Cell Metabolism

SWISS NETWORK FOR INTERDISCIPLINARY EDUCATION IN CHEMICAL BIOLOGY

Multilayered omics reveal sex- and depot-dependent adipose progenitor cell heterogeneity

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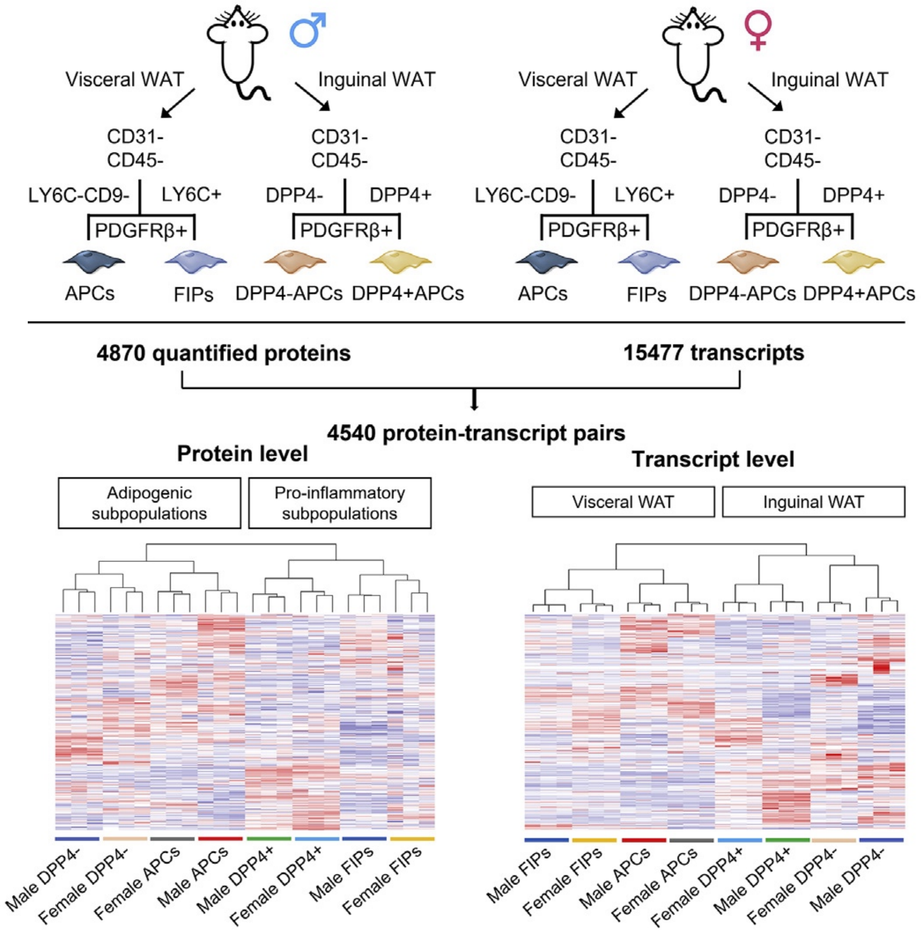

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Research Background

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White adipose tissue (WAT) and adipose progenitor cells(APCs)

WAT is a type of adipose (fat) tissue primarily responsible for **energy storage**

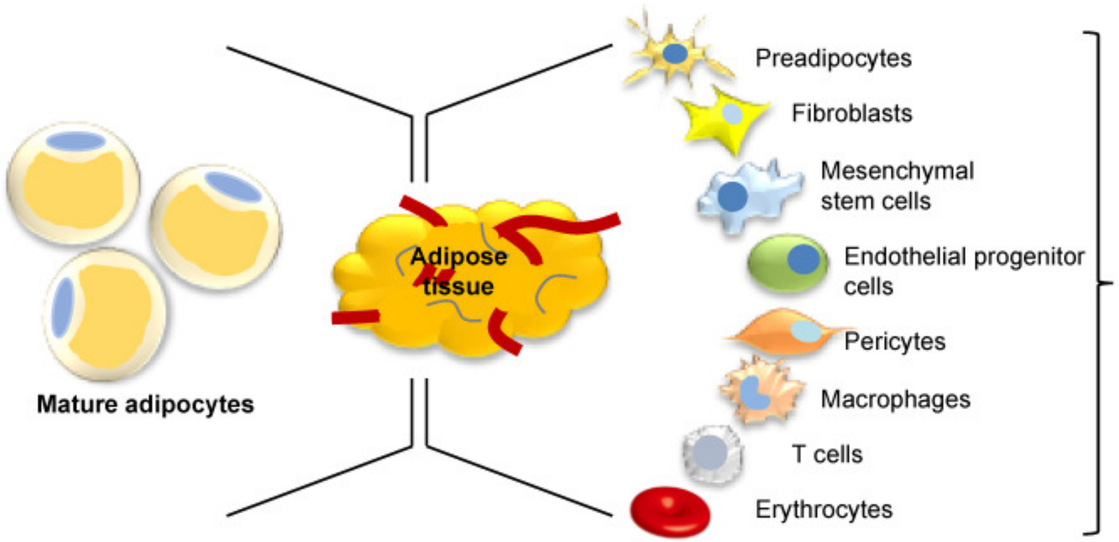

Fig1. Composition of adipose tissue.

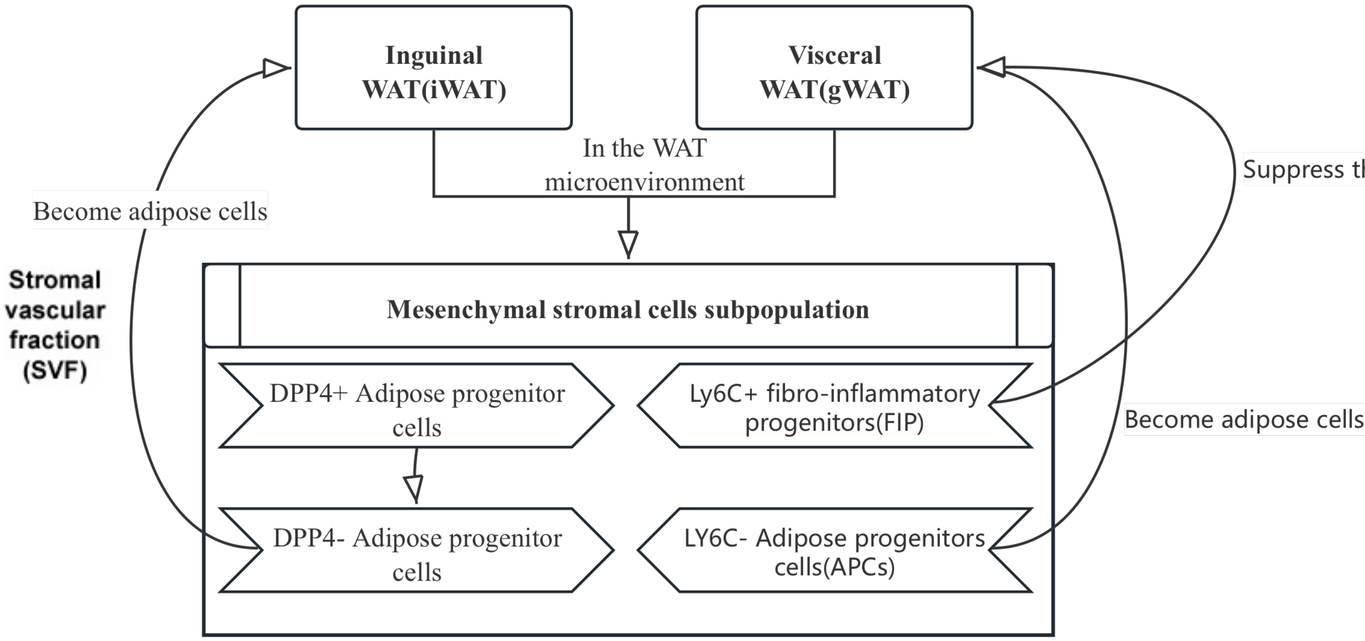

Fig2. The subpopulations of the cells in WAT

Trevor, Lucy V et al. “Adipose Tissue: A Source of Stem Cells with Potential for Regenerative Therapies for Wound Healing.” *Journal of clinical medicine* vol. 9,7 2161. 8 Jul. 2020,

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Previous efforts

✤The heterogeneity of mesenchymal stromal cell has been reported by **scRNA-seq**(Burl, Goldberg et al. 2018)

✤Joffin et al. identified significant **WAT-depot-differences** in the heterogeneity of PDGFRb+ progenitor cells. Karastergiou et al. found WAT expansion occurs in a **sex- and depot dependent manner**

✤High-fat diet (HFD) with **male APCs showing resistance** of adipogenesis in iWAT and activation in gWAT, while female APCs exhibit adipogenesis in both depots (Joffin et al., 2021; Shao et al., 2021)

✤Previous transcriptomics and follow-up function studies illustrates APCs and FIPs in gWAT **have different functions** (Hepler et al., 2018; Shan et al., 2020)

✤Shinde and McGaha et al. Illustrated **aryl hydrocarbon receptor(Ahr)** plays an important role in regulating inflammatory responses, and Ardite et al. reported **GSH metabolism** is essential for cell differentiation.

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Current problems

Why **transcriptomic alone** is not sufficient to describe the heterogeneity?

