Supporting Information

Identification of histone H3 and H4 amino acid residues important for the regulation of arsenite stress signaling in *Saccharomyces cerevisiae*

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Figure S1

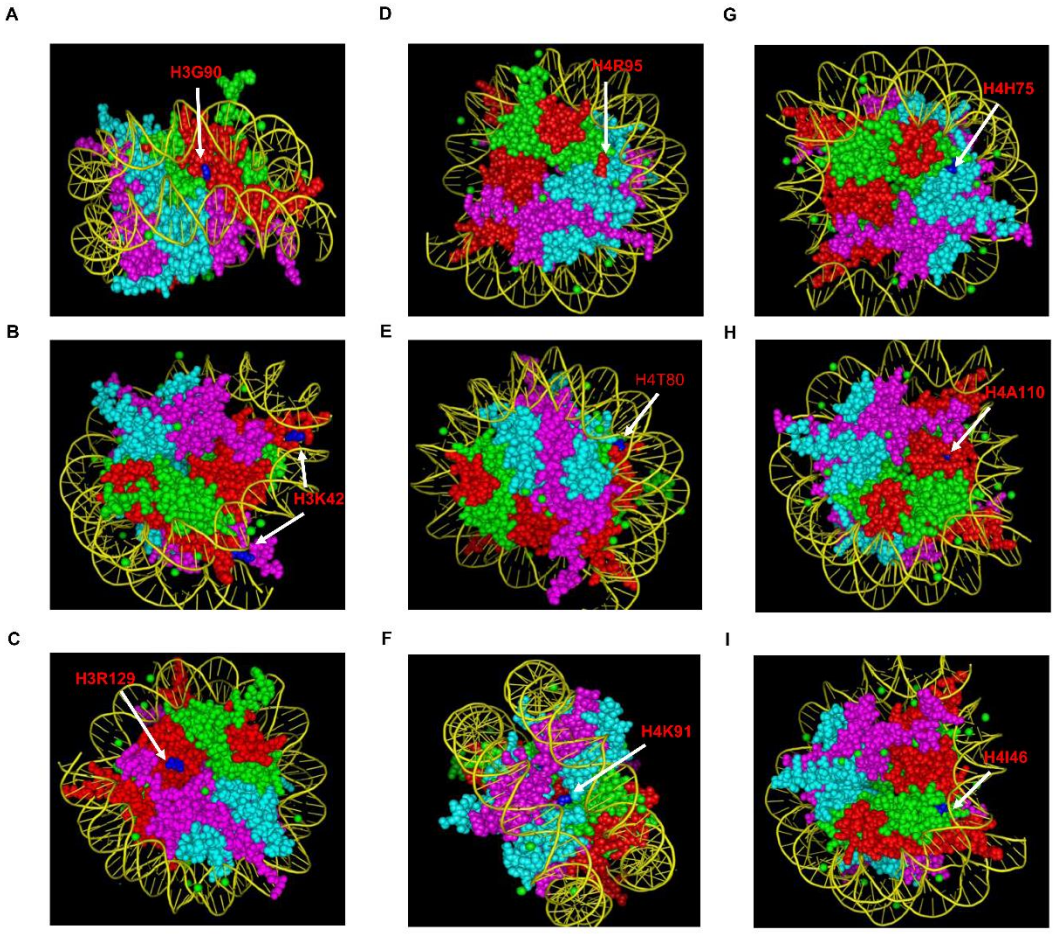

Figure S1: Nucleosome positions of important histone H3 and H4 residues

Nucleosome positions of important amino acid residues are depicted using PyMOL software. In nucleosome Histone proteins (H3-red, H4-green, H2A- magenta, H2B- cyan) and DNA (Yellow) are shown. (A) H3G90, (B) H3K42, (C) H3R129 are depicted in blue colour. (D) H4R95 amino acid residue is shown by red. (E) H4T80, (F) H4K91, (G) H4H75, (H) H3A110 and (I) H4I46 are shown in blue colour.

Figure S2

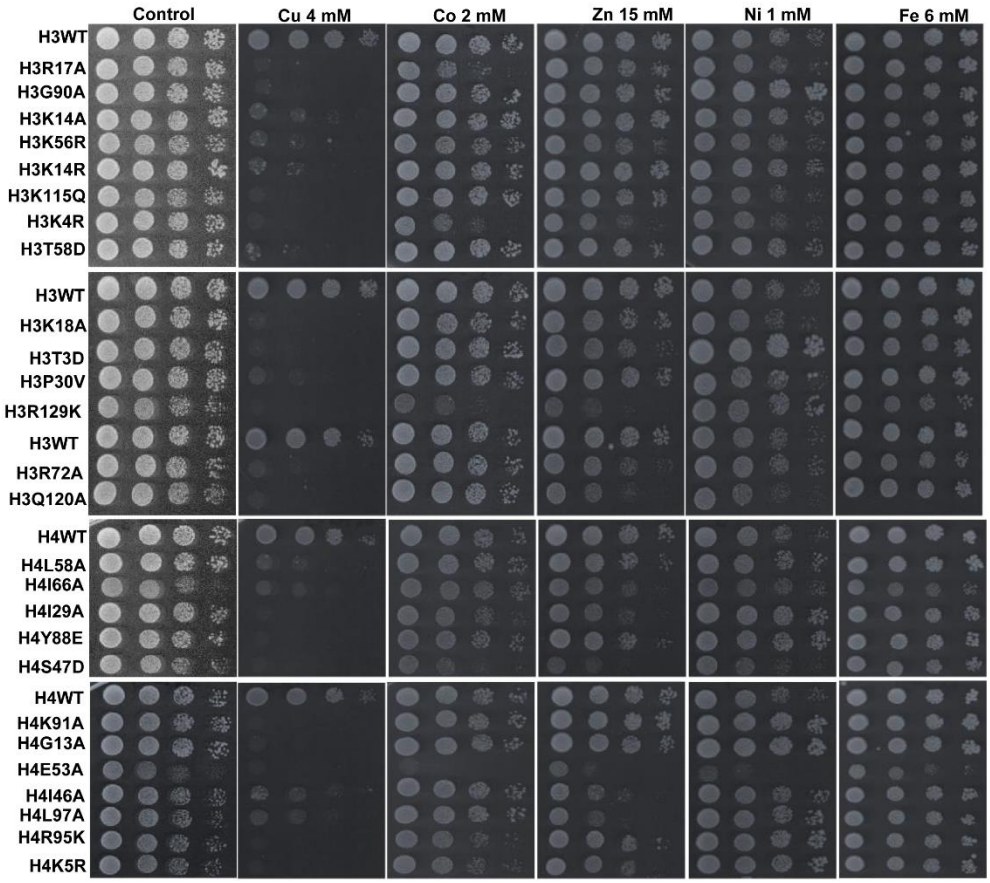

Figure S2: Effect of other metals on arsenite sensitive histone mutants

The spot sensitivity assay of arsenite sensitive H3 and H4 mutants in the presence of copper 4mM, cobalt 2mM, zinc 15mM, and iron 6mM. O/N grown Cells were serially diluted as described in materials and methods and 3 microliters of each diluted cell suspension was spotted onto SC agar plates containing different metals as indicated in the figure.

