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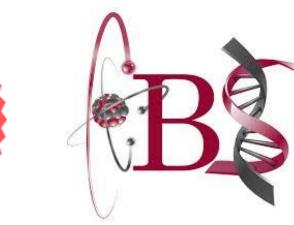
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Manipulations of GSH content modulate [Ca2+]i homeostasis in rat astroglioma cells





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Oxidative stress is an aggravating factor of all neurological disorders, including neurodegenerative diseases. It is due to an exaggerated accumulation of reactive oxygen species (ROS) concomitant to decreased reduced glutathione (GSH) levels; the main antioxidant of brain cells produced by astrocytes in large amounts. Cell death triggered by oxidative damage results from intracellular calcium overload which is deleterious to cell activity. The mechanisms involved in these calcium increases are not fully understood. Here, we have thus investigated whether GSH could protect neurons against oxidative stress through the modulation of calcium homeostasis. For this, GSH cell content was manipulated by modulating its metabolism. To investigate the link between GSH content and calcium homeostasis, we pre-treated rat astroglioma cells (C6) with sulforaphane (SFN) to increase intracellular GSH levels, or buthionine sulfoximine (BSO) to decrease them, during 24 hours for both treatments.

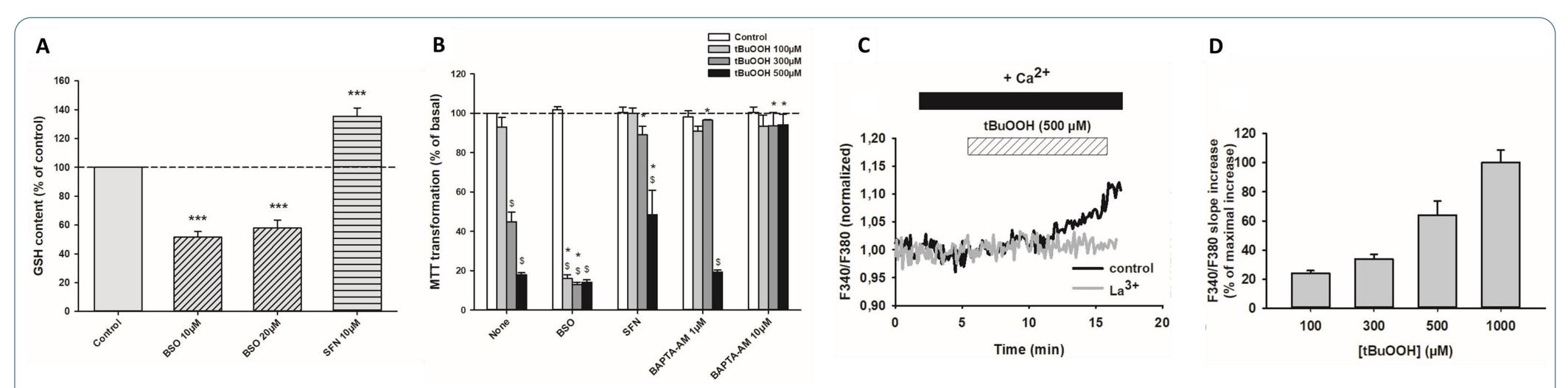


Figure 1. Manipulation of GSH content alters cell resistance to oxidative stress elicited by tBuOOH. 10 μ M SFN or BSO were applied directly in the culture medium 24 hours before experimentation. 100 μ M lanthanum blocked the Ca²⁺ increase elicited by 500 μ M tBuOOH.

