15 Juin 2023

Contribution des cellules souches à la croissance musculaire chez le porc

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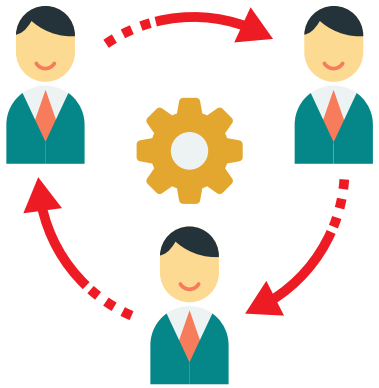
INRAE UMR-1348 PHYSIOLOGIE, ENVIRONNEMENT ET GÉNÉTIQUE POUR L'ANIMAL ET LES SYSTÈMES D'ÉLEVAGE (PEGASE)

L'INSTITUT agro Rennes Angers

Journées Muscle et Qualités / 15-16 juin 2023

Présentation

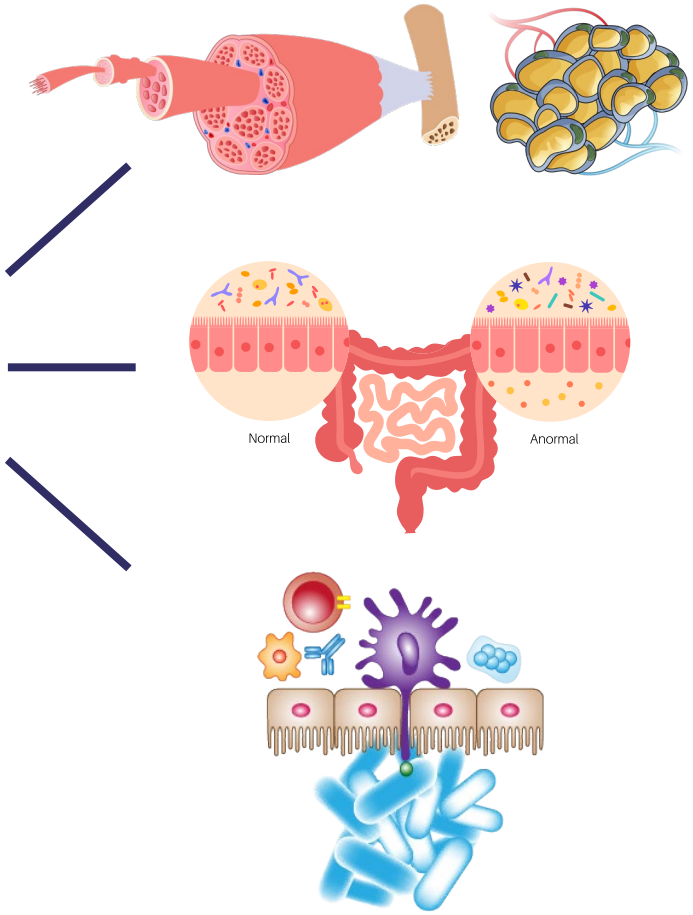
L'UMR PEGASE en chiffres



120
collaborateurs

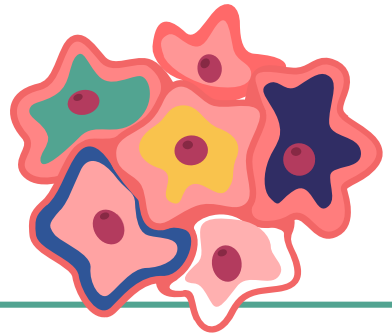
4
Équipes de recherche

1
Equipe Biologie des fonctions

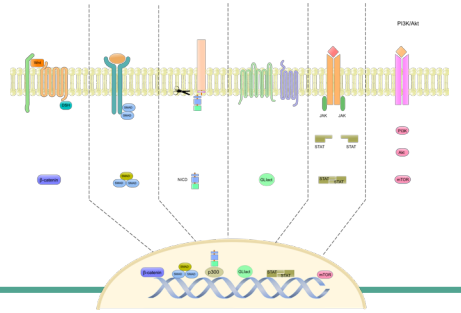


Objectifs de recherche

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.

