

# HUMAN FETAL LYMPHOPOIESIS

from thymus-colonizing cells  
to hematopoietic architecture

Pr Bruno CANQUE

Ecole Pratique des Hautes Etudes

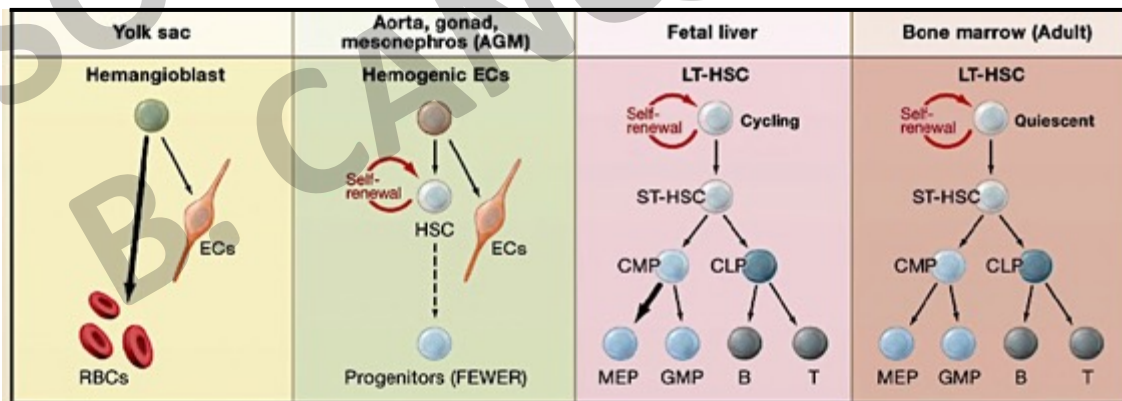
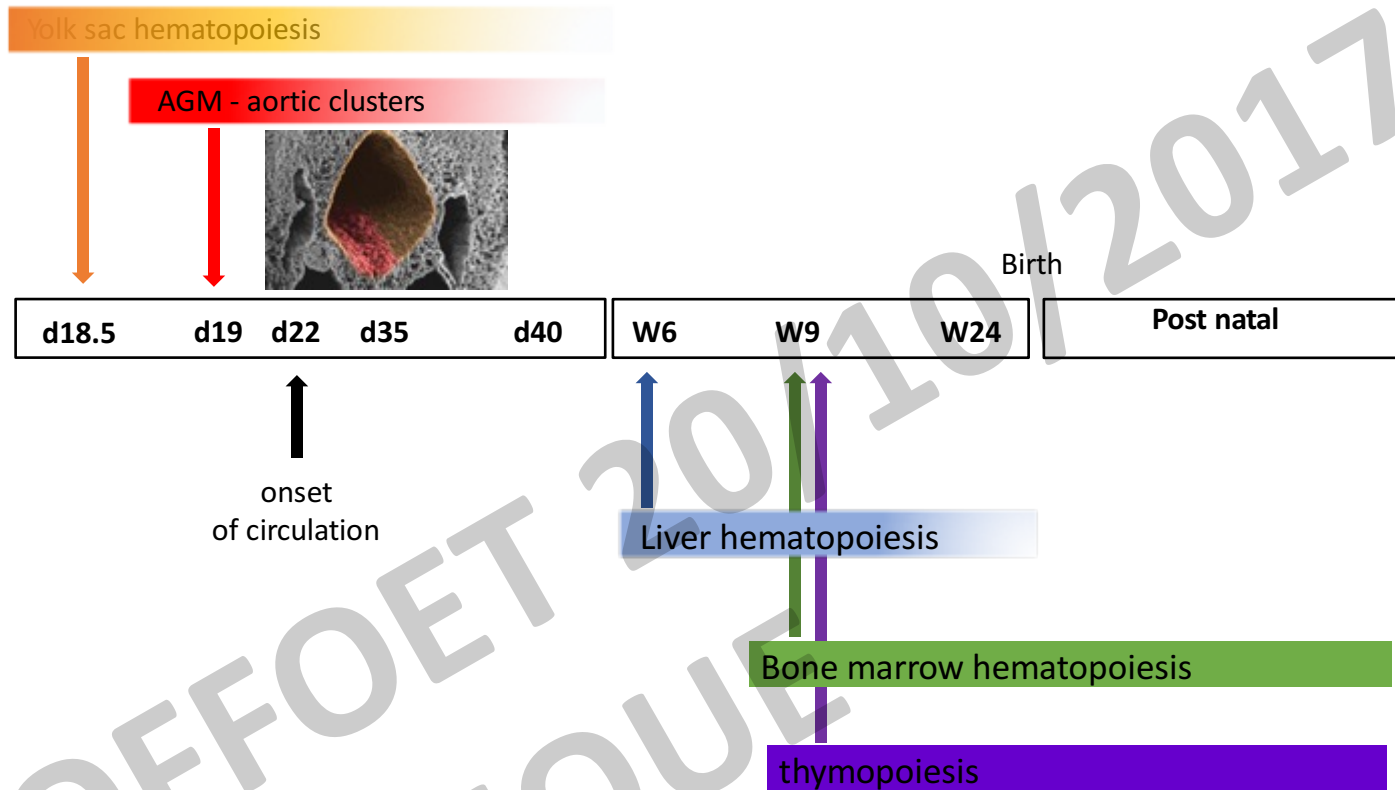
UMR INSERM-UPVII-EPHE 1126

Institut Universitaire d'Hématologie – Hôpital Saint Louis

# Human fetal hematopoiesis



# Hematopoietic development in humans



From Orkin & Zon



From Tavian et al.

Thymus colonization

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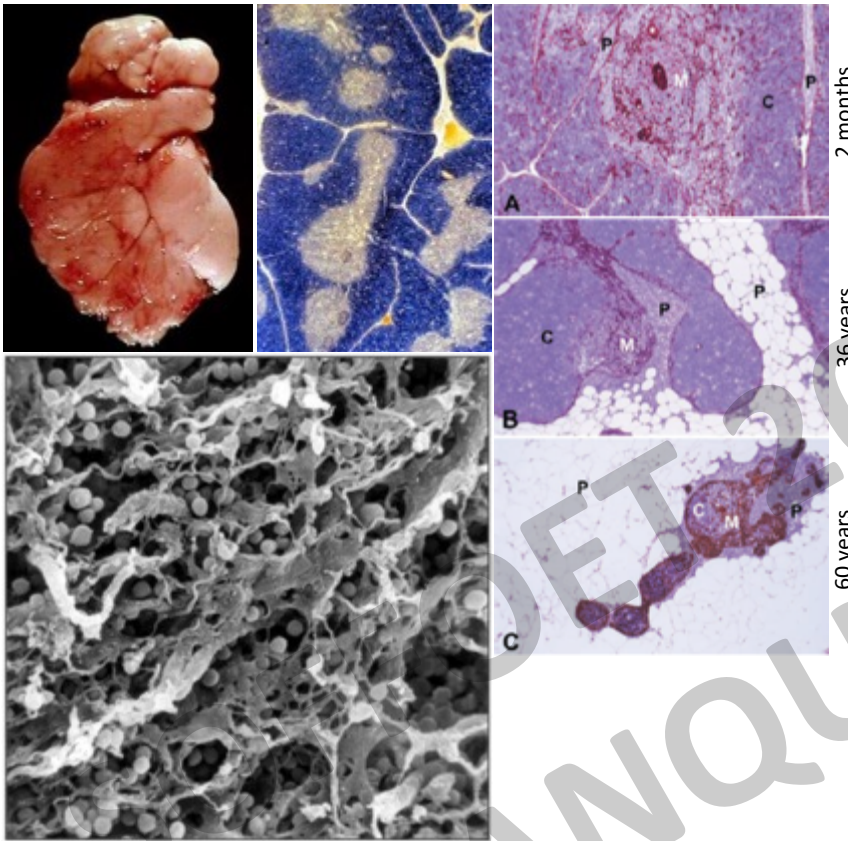
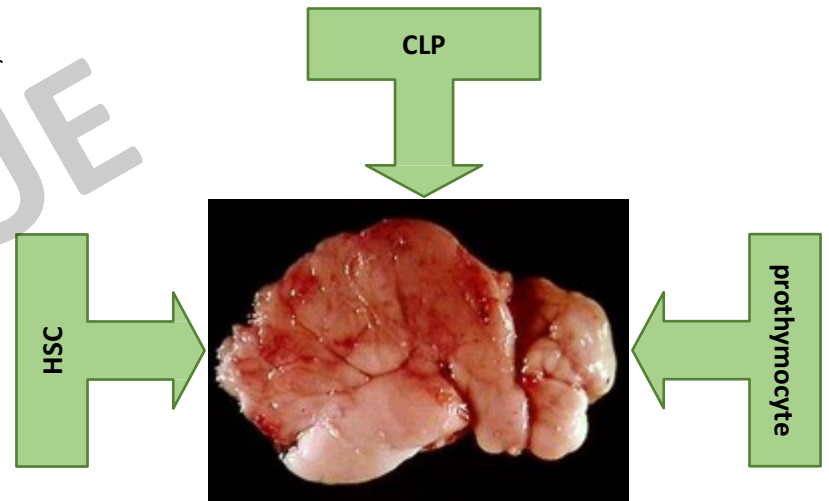


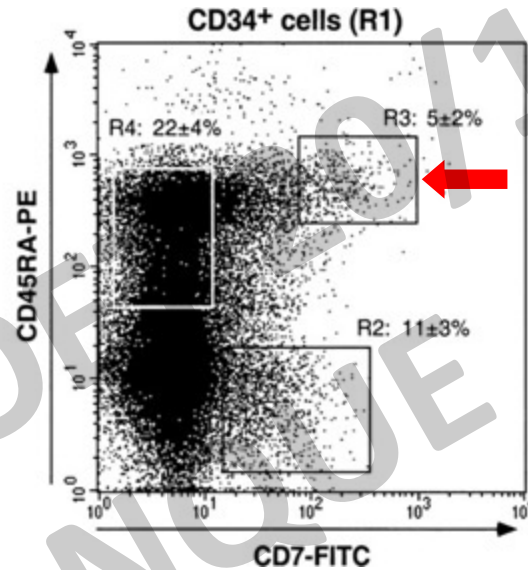
Figure 7-9 Immunobiology, 6/e. © Garland Science 2005



## Characterization of dendritic cell differentiation pathways from cord blood $CD34^+CD7^+CD45RA^+$ hematopoietic progenitor cells

Bruno Canque, Sandrine Camus, Aïi Dalloul, Edmond Kahn, Micaël Yagello, Colette Dezutter-Dambuyant, Daniel Schmitt, Christian Schmitt, and Jean Claude Gluckman

*Blood* 2000, 96:3748

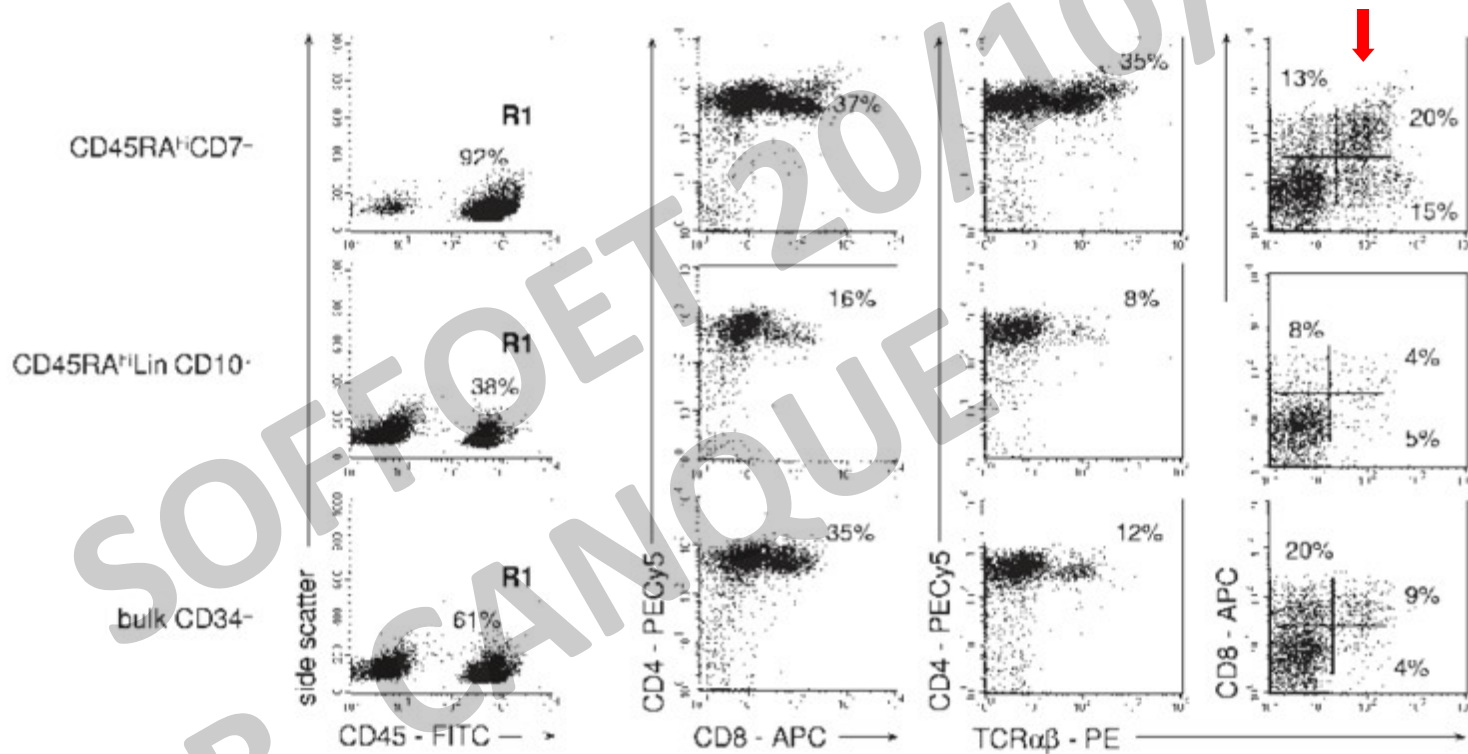


A. Galy, M. Travis, D. Cen, B. Chen, Human T, B, natural killer, and dendritic cells arise from a common bone marrow progenitor cell subset. *Immunity* **3**, 459-473 (1995).

Q. L. Hao *et al.*, Identification of a novel, human multilymphoid progenitor in cord blood. *Blood* **97**, 3683-3690 (2001).

