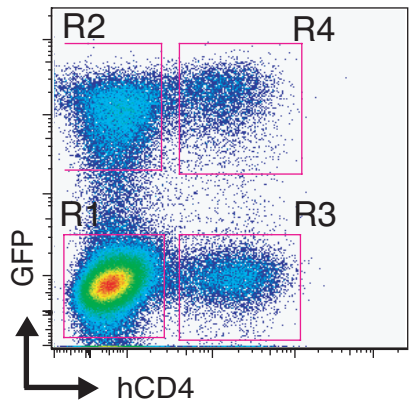
Supporting Information

Feng *et al.* 10.1073/pnas.0711961105



R1, uninfected
R2, PU.1 infected
R3, C/EBPα infected
R4, PU.1 and C/EBPα co-infected

Fig. S1. FACS plot of NIH 3T3 cells infected with PU.1-GFP and C/EBPα viruses for 7 days. The gates show uninfected, singly infected, and doubly infected cells.

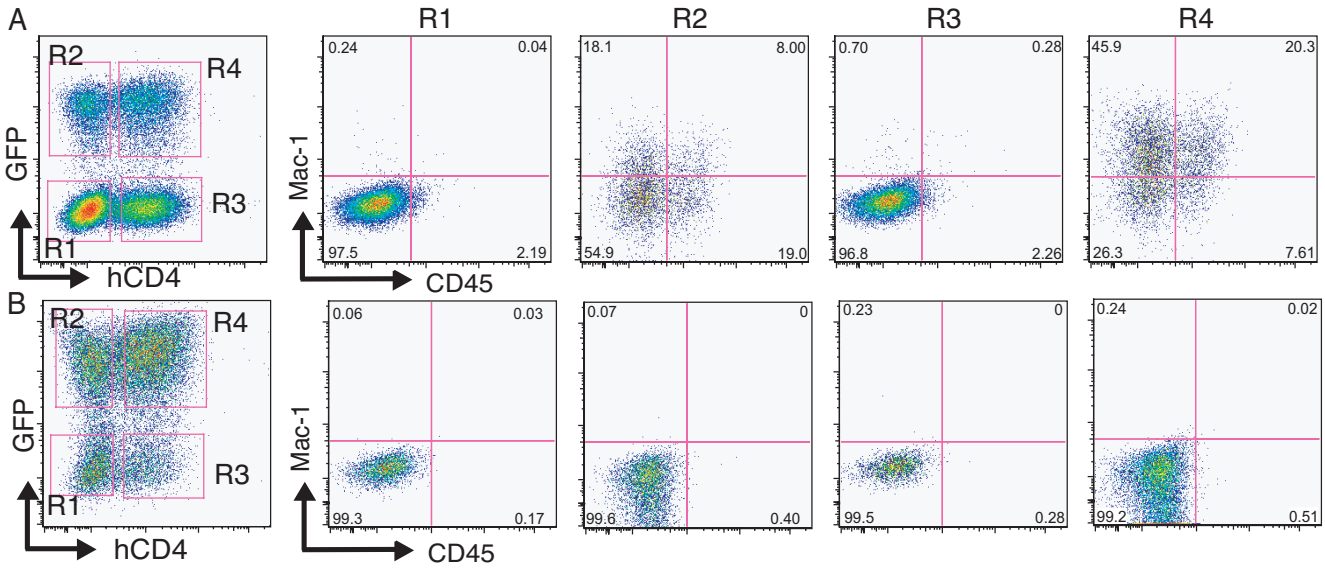


Fig. S2. FACS plots of NIH 3T3 cells coinfected with PU.1-GFP and C/EBPβ hCD4 (*A*) and control empty viruses (*B*). The gates in the plot at the far left show uninfected cells (R1), single GFP infected (R2), single hCD4 infected (R3), and double infected cells (R4). The other plots show Mac-1 and CD45 expression in the four gates selected.

