# Overexpression of *SET* is a recurrent event associated with poor outcome and contributes to protein phosphatase 2A inhibition in acute myeloid leukemia

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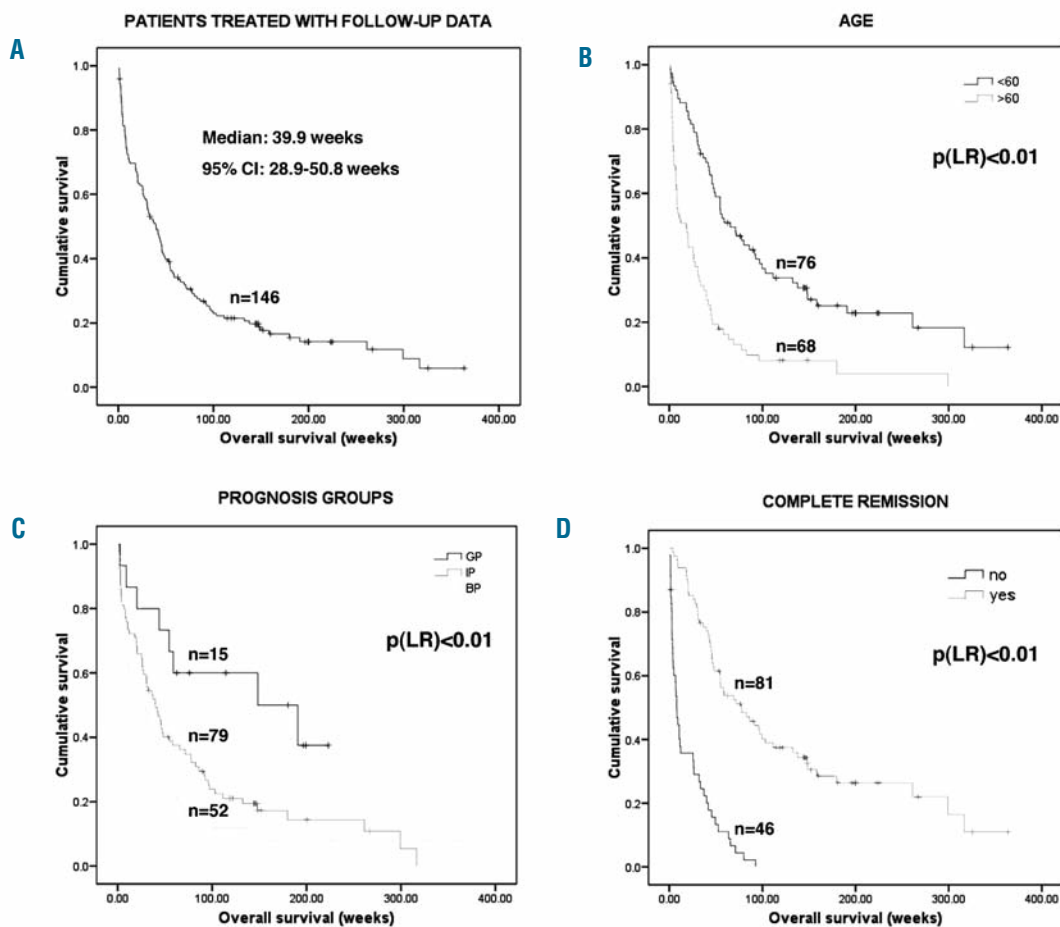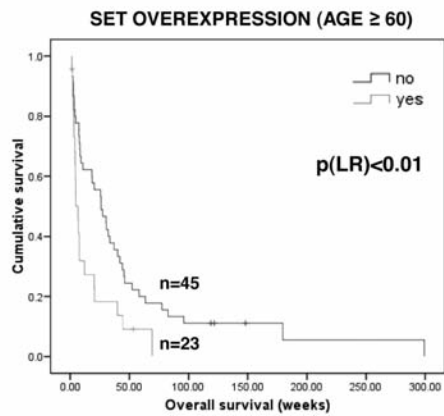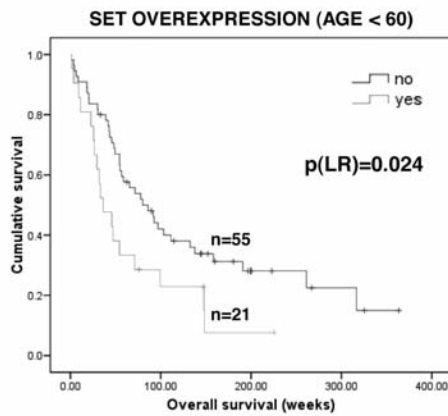
Online Supplementary Figure S1. Kaplan-Meier analyses of overall survival in the cohort of 146 treated patients with available clinical follow-up data. (A) Survival graph of the global cohort. An inferior outcome was observed in patients older than 60 years (B), patients included in the intermediate cytogenetic group (C), and patients who did not achieve a complete remission (D).