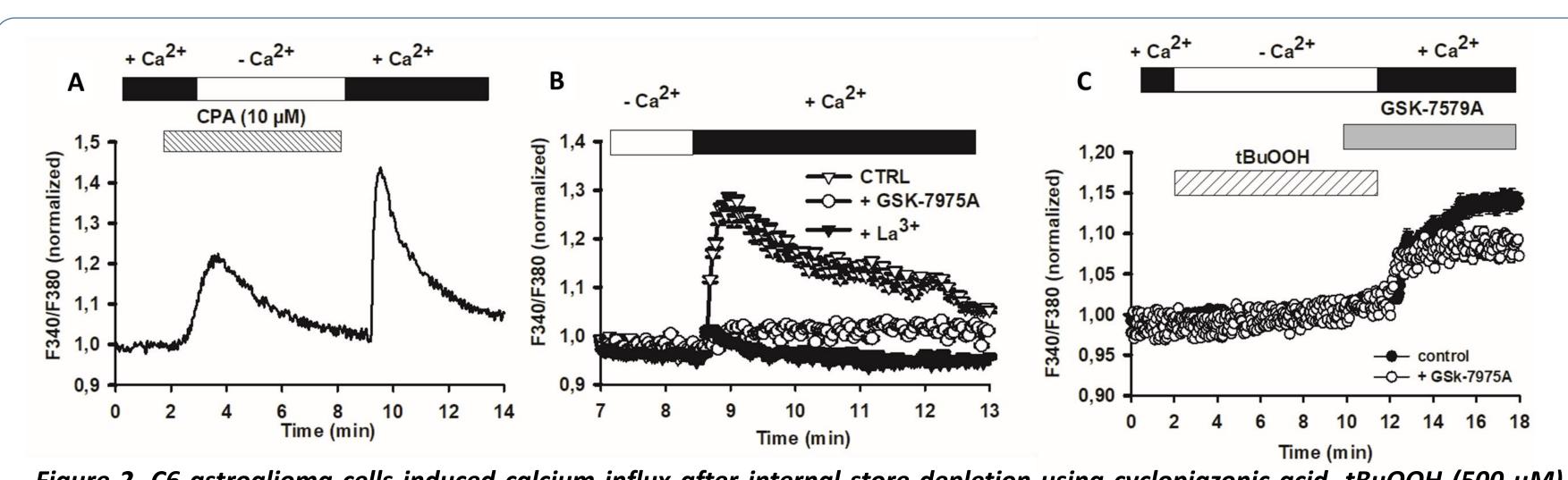


Figure 2. C6 astroglioma cells induced calcium influx after internal store depletion using cyclopiazonic acid. tBuOOH (500 μΜ) elicited independent-Store Operated Calcium Influx.

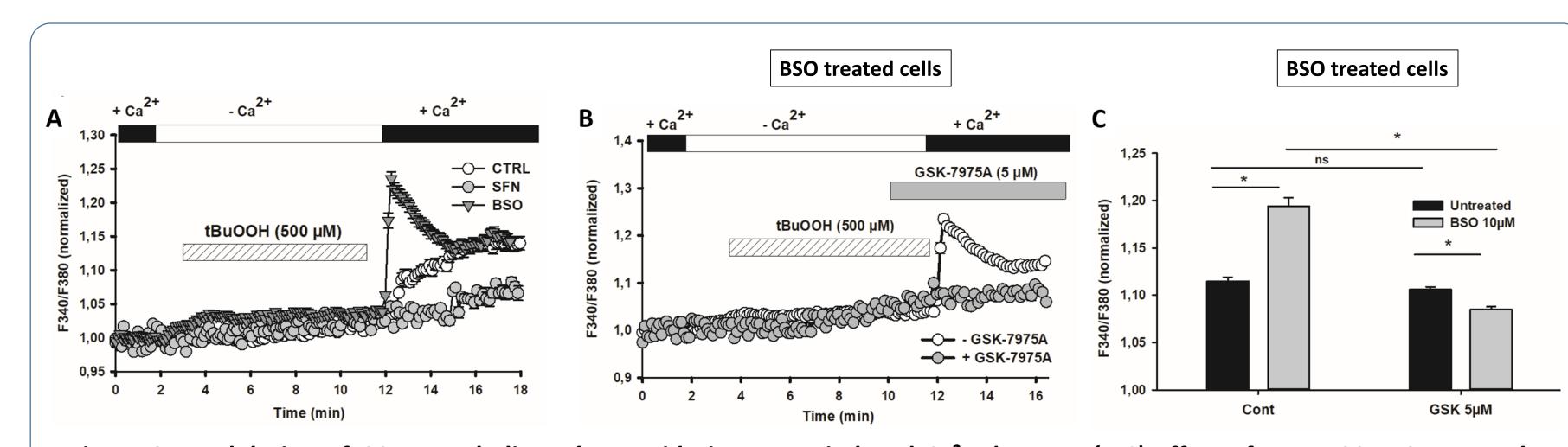


Figure 3. Modulation of GSH metabolism alters oxidative stress induced Ca²⁺ changes. (B,C) Effect of 5 μ M GSK-7975A on the tBuOOH-induced [Ca2+]i increase in BSO-pretreated cells.

