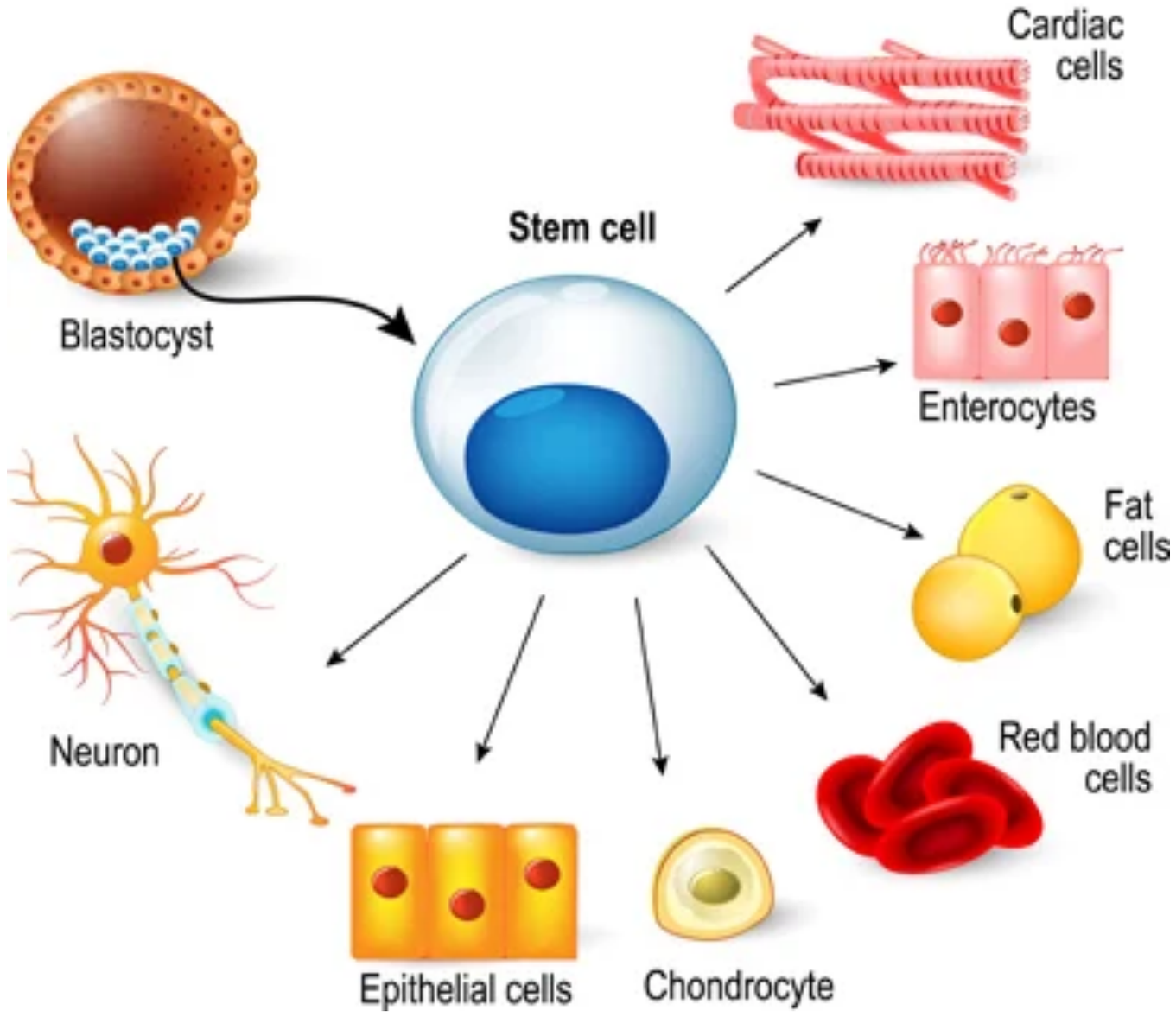
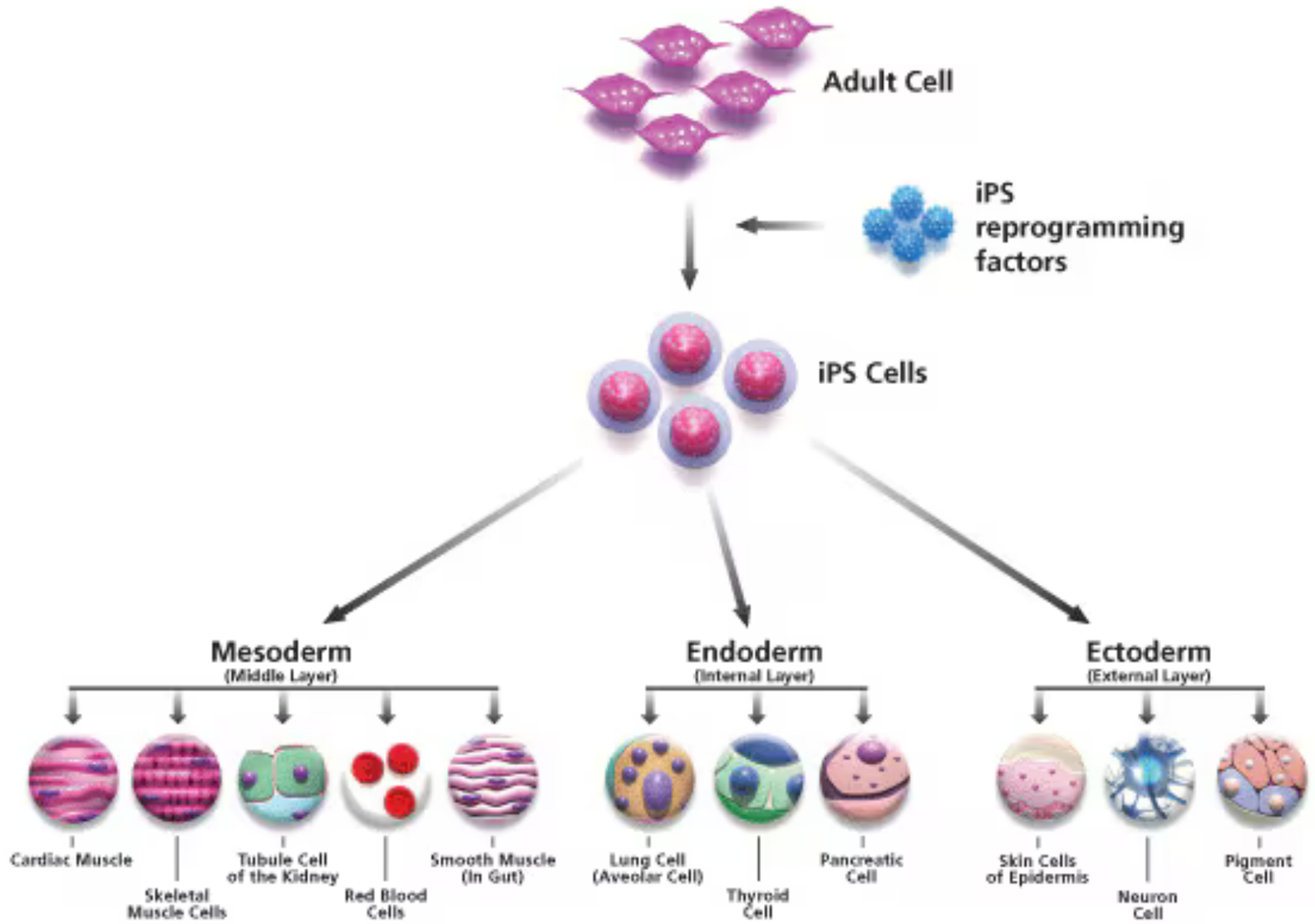
Metabolic reprogramming for stemness and differentiation

Stem cells are toti/multi-potent cells



The ability of stem cells to expand and give rise to specialized progeny underlies development, tissue regeneration, and normal homeostatic organismal function.

iPS cells are experimental surrogate for embryonic SC



WHAT ARE STEM CELLS?

Master Cells

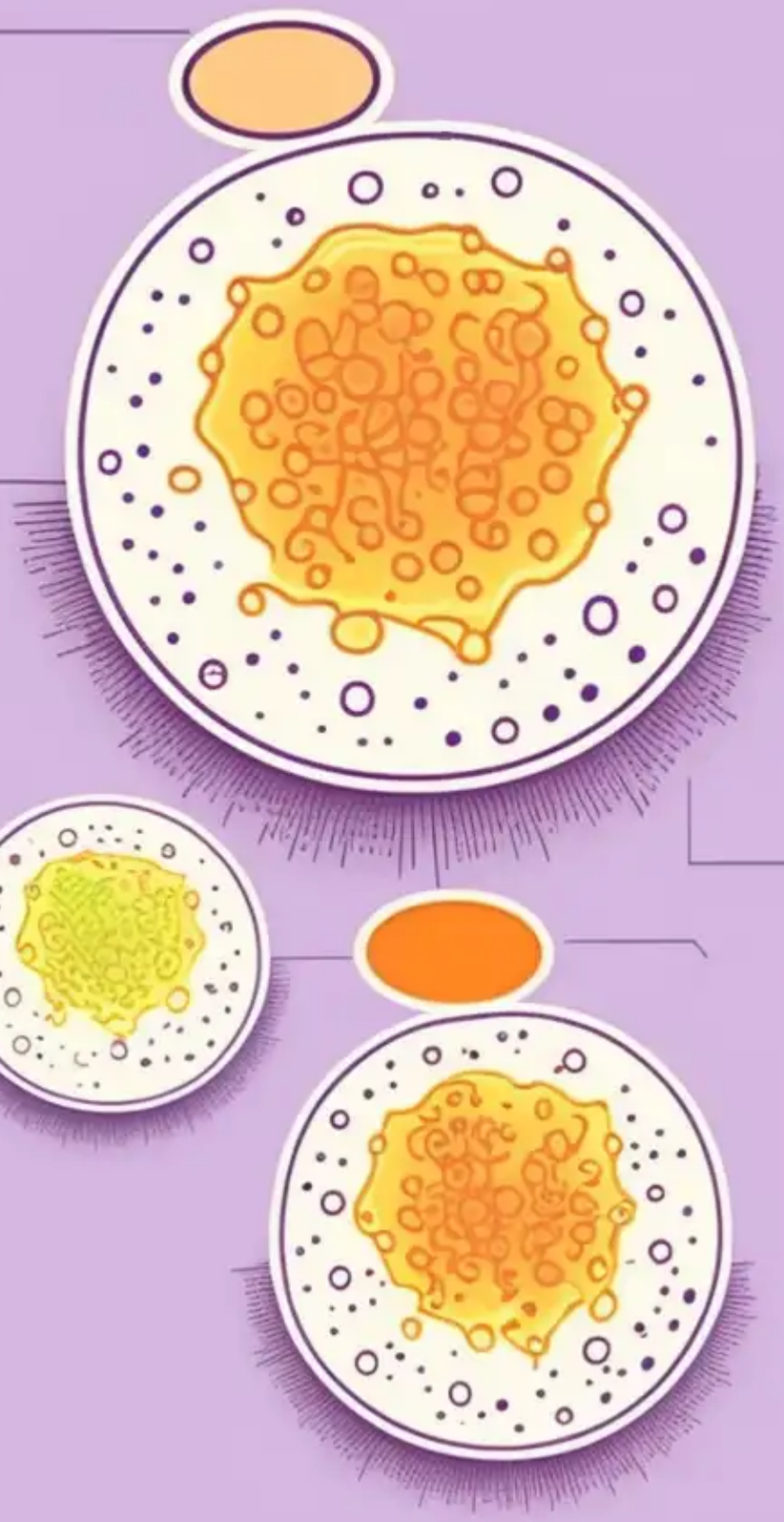
Stem cells are the body's master cells, with the potential to form many different cell types.

Self Renewal

They can self-renew and divide into daughter cells, either new stem cells or specialized cells. Stem cells provide new cells for the body as it grows and replaces specialized cells lost through normal wear and tear.

Differentiation

Differentiation is the process by which a cell specializes, acquiring the ability to perform certain functions and failing to develop



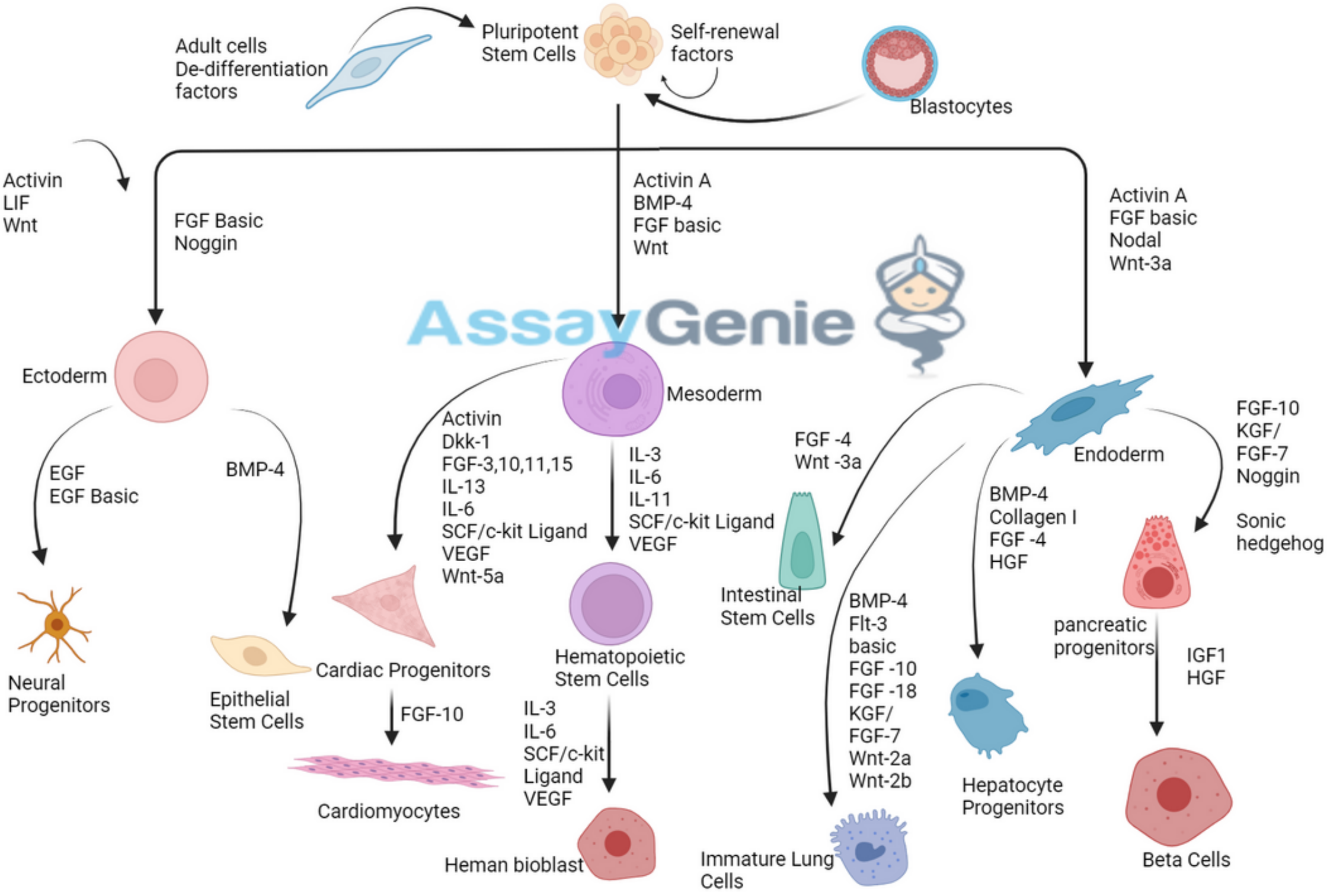
In detail

In developmental biology, differentiation refers to the process by which a less specialized cell matures into a more distinct form and function. At the same time, in medicine, it is used to determine which disease a patient is suffering from among those with similar symptoms. It can also refer to an increase in morphological or chemical heterogeneity or the act or process of differentiating; development from the one to the many, the simple to the complex, or the homogeneous to the heterogeneous.

Stem Cell Research

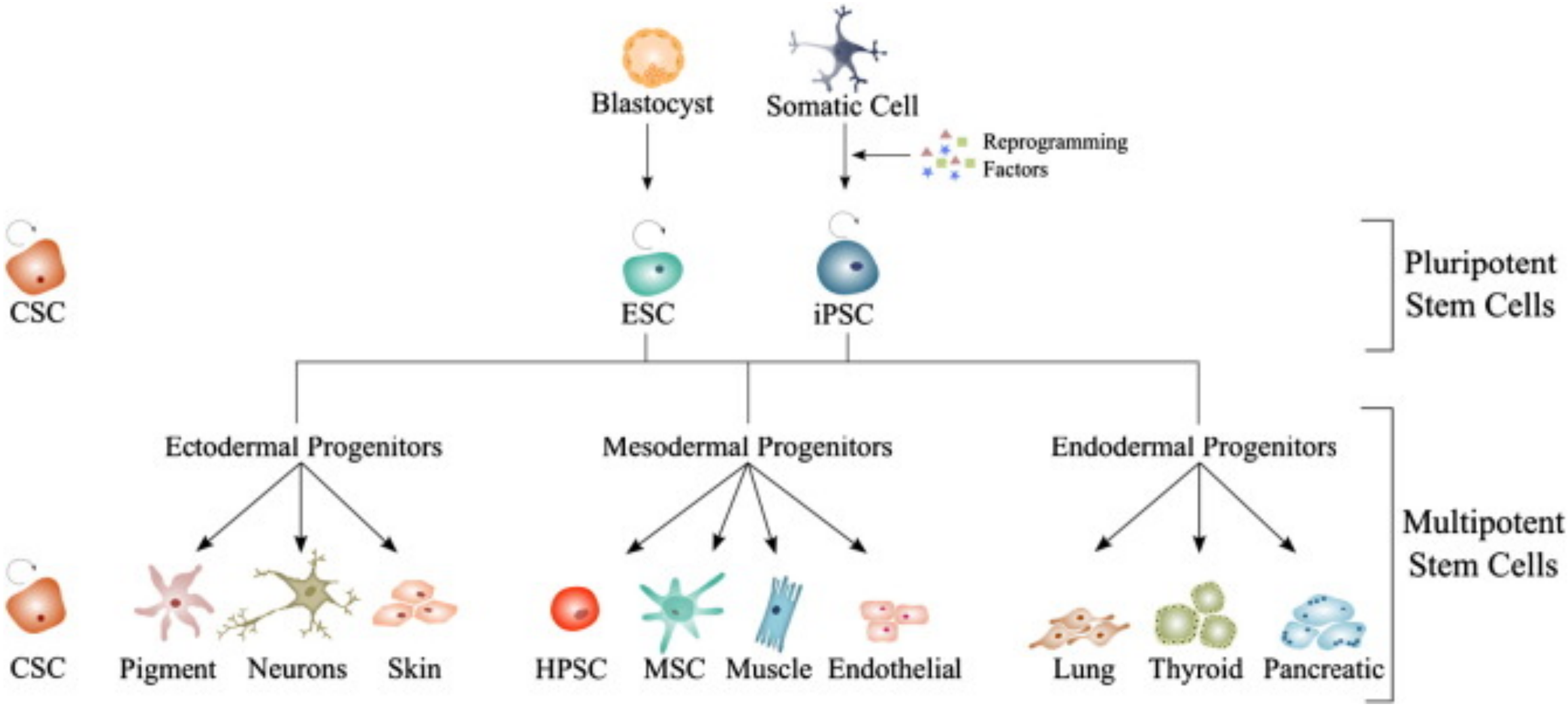
One of the most promising applications of stem cells is in regenerative medicine, where stem cells are used to repair or replace damaged tissues and organs. For example, stem cells have been found in multiple peer-reviewed studies to reduce inflammation and modulate the immune system, both of which may be beneficial for a variety of

Cells change phenotype and function upon differentiation



SC evolve and face different demands/environments

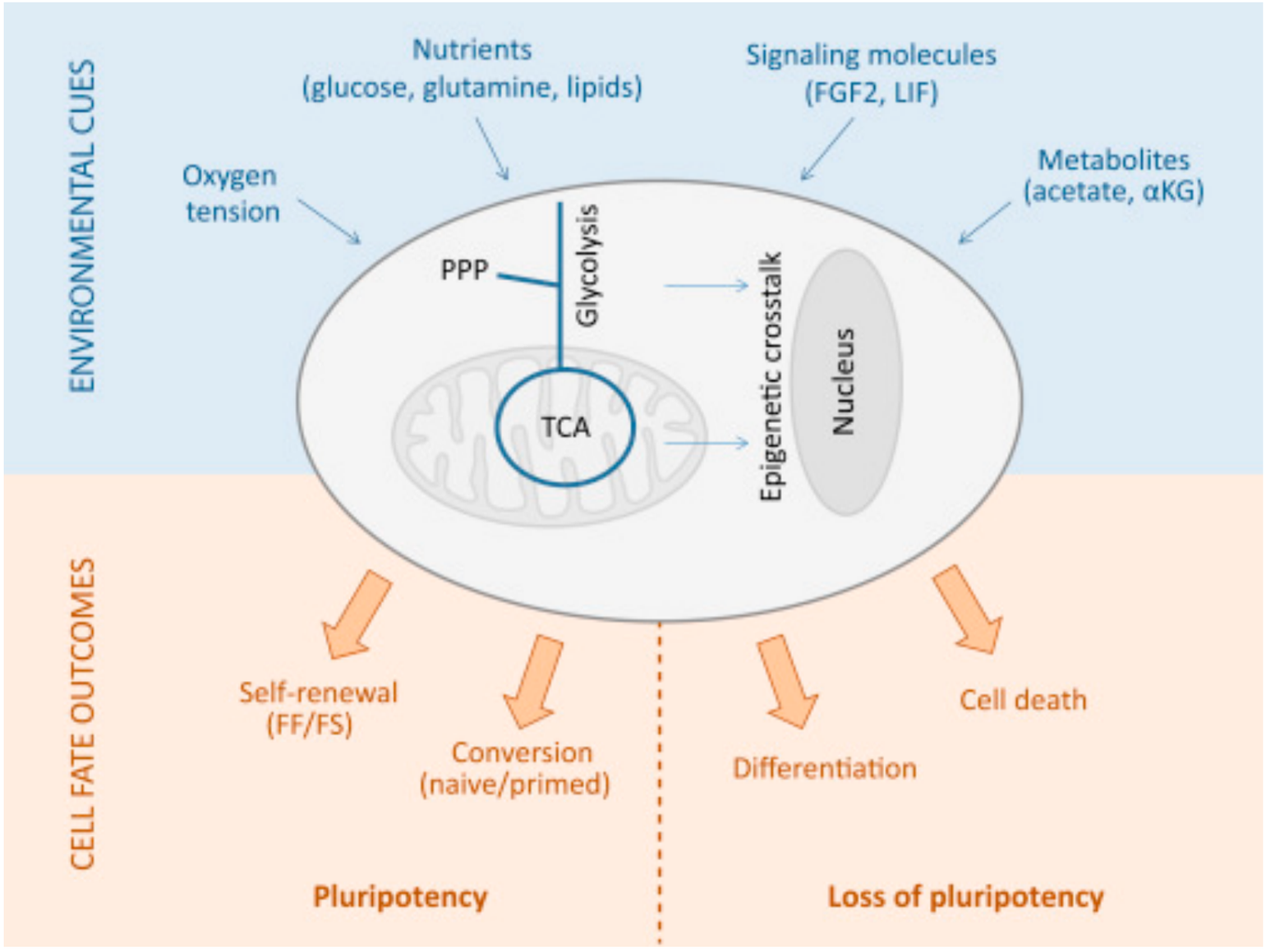
Stem cells have two jobs: to self-renew and to differentiate.



Stemness: maintaining properties and “youth”. Establish a “niche”.

Differentiation: change function and location. Widespread reprogramming of gene expression.

SC evolve and face different demands/environments



Mlody & Prigione, ***Cell Stem Cell****, 2016*

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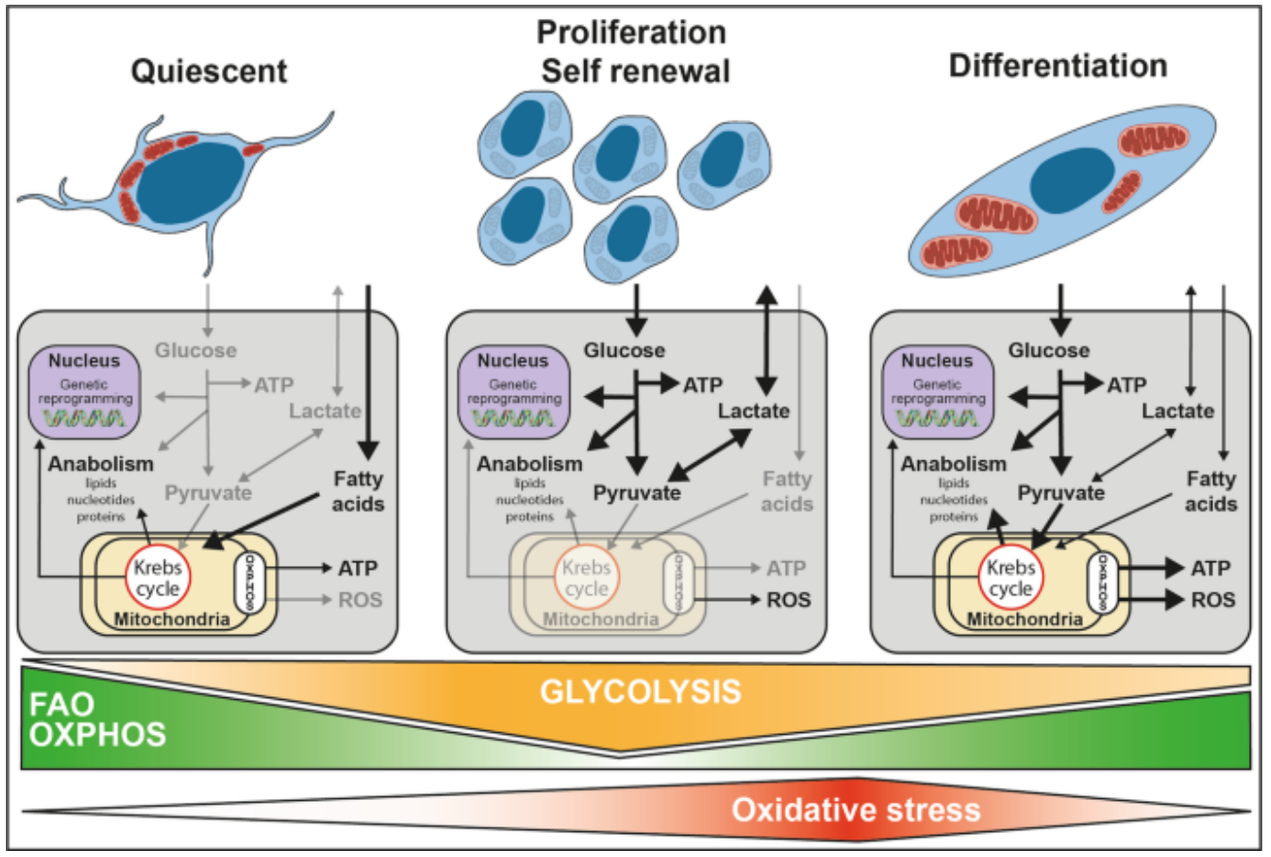


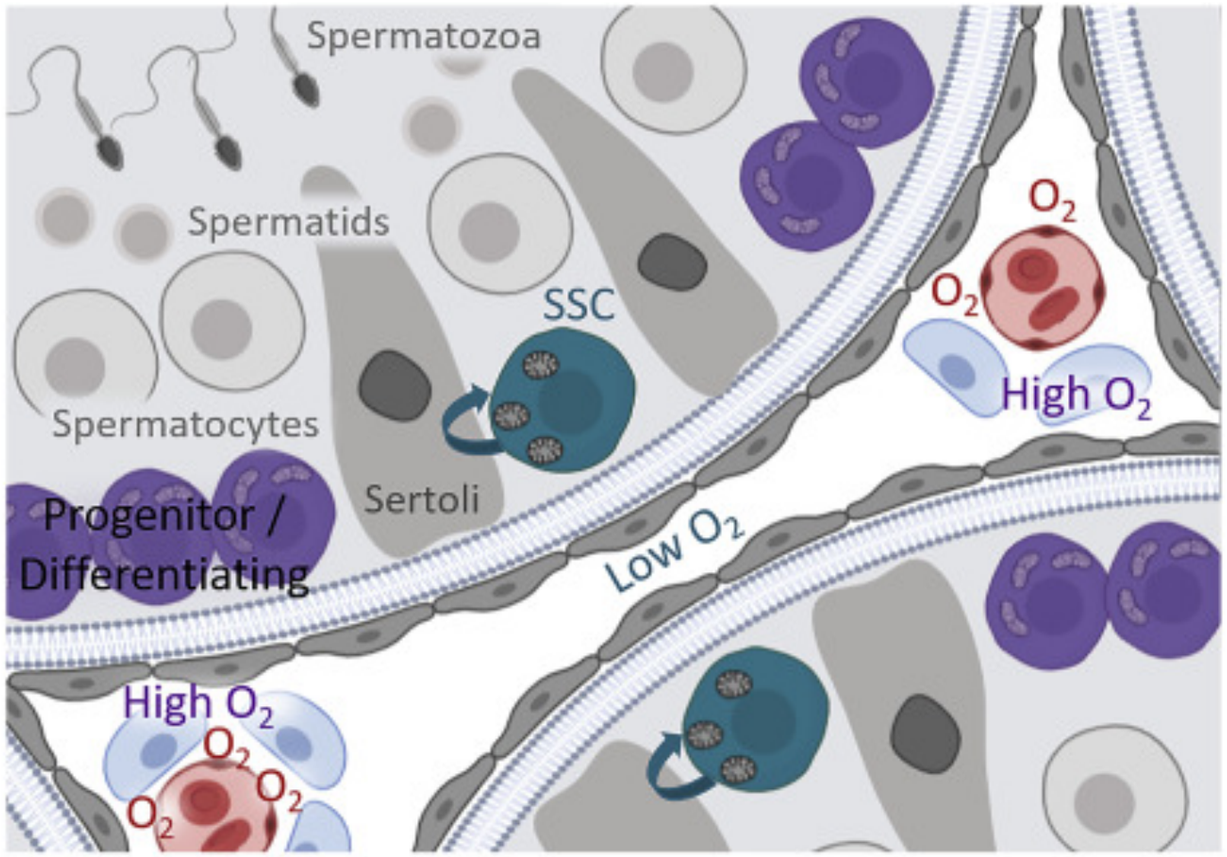
Relaix et al, ***Nat Comm****, 2021*

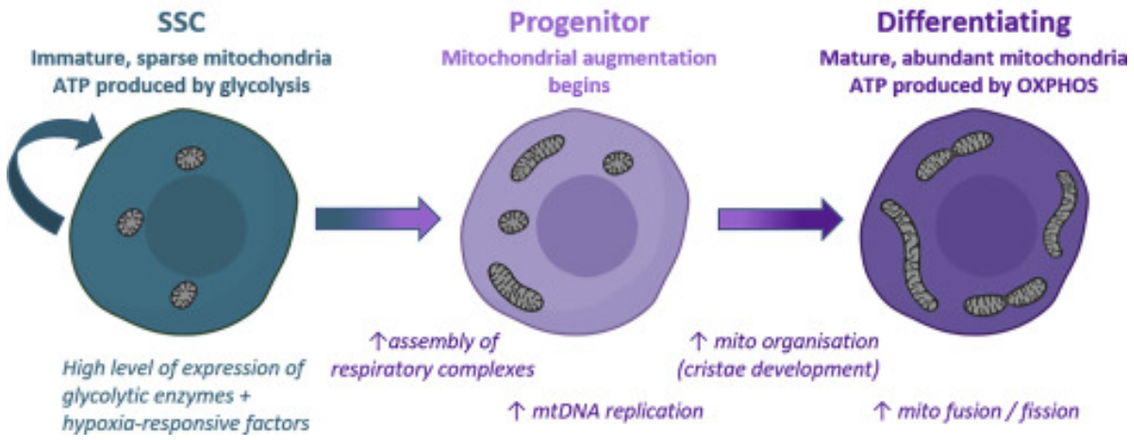
Cells of different lineages will have cell-type-specific functions, which may increase reliance on certain metabolic pathways, or reside in unique microenvironments that dictate nutrient availability.

Beyond meeting cell demands, metabolites also serve regulatory roles, influencing signaling pathways and chromatin modifications that ultimately modulate gene expression programs.

Collectively, cell state, lineage, and location collaborate to determine the metabolic preferences and requirements of a given cell. Underscoring the importance of metabolic fine tuning to meet cell-type-specific functions, metabolic dysregulation is causative in developmental pathologies such as inborn errors of metabolism, malignancy, and other diseases.

SC evolve and face different demands/environments





*Lord & Nixon, **Dev Cell**, 2020*

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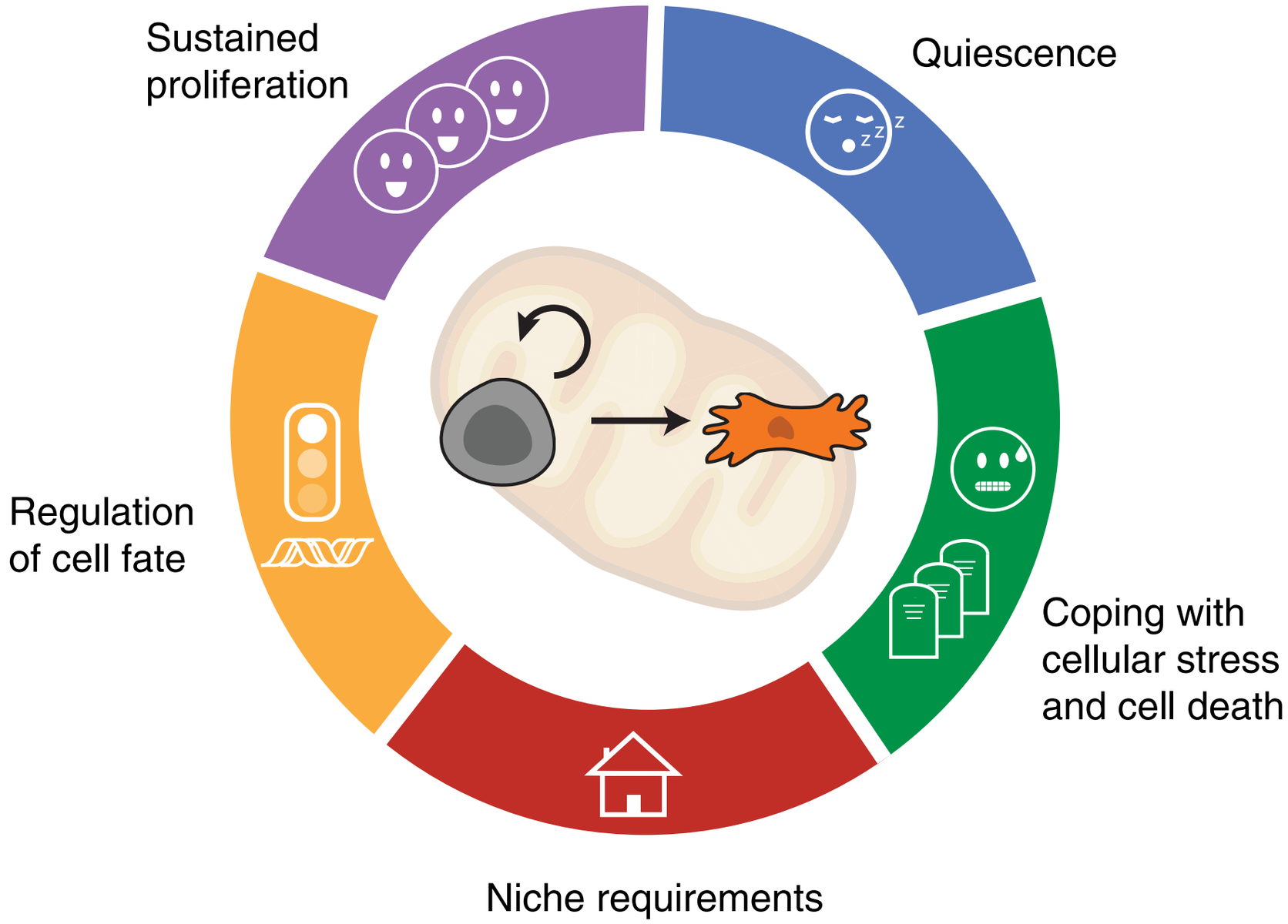
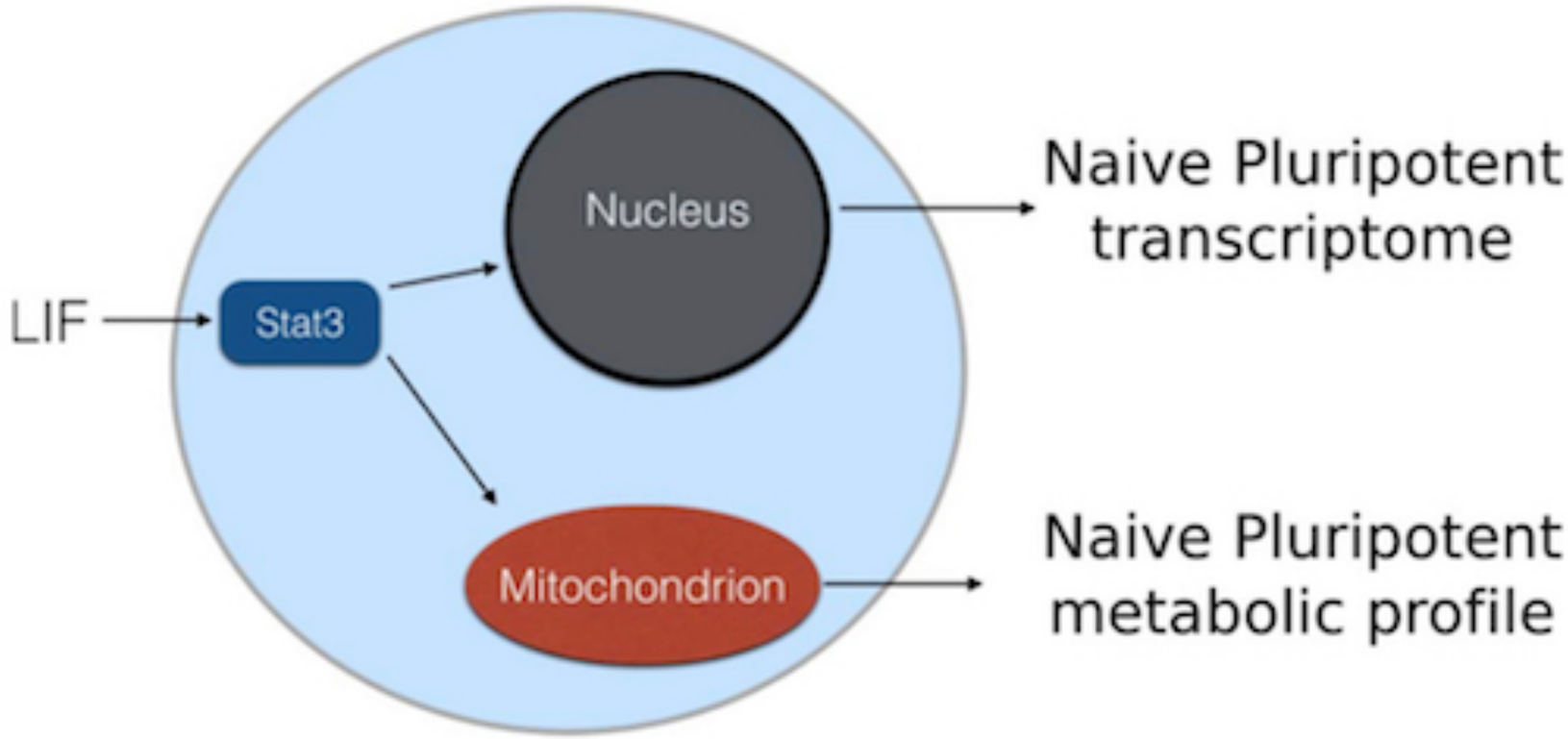
Metabolism supports both stemness and differentiation



Cell metabolism is emerging as an additional determinant of stem cell function.

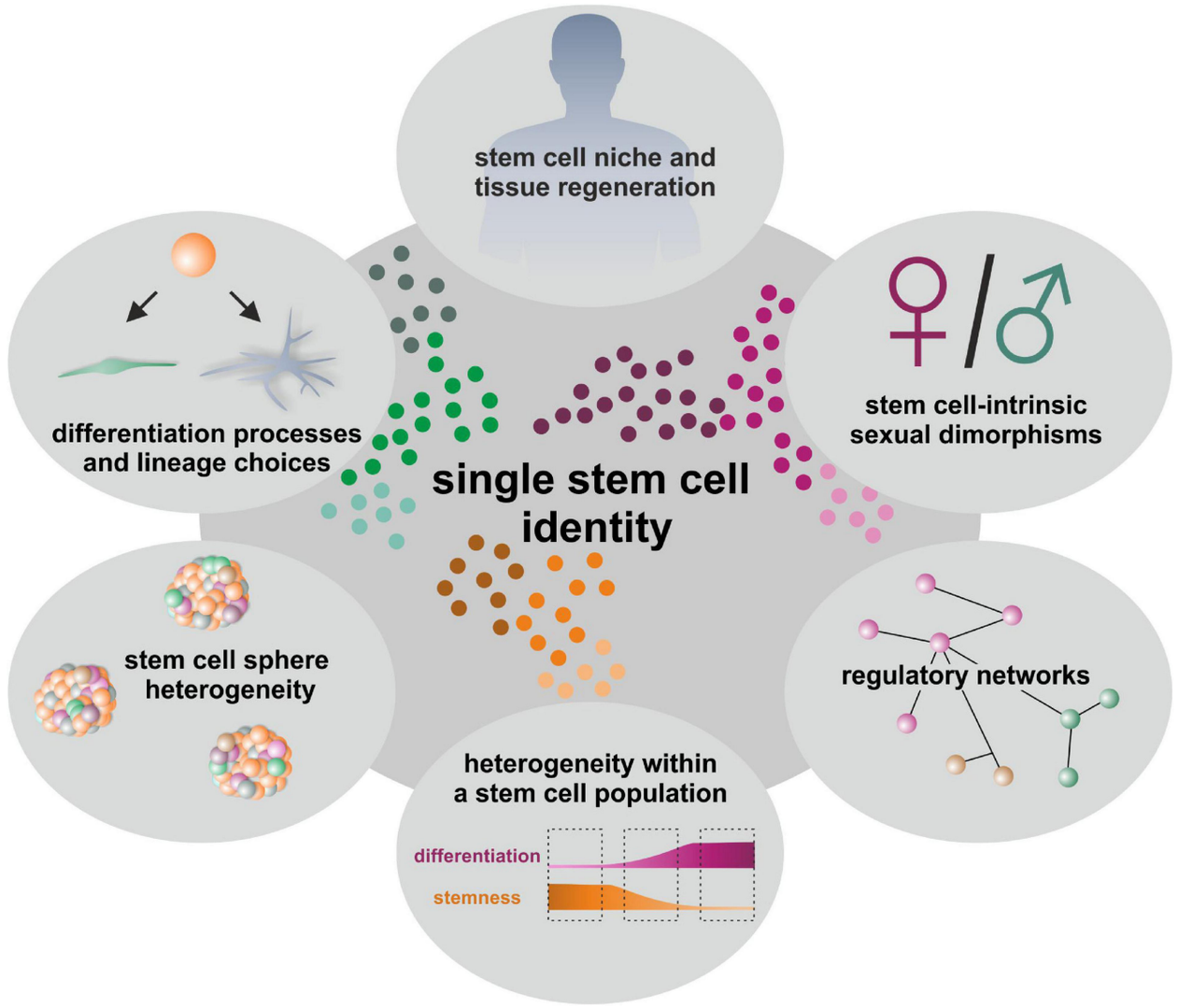
Jackson & Finley, ***Cell Stem Cell****, 2024*

Metabolism supports both stemness and differentiation



Maintenance of stemness (or cell reprogramming) requires both signals to the nucleus (inc: EPIGENOME REMODELING) and to metabolic hubs (METABOLISM REWIRING)

CAVEAT: not all SCs are the same

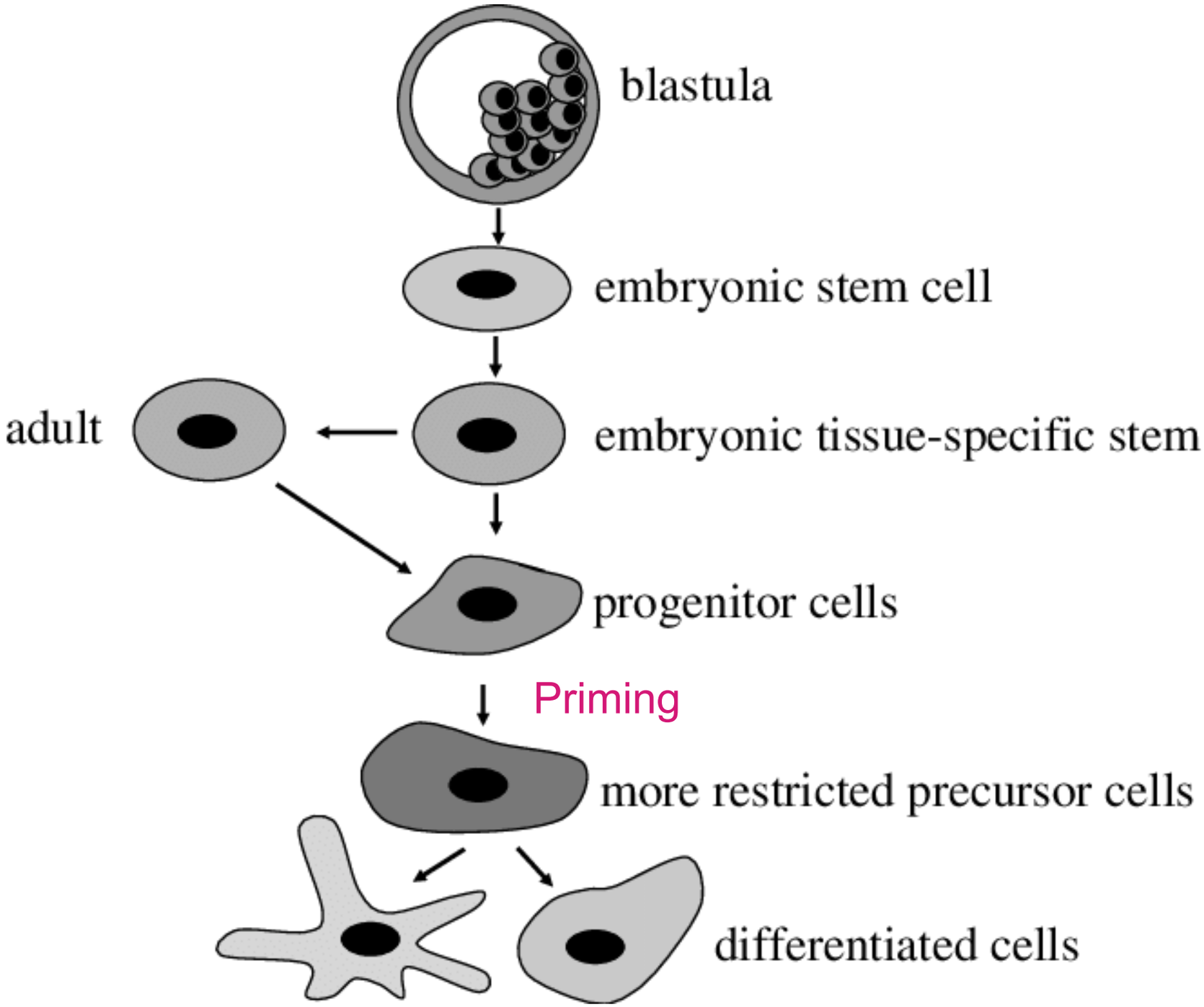


Embryonic stem cells isolated from the inner cell mass of the pre-implantation blastocyst are highly proliferative and undergo rapid cell division both in the pluripotent state and upon the induction of multi-lineage differentiation.

Tissue-resident stem cells found in the intestine are maintained in both highly proliferative and more dormant, metabolically quiet states.

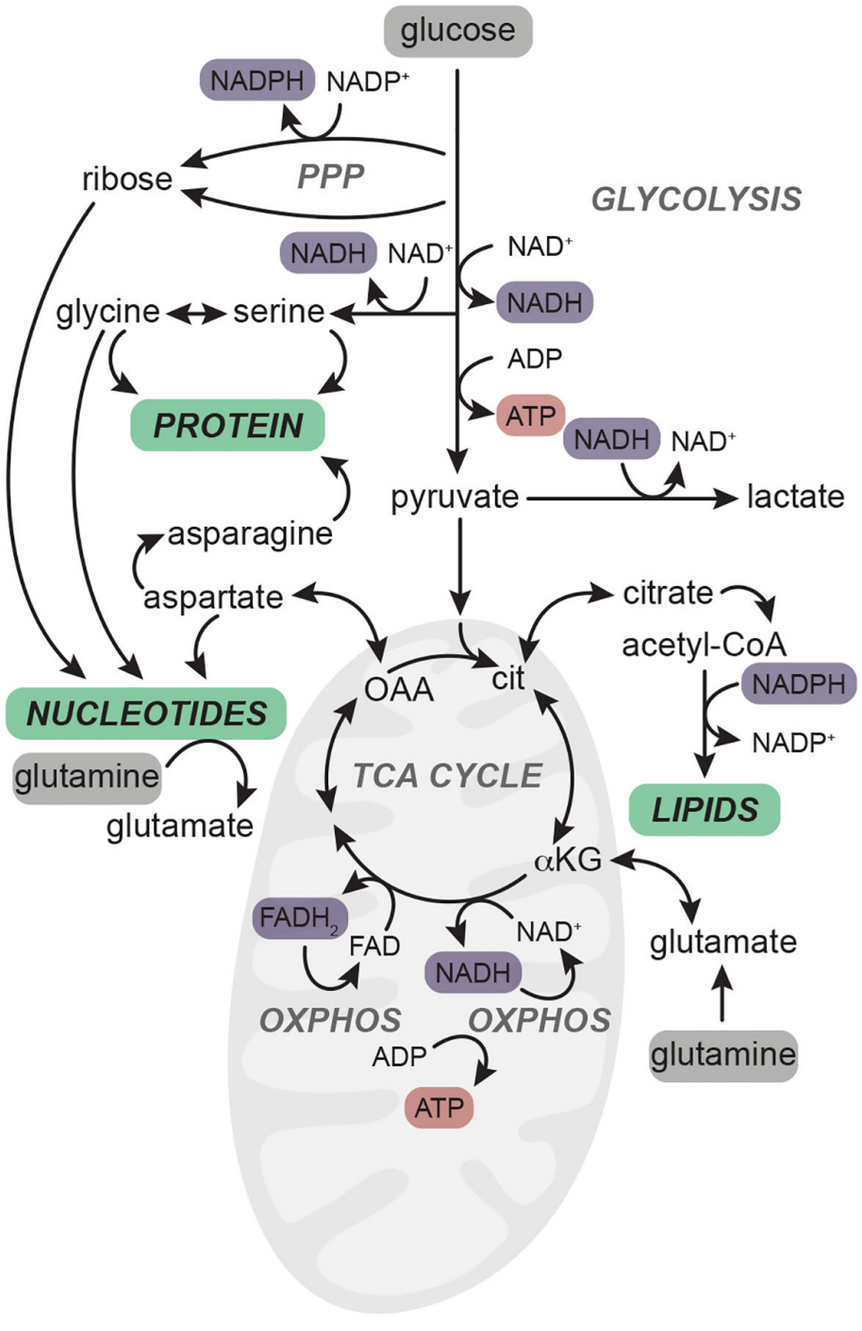
In other tissue compartments such as the skin, muscle, brain, and hematopoietic system, typically quiescent stem cell populations are rapidly mobilized in response to normal physiologic cues or in the context of tissue regeneration.

Embryonic vs Progenitor Stem Cells (ESC/PSC)



Role 1: metabolism supports cell proliferation

Like cancer cells, a key feature of stem cells is the capacity for rapid proliferation. Both cell types have therefore evolved metabolic strategies to support proliferation, many of which are shared between stem cells and cancer cells.



Intlekofer & Finley, ***Nat Metab****, 2019*

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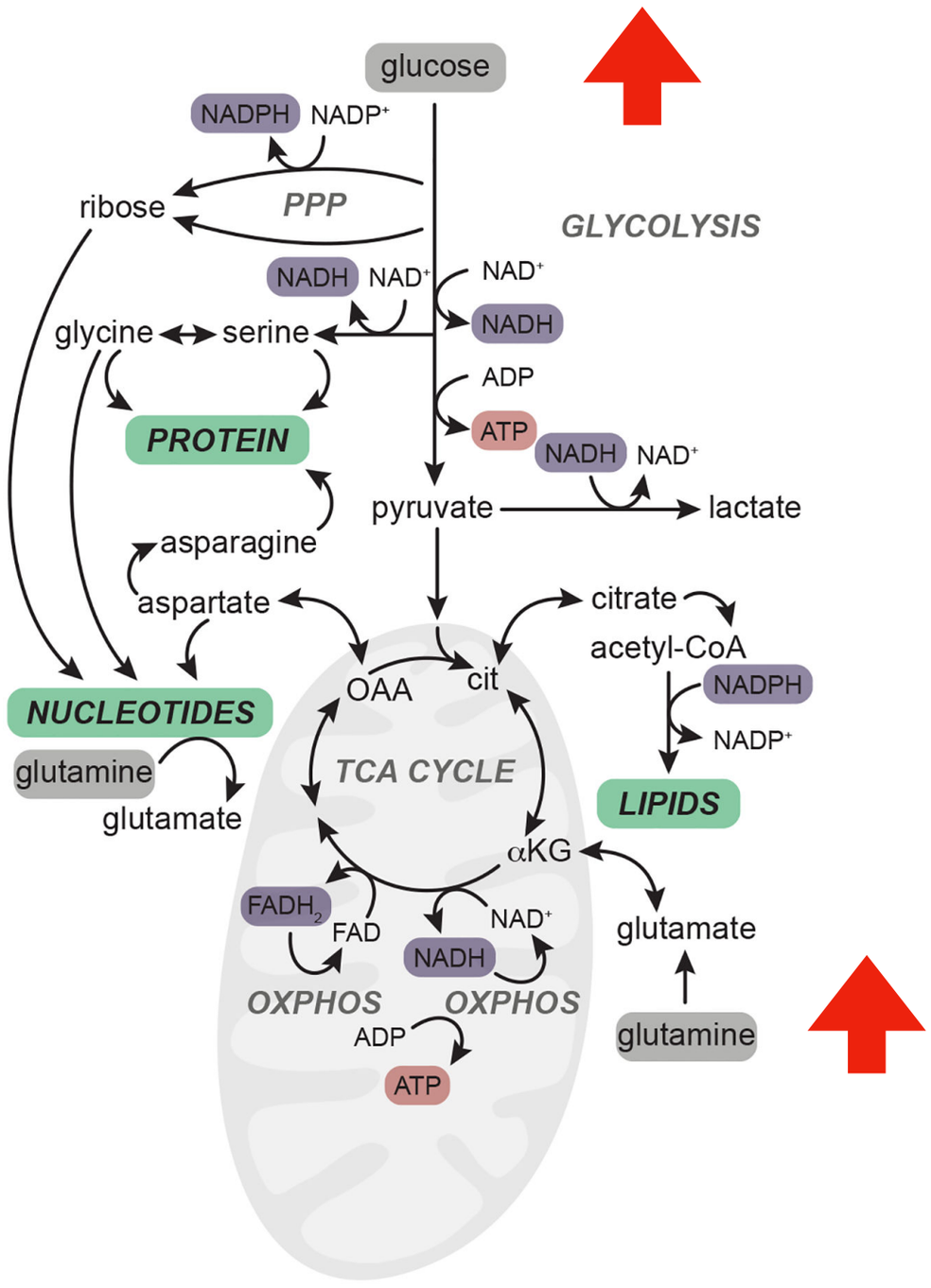


Uptake of both GLC and GLN is generally increased to sustain biomass production.

Note: Naive PSC (ESC) are totally independent on GLN and can grow in absence of GLN (prototrophs).

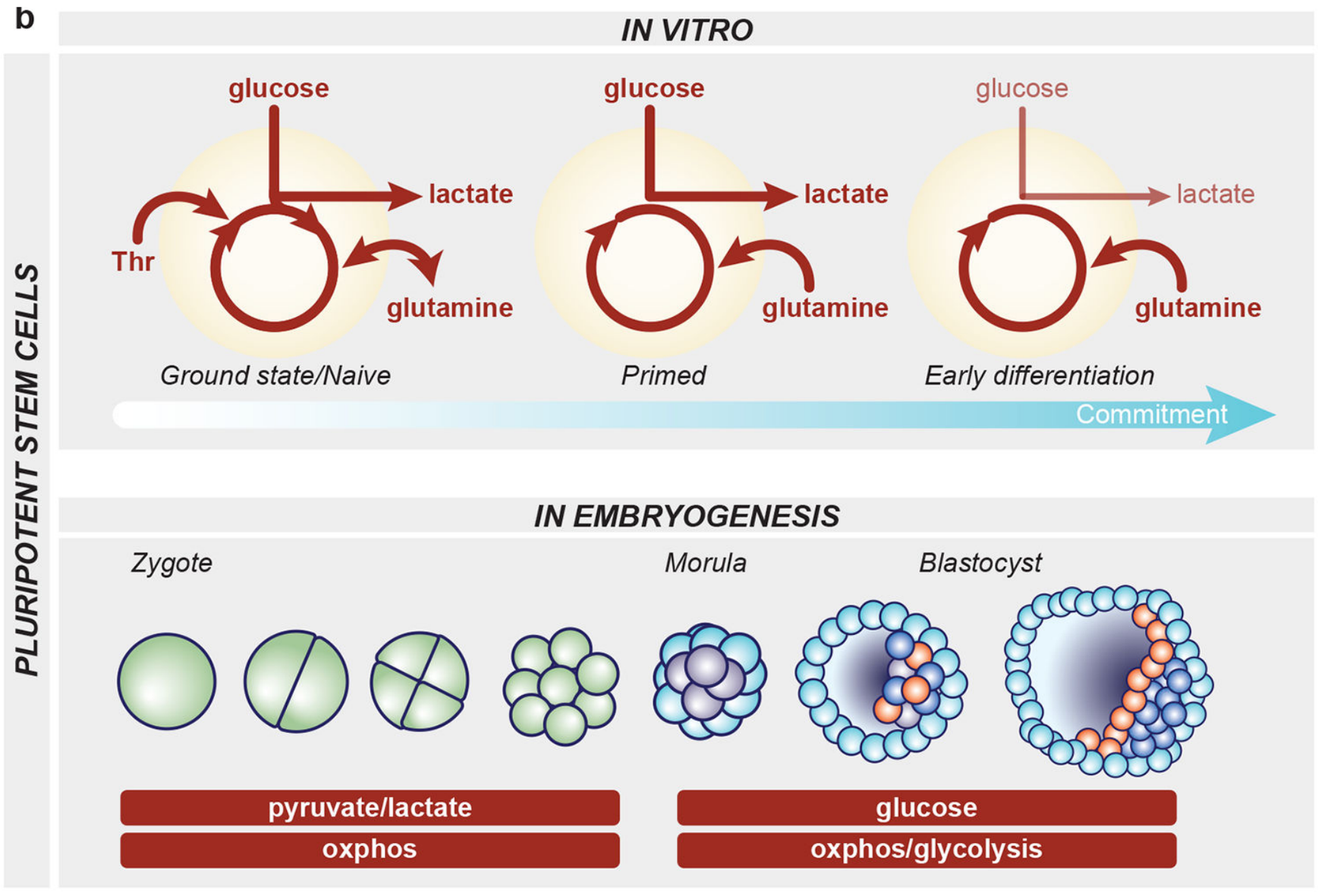
Note #2: Primed PSC also uptake Serine and cannot grow in absence (auxotrophs).

Intlekofer & Finley, ***Nat Metab****, 2019*

Role 1: metabolism supports cell proliferation

Continuous cell proliferation requires a net increase in biomass.

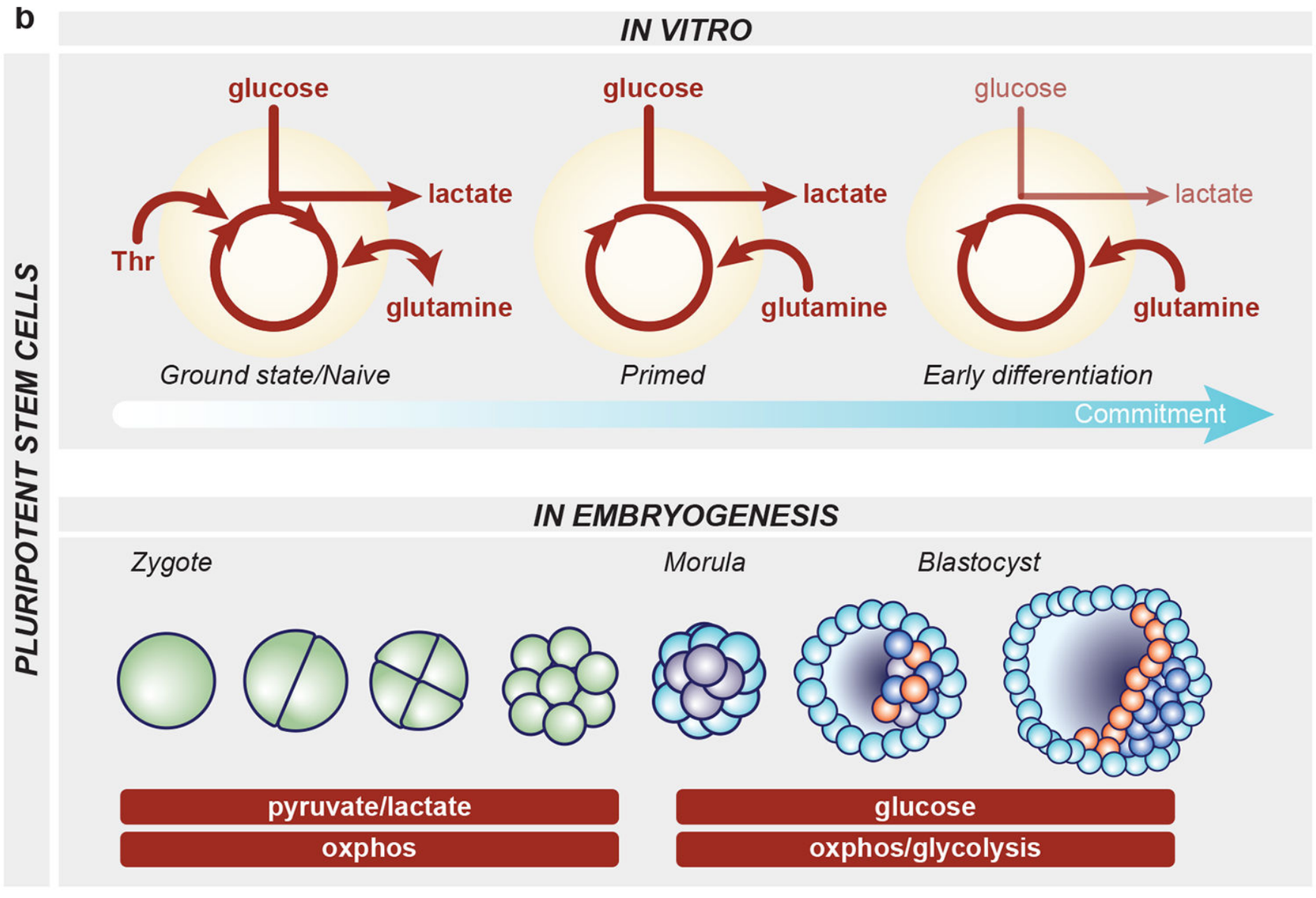
Accordingly, nutrients must be taken up and catabolized to generate the building blocks for macromolecules such as proteins, nucleic acids, and cellular membranes.



Role 1: metabolism supports cell proliferation

PSCs exhibit a variety of metabolic strategies depending on the culture condition or stage of differentiation.

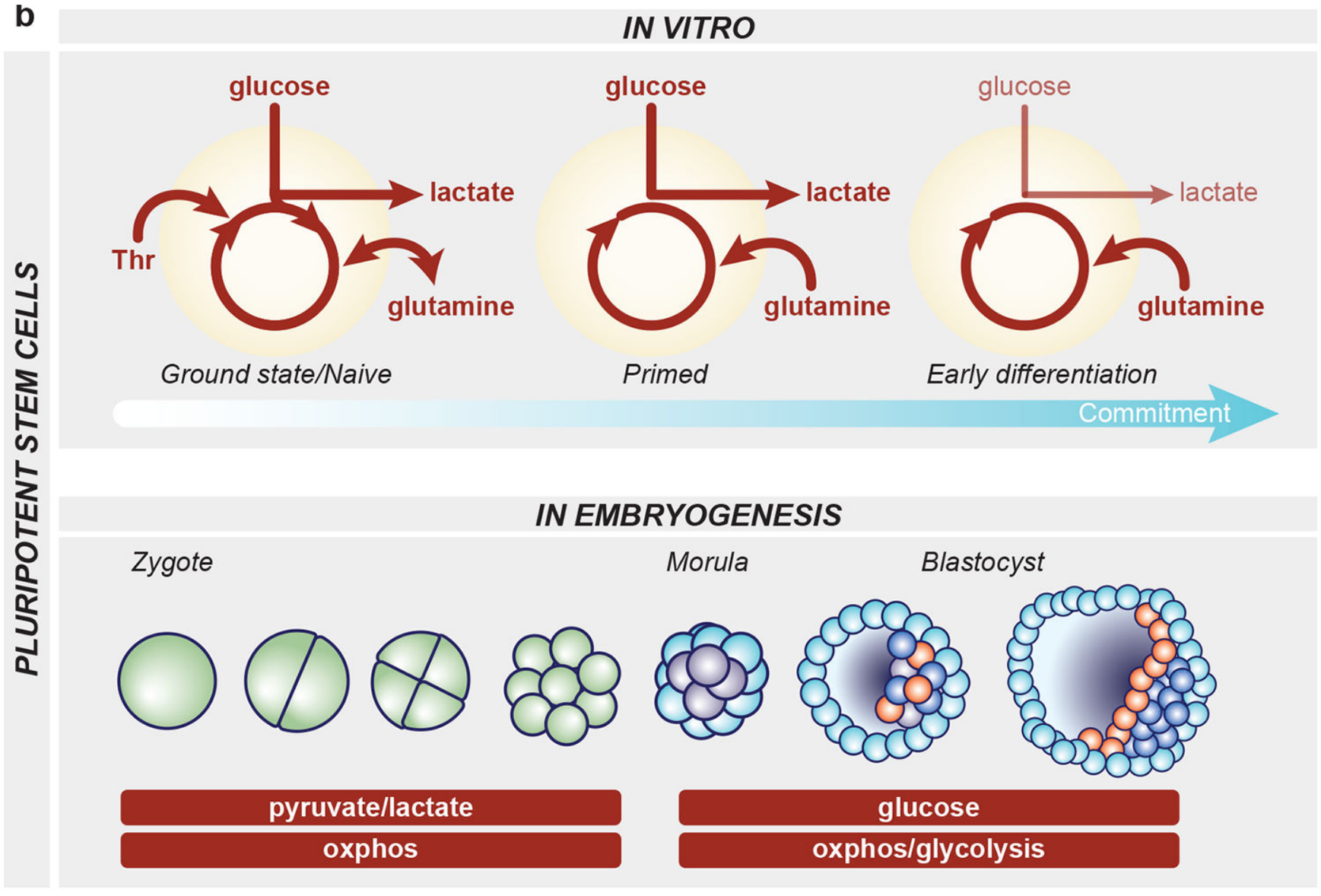
Both naïve and primed PSCs in culture exhibit high consumption of glucose and glutamine as well as extensive production of lactate. Upon differentiation, the proliferative hallmark of aerobic glycolysis decreases along with proliferation rate.



Role 1: metabolism supports cell proliferation

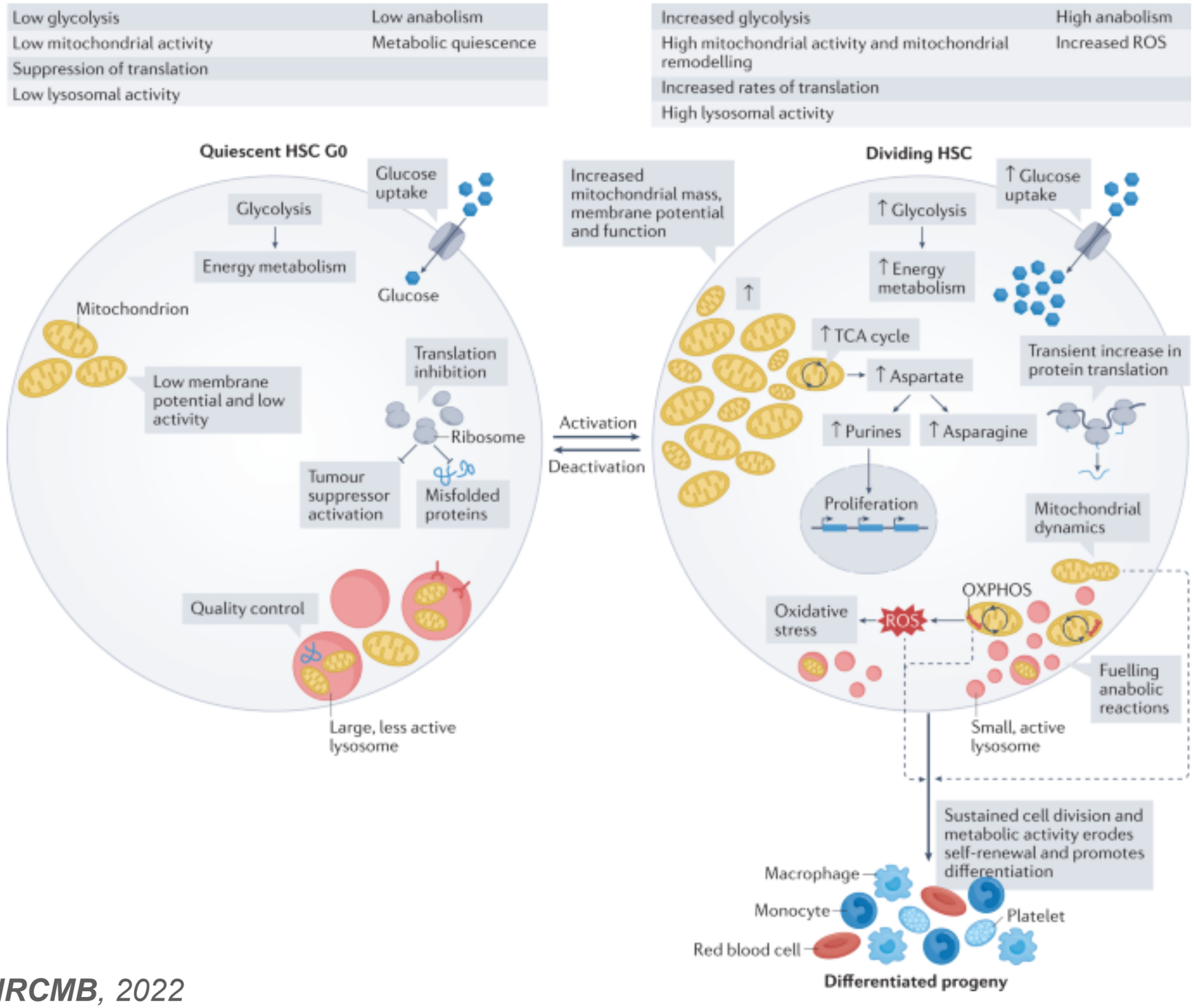
Studies of embryos developing ex vivo have identified two major metabolic stages:

- from zygote to morula, embryos are dependent upon the moncarboxylates pyruvate and lactate and are highly oxidative;
- from the morula stage, embryos begin to use glycolysis to fuel metabolic pathways and the trophectoderm exhibits features consistent with oxidative phosphorylation while the inner cell mass may rely more on aerobic glycolysis.



