LEGEND TO SUPPLEMENTARY FIGURES

Supplementary Fig. 1 Pin1 is present in the transcriptional complex binding to CD38 in HL-60 cells

HL-60 cells were treated with vehicle (DMSO) or ATRA (1 μM) for 2 hours. Chromatin immuno-precipitation (CHIP) assays were performed using anti-RARα, anti-Pin1 antibodies or the relative IgG control. In these experiments, the target DNA (**CD38-RARE**) is part of the *CD38* gene containing a functional RARE (intron 1). A portion of the CD38 coding region (**CD38-Coding**) was used as a negative control of the experiment . Input = amplification of the CD38 bands in the chromatin extracts of HL-60 cells before immuno-precipitation. The expected 81 (**CD38-RARE**) and 75 (**CD38-Coding**) base-pair bands amplified are indicated by arrows on the left. Amplification of the CD38 bands in chromatin extracts of human placenta were used as positive controls for the experiment. CHIP assays were performed with a commercial kit (catalog No. 17-295, Millipore, Vimodrone, Italy) using rabbit polyclonal anti-RARα (SC551X, Santa Cruz Biotechnologies, San Diego, CA) and anti-Pin1 (catalog No. 07-091, Millipore) antibodies. The amplimers used for the amplifications of the CD38 gene sequence containing the retinoid-responsive element (Kishimoto, H., Hoshino, S., Ohori, M., Kontani, K. *et al.*, J. Biol Chem, 1998, 273: 15429-15434) were: 5'TTCCGCCCGCTGTCTCT3', corresponding to nucleotide 729-745 of intron 1; 5'CCTACCACAGGCTTTGCAAAC3', complementary to nucleotide 789-809 of intron 1. The amplimers used for the amplification of the CD38 coding region were: 5'CCTGCTGCCGGCTCTCTAG3', corresponding to nucleotide 41-59 of exon 1 (numbering from the first ATG codon); 5'GCACCACGACGAGGATCAG3', complementary to nucleotide 97-115 of exon 1.

Supplemntary Fig. 2 Pin1 overexpression does not alter the steady-state levels of RXRα

(**A**) COS-7 cells were transfected with the RARα cDNA (0.1 μg), a RARE-tk-Luc reporter (1 μg) and the renilla luciferase normalization plasmid (0.5 μg), in the presence or absence of the Pin1 cDNA (0.4 μg) and/or RXRα (0.1 μg). Cells were treated with vehicle (DMSO) or ATRA (1 μM). The activity of firefly luciferase was measured in cell extracts and normalized for transfection efficiency using renilla luciferase. Results are expressed in fold induction, which is calculated as the ratio of the luciferase activity measured in extracts of cells treated with ATRA and the corresponding DMSO control. Each value is the mean ± S.D. of duplicate transfections. (**B**) COS 7 cells were co-transfected and treated as in (**A**). Twentyfour hours after transfection, cells were treated with vehicle (DMSO) or ATRA for further 24 hours. Western blot analyses were conducted on cell extracts with anti-RARα, anti-RXRα, anti-Pin1 and anti-β-actin antibodies.

Supplementary Fig. 3 Pin1 silencing alters myeloid differentiation markers in NB4 and HL-60 cells

NBT-reductase activity (*pR-PIN-NB4*, *pR-NB4*) or the percentage of NBT-reductase-positive cells (*pR-PIN-HL-60*, *pR-HL-60*) was determined after treatment of the blasts with vehicle or the indicated concentrations of ATRA for 4 days. Each value is the mean ± S.D. of three biological replicates consisting of independent cell cultures. The results are representative of two experiments. Aliquots of the same cell populations were also subjected to FACS analysis for the indicated surface markers. The percentage of CD11b-, CD11c- or CD38-positive cells and the corresponding mean cell-associated fluorescence (MAF) are shown by the bar graphs. The bar graphs summarize the results of one representative experiment.

Supplementary Fig. 4 Pin1 silencing alters the growth of NB4 cells

(A) The panel shows the growth curves of *pR-NB4* and *PR-PIN-NB4* cells. Each time point is the mean ± S.D. of three replicate cultures. (**B**) The *pR-NB4* and *PR-PIN-NB4* cell lines were treated for the amount of time and in the experimental conditions indicated. The number of viable

cells was determined manually after staining with trypan blue. Up until 3 days, the viability of *pR-PIN-NB4* and *pR-NB4* cells was over 90% in all the experimental conditions. Each time point is the mean ± S.D. of three replicate cultures. *Significantly lower relative to the corresponding vehicle-treated group (Student t-test, $p<0.01$).

