Deciphering HoxA9 function in multipotential progenitor biology and B cell development

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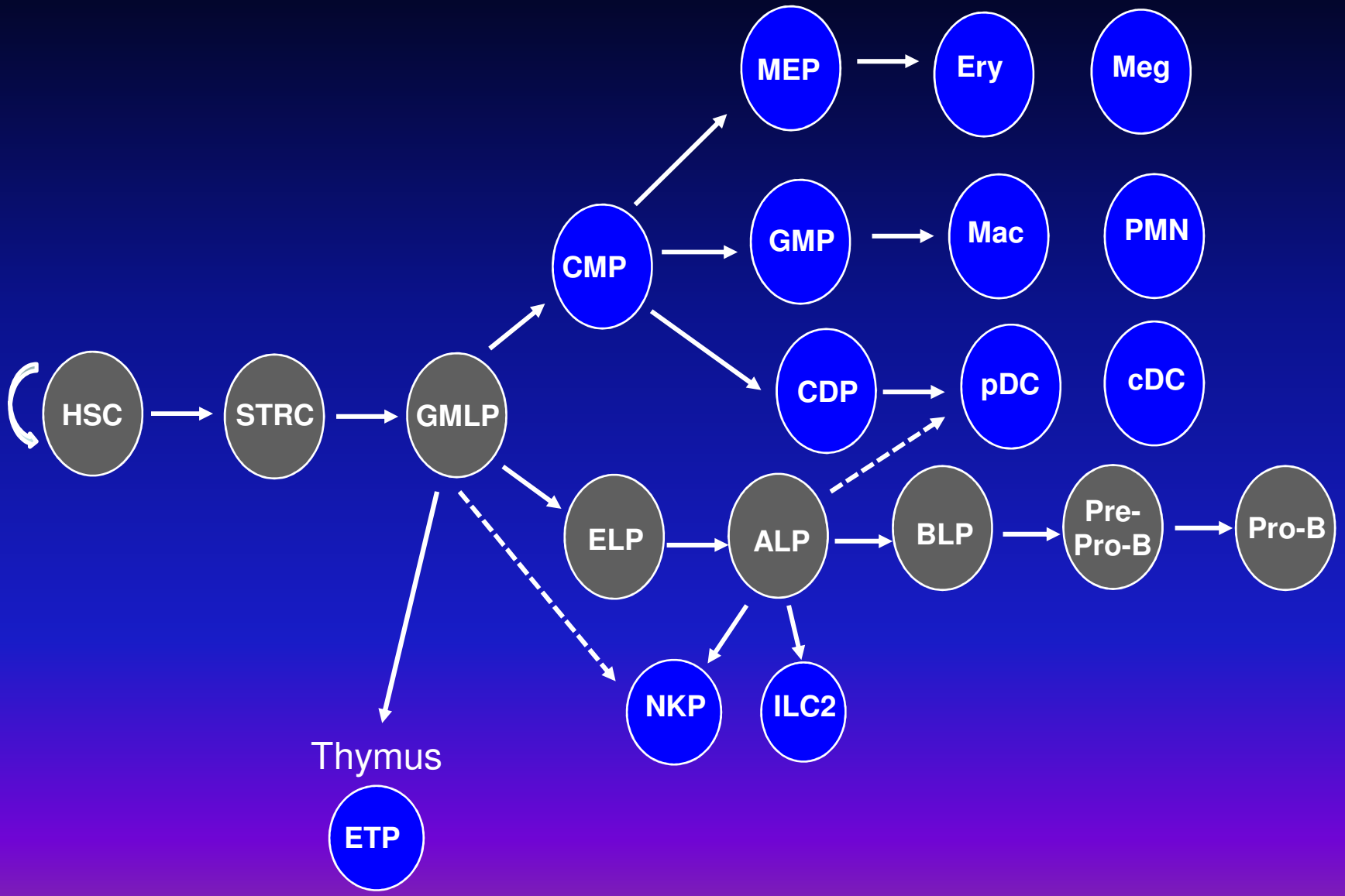
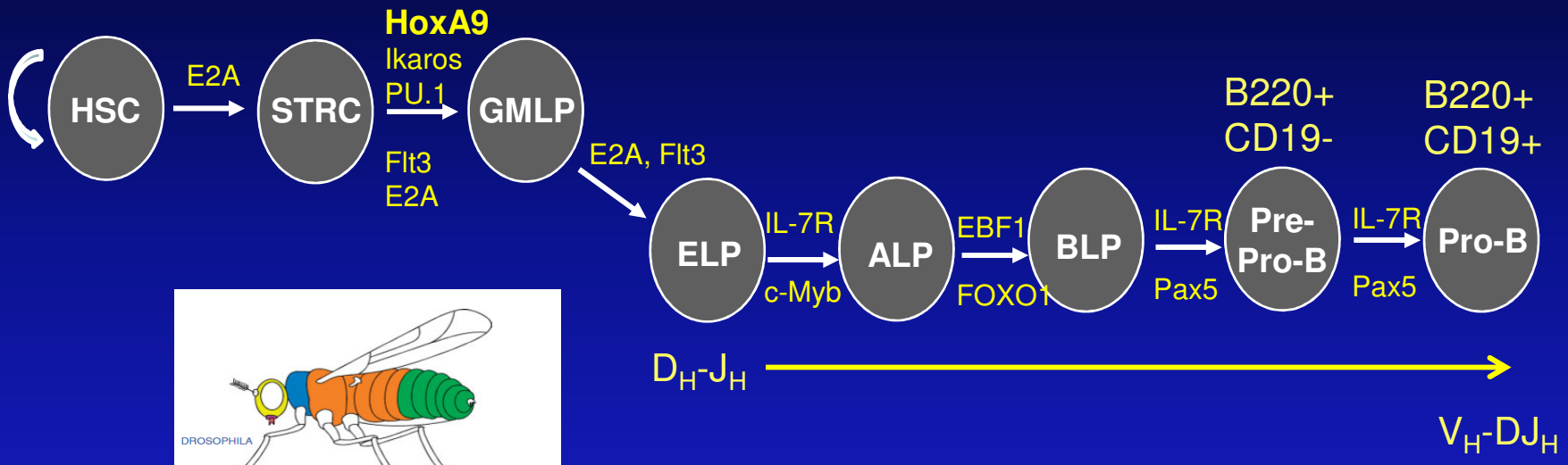
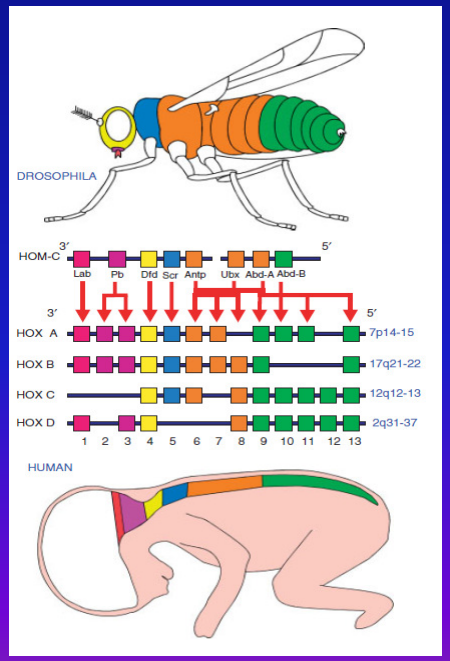
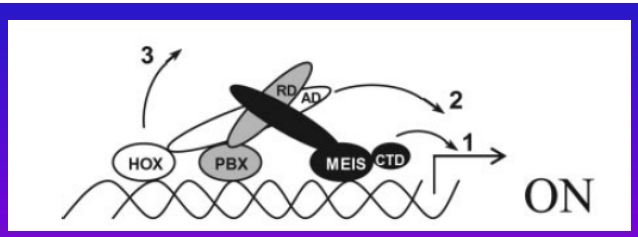
Overview of adult hematopoiesis in the mouse



