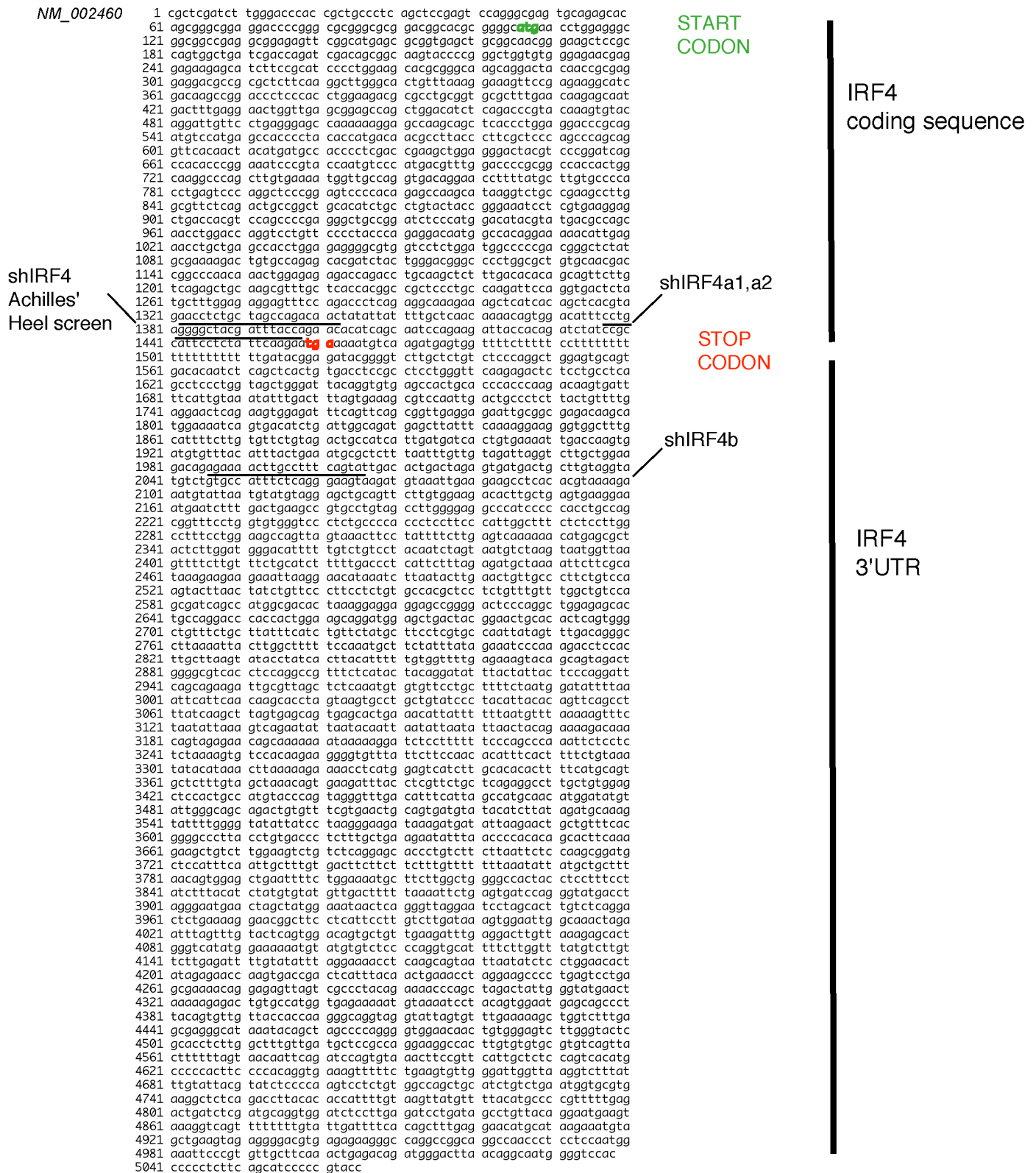


2007-11-12040B_Supplementary Figure 1



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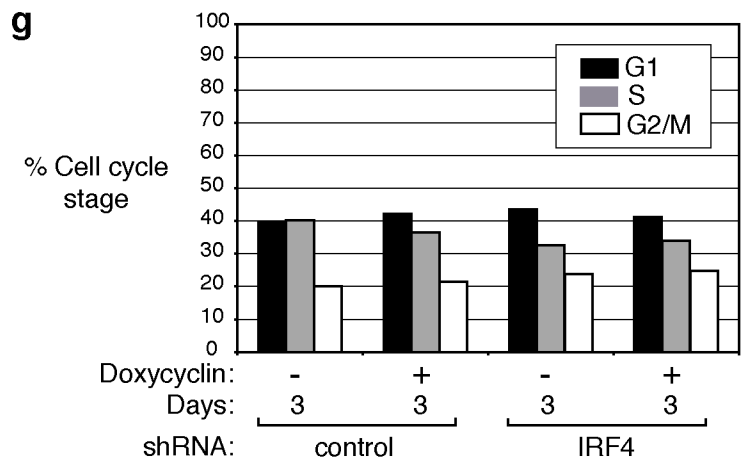
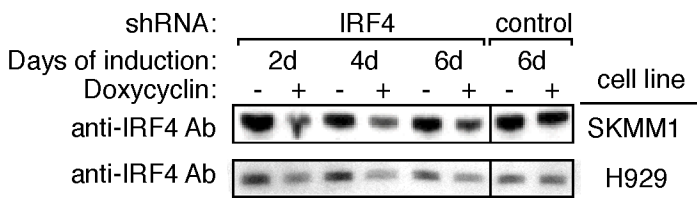
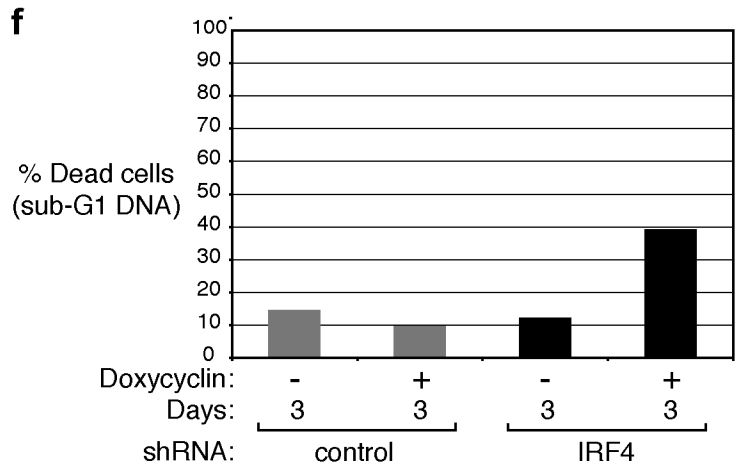
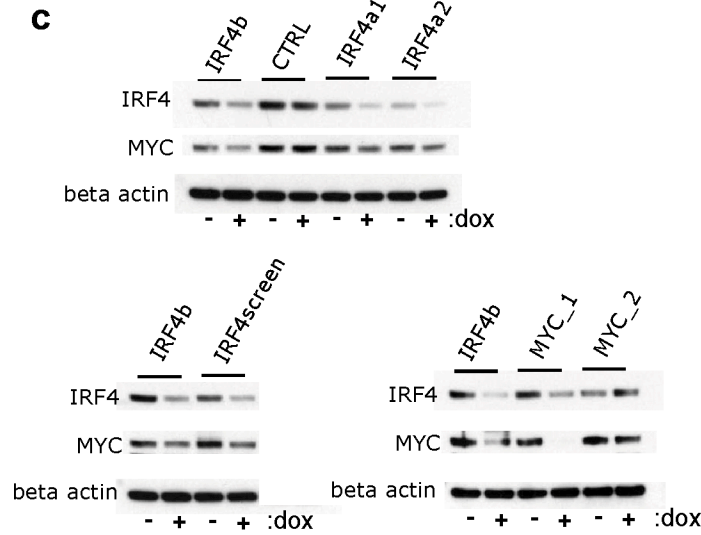
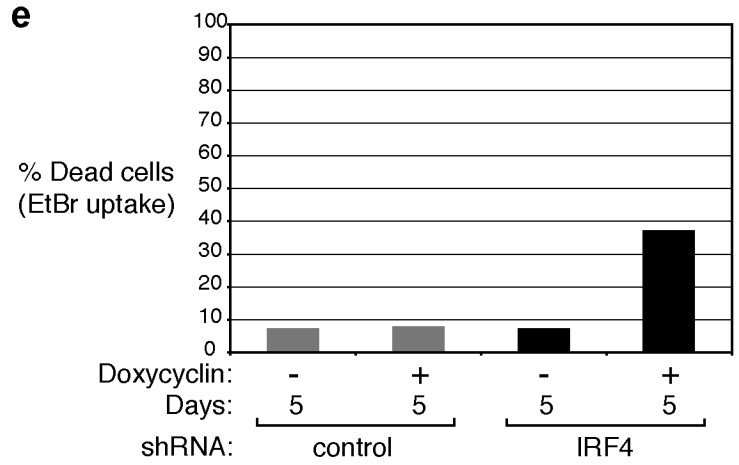
