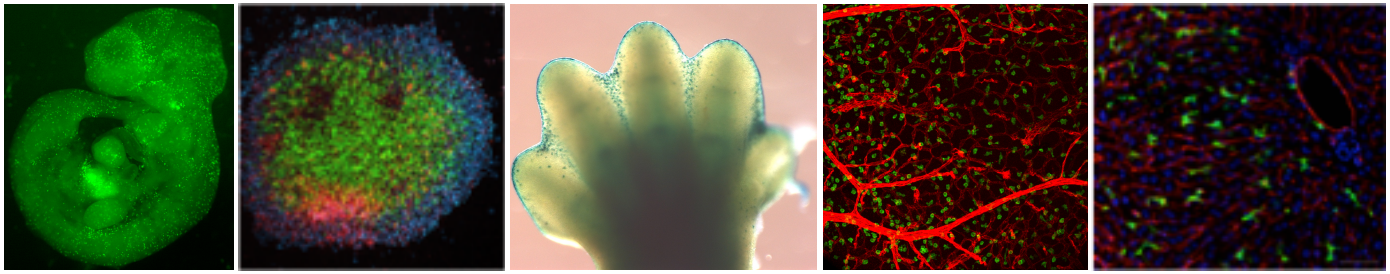


Gomez Perdiguero Lab, « Macrophages and Endothelial cells »,
CNRS UMR3738

Comprendre l'hétérogénéité des macrophages tissulaires et son impact lors de la réparation tissulaire

Visioconference organisée par l'AAEIP, l'AUF et la DDE

20-06-2018

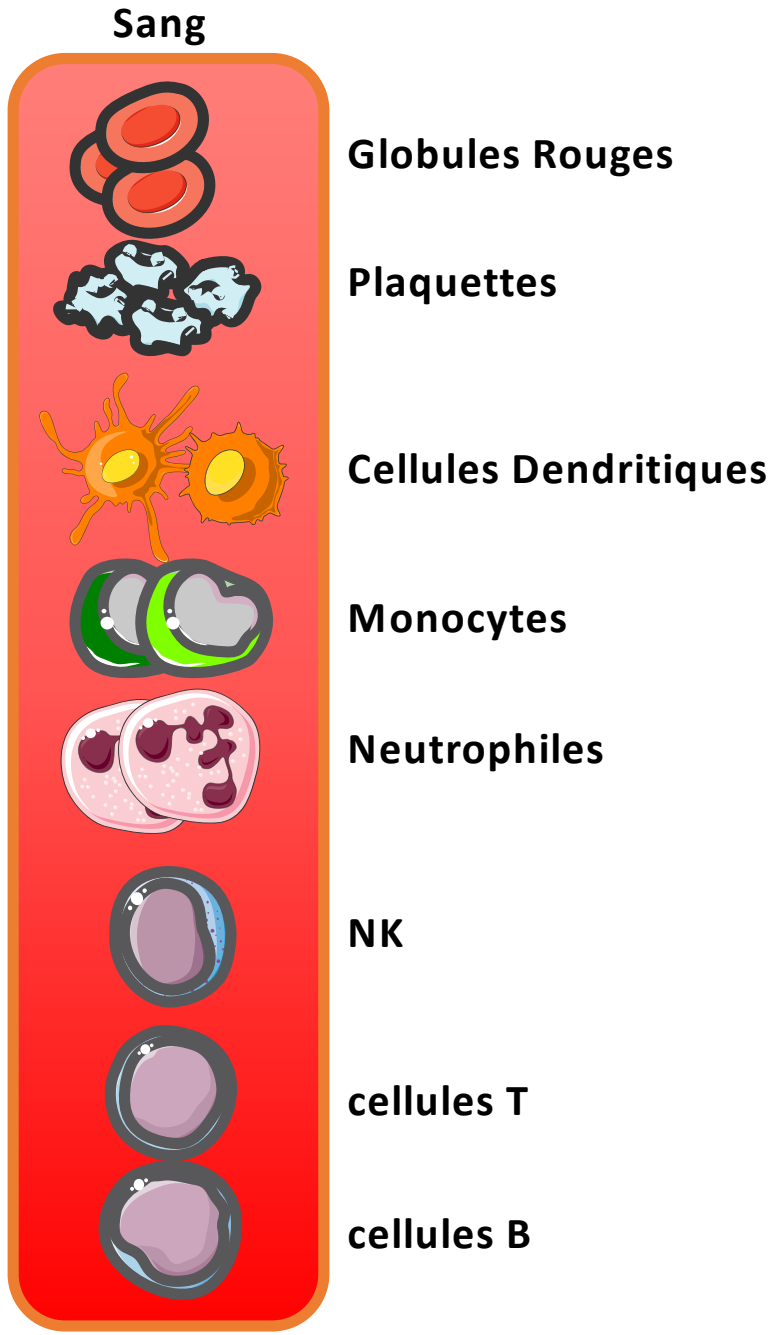
Comprendre l’hétérogénéité des macrophages tissulaires

1- Concepts généraux sur l’hématopoïese

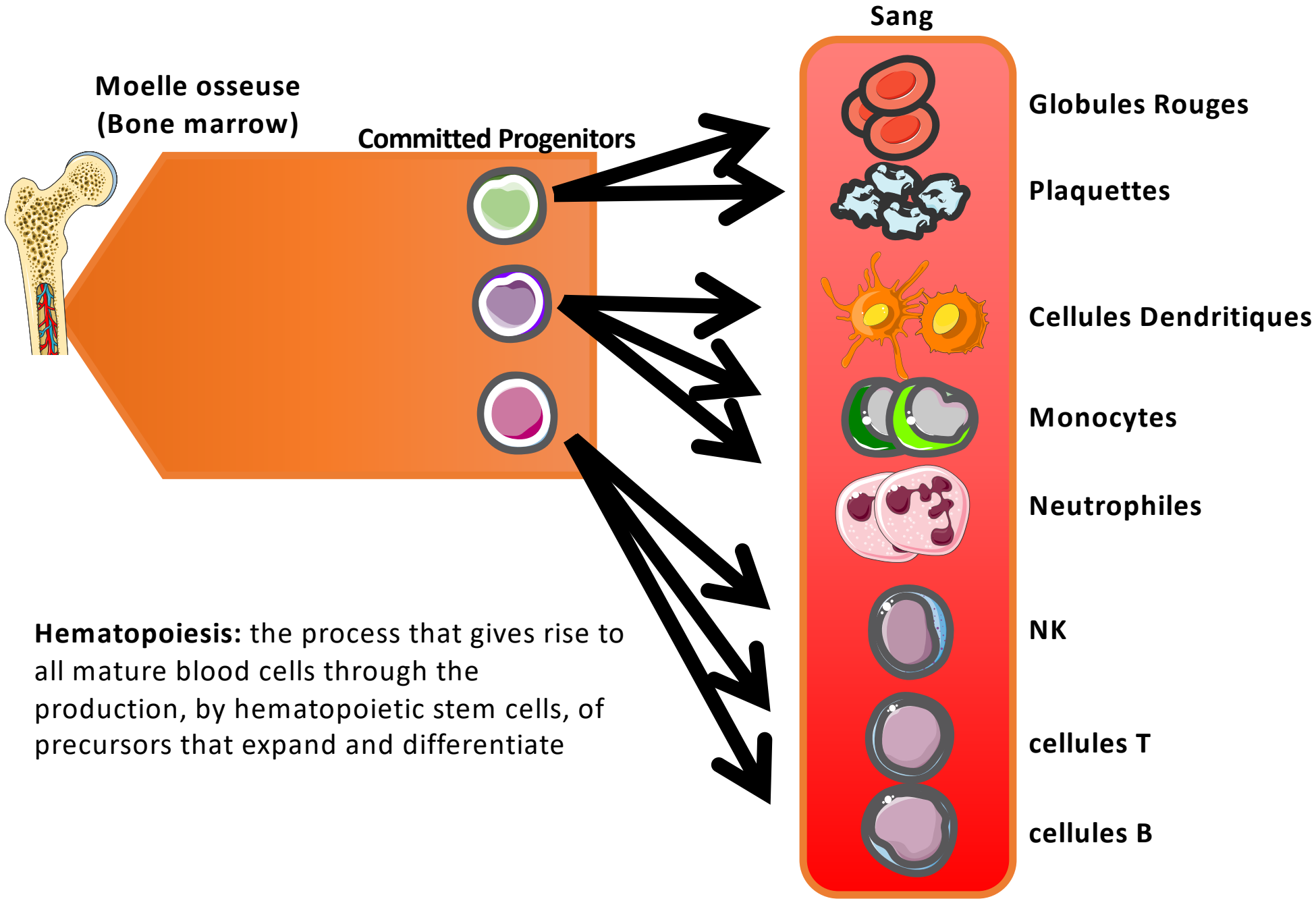
2- Développement du système hématopoïétique

3- Macrophages tissulaires résidents

Le système hématopoïetique adulte

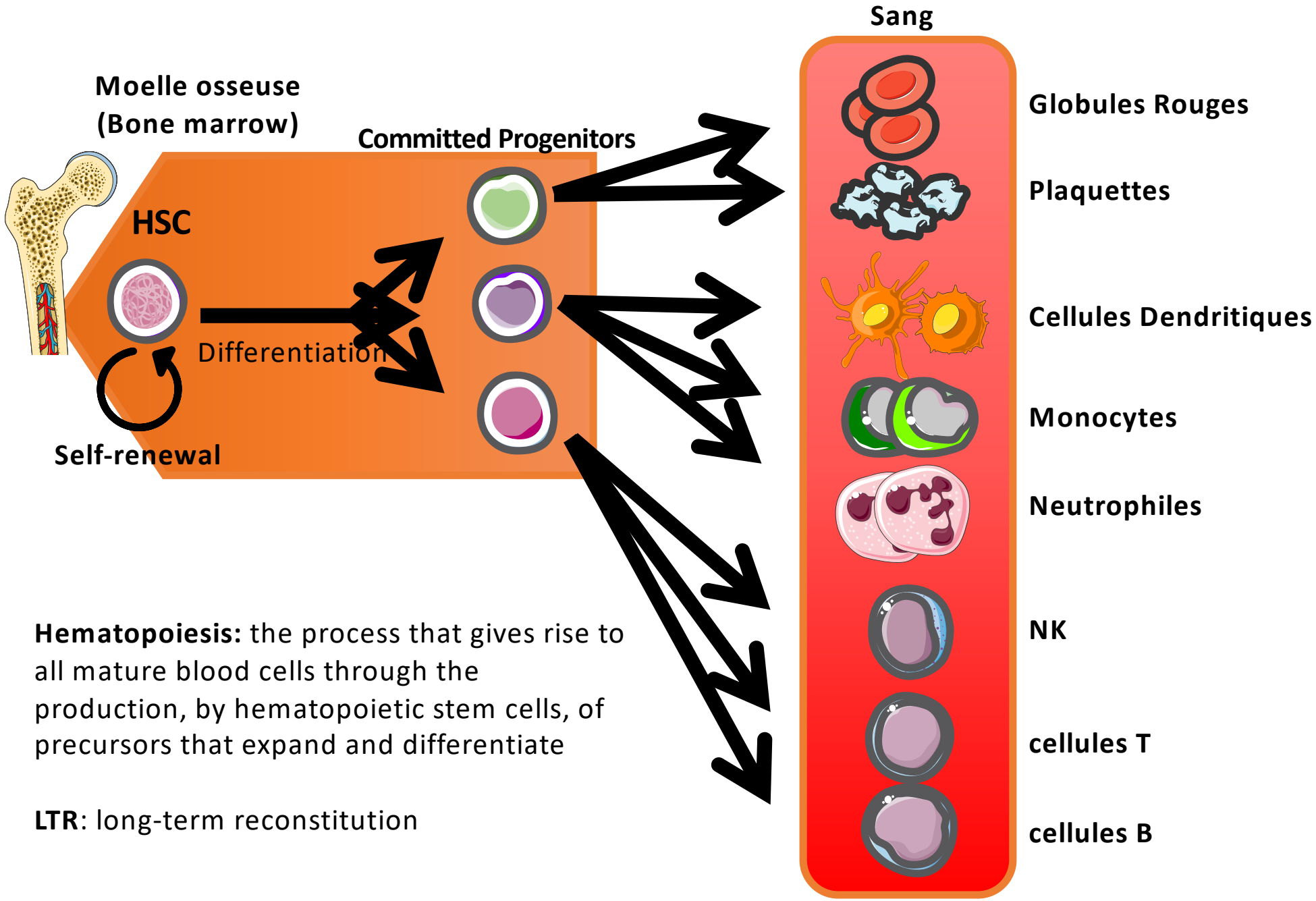


Le système hématopoïetique adulte



Hematopoiesis: the process that gives rise to all mature blood cells through the production, by hematopoietic stem cells, of precursors that expand and differentiate

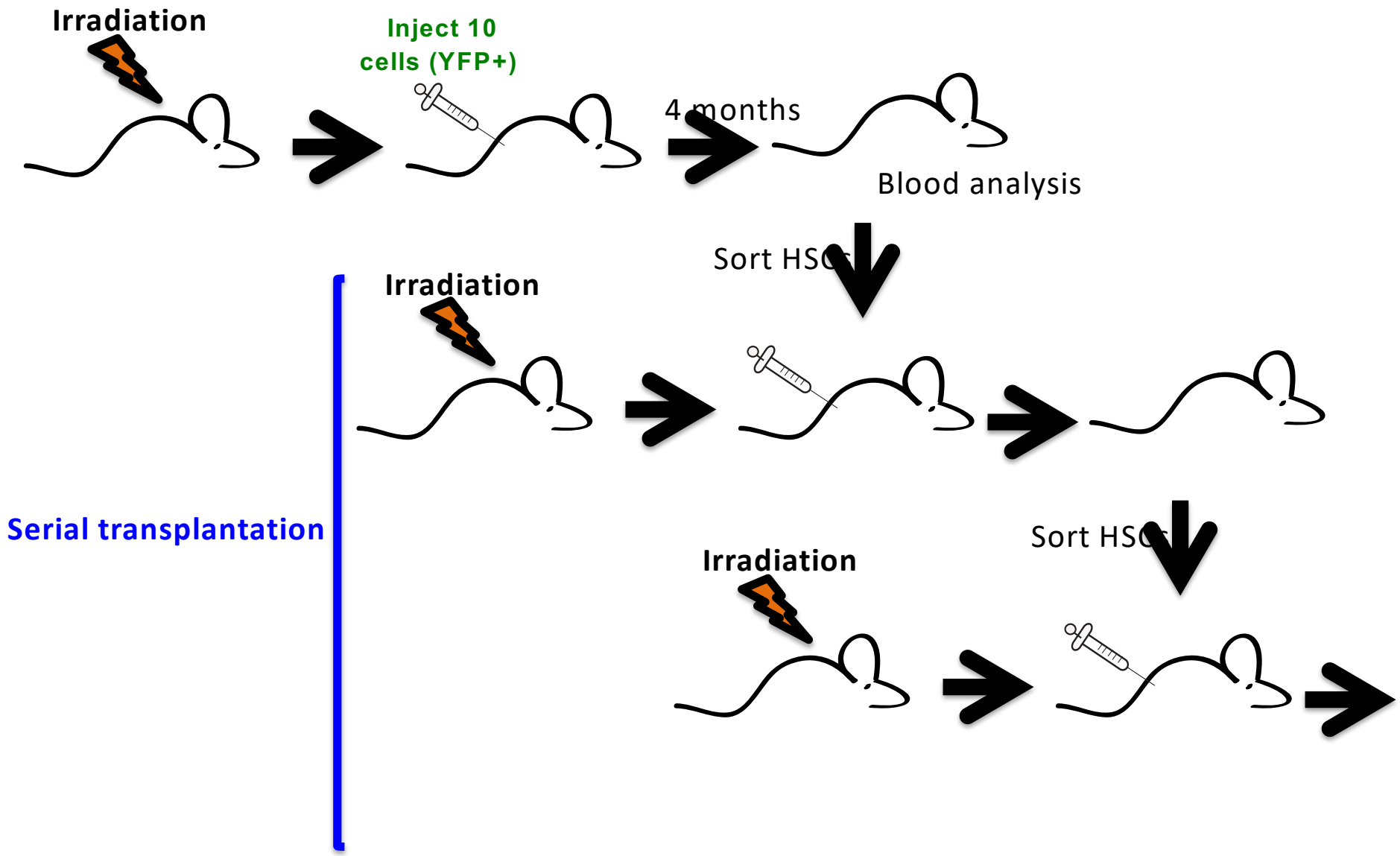
Le système hématopoïetique adulte



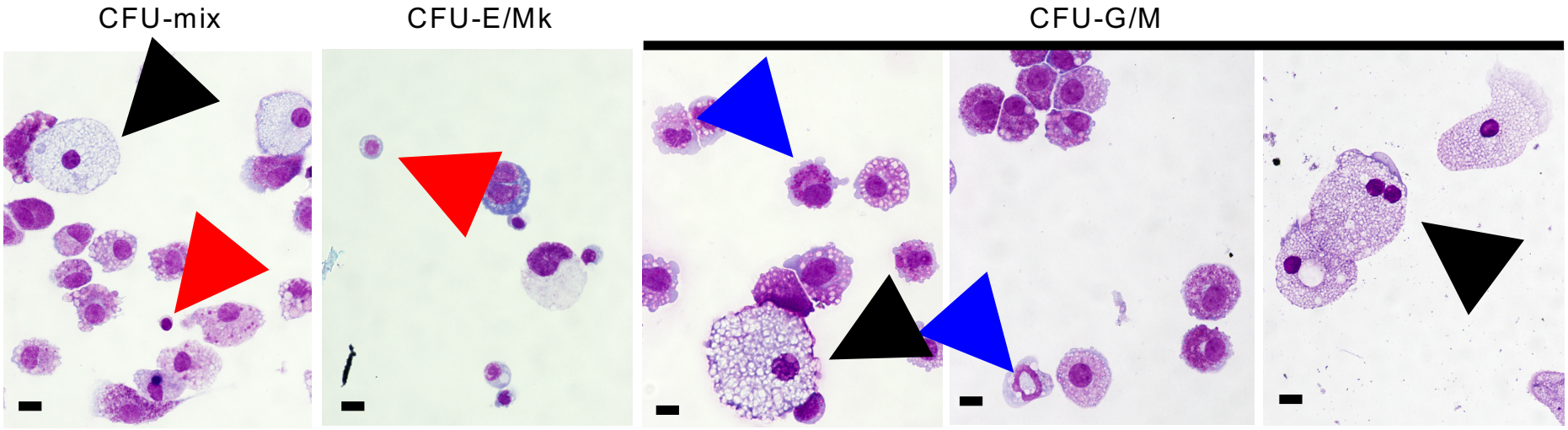
Hematopoiesis: the process that gives rise to all mature blood cells through the production, by hematopoietic stem cells, of precursors that expand and differentiate

LTR: long-term reconstitution

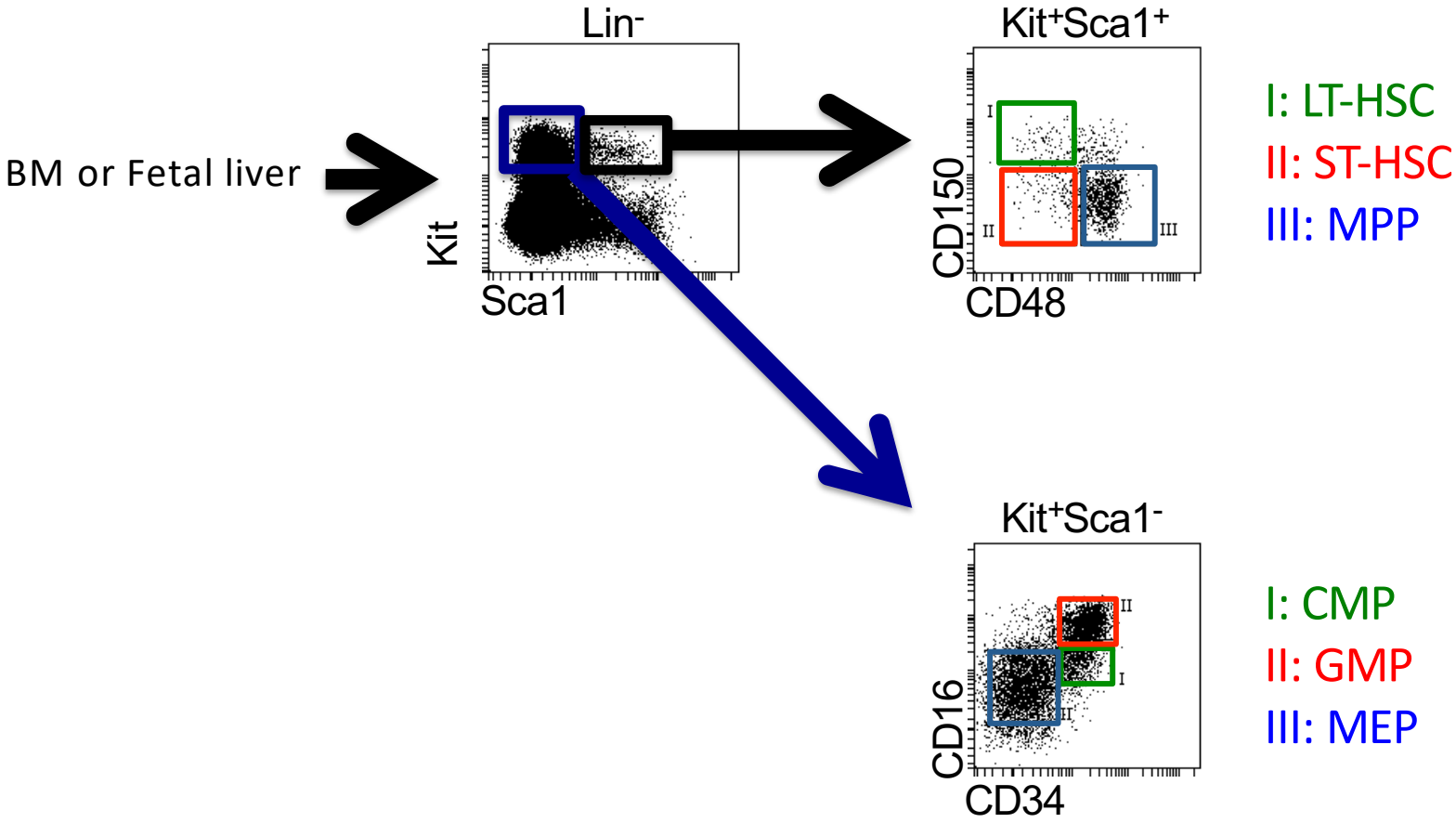
Defining HSCs: LTR in transplantation assays



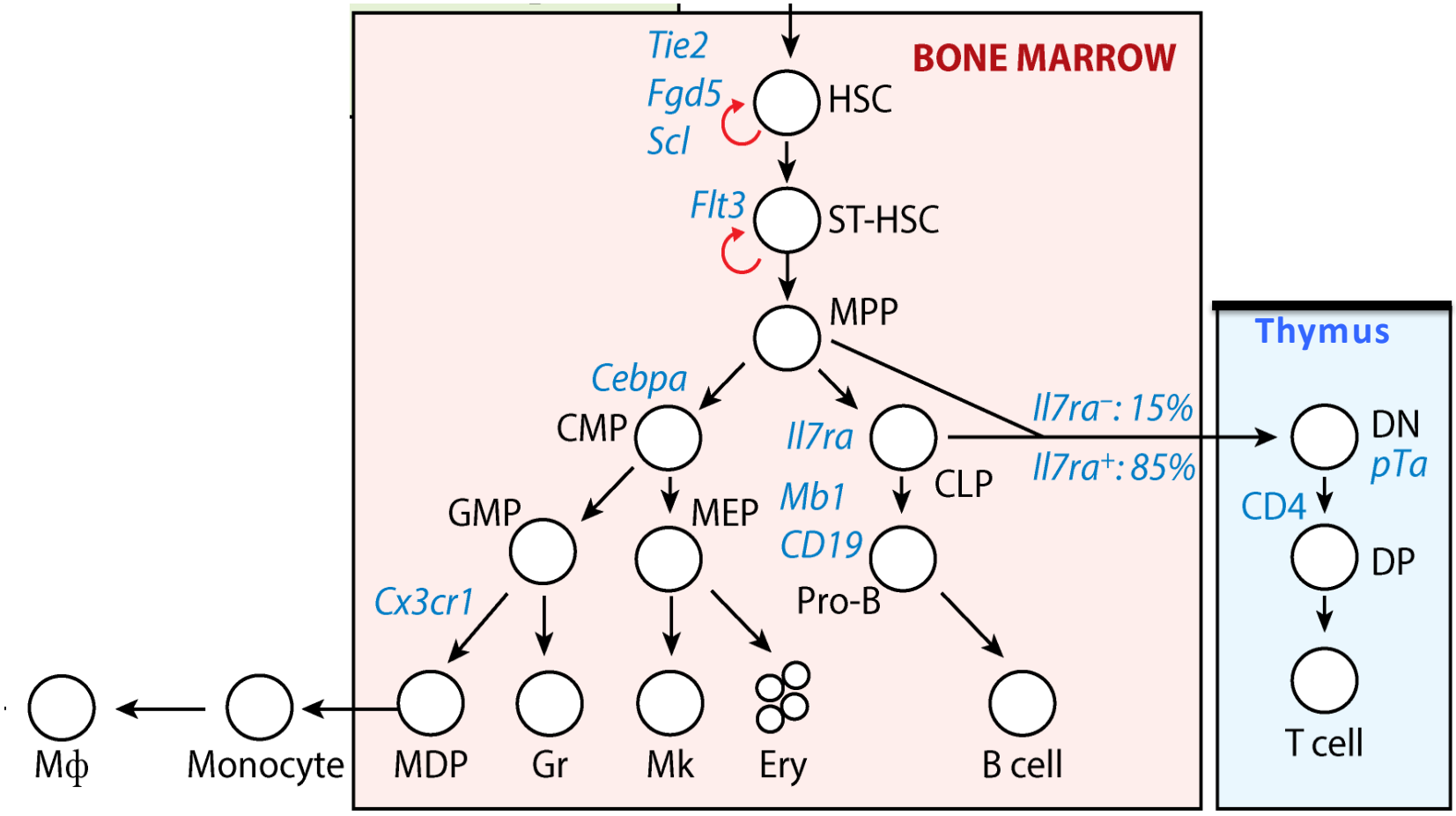
Progenitor potential: Colony-forming assays



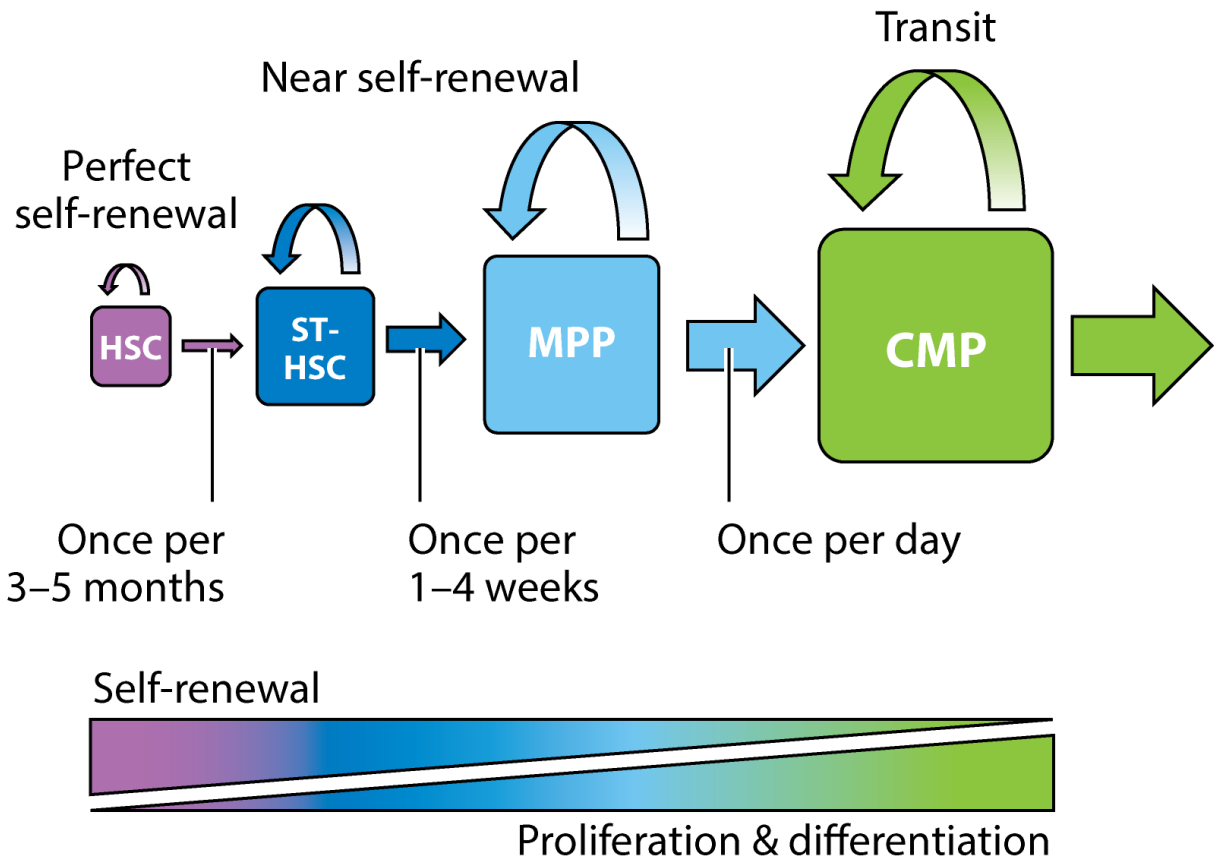
Defining HSCs: Phenotypic characterisation