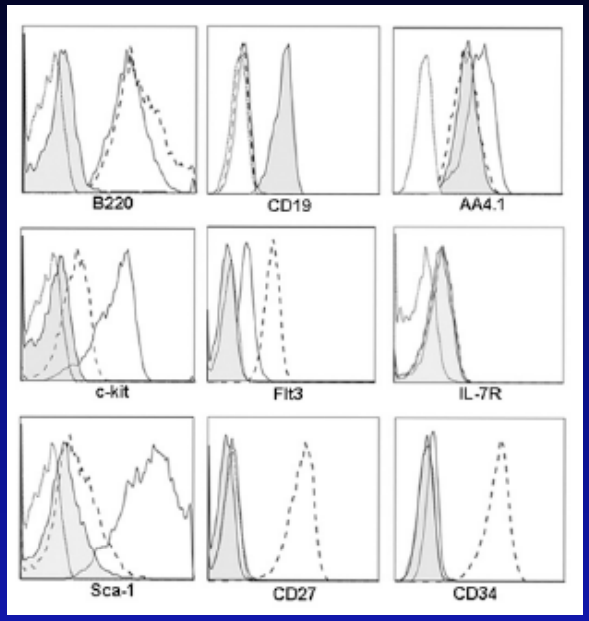
Sequentially-acting regulatory circuits orchestrate B cell fate specification and commitment

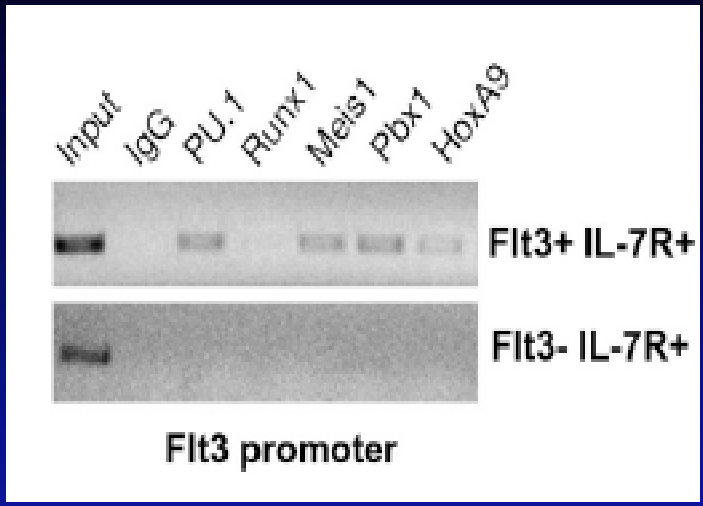




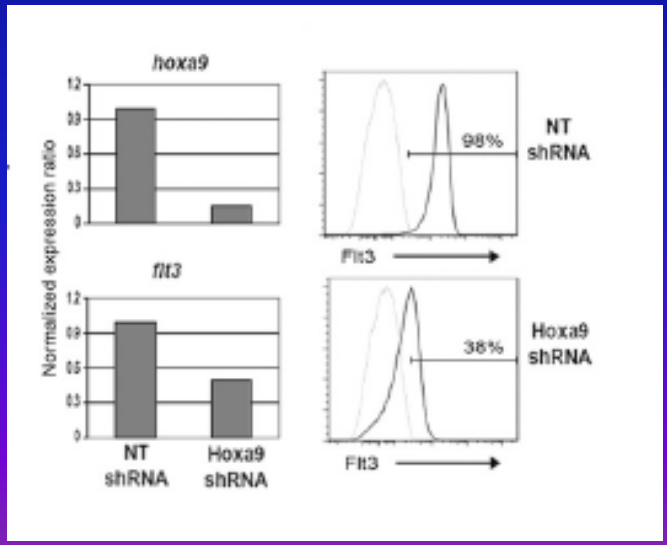


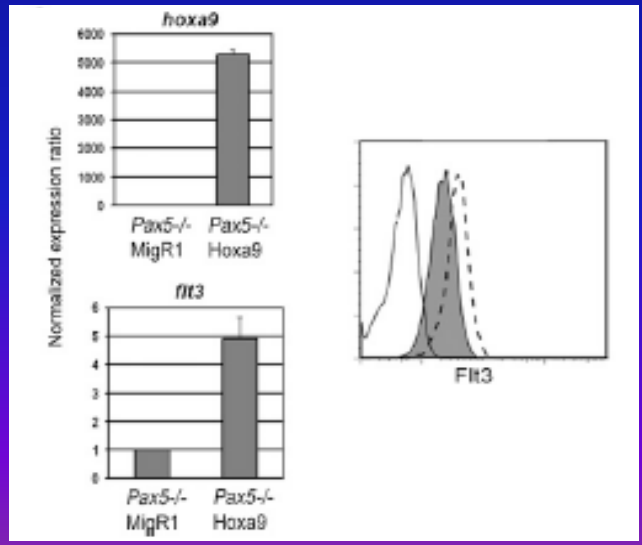
Hoxa9 regulates Flt3 in lymphohematopoietic progenitors



