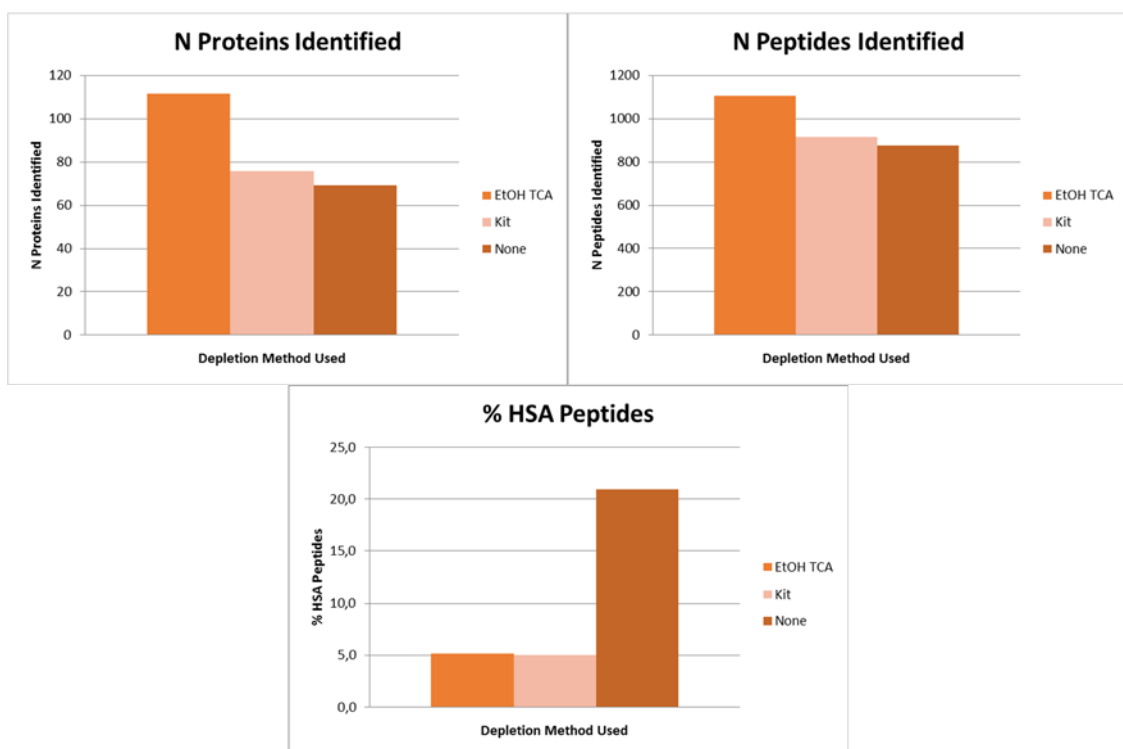


# 1. Annex I: Supplementary Material of Chapter I.

**Annex I. Supplementary data I.** HSA depletion results after comparing two strategies: TAC/EtOH chemical depletion and the commercial Thermo HSA depletion kit.

Depletion Method	EtOH/TCA				HSA Kit				None					
	S1	S2	S3	Average	S3 (HSA)	S1	S2	S3	Average	S3 (HSA)	S1	S2	S3	Average
<b>Serum Most Abundant Proteins &amp; Immunoglobulins</b>														
Acid-1-Glycoprotein	10	6	5	7	2	13	7	6	9	2	15	10	9	11
HSA	63	55	53	<b>57</b>	<b>253</b>	37	46	54	<b>46</b>	<b>207</b>	185	167	195	<b>182</b>
Apolipoprotein A1	18	11	11	13	39	20	25	24	23	18	31	28	29	29
Apolipoprotein A2	1	1	1	1	6	3	7	5	5	1	3	5	4	4
Apolipoprotein A3														
Apolipoprotein B	44	41	34	40	8				27	2	17	12	8	12
Ceruloplasmin	35	40	28	34			23	31		3	24	20	18	21
Complement C1q	5	6	9	7						6	3	2	3	3
Complement C3	67	60	76	68	14	24	24	41	30	66	56	42	54	51
Complement C4	43	34	36	38		5	3	7	5	37	26	15	21	21
Fibrinogen				1			4	4	4					
Haptoglobin	27	28	24	26	5	43	53	37	44	5	27	20	19	22
IgAs	15	17	26	<b>19</b>	<b>3</b>	22	28	24	<b>25</b>	13	12	7	10	<b>10</b>
IgDs														
IgGs	93	76	78	<b>82</b>	<b>12</b>	94	82	83	<b>86</b>	<b>40</b>	36	35	24	<b>32</b>
IgMs	34	25	29	<b>29</b>	<b>4</b>	31	14	22	<b>22</b>	20	24	3	20	<b>16</b>
Plasminogen	24	16	24	21	2					25	11	3	13	9
Prealbumin	6	6	8	7		8	10	10	9	2	1	1	1	1
Transferrin	40	55	44	46	34	72	103	81	85	12	41	48	38	42
α-1-Antitrypsin	25	18	12	18	13	28	15	20	21	2	20	9	11	13
α-2-Macroglobulin	59	70	71	67	10	86	55	73	71	21	53	51	51	52
<b>N of Proteins Identified</b>	<b>113</b>	<b>109</b>	<b>113</b>	<b>112</b>	<b>52</b>	<b>76</b>	<b>77</b>	<b>74</b>	<b>76</b>	<b>81</b>	<b>75</b>	<b>63</b>	<b>70</b>	<b>69</b>
<b>N of Peptides Identified</b>	<b>1136</b>	<b>1089</b>	<b>1087</b>	<b>1104</b>	<b>632</b>	<b>914</b>	<b>921</b>	<b>907</b>	<b>914</b>	<b>894</b>	<b>962</b>	<b>765</b>	<b>897</b>	<b>875</b>
% HSA Peptides	5,6	5,1	4,9	<b>5,2</b>	<b>40,0</b>	4,0	5,0	6,0	<b>5,0</b>	<b>23,2</b>	19,2	21,8	21,7	<b>20,9</b>
% IgG Peptides	8,2	7,0	7,2	<b>7,4</b>	<b>1,9</b>	10,3	8,9	9,2	<b>9,4</b>	<b>4,5</b>	3,7	4,6	2,7	<b>3,7</b>
% IgA Peptides	1,3	1,6	2,4	<b>1,8</b>	<b>0,5</b>	2,4	3,0	2,6	<b>2,7</b>	<b>1,5</b>	1,2	0,9	1,1	<b>1,1</b>
% IgM Peptides	3,0	2,3	2,7	<b>2,7</b>	<b>0,6</b>	3,4	1,5	2,4	<b>2,4</b>	<b>2,2</b>	2,5	0,4	2,2	<b>1,7</b>

After performing nanoLC-MALDI-TOF/TOF analysis of the 3 sera (S1, S2 and S3), under the 3 depletion conditions (EtOH/TCA, HSA kit and none), protein identifications were attained using the ProteinPilot software under the same searching and processing modes. The precedent table shows some of this information, particularly the most abundant serum/plasma proteins as well as the most frequent immunoglobulins. Besides, it indicates likewise the total number of proteins and peptides identified and from them, the % of the peptides which appertained to HSA and immunoglobulins. As it can be observed, the number of proteins identified is higher when using the EtOH/TCA depletion method (average: 112) than when using the HSA kit (76) or none of them (69). The same trend is followed with the number of peptides (1104 vs 914 vs 875, respectively). Hence, using the EtOH/TCA method, we could achieve a much better identification than when using either the other commercial method or none. Moreover, both depletion methods (the chemical and the commercial) reveal a decreased presence of HSA peptides in comparison with the total number of peptides (around 5%) when comparing them with the non-depleted samples (20.9%). These outcomes are illustrated in the following 3 charts.



There is also a noticeable peptide number increase, considering the different immunoglobulins and the rest of the most abundant proteins, when any of the depletion methods are conducted. This entails a wider sequence coverage of the proteins identified, improving as well the LC-MS/MS analysis. Furthermore, the phases where the HSA was supposed to remain on the two depletions studied were also analyzed for one of the serum samples (S3). In the corresponding protocols, for the EtOH/TCA method, this phase is the supernatant; while in the HSA kit, it is the elution phase, both named in the table as S3 (HSA). A higher percentage and number of HSA peptides is noticed when performing the EtOH/TCA method (253 peptides, 40%) in comparison with the other depletion method (207 peptides, 23.2%) or no depletion. In contrast, the total number of identified proteins and peptides is larger when the commercial method is carried out (81 proteins and 894 peptides vs 52 proteins and 632 peptides). From this data, it is presumed that with the commercial depletion, a greater number of proteins was also depleted, while the proportion of HSA peptides was almost 2 times lower than with the EtOH/TCA method. All in all, EtOH/TCA depletion method seems to be effective considering that it lowers down the number and proportion of HSA peptides while it also enriches the protein identification of the samples with a greater number of proteins and peptides. A similar propensity is followed when considering the commercial kit, although it is conformed in a smaller degree. In addition, the supposed remaining HSA phase contains more HSA (in terms of peptides and proportion of peptides) and a lower number of other different proteins and peptides when using the EtOH/TCA method than the commercial kit.

**Annex I. Supplementary data II.** Proteins commonly and exclusively identified by nanoLC-MALDI-TOF/TOF and nanoLC-ESI-tTOF.

This data is included in an excel in the attached CD.

**Annex I. Supplementary data III.** Intra- and inter-plate protein variability calculated with the triplicate measurements of the RA pool.

SAA1 (RA pool)	I	[SAA1] (ng/mL)	[SAA1] plasma (µg/mL)	Average Intraplate	CV Intraplate	CV Interplate
P1	0,8386	229,3434817	4,586869634	4,82	9,06	17,65
	0,9511	265,9772906	5,319545813			
	0,8319	227,1380914	4,542761829			
P2	0,8548	169,1244231	3,382488461	3,34	11,03	
	0,9229	184,382656	3,687653119			
	0,7578	147,6916248	2,953832496			
P3	0,8843	190,6947144	3,813894288	4,13	7,84	
	0,9473	205,9976234	4,119952467			
	1,0155	223,0740555	4,46148111			
P4	0,9487	198,2143388	3,964286775	3,46	14,17	
	0,8429	172,0901336	3,441802673			
	0,7482	149,1919068	2,983838136			
P5	0,8991	203,717042	4,07434084	4,66	10,86	
	1,0737	248,7182108	4,974364215			
	1,0642	246,2232027	4,924464055			

AACT (RA pool)	I	[AACT] (pg/mL)	[AACT] plasma (µg/mL)	Average Intraplate	CV Intraplate	CV Interplate
P1	0,8886	1368,921627	1368,921627	1380,34	5,29	17,41
	0,9539	1458,36996	1458,36996			
	0,8487	1313,722463	1313,722463			
P2	0,9357	2196,250267	2196,250267	2064,43	8,09	
	0,8071	1876,621035	1876,621035			
	0,9053	2120,432643	2120,432643			
P3	0,8837	1742,486026	1742,486026	1605,11	7,53	
	0,776	1514,969746	1514,969746			
	0,7964	1557,881751	1557,881751			
P4	0,8324	1951,227758	1951,227758	1821,58	7,73	
	0,785	1841,628175	1841,628175			
	0,7122	1671,878705	1671,878705			
P5	0,7871	1391,903622	1391,903622	1394,00	0,28	
	0,7869	1391,562893	1391,562893			
	0,791	1398,544584	1398,544584			

HPT (RA pool)	I	[HPT] (µg/mL)	[HPT] plasma (µg/mL)	Average Intraplate	CV Intraplate	CV interplate
P1	1,2388	6441,219701	1610,304925	1295,32	34,39	20,44
	0,5236	864,9531348	216,2382837			
	1,1578	3921,346756	980,336689			
P2	0,9585	2392,976219	598,2440547	523,82	13,84	
	0,8119	1813,759446	453,4398615			
	0,8841	2079,153804	519,788451			
P3	1,035	2554,200939	638,5502347	609,21	6,55	
	1,0271	2501,171644	625,292911			
	0,985	2255,192293	563,7980733			
P4	1,0251	2881,509306	720,3773266	335,86	0,38	
	0,6996	1347,099631	336,7749078			
	0,6971	1339,800737	334,9501842			
P5	1,0052	2120,011428	530,002857	498,43	6,75	
	0,9372	1852,172078	463,0430194			
	0,9786	2008,957099	502,2392747			

A1AG (RA pool)	I	[A1AG] (µg/mL)	[A1AG] plasma (µg/mL)	Average Intraplate	CV Intraplate	CV interplate
P1	1,0764	780,9959768	780,9959768	1203,58	49,65	31,39
	1,8004	1626,165028	1626,165028			
	-	-	-			
P2	1,1683	801,8279348	801,8279348	844,13	7,09	
	1,2551	886,4236038	886,4236038			
	3,2442	4363,631103	4363,631103			
P3	1,8056	1373,513879	1373,513879	1392,99	19,59	
	2,0678	1675,107348	1675,107348			
	1,5679	1130,352851	1130,352851			
P4	0,995	711,0890653	711,0890653	834,91	13,73	
	1,1572	856,2734995	856,2734995			
	1,2448	937,3576705	937,3576705			
P5	1,4128	1159,070883	1159,070883	2104,78	63,54	
	2,6412	3050,482625	3050,482625			
	2,833	-	-			

**Annex I. Supplementary data IV.** Statistics for each protein measured by ELISA with or without median normalization of the data and/or outlier removal.

