

Supplemental Data

Supplementary Table S1. Supplements and growth factors used during differentiation of human iPS cells into motor neurons

Human iPS cells to NEPs (day 0 to day 6)			
Growth factor	Concentration	Role	Source
CHIR99021	3 μ M	Wnt signaling activator	StemCell
SB431542	2 μ M	TGF- β /Activin/NODAL signaling inhibitor	Sigma Aldrich
DMH-1	2 μ M	BMP signaling inhibitor	Tocris
NEPs to MNPs (day 6 to day 12)			
Growth factor	Concentration	Role	Source
CHIR99021	1 μ M	Wnt signaling activator	StemCell
SB431542	2 μ M	TGF- β /Activin/NODAL signaling inhibitor	Sigma Aldrich
DMH-1	2 μ M	BMP signaling inhibitor	Tocris
Retinoic acid	0.1 μ M	Nuclear receptors RARs activator	Acros Organics
Purmorphamine	0.5 μ M	SHH signaling agonist	Millipore Sigma
MNPs to early MNs (day 12 to day 18)			
Growth factor	Concentration	Role	Source
Retinoic acid	0.5 μ M	Nuclear receptors RARs activator	Acros Organics
Purmorphamine	0.1 μ M	SHH signaling agonist	Millipore Sigma
Early MNs to mature MNs (day 18 to day 28)			

Growth factor	Concentration	Role	Source
Retinoic acid	0.5 μ M	Nuclear receptors RARs activator	Acros Organics
Purmorphamine	0.1 μ M	SHH signaling agonist	Millipore Sigma
Compound E	0.1 μ M	Notch signaling inhibitor	Millipore Sigma
IGF-1	10 ng/ml	PI3K/Akt and Ras/ERK pathway activator	Sigma Aldrich
BNDF	10 ng/ml	TrkB tyrosine kinase receptor activator	R&D systems
CNTF	10 ng/ml	Neurotrophic and survival factor for embryonic motor neurons	R&D systems

Supplementary Table S2. Primer sequences of genes quantified by qPCR

Gene Name	Primer Sequence	
	Forward (5'-3')	Reverse (5'-3')
<i>GAPDH</i>	CCCTTCATTGACCTCAACTACA	ATGACAAGCTTCCCGTTCTC
<i>β2-MICROGLOBULIN</i>	CCAGCGTACTCCAAAGATTCA	TGGATGAAACCCAGACACATAG
<i>SOX2</i>	GCTACAGCATGATGCAGGACCA	TCTGCGAGCTGGTCATGGAGTT
<i>POU5F14</i>	CCTGAAGCAGAAGAGGATCAC	AGATGGTCGTTTGGCTGAATA
<i>NANOG</i>	TGAAATCTAAGAGGTGGCAGAA	CCTGGTGGTAGGAAGAGTAAAG
<i>SOX1</i>	AATGTAGTAAGGCAGGTCCAAG	GGTGGTGGTGGTAATCTCTTT
<i>NES</i>	CACTTCAGTTTAGAGGCTAAGG	CCCTCTATGGCTGTTTCTTTCT
<i>OLIG2</i>	CAGAAGCGCTGATGGTCATA	CTCCCAAATCAACGAGAGACA
<i>CHAT</i>	GGAGTAAGAAAGCAACCAGAGA	CACAAAGAAGGGAGACCTACAG

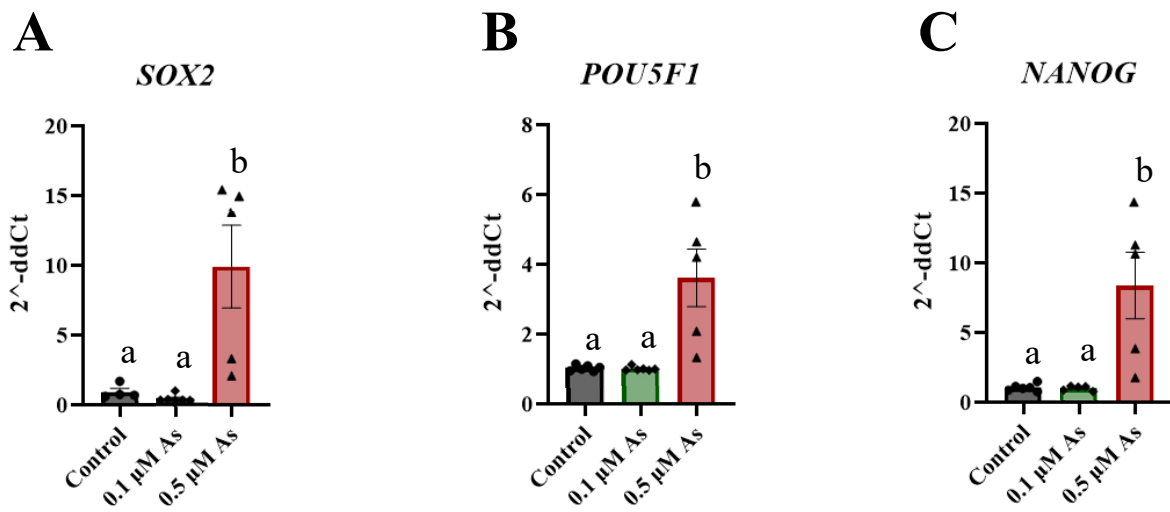
Supplementary Table S3. Correlation between transcript levels of *SOX2*, *POU5F1* and *NANOG* in human iPS cells exposed to 0, 0.1 or 0.5 μ M arsenic for six days.