Defining the hematopoietic tree: one example

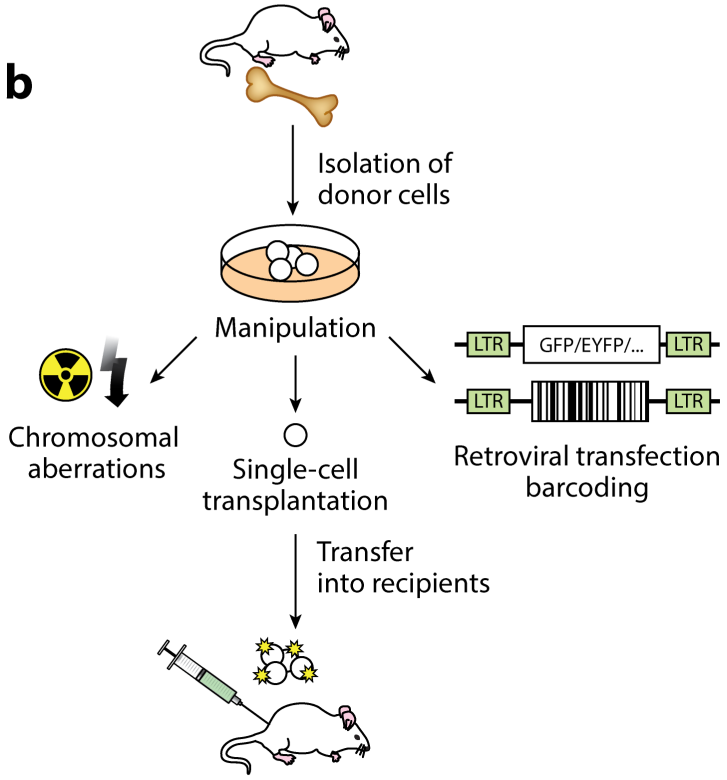


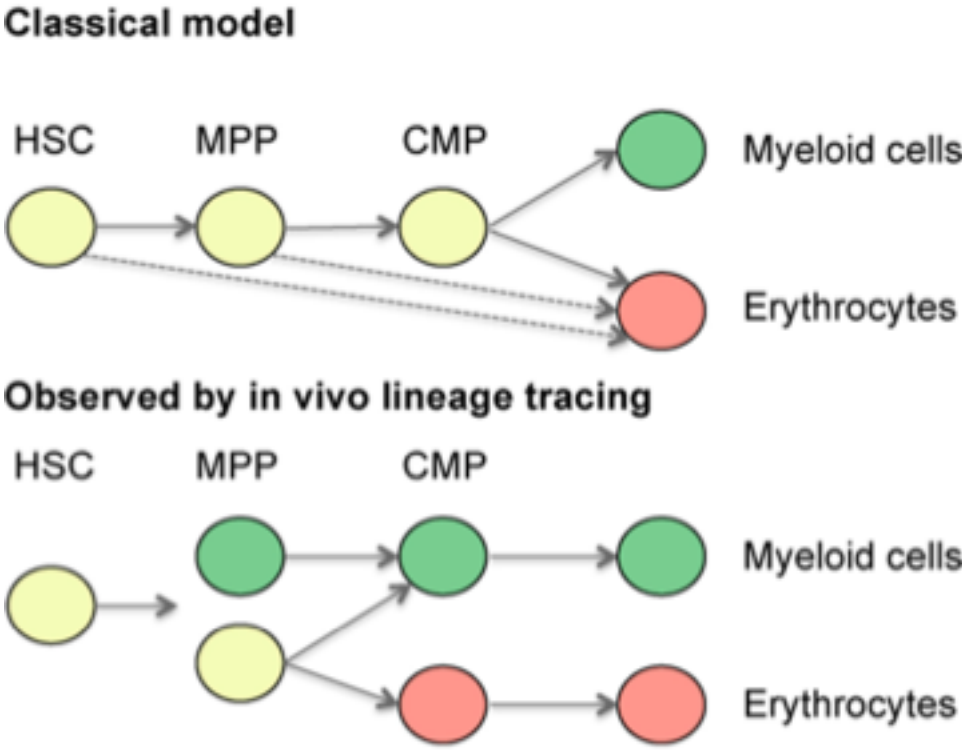
Kinetics of HSC self-renewal vs differentiation *in vivo*



Busch et al., Nature 2015

New quantitative approaches to address single-cell HSC fate

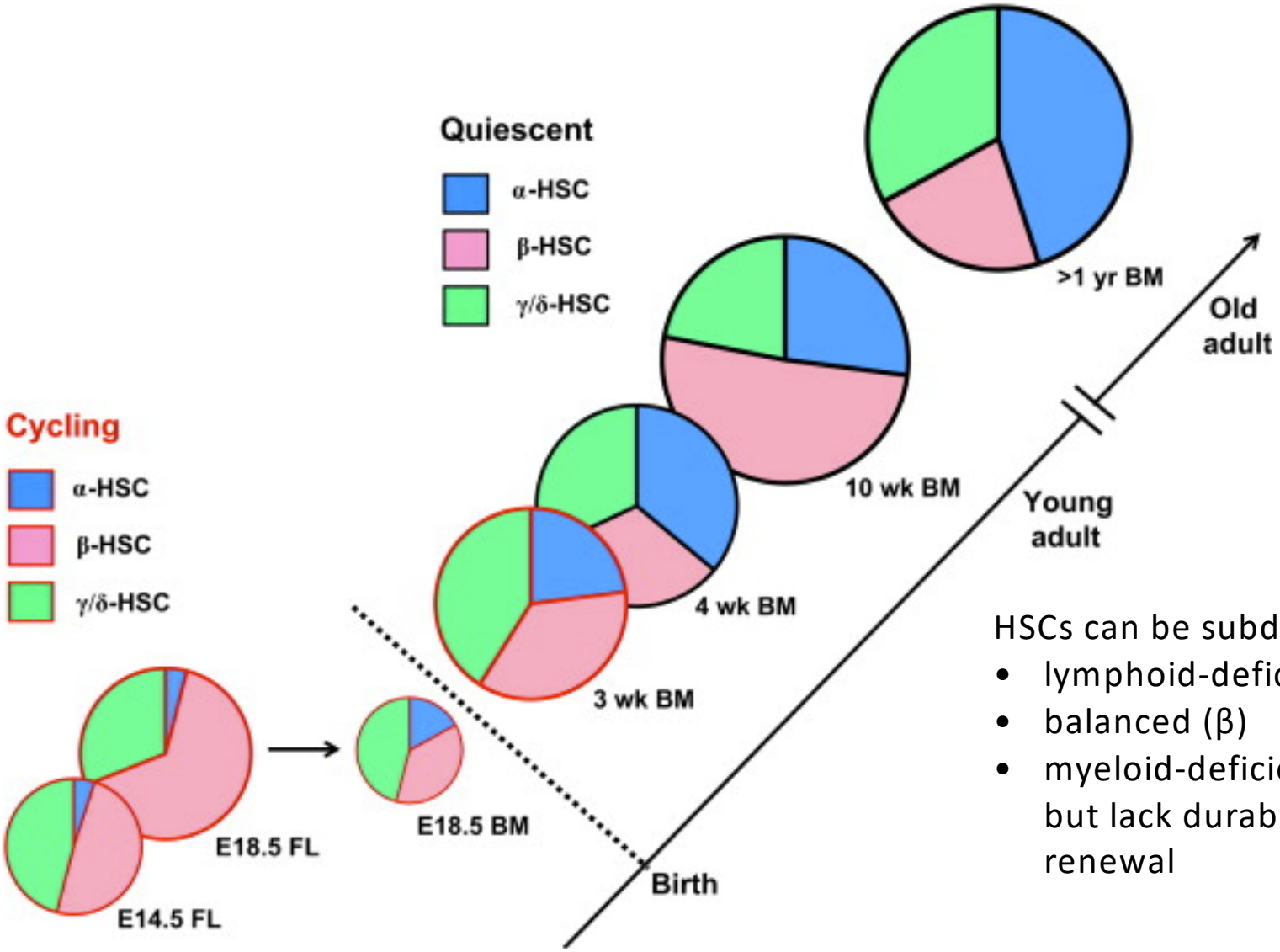




Höfer T, et al. 2016.
Annu. Rev. Immunol. 34:449–78

Peiré et al., Cell 2015

HSCs change throughout life



HSCs can be subdivided into:

- lymphoid-deficient (α)
- balanced (β)
- myeloid-deficient (γ+δ) but lack durable self-renewal

Benz et al., Cell Stem Cell, 10 (2012)

Comprendre l’hétérogénéité des macrophages tissulaires

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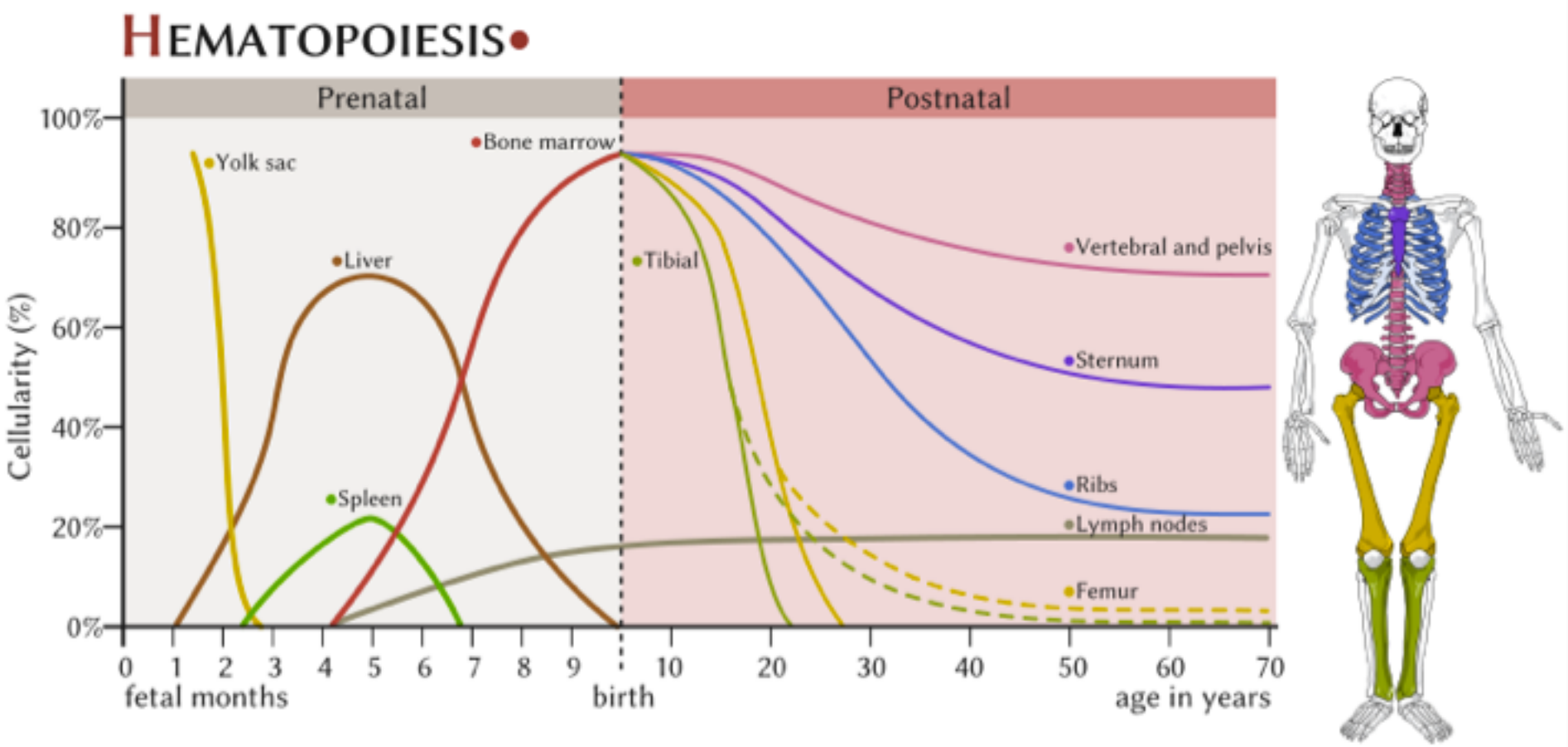
2- Développement du système hématopoïétique

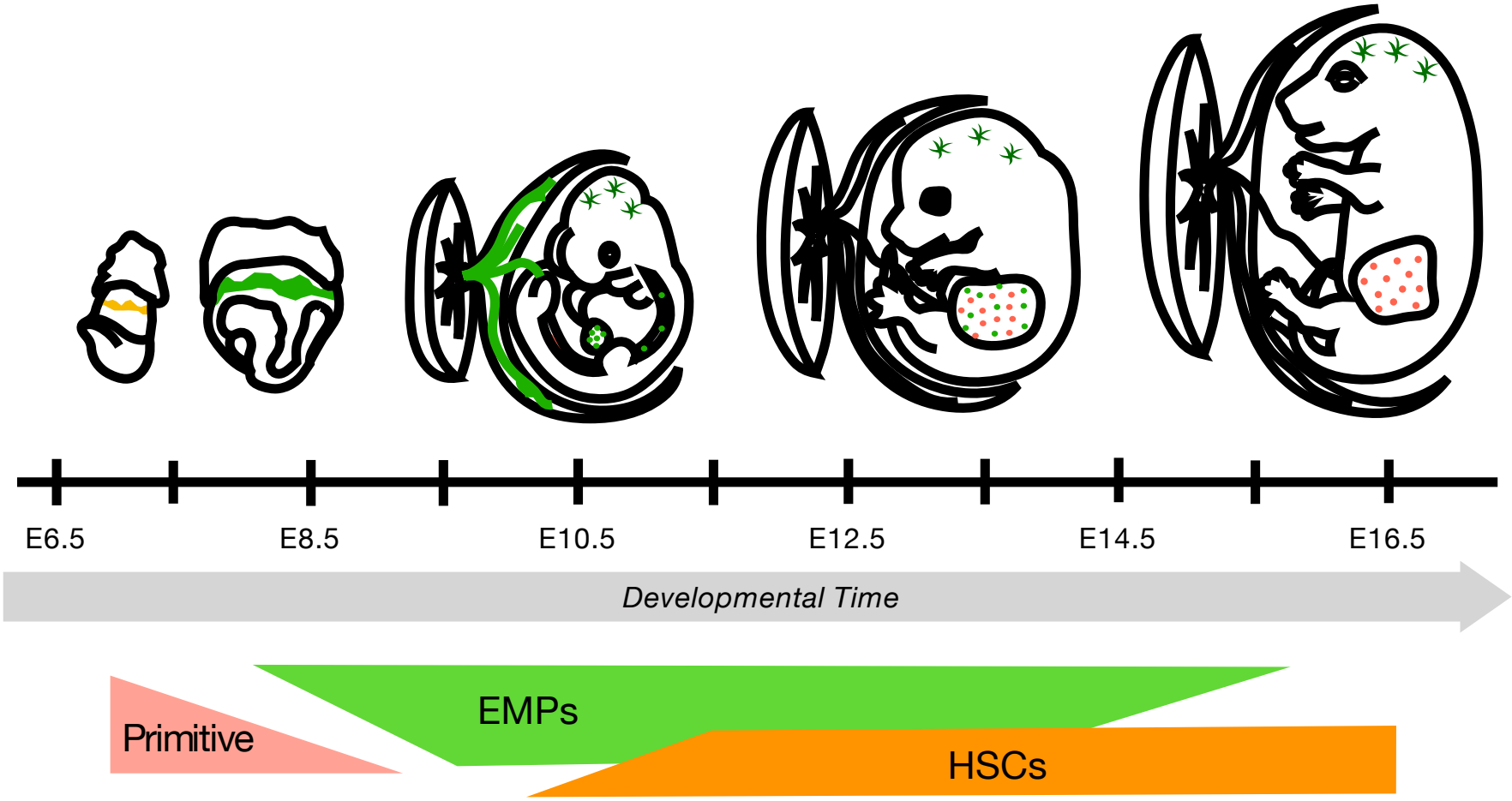
3- Macrophages tissulaires résidents

Développement du système hématopoïetique



Michał Komorniczak

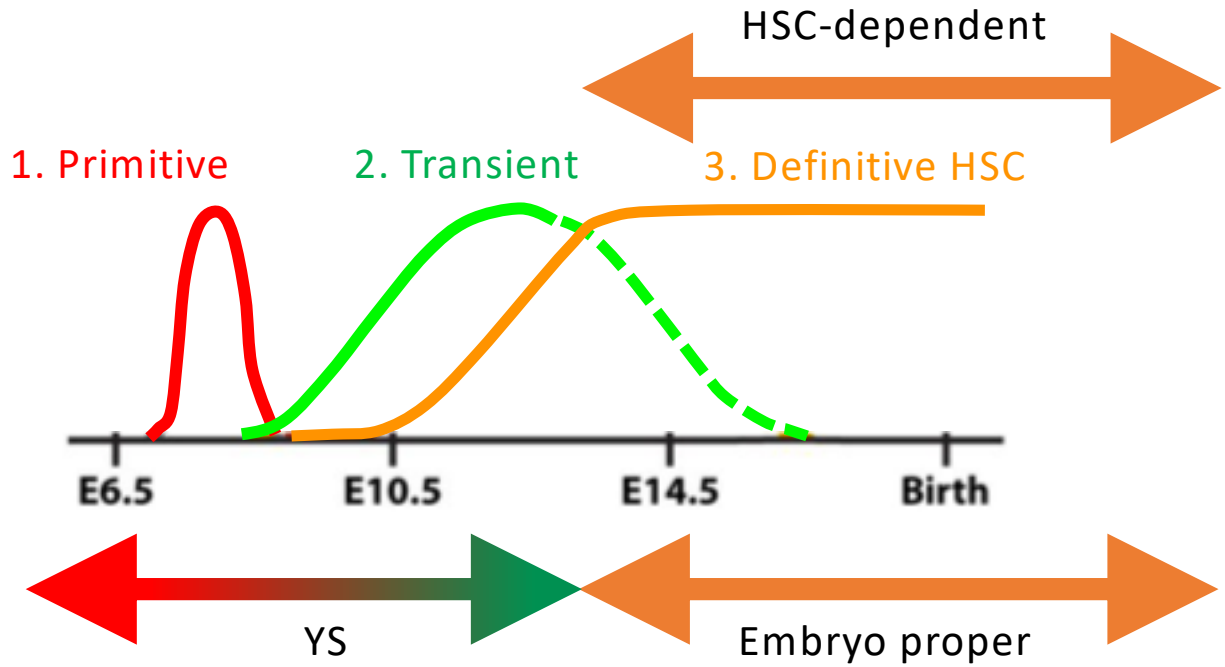
Les progéniteurs hématopoïétiques sont produits lors de trois vagues successives pendant le développement embryonnaire



EMP: Progéniteur Erythro-Myeloide

HSC: Cellule Souche Hématopoïétique

Les progéniteurs hématopoïétiques sont produits lors de trois vagues successives pendant le développement embryonnaire



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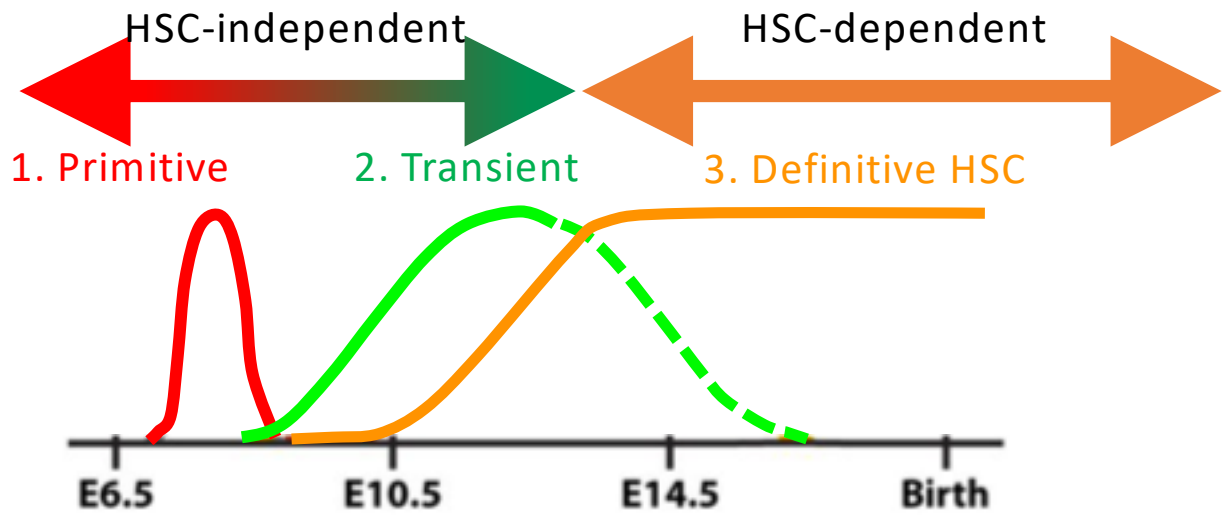

Site of emergence	YS	Embryo proper
Mechanism of emergence	Mesoderm	EHT
Site of expansion/diff.	YS	Fetal Liver
Diff. potential	Monopotent	Multipotent
Prolif. potential	Limited proliferation	Self-renewal

Différences entre HSCs and EMPs

EMPs have no lymphoid potential and cannot engraft irradiated recipient
EMPs do not self-renew

EMPs arise from endothelial cells in the yolk sac

Zovein et al., Cell Stem Cell 2008
Chen et al., Cell Stem Cell 2011
Frame et al., Stem Cells 2016
Kasaii et al., Sci Rep 2017

EMPs emerge both in the YS blood islands and the immature vascular plexus (venous and arterial)

EMPs do not require Notch signalling or blood flow

Bertrand et al., Blood 2010
Kasaii et al., Sci Rep 2017

Différences entre HSCs and EMPs

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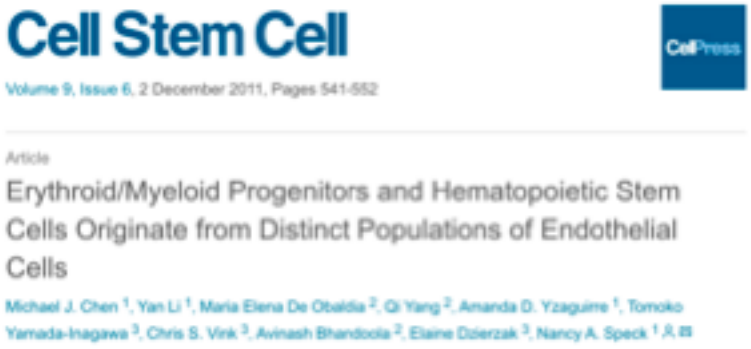
Kasaii et al., Sci Rep 2017

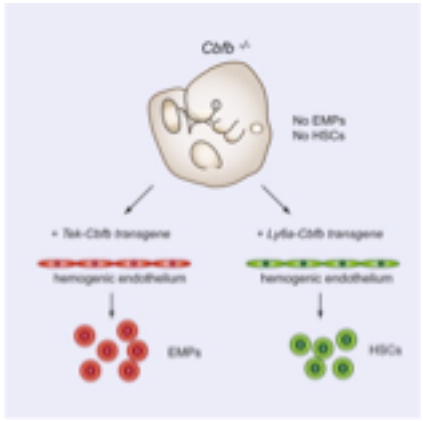
EMPs are sufficient to support fetal live until birth (erythrocyte production)

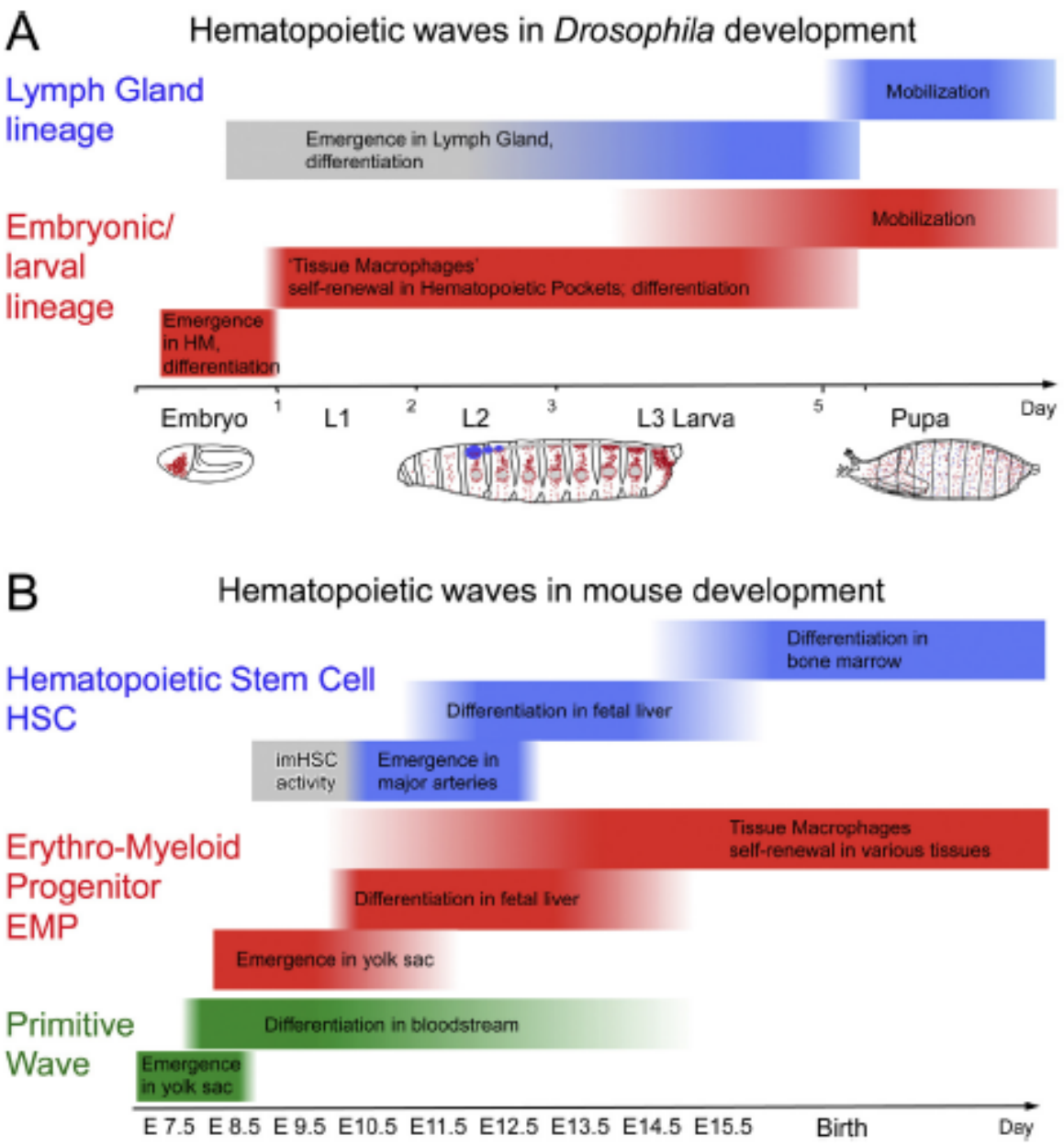

Cell Stem Cell
CellPress
Volume 9, Issue 6, 2 December 2011, Pages 541-552

Article

Erythroid/Myeloid Progenitors and Hematopoietic Stem Cells Originate from Distinct Populations of Endothelial Cells

Michael J. Chen [1], Yan Li [1], Maria Elena De Obaldia [2], Qi Yang [2], Amanda D. Yzaguirre [1], Tomoko Yamada-Inagawa [3], Chris S. Vink [3], Avinash Bhandoola [2], Elaine Dzierzak [3], Nancy A. Speck [1,4]





K.S. Gold, K. Brückner / Seminars in Immunology 27 (2015) 357–368

Comprendre l’hétérogénéité des macrophages tissulaires

1- Concepts généraux sur l’hématopoïese

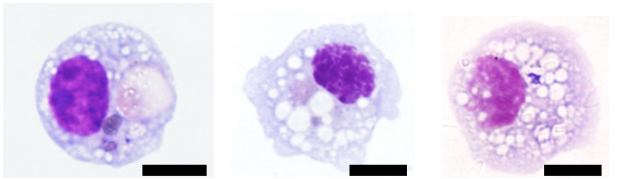
2- Développement du système hématopoïétique