SAA1 Group Comparison	Median Normalization			Median Normalization without outliers			No Normalization		
	Kruskal-Wallis: < 0.0001	Mann Whitney		Kruskal-Wallis: < 0.0001	Mann Whitney		Kruskal-Wallis: < 0.0001	Mann Whitney	
HD vs RA	-151.2	****	<0.0001	-154.7	****	<0.0001	-151.2	***	<0.0001
HD vs SLE	-90.81	****	<0.0001	-94.41	****	<0.0001	-90.31	***	<0.0001
HD vs PsA	-88.91	****	<0.0001	-87.14	****	<0.0001	-87.63	***	<0.0001
RA vs SLE	60.38	***	<0.0001	60.26	***	<0.0001	60.92	***	<0.0001
RA vs PsA	62.29	***	<0.0001	67.53	***	<0.0001	63.61	***	<0.0001
SLE vs PsA	1.905	ns	0.9606	7.269	ns	0.7417	2.684	ns	0.8921

SAA1 Group Comparison	Median Normalization			Median Normalization without outliers			No Normalization		
	Kruskal-Wallis: < 0.0001	Mann Whitney		Kruskal-Wallis: < 0.0001	Mann Whitney		Kruskal-Wallis: < 0.0001	Mann Whitney	
HD vs HA-RA	-181.5	****	<0.0001	-184,9	****	<0.0001	-181.3	****	<0.0001
HD vs LA-RA	-117.5	****	<0.0001	-121	****	<0.0001	-117.7	****	<0.0001
HD vs HA-SLE	-105.1	****	<0.0001	-108,7	****	<0.0001	-108.5	****	<0.0001
HD vs LA-SLE	-76.86	*	<0.0001	-80,46	**	<0.0001	-72.56	*	<0.0001
HD vs HA-PsA	-112.3	****	<0.0001	-111,1	****	<0.0001	-115.5	****	<0.0001
HD vs LA-PsA	-66.13	ns	0.0006	-63,82	ns	0.0006	-60.48	ns	0.0015
HA-RA vs LA-RA	64.03	**	<0.0001	63,86	**	<0.0001	63.63	**	0.0001
HA-SLE vs LA-SLE	28.26	ns	0.2538	28,26	ns	0.2538	35.97	ns	0.1454
HA-PsA vs LA-PsA	46.15	ns	0.0549	47,26	ns	0.046	55.01	ns	0.0209
HA-RA vs HA-SLE	76.37	*	0.0002	76,16	*	0.0002	72.81	*	0.0004
HA-RA vs HA-PsA	69.21	*	0.0006	73,8	*	0.0002	65.86	ns	0.0011

AACT Group Comparison	Median Normalization			No Normalization		
	Kruskal-Wallis: < 0.0001	Mann Whitney		Kruskal-Wallis: < 0.0001	Mann Whitney	
HD vs RA	-132.6	****	<0.0001	-122.9	***	<0.0001
HD vs SLE	-85.79	****	<0.0001	-78.11	***	<0.0001
HD vs PsA	-83.58	****	<0.0001	-75.80	***	<0.0001
RA vs SLE	46.84	*	0.0022	44.83	*	0.0042
RA vs PsA	49.05	*	0.0007	47.14	*	0.0015
SLE vs PsA	2.219	ns	0.7979	2.306	ns	0.8471

AACT Group Comparison	Median Normalization			No Normalization		
	Kruskal-Wallis: < 0.0001	Mann Whitney		Kruskal-Wallis: < 0.0001	Mann Whitney	
HD vs HA-RA	-173.5	****	<0.0001	-162.4	****	<0.0001
HD vs LA-RA	-85.55	***	<0.0001	-77.47	***	<0.0001
HD vs HA-SLE	-122.5	****	<0.0001	-107.6	****	<0.0001
HD vs LA-SLE	-49.11	ns	0.0147	-48.67	ns	0.0207
HD vs HA-PsA	-115.1	****	<0.0001	-99.53	***	<0.0001
HD vs LA-PsA	-52.03	ns	0.0021	-52.08	ns	0.003
HA-RA vs LA-RA	87.96	****	<0.0001	84.94	***	<0.0001
HA-SLE vs LA-SLE	73.38	ns	0.0042	58.89	ns	0.0196
HA-PsA vs LA-PsA	63.09	ns	0.0051	47.45	ns	0.0418
HA-RA vs HA-SLE	51.02	ns	0.012	54.86	ns	0.0109
HA-RA vs HA-PsA	58.39	ns	0.0015	62.89	ns	0.002

HPT Group Comparison	Median Normalization			No Normalization		
	Kruskal-Wallis: < 0,0001	Mann Whitney		Kruskal-Wallis: < 0,0001	Mann Whitney	
HD vs RA	-87.91	****	<0.0001	-81,28	****	<0.0001
HD vs SLE	-38.23	ns	0.0241	-35,07	ns	0.0337
HD vs PsA	-71.83	***	0.0002	-68,28	**	0.0006
RA vs SLE	49.68	*	0.0019	46,21	*	0.0034
RA vs PsA	16.08	ns	0.3764	13	ns	0.5092
SLE vs PsA	-33.61	ns	0.0635	-33,21	ns	0.0696

HPT Group Comparison	Median Normalization			No Normalization		
	Kruskal-Wallis: < 0,0001	Mann Whitney		Kruskal-Wallis: < 0,0001	Mann Whitney	
HD vs HA-RA	-125.4	***	< 0.0001	-114.7	****	<0.0001
HD vs LA-RA	-44.09	ns	0.0171	-42.27	ns	0.0327
HD vs HA-SLE	-40.45	ns	0.0631	-72	*	0.0007
HD vs LA-SLE	-36.01	ns	0.0758	1.86	ns	0.9388
HD vs HA-PsA	-120.5	***	< 0.0001	-142	****	<0.0001
HD vs LA-PsA	-23.2	ns	0.5039	5.397	ns	0.5087
HA-RA vs LA-RA	81.31	***	< 0.0001	72.39	**	0.0001
HA-SLE vs LA-SLE	4.438	ns	0.8663	73.86	ns	0.0025
HA-PsA vs LA-PsA	97.28	**	0.0013	147.4	****	<0.0001
HA-RA vs HA-SLE	84.95	**	0.0002	42.65	ns	0.0553
HA-RA vs HA-PsA	4.928	ns	0.8106	-27.3	ns	0.1653

A1AG Group Comparison	Median Normalization			No Normalization		
	Kruskal-Wallis: < 0,0001	Mann Whitney		Kruskal-Wallis: 0,0003	Mann Whitney	
HD vs RA	-75.16	****	<0.0001	-57.73	***	0.0001
HD vs SLE	-85.63	****	<0.0001	-69.05	***	0.0003
HD vs PsA	-55.64	*	0.0009	-41.44	ns	0.0185
RA vs SLE	-10.47	ns	0.499	-11.32	ns	0.3942
RA vs PsA	19.53	ns	0.1921	16.29	ns	0.2806
SLE vs PsA	30	ns	0.092	27.61	ns	0.1567

A1AG Group Comparison	Median Normalization			No Normalization		
	Kruskal-Wallis: < 0,0001	Mann Whitney		Kruskal-Wallis: 0,0005	Mann Whitney	
HD vs HA-RA	-97.75	****	<0.0001	-77.49	***	< 0.0001
HD vs LA-RA	-50.17	ns	0.0063	-35.85	ns	0.0526
HD vs HA-SLE	-94.34	***	0.0001	-60.21	ns	0.0145
HD vs LA-SLE	-77.82	**	0.0002	-76.98	*	0.0008
HD vs HA-PsA	-74.44	*	0.001	-40.94	ns	0.0468
HD vs LA-PsA	-37.31	ns	0.0377	-41.93	ns	0.0711
HA-RA vs LA-RA	47.58	ns	0.0096	41.64	ns	0.028
HA-SLE vs LA-SLE	16.52	ns	0.4363	-16.77	ns	0.5808
HA-PsA vs LA-PsA	37.13	ns	0.1683	-0.9885	ns	0.9472
HA-RA vs HA-SLE	3.411	ns	0.9253	17.29	ns	0.5717
HA-RA vs HA-PsA	23.31	ns	0.3203	36.55	ns	0.0641



**Annex I. Supplementary data V. SAA1, AACT, HPT and A1AG ELISA data normalization** including the n factor determination and the data information of each of the groups analyzed.

SAA1 Normalization	Median	n factor (median)
All Plates	872,53	
P1	890,37	0,98
P2	835,07	1,04
P3	1092,01	0,80
P4	868,60	1,00
P5	750,15	1,16

SAA1	HD	AR	SLE	PsA
Number of values	79	167	79	77
<b>[SAA1] in plasma samples after Median Normalization &amp; Outlier Removal</b>				
Minimum	84,15	139	95,8	123,4
25% Percentile	264,1	769,7	503,2	390,6
Median	357,9	1445	774,3	883,8
75% Percentile	570,9	3343	1441	1369
Maximum	3528	25671	11718	5903
Mean	505,9	3223	1476	1154
Std. Deviation	461,6	4406	2083	1176
Std. Error of Mean	51,93	341	234,4	134,1
Lower 95% CI	402,5	2550	1010	886,5
Upper 95% CI	609,3	3896	1943	1421

SAA1	HD	HA-RA	LA-RA	HA-SLE	LA-SLE	HA-PsA	LA-PsA
Number of values	79	88	79	39	40	38	39
<b>[SAA1] in plasma samples after Median Normalization &amp; Outlier Removal</b>							
Minimum	84,15	247,8	139	249,9	95,8	231,2	123,4
25% Percentile	264,1	920,9	583	522,5	459,8	549,3	364,5
Median	357,9	2199	1159	881,2	703,6	1142	742,1
75% Percentile	570,9	4887	2003	1606	1133	1768	982
Maximum	3528	25671	15286	11718	6693	5903	2986
Mean	505,9	4319	2001	1882	1081	1477	838,8
Std. Deviation	461,6	5241	2795	2675	1174	1493	625,2
Std. Error of Mean	51,93	558,7	314,4	428,4	185,6	242,2	100,1
Lower 95% CI	402,5	3209	1375	1015	705,1	985,7	636,2
Upper 95% CI	609,3	5430	2627	2750	1456	1967	1042

AACT Normalization	Median	n factor (median)
All Plates	725,78	
P1	636,85	1,14
P2	824,89	0,88
P3	746,56	0,97
P4	863,79	0,84
P5	649,27	1,12

AACT	HD	AR	SLE	PsA
Number of values	90	170	80	80
<b>[AACT] in plasma samples (<math>\mu\text{g/mL}</math>) after Median Normalization</b>				
Minimum	281,3	233,9	26,67	398,2
25% Percentile	510,7	653,3	603,6	599,9
Median	581,7	821	736,3	723,2
75% Percentile	680,5	1048	861,3	826,2
Maximum	992,5	2556	2080	1290
Mean	602,2	897,3	760,1	741,2
Std. Deviation	144,7	360,5	273,5	184,3
Std. Error of Mean	15,25	27,65	30,57	20,61
Lower 95% CI	571,9	842,7	699,2	700,2
Upper 95% CI	632,5	951,8	821	782,2

