Differential cleavage of LOX by BMP1 and ADAMTS2/14 regulates collagen binding through a tyrosine sulfate domain

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Supplementary Material

Supplementary Figures 1 to 5.

Supplementary Figure 1. Generation of HEK293 cells overexpressing ADAMTS2, ADAMTS3, ADAMTS14 and BMP1. Clones with inducible expression of the indicated proteases were incubated for 48 hours in the absence (basal) or presence of 1 μg/ml doxycycline (Dox). Secretion in the conditioned medium was evaluated by immunoblotting using specific antibodies. The blots shown correspond to representative experiments performed twice with two independent preparations.

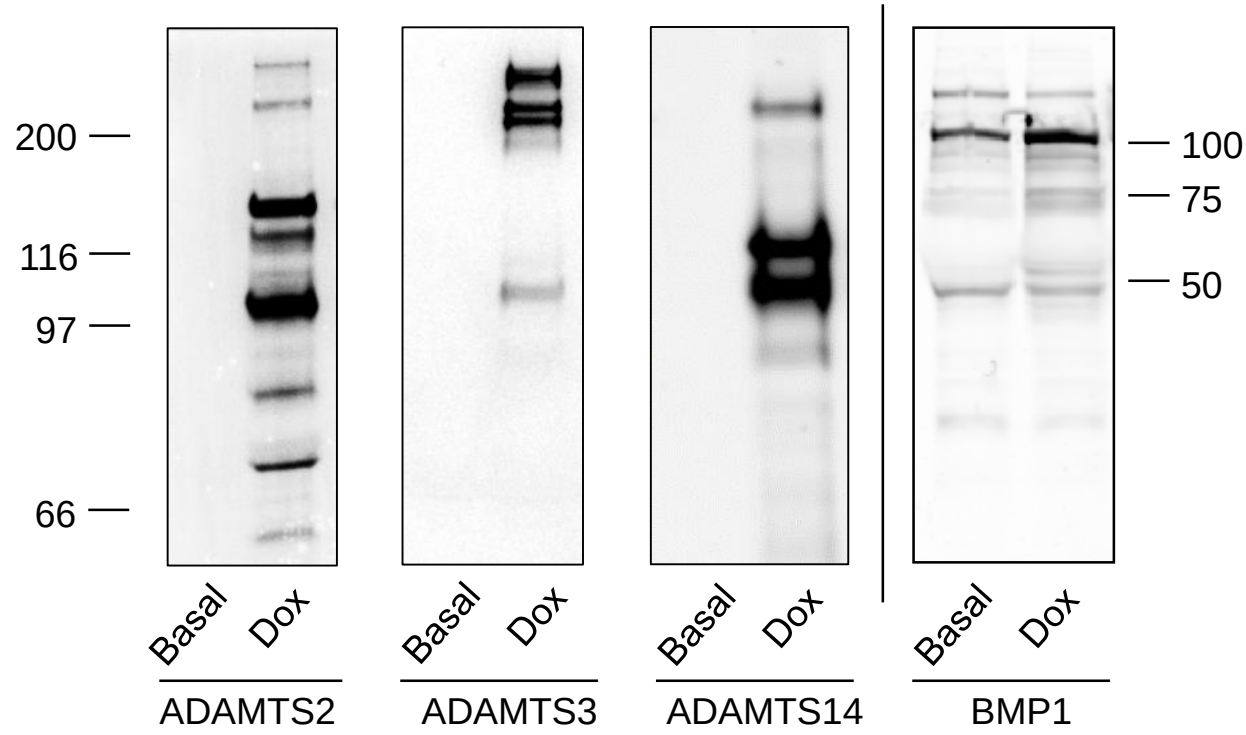
Supplementary Figure 2. Enrichment of supernatants treated with proteases in mature LOX forms for proteomic analysis. LOX-containing supernatants were treated with the indicated proteases for 2 hours and then filtered through a 50 kDa cutoff centrifugal filter. Aliquots of the inputs and the flow-through were fractioned by SDS-PAGE and stained with Coomassie Blue (upper panel) or analyzed by immunoblotting (lower panel) using an anti-LOX antibody. Stained gel and blot shown correspond to representative experiments performed twice with two independent preparations.

