

Epigenetic Regulation of Rapid Recall Response of Memory CD4+ T-cells

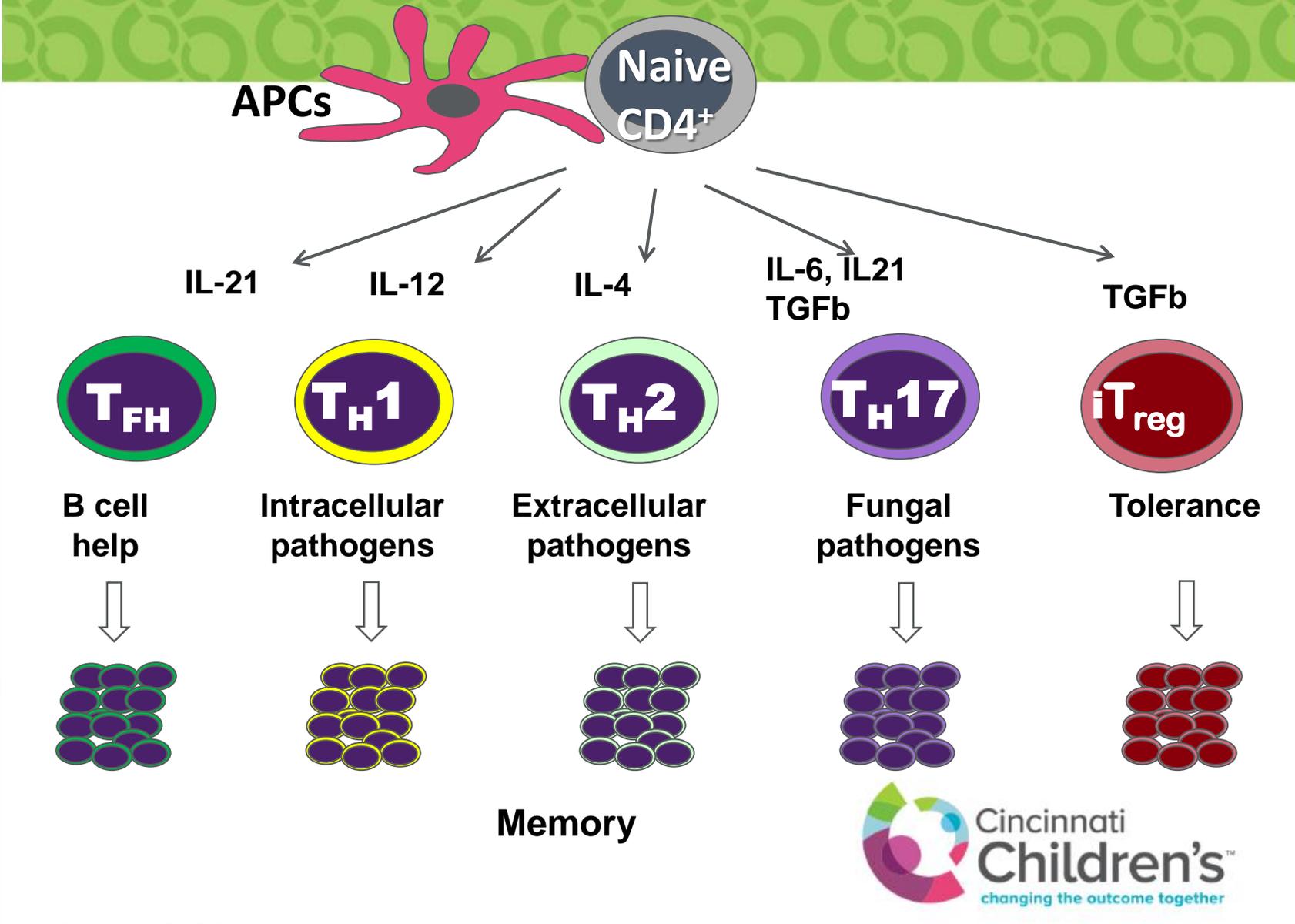
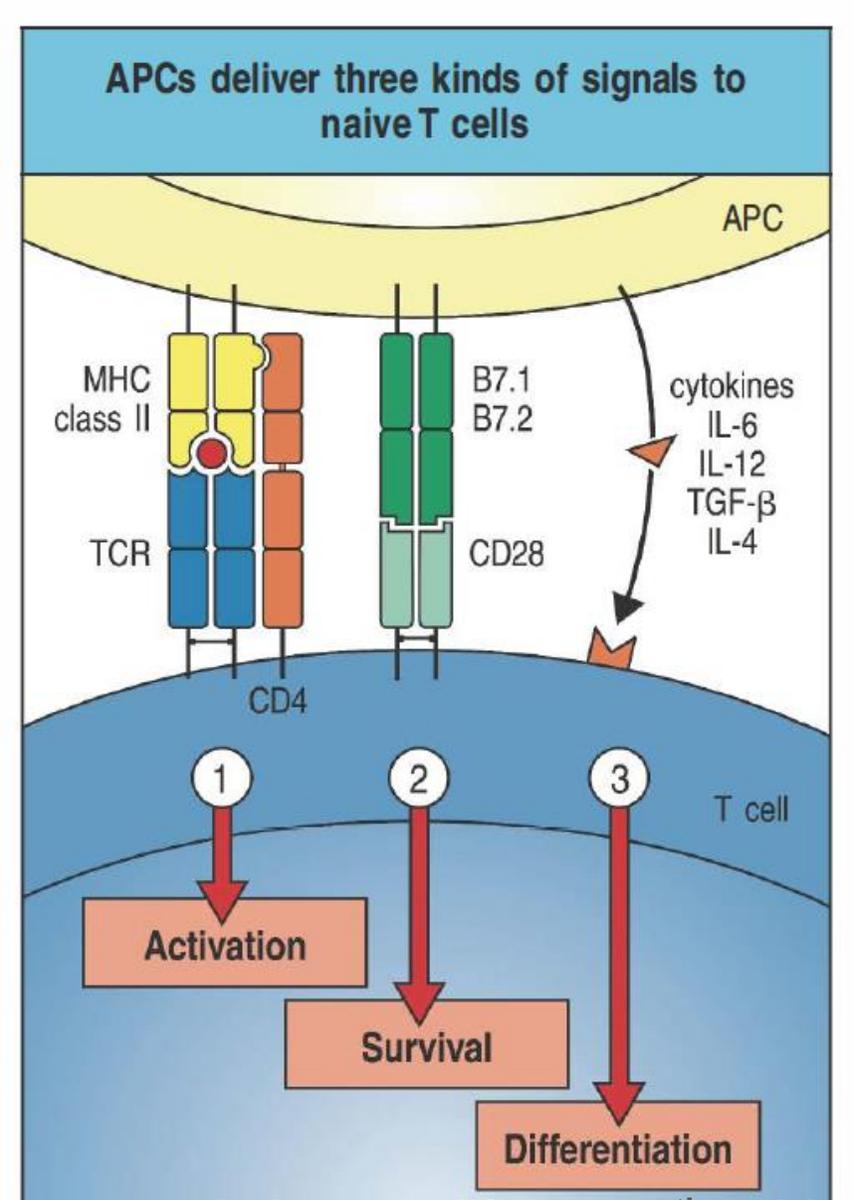
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August 16th, 2019



