HOXA/PBX3 knockdown impairs growth and sensitizes cytogenetically normal acute myeloid leukemia cells to chemotherapy

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Online Supplementary Design and Methods

Co-immunoprecipitation assays

Total cell protein samples (500 μg) were co-immunoprecipited with V5, Xpress or HA antibodies (1 μg) overnight at 4°C with gentle rotation. Protein complexes (50 μL aliquots) were purified by binding to Protein G Dynabeads® (Invitrogen) for 15 min at room temperature with gentle rotation. Complexes were then washed in RIPA x3, denatured in loading buffer containing β-mercaptoethanol, heated to 70°C to release the bound magnetic beads and subjected to sodium dodecylsulfate polyacrylamide gel electrophoresis. HOXA:TALE interactions were determined by blotting and antibody incubation for respective epitopes as previously described.[1] Endogenous HOXA6/A9:TALE complexes in acute myeloid leukemia (AML) cells were detected by obtaining total cell protein (500 μg) by standard methods and co-immunoprecipitation using HOXA6 (Sigma-Aldrich), HOXA9, MEIS1-3 (Upstate, Billerica, MA), PBX1-3 (Santa-Cruz Biotechnology, Santa Cruz, CA, USA) or immunoglobulin control antibodies with 50 μL of Protein G Dynabeads® as detailed above. Western blotting was carried out using HOXA6 (1:1000, Abcam, Cambridge, UK), HOXA9 (1:440), PBX1-3 (1:500) or MEIS1-3 (1:200) primary antibodies as above followed by horseradish peroxidase-conjugated rabbit antimouse, or goat anti-rabbit secondary antibodies (1:5000, 1:2000 respectively, both Dako) and signal detection.[1]

Microarray data profiling

The AmpliChip Leukemia, custom designed for stage 2 of the MILE study,[2] containing 1480 leukemia-associated probe sets was used to identify altered expression following *HOXA6* or *HOXA9* knockdown. Affymetrix® CEL files generated from the AmpliChip Leukemia (Roche Molecular Systems, Pleasanton, CA, USA), custom designed MILE study[2] were analyzed to stringent criteria. All CEL files were imported using a custom chip definition file, into the Partek Genomic Suite (St. Louis, MO, USA) and standardized using RMA background normalization, quantile normalization and median polish probe summarization.[3,4] ANOVA comparing non-silenced control and *HOXA6* or *HOXA9* knockdown was followed by identification of fold-changes ≥2; false discovery rate-adjusted *P*-values <0.05 were used.

Gene expression analysis

TALE genes were examined using SYBR probe-based chemistry with validated assay reagents and *18S* rRNA as an endogenous control (primer sequences available on request). Total RNA obtained from PBX3 or MEIS1 knockdown or respective non-silencing controls was converted to cDNA prior to quantitative real-time polymerase chain reaction (RQ-PCR) analysis. Relative expression values are represented as percentage of appropriate controls using the $\Delta\Delta C_T$ method.

Virus-mediated infection

Vesicular stomatitis virus-pseudotyped retroviruses were produced using 293GPE cells[5] as previously described.[6] Lentiviruses were produced using 293FT cells (Invitrogen) co-transfected with psPAX DNA (packaging), pMD2G DNA (envelope), and 5 μg of each *HOX/TALE* shRNA DNA using Lipofectamine™ 2000 (Invitrogen) and standard protocols.

Virus-containing supernatants were harvested, centrifuged at 3000 rpm for 5 min at +4°C to pellet cell debris and filtered using 0.2 μm filters. For each infection 400 μL of target cells (1.25 x 10^6/ mL) were spinoculated with 600 μL virus, 6 μg/mL polybrene at 1800 rpm for 45 min at 4°C. Infected cells were cultured for 16 h then selected in puromycin (Sigma-Aldrich) for 72 h at IC_{90} concentrations (0.25 μg/mL for U937 cells, 0.095 μg/mL for OCI AML3 cells). Cultures were subsequently maintained in media lacking puromycin. Puromycin-selected cells were washed with phosphate-buffered saline (PBS) x3 and analyzed by fluorescence microscopy/flow cytometry for green fluorescent protein expression.

Morphological analysis

Cells were cytospun onto glass slides at 400 x g for 5 min. Slides were stained with Accustain® Wright-Giemsa Stain (Sigma-Aldrich) to determine gross cell morphology. Images were captured at 200X and 100X using an inverted microscope (CKX41) with attached digital camera (E620), both from Olympus (Essex, UK). Morphological examinations were made by an independent hematologist in a blinded manner.

Measurement of colony-cell number

Methylcellulose was melted using pre-warmed media to obtain colony-forming cells. Following washing, with PBS x3, supernatants were carefully removed and 50 μL aliquots obtained for cell number assay by CellTiter-Glo® using the manufacturer's protocol.

References

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Online Supplementary Table S1. shRNA targeting sequences. The nucleotide sequence of up to three shRNA (a-c) and respective vectors used to target *HOXA6*, *A9*, *PBX3* or *MEIS1* expression. pGipz and pSM2 shRNA miR vectors (Open Biosystems, Thermo Fisher Scientific, Huntsville, AL, USA), NKI library pRSC vectors[7] and pLKO1 from the TRC library designed by The RNAi Consortium.[8] Non-targeting sequences within the vectors served as expression controls and MSCV-gfp was used as a positive control. Homology searches indicated that none of the shRNA used has notable comparable homology to another human gene or local miRNA. Since one or two mismatches are in use for RNAi-rescue experiments, this indicates a high level of specificity, which along with the use of multiple shRNA sequences per gene reduces the potential for off-target effects. Processing of dsRNA in human cells for entry into the RISC silencing complex requires splicing of 21 nucleotide-length (19-basepair stem length) dsRNA by DICER.[9,10] Shorter stem lengths do not result in DICER activity.[11]

