# Supplementary Information Guide

# Population Snapshots Predict Early Hematopoietic and Erythroid Hierarchies

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**Contents:**



**Supplementary Table 1 Lineage-specific marker genes**
List of marker genes for mature cells of each lineage and multipotent progenitors. These genes were used for visualizing cell types on SPRING plots and for identifying the most mature cells for Population Balance Analysis. Marker gene lists are sample-specific due to sample-to-sample differences in the detected lineages and the maturity of these lineages.

**Supplementary Table 2 Genes correlating with branching computationally predicted fate probabilities in hematopoietic progenitor cells.** The table shows the process by which genes were selected, as well as their identity. Column 1 (Cell subset) shows the PBA gates used, and for each cell subset, Column 2 (Lineage bias score) indicates the score to which the genes identified were correlated. Columns 3-5 give the Gene symbol, its Correlation to the lineage bias score in the cell subset, and the P-value of the correlation. For example, "P(Er)*P(Ba) > 0.07" identifies cells computationally predicted to reside close to the Erythroid-Basophil branch point, as measured by the product of the PBA scores P(Er) and P(Ba). Within this subset genes were tested for correlation against the PBA score "P(Er)-P(Ba)". Thus genes with positive correlation to this score were Erythroid biased and genes with negative correlation were Basophil biased.

**Supplementary Table 3 Dynamic changes in gene expression along the erythroid trajectory**

List of significantly varying genes in the basal BM dataset. For each gene, maximal and minimal expression levels, and the corresponding locations along the MPP to erythroid progression, are shown. See methods section for calculation of FDR-corrected p value.

**Supplementary Table 4 Gene set enrichment analysis (GSEA) of dynamic gene clusters**

Genes assigned to each dynamic cluster (sheet 1), and GSEA results for signaling pathways and Gene Onotology terms (sheet 2) and transcription factor targets (sheet 3). Dynamic gene clusters are numbered as in **Extended Data Fig. 5e**. In the GSEA tables (sheets 2 and 3), enriched gene set titles (column B), corresponding corrected p value (column C) and gene hits (column D) are shown.

**Supplementary Table 5 Genes differentially expressed in stress**

Genes differentially expressed (DE) in each of the MPP, EBMP, EEP, CEP, and ETD stages are shown, in two separate sheets for eBM and FL, respectively. See methods section for calculation of the DE score (column C).

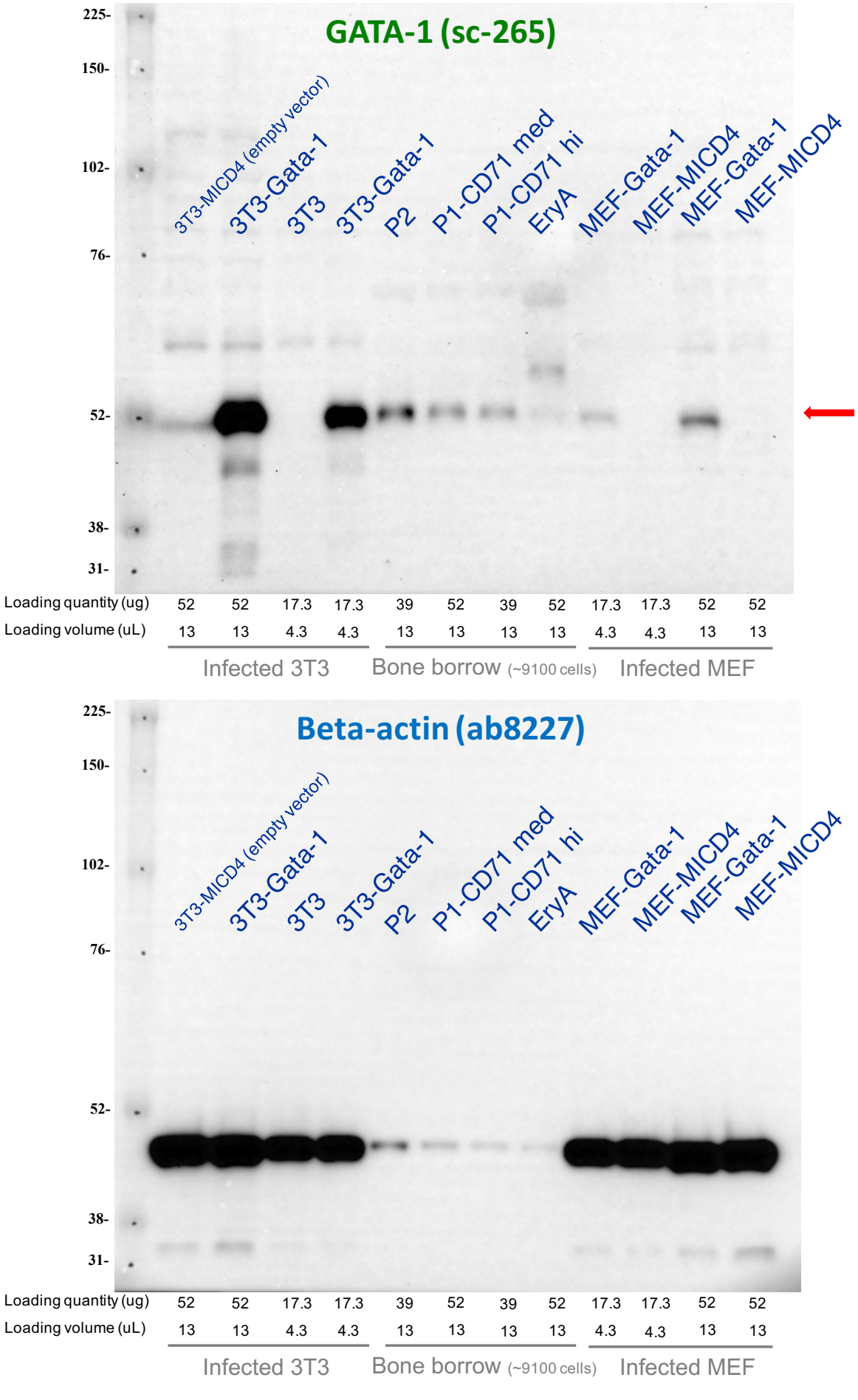
**Supplementary Table 6 Genes correlated with progression through the CEP stage**

