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Electronic supplementary information

Tamoxifen-like metallocifens target thioredoxin system determining mitochondrial impairment leading to apoptosis in Jurkat cells

Valeria Scalcon,[a]* Michèle Salmain,[b]* Alessandra Folda,[a] Siden Top,[b] Pascal Pigeon,[b,c] Hui Zhi Shirley Lee,[b,c] Gérard Jaouen,[b,c] Alberto Bindoli,[d] Anne Vessières,[b]**and Maria Pia Rigobello[a]**

a) Dipartimento di Scienze Biomediche, Università di Padova, Via Ugo Bassi 58/b, 35131 Padova, Italy.

b) Sorbonne Universités, UPMC Univ Paris 06, CNRS, Institut Parisien de Chimie Moléculaire (IPCM), F-75005 Paris, France

c) Chimie ParisTech, 11 rue Pierre et Marie Curie, F-75005 Paris, France

d) Istituto di Neuroscienze (CNR) Sezione di Padova, c/o Dipartimento di Scienze Biomediche, Via Ugo Bassi 58/b, 35131 Padova, Italy.

* Valeria Scalcon and Michèle Salmain contributed equally to the work

** Anne Vessières and Maria Pia Rigobello: co-corresponding authors

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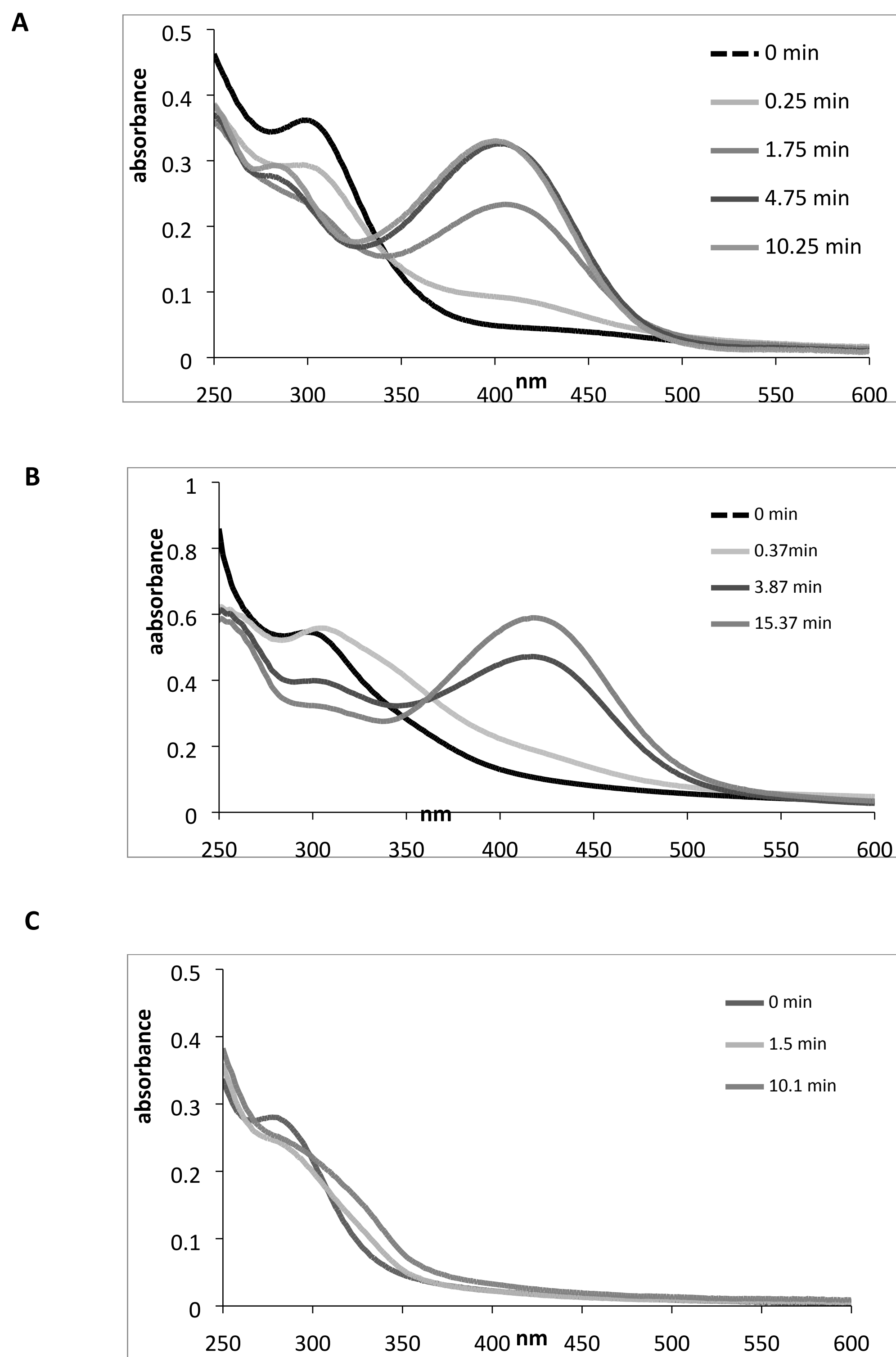

