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Supplemental Information

C/EBP-Induced Transdifferentiation Reveals Granulocyte-Macrophage Precursor-like Plasticity of B Cells

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Supplemental Figures

Figure S1

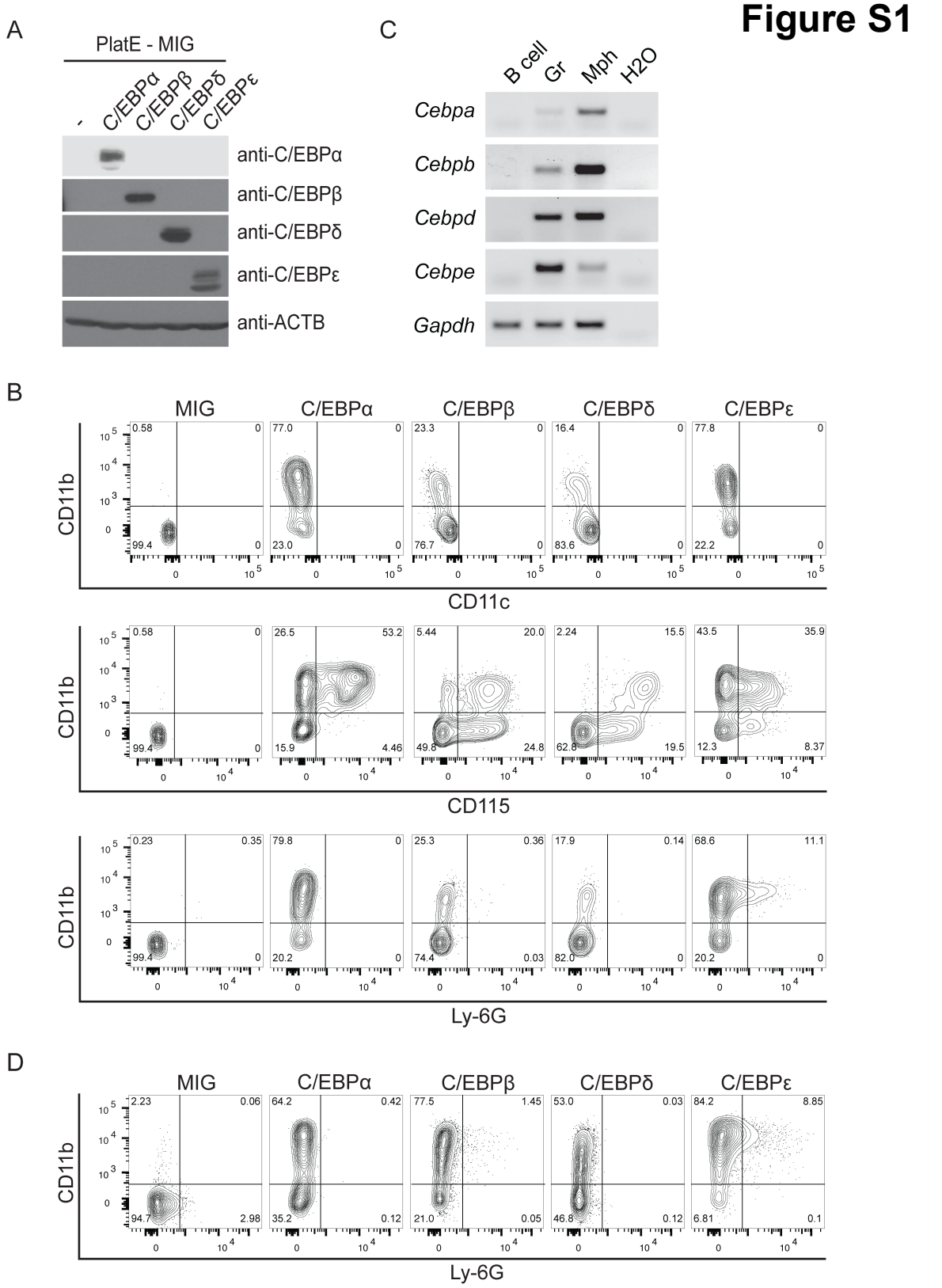
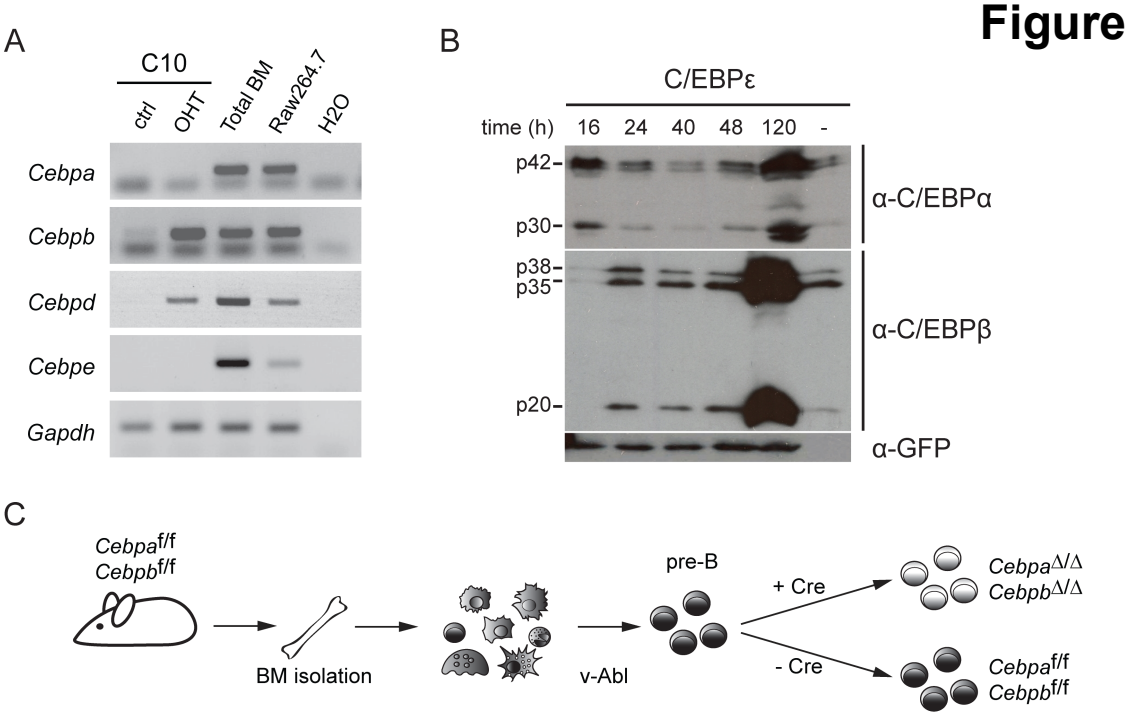
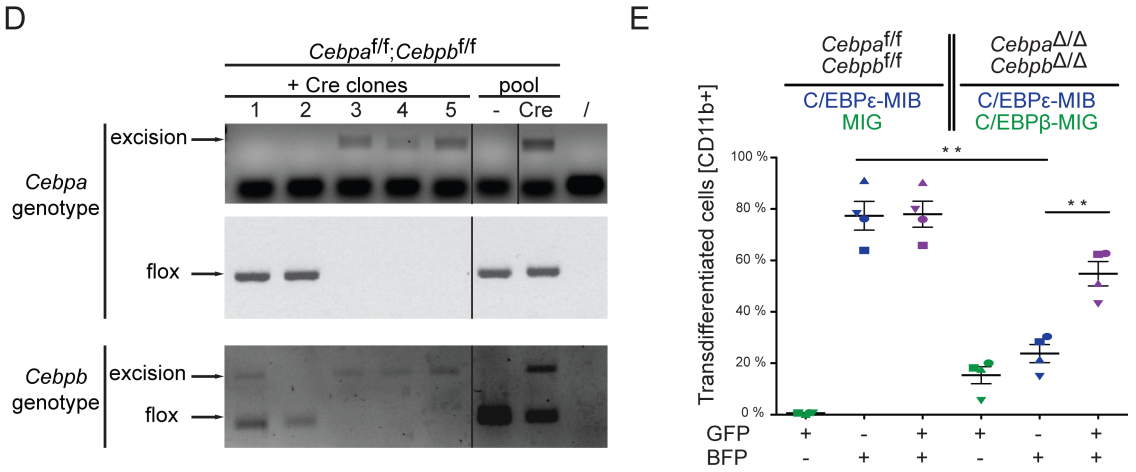


