

# Supplemental Figures

## **Evaluation of therapeutic collagen-based biomaterials in the infarcted mouse heart by extracellular matrix targeted MALDI imaging mass spectrometry**

Cassandra L. Clift<sup>1</sup>, Sarah McLaughlin<sup>2</sup>, Marcelo Muñoz<sup>2</sup>, Erik J. Suuronen<sup>2</sup>, Benjamin H. Rotstein,<sup>3</sup> Anand Mehta<sup>1</sup>, Richard R. Drake<sup>1</sup>, Emilio I. Alarcon<sup>2,3</sup>, Peggi M. Angel<sup>1\*</sup>

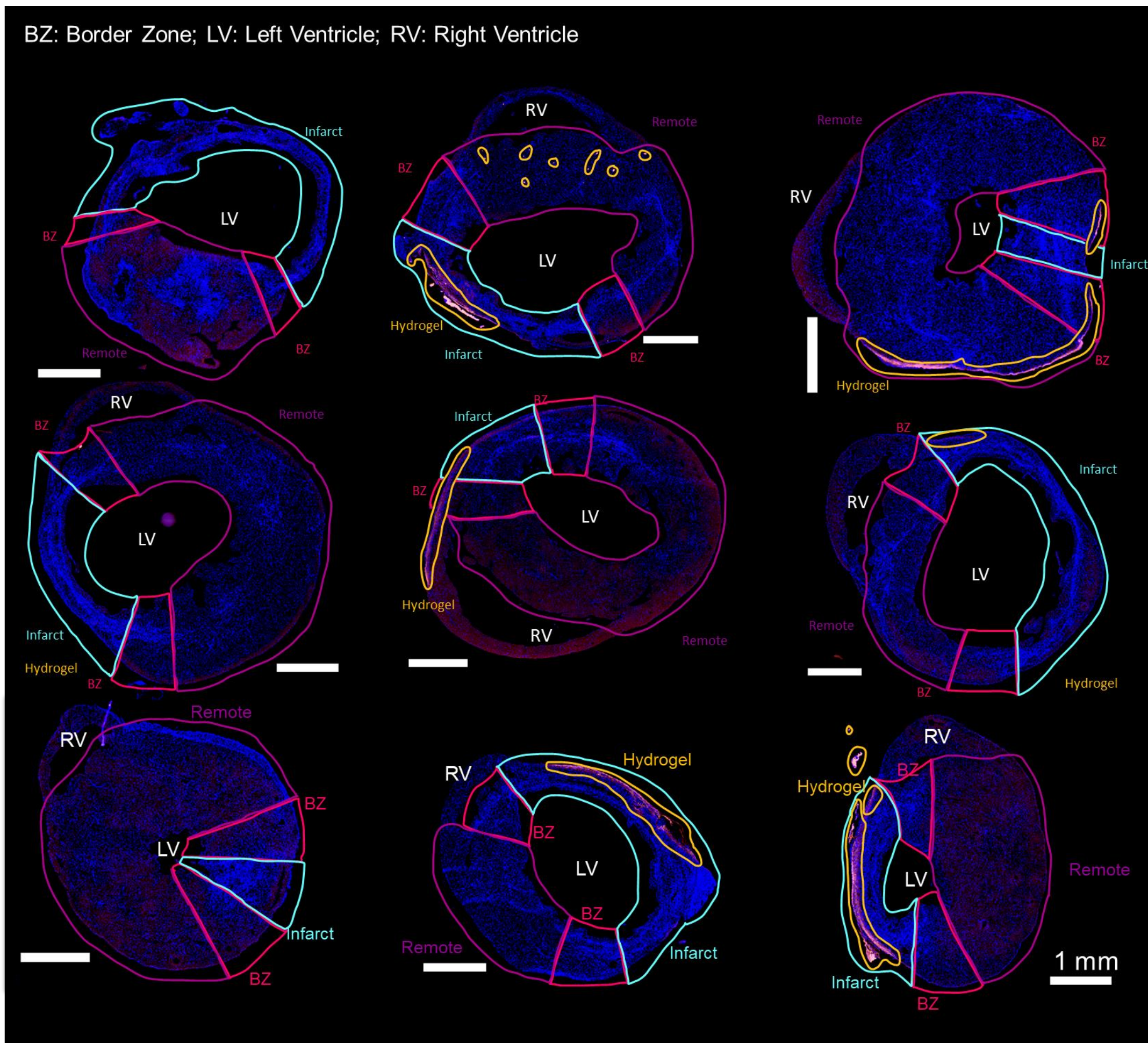
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**Supplementary Fig. 1:** Representative fluorescence microscopy imaged heart tissue sections with ROI annotations from all mice included in this study (n=3 per treatment group). All mice were confirmed to have a Left Ventricular Ejection Fraction of <50% for initial injury. representative annotated tissue section for each biological replicate shown across three patient categories: PBS injected control, rhCI hydrogel, and rhCIII hydrogel. Annotated regions include HIS (hydrogel injection site), Infarct (region of myocardial infarction, identified via previous studies (McLaughlin, 2019); BZ (border zone, region immediately adjacent to infarct), and remote (region furthest from infarct). LV: Left Ventricle. RV: Right Ventricle. Scale bar is 1 mm.

