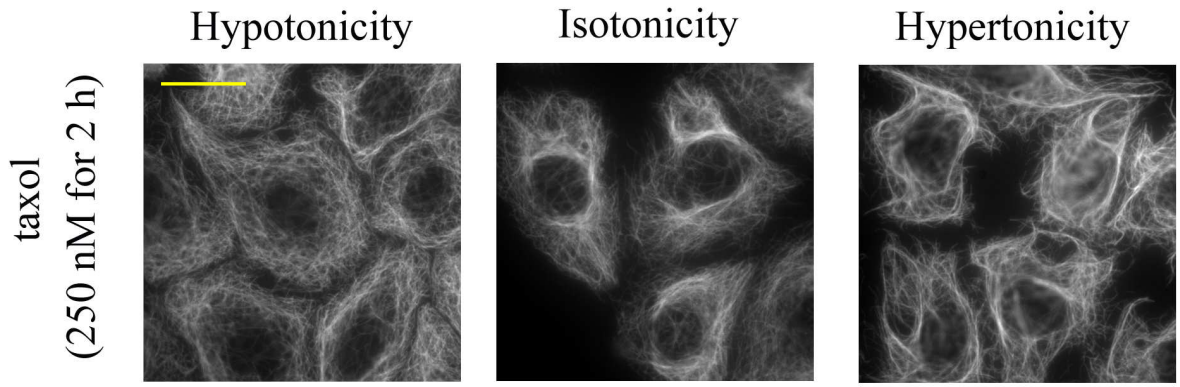
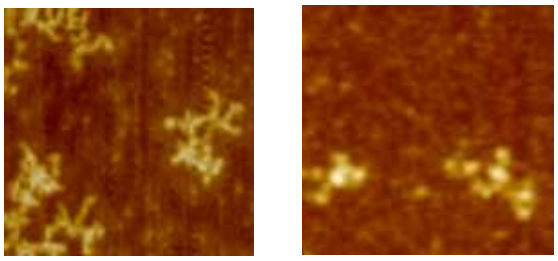


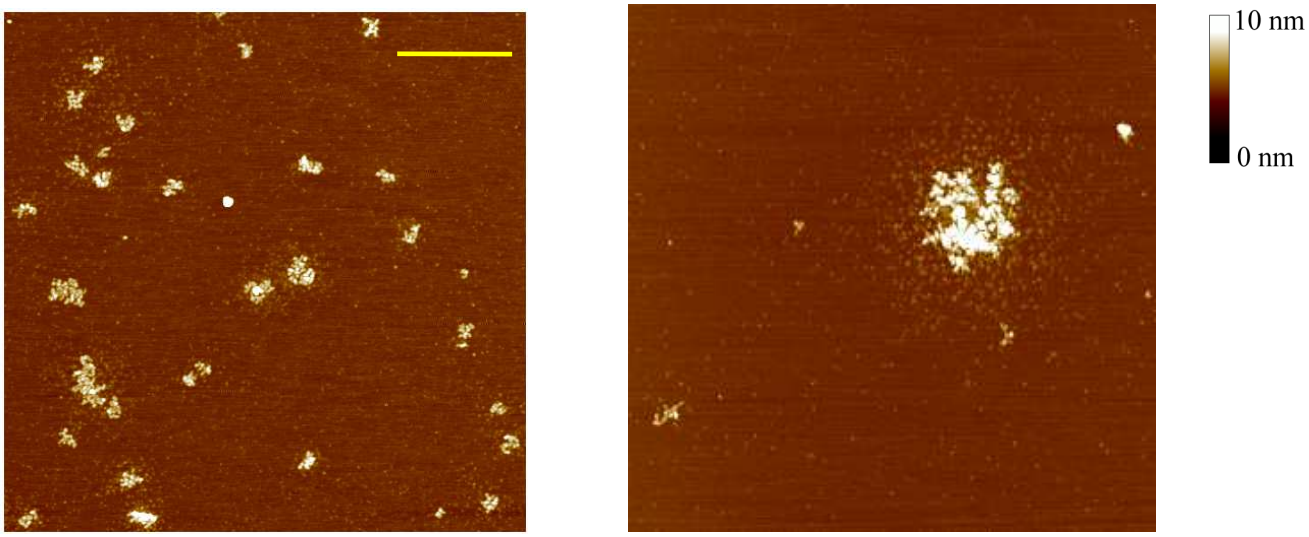
Figure S1: Hypertonicity favors the formation of MT bundles in taxol treated cells.
NRK cells were treated with taxol for 2 h under various extracellular osmolarities adjusted via NaCl addition. Hypotonicity, 220 mosmol/kg; Isotonicity, 320 mosmol/kg; hypertonicity, 520 mosmol/kg. NRK cells were labeled with anti-tubulin. As expected, taxol induces MT stabilization and leads to the formation of MT bundles under isotonic condition. MT bundling is more significant under hypertonic exposure while this tendency is less marked under hypotonic exposure.
Scale bar: 15 µm.



