Supplemental Material

Association between Arsenic Suppression of Adipogenesis and Induction of CHOP10 via the Endoplasmic Reticulum Stress Response

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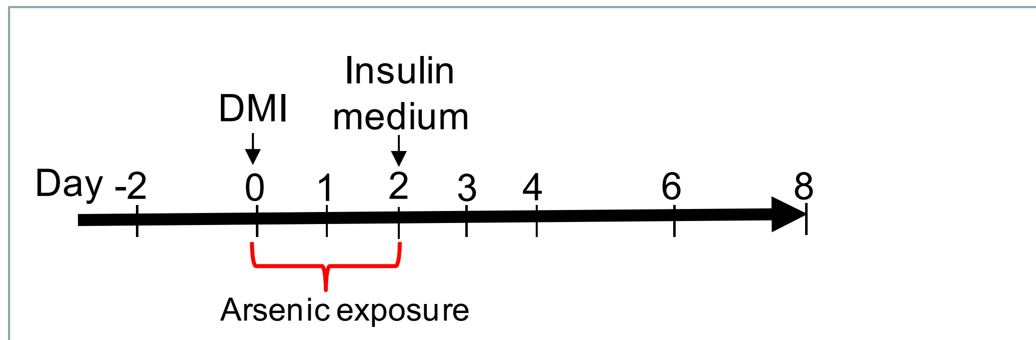
Methods

Immunofluorescence microscopy. Briefly, 3T3-L1 preadipocytes were plated onto coverslips in 6-well plates and induced to differentiate using the DMI protocol 1 day after 100% confluence as achieved in the presence or absence of arsenic. At 16 h, cell monolayers were washed with cold phosphate buffered saline (PBS) and fixed for 10 min at room temperature in 2% (v/v) formaldehyde. After washing in PBS, cells were permeabilized in 1% (v/v) Triton X-100 in PBS, washed and incubated with 10% goat serum (sc-2043, Santa Cruz) in PBS for 1 h at room temperature. Cells were incubated with mouse monoclonal IgG_{2a} against C/EBPβ (sc-7962; 1:500, Santa Cruz) in 10% goat serum in PBS for 16 h at 4°C and subsequently with Rhodamine-linked goat-anti-mouse IgG-R (sc-2092, 1:50, Santa Cruz) for 45 min at room temperature. After PBS washing, the coverslips were mounted with the Prolong Gold antifade reagent with DAPI (P36931, Invitrogen) onto microscope slides and examined using an Axio Observer Z1 fluorescence microscope (Carl Zeiss, Inc. Oberkochen, Germany). Image analysis on the number and intensity of fluorescent aggregation was performed using a wavelet-based, Microsoft Windows-compatible computer program developed by Larry S. Barak at Duke University (Kapur et al. 2009).