For each gene expressed in the CEP stage, this table lists the “Linearity score”, a measure designed to identify genes that steadily increase or decrease with progression along the differentiation ordering (see Methods section “Identifying genes that change steadily in the CEP stage”). Large positive scores indicate genes that are strongly up-regulated in a linear fashion throughout the CEP stage; large negative scores indicate genes that are down-regulated linearly; and scores close to 0 indicate genes whose expression changes little or in a non-linear fashion.

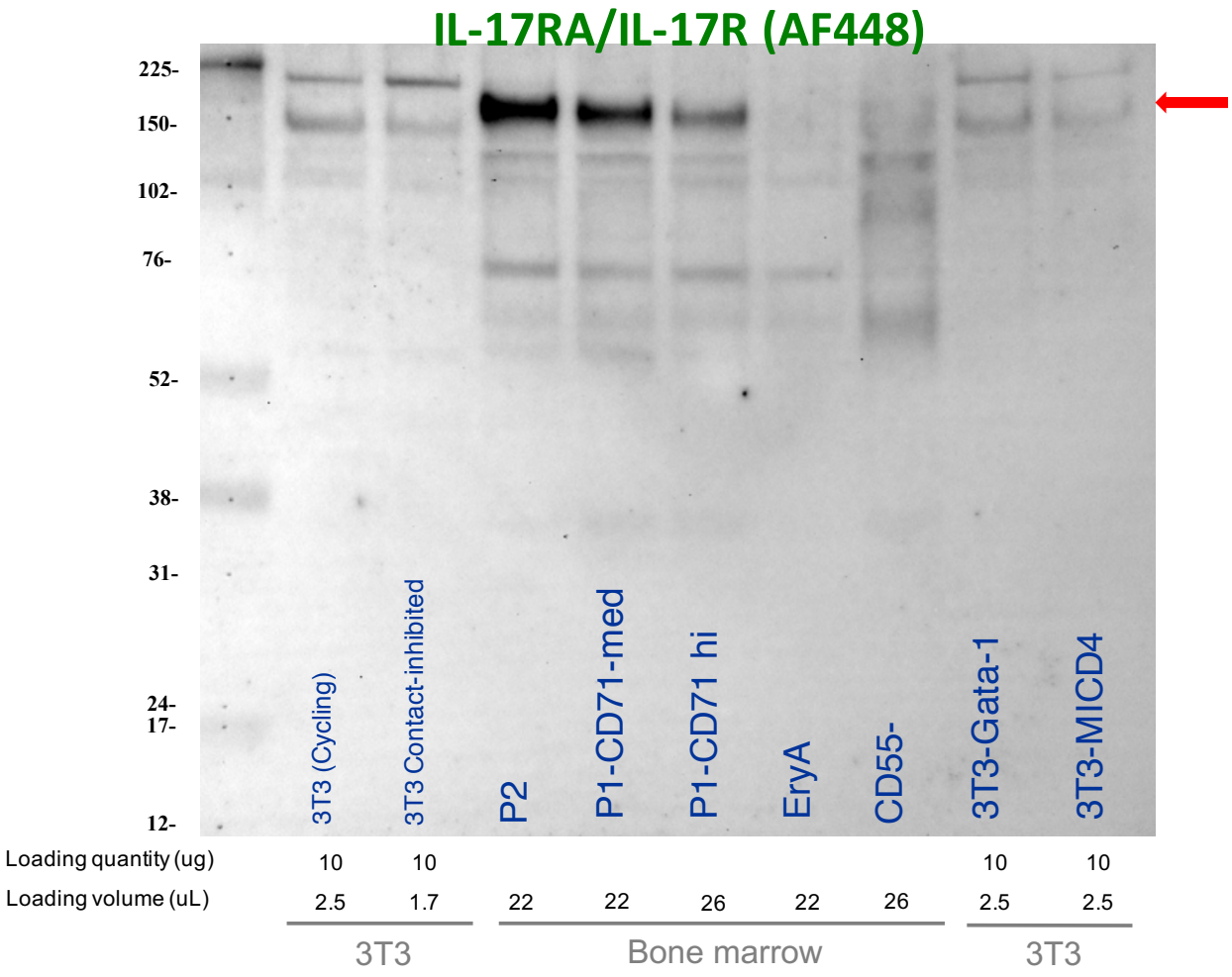
**Supplementary Table 7 Modifications to the inDrops protocol**

