

Extracting proteins from mammalian cell membranes with SMA

Sharing some of our 'interesting'
experiences so far in 2 short
stories

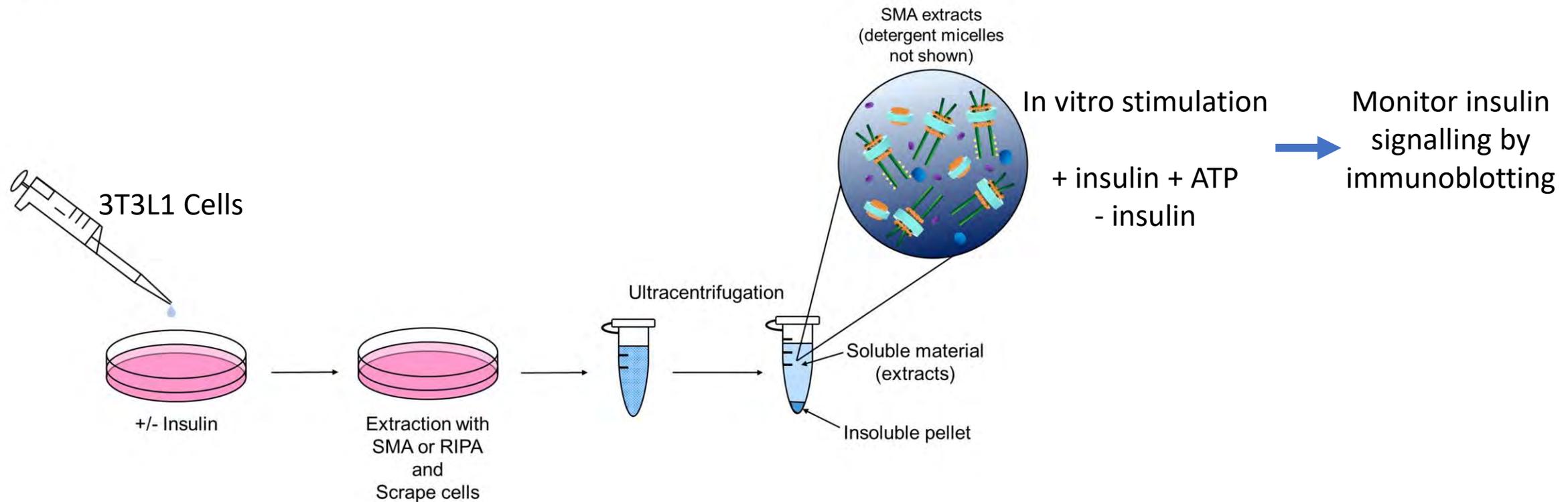
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Short Story 1: Insulin receptor signalling

- Question: Can we isolate functional insulin receptor in nanodiscs?
- To develop a simple assay to monitor membrane protein function in 'different flavoured' copolymer nanodiscs.



The insulin signalling cascade

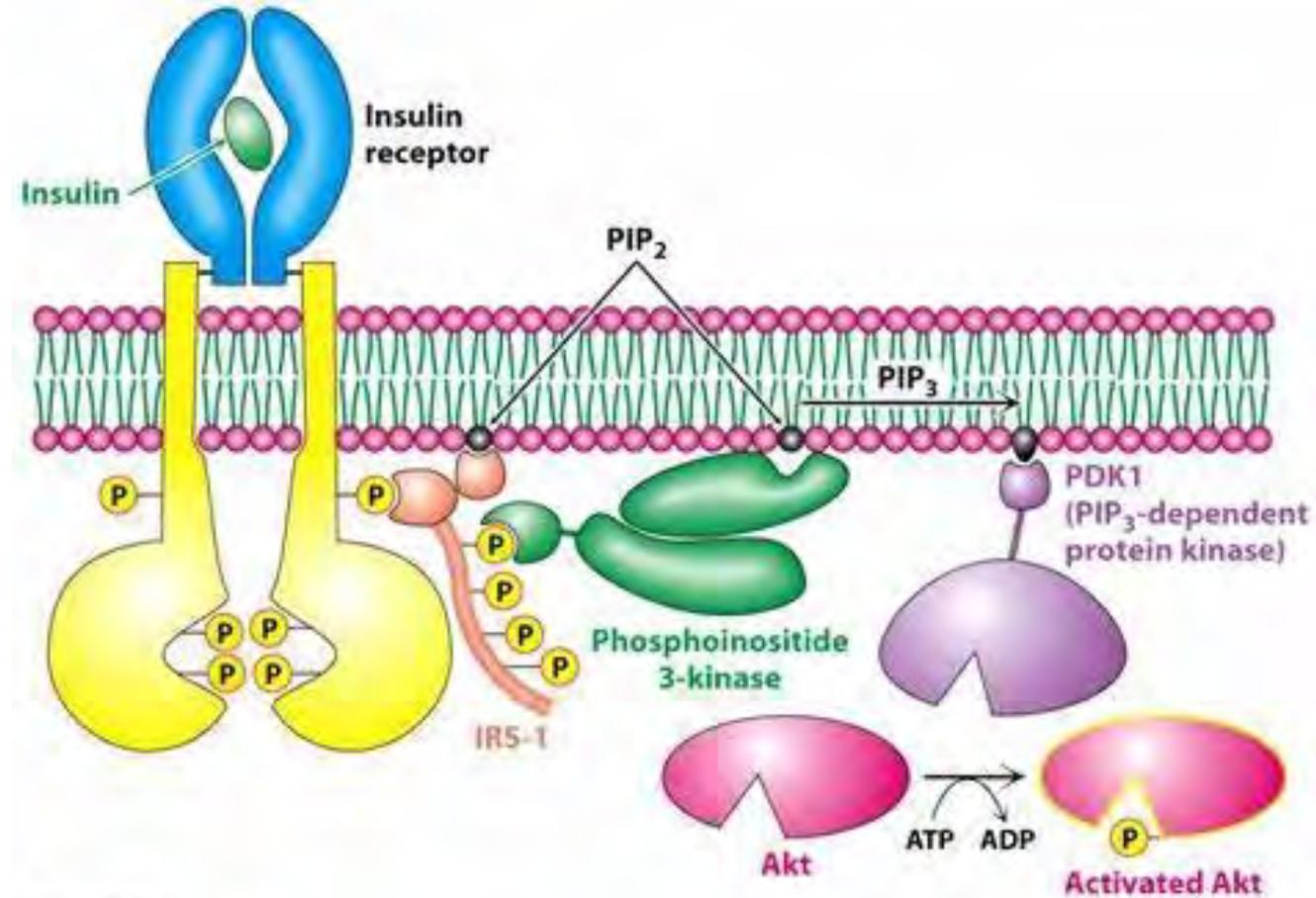
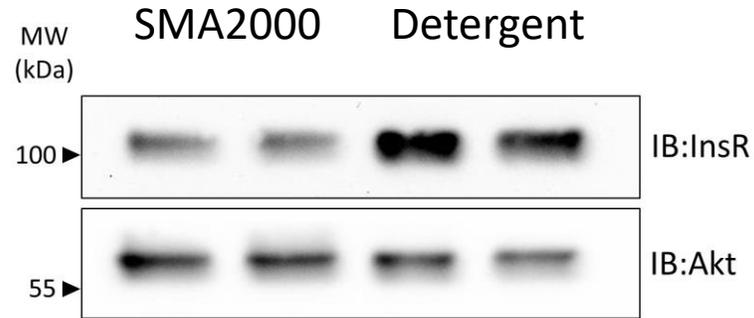