What is the **proteome basis** of the sex- or depot- dependent heterogeneity of subpopulations?

What is the molecular basis of iWAT expansion occurring in a **sex-dependent manner**?

What is the proteomics basis of APCs and FIPs **functioned differently** in gWAT?

How does **AhR** regulate FIPs and how does **GSH metabolism** regulate APCs?

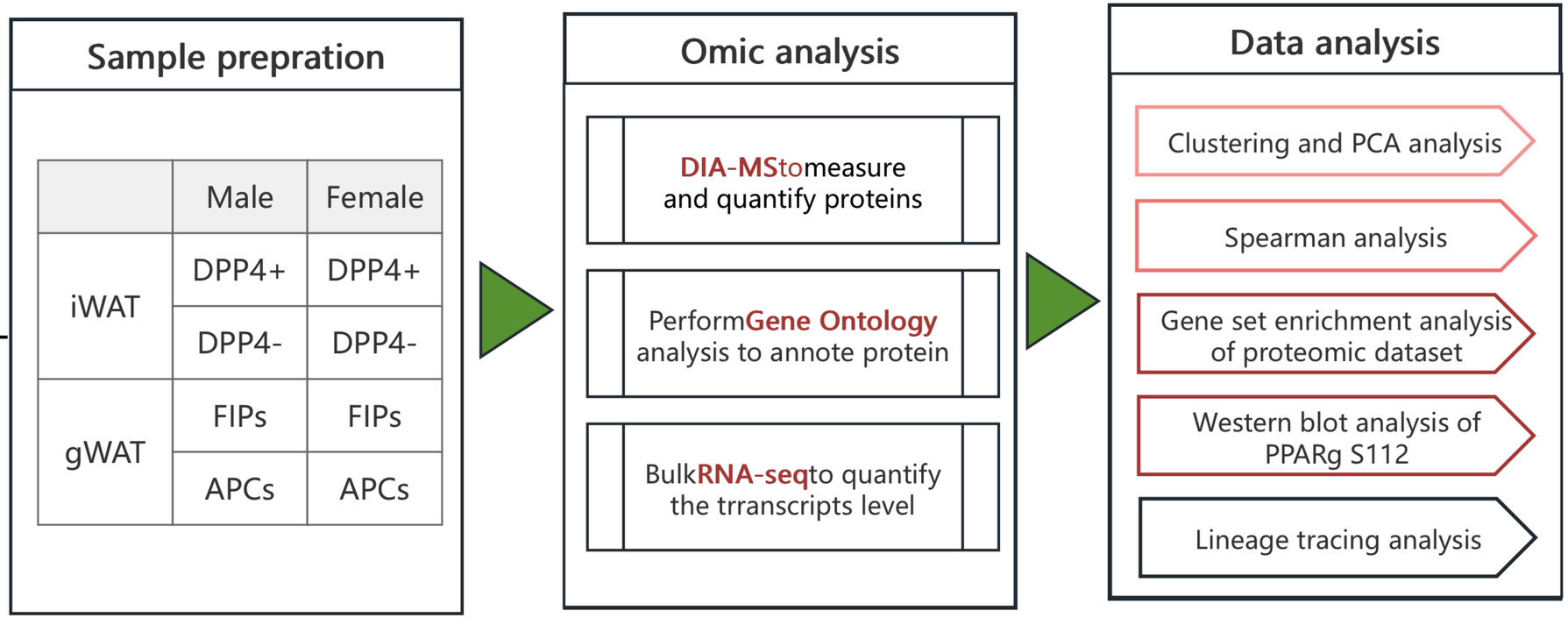

Fig1. Workflow of the Whole research

Why transcriptomic alone is not sufficient to describe the heterogeneity?

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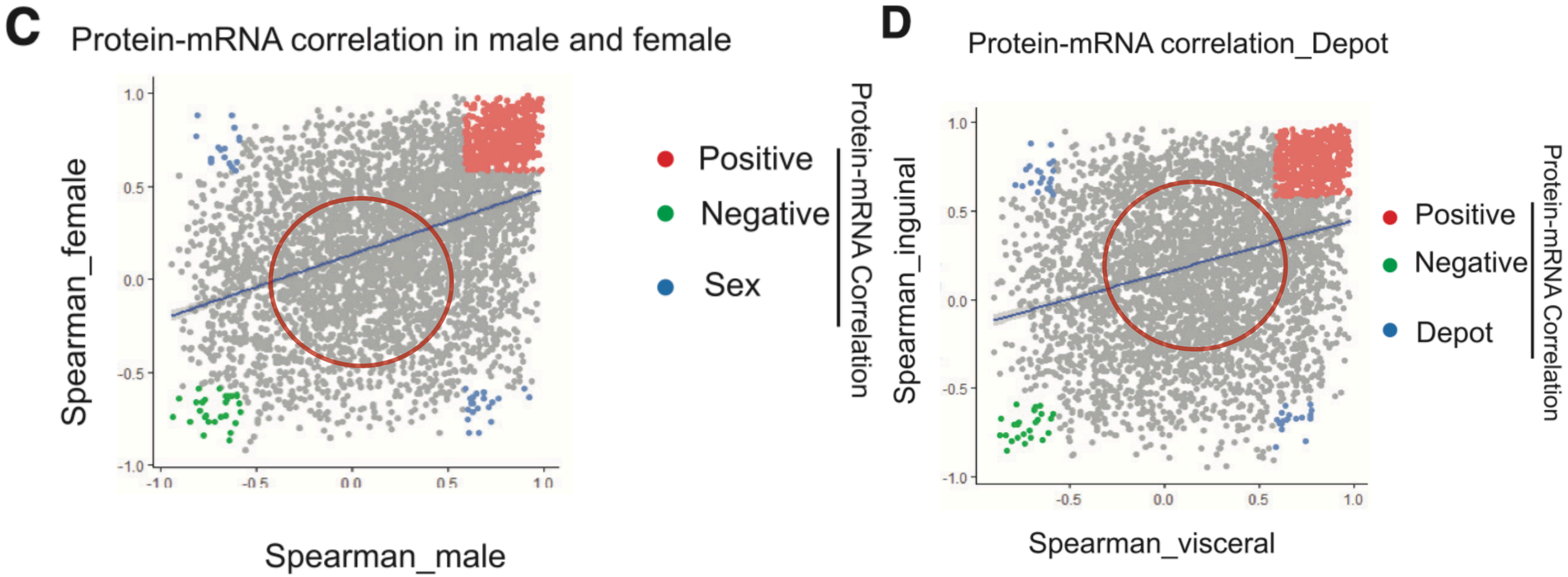

Fig1. Correlation of Spearman's rho in male versus female populations(Left) and Visceral versus Inguinal(Right)

This correlation has a difference dependent on sex and depot but most pairs don't have a significant correlation

Why transcriptomic alone is not sufficient to describe the heterogeneity?

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A moderate protein-mRNA correlation was observed in APC-to-FIP

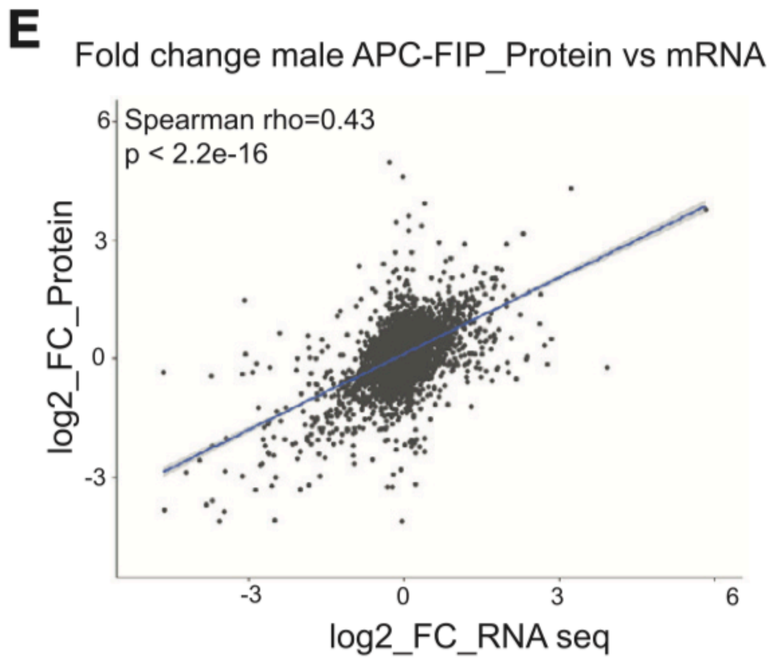

Fig1. Correlation between Fold changes from APC to FIP of Proteins and mRNA

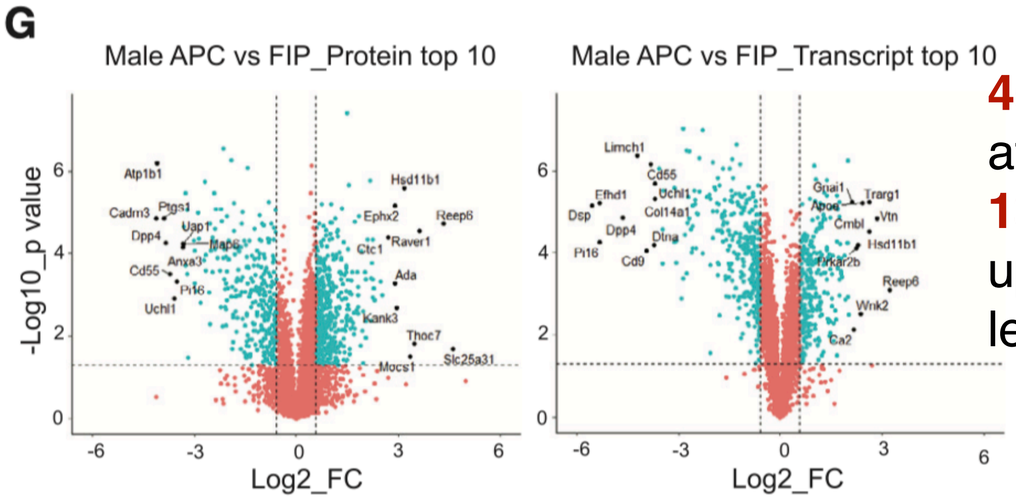

Fig2. Volcano plot depicting differences between male APCs and FIPs at the protein level (left) and transcript level (right)

