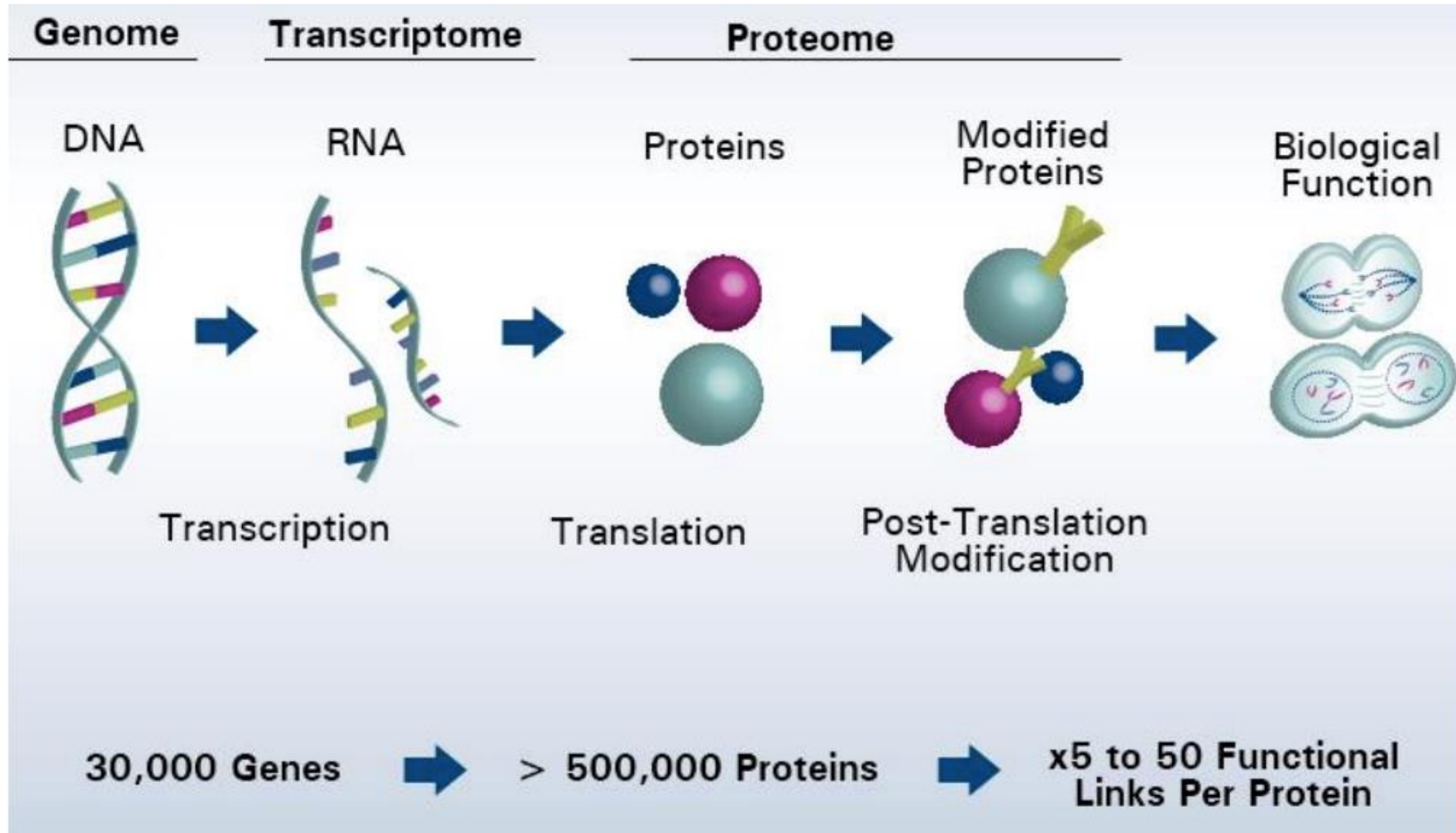


Quantitative Proteomics and iTRAQ/TMT

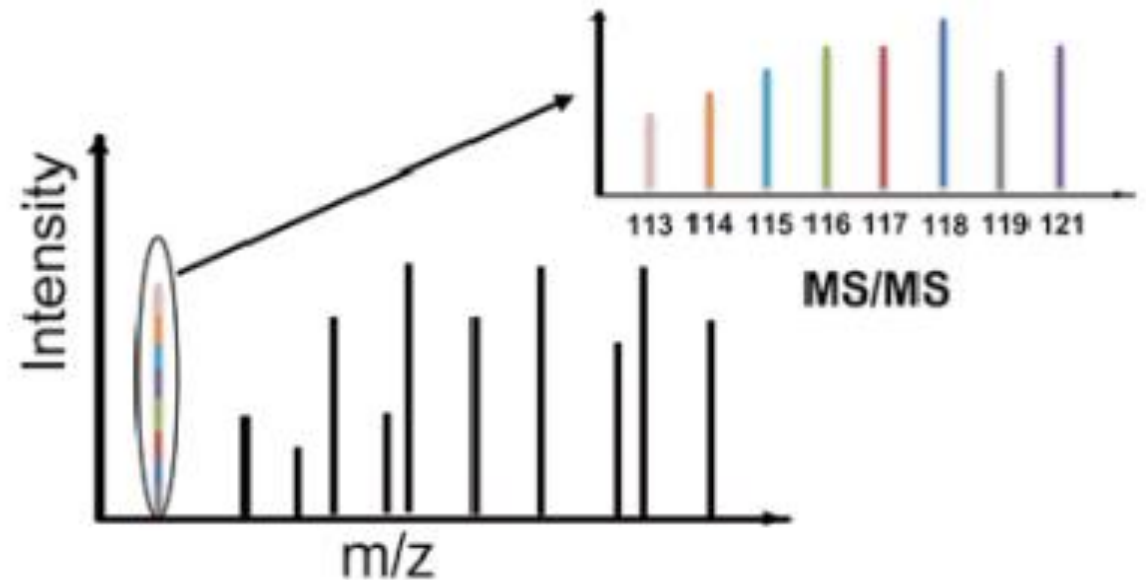
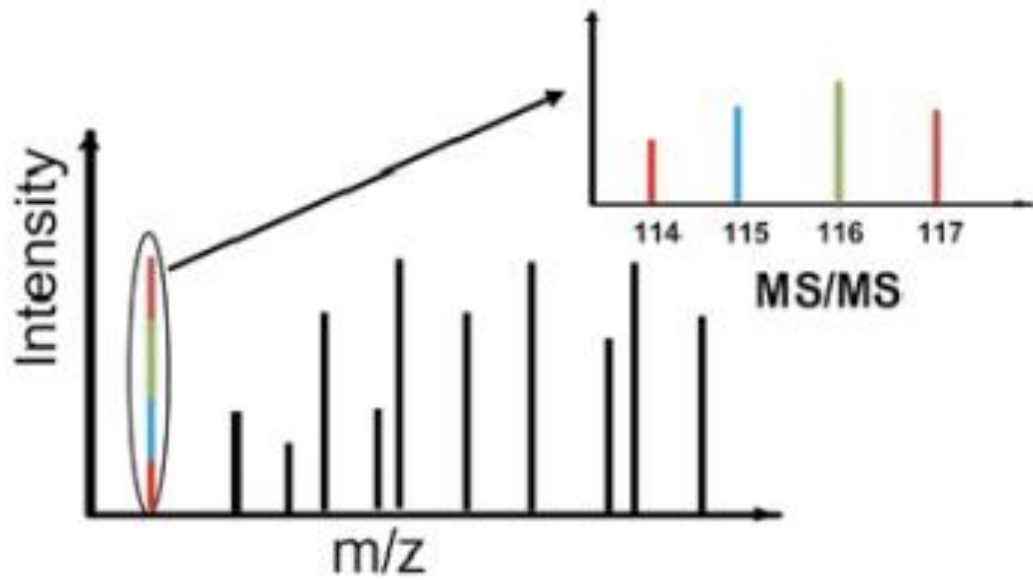
By: Miles Giehlbrock and
Praneeth Venigalla

What is Quantitative Proteomics?

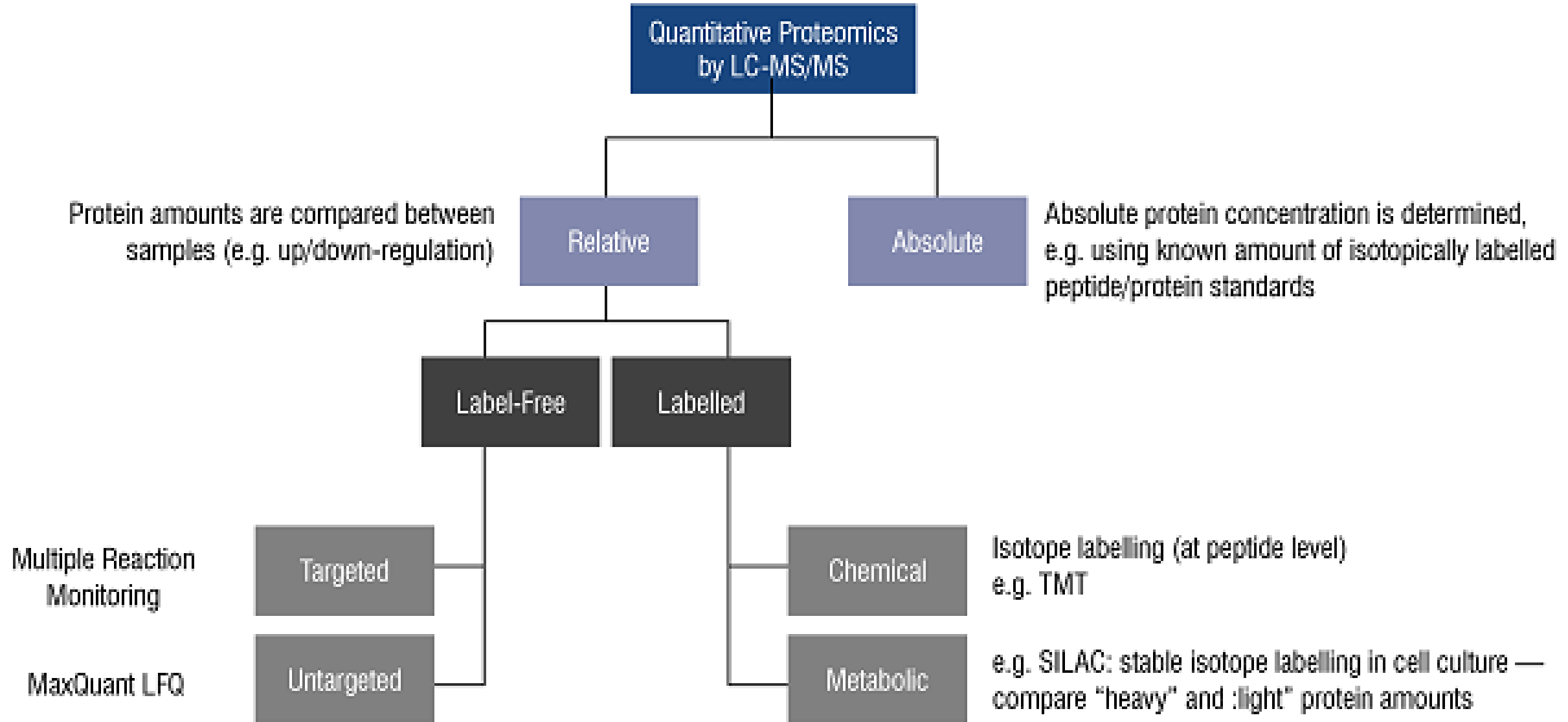


- Definition: focuses on **identifying** and **quantifying** the entire protein
- measure the relative or absolute abundance of proteins across different conditions, time points, or treatments.

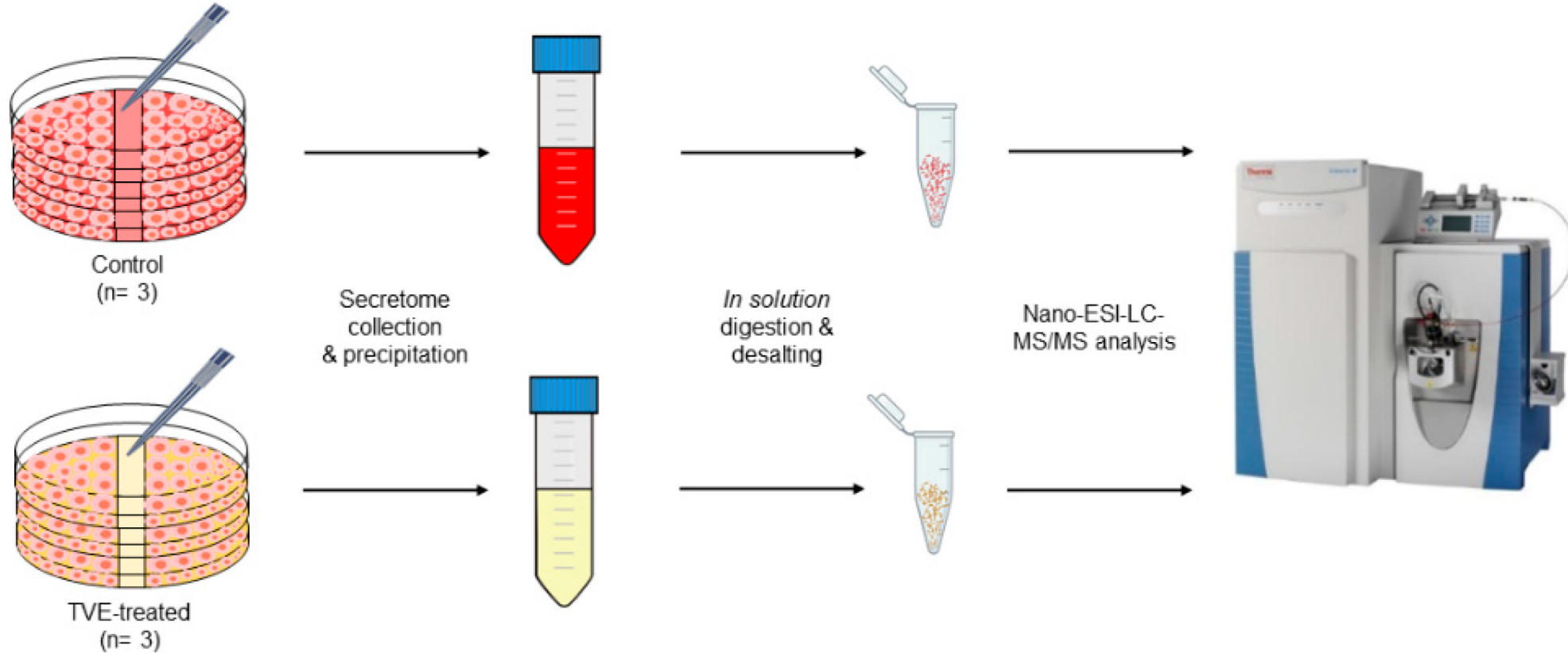
Why is it important to quantify proteins in a sample?



What are some ways to quantify proteins in a sample?

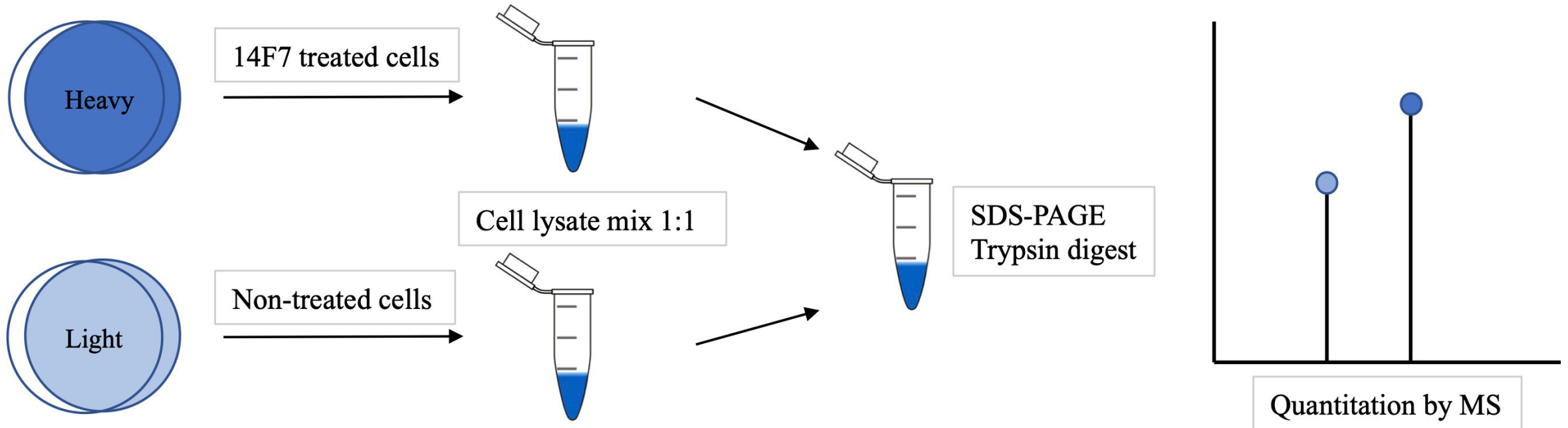


Review: Label-Free



Measured directly from the raw MS data without the use of any stable isotope labeling

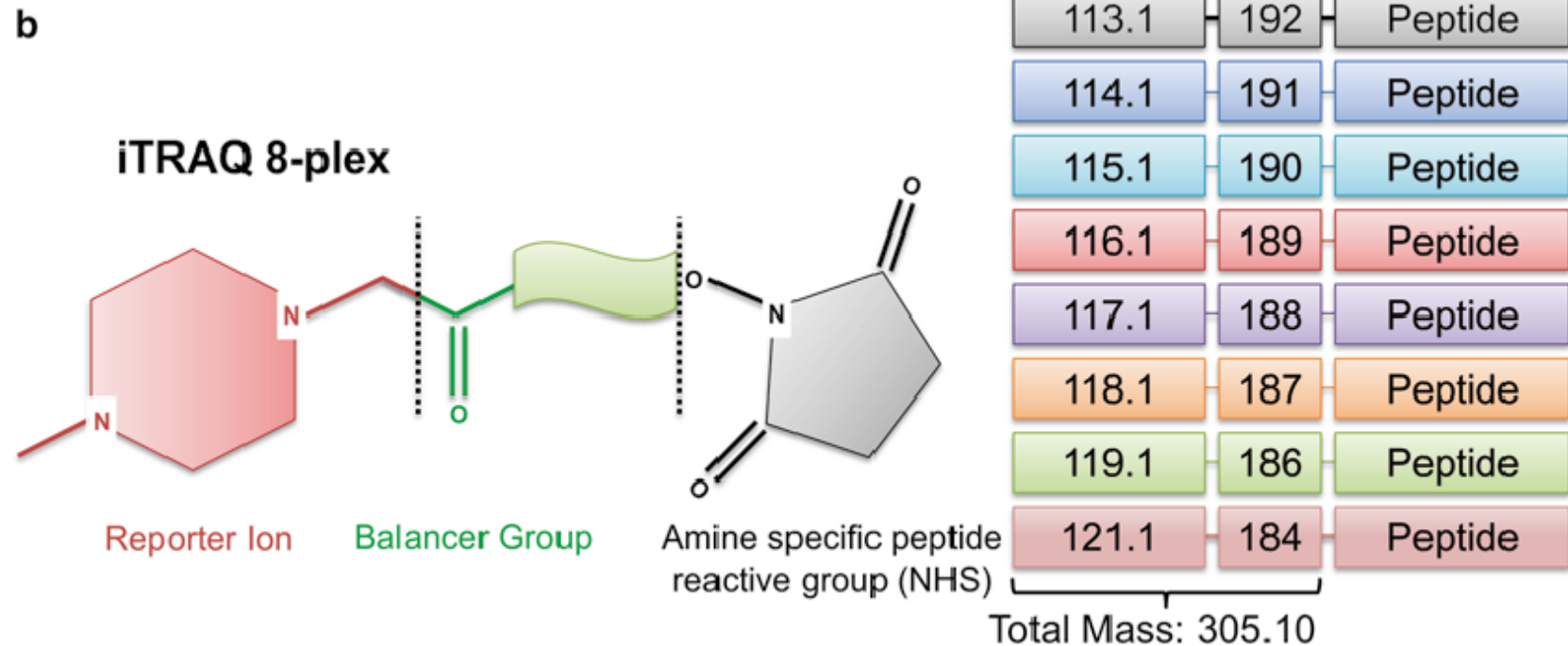
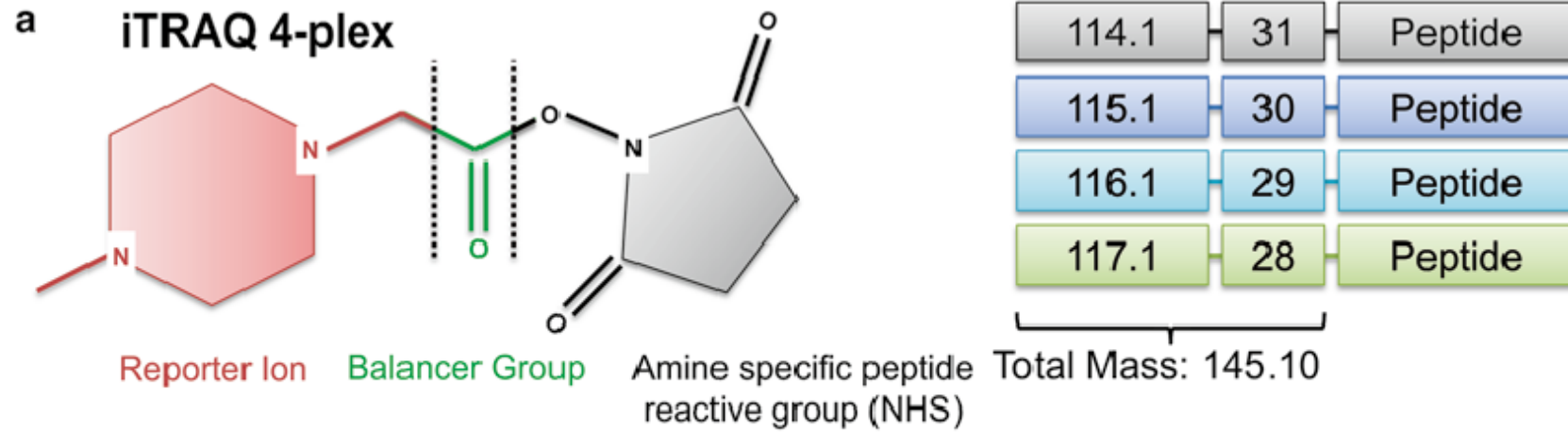
Review: SILAC



