Supplemental Inventory

GSK-3 promotes conditional association of CREB and its co-activators with MEIS1 to facilitate HOX-mediated transcription and oncogenesis

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Supplemental Data

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Supplemental Data

Supplemental Figure 1. Wang et al.

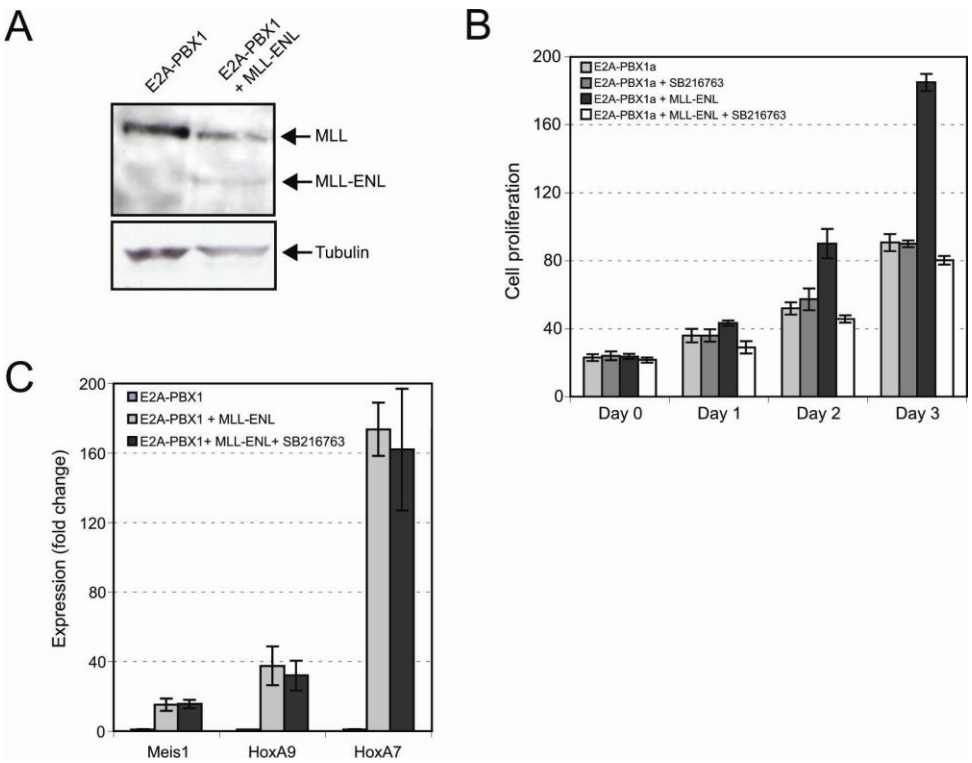

Figure S1, related to Figure 2. MLL-induced cell proliferation, but not MLL transcriptional activity, is dependent on GSK-3.

(A) Western blot analysis demonstrates expression of exogenous MLL-ENL in E2A-PBX1 immortalized murine myeloid progenitors.
(B) Cell numbers were determined at the indicated days (below) for E2A-PBX1-immortalized myeloid progenitors with or without co-expressed MLL-ENL grown in the presence or absence of 10 μM SB216763. The enhanced proliferation induced by MLL-ENL was abrogated by SB216763, which did not affect baseline proliferation induced by E2A-PBX1.
(C) Expression of MLL target genes (indicated below) was quantified by PCR in E2A-PBX1-immortalized myeloid progenitors with or without co-expressed MLL-ENL grown in the presence or absence of SB216763 (20 μM). In contrast to the growth inhibition in panel B, expression of *Meis1* and *Hox* genes induced by MLL-ENL was not affected by GSK-3 inhibition.
Error bars indicate ±SEM.

Supplemental Figure 2. Wang et al.

