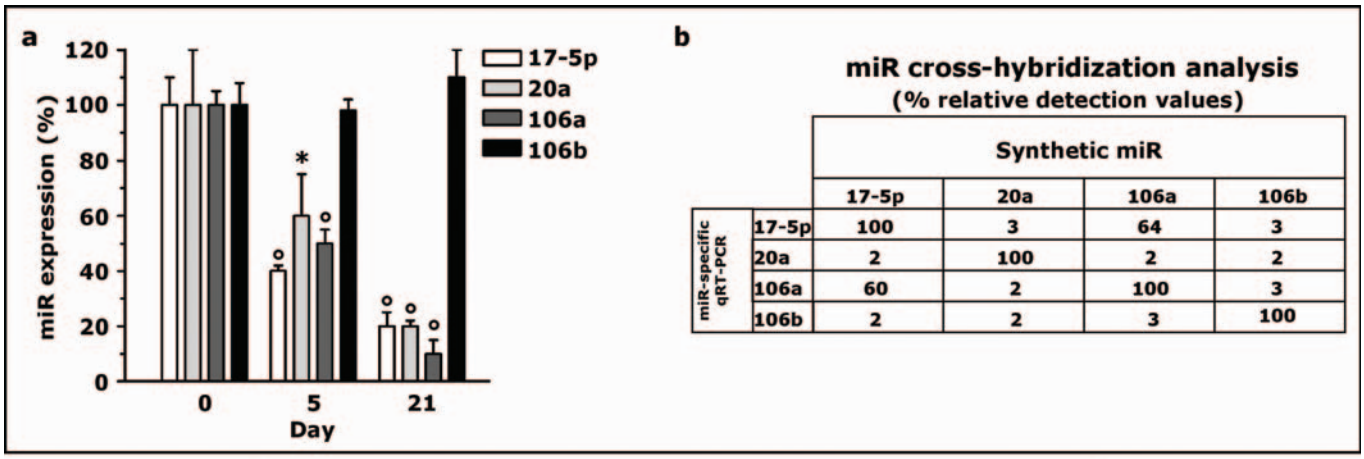

Figure S1 miR-17-5p/20a/106a expression in Mo and E cultures and cross-hybridization studies among miR-17-5p/20a/106a/106b. (**a**) miR-qRT-PCR analysis of miR-17-5p, -20a, -106a and -106b (normalized to U6) in CD34+ HPCs (day 0) and at sequential days of Mo culture (mean ± s.d., n=3). Percent expression values are shown. * $P < 0.05$, ° < 0.001. (**b**) miR-qRT-PCR analysis of RNA from Jurkat cells transfected with synthetic miR-17-5p, -20a, -106a or -106b (160 nM) at 48 hr post-transfection. miR-specific assays were used for detection of each miRNA. Percent values are indicated. (**c**) Northern blot of miR-17-5p, -20a and -106a expression (left) and Western blot analysis of AML1 expression (right) in CD34+ HPCs (day 0) and unilineage E culture at sequential culture days. Representative experiments out of three are presented.

