Supporting Information

Off-target inhibition of human dihydroorotate dehydrogenase (*h*DHODH) highlights challenges in the development of Fat mass and obesity-associated protein (FTO) inhibitors

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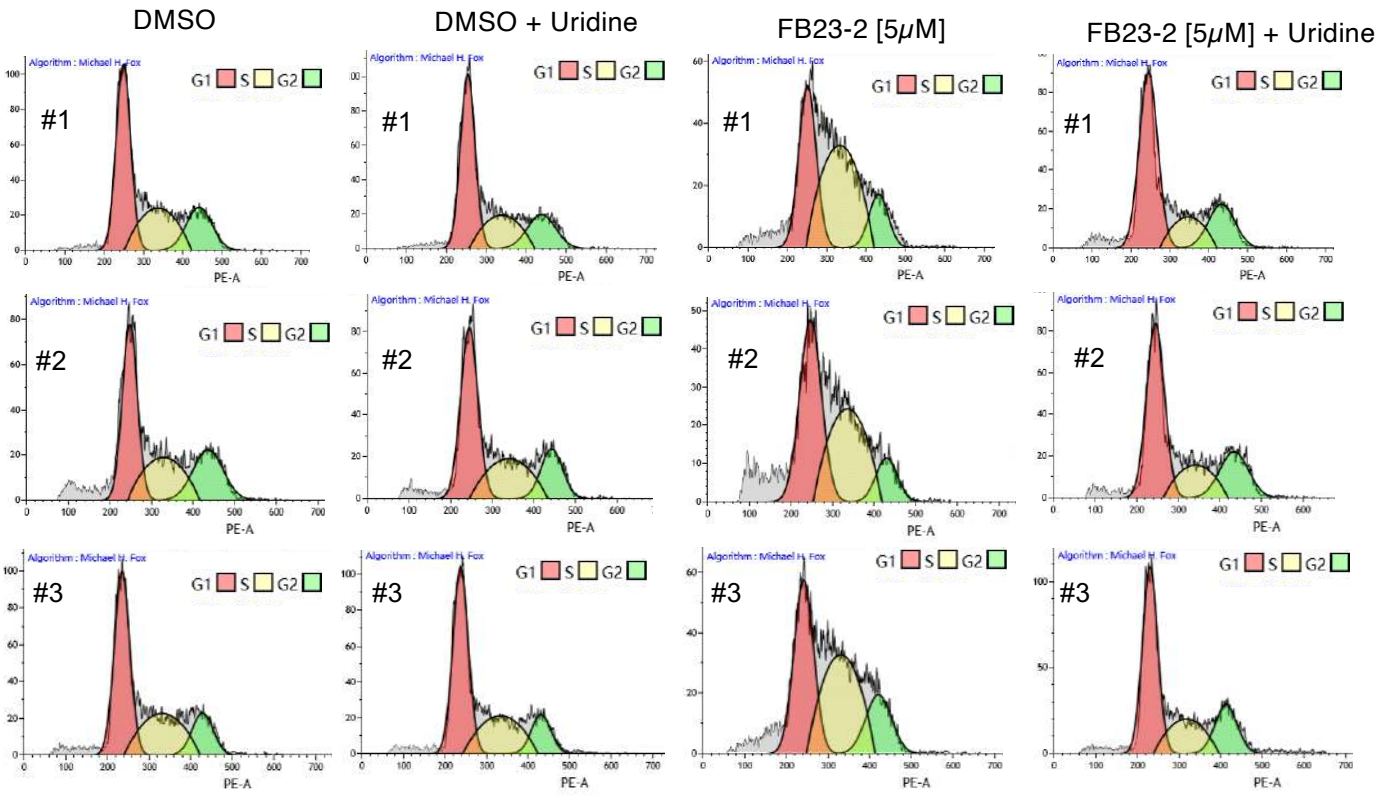

Figure S1. Cell cycle analysis of FB23-2 treated K562 cells. Cell cycle distribution of K562 cells analyzed 48 h following treatment with 5 μM FB23-2, in the presence or absence of 100 μM uridine in the culture medium. DMSO-treated cells served as the control.