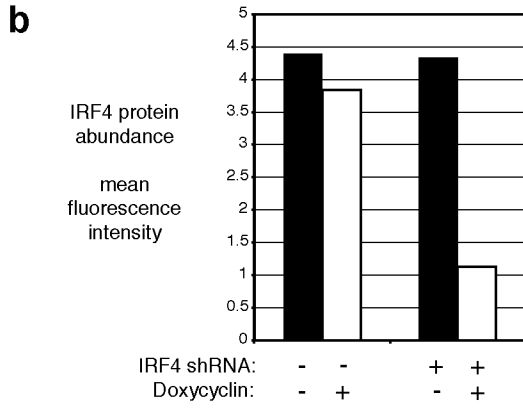
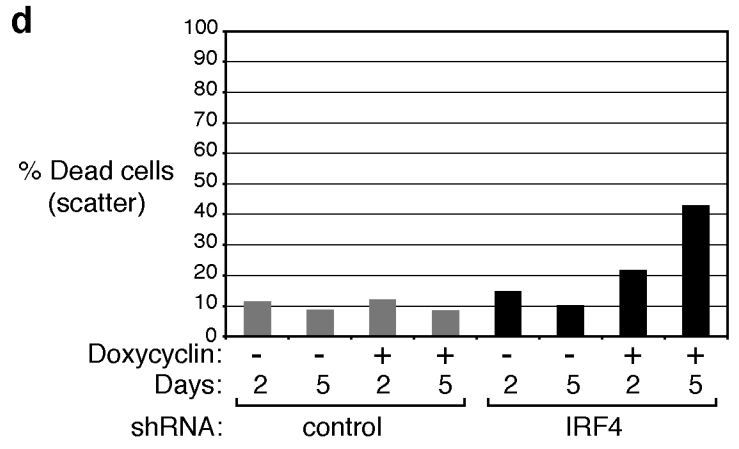
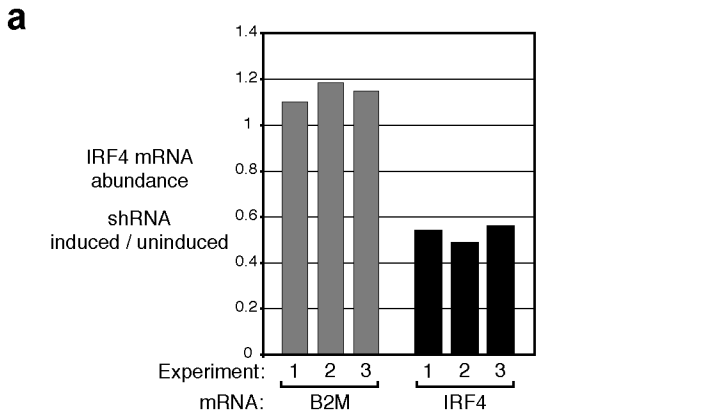
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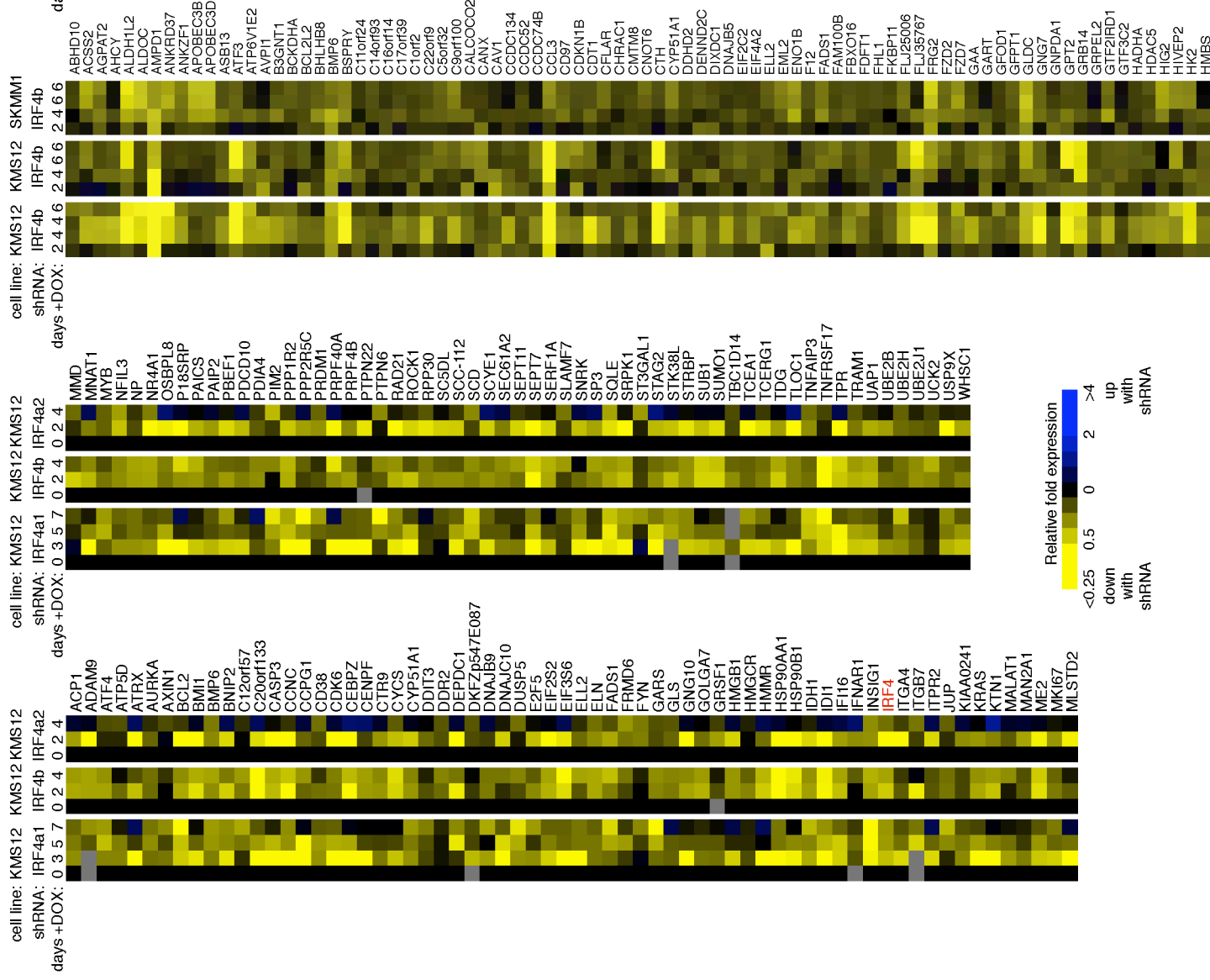
shCTRL (luciferase)- 5' *AGCTT*AAAAAGTGGATTCGAGTCGTCTTAATCTCTTGAATTAAGACGACTCGAAATCCACGG