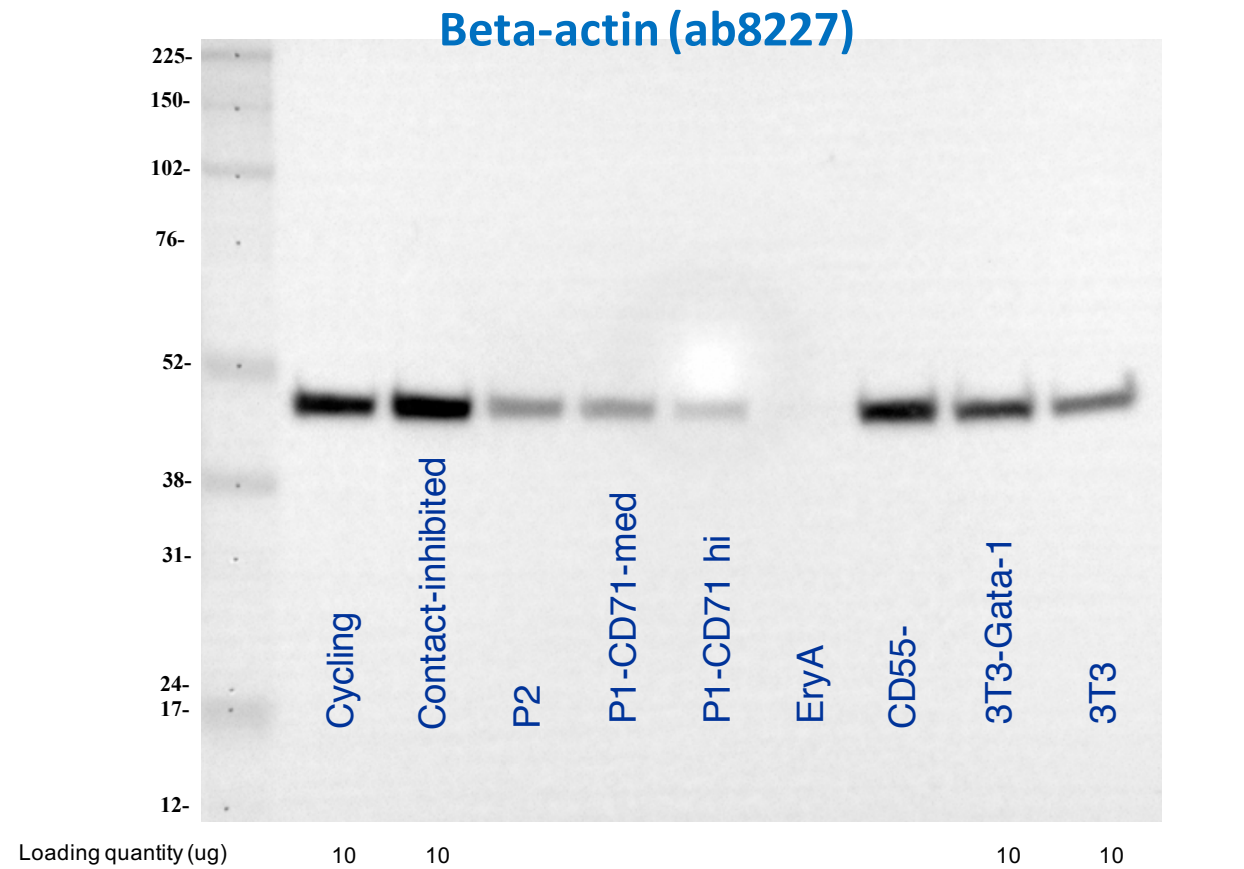
Detailed list of changes to the inDrops protocol by sample.

