Figure 2-Figure Supplement 7

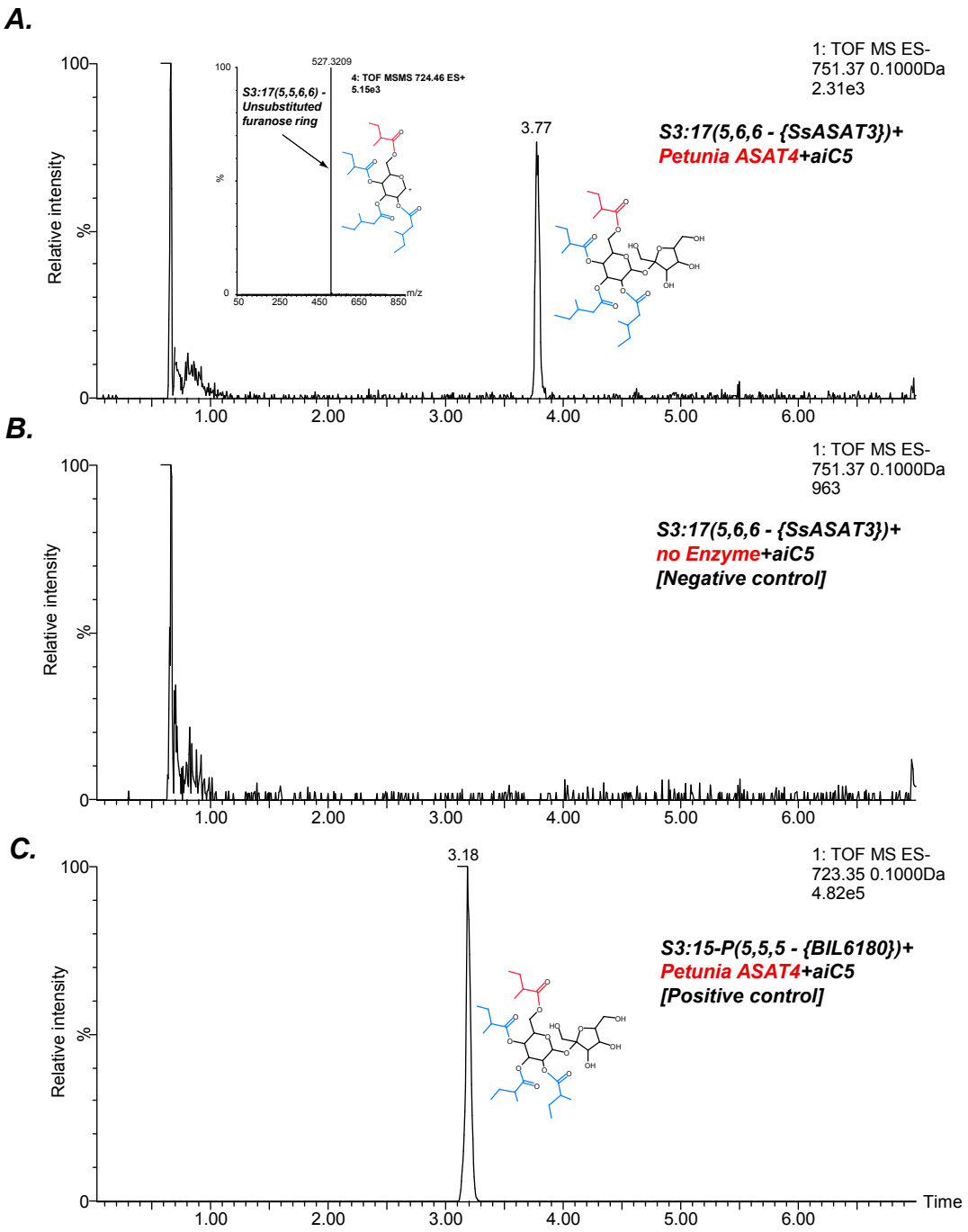

Figure 2-Figure Supplement 7: SsASAT3 acylates at the R3 position. An indirect assessment of SsASAT3 positional specificity using Petunia ASAT4, which acylates at the pyranose R6 position (A) The chromatogram shows that Petunia ASAT4, which is known to acylate at the R6 position, can acylate the tri-acylated sugar S3:17(5,6,6) produced by SsASAT3 using the SsASAT2 product S2:10(5,5) . The inset shows positive mode data suggesting all four chains are on the same ring. Given R6 is the only free hydroxyl group available on the pyranose ring in S3:17(5,6,6), and since SsASAT1 and SsASAT2 acylate R2 and R4 positions, the only position SsASAT3 can acylate without inhibiting PaASAT4 is R3. (B) Negative control with no enzyme (C) Positive control with S3:15-P(5,5,5) purified from BIL6180, with R2, R3 and R4 positions substituted by C5 chains based on NMR data (unpublished data).