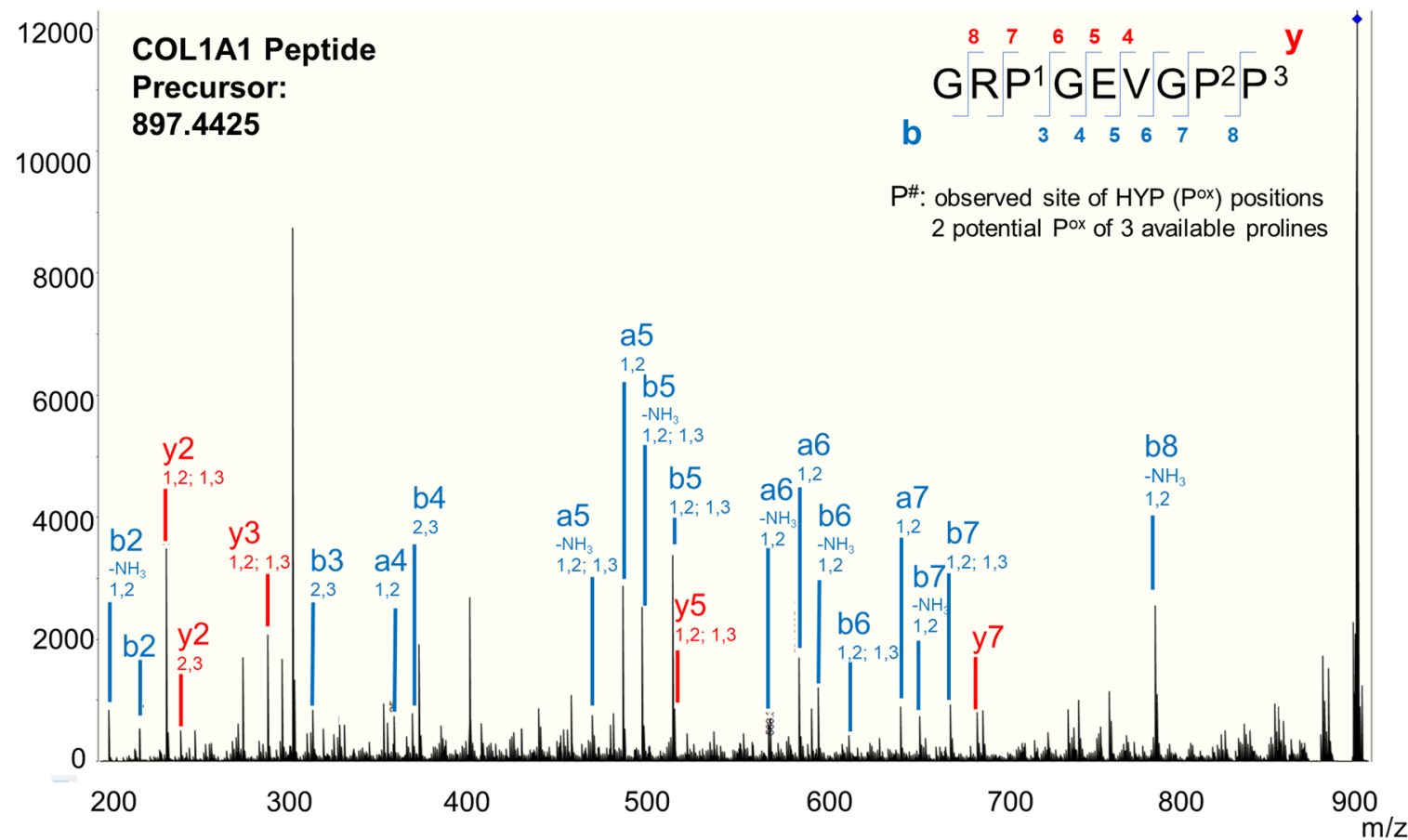
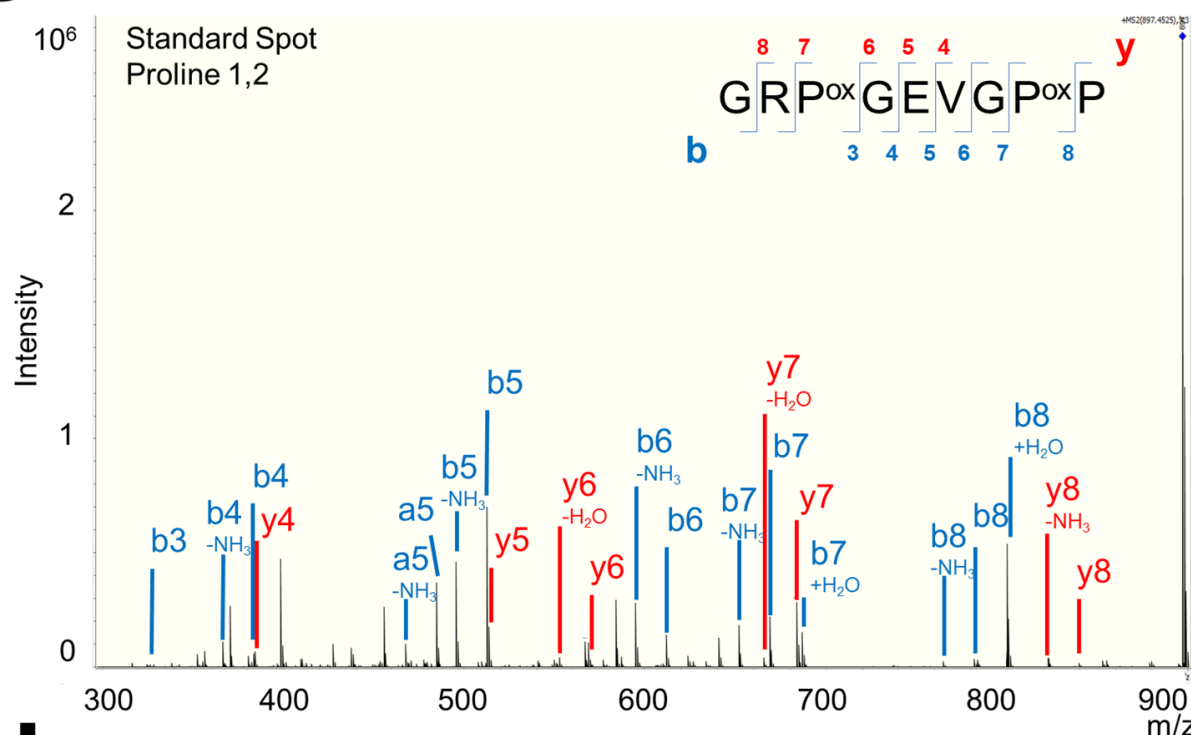
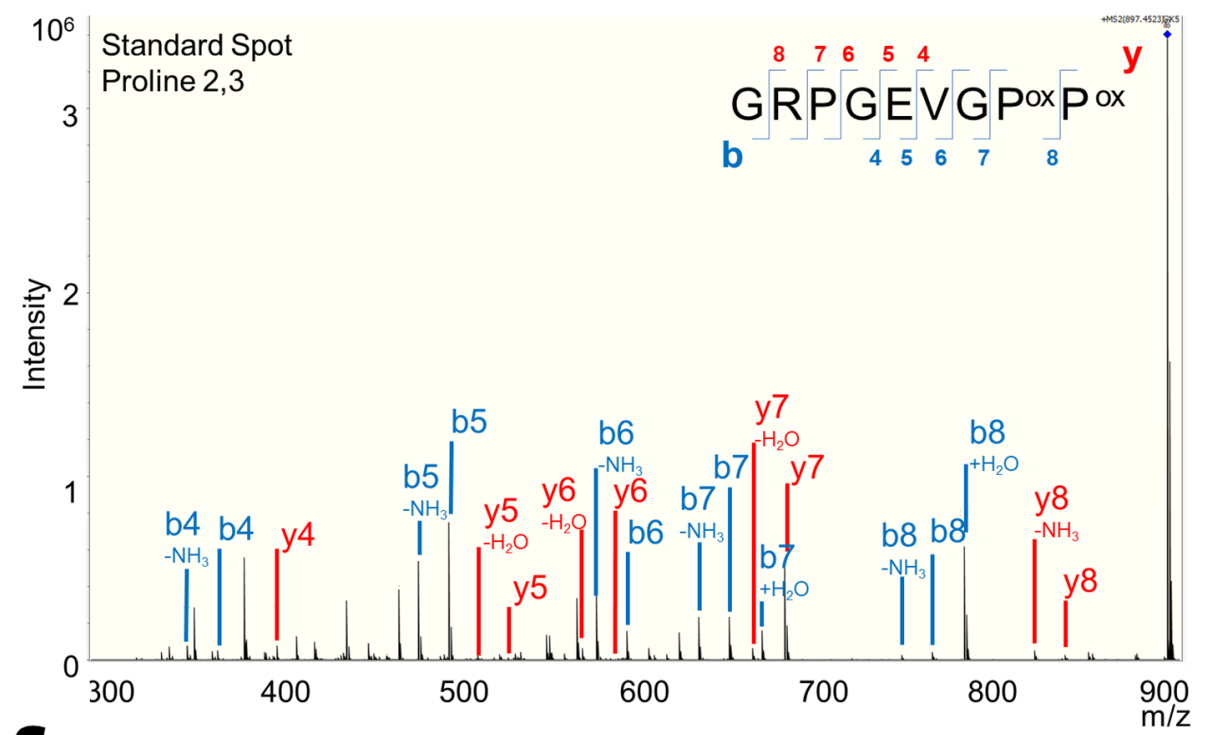
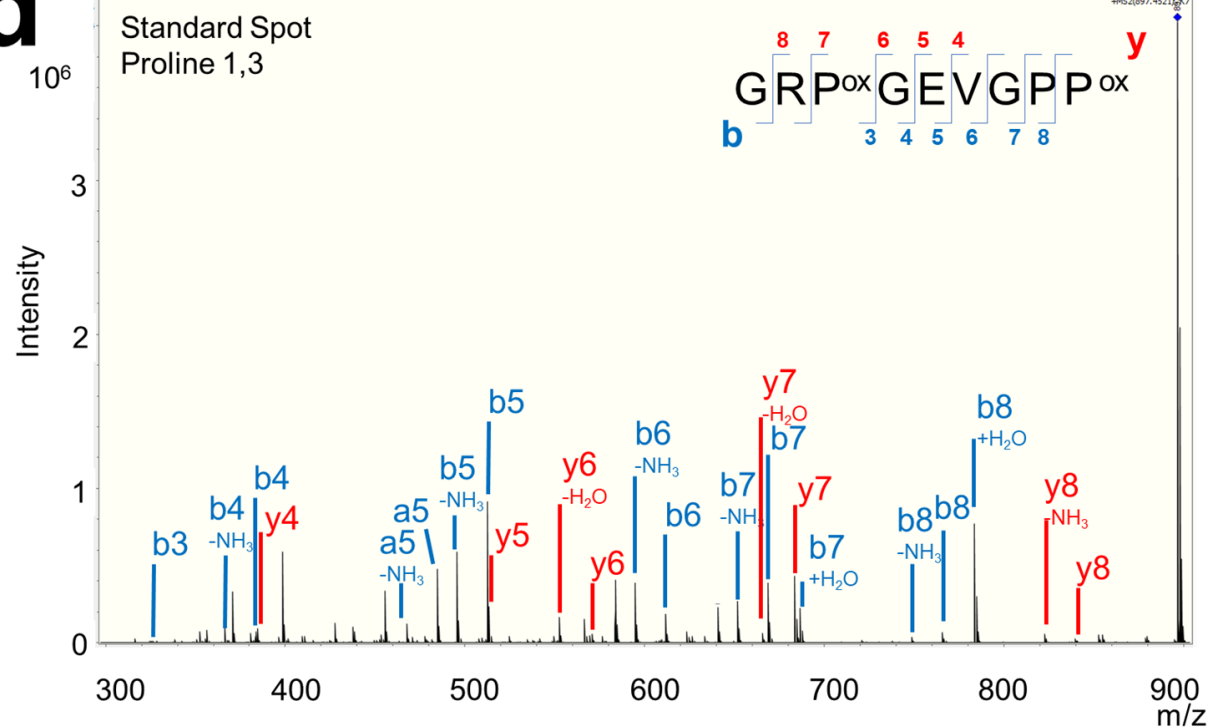
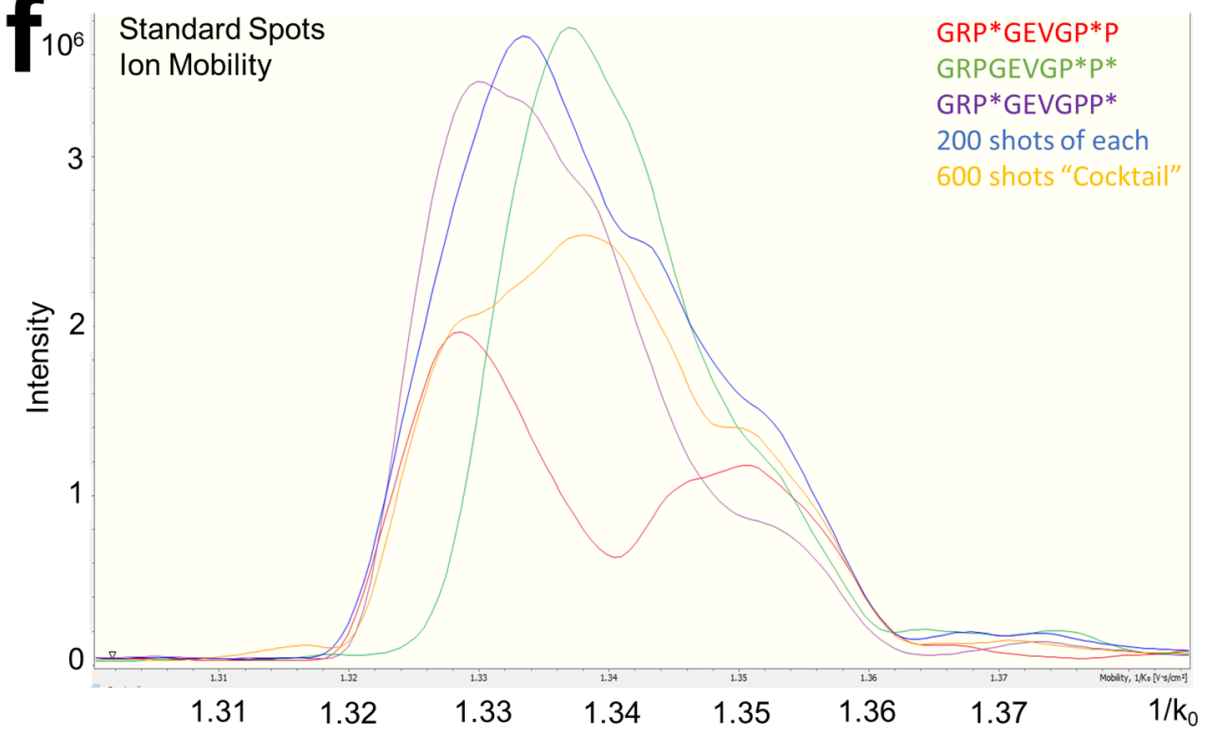