Figure S2





F

upregulated

Ahr	*Csf1r*	*Il18r1*	*Notch2*
Arhgdib	*Csf2rb*	*Il1rn*	*Nt5e*
Btnl1	*Ctsc*	*Il28a*	*Pecam1*
Ccl12	*Cybb*	*Il33*	*Plaur*
Ccl24	*Ddx58*	*Irak2*	*Runx3*
Ccl3	*Entpd1*	*Irak3*	*S100a9*
Ccl6	*Ets1*	*Irf7*	*Sele*
Ccl9	*Fcgr1*	*Itgal*	*Sell*
Ccr5	*Fcgr2b*	*Itgam*	*Ski*
Ccrl2	*Gfi1*	*Kir3dl1*	*Smad3*
Cd4	*Gpi1*	*Klra7*	*Stat3*
Cd44	*Gpr183*	*Lair1*	*Tagap*
Cd55	*H2-Ea-ps*	*Lilrb3*	*Tgfbi*
Cd69	*Hcst*	*Lilrb4*	*Tlr1*
Cdkn1a	*Hfe*	*Ltb4r1*	*Trem1*
Ceacam1	*Hlx*	*Mbl2*	*Tyrobp*
Cfb	*Ifih1*	*Muc1*	*Vcam1*
Csf1	*Ifitm1*	*Nfil3*	*Vtn*

G

downregulated

Aicda	*Ciita*	*Klrc3*	*Tnfrsf13c*
Bcl3	*Cmklr1*	*Lta*	*Traf4*
Btla	*Cxcr2*	*Ltb*	*Xcr1*
C1qa	*Cxcr6*	*Masp1*	
C1s	*Fas*	*Masp2*	
C8a	*Fn1*	*Pax5*	
Ccr6	*Icam2*	*Pdcd1lg2*	
Ccr7	*Ifna2*	*Pla2g2a*	
Ccr8	*Ifng*	*Pou2f2*	
Cd160	*Ikzf4*	*Rag1*	
Cd163	*Il10*	*Rag2*	
Cd2	*Il17b*	*Rorc*	
Cd22	*Il20*	*Slamf1*	
Cd244	*Il22*	*Slamf7*	
Cd40	*Il7r*	*Tfrc*	
Cd74	*Irf4*	*Tgfb3*	
Cd79b	*Irf8*	*Tigit*	
Cfd	*Klrb1*	*Tnfaip6*	