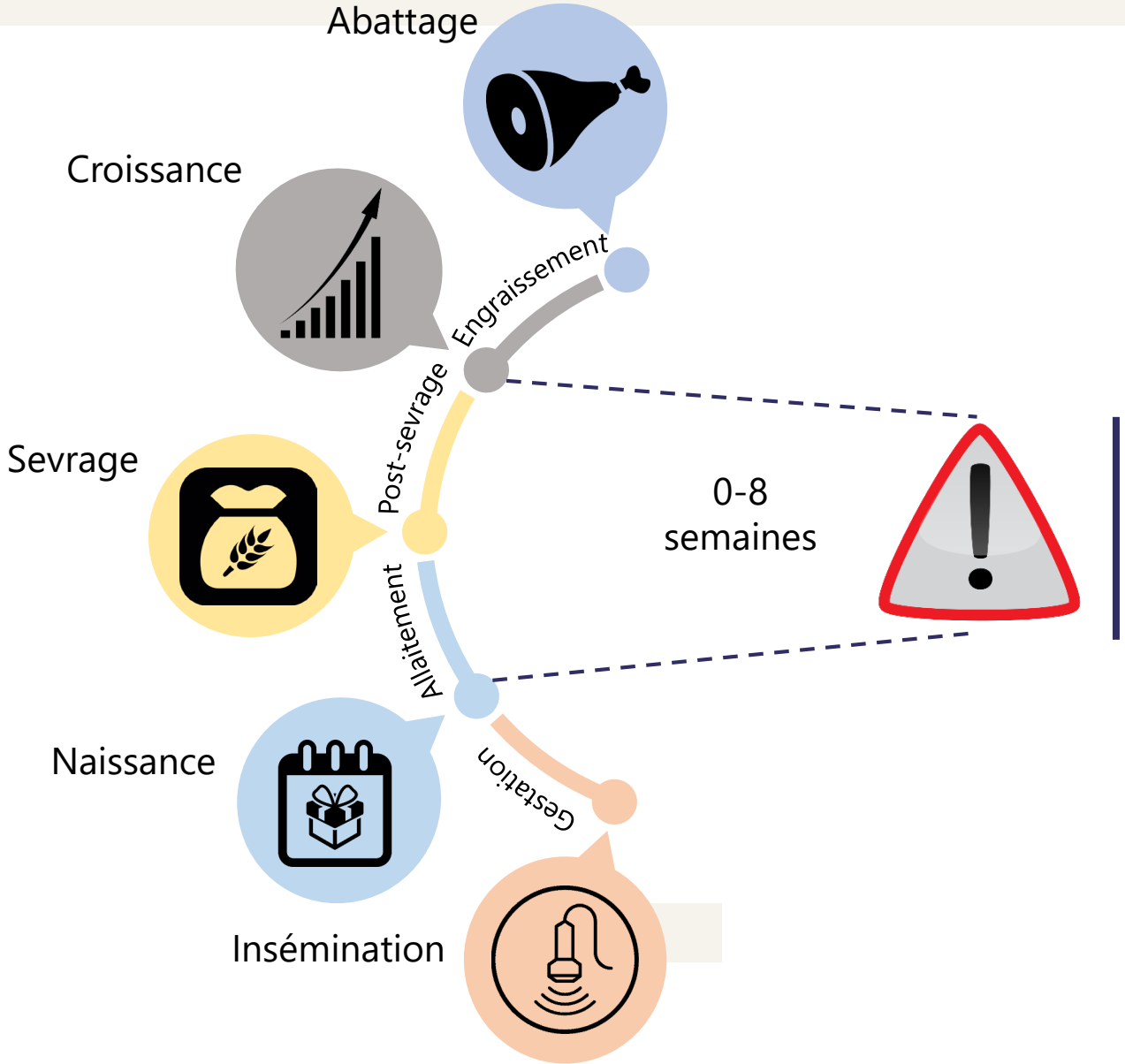


Exploration de la diversité cellulaire au cours des premières semaines de vie



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

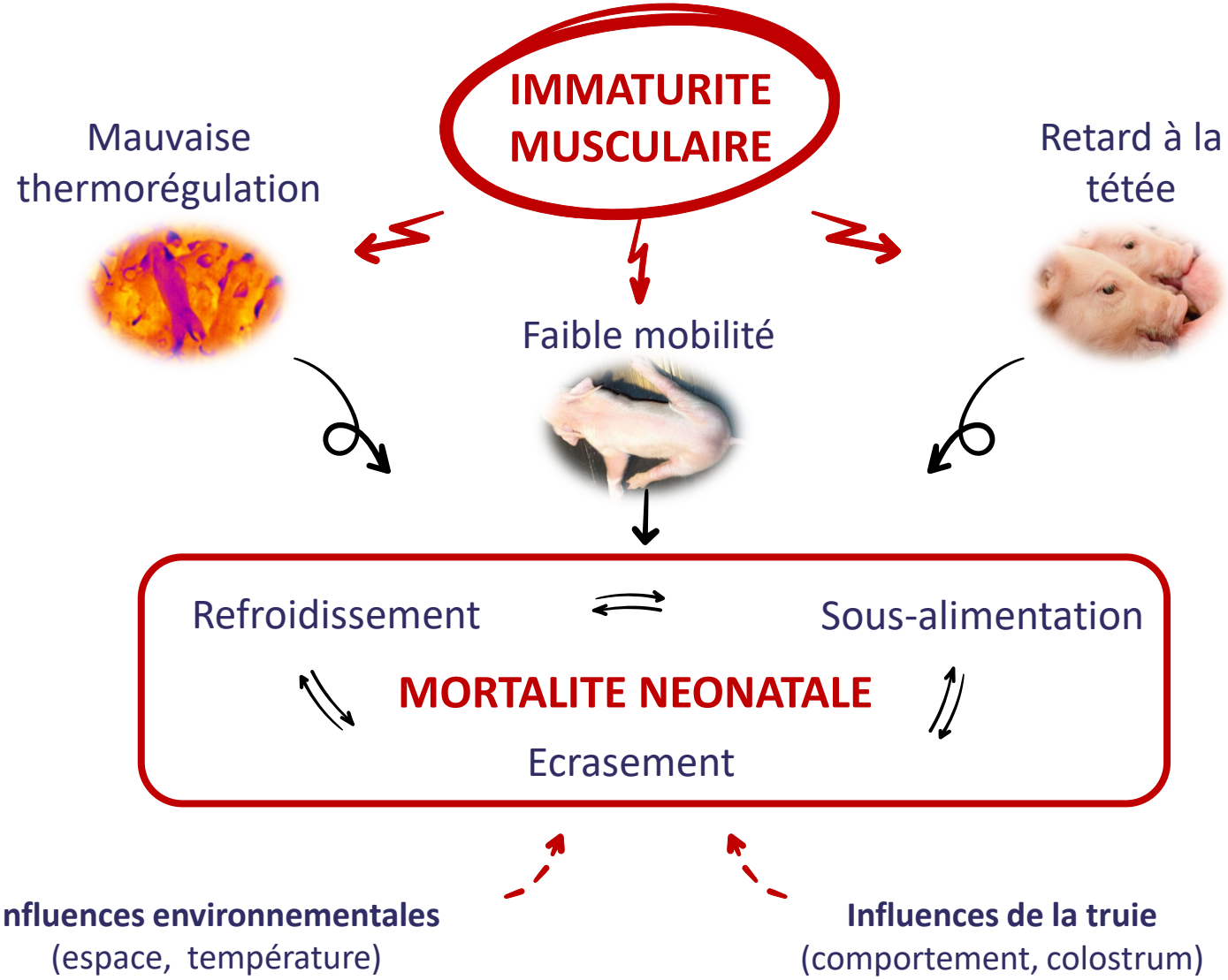
- races
- lignées

POIDS VIF A LA NAISSANCE

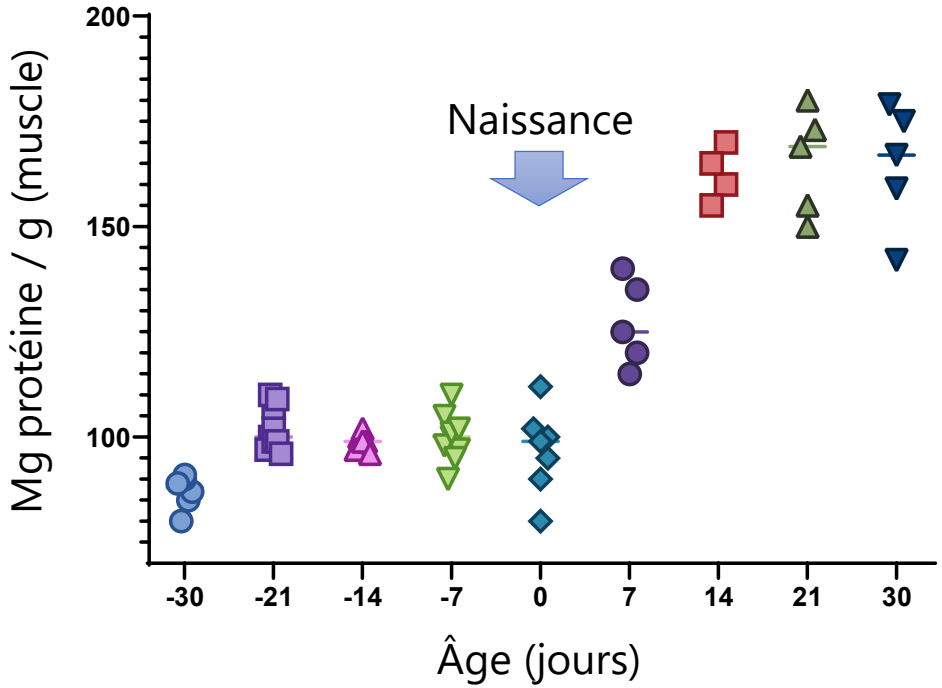
- variabilité
- (im)maturité
- survie

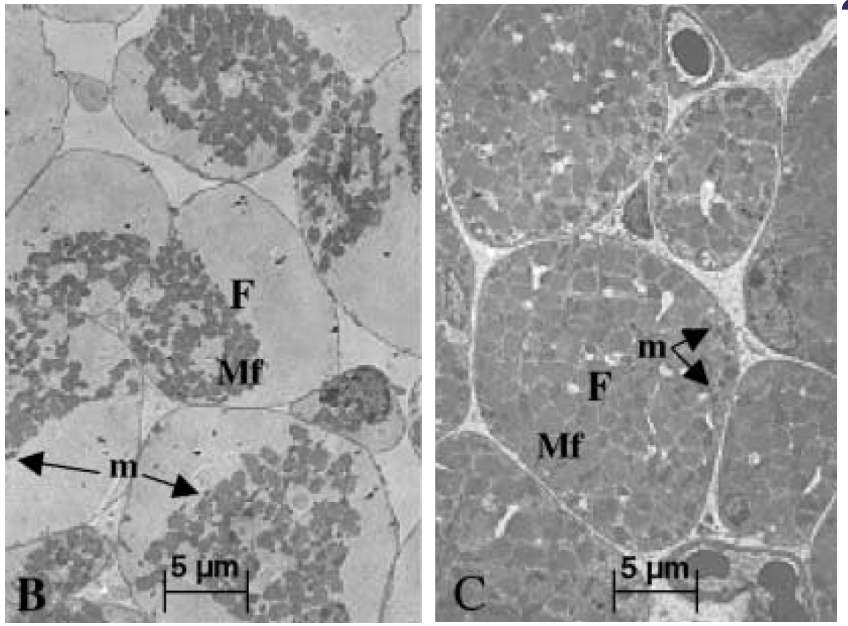
Vitalité néo-natale

Fonction musculaire et survie néo-natale



Croissance et maturation musculaire chez le porc





Naissance 7 jours

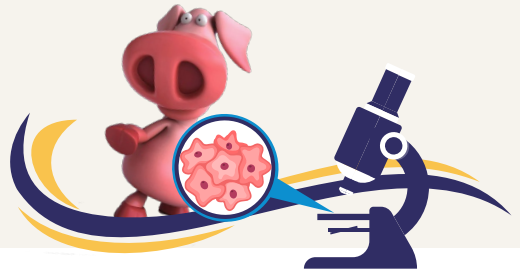
Forte synthèse protéique après la naissance

Forte maturation contractile et Métabolique après la naissance

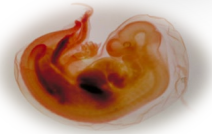
Maturité musculaire déterminante pour la survie

Lefaucheur et al., 1986

Développement précoce du tissu musculaire



0j — 1er tiers gestation



- 1ère génération de fibres
- Hyperplasie
- Différenciation

45j — 2ème tiers gestation

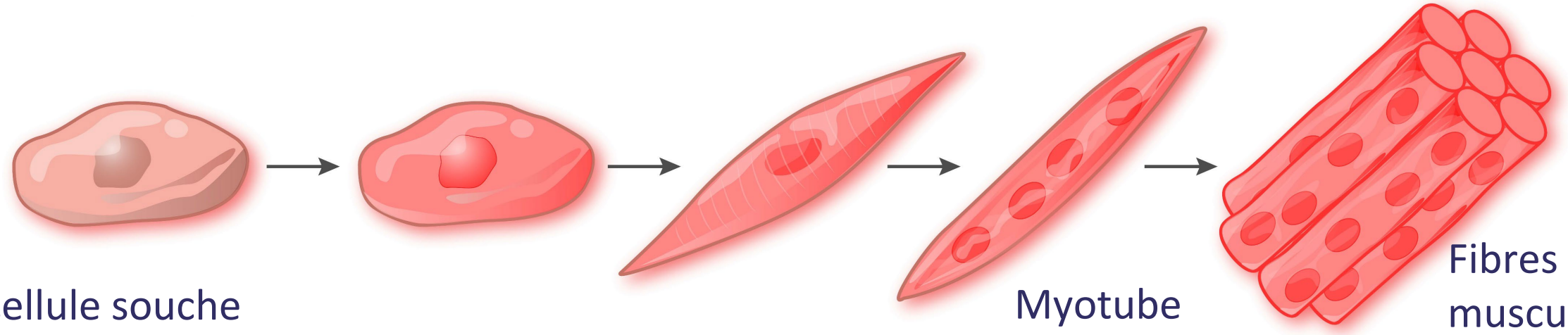
- 2ème génération de fibres
- Hyperplasie
- Différenciation