## Molecular characterization of early human T/NK and B-lymphoid progenitor cells in umbilical cord blood

Rima Haddad, Philippe Guardiola, Brigitte Izac, Christelle Thibault, Jerry Radich, Anne-Lise Delezoide, Claude Baillou, François M. Lemoine, Jean Claude Gluckman, Françoise Pflumio, and Bruno Canque



CD45RA<sup>+</sup>CD7<sup>+</sup> HPCs display thymus-colonizing activity and T potential







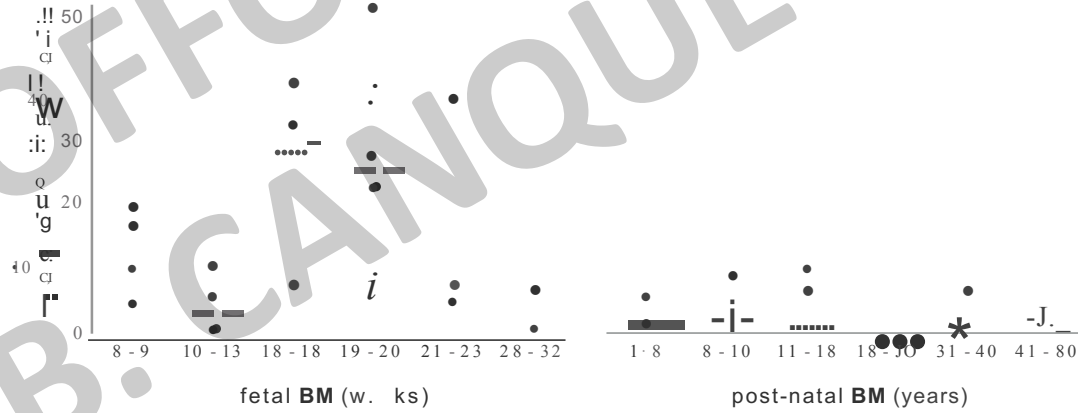
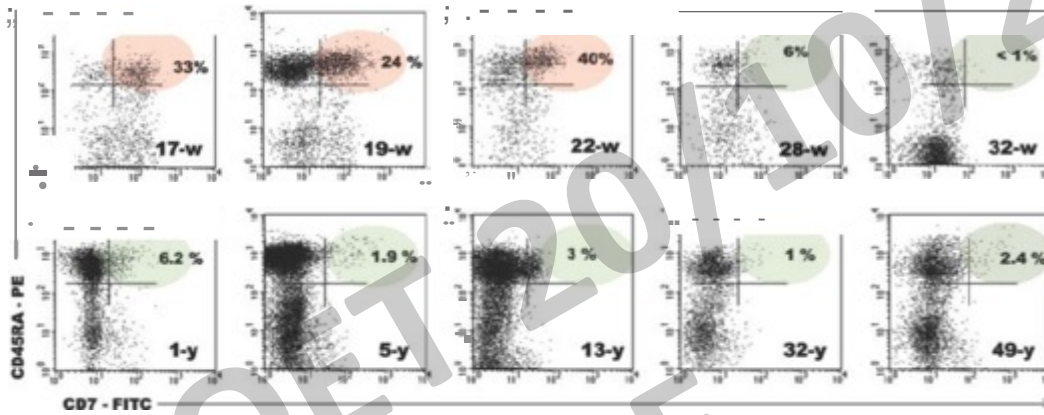
# Dynamics of Thymus-Colonizing Cells during Human Development

Rima Haddad,<sup>1</sup> Fabien Guilhot,<sup>1</sup> Emmanuelle S.,<sup>1</sup>  
Frederic Jourquin,<sup>1</sup> Nicias Setterblad,<sup>2</sup>  
Edmond Karim,<sup>1</sup> Micael Yagel,<sup>3</sup> Cecile Schiffrer,<sup>1</sup> Isabel  
de Andrade-Schmutz,<sup>4</sup> Marina Cavazzana-Calvo,<sup>5</sup>  
Jean Claude Gluckman,<sup>1</sup> Anne-Lise Oelze,<sup>6</sup>  
Françoise Pflieger,<sup>6</sup> and Bruno Canque<sup>1\*</sup>

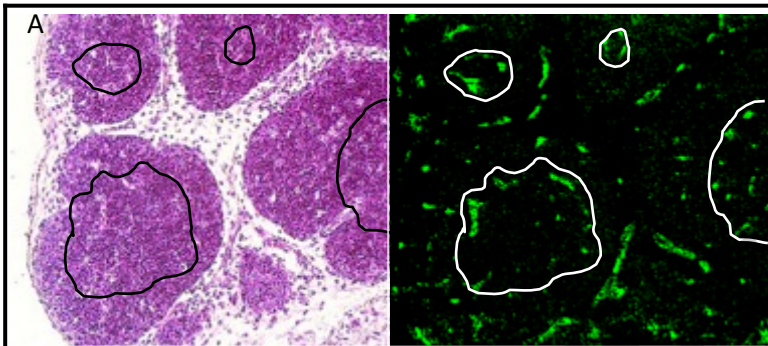
## Summary

Here, we identify fetal bone marrow (BM)-derived CD34<sup>+</sup>CD45RA<sup>+</sup>CD7<sup>+</sup> hematopoietic progenitors as thymus colonizing cells. This population, virtually absent from the fetal liver (FL), emerges in the BM by development weeks 8–9, where it accumulates throughout the second trimester, to finally decline around birth. Based on phenotypic, molecular, and functional criteria, we demonstrate that CD34<sup>+</sup>CD45RA<sup>+</sup>CD7<sup>+</sup> cells represent the direct precursors of the most mature CD34<sup>+</sup>CD1a<sup>+</sup> fetal thymocytes that follow a similar dynamic pattern during fetal and early postnatal development. Histological analysis of fetal thymuses further reveals that early migrants predominantly localize in the perivascular areas of the cortex, where they form a lymphostromal complex with thymic epithelial cells (TECs) driving their rapid specification toward the T lineage. Finally, using an ex vivo xenogeneic thymus colonization assay, we show that BM-derived CD34<sup>+</sup>CD45RA<sup>+</sup>CD7<sup>+</sup> progenitors are selectively recruited into the thymus parenchyma in the absence of exogenous cytokines, where they adopt a definitive T cell fate.

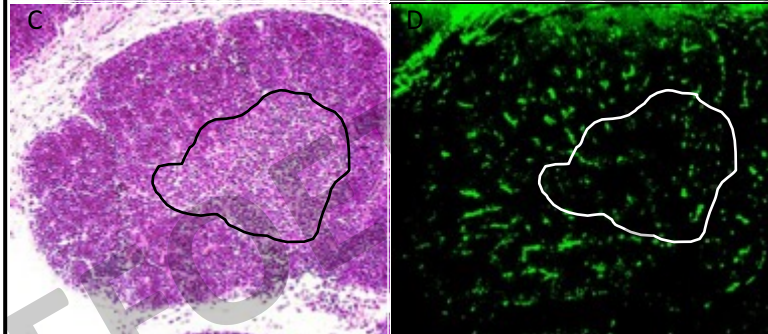
# Dynamics of Thymus-Colonizing Cells during Human Development



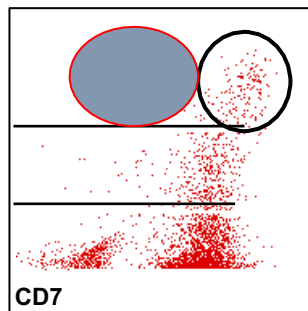
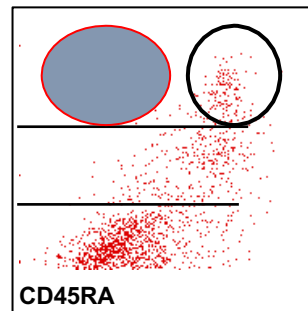
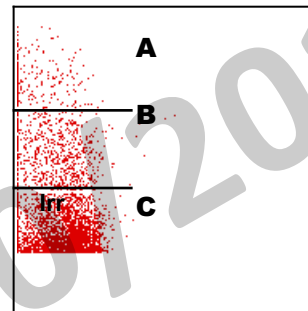
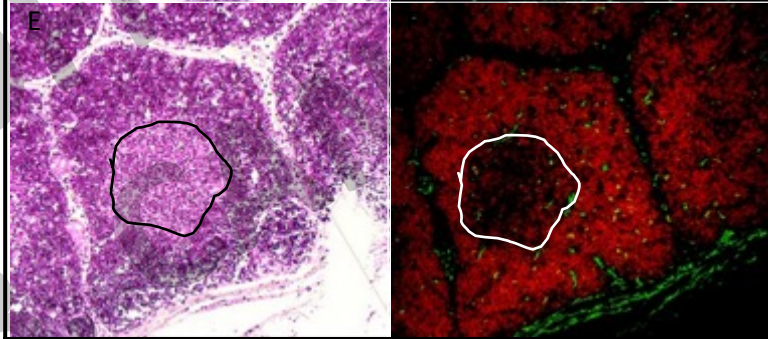
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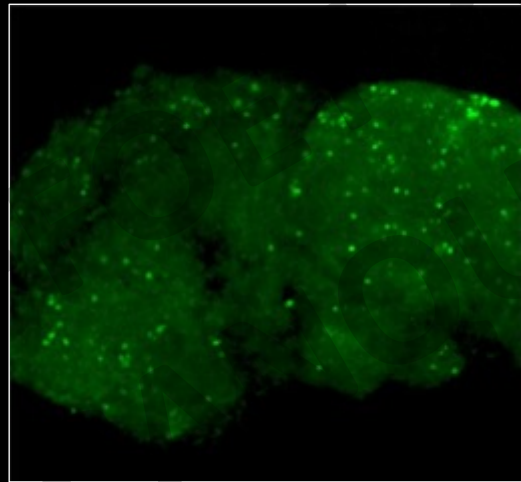
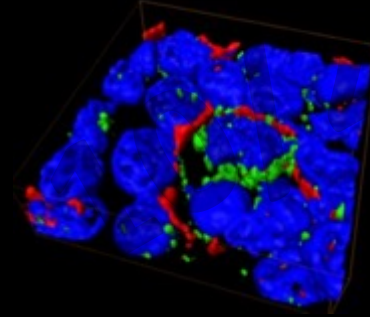
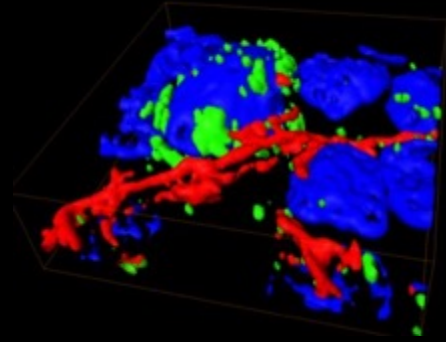


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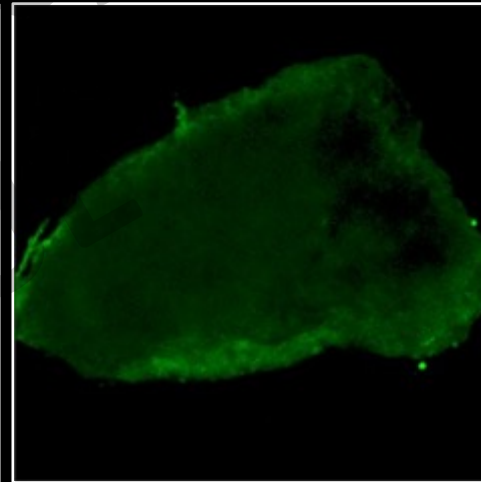


STAGE - III





CD34<sup>hi</sup>CD45RA<sup>hi</sup>CD7<sup>+</sup>



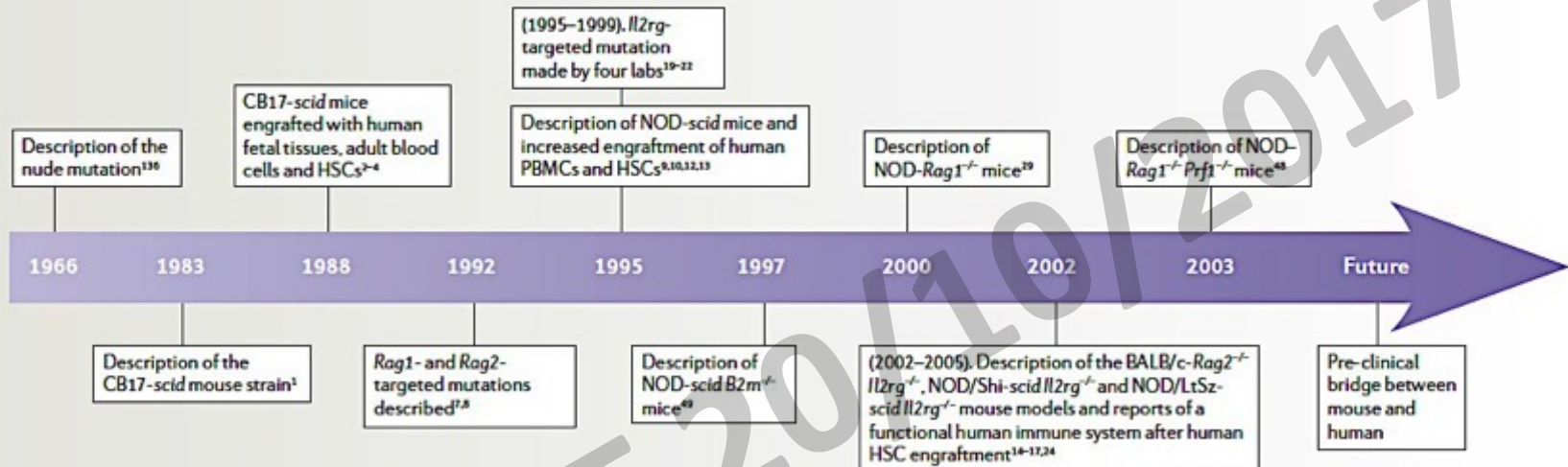
CD34<sup>hi</sup>CD45RA<sup>int</sup>CD7<sup>-</sup>

# In vivo modelization of human hematopoiesis:

Building the NSG-UCB model

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## Timeline | Important events in the development of humanized mice



B2m,  $\beta_2$ -microglobulin; HSC, haematopoietic stem cell; *Il2rg*, Interleukin-2 receptor  $\gamma$ -chain; NOD, non-obese diabetic; PBMC, peripheral-blood mononuclear cell; *Prf1*, perforin 1; *Rag*, recombination-activating gene; *scid*, severe combined immunodeficiency.

From Shultz et al. *Nat Rev Immunol* 2007

### Development of a Human Adaptive Immune System in Cord Blood Cell-Transplanted Mice

Elisabetta Traggiai,<sup>1\*</sup> Laurie Chicha,<sup>1\*</sup> Luca Mazzucchelli,<sup>2</sup> Lucio Bronz,<sup>3</sup> Jean-Claude Piffaretti,<sup>4</sup> Antonio Lanzavecchia,<sup>1</sup> Markus G. Manz<sup>1†</sup>

Science - 2 april 2004

### Human Lymphoid and Myeloid Cell Development in NOD/LtSz-*scid* *IL2R $\gamma$ <sup>null</sup>* Mice Engrafted with Mobilized Human Hemopoietic Stem Cells<sup>1,2</sup>

Leonard D. Shultz,<sup>\*</sup> Bonnie L. Lyons,<sup>\*</sup> Lisa M. Burzenski,<sup>\*</sup> Bruce Gott,<sup>\*</sup> Xiaohua Chen,<sup>†</sup> Stanley Chaleff,<sup>‡</sup> Malak Kotb,<sup>‡</sup> Stephen D. Gillies,<sup>§</sup> Marie King,<sup>¶</sup> Julie Mangada,<sup>¶</sup> Dale L. Greiner,<sup>¶</sup> and Rupert Handgretinger<sup>3†</sup>

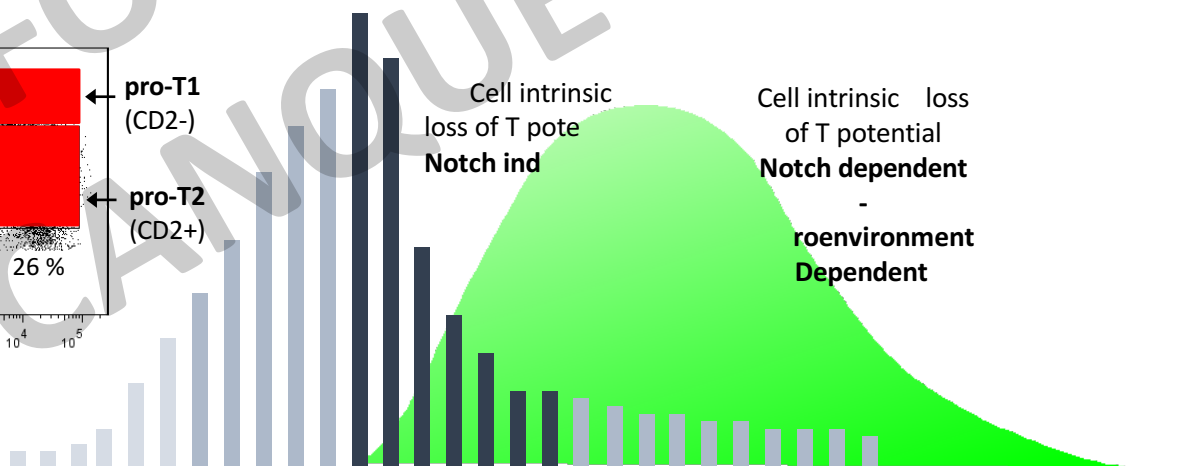
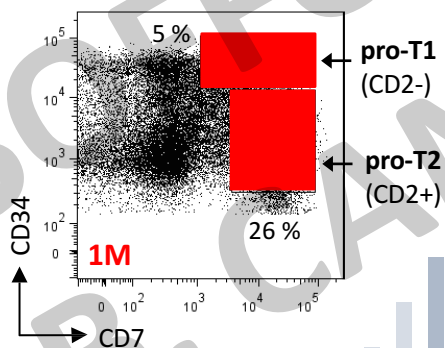
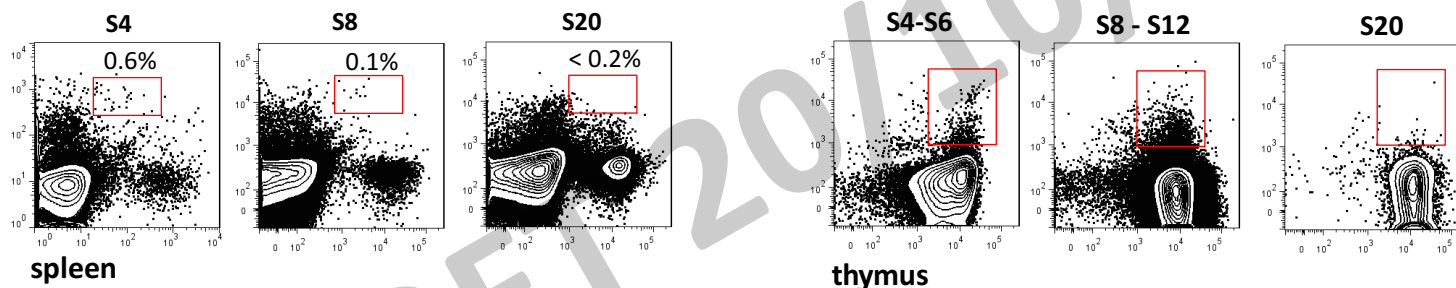
J Immunol - february 2005