- Cells are grown in a medium containing either 'light' or 'heavy' versions of specific amino acids
- Resulting proteins are then combined and analyzed by MS
- Direct comparison of protein abundance between conditions

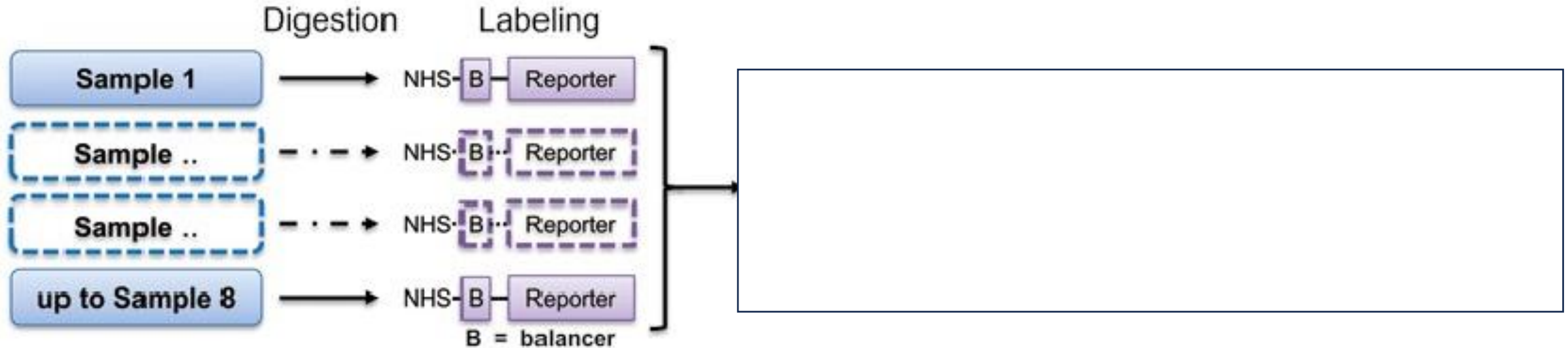
What are isobaric tags?

- Each isobaric tag consists of a reporter group, a balance group, and a peptide-reactive group
- The total mass of each tag is identical
- fragmentation during MS/MS releases reporter ions of different masses



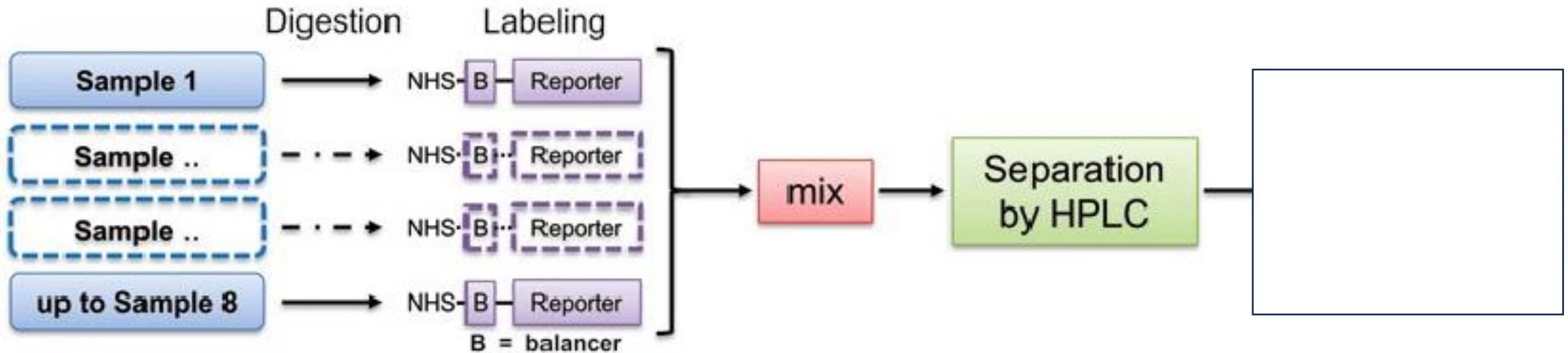
How does an iTRAQ assay work?

Each set of digested peptides from different samples is labeled with a distinct iTRAQ reagent



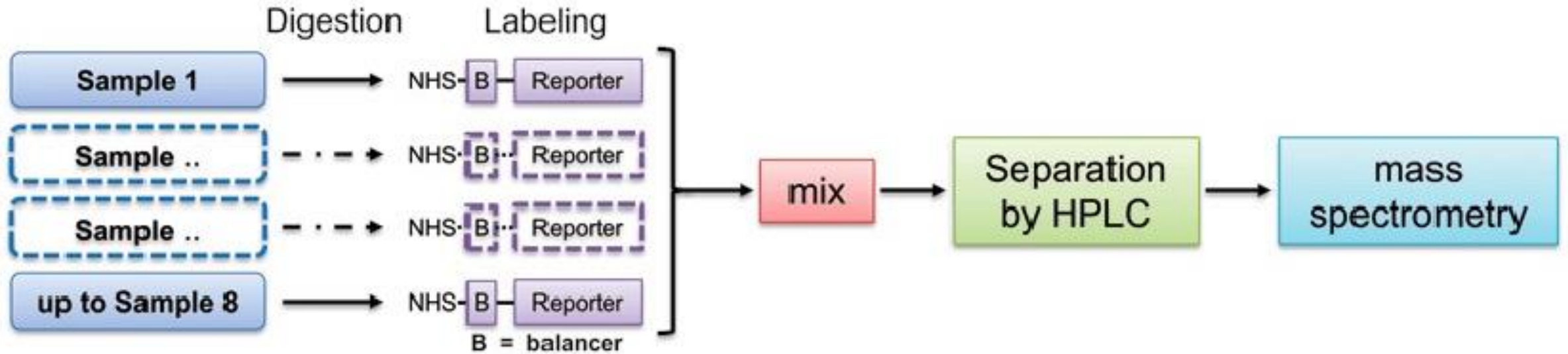
Isobaric **T**ags for **R**elative and **A**bsolute **Q**uantitation

How does an iTRAQ assay work?

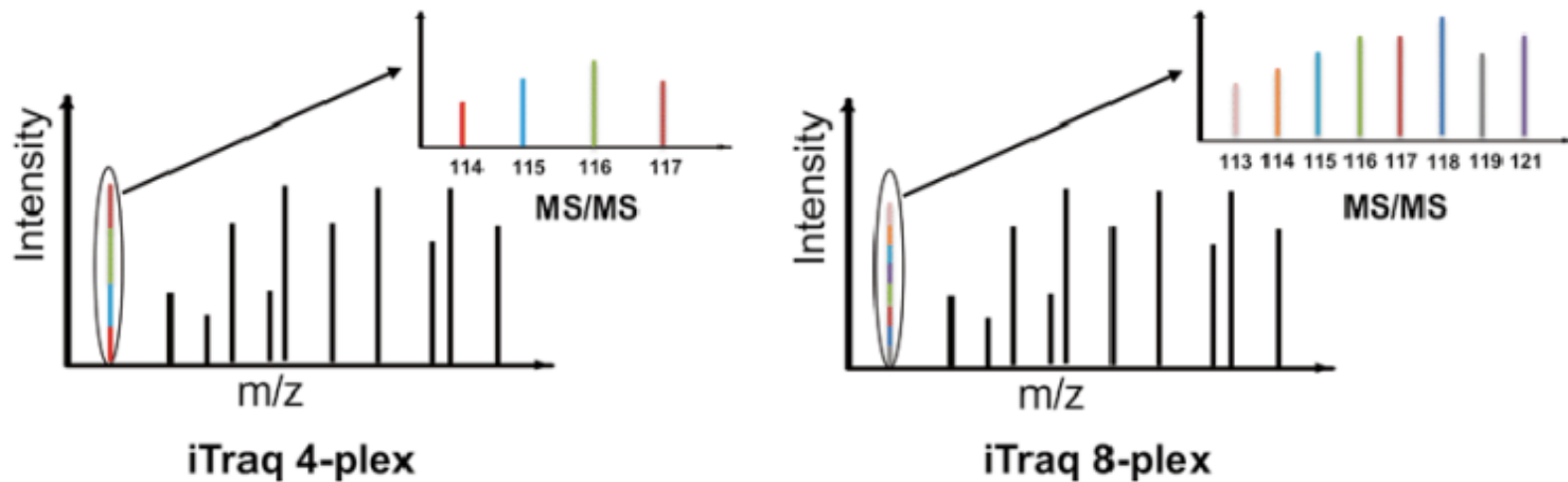


- Peptides from all samples are mixed together
- Separated by liquid chromatography (LC) to reduce sample complexity

How does an iTRAQ assay work?

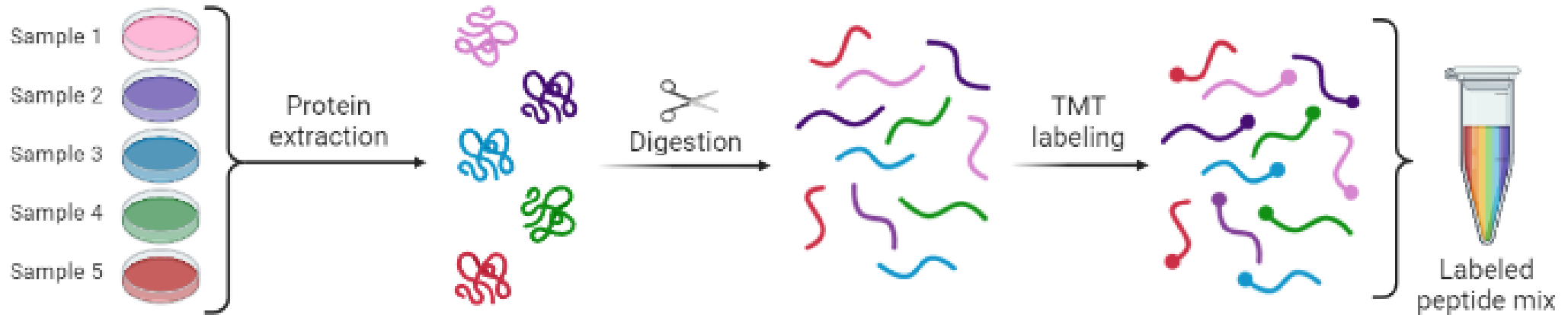


b Quantitation by iTRAQ reporter ions

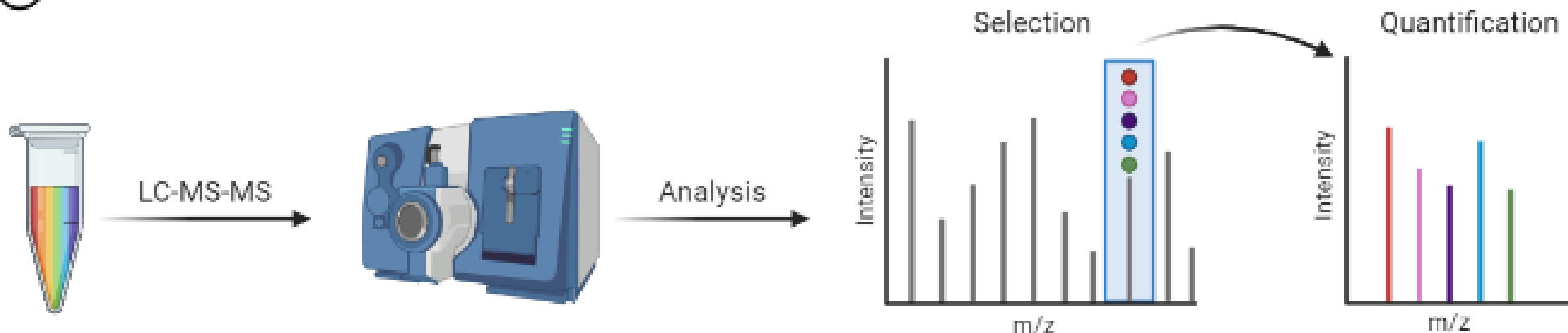


How does Tandem Mass Tags (TMT) labelling work?

1 TMT labeling protocol



2 Data collection and analysis



Pros

Cons

TMT

- **High Multiplexing Capacity:** 18
- **Improved Sensitivity**
- **Compatibility:** Compatible with a wide range of samples and complex biological matrices.
- **Data Quality:** more recent advancements in TMT reagents and mass spectrometry technology.

- **Cost:** Generally more expensive than iTRAQ,
- **Ratio Compression**
- **Complexity:** requires specialized software and expertise.

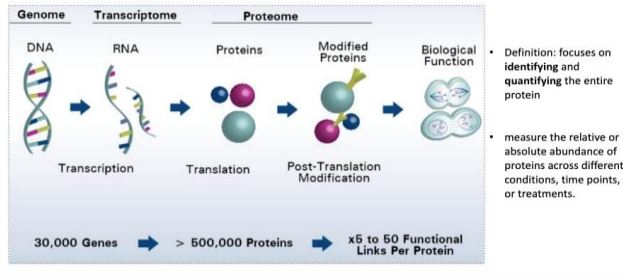
iTRAQ

- **Cost-Effective:** Less expensive than TMT
- **Flexibility:** Available in 4-plex and 8-plex versions,
- **Established Methodology:** Widely used and well-understood
- **Compatibility:** Suitable for many types of biological samples

- **Lower Multiplexing Capacity:** Limited to a maximum of 8-plex
- **Sensitivity Issues:** Can be less sensitive than TMT, **Chemical Noise**
- **Ratio Compression:** Like TMT, iTRAQ also suffers from ratio compression

Summary

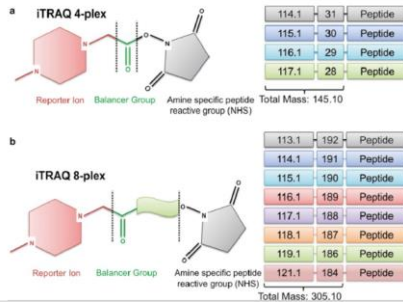
What is Quantitative Proteomics?



We use Quantitative Proteomics to help use identify protein expression levels in samples

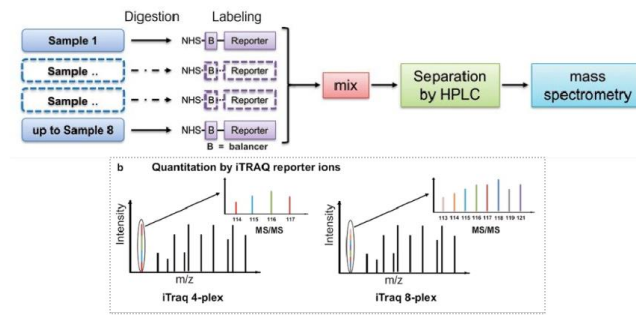
What are isobaric tags?

- Each isobaric tag consists of a reporter group, a balance group, and a peptide-reactive group.
- The total mass of each tag is identical.
- Fragmentation during MS/MS releases reporter ions of different masses.




Isobaric tags are molecules that are the same mass that separate into reporter ions of different masses

How does an iTRAQ assay work?



TMT and iTRAQ are methods that use isobaric tags to quantify protein levels

Omic approach to reveal the effects of obesity on the protein profiles of the exosomes derived from different adipose depots

Minting Chen¹ · Fan Zhang² · Baisen Chen¹ · Condon Lau³ · Keyang Xu¹ · Tiejun Tong⁴ · Chuying Huo¹ ·
Quanbin Han¹ · Tao Su² · Hiu Yee Kwan^{1,5} 

About the Author

- **Hiu Yee KWAN**
- B.Sc. (Hons.) degree in Biology, Hong Kong University.
- MA degree in Language Studies, The City University of Hong Kong.
- PhD degree in Physiology, Faculty of Medicine, The Chinese University of Hong Kong.
- Postdoctoral training, Department of Nutritional Sciences & Toxicology, University of California, Berkeley, USA.



Research Question: What are the cargo contents of exosomes from different adipose tissues under obesity conditions?



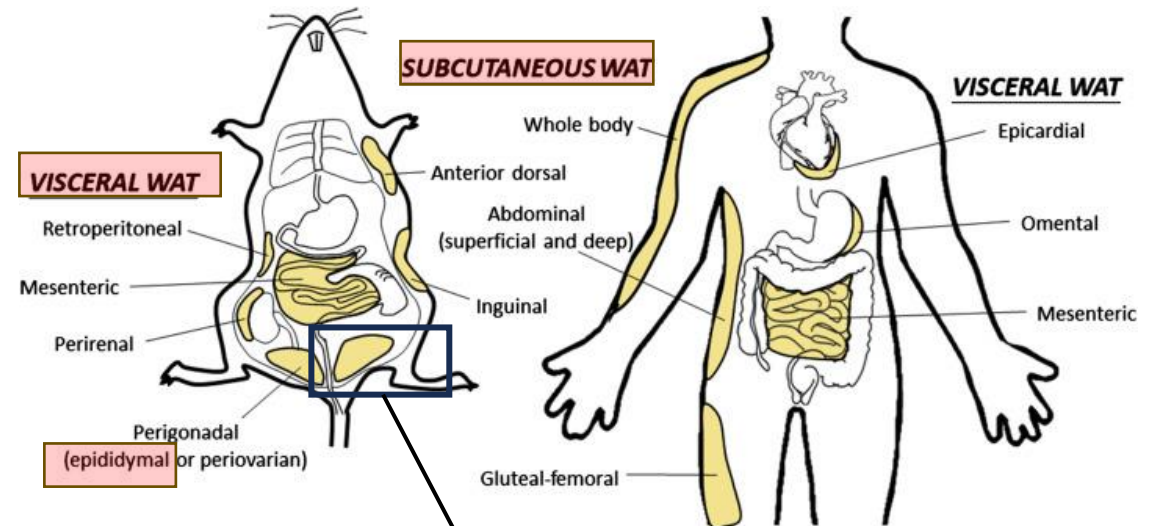
What are exosomes? And what are SAT-exos, VAT-exos, EAT-exos?



Exosomes

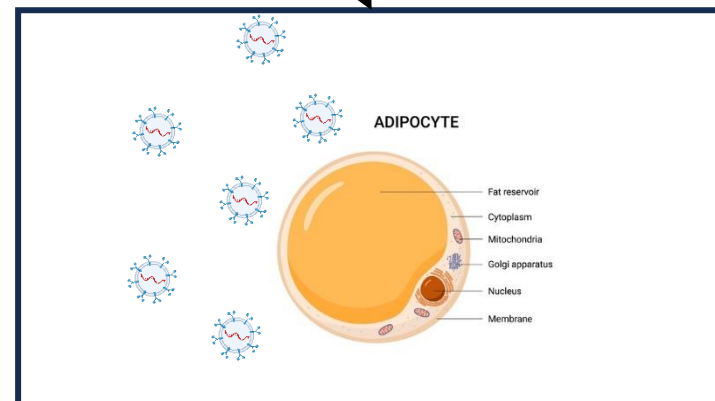
- Nanometer sized micro vesicles
- Secreted by cells
- Used for cell to cell signaling
- Packed with proteins

What are exosomes? And what are SAT-exos, VAT-exos, EAT-exos?