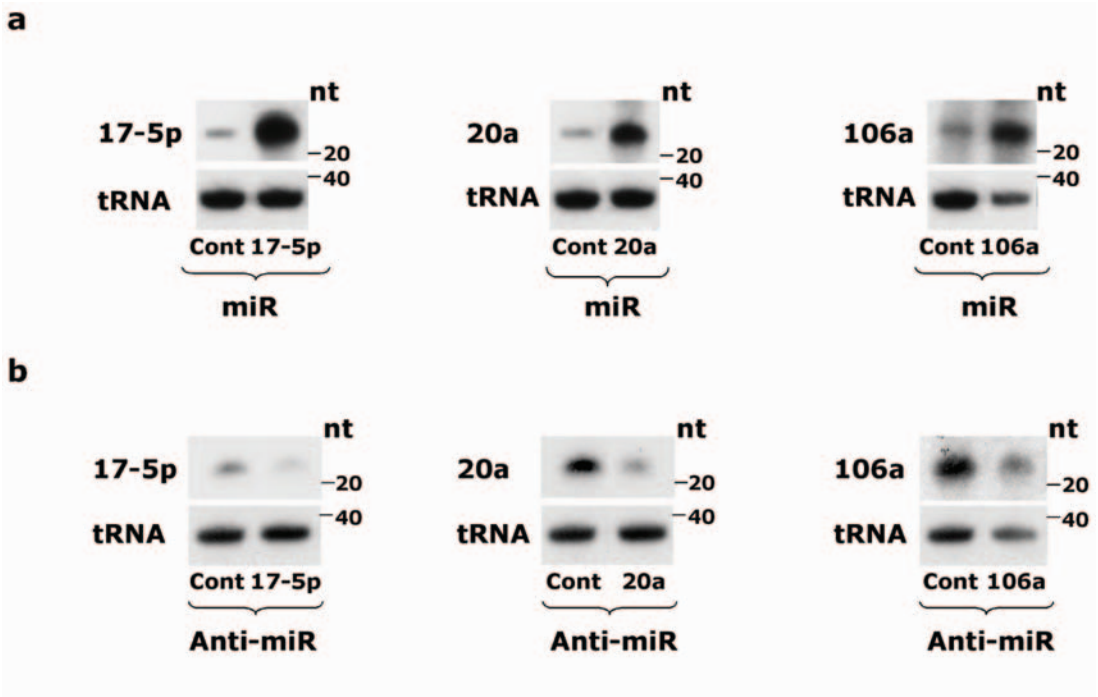

Figure S2 Overexpression of miR-17-5p, -20a and -106a or anti-miR oligonucleotides recognizing these miRs in Jurkat cell line. (**a**) Northern blot analysis of RNA from Jurkat cells transfected with miR-17-5p, -20a and -106a or a control scrambled oligonucleotide (Cont) (160 nM) at 48 hr post-transfection. Met-tRNA was used as a loading control. A representative experiment out of three is presented. (**b**) Northern blot analysis of RNA from Jurkat cells transfected with anti-miR oligonucleotides recognizing miR-17-5p, -20a and -106a (160 nM) at 48 hr post-transfection. A representative experiment out of three is shown.

