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Supplemental Data

An E2F1-Dependent Gene Expression Program that Determines the Balance between Proliferation and Cell Death

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Supplementary Figure 1

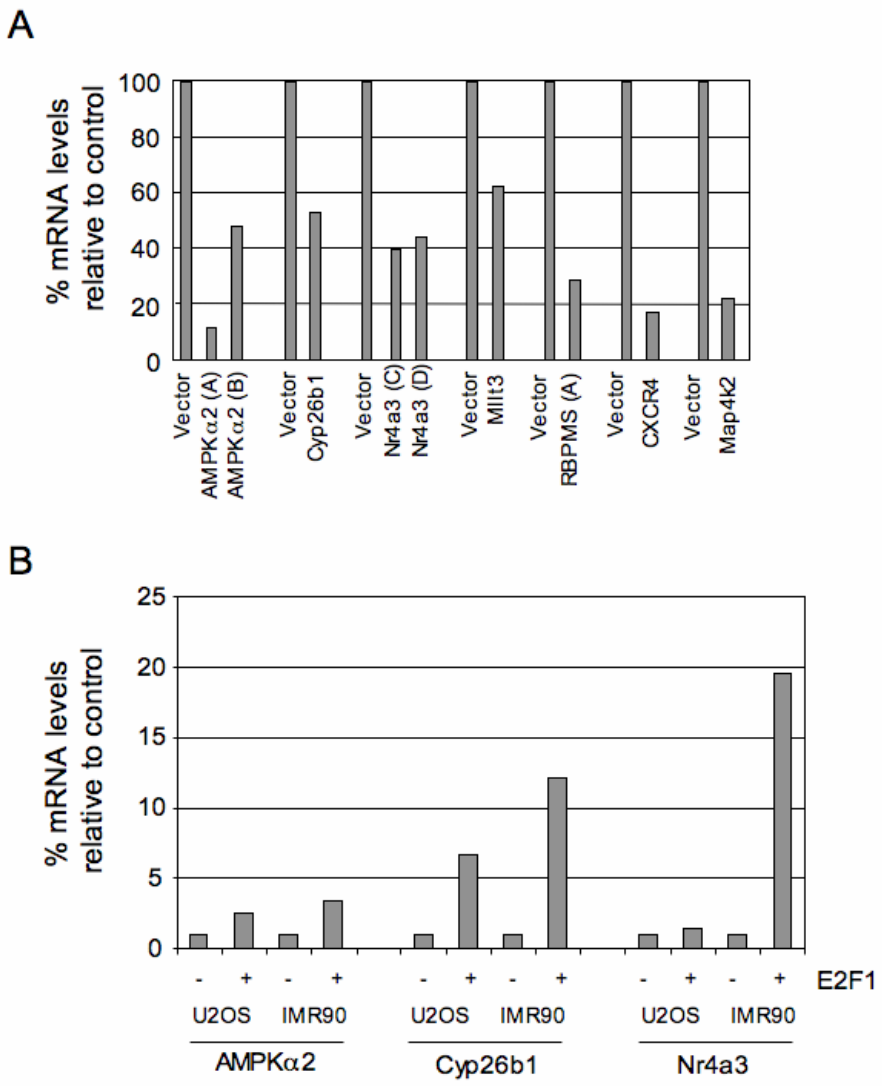

Figure S1. Expression levels in shRNA stable cell lines and E2F1 induction in U2OS and IMR90 cells.

A. Quantitative real-time PCR was used to measure mRNA levels of each gene in the knockdown cell lines compared with the levels measured in asynchronously growing control cells. Primers used for this analysis are listed in supplemental data.

B. U2OS and IMR90 cells were serum-deprived for 48 hours, infected with control or E2F1 expressing adenovirus (3 moi U2OS, 20 moi IMR90) and mRNA harvested 24 hours post-infection for real time PCR analysis of AMPKa2, Cyp26b1 and Nr4a3 fold changes caused by E2F1 expression.

