

# Found candidates receptor ligand pairs in self-renewal satellite cell

Collaboration with Penney GILBERT

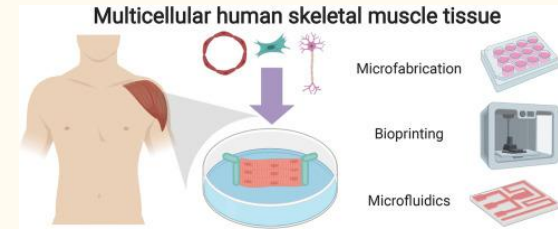
Analysis : Legrand's team :

- **Dr. Penney M. Gilbert**
- PhD Erik JACQUES
- RA Nitya GULATI
- Trainee Saif RJAIBI
- **Dr. Fabien LE GRAND**
- IE Johanna GALVIS-LACROUX
- IE William JARASSIER
- IE Pauline MOULLÉ

# Penney GILBERT project

Construct miniaturized versions of human skeletal muscle tissue in a dish for:

- Drug discovery effort
- Fundamental studies
  - muscle stem cell mediated repair
  - Duchenne muscular dystrophy
  - exercise ...



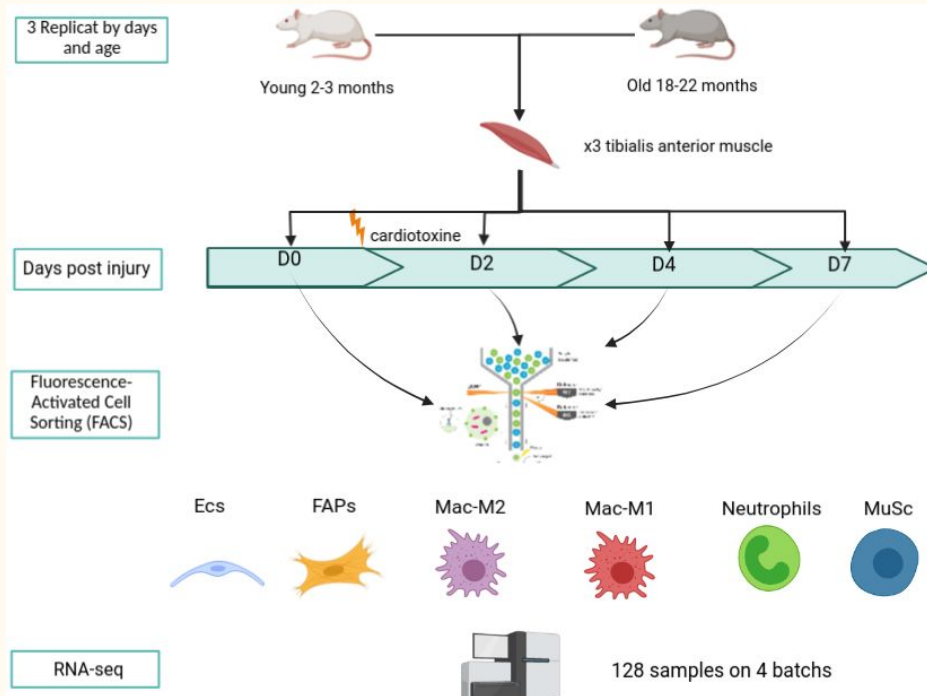
Louise A. Moyle, Erik Jacques, Penney M. Gilbert, Engineering the next generation of human skeletal muscle models: From cellular complexity to disease modeling, *Current Opinion in Biomedical Engineering*

Objective collaboration:

- ❖ Test the power of an in silico --> in vitro pipeline for discovery of satellite cell modulators

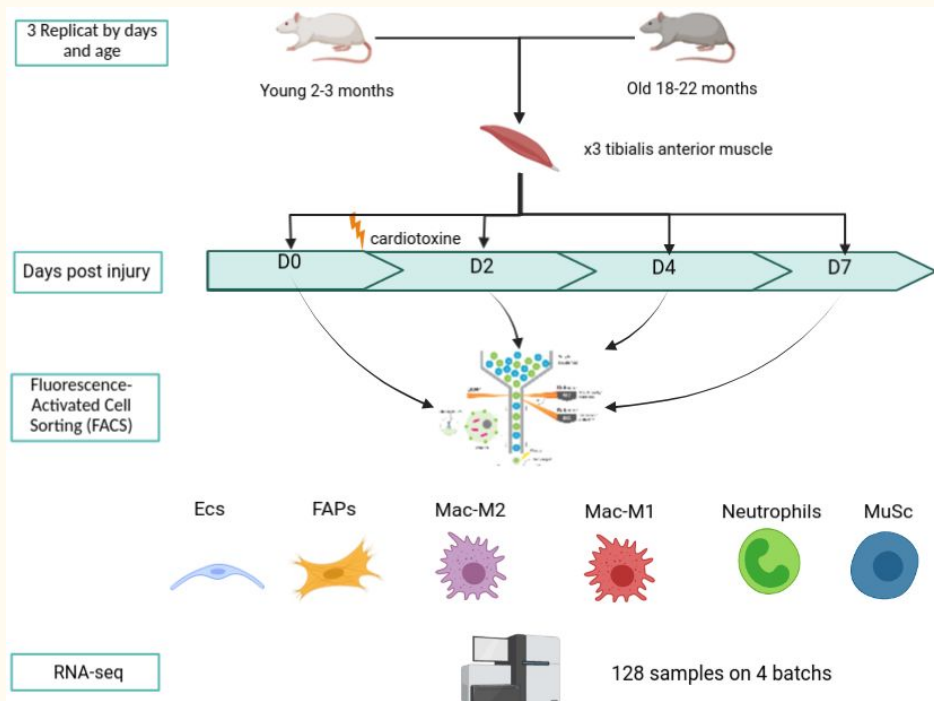
# Le Grand data

With the collaboration of Dr. Bénédicte CHAZAUD and Dr. Jérôme FEIGE teams RNA-seq data following this design experiment are available:



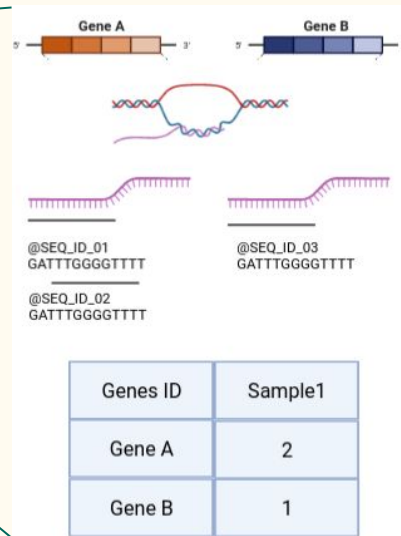
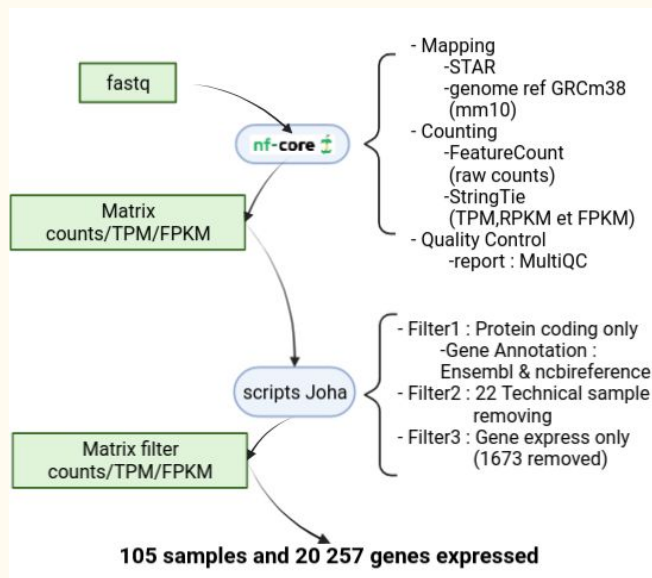
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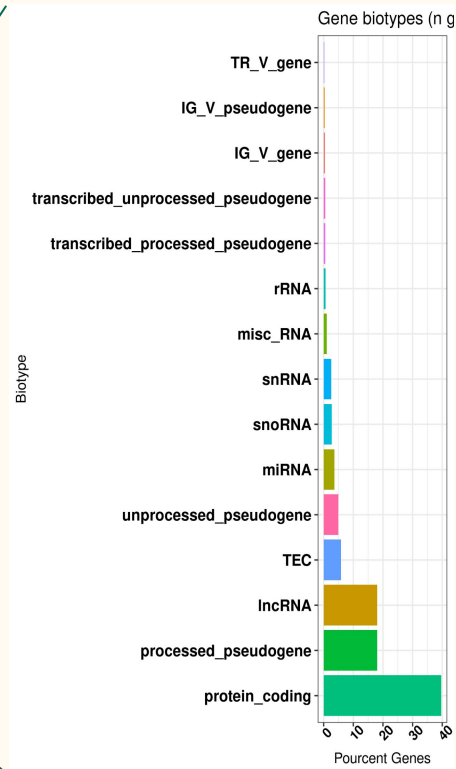
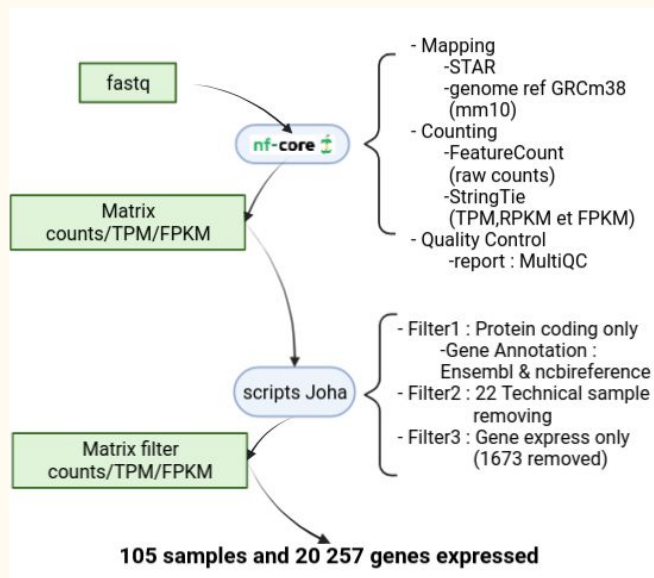