Role 1: metabolism supports cell proliferation

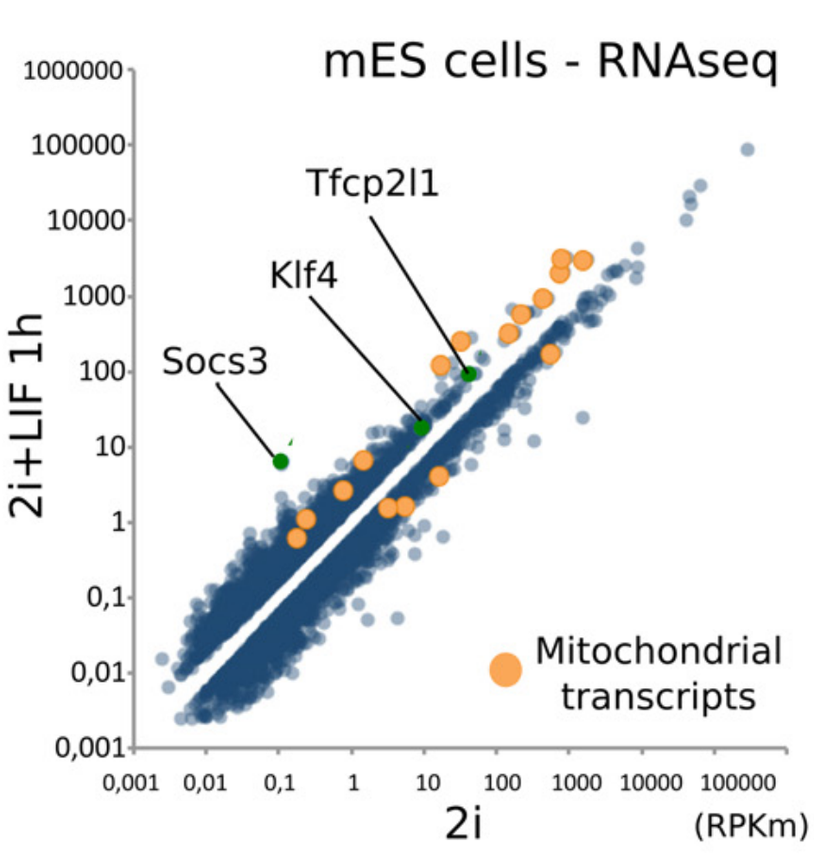
Aerobic glycolysis and mitochondrial respiration supports accelerated proliferation

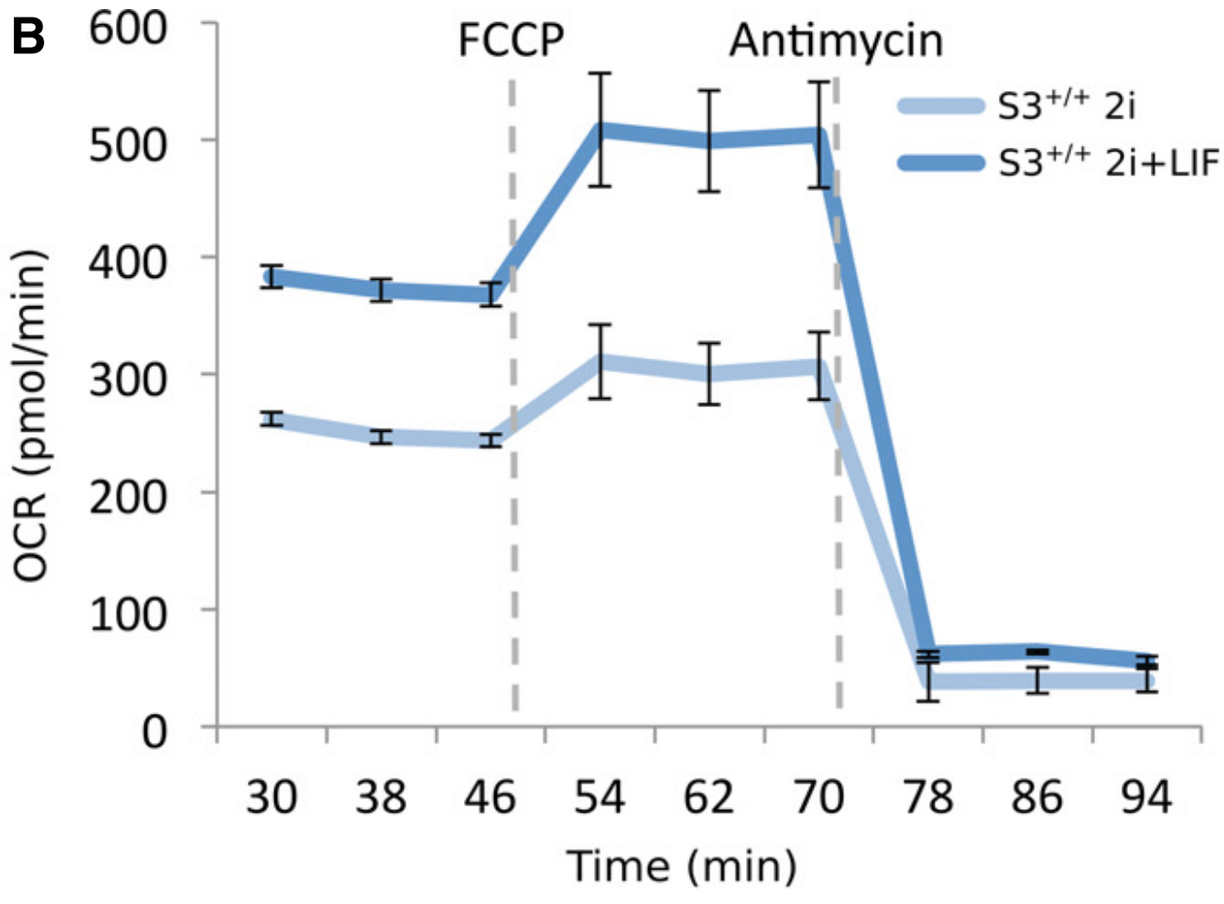


*Meacham et al, **NRCMB**, 2022*

Stat3 promotes mitochondrial transcription and oxidative respiration during maintenance and induction of naive pluripotency

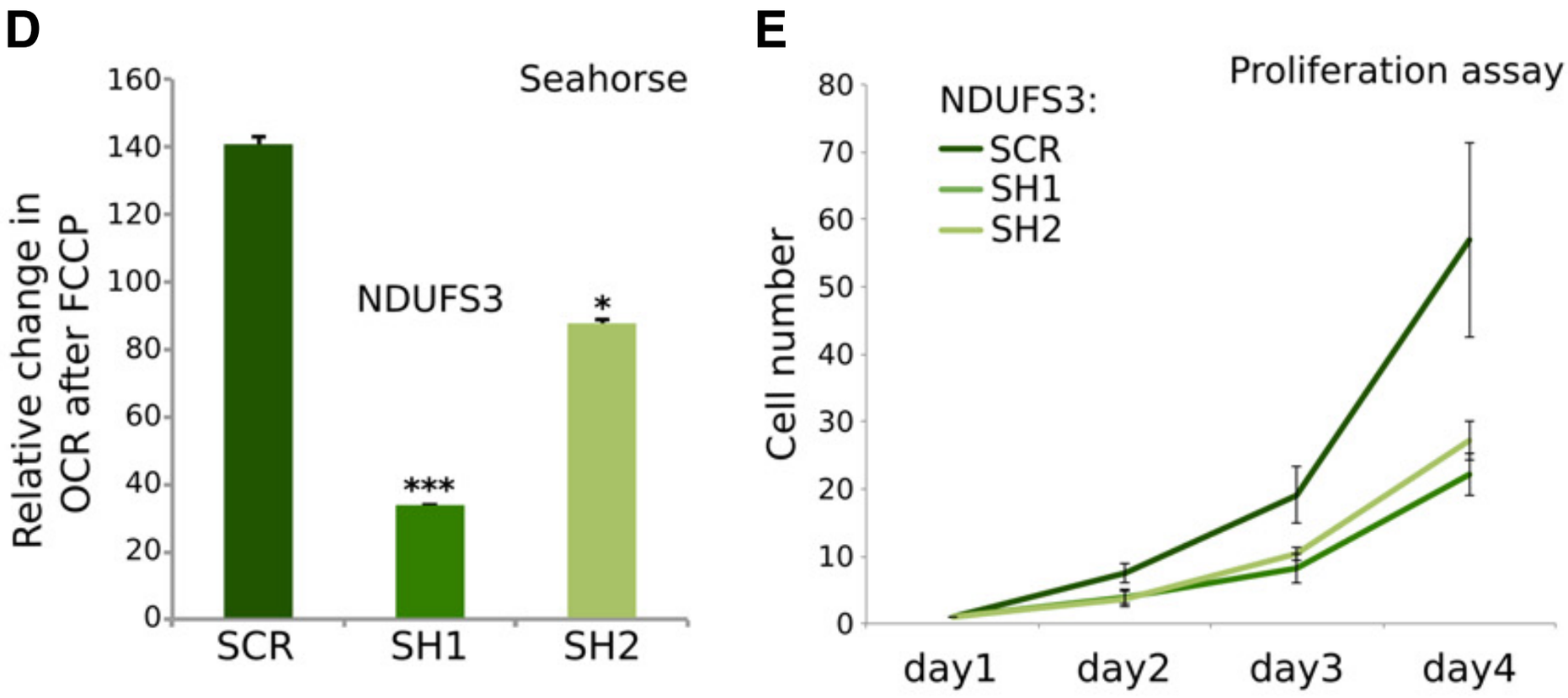
Elena Carbognin[1,†], Riccardo M Betto[1,†], Maria E Soriano[2], Austin G Smith[3,4,*] & Graziano Martello[1,**]





*Carbognin et al, **EMBO J**, 2016*

Mitochondrial respiration supports SC proliferation



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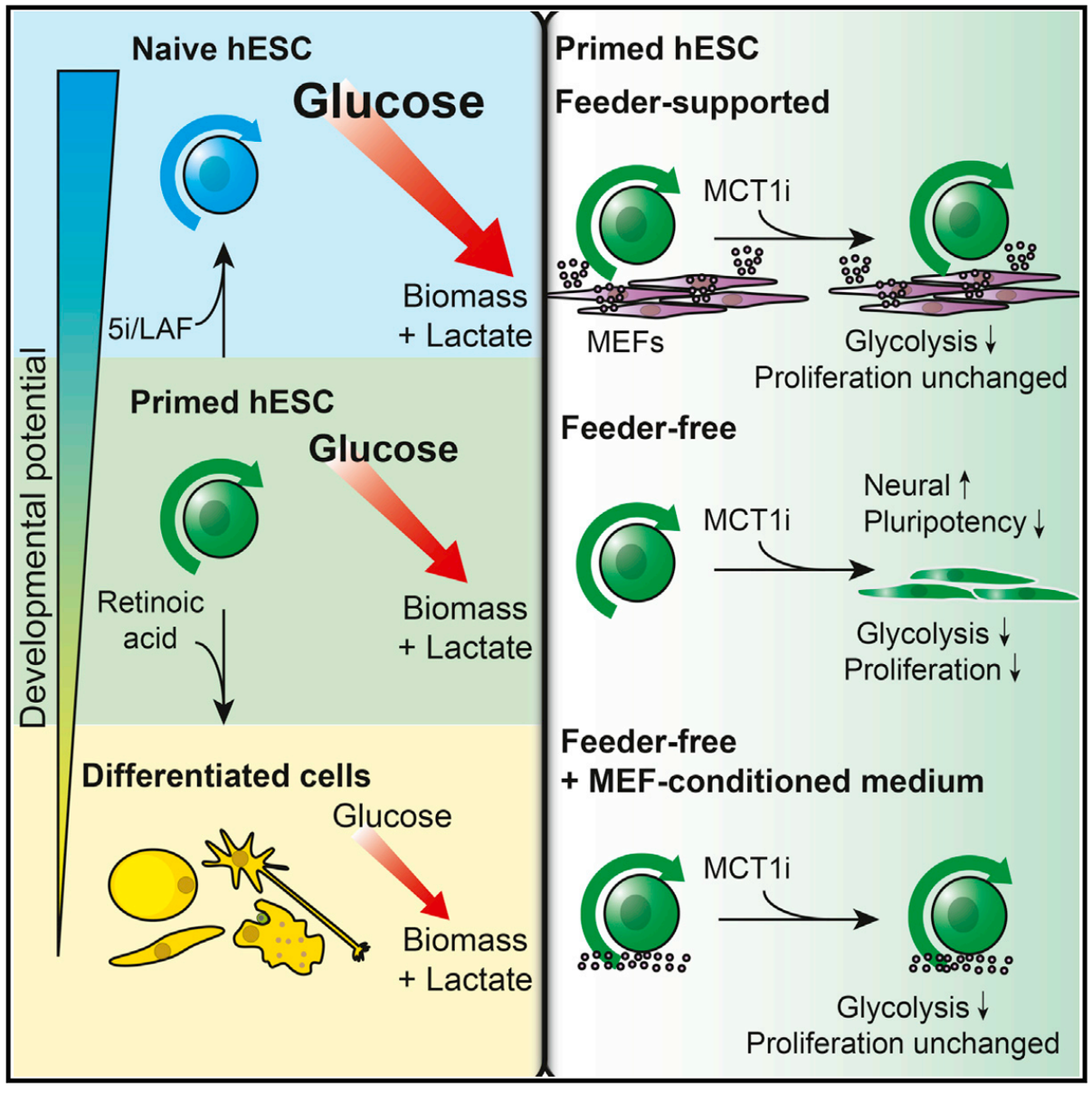
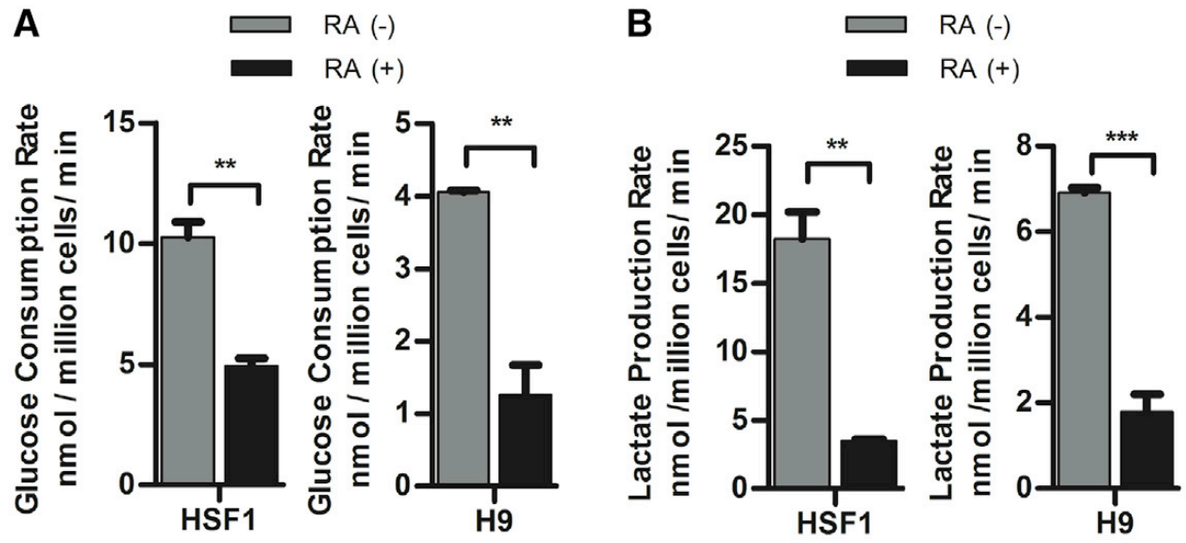
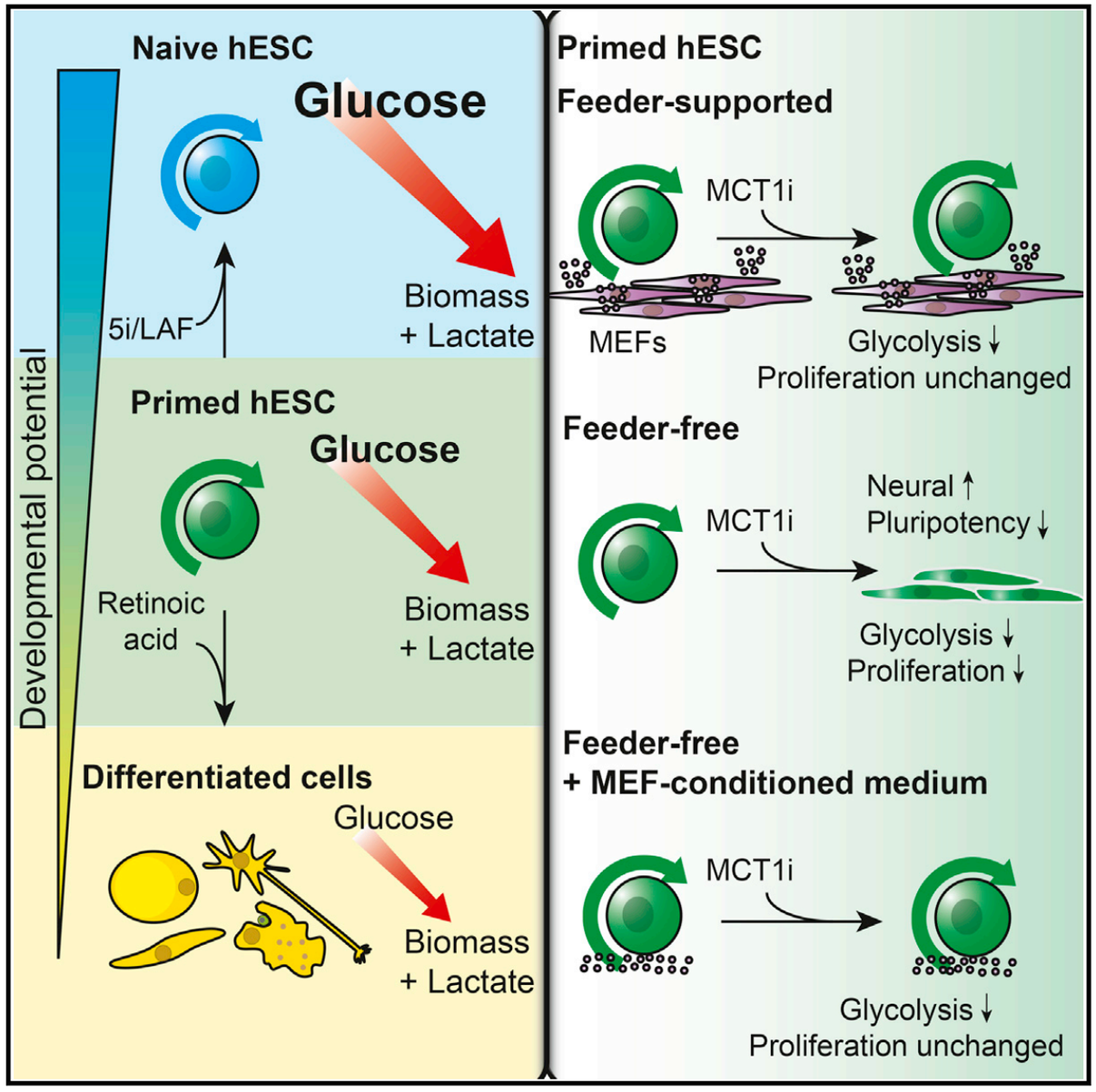
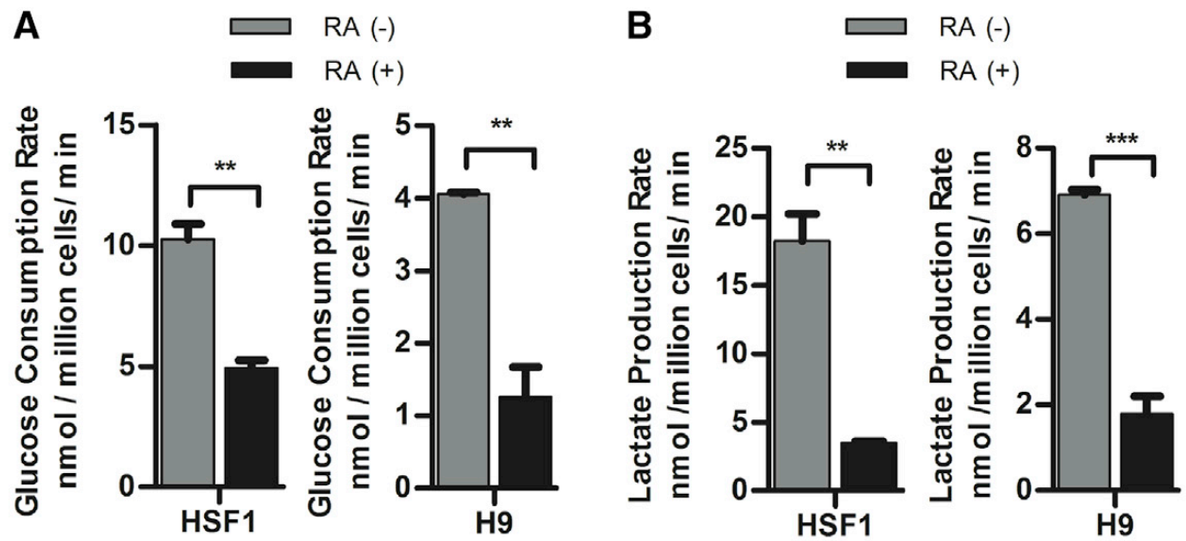
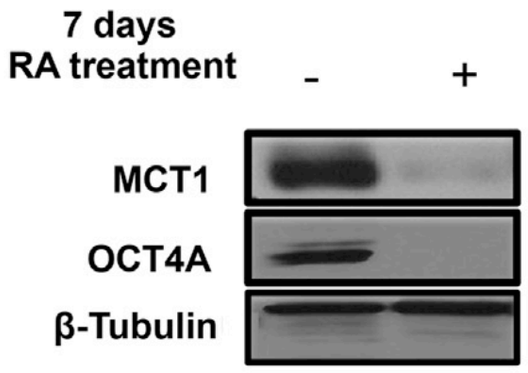


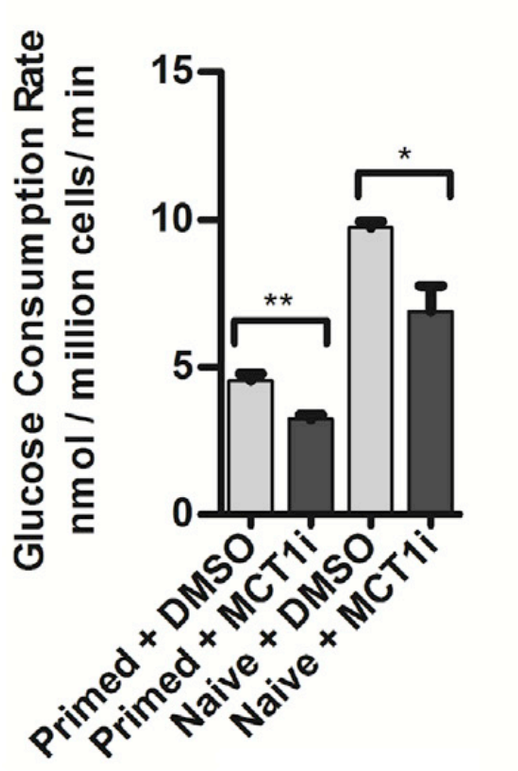


Gu et al, ***Cell Stem Cell****, 2016*









Gu et al, ***Cell Stem Cell****, 2016*

Role 1: metabolism supports cell proliferation

Aerobic glycolysis and mitochondrial respiration supports accelerated proliferation

In general, PSCs cultured in the naive state have higher basal respiration relative to their post-implantation counterparts, and naive mouse PSCs preferentially incorporate glucose-derived pyruvate into TCA cycle intermediates.

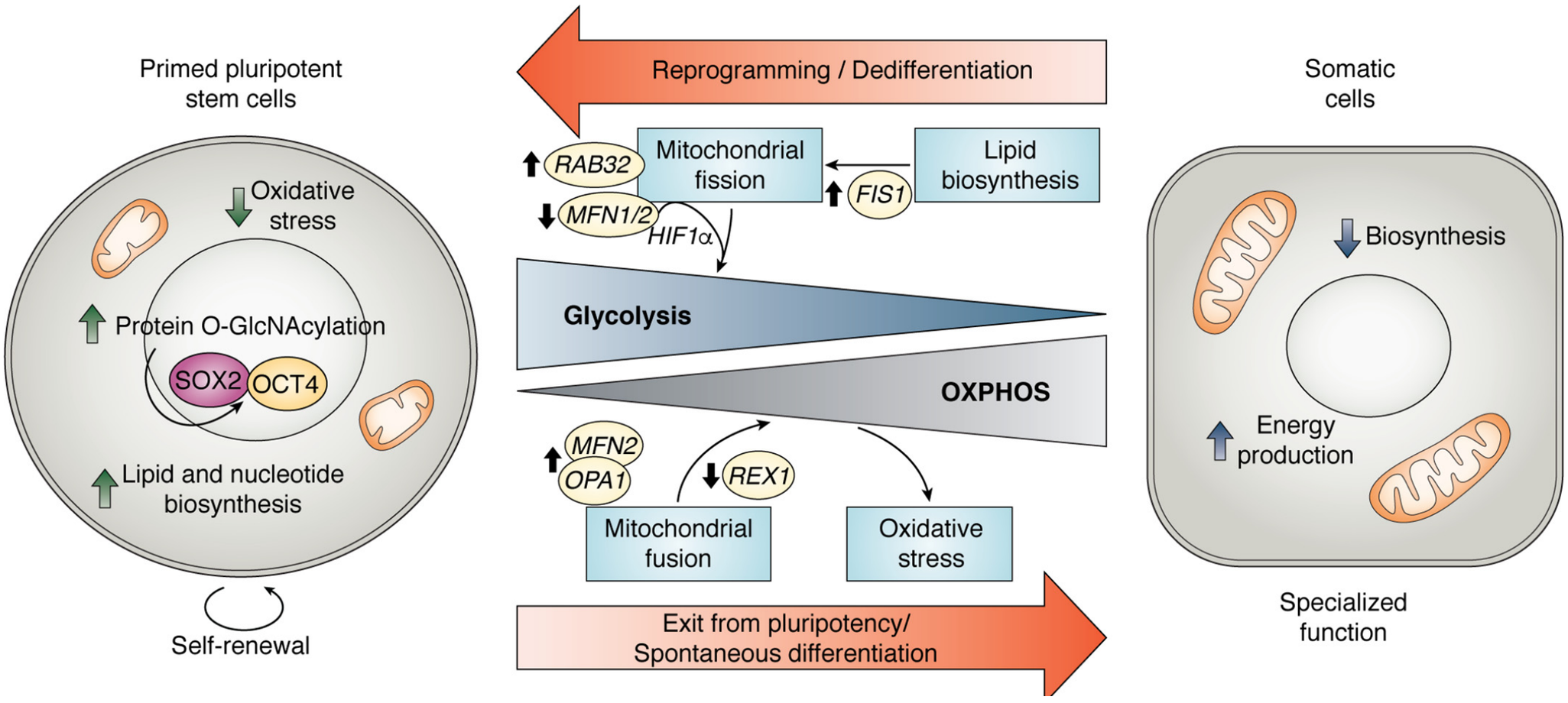
Increased respiration enables optimal proliferation in naive ESCs and may even be required for entry into the naive pluripotent state.

However, **the precise outputs of respiration that benefit naive PSCs remain unknown.**

Role 1: metabolism supports cell proliferation

Aerobic glycolysis and mitochondrial respiration supports accelerated proliferation

As PSCs exit the naive pluripotent state, they transiently decrease glucose oxidation in the TCA cycle and increase lactate secretion.

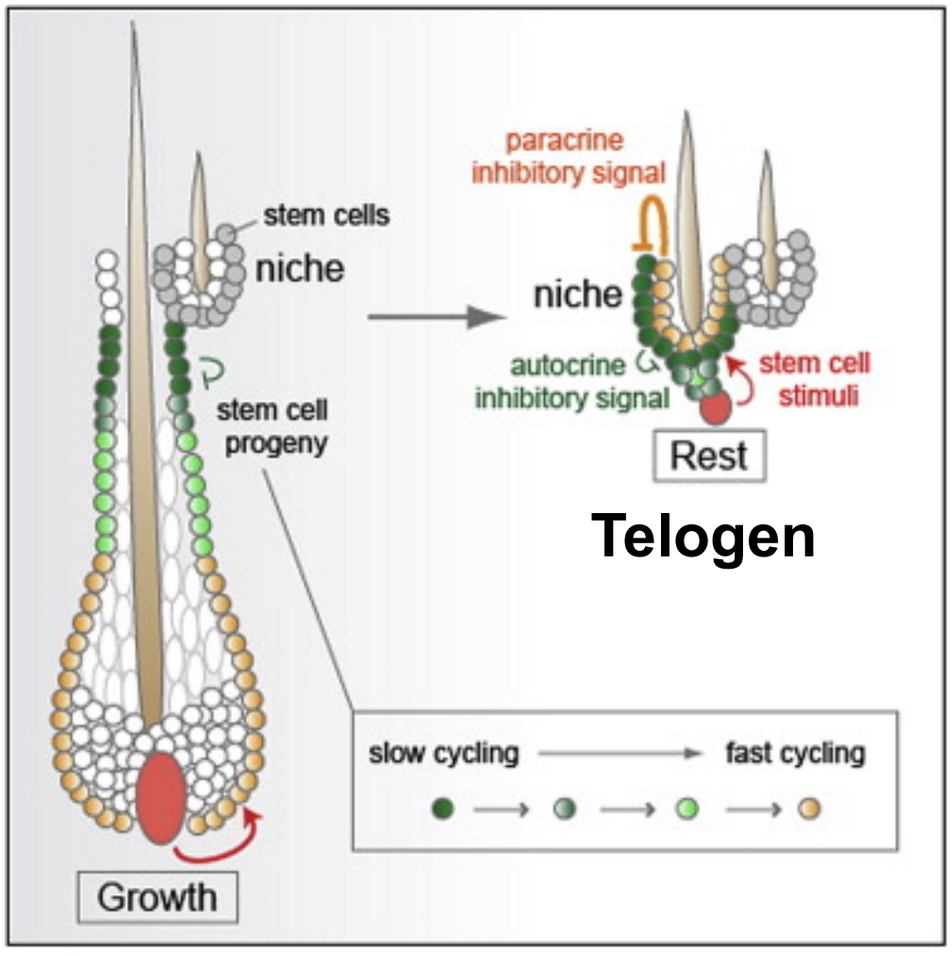


Aerobic glycolysis phenotype is reversed upon further differentiation, which is often accompanied by decreased glucose flux through glycolysis and decreased lactate secretion.

Aerobic glycolysis is not universally downregulated during differentiation, however; *ex: human PSCs maintain high glycolytic flux when differentiated to ectoderm, but not mesoderm and endoderm.*

Lactate dehydrogenase activity drives hair follicle stem cell activation

Aimee Flores[1,2,3], John Schell[4], Abigail S. Krall[5], David Jelinek[1], Matilde Miranda[1], Melina Grigorian[6], Daniel Braas[5,7], Andrew C. White[8], Jessica L. Zhou[9], Nicholas A. Graham[5,9], Thomas Graeber[5,10], Pankaj Seth[11], Denis Evseenko[12], Hilary A. Coller[1,2,3,13,14], Jared Rutter[4,15], Heather R. Christofk[2,5,7,13,14,16] and William E. Lowry[1,2,3,13,16]



Telogen

Anagen

*Flores et al, **NCB**, 2016*

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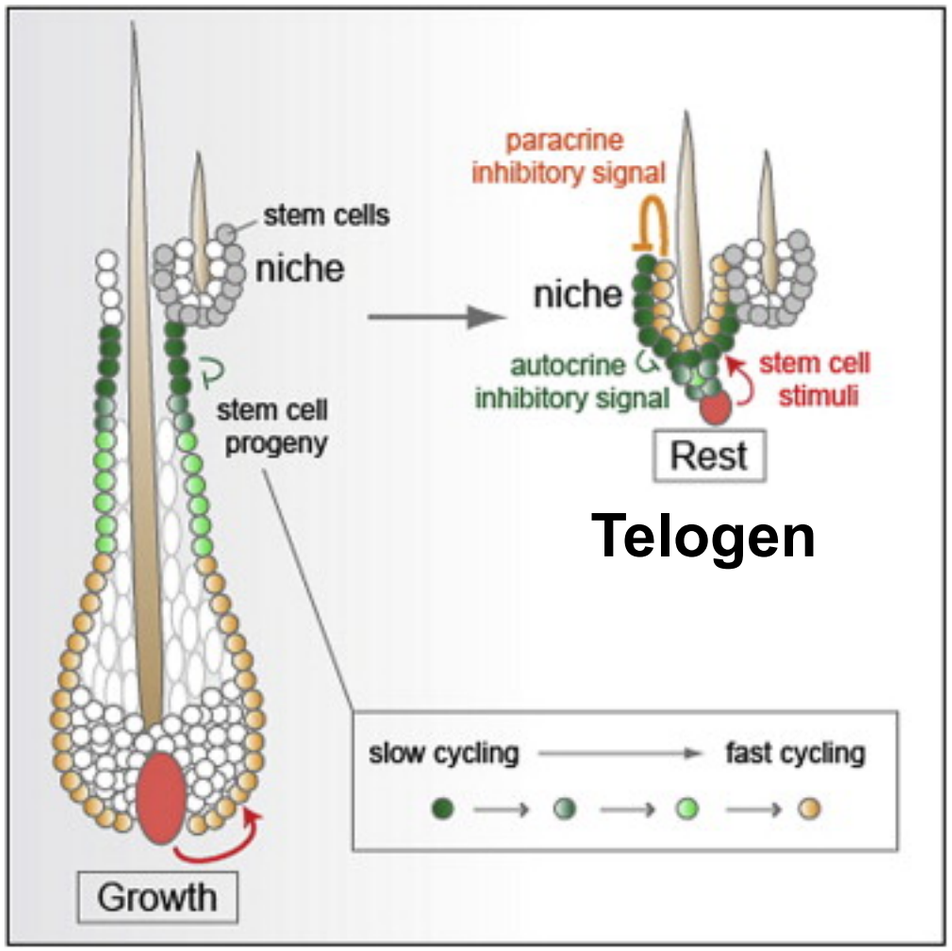

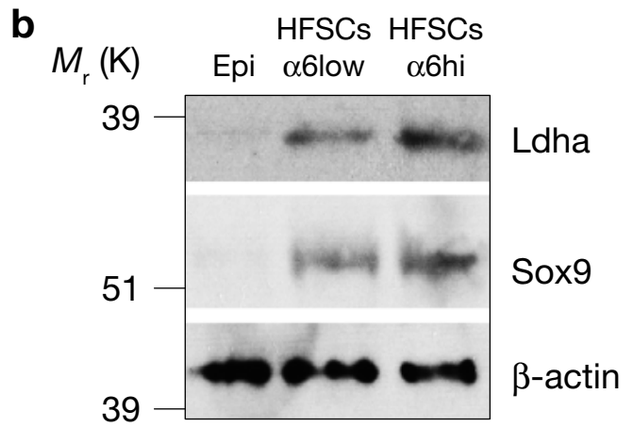

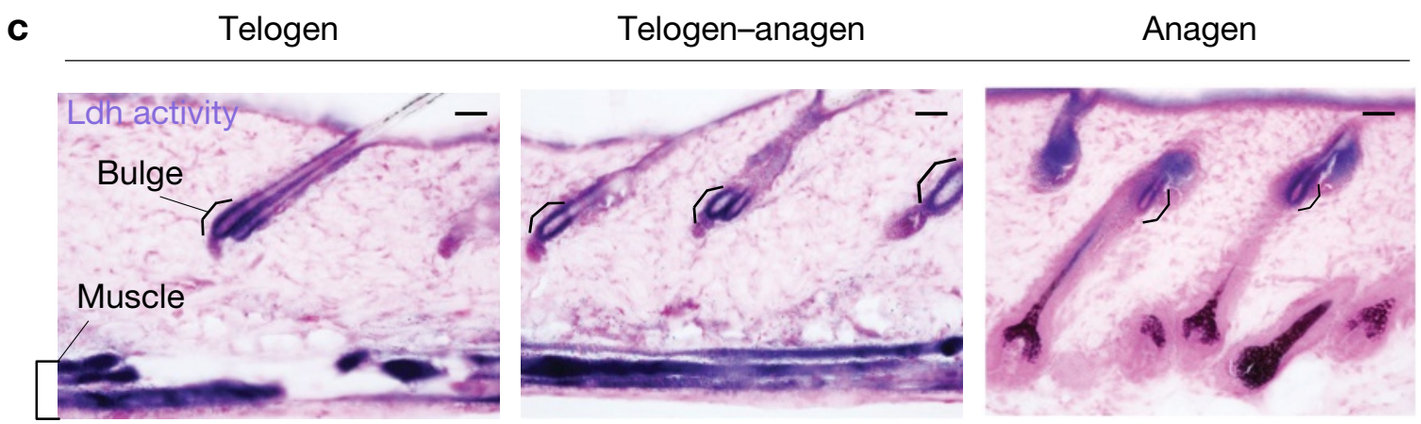

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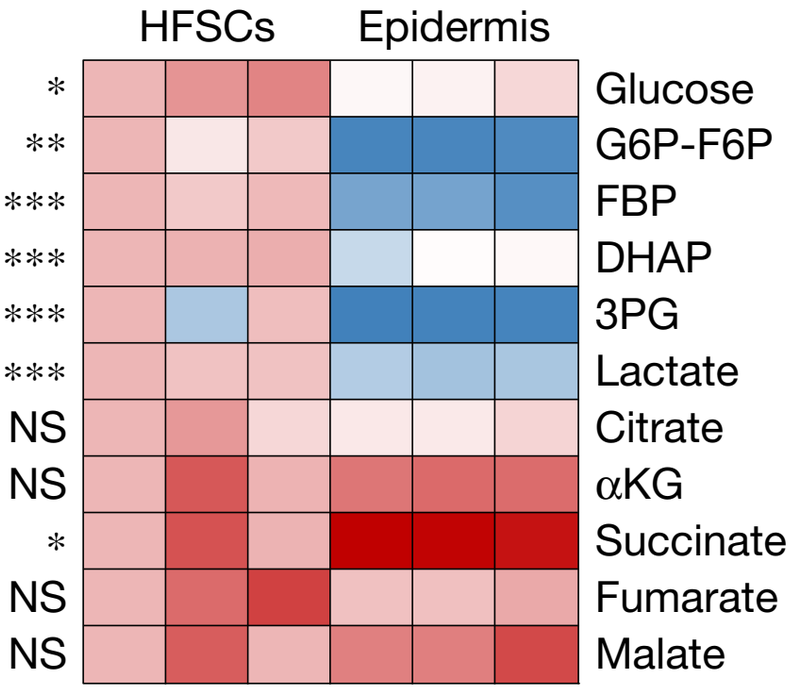

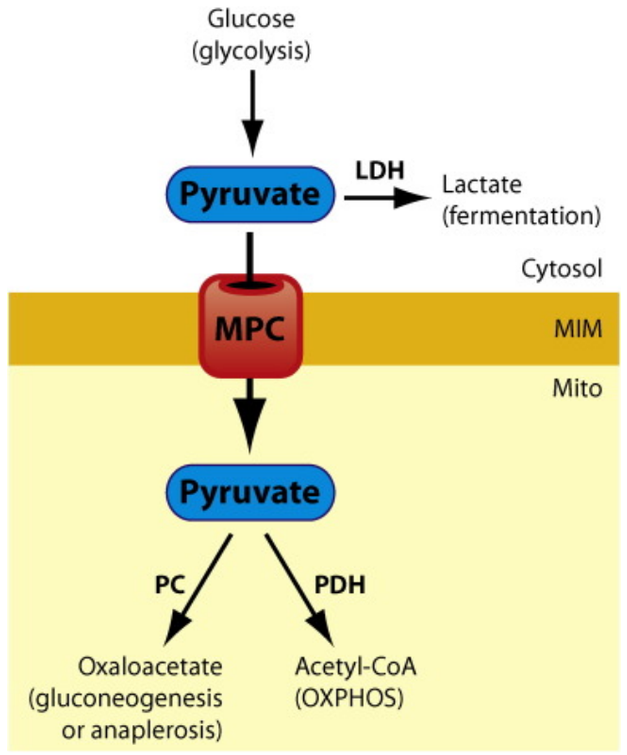

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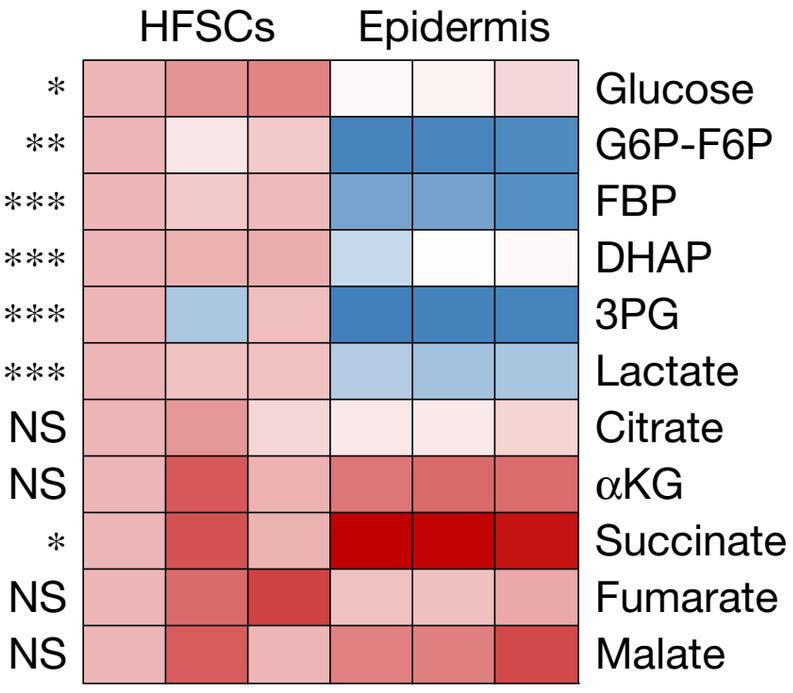

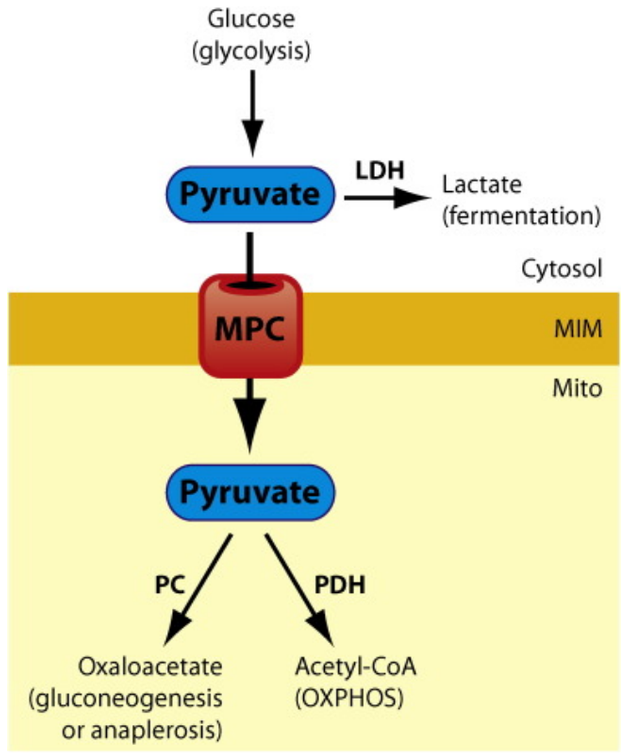

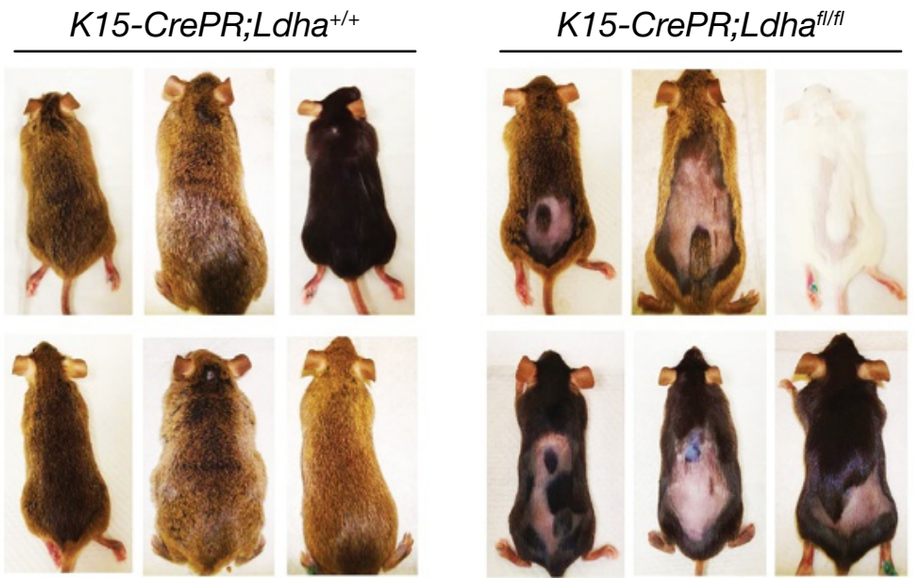

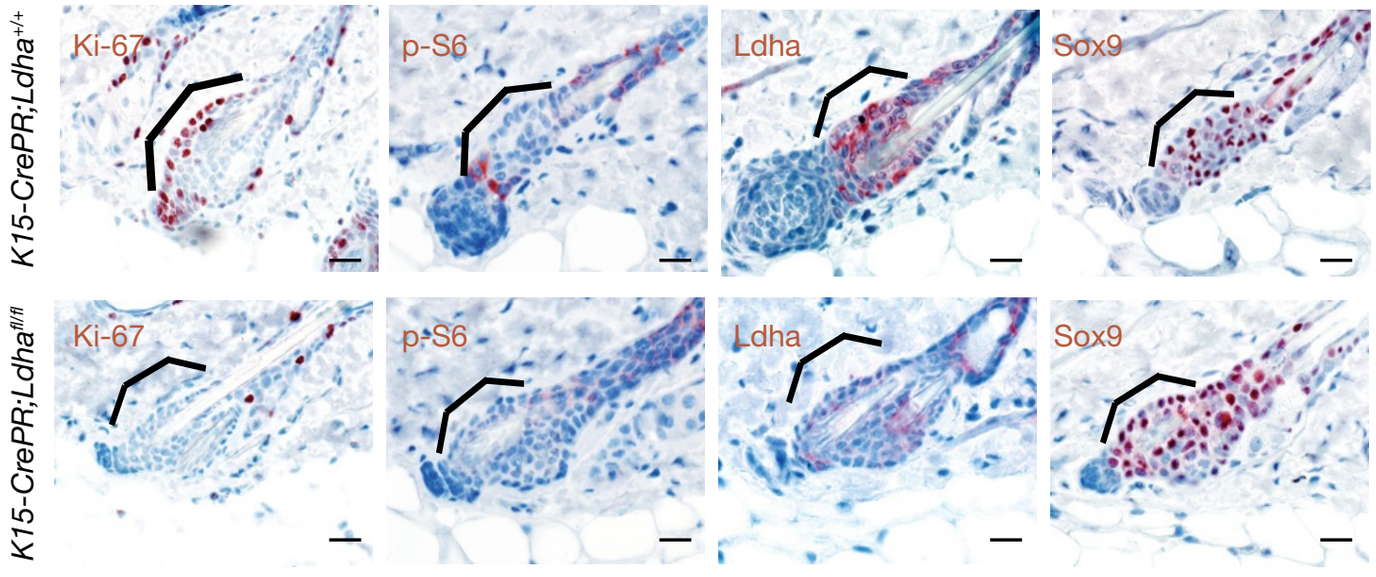

*Flores et al, **NCB**, 2016*

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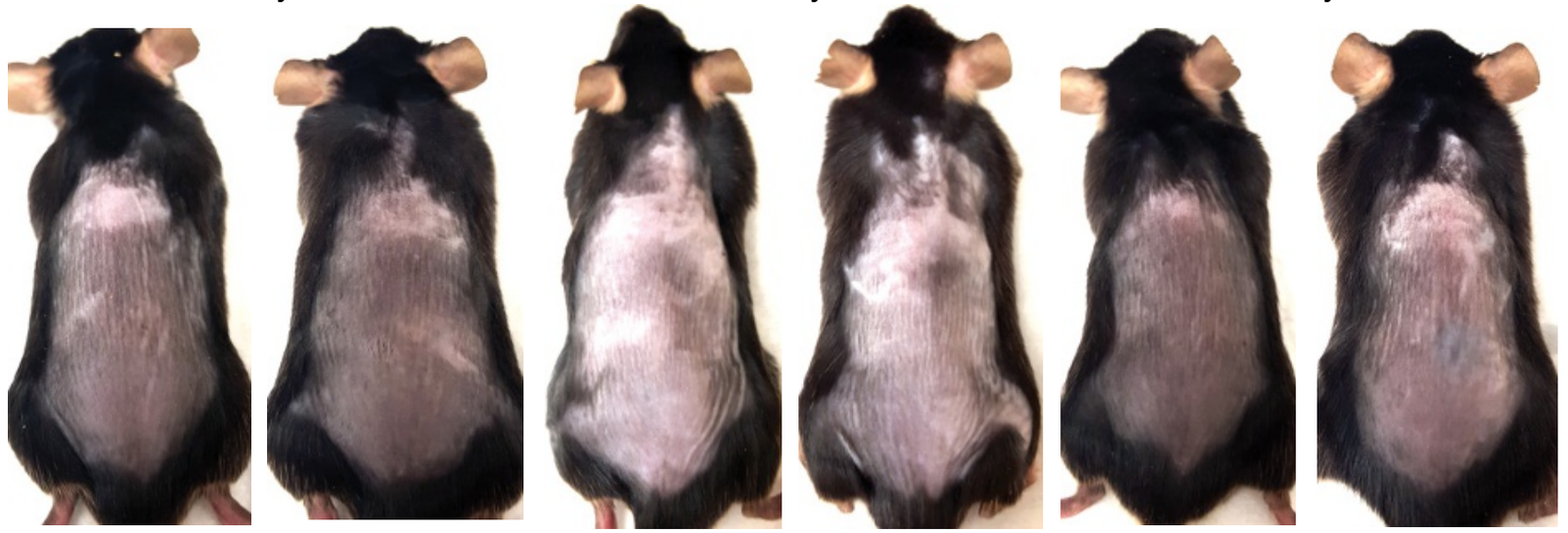
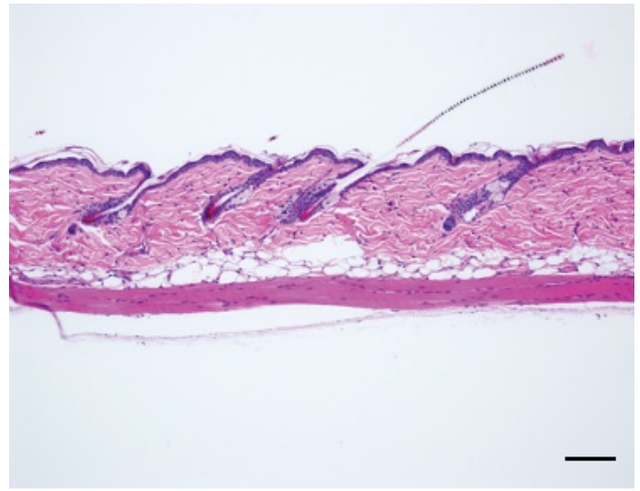
3 days 8 days 12 days

Vehicle





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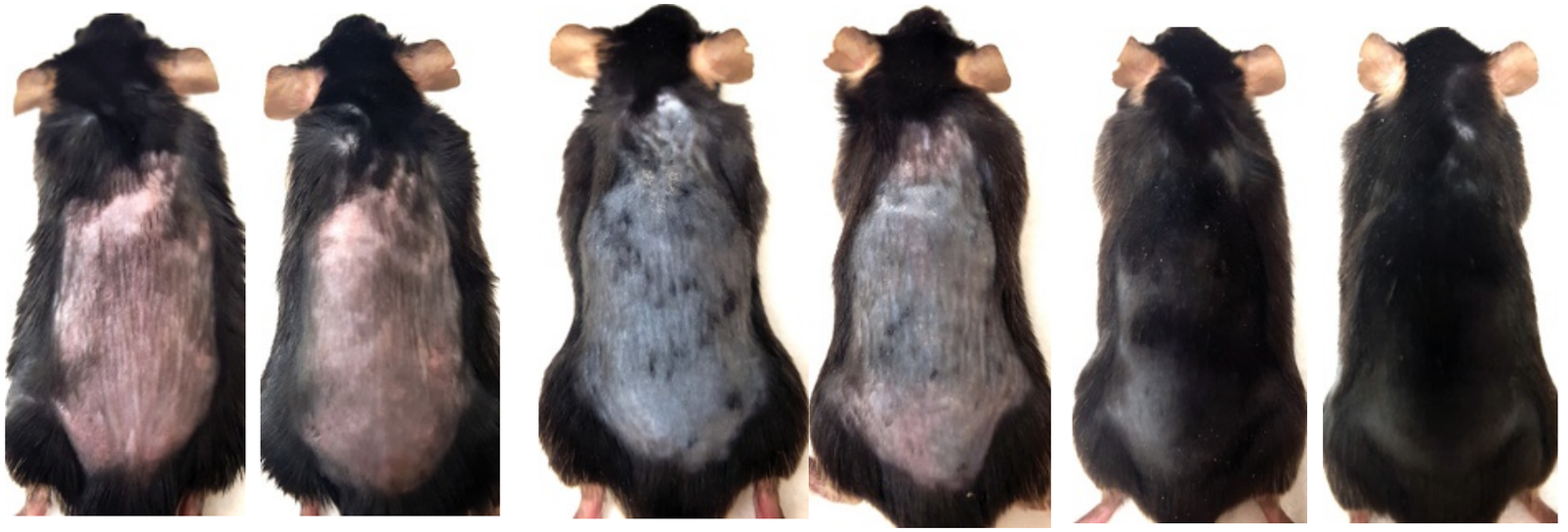

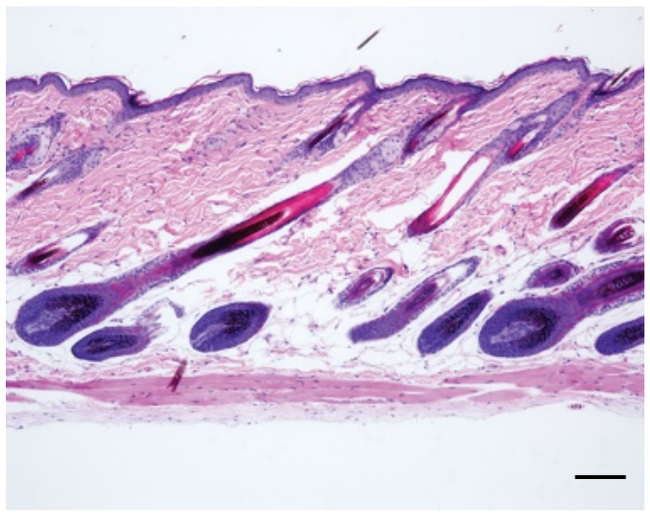

Flores et al, ***NCB****, 2016*

a

3 days

8 days

12 days

Vehicle

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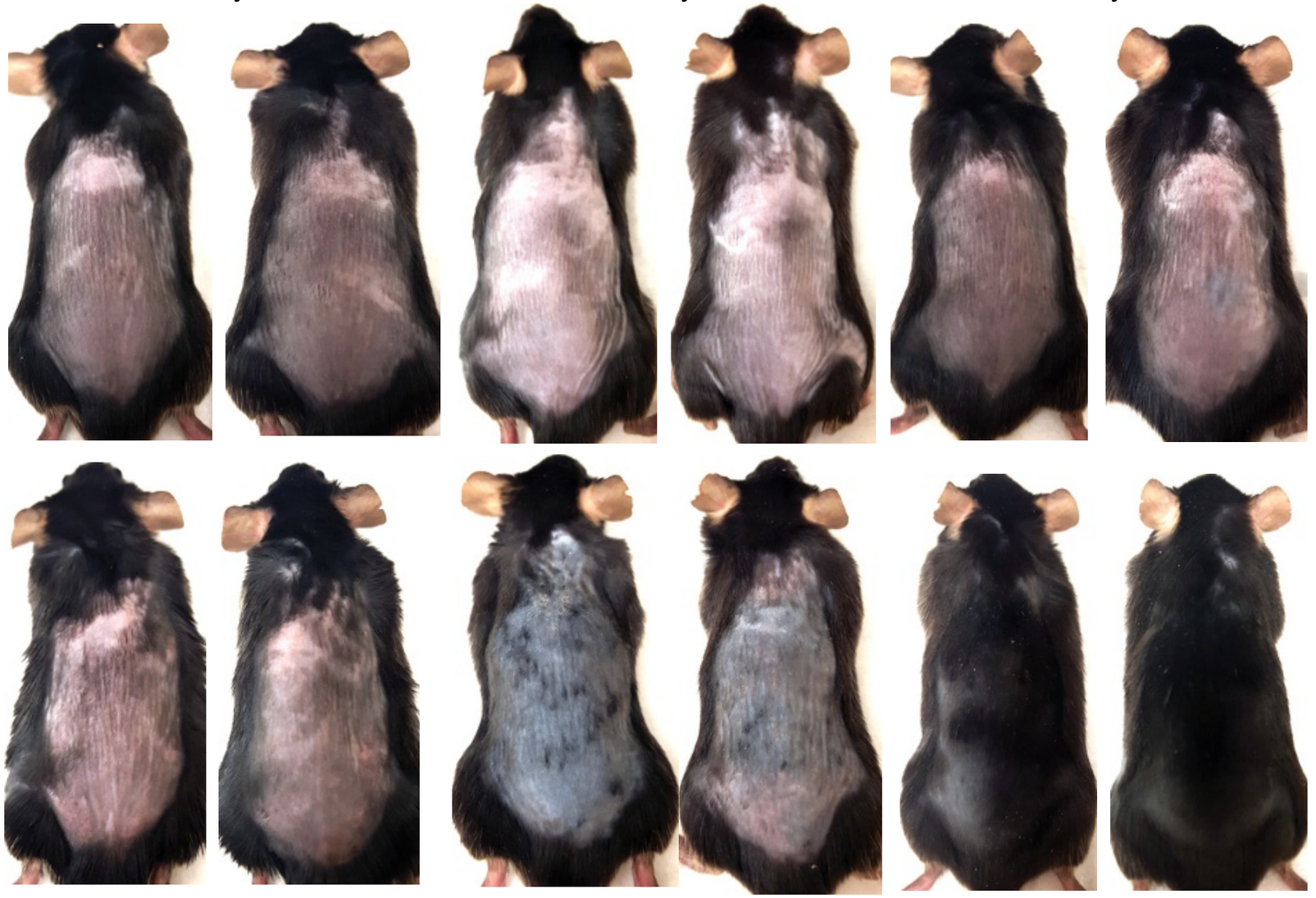
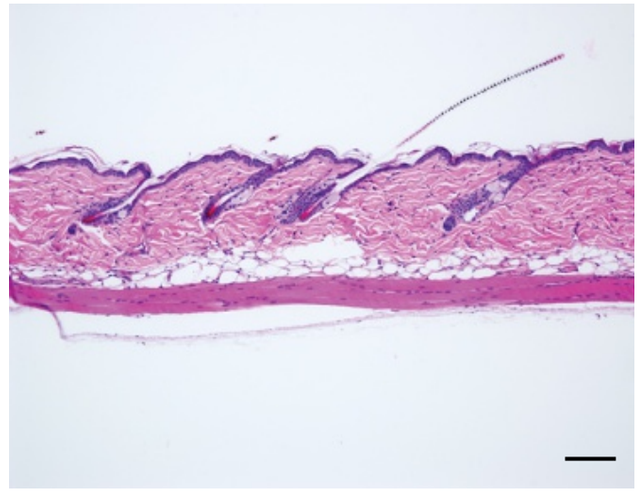
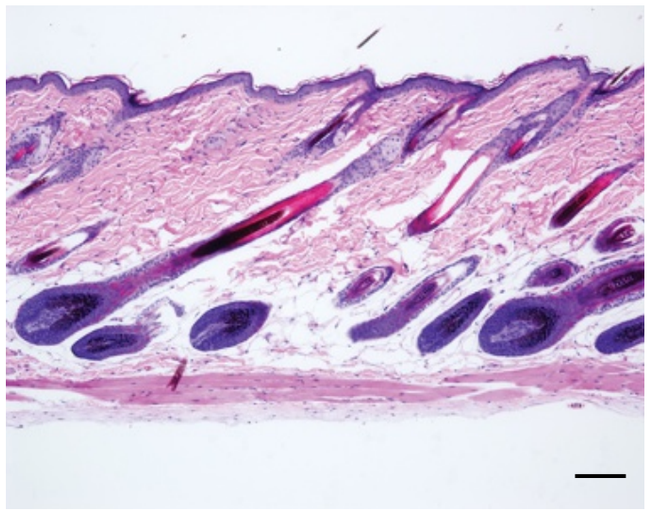
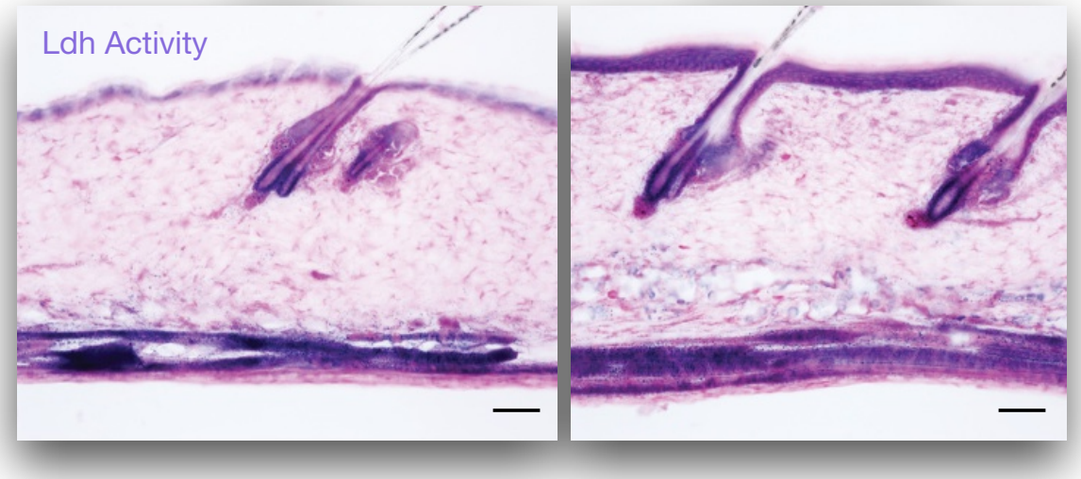




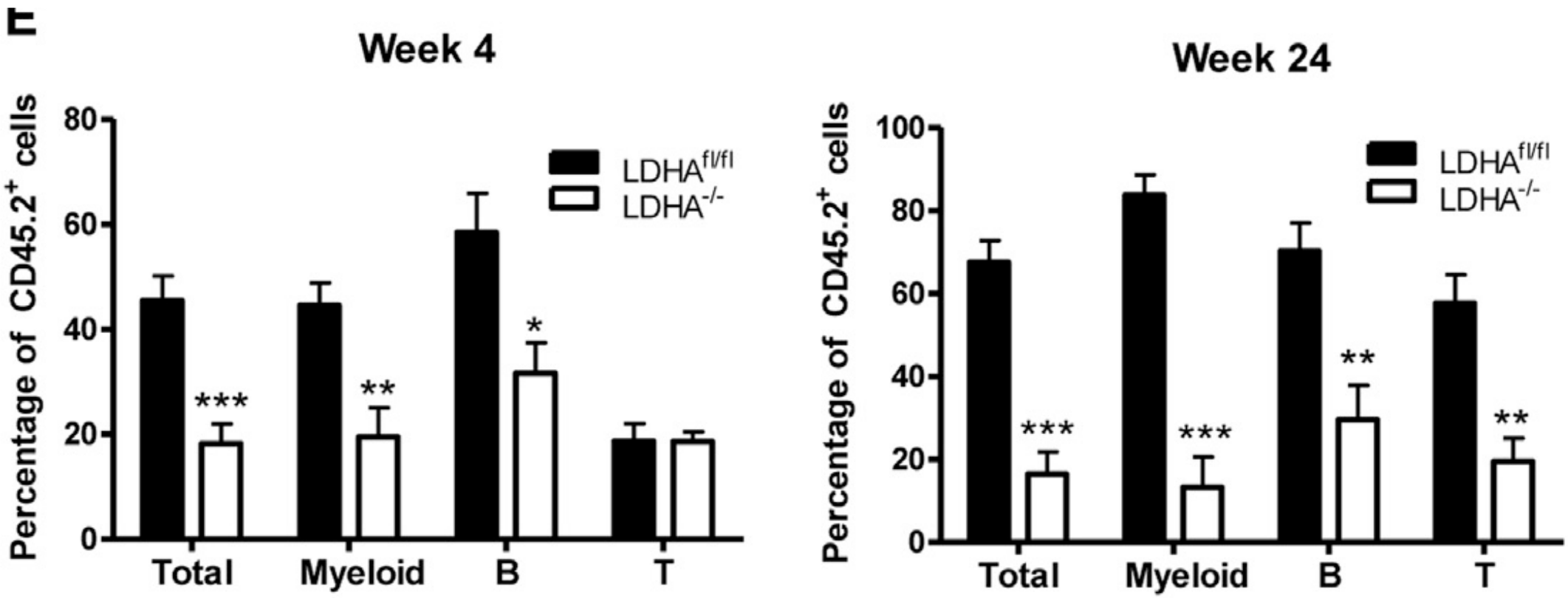




*Flores et al, **NCB**, 2016*

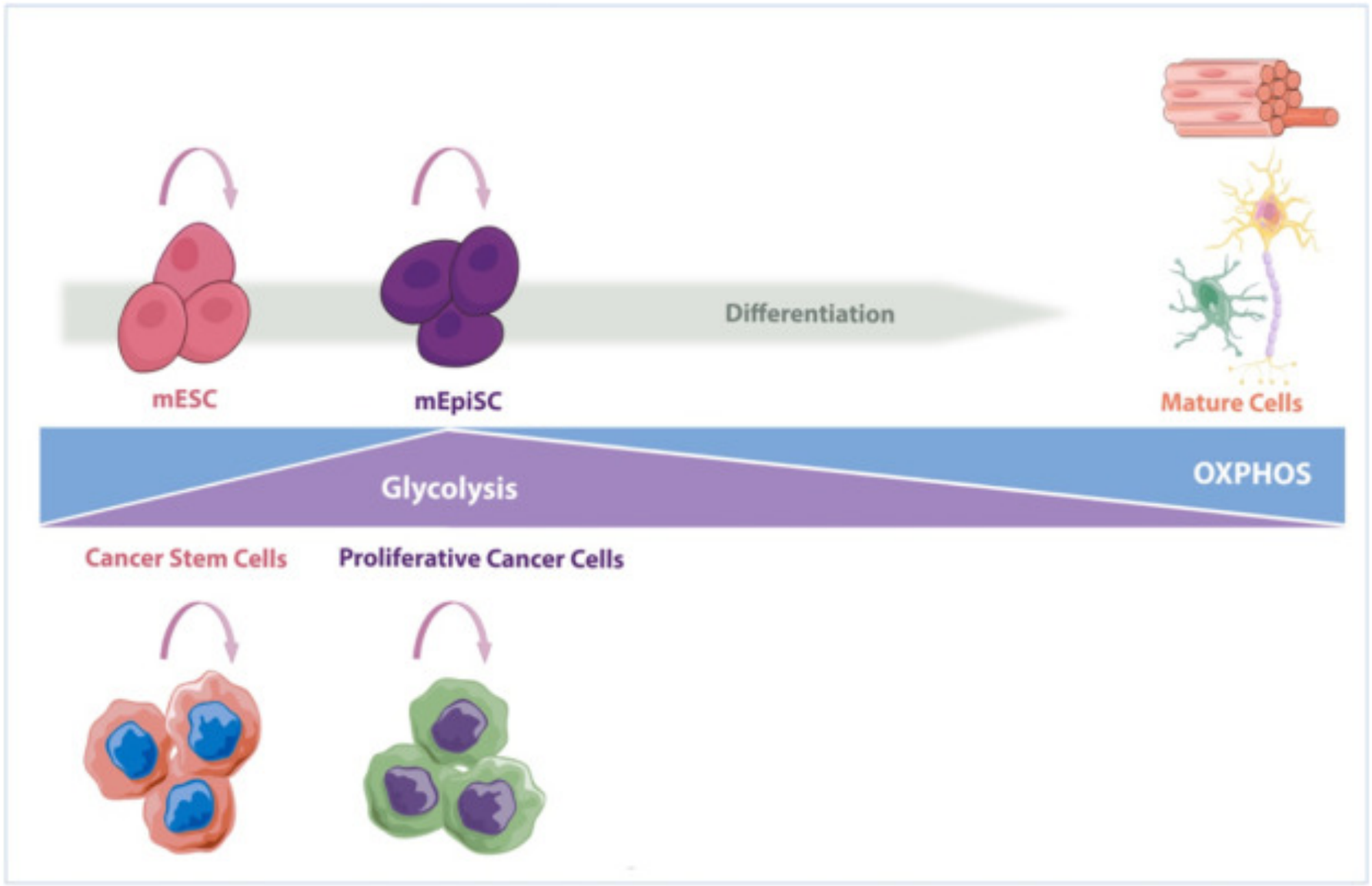
Cell-State-Specific Metabolic Dependency in Hematopoiesis and Leukemogenesis

Ying-Hua Wang,[1,2,3] William J. Israelsen,[4] Dongjun Lee,[1,2,3] Vionnie W.C. Yu,[1,2,3] Nathaniel T. Jeanson,[1,2,3] Clary B. Clish,[6] Lewis C. Cantley,[7] Matthew G. Vander Heiden,[4,5] and David T. Scadden[1,2,3,*]



*Wang et al, **Cell**, 2014*

Stage-specific reliance on glycolysis

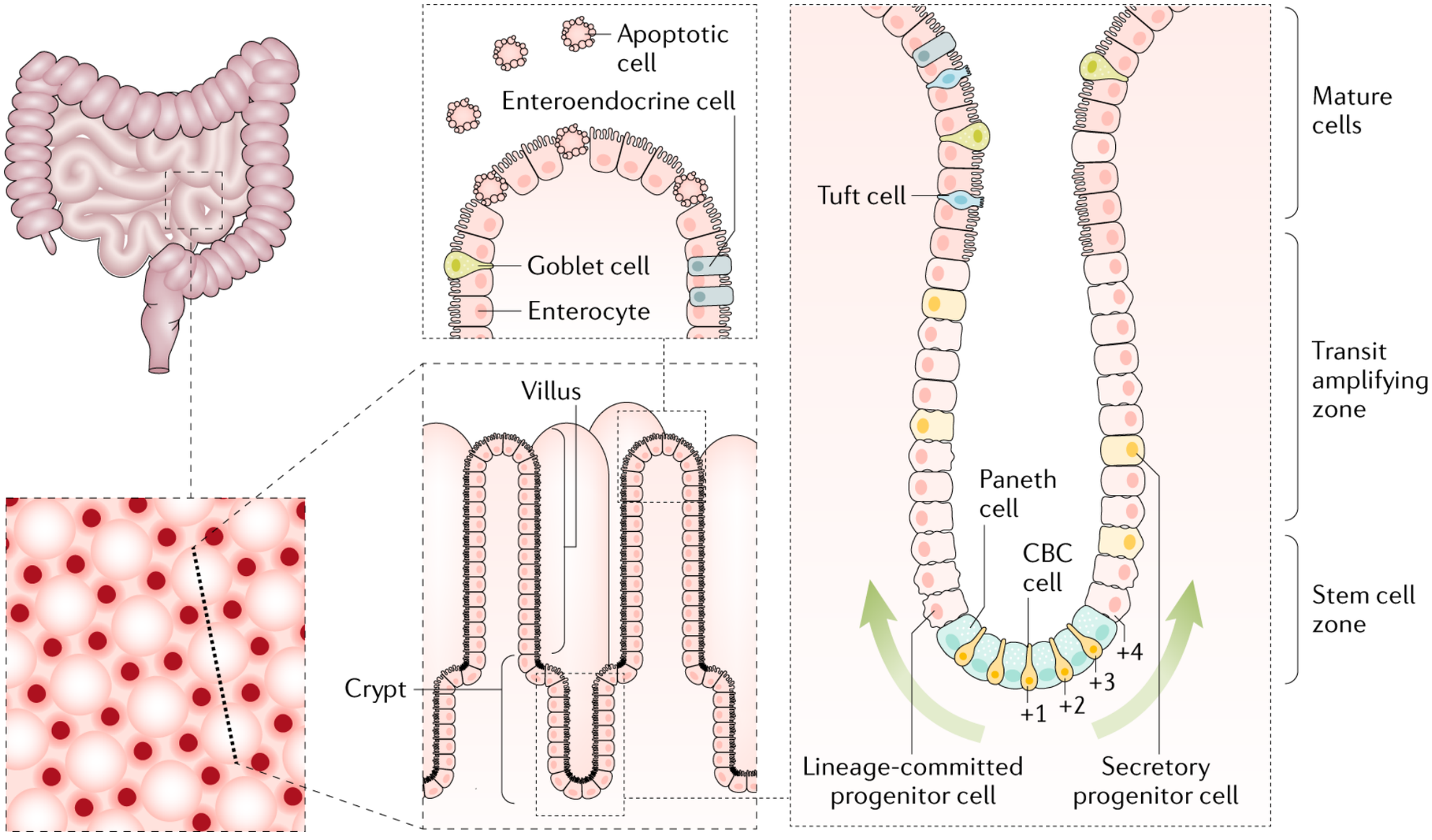


Accordingly, inhibition of glycolysis is not as detrimental for naive PSCs as it is for primed PSCs

Mlody & Prigione, ***Cell Stem Cell****, 2016*

Role 1: metabolism supports cell proliferation

PSC are often characterized by preference for non-canonical carbon substrates (e.g.: fatty acids).

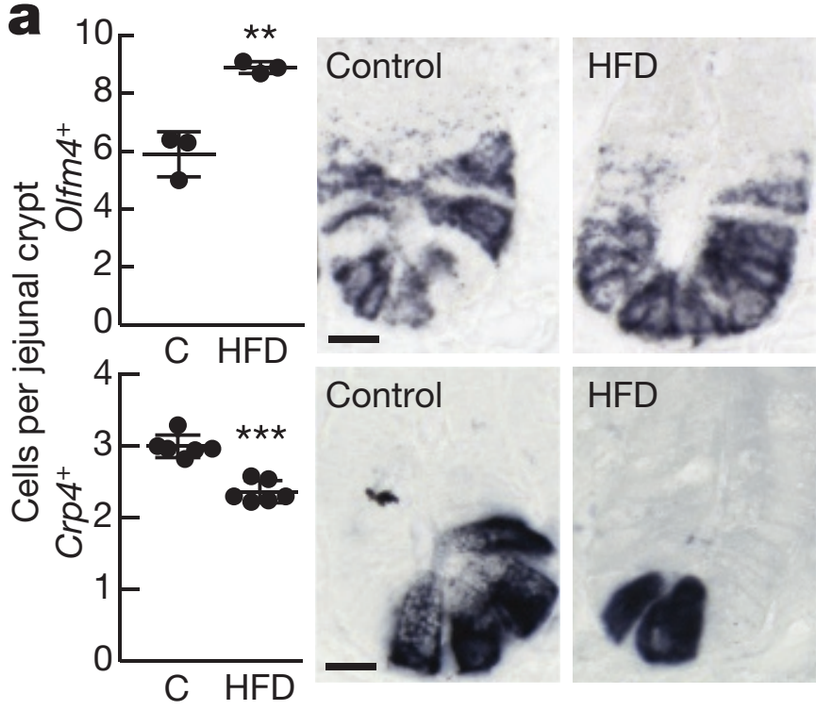


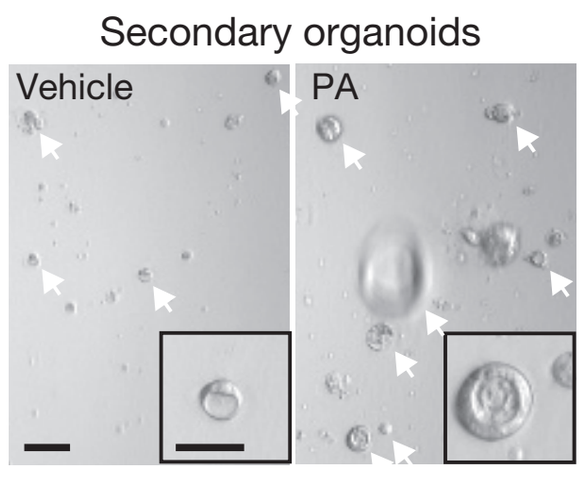
Fatty acid oxidation enhances SC function

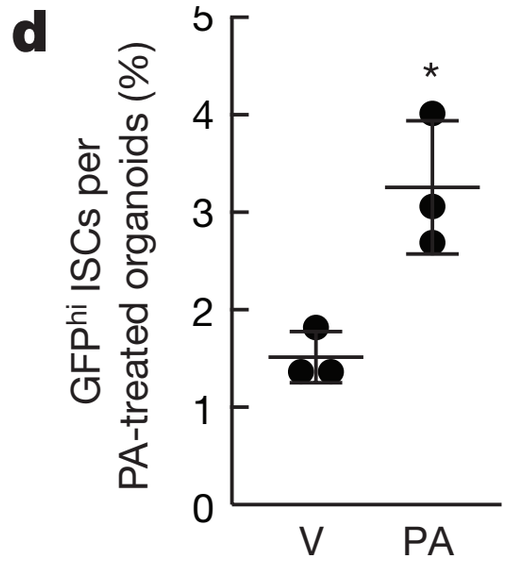
PSC are often characterized by preference for non-canonical carbon substrates (e.g.: fatty acids).

High-fat diet enhances stemness and tumorigenicity of intestinal progenitors

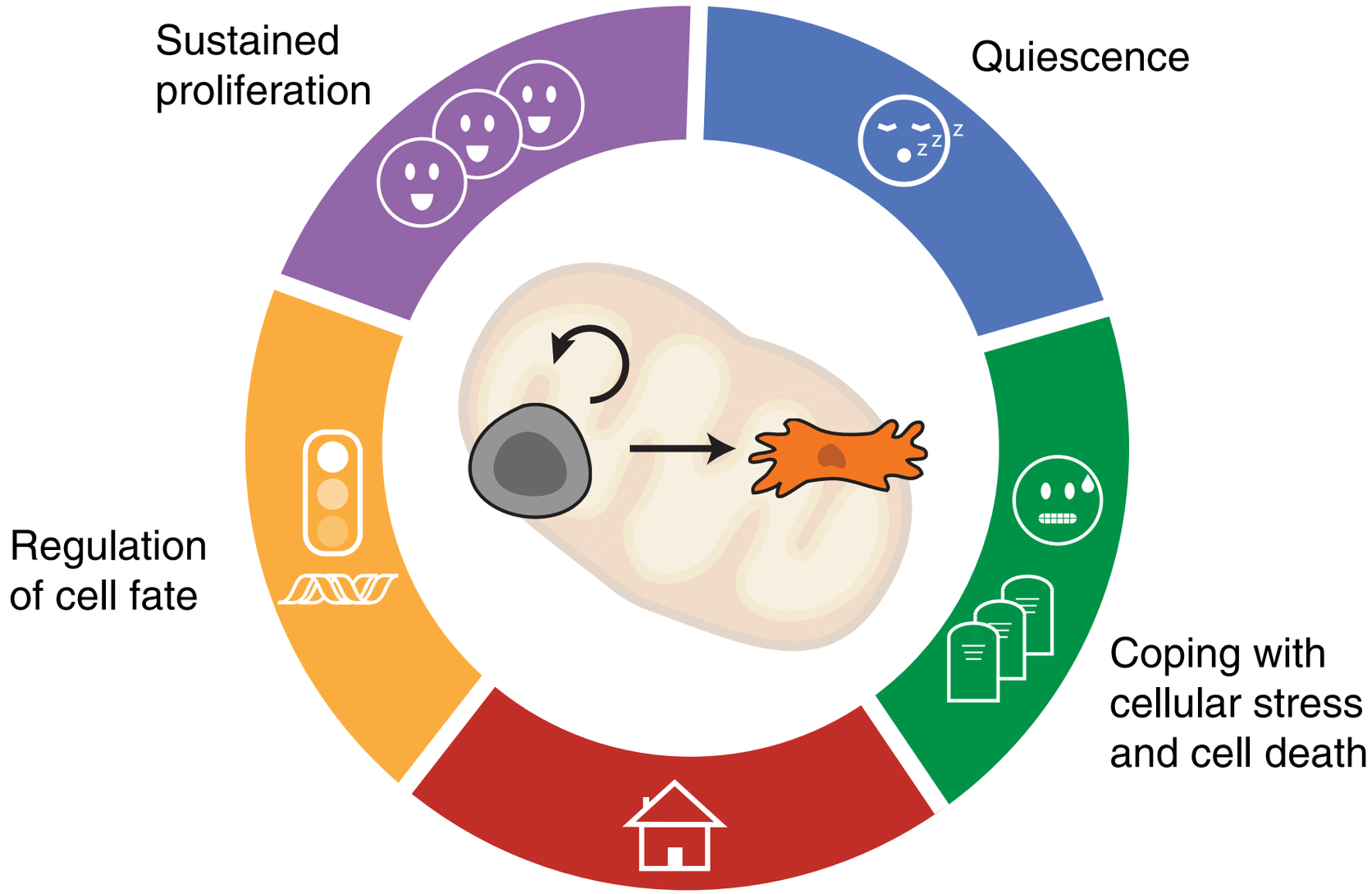
Semir Beyaz[1,2*], Miyeko D. Mana[1*], Jatin Roper[1,3*], Dmitriy Kedrin[1,4], Assieh Saadatpour[5], Sue-Jean Hong[6], Khristian E. Bauer-Rowe[1], Michael E. Xifaras[1], Adam Akkad[1], Erika Arias[1], Luca Pinello[5], Yarden Katz[7], Shweta Shinagare[1], Monther Abu-Remaileh[1,6], Maria M. Mihaylova[1,6], Dudley W. Lamming[8], Rizkullah Dogum[1], Guoji Guo[2], George W. Bell[6], Martin Selig[4], G. Petur Nielsen[4], Nitin Gupta[9], Cristina R. Ferrone[4], Vikram Deshpande[4], Guo-Cheng Yuan[5], Stuart H. Orkin[2], David M. Sabatini[1,6,7] & Ömer H. Yilmaz[1,4,7]







Role 2: regulation/adaptation to quiescence



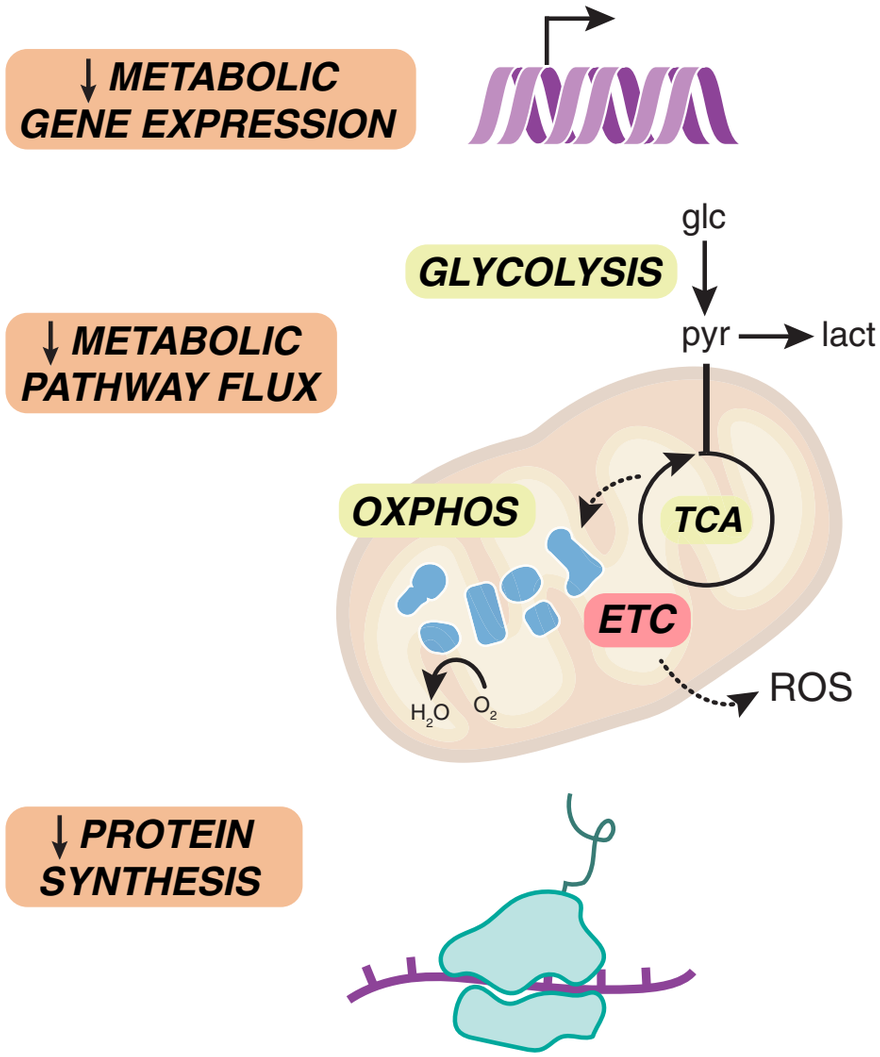
Cell metabolism is emerging as an additional determinant of stem cell function.

Jackson & Finley, ***Cell Stem Cell****, 2024*

Role 2: regulation/adaptation to quiescence

In addition to supporting the demands of cellular proliferation, metabolism can support the maintenance of non-dividing stem cell states.