Figure S3

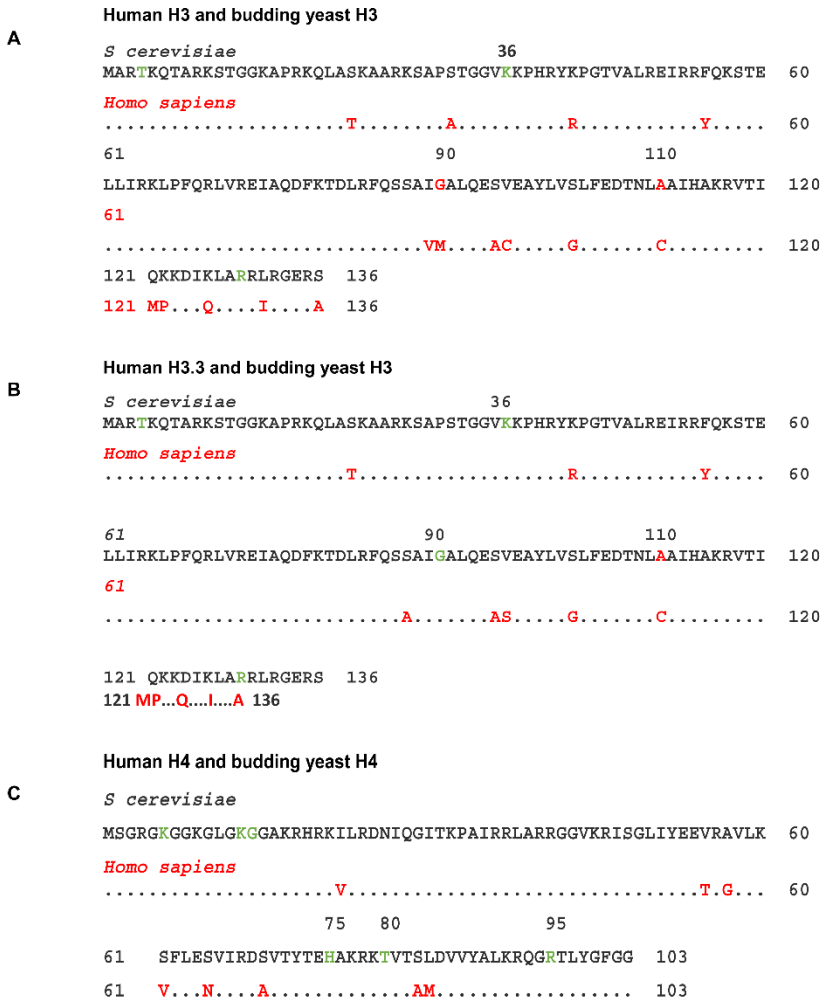

Figure S3: Amino acid sequence alignment of *S cerevisiae* H3 and human histones.

(A) Figure shows the result of Blastp tool of NCBI. Histone H3 sequence of *S cerevisiae* from SGD (*Saccharomyces cerevisiae* genome database) and H3.3 (P84243-1) of human were used for Blastp analysis. (B) Histone H3 sequence of *S cerevisiae* from SGD (*Saccharomyces cerevisiae* genome database) and H3 (P68431-1) of human were used for Blastp analysis. (C) Histone H4 sequence of S cerevisiae from SGD and H4 (P62805) of human were used for Blastp analysis. The important residues are highlighted in green colour which were found to be conserved in yeast and human. Residues which are not conserved are shown in red colour.

Figure S4

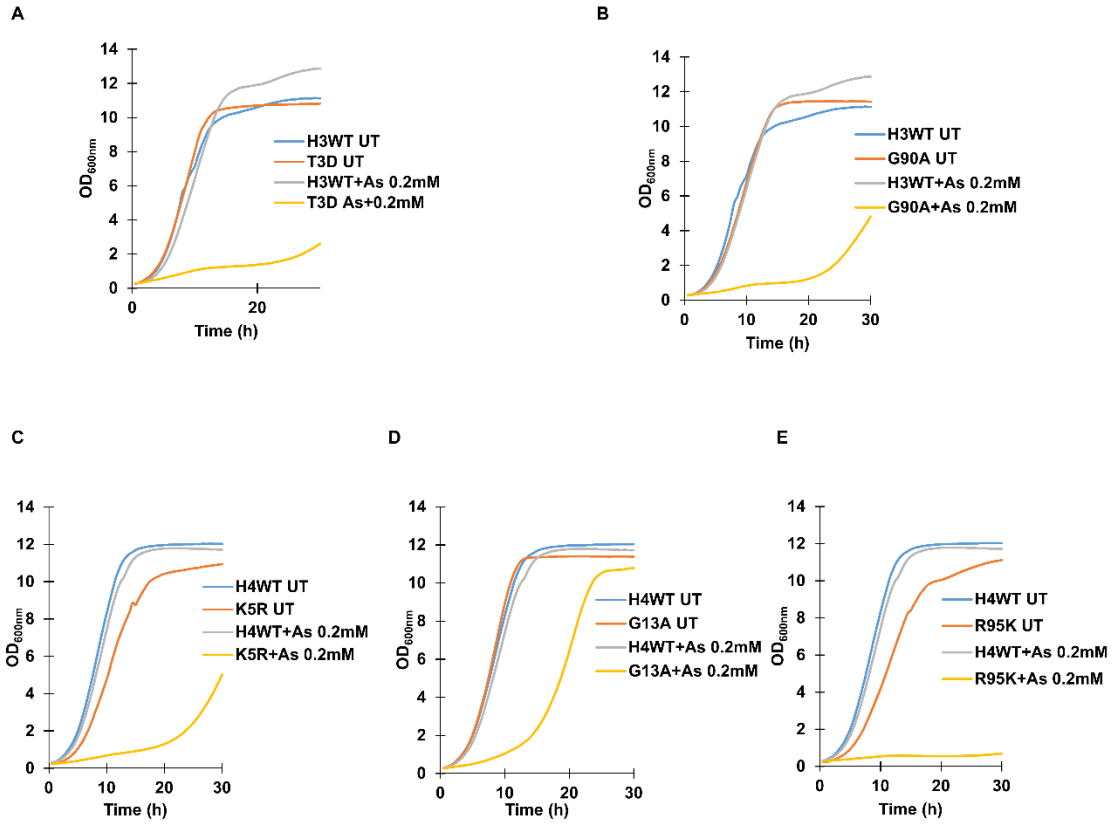

Figure S4: Effect of arsenic trioxide on the growth H3 and H4 histone mutants (H3T3D, H3G90A, H4K5R, H4G13A, and H4R95K) in liquid media

(A, and B) Growth curve analysis of arsenite sensitive histone H3 mutant cells in the presence and absence of 0.2 mM arsenic trioxide. (C, D, and E). Growth curve analysis of arsenite sensitive histone H4 mutant cells in the presence and absence of 0.2 mM arsenic trioxide.

Figure S5

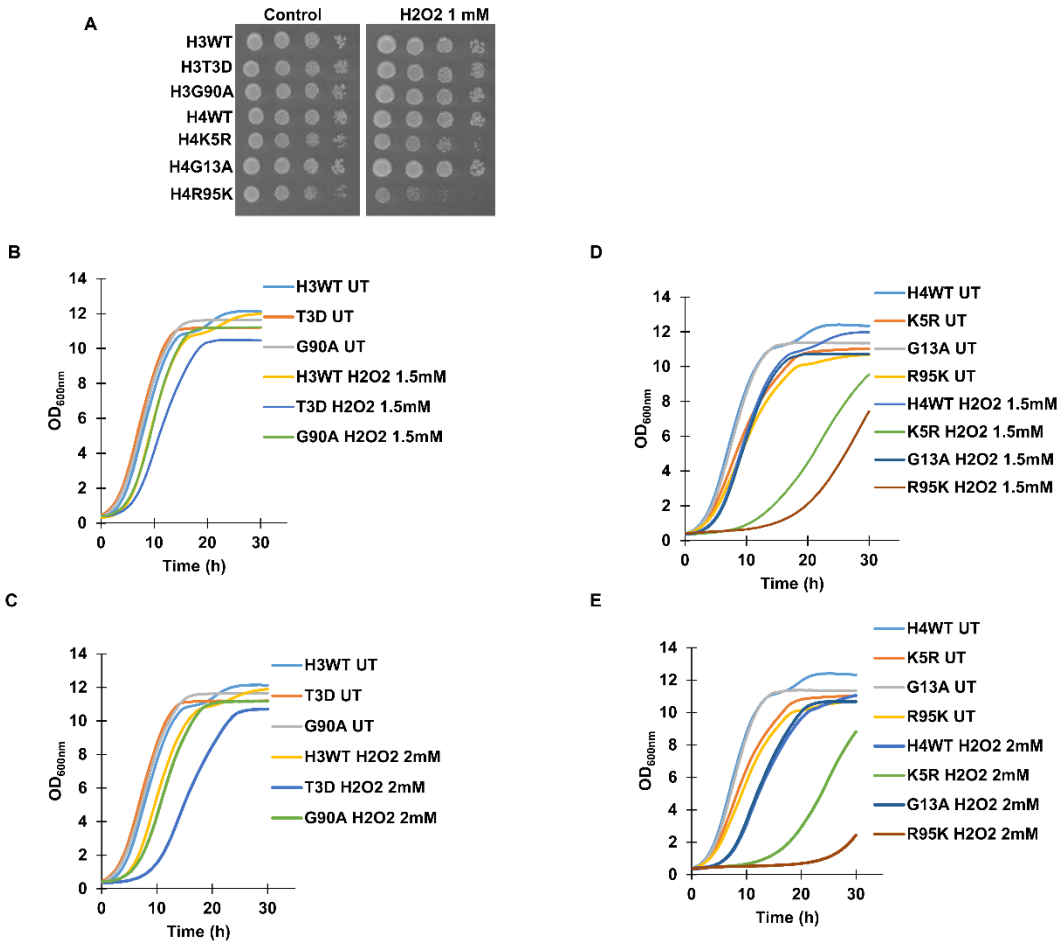

Figure S5: Effect of hydrogen peroxide in arsenite sensitive histone mutants

(A) Spot sensitivity assay of histone H3 and H4 mutants in the presence of 1 mM hydrogen peroxide. (B and C) are the growth curve analysis of H3 mutants in the presence of 1.5 mM and 2 mM hydrogen peroxide. (D and E) are the growth curve analysis of H4 mutants in the presence of 1.5 mM and 2 mM hydrogen peroxide.