Table S1. Primer sequences for Real-time RT-qPCR

Abbreviations	Gene name	Forward (5' - 3')	Reverse (5' - 3')
18S	mouse 18S ribosomal RNA	CGAACGTCTGCCCTATCAACTT	CCGGAATCGAACCCTGATT
Adrp	mouse Adipose differentiation-related protein	GGTGATGGCAGGCGACAT	CCATCGGACACTTCCTTAAAGG
Atf4	mouse Activating transcription factor 4	CTCAGACAGTGAACCCAATTGG	GGCAACCTGGTCGACTTTTATT
Adiposin	mouse Adiposin	GCTATCCCAGAATGCCTCGTT	TTCCACTTCTTTGTCCTCGTATTG
Cd36	mouse Cluster of differentiation 36	CAGAGTTCGTTATCTAGCCAAGGAA	CATTGGGCTGTACAAAAGACACA
Cebpα	mouse CCAAT-enhancer-binding protein α	CGCAAGAGCCGAGATAAAGC	CGGTCATTGTCACTGGTCAACT
Cebpβ	mouse CCAAT-enhancer-binding protein β	AAGCTGAGCGACGAGTACAAGA	GTCAGCTCCAGCACCTTGTG
Cebpδ	mouse CCAAT-enhancer-binding protein δ	GCCGTGCCCACCCTAGA	CGCTTTGTGGTTGCTGTTGA
Cebp ε	mouse CCAAT/enhancer binding protein ε	CGGCCATTCGCCTATCC	GCCCCAAAGCCTTCCTATCT
Chop10	mouse C/EBP homologous protein	CCTTTATTCTGCGGAGCTGAA	AGGGCCACAGAAGGATGGA
Gadd45a	mouse growth arrest and DNA-damage-inducible 45 α	GACGACGACCGGGATGTG	AGCAGAACGCACGGATGAG
Lpl	mouse lipoprotein lipase	GGACTGAGGATGGCAAGCAA	GCCACTGTGCCGTACAGAGA
Pparγ1	mouse Peroxisome proliferator-activated receptor γ1	GGGCTGAGGAGAAGTCACAC	TGGTTCACCGCTTCTTTCA
Pparγ2	mouse Peroxisome proliferator-activated receptor γ2	TGCTGTTATGGGTGAAACTCTG	CTGTGTCAACCATGGTAATTTCTT
Rxr α	mouse retinoid X receptor α	GCCGGCCTCTGACTGTGA	GCACCACAATGTCCCAGTGA
Srebp1	mouse Sterol regulatory element-binding protein 1	AGCCCACAATGCCATTGAG	CAGGTCTTTGAGCTCCACAATCT
sXbp1	mouse spliced X-box binding protein 1	GAGTCCGCAGCAGGTG	GTGTCAGAGTCCATGGGA
Xbp1	mouse X-box binding protein 1	ACGGCCTTGTGGTTGAGAAC	AGGATCCAGCGTGTCCATTC
LPL	human lipoprotein lipase	TCCGCGTGATTGCAGAGA	GCTCGTGGGAGCACTTCACT
CD36	human Cluster of differentiation 36	AGAGTTCGTTTTCTAGCCAAGGAA	TTGGGCTGCAGGAAAGAGACT
PPAR1	human Peroxisome proliferator-activated receptor γ1	GGCCGCAGATTTGAAAGAAG	TCGTTTGAGAAAATGGCCTTGT
PPAR2	human Peroxisome proliferator-activated receptor γ2	CAAACCCCTATTCCATGCTGTT	GCTTTCTGGGTCAATAGGAGAATC
18S	human 18S ribosomal RNA	CGCCCCAGCACTTTGG	TTACCAGCGGATGGATGGA

DMI protocol for 3T3-L1 cells:



DMIRI protocol for mouse ADSVFCs and human ADSCs:

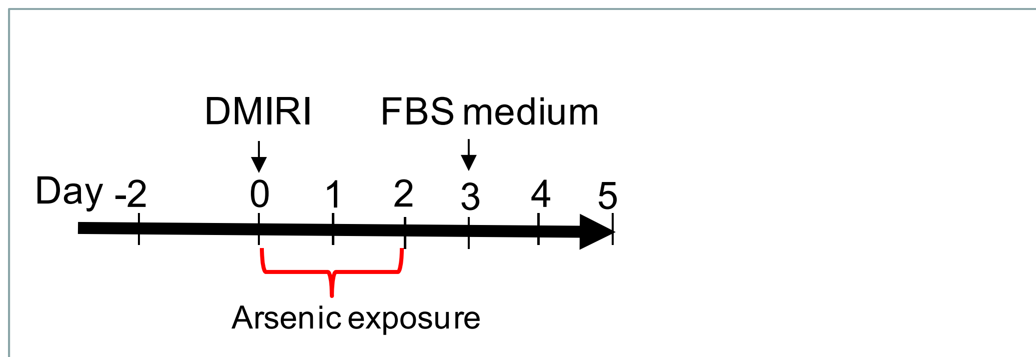


Figure S1. Protocols for adipogenic differentiation and arsenic exposure in 3T3-L1 cells, mouse ADSVFCs and human ADSCs. Cells were differentiated 1 day after confluence (designated as day 0) by replacing growth medium with differentiation medium as indicated (Black arrows). DMI: 1 μM dexamethasone, 0.5 mM IBMX and 1 μg/ml insulin in DMEM with 10% FBS; Insulin medium, 1 μg/ml insulin in DMEM with 10% FBS; DMIRI: DMI + 1 μM rosiglitazone and 125 μM indomethacin in DMEM with 10% FBS; FBS medium, DMEM with 10% FBS. Arsenicals were added during the first two days of adipogenic differentiation.

