June 29-July 1, 2015
ENCODE 2015:
Research Application and Users Meeting

Selection and function of signal-dependent enhancers: The Macrophage as a Case Study

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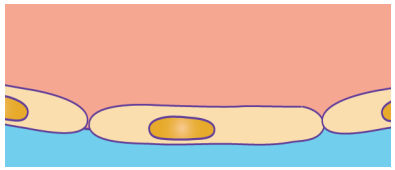
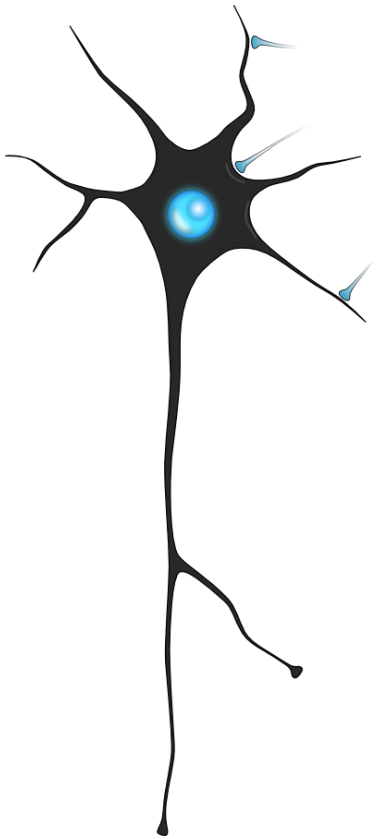
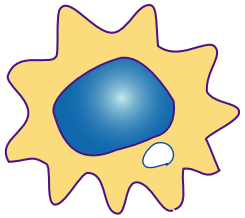
Different cell types arise from differential transcription of the same genome



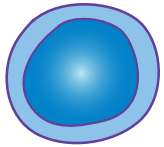
Endothelial cells



Neurons



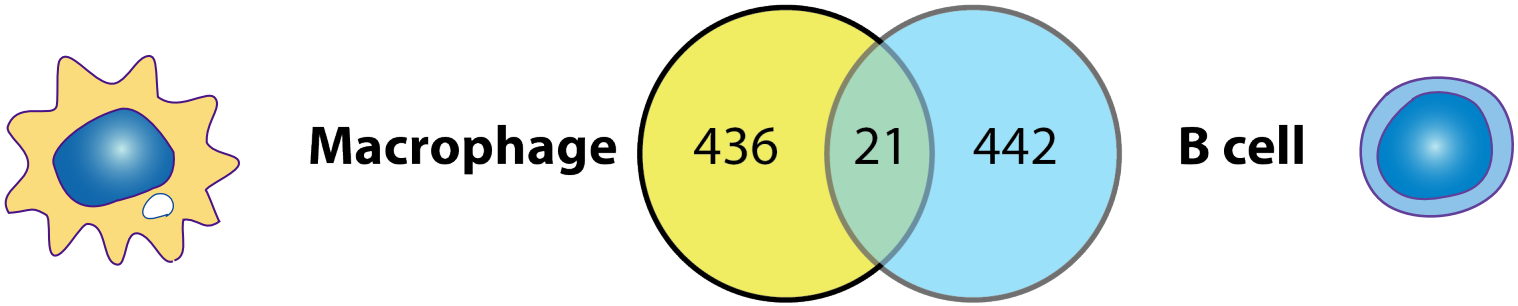
Macrophages



B cells

Transcriptional responses to the same signal can be cell type-specific

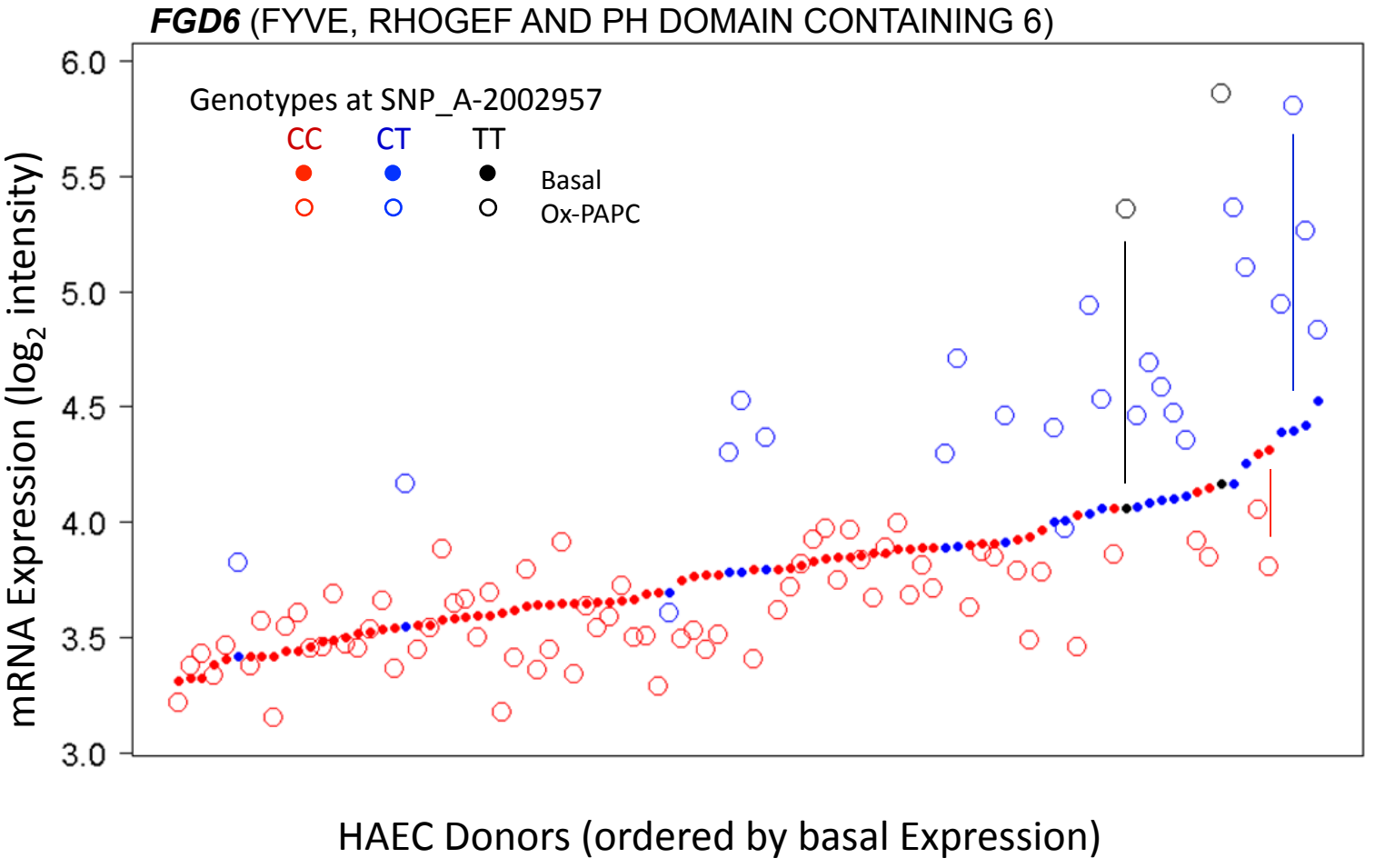
mRNAs increased > 4-fold 1h after TLR4 ligation



	Macrophage	B cell
GO term	p value	p value
Immune response	1e-36	> 0.05
Chemotaxis	1e-12	> 0.05
Protein folding	> 0.05	1e-10
RNA processing	> 0.05	1e-9
DNA replication	> 0.05	8e-3