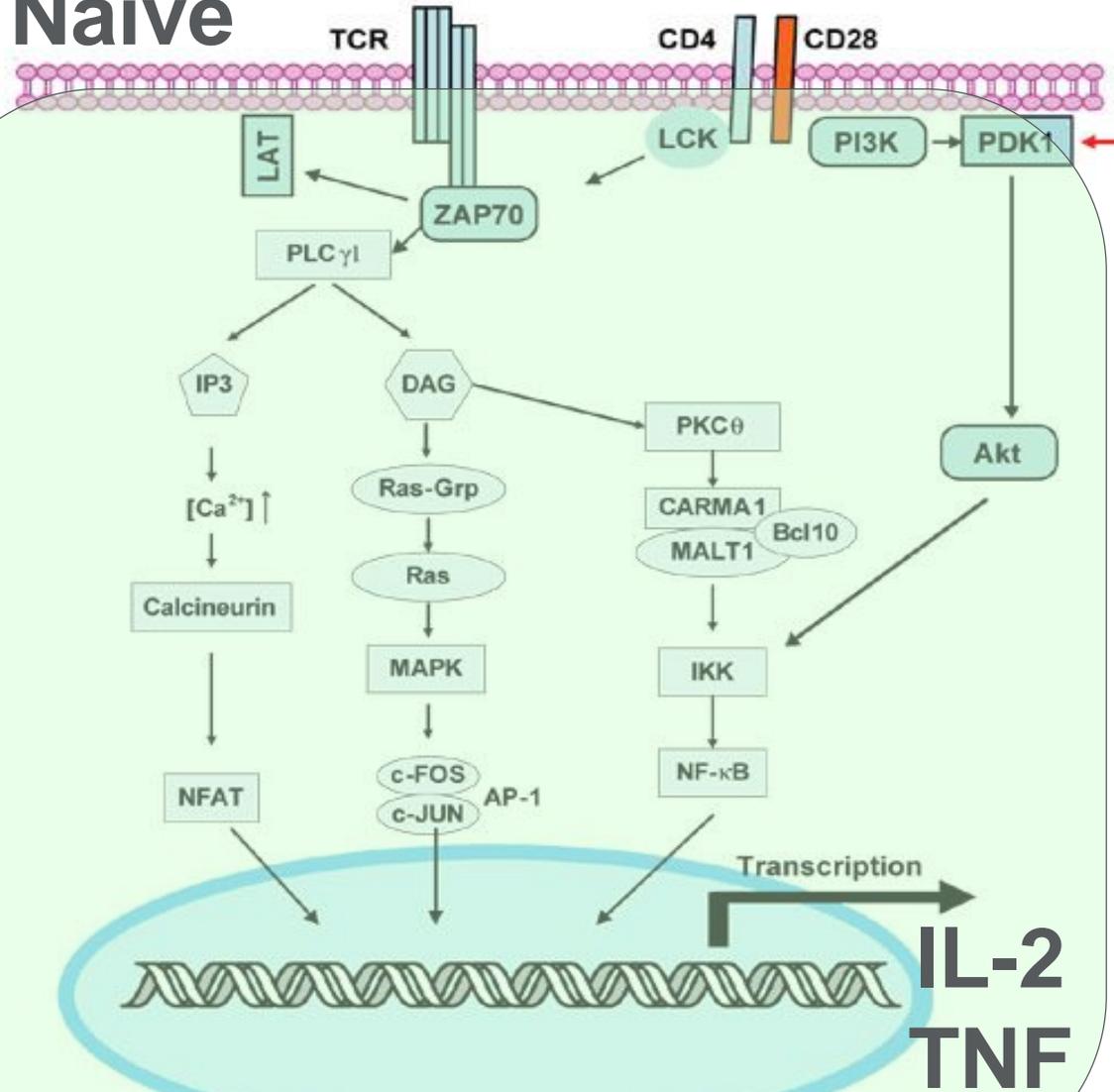
T-cell Response and CD4+ T-cell Differentiation



