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p73 regulates serine biosynthesis in cancer

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Key words

Serine, glucose, cancer metabolism, p73, GLS-2, lung adenocarcinoma.

Abbreviation list

Phosphoglycerate dehydrogenase (PHGDH); tricarboxylic acids (TCA); M2 isoform of pyruvate kinase (PKM2); non-small-cell lung carcinoma (NSCLC); phospho-serine aminotransferase 1 (PSAT-1); phospho-serine phosphatase (PSPH); cytochrome C oxidase subunit 4 (Cox4i1), Glutaminase 2 (GLS-2), serine/glycine biosynthesis (SGBS).

Abstract *[225 words]*

Activation of serine biosynthesis supports growth and proliferation of cancer cells. Human cancers often exhibit overexpression of phosphoglycerate dehydrogenase (PHGDH), the metabolic enzyme that catalyses the reaction that diverts serine biosynthesis from glycolytic pathway. By refueling serine biosynthetic pathways, cancer cells sustain their metabolic requirements, promoting macromolecule synthesis, anaplerotic flux and ATP. Serine biosynthesis intersects glutaminolysis and together with this pathway provides substrates for production of antioxidant GSH. In human lung adenocarcinomas we identified a correlation between serine biosynthetic pathway and p73 expression. Metabolic profiling of human cancer cell line revealed that TAp73 activates serine biosynthesis, resulting in increased intracellular levels of serine and glycine, associated to accumulation of glutamate, TCA anaplerotic intermediates and GSH. However, at molecular level p73 does not directly regulate serine metabolic enzymes, but transcriptionally controls a key enzyme of glutaminolysis, glutaminase-2 (GLS-2). p73, through GLS-2, favors conversion of glutamine in glutamate, which in turn drives serine biosynthetic pathway. Serine and glutamate can be then employed for GSH synthesis thus, the p73-dependent metabolic switch enables potential response against oxidative stress. In knockdown experiment indeed TAp73 depletion completely abrogates cancer cell proliferation capacity in serine/glycine-deprivation, supporting the role of p73 to help cancer cells under metabolic stress. These findings implicate p73 in regulation of cancer metabolism and suggest that TAp73 influences glutamine and serine metabolism, affecting GSH synthesis and determining cancer pathogenesis.

Introduction *[Main text 2734 words]*

Cancer cells alter their metabolism promoting macromolecules biosynthesis to sustain rapid growth. Oncogenic mutations progressively switch down oxidative phosphorylation privileging the conversion of glucose in lactate through the aerobic glycolysis[1]. Although aerobic glycolysis works with low energetic efficiency (2 moles ATP/mole glucose), it allows relevant inflow of glucose in the cell metabolism, whereby supplying cancer cells of carbons required for biomass accumulation (for review see Lunt and Vander Heiden[2]). Phosphoglycerate can be converted from glycolysis into serine following a 3-step reaction (**Fig. 1a**). Among the anabolic pathways branching from glycolysis, serine biosynthetic pathway is acquiring increasing interest. The metabolic enzyme, that catalyses the first reaction of this pathway, phosphoglycerate dehydrogenase (PHGDH), is overexpressed in 70% of the ER-negative breast cancers[3]. Human *PHGDH* gene, indeed, is located in a region of chromosome 1p, which shows high frequency of amplification in breast cancer and melanomas[3, 4]. Repression of PHGDH in breast cancer cells causes a strong decrease of tumor proliferation in xenografts [3], while its ectopic expression in mammary epithelial cells predisposes to cancer transformation[4]. Activation of serine biosynthetic pathway has a multitude of metabolic consequences. Serine is a non-essential amino-acid, which represents a precursor for macromolecules, such as other amino-acids, GSH, lipids and nucleotides[5, 6]. In addition, serine biosynthetic pathway contributes approximately 50% of the cellular anaplerotic flux of glutamine into tricarboxylic acid (TCA) cycle. Glutamate, derived from glutamine, is used as donor of an amino-group for the intermediate 3-phosphopuyruvate, generating the TCA intermediate α-ketoglutarate[3] (**Figs. 1a, S1**) and serine. Serine also acts as allosteric activator of the M2 isoform of pyruvate kinase (PKM2), tuning its enzymatic activity[7, 8]. PKM2 produces pyruvate in the last step of glycolysis[9]. Interestingly, M2 isoform of PK is predominantly expressed in cancer cells and its expression specifically associates with aerobic glycolysis and conversion of pyruvate in lactate[10, 11]. Serine acts as sensor of the cancer cell metabolic status. When serine is abundant, PKM2 full activation promotes consumption of glucose through the aerobic glycolysis. Upon serine deprivation PKM2 activity is reduced, pyruvate is shunt to a fuel-efficient mode in the mitochondria and glycolysis diverges towards serine biosynthetic pathway to sustain cell proliferation. Serine is therefore a crucial switch that by an allosteric regulation of PKM2[7] in tumor cells modulates the flux of glycolytic intermediates, leading to sustained mTORC1 activity

necessary to cell proliferation [8]. At the molecular level, activation of serine biosynthesis is regulated by a sensor of non-essential amino acid starvation, through General Control Nonderepressible 2 kinase - Activating Transcription Factor 4 (GCN2-ATF4) pathway. In amino acid starved cells GCN2-ATF4 directly activates transcription of all three enzymes that lead to the synthesis of serine from glycerate-3-phosphate[12, 13]. Here, we investigated additional mechanisms, which contribute to regulate the expression of enzymes involved in amino acid metabolism.