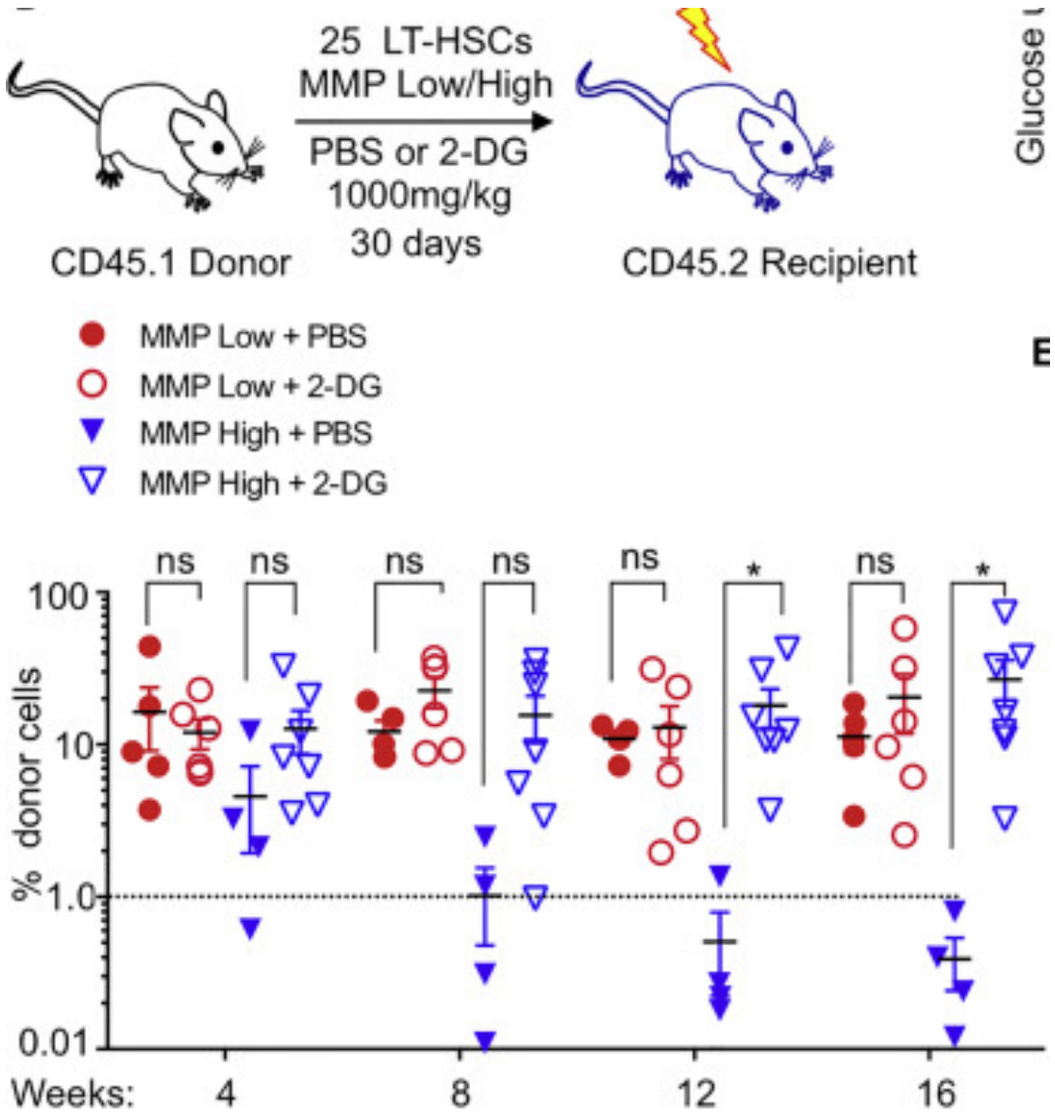
Many tissue stem cells undergo periods of quiescence, which is defined as temporary cell cycle exit into the G0 state that can be reversed in response to external stimuli. Tight control over quiescence is critical for normal stem cell homeostasis, and inappropriate proliferation can lead to exhaustion of the stem cell compartment.



Jackson & Finley, ***Cell Stem Cell****, 2024*

Role 2: regulation/adaptation to quiescence

Consistent with cell cycle exit, **quiescence has historically been considered a metabolically inert state**, where decreased cellular proliferation is thought to be accompanied by **decreased demand for the products of anabolic metabolism.**



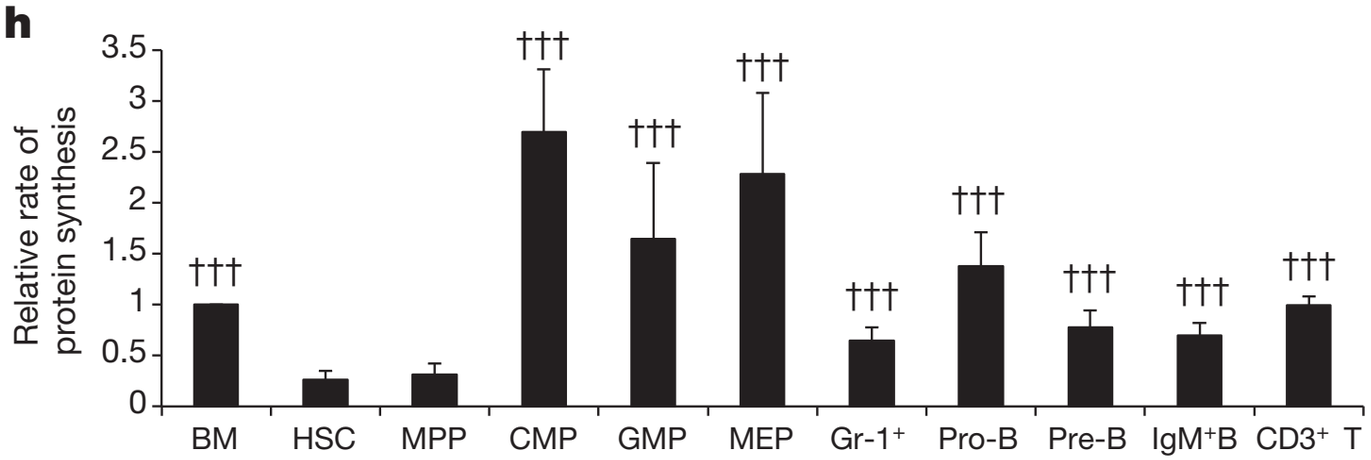
Glycolytic Inhibition Enhances HSC Long-Term Competitive Repopulation Activity *In Vivo*

Jiang et al, ***Cell Stem Cell****, 2020*

Role 2: regulation/adaptation to quiescence

Which metabolic outputs are truly reduced during quiescence is largely an open question, but many studies point to reduced protein synthesis as a common feature of quiescent stem cells. Decreased translation — among the most costly bioenergetic cellular processes — has been observed in PSCs, HSCs, NSCs, HFSCs, and MuSCs. Across these cell types, the specific benefit of low translation for quiescence remains unclear.

However, forced protein synthesis by genetic perturbations causes stem cells to exit quiescence and activate at the expense of self-renewal, leading to the eventual depletion of the stem cell pool.



Signer et al, ***Nature****, 2013*

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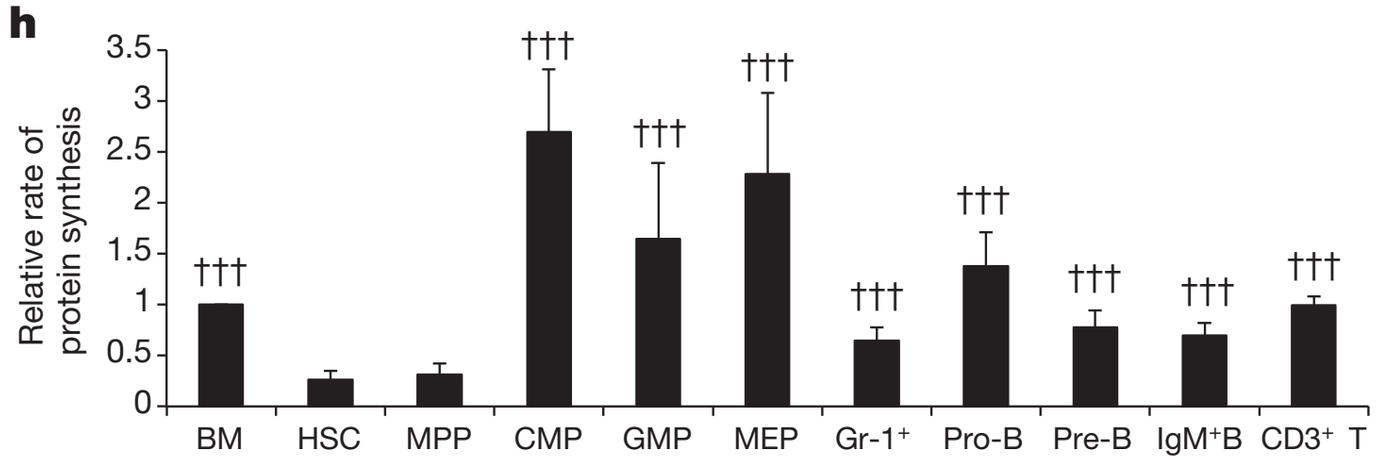
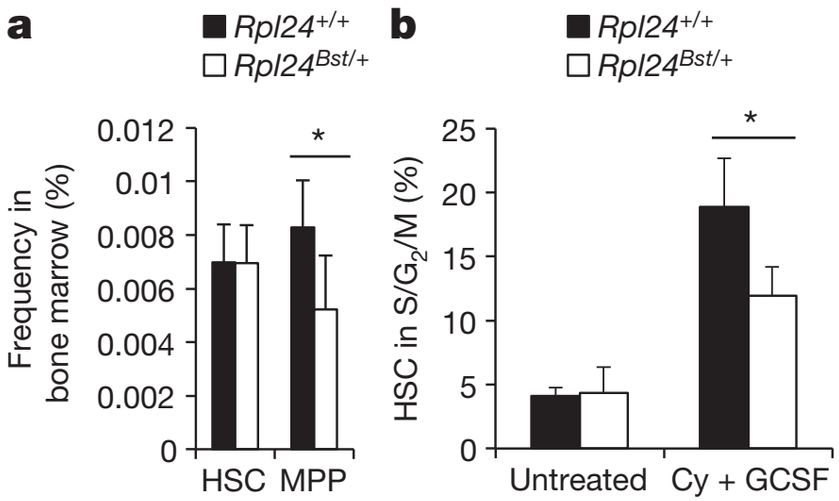




Rpl24$^{Bst/1}$ mice have a hypomorphic mutation in the Rpl24 ribosome subunit, reducing protein synthesis in multiple cell types by 30% in culture. Adult Rpl24$^{Bst/1}$ mice show relatively mild phenotypes: they are 20% smaller than wild-type mice and have mild pigmentation and skeletal abnormalities.

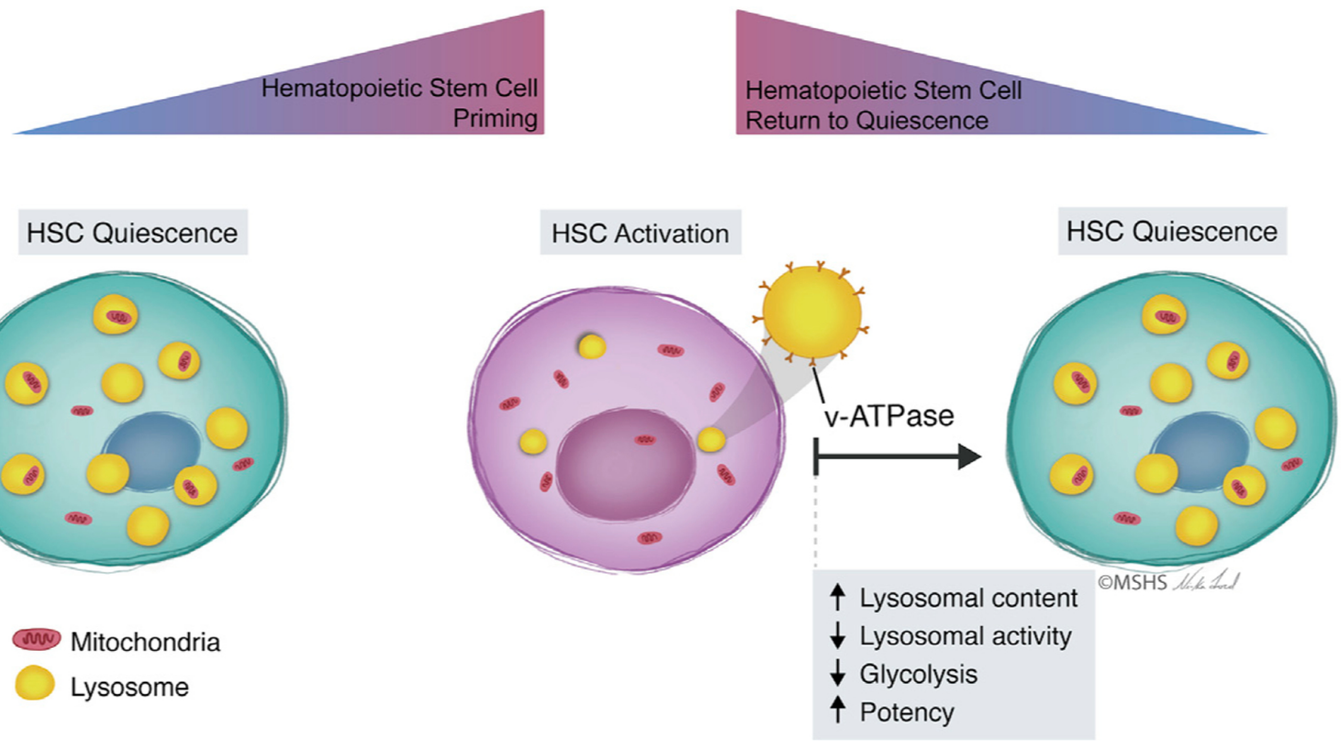
*Signer et al, **Nature**, 2013*

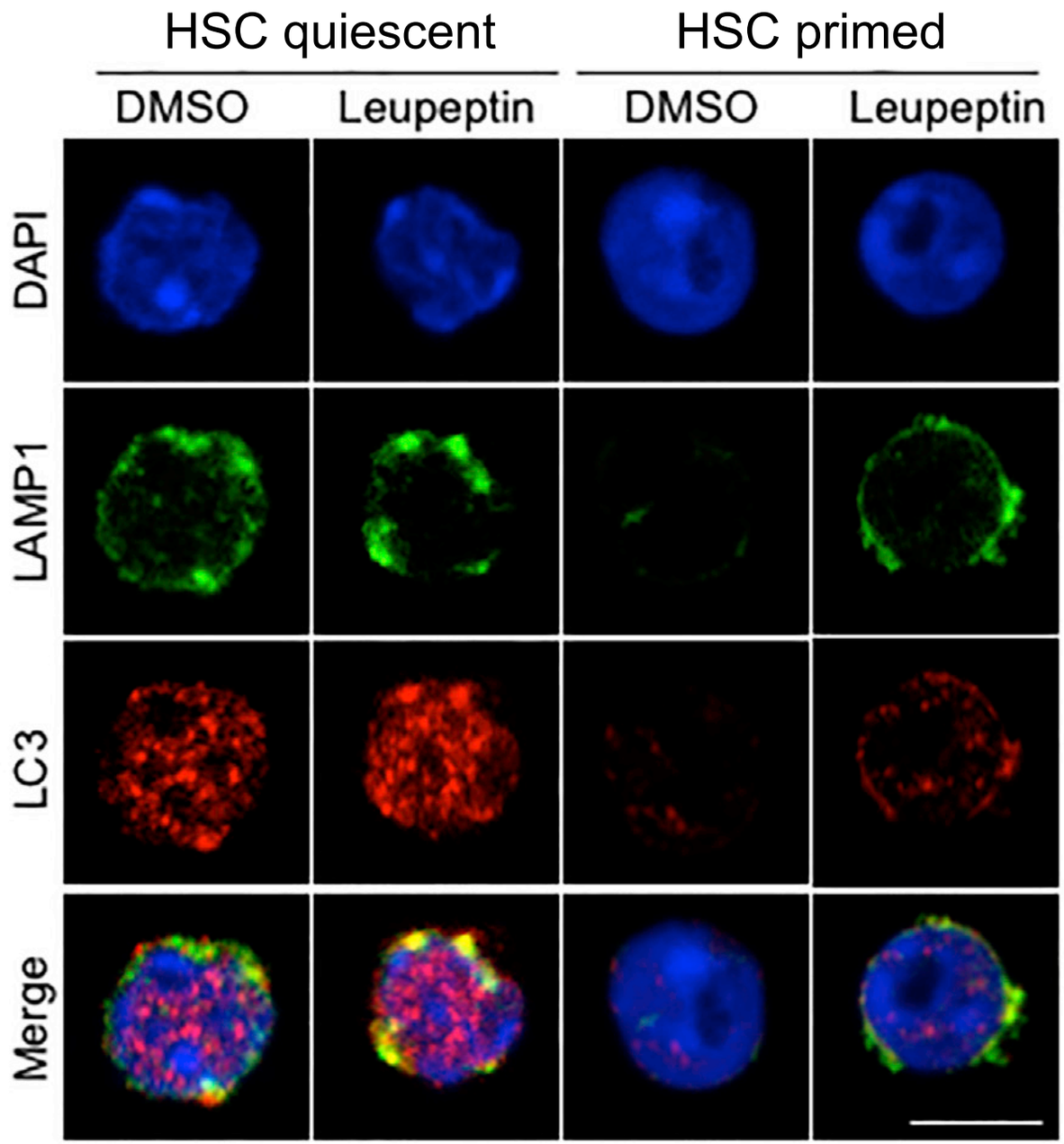
Role 2: regulation/adaptation to quiescence

In addition to decreased expression of metabolic genes, **quiescent stem cells have higher expression of genes involved in autophagy.**

In quiescence, autophagy — while incompatible with net biomass generation — might liberate nutrients that allow cell survival while in a metabolically low or isolated state.

Similarly, autophagy can be utilized by non-dividing cells to remove dysfunctional organelles or cellular debris that might otherwise be passed along to dividing progeny.

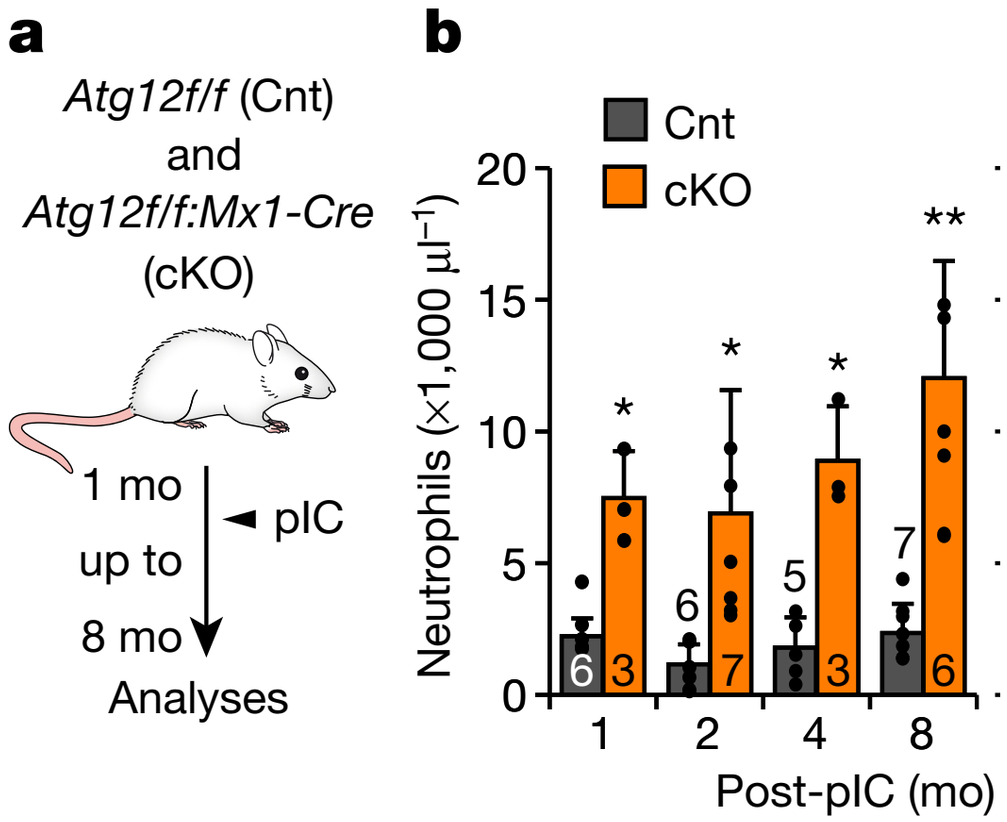




Liang et al, ***Cell Stem Cell****, 2020*

Autophagy maintains the metabolism and function of young and old stem cells

Theodore T. Ho[1], Matthew R. Warr[1], Emmalee R. Adelman[2], Olivia M. Lansinger[1], Johanna Flach[1], Evgenia V. Verovskaya[1], Maria E. Figueroa[2]† & Emmanuelle Passegué[1]†

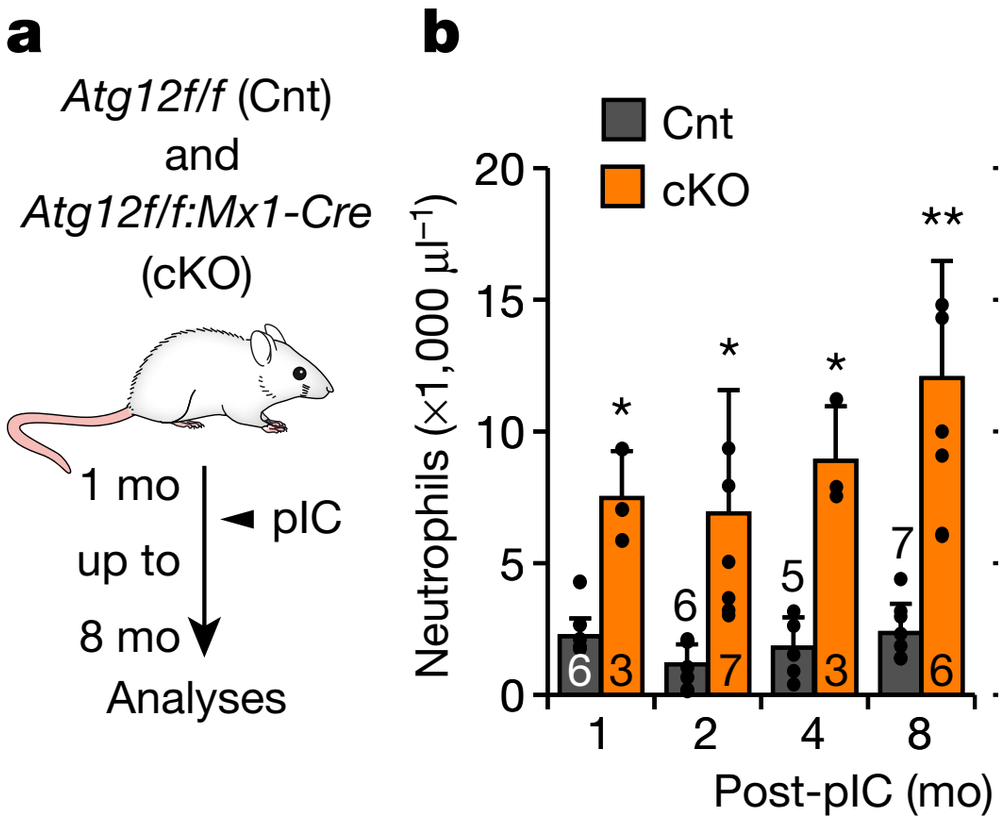


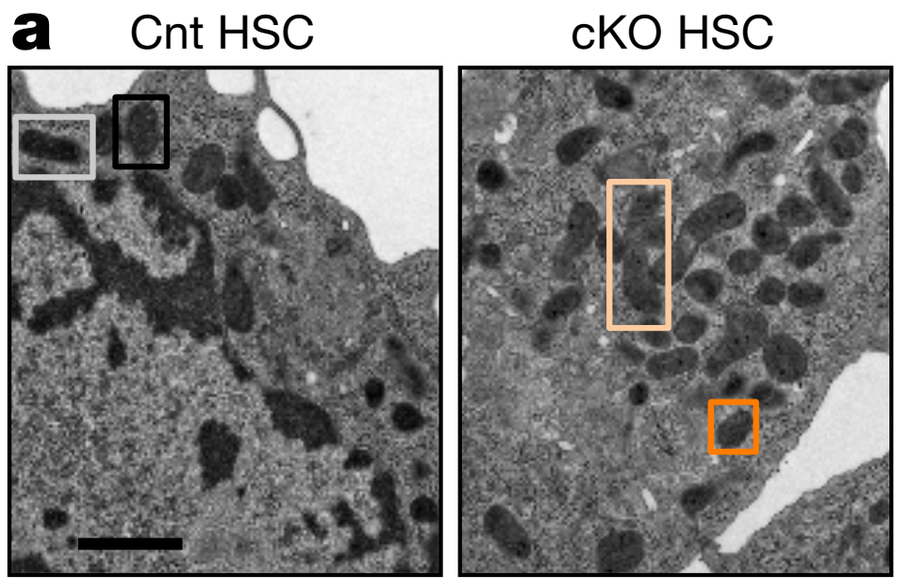
Impaired autophagy halts proper repopulation of the BM and promotes HSC differentiation

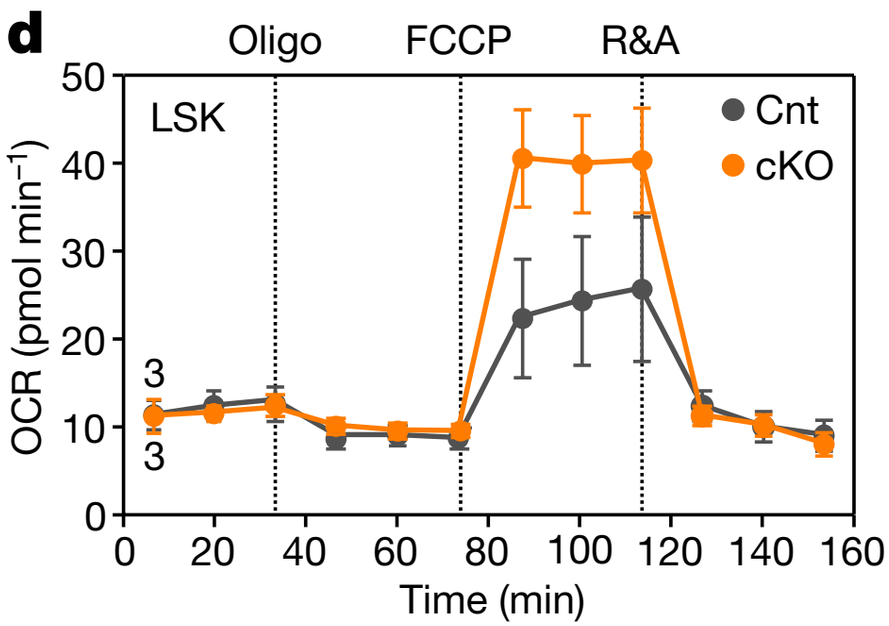
Ho et al, ***Nature****, 2017*

Autophagy maintains the metabolism and function of young and old stem cells

Theodore T. Ho[1], Matthew R. Warr[1], Emmalee R. Adelman[2], Olivia M. Lansinger[1], Johanna Flach[1], Evgenia V. Verovskaya[1], Maria E. Figueroa[2]† & Emmanuelle Passegué[1]†







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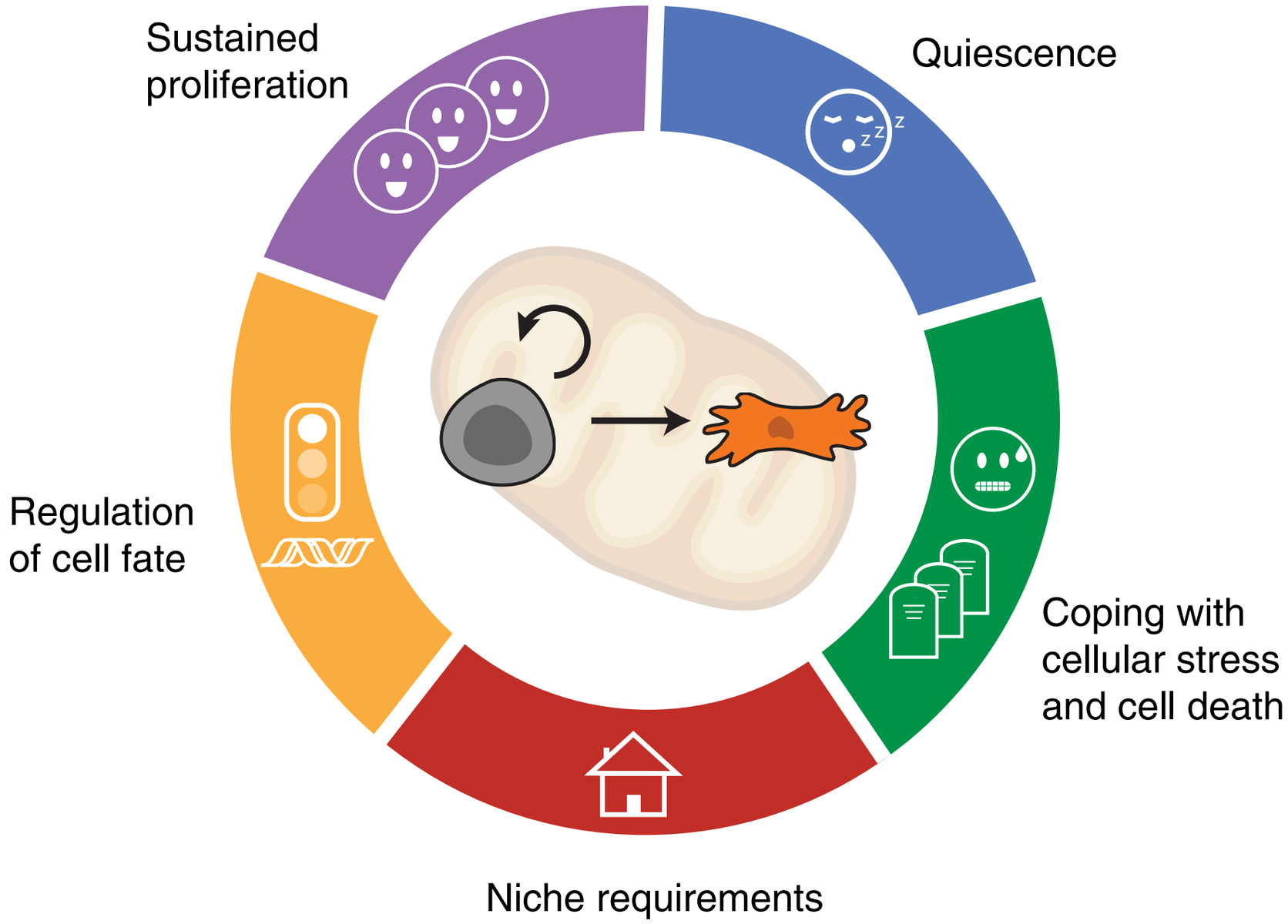
Ho et al, ***Nature****, 2017*

Role 3: resistance to metabolic stress and cell death



Cell metabolism is emerging as an additional determinant of stem cell function.

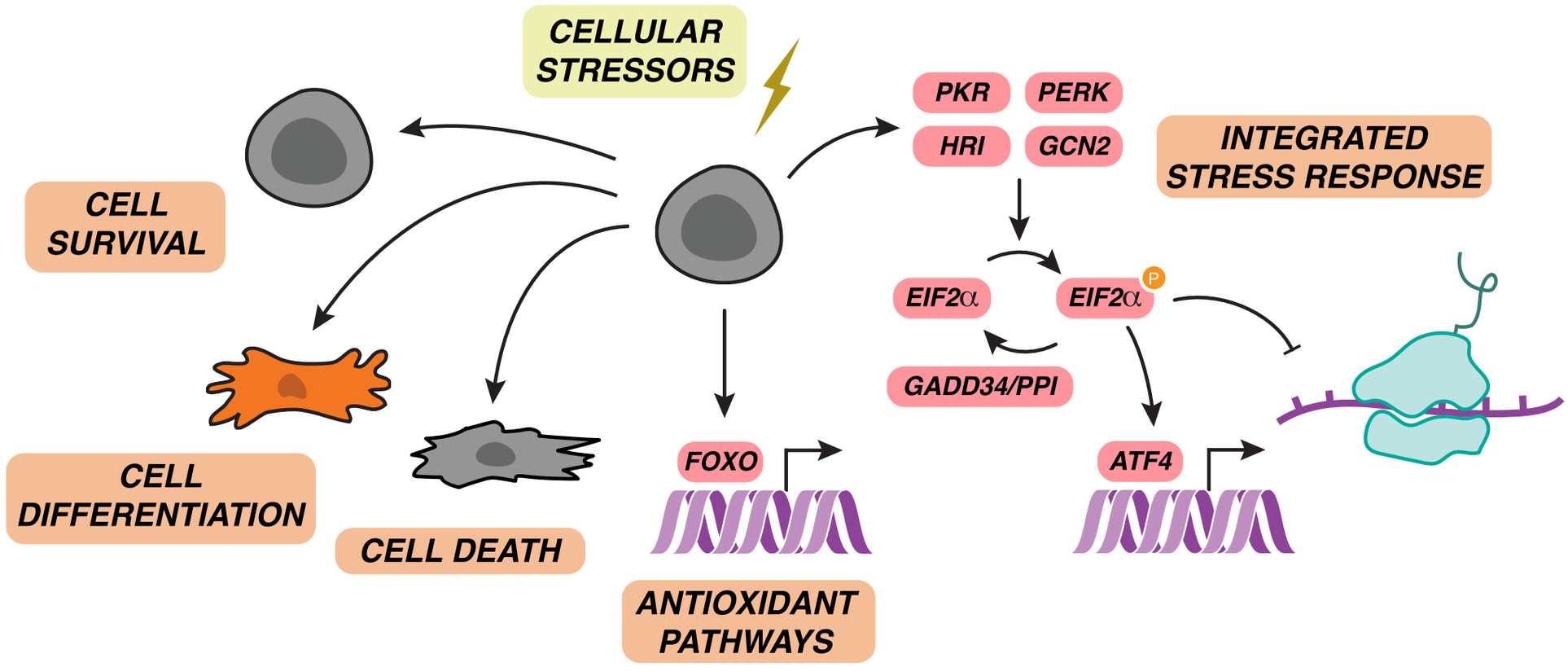
Jackson & Finley, ***Cell Stem Cell****, 2024*

Role 3: resistance to metabolic stress and cell death

As reservoirs for tissue regeneration in settings of injury, stem cells must be able to withstand some degree of cellular stress.

Strikingly, viable tissue stem cells capable of engraftment have been iso- lated up to four days postmortem, suggesting that stem cells are uniquely positioned to resist external stressors.

At the same time, precautions are taken to ensure defective stem cells do not contribute to the progenitor pool and so appropriate control of cell death programs is critical for normal development and tissue homeostasis.



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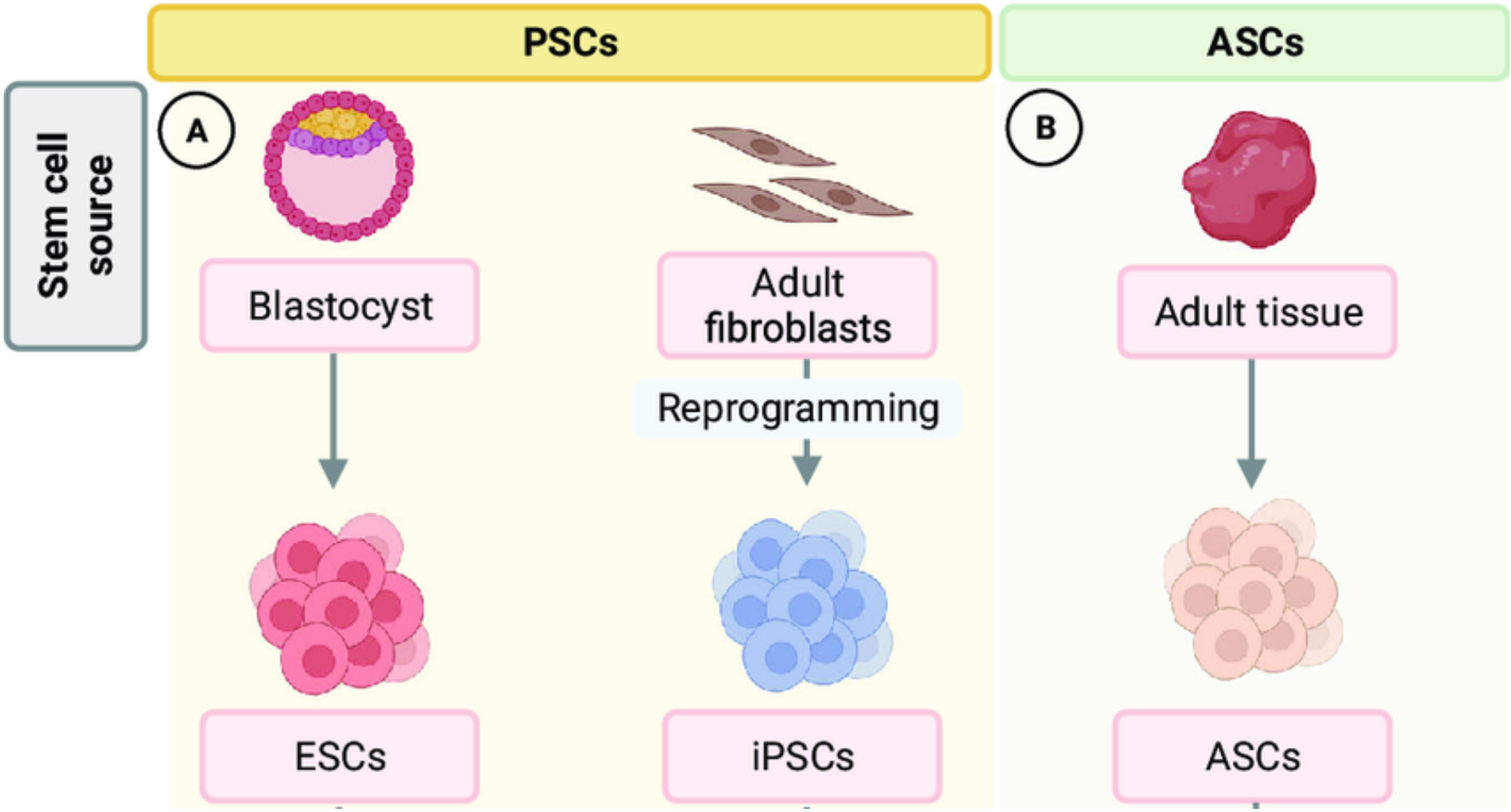

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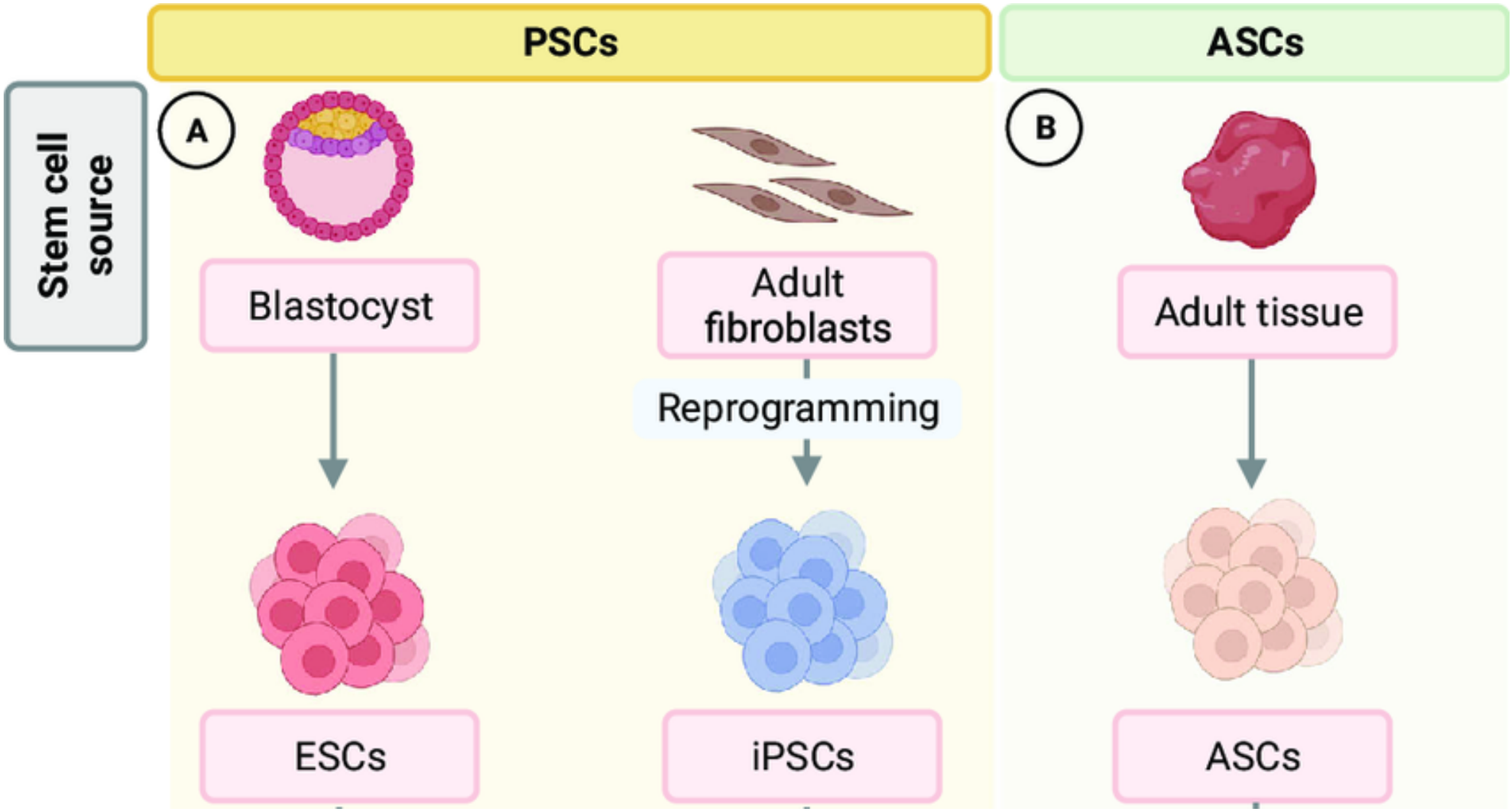


Fast replicating

Long lasting

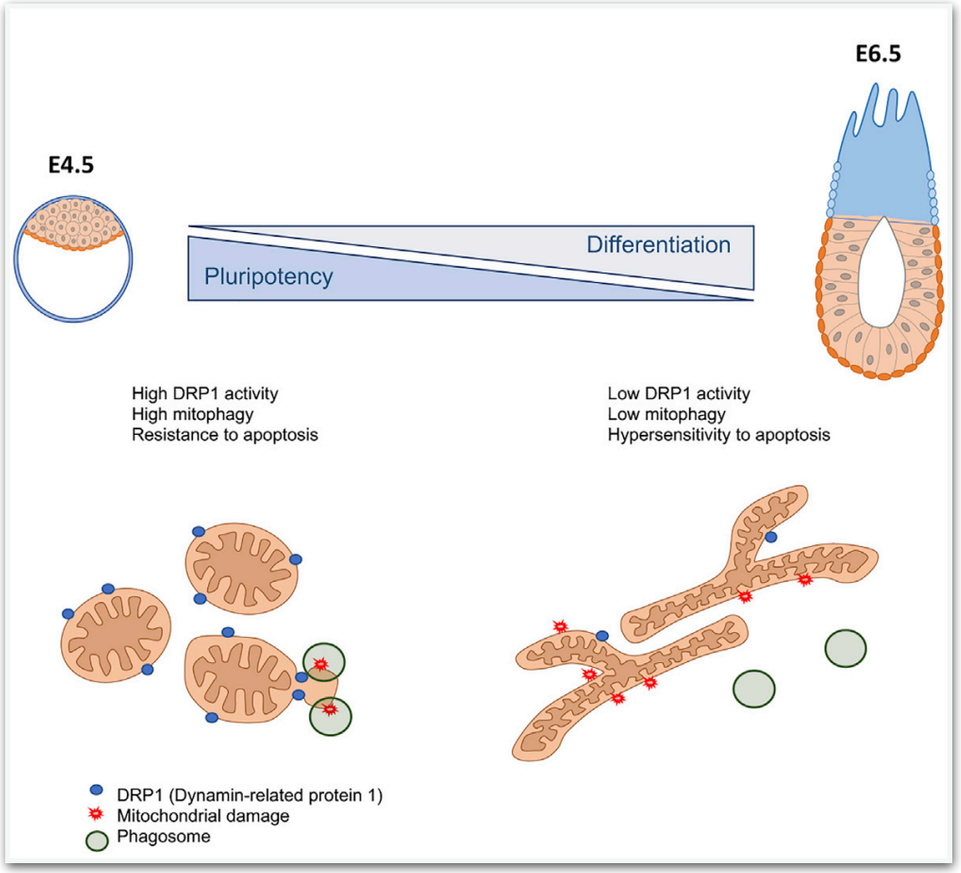
Metabolic reprogramming confers:

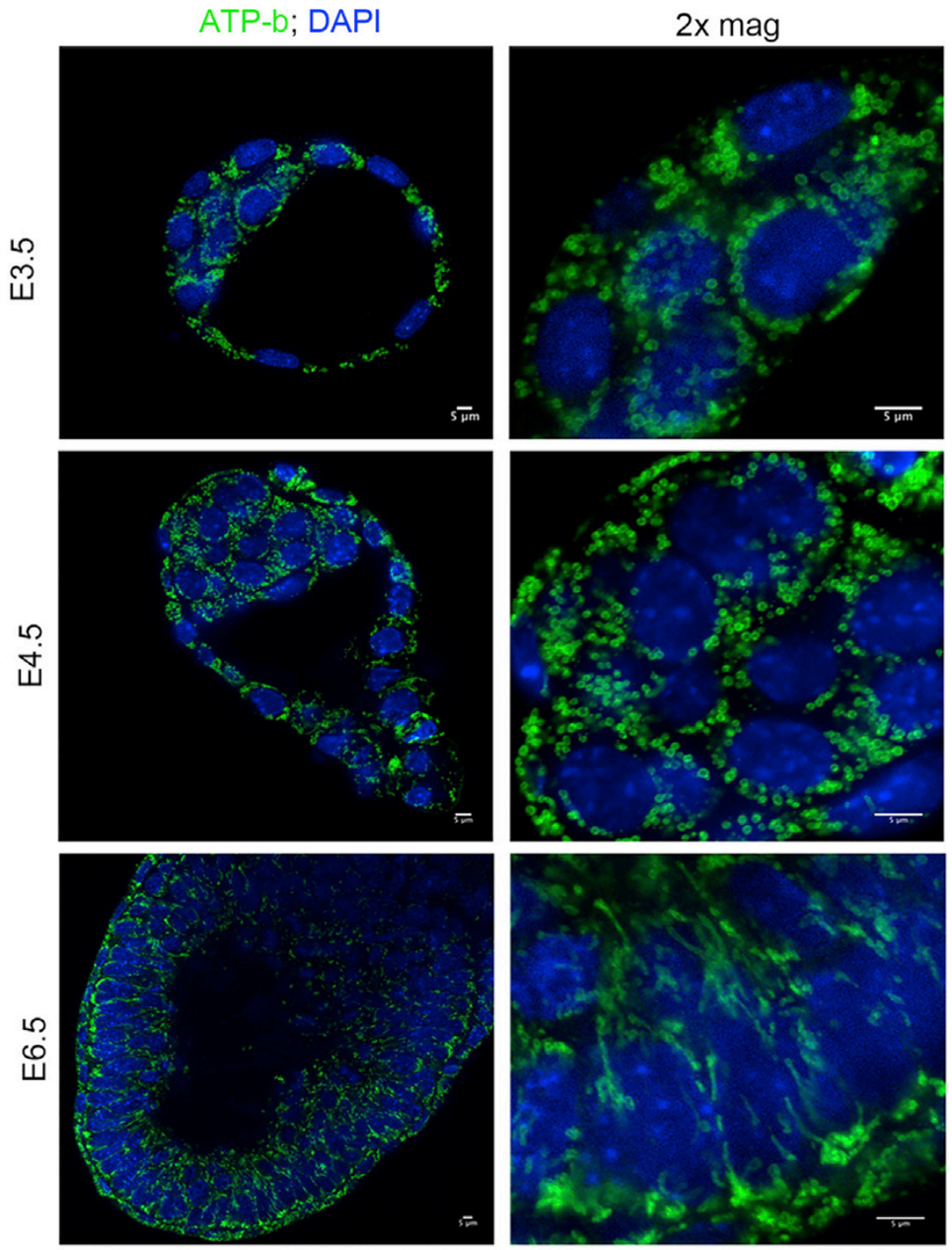
Resistance to cell death

Resistance to damage

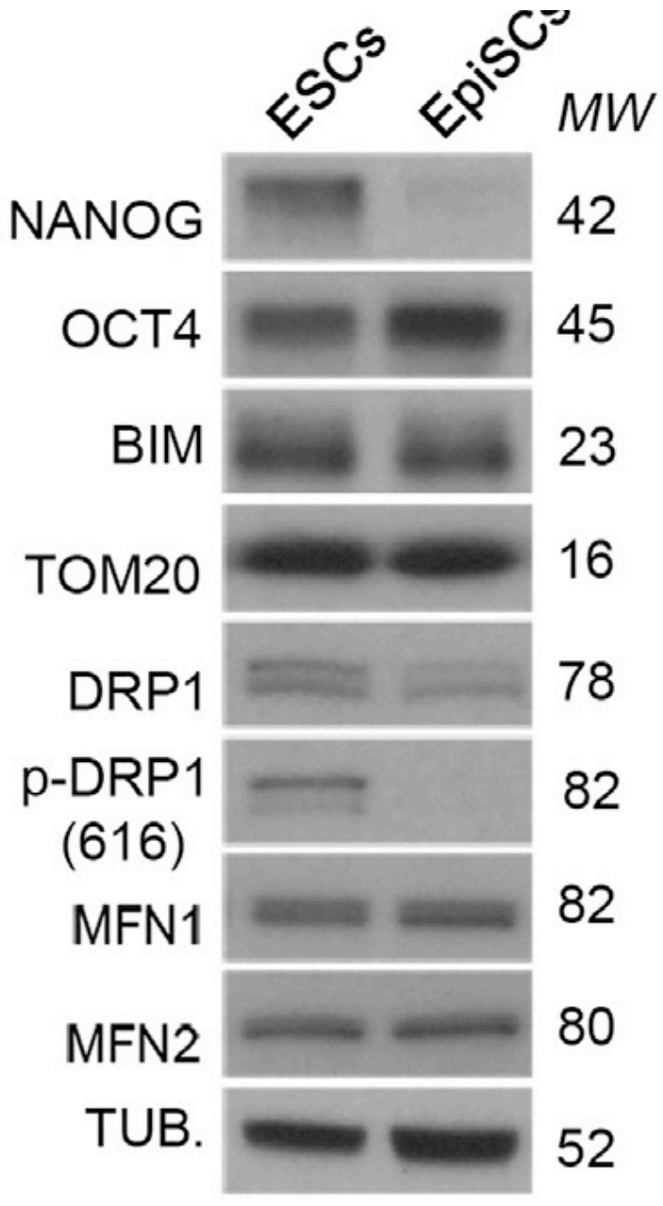
DRP1 levels determine the apoptotic threshold during embryonic differentiation through a mitophagy-dependent mechanism

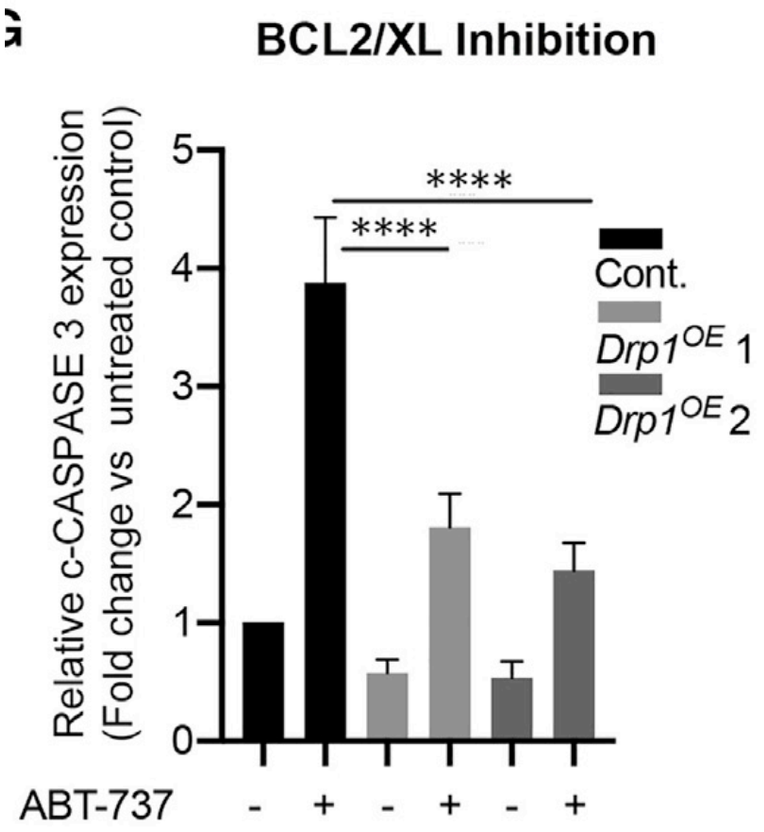
Barbara Pernaute,[1,8,9,10] Salvador Pérez-Montero,[1,8] Juan Miguel Sánchez Nieto,[1,8,11] Aida Di Gregorio,[1] Ana Lima,[1] Katerina Lawlor,[1] Sarah Bowling,[1] Gianmaria Liccardi,[2] Alejandra Tomás,[3] Pascal Meier,[2] Hiromi Sesaki,[4] Guy A. Rutter,[3,5,6] Ivana Barbaric,[7] and Tristan A. Rodríguez[1,12,*]





Pernaute et al, ***Dev Cell****, 2022*

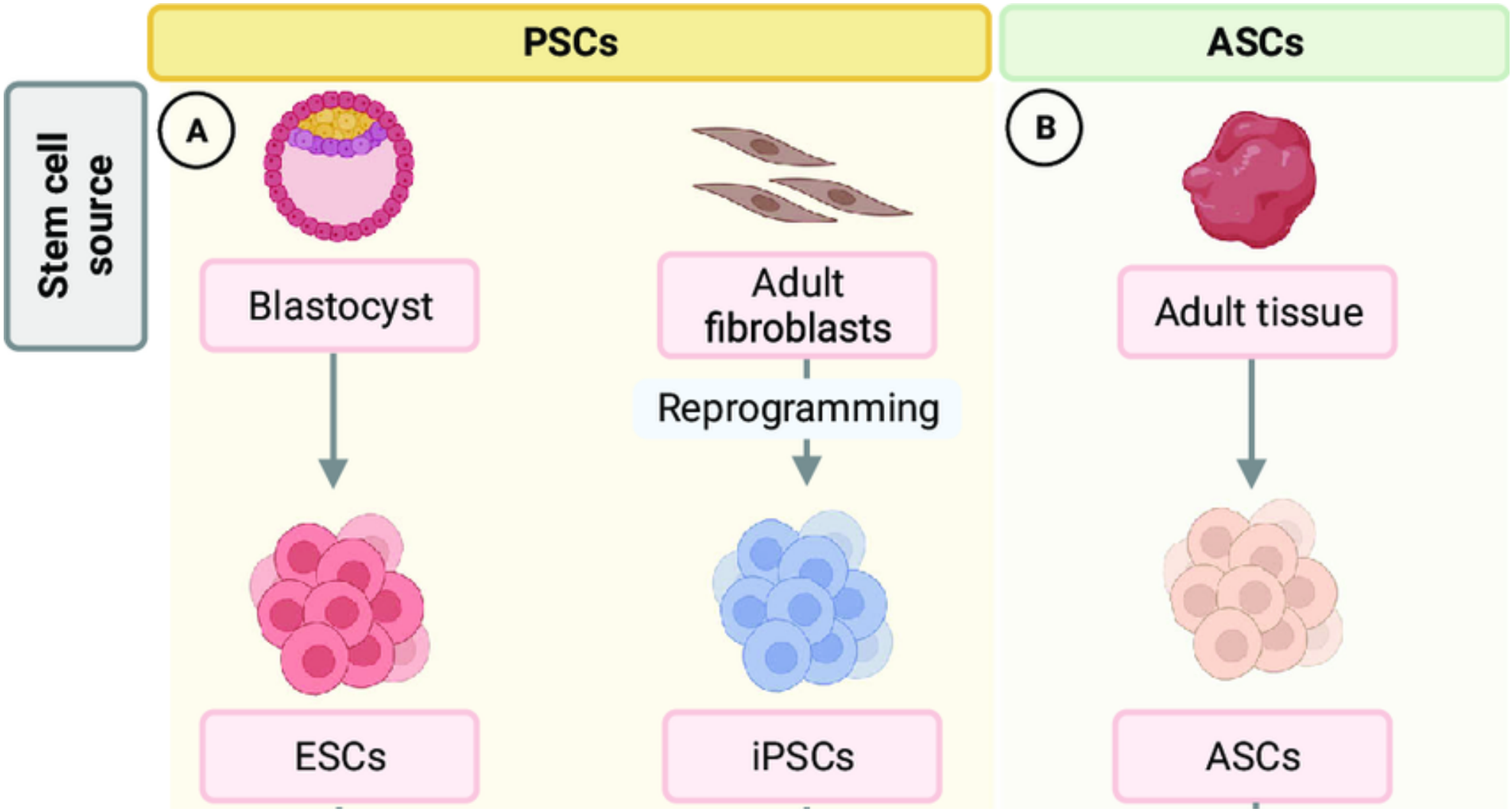




DRP1 over expression inhibits the apoptotic response during the onset of PSC differentiation

Pernaute et al, ***Dev Cell****, 2022*

Role 3: resistance to metabolic stress and cell death



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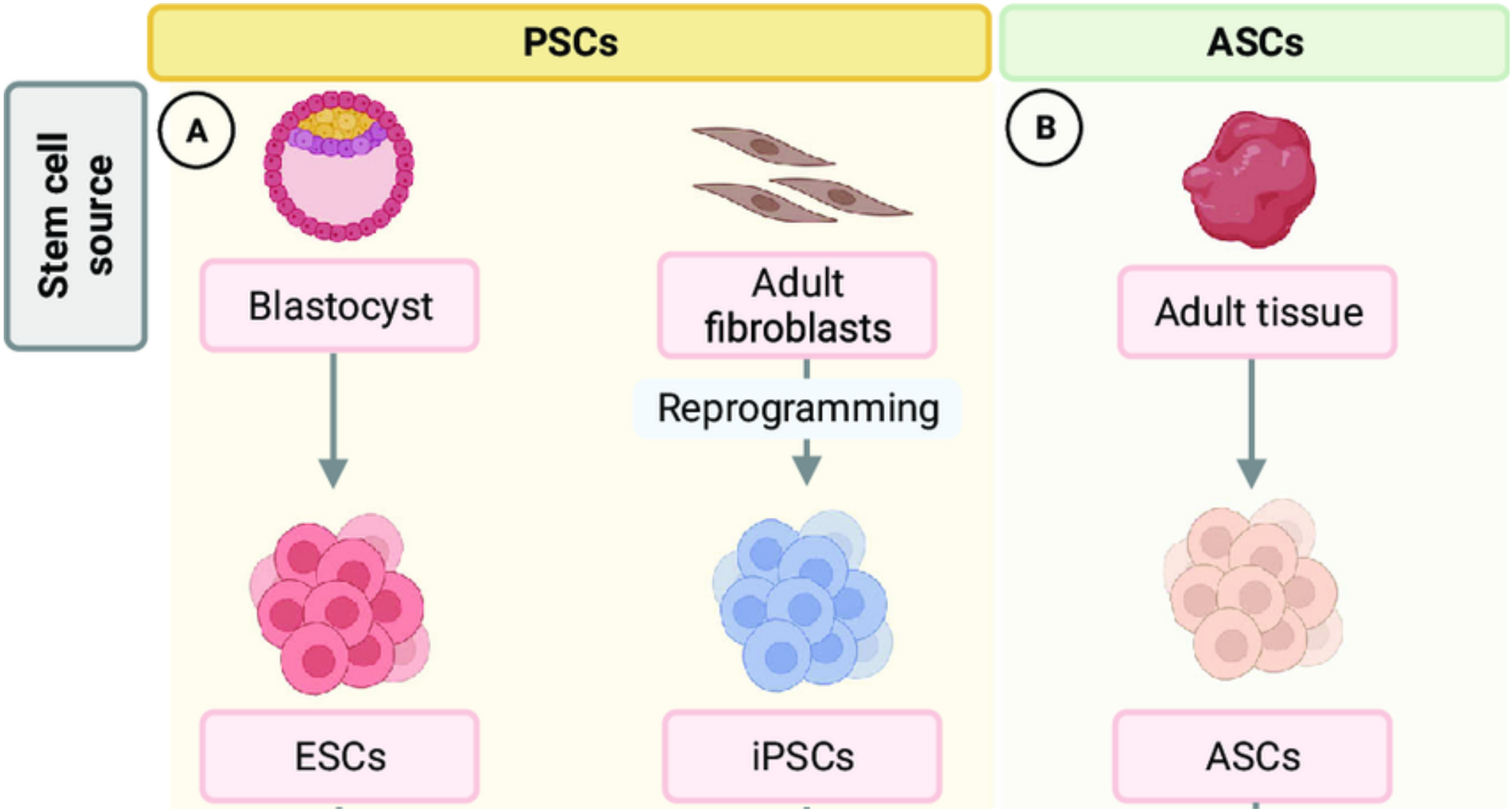


Fast replicating

Long lasting

Metabolic reprogramming confers:

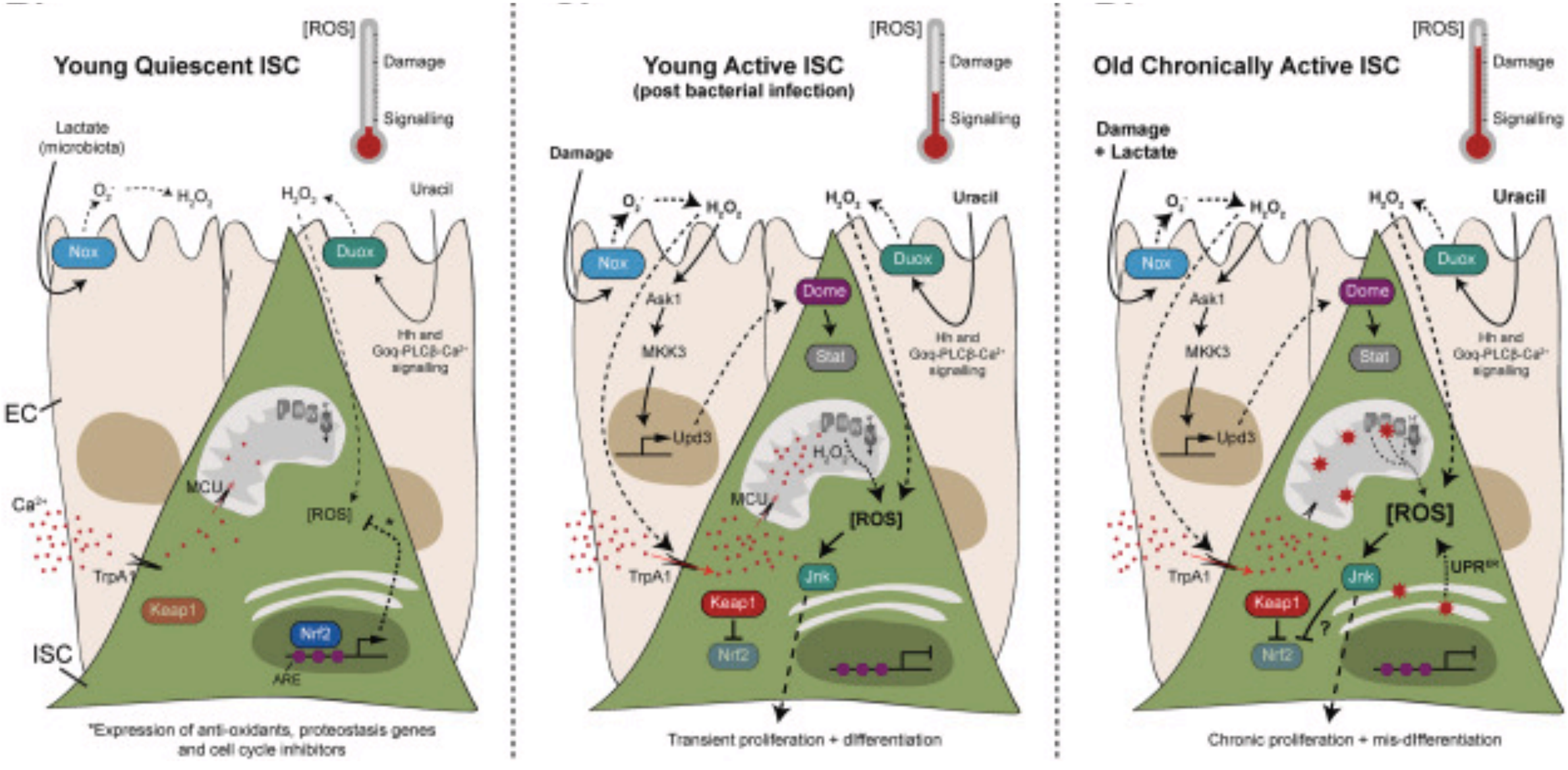
Resistance to cell death

Resistance to damage

Role 3: resistance to metabolic stress and cell death

ROS are endogenous toxic metabolites. Their levels must be finely monitored and tend to accumulate with aging.

Alterations in ROS levels cause both failure of stem cell self-renewal and impaired differentiation, suggesting that tight regulation of ROS is critical for stem cell function.

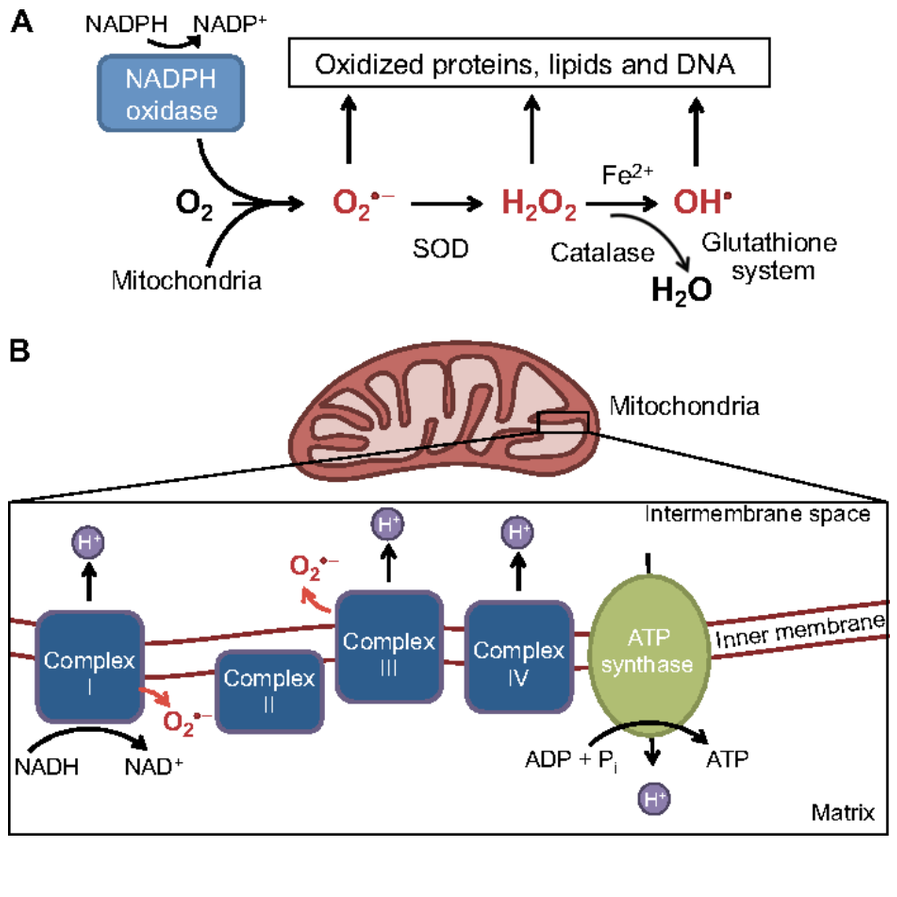


Role 3: resistance to metabolic stress and cell death

Because ROS can be genotoxic, many stem cell populations moderate its production to ensure genetic integrity in naive progenitors.

Mitochondrial ETC complexes I and III are a major source of ROS production in mammalian cells; therefore, decreased electron deposition into the ETC is one method of controlling cellular ROS levels.

Increased ROS levels are correlated with increased respiration and stem cell dysfunction in a variety of genetic models, and antioxidant treatment rescues stem cell defects seen in settings of altered respiration.

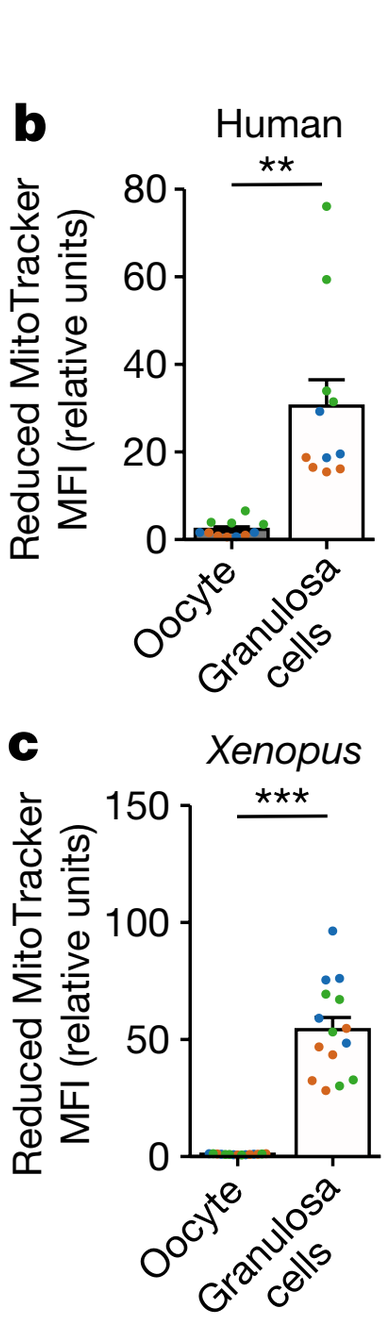


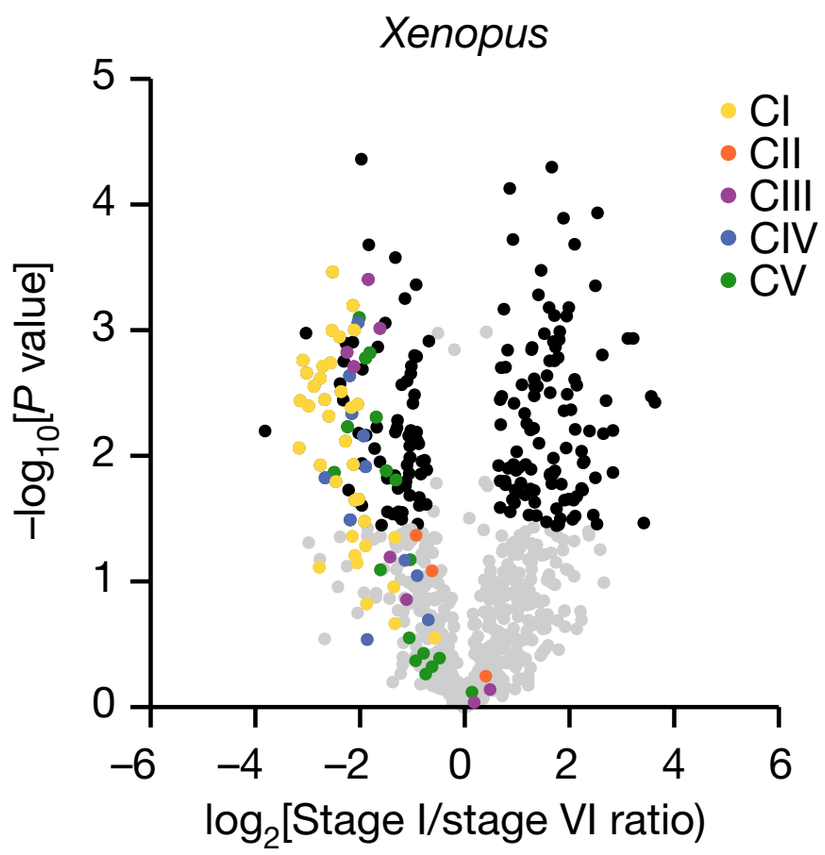
BIOLOGICAL CHEMISTRY

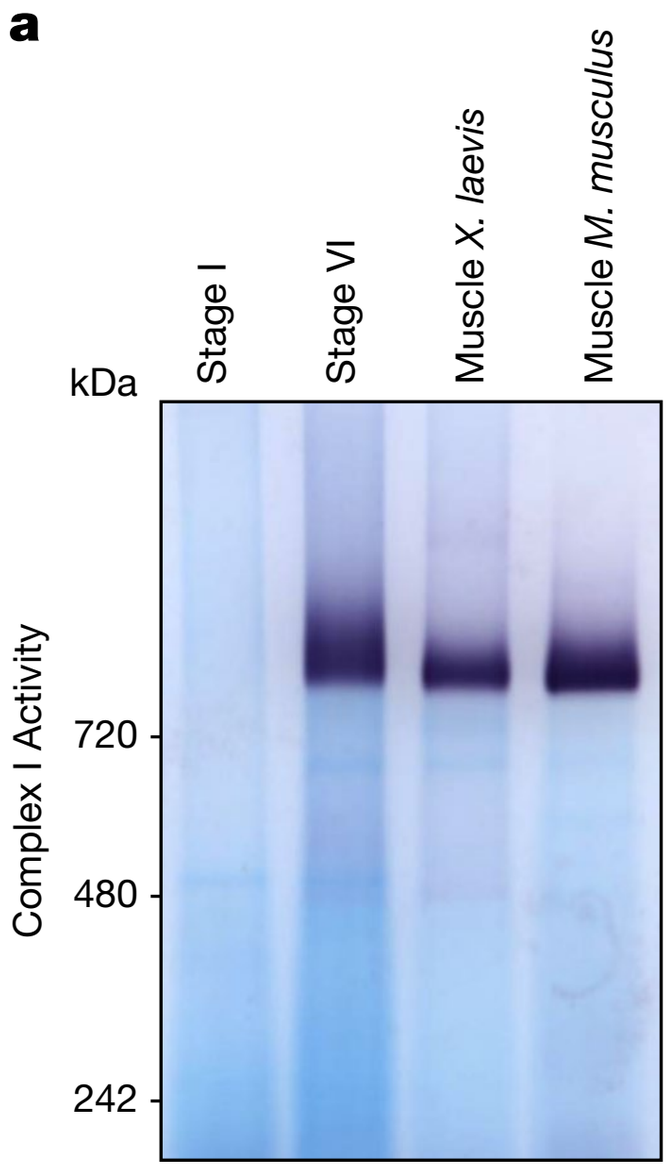
Oocytes live longer than most cells in the body. How?

Article

Oocytes maintain ROS-free mitochondrial metabolism by suppressing complex I

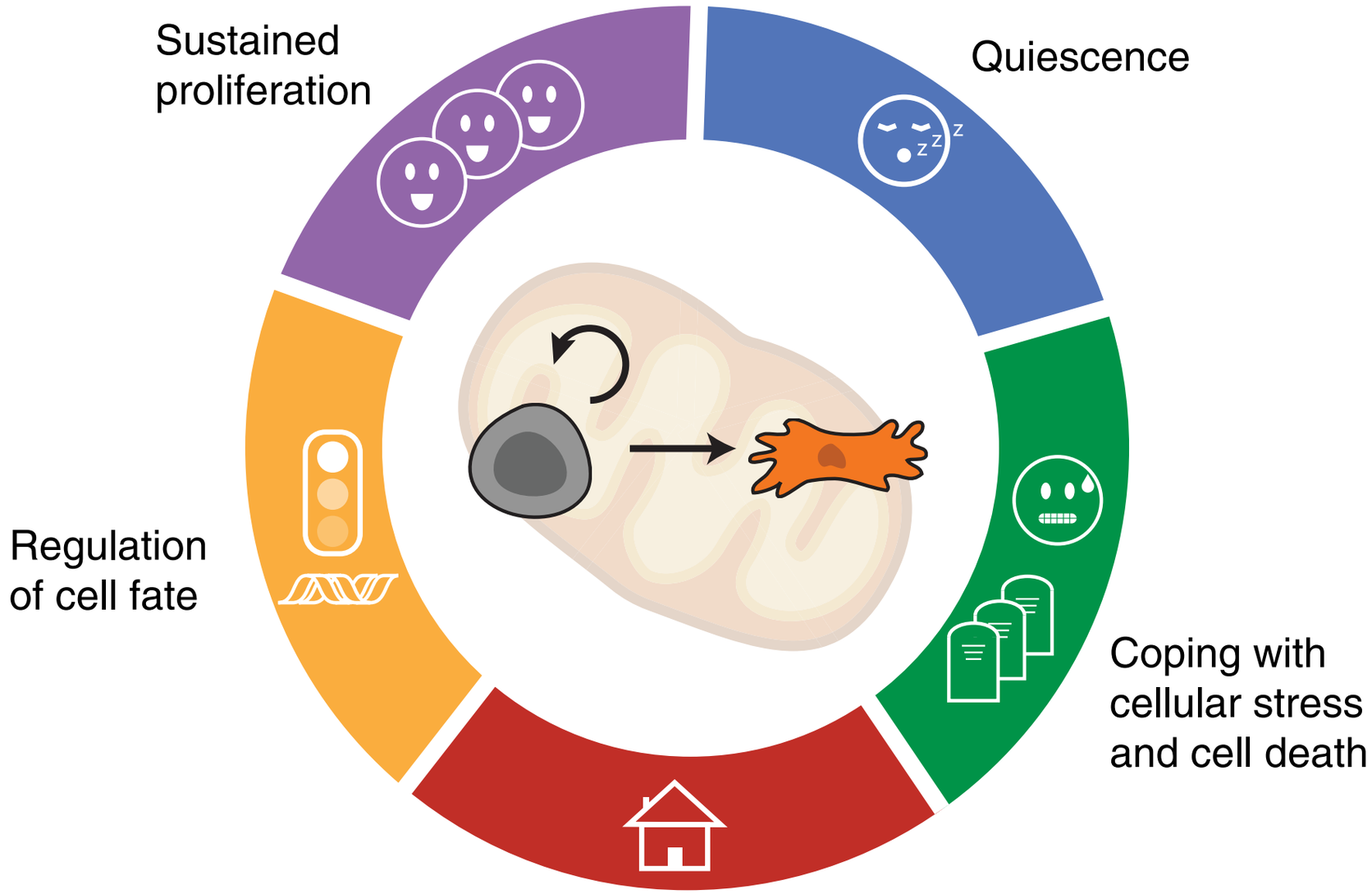






Rodriguez-Nuevo et al, ***Nature****, 2022*

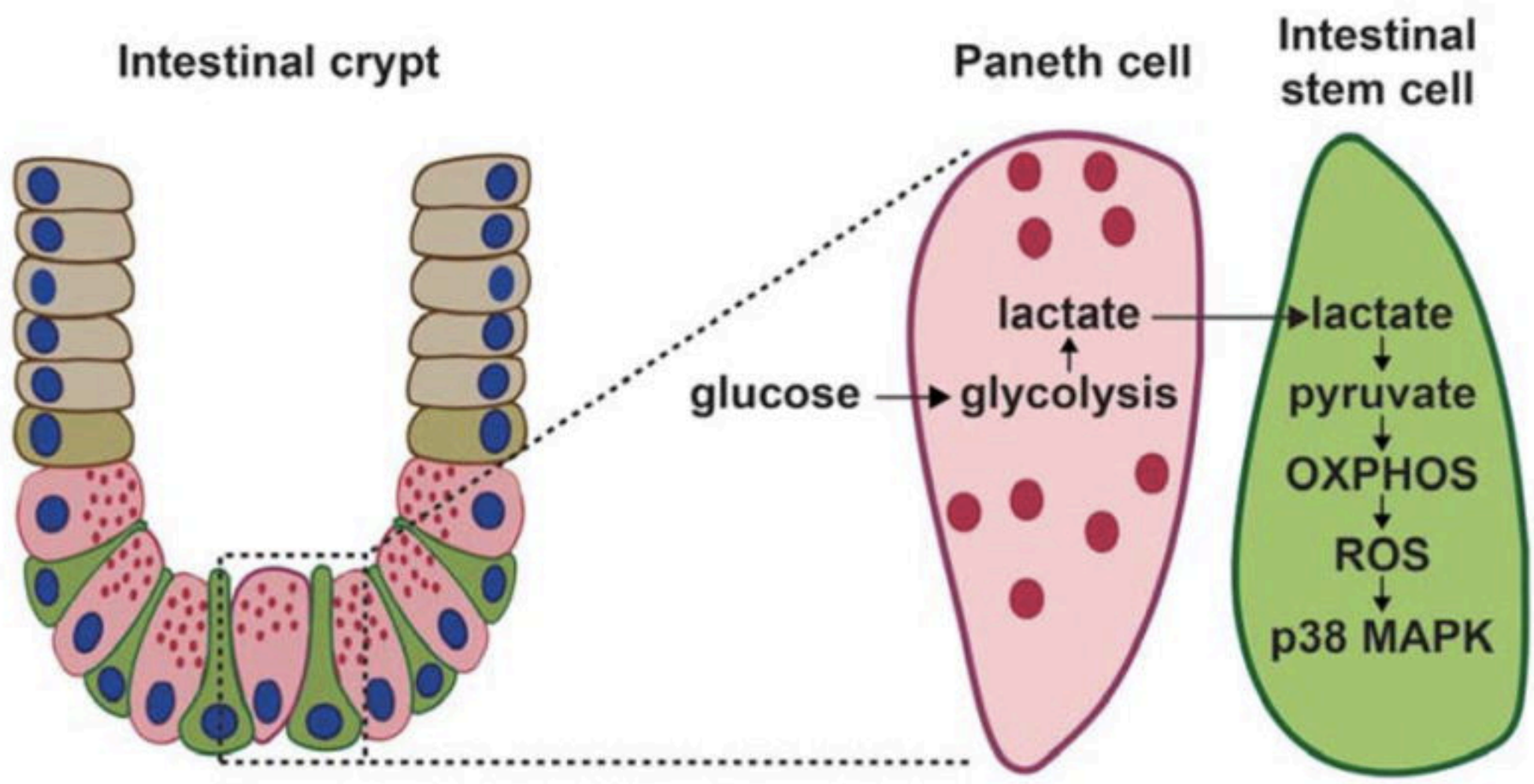
Role 4: establish/adapt to stem cell niche



Cell metabolism is emerging as an additional determinant of stem cell function.