Naked mRNA mRNP (mRNA:YB-1)

A

Without PEG 35K **PEG 35 K, 12%**



B **C**

Figure S2: Macromolecular crowding promotes the assembly of mRNP particles into granules.

(A) mRNP particles (YB-1:mRNA complexes) observed by AFM . The mRNP particles were obtained by mixing 2Luc RNA (5 µg/ml) and YB-1 (1.5 µM) in 20 mM Tris-HCl pH 7.4, 50 mM KCl.
We observe the typical beads-on-string structure of the YB-1:mRNA complexes. Scanned area : 200×200 nm².
(B) mRNP particles at lower magnification.
(C) Same as (B) but in the presence of 12 % (w/v) PEG 35K. Large granules made of mRNP particles are now observed, most probably due to excluded volume interactions. In agreement with this, no aggregation is observed with PEG 1K (data no shown). Under the same conditions but in the absence of YB-1, the PEG 35K concentration required to trigger granule formation increases (~20% (see fig. 1A)).
Scale bar: 0.5 µm.

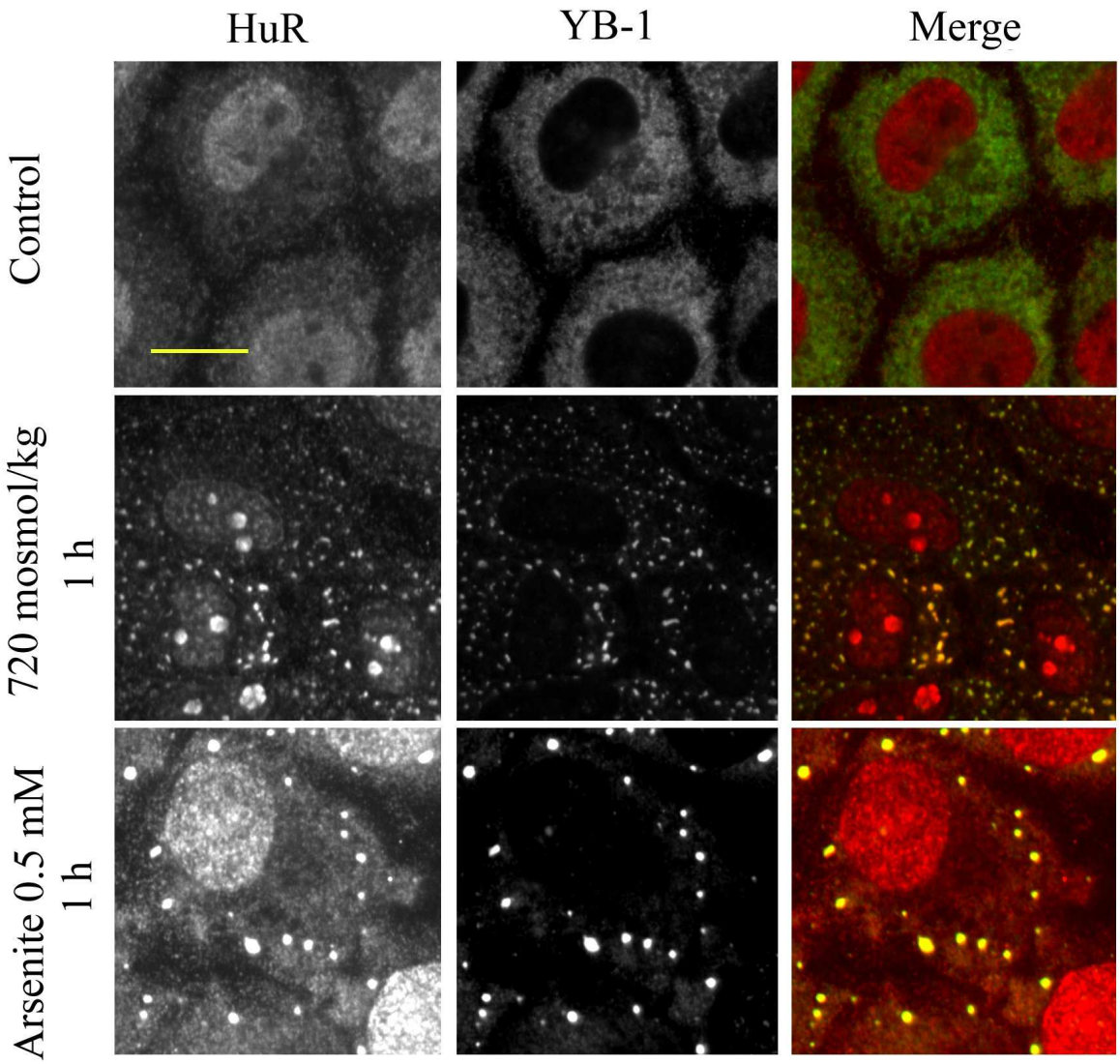


Figure S3: YB-1 is a cytoplasmic marker of mRNA which allows to visualize SGs in arsenite- and hypertonic-stressed cells. Under control condition, YB-1 is homogenously distributed in the cytoplasm while HuR, another SG marker, is rather located in the nucleus. After hypertonic- or arsenite-mediated stresses, these two proteins are co-localized in SGs. Scale bar: 15 µm.

