

Quantitative Glyco-proteomics detects macrophage release of inflammatory mediators following activation

Overview

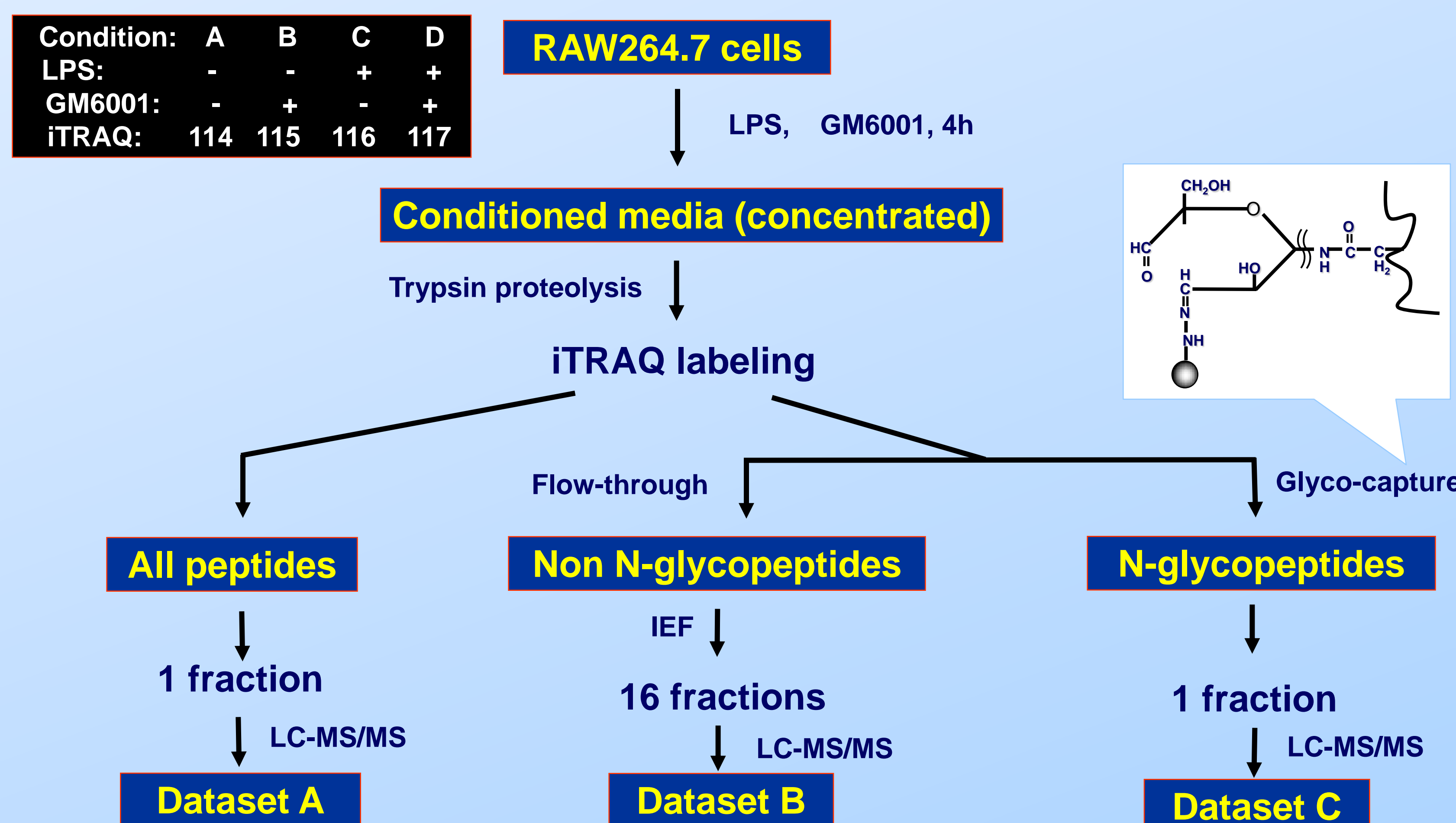
- Protein release following lipopolysaccharide (LPS)-mediated inflammatory stimulation of mouse macrophages (RAW264.7 cell line) was analyzed with a combinatorial proteomics platform using iTRAQ-based quantitation and glycopeptide enrichment.
- The iTRAQ-based quantitative proteomics profiling and evaluation of protein release in the presence and absence of a metalloprotease inhibitor allows secreted proteins to be distinguished from proteins proteolytically “shed” from the macrophage cell membrane.
- Application of glycopeptide capture enriched for shed and secreted proteins, and enhanced detection of the low abundance inflammatory mediators that have potential implications for inflammatory diseases such as atherosclerosis.

