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Supporting Information for

Arsenic toxicity is regulated by queuine availability and oxidation-induced reprogramming of the human tRNA epitranscriptome

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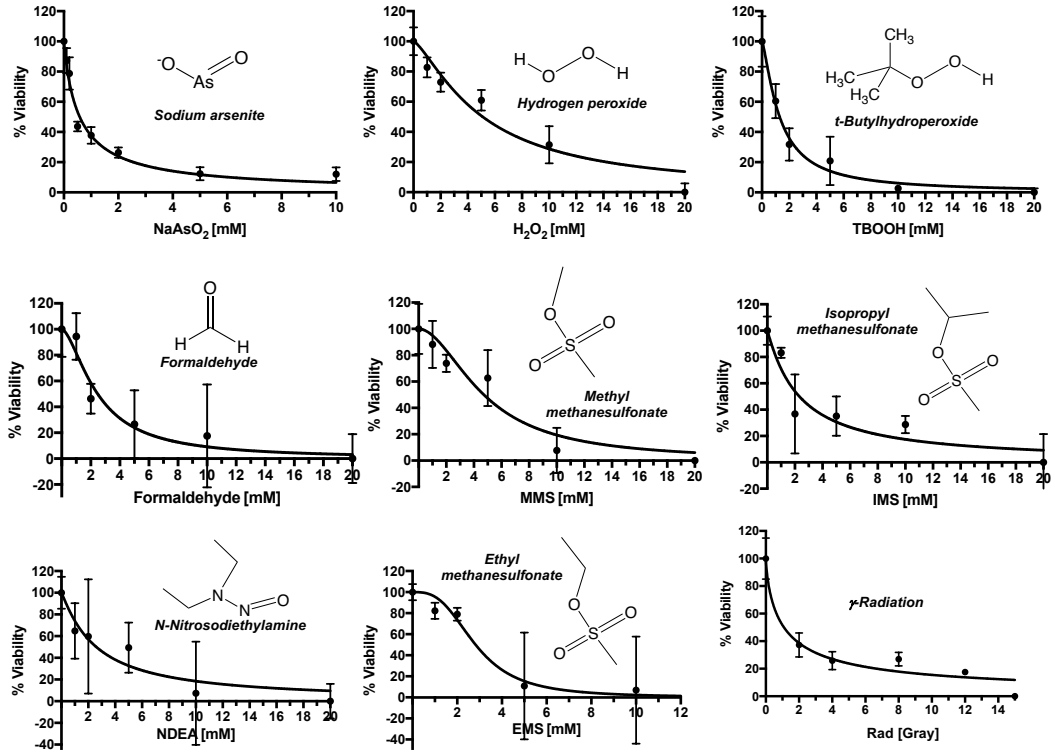
This PDF file includes:

Supplementary Figures

A



B

Toxicant	LD_{20} (mM)	LD_{50} (mM)
H_2O_2	1.8 [-0.1 / +0.2]	5.0 [-0.9 /+0.9]
MMS	2.3 [-0 / +2.7]	4.7 [-0 /+2.4]
EMS	1.8 [-0.7 / +3.2]	2.8 [-0.6 /+2.5]
IMS	0.6 [-0.3 / +0.4]	2.3 [-0.9 /+2.8]
FORM	1.1 [-0.2 / +1.4]	2.4 [-0.6 /+2.7]
$NaAsO_2$	0.1 [-0 / +0.1]	0.6 [-0.2 /+0.1]
NDEA	0.7 [-0.7 / +3.7]	2.6 [-2.4 /+2.2]
TBOOH	0.5 [-0.2 / +0.3]	1.3 [-0.3 /+0.8]
Rad	0.2 [-0.2 /+0]	1.1 [-0.9 /+1.9]

Figure S1. Dose-response data for treatment of HepG2 cells with toxicants. (**A**) Dose-response curves for treatment of HepG2 cells with toxicants. (**B**) Calculated lethal doses (LD) from dose-response curves shown in panel **A**.