The transcription factors of the p53 family exert control of different aspects of the cell biology. In response to distinct upstream inducers[14] p53 itself regulates a relevant number of subsets of genes[15]; these include recently discovered biochemical pathways, including, for example, the silencing of repeats and noncoding RNA[16], the connection of IL-7Ra to telomere erosion[17] and the metabolism of the cell[18], by regulating mevalonate[19] or serine[20]. This intense gene expression results in a very fine regulation of life[21] and death[22, 23] of the individual cell, or its senescence[24, 25]. Although p53 and its family members, namely p63 and p73, diverged during evolution, their interaction and interplay is in fact very evident and relevant[26]. p73 is, indeed, involved in tumor-suppression[27-30]. The use of alterative promoters within *TP73* gene allows the generation of two different transcripts, a transactivation domain-containing isoform (TAp73) and a N-terminal truncated isoform (ΔNp73)[31, 32]. The regulation of the protein half-life is crucial to the function of p73[33-35] and therefore for the *in vivo* progression of cancer. Experiments performed in conditional null mice for TAp73 ($TAp73^{-/-}$) show an increased susceptibility to spontaneous and carcinogen-induced tumors with a peculiar induction of lung adenocarcinomas [36], suggesting a tumor suppression role. Conversely ΔNp73 exerts oncogenic functions[37, 38]. *In vivo* tumor models, generated from $\Delta Np73^{-/-}$ MEF, present strong impairment of tumor formation and proliferation[39, 40]. Although *TP73* gene is rarely mutated in human cancers, there is increasing evidence that an altered ratio between TA and ΔN isoforms affects tumor progression[41]. p53 has been implicated in protection of cancer cell during serine starvation. p53-dependent p21 activation promotes cell survival by channeling serine stores towards GSH synthesis. Cells lacking p53 failed to arrest when serine-starved. This resulted in oxidative stress, reduced viability and severely impaired proliferation[20]. Thus, p53 coordinates a metabolic reprogramming of cancer cells to respond to metabolic stress. Our work uncovers p73 function in serine metabolism regulation of human cancer cells. Here we show that p73 isoforms influence serine metabolism in cancer cells. Firstly, bioinformatics assessment of cancer dataset(s)

unveiled a negative correlation between TAp73 expression and expression levels of serine biosynthesis enzymes. Metabolic profiling of human cancer cell line demonstrated the ability of TAp73 to regulate serine and glycine level. Mechanistically, we suggest that TAp73 promotes serine biosynthesis through transcriptional upregulation of the glutaminase-2 enzyme (GLS-2), resulting in accumulation of intracellular GSH. Strikingly, TAp73 depletion impairs cell proliferation during serine starvation suggesting that TAp73 plays a fundamental role in supporting cancer cells under metabolic/oxidative stress. In summary, altered expression of p73 in cancer cells might contribute to serine biosynthesis dysregulation, affecting cancer pathogenesis.

Results and Discussion

Expression of p73 isoforms correlate with serine biosynthesis pathway in human cancer

Recently, TAp73 has emerged as a key tumor suppressor of lung adenocarcinoma. TAp73$^{-/-}$ mice showed a predominant formation of spontaneous lung adenocarcinoma [42]. Moreover, in human lung adenocarcinomas a TAp73 downregulation has been described contextually to a ΔNp73 upregulation[36]. Thus, it suggested that altered TAp73/ΔNp73 ratio plays a role in cancer pathogenesis[43]. Notably, very often in this specific lung cancer subtype, p53 resulted inactivated[44]. Given that, to investigate a possible role for p73 in metabolism of cancer cells, we firstly performed an analysis on a human lung adenocarcinoma dataset (Khono et al, GSE31210)[45], which included 226 biopsies from stage I and II human lung adenocarcinomas. Specific probe for TAp73 and for ΔNp73 isoforms allowed clustering of tumors in different groups: TAp73 positive tumors or ΔNp73 positive tumors. Here, to gain insights into the possible involvement of p73 in metabolism, we tested whether TAp73 or ΔNp73 expression was correlated to specific metabolic pathways or cellular processes by gene set enrichment analysis (GSEA). Pathway signatures for standard metabolic pathways and related processes were extracted from gene ontology (GO terms). All enzymes in the serine biosynthesis pathway were combined into a gene signature describing “serine biosynthesis” (SBS). A second signature additionally includes enzymes used for the serine-to-glycine reaction (“serine/glycine biosynthesis”, SGBS). After clustering the samples using expression of TAp73 and ΔNp73 specific probe sets, we looked for elevated or reduced expression of the pathway signatures within the relevant clusters (enrichment of a cluster in samples associated with significant positive signature scores, or significant negative signature

scores for a pathway signature, respectively; see methods). Interestingly, we found a significant association between TAp73 expression and reduced activity of the serine/glycine biosynthesis pathway, while ΔNp73 expression was associated with an elevated activity (**Table 1**). Remarkably, in our analysis TAp73 and ΔNp73 showed clear opposite behavior, thus supporting the hypothesis of adversarial functions of the two isoforms in lung cancer pathogenesis. TAp73 indeed appeared to counteract tumor progression, associating with low activity of pathways involved in proliferation (DNA packaging, chromosome segregation and G1/S transition), and associating with reduced activity of cancer metabolism (glycolysis and serine biosynthesis). Conversely, ΔNp73 appeared to positively correlate with their activation (**Fig. 1a**). Next, we performed a Kaplan-Meier estimate analysis to assess the impact of serine synthesis on survival outcome. Patient datasets were clustered according to the expression of all genes involved in the SGBS pathway. In the lung adenocarcinoma dataset (Khono et al., GSE31210), expression of the pathway enzymes was highly inter-correlated and clustering clearly defined one group with an overall low expression of all enzymes and one group with an overall high expression (**Supp. Fig S2a**). Survival analysis showed that a high SGBS pathway function was predictive of poor survival (**Fig. 1b, left-upper panel**). Additional datasets of different cancer types confirmed the prediction power of a high serine/glycine biosynthesis signature for poor patient survival (**Figs. 1b, S2b-e**). These data suggest that an altered TAp73/ΔNp73 ratio might play a role in cancer pathogenesis, through the modulation of the poor prognostic SGBS pathway.