AACT	HD	HA-RA	LA-RA	HA-SLE	LA-SLE	HA-PsA	LA-PsA
Number of values	90	91	79	40	40	40	40
<b>[AACT] in plasma samples (<math>\mu\text{g/mL}</math>) after Median Normalization</b>							
Minimum	281,3	437,9	233,9	26,67	348,1	398,2	446,2
25% Percentile	510,7	759	574,7	638,3	530,1	652,9	581,6
Median	581,7	916,7	719,5	794,9	695,1	789,6	675,1
75% Percentile	680,5	1182	957,7	985,5	799,5	900	755
Maximum	992,5	2556	1426	2080	1273	1290	1256
Mean	602,2	1015	762	838,2	682	797,2	685,2
Std. Deviation	144,7	391,5	264,8	326,3	180,3	198	152,3
Std. Error of Mean	15,25	41,04	29,8	51,59	28,51	31,3	24,08
Lower 95% CI	571,9	933,2	702,6	733,9	624,3	733,9	636,5
Upper 95% CI	632,5	1096	821,3	942,6	739,6	860,5	733,9

HPT Normalization	Median	n factor
All Plates	550,56	
P1	752,74	0,73
P2	578,48	0,95
P3	595,15	0,93
P4	489,87	1,12
P5	502,50	1,10

HPT	HD	AR	SLE	PsA
Number of values	90	167	80	80
<b>[HPT] in plasma samples (µg/mL) after Median Normalization</b>				
Minimum	50,18	38,19	26,66	150,6
25% Percentile	355,2	472,3	399,5	432,1
Median	468,7	592,8	507,5	564,5
75% Percentile	579,7	746,8	623,6	757
Maximum	1220	1949	1393	1933
Mean	468,5	671,8	520,8	648,4
Std. Deviation	171,1	344,2	228,9	358,4
Std. Error of Mean	18,03	26,64	25,6	40,07
Lower 95% CI	432,7	619,3	469,9	568,6
Upper 95% CI	504,4	724,4	571,8	728,2

HPT	HD	HA-RA	LA-RA	HA-SLE	LA-SLE	HA-PsA	LA-PsA
Number of values	90	90	77	40	40	40	40
<b>[HPT] in plasma samples (µg/mL) after Median Normalization</b>							
Minimum	50,18	286,3	38,19	46,17	26,66	288,5	150,6
25% Percentile	355,2	545,2	395,5	401,8	399,1	513,5	342,3
Median	468,7	635	537,3	502,8	511,9	677,1	499,1
75% Percentile	579,7	857	638,3	633,8	619,8	836,2	596,2
Maximum	1220	1949	1620	1393	888,9	1525	1933
Mean	468,5	767,6	559,9	540,2	501,4	746,3	550,5
Std. Deviation	171,1	370,8	272,5	262,2	191,4	339	354,5
Std. Error of Mean	18,03	39,08	31,06	41,46	30,26	53,6	56,05
Lower 95% CI	432,7	690	498	456,3	440,2	637,9	437,2
Upper 95% CI	504,4	845,3	621,7	624,1	562,6	854,7	663,9

A1AG Normalization	Median	n factor
All Plates	1170,94	
P1	945,37	1,24
P2	931,91	1,26
P3	1633,11	0,72
P4	782,02	1,50
P5	1926,83	0,61

A1AG	HD	AR	SLE	PsA
Number of values	87	158	74	77
<b>[A1AG] in plasma samples (<math>\mu\text{g/mL}</math>) after Median Normalization</b>				
Minimum	424,4	383,6	600,1	430,4
25% Percentile	771,5	956,8	947	942,3
Median	950,7	1279	1300	1163
75% Percentile	1232	1809	1945	1547
Maximum	3235	4973	3878	3721
Mean	1076	1486	1547	1359
Std. Deviation	524,2	795,4	763,8	705,7
Std. Error of Mean	56,2	63,28	88,79	80,42
Lower 95% CI	964,5	1361	1370	1199
Upper 95% CI	1188	1611	1724	1519

A1AG	HD	HA-RA	LA-RA	HA-SLE	LA-SLE	HA-PsA	LA-PsA
Number of values	87	83	75	35	39	38	39
<b>[A1AG] in plasma samples (<math>\mu\text{g/mL}</math>) after Median Normalization</b>							
Minimum	424,4	383,6	452,6	600,1	698,7	607,5	430,4
25% Percentile	771,5	1108	838,5	952,5	930,2	943,6	911,4
Median	950,7	1428	1120	1436	1292	1159	1179
75% Percentile	1232	1993	1651	2503	1823	1834	1447
Maximum	3235	4973	3866	3878	3118	3721	2096
Mean	1076	1630	1327	1698	1411	1555	1168
Std. Deviation	524,2	881	658,4	929,6	554,6	881,3	404,9
Std. Error of Mean	56,2	96,7	76,02	157,1	88,81	143	64,84
Lower 95% CI	964,5	1438	1176	1378	1231	1265	1037
Upper 95% CI	1188	1823	1479	2017	1591	1845	1300

**Annex I. Supplementary data VI.** Protein sequences and MRM measured peptides (in orange).

**AACT**

HPNSPLDEENLTQENQDRGTHVDLGLASANVDFAFSLYKQLVLKAPDKNVIFSPLSISTALAFSLGA  
HNTTLEILKGLKFNLTETSEAEIHQSFQHLLRTLNQSSDELQLSMGNAMFVKEQLSLLDRFTEDAKR  
LYGSEAFATDFQDSAAAKKLINDYVKNTRGKITDLIKDLDSQTMMVLVNYIFFKAKWEMPFDPOD  
THQSRFYLSKKKWVMPMMSLHHLTIPYFRDEELSCTVVELKYTGNASALFILPDQDKMEEVEAM  
LLPETLKRWRDSLEFR**EIGELYLPK**FSISRDYNLNDILLQLGIEEAFTSKADLSGITGARNLAVSQVVHK  
**AVLDVFEEGTEASAATAVK**ITLLSALVETRTIVRFNRPFMIIVPTDTQNIFFMSKVTPNKQA

**A1AG1**

QIPLCANLVPVITNATLDQITGKWFYIASAFRNEEYNKSVQEIQATFFYFTPNKTEDTIFLREYQTRQ  
DQCIYNTTYLNVQRENGTISR**YVGGQEHFAHLLLR**DTKTYMLAFDVNDEKNWGLSVYADKPETT  
**KEQLGEFYEALDCLRIPK/SDVVYTDWK**KDKCEPLEKQHEKERKQEEGES

**A1AG2**

QIPLCANLVPVITNATLDRLITGKWFYIASAFRNEEYNKSVQEIQATFFYFTPNKTEDTIFLREYQTRQ  
NQCFYNSSYLNVQRENGTVSRYEGGREHVAHLLFLRDTKTLMFSGSYLDDEKNWGLSFYADKPETT  
**KEQLGEFYEALDCLCIPR**SDVMYTDWKKDKCEPLEKQHEKERKQEEGES

**HPT ALPHA CHAIN / BETA CHAIN**

VDSGNDVTDIADDGCPKPPEIAHGYVEHSVRYQCKNYYKLRTGEGDVVYTLNDKKQWINKAVGDKL  
PECEADDGCPKPPEIAHGYVEHSVRYQCKNYYKLRTGEGDVVYTLNNEKQWINKAVGDKLPECEAV  
CGKPKNPANPVQ /  
RILGGHLDAGSFPWQAKMVSHHNLTTGATLINEQWLLTTAKNLFNLHSENATAKDIAPTLTLYVGK  
KQLVEIEKVVLPNYSQVDIGLIKQKQVSVNERVMPICLPSKDYAEVGR**VGIVSGWGR**NANFKFT  
DHLKYVMLPVADQDQCIRHYEGSTVPEKKTSPVGVQPILNEHTFCAGMSKYQEDTCYGDAGSA  
FAVHLEEDTWYATGILSFDKSCAVAEYGVYVK**VTSIQDWVQK**TIAEN

**SAA1**

**RSFFSFLGEAFD GAR**DMWRAYS DMREANYIGSDKYFHARGNYDAAKRGPGGVWAAEAISDARE  
NIQRFFGHGAEDSLADQAANEWGRSGKDPNHFRPAGLPEKY

## 2. Annex II: Supplementary Material of Chapter II.

**Annex II. Supplementary data I.** Discovery phase results. Number of endogenous peptides and their corresponding unique proteins identified in the secretomes of human articular cartilage.

Unique proteins corresponding to the endogenous peptides identified in this work.								
Protein name	Number of neopeptides found in each cartilage secretome sample							
	UZ1	WZ1	UZ2	WZ2	UZ3	WZ3	N1	N2
[Pyruvate dehydrogenase [acetyl-transferring]]-phosphatase 1, mitochondrial			2					
Adipocyte enhancer-binding protein 1	2	4						
Aggrecan core protein			4		2		5	2
Annexin A1	2			2	2			
Apolipoprotein A-I	2	9						
Apolipoprotein A-IV	6	22						
Apolipoprotein C-III		3						
Apolipoprotein E	21	23	3	4			2	
Arginase-1				2				
Augurin	5	4	4	4	2		2	3
Biglycan	7	17	2		5	2	2	
Cartilage intermediate layer protein 1		16	7	9	4		14	6
Cartilage intermediate layer protein 2	2	6	3	5	2			2
Cartilage oligomeric matrix protein	23	35	47	28	17	6	41	25
Chitinase-3-like protein 2		3		2				
Chondroadherin			3	2				
Clusterin	23	56	26	25	12	6	18	21
Coagulation factor V		2						
Collagen alpha-1(I) chain					5	2		
Collagen alpha-1(II) chain	7		13	4	9	3	6	
Collagen alpha-1(III) chain	2		3		5	2	6	
Collagen alpha-1(IV) chain				2				
Collagen alpha-1(XXIV) chain		2	2	3				
Collagen alpha-1(XXV) chain					2			
Collagen alpha-2(I) chain				4	5			
Collagen alpha-2(IV) chain				3				
Collagen alpha-2(V) chain					5	2		
Collagen alpha-2(XI) chain					5			
Collagen alpha-5(IV) chain		2						
Collagen alpha-6(IV) chain			2					
Complement C1s subcomponent		3						
Complement C3	3	9	2					
Corneodesmosin				2				
Cubilin		3						
Decorin	13	34	2					
Dermcidin						2		
Desmocollin-1	2		3	7				
Desmoglein-1			3	2				
Fibrinogen alpha chain	28	48	9	27		3	5	
Fibrinogen beta chain	3	25		5				
Fibrinogen gamma chain	6	15		6	2			
Fibromodulin	7	16	5				4	2
Fibronectin	5	9	17	14	15	2	20	22
Glia-derived nexin	14	5	4					
Glyceraldehyde-3-phosphate dehydrogenase			4	10	5	2		
Heat shock 70 kDa protein 1A/1B	3							
Hemoglobin subunit alpha	14	15			17	8		
Hemoglobin subunit beta	18	18			24	24		
Hemoglobin subunit delta	8	3						
Hemoglobin subunit epsilon	2							
Histone H2B type 1-A			2					

Unique proteins corresponding to the endogenous peptides identified in this work.								
Protein name	Number of neopeptides found in each cartilage secretome sample							
	UZ1	WZ1	UZ2	WZ2	UZ3	WZ3	N1	N2
Inter-alpha-trypsin inhibitor heavy chain H2		9						
Keratin, type I cytoskeletal 10				3		4		
Keratin, type I cytoskeletal 24		2						
Keratin, type I cytoskeletal 9	6	3	20	16		6	8	12
Keratin, type II cytoskeletal 1			5					
Lactadherin				2			2	
Latent-transforming growth factor beta-binding protein 2	5	4			3		3	2
Loricrin	2							
Matrix Gla protein	3		2		4			2
Melanoma-derived growth regulatory protein		2						
Mimecan		2						
Mucin-17		2						
Multiple epidermal growth factor-like domains protein 10	2							
Myocyte-specific enhancer factor 2B				2				
Netrin-1	2	3						
Neuroblast differentiation-associated protein AHNAK			3	2				
Phospholipase A2, membrane associated		2	2					
Pleckstrin homology domain-containing family M member 2					2			
Plexin domain-containing protein 2			2					
Probable E3 ubiquitin-protein ligase HECTD4					2			
Procollagen C-endopeptidase enhancer 2		2						
Prolargin	16	61	26	19	7		10	12
Proteoglycan 4	12	15			5		4	
Protocadherin Fat 4		3						
Pyruvate kinase isozymes M1/M2		5		3	2		2	
Rap guanine nucleotide exchange factor-like 1				2				
Retinoic acid receptor responder protein 2		2						
Ribonuclease 4		2						
Salivary acidic proline-rich phosphoprotein 1/2						3		
Semaphorin-3D	2						2	2
Serine protease HTRA1	2	29	2	2	2			
Serine protease HTRA3		7						
Serum albumin			2	4				2
Serum amyloid A-1 protein			5	3			2	
Serum amyloid A-2 protein			3	2			2	
Short stature homeobox protein 2	3							
SPARC-related modular calcium-binding protein 2		6						
Stromelysin-1			5				2	
Synovial phospholipase-A2					2			
Target of Nesh-SH3		4						
Tenascin		3						
Threonine aspartase 1				2				
Thrombospondin-3	2	6	7	6			4	2
Thrombospondin-4	11	12	18	9			13	7
Transforming growth factor-beta-induced protein ig-h3		2		2				
Transthyretin	18	10	2					
Tubulin beta-4B chain					2			
Tumor necrosis factor receptor superfamily member 11B	5	8		2				2
Vimentin	15				3		11	
Vitronectin		2						



