**Supplemental Figure S1.** Changes in chromatin organization upon neutrophil differentiation.

(A) Wright-Giemsa stain of primary human neutrophils isolated from peripheral blood.

(B) Read normalized HiC matrices, each pixel represents normalized reads between chromosomes for H1 hESC on left and unstimulated human neutrophils on right.

(C) Ratio of total base pairs in the B compartment and total base pairs in the A compartment for H1 hESC (teal) and human neutrophils (blue).

(D) Significant interchromosomal interactions broken down by compartment membership of anchor fragments for H1 hESCs (top) and human neutrophils (bottom).

(E) Scatterplot comparing PC1 values of H1 hESCs and neutrophils at 40kb resolution with density contour plot overlaid in white. Regions that flip from the B to A compartments and have PC1 differences greater than two standard deviations of the genome-wide mean PC1 difference are colored by cell type: teal = H1 hESC; blue = neutrophil.

(F) Metascape gene functional analysis for genes in H1 hESC-specific PC1 positive regions highlighted in E. See supplemental table 2 for gene functional group designations.

(G) Metascape gene functional analysis for genes in neutrophil-specific PC1 positive regions highlighted in E. See supplemental table 2 for gene functional group designations.
Supplemental Figure S2. Stimulation-specific ΔPC1 domains are independently regulated and contain distinct genes sets.

(A) Venn diagram illustrating the overlap between *E. coli* ΔPC1 and PMA ΔPC1 domains and their respective genes defined during *E. coli* co-culture and PMA activation. Genes within 100kb of ΔPC1 domains were considered.

(B) Metascape analysis for gene sets defined in A. Summary groups are marked by enlarged points, complete metascape results can be found in Supplemental table 2.

(C) Log2( *E.coli* co-culture / PMA-activated neutrophil FPKM ) gene expression values for genes sets defined in A. ** Wilcoxon rank-sum test p-value: <1E-10
**Supplemental Figure S3.** *CXCL8* and PC1 (-) control dual color FISH.

(A) FISH results showing the distance of the *CXCL8* locus and a control PC1 (-) FISH probe from the nuclear edge in unstimulated and *E. coli* co-cultured neutrophils. Wilcoxon rank sum test p-values: *=0.0002; +=0.2714.
**Supplemental Figure S4.** *E. coli* ∆PC1 domains lose dense intra-subdomain chromatin contacts in favor of long-range regulatory Interactions.

(A) *E. coli* ∆PC1 domain surrounding the CCL cluster. From top to bottom: PC1 differential (*E. coli* co-cultured – unstimulated PC1); log2(*E. coli* co-cultured/unstimulated) differential HiC contact matrix; protein coding genes; unstimulated CTCF, SMC3, and H3K27ac ChIP-seq, RNA-seq, and chromatin interactions; PC1 differential (*E. coli* co-cultured – unstimulated); *E. coli* co-cultured CTCF, SMC3, and H3K27ac ChIP-seq, RNAseq, and chromatin interactions. Only chromatin interactions with a logP value of <= -50 are shown.

(B) As in A for the SGK1 locus.

(C) As in A for the CD83 locus.

(D) As in A for the FDG4 locus.

(E) As in A for the NR4A3 locus.
Supplemental Figure S5. Cell type-specific chromatin loops are associated with cell type-specific SMC3 and H3K27ac.

(A) Log$_2$( normalized $E$. coli co-cultured / unstimulated ) SMC3 ChIP-seq signal at all SMC3 peaks genome wide, at SMC3 peaks at unstimulated neutrophil-specific interaction anchors, and at SMC3 peaks at $E$. coli co-culture-specific interaction anchors.

(B) As in A, for H3K27ac ChIP-seq peaks and ChIP-seq signals.

(C) As in A, for CTCF ChIP-seq peaks and ChIP-seq signals.

(D) Log$_2$ ratio ($E$. coli co-cultured / unstimulated) of normalized ChIP-seq and RNA-seq signals at enhancers that amass polyadenylated transcripts and all enhancers genome-wide.

Wilcoxon rank sum test: ** <2E-16; * <1E-4; x = 2E-3; + not significant
Supplemental Figure S6. TFE3 translocation to the neutrophil nucleus upon microbial encounter.

(A) Immunofluorescence demonstrates a cytoplasmic localization of TFE3 in unstimulated neutrophils. DNA visualized in green, TFE3 protein in red.