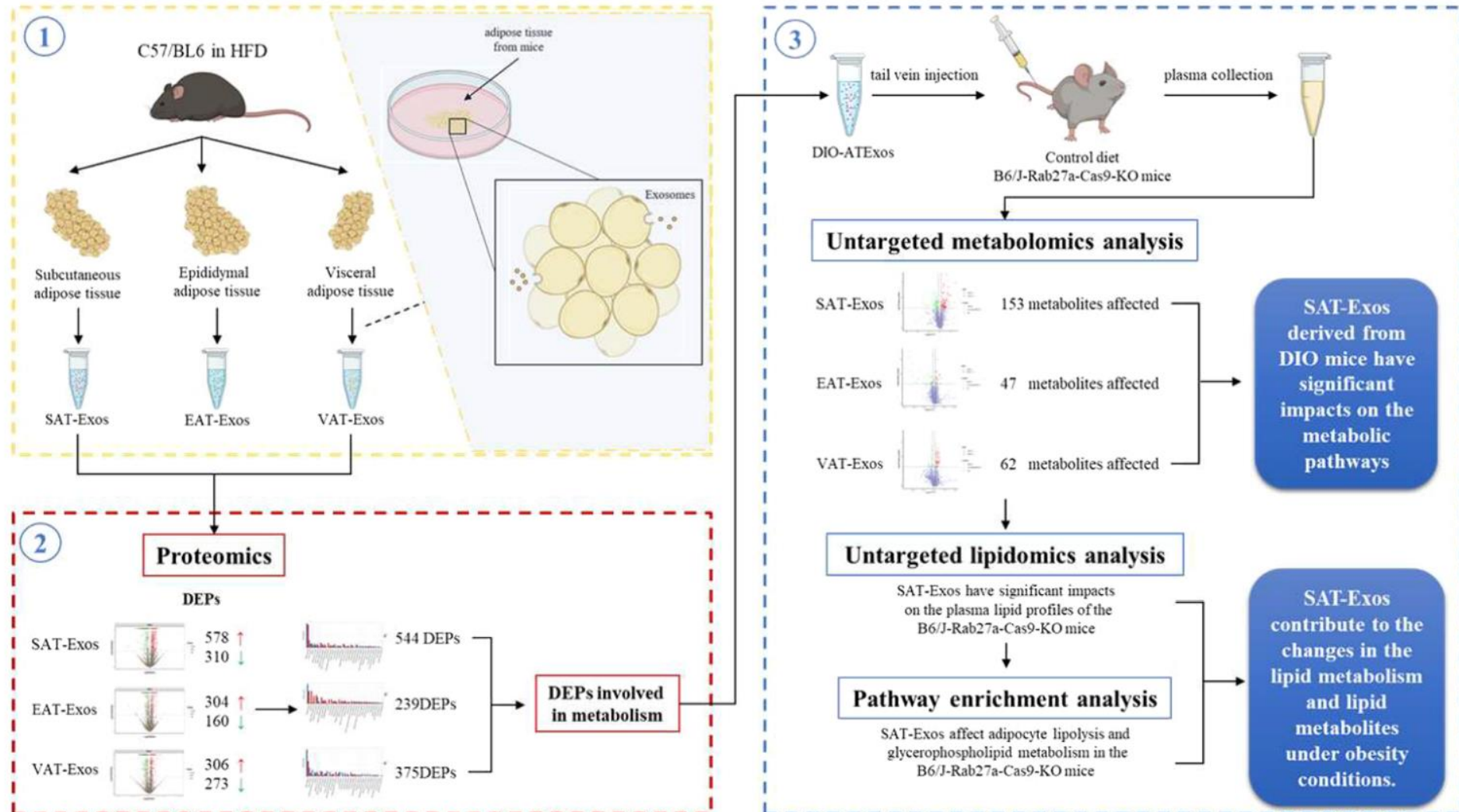
Exosomes

- Nanometer sized micro vesicles
- Secreted by cells (in this case adipocytes)
- Used for cell to cell signaling
- **Packed with proteins**



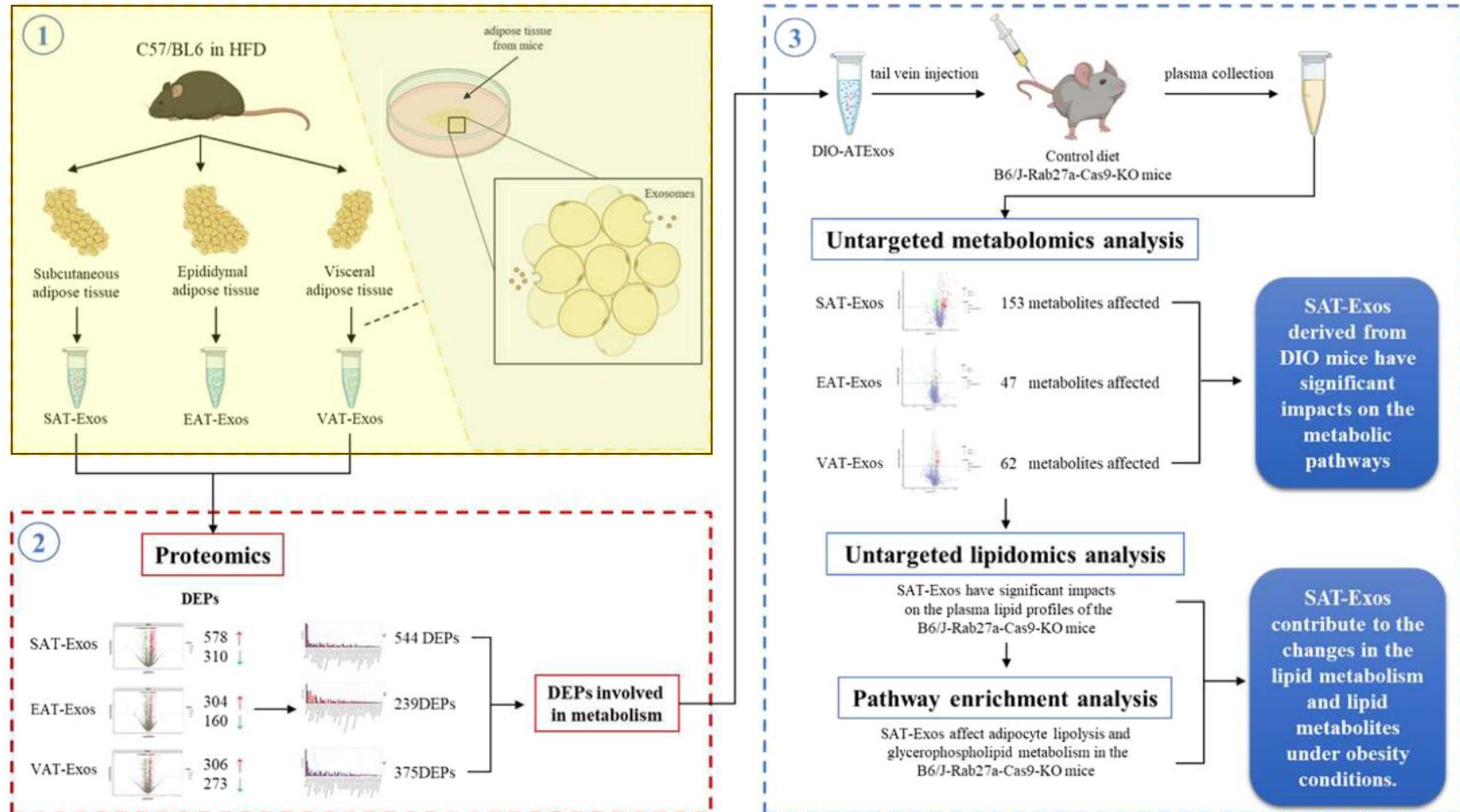
How did the researchers set up their research strategy?

c



How did the researchers set up their research strategy?

c



Model Organism

Male C57BL/6 mice (4–5 weeks old)

Two Groups:

- Control diet D12450J Research Diet
- High fat diet D12762 Research Diet

Overfed for 8 weeks to induce obesity

Were a source of exosomes under different obesity conditions



Model Organism

Male C57BL/6 mice (4–5 weeks old)

Two Groups:

- Control diet D12450J Research Diet
- High fat diet D12762 Research Diet

Overfed for 8 weeks to induce obesity

Were a source of exosomes under different obesity conditions

Were basically little exosome factories



Exosome Purification

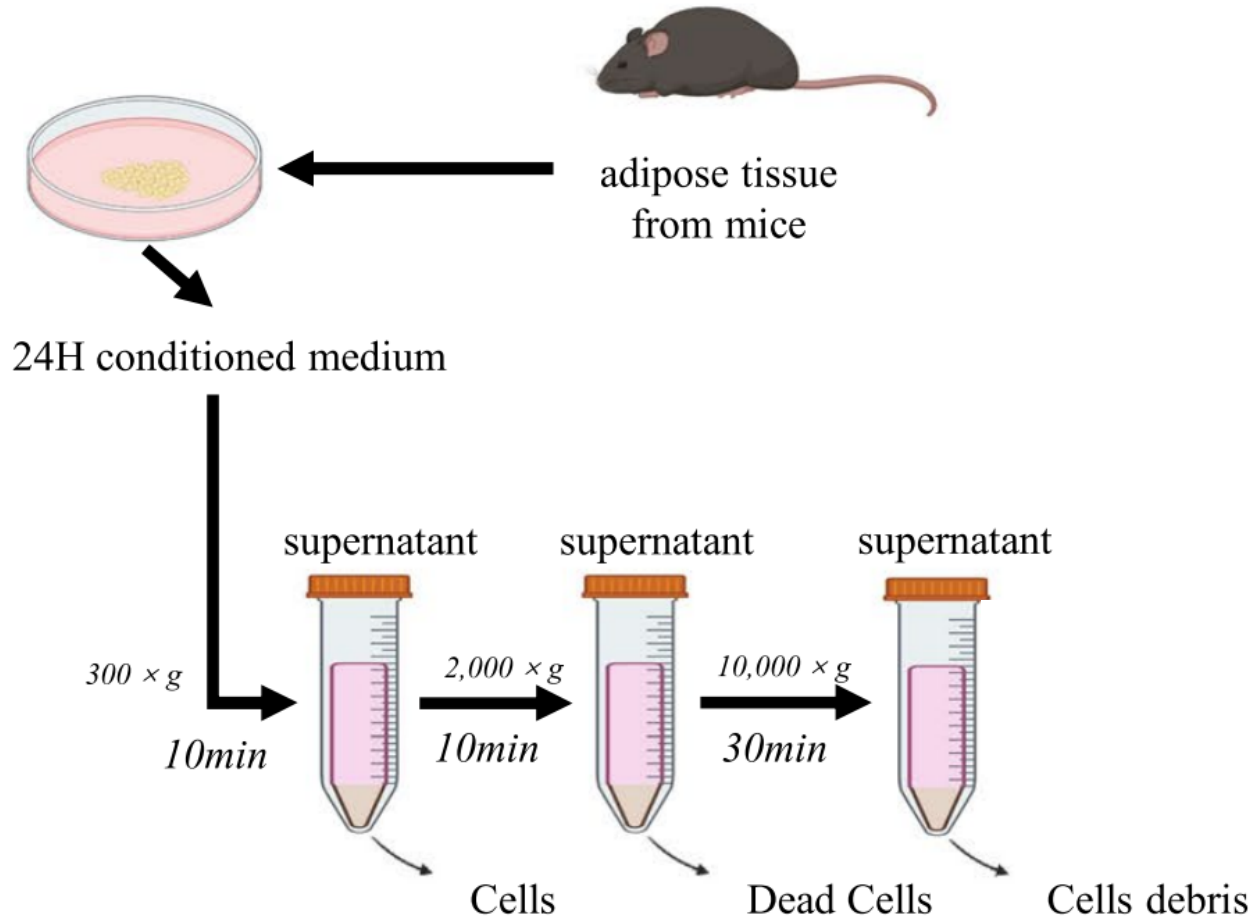


adipose tissue
from mice

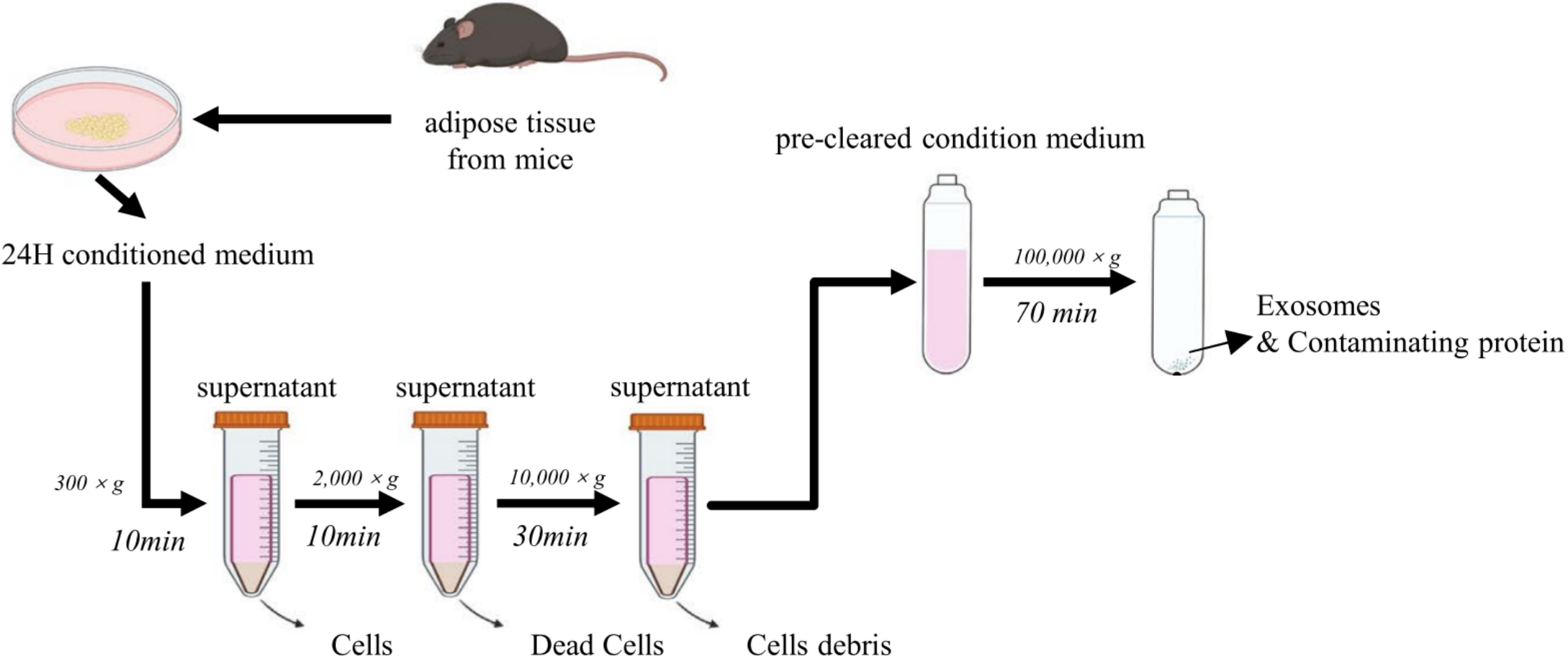
Exosome Purification



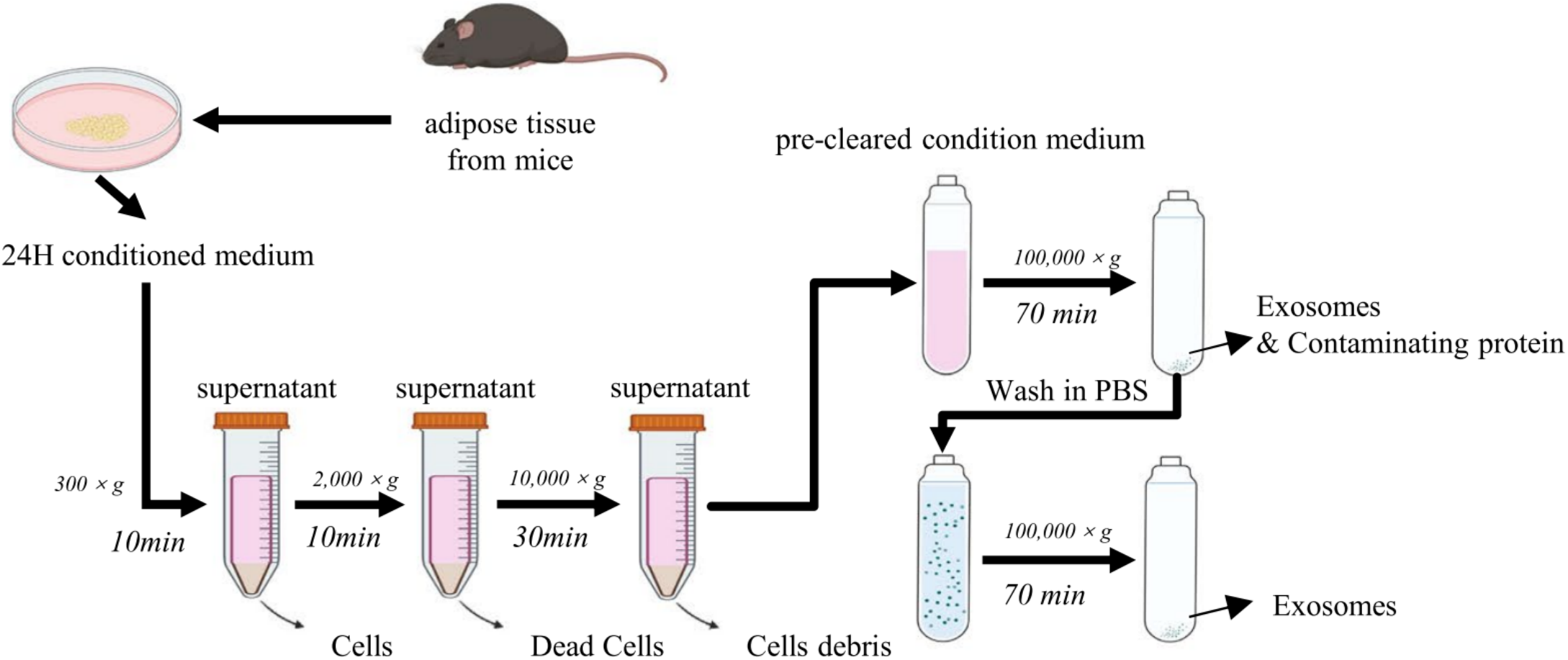
Exosome Purification



Exosome Purification

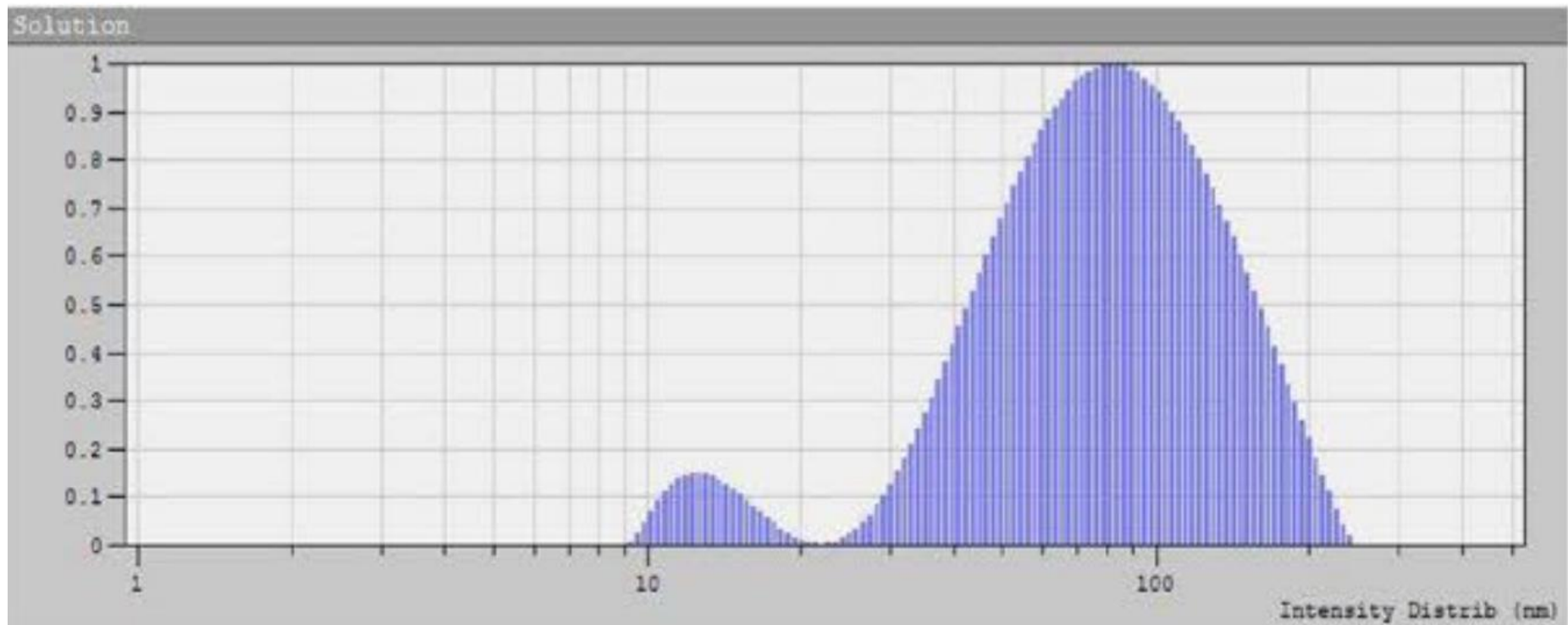


Exosome Purification



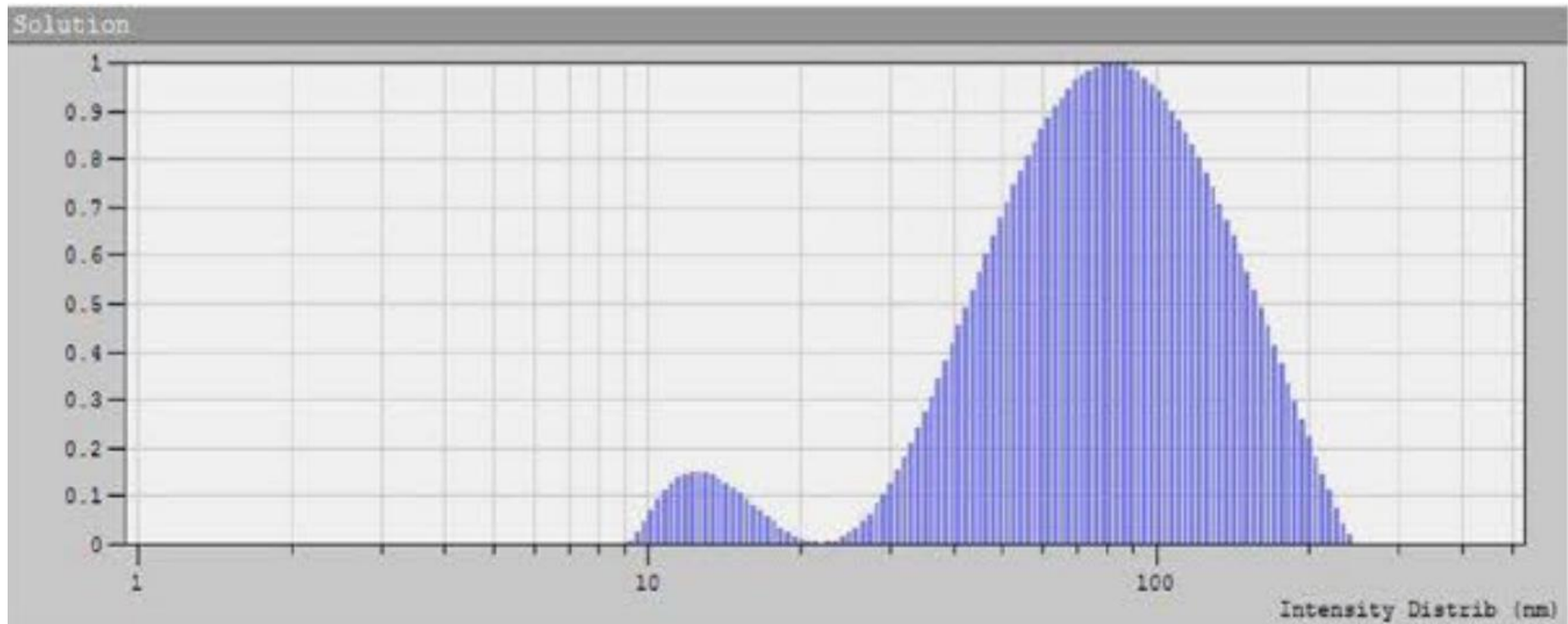
Exosome Isolation Confirmation

Dynamic Light Scattering Analysis (DLS)



Exosome Isolation Confirmation

Dynamic Light Scattering Analysis (DLS)



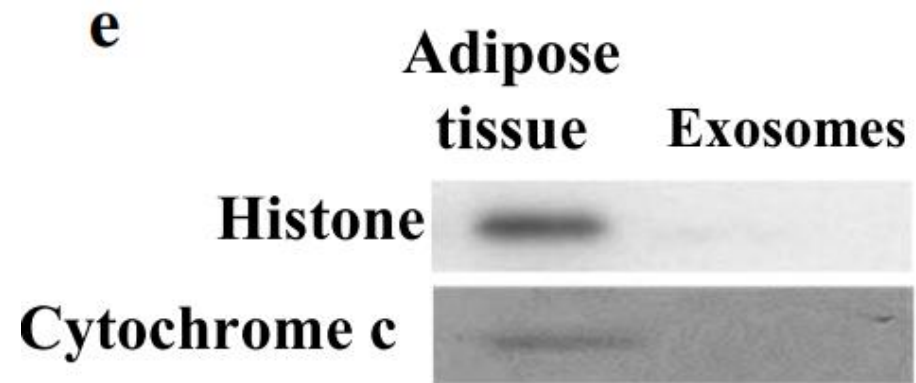
Smaller
Exosomes



Larger
Exosomes

Exosome Isolation Confirmation

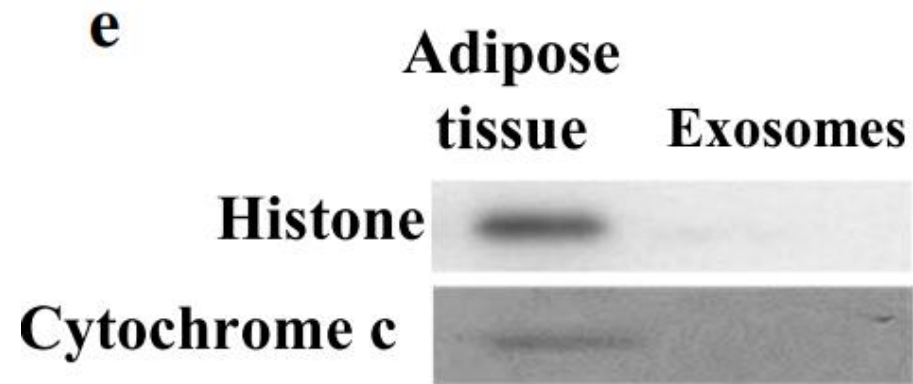
Western Blots



Confirms the Exosomes were separated from adipocytes

Exosome Isolation Confirmation

Western Blots

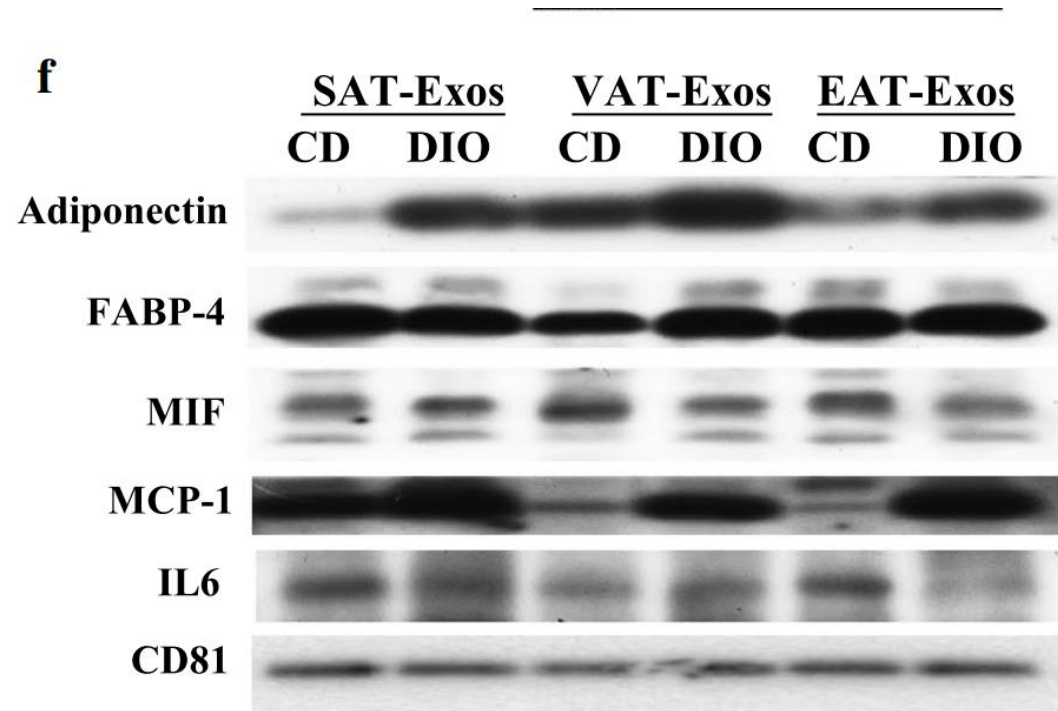


Confirms the Exosomes were separated from adipocytes



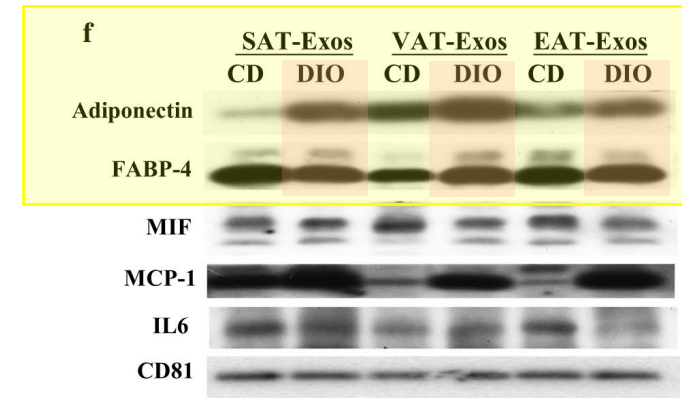
Confirms the culture media was exosome free

Inflammatory Adipokine Markers



CD = Control Diet
DIO = Diet Induced Obesity

Circulation Analysis



Confirms that Adipocyte – Derived Exosomes circulate in blood

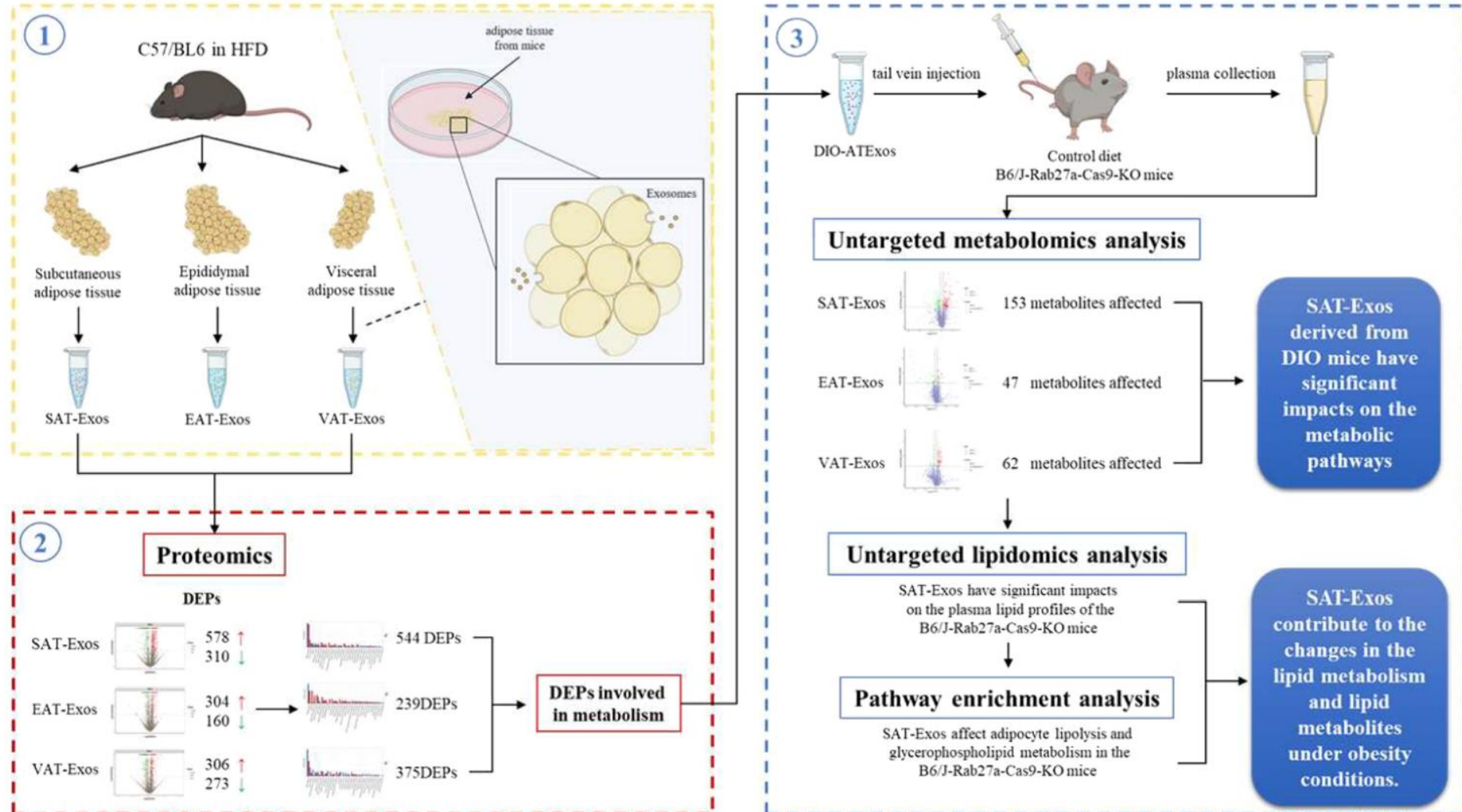


Western Blot Detection

- + Adiponectin
- + FABP-4
- MIF
- MCP-1
- IL6
- CD81

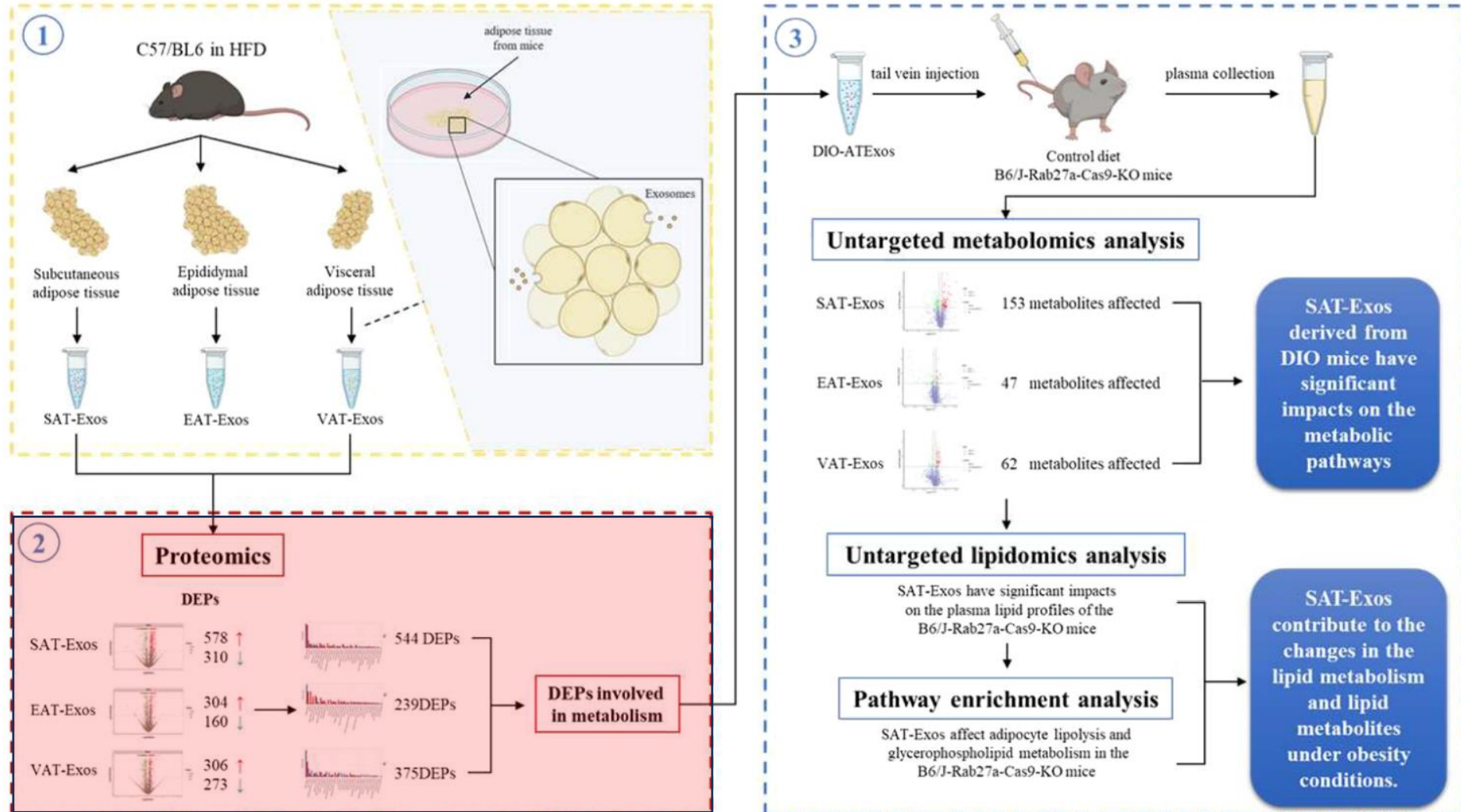
Workflow

c



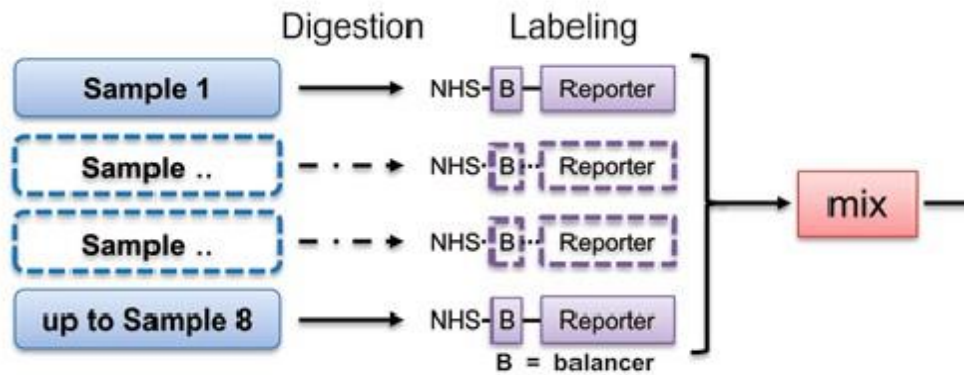
Workflow

c



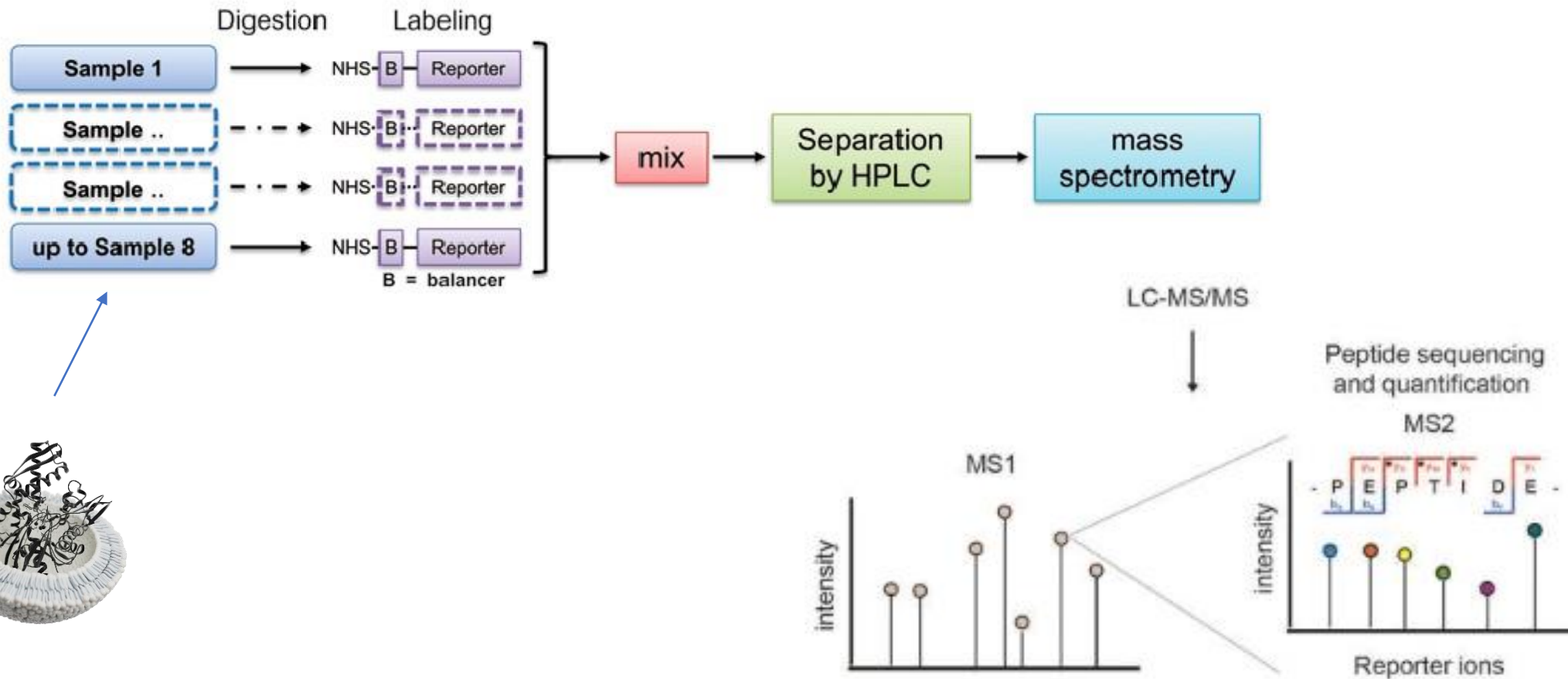
iTRAQ-based quantitative proteomic methods

a iTRAQ Workflow



iTRAQ-based quantitative proteomic methods

a iTRAQ Workflow



What is the pathway analysis of the detected exosomal proteins?