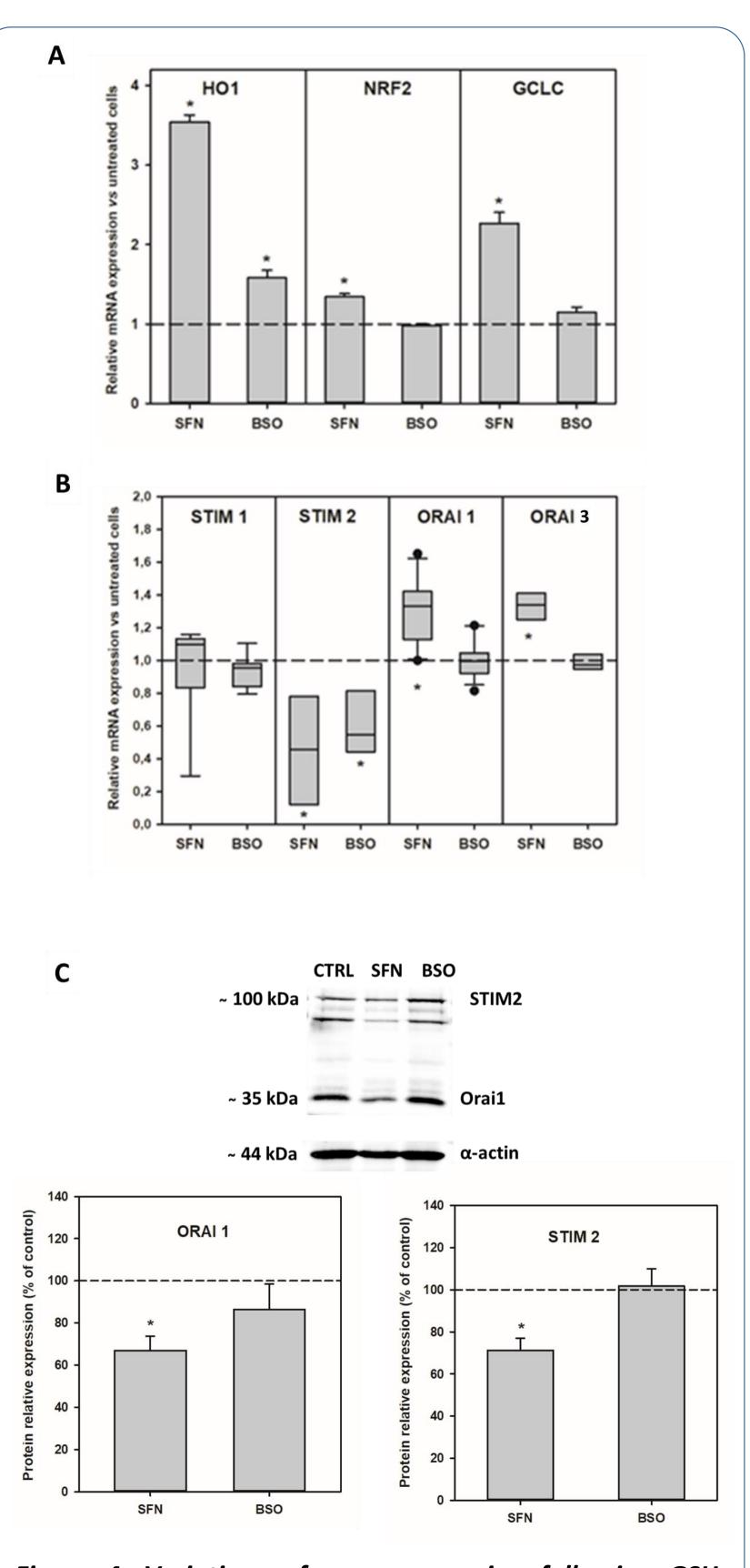