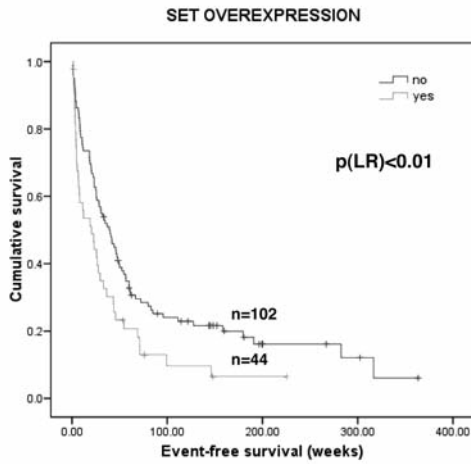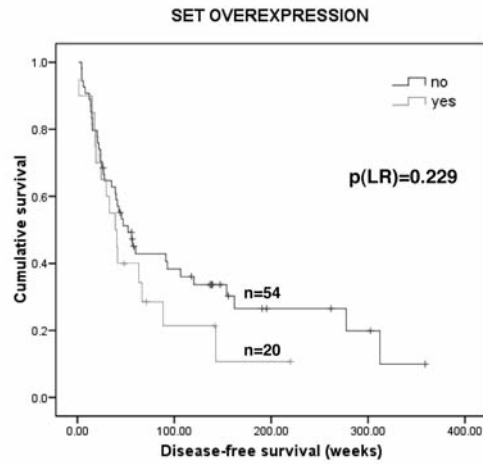
Online Supplementary Figure S2. Kaplan-Meier analysis in a series of 146 patients with AML. (A) Event-free survival. (B) Disease-free survival.
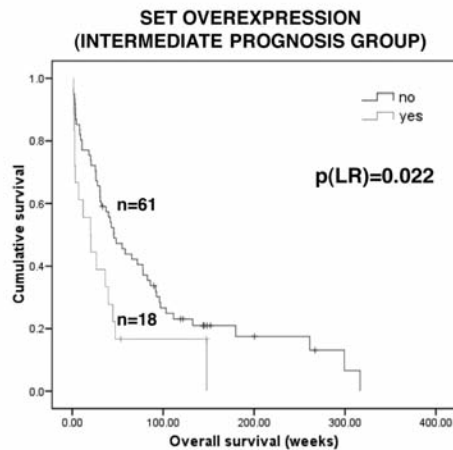

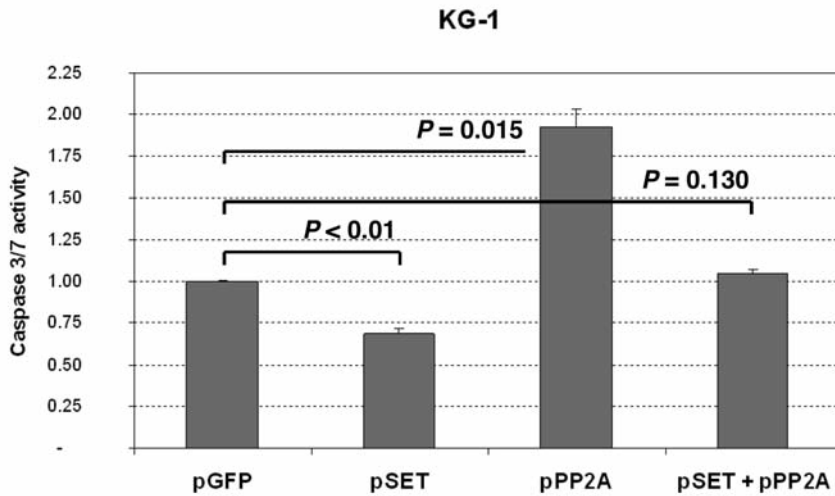
Online Supplementary Figure S3. Kaplan-Meier analyses of overall survival for SET overexpression in a series of 146 patients with AML. (A) Analyses of age-stratified patients. (B) Analyses of patients included in the intermediate prognosis group.