454 genes upregulated at the protein level, only **139** were also upregulated at transcript level

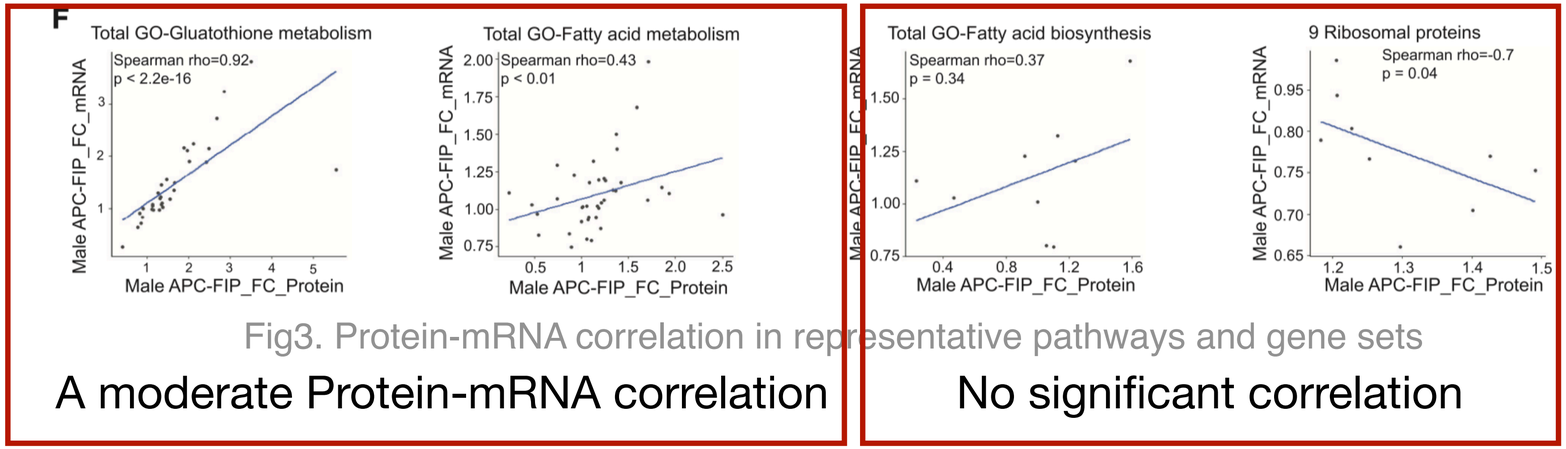

Fig3. Protein-mRNA correlation in representative pathways and gene sets

A moderate Protein-mRNA correlation

No significant correlation

Transcriptomic alone is not sufficient to describe or predict the expression of proteins

What is the proteomic basis of the sex- or depot-dependent heterogeneity of subpopulations?

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gWAT APCs were more similar to **gWAT FIPs** at transcript level,
gWAT APCs were more similar to **iWAT DPP4- cell** at protein level

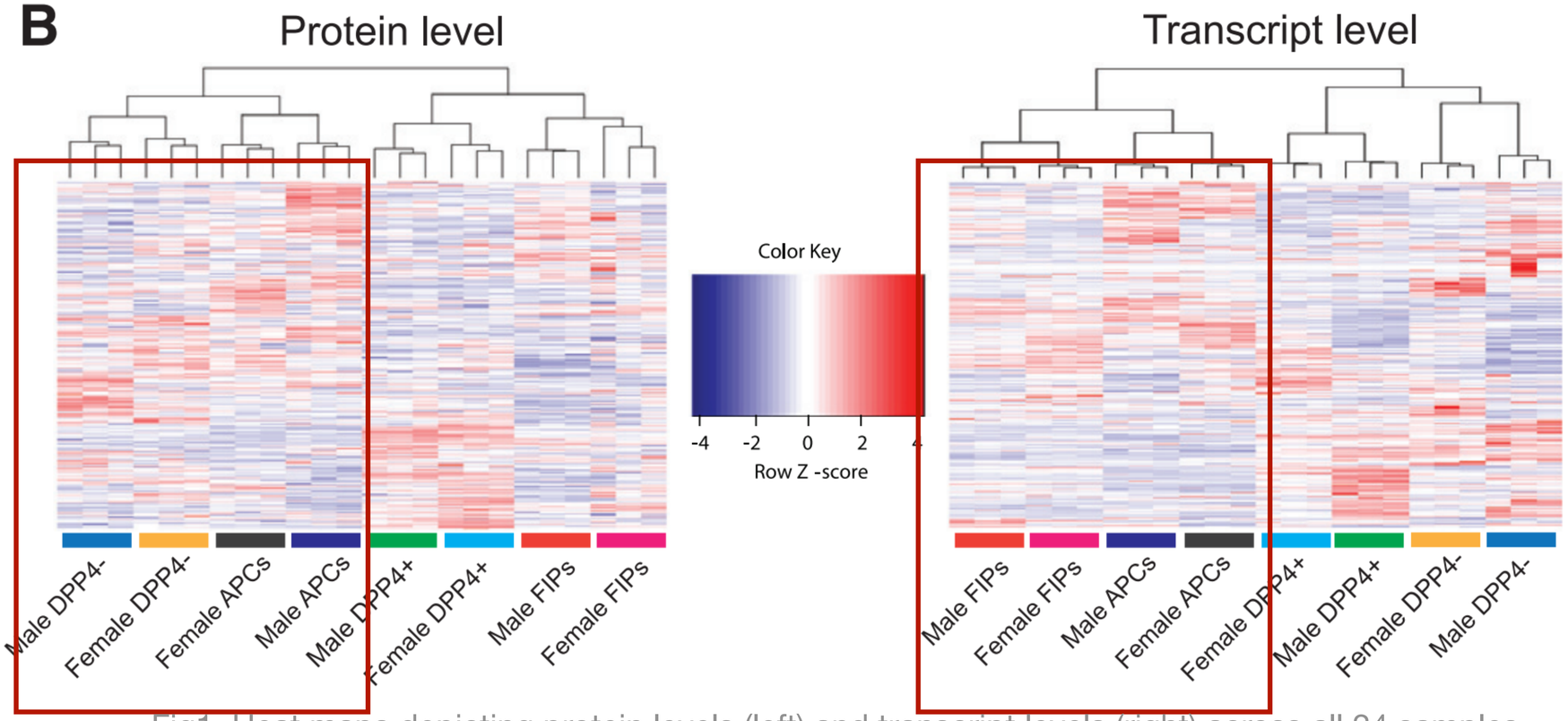

Fig1. Heat maps depicting protein levels (left) and transcript levels (right) across all 24 samples

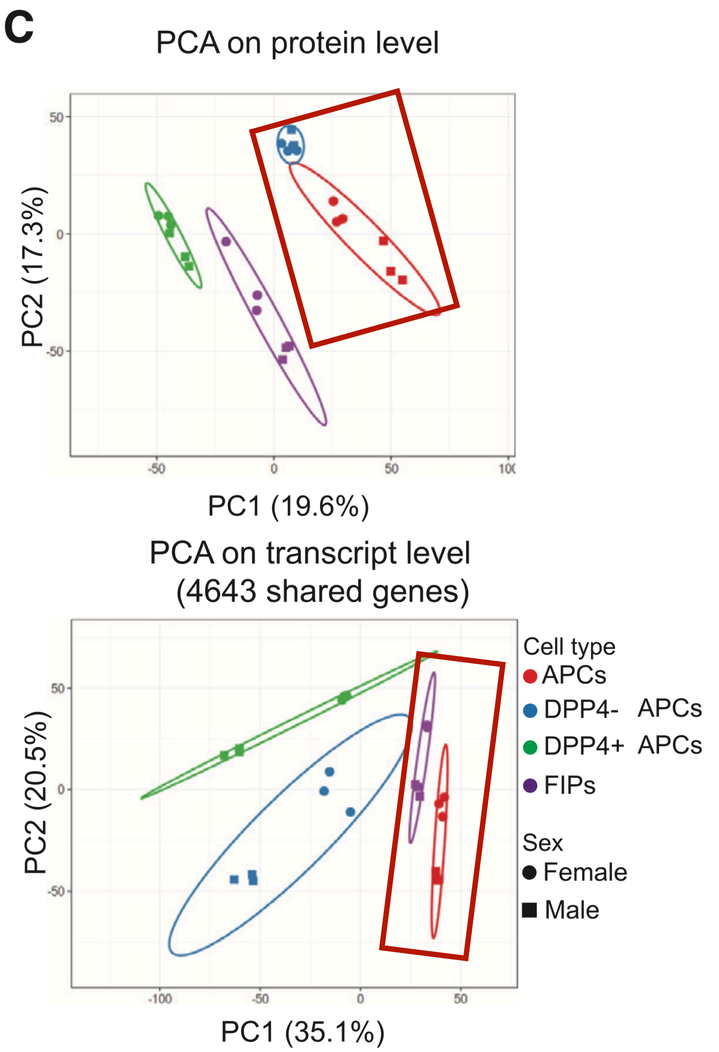

Fig2.Principal component analysis based on proteomics (up) and transcriptomics (down) data

The difference in protein expression explains the similar function in different depot

What is the proteomic basis of the sex- or depot-dependent heterogeneity of subpopulations?

