RT-PCR primers

h-Egr-1
forward 5'-GACCCGTTCGGATCCTTTCC-3'
reverse 5'-GCCACAAGGTGTTGCCACTG-3'

h-Actin
forward 5'-GGCGGCAACACCATGTACCCT-3'
reverse 5'-AGGGGCCGGACTCGTCATACT-3'

h-EGFR
forward 5' AAC ACC CTG GTC TGG AAG TAC 3'
reverse 5' ACA CCA GTT GAG CAG GTA CTG 3'

h-p53
forward 5' TGGAAACTACTTCCTGAAAACAAC 3'
reverse 5' AGTCAGGCCCTTCTGTCTTG 3'

Supplement Figure S1. Sequence of primers used for RT-PCR. Human Egr-1, actin and EGFR primers were designed using Primer3 software. Human p53 primers were from: Avery-Kiejda KA. et al, Clin. Cancer Res. 2008; 14:1659-68.

ChIP primers

h-*EGR1* promoter, p53 binding site A3 [1]
forward 5'-GGCCCCGGCGGCGGCTAGAGCTCTAGGCTT-3'
reverse 5'-GCGGCTCCCCAAGTTCTGCG-3'

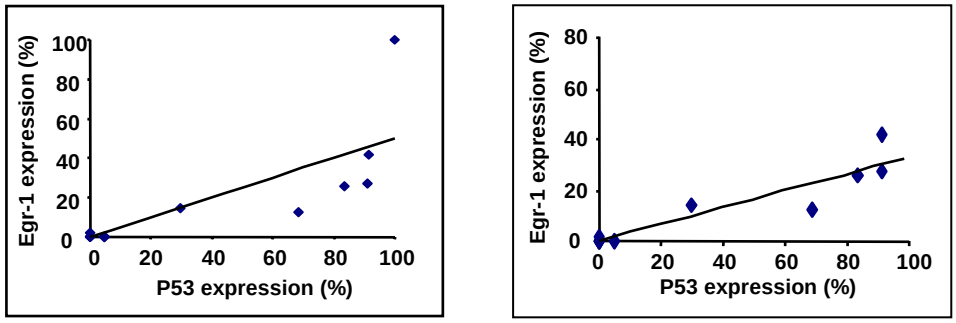
h-*EGR1* promoter, p53 binding site A2 [1]
forward 5'-TGACAGCGATAGAACCCCGGCCCGACTCGC
reverse 5'-CGTTGCCCCTCCCTCCGCCTTCTTCCCTCC

h-*TP53* promoter, Egr-1 binding site [2]
forward 5'-TGGGAGTTGTAGTCTGAACGCTTC-3'
reverse 5'-GAGAAGCTCAAAACTTTTAGCGCC-3'

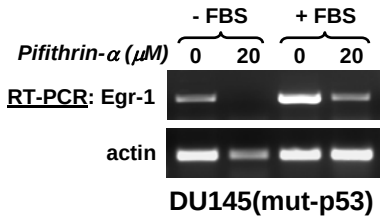
h-*EGFR* promoter, Egr1-Binding site (EBS)
forward 5'-GGACCCGAATAAAGGAGCAG-3'
reverse 5'-GAGGAGAATGCGAGGAGGAG-3'

Supplement Figure S2. Sequence of primers used in ChIP experiments. The first three primer pairs (*EGR1* promoter and *TP53* promoter) were from the indicated references [1] and [2].
The EBS in the *EGFR* promoter was described in [3]. Primers were designed using Primer3 software to amplify the DNA fragment surrounding the EBS.