Jackson & Finley, ***Cell Stem Cell****, 2024*

Role 4: establish/adapt to stem cell niche

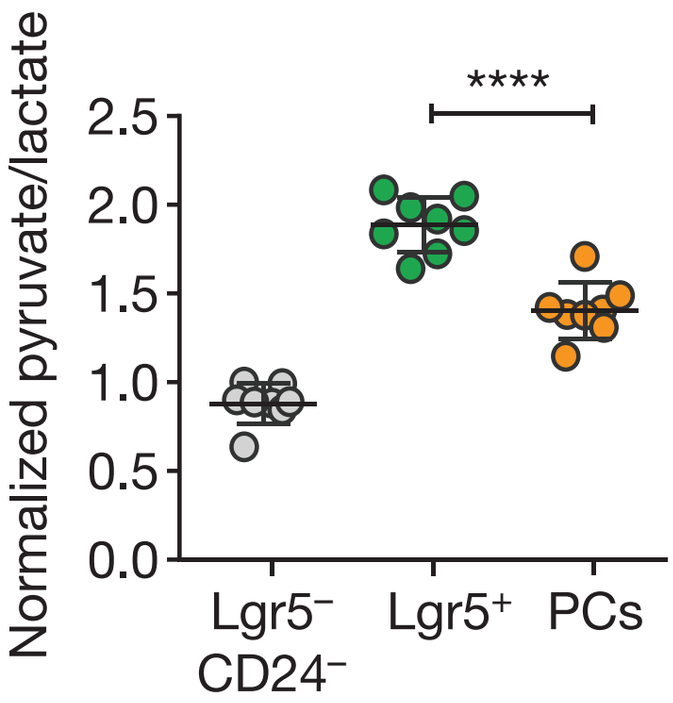


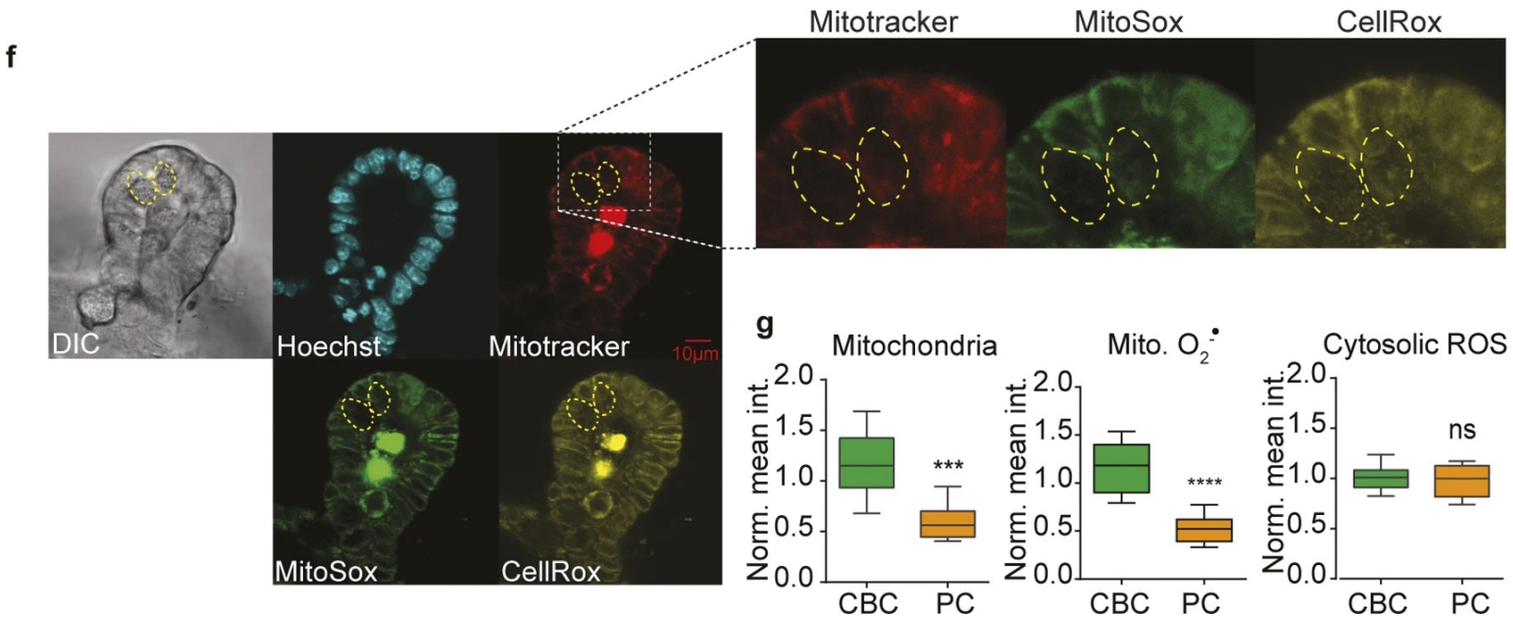
Metabolic differences between stem cells and their supporting cells may drive their distinct functions.

Roper & Yilmaz, ***Cell Metab****, 2017*

Interplay between metabolic identities in the intestinal crypt supports stem cell function

Maria J. Rodríguez-Colman[1], Matthias Schewe[2], Maaike Meerlo[1], Edwin Stigter[1], Johan Gerrits[3], Mia Pras-Raves[3], Andrea Sacchetti[2], Marten Hornsveld[1], Koen C. Oost[1], Hugo J. Snippert[1], Nanda Verhoeven-Duif[3], Riccardo Fodde[2] & Boudewijn M.T. Burgering[1]





Rodriguez-Colman et al, ***Nature****, 2017*

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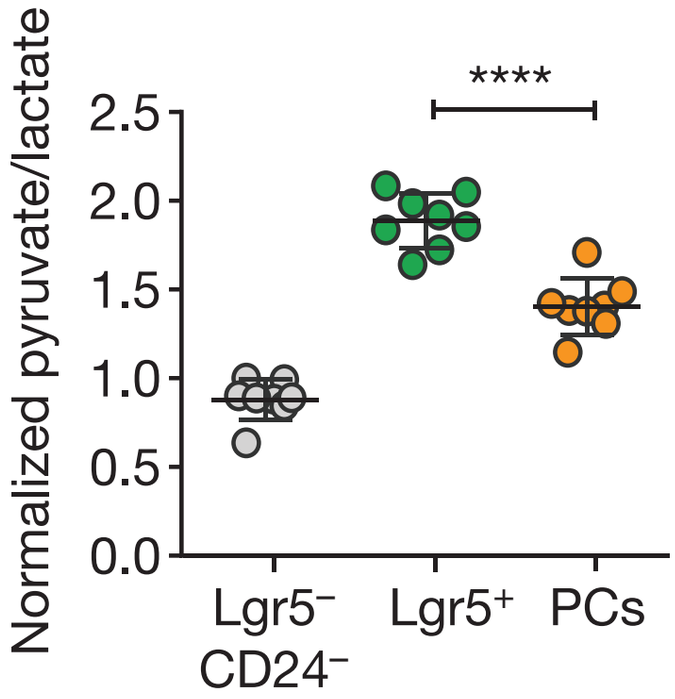
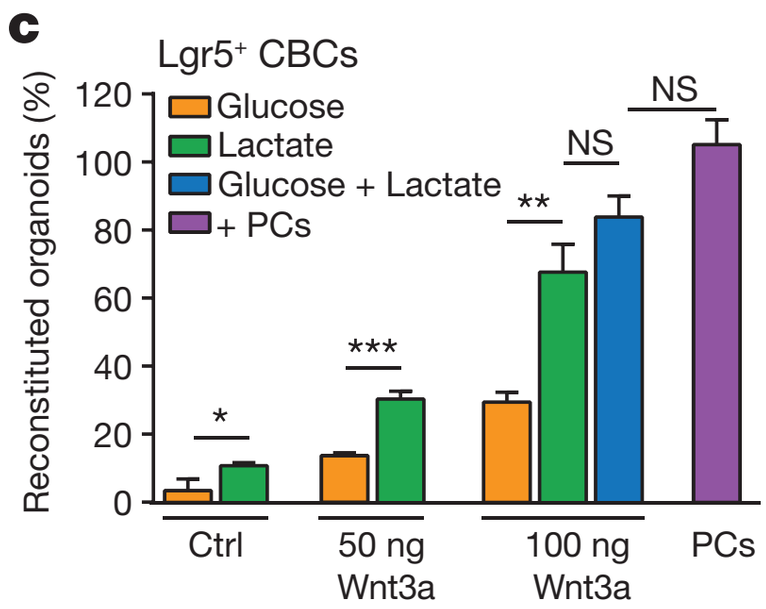
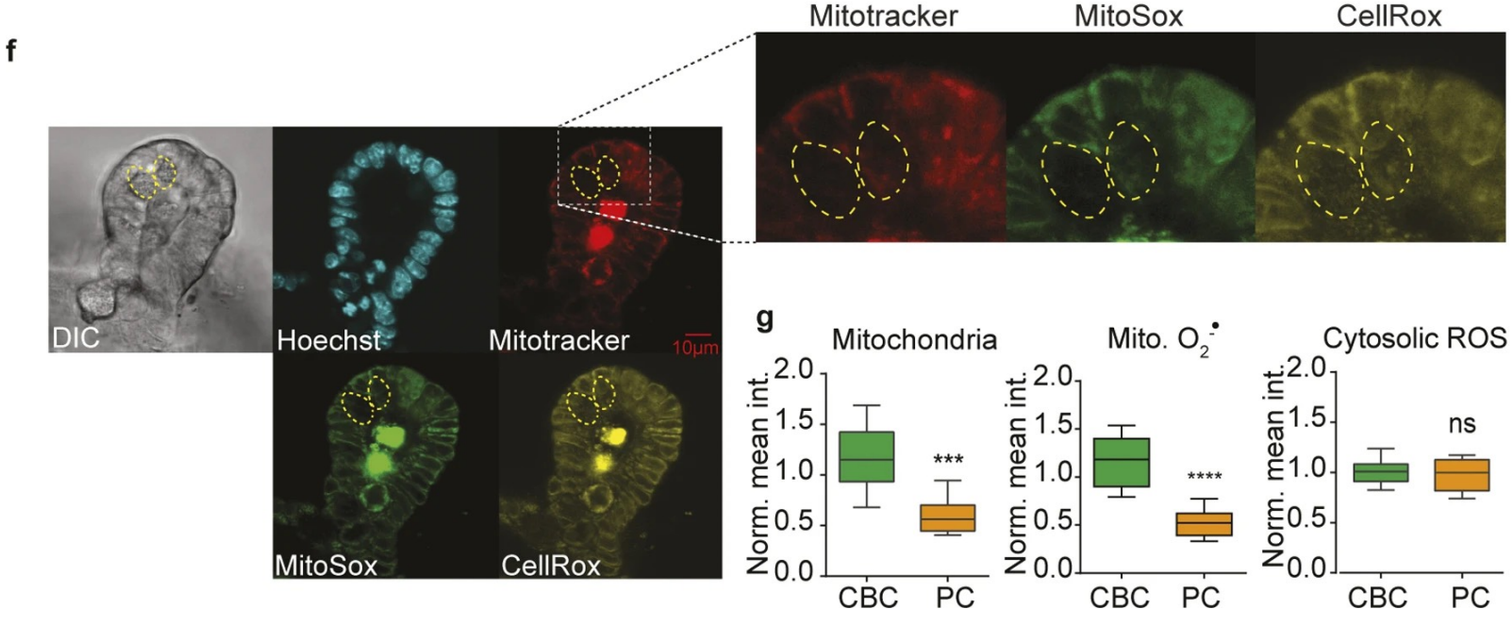
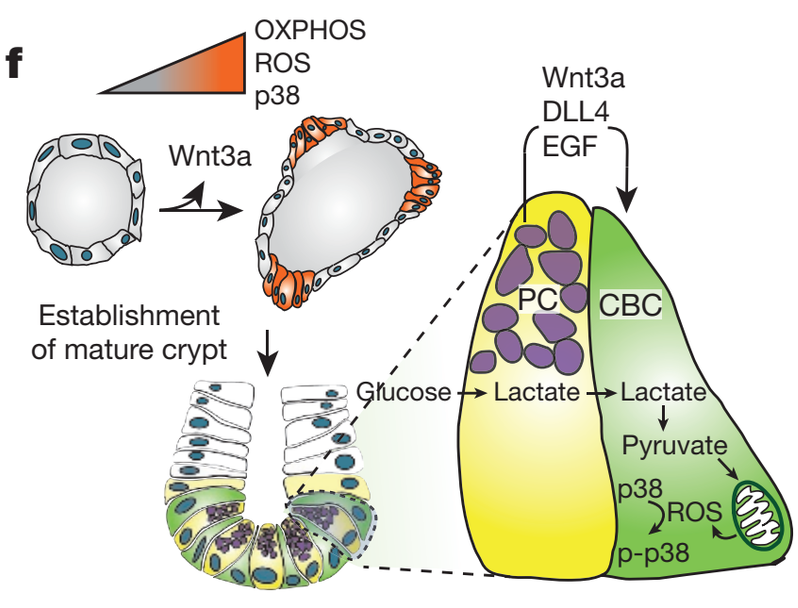


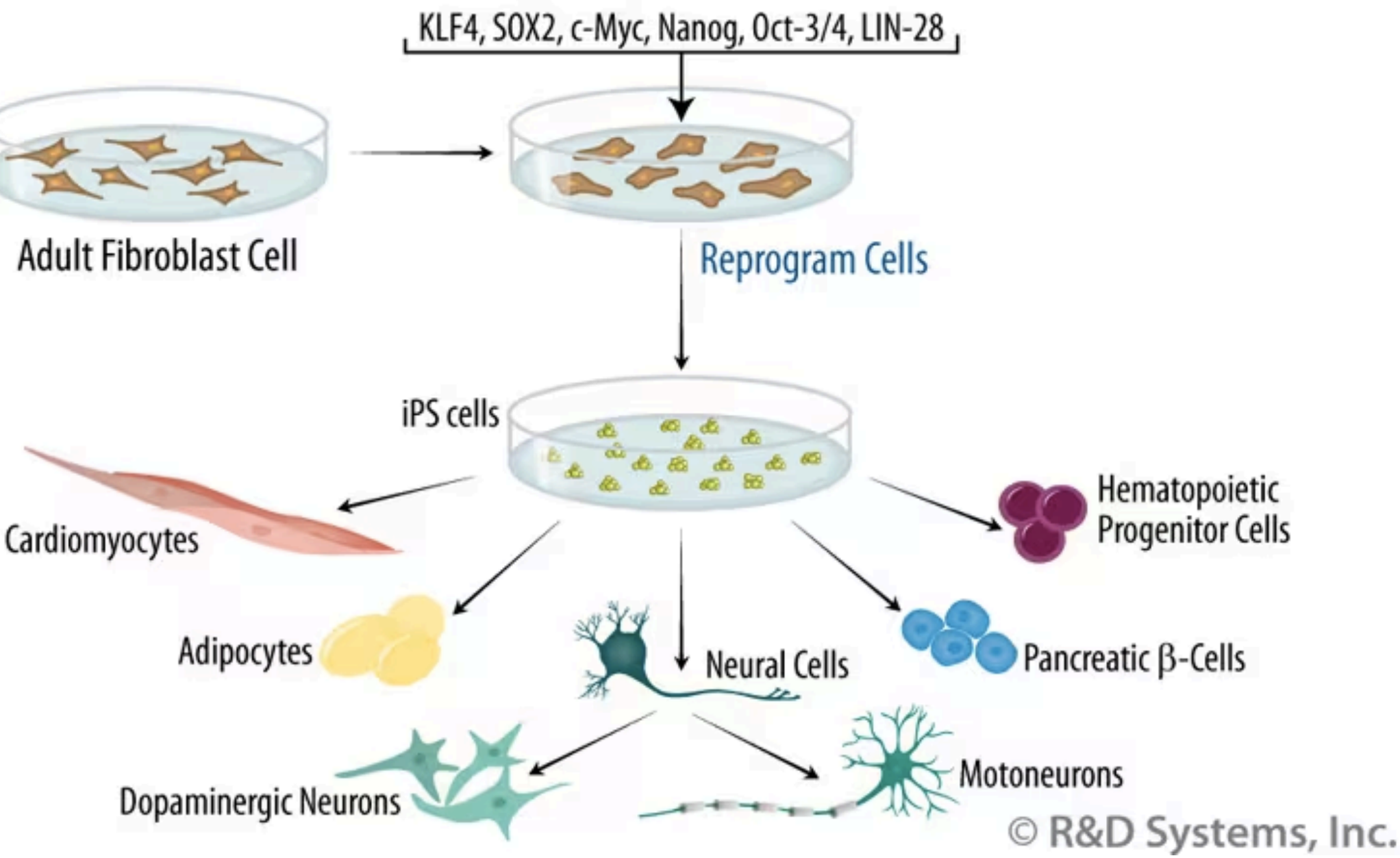






Rodriguez-Colman et al, ***Nature****, 2017*

Induced Pluripotent Stem Cells are ESC/PSC surrogates



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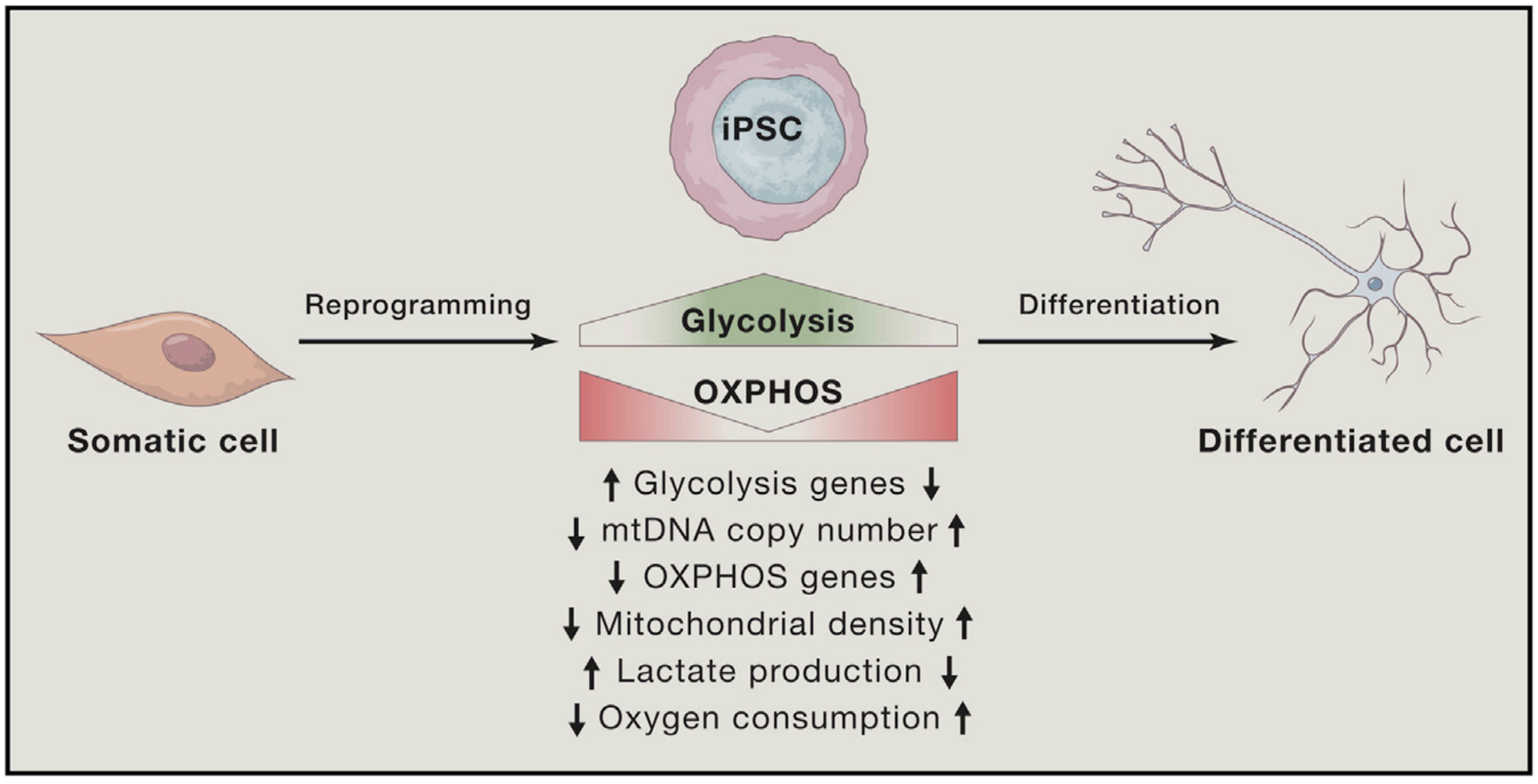
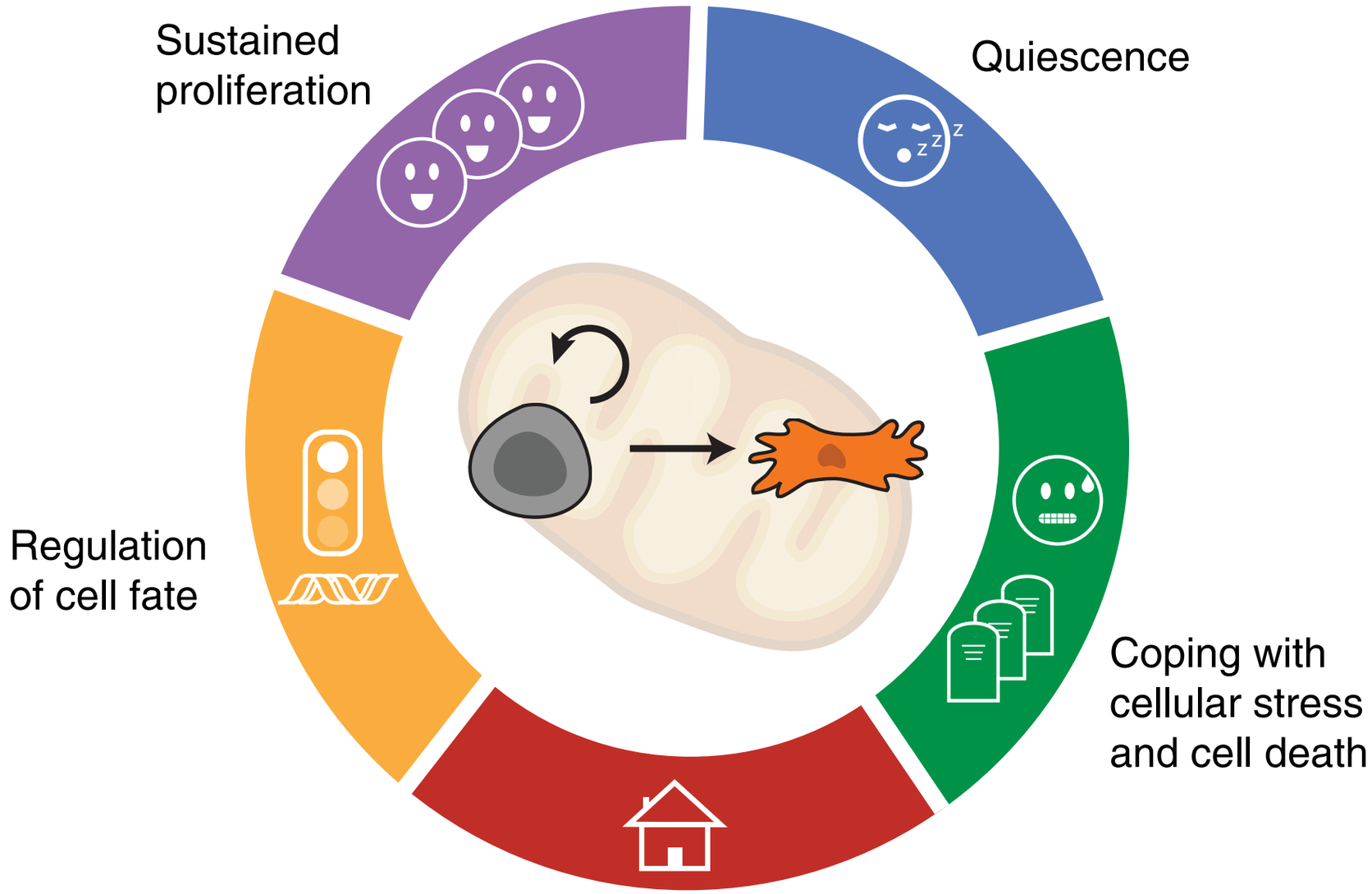


During cellular reprogramming, somatic cells exhibit an increase in the rate of glycolysis through an increase in the expression of glycolytic genes and an upregulation of glycolytic pathways. Concomitantly, mitochondrial metabolism is decreased through a downregulation of mitochondrial genes and reduced mitochondrial density, leading to decreased oxygen consumption. These changes, which are necessary to sustain a pluripotent state, are reversed when iPSCs are differentiated, allowing cells to acquire specialized functions.

Role 5: dictate cell fate decisions



Cell metabolism is emerging as an additional determinant of stem cell function.

Jackson & Finley, ***Cell Stem Cell****, 2024*

Role 5: dictate cell fate decisions

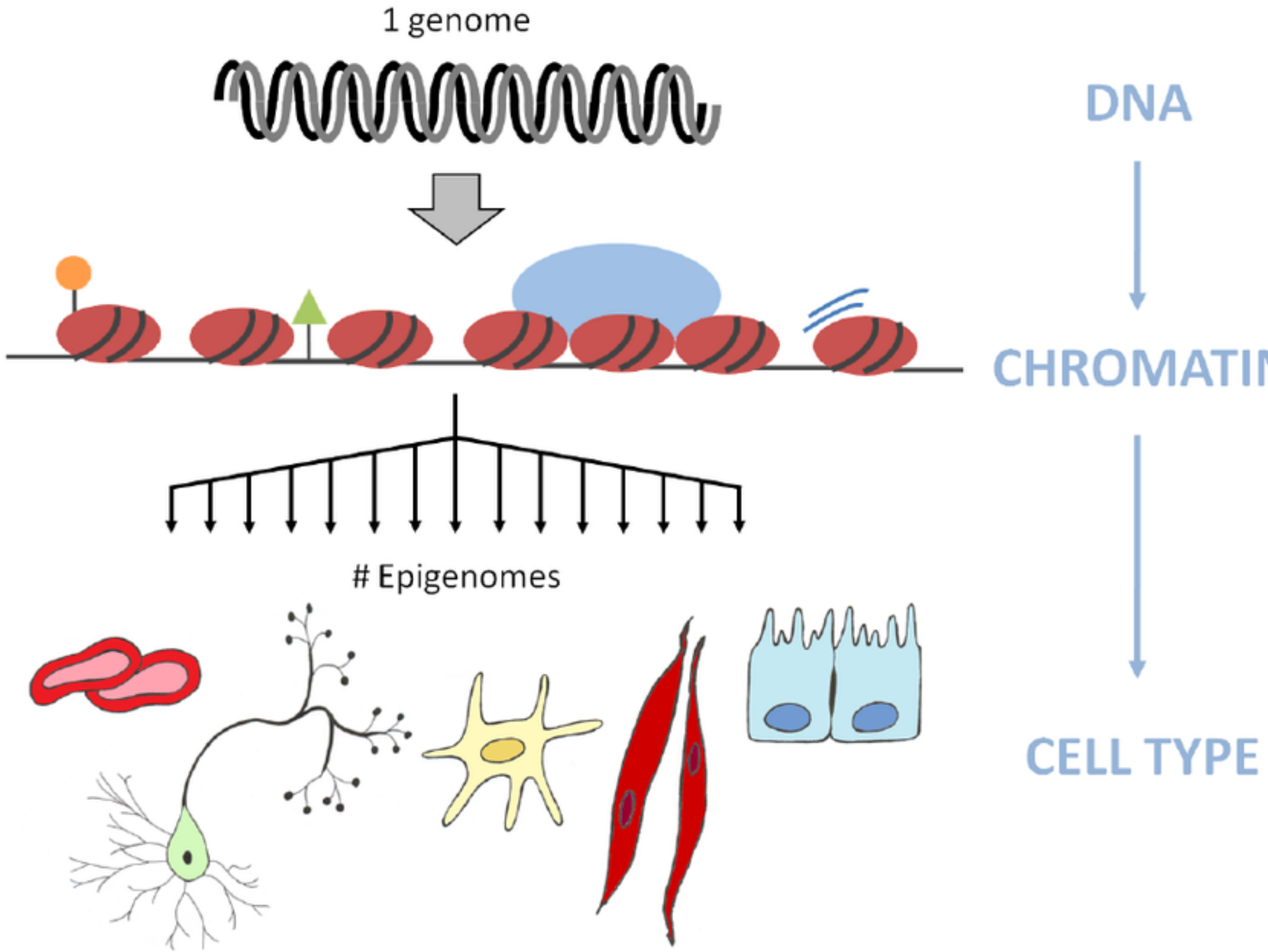
In addition to meeting the demands of proliferation and resisting cellular stressors, stem cells must retain the capacity for differentiation.

Stem cell differentiation is principally controlled at the level of transcription, where cell state transitions are coordinated by the activity of chromatin remodelers and the binding of lineage-specific transcription factors.

Notably, metabolites are the chemical precursors for post-translational modifications of histones and nucleic acids.

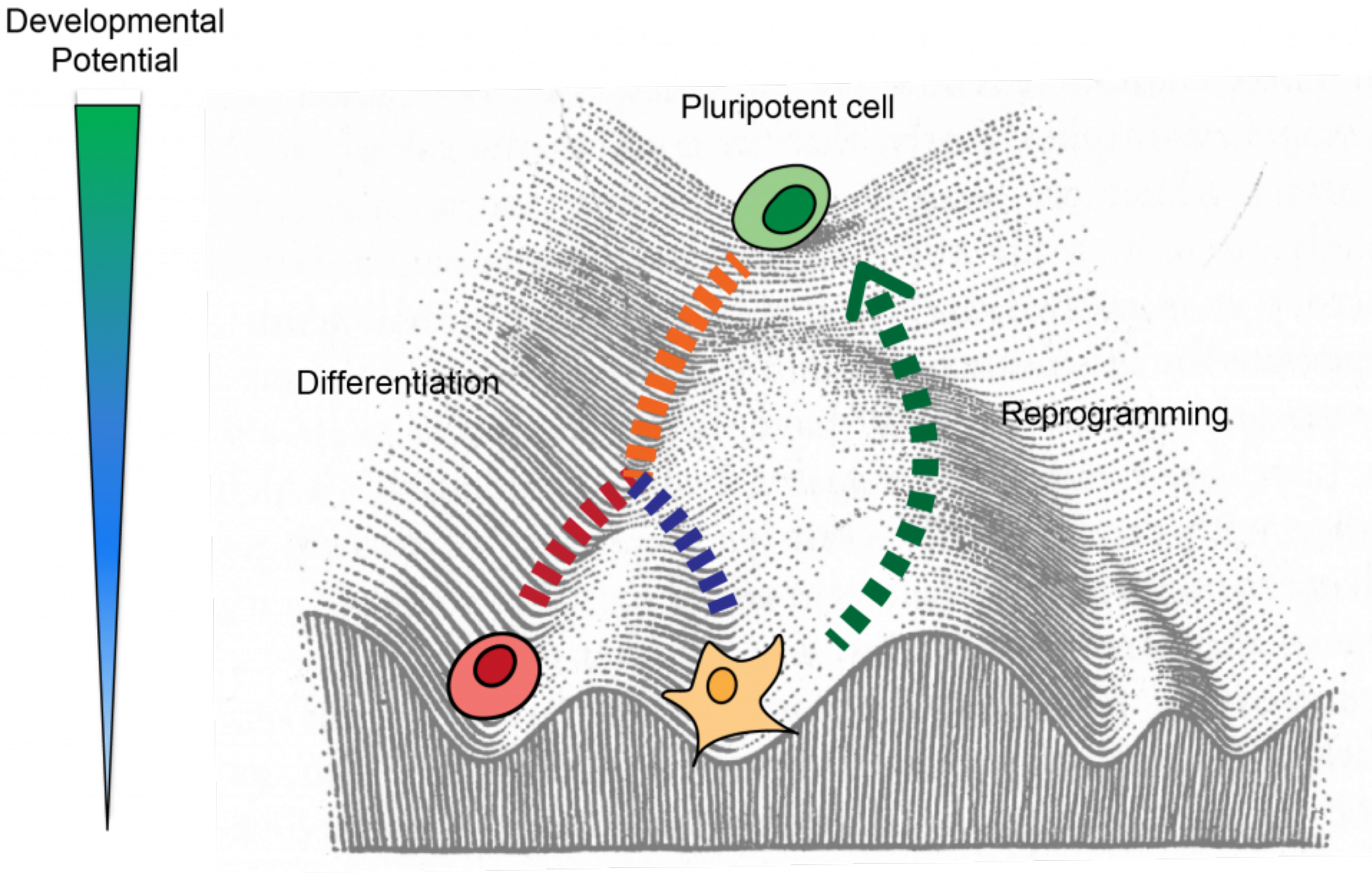
This link between metabolites and chromatin has led to the hypothesis that metabolic fluctuations can alter the distribution of chromatin and DNA modifications. In this scenario, metabolites serve not just as substrates for growth or viability, but also as signals that influence gene expres- sion programs to alter cell fate.

Epigenetic organization dictates cell identity



By regulating gene expression (positively and negatively), chromatin organization primes PSC for differentiation and enables the activation of lineage-restricted genes.

Stem Cell differentiation is regulated by the Waddington paradigm



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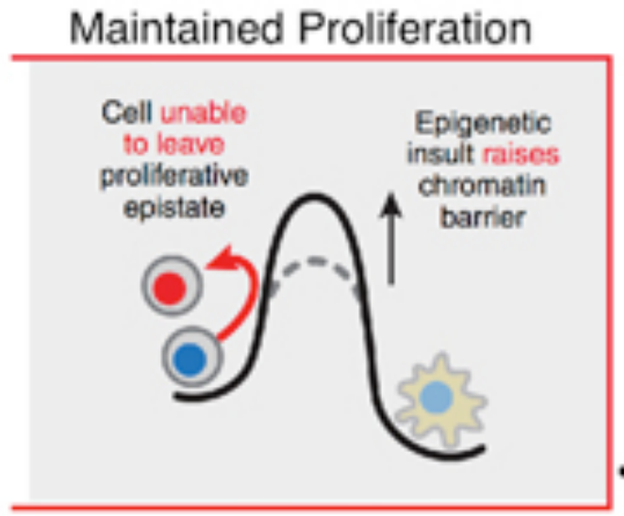
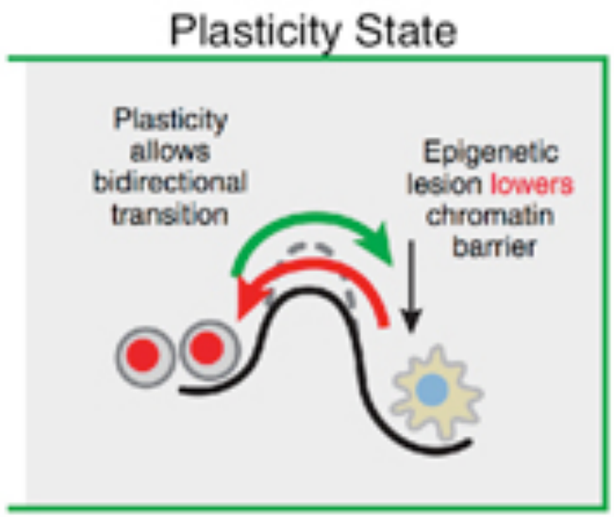
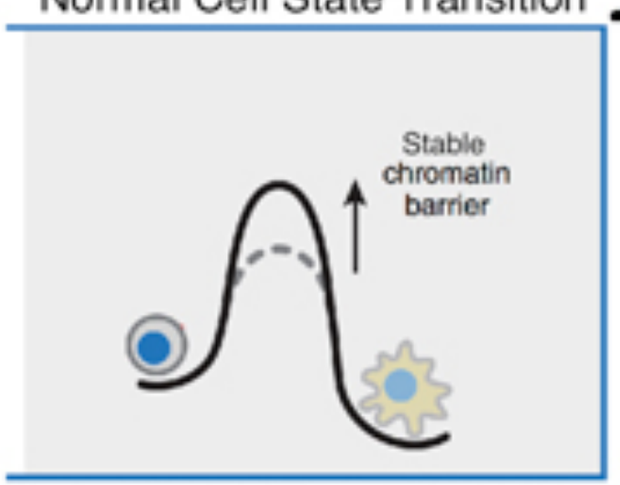
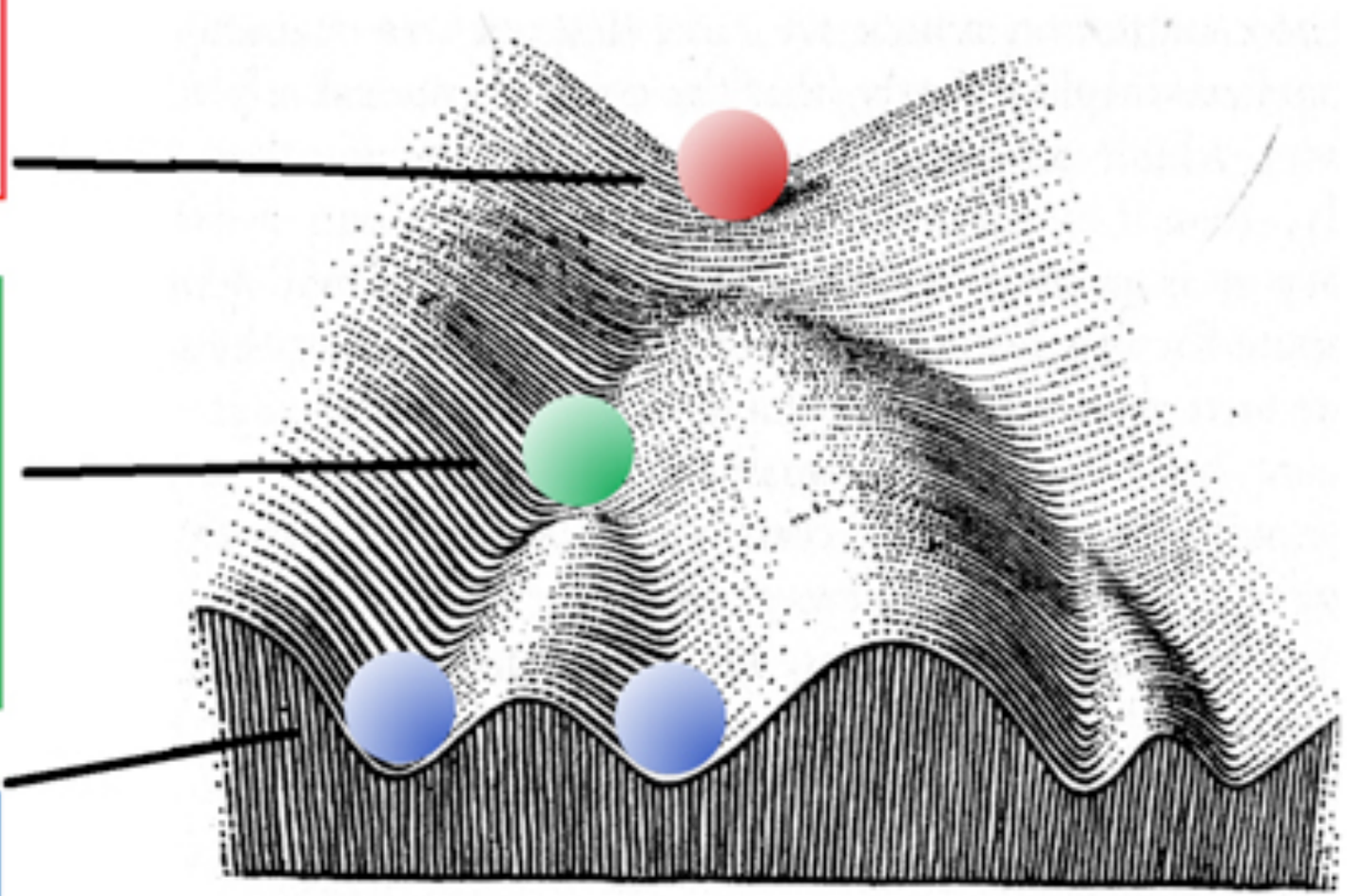






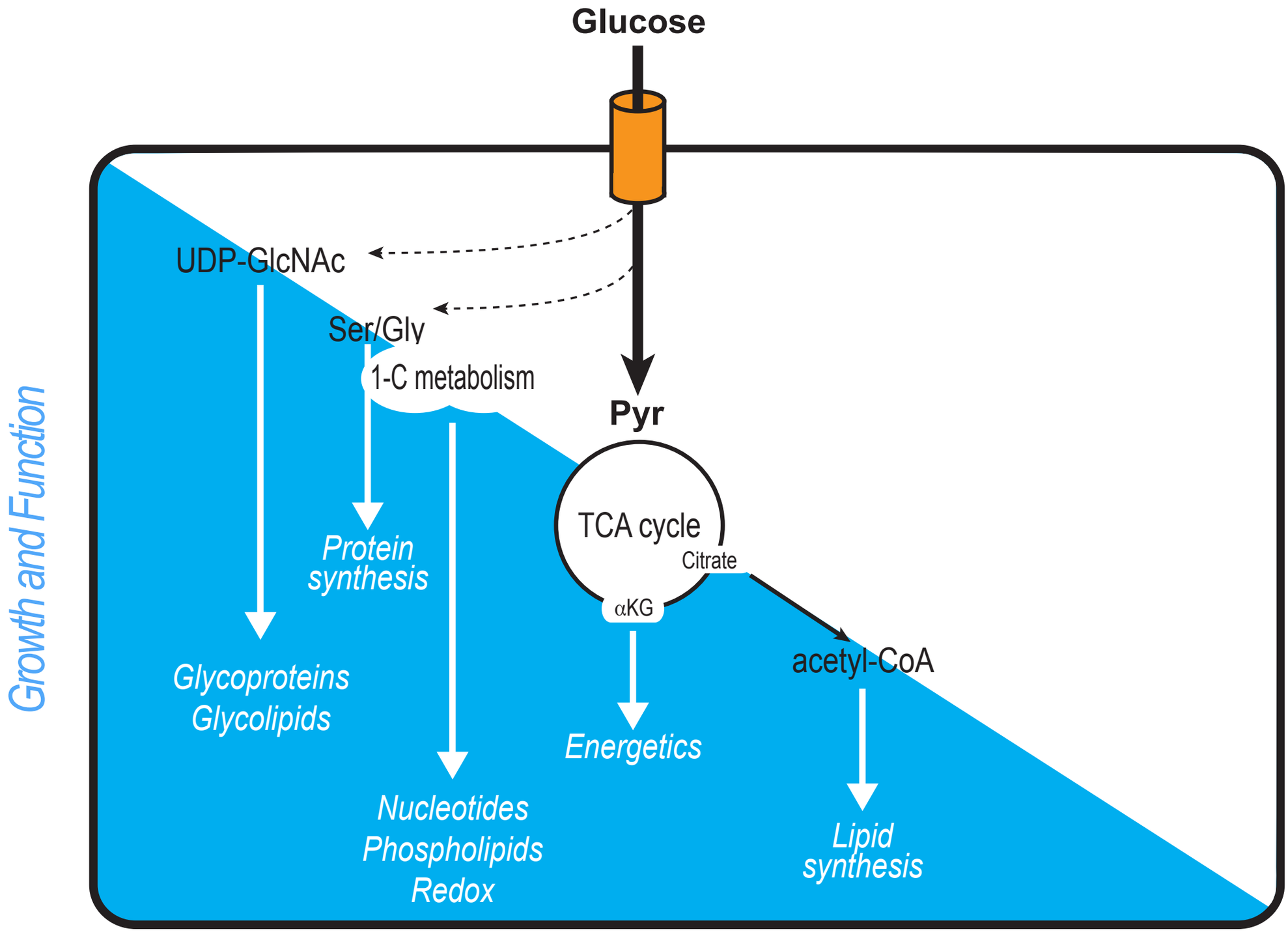
Flavahan WA et al. Science 357, 2017 - review



Conrad Waddington 1957

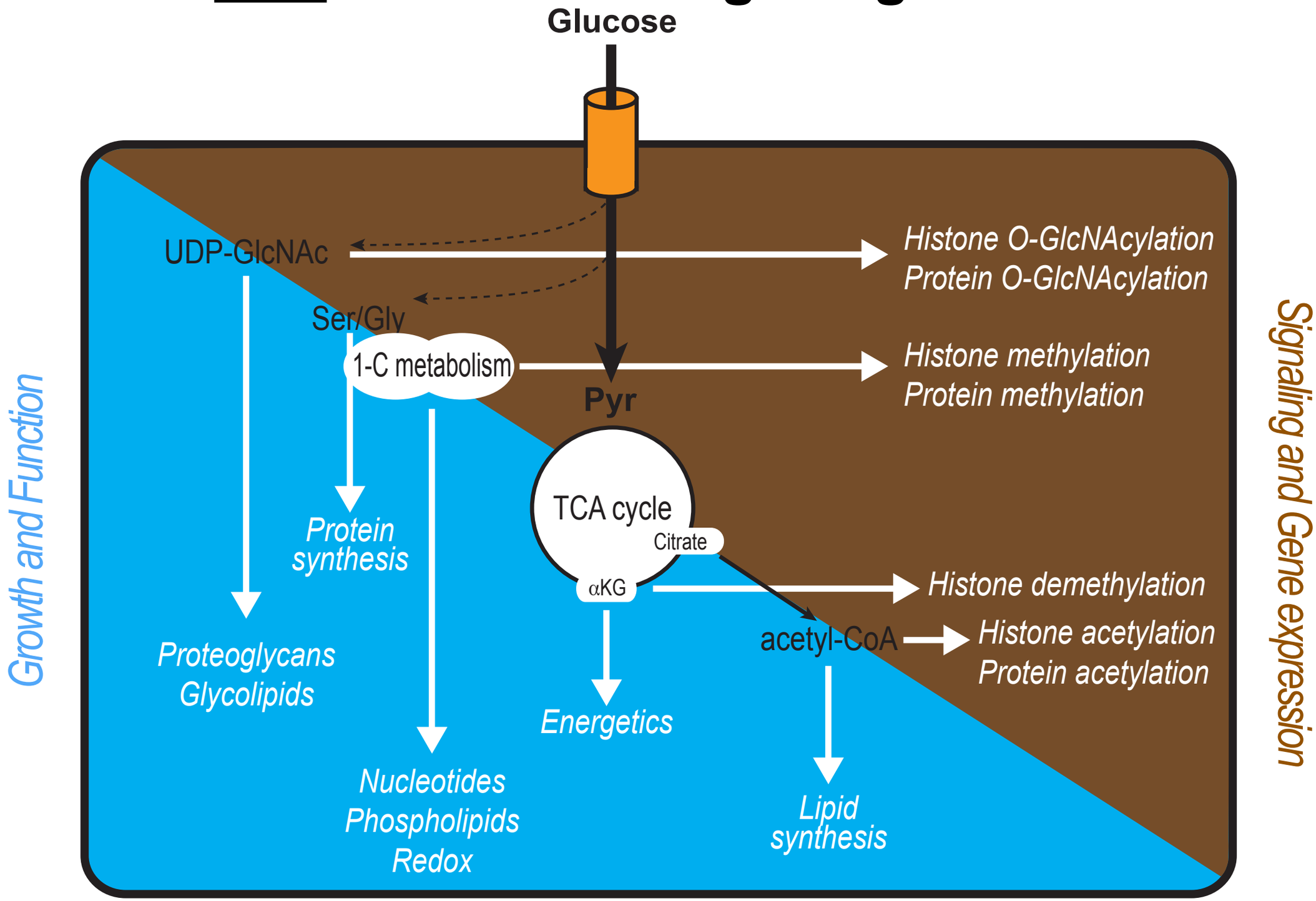
How metabolic regulation contributes to heighten (or lower) chromatin barrier?

Metabolites provide biomass / energy



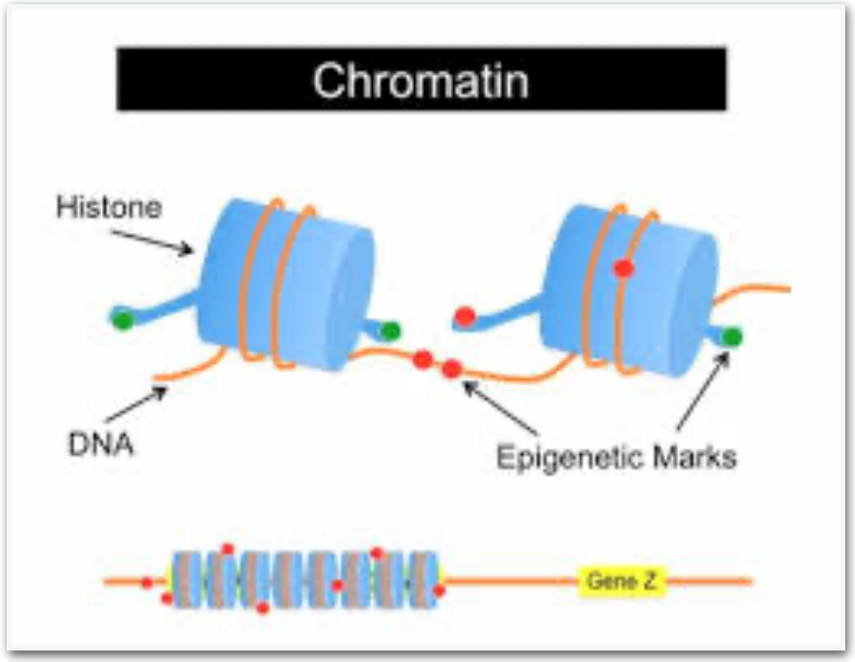
Carrer & Wellen, Curr Opin Biotechnol, 2015

Metabolites provide biomass / energy
AND contribute to signaling events



Carrer & Wellen, Curr Opin Biotechnol, 2015

Chromatin status is dictated by epigenetic marks



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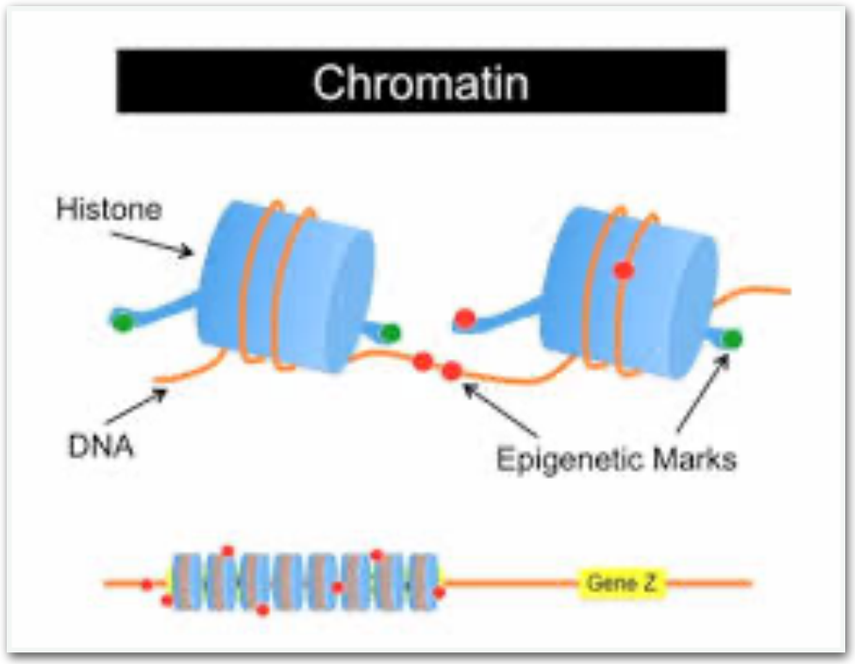
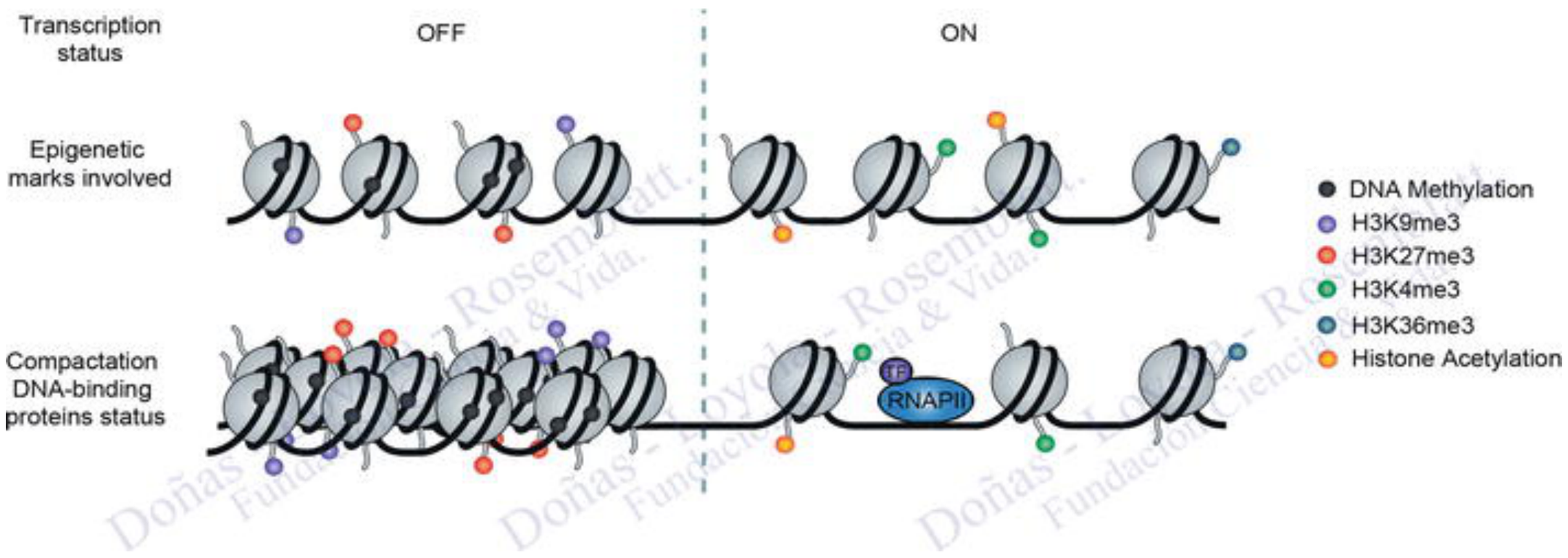
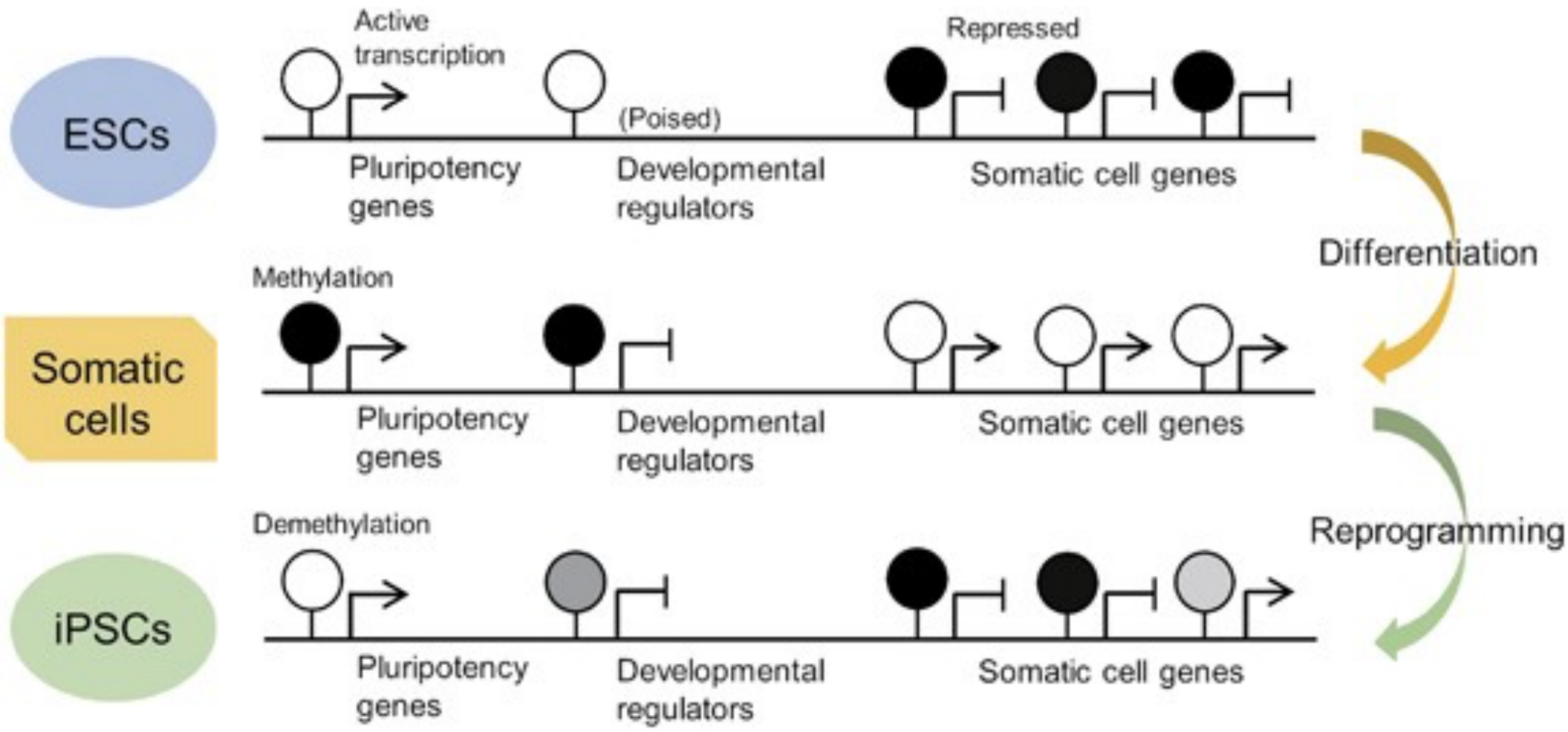
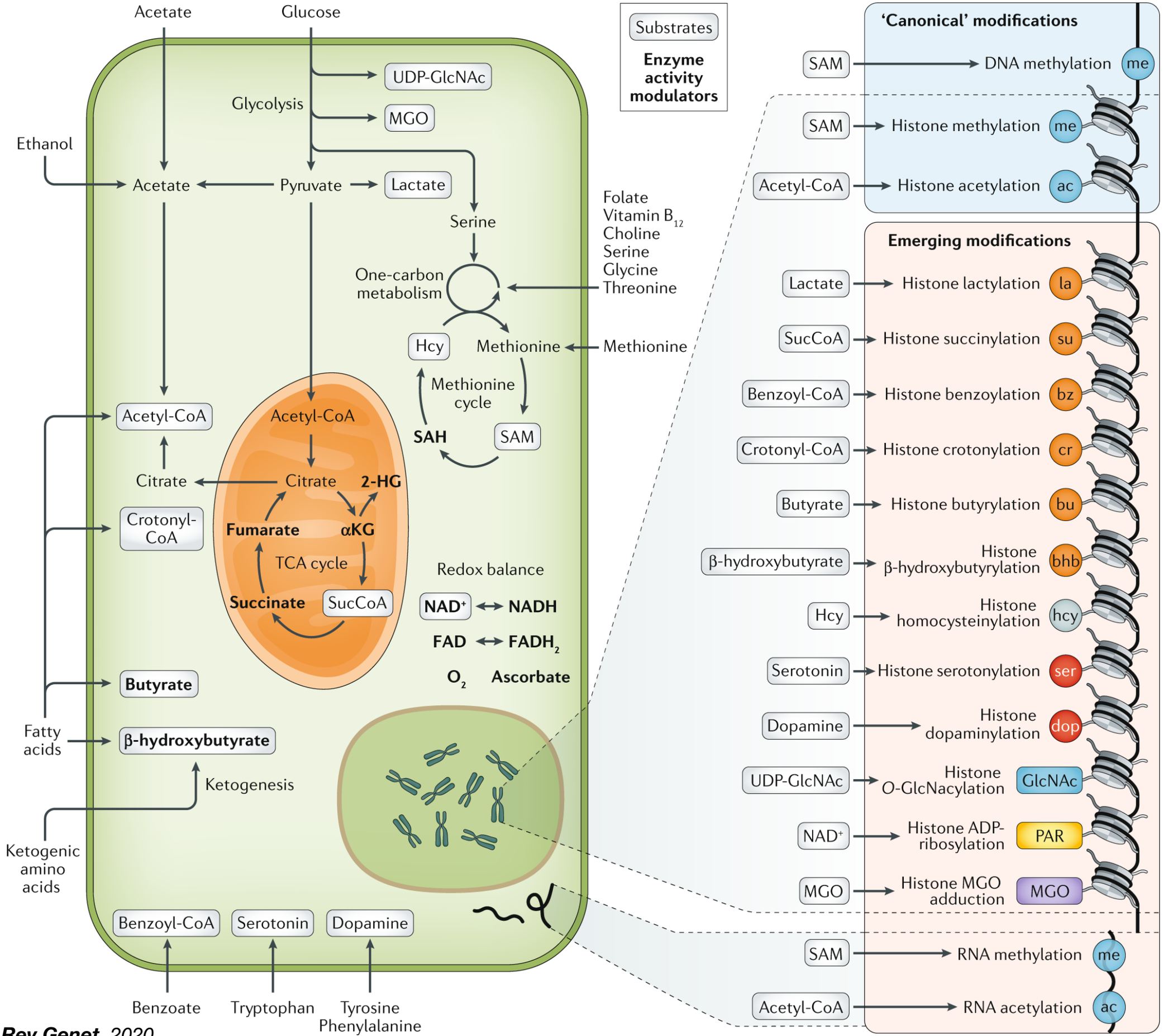




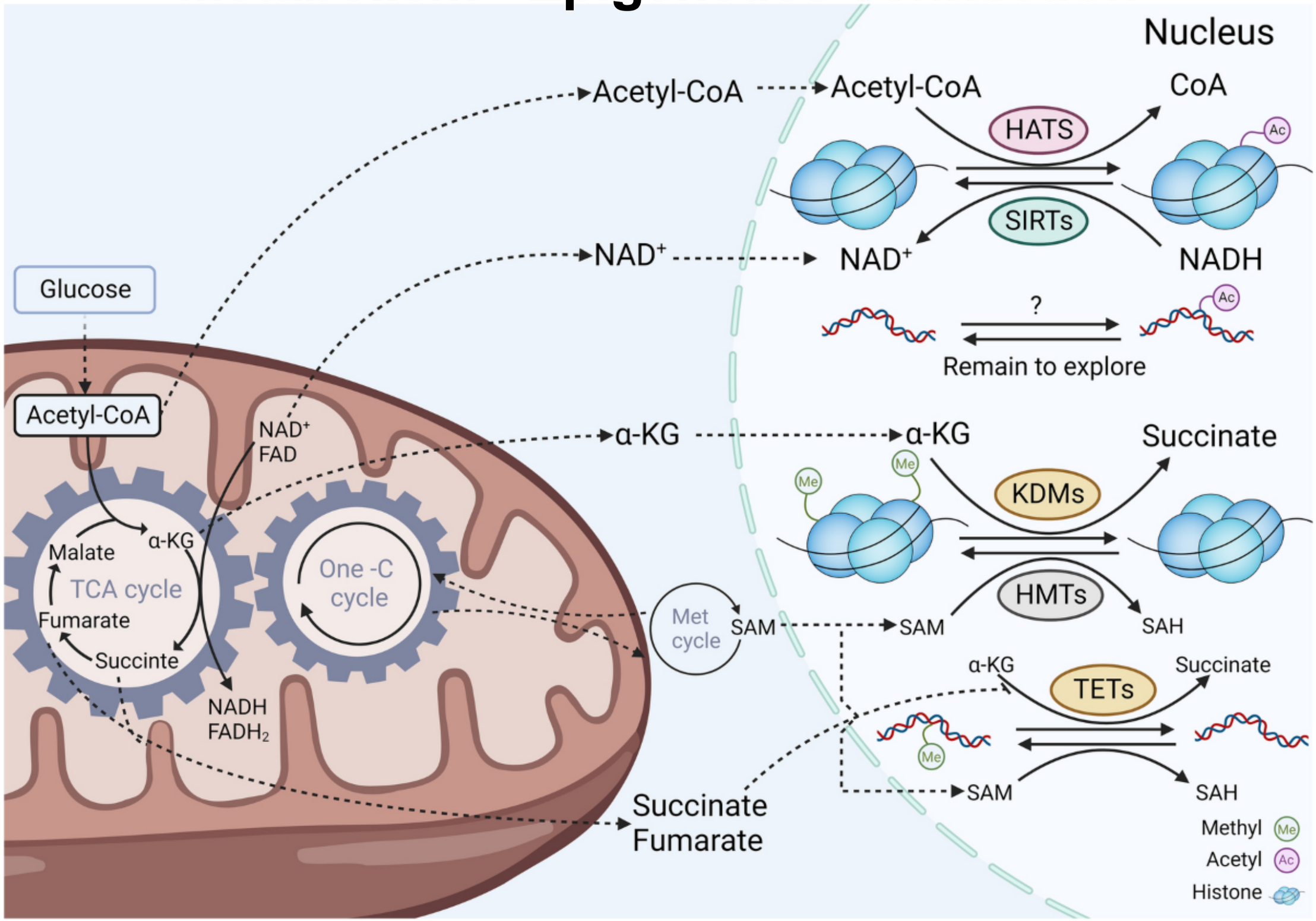
Chromatin reprogramming enables transcription at either pluripotency or lineage-specification genes



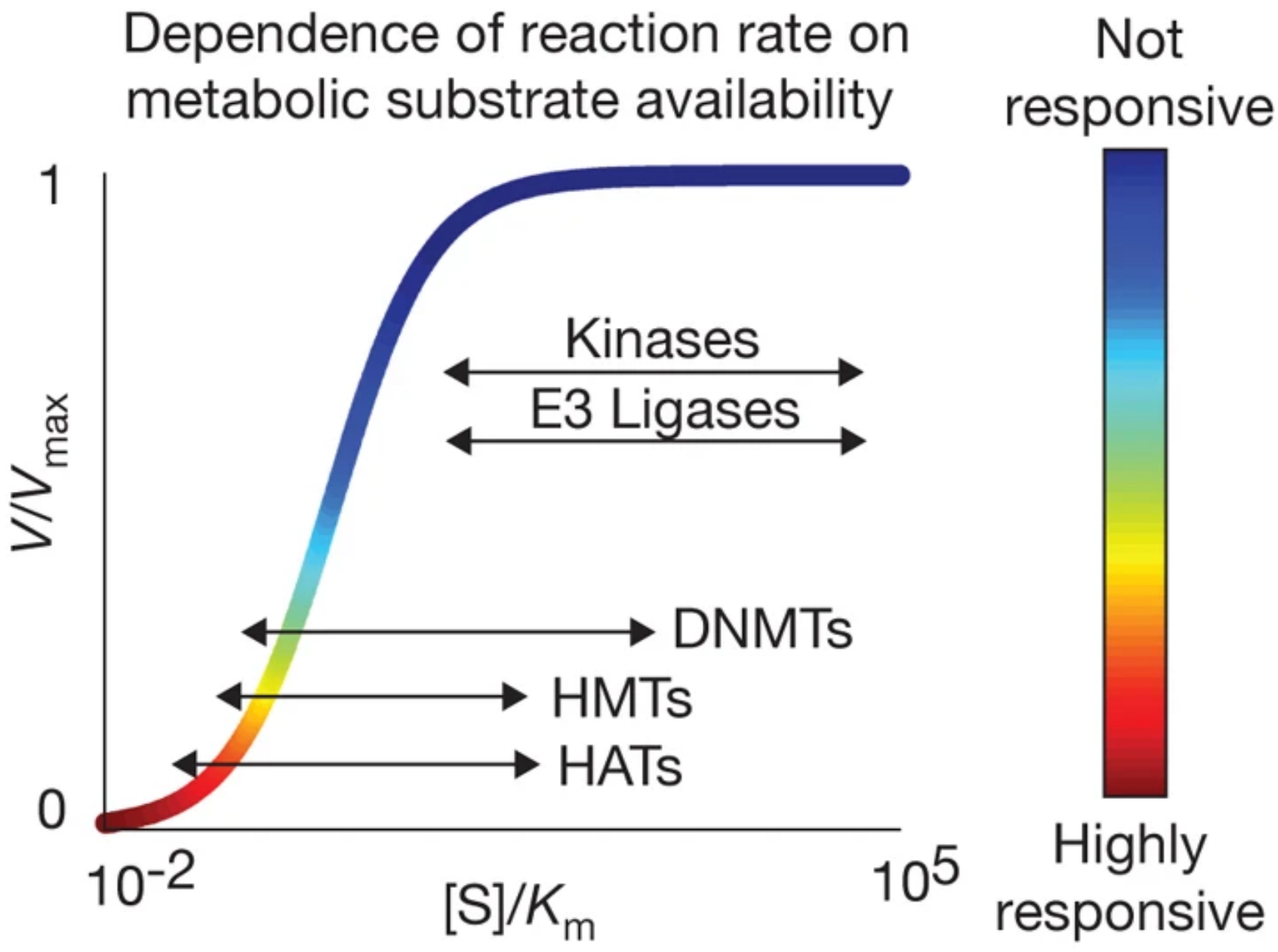


Dai et al, ***Nat Rev Genet****, 2020*

Metabolism - Epigenetics connection

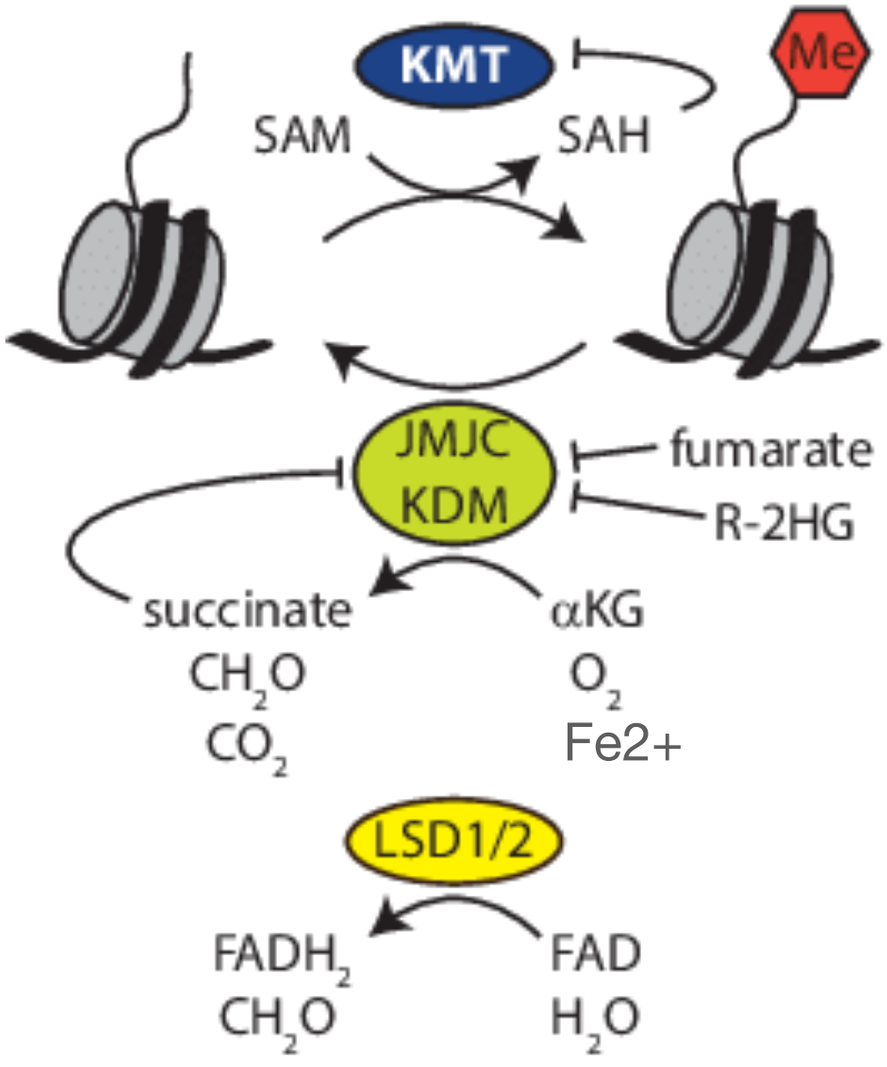


Metabolism - Epigenetics dependencies



*Reid et al, **NCB**, 2017*

Histone methylation depends on metabolites availability



KMT: Lysine (K) Methyl Transferase - *writer - deposits methyl moieties on DNA/proteins*

KDM: Lysine (K) De-Methylase - *eraser - removes methyl moieties from DNA/proteins*

JMJC: Jimonji C domain; found in aKG-depedent dioxygeneses (OGDG) - *erasers - remove methyl moieties from DNA/proteins*

LSDs: Lysine-Specific histone Demethylases - *erasers - remove methyl moieties from proteins*

Intracellular α–ketoglutarate maintains the pluripotency of embryonic stem cells

Bryce W. Carey[1]*, Lydia W. S. Finley[2]*, Justin R. Cross[3], C. David Allis[1] & Craig B. Thompson[2]

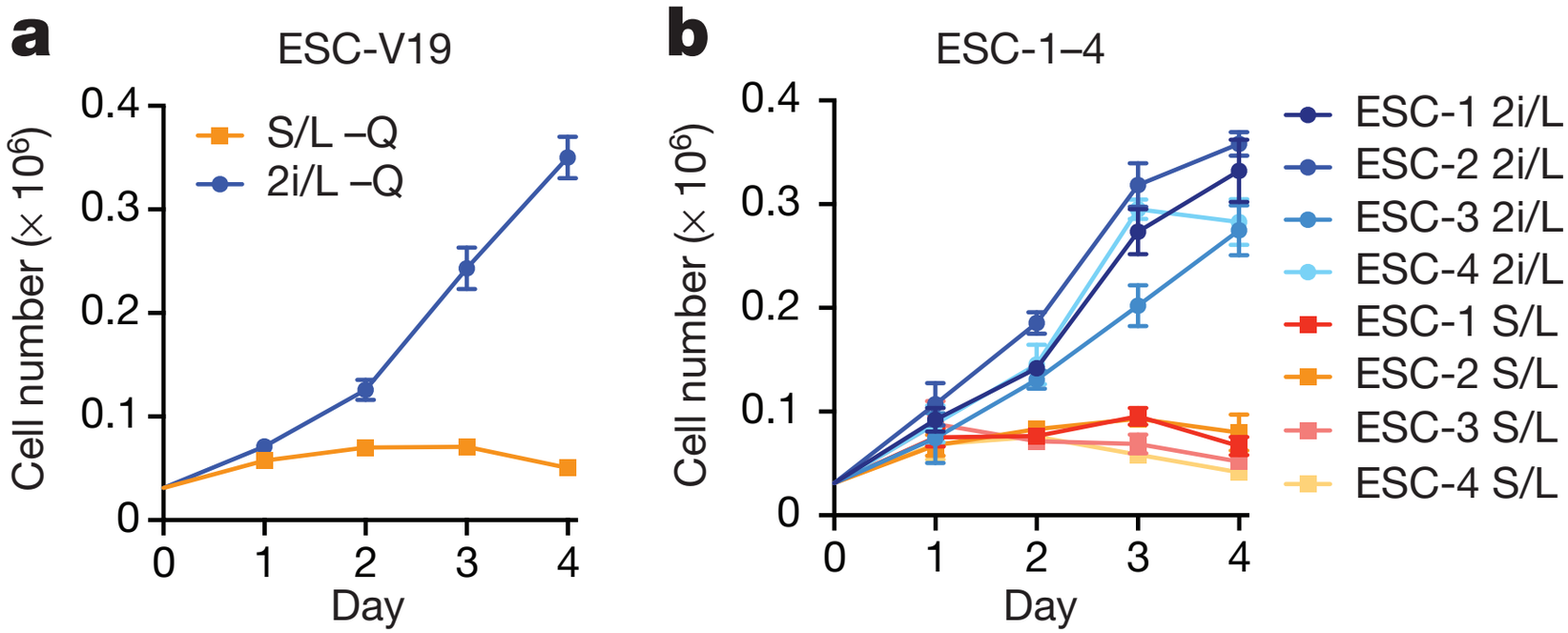
Mouse ES cells can be maintained in two medium formulations:

a serum-free medium reported to support a cellular phenotype that mimics ‘naive’ epiblast cells of the inner cell mass (containing GSK-3b and MAPK/ ERK inhibitors (2i)/leukaemia inhibitory factor (LIF), hereafter 2i/L)

or a serum-based medium that supports the proliferation of a more committed ES cell phenotype (serum/LIF, hereafter S/L).