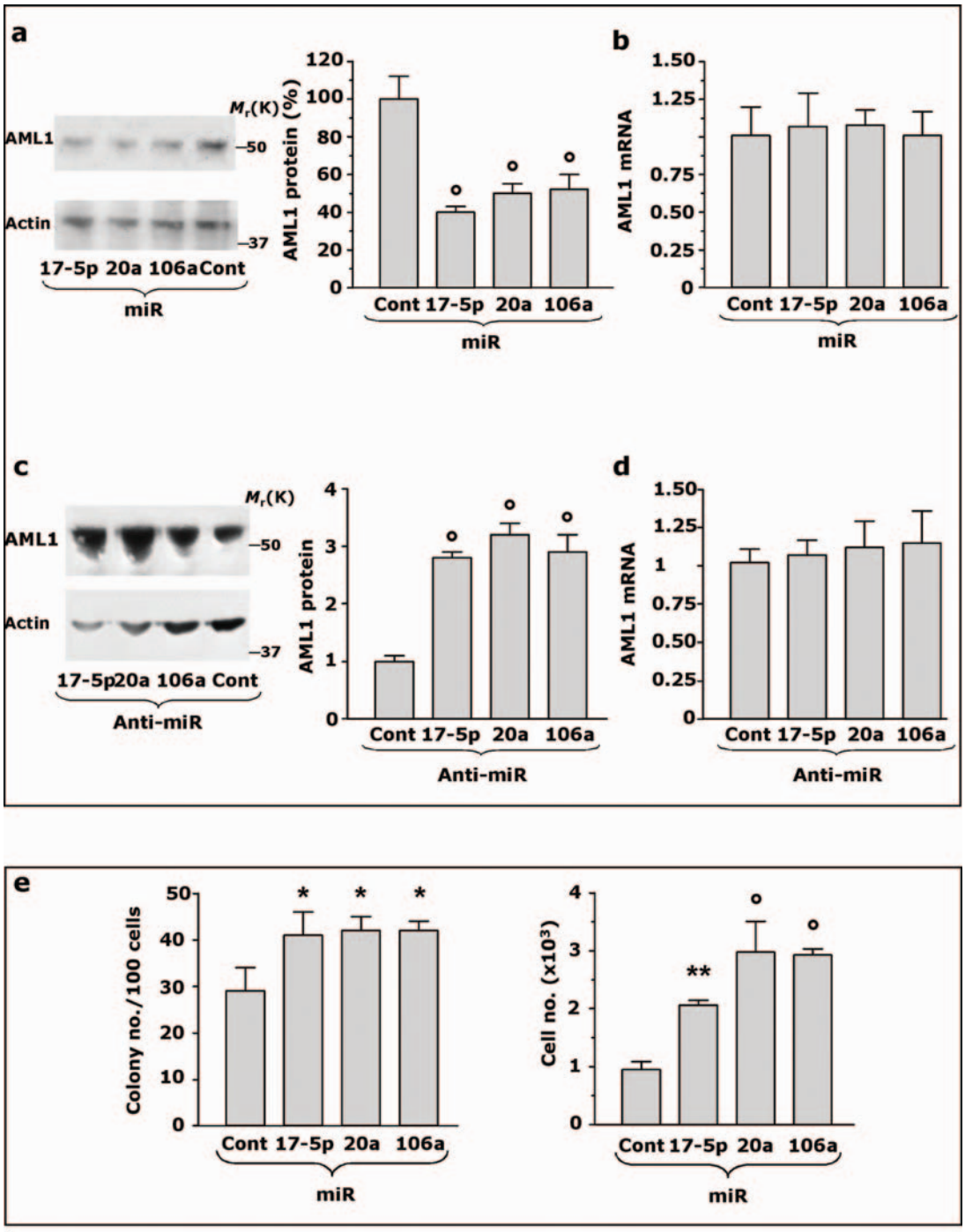

Figure S3 AML1 expression is translationally regulated by miR-17-5p, -20a and -106a in Jurkat cell line. (**a**) *Left panel.* AML1 protein expression by Western blot in Jurkat cells transfected with miR-17-5p, -20a and -106a or a control scrambled oligonucleotide (Cont) (160 nM), 72 hr post-transfection. Actin was used for normalization. A representative experiment out of three is shown. *Right panel.* Quantification of Western blot analysis from three independent experiments. Percentage of normalized AML1 is indicated; mean ± s.d., n = 3. (**b**) AML1 mRNA, measured by Real-Time PCR, in Jurkat cells transfected with miRs; mean ± s.d., n = 3. (**c**) *Left panel.* AML1 protein expression by Western blot in Jurkat cells transfected with anti-miR oligonucleotides recognizing miR-17-5p, -20a and -106a or a control anti-miR oligonucleotide (Cont) (160 nM) at 72 hr post-transfection. A representative experiment out of three is presented. *Right panel.* Quantification of Western blot analysis of AML1 protein in three independent experiments (fold induction of normalized values, mean ± s.d., n = 3). (**d**) AML1 mRNA level in Jurkat cells transfected with anti-miR oligonucleotides (mean ± s.d., n = 3). (**e**) Overexpression of miR-17-5p, -20a and -106a (300 nM) in Mo culture affects Mo colony formation. *Left panel.* Number of Mo colonies generated per 100 cells seeded (mean ± s.d., n = 3). *Right panel.* Number of cells per colony (mean ± s.d., n = 3). * P < 0.05, ** < 0.01, ° < 0.001 when compared to control groups.

