Article

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BRD4 directs hematopoietic stem cell development and modulates macrophage inflammatory responses

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Chae Dong Hoon

Introduction

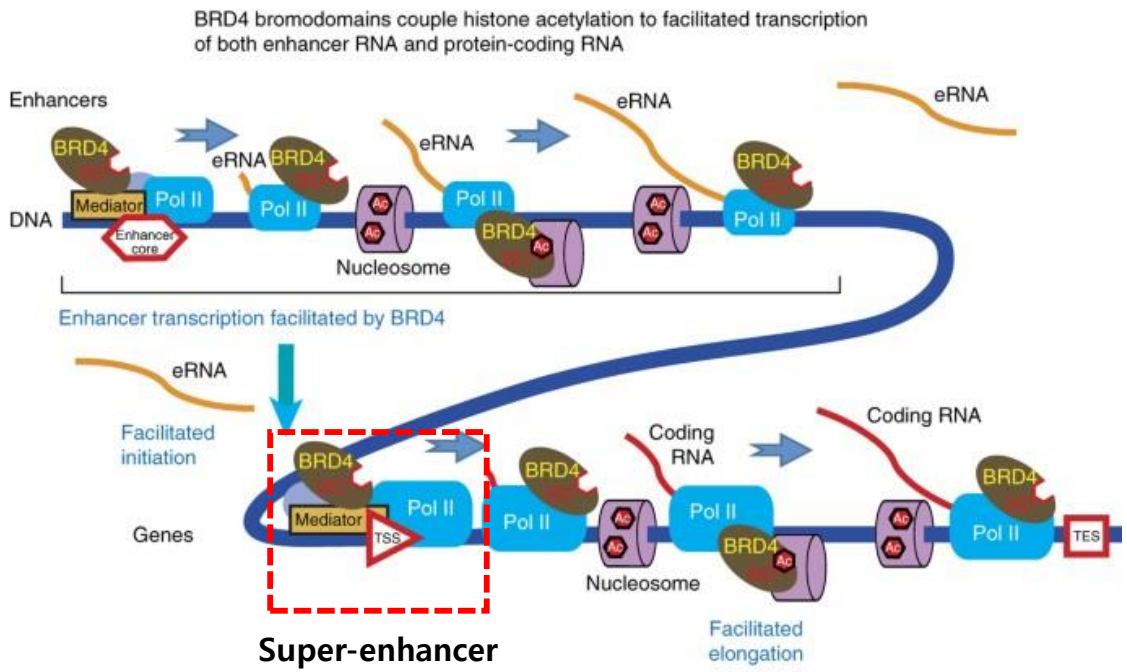
BRD4

- ✓ A bromodomain and extra terminal**(BET) family** that bind to **acetylated histones and regulates transcription**
- ✓ Occupy **various regions** of the genome
- ✓ Component of **super-enhancer(SE)** in normal and cancer cell

-> help to define cell type and linage specificity

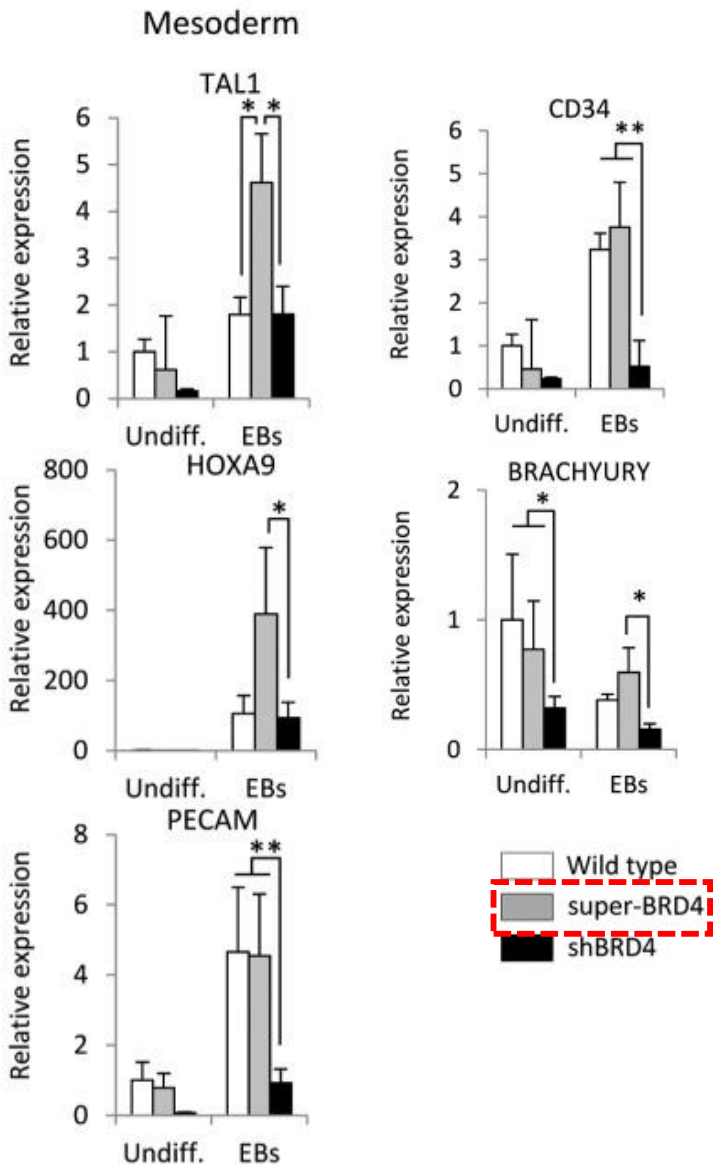
- ✓ SE is large regulatory DNAs, enriched with RNA polymerase II(Pol II) and acetylated histone (H3K27ac)
- ✓ BRD4 is associated **to hematopoietic differentiation of embryonic stem cells**
- ✓ There is no significant show that BRD4 is required for **hematopoiesis and inflammatory responses**