Figure S6

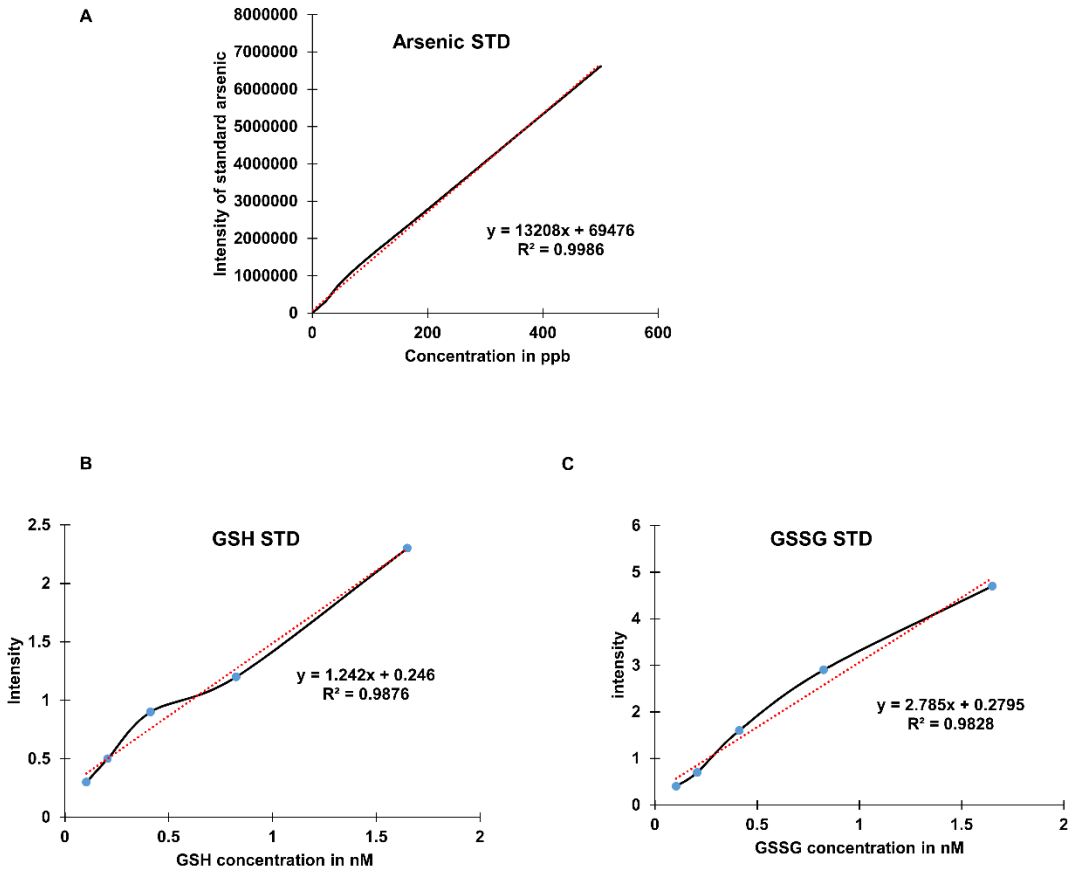

Figure S6: Standard curves of arsenic, GSH, and GSSG measurement.

(A) The graph shows the standard curve used for yeast intracellular arsenic quantification using ICP MS. (B) Graph shows the standard curve used for yeast intracellular total GSH quantification. (C) Graph shows the standard curve used for yeast intracellular total GSH quantification.

Figure S7

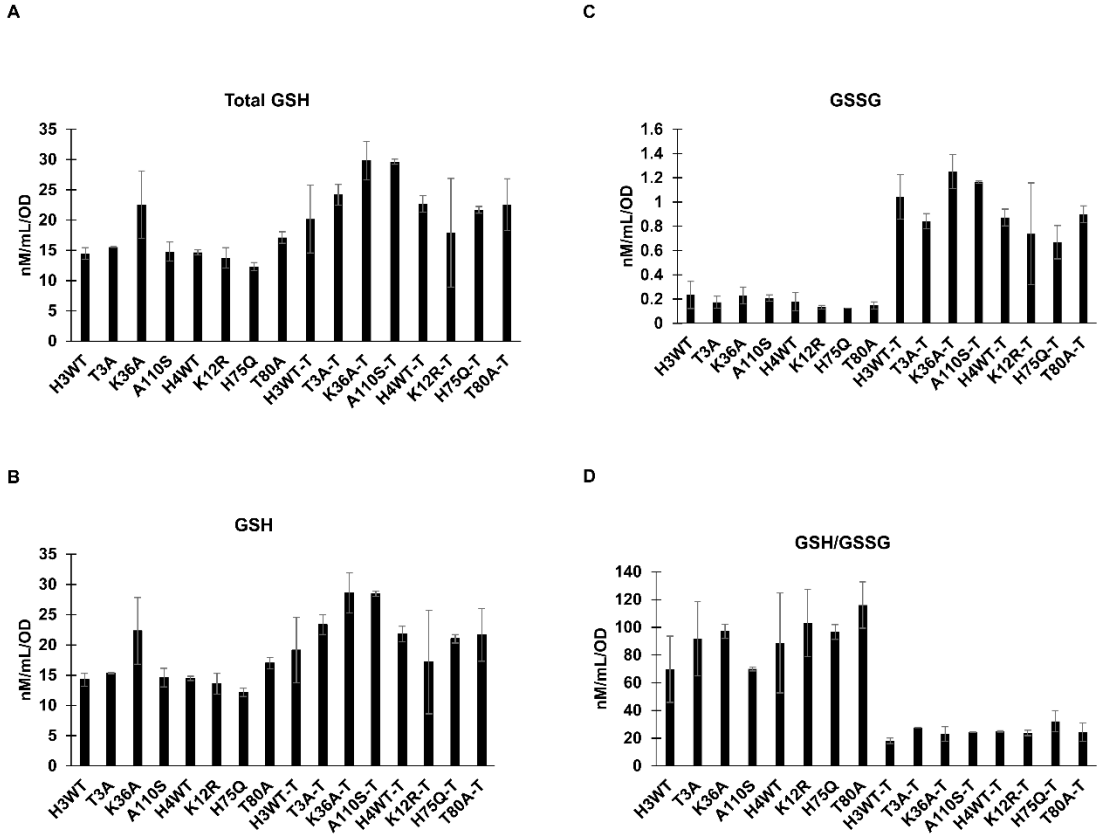

Figure S7: GSH and GSSG measurement in the arsenite resistant mutants.

(A, B, C, and D) bar graphs representing the total GSH, GSH, GSSG and GSH/GSSG ratio in the arsenite resistant H3 and H4 mutants treated with 0.8mM arsenic for 6 hours along with wild type cells.