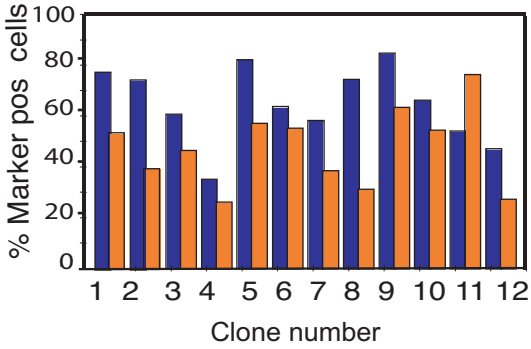


Fig. S3. Experiment showing that subclones of NIH 3T3 cells are susceptible to transcription factor-induced conversion. Shown is Mac-1 (blue bars) and CD45 (orange bars) expression in NIH 3T3 subclones coexpressing PU.1 and C/EBPα viruses 7 days after infection. None of the subclones expressed Mac-1 or CD45 before infection.

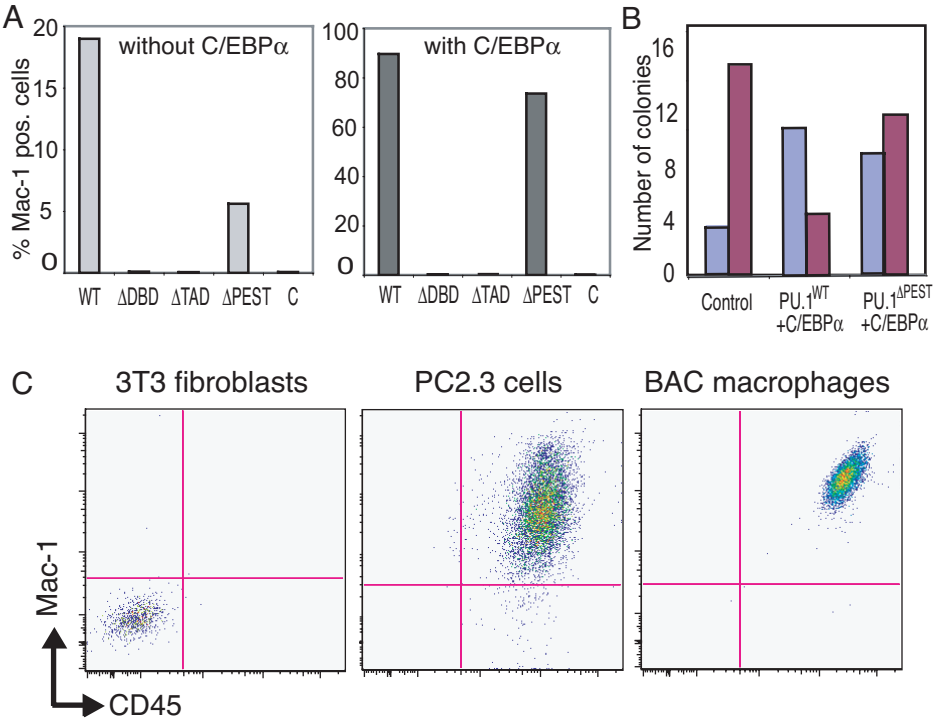


Fig. S4. PU.1 domains required for Mac-1 up-regulation and growth of colonies infected with PU.1 viruses. (*A*) Up-regulation of Mac-1 in 3T3 cells infected with wild-type PU.1 (WT), a deletion in the DNA binding domain (DBD), in the transcativation domain (ΔTAD), and in the PEST domain (ΔPEST), in the absence or presence of C/EBPα coinfection. (*B*) Uninfected control or doubly infected cells were sorted and seeded singly into 96-well plates. Colony formation was scored 10 days after seeding. The number of colonies with $<$500 cells are indicated by blue bars, and colonies with $>$500 cells are indicated by purple bars. (*C*) FACS profiles of PC2.3 cells and control cells showing expression of Mac-1 and CD45.