Differentially expressed proteins under control vs obese conditions

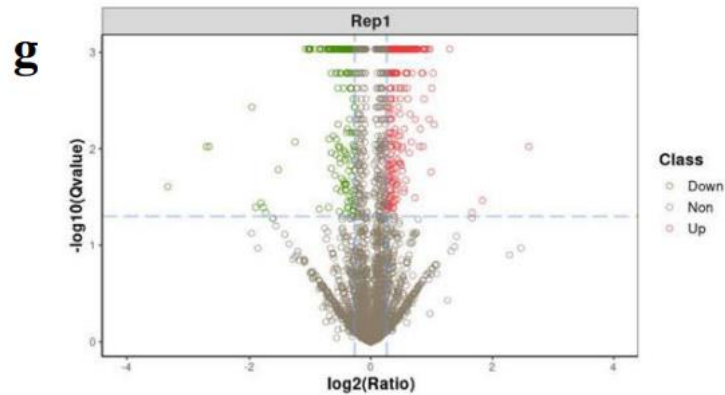


Index	Pathway	Proteins with pathway annotation (4049)	Pathway ID
1	Metabolic pathways	545 (13.46%)	ko01100
2	Pathways in cancer	187 (4.62%)	ko05200
3	Endocytosis	170 (4.2%)	ko04144
4	PI3K-Akt signaling pathway	165 (4.08%)	ko04151
5	Human papillomavirus infection	155 (3.83%)	ko05165
6	Focal adhesion	124 (3.06%)	ko04510
7	Human immunodeficiency virus 1 infection	121 (2.299%)	ko05170
8	Epstein–Bar virus infection	120 (2.96%)	ko05169

What proteins are enriched in exosomes from obese mice?

Differentially expressed proteins under control vs obese conditions

EAT-Exos

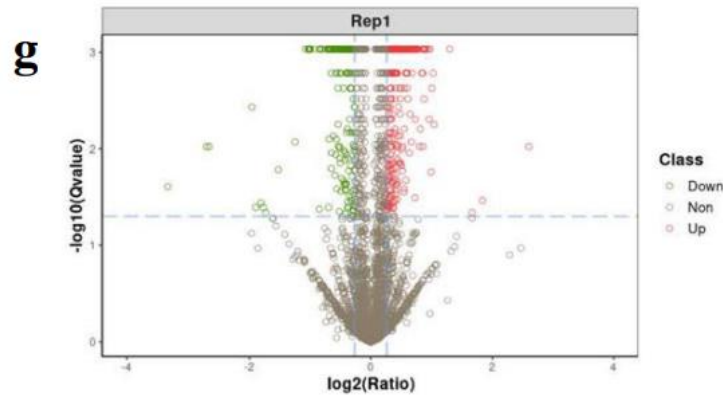


304 proteins were upregulated
186 were downregulated

What proteins are enriched in exosomes from obese mice?

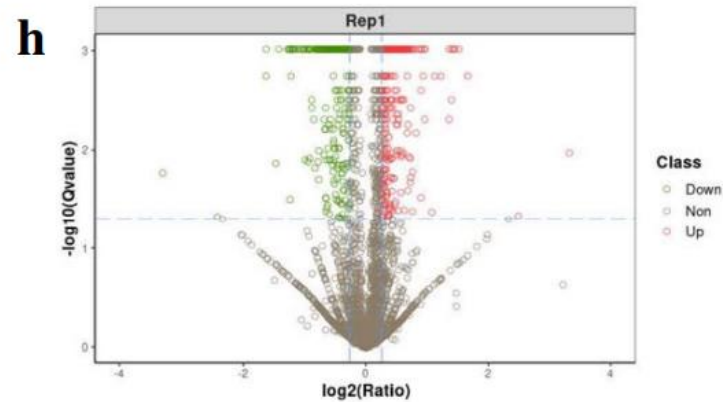
Differentially expressed proteins under control vs obese conditions

EAT-Exos



304 proteins were upregulated
186 were downregulated

VAT-Exos

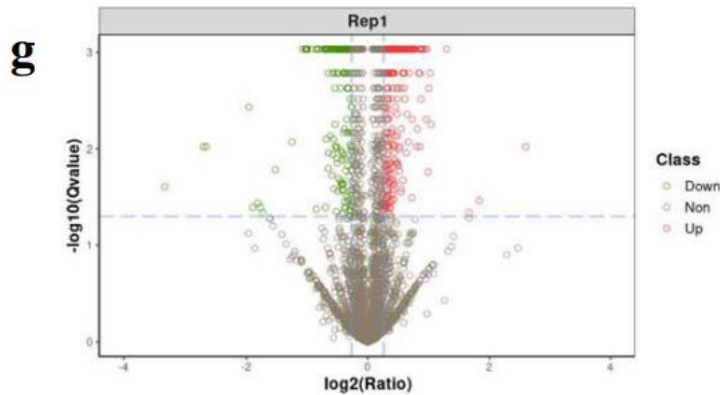


306 proteins were upregulated
273 were downregulated

What proteins are enriched in exosomes from obese mice?

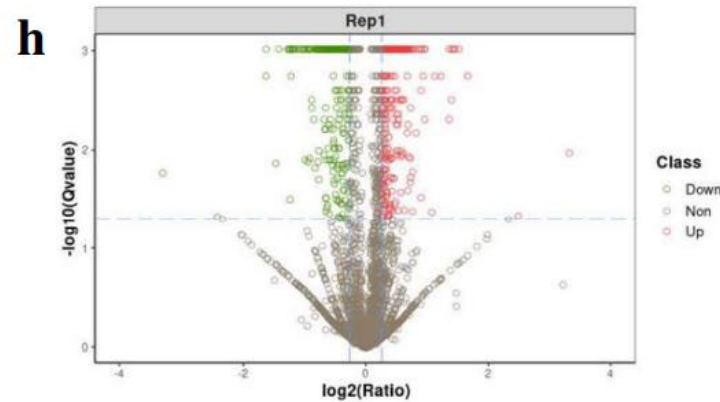
Differentially expressed proteins under control vs obese conditions

EAT-Exos



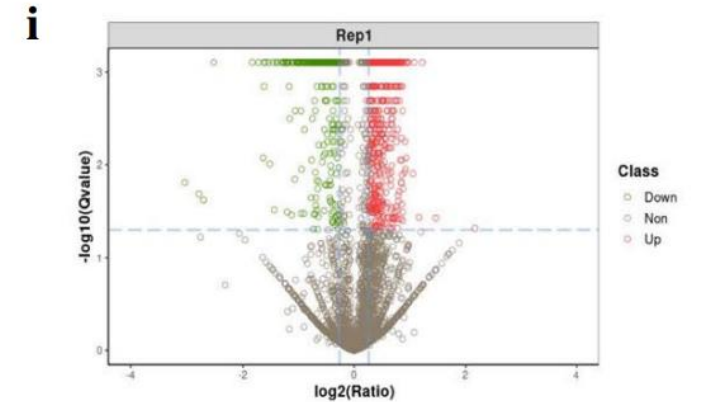
304 proteins were upregulated
186 were downregulated

VAT-Exos



306 proteins were upregulated
273 were downregulated

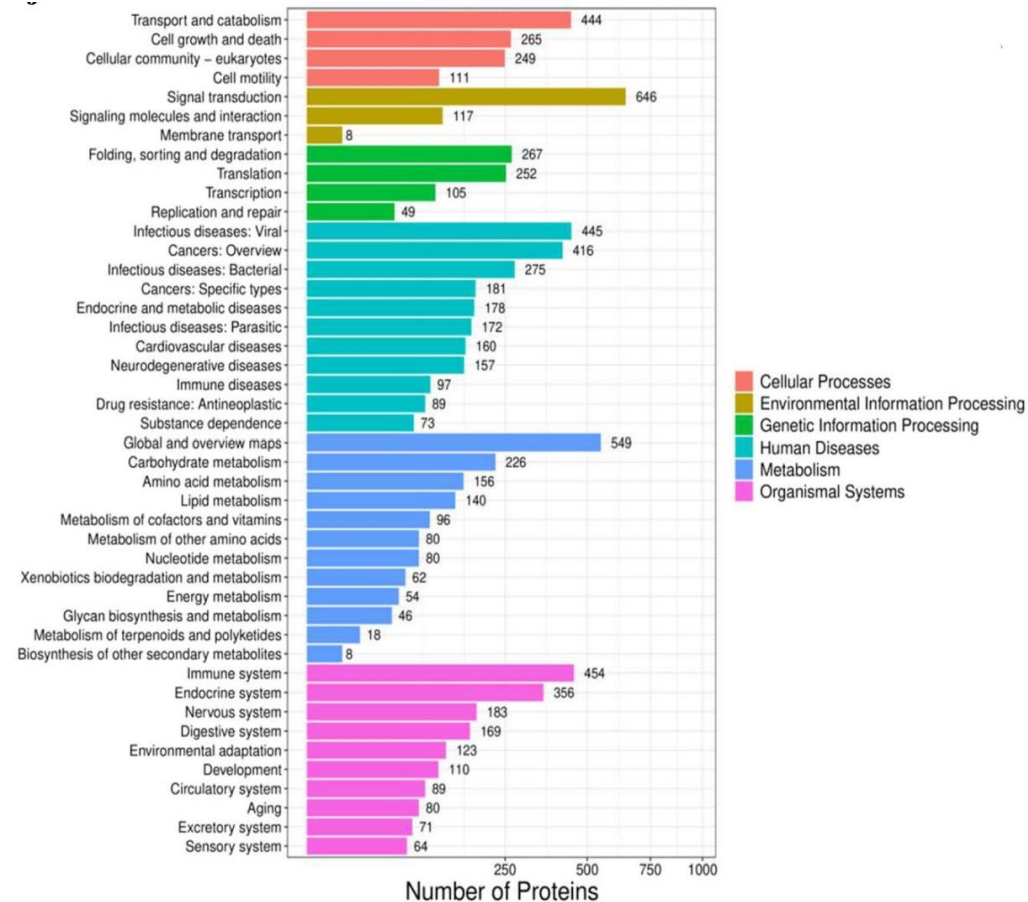
SAT-Exos



578 proteins were upregulated
310 were downregulated

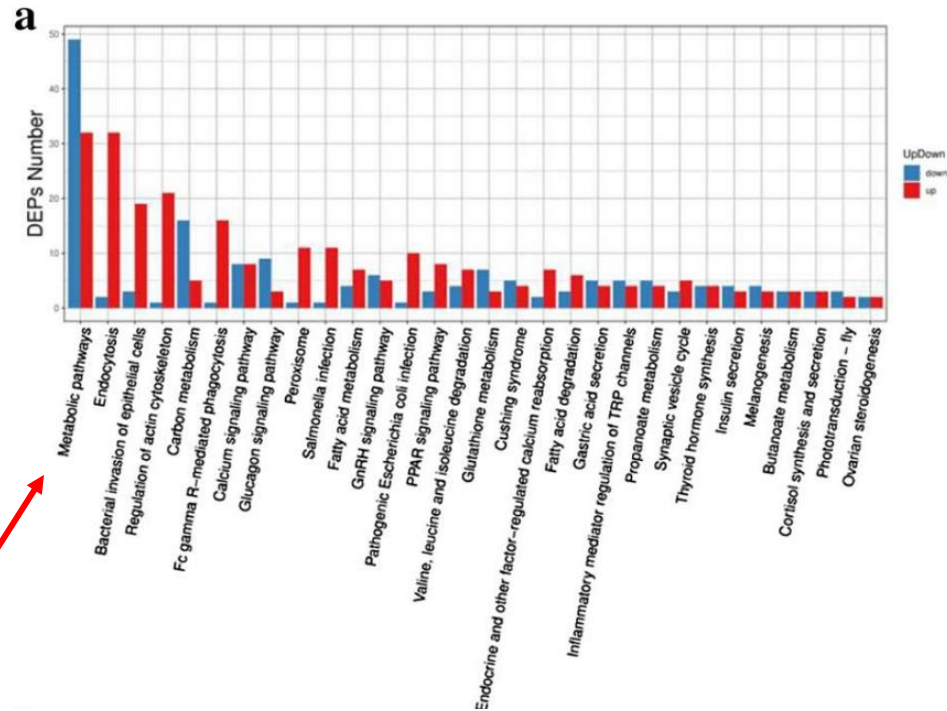


What are the GO terms associated with proteins enriched in exosomes from obese mice?

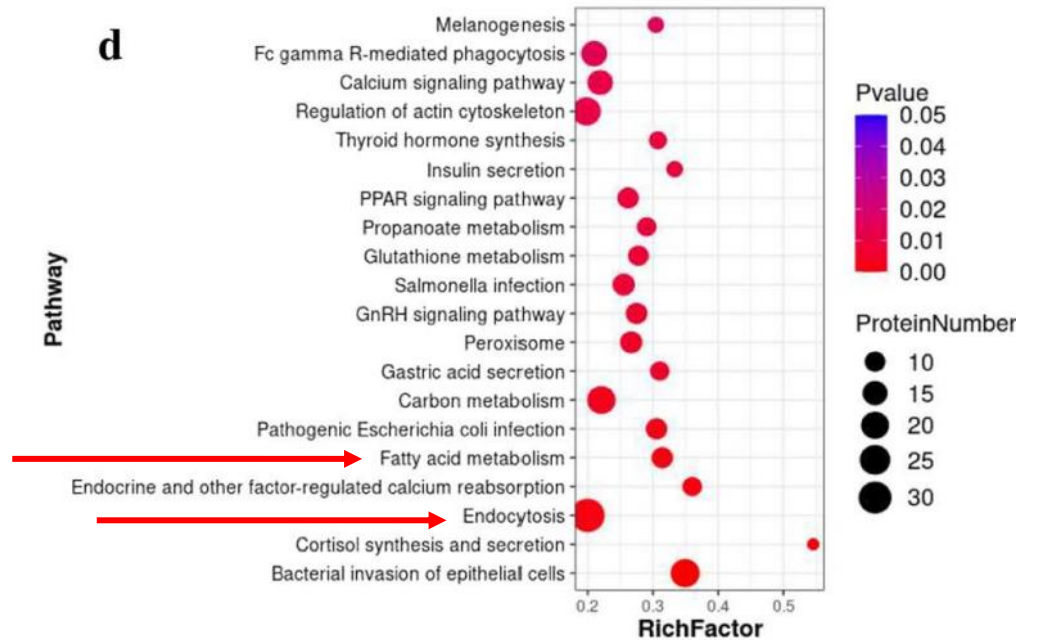


How does obesity affect metabolic proteins?

Protein Profiles in Control vs High Fat Diet - Epididymal Adipose Tissue Exosomes



Enriched GO Terms

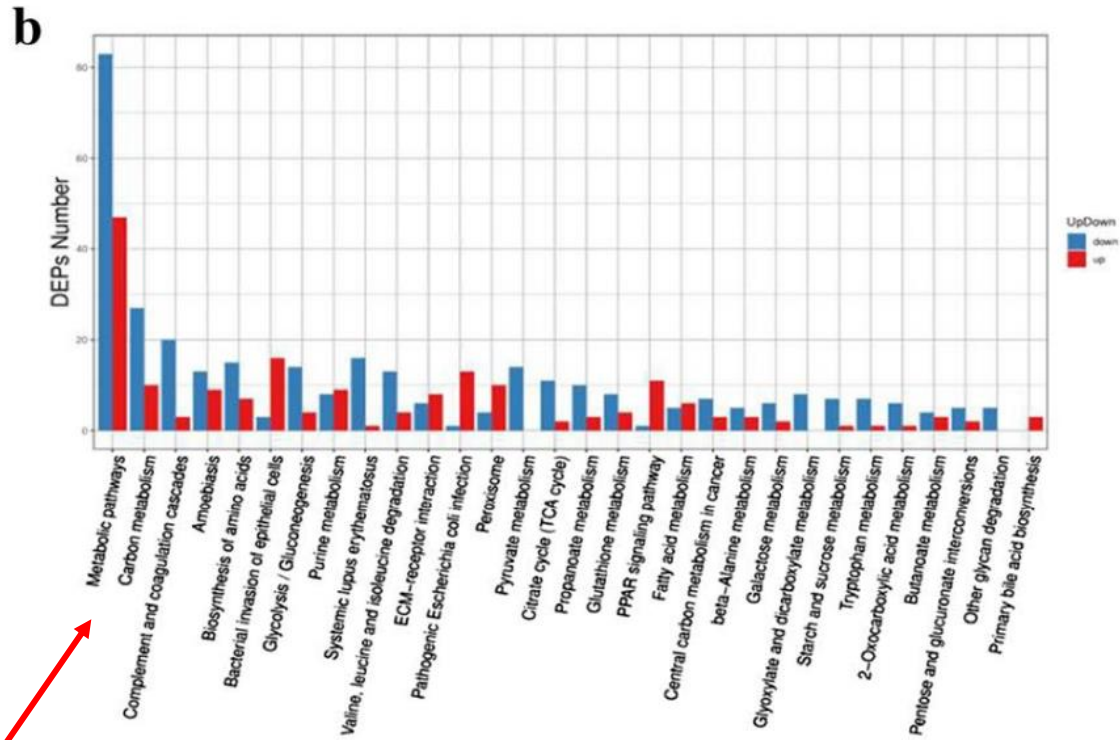


Rich Factor =

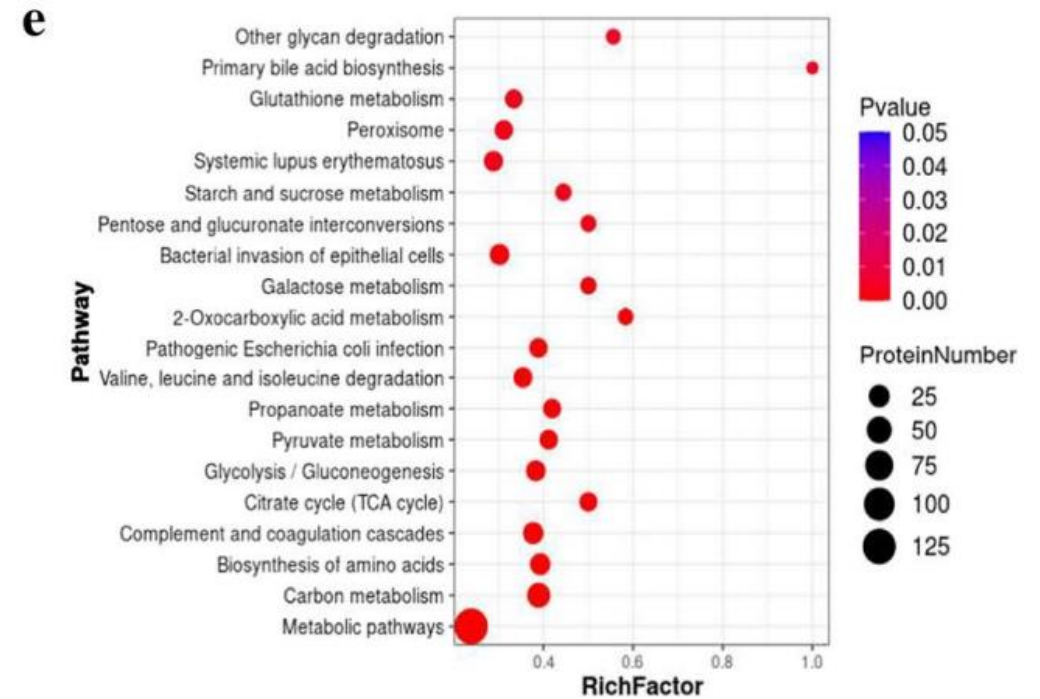
$$\frac{\text{Number of DEPs in pathway}}{\text{Total number of DEPs in dataset} \times \left(\frac{\text{Number of proteins in pathway}}{\text{Total number of proteins in dataset}} \right)}$$

How does obesity affect metabolic proteins?