Supplementary Figure 2

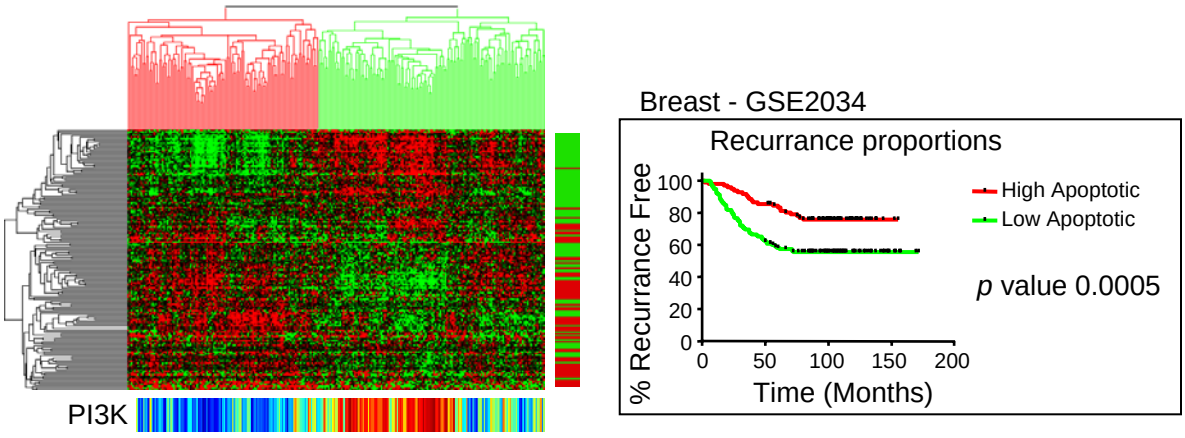

Figure S2. Analysis of E2F1 target gene expression in human cancers.

Breast tumors (GSE2034) were analyzed as described in Figure 5 and are also segregated into two major groups defined by high or low expression levels of the PI3K repressed and non-repressed E2F1 targets. PI3K gene expression signatures were used to predict the activation state of the PI3K pathway (blue representing low activity and red, high activity) and are displayed below the clustered genes. The tumors are separated into two groups based on their expression of PI3K repressed E2F1 targets, again designated either ‘high apoptotic’ or ‘low apoptotic’ and patients were separated using Kaplan-Meier curves for the recurrance (rather than survival) outcomes.

Supplementary Table 1

A

E2F1 Target Genes Not Repressed by PI3K signaling

Gene Ontology	BF	p value
GO:0007049 [5]: cell cycle	44	< 0.0001
GO:0000067 [6]: DNA replication and chromosome cycle	35	< 0.0001
GO:0008283 [4]: cell proliferation	35	< 0.0001
GO:0000278 [6]: mitotic cell cycle	28	< 0.0001
GO:0006260 [6]: DNA replication	27	< 0.0001
GO:0007067 [8]: mitosis	26	< 0.0001
GO:0000087 [7]: M phase of mitotic cell cycle	26	< 0.0001
GO:0000279 [6]: M phase	24	< 0.0001
GO:0006259 [5]: DNA metabolism	23	< 0.0001
GO:0000280 [7]: nuclear division	22	< 0.0001
GO:0000910 [5]: cytokinesis	16	< 0.0001
GO:0043283 [4]: biopolymer metabolism	15	< 0.0001
KEGG Pathway	**BF**	**p value**
path:hsa04110: Cell cycle	18	< 0.0001
path:hsa00240: Pyrimidine metabolism	10	< 0.0001
path:hsa00230: Purine metabolism	4	0.0002
TRANSFAC	**BF**	**p value**
V$E2F1_Q6_01	22	< 0.0001
V$E2F_02: E2F	14	< 0.0001
V$E2F_Q3: E2F	14	< 0.0001
V$E2F4DP1_01: E2F-4:DP-1 heterodimer	14	< 0.0001
V$E2F_Q4_01	14	< 0.0001
V$NFY_01: nuclear factor Y (Y-box binding factor)	14	< 0.0001
V$E2F1_Q4_01	13	< 0.0001
V$E2F_03	13	< 0.0001
V$E2F1DP1_01: E2F-1:DP-1 heterodimer	12	< 0.0001
V$E2F1_Q6: E2F-1	10	< 0.0001
V$E2F1DP2_01: E2F-1:DP-2 heterodimer	10	< 0.0001
V$E2F_Q6_01	10	< 0.0001
V$E2F4DP2_01: E2F-4:DP-2 heterodimer	9	< 0.0001
V$NFY_Q6: nuclear factor Y (Y-box binding factor)	9	< 0.0001
V$E2F1DP1RB_01: Rb:E2F-1:DP-1 trimeric complex	8	< 0.0001