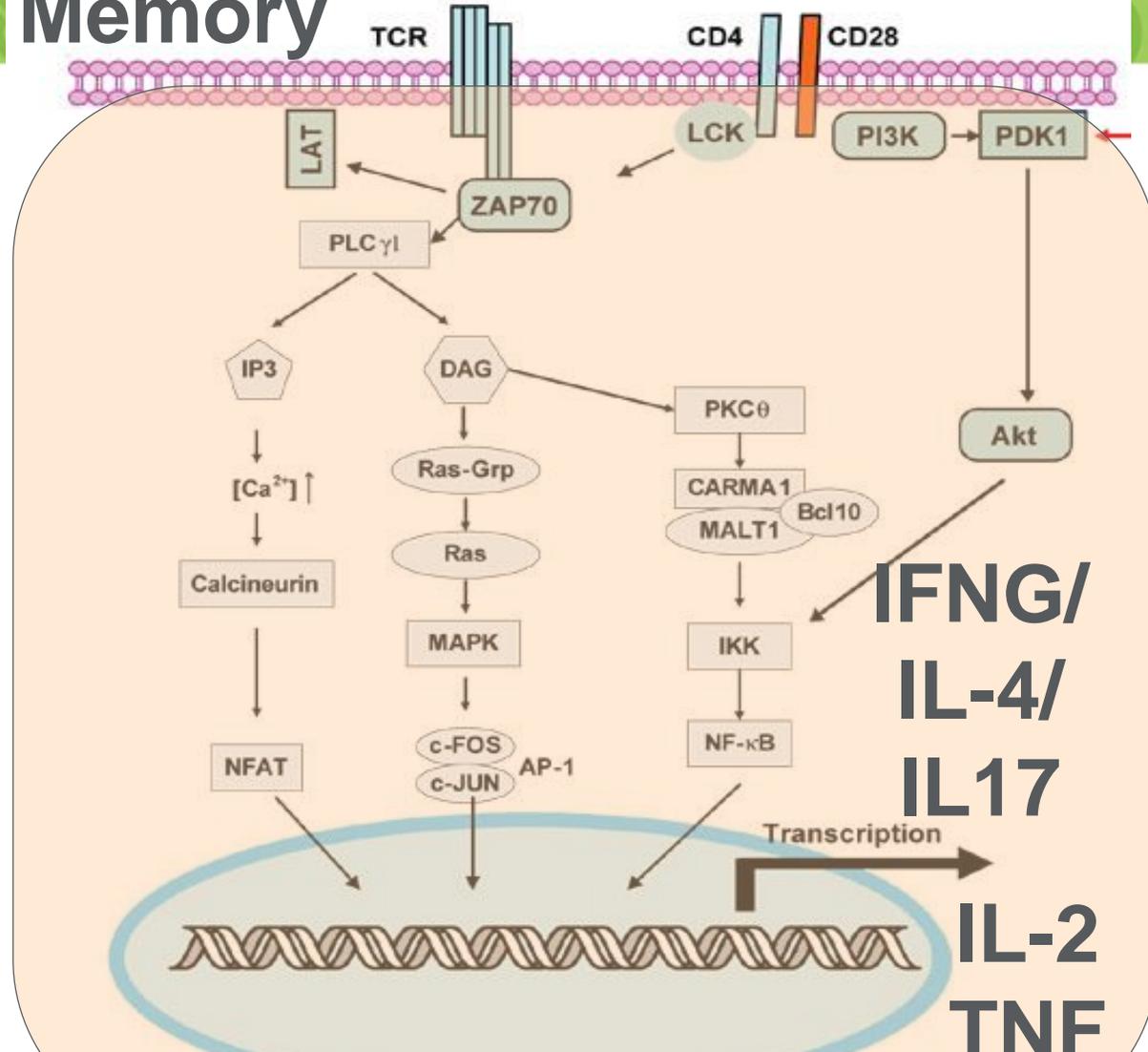
Janeway's Immunobiology, 9th edition, Figure 9.22

