## SUPPLEMENTAL MATERIALS

### SUPPLEMENTAL TABLES

**Table S1:** Select published overall formation constants ($K_f$) of 8-hydroxiquinoline with $Cu^{II}$ or $Zn^{II}$.

| **Reference** | Steger *et al*., (1973) (1) | Albert *et al*., (1953) (2) | Maley *et al*., (1949) (3) | Tardito *et al*., (2012) (4) | Ferrada *et al*., (2007) (5) |
|---|---|---|---|---|---|
| **log$K_f$ ($Cu^{II}$)** | n.d. | 23.4 | 29 | 33.57 | 10.9* |
| **log$K_f$ ($Zn^{II}$)** | 9.49 | 17.2 | 20.81 | n.d. | 9.3* |
| **Conditions** (solvent, temperature, ionic strength *I*) | 60% v/v Dioxane-water; $I_{NaClO_4}$ = 0.1M; 25°C | 0.1N KOH; 20°C | 70% v/v water-dioxane; 25°C | DMSO:water 1:1 (w/w); $I_{KCl}$=0.1M; 25°C | Barth's solution; 25°C |

*reported as conditional equilibrium constants

n.d. : not determined

### REFERENCES for Table S1


1. **Steger HF, Corsini A.** 1973. Stability of metal oxinates — I Effect of ligand basicity. Journal of Inorganic and Nuclear Chemistry **35:**1621-1636.
2. **Albert A, Gibson MI, Rubbo SD.** 1953. The influence of chemical constitution on antibacterial activity. VI. The bactericidal action of 8-hydroxyquinoline (oxine). British journal of experimental pathology **34:**119-130.
3. **Maley L, Mellor D.** 1949. The Relative Stability of Internal Metal Complexes. I. Complexes of 8-Hydroxyquinoline, Salicylaldehyde, and Acetylacetone. Australian Journal of Scientific Research **2:**92.
4. **Tardito S, Barilli A, Bassanetti I, Tegoni M, Bussolati O, Franchi-Gazzola R, Mucchino C, Marchiò L.** 2012. Copper-dependent cytotoxicity of 8-hydroxyquinoline derivatives correlates with their hydrophobicity and does not require caspase activation. Journal of Medicinal Chemistry **55:**10448-10459.
5. **Ferrada E, Arancibia V, Loeb B, Norambuena E, Olea-Azar C, Huidobro-Toro JP.** 2007. Stoichiometry and conditional stability constants of Cu(II) or Zn(II) clioquinol complexes; implications for Alzheimer's and Huntington's disease therapy. NeuroToxicology **28:**445-449.

**Table S2:** Partial ($k_1$ and $k_2$) and overall formation constants ($K_f$) of 8HQ, CQ, NQ and HCA determined by UV/Vis titration in HEPES buffer at pH7.4. The maximal errors are <5% for $k_1$, <3% for $k_2$ and <15% for $K_f$.

| **Ligand** | 8-Hydroxyquinoline (8HQ) | | | Clioquinol (CQ) | | | Nitroxoline (NQ) | | | 8-hydroxyquinoline-2-carboxylic acid (HCA) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Constants** | $\log k_1$ | $\log k_2$ | $\log K_f$ | $\log k_1$ | $\log k_2$ | $\log K_f$ | $\log k_1$ | $\log k_2$ | $\log K_f$ | $\log K_f$ |
| **Cu(II)** | 4.74 | 4.49 | 9.22 | 3.31 | 2.1 | 8.15 | 4.59 | 3.49 | 8.07 | 6.47* |
| **Zn(II)** | 4.84 | 4.24 | 9.08 | 3.89 | 4.85 | 8.75 | 4.83 | 3.06 | 7.89 | 7.35* |

* HCA forms only a 1:1 complex. Hence we can only report the overall formation constant $K_f$.