2i/L: NAIVE

S/L: COMMITTED



Committed/Primed cells are GLN auxotrophs.

*Carey et al, **Nature**, 2015*

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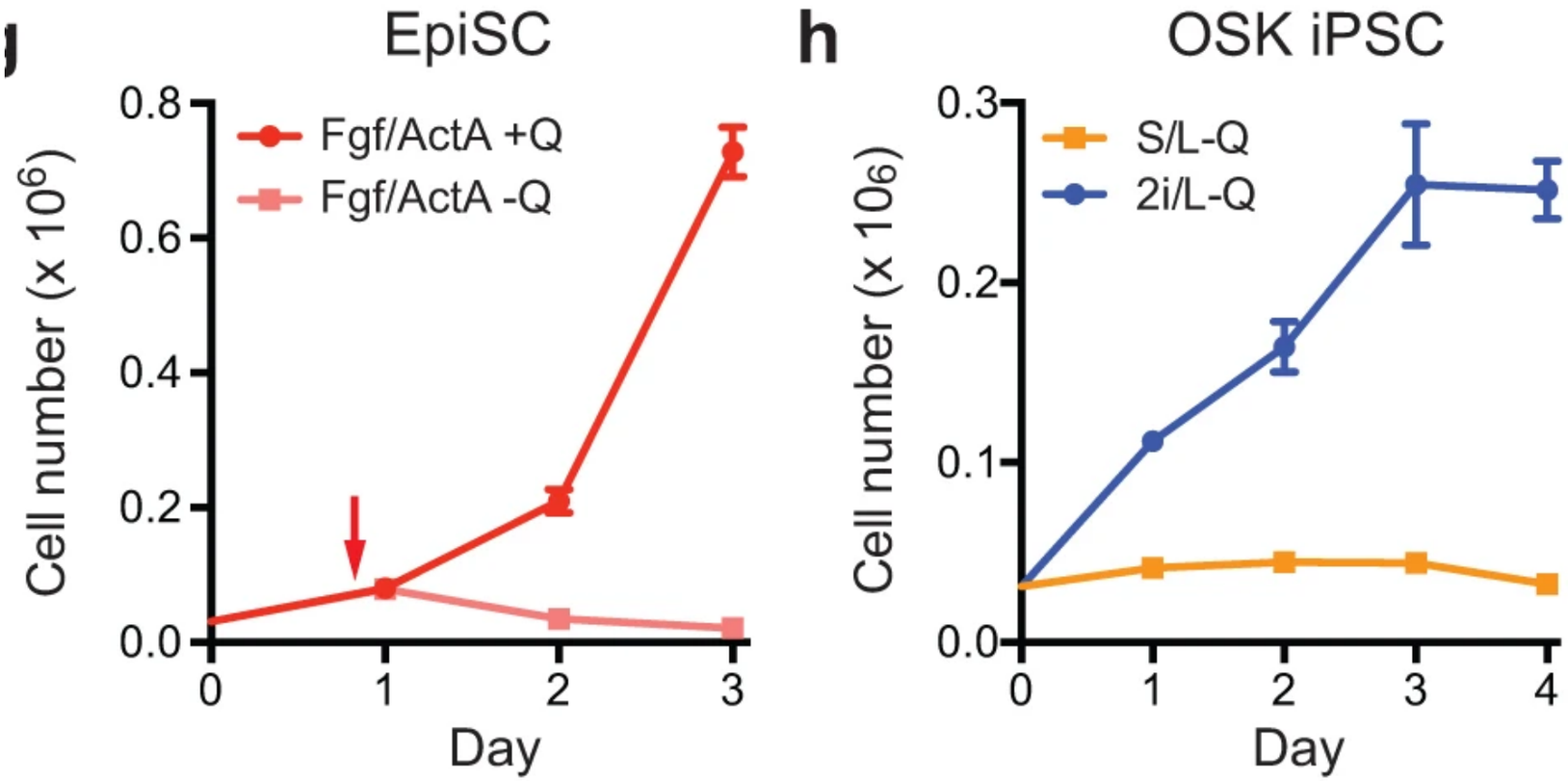
2i/L: NAIVE

S/L: COMMITTED

EpiSC: EPIBLAST STEM CELLS (PRIMED)



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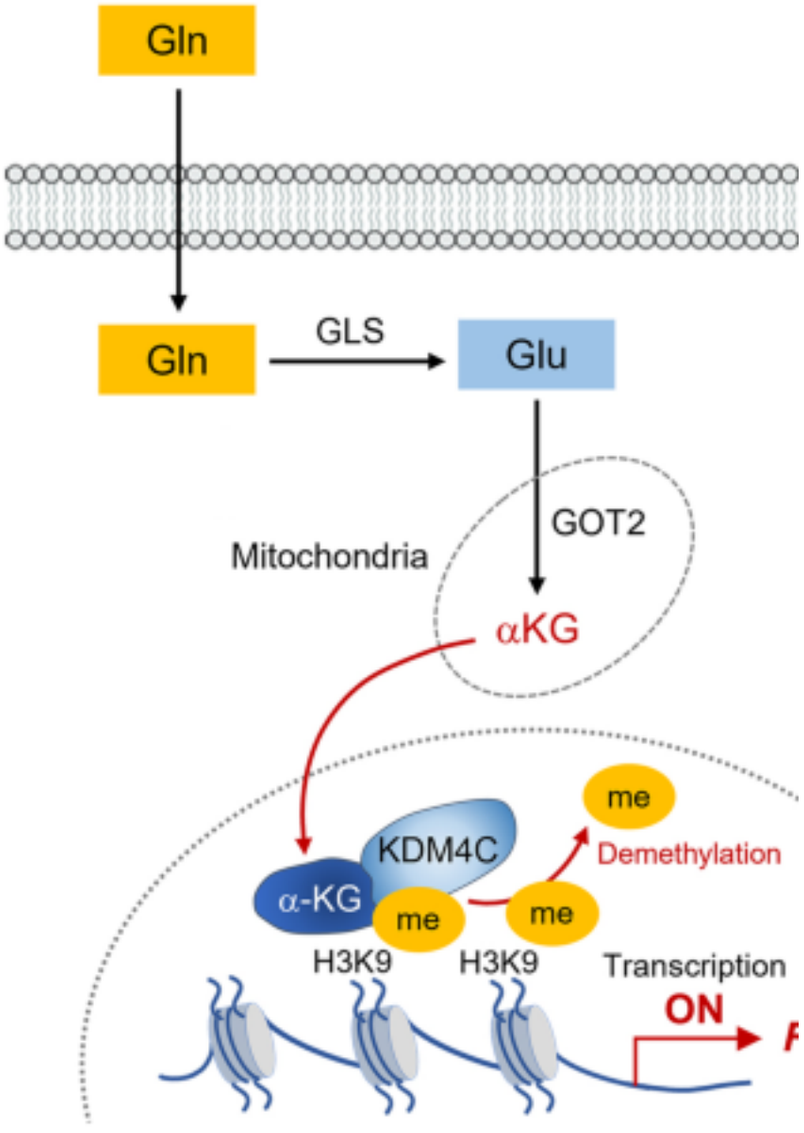
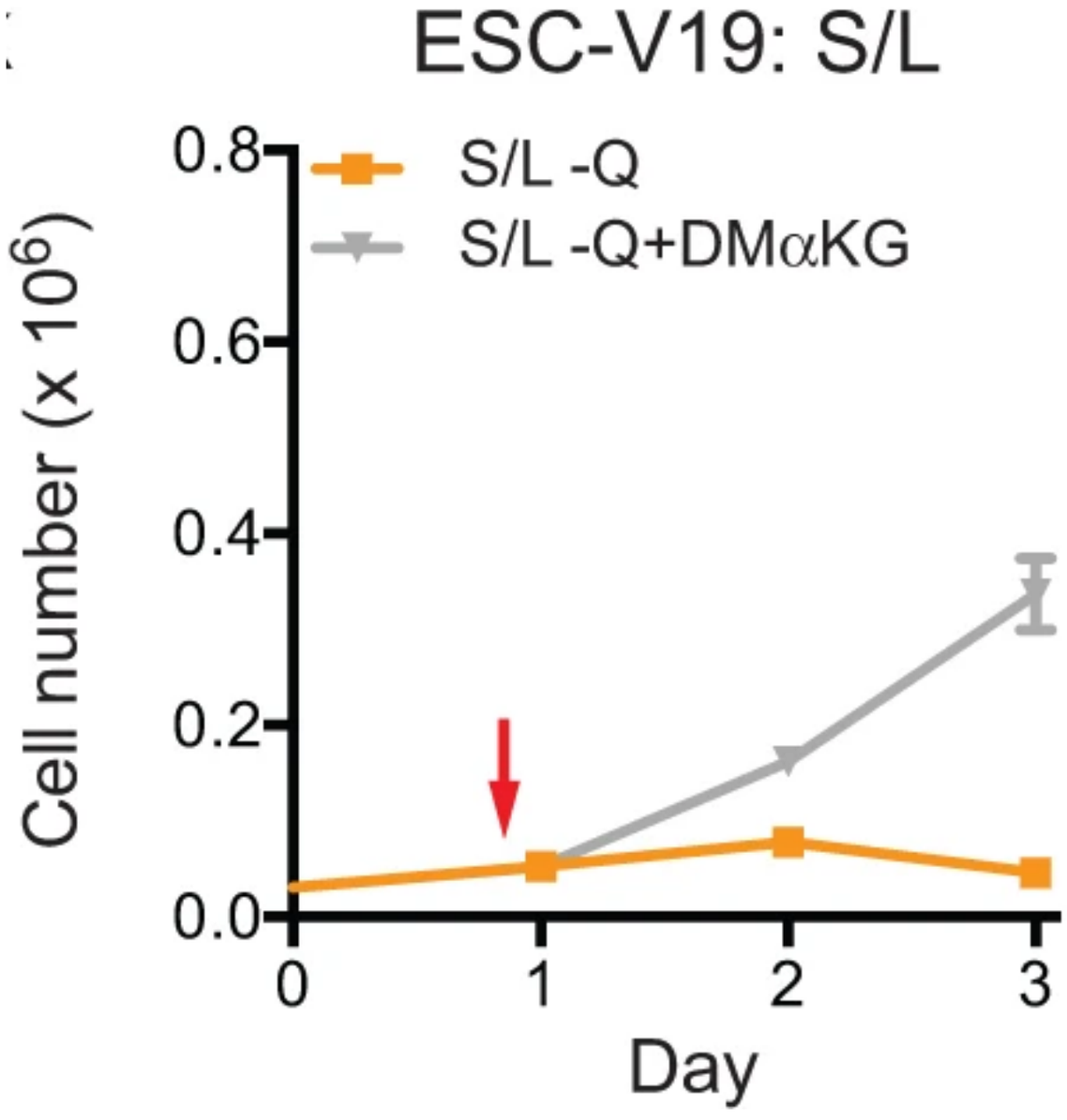
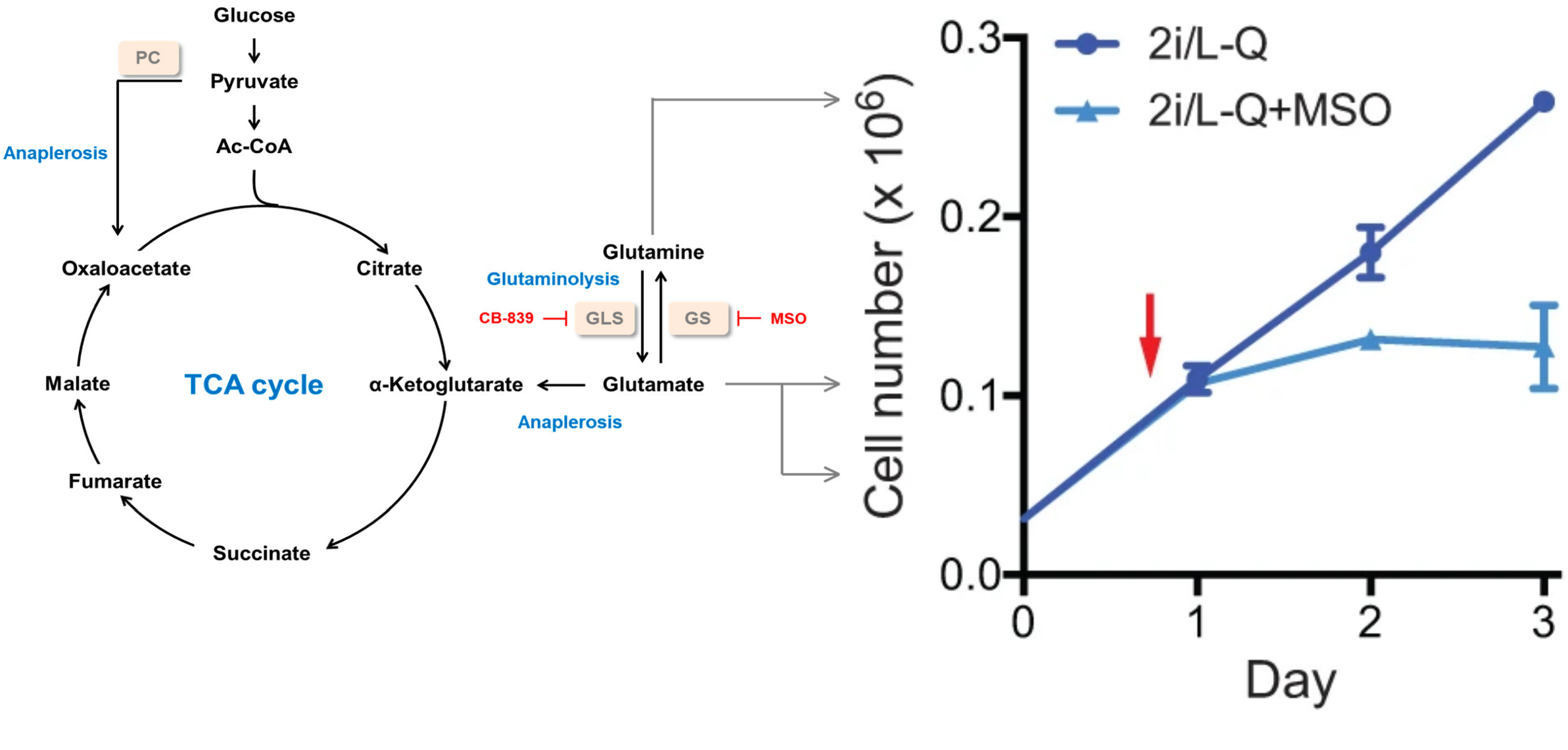




aKG is important for SC proliferation

Carey et al, ***Nature****, 2015*

Why naive cells can grow without GLN?

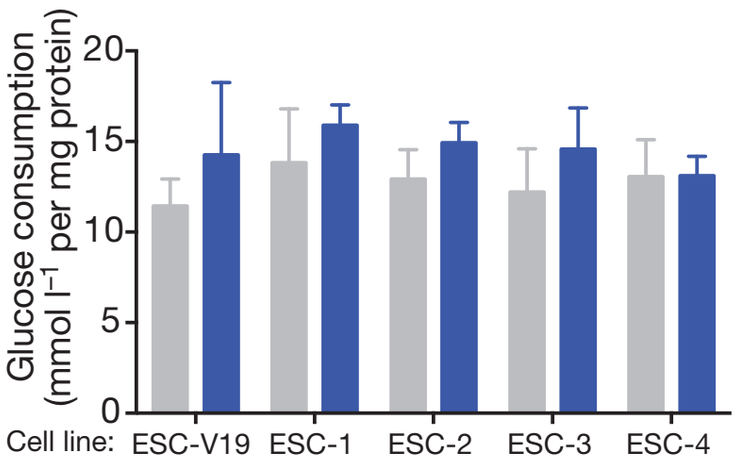


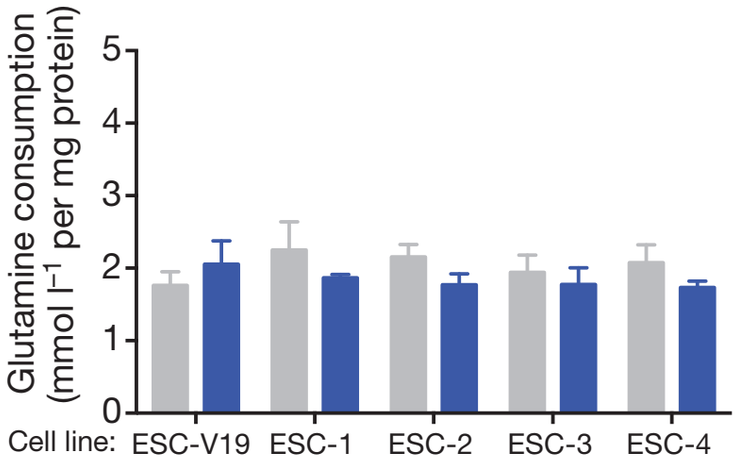
Naive SC can synthesize their own GLN

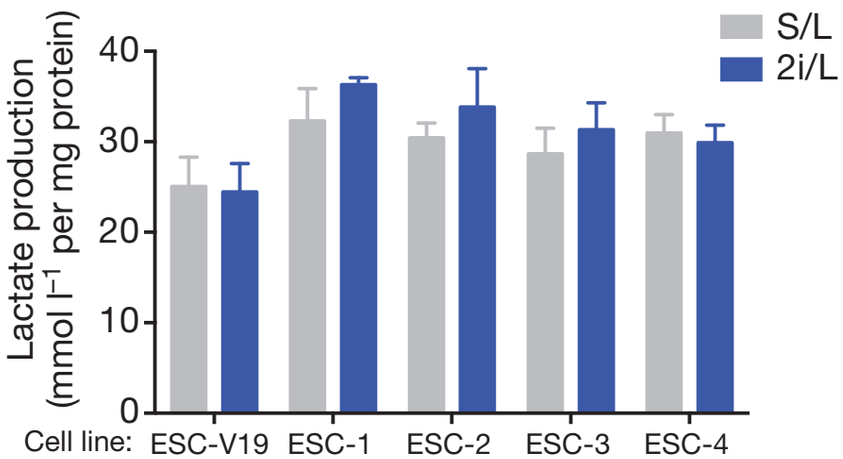
Carey et al, ***Nature****, 2015*

Do naive and primed SCs use different carbon sources to sustain growth?

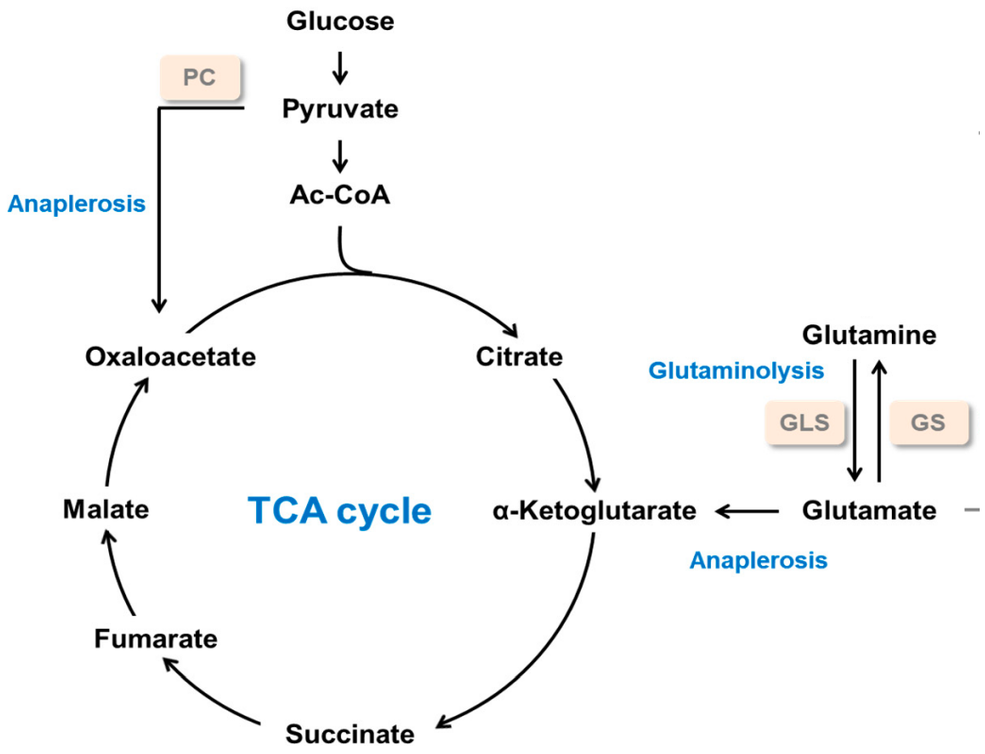
a



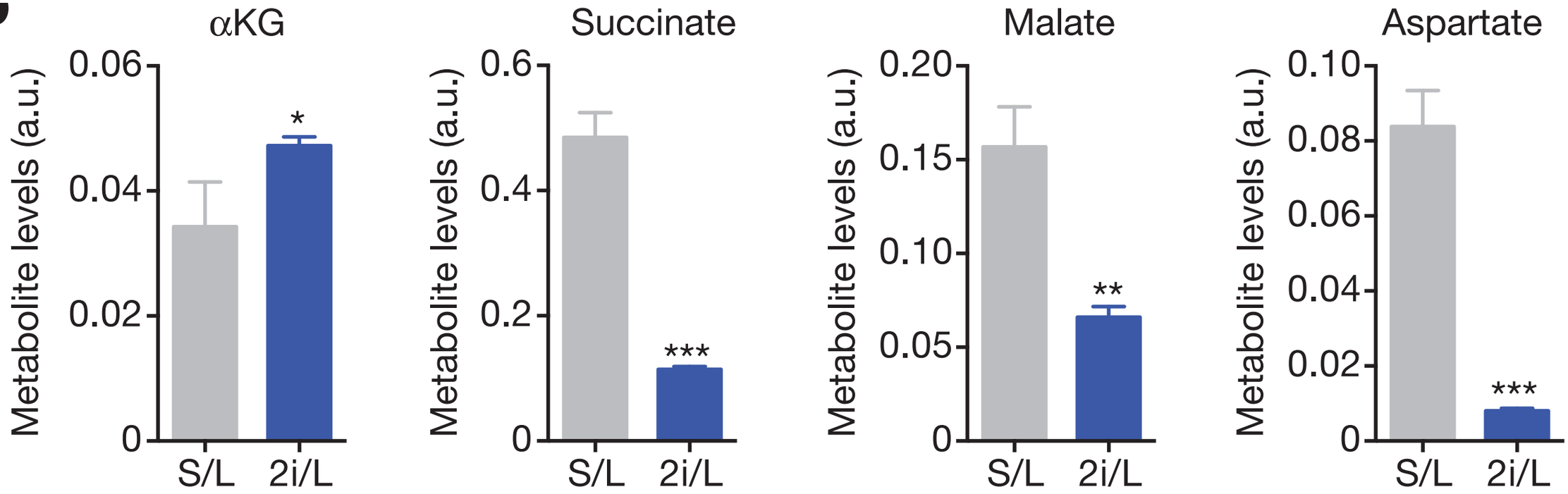




Carey et al, ***Nature****, 2015*

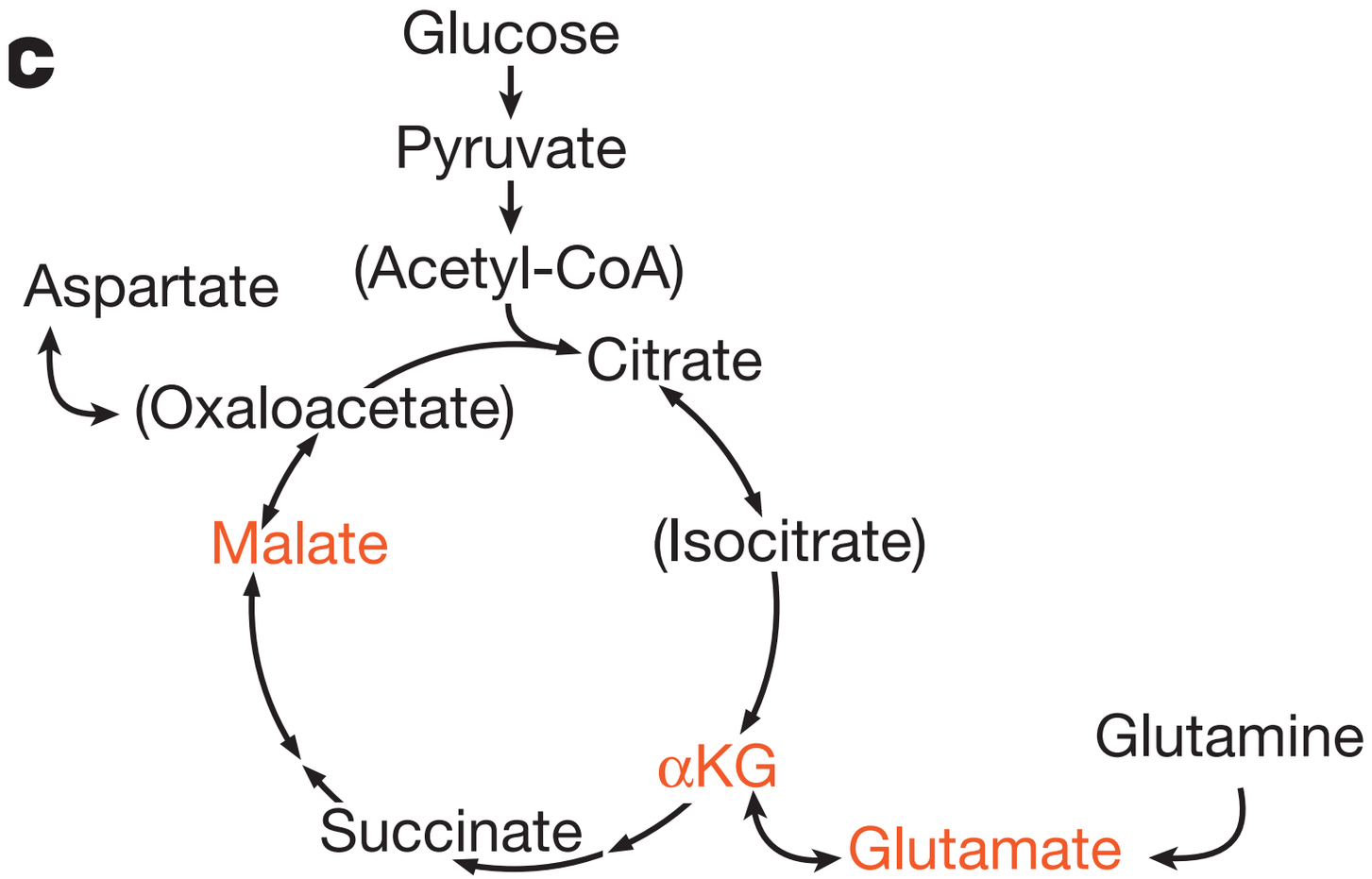


Oxidation of glucose and glutamine via the mitochondrial TCA cycle provides a critical source of the biosynthetic precursors required for cell proliferation.



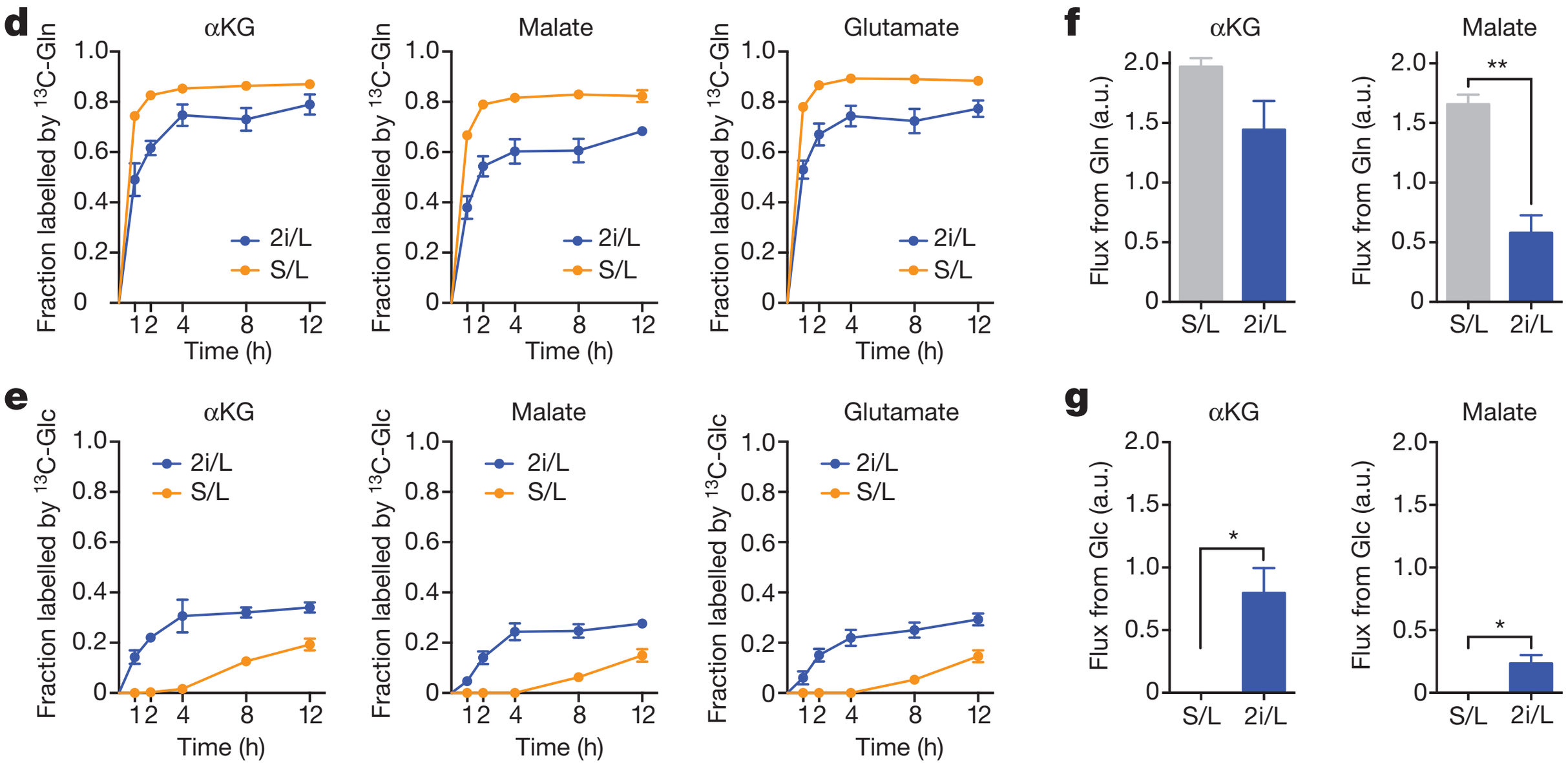
*Carey et al, **Nature**, 2015*

What is happening when SC commit?

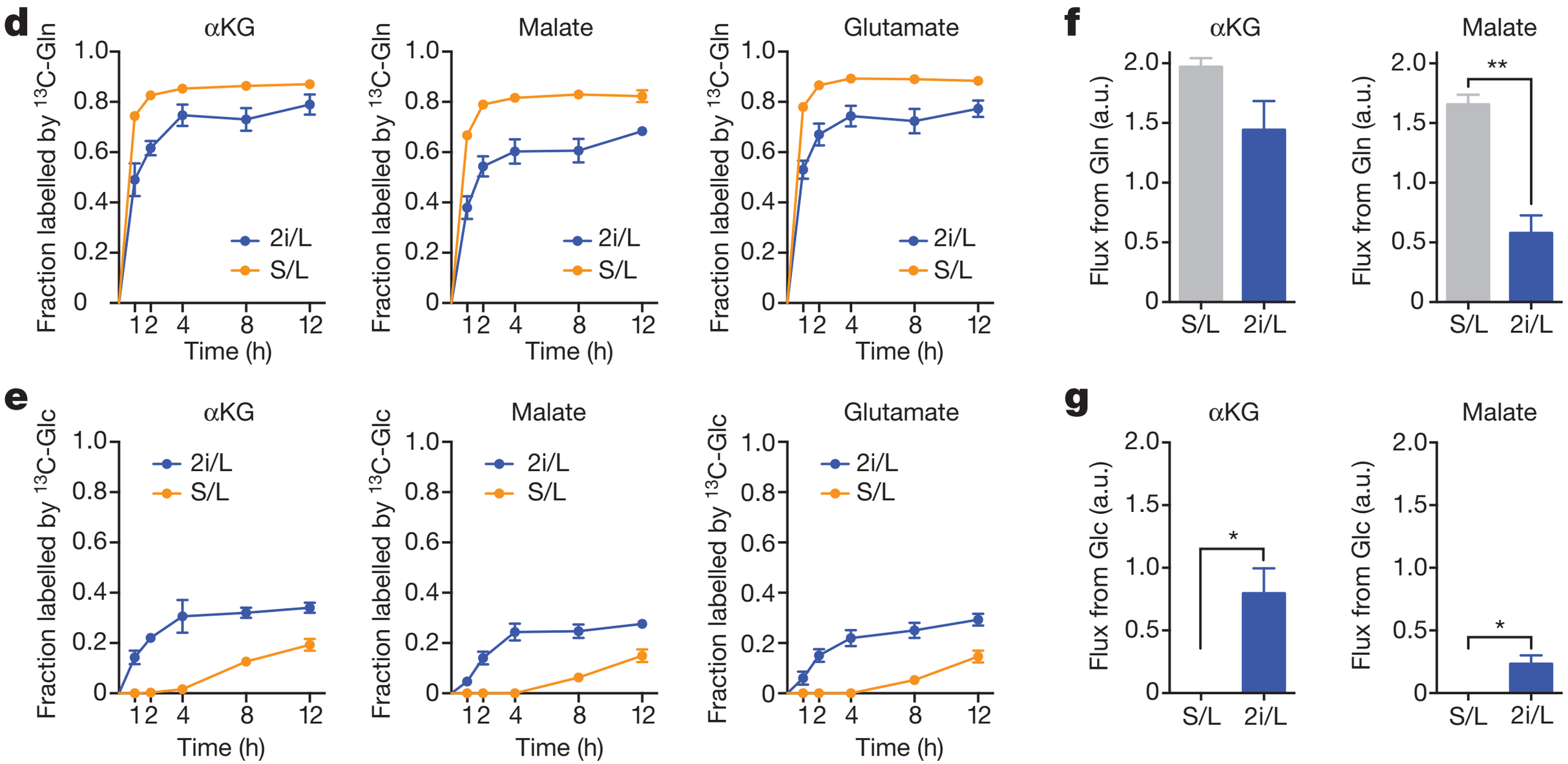


In most cells, glutamine is catabolized to aKG to support TCA cycle anaplerosis.

Carey et al, ***Nature****, 2015*

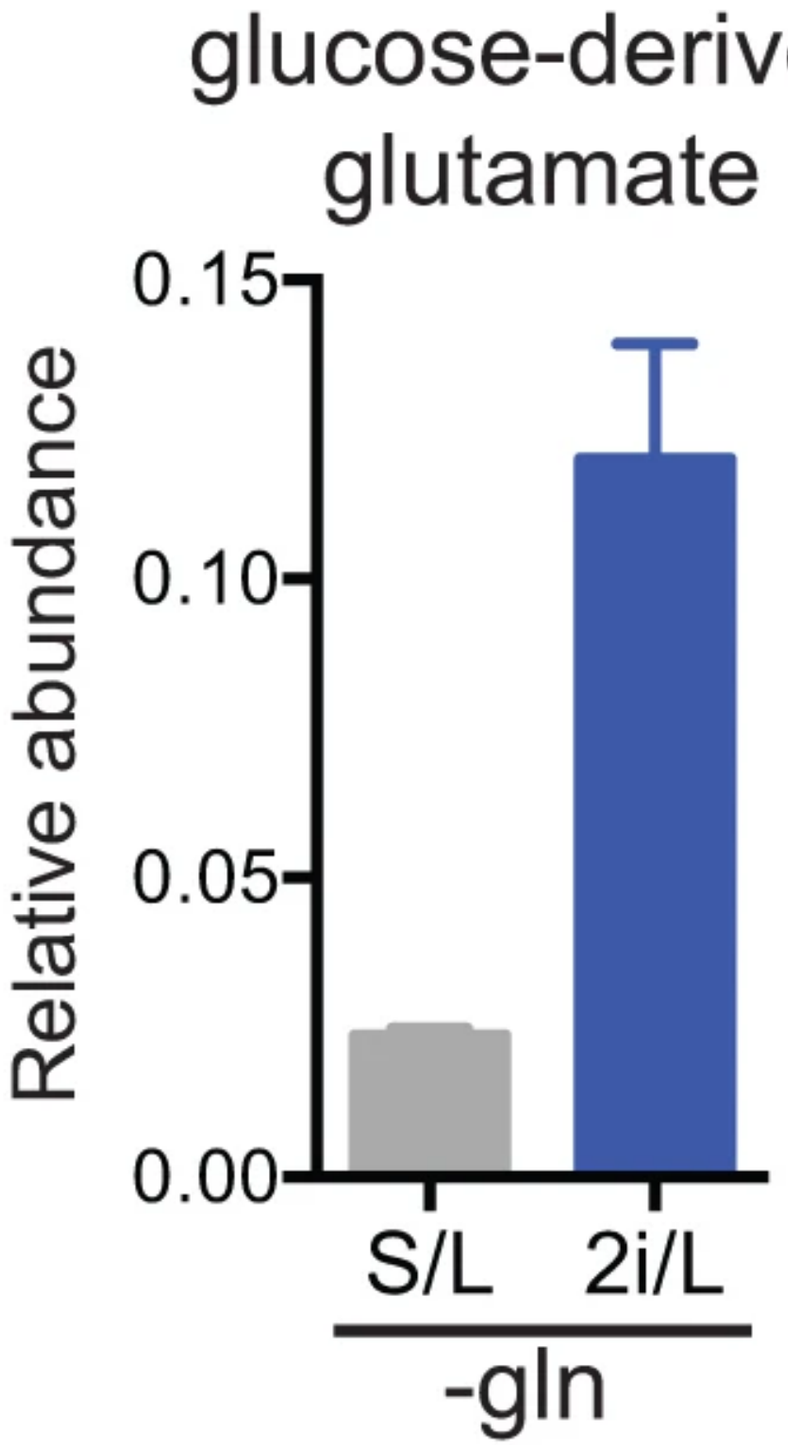


*Carey et al, **Nature**, 2015*



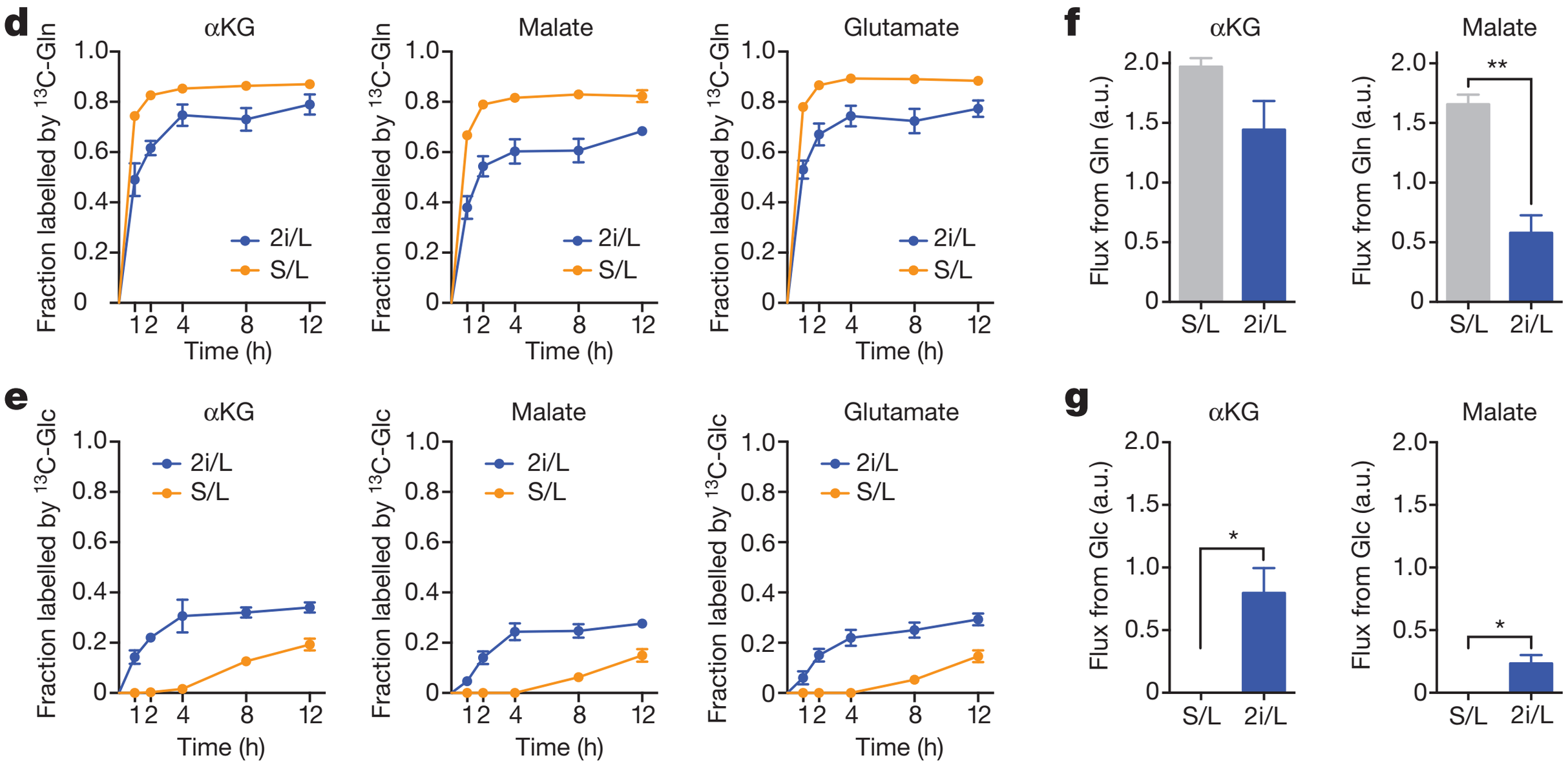
Naive SCs rely more on GLC oxidation for the generation of TCA/anabolic metabolites.

Carey et al, ***Nature****, 2015*



Consequently, during conditions of glutamine depletion, cells cultured in 2i/L medium were able to use glucose-derived carbons to maintain elevated glutamate pools sufficient to support cell growth.

Carey et al, ***Nature****, 2015*



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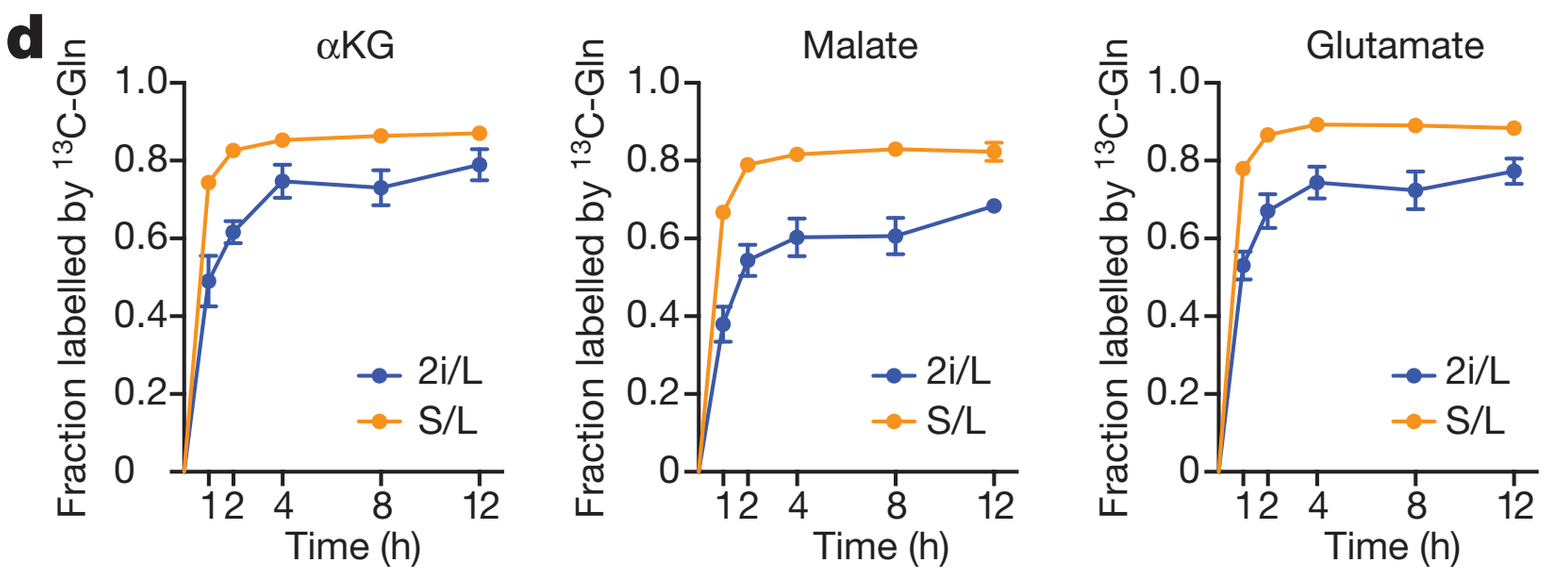
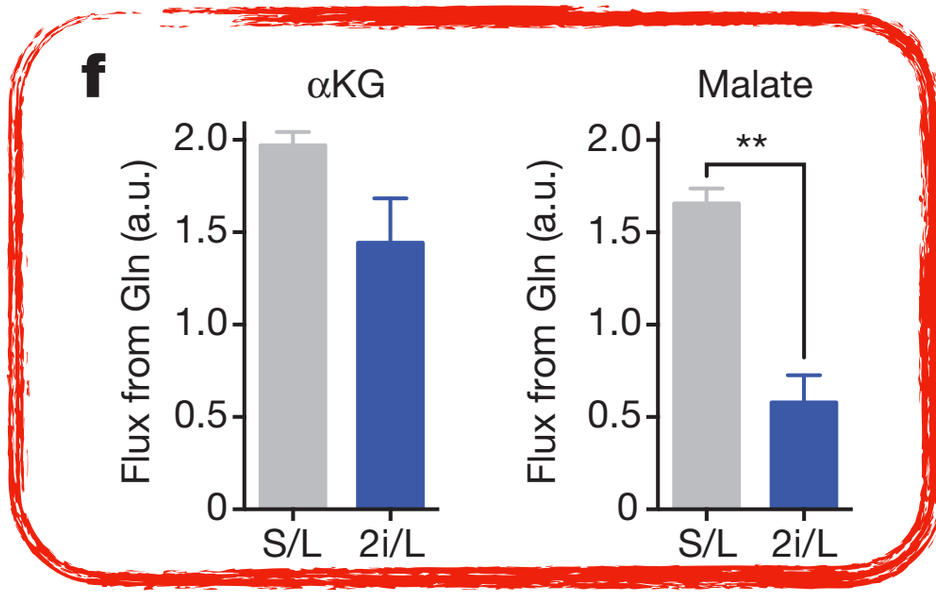
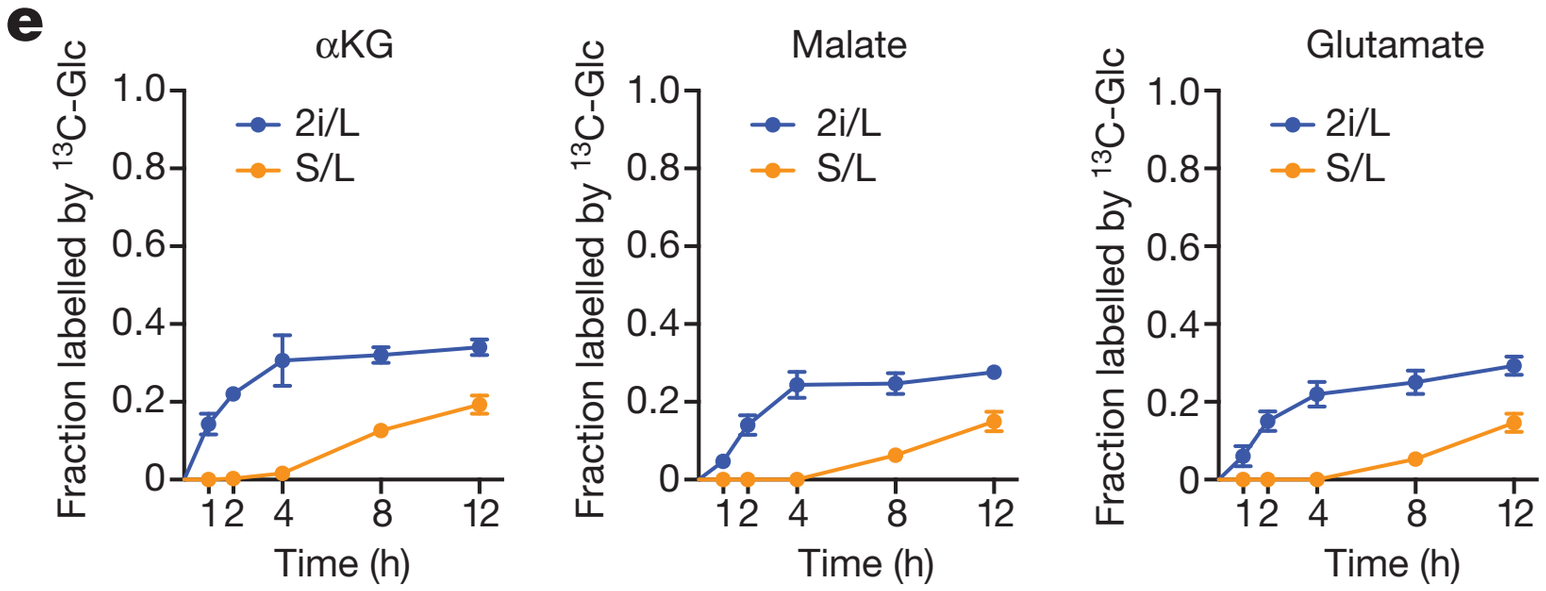
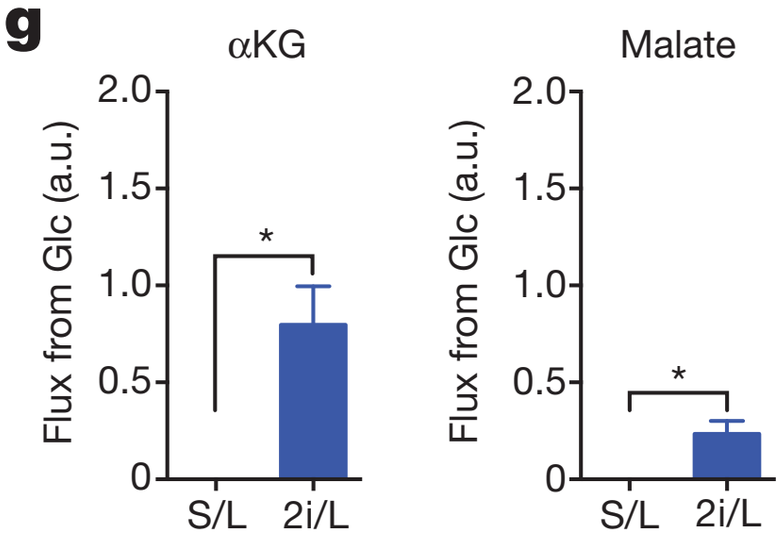
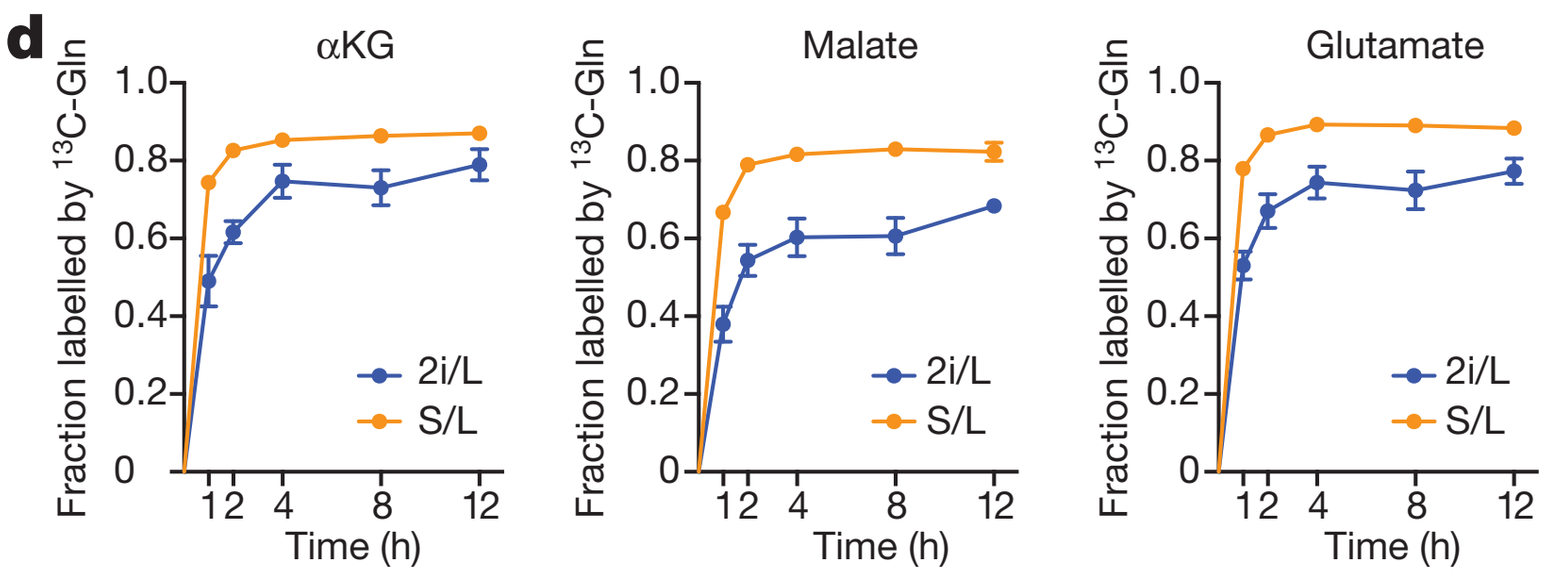


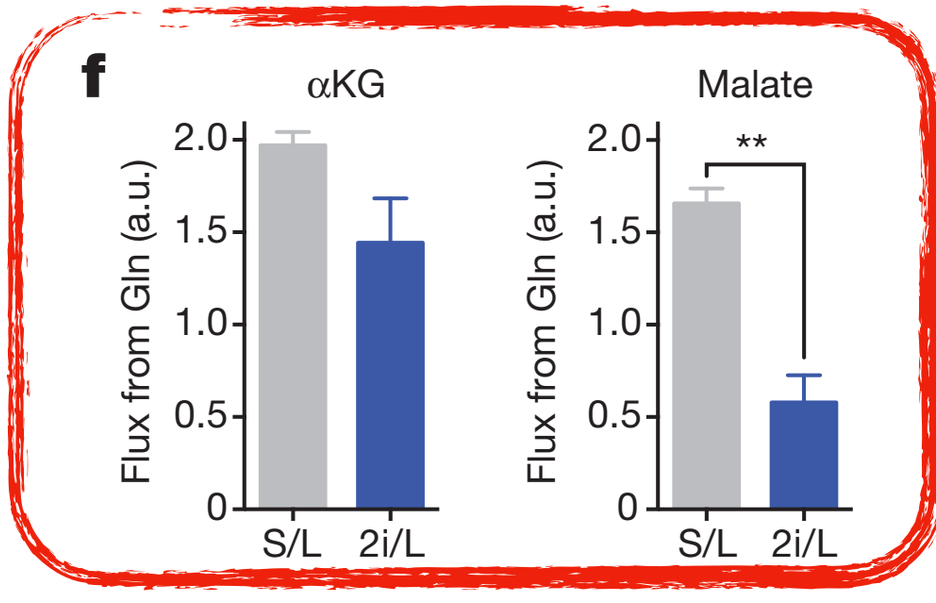


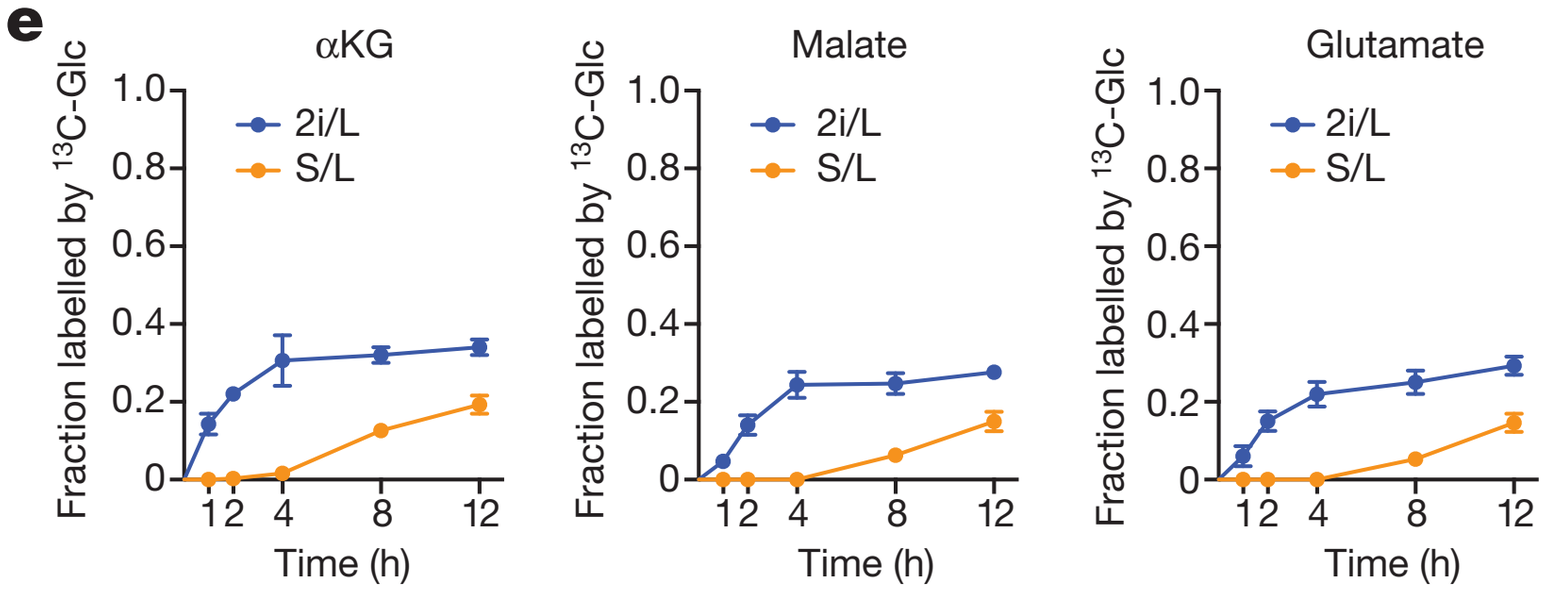


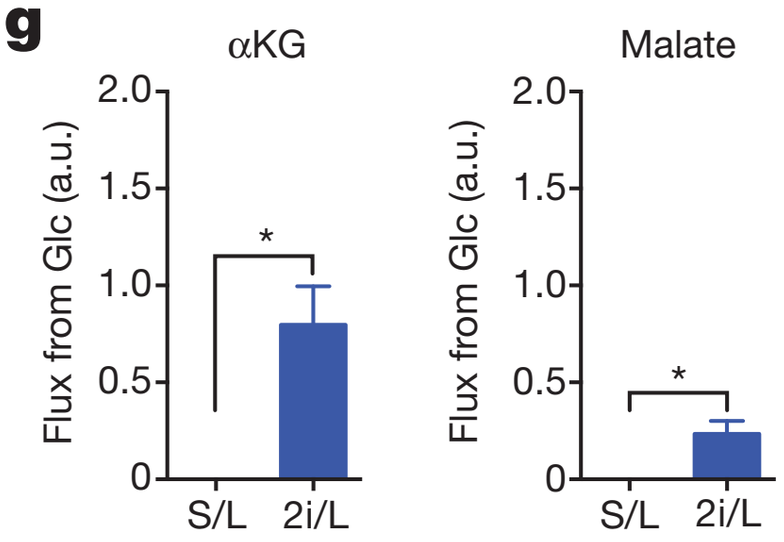


Carey et al, ***Nature****, 2015*





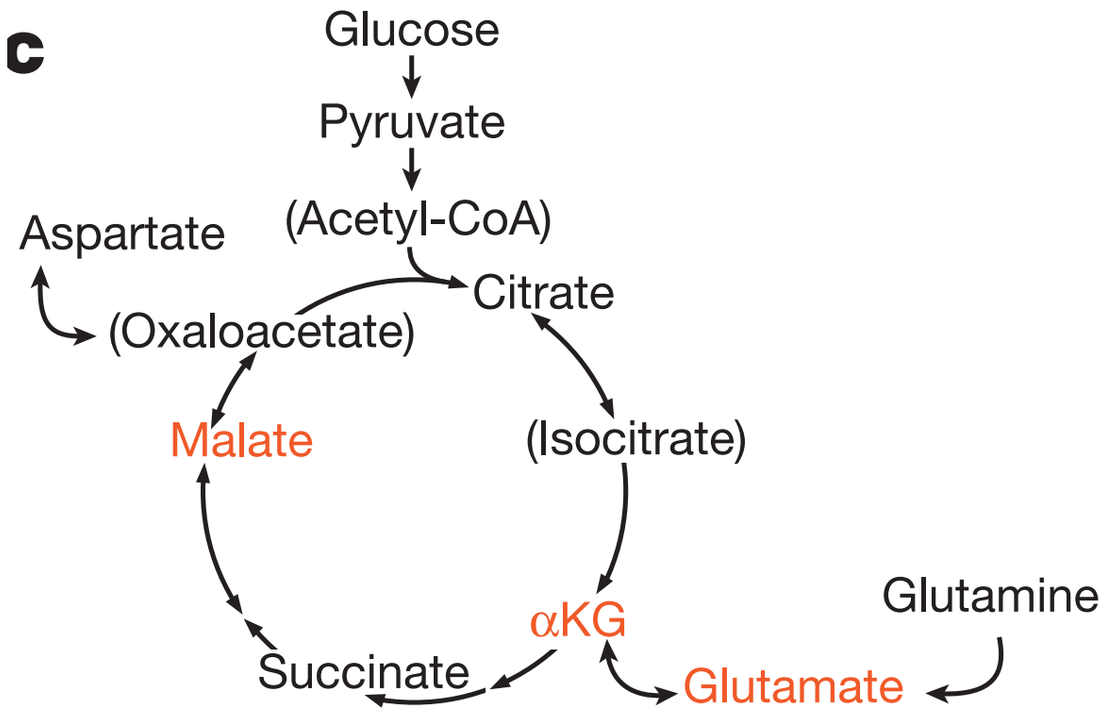


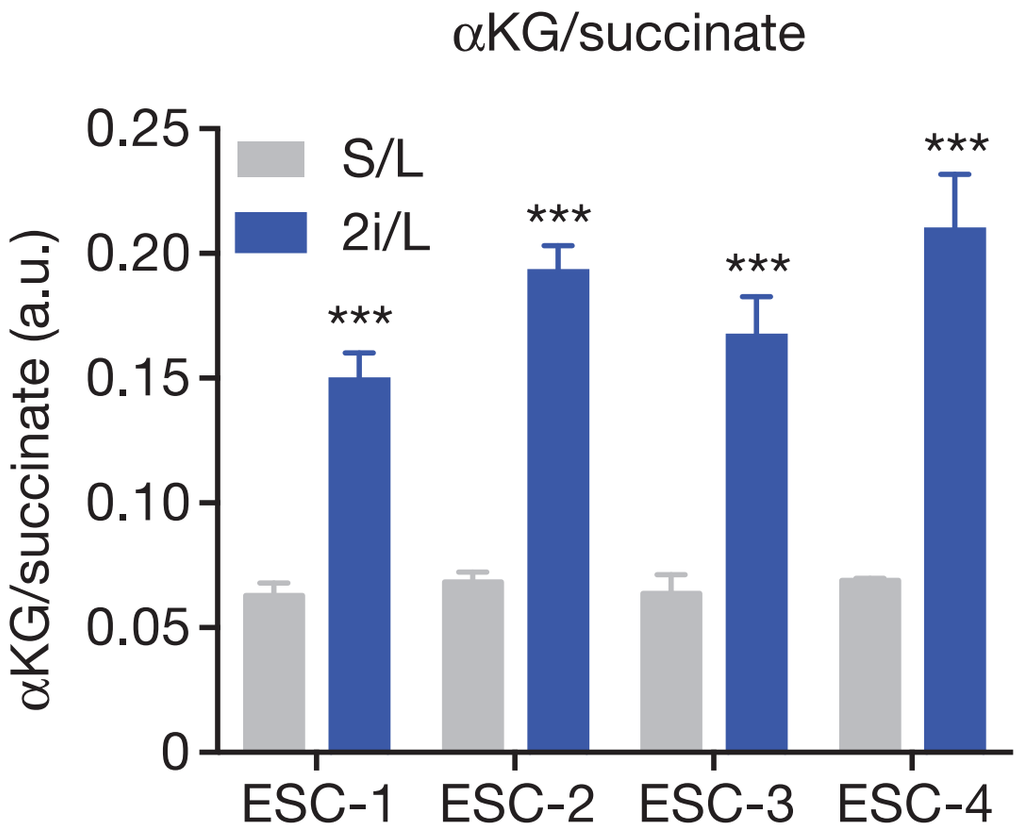


Diminished glutamine entry into the TCA cycle, coupled with the observed efflux of glucose-derived carbons from the TCA cycle as glutamate, suggested that **cells cultured in 2i/L might not be oxidizing all the aKG** produced from glutamine in the mitochondria.

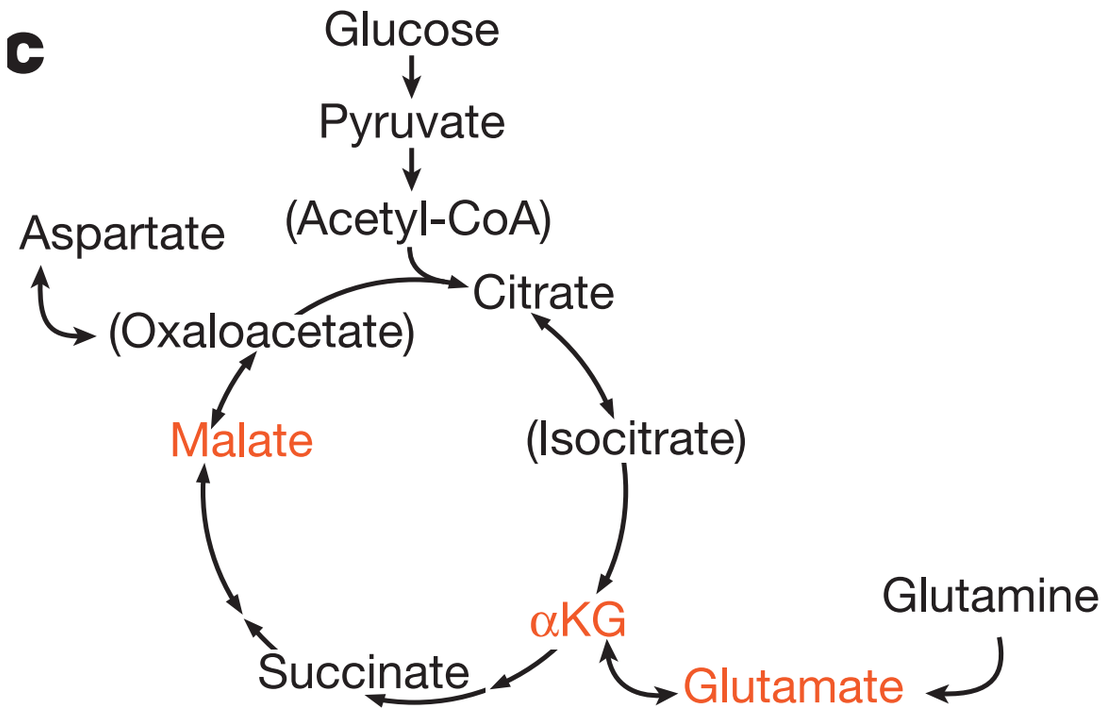
*Carey et al, **Nature**, 2015*

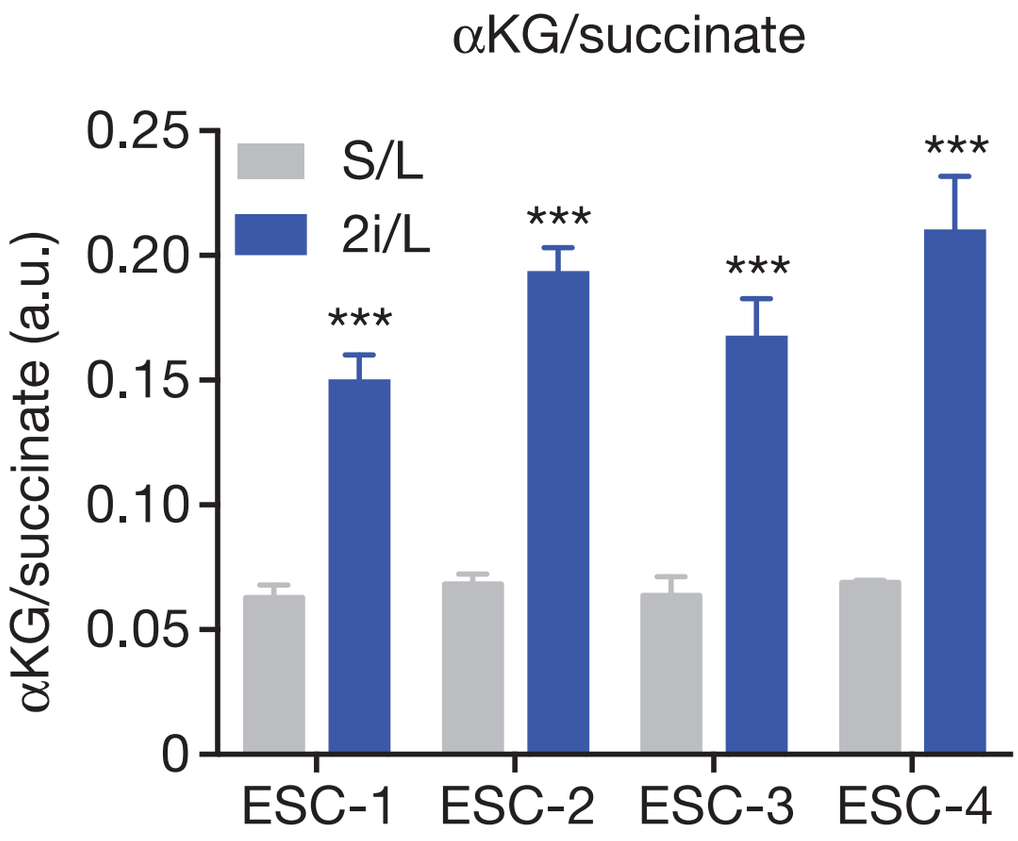
c





Carey et al, ***Nature****, 2015*

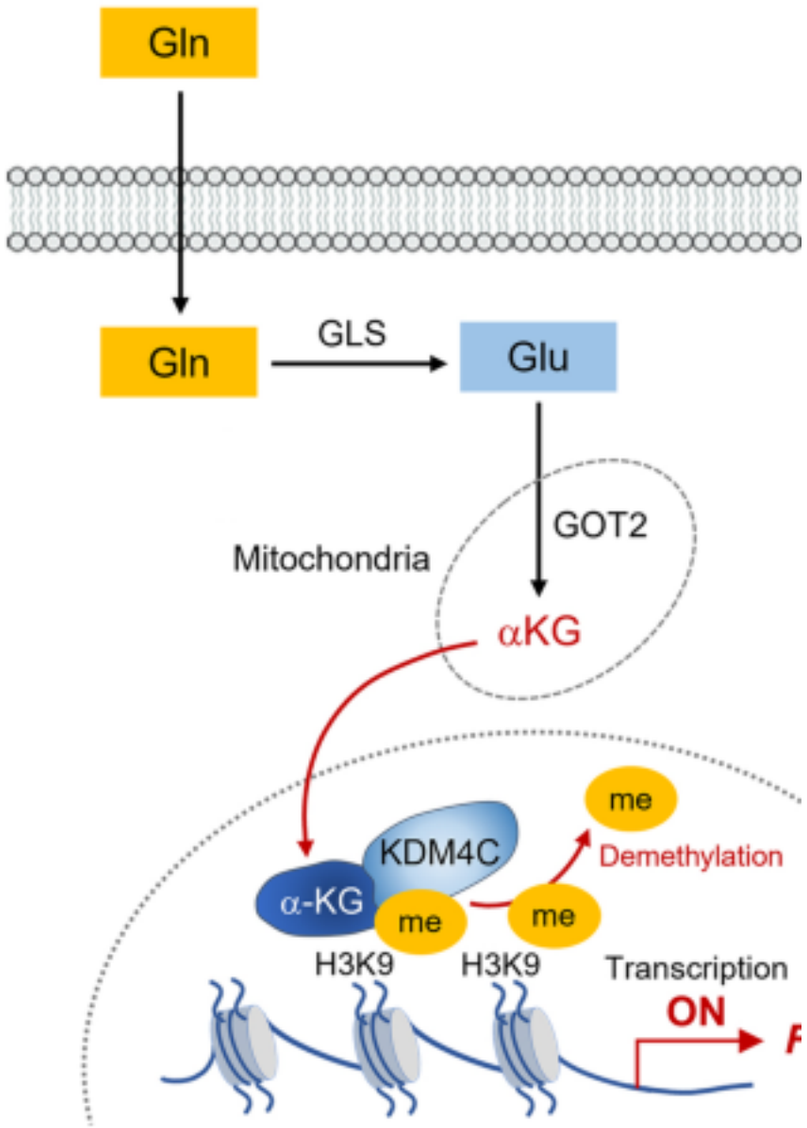


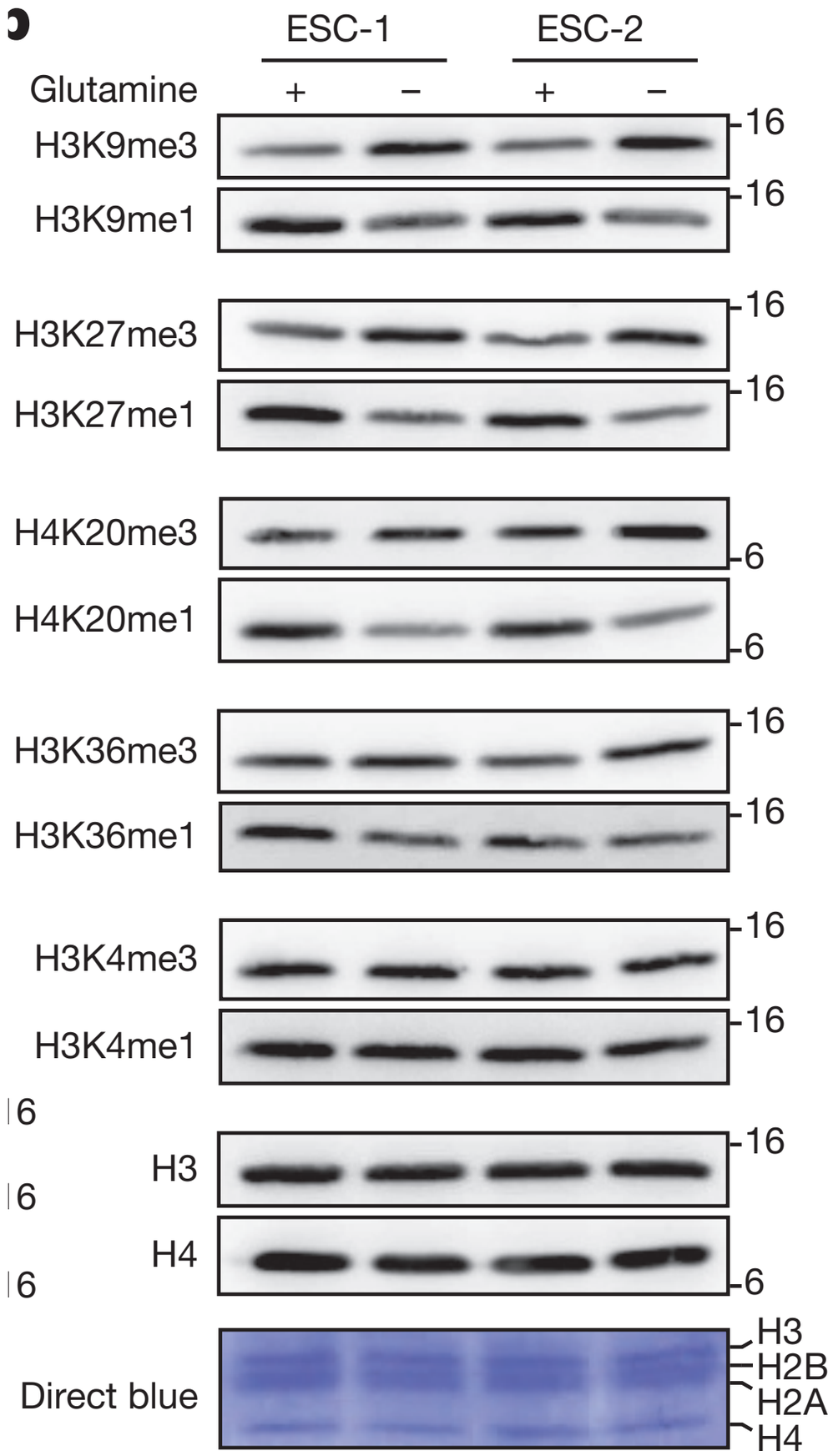


Naive cells do not oxidize all their aKG (in the mitochondria).