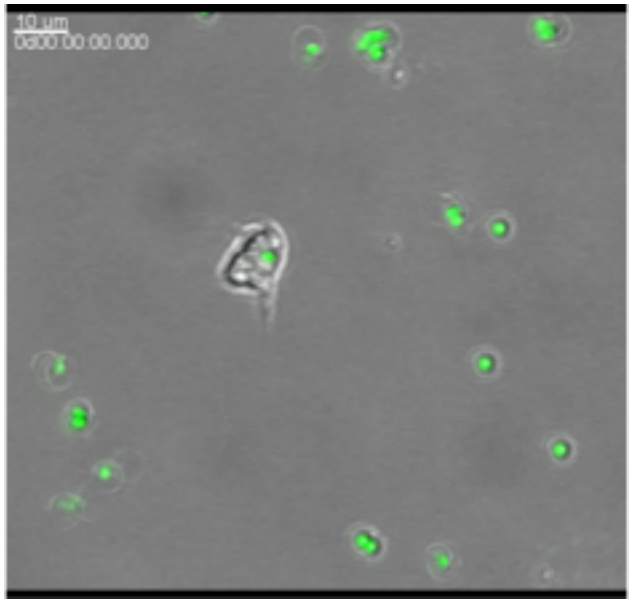
3- Macrophages tissulaires résidents

Définition d'un macrophage tissulaire résident

Phagocytes described by Ellie Metchnikoff at the end of 19th century

Liver Lung Skin



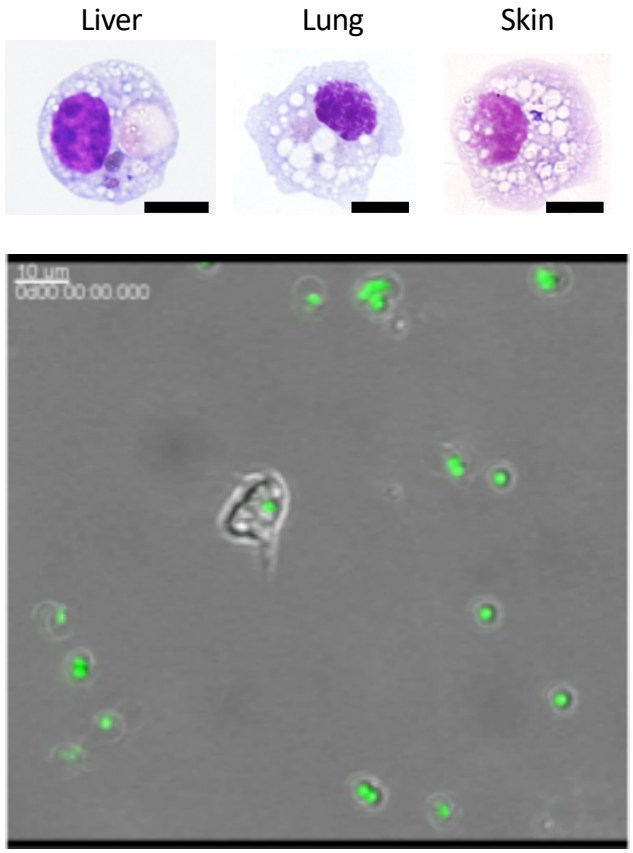


- Highly Phagocytic cells
- Sessile innate immune cells

Organ	Cell type
Brain	Microglia
Epidermis	Langerhans cells
Liver	Kupffer cells
Spleen	Red pulp macrophages
Lung	Alveolar macrophages
…	

Définition d'un macrophage tissulaire résident

Phagocytes described by Ellie Metchnikoff at the end of 19th century



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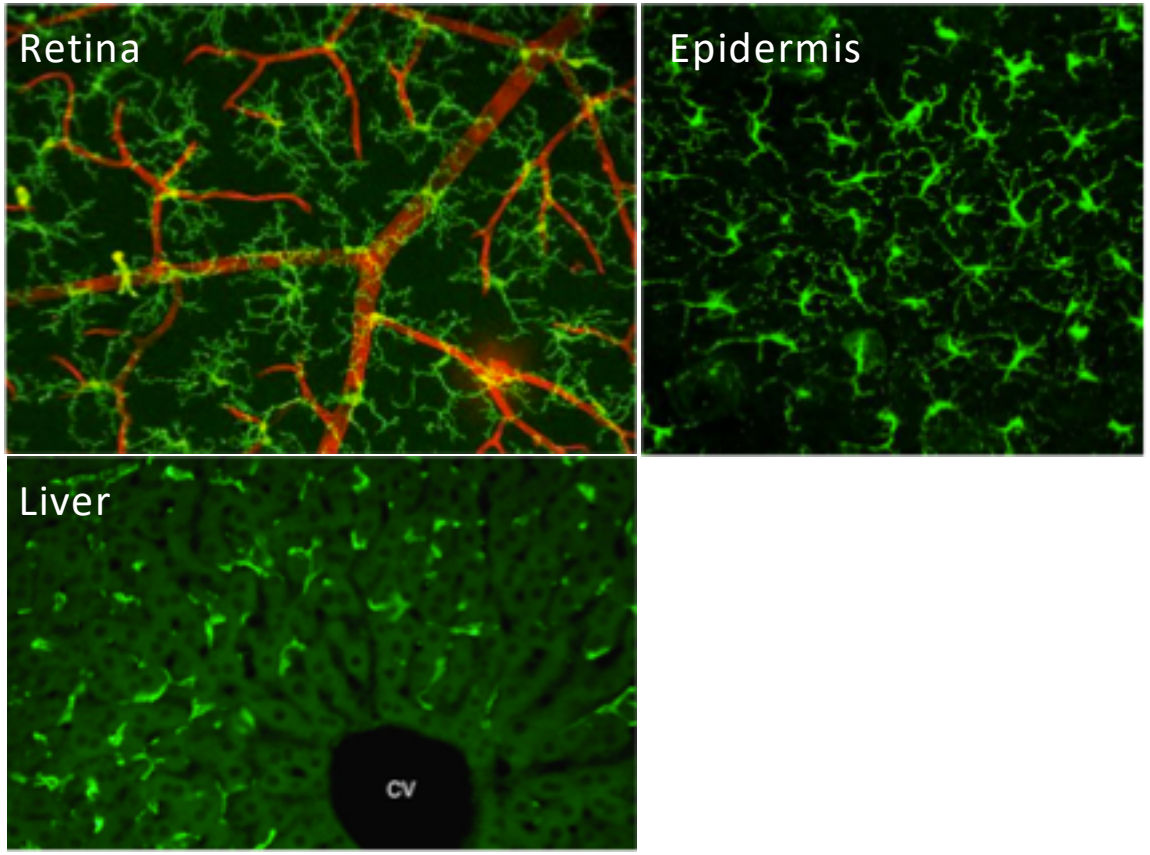
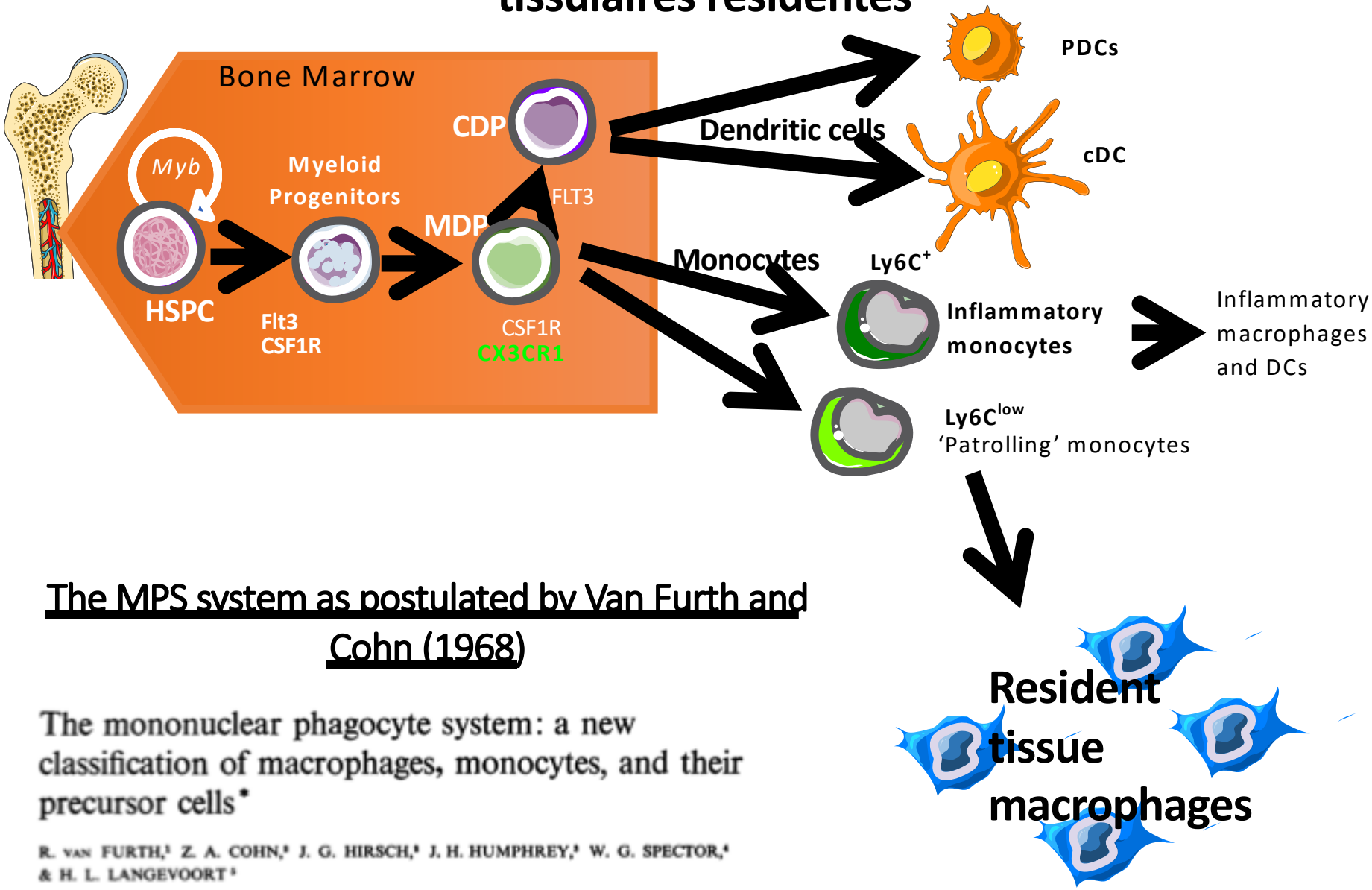


Ontogenèse du système des phagocytes mononucléaires: cellules tissulaires résidentes

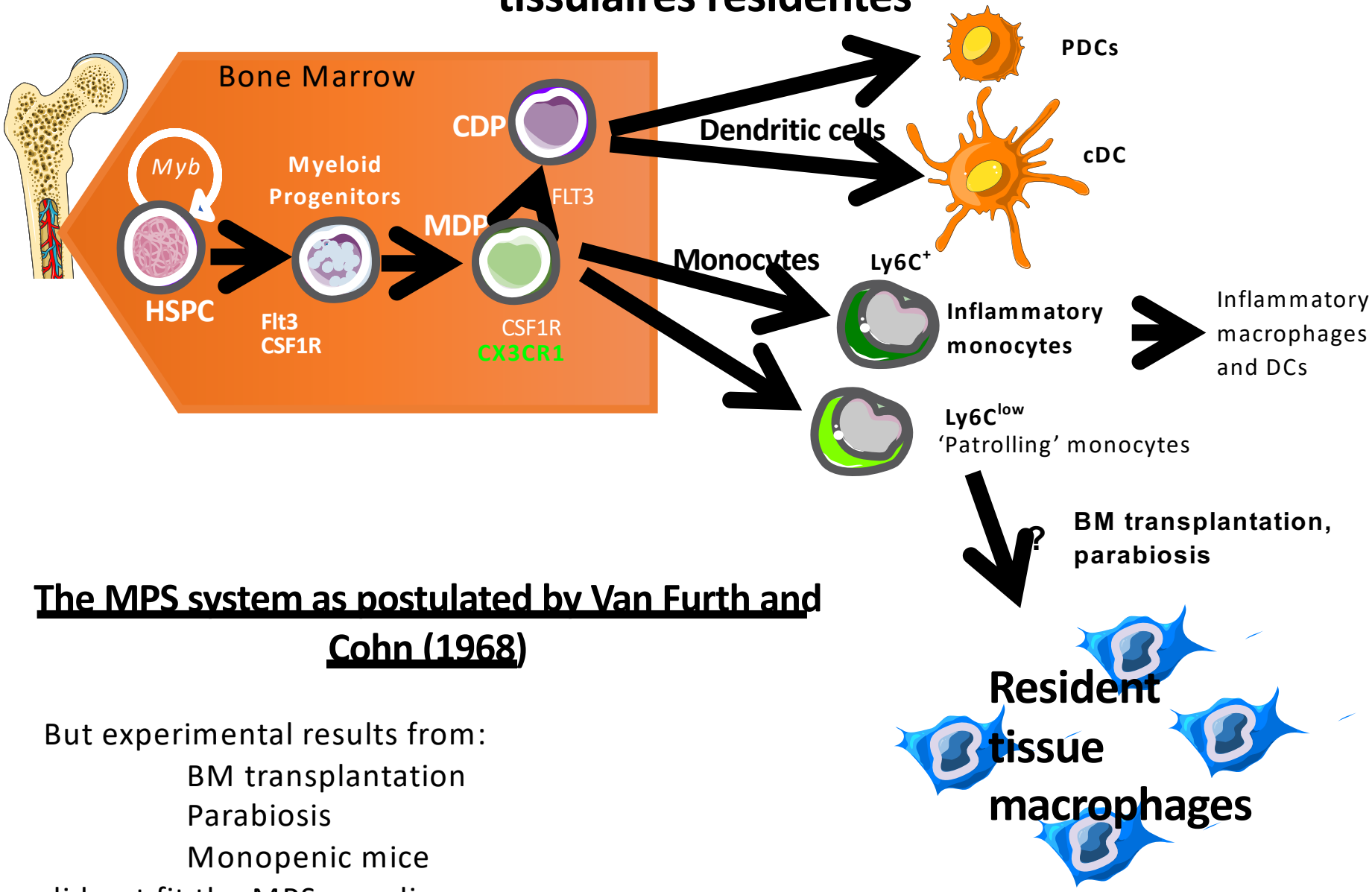


The MPS system as postulated by Van Furth and Cohn (1968)

The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells*

R. VAN FURTH,[1] Z. A. COHN,[2] J. G. HIRSCH,[2] J. H. HUMPHREY,[3] W. G. SPECTOR,[4] & H. L. LANGEVOORT[5]

Ontogenèse du système des phagocytes mononucléaires: cellules tissulaires résidentes



The MPS system as postulated by Van Furth and Cohn (1968)

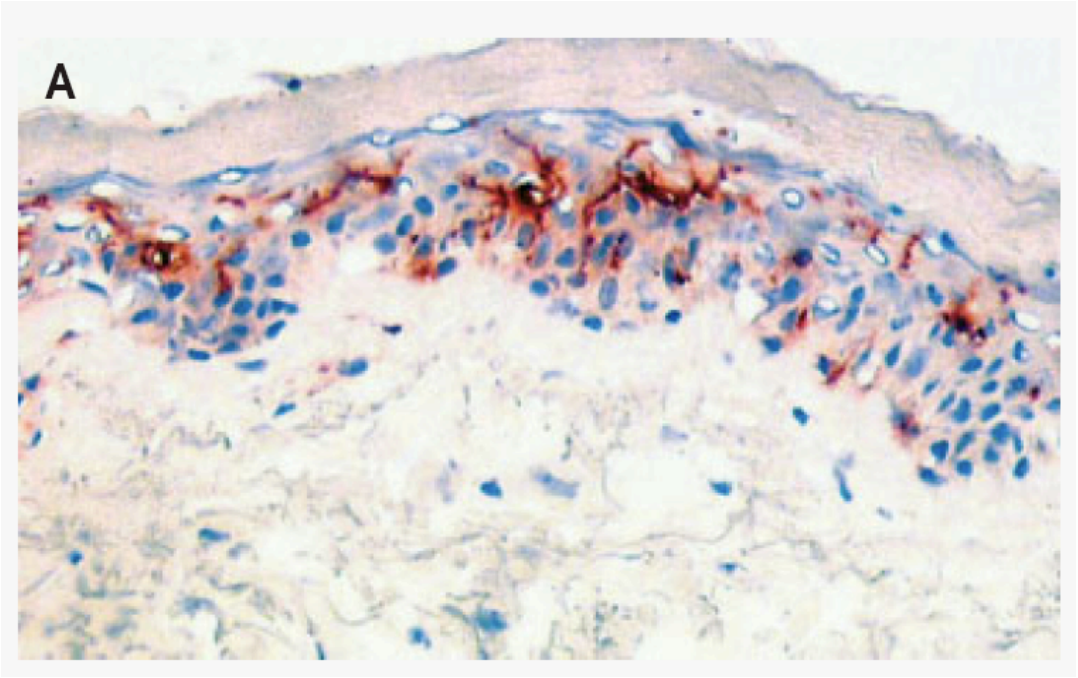
But experimental results from:

- BM transplantation
- Parabiosis
- Monopenic mice

did not fit the MPS paradigm

Les progéniteurs de la moelle osseuse ne remplacent pas les macrophages de la peau

Langerhans' cells detected in the epidermis of the allograft 4.5 years and 10 years after transplantation are of donor origin



Kanitakis et al., NEJM 2004
Kanitakiis et al., Experimental Dermatology 2011

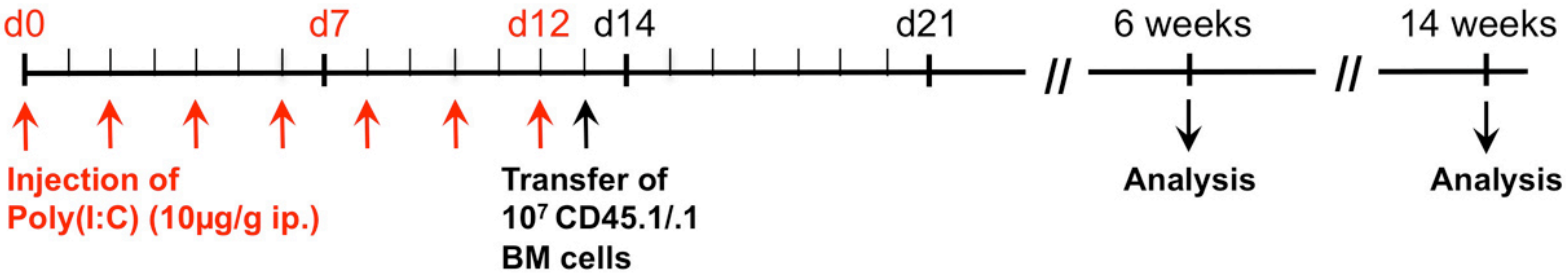
Can we test the validity of the MPS Paradigm?

- Do Resident Macrophages continuously renew from circulating precursors that arise in the adult BM from HSCs?

Methods

- Transplantation
- Fate mapping / Pulse labelling
- (Long term) single- cell tracking
- Potential assays at the population or single-cell based

Délétion conditionnelle de *Myb* dans les souris adultes *MxCre; Myb*$^{flox/flox}$



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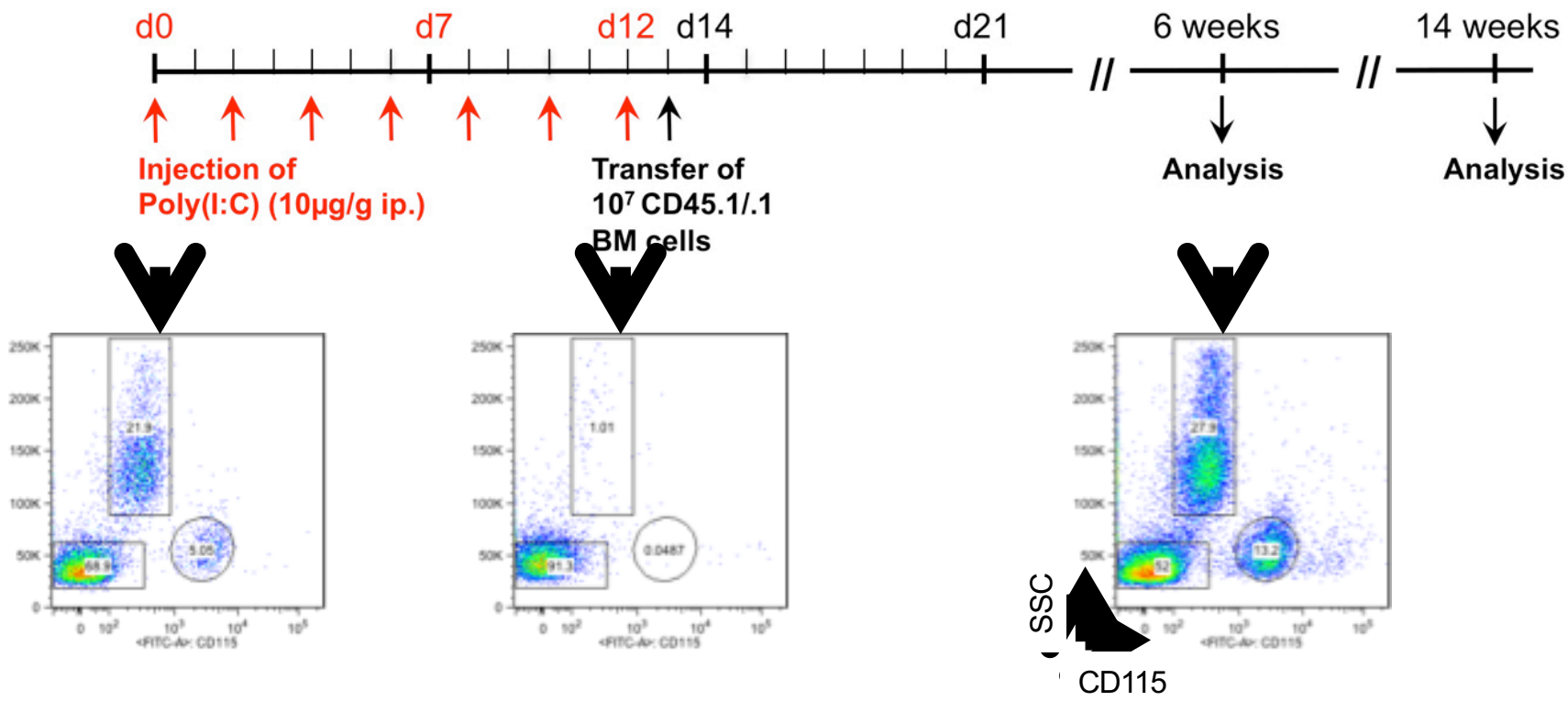
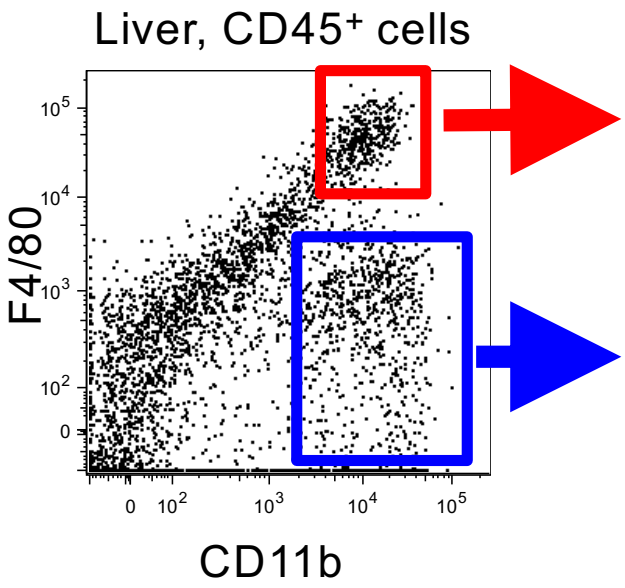
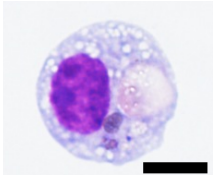


Cellules myeloides dans les tissus adultes

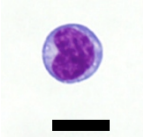


F4/80^{bright} cells

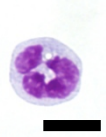


CD11b^{high} F4/80^{low} cells

Monocytes



Neutrophils

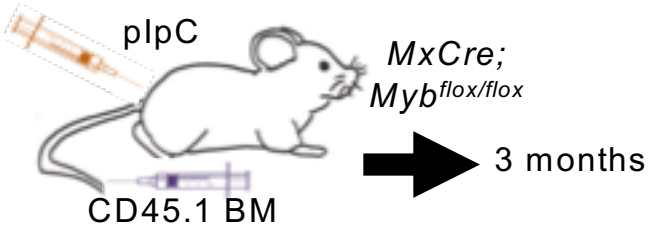


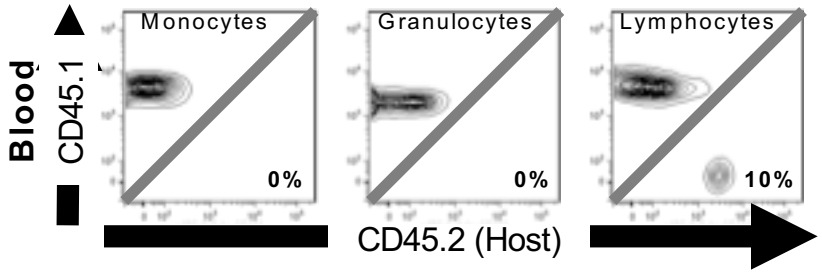
Eosinophils

Dendritic cells

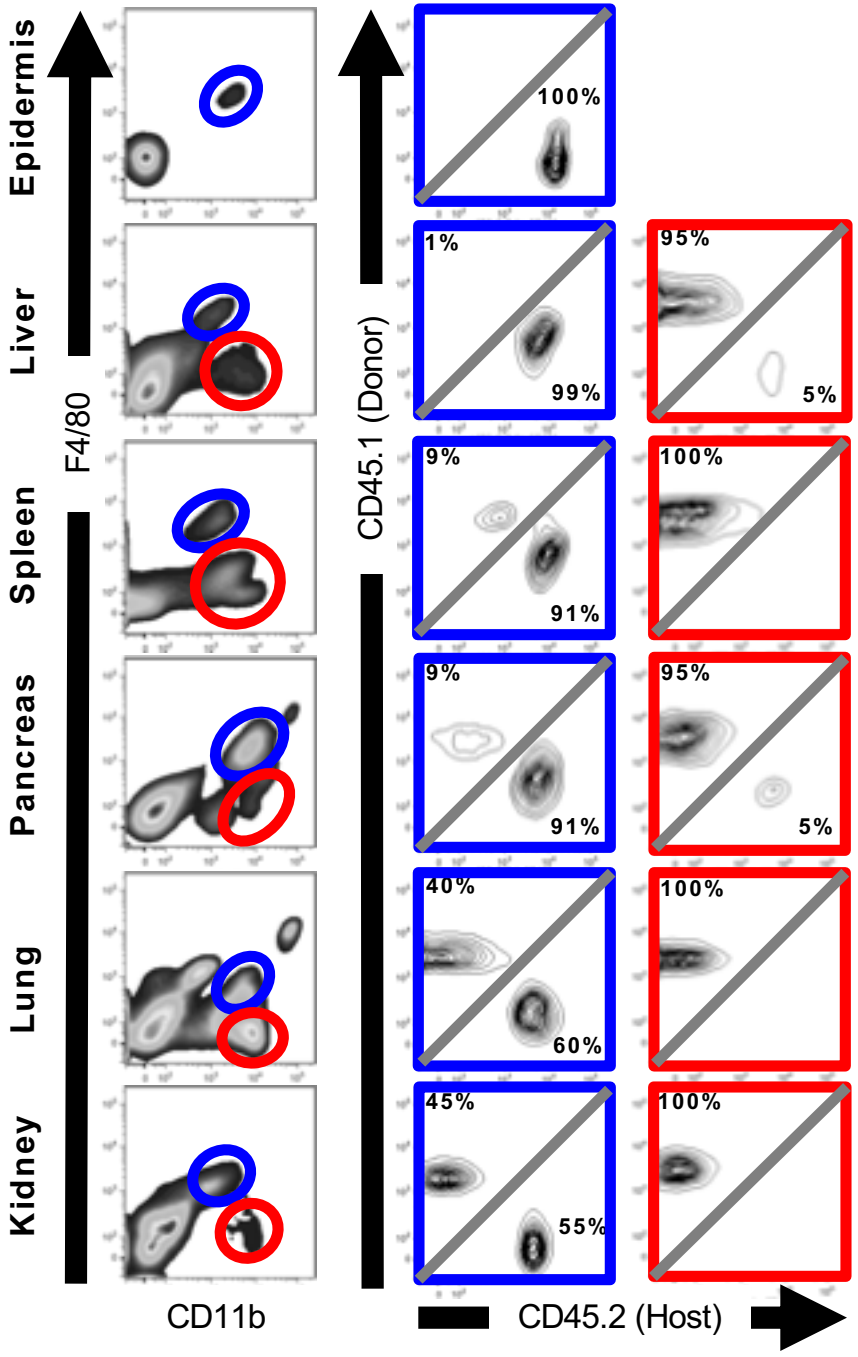
Délétion conditionnelle de *Myb* dans les souris adultes *MxCre; Myb*$^{flox/flox}$

Host F4/80^{bright} macrophages persist in adult tissues in the absence of MYB and independently of HSCs,
even in the presence of wt donor HSCs



