Untreated

■siControl ■siChe-1

LAL-B



Expanded View Figures

Figure EV1. Che-1 silencing slightly affects cell viability. Related to Fig 1.

- A WB analysis of Che-1 silencing performed 24, 36, and 48 h postnucleoporation in NALM-6, LAL-B, and LAL-B #2 cell lines.
- B Viability assay performed in NALM-6, LAL-B, and LAL-B #2 cell lines treated as in (A). Data are presented as the means and standard deviation (SD) from three independent experiments. * $P \le 0.05$; *** $P \le 0.001$ by Student's *t*-test.

Source data are available online for this figure.





Figure EV2.

Α

Figure EV2. Che-1 binds c-Myc promoter and is involved in c-Myc-dependent tumorigenesis. Related to Fig 4.

- A Genome Browser Screenshot of Che-1 promoter, showing E-box conservation among human and mouse.
- B qRT–PCR experiment performed in P493-6 cell line untreated (–tet) or treated with 0.1 μ g/ml tetracycline for 72 h (+tet). Values were normalized with actin mRNA levels using the $\Delta\Delta C_t$ method. Error bars represent the standard error of three different experiments.
- C Cross-linked chromatin derived from P493-6 cells untreated or treated with 0.1 µg/ml tetracycline for 72 h was immunoprecipitated with c-Myc antibody or in the absence of antibody and analyzed by qRT–PCR (ChIP–qPCR). Data are expressed as a percentage of input. Error bars represent the standard error of three different experiments.
- D c-Myc binds Che-1 promoter in P493-6 cell line. Data from Amati's group deposited at GSE51011, UCSC Genome Browser Screenshot showing only portion of MACS2generated bigWig.
- E Che-1 expression (RPKM) increasing along with tumorigenesis. RNA-seq processed data from Sabo et al [20] Data are presented as the means and SD from three independent experiments (GSE51011).

Data information: * $P \le 0.05$; ** $P \le 0.01$ by Student's *t*-test. Source data are available online for this figure.



Figure EV3. c-Myc silencing affect cell viability and proliferation with a major effort. Related to Fig 5.

- A WB analysis of P493-6 cells untreated (-tet), treated with tetracycline for 72 h (+tet) and 4 h after release. β-actin was used as loading control.
- B WB and proliferation assay of c-Myc silencing performed 24, 36, and 48 h postnucleoporation in NALM-6, LAL-B, and LAL-B #2 cell lines. Data are presented as the means and standard deviation (SD) from three independent experiments.
- C Viability assay performed in NALM-6, LAL-B, and LAL-B #2 cell lines treated as in (B). Data are presented as the means and standard deviation (SD) from three independent experiments.
- D Che-1/c-Myc transcript correlation in the "Exp B Cells" dataset comprising of several hematological samples.
- E Gene Ontology enrichment for the 1,372 gene Che-1/c-Myc-r-PLUS overlap showed in Fig 5H.

Data information: * $P \le 0.05$; ** $P \le 0.01$; *** $P \le 0.01$ by Student's t-test. Source data are available online for this figure.



Figure EV4. Che-1 and c-Myc cooperate in controlling proliferation. Related to Fig 6.

- A WB analysis of NALM-6 and LAL-B cell lines nucleoporated with siRNA targeting Che-1 (siChe-1), c-Myc (si c-Myc), or with control siRNA (siControl), and subjected to cell number analysis (Fig 6A). β-actin was used as loading control.
- B Cell number analysis performed on P493-6 cells transiently transfected either with siRNA targeting Che-1 (siChe-1) or with control siRNA (siControl), and untreated or treated with tetracycline for 72 h. Data are presented as the means ± SD from three independent experiments.
- C WB analysis of P493-6 cells treated as in (B).
- D WB analysis of NALM-6 and LAL-B cell lines treated as in (A), and subjected to RNA-seq experiment (Fig 6B).
- E, F qRT–PCR analysis performed in P493-6 cells treated as in (B), to validate the downregulation of some genes (Cyclin B1, Cdk1, Foxm1) emerged from the RNA-seq experiment. Error bars represent the standard error of three different experiments.
- G LAL-B #2 cells were nucleoporated with Control siRNA (siControl) in combination with a Myc-tagged empty vector (Empty vector) or with siRNA c-Myc (si c-Myc) in combination with empty vector, or with si c-Myc and Myc-tagged Che-1 expressing vector (Myc-Che-1), and harvested after 36 h. WB analysis of TCEs with the indicated Abs is shown. It is representative of three independent experiments.

Data information: ** $P \le 0.01$; *** $P \le 0.001$ by Student's *t*-test. Source data are available online for this figure.



Figure EV5. Significant c-Myc motifs emerged from Centdist ChIP-seq MACS2 analysis. Related to Fig 7.