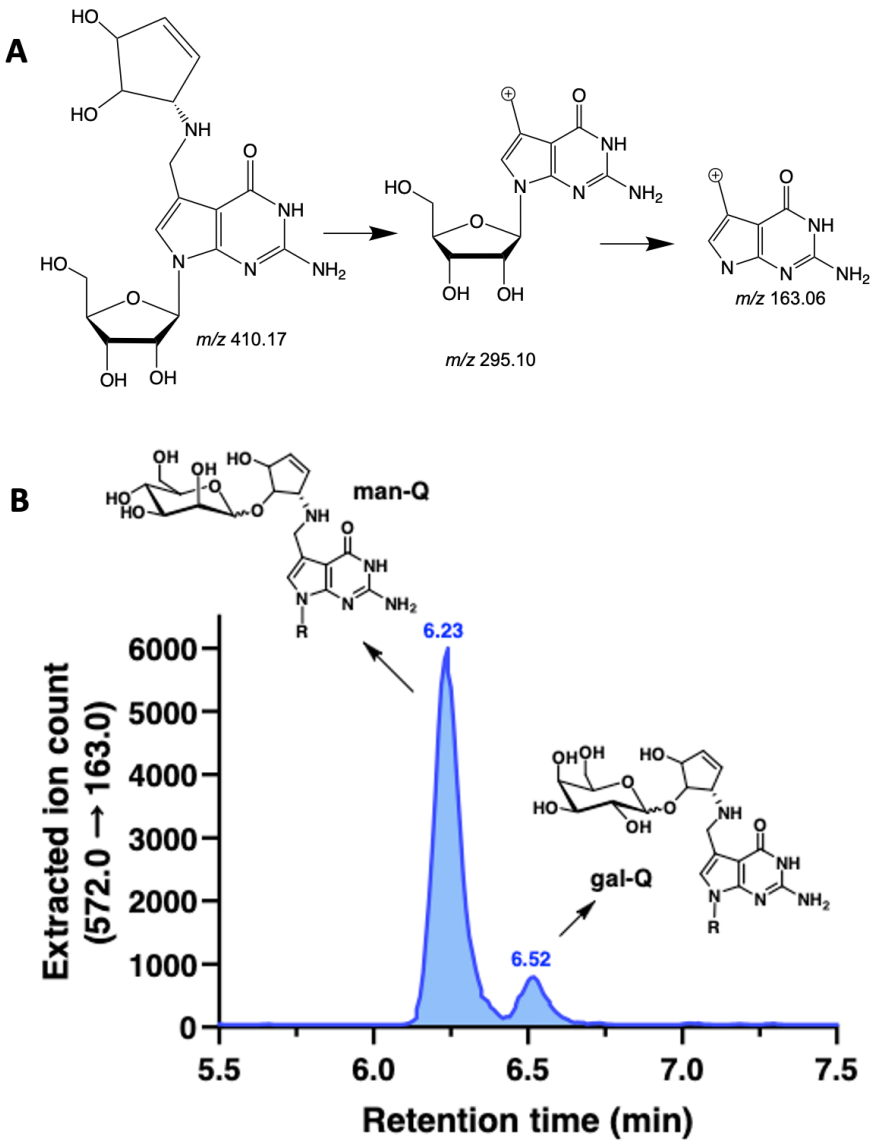


Figure S2. Identification and quantification of gal-Q and man-Q by LC-MS/MS. **(A)** The glycosylated derivatives of Q were identified on the basis of two CID transitions: *m/z* 572.22 to 295.10 and *m/z* 295.10 to 163.06. **(B)** The order of elution by reversed-phased (C18) HPLC and relative quantities are similar to values observed by Carell and coworkers (1).

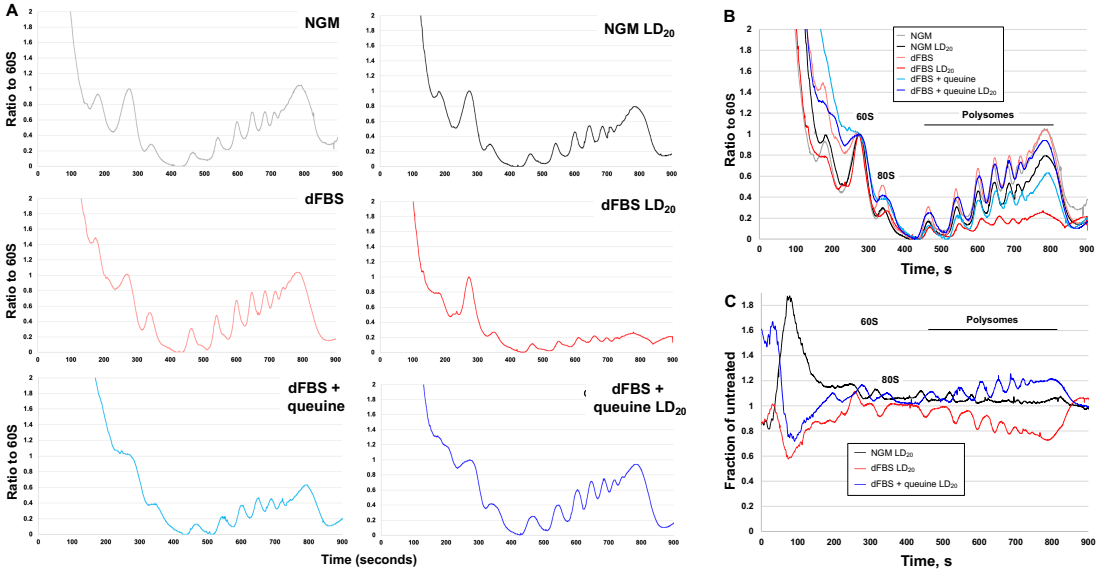


Figure S3. Arsenite-induced increases in Q in tRNA influence global translation. Individual (**A**) and combined (**B**) graphs of polysome analysis of translating mRNA from HepG2 cells grown in media containing normal growth media (NGM), dFBS, or dFBS+queuine untreated and after exposure to LD_{20} doses of $NaAsO_2$. These are representative of biological replicates (N = 3). (**C**) LD_{20} polysome traces from above were normalized to their media specific untreated sample and replotted.