Figure S8

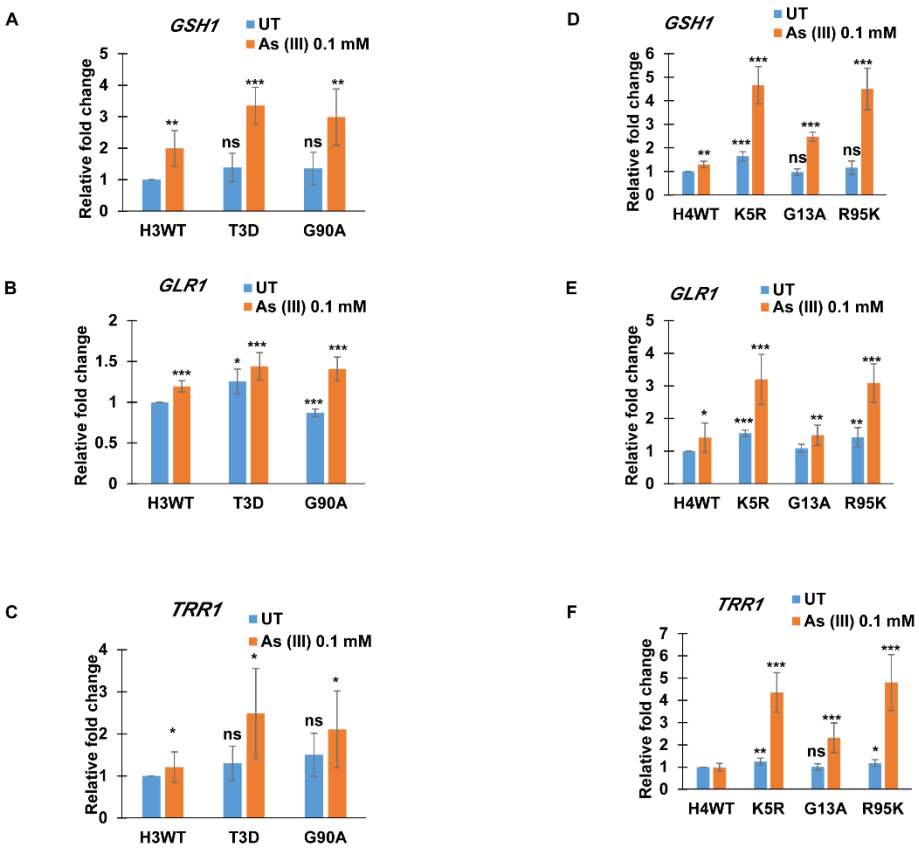

Figure S8: mRNA analysis of genes involved in the maintenance of GSH/GSSG ratio

(A) Bar graphs showing the mRNA expression of *GSH1* gene in arsenite sensitive histone H3 mutants treated with arsenite 0.1mM for 6 hours. (B) Bar graph show the mRNA expression of *GLR1* gene in arsenite sensitive histone H3 mutants treated with arsenite 0.1mM for 6 hours. (C) Bar graph show the mRNA expression of *TRR1* gene in arsenite sensitive H3 histone mutants treated with arsenite 0.1mM for 6 hours. (D, E, and F) are the bar graph of genes *GSH1*, *GLR1* and *TRR1* in histone H4 mutants treated with arsenite 0.1mM for 6 hours. Statistical analysis was performed using a t test (*P <0.05, **P <0.001, ***P< 0.0001) ns indicates the non-significance.

Figure S9

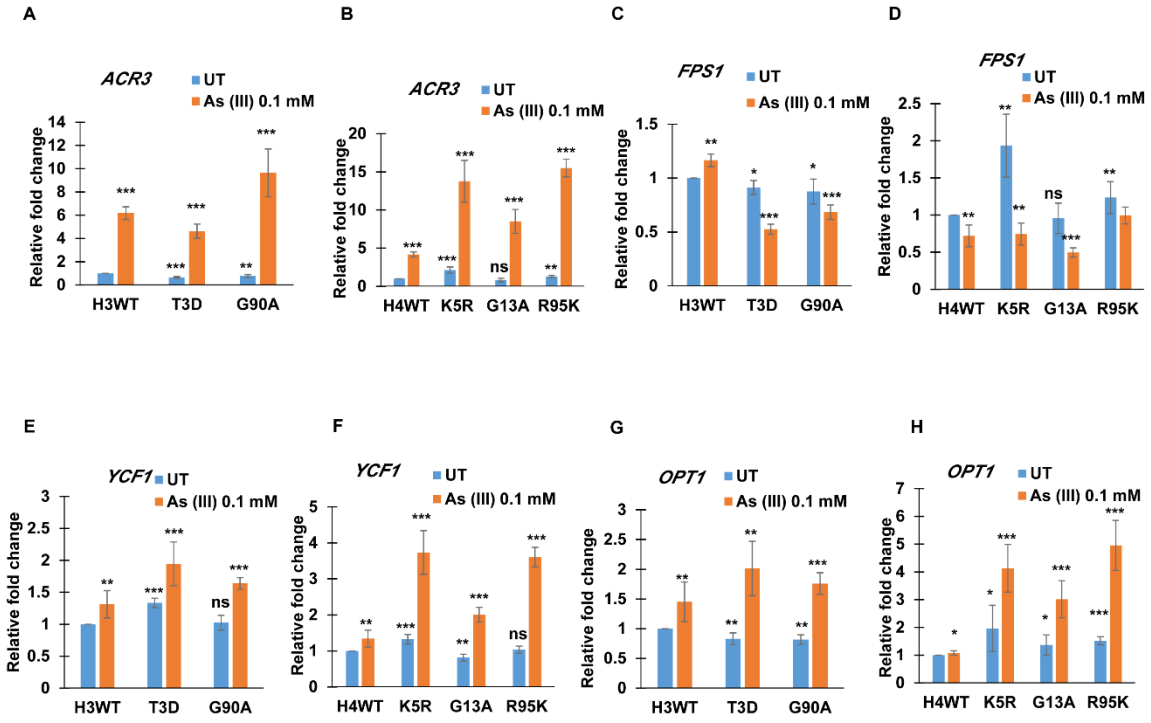

Figure S9: Gene expression analysis of transporter genes involved in arsenite toxicity in histone mutants.

(A and B) mRNA expression analysis of *ACR3* gene in the H3 and H4 mutants in the presence of arsenite 0.1mM for 6 hours treatment. (C and D) bar graph shows the mRNA expression of *FPS1* gene in H3 and H4 mutants in the presence of arsenite 0.1mM for 6 hours. (E and F) mRNA expression of *YCF1* gene in the H3 and H4 mutants in the presence of arsenite 0.1mM for 6 hours treatment. (G and H) graph represents the mRNA expression of *OPT1* gene in the H3 and H4 mutants in the presence of arsenite 0.1mM for 6 hours. Statistical analysis was performed using a t test (*P <0.05, **P <0.01, ***P< 0.0001) ns indicates the non-significance.