Introduction

Macrophages are critical regulators of immune and inflammatory responses that can determine disease pathogenesis. Stimulation following injury or microbial insult leads to macrophage protein secretion and proteolytic release from the cell surface. Mediators released by activated macrophages thus represent candidate proteins for biomarker discovery.

To identify proteins secreted or shed by activated macrophages, 4-plex, iTRAQ-based quantitative proteomics approaches were used to analyze macrophage proteins released into the media during inflammatory stimulation with lipopolysaccharide (LPS). Analysis of changes in protein release in the presence or absence of a metalloprotease inhibitor (GM6001) allows distinction between secreted and shed proteins. Application of glycoprotein capture to further enrich for secreted and shed proteins, together with the quantitative proteomics, allowed detection of less abundant proteins, including many cell surface proteins that regulate macrophage migration, phagocytosis, and antigen presentation.

Experimental Procedures

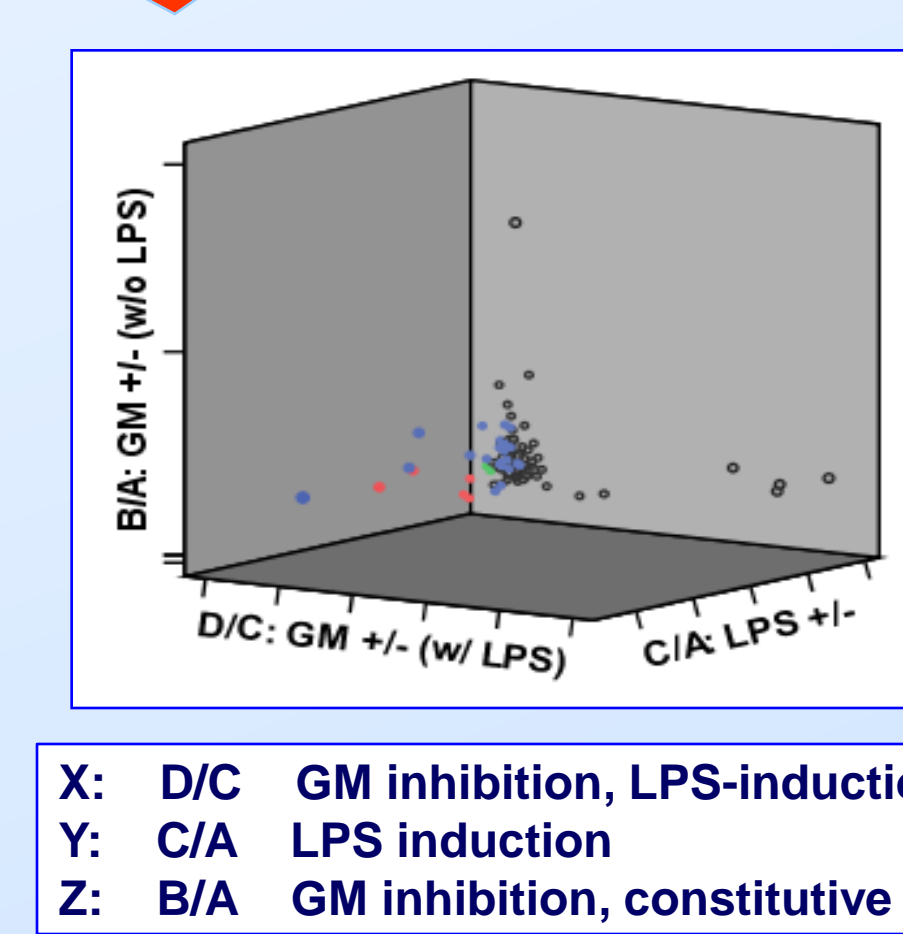


Results & Discussions

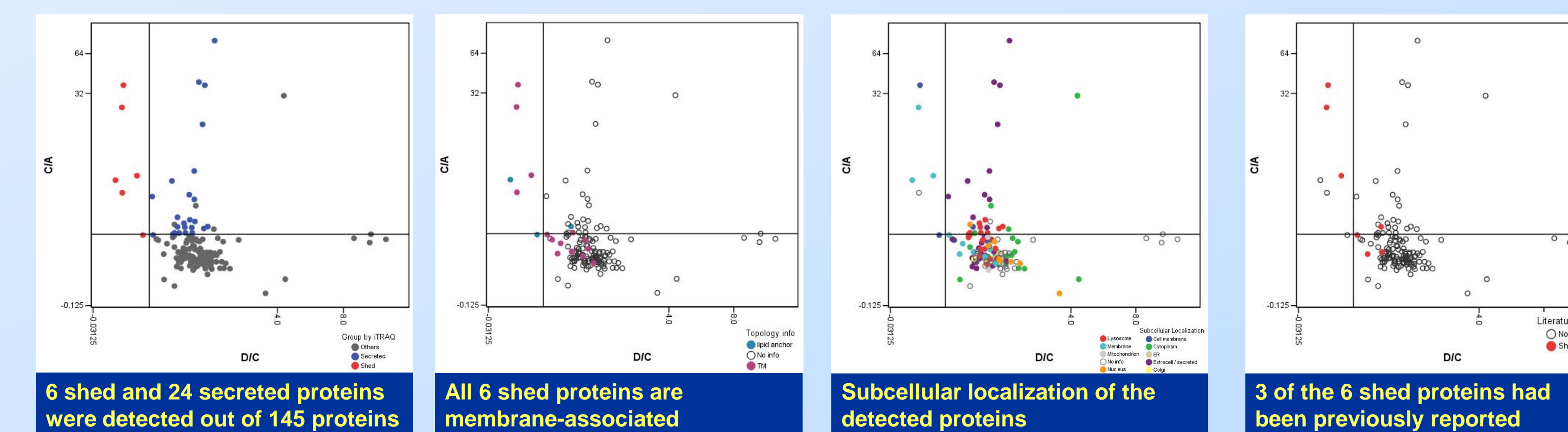
I. A total of 628 proteins were identified and quantified, including 71 secreted and 30 shed proteins following LPS stimulation.

	Dataset A	Dataset B	Dataset C	Unique protein IDs	Cutoff threshold
Total proteins	145	508	112	628	
Secreted proteins	24	43	15	71	D/C >0.6, C/A > 2.0, C/B > 1.85
Shed proteins	6	7	23	30	D/C <0.6, C/A > 2.0, C/B > 1.85