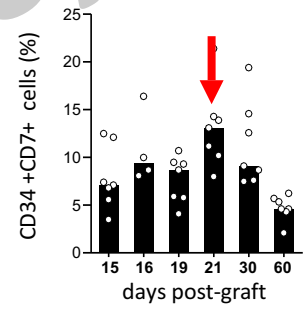
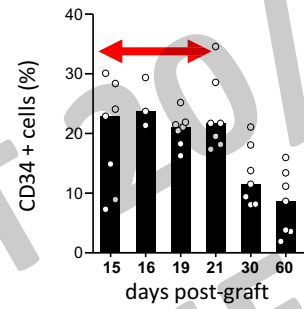
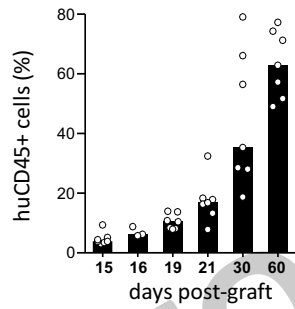
# Dynamics of Human Prothymocytes and Xenogeneic Thymopoiesis in Hematopoietic Stem Cell-Engrafted Nonobese Diabetic-SCID/IL-2 $\gamma$ <sup>null</sup> Mice

Journal of immunology 2012, 189:1648

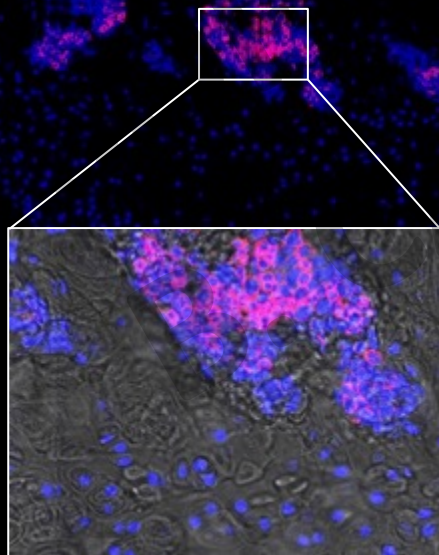
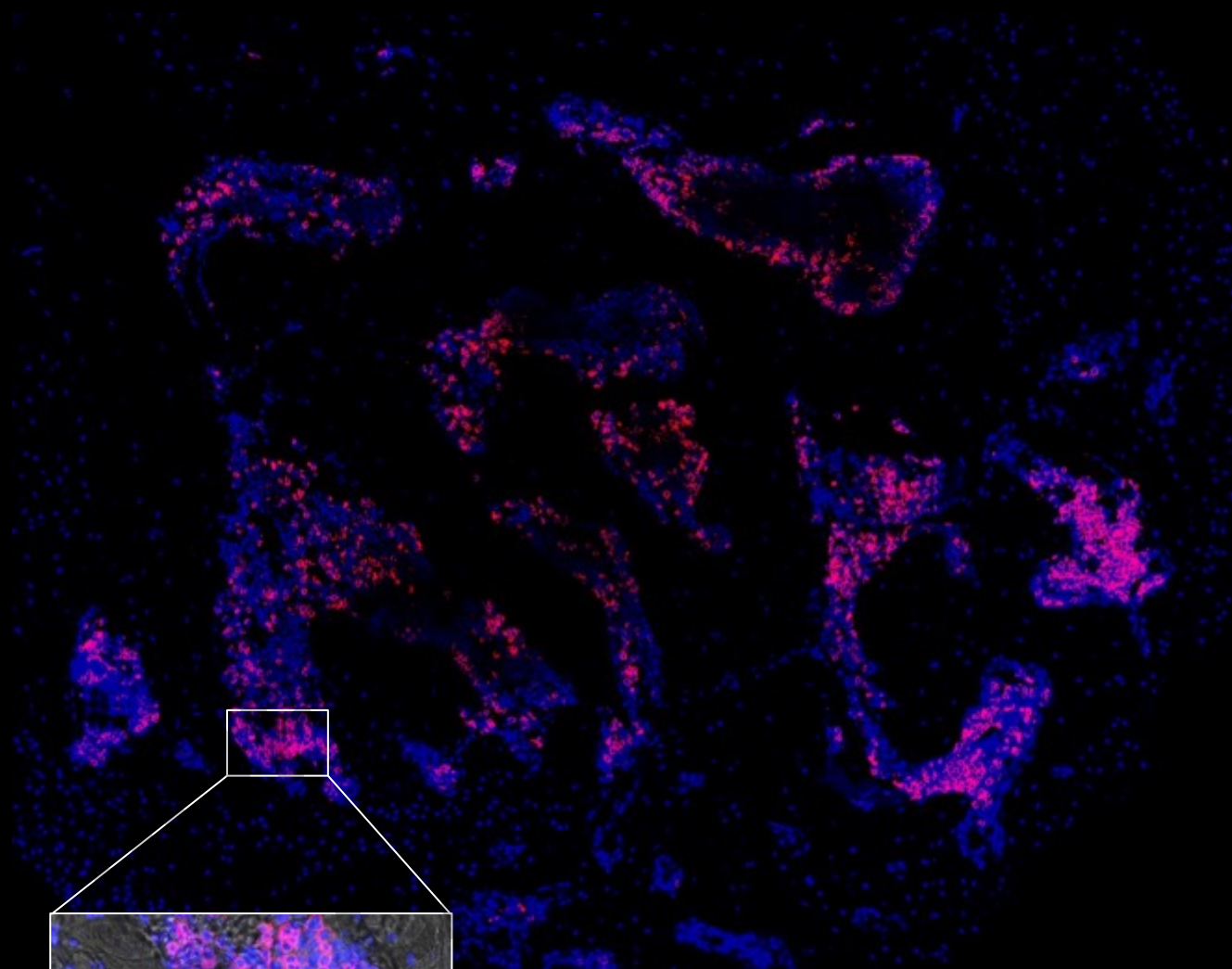
Véronique Parietti,<sup>\*,1,2</sup> Elisabeth Nelson,<sup>\*,1,2</sup> Gladys Telliam,<sup>\*,1,2</sup> Sandrine Le Noir,<sup>3</sup> Marika Pla,<sup>4</sup> Marc Delord,<sup>2</sup> Valérie Vanneau,<sup>2</sup> Mahmood Mohtashami,<sup>2</sup> Elizabeth A. Macintyre,<sup>3</sup> Jean Claude Gluckman,<sup>\*,1,2</sup> Vahid Asnafi,<sup>3</sup> Juan-Carlos Zúñiga-Pflücker,<sup>2</sup> Jérôme Larghero,<sup>3</sup> and Bruno Canque<sup>\*,1,2</sup>





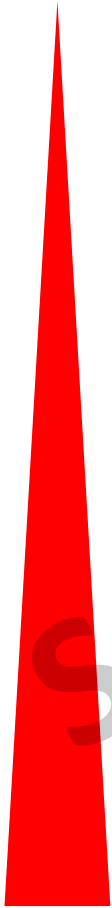


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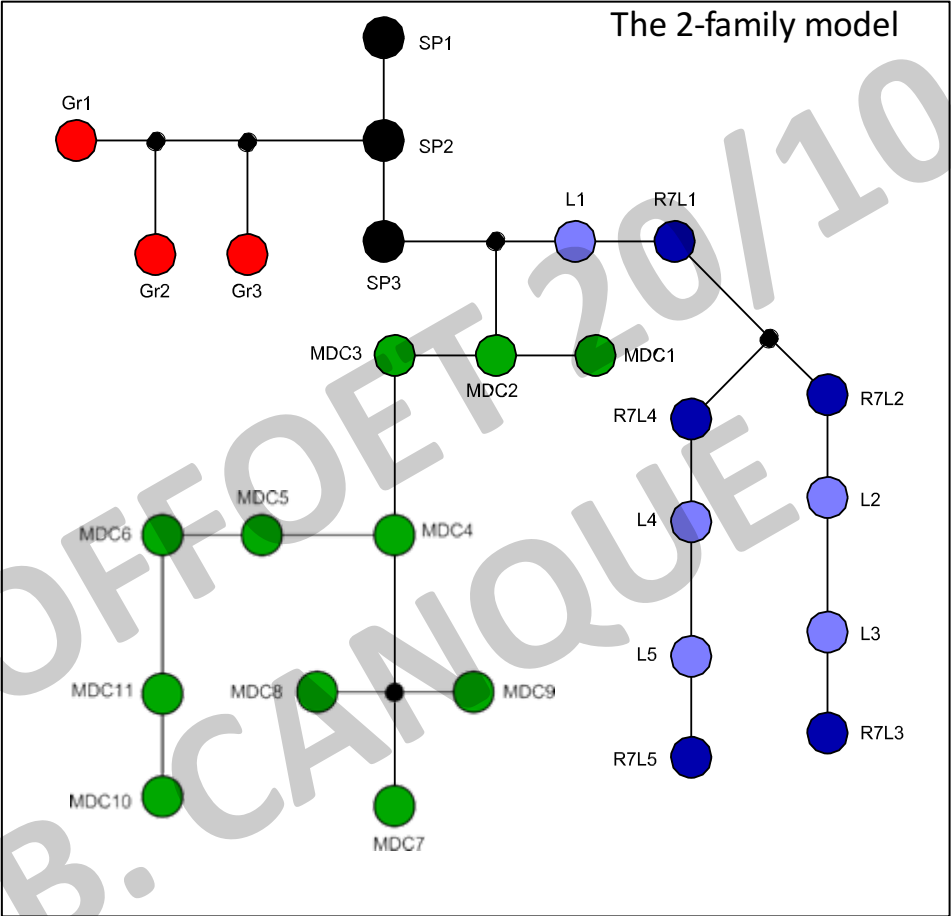


- NSG-UCB week-3
- Femur
- hu CD45

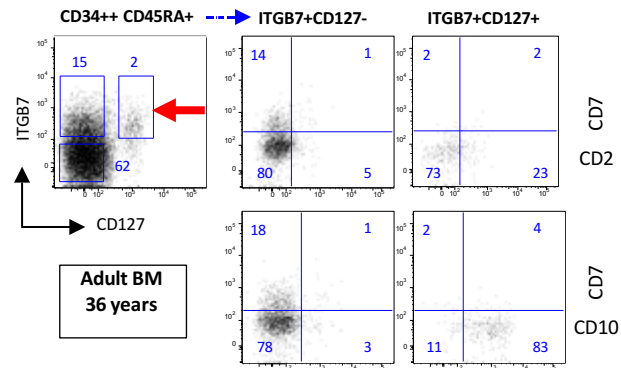
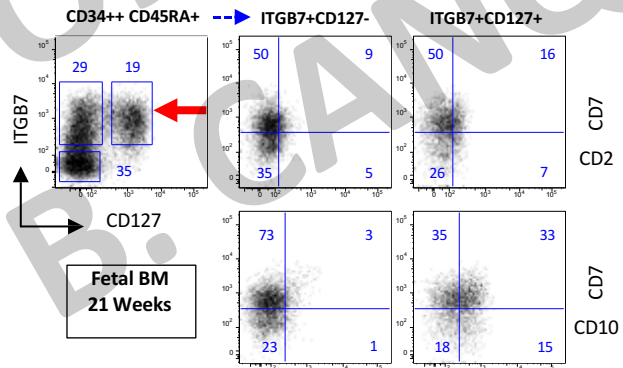
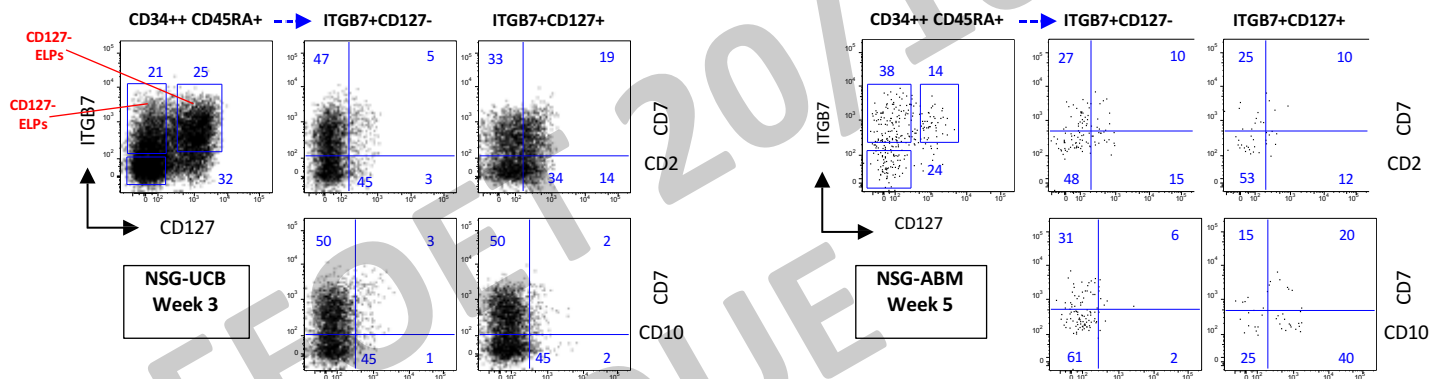
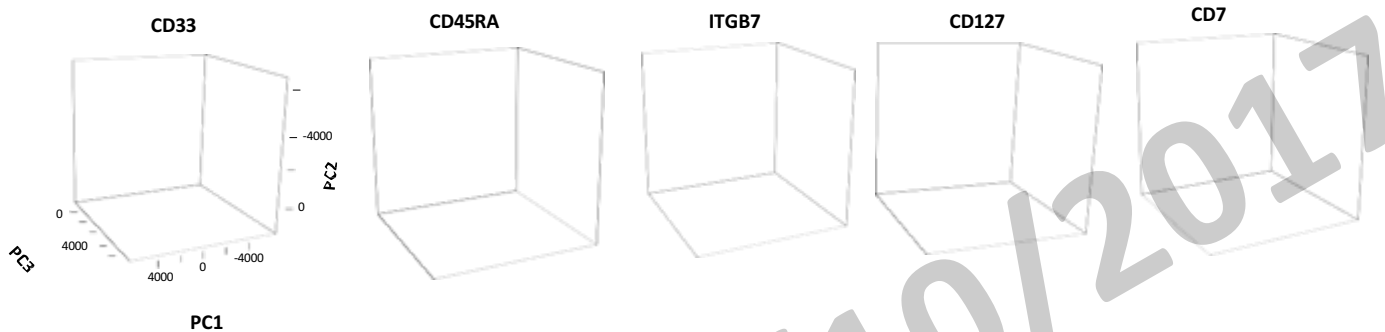
# Phenotypic roadmapping