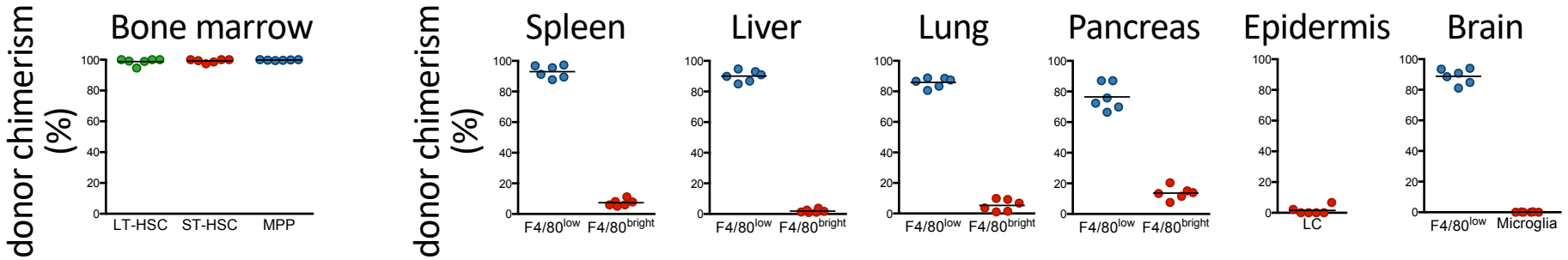


Schulz*, Gomez Perdiguero*, *et al.*, ***Science***, 2012



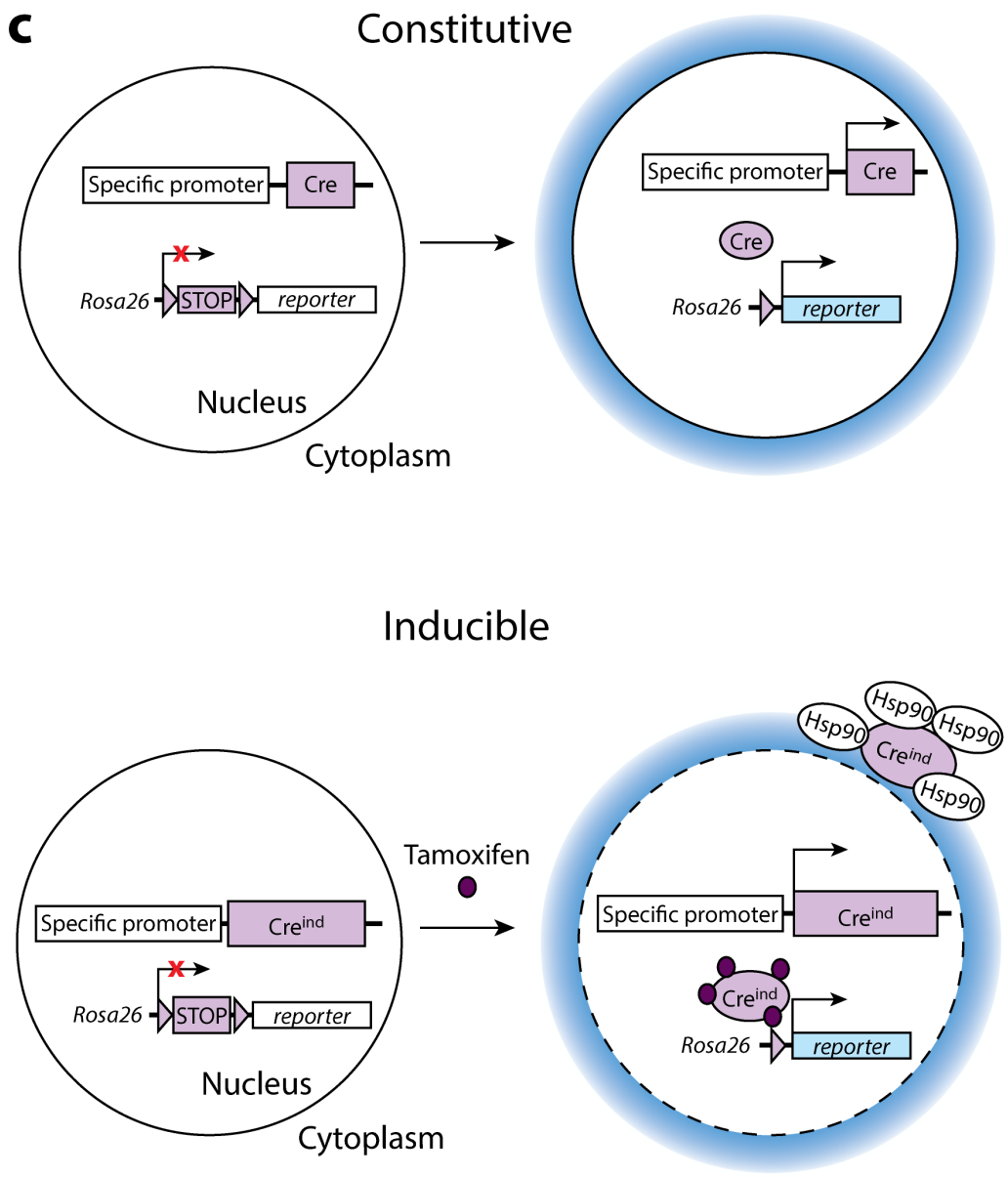
Les macrophages résidents ne dérivent pas de HSCs adultes

Non irradiation Chimera



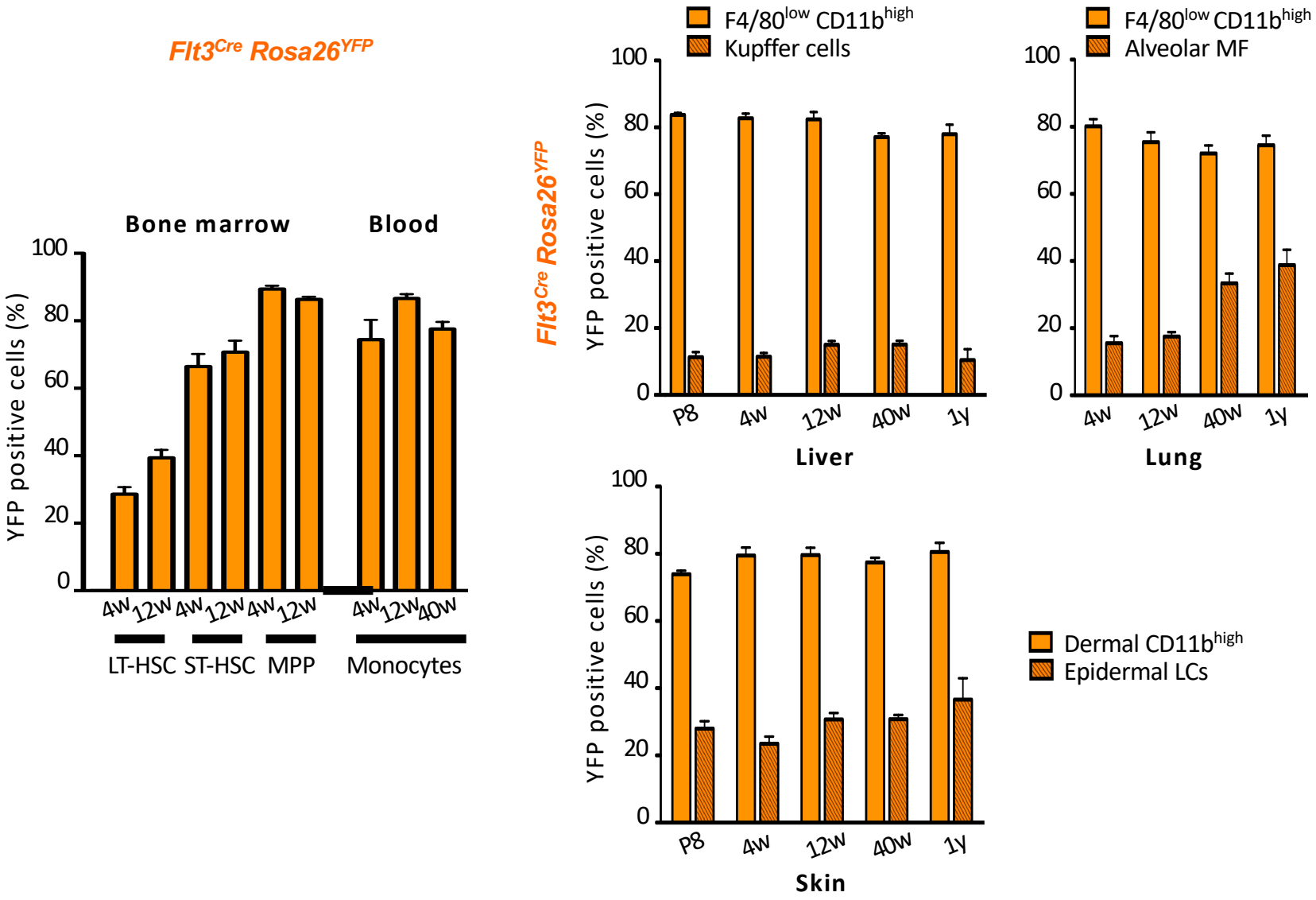
Gomez Perdiguero*, Klapporth* *et al.*, ***Nature***, 2015

Fate mapping reposant sur les systèmes Cre/Lox



Les macrophages résidents ne dérivent pas de HSCs adultes

Fate mapping of HSC-derived cells



Gomez Perdiguero*, Klapporth* *et al.*, ***Nature***, 2015

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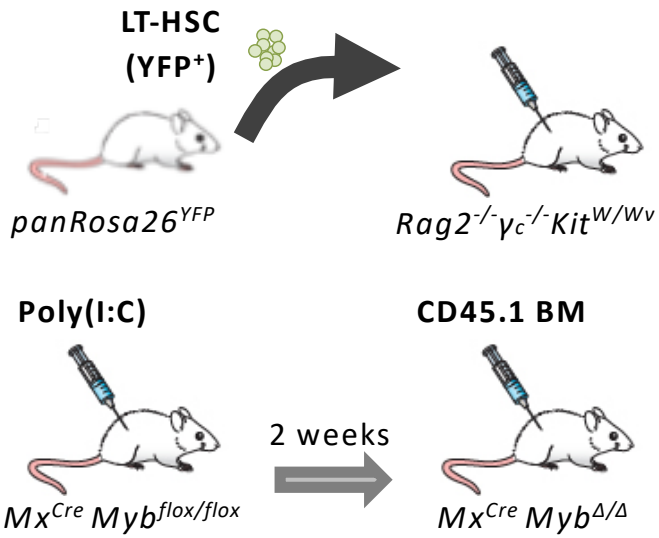


Schulz*, Gomez Perdiguero*, *et al.*, ***Science***, 2012

Gomez Perdiguero*, Klapporth* *et al.*, ***Nature***, 2015

Fate mapping of HSC-derived cells

Flt3^{Cre} Rosa26^{YFP}	Schulz*, Gomez Perdiguero*, *et al.*, ***Science***, 2012
S100a4^{Cre} Rosa26^{YFP}	Hashimoto *et al.*, ***Immunity***, 2013
CX$_3$Cr1$^{Cre\ and\ CreERT2}$ Rosa26^{YFP}	Yona *et al.*, ***Immunity***, 2013
Kit$^{Mer\text{-}iCre\text{-}Mer}$ Rosa26^{YFP}	Sheng *et al.*, ***Immunity***, 2015

Parabiosis

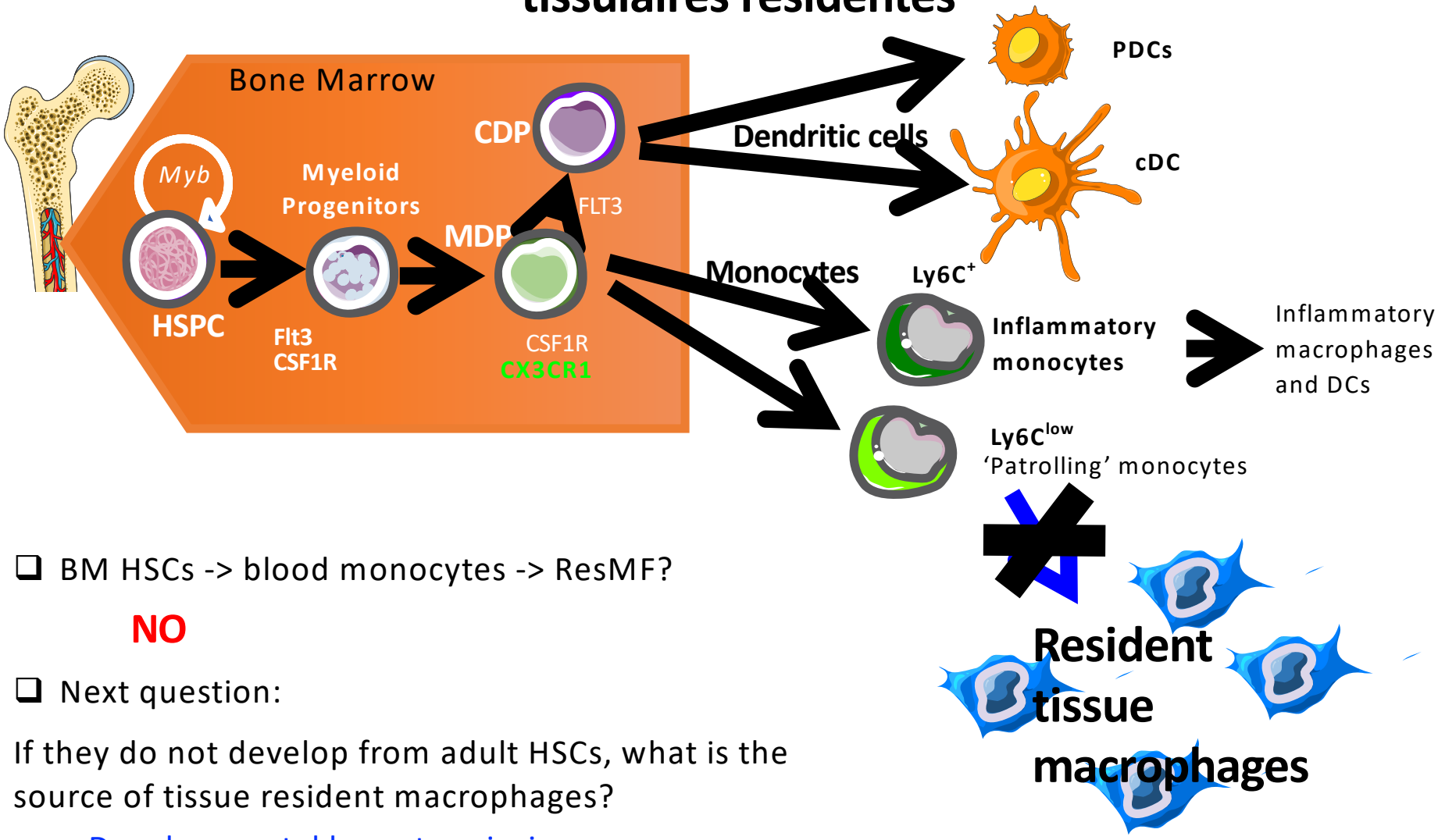
Hashimoto *et al.*, ***Immunity***, 2013

Tissue resident macrophages do not equilibrate with blood monocytes or BM progenitors in all tissues but … the intestinal lamina propria

what about other mucosal macrophages?

Varol *et al.*, ***JEM***, 2007
Bain *et al.* ***Nat Immunol*** 2014

Ontogenèse du système des phagocytes mononucléaires: cellules tissulaires résidentes



- BM HSCs -> blood monocytes -> ResMF?

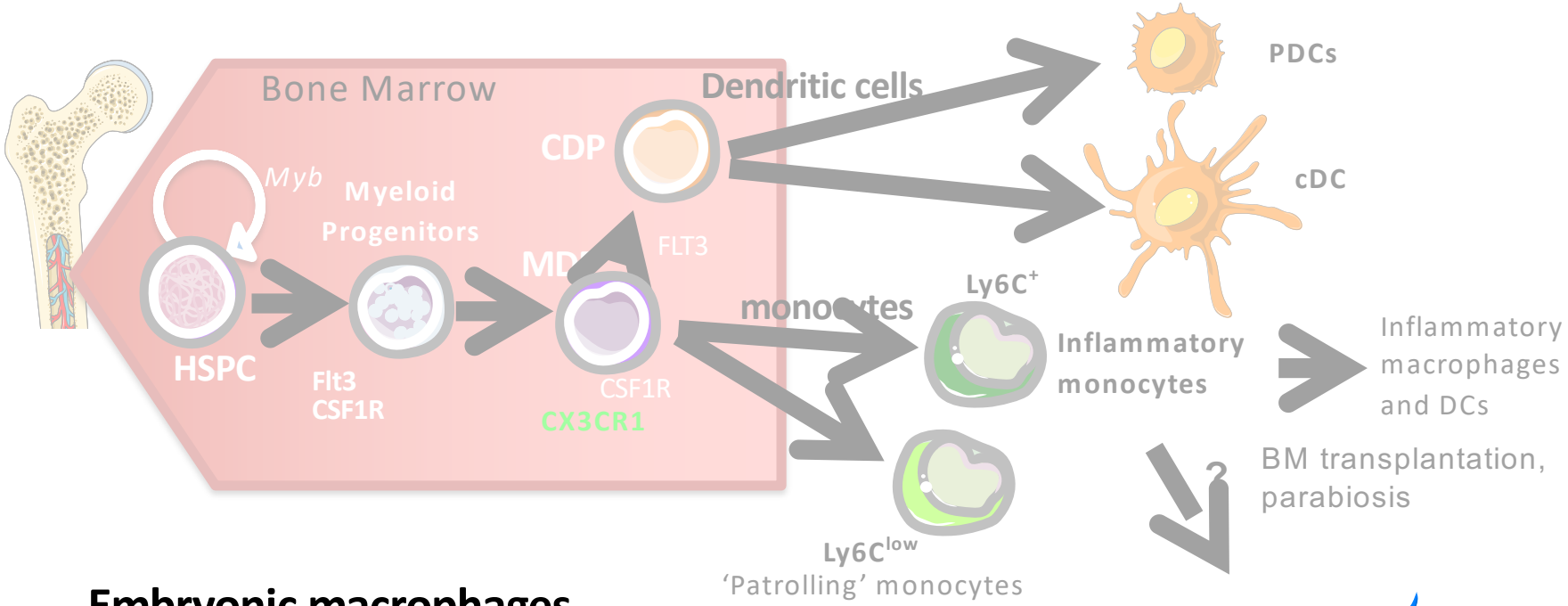
NO

- Next question:

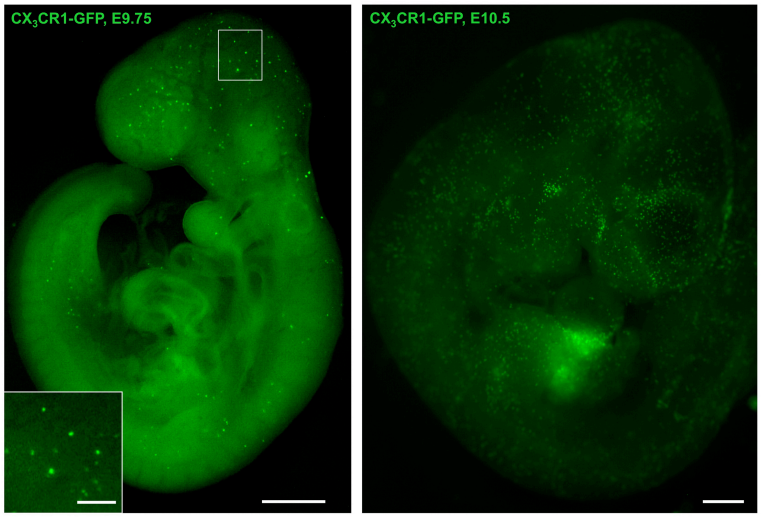
If they do not develop from adult HSCs, what is the source of tissue resident macrophages?

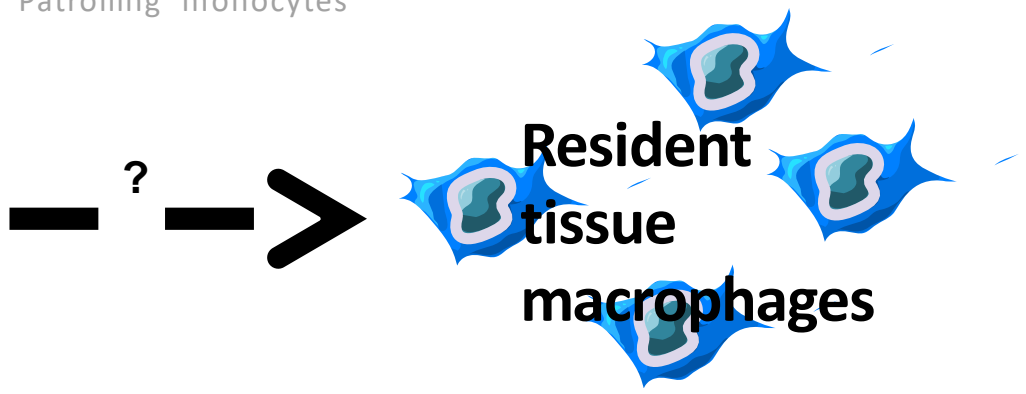
Developmental hematopoiesis

Ontogenèse du système des phagocytes mononucléaires



Embryonic macrophages





Ontogenèse du système des phagocytes mononucléaires

Macrophages emerge in the embryo before HSCs

Moore & Metcalf, Br J Haematol 1970
Takahashi *et al.*, JLB 1989
Naito *et al.*, JLB 1990

Ashwell *et al.*, Brain Res. Dev Brain Res 1991
Sorokin *et al.*, Anat. Rec. 1992

Cuadros *et al.*, Development 1992
Alliot *et al.*, Brain Res. Dev Brain Res 1999
Herbomel *et al.*, Development 1999

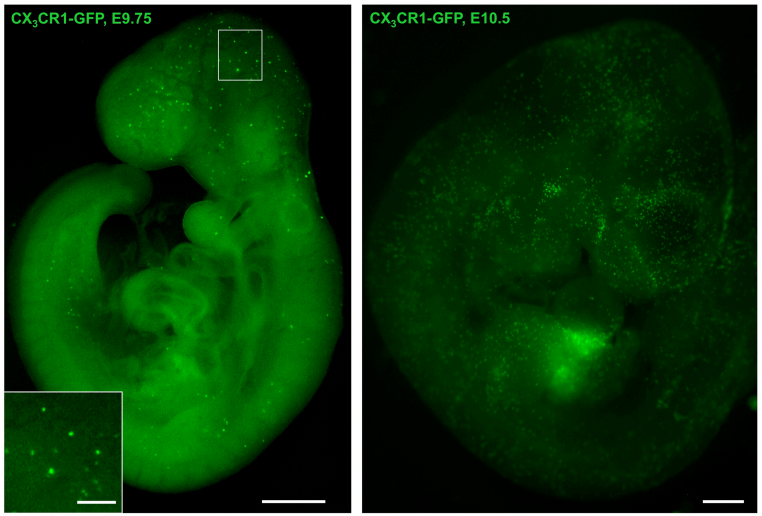
Bertrand *et al.*, Blood 2005

Fetal HSC

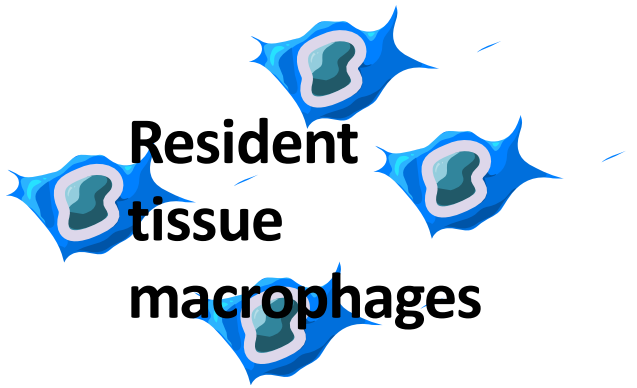
Hematopoietic
Stem
Cell

?

Embryonic macrophages

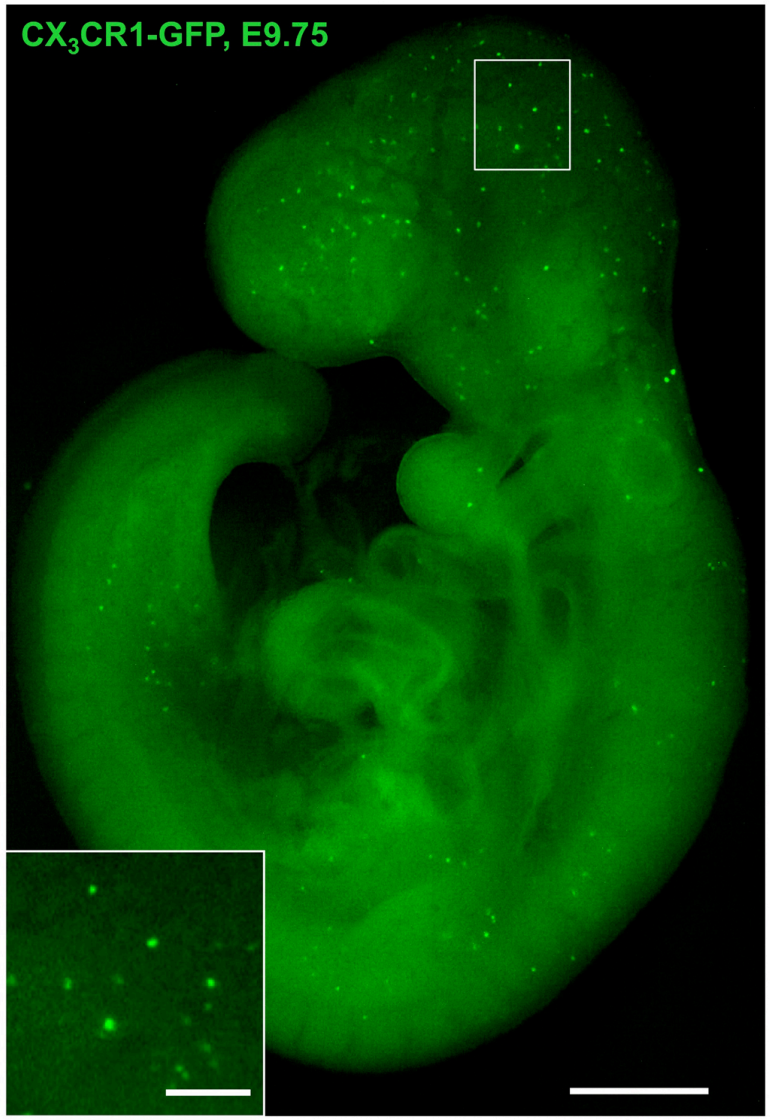


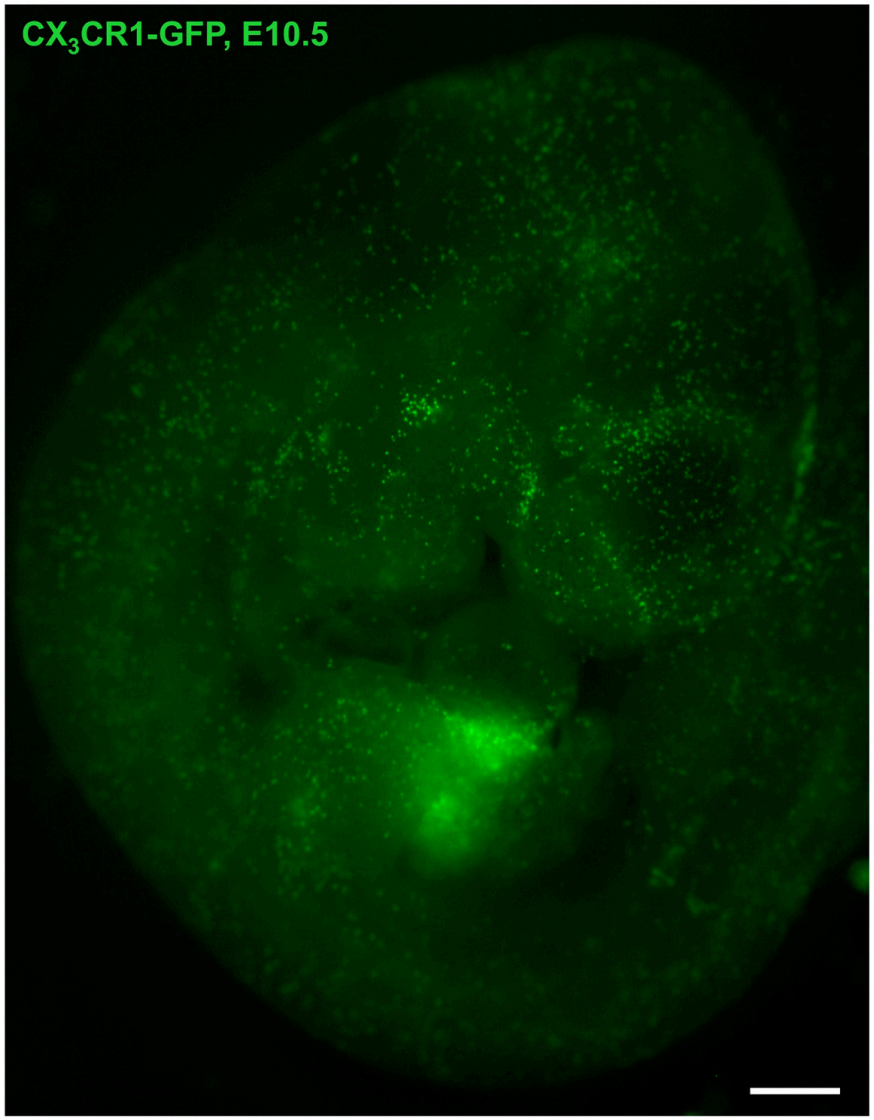
?