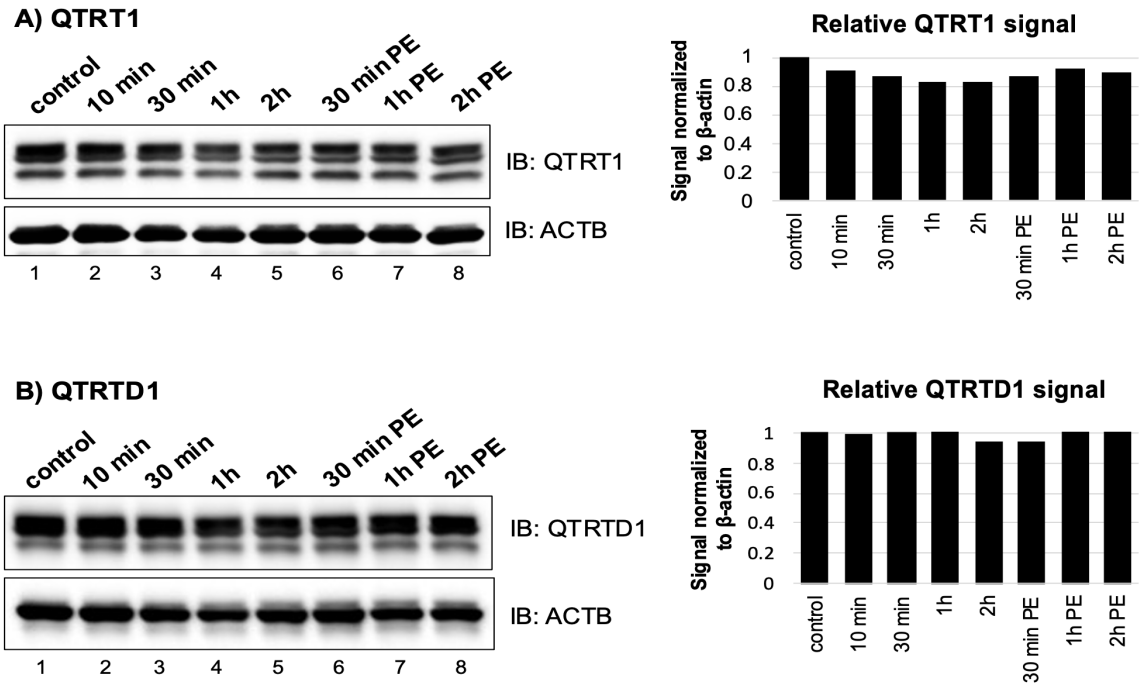


Figure S4. QTRT1 and QTRTD1 expression levels do not change in response to $NaAsO_2$. Western blot detection of QTRT1 (**A**) and QTRTD1 (**B**) expression in HepG2 cells during (10 min, 30 min, 1 h, 2 h) and post a 2 h-exposure (PE) (30 min, 1 h, 2 h) to an LD_{70} dose of $NaAsO_2$.

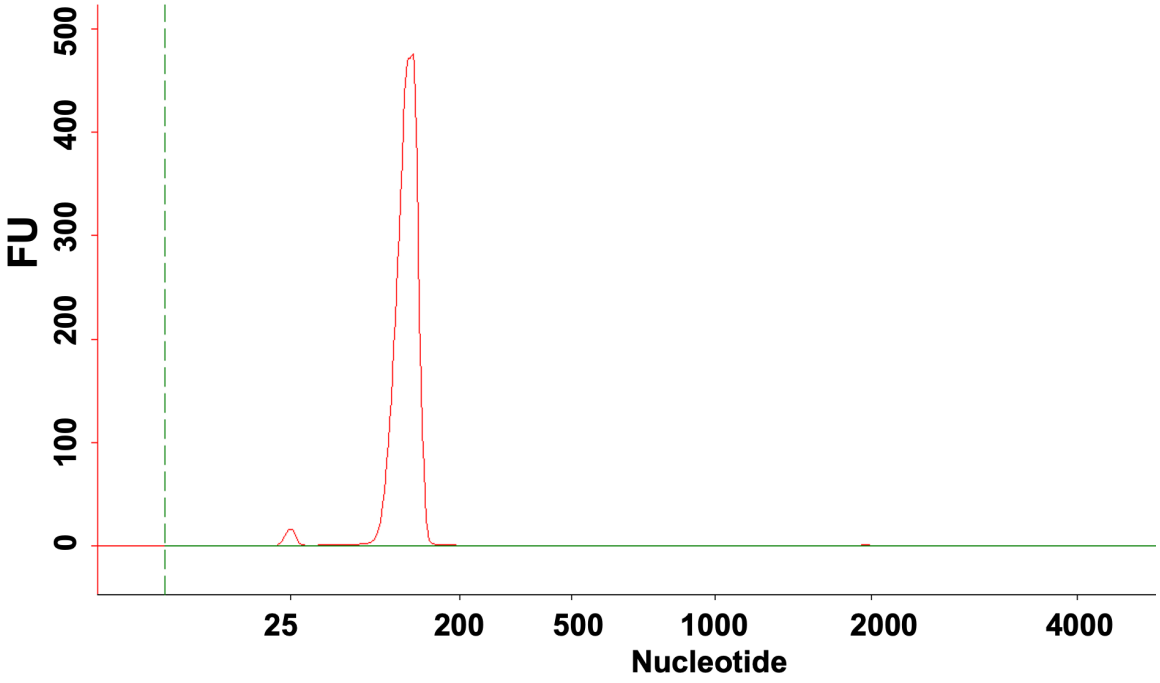


Figure S5: Representative bioanalyzer electropherogram of a tRNA fraction enriched by fractional precipitation of total RNA. The signal at 25 nt represents an internal size standard.

Table S1. STRING pathway analysis of proteins up-regulated in response to $NaAsO_2$. Proteins in bold font represent the glycolysis and pentose phosphate pathway enzymes selected for analysis.

KEGG description	Number Identified	Number in background	False discovery rate	Protein name
Carbon metabolism	9	116	3.19E-09	ADH5, **ALDOA**, **ENO1**, **GAPDH,** IDH1, **PGK1**, PHGDH, **TALDO1**,TPI1
Biosynthesis of amino acids	8	72	3.19E-09	**ALDOA, ENO1, GAPDH**, IDH1, **PGK1**, PHGDH, **TALDO1**, TPI1
Glycolysis/ Gluconeogenesis	7	68	3.27E-08	ADH5**, ALDOA**, **ENO1, GAPDH**, LDHA, **PGK1**, TPI1
Metabolic pathways	16	1250	1.85E-06	ADH5, **ALDOA**, ATIC, **ENO1**, FASN, **GAPDH**, GART, IDH1, LDHA, MTHFD1, NME1, PAICS, **PGK1**, PHGDH, **TALDO1**, TPI1
Antigen processing and presentation	5	66	2.40E-05	HSP90AA1, HSPA2, HSPA6, HSPA8, PSME2
Estrogen signaling pathway	6	133	2.83E-05	CALM1, HSP90AA1, HSPA2, HSPA6, HSPA8, KRT35
Protein processing in endoplasmic reticulum	6	161	7.04E-05	HSP90AA1, HSPA2, HSPA6, HSPA8, MBTPS1, SEC23A
HIF-1 signaling pathway	5	98	9.47E-05	**ALDOA, ENO1, GAPDH, LDHA, PGK1**

Table S2. STRING pathway analysis of proteins down-regulated in response to $NaAsO_2$