Cell sequenced	D0	D2	D4	D7
ECs				
FAPs				
Mac-M1				
Mac-M2				
Neutrophils				
MuSc				

# Pretreatment RNA-seq data



# Pretreatment RNA-seq data



# Natmi zoom

Network Analysis Toolkit for Multicellular Interaction (NATMI) *Hou, R., Denisenko, E., Ong, H.T. et al. Predicting cell-to-cell communication networks using NATMI. Nat Commun 11, 5011 (2020).*

→ Develop for single cell sequencing

Adaptation for bulk RNA sequencing :

- Considerate one replicate like one cell

Software base on:

- Expression table with TPM value
- Database Ligand Receptor ConnectomeDB2020

→ Interaction relevant if the receptor and the ligand expressed  $\geq 0.6$  ratio cell expression

# Natmi zoom

## Results informations:

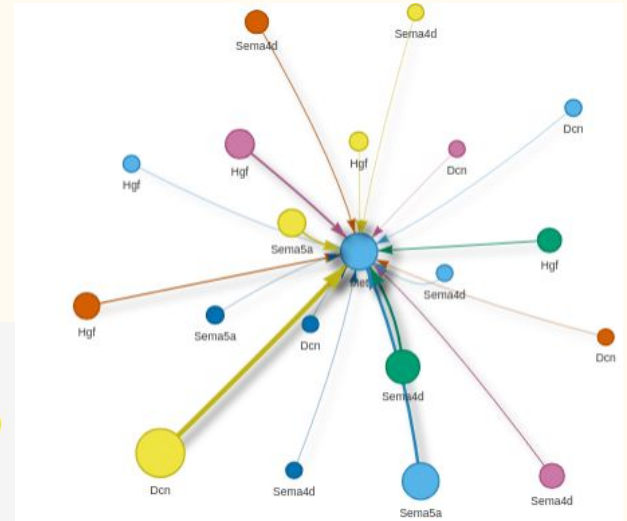
- For receptor and ligand:
  - Type cell
  - Symbol gene
  - Detection rate or percent of replicate with expression
- For receptor, ligand and edge (product ligand receptor value):
  - Average expression value - meanTPM
  - Derived specificity of average expression value

## Exemple representation of results:

→ All interaction in D2 for young expression with Met in satellite cell

Cell types:

- Antiinflammatory Macrophages (M2)
- Endothelial cells (ECs)
- Fibroadipogenic Progenitors (FAPs)
- Neutrophils (Neutro)
- Proinflammatory Macrophages (M1)
- Satellite cells/MuSC (sCs)





# Collaboration :

## **Penney Gilbert team**

### Objective:

- Discovery satellite cells modulators
  - Focus on
    - Boosting in self-renewal states
    - Boosting in quiescence states
  - Factors that we could 'add into' the system easily (ECM, GFs, cytokines, etc)
- Receptor doesn't change much, and then suddenly a ligand becomes accessible

# Collaboration :

## Penney Gilbert team

### Objective:

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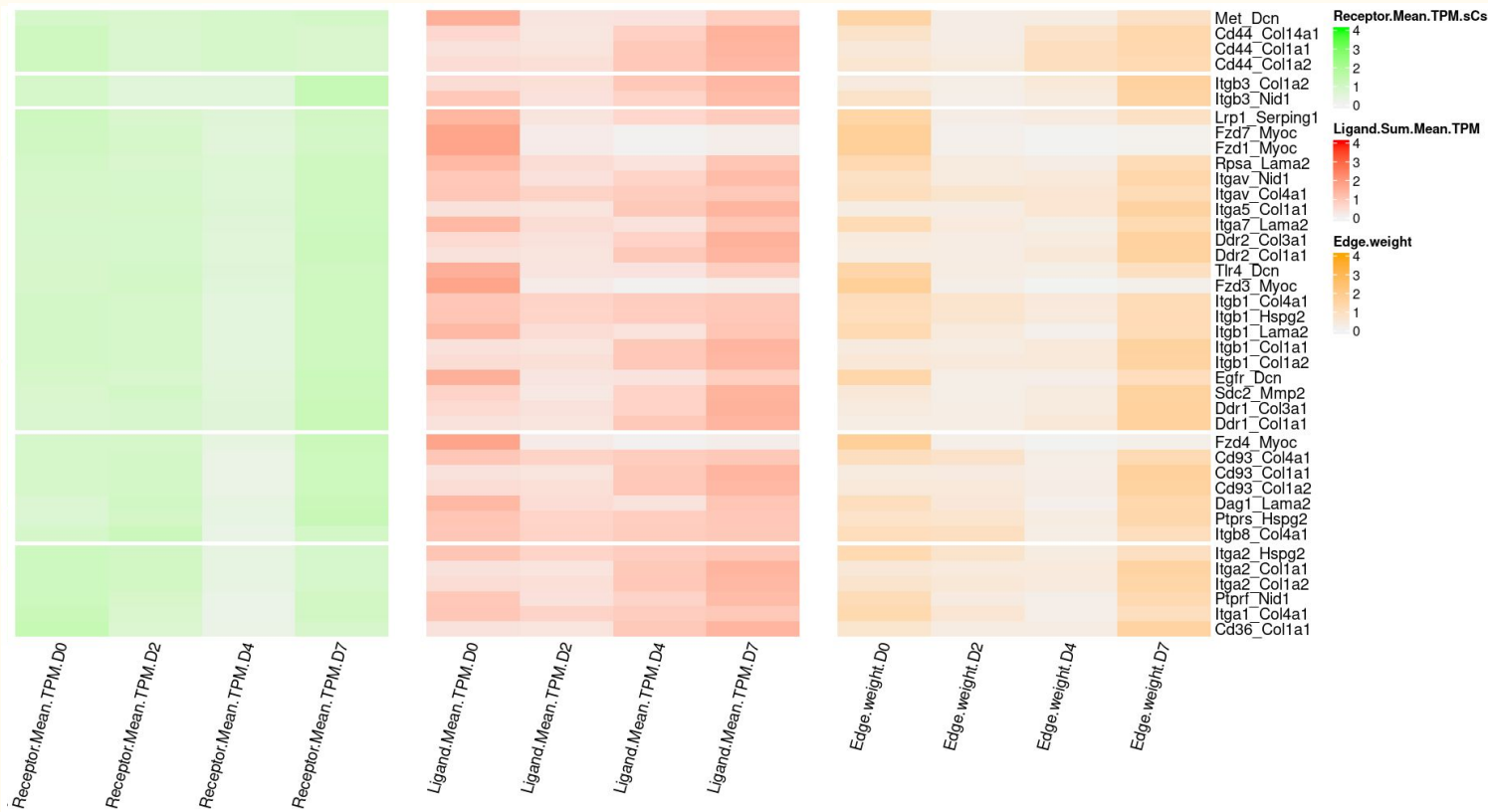
## Le Grand team - Bioinformatique point of view

### Analyse Data

- Focus on Young RNA-seq data
- Explore Natmi result - Receptor-Ligand results
- Focus on
  - Receptor expressed by satellite cells
  - Receptor expressed all days and replicats
  - Ligand expressed by all type cells
- Split analysis on two:
  - 'n' for "boosting in self-renewal states" :
    - Expression  $D0 < D2$
    - Expression  $D7 < D4$
  - 'U' for "boosting in quiescence states"
    - Expression  $D0 > D2$
    - Expression  $D7 > D4$

