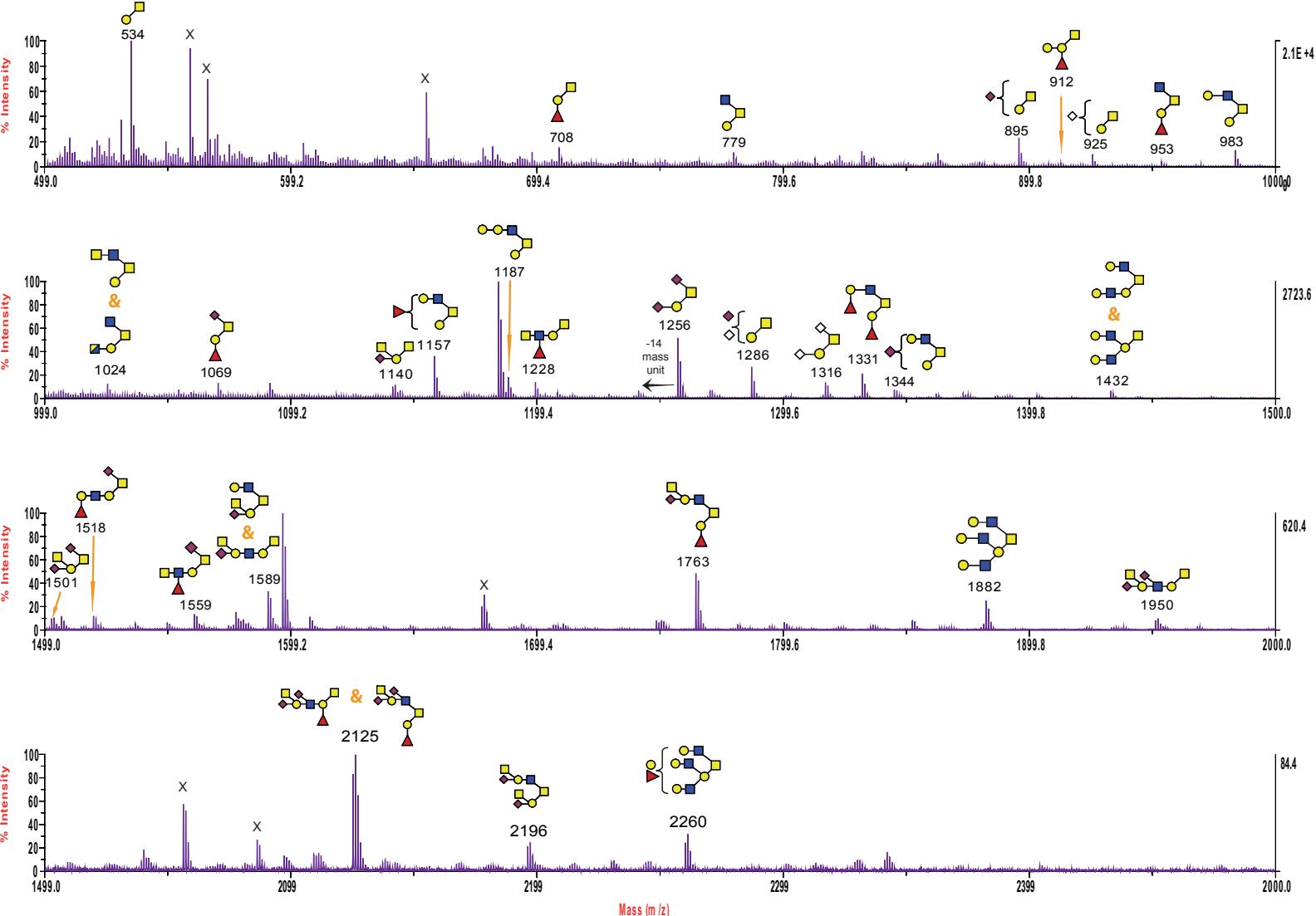
**Supplementary Figure 1. MALDI-TOF-MS spectra of O-glycans from the kidneys of wild type and knockout mice.** The glycomic profiles of reduced and permethylated O-glycans  $[M+Na]^+$  detected in the kidneys of (A) wild type, (B) C2GnT2 knockout, (C) C2GnT3 knockout and (D) C2GnT triple knockout mice. Cartoon structures assigned were based on mass spectrometric data and knowledge of biosynthetic pathways for the selected range of  $m/z$  1000-2000 except for (D),  $m/z$  500-1000. Only two glycan structures were detected in (D). X; impurities.

Key: ■ GalNAc ■ GlcNAc ■ HexNAc ■ Gal ■ Man ■ Fuc ■ NeuAc ■ NeuGc



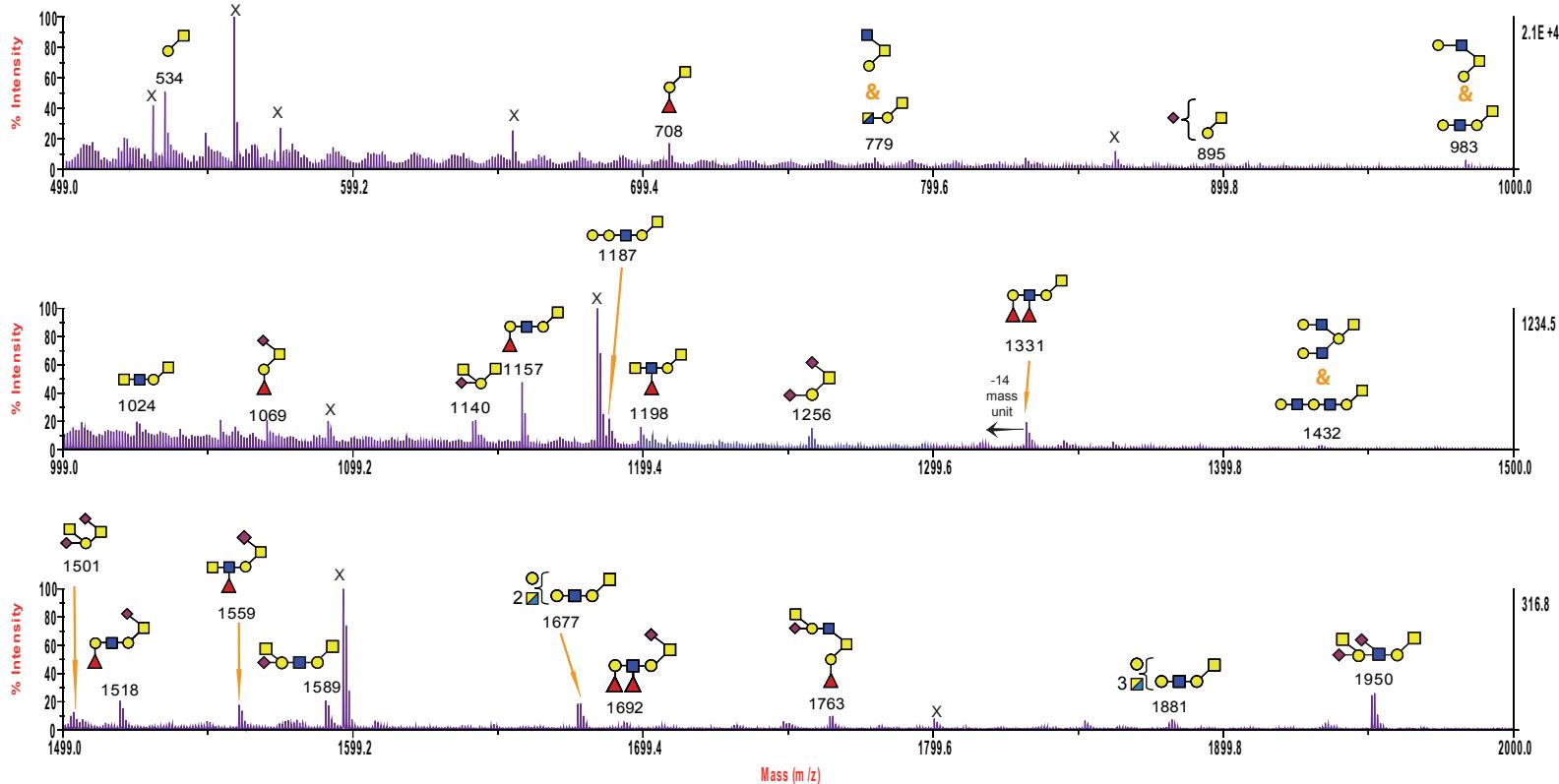
**Supplementary Figure 2. MALDI-TOF-MS spectra of O-glycans from the thymus of wild type and knockout mice.** The glycomic profiles of reduced and permethylated O-glycans  $[M+Na]^+$  detected in the thymus of (A) wild type and (B) C2GnT3 knockout mice. Cartoon structures assigned were based on mass spectrometric data and knowledge of biosynthetic pathways for the selected range of  $m/z$  1000-2000. X; impurities.

