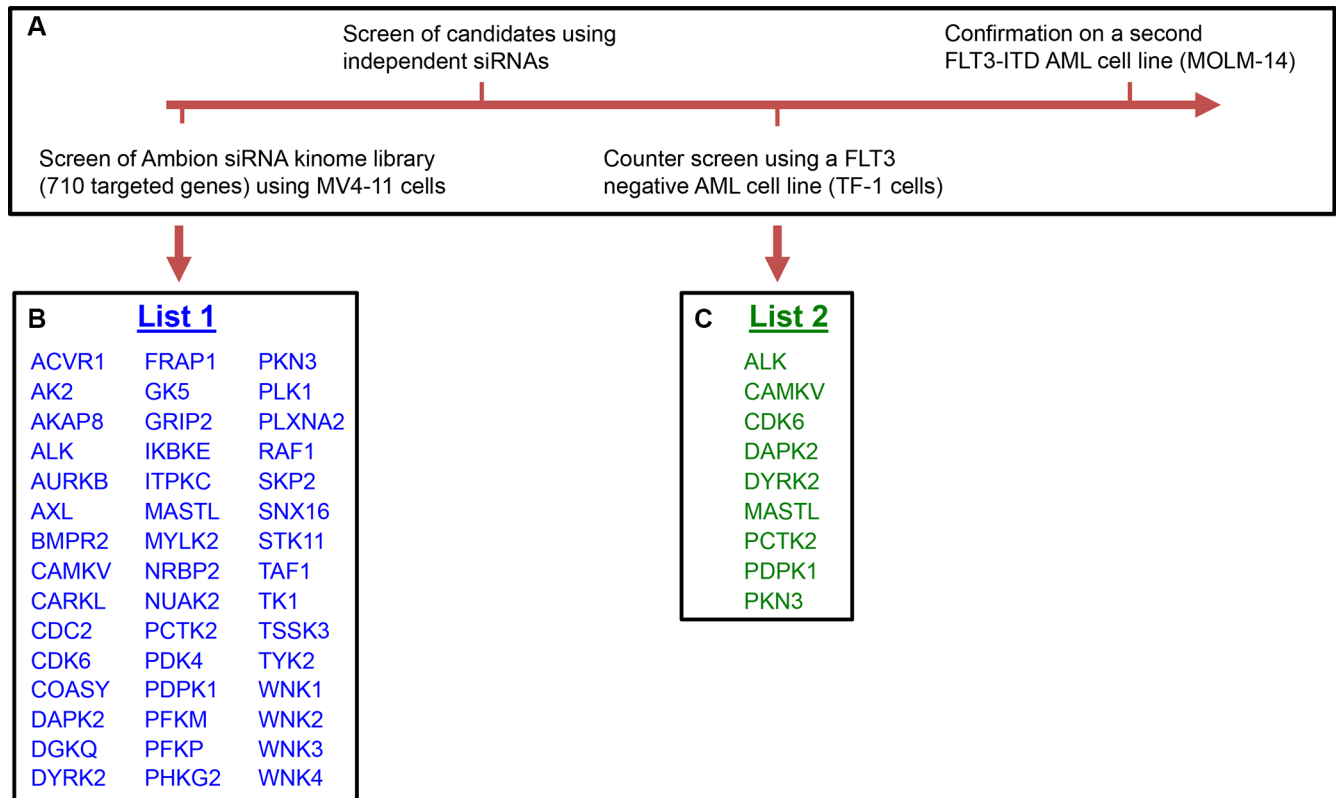