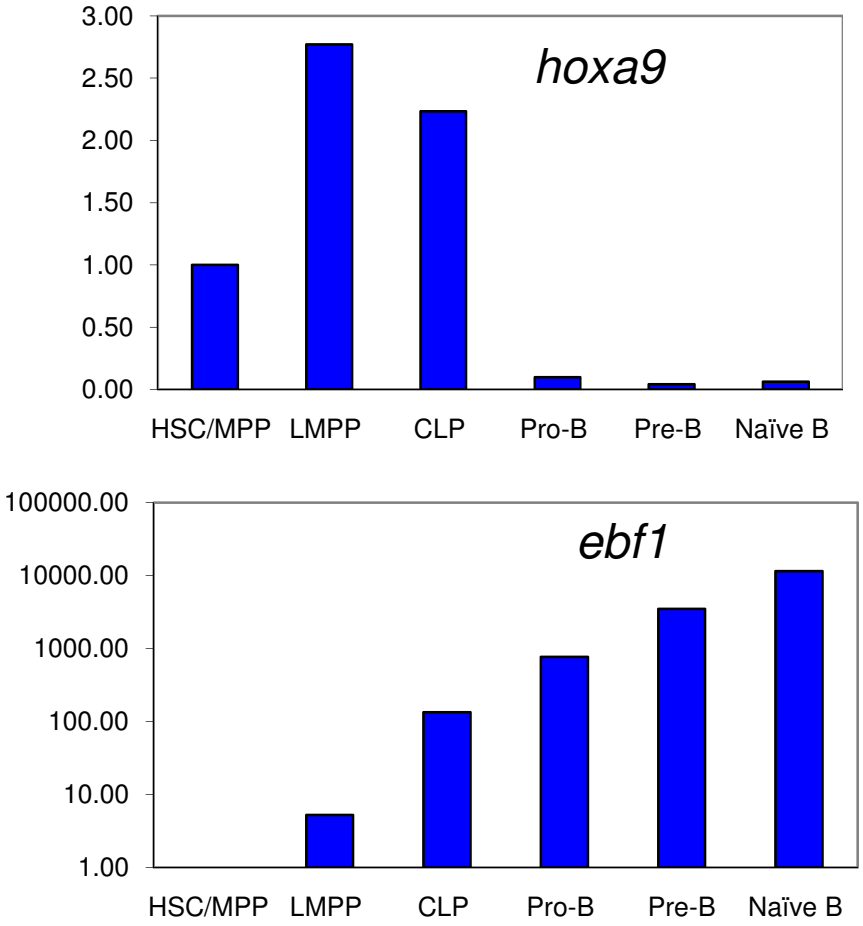


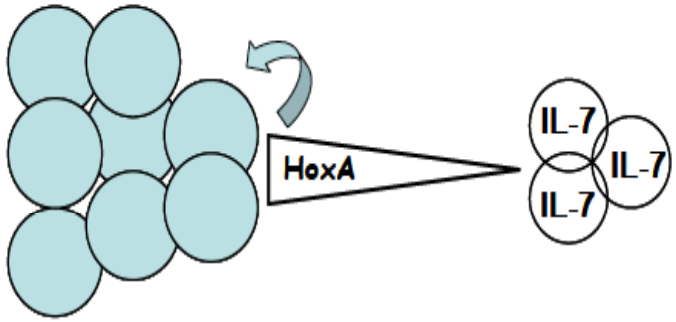
Gwin and Medina, JI, 2010



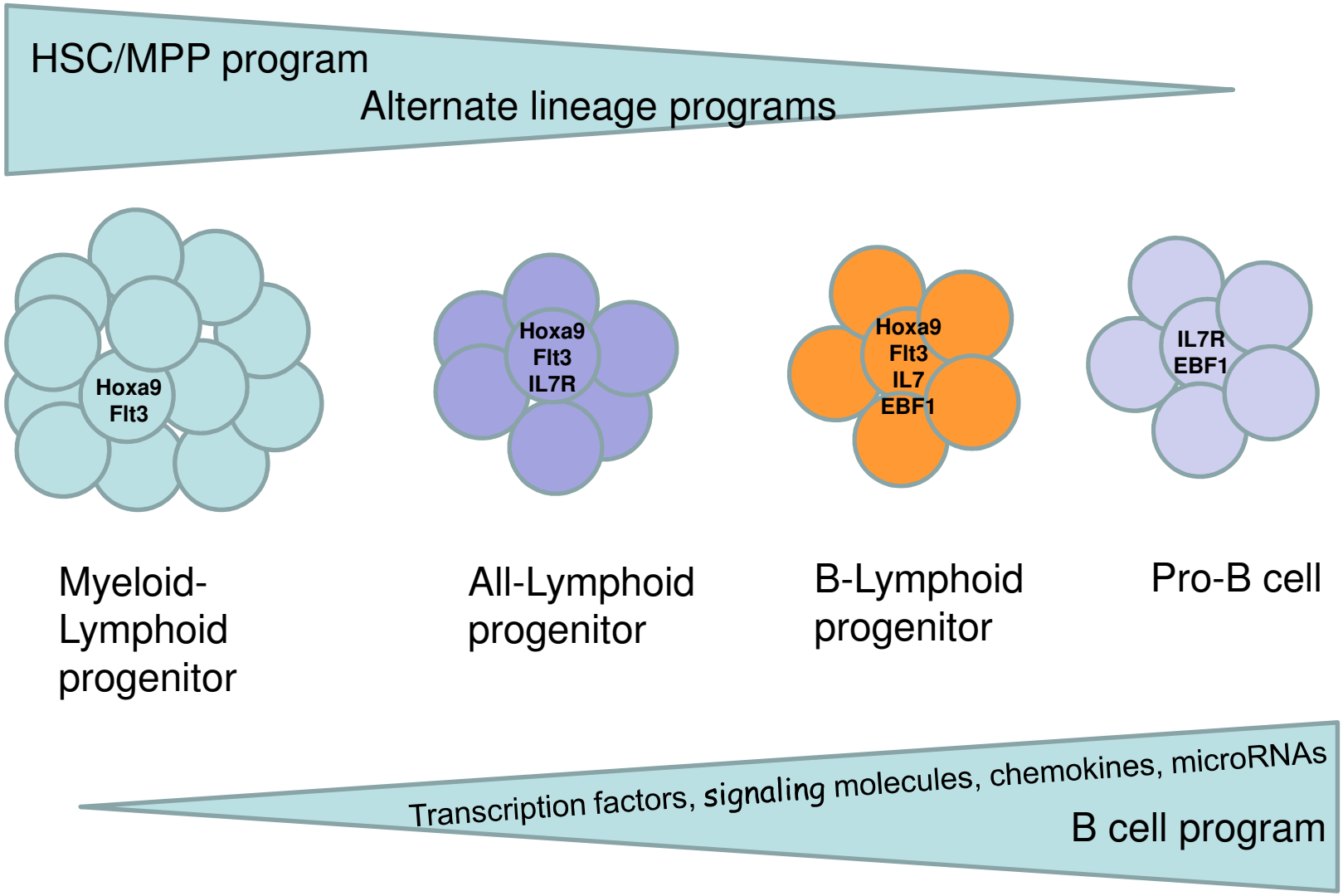


Inverse expression of HoxA9 and EBF1 in lymphoid/B cell precursors

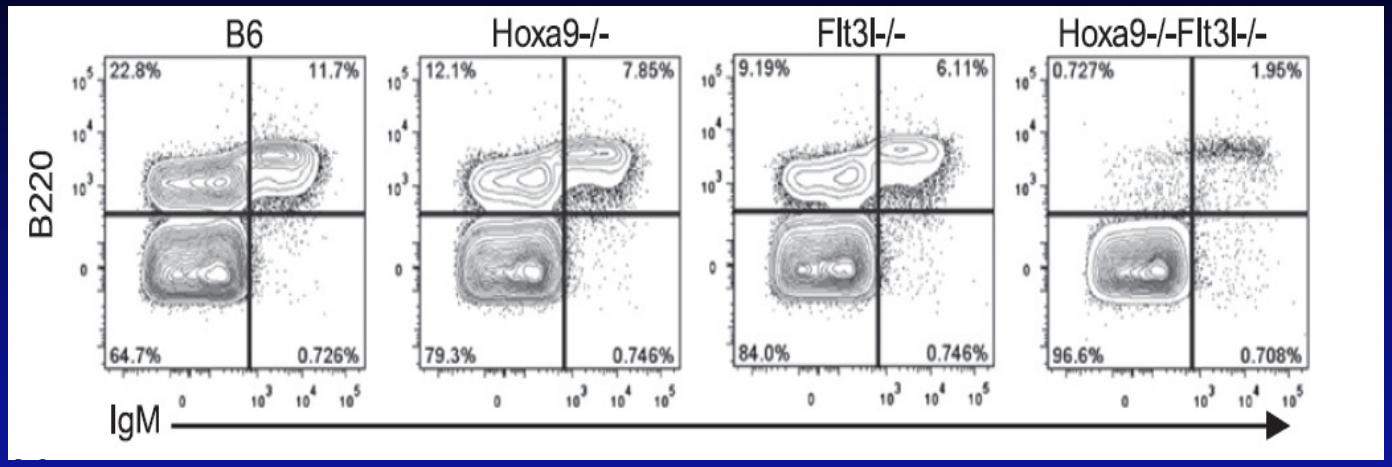


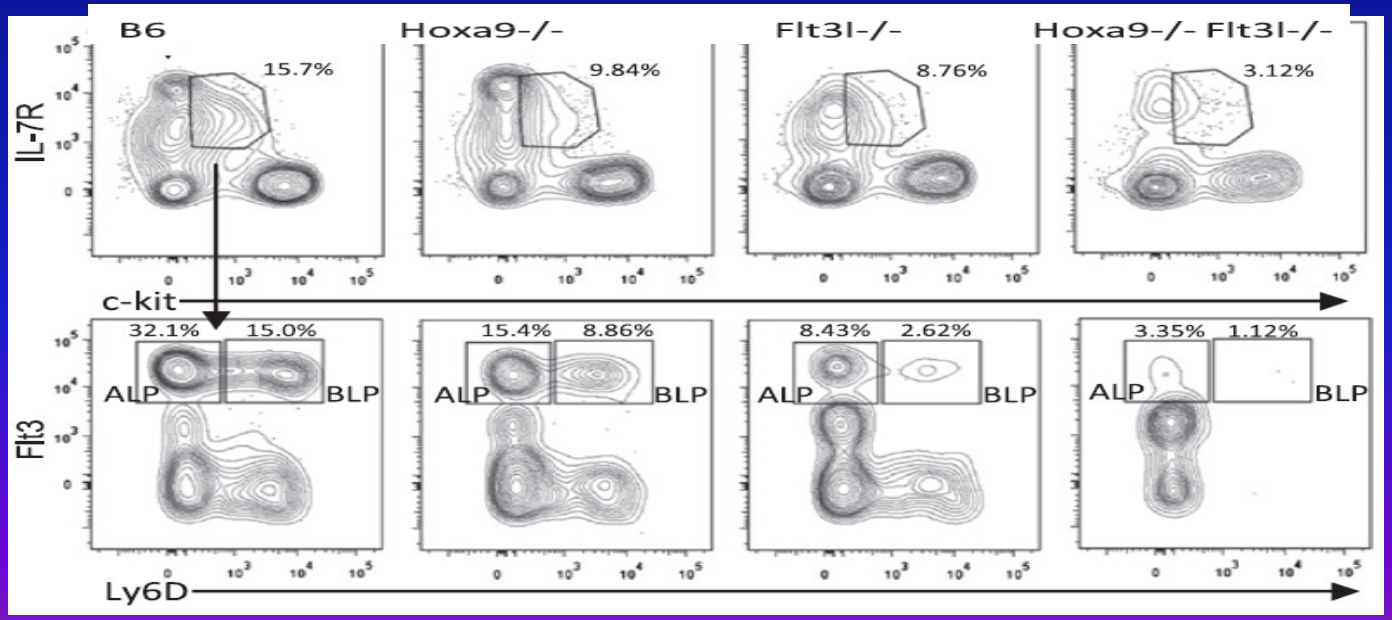


HSC/MPP → Pro-B
CLP/BCP


HSC/MPP program
Alternate lineage programs
Hoxa9
Flt3
Hoxa9
Flt3
IL7R
Hoxa9
Flt3
IL7
EBF1
IL7R
EBF1
Myeloid-
Lymphoid
progenitor
All-Lymphoid
progenitor
B-Lymphoid
progenitor
Pro-B cell
Transcription factors, signaling molecules, chemokines, microRNAs
B cell program

