Arsenite-induced mitochondrial superoxide formation: time and concentration requirements for the effects of the metalloid on the endoplasmic reticulum and mitochondria

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Supplemental Figure 1. The effect of the short-term co-exposure to arsenite and ATP, or Cf, on Ca^{2+}-dependent homeostasis

RP-cells pre-loaded for 30 min with Fluo-4-AM (A), or Rhod-2-AM (B), were exposed for 5 min to 2-APB (50 μM), Ry (20 μM) or Ru360 (10 μM), and treated for a further 10 min with 2.5 μM arsenite, with or w/o ATP, or Cf. Cells were then analyses for their fluorescence responses. Results represent the means ± SD calculated from at least three separate experiments. *$P < 0.05$, **$P < 0.01$, as compared to untreated cells (one-way ANOVA followed by Dunnet's test).

Supplemental Figure 2. NADPH-oxidase is not involved in Ca^{2+}-dependent ROS formation induced by arsenite

(A) RP-cells pre-loaded with DHR (30 min), were exposed for 5 min to the vehicle, apocynin (10 μM) or DPI (1 μM) and for a further 10 min to the arsenite alone or associated with ATP. In some experiments, the cells were exposed for 30 min to PMA (100 ng/ml) in the absence or presence of apocynin or DPI. After treatments, the cells were analysed for DHR-fluorescence. (B) The cells treated as indicated in A were analysed for phospho p47 ^{phox} expression. The blot, representative of three separate experiments, was re-probed for p47 ^{phox}. Results represent the means ± SD calculated

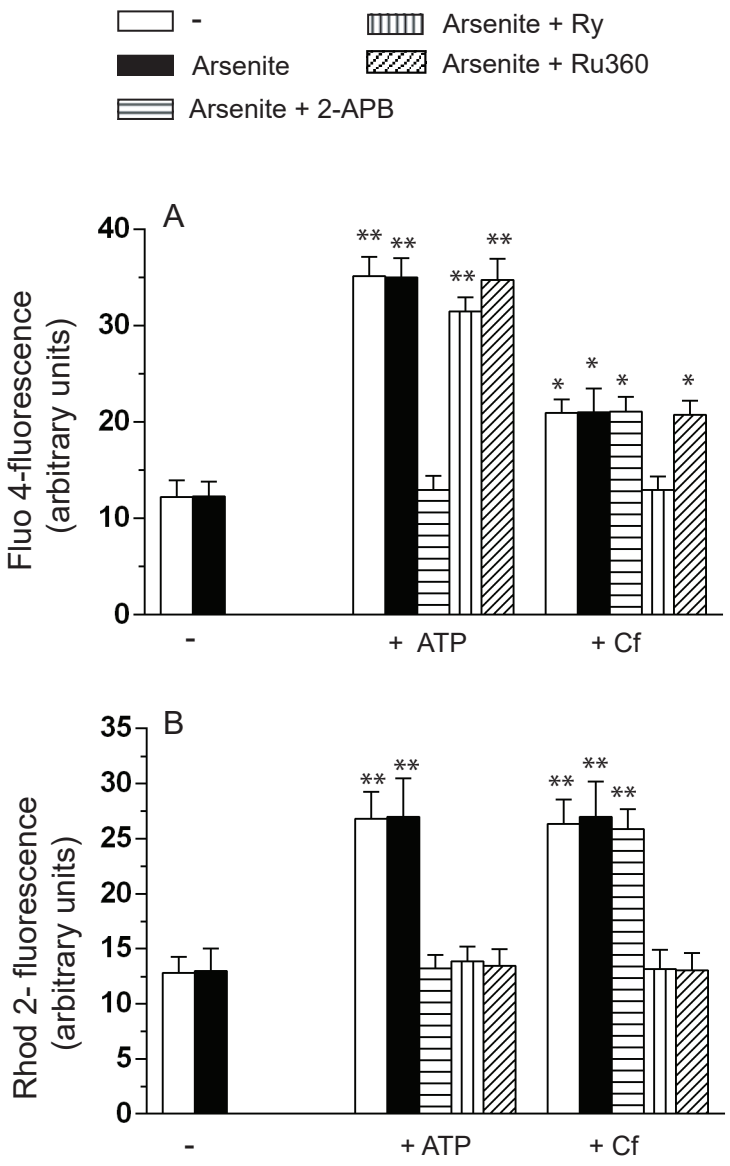
from at least three separate experiments. *$P < 0.05$, **$P < 0.01$, as compared to untreated cells (H two-way ANOVA followed by Bonferroni's test; I one-way ANOVA followed by Dunnet's test).