Protein Profiles in Control vs High Fat Diet - Visceral Adipose Tissue Exosomes



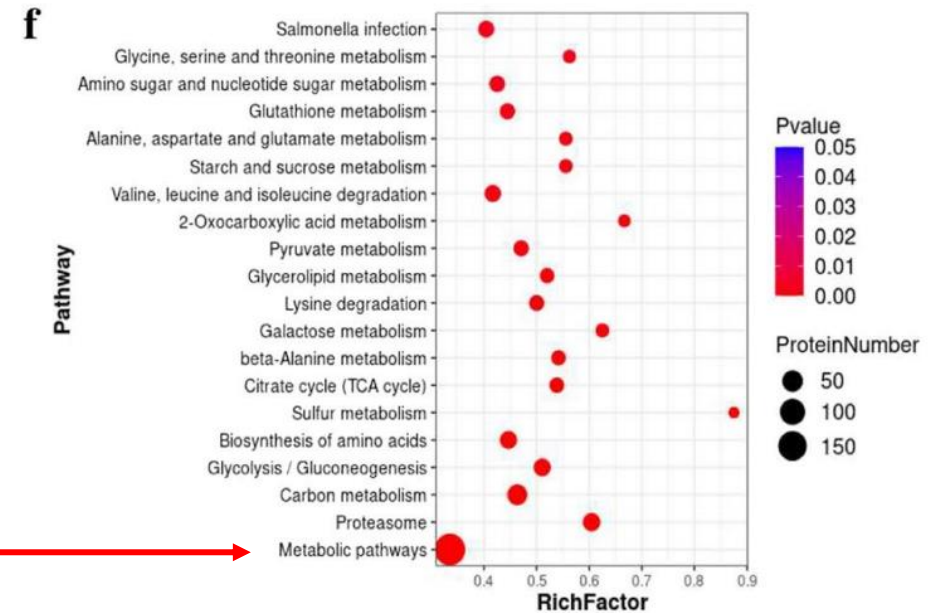
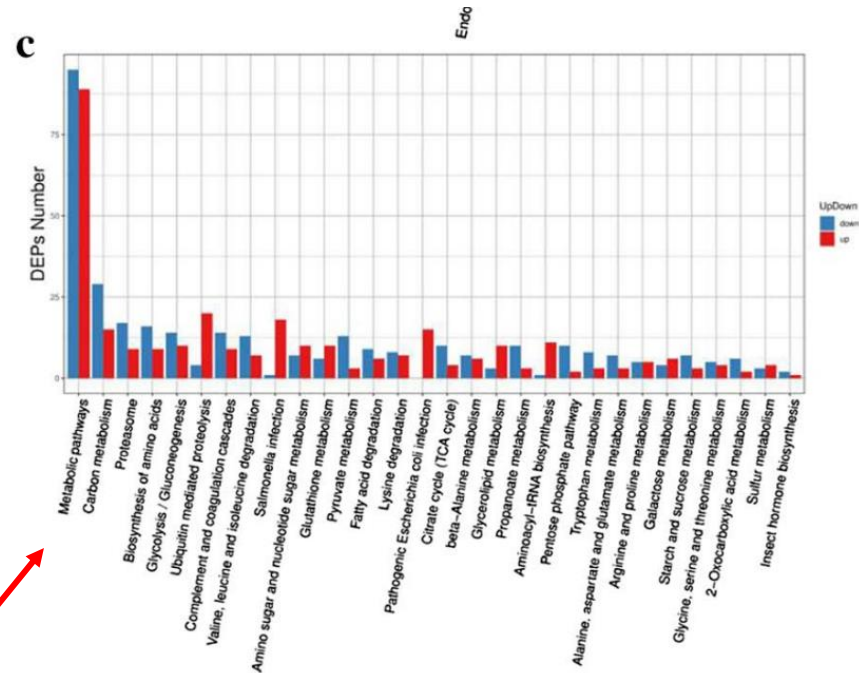
Enriched GO Terms



How does obesity affect metabolic proteins?

Protein Profiles in Control vs High Fat Diet - Subcutaneous Adipose Tissue Exosomes

Enriched GO Terms

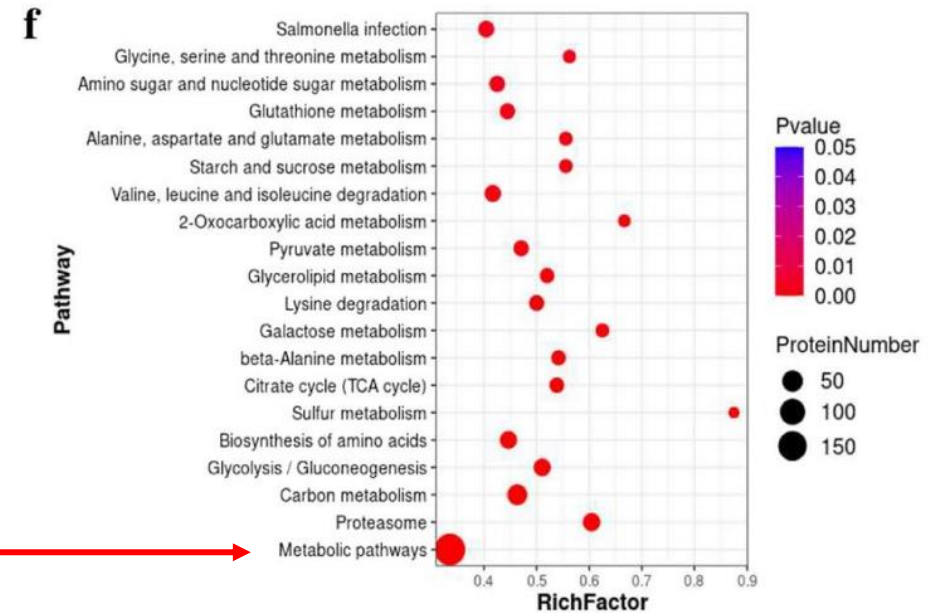
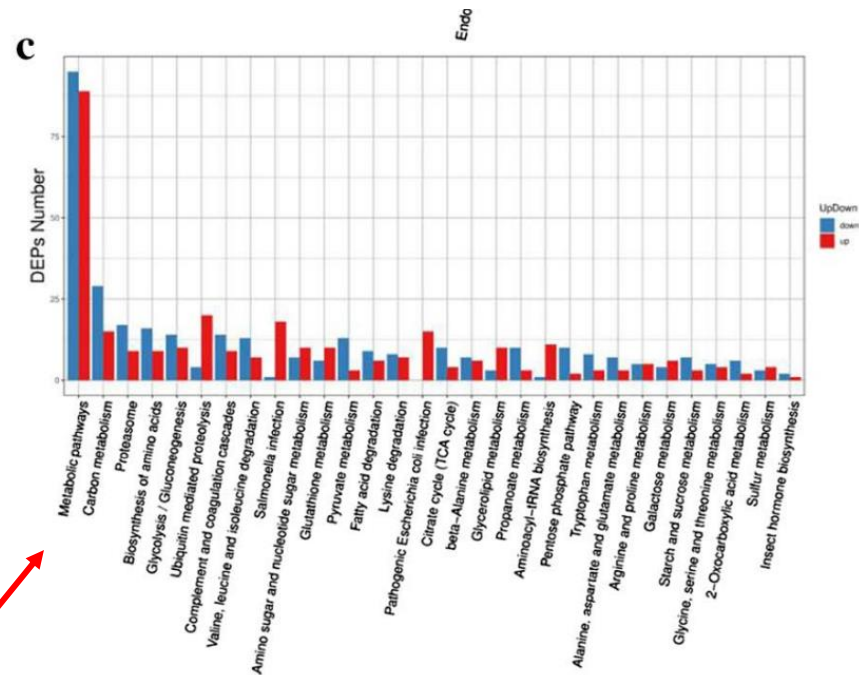


How does obesity affect metabolic proteins?

Protein Profiles in Control vs High Fat Diet - Subcutaneous Adipose Tissue Exosomes

DEPs were had a 2-fold increase – 0.3-fold reduction

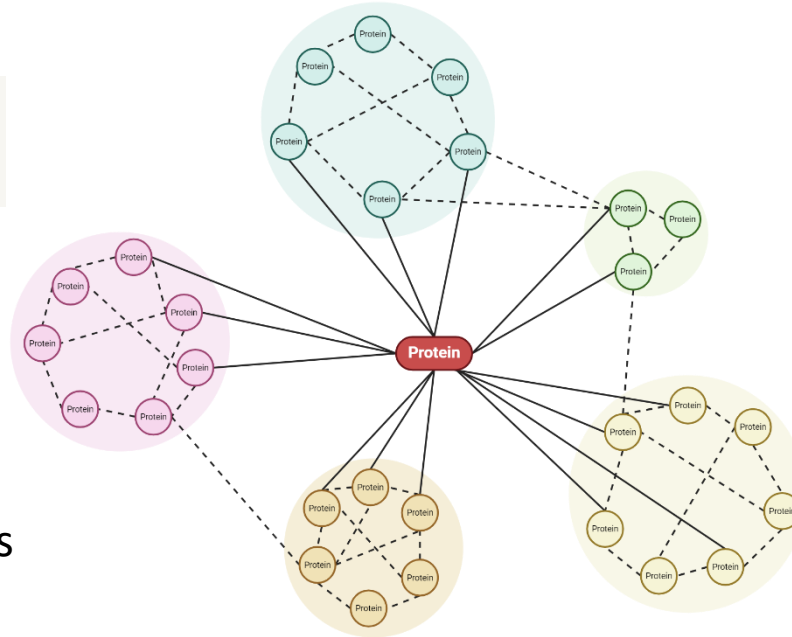
Enriched GO Terms



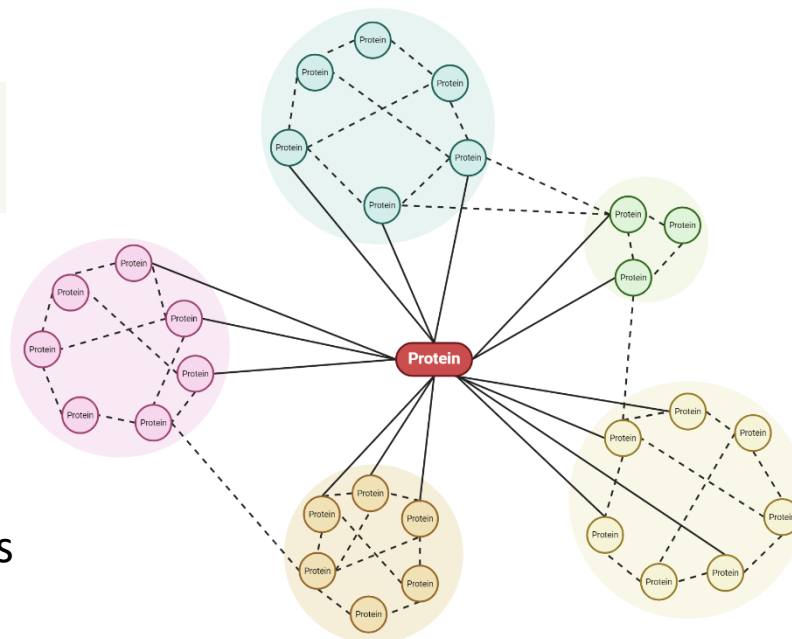
What is the expression of ARF1 and MAP3K in SAT and VAT-exos



Protein-Protein
Interaction Analysis



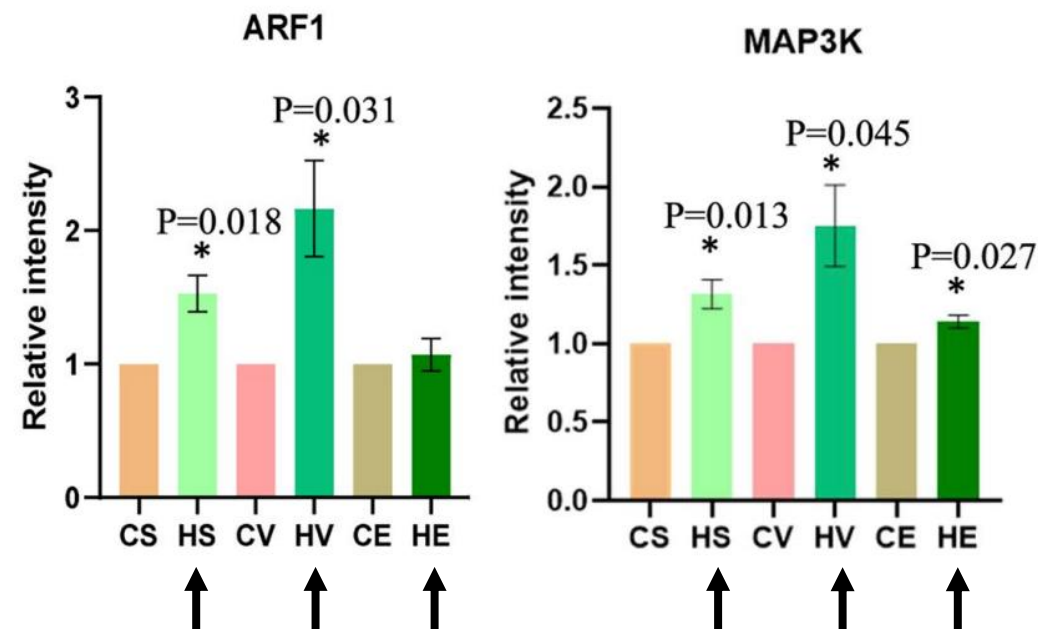
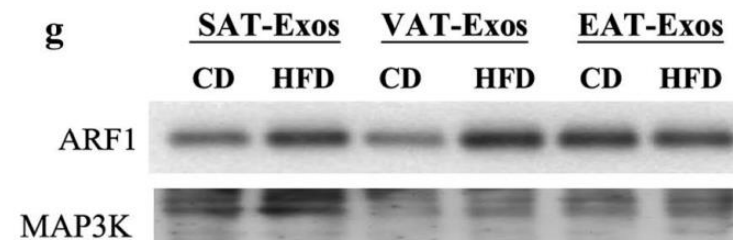
What is the expression of ARF1 and MAP3K under obese conditions?



Protein-Protein
Interaction Analysis

Highlighted Differentially Expressed Proteins in
SAT-Exos:

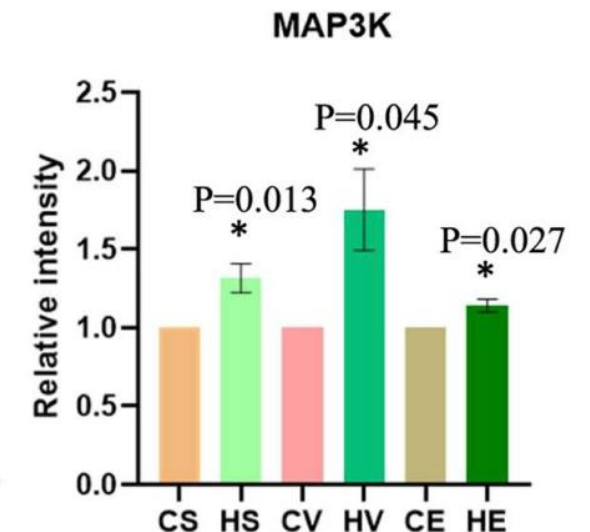
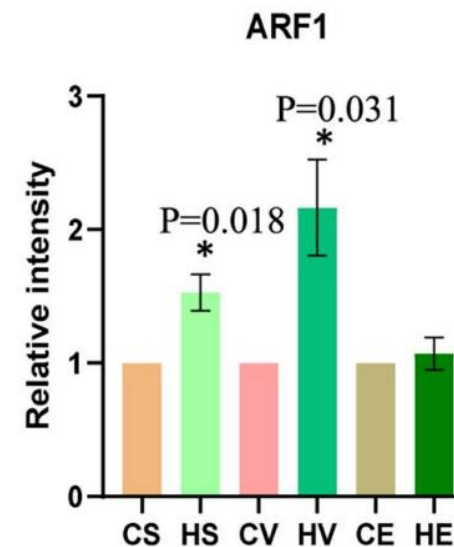
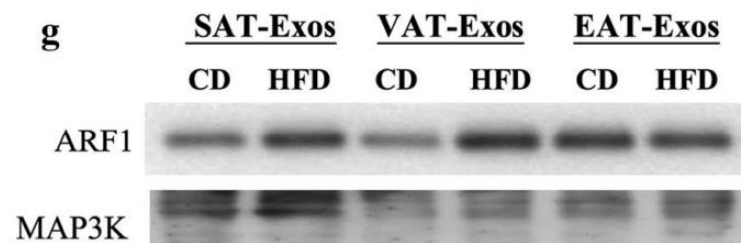
- ADP-ribosylation factor 1 (ARF1)
- mitogen-activated protein kinase kinase kinase 3 (MAP3K)



What is the expression of ARF1 and MAP3K in SAT and VAT-exos

Highlighted Differentially Expressed Proteins:

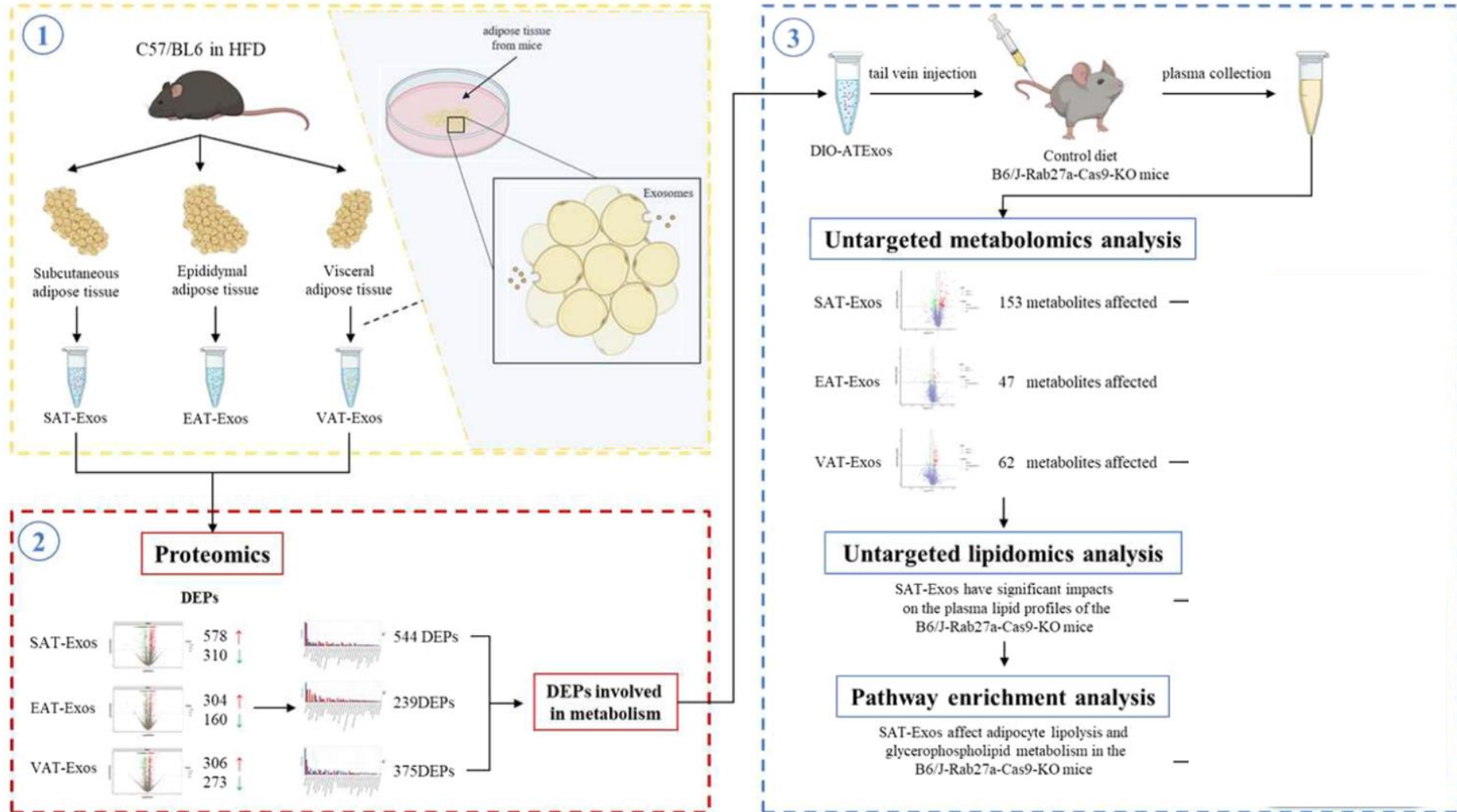
- ADP-ribosylation factor 1 (ARF1)
- mitogen-activated protein kinase kinase kinase 3 (MAP3K)



Main Takeaway: Obesity has a more prominent effect on the proteins in the SAT-Exos than those in EAT-Exos and VAT-Exos. The DEPs in the EAT-Exos are involved in different biological functions, while those in SAT-Exos and VAT-Exos are mainly involved in metabolism.