**SUPPLEMENTAL FIGURES**

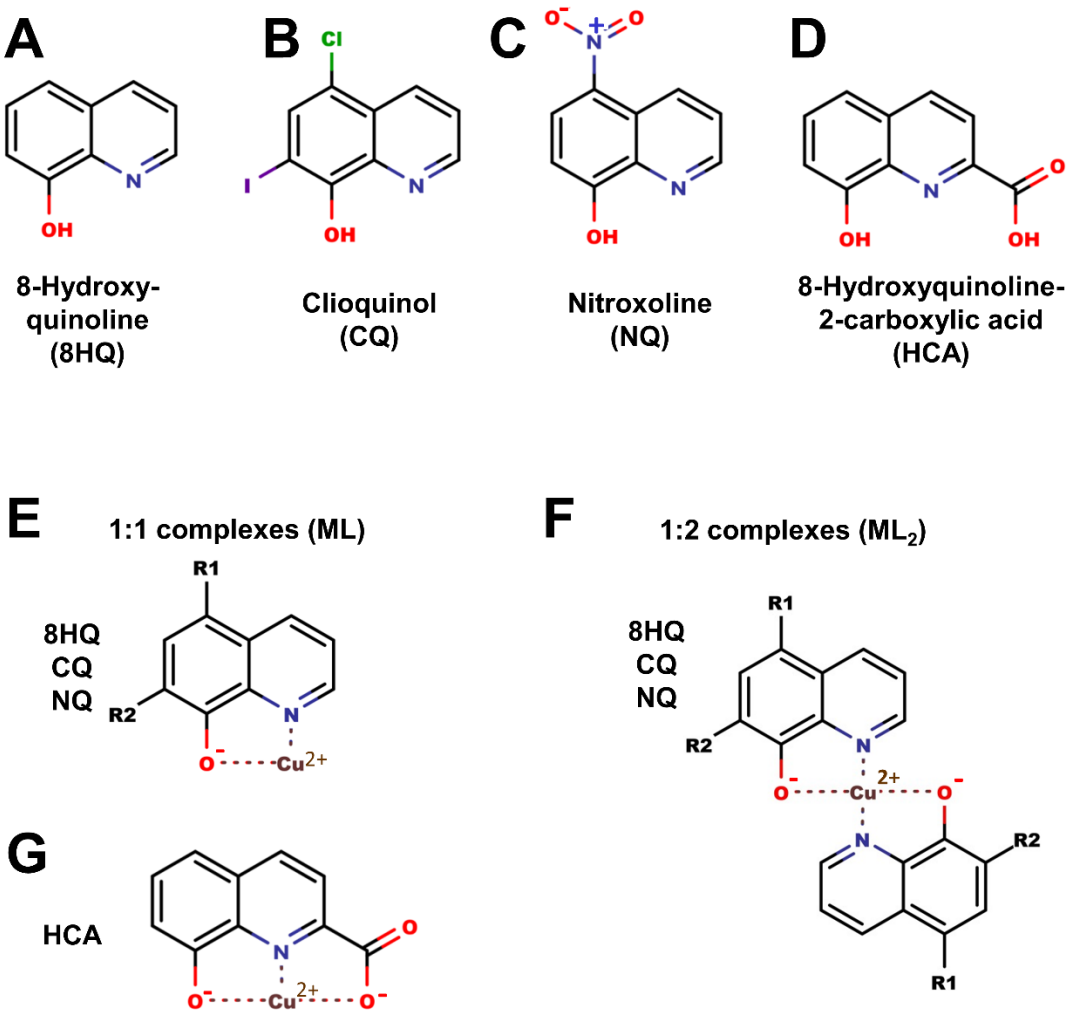


**Figure S1:** Molecular structures of select 8-hydroxyquinolines and their metal-complexes. (A) 8-hydroxyquinoline (8HQ). (B) Clioqionol (CQ). (C) Nitroxoline (NQ). (D) 8-hydroxyquinoline-2-carboxylic acid (HCA). (E) Generalized structural representation of the 1:1 metal complexes of 8HQ, CQ and NQ **(F)** Generalized model of the 1:2 complex of 8HQ, CQ or NQ. **(G)** The 1:1 copper complex of HCA. Please note that the Cu ion in these complexes coordinates ligands in a tetrahedral or octahedral geometry. Besides 8-hydroxyquinoline ligands, solvent molecules (e.g. $H_2O$) or ions (e.g. $Cl^-$, $OH^-$) may also be incorporated to accommodate these geometries but are not shown here. R1 and R2 are synonymous of the respective sidegroups found in 8HQ (R1, R2:H), CQ (R1: Cl; R2: I) or NQ (R1: $NO_2$; R2: H).

