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Contribution des cellules souches à la croissance musculaire chez le porc

Frederic Dessauge

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15 Juin
2023

Contribution des cellules souches à la croissance musculaire chez le porc

DESSAUGE Frédéric

INRAE

UMR-1348

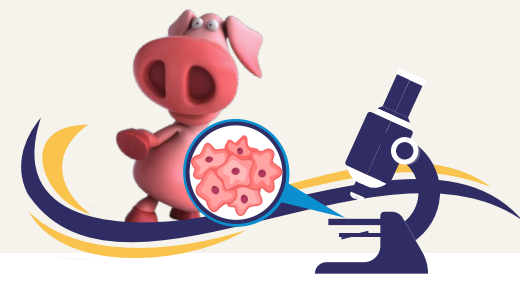
PHYSIOLOGIE, ENVIRONNEMENT
ET GÉNÉTIQUE POUR L'ANIMAL
ET LES SYSTÈMES D'ÉLEVAGE (PEGASE)

**L'INSTITUT
agro** Rennes
Angers

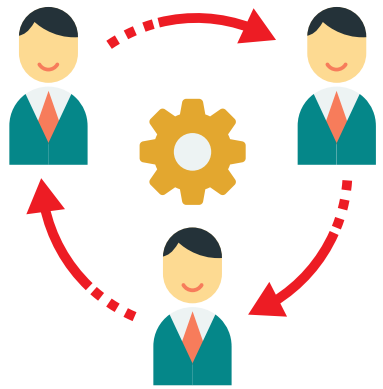
Journées Muscle et Qualités / 15-16 juin 2023



Présentation



L'UMR PEGASE en chiffres



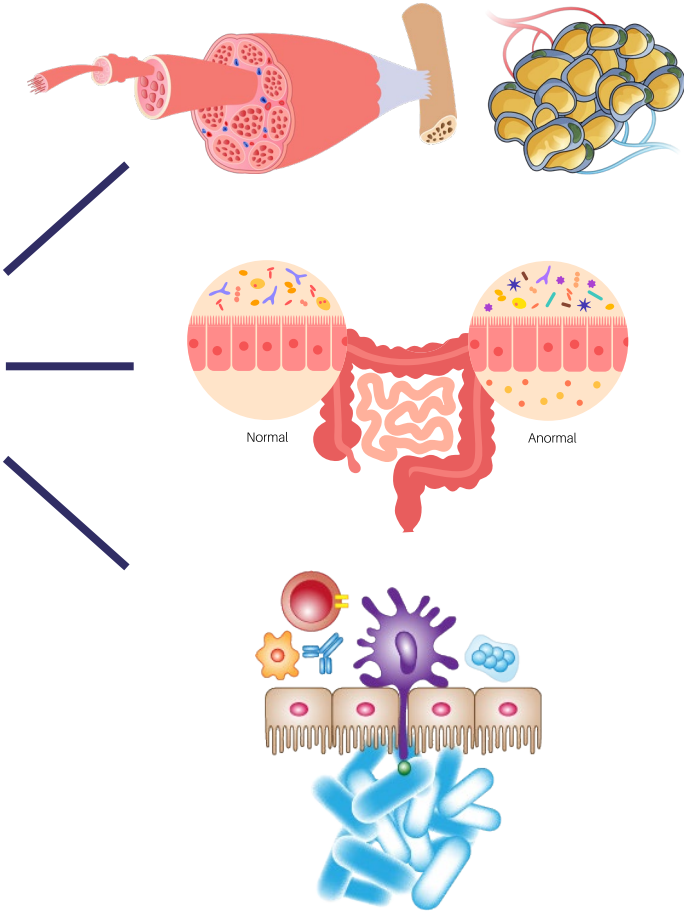
120
collaborateurs



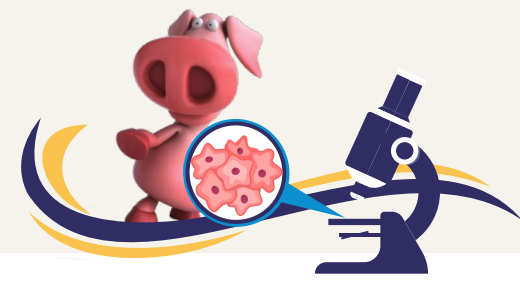
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Équipes de recherche



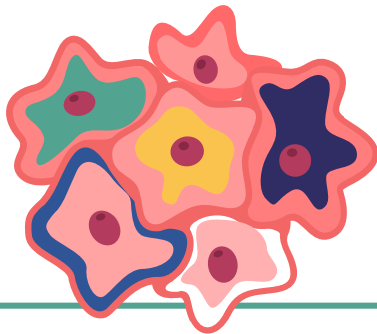
1
Equipe Biologie des
fonctions



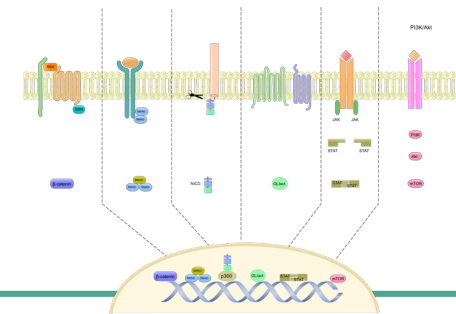
Objectifs de recherche



Décrire comment des mécanismes biologiques spécifiques contrôlent la croissance et le développement des tissus musculaire et adipeux et contribuent à terme à la construction des qualités de la viande.

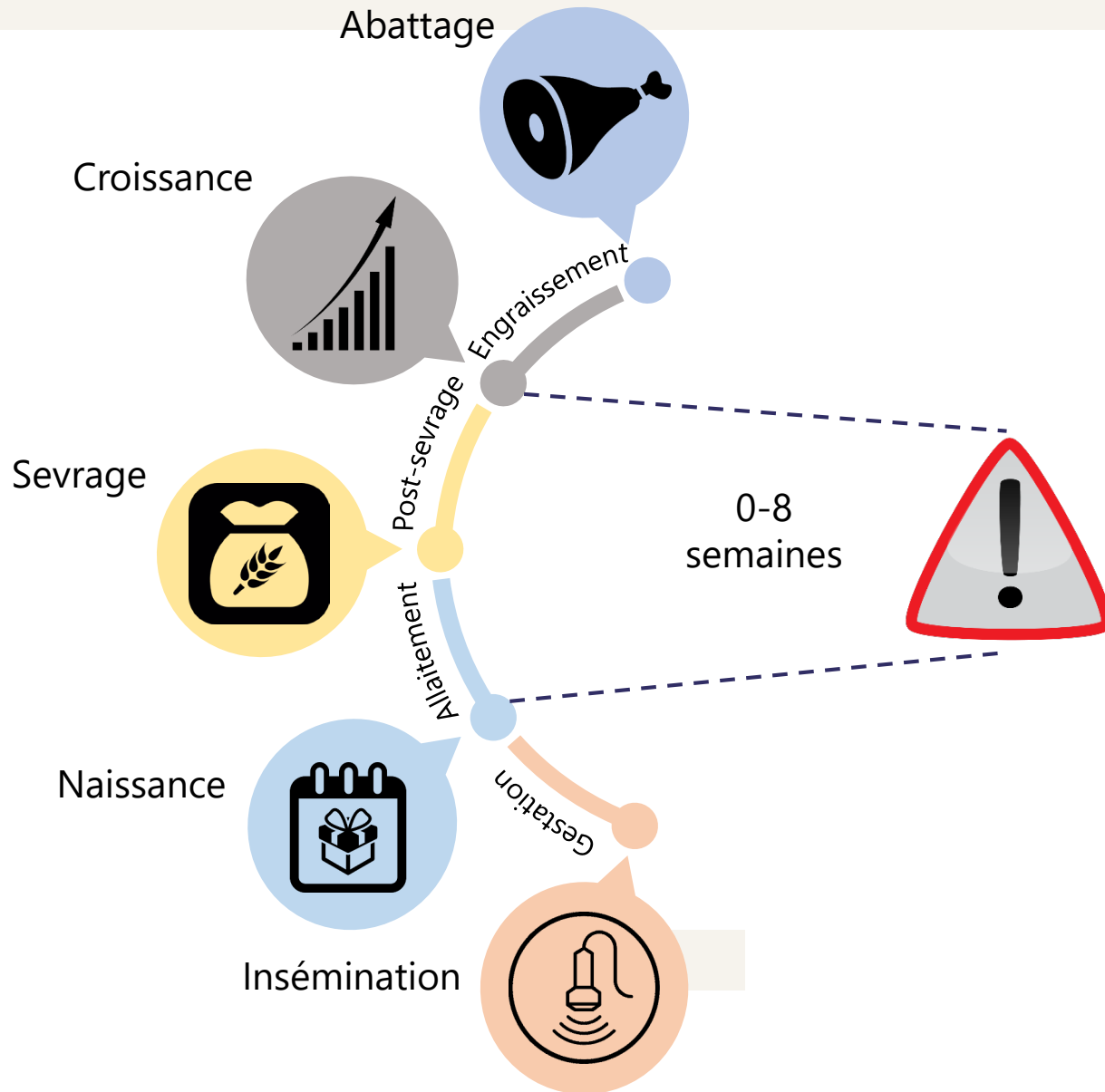
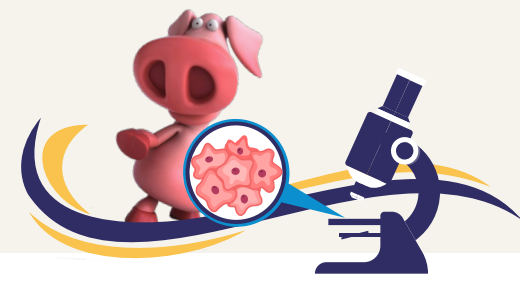


Exploration de la diversité cellulaire au cours des premières semaines de vie



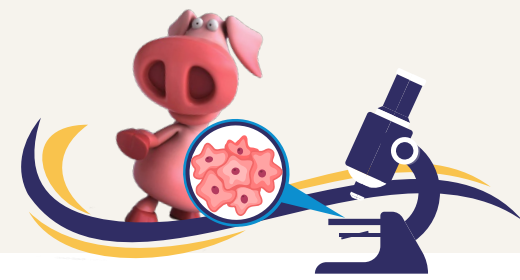
Dynamique des voies moléculaires et cellulaires contrôlant les dépôts de masse maigre et grasse

