nature genetics

SUPPLEMENTARY INFORMATION

https://doi.org/10.1038/s41588-020-0643-0

In the format provided by the authors and unedited.

CTCF is dispensable for immune cell transdifferentiation but facilitates an acute inflammatory response

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Supplementary Notes

Supplementary Note 1. Transdifferentiation of human B cells into macrophages

In this study, we used a system consisting of a B leukemia cell line (BLaER) that can be efficiently converted by exogenous C/EBPα expression into functional induced macrophages (iMacs) with only one cell division on average (**Fig.1a**)[1]. Given that C/EBPα exerts its lineage-determining capacity by predominantly binding to distal enhancers[2,3], we reasoned that our transdifferentiation system would heavily rely on long-range transcriptional control and therefore on CTCF-mediated 3D genome organization. BLaER cells expressing C/EBPα fused to the estrogen receptor were treated with ß-estradiol (ß-est), converting cells to phagocytic iMacs 168 hours post-induction (hpi) (**Fig.1a**).

Supplementary Note 2. Chromatin compartmentalization dynamics during transdifferentiation of B cells into macrophages

First, we studied the A/B chromatin compartmentalization dynamic on the basis of the first eigenvector values of a principal component analysis on the Hi-C correlation matrix (PC1 values), showing local transitions between compartments (**Fig.1b-c**). Overall, A-B compartmentalization was highly reproducible between biological duplicates and revealed changes across the genome over time (**Extended Data Fig.1a-b**). PCA highlighted a progressive rewiring of genome compartmentalization during transdifferentiation (**Fig.1d**). Although most regions assigned to either the A (40%) or B (60%) compartment remained essentially constant throughout transdifferentiation (**Extended Data Fig.1c**), around 14% were dynamic (**Fig.1e**). Of these, ~25% switched from B-to-A, ~40% from A-to-B and the remaining regions showed transient transitions (A-B-A and B-A-B) (**Fig.1e**). Genes associated to regions exhibiting a B-to-A switch were upregulated on average and found to be enriched for ‘immune system’-related functions whereas genes in A-to-B switching domains were downregulated and enriched for ‘transcription’-related processes (**Fig.1f**, **Extended Data Fig.1d**). In contrast, expression of genes in A-B-A regions remained unchanged, while genes in B-A-B domains were slightly upregulated

(**Fig.1f**), raising the possibility of a 'memory effect' of regions that have transiently moved into the A compartment.

Supplementary Note 3. Auxin-inducible degron approach to deplete CTCF during transdifferentiation of B cells into macrophages

We used CRISPR/Cas9 technology to introduce into BLaER cells, a transgene encoding the Tir1 F-box protein from *Oryza sativa* and targeted the stop codon of both CTCF alleles to introduce a 68-amino acid version of the auxin-inducible degron (mAID) tag together with a mCherry cassette. Addition of auxin to culture medium then triggers proteasome-dependent CTCF degradation also resulting in a loss of $mCherry^{+}$ fluorescence (**Fig.2b-c**).

Supplementary Table 1. Sequences of oligonucleotides used for qRT-PCR

Oligonucleotide	Sequence
IL6_Forward	AGTGAGGAACAAGCCAGAGC
IL6_Reverse	ATTTGTGGTTGGGTCAGGGG
IL1B_Forward	CGCCAGTGAAATGATGGCTT
IL1B_Reverse	ATCCAGAGGGCAGAGGTCC
TNF_Forward	ACTTTGGAGTGATCGGCCC
TNF_Reverse	CATTGGCCAGGAGGGCATT
CCL2_Forward	ATCAATGCCCCAGTCACCTG
CCL2_Reverse	TCTCCTTGGCCACAATGGTC
CCL1_Forward	CGGAGCAAGAGATTCCCCTG
CCL1_Reverse	TGCCTCTGAACCCATCCAAC
CCL5_Forward	TCATTGCTACTGCCCTCTGC
CCL5_Reverse	CACACTTGGCGGTTCTTTCG
CSF3_Forward	GAGTGTGCCACCTACAAGCT
CSF3_Reverse	CCGCTATGGAGTTGGCTCAA
GAPDH_Forward	CAGCCTCAAGATCATCAGCA
GAPDH_Reverse	TGTGGTCATGAGTCCTTCCA

Supplementary References

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