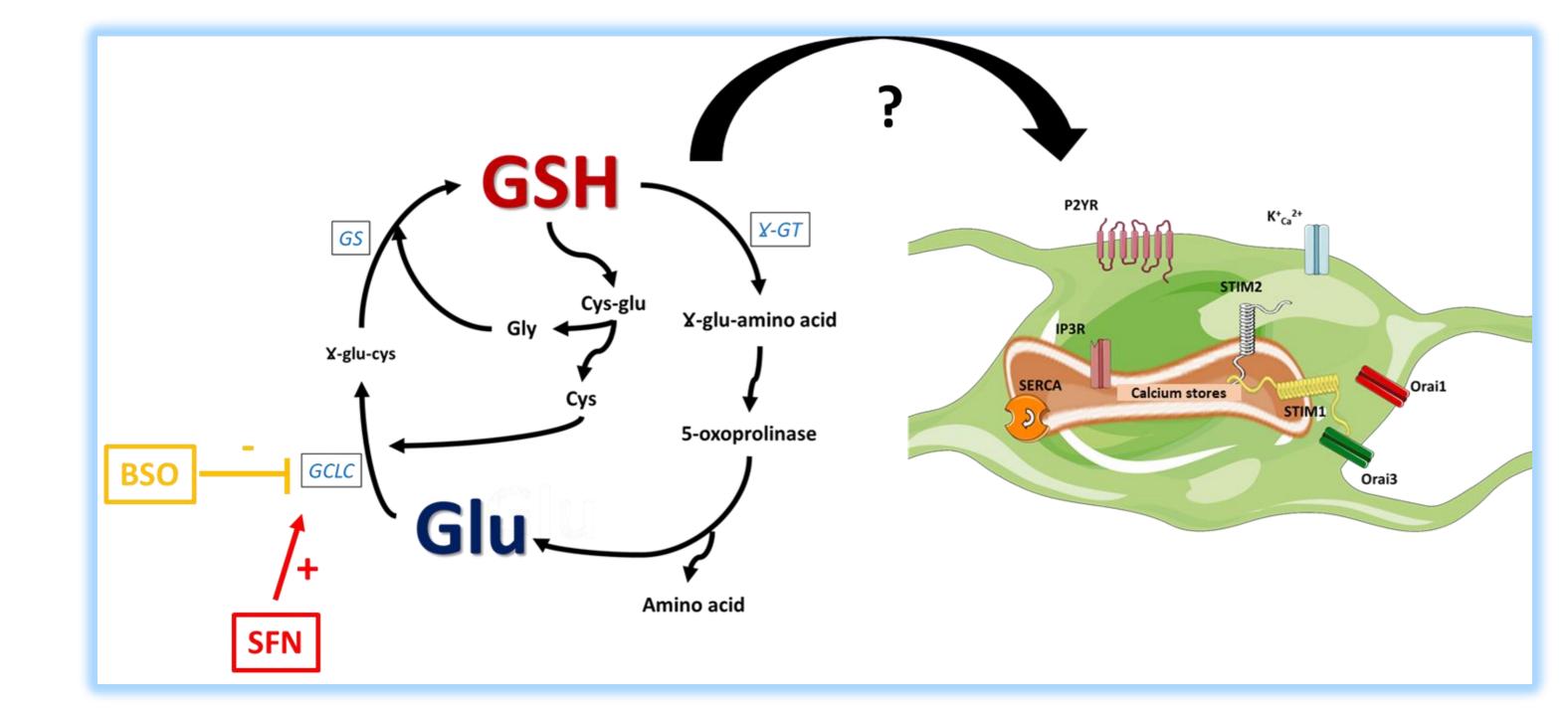