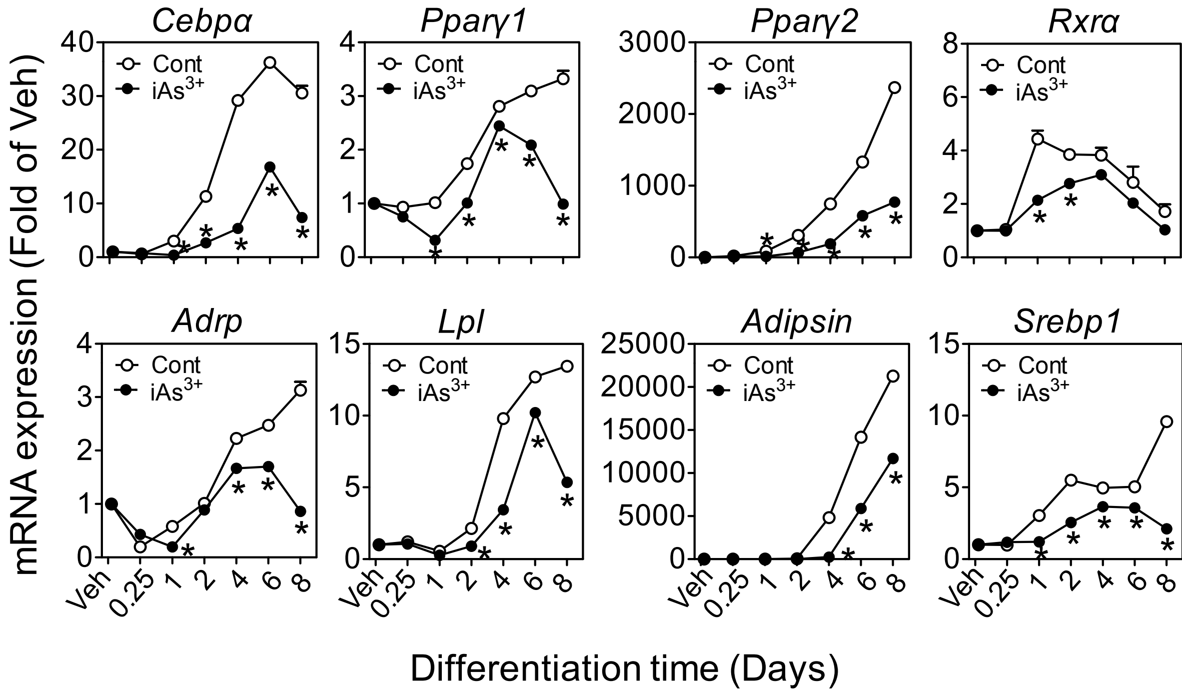


Figure S2. Inhibitory effect of iAs^{3+} exposure on mRNA expression of adipogenic genes during adipogenesis induced by hormonal cocktail DMI. Cells were differentiated with DMI protocol alone (Control, Cont) or with 5 μM iAs^{3+} added during the first 48 h of differentiation. Vehicle (Veh) indicates the 3T3-L1 cells without DMI treatment, * $p < 0.05$ iAs^{3+}-treated vs. Cont with the same treatment.