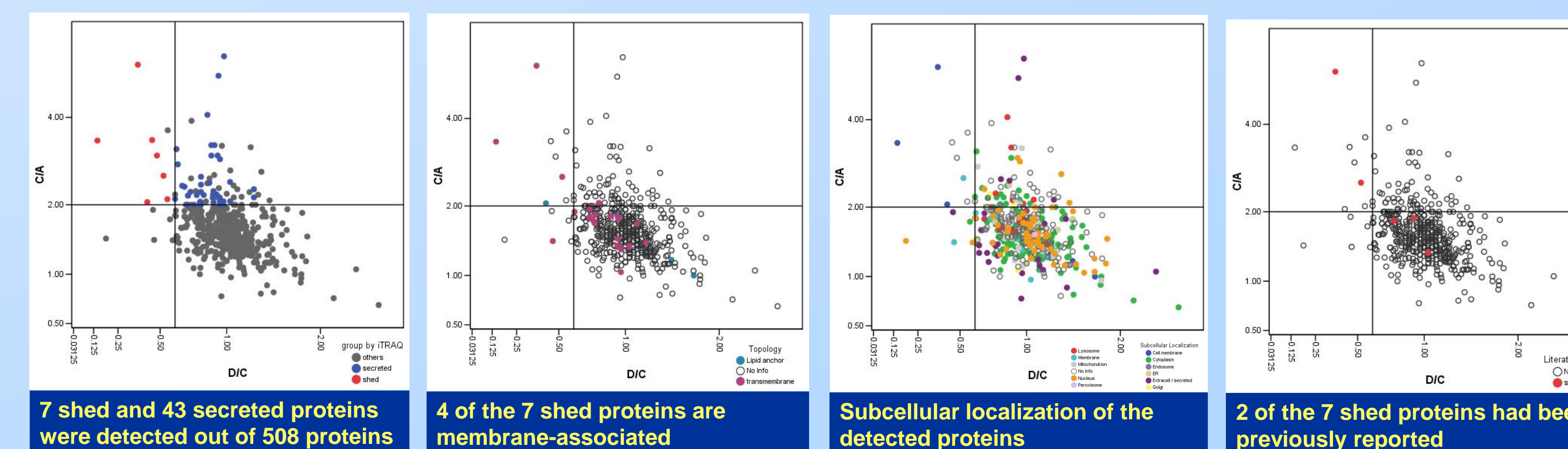
Gene Symbol	114 (A)	115 (B)	116 (C)	117 (D)	C/A	D/C	B/A
Dpep2					25.00	0.25	0.67
Scn11					37.00	0.26	0.31
Sdc4					5.08	0.27	0.50
Tnf	0.13	0.04	0.68	0.17	5.08	0.26	0.31
H2-D1	0.22	0.11	0.44	0.22	1.95	0.51	0.50
Sirpa	0.21	0.09	0.42	0.27	1.96	0.65	0.42
Lrp1	0.17	0.09	0.35	0.38	2.06	1.09	0.53
Atad1	0.18	0.10	0.36	0.35	2.00	0.97	0.56
Alas1	0.16	0.17	0.33	0.33	2.07	1.00	1.07
Dnase2a					37.00	1.19	1.00
Hexa	0.14	0.14	0.33	0.39	2.38	1.19	1.00
Scamp1	0.10	0.14	0.46	0.30	4.70	0.64	1.47
Ccl4	0.01	0.02	0.37	0.60	37.00	1.62	2.00
Lcn2	0.07	0.05	0.45	0.43	6.43	0.98	0.71
Cd14	0.15	0.09	0.37	0.39	2.40	1.06	0.60
Spp1	0.12	0.11	0.37	0.39	3.00	1.06	0.92
Lgmn	0.13	0.11	0.35	0.41	2.62	1.18	0.95
Ctsh	0.16	0.13	0.32	0.39	2.07	1.21	0.96
Cst3	0.08	0.02	0.39	0.51	4.88	1.28	0.25
Lipa	0.12	0.08	0.35	0.45	2.83	1.29	0.67
Tnc	0.14	0.07	0.34	0.45	2.36	1.33	0.50
Ctsh	0.15	0.14	0.31	0.41	2.07	1.34	0.93
Ccl3	0.05	0.01	0.39	0.55	7.80	1.38	0.20
Plasp	0.08	0.12	0.34	0.47	4.43	1.59	1.57
Fli2	0.11	0.16	0.30	0.42	2.73	1.40	1.45
Cxcl2	0.01	0.01	0.39	0.59	39.00	1.49	1.00
Lil	0.02	0.02	0.37	0.58	18.00	1.57	1.00