Brain Microglia
Ginhoux *et al.*, Science 2010

Les macrophages CX3CR1^{gfp} colonisent l'embryon E10.5



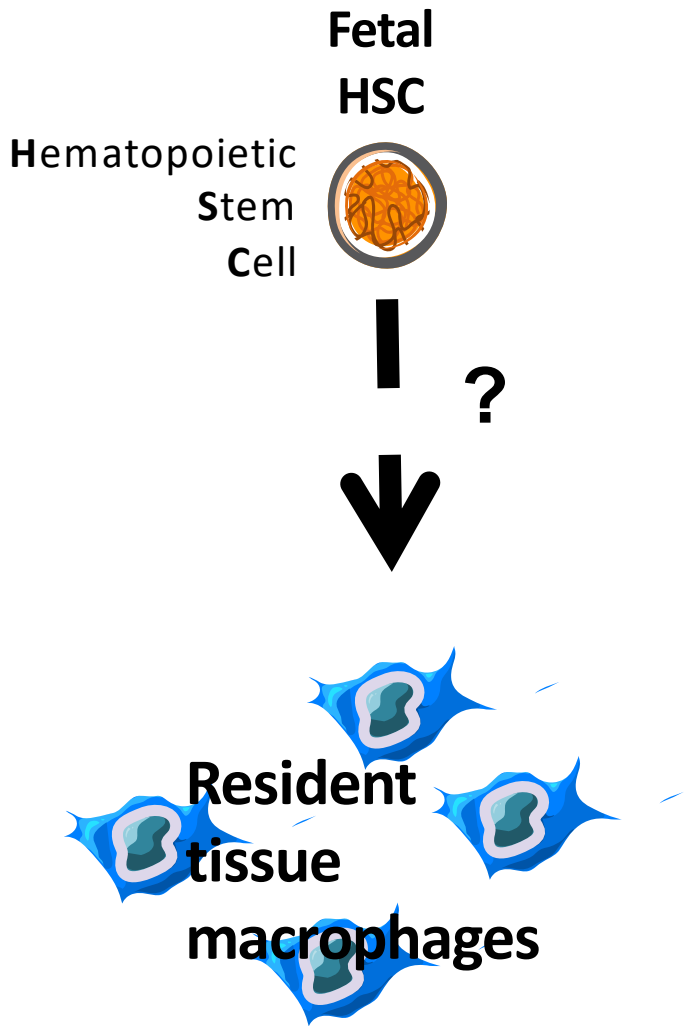


CX3CR1^{gfp}

Ontogenèse du système des phagocytes mononucléaires

Do adult resident Macrophages derive from fetal HSCs?

- Analysis of mutant embryos lacking HSCs
- Pulse labelling of fetal HSCs



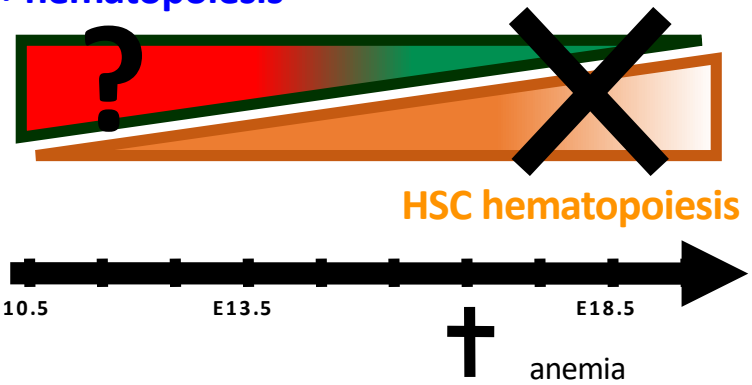
Les Macrophages se développent dans l'absence de HSCs fœtales

Myb$^{-/-}$

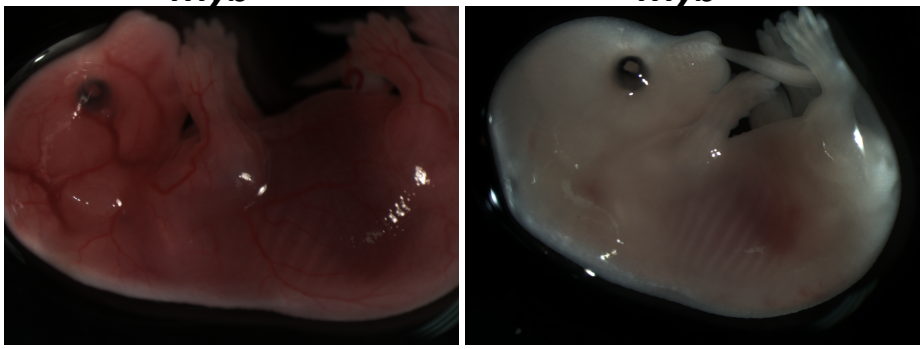
Y. Mukouyama et al., Curr Biol 9, 833 (1999).

M. L. Mucenski et al., Cell 65, 677 (1991).
R. Sumner, et al, Oncogene 19, 3335 (2000).

« YS » hematopoiesis



Myb$^{+/+}$ *Myb*$^{-/-}$



***Myb*$^{-/-}$ lack HSC and definitive erythropoiesis**

Les Macrophages se développent dans l'absence de HSCs fœtales

Myb$^{-/-}$

Y. Mukouyama et al., Curr Biol 9, 833 (1999).
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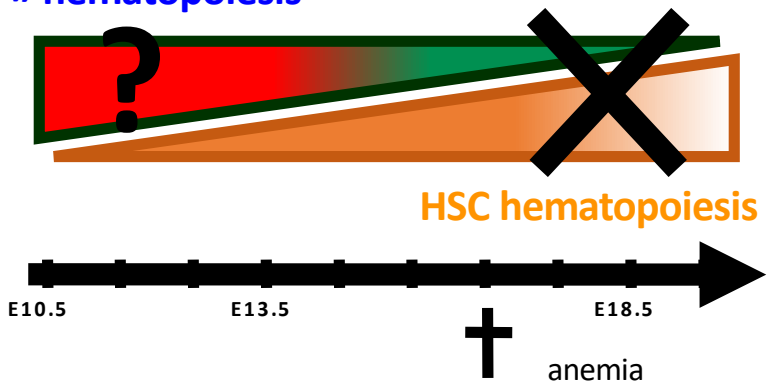
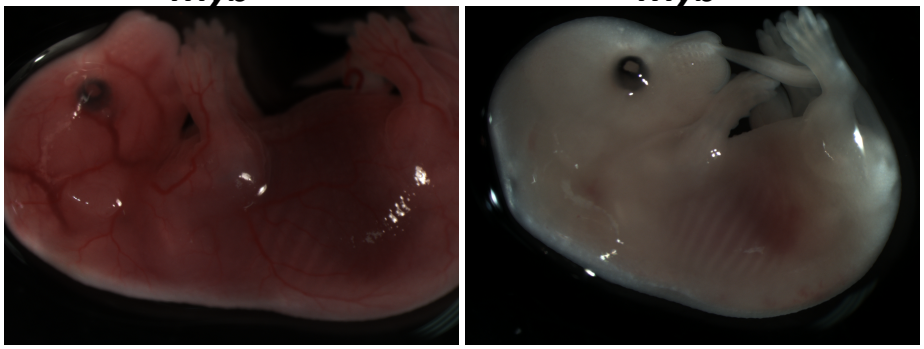
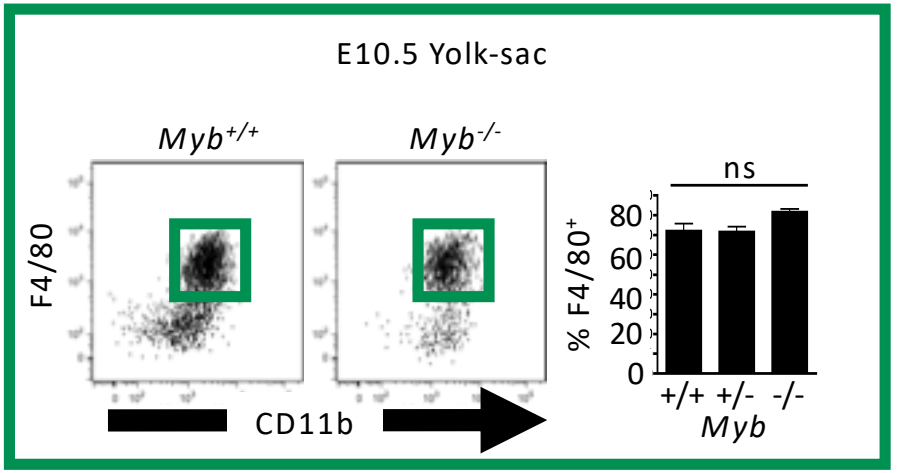
« YS » hematopoiesis

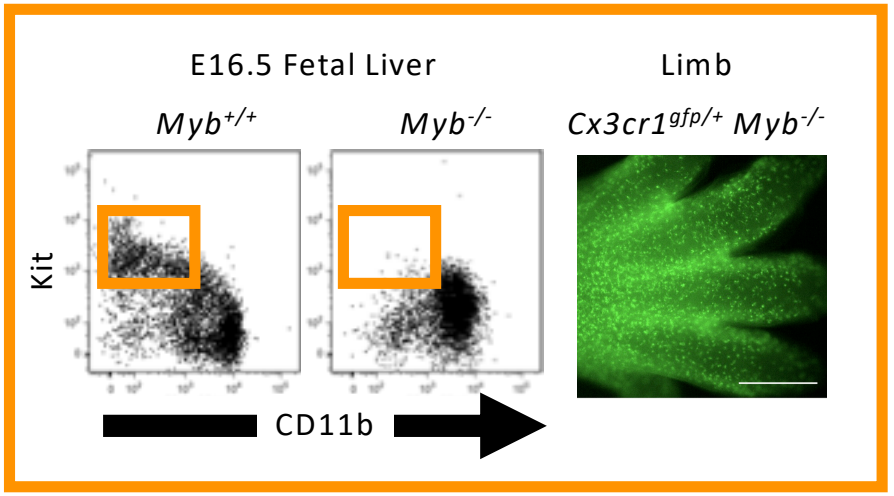


Myb$^{+/+}$ *Myb*$^{-/-}$



Myb$^{-/-}$ lack HSC and definitive erythropoiesis





F4/80^{bright} macrophages are *Myb*-independent

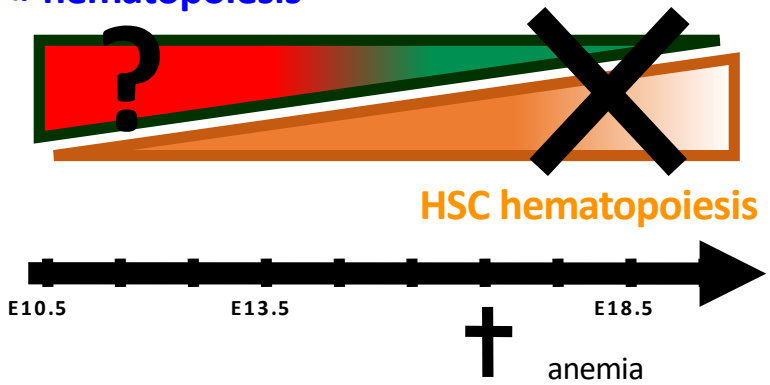
Schulz*, Gomez Perdiguero*, *et al.*, ***Science***, 2012

Les Macrophages se développent dans l'absence de HSCs fœtales

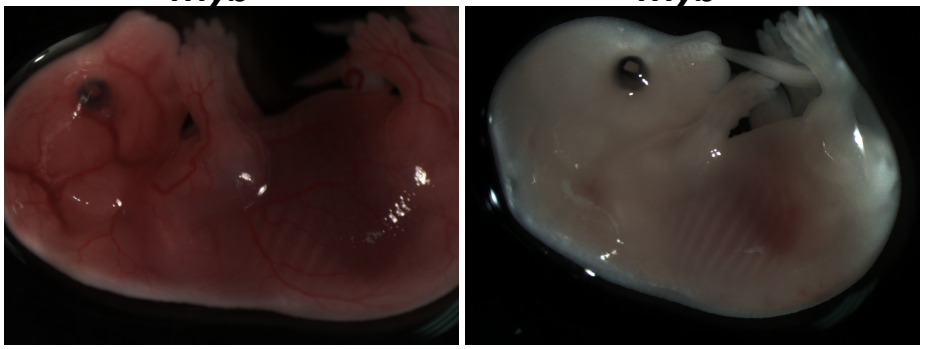
***Myb*$^{-/-}$**

Y. Mukouyama et al., Curr Biol 9, 833 (1999).
M. L. Mucenski et al., Cell 65, 677 (1991).
R. Sumner, et al, Oncogene 19, 3335 (2000).

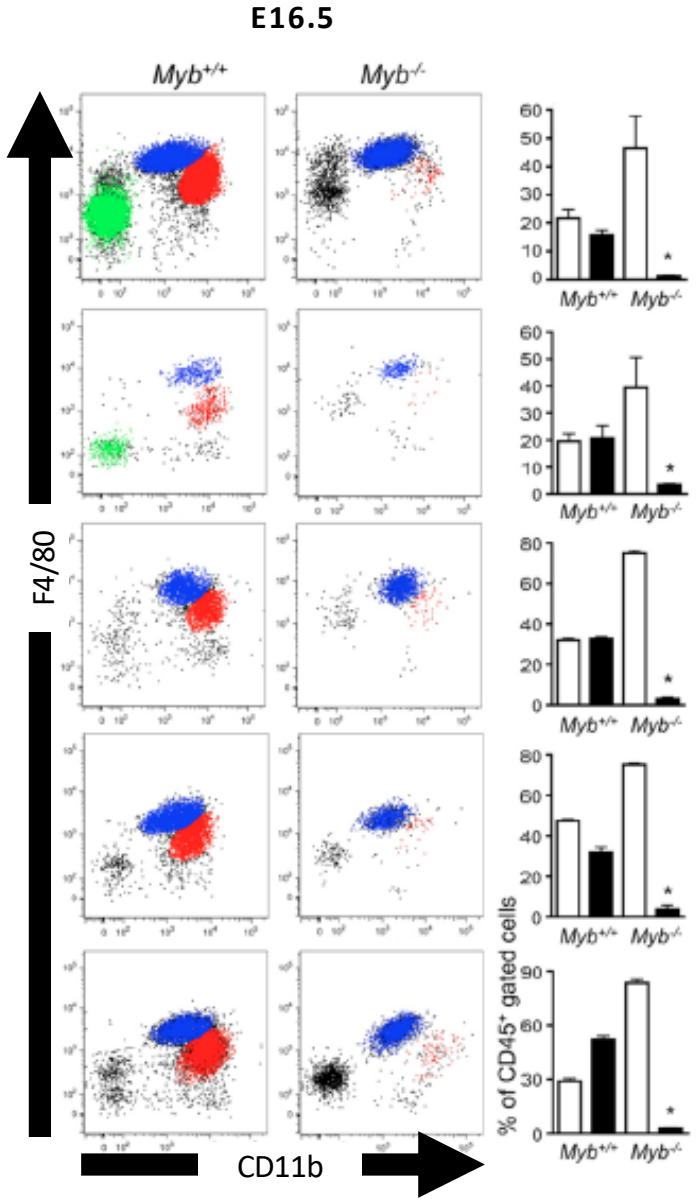
« YS » hematopoiesis



***Myb*$^{+/+}$** ***Myb*$^{-/-}$**



F4/80^{bright} macrophages are *Myb*-independent



Schulz*, Gomez Perdiguero*, *et al.*, ***Science***, 2012

Evaluation de la contribution des HSCs fœtales aux macrophages résidents

Tie-2 is an TK receptor for Angiopoietin-1 and -2 expressed by

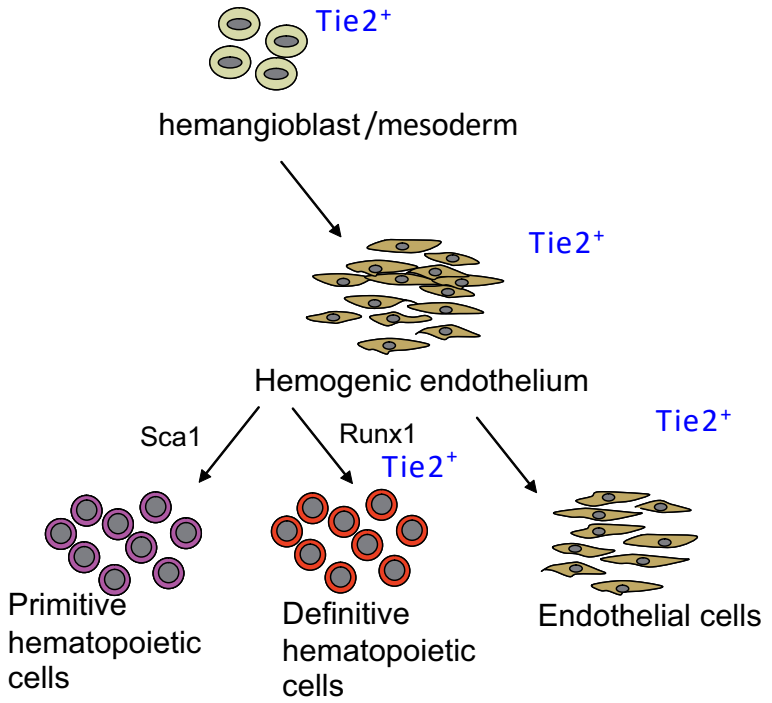
- endothelial cells
- Bone marrow HSCs
- Short lived myeloid progenitors

Tie-2 is expressed during embryonic development by

- YS mesoderm (as early as E6.5)
- endothelial cells & HSCs (AGM and FL)
- "hemangioblast" and/or hemogenic endothelium

Tie-2 is **not** expressed during embryonic development by

- E10.5 YS macrophages
- E16.5 lung, skin and kidney macrophages

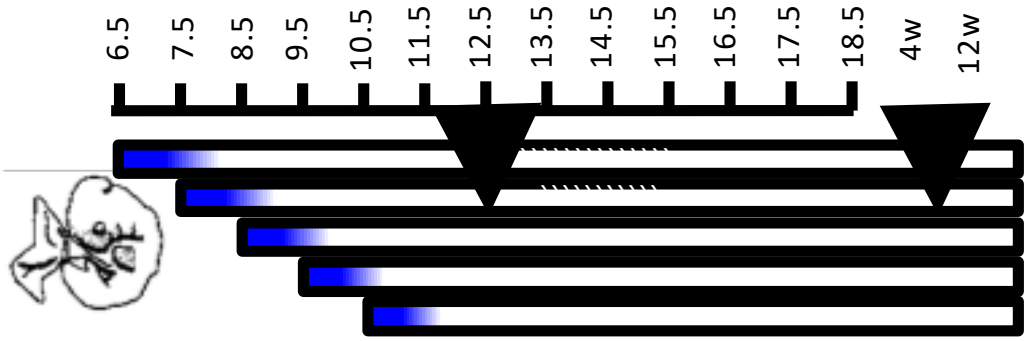


Hueno & Weissman, Int. J. Dev. Biol. (2010)
Cumano & Godin, Annual Review of Immunology (2007)

Pulse-Labeling of Tie2+ progenitors

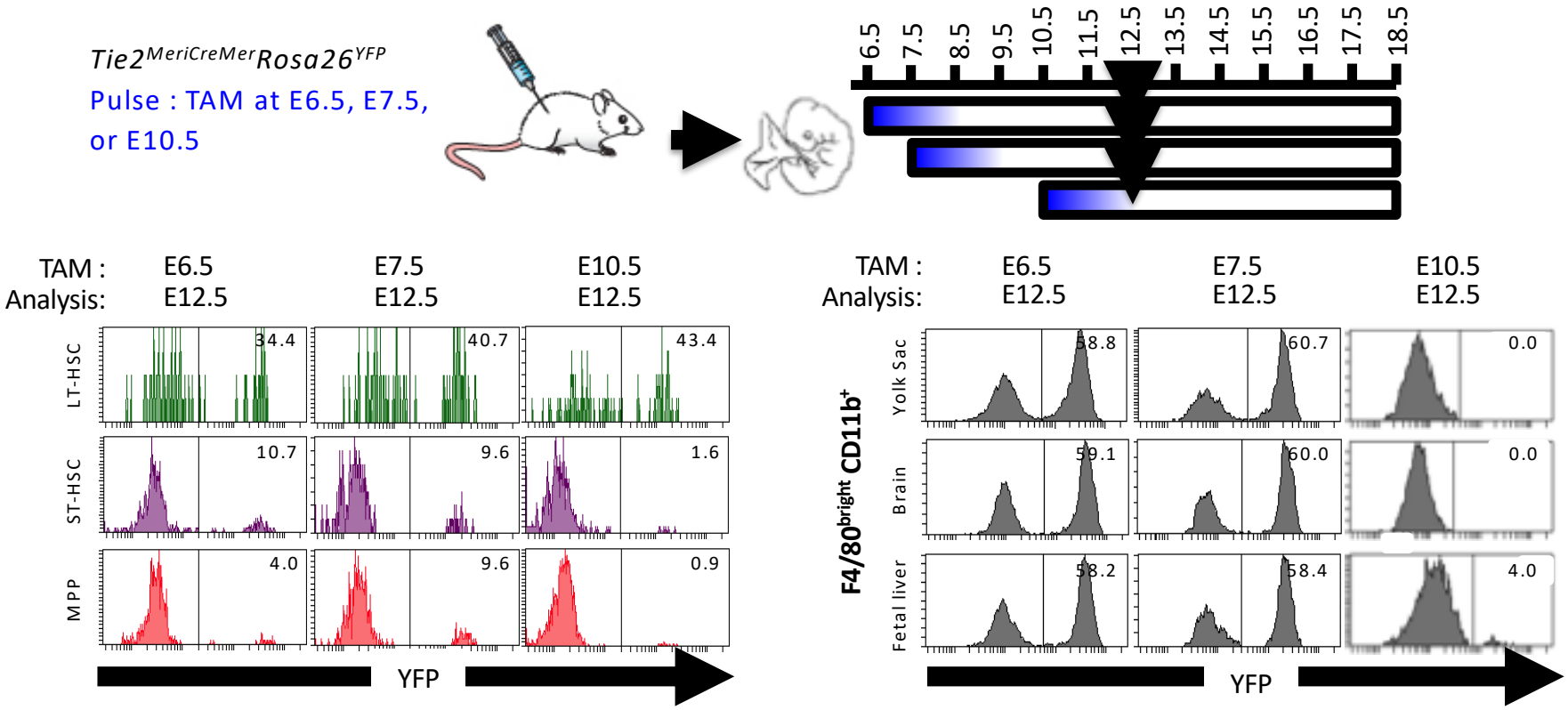
Tie2^{MeriCreMer} *Rosa26*^{YFP}

Pulse at E6.5, 7.5, 8.5, 9.5 or 10.5



Kay Klapporth, Katrin Busch & Hans-Reimer Rodewald

Les progéniteurs des macrophages résidents sont indépendants des HSCs.



Tissue resident macrophages progenitors:

- **arise from *Tie2*+ cells before E10.5**
- **are distinct from dHSCs**

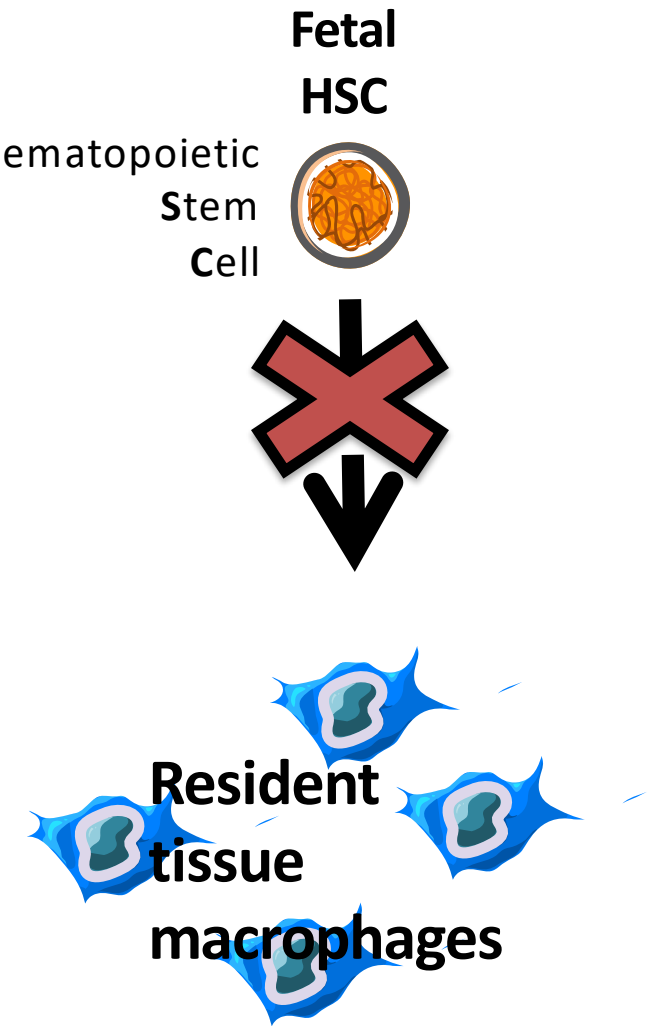
Gomez Perdiguero*, Klapporth* *et al.*, ***Nature***, 2015

Ontogenèse du système des phagocytes mononucléaires

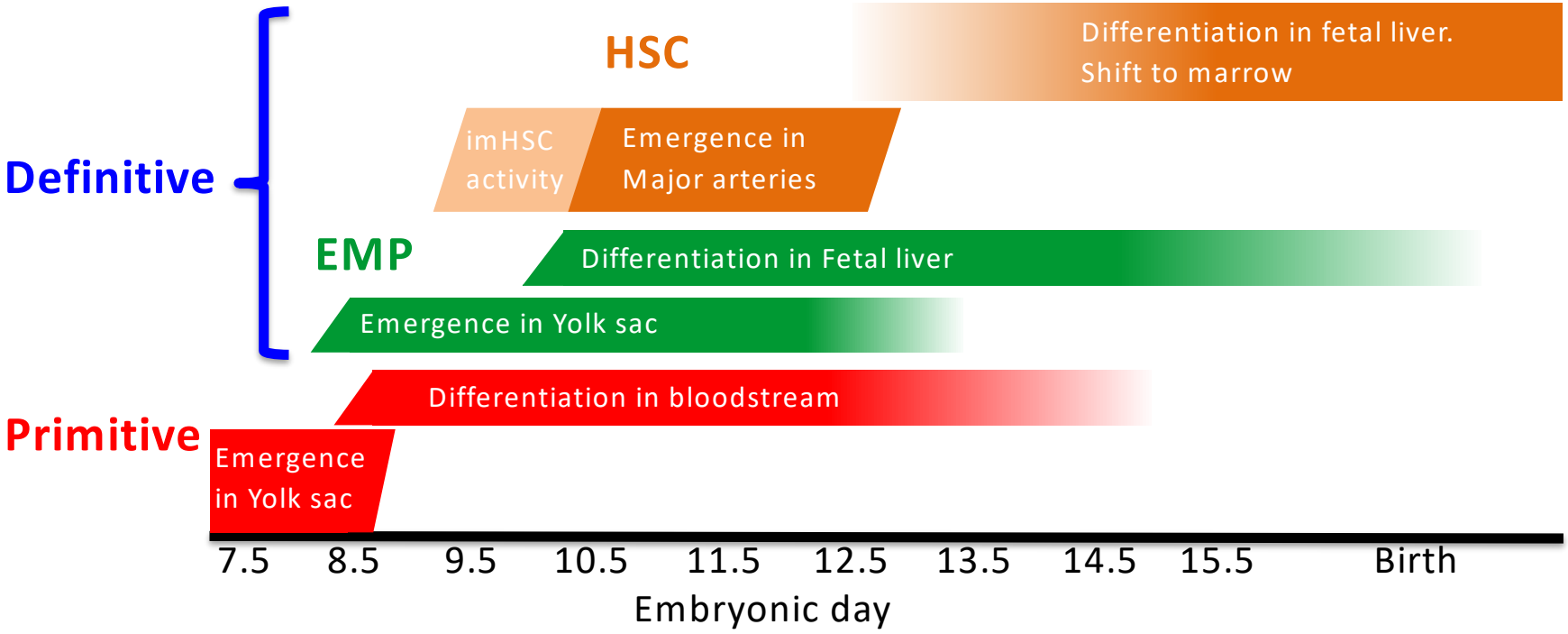
Do adult resident Macrophages derive from fetal HSCs?