# Top 40 candidates receptor-ligand

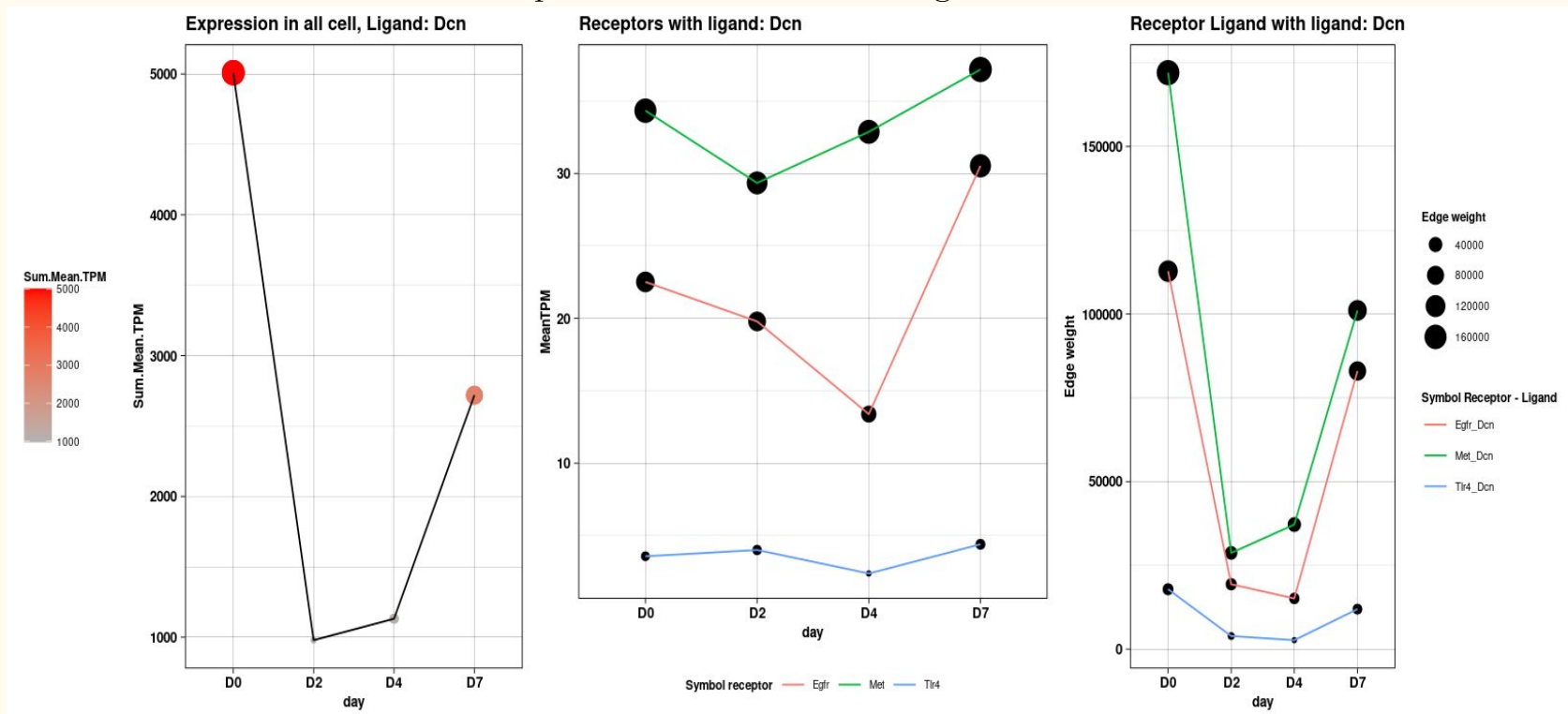
## Boosting in quiescence states



# Candidates receptor-ligand

Boosting in quiescent state: **zoom couple Met\_Dcn**

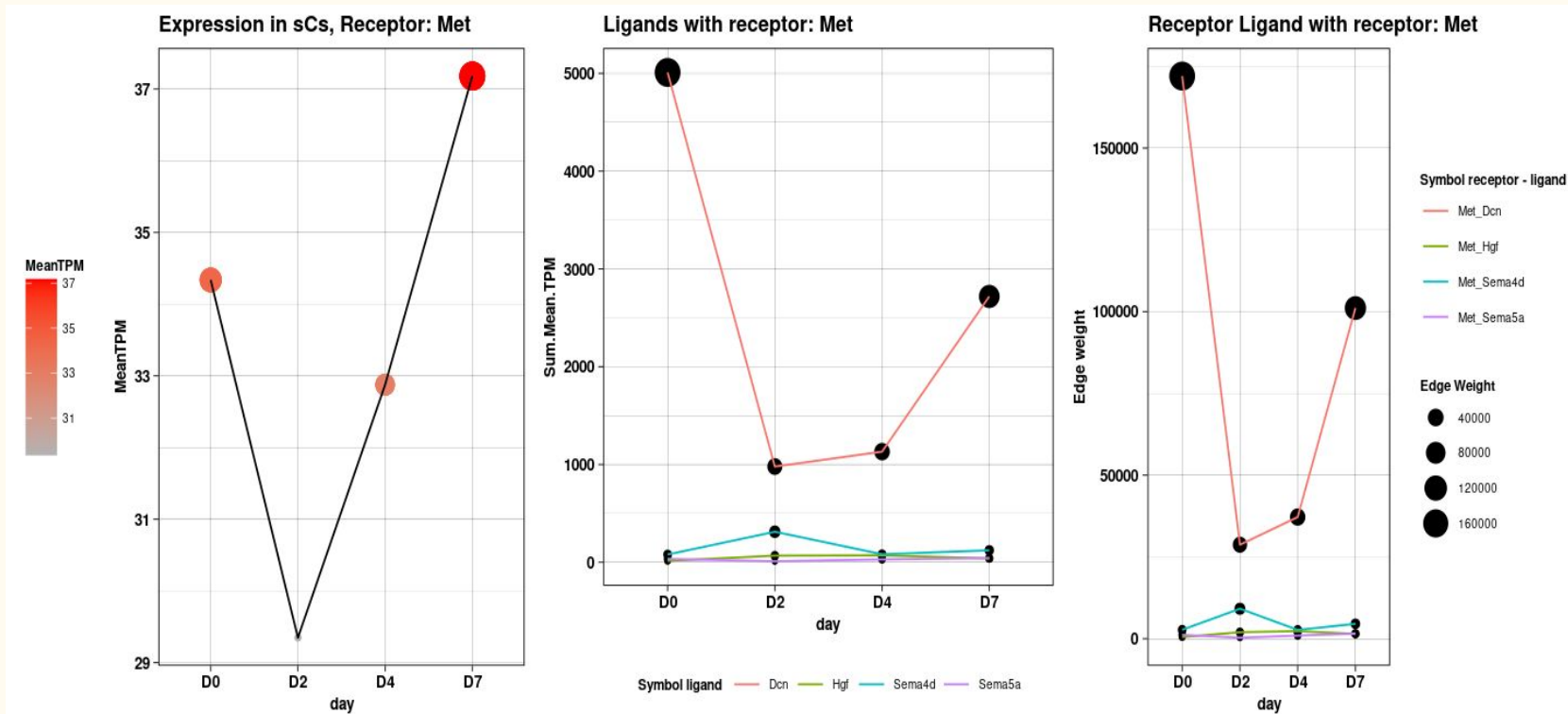
Explore all interaction with ligand Dcn



# Candidates receptor-ligand

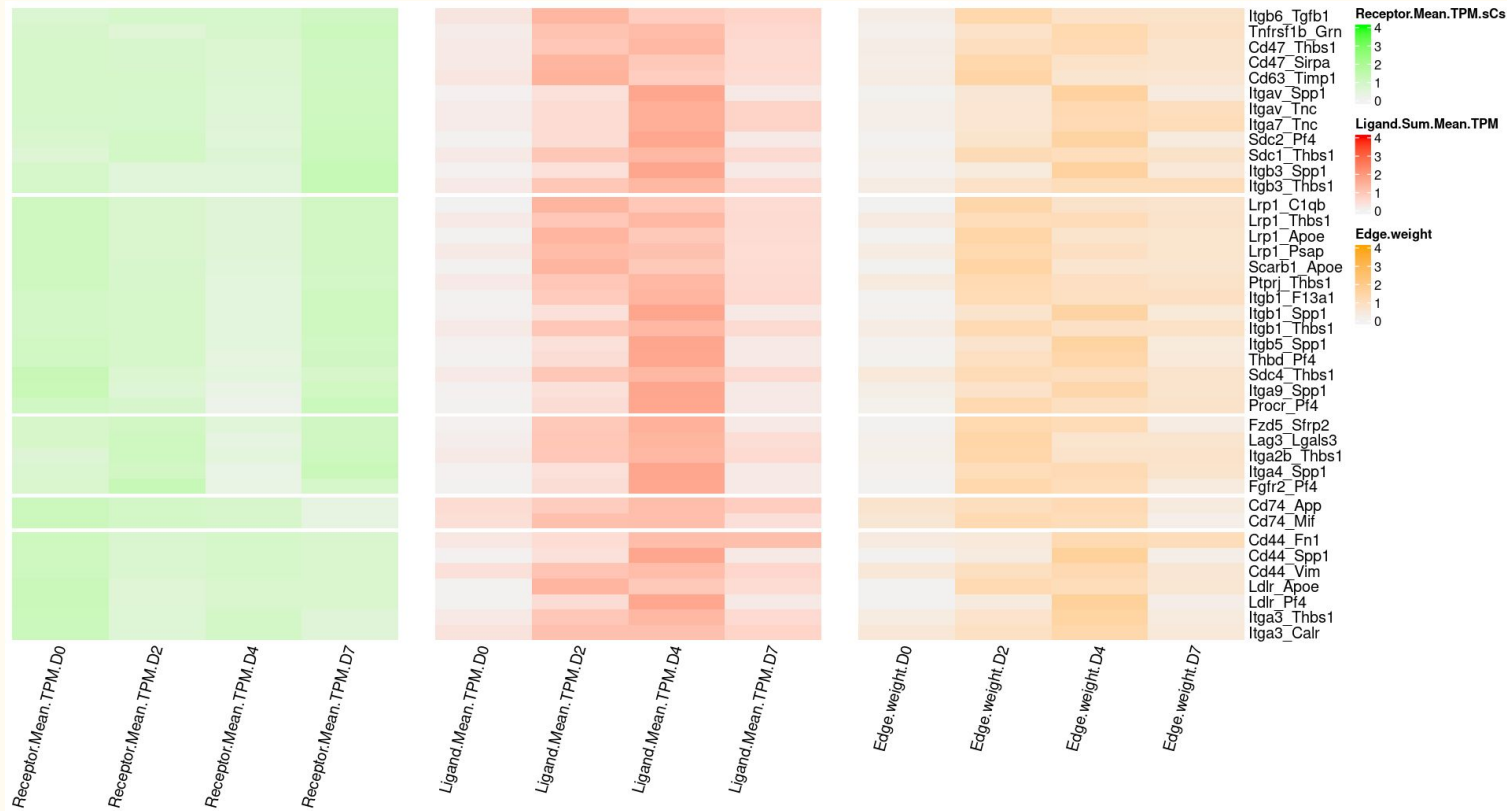
Boosting in quiescent state: **zoom couple Met\_Dcn**

Explore all interaction with receptor Met