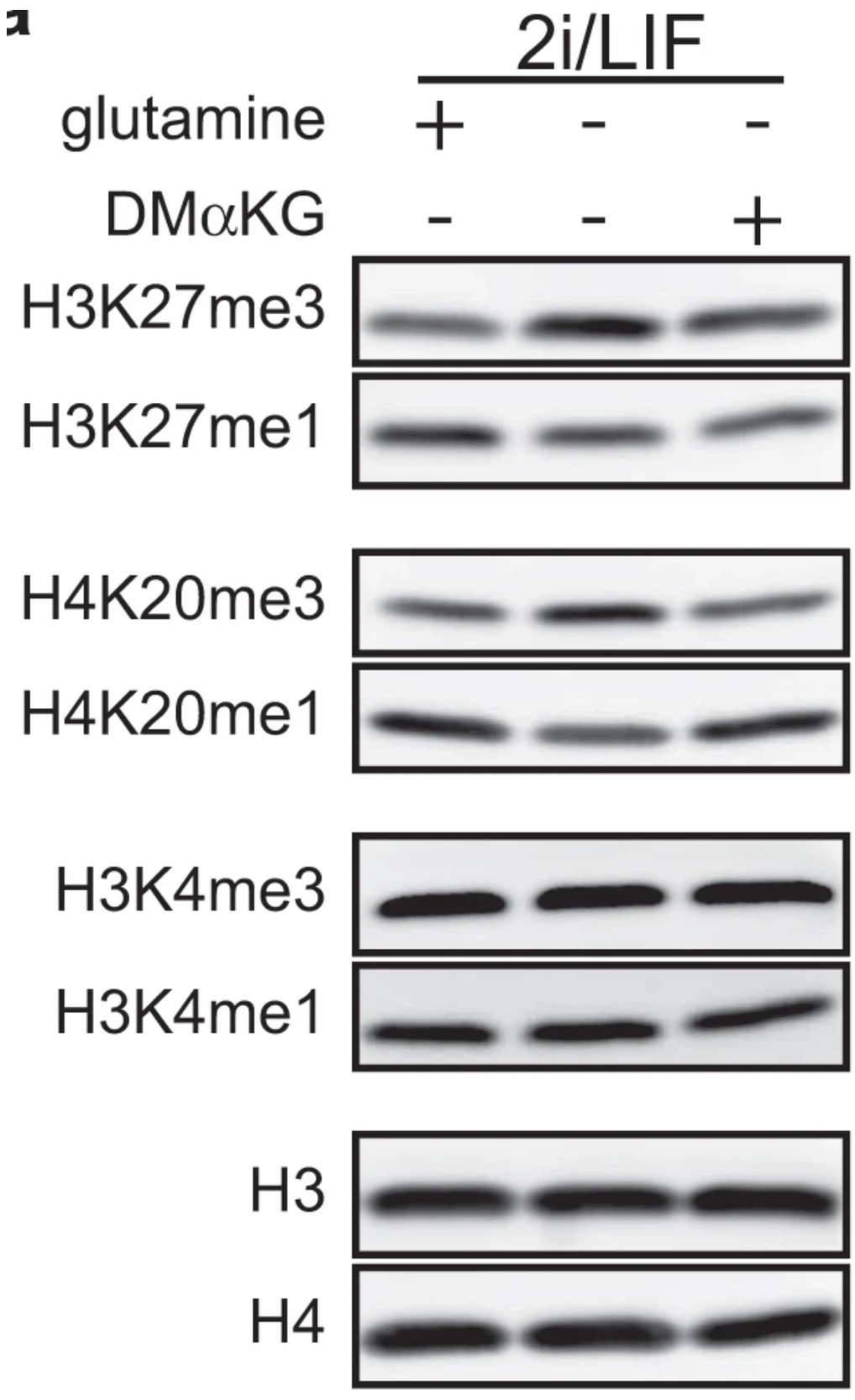
Carey et al, ***Nature****, 2015*

What does aKG do?





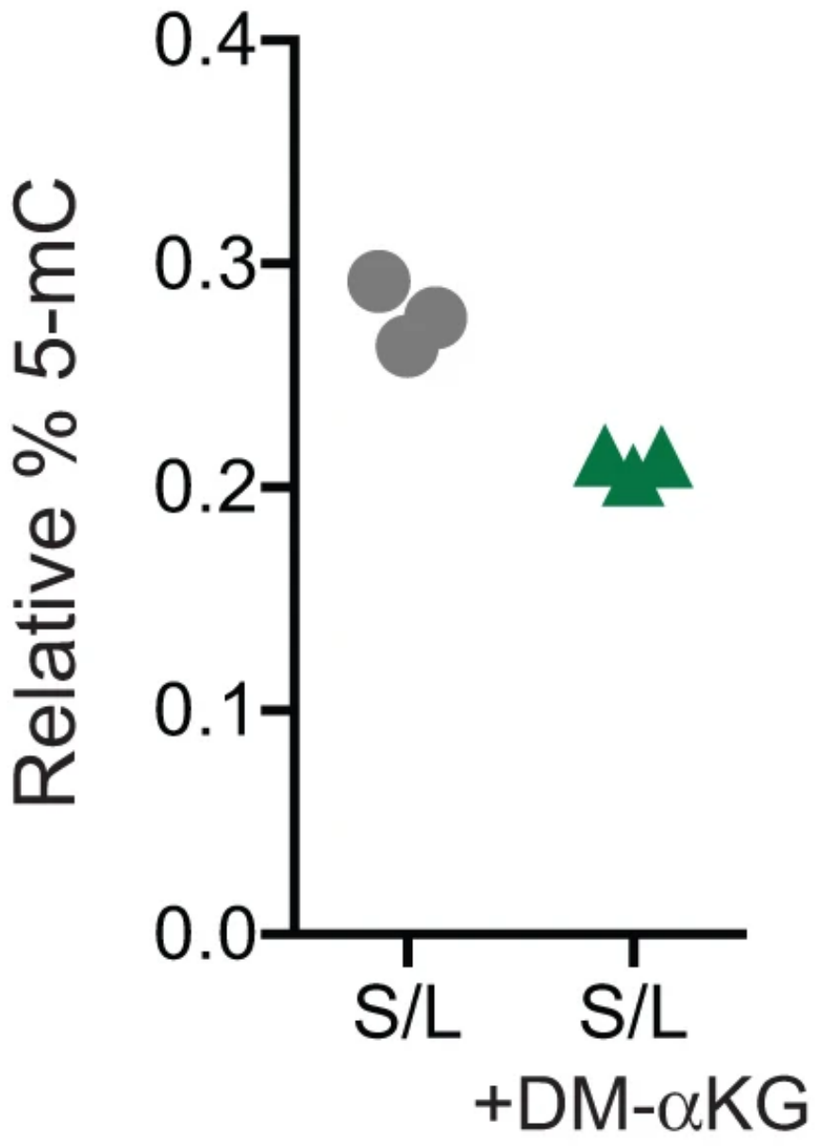
Carey et al, ***Nature****, 2015*



Changes in histone methylation could be accounted for by the decline in glutamine-dependent aKG

Carey et al, ***Nature****, 2015*

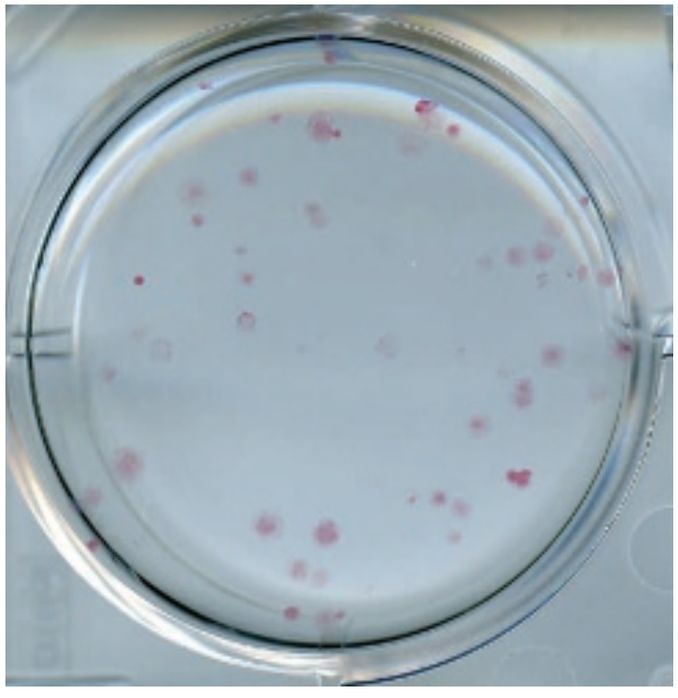
Methylation of certain histone lysines, including H3K27, are actively suppressed by aKG-dependent histone demethylases in ES cells maintained in 2i/L medium



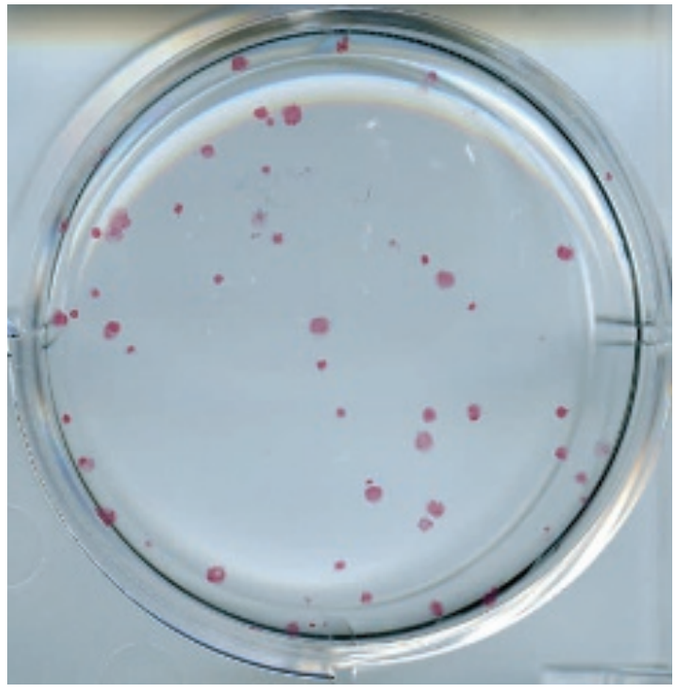
aKG promotes also DNA demethylation (commonly observed in ESC)

Carey et al, ***Nature****, 2015*

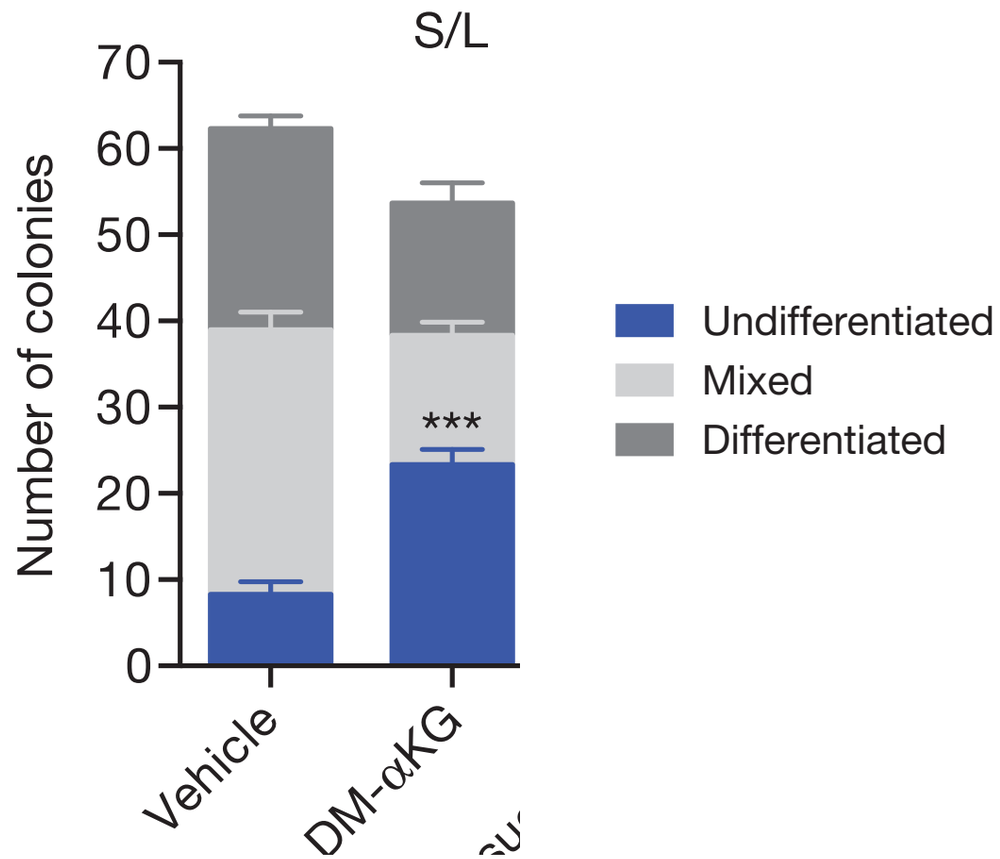
Do aKG levels influence SC fate decisions?



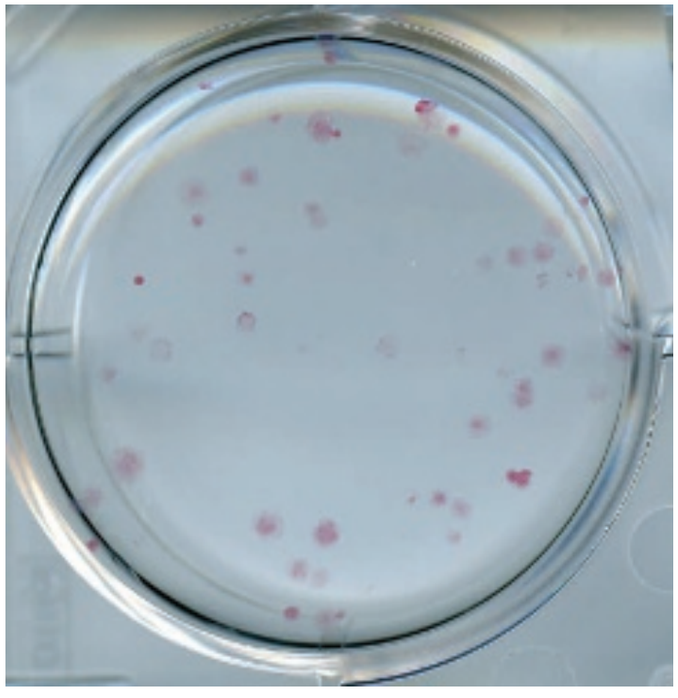
S/L+DMSO



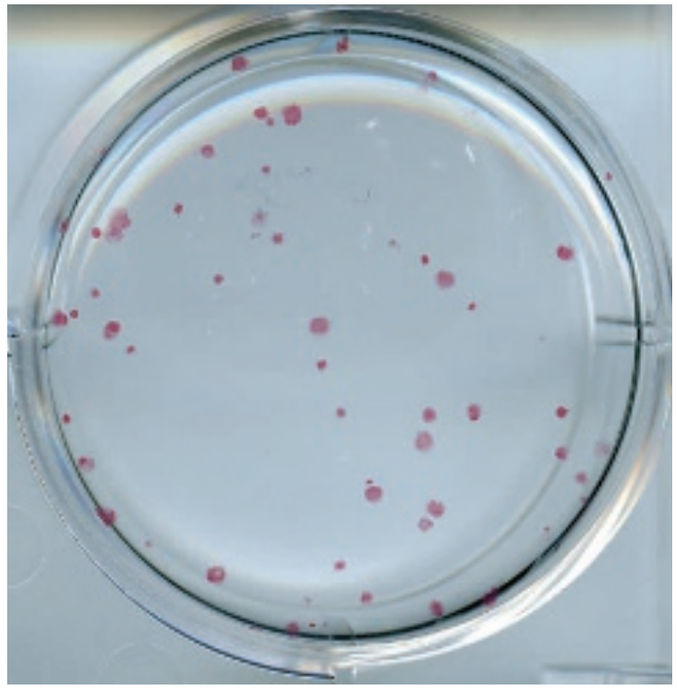
S/L+DM-αKG



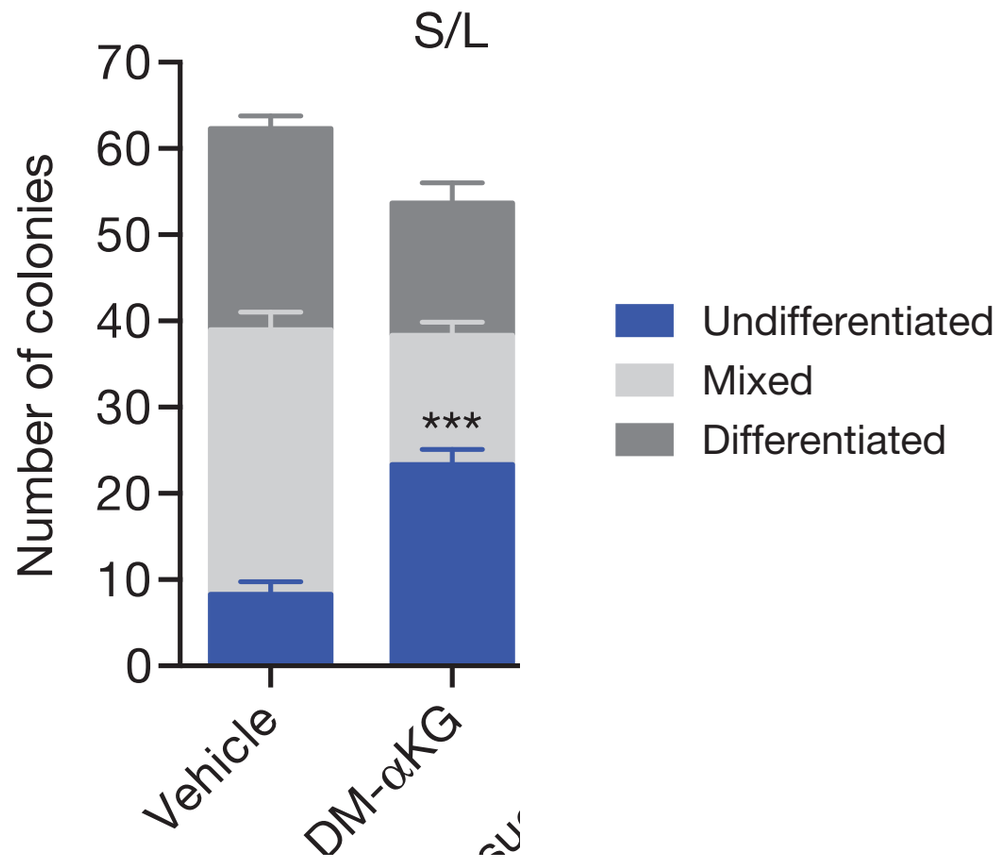
Carey et al, ***Nature****, 2015*

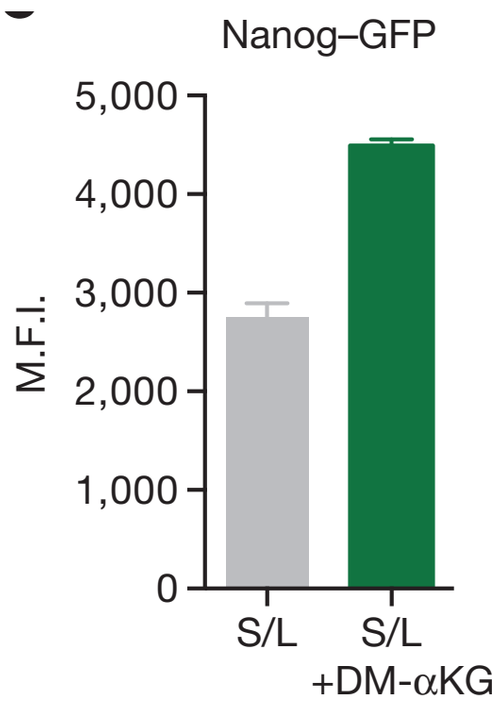


S/L+DMSO

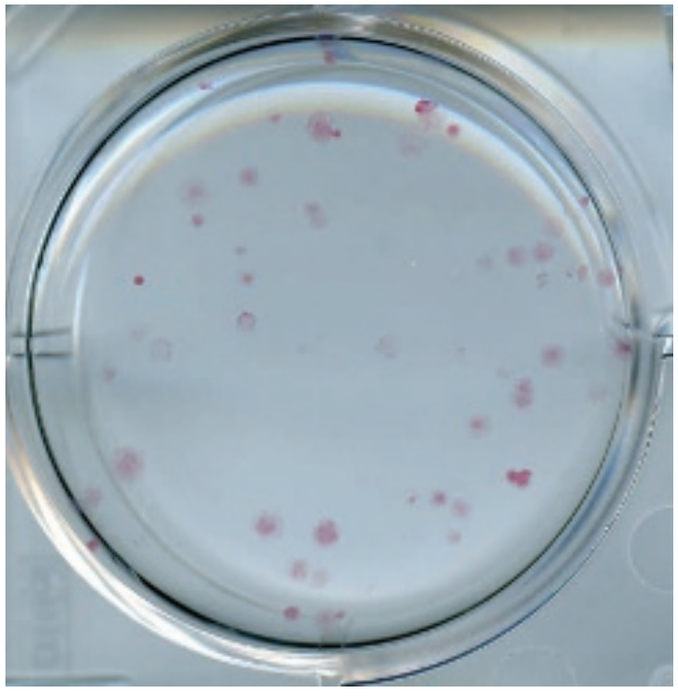


S/L+DM-αKG

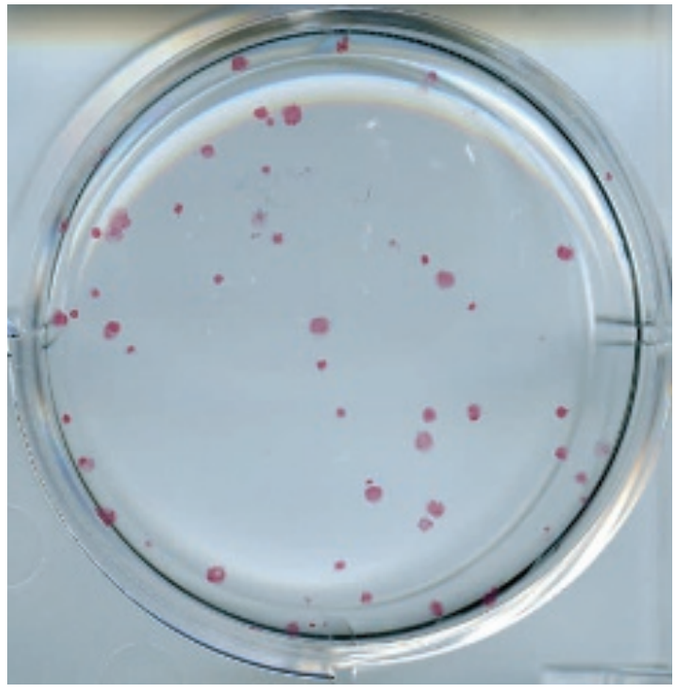




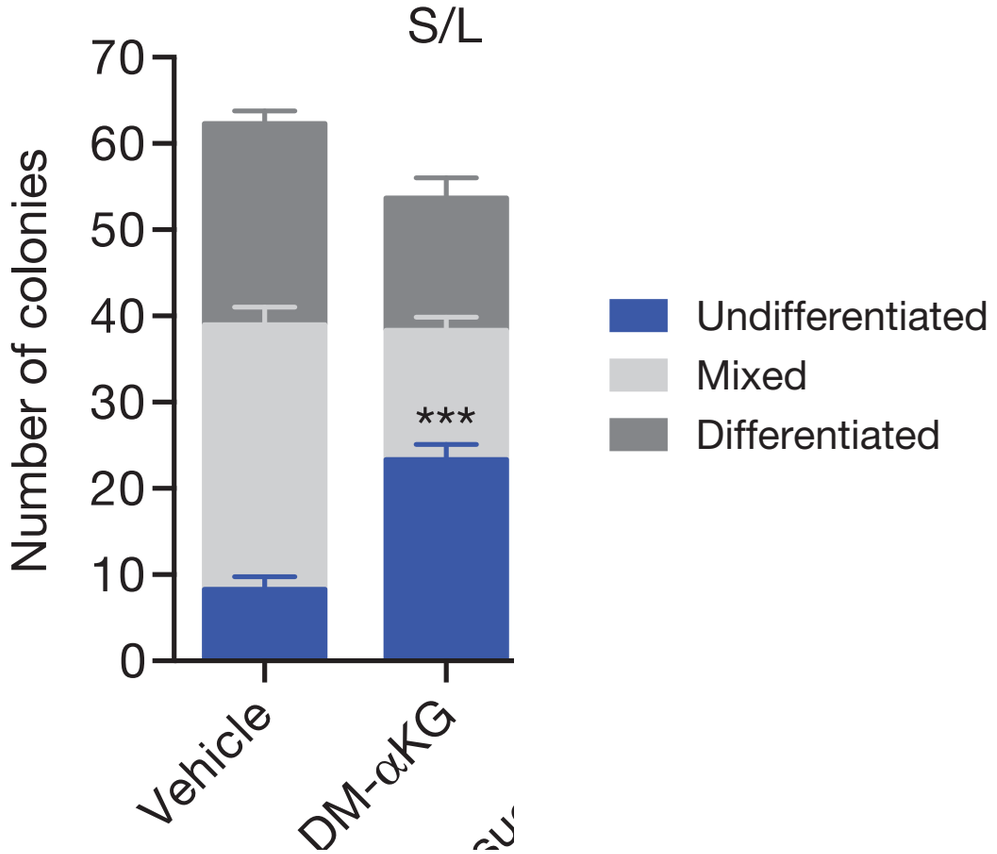
Carey et al, ***Nature****, 2015*

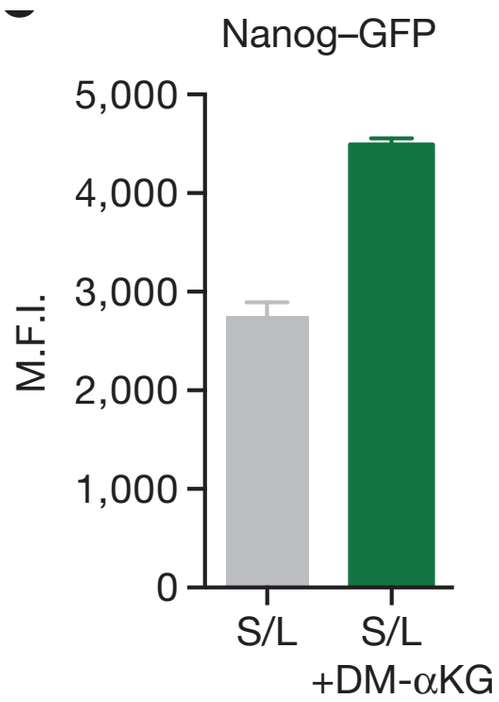


S/L+DMSO



S/L+DM-αKG





aKG promotes the expression of stemless-related genes (through demethylation of repressive markers) and enables self-renewal of ES cells *in vitro*.

Carey et al, ***Nature****, 2015*

These data demonstrate that the cellular aKG/succinate ratio contributes to the ability of ES cells to suppress differentiation.

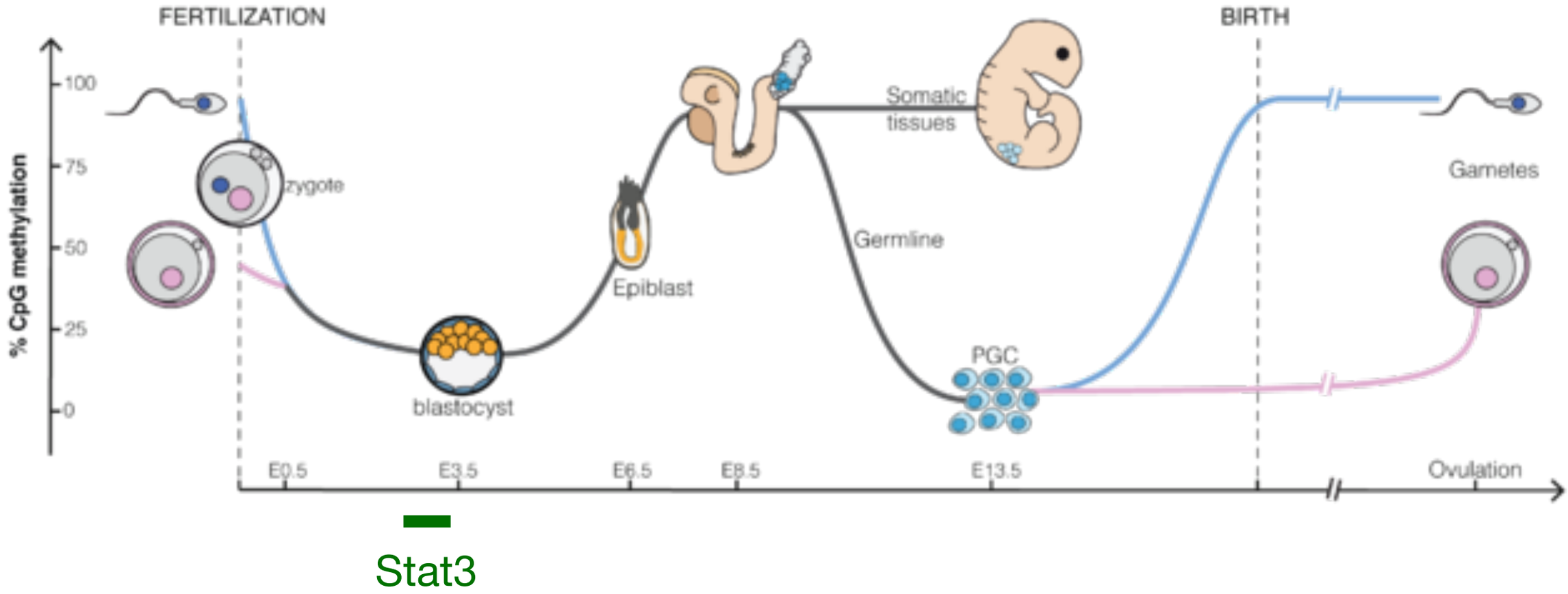
The rewiring of cellular metabolism by inhibitors of GSK3b and MAPK/ERK signaling results in a reprogramming of glucose and glutamine metabolism; in turn, this leads to accumulation of aKG and favors demethylation of repressive chromatin marks such as DNA methylation and H3K9me3, H3K27me3 and H4K20me3

Can they rule out chromatin-independent effects of aKG?

Can they rule out aKG-independent effects of Q starvation?

Metabolic control of DNA methylation in naive pluripotent cells

Riccardo M. Betto[1], Linda Diamante[1], Valentina Perrera[1,14], Matteo Audano[2], Stefania Rapelli[3,4], Andrea Lauria[3,4], Danny Incarnato[3,5,15], Mattia Arboit[1], Silvia Pedretti[2], Giovanni Rigoni[6,16], Vincent Guerineau[7], David Touboul[7], Giuliano Giuseppe Stirparo[8], Tim Lohoff[8], Thorsten Boroviak[9,10,11,12], Paolo Grumati[13], Maria E. Soriano[6], Jennifer Nichols[8,9], Nico Mitro[2] ✉, Salvatore Oliviero[3,4] ✉ and Graziano Martello[1,6] ✉



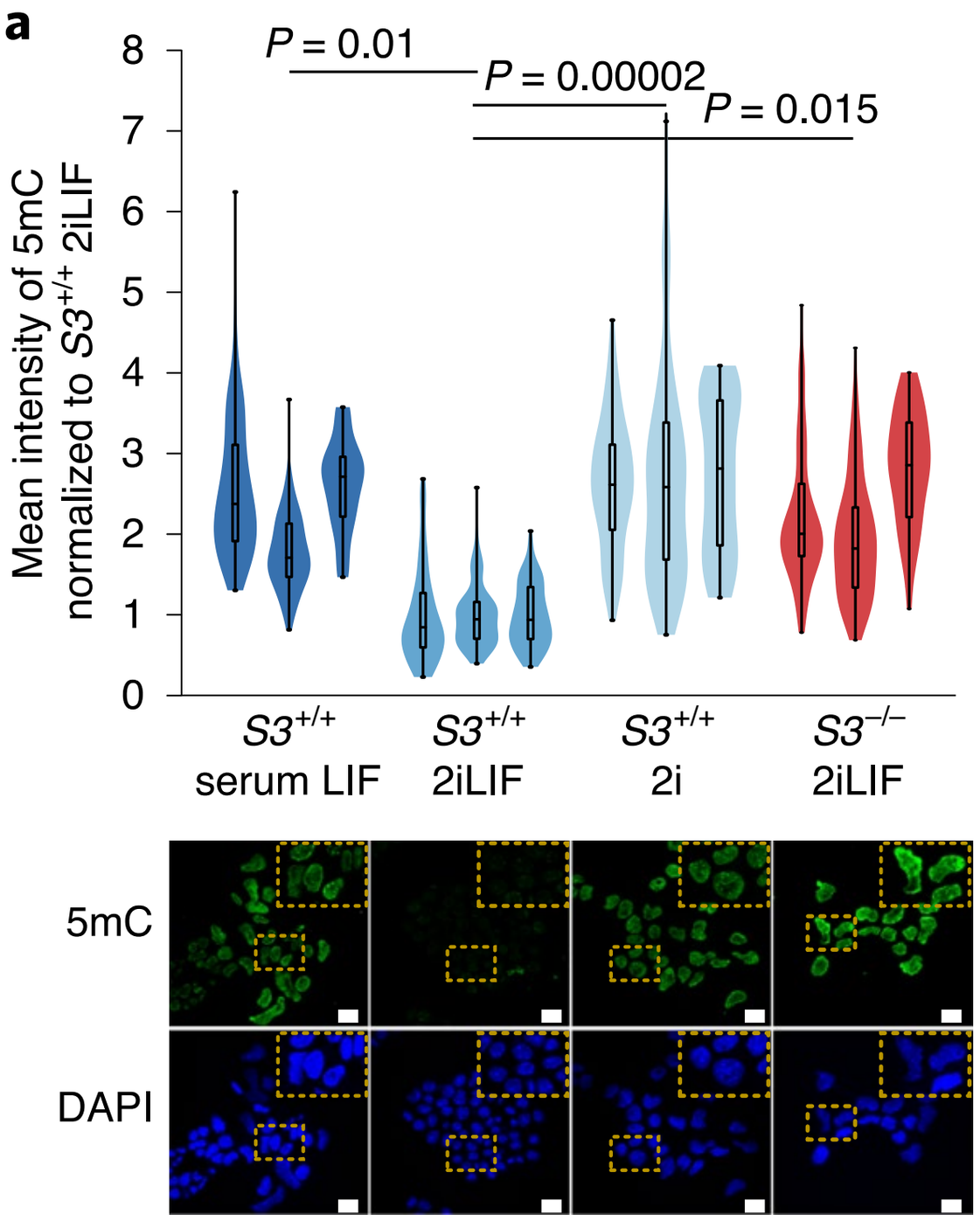
Betto et al, ***Nat Genet****, 2021*

Can Stat3 activation explain DNA demethylation?

What is the mechanism?

2i/LIF: NAIVE

2i: COMMITTED

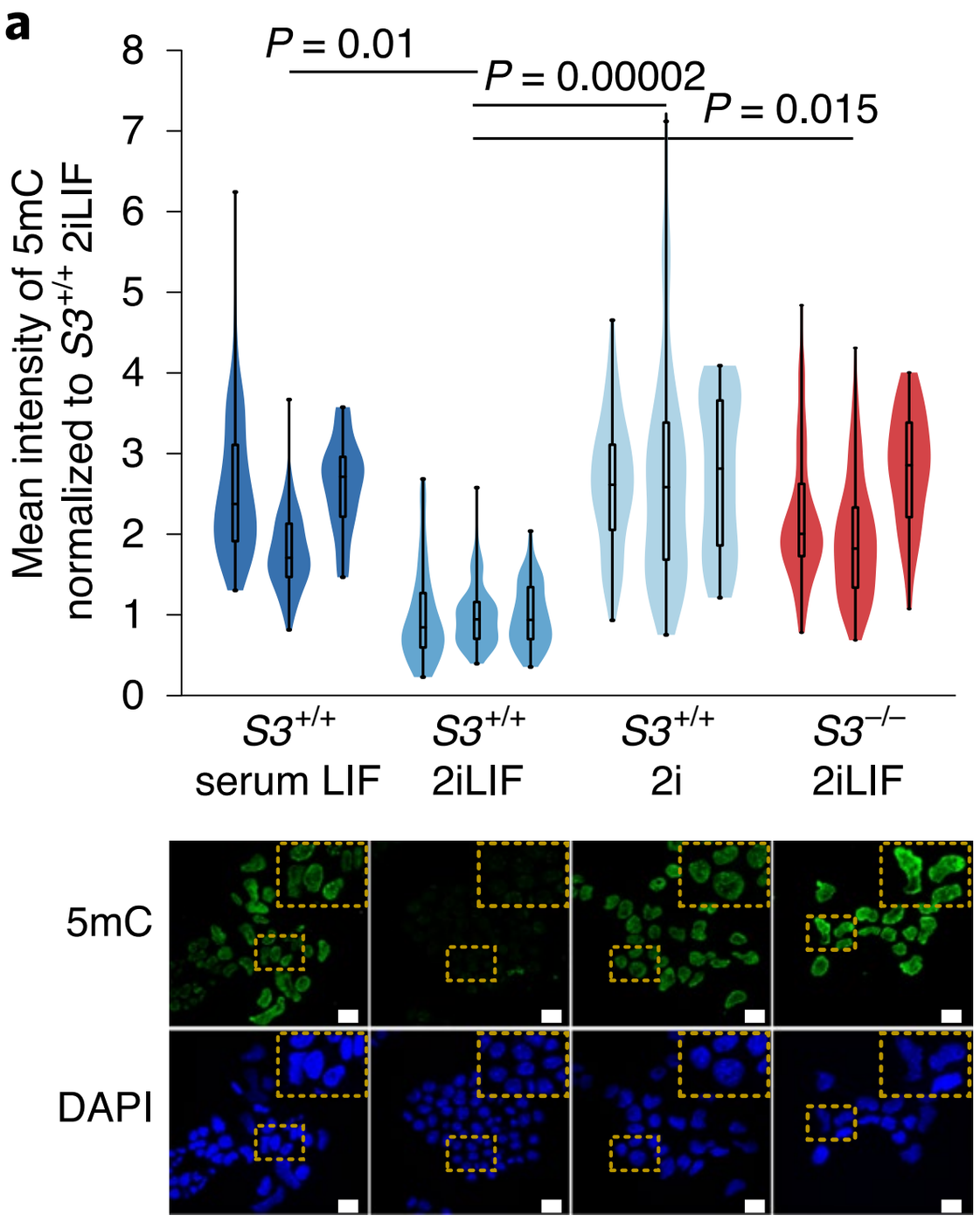


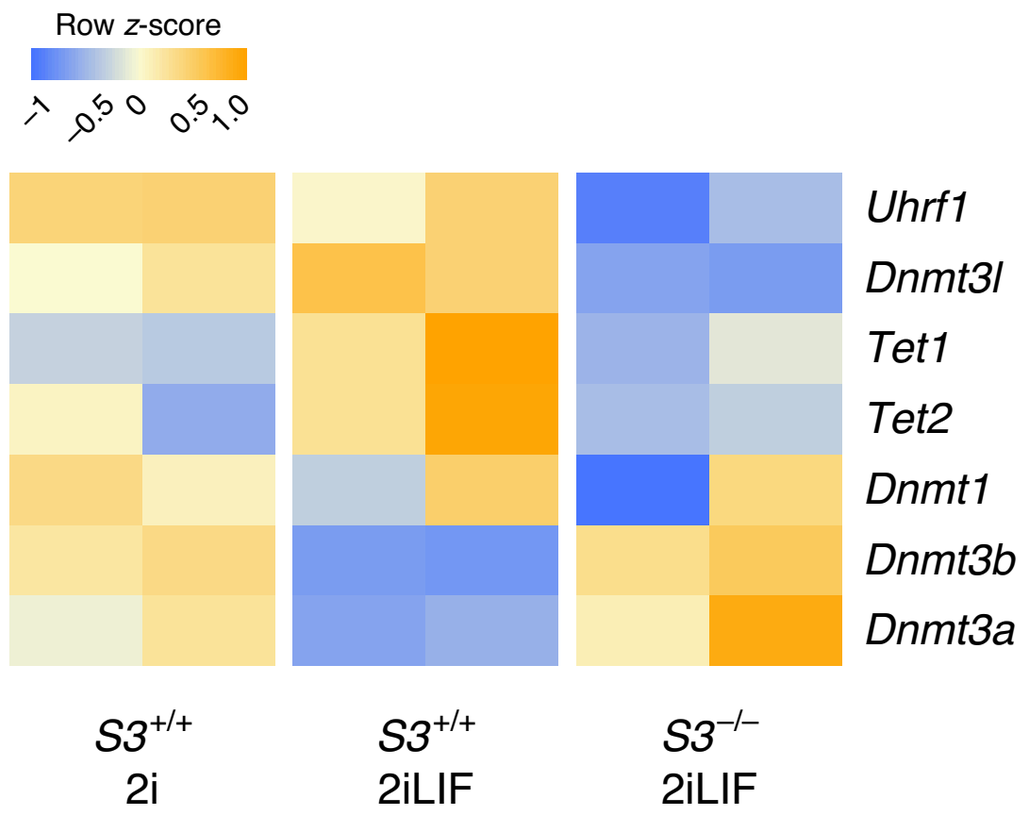
Betto et al, ***Nat Genet****, 2021*

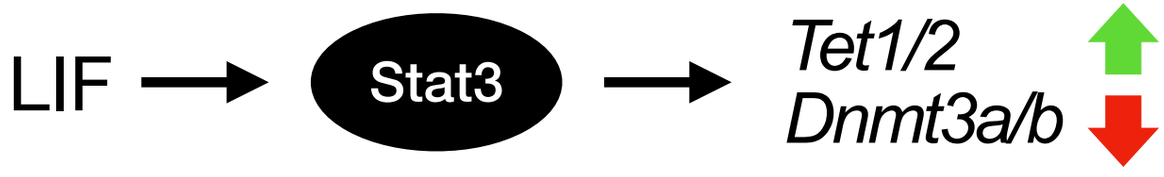
2i/LIF: NAIVE

2i: COMMITTED

How?



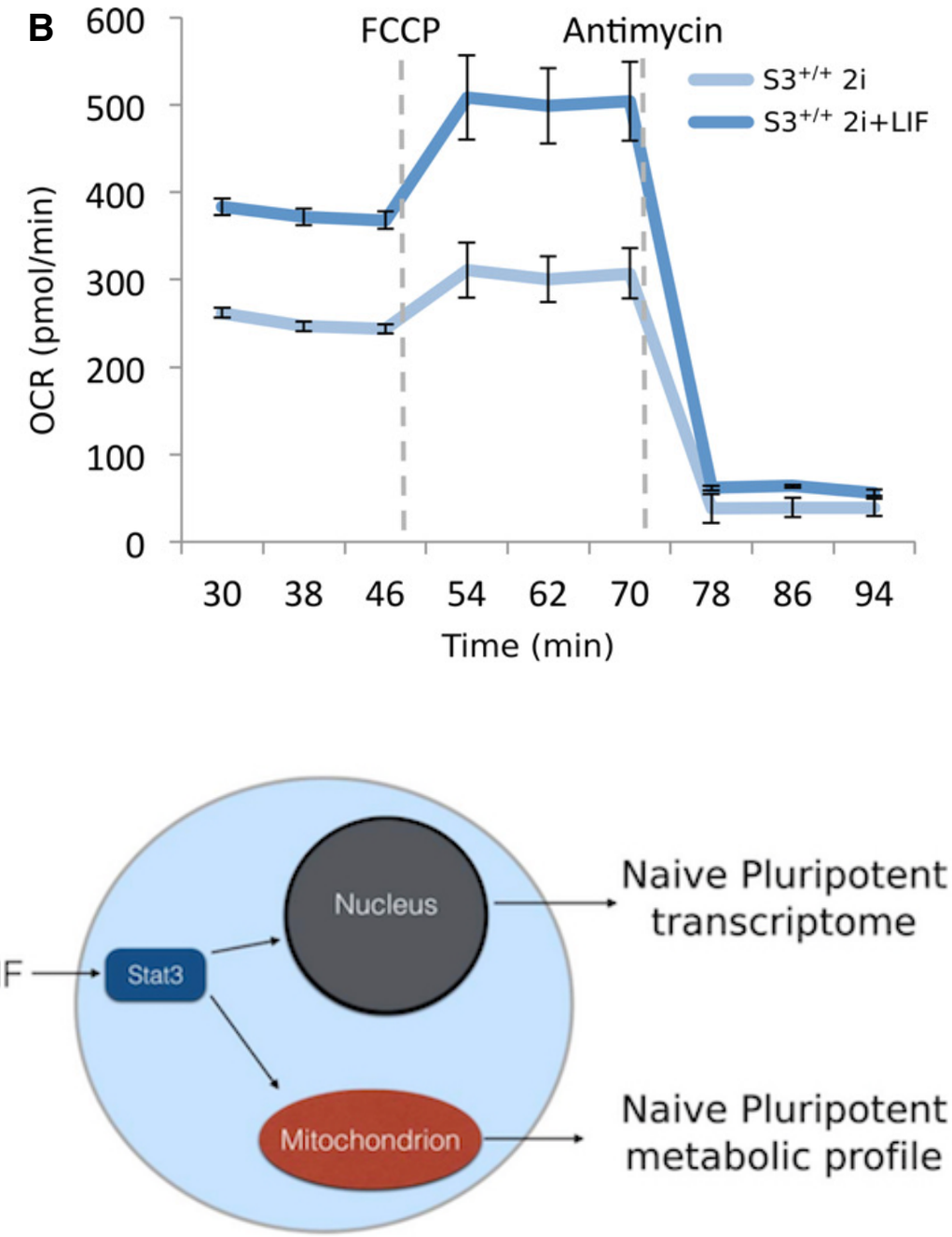




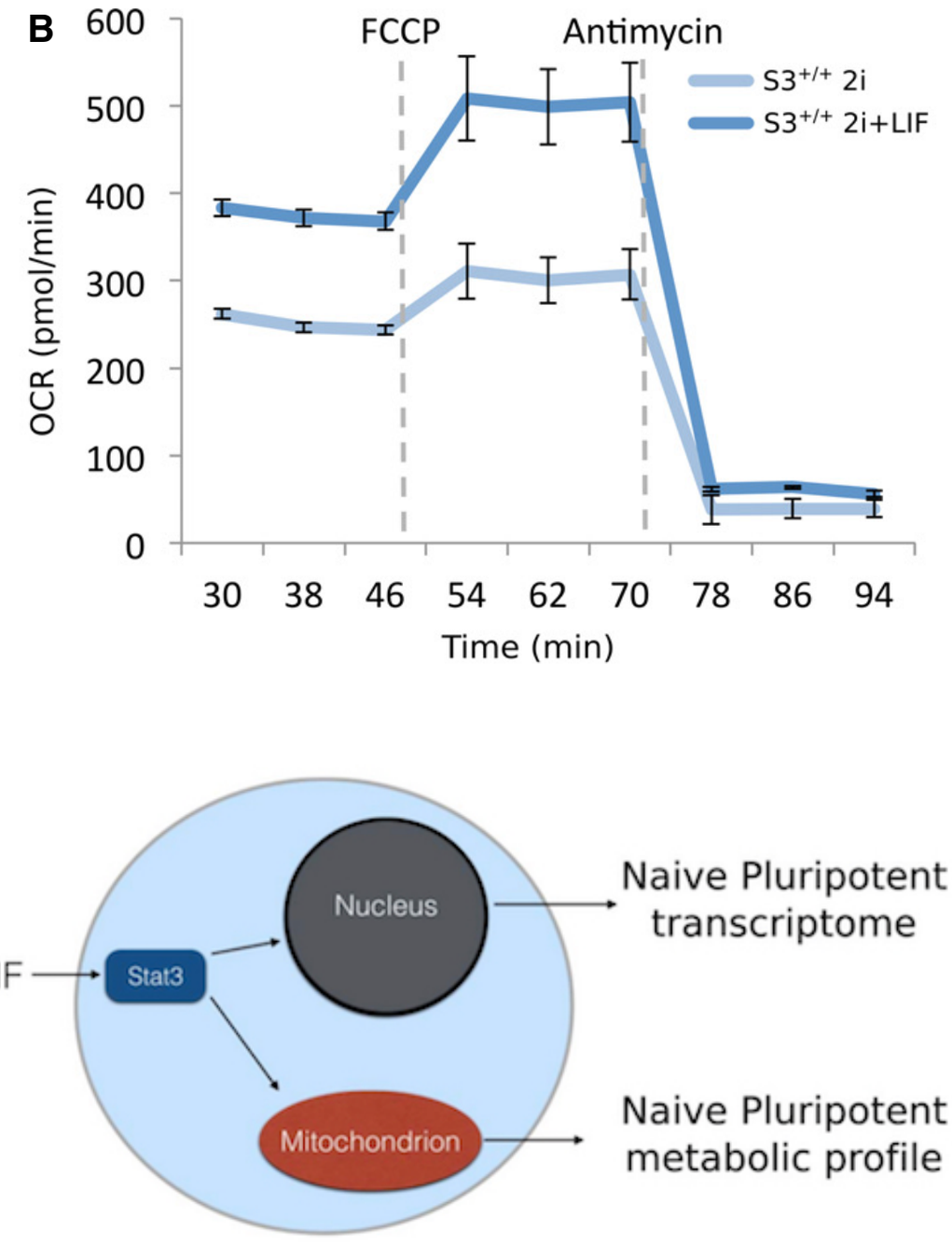
Betto et al, ***Nat Genet****, 2021*

No binding sites for STAT3 near the Dnmt or Tet promoters….

Indirect mechanism?

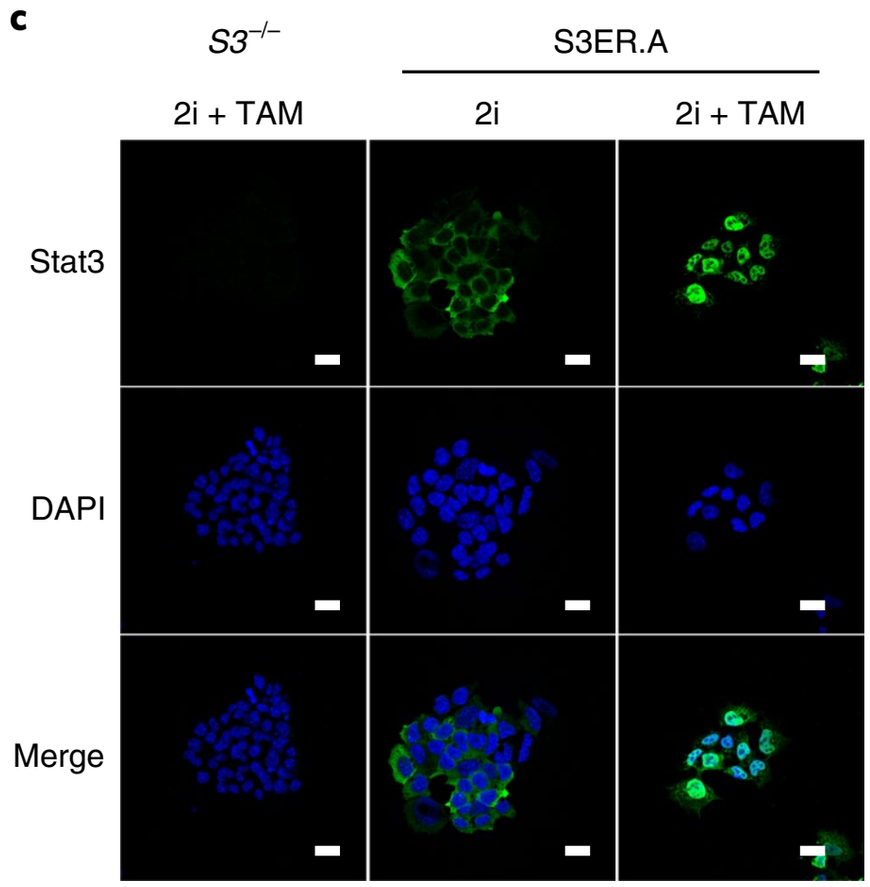


*Carbognin et al, **EMBO J**, 2016*



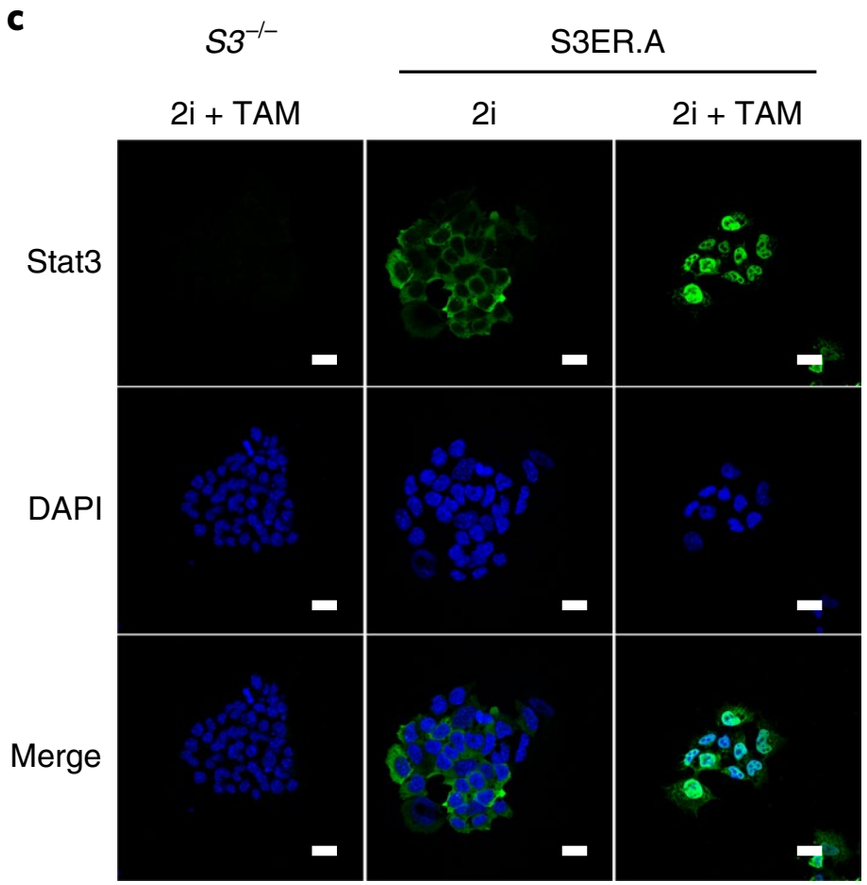
Is the nuclear or mitochondrial STAT3 protein that promotes DNA demethylation?

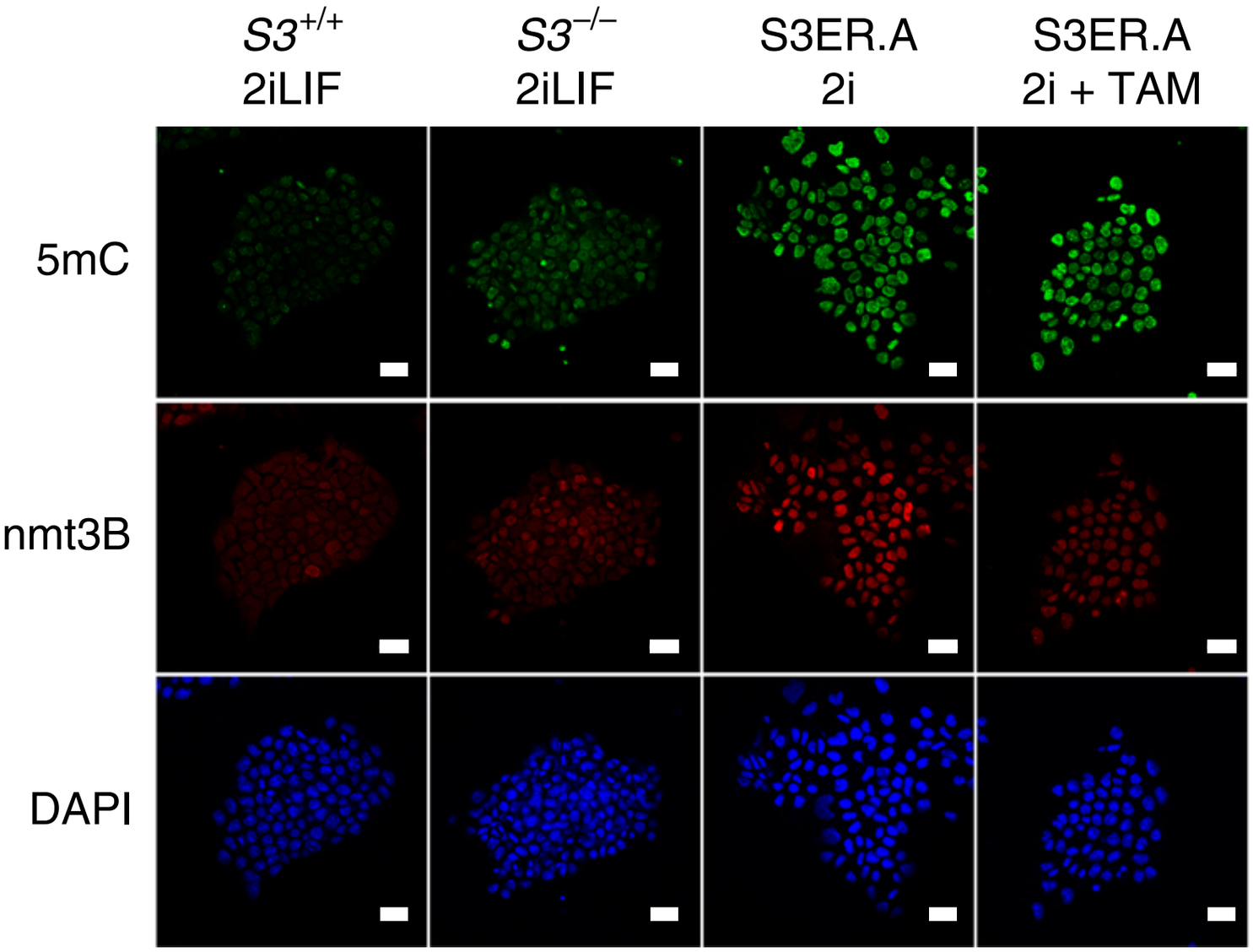
*Carbognin et al, **EMBO J**, 2016*



They generated a Stat3 construct fused to an estrogen receptor domain (S3ER) that localizes to the nucleus following tamoxifen (TAM) treatment (in S3−/− cells)

Betto et al, ***Nat Genet****, 2021*





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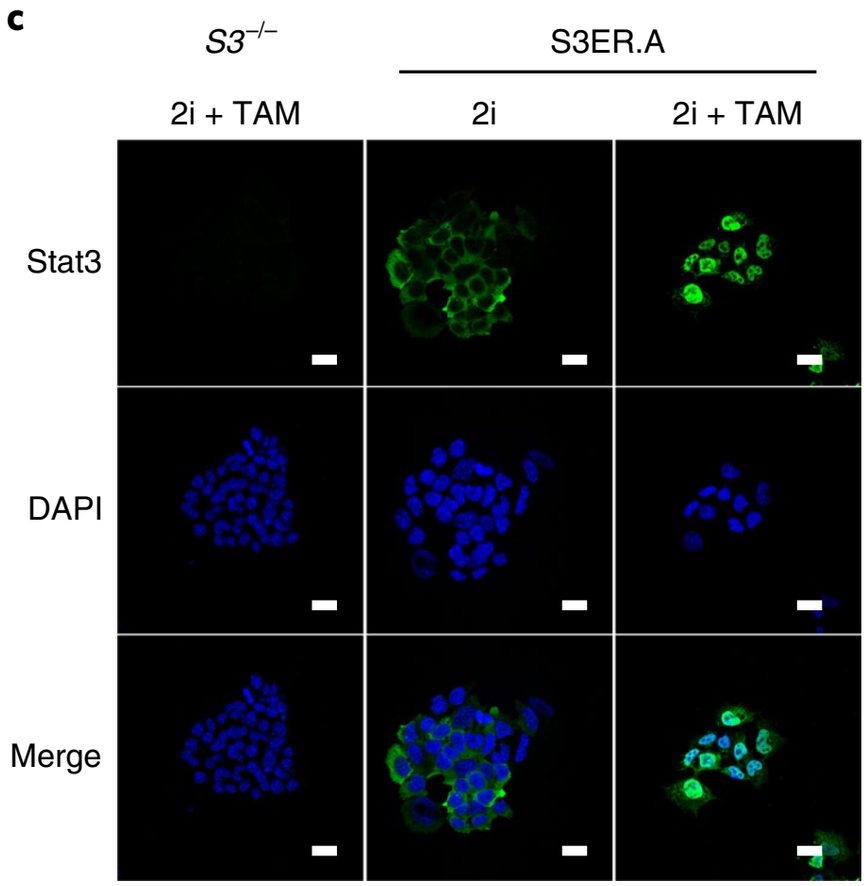

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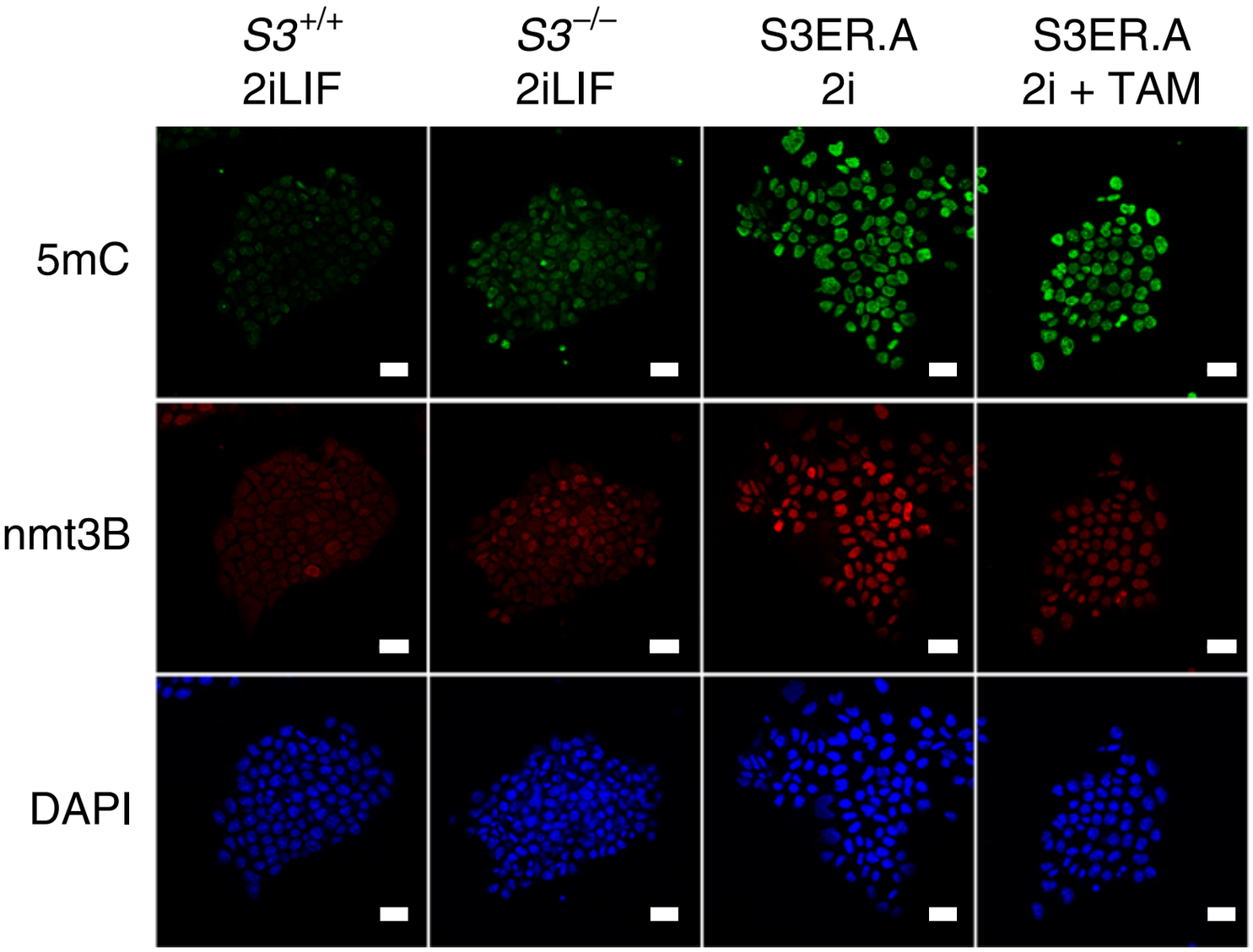
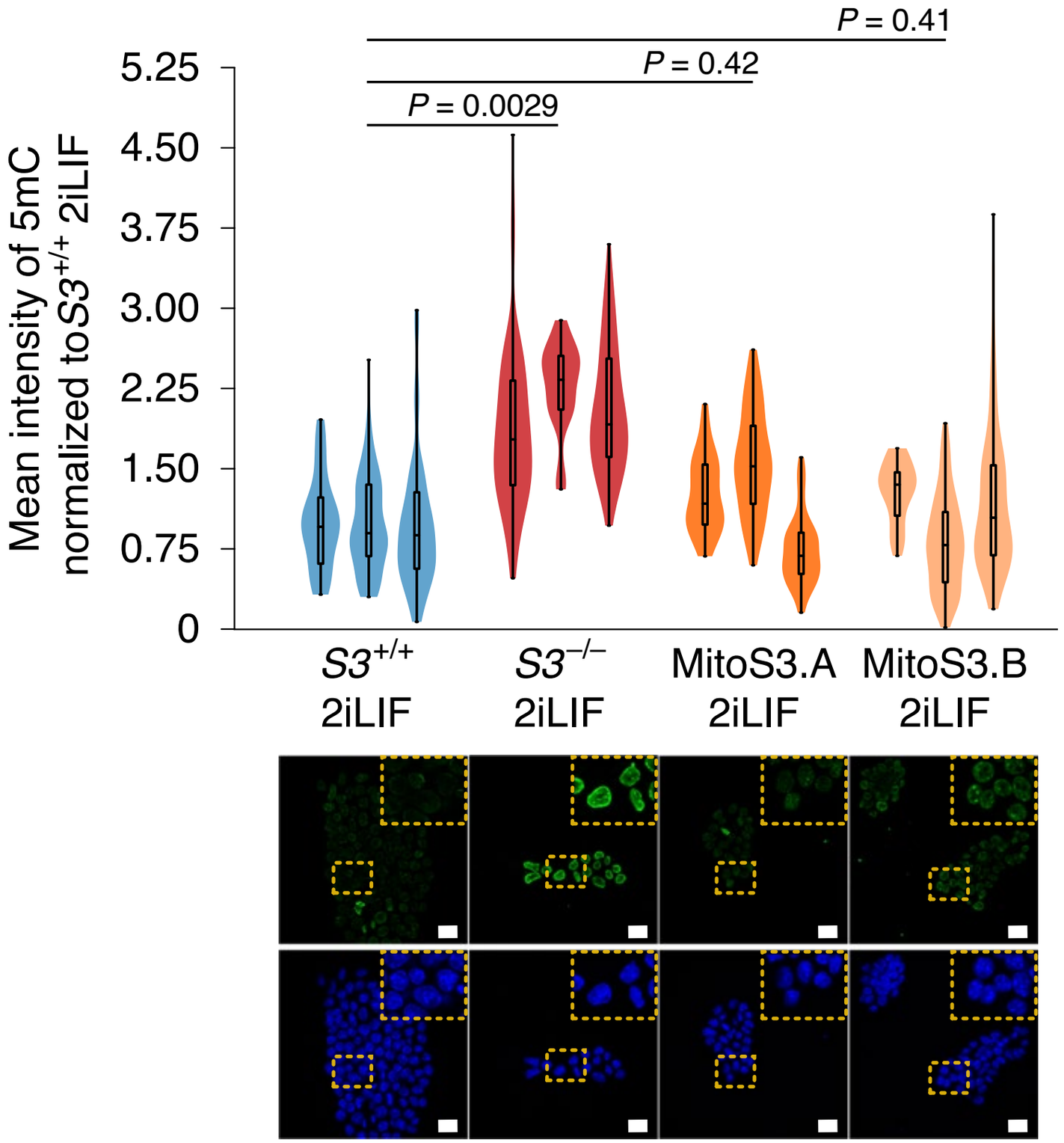
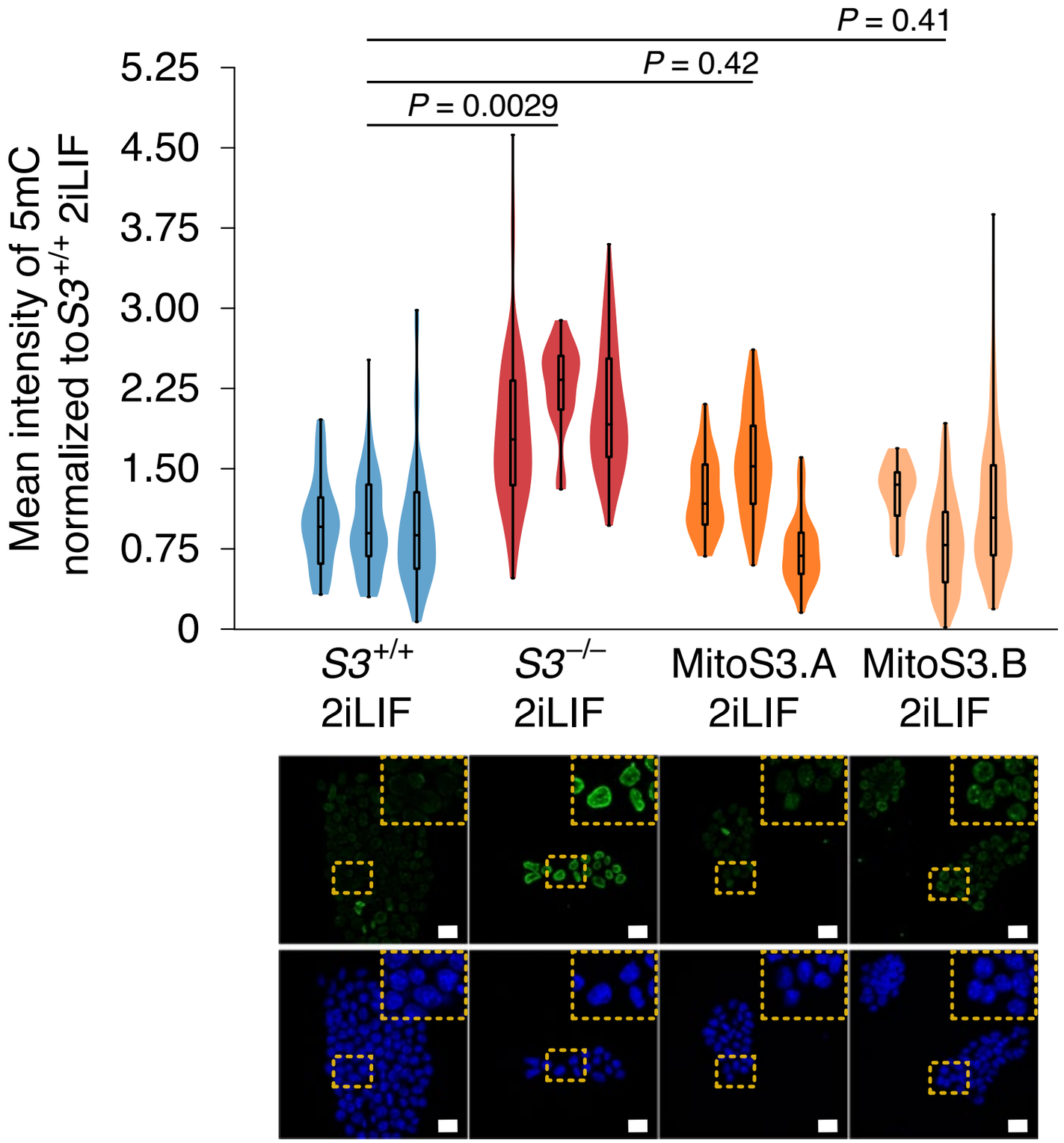


Nuclear STAT3 does not impact DNA methylation

Betto et al, ***Nat Genet****, 2021*



Betto et al, ***Nat Genet****, 2021*

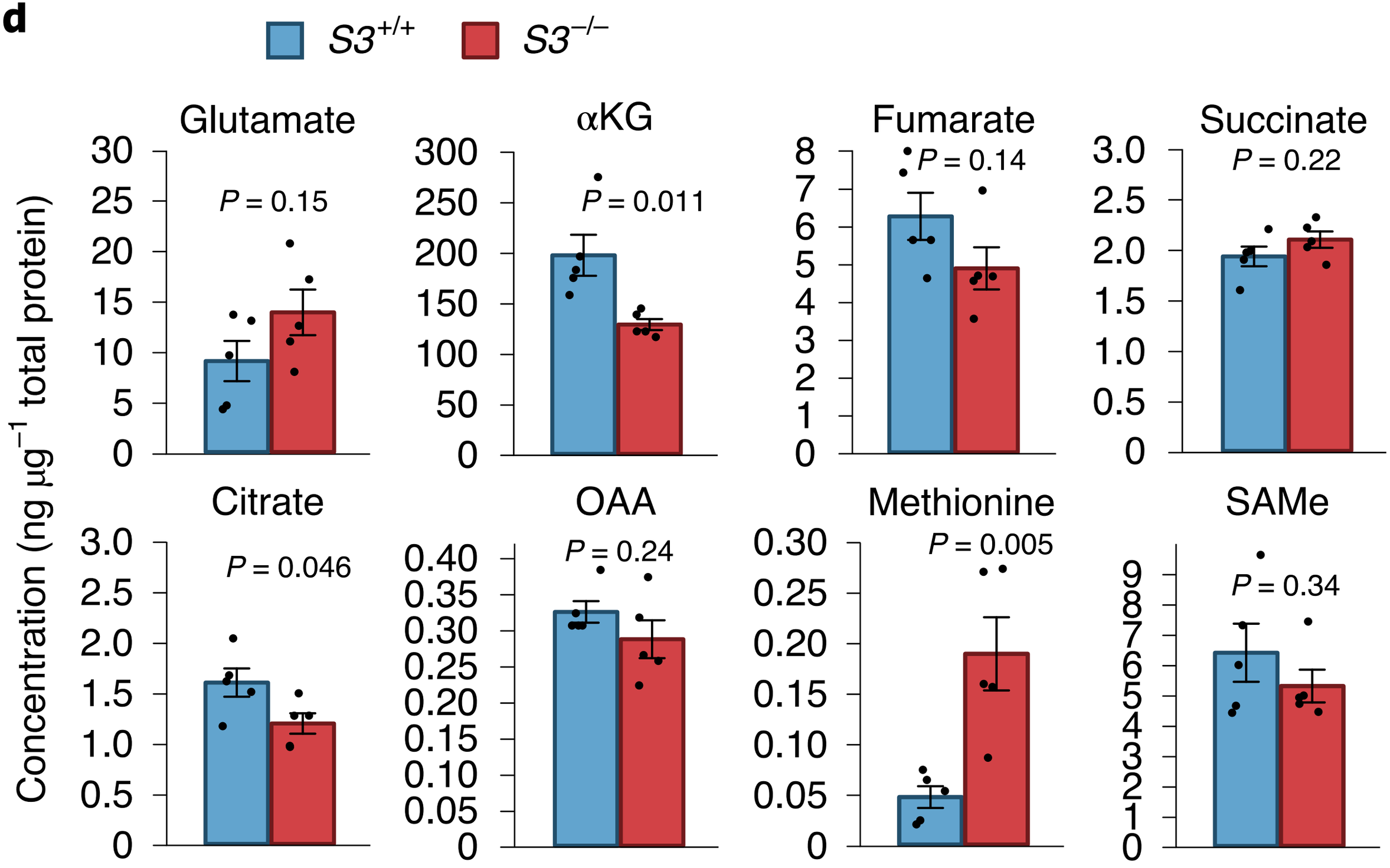


Expression of mitochondrial STAT3 lowers DNA methylation (in S3-/- cells)

Betto et al, ***Nat Genet****, 2021*

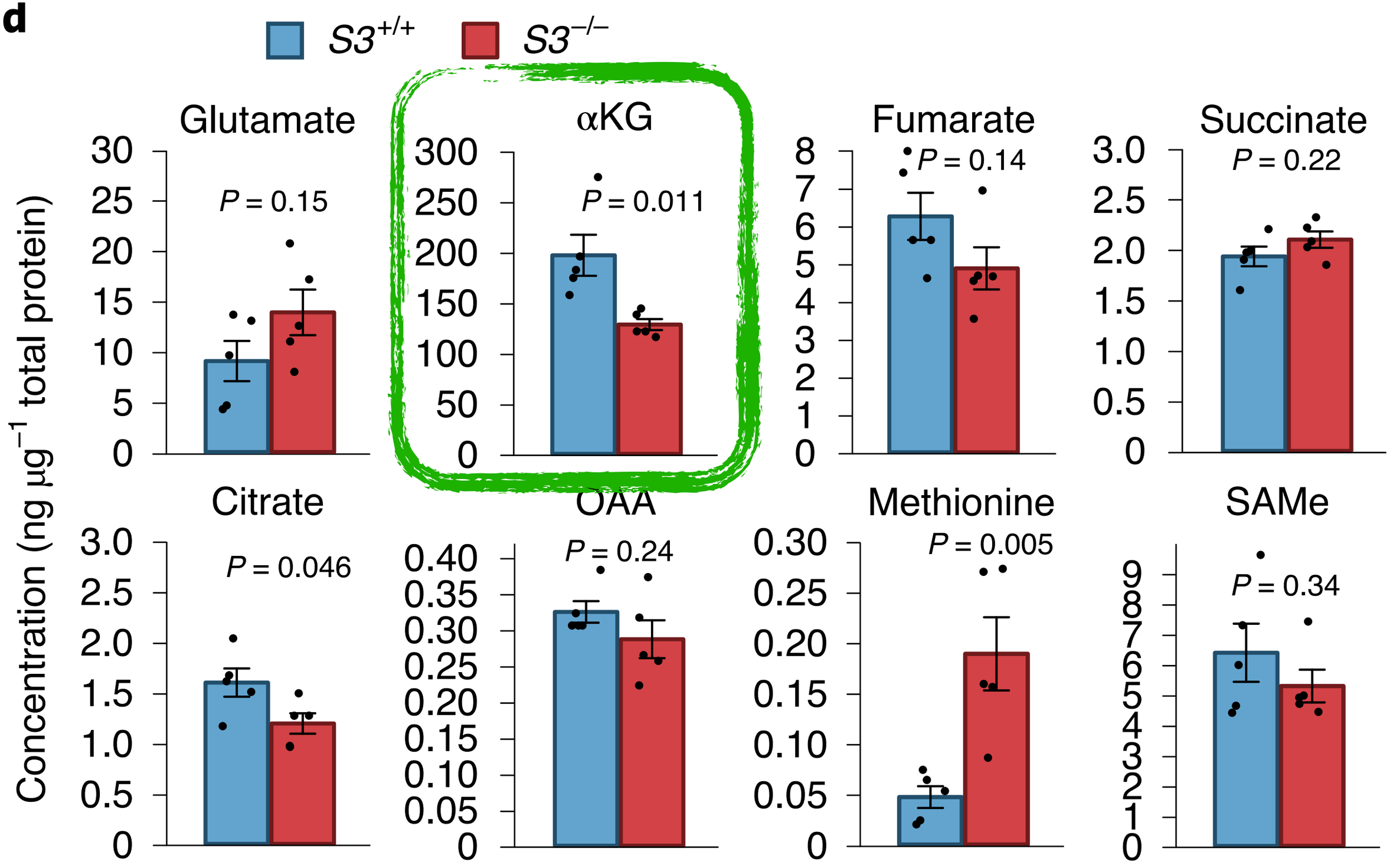
Is STAT3 effect on DNA methylation dependent on metabolic rewiring?

d



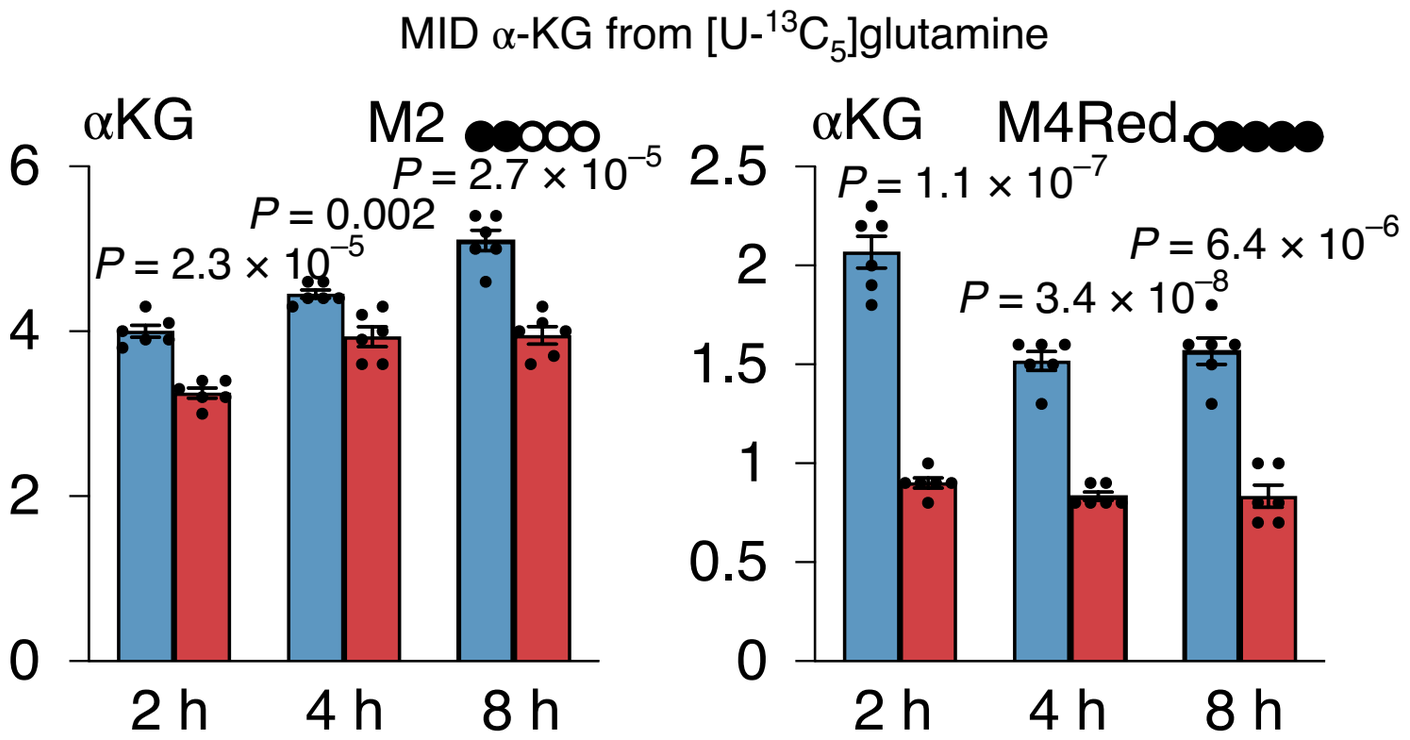
Betto et al, ***Nat Genet****, 2021*

d

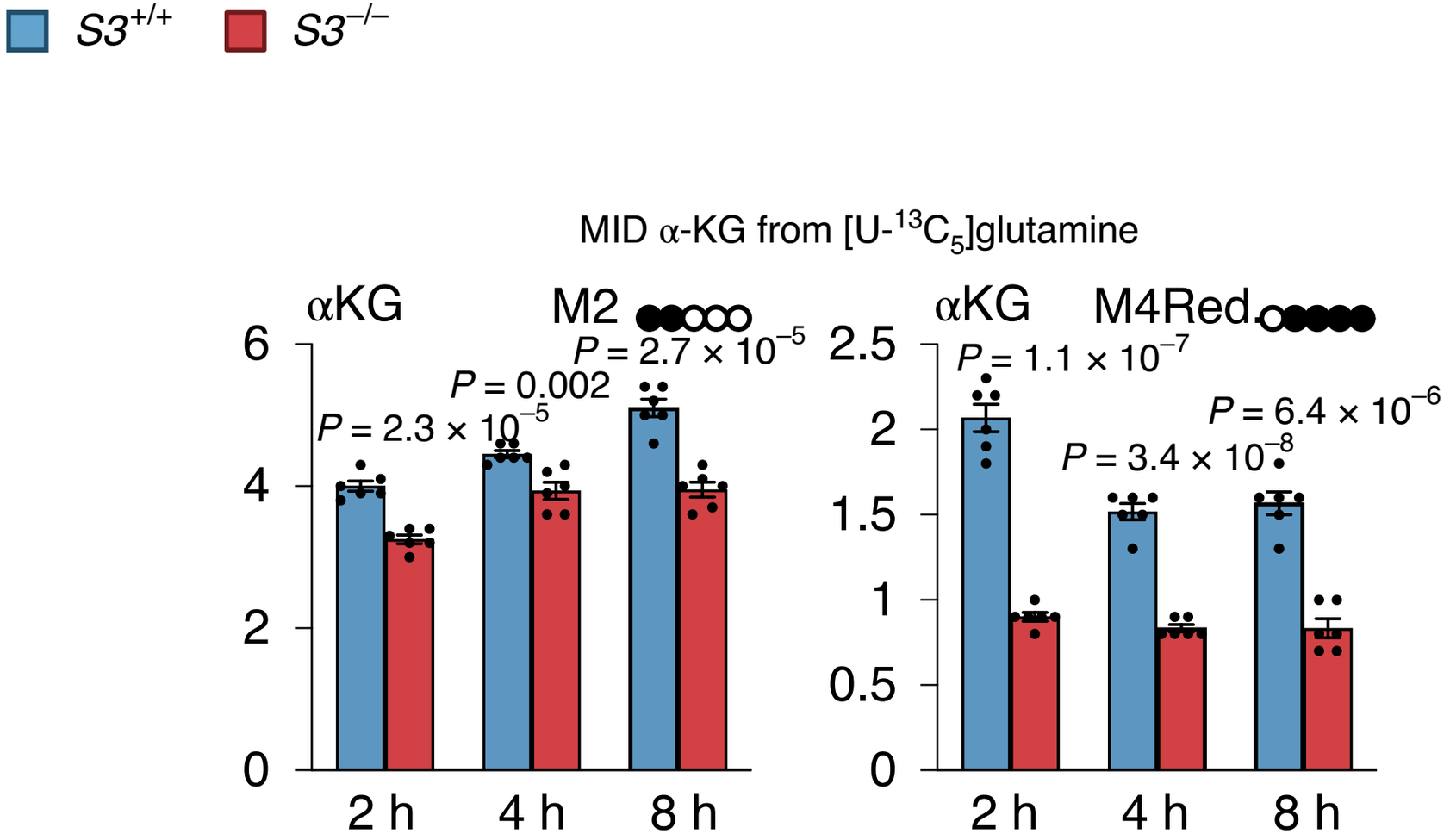


Betto et al, ***Nat Genet****, 2021*

$S3^{+/+}$ $S3^{-/-}$



Betto et al, ***Nat Genet****, 2021*



Expression of STAT3 enhances glutamine-to-aKG conversion

Betto et al, ***Nat Genet****, 2021*

Betto et al, ***Nat Genet****, 2021*

Is that dependent on mitochondrial STAT3?