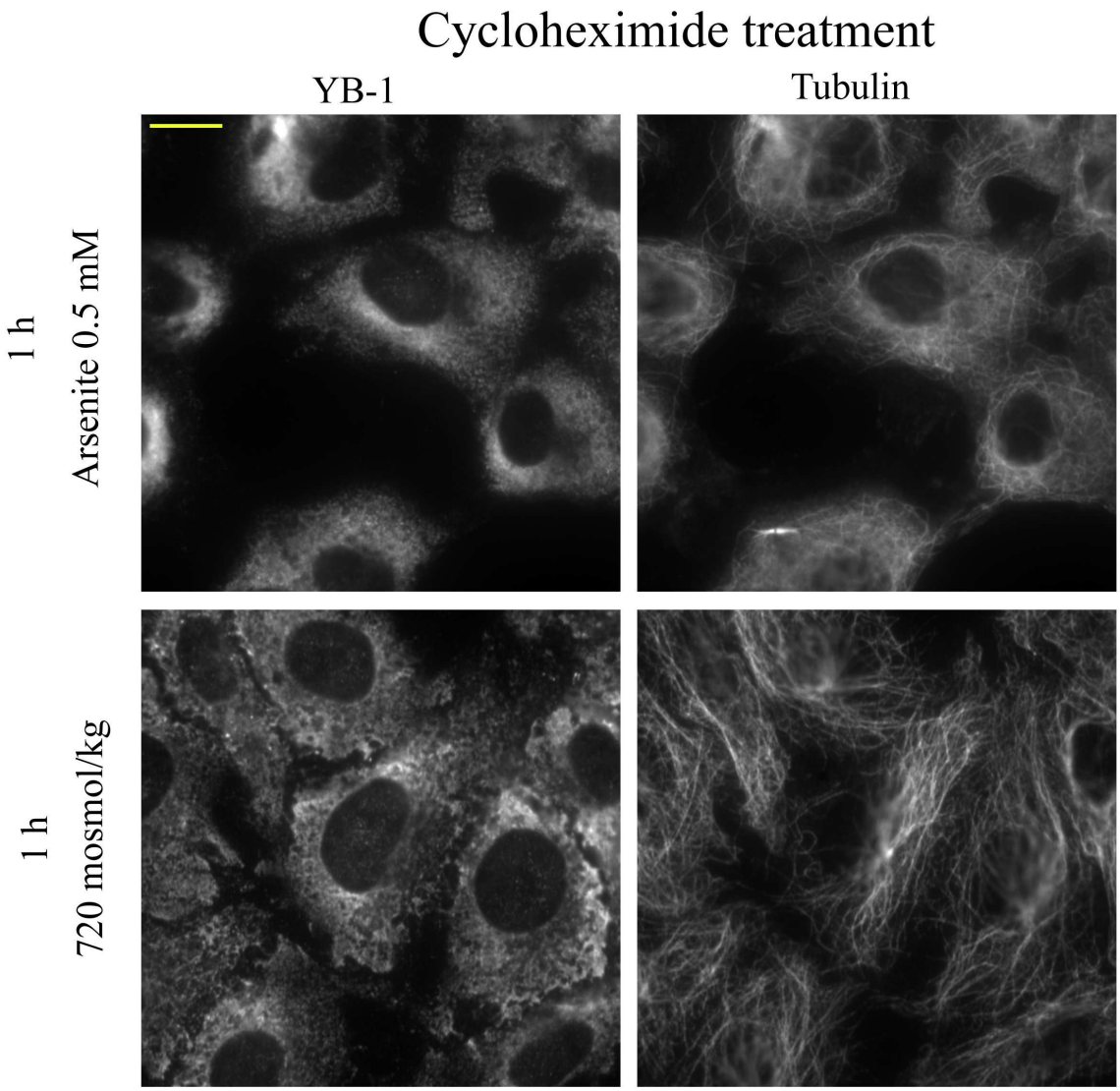


Figure S4: Cycloheximide (10 µg/ml) inhibits SG formation in both arsenite- and hypertonic-stressed cells. NRK cells were stained with anti-YB1 and anti-tubulin for the observation of mRNA and MTs respectively. Let us notice that MTs keep their tendency to form bundles after hypertonic treatment. Scale bar: 10 µm.