Memory cells respond differently to TCR/CD28 signal

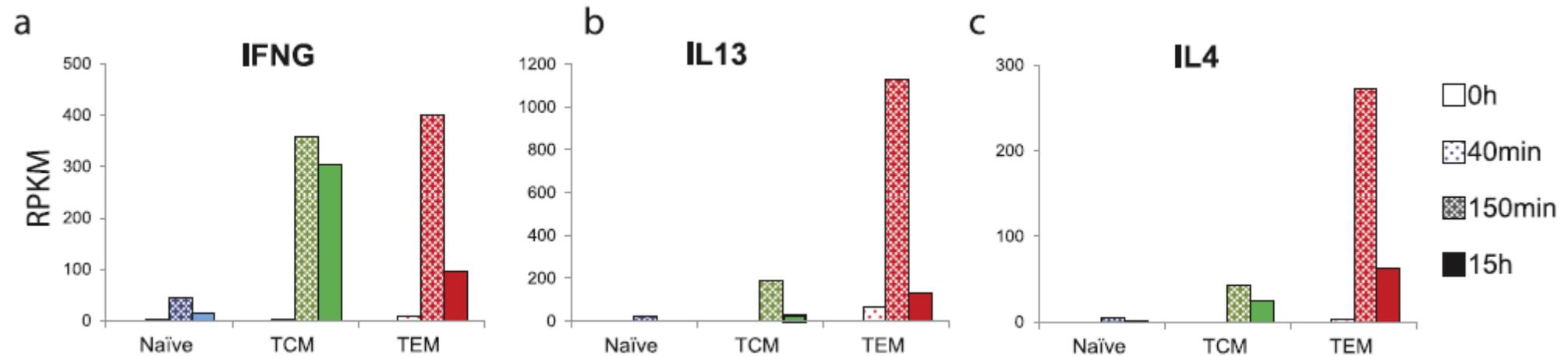
Naive



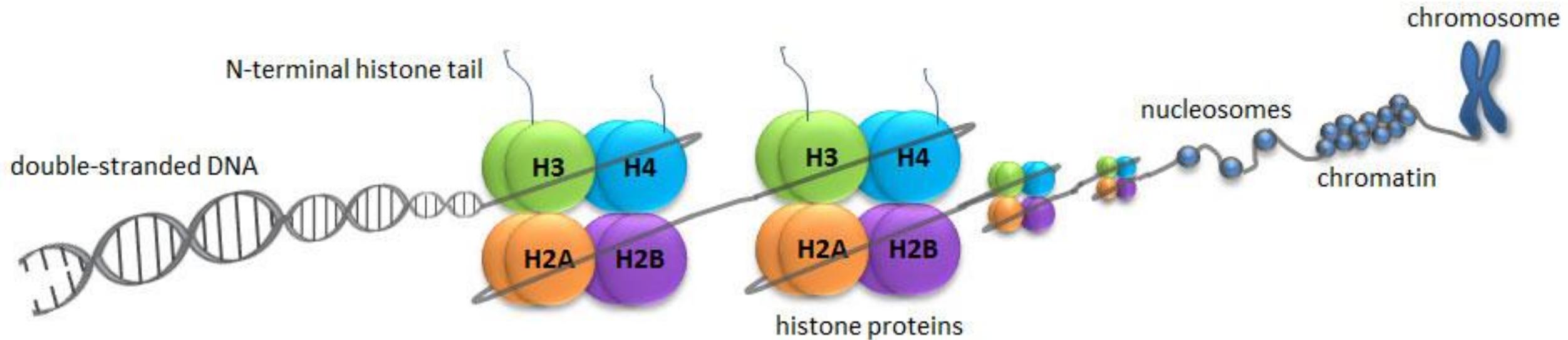
Memory



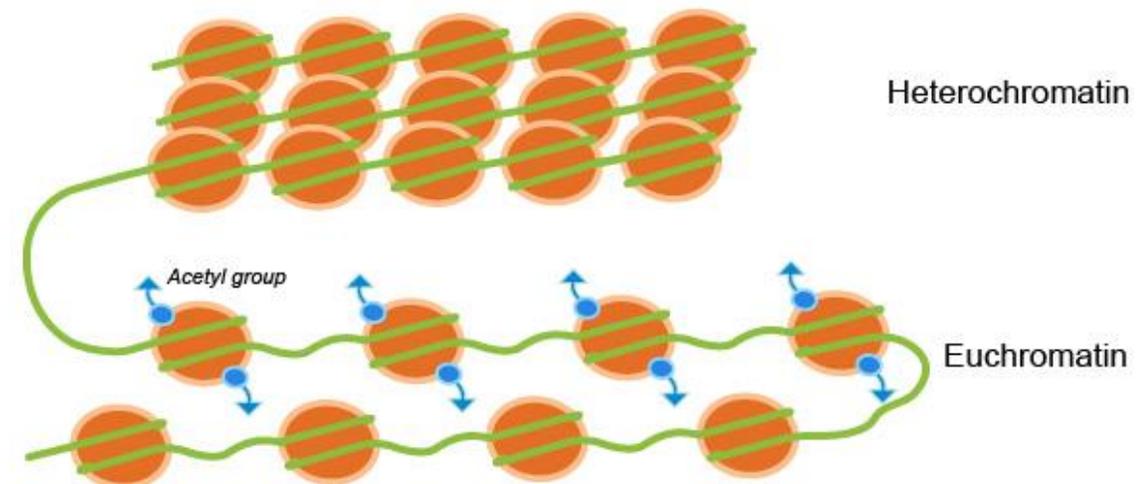
Cytokine gene mRNA transcript expression during T helper-cell activation



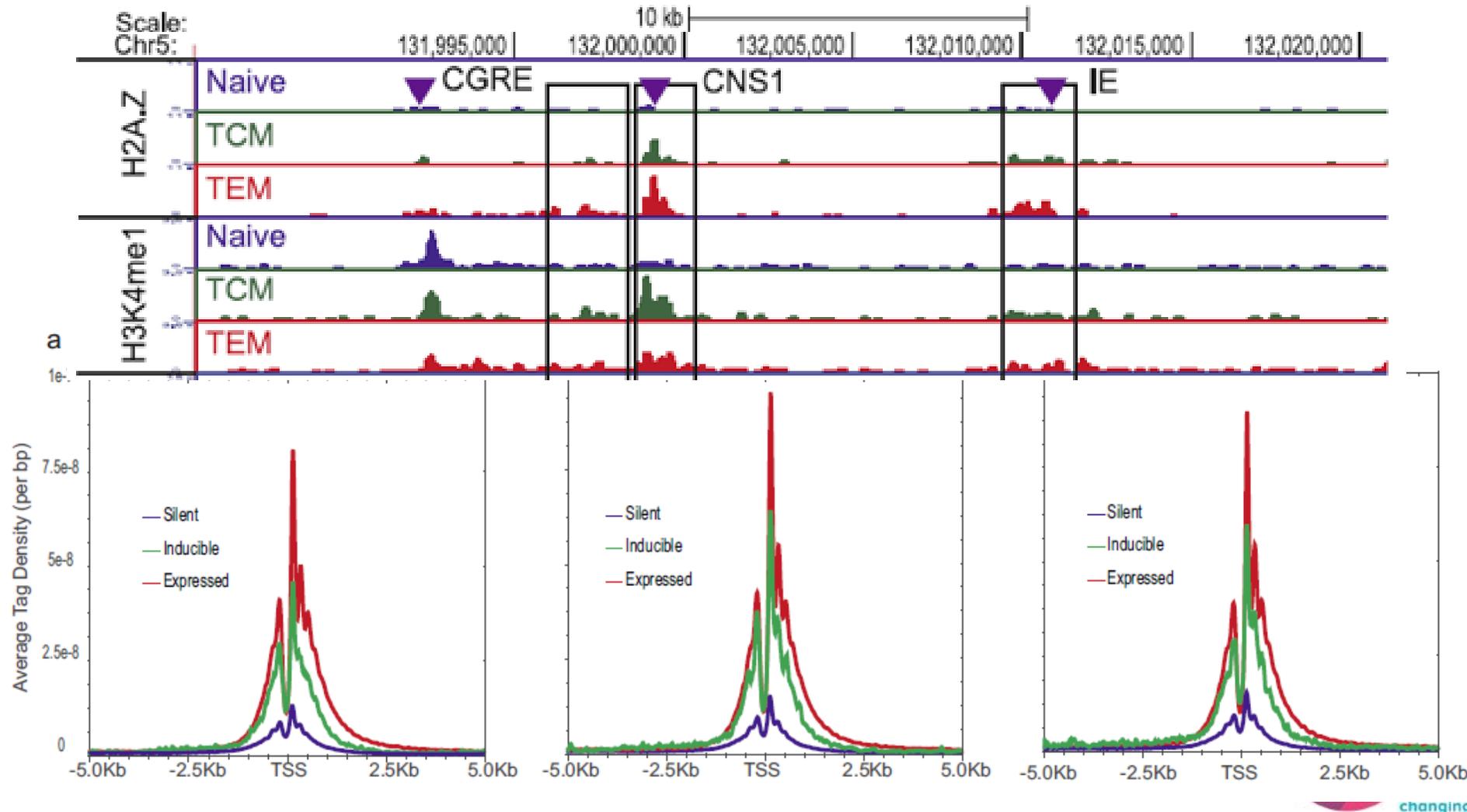
Intracellular DNA organization: histones and chromatin