Figure S3

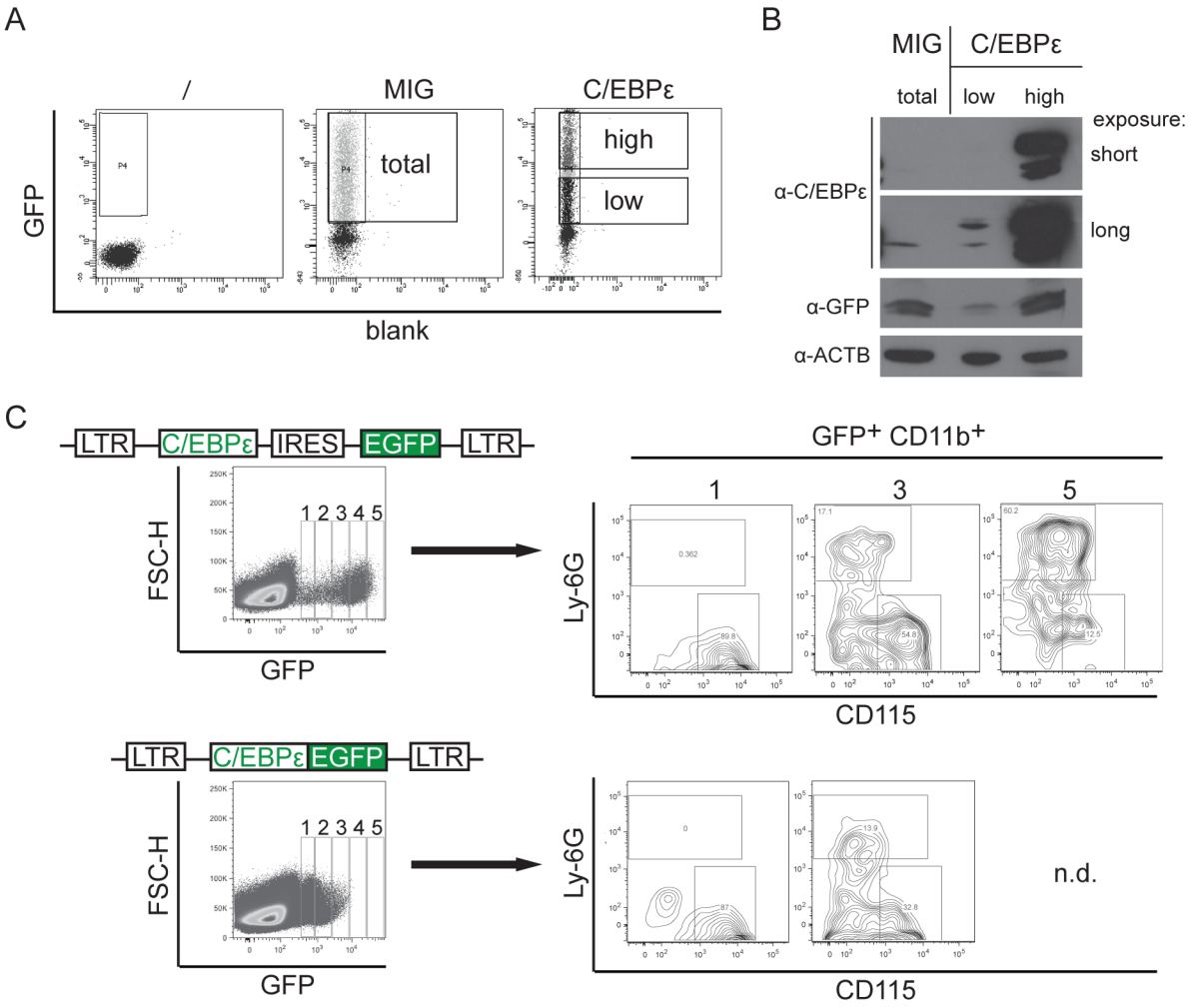

A
/
MIG
C/EBPε
GFP
total
high
low
blank
B
MIG
C/EBPε
total
low
high
exposure:
short
long
α-C/EBPε
α-GFP
α-ACTB
C
LTR
C/EBPε
IRES
EGFP
LTR
FSC-H
GFP
1 2 3 4 5
GFP+ CD11b+
1
3
5
Ly-6G
CD115
n.d.