Target sequences of each shRNA are overlined on the human IRF4 mRNA sequence and shown in italics in the shRNA sequence. The control shRNA targets the coding region of the luciferase gene.

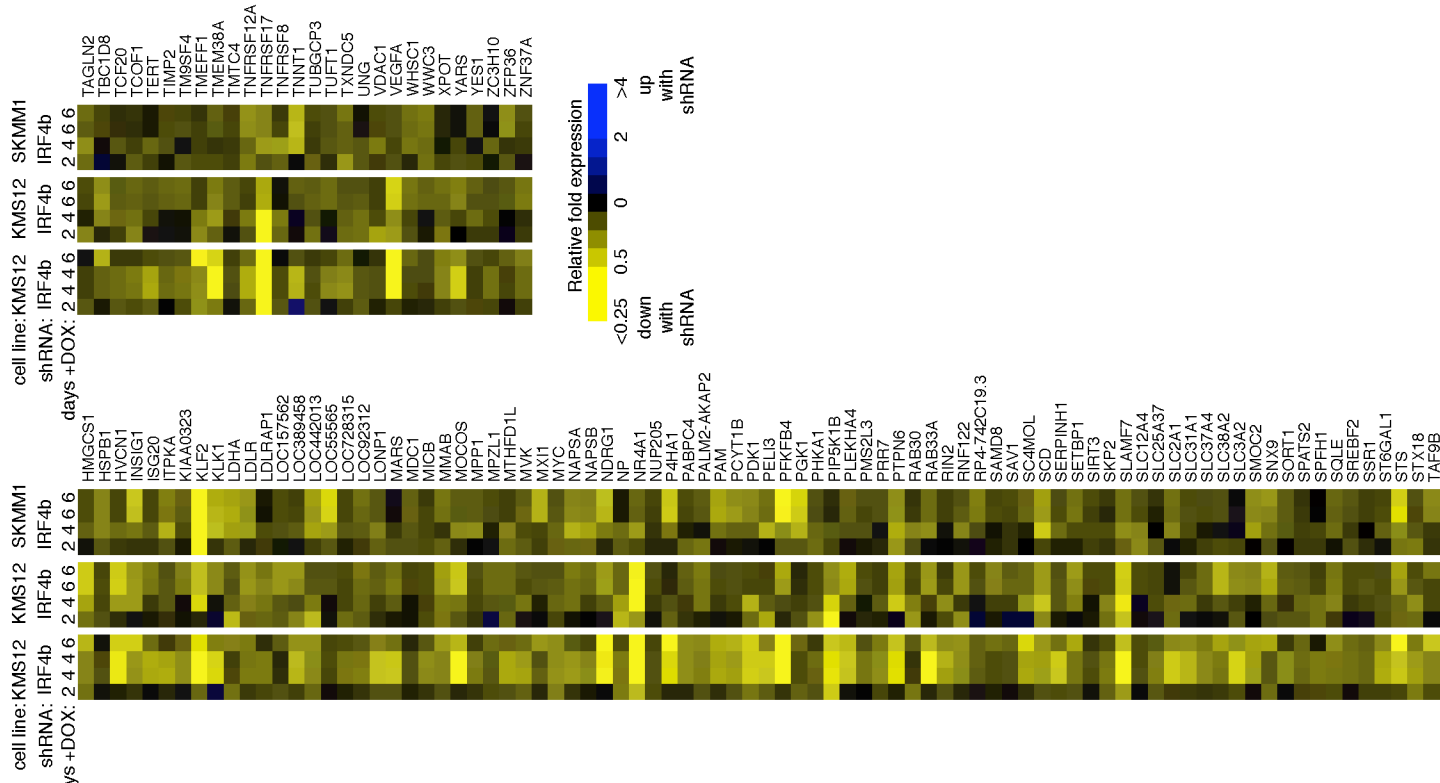


