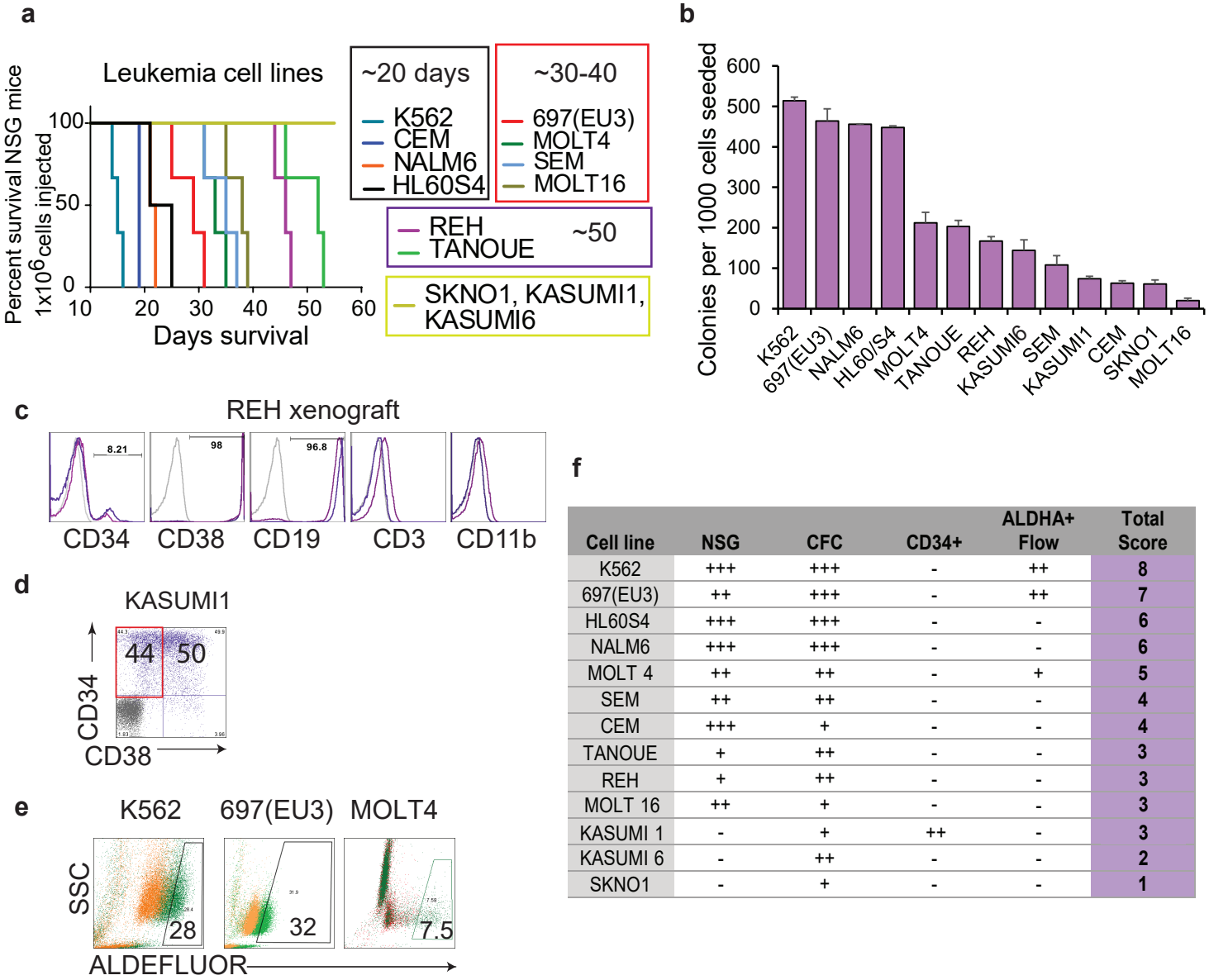

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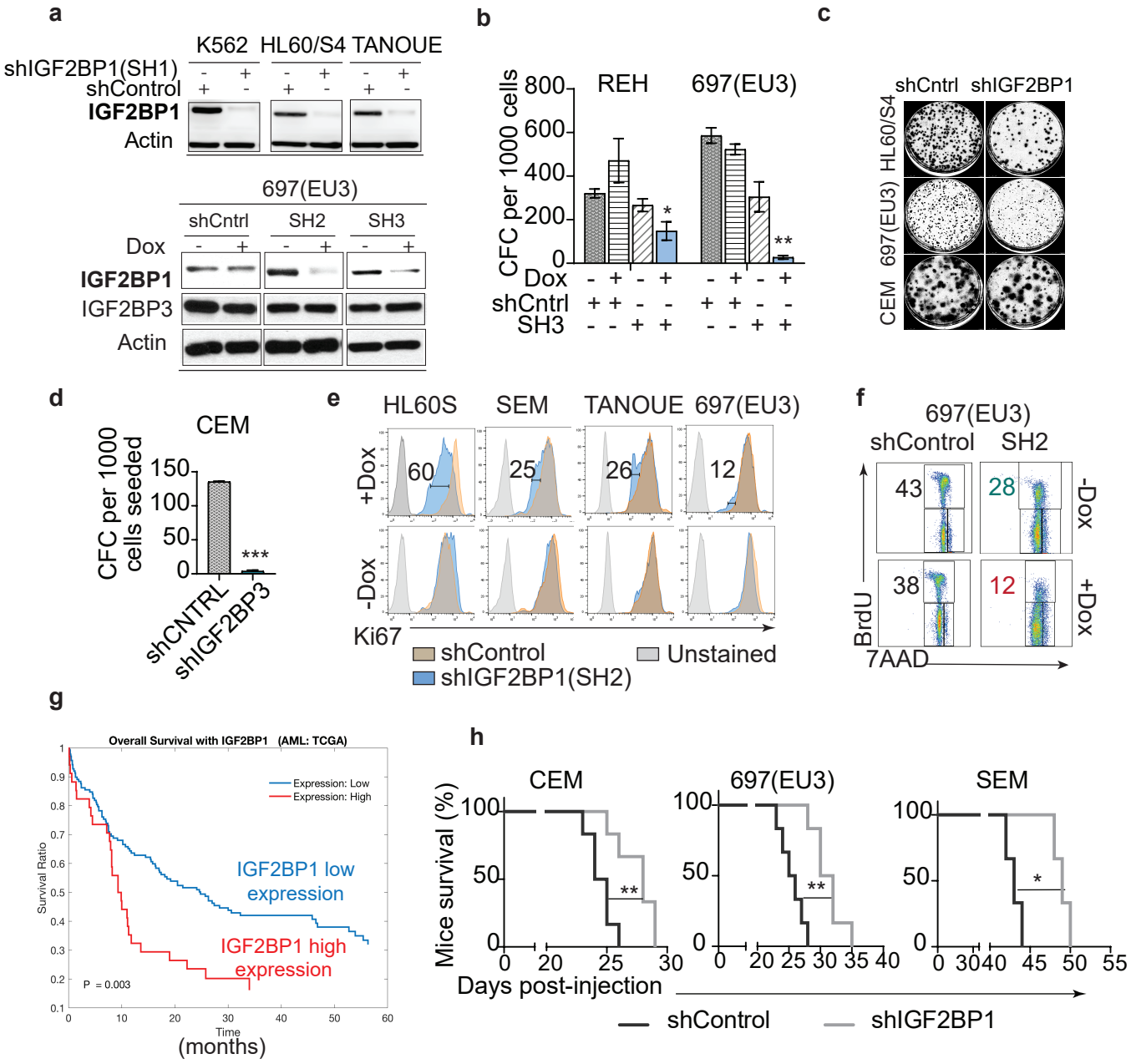
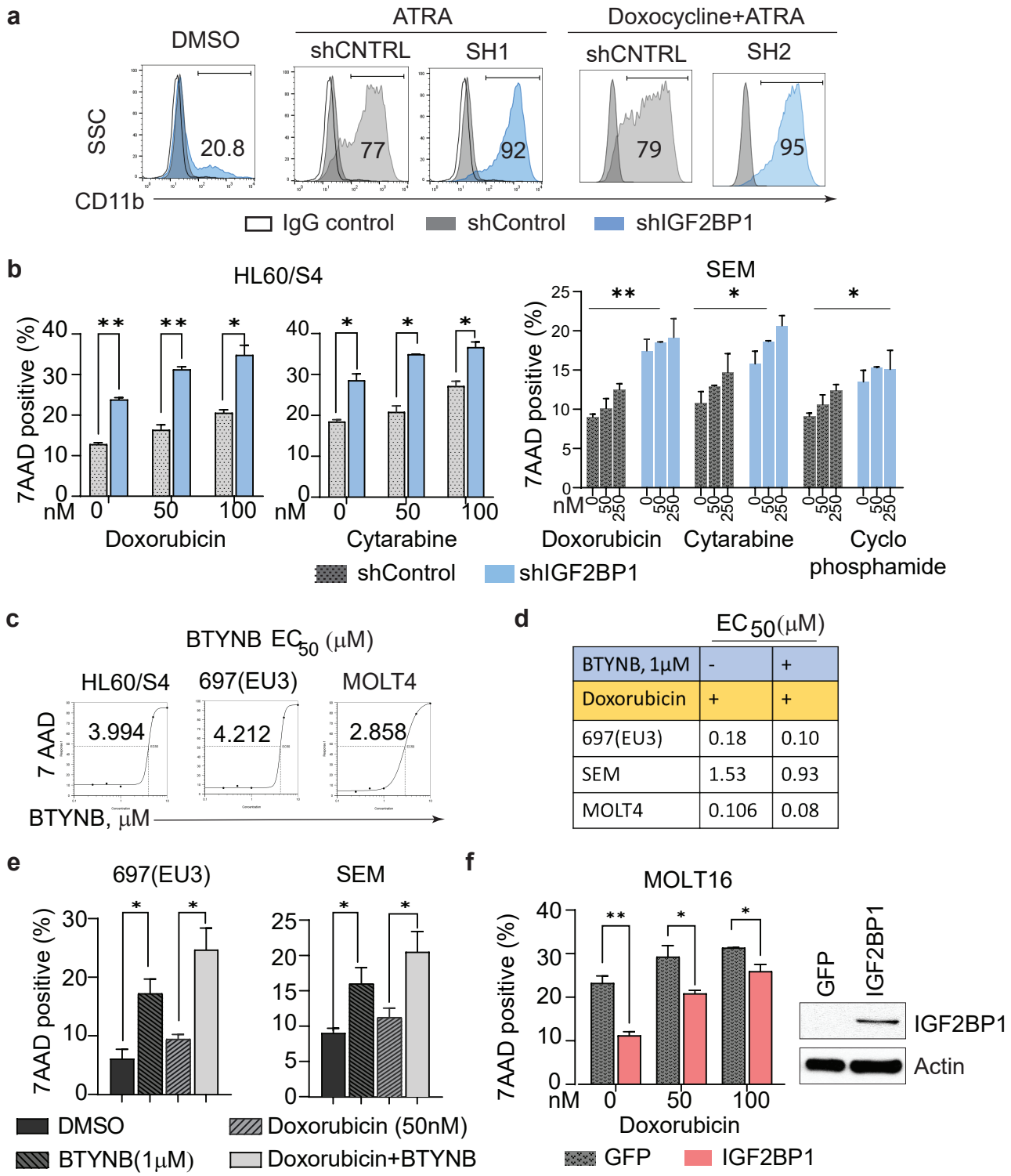
Cell line	NSG	CFC	CD34+	ALDHA+ Flow	Total Score
K562	+++	+++	-	++	8
697(EU3)	++	+++	-	++	7
HL60S4	+++	+++	-	-	6
NALM6	+++	+++	-	-	6
MOLT 4	++	++	-	+	5
SEM	++	++	-	-	4
CEM	+++	+	-	-	4
TANOUE	+	++	-	-	3
REH	+	++	-	-	3
MOLT 16	++	+	-	-	3