Contexte



Période critique pour l'élevage du porc

Le porcelet, un animal fragile



ALIMENTATION

- phase lactée
- sevrage

ENVIRONNEMENT

- température
- pathogènes
- exercice physique



GENETIQUE

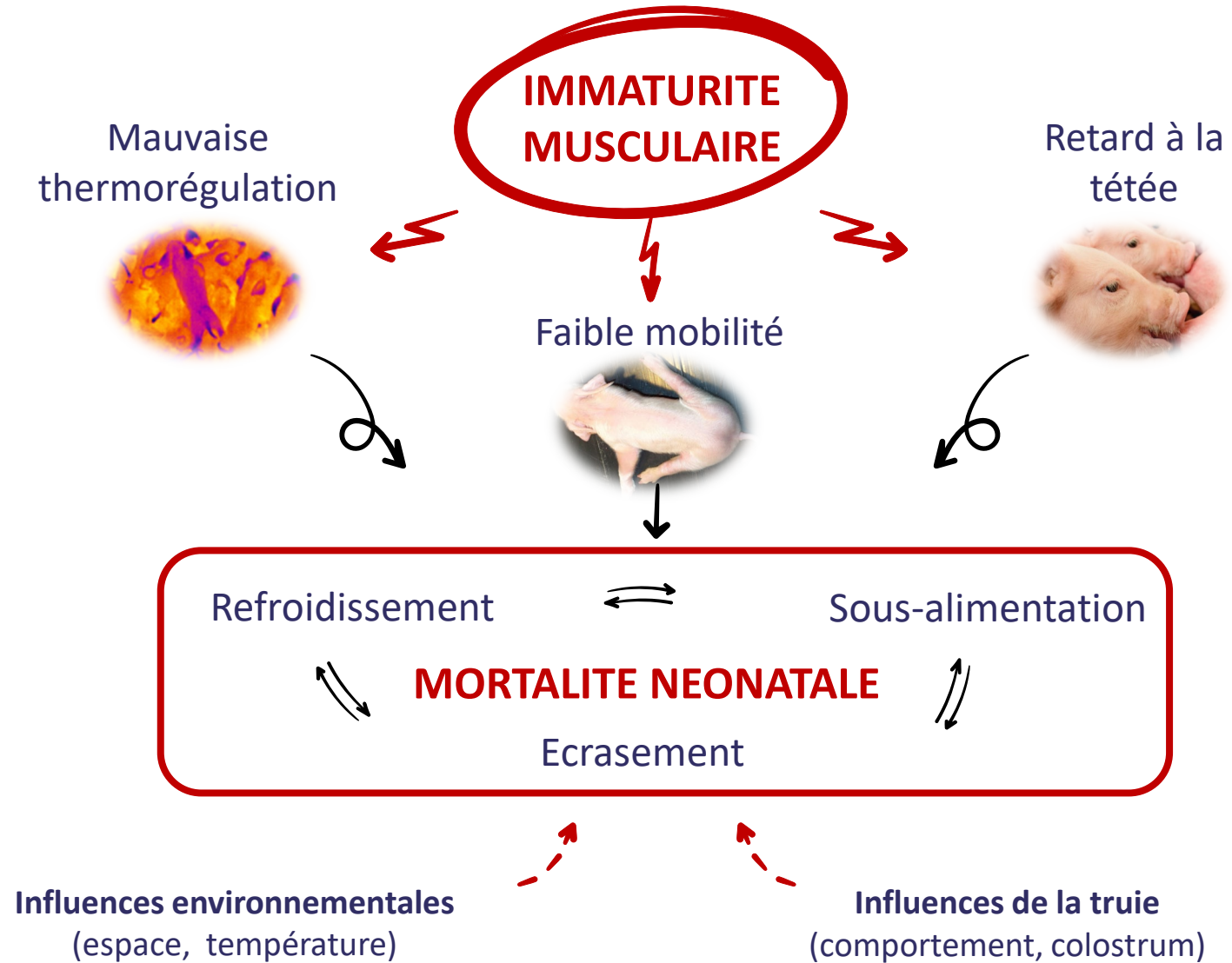
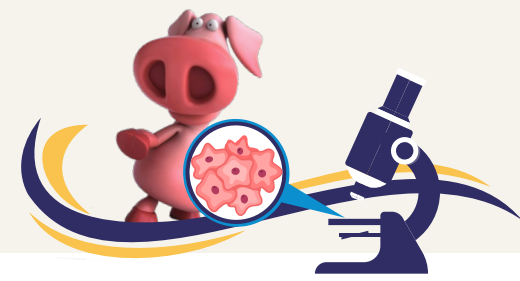
- races
- lignées

POIDS VIF A LA NAISSANCE

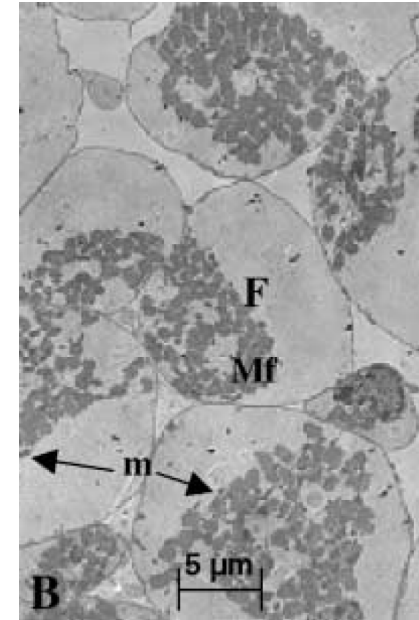
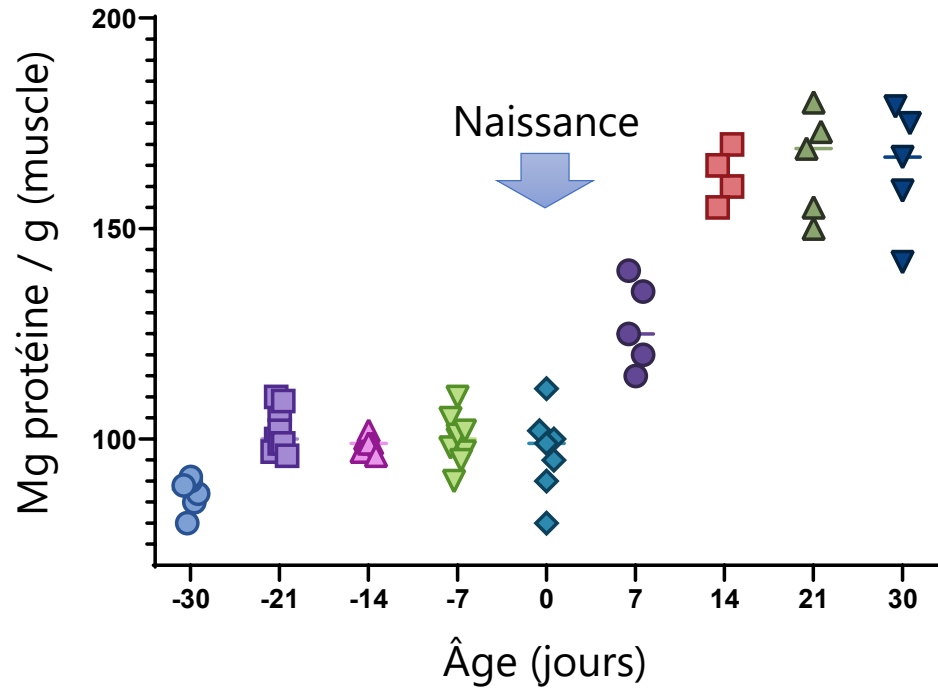
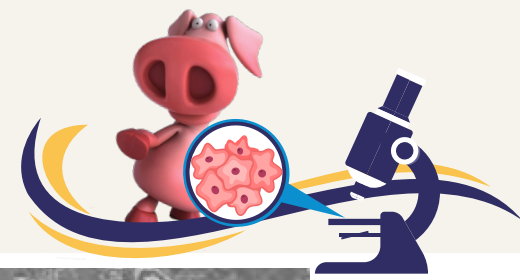
- variabilité
- (im)maturité
- survie

Vitalité néo-natale

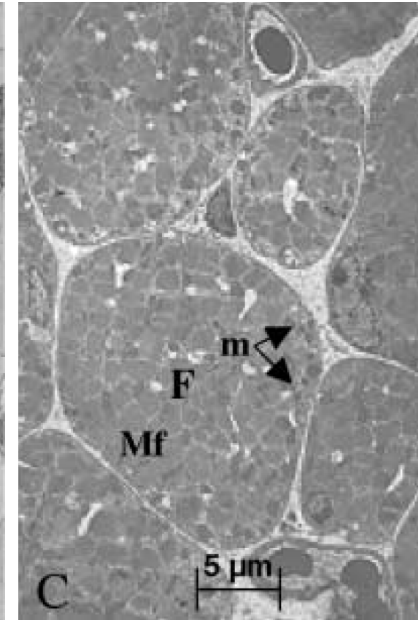
Fonction musculaire et survie néo-natale



Croissance et maturation musculaire chez le porc



Naissance



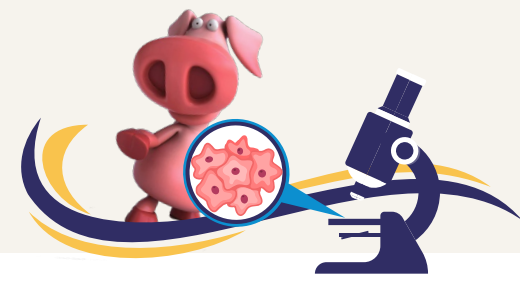
7 jours

Forte synthèse protéique après la naissance

Forte maturation contractile et Métabolique après la naissance

Maturité musculaire déterminante pour la survie

Développement précoce du tissu musculaire



0j

1^{er} tiers gestation

- 1^{ère} génération de fibres
- Hyperplasie
- Différenciation

45j

2^{ème} tiers gestation

- 2^{ème} génération de fibres
- Hyperplasie
- Différenciation

90j

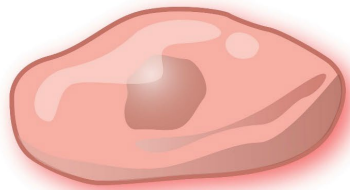
3^{ème} tiers gestation

- Nombre total de fibres fixé
- Hypertrophie

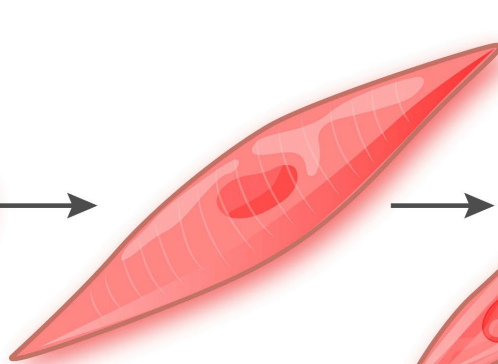
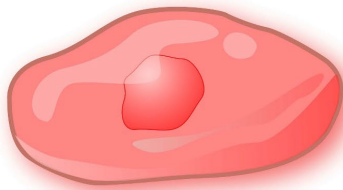
114j

Naissance

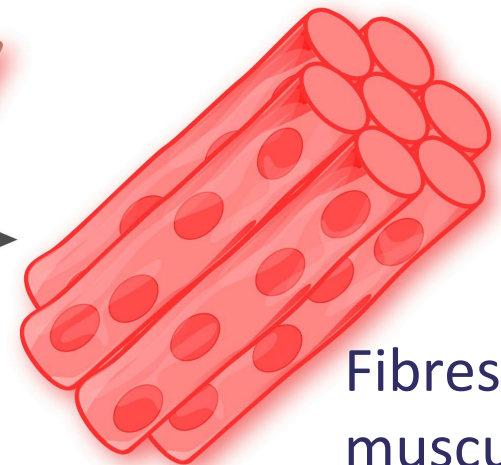
- 3^{ème} génération de fibres
- Hypertrophie