**a**

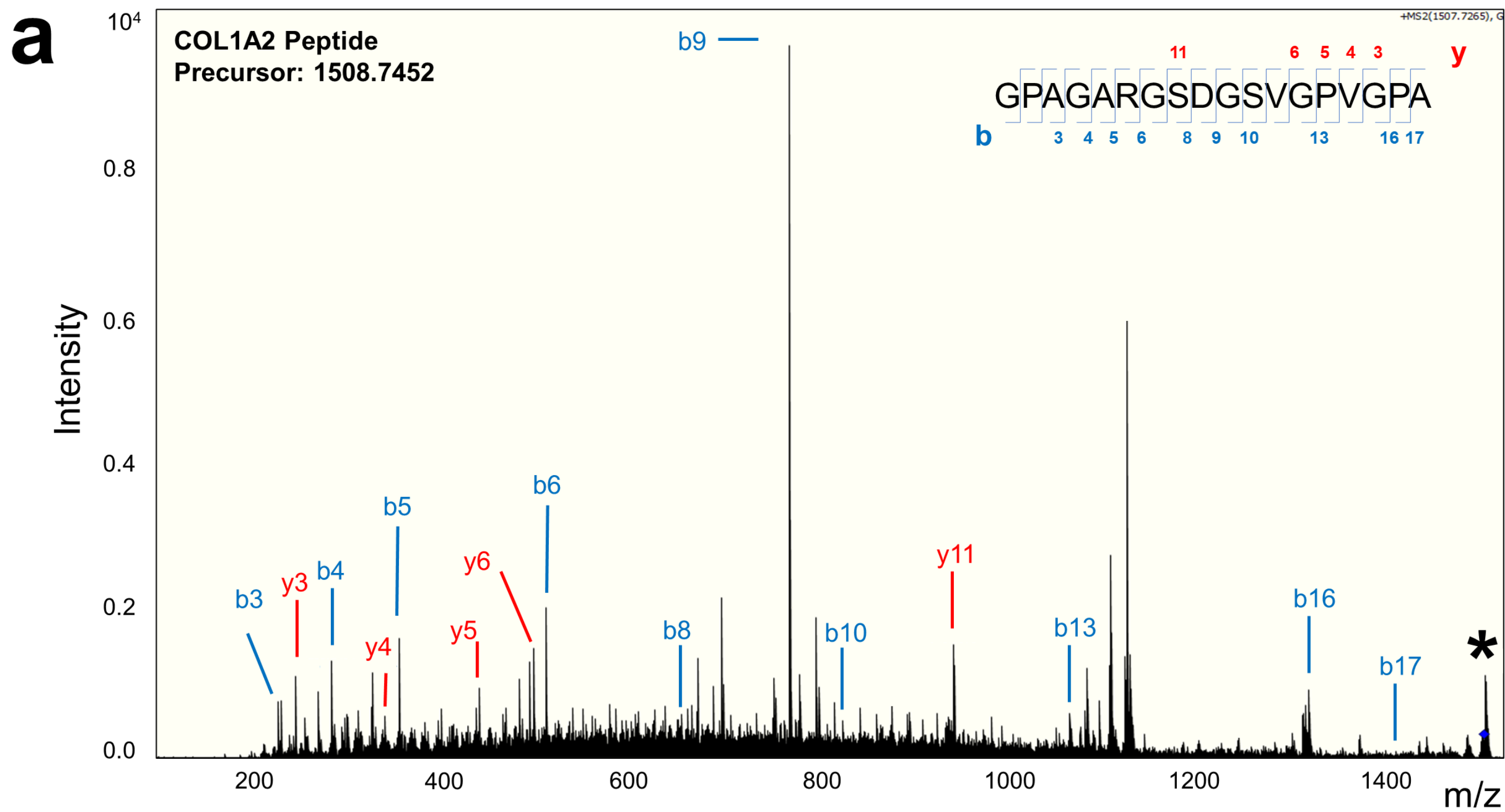
HYP (P <sup>ox</sup> ) Position			b	897.4425	y	HYP (P <sup>ox</sup> ) Position		
1,2	1,3	2,3				1,2	1,3	2,3
--	--	--	1	G	9	--	--	--
214.130	214.130	214.130	2	R	8	840.421	840.421	840.421
327.178	327.178	311.183	3	P <sup>1</sup>	7	684.320	684.320	684.320
384.200	384.199	368.204	4	G	6	571.272	571.272	587.267
513.242	513.242	497.247	5	E	5	514.251	514.251	530.246
612.310	612.310	596.315	6	V	4	385.208	385.208	401.203
669.332	669.332	653.337	7	G	3	286.140	286.140	302.135
782.380	766.384	766.384	8	P <sup>2</sup>	2	229.118	229.118	245.113
--	--	--	9	P <sup>3</sup>	1	116.071	132.066	132.066