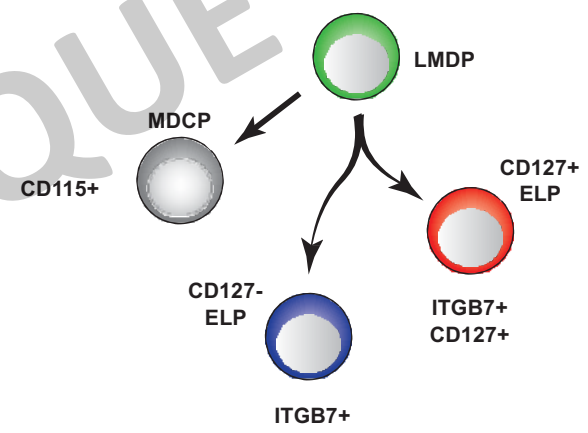
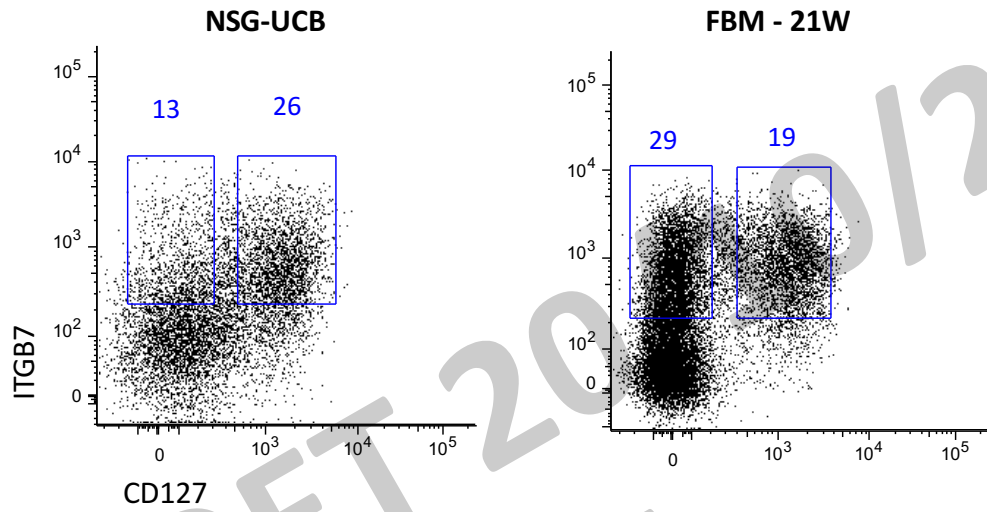


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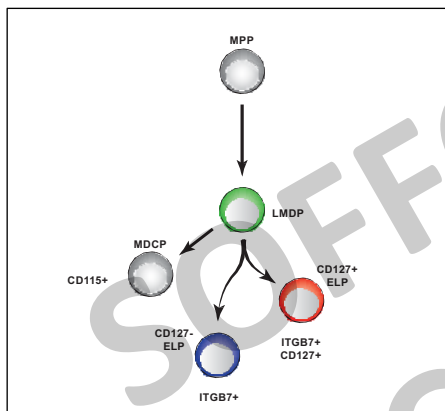
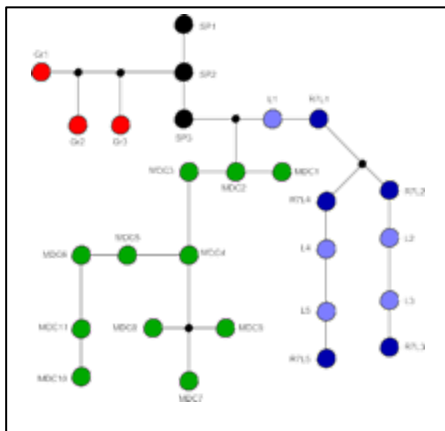
- > 80 surface markers
- 14-16 antibody panels
- 27 populations
- 28 parameters
- Minimum spanning tree





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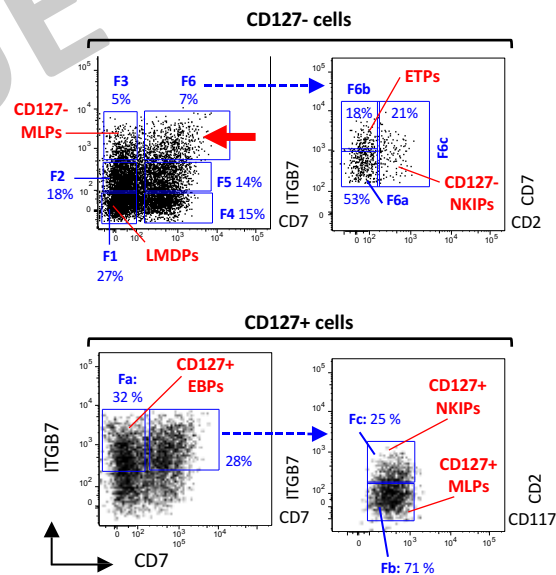
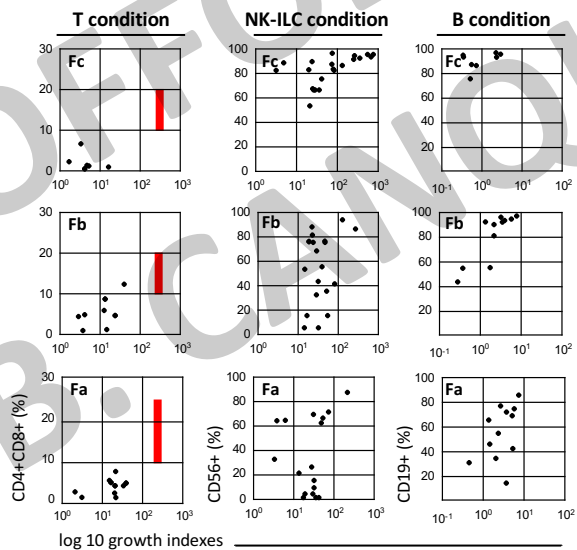
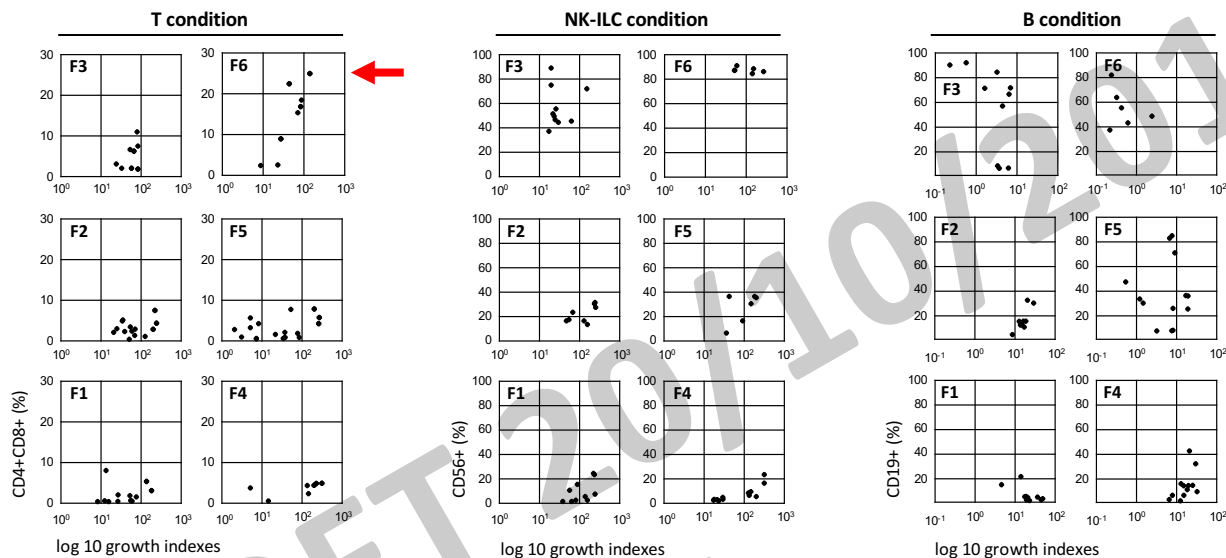


# Functional characterization of ELPs

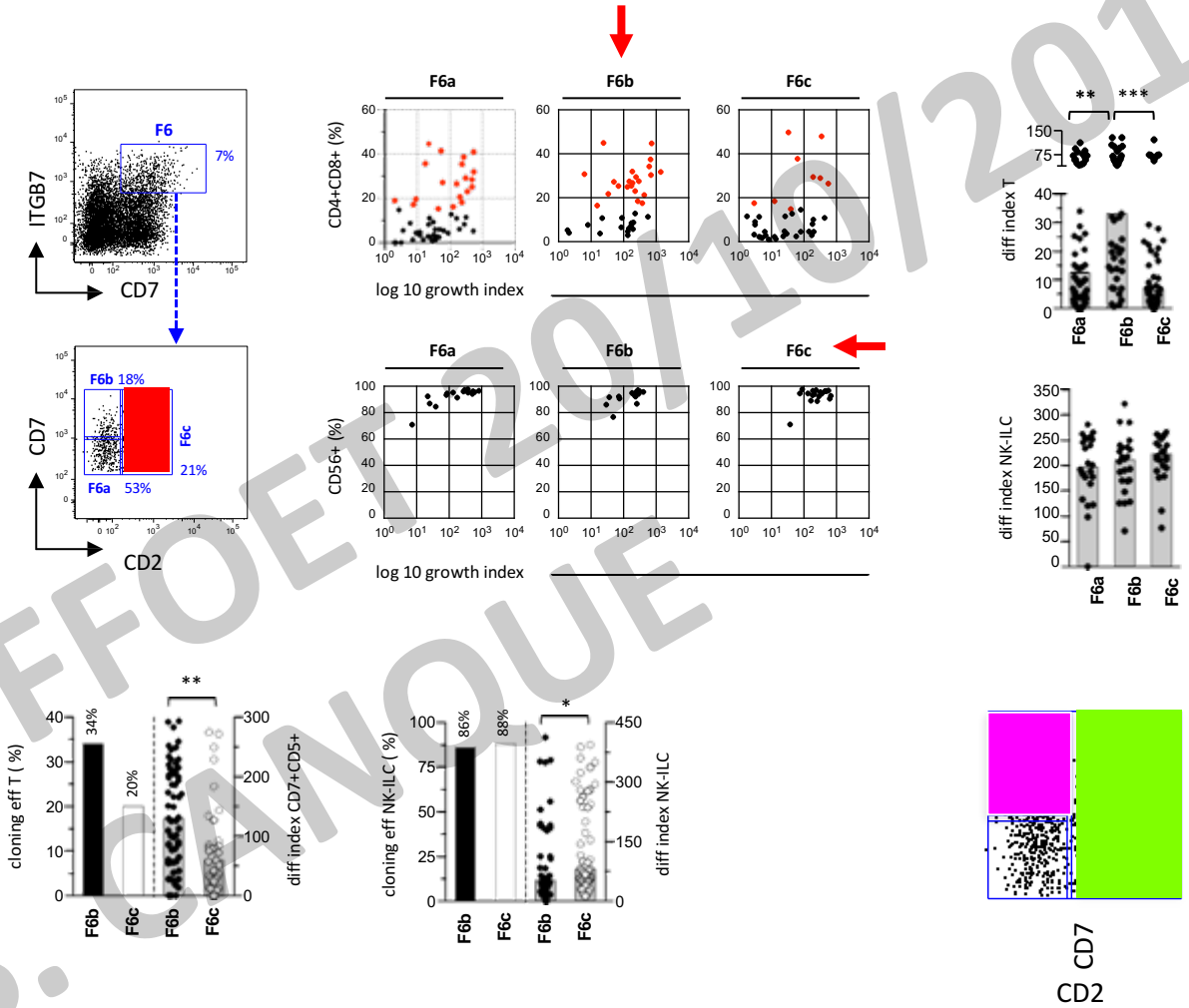
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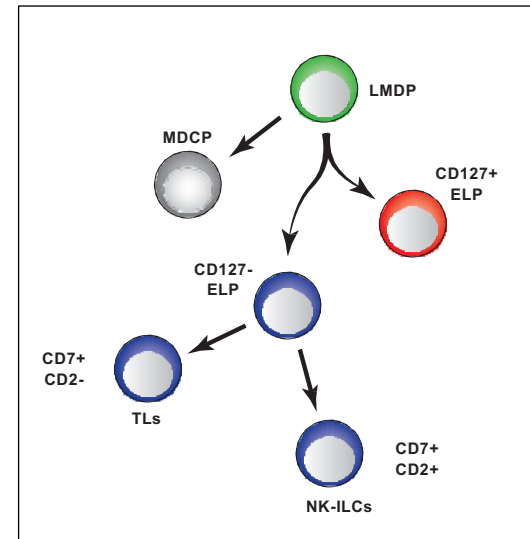
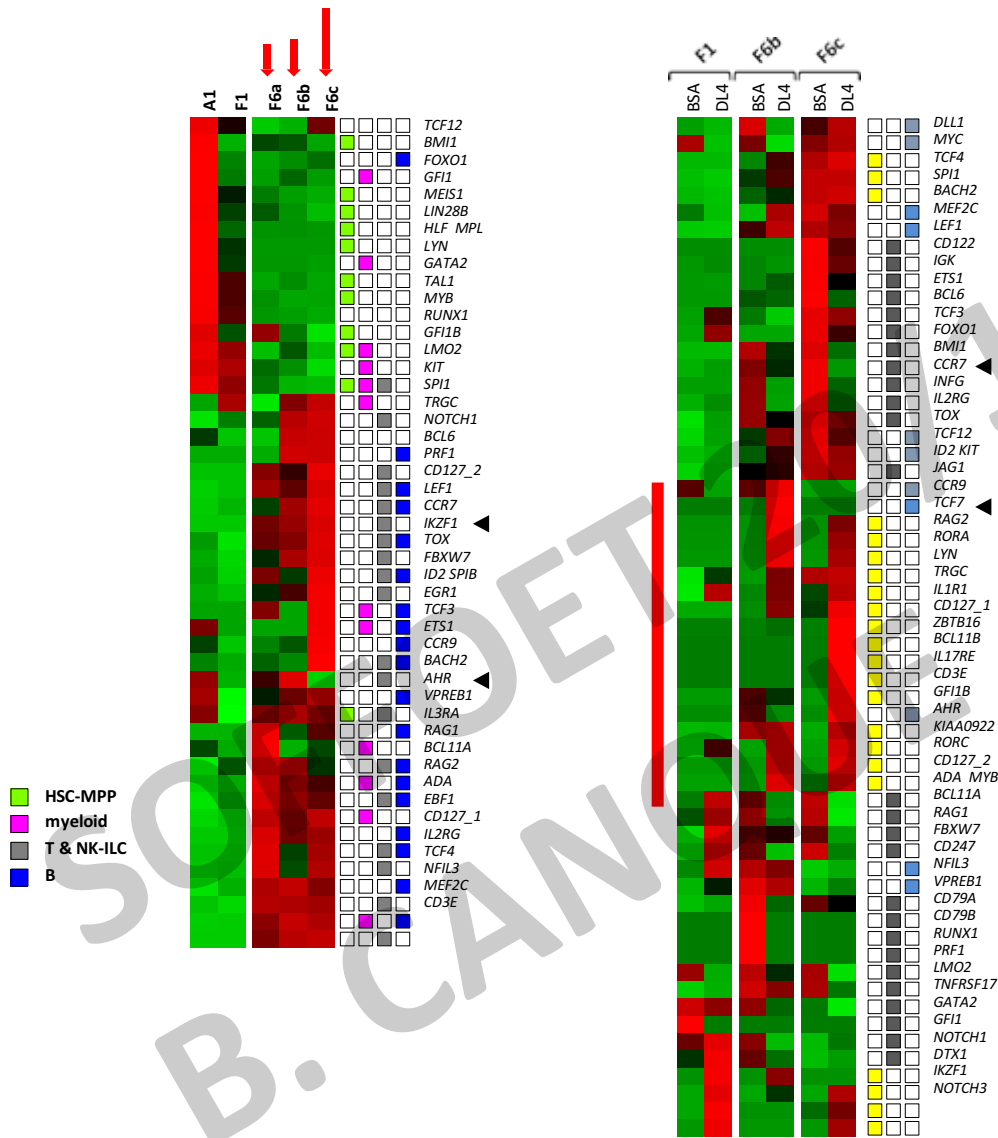
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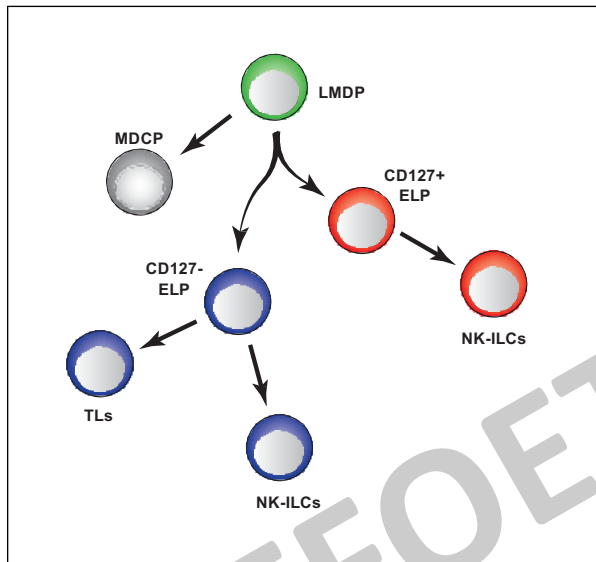
# Early divergence between ETPs and NKIPs