# Top 40 candidates receptor-ligand

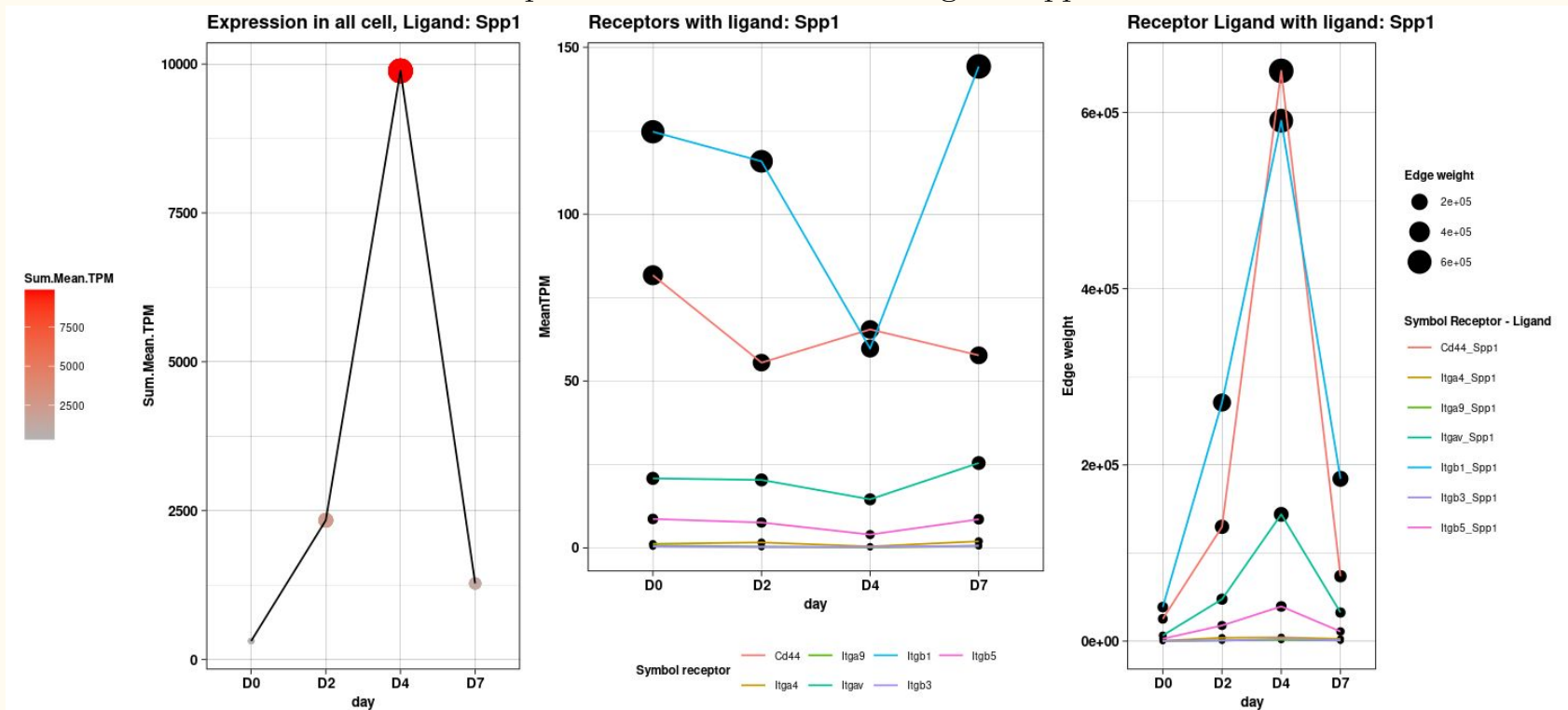
## Boosting in self-renewal states



# Candidates receptor-ligand

Boosting in self-renewal states : zoom couple Cd44\_Spp1

Explore all interaction with migand Spp1

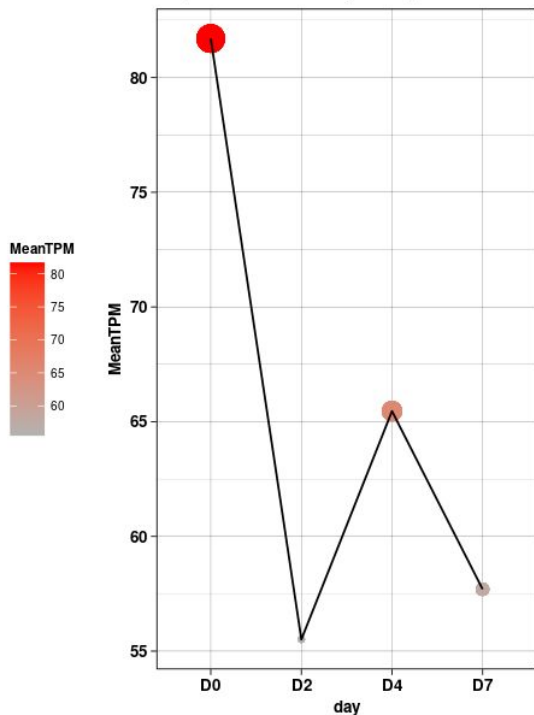


# Candidates receptor-ligand

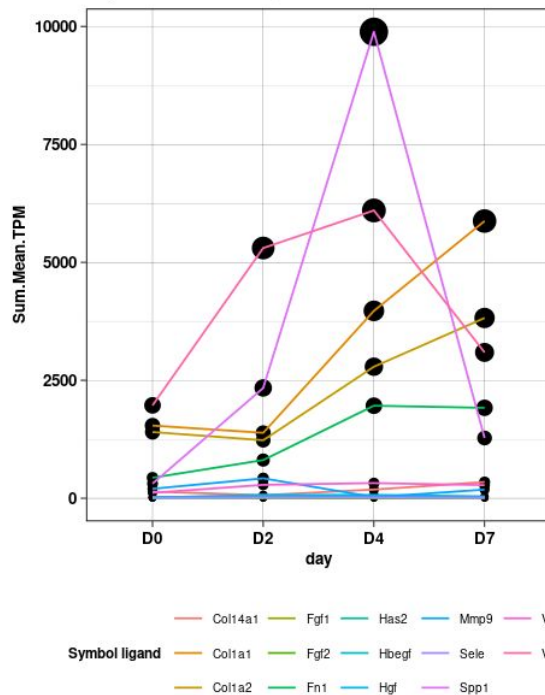
Boosting in self-renewal states : zoom couple Cd44\_Spp1