Betto et al, ***Nat Genet****, 2021*

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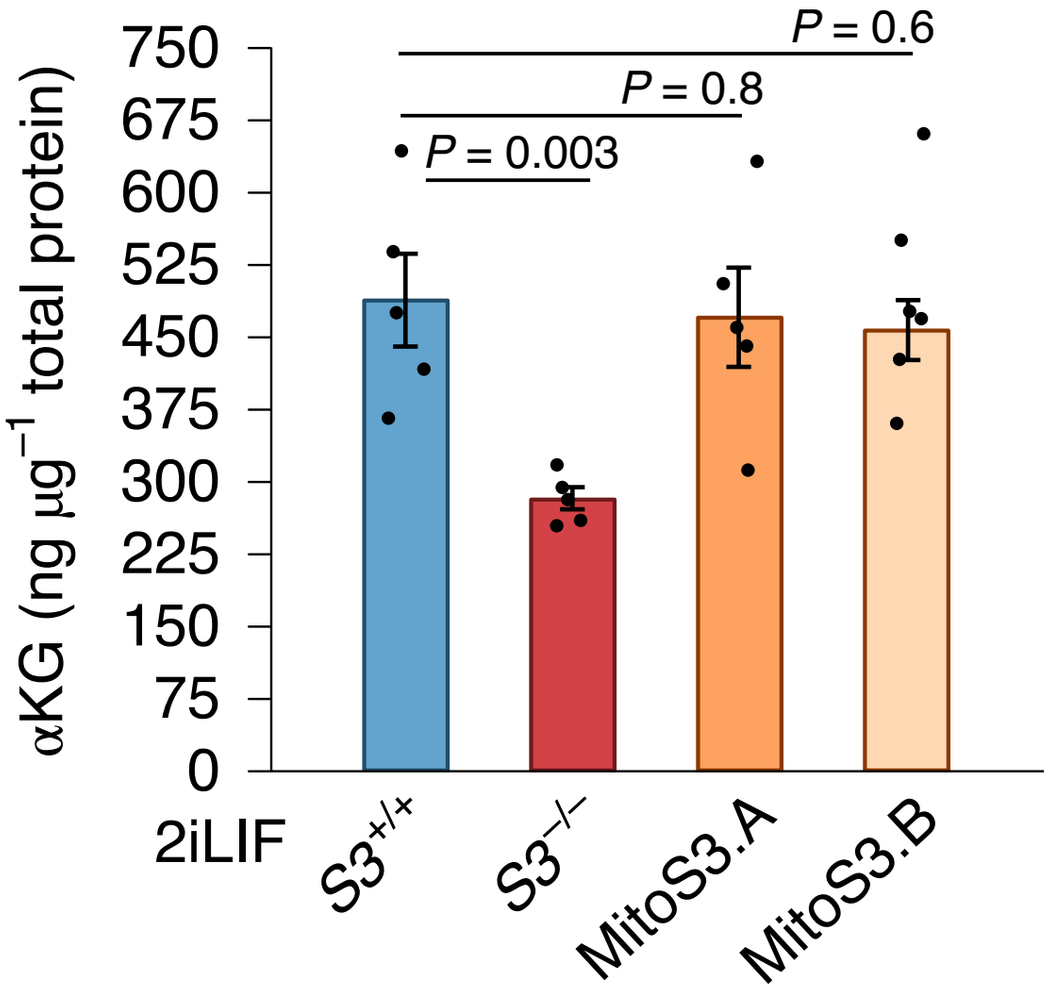
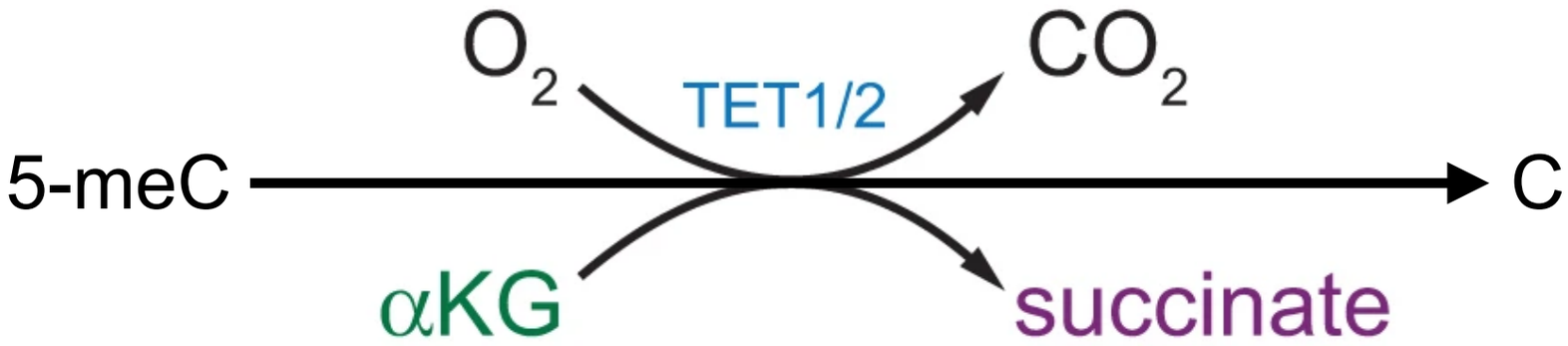
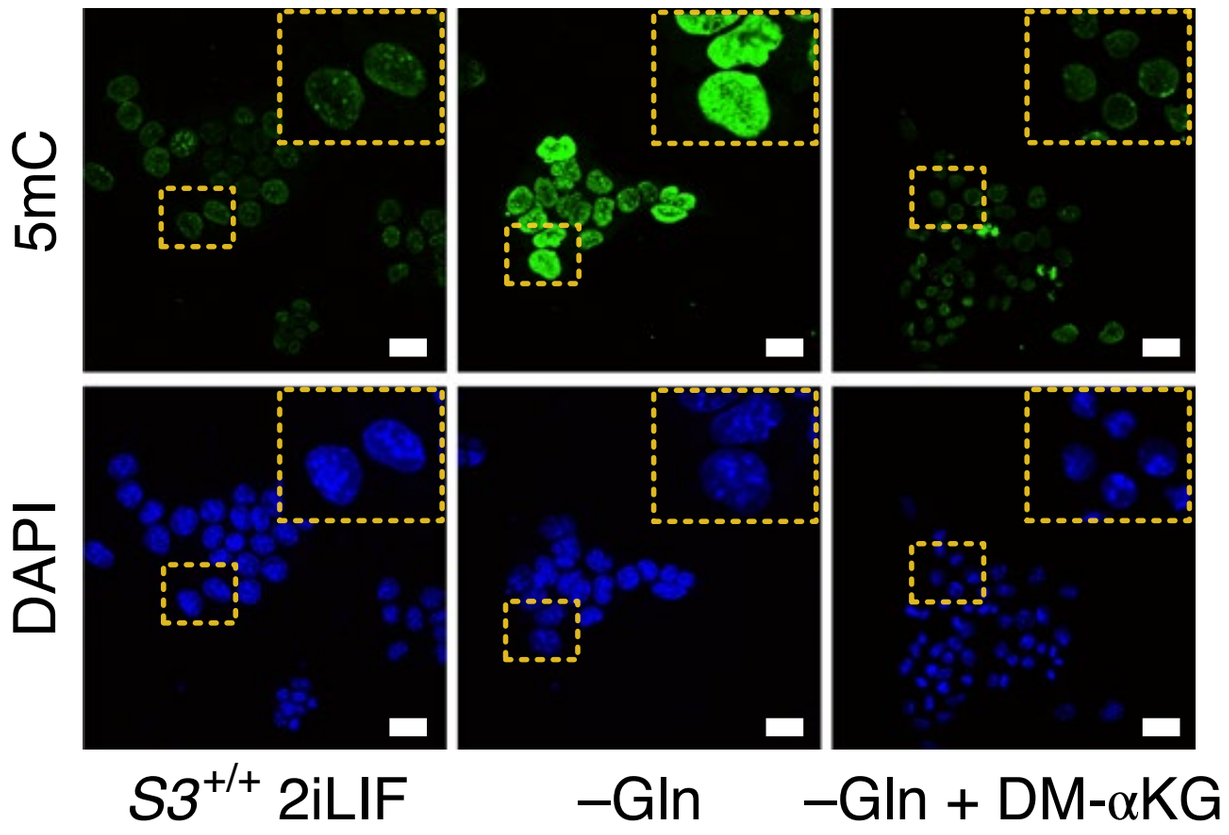


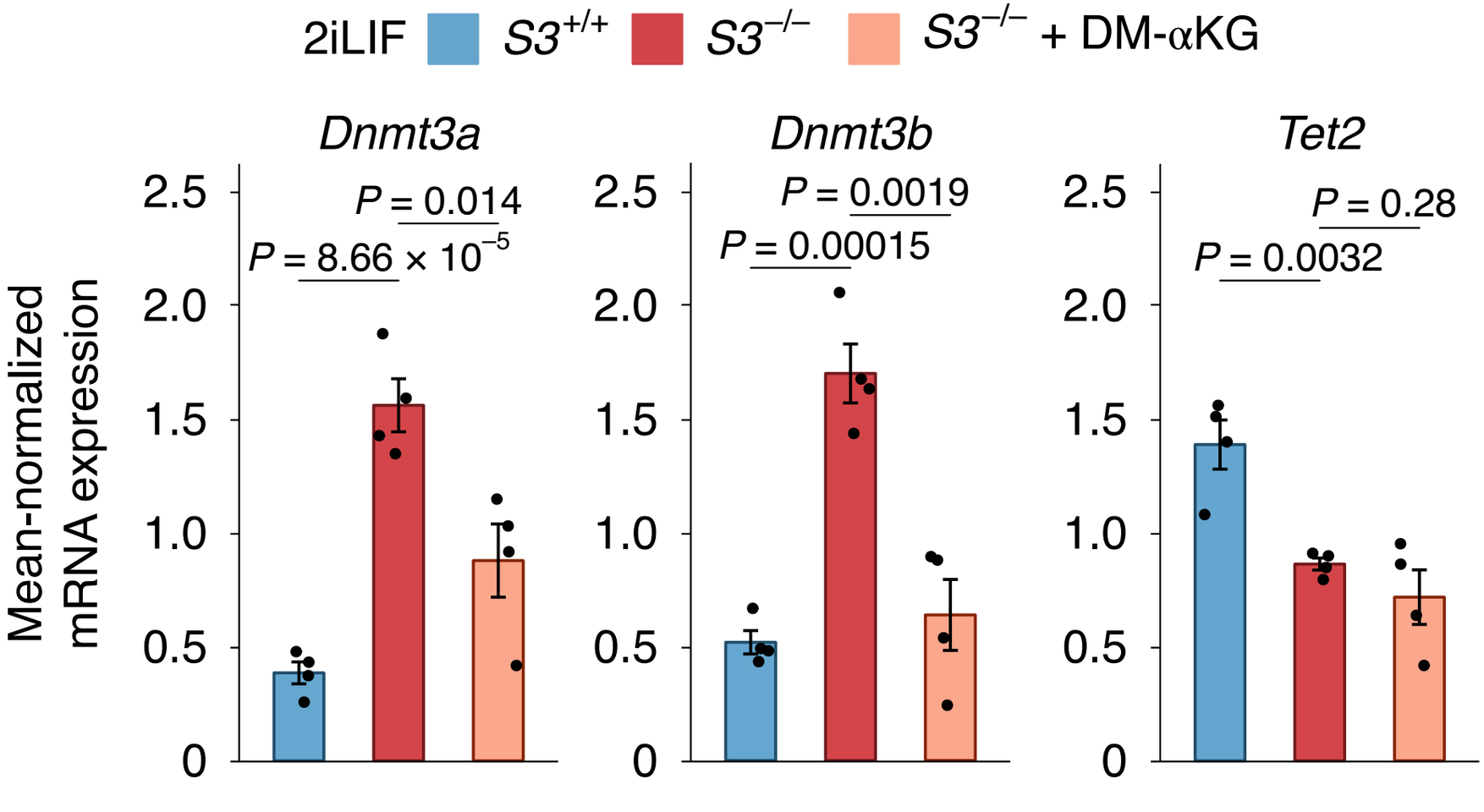
Betto et al, ***Nat Genet****, 2021*

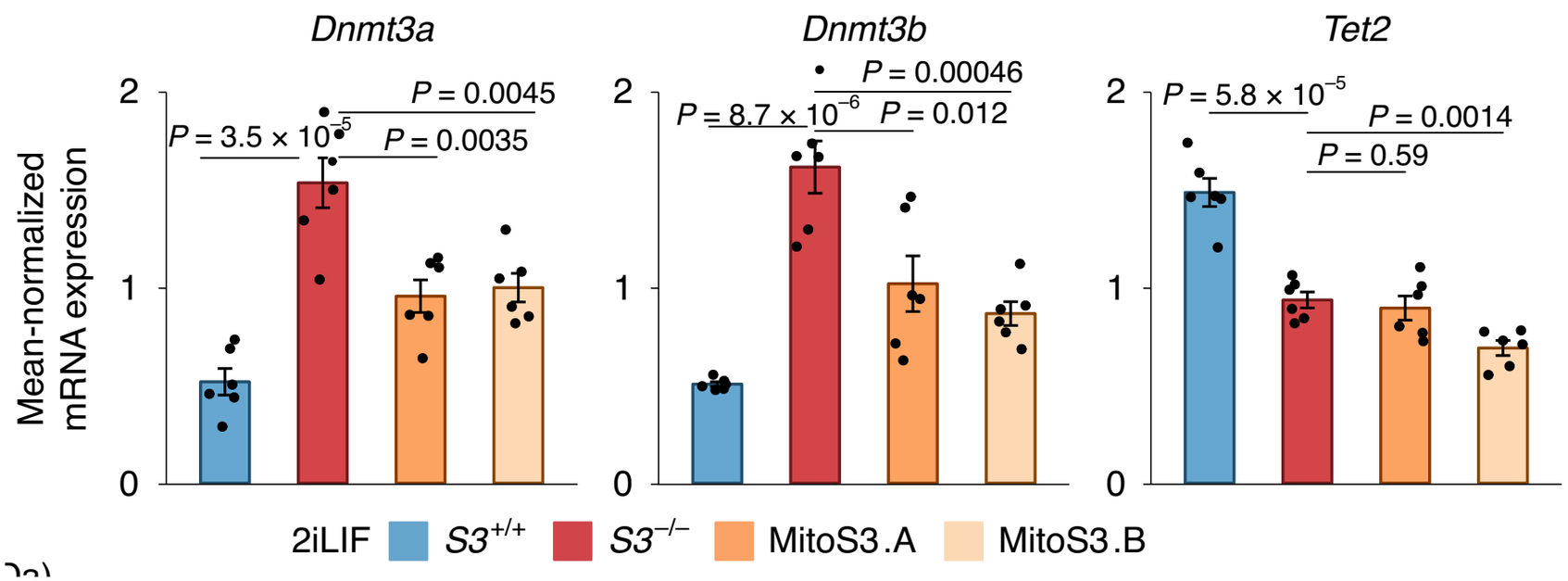
Is STAT3 effect on DNA methylation dependent on metabolic rewiring?





Betto et al, ***Nat Genet****, 2021*

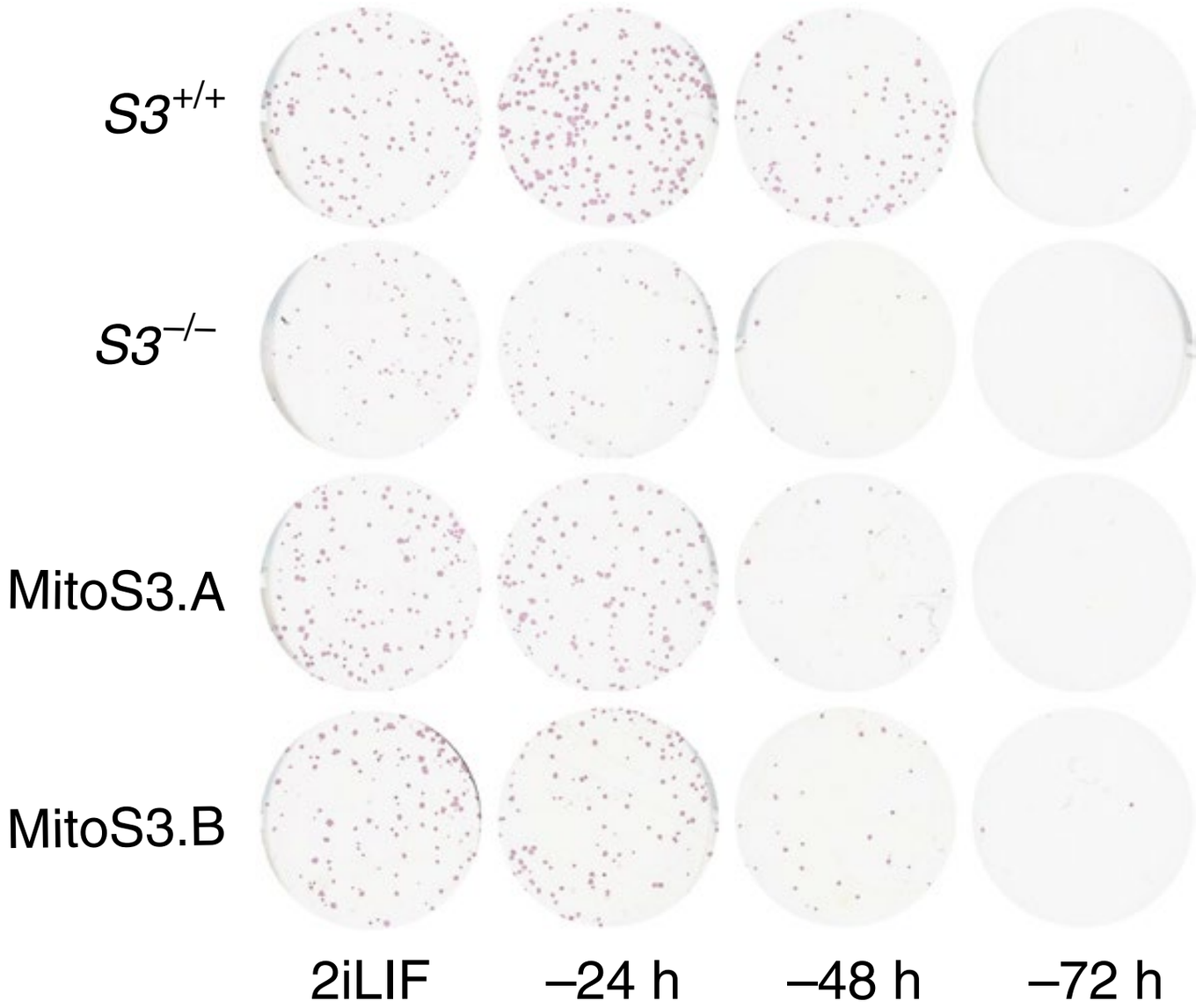




Betto et al, **Nat Genet**, 2021

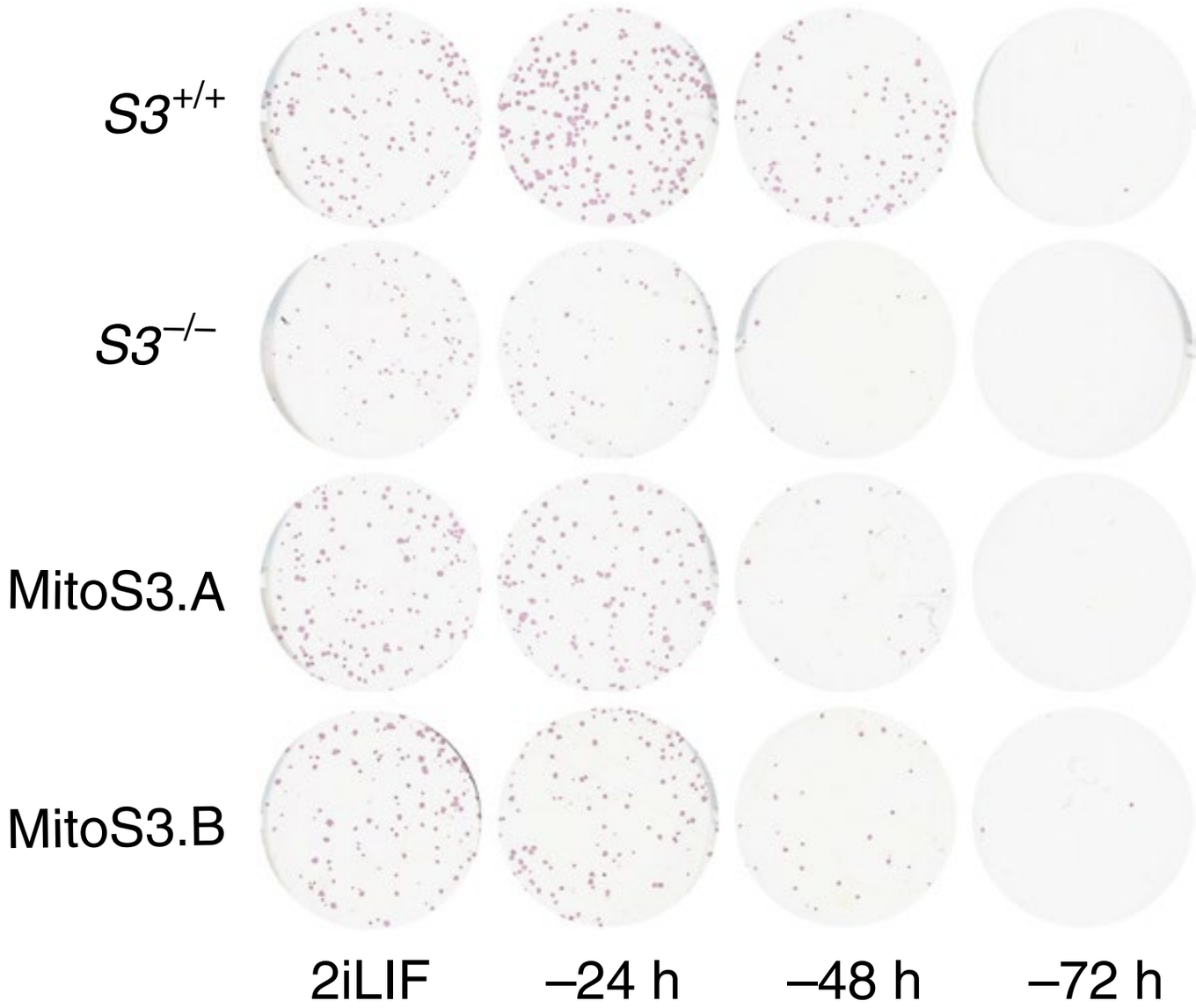
What is the impact of mitoSTAT3 (aKG) on ESC differentiation?

LIF-Stat3 axis stabilize pluripotency and/or slow down differentiation



Betto et al, ***Nat Genet****, 2021*

LIF-Stat3 axis stabilize pluripotency and/or slow down differentiation



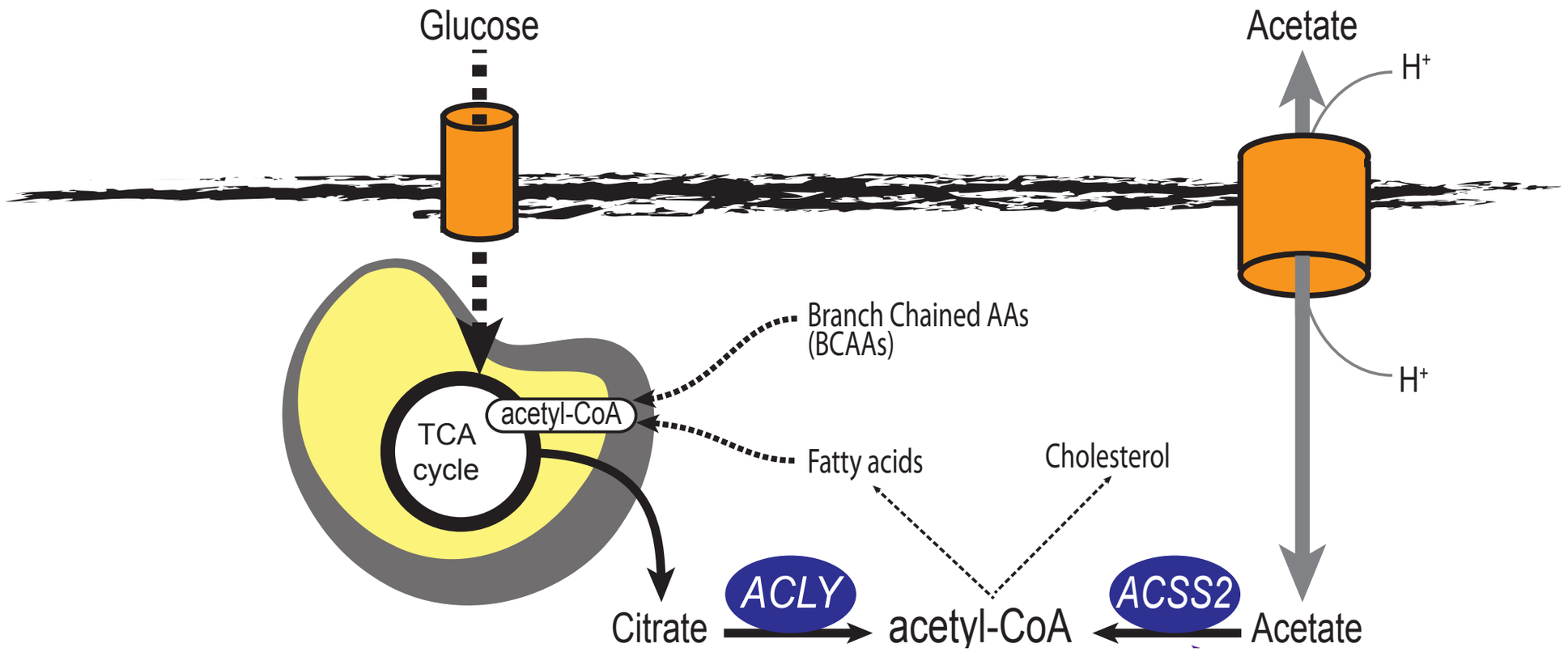
Alkaline Phosphates (AP) positive clones

Betto et al, ***Nat Genet****, 2021*

Together:

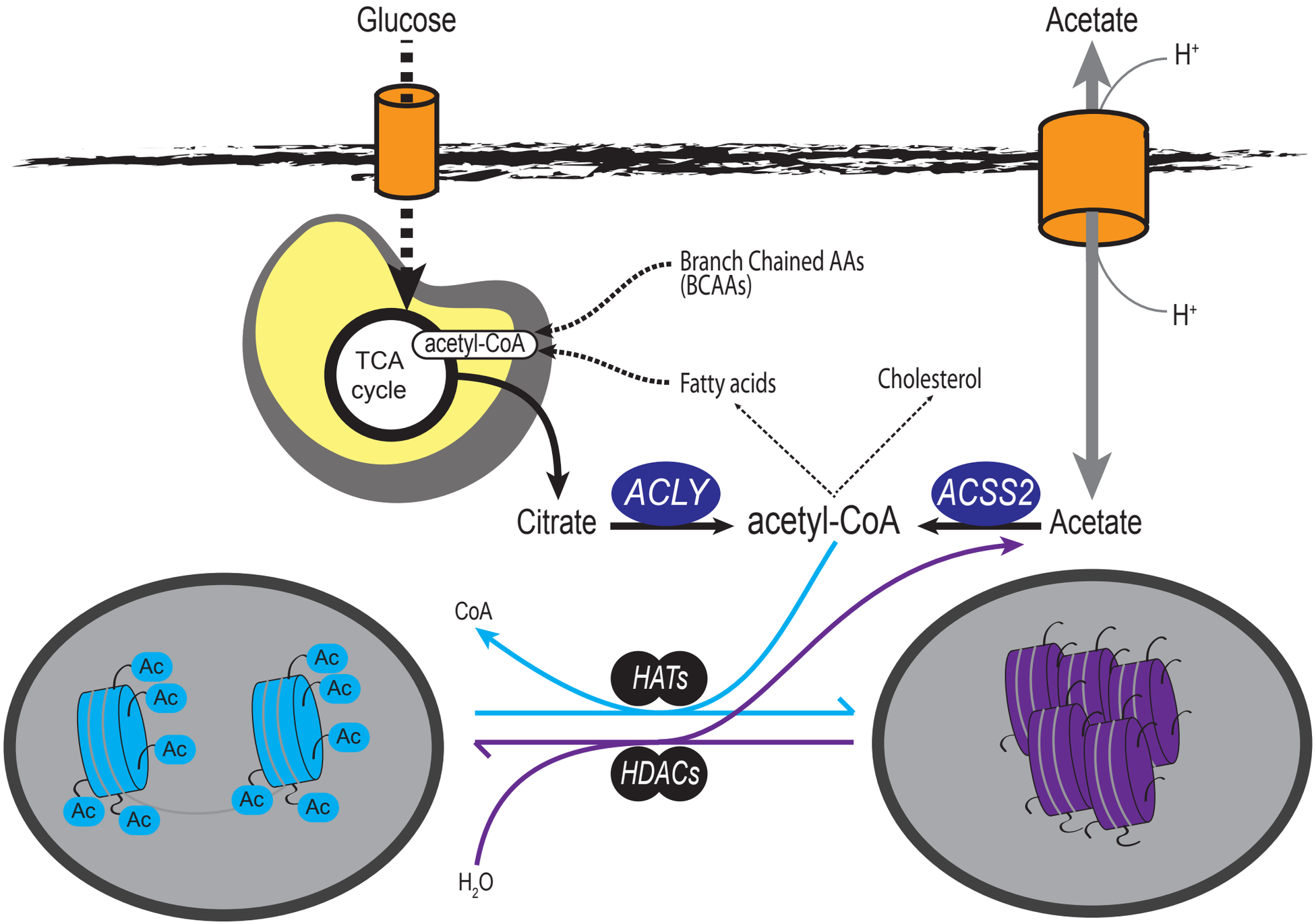
- Signaling events maintain stemness through metabolic rewiring
- Key is elevation of aKG in naive cells
- Increased aKG promotes global levels of hypomethylation (DNA + histones)
- aKG promotes the removal of repressive markers and expression of pluripotency genes

Acetyl-CoA levels are in equilibrium with histone acetylation



Carrer & Wellen, Curr Opin Biotechnol, 2015

Acetyl-CoA levels are in equilibrium with histone acetylation

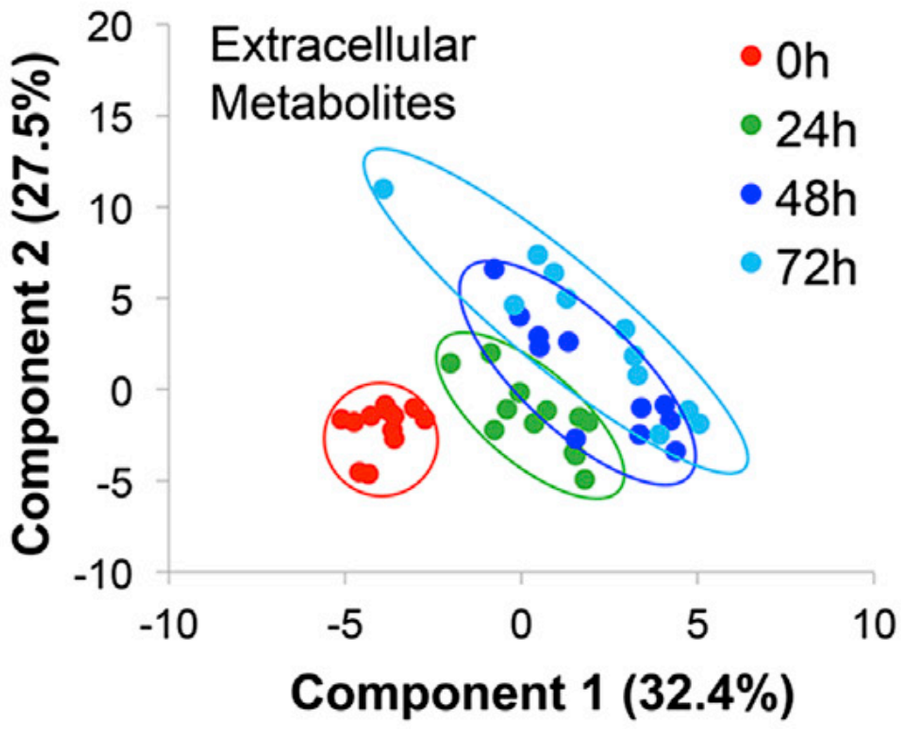


Carrer & Wellen, Curr Opin Biotechnol, 2015

Glycolysis-Mediated Changes in Acetyl-CoA and Histone Acetylation Control the Early Differentiation of Embryonic Stem Cells

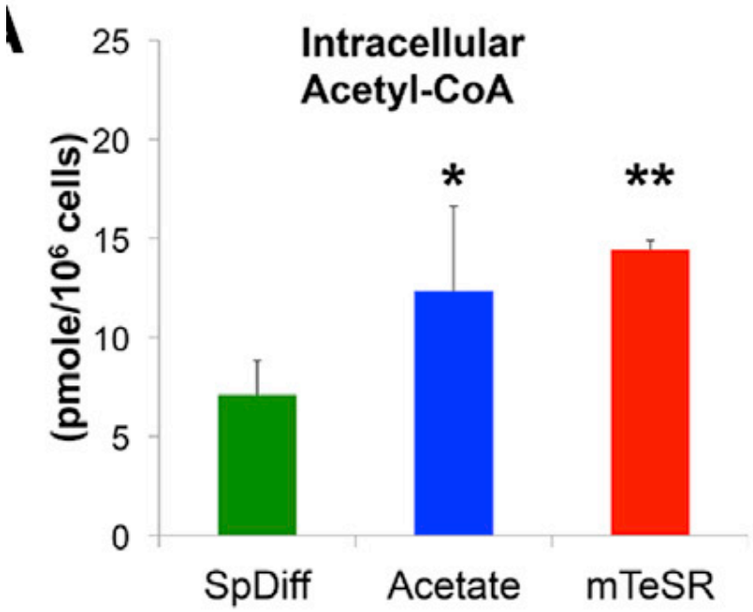
Arieh Moussaieff,[1,2,3,4,5,*] Matthieu Rouleau,[6,7] Daniel Kitsberg,[1,2] Merav Cohen,[1,2] Gahl Levy,[1] Dinorah Barasch,[5] Alina Nemirovski,[5] Shai Shen-Orr,[8] Ilana Laevsky,[8] Michal Amit,[8] David Bomze,[1,2] Bénédicte Elena-Herrmann,[9] Tali Scherf,[10] Malka Nissim-Rafinia,[2,11] Stefan Kempa,[12] Joseph Itskovitz-Eldor,[8] Eran Meshorer,[2,11] Daniel Aberdam,[3,4,13] and Yaakov Nahmias[1,2,13,*]

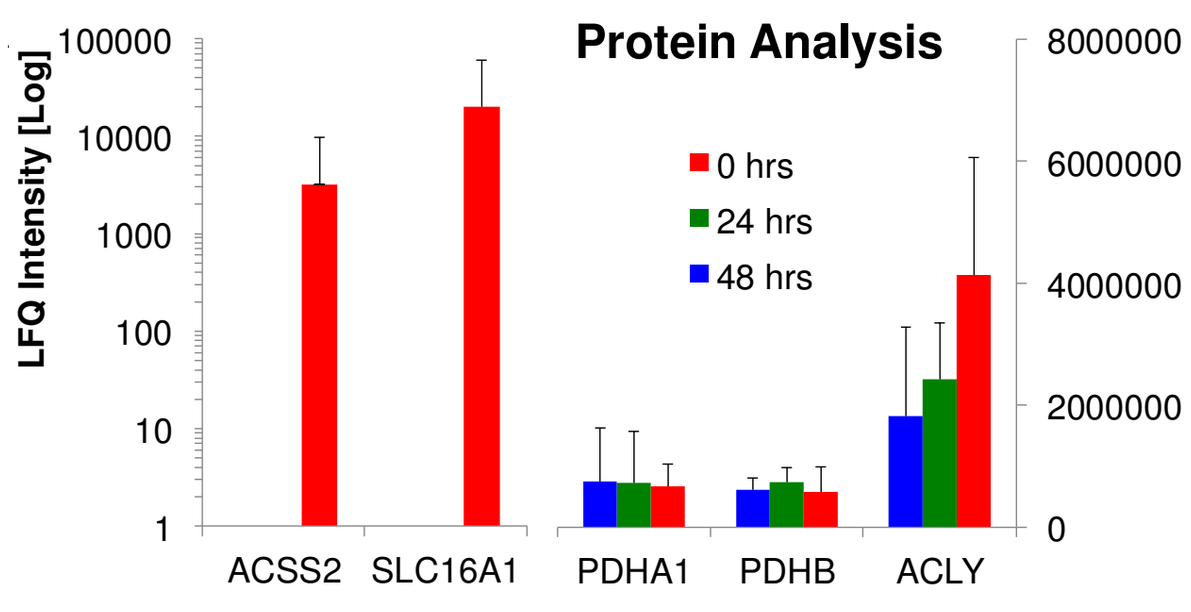
What are the metabolic changes that characterize exit from pluripotency?



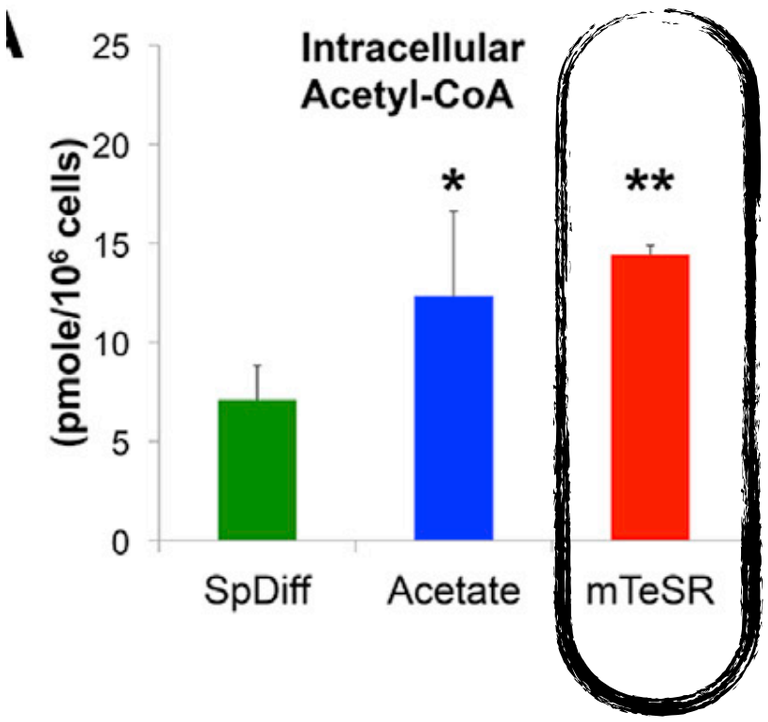
rFGF REMOVAL

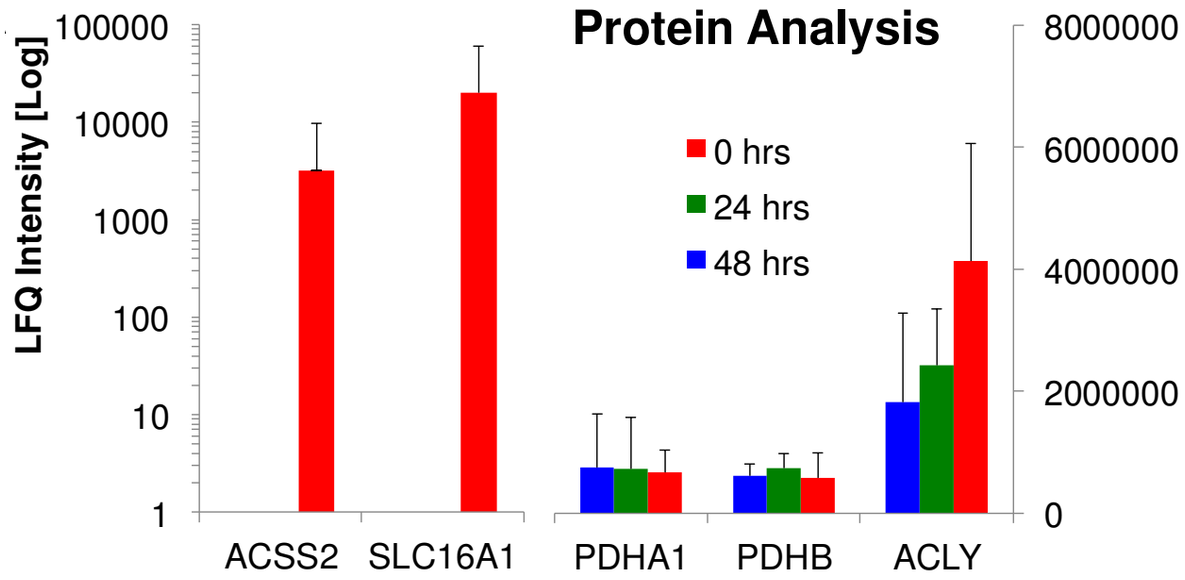
Moussaieff et al, ***Cell Metab****, 2015*



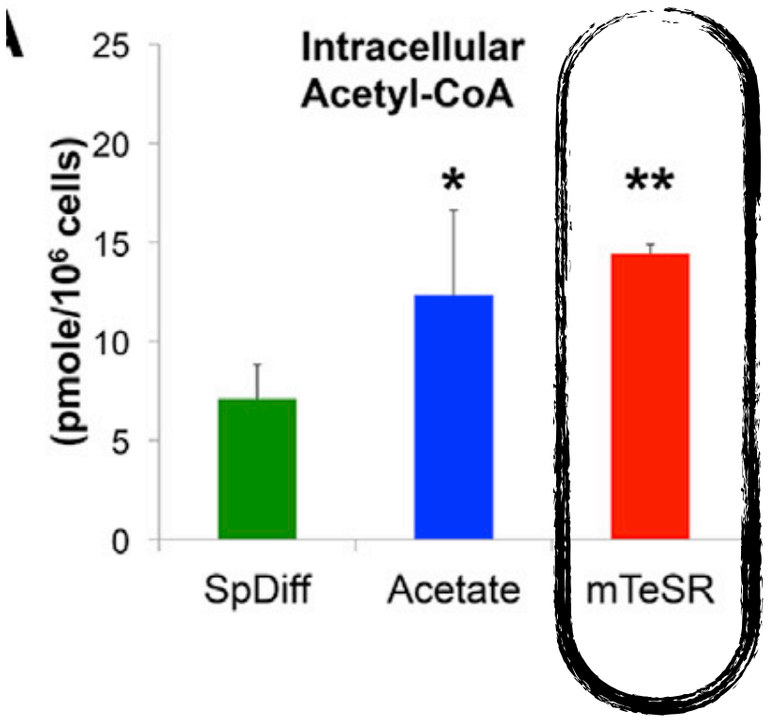


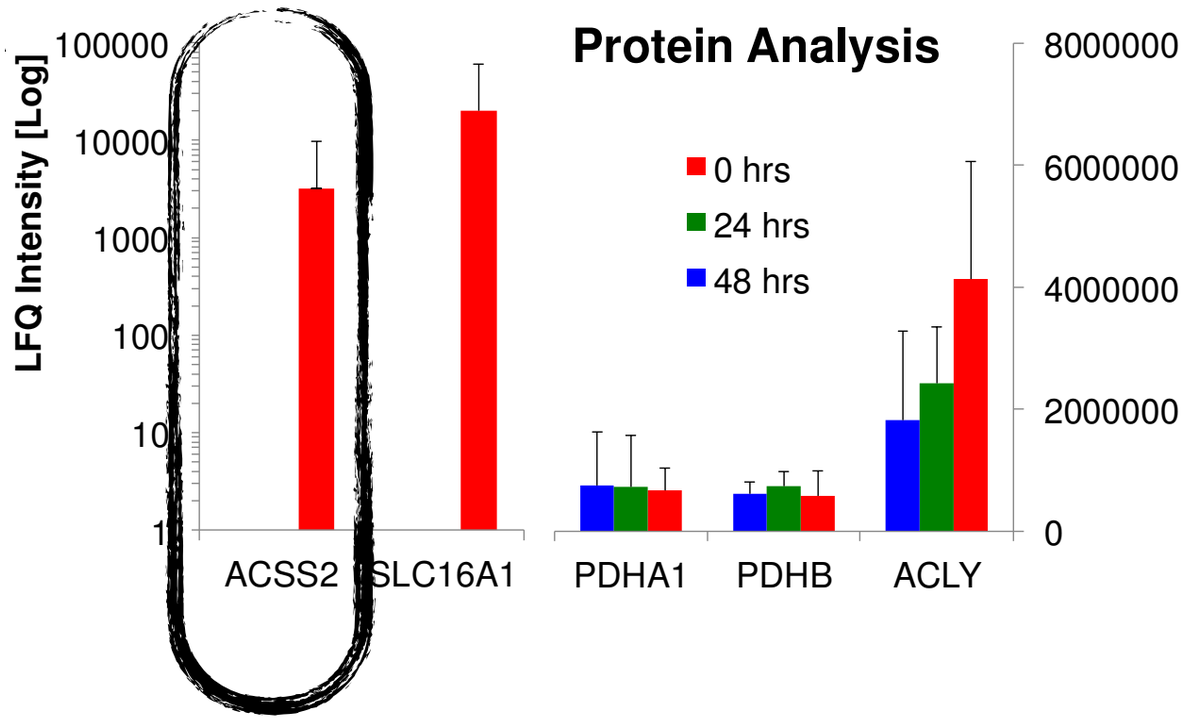
Moussaieff et al, ***Cell Metab****, 2015*



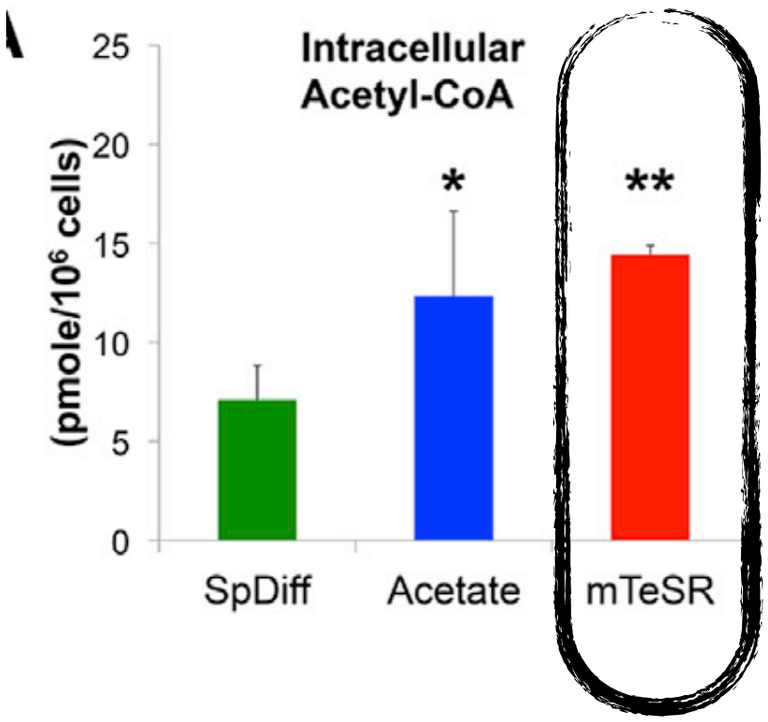


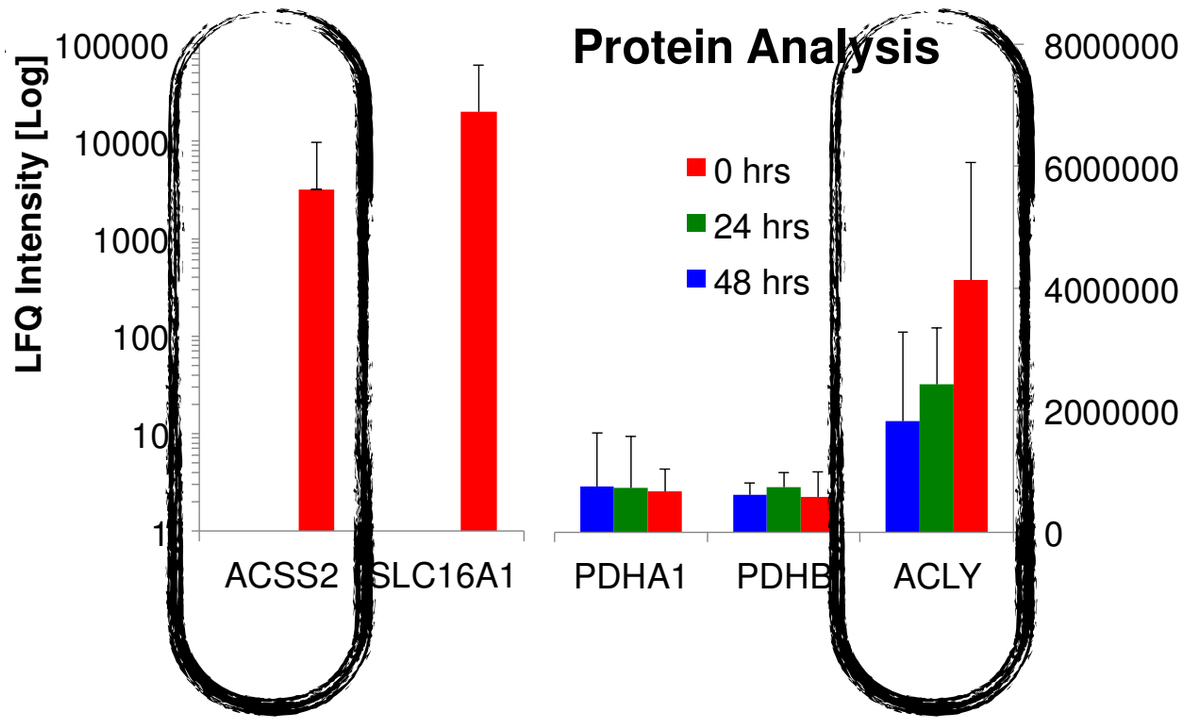
Moussaieff et al, ***Cell Metab****, 2015*



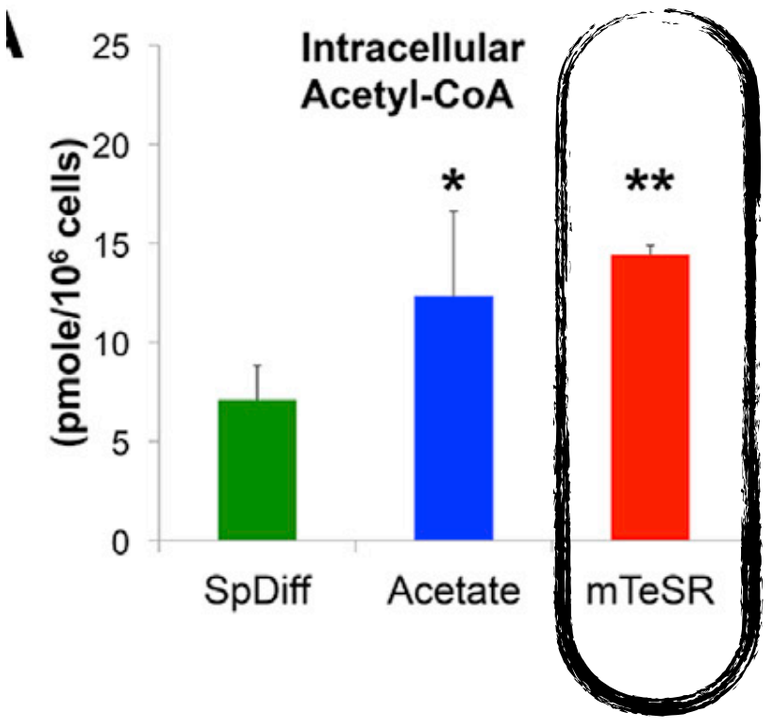


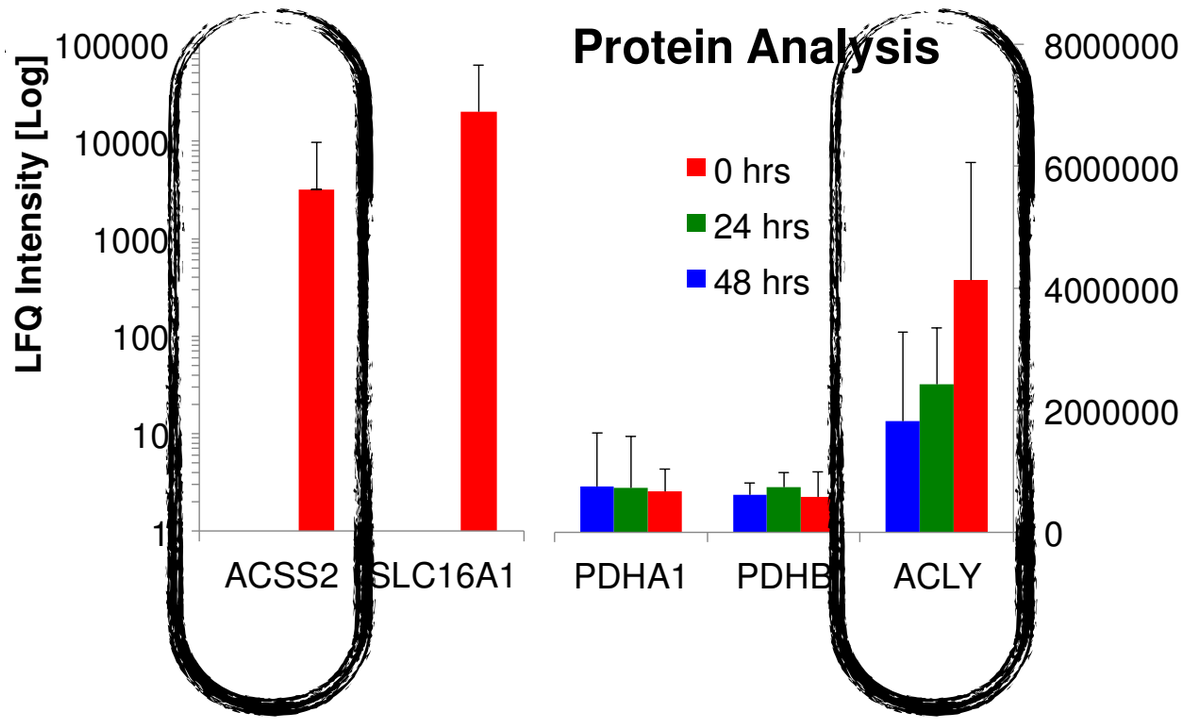
Moussaieff et al, ***Cell Metab****, 2015*





Moussaieff et al, ***Cell Metab****, 2015*



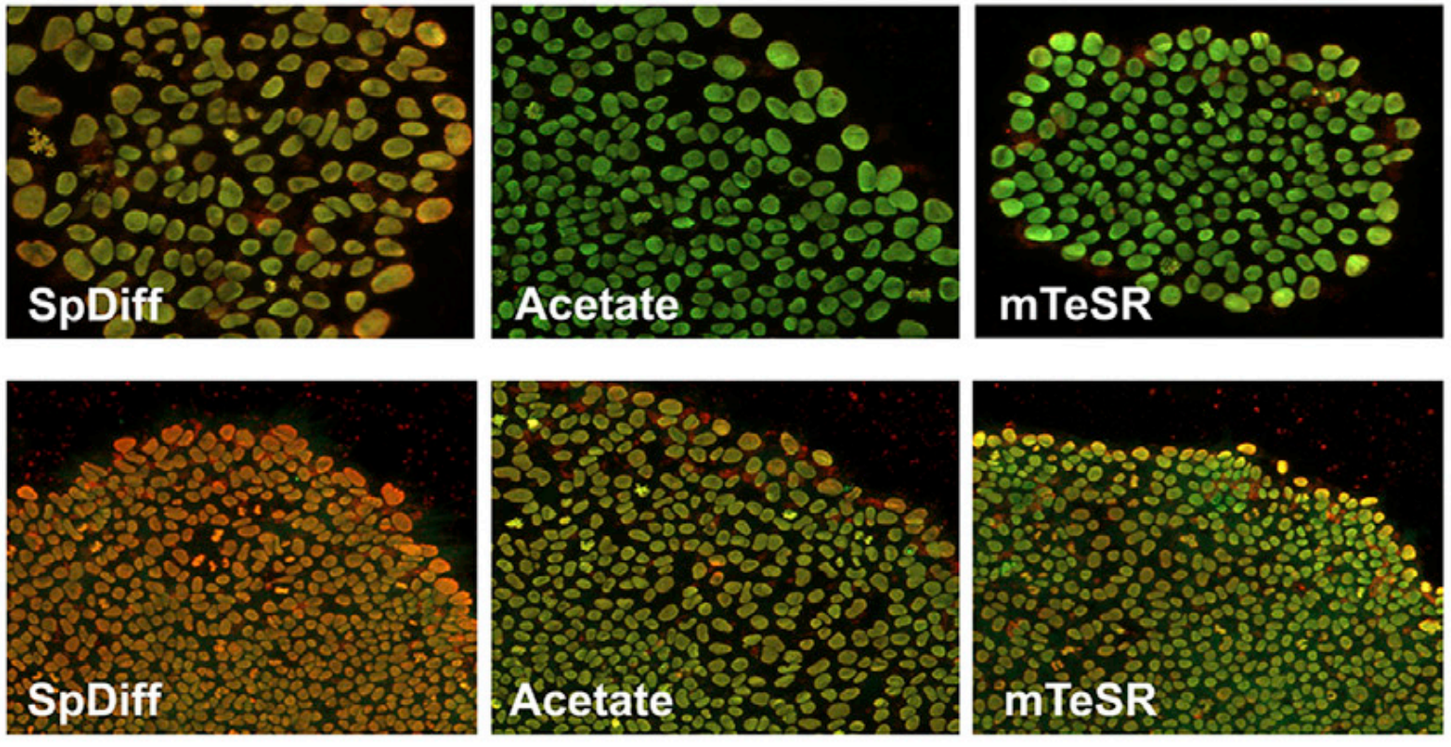


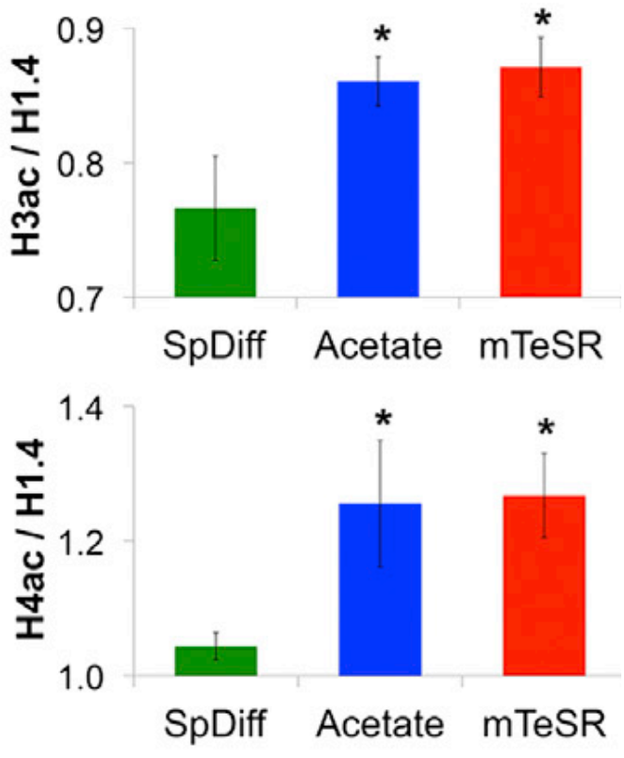
Acetyl-CoA production is higher in pluripotent naive cells (and rapidly drops upon initiation)

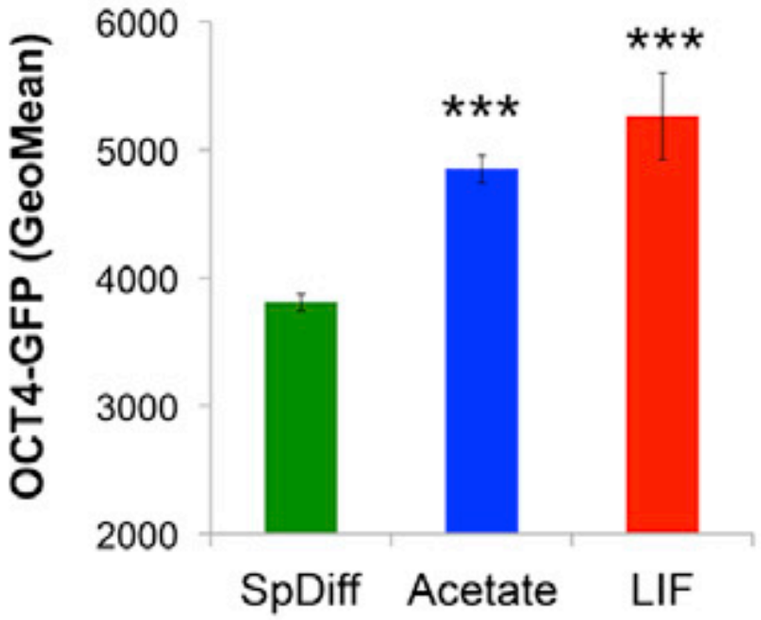
Moussaieff et al, ***Cell Metab****, 2015*

What is the impact of acetyl-CoA rewiring for the epigenome?

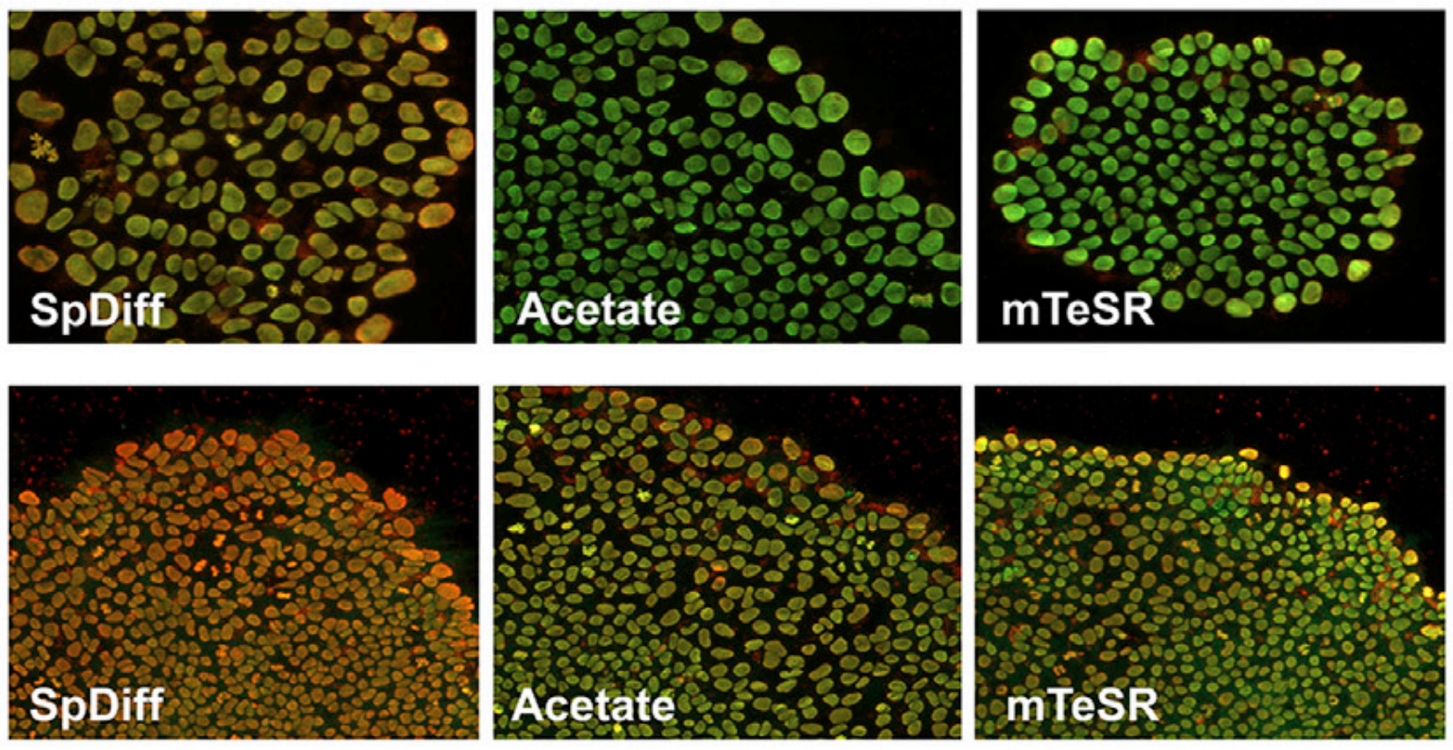
And for pluripotency?

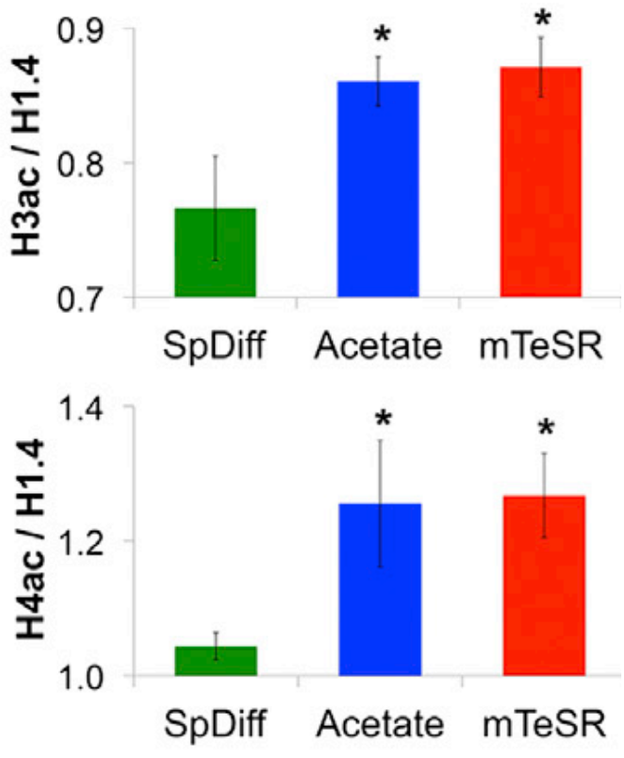


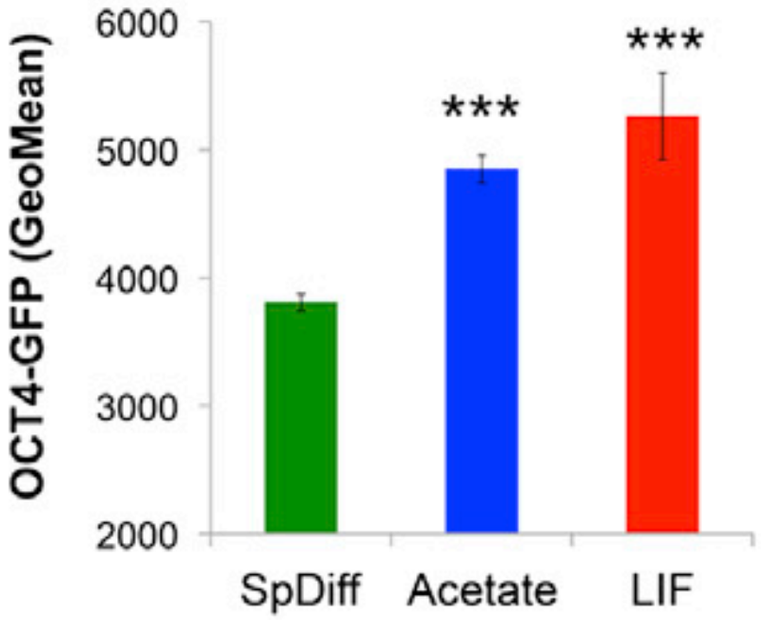




Moussaieff et al, ***Cell Metab****, 2015*



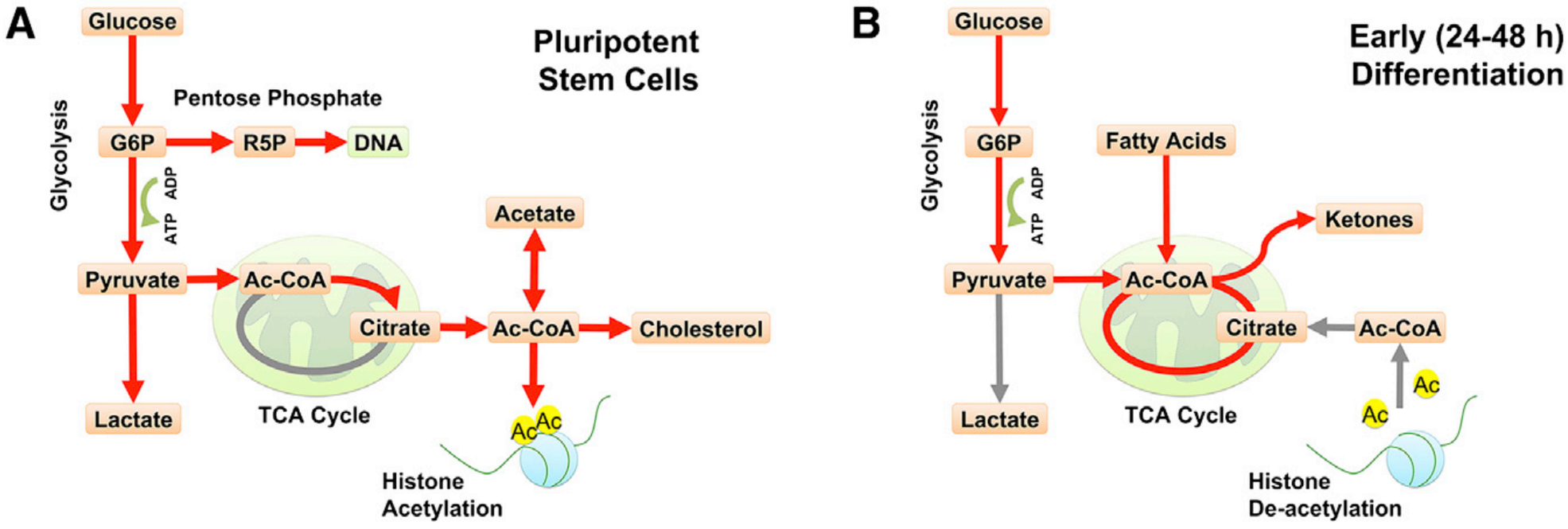




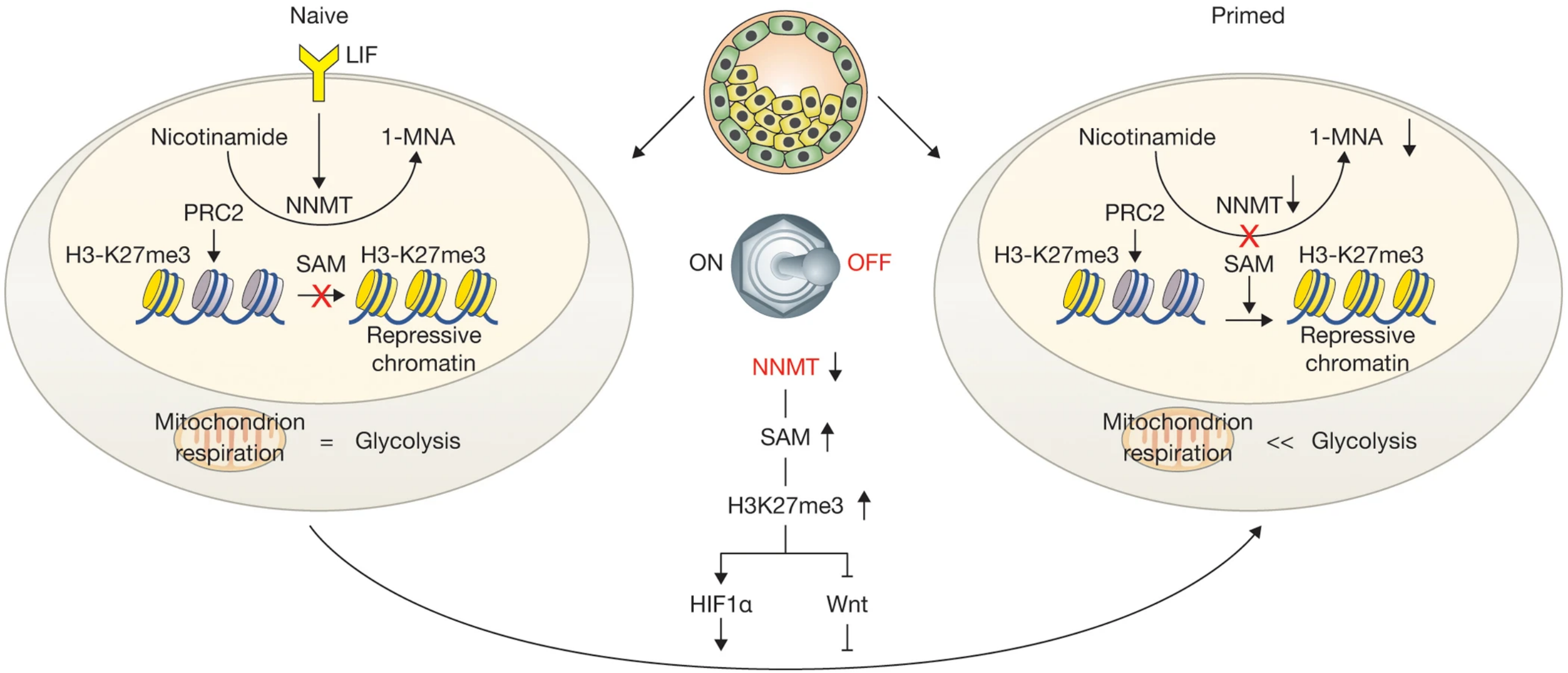
Decline in acetyl-CoA abundance is associated with decrease of global levels of histone acetylation.