KEGG description	Number of identified genes	Number of genes in background	False discovery rate	Protein name
Ribosome	10	130	4.29E-11	RPL26, RPL31, RPL35, RPL6, RPLP0, RPS10, RPS16, RPS18, RPS2, RPS7
Oxidative phosphorylation	4	131	0.0063	ATP5B, ATP5D, ATP5E, ATP5G1
Metabolic pathways	10	1250	0.0063	ACAT1, ACSL4, APRT, ATP5B, ATP5D, ATP5E, ATP5G1, CES1, GLUD1, HSD17B2
Spliceosome	4	130	0.0063	DDX5, HNRNPC, RBMX, U2AF2
Thermogenesis	5	228	0.0063	ACSL4, ATP5B, ATP5D, ATP5E, ATP5G1
Alzheimer's disease	4	168	0.0063	ATP5B, ATP5D, ATP5E, ATP5G1
Parkinson's disease	4	142	0.0063	ATP5B, ATP5D, ATP5E, ATP5G1
Huntington's disease	4	193	0.0089	ATP5B, ATP5D, ATP5E, ATP5G1

Table S3. Isoacceptor and Total Codon Frequency data for genes encoding arsenite up-regulated glycolysis proteins. The data correspond to **Figure 5C**.

Isoacceptor Codon Frequencies							
Codon	***GAPDH***	***TALDO1***	***ENO1***	***PKM***	***ALDOA***	***TKT***	***PGK1***
AsnAAC	0.7895	0.7000	0.5833	0.5882	0.6875	0.6500	0.5217
AsnAAT	0.2105	0.3000	0.4167	0.4118	0.3125	0.3500	0.4783
AspGAC	0.6000	0.6667	0.5385	0.5172	0.6429	0.8000	0.5652
AspGAT	0.4000	0.3333	0.4615	0.4828	0.3571	0.2000	0.4348
HisCAC	0.4000	0.8333	0.5000	0.5000	0.6667	0.7059	0.6000
HisCAT	0.6000	0.1667	0.5000	0.5000	0.3333	0.2941	0.4000
TyrTAC	0.5556	0.7778	0.6364	0.8889	0.6154	0.7059	0.5000
TyrTAT	0.4444	0.2222	0.3636	0.1111	0.3846	0.2941	0.5000
Total Codon Frequencies							
Codon	***GAPDH***	***TALDO1***	***ENO1***	***PKM***	***ALDOA***	***TKT***	***PGK1***
AsnAAC	0.0446	0.0207	0.0322	0.0176	0.0263	0.0208	0.0287
AsnAAT	0.0119	0.0089	0.0230	0.0123	0.0119	0.0112	0.0263
AspGAC	0.0357	0.0414	0.0322	0.0265	0.0215	0.0449	0.0311
AspGAT	0.0238	0.0207	0.0276	0.0247	0.0119	0.0112	0.0239
HisCAC	0.0119	0.0148	0.0069	0.0106	0.0191	0.0192	0.0072
HisCAT	0.0179	0.0030	0.0069	0.0106	0.0095	0.0080	0.0048
TyrTAC	0.0149	0.0207	0.0161	0.0141	0.0191	0.0192	0.0048
TyrTAT	0.0119	0.0059	0.0092	0.0018	0.0119	0.0080	0.0048

Table S4: Composition of tRNA digestion enzyme master mix.

Reagent (stock concentration)	Amount added (µL)
$MgCl_2$ (100 mM)	2.5
Tris (1 M, pH 8)	5
Coformycin (0.1 mg/mL)	0.5
Deferoxamine (100 mM)	0.75
Butylated hydroxytoluene (BHT; 100 mM)	0.15
^{15}NdA (1 µM)	5
H_2O	0.85
Benzonase (5 U/µL)	2.25
Calf intestinal alkaline phosphatase (17 U/µL)	1.5
Phosphodiesterase (PDE) I (0.1 U/µL)	1.5

Table S5. Writer and target mRNA data

Gene	Fold-change (log_2)	p-value	p-adj
ALDOA	0.75	6.56E-02	2.81E-01
GAPDH	0.25	3.33E-01	6.61E-01
PGK1	0.18	4.10E-01	7.24E-01
ENO1	0.14	5.92E-01	8.33E-01
PKM	0.10	7.45E-01	9.06E-01
TKT	0.08	7.46E-01	9.06E-01
TALDO1	0.88	2.23E-03	2.96E-02
QTRT1	-0.29	2.68E-01	6.00E-01
QTRT2	0.02	9.27E-01	9.77E-01
ALKBH8	0.03	9.27E-01	9.77E-01
TRMO	0.78	1.46E-02	1.08E-01

Supplemental Extended Methods

Cell culture conditions. Hepatocellular carcinoma HepG2 cells were obtained from ATCC (HB-8065, Manassas, VA). The cells were cultured in a normal growth media (NGM) consisting of Dulbecco's modified Eagle's medium, low glucose with glutamine and pyruvate (Gibco, Waltham, MA), supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich, St. Louis, MO), 1X MEM non-essential amino acids (Gibco, Waltham, MA) and 1X penicillin-streptomycin solution (Gibco, Waltham, MA) at 37 °C in 5% CO_2 atmosphere. Q-depleted HepG2 cells were generated by culturing the cells in medium in which the 10% FBS component was replaced with dialyzed fetal bovine serum (dFBS) at 10%, with growth occurring for at least 10 passages while maintaining the cells at 70-80% confluency. Cells were also grown in dFBS supplemented with 1 μM queuine hydrochloride (Toronto Research Chemicals, North York, ON) (dFBS+Q-base) for 24 h prior to harvesting the cells. During treatment and replacement of growth media, the cells remained in their designated cell culture medium (NGM, dFBS or dFBS+Q-base). Mitochondria from HepG2 cells were purified using the Qproteome Mitochondria Isolation kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Briefly, HepG2 cells (75% confluent 150 mm dish) were washed with 0.9% NaCl solution (1 ml) and resuspended in ice-cold lysis buffer (1 ml). After incubation on an end-over-end shaker for 10 min at 4 ºC, the lysate was centrifuged at 1000 x g for 10 min at 4 ºC. The resulting pellet was washed one more time with lysis buffer before it was disrupted in ice-cold Disruption buffer (1.5 ml) and centrifuged at 1000 x g for 10 min at 4 ºC. The supernatant was transferred to a clean tube and centrifuged at 6000 x g for 10 min at 4 ºC to pellet the mitochondria. Mitochondria were further purified using the provided Mitochondria purification buffer according to the manufacturer's instructions before they were resuspended in TRI-Reagent (500 µl) for total RNA isolation.