p73 affects serine biosynthesis in cancer cells

To further investigate p73 involvement in regulation of serine biosynthesis, we next evaluated serine level upon TAp73 overexpression. TAp73β SaOs Tet-On inducible cell line allowed to finely monitor metabolites an early time point of TAp73 overexpression; thus, we avoided to encounter secondary metabolic changes due to p73 cell cycle arrest and apoptosis functions. By 24h, cells have undergone a degree of G1 arrest and apoptosis, as assessed by the appearance of sub-G1 population (**Supp. Fig. S3a,c**). Hence, all functional experiments with TAp73 overexpression were performed before this time. Interestingly, we observed a statistically significant increase of intracellular serine and glycine after 8h of TAp73 overexpression. Consistently with activation of serine biosynthesis, we identified increase of glutamate, α-ketoglutarate and succynil-CoA (**Fig. 1c**). Accumulation of these metabolites was consistent with activation of anaplerotic flux

derived from glutaminolysis, which intersects serine biosynthesis in the glutamate deamination reaction (**Fig. 1a**). α-ketoglutarate analysis was the only measurement, which, although showing a consistent trend, did not produce a statistically significant p value due to high variability of the technical replicates. Finally, TAp73 overexpression strikingly promoted also accumulation of GSH (**Fig. 1c**), suggesting that serine synthesis is at least partially addressed to counteract oxidative stress. This suggests a parallelism between p73 and p53, which is employed in diverging serine to GSH production during serine depletion[20] and in promoting glutamine/glutamate conversion to accumulate anti-oxidant GSH[46]. Overall, this metabolic analysis suggests a positive role of TAp73 in metabolism through the serine biosynthesis pathway.

p73-dependent modulation of serine biosynthetic enzymes (PHGDH, PSAT-1, PSPH)

To investigate the possible mechanism for p73's role in regulation of the serine biosynthetic pathway, we used Matinspector software (Genomatix)[47] to screen a region of about 1000 bp upstream the transcriptional start site (TSS, +1) of the human PHGDH, PSAT-1 and PSPH genes for p53-like binding sites (p53BS). We identified high score putative p53BSs in the human promoter region of all of the three enzymes. We found a p53BS located at -574/-578 from the transcriptional start (TSS) in PHGDH promoter, at -592/-596 in PSAT-1 promoter and at -1123/-1123 in PSPH promoter (**Fig. 2a**). We therefore evaluated p73 capacity to regulate serine enzyme expression. Short-time induction of exogenous TAp73 in SaOs-2 Tet-On cells did not reveal any relevant changes of PHGDH, PSAT-1 and PSPH mRNA (**Fig 2b and sup. Fig. S4a-c**). Conversely, ΔNp73 overexpression in SaOs Tet-On cells led to a progressive increase of PHDGH and PSAT-1 mRNA respectively up to a 2,3-fold and 2.2 increase, whereas ΔNp73 did not appear to be able to modulate PSPH mRNA as evaluated (**Supp. Fig. S2d-f**). To further investigate p73-dependent regulation of serine biosynthetic enzymes we analyzed PHGDH, PSAT-1, PSPH mRNA levels in selective TAp73-knockdown H1299 cells. Notably the p53-null cell line, H1299 expresses relevant level of TAp73 (qPCR Ct=24), but very low level of ΔNp73 (qPCR Ct=31) (**Supp. Fig. S5a**). Interestingly TAp73 depletions resulted in a significant reduction of PHGDH, PSAT-1 expression suggesting an involvement of TAp73 in their regulation (**Fig. 2c**). However, p73 was not directly able to transactivate p53BSs within the promoters of serine biosynthetic enzymes, as assessed by luciferase reporter gene assay (**Fig. 2d**). This latter

experiment, together with TAp73 overexpression experiment, suggests that changes in PHGDH and PSAT-1 expression levels are indirect consequences of p73 functions, which potentially can be related to metabolic pathway activation, but independent by direct transcriptional control. PHGDH, PSAT-1 and PSPH expression indeed are strictly regulated by the GCN2-ATF4 pathway, which acts as sensor of amino acid (serine and glutamine) deprivation (**Supp. Fig. S1**). Hence, we checked whether ATF-4 expression level was altered in TAp73-knockdown H1299. Consistently with our hypothesis ATF-4 was significantly downregulated after 48h of TAp73 silencing (**Fig. 2e**), suggesting that modulation of serine biosynthetic enzymes by p73 might be at least partially associated to ATF-4 pathway alteration. These data suggest that TAp73 activates serine biosynthesis, playing an indirect control on the expression levels of these metabolic enzymes.

p73 transcriptionally controls GLS-2 expression

If p73 does not directly regulates serine enzyme expression, might p73 be regulating enzymes of related metabolic pathway? The metabolic profiling analysis indicated that, consistently with serine biosynthesis activation, p73 also promoted conversion of glutamine into glutamate to refuel the TCA cycle anaplerotic flux (**Fig. 1c**). Given that, we next asked whether p73 indirectly pushed serine biosynthesis by promoting the glutaminolysis pathway. The cellular glutamine conversion rate is regulated by the expression of glutaminase enzymes. To test this hypothesis we overexpressed for 24h TAp73 in H1299 and SaOs-2 Tet-On cells and analyzed mRNA level of the mitochondrial glutaminase isoform (GLS-2). As shown in **figure 3b,c,** TAp73 significantly induced GLS-2 mRNA at about 7.5-fold in H1299 and 3-fold increase in TAp73 SaOs-2 Tet-On. However, selective knockdown on endogenous TAp73 in H1299 does not affect GLS-2 expression level. This basically suggests that additional transcriptional factors control basal expression of GLS-2 (**Supp. Fig. S5b**). *In silico* promoter analysis by Matinspector identified a p53BS at -787/-791 from the transcriptional start of the GLS-2 human gene (**Fig. 3a**). To check whether the p73-dependent GLS-2 induction was due to direct binding of p73 on this responsive element, we then performed a chromatin immunopreciptiation (ChIP) assay in TAp73 SaOs-2. HA antibody specifically pulled down the DNA fragment containing the potential p53BS element in the GLS2 promoter, showing a significant enrichment of the promoter binding

of 3,5-fold compared to the IgG (**Fig. 3d**). These results together demonstrate that p73 binds a p53 DNA-binding element in the human GLS2 gene promoter. To investigate if the binding of p73 on GLS-2 promoter was functional we performed a luciferase gene reporter assay using the human GLS-2 promoter fragment including this p53-BS. After 24h of transfection luciferase activity confirmed TAp73 ability to transactivate GLS-2 promoter (**Fig. 3e**). These data definitely prove that TAp73 transcriptionally controls the expression of GLS-2.

