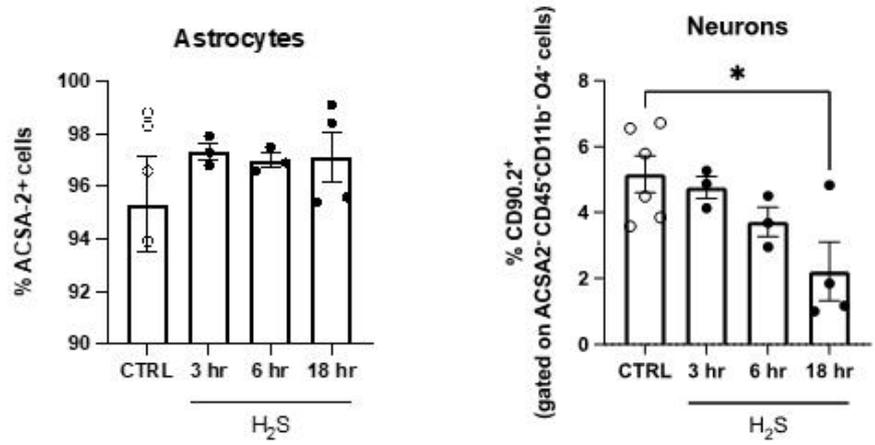
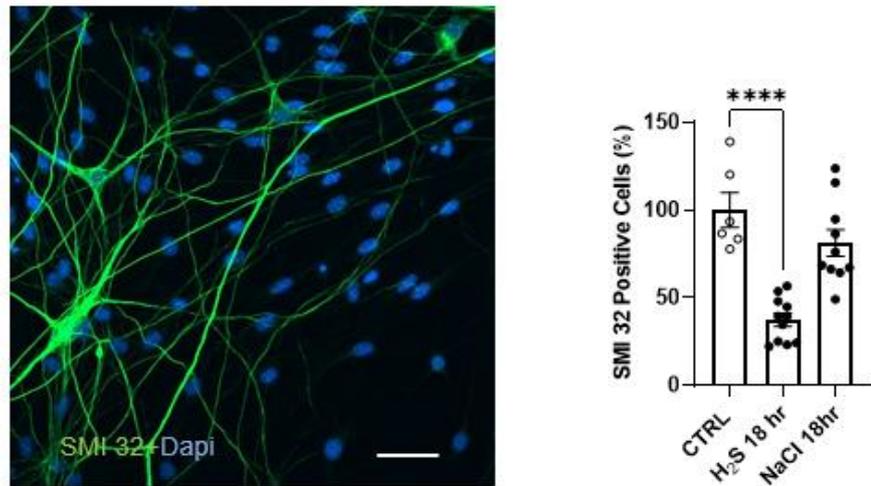
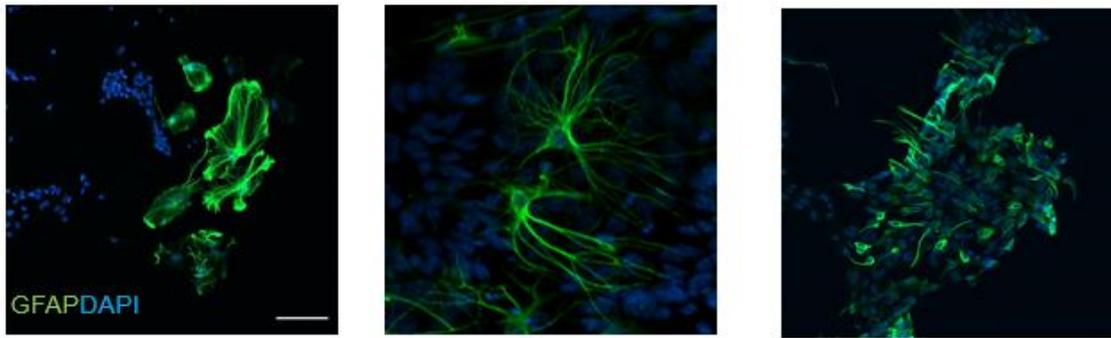
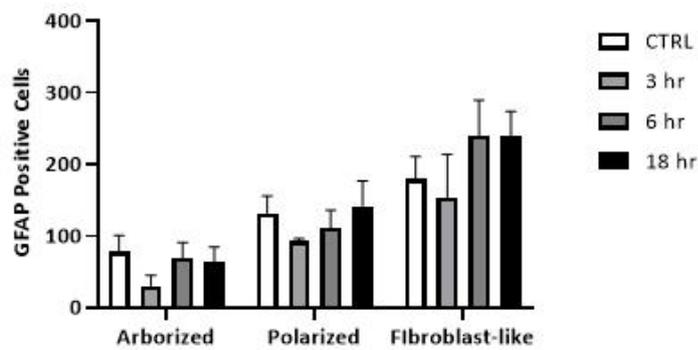
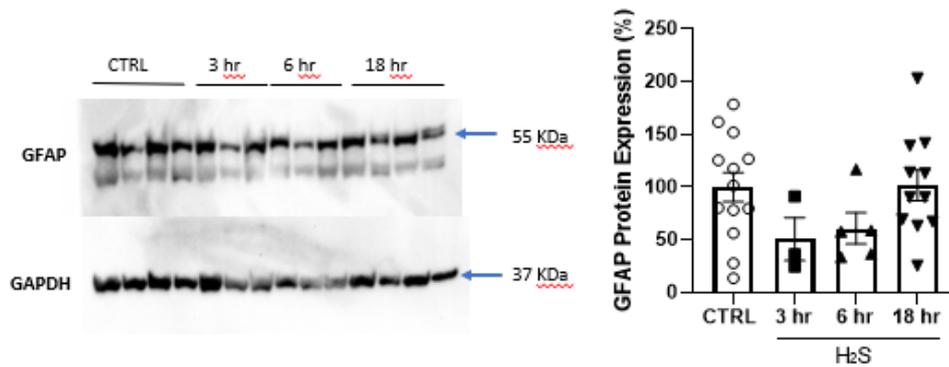