Proliferation/MTT assay and dose-response curves. HepG2 cells grown in cell culture medium (NGM, dFBS or dFBS + Q-base) were seeded in 96-well plates at a density of $1.8x10^4$ cells per well and incubated at 37 °C in 5% CO_2 atmosphere. To determine the dose-response curves, cells were treated with various concentrations of indicated toxicant, sodium arsenite ($NaAsO_2$), hydrogen peroxide (H_2O_2), t-butylhydroperoxide (TBOOH), methyl methansulfonate (MMS), ethyl methansulfonate (EMS), iso-propyl methanesulfonate (IMS), and N-Nitrosodiethylamine (NDEA) (Sigma-Aldrich, St. Louis, MO) for 2 h. Ionizing radiation (γ-radiation; Rad) was delivered by an X-ray generator (CP160 Faxitron, Tucson, AZ) at a dose rate of 1.48 Gy per min. After all treatments, the medium was replaced with fresh growth medium and, at specified times after treatment, cells were analyzed using the MTT assay. Briefly, 10 μL of 3,[4,5-dimethylthiazol-2-yl-]diphenyltetrazolium bromide (MTT; 5 mg/ml; Sigma-Aldrich, St. Louis, MO) was added to each well containing a volume of 100 μL of growth medium and the plates incubated for 4 h at 37 °C in 5% CO_2 atmosphere. Medium and dye were removed and 200 μL of DMSO was added to each well to dissolve the formazan salts. Plates were covered and incubated on an orbital shaker for 30 min at ambient temperature. Absorbance at 570 nm was measured using a Synergy H1 Microplate reader (Tecan, Maennedorf, Zurich). Dose-response curves were plotted using Prism software (GraphPad Prism 6 software, San Diego, CA) and a non-linear curve fit. Data for LD_{20} and LD_{50} doses are noted in **Dataset S1**. Student T-tests were performed using GraphPad Prism 6 software.

RNA extraction and purification. Total RNA was extracted from HepG2 cells using TRIzol reagent (ThermoFisher Scientific, Waltham, MA) according to the manufacturer's instructions. Briefly, approximately 15 million cells for each extraction were lysed and homogenized in 3 ml of TRIzol reagent. 0.2 mL of chloroform (Fisher Scientific, Waltham, MA) per 1 mL of TRIzol reagent used for lysis was added to the sample and vortexed for 15 s. Sample was incubated for 3 min prior to centrifugation at 12,000 x g for 15 min at 4 °C. The aqueous phase was transferred to a new Eppendorf tube and 0.5 mL of Isopropanol (Fisher Scientific, Waltham, MA) per 1 mL of TRIzol reagent used for lysis was added and mixed gently by inverting the tube several times. Sample was incubated at ambient temperature for 10 min prior to centrifugation at 12,000 x g for 10 min at 4 °C. Supernatant was discarded and RNA pellet washed with 75 % (v/v) ethanol and centrifuged at 7,500 x g for 10 min at 4 °C. Supernatant was removed and RNA was air dried and

then resuspended in an appropriate amount of RNAse-free water (Hyclone, Marlborough, MA). Samples were flash-frozen in liquid nitrogen and stored at -80 °C.

tRNA purification and sample preparation. Purification of tRNA from total RNA was carried out by fractional precipitation using the Quick-RNA MiniPrep kit (Zymo Research, Irvine, California) according to the manufacturer's instructions. Briefly, total RNA (100 µg) in water (100 µL) was mixed with 2 volumes of a 1:1 mixture of RNA Lysis Buffer and ethanol (100%). The resulting mixture was loaded onto a Zymo-Spin column and centrifuged for 30 s at 16,000 x g to remove RNAs >200 nt. The flow-through was mixed with 1 volume of ethanol (100%) and transferred to a new column on which the tRNA-enriched fraction was captured by centrifuging for 30 s at 16,000 x g. The column was washed once with RNA Prep Buffer (400 µl) and twice with RNA Wash Buffer (500 µL) by centrifuging at 16,000 x g for 1 min. The tRNA-enriched fraction was then eluted by the addition of water (30 µL) and centrifugation at 16,000 x g for 1 min. RNA concentration was determined using a NanoDrop 1000 spectrophotometer (ThermoFisher Scientific, Waltham, MA) and the purity of the fraction was assessed using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) (**Supplementary Fig. S5**). tRNA hydrolysis to ribonucleosides was achieved by mixing tRNA (6 µg in 30 µL H_2O) with digestion enzyme master mix (20 µL; **Supplementary Table S4**) and incubating the mixture at 37 °C for 6 h. Enzymes were subsequently removed by filtration through 10 kDa MWCO spin columns (VWR, Radnor, PA) before concentrating the ribonucleoside mixture to dryness a SC110 SpeedVac (ThermoFisher Scientific, Waltham, MA).