Target Gene		shRNA Sequence	Vector	Target Homology	Gene Homology	RefSeq homology	TALE/HOX homology
HOXA6	a	CTCGTGTTTCTATTCTGAT	pGipz miR	19/19	16/16	14/14	< 14/14
	b	CTCGTGTTTCTATTCTGAT	pSM2 miR	19/19	16/16	14/14	< 14/14
HOXA9	a	GCATTTAAGTCTGTCCATT	pGipz miR	19/19	16/16	< 14/14	< 14/14
	b	GCATTTAAGTCTGTCCATT	pSM2 miR	19/19	16/16	< 14/14	< 14/14
PBX3	a	GCGAACTCATACAAACCAATA	pLKO1	21/21	16/16	< 14/14	< 14/14
	b	GCTAATGAGACTGGACAATAT	pLKO1	21/21	16/16	15/15	< 15/15
	c	CACACAGAACTGGAGAAATAT	pLKO1	21/21	16/16	< 16/16	< 16/16
MEIS1	a	CGGTATATTAGCTGTTTGA	pGipz miR	19/19	15/15	< 14/14	< 14/14
	b	GGGGGAAGGCTGCAAAGTA	pRSC	19/19	16/16	15/15	< 15/15
	c	GGTACGACGATCTACCCCA	pRSC	19/19	16/16	< 16/16	< 16/16
None		ATCTCGCTTGGGCGAGAGTAAG	pGipz	None	15/15	15/15	< 15/15
None		ATCTCGCTTGGGCGAGAGTAAG	pSM2	None	15/15	15/15	< 15/15
None		TACAACAGCCACAACGTCTAT	pLKO1	None	15/15	15/15	< 15/15
None		ATCTCGCTTGGGCGAGAGTAAG	pRSC	None	15/15	15/15	< 15/15
None		CAACAAGATGAAGAGCACCAA	pLKO1-SHC002 control	None	16/16	16/16	< 15/15

Target Homology	Homology stretch to mRNA of intended target gene
Gene Homology	Best homology stretch to any other human gene
RefSeq homology	Best homology stretch to any other human RefSeq (curated) gene mRNA
TALE/HOX homology	Best homology stretch to any other human TALE/HOX gene

MEIS1/2/3	no miRNA in locus	
PBX1/2/3/4	no miRNA in locus	
HOXA	mir196b	no homology of any used shRNA to miRNA seed sequence
HOXB	mir3185, mir10a, mir196a1	no homology of any used shRNA to miRNA seed sequence
HOXC	mir615, mir196a2	no homology of any used shRNA to miRNA seed sequence
HOXD	mir10b	no homology of any used shRNA to miRNA seed sequence
		Databases used: www.pictar.org and UCSC Genome

Online Supplementary Table S2. *HOXA/TALE* signature in the MILE AML dataset. Differentially expressed *HOXA/TALE* genes identified between the combined favorable risk AML group (MILE 9-11) consisting of inv(16)/t(16;16), t(8;21) and t(15;17), and the intermediate risk AML group (MILE-13) defined as cytogenetically normal (CN-AML) + other abnormalities not 11q23. The signature was identified using false discovery rate-adjusted significant difference *P* values ≤ 0.0000005 and fold-changes as indicated where expression is higher in intermediate *vs*. favorable AML. **PBX4* and *MEIS3* were absent from the array.

Probeset ID	symbol	p-value(MILE Class)	p-value(9-11 v 13)	Ratio(9-11 v 13)	Fold-Change(9-11 v 13)	F(MILE Class)	SS(MILE Class)	SS(Error)	F(Error)
GC07M027168_at	HOXA9	0	4.20E-45	0.0528589	-18.9183	92.395	1584.91	2584.47	1
GC07M027113_at	HOXA3	2.21E-31	1.16E-32	0.588083	-1.70044	57.2459	48.0101	126.359	1
GC07M027147_at	HOXA5	1.24E-30	2.04E-30	0.163648	-6.11068	55.6543	592.006	1602.67	1
GC07M027159_at	HOXA7	2.75E-27	3.60E-28	0.374823	-2.66793	48.6956	167.122	517.085	1
GC07M027134_at	HOXA4	1.24E-25	1.74E-26	0.608473	-1.64346	45.3409	42.9681	142.782	1
GC02P066660_at	MEIS1	1.05E-33	8.72E-25	0.254845	-3.92395	62.2482	483.807	1171.02	1
GC07M027151_at	HOXA6	3.81E-21	6.39E-23	0.483242	-2.06936	36.5132	88.194	363.92	1
GC07M027099_at	HOXA1	1.88E-19	5.75E-20	0.506305	-1.97509	33.2817	83.0035	375.758	1
GC09P128508_at	PBX3	1.76E-19	2.96E-18	0.401912	-2.48811	33.3375	165.89	749.731	1
GC07M027106_at	HOXA2	3.35E-09	1.31E-10	0.770449	-1.29794	14.7899	11.5157	117.312	1
GC07M027220_at	HOXA11	6.78E-06	3.01E-07	0.724379	-1.38049	9.16024	17.4505	287.025	1

Online Supplementary Table S3. Cytogenetic and *HOXA/TALE* expression status in a cohort of AML patients. Tabulated cytogenetic and RQ-PCR values for *HOXA6*, *HOXA9*, *MEIS1* and *PBX3* in a cohort of samples (n=37) from anonymized AML patients. Mutation status was confirmed by PCR or pyrosequencing for favorable translocations or *NPM1/FLT3* mutations. Relative gene expression was determined by target C_T values being corrected to endogenous *18S* rRNA controls.