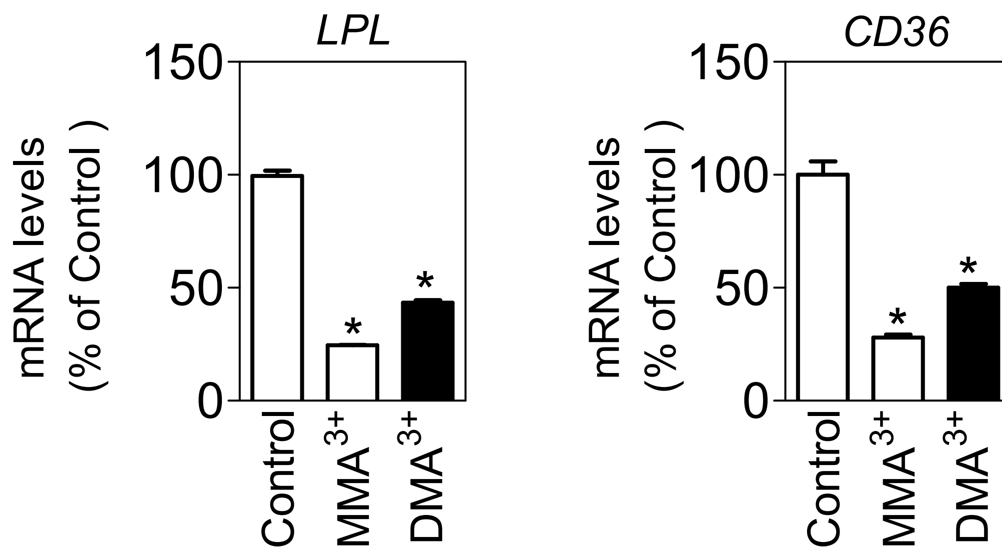


Figure S3. MMA^{3+} and DMA^{3+} suppress adipogenic gene expression in human ADSCs. The cells were cultured to 95% confluence and differentiated for 5 days using DMIRI protocol, followed by an immediate measurement of mRNA expression. DMIRI protocol, cells were differentiated by replacing growth medium with DMIRI differentiation medium containing 1 μM dexamethasone, 0.5 mM IBMX, 1 μg/ml insulin, 1 μM rosiglitazone and 125 μM indomethacin in DMEM with 10% FBS. After three days the medium was changed to DMEM with 10% FBS and maintained for 2 additional days. MMA^{3+} (0.2 μM) or DMA^{3+} (2 μM) was added during Day 1 and 2 of differentiation. * $p < 0.05$ Arsenic-treated vs. Control.

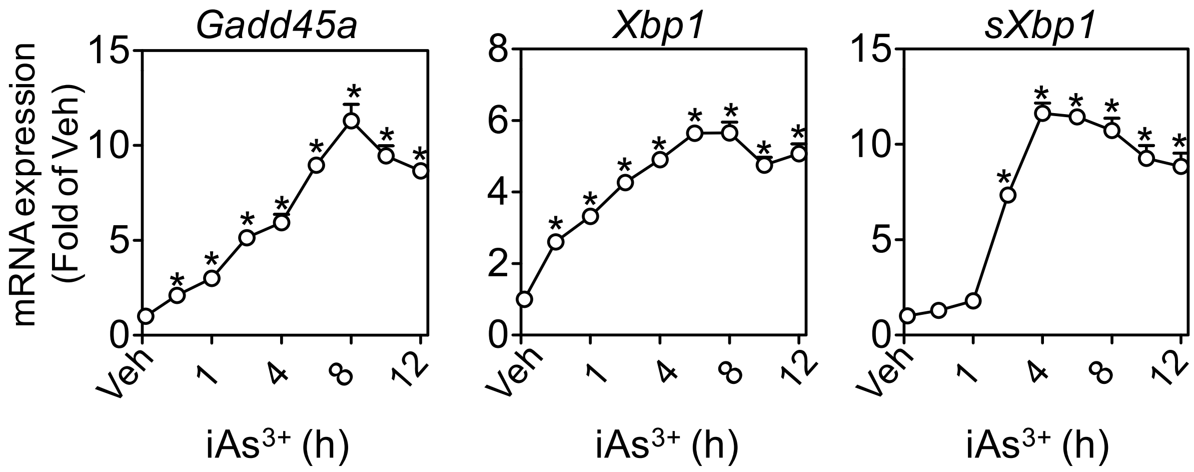


Figure S4. Effect of iAs^{3+} on the mRNA expression of UPR genes in 3T3-L1 cells. Cells were exposed to 5 μM iAs^{3+} in DMEM with 10% CS for the indicated time. n = 3. * $p < 0.05$ iAs^{3+}-treated vs. Vehicle (Veh).