KASUMI 1	-	+	++	-	3
KASUMI 6	-	++	-	-	2
SKNO1	-	+	-	-	1

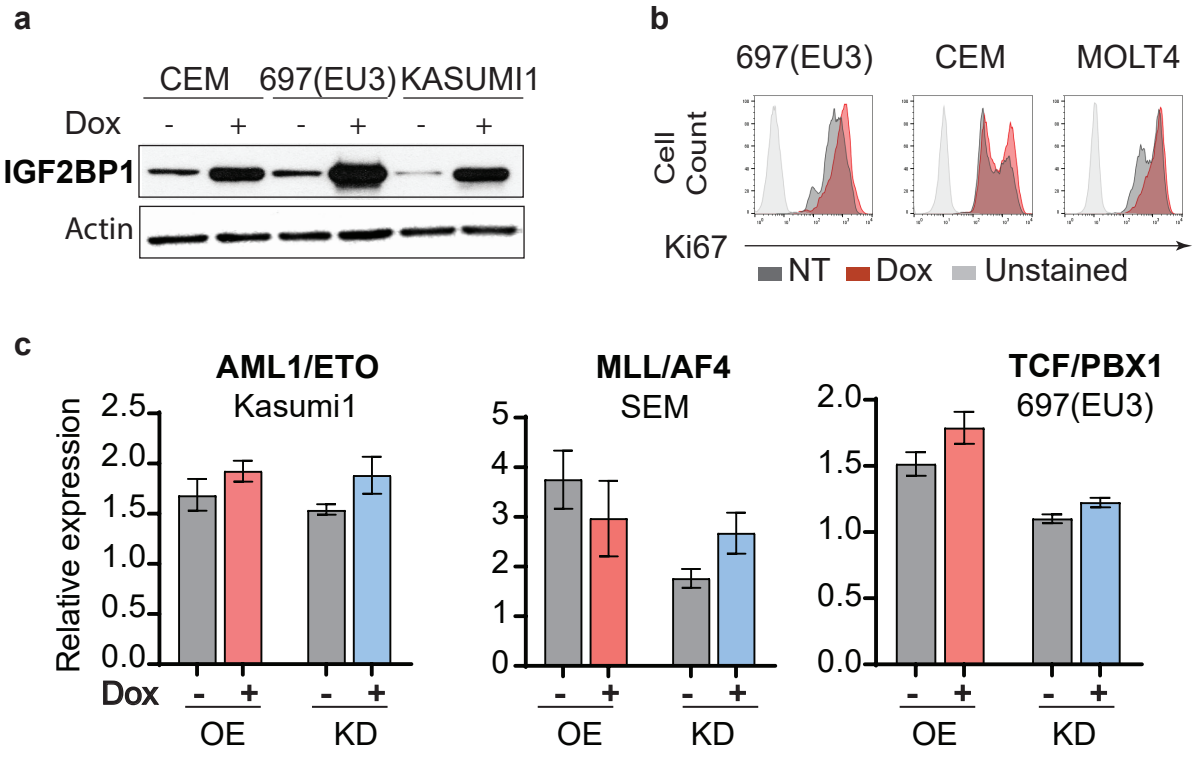
Supplementary Figure 1. Assessment of cancer stem cell properties in leukemia cell lines.
a, Kaplan-Meier survival analysis of NSG mice injected with 1x10⁶ genetically unmodified, original cell lines. Cell lines were grouped by tumorigenicity: very high tumorigenicity – mice lethality ~20 days post-injection or sooner (K562, CEM, NALM6, HL60/S4); high tumorigenicity – mice lethality ~ 30-40 days post-injection (697(EU3), MOLT4, SEM, and MOLT16); medium tumorigenicity- mice lethality ~ 50-60 days post-injection (REH, TANOUE); low or absent tumorigenicity - more than 3 months or never (SKNO1, KASUMI1, KASUMI6); **b**, CFC assay of original cell lines (n=3, ± SEM); **c,** Flow cytometric analysis of NSG mouse bone marrow stained with the human hematopoietic stem cell (CD34 and CD38), B-cell (CD19), T-cell (CD3), and myeloid (CD11b) markers, REH xenograft (n=2); **d**, Flow cytometric analysis of CD34 and CD38 expression in KASUMI1 in vitro cultures, gated CD34+/CD38- subpopulation. **e**, ALDEFLUOR staining of three cell lines with gated ALDH+ populations. **f**, Cumulative scores for the stem cell properties based on the conducted assays.