**Extended Data Figure 5b**



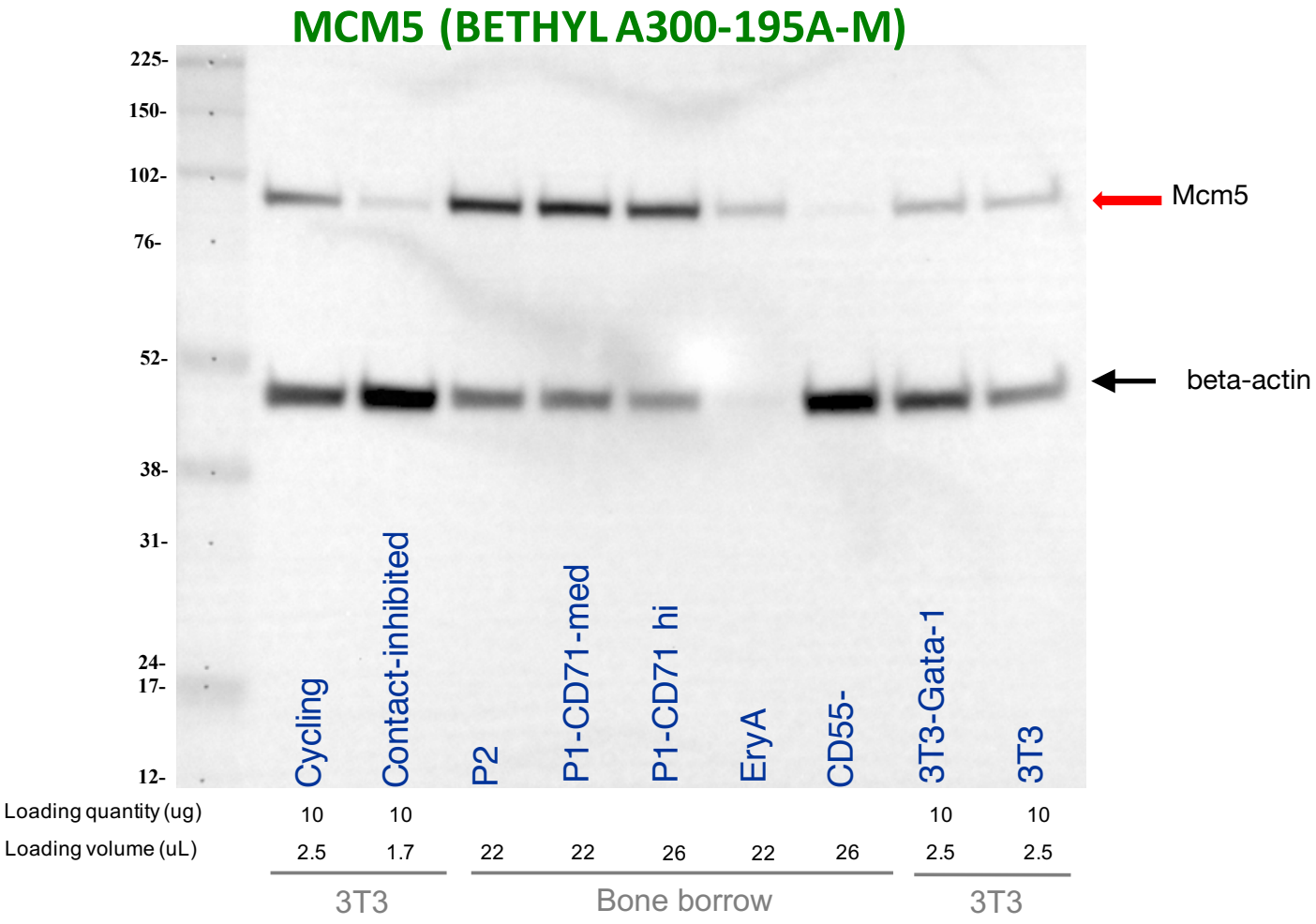
**Extended Data Figure 9d**



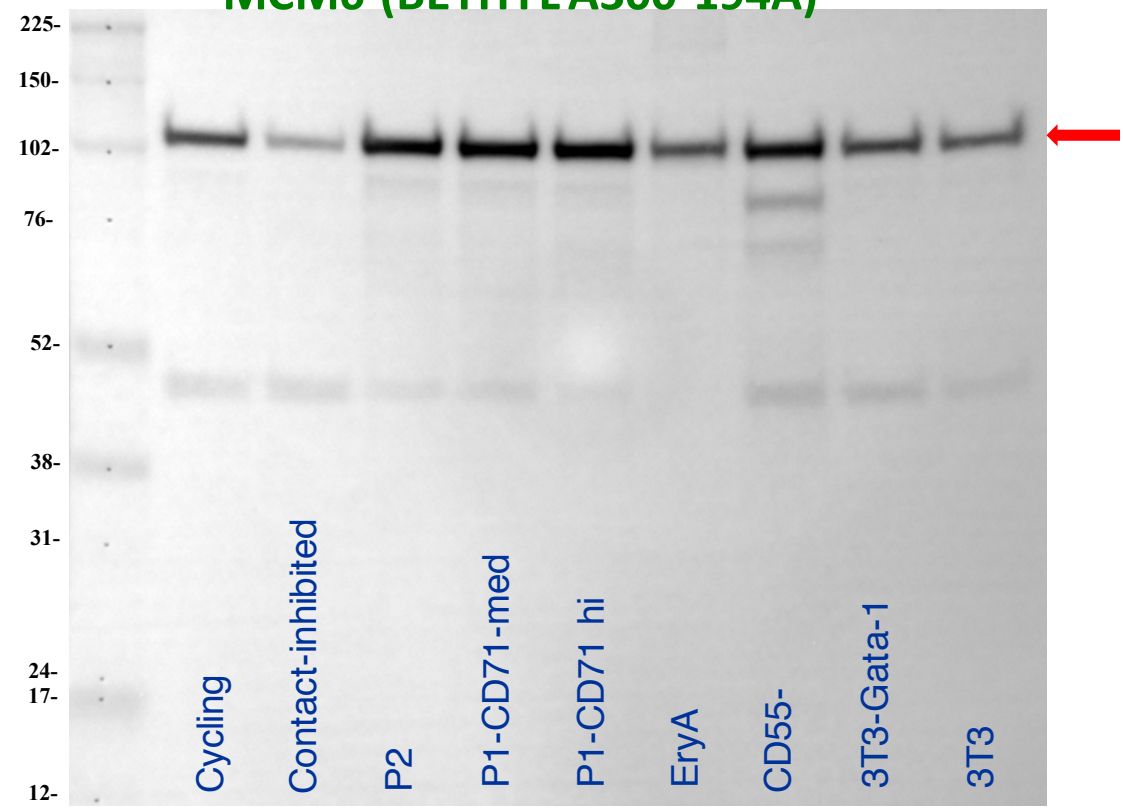



**Extended Data Fig. 10h:**

The same membrane as in Extended Data Fig. 9d was also probed for Mcm5, Mcm6, PCNA, Mcm2. (see above, for actin probe; the membrane was probed for Mcm5 without stripping the actin probe, which means that the actin bands are still visible following probing for Mcm5, below)