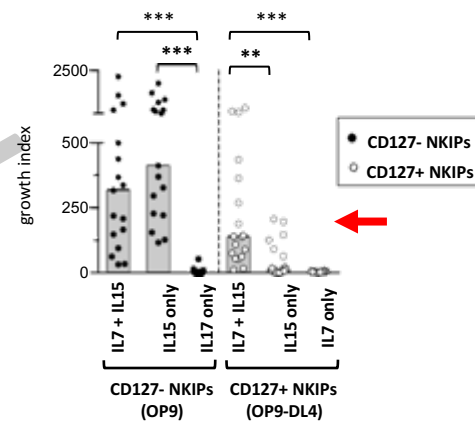
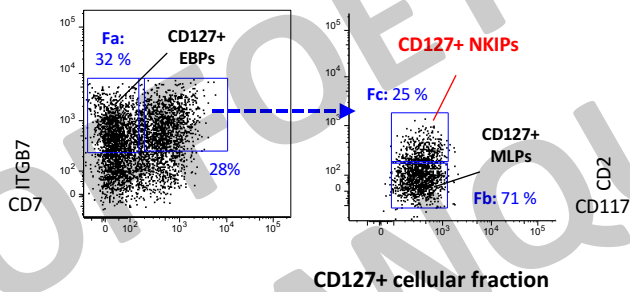
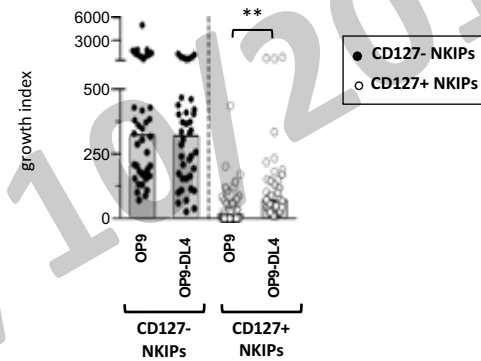
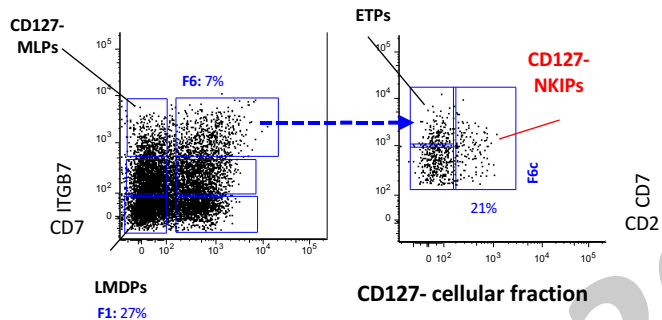
SOFFO EN 2017  
 B. CAVALLOU

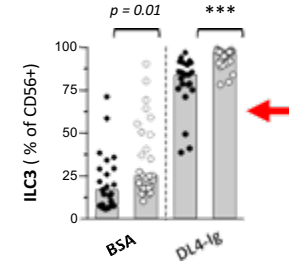
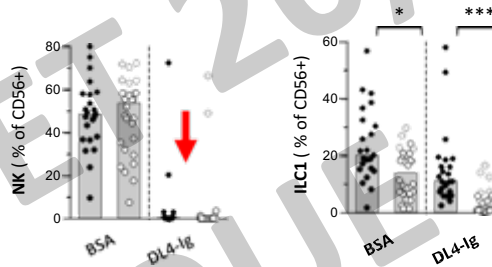
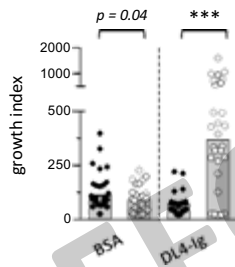
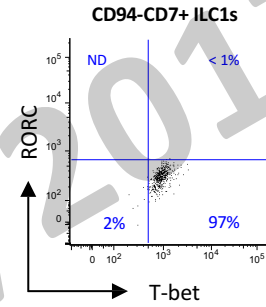
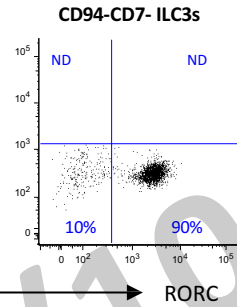
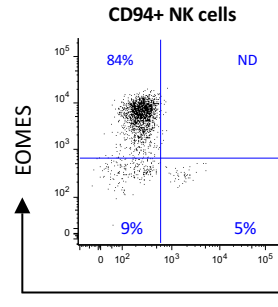
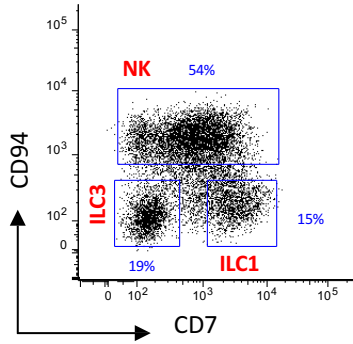


# Functional characterization of hu-NKIPs

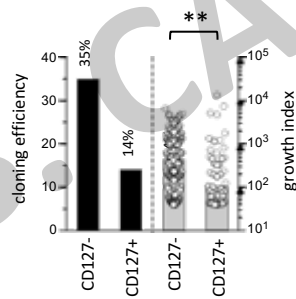


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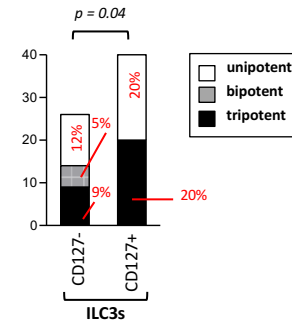
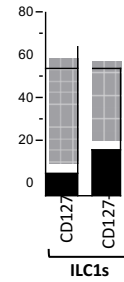
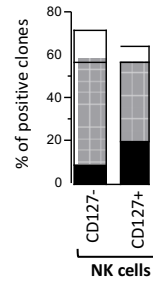


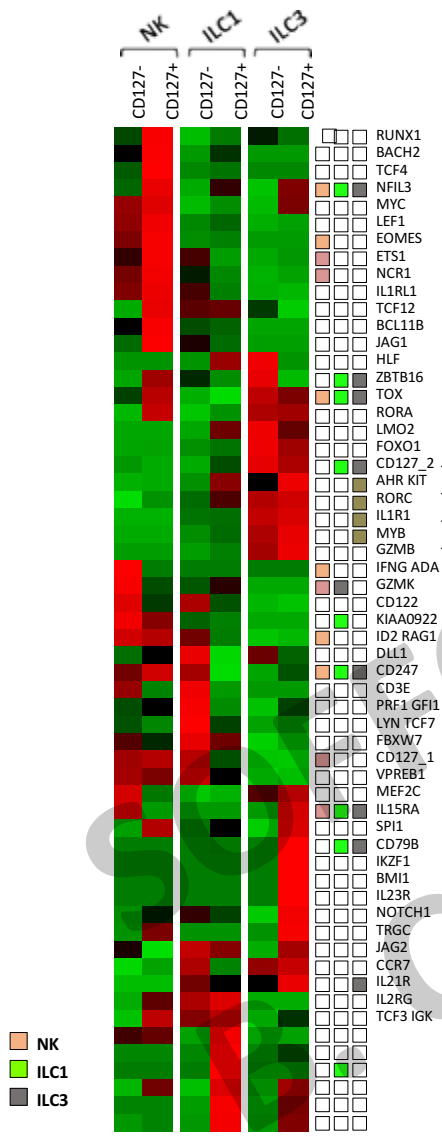


● CD127- NKIPs ○ CD127+ NKIPs

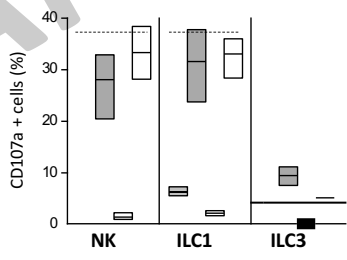
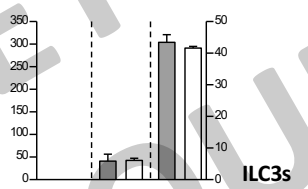
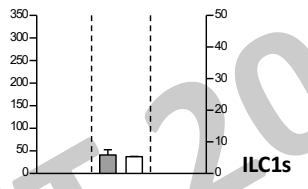
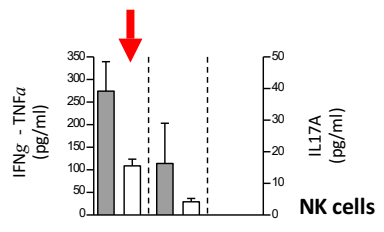


growth index

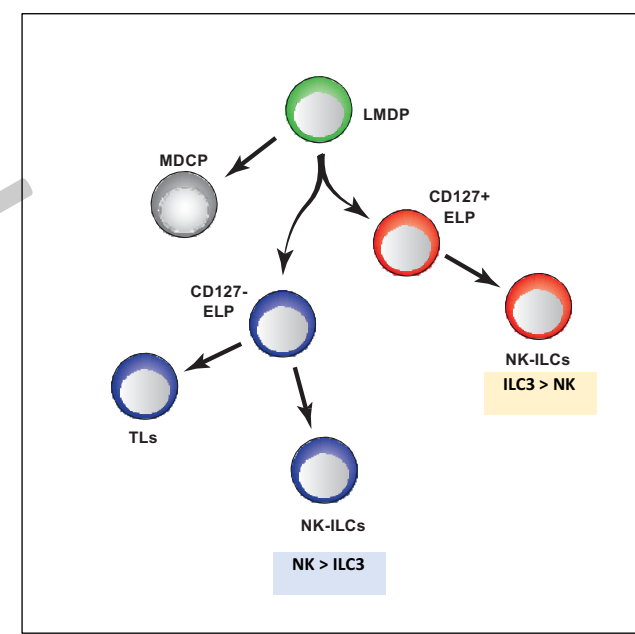




- RUNX1
- BACH2
- TCF4
- NFIL3
- MYC
- LEF1
- EOMES
- ETS1
- NCR1
- IL1RL1
- TCF12
- BCL11B
- JAG1
- HLF
- ZBTB16
- TOX
- RORA
- LMO2
- FOXO1
- CD127\_2
- AHR KIT
- RORC
- IL1R1
- MYB
- GZMB
- IFNG ADA
- GZMK
- CD122
- KIAA0922
- ID2 RAG1
- DLL1
- CD247
- CD3E
- PRF1 GF11
- LYN TCF7
- FBXW7
- CD127\_1
- VPREB1
- MEF2C
- IL15RA
- SPI1
- CD79B
- IKZF1
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- IL23R
- NOTCH1
- TRGC
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- CCR7
- IL21R
- IL2RG
- TCF3 IGHK

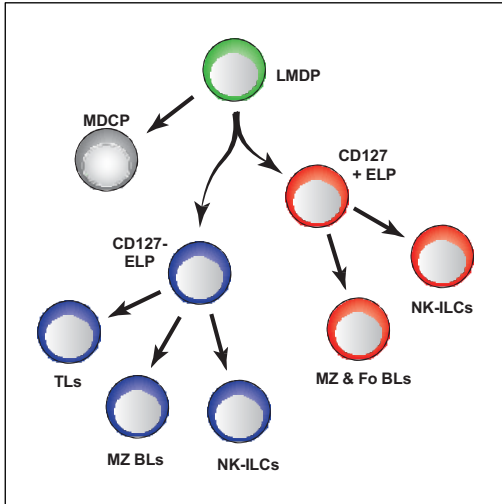


CD127- NK-ILCs  
CD127+ NK-ILCs



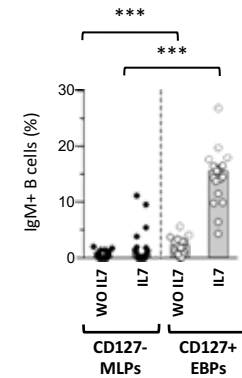
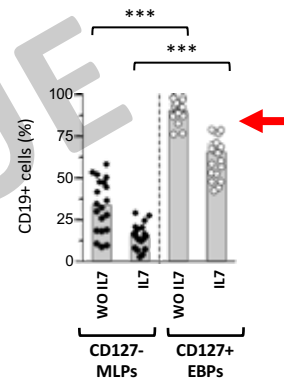
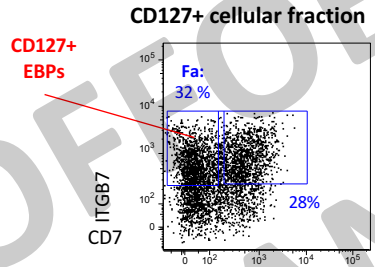
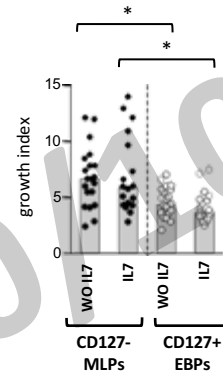
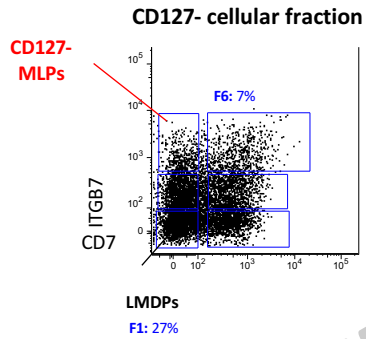