Explore all interaction with receptor Cd44

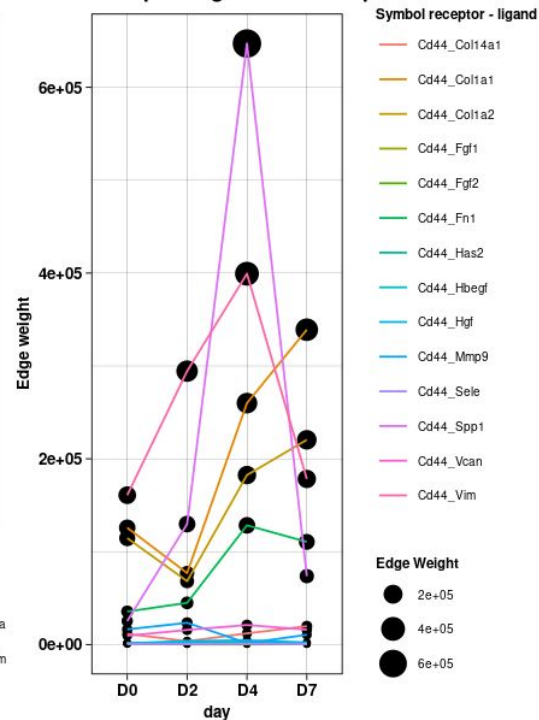
Expression in sCs, Receptor: Cd44



Ligands with receptor: Cd44



Receptor Ligand with receptor: Cd44





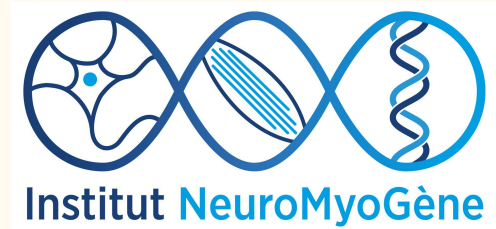
# RNA-seq analysis of 6 cell types in regeneration muscle tissue between young and old mice

Partenariat des équipes de:

- Dr. Fabien LE GRAND
- Dr. Bénédicte CHAZAUD
- Dr. Jérôme FEIGE

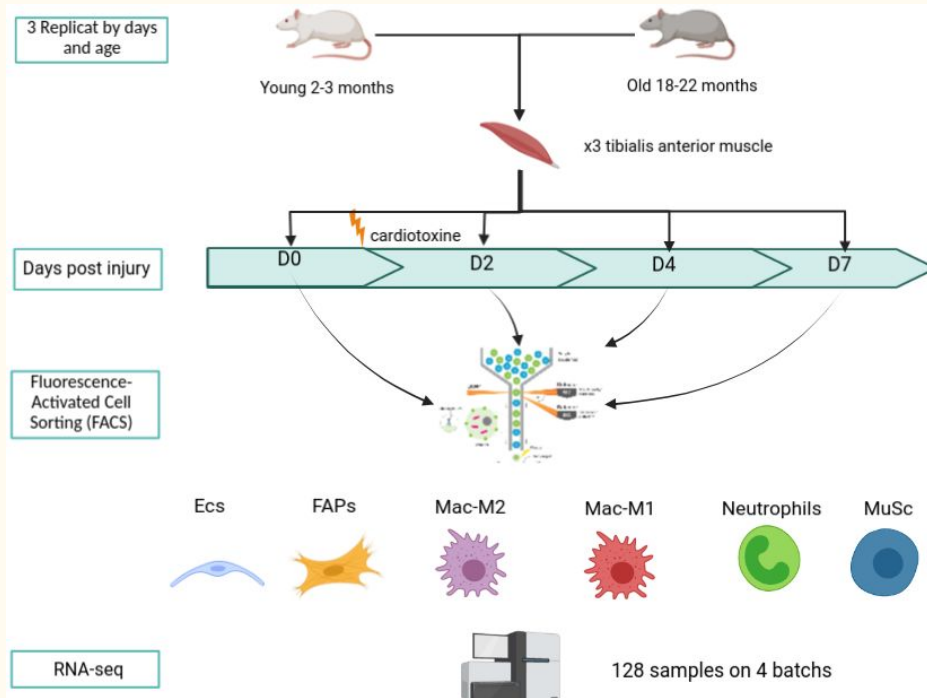
Contributeurs Principaux:

- PhD Hoang DIEU-HUONG
- IE Johanna GALVIS-LACROUX
- IE William JARASSIER
- IE Pauline MOULLÉ



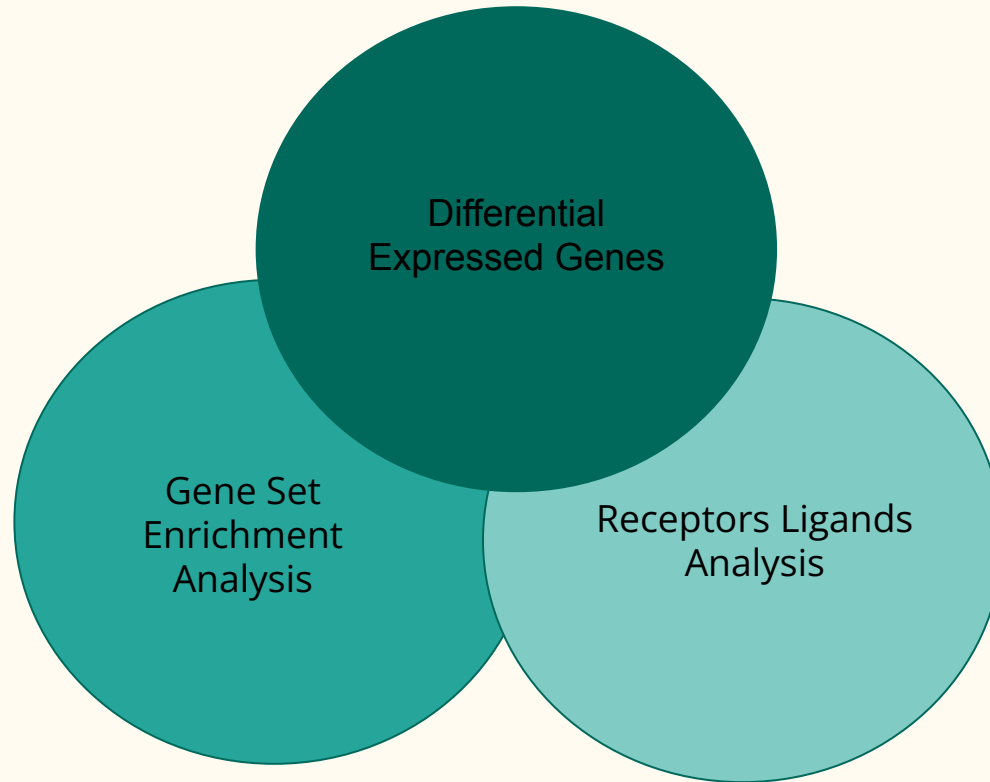
# Objective analysis - experimental design

Found difference between expression Old and Young mice in regeneration muscle tissue.



Cell sequenced	D0	D2	D4	D7
ECs				
FAPs				
Mac-M1				
Mac-M2				
Neutrophils				
MuSc				