		Translocation/	Gene Expression (C_T corrected for 18S rRNA)			
Local Code	CytoRisk	Mutation	HoxA6	HoxA9	Meis1	Pbx3
4922	Favourable	t(8;21)	20.97	27.93	27.33	24.06
41132	Favourable	t(8;21)	28.13	32.14	34.82	24.03
4711	Favourable	t(15;17)	27.52	30.78	31.48	22.71
418	Favourable	t(8;21)	26.48	30.80	28.91	25.52
514	Favourable	t(8;21)	26.15	33.02	31.63	24.65
9001	Favourable	t(8;21)	31.32	32.57	32.97	23.81
10272	Favourable	t(15;17)	32.23	32.17	34.29	25.78
11070	Favourable	t(15;17)	31.07	29.00	33.69	23.64
11156	Favourable	t(15;17)	28.74	27.58	28.01	23.67
8293	Intermediate	NPM1/FLT3-ITD	20.84	20.89	25.33	30.15
8552	Intermediate	NPM1	20.80	21.21	21.32	20.29
9305	Intermediate	wt	24.66	24.71	23.96	25.63
9313	Intermediate	wt	18.81	12.96	22.77	24.17
9332	Intermediate	NPM1	21.18	23.04	22.35	25.70
9356	Intermediate	wt	24.91	25.56	23.62	28.05
9398	Intermediate	wt	19.53	15.33	25.91	23.52
9441	Intermediate	NPM1/FLT3-TKD	17.21	13.82	23.39	24.53
9529	Intermediate	wt	22.90	18.24	27.89	24.87
10134	Intermediate	NPM1	19.16	20.24	19.34	20.23
10248	Intermediate	FLT3-ITD	23.82	18.36	26.41	25.33
10365	Intermediate	NPM1	16.92	12.47	17.57	19.41
10380	Intermediate	NPM1	19.03	19.97	21.60	20.11
10383	Intermediate	wt	20.31	17.27	25.15	25.62
10510	Intermediate	NPM1	22.35	18.71	24.96	24.16
10574	Intermediate	NPM1	16.84	12.67	22.07	21.11
10584	Intermediate	NPM1	18.11	11.72	21.72	19.50
10637	Intermediate	wt	19.62	12.20	23.50	24.30
11089	Intermediate	wt	28.71	33.31	32.29	25.14
11093	Intermediate	wt	18.46	13.44	19.75	21.22
11099	Intermediate	wt	11.23	8.49	14.94	15.67
11110	Intermediate	NPM1/FLT3-ITD	19.19	19.44	21.44	20.58
11112	Intermediate	NPM1	19.05	20.56	20.99	19.69
11119	Intermediate	wt	23.74	23.54	22.76	19.97
11146	Intermediate	wt	20.92	21.13	24.27	22.72
11155	Intermediate	NPM1/FLT3-ITD	18.28	19.15	20.23	18.65
11165	Intermediate	NPM1	19.30	19.19	20.42	20.83
11177	Intermediate	NPM1	19.56	24.04	22.09	21.75

Online Supplementary Table S4. Table of estimated copies for AML cell lines and CN-AML samples. Representative gene expression values of *HOXA6*, *HOXA9*, *PBX3* and *MEIS1* in OCI-AML3, U937 cell lines and CN-AML patients' samples (CN-AML1-5) subjected to *HOXA/TALE* knockdown. C_T values, corrected for *18S* RNA loading, for 50 ng equivalents of RNA and corresponding copies are presented. Copy numbers were obtained following standard curve generation that indicated a C_T of 35 was equivalent to 10 copies of the gene. Values obtained are based on the principle that a two-fold increase in expression is obtained within each PCR cycle.

	OCI-AML3		U937		CN-AML-1		CN-AML-2		CN-AML-3		CN-AML-4		CN-AML-5	
	C_T Value	Copies	C_T Value	Copies	C_T Value	Copies	C_T Value	Copies	C_T Value	Copies	C_T Value	Copies	C_T Value	Copies
HOXA6	25.9	5.56.E+03	26.6	3.47E+03	22.6	5.47E+04	21.9	9.07E+04	22.7	5.20E+04	20.9	1.7E+05	24.6552	1.3E+04
HOXA9	25.1	9.69.E+03	26.2	4.52E+03	24.0	1.99E+04	23.2	3.48E+04	24.5	1.42E+04	21.1	1.5E+05	24.7099	1.3E+04
PBX3	27.1	2.32.E+03	27.7	1.59E+03	23.7	2.51E+04	22.6	5.30E+04	22.9	4.25E+04	22.7	5.0E+04	23.9611	2.1E+04
MEIS1	27.1	2.37.E+03	26.8	3.00E+03	23.9	2.18E+04	22.9	4.48E+04	22.3	6.62E+04	24.3	1.7E+04	25.6295	6.6E+03

Online Supplementary Table S5. Differentially expressed genes in *HOXA6/HOXA9* knockdown AML cell lines. Gene lists obtained following knockdown of either *HOXA6* or *HOXA9* in OCI-AML3 or U937 cells. Significant differences in gene expression, compared to non-silenced control, were determined by AmpliChip Leukemia, ($P \leq 0.05$ and fold change ≥ 2). (A) Overlapping *HOXA6/A9* and (B) *HOX* gene or cell line-specific gene lists are presented.

A

OCI AML3 shHOXA6 and shHOXA9 overlapping genes							
Probe-set ID	**Gene Symbol**	**Ratio (NSC *vs.* A6)**	**Fold-Change(NSC *vs.* A6)**	**Description (NSC *vs.* A6)**	**Ratio(NSC *vs.* A9)**	**Fold-Change(NSC *vs.* A9)**	**Description (NSC *vs.* A9)**
201029_s_copy3_at	CD99	0.482697	-2.07169	NSC down *vs.* A6	0.459514	-2.17621	NSC down *vs.* A9
205653_copy2_at	CTSG	2.12208	2.12208	NSC up *vs.* A6	0.4962	-2.01532	NSC down *vs.* A9
213150_copy3_at	HOXA10	0.317046	-3.15412	NSC down *vs.* A6	0.39794	-2.51294	NSC down *vs.* A9
214464_copy1_at	CDC42BPA	0.427272	-2.34043	NSC down *vs.* A6	0.462212	-2.16351	NSC down *vs.* A9
225563_copy3_at	PAN3	0.451912	-2.21282	NSC down *vs.* A6	0.453013	-2.20744	NSC down *vs.* A9
U937 shHOXA6 and shHOXA9 overlapping genes							
Probeset ID	**Gene Symbol**	**Ratio(A6 vs. NSC)**	**Fold-Change(A6 vs. NSC)**	**Description (A6 vs. NSC)**	**Ratio(A9 vs. NSC)**	**Fold-Change(A9 vs. NSC)**	**Description (A9 vs. NSC)**
203611_copy2_at	TERF2	0.457042	-2.18798	A6 down vs NSC	0.494624	-2.02174	A9 down vs NSC
209771_x_at	CD24	4.4529	4.4529	A6 up vs NSC	4.06334	4.06334	A9 up vs NSC
211560_s_at	ALAS2	2.73661	2.73661	A6 up vs NSC	3.0661	3.0661	A9 up vs NSC
212022_s_at	MKI67	0.358474	-2.7896	A6 down vs NSC	0.392852	-2.54549	A9 down vs NSC
216379_x_at	CD24	3.84483	3.84483	A6 up vs NSC	3.88345	3.88345	A9 up vs NSC
224707_copy2_at	C5orf32	2.24653	2.24653	A6 up vs NSC	2.69366	2.69366	A9 up vs NSC