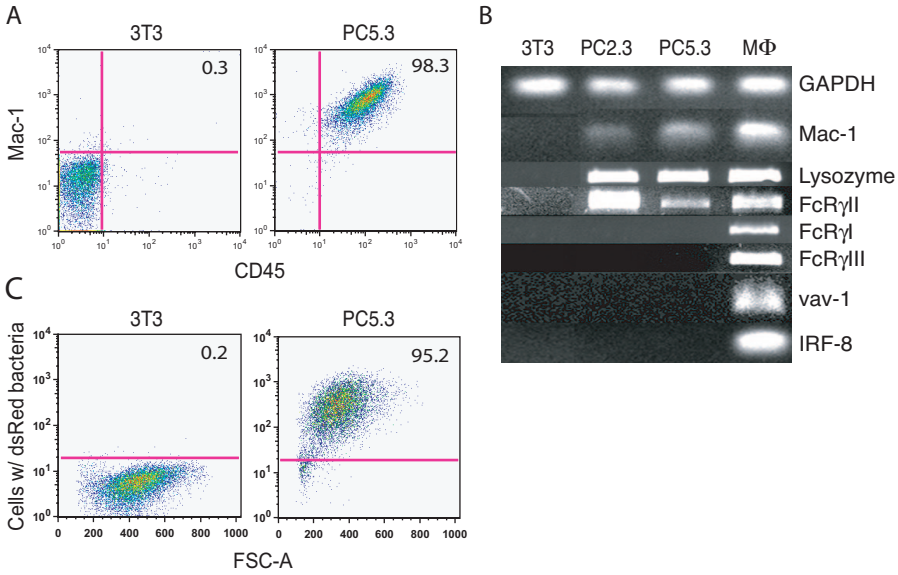


Fig. S5. Characterization of PC5.3 cells. (*A*) FACS profiles showing Mac-1 and CD45 expression. (*B*) Gene expression analysis by RT-PCR relative to GAPDH in several cell types, using the RAW macrophage cell line as a control. (*C*) FACS-based phagocytosis assay using dsRed *E. coli*. The numbers show the percentage of cells that have taken up the bacteria.

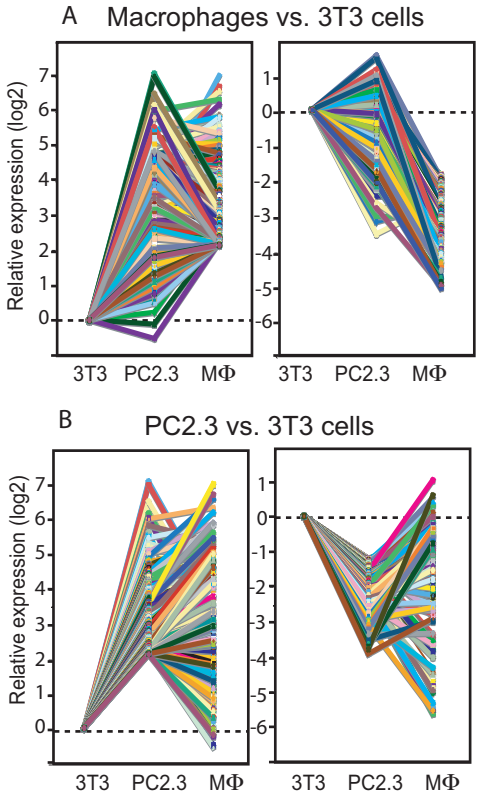


Fig. S6. Relative gene expression in 3T3 versus PC2.3 and BAC macrophage cells based on oligonucleotide cDNA arrays. (*A*) The diagrams show the 250 most highly and most lowly expressed genes in macrophages versus 3T3 cells and the corresponding relative levels in PC2.3 cells. (*B*) As in *A*, but showing the 250 most up- or down-regulated genes in PC2.3 cells.