**b**

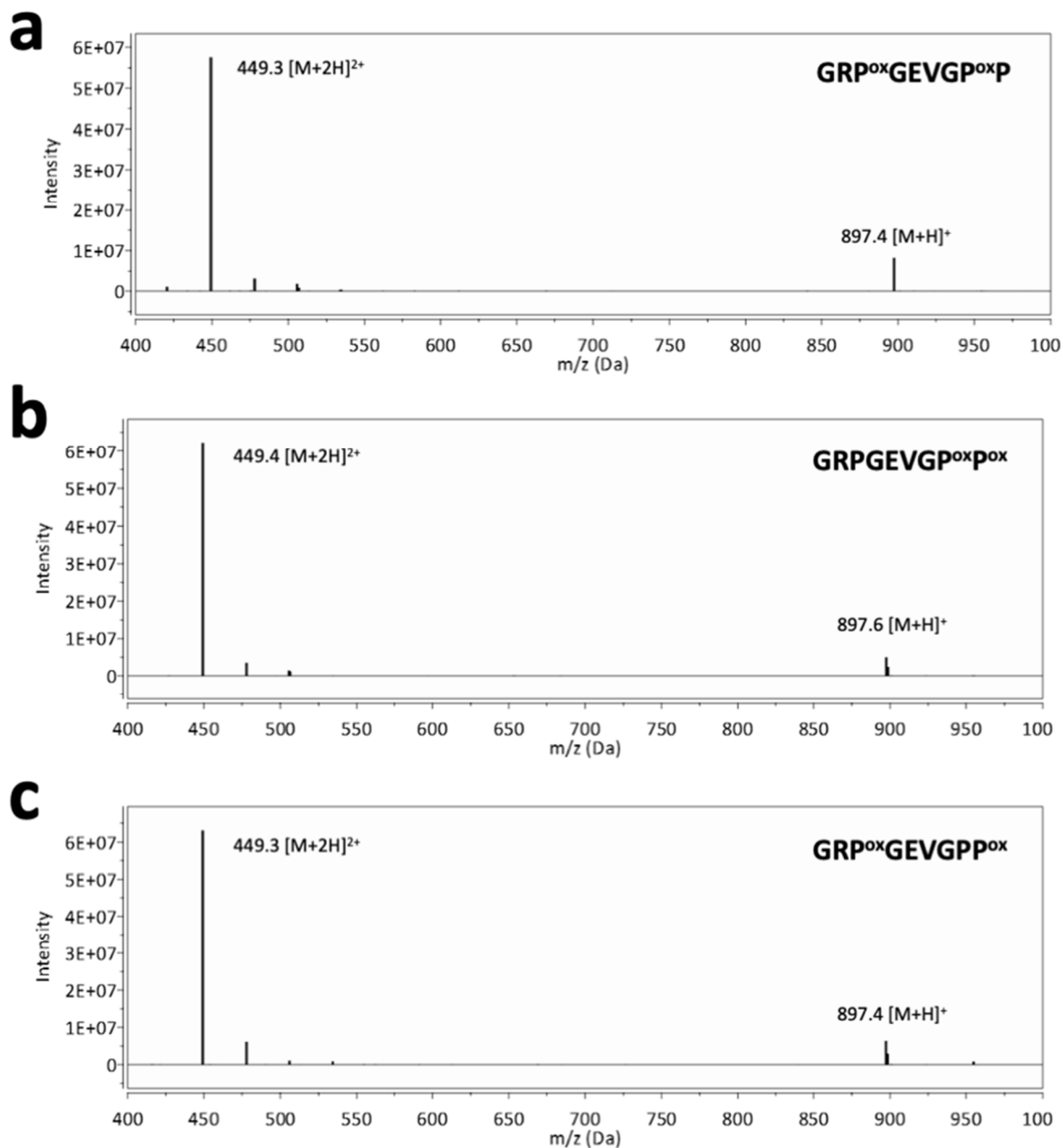
## On-Tissue MS/MS

**c****e****d****f**

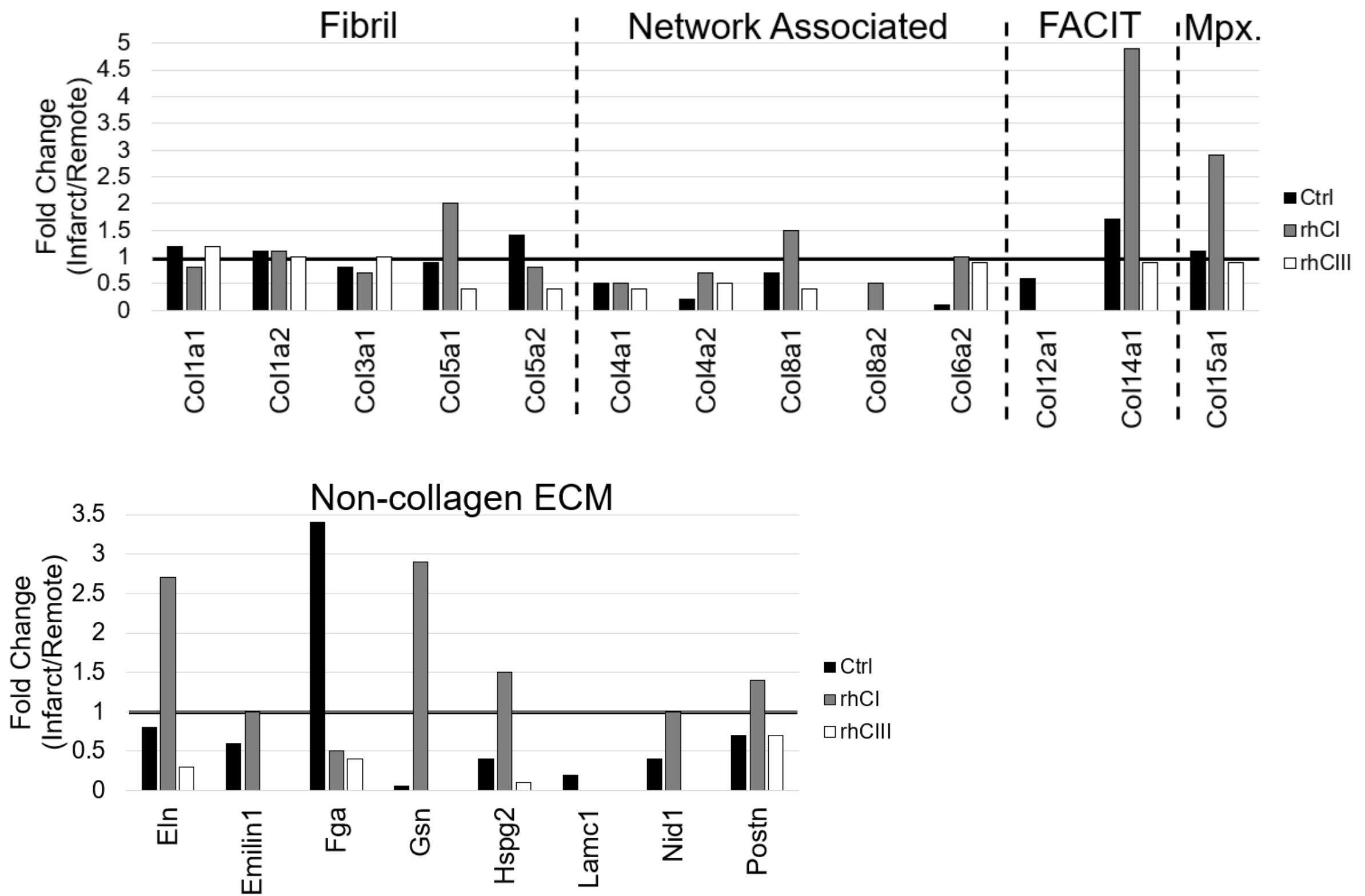
**Supplementary Figure S2. On-tissue MALDI-IMS provides evidence of peptide sequence validation and hydroxyproline site occupancy.** **a.** Table showing potential fragment ion m/z depending on hydroxyproline site localization positions of P1-P2, P2-P3, or P1-P3. **b.** Annotated MALDI-MS/MS spectra produced by CID fragmentation on tissue. Identified b (blue) and y (red) ions are shown. Precursor ion is 897.4425 m/z, corresponding to a hydroxyproline containing COL1A1 peptide. P#: observed site of HYP (P<sup>ox</sup>) positions. There are 2 potential P<sup>ox</sup> sites out of 3 available prolines within the sequence. **c-e.** Annotated MALDI-MS/MS spectra produced by CID fragmentation of synthesized peptide standards of the three potential HYP site localizations (**Supp. Fig S4**). **f.** Ion Mobility study of the three synthesized peptides standards shown in **c-e** (and **Supp. Fig. S4**). An additional study was done of the three combined standards spotted ("cocktail") as well as on-target summed acquisition of the three standards spotted separately. 600 shots were taken for each acquisition.