Figure S4

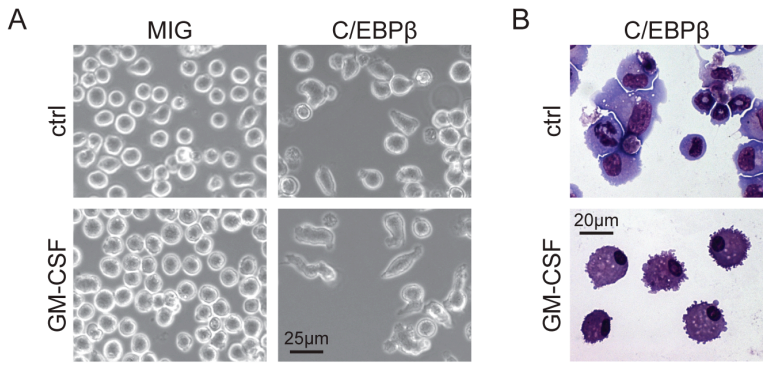


Figure S5

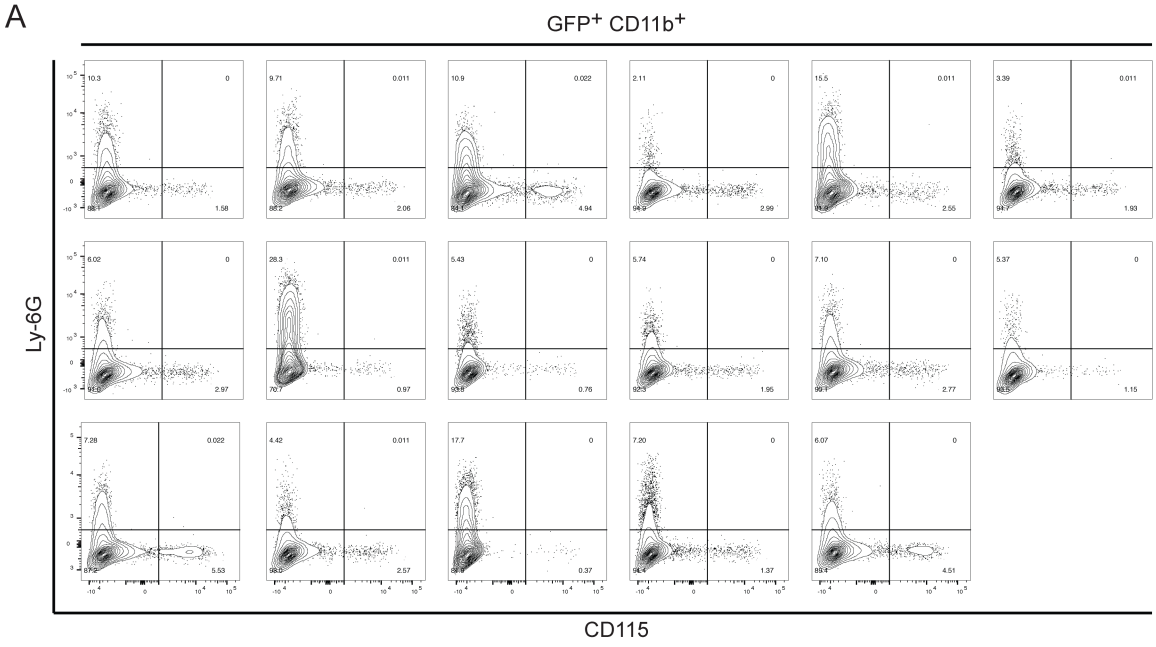


Figure S1: Lympho-myeloid conversion potential of C/EBP family members,

Related to Figure 1

(**A**) Immunoblot analysis of C/EBP family members of total protein lysates from Plat-E cells 72 h after transduction with C/EBP-MIG constructs. Untransduced cells served as control and ACTB as housekeeping gene. (**B**) Flow cytrometric analysis of B cells transduced with individual C/EBPs focusing on macrophage ($CD11b^+CD115^+$), dendritic ($CD11b^+CD11c^+$) and granulocytic ($CD11b^+Ly\text{-}6G^+$) markers 4 days after transduction. (**C**) Semi-quantitative RT-PCR analysis of C/EBP expression in murine primary FACS-purified B cells ($CD19^+$), granulocytes ($CD11b^+Ly\text{-}6G^+$) and macrophages ($CD11b^+CD115^+$). *Gapdh* served as housekeeping gene. (**D**) Flow cytometric analysis of C/EBP-transduced GFP^+-gated HAFTL1 cells 4 days after induction.

Figure S2: The role of endogenous *Cebpa* and *Cebpb* on transdifferentiation,

Related to Figure 3

(**A**) Semi-quantitative RT-PCR expression analysis of endogenous C/EBP family members in C10 cells treated with OHT for 4 days and non-induced controls (Bussmann et al. 2009). RNA from total bone marrow or monocytic Raw264.7 cells served as controls. *Gapdh* was used as housekeeping gene. (**B**) Expression kinetics of endogenous C/EBPα, and β protein in C/EBPε-transduced B cells based on immunoblot analysis. (**C**) Workflow for the generation of *Cebpa* and *Cebpb* double knockout v-Abl immortalized B cells and isogenic controls derived from a mouse with homozygous floxed *Cebpa* and *Cebpb* alleles. Deletion of *Cebpa* and *Cebpb* by incubating cells with recombinant Cre-recombinase. **(D)** *Cebpa* and *Cebpb* genotypes of established Cre-incubated cell clones was assessed by PCR. Pools of Cre-treated and untreated cells served as controls. Clone 4 was selected for further experiments.