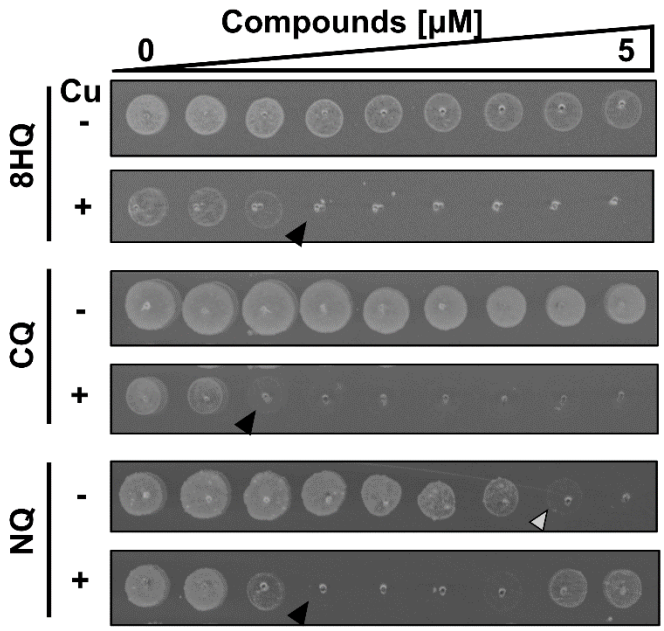


**Figure S2:** Bactericidal activity of 8-hydroxyquinolines. Samples were obtained from dose response curves shown in Figure 1 D, E, and F, and spotted on Middlebrook 7H10 agar plates to demonstrate bactericidal action of 8HQ, CQ and NQ. Black and grey arrowheads point towards the MICs in the presence (+) or absence (-) of 7.5 µM $CuSO_4$ (Cu) and match the respective MICs from the dose response curve assay (Fig. 1D, E, F, respectively). The concentration differential of compounds between individual spots is 2-fold with the maximum concentration shown being 5 µM.

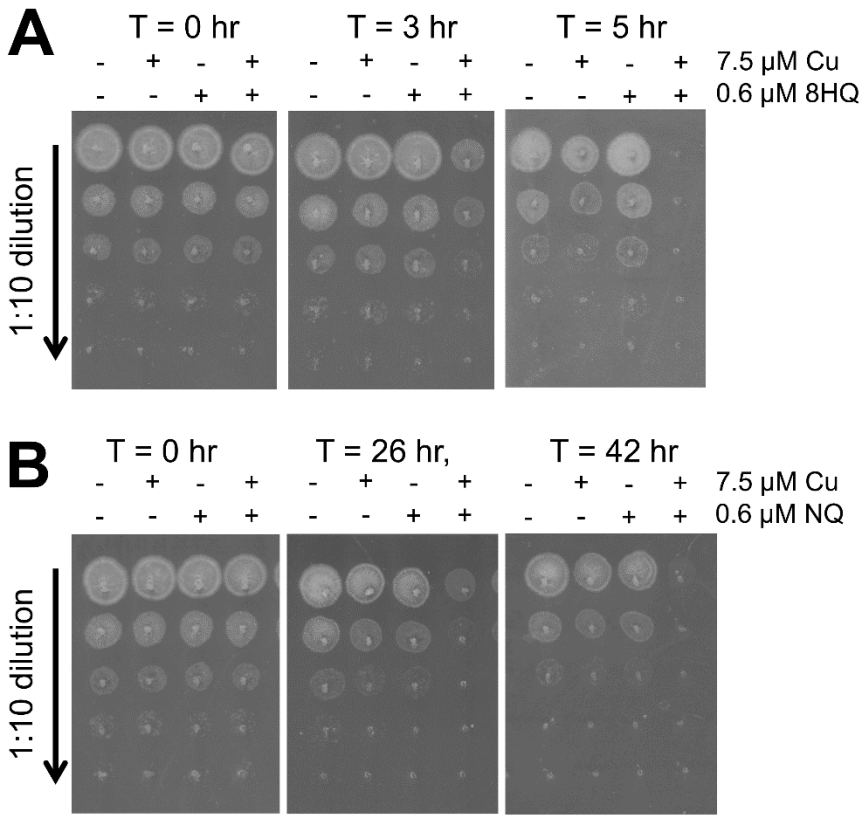


**Figure S3:** Time to kill assay on Mtb mc$^2$6230 for **(A)** 8HQ and **(B)** NQ. Aliquots of samples receiving the indicated treatment were pulled at specific time points over the course of 48h and then spotted on Middlebrook 7H10 agar plates. Only time points at which significant changes in regards to growth were noted are shown.