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556 metabolism-related proteins were distinguished by clustering.
Further indicating the difference of metabolism among these subpopulations

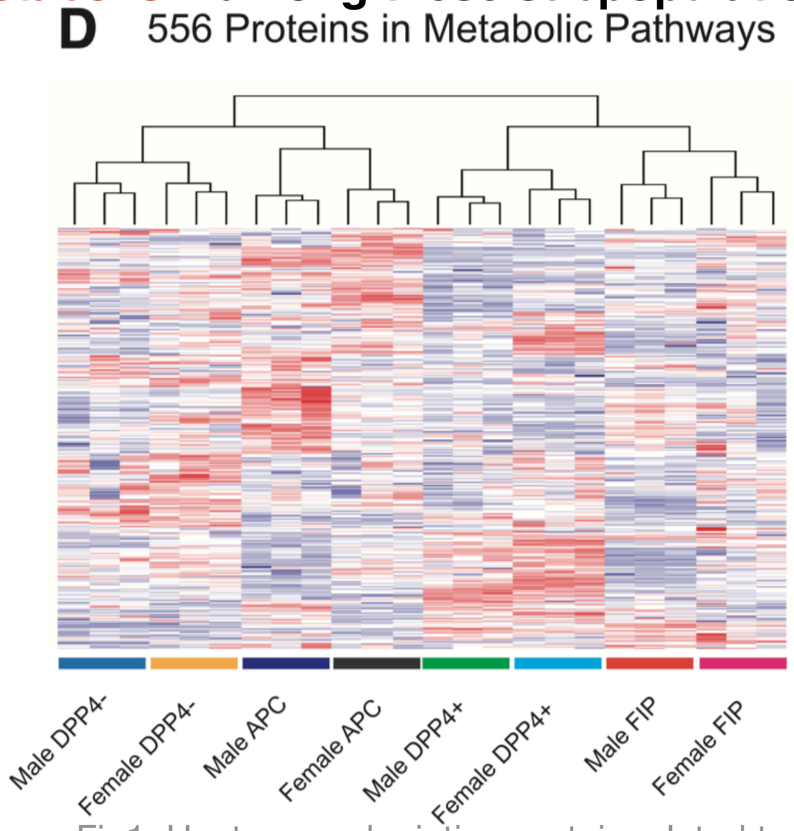

Fig1. Heat maps depicting protein related to metabolic pathway

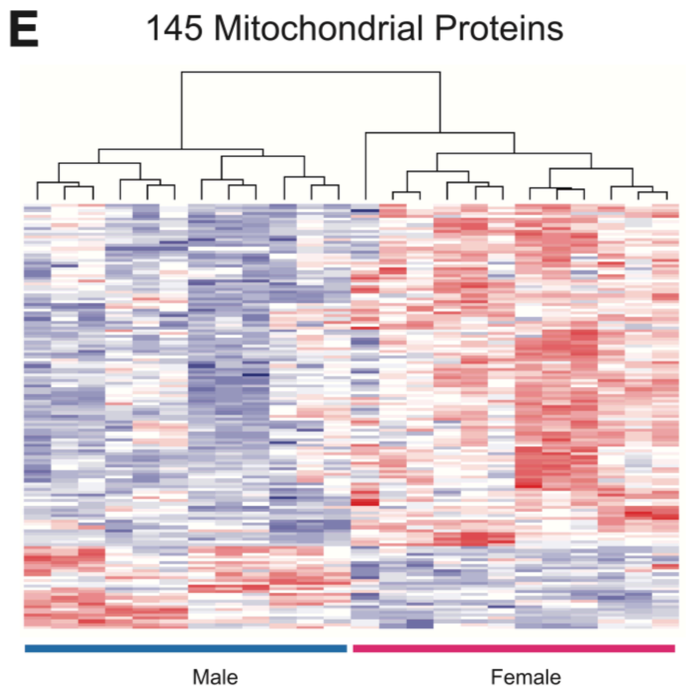

Fig2. Heat maps depicting protein annotated as Mitochondrial related

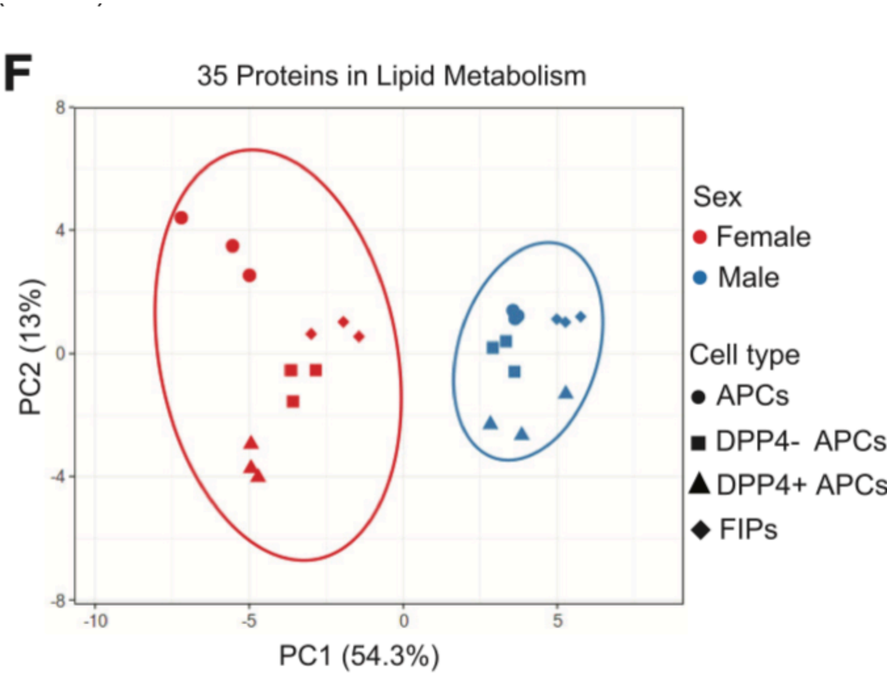

Fig3. PCA analysis for protein annotated as Lipid metabolism related

The expression of **mitochondrial proteins and regulators of lipid metabolism** might contribute to the **Sex-dependent heterogenity**

Protein expression explain the depot-dependent heterogeneity as well as the sex-dependent heterogeneity

What is the molecular basis of iWAT expansion occurring in a sex-dependent manner?

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GSEA revealed that several pathways, including **estrogen response**, **TNF-α signaling**, and **hypoxia**, were significantly enriched in the APCs, showing strong sex-specific expression patterns.

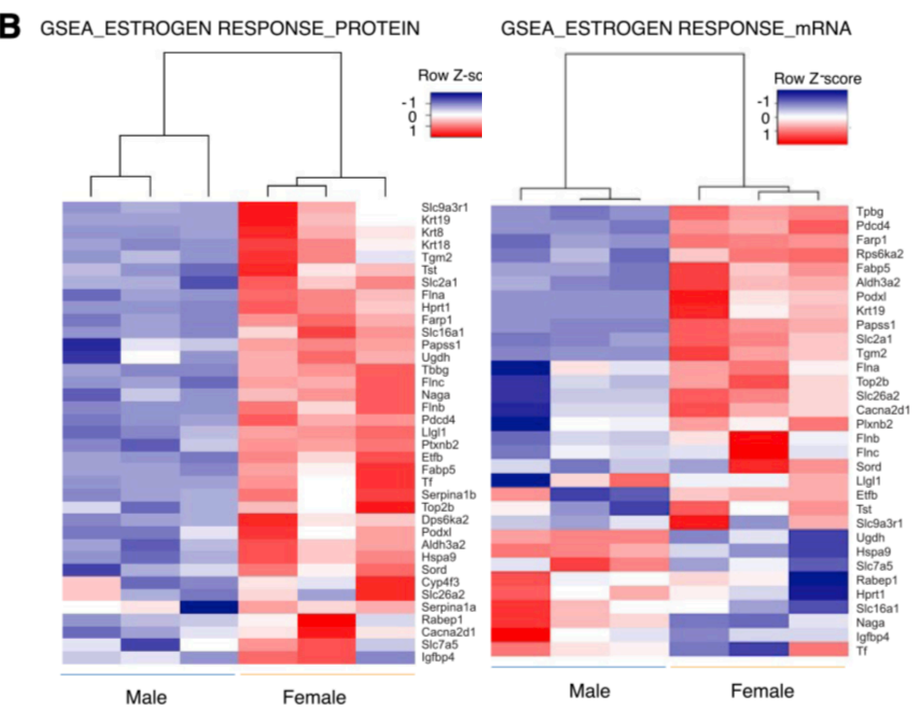

Fig1. Heat maps depicting protein expression (left) and mRNA expression (right) of an "estrogen response"