2007-11-12040B_Supplementary Figure 3

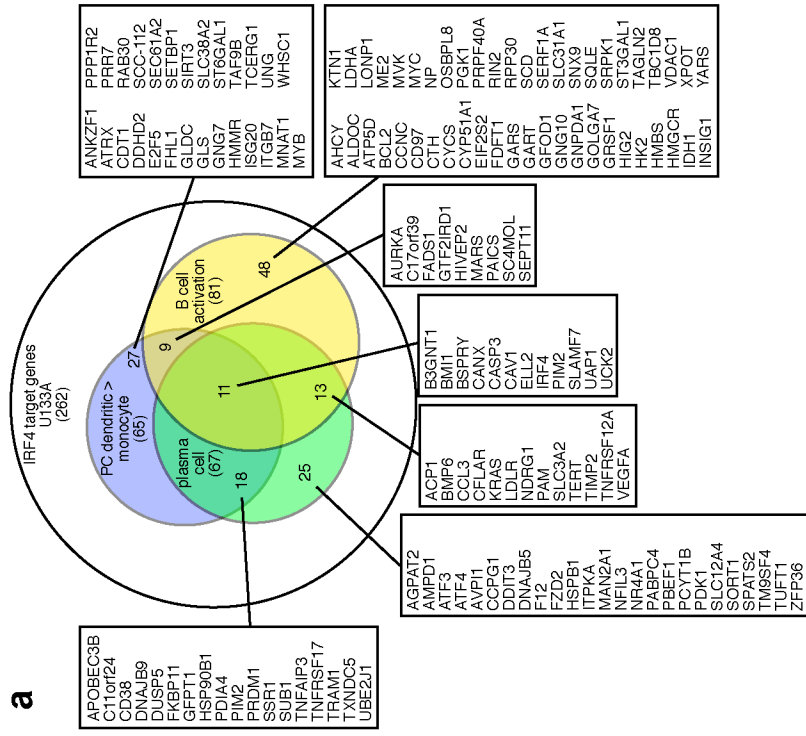
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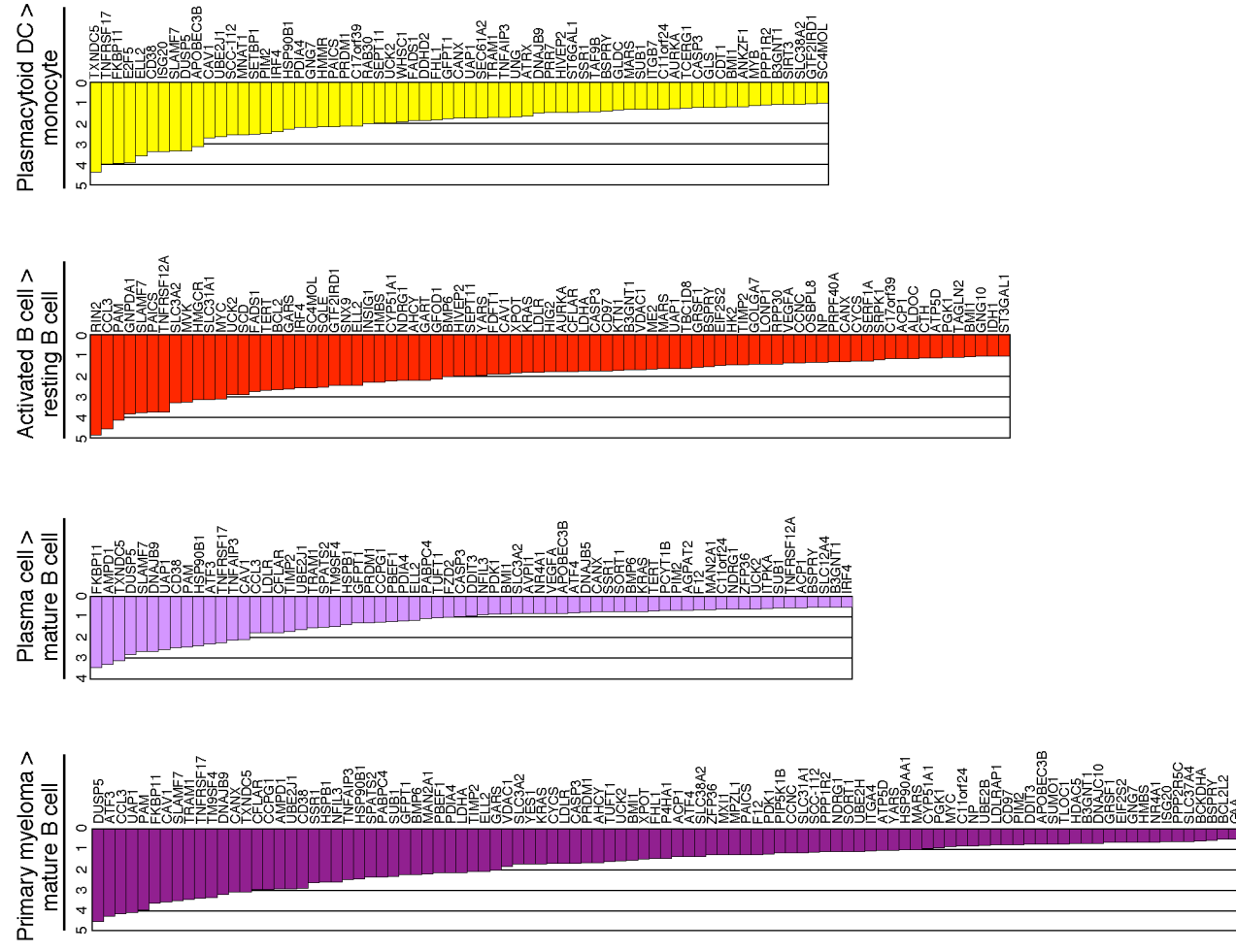


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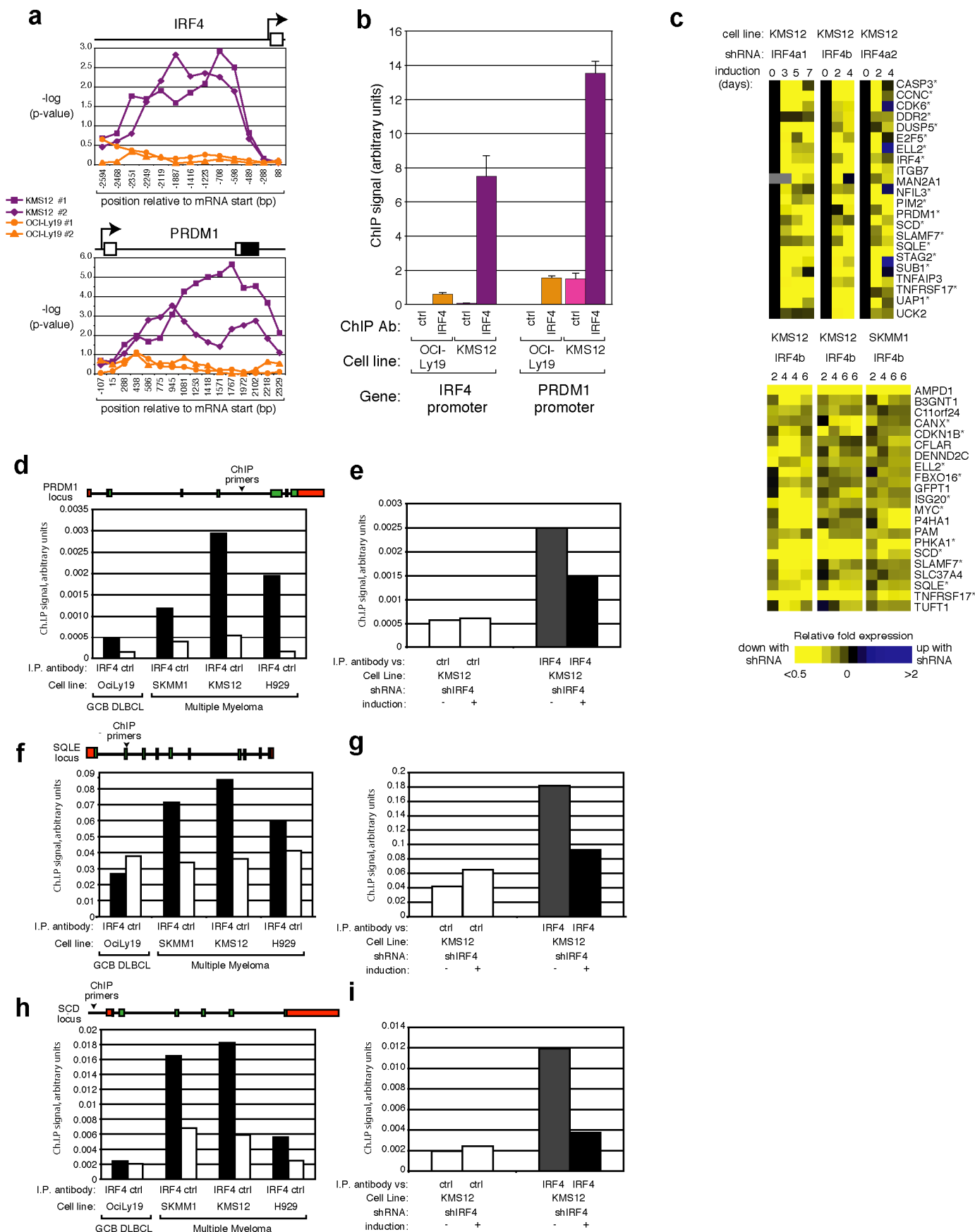