(B) Immunofluorescence of neutrophils co-cultured with E. coli for 1 hour. Arrow points to an activating neutrophil undergoing delobulation with nuclear TFE3. Colors as in (A).

(C) Immunofluorescence of neutrophils co-cultured with E. coli for 3 hours demonstrating E. coli co-culture dependent nuclear localization of TFE3 in neutrophils following microbial encounter.
Supplemental Figure S7. SMC3-amassed enhancers form de novo chromatin loops with inflammatory gene promoters.

(A) Example of *E. coli* co-culture-induced SMC3 binding at a pre-existing enhancer resulting in increased interaction strength between the enhancer and the *LIF* and *OSM* gene locus, resulting in their transcriptional up-regulation. CTCF, SMC3, and H3K27ac ChIP-seq, and RNAseq signals, chromatin interactions, and changes in PC1 scores are shown for unstimulated neutrophils in blue, and *E. coli* co-cultured neutrophils in green. Arrows show example genes, asterixis show position of loop anchors connecting SMC3-amassed enhancers to their target genes in *E. coli* co-cultured neutrophils.

(B) As in A, for the *SCOS3/PGS1* locus, illustrating de novo loop formation between an SMC3-amassed enhancer cluster and their target genes.

(C) As in B, for the *WSB1* gene locus.

(D) As in B for the *CCRL2* gene locus.
Supplemental Figure S8. Pre-existing and de novo formed enhancers both rely on cohesin recruitment to activate gene expression.

(A) Log$_2$ differential (E. coli co-cultured / unstimulated FPKM) gene expression cumulative distribution frequency plots for all genes, genes linked to any H3K27ac-defined enhancer specific to E. coli co-cultured neutrophils, and for genes linked to SMC3-amassed H3K27ac-defined enhancers specific to E. coli co-cultured neutrophils.

(B) As in A, for enhancers shared between unstimulated and E. coli co-cultured neutrophils.

(C) As in A, for enhancers identified only in unstimulated neutrophils.
Denholtz Supplemental Figure S2

A

Domains // genes

E. Coli ΔPC1

PMA ΔPC1

110 // 433

128 // 590

169 // 598

B

E. Coli ΔPC1 - specific

Shared ΔPC1

PMA ΔPC1 - specific

C

Log2 (E. coli / PMA FPKM)

E. Coli - specific

Shared

PMA - specific
Distance from nuclear edge (um)

CXCL - Unstimulated
PC1 (-) control

n = 190 97 147 85

E. coli co-cultured
**Figures A-D**

**A**
- Log2 (E. coli / US SMC3)
- Genomes-specific and US-specific data points.
- Log2 values range from -6 to 6.

**B**
- Log2 (E. coli / US H3K27ac)
- Genomes-specific and US-specific data points.
- Log2 values range from -6 to 6.

**C**
- Log2 (E. coli / US CTCF)
- Genomes-specific and US-specific data points.
- Log2 values range from -6 to 6.

**D**
- Log2 (E. coli / US normalized signal)
- Data points for SMC3, CTCF, H3K27ac, polyA RNA separately.
- Log2 values range from -6 to 6.
Denholtz Supplemental Figure S7

A

CTCF
SMC3
H3K27ac
RNAseq

Interactions

ΔPC1
(E. coli – Unstim.)

CTCF
SMC3
H3K27ac
RNAseq

SMC3-amassed enhancers

Interactions

B

C

D

LIF
OSM
SOCS3
PGS1
WSB1
CCRL2

Chr 22 (Mb)

Chr 17 (Mb)

Chr 17 (Mb)

Chr 3 (Mb)
Denholtz Supplemental Figure S8

A. Genes linked to E. coli co-culture specific enhancers

- Genes linked to E. coli co-culture specific SMC3-amassed enhancers
- Genes linked to any E. coli co-culture specific enhancer
- All genes

KS test p-value
- 1.1E-4
- 1.1E-5
- 6.5E-4

B. Genes linked to shared enhancers

- Genes linked to shared SMC3-amassed enhancers
- Genes linked to any shared enhancer
- All genes

KS test p-value
- 4.2E-5
- 0.019
- 0.111

C. Genes linked to unstimulated-specific enhancers

- Genes linked to unstimulated-specific SMC3-amassed enhancers
- Genes linked to any unstimulated-specific enhancer
- All genes

KS test p-value
- 7.0E-3
- 0.407
- 0.290