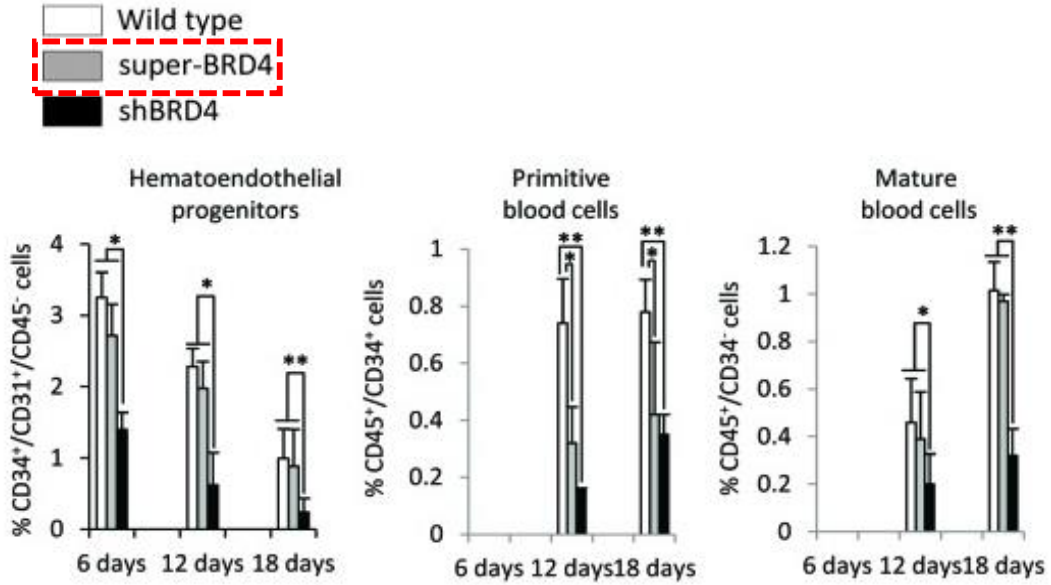
***Scheme of Action of BRD4**



Nature Structural & Molecular Biology volume 21, pages1047–1057 (2014)

Pre-study

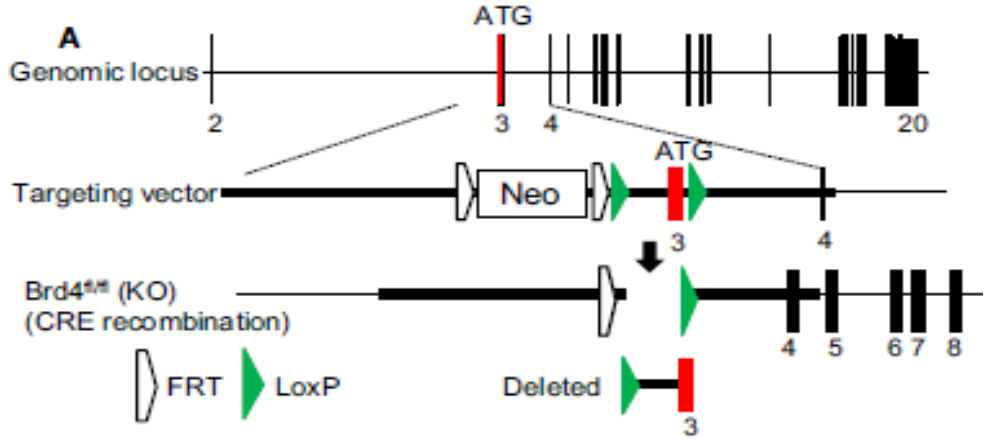




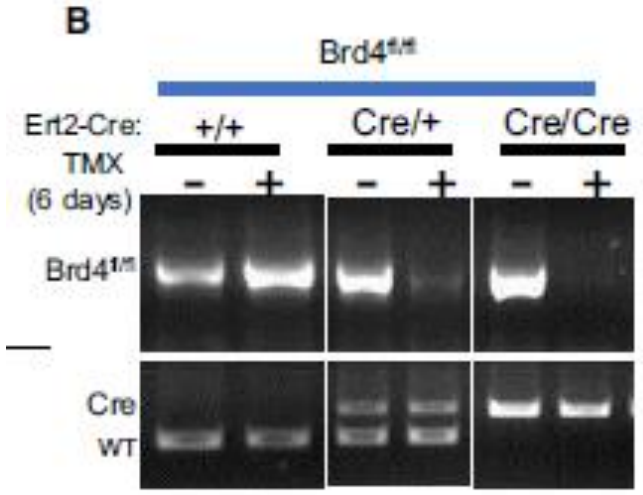
Epigenetics. 2014 Apr;9(4):566-78

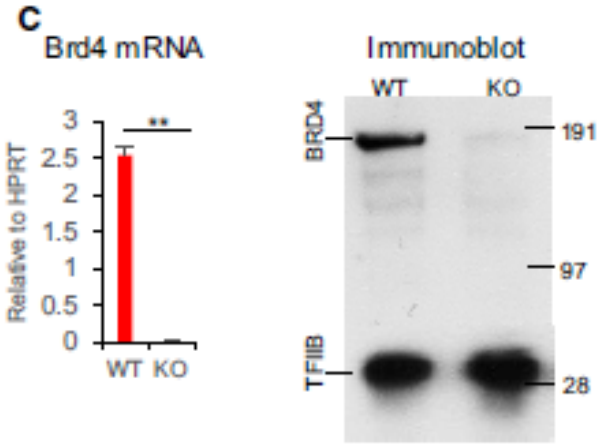
- ✓ BRD4 expression affected mesodermal markers during spontaneous differentiation of ESCs
- ✓ Downregulation of BRD4 impairs differentiation potential of hematopoietic progenitors from ESCs

Brd4 deletion blocks hematopoietic stem cell development



BRD4 gene / Cre system





*Vav-Cre mouse

-specific in cells of the hematopoietic system

*Ert2-Cre mouse

*Tamoxifen(TMX)

✓ Ert2-Cre-based deletion show depletion of BRD4 mRNA and protein *in vitro*

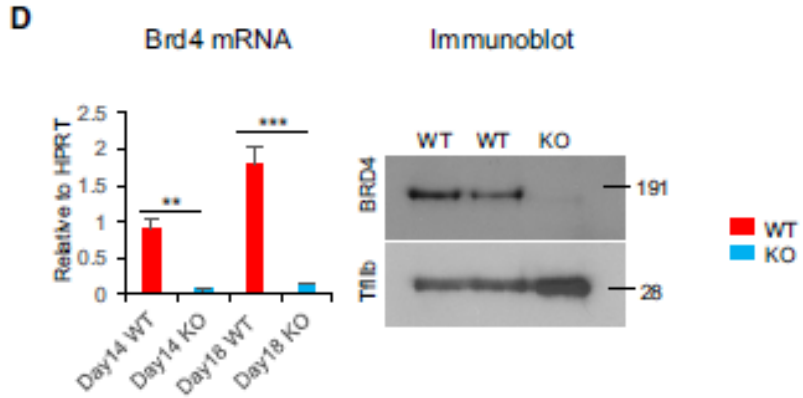
Vav-Cre-based Brd4 deletion blocks hematopoietic stem cell development