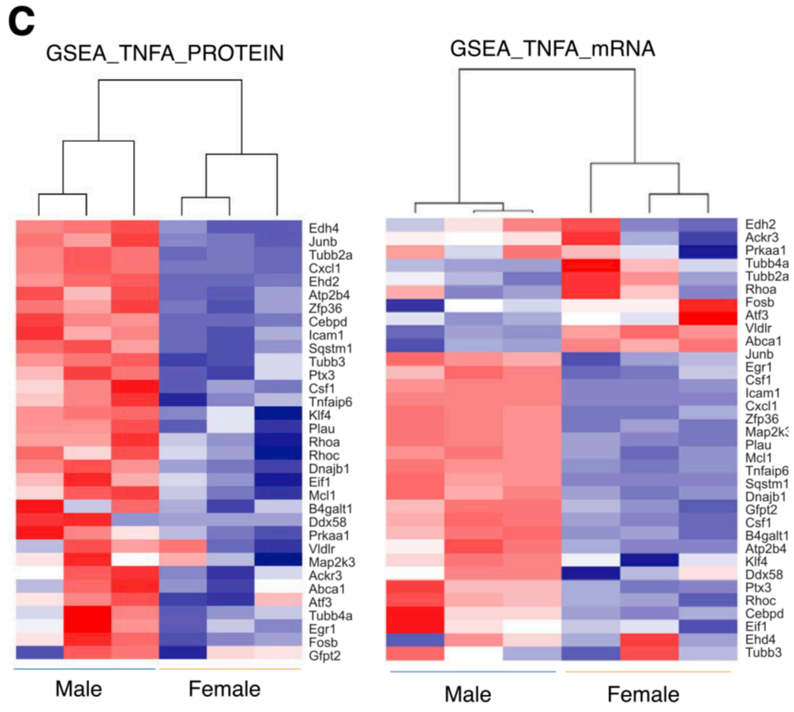

Fig2. Heat maps depicting protein expression (left) and mRNA expression (right) of an "TNFa signaling'

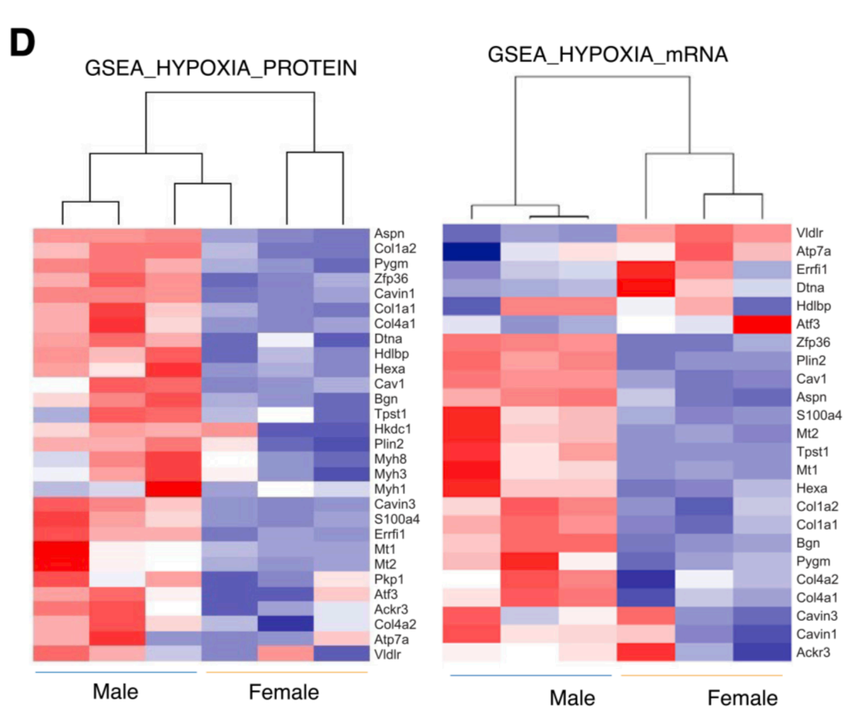

Fig3. Heat maps depicting protein expression (left) and mRNA expression (right) of an ''hypoxia"

The protein related to **estrogen response** might contribute to adipogenesis while the proteins related to **TNF-α signaling and hypoxia** might suppress this process.

What is the molecular basis of iWAT expansion occurring in a sex-dependent manner?

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Previous studies illustrate the hypoxia suppress the APCs differentiating through **PPARγ phosphorylation**

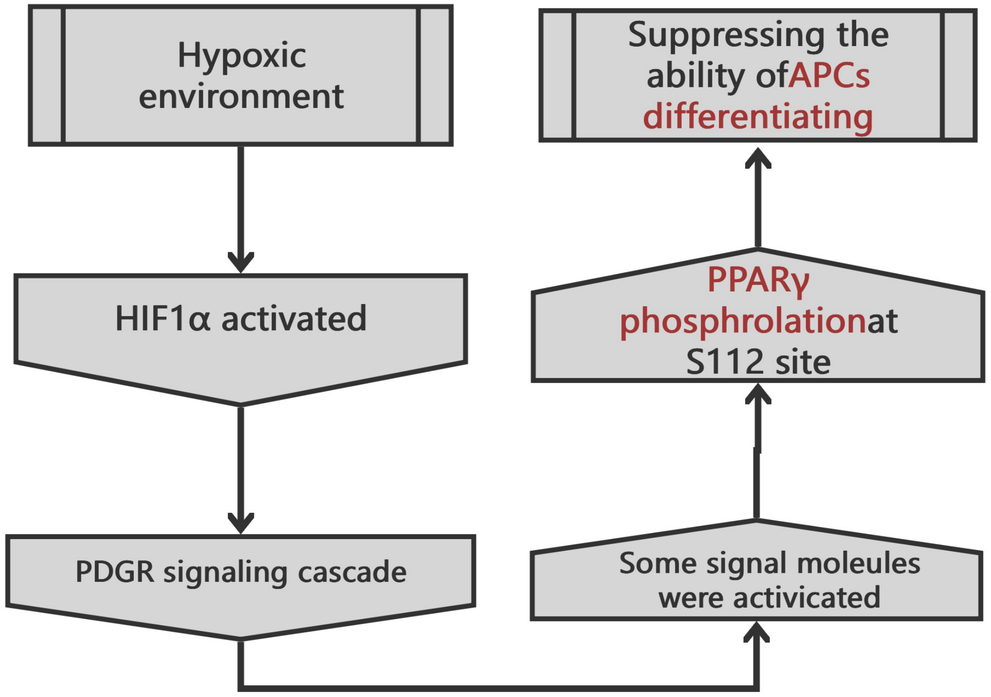

Fig1. Signal pathway of PPARγ phosphorylation

Diet-induced-Obesity leads to a hypoxic environment, using Westernbloting to test the phosphorylation level

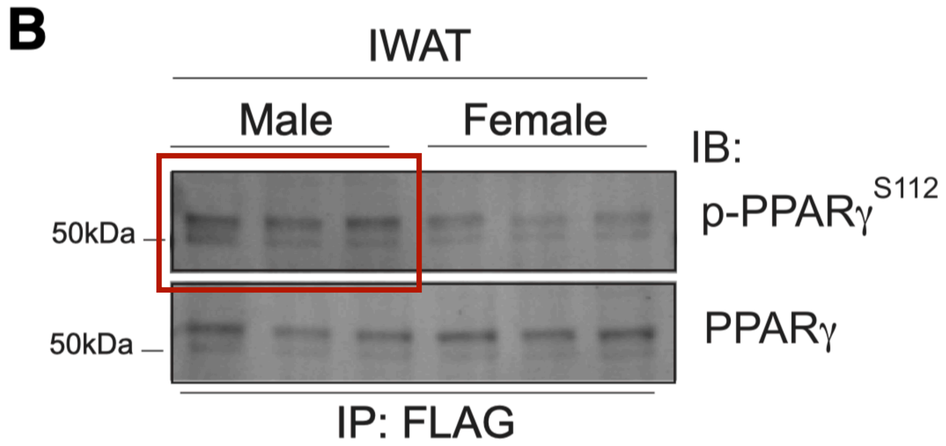

Fig2. Western blot analysis of PPARγ S112 phosphorylation in male and female

Such phosphorylation **has a higher level in male iWAT**, which partially explains the lower adipogenesis level in male

What is the molecular basis of iWAT expansion occurring in a sex-dependent manner?

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To further Ilustrate the sex-dependent difference in APCs differentiation related to PPARγ, authors introduced a new model named **Mural-Chase model** to trace the fate of APCs.