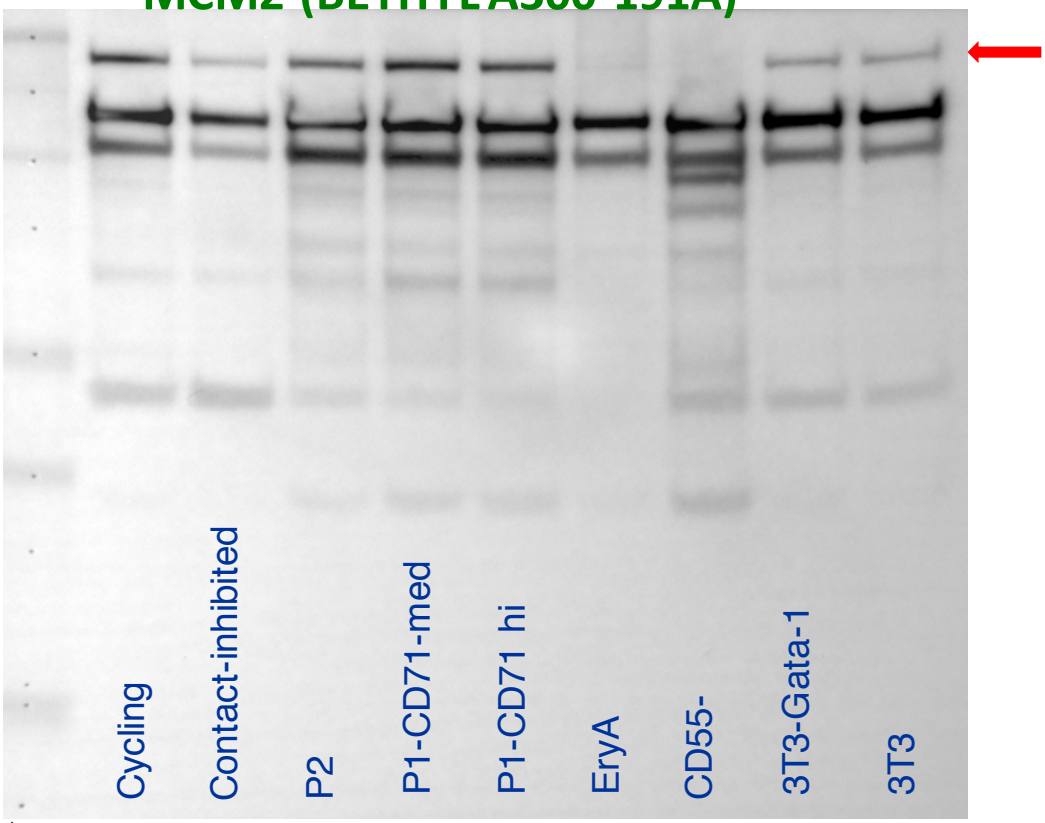


## MCM6 (BETHYL A300-194A)



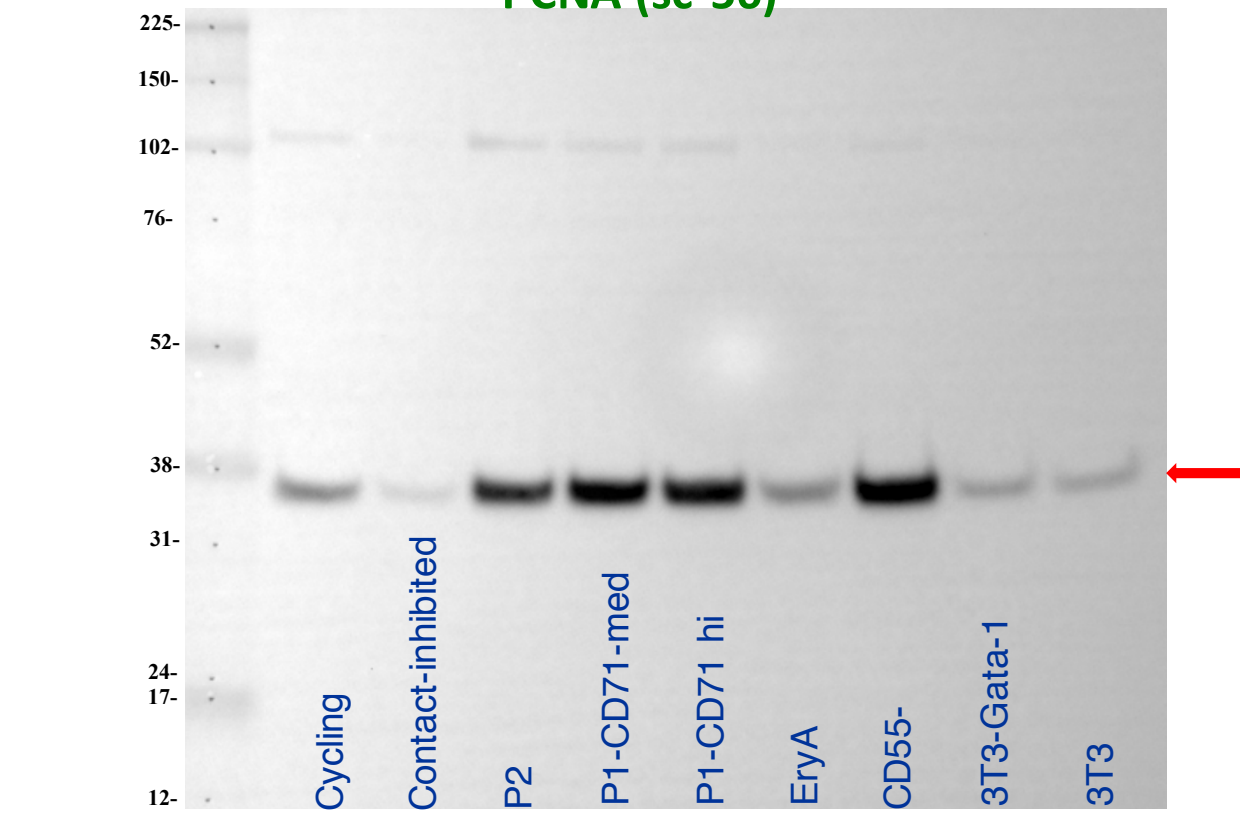

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Loading quantity (ug) | 10 | 10 | | | | | | 10 | 10 |
| Loading volume (uL) | 2.5 | 1.7 | 22 | 22 | 26 | 22 | 26 | 2.5 | 2.5 |
| | 3T3 | | Bone borrow | | | | | 3T3 | |

## MCM2 (BETHYL A300-191A)




| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Loading quantity (ug) | 10 | 10 | | | | | | 10 | 10 |
| Loading volume (uL) | 2.5 | 1.7 | 22 | 22 | 26 | 22 | 26 | 2.5 | 2.5 |
| | 3T3 | | Bone borrow | | | | | 3T3 | |

# PCNA (sc-56)


225-
150-
102-
76-
52-
38-
31-
24-
17-
12-
Cycling
Contact-inhibited
P2
P1-CD71-med
P1-CD71 hi
EryA
CD55-
3T3-Gata-1
3T3
Loading quantity (ug)
10
10
10
10
Loading volume (uL)
2.5
1.7
22
22
26
22
26
2.5
2.5
3T3
Bone borrow
3T3

## Supplementary Table 1

| Sample | E | Ba | Meg | MPP | Ly | D | M | G | Eo | NK |
|---|---|---|---|---|---|---|---|---|---|---|
| bBM | Hbb-bt, Hba-a2, Hba-a1, Alas2, Bpgm | Lmo4, Ifitm1, Ly6e, Srgn | Pf4, Itga2b, Vwf, Pbx1, Mef2c | Hlf, Gcnt2 | Cd79a, Igll1, Vpreb3, Vpreb1, Lef1 | H2-Aa, Cd74, H2-Eb1, H2-Ab1, Cst3 | Csf1r, Ly6c2, Ccr2 | Lcn2, S100a8, Ltf, Lyz2, S100a9 | NA | NA |