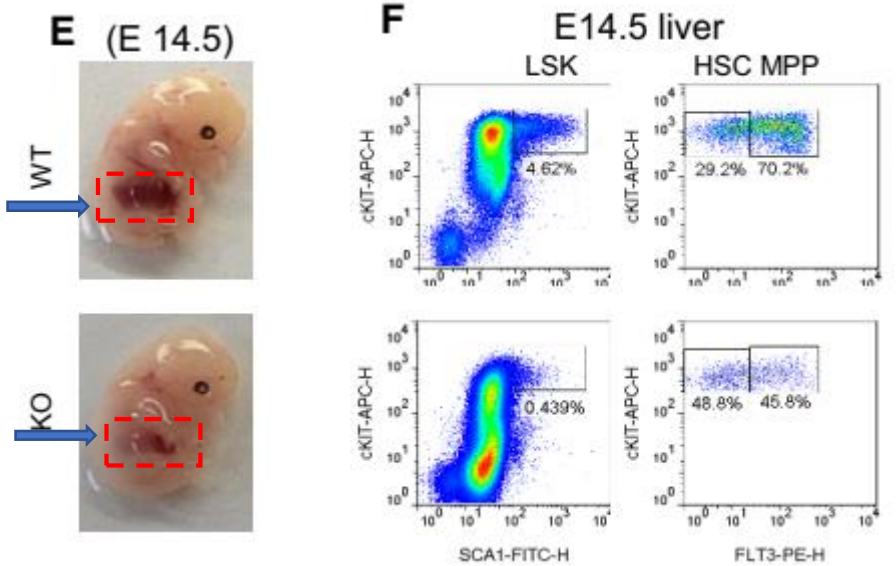
*Vav-Cre mouse

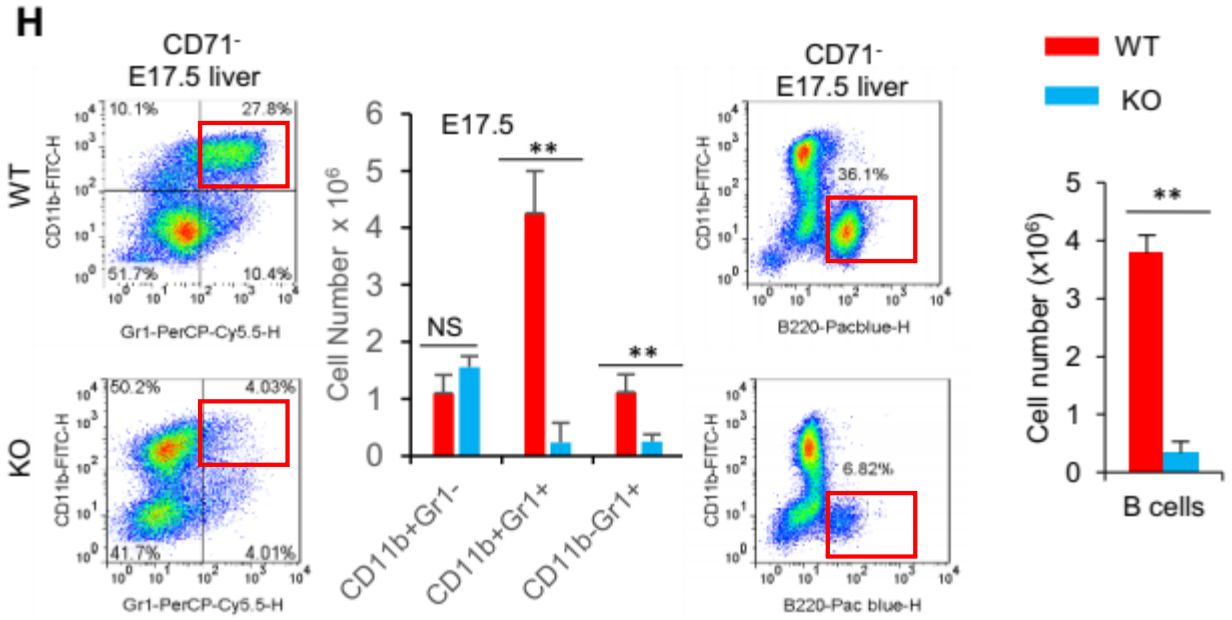
*Cr-1 CD11b – myeloid marker

*B220(CD45R) – B cell marker

✓ **BRD4 deletion using Brd4$^{fl/fl}$ Vav-cre mice**







✓ BRD4 KO embryo is distinguished from WT by reduced size and pale hue of fetal liver

✓ **Lin$^-$Sca$^+$ckit$^+$(LSK) population, Hematopoietic stem cell(HSC) and multipotent progenitor(MPP)** are lower in KO embryo than WT

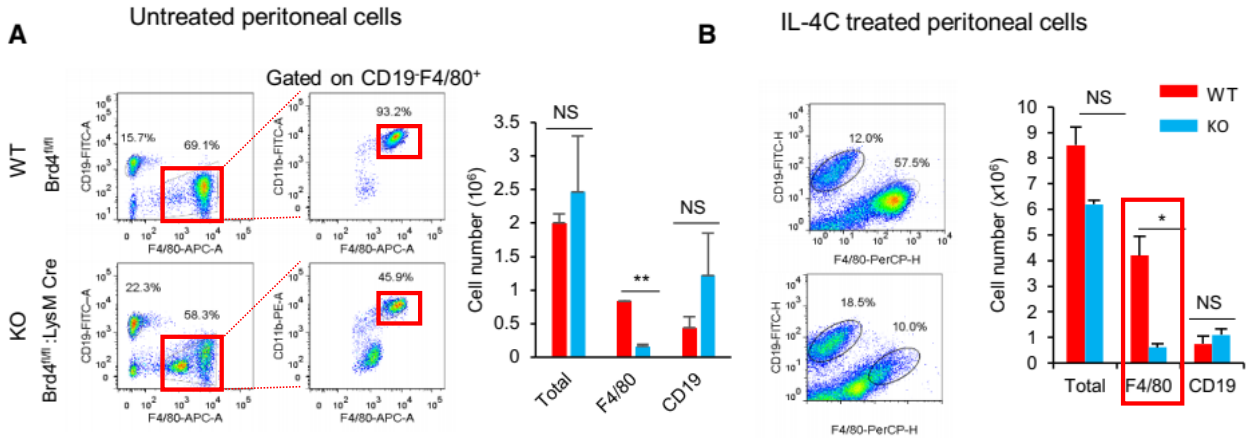
✓ Differentiation into **myeloid and lymphoid lineage** is reduced in KO embryo