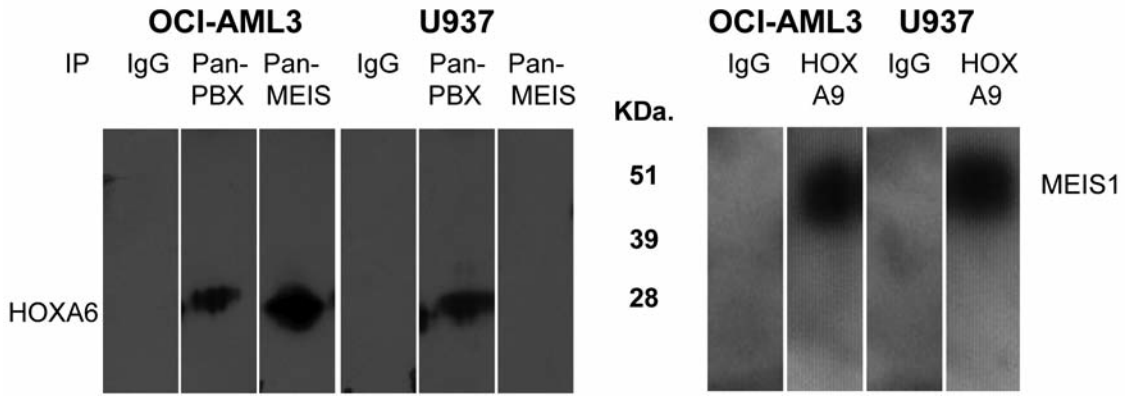
B

OCI AML3 shHOXA6				
Probeset ID	**Gene Symbol**	**Ratio(NSC vs. A6)**	**Fold-Change(NSC vs. A6)**	**Description (NSC vs. A6)**
1553043_a_at	CD300LF	0.42456	-2.35538	NSC down vs A6
201029_s_copy1_at	CD99	0.484295	-2.06486	NSC down vs A6
201029_s_copy3_at	CD99	0.482697	-2.07169	NSC down vs A6
205653_copy2_at	CTSG	2.12208	2.12208	NSC up vs A6
207332_s_at	TFRC	2.06588	2.06588	NSC up vs A6
209732_copy3_at	CLEC2B	2.39019	2.39019	NSC up vs A6
210895_s_at	CD86	0.228794	-4.37074	NSC down vs A6
213150_copy3_at	HOXA10	0.317046	-3.15412	NSC down vs A6
214464_copy1_at	CDC42BPA	0.427272	-2.34043	NSC down vs A6
214575_s_at	AZU1	2.05883	2.05883	NSC up vs A6
215646_s_at	VCAN	0.141688	-7.05776	NSC down vs A6
219010_at	C1orf106	2.26256	2.26256	NSC up vs A6
219463_copy1_at	C20orf103	2.48387	2.48387	NSC up vs A6
219463_copy2_at	C20orf103	3.14053	3.14053	NSC up vs A6
219463_copy3_at	C20orf103	2.68715	2.68715	NSC up vs A6
221766_s_copy1_at	FAM46A	0.428529	-2.33356	NSC down vs A6
224782_copy3_at	ZMAT2	2.18532	2.18532	NSC up vs A6
225563_copy3_at	PAN3	0.451912	-2.21282	NSC down vs A6
226364_at	HIP1	0.478865	-2.08827	NSC down vs A6
229622_copy3_at	FAM132B	0.324904	-3.07783	NSC down vs A6
AFFX-r2-Bs-dap-3_at	---	2.75081	2.75081	NSC up vs A6
AFFX-r2-Bs-dap-5_at	---	2.42078	2.42078	NSC up vs A6
AFFX-r2-Bs-thr-3_s_at	---	4.0725	4.0725	NSC up vs A6

U937 shHOXA6				
Probeset ID	**Gene Symbol**	**Ratio(NSC vs. A6)**	**Fold-Change(NSC vs. A6)**	**Description (NSC vs. A6)**
203372_s_copy1_at	SOCS2	0.490198	-2.03999	A6 down vs NSC
203611_copy2_at	TERF2	0.457042	-2.18798	A6 down vs NSC
205453_copy3_at	HOXB2	0.453837	-2.20343	A6 down vs NSC
205837_s_at	GYPA	2.13563	2.13563	A6 up vs NSC
206655_s_at	GP1BB /// SEPT5	2.24	2.24	A6 up vs NSC
209732_copy1_at	CLEC2B	2.30623	2.30623	A6 up vs NSC
209771_x_at	CD24	4.4529	4.4529	A6 up vs NSC
211560_s_at	ALAS2	2.73661	2.73661	A6 up vs NSC
211820_x_at	GYPA	2.26804	2.26804	A6 up vs NSC
211821_x_at	GYPA	2.12875	2.12875	A6 up vs NSC
212022_s_at	MKI67	0.358474	-2.7896	A6 down vs NSC
216379_x_at	CD24	3.84483	3.84483	A6 up vs NSC
221577_x_copy1_at	GDF15	0.291965	-3.42507	A6 down vs NSC
221577_x_copy2_at	GDF15	0.188384	-5.30831	A6 down vs NSC
221577_x_copy3_at	GDF15	0.267764	-3.73463	A6 down vs NSC
224707_copy2_at	C5orf32	2.24653	2.24653	A6 up vs NSC
225386_s_copy1_at	HNRPLL	2.66406	2.66406	A6 up vs NSC
225386_s_copy2_at	HNRPLL	2.20949	2.20949	A6 up vs NSC
225386_s_copy3_at	HNRPLL	2.25902	2.25902	A6 up vs NSC