90j — 3ème tiers gestation

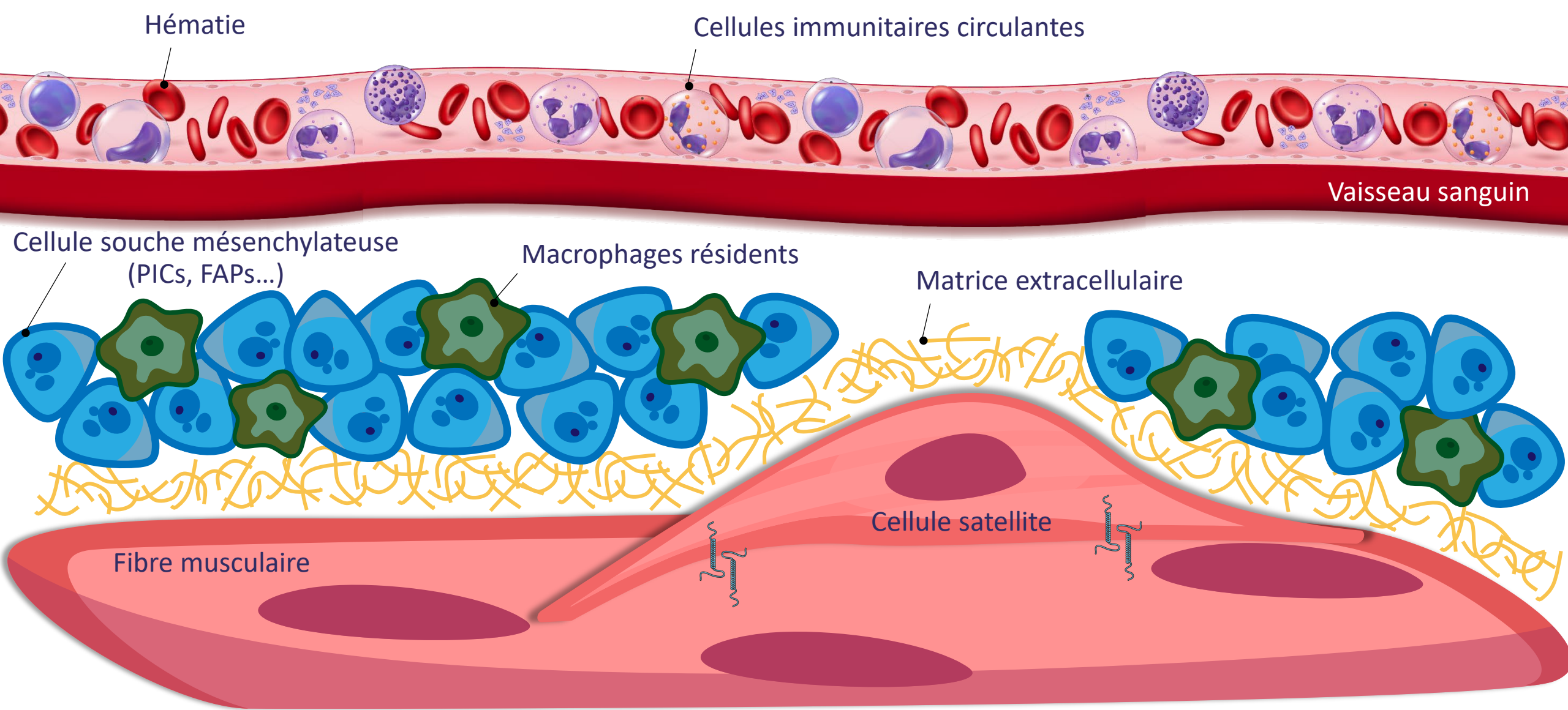
- Nombre total de fibres fixé
- Hypertrophie

114j — Naissance

- 3ème génération de fibres
- Hypertrophie



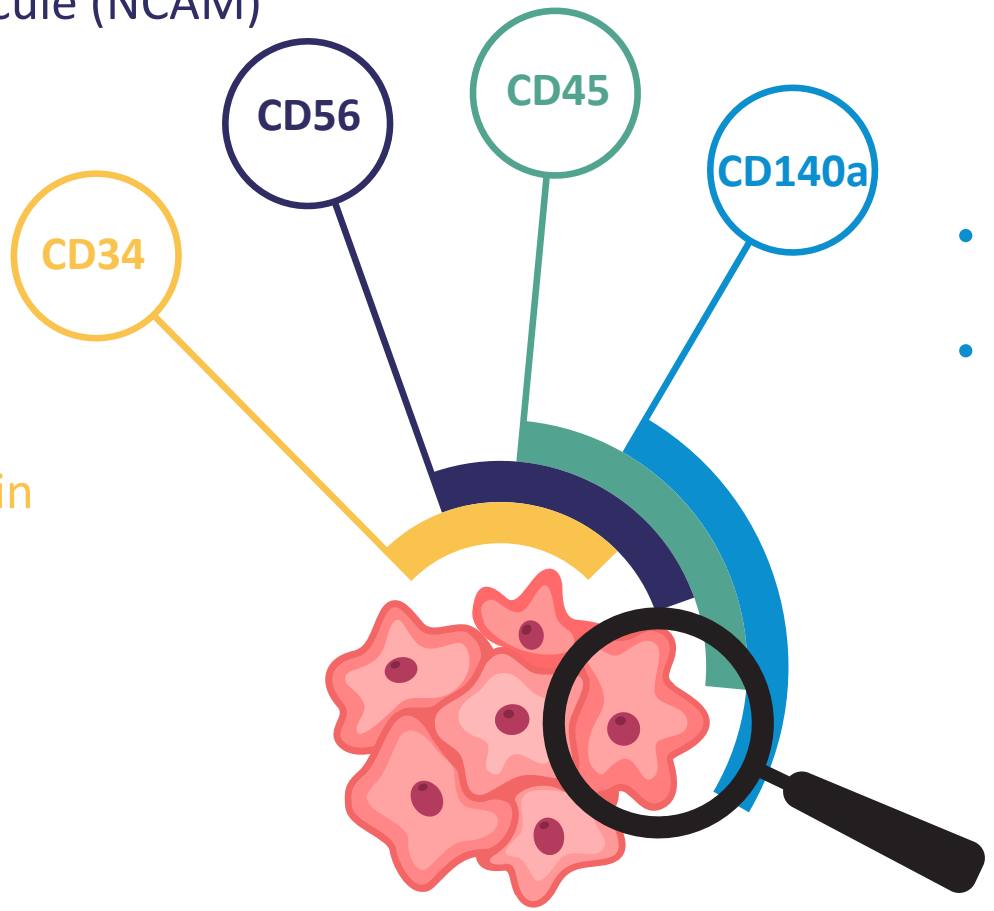
Une diversité de cellules souches dans le muscle



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

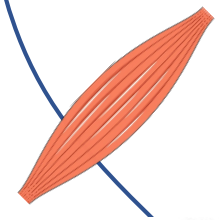
Perruchot *et al.*, 2019

Diminution du réservoir de cellules souches avec l'âge

Nécessité de travailler dans les premiers jours de vie

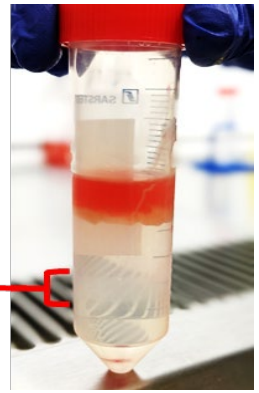
Stratégie expérimentale

Porcelet J7 (3-3,2kg)