Supplementary Figure 3. 2D-electrophoresis/immunoblotting analysis of BMP1 and ADAMTS proteolysis of wild type and tyrosine mutant LOX constructs. Blots show either the precursor **(A)** or the forms processed by BMP1 **(B)** or ADAMTS2 **(C)** for wild type (WT) and mutant (Mut) LOX proteins. Note that isoelectric point (pI) of precursor and BMP1-processed forms shift to values significantly more basic in the mutant (red arrows), whereas it remains invariable for wild type and mutant upon ADAMTS2 cleavage (red arrowheads). The blots shown correspond to representative experiments performed twice with two independent preparations.

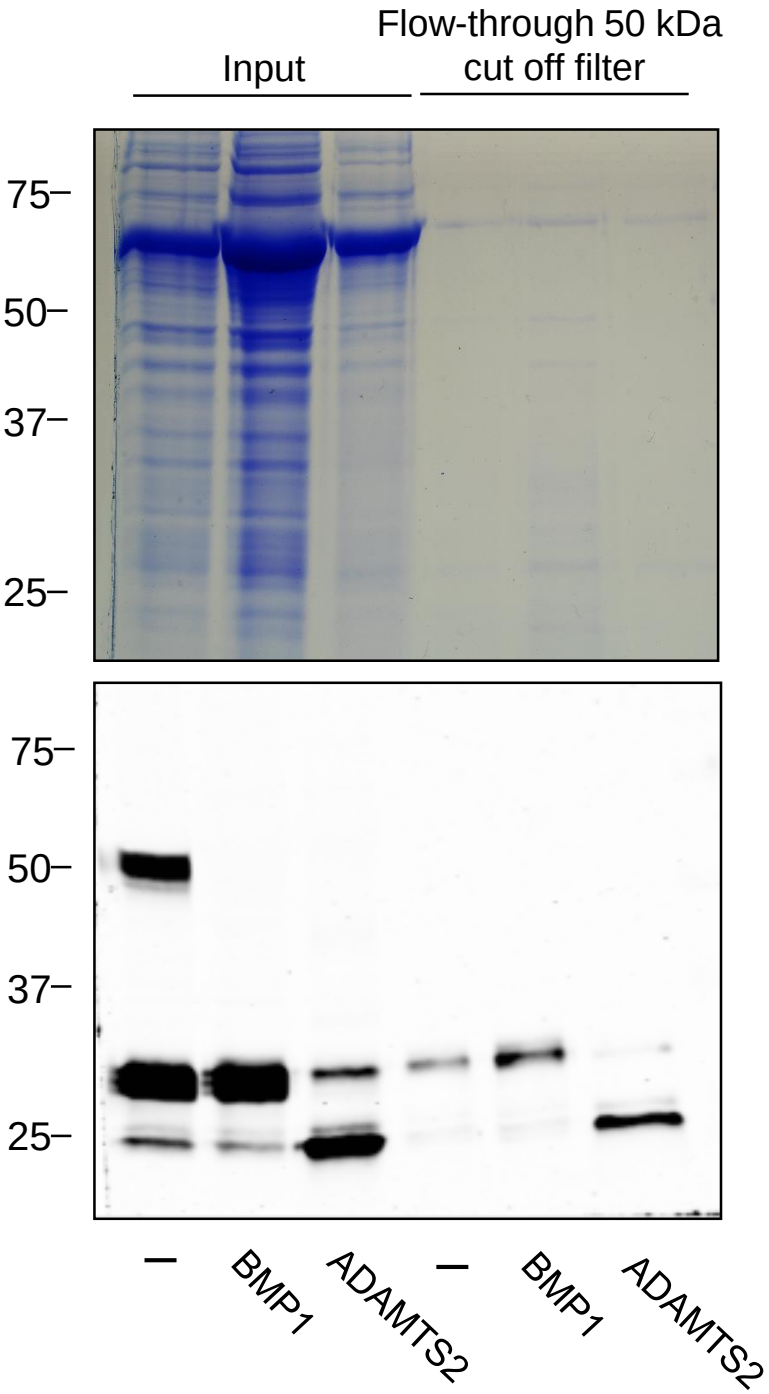
Supplementary Figure 4. Proteomic characterization of BMP1-processed LOX-GFP protein. LOX-GFP supernatants were incubated with BMP1 and proteolytic fragments immunoprecipitated with GFP-Trap agarose beads as described under Experimental Procedures. **A)** Immunoprecipitates were analyzed by immunoblotting with an anti-GFP antibody or stained for total protein with Coomassie Blue. Gel bands matching LOX-GFP precursor (1) and processed forms (2 and 3) were excised from the gel and digested with sequencing-grade AspN, and resulting peptides analyzed by Parallel Reaction Monitoring (PRM). The panel also shows extracted ion chromatograms (XIC) of precursor ion 651.8 m/z (-2), corresponding to the monosulfonated peptides. Note that the intensity of the chromatogram signals parallels that observed in the corresponding bands stained with anti-GFP antibody by immunoblotting. XIC chromatograms clearly suggested the presence of two different 651.8 (-2) m/z precursor ions, eluting at very close but different retention times. **B)** Analysis of MS2 spectra as shown in Supplementary Figure 5 confirmed the identity of peak 1 with the peptide DDNPYYNY**Y** modified in the last position while peak 2 corresponded to the same peptide sulfonated in the previous doublet, either DDNP**Y**YNYY (Tyr183) or DDNPY**Y**NYY (Tyr184). These two options could not be distinguished due to the lack of specific fragment ions.