# Steps of analyse



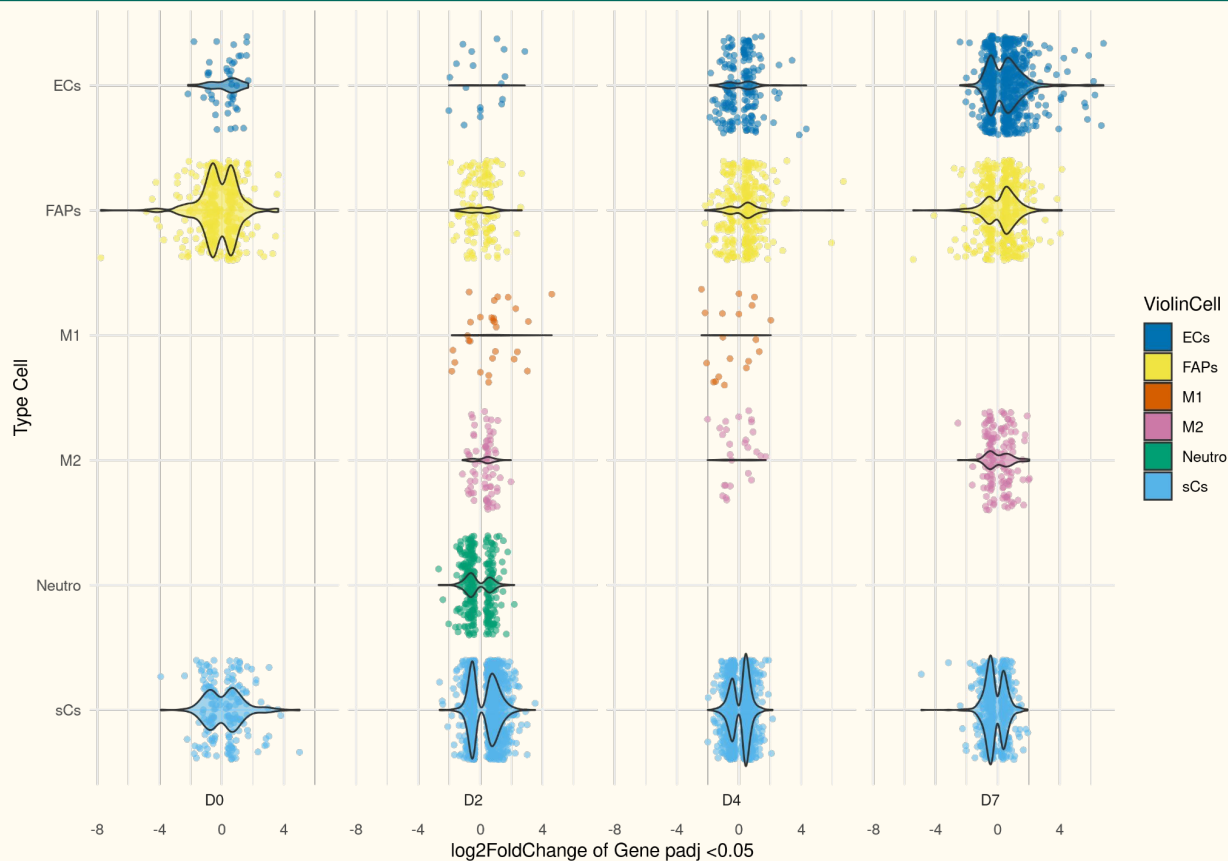
# Steps of analyse

1



- On gene with on less 3 samples with raw count  $\geq 5$  (2 460 gene remove)
- DEG Old-Young for each 18 conditions TypeCell.DayPostInjury
- 25% (5902) genes are  $\text{padj} < 0.05$  on less one condition

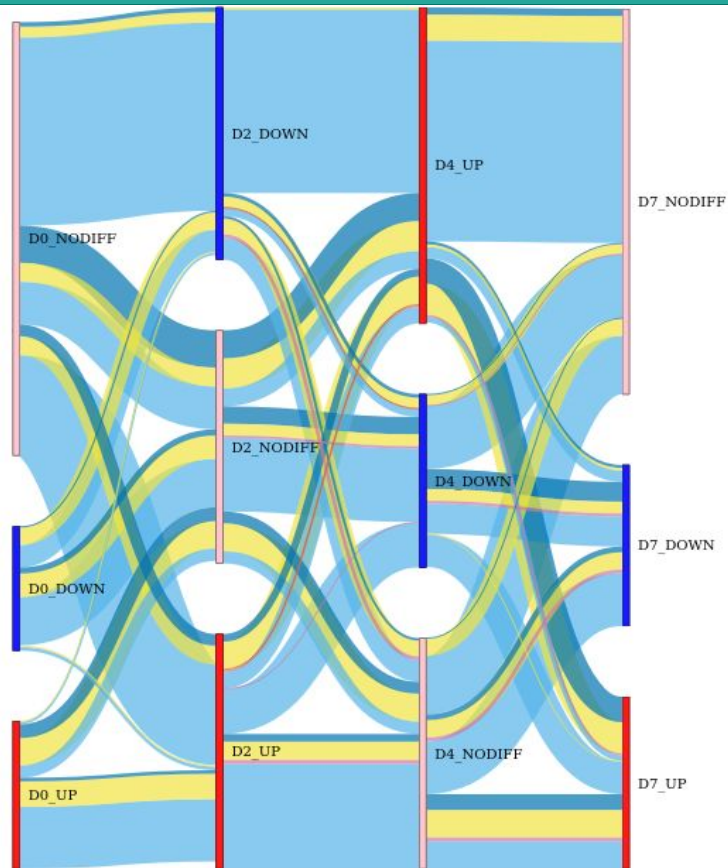
# 1 Differential Expressed Genes



Proportion of genes DEG signi

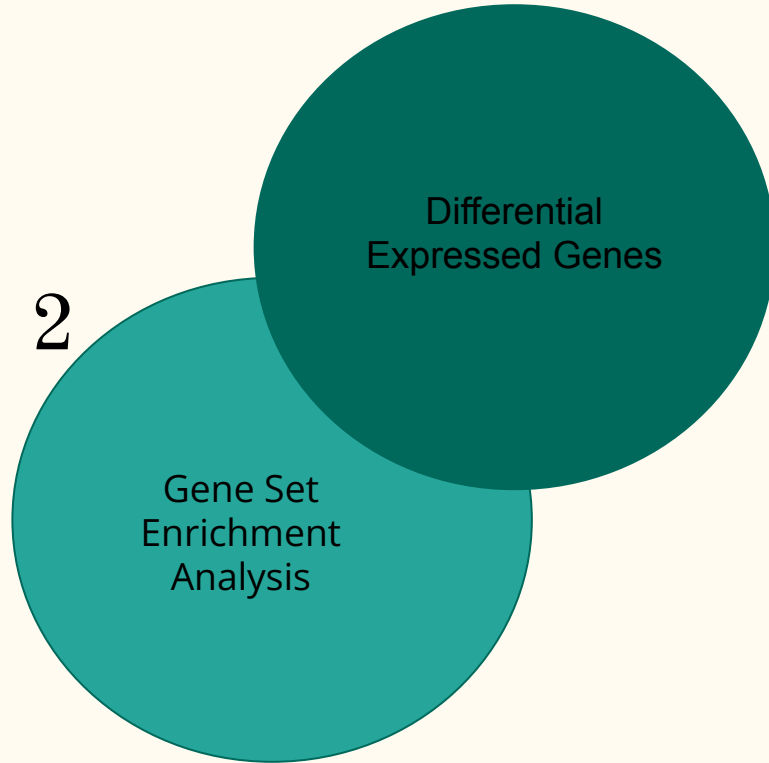
	ECs	FAPs	sCs	M1	M2	Neutro
D0	1.6	8.7	8.1	NA	NA	NA
<b>D2</b>	1.0	4.0	32.3	0.5	1.6	5.3
D4	4.7	6.6	23.4	0.3	0.6	NA
D7	10.4	7.0	10.1	NA	2.4	NA

# 1 Differential Expressed Genes



symbol	nbDa yDE	Dynamic	id	day	type	padj	log2Fo ldChan ge	meanCount Normalized Young	meanCou ntNormali zedOld
C4b	3	D2_UP.D4_ UP.D7_UP	ENSMUSG0000 0073418	D2	M2	2.2681428807 9248e-10	1.9075	67.5574	267.4453
C4b	3	D2_UP.D4_ UP.D7_UP	ENSMUSG0000 0073418	D4	M2	0.0003253618 3106648	0.963	317.4045	656.2569
C4b	3	D2_UP.D4_ UP.D7_UP	ENSMUSG0000 0073418	D7	M2	1.1287827149 5032e-15	1.3087	594.9828	1508.9978
C4b	2	D2_UP.D4_ UP	ENSMUSG0000 0073418	D2	M1	8.9056336779 2302e-8	1.9639	145.8828	604.7223
C4b	2	D2_UP.D4_ UP	ENSMUSG0000 0073418	D4	M1	0.0112502470 567319	0.8397	520.7154	1004.215
C4b	1	D2_UP	ENSMUSG0000 0073418	D2	Neutr o	0.0016811776 2706364	1.7635	42.3539	170.0887
C4b	1	D2_UP	ENSMUSG0000 0073418	D2	sCs	0.0000276347 552724158	1.5361	92.4862	293.1857
C4b	1	D4_UP	ENSMUSG0000 0073418	D4	FAPs	1.3517627461 7256e-9	0.9665	1399.785	2811.6533
C4b	1	D7_UP	ENSMUSG0000 0073418	D7	ECs	0.0029271505 22015 95	3.2702	1.9394	26.72