**Annex II. Supplementary data II. Targeted proteomics design. MRM mass spectrometry transitions analyzed in this work, and settings for their analysis.**

Transitions and settings for MRM analysis.							
Q1 m/z	Q3 m/z	RT	Compound name	DP (volts)	CE (volts)	EP	CXP
565.788	687.331	20	sp P51888 PRELP_HUMAN.DSNKIETIPN.+2b6.light	72.4	29.2	10	15
565.788	788.378	20	sp P51888 PRELP_HUMAN.DSNKIETIPN.+2b7.light	72.4	29.2	10	15
565.788	901.463	20	sp P51888 PRELP_HUMAN.DSNKIETIPN.+2b8.light	72.4	29.2	10	15
568.795	687.331	20	sp P51888 PRELP_HUMAN.DSNKIETIPN.+2b6.heavy	72.4	29.2	10	15
568.795	788.378	20	sp P51888 PRELP_HUMAN.DSNKIETIPN.+2b7.heavy	72.4	29.2	10	15
568.795	901.463	20	sp P51888 PRELP_HUMAN.DSNKIETIPN.+2b8.heavy	72.4	29.2	10	15
483.232	763.398	18.5	sp P51888 PRELP_HUMAN.SDGVFKPDT.+2y7.light	66.3	26.3	10	15
483.232	460.24	18.5	sp P51888 PRELP_HUMAN.SDGVFKPDT.+2y4.light	66.3	26.3	10	15
486.239	769.412	18.5	sp P51888 PRELP_HUMAN.SDGVFKPDT.+2y7.heavy	66.3	26.3	10	15
486.239	466.254	18.5	sp P51888 PRELP_HUMAN.SDGVFKPDT.+2y4.heavy	66.3	26.3	10	15
499.233	823.394	19	sp P51888 PRELP_HUMAN.SSDLENVPH.+2y7.light	67.5	26.8	10	15
499.233	708.367	19	sp P51888 PRELP_HUMAN.SSDLENVPH.+2y6.light	67.5	26.8	10	15
502.741	830.412	19	sp P51888 PRELP_HUMAN.SSDLENVPH.+2y7.heavy	67.5	26.8	10	15
502.741	715.385	19	sp P51888 PRELP_HUMAN.SSDLENVPH.+2y6.heavy	67.5	26.8	10	15
546.793	864.469	23	sp P51888 PRELP_HUMAN.DLENVPHLR.+2y7.light	71	28.5	10	15
546.793	735.426	23	sp P51888 PRELP_HUMAN.DLENVPHLR.+2y6.light	71	28.5	10	15
546.793	621.383	23	sp P51888 PRELP_HUMAN.DLENVPHLR.+2y5.light	71	28.5	10	15
546.793	571.272	23	sp P51888 PRELP_HUMAN.DLENVPHLR.+2b5.light	71	28.5	10	15
551.798	874.477	23	sp P51888 PRELP_HUMAN.DLENVPHLR.+2y7.heavy	71	28.5	10	15
551.798	745.434	23	sp P51888 PRELP_HUMAN.DLENVPHLR.+2y6.heavy	71	28.5	10	15
551.798	631.391	23	sp P51888 PRELP_HUMAN.DLENVPHLR.+2y5.heavy	71	28.5	10	15
551.798	571.272	23	sp P51888 PRELP_HUMAN.DLENVPHLR.+2b5.heavy	71	28.5	10	15
626.302	876.457	23.5	sp P02751 FINC_HUMAN.SSGSGPFTDVRAA.+2y8.light	76.8	31.4	10	15
626.302	779.405	23.5	sp P02751 FINC_HUMAN.SSGSGPFTDVRAA.+2y7.light	76.8	31.4	10	15
626.302	632.336	23.5	sp P02751 FINC_HUMAN.SSGSGPFTDVRAA.+2y6.light	76.8	31.4	10	15
628.305	880.464	23.5	sp P02751 FINC_HUMAN.SSGSGPFTDVRAA.+2y8.heavy	76.8	31.4	10	15
628.305	783.412	23.5	sp P02751 FINC_HUMAN.SSGSGPFTDVRAA.+2y7.heavy	76.8	31.4	10	15
628.305	636.343	23.5	sp P02751 FINC_HUMAN.SSGSGPFTDVRAA.+2y6.heavy	76.8	31.4	10	15
676.826	876.457	23.5	sp P02751 FINC_HUMAN.TSSGSGPFTDVRAA.+2y8.light	80.5	33.2	10	15
676.826	632.336	23.5	sp P02751 FINC_HUMAN.TSSGSGPFTDVRAA.+2y6.light	80.5	33.2	10	15
676.826	531.289	23.5	sp P02751 FINC_HUMAN.TSSGSGPFTDVRAA.+2y5.light	80.5	33.2	10	15
678.829	880.464	23.5	sp P02751 FINC_HUMAN.TSSGSGPFTDVRAA.+2y8.heavy	80.5	33.2	10	15
678.829	636.343	23.5	sp P02751 FINC_HUMAN.TSSGSGPFTDVRAA.+2y6.heavy	80.5	33.2	10	15
678.829	535.296	23.5	sp P02751 FINC_HUMAN.TSSGSGPFTDVRAA.+2y5.heavy	80.5	33.2	10	15
581.285	975.499	29.5	sp P81605 DCD_HUMAN.DAVEDLESVGK.+2y9.light	73.5	29.8	10	15
581.285	876.431	29.5	sp P81605 DCD_HUMAN.DAVEDLESVGK.+2y8.light	73.5	29.8	10	15
581.285	747.388	29.5	sp P81605 DCD_HUMAN.DAVEDLESVGK.+2y7.light	73.5	29.8	10	15
585.292	983.513	29.5	sp P81605 DCD_HUMAN.DAVEDLESVGK.+2y9.heavy	73.5	29.8	10	15
585.292	884.445	29.5	sp P81605 DCD_HUMAN.DAVEDLESVGK.+2y8.heavy	73.5	29.8	10	15
585.292	755.403	29.5	sp P81605 DCD_HUMAN.DAVEDLESVGK.+2y7.heavy	73.5	29.8	10	15
564.768	814.405	15	sp P81605 DCD_HUMAN.ENAGEDPGLAR.+2y8.light	72.3	29.2	10	15
564.768	628.341	15	sp P81605 DCD_HUMAN.ENAGEDPGLAR.+2y6.light	72.3	29.2	10	15
564.768	513.314	15	sp P81605 DCD_HUMAN.ENAGEDPGLAR.+2y5.light	72.3	29.2	10	15
569.772	824.414	15	sp P81605 DCD_HUMAN.ENAGEDPGLAR.+2y8.heavy	72.3	29.2	10	15
569.772	638.35	15	sp P81605 DCD_HUMAN.ENAGEDPGLAR.+2y6.heavy	72.3	29.2	10	15
569.772	523.323	15	sp P81605 DCD_HUMAN.ENAGEDPGLAR.+2y5.heavy	72.3	29.2	10	15

Transitions and settings for MRM analysis.							
Q1 m/z	Q3 m/z	RT	Compound name	DP (volts)	CE (volts)	EP	CXP
638.326	976.521	19.5	sp P08493 MGP_HUMAN.NANTFISPPQR.+2y8.light	77.7	31.8	10	15
638.326	875.473	19.5	sp P08493 MGP_HUMAN.NANTFISPPQR.+2y7.light	77.7	31.8	10	15
638.326	728.405	19.5	sp P08493 MGP_HUMAN.NANTFISPPQR.+2y6.light	77.7	31.8	10	15
638.326	528.289	19.5	sp P08493 MGP_HUMAN.NANTFISPPQR.+2y4.light	77.7	31.8	10	15
643.33	986.529	19.5	sp P08493 MGP_HUMAN.NANTFISPPQR.+2y8.heavy	77.7	31.8	10	15
643.33	885.482	19.5	sp P08493 MGP_HUMAN.NANTFISPPQR.+2y7.heavy	77.7	31.8	10	15
643.33	738.413	19.5	sp P08493 MGP_HUMAN.NANTFISPPQR.+2y6.heavy	77.7	31.8	10	15
643.33	538.297	19.5	sp P08493 MGP_HUMAN.NANTFISPPQR.+2y4.heavy	77.7	31.8	10	15
545.786	875.473	19.5	sp P08493 MGP_HUMAN.NTFISPPQR.+2y7.light	70.9	28.5	10	15
545.786	728.405	19.5	sp P08493 MGP_HUMAN.NTFISPPQR.+2y6.light	70.9	28.5	10	15
545.786	615.321	19.5	sp P08493 MGP_HUMAN.NTFISPPQR.+2y5.light	70.9	28.5	10	15
550.79	885.482	19.5	sp P08493 MGP_HUMAN.NTFISPPQR.+2y7.heavy	70.9	28.5	10	15
550.79	738.413	19.5	sp P08493 MGP_HUMAN.NTFISPPQR.+2y6.heavy	70.9	28.5	10	15
550.79	625.329	19.5	sp P08493 MGP_HUMAN.NTFISPPQR.+2y5.heavy	70.9	28.5	10	15
513.303	825.519	25.5	sp P49747 COMP_HUMAN.AEPGIQLKAV.+2y8.light	68.5	27.3	10	15
513.303	728.466	25.5	sp P49747 COMP_HUMAN.AEPGIQLKAV.+2y7.light	68.5	27.3	10	15
513.303	558.361	25.5	sp P49747 COMP_HUMAN.AEPGIQLKAV.+2y5.light	68.5	27.3	10	15
516.812	832.536	25.5	sp P49747 COMP_HUMAN.AEPGIQLKAV.+2y8.heavy	68.5	27.3	10	15
516.812	735.484	25.5	sp P49747 COMP_HUMAN.AEPGIQLKAV.+2y7.heavy	68.5	27.3	10	15
516.812	565.378	25.5	sp P49747 COMP_HUMAN.AEPGIQLKAV.+2y5.heavy	68.5	27.3	10	15
513.303	855.493	22	sp P49747 COMP_HUMAN.AVAEPIQLK.+2y8.light	68.5	27.3	10	15
513.303	784.456	22	sp P49747 COMP_HUMAN.AVAEPIQLK.+2y7.light	68.5	27.3	10	15
513.303	655.414	22	sp P49747 COMP_HUMAN.AVAEPIQLK.+2y6.light	68.5	27.3	10	15
516.812	862.511	22	sp P49747 COMP_HUMAN.AVAEPIQLK.+2y8.heavy	68.5	27.3	10	15
516.812	791.474	22	sp P49747 COMP_HUMAN.AVAEPIQLK.+2y7.heavy	68.5	27.3	10	15
516.812	662.431	22	sp P49747 COMP_HUMAN.AVAEPIQLK.+2y6.heavy	68.5	27.3	10	15
628.852	930.5	20	sp P49747 COMP_HUMAN.VLNQGREIVQT.+2y8.light	77	31.5	10	15
628.852	802.442	20	sp P49747 COMP_HUMAN.VLNQGREIVQT.+2y7.light	77	31.5	10	15
628.852	797.426	20	sp P49747 COMP_HUMAN.VLNQGREIVQT.+2b7.light	77	31.5	10	15
628.852	910.51	20	sp P49747 COMP_HUMAN.VLNQGREIVQT.+2b8.light	77	31.5	10	15
632.36	930.5	20	sp P49747 COMP_HUMAN.VLNQGREIVQT.+2y8.heavy	77	31.5	10	15
632.36	802.442	20	sp P49747 COMP_HUMAN.VLNQGREIVQT.+2y7.heavy	77	31.5	10	15
632.36	804.444	20	sp P49747 COMP_HUMAN.VLNQGREIVQT.+2b7.heavy	77	31.5	10	15
632.36	917.528	20	sp P49747 COMP_HUMAN.VLNQGREIVQT.+2b8.heavy	77	31.5	10	15
525.249	805.42	26	sp O75339 CILP1_HUMAN.DEGDTFPLR.+2y7.light	69.4	27.8	10	15
525.249	748.399	26	sp O75339 CILP1_HUMAN.DEGDTFPLR.+2y6.light	69.4	27.8	10	15
525.249	633.372	26	sp O75339 CILP1_HUMAN.DEGDTFPLR.+2y5.light	69.4	27.8	10	15
530.253	815.429	26	sp O75339 CILP1_HUMAN.DEGDTFPLR.+2y7.heavy	69.4	27.8	10	15
530.253	758.407	26	sp O75339 CILP1_HUMAN.DEGDTFPLR.+2y6.heavy	69.4	27.8	10	15
530.253	643.38	26	sp O75339 CILP1_HUMAN.DEGDTFPLR.+2y5.heavy	69.4	27.8	10	15
624.323	794.416	27.5	sp O75339 CILP1_HUMAN.NLEPRTGFLSN.+2y7.light	76.6	31.3	10	15
624.323	638.314	27.5	sp O75339 CILP1_HUMAN.NLEPRTGFLSN.+2y6.light	76.6	31.3	10	15
624.323	768.4	27.5	sp O75339 CILP1_HUMAN.NLEPRTGFLSN.+2b7.light	76.6	31.3	10	15
624.323	915.468	27.5	sp O75339 CILP1_HUMAN.NLEPRTGFLSN.+2b8.light	76.6	31.3	10	15
627.831	801.433	27.5	sp O75339 CILP1_HUMAN.NLEPRTGFLSN.+2y7.heavy	76.6	31.3	10	15
627.831	645.332	27.5	sp O75339 CILP1_HUMAN.NLEPRTGFLSN.+2y6.heavy	76.6	31.3	10	15
627.831	768.4	27.5	sp O75339 CILP1_HUMAN.NLEPRTGFLSN.+2b7.heavy	76.6	31.3	10	15
627.831	915.468	27.5	sp O75339 CILP1_HUMAN.NLEPRTGFLSN.+2b8.heavy	76.6	31.3	10	15
733.86	964.473	33.5	sp O75339 CILP1_HUMAN.STATAAQTDLNFIN.+2y8.light	84.6	35.3	10	15
733.86	735.367	33.5	sp O75339 CILP1_HUMAN.STATAAQTDLNFIN.+2y6.light	84.6	35.3	10	15
733.86	847.379	33.5	sp O75339 CILP1_HUMAN.STATAAQTDLNFIN.+2b9.light	84.6	35.3	10	15
733.86	1.074.506	33.5	sp O75339 CILP1_HUMAN.STATAAQTDLNFIN.+2b11.light	84.6	35.3	10	15
737.368	971.491	33.5	sp O75339 CILP1_HUMAN.STATAAQTDLNFIN.+2y8.heavy	84.6	35.3	10	15
737.368	742.384	33.5	sp O75339 CILP1_HUMAN.STATAAQTDLNFIN.+2y6.heavy	84.6	35.3	10	15
737.368	847.379	33.5	sp O75339 CILP1_HUMAN.STATAAQTDLNFIN.+2b9.heavy	84.6	35.3	10	15
737.368	1.081.523	33.5	sp O75339 CILP1_HUMAN.STATAAQTDLNFIN.+2b11.heavy	84.6	35.3	10	15