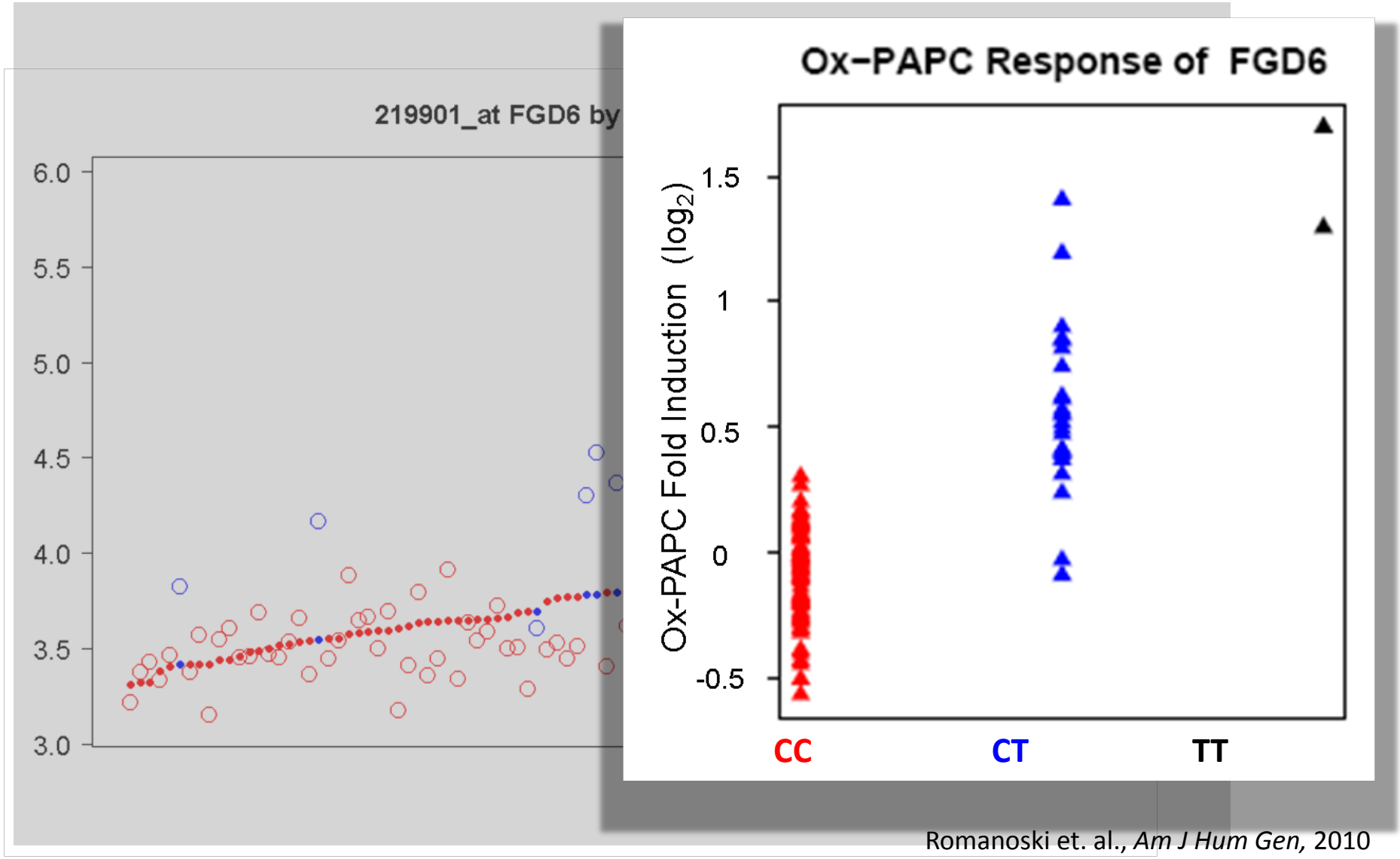
Nathan Spann and Cody Diehl

Signal-dependent responses of the same cell type can vary among individuals



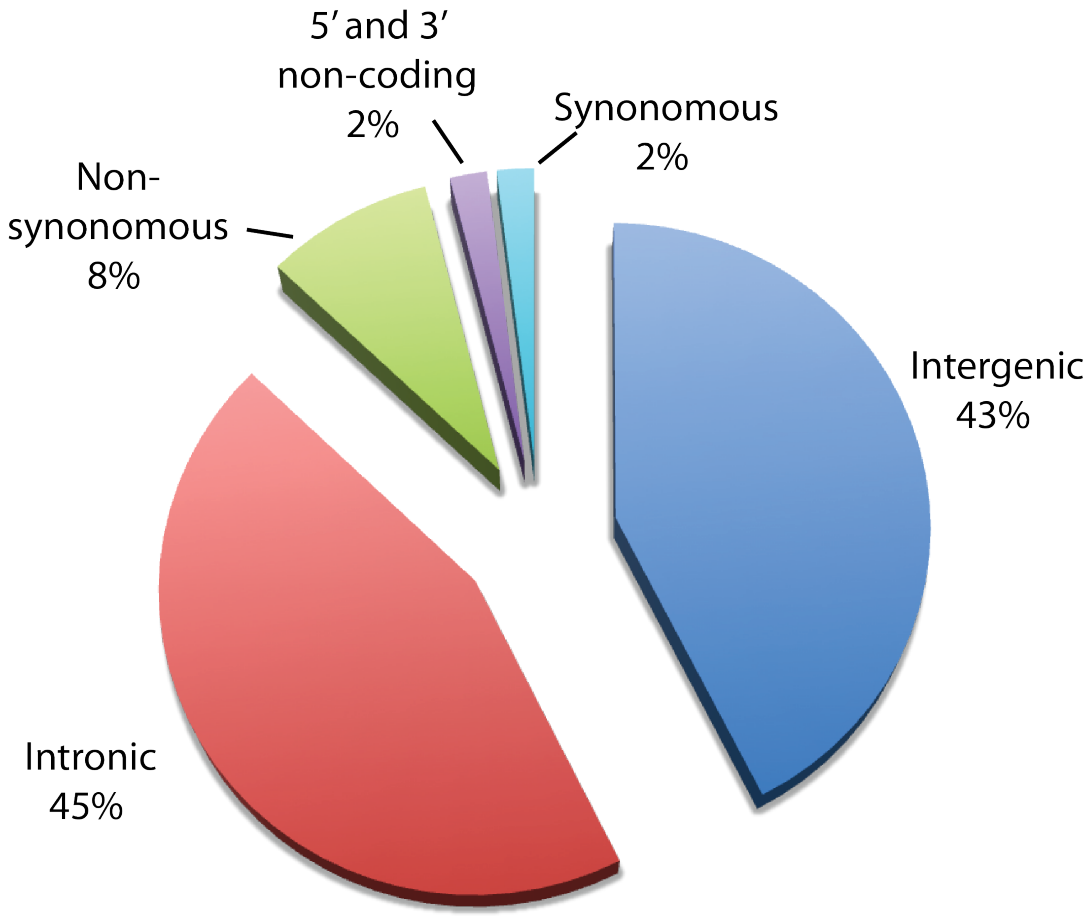
Romanoski et. al., *Am J Hum Gen,* 2010

The FGD6 response to Ox-PAPC is associated to a local DNA variant



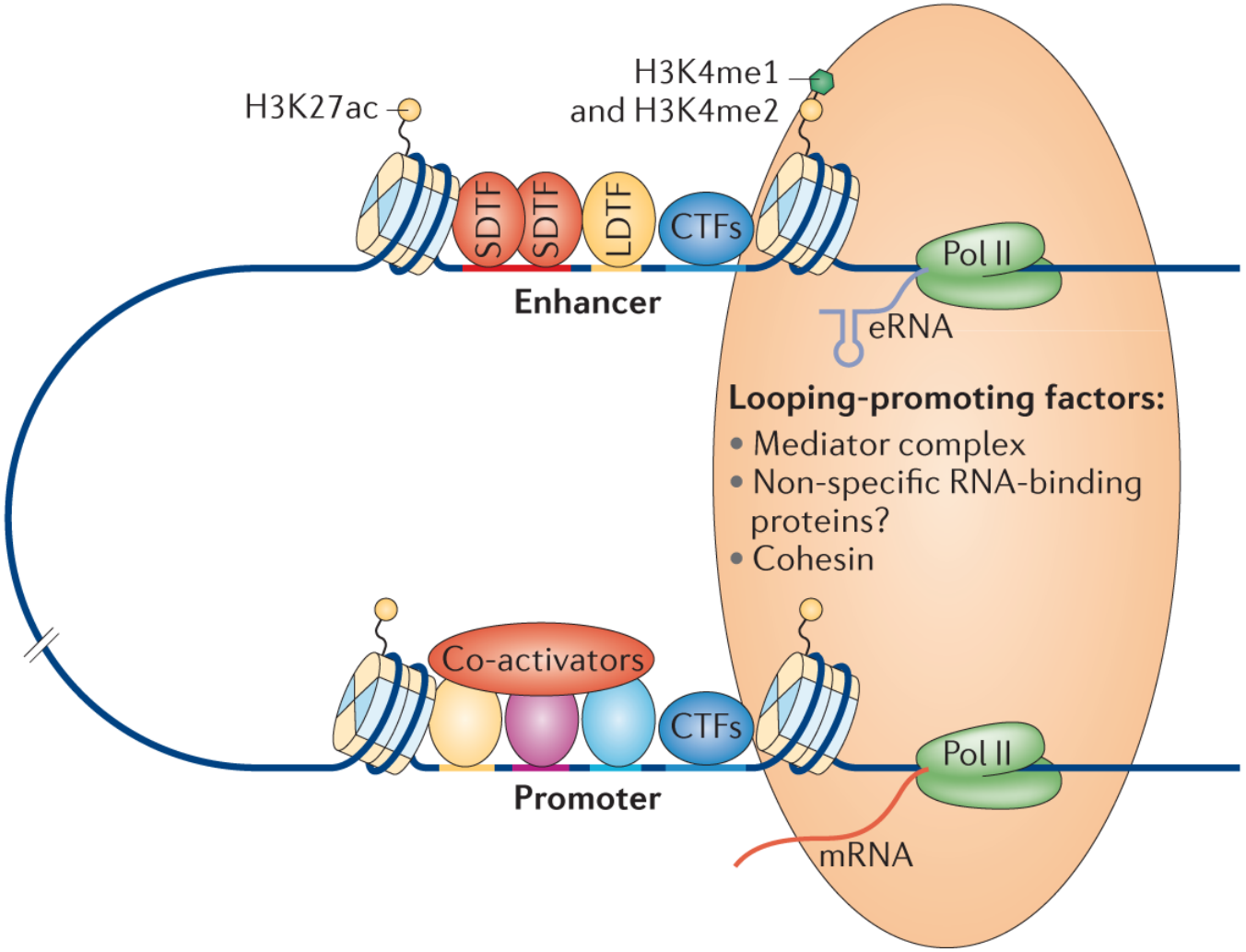
Romanoski et. al., *Am J Hum Gen,* 2010

Most GWAS disease/trait-associated SNPs reside in non-coding regions of the genome



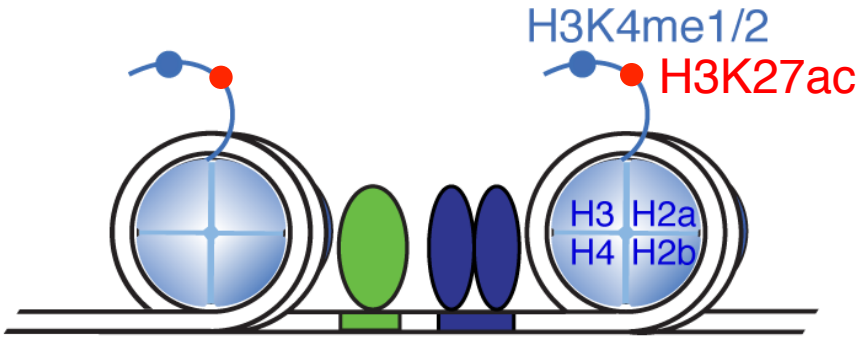
Hindorff et al., Proc Natl Acad Sci U S A. 2009;106:9362-7

Enhancer/promoter interactions establish cell-specific and signal-dependent gene expression



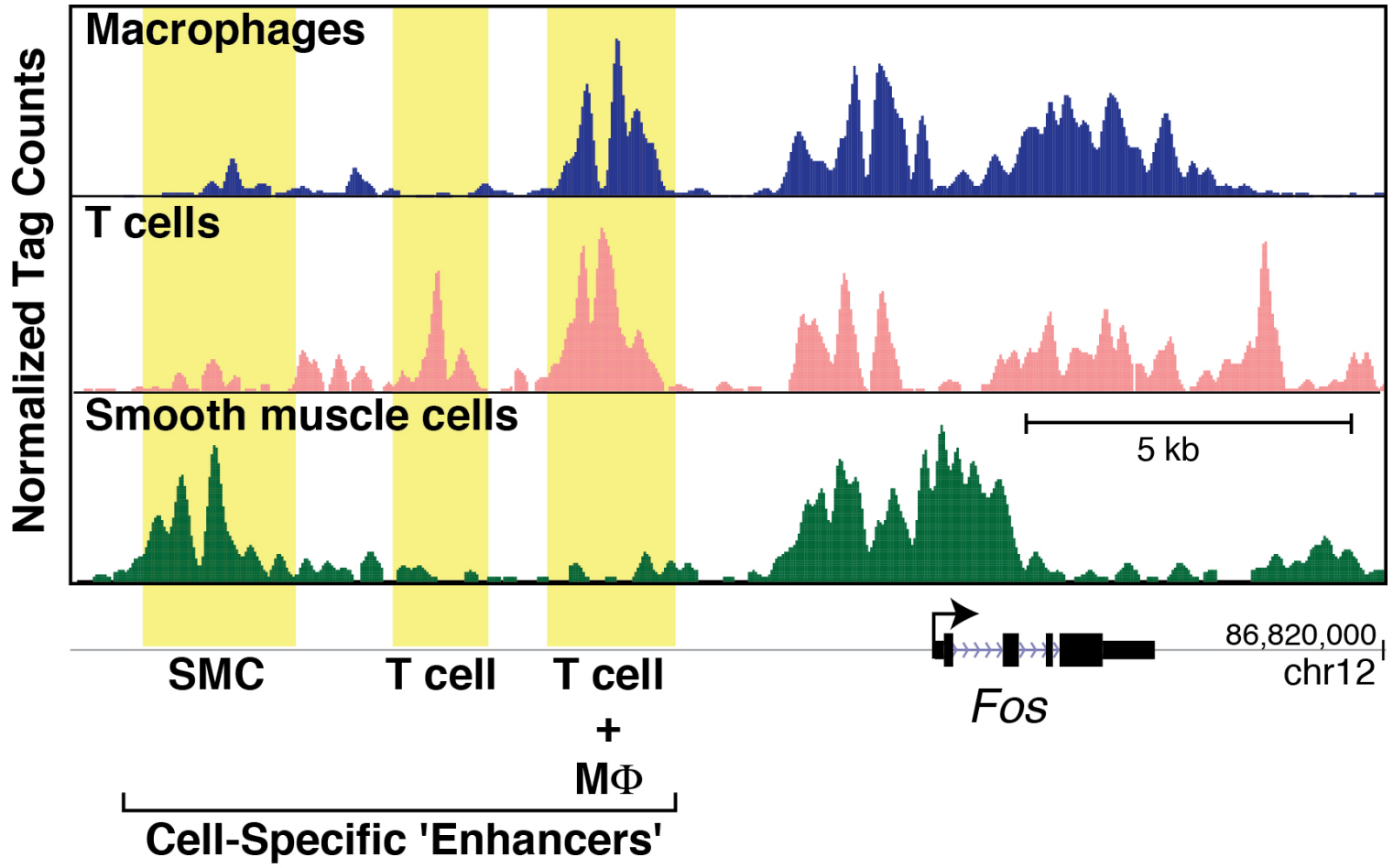
Heinz et al., Nature Reviews Molecular and Cell Biology, 2015

Enhancers are major determinants of cell-specific and signal-dependent gene expression



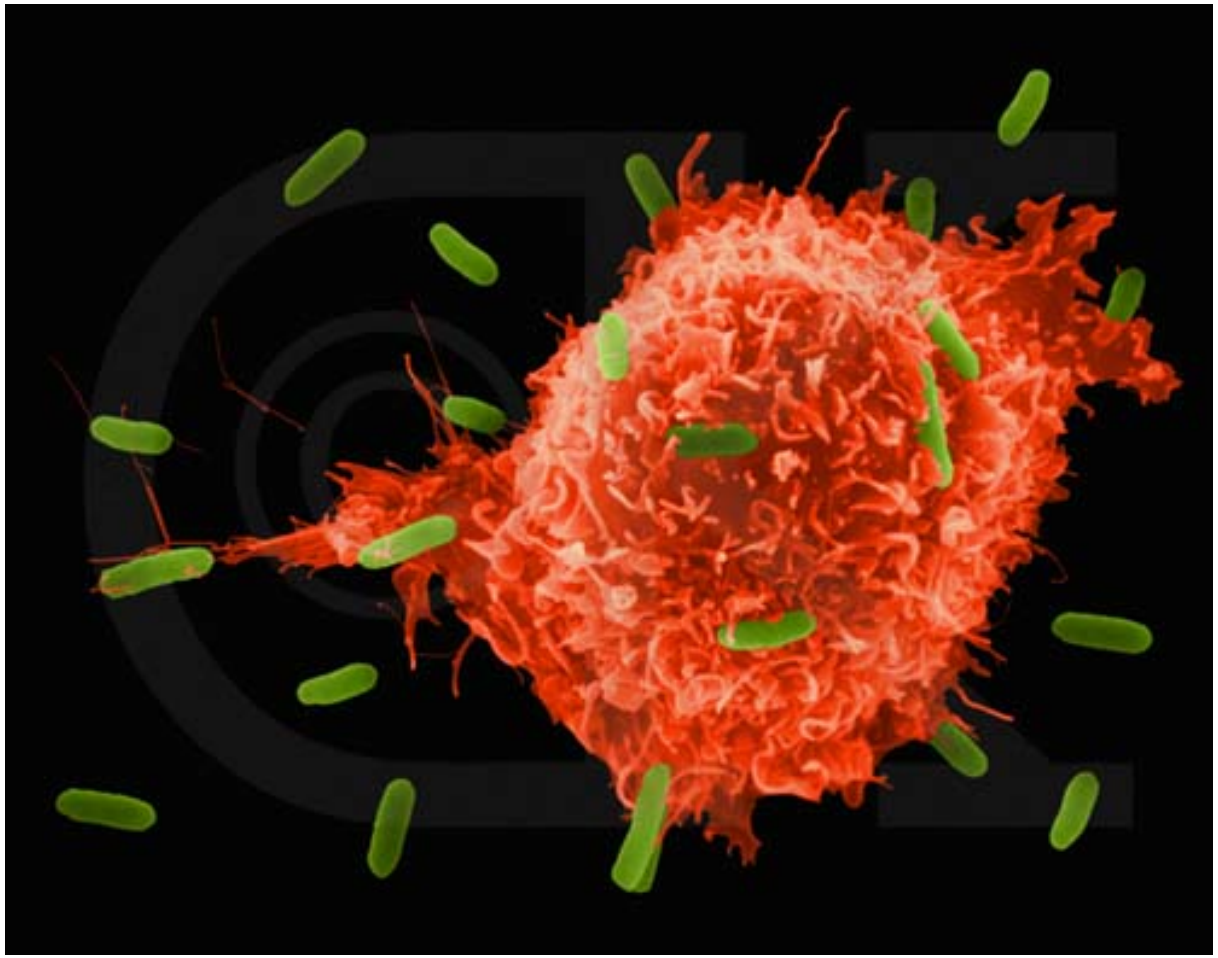
- Act at a distance from promoters to 'Enhance' gene transcription
- Function determined by sequence-specific transcription factors
- Exhibit a distinct epigenetic signature; H3K4me1/2 > H3K4me3, H3K27ac
- ~ 1 million predicted in the human genome