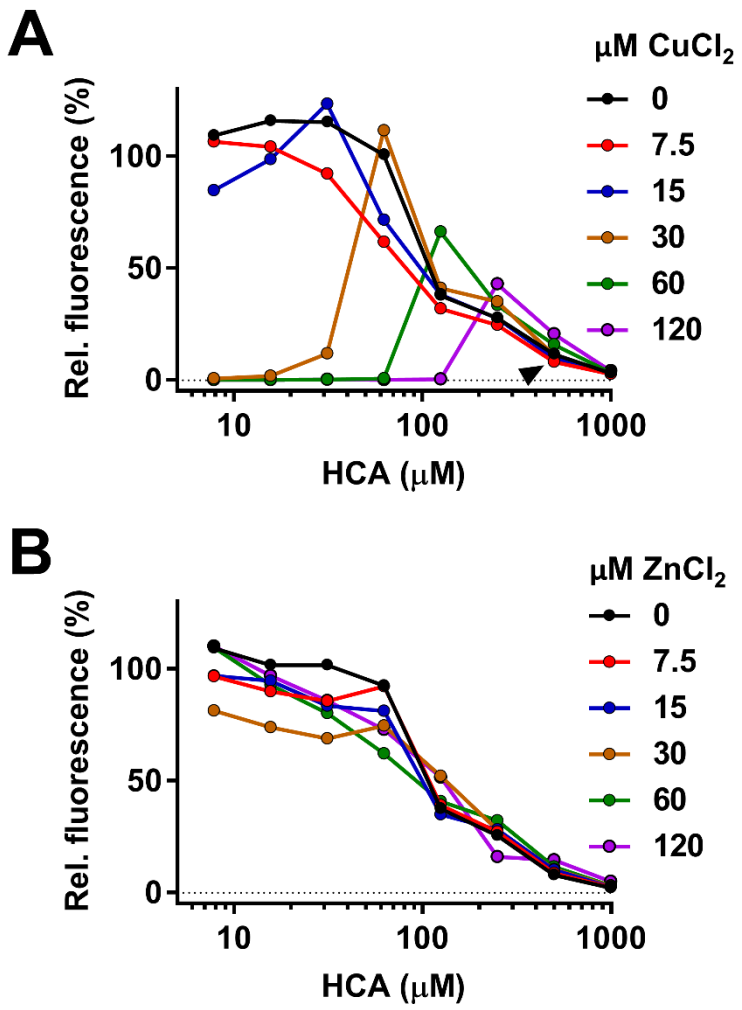


**Figure S4.** Dose response matrices for 8-hydroxyquinoline-2-carboxylic acid (HCA) in combination with **(A)** copper chloride (Cu) or **(B)** zinc chloride (Zn). Individual data points represent the mean fluorescence (resazurin dye was used) of three replicates from the same assay plate. Error bars represent standard deviation of at least 3 sample replicates. Error bars indicate standard deviation. All values were background subtracted and normalized to their respective controls. The presented data are representative of at least two independent repeats.