| eBM | Hbb-bt, Hba-a2, Hba-a1, Alas2, Bpgm | Lmo4, Ifitm1, Ly6e, Srgn | Pf4, Itga2b, Vwf, Pbx1, Mef2c | Hlf, Gcnt2 | Cd79a, Igll1, Vpreb3, Vpreb1, Lef1 | NA | Csf1r, Ly6c2, Ccr2 | Lcn2, S100a8, Ltf, Lyz2, S100a9 | NA | NA |
| FL | Hbb-bt, Hba-a2, Hba-a1, Alas2, Bpgm | Lmo4, Ifitm1, Ly6e, Srgn | Pf4, Itga2b, Vwf, Pbx1, Mef2c | Hlf, Gcnt2 | Ccr9, Mzb1, Lsp1, Flt3, Ccr7, Lck | NA | Csf1r, Ly6c2, Ccr2 | Elane, Mpo | NA | NA |
| P1-P5 | Car2, Rhd, Tfrc | Cpa3, Lmo4, Srgn, Cd53 | Pf4, Itga2b, Vwf, Pbx1, Mef2c | Hlf, Gcnt2 | NA | NA | Csf1r, Ly6c2, Ccr2 | Elane, Prtn3 | Epx, Prg2 | NA |
| Paul et al. | Alad, Hba-a2, Rhd, Cpox | Prss34, Alox5, Mcpt8, Lmo4 | Pf4, Vwf, Pbx1 | Hlf, Gcnt2 | Dntt, Flt3 | H2-Aa, Cd74, H2-Eb1, H2-Ab1, Cst3 | Csf1r, Ly6c2, Ccr2, F13a1, Slpi | Mpo, Elane, Gstm1 | NA | Ccl5, Shisa5, Lck, Ctsw, Nkg7 |
| Nestorowa et al. | Hbb-bt, Hba-a2, Hba-a1, Alas2, Bpgm | Lmo4, Ifitm1, Ly6e, Srgn | Pf4, Itga2b, Vwf, Pbx1, Mef2c | Hlf, Gcnt2, Procr | Cd79a, Igll1, Vpreb3, Vpreb1, Lef1 | NA | Csf1r, Ly6c2, Ccr2 | Mpo, Elane | NA | NA |