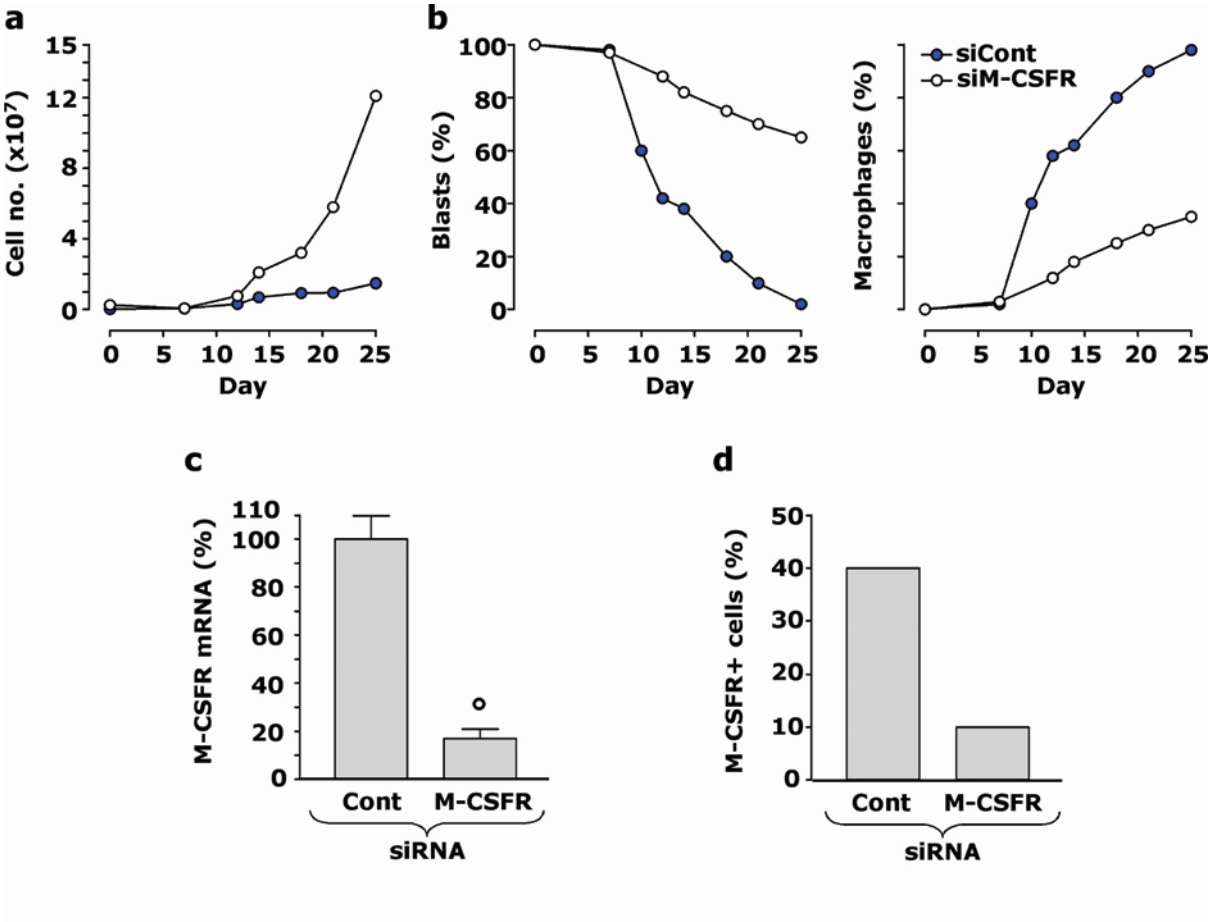

Figure S4 Inhibition of M-CSFR expression by small-interfering RNA (siRNA) treatment affects monocytopoiesis. A representative experiment out of three is presented (except for panel c). (**a**) Growth curve of Mo culture cells transfected with siRNA targeting M-CSFR mRNA (siM-CSFR) or a control oligonucleotide (siCont). (**b**) Wright-Giemsa staining of transfected Mo at various days of differentiation: percentage of blasts (left) and macrophages (right) is shown. (**c**) M-CSFR mRNA expression by Real-Time PCR in transfected cells at 72 hr post-transfection (mean ± s.d., n = 3). ° $P < 0.001$ when compared to control. (**d**) FACS analysis of M-CSFR expression in transfected cells on day 10 of Mo culture. Percent values of M-CSFR cells are presented.