Figure 4. Variations of gene expression following GSH metabolic manipulations. (A) mRNA expressions of Nrf2, Heme Oxygenase 1 (HO-1) and glutamate cysteine ligase catalytic subunit (GCLC) genes (B) mRNA expressions of SOCE proteins genes, including STIM1, STIM2, Orai1 and Orai3. Gene expression were normalized to b-actin. (C) STIM and Orai protein expression normalized to a-actin.



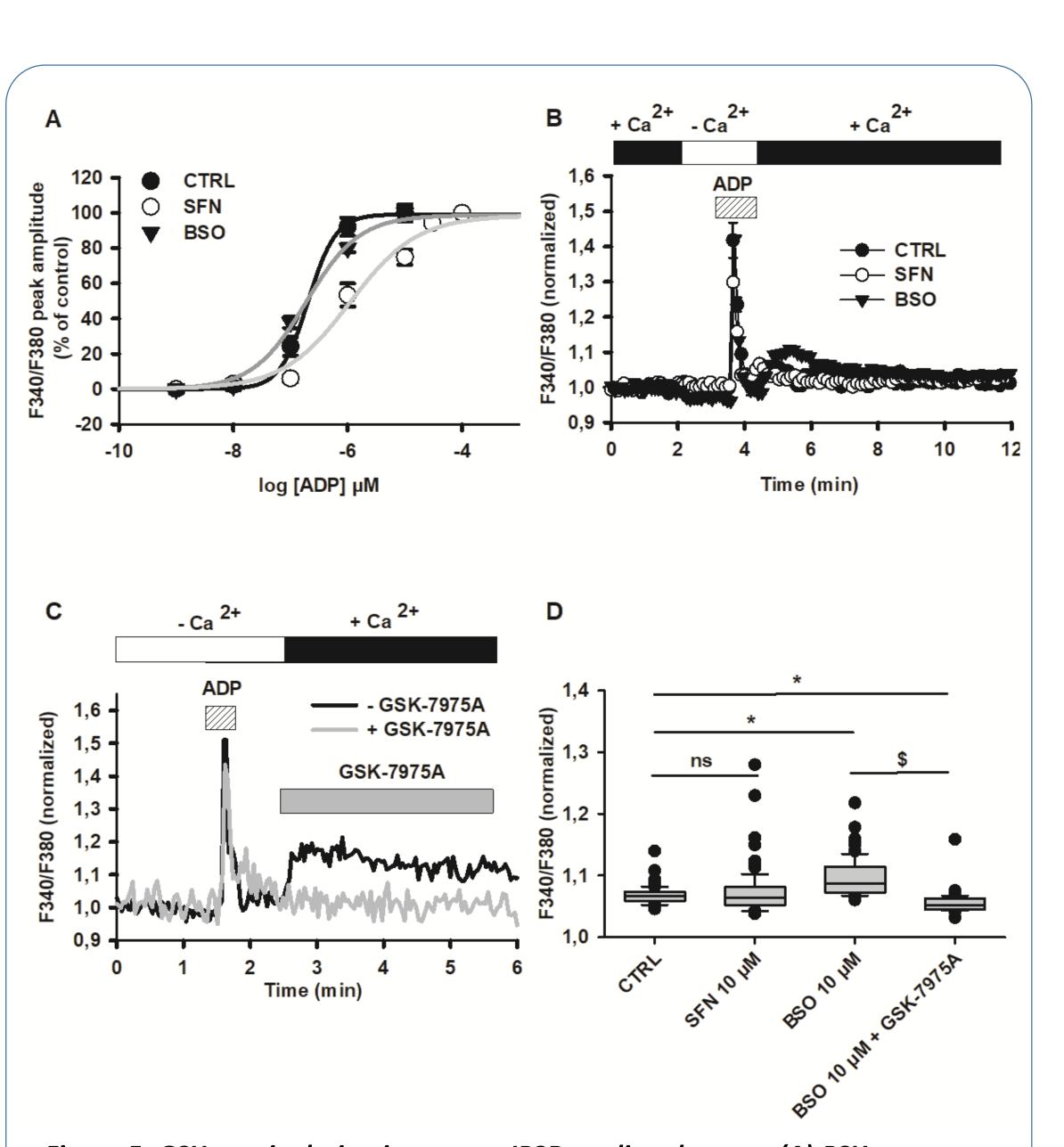


Figure 5. GSH manipulation impact on IP3R mediated stores. (A) P2Y receptors were stimulated by increasing concentrations of ADP (1, 10, 100 nM and 1, 10 μ M) in the absence of extracellular calcium and (D) the peak amplitude was determined (C) 5 μ M of GSK-7975A were used 3 minutes prior extracellular calcium reinstatment.