Transitions and settings for MRM analysis.							
Q1 m/z	Q3 m/z	RT	Compound name	DP (volts)	CE (volts)	EP	CXP
794.36	787.42	22.5	sp P10909 CLUS_HUMAN.ASHTSDSDVPSGVTEV.+2y8.light	89	37.5	10	15
794.36	688.351	22.5	sp P10909 CLUS_HUMAN.ASHTSDSDVPSGVTEV.+2y7.light	89	37.5	10	15
794.36	801.301	22.5	sp P10909 CLUS_HUMAN.ASHTSDSDVPSGVTEV.+2b8.light	89	37.5	10	15
794.36	900.369	22.5	sp P10909 CLUS_HUMAN.ASHTSDSDVPSGVTEV.+2b9.light	89	37.5	10	15
797.367	793.433	22.5	sp P10909 CLUS_HUMAN.ASHTSDSDVPSGVTEV.+2y8.heavy	89	37.5	10	15
797.367	694.365	22.5	sp P10909 CLUS_HUMAN.ASHTSDSDVPSGVTEV.+2y7.heavy	89	37.5	10	15
797.367	801.301	22.5	sp P10909 CLUS_HUMAN.ASHTSDSDVPSGVTEV.+2b8.heavy	89	37.5	10	15
797.367	900.369	22.5	sp P10909 CLUS_HUMAN.ASHTSDSDVPSGVTEV.+2b9.heavy	89	37.5	10	15
843.894	787.42	26	sp P10909 CLUS_HUMAN.ASHTSDSDVPSGVTEVV.+2y8.light	92.6	39.2	10	15
843.894	801.301	26	sp P10909 CLUS_HUMAN.ASHTSDSDVPSGVTEVV.+2b8.light	92.6	39.2	10	15
843.894	900.369	26	sp P10909 CLUS_HUMAN.ASHTSDSDVPSGVTEVV.+2b9.light	92.6	39.2	10	15
846.901	793.433	26	sp P10909 CLUS_HUMAN.ASHTSDSDVPSGVTEVV.+2y8.heavy	92.6	39.2	10	15
846.901	801.301	26	sp P10909 CLUS_HUMAN.ASHTSDSDVPSGVTEVV.+2b8.heavy	92.6	39.2	10	15
846.901	900.369	26	sp P10909 CLUS_HUMAN.ASHTSDSDVPSGVTEVV.+2b9.heavy	92.6	39.2	10	15
722.359	1142.62	31.5	sp P10909 CLUS_HUMAN.GEDQYYLRVTTV.+2y9.light	83.8	34.9	10	15
722.359	1.014.562	31.5	sp P10909 CLUS_HUMAN.GEDQYYLRVTTV.+2y8.light	83.8	34.9	10	15
722.359	756.284	31.5	sp P10909 CLUS_HUMAN.GEDQYYLRVTTV.+2b6.light	83.8	34.9	10	15
725.366	1.148.634	31.5	sp P10909 CLUS_HUMAN.GEDQYYLRVTTV.+2y9.heavy	83.8	34.9	10	15
725.366	1.020.576	31.5	sp P10909 CLUS_HUMAN.GEDQYYLRVTTV.+2y8.heavy	83.8	34.9	10	15
725.366	756.284	31.5	sp P10909 CLUS_HUMAN.GEDQYYLRVTTV.+2b6.heavy	83.8	34.9	10	15
718.367	589.356	24.5	sp P07093 GDN_HUMAN.SEDGTKASAATTAIL.+2y6.light	83.5	34.7	10	15
718.367	776.342	24.5	sp P07093 GDN_HUMAN.SEDGTKASAATTAIL.+2b8.light	83.5	34.7	10	15
718.367	847.379	24.5	sp P07093 GDN_HUMAN.SEDGTKASAATTAIL.+2b9.light	83.5	34.7	10	15
718.367	918.416	24.5	sp P07093 GDN_HUMAN.SEDGTKASAATTAIL.+2b10.light	83.5	34.7	10	15
721.876	596.373	24.5	sp P07093 GDN_HUMAN.SEDGTKASAATTAIL.+2y6.heavy	83.5	34.7	10	15
721.876	776.342	24.5	sp P07093 GDN_HUMAN.SEDGTKASAATTAIL.+2b8.heavy	83.5	34.7	10	15
721.876	847.379	24.5	sp P07093 GDN_HUMAN.SEDGTKASAATTAIL.+2b9.heavy	83.5	34.7	10	15
721.876	918.416	24.5	sp P07093 GDN_HUMAN.SEDGTKASAATTAIL.+2b10.heavy	83.5	34.7	10	15
471.277	826.54	24	sp P07093 GDN_HUMAN.AVAQTDLKEPLKV.+3y7.light	65.5	23.2	10	15
471.277	713.456	24	sp P07093 GDN_HUMAN.AVAQTDLKEPLKV.+3y6.light	65.5	23.2	10	15
471.277	585.361	24	sp P07093 GDN_HUMAN.AVAQTDLKEPLKV.+3y5.light	65.5	23.2	10	15
471.277	586.283	24	sp P07093 GDN_HUMAN.AVAQTDLKEPLKV.+3b6.light	65.5	23.2	10	15
473.616	833.557	24	sp P07093 GDN_HUMAN.AVAQTDLKEPLKV.+3y7.heavy	65.5	23.2	10	15
473.616	720.473	24	sp P07093 GDN_HUMAN.AVAQTDLKEPLKV.+3y6.heavy	65.5	23.2	10	15
473.616	592.378	24	sp P07093 GDN_HUMAN.AVAQTDLKEPLKV.+3y5.heavy	65.5	23.2	10	15
473.616	586.283	24	sp P07093 GDN_HUMAN.AVAQTDLKEPLKV.+3b6.heavy	65.5	23.2	10	15
515.769	576.314	21	sp P02458 CO2A1_HUMAN.AGPPGPVGPAGGP.+2b7.light	68.7	27.4	10	15
515.769	633.336	21	sp P02458 CO2A1_HUMAN.AGPPGPVGPAGGP.+2b8.light	68.7	27.4	10	15
515.769	708.367	21	sp P02458 CO2A1_HUMAN.AGPPGPVGPAGGP.+2y9.light	68.7	27.4	10	15
515.769	651.346	21	sp P02458 CO2A1_HUMAN.AGPPGPVGPAGGP.+2y8.light	68.7	27.4	10	15
518.776	576.314	21	sp P02458 CO2A1_HUMAN.AGPPGPVGPAGGP.+2b7.heavy	68.7	27.4	10	15
518.776	633.336	21	sp P02458 CO2A1_HUMAN.AGPPGPVGPAGGP.+2b8.heavy	68.7	27.4	10	15
518.776	714.381	21	sp P02458 CO2A1_HUMAN.AGPPGPVGPAGGP.+2y9.heavy	68.7	27.4	10	15
518.776	657.36	21	sp P02458 CO2A1_HUMAN.AGPPGPVGPAGGP.+2y8.heavy	68.7	27.4	10	15
650.344	620.34	19	sp P02458 CO2A1_HUMAN.AGPSGPRGPPGPVGP.+2y7.light	78.5	32.3	10	15
650.344	523.287	19	sp P02458 CO2A1_HUMAN.AGPSGPRGPPGPVGP.+2y6.light	78.5	32.3	10	15
650.344	680.347	19	sp P02458 CO2A1_HUMAN.AGPSGPRGPPGPVGP.+2b8.light	78.5	32.3	10	15
653.351	626.354	19	sp P02458 CO2A1_HUMAN.AGPSGPRGPPGPVGP.+2y7.heavy	78.5	32.3	10	15
653.351	529.301	19	sp P02458 CO2A1_HUMAN.AGPSGPRGPPGPVGP.+2y6.heavy	78.5	32.3	10	15
653.351	680.347	19	sp P02458 CO2A1_HUMAN.AGPSGPRGPPGPVGP.+2b8.heavy	78.5	32.3	10	15

**Annex II. Supplementary data III.** Quantification data obtained for the panel of peptides analyzed by MRM mass spectrometry. Results are expressed in peak area ratios of abundance (light/heavy peptides), with a confidence level of  $p < 0.05^*$ .