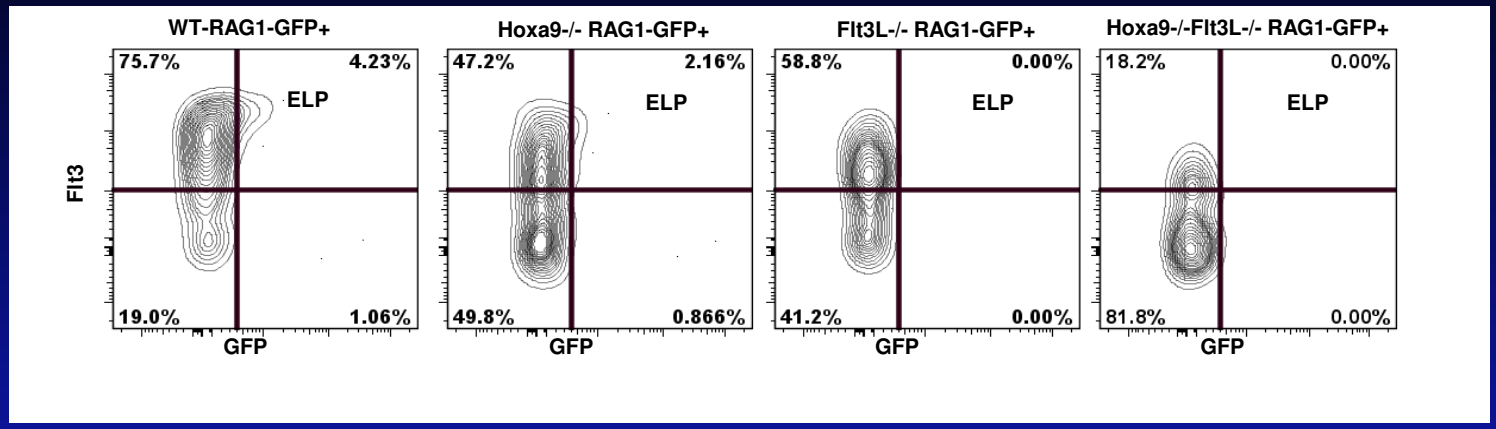
hoxa9-/- flt3l-/- mice exhibit a severe lympho-hematopoietic block

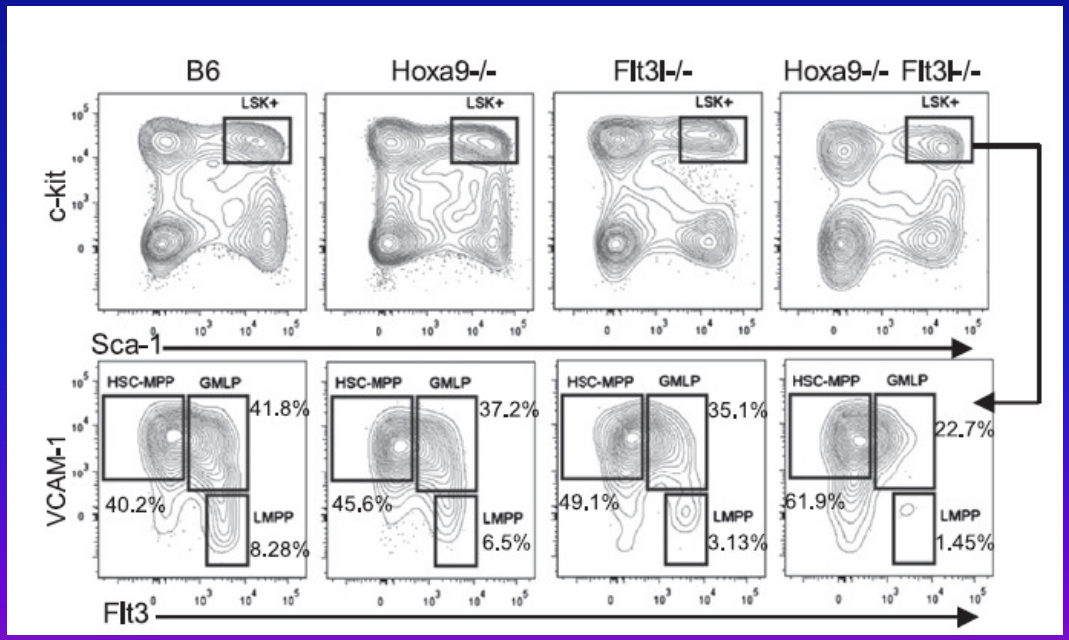


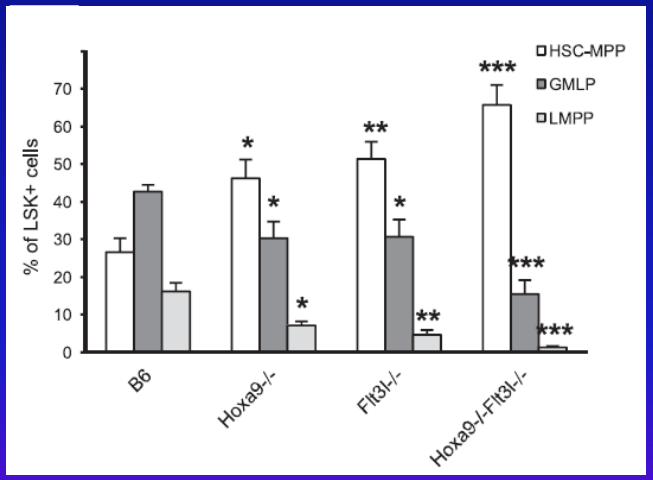


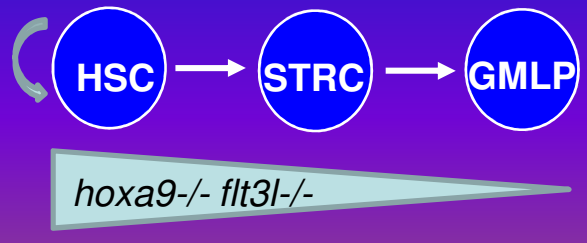
Gwin and Medina, JI, 2013

HoxA9 and Flt3 signaling function together to establish the multipotential progenitor pool competent to undergo lymphoid priming