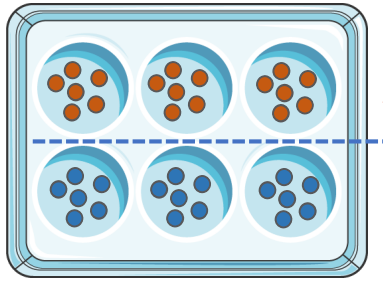


Isolement des cellules musculaires à partir du *longissimus dorsi*

Anneau de cellules satellites



0,5 x 10^5 cell / g



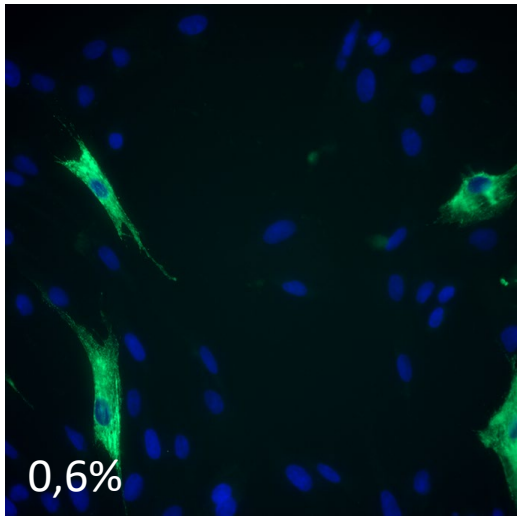
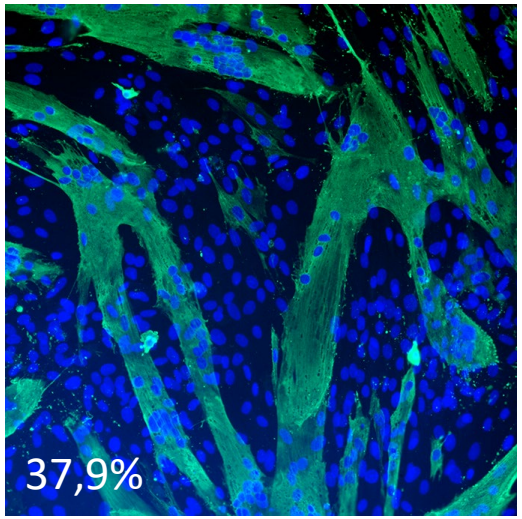
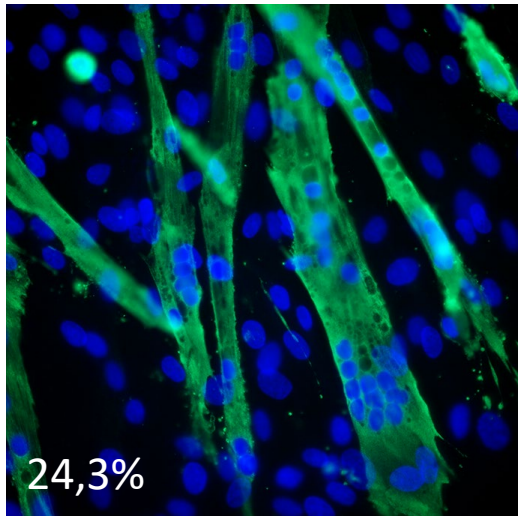
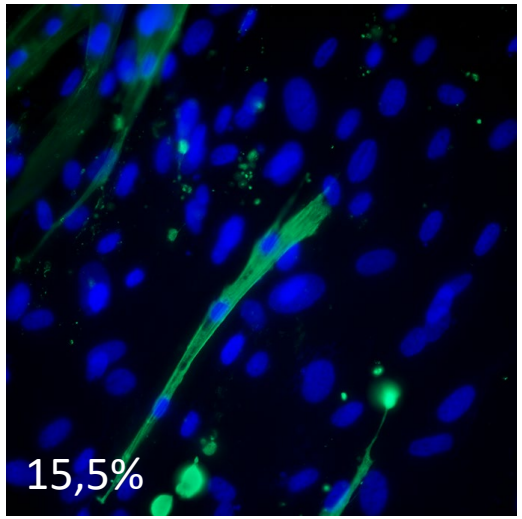
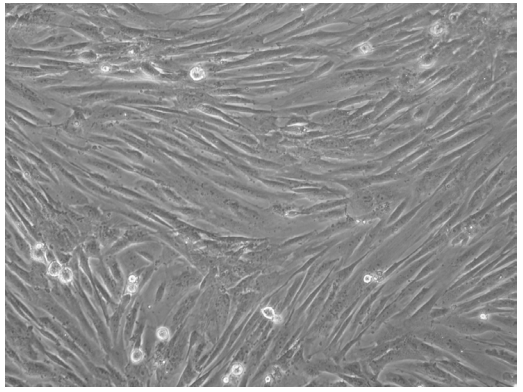
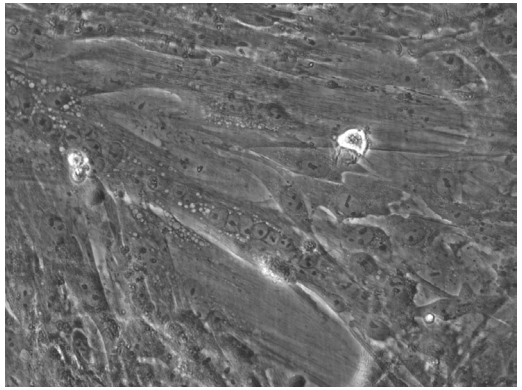
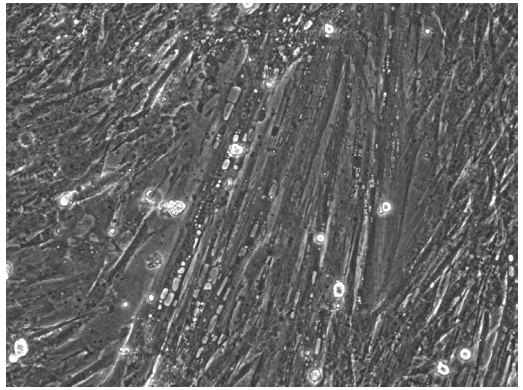
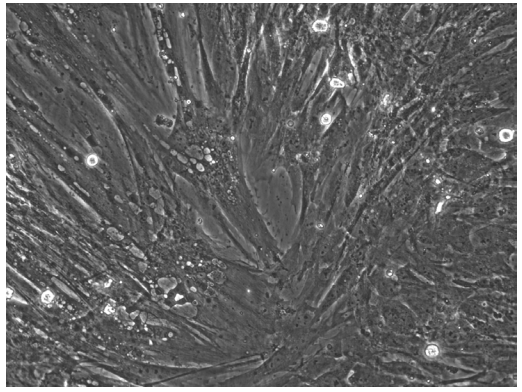
Prolifération puis différentiation en condition myogénique ou adipogénique

Phénotypage par cytométrie en flux

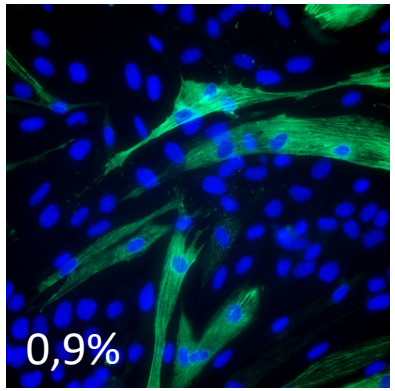
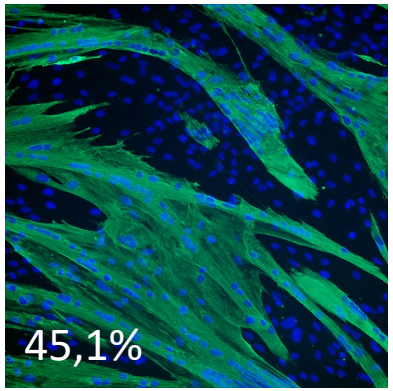
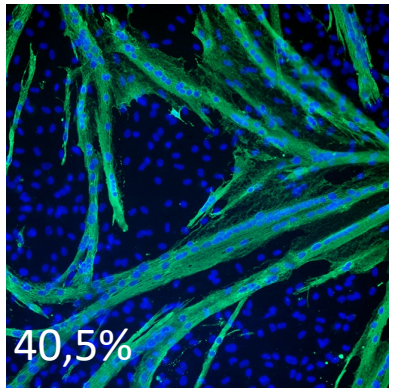
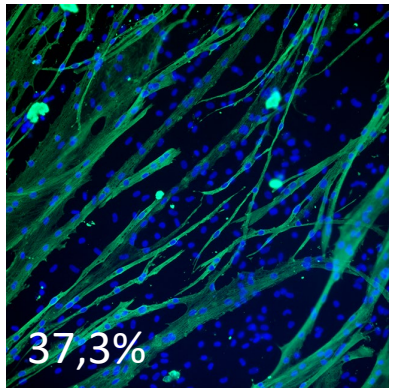
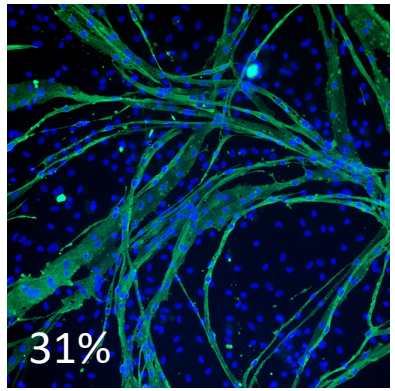
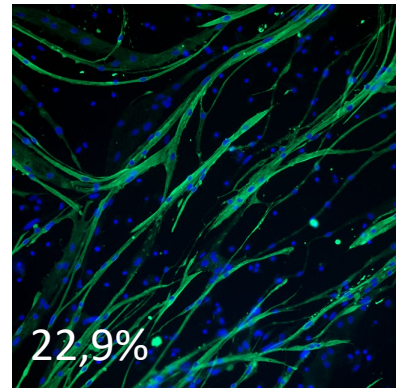
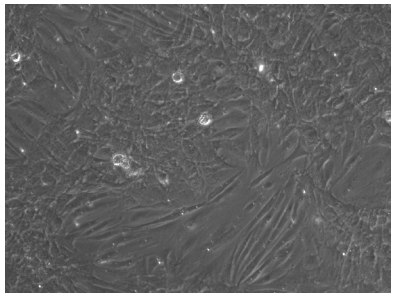
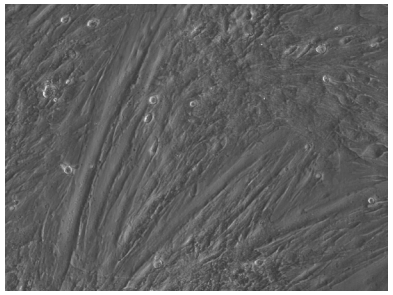
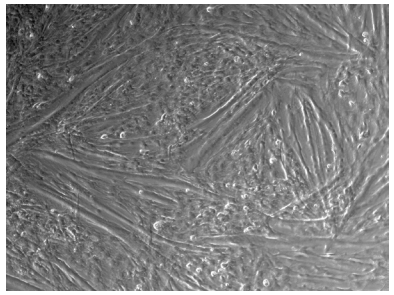
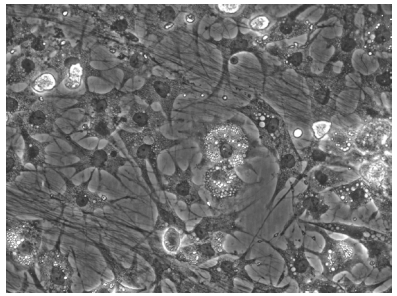
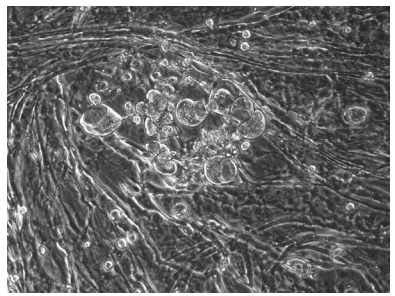
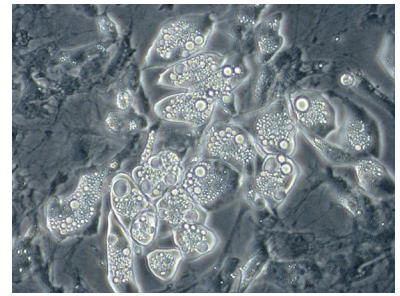
Analyse de genes par qPCR