Supplementary Figure 5. Analysis of tyrosine sulfation of AspN-digested peptide DDNPYYNYY by PRM. MS2 spectra of peaks 1 and 2 from precursor and BMP1-processed bands showed that they were almost identical. Nevertheless, a more detailed analysis in the context of different versions of monosulfonated synthetic DDNPYYNYY peptides revealed subtle differences that aimed to locate the sulfuryl modification in a given tyrosine residue. This is shown for synthetic peptides *versus* peaks 1 and 2 of band 2 in the m/z ranges 100-300 **(A, B)**, 300-575 **(C, D)**, 500-700 **(E, F)** and 700-1300 m/z **(G, H)**. Identical results were obtained for bands 1 and 3. Of particular interest is the

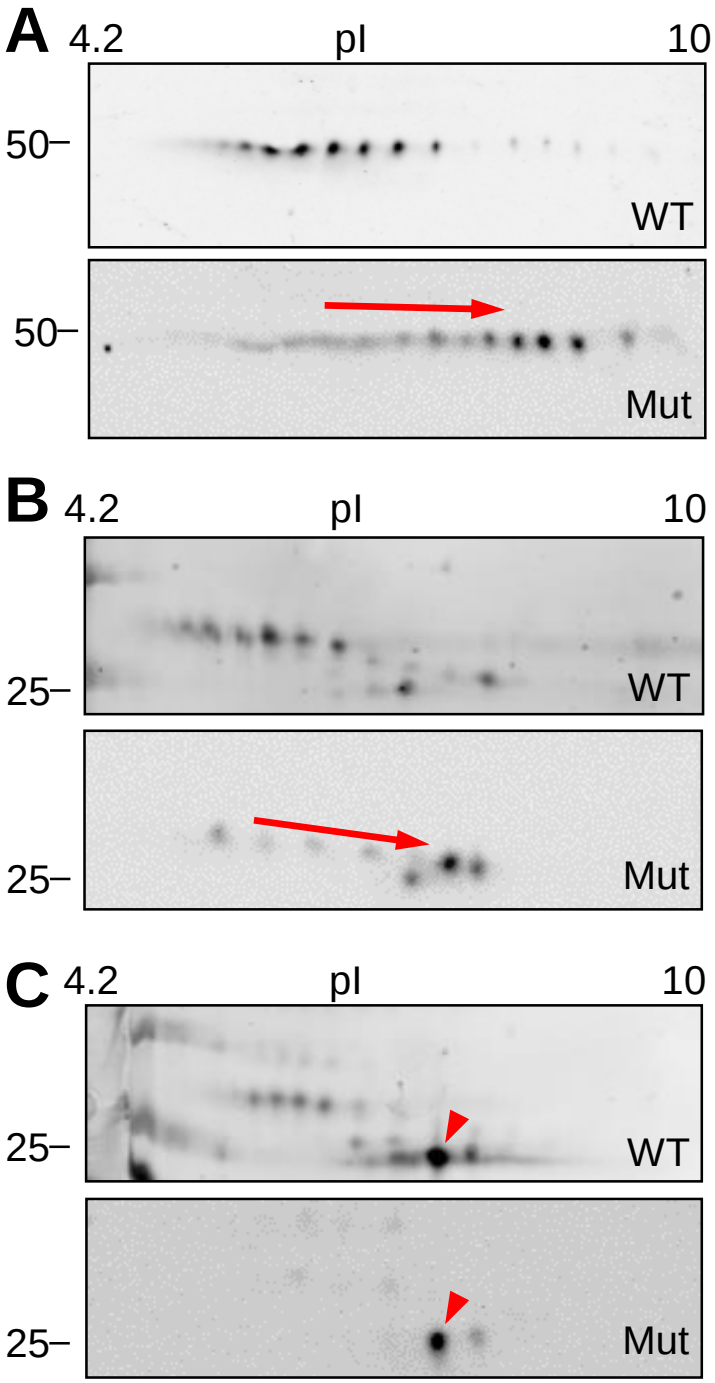
presence of fragment ions 260.05 and 180.08 m/z (-1) in peaks 1 and 2, respectively. By comparison with synthetic peptides, these fragment ions clearly distinguished sulfation at Tyr187 in peak 1 from the same modification at other positions. Similarly, enhanced intensity of signal at 440 m/z pointed out in the same direction. Finally, signal corresponding to fragment ion 731.3 m/z was found that clearly distinguished both MS spectra. While this analysis did not permit to identify the molecular species corresponding to this fragment ion, based on the spectra obtained with the synthetic peptides, it clearly distinguished the pair DDNPYYNY**Y**/DDNPYYN**Y**Y from DDNPY**Y**NYY/DDNP**Y**YNYY. Taking together, these results allowed assigning peak 1 to the peptide harboring the modification in Tyr187, whereas peak 2 corresponds to the peptide modified either in Tyr183 or Tyr184.