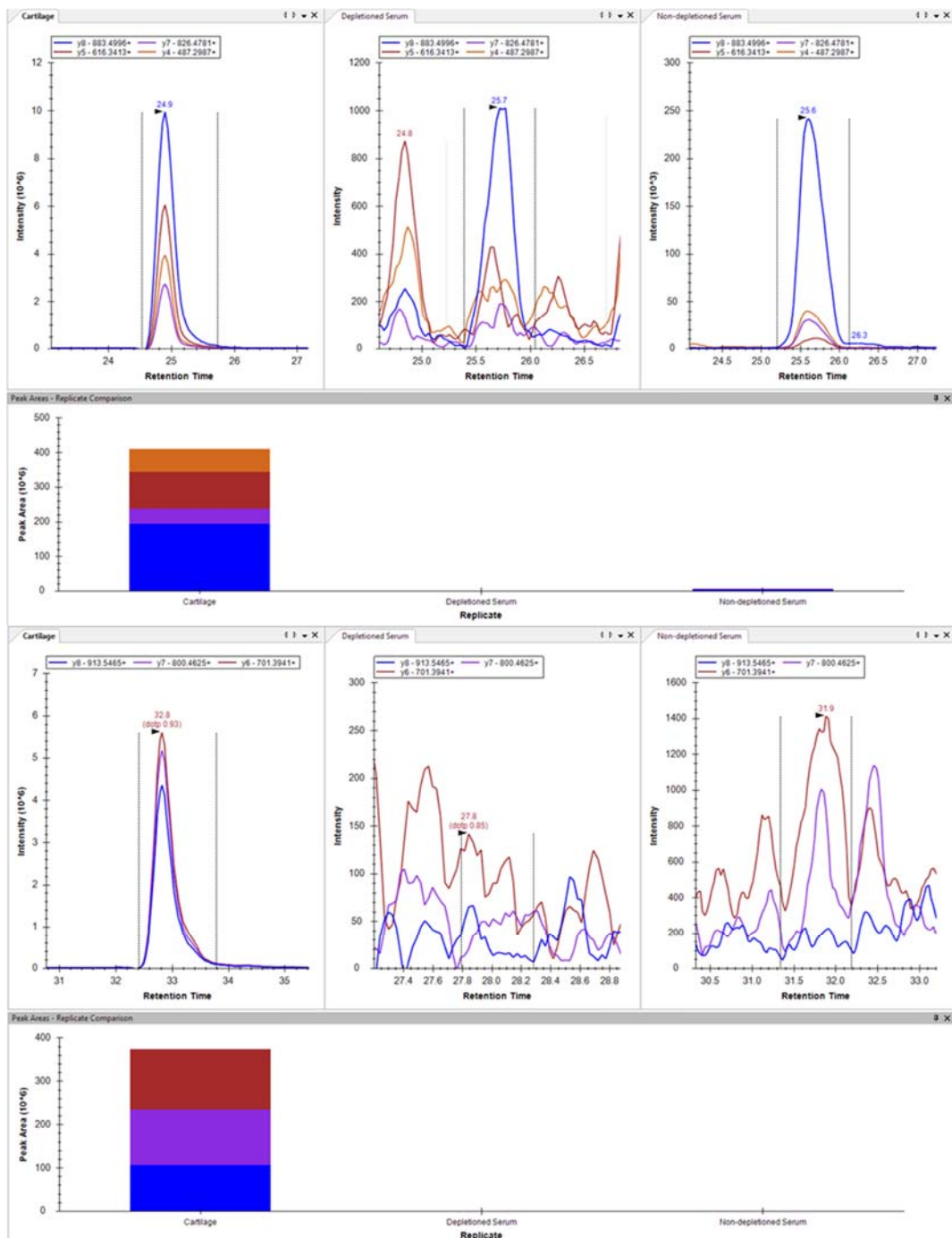
This data is included in the excel in the attached CD.

**Annex II. Supplementary data IV.** Serum and synovial fluid (SF) proteins whose neopeptides were selected for MRM method development.

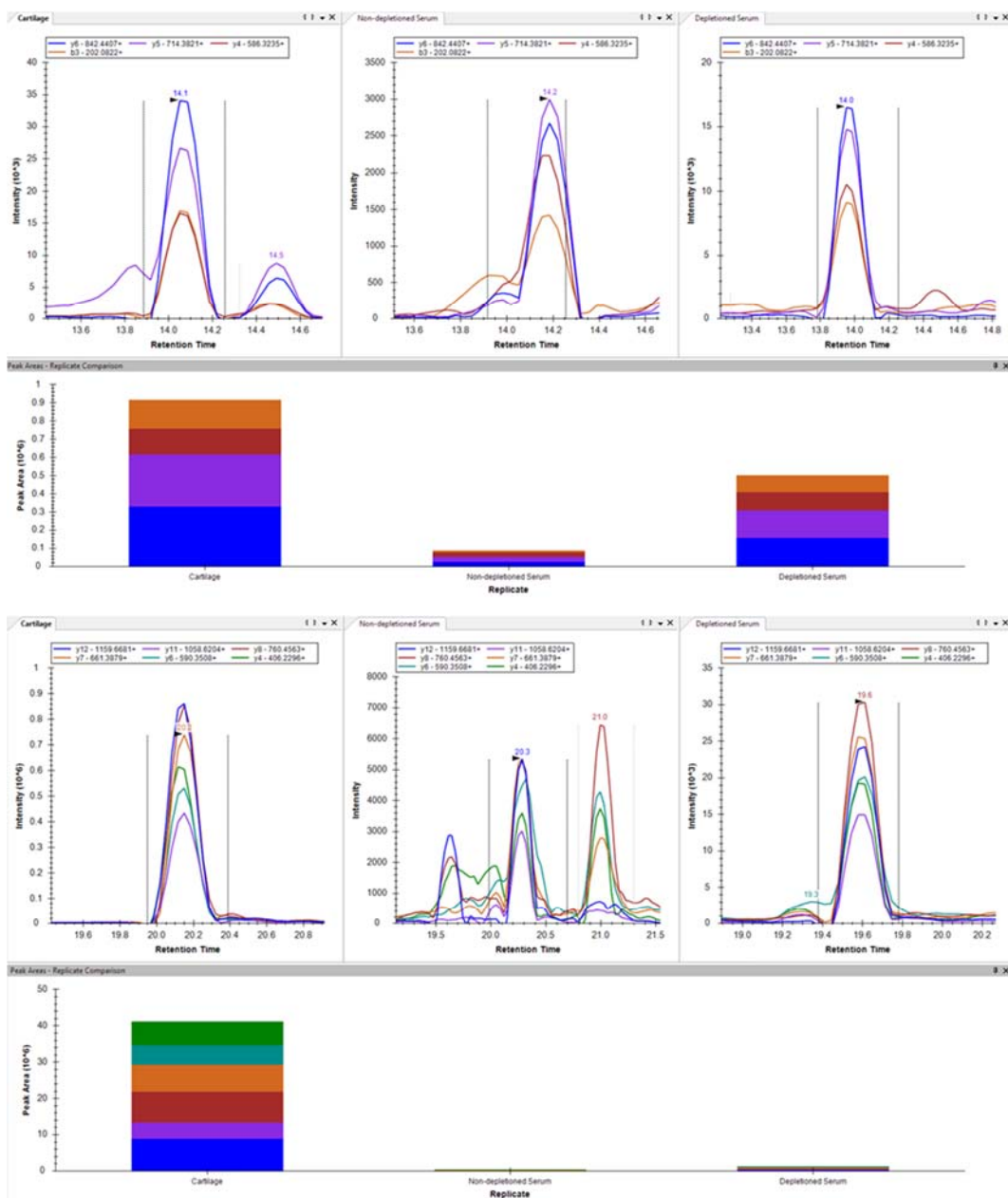
<b>Serum/SF neopeptide appertaining proteins selected for MRM development</b>	
Actin, cytoplasmic 1	Histone H2B type 1-O
Alpha-2-HS-glycoprotein	Histone H2B type 2-E
Alpha-2-macroglobulin	Insulin-like growth factor II
Apolipoprotein A-I	Inter-alpha-trypsin inhibitor heavy chain H1
Apolipoprotein A-II	Inter-alpha-trypsin inhibitor heavy chain H4
Apolipoprotein A-IV	Matrix gla protein
Apolipoprotein C-III	Kininogen-1
Apolipoprotein E	Osteopontin
Caldesmon	Plasminogen
Cathepsin g	Plexin domain-containing protein 2
Clusterin	PDZ and LIM domain protein 1
Coagulation factor V	Protein S100-A8
Collagen alpha-1(I) chain	Protein S100-A9
Collagen alpha-2(I) chain	Septin-2
Complement C3	Serglycin
Dermcidin	Thymosin beta-4
Extracellular matrix protein 1	Transcription factor maf
Fibrillin-1	Transforming growth factor beta-1- induced transcript 1 protein
Fibronectin	
Filamin-a	Transgelin-2
Frizzled-2	Sulfhydryl oxidase 1
Gelsolin	Transthyretin
Heat shock protein beta-1	Vitronectin

### 3. Annex III: Supplementary Material of Chapter III.

**Annex III. Supplementary data I.** MRM chromatograms showing the presence of CILP1-IVG and CILP1-TFL in a cartilage digest sample (at 24.9min and 32.8 min respectively), confirmed with a high confidence (greater than 98%) by peptide identification search using the ProteinPilot software, whereas they are not detected and/or identified in the depleted and/or non-depleted serum digests.



**Annex III. Supplementary data II.** MRM chromatograms showing the presence of PRG4-GGS and PRG4-GFG in a cartilage digest sample (at 14.1min and 20.2min respectively), confirmed with a high confidence (99%) by peptide identification search using the ProteinPilot software, whereas they are hardly detected and no identified in the non-depleted serum digests. In the case of depleted serum digests, the peptides and analogous proteins are detected by MRM, however this was not sufficient to achieve protein and/or peptide identification when MS/MS spectra were searched in the ProteinPilot software.



**Annex III. Supplementary data III.** First iMALDI PRG4-GFG response curve with 8 points made in triplicates from 0 to 2500 fmol, used for assay validation. Light (L/NAT) and heavy (H/SIS) peak intensities and their corresponding L/H ratios, including the average and coefficient of variation (CV) data of each triplicate measurements.

iMALDI PRG4-GFG Response Curve	L/NAT (I)	H/SIS (I)	H/L ratio (I)	H/L ratio (I)	
				Average	CV
0	6539				
0	6617				
0	1974				
50	2967	403	0,14	0,15	15,90
50	6941	1266	0,18		
50	7209	1049	0,15		
250	5177	2026	0,39	0,35	10,14
250	3714	1232	0,33		
250	3054	1002	0,33		
500	828	526	0,64	0,66	5,46
500	1956	1251	0,64		
500	2180	1525	0,70		
750	2412	2213	0,92	0,97	4,55
750	2725	2721	1,00		
750	1424	1407	0,99		
1000	3374	4352	1,29	1,40	9,06
1000	2758	3767	1,37		
1000	1683	2587	1,54		
1500	2157	4347	2,02	1,96	5,80
1500	1322	2697	2,04		
1500	2998	5492	1,83		
2500	2024	6767	3,34	3,21	5,46
2500	1968	6429	3,27		
2500	1197	3602	3,01		



**Annex III. Supplementary data IV. iMALDI PRG4-GFG assay intra-day and inter-day variability and precision measurements of low (117.5 fmol), medium (470 fmol) and high (2350 fmol) concentrations made with 5 replicates.**

PRG4-GFG	LOQ: 47 fmol	LOW (2.5x LOQ): 117.5 fmol			MEDIUM (10x LOQ): 470 fmol			HIGH (50x LOQ): 2350 fmol			Precision / Intraday %CV		
Day	Replicate Number	NAT LOW (I)	SIS LOW (I)	SIS/NAT ratio (LOW)	NAT MED (I)	SIS MED (I)	SIS/NAT ratio (MED)	NAT HIGH (I)	SIS HIGH (I)	SIS/NAT ratio (HIGH)	%CV LOW (n=5)	%CV MED (n=5)	%CV HIGH (n=5)
1	1	4265	981	0.23	2874	2185	0.76	2974	9818	3.30	4.33	3.71	12.25
	2	6395	1584	0.25	4413	3500	0.79	2607	9666	3.71			
	3	6162	1382	0.22	5587	4077	0.73	2478	8374	3.38			
	4	3091	711	0.23	3469	2747	0.79	1706	5971	3.50			
	5	2008	491	0.24	2759	2195	0.80	3050	8036	2.63			
2	1	5236	1637	0.31	2003	1742	0.87	2430	8469	3.49	6.45	2.85	1.95
	2	4409	1272	0.29	4148	3590	0.87	2754	9909	3.60			
	3	4960	1342	0.27	5685	5150	0.91	2592	9026	3.48			
	4	4053	1207	0.30	4163	3819	0.92	1592	5526	3.47			
	5	5008	1346	0.27	4555	3936	0.86	2220	7570	3.41			
3	1	3200	956	0.30	3063	2459	0.80	1701	6218	3.66	4.30	4.32	12.88
	2	3706	1192	0.32	3013	2291	0.76	2431	7528	3.10			
	3	4908	1501	0.31	2715	2204	0.81	1114	3017	2.71			
	4	2675	804	0.30	2896	2396	0.83	2453	7335	2.99			
	5	4273	1221	0.29	3605	2697	0.75	2129	5750	2.70			
4	1	3287	730	0.22	4098	2948	0.72	2537	7794	3.07	4.84	4.06	5.32
	2	6201	1531	0.25	3014	2200	0.73	1841	6097	3.31			
	3	3932	923	0.23	2971	2214	0.75	1234	3752	3.04			
	4	5189	1149	0.22	2917	1948	0.67	3371	9895	2.94			
	5	6971	1554	0.22	4883	3498	0.72	3955	11461	2.90			
5	1	4031	1251	0.31	1708	1470	0.86	1886	5716	3.03	14.03	6.95	3.53
	2	6148	2078	0.34	3995	3463	0.87	1551	4820	3.11			
	3	3467	977	0.28	5600	4853	0.87	1865	5884	3.15			
	4	4185	1407	0.34	3105	2361	0.76	2645	8790	3.32			
	5	7464	1771	0.24	6202	4716	0.76	2471	7972	3.23			
Interday % CV		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	7.18	6.87	8.36	7.43			
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	11.44	8.36	10.30	7.33			
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	8.95	6.49	10.08				