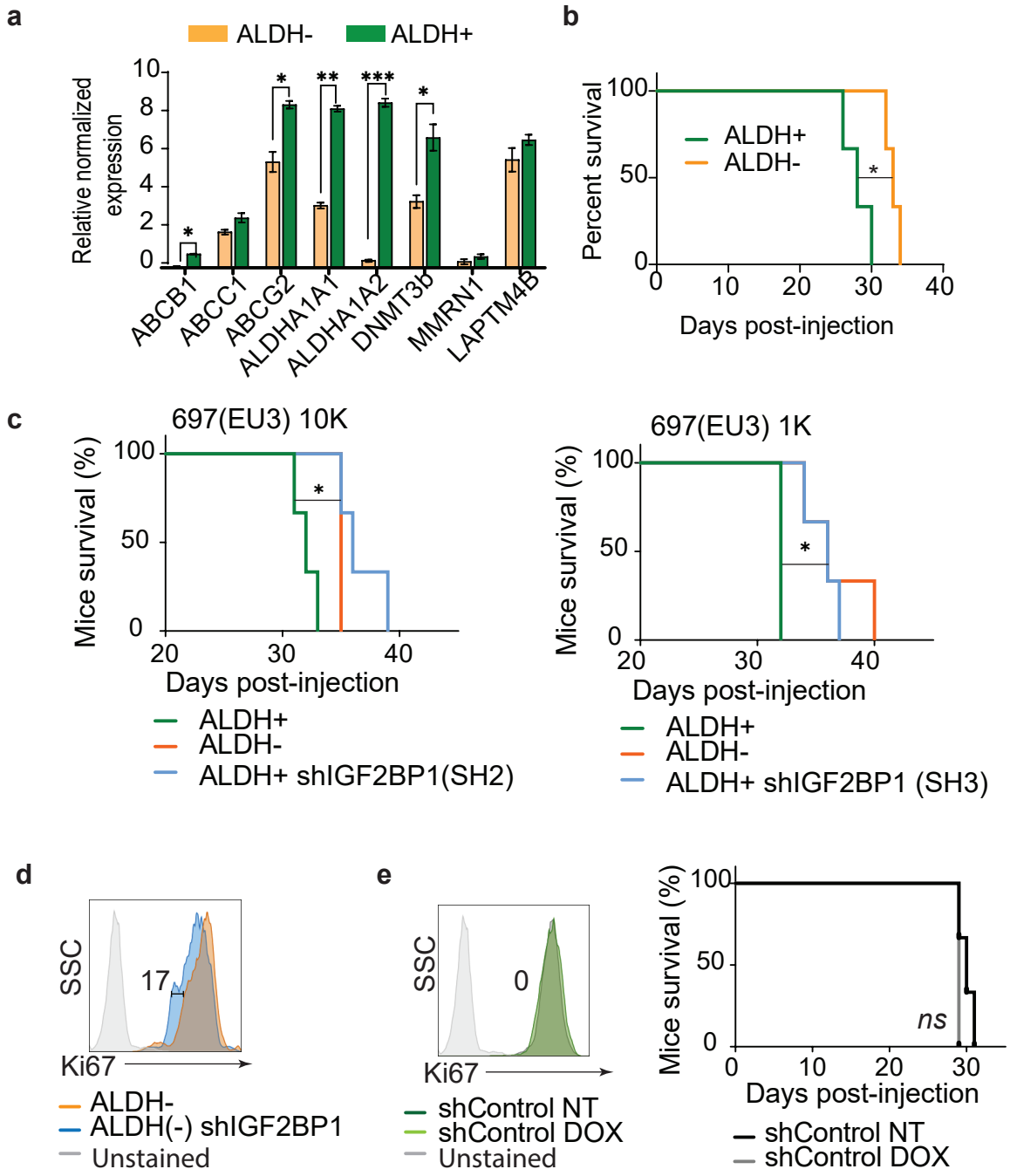
Supplementary Figure 2. IGF2BP1 loss-of-function reduces leukemia cell growth and tumorigenesis. **a,** Western blot analysis of indicated proteins in cells expressing constitutive shIGF2BP1(SH1), doxycycline-inducible shIGF2BP1 (SH2 and SH3), and shControls. **b,** Colony-Forming Cell (CFC) assay for leukemia cell lines with doxycycline-inducible shIGF2BP1(SH3) knockdown and shControl (t-test, *P<0.05, **P<0.01, n=3, ± SEM); **c,** A phase contrast photograph of CFC assays shIGF2BP1(SH1) knockdown and shControl; **d,** CFC assay for CEM leukemia cell line with shIGF2BP3 knockdown and a scrambled shControl (t-test, ***P<0.001, n=3, ± SEM); **e,** Flow cytometric analysis of Ki67 staining in selected leukemia cell lines with doxycycline-induced expression of shIGF2BP1(SH2) or shControl; **f,** Representative flow cytometric analysis of BrdU staining in 697(EU3) with doxycycline-inducible IGF2BP1(SH2) knockdown; **g,**The overall survival of AML patients with high (red) and low (blue) levels of IGF2BP1 expression, TCGA database; **h,** Kaplan-Meier survival analysis of mice transplanted with B- and T-cell leukemia cells with and without stable IGF2BP1(SH1) knockdown (Mantel-Cox test, **P=0.0062 (CEM, *n*=6), **P=0.0012 (697(EU3), *n*=6), *P=0.0246 (SEM, *n*=3)).