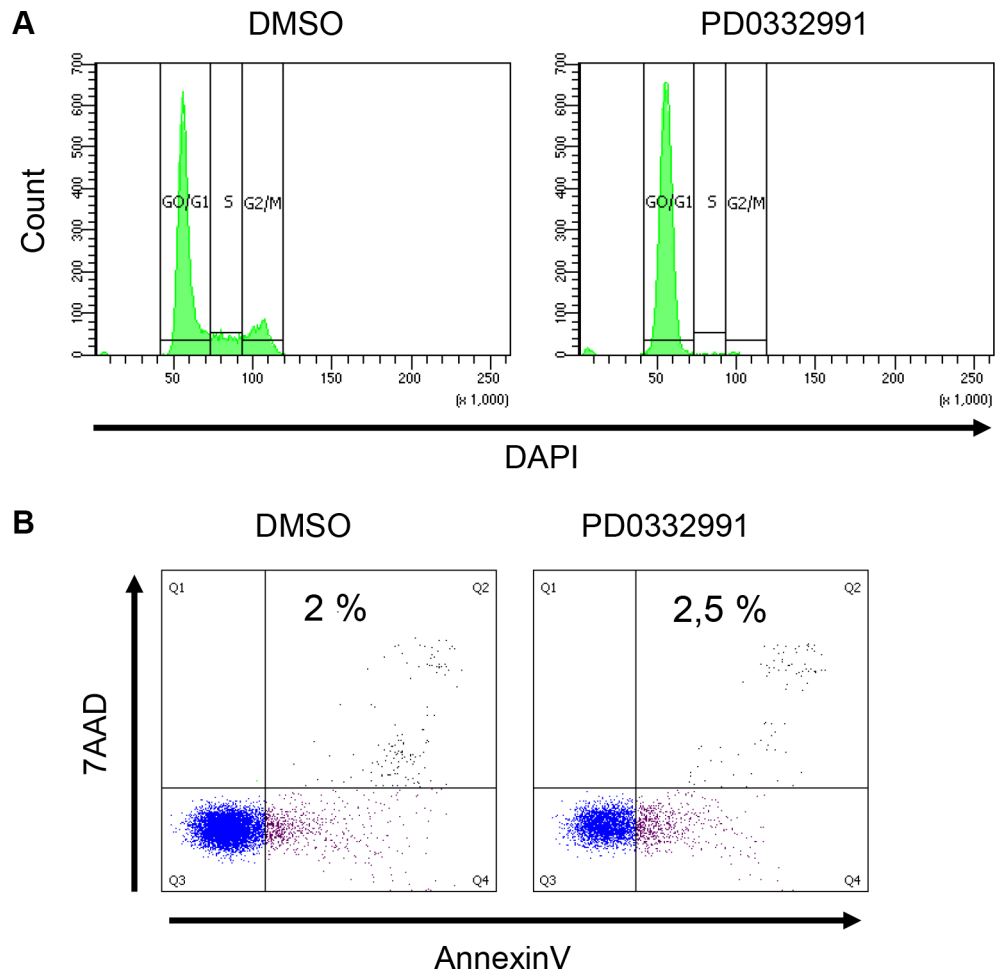