Cell-specific selection of enhancers

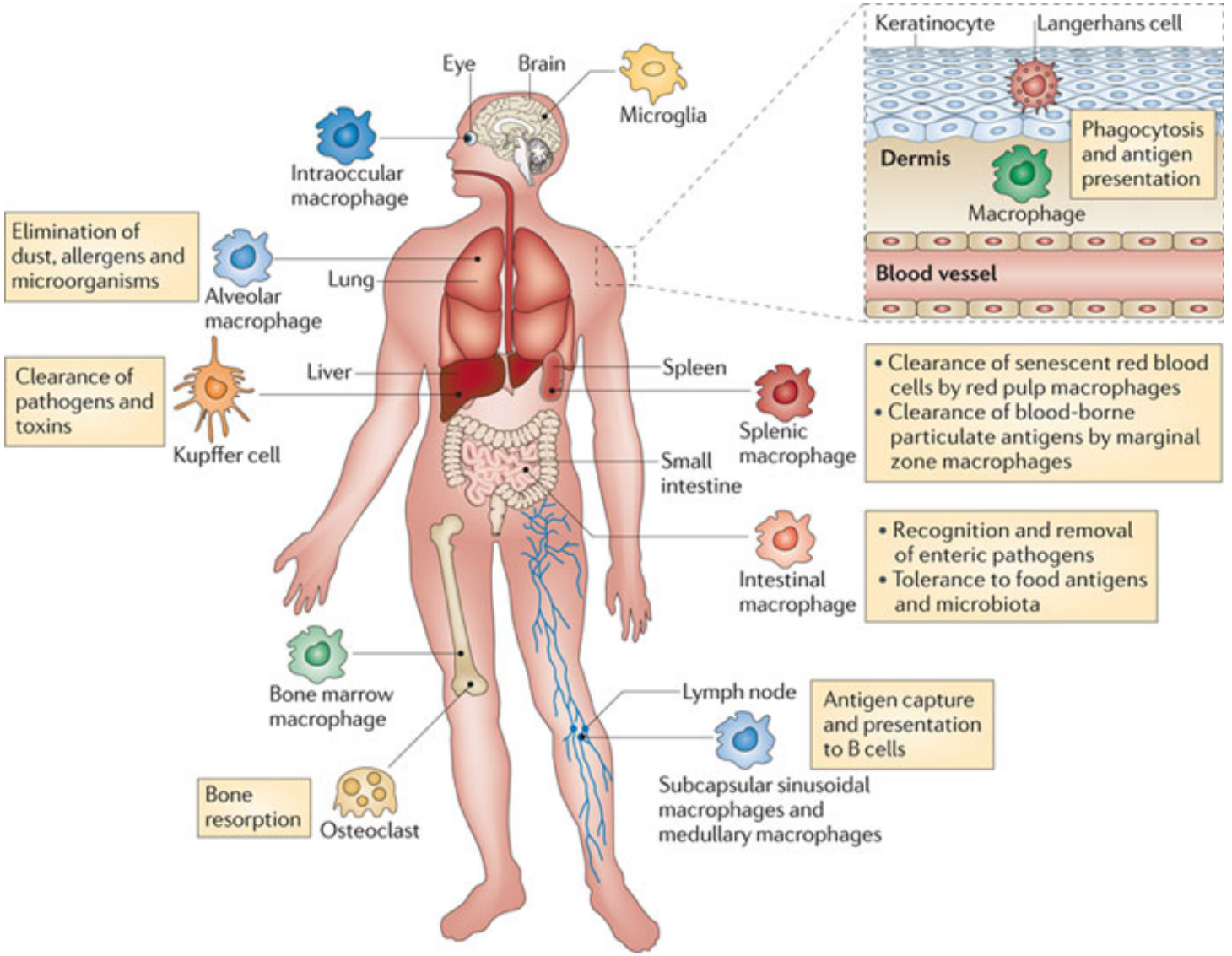


From ENCODE - Combined H3K4me3 and H3K4me1

Macrophages play essential roles in the response to infection and injury



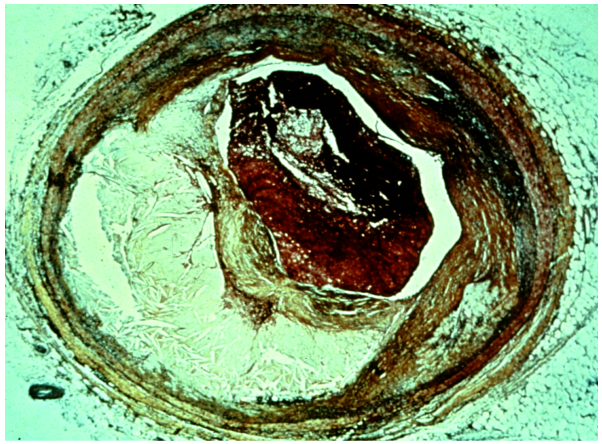
Specialized homeostatic functions of resident tissue macrophages



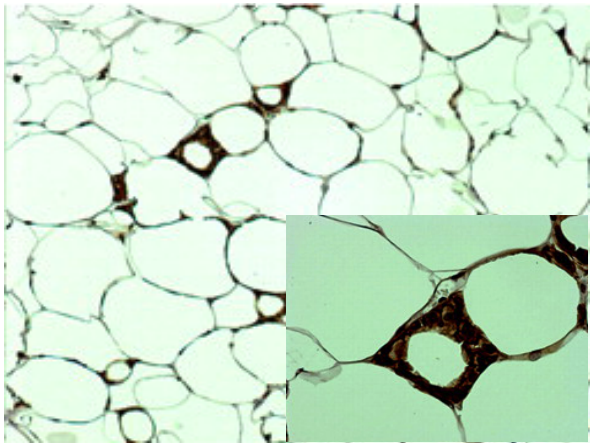
PJ Murray, TA Wynn, Nature Reviews Immunology 2011

Roles of macrophages in human disease

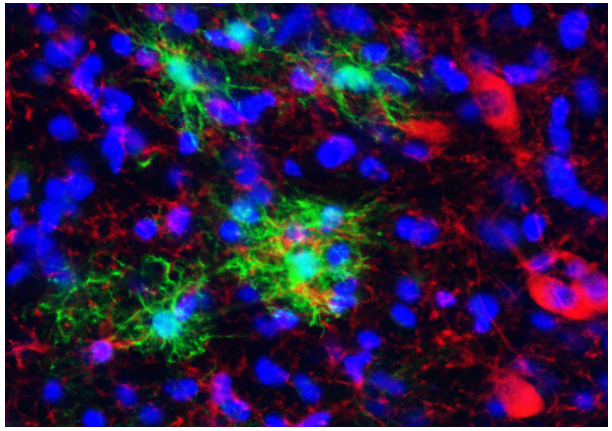
Macrophage foam cells
in atherosclerosis



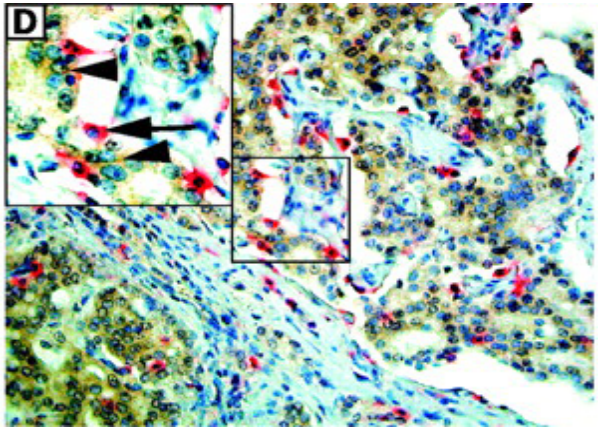
Adipose tissue macrophages
in insulin resistance



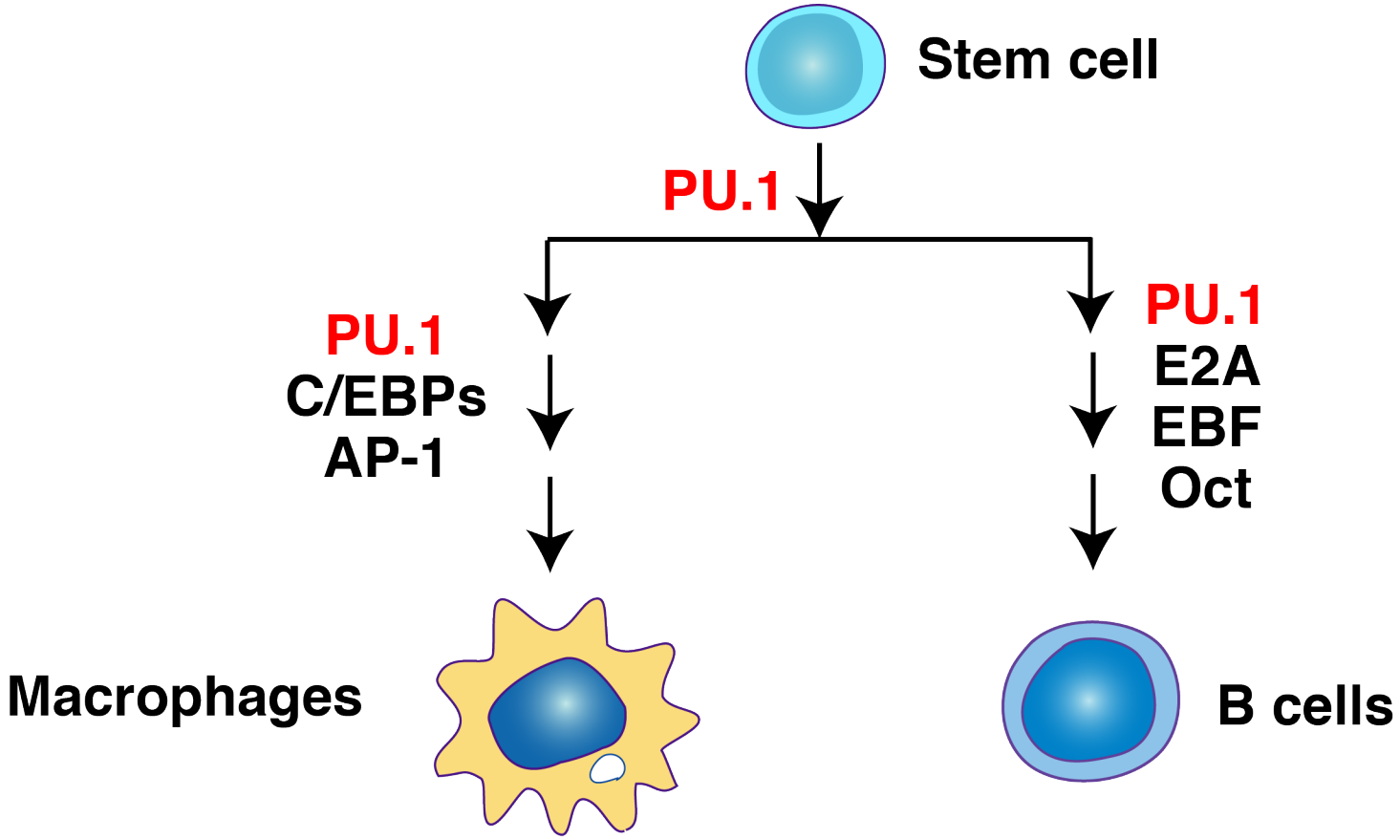
Activated microglia in
neurodegenerative disease



Tumor-associated macrophages
in cancer



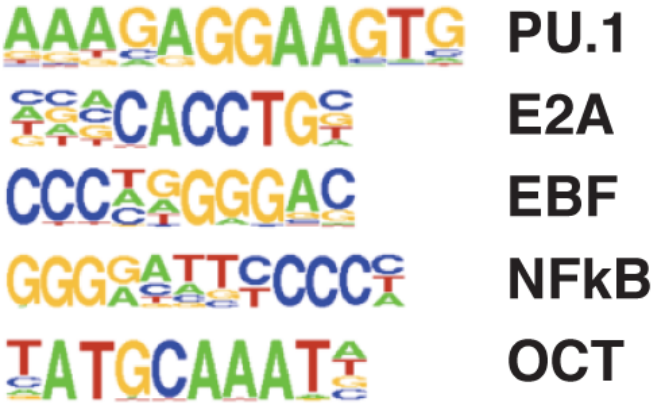
PU.1 is required for both macrophage and B cell differentiation



Enriched motifs at macrophage and B-cell-specific PU.1 binding sites

Macrophage-specific PU.1 binding sites

B-cell-specific PU.1 binding sites



Heinz et al, Molecular Cell 2010

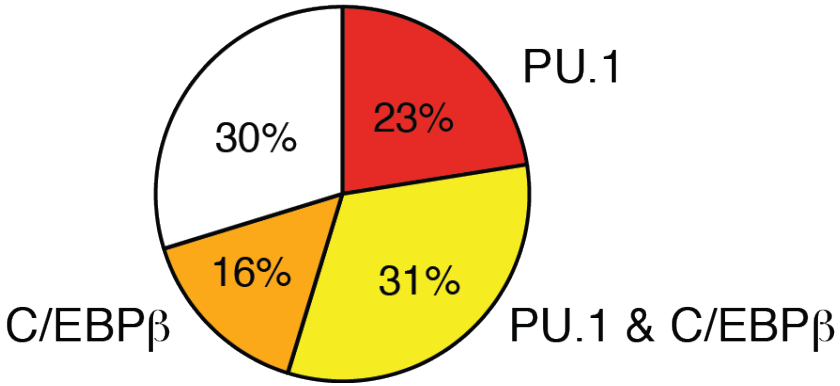
PU.1 and C/EBPβ occupy the majority of the enhancer-like regions in macrophages