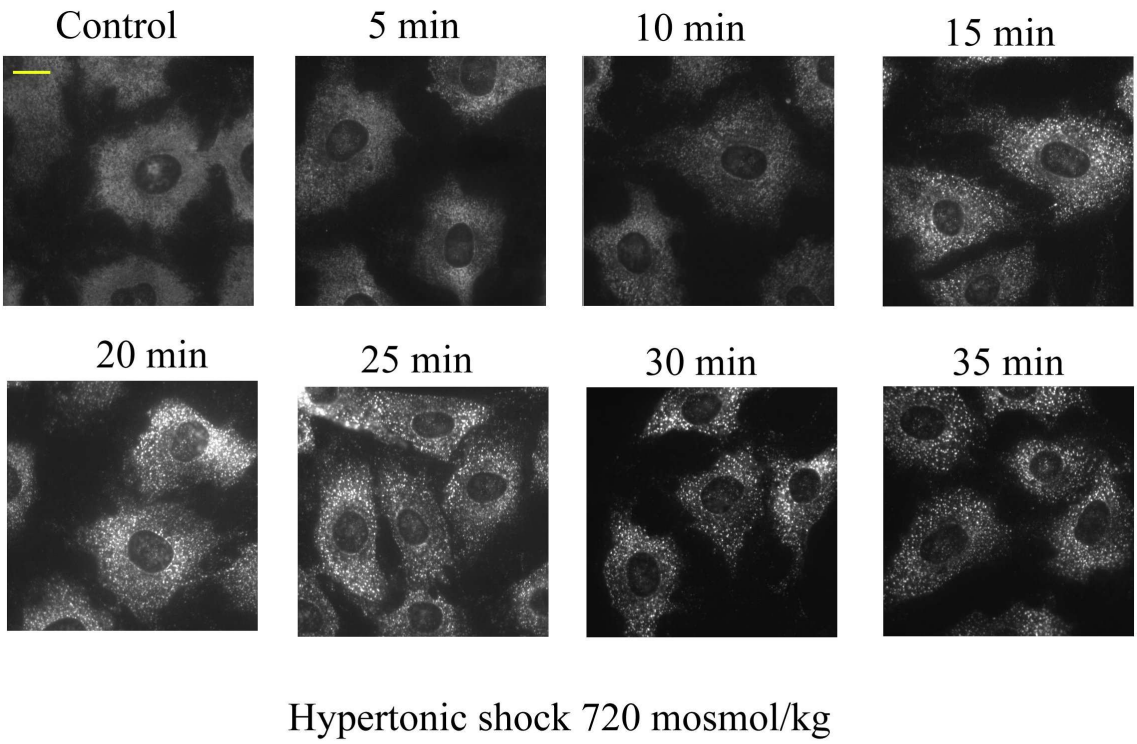


Figure S5: Kinetics of SG assembly in NRK cells during hypertonic stress. Cells were fixed at varying times and labeled with anti-YB1. After 15 min of hypertonic stress, we observe the appearance of SGs. Scale bar: 10 µm.