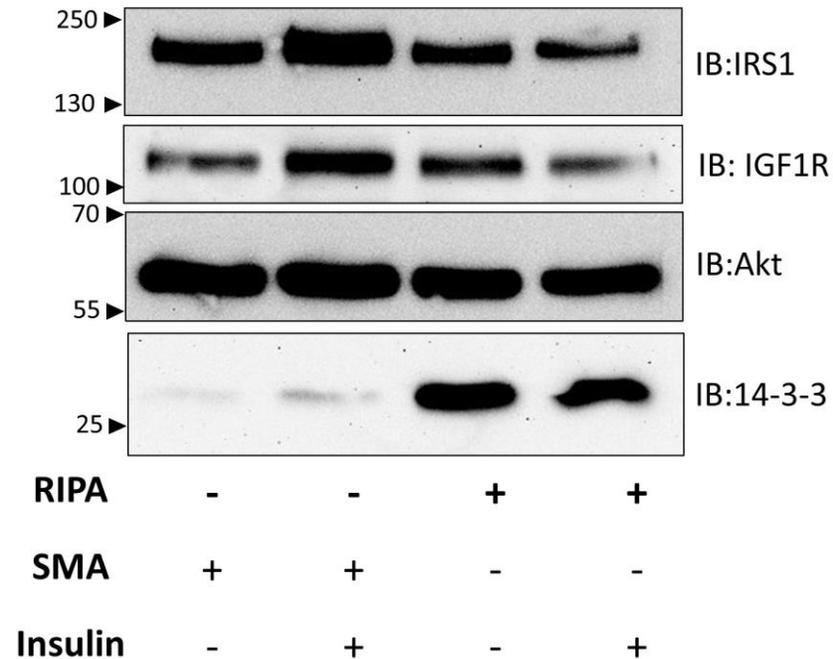
Figure 14.21
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What is in the supernatants following centrifugation?