Cellule souche

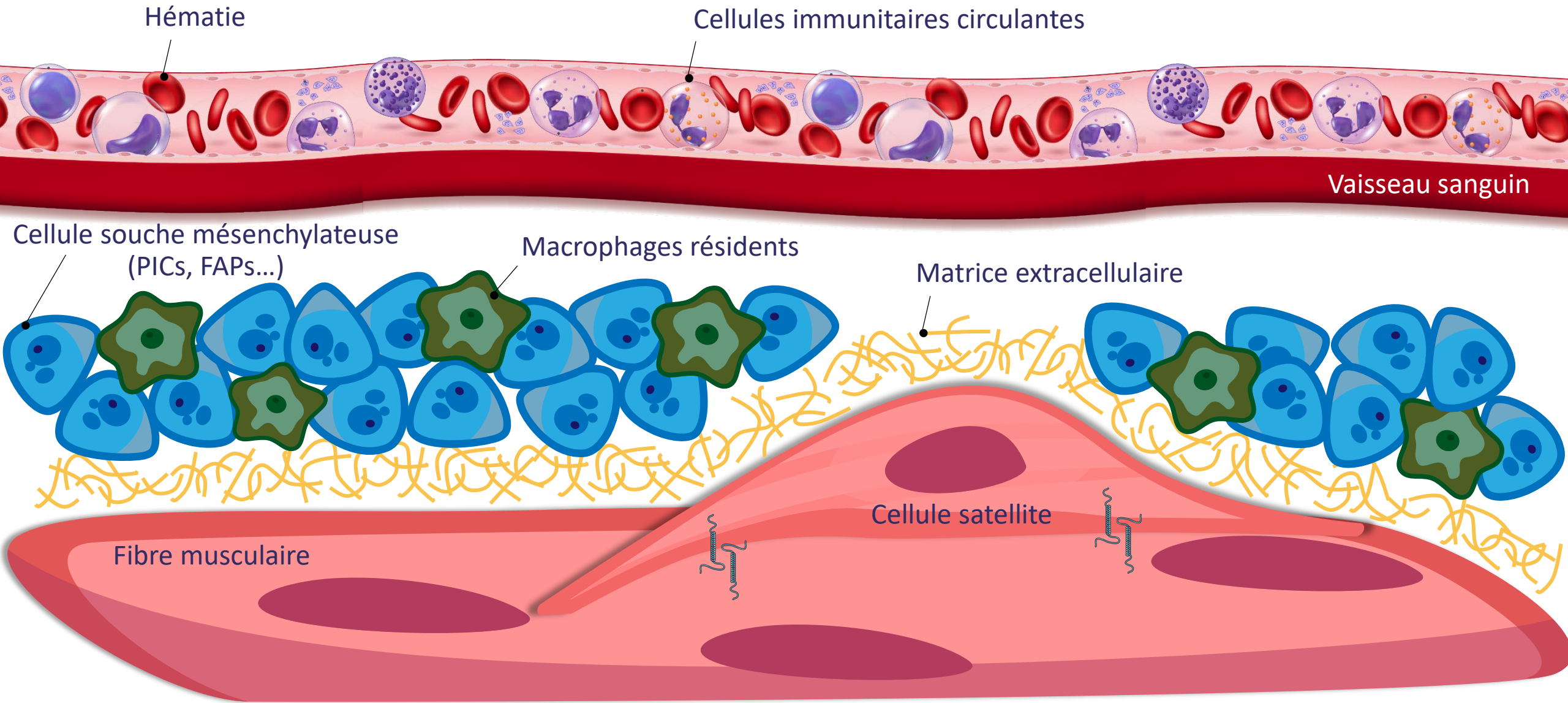
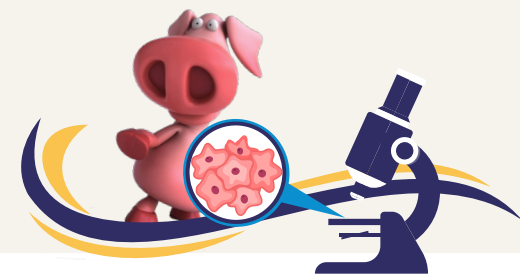


Myotube

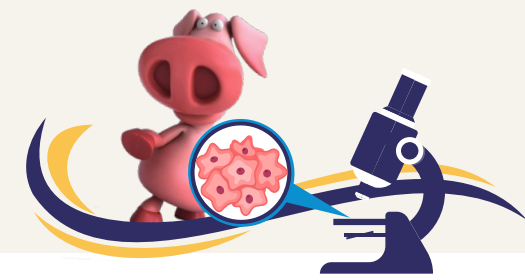


Fibres musculaires

Une diversité de cellules souches dans le muscle



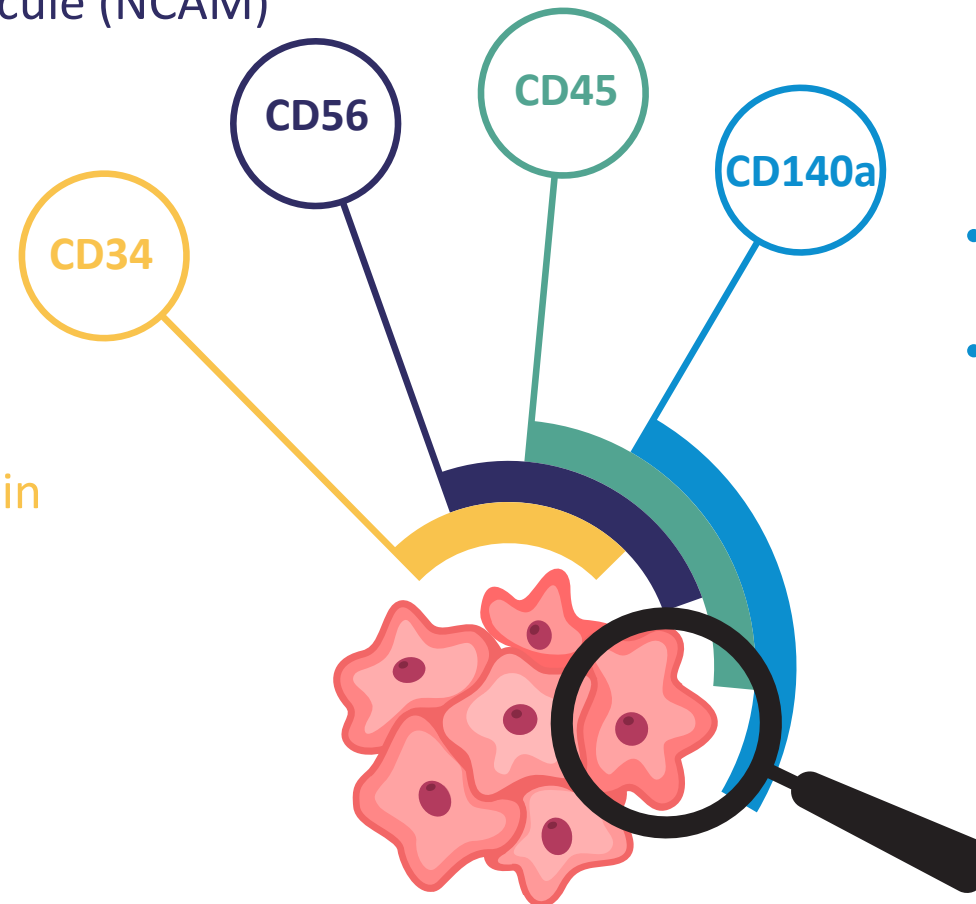
Des cellules souches, des marqueurs de surface



- Marqueur myogénique
- Neural Cell Adhesion Molecule (NCAM)

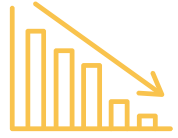
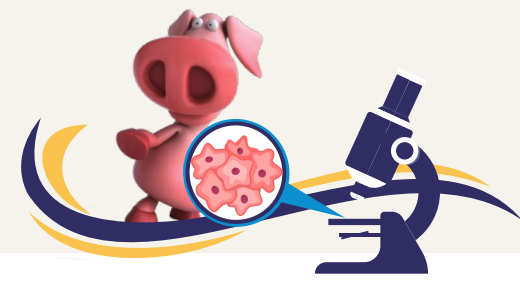
- Marqueur cellules hématopoïétiques
- Protein Tyrosine Phosphatase

- Marqueur cellules souches hématopoïétiques
- Phospho-Glycoprotein Protein



- Marqueur cellules fibro-adipogéniques
- Platelet-Derived Growth Factor Recept A

Des marqueurs de surface qui évoluent chez l'adulte



Régime différencié (fibres, gras)

↳ *Perruchot et al., 2021*



Supplémentation acides aminés

↳ *Castellano et al., 2017*



Statut sanitaire

↳ *Quemener et al., 2022*



Statut Redox

↳ *Perruchot et al., 2019*



Diminution du réservoir de cellules souches avec l'âge

↳
Nécessité de travailler dans les premiers jours de vie

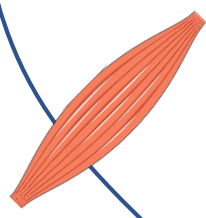


Stratégie expérimentale

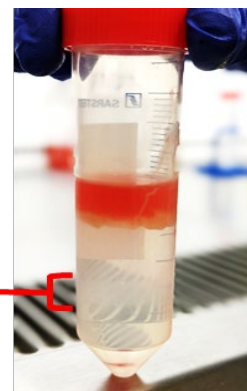


Porcelet J7 (3-3,2kg)

Isolement des cellules musculaires à partir du *longissimus dorsi*

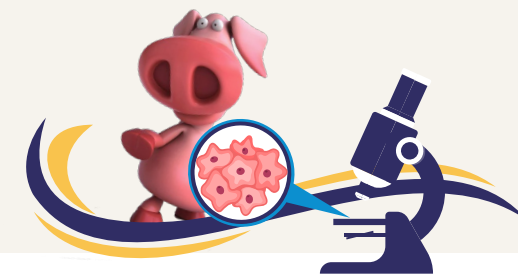


Anneau de cellules satellites

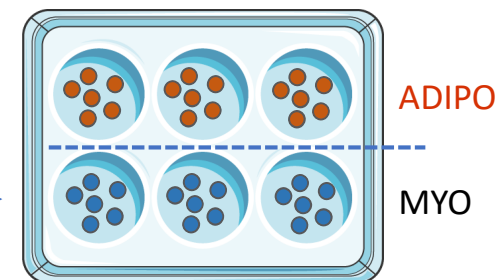


$0,5 \times 10^5$ cell / g

Phénotypage par cytométrie en flux



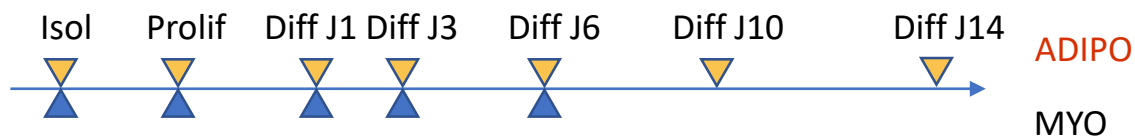
Analyse de gènes par qPCR



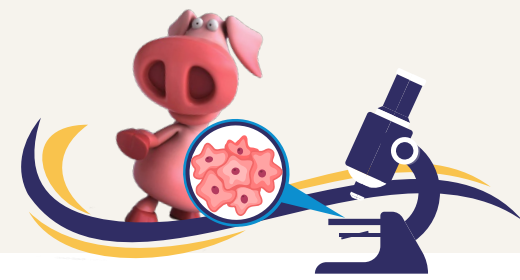
Prolifération puis différentiation en condition myogénique ou adipogénique



Mesure fusion par microscopie



Cellules satellites en condition myogénique



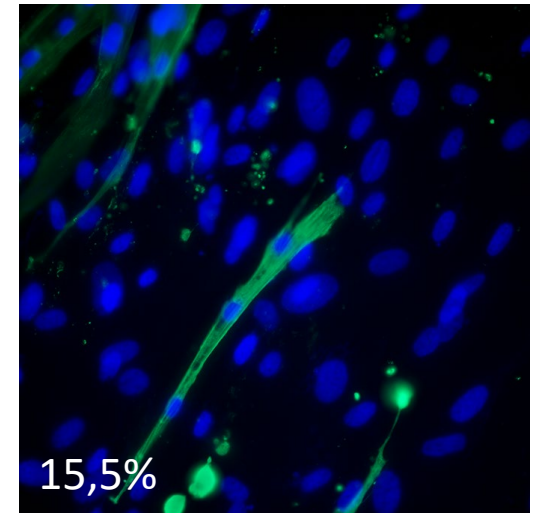
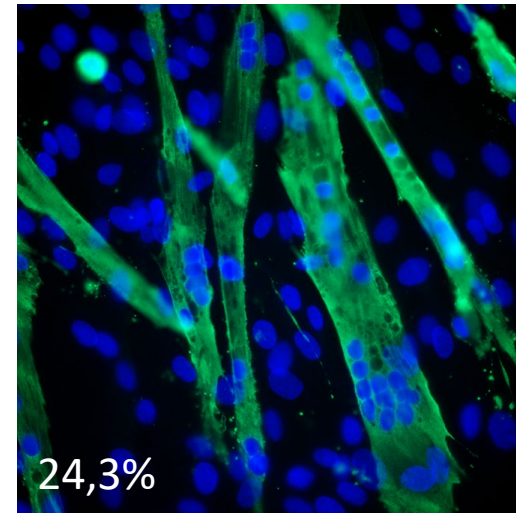
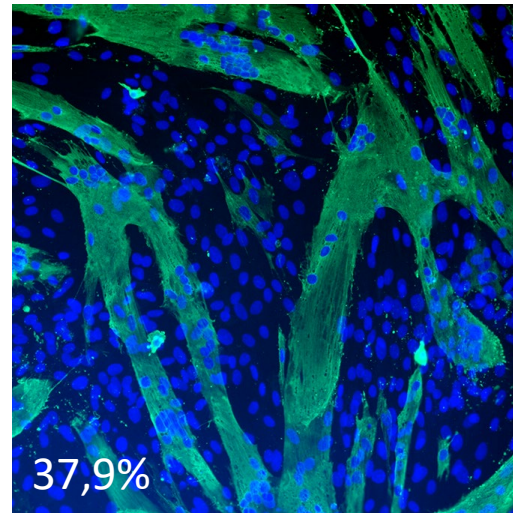
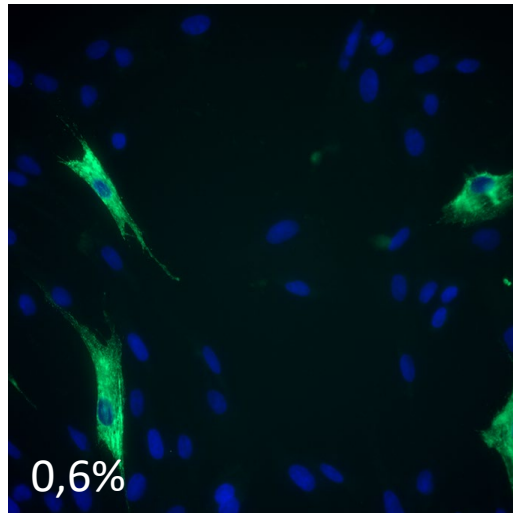
Prolif