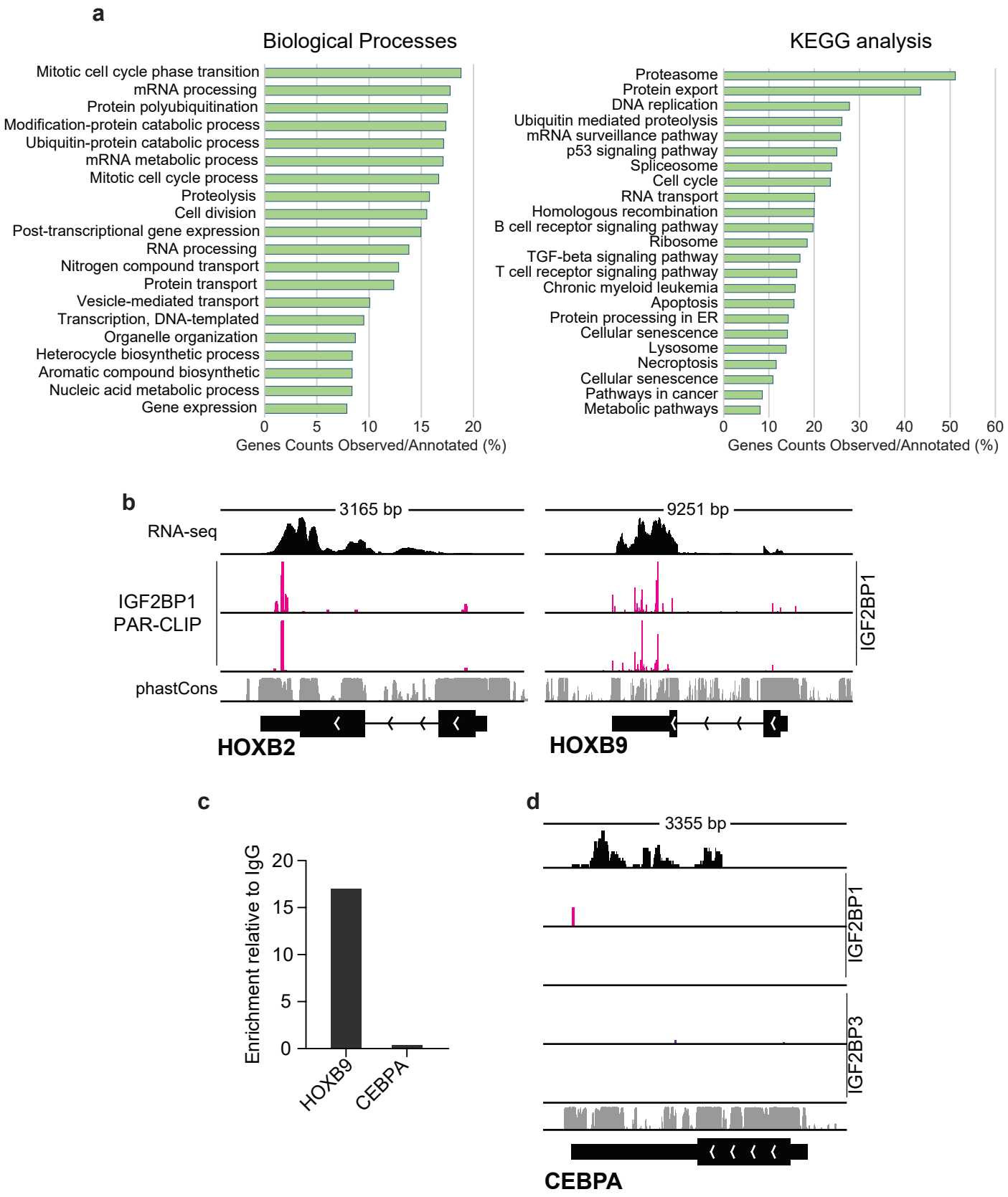
Supplementary Figure 3. IGF2BP1 loss-of-function increases sensitivity of leukemia cells to chemotherapeutic drugs. a, Flow cytometric analysis of CD11b expression after DMSO or all-trans retinoid acid (ATRA) HL60/S4 treatments, day 4. Left graph: spontaneous differentiation upon shIGF2BP1(SH1) expression; Right graphs: ATRA treatments. All differentiations are compared to shControl treated with DMSO; **b**, Cell viability HL60/S4 and SEM expressing shIGF2BP1(SH1) 24 hrs post-treatment (t-tests, *P<0.05, **P<0.01, n=3, ± SEM); **c**, EC_{50} of BTYNB (IGF2BP1 inhibitor) in indicated leukemia cell lines, day 4; **d,** Table EC50 for doxorubicin (50nM) only, and combined doxorubicin (50nM)/BTYNB(1μM) treatments in indicated cell lines; **e**, Cell viability assessment of 697(EU3) and SEM cells treated with DMSO, BTYNB (1 μM), doxorubicin (50 nM) and combined doxorubicin/BTYNB components, 24 hrs (t-test, *P<0.05, n=3, ± SEM); **f**, Dose-response assessment of cell viability in MOLT16 with forced expression of IGF2BP1 and GFP control, 24 hrs post doxorubicin treatment (t-test, *P<0.05, **P<0.01, n=3, ± SEM). Right panel: Western blot for IGF2BP1 and beta-actin.