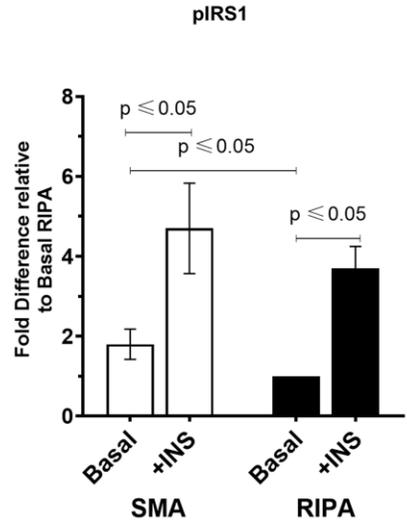
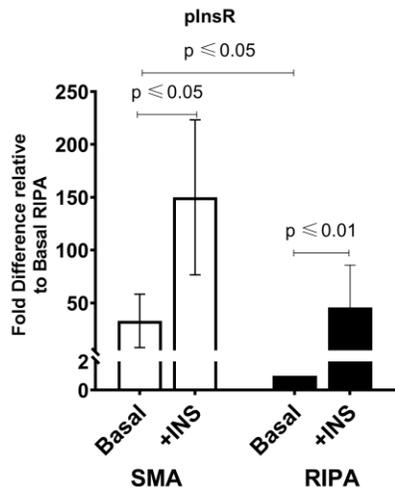
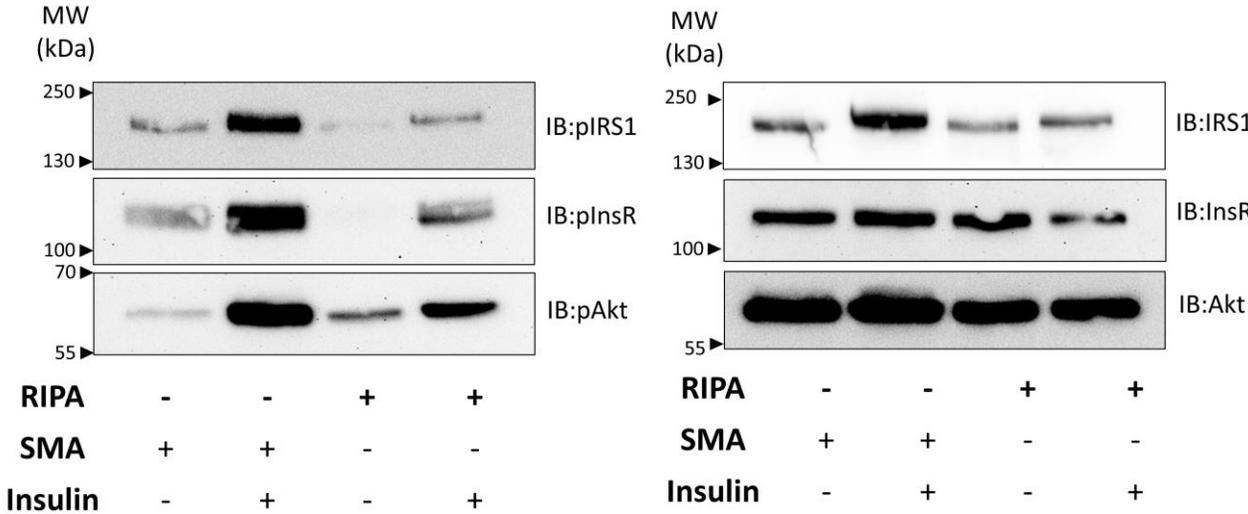
Everything we expected



Except.....



SMA2000 extraction from cells with no insulin added results in InsR and IRS1 phosphorylation



Conclusions from Story 1

- SMA2000 extraction results in unexpected autophosphorylation of a sub-population of the InsR and phosphorylation of IRS1.
- Other polymers do the same (Xiran25010 and XiranSL30010).
- Causes issue in development of assay.
- Do not see Akt activation upon SMA extraction (spatial constraints).
- Do not have evidence that *in vitro* addition of insulin results in further phosphorylation of InsR. Other polymers may be more suitable.
- SMA extraction has some 'weird effects' on some cytoplasmic proteins (14-3-3 and GAPDH).

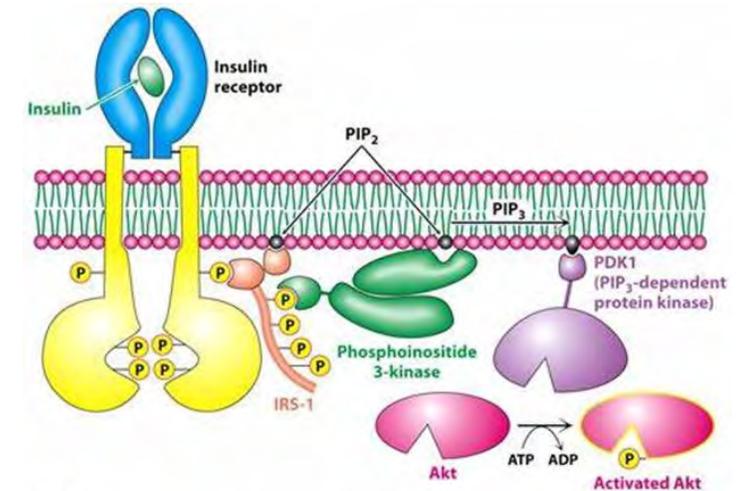
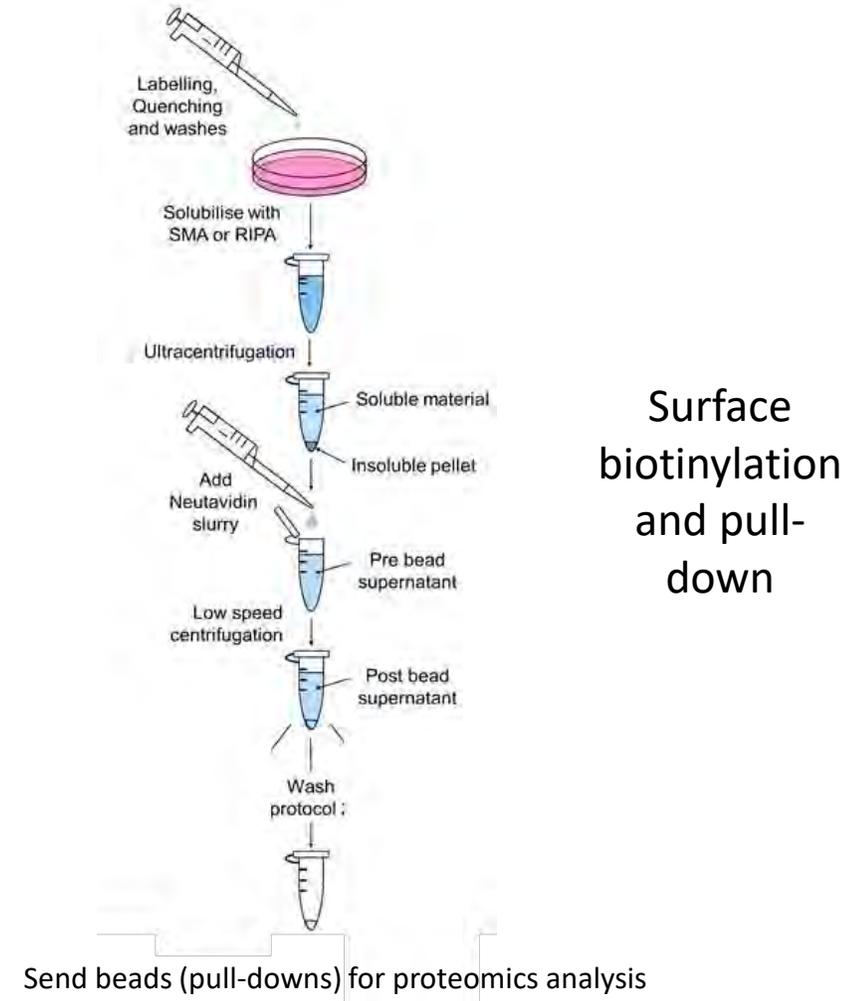