Fig.S1. **UV-Vis spectral monitoring of enzymatic oxidation of Fc-OH-Tam (A), Rc-OH-Tam (B), or OH-Tam[3] (C).** Each compound was mixed with HRP (44 nM) and H_2O_2 (200 µM) in 0.2 M Tris-HCl, 1 mM EDTA pH 8.1 and the UV-Vis spectra were recorded at the indicated times. Gradual appearance of a peak at 402 nm in the **Fc-OH-Tam** spectra (A) and a peak at 418 nm in the **Rc-OH-Tam** spectra **(B)** are assigned to the formation of the quinone methide derivatives. Disappearance of the peak at 279 nm for **OH-Tam[3] (C)** is assigned to its progressive oxidation without conversion to the quinone methide. (see Experimental Section for details)

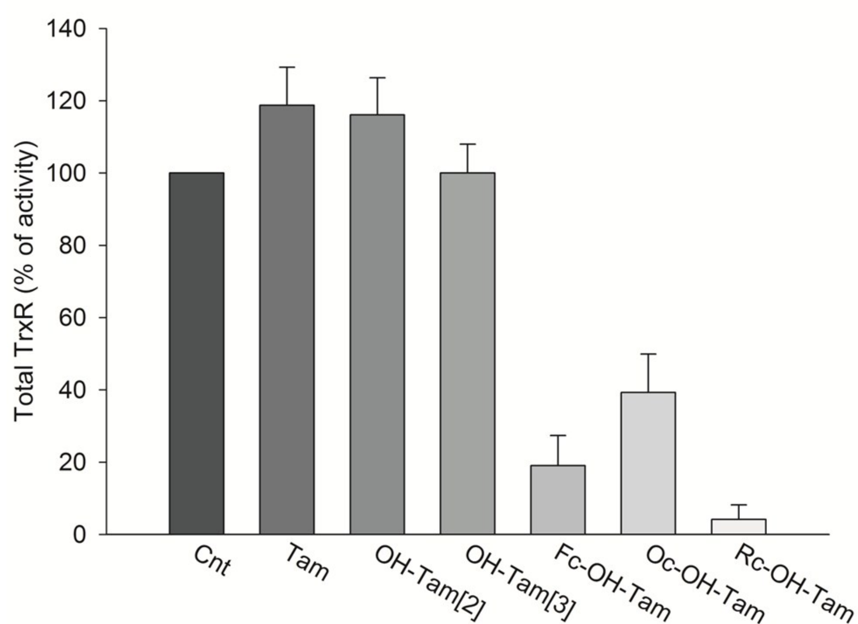

Fig. S2. Total TrxR activity in cell lysates. TrxR activity was determined after treatment of Jurkat cells (2 x 10^6) for 18 h with the indicated compounds (15 µM) following the procedure reported in the Experimental Section.