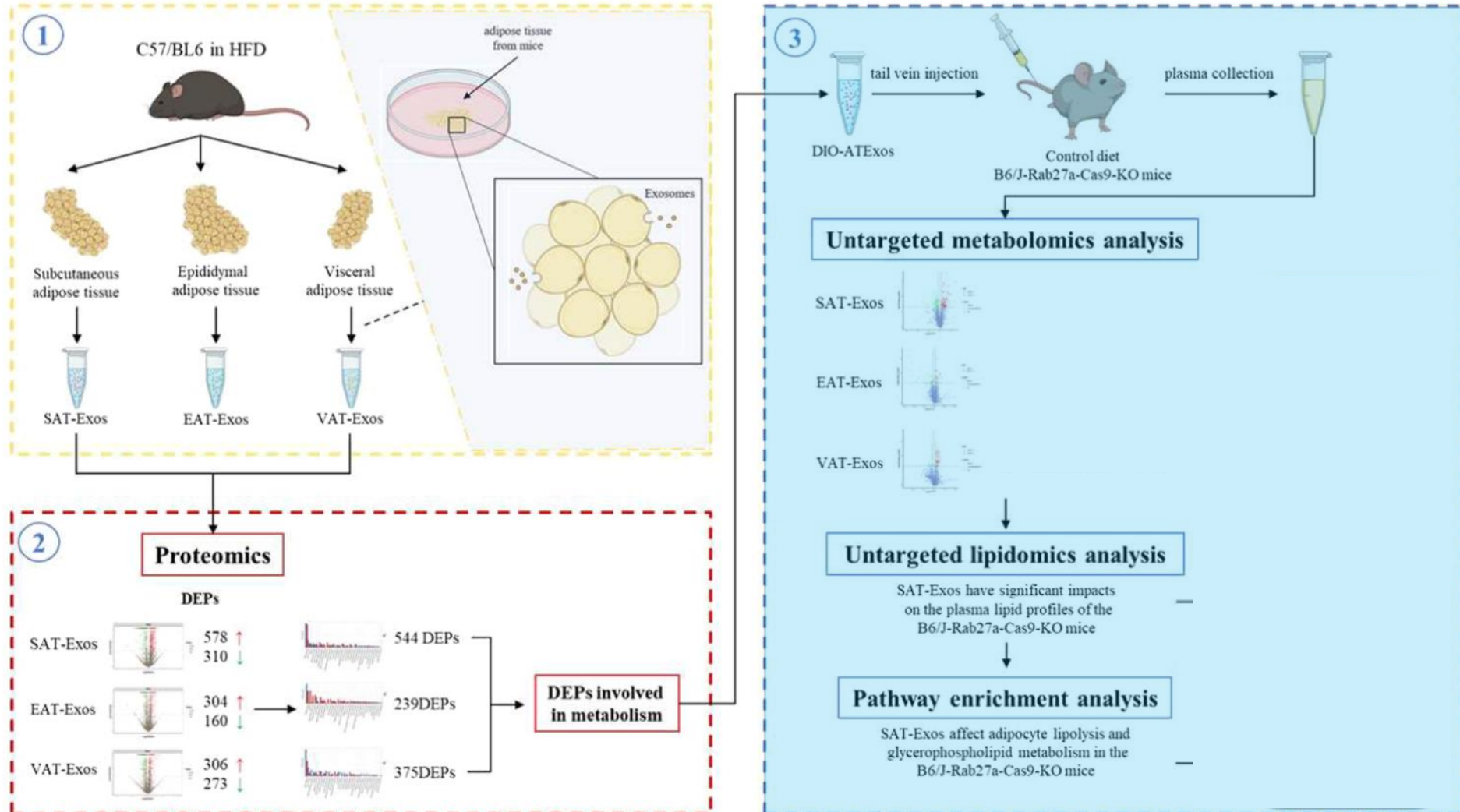
Workflow

c

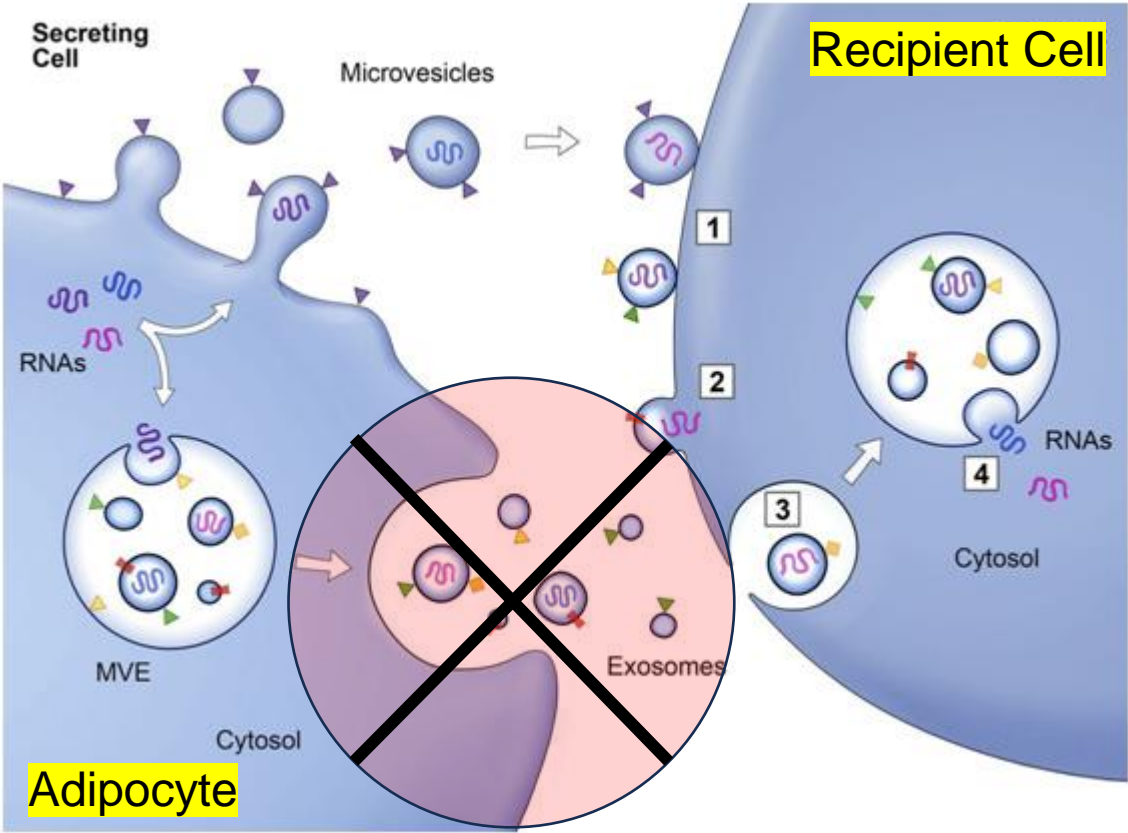


Workflow

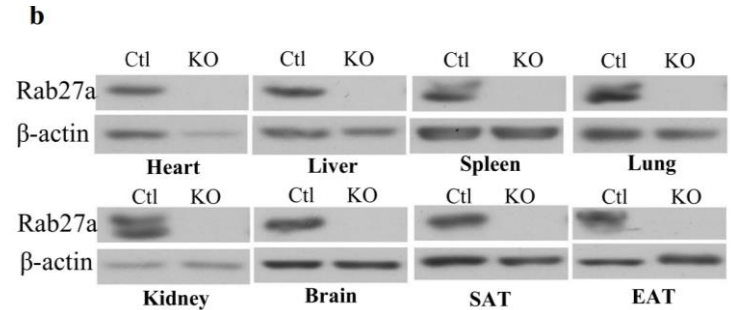
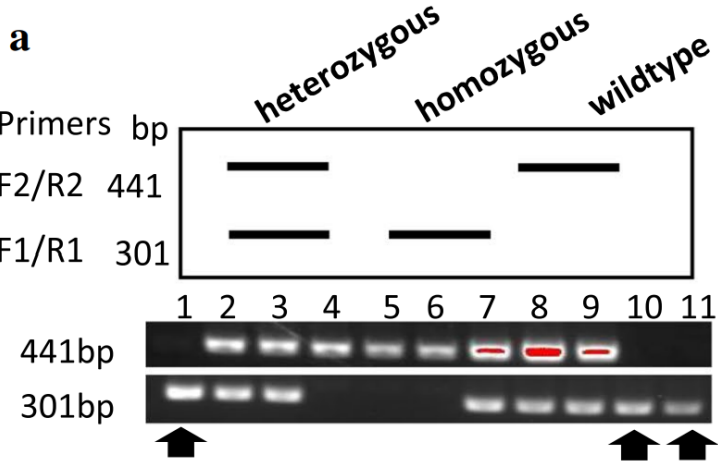
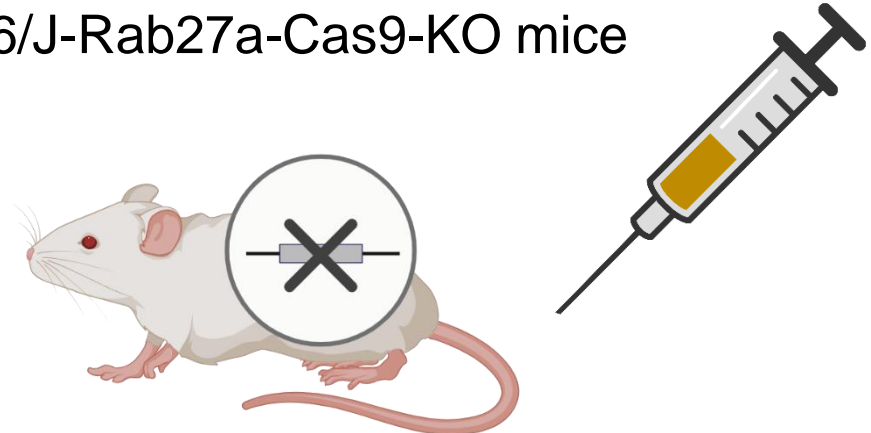
c



Model Organism



B6/J-Rab27a-Cas9-KO mice

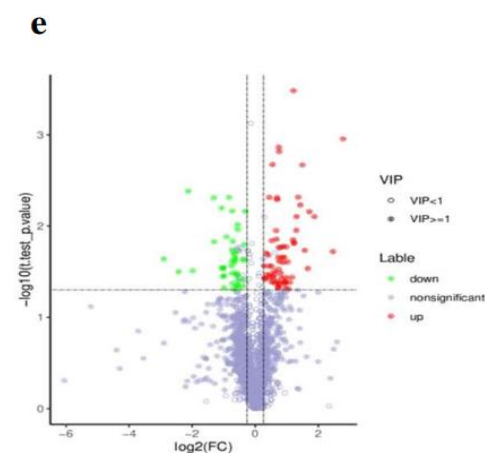
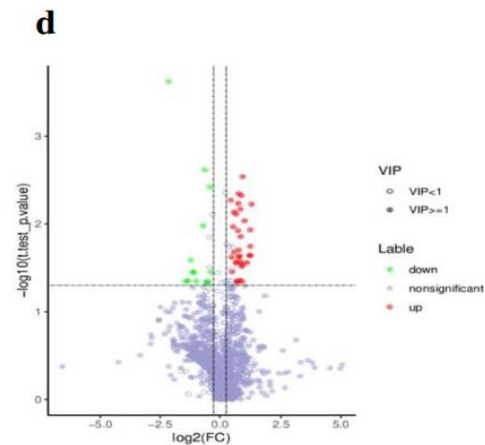
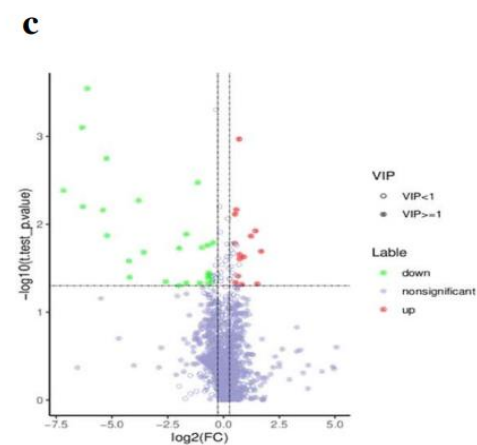


Untargeted Metabolic Analysis

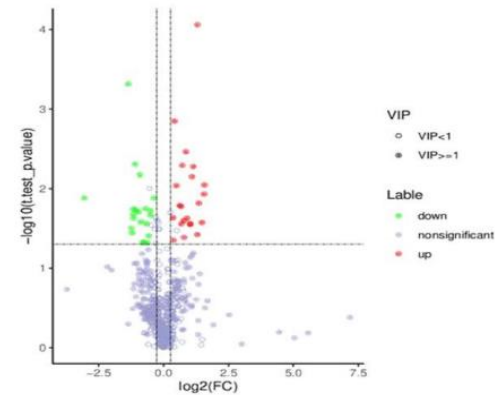
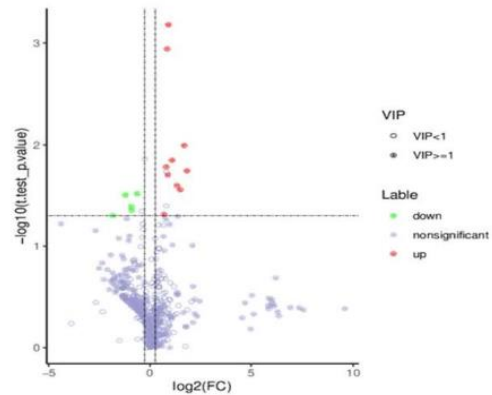
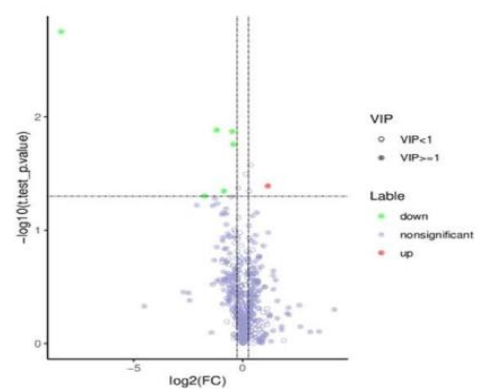
Differential Metabolite Analysis

HPLC + Mass Spectrometry

Positive Ion Mode



Negative Ion Mode



EAT-Exos

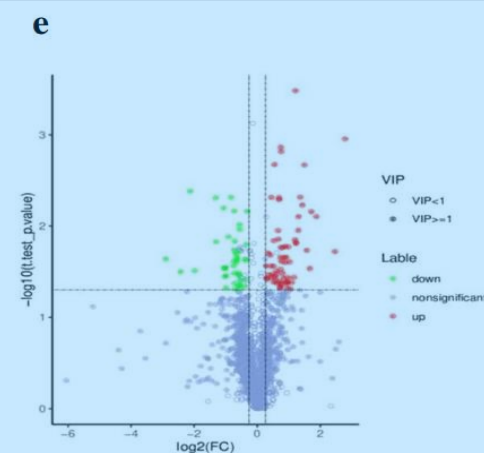
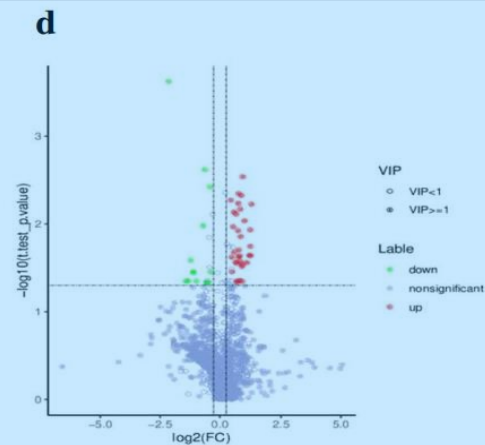
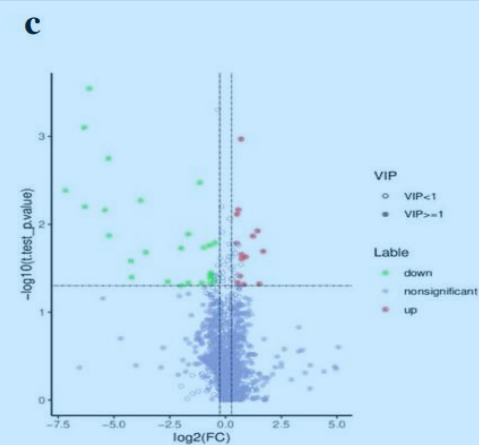
VAT-Exos

SAT-Exos

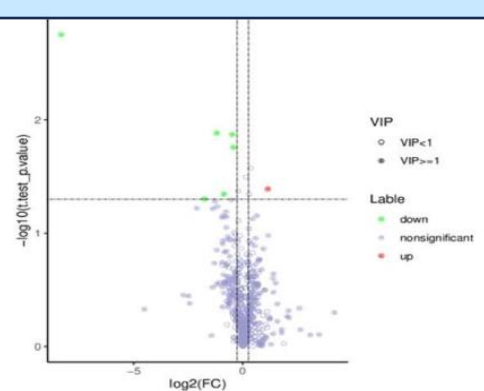
Untargeted Metabolic Analysis

Differential Metabolite Analysis HPLC + Mass Spectrometry

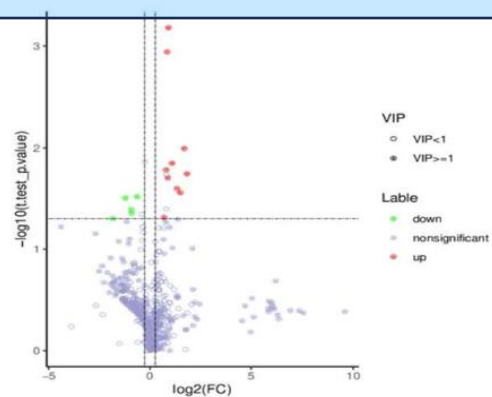
Positive Ion Mode



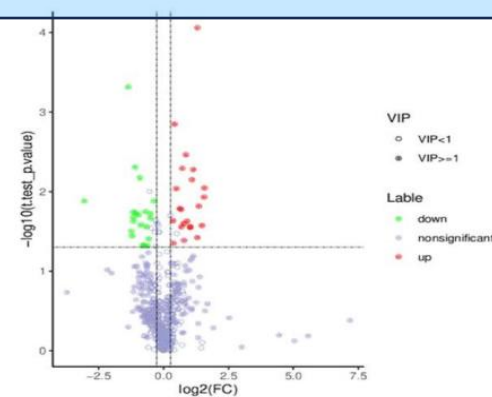
Negative Ion Mode



EAT-Exos



VAT-Exos

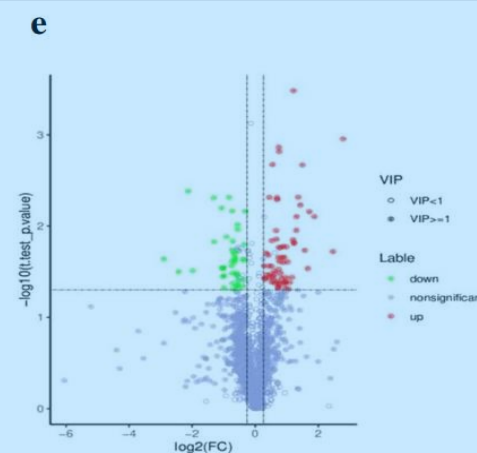
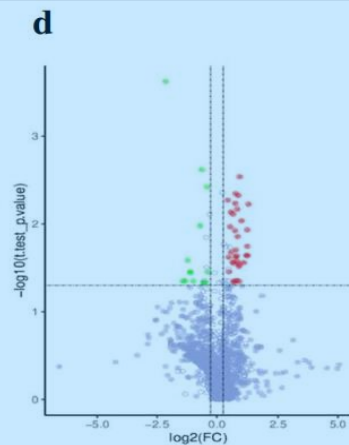
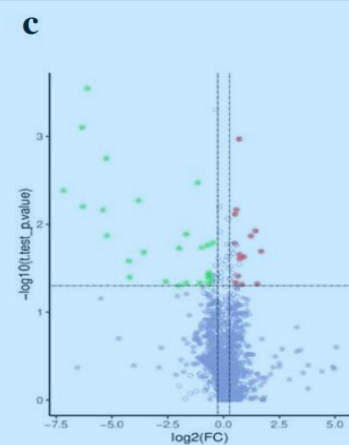


SAT-Exos

Untargeted Metabolic Analysis

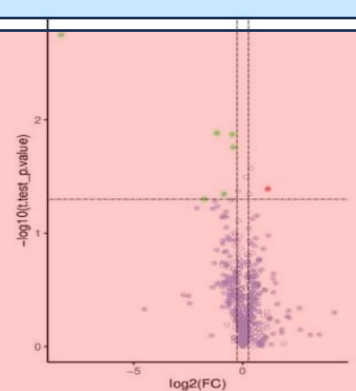
Differential Metabolite Analysis HPLC + Mass Spectrometry