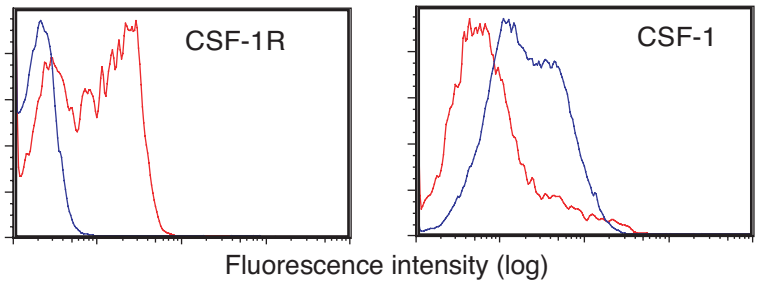


Fig. S7. FACS analysis of CSF-1R and CSF-1. Blue lines, 3T3 cells; red lines, PC2.3 cells.

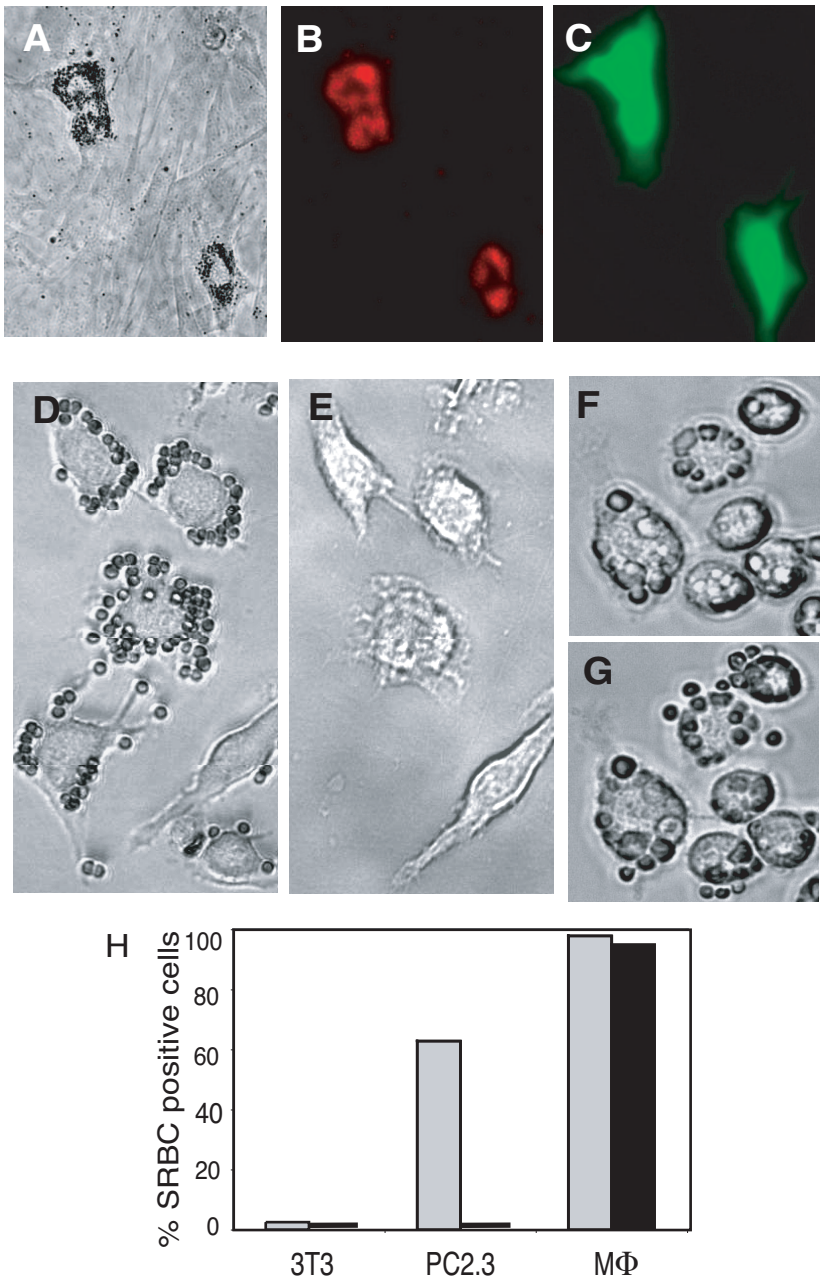


Fig. S8. Phagocytic capacity of PC2.3 cells. (*A–C*) Brightfield and fluorescent images of primary mouse embryo fibroblasts infected with PU.1-EGFP and C/EBPa-hCD4 viruses for 11 days, incubated with red fluorescent beads, and washed. (*B*) Red fluorescent beads. (*C*) GFP fluorescence. (*D–G*) phagocytosis of sheep red blood cells (SRBCs). (*D*) PC2.3 cells incubated with antibody coated SRBCs, showing formation of rosettes. (*E*) Cells in *D* after treatment with lysis buffer to remove noninternalized SRBCs. (*F* and *G*) BAC macrophages incubated with opsonized SRBCs before and