Key: ■ GalNAc ■ GlcNAc ■ HexNAc ○ Gal ● Man ▲ Fuc ♦ NeuAc ◇ NeuGc



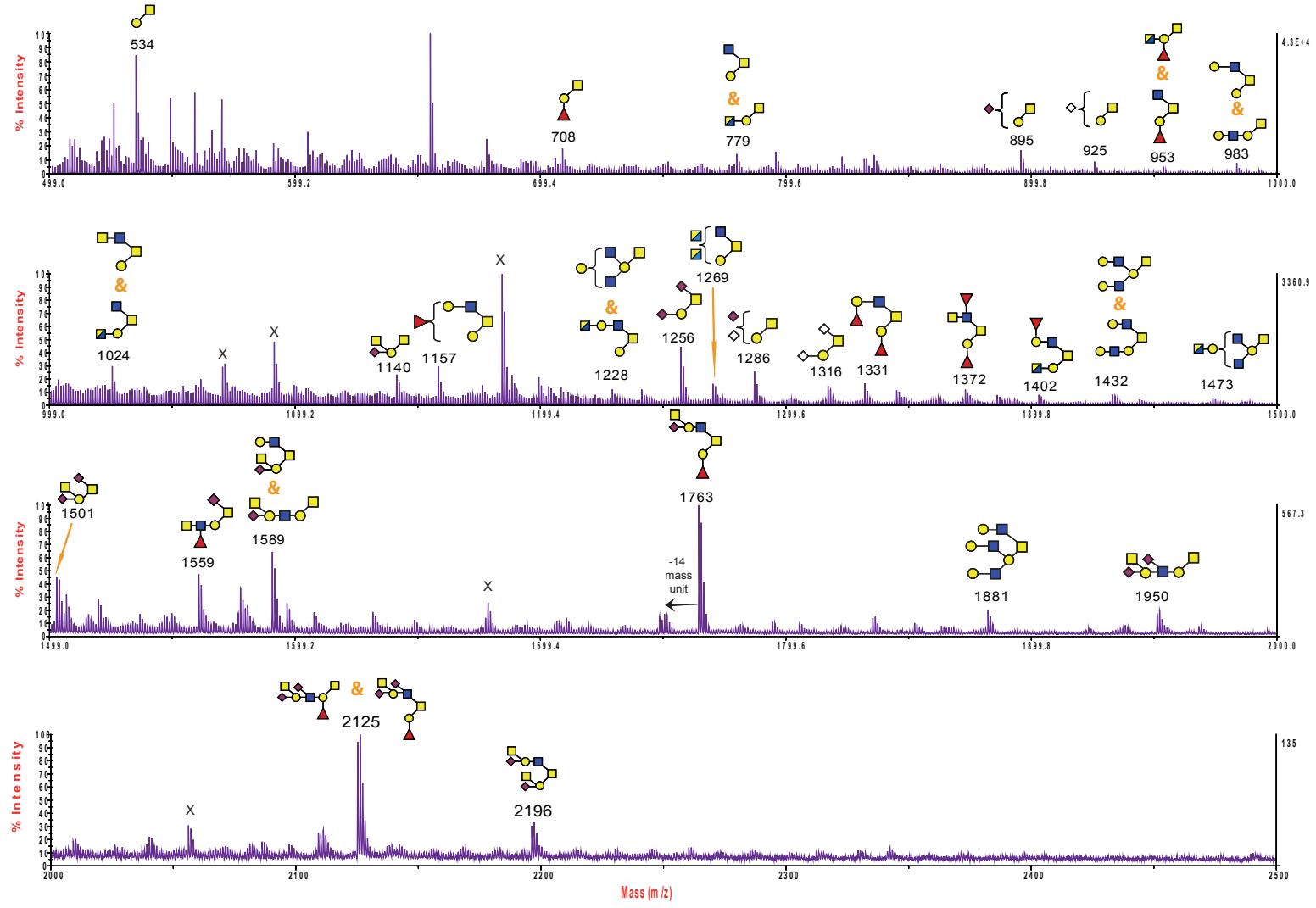
**Supplementary Figure 3. The full range MALDI-TOF-MS spectra of permethylated O-glycans from the colon of wild type mice.** Data were acquired in the positive ion mode  $[M+Na]^+$ . Cartoon structures assigned were based on mass spectrometric data and knowledge of O-glycan biosynthetic pathways. For high-mass glycans especially  $m/z$  above 2000, only the major sequences are annotated. The black arrow showing signal with 14 mass unit lower than the glycan at  $m/z$  1256 corresponds to the underpermethylation of that glycan. All unannotated signals with similar interval from annotated glycan peaks correspond to their respective underpermethylated form. X; impurities.