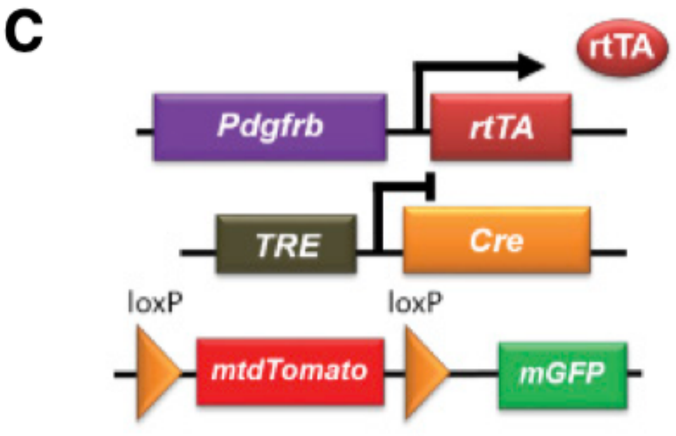

Fig1. The Mural-Chaser Model

Here use a Rosiglitazone treatment to **accelerate the PPARγ-Pdgfrb activation** and Trametinib to **inhibit the phosphorylation**

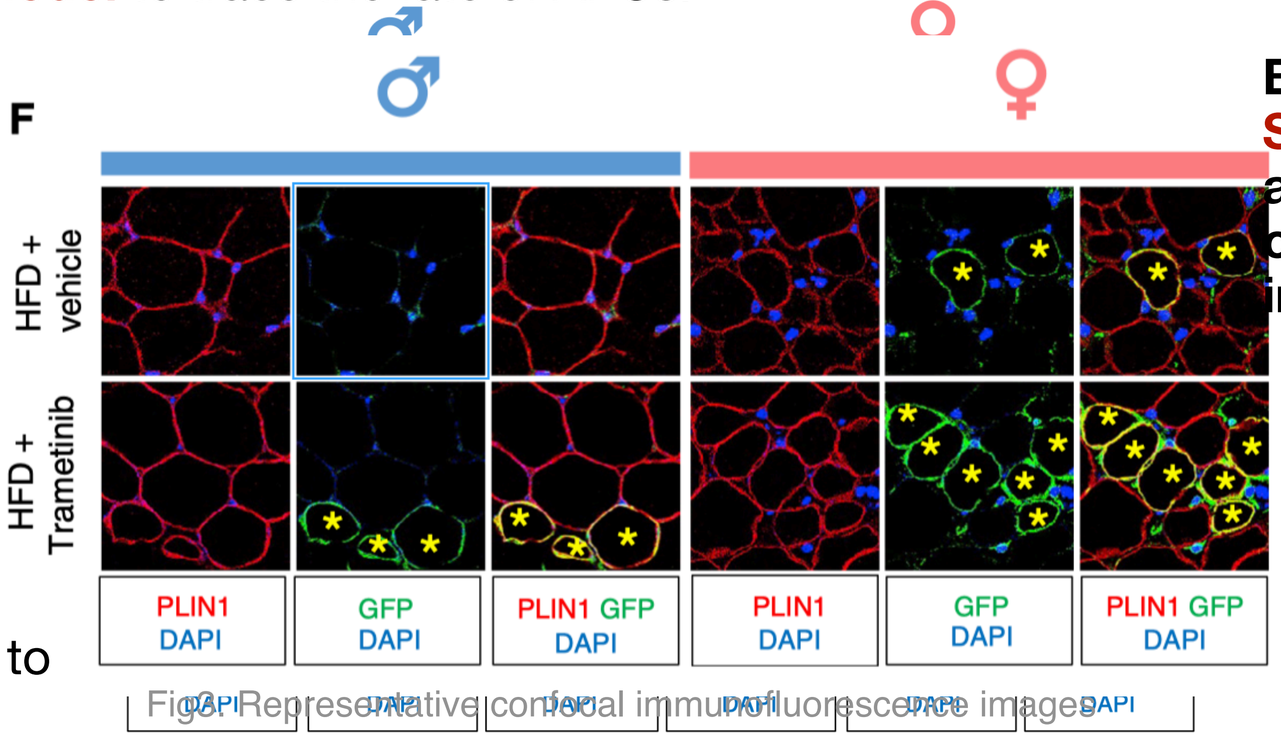

Fig2. Representative confocal immunofluorescence images

By inhibiting PPARγ S122 phosphorylation, PPARγ activation moting apogenesis adipogenesis in male ct in female can be significantly increased

PPARγ phosphorylation underlies sex differences in iWAT expansion (APC differentiation).

What is the proteomics basis of APCs and FIPs functioned differently in gWAT?

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The authors obtained the differential expression data of APCs and FIPs through proteomic analysis and used GSEA to enrich their functional pathways.

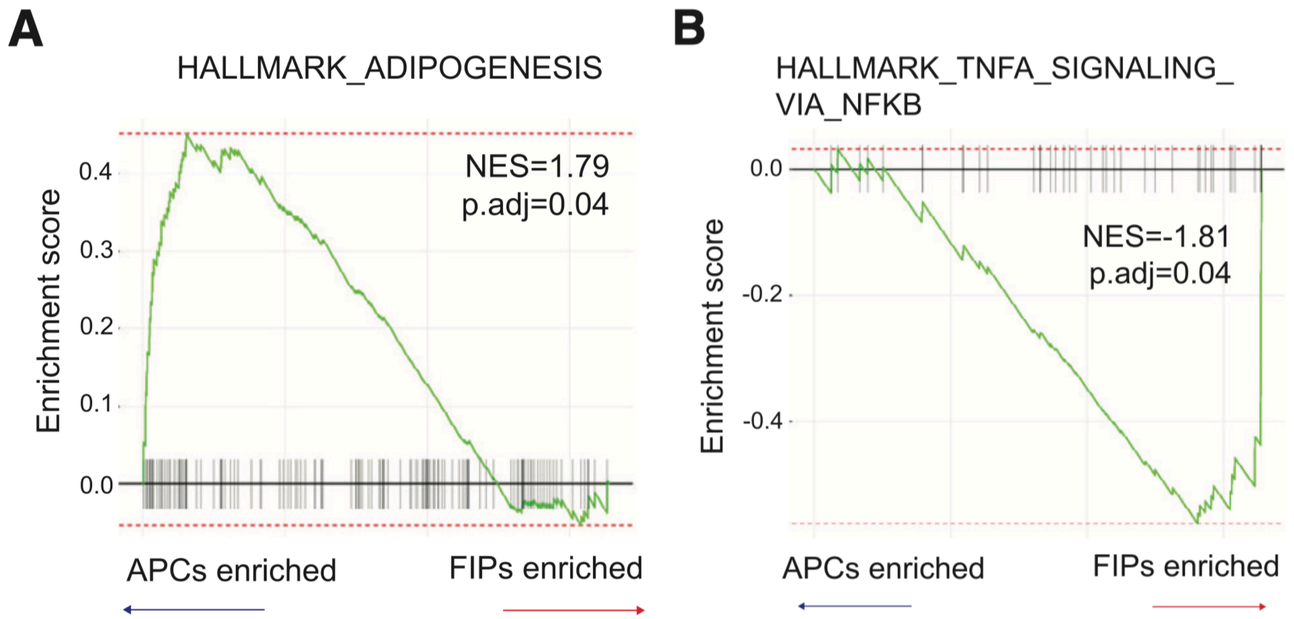

Fig1. Gene set enriched analysis (GSEA) based on proteomic data.
Male APCs are enriched in ‘’adipogenesis” signature(Left)
Male FIPs enriched in TNF-α signaling signature(Right)

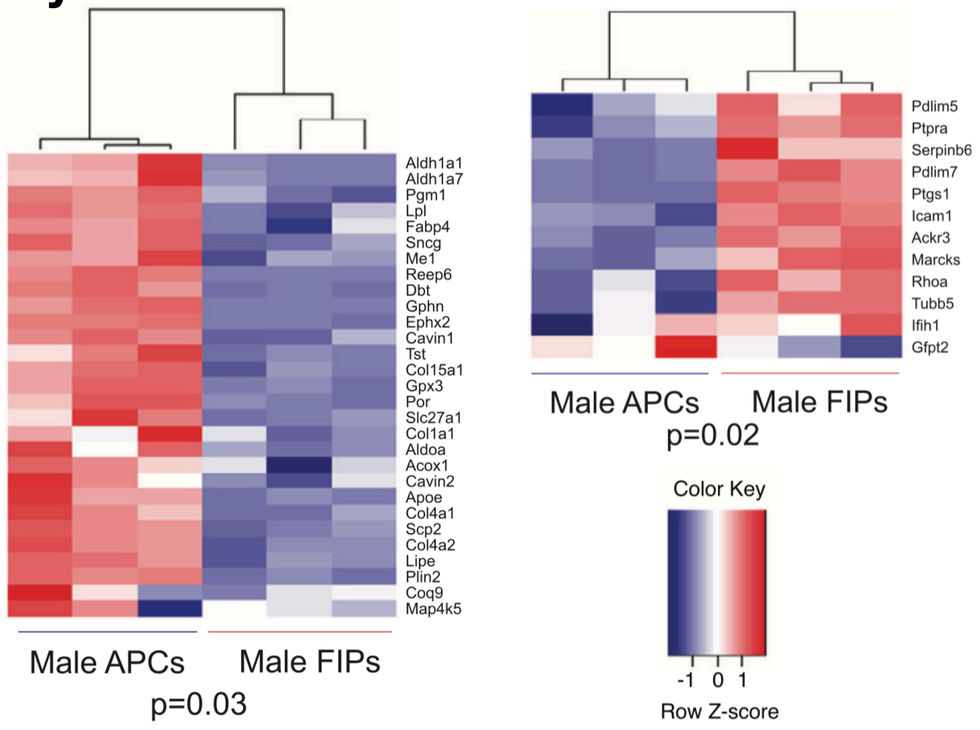

Fig1. Heat map depicting the expression of leading-edge subset of genes.