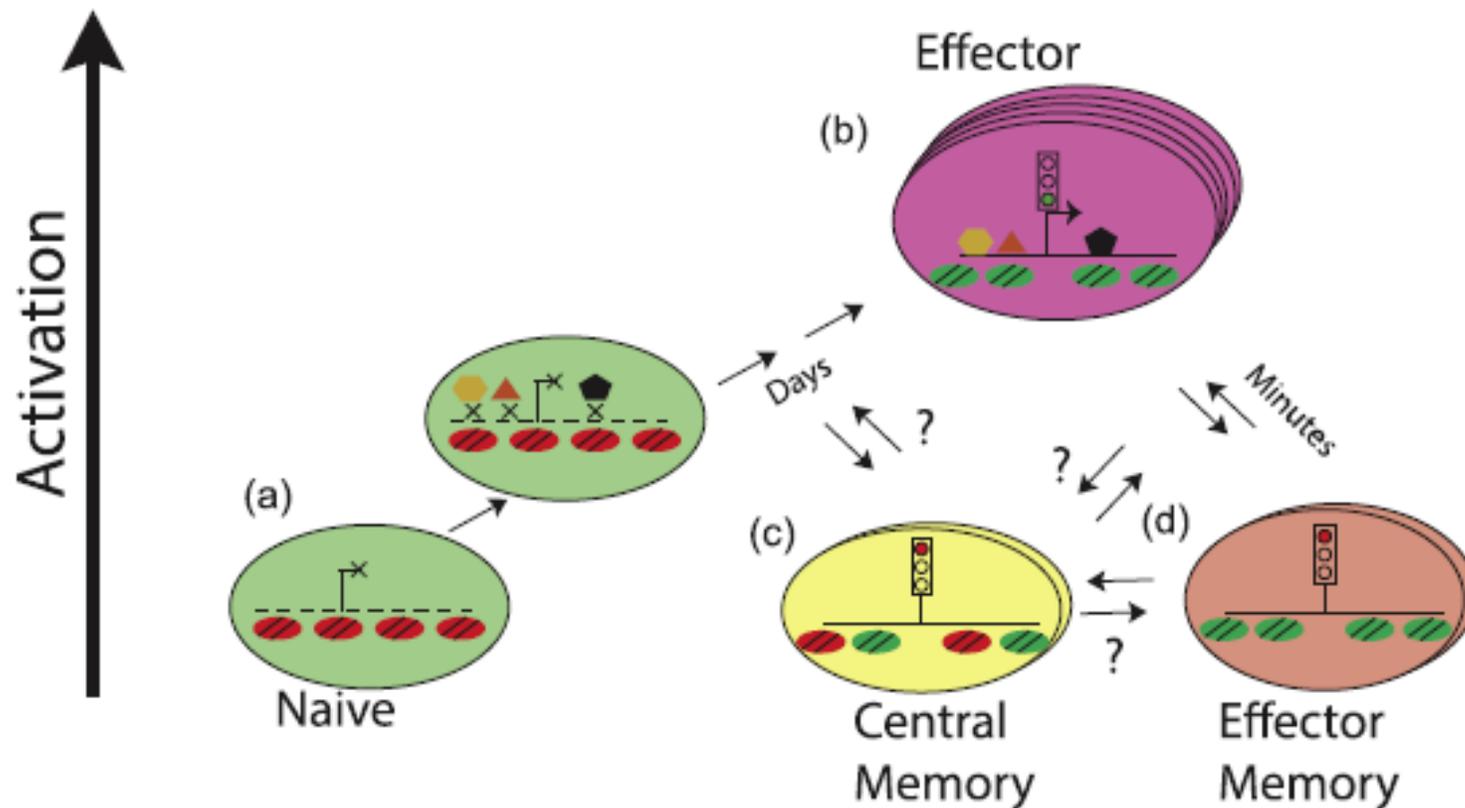
Epigenetics: heritable changes in phenotype or gene expression caused by mechanisms other than changes in underlying DNA sequence



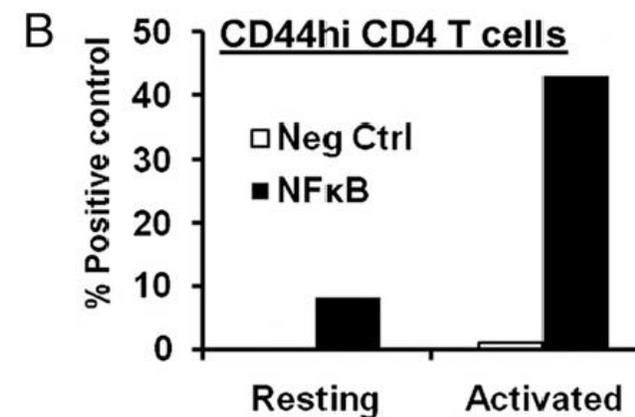
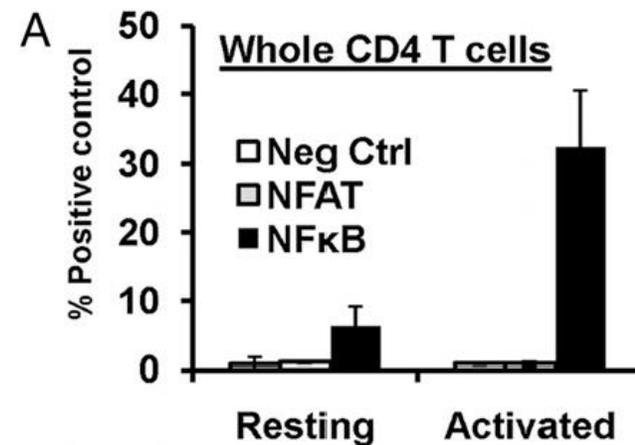
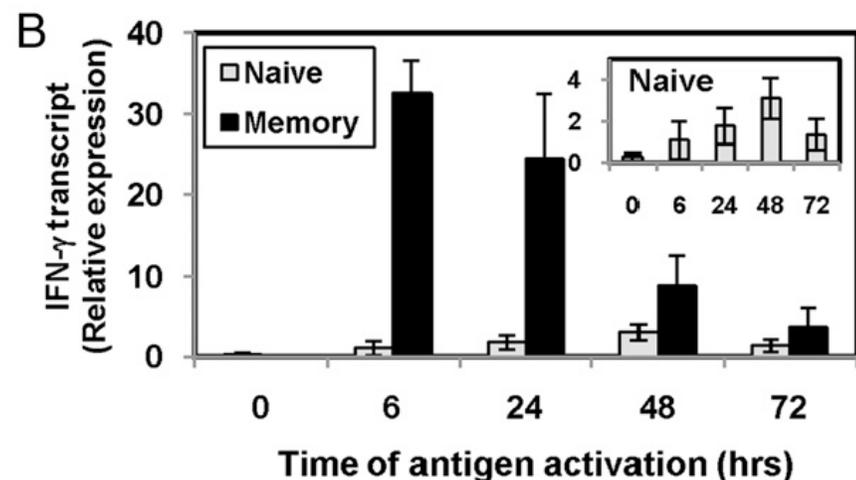
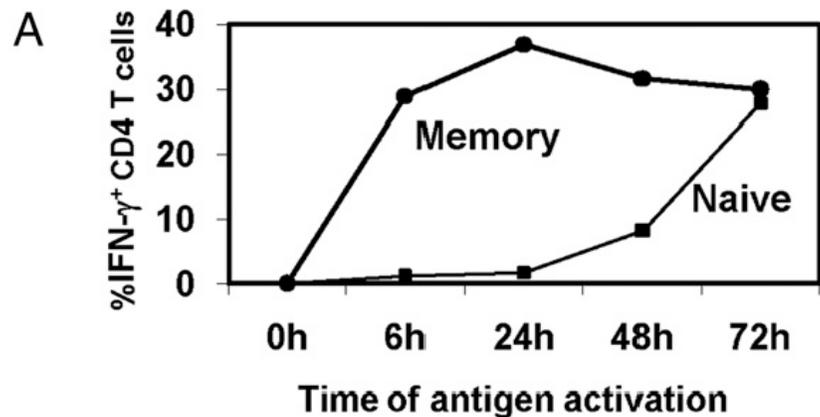
Inducible genes are modified prior to induction: “poised”