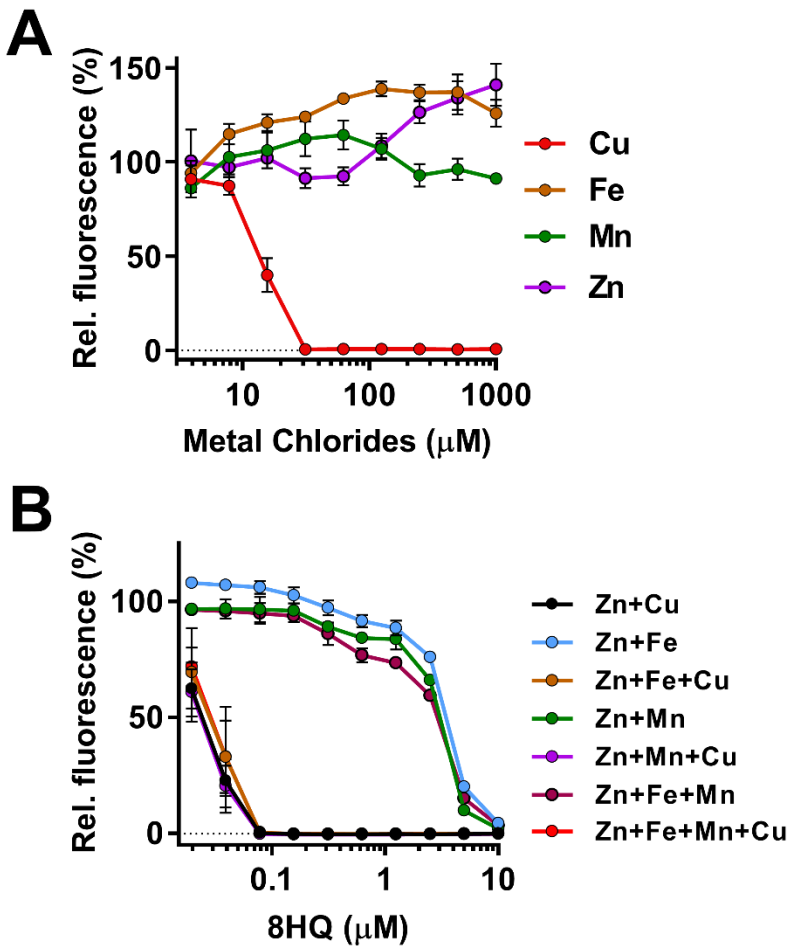


**Figure S5**. **(A)** Sensitivity of Mtb mc$^2$6230 to various metal chlorides in HdB medium. **(B)** Metal competition assay to demonstrate robustness of 8HQ's copper related anti-Mtb properties in the presence of other metals at physiologically relevant concentrations. Zn, Fe and Mn are the most likely in vivo competitors of Cu based on their affinity for 8HQ (see binding constants ($K_f$) from Table S2) and their abundance in humans.

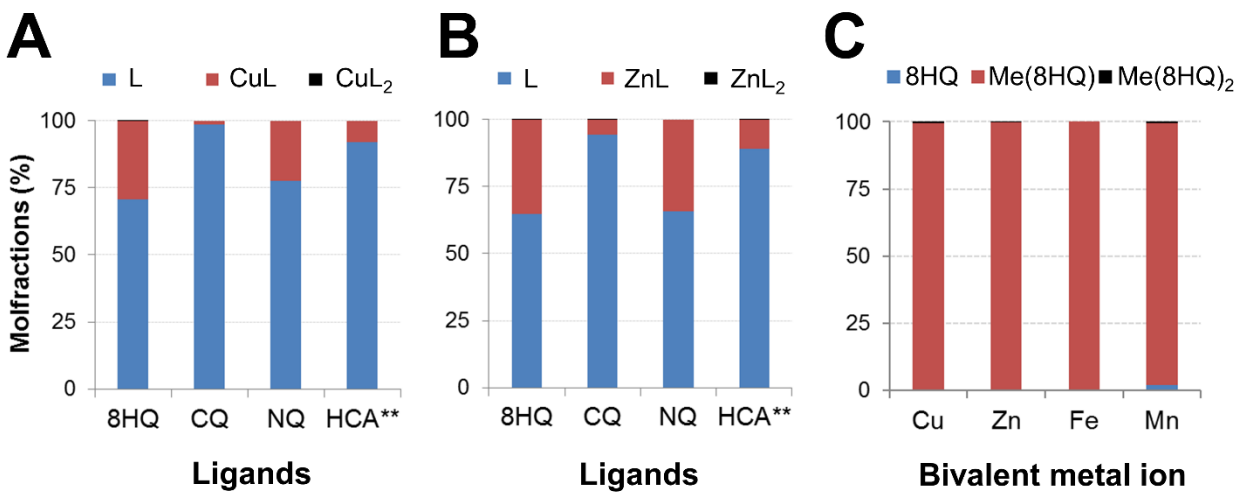


**Figure S6.** Calculated molfractions of 8-hydroxyquinoline (8HQ), Clioquinol (CQ), Nitroxoline (NQ) at 0.16 µM (approximate MIC concentration, see Fig. 1) in the presence of **(A)** 7.5 µM Cu or **(B)** Zn ions and for 8-Hydroxyquinoline-2-carboxylic acid (**HCA) at 60 µM with 30 µM of metals present. **(C)** Molfractions for 8HQ in the presence of various bivalent metal ions (M) (Cu, Zn, Fe or Mn). The molfractions shown in panel (A) and (B) are based on $K_f$ values listed in Table S2. Molfractions in panel C were calculated using $K_f$ values taken from Albert et al. 1953 (Table S1). Calculations were performed using the BINDSIM tool, which is available online at www.supramolecular.org.