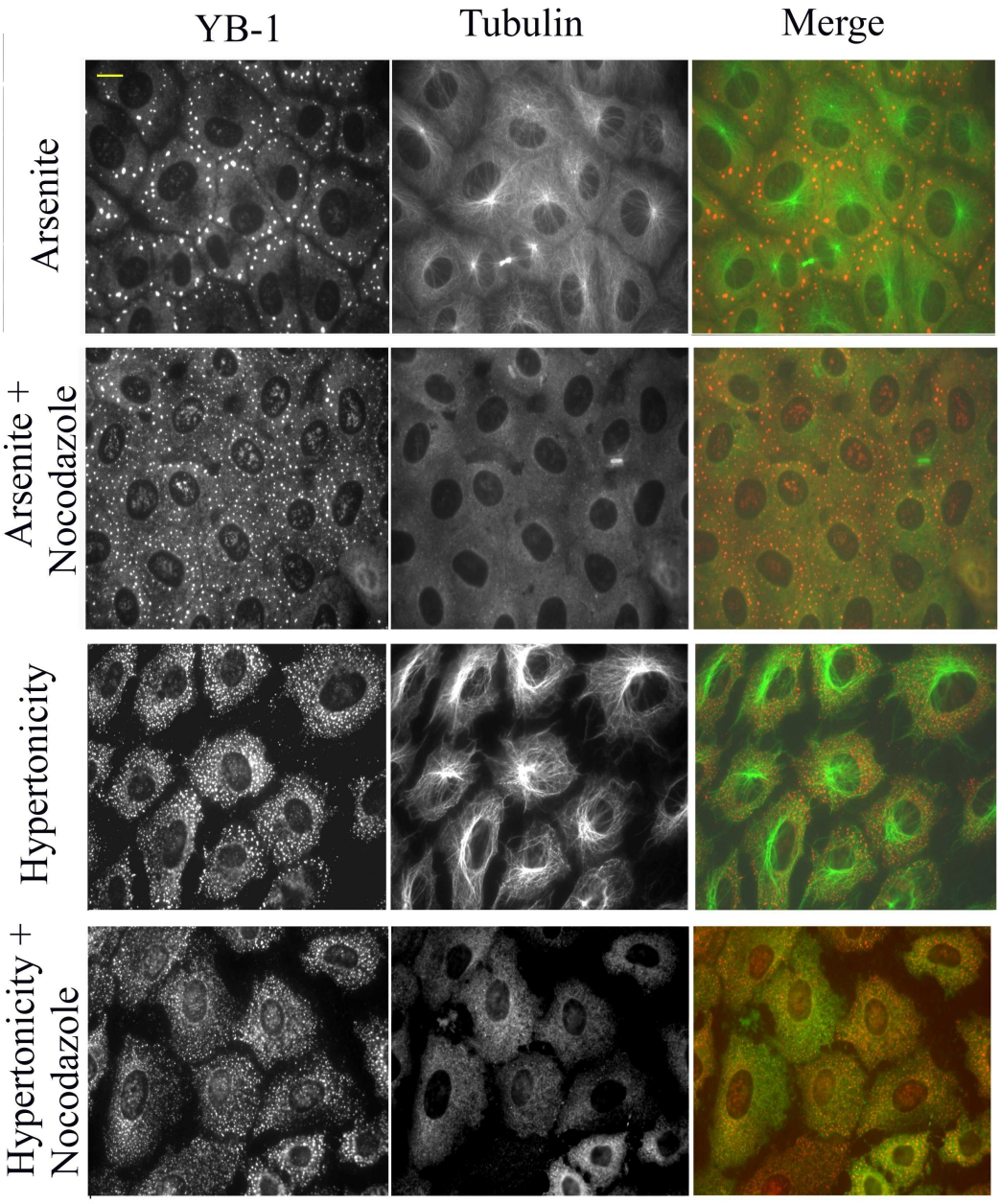