Macrophage H3K4me1 Peaks

Enriched motifs

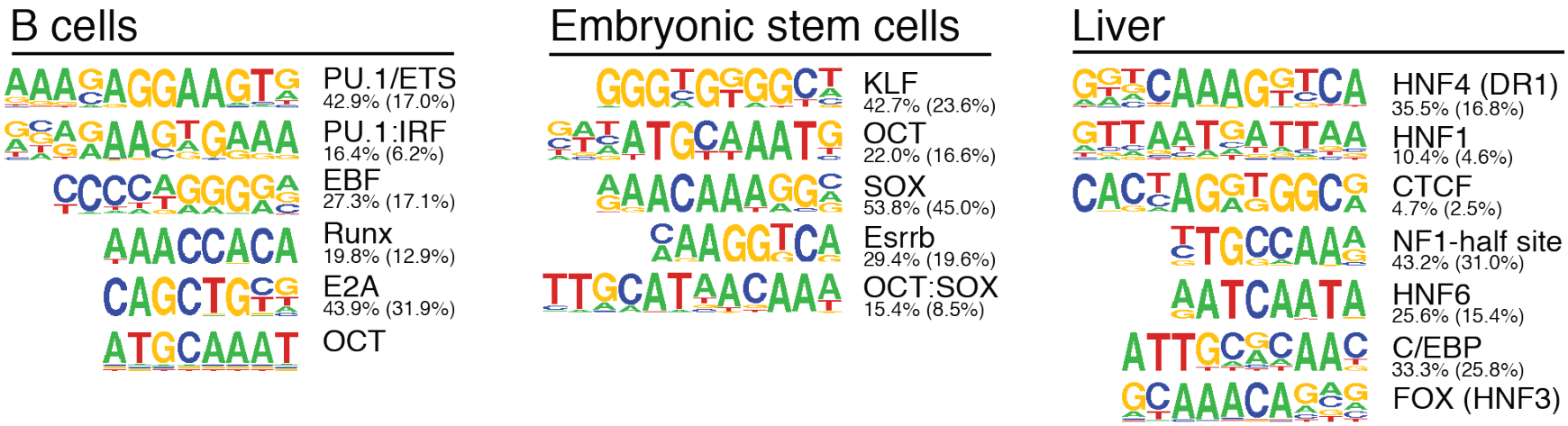
Macrophage H3K4me1 Peaks

PU.1 and C/EBPβ ChIP-Seq



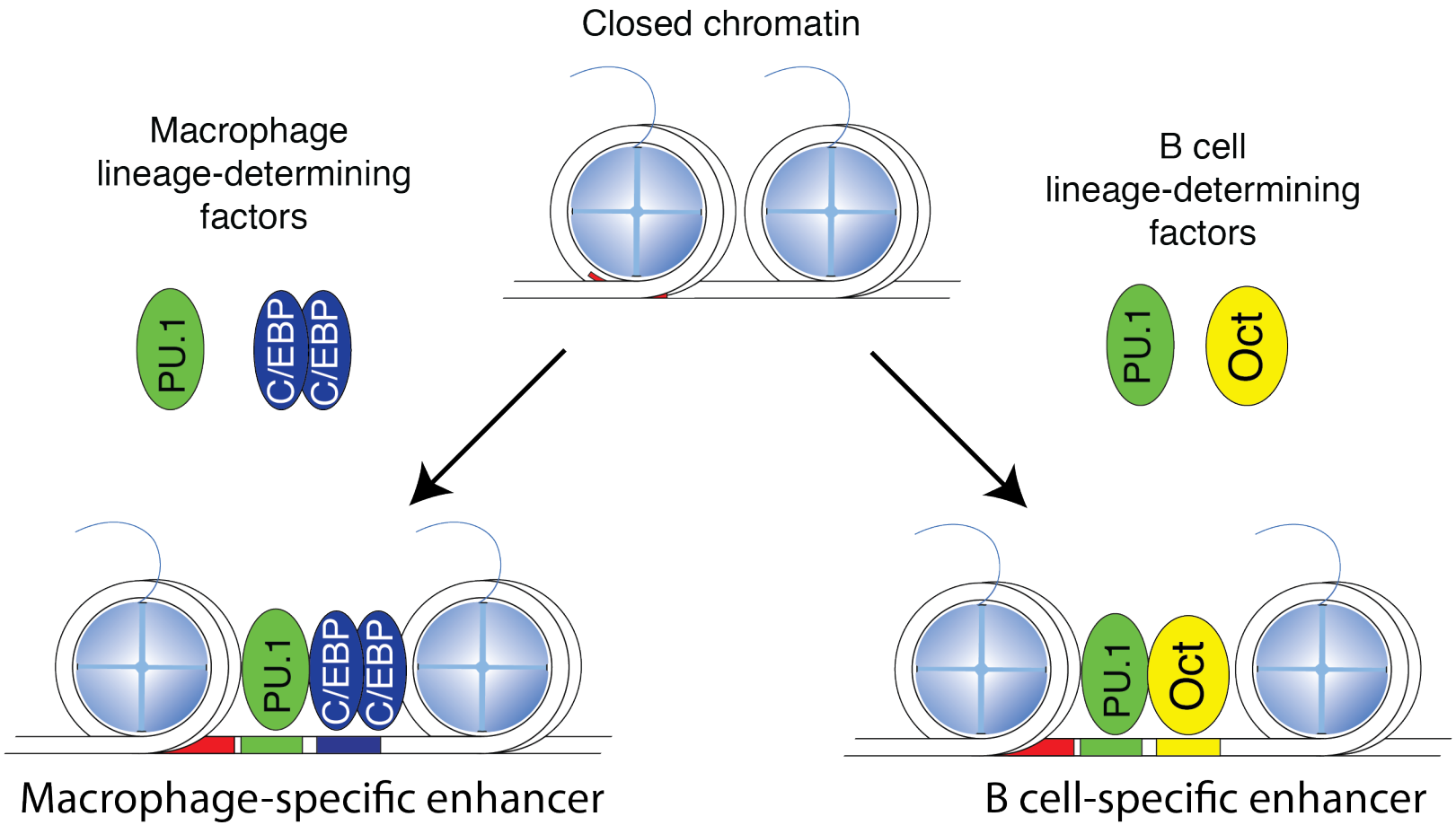
Heinz, et al., Mol. Cell 2010

Motifs for lineage determining TFs are enriched in H3K4me1 marked regions of the genome



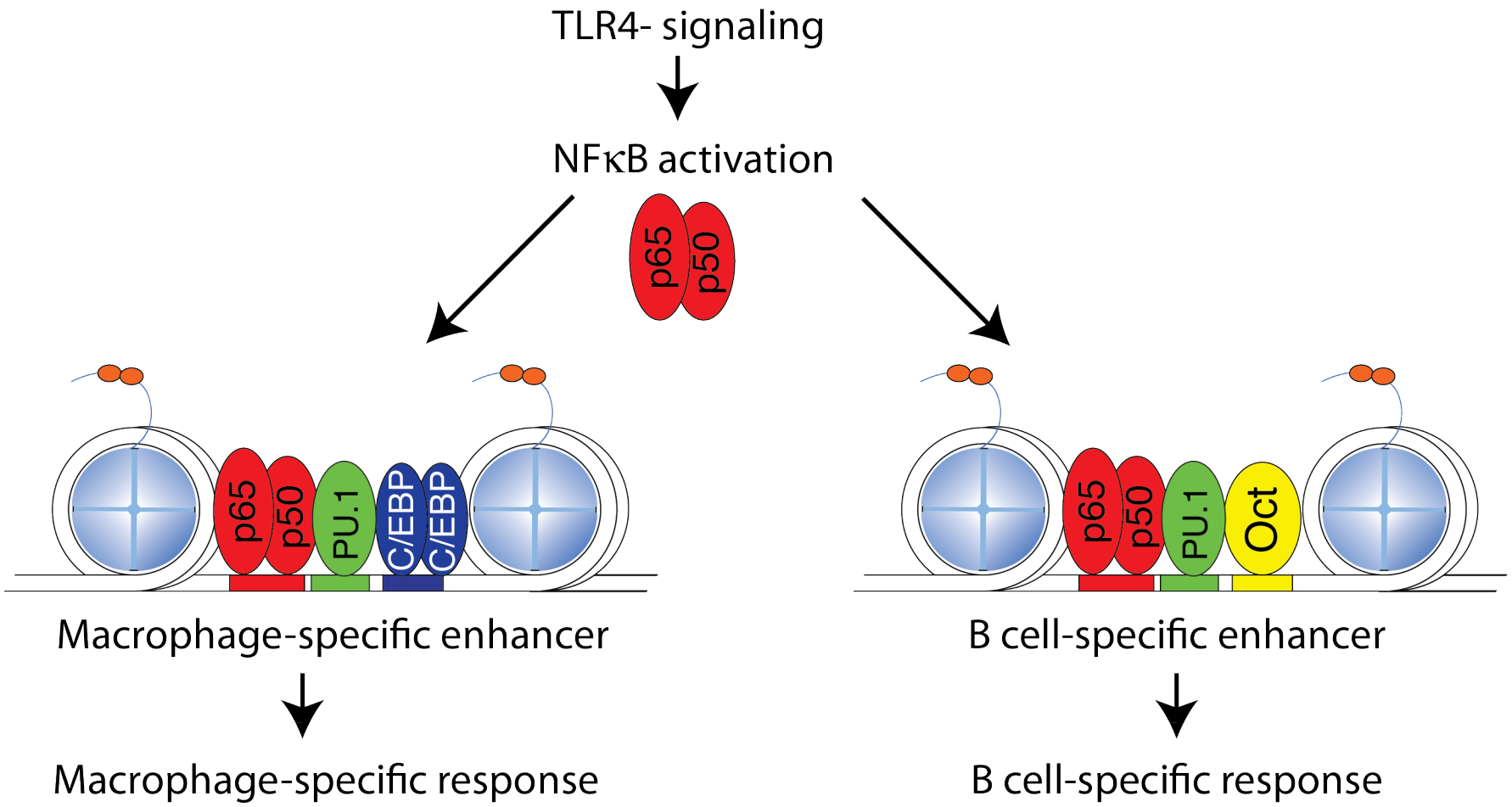
Motifs from ENCODE H3K4me1 data; Heinz et al, Molecular Cell 2010

Selecting macrophage and B cell-specific enhancers



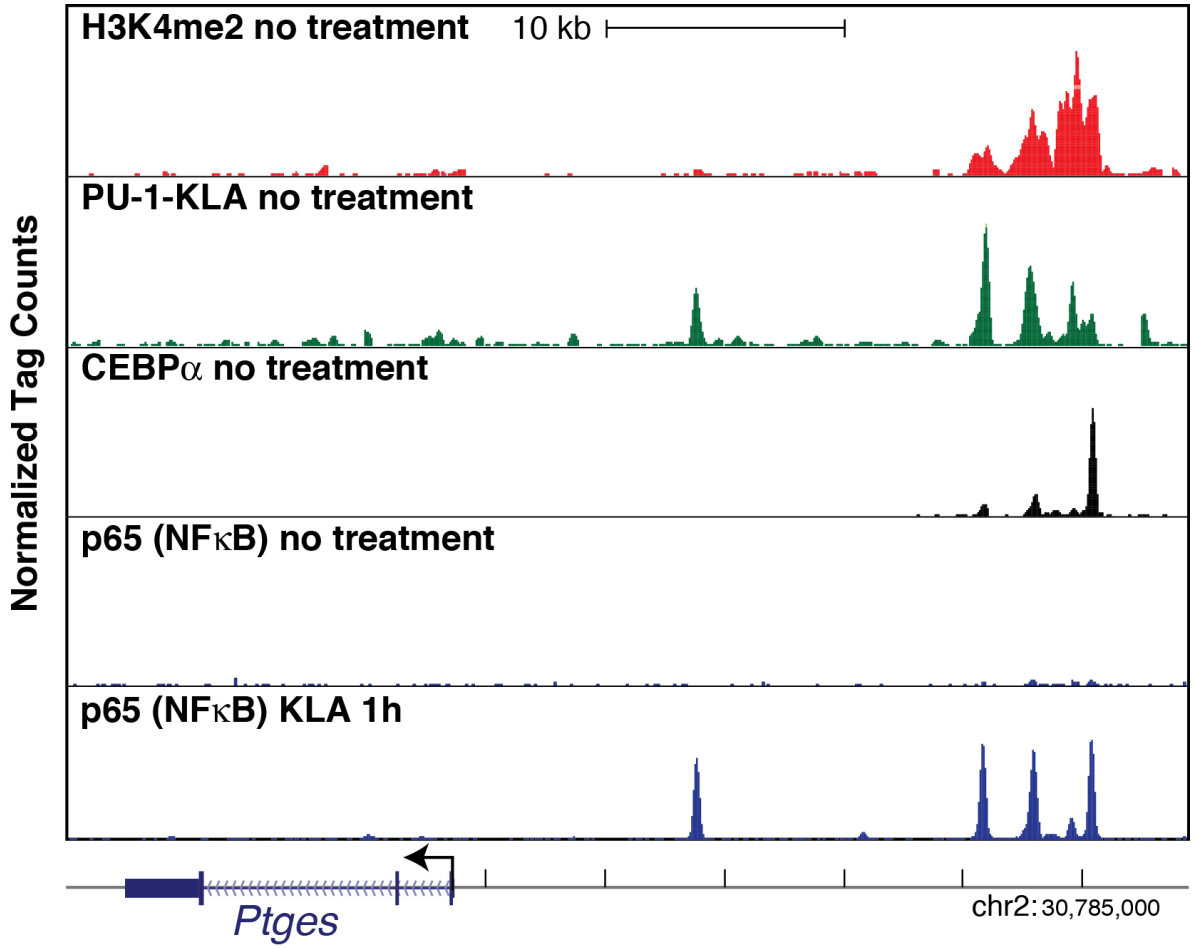
Sven Heinz, Chris Benner, Mol Cell. 2010;38:576-89

Selecting macrophage and B cell-specific enhancers



Sven Heinz, Chris Benner, Mol Cell. 2010;38:576-89

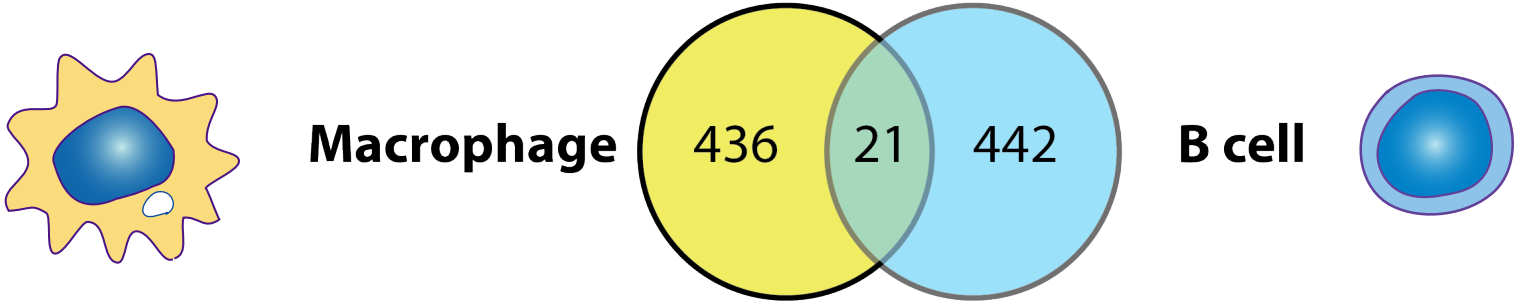
Binding of p65 (NFkB) to poised enhancer like regions near *Ptges*



Kaikkonen et al., Mol Cell 2013

Transcriptional responses to the same signal can be cell type-specific

mRNAs increased > 4-fold 1h after TLR4 ligation



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Nathan Spann and Cody Diehl

A collaborative – hierarchical model for enhancer selection and activation

Cell Fate-determining factors (i.e, lineage-determining TFs)

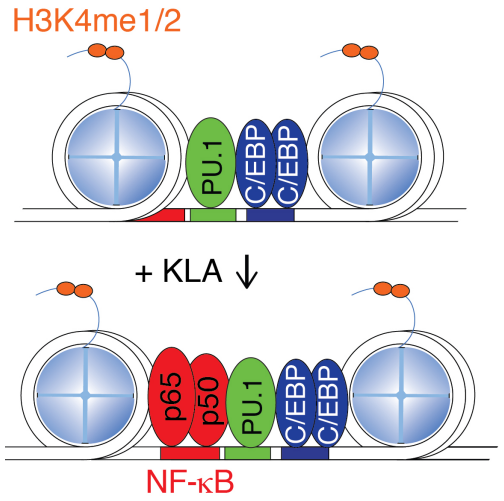
- Expressed in cell-restricted combinations
- Collaborate with each other and additional factors to bind to DNA and initiate nucleosome remodeling
- Establish cellular identity and exhibit reprogramming potential

Cell State-determining factors (i.e., Signal-dependent TFs)

- Broadly expressed
- Primarily localize to pre-existing 'primed'-enhancers
- Confer responsiveness to internal and external signals