p73 sustains cancer cell growth in serine deprivation

TAp73/GLS-2 axis can explain how p73 affects glutamine/glutamate conversion reaction and the related metabolic pathways. Glutamate accumulation might produce acceleration of serine biosynthesis, resulting in increased level of serine and glycine (**Fig 1c**). A common employment of glutamate and glycine in cellular metabolism is the biosynthesis of antioxidant GSH. As matter of fact, TAp73 overexpressing cells showed also increased level of intracellular GSH (**Fig. 1c**). Similarly to p53[20, 46, 48], TAp73 might therefore drive GSH synthesis to counteract oxidative stress and to sustain cell viability during metabolic stress condition (**Supp. Fig. 1**). Indeed, the capacity of cancer cells to deal with serine starvation relays to oxidative stress response. To prove this hypothesis we assessed the contribution of TAp73 to cell proliferation during serine and glycine (Ser/Gly) depletion in the p53-null cell line, H1299. TAp73 knockdown impaired cell proliferation already when cultured in complete medium. However when we switched in Ser/Gly-deprived medium, TAp73 knockdown cells completely lost their proliferation capacity (8% BrdU positive cells, compared to 38% of the control cells in Ser/Gly deprivation and 24% of TAp73 knockdown cells in compete medium) (**Fig. 4a**). Cell cycle profile analysis clarified that in Ser/Gly deprivation TAp73 knockdown promoted a strong G1 phase block (75%), associated to a consistent reduction of proliferating phases (S and G2 phases) (**Fig. 4b**), however no apoptotic effect were observed in our experimental condition (24h Ser/Gly deprivation) (**Fig. S5**). This data demonstrate that TAp73 plays a crucial role in metabolic stress response of cancer cells. p73, similarly to p53, helps cancer cells to face serine starvation, probably preserving cellular anti-oxidant capacity

p73/GLS-2 axis represents a prognostic factor in cancer patients

To evaluate the clinical relevance of TAp73/GLS-2 axis for human cancers, we assessed the expression levels of TAp73 and GLS-2 in lung carcinoma samples. We extracted total RNA form fresh specimens of lung carcinoma obtained from surgery of cancer patients. By using real time qPCR we calculated Ct values, normalized on the house keeping gene, for TAp73 and GLS-2 mRNA. Plotting Ct values of each sample we identified a positive correlation between TAp73 and GLS-2 with a Pearson correlation factor R=0.4 (**Fig. 4d**). The identification of a direct correlation between these two genes suggested the existence of this axis in human cancers. However, we further investigated the clinical relevance of TAp73-dependent GLS-2 regulation, evaluating the biological consequence of TAp73-GLS-2 axis disruption in patients. We selected a human lung adenocarcinoma dataset (GSE311210) and we split the samples in two cohorts. The first cohort included all the samples to maximize positive correlation between p73 and GLS-2, while in the second cohort all the other samples were included (**Fig. S6a**). Thus, we clustered the dataset in two biological groups: one where p73/GLS2 axis was present (p73/GLS-2 interaction), and second in which it was absent (p73/GLS-2 NO interaction). Strikingly, the cohort with "p73/GLS-2 interaction" showed significant worse survival outcome compared to the cohort "p73/GLS-2 NO interaction" (**Fig. 4d**). Comparable trend was confirmed in two additional lung adenocarcinoma datasets (**Fig. S.6b,c**). This data suggests that retention of TAp73/GLS-2 axis in cancer represents a negative prognostic factor, supporting the finding that TAp73 regulating GLS-2 sustains cancer cells adaptation to stress condition.

In conclusion, our work associates p73 function to cancer metabolism, in particular serine metabolism. Overall our findings add novel insights in the regulation of cancer metabolism, enabling identification of p73 isoforms as potential biomarkers and targets for novel approaches in diagnosis, prognosis and therapy.

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COMPETING FINANTIAL INTERESTS

The authors declare no competing financial interests.

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Figure legends

Figure 1. Serine biosynthetic pathway in cancer cells. (**a**) Schematic representation of serine biosynthetic pathway. 3-phosphoglycerate precursors derive from glycolysis, PSAT-1 involves glutamate, derived from glutaminolysis (glutamine to glutamate conversion catalyzed by GLS-2), to transfer an amino-group on phosphopyruvate intermediate, generating α-ketoglutarate, which refuels TCA cycle. Glycine is a precursor of nucleotides and amino acids. Both glutamate and glycine are required for GSH synthesis. (**b**) Effect on survival of the serine biosynthetic enzymes status. Clinical follow up data of lung, breast, colon and prostate cancer datasets were censored for survival. Kaplan–Meier analysis with suppressed censoring showed a significant trend toward poor survival in the high "serine biosynthetic pathway" group. (**c**) TAp73 induces the synthesis of the serine metabolites. TAp73β SaOs-2 Tet-On cells were used to assess metabolic profiling analysis by mass spectrometry. After 8h of TAp73β induction in SaOs-2 Tet-On cells with doxycycline (Doxy), serine, glycine, α-ketoglutarate, succynil-CoA and GSH intracellular levels were measured by using a GC/MS approach, glutamate was measured by using a LC/MS approach.

Figure 2. p73-dependent PHGDH, PSAT-1 and PSPH expression. (**a**) Schematic representation of promoter regions of PHGDH, PSAT-1 and PSPH human gene. p53 binding sites are depicted with the core sequence of responsive element. Score indicates the matrix similarity. (**b**) Real time qPCR to evaluate PHGDH, PSAT-1 and PSPH in SaOs-2 cells, carrying Tet inducible-expression system, were analyzed at different time points of TAp73α (d, n=3 biological replicates) induction by doxycycline (Doxy) treatment. (**c**) Expression of PHGDH, PSAT-1 and PSPH was assessed by qPCR 48h after TAp73-knockdown in H1299 cell line. (**d**) PHGDH, PSAT-1 and PSPH promoter activity was evaluated by luciferase reporter gene assay in H1299 cells after 24h of TAp73 or ΔNp73 overexpression. (**e**) ATF-4 mRNA level was evaluated by qPCR 48h after TAp73-knockdown in H1299 cells. All the data represent mean ± SD of three different experiments.