Mesure fusion par microscopie

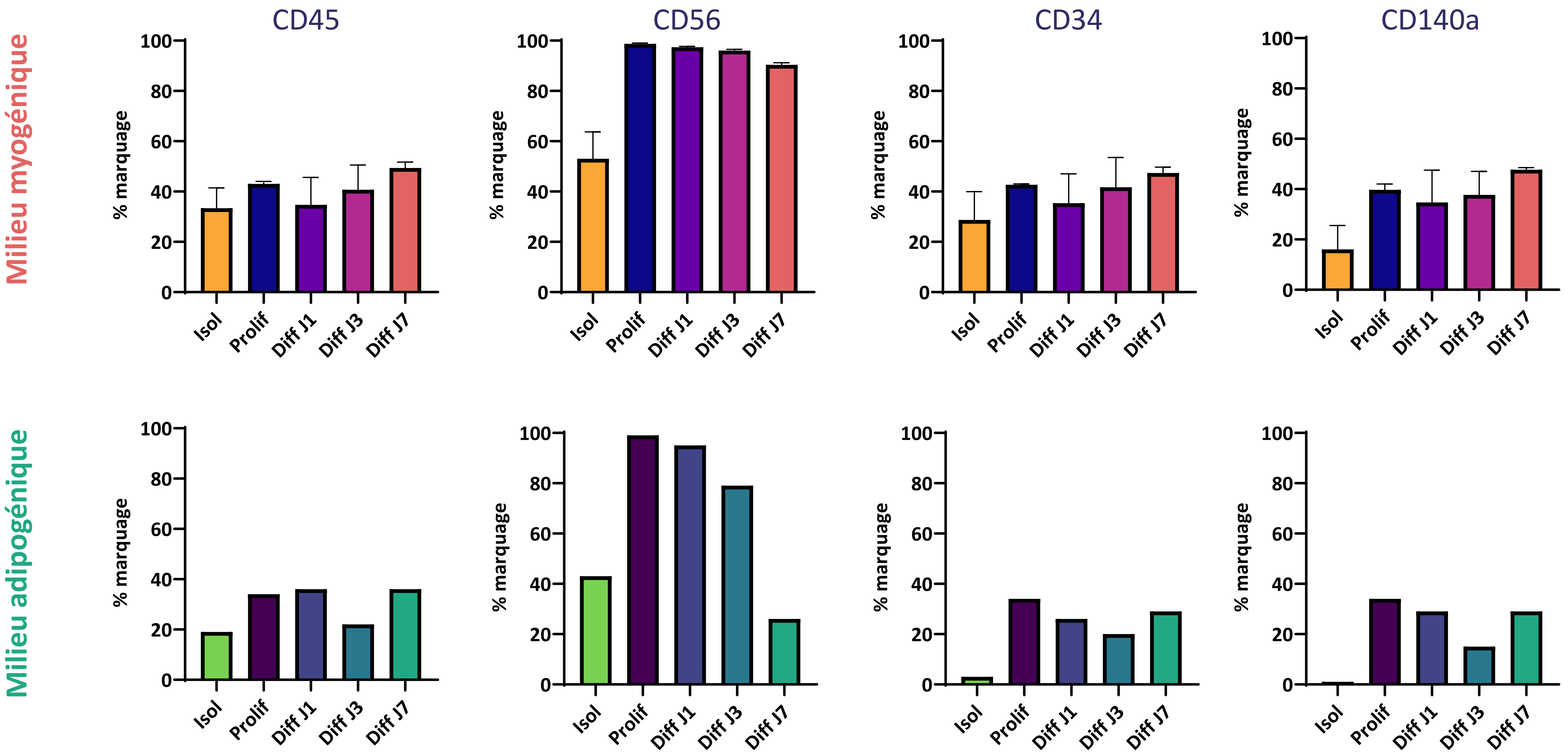
Cellules satellites en condition myogénique

	Prolif	Diff J1	Diff J3	Diff J6
Marquage MF20				
Contraste de phase				

Cellules satellites en condition adipogénique

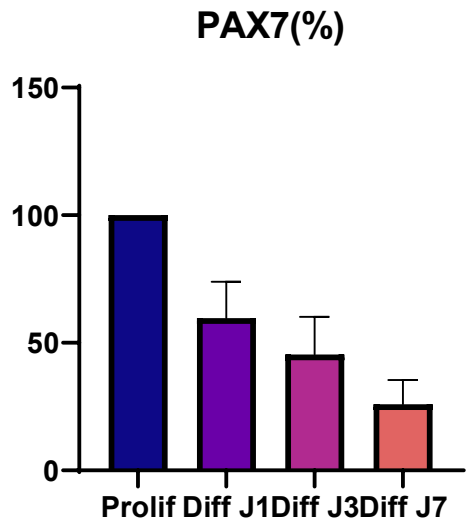
Prolif	Diff J1	Diff J3	Diff J6	Diff J10	Diff J14
					
					

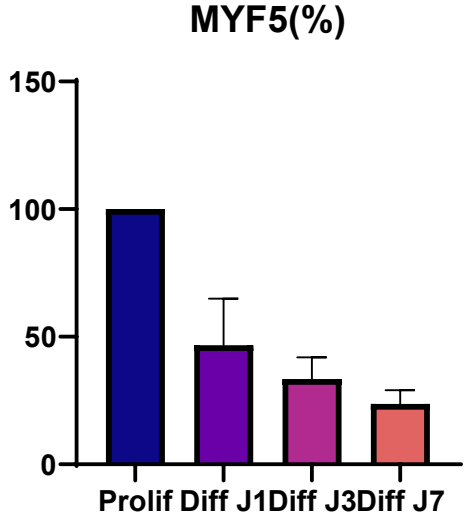
Evolution des marqueurs de surface (cytométrie)

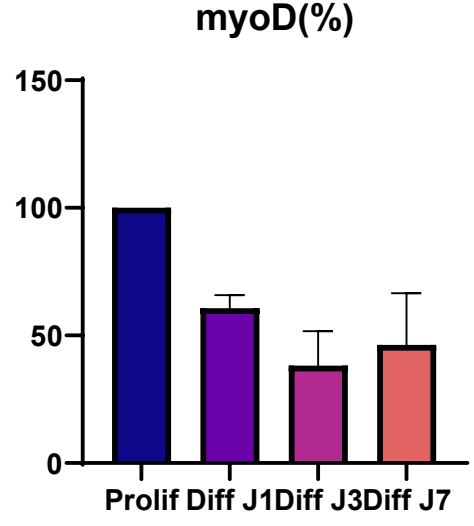


Expression des marqueurs myogéniques (qPCR)

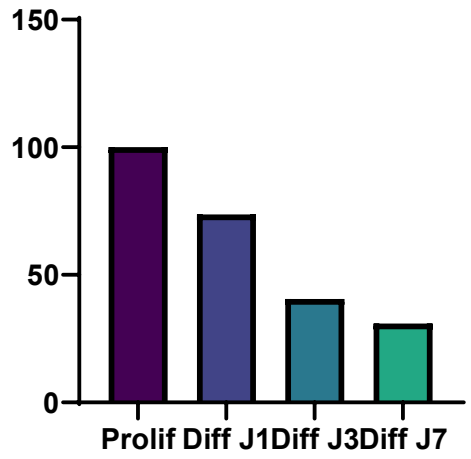
Milieu myogénique

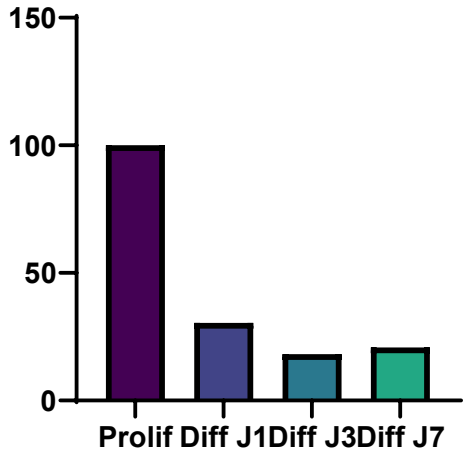


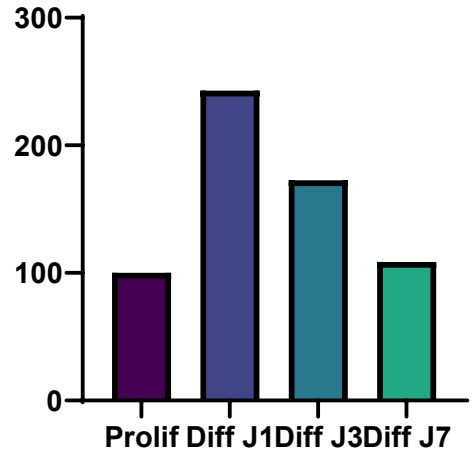




Milieu adipogénique

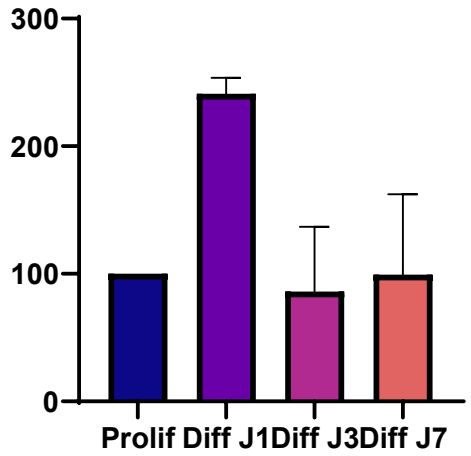


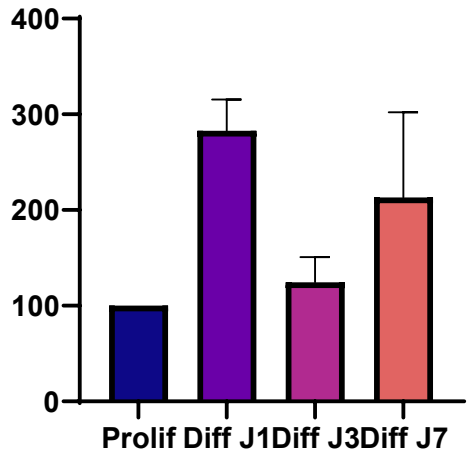


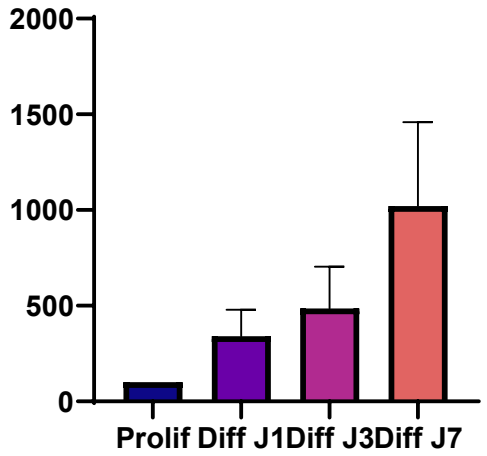


Expression des marqueurs myogéniques (qPCR)