Resident Macrophages develop AND are maintained in the absence of *Myb* and functional HSCs

Adult resident macrophages progenitors arise from *Tie2*$^{+}$ cells before E10.5 and are distinct from dHSCs

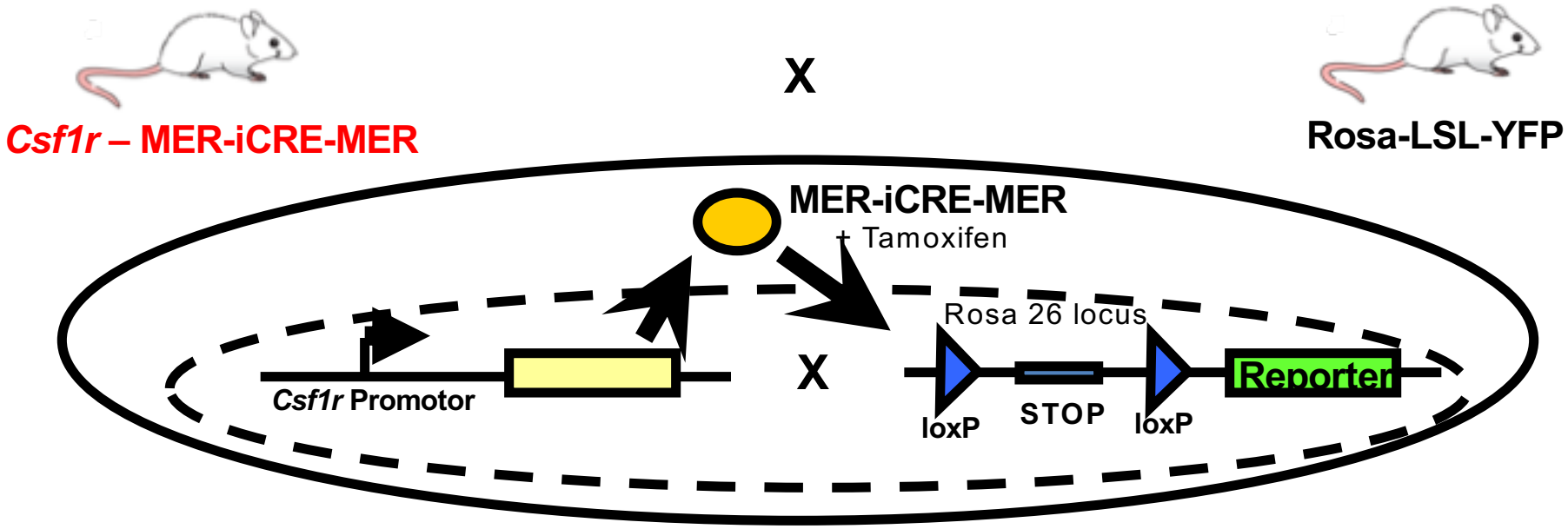


Les macrophages résidents dérivent de progéniteurs du sac vitellin

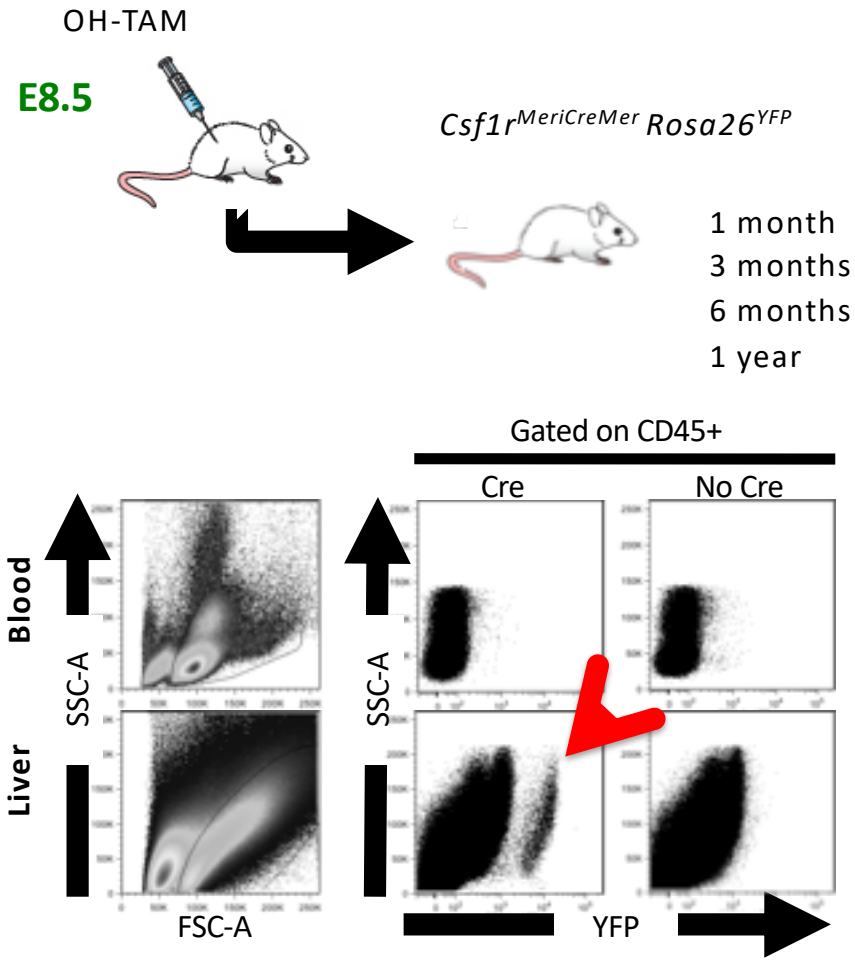


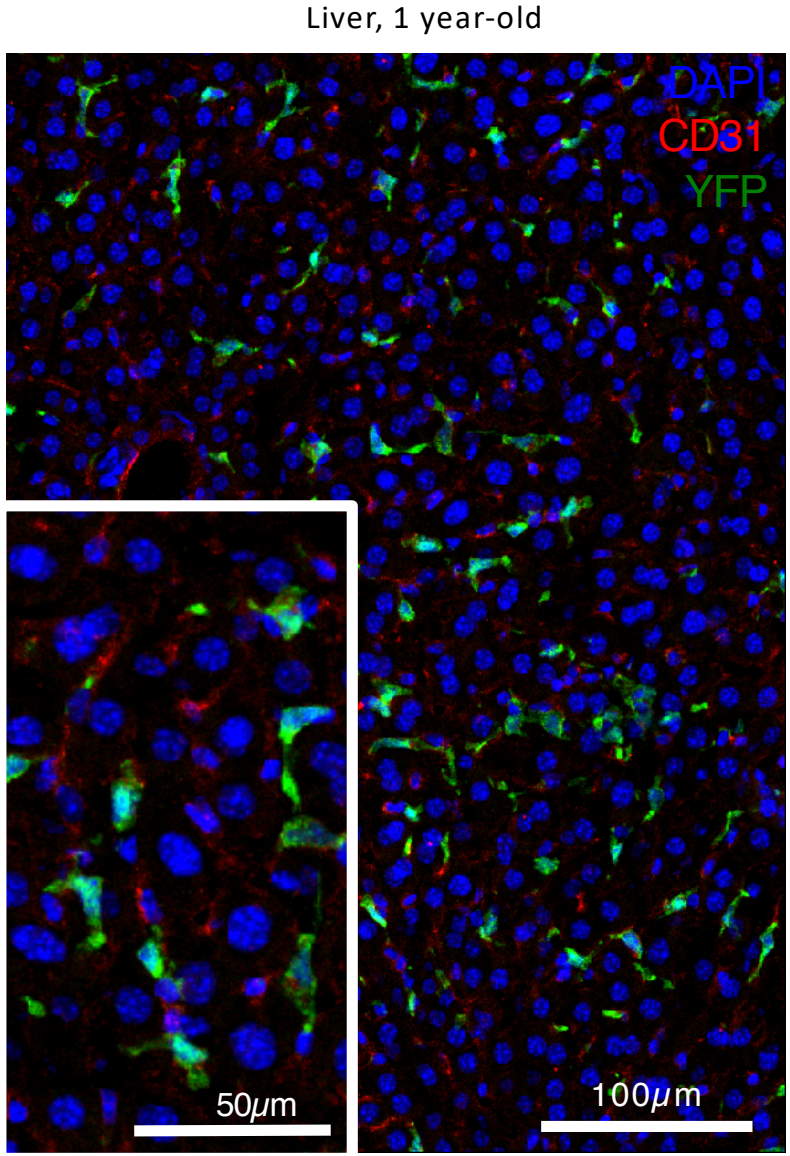
Can we label resident macrophages WITHOUT labelling HSC?
And if so, what YS progenitors are we labelling?

Marquage génétique des macrophages et leurs progéniteurs *in vivo*



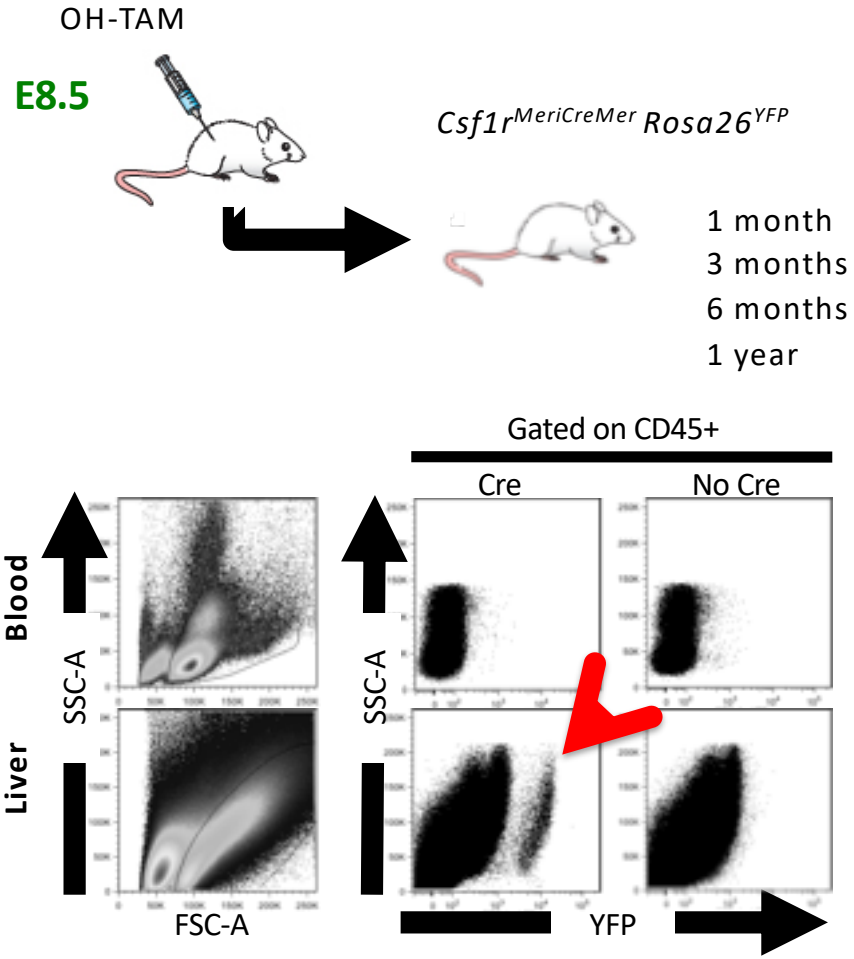
Les progéniteurs *Csf1r*+ présents à E8.5 donnent naissance aux macrophages F4/80bright





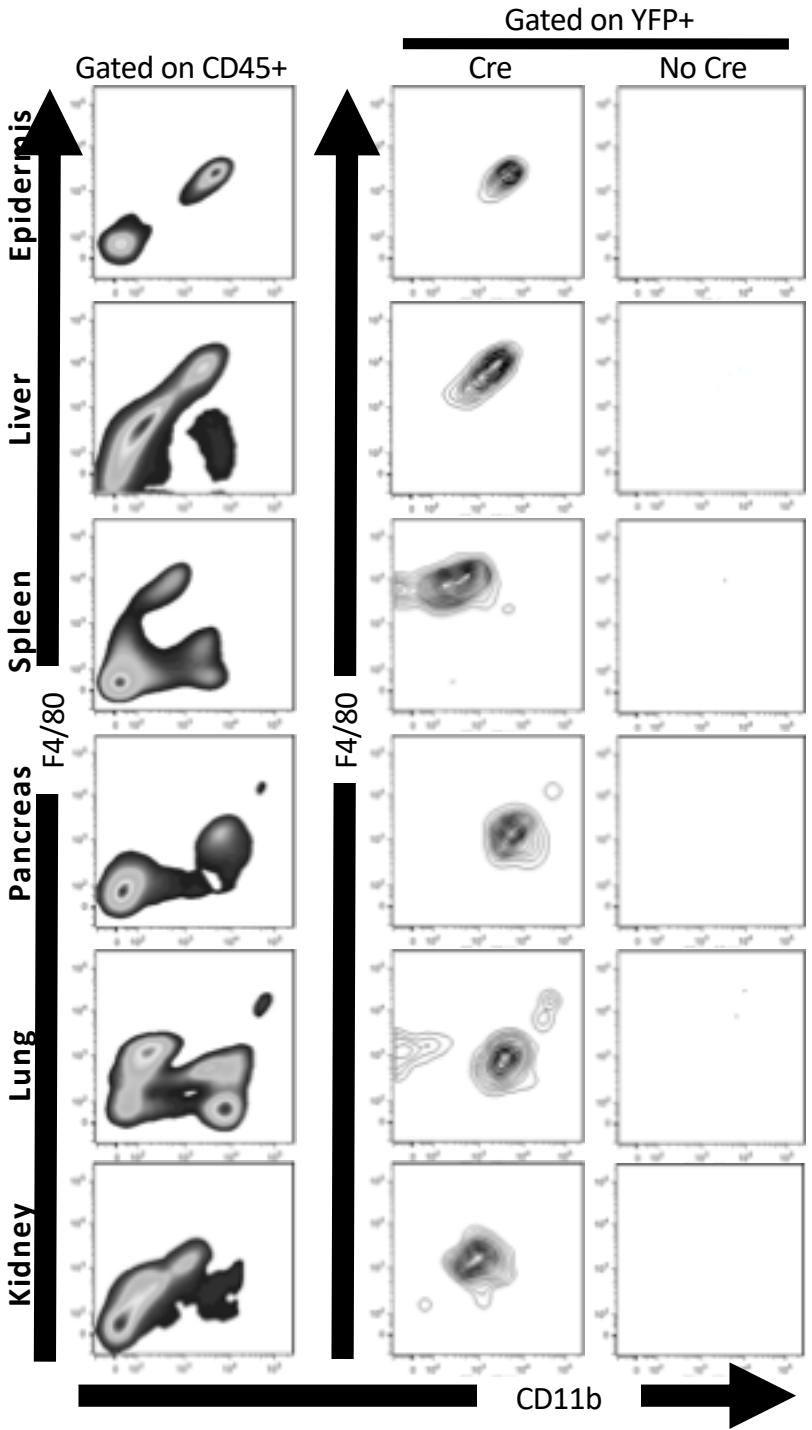
Schulz*, Gomez Perdiguero*, *et al.*, ***Science***, 2012

Les progéniteurs *Csf1r*+ présents à E8.5 donnent naissance aux macrophages F4/80bright

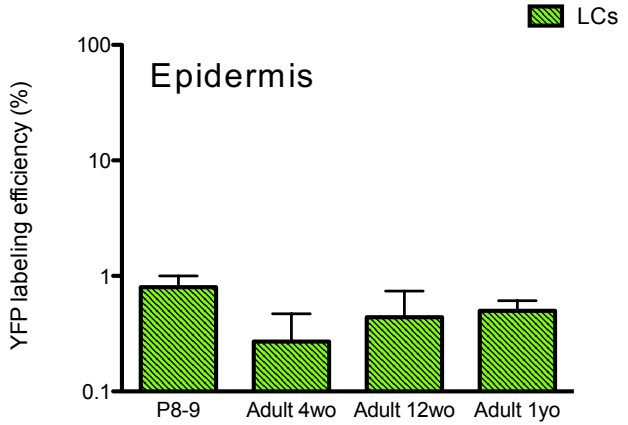


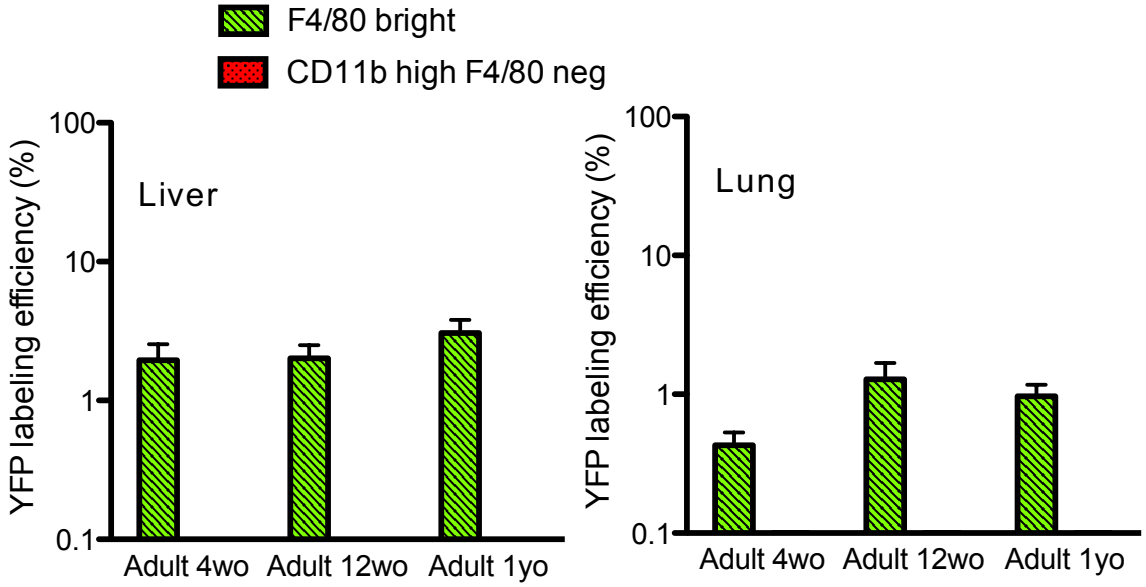
Myb-independent F4/80bright macrophages in adults tissues originate from progenitors that express ***Csf1r*** at E8.5-9.5

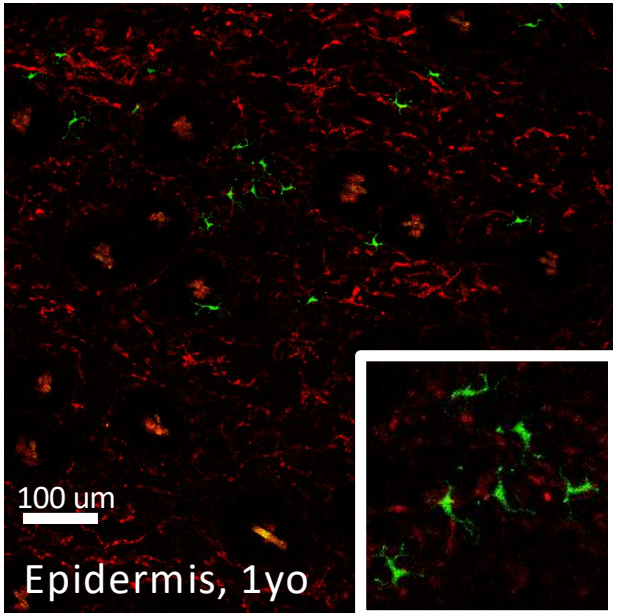
Schulz*, Gomez Perdiguero*, *et al.*, ***Science***, 2012



Maintien des macrophages dérivés de progéniteurs *Csf1r+* présents à E8.5

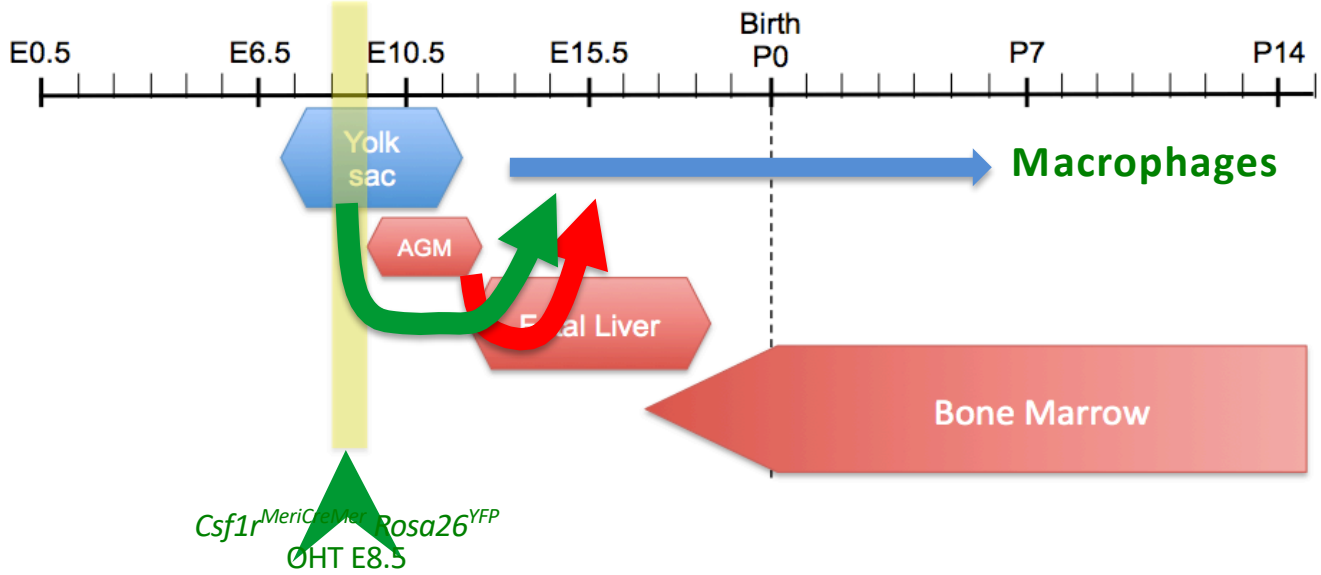






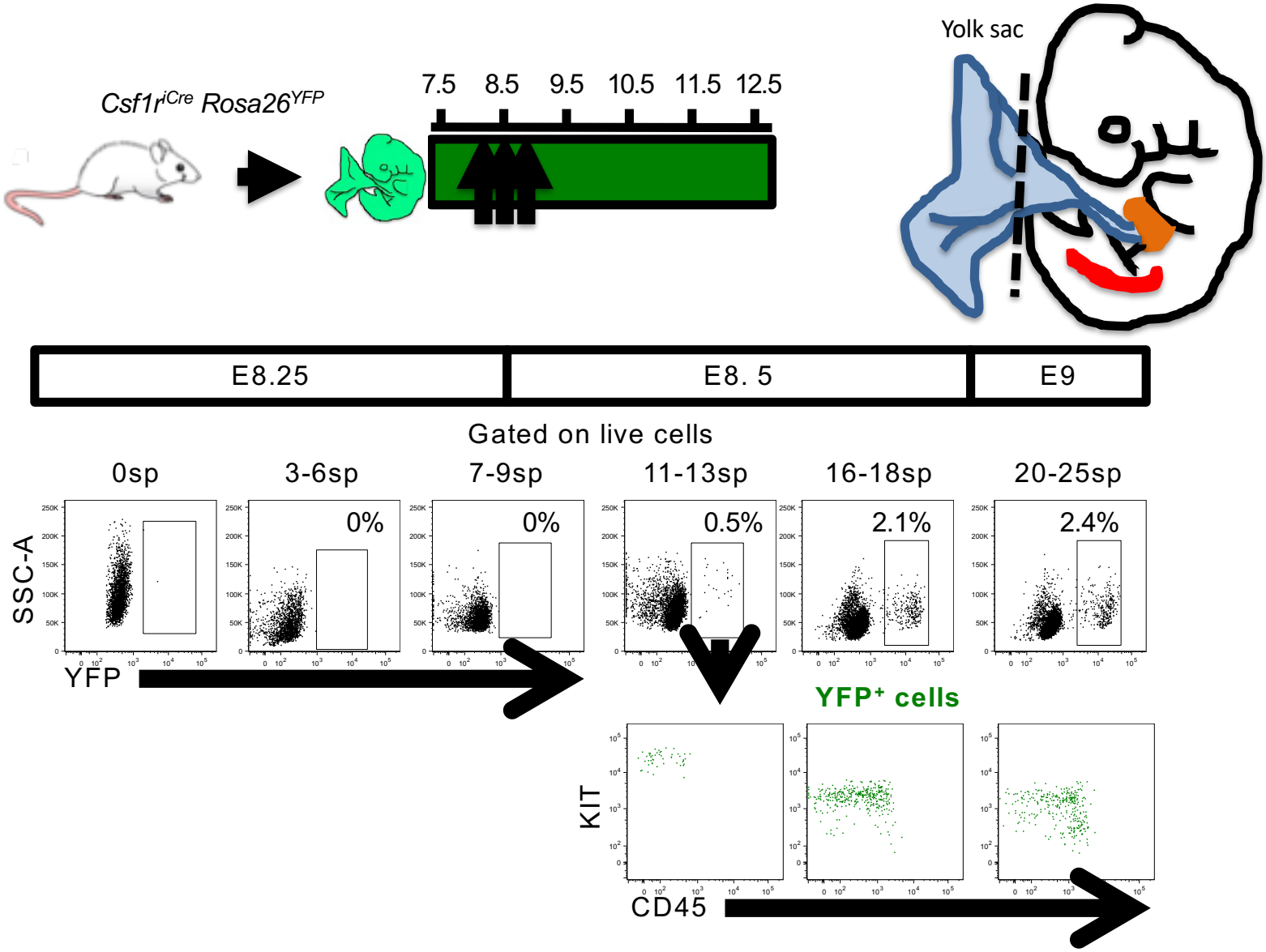
Elisa Gomez Perdiguero

Identification des progeniteurs *Csf1r*$^{+}$

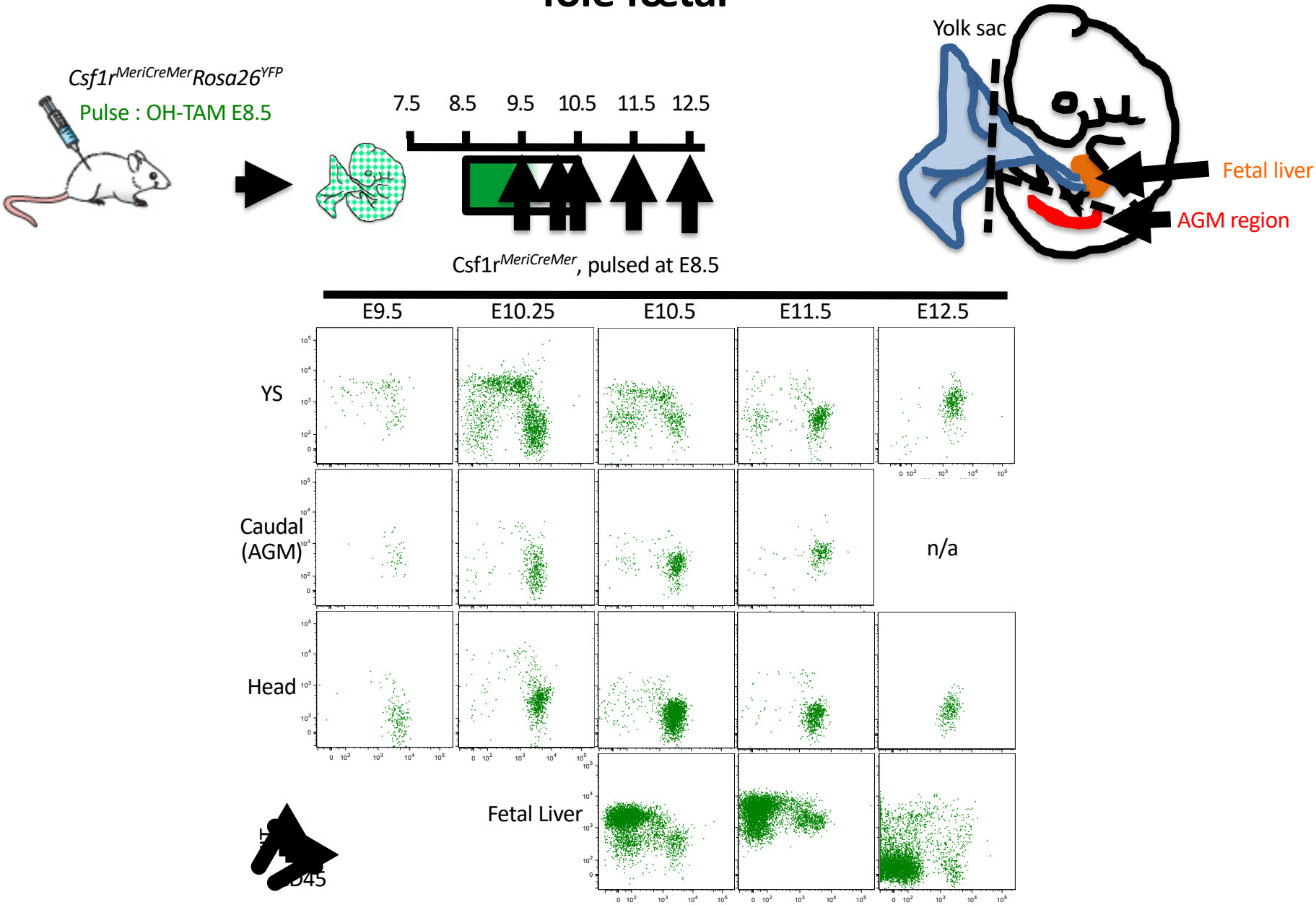


i.e. **What are we labelling when we inject tamoxifen at E8.5?**

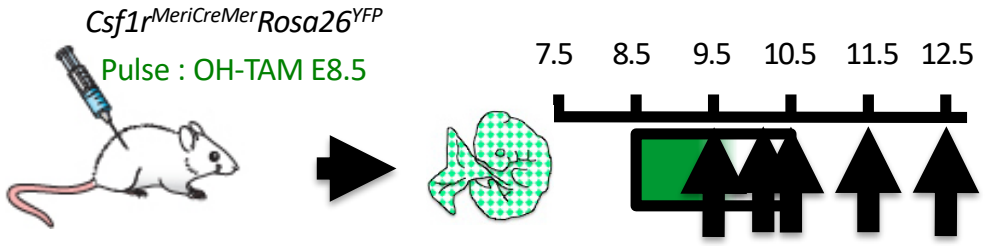
Les progéniteurs *Csf1r*$^{+}$ sont originaires du sac vitellin à E8.5