Figure 3. p73 transcriptionally controls GLS-2 expression. (**a**) Schematic representation of promoter regions of *GLS-2*, human gene. p53 binding sites are depicted with the core sequence of responsive element. Score indicates the matrix similarity. (**b**) H1299 cells were transfected for 24h with TAp73 expressing or empty control vectors. mRNA level of GLS-2 was quantified by real time qPCR. (**c**) SaOs-2 Tet-On cells were analyzed after 24h of TAp73α (c, n=3 biological replicates) to evaluate by real time qPCR GLS-2 mRNA level. (**d**) ChIP performed in TAp73 SaOs-2 Tet-On cell after 24h of doxycycline show that p73 binds p53-like responsive element in GLS-2 promoter. p53-like responsive element in MDM2 promoter was used as positive control. (**e**) GLS-2 promoter activity was evaluated by luciferase reporter gene assay in H1299 cells after 24h of TAp73 or ΔNp73 overexpression. All the data represent mean ± SD of three different experiments.

Figure 4. TAp73 helps cancer cells in serine starvation and TAp73/GLS-2 interaction predict negative survival in cancer patients. (**a**) H1299 control-transfected and siTAp73-transfected cells were grown for 24h in complete medium (containing serine and glycine) or equivalent medium lacking these amino-acids. BrdU incorporation assay performed after 1h pulse with BrdU analog (EdU) measured cell proliferation. (**b**) Cell cycle profile of H1299 transfected cells was evaluated by propidium iodide staining and FACS analysis. (**c**) qPCR on fresh specimens of human lung carcinoma showed direct correlation between the mRNA levels of TAp73 and GLS-2 (Pearson correlation factor R=0.4, n=11). (**d**) Positive p73/GLS-2 correlation (p73/ GLS-2 Interaction) represents a negative prognostic factor for patient survival. A lung adenocarcinoma dataset (GSE31210) was split in two cohorts "p73/ GLS-2 Interaction" and "p73/ GLS-2 NO Interaction" on the basis of existence or not of p73/GLS-2 correlation. Survival estimation showed different prediction for the two cohorts (n=226, p=0.007).

Pathway	Positive Enrichment		Negative Enrichment	
	TAp73	ΔNp73	TAp73	ΔNp73
Glycolysis	not sign	**1,73E-006**	**1,39E-002**	not sign
Electron transport chain	not sign	**5,63E-004**	**2,91E-002**	not sign
Serine biosynthesis	not sign	**3.65E-003**	**0.01970142**	not sign
DNA packaging	not sign	**3,07E-006**	**1.77E-002**	not sign
Chromosome segregation	not sign	**1,36E-005**	**3,56E-002**	not sign

Table1. Gene Set Enrichment Analysis (GSEA) highlights association between p73 and serine biosynthesis pathway. Lung adenocarcinomas samples from GSE31210 array data set have been clustered in group expressing TAp73 (TAp73 columns) or ΔNp73 (ΔNp73 columns). Associations between TAp73 or ΔNp73 expression and different group of genes involved in specific pathways have been analyzed. Red and bleu bold p values respectively indicate statically significant positive or negative associations (enrichment). Not sign indicates associations not statically significant. Red square highlights pathway of interest.