Figure S10

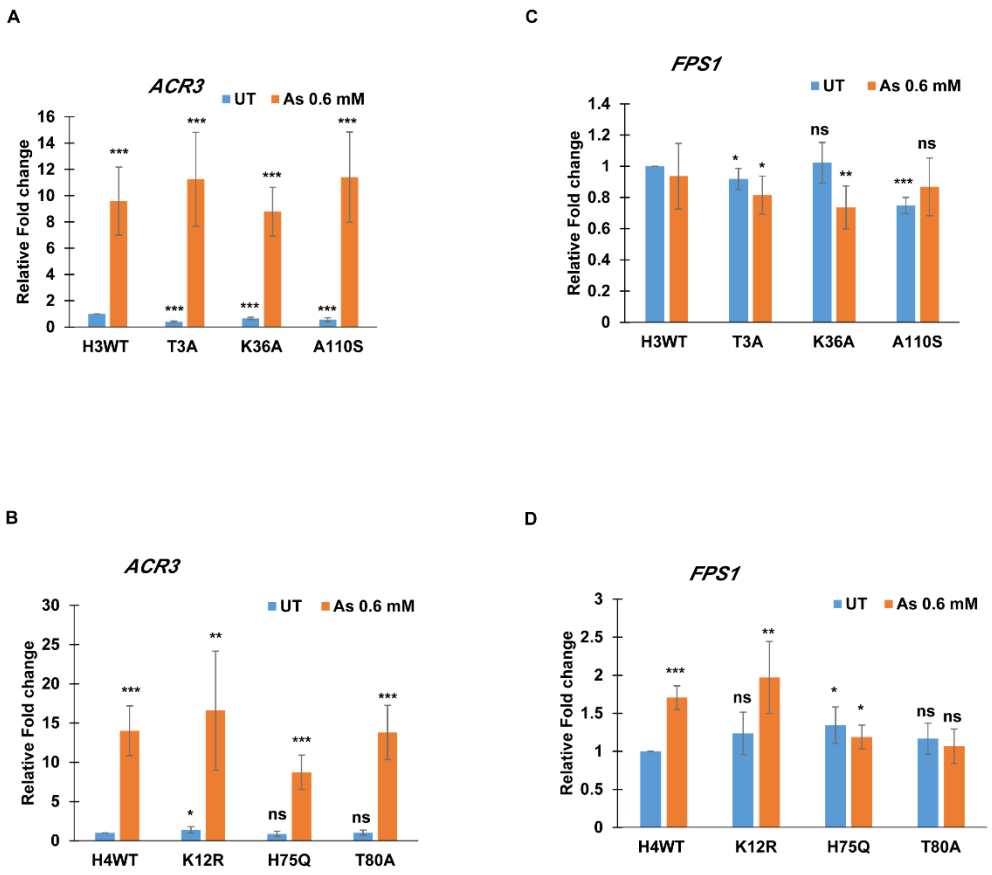

Figure S10: Gene expression analysis of *ACR3* and *FPS1* in H3/H4 arsenic resistant mutants

The bar graph (A) and (B) show the expression of *ACR3* gene in H3 and H4 mutants in the absence and presence of arsenite 0.6 mM treated for 3 hours respectively. The bar graph (C) and (D) show the expression of *FPS1* gene in H3 and H4 mutants in the absence and presence of arsenite 0.6 mM treated for 3 hours respectively. UT indicates untreated (control). Statistical analysis was performed using a t test (*P <0.05, **P <0.001, ***P< 0.0001) ns indicates the non-significance.

Figure S11

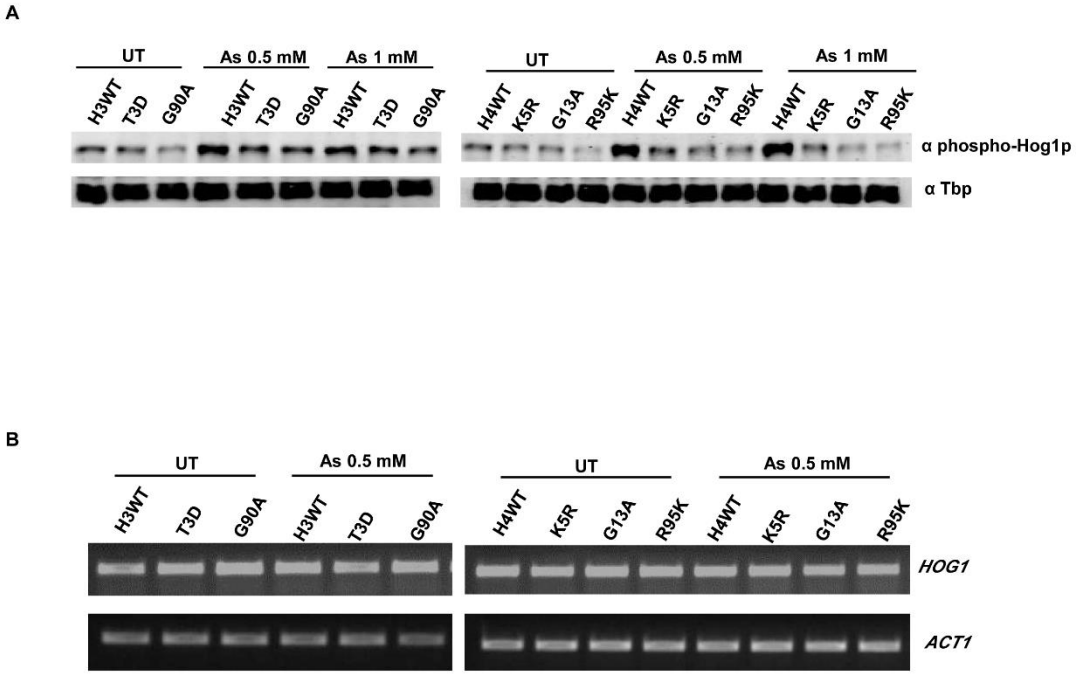

Figure S11 Analysis of Hog1 phosphorylation and expression in arsenite sensitive mutants.

(A) Western blots represent the Hog1 phosphorylation in H3 and H4 arsenite sensitive mutants treated with higher concentrations of arsenic 0.5 and 1mM for 30 minutes. Tbp was used as loading control. (B) Gene expression analysis of *HOG1* using specific primer by semi quantitative PCR. *ACT1* used as control gene.

Figure S12

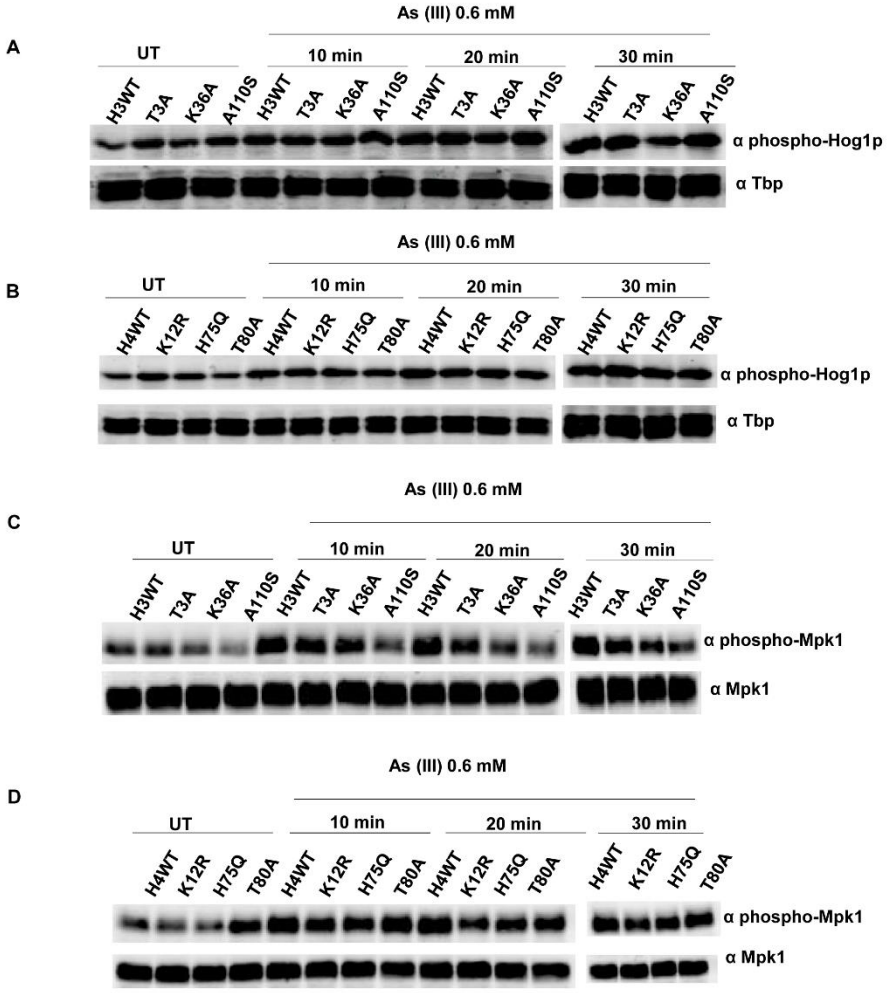

Figure S12: Analysis of Hog1 and Slt2 activation (phosphorylation) in arsenite resistant mutants.

(A and B) The western blot represents the phosphorylation level of Hog1 by using anti phospho-p38 antibody in H3 and H4 mutants. (C and D) The western blot represent the phosphorylation of Slt2 probed with anti-phospho-p44/42 and Mpk1 antibody in H3 and H4 mutants respectively. Tbp antibody is used as loading control.