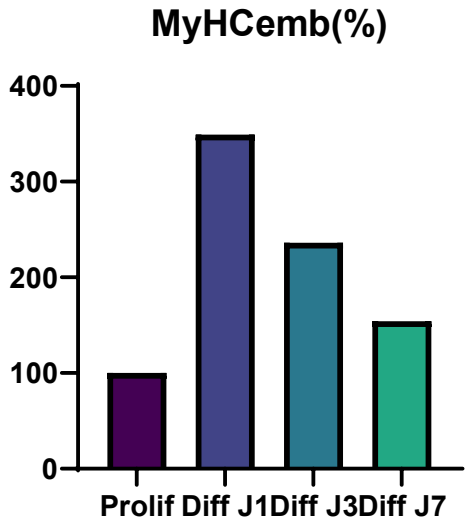
Milieu myogénique

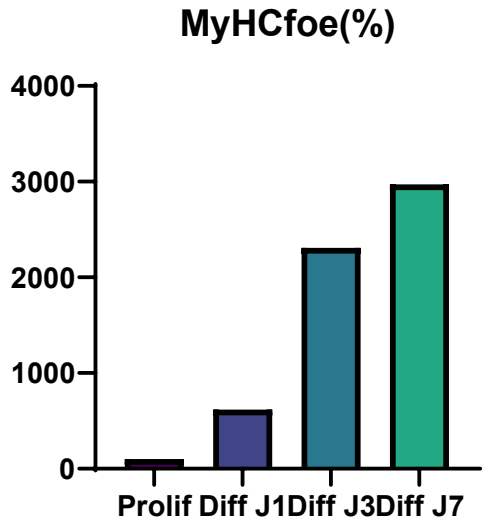


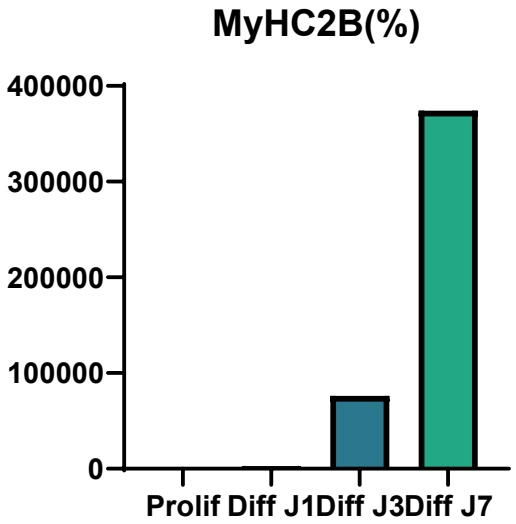




Milieu adipogénique

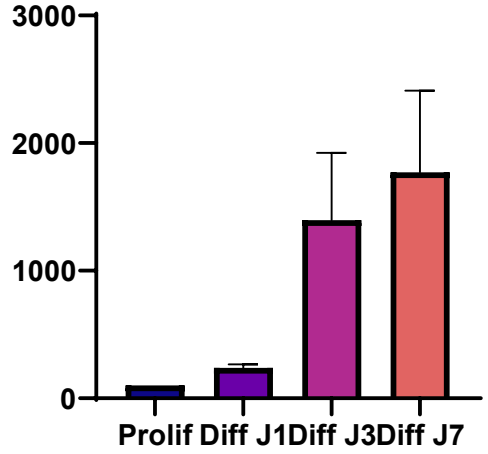


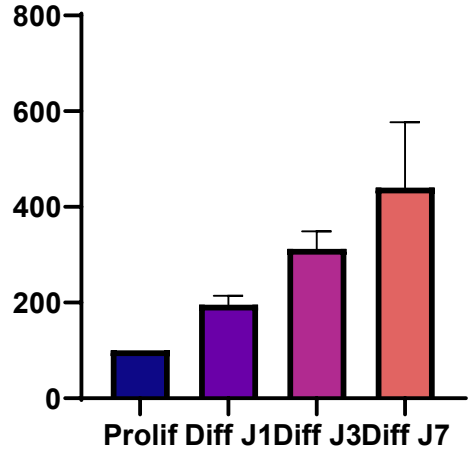


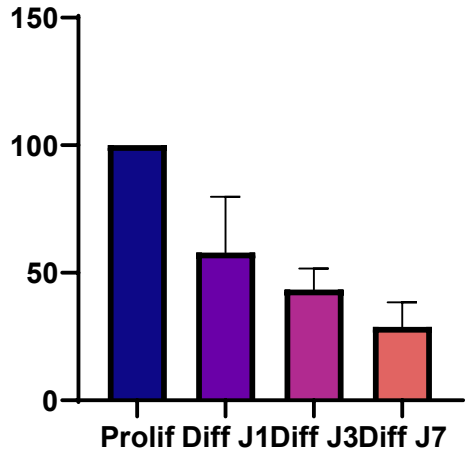


Expression des marqueurs adipogéniques (qPCR)