B

E2F1 Target Genes Repressed by PI3K signaling

Gene Ontology	BF	p value
GO:0016055 [6]: Wnt receptor signaling pathway	2	0.003
GO:0007049 [5]: cell cycle	1	0.010
GO:0007222 [7]: frizzled signaling pathway	1	0.011
GO:0015871 [6]: choline transport	1	0.014
GO:0030511 [6]: positive regulation of TGF beta receptor sig	1	0.014
GO:0007379 [4]: segment specification	1	0.014
GO:0006793 [5]: phosphorus metabolism	1	0.017
GO:0006796 [6]: phosphate metabolism	1	0.017
KEGG Pathway	**BF**	**p value**
path:hsa00760: Nicotinate and nicotinamide metabolism	2	0.001
path:hsa04910: Insulin signaling pathway	2	0.002
path:hsa00562: Inositol phosphate metabolism	2	0.002
path:hsa00061: Fatty acid biosynthesis (path 1)	1	0.007
path:hsa04810: Regulation of actin cytoskeleton	1	0.007
path:hsa04310: Wnt signaling pathway	0	0.012
TRANSFAC	**BF**	**p value**
V$E2F1_Q3_01	14	< 0.0001
V$KROX_Q6	9	< 0.0001
V$E2F1_Q6: E2F-1	8	< 0.0001
V$E2F1_Q6_01	5	< 0.0001
V$SP3_Q3	5	0.0001
V$MYCMAX_B: c-Myc:Max binding sites	5	0.0002
V$E2F1_Q4: E2F-1	5	0.0002
V$E2F_Q3_01	4	0.0005
V$E2F1DP1_01: E2F-1:DP-1 heterodimer	4	0.0006
V$LXR_Q3	4	0.0007
V$LXR_DR4_Q3	3	0.0008

Table S1. Gene annotation, KEGG pathway and TRANSFAC analysis of PI3K repressed and non-repressed E2F1 target genes.

We analyzed gene annotation of these genes identified by fold-induction in Excel using the GATHER resource which displays representation of GO annotations, a Bayes Factor (BF) and a *p value* based on the level of that GO terms presence in the gene set. This analysis demonstrated that the set of non-PI3K repressed E2F target genes are significantly overrepresented for cell cycle (37/117), DNA replication, cell proliferation and mitotic genes. Furthermore, TRANSFAC analysis of these gene promoters indicated, as expected, that E2F binding motifs are significantly overrepresented in this list of genes (94/117). In contrast, the E2F1 target genes whose expression is blocked by PI3K signaling do not fall into any single category based on GO annotations. This indicates that these gene products may play roles in diverse biological

processes, or that not enough is known about the function of these genes to designate GO terms to them. We find, however, that approximately 89% of these genes are predicted to have E2F binding motifs in their promoters, indicating that E2F is likely to directly transcribe these genes.