Model for rapid recall of T-cell activation



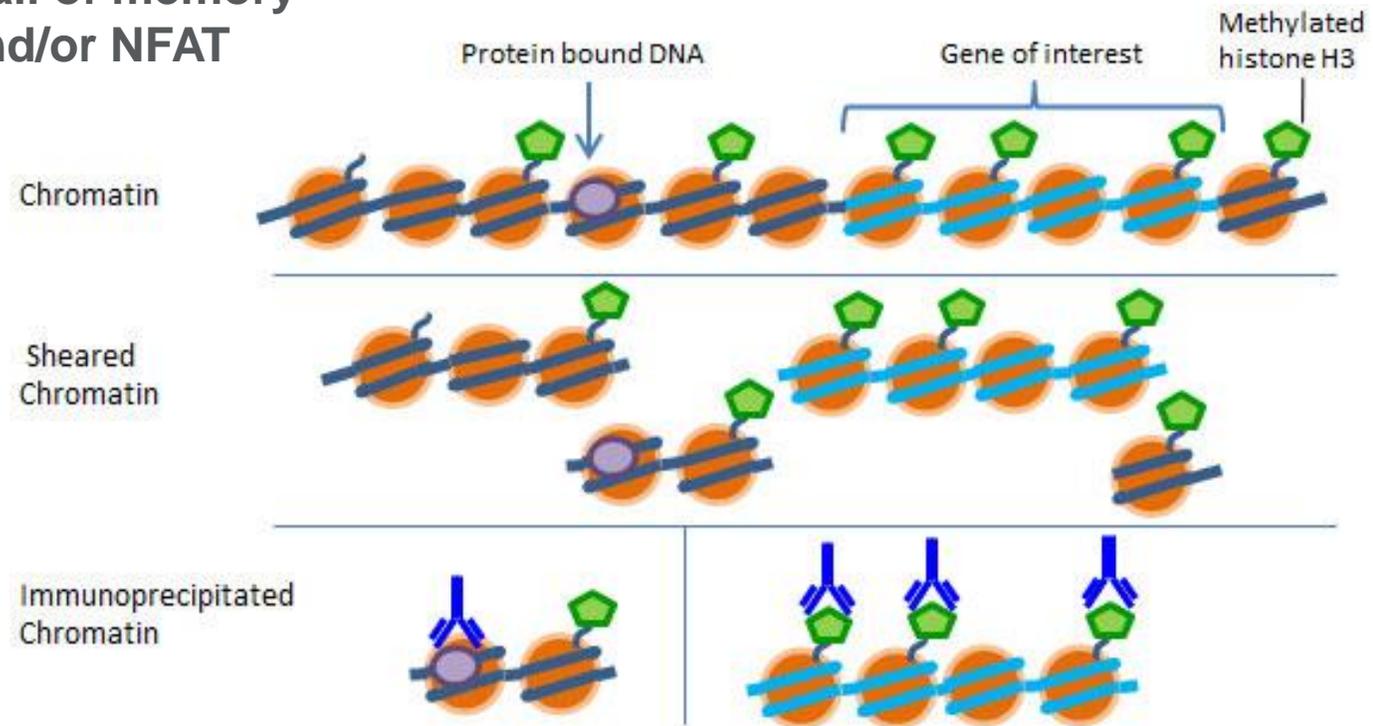
IFN γ and NF κ B in Murine Memory Cells



Hypothesis and Experimental approach

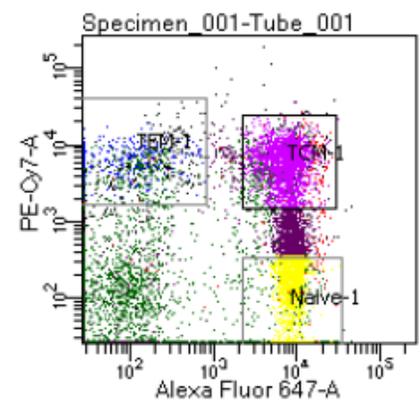
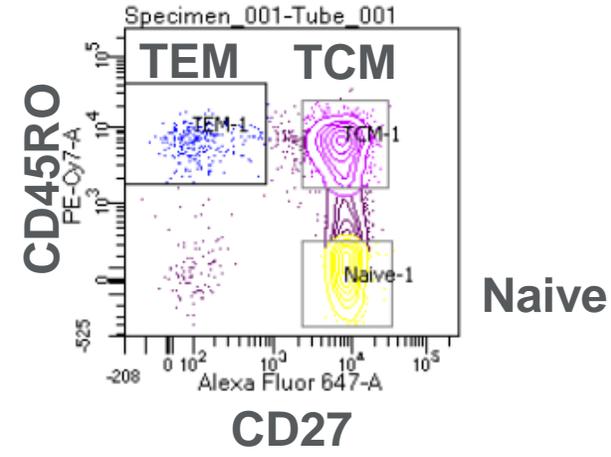
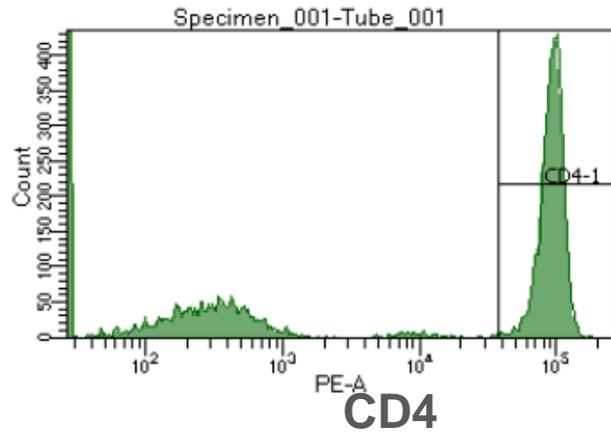
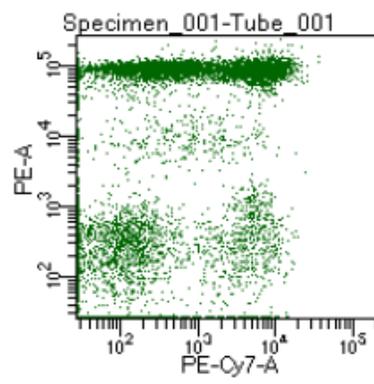
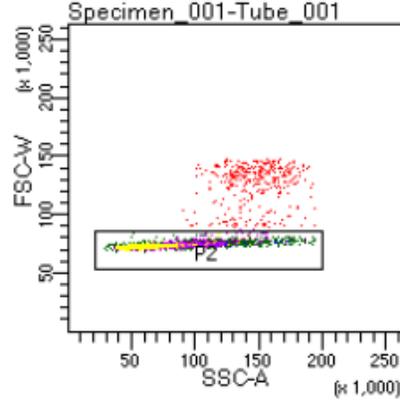
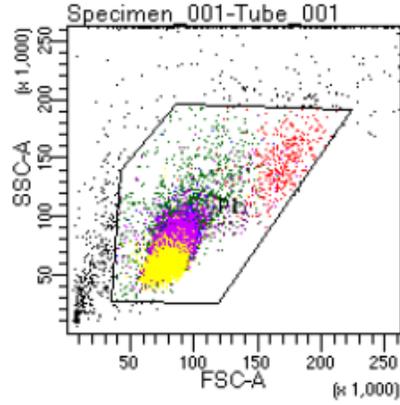