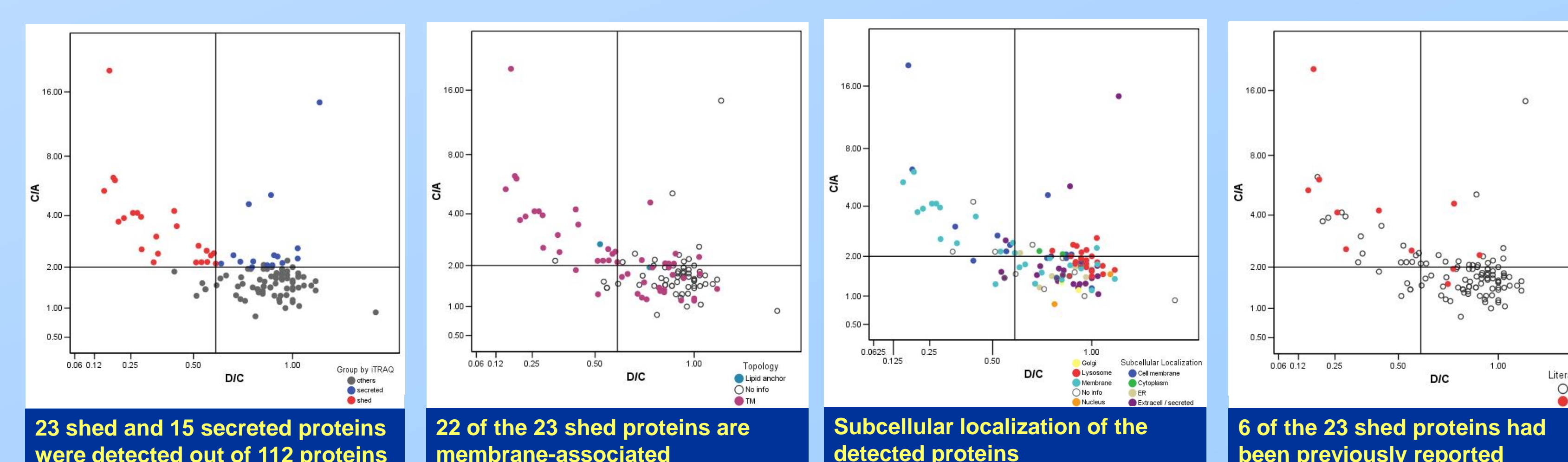
II. Secreted and shed proteins have distinct iTRAQ quantitative patterns (dataset A).