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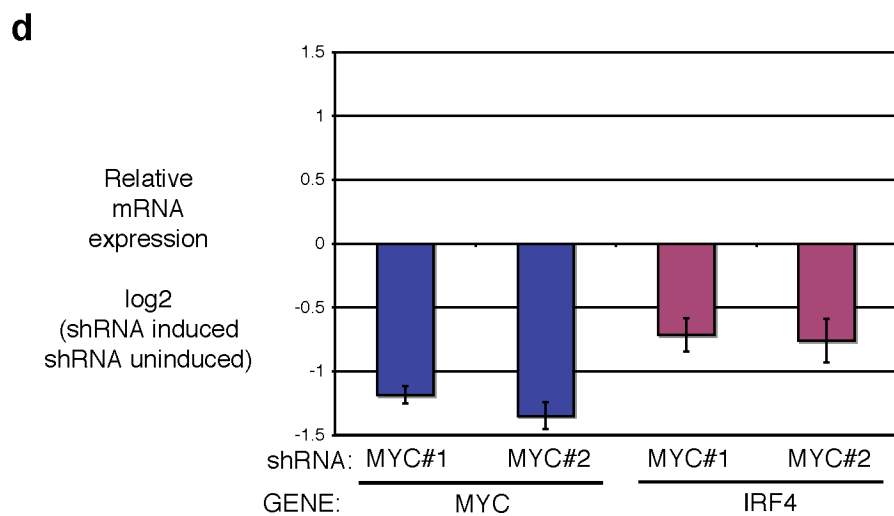
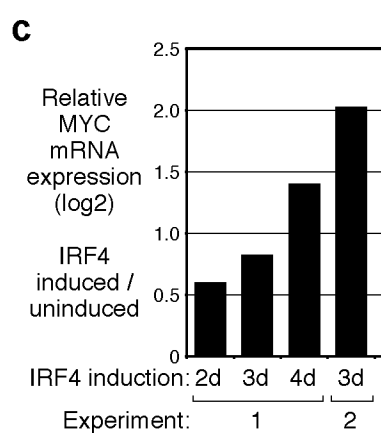
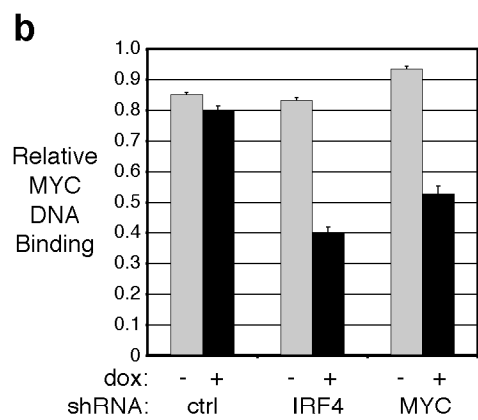
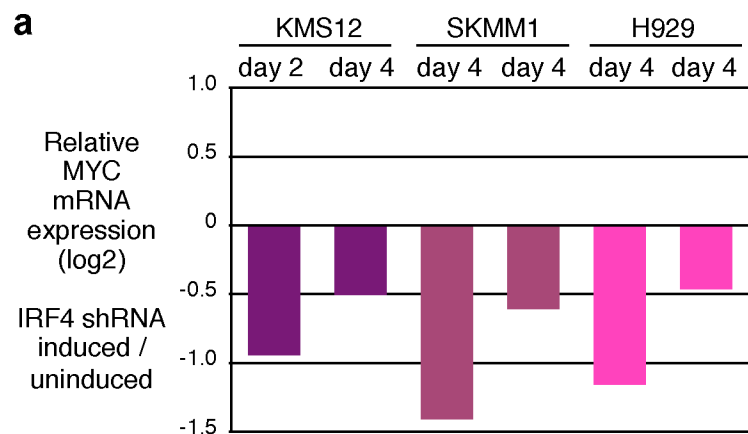
Cell type A:
Cell type B:
expression
A - B (log2):



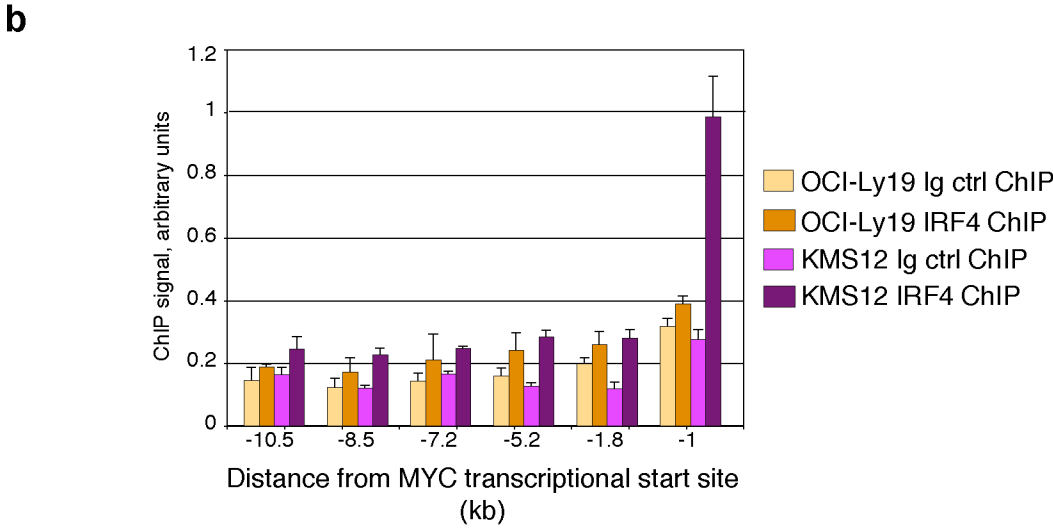
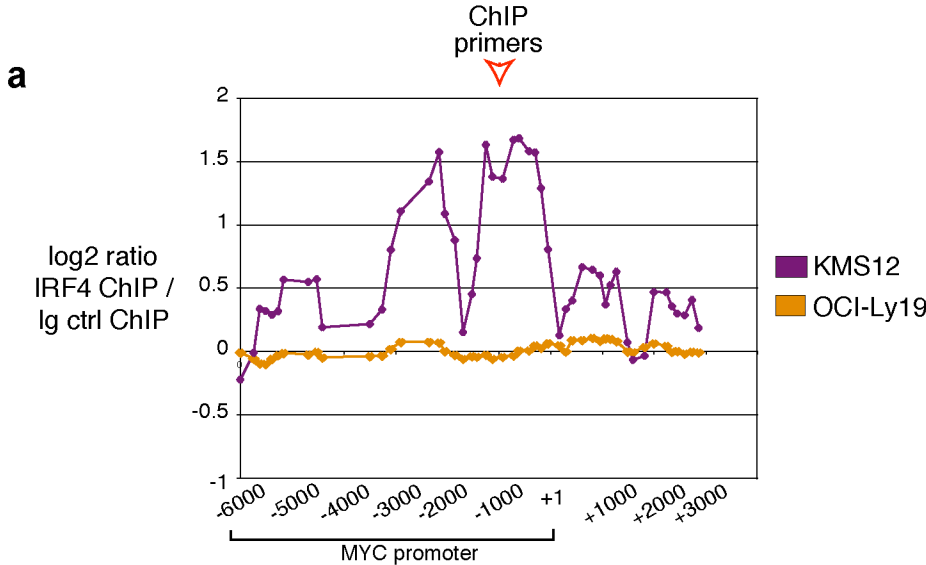
2007-11-12040B_Supplementary Figure 5



