SUPPLEMENTARY INFORMATION

Supplementary Methods

Microscopic visualisation of lysosomes by acridine orange staining. SK-N-MC cells were incubated with AO that accumulates in lysosomes to induce a red lysosomal fluorescence and a weak green cytosolic fluorescence following blue light excitation (1). Cells were incubated for 15 min/37°C with AO (20 μM), washed 3 times with PBS at room temperature and then incubated for 30 min/37°C with either Cu[Dp44mT], Fe[Dp44mT]$_2$, Cu(II), Fe(III) or Dp44mT (all at 25 μM). Samples were examined with a Zeiss Axio Observer.Z1 fluorescence microscope (Zeiss, Oberkochen, Germany) equipped with FITC and Texas Red filters. Images were captured with an AxioCam camera and AxioVision Rel. 4.7 Software (Zeiss).

Assessment of lysosomal membrane permeability. Lysosomal AO red fluorescence was measured (FL4 channel) using a FACSCalibur Flow Cytometer (Becton Dickinson, Franklin Lakes, NJ) equipped with a 488 nm argon laser.

Lysosomal permeability was also examined by following lysosomal cathepsin D release using a conjugate of pepstatin A and BODIPY FL (Invitrogen) that selectively binds to cathepsin D (2). Lysotracker® red (Invitrogen, Carlsbad, CA) was used to confirm co-localization of cathepsin D with lysosomes. Cells were incubated with Cu[Dp44mT], Dp44mT, or Cu(II) (at 25 μM) for 0.5 h/37°C and then pepstatin A-BODIPY FL conjugate (1 μM) and Lysotracker® Red (75 nM) were added and incubated for 0.5 h/37°C. The fluorescence microscope and camera system described

above was used to examine green (excitation, 495 nm, emission, 516 nm) and red (excitation, 577 nm, emission, 592 nm) fluorescence.

SUPPLEMENTARY REFERENCES

1. Persson HL, Kurz T, Eaton JW, Brunk UT (2005) Radiation-induced cell death: importance of lysosomal destabilization. *Biochem J* 389:877-884.
2. Chen CS, Chen WN, Zhou M, Arttamangkul S, Haugland RP (2000) Probing the cathepsin D using a BODIPY FL-pepstatin A: applications in fluorescence polarization and microscopy. *J Biochem Biophys Methods* 42:137-151.

SUPPLEMENTARY FIGURE LEGENDS

Fig. S1 Effect of incubation time on ^{64}Cu accumulation or ^{59}Fe release from prelabeled: **(A and B)** SK-N-MC cells or **(C and D)** HUVECs. Cells were prelabeled for 3 h/37°C with ^{64}Cu (10 μCi) or ^{59}Fe-transferrin (^{59}Fe-Tf; 0.75 μM), washed on ice and reincubated with medium (control) or the chelators (25 μM) for 0.5, 1, 3 or 6 h/37°C. **(E and F)** Effect of chelator concentration on ^{64}Cu or ^{59}Fe release from SK-N-MC cells as a function of concentration. Cells were prelabeled as above and reincubated at 37°C with medium (control) or the chelators (0.025-500 μM) for 3 h/37°C. Results are mean ± SD (3 experiments).

Fig. S2 Graph showing quantification of loss of AO red fluorescence by Cu[Dp44mT] (25 μM) after 0.5 h/37°C incubation as determined by flow cytometry. The $Fe[Dp44mT]_2$ complex, Dp44mT, $CuCl_2$ or $FeCl_3$ (at 25 μM), had no significant effect relative to the control. Results are mean ± SD (3 experiments). *** *vs*. control, $p<0.001$.

Fig. S3 The Cu chelators BCS or TM, their Cu complexes or $CuCl_2$ (0.01 – 500 μM) have no effect on cellular viability of SK-N-MC cells after a 72 h/37°C incubation.

Fig. S4 NAC (5 mM) preserves lysosomal integrity when co-incubated with Cu[Dp44mT] (25 μM; 0.5 h/37°C) as indicated by the retention of red vesicular staining by the lysosomotropic dye, acridine orange. Results are a typical of 3 performed. Scale bar: 50 μm.