Supplemental Figures

Multiplexed Imaging Mass Spectrometry of the Extracellular Matrix using Serial Enzyme Digests from Formalin-Fixed Paraffin Embedded Tissue Sections

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Co-Authors: Cassandra L. Clift, Richard R. Drake, Anand Mehta, Peggi M. Angel

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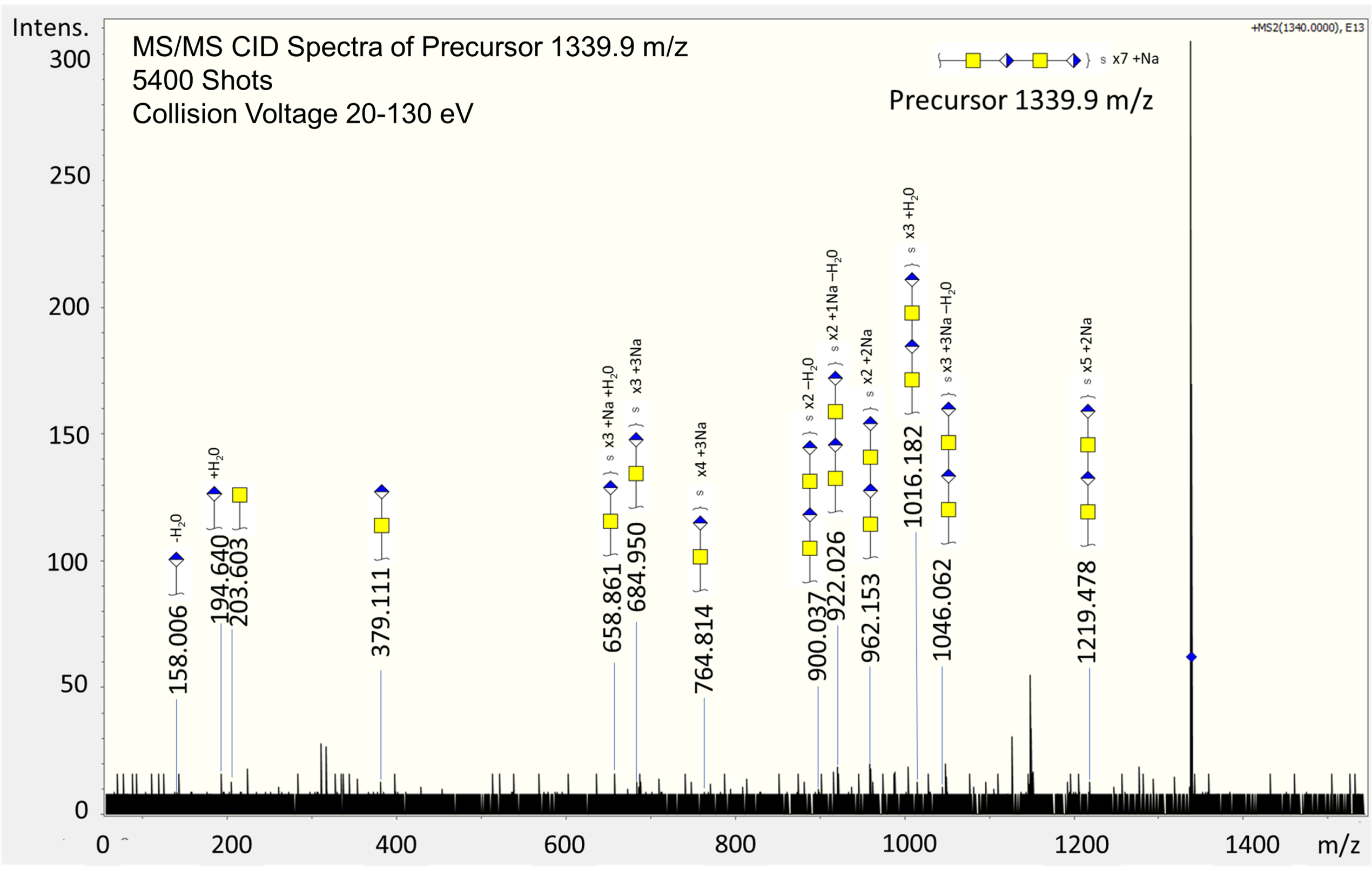
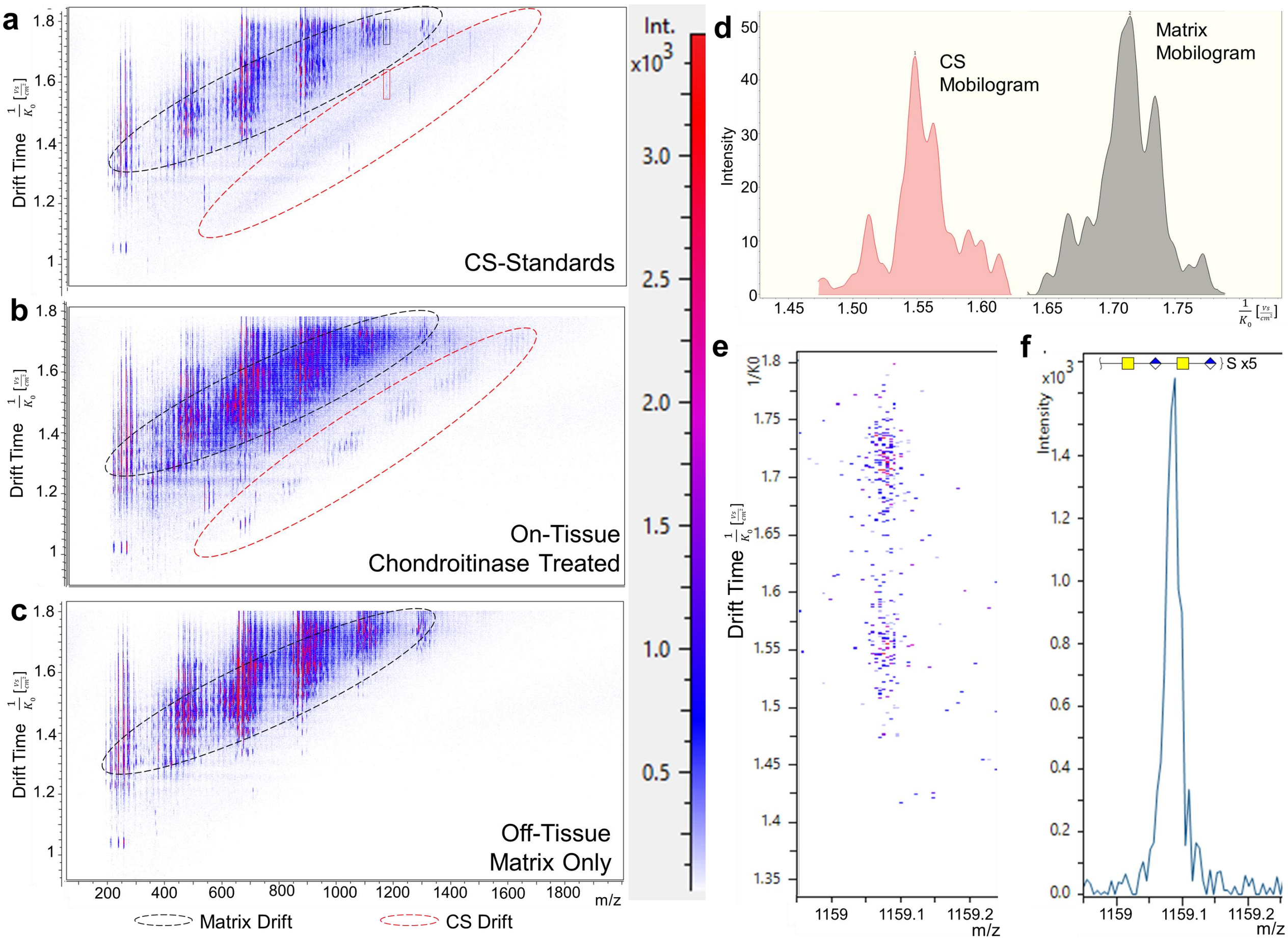