Figure S13

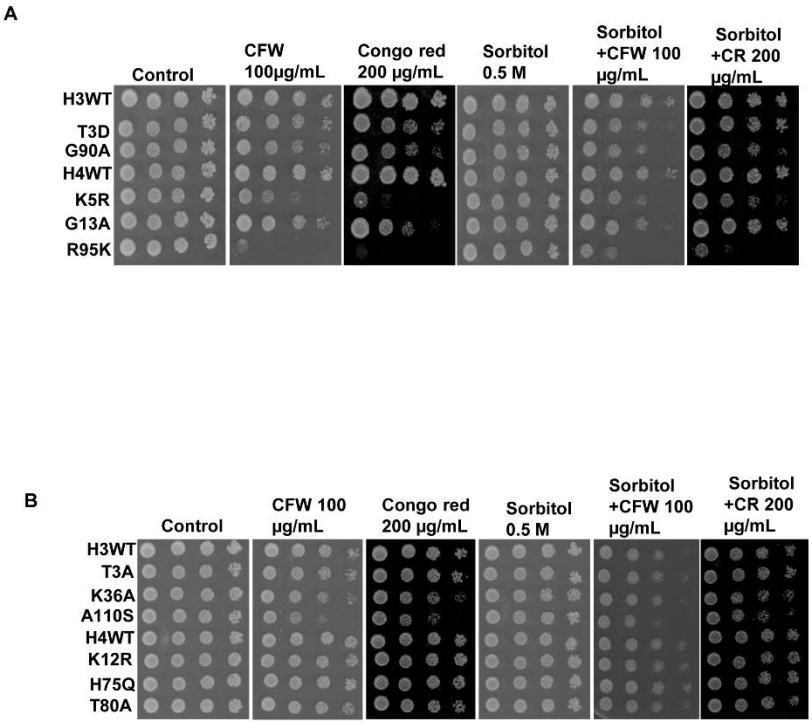

Figure S13: The effect of cell-wall perturbing agents on the growth of arsenite sensitive and resistant histone mutants.

(A) Spot sensitivity assay of arsenite sensitive H3 and H4 mutants in the presence of CFW, Congo red, and supplemented with sorbitol. (B) Spot sensitivity assay of arsenite resistant H3 and H4 mutants in the presence of CFW, Congo red, and supplemented with sorbitol.

Figure S14

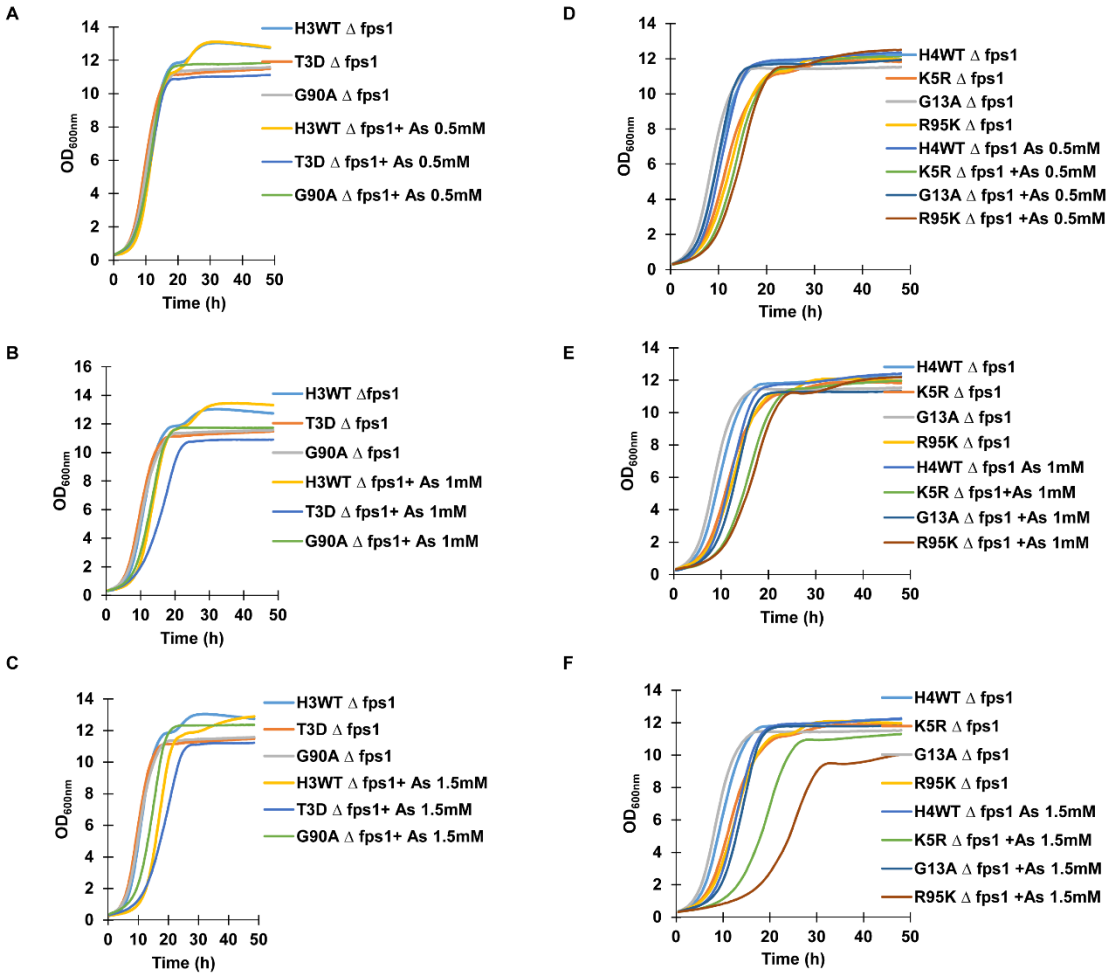

Figure S14: Effect of *FPS1* deletion on arsenite sensitive histone mutants.

(A, B, and C) the growth curves *FPS1* deleted H3 mutants in the presence of arsenic trioxide 0.5mM, 1mM, and 1.5mM concentrations. (D, E, and F) the growth curves represent, the growth of *FPS1* deficient H4 mutants in the presence of arsenic trioxide 0.5mM, 1mM, and 1.5mM concentrations.

Figure S15

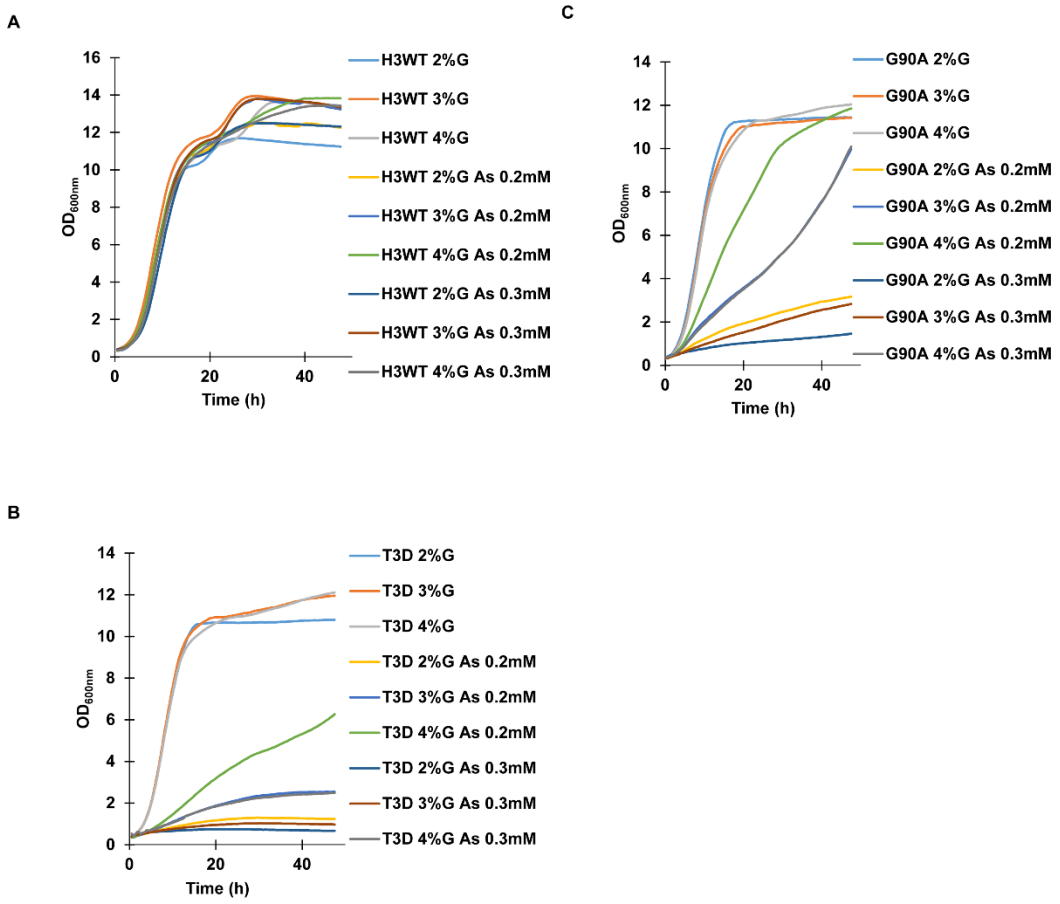

Figure S15: Effect of glucose concentration on arsenite sensitive H3 histone mutants.