(**E**) Quantification of transdifferentiated cells ($CD11b^+$, related to Figure 3C) from four independent experiments. ±SEM, **p<0.01. (**F**) Nanostring analysis showing upregulated genes 24 h after C/EBPε transduction in GFP^+-sorted cells compared to control B cells (MIG-transduced). Genes that were not upregulated in $Cebpa^{\Delta/\Delta}$;$Cebpb^{\Delta/\Delta}$ cells are marked in red. (**G**) Downregulated genes in GFP^+ FACS-purified control cells transduced for one day with C/EBPε compared to empty vector. Genes refractory to down-regulation in $Cebpa^{\Delta/\Delta}$;$Cebpb^{\Delta/\Delta}$ cells are marked in red. See also Table S1.

Figure S3: Effect of C/EBPε dosage on transdifferentiated cell type outcome, Related to Figure 4

(**A**) Sorting scheme of GFP^+ Plat-E cells transduced with empty vector control (MIG) or C/EBPε-MIG for 72 h. (**B**) Immunoblot detection of C/EBPε, GFP and ACTB in protein lysates of 1×10^6 FACS-purified cells from GFP-fractions in (A). Two exposure times (short and long) are shown. (**C**) B cell were transduced with pMSCV-based vectors either containing bi-cistronic C/EBPε-IRES-EGFP (top panels) or C/EBPε-EGFP fusion (bottom panels) transgenes. Transduced cells were classified according to GFP intensity and analysed for $CD11b^+$Ly-$6G^+$ and $CD11b^+CD115^+$ fractions.

Figure S4: Myeloid signalling-response of transdifferentiated bi-potential cells,

Related to Figure 5

(**A**) Bright field images of long-term C/EBPβ-transduced cells, untreated or treated with 10 ng/ml GM-CSF for 24 h. Empty vector (MIG) transduced B cells served as control. (**B**) Subsequent cytospin preparations and May-Grünwald-staining after 16 days of culture.

Figure S5: Persistence of the GMP-like phenotype after serial subcloning,

Related to Figure 5

Analysis of 17 subclones derived from a GMP-like parental clone presented in Figure 5G,H. Flow cytometric analysis of CD115 versus Ly-6G in the GFP+CD11b+-gated population indicates persistence of the GMP-like potential.

Supplemental Tables

Table S1: Gene expression signature in C/EBP-transduced B cells,

Related to Figure 2, 3

Nanostring analysis (gene set GXA-MIM) of GFP^+ B cells (*Cebpa*$^{f/f}$;*Cebpb*$^{f/f}$) transduced with individual C/EBPs for 24 h. C/EBPε-infected *Cebpa*$^{\Delta/\Delta}$;*Cebpb*$^{\Delta/\Delta}$ B cells (B-DKO) were included for comparison. MIG-transduced cells served as control. Normalized gene expression values are shown.

Table S2: List of primer sequences,

Related to Experimental Procedures, Figure 4, Figure S1, S2

Oligonucleotide	Sequence 5'-3'
Cebpa FW	GCCAGTTGGGGCACTGGGTG
Cebpa RV	CCGCGGCTCCACCTCGTAGA
Cebpb FW	GCGTTCATGCACCGCCTGCT
Cebpb RV	TAGGCCAGGCAGTCGGGCTC
Cebpd FW	AGAACCCGCGGCCTTCTAC
Cebpd RV	ATGTAGGCGCTGAAGTCGAT
Cebpe FW	CACACTGCGGGCAGACAG
Cebpe RV	GTGCCTTGAGAAGGGGACT
Gapdh FW	AATGTGTCCGTCGTGGATCTGA
Gapdh RV	GATGCCTGCTTCACCACCTTCT
Cebpa flox FW	TGGCCTGGAGACGCAATGA
Cebpa flow RV	CGCAGAGATTGTGCGTCTTT
Cebpa Δ FW	GCCTGGTAAGCCTAGCAATCCT
Cebpa Δ RV	TGGAAACTTGGGTTGGGTGT
Cebpb flox/Δ FW	GAGCCACCGCGTCCTCCAGC
Cebpb flox RV	GGTCGGTGCGCGTCATTGCC
Cebpb Δ RV	AGCAGAGCTGCCCCGGCAAA
DQ52 FW1	ACGTCGACTTTTGYAAGGGATCTACTACTGT
DFS FW2	ACGTCGACGCGGACGACCACAGTGCAACTG
JH4A RV	GGGTCTAGACTCTCAGCCGGCTCCCTCAGGG