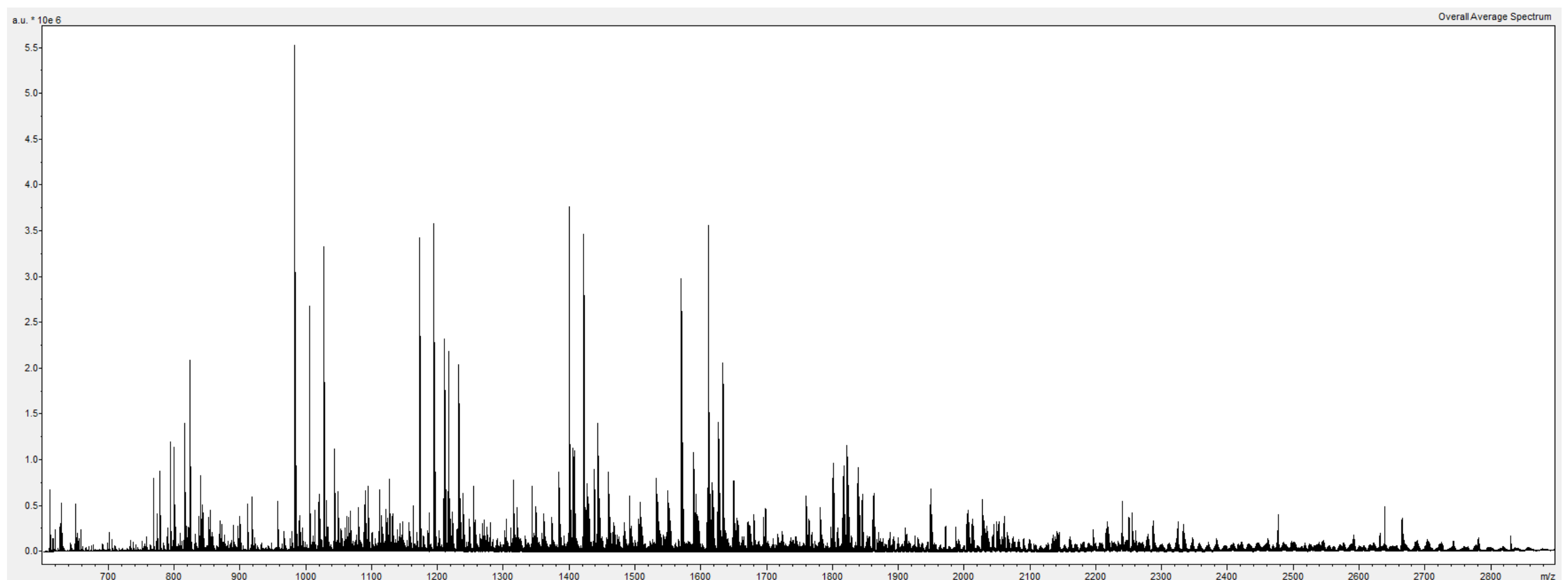
**Supplementary Figure S3. On-tissue MALDI-IMS provides evidence of peptide sequence validation and hydroxyproline site occupancy. a.** Annotated MALDI-MS/MS spectra produced by CID fragmentation. Identified b (blue) and y (red) ions are shown. Precursor ion is 1508.7452 m/z, corresponding to an unmodified COL1A2 peptide.



Supplemental Figure S4. Confirmation of the synthesized peptide mass. a. Mass Spectrometry data of the synthesized peptide GRP<sup>ox</sup>GEVGP<sup>ox</sup>P, b. synthesized peptide GRPGEVGP<sup>ox</sup>P<sup>ox</sup>, and c. synthesized peptide GRP<sup>ox</sup>GEVGPP<sup>ox</sup>. Mass Spectrometry analysis was performed in a Waters ACQUITY UPLC XEVO TQD with a column 2.1x100 mm BEH C8, and a flow rate of 0.5 mL/min, a gradient of 2% to 95% of Acetonitrile in 9 minutes, and constant 0.1% of Formic Acid.

**a**

**Supplemental Figure S5.** Protein Fold Change intensity values of Infarct/Remote ROIs for each treatment group (PBS control, rhCI hydrogel, rhCIII hydrogel). Raw values can be seen in **Supplemental Table S3**. Proteins are organized by collagen subtype (FACIT: Fibril associated collagen with interrupted triple helices; Mpx: Multiplexin) and non-collagen proteins.



**Supplemental Figure S6.** Representative collagenase spectra. MALDI IMS analysis by 7.0 Tesla solariX™ Legacy FT-ICR (Bruker Scientific, LLC) operated in positive ion broadband mode over m/z range 600-2500. A transient length of 1.050 ms was used with a resolving power of 29000 calculated at 1400 m/z. Laser settings used were 200 shots/pixel with a 75  $\mu\text{m}$  step size.

# Supplemental Tables

## **Evaluation of therapeutic collagen-based biomaterials in the infarcted mouse heart by extracellular matrix targeted MALDI imaging mass spectrometry**

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**Supplemental Table S1:** MALDI-IMS mapped human hydrogel peptides, showing both peptides that are unique to the human proteome as well as those that share mouse and human homology sequences. For those with human specific sequences, the corresponding mouse peptide and m/z is shown.

Protein	Sequence	HYP	Centroid [m/z]	Human + Mouse Homology/Isobaric	Human Specific	Corresponding Mouse m/z (Sequence)
COL3A1	GPP <sup>ox</sup> GPTGPG	+	752.3231			766.3730 (GPPGPTGPA)
COL3A1	LQGLP <sup>ox</sup> GTG	+	758.4520	+		758.4520 (LQGIPGTG)
COL3A1	GPP <sup>ox</sup> GLAGAP <sup>ox</sup>	+	768.3922			798.3992 (GPPGTAGIP)
COL3A1	GPAGFP <sup>ox</sup> GAP <sup>ox</sup>	+	802.3981	+		
COL3A1	GPP <sup>ox</sup> GINGSP <sup>ox</sup>	+	827.4826			828.3482 (GPPGNNGSP)
COL3A1	GPP <sup>ox</sup> GTAGFP <sup>ox</sup>	+	832.4206	+		
COL3A1	GPP <sup>ox</sup> GEP <sup>ox</sup> GQA	+	841.4255	+		
COL3A1	GPAGIP <sup>ox</sup> GFP <sup>ox</sup>	+	844.4086		+	862.3764 (GPAGMP <sup>ox</sup> GFP <sup>ox</sup> )
COL3A1	GPAGIP <sup>ox</sup> GFP <sup>ox</sup>	+	844.4096			862.3764 (GPAGMPGFP)
COL3A1	GAP <sup>ox</sup> GFRGPA	+	845.4069		+	845.4264 (GPSGFRGPA)
COL1A2	GKEGPVGLP <sup>ox</sup>	+	869.4550	+		
COL1A1	GRP <sup>ox</sup> GEAGLP <sup>ox</sup>	+	885.4538	+		
COL1A1	GSP <sup>ox</sup> GFQGLP <sup>ox</sup>	+	891.4158	+		
COL1A1	GRP <sup>ox</sup> GEVGP <sup>ox</sup> P	+	897.4448	+		
COL1A2	GERGVVGPQ		898.4464	+		
COL1A1	GQRGVVGLP <sup>ox</sup>	+	898.5197	+		
COL1A2	GPP <sup>ox</sup> GFQGLP <sup>ox</sup>	+	901.4262	+		
COL3A1	VAVGGLAGYP <sup>ox</sup>	+	919.4372		+	
COL3A1	GQP <sup>ox</sup> GVMGFP <sup>ox</sup>	+	921.4389			
COL1A2	GNIGPVGAAGAP <sup>ox</sup>	+	996.4044		+	998.4901 (GNIGPTGAAGAP)
COL1A1	GPP <sup>ox</sup> SAGFDFS	+	997.4646		+	999.4054 (GPPSSGGYDFS)
COL3A1	IAGITGARGLA		999.5115	+	+	999.5115 (IAGLTGARGLA)
COL1A2	GARGSDGSVGPV		1058.5198	+		
COL3A1	SGVAVGGLAGYP <sup>ox</sup>	+	1063.5199		+	
COL3A1	GPLGIAGITGAR		1082.6426	+	+	1082.6426 (GPLGLAGITGAR)
COL3A1	GP <sup>ox</sup> PGTAGFP <sup>ox</sup> GSP <sup>ox</sup>	+	1089.5523	+		
COL1A2	GSRGFP <sup>ox</sup> GADGVA	+	1106.4586	+		
COL1A2	GRTGEVGAVGP <sup>ox</sup> P	+	1112.5001		+	
COL1A1	GPPSAGFDFS		1128.5681		+	1130.4789 (GPPSSGGYDFS)
COL1A2	FGYDGFYR		1139.4483		+	1137.5000 (FGFEGDFYR)
COL3A1	GPLGIAGITGARG		1139.6670	+	+	1139.6670 (GPLGLAGITGARG)
COL1A1	GPPGPPSAGFDF		1145.4746		+	1147.5055 (GPPGPPSSGGYDF)
COL1A2	GGGYDFGYDGDF		1269.5981		+	1267.4902 (GGGYDFGFEGDF)
COL1A1	GAVGPAGKDGEAGAQ		1284.5619	+		
COL1A2	GP <sup>ox</sup> KGEIGAVGNAGPA	+	1310.6298		+	1362.7012 (GPKGELGPVGNPGPA)
COL3A1	GPLGIAGITGARGLA		1323.8029	+	+	1323.8029 (GPLGLAGITGARGLA)
COL1A2	VSGGGYDFGYDGDF		1455.6838		+	1453.5907 (VSGGGYDFGFEGDF)
COL1A1	GPPSAGFDFSFLPQ		1466.7614		+	1468.6743 (GPPSSGGYDFSFLPQ)
COL1A2	GPAGARGSDGSVGPVGP		1508.7727	+		
COL1A2	GPDGNKGEP <sup>ox</sup> GVVAVGTAGPS	+	1838.8609		+	1726.7991 (GPDGNKGEAGAVGAPGSAGAS)