Effects of natural genetic variation support a collaborative/hierarchical model

C57BL/6

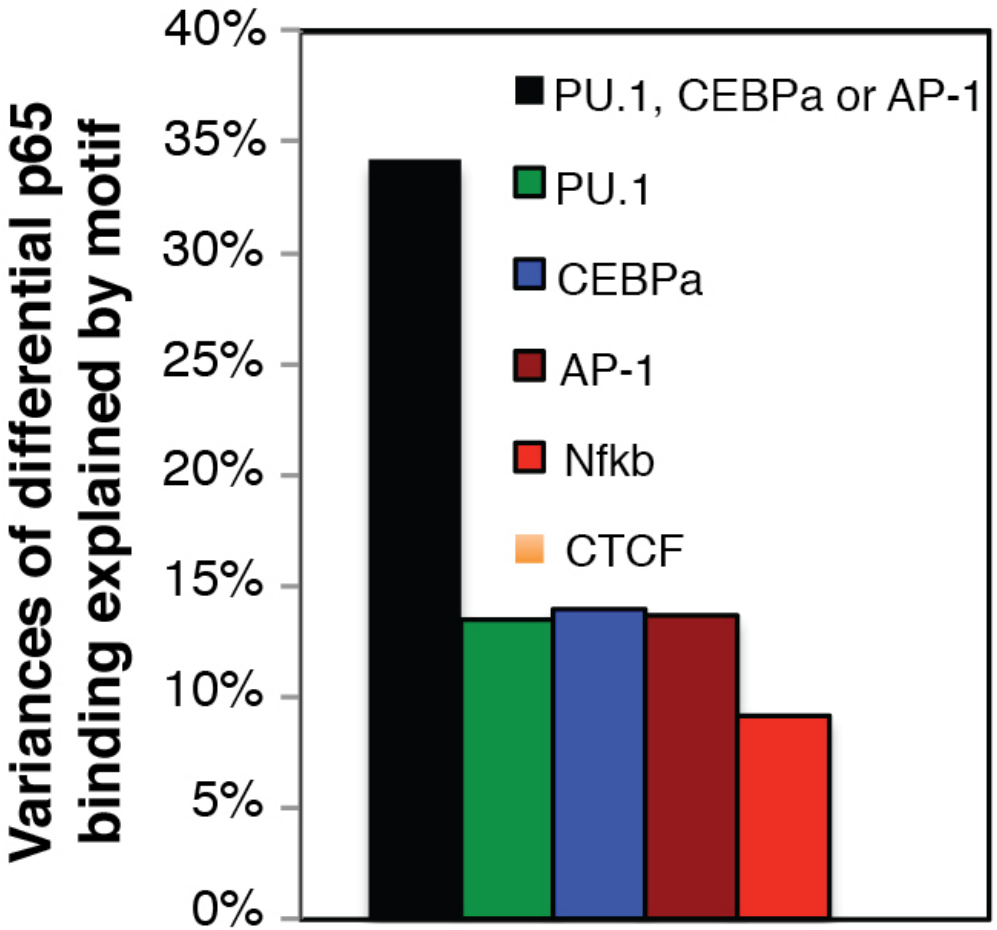


BALBc ~4m SNPS

- Mutations in PU.1 motifs reduce nearby binding of C/EBP
- Mutations in C/EBP motifs reduce nearby binding of PU.1
- Mutations in NFκB motifs rarely reduce binding of nearby PU.1 or C/EBP
- Mutations in PU.1 or C/EBP motifs frequently reduce binding of NFκB

Casey Romanoski, Sven Heinz; Nature 2013;503:487-92

Effect of motif mutations on p65 binding



Casey Romanoski, Sven Heinz; Nature 2013;503:487-92

A collaborative – hierarchical model for enhancer selection and activation

- Supported by gain and loss of function experiments and effects of mutations in transcription factor binding sites (e.g., Heinz et al., Mol Cell, 2010, Heinz et al., Nature 2013, Kaikkonen et al., Mol. Cell, 2013)

- Model is vastly oversimplified and has poor predictive power

- Fails to explain how new enhancers are selected

- Fails to account for functions of the majority of transcription factors expressed in macrophages

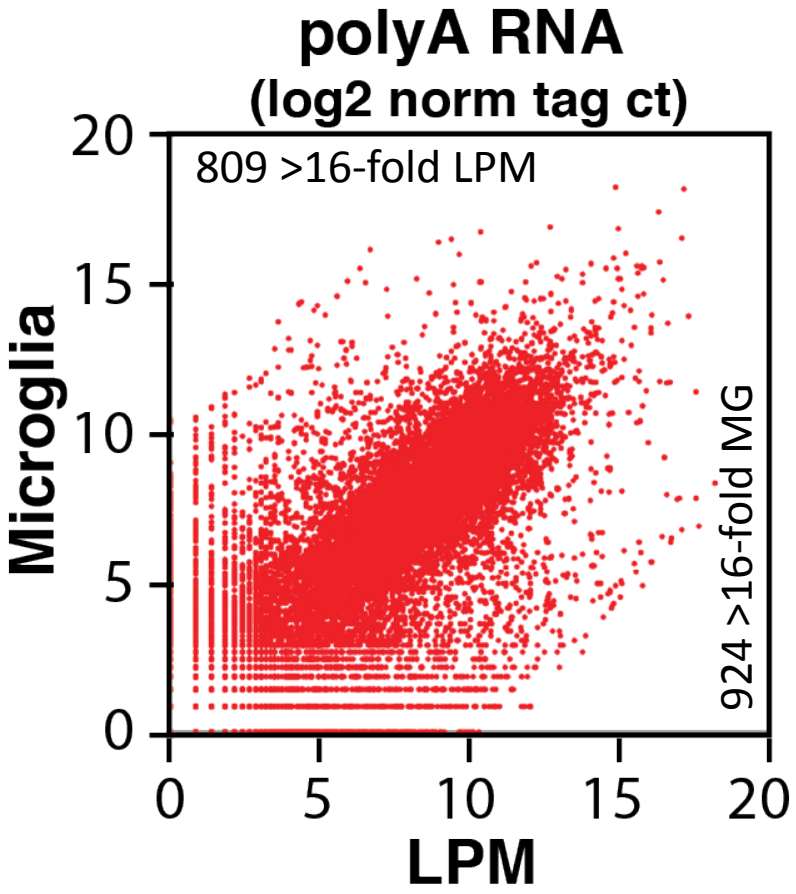
- Relevance to in vivo populations of macrophages is unclear

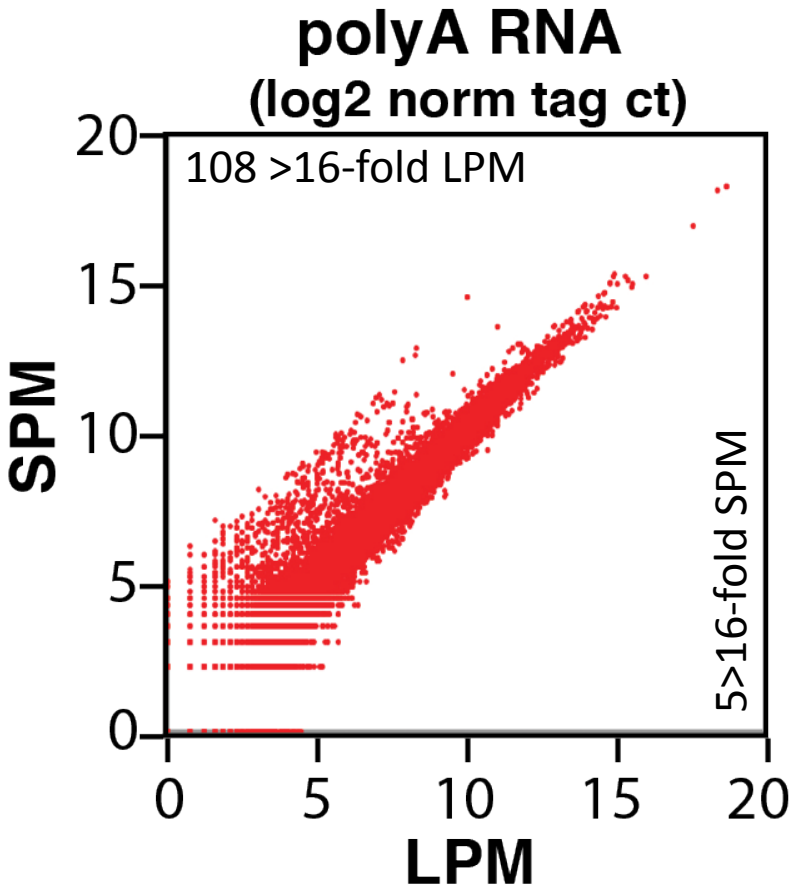
A comparison of tissue resident macrophage subsets

Cell type	Environment
Microglia (MG)	Brain/TGFβ
Large perit. MΦ (LPM - MHCII^{low})	Peritoneum RA
Small perit. MΦ (SPM - MHCII^{med})	

Gosselin, Link, Romanoski, et al., Cell 159:1327, 2014

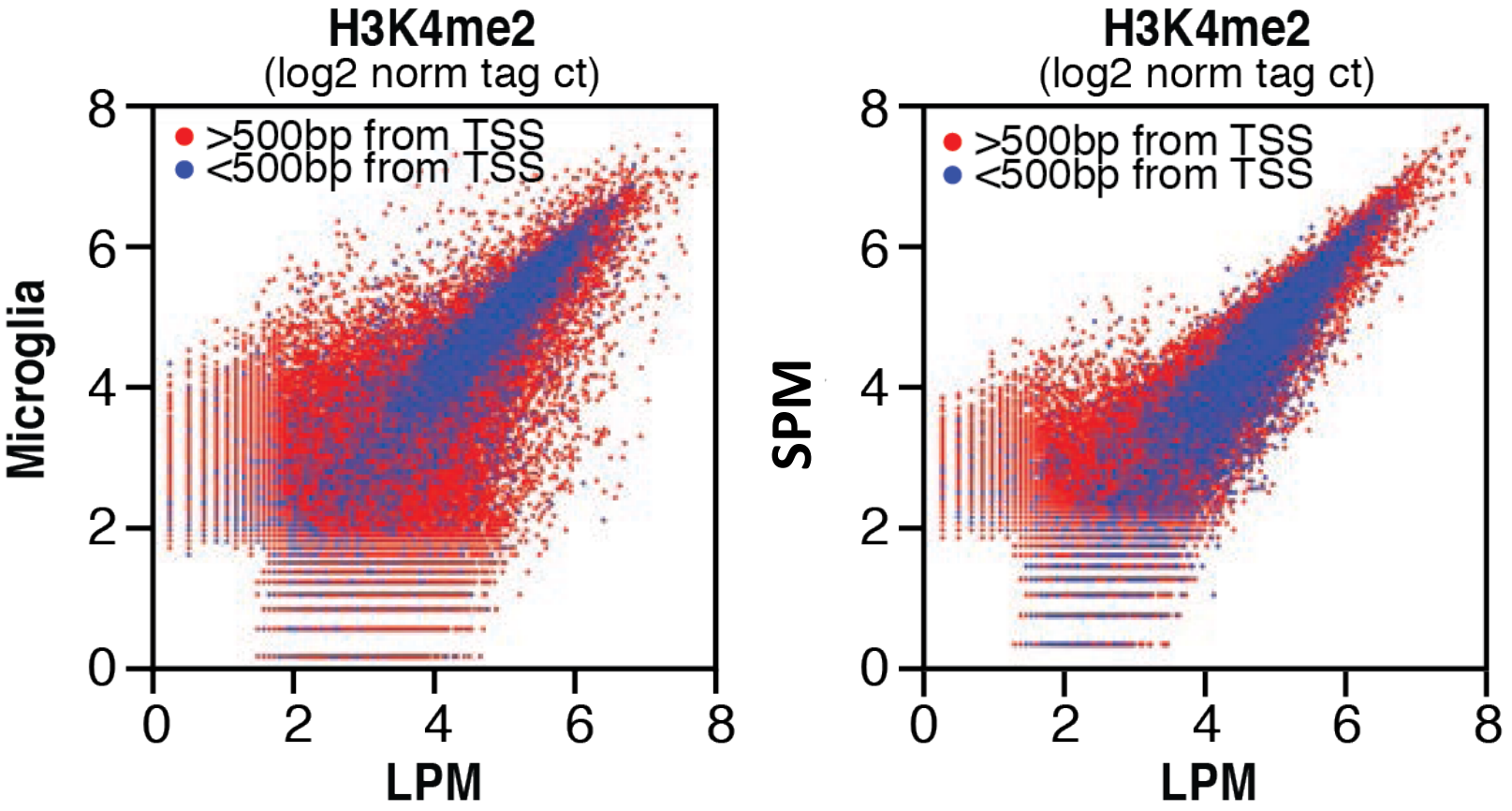
Macrophage transcriptomes can be highly divergent dependent on tissue of residence





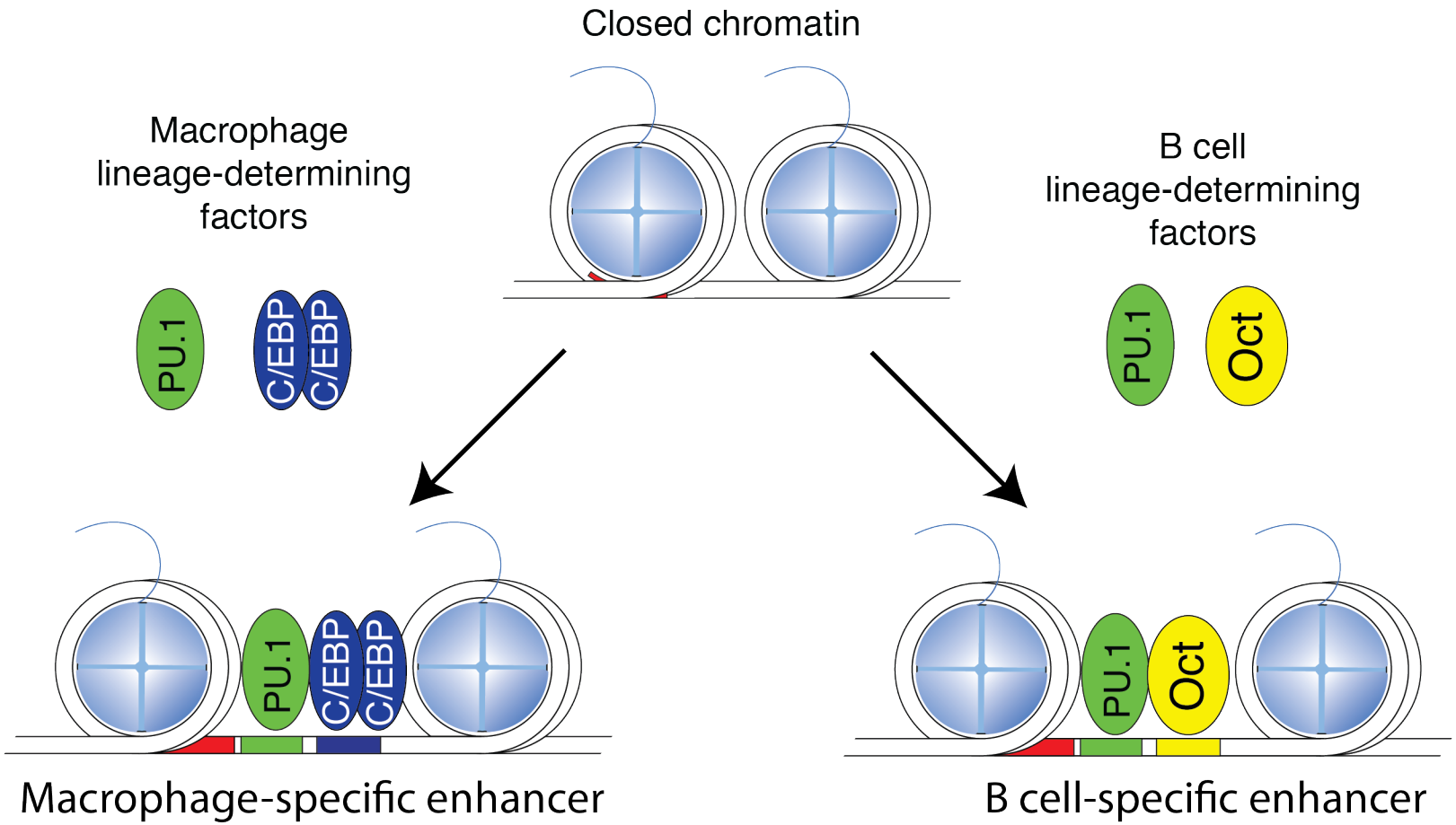
Gosselin, Link, Romanoski, et al., Cell 2014

