Supplemental File A: Methods and rationale

Apart from the cell types used, the screening protocol employed in this study differed markedly from the Wills *et al.* method. We directly injected test compounds onto cells during the screening assay rather than use a protracted pre-screen exposure. By measuring OCR before the addition of test chemicals, corrections for well-to-well variations in OCR resulting from cell seeding and plate edge effects were made possible, allowing us to assess mitochondrial function using change in normalized respiration instead of absolute OCR values. Pilot experiments conducted prior to screening revealed that longer pre-screen exposures only marginally increased effects on respiration and the number of active chemicals (data not shown). Additionally, we excluded the use of oligomycin as an injection reagent in the RSA protocol. The main purpose of oligomycin in typical respirometric mitochondrial testing is to determine coupling efficiency, i.e. the fraction of mitochondrial respiration leading to ATP synthesis. For HTT screening purposes, the absolute coupling efficiency of HepG2 cells is less important than relative coupling efficiency before and after test compound injection. Uncouplers decrease coupling efficiency by increasing proton leak and are readily identified with the tiered testing approach presented herein. Furthermore, putative ATP synthase inhibitors cannot be identified in the presence of oligomycin and omission of this injection step allowed the identification 29 such chemicals in this study.