Figure 14.21
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Short Story 2: Surface Smalpome

- Aim: To compare SMA extracted surface proteome with detergent extracted surface proteome. (TMT quantitative approach)
- Is there evidence that SMA is more selective in the proteins it extracts from The PM than detergent (e.g. are proteins associated with lipid rafts less efficiently extracted by SMA)?
- Is there evidence that protein complex integrity (ie membrane proteins with cytoplasmic proteins) is maintained in nanodiscs?
- Story on performing proteomics on SMA extracted samples so far?

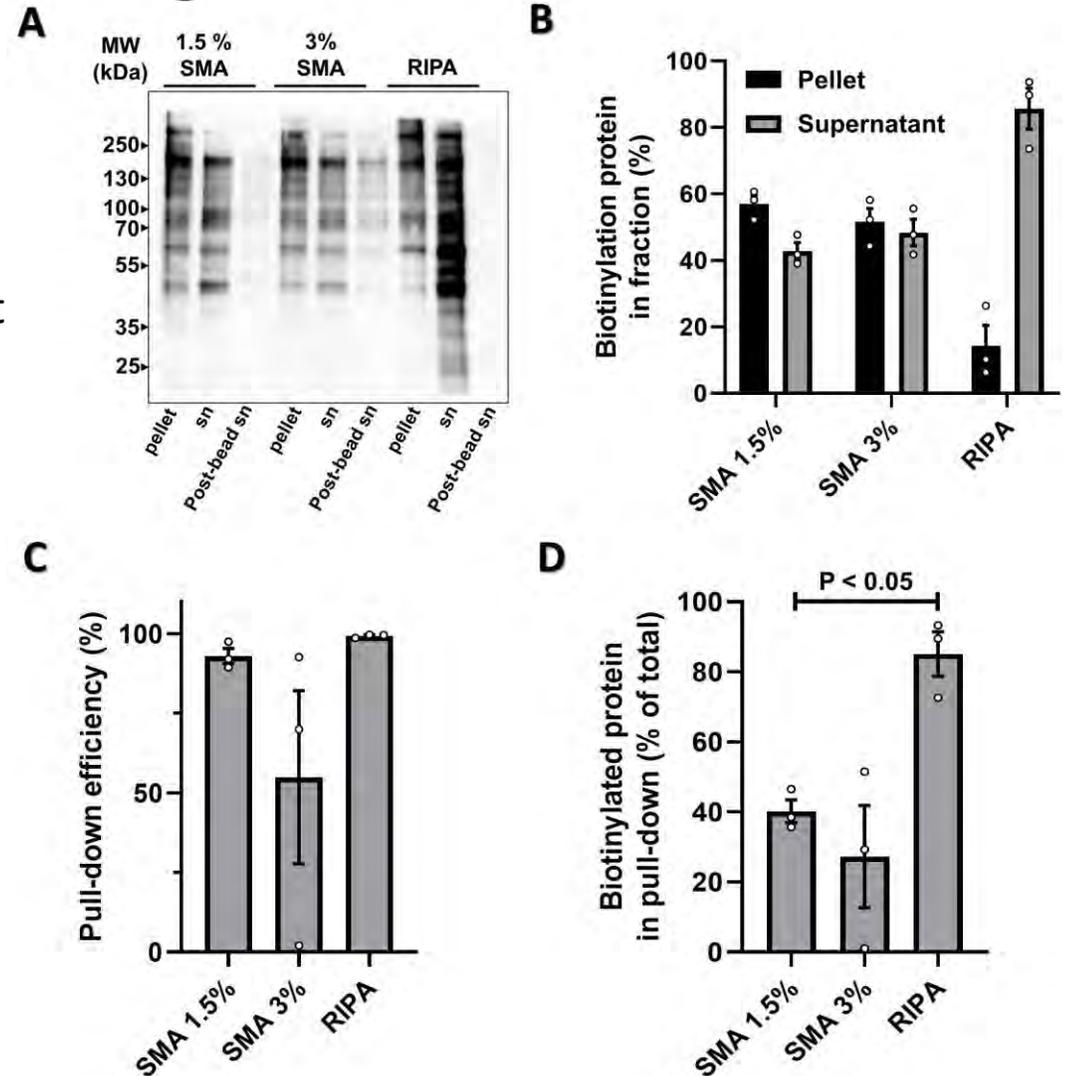


SMA2000 is not as efficient at solubilising PM proteins as detergent

- Are biotinylated proteins in the SMA extract supernatants? ✓
- Are biotinylated proteins from SMA supernatants efficiently pulled-down? ✓