Key: ■ GalNAc ■ GlcNAc ■ HexNAc ○ Gal ● Man ▲ Fuc ♦ NeuAc ◇ NeuGc



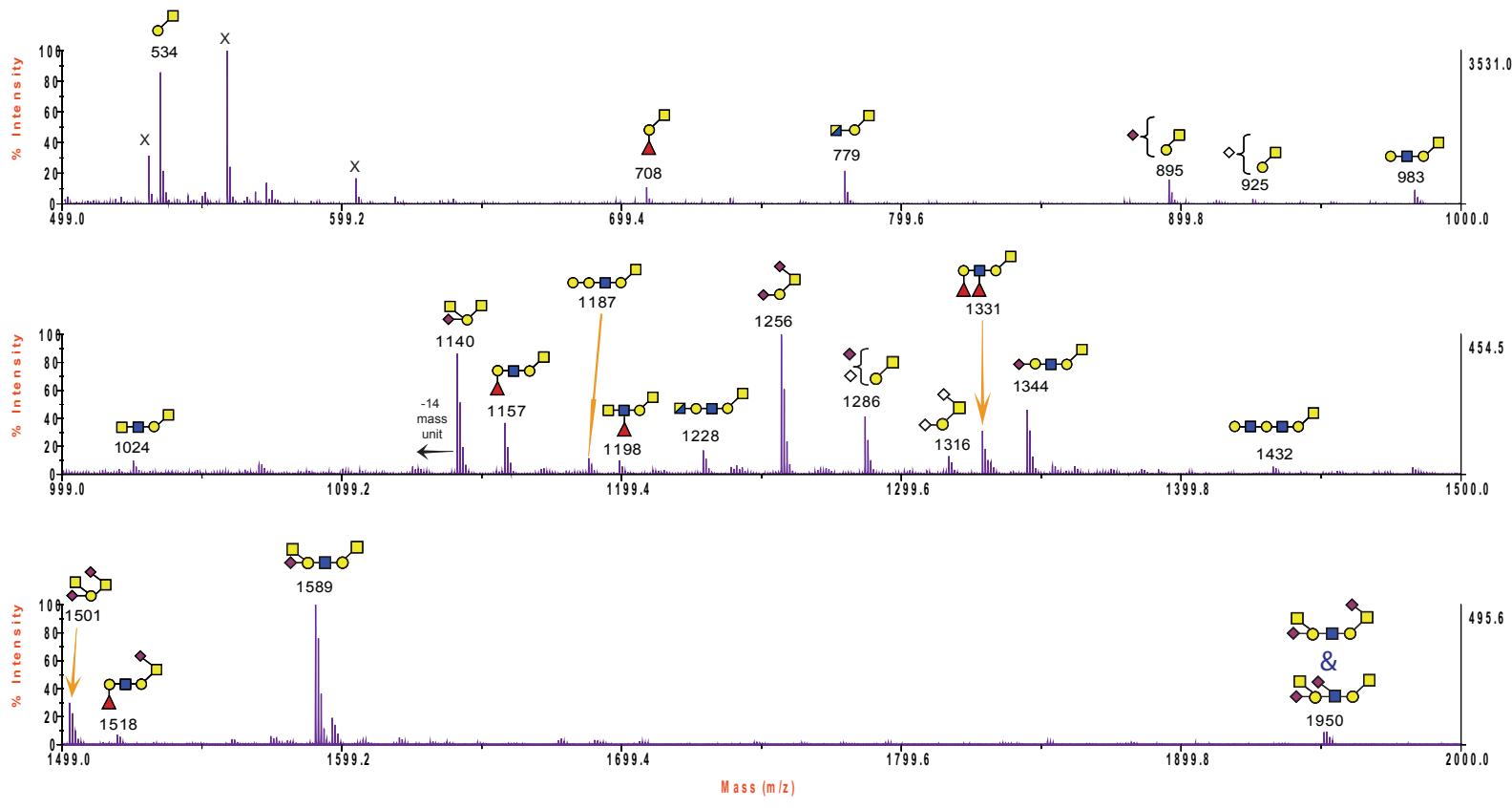
**Supplementary Figure 4. The full range MALDI-TOF-MS spectra of permethylated O-glycans from the colon of C2GnT2 knockout mice.** Data were acquired in the positive ion mode  $[M+Na]^+$ . Cartoon structures assigned were based on mass spectrometric data and knowledge of O-glycan biosynthetic pathways. The black arrow showing signal with 14 mass unit lower than the glycan at  $m/z$  1331 corresponds to the underpermethylation of that glycan. All unannotated signals with similar interval from annotated glycan peaks correspond to their respective underpermethylated form. X; impurities.

Key: ■ GalNAc ■ GlcNAc ■ HexNAc ○ Gal ● Man ▲ Fuc ♦ NeuAc ◇ NeuGc



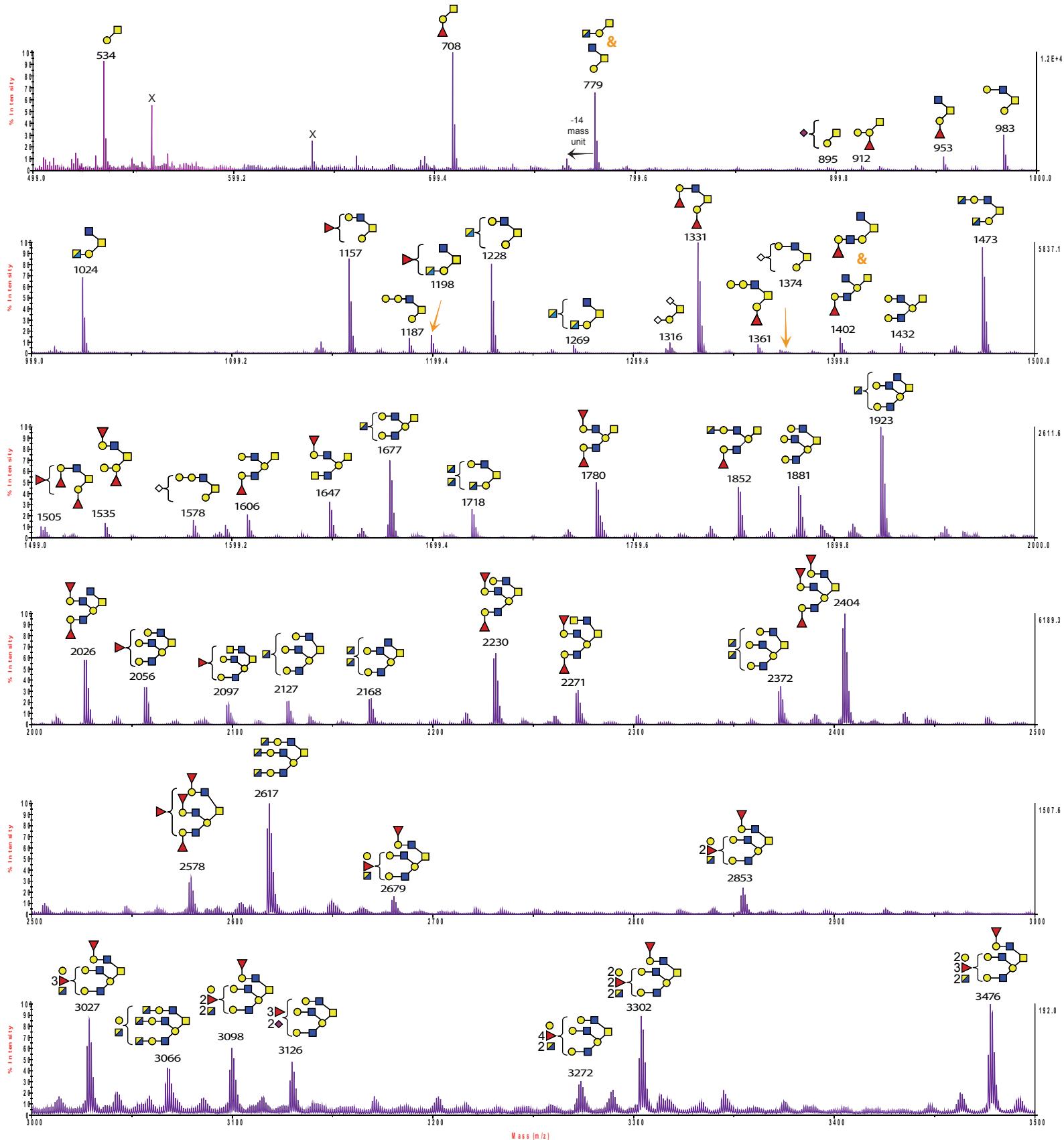
**Supplementary Figure 5. The full range MALDI-TOF-MS spectra of permethylated O-glycans from the colon of C2GnT3 knockout mice.** Data were acquired in the positive ion mode  $[M+Na]^+$ . Cartoon structures assigned were based on mass spectrometric data and knowledge of O-glycan biosynthetic pathways. For high-mass glycans especially  $m/z$  above 2000, only the major sequences are annotated. The black arrow showing signal with 14 mass unit lower than the glycan at  $m/z$  1763 corresponds to the underpermethylation of that glycan. All unannotated signals with similar interval from annotated glycan peaks correspond to their respective underpermethylated form. X; impurities.