Supplementary Figure 1

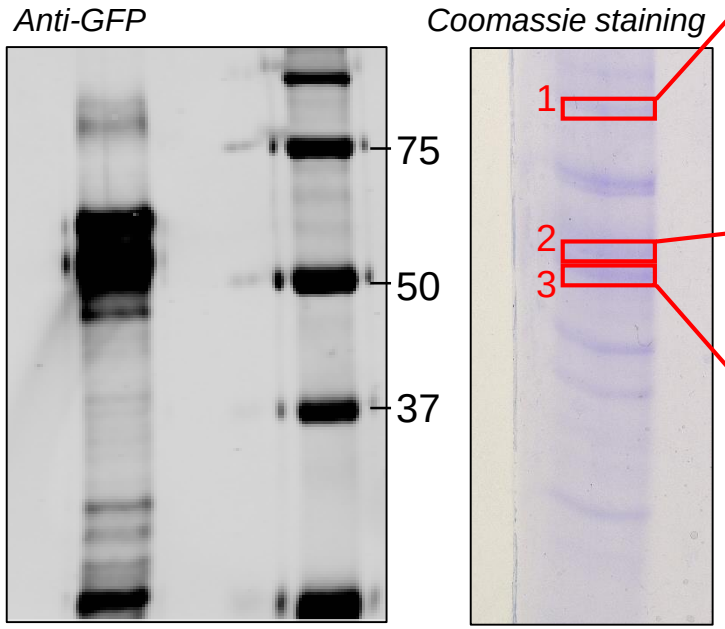


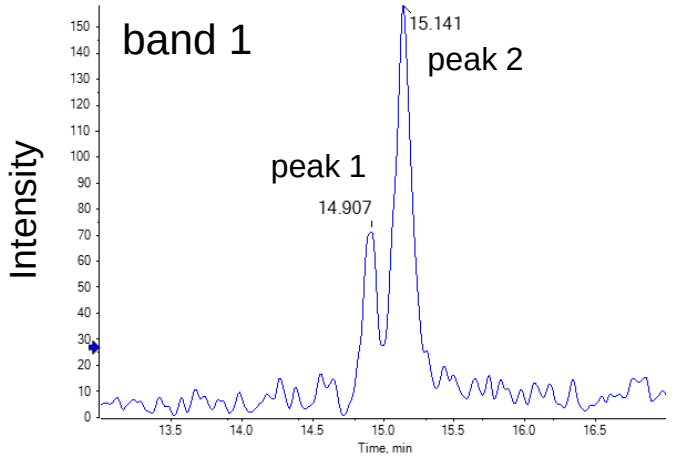
Supplementary Figure 2