**Supplemental Table S2:** Significantly Differentially Expressed human hydrogel derived peptides (ROC AUC); infarct ROI comparisons.

<b>Groups</b>	<b>ROI</b>	<b>Protein</b>	<b>Peptide</b>	<b>Centroid [m/z]</b>	<b>AUC</b>
rhCI-Ctrl	Infarct	COL1A1	GRP <sup>ox</sup> GEVGP <sup>ox</sup> P	897.445	0.605
rhCI-Ctrl	Infarct	COL1A2	GSRGFP <sup>ox</sup> GADGVA	1106.459	0.668
rhCI-Ctrl	Infarct	COL1A1	GPPSAGFDFSF	1128.568	0.689
rhCI-Ctrl	Infarct	COL1A2	GPAGARGSDGSPVGPVGP	1508.773	0.673
rhCIII-Ctrl	Infarct	COL3A1	GPP <sup>ox</sup> GTAGFP <sup>ox</sup>	832.421	0.610
rhCIII-Ctrl	Infarct	COL3A1	GP <sup>ox</sup> PGTAGFP <sup>ox</sup> GSP <sup>ox</sup>	1089.552	0.671

**Supplemental Table S3:** Proteins identified via LC-MS/MS analysis on infarcted and remote regions of tissue for PBS Ctrl, rhCI hydrogel, and rhCIII hydrogel treated samples. Protein level Fold Change values (Infarct/Remote) were used to create hierarchical cluster in Fig 4b, with the exception of those with INF (infinity) values. Proteins were identified with a minimum of 2 peptides and a protein threshold of 99% with less than 1% FDR.

		Total Spectral Count									
Accession Number	Alternate ID	Molecular Weight	Fold Change by Sample (Ctrl I/R)	Fold Change by Sample (rhCI I/R)	Fold Change by Sample (rhCIII I/R)	Ctrl_Infarc t	rhCIII_Infarct	rhCIII_Remote	rhCI_Infarc t	rhCI_Remote	
<b>Bio View identified Proteins (33)</b>											
40S ribosomal protein SA OS=Mus musculus OX=10090 GN=Rpsa PE=1 SV=4	Rpsa	33 kDa	0 INF	0 INF	INF	2			1		
60 kDa heat shock protein, mitochondrial OS=Mus musculus OX=10090 GN=Hspd1 PE=1 SV=1	Hspd1	61 kDa	0 INF	0 INF	INF	2					
Basement membrane-specific heparan sulfate proteoglycan core protein OS=Mus musculus OX=10090 GN=Hspg2 PE=1 SV=1	Hspg2	398 kDa	0.4	1.5	0.1	6	1	6	11	7	
Biglycan OS=Mus musculus OX=10090 GN=Bgn PE=1 SV=1	Bgn	42 kDa	0 INF	0 INF	INF	0			2		
Collagen alpha-1(I) chain OS=Mus musculus OX=10090 GN=Col1a1 PE=1 SV=4	Col1a1	138 kDa	1.2	0.8	1.2	354	287	211	236	275	
Collagen alpha-1(III) chain OS=Mus musculus OX=10090 GN=Col3a1 PE=1 SV=4	Col3a1	139 kDa	0.8	0.7	1	105	110	97	72	107	
Collagen alpha-1(V) chain OS=Mus musculus OX=10090 GN=Col4a1 PE=1 SV=4	Col4a1	161 kDa	0.5	0.5	0.4	9	7	14	8	16	
Collagen alpha-1(V) chain OS=Mus musculus OX=10090 GN=Col5a1 PE=1 SV=2	Col5a1	184 kDa	0.9	2	0.4	14	4	9	12	6	
Collagen alpha-1(VIII) chain OS=Mus musculus OX=10090 GN=Col8a1 PE=1 SV=3	Col8a1	74 kDa	0.7	1.5	0.4	9	4	10	9	6	
Collagen alpha-1(XI) chain OS=Mus musculus OX=10090 GN=Col11a1 PE=1 SV=2	Col11a1	181 kDa	INF	INF	INF	3	0		2	0	
Collagen alpha-1(XII) chain OS=Mus musculus OX=10090 GN=Col12a1 PE=2 SV=3	Col12a1	340 kDa	0.6 INF	0.6 INF	0	2		1	3	0	
Collagen alpha-1(XIV) chain OS=Mus musculus OX=10090 GN=Col14a1 PE=1 SV=2	Col14a1	193 kDa	1.7	4.9	0.9	3	1	1	5	1	
Collagen alpha-1(XV) chain OS=Mus musculus OX=10090 GN=Col15a1 PE=1 SV=2	Col15a1	140 kDa	1.1	2.9	0.9	2	1	1	3	1	
Collagen alpha-2(I) chain OS=Mus musculus OX=10090 GN=Col1a2 PE=1 SV=2	Col1a2	130 kDa	1.1	1.1	1	506	367	328	347	313	
Collagen alpha-2(IV) chain OS=Mus musculus OX=10090 GN=Col4a2 PE=1 SV=4	Col4a2	167 kDa	0.2	0.7	0.5	3	3	5	7	10	
Collagen alpha-2(V) chain OS=Mus musculus OX=10090 GN=Col5a2 PE=1 SV=1	Col5a2	145 kDa	1.4	0.8	0.4	17	4	9	12	14	
Collagen alpha-2(VI) chain OS=Mus musculus OX=10090 GN=Col6a2 PE=1 SV=3	Col6a2	110 kDa	0.1	1	0.9	1	1	1	1	1	
Collagen alpha-2(VIII) chain OS=Mus musculus OX=10090 GN=Col8a2 PE=2 SV=2	Col8a2	67 kDa	INF	0.5	0	4	0	1	1	2	
EMILIN-1 OS=Mus musculus OX=10090 GN=Emilin1 PE=1 SV=1	Emilin1	108 kDa	0.6	1	0	2		1	2	2	
Elastin OS=Mus musculus OX=10090 GN=Ein PE=1 SV=2	Ein	72 kDa	0.8	2.7	0.3	32	4	13	30	11	
Fibrinogen alpha chain OS=Mus musculus OX=10090 GN=Fga PE=1 SV=1	Fga	87 kDa	3.4	0.5	0.4	6	1	2	1	2	
Fibrinogen beta chain OS=Mus musculus OX=10090 GN=Fgb PE=1 SV=1	Fgb	55 kDa	INF	INF	INF	2		0	1		
Fibrinogen gamma chain OS=Mus musculus OX=10090 GN=Fgg PE=1 SV=1	Fgg	49 kDa	INF	INF	INF			0	3		
Fibronectin OS=Mus musculus OX=10090 GN=Fn1 PE=1 SV=4	Fn1	273 kDa	INF	INF	INF	1			9		
Galectin-3 OS=Mus musculus OX=10090 GN=Lgals3 PE=1 SV=3	Lgals3	28 kDa	INF	INF	INF	1			2		
Gelsolin OS=Mus musculus OX=10090 GN=Gsn PE=1 SV=3	Gsn	86 kDa	0.06	2.9	INF	1	0	9	3	1	
Laminin subunit alpha-2 OS=Mus musculus OX=10090 GN=Lama2 PE=1 SV=2	Lama2	344 kDa	0 INF	0 INF	INF	4		4	1	0	
Laminin subunit gamma-1 OS=Mus musculus OX=10090 GN=Lamc1 PE=1 SV=2	Lamc1	177 kDa	0.2 INF	0.2 INF	INF	1		3	1		
Microfibril-associated glycoprotein 4 OS=Mus musculus OX=10090 GN=Mfap4 PE=1 SV=1	Mfap4	29 kDa	INF	INF	INF	2			1		
Nidogen-1 OS=Mus musculus OX=10090 GN=Nid1 PE=1 SV=2	Nid1	137 kDa	0.4	1	0	2		3	1	1	
Periostin OS=Mus musculus OX=10090 GN=Postn PE=1 SV=2	Postn	93 kDa	0.7	1.4	0.7	13	3	4	10	7	
Tenascin OS=Mus musculus OX=10090 GN=Tnc PE=1 SV=1	Tnc	232 kDa	INF	INF	INF	2			2		
Transforming growth factor-beta-induced protein ig-h3 OS=Mus musculus OX=10090 GN=Tgfb1 PE=1 SV=1	Tgfb1	75 kDa	INF	INF	INF	0			2		