**Supplementary Table 2: List of transcription factors, receptors and chromatin modifiers correlated with fate bias at key decision points.**

| Cell subset | Lineage bias score | Gene symbol | Correlation | P-value |
|---|---|---|---|---|
| P(Er)*P(Ba) > 0.07 | P(Er)-P(Ba) | Lmo4 | -0.58 | 1.00E-21 |
| P(Er)*P(Ba) > 0.07 | P(Er)-P(Ba) | Gata2 | -0.44 | 1.00E-11 |
| P(Er)*P(Ba) > 0.07 | P(Er)-P(Ba) | Cebpa | -0.33 | 1.00E-06 |
| P(Er)*P(Ba) > 0.07 | P(Er)-P(Ba) | Klf1 | 0.33 | 1.00E-06 |
| P(Er,Ba)*P(Mk) > 0.02 | P(Er,Ba)-P(Mk) | Pbx1 | -0.49 | 1.00E-33 |
| P(Er,Ba)*P(Mk) > 0.02 | P(Er,Ba)-P(Mk) | Mpl | -0.38 | 1.00E-19 |
| P(Er,Ba)*P(Mk) > 0.02 | P(Er,Ba)-P(Mk) | Mef2c | -0.27 | 1.00E-10 |
| P(Er,Ba)*P(Mk) > 0.02 | P(Er,Ba)-P(Mk) | Myb | 0.28 | 1.00E-10 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Gfi1 | -0.51 | 1.00E-80 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Cebpa | -0.42 | 1.00E-52 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Baz1a | -0.39 | 1.00E-44 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Cebpb | -0.36 | 1.00E-36 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Mybl2 | -0.35 | 1.00E-35 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Spi1 | -0.34 | 1.00E-33 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Irf8 | -0.34 | 1.00E-32 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Cebpe | -0.33 | 1.00E-31 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Xbp1 | -0.33 | 1.00E-31 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Cited4 | -0.33 | 1.00E-30 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Hmgn2 | -0.29 | 1.00E-24 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Ostf1 | -0.28 | 1.00E-23 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Cbx4 | -0.28 | 1.00E-22 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Atf4 | -0.28 | 1.00E-22 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Csf1r | -0.28 | 1.00E-22 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Gtf2e2 | -0.27 | 1.00E-20 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Arhgap17 | -0.26 | 1.00E-20 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Mtf1 | -0.26 | 1.00E-19 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Hmga1 | -0.26 | 1.00E-18 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Mcm5 | -0.26 | 1.00E-18 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Cited2 | -0.25 | 1.00E-18 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Mef2d | -0.25 | 1.00E-18 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Lyl1 | -0.25 | 1.00E-18 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Gata2 | 0.45 | 1.00E-59 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Gata1 | 0.38 | 1.00E-42 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Zfpm1 | 0.36 | 1.00E-37 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Pbx1 | 0.31 | 1.00E-27 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Mpl | 0.29 | 1.00E-23 |
| P(Ba,Er,Mk)*P(G,D,Mo,Ly) > 0.07 | P(Ba,Er,Mk)-P(G,D,Mo,Ly) | Ldb1 | 0.28 | 1.00E-22 |