An essential pathway links FLT3-ITD, HCK and CDK6 in acute myeloid leukemia

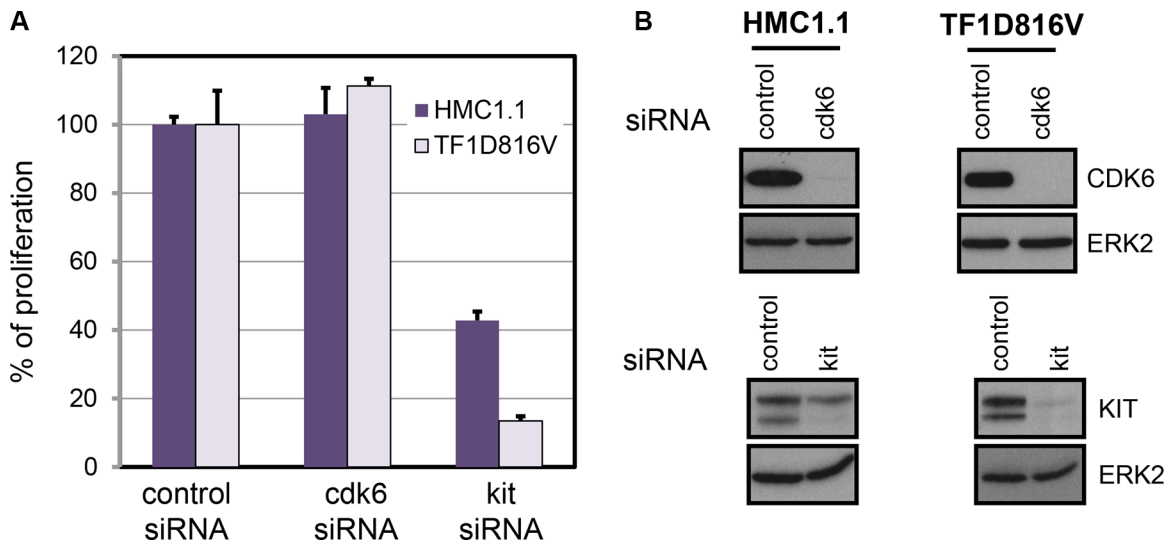
Supplementary Materials



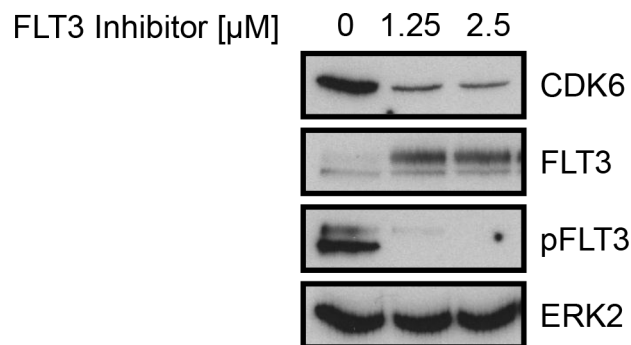
Supplementary Figure S1: Screening strategy. (A) Description of the major steps of the screen. (B) List of genes that reduced proliferation of MV4-11 cells by at least 60% in the original screen. (C) List of genes that were confirmed to inhibit MV4-11 cells but that did not inhibit TF-1 cells.



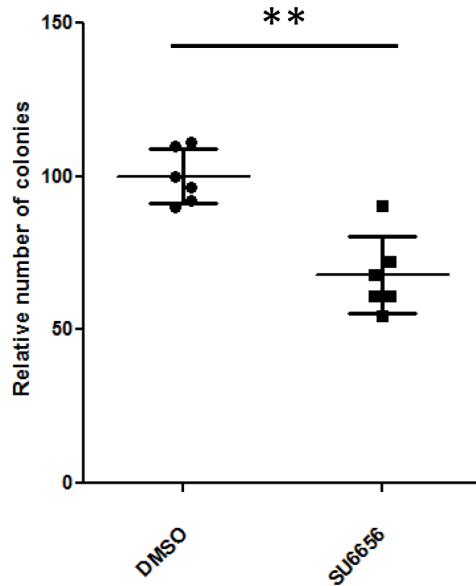
Supplementary Figure S2: Effect of PD0332991 on MV4-11 cell cycle and cell death. (A) Cell treated with PD0332991 are blocked in the G1 phase of the cell cycle. Flow cytometry analysis of MV4-11 cells treated with PD0332991 (0,5 μ M) for 48 hours and stained with DAPI. (B) Analysis of cell death by flow cytometry. MV4-11 cells treated with PD0332991 for 48 hours were stained with 7AAD and Annexin V. The percentage of double positive dead cells is indicated.



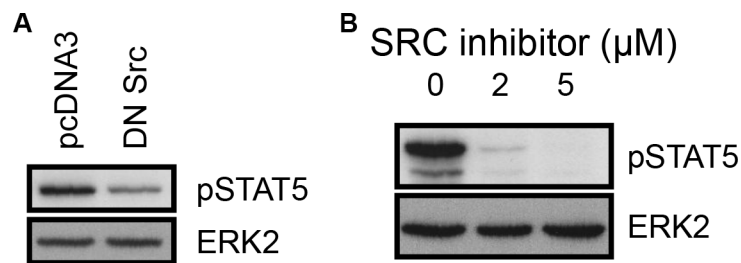
Supplementary Figure S3: KIT mutated cell lines are not dependent on CDK6 expression. (A) Thymidine incorporation assays were used to evaluate proliferation of HMC1.1 and TF1D816V cells treated with the indicated siRNAs. The histogram shows a representative result from three independent experiments. (B) Expression of CDK6 and KIT proteins in cells treated with control-, cdk6-, or kit- siRNAs as indicated were controlled by western blot. As a control, expression of ERK2 in the same lysates is shown below each panel.