Figure 1, *Amelio et al.*

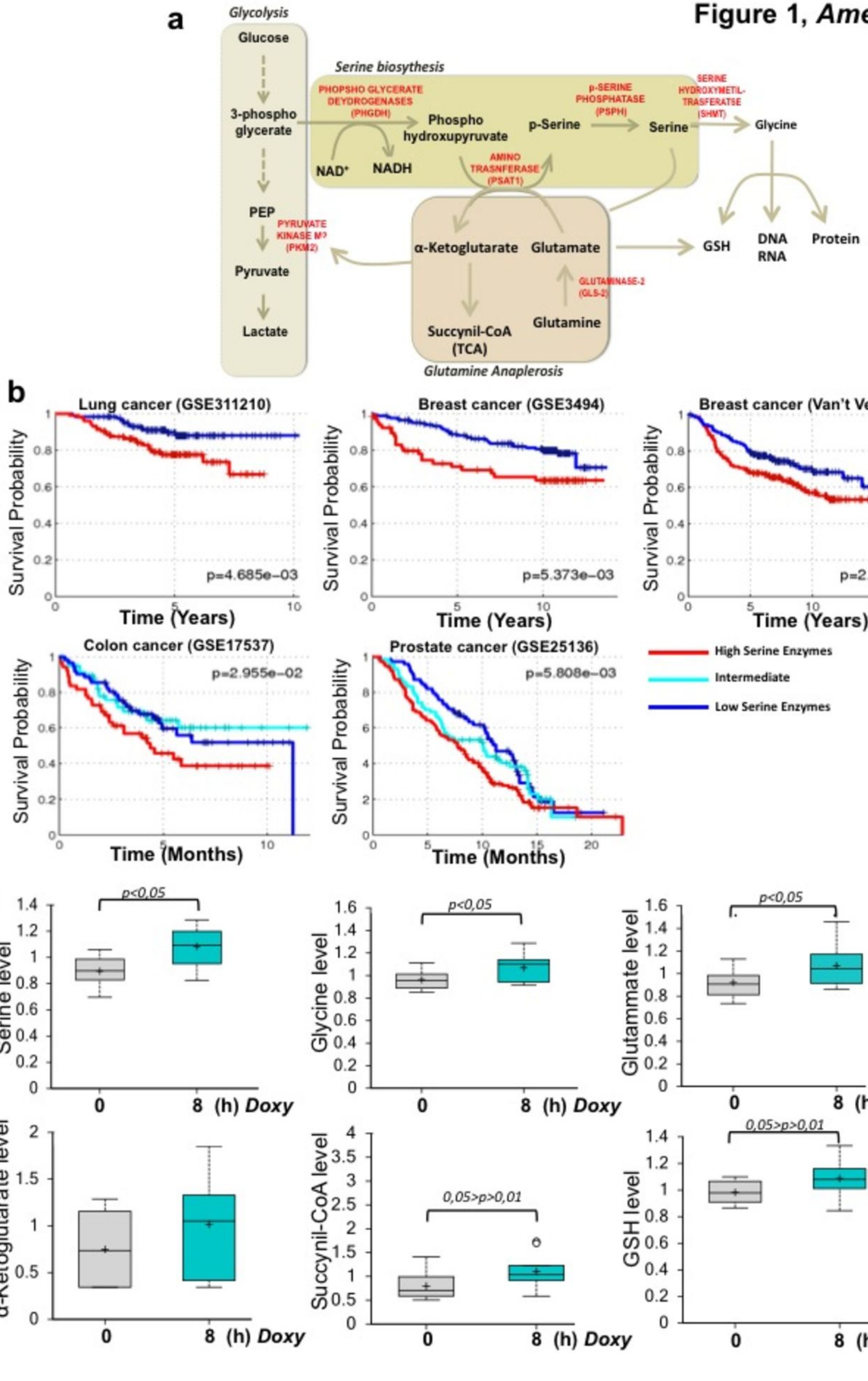
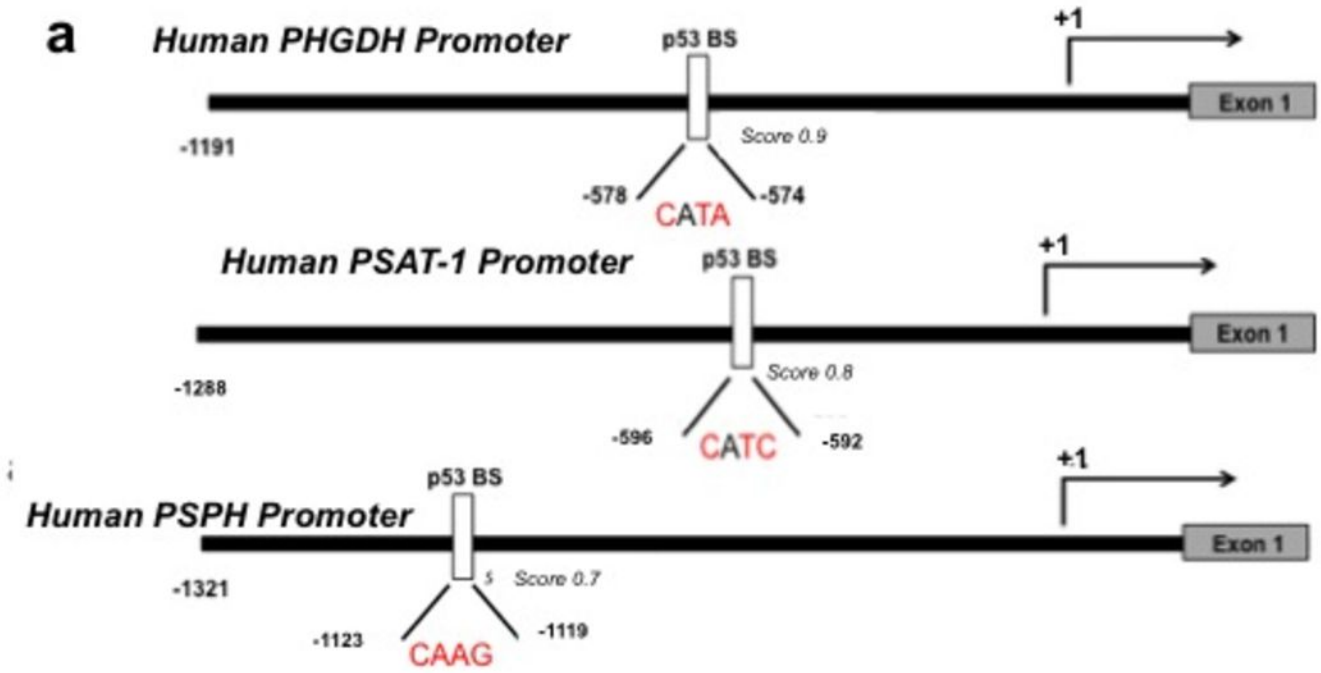