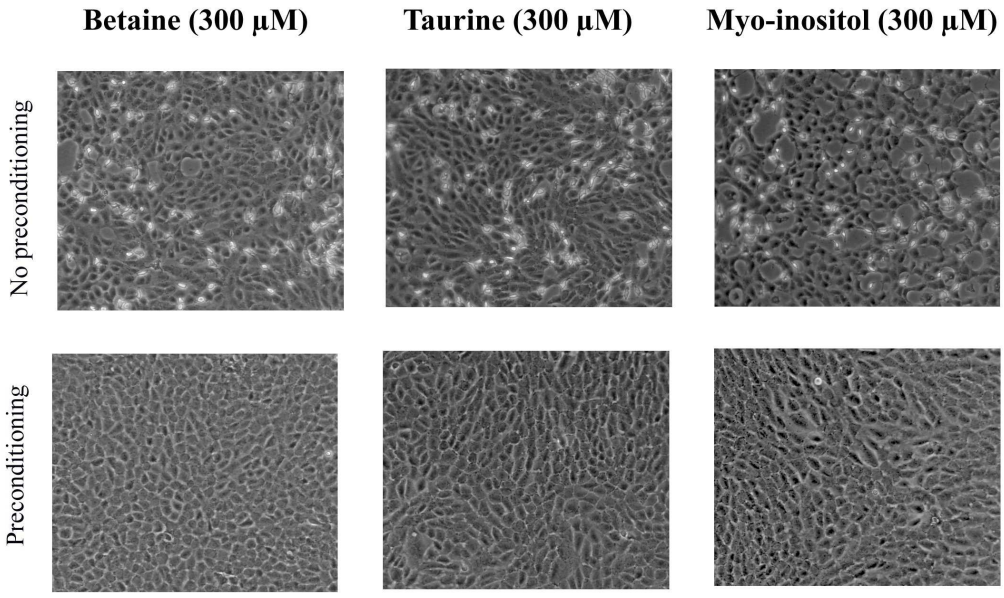