Hypothesis: the epigenetically encoded rapid recall of memory CD4+ T-cells is mediated by activation of NF κ B and/or NFAT

- Isolate blood from human donor
- Isolate PBMCs
- Negative selection for CD4+ T-cells
- Cell sorting
- Activation
- Chip-seq for NF κ B and NFAT
- (data already collected: ATAC Seq)
- Data analysis



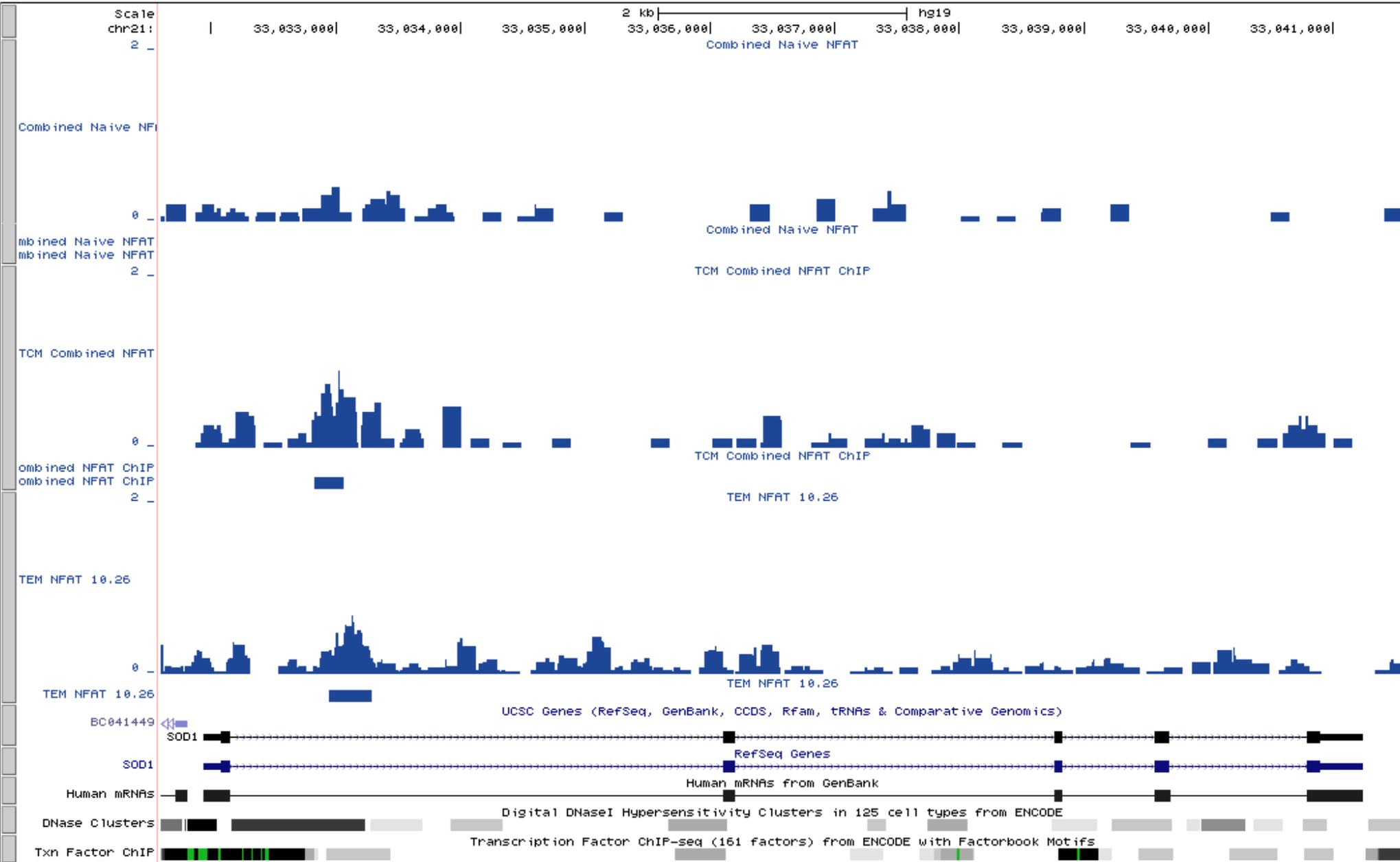
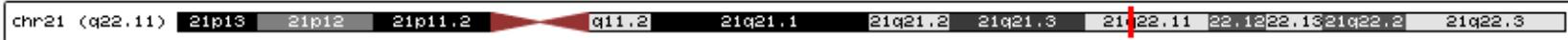
Immunoprecipitated DNA can be used for ChIP-PCR, ChIP-on-chip or ChIP-seq