Brd4 deletion compromises development and proliferation of resident peritoneal macrophages

✓ For the role of Brd4 in macrophage, check the resident macrophage that originate from **yolk-sac progenitor**

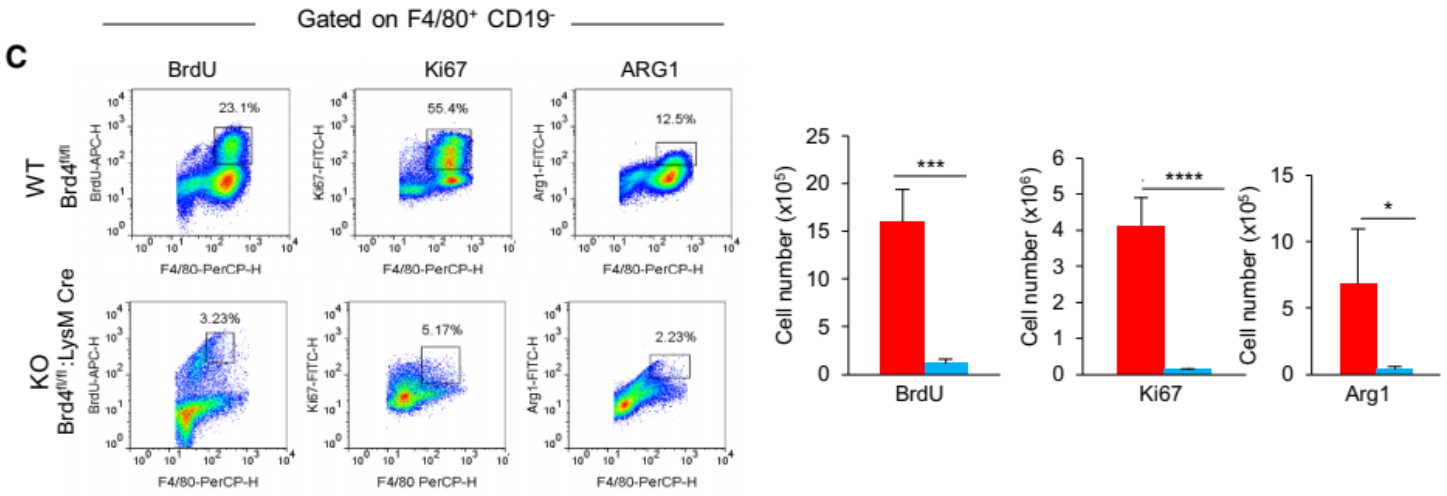
***LysM-Cre mouse**

-myeloid-specific



✓ Peritoneal macrophages respond to the Th2 cytokine IL-4 and undergo proliferation

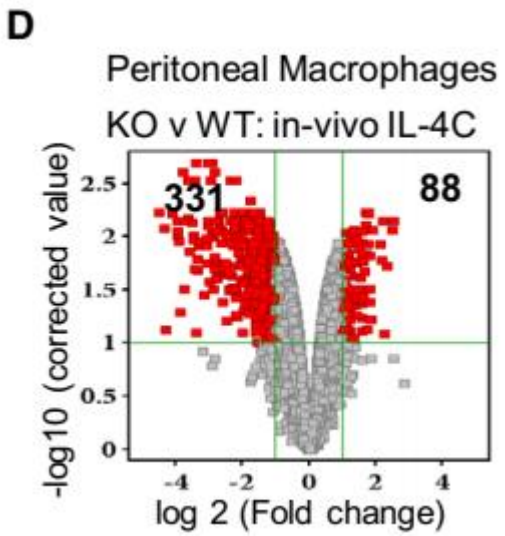
-> IL-4C complex treatment show no increase of number of macrophage in KO mice



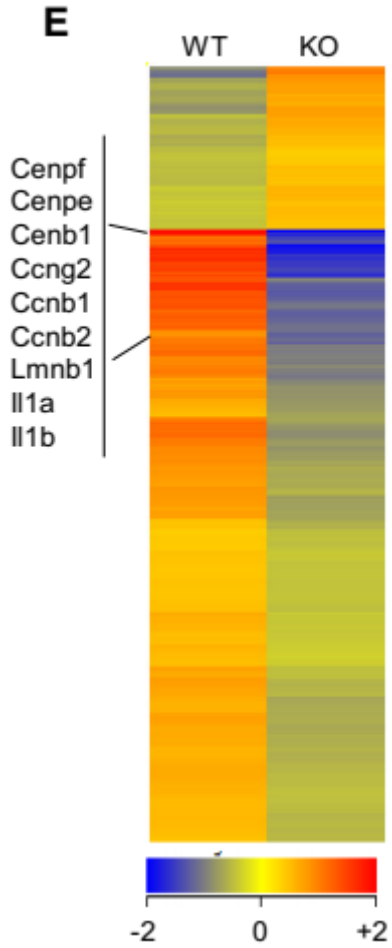
✓ Analysis of BrdU incorporation, Ki-67, and ARG1 expression confirmed **reduced cell division** in Brd4 KO macrophages

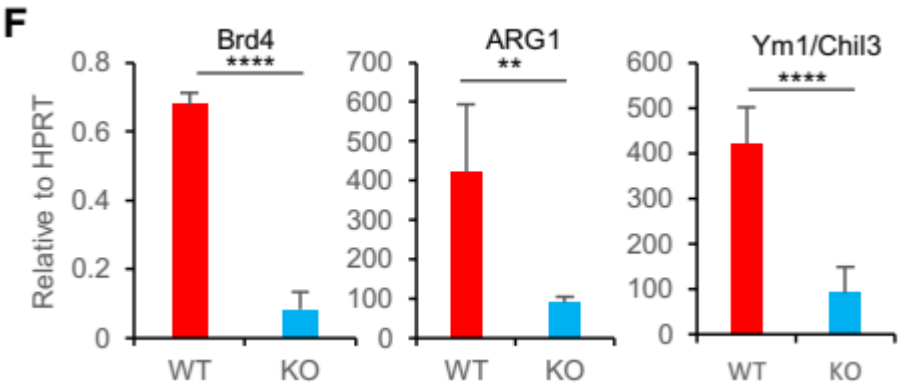
=> Resident macrophage require BRD4 for full differentiation and IL-4-induced proliferation