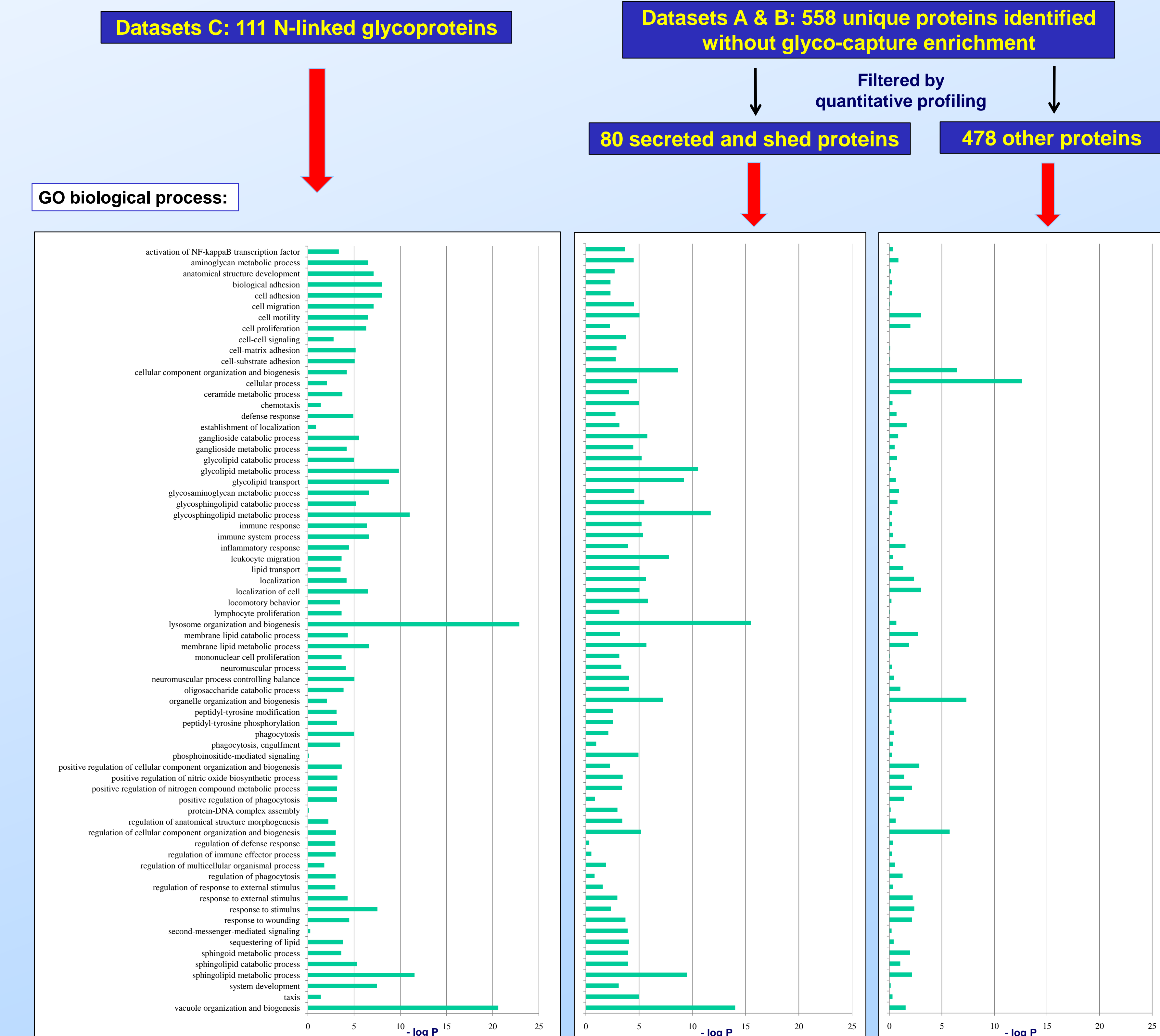
III. IEF fractionation identifies more total proteins, but not more shed proteins (dataset B).