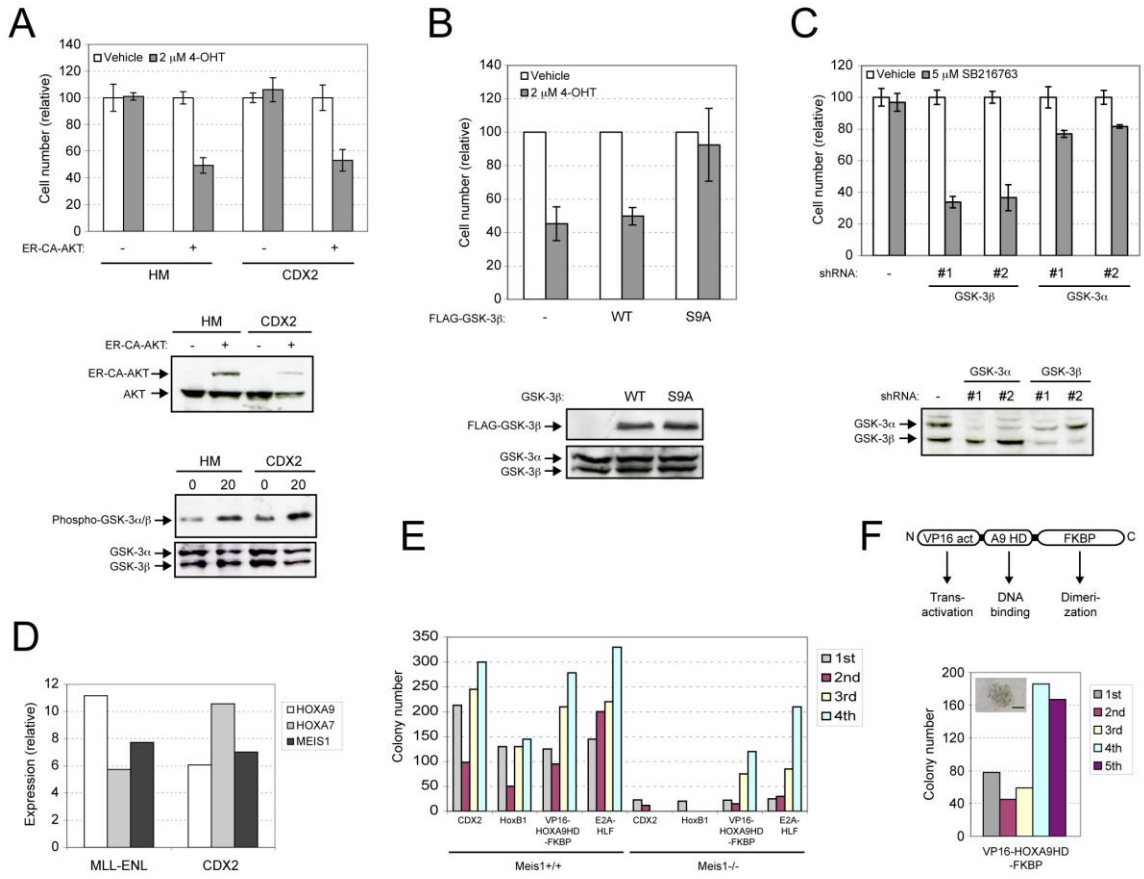

Figure S2, related to Figure 3. CDX2-induced cell proliferation is dependent on GSK-3 and MEIS1.

(A) Murine myeloid progenitors transformed by HOXA9/MEIS1 (HM) or CDX2 were transduced with retroviral vector expressing ER-CA-AKT. Bar graph shows cell numbers determined after two days culture with (+) or without (-) 4-hydroxy-tamoxifen. Data are expressed relative to untreated cells. Western blot analysis demonstrates expression of ER-CA-AKT (middle panel), and increased phosphorylation of GSK-3β (lower panel) confirming activation of AKT following treatment with 4-hydroxy-tamoxifen (2 μM) for 20 hours.
(B) Cell counts (bar graph) were determined after 3 days treatment with 4-hydroxy-tamoxifen and are expressed relative to untreated cells. Western blot analysis (lower panel) shows expression of exogenous wild type (WT) or mutant (S9A) GSK-3β in CDX2-immortalized cells expressing ER-CA-AKT.
(C) Cell counts (bar graph) were determined after 3 days culture in the presence or absence of 5 μM SB216763 and are expressed relative to untreated cells. Western blot analysis (lower panel) shows expression of GSK-3 isoforms in CDX2-immortalized cells transduced with knockdown constructs for GSK-3α or GSK-3β.

(D) Expression levels of *HoxA9*, *HoxA7* and *Meis1* are shown for mouse myeloid progenitors transformed by MLL-ENL or CDX2. Results of a representative experiment are shown as fold-change compared to expression levels in c-kit^{+} bone marrow cells.
(E) Fetal liver progenitors from wild type (Meis1$^{+/+}$) or mutant (Meis1$^{-/-}$) mice were transduced with the indicated genes (below) and subjected to serial replating in methylcellulose cultures. Colony numbers determined at each round of a representative experiment are shown.
(F) Murine myeloid progenitors (c-kit^{+} bone marrow cells) were transduced with VP16-HOXA9-FKBP (an activated form of HOXA9 shown schematically) and subjected to serial replating in methylcellulose cultures. Colony numbers determined at each round of a representative experiment are shown. Inset shows representative colony (Scale = 100 μm).
Error bars indicated ± SEM of triplicate analyses.