Supplemental Data

Open Biosystems shRNA constructs used in this study.

v2HS_57638	Prkaa2	CCGTTGATCTGTCTCTAGTT
v2HS_57640	Prkaa2	CAATATCTTTAATAGGTTAA
v2HS_57641	Prkaa2	CATGTAACAGGTAACCACAA
v2HS_35782	Cyp26b1	CGGGCATTATTTGGGTTTAA
v2HS_203095	NR4A3	CACTGAGCATGATCACAGAA
v2HS_203384	NR4A3	CTGAGTTACAGAGTAATTAA
v2HS_239201	NR4A3	CTAATTGCTGCAAAGTATAA
v2HS_239519	NR4A3	CTGTTTGCTTCCATAAACAA
v2HS_48465	Mllt3	CCTCCACCACCCTTACTAAA
v2HS_77739	RBPMS	CAATCTGTCTTGTGGGTATT
v2HS_77740	RBPMS	CTACTAGAAGGACGAACAAT
v2HS_56171	NR2F1	AACATTATGGGCATCGAGAA
v2HS_248986	NR2F1	GAGCTACAAAGCATGGGAAA
v2HS_70755	CBX7	CCCAGGGAATGGAATCTAGA
v2HS_70751	CBX7	GAGACTGTATAGCATCTATT
v2HS_96071	LPHN1	CAGCCGCCTGGACAACATTA
v2HS_172392	CXCR4	CAGCTGTTTATGCATAGATA
v2HS_172639	ATF3	GCTGGCTACTGTCTATTAAA
v2HS_172635	ATF3	CTCTTTATCCAACAGATAAA
v2HS_47611	MAPKKKK4	CTGGGAAACACGGTATTTAA

Real-time PCR primers used in this study:

Probe Name	**Sequence**
Prkaa2.Hs.For	ATCCGAAGTCAGAGCAAACC
Prkaa2.Hs.Rev	TTTTCACGTAATTGCCAGTCA
Cyp26b1.Hs.For	TCATTGAGAGCAGCAAGGAG
Cyp26b1.Hs.Rev	GCAGCTGCATGATGAGTGA
Nr4a3.Hs.For	CGTCGAAACCGATGTCAGTA
Nr4a3.Hs.Rev	TAATGGGCTCTTTGGTTTGG
Rbpms.Hs.For	GAAGGACCGGGAAGATGAA
Rbpms.Hs.Rev	AGATAGAGCTCCCGAGGTTTG
Mllt3.Hs.For	ACACAACACCCCAGCAAAC
Mllt3.Hs.Rev	GTACGAACACCATCCAGTCG
Gapdh.Hs.For	GGGAAACTGTGGCGTGAT
Gapdh.Hs.Rev	GGAGGAGTGGGTGTCGCTGTT

Prkaa2.Rn.For	CCAAGTGATCAGCACTCCAA
Prkaa2.Rn.Rev	CAGTAGTCCACGGCAGACAG
Cyp26b1.Rn.For	GAGAGACTGGTCACTGGTTGC
Cyp26b1.Rn.Rev	GTTCGCCCAGTAGGATCTTG
Nr4a3.Rn.For	GACTCTCCCCCAATCCAGA
Nr4a3.Rn.Rev	TTGAGGGTCCAGGCTCAG
Rbpms.Rn.For	AGCCGAGAAGGAGAACACC
Rbpms.Rn.Rev	AATGGCCTGAAGAGCAGGTA
Mllt3.Rn.For	CGAGGAGGAGTCTGATGAGG
Mllt3.Rn.Rev	AGAGGCAGAGCTGCTTTCAC
CDKN1B.Rn.For	GGGTCTCAGGCAAACTCTGA
CDKN1B.Rn.Rev	TCTGTTGGCCCTTTTGTTTT
Gapdh.Rn.For	ATGACAACTTTGGCATCGTG
Gapdh.Rn.Rev	GGATGCAGGGATGATGTTCT