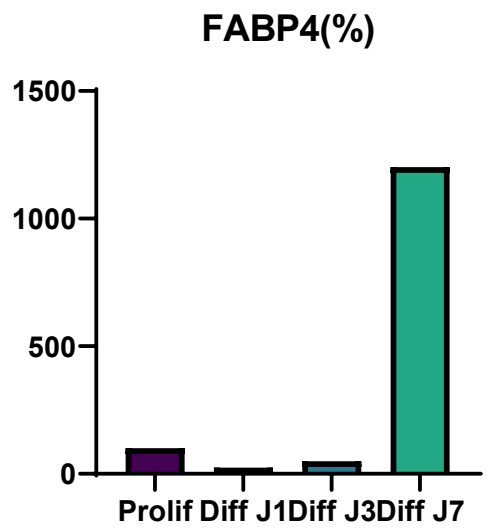
Milieu myogénique

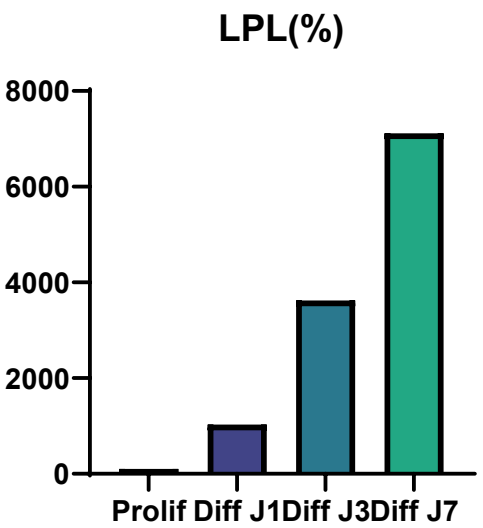


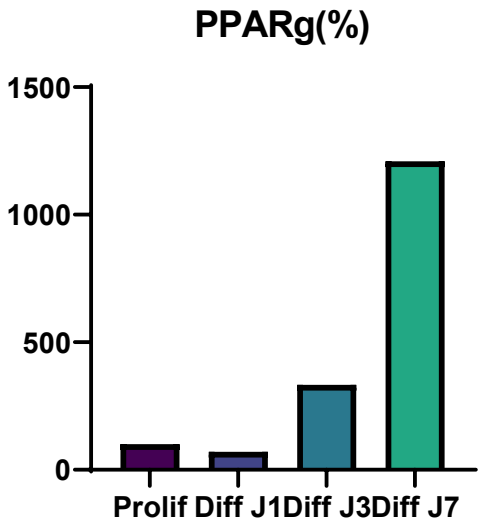




Milieu adipogénique



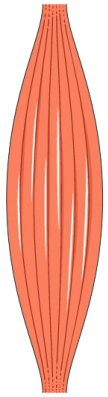




Stratégie de tri

muscle

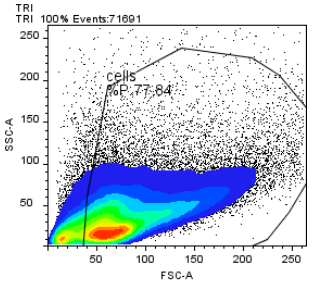
60 g



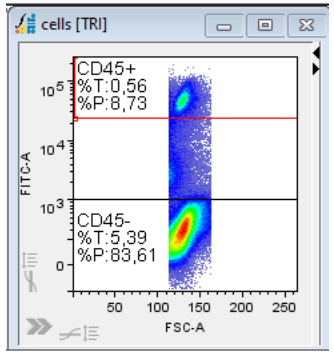
Cellules isolées

10 x 10^6 cellules

5 x 10^6 cellules



Population	Event	% Tot.	% Par.
TRI	71691,00	100,00	100,00
cells [TRI]			77,84

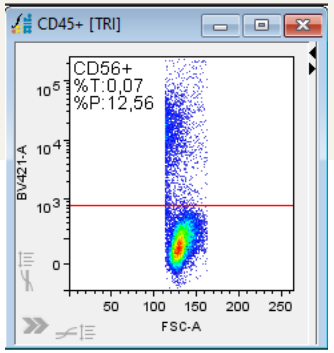


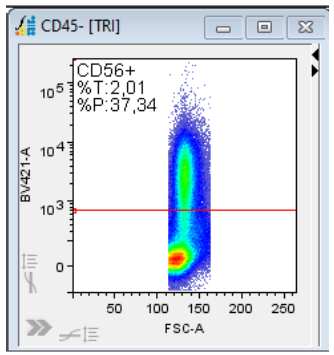
CD45+

9%

CD45-

84%





CD45+/CD56+

13%

CD45+/CD56-

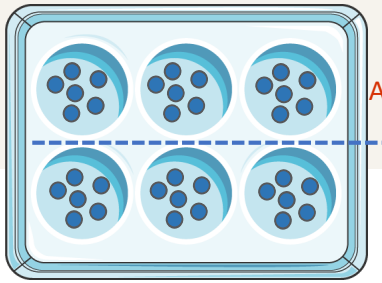
87%

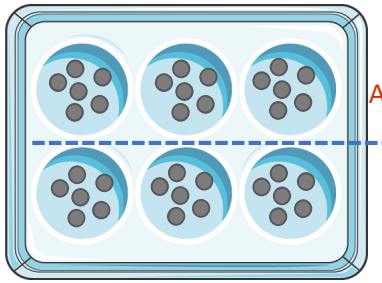
CD45-/CD56+

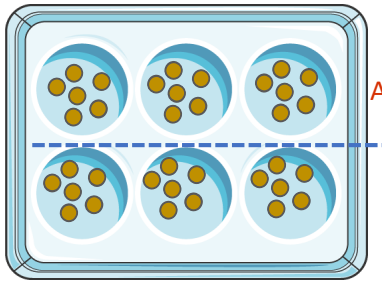
37%

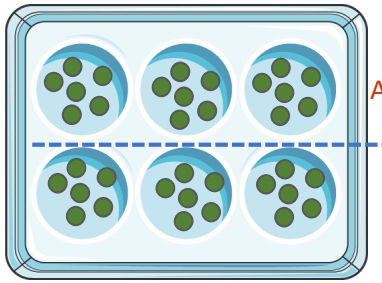
CD45-/CD56-

63%

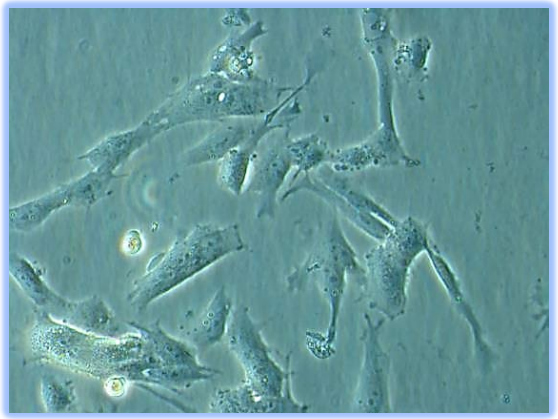
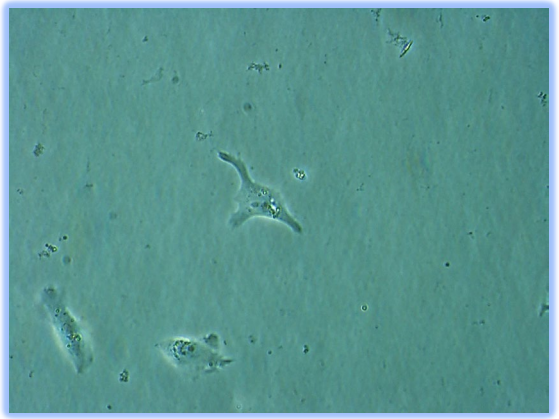
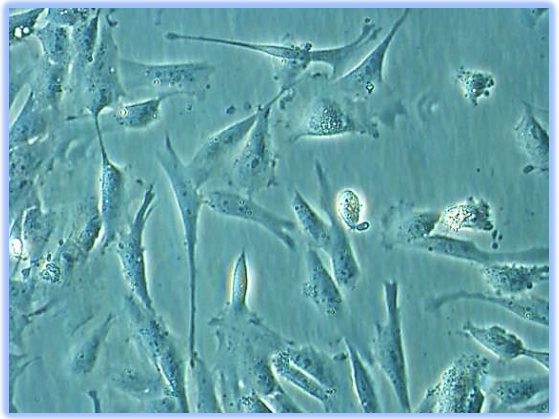
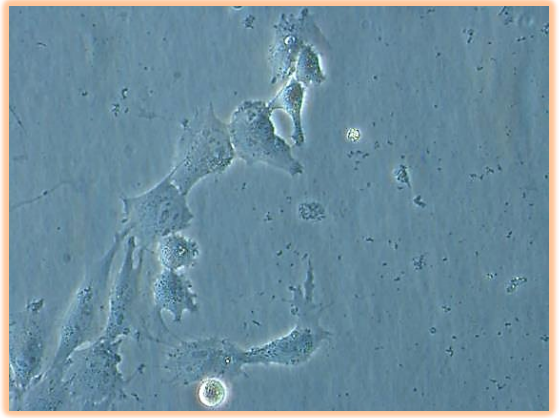
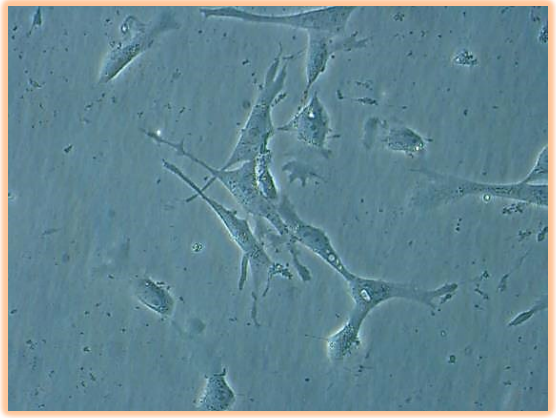
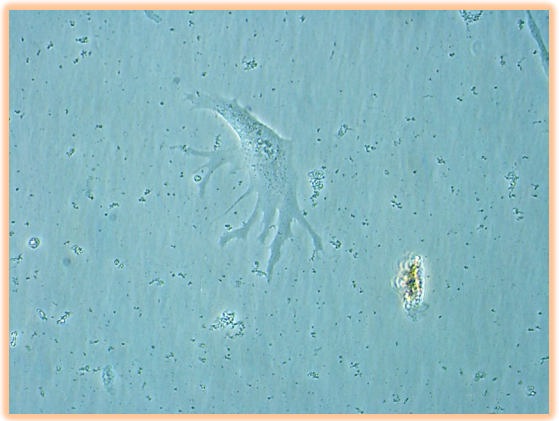
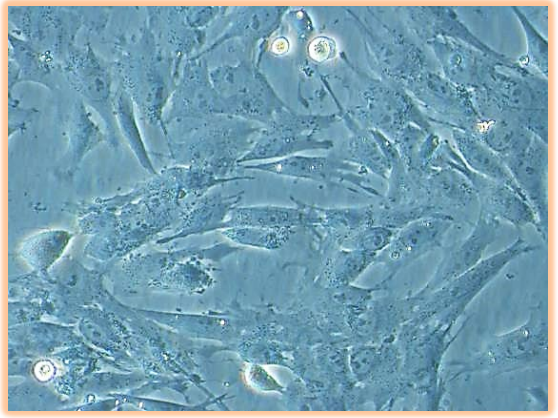






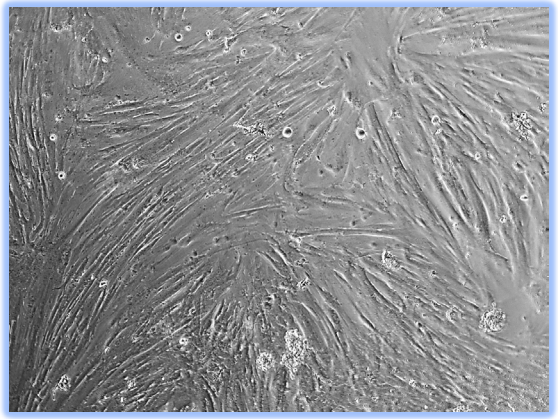
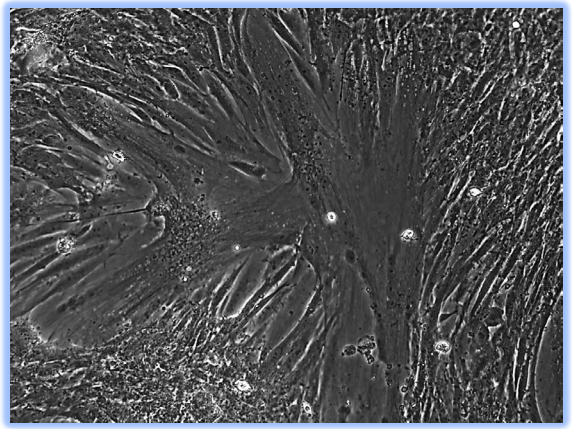
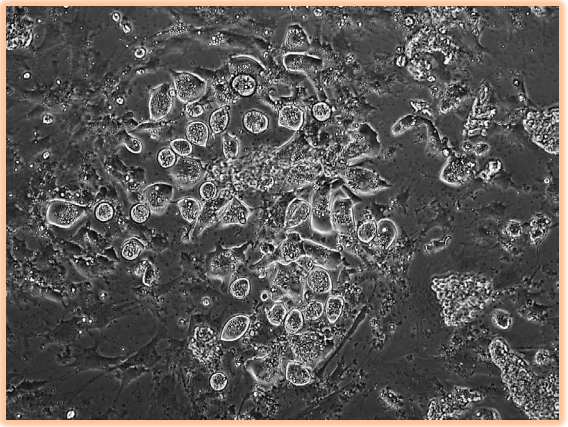


Culture des populations triées

	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				

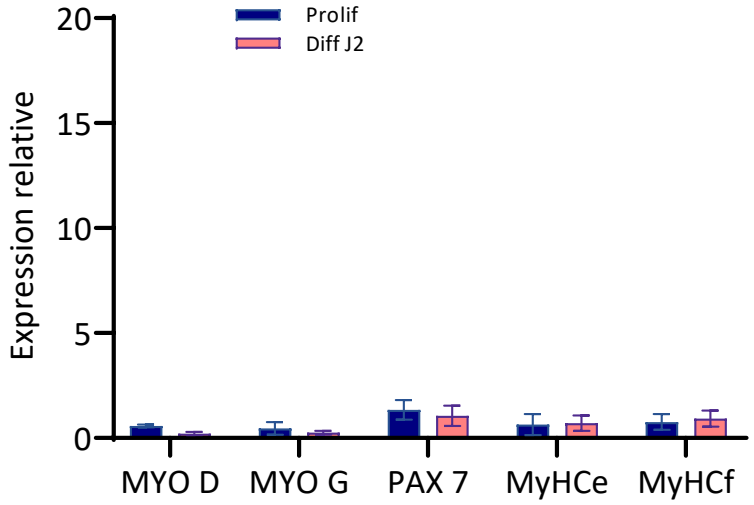
Prolifération J4 (objectif 40)