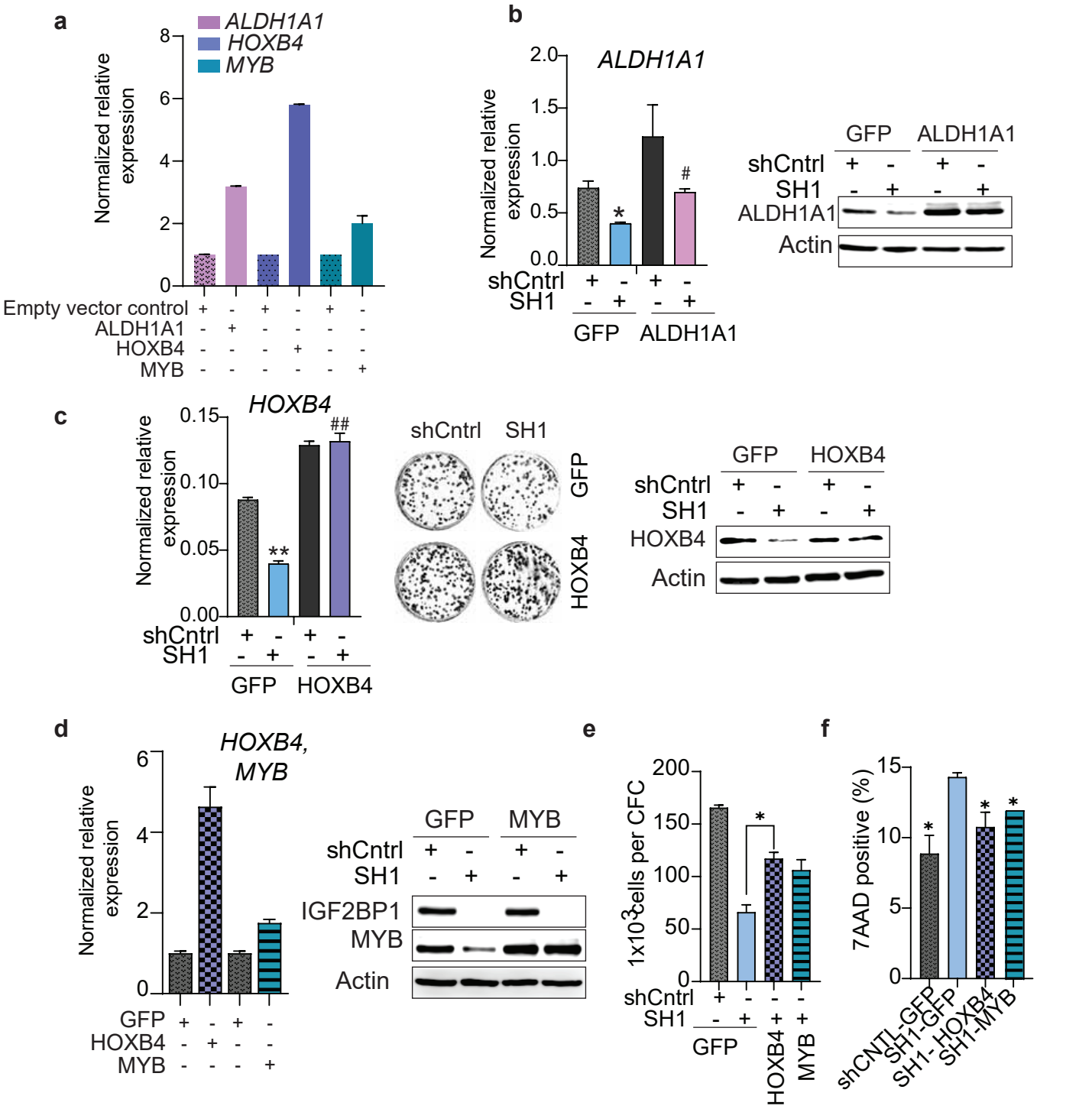
Supplementary Figure 4. IGF2BP1 gain-of-function effect on cell proliferation and expression of fusion transcripts. a, Representative Western blot analysis of IGF2BP1 expression in cell lines with doxycycline-inducible overexpression of IGF2BP1; **b,** Flow cytometric analysis of Ki67 staining in selected leukemia cell lines with IGF2BP1 overexpression (red); **c,** qPCR analysis of indicated fusion transcripts with doxycycline-inducible overexpression (OE) and knockdown (KD) of IGF2BP1 with SH2.