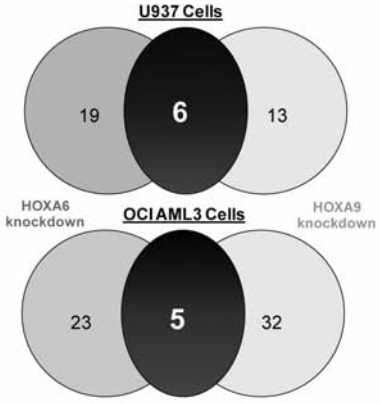
OCI AML3 shHOXA9				
Probeset ID	**Gene Symbol**	**Ratio(NSC vs. A9)**	**Fold-Change(NSC vs. A9)**	**Description (NSC vs. A9)**
1557480_a_at	DYSFIP1	0.479955	-2.08353	NSC down vs A9
1557480_a_at	DYSFIP1	0.479955	-2.08353	NSC down vs A9
200839_s_copy1_at	CTSB	2.23887	2.23887	NSC up vs A9
201029_s_copy3_at	CD99	0.459514	-2.17621	NSC down vs A9
201417_copy1_at	SOX4	2.16425	2.16425	NSC up vs A9
201743_at	CD14	2.11051	2.11051	NSC up vs A9
201876_copy2_at	PON2	0.477749	-2.09315	NSC down vs A9
201923_copy2_at	PRDX4	0.441369	-2.26568	NSC down vs A9
201923_copy3_at	PRDX4	0.393466	-2.54151	NSC down vs A9
203335_at	PHYH	0.416547	-2.40069	NSC down vs A9
203936_s_copy1_at	MMP9	2.20413	2.20413	NSC up vs A9
205627_at	CDA	0.427247	-2.34057	NSC down vs A9
205653_copy1_at	CTSG	0.34869	-2.86788	NSC down vs A9
205653_copy2_at	CTSG	0.4962	-2.01532	NSC down vs A9
205653_copy3_at	CTSG	0.376923	-2.65306	NSC down vs A9
205789_at	CD1D	2.68977	2.68977	NSC up vs A9
206488_s_at	CD36	2.60929	2.60929	NSC up vs A9
209395_at	CHI3L1	2.1121	2.1121	NSC up vs A9
209905_copy2_at	HOXA9	0.374406	-2.6709	NSC down vs A9
209905_copy3_at	HOXA9	0.388789	-2.57209	NSC down vs A9
213150_copy3_at	HOXA10	0.39794	-2.51294	NSC down vs A9
214464_copy1_at	CDC42BPA	0.462212	-2.16351	NSC down vs A9
214511_x_at	FCGR1B	0.495832	-2.01681	NSC down vs A9
219519_s_at	SIGLEC1	2.51892	2.51892	NSC up vs A9
219689_at	SEMA3G	2.05427	2.05427	NSC up vs A9
225285_at	BCAT1	0.429483	-2.32838	NSC down vs A9
225344_at	NCOA7	0.484528	-2.06387	NSC down vs A9
225563_copy3_at	PAN3	0.453013	-2.20744	NSC down vs A9
226517_at	BCAT1	0.383757	-2.60581	NSC down vs A9
226876_at	FAM101B	0.297457	-3.36183	NSC down vs A9
227949_copy1_at	PHACTR3	0.384103	-2.60347	NSC down vs A9
229622_copy1_at	FAM132B	0.431148	-2.31939	NSC down vs A9
229622_copy2_at	FAM132B	0.481986	-2.07475	NSC down vs A9

U937 shHOXA9				
Probeset ID	**Gene Symbol**	**Ratio(A9 vs. NSC)**	**Fold-Change(A9 vs. NSC)**	**Description (A9 vs. NSC)**
203372_s_copy1_at	SOCS2	2.52453	2.52453	A9 up vs NSC
203611_copy2_at	TERF2	3.61537	3.61537	A9 up vs NSC
205453_copy3_at	HOXB2	0.494624	-2.02174	A9 down vs NSC
205837_s_at	GYPA	0.464547	-2.15263	A9 down vs NSC
206655_s_at	GP1BB /// SEPT5	2.06828	2.06828	A9 up vs NSC
209732_copy1_at	CLEC2B	4.06334	4.06334	A9 up vs NSC
209771_x_at	CD24	3.0661	3.0661	A9 up vs NSC
211560_s_at	ALAS2	0.392852	-2.54549	A9 down vs NSC
211820_x_at	GYPA	0.400824	-2.49486	A9 down vs NSC
211821_x_at	GYPA	3.88345	3.88345	A9 up vs NSC
212022_s_at	MKI67	3.41245	3.41245	A9 up vs NSC
216379_x_at	CD24	2.69366	2.69366	A9 up vs NSC
221577_x_copy1_at	GDF15	3.84289	3.84289	A9 up vs NSC



Online Supplementary Figure S1. Identification of HOXA6/HOXA9:TALE protein interactions. Representative co-immunoprecipitation (IP) studies demonstrate that HOXA6 forms a high affinity complex with PBX and can interact with MEIS in OCI-AML3 and U937 AML cells (left panel). Similar results were obtained for HOXA9 and PBX (*data not shown*). The HOXA9:MEIS1 interaction was used as a positive control (right panel).