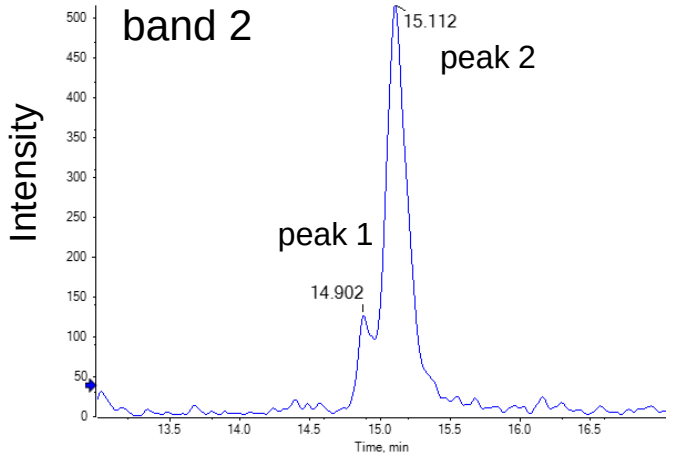


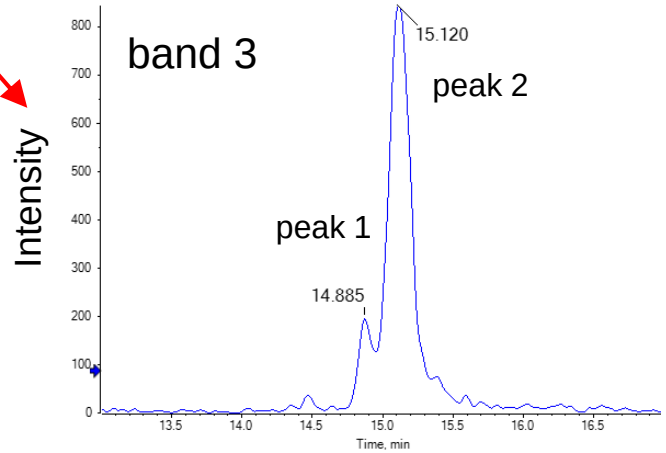
Supplementary Figure 3

A IP-anti-GFP: BMP1-cleaved LOX/GFP