# Characterization of hu EBPs & proB cells

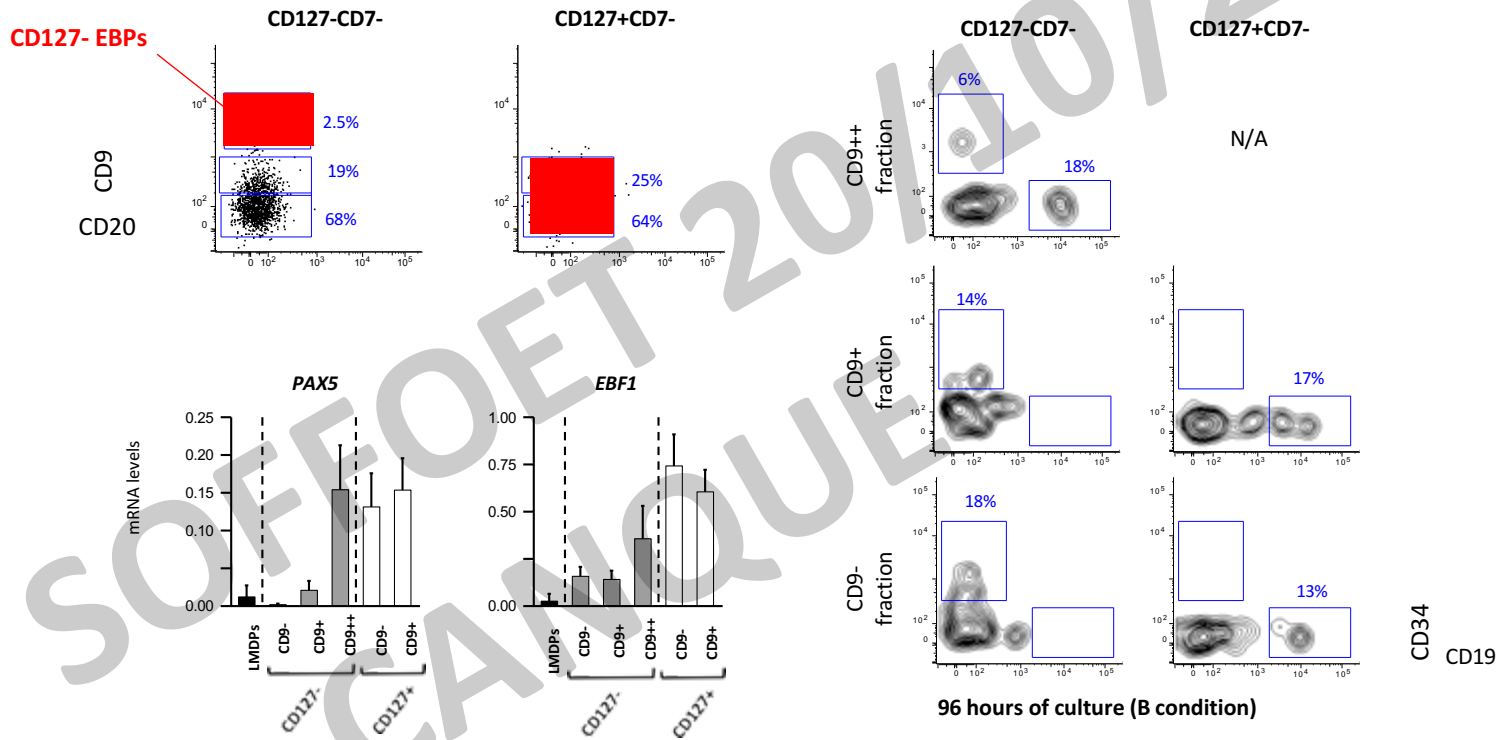


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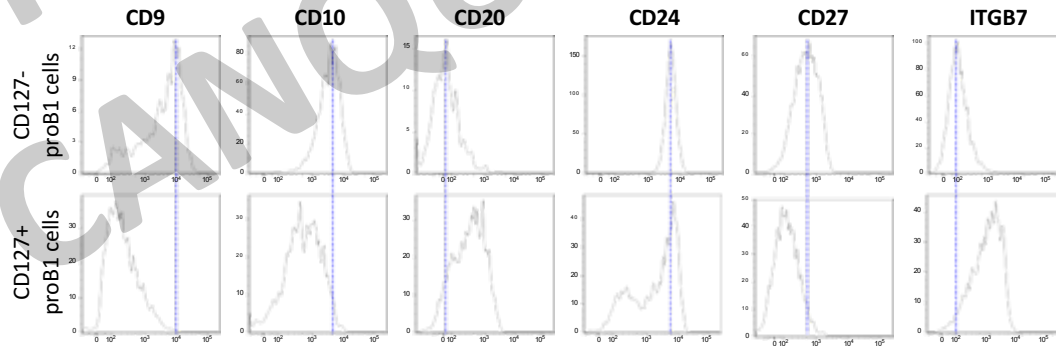
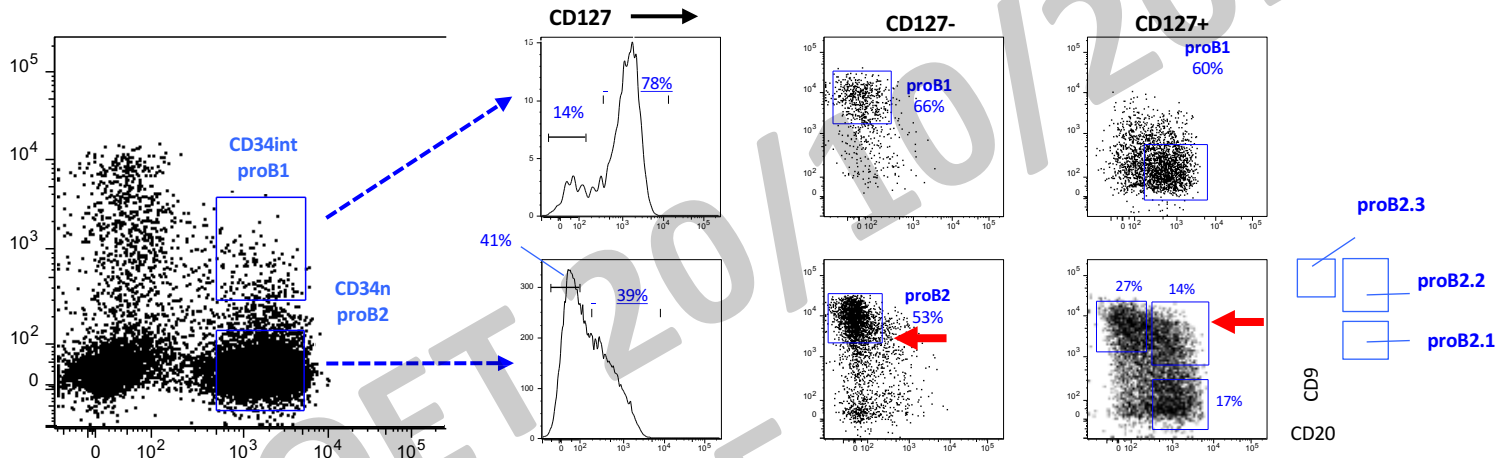
# Comparative assessment of the B potential



# Identification of human EBPs

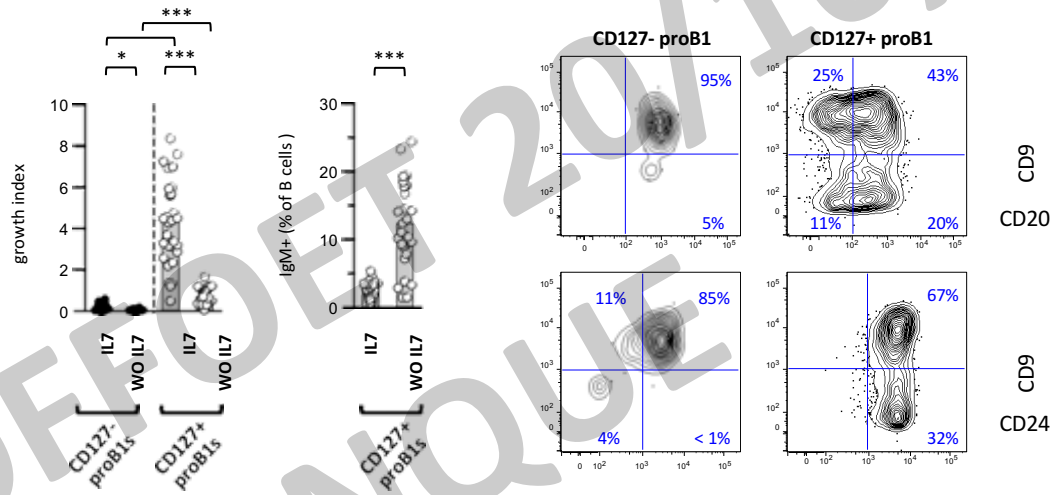


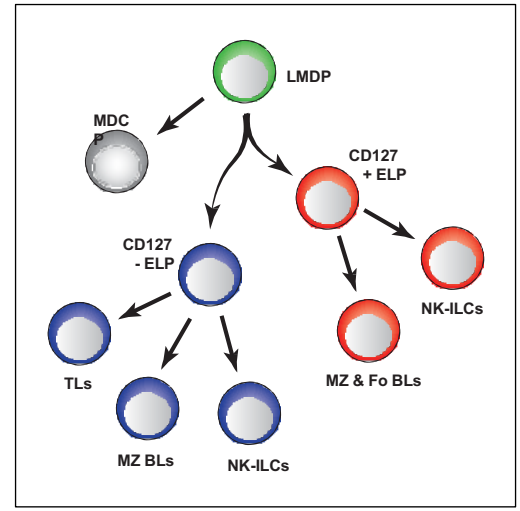
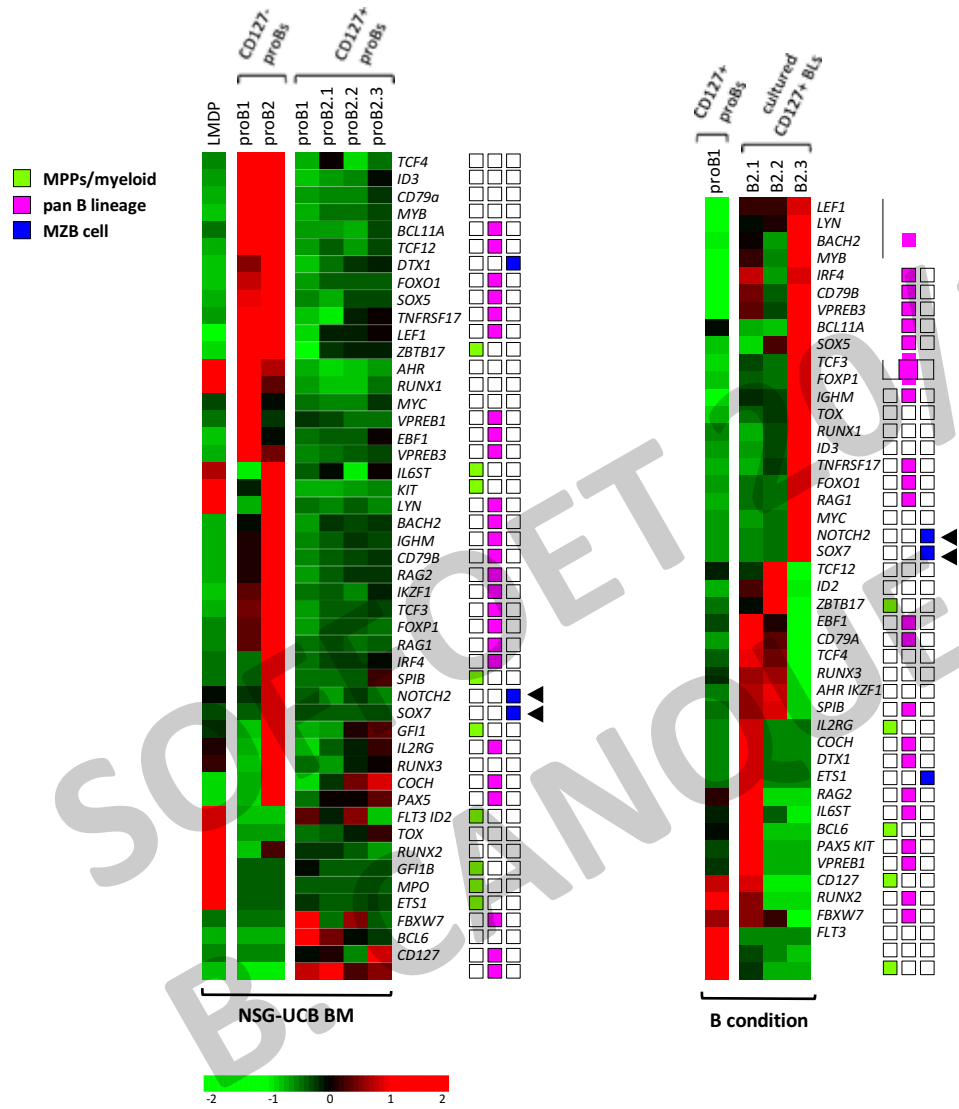
# Phenotype of human proB cells



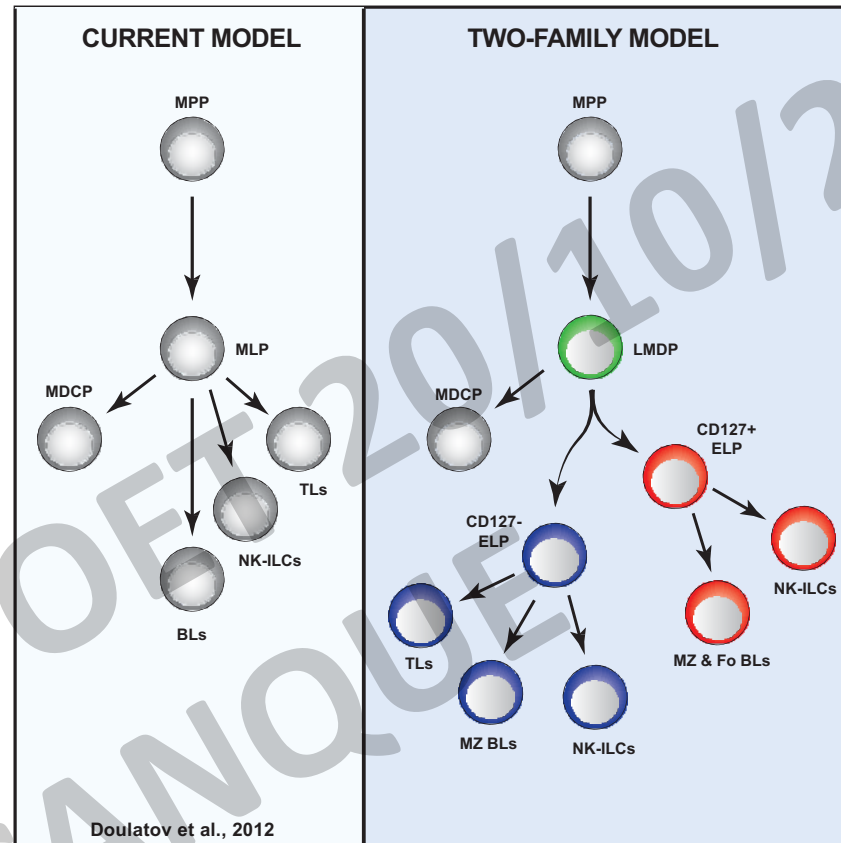
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# Functional characterization of hu proB cells





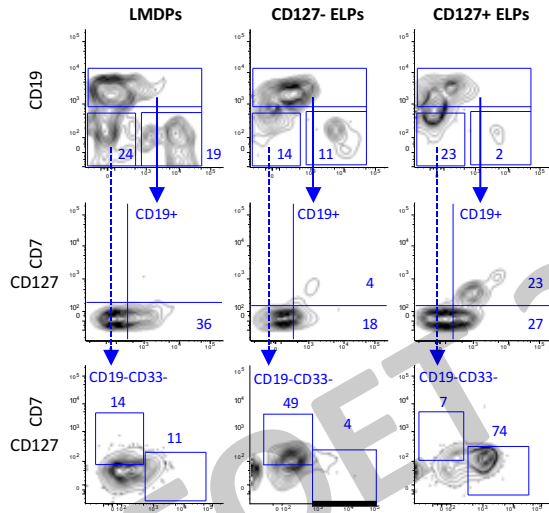
# In vivo validation



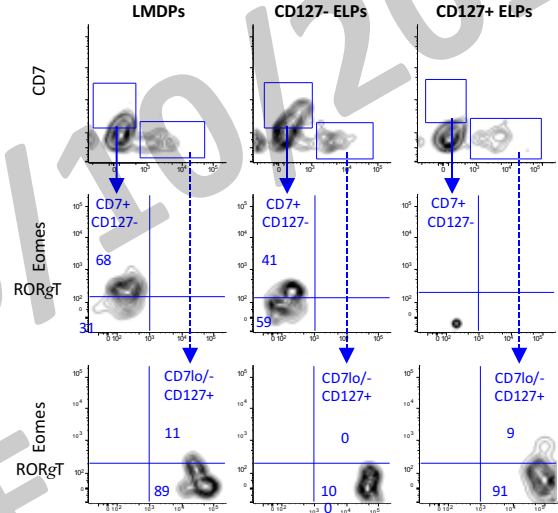
Impact of Notch1 signaling on ELP emergence & specification  
Analysis of precursor – successor relationships



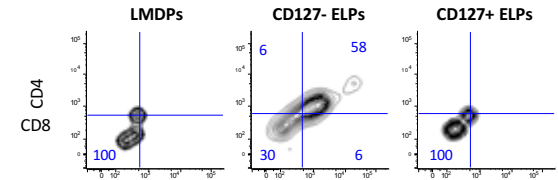
**bone marrow (hu-CD45+ cells)**



**spleen (hu-CD45+CD19-CD33- cells)**

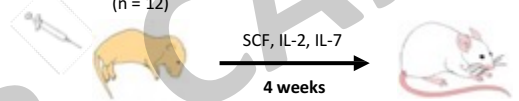


**thymus (hu-CD45+ cells)**

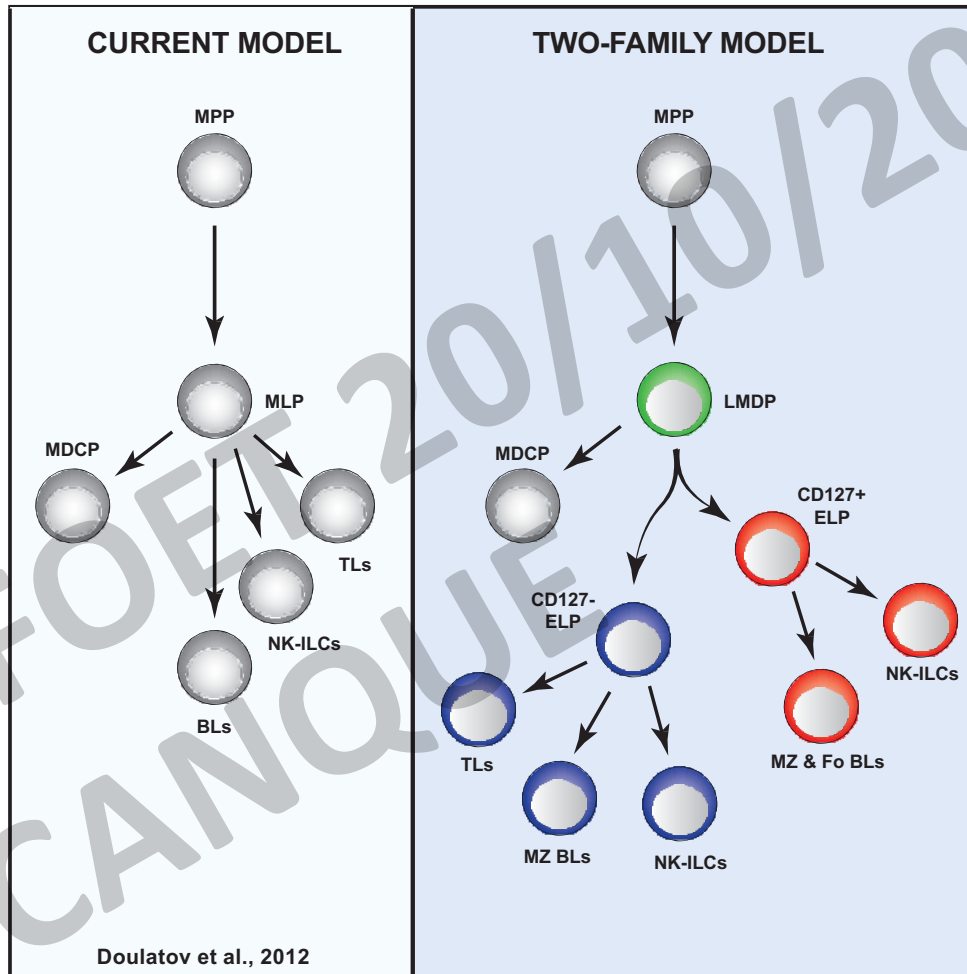


LMDPs, CD127- and CD127+ ELPs from NSG-UCB BM (n = 12)