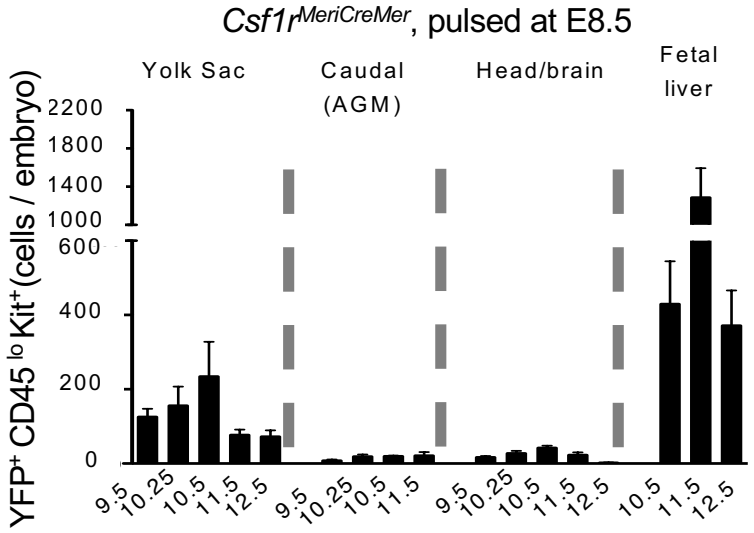


Les progéniteurs *Csf1r*$^{+}$ sont originaires du sac vitellin et colonisent le foie fœtal

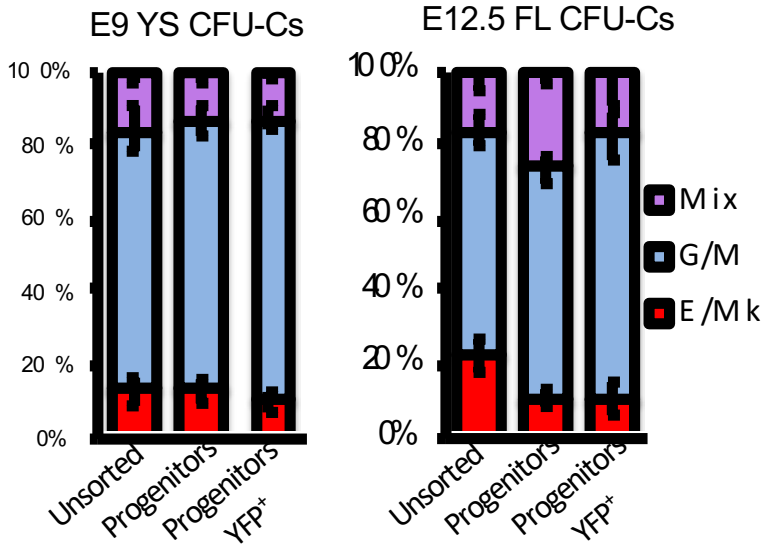


Les progéniteurs *Csf1r*$^{+}$ sont originaires du sac vitellin et colonisent le foie fœtal



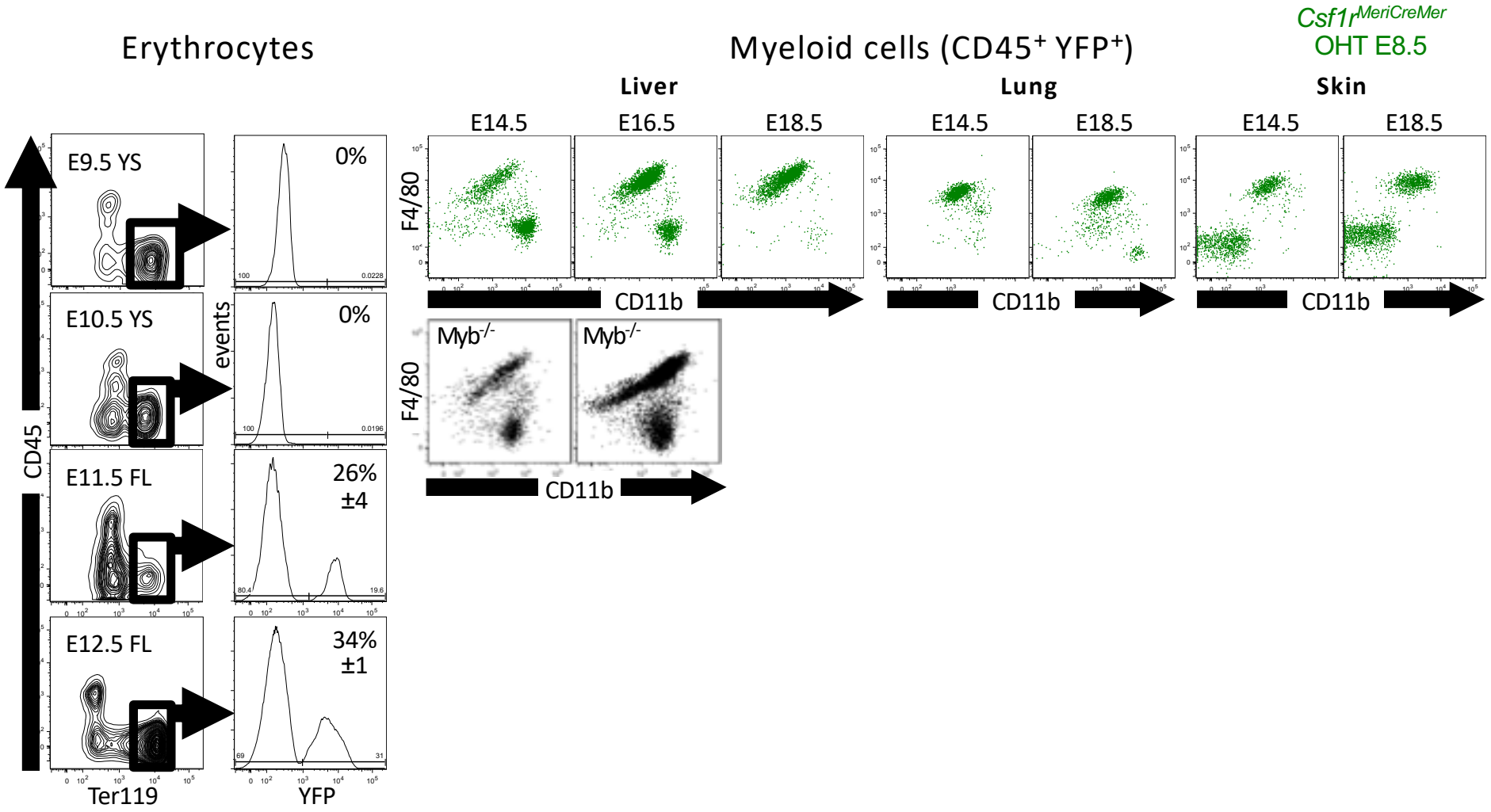


Expansion in the fetal liver



Erythro and myeloid potential

Les progéniteurs *Csf1r*$^{+}$ se différencient en érythrocytes, monocytes, granulocytes, mastocytes et macrophages F4/80^{bright}



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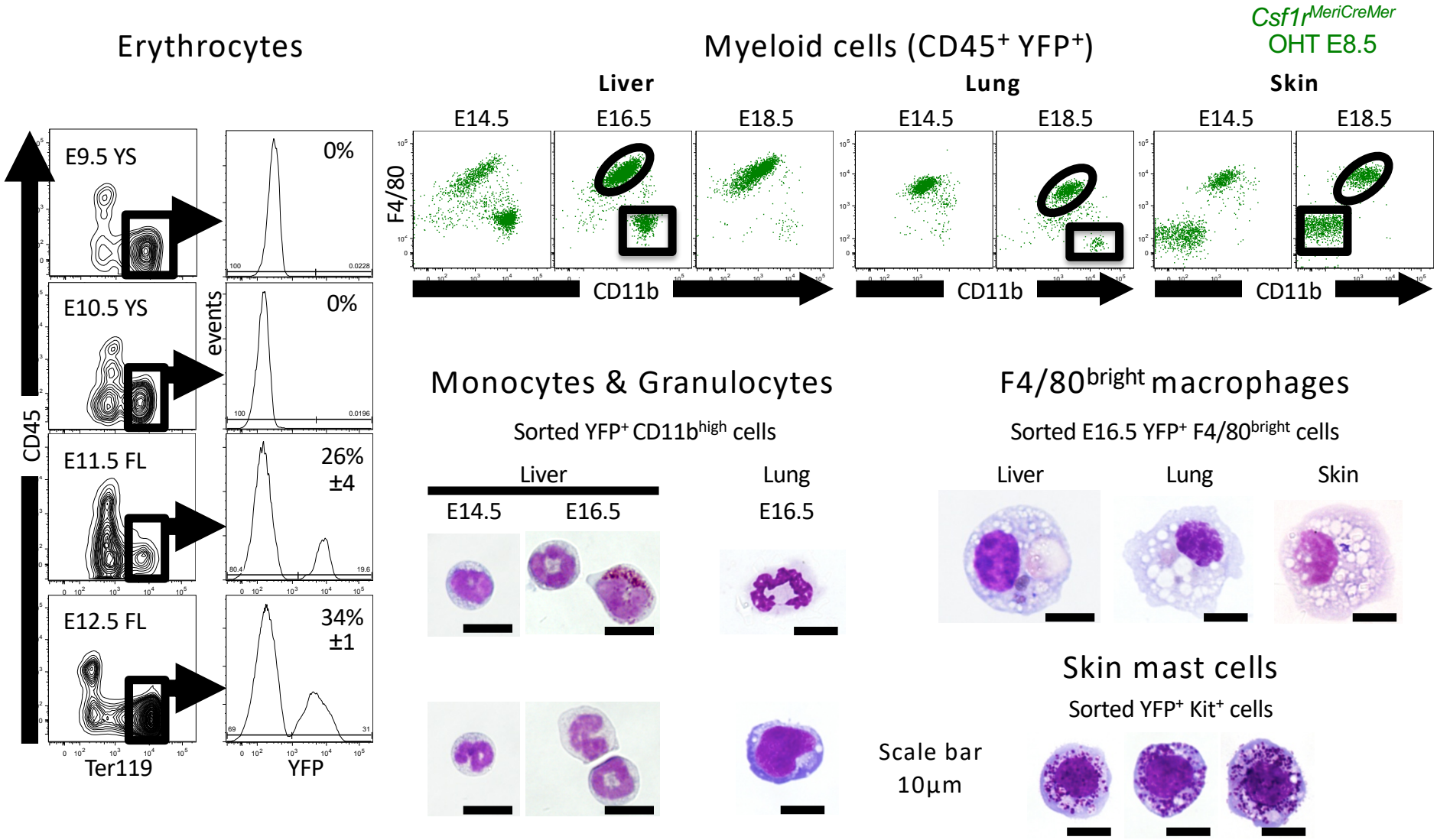
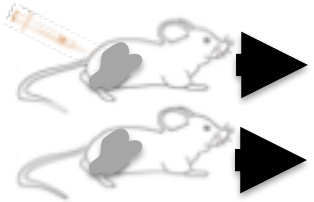
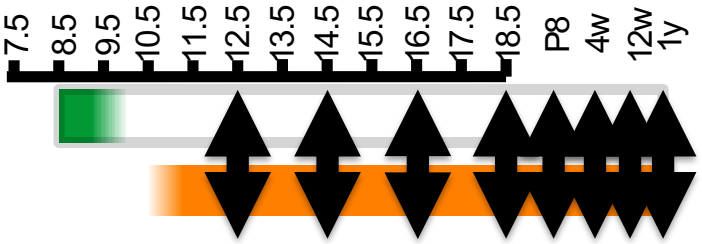


Les progéniteurs *Csf1r*$^{+}$ sont les principaux producteurs des cellules myéloïdes dans le foie fœtal jusqu'à E16.5

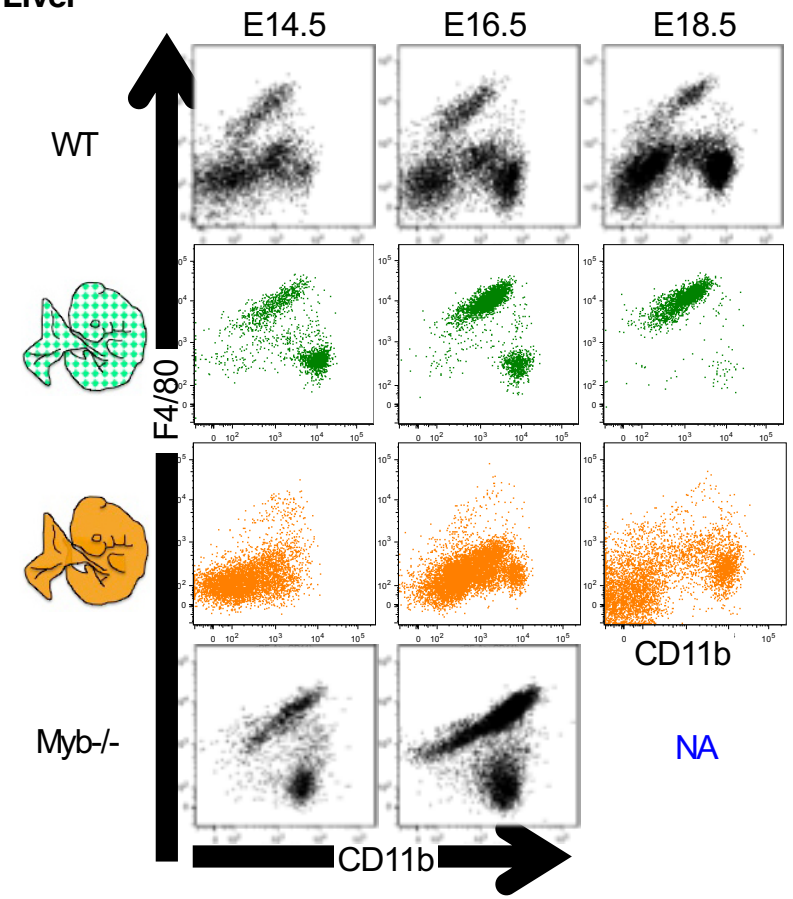
Csf1r-Mer-iCre-Mer
x *Rosa26 LSL-YFP*
Pulse : OH-TAM E8.5

Flt3-Cre x *Rosa26 LSL-YFP*

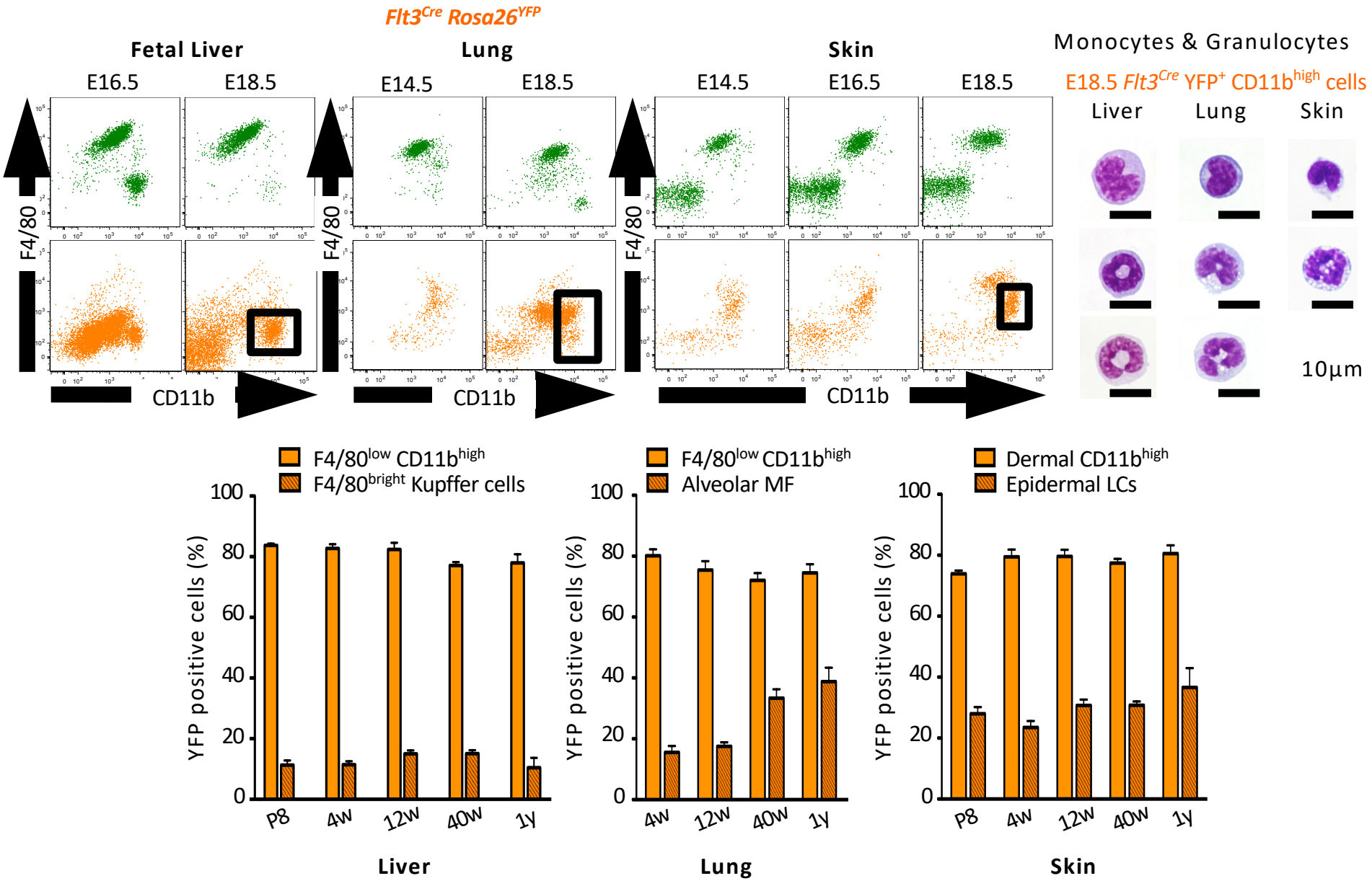




Fetal Liver

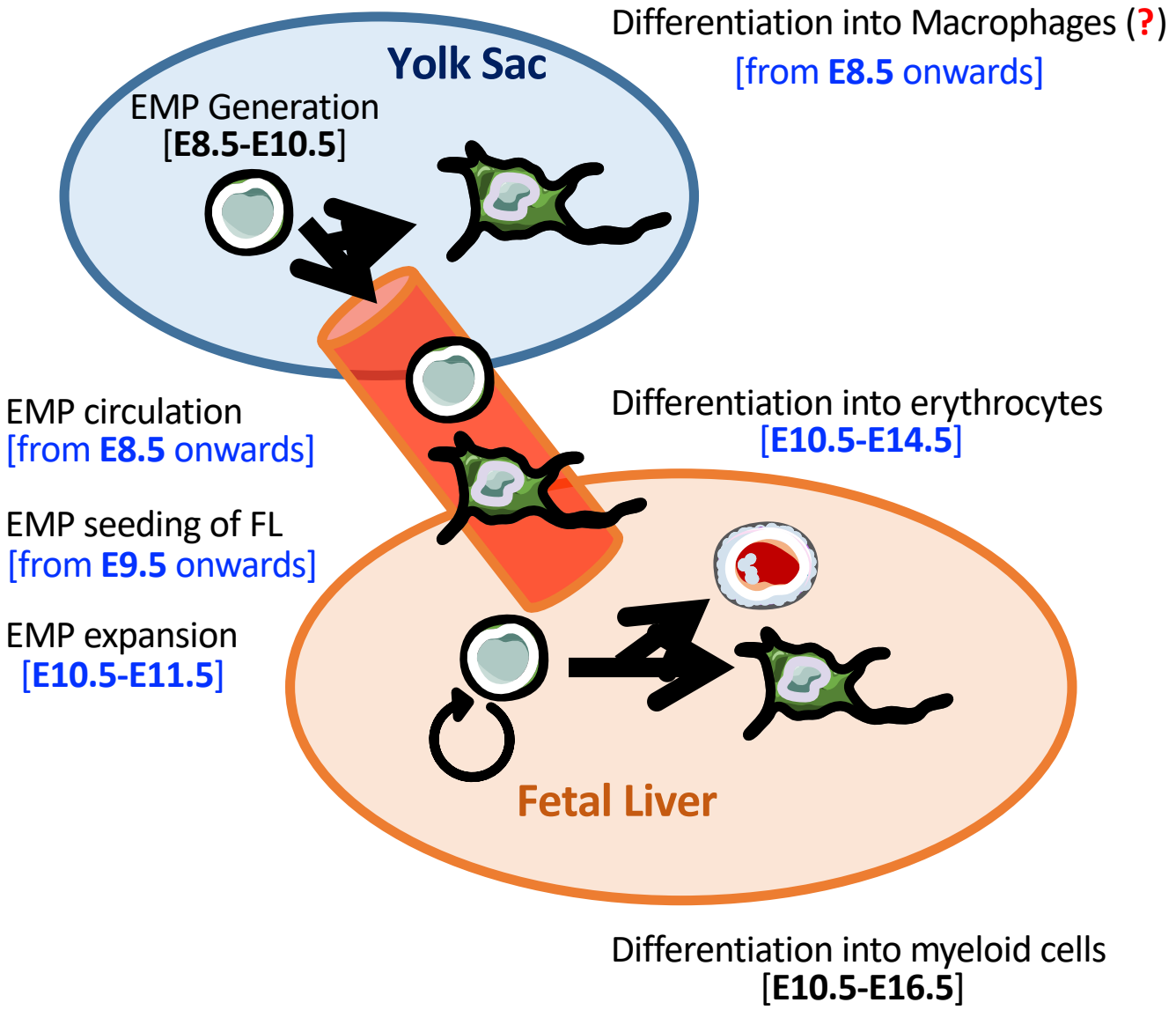


Fetal liver HSCs derived (*Flt3*$^{+}$) precursors do not replace (YS-derived) F4/80^{bright} macrophages



Gomez Perdiguero, *et al*., Nature ***2014***

Les progéniteurs *Csfr1*$^{+}$ sont des progéniteurs erythro-myéloïdes (EMPs)



Les progéniteurs *Csfr1*$^{+}$ sont des progéniteurs erythro-myéloïdes (EMPs)