Supplementary Fig. 5 Effects of Pin1 inhibition by PiB in NB4 and HL-60 cells

Left panels: NB4 cells were treated with the indicated concentrations of PiB and/or ATRA for 2 days as indicated. Cell extracts (50 μg of protein) were subjected to Western blot analysis with anti-STAT-1 antibodies recognizing the total (STAT-1) or the tyrosine-phosphorylated and active (STAT-1-P) pools of the protein, anti-cEBPβ and anti-β-actin antibodies. Right panels: Cell extracts of HL-60 cells treated for 2 days as indicated were subjected to Western blot analysis with anti-STAT-1, anti-cEBPβ and anti-β-actin antibodies.

Supplementary Fig. 6 Pin1 inhibition by PiB stimulates the differentiating and anti-proliferative effects of ATRA in PR-9 cells expressing PML-RARα

(**A**) The Western blot shows that the expression of PML-RARα is induced after treatment with 100 μM Zinc sulfate (Zn^{++}) for 24 hours. Co-treatment with Zn^{++} and ATRA causes degradation of PML-RARα. Actin is used to show that equivalent amounts of proteins were loaded on the gel. (**B**) PR-9 cells were treated for 24 hours with the indicated compounds along with Zn^{++} to induce the expression of PML-RARα. Western blot analyses were conducted with anti- RARα and anti-β-actin antibodies. C = NB4 control cell extracts. The asterisk on the left indicates a non-specific band recognized by the anti-RARα antibody. (**C**) The number of NBT-reductase-positive cells was determined after treatment of PR-9 cells. Each value is the mean ± S.D. of three replicate cultures. * Significantly higher relative to the group treated with ATRA alone (Student T-test, $p<0.01$). (**D**) PR-9 cells were treated for 4 days with the indicated compounds in the presence of Zn^{++} to induce

the expression of PML-RARα. The number of viable cells was counted manually after staining with trypan blue. Each value is the mean ± S.D. of three replicate cultures.

* Significantly lower relative to the groups treated with ATRA or PIB alone (Student t-test, $p<0.01$).

Supplementary Fig. 7 Effect of Pin1 inhibition on the NB4 cell cycle

Representative FACS analyses of NB4 cells seeded at 150,000 cells / ml and treated with vehicle (C) or the indicated compounds and combinations thereof, after staining with propidium iodide as described (Valli C, Paroni G, Di Francesco AM, Riccardi R, *et al.* Mol Cancer Ther. 2008; 7: 2941-2954). The percentage of cells in the G1, S and G2/M phase of the cycle is shown in bold characters inside each panel. The results are representative of two replicate cultures and the percentage of cells in each phase of the cell cycle determined in each experimental group does not vary significantly between replicates.

Supplementary Fig. 8 Pin1 inhibition by PiB does not sensitize ATRA-resistant NB4.007 and HL-60R cells to the retinoid

The ATRA-resistant NB4.007 (**A**) and HL-60R (**B**) cell lines were treated for four days as indicated. The number of viable cells was determined manually after staining with trypan blue (left panels), while the number of NBT-reductase-positive cells was determined after challenge with NBT and 4α-phorbol 12-myristate 13-acetate for 30 minutes (right panels). Each value is the mean ± S.D. of three replicate cultures.

Supplementary Fig. 9 Effects of Pin1 inhibition by PiB in freshly isolated AML cells

Right upper panel: Blasts from patient 2 (500,000 cells/ml) were treated with the indicated concentrations of ATRA and/or PiB for 4 days. Cell extracts were subjected to Western blot analysis with anti-cEBPβ, anti-STAT-1, and anti-β-actin antibodies. Left upper panel: Blasts from

patient 3 (500,000 cells/ml) were treated with the indicated concentrations of ATRA and/or PiB for 4 days. The amount of NBT-reductase activity is shown on the left. Each value is the mean $\pm$ S.D. of three replicate cultures. * Significantly higher relative to the group treated with ATRA (Student t-test, $p<0.01$). Western blots performed with anti-Pin1 and anti-β-actin antibodies are also shown. Lower graphs and panels: Blasts (500,000 cells/ml) from patient 5 were treated as indicated for 4 days. The percentage of CD11c-positive cells along with the corresponding MAF (numbers in parenthesis) is shown in the bar-graph. The levels of STAT-1, CEBPβ and β-actin proteins were determined by Western blot analysis.