FACS analysis: BM, THY, SPLEEN



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Impact of Notch1 signaling on ELP emergence & specification  
 Analysis of precursor – successor relationships

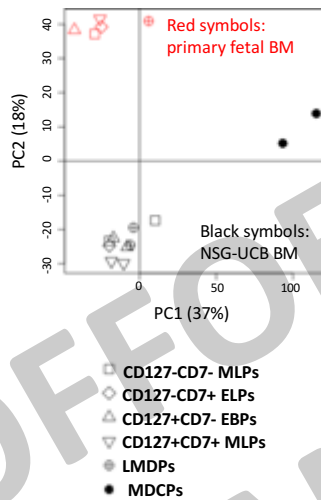
# Genome-wide transcriptional profiling

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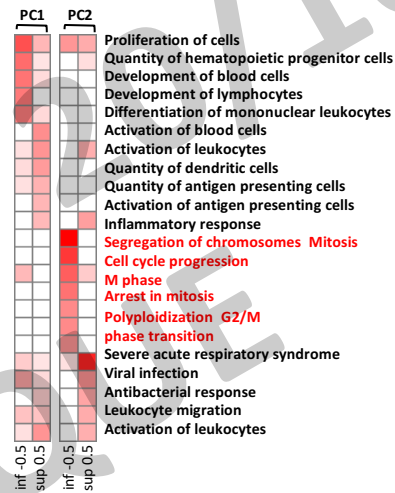
# Comparative analyses

## NSG-UCB versus Fetal Bone Marrow-1

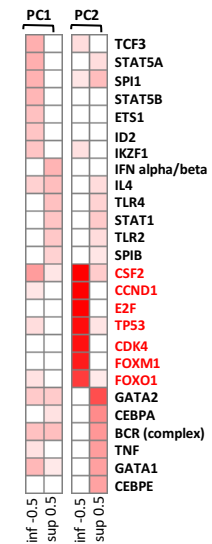
Principal component analysis



Diseases and biological functions

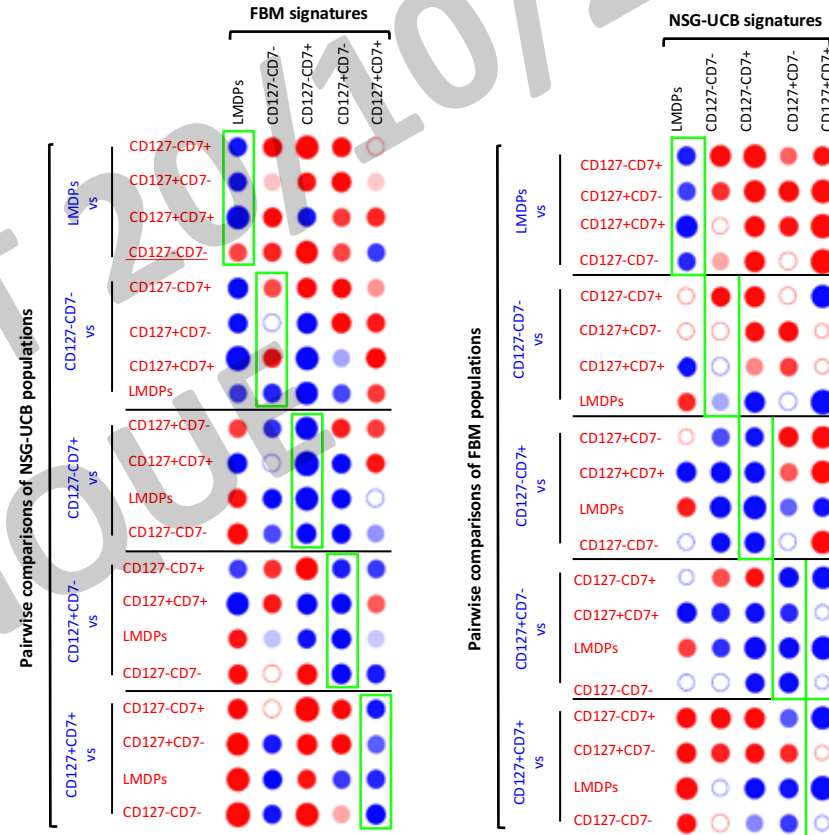
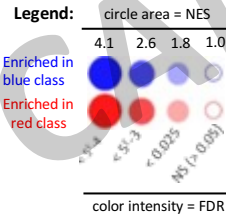
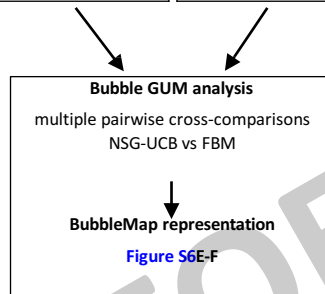
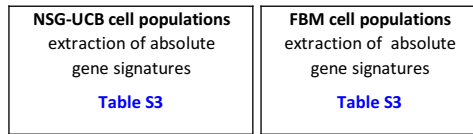


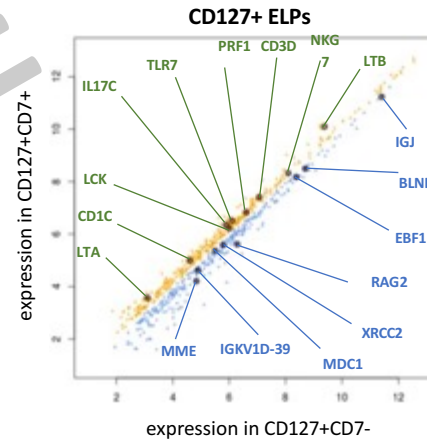
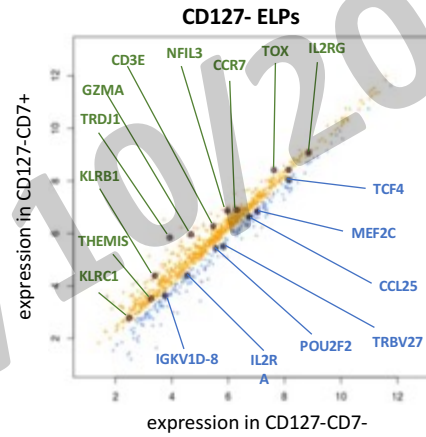
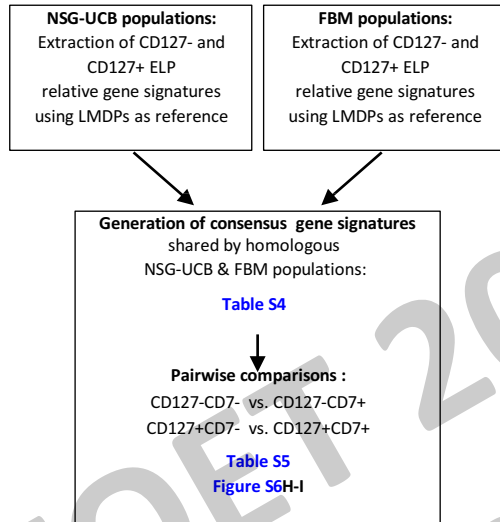
Upstream regulators



# Comparative analyses

## NSG-UCB versus Fetal Bone Marrow-2

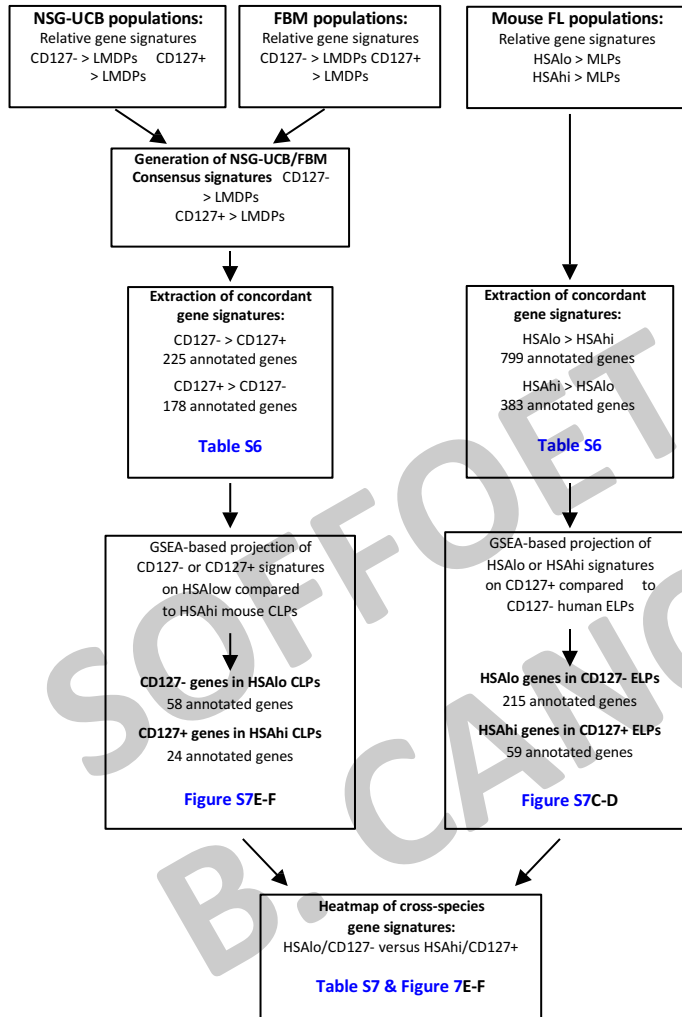




Comparative analyses  
Family-specific gene  
signatures

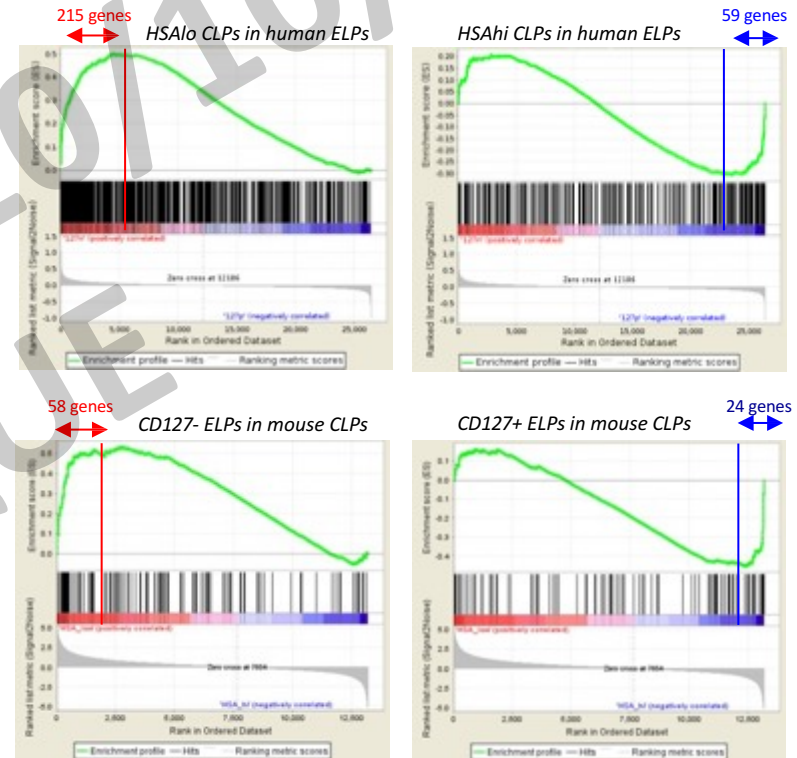
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# Cross-species analyses: human ELPs versus mouse CLPs



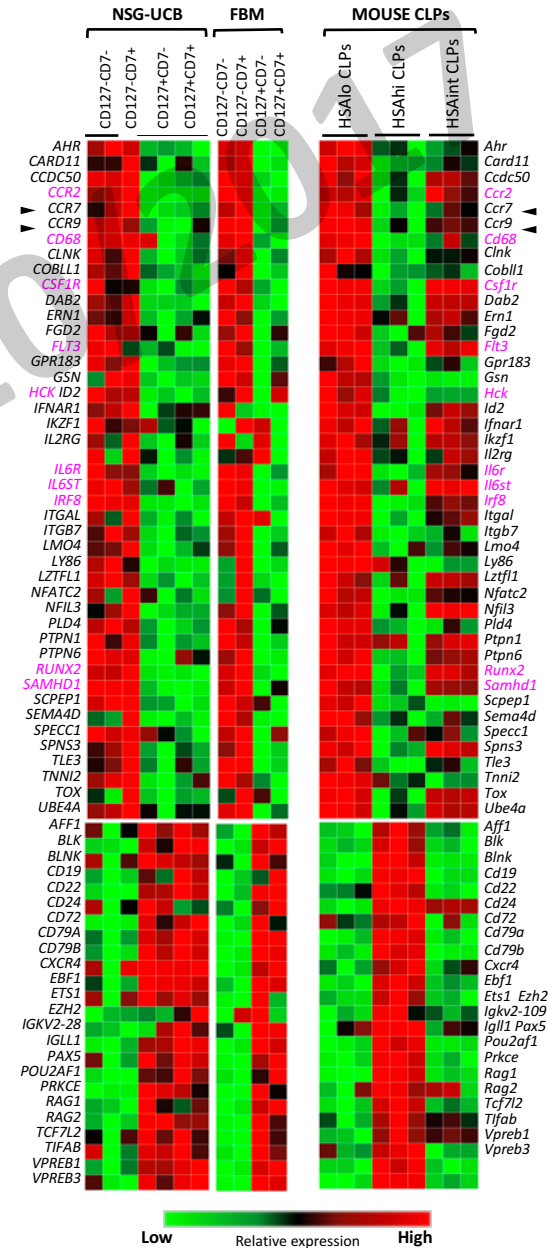
## Asynchronous lineage priming determines commitment to T cell and B cell lineages in fetal liver

Claire Berthault<sup>1-3,6</sup>, Cyrille Ramond<sup>1,2,4,6</sup>, Odile Birlen-Defranoux<sup>1-3</sup>, Guillaume Soubigou<sup>5</sup>, Sylvestre Chea<sup>1-3</sup>, Rachel Golub<sup>1-3</sup>, Pablo Pereira<sup>1-3</sup>, Paulo Vieira<sup>1-3,6</sup> & Ana Cumano<sup>1-3,6</sup>