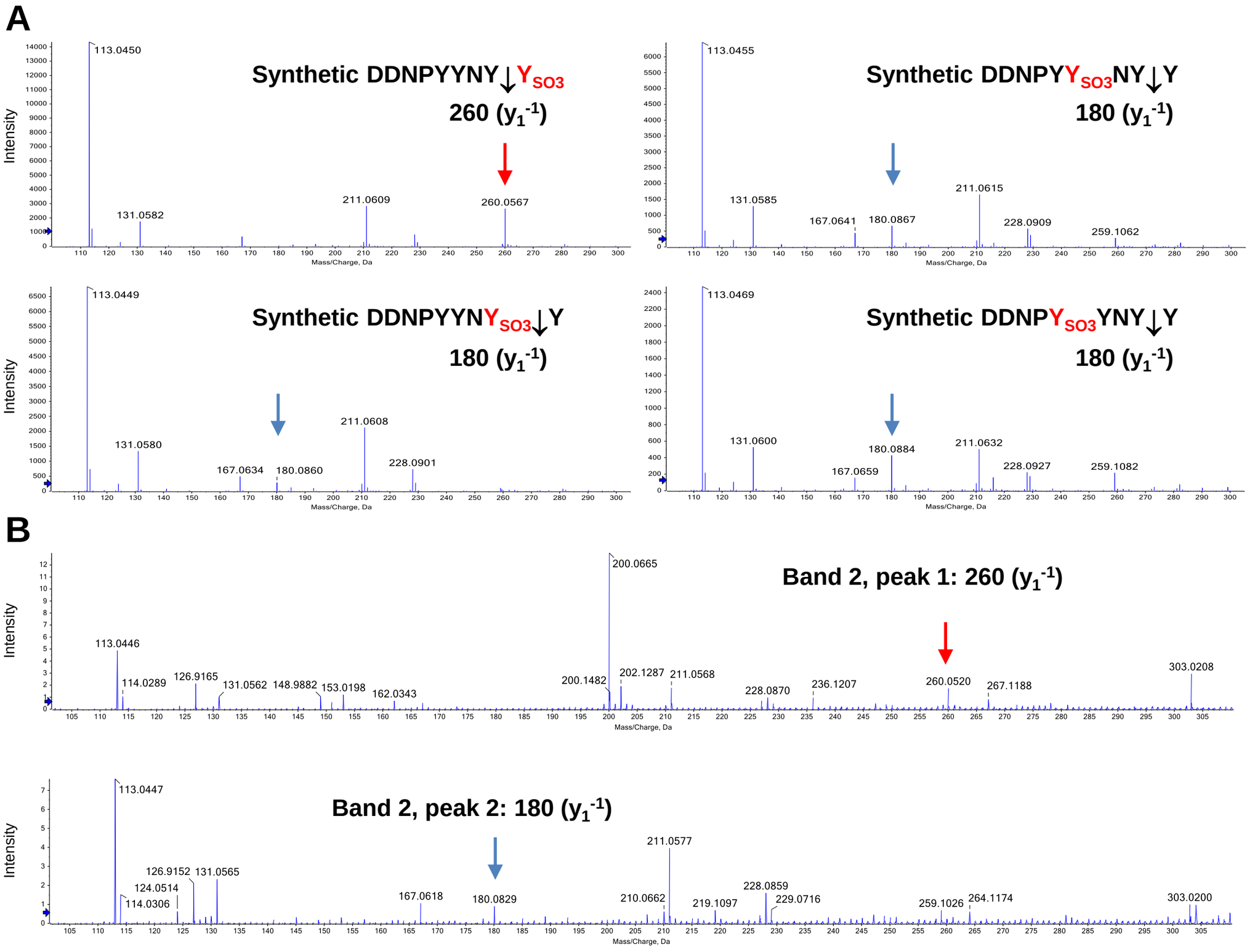


B

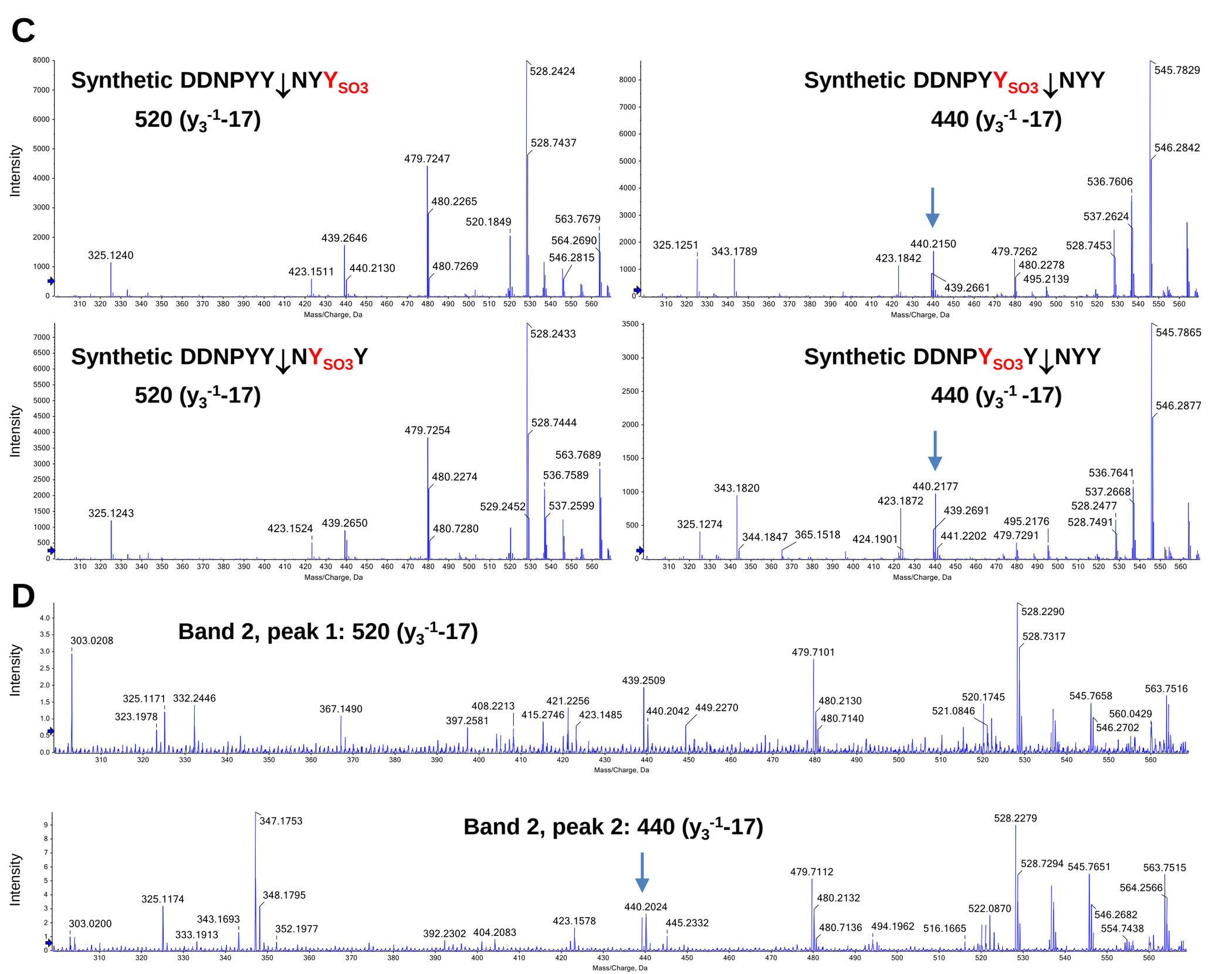
PEAK 1: DDNPYYNYY_{SO3}