Supplemental Figure 3. Short-term exposure to arsenite/ATP or Cf did not change the redox state of Trx2

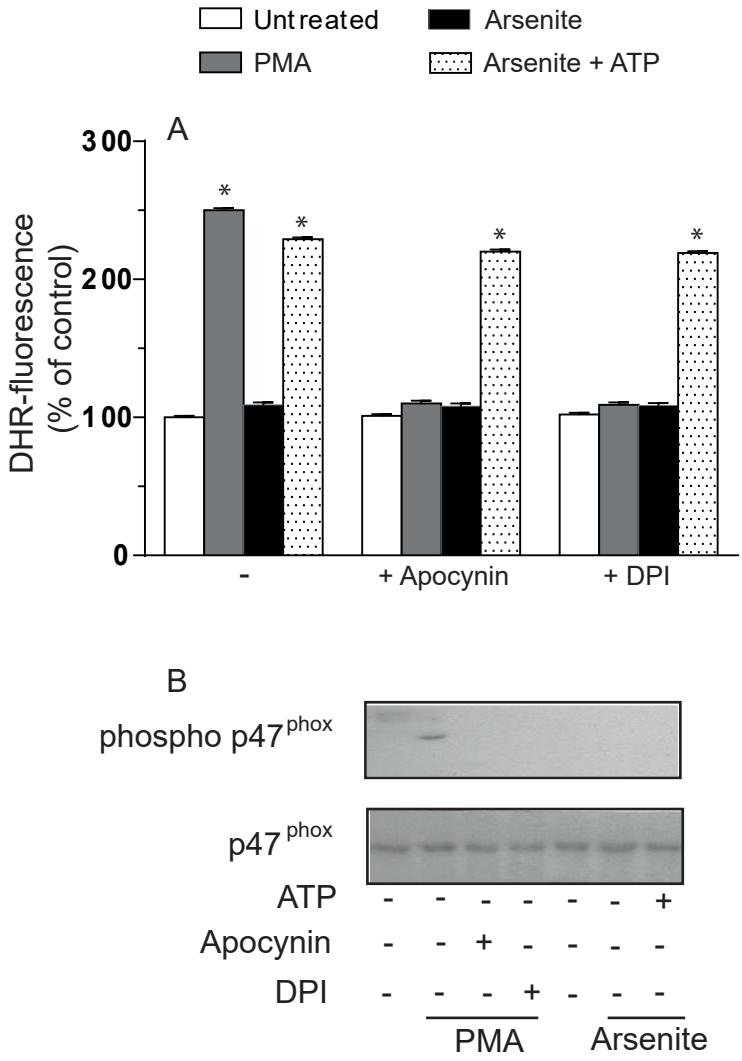
RP-cells (2.5×10^6), treated for 10 min as detailed below, were processed for the analysis of reduced, partially oxidized and fully oxidized Trx2 thiol groups. The redox state of Trx2 was measured by urea-PAGE under non-reducing conditions. (a) control; (b) 2.5 μM arsenite; (c) arsenite and ATP (d) arsenite and Cf (e), 0.5 mM diamide (60 min), (f) 1 mM diamide (60 min), (g) control, (h) mobility standard.

Supplemental Figure 4. Short-term exposure to arsenite/ATP or Cf is associated with the induction of DNA single-strand breakage.