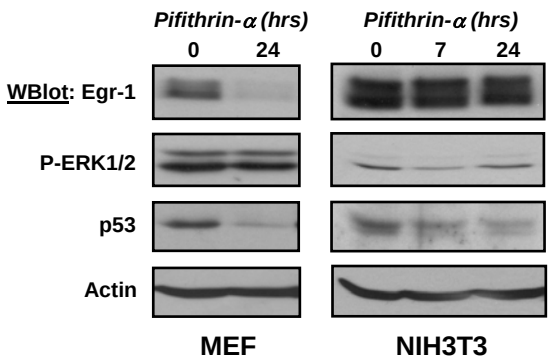
[1] Yu J, Baron V, Mercola D, Mustelin T, Adamson ED. A network of p73, p53 and Egr1 is required for efficient apoptosis in tumor cells. Cell Death Differ 2007;14:436-46.
[2] Krones-Herzig A, Mittal S, Yule K*, et al.* Early growth response 1 acts as a tumor suppressor in vivo and in vitro via regulation of p53. Cancer Res 2005;65:5133-43.
[3] Nishi H, Nishi KH, Johnson AC. Early Growth Response-1 gene mediates up-regulation of epidermal growth factor receptor expression during hypoxia. Cancer Res 2002;62:827-34.



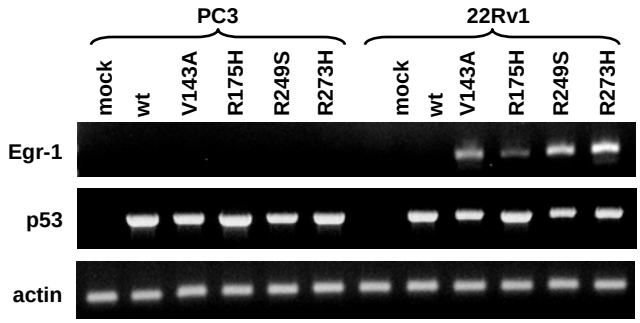
Supplement Figure S3: Correlation between Egr-1 and p53 expression in prostate cells. Protein expression was quantified by densitometric analysis of the autoradiograms. Egr-1 expression was plotted against p53 expression for each cell line. Correlation coefficient R=0.602 (left panel). When M12 cells were considered outsider and removed, the correlation coefficient was R=0.865 (right panel).



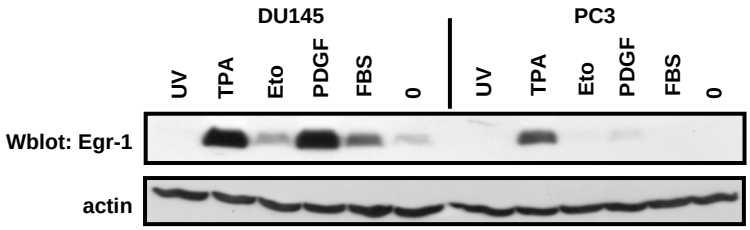
Supplement Figure S4: DU145 cells were treated with Pifithrin-α (20 μM) for 16 hrs, followed by FBS stimulation for 1 hr. Total RNA was purified from the cells and Egr-1 mRNA level was measured by semi-quantitative RT-PCR. Results were analyzed on 2% agarose gels containing Ethidium Bromide.



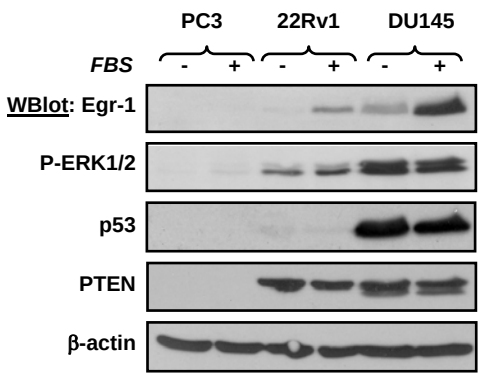
Supplement Figure S5: Effect of p53 inhibitor pifithrin-α on Egr-1 protein expression in mouse fibroblasts. Mouse Embryo Fibroblasts (MEF) were a gift from Prof. Dan Mercola (UC Irvine) and have been described in (Krones-Herzig et al, PNAS 2003; 100:3233). These cells were used at late passage (P44) and contain mutant p53. NIH3T3 cells, in contrast, contain wild-type p53. Cells were treated with 30 μM pifithrin-α for the indicated times, lysed, and analyzed by Western Blot. It can be noted that in contrast to human prostate cancer cells, pifithrin-α decreased the level of p53 in both fibroblast cell lines.