# Steps of analyse



→ Algorithme : fGEA use in R

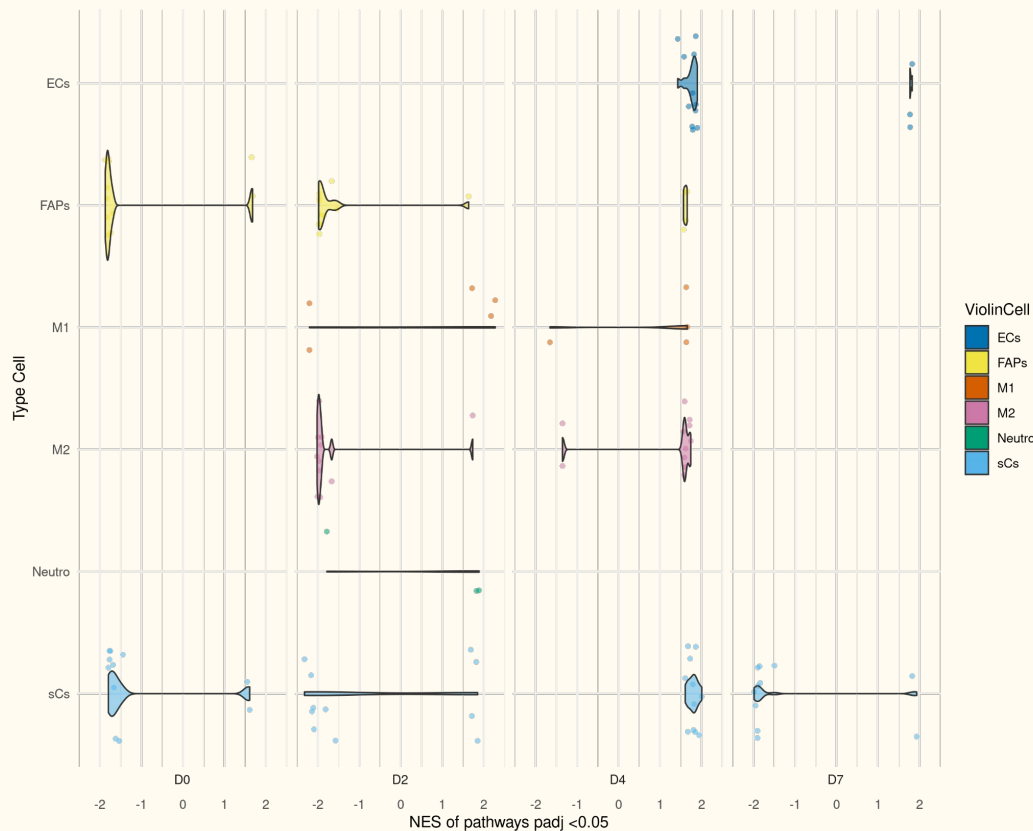
→ Database:

◆ Genes Set from Molecular Signatures Database (MSigDB) species *Mus musculus*

◆ category C2 subcategory = Canonical pathways  
Reactome database

- On each days and cellType
- On “all” genes
- Sort by signed of  $\log_2\text{FoldChange} * -\log_{10}(\text{padj})$
- Approx 3 000 genes with  $\text{padj} = \text{NA}$

# 2 Gene Set Enrichment Analysis

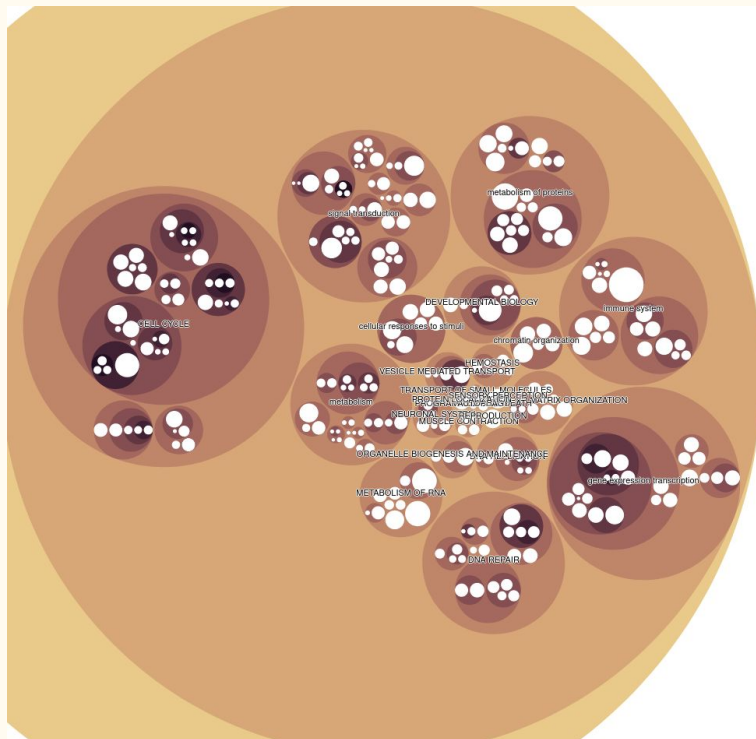


Proportion of pathway DE signi

	ECs	FAPs	sCs	M1	M2	Neutro
D0	0	71	72	NA	NA	NA
D2	0	78	384	5	11	3
D4	115	4	325	4	18	NA
D7	3	0	20	NA	0	NA



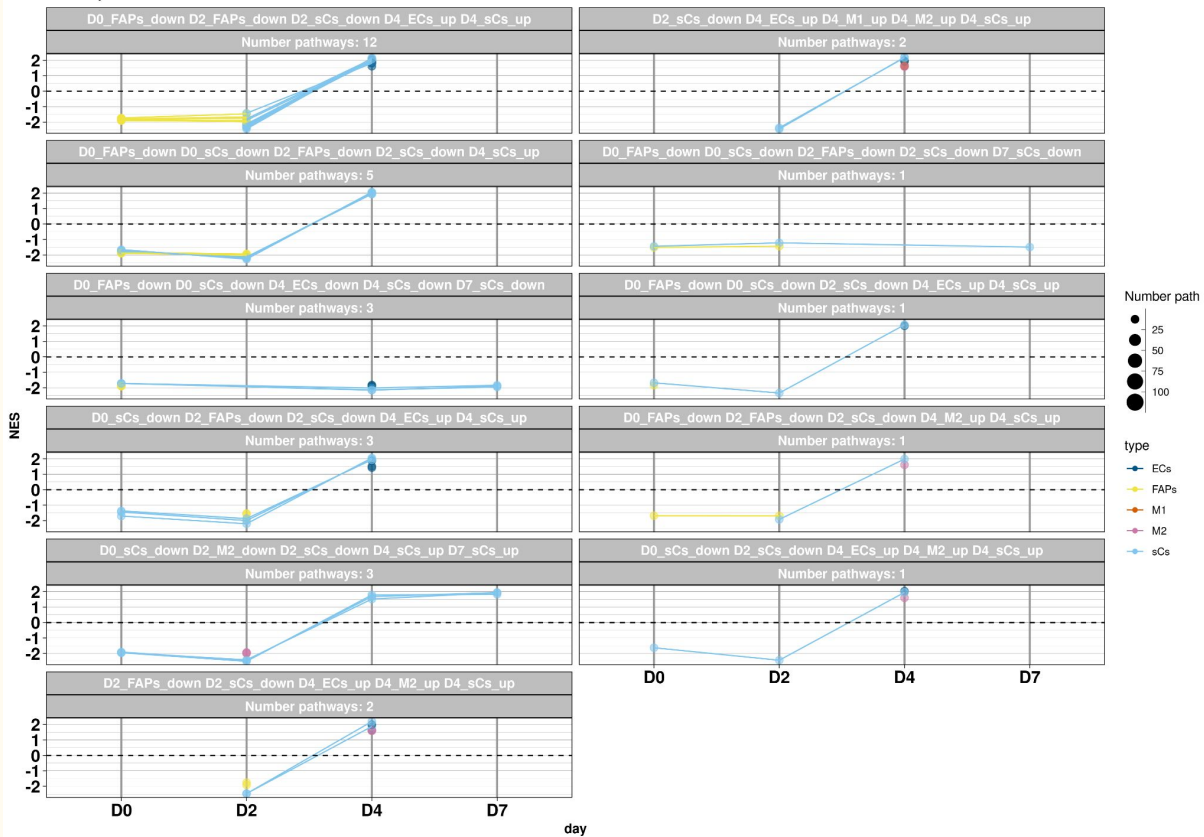
# 2 Gene Set Enrichment Analysis



AUTOPHAGY						
IN REACTOME HIERARCHIE	AUTOPHAGY	MACROAUTOPHAGY	SELECTIVE AUTOPHAGY	AGGREPHAGY		
CELL CYCLE						
IN REACTOME HIERARCHIE	CELL CYCLE	CELL CYCLE CHECKPOINTS	G1 S DNA DAMAGE CHECKPOINTS	P53 DEPENDENT G1 S DNA DAMAGE CHECKPOINT	P53 DEPENDENT G1 DNA DAMAGE RESPONSE	STABILIZATION OF P53
IN REACTOME HIERARCHIE	CELL CYCLE	CELL CYCLE CHECKPOINTS	G2 M CHECKPOINTS	ACTIVATION OF ATR IN RESPONSE TO REPLICATION STRESS		
IN REACTOME HIERARCHIE	CELL CYCLE	CELL CYCLE CHECKPOINTS	G2 M CHECKPOINTS	G2 M DNA DAMAGE CHECKPOINT		