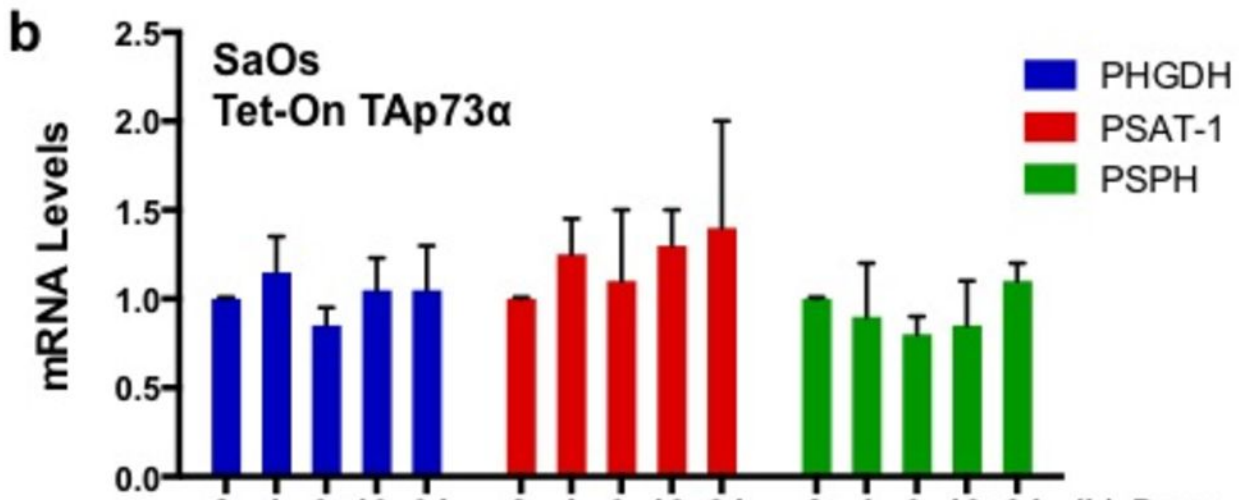
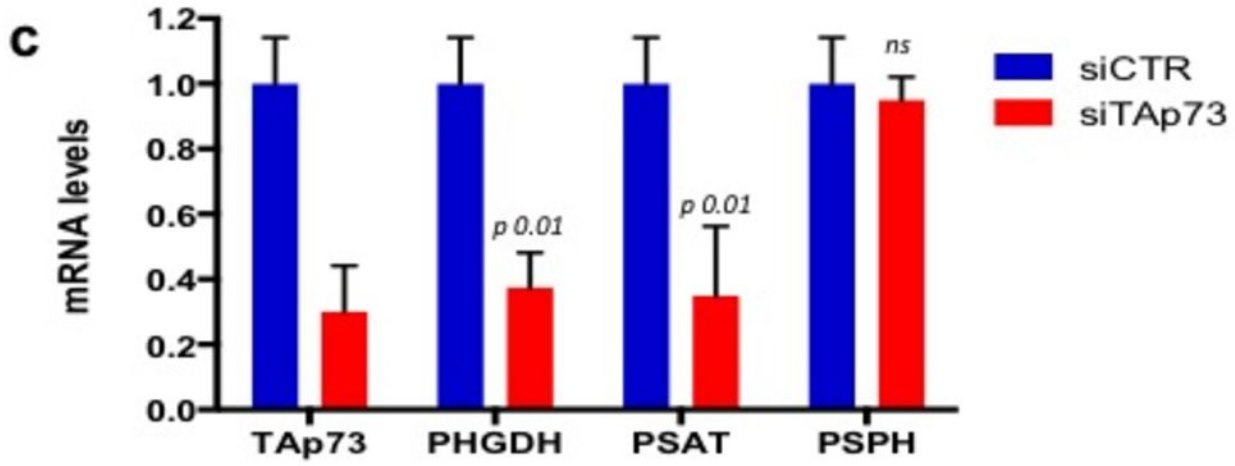
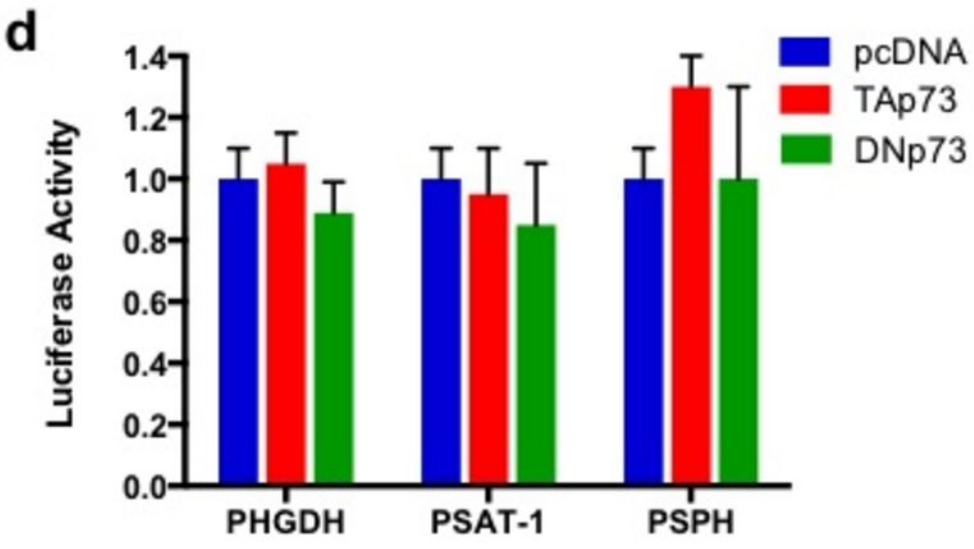