Diff J1

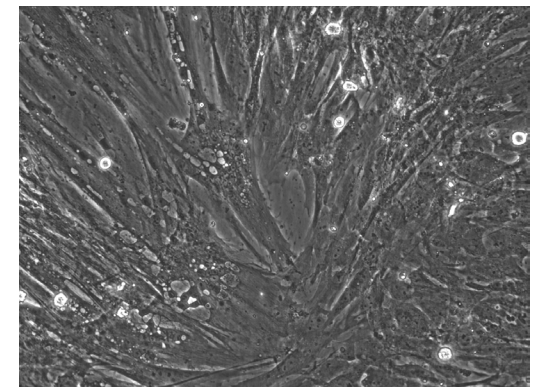
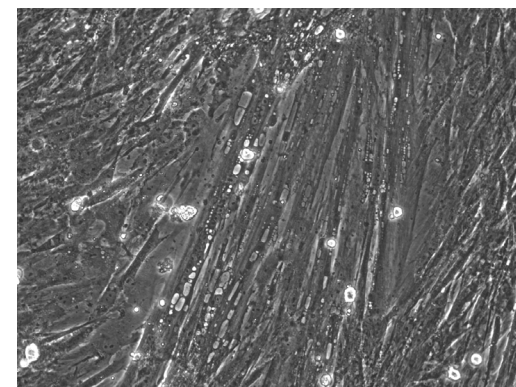
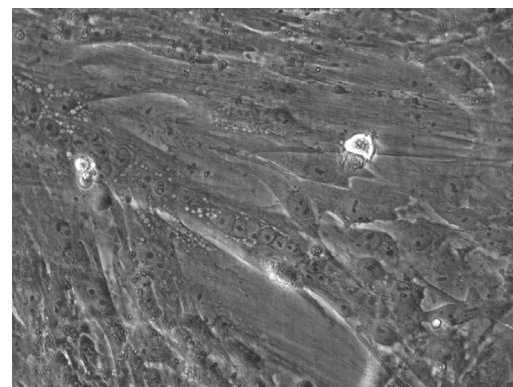
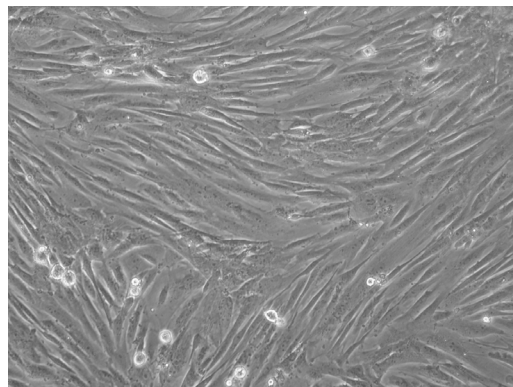
Diff J3

Diff J6

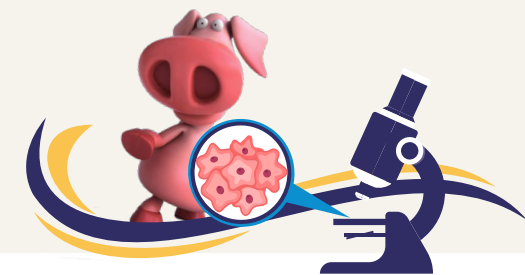
Marquage
MF20



Contraste
de phase



Cellules satellites en condition adipogénique



Prolif

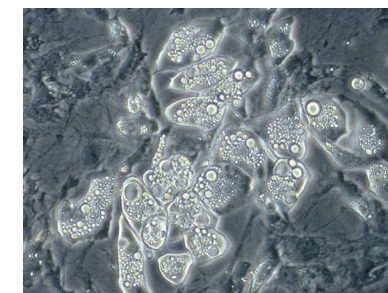
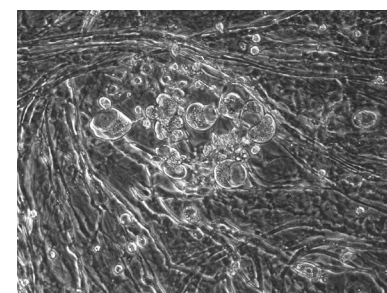
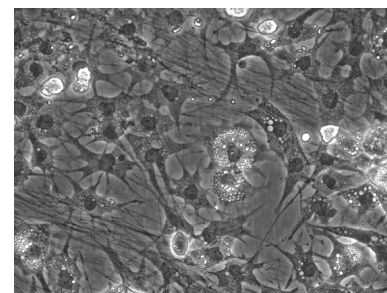
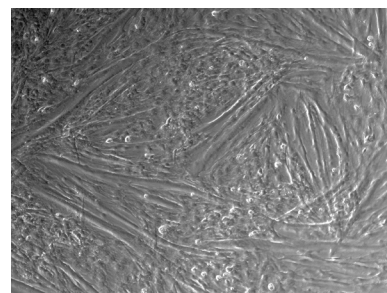
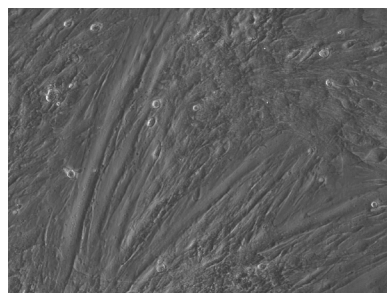
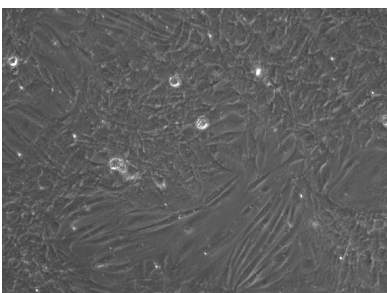
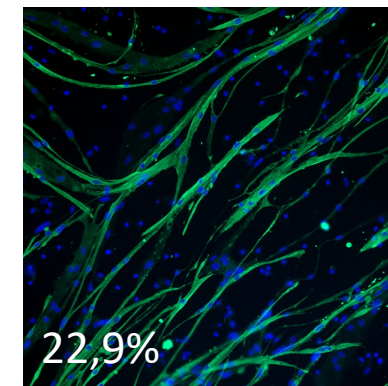
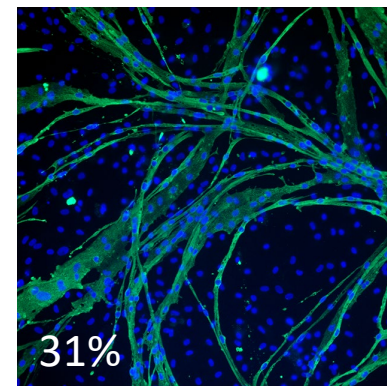
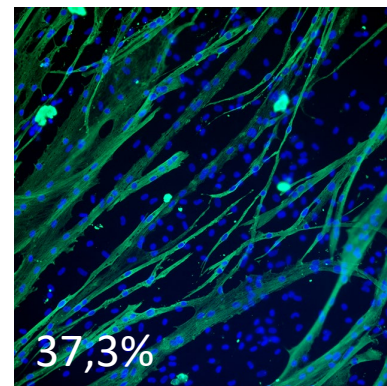
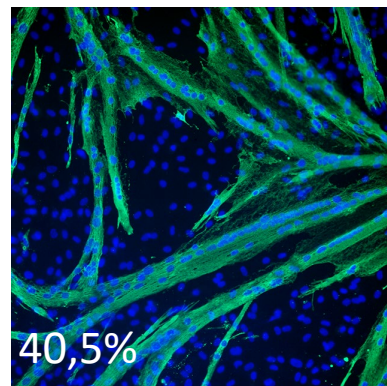
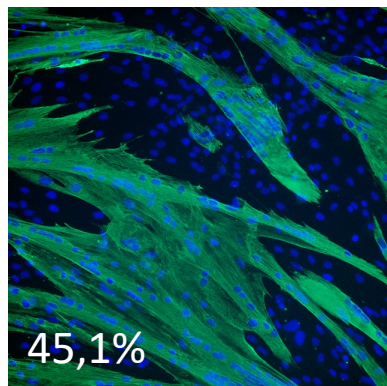
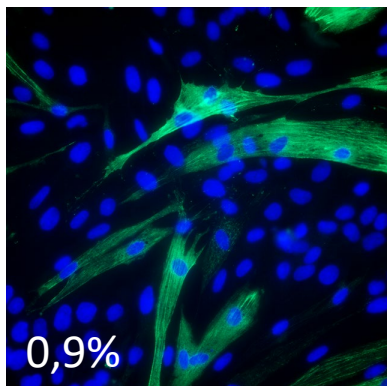
Diff J1