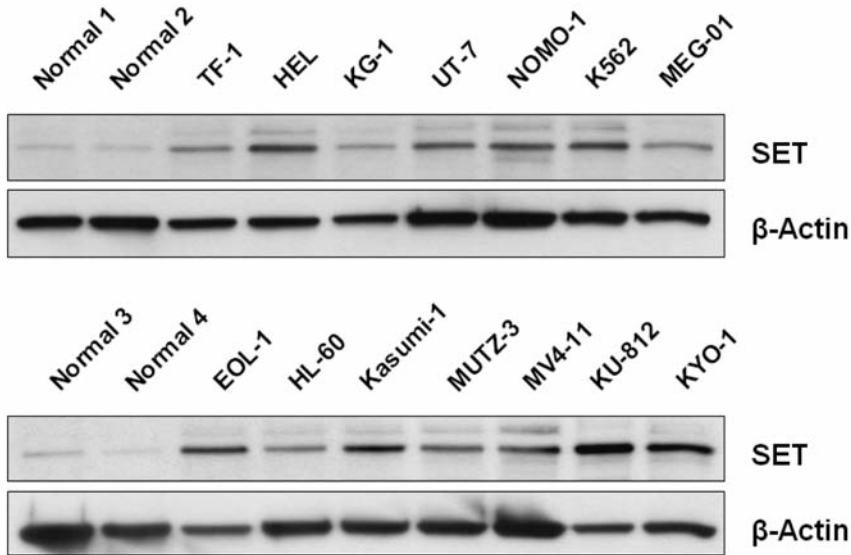
**Online Supplementary Figure S4.** Western blot analysis showing SET expression in 13 AML cell lines. Protein extract of the CML cell line K562 was included as a positive control for SET over-expression.



**Online Supplementary Figure S5.** SET inhibits the caspase-dependent apoptosis induced after PP2Ac overexpression. Caspase 3/7 assays in KG-1 cells transfected with SET, PP2Ac or both SET and PP2Ac. KG-1 cells transfected with an empty vector were used as a control.
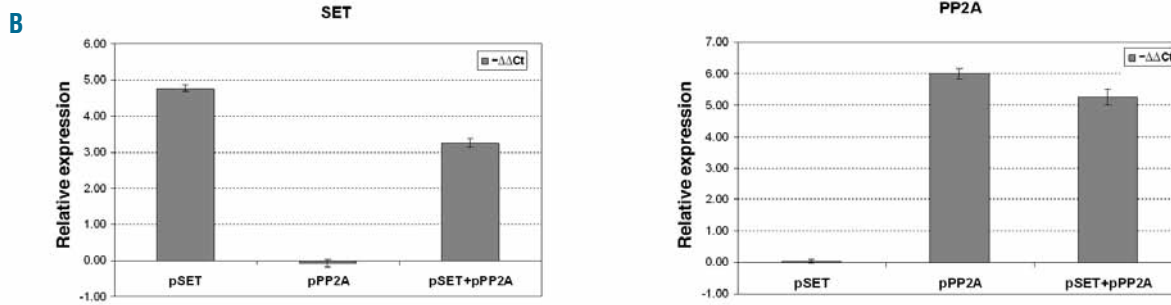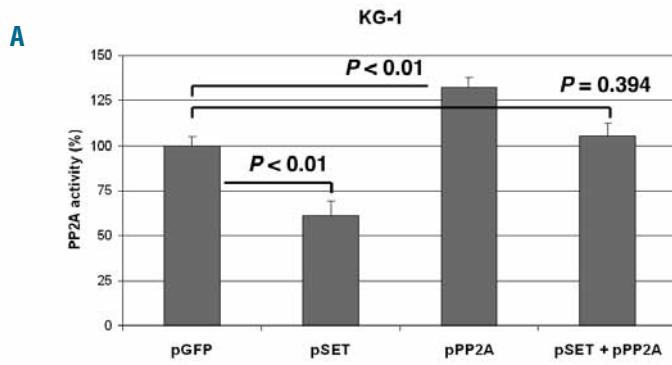
Online Supplementary Figure S6. SET inhibits the PP2A activity induced after PP2Ac overexpression. (A) PP2A phosphatase activity assays in KG-1 cells transfected with SET, PP2Ac, and both SET and PP2Ac. KG-1 cells transfected with an empty vector were used as controls. (B) Real-time RT-PCR showing SET and PP2Ac expression after transfection with SET, PP2Ac or both SET and PP2Ac. KG-1 cells transfected with an empty vector were used as controls.