LC-MS/MS analysis of modified ribonucleosides. Dry ribonucleoside mixtures were re-dissolved in 5 mM ammonium acetate (pH 5.3, 25 µL) and resolved on a Synergi Fusion reversed-phase HPLC column (100x2 mm, 2.5 µm particle size, 100 Å pore size; Phenomenex, Torrance, CA). The following gradient of acetonitrile in 5 mM ammonium acetate (pH 5.3) at a flow rate of 0.35 ml/min and 35 °C was used for elution: 0-1 min, 0%; 1-10 min, 0-10%, 10-14 min, 10-40%, 14-15min, 40-80%, with an injection volume of 10 µL. The HPLC column was coupled to a 6490 Triple Quadrupole LC/MS spectrometer (Agilent Technologies, Santa Clara, CA) with an electrospray ionization source that was operated in positive ion mode with the following parameters for voltages and source gas: gas temperature, 200 °C; gas flow, 11 L/min; nebulizer, 20 psi; capillary voltage, 1800 V; sheath gas heater, 300; and sheath gas flow, 12. The first and third quadrupoles (Q1 and Q3) were fixed to unit resolution and the modifications were quantified by pre-determined molecular transitions. The dwell time was 500 ms, the fragmentor voltage was 380 V, and the cell accelerator voltage was 7 V for each nucleoside. The retention time, *m/z* of the transmitted parent ion, *m/z* of the monitored product ion, and collision energy of each modified nucleoside are as follow: ac^4C, 7.25 min, *m/z* 286 → 154, 4 V; acp^3U, 2.2 min, *m/z* 346 → 214, 10 V; Cm, 4.6 min, *m/z* 258 → 112, 4 V; D, 1.5 min, *m/z* 247 → 115, 4 V; Gm, 7.0 min, *m/z* 298 → 152, 6 V; I, 5.0 min, *m/z* 269 → 137, 4 V; i^6A, 8.1 min, *m/z* 336 → 204, 12 V; m^1A, 4.0 min, *m/z* 282 → 150, 16 V; m^1G, 6.9 min, *m/z* 298 → 166, 8 V; m^1I, 6.7 min, *m/z* 283 → 151, 6 V; $m^2{}_2G$, 8.7 min, *m/z* 312 → 180, 10 V; m^2G, 7.3 min, *m/z* 298 → 166, 6 V; m^3C, 3.7 min, *m/z* 258 → 126, 4 V; m^5C, 4.0 min, *m/z* 258 → 126, 10 V; m^5U, 5.3 min, *m/z* 259 → 127, 6 V; m^5Um, 8.0 min, *m/z* 273 → 127, 4 V; m^6t^6A, 10.4 min, *m/z* 427 → 295, 8 V; m^7G, 4.6 min, *m/z* 298 → 166, 10 V; mcm^5s^2U, 9.5 min, *m/z* 333 → 201, 4 V; mcm^5U, 8.0 min, *m/z* 317 → 185, 4 V; ms^2t^6A, 11.3 min, *m/z* 459 → 327, 10 V; Q, 6.3 min, *m/z* 410 → 295, 10 V; t^6A, 8.9 min, *m/z* 413 → 281, 8 V; Um, 6.0 min, *m/z* 259 → 113, 4 V; Y, 1.6 min, *m/z* 245 → 191, 8 V. Modified ribonucleosides were identified using synthetic standards or by HPLC retention time and collision-induced dissociation (CID) fragmentation patterns. Biological triplicates of both exposed and unexposed control cells were used for each toxicant. Mass spectrometric peak area for each modification was corrected for variation in the quantity of input tRNA by dividing the peak area by the total UV-Vis absorbance peak area (in-line Agilent were detector) for the four canonical ribonucleosides. Fold-change values for ribonucleosides (relative to the average of the control samples) were calculated for each dose and time point and significant changes were determined by Student's t-test ($p \leq 0.05$), with Heat maps generated using the $\log_2$ fold-change in GraphPad Prism 6 software. See **Dataset S1** for data.

Labeling mitochondria with MitoTracker Green. To label the mitochondria, HepG2 cells were grown in NGM, dFBS, and dFBS + Q-base and seeded in 12-well dishes at a density of $1.4x10^5$ per well and incubated in a humidified CO_2 incubator overnight. Cells were treated with an LD_{20} and LD_{50} of $NaAsO_2$ (Sigma-Aldrich, St. Louis, MO) in culture medium for 2 h, followed by replacement with fresh growth medium and continued incubation for 6 h. Growth medium was removed and staining solution (media without FBS) containing 200 nM of MitoTracker green FM (ThermoFisher Scientific, Waltham, MA) was added to the cells and incubated for a total of 30 min at 37 °C. After 20 min, 2 drops of Nuc Blue Live (ThermoFisher Scientific, Waltham, MA) per ml of media was added for 10 min to stain the nuclei. Cells were washed with PBS three times to remove excess stain and kept in PBS during microscopy. Cells were immediately imaged at either 20x or 40x magnification using the EVOS fluorescent Imaging System (ThermoFisher Scientific, Waltham, MA). MitoTracker green FM was detected with the GFP LED light cube and Nuc Blue Live Stain with the DAPI LED light cube. The fluorescence intensity was quantified using ImageJ software (National Institutes of Health, version 1). Student T-tests were performed using GraphPad Prism 6 software.