Supplementary Figure S4: CDK6 expression level is dependent on the catalytic activity of FLT3-ITD. CDK6 protein expression was evaluated by western blot in MV4-11 cells treated with SU11248 for 48 hours. Inhibition of FLT3 kinase activity was controlled using a FLT3-Y591 phospho-specific antibody (pFLT3). ERK2 expression is shown as a lysate control.



Supplementary Figure S5: FLT3-ITD-mediated colony formation is dependent on SRC-family kinases. Colony forming assay analysis of wild-type murine bone-marrow transduced with FLT3-ITD retroviruses in the presence of SU6656 (2 μ M) or DMSO as a control. Cells were seeded in methylcellulose Methocult M3231 medium. Colonies were counted two weeks later. $**P \leq 0,01$, Mann-Whitney test.



Supplementary Figure S6: STAT5 activation is dependent on SFKs. (A) Expression of phosphorylated STAT5 (pSTAT5) in MV4-11 cells transfected with a control (pcDNA3) or a dominant-negative SRC (DN Src) plasmid. (B) pSTAT5 protein expression in MV4-11 cells treated with SRC family kinase inhibitor SU6656 or DMSO control (0) for 16 hours. As a loading control, expression of ERK2 in the same membranes is shown below each panel. Representative western blots from three independent experiments are shown.

Supplementary Table S1: List of the 710 genes targeted by the siRNA library. See Supplementary_Table_S1

Supplementary Table S2: AML samples used in the study^a

Patient no.	Age	FAB ^b	FLT3	blasts %	Sample origin
1	49	M4	ITD	73	peripheral blood
2	56	M5	ITD	89	peripheral blood
3	47	M5	ITD	91	peripheral blood
4	62	M4	ITD	67	peripheral blood
5	48	M5	ITD	91	peripheral blood
6	20	M4	ITD	67	peripheral blood
7	31	M5	ITD	95	peripheral blood
8	54	M2	ITD	66	peripheral blood
9	58	M4	ITD	82	peripheral blood
10	75	M5	ITD	84	bone marrow

^aAll samples had a normal karyotype.

^bFrench American British classification.

Supplementary Table S3: List of siRNAs used in the study

Targeted gene	Species	siRNA sequences (5' to 3')	Sources
CDK6	Human	GGCAAAGACCUACUUCUGAAGUGUU GACCACUACUUGGAUAAAGUCCA ACCGAGUAGUGCAUCGCGAUCUAAA	Invitrogen ^a
CDK4	Human	GAAAUUGGUGUCGGUGCCUAUGGGA GCACAGUUCGUGAGGUGGCCUUUACU UUACCUUGAUCUCCCGGUCAGUUCG	Invitrogen ^a
FYN	Human	GAAGCCCGCUCCUUGACAA GGAGAGACAGGUUACAUC CGGAUUGGCCCGAUUGAUA GGACUCAUAUGCAAGAUUG	Dharmacon ^b
LYN	Human	GCGACAUGAUUAAACAUA GUGAUGUUAUUAAGCACUA GAGAUCCAACGUCCAUAUA UUACAUCUCUCCACGAAUC	Dharmacon ^b
SRC	Human	GGGAGAACCUCUAGGCACA GCAGAGAACCCGAGAGGGA CCAAGGGCCUCAACGUGAA GCAGUUGUAUGCUGUGGUU	Dharmacon ^b
HCK	Human	GCCAUCAACUUUGGCUCUUCACCA AAACUGUCGGUGCCUGCAUGUCUU GGACAUCAUCGUGGUUGCCUGUAU	Invitrogen ^a
FLT3	Human	CAAGAAACGACACCGGAUAUU GAAUUUAAGUCGUGUGUUCUU GCAAUGAUUUUGGGACUAUU CGCAACAGCUUAUGGAAUUUU	Dharmacon ^b
KIT	Human	GGCCGACAAAAGGAGAUCU	Qiagen
Control ^c		UUCUCCGAACGUGUCACGU	Qiagen

^aStealth siRNAs.

^bOn-TARGETplus SMART pool siRNAs.

^cNon targeting siRNA.