(A, B, and C) Growth curves represent the effect of glucose concentration (2%, 3% and 4%) in the presence of arsenite 0.2mM and 0.3mM on histone H3 mutants.

Figure S16

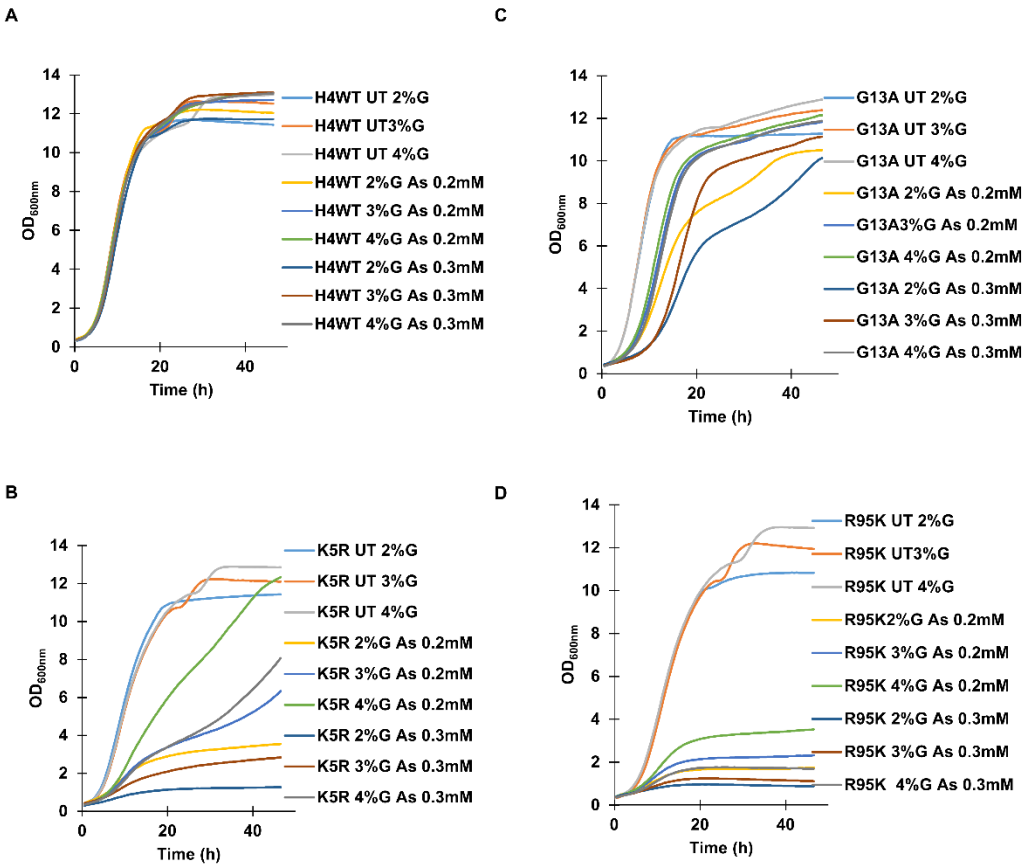

Figure S16: Effect of glucose concentration on arsenite sensitive H4 histone mutants.

(A, B, C and D), Growth curves represent the effect of glucose concentration (2%, 3% and 4%) in the presence of arsenite 0.2mM and 0.3mM on histone H4 mutants.

Table S1. List of Histone H3 and H4 mutants used in this study

H3_wild-type	*H3-L70A*	*H3-K42R*	*H3-S28D*	*H3-Δ4-15*	*H4-K59A*	*H4-R19K*
H3-A1S	*H3-V71A*	*H3-K56R*	*H3-S31D*	*H3-Δ4-20*	*H4-S60A*	*H4-R23K*
H3-R2A	*H3-R72A*	*H3-K64R*	*H3-S57D*	*H3-Δ4-30*	*H4-L62A*	*H4-R35K*
H3-T3A	*H3-E73A*	*H3-K79R*	*H3-S86D*	*H3-Δ4-35*	*H4-E63A*	*H4-R36K*
H3-K4A	*H3-I74A*	*H3-K115R*	*H3-S87D*	*H3-Δ28-31*	*H4-S64A*	*H4-R39K*
H3-Q5A	*H3-A75S*	*H3-K121R*	*H3-S102D*	*H3-Δ32-35*	*H4-V65A*	*H4-R40K*
H3-T6A	*H3-Q76A*	*H3-K122R*	*H3-S135D*	**H4_wild type**	*H4-I66A*	*H4-R55K*
H3-A7S	*H3-D77A*	*H3-K125R*	*H3-Y99E*	*H4-S1A*	*H4-R67A*	*H4-R67K*
H3-R8A	*H3-F78A*	*H3-K4Q*	*H3-Y41F*	*H4-G2A*	*H4-D68A*	*H4-R78K*
H3-K9A	*H3-K79A*	*H3-K9Q*	*H3-Y99F*	*H4-R3A*	*H4-S69A*	*H4-R92K*
H3-S10A	*H3-T80A*	*H3-K14Q*	*H3-P30V*	*H4-G4A*	*H4-V70A*	*H4-R95K*
H3-T11A	*H3-D81A*	*H3-K18Q*	*H3-P38V*	*H4-K5A*	*H4-T71A*	*H4-D24N*
H3-G12A	*H3-L82A*	*H3-K23Q*	*H3-H39Q*	*H4-G6A*	*H4-T73A*	*H4-D68N*
H3-G13A	*H3-R83A*	*H3-K27Q*	*H3-K9,14,18,23R*	*H4-G7A*	*H4-E74A*	*H4-N25D*
H3-K14A	*H3-Q85A*	*H3-K36Q*	*H3-K9,14,18,23Q*	*H4-K8A*	*H4-H75A*	*H4-E52Q*
H3-A15S	*H3-S86A*	*H3-K37Q*	*H3-K9,14,18,23A*	*H4-G9A*	*H4-K77A*	*H4-E53Q*
H3-P16A	*H3-S87A*	*H3-K42Q*	*H3-Δ1-4*	*H4-L10A*	*H4-K79A*	*H4-E63Q*
H3-R17A	*H3-A88S*	*H3-K56Q*	*H3-Δ1-8*	*H4-G11A*	*H4-T80A*	*H4-E74Q*
H3-K18A	*H3-I89A*	*H3-K64Q*	*H3-Δ1-12*	*H4-K12A*	*H4-V81A*	*H4-Q27E*
H3-Q19A	*H3-G90A*	*H3-K79Q*	*H3-Δ1-16*	*H4-G13A*	*H4-T82A*	*H4-Q93E*
H3-L20A	*H3-A91S*	*H3-K115Q*	*H3-Δ1-20*	*H4-G14A*	*H4-S83A*	*H4-T30D*
H3-A21S	*H3-L92A*	*H3-K121Q*	*H3-Δ1-24*	*H4-A15S*	*H4-D85A*	*H4-T71D*
H3-S22A	*H3-E94A*	*H3-K122Q*	*H3-Δ1-28*	*H4-K16A*	*H4-V86A*	*H4-Δ17-24*
H3-K23A	*H3-S95A*	*H3-K125Q*	*H3-Δ1-32*	*H4-R17A*	*H4-V87A*	*H4-Δ20-23*
H3-A24S	*H3-V96A*	*H3-R2K*	*H3-Δ5-8*	*H4-H18A*	*H4-Y88A*	*H4-T82D*
H3-A25S	*H3-A98S*	*H3-R8K*	*H3-Δ5-12*	*H4-R19A*	*H4-A89S*	*H4-T96D*
H3-R26A	*H3-Y99A*	*H3-R17K*	*H3-Δ5-16*	*H4-K20A*	*H4-K91A*	*H4-S1D*
H3-K27A	*H3-L100A*	*H3-R26K*	*H3-Δ5-20*	*H4-I21A*	*H4-R92A*	*H4-S47D*
H3-S28A	*H3-V101A*	*H3-R40K*	*H3-Δ5-24*	*H4-L22A*	*H4-Q93A*	*H4-S60D*
H3-A29S	*H3-S102A*	*H3-R49K*	*H3-Δ5-28*	*H4-R23A*	*H4-G94A*	*H4-S64D*
H3-P30A	*H3-F104A*	*H3-R53K*	*H3-Δ5-32*	*H4-D24A*	*H4-R95A*	*H4-Δ21-24*
H3-S31A	*H3-E105A*	*H3-R63K*	*H3-Δ9-12*	*H4-N25A*	*H4-T96A*	*H4-Y72E*
H3-T32A	*H3-D106A*	*H3-R69K*	*H3-Δ9-16*	*H4-I26A*	*H4-L97A*	*H4-Y88E*
H3-G33A	*H3-T107A*	*H3-R72K*	*H3-Δ9-20*	*H4-Q27A*	*H4-Y98A*	*H4-Y98E*
H3-G34A	*H3-N108A*	*H3-R83K*	*H3-Δ9-24*	*H4-G28A*	*H4-G99A*	*H4-Y51F*
H3-V35A	*H3-L109A*	*H3-R128K*	*H3-Δ9-28*	*H4-I29A*	*H4-F100A*	*H4-Y88F*
H3-K36A	*H3-A110S*	*H3-R129K*	*H3-Δ9-32*	*H4-T30A*	*H4-G101A*	*H4-Y98F*
H3-K37A	*H3-A111S*	*H3-R131K*	*H3-Δ9-36*	*H4-K31A*	*H4-G102A*	*H4-H18Q*