BD FACSDiva 8.0.1



Tube: Tube_001

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█ P1	9,286	92.9	92.9
█ P2	8,943	96.3	89.4
█ CD4-1	5,218	58.3	52.2
█ TEM-1	259	5.0	2.6
█ TCM-1	2,048	39.2	20.5
█ Naive-1	1,732	33.2	17.3



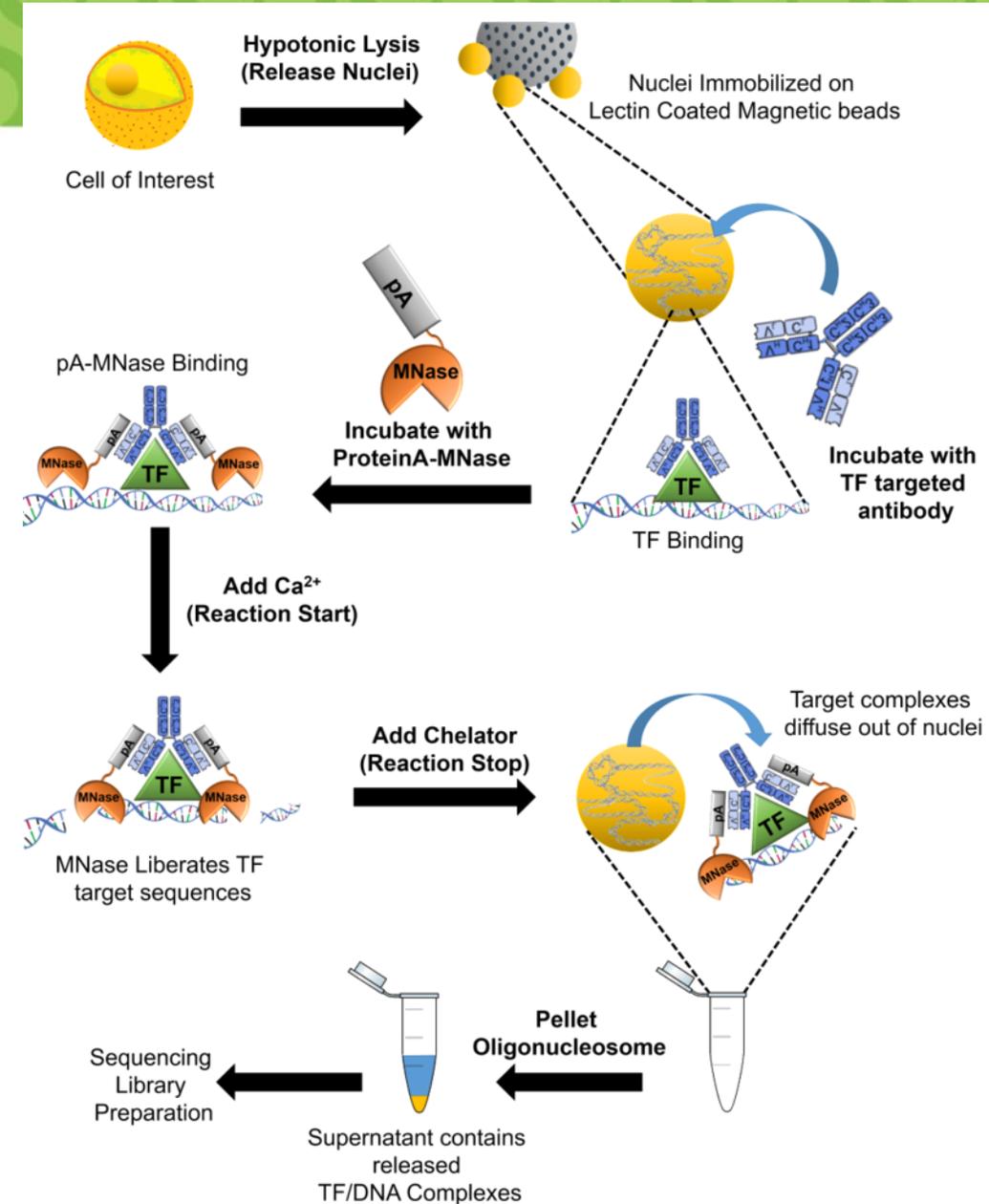
Naive

TCM

TEM

Future directions

- Further analysis of NF κ B and NFAT ChIPs
- ChIPs of multiple NFAT and NF κ B antibodies sent for sequencing
- Sample size issue, particularly for TEM cells – attempting to optimize Cut and Run



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