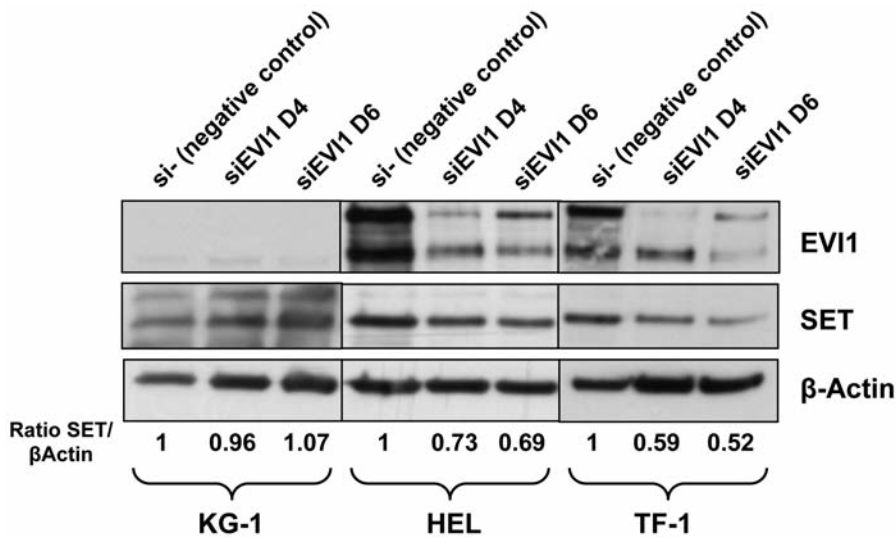


Online Supplementary Figure S7. Western blot analysis showing SET levels in the KG-1, HEL and TF-1 cell lines after EVI1 silencing. Densitometry analysis showing SET levels is included.
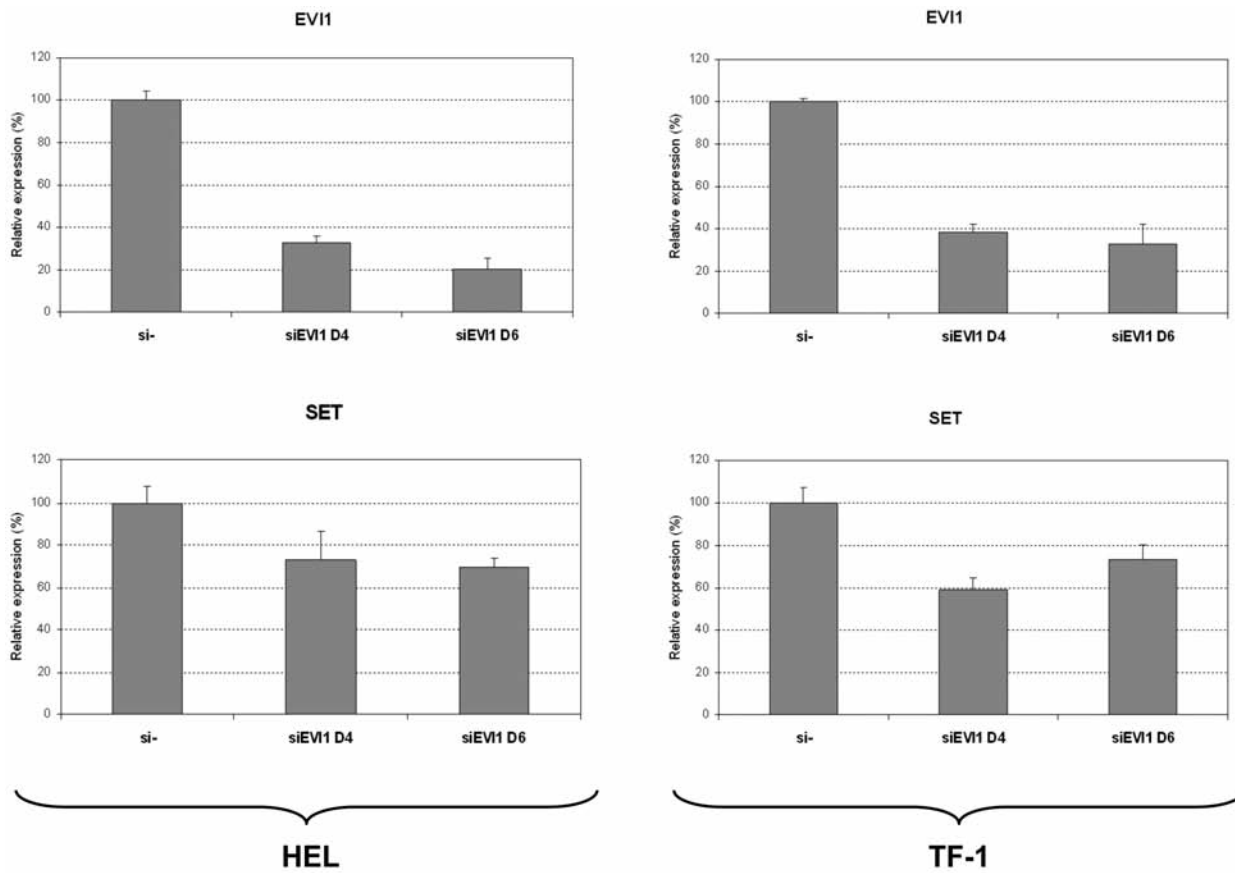