Key: ■ GalNAc ■ GlcNAc ■ HexNAc ■ Gal ■ Man ■ Fuc ■ NeuAc ■ NeuGc



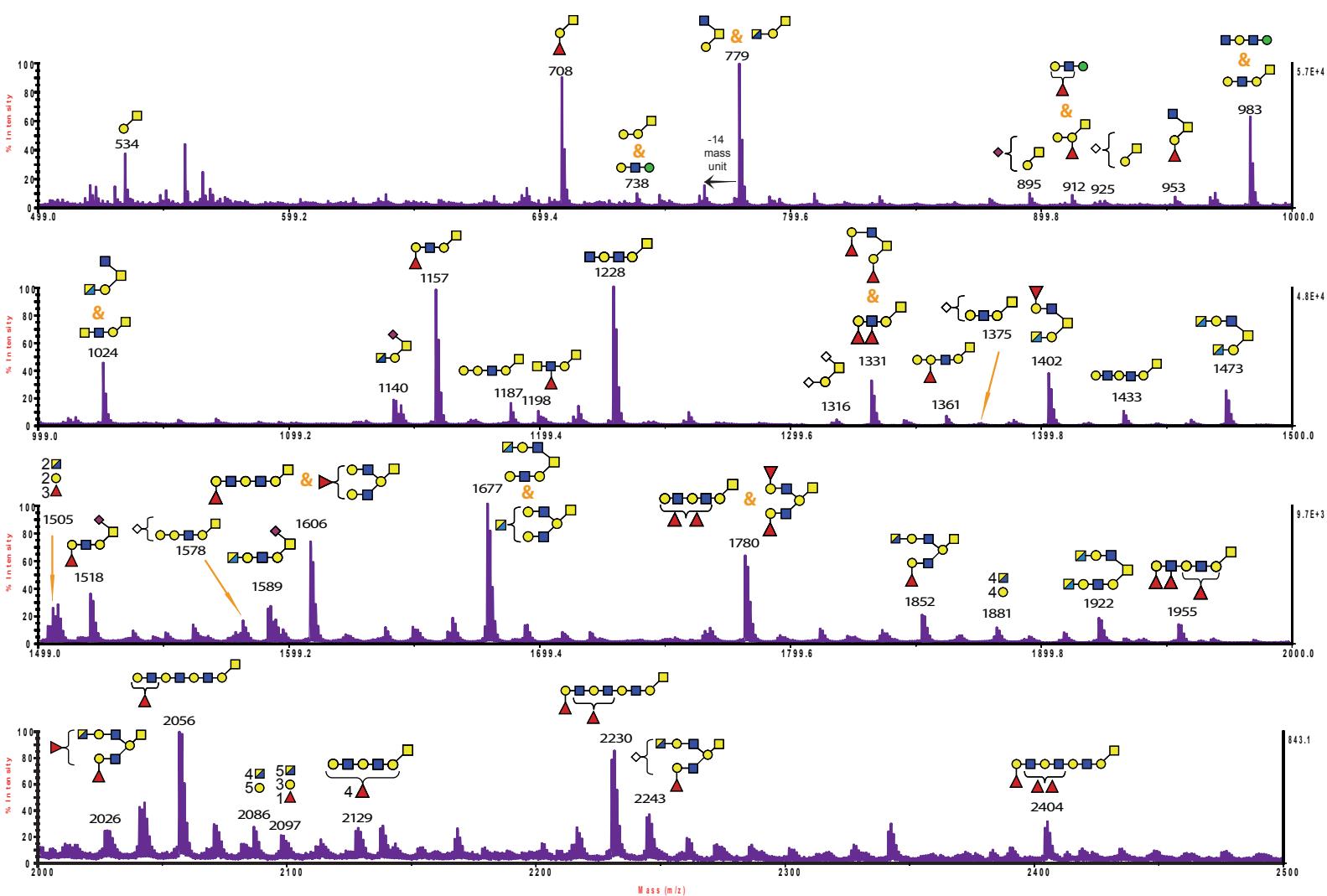
**Supplementary Figure 6. The full range MALDI-TOF-MS spectra of permethylated O-glycans from the colon of C2GnT triple knockout mice.** Data were acquired in the positive ion mode  $[M+Na]^+$ . Cartoon structures assigned were based on mass spectrometric data and knowledge of O-glycan biosynthetic pathways. The black arrow showing signal with 14 mass unit lower than the glycan at  $m/z$  1140 corresponds to the underpermethylation of that glycan. All unannotated signals with similar interval from annotated glycan peaks correspond to their respective underpermethylated form. X: impurities.