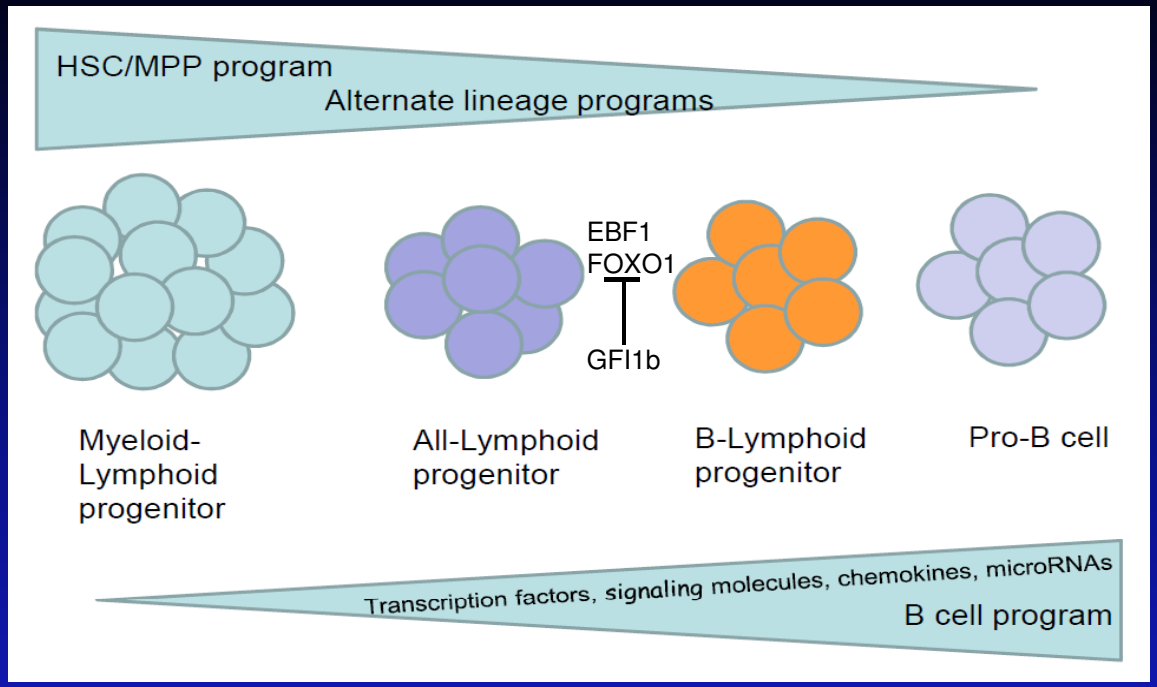


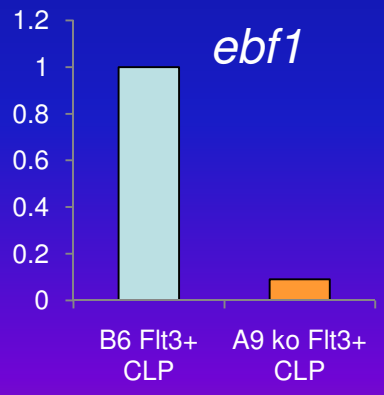


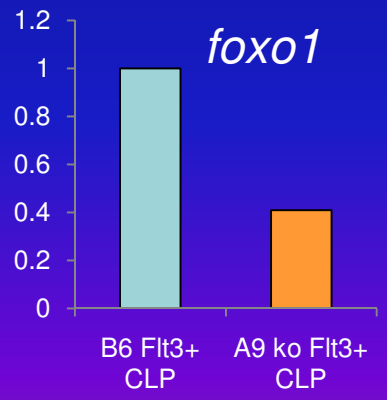


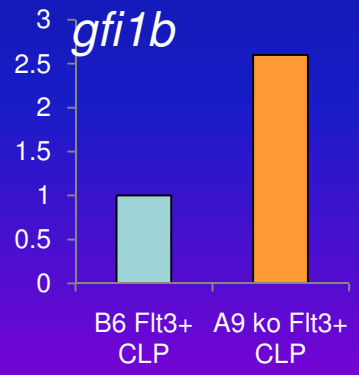


The role of HoxA9 in B cell fate specification.

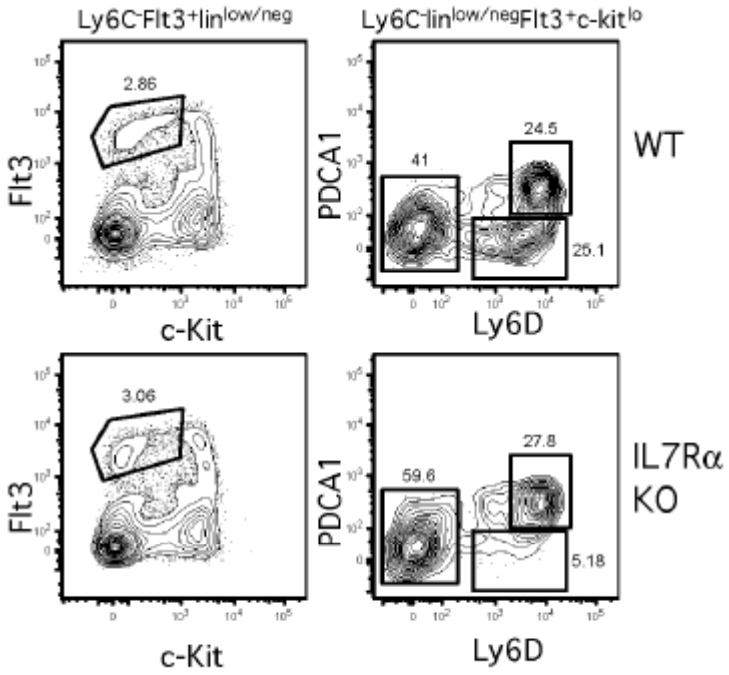


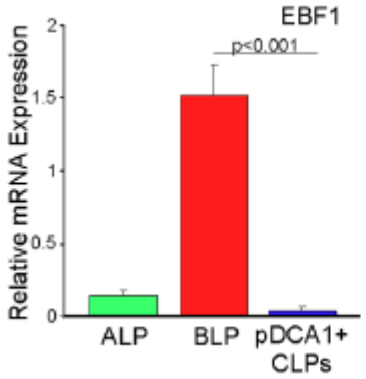


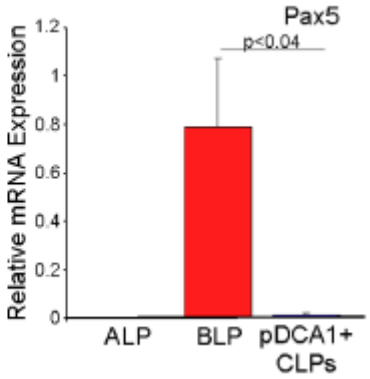




HoxA9-/- hematopoietic progenitors have impaired IL-7 signaling







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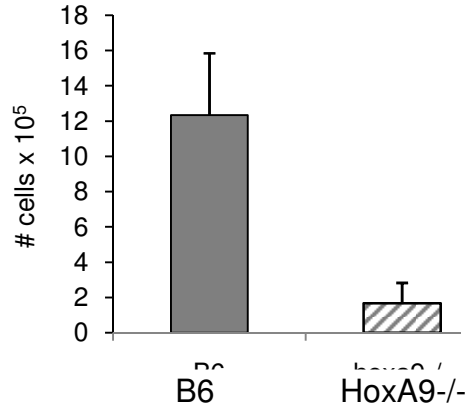
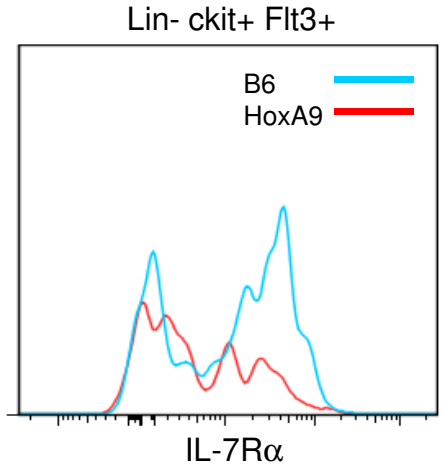
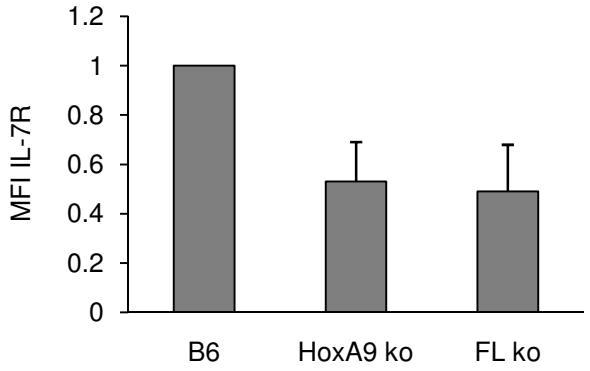
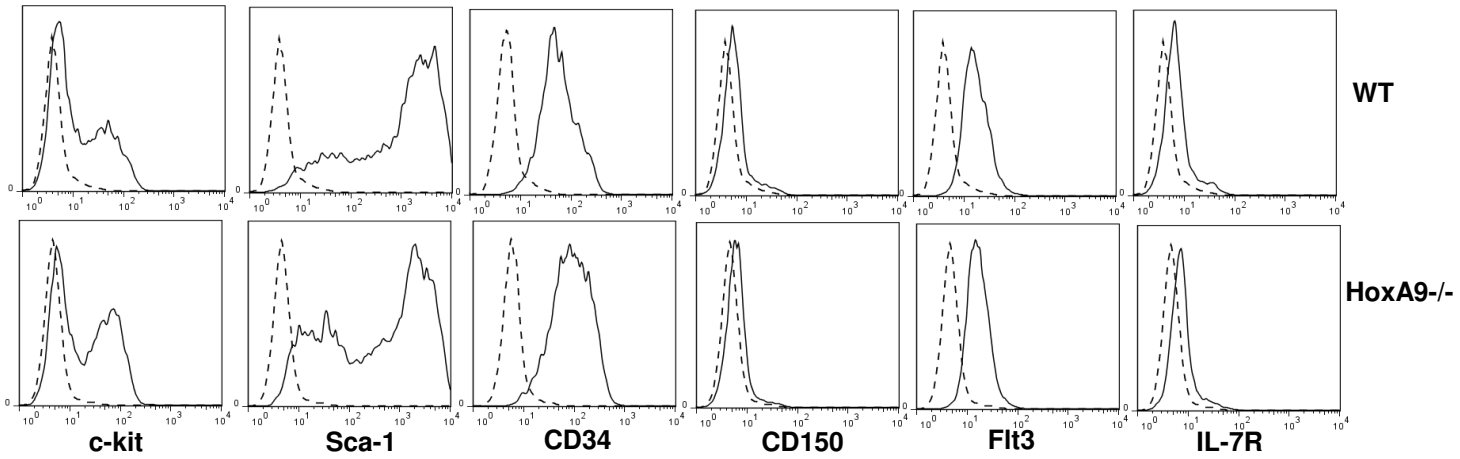