A



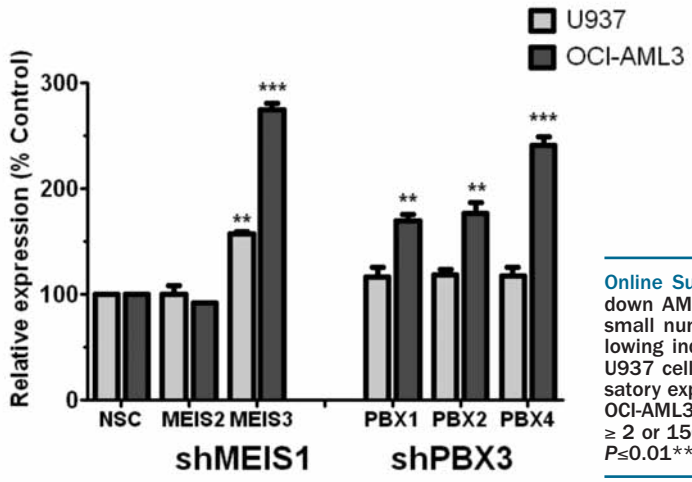
U937

Common probe sets	Class	Expression *vs.* NSC
TERF2	Telomere maintenance	Lower
CD24	Adhesion	Higher
ALAS2	Enzyme	Higher
MKi67	Proliferation	Lower
CD24	Adhesion	Higher
C5orf32	unknown	Higher

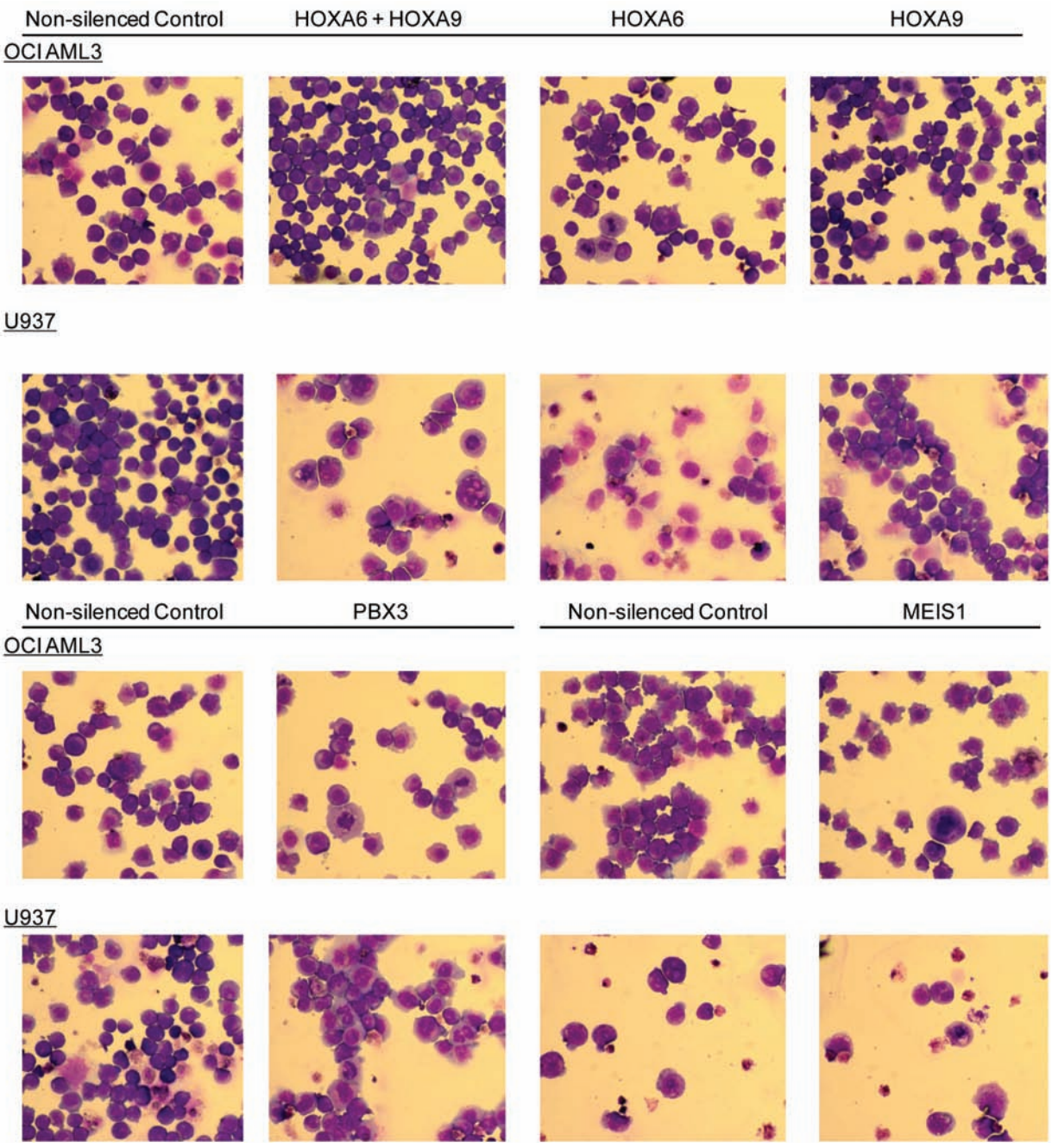
OCI-AML3

Common probe sets	Class	Expression *vs.* NSC
CD99	Adhesion	Higher
Cathepsin G	Protein decay	Lower (A6), higher (A9)
HOXA10	Transcription factor	Higher
CDC42BP α	Enzyme	Higher
PAN3	mRNA decay	Higher