Key: ■ GalNAc ■ GlcNAc ■ HexNAc ○ Gal ● Man ▲ Fuc ♦ NeuAc ◇ NeuGc



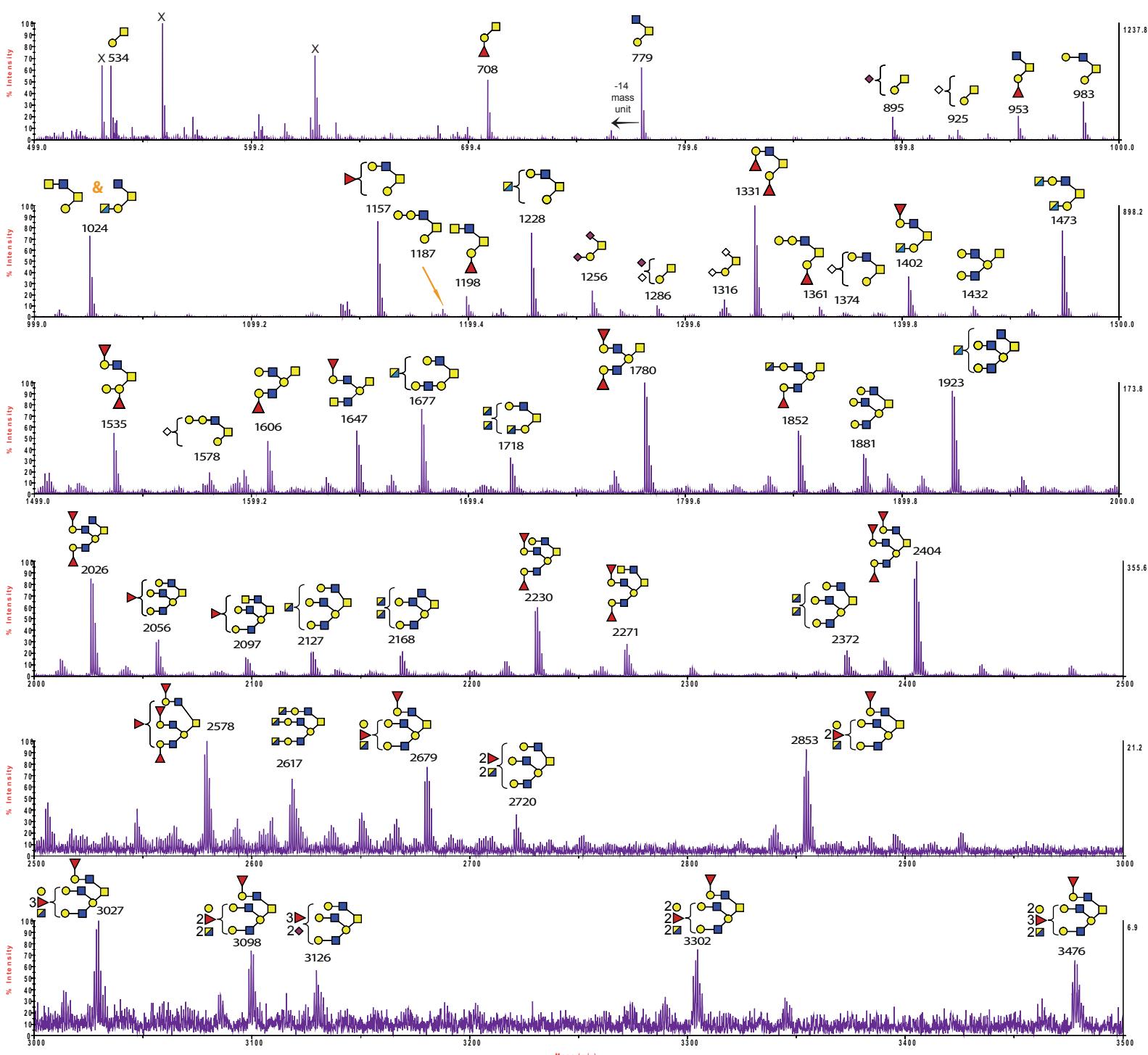
**Supplementary Figure 7. The full range MALDI-TOF-MS spectra of permethylated O-glycans from the stomach of wild type mice.** Data were acquired in the positive ion mode  $[M+Na]^+$ . Cartoon structures assigned were based on mass spectrometric data and knowledge of O-glycan biosynthetic pathways. For high-mass glycans especially  $m/z$  above 2000, only the major sequences are annotated. The black arrow showing signal with 14 mass unit lower than the glycan at  $m/z$  779 corresponds to the underpermethylation of that glycan. All unannotated signals with similar interval from annotated glycan peaks correspond to their respective underpermethylated form. X; impurities.

Key: ■ GalNAc ■ GlcNAc ■ HexNAc ■ Gal ■ Man ■ Fuc ■ NeuAc △ NeuGc



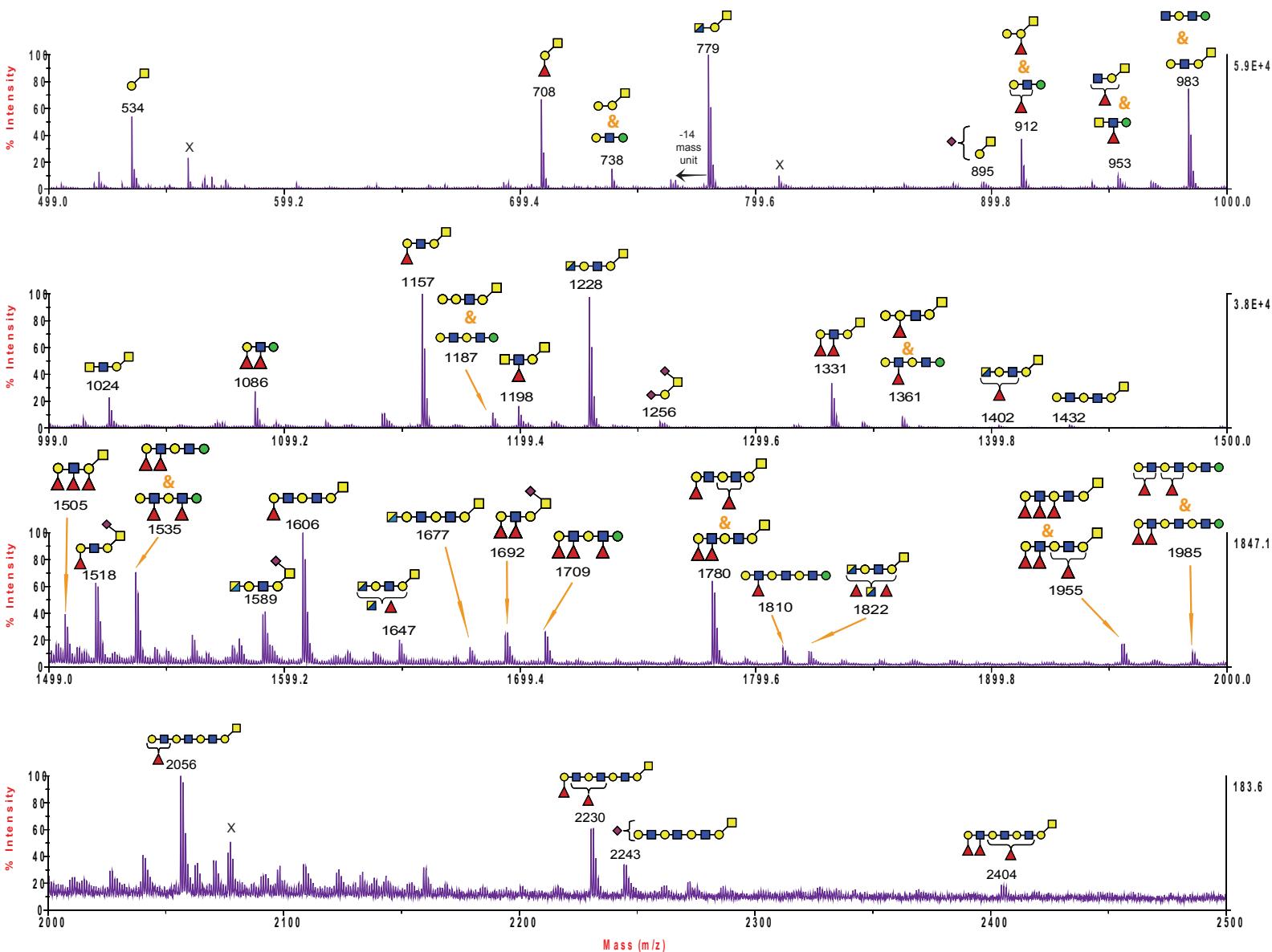
**Supplementary Figure 8. The full range MALDI-TOF-MS spectra of permethylated O-glycans from the stomach of C2GnT2 knockout mice.** Data were acquired in the positive ion mode  $[M+Na]^+$ . Cartoon structures assigned were based on mass spectrometric data and knowledge of O-glycan biosynthetic pathways. For high-mass glycans especially  $m/z$  above 2000, only the major sequences are annotated. The black arrow showing signal with 14 mass unit lower than the glycan at  $m/z$  779 corresponds to the underpermethylation of that glycan. All unannotated signals with similar interval from annotated glycan peaks correspond to their respective underpermethylated form. X; impurities.