Macrophage enhancer landscapes differ depending on their source



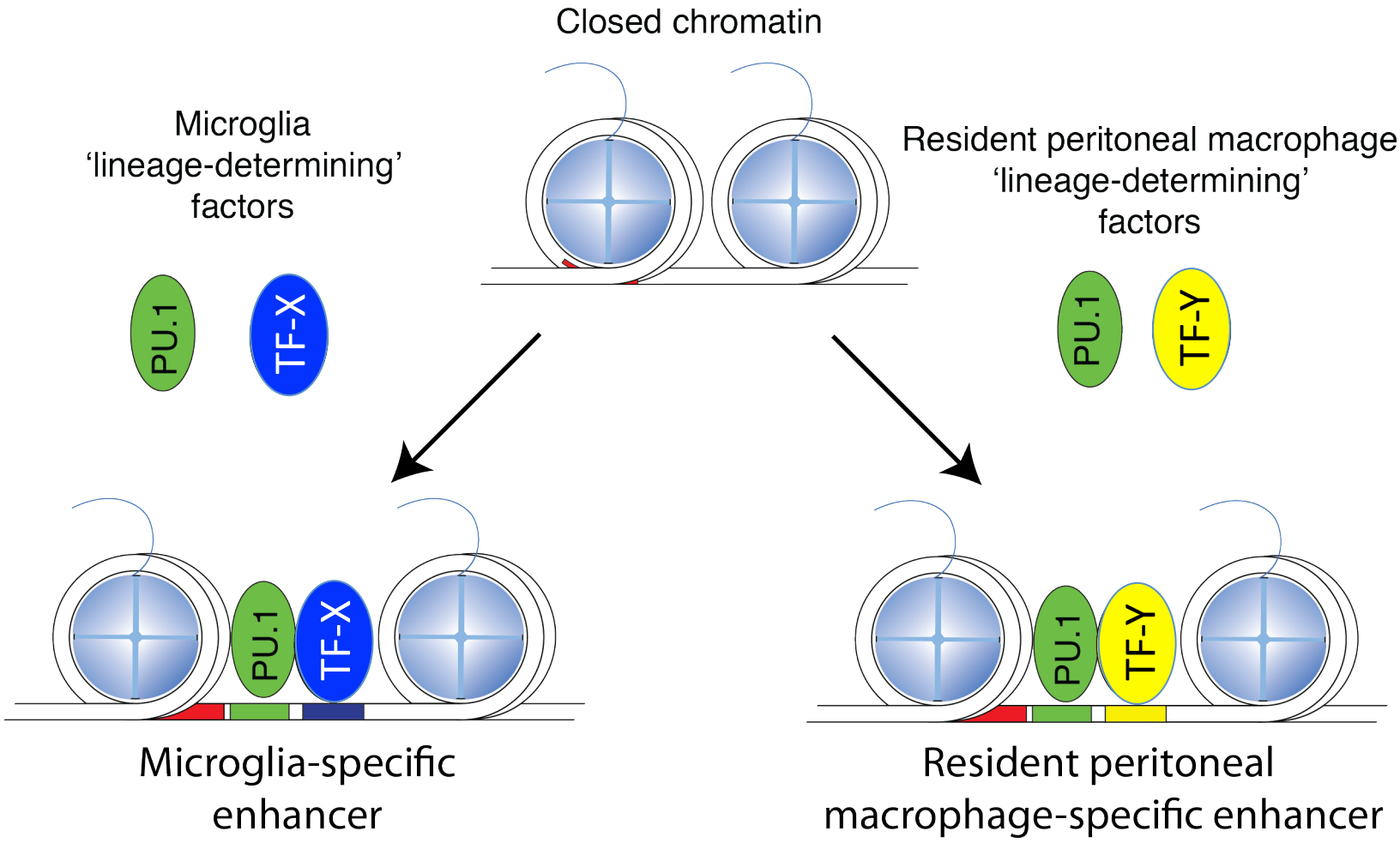
Gosselin, Link, Romanoski, et al., Cell 159:1327, 2014

Selecting macrophage and B cell-specific enhancers



Sven Heinz, Chris Benner, Mol Cell. 2010;38:576-89

Selecting microglia- and RPM-specific enhancers



What TFs collaborate with PU.1 to select microglia and RPM-specific enhancers?

- Strategy :
  - Use the vast natural genetic variation provided by inbred strains of mice as an in vivo 'mutagenesis screen'

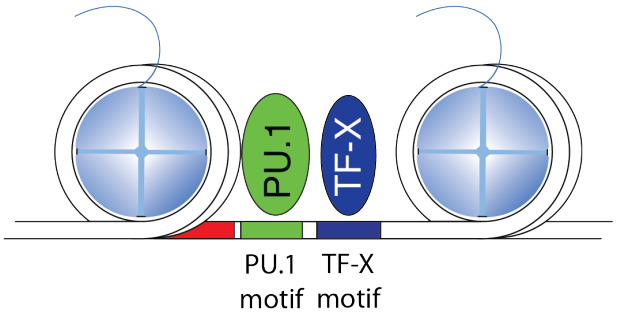
Inbred Strain	SNPs relative to C57BL/6J
BALB/cJ	~4 million
NOD/ShiLt/J	~5 million
SPRET/EiJ	~40 million

Use of natural genetic variation to identify motifs for collaborative TFs

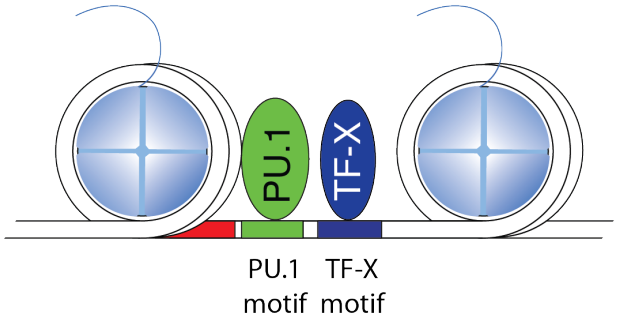
Transcription Factor X is a collaborative partner for PU.1 at a subset of PU.1 binding sites in the genome

Most genomic locations

Strain 1

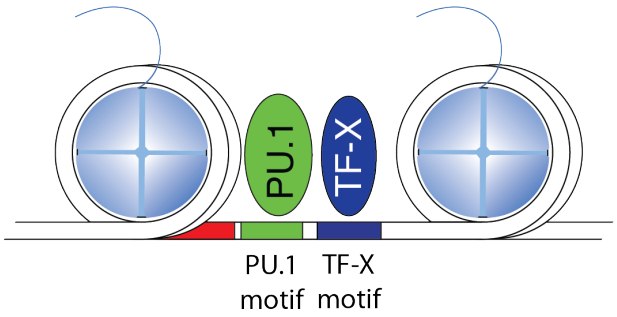


Strain 2

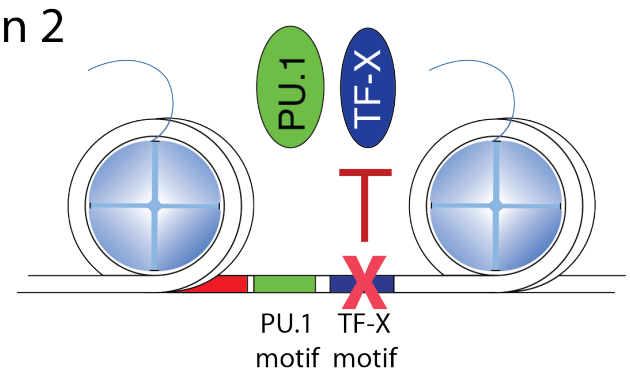


Informative genomic locations

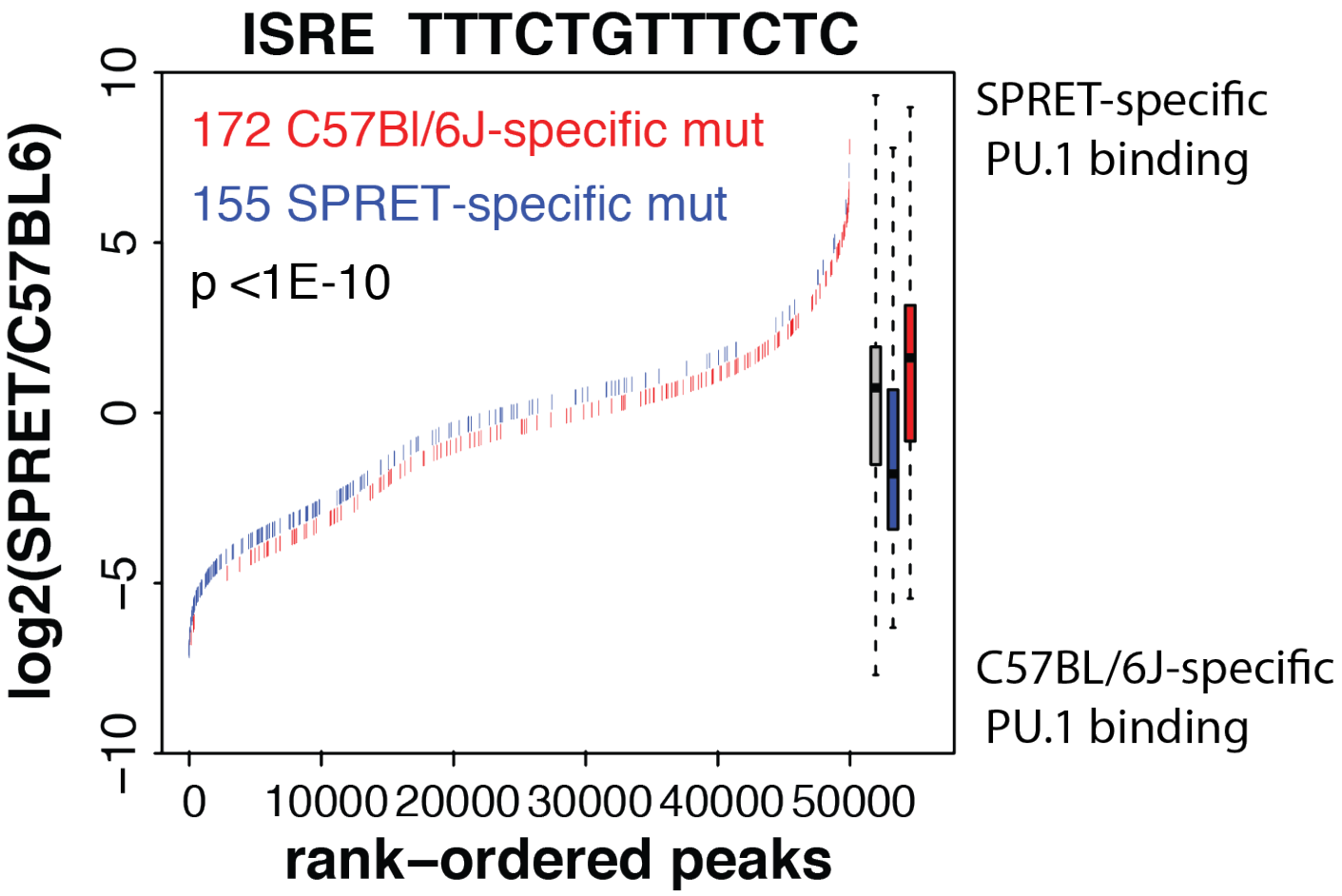
Strain 1



Strain 2

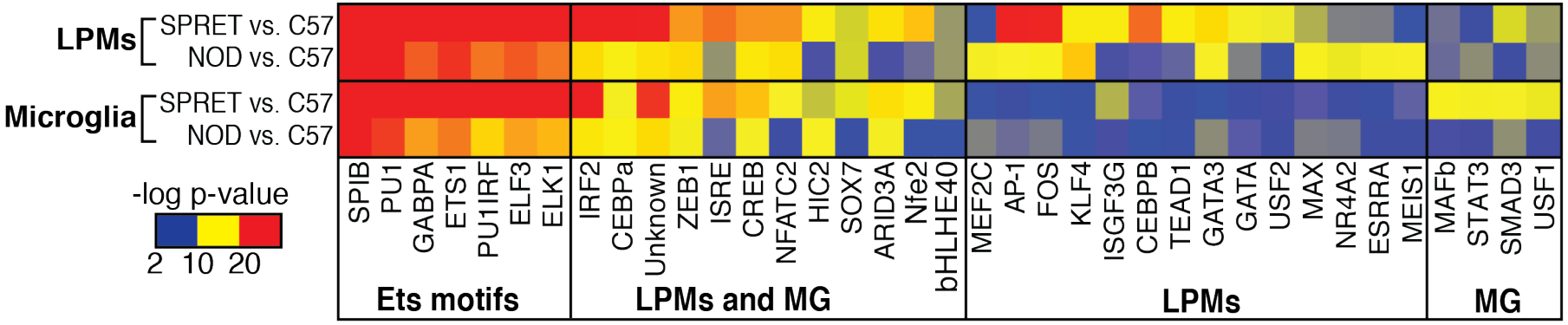


Mutations in nearby ISRE motifs are highly correlated with strain-specific PU.1 binding



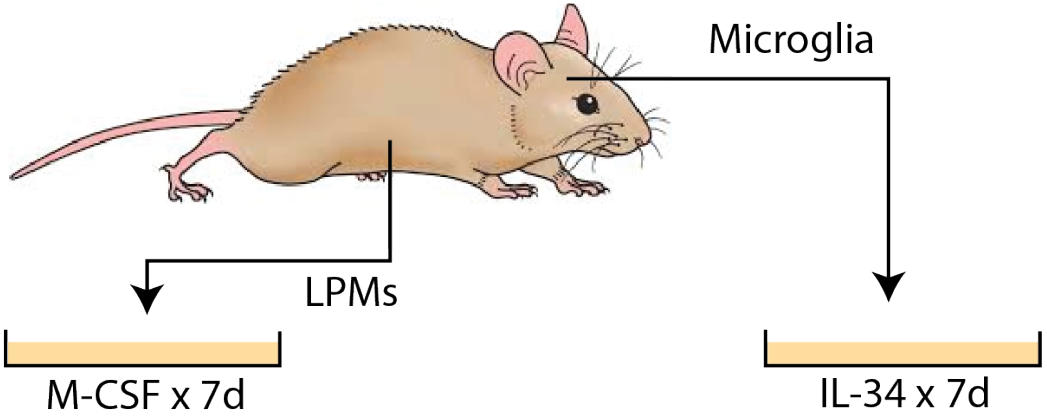
Gosselin, Link, Romanoski, et al., Cell 2014