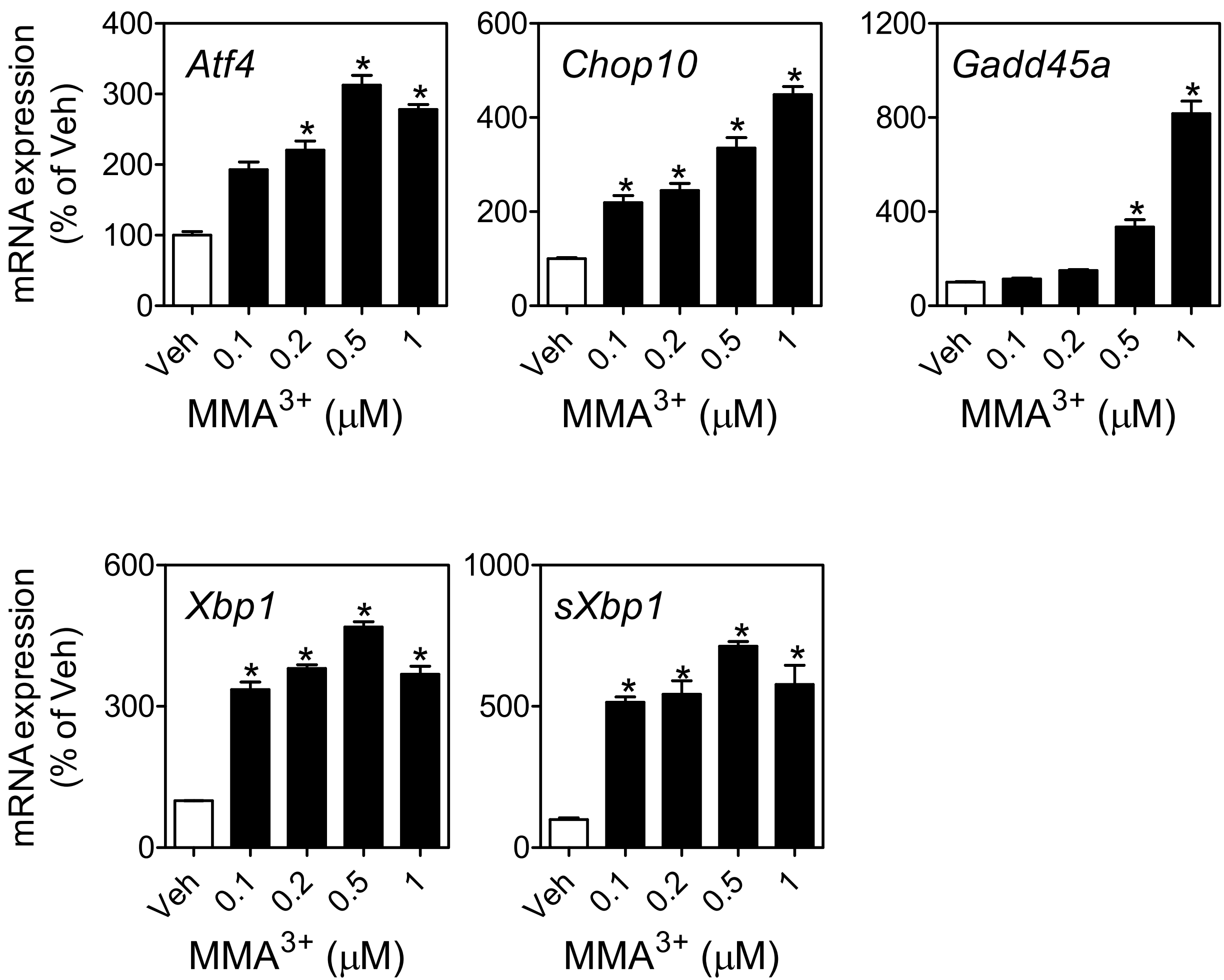


Figure S5. Effect of MMA^{3+} on the mRNA expression of UPR genes in 3T3-L1 cells. Cells were exposed to MMA^{3+} in DMEM with 10% CS for 6 h. n = 3. * $p < 0.05$ MMA^{3+}-treated vs. Vehicle (Veh).