Key: ■ GalNAc ■ GlcNAc ■ HexNAc ○ Gal ○ Man ▲ Fuc ♦ NeuAc ◇ NeuGc



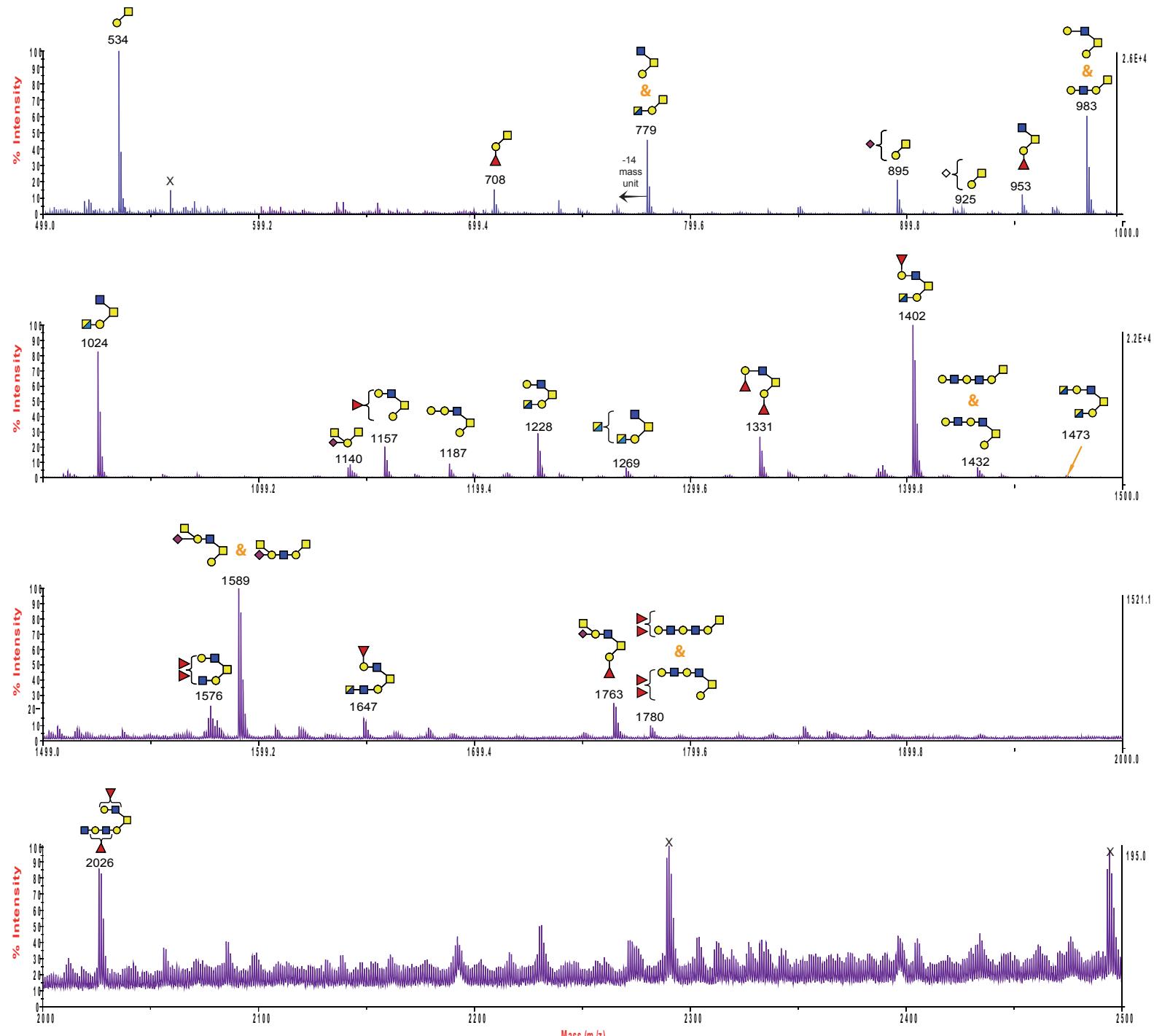
**Supplementary Figure 9. The full range MALDI-TOF-MS spectra of permethylated O-glycans from the stomach of C2GnT3 knockout mice.** Data were acquired in the positive ion mode  $[M+Na]^+$ . Cartoon structures assigned were based on mass spectrometric data and knowledge of O-glycan biosynthetic pathways. For high-mass glycans especially  $m/z$  above 2000, only the major sequences are annotated. The black arrow showing signal with 14 mass unit lower than the glycan at  $m/z$  779 corresponds to the underpermethylation of that glycan. All unannotated signals with similar interval from annotated glycan peaks correspond to their respective underpermethylated form. X; impurities.