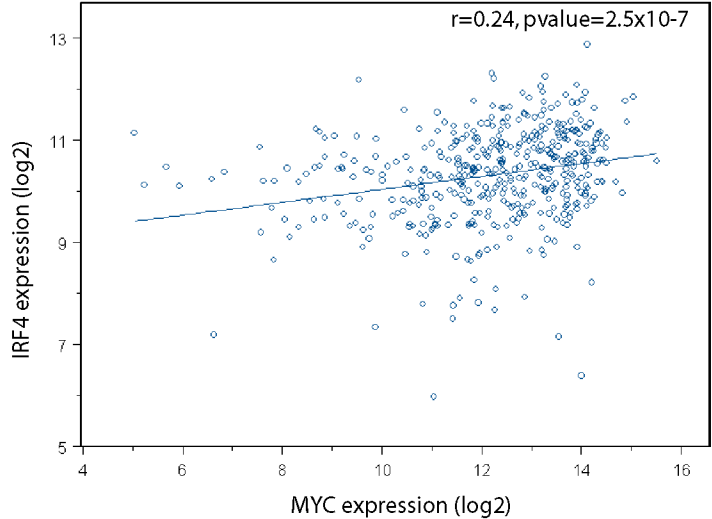
2007-11-12040B_Supplementary Figure 6



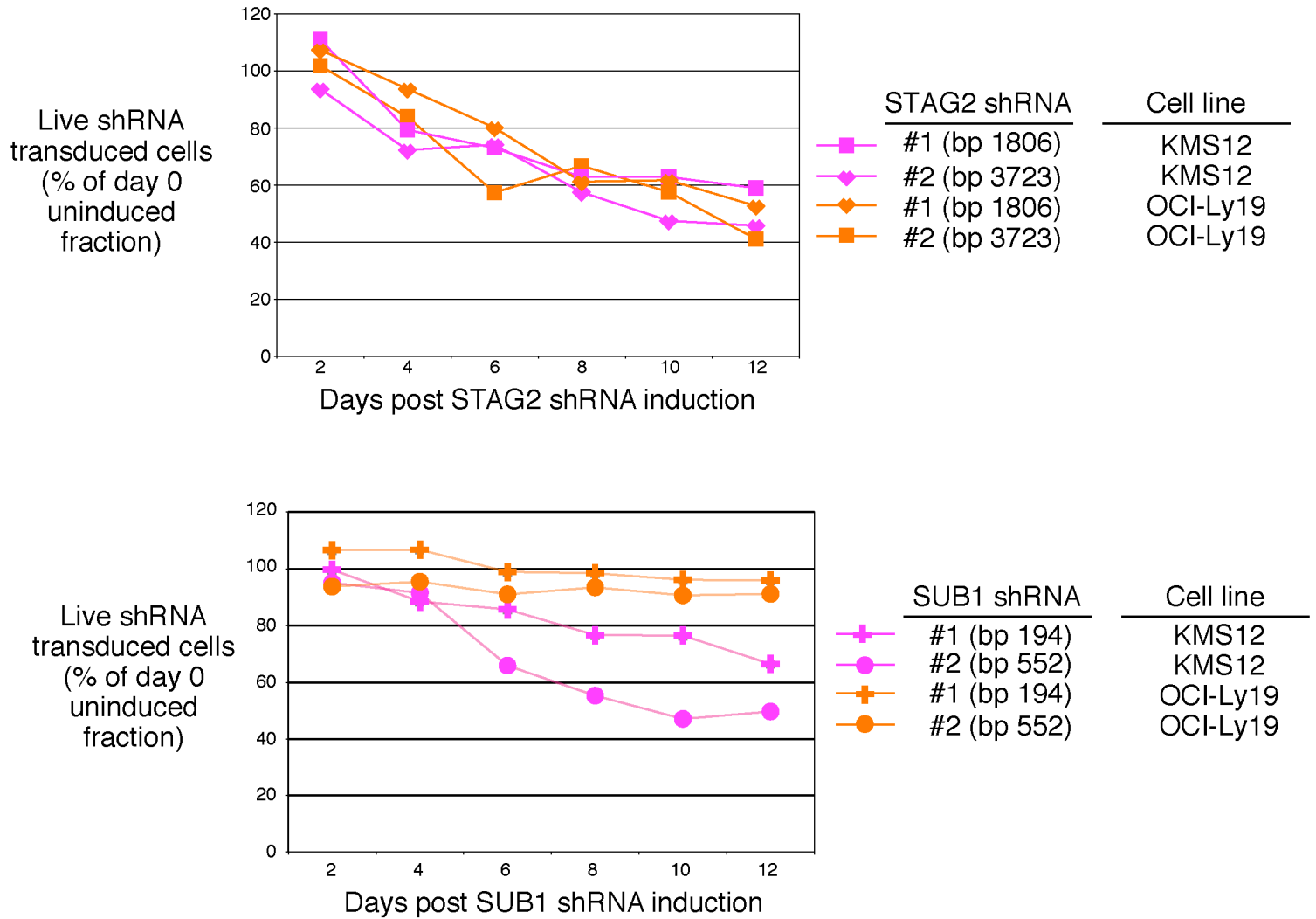
2007-11-12040B_Supplementary Figure 7



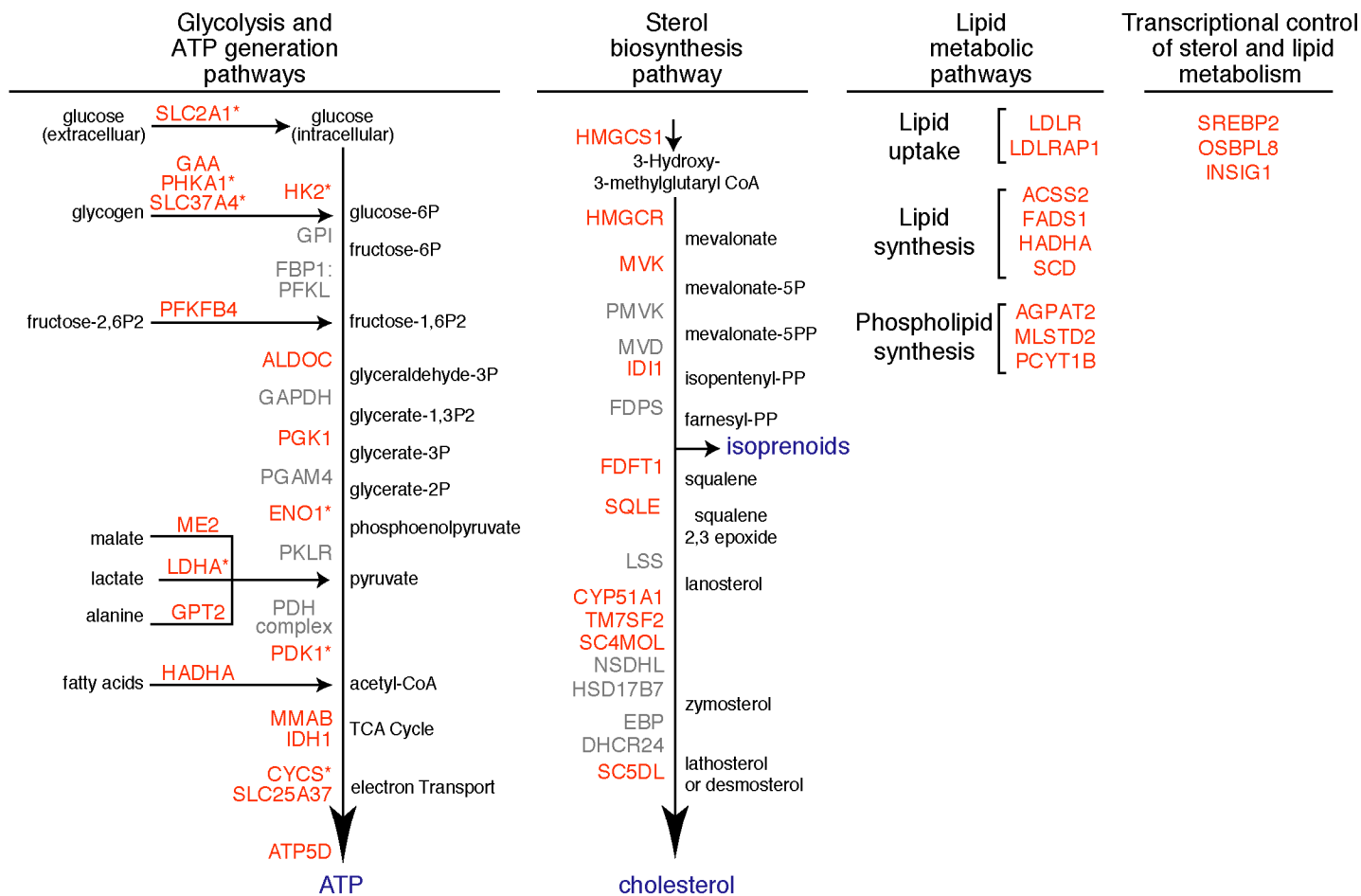
c Correlation between IRF4 and MYC expression in Primary MM Patient Samples



2007-11-12040B_Supplementary Figure 8



2007-11-12040B_Supplementary Figure 9



Supplementary Figure Legends

Supplementary Figure 1 IRF4 shRNAs. Target sequences for each shRNA are overlined on the human IRF4 mRNA sequence and are shown in italics in the shRNA sequences below the mRNA. The control shRNA targets the coding region of the luciferase gene.

Supplementary Figure 2 Modest knockdown of IRF4 mRNA and protein causes myeloma cell death without cell cycle arrest. **a,** The myeloma line SKMM1 was transduced in three independent experiments with an inducible IRF4 shRNA (shIRF4b, Supplementary Fig.1), and mRNA levels for beta-2-microglobulin (B2M, control) and IRF4 were quantitated at day 4 after dox induction, comparing induced and uninduced cultures. **b,** Myeloma cells were transduced with an inducible IRF4-target shRNA. IRF4 protein levels were measured by intracellular staining and flow cytometry, comparing cells bearing the shRNA (gfp+) and those without in the same culture under induced and uninduced conditions. **c,** Western blots of 3 myeloma lines bearing control shRNA or shIRF4b over a time course of shRNA induction. The myeloma line KMS12 was transduced with the control, IRF4-targeted, or MYC-targeted shRNAs described in the manuscript. Since these vectors bear a puromycin selection cassette, pure populations of these cells could be obtained after addition of puromycin to the media for one week. Cultures of cells with each shRNA were split into two wells and left untreated as controls or induced with doxycycline. Doxycycline and fresh media were added every two days as appropriate. Whole cell lysates from one million cells from each culture were western blotted for expression of IRF4 and MYC, with expression of beta actin used as a loading control. Similar induction of shIRF4 was performed in the myeloma lines H929 and SKMM1, and western blotting was done as above, showing the knockdown of the IRF4 protein. **d,** KMS12 myeloma cells were transduced with control or IRF4 shRNAs, and the accumulation of dead cells, determined by changes in forward- and side-scatter properties, was monitored over time after shRNA induction. **e,** As in **(d)** the accumulation of dead cells, determined by uptake of ethidium bromide (EtBr), measured after 5 days of shRNA induction. **f,** As in **(d)**, subG1 DNA content, determined by