Figure S6: Hypertonic-stressed cells displayed smaller and more homogenously distributed SGs than arsenite-stressed cells.
NRK cells were treated with arsenite (400 µM, 45 min) or exposed to hypertonicity (720 mosmol/kg, 45 min). These cells were pretreated or not with nocodazole (10 µM) for 1 h prior and during the indicated treatments to destabilize microtubules. In arsenite-treated cells, we observed that nocodazole induces the formation of considerably smaller SGs due to MT disruption. In addition, SGs which generally avoid the centrosomal region, were rather homogenously distributed. On the other, in hypertonic-stressed cells, nocodazole has a slight effect on SG size (slightly smaller) and no effect on their distribution. Scale bar: 10 µm.

A



B

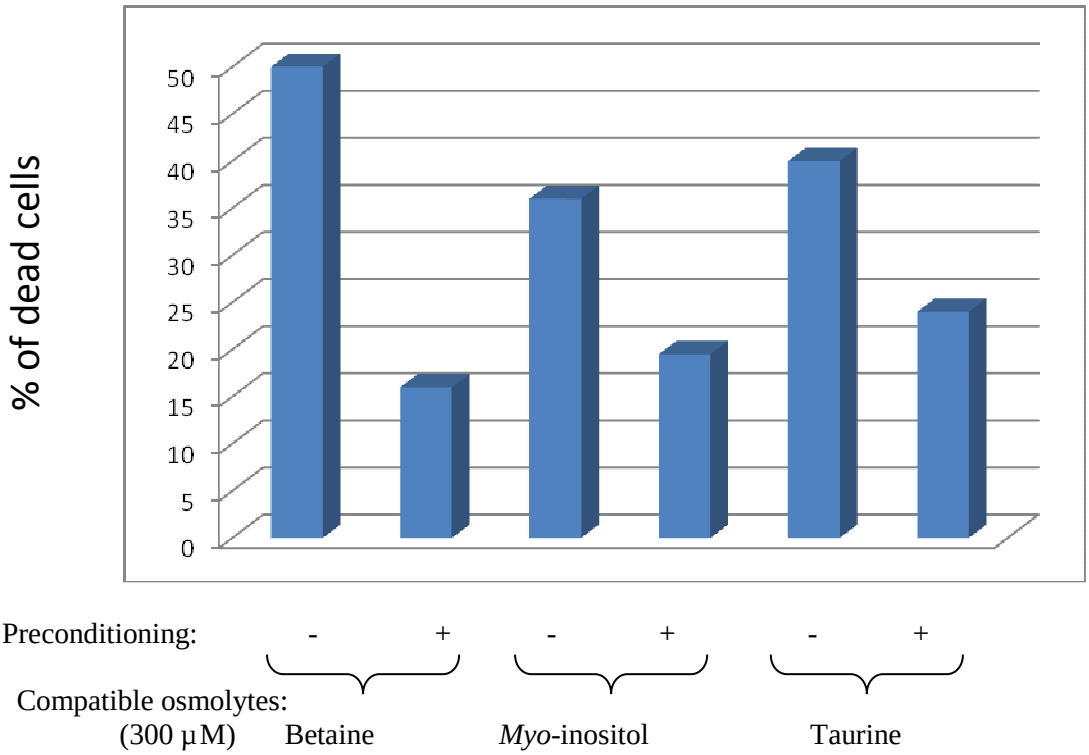


Figure S7: Hypertonic preconditioning (470 mosmol/kg, 24 h) in the presence of compatible osmolytes (300 µM) also increases the rate of cell survival. **A)** Phase contrast optical microscopy of control NRK cells or preconditioned for 24 h as indicated and exposed to a hypertonic shock (+ 400 mosmol/kg for 6 h). **B)** % of dead cells measured by trypan blue exclusion after 6 h of the indicated treatments.