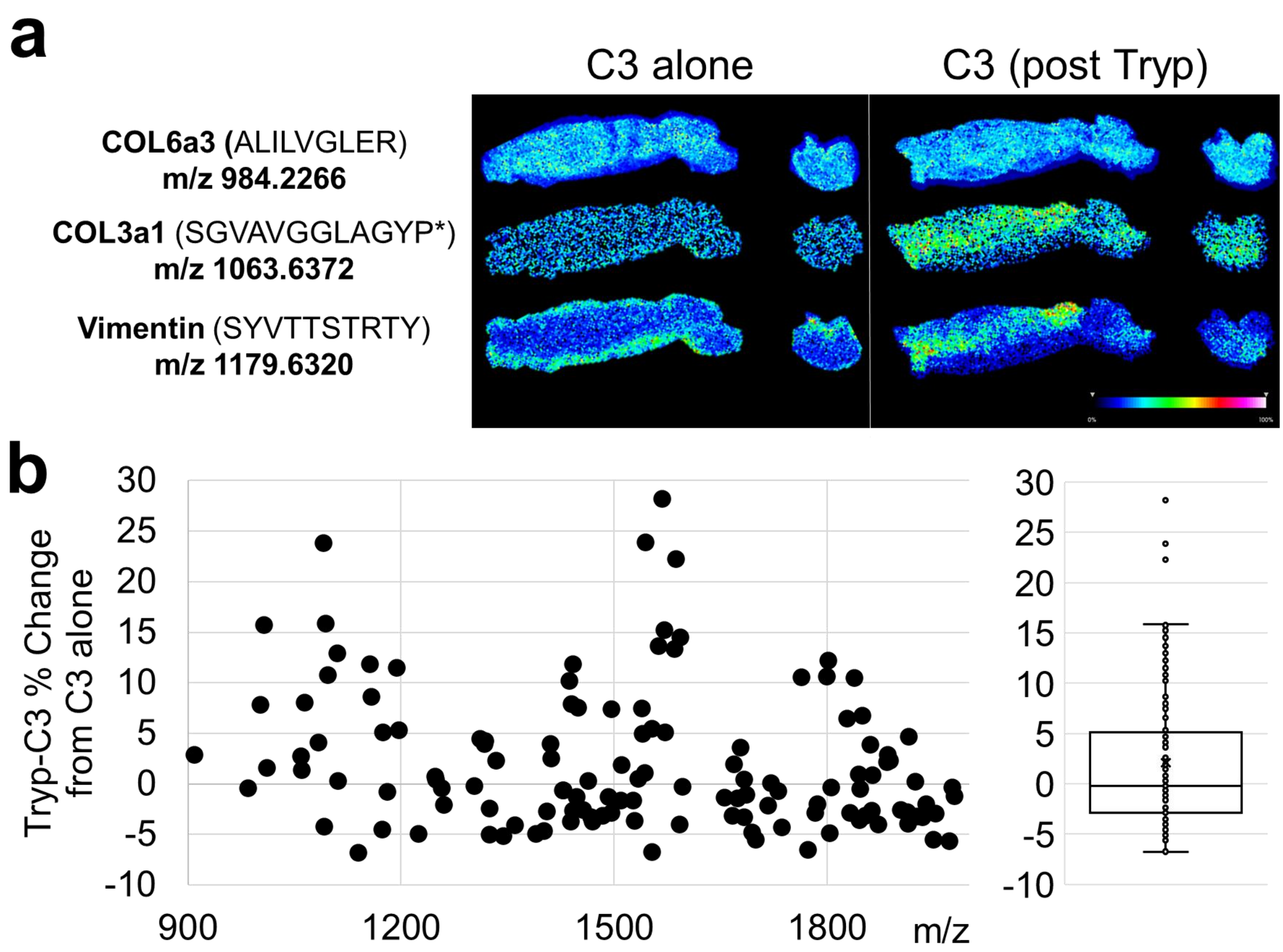
Supplemental Figure 1. Fragmentation of precursor m/z 1339.9 shows evidence of isobaric CS peak with matrix. Summed spectra of CID fragmentation across 5400 shots and increasing collision voltages between 20-130eV. Summed spectra shows loss of sulfation state, chain length, and chain formation with fragmented ions corresponding to GalNAc and GlcUA seen.