Brd4 deletion compromises development and proliferation of resident peritoneal macrophages



Cell proliferation -related gene



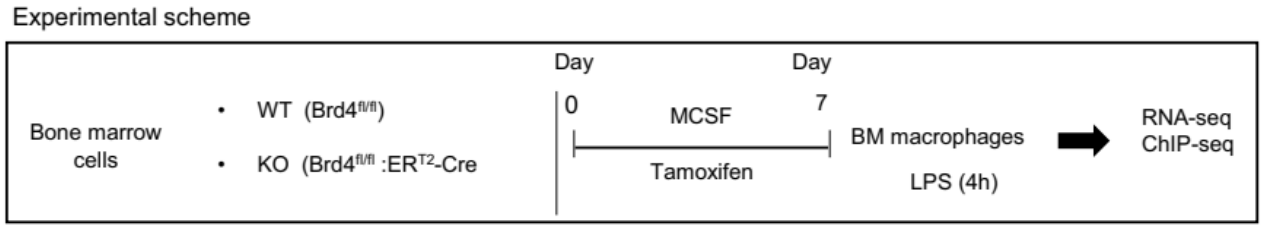


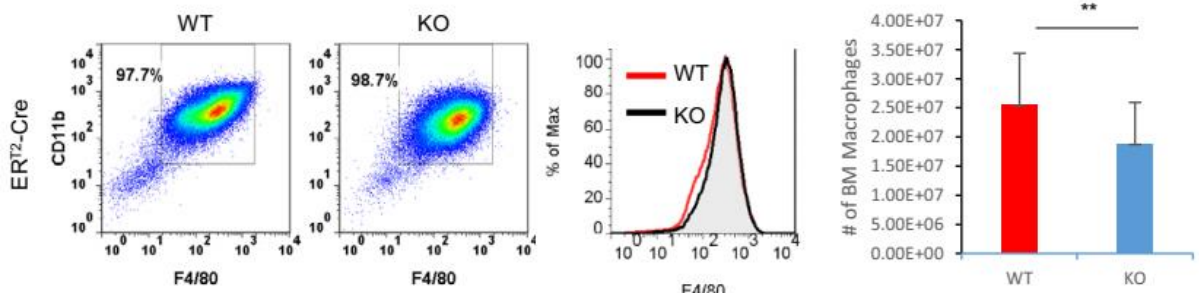
✓ Hierarchical clustering of downregulated genes revealed marked enrichment in categories related to **cell division and mitosis**

✓ **M2-specific genes** were downregulated in KO macrophages

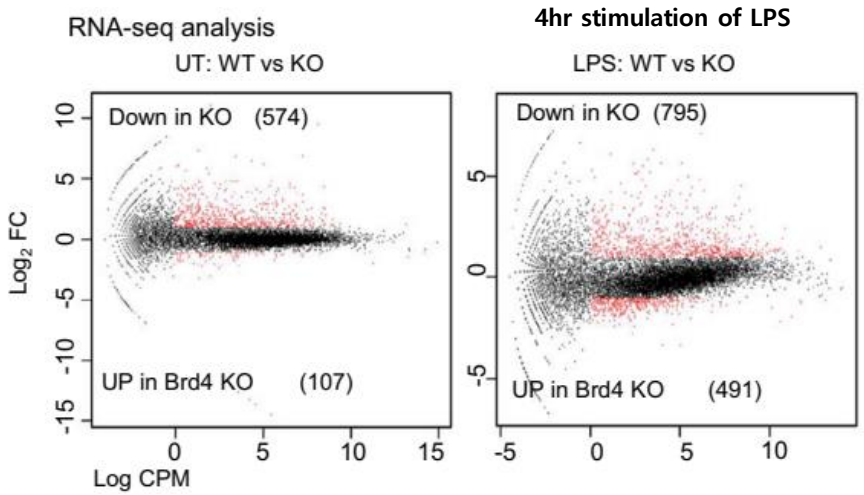
=>Brd4 deletion erase macrophage's ability to proliferate, while retaining many macrophage-specific traits

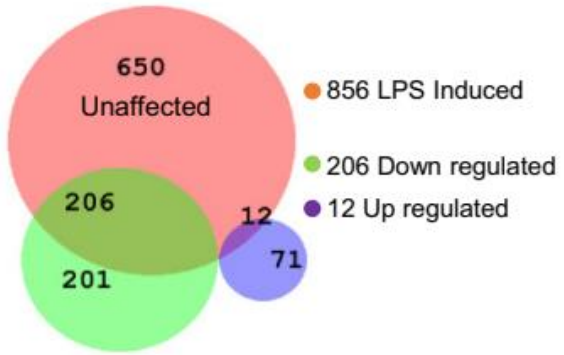
Brd4 deletion partially inhibits LPS-induced gene expression in BM-derived macrophages