Supplemental Figure 3. Wang et al.

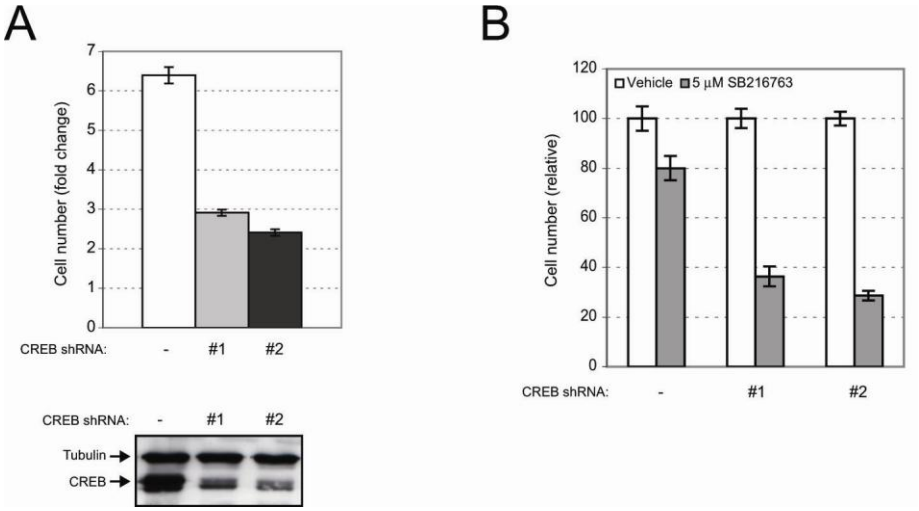

Figure S3, related to Figure 4. CREB modulates CDX2-immortalized cell proliferation and sensitivity to GSK-3 inhibition.

(A) Mouse myeloid progenitors immortalized by CDX2 were transduced with lentiviral vectors expressing *CREB* shRNAs, and then cultured in R20/20. Cell numbers were quantified on day 3 and expressed as fold change compared to day 0. Western blot analysis (lower panel) shows reduced CREB protein levels in knockdown cells.
(B) Mouse myeloid progenitors immortalized by CDX2 and expressing *CREB* shRNAs (indicated below) were cultured in the presence or absence of 5 μM SB216763. Cell numbers were quantified on day 2 and expressed as relative change compared to untreated cells. Error bars indicate ± SEM of triplicate analyses.

Supplemental Figure 4. Wang et al.

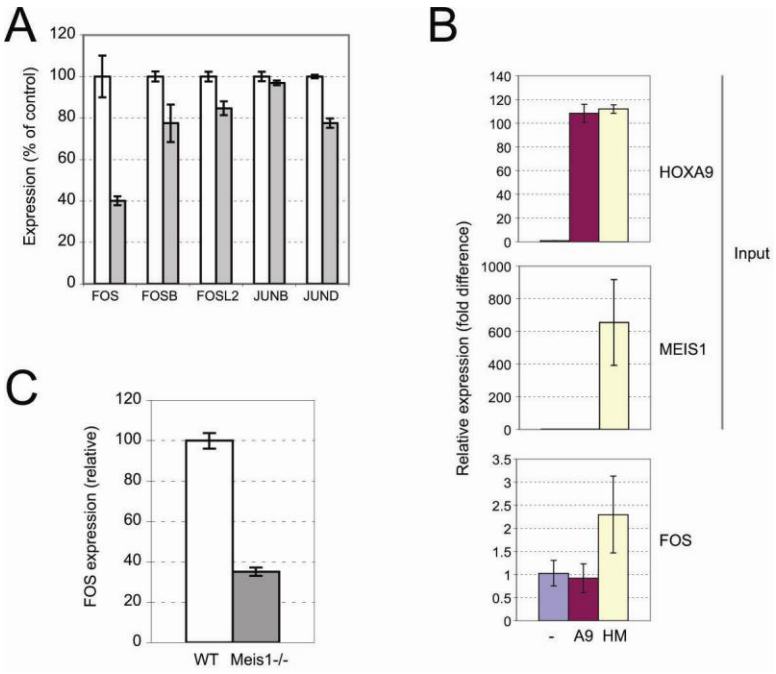

Figure S4, related to Figure 6. *FOS* expression level is regulated by GSK-3 and MEIS1.