Proteomic analysis further explains the different functions of the two subpopulations

What is the proteomics basis of APCs and FIPs functioned differently in gWAT?

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Ingenuity pathway analysis(IPS) shows 18 pathways were enhanced in APS while 4 were enhanced in FIPs

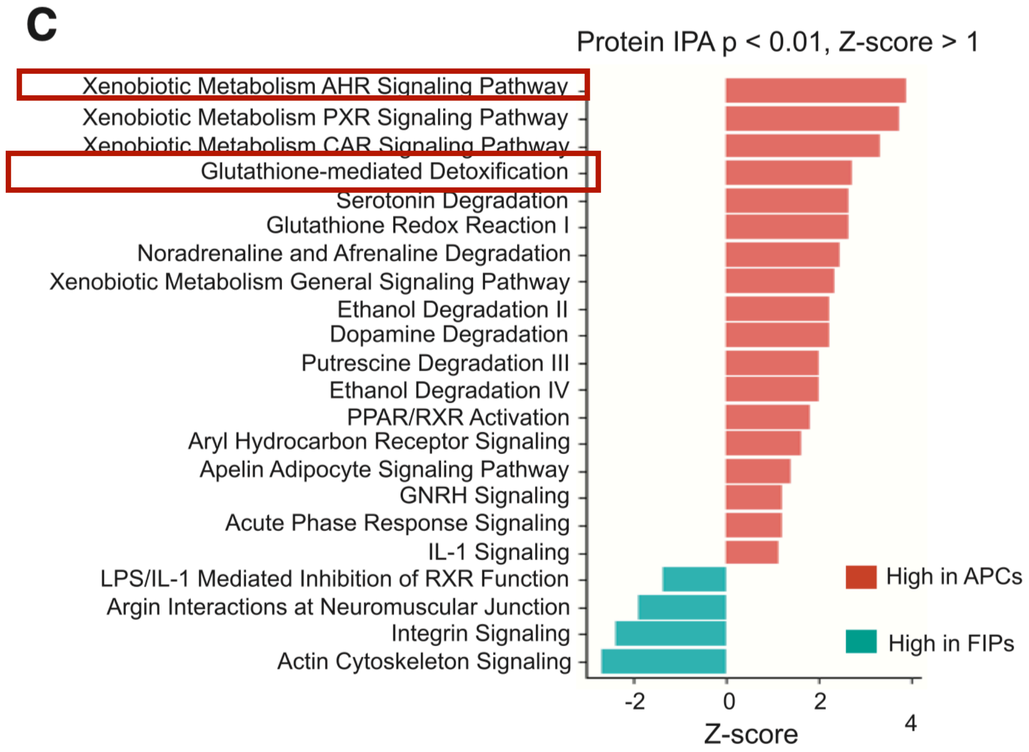

Fig1. Most significantly enriched pathways discriminating male APCs and FIPs identified by ingenuity pathway analysis (IPA)

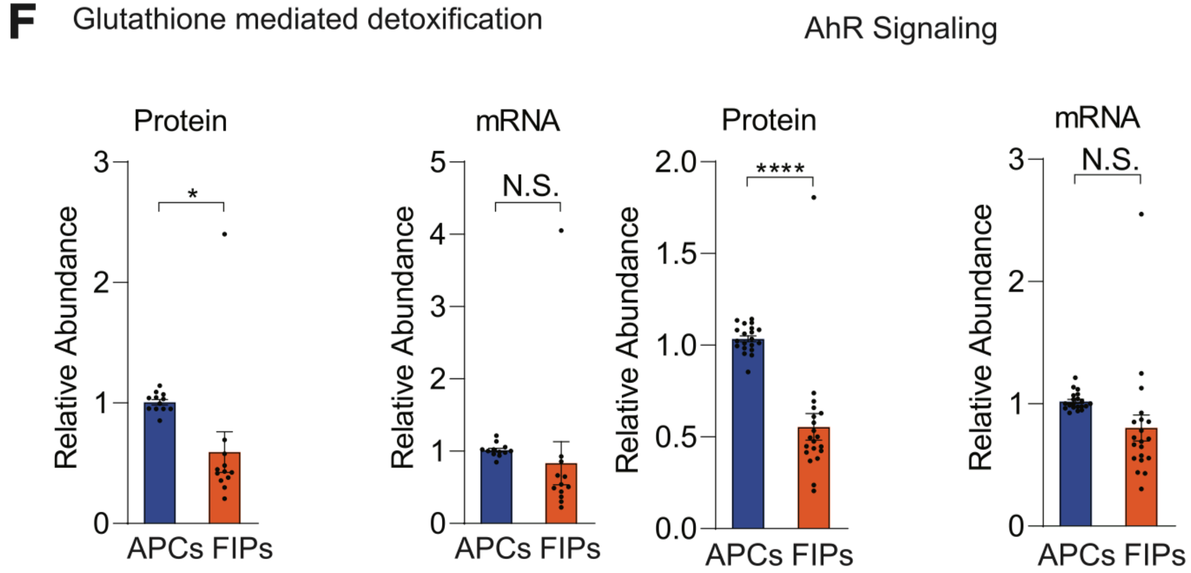

Fig2.Bar plot illustrate a more significant difference between the proteomic data of two subpopulations

Proteomics analysis has a stronger ability to capture differences in regulatory pathways

How does AhR regulate FIPs and how does GSH metabolism regulate APCs?

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Suppress the expression of AhR by CRISPR-Cas9

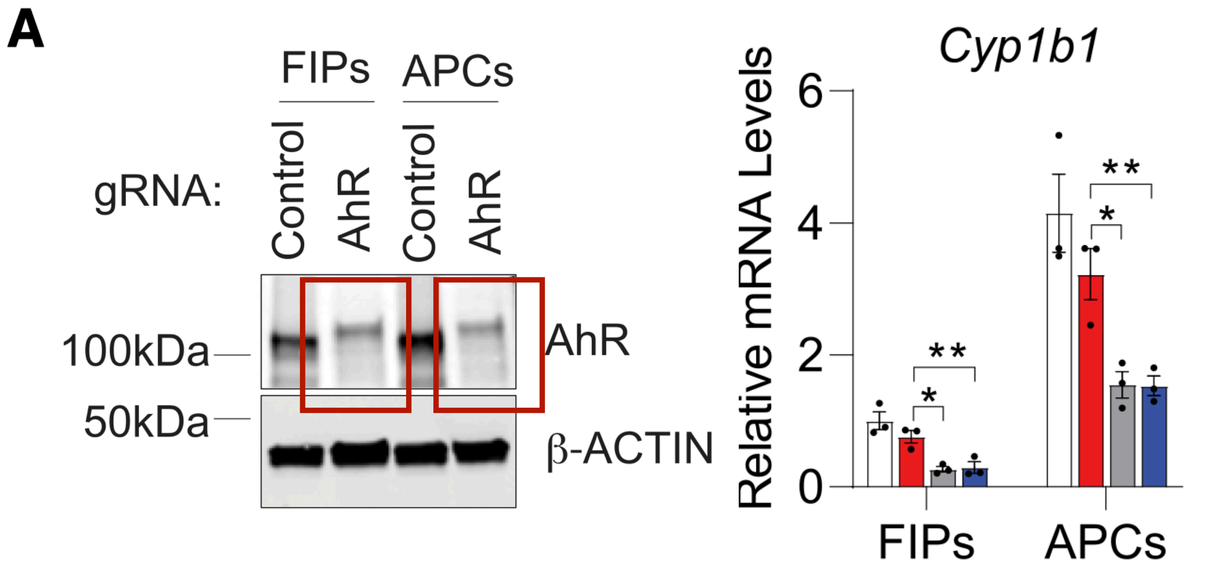

Fig1.Western blot of AhR(Left) and mRNA levels of Cyp1b1(Right) FIPs and APCs transduced with CRISPR lentivirus, Cyp1b1 is a target gene of AhR