**Online Supplementary Figure S8.** Relative expression of EVI1 and SET after EVI1 silencing in HEL and TF-1 cells.
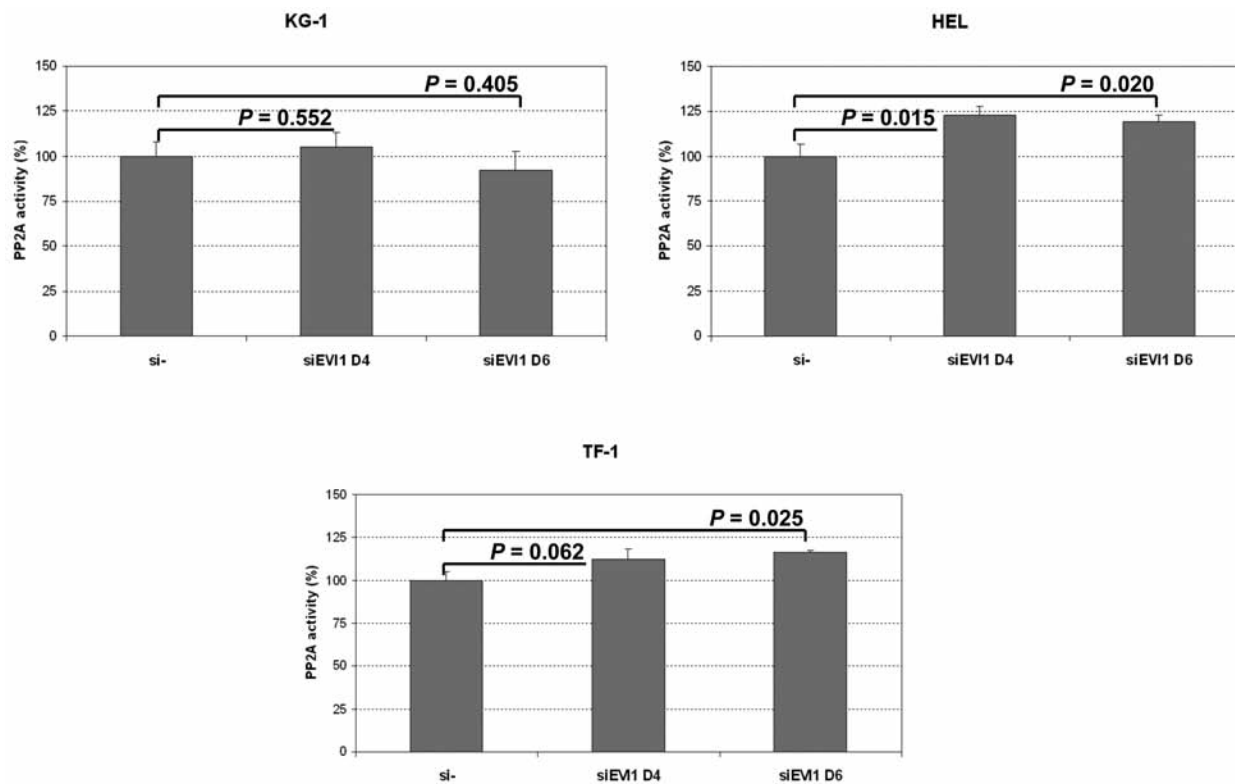


**Online Supplementary Figure S9.** PP2A phosphatase activity assays showing changes in PP2A activity in HEL and TF-1 cells after EVI1 silencing.