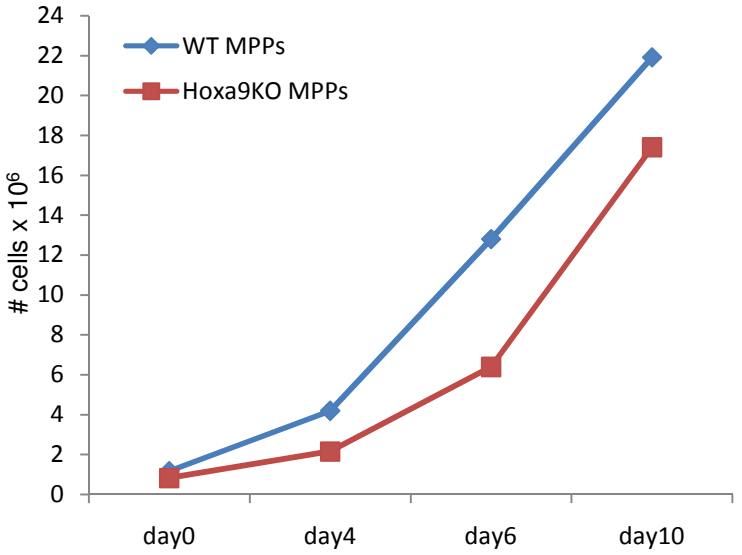


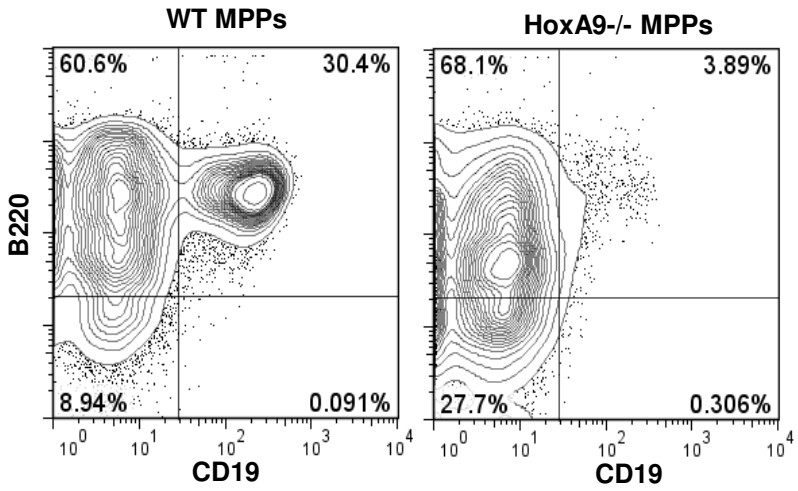


Culture model to identify HoxA9 target genes critical for B cell development

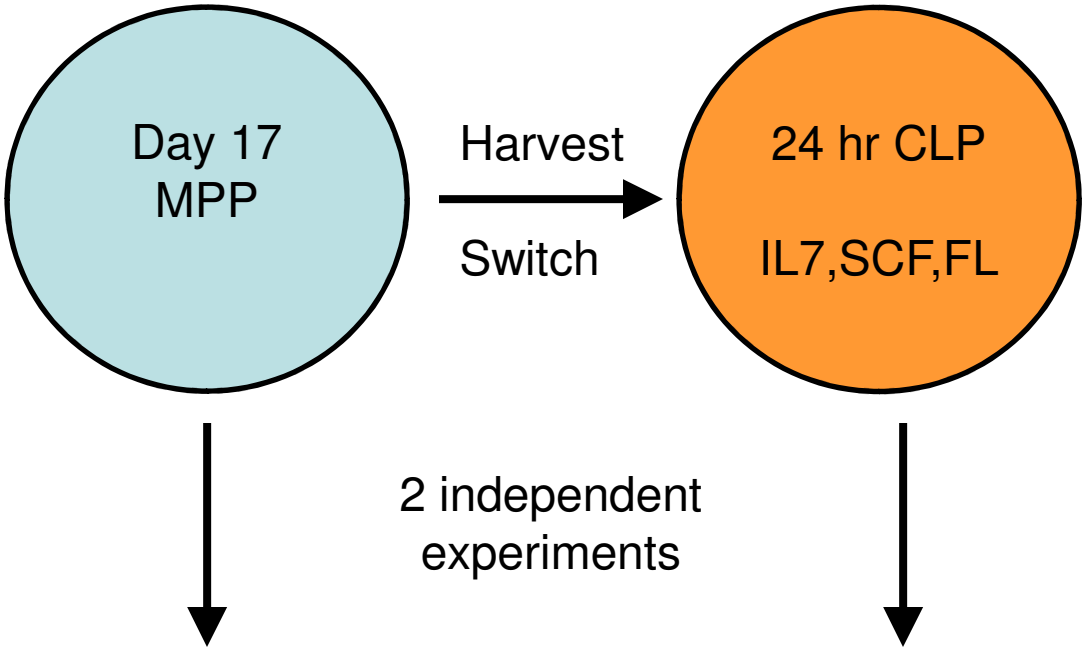
Lin- c-kit cells cultured for 17 days in SCF+FL+Tpo+IL-6







RNA-Seq Platform



Summary of RNA-Seq Platform

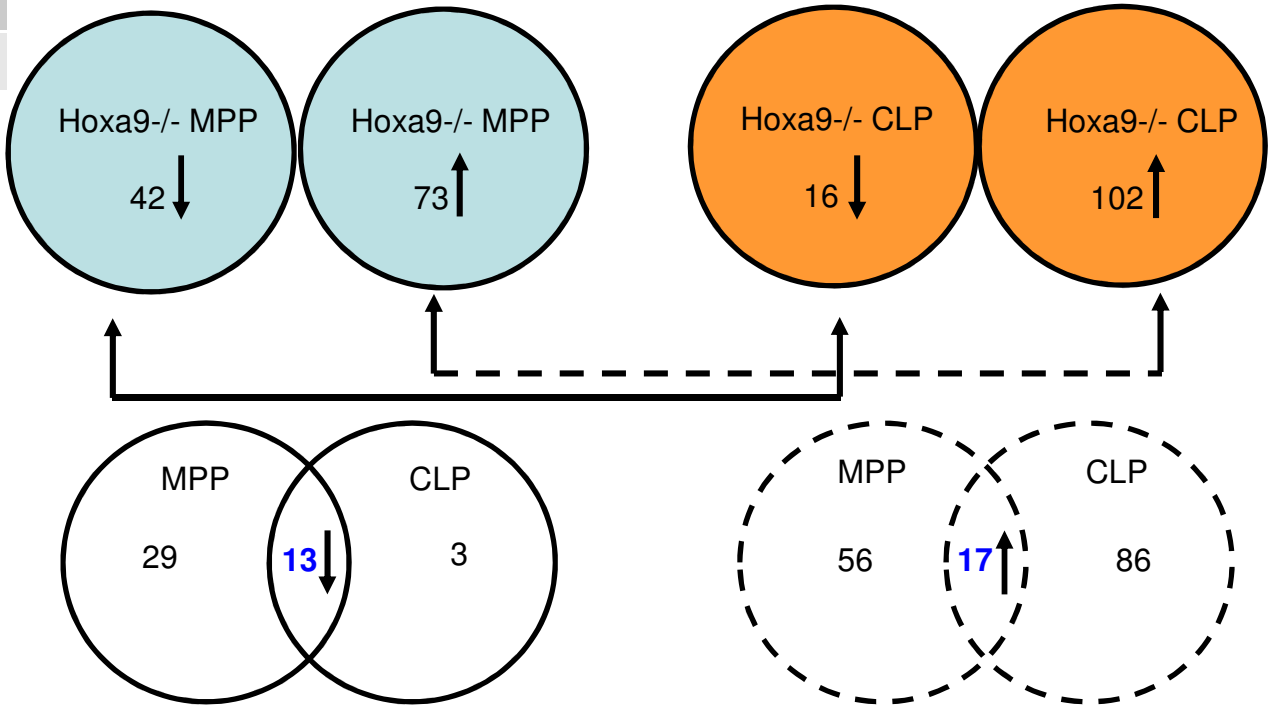
Sample	Total Reads
Hoxa9ko-CLP-1	89,662,470
Hoxa9ko-CLP-2	86,812,194
Hoxa9ko-MPP-1	79,030,174
Hoxa9ko-MPP-2	102,001,936
WT-CLP-1	108,111,316
WT-CLP-2	118,748,182
WT-MPP-1	98,958,636
WT-MPP-2	99,110,526