labeling with bromodeoxyuridine (BrdU) and counterstaining with propidium iodide (PI), measured after 3 days of shRNA induction. **g**, As in **(d)**, cell cycle distribution determined by labeling with BrdU and counterstaining with PI, measured after 3 days of shRNA induction and quantitated using Modfit software.

Supplementary Figure 3 Gene expression profiling reveals genes dependent on IRF4 expression in myeloma lines.

The KMS12 and SKMM1 myeloma lines expressing the TET repressor were transduced with inducible expression vectors bearing shRNAs targeting IRF4 or with a control shRNA (Supplementary Fig.1). Gene expression profiling was performed on cDNA microarrays comparing RNAs from cells bearing control shRNA (Cy3-yellow) or IRF4 shRNAs (Cy5-blue) **(a)**, or comparing RNAs from cells bearing IRF4 shRNA in the absence of doxycycline (Cy3-yellow) or IRF4 shRNAs (Cy5-blue) in the presence of doxycycline (dox) to induced shRNA expression **(b)**.

Exemplar array elements are shown for genes whose expression is reduced by > 1.3-fold in at least 50% of the induced time points. IRF4 is indicated in red. Color bar indicates relative fold change in gene expression; gray indicates data below confidence thresholds for spot size, architecture, and level above local background. **a**, Data from human lymphochip cDNA gene expression arrays. **b**, Data from Agilent two color (4x44K) gene expression arrays.

Supplemental Figure 4 Expression of IRF4 target genes in hematopoietic cell types.

a, Expression of the genes whose expression was reduced in myeloma lines by an shRNA against IRF4 was compared across various developmental systems on a Venn diagram depicting the overlap between plasmacytoid dendritic cells (PCDC), plasma cell, and activated B cell gene expression programs. Of the 308 IRF4 target genes, defined by gene expression analysis on Lymphochips and Agilent gene expression platforms (Supplementary Fig. 3), 262 are well-measured on Affymetrix gene expression arrays. Of these, 65 are more highly expressed in primary PCDC samples compared to primary monocytes (blue circle), 67 are more highly expressed in primary plasma cells vs. mature B cells (green circle), and 81 are induced between 1hr and 24hrs during activation of primary human B cells via BCR crosslinking (yellow circle). **b**, Gene expression

differences (log₂) are shown for the developmental comparisons shown in Fig. 2. and (a). Data cutoffs are as follows: primary myeloma vs. mature B, difference 1.4-fold, minimum log₂ spot signal of 5 for either sample; primary plasma cell vs. mature B, difference 1.4-fold, minimum log₂ spot signal of 5 for either sample; primary activated B cell vs. resting B cell, difference 2-fold, minimum log₂ spot signal of 7 for either sample; primary plasmacytoid dendritic cell vs. monocyte, difference 2-fold, minimum log₂ spot signal of 7 for either sample. See Methods for GEO data set references.