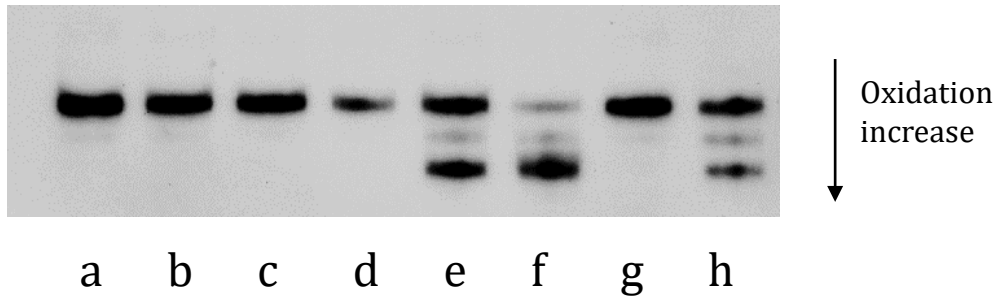
Representative micrographs of cells treated for 30 min with the vehicle (A), 2.5 μM arsenite (B), ATP (in the last 10 min, C), Cf (in the last 10 min, D), arsenite and ATP (E) or arsenite and Cf (F) and finally processed with the alkaline halo assay. (G) micrograph of cells exposed for 6 h to arsenite.



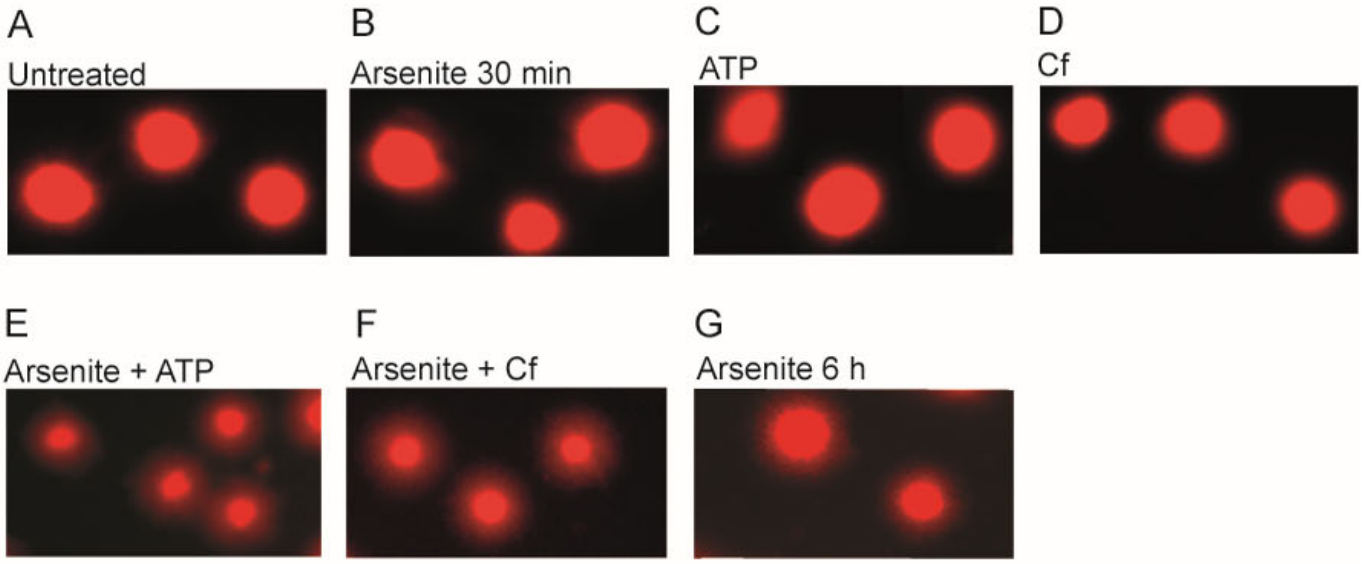
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4