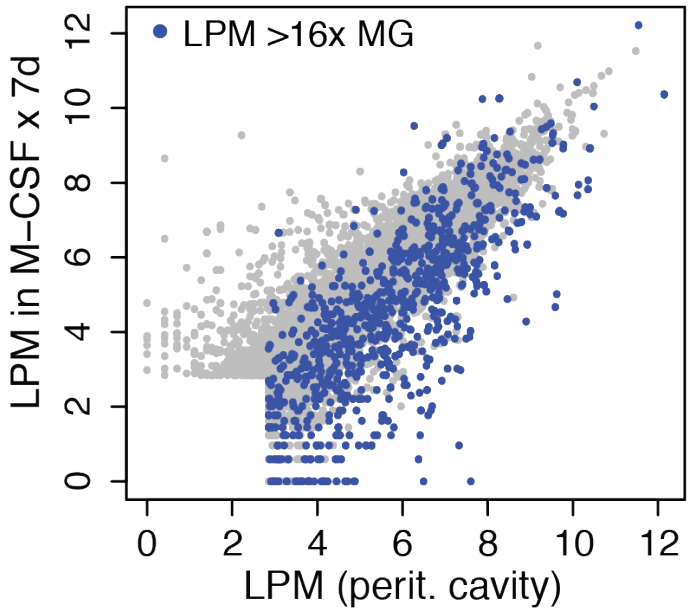
Discovery of TF motifs associated with common and subset-specific binding of PU.1

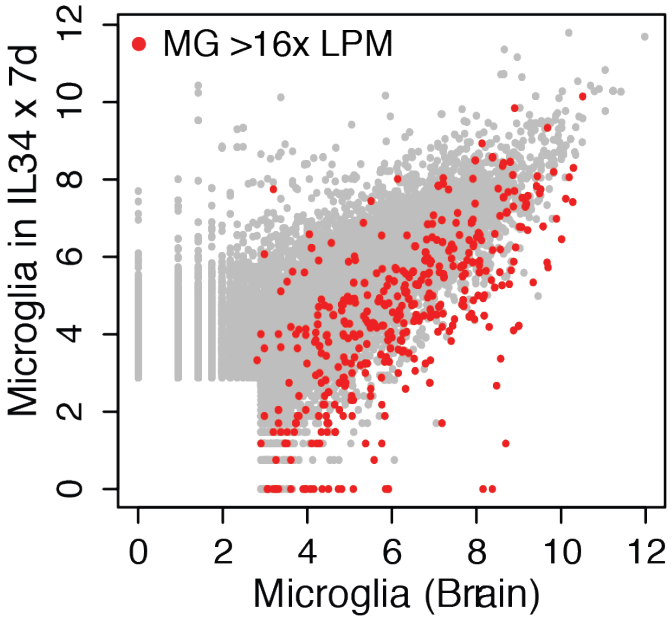


Gosselin, Link, Romanoski, et al., Cell 2014

Macrophage identities require constant environmental signals

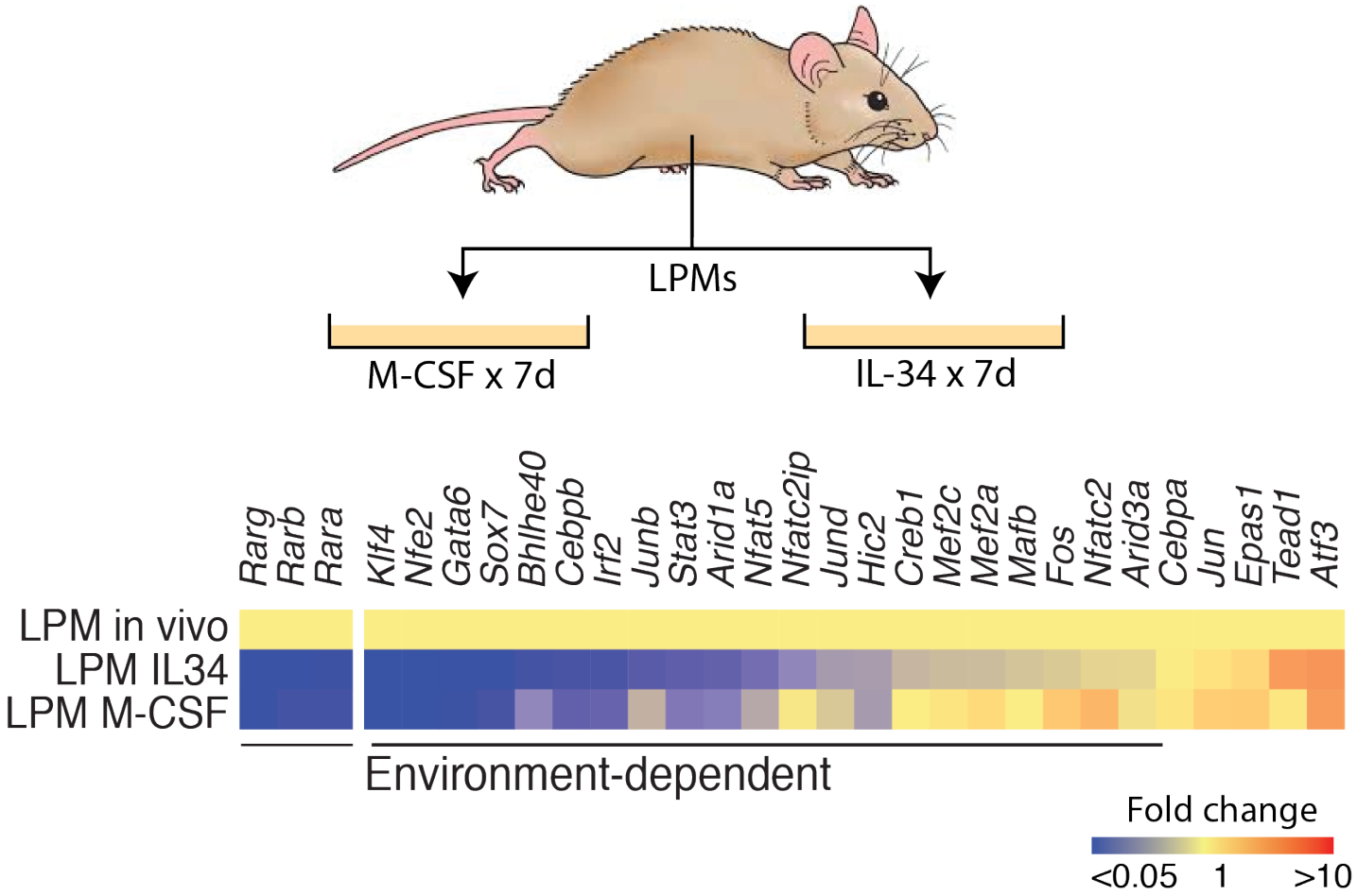






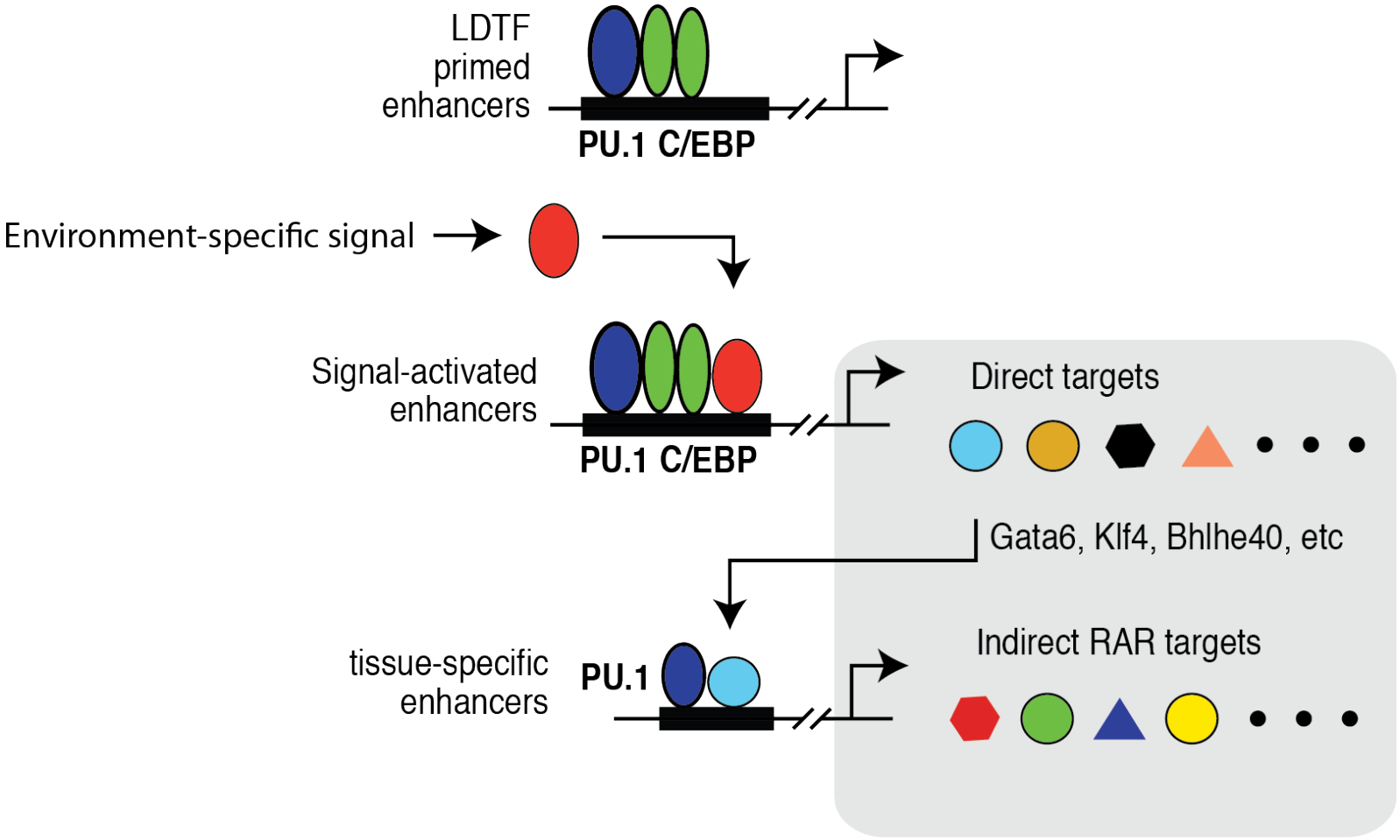
Gosselin, Link, Romanoski, et al., Cell 2014

Environment controls expression of TFs that collaborate with PU.1



Gosselin, Link, Romanoski, et al., Cell 2014

Environment drives selection and function of enhancers controlling macrophage identities

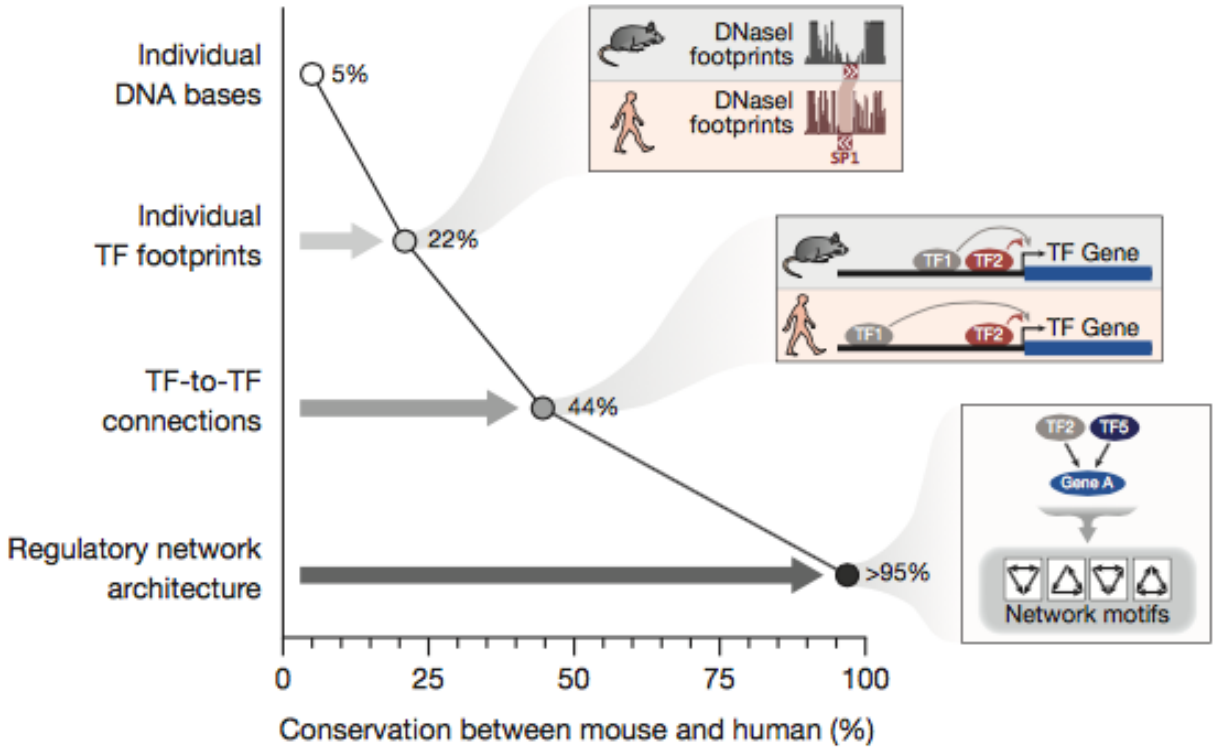


Gosselin, Link, Romanoski, et al., Cell 2014

ARTICLE

OPEN
doi:10.1038/nature13972

Conservation of trans-acting circuitry during mammalian regulatory evolution



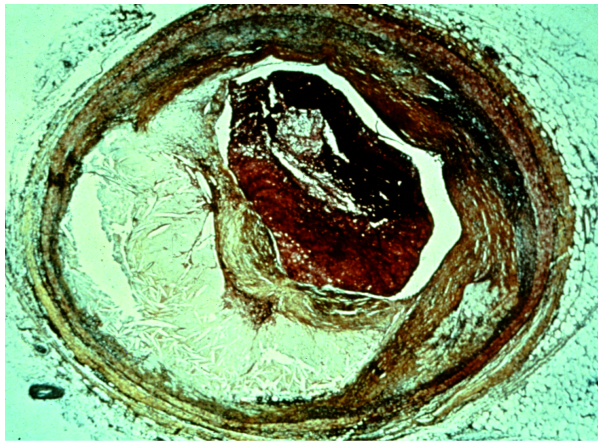
Stergachis et al., Nature 2014

Some take home points

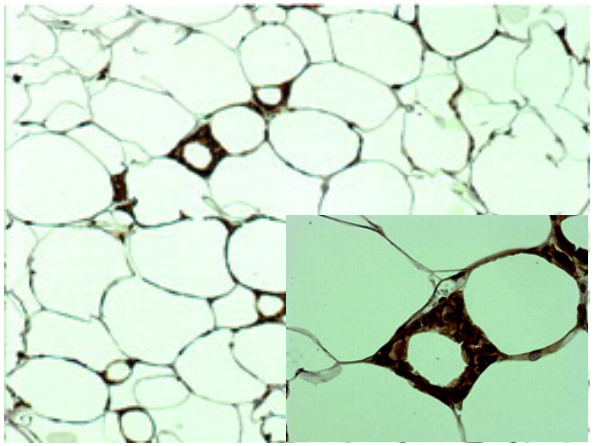
- Knowledge of the enhancer landscape of a cell reveals much about that cell's identity and regulatory potential
- Enhancer landscapes enable prediction of key lineage determining transcription factors and sites of action of signal-dependent factors
- Transcription factor binding maps inform analysis of genetic variation
- Natural genetic variation can be exploited to discover regulatory networks that drive cell-specific gene expression