## 2 Gene Set Enrichment Analysis

### Pathways with 5 conditions enriched

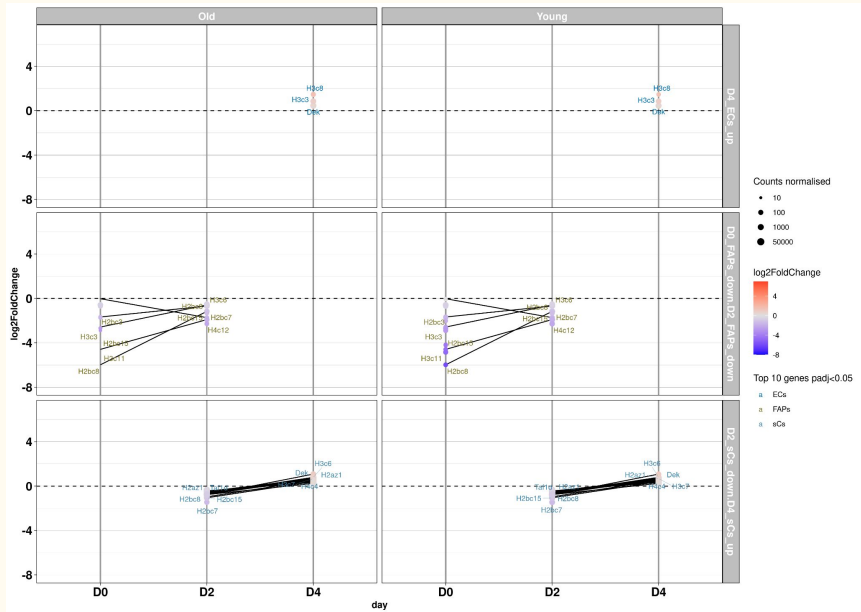
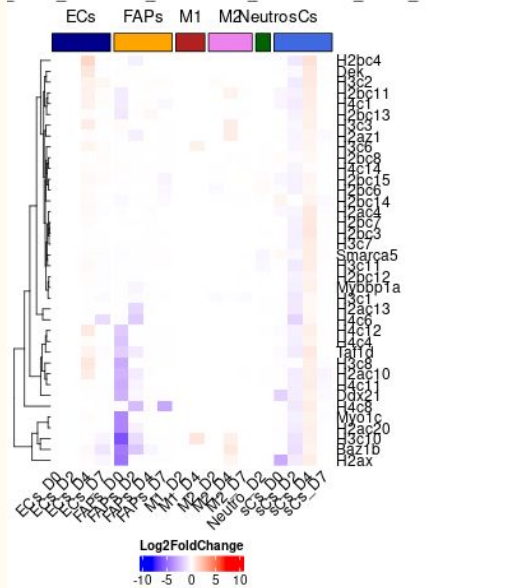


Numbers conditions enrichis	1	2	3	4
Numbers pathways	254	139	46	28

Numbers conditions enrichis	5	6	7	8
Numbers pathways	34	22	3	1

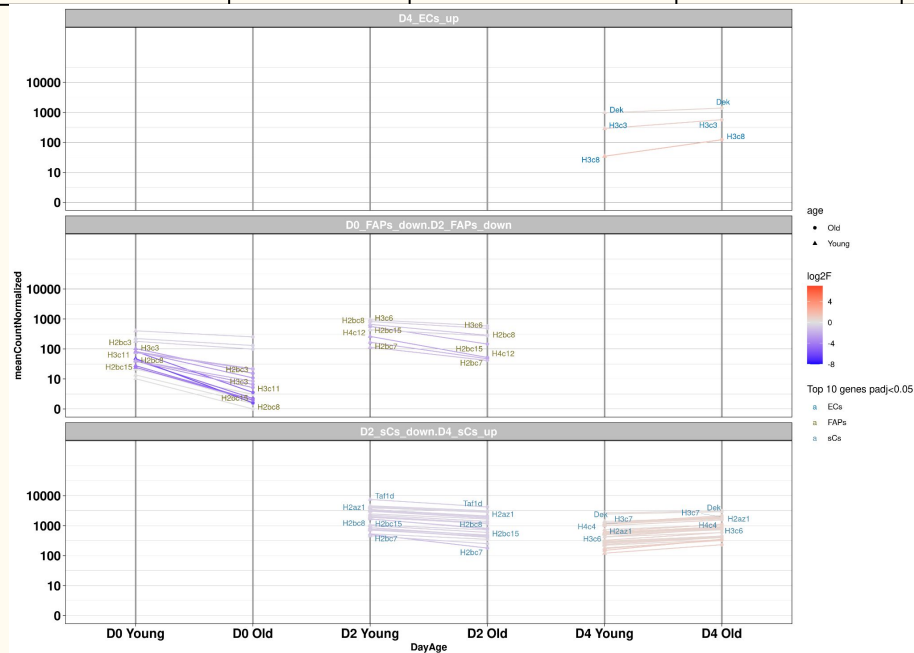
## 2 Gene Set Enrichment Analysis

CondSigni	NamePathway	link	DayTypeSens	padj	NES	NbPathWithDynamique
D0_FAPs_down D2_FAPs_down D2_sCs_down D4_EC_s_up D4_sCs_up	B WICH COMPLEX POSITIVELY REGULATES RRNA EXPRESSION	FigureSupp	D0_FAPs_down	0.00534289344241228	-1.83663650472637	12

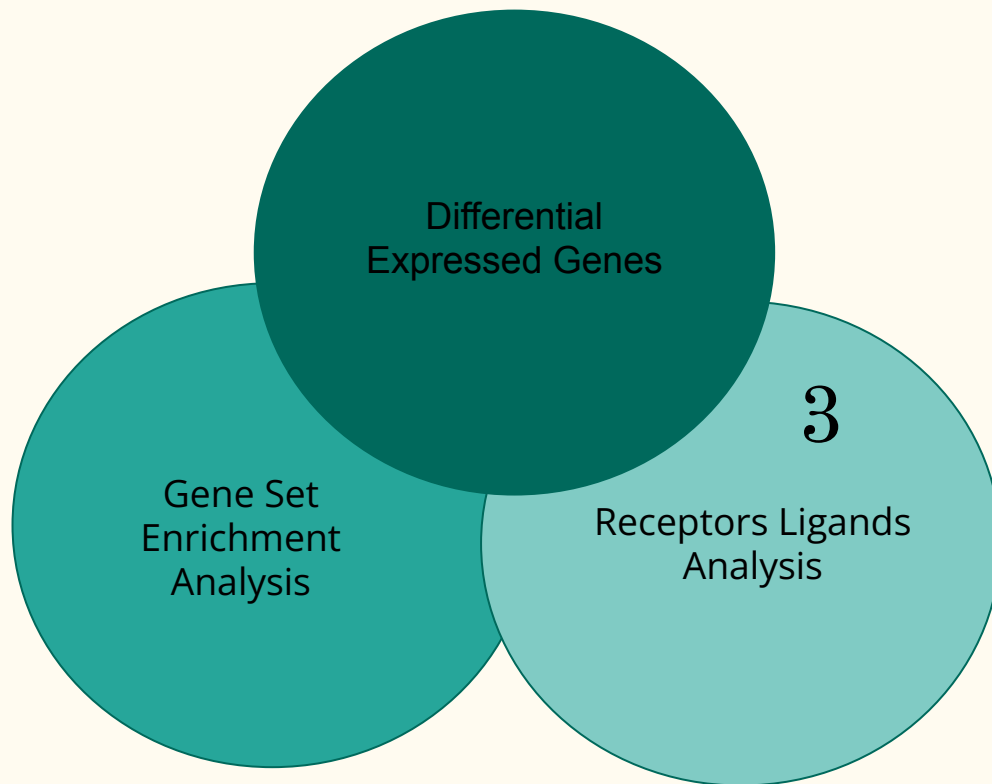


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CondSigni	NamePathway	link	DayTypeSens	padj	NES	NbPathWithDynamique
D0_FAPs_down D2_FAPs_down D2_sCs_down D4_EC_s_up D4_sCs_up	B WICH COMPLEX POSITIVELY REGULATES RRNA EXPRESSION	FigureSupp	D0_FAPs_down	0.00534289344241228	-1.83663650472637	12



# Steps of analyse



## Network Analysis Toolkit for Multicellular Interaction (NATMI)

Hou, R., Denisenko, E., Ong, H.T. et al. Predicting cell-to-cell communication networks using NATMI. *Nat Commun* 11, 5011 (2020).

→ Develop for single cell sequencing

Adaptation for bulk RNA sequencing :

- Considerate one replicate like one cell

Software base on:

- Expression table with raw Count  
Normalised value
- Database Ligand Receptor  
Connectome DB2020

# 3 Receptors ligands analysis

Available information by couple receptor ligand:

- Type Receptor
  - DEG info: padj,log2fodchange
  - Expression Young Old
  - Detection rate
- Day
- Type Ligand
  - DEG info: padj,log2fodchange
  - Expression Young Old
  - Detection rate
- Edge Infos
  - Expression weight
- Delta edge young old
  - Log2foldchange

- Simplification : groupe type cell → Receptor or Ligand DEG at less in one condition
- Try to curate receptor ligand with comparaison expression Ligand associated to a receptor