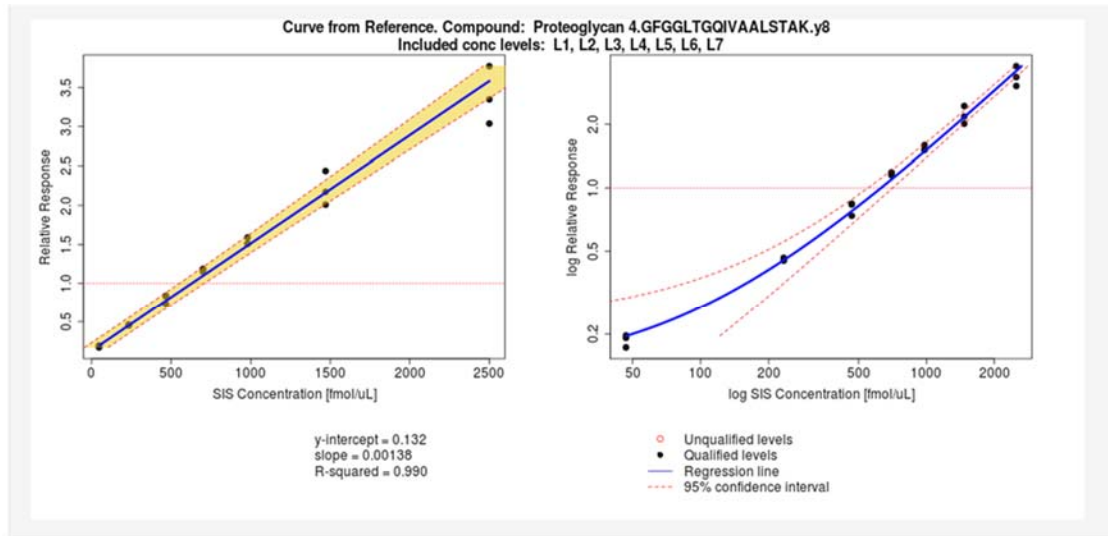
**Annex III. Supplementary data V.** iMALDI PRG4-GFG accuracy (% nominal) measurements of low (117.5 fmol), medium (470 fmol) and high (2350 fmol) concentrations made with 5 replicates.

PRG4-GFG Day	LLOQ: 47 fmol Replicate Number	LOW (2.5X LLOQ): 117.5 fmol			MEDIUM (10X LLOQ): 470 fmol			HIGH (50X LLOQ): 2350 fmol		
		SIS/NAT ratio (LOW)	NAT (fmol)	SPM SIS (fmol)	SIS/NAT ratio (MED)	SIS Spike	SPM SIS (fmol)	SIS/NAT ratio (HIGH)	NAT (fmol)	SPM SIS (fmol)
<b>1</b>	1	0,23	664,308	152,80	0,76	664,308	505,05	3,30	664,308	2193,07
	2	0,25	664,308	164,54	0,79	664,308	526,87	3,71	664,308	2463,06
	3	0,22	664,308	148,99	0,73	664,308	484,77	3,38	664,308	2244,92
	4	0,23	664,308	152,81	0,79	664,308	526,05	3,50	664,308	2325,08
	5	0,24	664,308	162,44	0,80	664,308	528,51	2,63	664,308	1750,29
<b>2</b>	1	0,31	664,308	207,69	0,87	664,308	577,75	3,49	664,308	2315,24
	2	0,29	664,308	191,65	0,87	664,308	574,94	3,60	664,308	2390,21
	3	0,27	664,308	179,74	0,91	664,308	601,79	3,48	664,308	2313,29
	4	0,30	664,308	197,83	0,92	664,308	609,41	3,47	664,308	2305,88
	5	0,27	664,308	178,55	0,86	664,308	574,03	3,41	664,308	2265,23
<b>3</b>	1	0,30	664,308	198,46	0,80	664,308	533,31	3,66	664,308	2428,38
	2	0,32	664,308	213,67	0,76	664,308	505,12	3,10	664,308	2057,14
	3	0,31	664,308	203,16	0,81	664,308	539,28	2,71	664,308	1799,12
	4	0,30	664,308	199,66	0,83	664,308	549,61	2,99	664,308	1986,42
	5	0,29	664,308	189,82	0,75	664,308	496,99	2,70	664,308	1794,16
<b>4</b>	1	0,22	664,308	147,53	0,72	664,308	477,89	3,07	664,308	2040,84
	2	0,25	664,308	164,01	0,73	664,308	484,90	3,31	664,308	2200,05
	3	0,23	664,308	155,94	0,75	664,308	495,04	3,04	664,308	2019,84
	4	0,22	664,308	147,10	0,67	664,308	443,63	2,94	664,308	1949,96
	5	0,22	664,308	148,09	0,72	664,308	475,89	2,90	664,308	1925,07
<b>5</b>	1	0,31	664,308	206,16	0,86	664,308	571,74	3,03	664,308	2013,35
	2	0,34	664,308	224,53	0,87	664,308	575,84	3,11	664,308	2064,45
	3	0,28	664,308	187,20	0,87	664,308	575,69	3,15	664,308	2095,87
	4	0,34	664,308	223,34	0,76	664,308	505,13	3,32	664,308	2207,66
	5	0,24	664,308	157,62	0,76	664,308	505,14	3,23	664,308	2143,21
		Average LOW SIS experimental (fmol)			Average MED SIS experimental (fmol)			Average HIGH SIS experimental (fmol)		
		180,13			529,77			2131,67		
		LOW % Nominal: (SIS experimental / SIS theoretical)*100			MED % Nominal: (SIS experimental / SIS theoretical)*100			HIGH % Nominal: (SIS experimental / SIS theoretical)*100		
		90,71			112,72			90,71		

**Annex III. Supplementary data VI.** Second iMALDI PRG4-GFG response curve with 8 points made in triplicates from 0 to 2500 fmol, used for sample measurement. Light (L/NAT) and heavy (H/SIS) peak intensities and their corresponding L/H ratios, including the average and coefficient of variation (CV) data of each triplicate measurements.

iMALDI PRG4-GFG	L/NAT (I)	H/SIS (I)	H/L ratio (I)	H/L ratio (I)	
				Average	CV
0	2506				
0	6365				
0	3273				
50	8013	1578	0,20	0,19	6,78
50	5917	1135	0,19		
50	5514	953	0,17		
250	5006	2278	0,46	0,46	1,61
250	5127	2387	0,47		
250	6349	2866	0,45		
500	4348	3628	0,83	0,80	7,23
500	5653	4759	0,84		
500	1764	1301	0,74		
750	4560	5249	1,15	1,17	1,59
750	4635	5472	1,18		
750	4587	5439	1,19		
1000	3995	6026	1,51	1,54	3,00
1000	2357	3560	1,51		
1000	903	1435	1,59		
1500	3628	7289	2,01	2,21	9,82
1500	2416	5891	2,44		
1500	3651	7932	2,17		
2500	2567	7798	3,04	3,39	10,88
2500	3020	11389	3,77		
2500	2830	9473	3,35		

**Annex III. Supplementary data VII.** Second iMALDI PRG4-GFG response curve representation with Qualis-SIS software of 8 points made in triplicates from 0 to 2500 fmol, used for sample measurement.



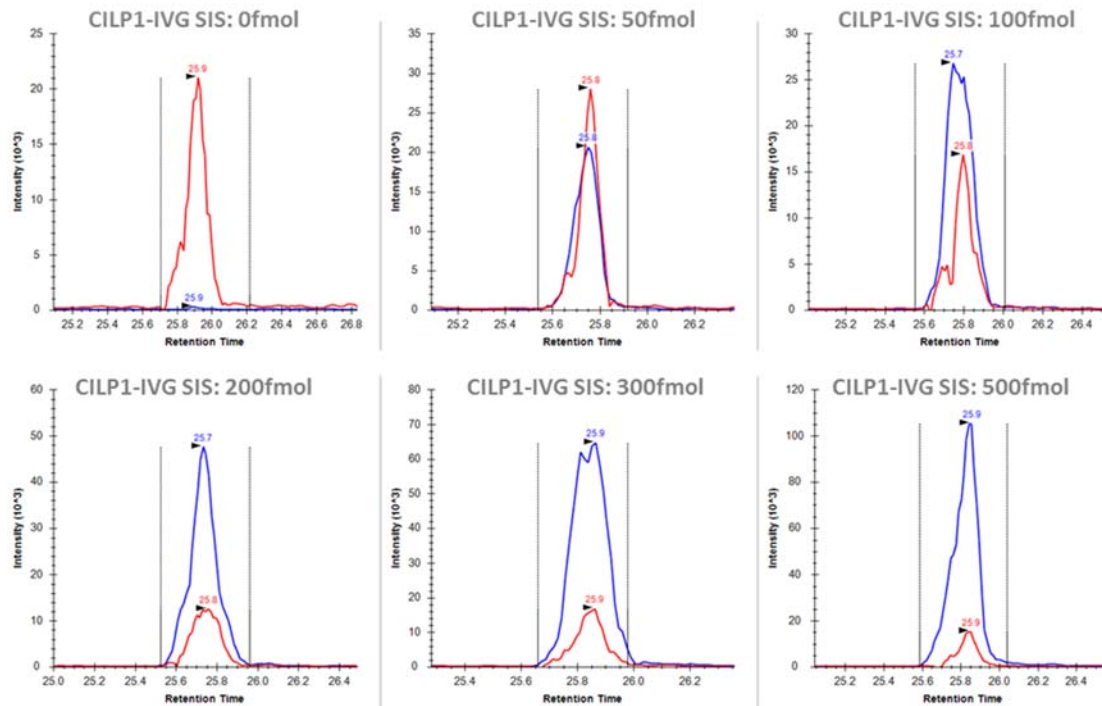
Criteria	Qualification Requirements
1 Precision Excluding LLOQ	av. <20% CV per level
2 Precision at LLOQ	av. <20% CV
3 Accuracy Excluding LLOQ	20%
4 Accuracy at LLOQ	20%
5 Min. No. of Levels	3 consecutive
6 No. of Acceptable Replicates/Level	per qualified level

Metric	Value
1 LLOQ (ng/mL)	6924.090
2 LLOQ (fmol/uL)	46.685
3 ULOQ (ng/mL)	370785.000
4 ULOQ (fmol/uL)	2500.000
5 Dynamic Range	53.550
6 Conc NAT in Reference (ng/mL)	84622.876
7 Conc NAT in Reference (fmol/uL)	570.566

**Annex III. Supplementary data VIII.** SPM and SCM measurements of the 38 serum samples analyzed using the validated iMALDI PRG4-GFG assay in fmol, fmol/ $\mu$ L serum,  $\mu$ g/mL serum. Difference (%) between the cited SPM and SCM measurements.