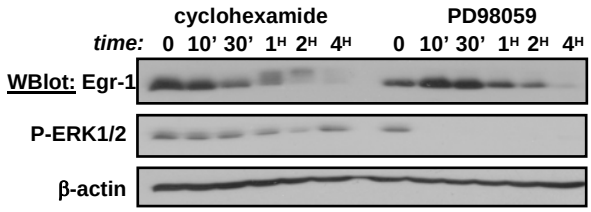
Supplement Figure S6: PC3 and 22Rv1 cells were transfected with wt-p53 or with the indicated mutant p53. Two days after transfection, total RNA was purified from the cells and mRNA levels were measured by semi-quantitative RT-PCR. Results were analyzed on 2% agarose gels containing Ethidium Bromide.



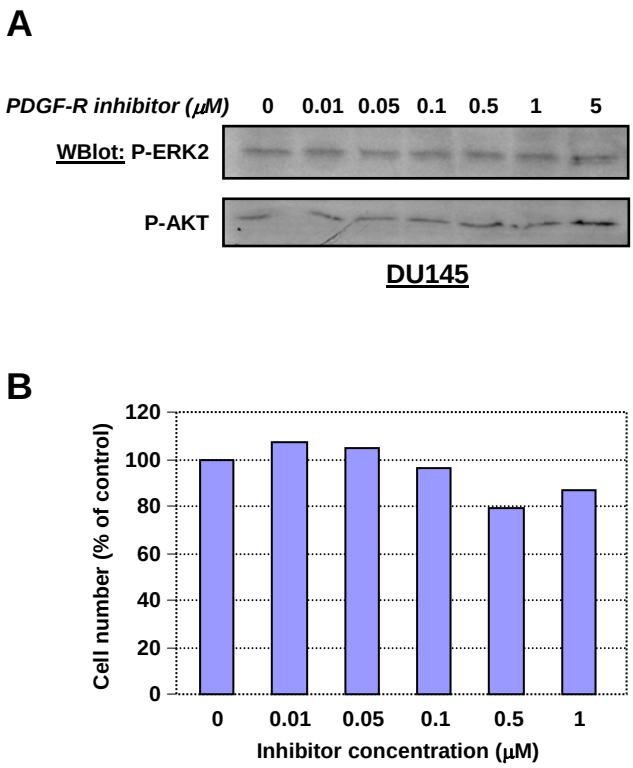
Supplement Figure S7: PC3 and DU145 cells were treated with various growth factors or stress for 1 hour (eto: etoposide; UV: ultra-violet; FBS: fetal bovine serum), lysed, and Egr-1 protein level was analyzed by western blot.
Egr-1 expression was low in PC3, and could be induced only by TPA, though to a lesser extent than in DU145.



Supplement Figure S8: Cells were treated for PBS for 1 hour to allow for maximum Egr-1 induction. Protein expression and phosphorylation of ERK1/2 were measured by western blot using specific antibodies. We observe that PC3 cells, which have almost undetectable amount of Egr-1, also display weak phosphorylation of ERK1/2. In addition, PC3 cells were “resistant” to Egr-1 induction .



Supplement Figure S9: Cells were treated with cyclohexamide or with MEK inhibitor PD98059 for the indicated times. The experiments were analyzed by western blot using the indicated antibodies. Phosphorylation of ERK1/2 is shown as control for PD98059 efficacy. Egr-1 half-life was calculated from two similar experiments, T1/2 = 30 min.



Supplement Figure S10: Lack of effect of PDGF-R inhibitor on protein phosphorylation and growth of DU145. DU145 cells were treated with increasing concentrations of PDGF-R inhibitor or with DMSO. **(A)** Cells were lysed after 4hrs and the phosphorylation of ERK1/2 and AKT was visualized by Western Blot. **(B)** After 48hrs, cells were counted using the Cell Coulter Multisizer as described in the Methods. The experiment shown is representative of two similar experiments, each run in duplicates.

PDGF-R inhibitor (TK inhibitor III) was purchased from Calbiochem (EMD Biosciences, San Diego, CA).