Figure 2, *Amelio et al.*









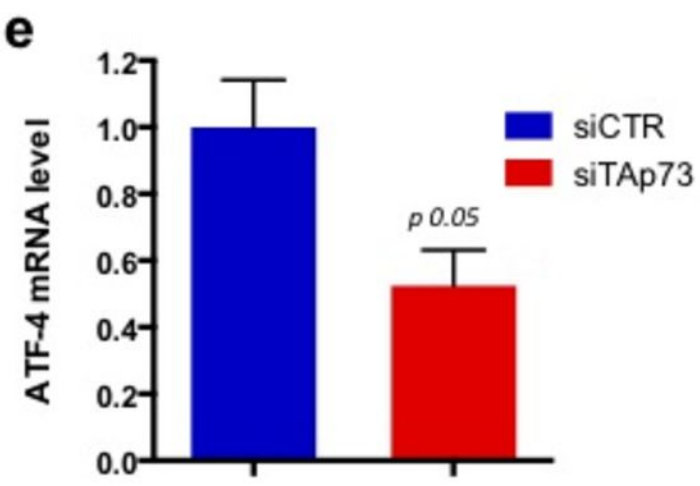
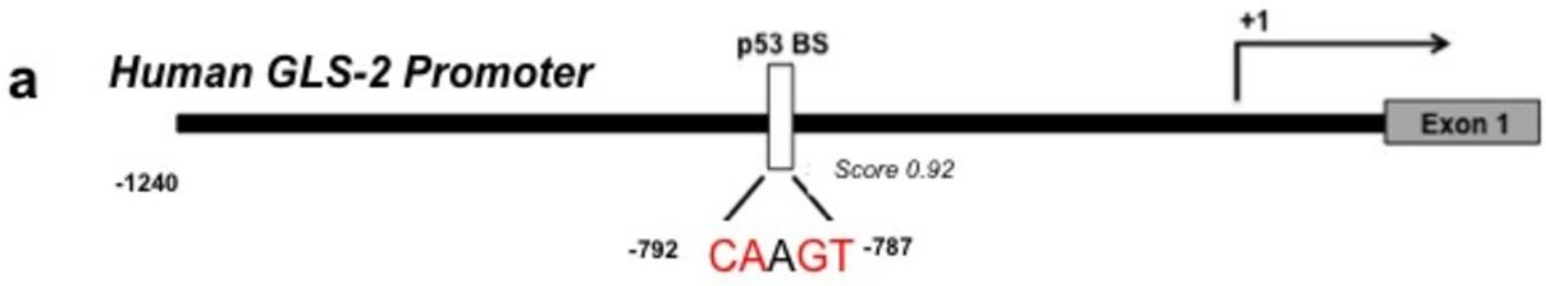
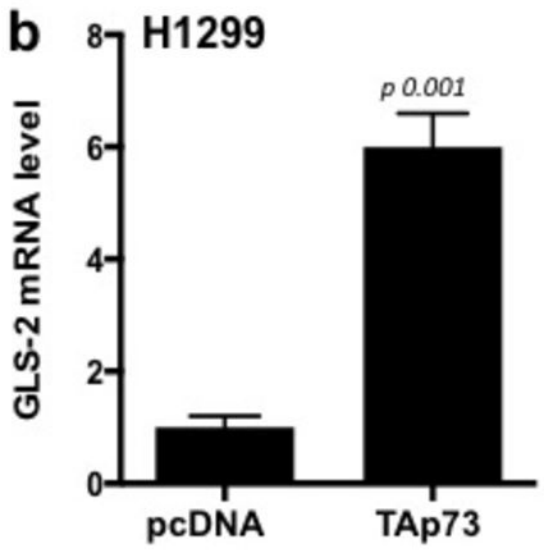
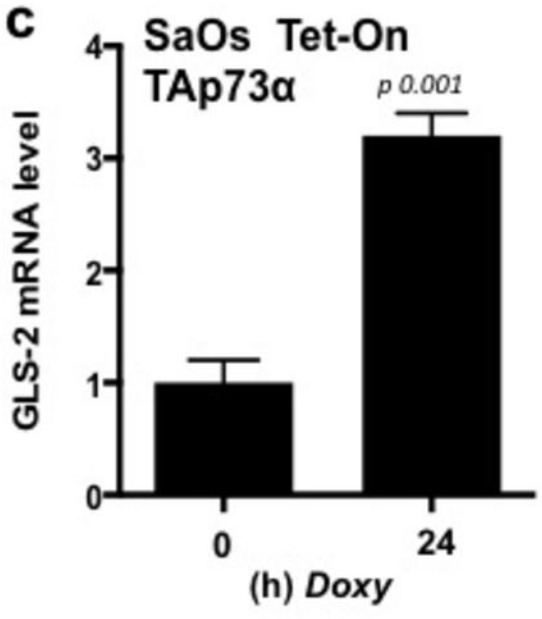
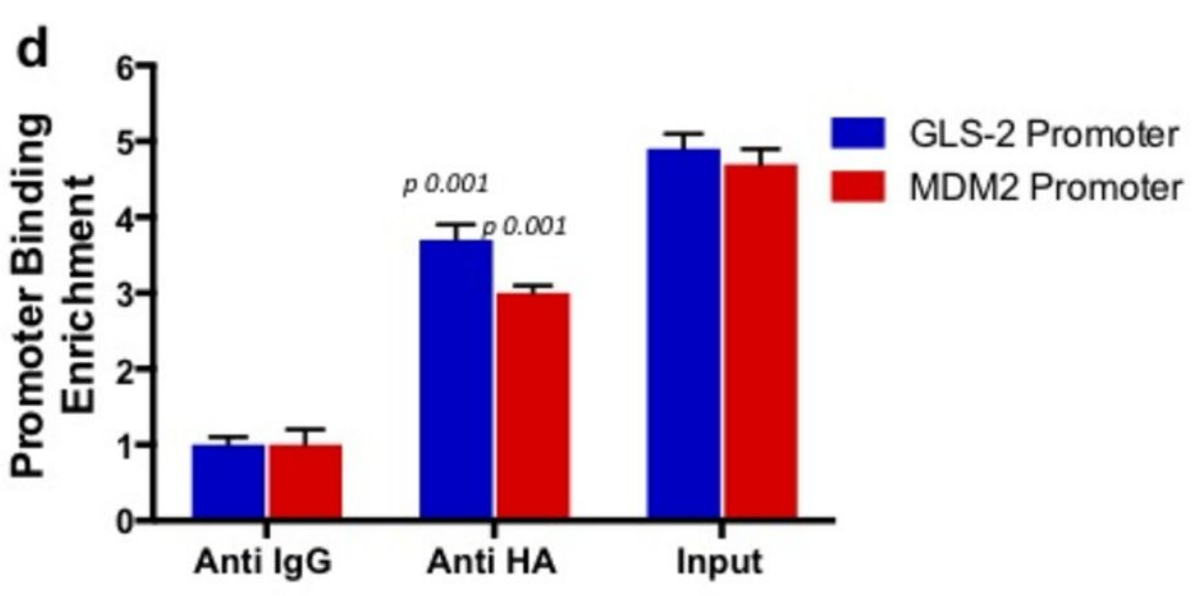


Figure 3, *Amelio et al.*









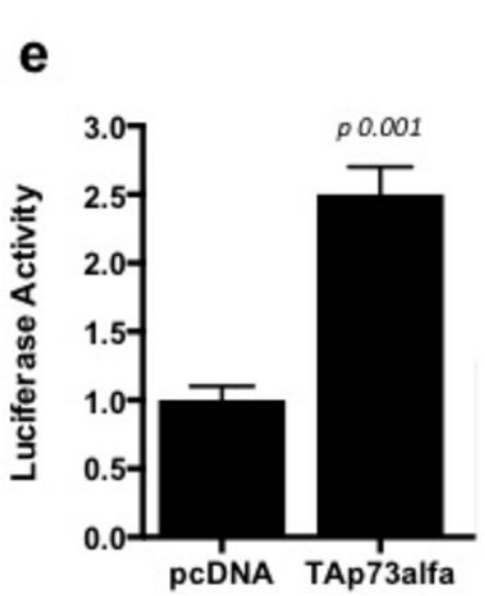


Figure 4, *Amelio et al.*