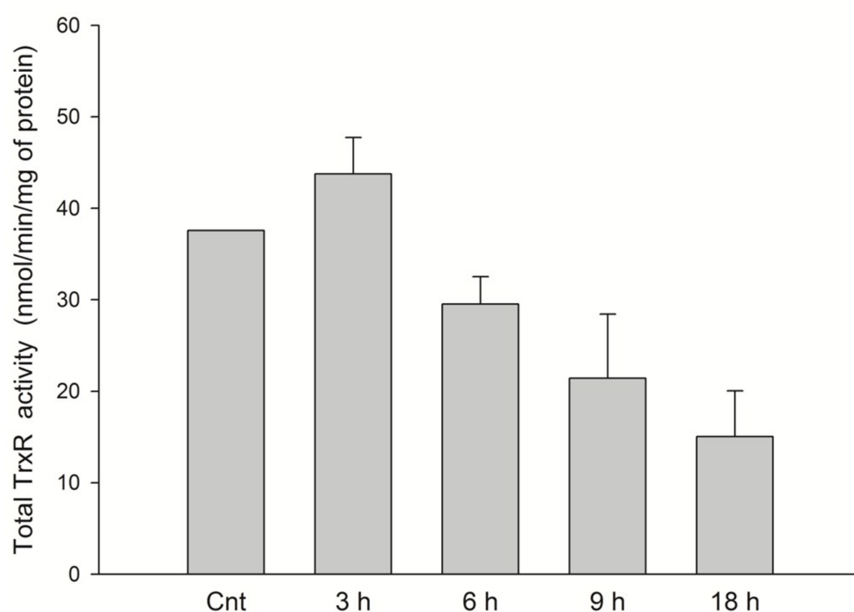

Fig. S3. Time scale of total TrxR activity in cell lysates after Fc-OH-Tam treatment. Jurkat cells (2 x 10^6) were treated for 3, 6, 9 or 18 h with 15 µM **Fc-OH-Tam**, then lysed and subjected to thioredoxin activity assay as described in Experimental Section.

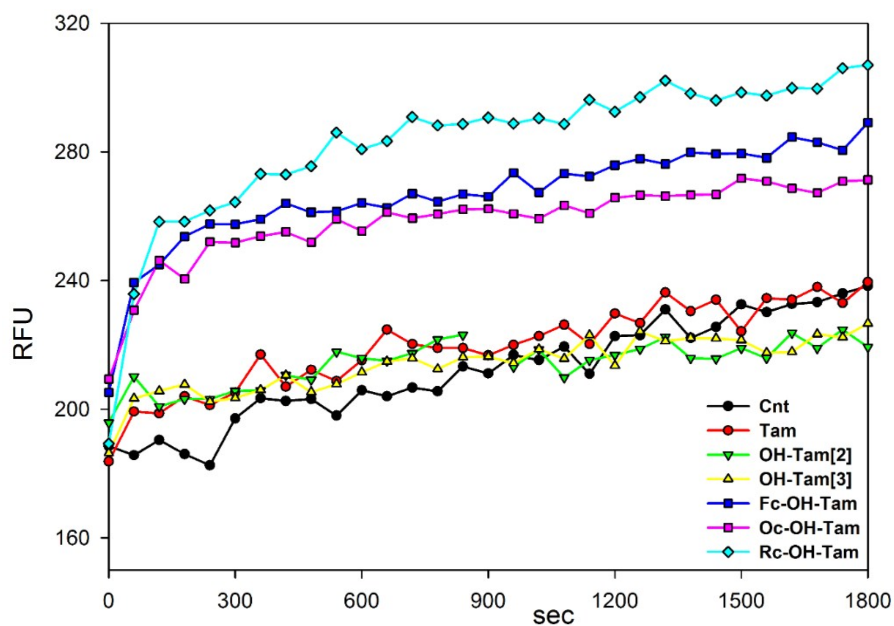

Fig. S4. Short-term ROS production of Jurkat cells after treatment with TLMs or organic tamoxifen derivatives. Jurkat cells (4 x 10^5/well) were incubated for 1 h with 15 µM DHR. Afterwards, the medium was removed and 10 µM compounds were added to the cells. Fluorescence increase was monitored on a plate reader as reported in Experimental Section.