Supplemental Figure 2. Ion mobility analysis of CS standards and tissue shows unique drift populations of matrix and CS species at isobaric peaks. **a.** Heat map of spotted CS standards. **b.** Heat map of shots taken on-tissue treated with chondroitinase ABC. **c.** Heat map of shots of matrix only, taken on the same chondroitinase ABC treated slide but off-tissue. **d.** Mobilograms for the CS and Matrix drifts shown in **(e)** for the isobaric peak shown in **(f)**. 10280 shots were summed for all TIMS experiments.



Supplemental Figure 3. Collagenase peptide imaging is compatible with pre-treatment of trypsin. A. Representative images of COLase3 digests either after antigen retrieval only (C3 alone) or after digestion with a trypsin. Three ECM peaks are shown: collagen 6a3 peptide (top), collagen 3a1 peptide (middle), and vimentin peptide (bottom**). B-D.** Quantification of the percent change in peak signal intensity for all COLase3 peptides identified trypsin incubation and analyte removal as compared to control. Each point represents a COLase3 peptide peak with a boxplot of the data shown to the right. P* represents hydroxyproline.



Supplemental Figure 4. Aortic Valve tissue sections used for reproducibility studies. a-b. Movat's Pentachrome staining of tissue sections with GAGs in blue, elastin in purple, and collagen in yellow. Tissue shown in **b** has portions of the outflow tract (OT) and aortic wall still attached. OT has minimal collagen staining. Aortic valve in **b** is highlighted by dotted line.