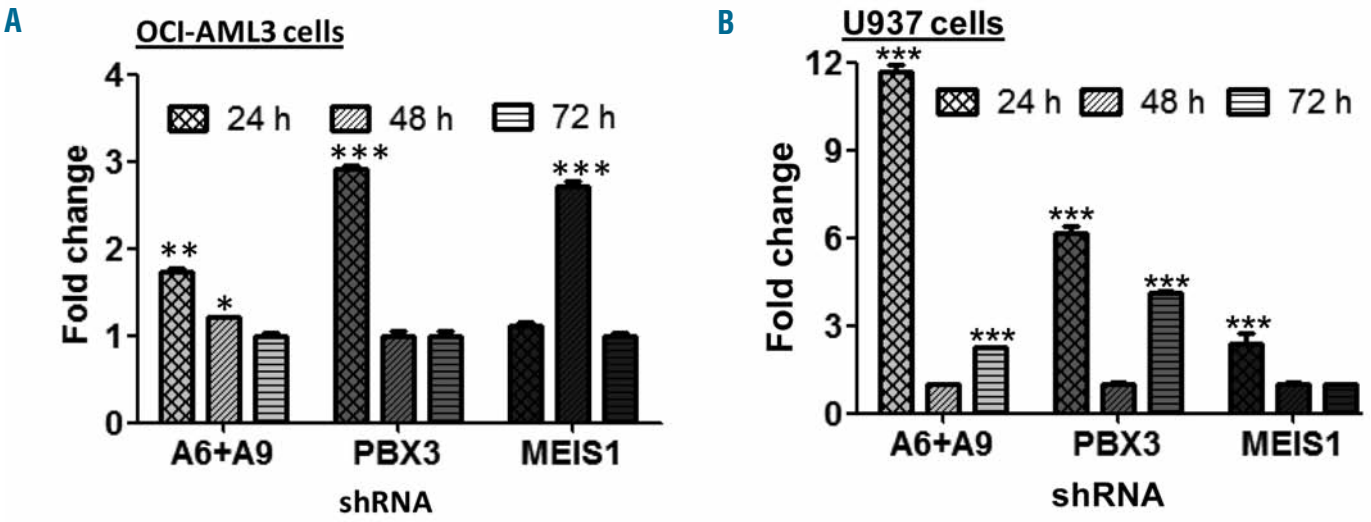
B



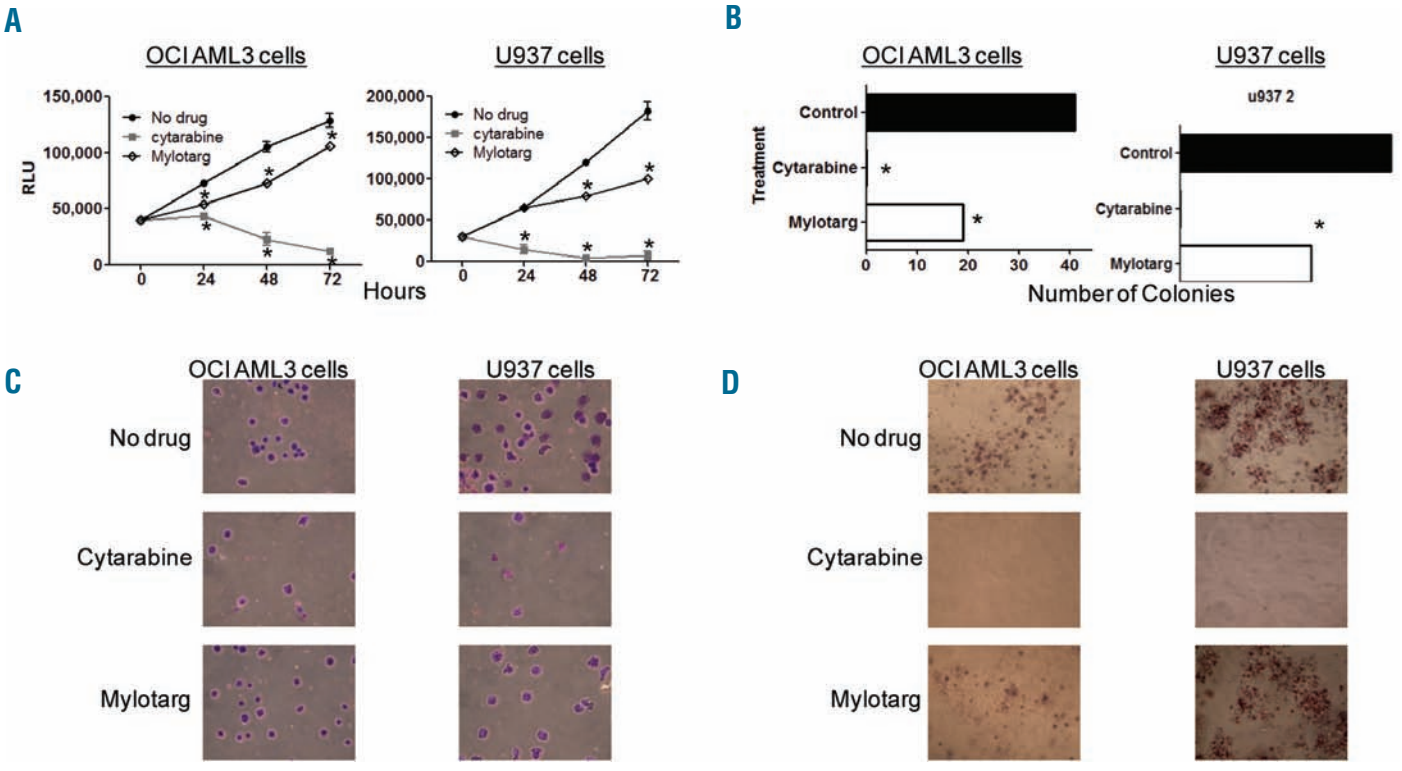
Online Supplementary Figure S2. Differential gene expression in knockdown AML cell lines. (A) Venn diagram and associated table depicting a small number of AmpliChip Leukemia genes differentially expressed following individual knockdown of *HOXA6* or *HOXA9* (72 h) in OCI-AML3 or U937 cells. (B) A histogram plot depicting no change or modest compensatory expression of *TALE* genes following knockdown of *PBX3* or *MEIS1* in OCI-AML3 or U937 cells. Significantly altered gene expression (fold-change ≥ 2 or 150%) was identified by ANOVA compared to non-silenced controls, $P \leq 0.01$** or $P \leq 0.001$***.