A**B**

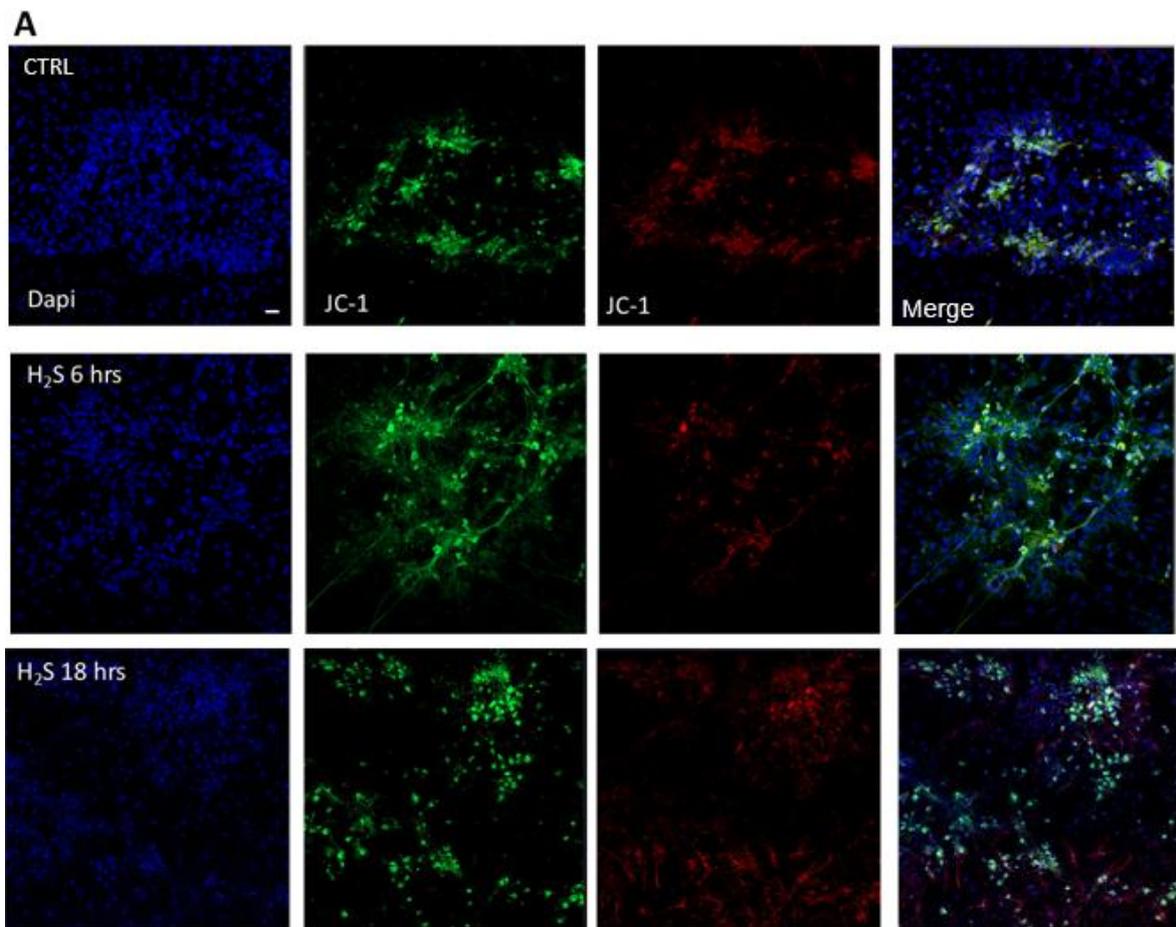
Supplementary Figure S1. (A) Percentage of ACSA-2+ cells (astrocytes) and CD90.2+ cells (neurons) under H₂S treatment. While ACSA-2+ were not affected by H₂S toxicity, the number of CD90.2+ cells decreased significantly at 18 h. (B) H₂S toxicity toward motor neurons was confirmed by direct counting of the SMI32+ neurons, an equimolar treatment with NaCl did not show any significant toxicity. All values were compared by using one-way ANOVA with * $p < 0.05$, **** $p < 0.0001$ (Scale Bar 20 μ m).