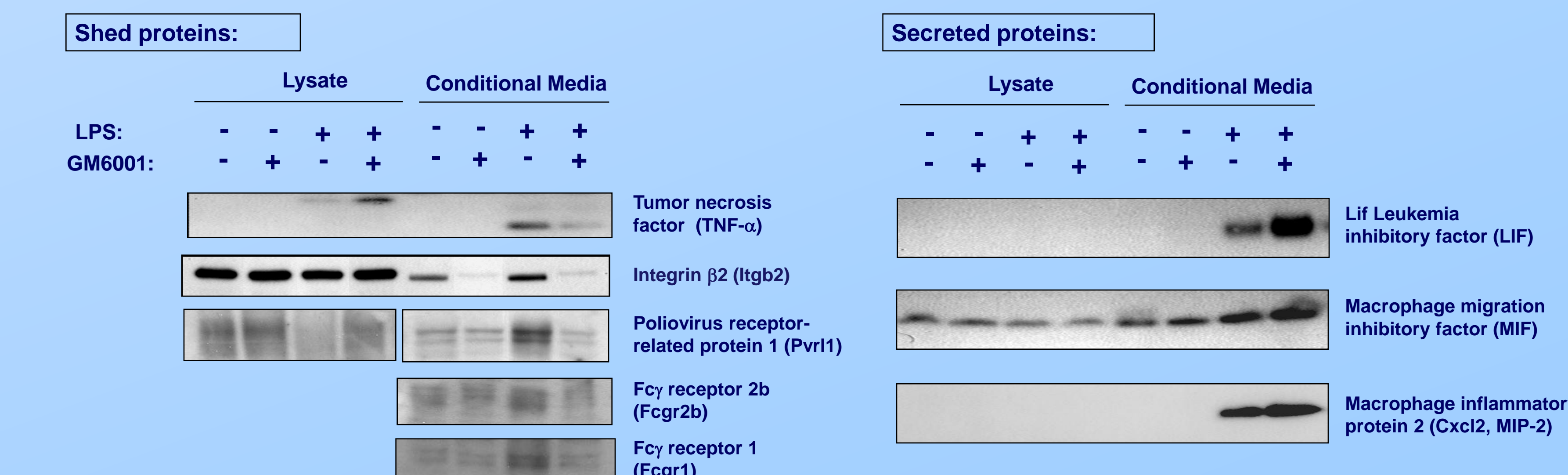
IV. Glyco-capture allows detection of more shed proteins (dataset C).



V. The shed and secreted proteins detected by quantitative profiling have similar gene ontology patterns to those identified by glyco-capture enrichment.



VI. Validation of selected targets:



Conclusions

- iTRAQ analysis of proteins released by mouse macrophages following LPS stimulation with and without a metalloprotease inhibitor demonstrates:
- Unique iTRAQ-based quantitative patterns that allow distinction between secreted and shed proteins
 - Glycopeptide enrichment leads to detection of less abundant shed and secreted proteins that are often glycosylated, and gene ontology analysis suggests these proteins may play important regulatory roles in inflammatory diseases such as atherosclerosis

Acknowledgement

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