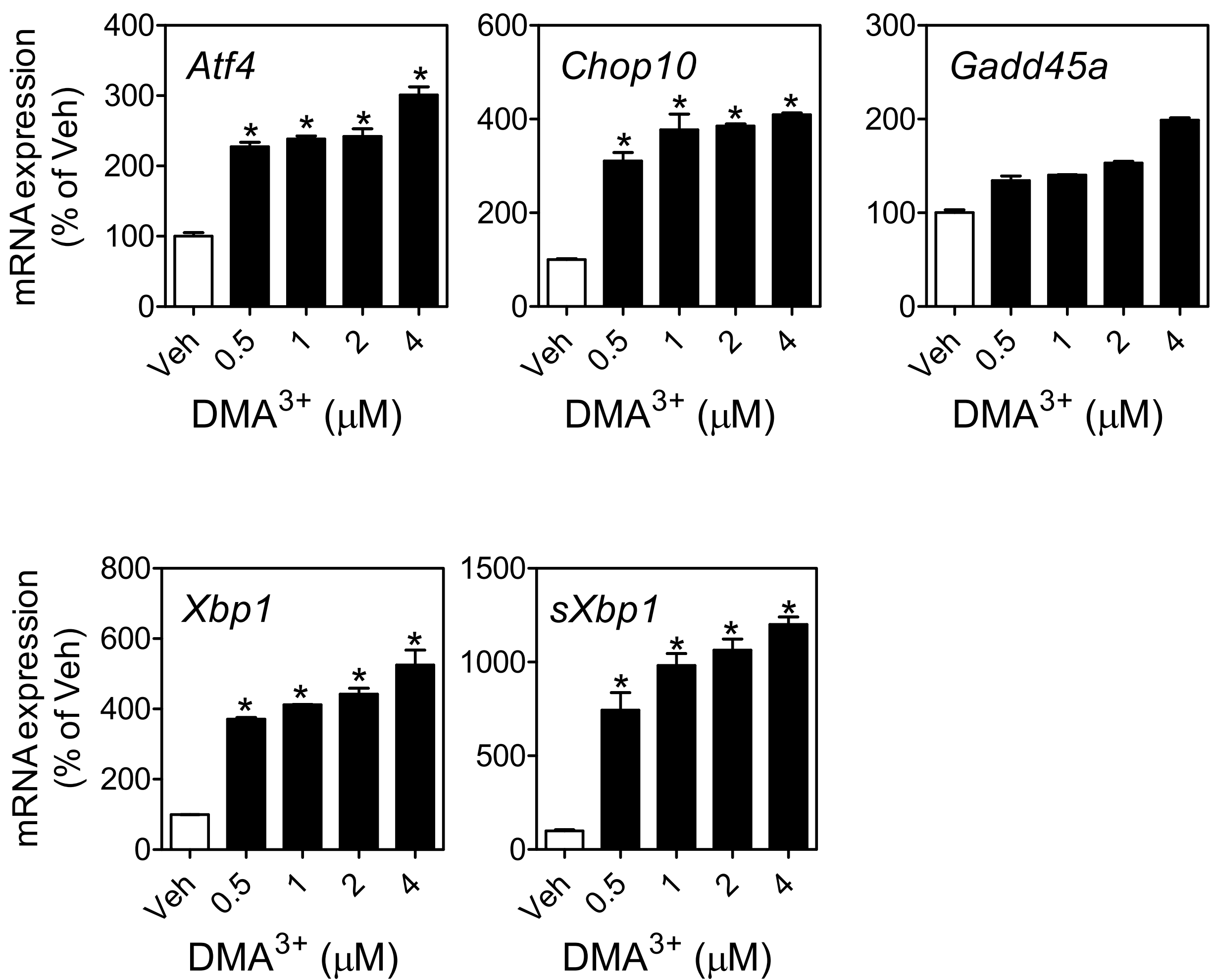


Figure S6. Effect of DMA^{3+} on the mRNA expression of UPR genes in 3T3-L1 cells. Cells were exposed to DMA^{3+} in DMEM with 10% CS for 6 h. n = 3. * $p < 0.05$ DMA^{3+}-treated vs. Vehicle (Veh).

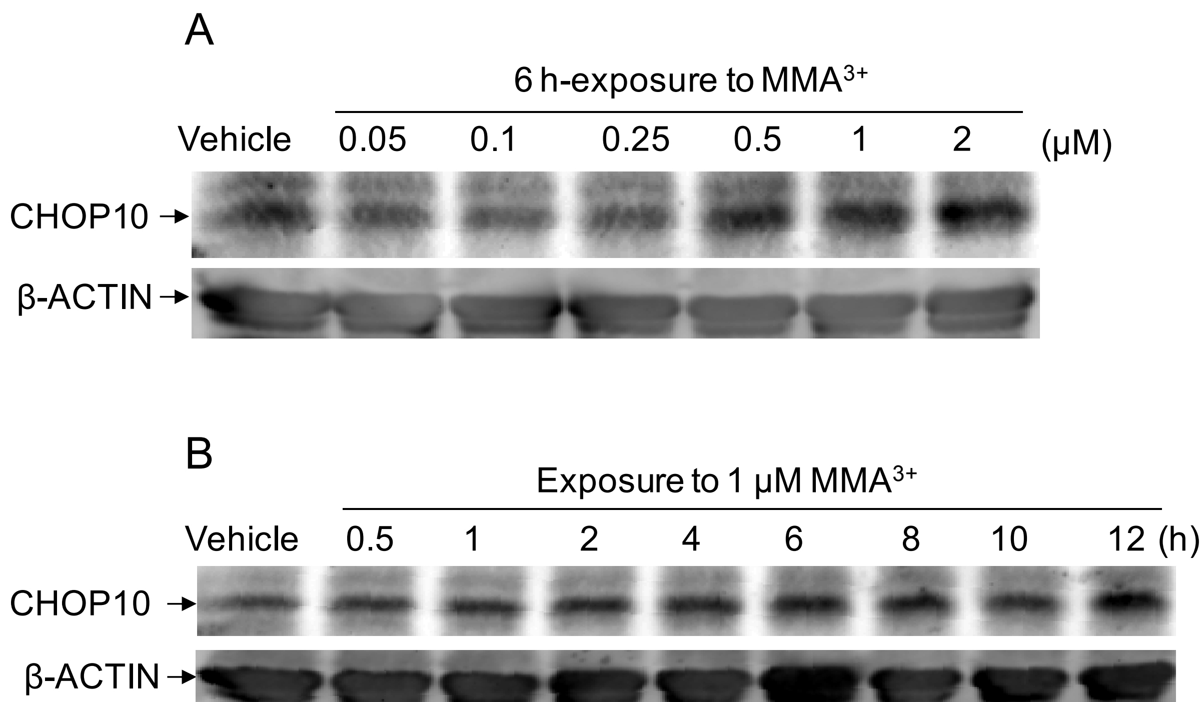


Figure S7. Concentration-response (A) and time course (B) of MMA^{3+}-induced protein expression of CHOP10 in 3T3-L1 preadipocytes. Cells were exposed to MMA^{3+} in DMEM with 10% CS.

References:

Kapur A, Zhao P, Sharir H, Bai Y, Caron MG, Barak LS, et al. 2009. Atypical responsiveness of the orphan receptor GPR55 to cannabinoid ligands. J Biol Chem 284(43): 29817-29827.