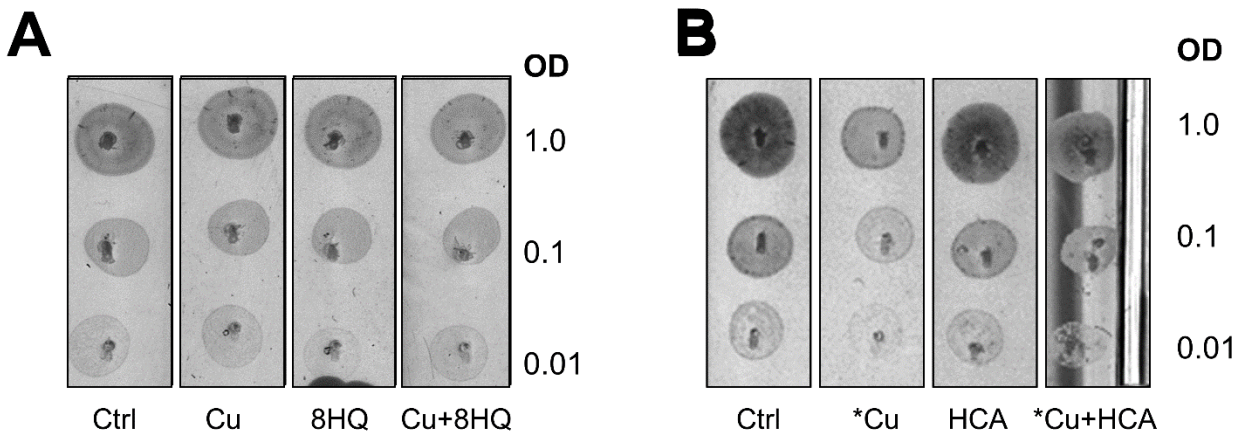


**Figure S7.** Viability assay of Mtb mc$^2$6230 samples used in the PhenGreenFL assay. Panels **(A)** and **(B)** correspond to samples shown in Figure 4A and 4B, respectively. Prior adding Phen Green FL dye to the treated and washed samples, an aliquot was pulled and diluted in 7H9 medium in 10-fold increments. From each dilution, 5µL were spotted on Middlebrook 7H10 agar plates as indicated. Samples were treated with (A) 2.5 µM Cu, 0.62 µM 8HQ or both and (B) 50 µM Cu (*Cu), 100 µM HCA or both.