Read length:50bp

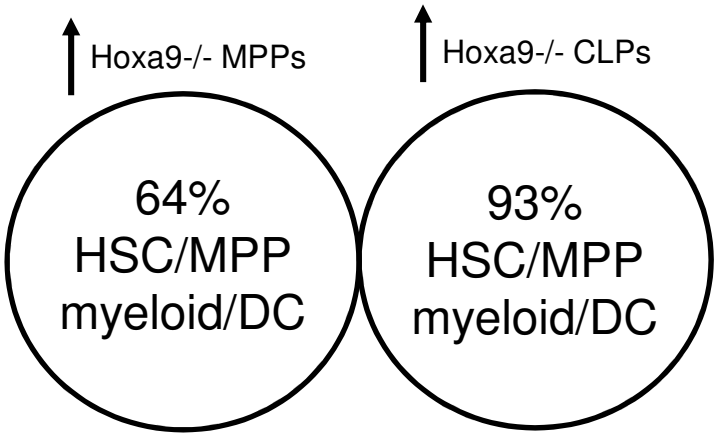
40-50X depth of coverage/gene

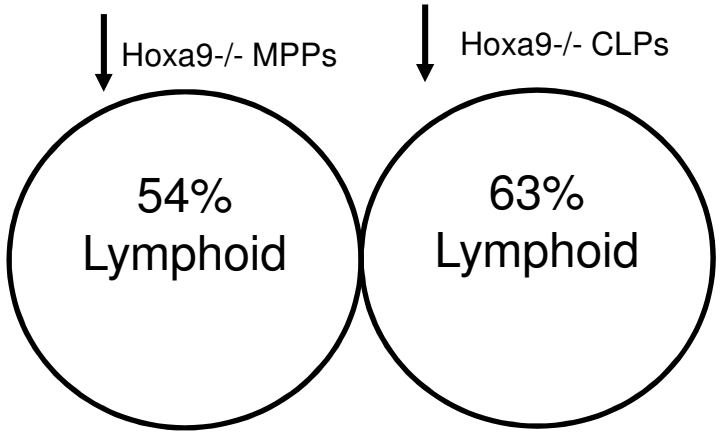
Differential expression cutoff: absolute fold change 1.4

False discovery rate of ≥0.1; p>0.05



Hoxa9-deficiency impairs lymphoid priming and downregulation of HSC/MPP and/or alternative lineage gene programs





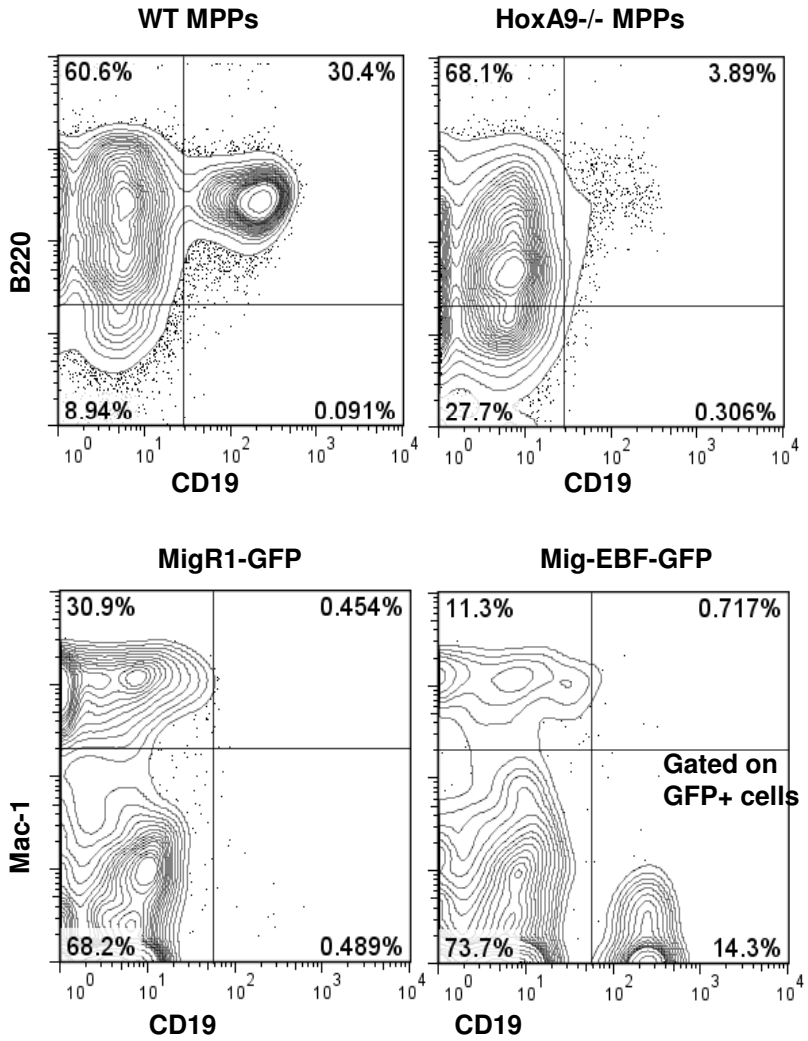
Shared upregulated

Lcn2 – lipocalin 2 – neutrophil
Mgam – maltase-glucoamylase - neutrophil
Clec4b – ATP binding cassette - DC
Adam23 – disintegrin and metallopeptidase - DC
Pglyrp1 – peptidoglycan recognition protein - neutrophil
Elane – elastase – macrophage/HSC/MPP
Abca13 – ATP binding cassette - neutrophil
Ehd3 – EH domain containing 3 – T cells
Dio2 – deiodinase – HSC/MPP
Mpo – myeloperoxidase - monocytes
Vcam1 – vascular cell adhesion molecule 1 – HSC/MPP
Dach1 – dachshund - neutrophil
Ppic – peptidylprolyl isomerase C – HSC/MPP
Rab38 – member of RAS family - macrophage
Mgl2 – macrophage galactose N-acetyl-galactosamine specific
Ikzf2 – ikaros zinc finger 2; Helios – HSC/T cells/T regs
Gata2 – HSC/MPP

Shared downregulated

Rag1 – recombinase activating gene – lymphocytes
Gfra1 – sperm stem cell self renewal - BCPs
Rag2 - recombinase activating gene - lymphocytes
Ppfia4 – protein tyrosine phosphatase – spl DC / Macs
Slc15a2 – H+/peptide transporter - BCPs
P2rx3 – purinergic receptor P2X - BCPs
Cdh17 – cadherin 17 - BCPs
Cecr2 – cat eye syndrome - BCPs
Gimap4 – GTPase - BCPs
Lin28b - regulator adult to fetal HSC; let7 biogenesis - BCPs
Cd27 – member of TNF receptor family – MPPs/BCPs
Igfbp3 – insulin growth factor 2 binding protein - BCPs