Supplementary Figure 5. IGF2BP1 expression supports leukemia cancer stem cell properties.
a, Quantitative PCR of ALDH$^-$ and ALDH$^+$ subpopulations (t-test, *P<0.05, **P<0.01, ***P<0.001, n=3, ± SEM); **b**, Kaplan-Meier survival analysis of NSG mice transplanted with 2.5x10^5 ALDH- and ALDH+ cells (Mantel-Cox test, *P=0.0245, n=5). **c,** Kaplan-Meier survival analysis of mice injected with indicated ALDH subpopulations and IGF2BP1 knockdown. The left panel: 1x10^4 cells, shIGF2BP1(SH2), *n*=3. The right panel: 1x10^3 cells, shIGF2BP1(SH3), *n*=3. **d,** Flow cytometric analysis of Ki67 staining of 697(EU3) ALDH$^-$ cells with and without doxycycline-induced shIGF2BP1(SH2); **e,** Flow cytometric analysis of Ki67 staining of 697(EU3) ALDH+ cells with and without doxycycline-induced shControl,and Kaplan-Meier survival analysis of mice injected with 1x10^3 697(EU3) ALDH+ cells with and without doxycycline-induced shControl, *n*=3, not significant.



Supplementary Figure 6. PAR-CLIP analysis of K562 CML cells.
a, Gene Ontology (GO) and KEGG pathway analysis for mRNAs bound with IGF2BP1 in PAR-CLIP assay; **b**, The representative screenshots of two independent PAR-CLIP experiments illustrating the amplification (vertical red bars) in IGF2BP1-mRNA-binding sites of HOXB2 and HOXB9; **c,** Quantitative analysis for HOXB9 and CEBPA mRNA enrichment in anti-IGF2BP1 cross-linking immunoprecipitations (CLIP) relative to anti-IgG immunoprecipitations (*n*=3); **d,**The screenshots of two independent PAR-CLIP experiments illustrating IGF2BP1 and IGF2BP3 fewer binding sites with CEBPA mRNA. IGF2BP1 (two upper panels, a red bar) and IGF2BP3 (two lower panels, a blue bar).



Supplementary Figure 7. The ectopic expression of stem cell genes rescues IGF2BP1 loss-of-function phenotype in leukemia. a, qPCR analysis of ALDH1A1, HOXB4, MYB expression in K562 transduced with indicated vectors for gene's overexpression; **b,** qPCR and Western blot analysis of ALDHA1A1 expression in 697(EU3) cells seeded for CFC assay, day 1 of CFC (t-test for shControl and SH1-GFP: *P<0.05, n=3, ± SEM), (t-test for SH1-*GFP* and SH1-*ALDH1A1*: #P<0.05, n=3, ± SEM); **c,** qPCR of *HOXB4* expression in HL60/S4 colonies, day 14 of CFC (t-test for shCntrl-GFP and SH1-GFP: **P<0.01, n=3, ± SEM), (t-test for SH1-GFP and SH1-HOXB4: ##P<0.01, n=3, ± SEM) (left); Western blot analysis of HOXB4 expression in cells seeded for CFC, day 1 of CFC (right). **d,** qPCR analysis of *HOXB4*, *MYB* over-expression and Western blot analysis of IGF2BP1 and MYB expression in K562 cells seeded for CFC assay, day 1 of CFC; **e**, CFC assays for K562 cells co-expressing constitutive shIGF2BP1 or shControl in combination with *GFP*, *HOXB4*, or *MYB*, day 14 (t-test, *P<0.05, n=3, ± SEM); **f**, Cell viability assay in K562 expressing indicated constructs (t-test, *P<0.05, n=3, ± SEM);