| | | | | |
|---|---|---|---|---|
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Gfi1 | -0.38 | 1.00E-20 |
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Xbp1 | -0.36 | 1.00E-18 |
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Baz1a | -0.34 | 1.00E-15 |
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Cebpa | -0.33 | 1.00E-15 |
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Cebpb | -0.32 | 1.00E-14 |
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Cited4 | -0.31 | 1.00E-13 |
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Cited2 | -0.3 | 1.00E-12 |
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Hmgn2 | -0.29 | 1.00E-11 |
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Cebpe | -0.28 | 1.00E-10 |
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Mcm5 | -0.27 | 1.00E-10 |
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Ostf1 | -0.26 | 1.00E-09 |
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Phf10 | -0.26 | 1.00E-09 |
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Dazap2 | -0.26 | 1.00E-09 |
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Hmga1 | -0.25 | 1.00E-09 |
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Satb1 | 0.43 | 1.00E-25 |
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Sox4 | 0.41 | 1.00E-22 |
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Mta3 | 0.41 | 1.00E-22 |
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Foxp1 | 0.26 | 1.00E-09 |
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Ebf1 | 0.26 | 1.00E-09 |
| P(Ly)*P(Mo,G,D) > 0.07 | P(Ly)-P(Mo,G,D) | Hlf | 0.26 | 1.00E-09 |
| P(Mo)*P(G) > 0.07 | P(Mo)-P(G) | Gfi1 | -0.5 | 1.00E-21 |
| P(Mo)*P(G) > 0.07 | P(Mo)-P(G) | Myb | -0.43 | 1.00E-15 |
| P(Mo)*P(G) > 0.07 | P(Mo)-P(G) | Cebpe | -0.38 | 1.00E-12 |
| P(Mo)*P(G) > 0.07 | P(Mo)-P(G) | Baz1a | -0.29 | 1.00E-07 |
| P(Mo)*P(G) > 0.07 | P(Mo)-P(G) | Irf8 | 0.74 | 1.00E-57 |
| P(Mo)*P(G) > 0.07 | P(Mo)-P(G) | Csf1r | 0.44 | 1.00E-16 |
| P(Mo)*P(G) > 0.07 | P(Mo)-P(G) | Pou2f2 | 0.3 | 1.00E-07 |
| P(Mo)*P(G) > 0.07 | P(Mo)-P(G) | Klf4 | 0.26 | 1.00E-05 |
| P(Mo)*P(G) > 0.07 | P(Mo)-P(G) | Aes | 0.25 | 1.00E-05 |

**Supplementary Table 7**

| Sample set | Sequence of RT primers on hydrogel beads | ExoI/HinfI treatment (step 137 in (1)) | PE2-N6 primer sequence (step 151 in (1)) | PCR primer sequences (steps 157 and 160 in (1)) | Custom sequencing primers? | Sequencing platform |
|---|---|---|---|---|---|---|
| Batch 1 of Kit+ samples: Basal bone marrow (bBM), +Epo bone marrow (eBM), fetal liver (FL) | 5'-CGATGACGTAATACGACTCACTATAGGGATACCACCATGGCTCTTTCCCTACACGACGCTCTTCCGATCT[bc1,8-11nt]GAGTGATTGCTTGTGACGCCTT[bc2,8nt]NNNNNNTTTTTTTTTTTTTTTTTTTV-3' (same as in (1, 2)) | 37 ºC for 60 min heat inactivation at 80 ºC for 10 min (same as in (2)) | 5' -TCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNN-3' (same as in (1)) | 5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGA-3'<br><br>5'-CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAAC-3' (same as in (2)) | No | HiSeq 2000 |
| Batch 2 of Kit+ samples | Same as above and in (1, 2) | 37 ºC for 30 min (same as in (1)) | Same as above and in (1) | 5' -CAAGCAGAAGACGGCATACGAGATXXXXXXCTCTTTCCCTACACGA-3'<br><br>5' -AATGATACGGCGACCACCGAGATCTACACGGTCTCGGCATTCCTGCTGAAC-3'<br><br>(same as in (1)) | Read1: 5'-GGCATTCCTGCTGAACCGCTCTTCCGATCT-3'<br><br>Index read: 5'-AGATCGGAAGAGCGTCGTGTAG-GGAAAGAG-3'<br><br>Read2: 5'-CTCTTTCCCTACACGACGCTCTTCCGATCT-3' (same as in (1)) | NextSeq 500 |
| FACS subsets P1, P1-CD71hi, P2, P3, P4, | 5'-CGATGACGTAATACGACTCACTATAGGGTGTCGGGTGCAG[bc1,8nt]GT | Same as above and in (1) | 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG | 5'-AATGATACGGCGACCACCGAGATCTACACXXXXXXXXTCGTCGGCAGCGTC-3' | No | NextSeq 500 |

| | | | | | | |
|---|---|---|---|---|---|---|
| P5 | CTCGTGGGCTCGGAGATGTGTATAAGAGACAG[bc2,8nt]NNNNNNTTTTTTTTTTTTTTTTTV-3’ | | NNNNNN-3’ | 5’-CAAGCAGAAGACGGCATACGAGATGGGTGTCGGGTGCAG-3’ | | |


1. Zilionis R, Nainys J, Veres A, Savova V, Zemmour D, Klein AM, et al. Single-cell barcoding and sequencing using droplet microfluidics. Nat Protoc. 2017;12(1):44-73.
2. Klein AM, Mazutis L, Akartuna I, Tallapragada N, Veres A, Li V, et al. Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells. Cell. 2015;161(5):1187-201.