EMP emergence

Bertrand, J ***Blood*** 2005
Gomez Perdiguero, E. ***Nature*** 2015

Frame , J.M. ***Stem Cells*** 2016
Kasaii, B. **Scientific reports** 2017

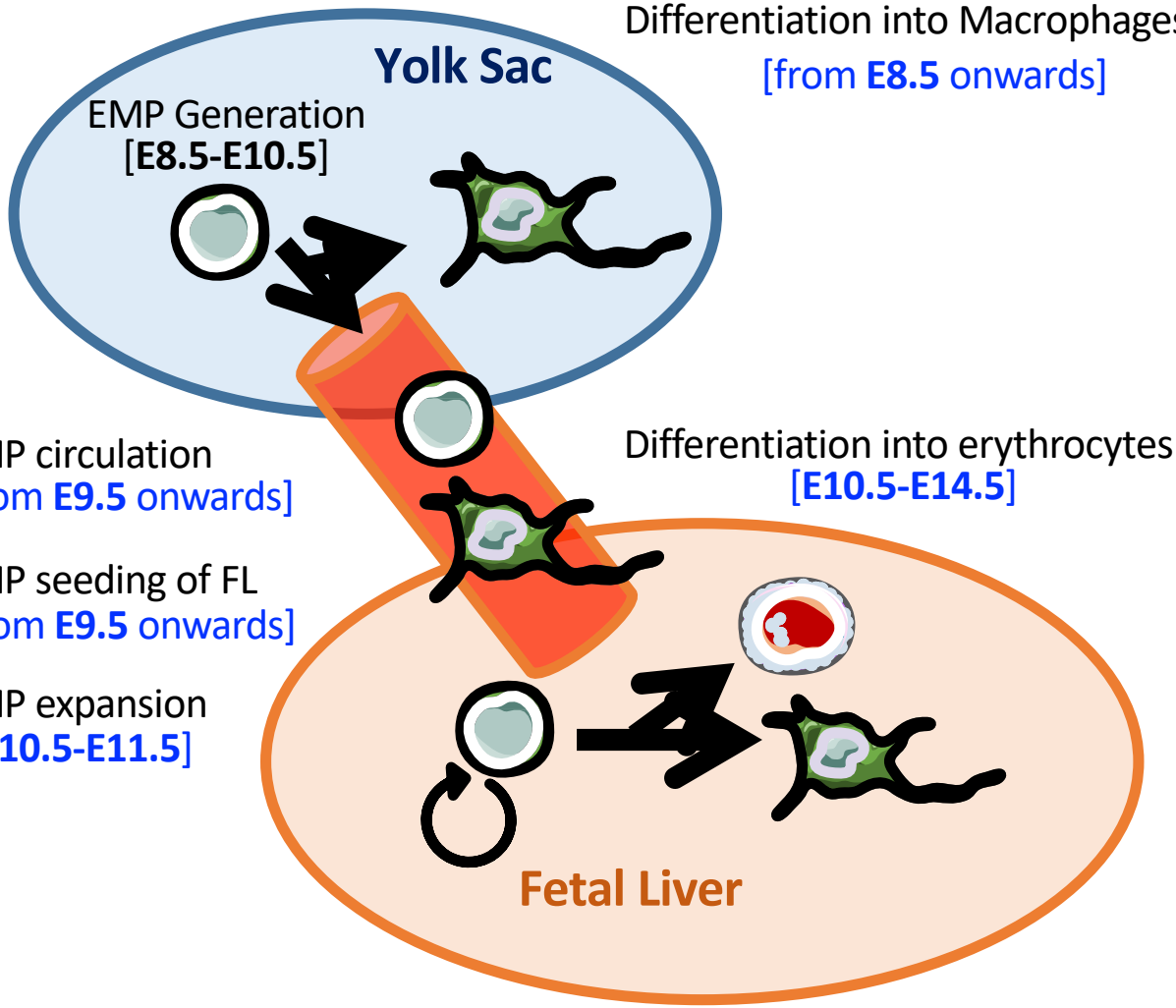
EMP phenotype

Bertrand, J ***PNAS*** 2005
Gomez Perdiguero, E. ***Nature*** 2015

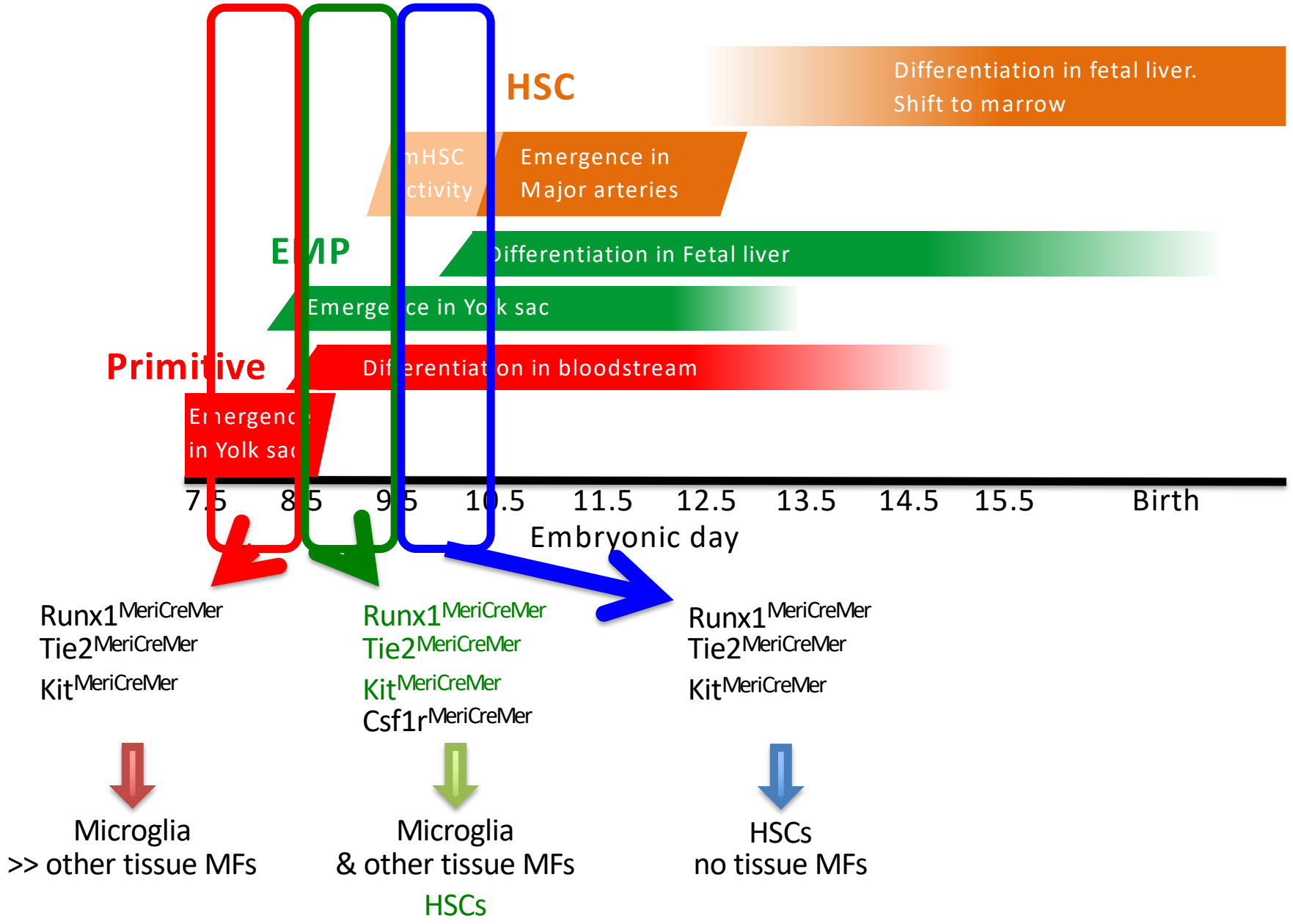
McGrath, K.E. ***Cell reports*** 2015

EMP function

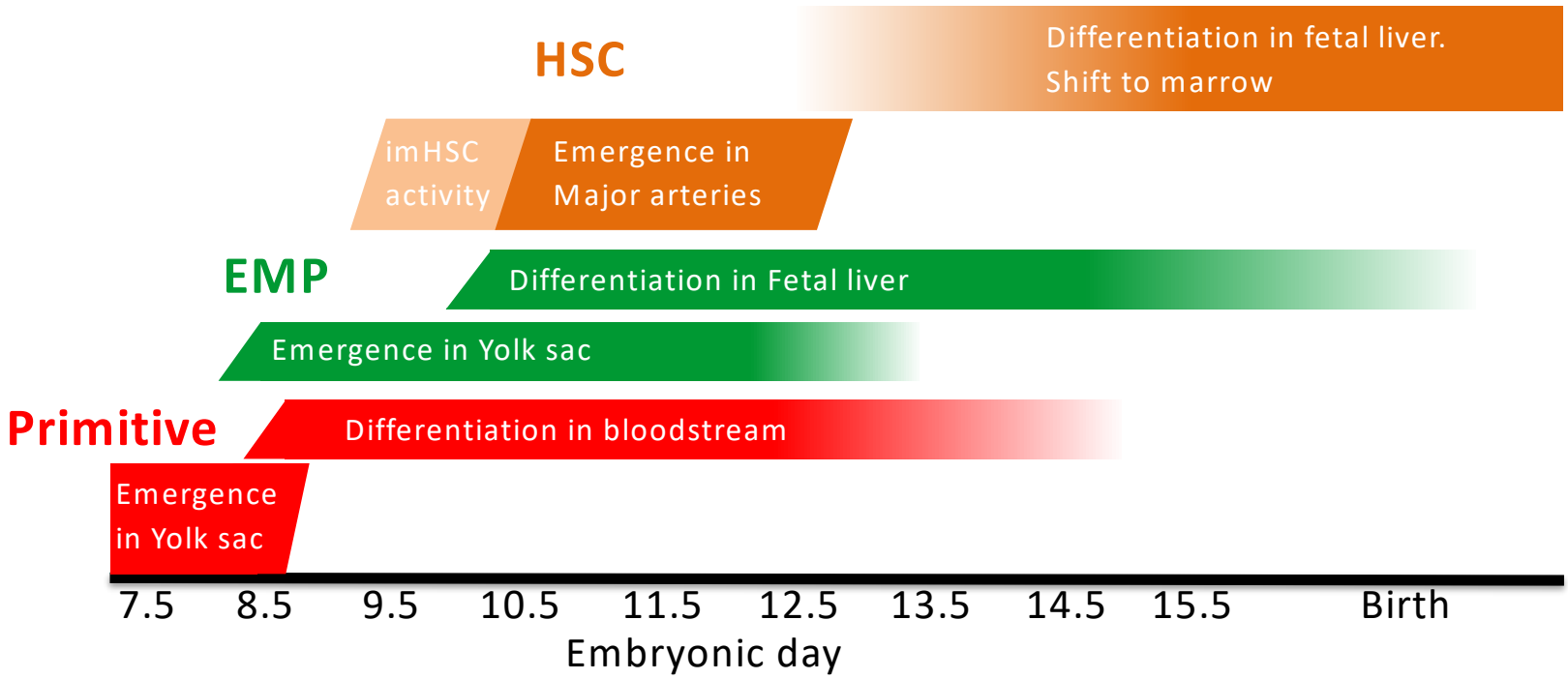
Chen, M.J. ***Cell Stem Cell*** 2011



Les macrophages résidents dérivent des YS-EMPs



Les macrophages résidents dérivent des YS-EMPs



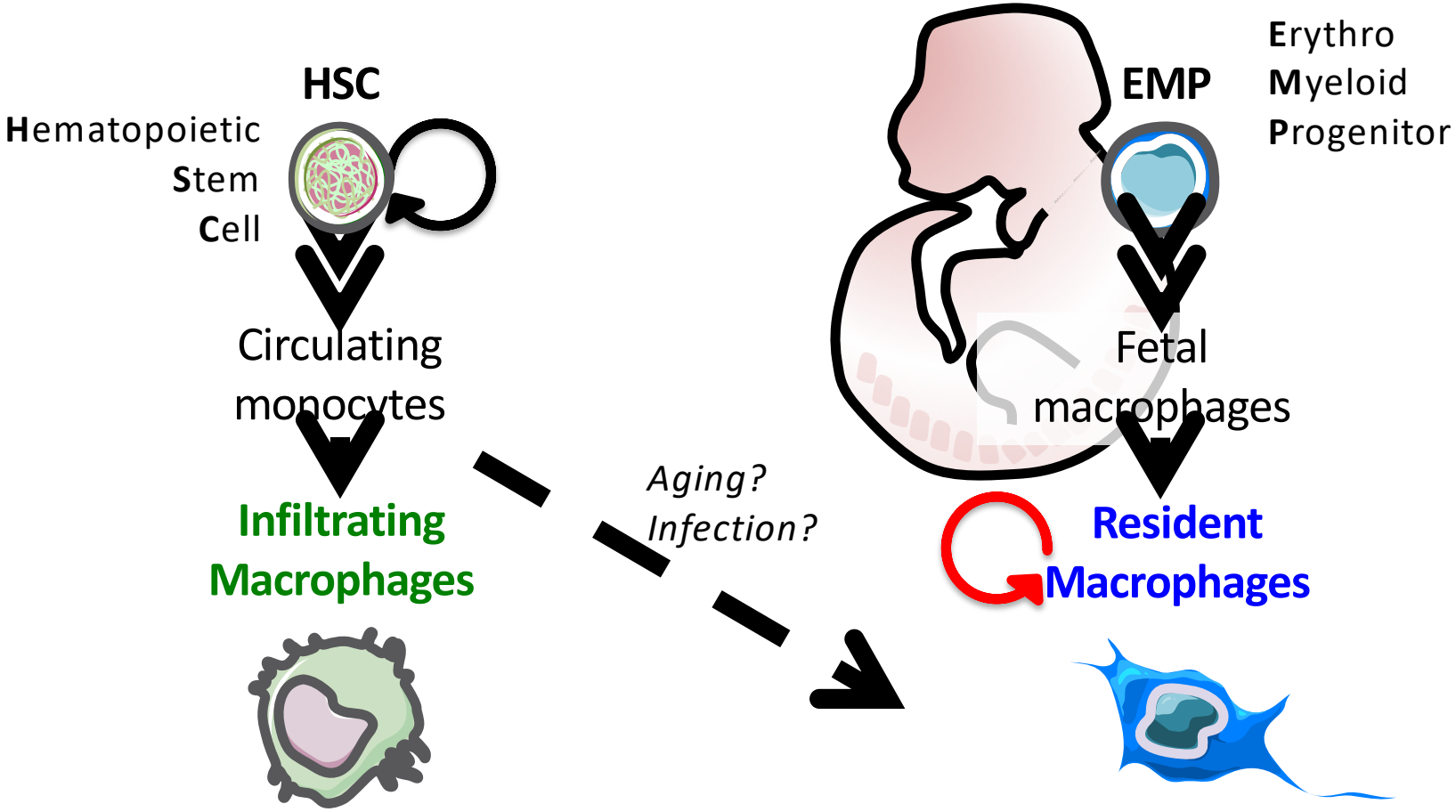
Gomez Perdiguero*, Klapporth* *et al.*, ***Nature***, 2015
Hoeffel *et al.*, ***Immunity*** 2015
McGrath *et al.*, ***Cell Report*** 2015
Sheng *et al.*, ***Immunity*** 2016

Microglia is “special” in all fate mapping models available so far ….

Due to a “kinetic” bias?
Due to a contribution of other YS-derived progenitors?

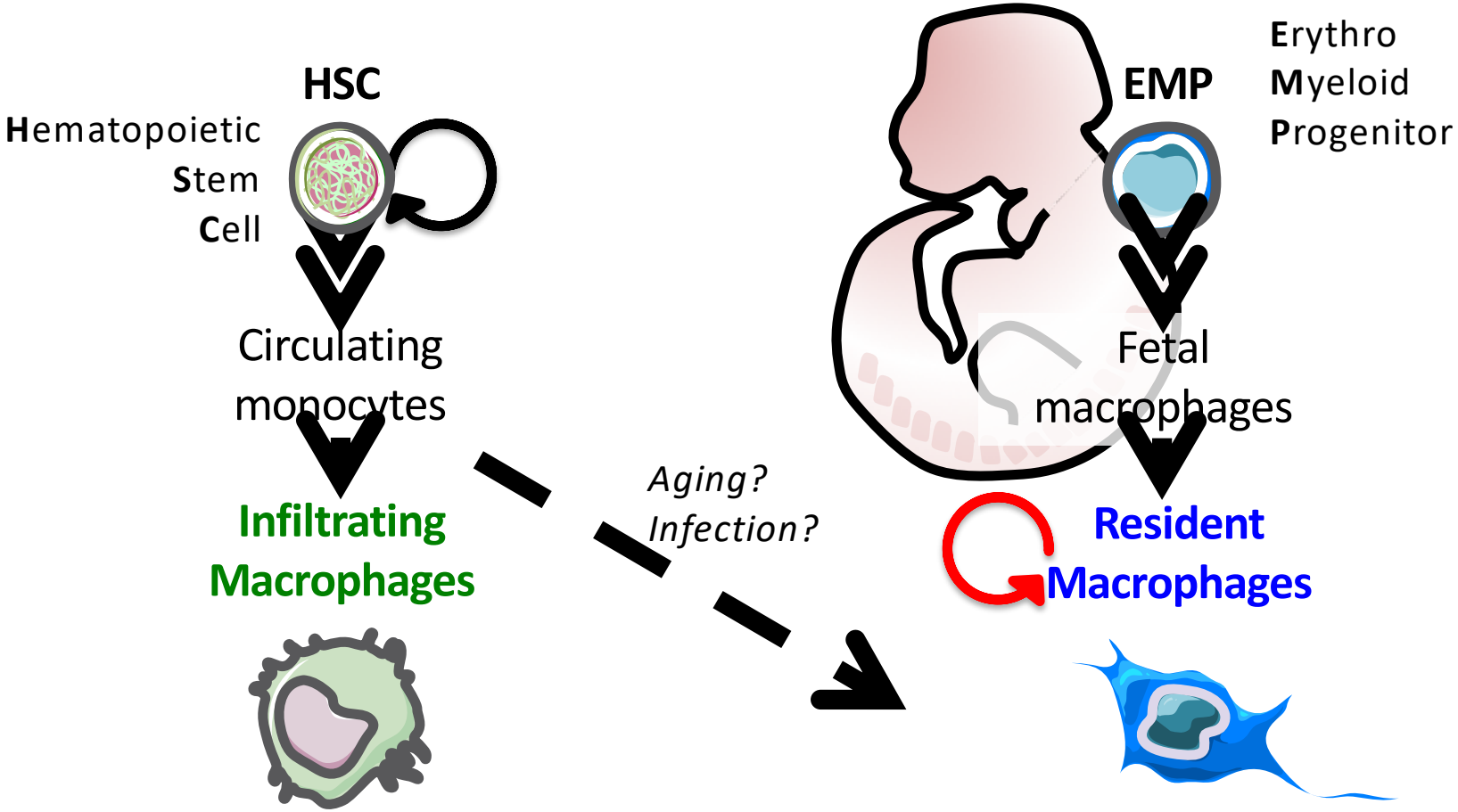
Is there a contribution of Primitive progenitors?

Deux lignages de macrophages coexistent dans les tissus adultes



➢ **Two independent lineages of macrophages coexist in most adult tissue (Resident versus infiltrating)** **[ontogeny]**

Deux lignages de macrophages coexistent dans les tissus adultes

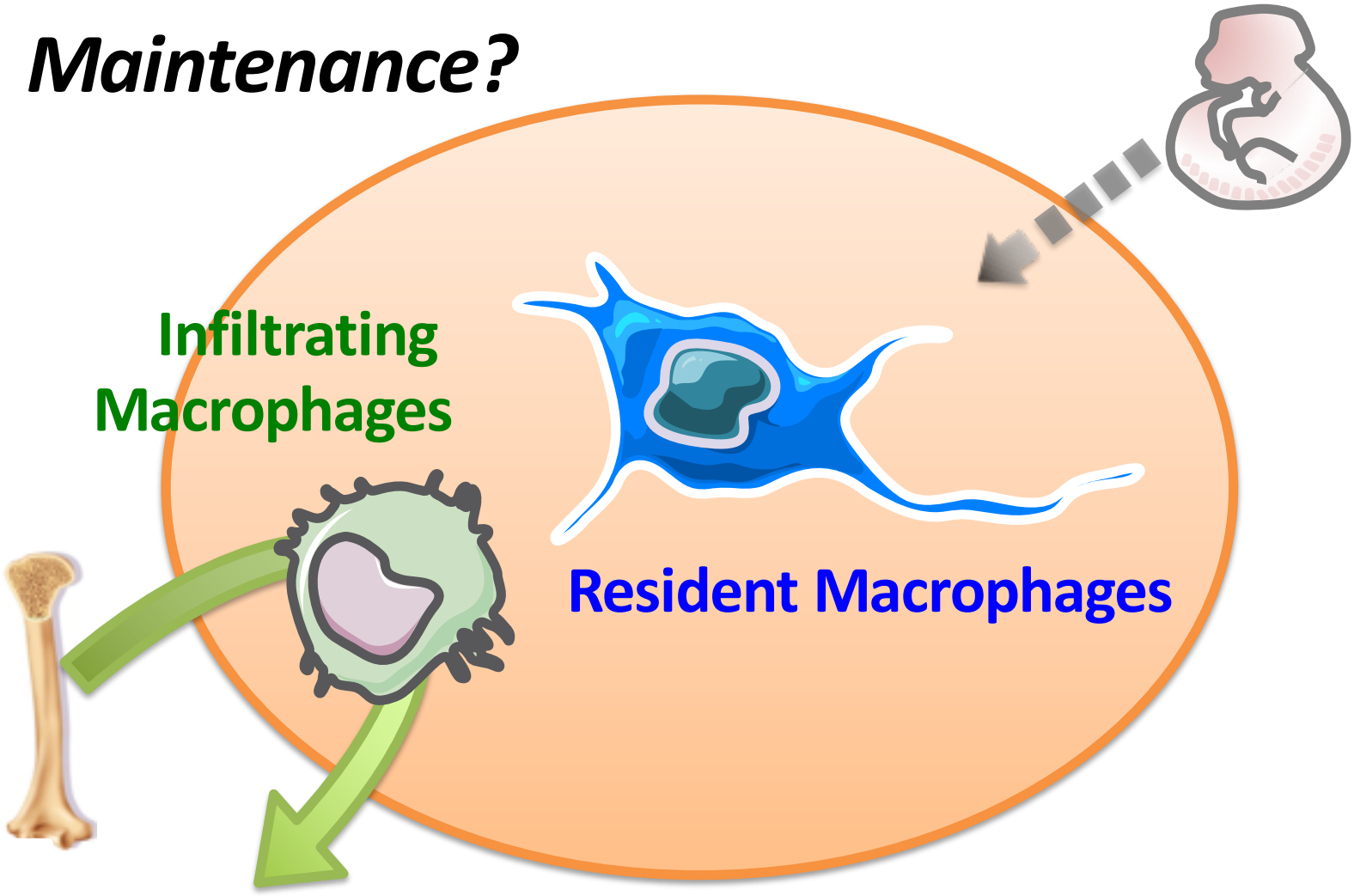


- **Two independent lineages of macrophages coexist in most adult tissue (Resident versus infiltrating)** **[ontogeny]**

- Macrophages have tissue-specific gene expression and epigenetic profiles **[environment]**

From fetal to adult resident macrophages: Tissue specialization

Maintenance?



Comment les macrophages résidents se maintiennent dans les tissus adultes?

Long-lived?

Proliferation?

Chorro *et al.*, ***JEM*** 2009
Ghigo *et al.*, ***JEM***, 2013

Lawson, *et al.*, ***Neuroscience***, 1992
Bruttger *et al.*, ***Immunity***, 2015

Jenkins, *et al.*, ***Science***, 2011
Davies *et al.*, ***Eur J Immunol***, 2015

Pforte *et al.*, ***Eur Resp J*** 1993
Hashimoto *et al.*, ***Immunity***, 2013

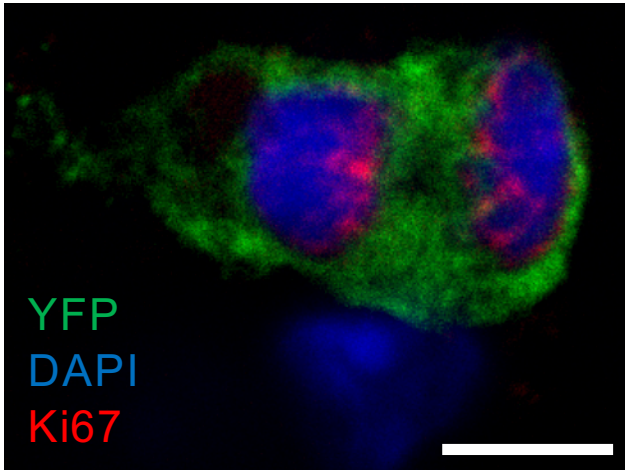
Bouwens, *et al.*, ***Hepatology,*** 1986
Yamada *et al.*, ***JLB***, 1990

Self-renewal?

Aziz *et al.*, ***Science***, 2009
Soucie *et al.*, ***Science***, 2016

Local Progenitor/Precursor?

Ghigo *et al.*, ***JEM***, 2013
Garceau *et al.*, ***BMC Biol***, 2015



Proliferating resident macrophage in the liver

Liver, 52 week-old mice
Csf1r-Mer-iCre-Mer
x *Rosa26 LSL-YFP*
Pulsed w OH-TAM at E8.5

Comment les macrophages résidents se maintiennent dans les tissus adultes?

Long-lived?

Proliferation?

Chorro *et al.*, ***JEM*** 2009
Ghigo *et al.*, ***JEM***, 2013

Lawson, *et al.*, ***Neuroscience,*** 1992
Bruttger *et al.*, ***Immunity***, 2015

Jenkins, *et al.*, ***Science,*** 2011
Davies *et al.*, ***Eur J Immunol***, 2015

Pforte *et al.*, ***Eur Resp J*** 1993
Hashimoto *et al.*, ***Immunity***, 2013

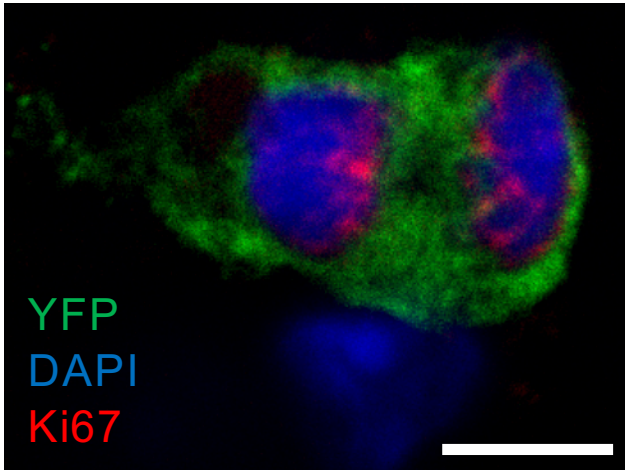
Bouwens, *et al.*, ***Hepatology,*** 1986
Yamada *et al.*, ***JLB***, 1990

Self-renewal?

Aziz *et al.*, ***Science***, 2009
Soucie *et al.*, ***Science***, 2016

Local Progenitor/Precursor?

Ghigo *et al.*, ***JEM***, 2013
Garceau *et al.*, ***BMC Biol***, 2015



Proliferating resident macrophage in the liver

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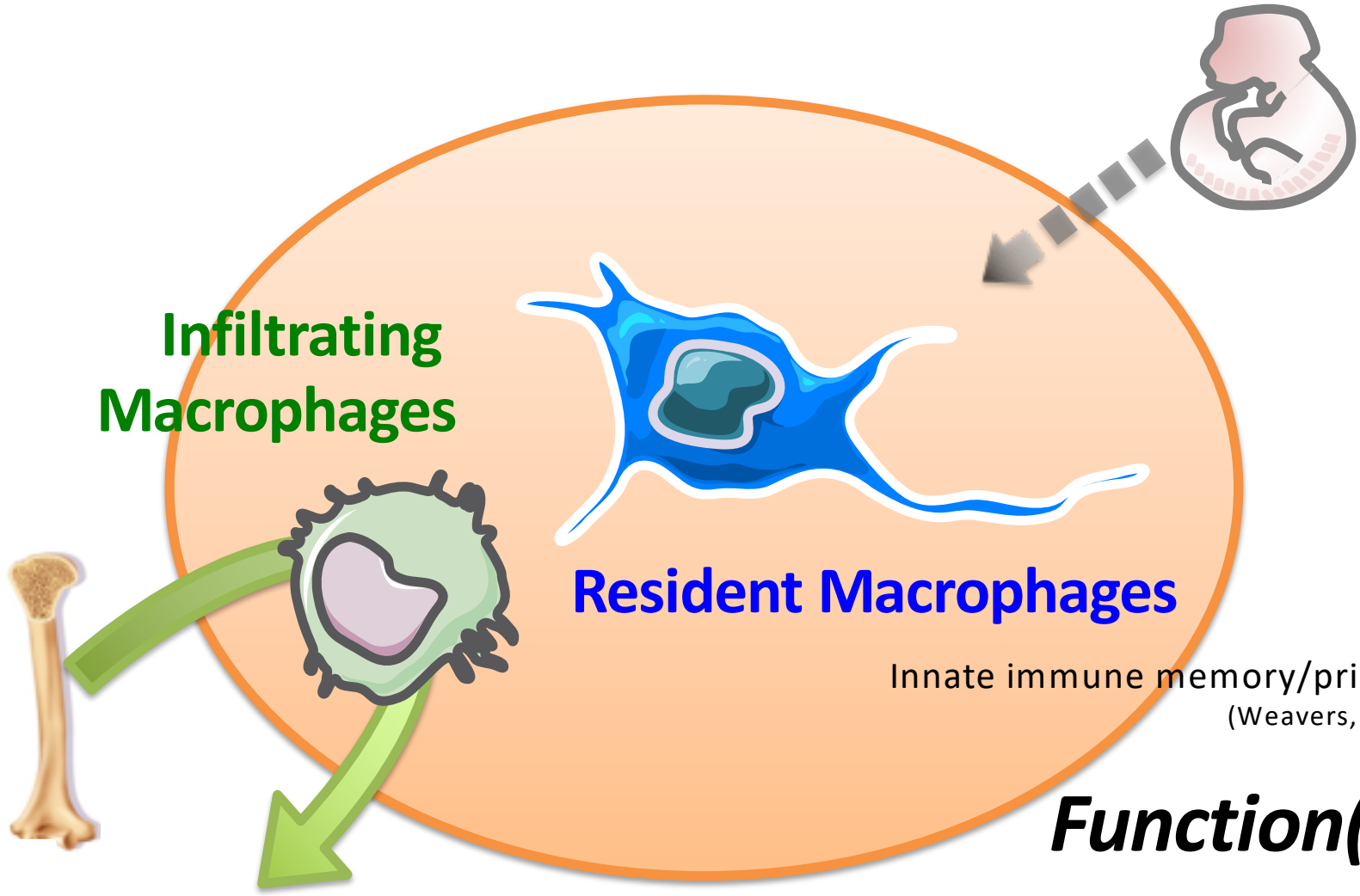
Replacement by HSC-derived cells?

Heart: Molawi *et al.*, ***JEM***, 2014
Lung: Gomez Perdiguero ***Nature*** 2015



Innate immune memory/priming?
(Weavers, Cell 2016)

Function(s)?

"Immune" sentinels	"Homeostasis" sentinels
Eat to defend	**Eat to repair**

Fonctions spécifiques du lignage chez les macrophages?

- Salamander and Zebrafish amputation models:

Impaired regeneration in when macrophages are depleted (permanent scar tissue, reduced mesenchymal proliferation and impaired neovascularisation)

(JW Godwin PNAS 2013)
(TA Petrie Development 2014)

- Mouse tissue repair/regeneration models:

Embryonic skin wound healing and Neonate heart regeneration are impaired when monocyte/macrophages are depleted
(permanent scar tissue, loss of tissue functionality and impaired neovascularisation)

(AB Aurora et al., JCI 2014)

Macrophages in **adult Skin wound healing** have opposing functions during the early inflammatory phase and the later stages. Monocyte/macrophages depletion in late stages leads to severe hemorrhage, endothelial cell apoptosis, vessel destabilization, and a failure in wound closure.

(T Lucas et al. J Immunol 2010)

In adult muscle injury, Macrophages promote muscle membrane repair and muscle fibre growth and regeneration.

(JG Tidball, J Physiol 2007)

Macrophages are necessary for epimorphic regeneration in African spiny mice

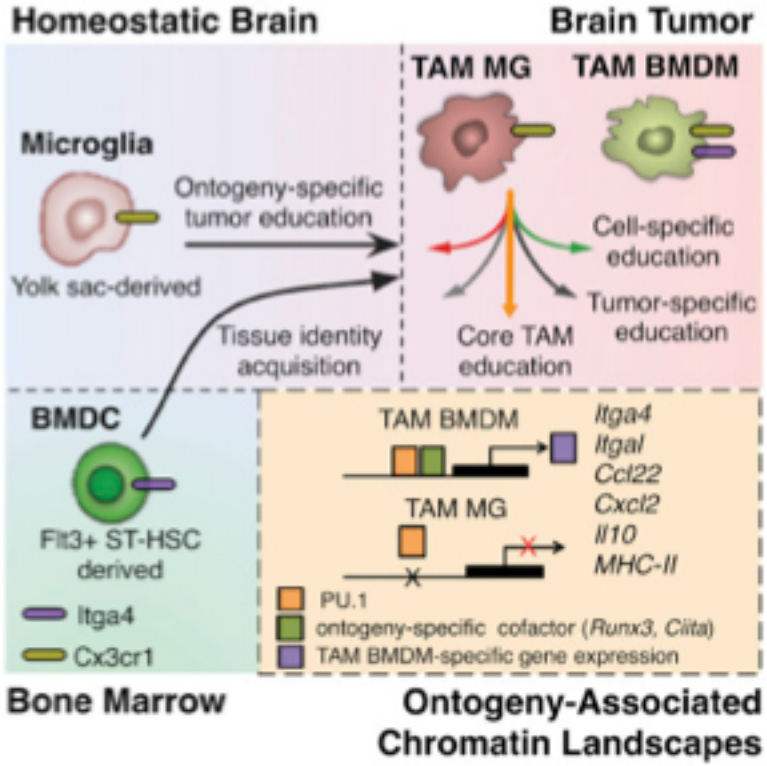
(J Simkin, eLIFE 2017)

Fonctions spécifiques du lignage chez les macrophages?

Resource

Cell Reports

Macrophage Ontogeny Underlies Differences in Tumor-Specific Education in Brain Malignancies

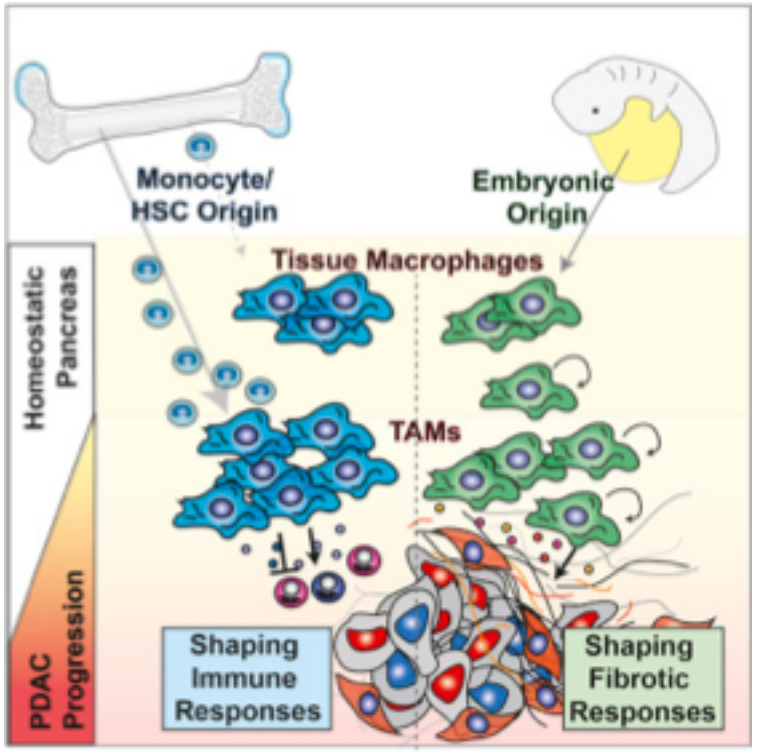


Bowman et al., Nov 2016 Cell Reports

Article

Immunity

Tissue-Resident Macrophages in Pancreatic Ductal Adenocarcinoma Originate from Embryonic Hematopoiesis and Promote Tumor Progression



Zhu et al., August 2017 Immunity

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