PEAK 2 { DDNPY_{SO3}YNYY or DDNPYY_{SO3}NYY

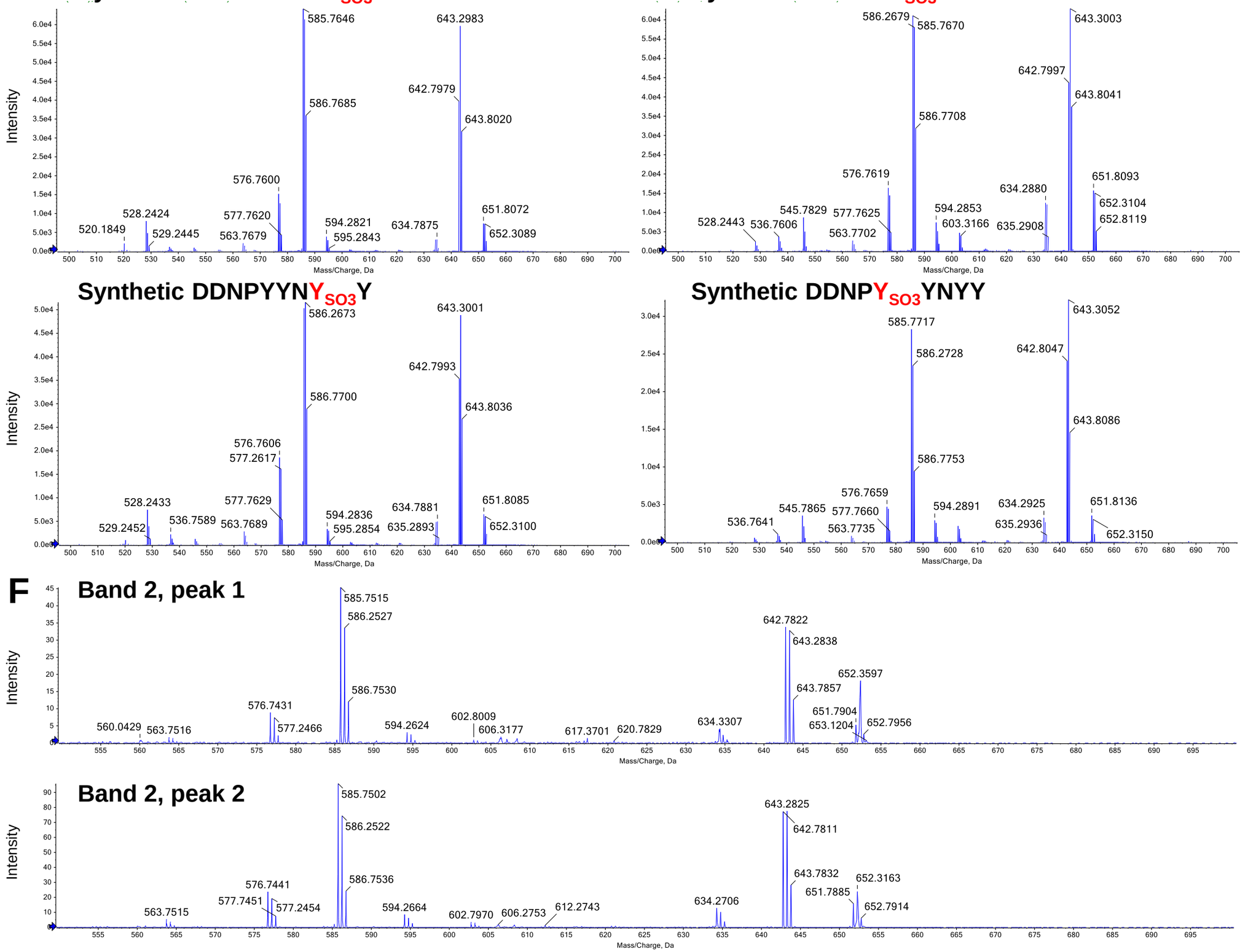
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 5 (con't)