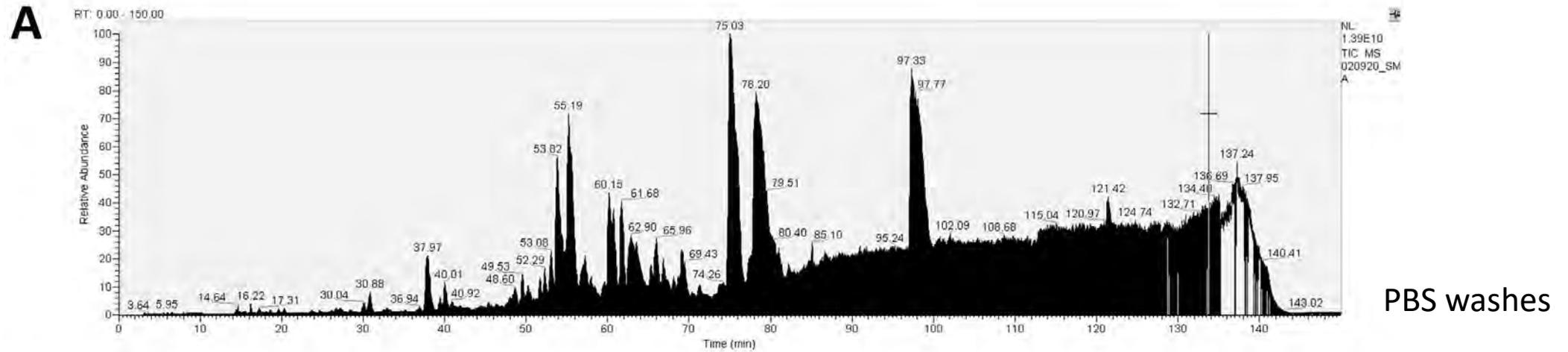
But only 40% in SMA extract supernatants.

85% in detergent extract supernatants.

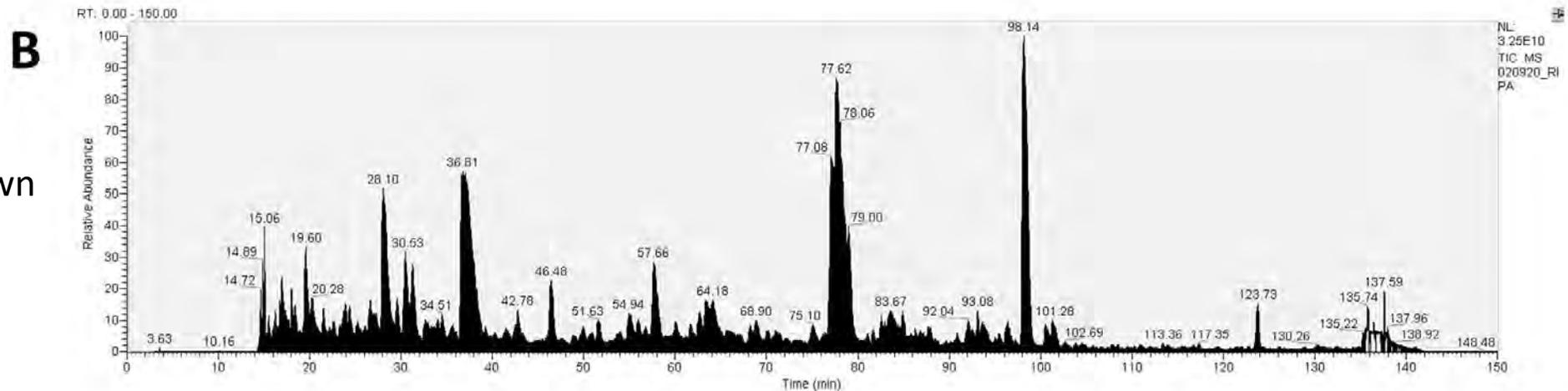


Presence of SMA interferes with proteomics analysis.