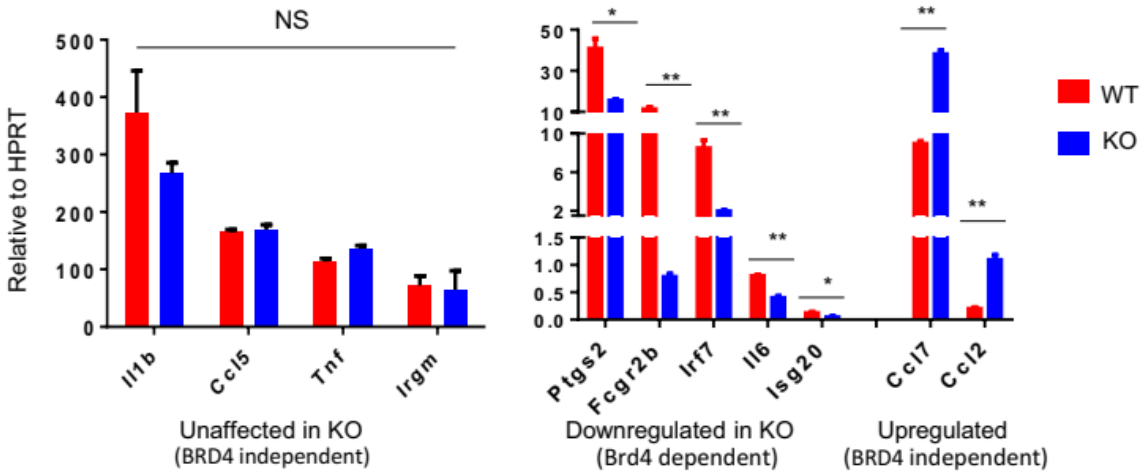




✓ Ert2-Cre KO culture shows similar yield and FACS analysis







✓ LPS treatment induced 868 genes in WT cells

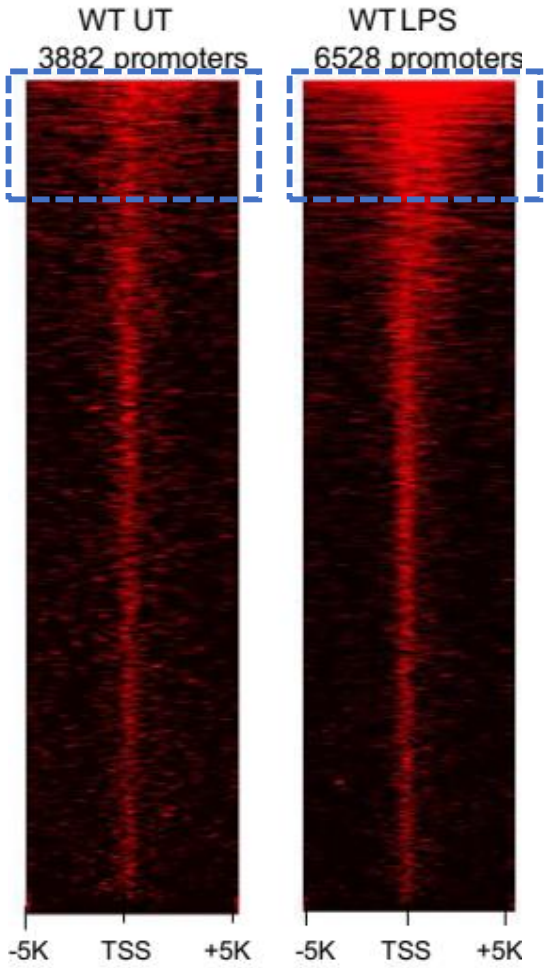
-> 206 genes were downregulated and 12 genes were upregulated in KO macrophages

=>Brd4 KO have **limited inhibition** of LPS-induced transcription

LPS treatment increases genome-wide BRD4 occupancy

*Transcription start site(TSS)

*Transcription end site(TES)



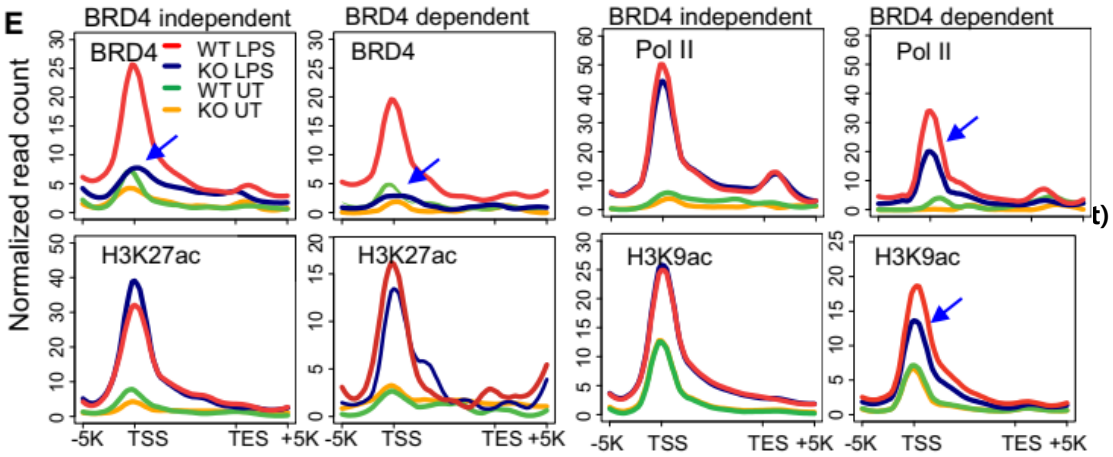
C Motif analysis at BRD4 binding sites:

UT

Factor	Logo	P value
PU.1		1E-283
AP1		1E-52
IRF1		1E-19

LPS

Factors	Logo	P value
PU.1		1E-274
AP1		1E-162
IRF1		1E-99
NFKB-p65		1E-44

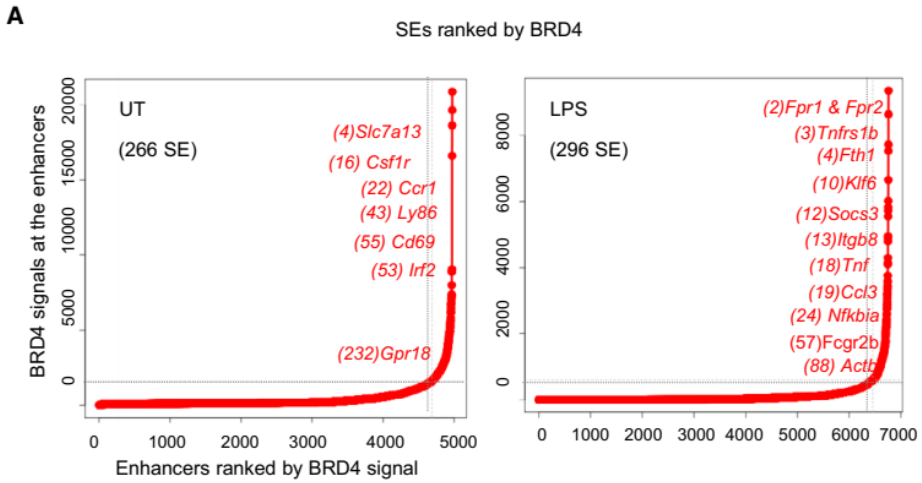


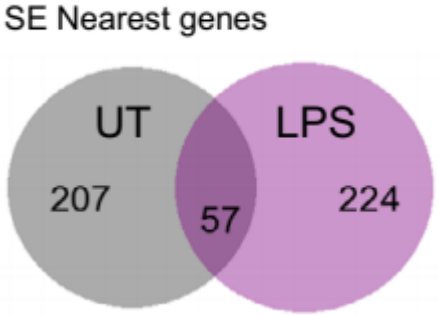
- ✓ 4 h of LPS stimulation markedly increased BRD4 signal intensity over the genic regions
- ✓ Motif analysis identified several transcription factor binding sites near the BRD4 occupied area both in untreated and in LPS-treated macrophages
- ✓ BRD4 is recruited to LPS-stimulated gene and BRD4 KO affect to reducing Pol II occupancy in KO macrophage