Understanding and modifying roles of macrophages in human disease

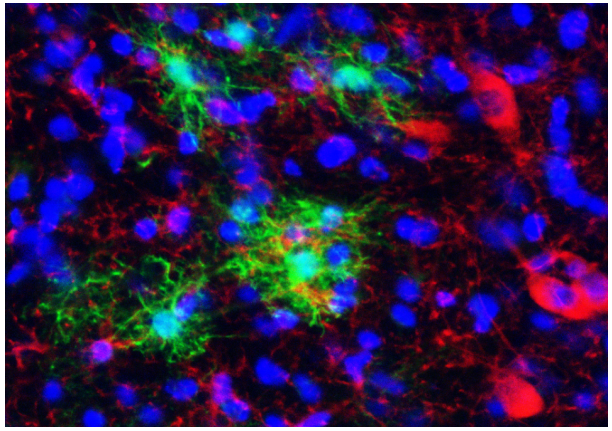
Macrophage foam cells in atherosclerosis



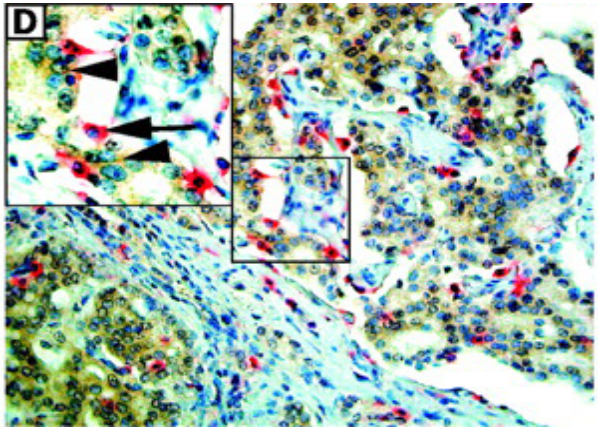
Adipose tissue macrophages in insulin resistance



Activated microglia in neurodegenerative disease



Tumor-associated macrophages in cancer



Going forward

- Improve methodology to define regulatory networks in specific cell types within complex tissues
- Determine effects of cell-autonomous and non-autonomous disease mechanisms on enhancer selection and function
- Consideration of regulatory networks as complex phenotypes for therapeutic modulation

Thanks!

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- Ty Troutman
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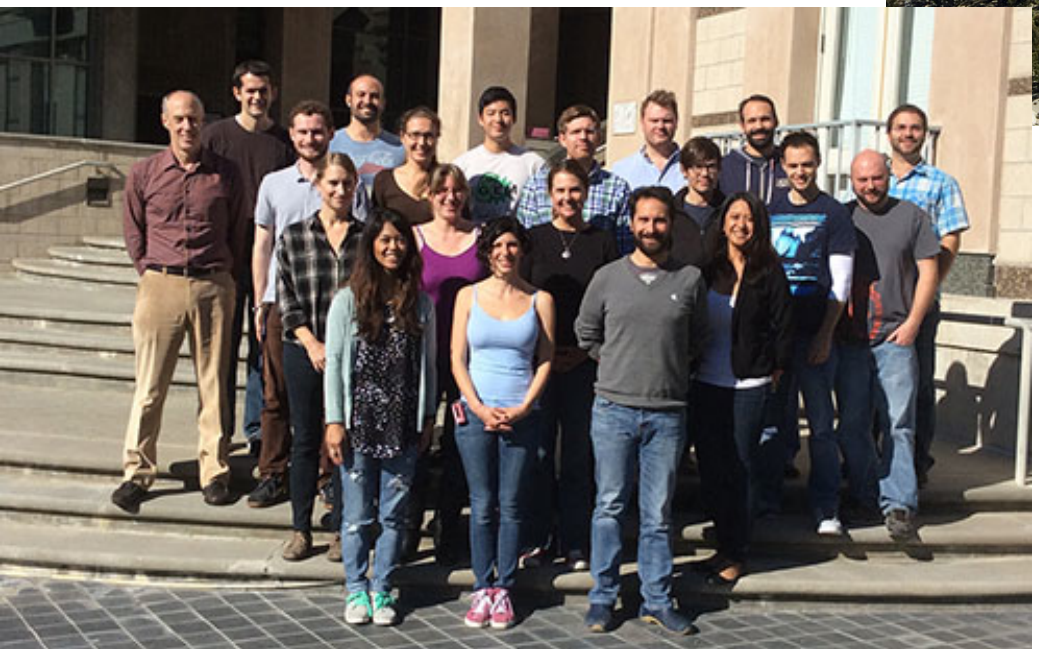

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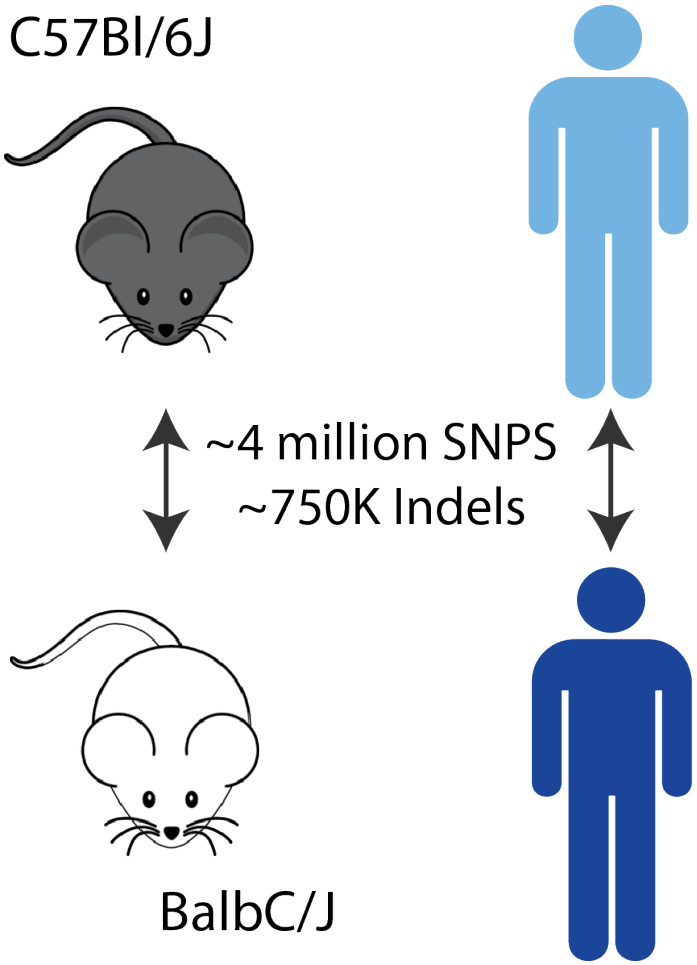
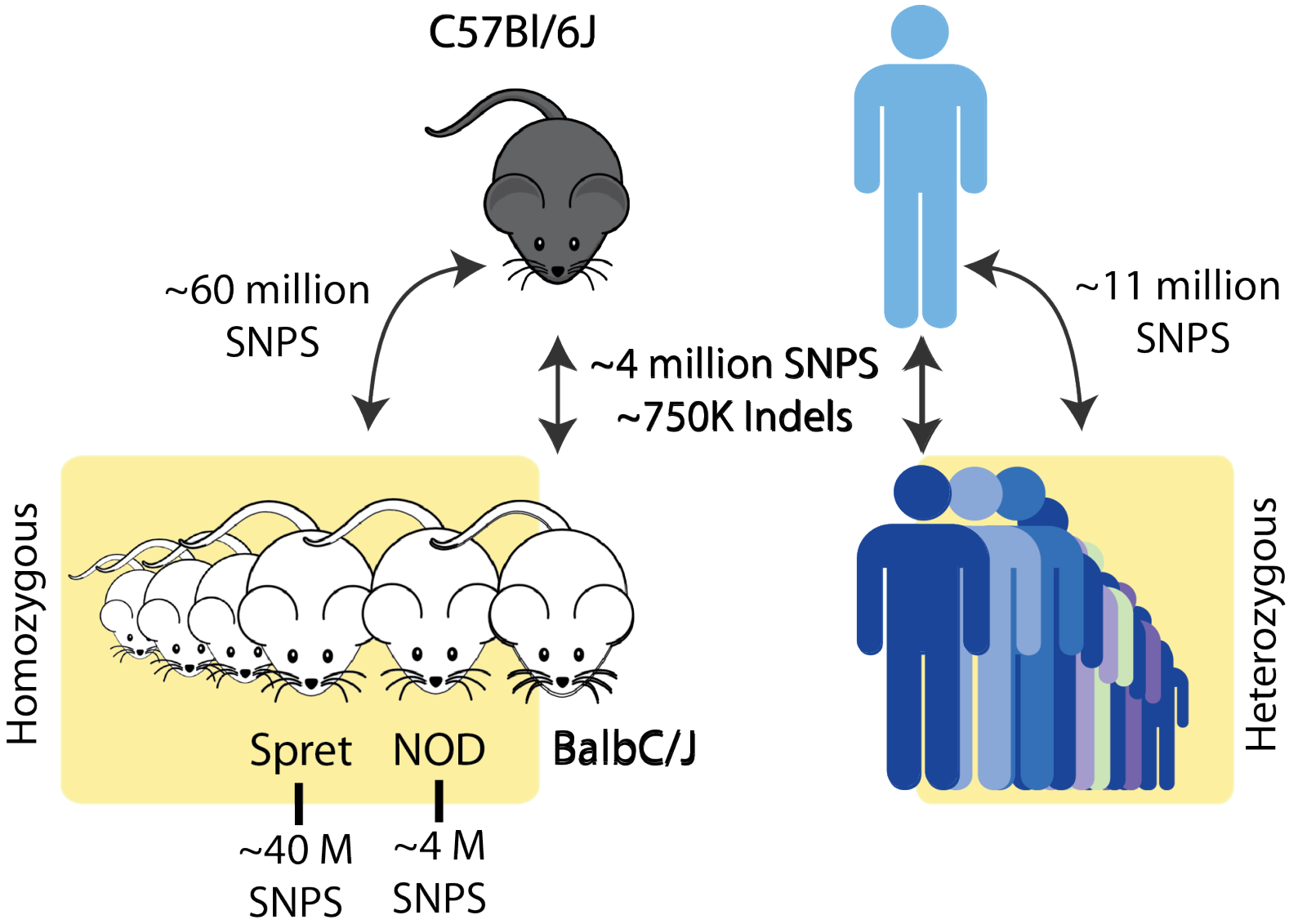
- Hanna Gardiner

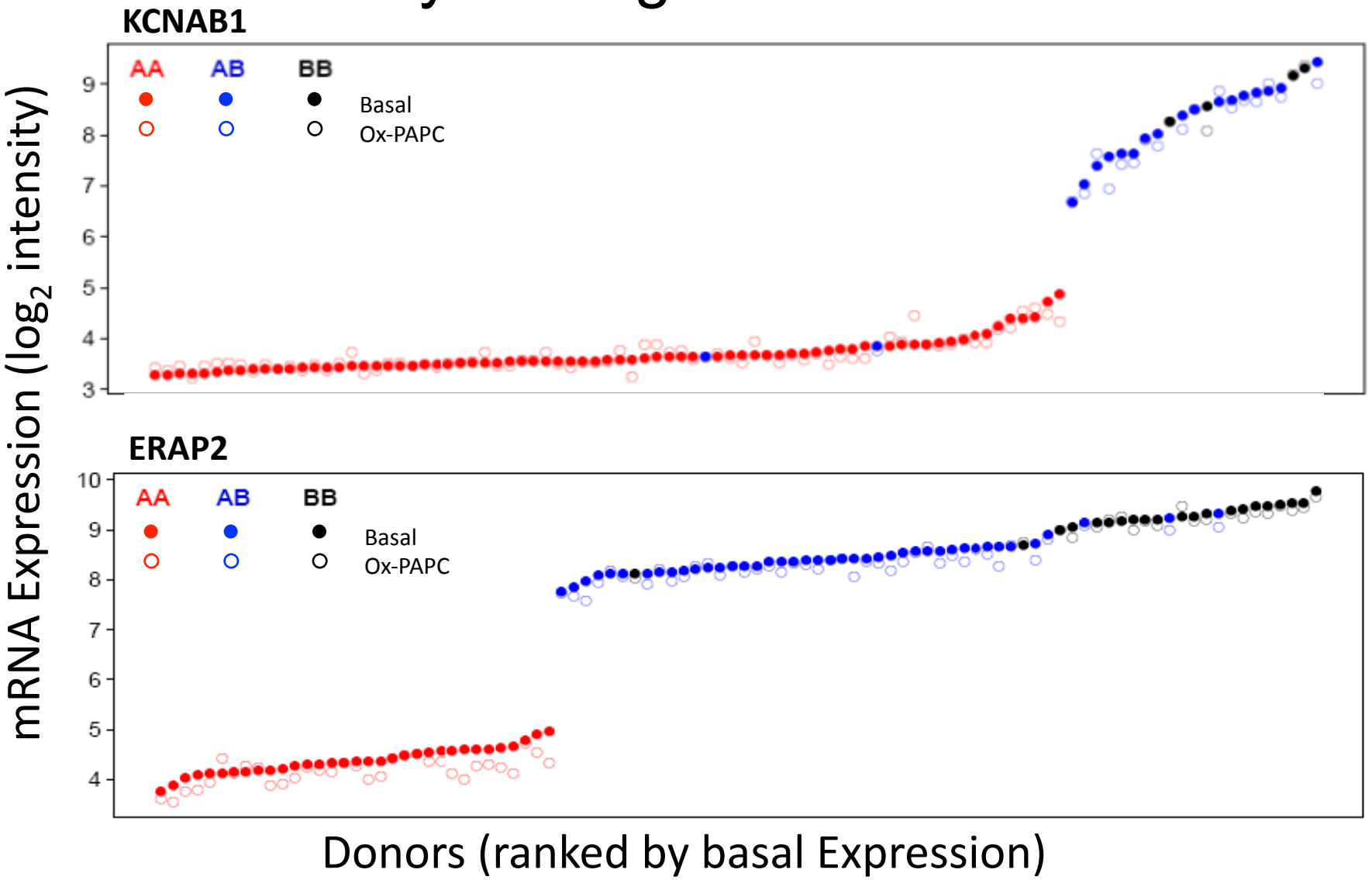
Exploiting natural genetic variation to understand enhancer selection and function



Exploiting natural genetic variation to understand enhancer selection and function



Gene expression in the same cell type can vary among individuals



Romanoski et. al., *Am J Hum Gen,* 2010