Correlation			
	<i>SOX2</i>	<i>POU5F1</i>	<i>NANOG</i>
<i>SOX2</i>	1	0.94	0.96
<i>POU5F1</i>	0.94	1	0.99
<i>NANOG</i>	0.96	0.99	1

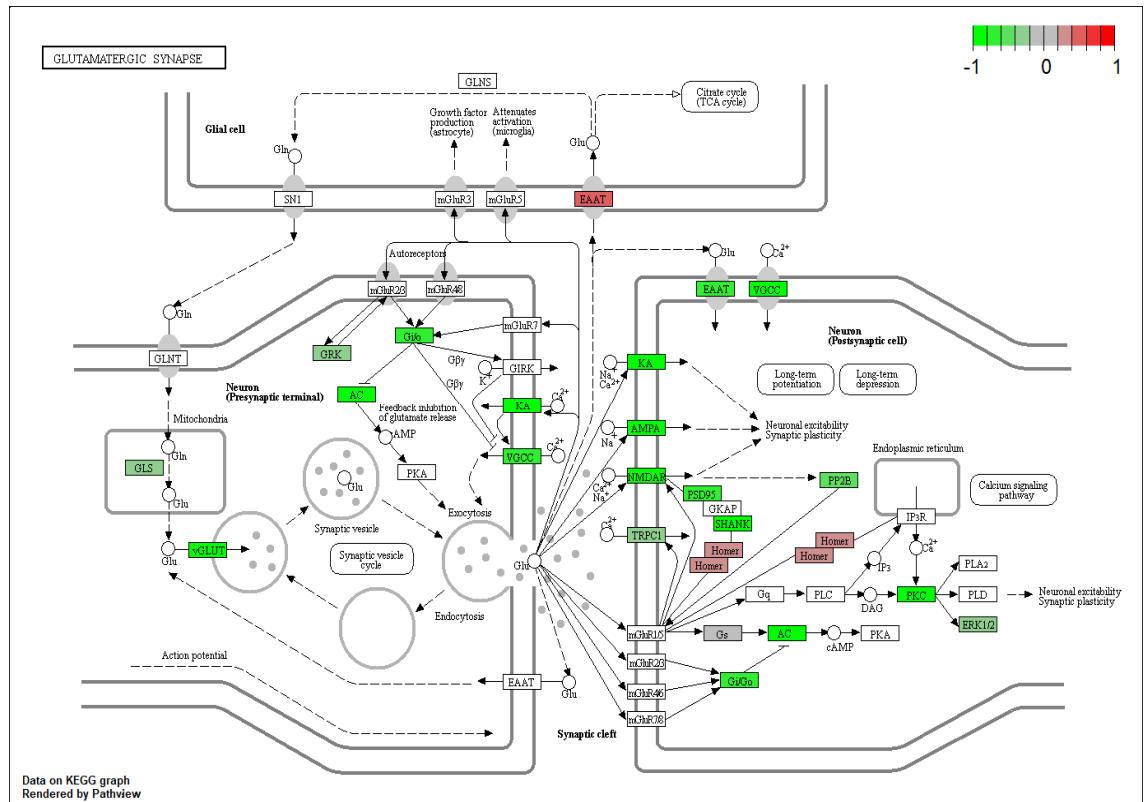
Supplementary Table S4. Correlation between transcript levels of *SOX2*, *SOX1* and *NES* in day 6 NEPs exposed to 0, 0.25, 0.5 and 0.75 μ M.

Correlation			
	<i>SOX1</i>	<i>SOX2</i>	<i>NES</i>
<i>SOX1</i>	1	0.78	0.78
<i>SOX2</i>	0.78	1	0.76
<i>NES</i>	0.78	0.76	1

Supplementary Figure S1. Arsenic increases transcript levels of pluripotency markers *SOX2*, *POU5F1* and *NANOG* in human iPS cells. Transcript levels of *SOX2* (A), *POU5F1* (B) and *NANOG* (C) were assessed by qPCR in human iPS cells exposed to 0, 0.1 or 0.5 μ M arsenic for six days. Fold change was determined using the ddCt method and results were normalized to the geometric mean of Gapdh and β 2-microglobulin. Statistical differences were determined using ANOVA followed by Tukey's multiple comparison test (*; $p \leq 0.05$) (n = 5-6 per exposure group).



C



Supplementary Figure S3. Arsenic exposure impairs ChAT protein levels in adult mice hippocampi. (A)

Representative images of 3D z-stacked images of MAP2 (yellow) and ChAT (blue) in hippocampi harvested from adult mice exposed to 100 ppb of arsenic for five weeks. (B) Relative fluorescence of MAP2 (left) and ChAT (right) was determined in ImageJ and is presented as integrated density value (IDV) \pm SE (n = 3-4 per exposure group). Statistical differences were determined using Student's t-test (*; $p \leq 0.05$).