=> BRD4 occupancy increase under LPS treatment and occupancy itself does not imply functional necessity

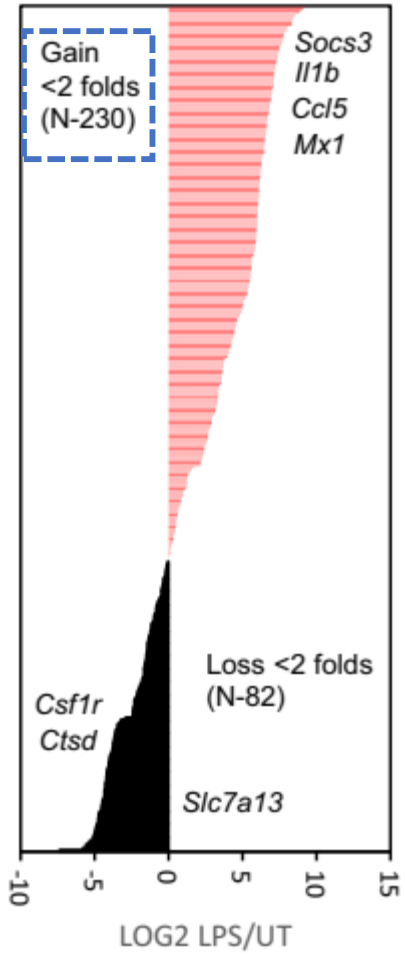
LPS stimulation triggers reorganization of super-enhancers

ChIP-seq





Genome-wide gain and loss of BRD4 SEs upon LPS stimulation

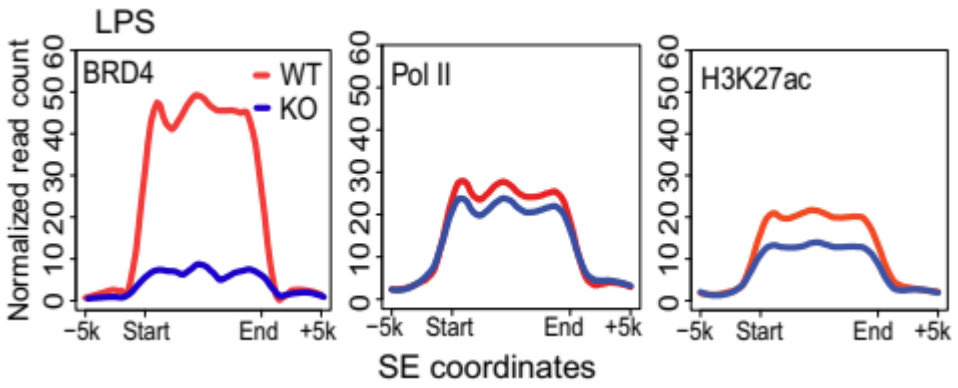


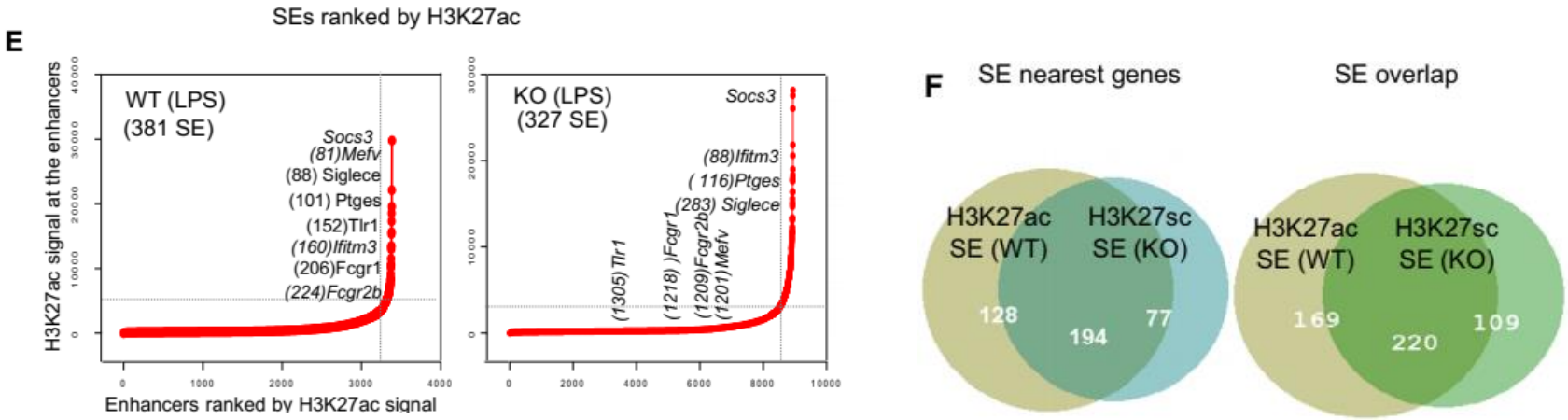
- ✓ SEs in untreated and LPS-treated macrophages were distinct, neighboring largely different sets of gene loci