H3-P38A	*H3-A114S*	*H3-R134K*	*H3-Δ13-16*	*H4-P32A*	*H4-K5R*	*H4-H75Q*
H3-H39A	*H3-K115A*	*H3-D77N*	*H3-Δ13-20*	*H4-A33S*	*H4-K8R*	*H4-K5,8,12,16R*
H3-R40A	*H3-V117A*	*H3-D81N*	*H3-Δ13-24*	*H4-R35A*	*H4-K12R*	*H4-K5,8,12,16Q*
H3-K42A	*H3-Q120A*	*H3-D106N*	*H3-Δ13-28*	*H4-R36A*	*H4-K16R*	*H4-Δ1-4*
H3-P43A	*H3-K121A*	*H3-E50Q*	*H3-Δ13-32*	*H4-L37A*	*H4-K20R*	*H4-Δ1-8*
H3-G44A	*H3-K122A*	*H3-E59Q*	*H3-Δ13-36*	*H4-A38S*	*H4-K31R*	*H4-Δ1-12*
H3-V46A	*H3-K125A*	*H3-E73Q*	*H3-Δ17-20*	*H4-G41A*	*H4-K44R*	*H4-Δ1-16*
H3-A47S	*H3-A127S*	*H3-E94Q*	*H3-Δ17-24*	*H4-G42A*	*H4-K59R*	*H4-Δ1-20*
H3-R49A	*H3-R128A*	*H3-E105Q*	*H3-Δ17-28*	*H4-V43A*	*H4-K77R*	*H4-Δ1-24*
H3-E50A	*H3-R129A*	*H3-E133Q*	*H3-Δ17-32*	*H4-K44A*	*H4-K79R*	*H4-Δ5-8*
H3-R53A	*H3-R131A*	*H3-Q5E*	*H3-Δ17-36*	*H4-I46A*	*H4-K91R*	*H4-Δ5-12*
H3-K56A	*H3-G132A*	*H3-Q19E*	*H3-Δ21-24*	*H4-S47A*	*H4-K5Q*	*H4-Δ9-12*
H3-S57A	*H3-E133A*	*H3-Q76E*	*H3-Δ21-28*	*H4-G48A*	*H4-K8Q*	*H4-Δ9-16*
H3-T58A	*H3-R134A*	*H3-Q85E*	*H3-Δ21-32*	*H4-L49A*	*H4-K12Q*	*H4-Δ9-20*
H3-E59A	*H3-S135A*	*H3-Q93E*	*H3-Δ21-36*	*H4-I50A*	*H4-K16Q*	*H4-Δ9-24*
H3-L60A	*H3-K4R*	*H3-T3D*	*H3-Δ25-28*	*H4-Y51A*	*H4-K20Q*	*H4-Δ13-16*
H3-L61A	*H3-K9R*	*H3-T6D*	*H3-Δ25-32*	*H4-E52A*	*H4-K31Q*	*H4-Δ13-20*
H3-R63A	*H3-K14R*	*H3-T11D*	*H3-Δ25-36*	*H4-E53A*	*H4-Δ15-18*	*H4-Δ13-24*
H3-K64A	*H3-K18R*	*H3-T32D*	*H3-Δ29-32*	*H4-V54A*	*H4-K59Q*	*H4-Δ17-20*
H3-L65A	*H3-K23R*	*H3-T58D*	*H3-Δ29-36*	*H4-R55A*	*H4-K77Q*	*fps1Δ H4WT*
H3-P66A	*H3-K27R*	*H3-T80D*	*H3-Δ33-36*	*H4-A56S*	*H4-K79Q*	
H3-Q68A	*H3-K36R*	*H3-S10D*	*H3-Δ1-20*	*H4-V57A*	*H4-R3K*	
H3-R69A	*H3-K37R*	*H3-S22D*	*H3-Δ1-28*	*H4-L58A*	*H4-R17K*	
fps1Δ H3WT	*fps1Δ H3T3D*	*fps1Δ H3G90A*	*fps1Δ H4K5R*	*fps1Δ H4G13A*	*fps1Δ H4R95K*	

Table S2. List of gene specific primers used in this study

S.No.	Gene	Primer Sequence (5'→ 3')
1	*ACT1*	**F:** CGTCGGTAGACCAAGACACC
		R: TGGGGCAACTCTCAATTCGT
2	*HXT1*	**F:** GAAAGTCAAGTGCAACCCGC
		R: CTACCGTCGTGGTGCTTCAT
3	*HXT3*	**F:** TGTGTTTTGCCTGGGCTTTG
		R: ACCCCATGATGCTGAACCAG
4	*HXT4*	**F:** CGCAGACGATCCAGCTGTTA
		R: AACCGACAGCAGTGAAAACG
6	*HXT9*	**F:** ACCGGGGCAATCAACTTTTAC
		R: TCATGGTCTATGGCGTCAGC
7	*ACR3*	**F:** GACGCTGAAGGTCATCCCAA
		R:ATCCACGCCAATGCTGTCAT
8	*FPS1*	**F:** TTGATCGGTGCCTTCACAGG
		R: CAGCGCAAATGTTCCTGCTT
9	*TRR1*	**F:**TATTTCTGCCTGTGCCGTGT
		R:TCGTTCTTCTCAGCACGCTT
10	*YCF1*	**F:** CAAGGCACATTAACGGCAGG
		R: GGCCATTCTTTGGGTGGTCT
11	*OPT1*	**F:** ACATGCCCGTCATTTCAGGT
		R: ATGCAGTGGACAAAAACGGC
12	*GSH1*	**F:** ATGGGCTGTTCGTGCTTACA
		R: TTTCCTTCGGAGTACGGTCG
13	*FPS1* deletion	**F:**CCAAGTACGCTCGAGGGTACATTCTAATGCATTAAAAGACCTGTGCGGTATTTCACACCG
		R: ATCAGTCTATATTATTTGTTTCTTTTTCTTGTCTGTTTTCAGATTGTACTGAGAGTGCAC
14	*HOG1*	**F:** GGATGCCTTGGCTCATCCTT
		R:TGGTCATCAAACGTGGCAGA