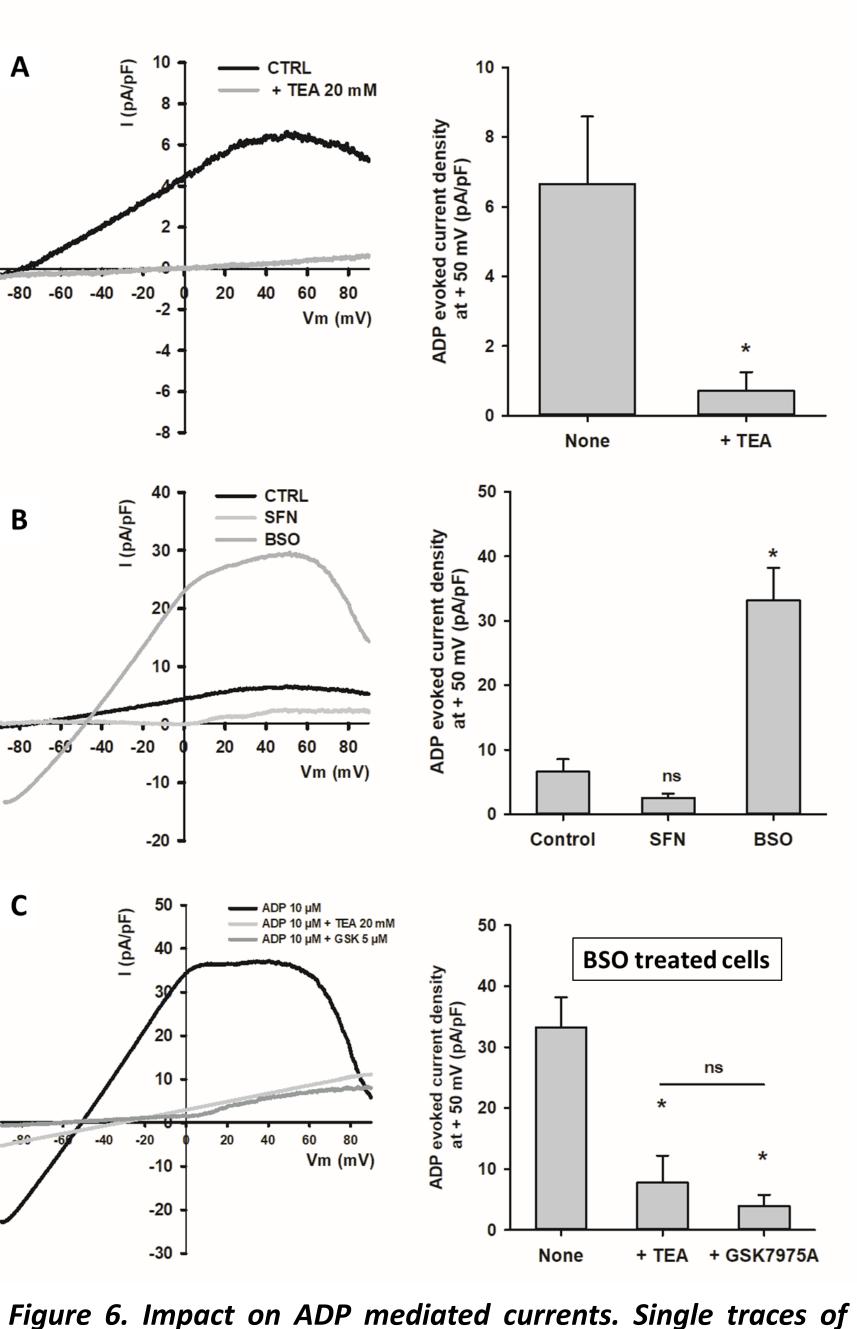


Figure 6. Impact on ADP mediated currents. Single traces of currents recorded after (A) 10 μ M ADP application on control cells either in the absence or in the presence of 20 mM TEA or not (B) 10 μ M ADP application on control, BSO- and SFN pretreated cells (C) BSO-treated cells with 20 mM TEA or 5 μ M GSK 7579A are shown. Currents were recorded by applying voltage ramps from -100 to 100 mV every 10 seconds.

We conclude that GSH cell content changes impact intracellular Ca2+ homeostasis in C6 glioma cells likely via sensitization/desensitization mechanisms of Calcium Release Activated Channels (CRAC). GSH depletion due to oxidative stress regulation could thus contribute to the alterations of Ca²⁺ homeostasis in astrocytes under pathological conditions.