Key: ■ GalNAc ■ GlcNAc ■ HexNAc ■ Gal ■ Man ■ Fuc ■ NeuAc △ NeuGc



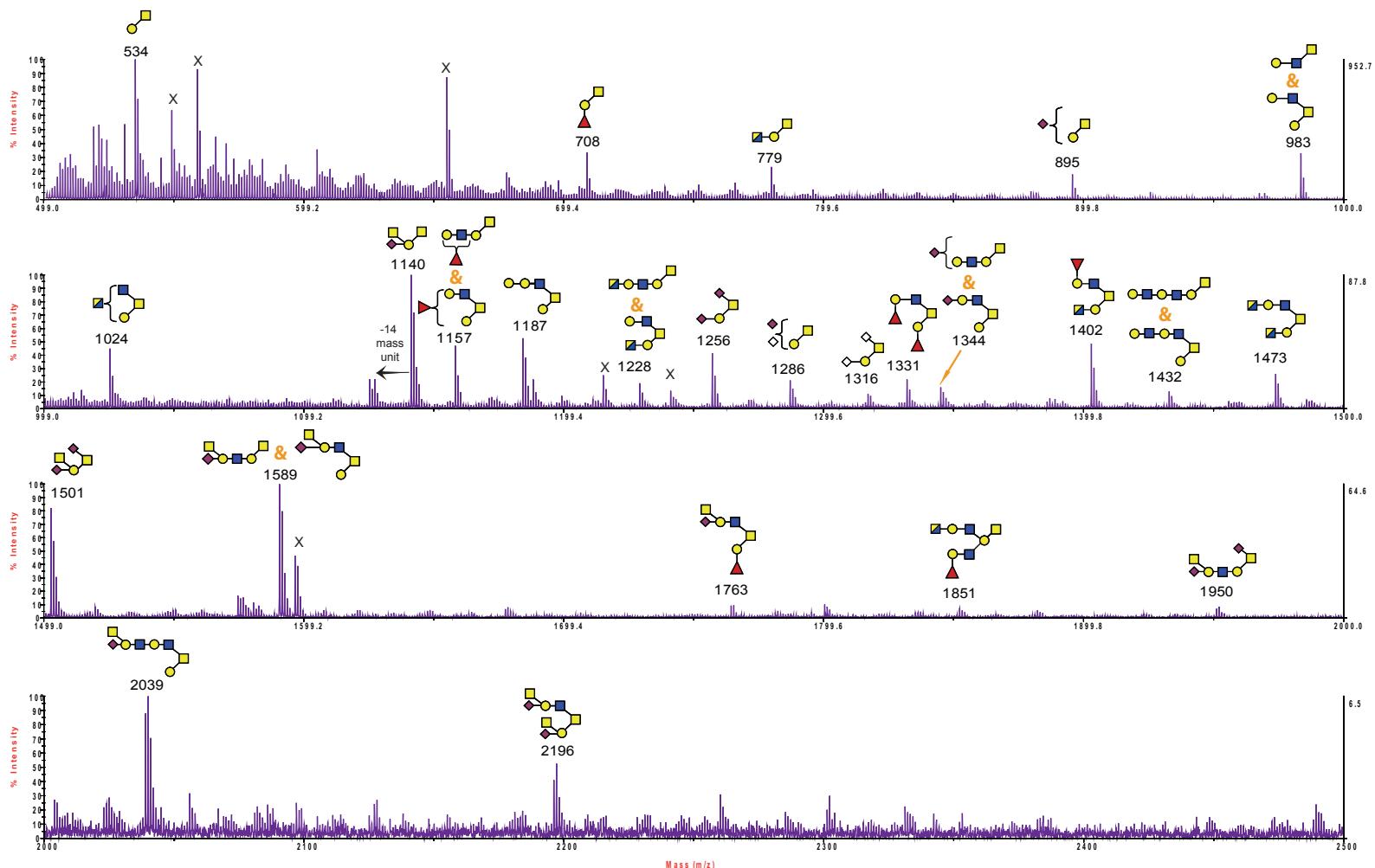
**Supplementary Figure 10. The full range MALDI-TOF-MS spectra of permethylated O-glycans from the stomach of C2GnT triple knockout mice.** Data were acquired in the positive ion mode  $[M+Na]^+$ . Cartoon structures assigned were based on mass spectrometric data and knowledge of O-glycan biosynthetic pathways. For high-mass glycans especially  $m/z$  above 2000, only the major sequences are annotated. The black arrow showing signal with 14 mass unit lower than the glycan at  $m/z$  779 corresponds to the underpermethylation of that glycan. All unannotated signals with similar interval from annotated glycan peaks correspond to their respective underpermethylated form. X; impurities.

Key: ■ GalNAc ■ GlcNAc ■ HexNAc ○ Gal ● Man ▲ Fuc ◆ NeuAc ◇ NeuGc



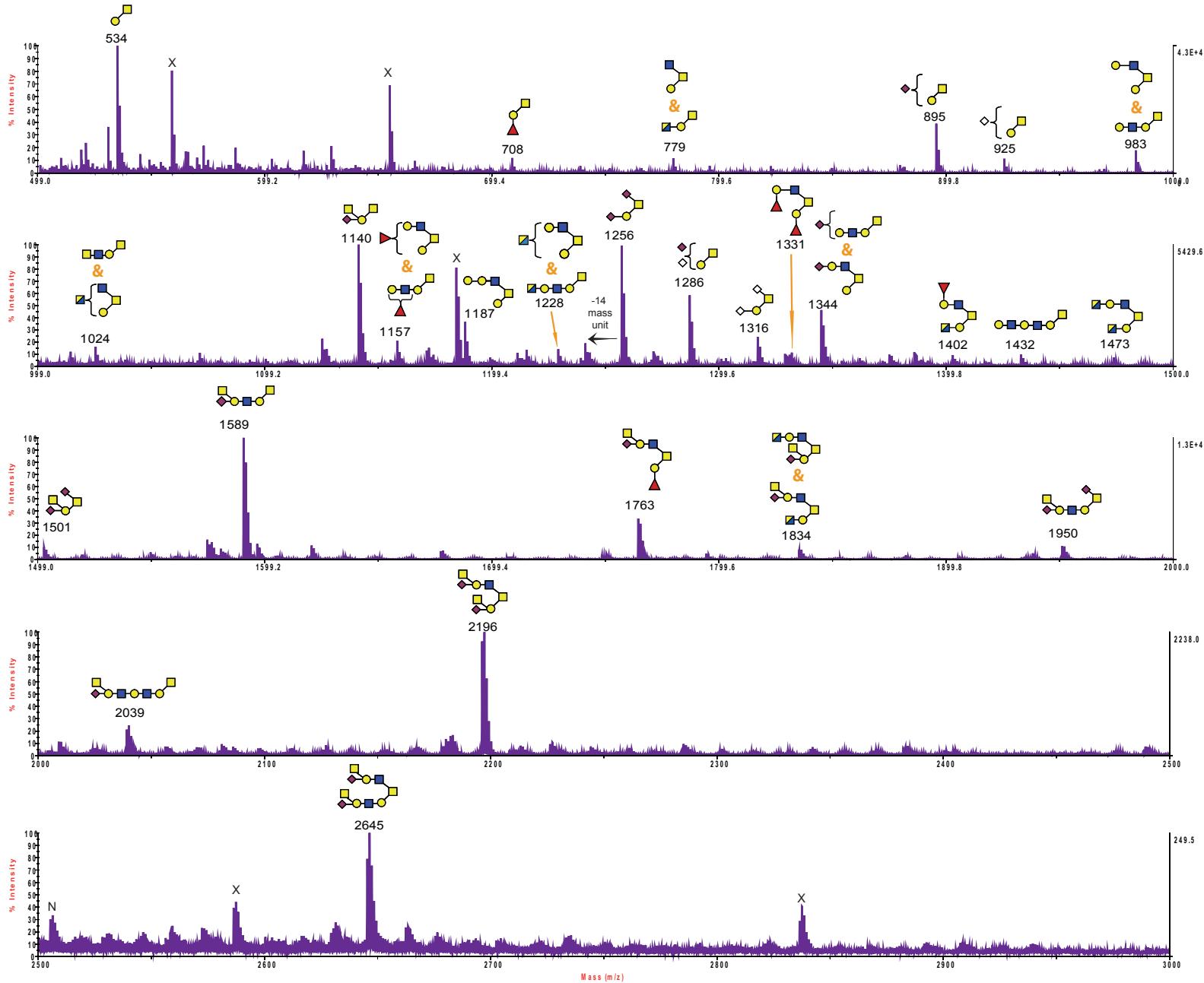
**Supplementary Figure 11. The full range MALDI-TOF-MS spectra of permethylated O-glycans from the small intestine of wild type mice.** Data were acquired in the positive ion mode  $[M+Na]^+$ . Cartoon structures assigned were based on mass spectrometric data and knowledge of O-glycan biosynthetic pathways. For high-mass glycans especially  $m/z$  above 2000, only the major sequences are annotated. The black arrow showing signal with 14 mass unit lower than the glycan at  $m/z$  779 corresponds to the underpermethylation of that glycan. All unannotated signals with similar interval from annotated glycan peaks correspond to their respective underpermethylated form. X; impurities.