- ✓ LPS-treated macrophages gained 230 new SEs

-> LPS stimulation triggered a large-scale SE reorganization

Brd4KO macrophages form alternative super-enhancers

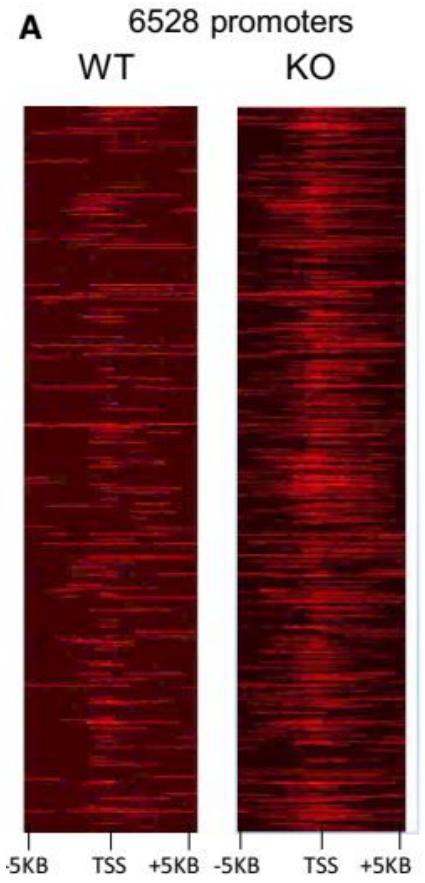


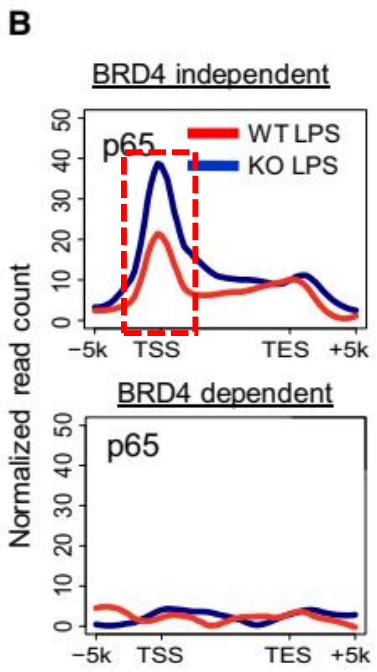


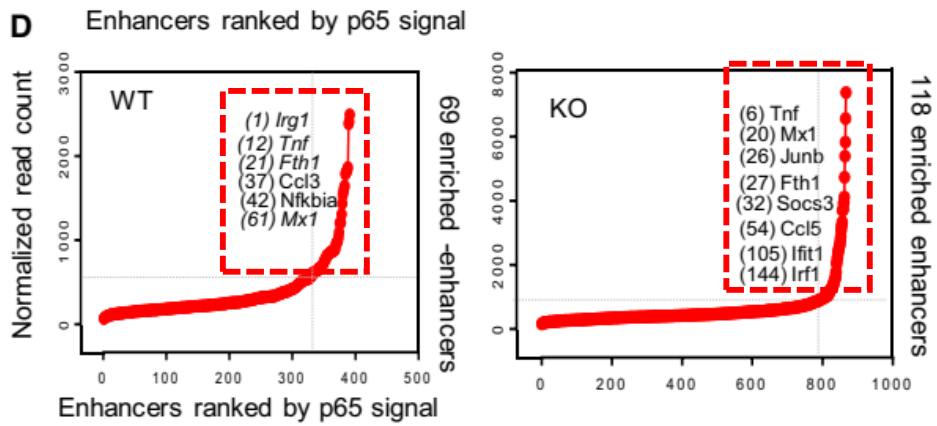
✓ SEs are assembled in KO macrophages without BRD4, and these SEs target a set of genes shared by WT macrophages

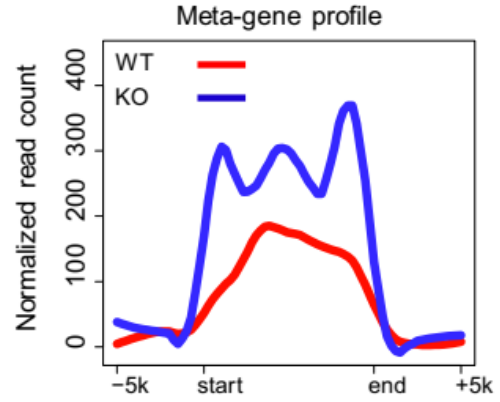
=> alternative SEs could support a significant fraction of LPS-induced transcription without BRD4

Increased NF-κB (p65) binding in Brd4KO macrophages





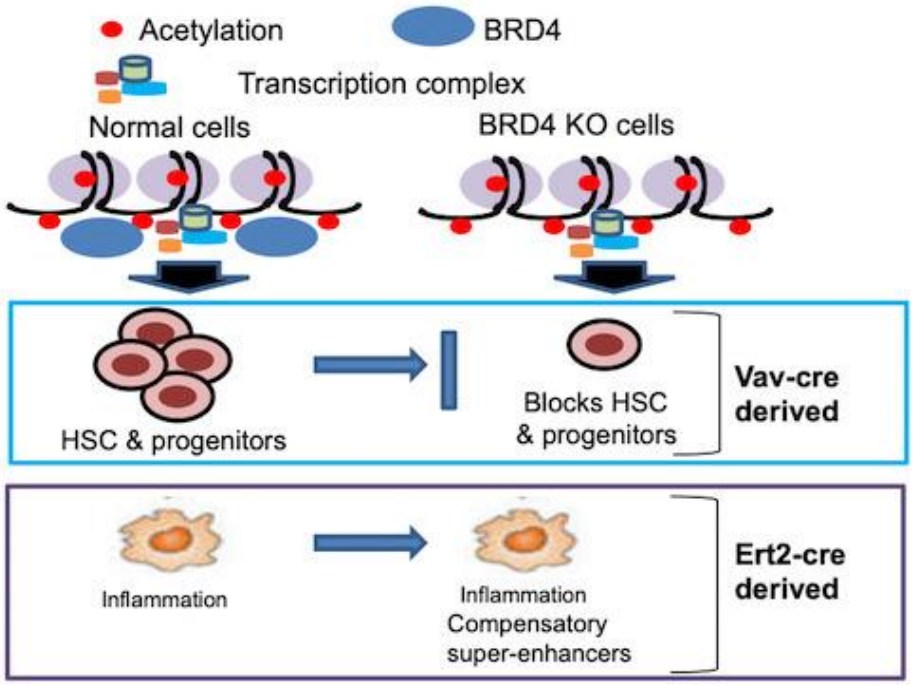




- ✓ NF-kB p65 binding was distinctly higher in KO macrophages than in WT macrophages at BRD4-independent region
- ✓ BRD4-dependent genes had virtually no p65 binding
- ✓ KO macrophages had greater p65 occupancy than WT cells in enhancer regions

=>increased NF-kB binding in KO macrophages allow KO macrophage to retain inflammatory responses

Conclusion



- BRD4 is required for development and proliferation of mouse hematopoietic stem cells.
- BRD4 is dispensable for macrophage differentiation and inflammatory response.
- BRD4 broadly occupies genic and intergenic regions of transcribed genes in both unstimulated and LPS-stimulated macrophages.
- Brd4 deletion is associated with increased NF-κB occupancy