Acetate/Acetyl-CoA supplementation induces pluripotency genes.

Moussaieff et al, ***Cell Metab****, 2015*

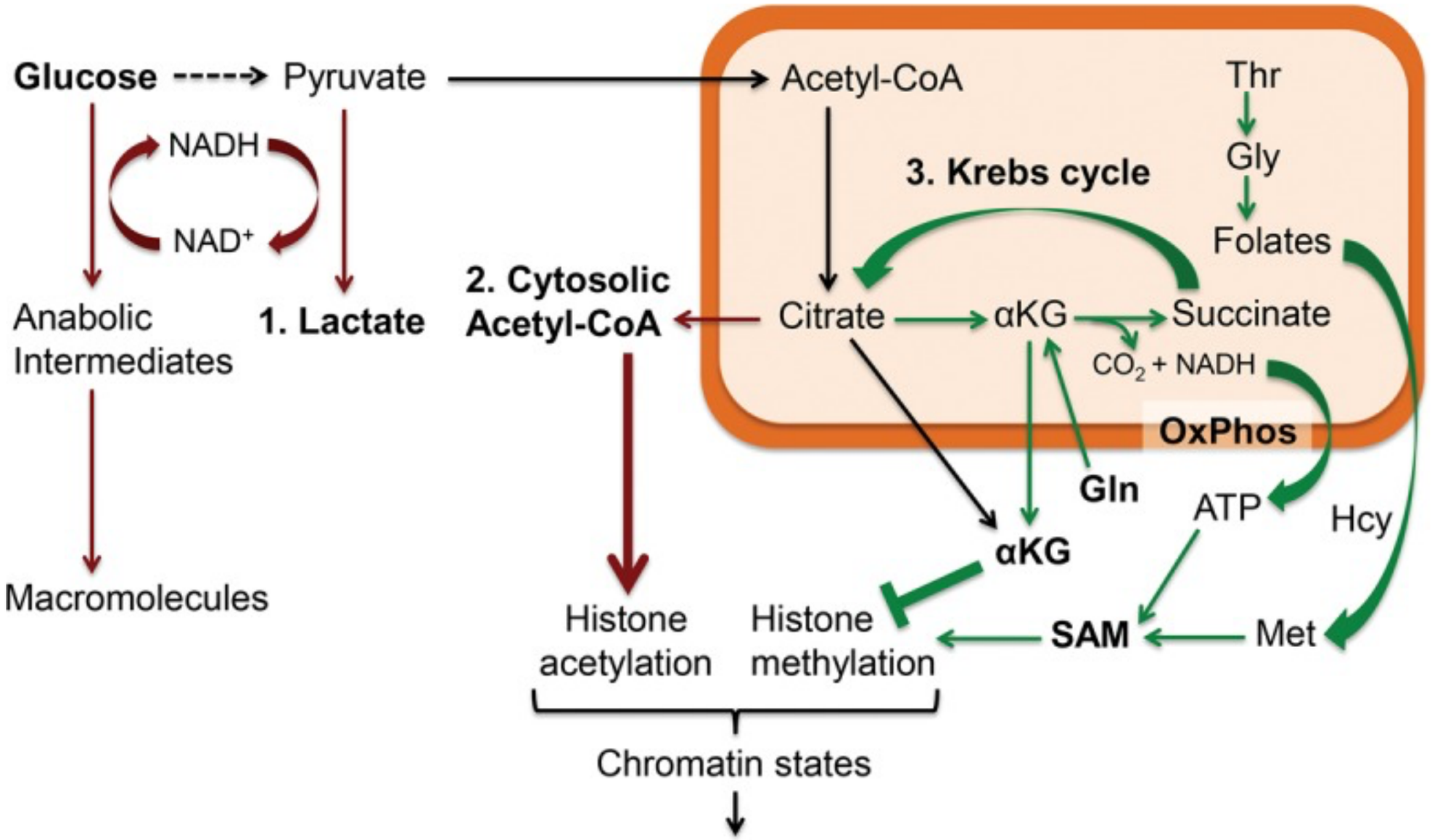


Moussaieff et al, ***Cell Metab****, 2015*



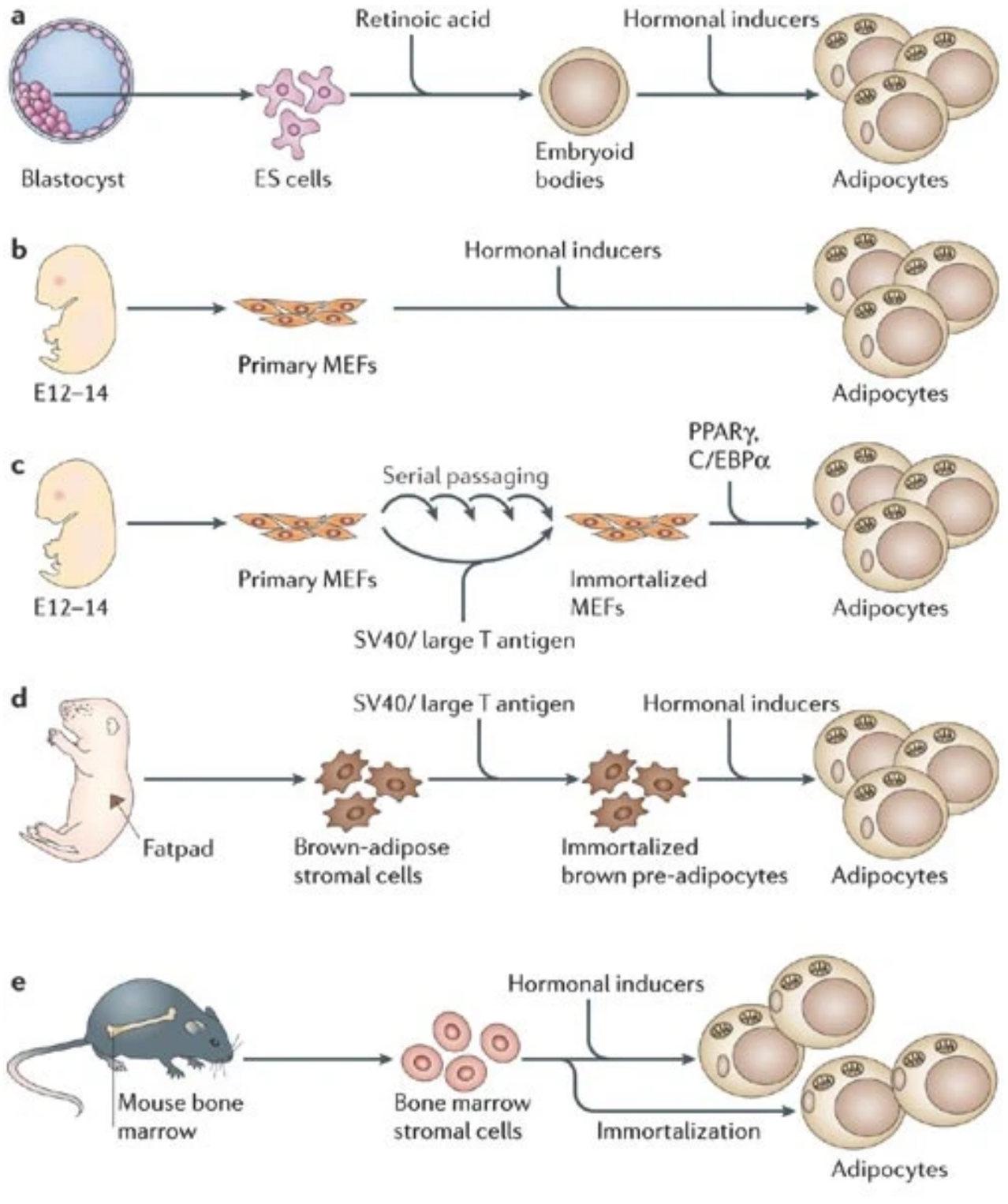
*Sperber et al, **NCB**, 2015*

Together:

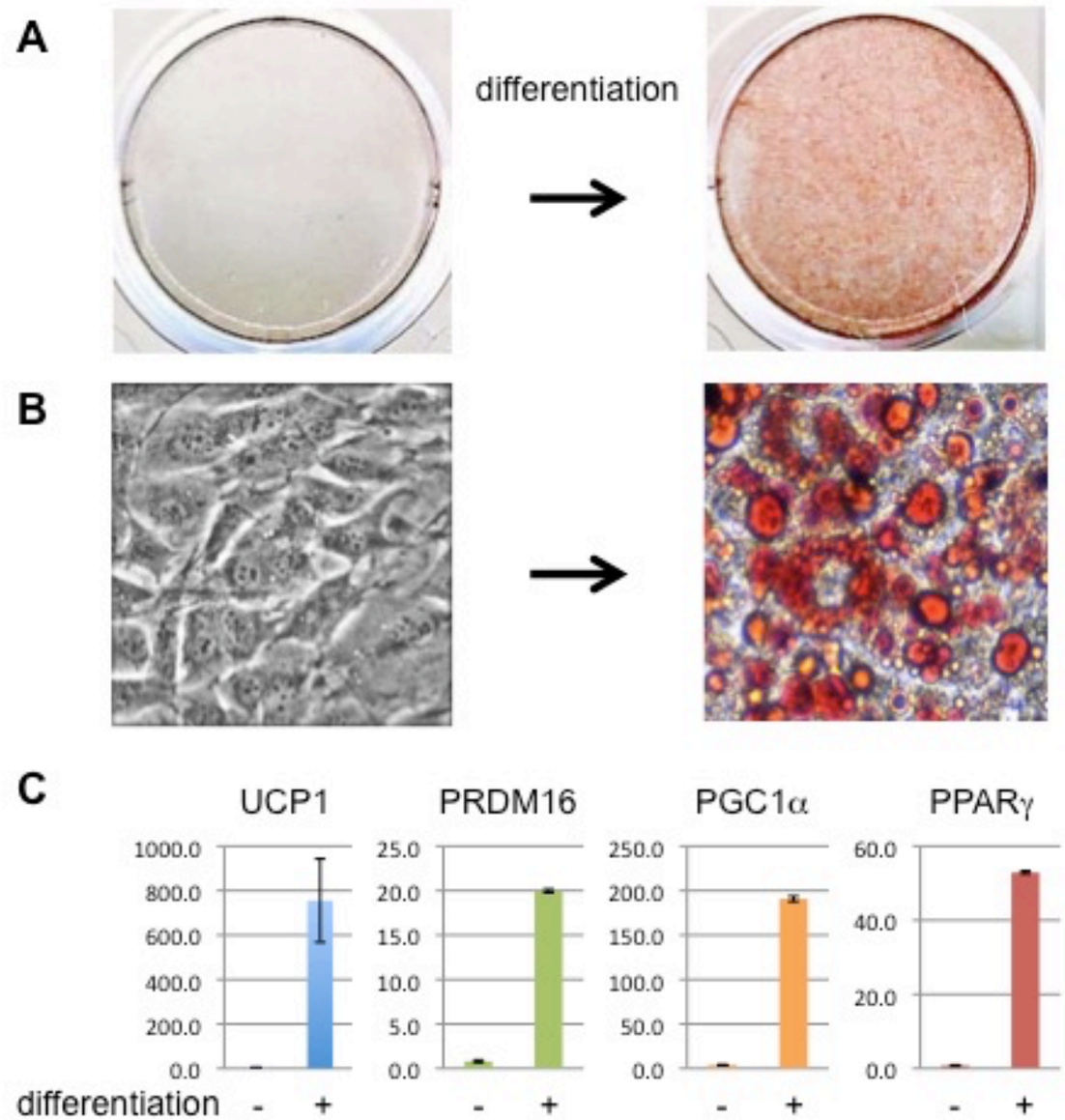

Glucose
Pyruvate
NADH
NAD+
Anabolic Intermediates
Macromolecules
1. Lactate
2. Cytosolic Acetyl-CoA
Acetyl-CoA
3. Krebs cycle
Citrate
αKG
Succinate
CO2 + NADH
OxPhos
Thr
Gly
Folates
Gln
ATP
Hcy
αKG
SAM
Met
Histone acetylation
Histone methylation
Chromatin states
Regulation of pluripotent states

Role of metabolism-epigenetic connection for adult cell differentation

Adipocyte differentiation

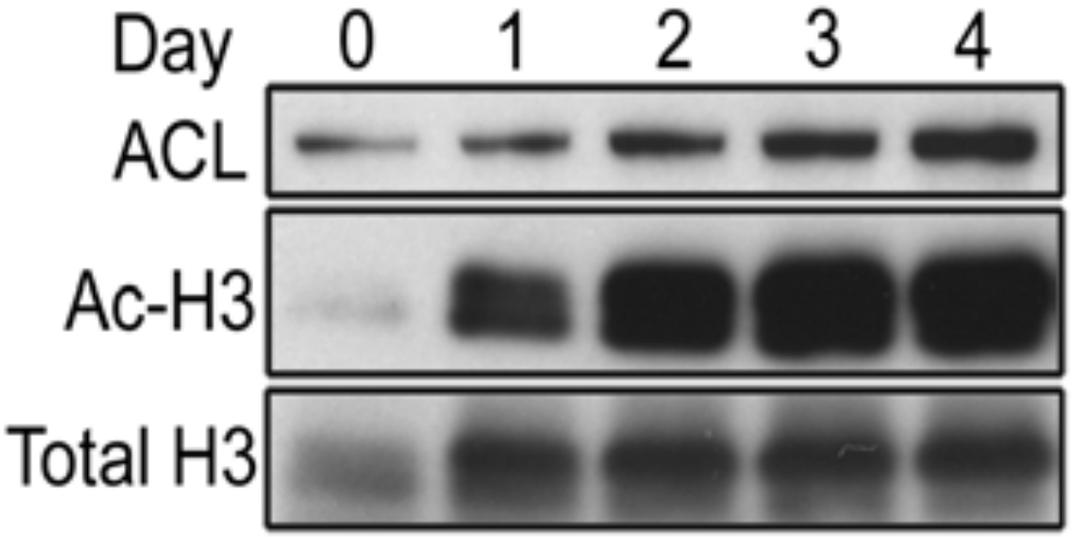


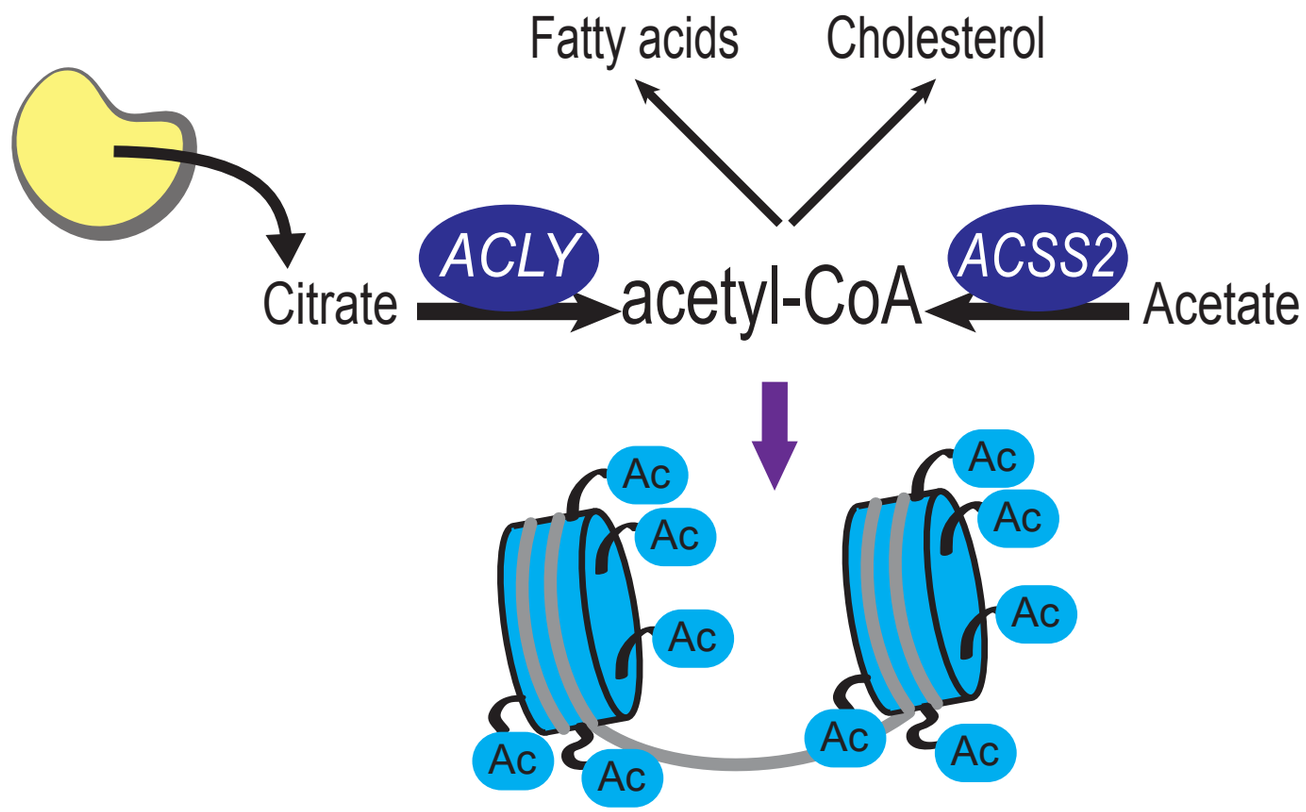
Adipocyte differentiation



ATP-Citrate Lyase Links Cellular Metabolism to Histone Acetylation

Kathryn E. Wellen,* Georgia Hatzivassiliou,*† Uma M. Sachdeva, Thi V. Bui, Justin R. Cross, Craig B. Thompson‡





Wellen et al, ***Science****, 2009*

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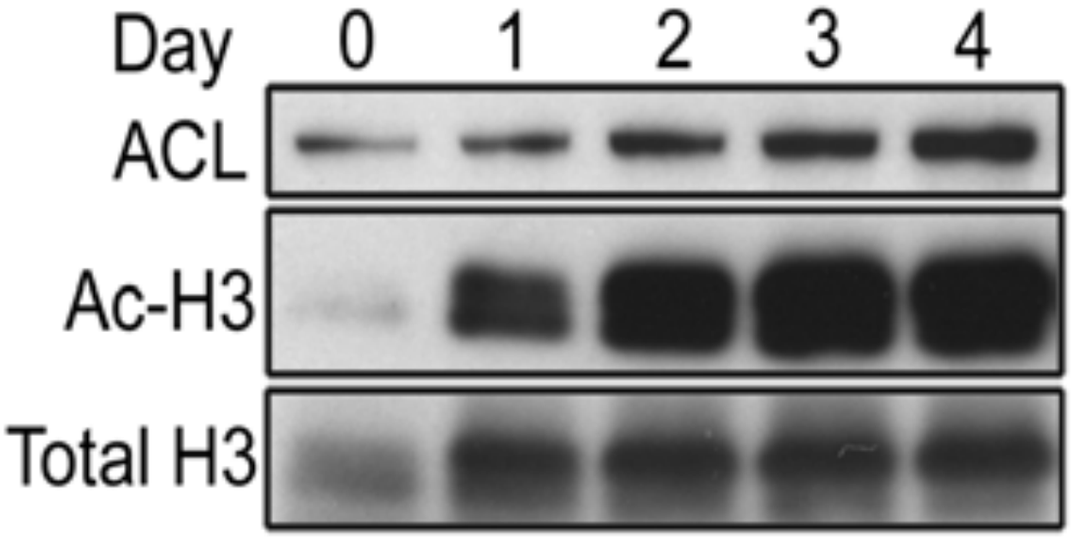
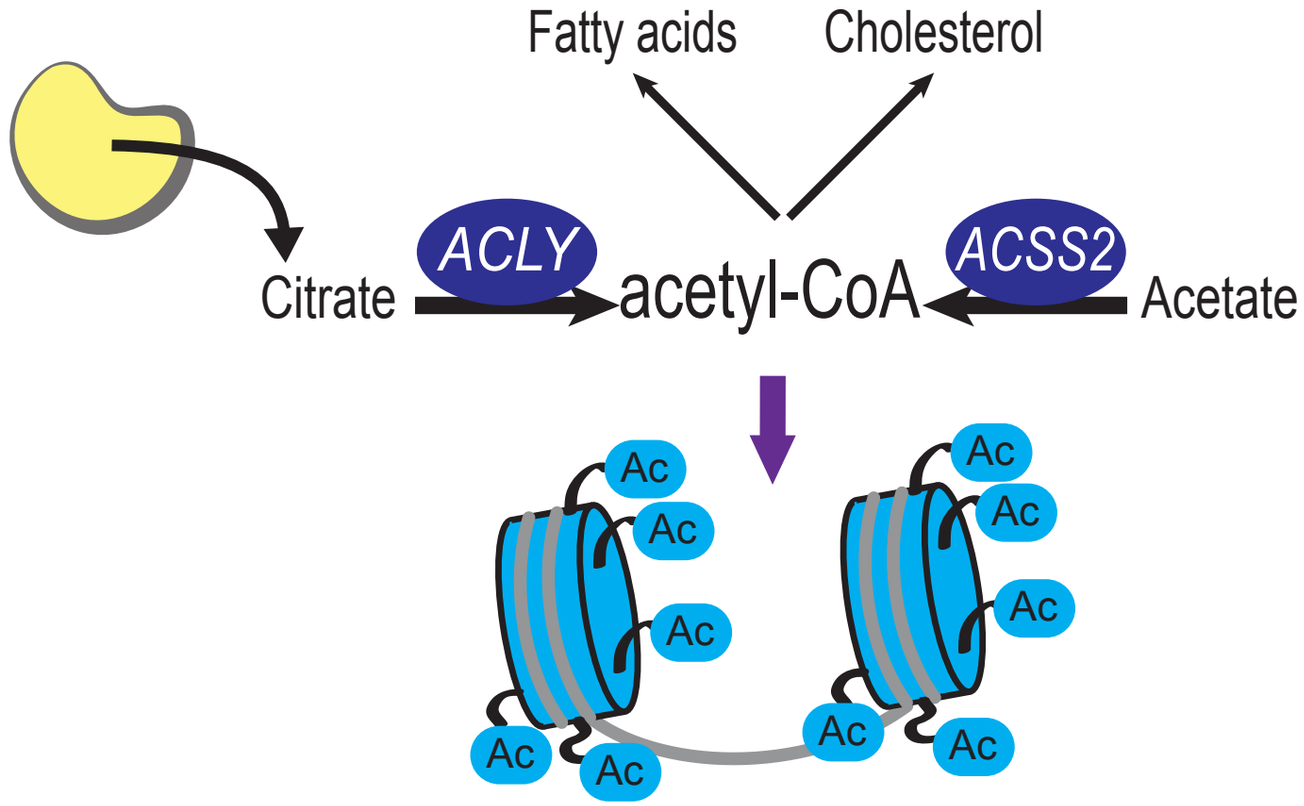


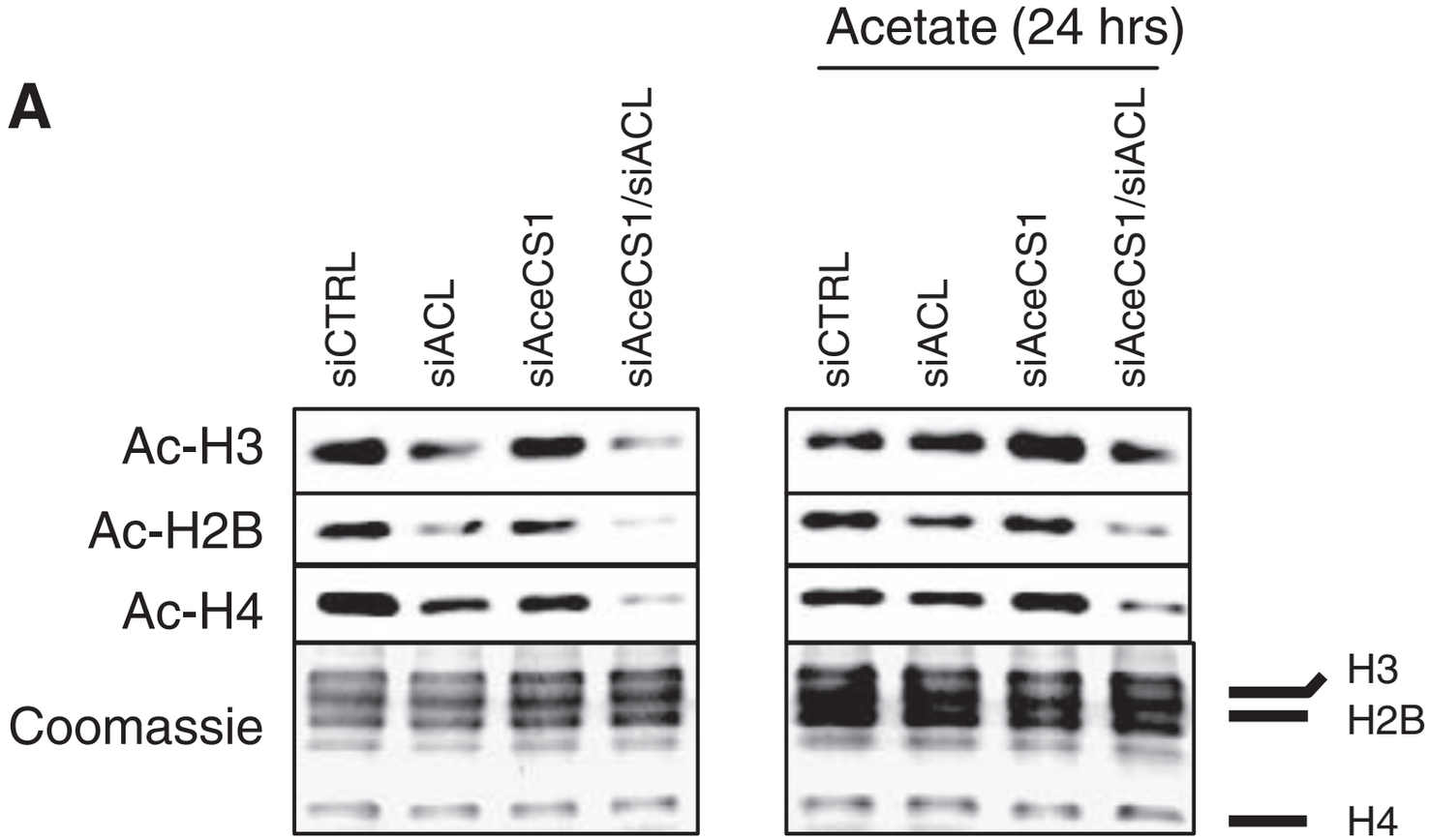


Histone acetylation (and ACLY expression) progressively increase during adipocyte differentiation *in vitro*.

Is that linked to acetyl-CoA availability??

Wellen et al, ***Science****, 2009*

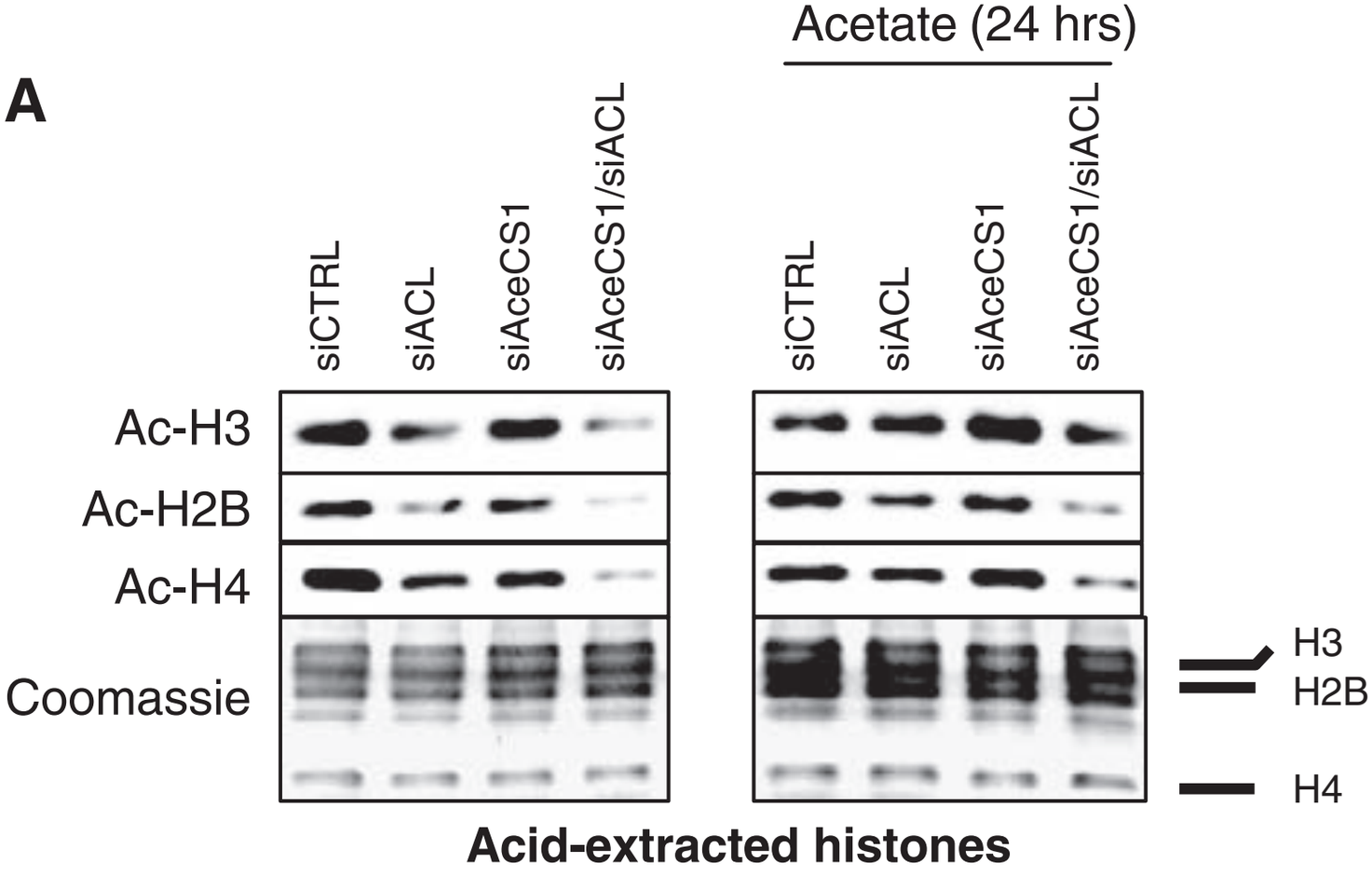
A



Acid-extracted histones

Wellen et al, ***Science****, 2009*

A

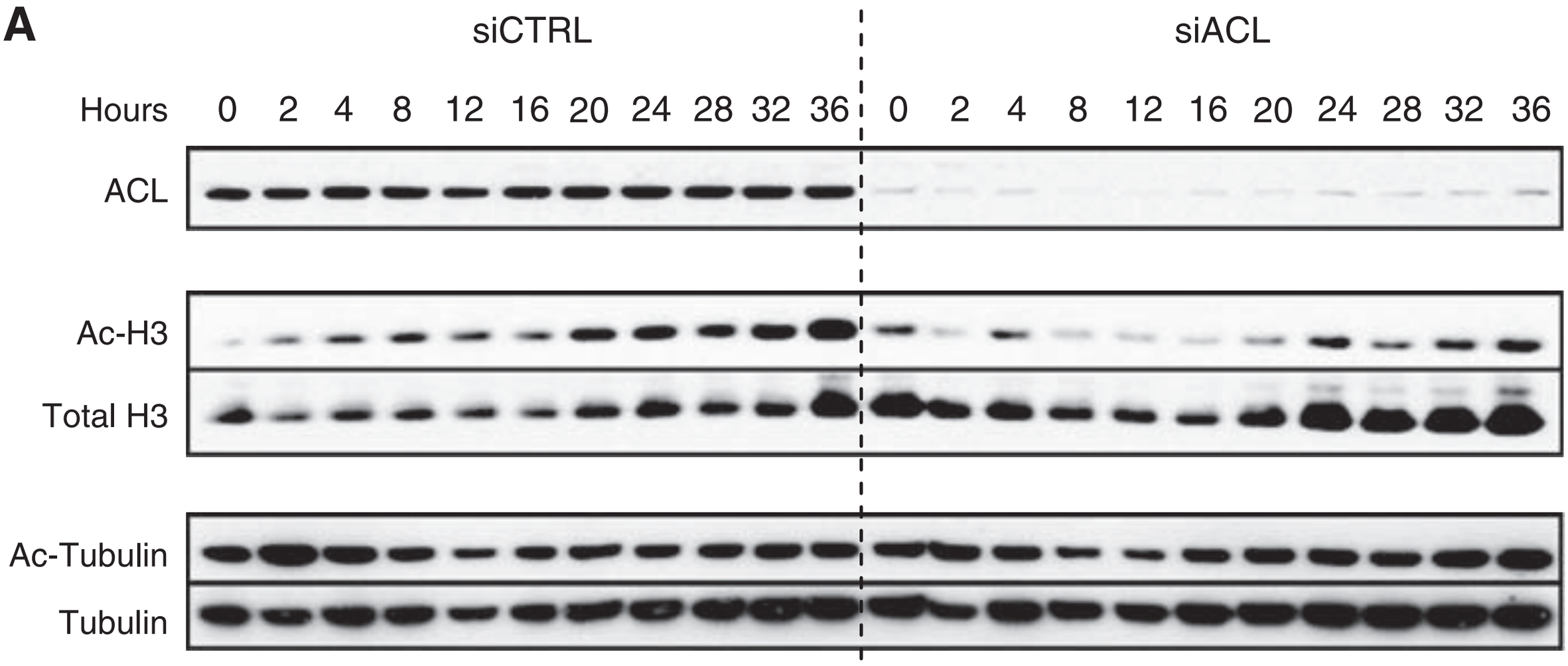


Acid-extracted histones

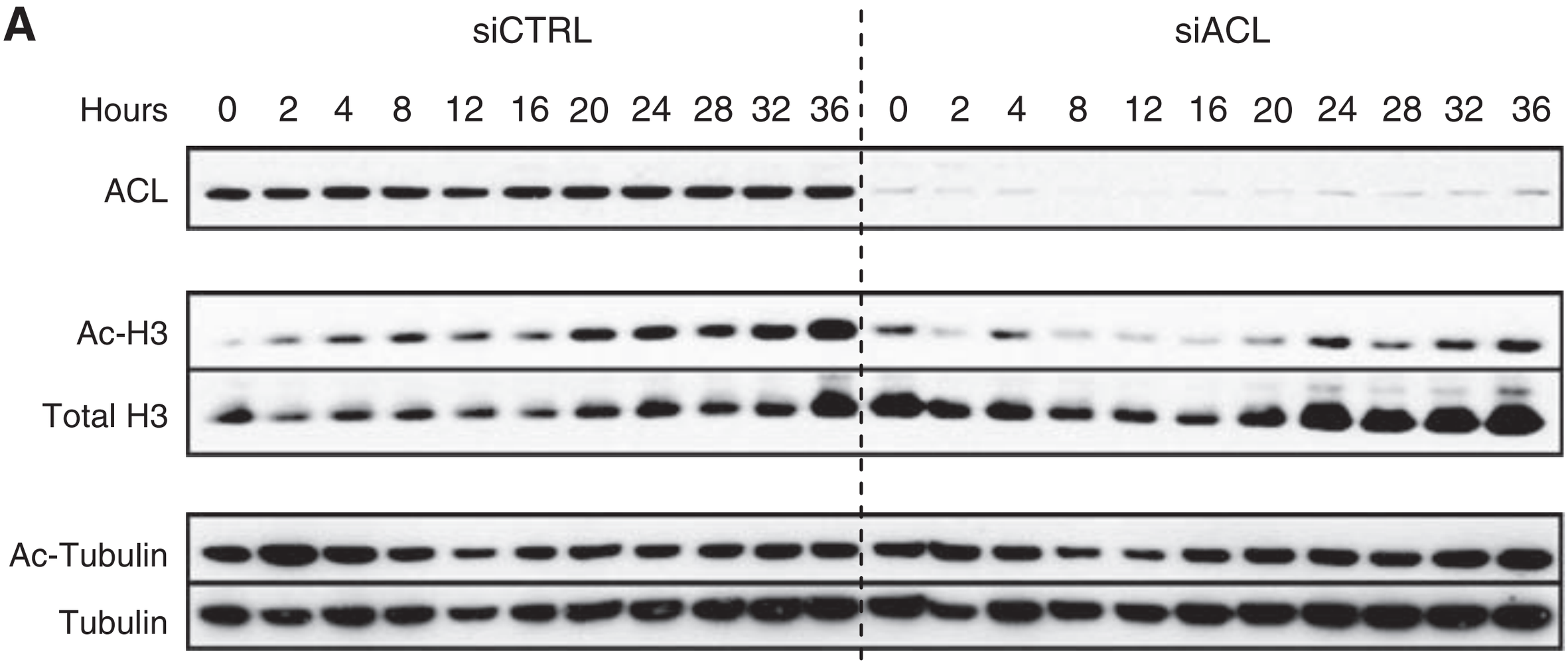
HCT116 cells

Is that true for differentiating adipocytes??

Wellen et al, ***Science****, 2009*

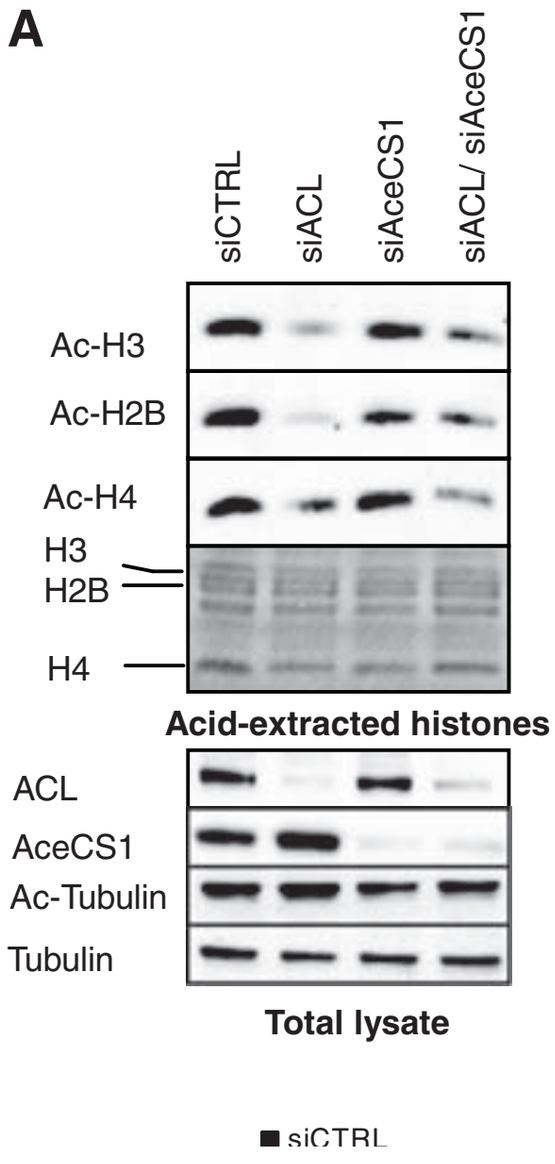


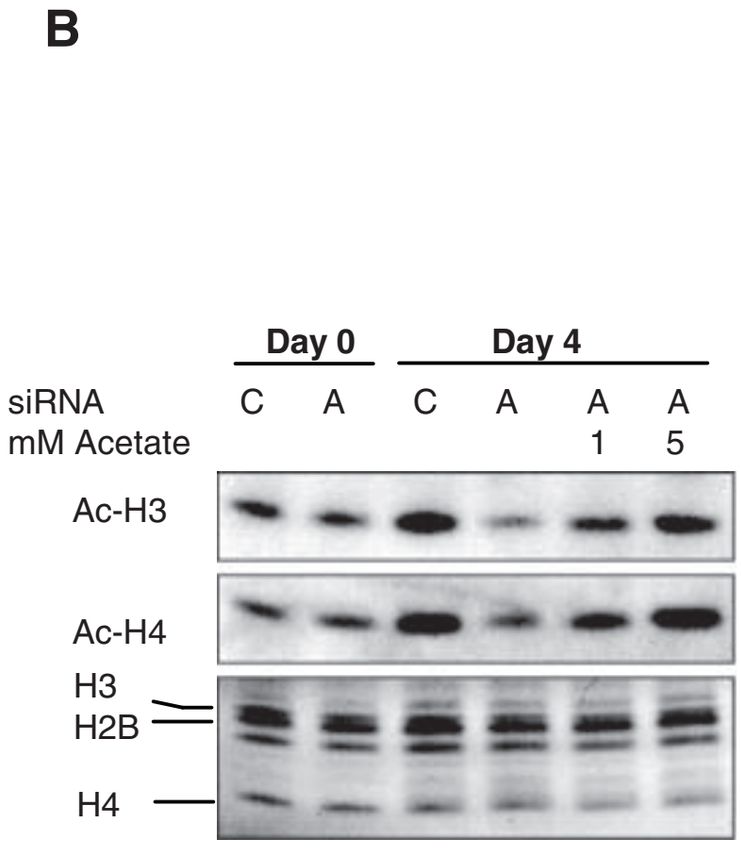
Wellen et al, ***Science****, 2009*

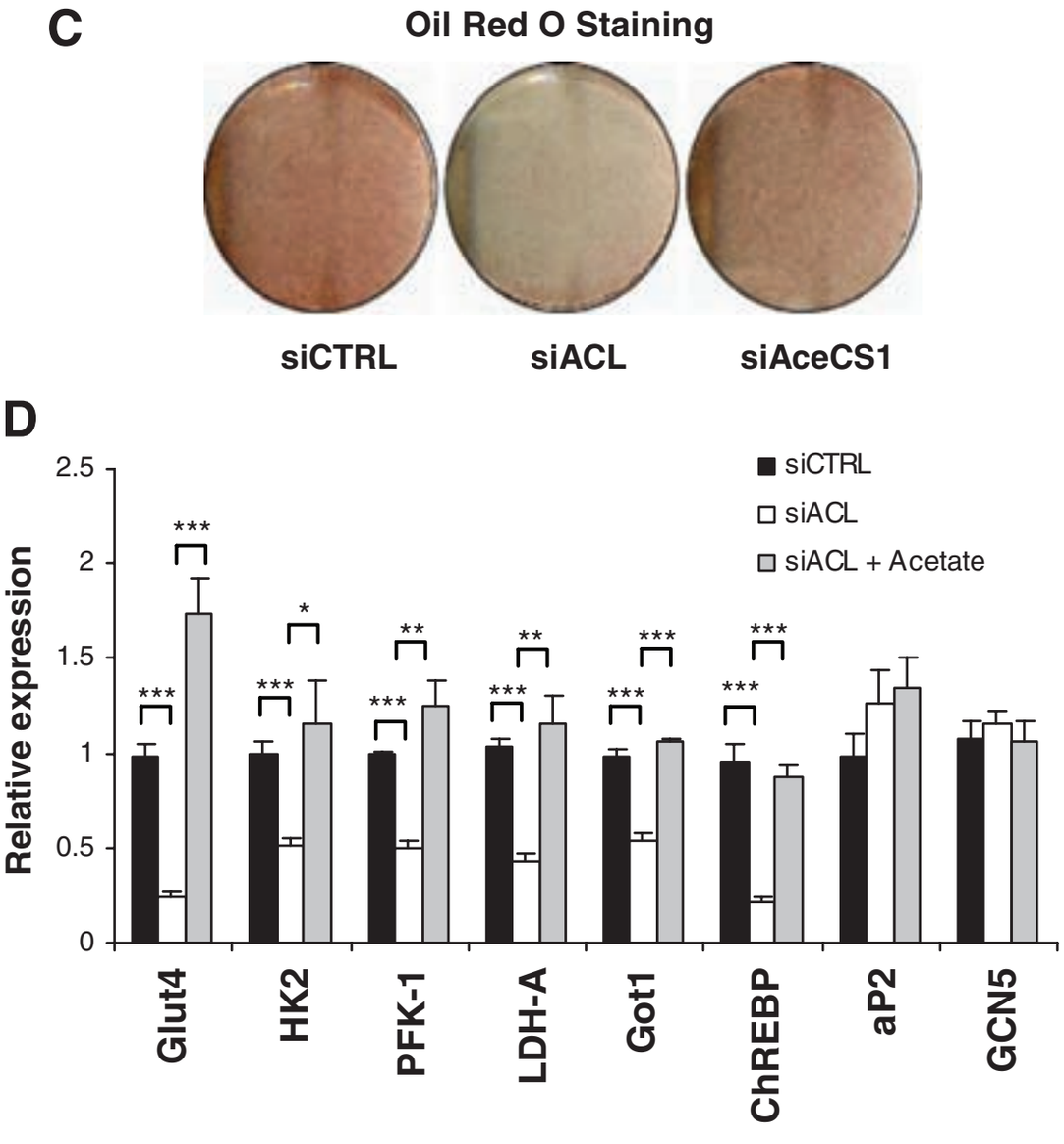


ACLY ablation inhibits differentiation-dependent histone hyperacetylation.

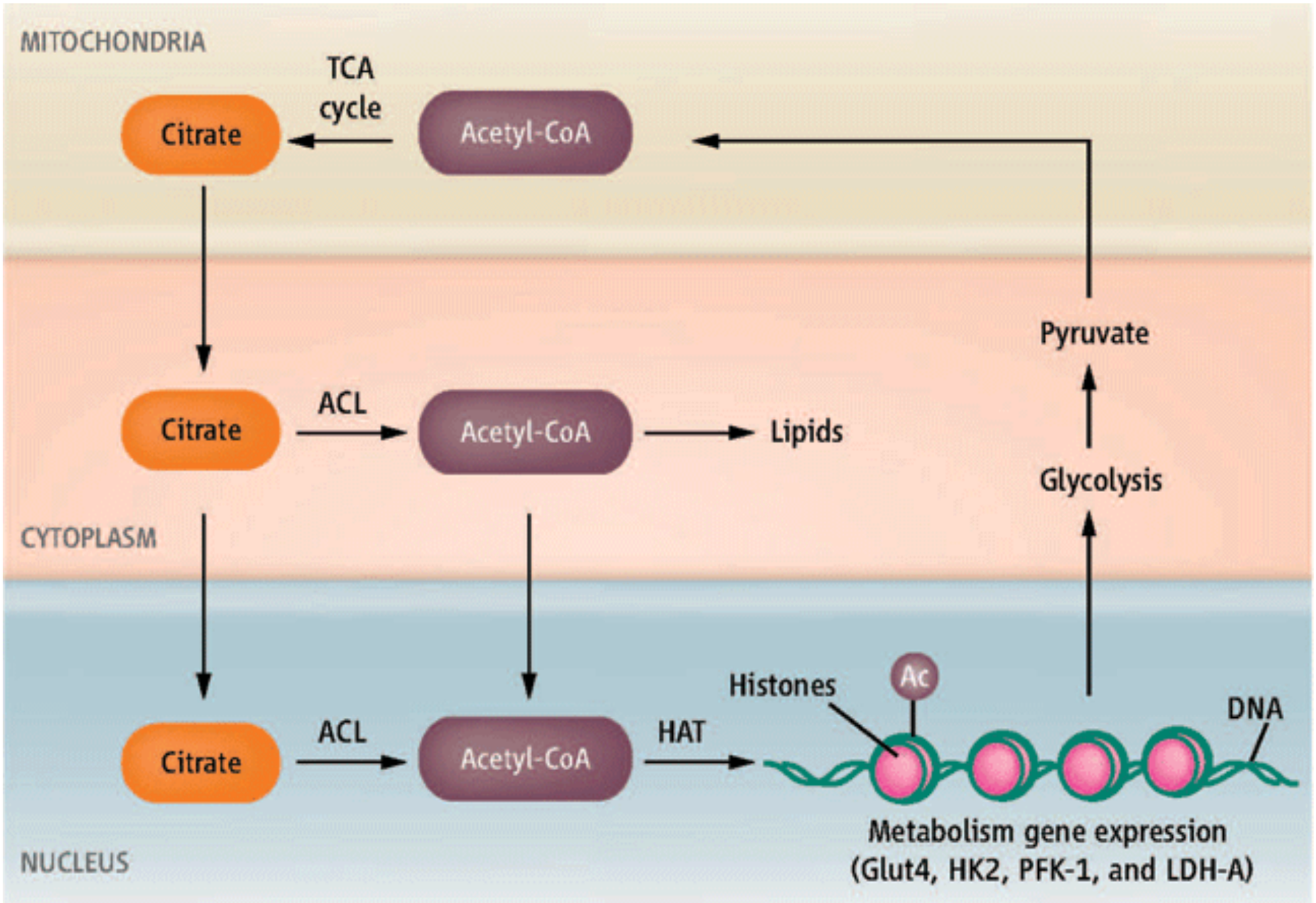
Wellen et al, ***Science****, 2009*







Wellen et al, ***Science****, 2009*

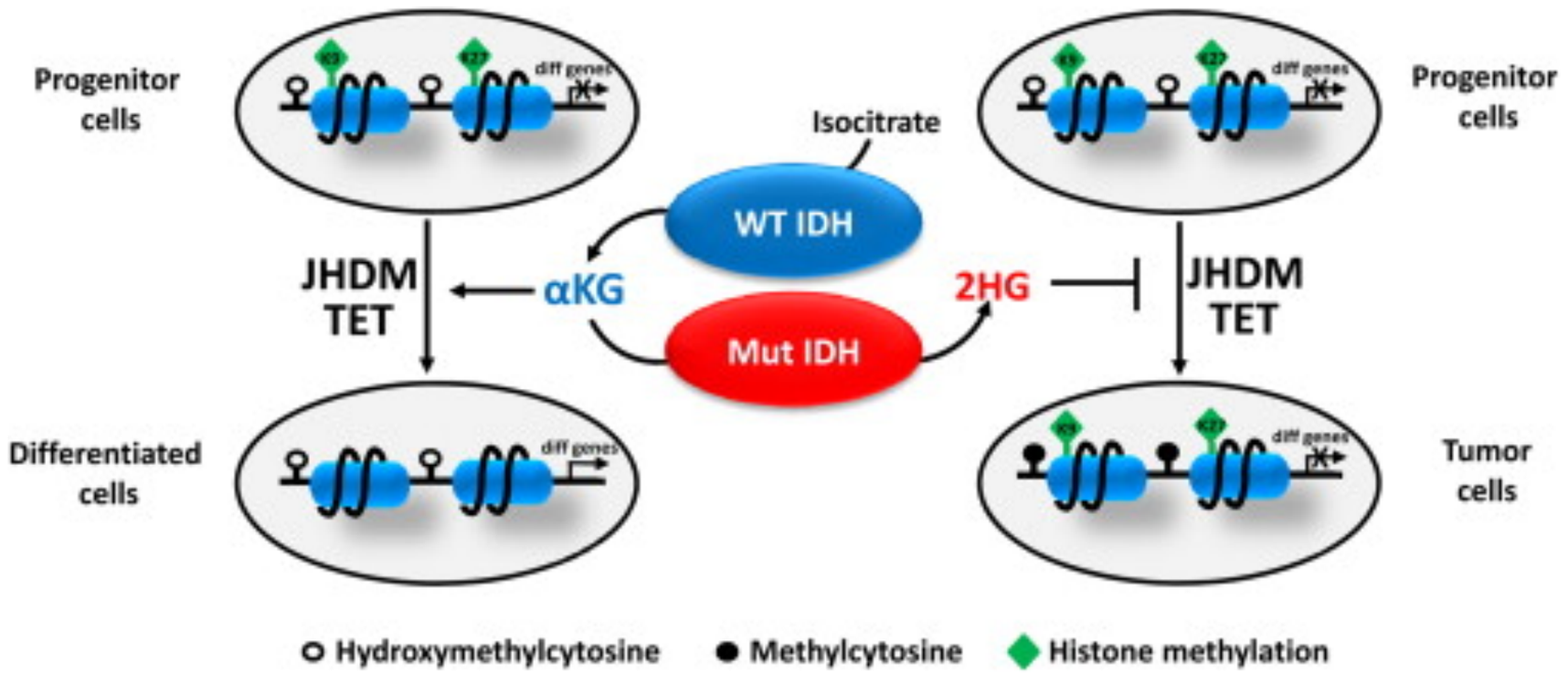


Rathmell & Newgard, ***Science****, 2009*
Wellen et al, ***Science****, 2009*

What about histone acetylation?
a-KetoGlutarate?

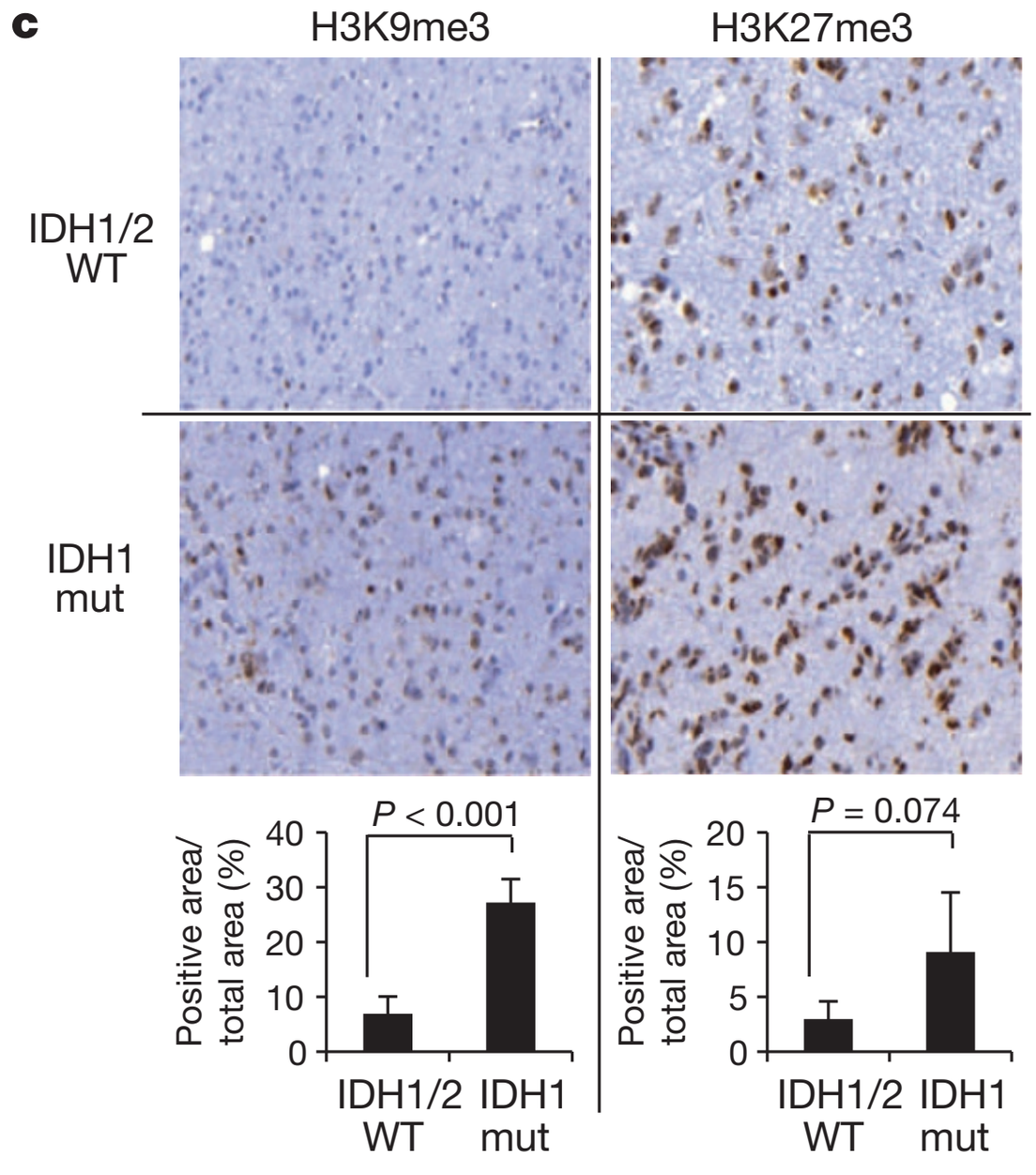
IDH mutation impairs histone demethylation and results in a block to cell differentiation

Chao Lu[1,2], Patrick S. Ward[1,2], Gurpreet S. Kapoor[3], Dan Rohle[4,5], Sevin Turcan[4], Omar Abdel-Wahab[4,6], Christopher R. Edwards[7], Raya Khanin[8], Maria E. Figueroa[9], Ari Melnick[9], Kathryn E. Wellen[2], Donald M. O'Rourke[3,10], Shelley L. Berger[7], Timothy A. Chan[4], Ross L. Levine[4,6], Ingo K. Mellinghoff[4,5,11] & Craig B. Thompson[1]



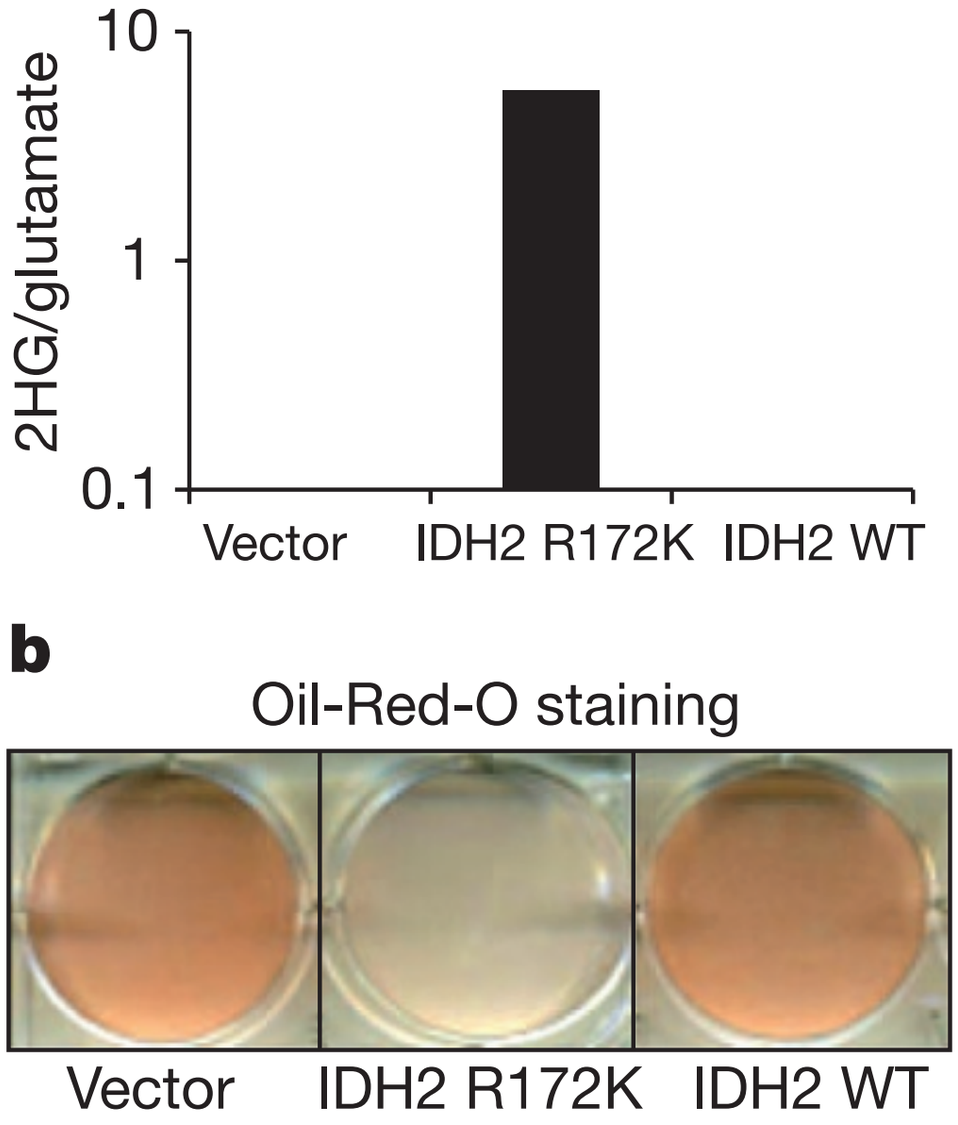
Lu et al, ***Nature****, 2012*

What about histone acetylation?
a-KetoGlutarate?

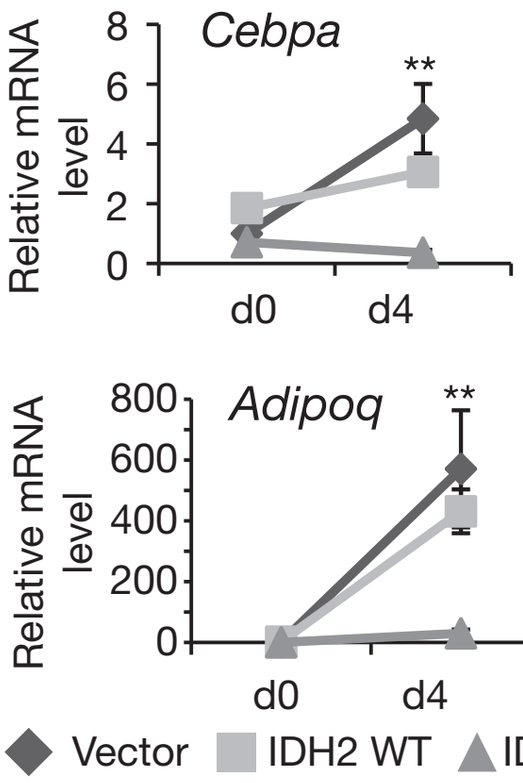


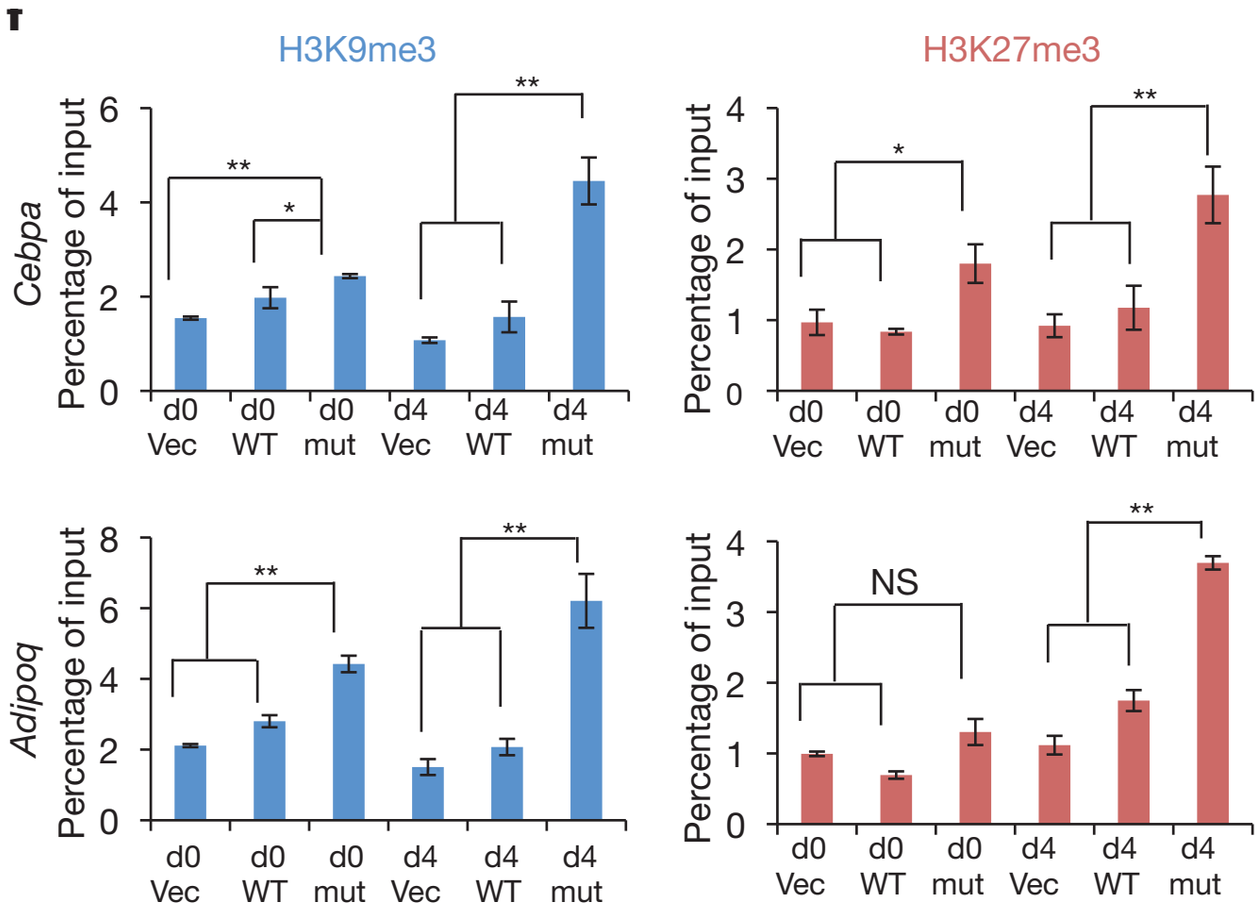
Oligodendroglioma

Lu et al, ***Nature****, 2012*



Lu et al, ***Nature****, 2012*





Gene expression

(qPCR)

Histone methylation

(ChIP-qPCR)

*Lu et al, **Nature**, 2012*

Differentiation
Oxidative Phosphorylation

Villus

Inactive
Fibroblasts

Guanylin
Uroguanylin

Enterocyte

Enteroendocrine
Cell

Goblet Cell

Paneth Cell

Intestinal
Stem Cell

Crypt

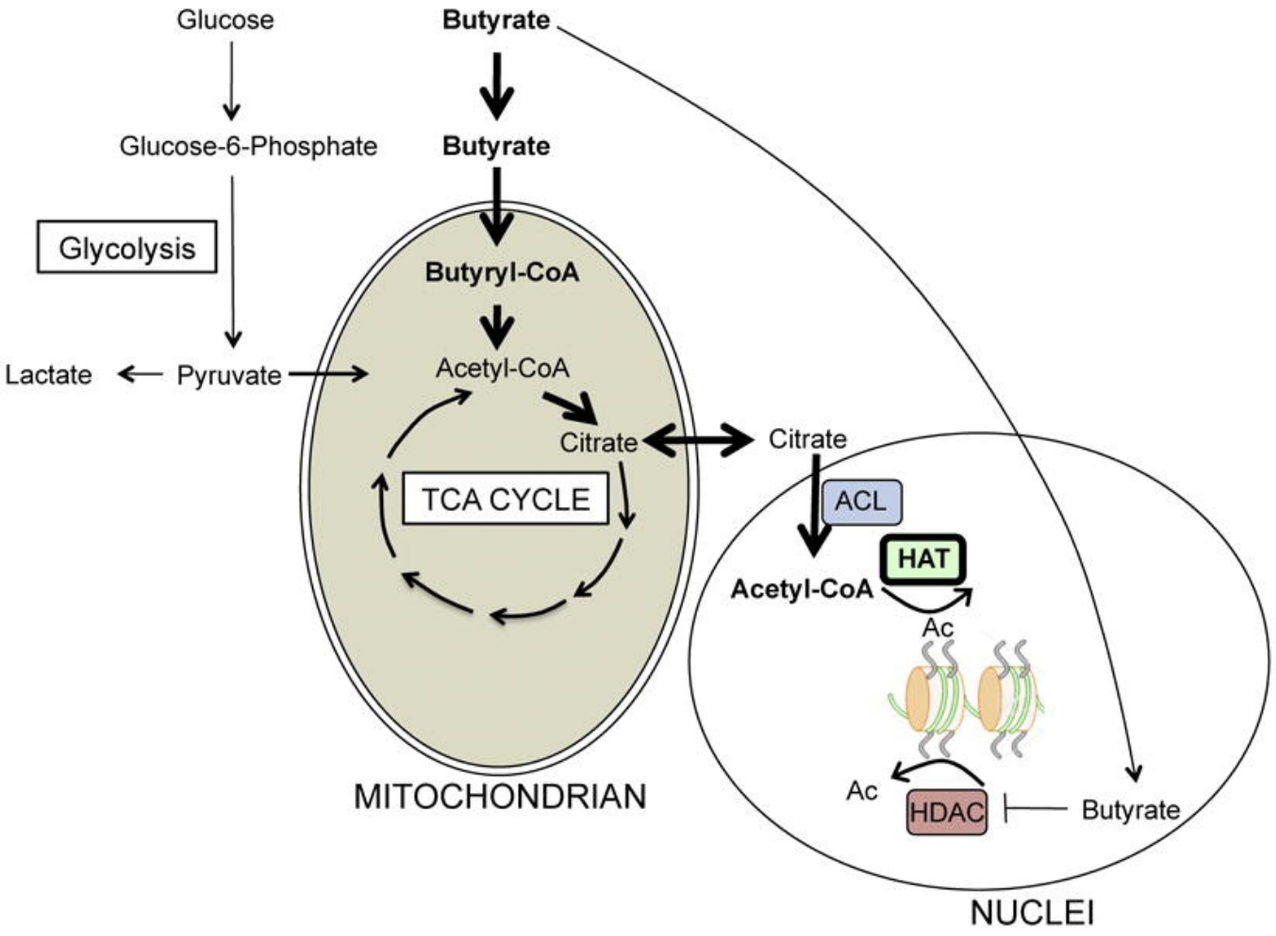
Proliferation
Anabolism

Paricryptal
Myofibroblast

Active
Fibroblasts

Colonocytes

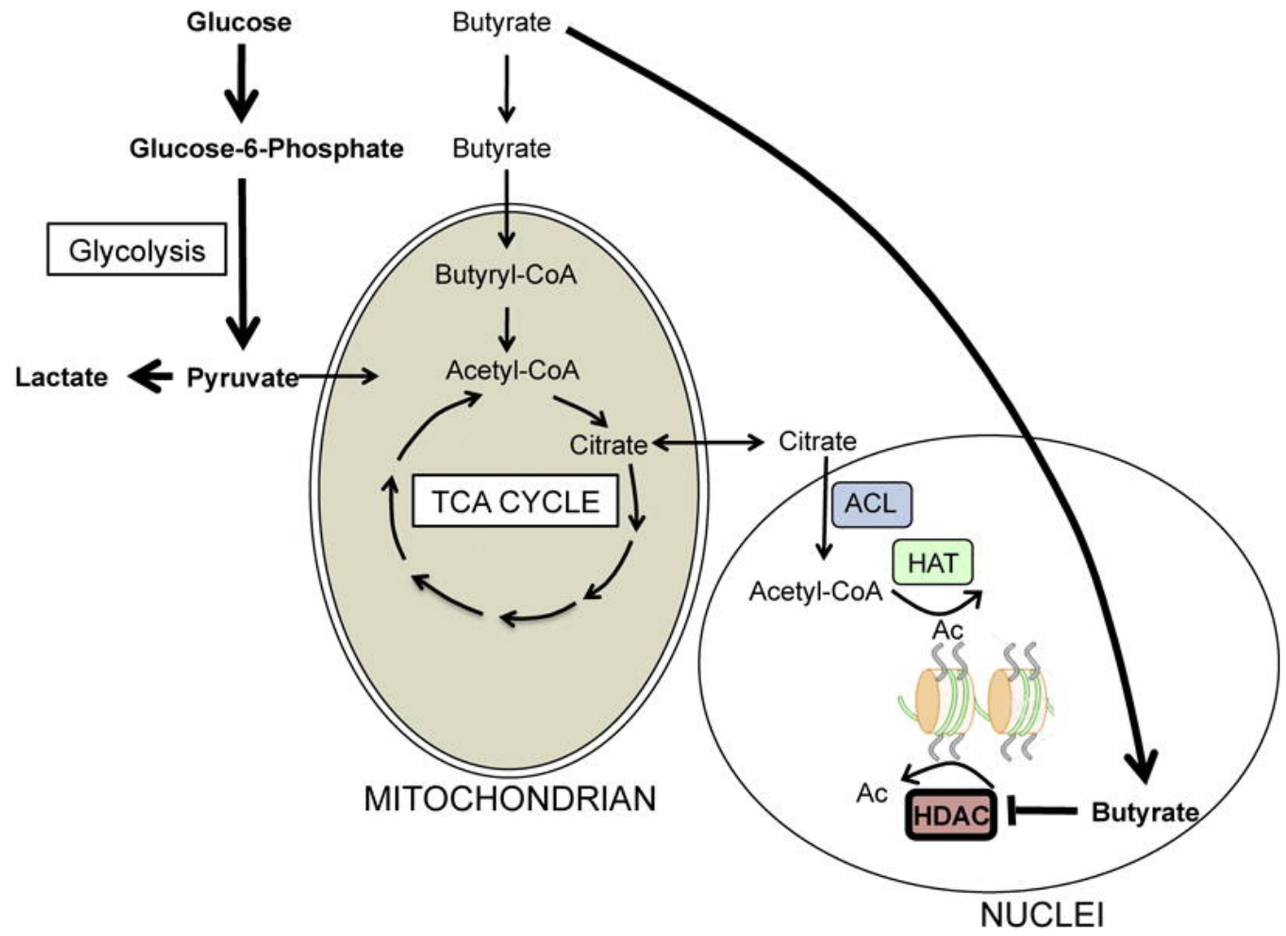
(differentiated)



- Butyrate Oxidation/Energetics
- HAT Activity
- Proliferation

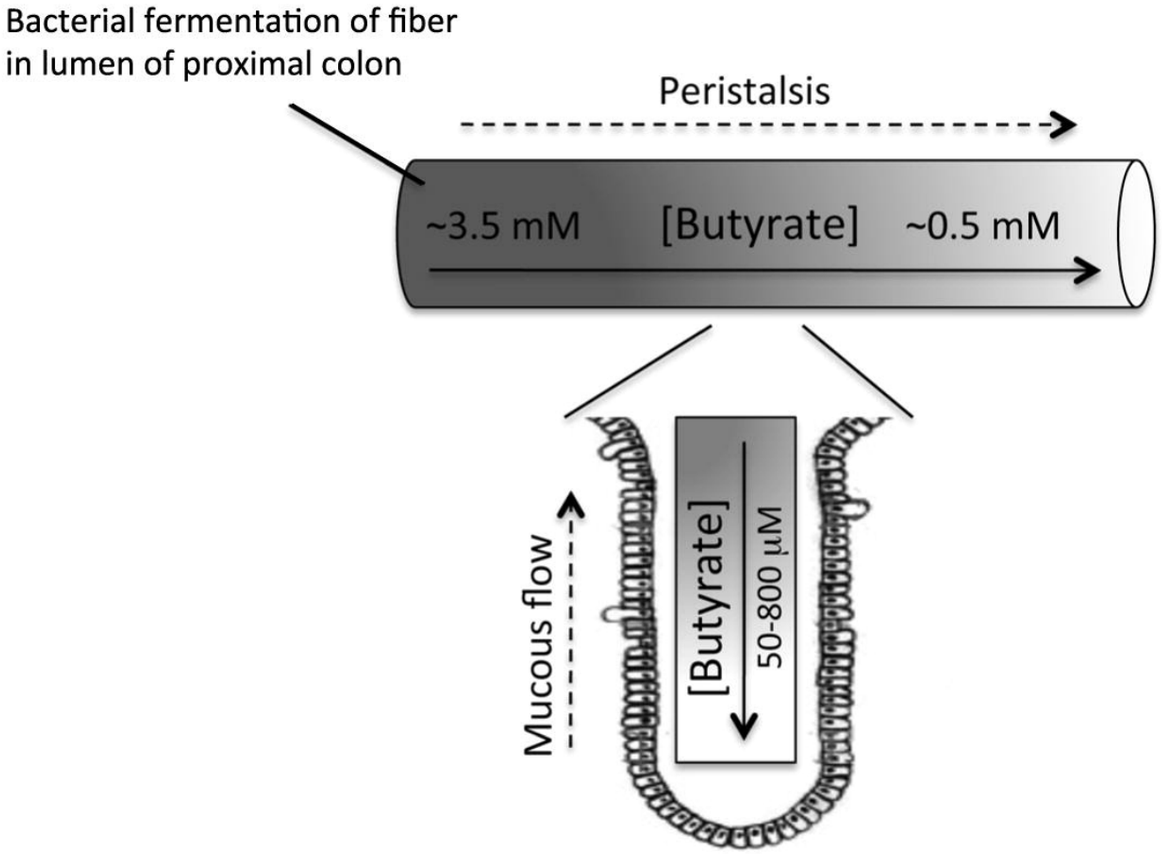
Proliferating colonocytes

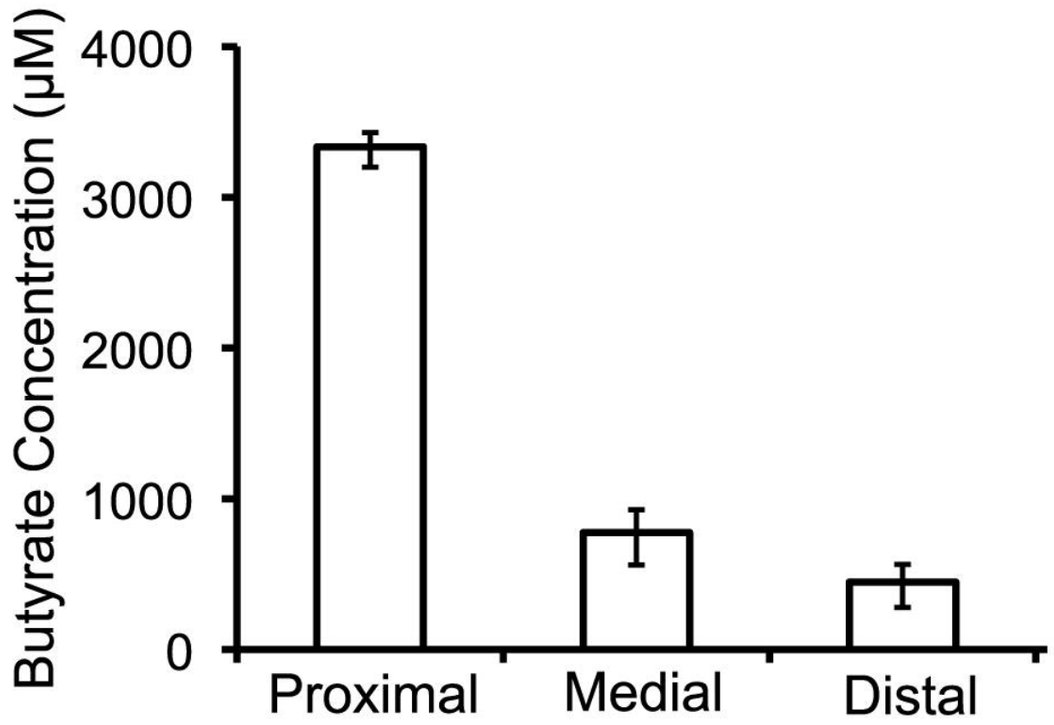
(undifferentiated)



- Butyrate Accumulation
- HDAC inhibition
- Apoptosis

Donohoe et al, ***Mol Cell****, 2012*





*Donohoe et al, **Mol Cell**, 2012*