Key: ■ GalNAc ■ GlcNAc ■ HexNAc ○ Gal ○ Man ▲ Fuc ♦ NeuAc ◇ NeuGc



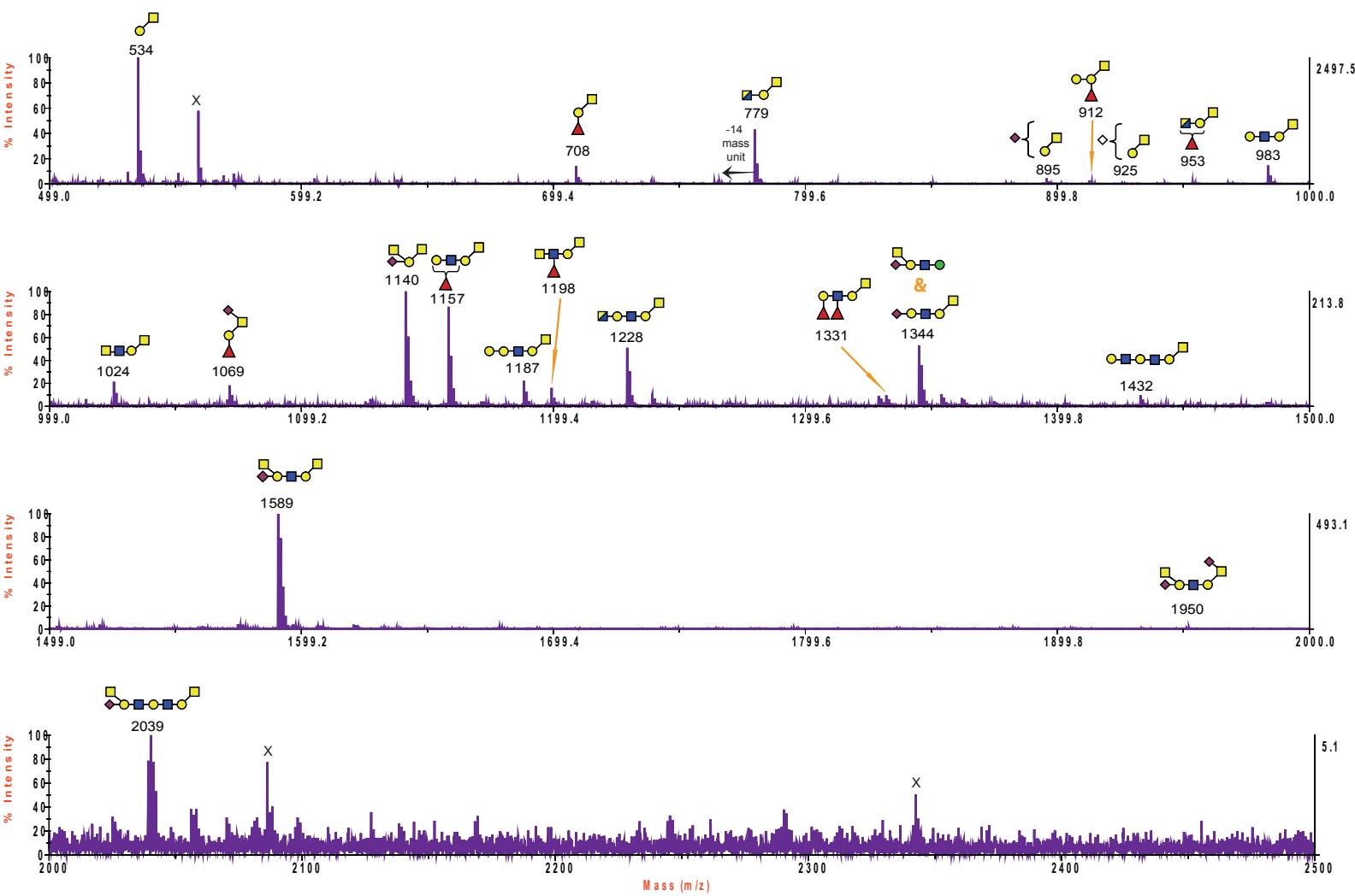
**Supplementary Figure 12. The full range MALDI-TOF-MS spectra of permethylated O-glycans from the small intestine of C2GnT2 knockout mice.** Data were acquired in the positive ion mode  $[M+Na]^+$ . Cartoon structures assigned were based on mass spectrometric data and knowledge of O-glycan biosynthetic pathways. For high-mass glycans especially  $m/z$  above 2000, only the major sequences are annotated. The black arrow showing signal with 14 mass unit lower than the glycan at  $m/z$  1140 corresponds to the underpermethylation of that glycan. All unannotated signals with similar interval from annotated glycan peaks correspond to their respective underpermethylated form. X: impurities.

Key: ■ GalNAc ■ GlcNAc ■ HexNAc ■ Gal ■ Man ■ Fuc ■ NeuAc △ NeuGc



**Supplementary Figure 13. The full range MALDI-TOF-MS spectra of permethylated O-glycans from the small intestine of C2GnT3 knockout mice.** Data were acquired in the positive ion mode  $[M+Na]^+$ . Cartoon structures assigned were based on mass spectrometric data and knowledge of O-glycan biosynthetic pathways. For high-mass glycans especially  $m/z$  above 2000, only the major sequences are annotated. The black arrow showing signal with 14 mass unit lower than the glycan at  $m/z$  1256 corresponds to the underpermethylation of that glycan. All unannotated signals with similar interval from annotated glycan peaks correspond to their respective underpermethylated form. X; impurities; N; residual N-glycans.

Key: ■ GalNAc ■ GlcNAc ▲ HexNAc ○ Gal ● Man ▲ Fuc ♦ NeuAc ◇ NeuGc



**Supplementary Figure 14. The full range MALDI-TOF-MS spectra of permethylated O-glycans from the small intestine of C2GnT triple knockout mice.** Data were acquired in the positive ion mode  $[M+Na]^+$ . Cartoon structures assigned were based on mass spectrometric data and knowledge of O-glycan biosynthetic pathways. For high-mass glycans especially  $m/z$  above 2000, only the major sequences are annotated. The black arrow showing signal with 14 mass unit lower than the glycan at  $m/z$  779 corresponds to the underpermethylation of that glycan. All unannotated signals with similar interval from annotated glycan peaks correspond to their respective underpermethylated form. X; impurities.

Key: ■ GalNAc ■ GlcNAc ■ HexNAc ○ Gal ● Man ▲ Fuc ♦ NeuAc ◇ NeuGc