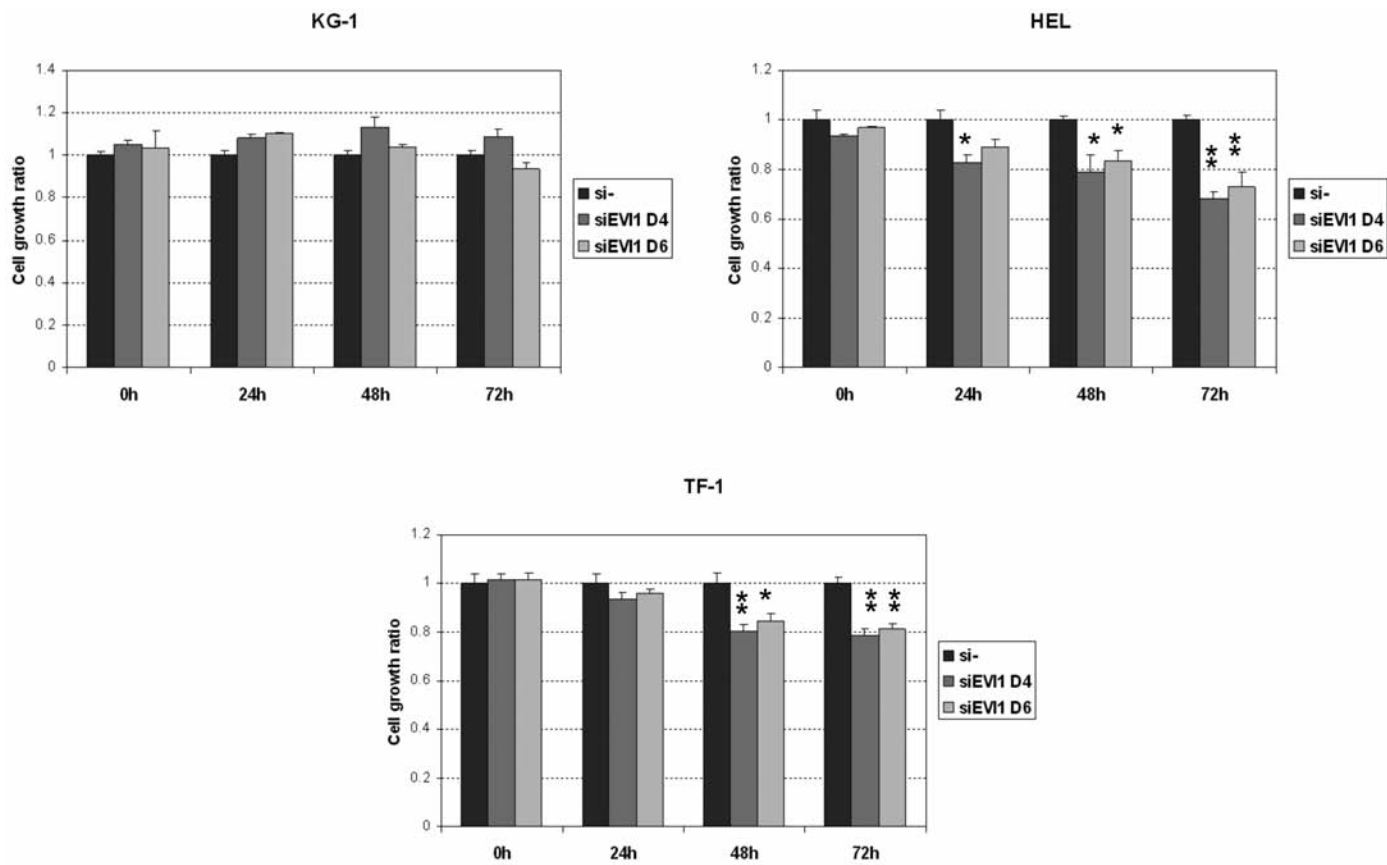
**Online Supplementary Figure S10.** Suppression of EVI1 induces decreased cell viability in SET-expressing AML cells. *P<0.05; **P<0.01.