after lysis, respectively. (*I*) Quantification of the data, with the percentage of rosette forming cells in gray and cells that ingested SRBCs in black.

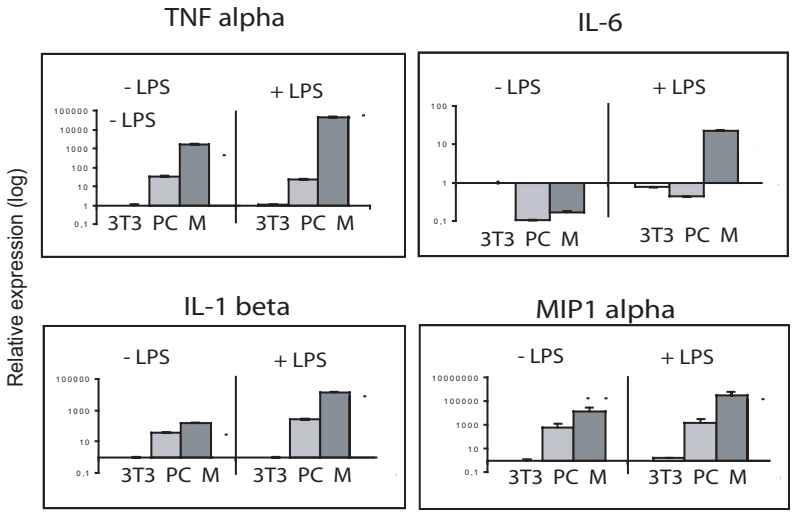


Fig. S9. Inflammatory response. NIH 3T3 cells and PC2.3 and BAC macrophages were treated with 1 μg/ml LPS for 6 h, and RNA was isolated. The data show quantitative RT-PCR results of triplicate samples for the expression of four cytokines relative to untreated 3T3 cells.

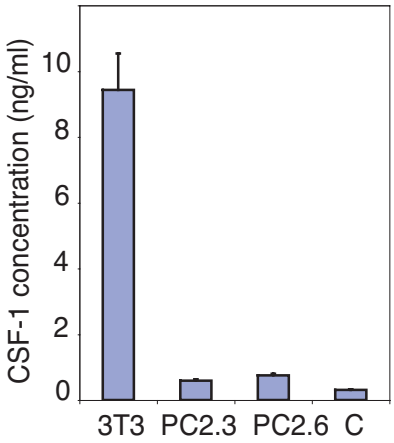


Fig. S10. CSF-1 production. NIH 3T3 cells and PC2.3 and BAC macrophages were washed and incubated overnight with growth medium, and the CSF-1 concentration in the supernatants was tested by RIA. Bars indicate standard deviation of triplicate samples.