Diff J3

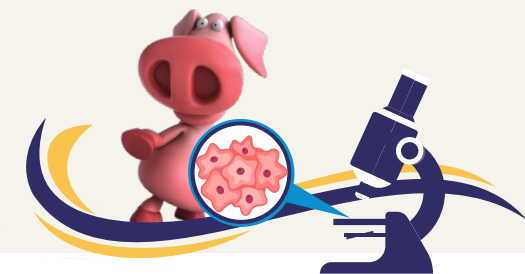
Diff J6

Diff J10

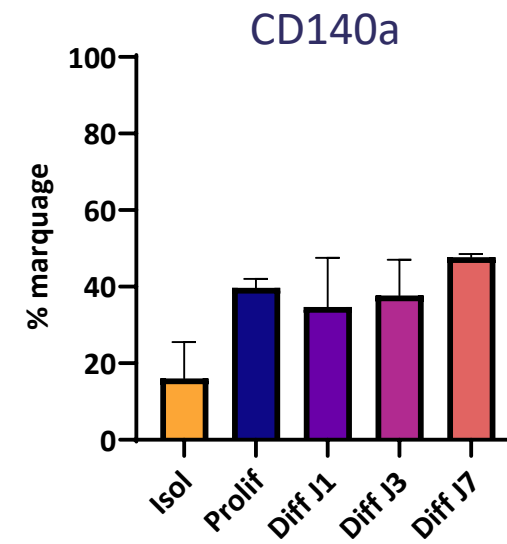
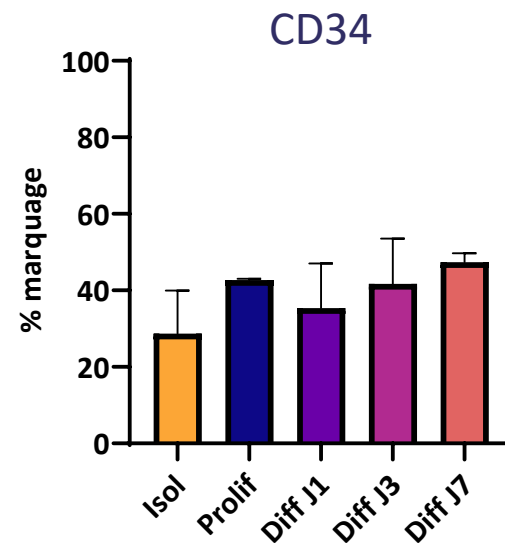
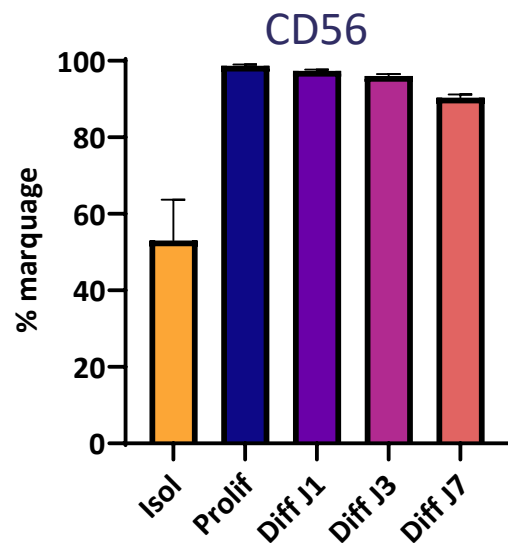
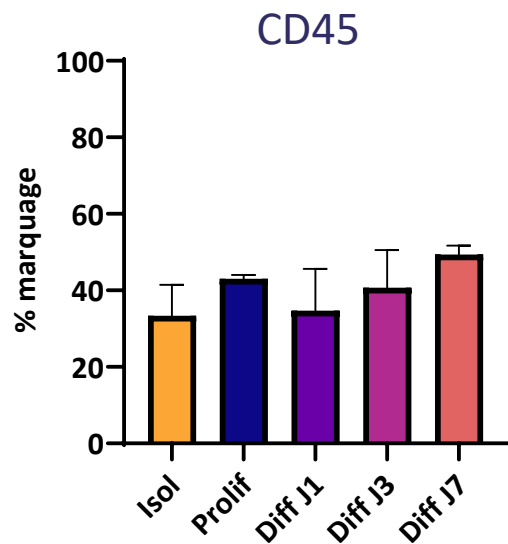
Diff J14



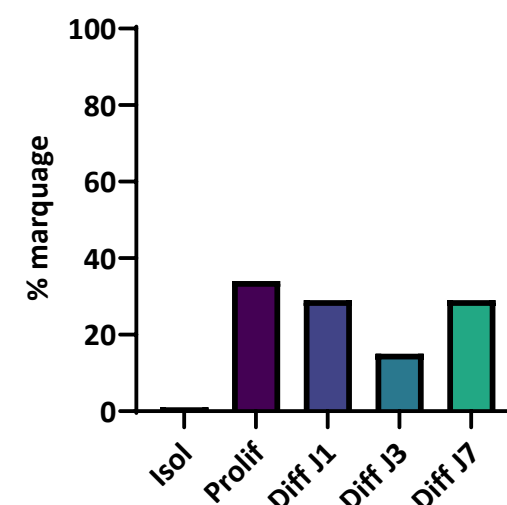
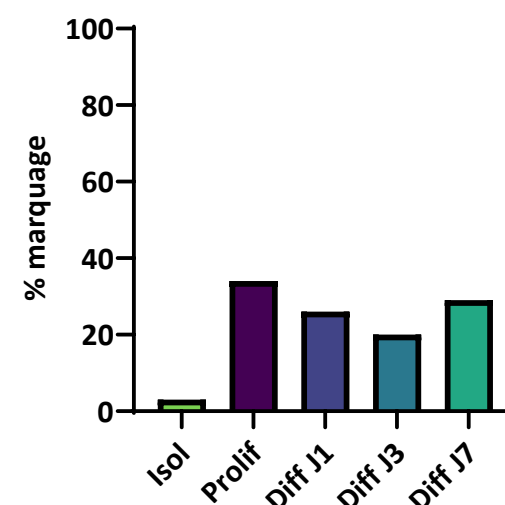
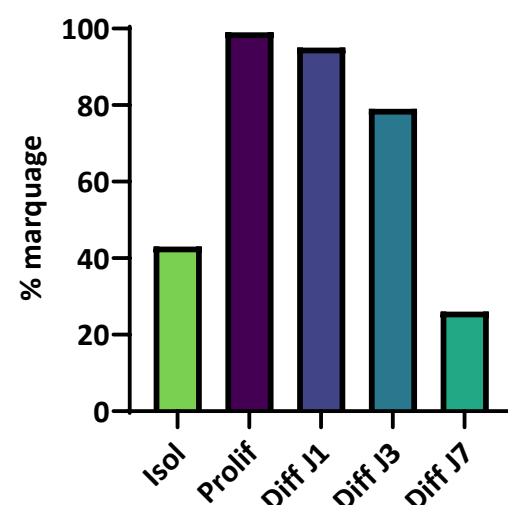
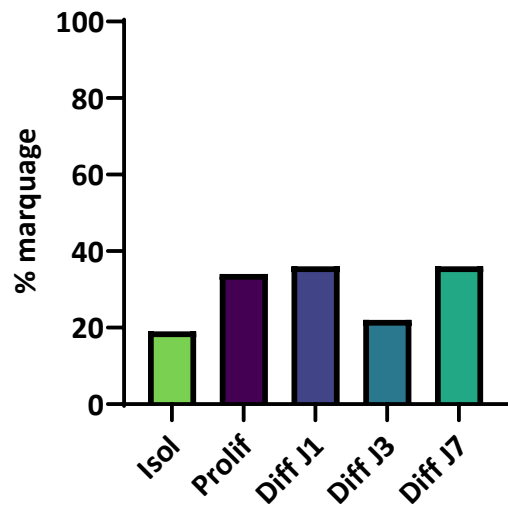
Evolution des marqueurs de surface (cytométrie)



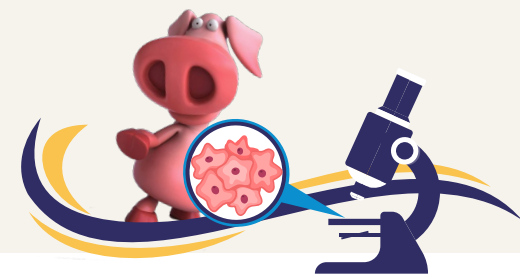
Milieu myogénique



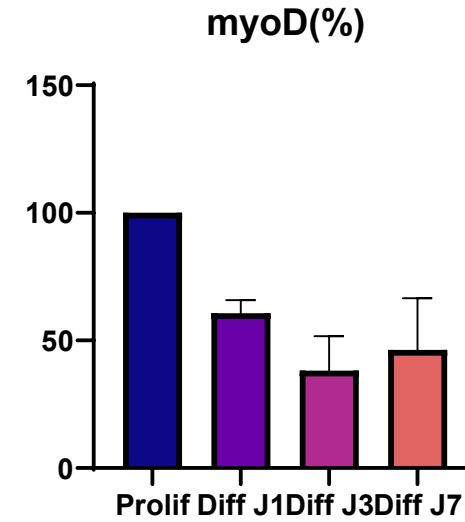
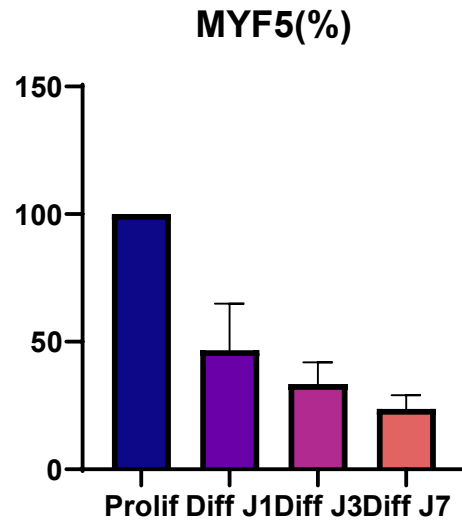
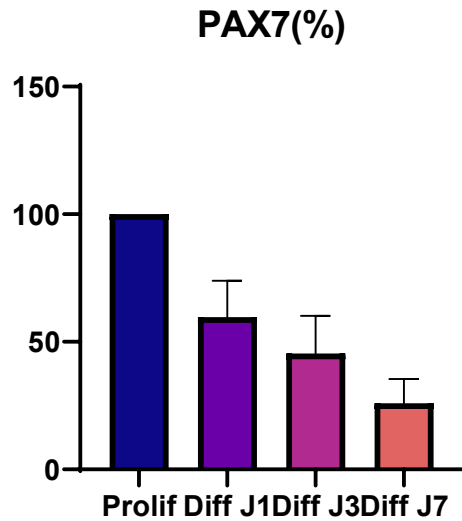
Milieu adipogénique



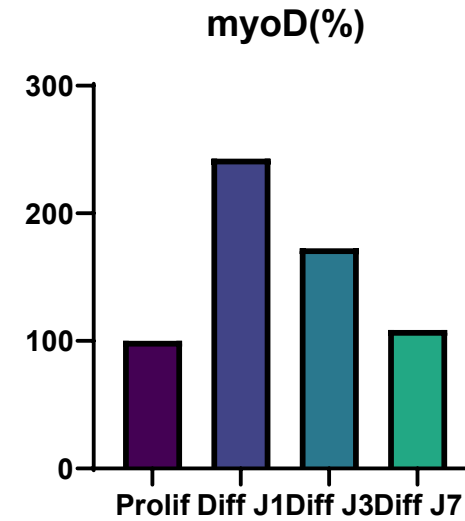
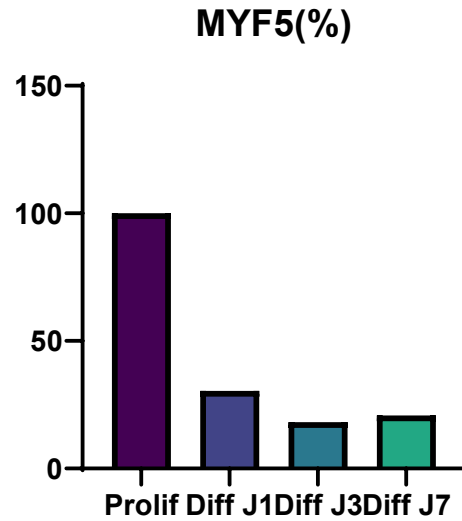
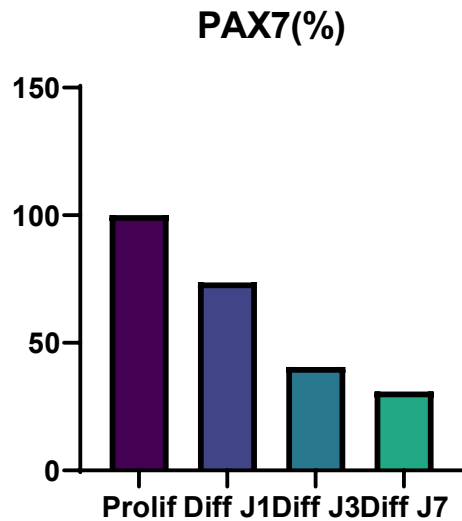
Expression des marqueurs myogéniques (qPCR)



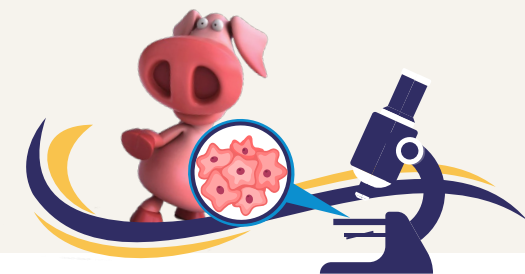
Milieu myogénique



Milieu adipogénique

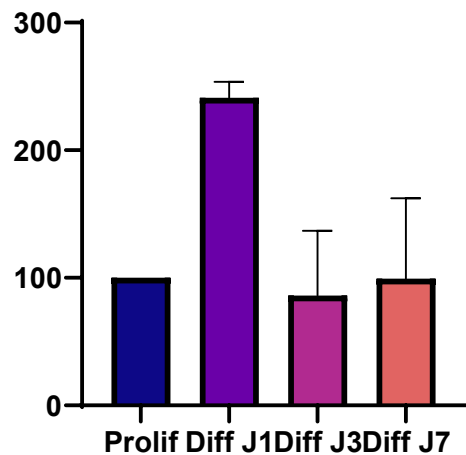


Expression des marqueurs myogéniques (qPCR)