**Supplemental Table S4:** Pathway annotations for non-collagen type proteins identified in LC-MS/MS experiments. Pathways were annotated in Scaffold v5 via reactome.org

Pathway	% of Displayed Proteins			Protein Accession Numbers
	Ctrl	rhCI	rhCIII	
Chondroitin sulfate biosynthesis	3.03	3.13	0.00	P28653
Collagen biosynthesis and modifying enzymes	42.42	43.75	63.64	P25318, Q61245
Collagen chain trimerization	42.42	43.75	63.64	P25318, Q61245
Degradation of the extracellular matrix	12.12	12.50	13.64	P54320, Q05793, P11276, P10493
Dermatan sulfate biosynthesis	3.03	3.13	0.00	P28653
ECM proteoglycans	30.30	31.25	31.82	Q05793, P11276, P28653, Q80YX1
Fibronectin matrix formation	3.03	3.13	0.00	P11276
Non-integrin membrane-ECM interactions	30.30	31.25	40.91	Q05793, P11276, Q61245



**Supplemental Table S5:** MALDI-mapped endogenous mouse ECM peptides identified via LC-MS/MS proteomics. \*Mouse peptides that share homology sequences with human peptides (+) were mapped via MALDI-ECM-IMS, but were not used for the endogenous mouse differential expression experiments (**Fig. 4c**). Lowercase 'm' represents oxidation of Methionine.