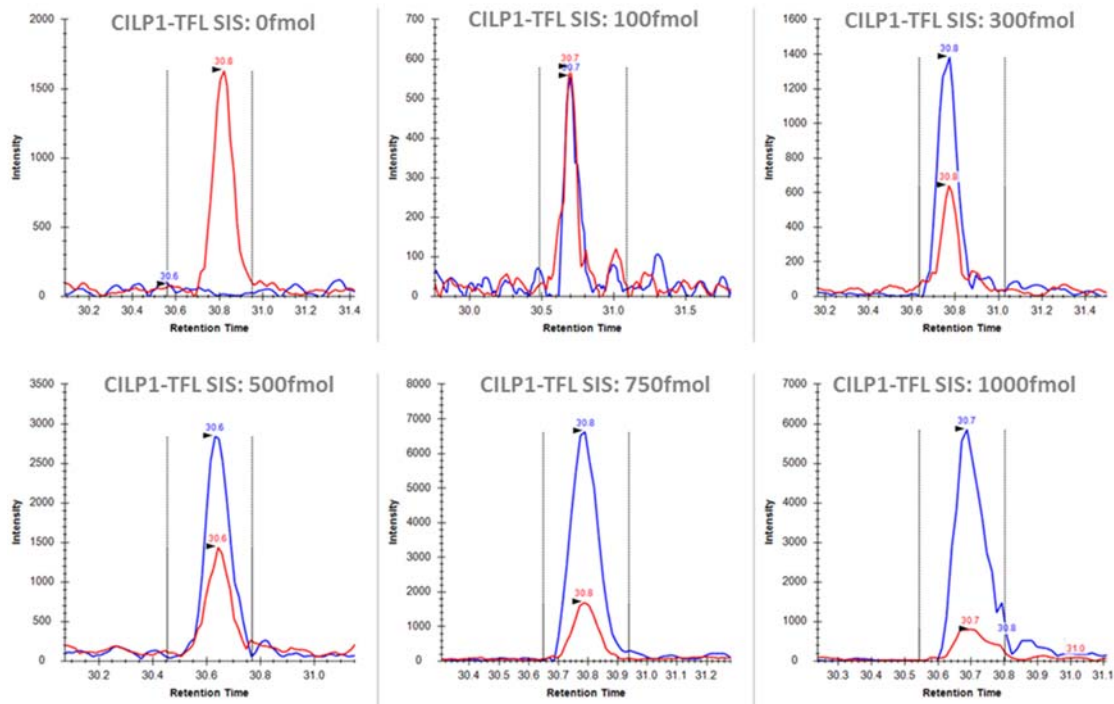
Sample ID	PRG4-GFG SPM			PRG4-GFG SCM			(SPM-SCM) /SCM*100
	fmol	fmol/ $\mu$ L serum	$\mu$ g/mL serum	fmol	fmol/ $\mu$ L serum	$\mu$ g/mL serum	
OA1	573,95	47,83	6,87	502,95	41,91	6,02	14,12
OA2	650,89	54,24	7,79	578,64	48,22	6,93	12,49
OA3	860,30	71,69	10,30	800,00	66,67	9,58	7,54
OA4	803,69	66,97	9,62	653,28	54,44	7,82	23,02
OA5	1340,91	111,74	16,06	1387,65	115,64	16,62	-3,37
OA6	422,28	35,19	5,06	360,04	30,00	4,31	17,29
OAR380	1049,68	87,47	12,57	1013,32	84,44	12,14	3,59
OAR405	1053,14	87,76	12,61	1017,49	84,79	12,18	3,50
OAR277	442,39	36,87	5,30	378,34	31,53	4,53	16,93
OAR330	663,88	55,32	7,95	592,42	49,37	7,09	12,06
OAR113	905,14	75,43	10,84	847,69	70,64	10,15	6,78
OAR2	790,34	65,86	9,46	722,59	60,22	8,65	9,38
OAR400	915,13	76,26	10,96	957,96	79,83	11,47	-4,47
OAR23	727,88	60,66	8,72	658,00	54,83	7,88	10,62
OAR387	753,56	62,80	9,02	684,04	57,00	8,19	10,16
OAR26	586,50	48,87	7,02	514,74	42,90	6,16	13,94
OAR265	767,53	63,96	9,19	699,06	58,26	8,37	9,79
OAR98	715,52	59,63	8,57	644,92	53,74	7,72	10,95
OAR34	1148,71	95,73	13,76	1133,64	94,47	13,58	1,33
OAR132	747,72	62,31	8,95	606,08	50,51	7,26	23,37
OAR74	736,73	61,39	8,82	666,69	55,56	7,98	10,50
OAR273	988,46	82,37	11,84	942,24	78,52	11,28	4,91
N07/546	783,55	65,30	9,38	716,35	59,70	8,58	9,38
N07/533	781,01	65,08	9,35	714,20	59,52	8,55	9,36
N07/1298	775,79	64,65	9,29	709,34	59,11	8,49	9,37
N07/1211	959,80	79,98	11,49	913,04	76,09	10,93	5,12
N1	862,13	71,84	10,32	802,51	66,88	9,61	7,43
N2	445,44	37,12	5,33	380,85	31,74	4,56	16,96
N3	405,93	33,83	4,86	344,58	28,72	4,13	17,80
N4	703,25	58,60	8,42	632,54	52,71	7,57	11,18
N5	489,01	40,75	5,86	421,67	35,14	5,05	15,97
N6	1053,04	87,75	12,61	1137,94	94,83	13,63	-7,46
N08/851	432,94	36,08	5,18	368,91	30,74	4,42	17,36
N06/1432	1539,27	128,27	18,43	1456,37	121,36	17,44	5,69
N07/1292	603,60	50,30	7,23	531,53	44,29	6,37	13,56
N06/1488	913,86	76,16	10,94	858,07	71,51	10,28	6,50
N07/1161	1628,68	135,72	19,50	1586,85	132,24	19,00	2,64
N07/618	539,02	44,92	6,46	469,16	39,10	5,62	14,89

**Annex III. Supplementary data IX.** SISCAPA-MRM CILP1-IVG response curve chromatogram representation and values of 6 points ranging from 0 to 500 fmol, made in duplicates. Light (NAT) and heavy (SIS) peak areas and their corresponding NAT/SIS ratios, including the average and coefficient of variation (CV) data of each duplicate measurements.



Protein Name	Peptide Sequence	Replicate Name	NAT Total Area	SIS Total Area	SIS/NAT ratio	Average SIS/NAT ratio	CV (%) SIS/NAT ratio
CILP1	IVGPLEVNVNR	500 fmol SIS-IVG (1)	53975	620972	11,50	10,11	19,44
CILP1	IVGPLEVNVNR	500 fmol SIS-IVG (2)	61470	536292	8,72		
CILP1	IVGPLEVNVNR	300 fmol SIS-IVG (1)	84691	390399	4,61	4,51	3,16
CILP1	IVGPLEVNVNR	300 fmol SIS-IVG (2)	80707	355794	4,41		
CILP1	IVGPLEVNVNR	200 fmol SIS-IVG (1)	90625	324576	3,58	3,61	1,30
CILP1	IVGPLEVNVNR	200 fmol SIS-IVG (2)	70734	258023	3,65		
CILP1	IVGPLEVNVNR	100 fmol SIS-IVG (1)	83027	179451	2,16	2,41	14,44
CILP1	IVGPLEVNVNR	100 fmol SIS-IVG (2)	59200	157060	2,65		
CILP1	IVGPLEVNVNR	50 fmol SIS-IVG (1)	58820	47630	0,81	0,90	13,94
CILP1	IVGPLEVNVNR	50 fmol SIS-IVG (2)	90184	88996	0,99		
CILP1	IVGPLEVNVNR	0 fmol SIS-IVG (1)	97850	1557	0,02	0,02	29,82
CILP1	IVGPLEVNVNR	0 fmol SIS-IVG (2)	83794	2046	0,02		

**Annex III. Supplementary data X.** SISCAPA-MRM CILP1-TFL response curve chromatogram representation and values of 6 points ranging from 0 to 500 fmol, made in duplicates. Light (NAT) and heavy (SIS) peak areas and their corresponding NAT/SIS ratios, including the average and coefficient of variation (CV) data of each duplicate measurements.



Protein Name	Peptide Sequence	Replicate Name	NAT Total Area	SIS Total Area	SIS/NAT ratio	Average SIS/NAT ratio	CV (%) SIS/NAT ratio
CILP1	TFLVGNLEIR	1000 fmol SIS-TFL (1)	3328	21150	6,36	4,91	41,46
CILP1	TFLVGNLEIR	1000 fmol SIS-TFL (2)	3691	12821	3,47		
CILP1	TFLVGNLEIR	750 fmol SIS-TFL (1)	5890	24856	4,22	3,66	21,70
CILP1	TFLVGNLEIR	750 fmol SIS-TFL (2)	4250	13163	3,10		
CILP1	TFLVGNLEIR	500 fmol SIS-TFL (1)	4447	11203	2,52	2,56	2,37
CILP1	TFLVGNLEIR	500 fmol SIS-TFL (2)	2872	7482	2,61		
CILP1	TFLVGNLEIR	250 fmol SIS-TFL (1)	2974	5612	1,89	1,62	23,72
CILP1	TFLVGNLEIR	250 fmol SIS-TFL (2)	3192	4293	1,34		
CILP1	TFLVGNLEIR	125 fmol SIS-TFL (1)	2984	2275	0,76	0,82	10,58
CILP1	TFLVGNLEIR	125 fmol SIS-TFL (2)	6237	5524	0,89		
CILP1	TFLVGNLEIR	0 fmol SIS-TFL (1)	6427	252	0,04	0,04	14,11
CILP1	TFLVGNLEIR	0 fmol SIS-TFL (2)	6075	291	0,05		

**Annex III. Supplementary data XI.** Concentration measurements of 12 serum samples analyzed using the validated SISCAPA-MRM CILP1-IVG and CILP1-TFL multiplex assay in fmol, fmol/ $\mu$ L serum,  $\mu$ g/mL serum and the corresponding average values.

Protein Name	Peptide Sequence	Replicate Name	NAT/SIS ratio	NAT (fmol)	NAT (fmol/ $\mu$ L serum)	NAT ( $\mu$ g/mL serum)	Average NAT ( $\mu$ g/mL serum)
CILP1	IVGPLEVNVR	HD 1	0,62	124,92	6,86	0,89	1,28
CILP1	IVGPLEVNVR	HD 2	0,74	147,54	8,11	1,06	
CILP1	IVGPLEVNVR	HD 3	0,56	112,44	6,18	0,80	
CILP1	IVGPLEVNVR	HD 4	0,92	184,48	10,14	1,32	
CILP1	IVGPLEVNVR	HD 5	1,09	218,20	11,99	1,56	
CILP1	IVGPLEVNVR	HD 6	1,41	282,30	15,51	2,02	
CILP1	IVGPLEVNVR	OA1	0,83	166,26	9,14	1,19	1,38
CILP1	IVGPLEVNVR	OA2	1,31	262,62	14,43	1,88	
CILP1	IVGPLEVNVR	OA3	1,22	243,50	13,38	1,74	
CILP1	IVGPLEVNVR	OA4	0,66	132,90	7,30	0,95	
CILP1	IVGPLEVNVR	OA5	0,68	136,34	7,49	0,98	
CILP1	IVGPLEVNVR	OA6	1,08	215,40	11,84	1,54	

	CILP1-IVG	OA	HD
Number of values		6	6
Minimum		0,9500	0,8000
25% Percentile		0,9725	0,8675
Median		1,365	1,190
75% Percentile		1,775	1,675
Maximum		1,880	2,020
Mean		1,380	1,275
Std. Deviation		0,3965	0,4601
Std. Error		0,1619	0,1878
Lower 95% CI		0,9639	0,7922
Upper 95% CI		1,796	1,758

Protein Name	Peptide Sequence	Replicate Name	NAT/SIS ratio	NAT (fmol)	NAT (fmol/ $\mu$ L serum)	NAT ( $\mu$ g/mL serum)	Average NAT ( $\mu$ g/mL serum)
CILP1	TFLVGNLEIR	HD 1	4,15	830,28	45,62	5,94	6,19
CILP1	TFLVGNLEIR	HD 2	4,93	986,36	54,20	7,06	
CILP1	TFLVGNLEIR	HD 3	3,66	731,68	40,20	5,24	
CILP1	TFLVGNLEIR	HD 4	5,47	1094,10	60,12	7,83	
CILP1	TFLVGNLEIR	HD 5	4,53	906,30	49,80	6,49	
CILP1	TFLVGNLEIR	HD 6	3,19	637,18	35,01	4,56	
CILP1	TFLVGNLEIR	OA1	4,12	824,36	45,29	5,90	8,76
CILP1	TFLVGNLEIR	OA2	9,57	1914,24	105,18	13,70	
CILP1	TFLVGNLEIR	OA3	4,34	867,52	47,67	6,21	
CILP1	TFLVGNLEIR	OA4	7,24	1447,22	79,52	10,36	
CILP1	TFLVGNLEIR	OA5	5,38	1076,52	59,15	7,71	
CILP1	TFLVGNLEIR	OA6	6,07	1213,64	66,68	8,69	

	CILP1-TFL	OA	HD
Number of values		6	6
Minimum		5,900	4,560
25% Percentile		6,133	5,070
Median		8,200	6,215
75% Percentile		11,20	7,253
Maximum		13,70	7,830
Mean		8,762	6,187
Std. Deviation		2,924	1,197
Std. Error		1,194	0,4885
Lower 95% CI		5,693	4,931
Upper 95% CI		11,83	7,442