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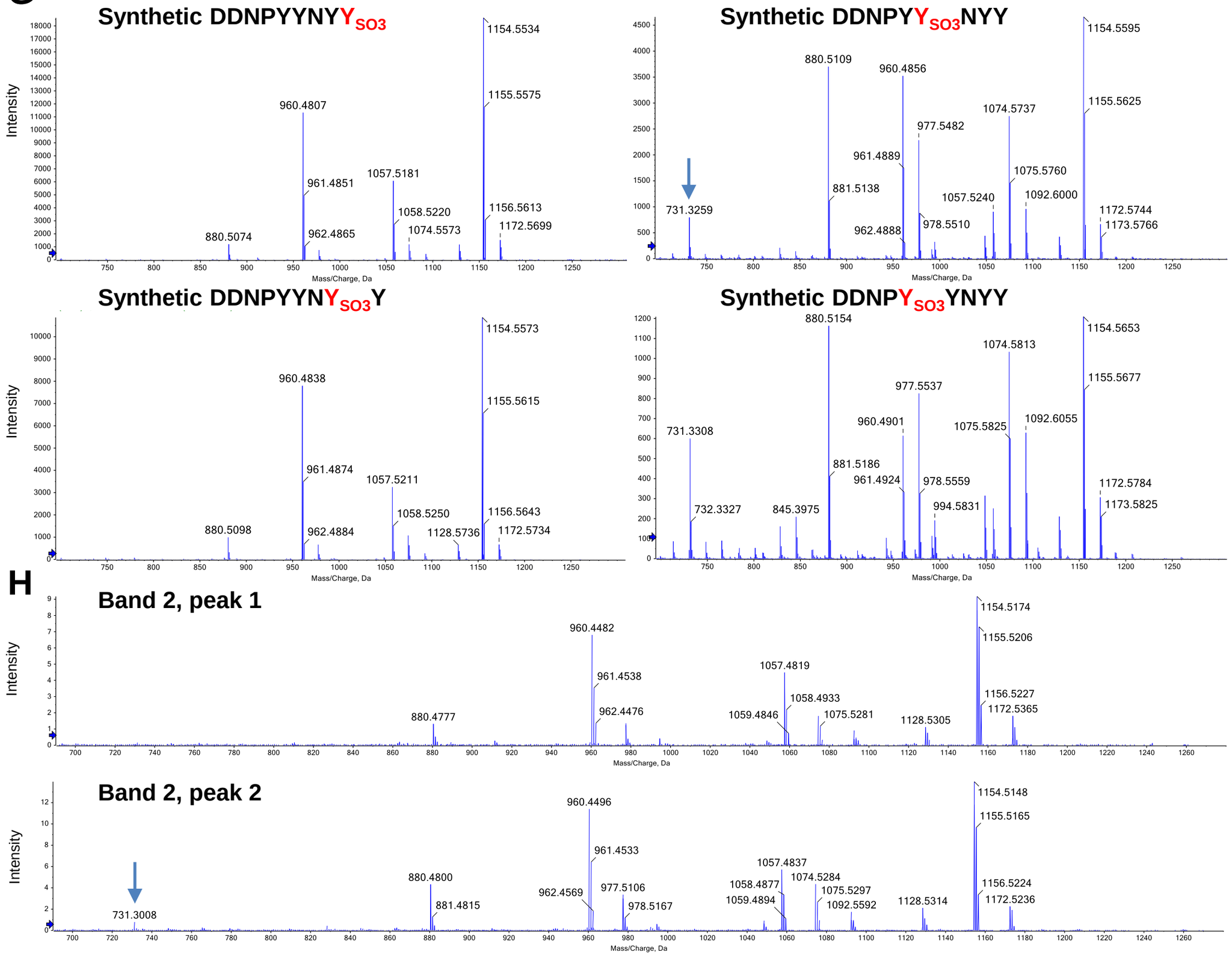

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