Centroid [m/z]	Protein name	Alternate IDs	Peptide sequence	Human Homology*	Peptide identification probability	Discriminant Score	Hyperscore	Nextscore
1,013.51	Collagen alpha-2(I) chain	Col1a2	GPAGAAAGATGARG		99.70%	4.96	23.1	14.1
1052.5848	Collagen alpha-2(I) chain	Col1a2	GSVGPVAVGPR		99.70%	3.91	25.5	13.3
1064.5161	Elastin	Eln	FGAGAGVPGFGAG		99.60%	4.55	25.9	15.8
1082.6319	Collagen alpha-1(I) chain	Col1a1	IAGQRGVVGLP <sup>ox</sup>	+	99.40%	3.07	25.5	14.6
1088.5332	Collagen alpha-2(I) chain	Col1a2	GPVGRGTGETGAS		99.70%	4.49	23.5	12.6
1095.5181	Collagen alpha-1(I) chain	Col1a1	GPAGQDGRP <sup>ox</sup> GPA		99.50%	4.37	22.3	18.6
1139.6534	Collagen alpha-1(III) chain	Col3a1	GPLGIAGLTGARG		99.70%	5.68	32.5	19.9
1154.6056	Collagen alpha-2(I) chain	Col1a2	GKEGPVGLP <sup>ox</sup> GID	+	99.60%	4.27	24.5	16.8
1179.5577	Collagen alpha-1(I) chain	Col1a1	GARGQAGVmGFP <sup>ox</sup>	+	98.90%	3.45	22.1	13.8
1187.5806	Collagen alpha-2(I) chain	Col1a2	GPRGEAGNIGFP <sup>ox</sup>		99.70%	4.28	27.4	17.1
1229.5912	Collagen alpha-1(I) chain	Col1a1	GFP <sup>ox</sup> GERGVQGP <sup>ox</sup>	+	98.30%	3.78	28.5	26.7
1231.5954	Gelsolin	Gsn	LVADENPFAQGA	+	99.70%	5.38	28.1	14.9
1266.6327	Collagen alpha-2(I) chain	Col1a2	GEP <sup>ox</sup> GPAGSVGPVAV		98.80%	3.62	27.8	27.8
1267.6393	Collagen alpha-1(III) chain	Col3a1	GPP <sup>ox</sup> GTAGIP <sup>ox</sup> GARGGA		98.40%	3.47	22.4	20.2
1277.6962	Collagen alpha-2(I) chain	Col1a2	GPAGSVGPVAVGPR		99.40%	4.32	32.3	18
1283.6340	Collagen alpha-2(I) chain	Col1a2	GPAGARGSDGSVGPV	+	97.60%	3.77	24.2	15.1
1302.6916	Collagen alpha-1(I) chain	Col1a1	GPIGPAGARGPAGPQ		99.70%	6.91	32.5	15.1
1311.5968	Collagen alpha-1(I) chain	Col1a1	GPAGFAGPP <sup>ox</sup> GADGQP	+	99.10%	2.94	27.1	27.1
1312.6131	Collagen alpha-2(I) chain	Col1a2	GPDGNKGEAGAVGAP <sup>ox</sup>		97.10%	3.67	22.9	14.8
1323.7746	Collagen alpha-1(III) chain	Col3a1	GPLGIAGLTGARGLA		99.50%	4.99	24.2	16.3
1325.5973	Collagen alpha-1(III) chain	Col3a1	GPTGPAGDKGDSGP <sup>ox</sup> P		97.80%	3.73	23.3	23.3
1349.6336	Collagen alpha-2(V) chain	Col5a2	GPAGPAGEP <sup>ox</sup> GKEGP <sup>ox</sup> P		99.70%	4.83	23.7	23.7
1349.6812	Collagen alpha-1(IV) chain	Col4a1	IRGDP <sup>ox</sup> GPP <sup>ox</sup> GVQGPA		99.00%	4.2	30.8	26.5
1354.5584	Collagen alpha-1(I) chain	Col1a1	GPPGEP <sup>ox</sup> GEP <sup>ox</sup> GGSGPM		99.70%	5.17	32.8	28.2
1362.6401	Collagen alpha-1(I) chain	Col1a1	GPAGQDGRP <sup>ox</sup> GPAGP <sup>ox</sup> P		99.10%	4.41	25.4	25.4
1383.6689	Collagen alpha-2(I) chain	Col1a2	GLP <sup>ox</sup> GADGRAGVMGP <sup>ox</sup> P	+	99.50%	4.52	27.6	27.6
1395.6979	Collagen alpha-1(I) chain	Col1a1	GSP <sup>ox</sup> GTPGPQGIAGQR		99.70%	5.37	26.7	17.7
1399.6638	Collagen alpha-2(I) chain	Col1a2	GLP <sup>ox</sup> GADGRAGVmGP <sup>ox</sup> P	+	99.70%	6.07	27.8	27.8
1400.6444	Collagen alpha-2(V) chain	Col5a2	GPNGEQGSAGIP <sup>ox</sup> GPF	+	99.70%	3.36	22.5	17.4
1407.7594	Collagen alpha-2(I) chain	Col1a2	GPRGEVGLP <sup>ox</sup> GLSGPV	+	99.70%	4.13	34.9	22.2
1421.6481	Collagen alpha-2(I) chain	Col1a2	GmTGFP <sup>ox</sup> GAAGRTGP <sup>ox</sup> P	+	97.90%	3.41	19.4	19.4
1442.7060	Collagen alpha-1(I) chain	Col1a1	GLQGMP <sup>ox</sup> GERGAAGLP <sup>ox</sup>	+	98.90%	4	25.9	21.4
1472.6915	Collagen alpha-1(III) chain	Col3a1	GRDGTP <sup>ox</sup> GGP <sup>ox</sup> GIRGMP <sup>ox</sup>		99.70%	5.37	26.8	24.7
1484.6726	Collagen alpha-2(I) chain	Col1a2	GPAGASGDRGEAGAAGPS		99.70%	7.06	34.4	14.3
1508.7454	Collagen alpha-2(I) chain	Col1a2	GPAGARGSDGSVGPVAVGPA	+	99.60%	4.9	37.3	20.7
1518.8025	Collagen alpha-2(I) chain	Col1a2	GPAGSVGPVAVGPRGSPS		99.70%	6.86	29.4	15.6
1524.7768	Collagen alpha-2(I) chain	Col1a2	GAAGATGARGLVGE <sup>ox</sup> GPA	+	99.70%	8.82	44.5	39.6
1528.6513	Collagen alpha-1(I) chain	Col1a1	GSP <sup>ox</sup> GSP <sup>ox</sup> GEQGPSGASGPA		99.70%	5.43	33.3	24.4
1534.7613	Collagen alpha-1(III) chain	Col3a1	GPP <sup>ox</sup> GTAGIP <sup>ox</sup> GARGGAGP <sup>ox</sup> P		99.70%	6.27	32.1	32.1
1534.8116	Collagen alpha-2(I) chain	Col1a2	IAGALGEP <sup>ox</sup> GPLGISGP <sup>ox</sup> P		99.70%	4.83	44.9	42.4
1566.7035	Collagen alpha-1(I) chain	Col1a1	GPP <sup>ox</sup> GP <sup>ox</sup> AGEKGS <sup>ox</sup> GADGPA	+	98.90%	3.38	25.2	22.9
1578.7985	Collagen alpha-2(I) chain	Col1a2	GPVGPAGVRSQGSQGP		97.20%	2.79	17.2	11.1
1579.7964	Tenascin	Tnc	LDNLSKITAQQQYE		99.70%	10.5	34.7	13.2
1580.8759	Collagen alpha-1(III) chain	Col3a1	GSP <sup>ox</sup> GPLGIAGLTGARGLA		99.20%	4.25	22.7	22.7
1584.7327	Collagen alpha-1(III) chain	Col3a1	GSP <sup>ox</sup> GGKGEMGPAGIP <sup>ox</sup> GAP <sup>ox</sup>	+	97.50%	3.49	26.3	24.5
1585.7720	Collagen alpha-1(I) chain	Col1a1	GANGAP <sup>ox</sup> GIAGAP <sup>ox</sup> GFP <sup>ox</sup> GAR	+	99.70%	3.8	24.2	16.4
1594.6656	Periostin	Postn	FAPSNEAWENLSD		99.70%	7.46	33	16.4
1625.7770	Collagen alpha-1(I) chain	Col1a1	GPP <sup>ox</sup> GSAGSP <sup>ox</sup> GKDGLNGLP <sup>ox</sup>		98.00%	3.71	24.3	22.1
1644.7140	Collagen alpha-2(I) chain	Col1a2	FQGPAGEP <sup>ox</sup> GEP <sup>ox</sup> GQTGP <sup>ox</sup> A	+	99.70%	4.79	24.7	23
1672.7752	Collagen alpha-2(I) chain	Col1a2	GPP <sup>ox</sup> GMTGFP <sup>ox</sup> GAAGRTGP <sup>ox</sup> P	+	99.70%	5.14	24.4	24.4
1674.9177	Collagen alpha-1(V) chain	Col5a1	GPP <sup>ox</sup> GEVIQPLPIQASR	+	99.70%	5.87	28.1	25.9
1698.8157	Collagen alpha-2(I) chain	Col1a2	GPRGDQGPVGRGTGETGAS		99.00%	4.49	20.6	11.6
1699.8184	Collagen alpha-2(I) chain	Col1a2	GSRGLP <sup>ox</sup> GADGRAGVmGP <sup>ox</sup> P	+	99.70%	5.25	27.1	27.1
1710.8774	Collagen alpha-1(I) chain	Col1a1	GPKGTAGEP <sup>ox</sup> GKAGERGLP <sup>ox</sup>		99.70%	8.41	33	16.9
1743.7608	Collagen alpha-1(V) chain	Col5a1	GTTGPTGQM GDP <sup>ox</sup> GERGP <sup>ox</sup> P		99.70%	4.4	20.7	20.7
1767.8624	Collagen alpha-2(I) chain	Col1a2	GSAGASGPGGLP <sup>ox</sup> GERGAAGIP <sup>ox</sup>		99.70%	5.26	31.4	22.2
1795.8098	Collagen alpha-1(I) chain	Col1a1	GPAGEKGS <sup>ox</sup> GADGPAGSP <sup>ox</sup> GTP		99.70%	4.95	24.7	24.7
1908.8939	Collagen alpha-1(I) chain	Col1a1	GEAGKP <sup>ox</sup> GEQGVPGDLGAP <sup>ox</sup> GPS	+	99.10%	4	26.9	26.9
1913.8475	Collagen alpha-1(I) chain	Col1a1	GLAGP <sup>ox</sup> PGESGREGSP <sup>ox</sup> GAEGSP <sup>ox</sup>		98.40%	2.73	17.7	17.7
2142.9943	Collagen alpha-2(I) chain	Col1a2	GP <sup>ox</sup> PGFVGEKGPSGEP <sup>ox</sup> GTAGAP <sup>ox</sup> GTA		99.70%	8.73	34.9	34.9
2278.0489	Collagen alpha-2(I) chain	Col1a2	GPQGFQGPAGEP <sup>ox</sup> GEP <sup>ox</sup> GQTGPAGPR		98.80%	3.59	39.9	32.8
2279.0152	Collagen alpha-1(I) chain	Col1a1	GPQGFQGPAGEP <sup>ox</sup> GEP <sup>ox</sup> GGSGPMGPR		99.00%	3.18	37.6	29

**Supplemental Table S6:** Significantly Differentially Expressed Endogenous Mouse Peptides (ROC AUC); whole heart and infarct ROI comparisons. AUC thresholds of this study were 0.6.

Centroid [m/z]	ppm	Alternate IDs	Peptide sequence	Ctrl-rhCI AUC whole heart	Ctrl-rhCIII AUC whole heart	rhCI-rhCIII AUC whole heart	Ctrl-rhCI AUC at Infarct	Ctrl-rhCIII AUC at Infarct	rhCI-rhCIII AUC at Infarct
1088.5332	7.83	Col1a2	GPVGRTGETGAS	<b>0.651</b>	0.532	<b>0.618</b>	0.508	0.550	0.548
1187.5806	7.42	Col1a2	GPRGEAGNIGFP <sup>ox</sup>	<b>0.600</b>	0.505	<b>0.608</b>	0.547	0.530	0.517
1302.6916	12.14	Col1a1	GPIGPAGARGPAGPQ	<b>0.630</b>	0.543	<b>0.619</b>	0.536	0.566	<b>0.748</b>
1349.6466	15.72	Col5a2	GPAGPAGEP <sup>ox</sup> GKEGP <sup>ox</sup> P	<b>0.683</b>	0.549	<b>0.628</b>	0.527	0.517	0.544
1534.7613	14.11	Col3a1	GPP <sup>ox</sup> GTAGIP <sup>ox</sup> GARGGAGP <sup>ox</sup> P	0.406	0.584	<b>0.662</b>	<b>0.600</b>	<b>0.800</b>	<b>0.710</b>
1698.8173	11.30	Col1a2	GPRGDQGPVGRTGETGAS	0.499	0.574	0.571	<b>0.610</b>	<b>0.744</b>	0.580
1743.7608	5.50	Col5a1	GTTGPTGQM GDP <sup>ox</sup> GERGP <sup>ox</sup> P	0.528	0.576	0.547	0.580	<b>0.680</b>	<b>0.607</b>
2142.9943	12.49	Col1a2	GP <sup>ox</sup> PGFVGEKGPSGEP <sup>ox</sup> GTAGAP <sup>ox</sup> GTA	0.539	0.564	0.526	<b>0.657</b>	<b>0.674</b>	0.543