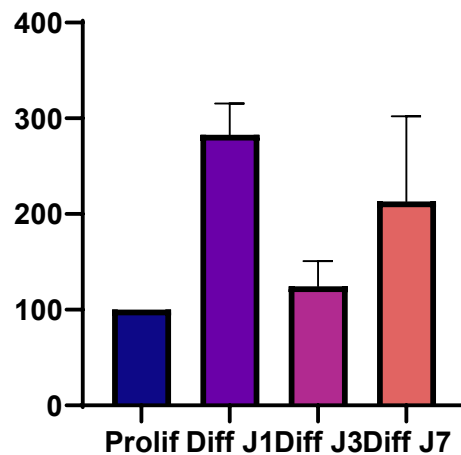


Milieu myogénique

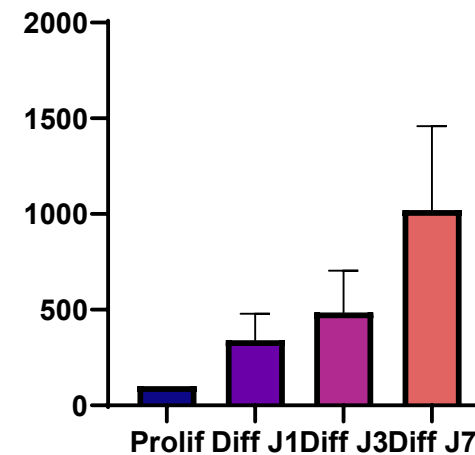
MyHCemb(%)



MyHCfoe(%)

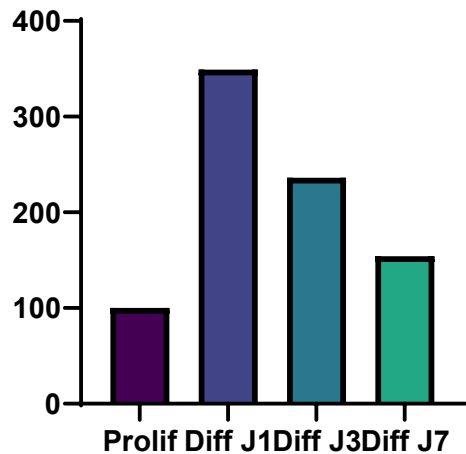


MyHC2A(%)

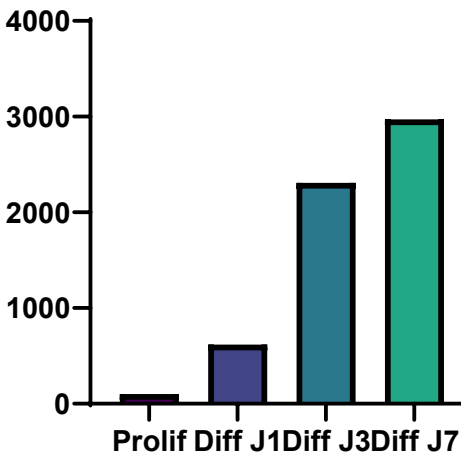


Milieu adipogénique

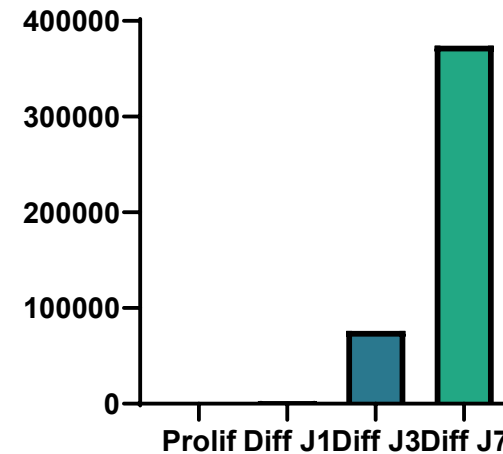
MyHCemb(%)



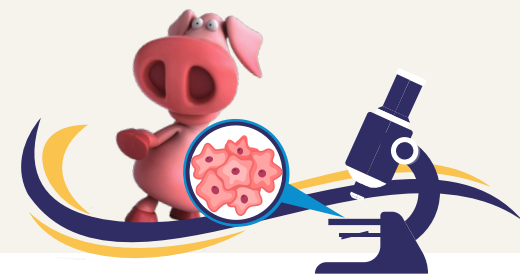
MyHCfoe(%)



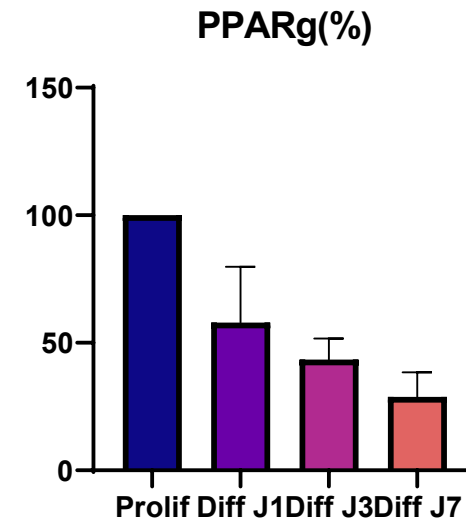
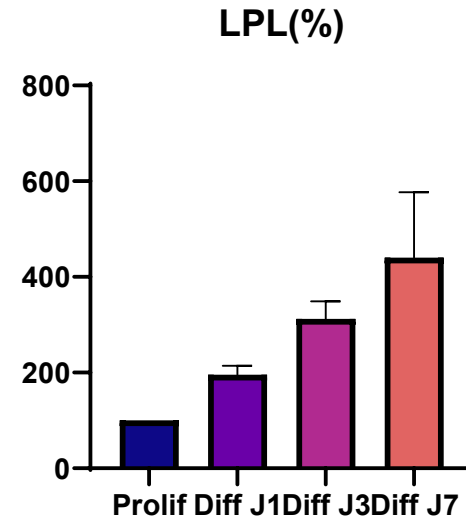
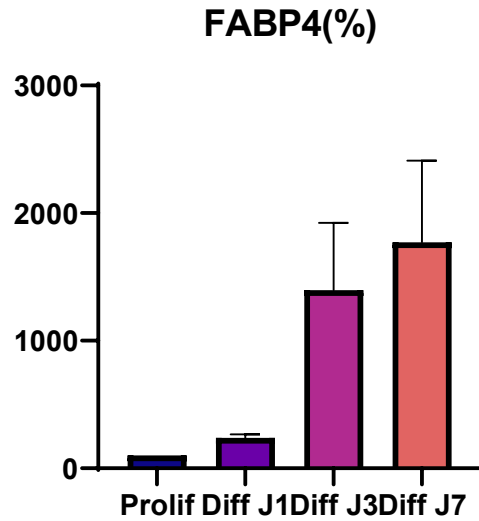
MyHC2B(%)



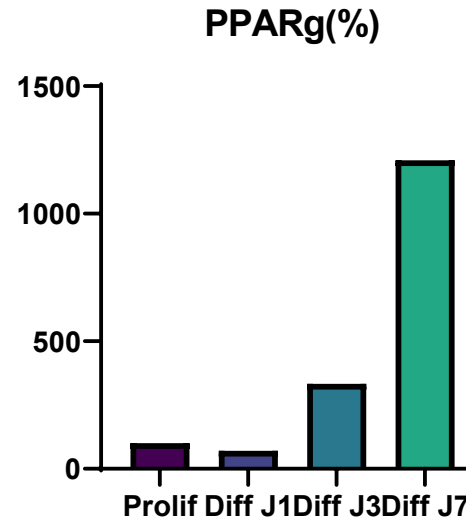
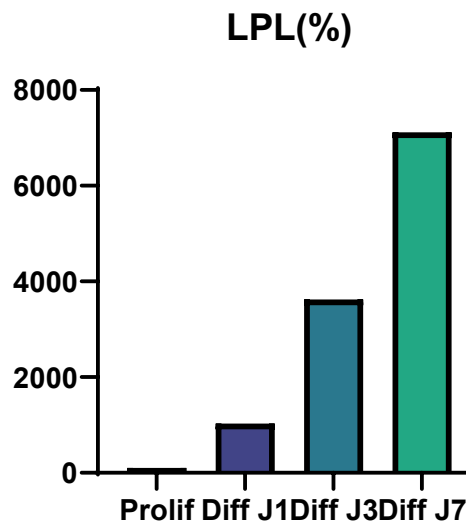
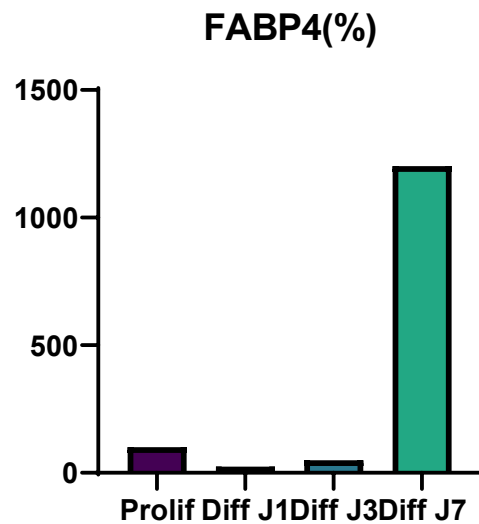
Expression des marqueurs adipogéniques (qPCR)



Milieu myogénique



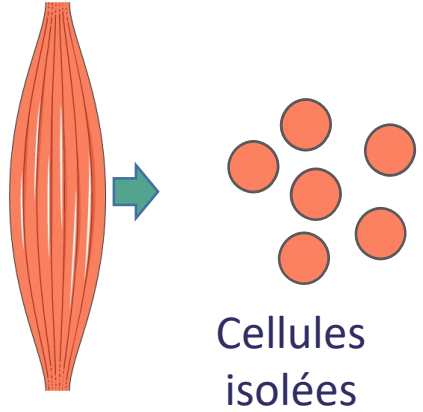
Milieu adipogénique



Stratégie de tri

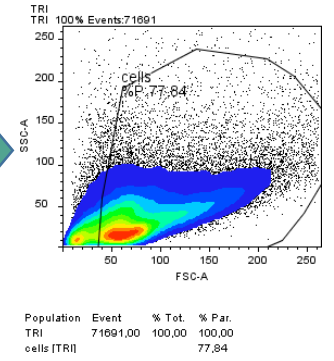


muscle
60 g

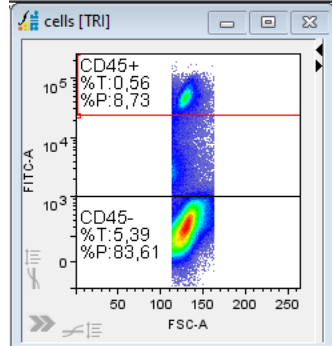


10 x 10⁶ cellules

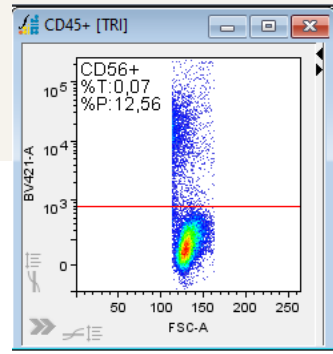
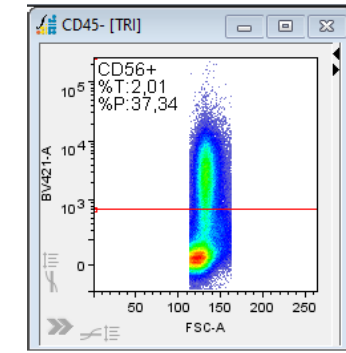
5 x 10⁶ cellules