**Online Supplementary Table S1.** Clinical and molecular characteristics of a series of 146 patients with AML at diagnosis and clinical follow-up data of those who received induction therapy.

| | N. (%) |
|---|---|
| **Sex** | |
| Male | 72 (49.3) |
| Female | 74 (50.7) |
| **Age** | |
| <60 years | 76 (52.8) |
| >60 years | 68 (47.2) |
| No data | 2 |
| **Complete remission** | |
| No | 46 (35.7) |
| Yes | 81 (64.3) |
| No data | 19 |
| **Diagnosis** | |
| AML-M0 | 12 (8.2) |
| AML-M1 | 36 (24.7) |
| AML-M2 | 36 (24.7) |
| AML-M4 | 22 (15) |
| AML-M5 | 23 (15.8) |
| AML-M6 | 10 (6.8) |
| AML-NOS | 7 (4.8) |
| **Secondary AML** | |
| No | 98 (67) |
| Yes | 16 (33) |
| No data | 32 |
| **Cytogenetic group** | |
| Good | 15 (10.2) |
| Intermediate | 79 (54.2) |
| Poor | 52 (35.6) |
| *SET* overexpression | |
| No | 102 (69.8) |
| Yes | 44 (30.2) |
| *SETBP1* overexpression | |
| No | 101 (73.7) |
| Yes | 36 (26.3) |
| No data | 9 |
| *EVI1* overexpression | |
| No | 94 (72.8) |
| Yes | 35 (27.2) |
| No data | 17 |
| *FLT3*-ITD | |
| No | 77 (77) |
| Yes | 23 (22) |
| No data | 46 |
| *NPM1* mutated | |
| No | 19 (38.8) |
| Yes | 30 (61.2) |
| No data | 97 |

**Online Supplementary Table S2.** Characteristics of the 16 patients with AML at diagnosis and PP2A data available whose SET levels were analyzed by western blot.

| Case | Sex | Age | FAB classification | AML type | Karyotype | SET OE | PP2A hyper-phosphorylation- | PP2Ac protein expression |
|---|---|---|---|---|---|---|---|---|
| P1 | F | 76 | AML-M2 | sAML | 46,XX | no | no | reduced |
| P2 | M | 66 | AML-M1 | sAML | 46,XY | no | no | reduced |
| P3 | F | 53 | AML-M4 | *de novo* | 46,XX | no | no | reduced |
| P4 | M | 41 | AML-M4 | *de novo* | ND | yes | yes | normal |
| P5 | F | 61 | AML-M4 | *de novo* | 46,XX | yes | yes | normal |
| P6 | M | 71 | AML-M1 | *de novo* | 46,XY | yes | yes | normal |
| P7 | F | 55 | AML-M0 | *de novo* | 46,XX | no | yes | normal |
| P8 | M | 55 | AML-M1 | *de novo* | 46,XY | no | yes | normal |
| P9 | M | 73 | AML-M4 | *de novo* | 46,XY | yes | yes | reduced |
| P10 | M | 43 | AML-M5 | *de novo* | 46,XY | yes | yes | normal |
| P11 | M | 64 | AML-M2 | *de novo* | 47,XY,+8 | no | yes | normal |
| P12 | F | 42 | AML-M5 | *de novo* | 48,XX,del(1)(p21), +4,+6,del(11)(q21), der(11)add(11)(p15) | no | no | reduced |
| P13 | F | 74 | AML-M1 | *de novo* | 46,XX | no | yes | normal |
| P14 | F | 69 | AML-M7 | *de novo* | 46,XX,add(3)(q?) | no | yes | normal |
| P15 | F | 69 | AML-M7 | *de novo* | 46,XX/46,XX,add(3)(q?) | yes | yes | normal |
| P16 | F | 64 | AML-M5 | sAML | 46,XX | no | yes | reduced |

*These cases are included in the report by Cristóbal et al. (2011).[12] OE: overexpression.*