Positive Ion Mode

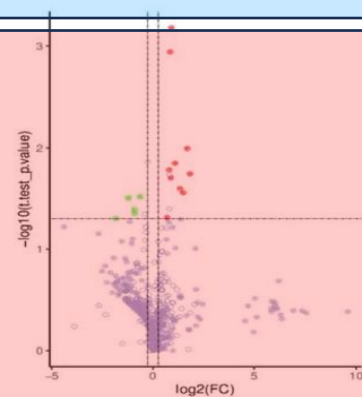


1858 detected compounds

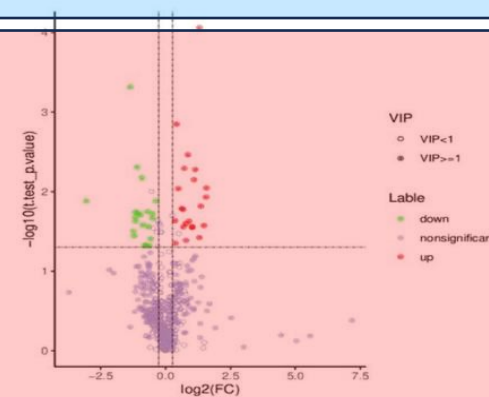
Negative Ion Mode



EAT-Exos



VAT-Exos

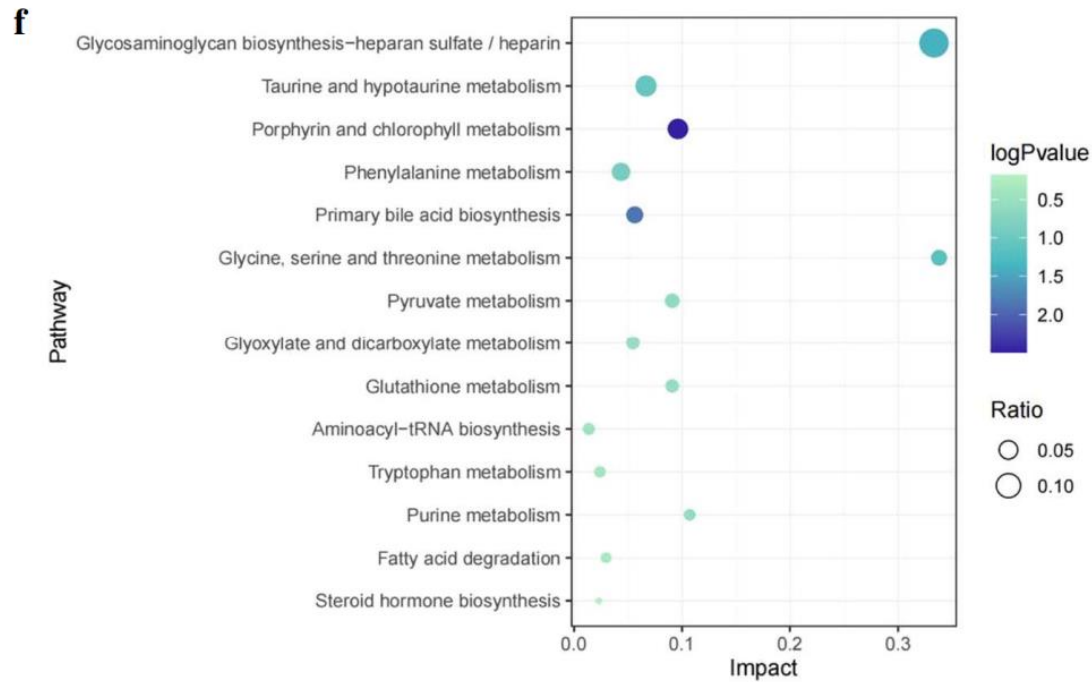


SAT-Exos

561 detected compounds

Untargeted Metabolic Analysis

Differential Metabolite Pathway Analysis



Untargeted Metabolomics:
screening all detectable **metabolites**
or lipids in your samples, measuring
the changes between two or more
different groups or under two or
more biological conditions

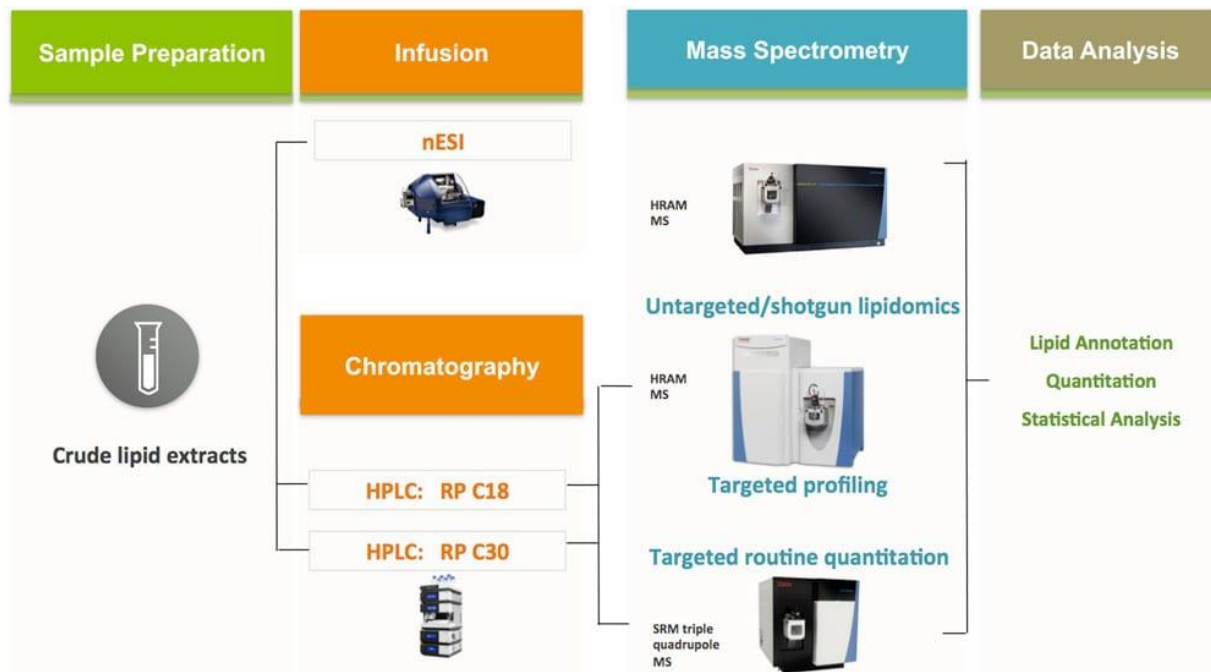
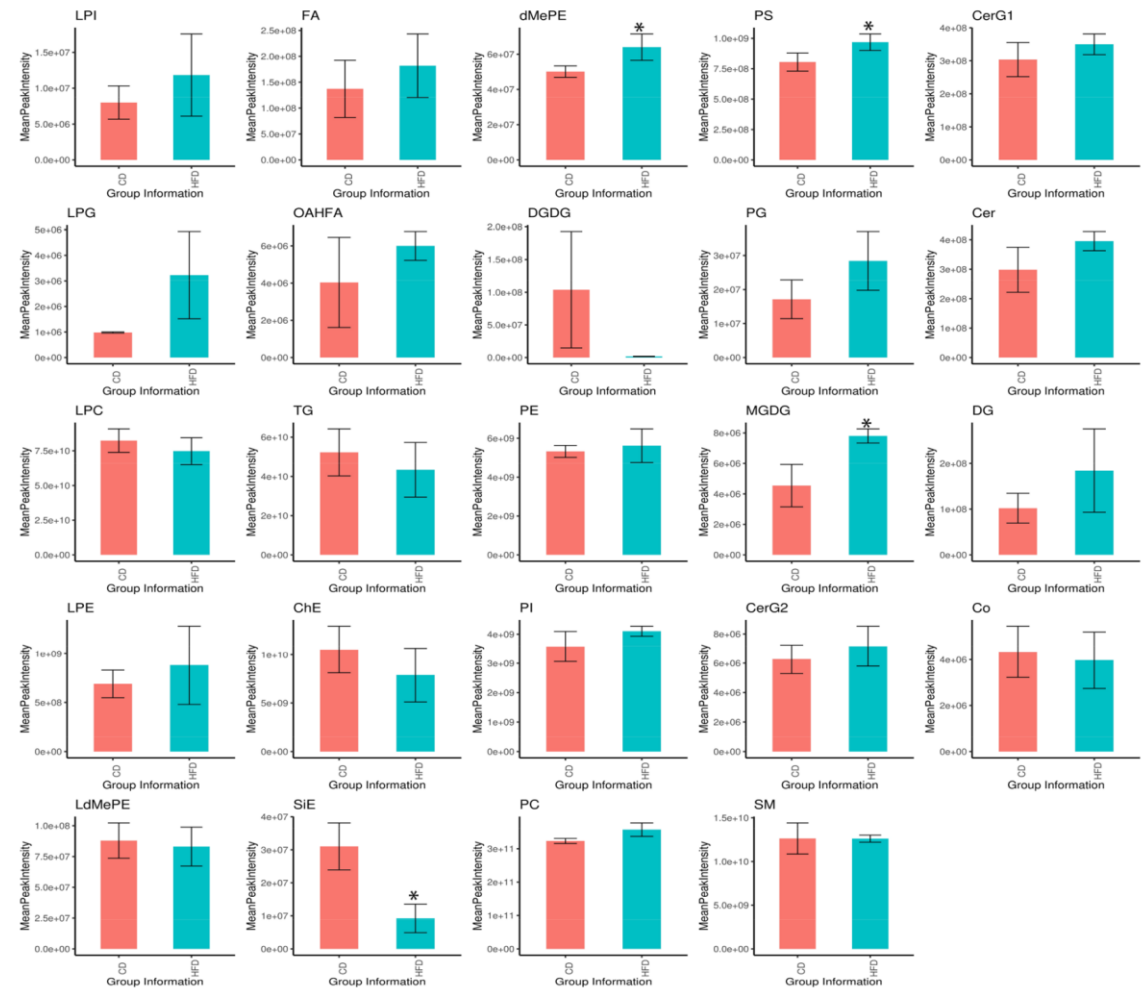
What is the pathway enrichment analysis of different metabolites in the Rab27 KOmouse after injection?

Differential Metabolite Analysis – Pathway Enrichment

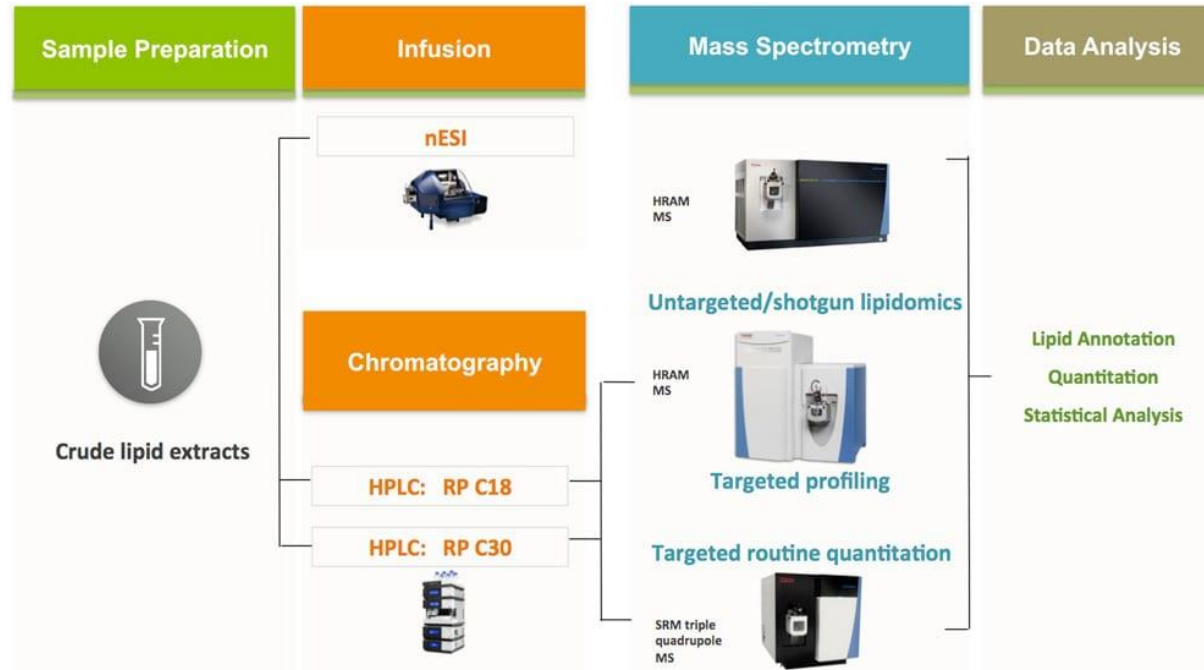
Group	Pathway	Count	All metabolites with pathway annotation (Count, All)	P-value	Pathway ID	Mode
EAT-Exos vs PBS	Circadian entrainment	1	9	0.003888944	map04713	pos
	Protein digestion and absorption	1	47	0.02016342	map04974	pos
	Neuroactive ligand-receptor interaction	1	52	0.02228738	map04080	pos
	Tyrosine metabolism	1	78	0.03326717	map00350	pos
	Tryptophan metabolism	1	81	0.0345271	map00380	pos
VAT-Exos vs PBS	Protein digestion and absorption	2	47	0.001167526	map04974	pos
	Intestinal immune network for IgA production	1	2	0.002162688	map04672	pos
	Th17 cell differentiation	1	4	0.004320931	map04659	pos
	Retinol metabolism	1	25	0.02671636	map00830	pos
	Pantothe and CoA biosynthesis	1	28	0.02987637	map00770	pos
	beta-Alanine metabolism	1	32	0.03407456	map00410	pos
	Glutathione metabolism	1	38	0.04033949	map00480	pos
	Phenylalanine, tyrosine and tryptophan biosynthesis	2	35	3.40E-05	map00400	neg
	Protein digestion and absorption	2	47	6.30E-05	map04974	neg
	2-Oxocarboxylic acid metabolism	2	134	0.000514138	map01210	neg
	Melanogenesis	1	6	0.001621972	map04916	neg
	Prolactin signaling pathway	1	11	0.002972007	map04917	neg
	Dopaminergic synapse	1	12	0.003241839	map04928	neg
	Thiamine metabolism	1	31	0.00835755	map04728	neg
	Aminoacy-tRNA biosynthesis	1	52	0.0139873	map00730	neg
	Phenylalanine metabolism	1	60	0.01612523	map00970	neg
	Tyrosine metabolism	1	78	0.02092201	map00360	neg
	Tryptophan metabolism	1	81	0.02171975	map00350	neg
	Ubiquinone and other terpenoid-quinone biosynthesis	1	92	0.02463989	map00130	neg
	Biosynthesis of amino acids	1	128	0.03414831	map01230	neg
SAT-Exos vs PBS	Tyrosine metabolism	4	78	4.78E-06	map00350	pos
	Protein digestion and absorption	3	47	4.32E-05	map04974	pos
	Neuroactive ligand-receptor interaction	2	52	0.002604142	map04080	pos
	Metabolic pathways	7	1706	0.009586724	map01100	pos
	Synaptic vesicle cycle	1	12	0.0173918	map04721	pos
	Pantothe and CoA biosynthesis	1	28	0.0401283	map00770	pos
	Mineral absorption	1	29	0.04153238	map04978	pos
	Thiamine metabolism	1	31	0.04433461	map00730	pos
	beta-Alanine metabolism	1	32	0.04573277	map00410	pos
	Phenylalanine metabolism	2	60	0.001058578	map00360	neg
	Melanogenesis	1	6	0.00485934	map04916	neg
	Prolactin signaling pathway	1	11	0.008891944	map04917	neg
	Dopaminergic synapse	1	12	0.009696633	map04728	neg
	Linoleic acid metabolism	1	28	0.02248901	map00591	neg
	Thiamine metabolism	1	31	0.02487034	map00730	neg
	Phenylalanine, tyrosine and tryptophan biosynthesis	1	35	0.02803703	map00400	neg
	Protein digestion and absorption	1	47	0.0374796	map04974	neg
	Fatty acid biosynthesis	1	50	0.03982683	map00061	neg
	Aminoacy-tRNA biosynthesis	1	52	0.04138868	map00970	neg
	Lysine degradation	1	54	0.04294815	map00310	neg

What are the changes in lipid sub-classes in the high fat diet-induced (DIO) mice?

Untargeted Lipidomics: screening all detectable lipids in your samples, measuring the changes between two or more different groups or under two or more biological conditions



What was the strategy to isolate lipid subclasses from DIO mice?



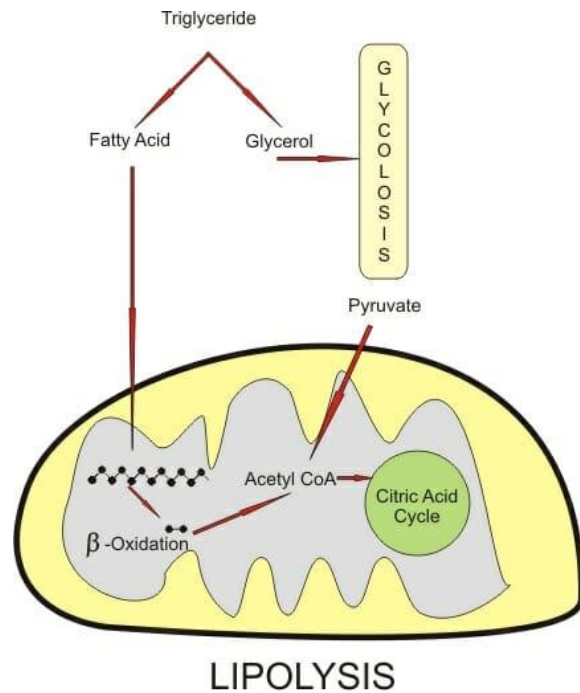
What is the pathway enrichment of differential lipids metabolites from Rab27 mice?

Table 3 The pathway enrichment analysis of differential lipid metabolites in B6/J-Rab27a-Cas9-KO mice after SAT-Exos injection

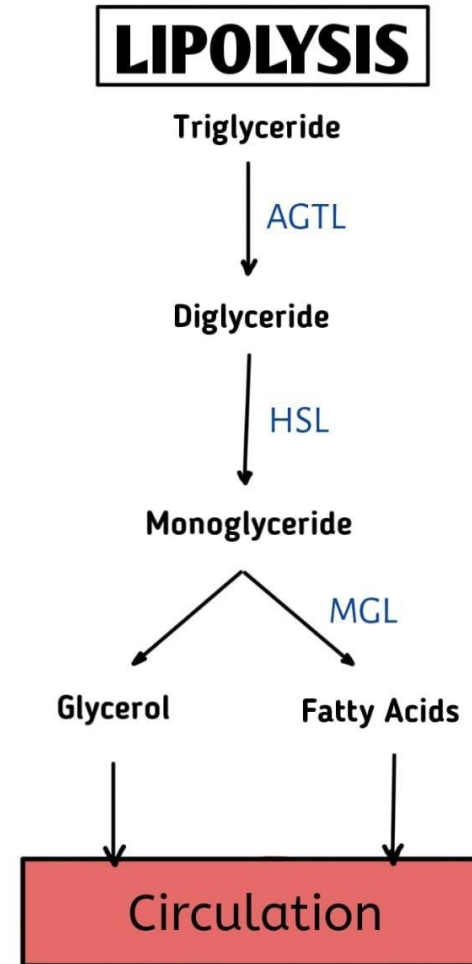
#	SAT-Exos_PBS-quant-identification				
	Pathway	Level 1	Level 2	Count	Pathway ID
1	Platelet activation	Organismal Systems	Immune system	1	map04611
2	Retrograde endocannabinoid signaling	Organismal Systems	Nervous system	1	map04723
3	Long-term depression	Organismal Systems	Nervous system	1	map04730
4	Serotonergic synapse	Organismal Systems	Nervous system	1	map04726
5	Necroptosis	Cellular Processes	Cell growth and death	1	map04217
6	Oxytocin signaling pathway	Organismal Systems	Endocrine system	1	map04921
7	Fc gamma R-mediated phagocytosis	Organismal Systems	Immune system	1	map04666
8	Vascular smooth muscle contraction	Organismal Systems	Circulatory system	1	map04270
9	Arachidonic acid metabolism	Metabolism	Lipid metabolism	1	map00590
10	Ovarian steroidogenesis	Organismal Systems	Endocrine system	1	map04913
11	Metabolic pathways	Metabolism	Global and overview maps	1	map01100
12	Inflammatory mediator regulation of TRP channels	Organismal Systems	Sensory system	1	map04750
13	Linoleic acid metabolism	Metabolism	Lipid metabolism	1	map00591
14	Biosynthesis of unsaturated fatty acid	Metabolism	Lipid metabolism	1	map01040
15	Regulation of lipolysis in adipocytes	Organismal Systems	Endocrine system	1	map04923
16	GnRH signaling pathway	Organismal Systems	Endocrine system	1	map04912
17	Ferroptosis	Cellular Processes	Cell growth and death	3	map04216
18	Glycerophospholipid metabolism	Metabolism	Lipid metabolism	1	map00564
19	Fc epsilon RI signaling pathway	Organismal Systems	Immune system	1	map04664
20	Aldosterone synthesis and secretion	Organismal Systems	Endocrine system	1	map04925

Results

SAT-Exosomes contribute to the lipid metabolism and lipid metabolites under obesity conditions.



Frank Boumprey M.D. 2009



References

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