(A) Bar graph depicts expression levels of AP-1 family genes following SB216763 treatment as determined by quantitative PCR. Data (mean of triplicate determinations) are expressed relative to untreated cells (average of duplicate analyses).
(B) Expression levels of *HOXA9*, *MEIS1* and *FOS* as determined by qPCR (± SEM of triplicate analyses) are shown for RCH-ACV (E2A-PBX1) cells transduced with HOXA9 (A9) or HOXA9+MEIS1 (HM).
(C) Bar graph depicts the expression levels of *FOS* in wild type or *Meis1*$^{-/-}$ fetal liver progenitors immortalized by E2A-HLF (± SEM of triplicate analyses).

Supplemental Table 1. Wang et al.

Table S1, related to Figure 1. Probe sets differentially expressed at least 1.5 fold following GSK-3 treatment of MLL leukemia cells.

This table is provided as a separate Excel file.

Supplemental Table 2. Wang et al.

Table S2, related to Figure 1. Gene sets down-regulated following GSK-3 inhibition in mixed lineage leukemia.*

Gene set name	FDR	Brief description of gene set
Cell Cycle		
SERUM_FIBROBLAST_CELL CYCLE	0	Cell-cycle dependent genes regulated following exposure to serum in a variety of human fibroblast cell lines
CELL_CYCLE	0	Progression of events that occur during replication (GO)
BRENTANI_CELL_CYCLE	0	Cancer related genes involved in the cell cycle(Broad institute)
GOLDRATH_CELLCYCLE	0	Cell cycle genes induced during antigen activation of CD8+ T cells
G1_TO_S_CELL_CYCLE_RE ACTOME	0	curated gene sets
CELL_CYCLE_CHECKPOINT	0.002971	Genes involved in cell cycle checkpoint
Differentiation		
LI_FETAL_VS_WT_KIDNEY_ DN	0	Genes differing in their mRNA expresssion between WTs and fetal kidneys HIGH
SASAKI_ATL_UP	0.00191	Highly expressed genes in ATL cells compared with normal CD4 and CD4 CD45RO T cells
GOLUB_ALL_VS_AML_UP	0.024989	Genes highly correlated with acute lymphoblastic leukemia (correlation P(g,c) > 0.30)
BRUNO_IL3_DN	0.020917	Annotated list of commonly downregulated genes in differentiation pathways
ZHAN_MULTIPLE_MYELOMA _SUBCLASSES_DIFF	0.016769	The 30 most differentially expressed genes in a comparison of MM1 and MM4 subgroups
Cancer and embryonic stem cell		
CHANG_SERUM_RESPONSE _UP	0	Core group of genes consistently up-regulated following exposure to serum (not cell-cycle dependent)
CANCER_UNDIFFERENTIAT ED_META_UP	0	Genes commonly upregulated in undifferentiated cancer relative to well-differentiated cancer
BRCA_PROGNOSIS_NEG	0	Genes whose expression is consistently negatively correlated with breast cancer outcomes - higher expression is associated with metastasis and poor prognosis
VANTVEER_BREAST_OUTC OME_GOOD_VS_POOR_DN	0	Poor prognosis marker genes in Breast Cancer (part of NKI-70) from Van't Veer et al 2002
CORDERO_KRAS_KD_VS_C ONTROL_DN	0	Genes upregulated in control vs kras knockdown in a human cell line
BASSO_REGULATORY_HUB S	1.74E-04	Genes that account for most of the interactions in the reconstructed regulatory networks from expression profiles in human B cells.
BREAST_DUCTAL_CARCINO MA_GENES	8.66E-04	Genes upregulated in breast tumors
CANCER_NEOPLASTIC_MET A_UP	0.002859	Sixty-seven genes commonly upregulated in cancer relative to normal tissue
BHATTACHARYA_ESC_UP	0.037914	Genes upregulated in undifferentiated human embryonic stem cells.
RAS_ONCOGENIC_SIGNATU RE	0.038112	Genes discriminating cells expressing activated H-Ras oncogene from control cells
HCC_SURVIVAL_GOOD_VS_ POOR_DN	0.031251	Genes highly expressed in hepatocellular carcinoma with poor survival.
ZUCCHI_EPITHELIAL_UP	0.031416	The 50 most upregulated genes in breast carcinoma as compared to normal mammary epithelium.
HOX/MEIS/MLL		