Culture des populations triées (CD45^{-})

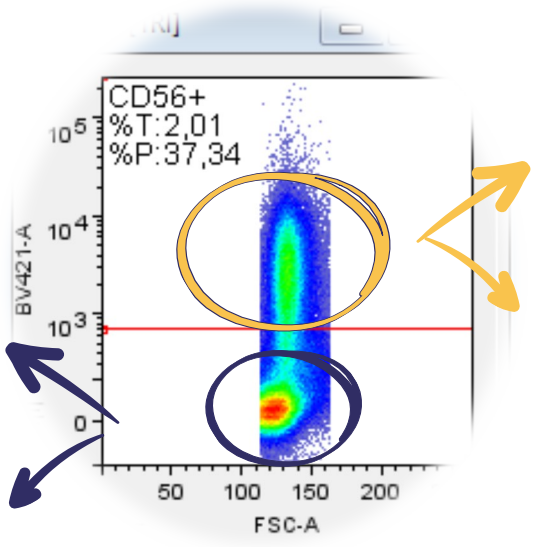
	CD45-/CD56-	CD45-/CD56+
Milieu myogénique		
Milieu adipogénique	DIFF + dans les 2 conditions 	DIFF ++ dans les 2 conditions 

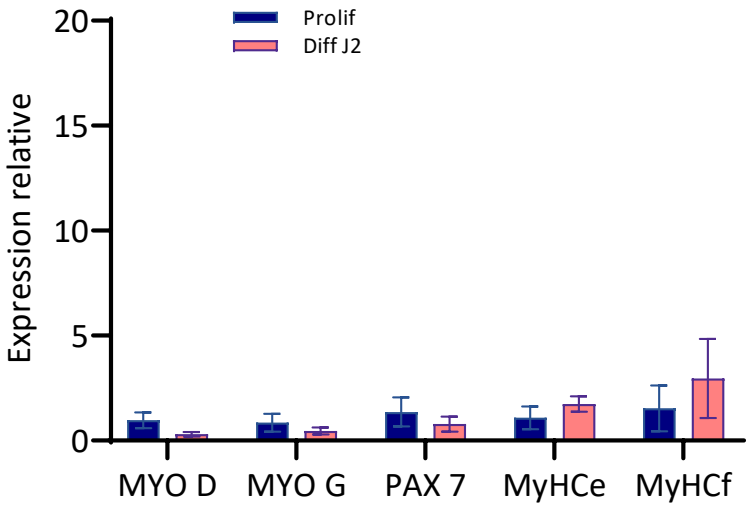
Expression des marqueurs myogéniques après tri

Milieu myogénique

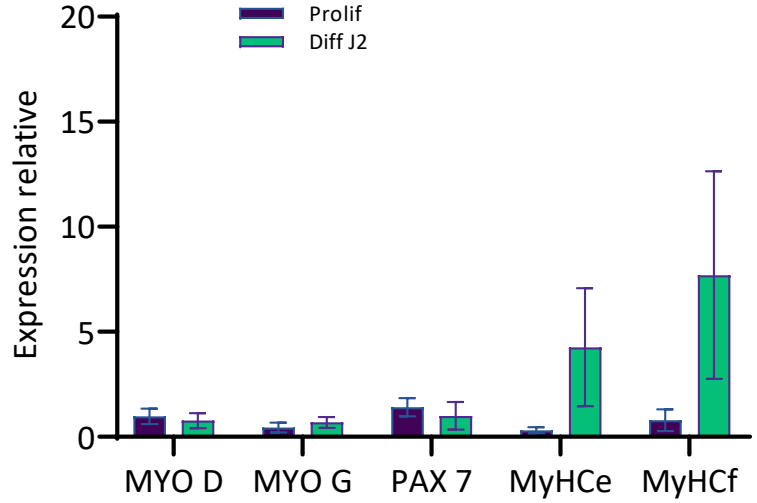


CD45-/CD56+

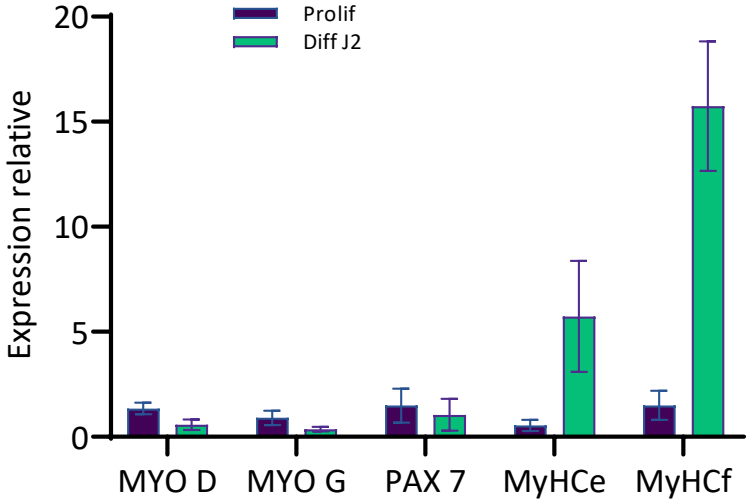




Milieu adipogénique



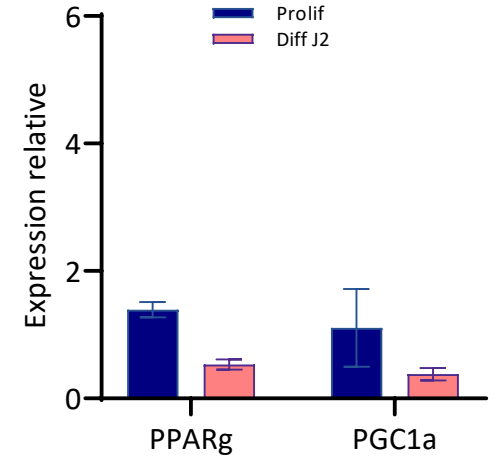
CD45-/CD56-

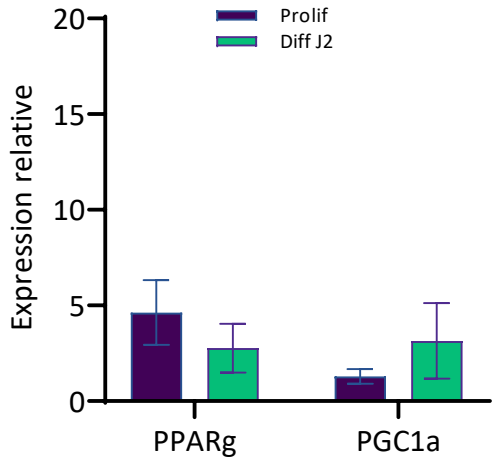


Expression des marqueurs adipogéniques après tri

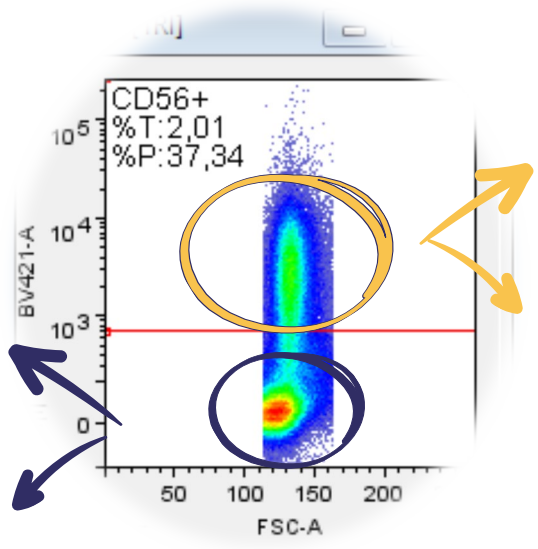
Milieu myogénique

Milieu adipogénique

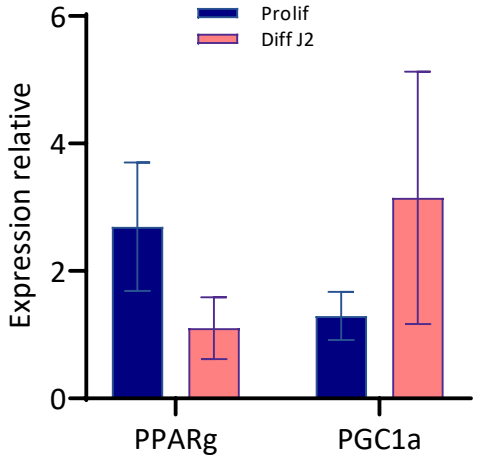


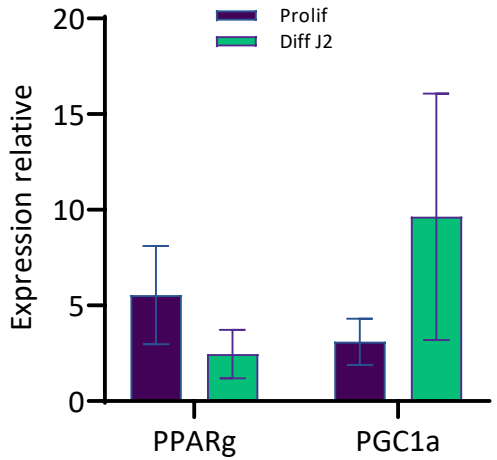


CD45-/CD56+



CD45-/CD56-





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 !)