SMA pull down

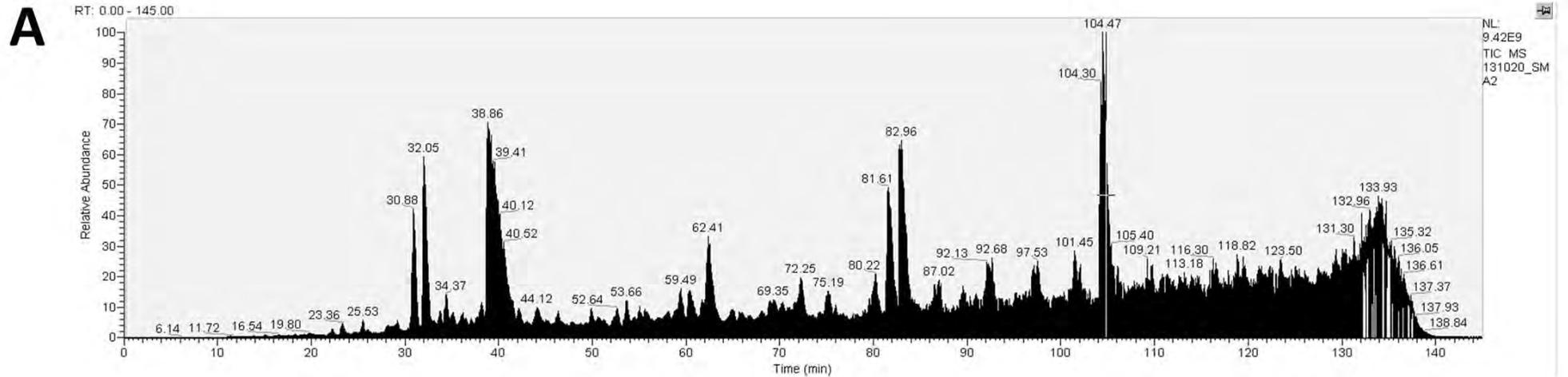


Detergent pull down

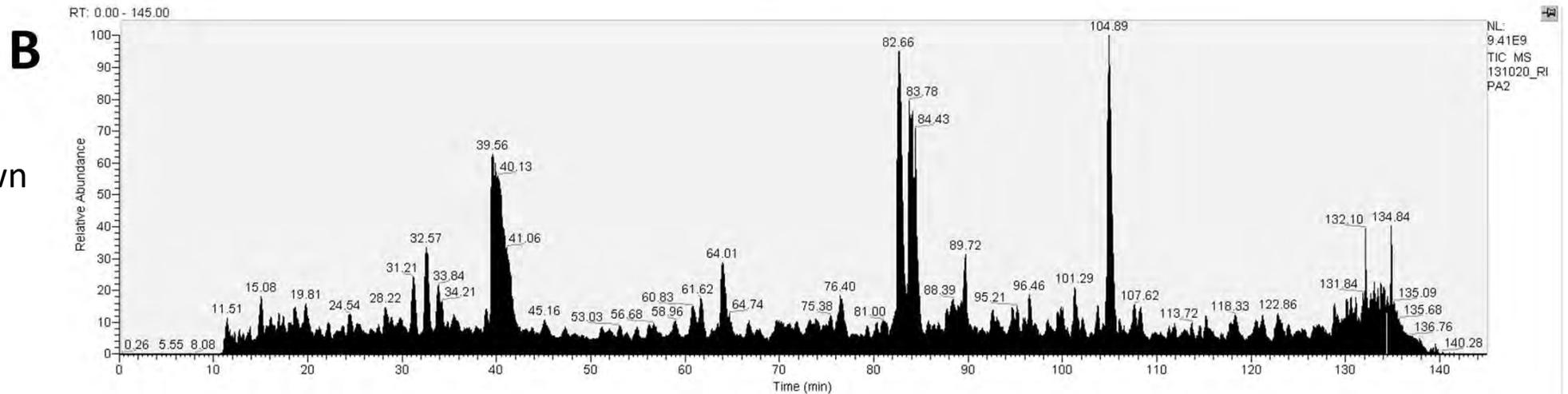


How did we diminish SMA interference? Washing in conditions promoting nanodisc disassembly (50mM CaCl₂) improve TIC profile