Analysis of mitochondrial function and energy metabolism. In order to assess mitochondrial function of live HepG2 cells and possible metabolic switching from mitochondrial respiration to glycolysis after treatment with $NaAsO_2$, we used the Seahorse XF24 extracellular flux analyzer kit (Agilent Technologies, Santa Clara, CA), similar to our previously reported study (2). Briefly, HepG2 cells were seeded at a concentration of 5×10^4 cells per well in a XF 24-well microplate overnight, allowing the cells to adhere. An XF sensor cartridge was hydrated with XF Calibrant overnight in a non-CO_2 incubator at 37 °C. After seeding (24 h), the cells were treated with LD_{20} and LD_{50} doses of $NaAsO_2$ for 2 h after which growth medium was replaced with 250 μL of fresh growth media and samples were incubated for 6 h. Assay medium was prepared according to manufacturer's instructions by supplementing Seahorse XF DMEM with 1 mM pyruvate, 2 mM glutamine and 10 mM glucose, and adjusted to pH 7.4. Cells were washed once with the assay medium and then 608 μL of assay media added to each well. The XF culture plate was equilibrated in non-CO_2 incubator at 37 °C for 1 h prior to the start of the assay. During this equilibration time, the assay compounds were prepared and added to the appropriate ports of the hydrated sensor cartridge: 4 μM oligomycin, an ATP synthase inhibitor; 5 μM FCCP (carbonyl cyanide-4-(trifluoromethoxyphenylhydrazone), positive control reagent that is an uncoupler of oxidative phosphorylation that depolarize the mitochondrial membrane and disrupts ATP synthesis, and 10 μM rotenone, a complex 1 inhibitor. The assay measures the real-time oxygen consumption rate (OCR) as an index of mitochondrial respiration and extracellular acidification rate (ECAR) as an index of glycolytic activity in HepG2 cells. Both assays were performed before and after injection with the three assay compounds. Using OCR and ECAR data points from the analysis, the cell energy phenotype was automatically generated using Wave software, which yielded an energy phenotype of the untreated versus treated HepG2 cells.

Measurement of intracellular ROS levels. To assess intracellular ROS levels of HepG2 cells grown in NGM, dFBS, and dFBS + Q-base in response to $NaAsO_2$ treatment, cells were seeded in 6-well dishes at a density of 7.2×10^5 per well and incubated in a humidified CO_2 incubator overnight. Cells were treated with LD_{20} and LD_{50} doses of $NaAsO_2$ for 2 h, followed by replacement with fresh growth media and incubation for 24 h. CellROX Orange Reagent (ThermoFisher Scientific, Waltham, MA) was then added directly to the cells at a concentration of 5 μM and incubated in a humidified CO_2 incubator at 37 °C for 20 min, followed by addition of 2 drops of Nuc Blue Live Stain (ThermoFisher Scientific, Waltham, MA) per ml of media and incubation for an additional 10 min to stain the nuclei. Cells were washed with PBS three times to remove excess stain and kept in PBS during microscopy. Cells were imaged at 20X magnification using the EVOS fluorescent Imaging System (ThermoFisher Scientific, Waltham, MA). CellRox Orange was detected with the RFP LED light cube and Nuc Blue Live Stain with the DAPI LED light cube. The fluorescence intensity was quantified using ImageJ software (National Institutes of Health, version 1). Student T-tests were performed using GraphPad Prism 6 software.

mRNA analysis. Cells were grown in normal growth medium (NGM) and were seeded at 3.5 x 10^6 cells in a 100 mm dish and incubated overnight at 37°C in 5% CO_2. Post overnight incubation, cells were treated with an LD_{20} dose (0.12 mM) of $NaAsO_2$ for 2 h. Next, media was exchanged and cells were further incubated for 6 h, and RNA was isolated using the Trizol-chloroform (Thermo Fisher, Waltham, MA catalog no. 15596026) protocol. The sequencing library was prepared using the NEBNext Poly(A) mRNA Magnetic isolation kit (New England Biolabs, Ipswich, MA, catalog no. E7490L) by following the manufacture supplied instructions. Sequencing was performed on the Illumina HiSeq platform at GeneWiz (Chelmsford, MA). The data quality was analyzed using the Phred quality score with the baseline set at Q30. The indexed human genome was acquired from Biostars (3). The reads were aligned using STAR (4). The differential gene expression (DGE) analysis was performed using DESeq2 with default parameters (5). The DGE analysis was visualized using R studio (http://www.rstudio.com/). The up- and down-regulated gene clusters were plotted as heatmaps using the Morpheus software package (https://software.broadinstitute.org/morpheus).

Polysome analysis. Sucrose gradients were prepared by filling a 14x95 mm polypropylene centrifuge tube to a height of 45 mm with 10% sucrose solution composed of 300 mM NaCl, 15 mM $MgCl_2$, 15 mM Tris-HCl pH 7.5, 0.1 mg/mL cycloheximide (~6 mL). Next, a 50% sucrose solution composed of 300 mM NaCl, 15 mM $MgCl_2$, 15 mM Tris-HCl pH 7.5, 0.1 mg/ml cycloheximide was gently added to the bottom of the ultracentrifuge tube slowly pushing the 10% sucrose solution upwards until to solution reached a height of 90 mm (~6 mL). These ultracentrifuge tubes were then put onto the Gradient Master 108 by Biocomp to mix the gradients and stored at 4 °C. After the cells were exposed to their experimental conditions, 100 µL of 10 mg/ml of cycloheximide was added to 10 mL of cell media. Cells were incubated for 3 min and then immediately put on ice. The plates were washed three times using 5 ml of 0.1 mg/mL cycloheximide in PBS. After the wash, 400 µl of cell lysis buffer (300 mM NaCl, 15 mM $MgCl_2$, 15mM Tris-HCl pH 7.5, 0.1 mg/ml cycloheximide, 1% Triton-X) was added and samples incubated for 10 min. The cells were removed from the plate using a cell scraper. The lysate solution was then added to a centrifuge tube and incubated on ice for 10 min. Next, samples were centrifuged at 4 °C for 10 min at 10,000 rpm (7,056 RCF). Then, 500 µL of cell lysate was laid on top of the previously made sucrose gradients. These were then placed in ultracentrifuge tube holders and lysis solution was added until the weights were equal. Samples were then centrifuged at 35,000 rpm (104,456 RCF) and 4 °C for 2.75 h. After centrifugation, samples were fractionated and analyzed using the ISCO density gradient fractionator model 185 and Teledyne ISCO UA-6 UV/VIS detector respectively (Teledyne Technologies International, Thousand Oaks, CA). The needle was assembled and attached to the syringe. A 60 % sucrose gradient was added to the syringe. Air was removed from this system before the needle was inserted through the bottom of sample's polypropylene tube. Next, 60 % sucrose solution was applied at a speed of 0.75 mL/min through the needle, gently pushing the sample upwards into the gradient fractionator with tubes collection fractions of 0.75 ml. Polysome profiles were collected using ISCO UA-6 UV-vis detector and Windaq software. These profiles begin as soon as the UV-vis detector senses the sample in the detector. Polysome profiles were scaled to the 80S peak and zeroed to the smallest detected value from the polysome (found between the 80S peak and the 1st polysome peak).