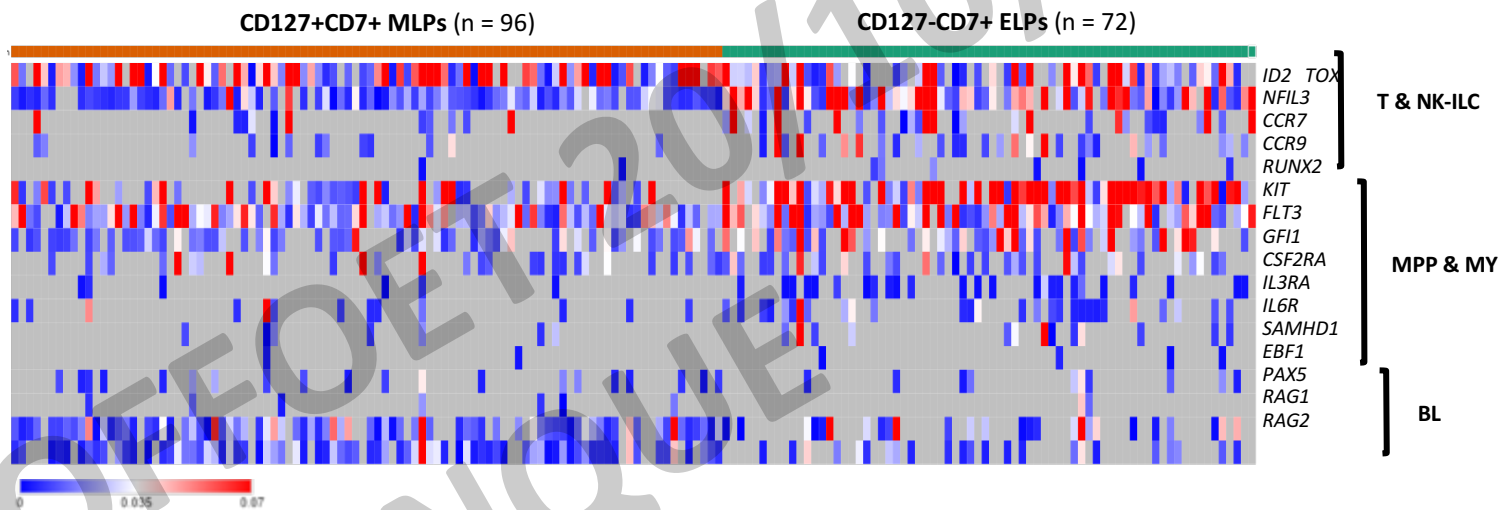
# Asynchronous lineage priming determines commitment to T cell and B cell lineages in fetal liver

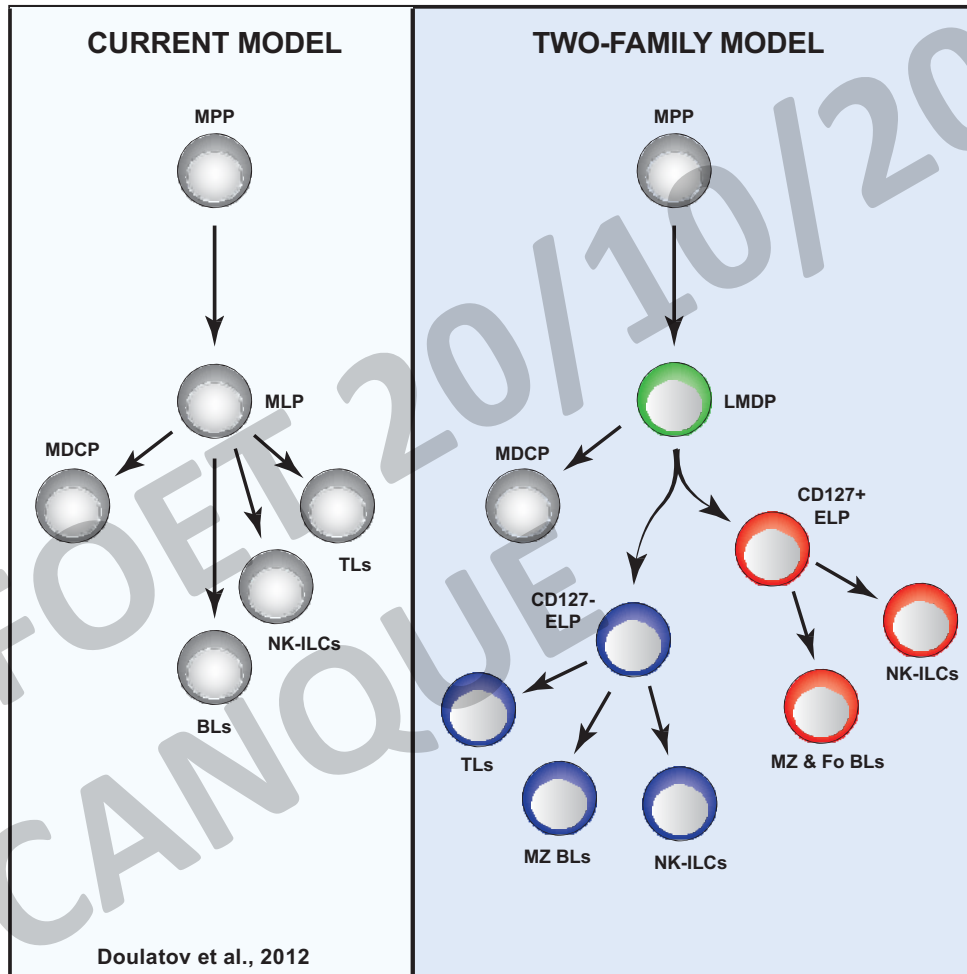
Claire Berthault<sup>1-3,6</sup>, Cyrille Ramond<sup>1,2,4,6</sup>, Odile Burlen-Defranoux<sup>1-3</sup>, Guillaume Soubigou<sup>5</sup>, Sylvestre Chea<sup>1-3</sup>, Rachel Golub<sup>1-3</sup>, Pablo Pereira<sup>1-3</sup>, Paulo Vieira<sup>1-3</sup> & Ana Cumano<sup>1-3</sup>





# Single-cell analyses





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# Molecular and Functional Characterization of Lymphoid Progenitor Subsets Reveals a Bipartite Architecture of Human Lymphopoiesis

Kutaiba Alhaj Hussen,<sup>1</sup> Thien-Phong Vu Manh,<sup>2</sup> Fabien Guimiot,<sup>3</sup> Elisabeth Néson,<sup>4</sup> Emna Chabaane,<sup>1</sup> Marc Delord,<sup>4</sup> Maxime Barbier,<sup>5</sup> Cléa Berthault,<sup>5</sup> Nicolas Dupuy,<sup>7</sup> Antonio José Alberdi,<sup>8</sup> Odile Burten-Defranoux,<sup>6</sup> Gerard Socié,<sup>7</sup> Jean Christophe Bories,<sup>9</sup> Jérôme Larghero,<sup>9</sup> Valérie Vanneaux,<sup>9</sup> Els Verhoeyen,<sup>10</sup> Thierry Vith,<sup>9</sup> Marc Dalod,<sup>2</sup> Jean Claude Guckman,<sup>1</sup> Ana Cumano,<sup>11</sup> and Bruno Canque<sup>1</sup>\*

<sup>1</sup>INSERM U26, Université Paris-Diderot, Laboratoire Pratique des Hautes Etudes/PSL Research University, Institut Universitaire d'Hématologie, Hôpital Saint-Louis, Paris, France

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<sup>4</sup>Institut de Systématique, Évolution, Biodiversité OS/EB, UMR 7205, CNRS, Muséum National d'Histoire Naturelle (MNHN), Université Pierre et Marie Curie, and École Pratique des Hautes Etudes (EPHE), Sorbonne Universités, Paris Sciences et Lettres, Paris, France

<sup>5</sup>Unité de Lymphopoïèse, Département d'immunologie, Institut Pasteur, Université Paris-Diderot, Sorbonne Paris Cité, Cellule Pasteur, INSERM U1223, Paris, France

<sup>6</sup>INSERM UMRS 1160, Institut Universitaire d'Hématologie, Université Paris-Diderot, Sorbonne Paris Cité; laboratoire d'immunologie et Histocompatibilité, AP-HP, Hôpital Saint-Louis, 75010 Paris, France

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<sup>9</sup>ICAI, International center for Infectiology Research, EVIA team, Université de Lyon, INSERM U1111, Lyon, France; and Centre Méditerranéen de Médecine Moléculaire (C3M), team "Contrôle métabolique des maladies" Inserm, U1065, 06204 Nice, France

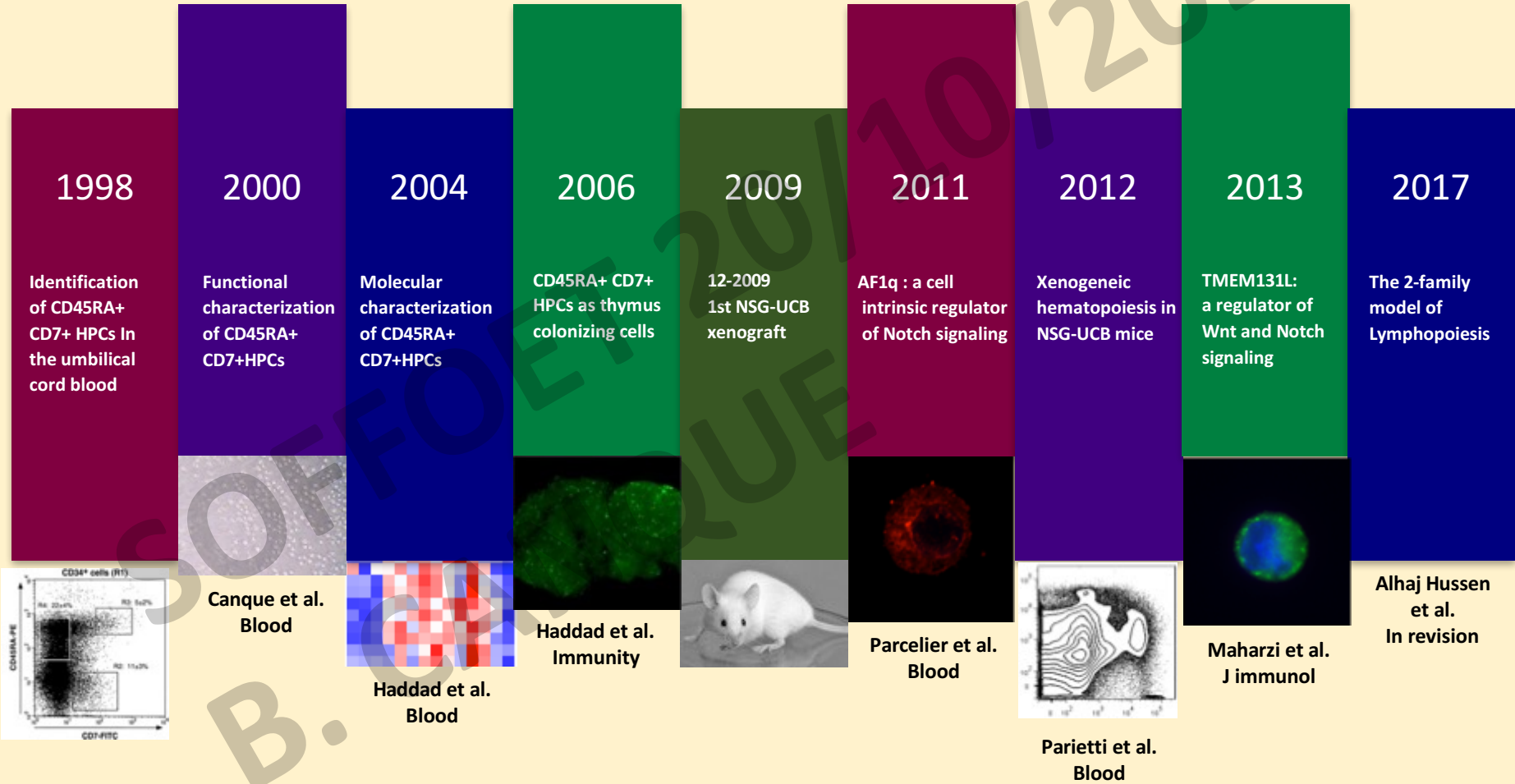
\* Lead Contact

\*Correspondence: [bruno.canque@ephe.sorbonne.fr](mailto:bruno.canque@ephe.sorbonne.fr)

<https://doi.org/10.1016/j.immuni.2017.09.009>

# 1998 - 2009: DESCRIPTIVE APPROACH

# 2010 - 2017: MODELIZING APPROACH

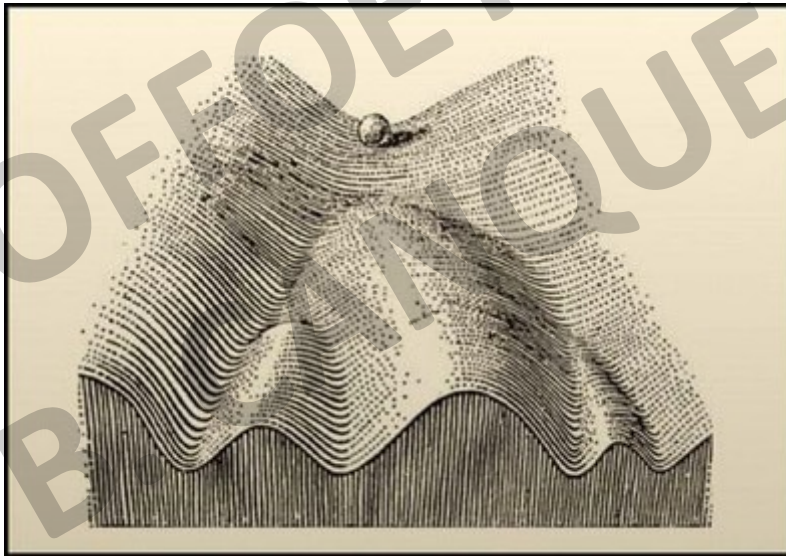
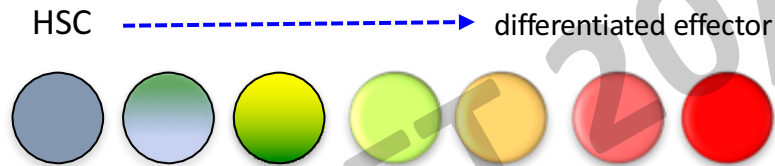


## CONCLUSIONS & PERSPECTIVES

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## An integrated approach of cell differentiation :

- Phenotypic level : OK
- Transcriptional : single-cell transcriptomic
- Epigenetic : ATAC-seq - ChIP-seq - Methy-seq
- Nuclear architecture : HiC - FISH



CH Waddington. Principles of embryology (1956)

# Humanized models of leukemogenic transformation : Myc – Bcl2

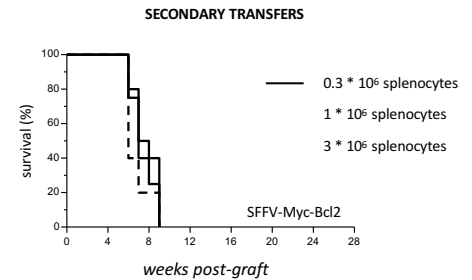
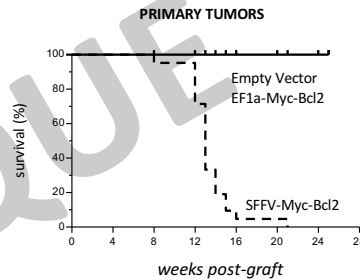
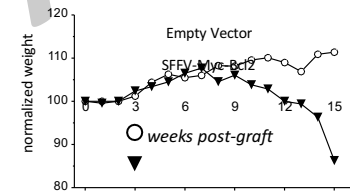
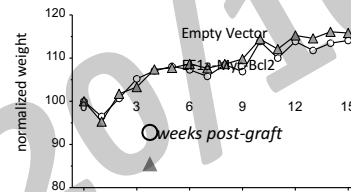
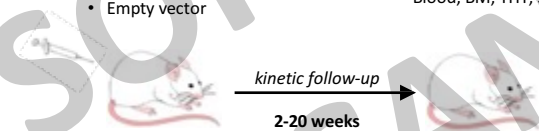


**CD34+ HPCs**

- EF1α-Myc-Bcl2
- SFFV-Myc-Bcl2
- Empty vector

**sacrifice**

- FACS analysis
- Blood, BM, THY, SPLEEN



Dupont et al., in preparation  
Collaboration with AXENIS



not too bad...  
pour une fois...

we're the best !  
surtout moi...





Kutaiba Alhaj Hussen

Elisabeth Nelson

Salomé Dupont

Emna Chaabane

Seydou Keita

Jean Claude Gluckman

Bruno Canque

JC Bories

Fabien Guimiot

Marc Delord

Nicolas Dulphy

Antonio Alberdi

Niclas Setterblad

Sophie Duchez

Christelle Doliger

Jerôme Larghero

Valérie Vanier

Gérard Socié

Ana Cumano

Claire Berthault

Odile Burlen-Defranoux

Marc Dalod

Thien Vu Manh

Thierry Wirth

Maxime Barbier

Nicolas Legrand

Erwan Corcuff