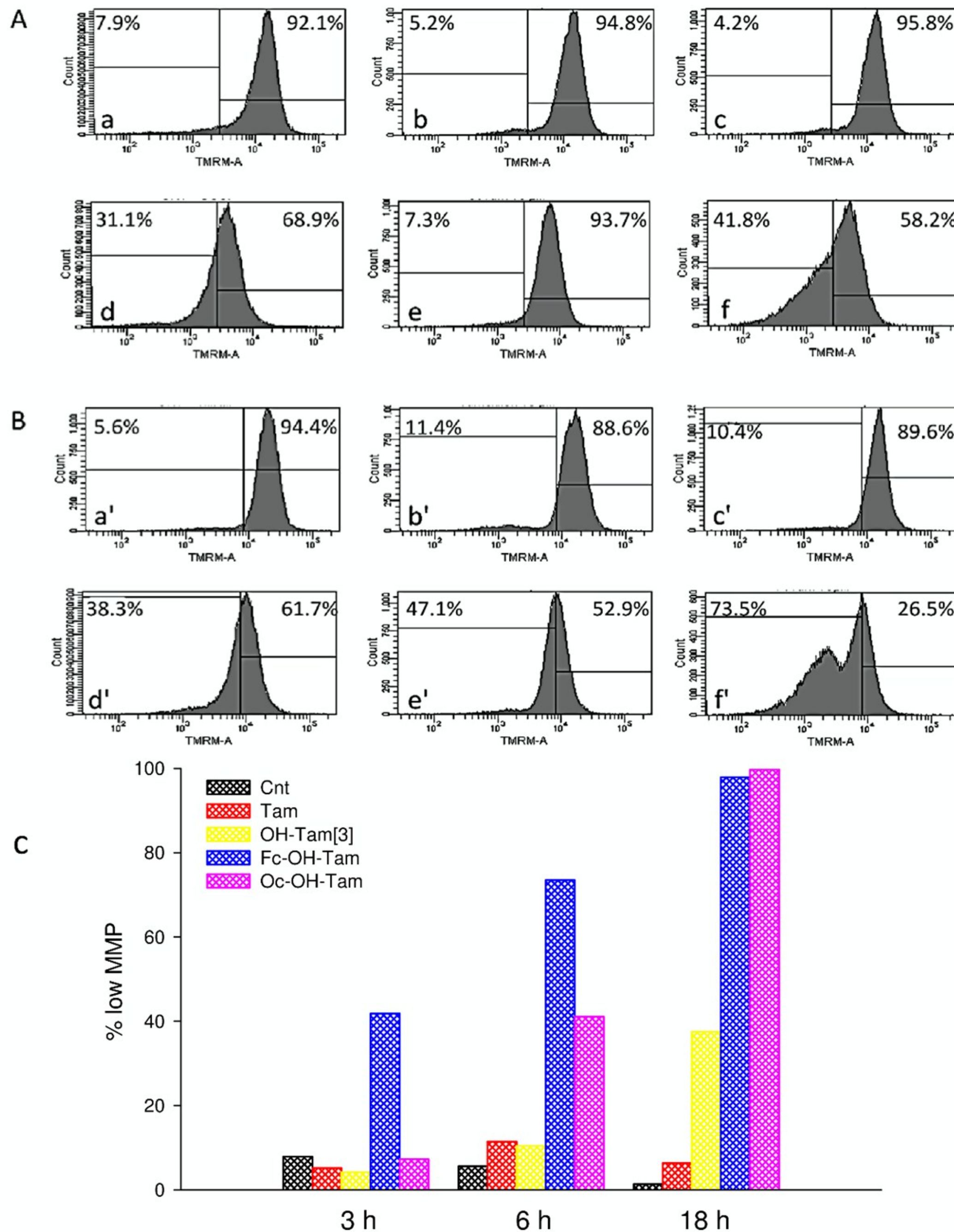

Fig. S5. Mitochondrial membrane potential (MMP) analysis of Jurkat cells after treatment with the various compounds at different incubation times. Cells (1 x 10^6) were treated for 3 h **(A)** or 6 h **(B)** with the indicated compounds (15 µM) and MMP was measured on 3 x 10^4 cells by flow cytometry analysis following TMRM fluorescence intensity (see Experimental Section). The two populations divided by the vertical gate correspond to cells with low MMP (left) and cells with high MMP (right). 1 µM carbonyl cyanide-m-chlorophenylhydrazone (CCCP), a classic uncoupling agent, was used as a positive control. a,a' **Cnt**; b,b' **Tam**; c,c' **OH-Tam[3]**; d,d' **CCCP**; e,e' **Oc-OH-Tam**; f,f' **Fc-OH-Tam**. **(C)** Percentage of Jurkat cells with low MMP in the range 3-18 h from the treatment with 15 µM compounds.