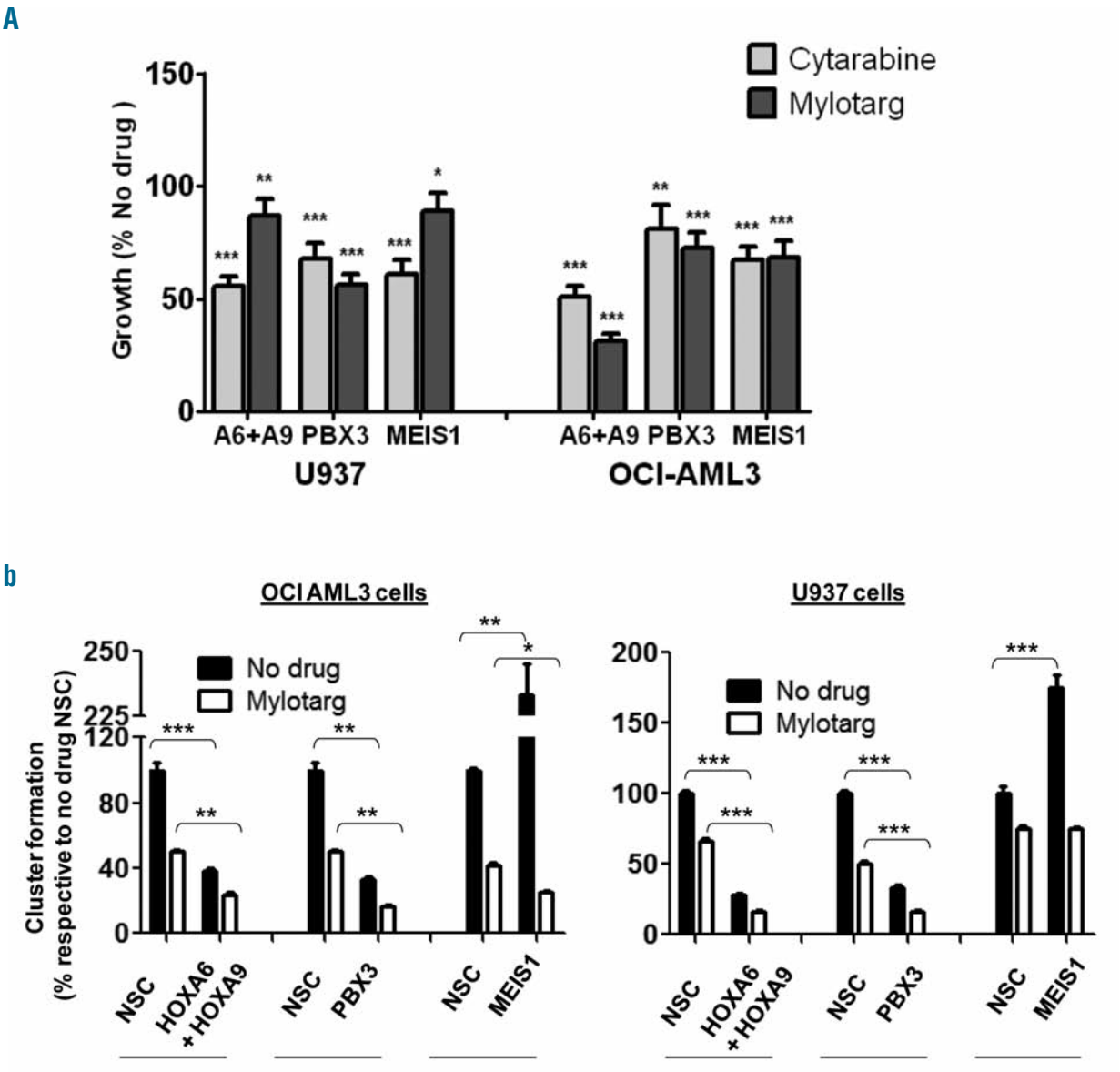
Online Supplementary Figure S3. Cellular morphology of treated AML cell lines. OCI-AML3 and U937 cell lines were treated with shRNA as labeled. Gene knockdown cells were selected in puromycin for 3 days, cytospins prepared at day 8 and Wright-Giemsa stained. Representative images were obtained at 200X using a light microscope (BH-2) and captured by an attached digital camera (DP25) and Cell^B imaging software, (all from Olympus, Essex, UK).



Online Supplementary Figure S4. Representative caspase 3/7 activity following *HOXA/TALE* knockdown. Representative histograms depicting a caspase 3/7 activity time-course measured by luminescence per 100 OCI-AML3 (A) or U937 (B) cells. Caspase 3/7 activity was measured at 24, 48 and 72 h following *HOXA/TALE* knockdown and fold-change values compared to non-silenced controls were obtained. Mean and standard deviation of triplicate experiments are plotted, **P*≤0.05, ***P*≤0.01, ****P*≤0.001.



Online Supplementary Figure S5. Chemotherapy-response of AML cell lines. (A) OCI-AML3 and U937 cell lines were treated in liquid culture with IC_{50} doses of cytarabine or Mylotarg for up to 72 h as indicated. Cell counts was evaluated by CellTiter-Glo®. (B) In addition the cell lines were treated and maintained in methylcellulose for up to 14 days and the number of colonies enumerated. (C, D) Images of treated cells were taken for morphology after 48 h in liquid culture (C) and INT staining after 10 days in methylcellulose (D). *P*≤0.05 is denoted by *. Representative morphology and colony images captured at 200X and 100X respectively using an inverted microscope (CKX41) with attached digital camera (E620) both from Olympus (Essex, UK).



Online Supplementary Figure S6. Combined chemotherapy and *HOX/TALE* knockdown in AML cell lines. (A) A histogram plot depicting percentage cell growth of OCI-AML3 and U937 cells. Cell numbers were quantified in liquid culture by measurement of ATP levels (CellTiter-Glo®) following pre-conditioning knockdown with the indicated shRNA (48 h) ± cytarabine or Mylotarg treatment for a further 24 h. Data from three independent experiments are presented as percentages of the respective no drug-treated controls. (B) OCI-AML3 and U937 cells pre-conditioned with *PBX3* or combined *HOXA6* + *HOXA9* shRNA were treated with Mylotarg (IC_{50} dose) and maintained in methylcellulose for up to 14 days. Clusters of <40 cells were enumerated and percentages compared to no drug and non-silencing controls are plotted. Data are representative of duplicate wells, n=2. Mean and standard deviation values are plotted, $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$.