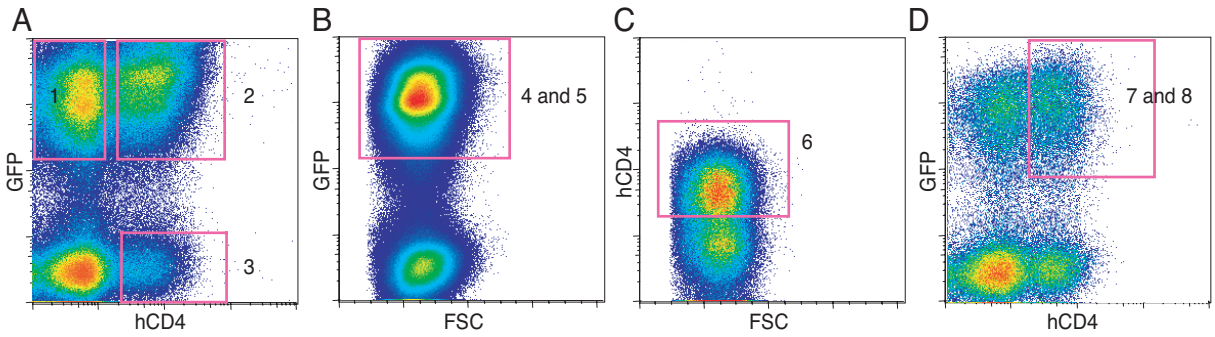


Fig. S11. (*A*) FACS plots of NIH 3T3 cells infected for 4 days with a combination of control vectors (*A*), PU.1 alone (*B*), C/EBPa alone (*C*), and a combination of PU.1 and C/EBPa (*D*). The numbers next to the red squares indicate gates used to sort fractions for isolation of mRNA analyzed in Fig. 5*D* (profiles of fraction 5 and 8 cells are not shown). FSC, forward scatter.

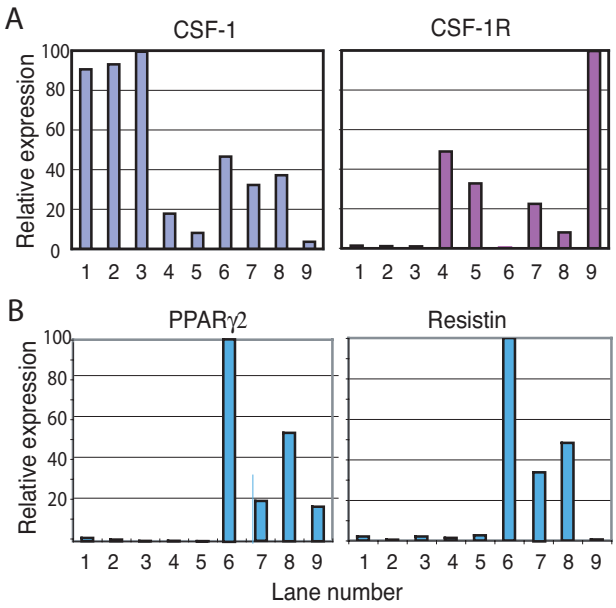


Fig. S12. Quantification of the data shown in Fig. 5*D*. Gels were scanned by densitometry. Values were plotted relative to the highest number obtained.

Table S1. Number of genes that are up or down-regulated at least 2-fold in BAC1.2F5 macrophages relative to NIH 3T3 cells

Variables	Up-regulated in BAC MΦ	Down-regulated in BAC MΦ
No. of genes	960	1,052
Fold change	5.3	0.34
No. of genes also changed in PC2.3 cells	620	400
Fold change	3.9	0.38

Comparisons of relative gene expression, based on two-color cDNA array analysis, were made for BAC macrophages versus 3T3 cells. The table shows the number of genes that are up- or down-regulated at least 2-fold in BAC1.2F5 macrophages relative to NIH 3T3 cells. The table also indicates the average fold expression changes.

Table S2. Number of genes that are up or down-regulated at least 2-fold in PC2.3 cells relative to NIH 3T3 cells

Variables	Up-regulated in PC2.3 cells	Down-regulated in PC2.3 cells
No. of genes	954	400
Fold change	5.3	0.38
No. of genes also changed in BAC cells	597	231
Fold change	5.6	0.49

Comparisons of relative gene expression, based on two-color cDNA array analysis, were made for PC2.3 cells versus 3T3 cells. The table shows the number of genes that are up- or down-regulated at least 2-fold in PC2.3 cells relative to NIH 3T3 cells. The table also indicates the average fold expression changes.