HESS_HOXAANMEIS1_UP	0	Genes upregulated in Hoxa9/Meis1 transduced cells vs control
ROSS_FAB_M7	0.043688	Genes upregulated in AML samples of the FAB class M7 including t(15 17)[PML-RARalpha] t(8 21)[AML1-ETO] inv(16) [CBFbeta-MYH11] MLL chimeric fusion genes
CHEN_HOXA5_TARGETS_DN	0.039237	Genes induced by HoxA5 expression.
LEI_MYB_REGULATED_GENES	0.020382	Myb-regulated genes
MLL-LSC_Somervaille	0	MLL leukemia stem cell associated genes
CREB/cAMP		
ADIP_DIFF_CLUSTER2	0	Strongly upregulated at 16 hours during differentiation of 3T3-L1 fibroblasts into adipocytes (cluster 2)
ADIP_DIFF_CLUSTER3	0.042108	Same as above (cluster 3)
ADIP_DIFF_CLUSTER4	0	Same as above (cluster 4)
ADIP_DIFF_CLUSTER5	0	Same as above (cluster 5)
IDX_TSA_UP_CLUSTER3	0	Strongly up-regulated at 16-24 hours during differentiation of 3T3-L1 fibroblasts into adipocytes (cluster 3)
IDX_TSA_UP_CLUSTER4	0.029147	Up-regulated from 8-48 hours during differentiation of 3T3-L1 fibroblasts into adipocytes as above (cluster 4)
IDX_TSA_UP_CLUSTER5	0.029744	Up-regulated at 48-96 hours during differentiation of 3T3-L1 fibroblasts into adipocytes as above (cluster 5)
GN_CAMP_GRANULOSA_UP	0.005582	Up-regulated in human granulosa cells by the gonadotropins LH and FSH, as well as by cAMP-stimulator forskolin
FSH_OVARY_MCV152_UP	0.028301	Up-regulated in ovarian epithelial cells (MCV152) 72 hours following FSH treatment, compared to untreated
XU_CBP_DN	0.027185	Fifty-nine probe sets downregulated at least 17-fold in CBP null B cells
FSH_GRANULOSA_UP	0.016507	up-regulated in human granulosa cells stimulated with follicle stimulation hormone (FSH)
LH_GRANULOSA_UP	0.016489	Up-regulated in human granulosa cells stimulated with luteinizing hormone (LH)

* Gene sets identified by GSEA to be down-regulated following GSK-3 inhibition of the RS4;11 mixed lineage leukemia cell line are shown along with their respective false discovery rates (FDR). Gene sets are organized into functional groups (bold type) based on their annotations (right hand column).

Supplemental Experimental Procedures

VP16-HOXA9-FKBP construct

The constitutively active VP16-HOXA9-FKBP transformation construct was generated by PCR and standard cloning techniques. It consisted of the HOXA9 DNA binding homeodomain fused at its amino terminus with the VP16 transcriptional activation domain and containing at its carboxyl terminus a single FKBP dimerization module (Pc4-FM2E) (Ariad Pharmaceuticals) that mediates spontaneous dimerization.

shRNA sequences used in this study

shRNA sequences used for knock-down studies.	
shRNA	**Sequence**
CREB-shRNA1-Forward	TGCCAAGGATTGAAGAAGAATTCAAGAGATTCTTCTTCAATCCTTGGCTTTTTTC
CREB-shRNA1-Reverse	TCGAGAAAAAAGCCAAGGATTGAAGAAGAATCTCTTGAATTCTTCTTCAATCCTTGGCA
CREB-shRNA2-Forward	TGCCACAGATTGCCACATTATTCAAGAGATAATGTGGCAATCTGTGGCTTTTTTC
CREB-shRNA2-Reverse	TCGAGAAAAAAGCCACAGATTGCCACATTATCTCTTGAATAATGTGGCAATCTGTGGCA
CREB-shRNA3-Forward	TGCCGGGTACTACCATTCTATTCAAGAGATAGAATGGTAGTACCCGGCTTTTTTC
CREB-shRNA3-Reverse	TCGAGAAAAAAGCCGGGTACTACCATTCTATCTCTTGAATAGAATGGTAGTACCCGGCA