SMA pull down

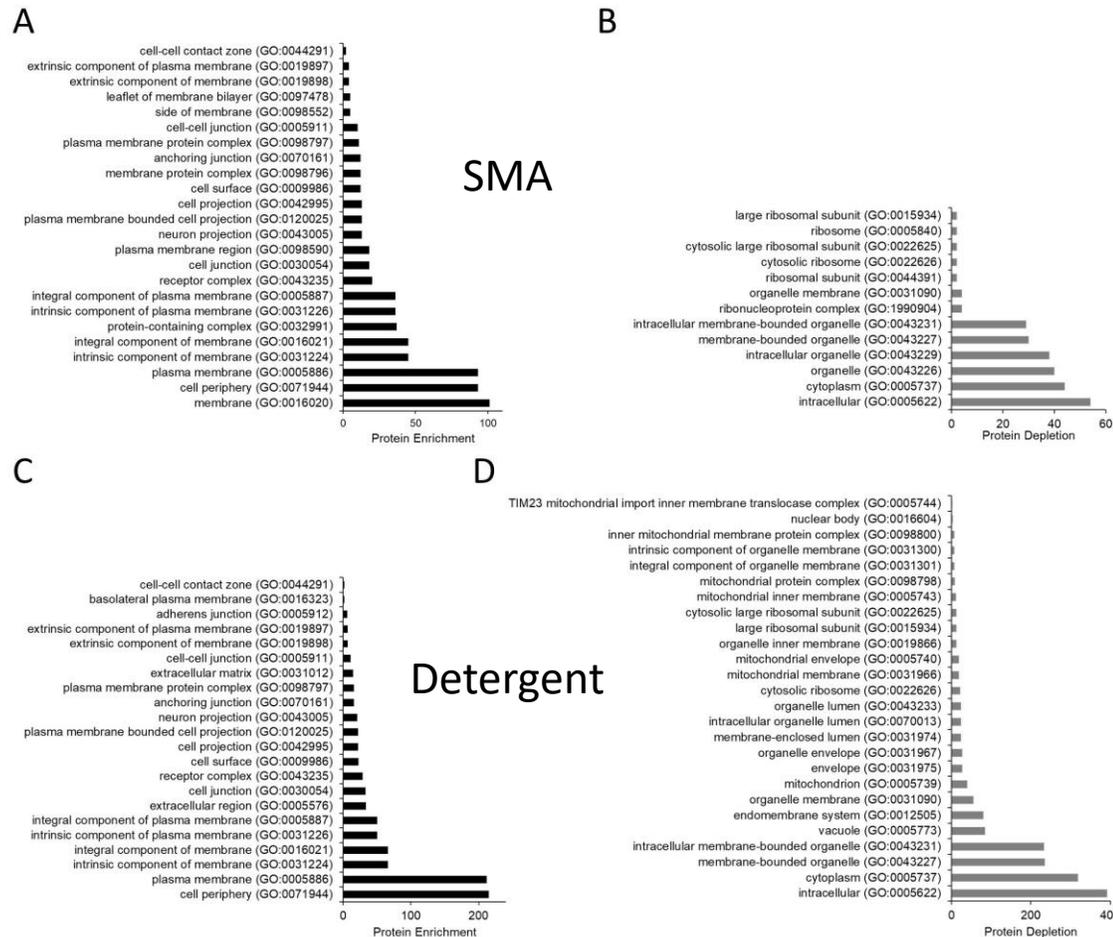


Detergent pull down



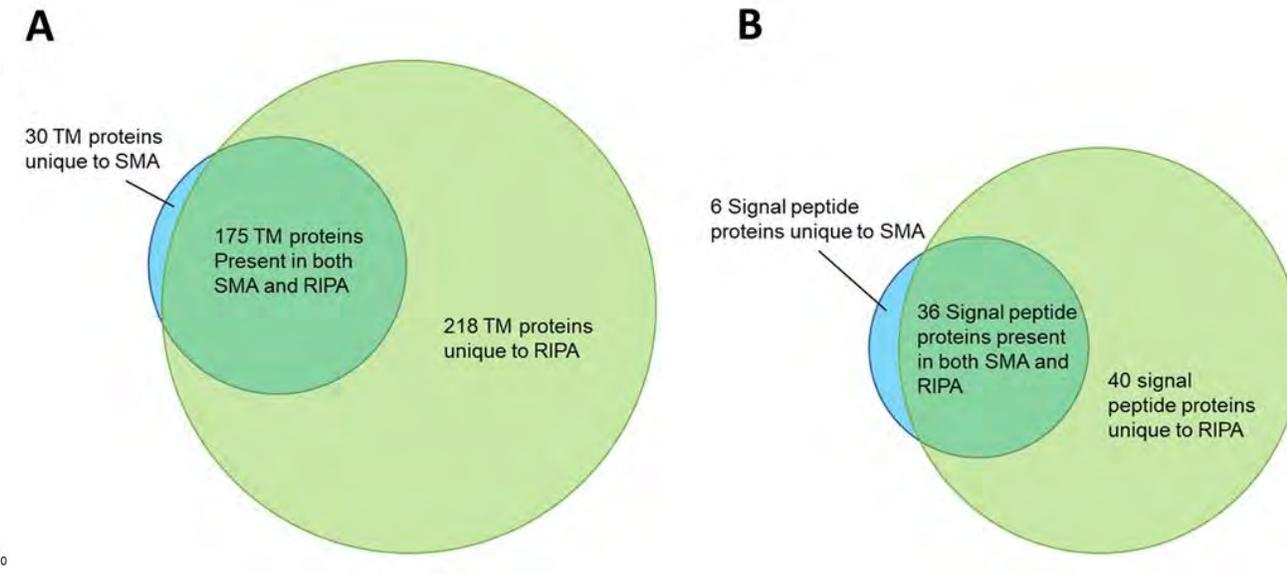
Samples are highly enriched for Transmembrane proteins and those containing Signal Peptides

PANTHER GO analysis



371 proteins detected with high confidence in SMA pull-downs. 67% TM or SP

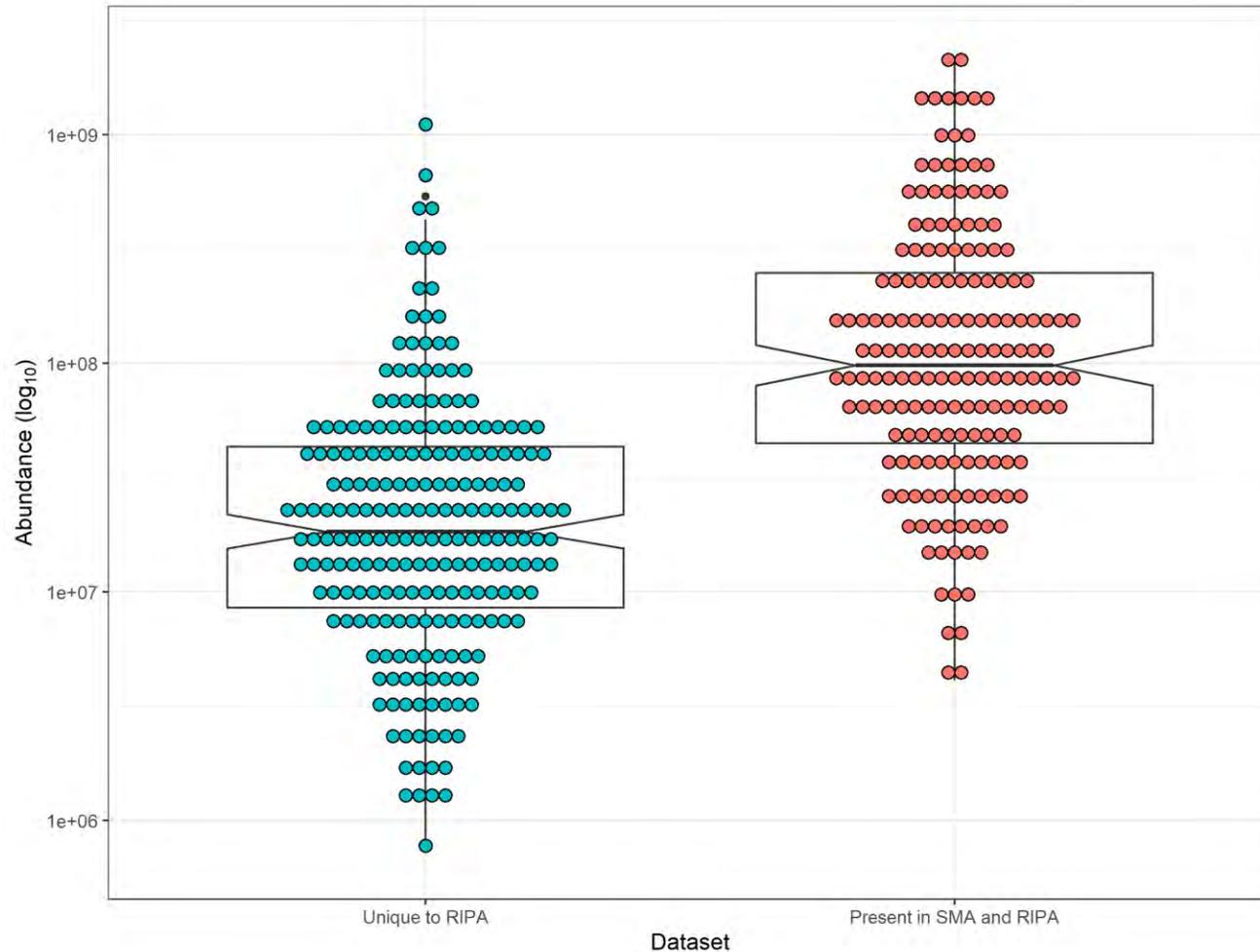
1368 proteins detected with high confidence in detergent pull-downs. 34% with TM or SP