CD45+
9%



CD45-
84%

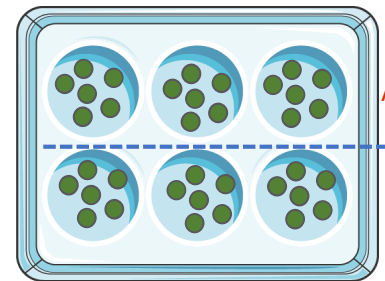
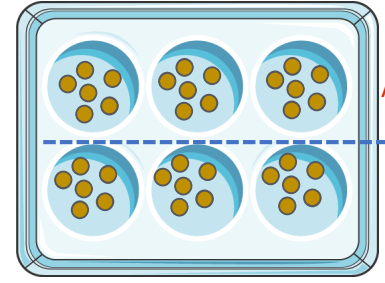
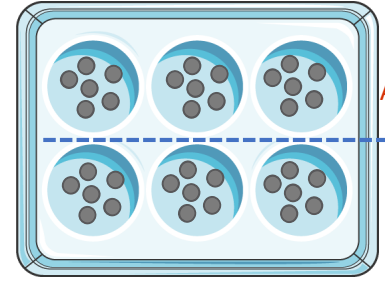
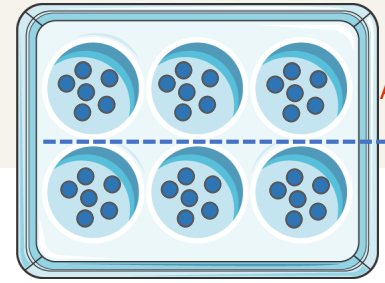


CD45+/CD56+
13%

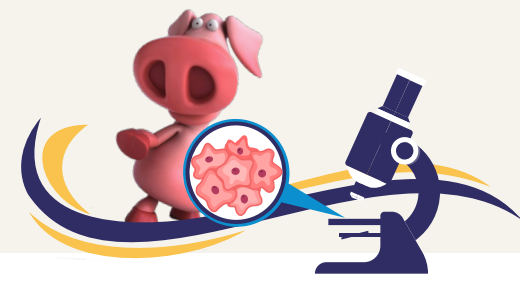
CD45+/CD56-
87%

CD45-/CD56+
37%

CD45-/CD56-
63%



Culture des populations triées



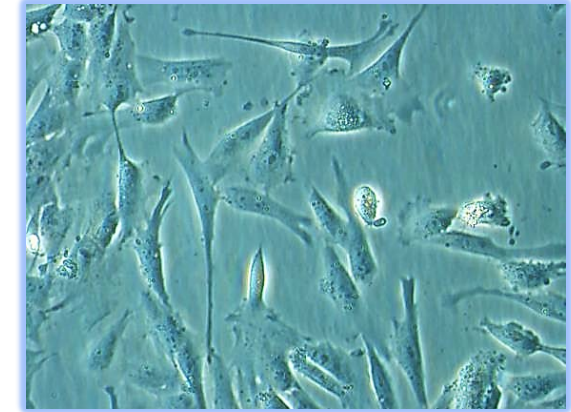
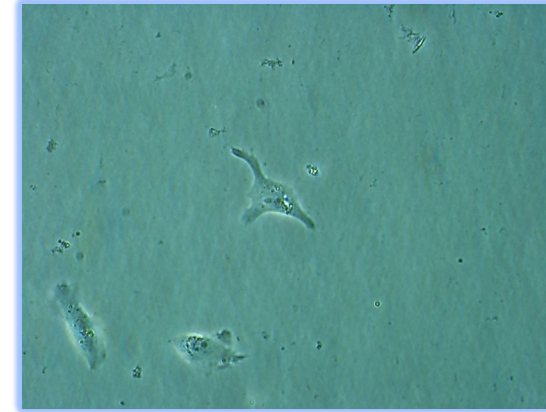
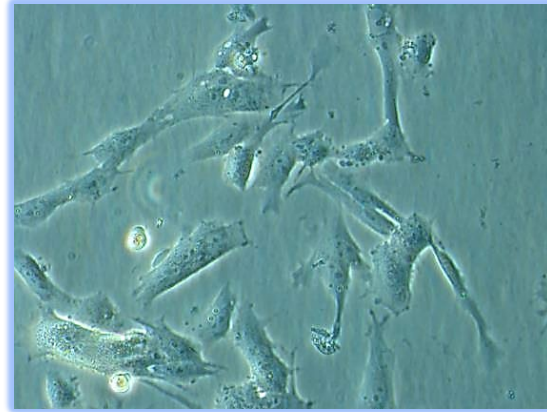
CD45-/CD56-

CD45-/CD56+

CD45+/CD56-

CD45+/CD56+

Milieu myogénique



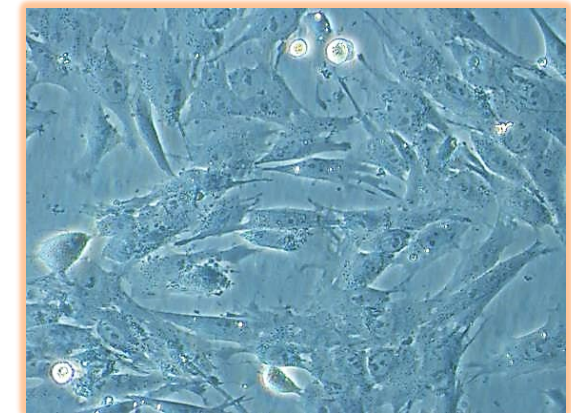
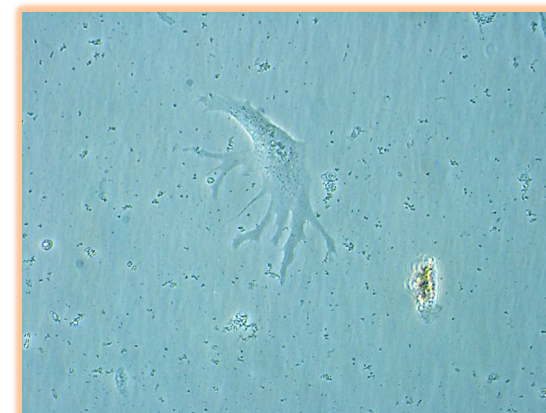
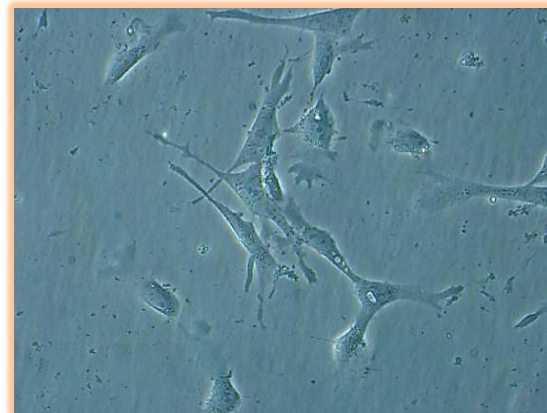
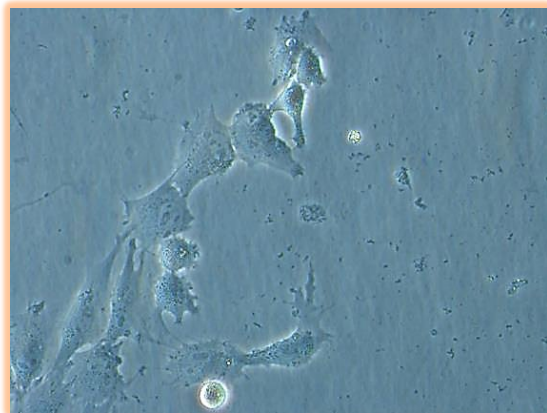
Prolif + dans les 2 conditions

Prolif ++ dans les 2 conditions

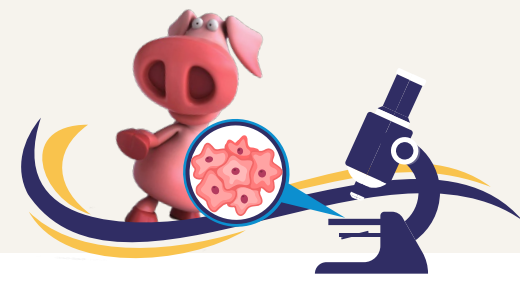
Peu de prolif et cellules dendritiques

Prolif +++ dans les 2 conditions

Milieu adipogénique



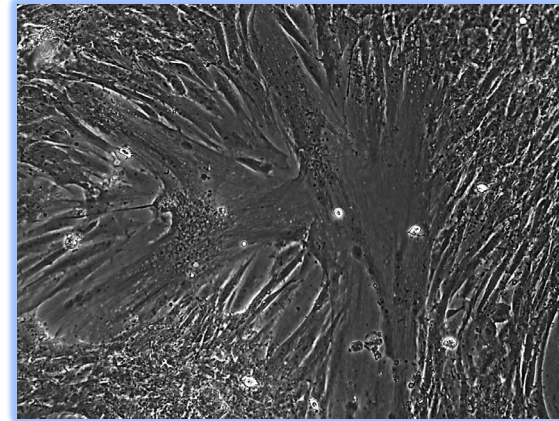
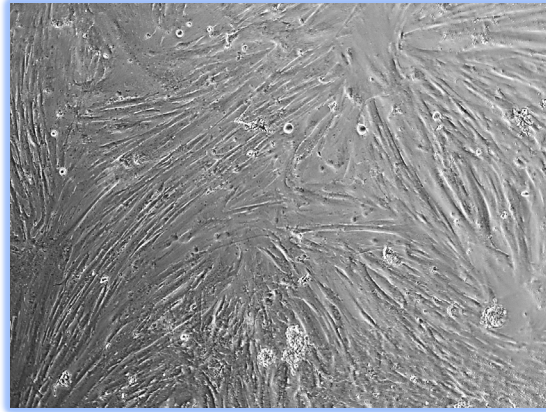
Culture des populations triées (CD45⁻)



CD45⁻/CD56⁻

CD45⁻/CD56⁺

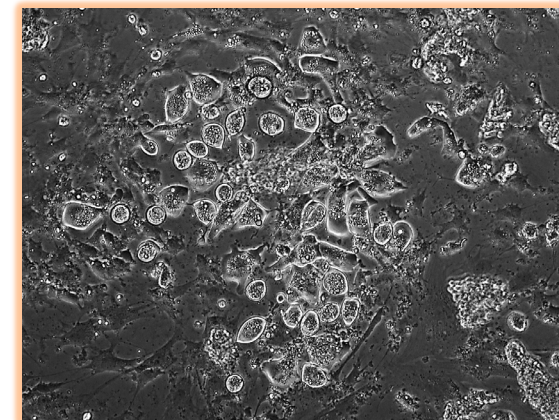
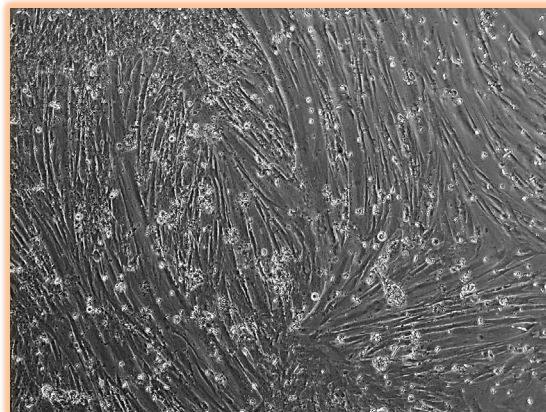
Milieu myogénique