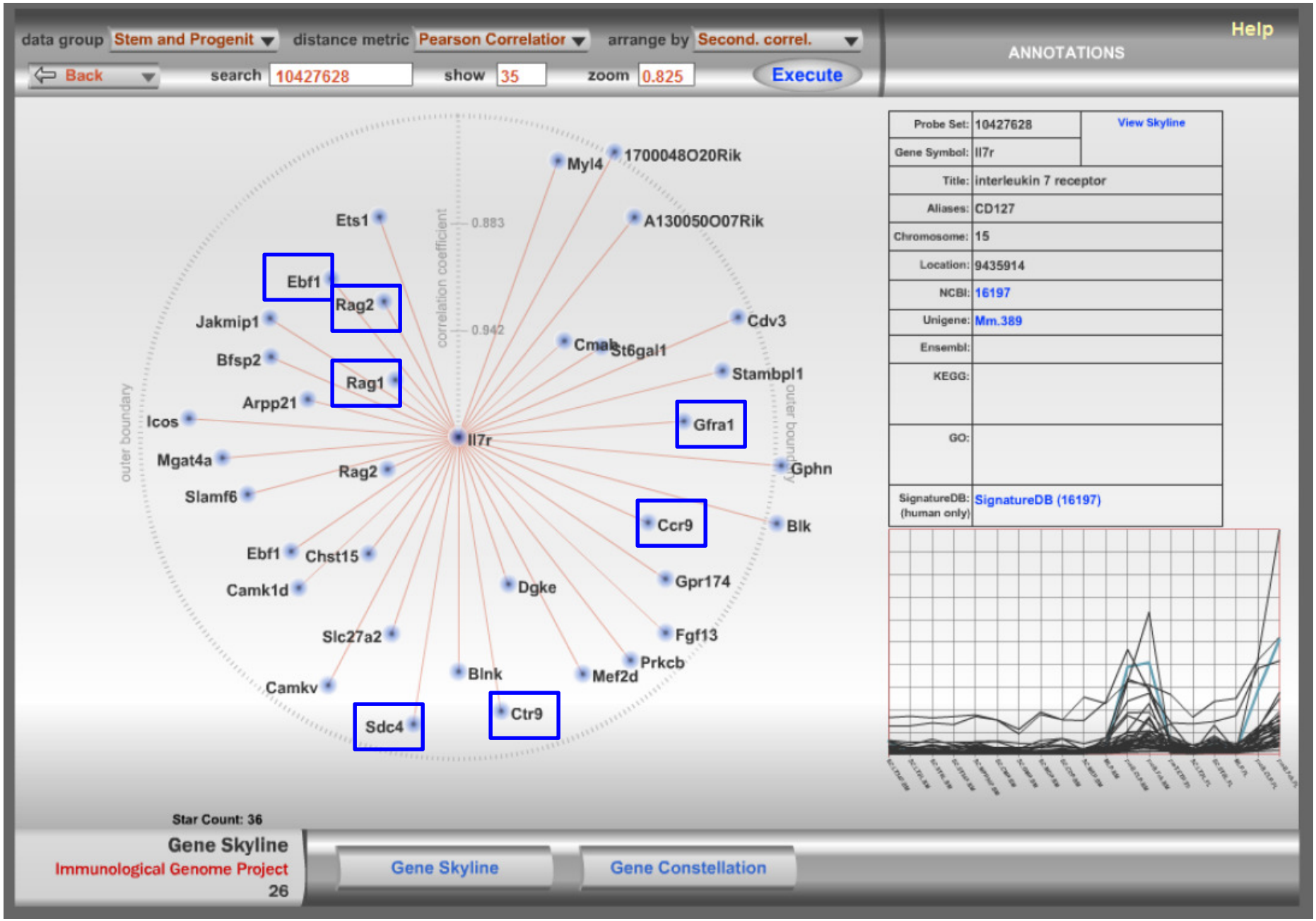
Ectopic expression of EBF1 is sufficient to restore the generation of BCPs from HoxA9-/- MPPs



17 day MPP switched to SCF+FL+IL7
and cultured for an additional 6-8 days

Hoxa9-/- d17 MPP in SCF+FL+IL7
6-8 days after retroviral transduction

Linking RNA-Seq and Microarray data to the IL-7R signaling pathway

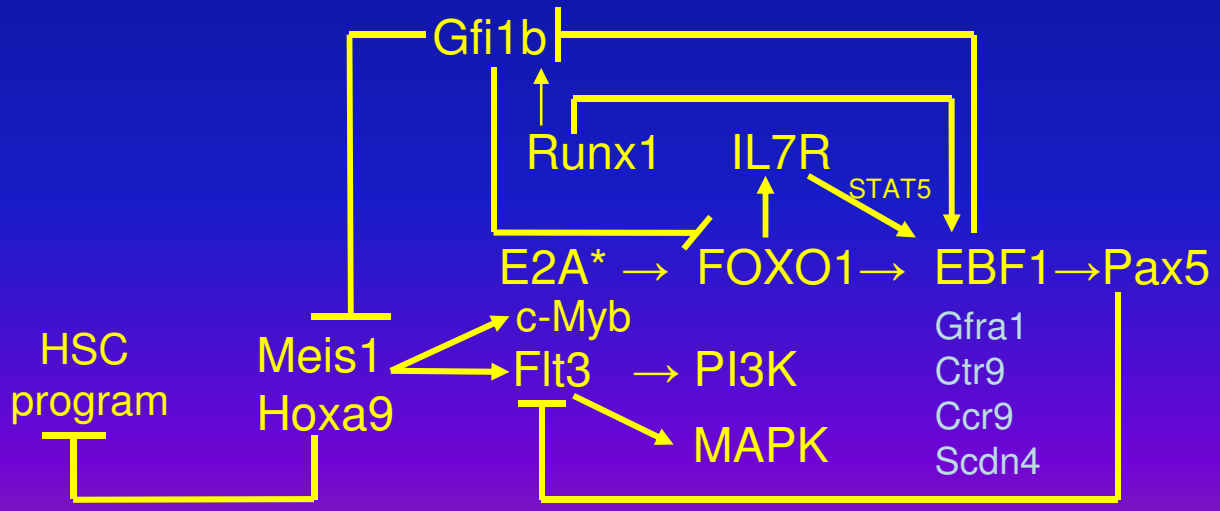


Conclusions:

We developed a short-term in vitro culture model to expand MPPs from WT and HoxA9-/- mice that will be informative in the identification, characterization, and validation of HoxA9 regulated genetic circuits in early hematopoiesis.

RNA-Seq confirmed decreased lymphoid priming in MPPs and under B cell differentiation conditions due to HoxA9 deficiency independent of Flt3 signaling.

HoxA9 deficiency impairs IL-7R expression and signaling – enforced expression of EBF1 can bypass the IL-7 signaling defect.



Underway:

1. Identify novel HoxA9 target genes : compare results to microarray platform of genes upregulated in EBF1-/- multipotent cells that express endogenous Hoxa9
   - underexpressed in Hoxa9-/- MPPs in RNA-Seq platform (transcriptional activation)
   - overexpressed in EBF1-/- MPPs in Affymetrix Array
   Chd17, CD27, CCR9, Ppfia4, Sorcs2, RAG2, GPR25, Jup, Igf2bp3

   - overexpressed in Hoxa9-/- MPPs in RNA-Seq platform (transcriptional repressor)
   - underexpressed in EBF1-/- MPPs in Affymetrix Array
   Mgst1, Tgm2, Enpp4, Plekha8, Muc13, Cpne2, Tmem176a, Mreg, Adcy6, STAT4, Sulf2, SpiB

2. Identify novel EBF1 target genes : compare results to microarray platform of genes underexpressed in EBF1-/- multipotent cells to genes underexpressed in HoxA9-deficient MPPs or CLPs
   P2rx3, RAG1, Rgs8, Cecr2, Egfl7, Uaca, Lax1, Dntt, Slc15a2, Gimap4

3. Perform gain-of-function and loss-of-function experiments in WT or HoxA9-/- MPPs to evaluate gene function in B cell development followed by molecular validation.

4. Determine if HoxA9 target genes are relevant to leukemogenesis.

Acknowledgements

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