A**B**

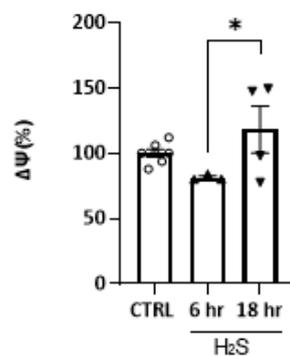
100
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**C**

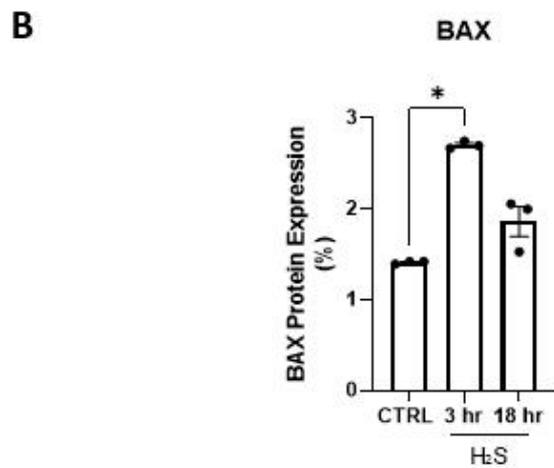
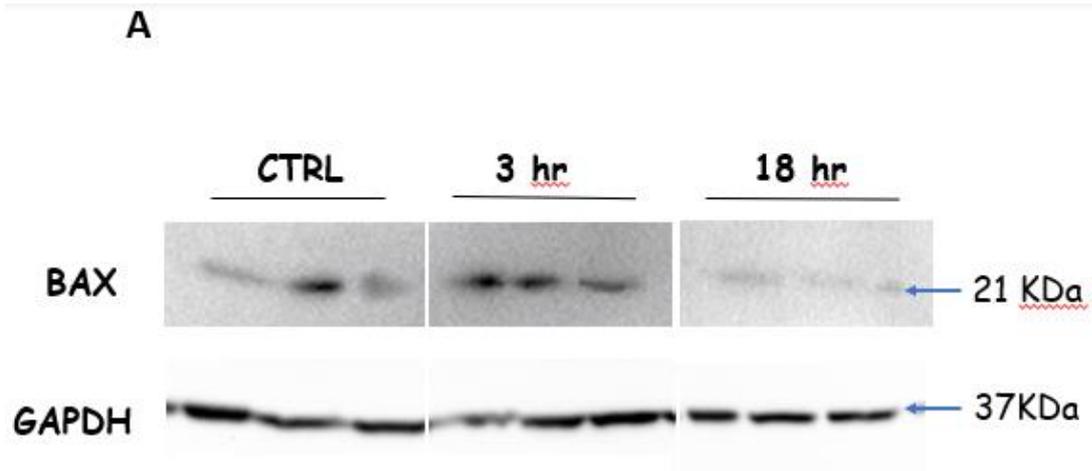
Supplementary Figure S2. (A) Representative images of fibroblast-like (1), arborized (2) and polarized (3) astrocytes morphologies after GFAP (green) staining. (B) The different shapes were quantified by direct counting of the GFAP+ cells in Control (Ctrl) and H₂S treated cultures at 3, 6 and 18 h. (C) Representative western blot of the GFAP expression levels at the indicated times. Data are expressed as mean \pm SEM (Scale Bar 20 μ m).