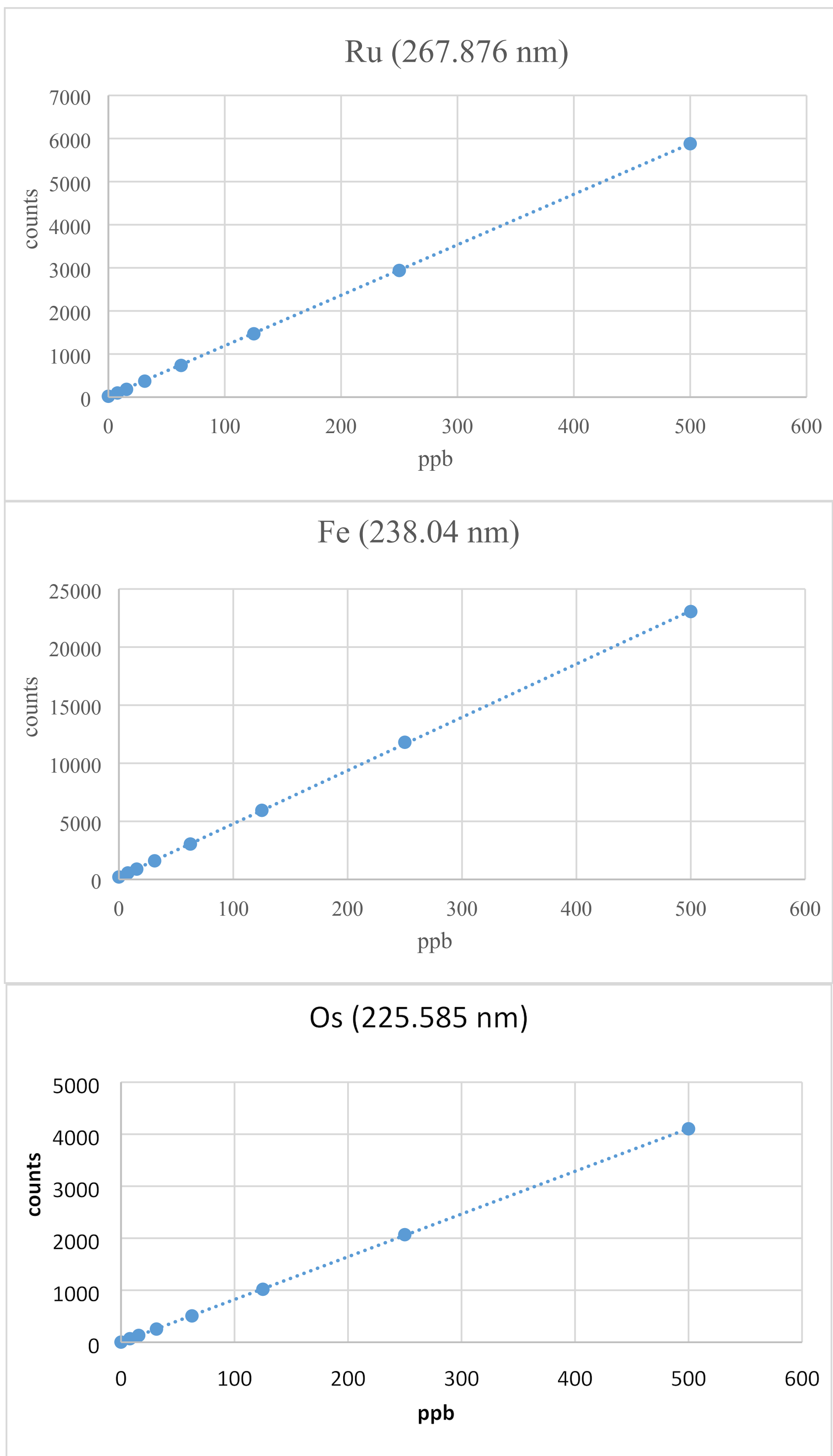

Fig. S6. Calibration curves for Ru, Fe, Os in the range (7.8 – 500 ppb) measured by ICP-OES