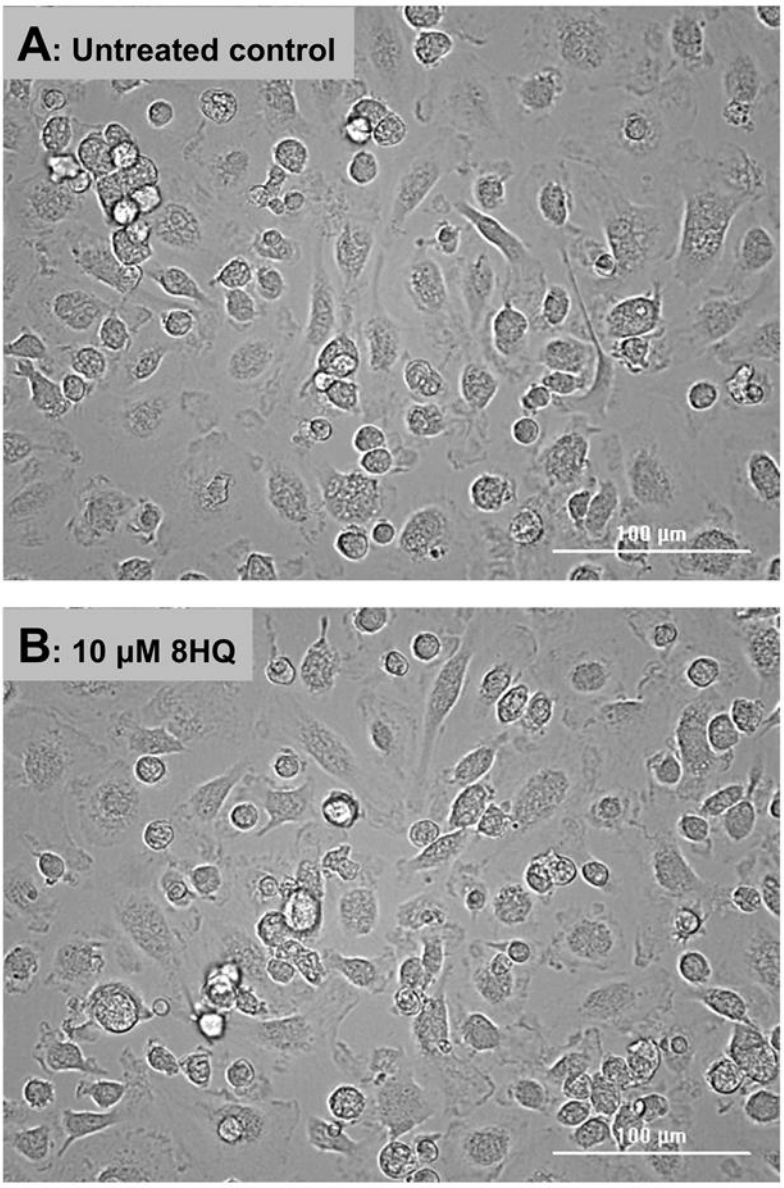


**Figure S8:** Peritoneal macrophages **(A)** untreated or **(B)** treated for 48h with 10 µM 8-hydroxyquinoline (8HQ). Images were taken using the Cytation 3 imaging reader (BioTek) equipped with a 20x objective in Brightfield mode. The reader is controlled by Gen5 software. Images were cropped and minimally processed using Microsoft PowerPoint 2016 software.

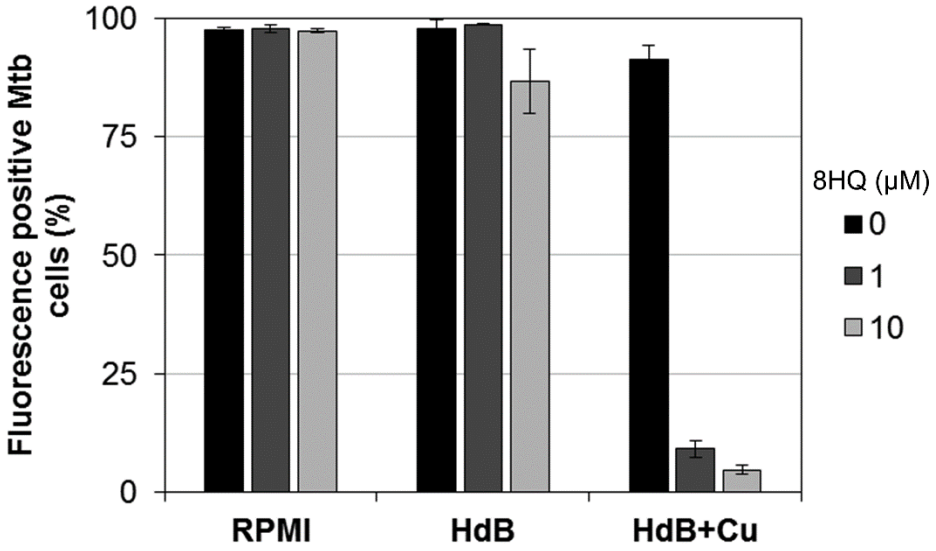


**Figure S9:** Fluorescein diacetate (FDA) viability stain and flow cytometry of Mtb mc²6230 exposed to 8HQ in cell culture medium (RPMI 1640) with 10%FBS as source of copper ions, or HdB medium without or with (+Cu) 7.5 µM $CuSO_4$. Samples were stained with FDA after 48h treatment with 8HQ.