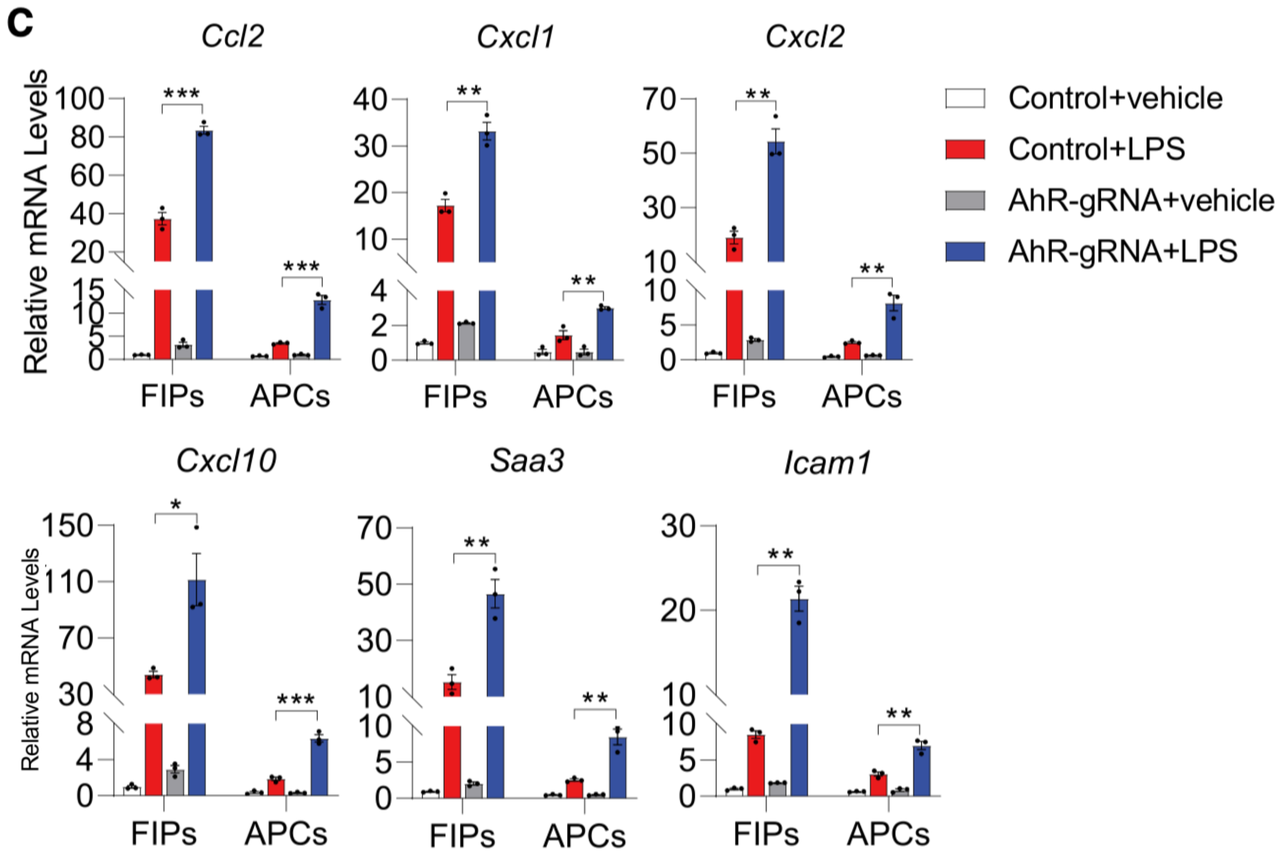

Fig2. mRNA levels of pro-inflammatory genes in cultures of FIPs and APCs

AhR regulates the inflammatory through inhibiting the expression of pro-inflammatory genes

How does AhR regulate FIPs and how does GSH metabolism regulate APCs?

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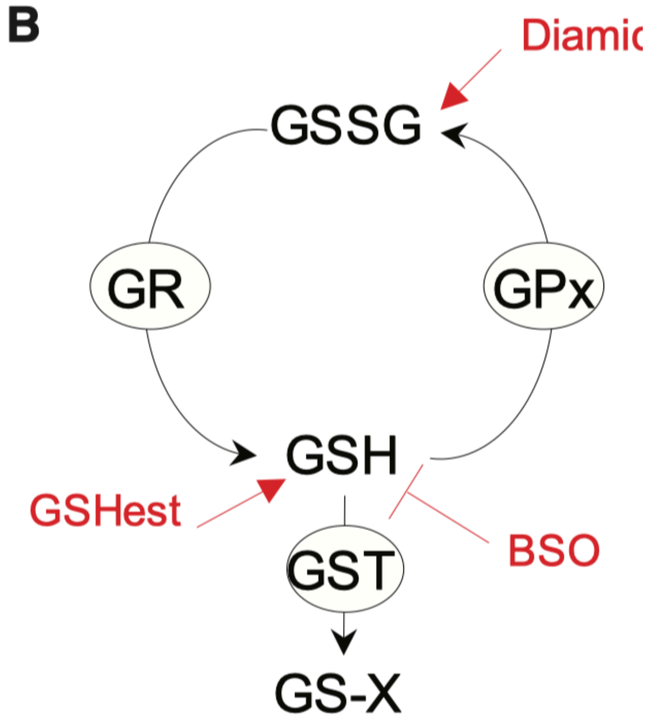
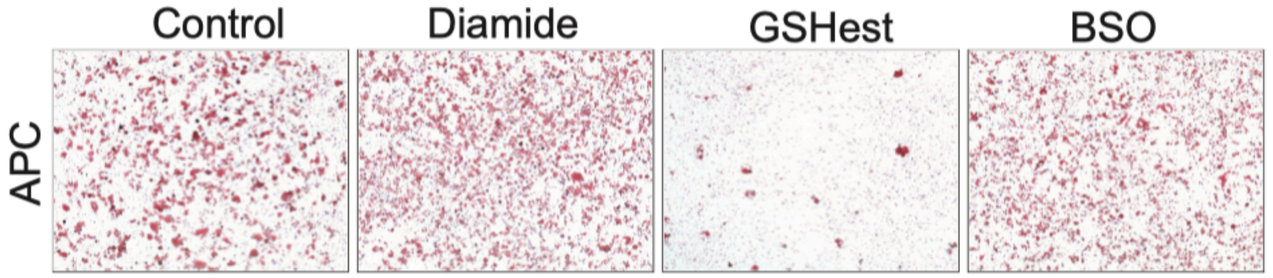


Fig1. Schematic diagram of the effects of different complex in the glutathione redox pathway



Higher GSH/ GSSG ratio, Less differentiation

Fig2. Representative bright-field images of Oil Red O-stained cultures

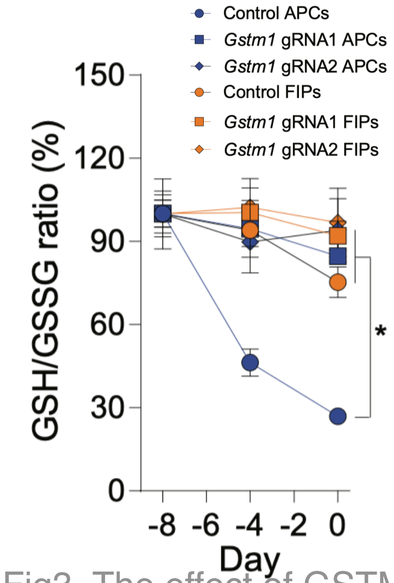


Fig3. The effect of GSTM1 on ratio of GSH/GSSG

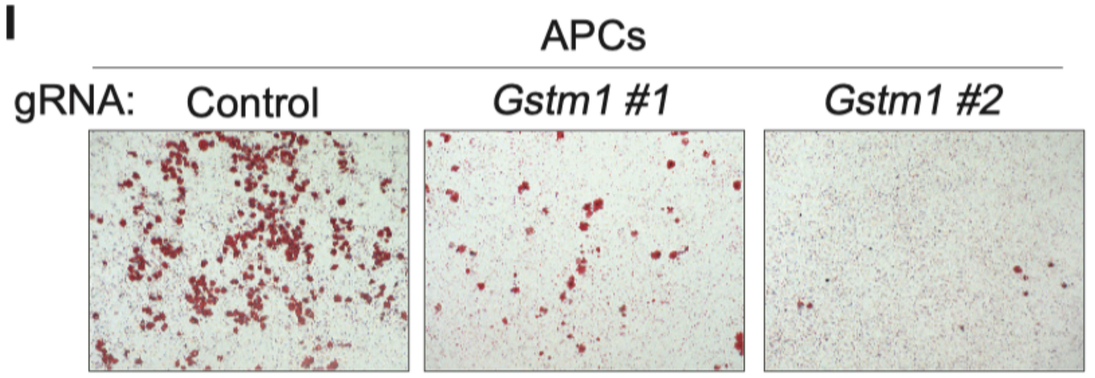


Fig4. Representative bright-field images of Oil Red O-stained cultures of differentiated APCs transduced with the indicated CRISPR lentivirus

GSTM1 regulate the APCs differentiation through maintaining a lower level of GSH/GSSG ratio

Conclusion

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Key Results

- **Transcriptomic alone** is not sufficient to describe or predict the expression of proteins of adipose progenitors
- Proteomic analysis explain the **depot- and sex-heterogeneity of APCs**
- **PPARγ phosphorylation** underlies sex differences in iWAT expansion (APC differentiation).
- Proteomic analysis further explains the **different functions** of the FIPs and APCs in gWAT
- AhR regulates the inflammatory through **inhibiting the expression of pro-inflammatory genes**
- GSTM1 regulate the APCs differentiation **through maintaining a lower level of GSH/GSSG ratio**

Main achievement

- Better understand the functional differences of adipose progenitor cells
- Provide a reference for other tissue heterogeneity studies.
- Pointed out the advantages of proteomic analysis

Limitations

- Tissue processing may affect gene expression results
- Incomplete coverage of all cell subpopulations
- Restricted to iWAT and gWAT in steady-state conditions

Questions

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What are the relationships between APCs, WAT, and mesenchymal stromal cells?

How does the Mural-Chase model work ?