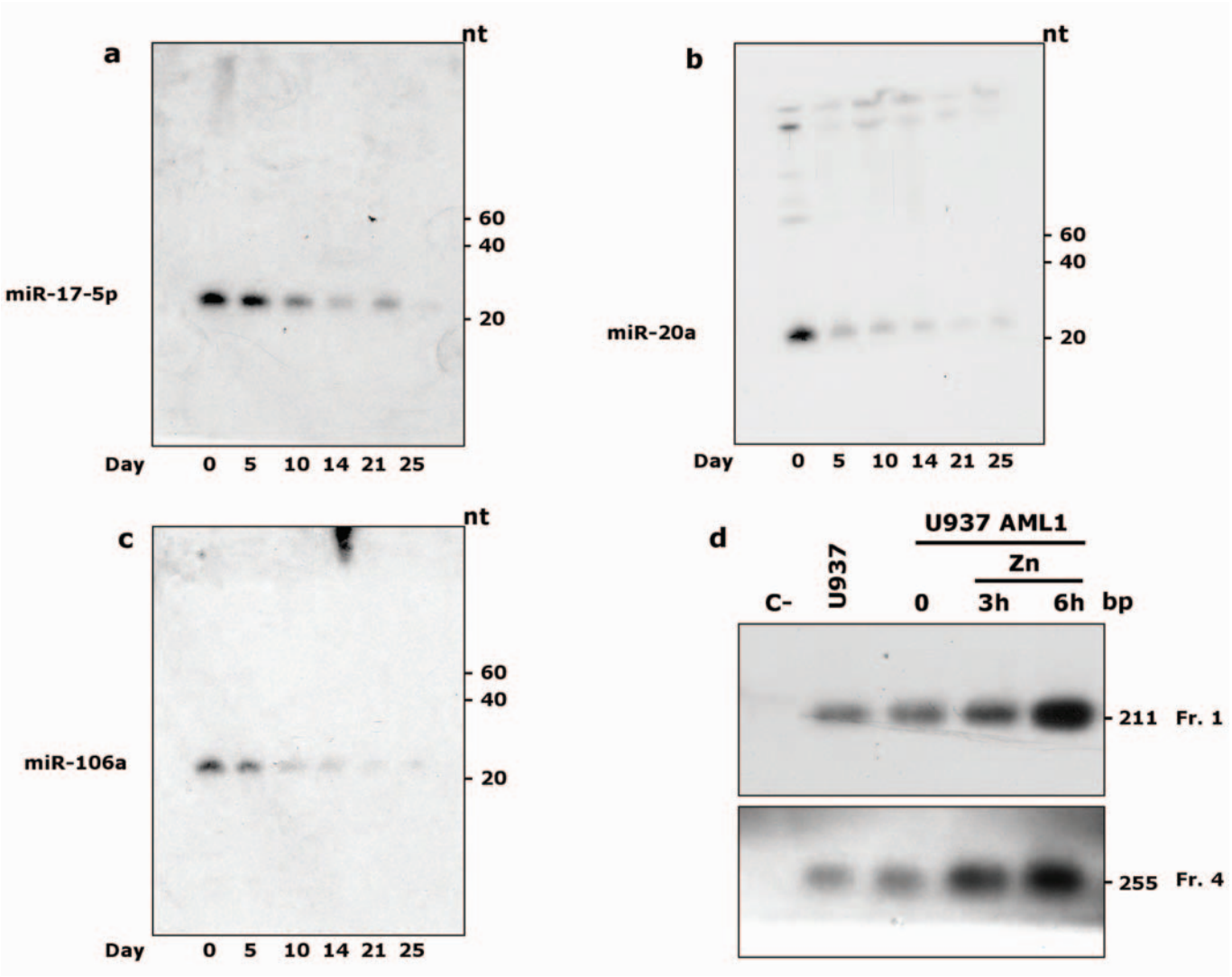

Figure S5 Full length scan of gels showed in Figure 1 (panels a-c) and Figure 7 (panel d).

Gene Symbol	Gene Id	Gene Accession	Scale	Pool Catalog Number	Duplex Catalog Number	Sequence
RUNX1	861	NM_001754 (RUNX1)	5 nmol	L-003926-00	J-003926-05	UGACAACCCUCUCUGCAGA
RUNX1	861	NM_001754 (RUNX1)	5 nmol	L-003926-00	J-003926-06	GAAACUAGAUGAUCAGACC
RUNX1	861	NM_001754 (RUNX1)	5 nmol	L-003926-00	J-003926-07	CGAUAGGUCUCACGCAACA
RUNX1	861	NM_001754 (RUNX1)	5 nmol	L-003926-00	J-003926-08	CAAAUGAUCUGGUGGUUAU
CSF1R	1436	NM_005211 (CSF1R)	5 nmol	L-003109-00	J-003109-10	GGAAGAUCAUCGAGAGCUA
CSF1R	1436	NM_005211 (CSF1R)	5 nmol	L-003109-00	J-003109-11	GGUGAAGGAUGGAUACCAA
CSF1R	1436	NM_005211 (CSF1R)	5 nmol	L-003109-00	J-003109-12	GUAACGUGCUGUUGACCAA
CSF1R	1436	NM_005211 (CSF1R)	5 nmol	L-003109-00	J-003109-13	CCAGCAGCGUUGAUGUUAA