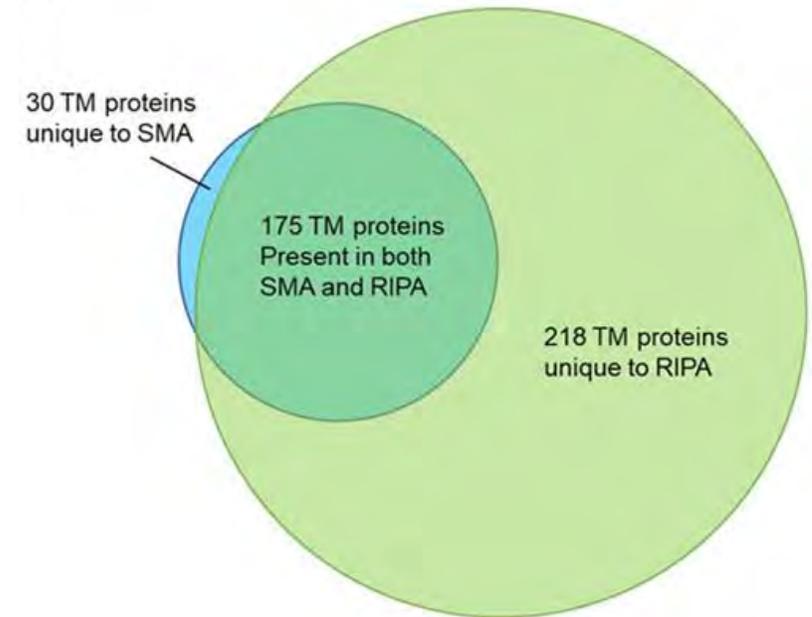
Enrichment

Depletion

No strong Evidence for selectivity: The most abundant proteins from the RIPA pull-downs are also in the SMA pull-downs.



A



Other observations

- GPI anchored proteins are not apparently depleted in SMA pull-downs 10/247 (4%) and 15/470 (3.2%) in detergent.

- Proteins only detected/highly enriched in SMA pull-downs are mostly multi-spanning

Uniprot code	Membrane protein (Gene name)	No. TM domains
Q8R0I4 ^a	Tm2d2	2
Q8VI59 ^a	Pcnx3	13
B2RWU5 ^a	Abca7	11
Q8K2Y3 ^a	Eva1b	1
Q5Y5T2 ^a	Zdhhc18	4
Q61469 ^a	Plpp1	6
Q3TMA0 ^a	Slc16a3	12
Q61091 ^a	Fzd8	7
Q542F3 ^a	Slc19a1	11
A2AW86 ^a	Ly75	1
Q5U647 ^a	Slc1a5	9
Q6PIX5 ^a	Rhbdf1	7
Q8BY89	Slc44a2 Ctl2	10
Q8C8K1 ^a	Ephb4	1
A0A0R4J0A9 ^a	Lrp6	1
Q8CC06 ^a	Itga6	1
Q3TRK9 ^b	Slc16a1	10
Q02013 ^b	Aqp1	6
Q8C145 ^b	Slc39a6	6
G5E829 ^b	Atp2b1	10

Conclusions from story 2....so far

- SMA does not solubilise PM of mammalian cells as effectively as detergent.
- We are confident that quality of proteomics data from SMA pull-downs is now good enough for TMT labelling.
- Is there evidence that SMA is more selective in the proteins it extracts from the PM (e.g. are proteins associated with lipid rafts less efficiently extracted by SMA)?  Too early to say. Need quantitative proteomics to see subtle differences. Intriguing that some proteins (Multi TM) uniquely detected in SMA pull-downs.
- Is there evidence that protein complex integrity (ie membrane proteins with cytoplasmic proteins) is maintained in nanodiscs? 
The washes disassemble the nanodiscs so not possible to address this question

Acknowledgements

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