Various Nucleolar Stress Inducers Result in Highly Distinct Changes in Water, Dry Mass and Elemental Content in Cancerous Cell Compartments: Investigation Using a Nano-Analytical Approach.

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Legends of supplementary figures

Figure S1I. 3D visualization of UBF (blue), and fibrillarin (red) in HeLa cells stably expressing H2B-GFP (green) by confocal microscopy. The upper half of each cell was removed to show the internal 3D distribution of fluorescence. A) control cells; B) cells treated with CX-5461 for 30 h. Scale bar is 5 μ m.



Figure S2. Illustrative examples of Scanning Transmission Electron Microscopy images of ultrathin cryo-sections of Hela H2B-GFP. (A) Control cell. (B) Cell treated with CX-5461. (C) Cell treated with DRB. Cell treated with DAM. Images were obtained without cryo-protectant, chemical fixative or heavy metal staining. Different organelles are identified such as nucleolus (NU) in the nucleus limited with nuclear envelope (NE) and mitochondria (MIT) in the cytoplasm. Note the tubular structure of mitochondria with cristae in all the conditions. Scale bar is 2 µm for images A, B, C and D. (E) The diameter of 324 to 487 mitochondria was measured on images of ultrathin cryo-sections of control cells and of cells treated with CX-5461, DRB or DAM by using Image J software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2018). P values, compared to control, were calculated using a two-tailed Student's-test unpaired with equal variance.



Figure S3. Quantification of the main elements (N, P, K, Na, Cl, and S) by energy dispersive X-ray spectrometry (EDXS) in regions of interest (ROI) of cell compartments in control cells and cells treated with CX-5461, DRB, or DAM. For clarity, data are presented as the percentage relative to control cells (100%) for each element: A) nitrogen, B) potassium, C) phosphorus, D) sodium, E) chloride, and F) Sulphur. Condensed chromatin (cond.chr.), nucleolar dense component (DFC), nucleolar granular component (GC), nucleolar fibrillar centers (FC), nucleoplasm (nucleopl.), cytosol, and mitochondria (mitoch.). Data are the means of three separate experiments +/- 1 standard deviation.