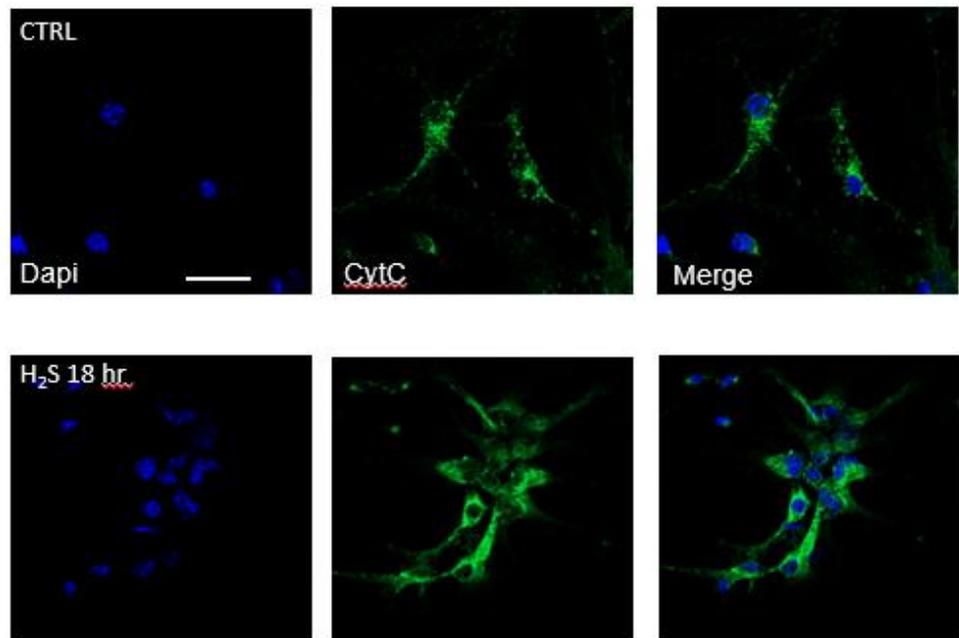
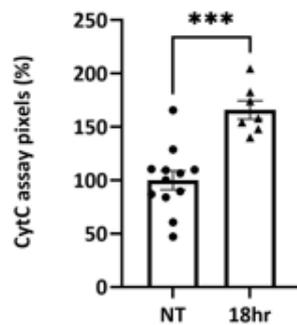
B



Supplementary Figure S3. (A) Immunofluorescence of spinal cord culture in Control (upper panels) and under H₂S toxicity (lower panels, 6 h and 18 h treatment). The cultures were challenged at different times with H₂S 200 μM, and then loaded with the mitochondrial membrane potential dye JC-1, fixed and probed with DAPI. (B) Quantitative analyses of the ratio between the JC-1 aggregate (red) and the JC-1 monomer (green) is presented as a percentage normalized to the non-treated cultures. Data are expressed as mean ±SEM of three fields from three separate experiments. The values were compared by using one-way ANOVA with * $p < 0.05$ (Scale bar 20 μm).



Supplementary Figure S4. H₂S treatment provoked a time-dependent increase of BAX reaching significance at three hours of 200 μ M H₂S incubation. (A) Representative western blot showing the Bax increase after three hours of H₂S treatment, and a subsequent decrease after 18 h. (B) Data are the result of three independent experiments mean \pm SEM. All values were compared by using one-way ANOVA with * $p < 0.05$.

A**B**

Supplementary Figure S5. H₂S treatment provoked the release of cytochrome c after 18 h of incubation. H₂S 200 μ M was incubated for 18 h, the cells were then fixed and probed with cytochrome c (green) and Dapi. Confocal images were captured with a 63X objective from three different cultures. Six images/cultures for each group were taken and the pixels were quantified using Image J (Fiji software). (A) Representative images of Control (Ctrl) and treated cultures (18 h). (B) The graph indicates the cytosolic levels (in pixels) of cytochrome c reaching significance at 18 h. The values of the pixels are presented as percentage normalized to the non-treated values as mean \pm SEM (B). All values were compared by using one-way ANOVA with *** $p < 0.001$ (Scale Bar 20 μ m).