Proteomics. Biological triplicates of $NaAsO_2$-exposed and control HepG2 cells were lysed on ice using cold urea lysis buffer (1 mL per 15 cm dish; 8 M urea and 1 mM sodium orthovanadate, Na_3VO_4) by pipetting up and down. The protein concentration was subsequently determined using a BCA assay. In preparation for LC-MS, protein disulfides were reduced by adding DTT to a final concentration of 10 mM and incubating at 56 °C for 1 h. Samples were cooled to 23 °C and alkylated using iodoacetamide at a final concentration of 55 mM for 1 h in the dark while rotating. Ammonium bicarbonate (NH_4HCO_3) (pH 8) was then added to the samples to dilute urea to a final concentration of <1 M. Protein was then digested using trypsin (Promega, Madison, WI) at an enzyme-to-protein ration of 1:50 by rotating overnight at ambient temperature. After digestion, formic acid was added to a final concentration of 5 % and samples were desalted using Pierce Peptide desalting spin columns (ThermoFisher Scientific, Waltham, MA*)* according to the

manufacturer's instructions. Desalted peptide solutions were divided into 400 µg aliquots, concentrated to ~1-5 µL, and frozen at -80 °C. Peptide digests were labeled with TMT-6plex reagents (ThermoFisher Scientific, Waltham, MA) as follows: TMT-126 – control 1; TMT-127 – control 2; TMT-128 – control 3; TMT-129 – $NaAsO_2$-treated 1; TMT-130 - $NaAsO_2$-treated 2; TMT-131 - $NaAsO_2$-treated 3. The samples were dissolved in 150 mM triethylammonium bicarbonate (TEAB, 100 µL) by vortexing for 1 min and subsequent centrifugation for 1 min at 15,500 x g. Anhydrous acetonitrile (30 µl) was added to each TMT tube followed by vortexing for 1 min and centrifugation for 1 min at 15,500 x g. The reconstituted TMT reagents were then transferred to the appropriate sample tubes, vortexed for 1 min and centrifuged for 1 min at 15,500 x g. The samples were then incubated at room temperature for 1 h. After TMT-labelling the samples were combined and dried by vacuum centrifugation. Dried peptides were resuspended in 50 µL 0.1% formic acid and 6 µL were loaded on a precolumn and separated by reverse-phase HPLC using an EASY-nLC1000 (ThermoFisher Scientific, Waltham, MA) over 140 min at 200 nL/min with a gradient at 5 min from 10-30% Buffer B (80% acetonitrile, 0.1% formic acid) for 100 min, followed by a 14 min gradient up to 40% B, 5 min to 60% B and 2 min to 100% B where the buffer was held for 10 min before a re-equilibration at 0% B. The eluent was directly injected into an Orbitrap QExactive mass spectrometer (ThermoFisher Scientific, Waltham, MA) with electrospray ionization and operated in a data-dependent mode. The parameters for the full scan MS: resolution of 70,000 across 350-2000 *m/z*, AGC target at $3e^6$, and maximum IT 50 ms. The full MS scan was followed by MS/MS for the top 10 precursor ions in each cycle with an NCE of 32 and dynamic exclusion of 30 s. Resolution was set at 35,000, AGC $1e^5$, maximum IT 300 ms, isolation window 2 *m/z*, and minimum AGC target at $1e^3$. Raw mass spectral data files (.raw) were searched using Proteome Discoverer 2.1 (ThermoFisher Scientific, Waltham, MA) and Mascot version 2.4.1 (Matrix Science) against the human SwissProt database. Mascot search parameters were: 15 ppm mass tolerance for precursor ions; 0.015 Da fragment ion mass tolerance; 2 missed cleavages of trypsin; fixed modifications were carbamidomethylation of cysteine and TMT 6plex modification of lysines and peptide N-termini; variable modifications were methionine oxidation, tyrosine phosphorylation, and serine/threonine phosphorylation. Only the peptides with a Mascot score greater than or equal to 25 and an isolation interference less than or equal to 30 were included in the quantitative data analysis. TMT quantification was obtained using Proteome Discoverer and isotopically corrected as per manufacturer's instructions. The TMT values were normalized to the mean relative protein quantification ratios obtained from a total protein analysis. Without fractionation, we were able to identify 544 proteins, 286 of which satisfied the filtering criteria. Proteomics data has been submitted to the PRIDE repository under submission number 1-20211230-6555 on 30 Dec 2021.

***Codon analytics*.** Human cDNAs, which in some cases included multiple splice variants, for all known proteins were identified in GenBank and parsed to contain the complete coding sequence from start to stop codon. The resulting sequences were analyzed for codon isoacceptor frequency using the previously described Gene Specific Codon Usage (GCSU) algorithm.(6-9) Briefly, each of the 64 codons was counted for each sequence. The resulting gene sequence specific codon count data was used to determine the isoacceptor codon frequencies for each amino acid, with the summed frequency for each of the codons specific to each amino acid adding to 1.0, and the sum of the 21 isoacceptor codon frequencies in a gene adding to 21.0. The total codon frequency for each codon in a gene was also analyzed using GSCU. Total codon frequency measurers were determined by dividing the number of times a specific codon was found in a gene by the total number of codons in the gene, with the sum of all total codon frequencies in a gene adding to 1.0. Statistical analysis of specific gene lists to identify codon bias was determined using the t-statistic.

Supplementary References

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