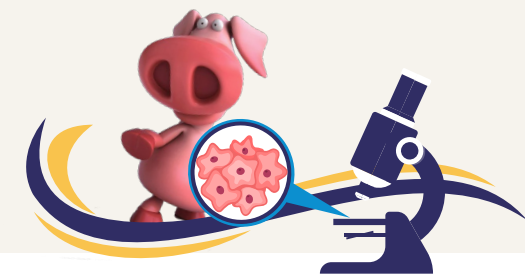
DIFF + dans les 2 conditions

DIFF ++ dans les 2 conditions

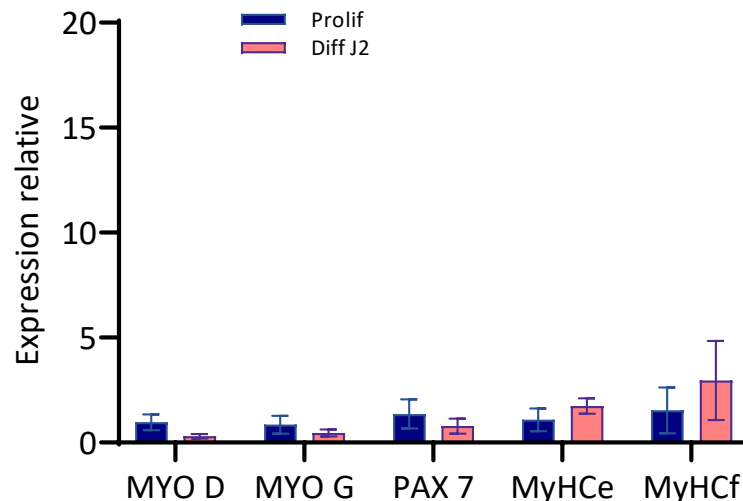
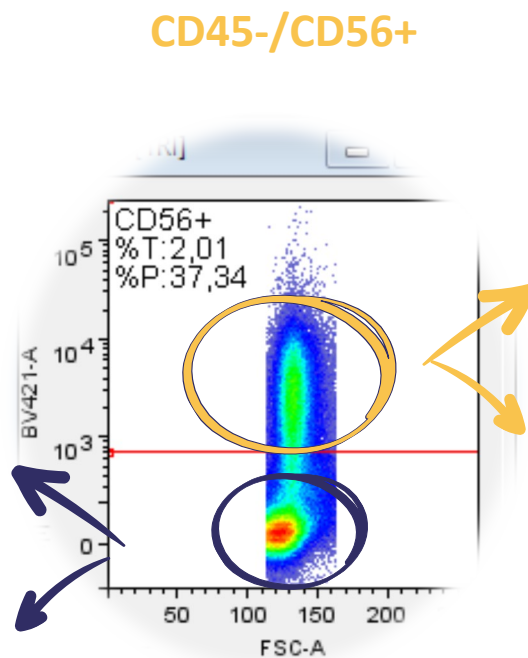
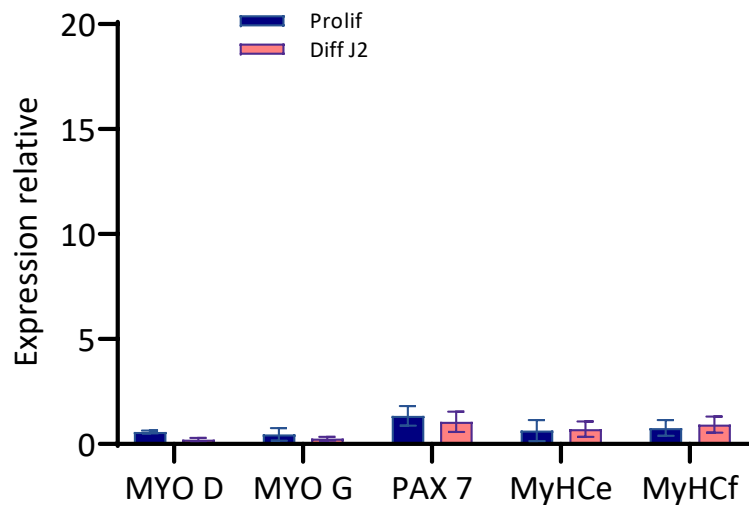
Milieu adipogénique



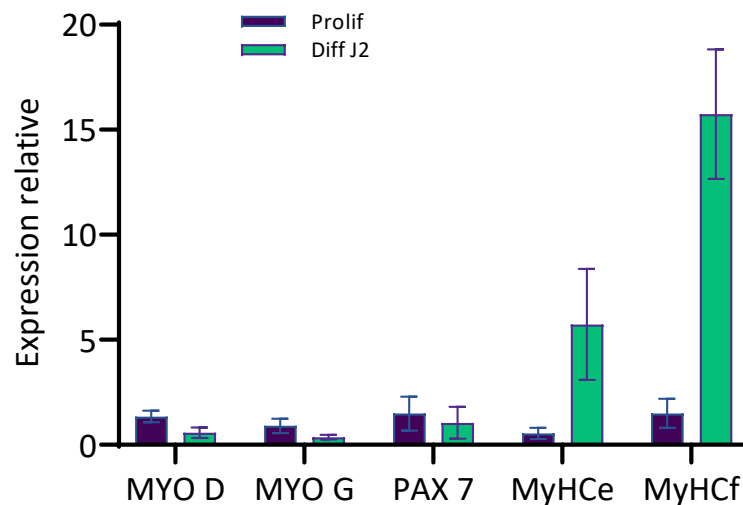
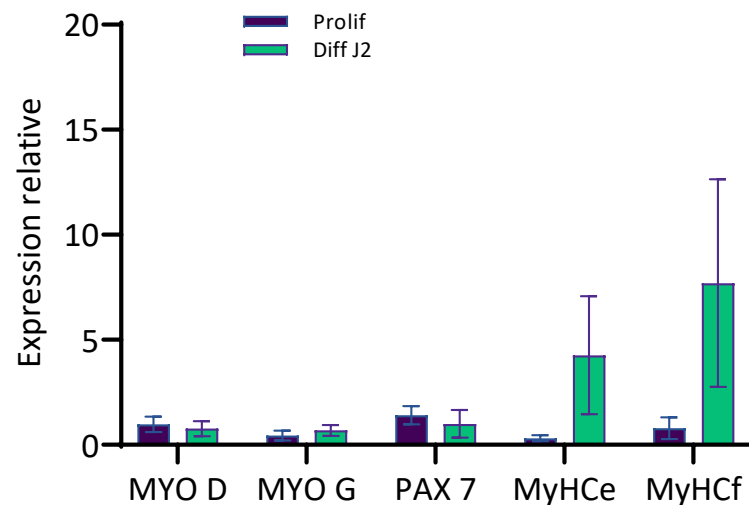
Expression des marqueurs myogéniques après tri



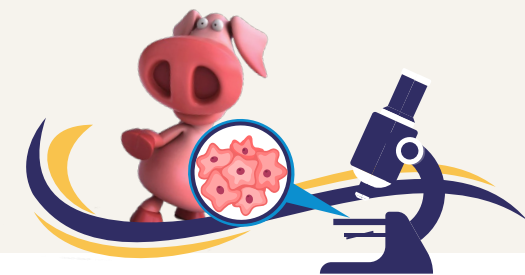
Milieu myogénique



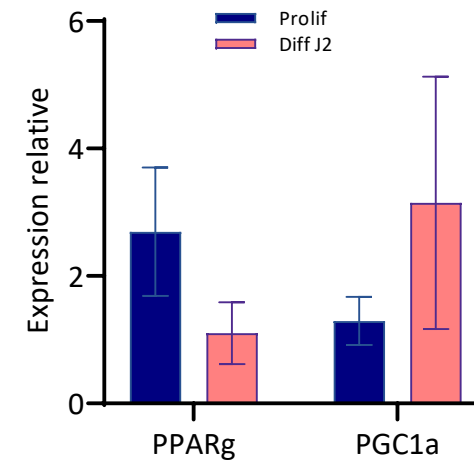
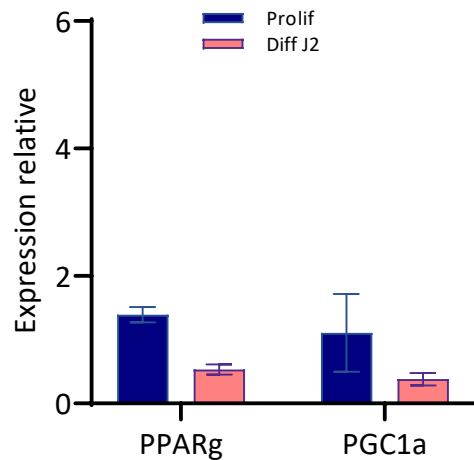
Milieu adipogénique



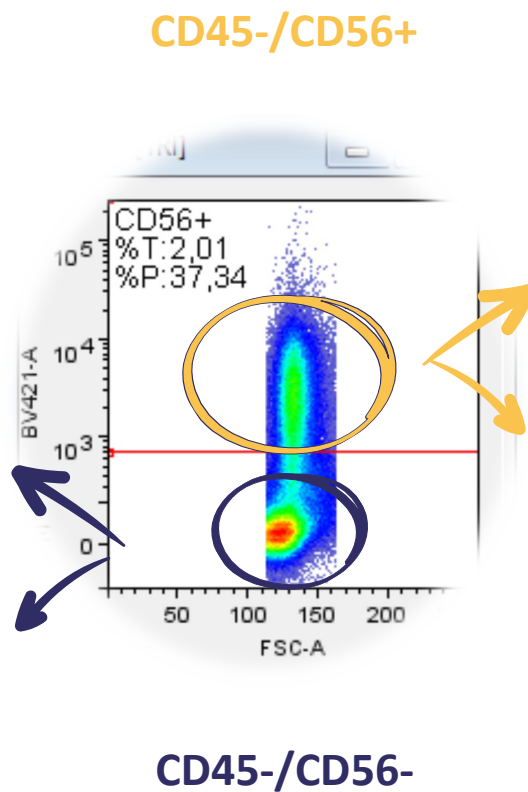
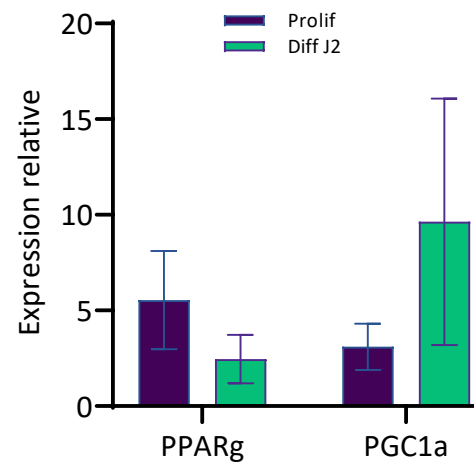
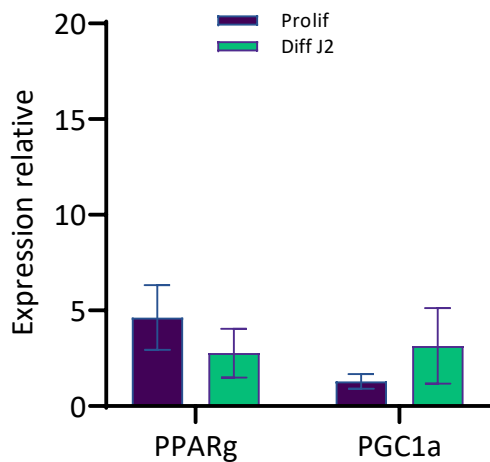
Expression des marqueurs adipogéniques après tri



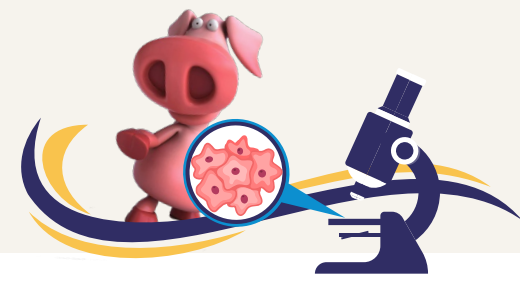
Milieu myogénique



Milieu adipogénique



Conclusions



- ❗ Capacité à faire différencier les cellules souches en fibres musculaires et en adipocytes fonctionnels
- ❗ Expression des marqueurs de surface altérée par la culture primaire
- ❗ Tri cellulaire = méthodologie complexe pour caractériser les différentes populations cellulaires du muscle
- ❗ Relevance du marqueur myogénique CD56
- ❗ Le milieu adipogénique: un milieu prometteur (expression de myosines adultes !)