Supplementary Figure 5 Chromatin immunoprecipitation (ChIP) confirmation of direct IRF4 target genes in human myeloma. **a**, Direct IRF4 genes were identified by ChIP-CHIP analysis on Agilent promoter arrays (-7.5 to +2 kb), comparing material immunoprecipitated with control or anti-IRF4 antisera from the KMS12 myeloma line vs. the IRF4-negative OCI-Ly19 lymphoma line. Representative data traces for 2 IRF4 target genes, IRF4 and PRDM1 are shown, plotting the negative log of the p-value for IRF4 binding to each ChIP-CHIP probe vs. the transcriptional start of the gene (human genome build 17, Supplementary Table 2). **b**, ChIP performed as in Figure 2d using PCR primers at the 5' ends of IRF4 and PRDM1. SYBR green QPCR ChIP assays were performed on independently derived chromatin from KMS12 (purple bars) and OCI-ly19 (orange bars). Error bars are s.d. from triplicate measurements. Values are normalized signal from immunoprecipitated material divided by input DNA signal (pre-i.p.) in arbitrary units. **c**, upper panel: Expression of IRF4 direct targets is affected by IRF4-targeting shRNAs. The KMS12 line expressing the TET repressor with inducible expression vectors bearing shRNAs targeting IRF4 (Cy5-blue) or a control shRNA (Cy3-yellow) were analyzed by gene expression profiling on human Lymphochip cDNA microarrays comparing RNAs from cells in the absence or presence of doxycycline over time. lower panel: Data from Agilent two color gene expression arrays comparing RNA from cells bearing IRF4 shRNA in the absence of doxycycline (Cy3-yellow) or in the presence of doxycycline (Cy5-blue) to induce shRNA expression. Exemplar array elements are shown for genes whose expression is reduced by > 1.3-fold in at least 50% of the induced time points. Color bar indicates relative fold change in gene expression vs. control; gray indicates data below confidence thresholds. *:ChIP-confirmed direct IRF4 targets. **d**,

Immunoprecipitation of cross-linked chromatin from 3 myeloma lines and one lymphoma line were immunoprecipitated with control anti-serum (open bar) or polyclonal anti-IRF4 antiserum (black bar) and a region of human PRDM1 intron 4, shown on the cartoon (boxes, exons; red, noncoding; green, coding; arrow, approximate position of primers), was amplified using specific primers. The amount of amplification indicating IRF4 binding near this region is shown for each sample/antiserum combination, and is normalized to the amount of input DNA. **e**, ChIP analysis of PRDM1 intron 4 in the KMS12 myeloma line with an shRNA targeting IRF4 under induced and uninduced conditions, using control (open bars) or IRF4 anti-serum (shaded bars). **f**, ChIP as in (**d**) analyzing a fragment from the SQLE locus. **g**, ChIP as in (**e**) showing the IRF4 dependence of the ChIP signal for this region in the SQLE gene. **h**, ChIP as in (**d**) analyzing a fragment from the SCD locus. **i**, ChIP as in (**e**) showing the IRF4 dependence of the ChIP signal for this region in the SCD gene.

Supplementary Figure 6 MYC is a direct IRF4 target gene.

a, Expression of MYC measured after shIRF4 induction in myeloma lines. The KMS12, SKMM1 and H929 myeloma lines expressing the TET repressor were transduced with inducible expression vectors bearing shRNAs targeting IRF4 or with a control shRNA (Supplementary Fig.1). Gene expression profiling was performed on cDNA microarrays comparing RNAs from cells bearing IRF4 shRNA uninduced or IRF4 shRNAs in the presence of doxycycline to induce shRNA expression. Expression differences (induced/uninduced) from the array element for human MYC are shown. Data from human lymphochip cDNA gene expression arrays, GEO:GSE8958. **b**, Nuclear extracts from cells in (**a**) were assayed for relative MYC DNA binding activity by ELISA. Error bars indicate the s.d. of duplicate measurements. **c**, Several independent transduction of an IRF4 expression vector into lymphoma line OCI-Ly7 were performed and MYC expression was measured using human Lymphochips (as in Fig.3, comparing the difference between mock transduced and IRF4-expressing cells, GEO:GSE6337). **d**, Two independent shRNAs targeting the 3'UTR of MYC were transduced into KMS12 cells expressing the TET repressor, and gene expression profiling was performed on cDNA microarrays (see text) comparing RNAs from cells bearing IRF4 shRNA uninduced or

IRF4 shRNAs in the presence of doxycycline to induce shRNA expression (4 days). Expression differences (induced/uninduced, average of 3 array elements, error bars = s.d.) for human MYC and IRF4 are shown.

Supplementary Figure 7 ChIP analysis of human MYC and its correlation with IRF4 in myeloma patient samples. **a**, ChIP analysis on Agilent promoter arrays, comparing material immunoprecipitated with control or anti-IRF4 antisera from the KMS12 myeloma line vs. the IRF4-negative OCI-Ly19 lymphoma line. Shown is log₂ ratio of binding to each element (IRF4 i.p. / control sera i.p.), plotted over the MYC locus relative to the transcriptional start site (+1). **b**, IRF4 binding to the MYC promoter was further confirmed and mapped by designing primers to regions of potential IRF4 binding and performing individual ChIP assays (SYBR green QPCR) on independently derived chromatin from the KMS12 myeloma line (purple bars) and the OCI-Ly19 lymphoma line (orange bars). Error bars are s.d. from triplicate measurements. Values shown are normalized signal from immunoprecipitated material divided by input DNA signal (pre-i.p.). **c**, Positive correlation between expression of IRF4 and MYC in 451 primary myeloma samples (GSE2658).

Supplementary Figure 8 Toxicity of shRNA knockdown of STAG2 and SUB1. Knockdown of STAG2 is toxic to a myeloma (KMS12) and a lymphoma cell line (OCI-Ly19), while knockdown of SUB1 is only toxic to the myeloma line. Inducible shRNAs against STAG2 and SUB1 were retrovirally transduced into cell lines with the TET repressor, and shRNA expression was induced with doxycycline. Survival of cells shRNA⁺ (gfp⁺) cells was monitored by flow cytometry, and the fraction of gfp⁺ cells was normalized to the fraction of gfp⁺ cells at day 0 (pre doxycycline induction). Base pair (bp) numbers indicate the start of the shRNA homology from the transcriptional start of the gene (+1).

Supplementary Figure 9

Glycolytic, sterol, and lipid synthesis pathways are shown with IRF4 target genes depicted in red (Supplementary Table 2 and Supplementary Fig.3). MYC target genes are depicted with a *.