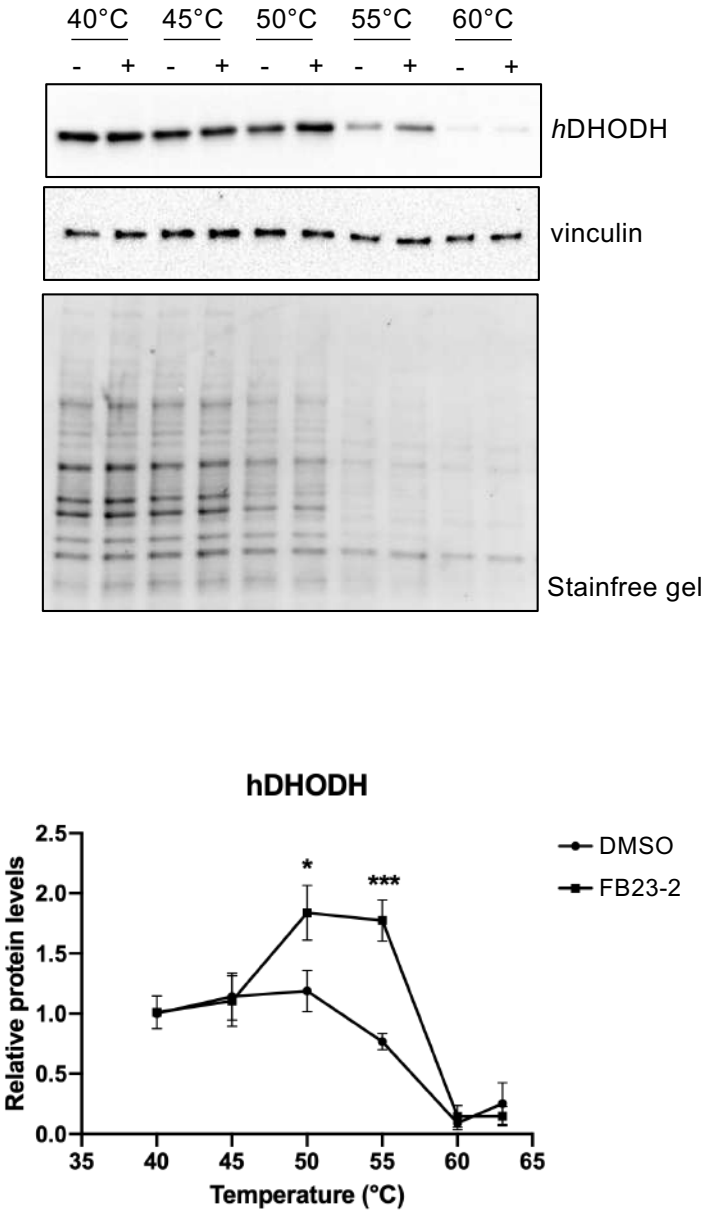

Figure S2. CETSA assay with *h*DHODH in K562 cells. Upper panel, representative Western blot analysis demonstrating the stabilizing effect of FB23-2 on *h*DHODH at various temperatures in K562 cells. Lower panel, graph illustrating the quantified levels of *h*DHODH relative to total proteins from three replicates. Data are represented as mean ± SD. *P < 0.05, ***P < 0.001.

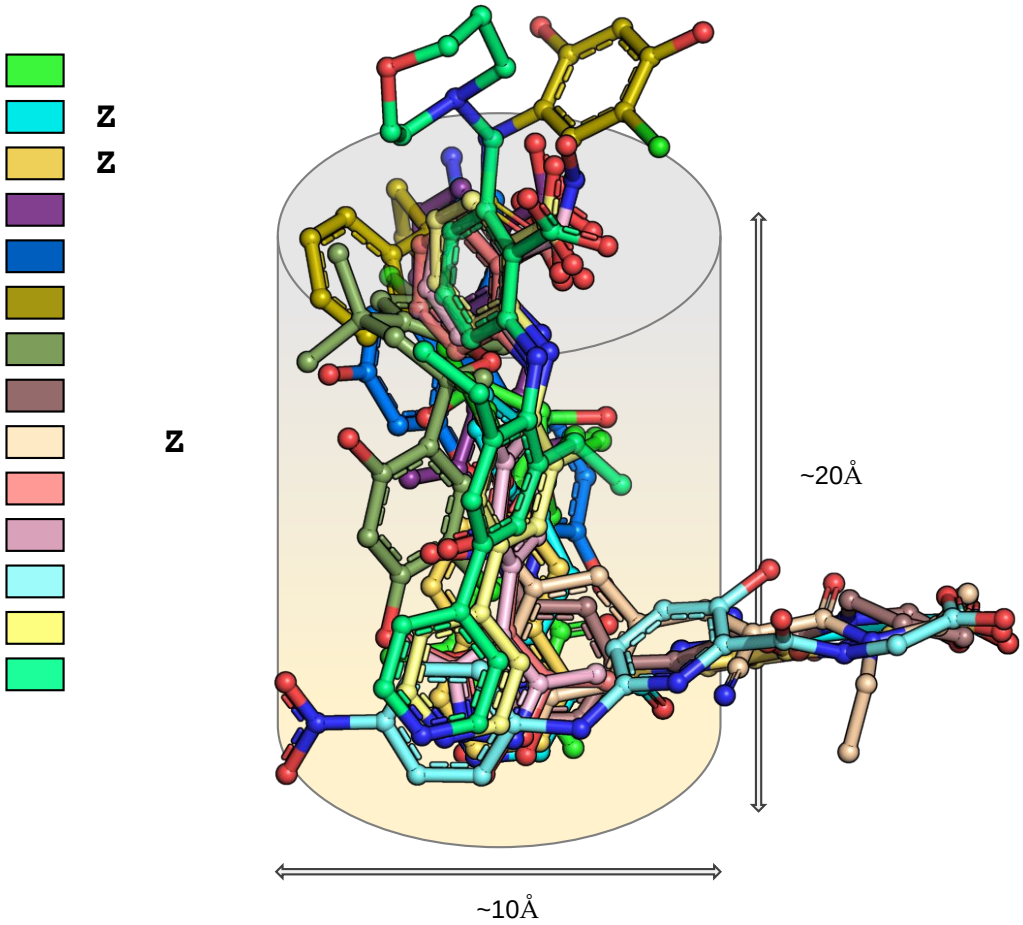

Figure S3. Superposed three-dimensional structures of representative FTO-inhibitors solved in complex with FTO. For each compound, the corresponding PDB code of the FTO complex is shown in the color-code legend. The 20Åx10Å cylinder-shaped cleft of hDHODH is shown as reference. According to docking analysis, the compounds indicated in bold (Supplementary Table I), due to their size/conformations, would make severe steric clashes with hDHODH and are therefore predicted as selective for FTO.