

## SUPPLEMENTARY FIGURES

**Supplementary Figure S1. Effect of EDK-87 and EDK-88 on primary sample viability.** AML patient and healthy blood samples were treated at the indicated concentrations with EDK-87 and EDK-88 for 72h. Frequency of live cells is represented. Results are the same as Figure 1E separated by sample.

**Supplementary Figure S2. EDK-87 and EDK-88 are effective *in vivo*.** Representative luminescence images of experimental mice, referred to Figure 2; **A.** MonoMac-1 and **B.** KG-1 cells. **C.** HL60, KG1 and SKM1 cells and their AraC resistant (AraC<sup>R</sup>) counterparts were stained with LysoTracker Green and analyzed by Flow cytometry. HL60 and MonoMac1 cells were treated with indicated concentrations of EDK87 and 50  $\mu$ M chloroquine for 24h. Presence of LC3-I and -II was assessed by Western blotting and normalized to housekeeping protein GAPDH. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$  in one-way ANOVA comparing with untreated control, except (F), paired t-test.

**Supplementary Figure S3. EDK-87 and EDK-88 induce lysosomal and autophagic compartment expansion.** KG1 and MonoMac1 cells were treated with EDK-87 or EDK-88 at 10  $\mu$ M for 24 h, stained with LysoTracker Deep Red and analyzed by **A.** Flow cytometry and **B.** confocal microscopy (scale bar = 10  $\mu$ m). **C.** LysosomalMetriq-transduced KG1 cells were treated with the compounds at 5 and 10  $\mu$ M for 24h and the red/green fluorescence was measured by flow cytometry. **D.** displays representative membranes and **E.** quantified LC3-II/GAPDH bands. **F.** HL60, KG1 and MonoMac1 cells were treated for 24h with 10  $\mu$ M EDK-87 or EDK-88, stained with CytoID and acquired in a flow cytometer. **G.** HL60, KG1 and MonoMac1 cells were treated for 48h with 10  $\mu$ M EDK-87 or EDK-88 alone or in combination with 500  $\mu$ M 3-methyladenine (3MA) and viability was analyzed by flow cytometry. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$  in one-way ANOVA comparing with untreated control, except (F), paired t-test.

**Supplementary Figure S4. EDK-87 and EDK-88 induce lysosomal membrane permeabilization A.** Gal3-GFP-transduced HL60 cells were treated with indicated compounds at 10 and 20  $\mu$ M for 24h. Cells with galectin 3 puncta were counted by confocal microscopy. Left panel shows representative galectin 3 puncta images (scale bar = 10  $\mu$ m), right panel shows quantification of puncta-containing cells. **B.** KG1 and MonoMac1 cells loaded with Dextran-Rhodamine B were treated with EDK compounds at 10  $\mu$ M for 24h and analyzed by confocal microscopy (scale bar = 10  $\mu$ m). **C.** KG1 and MonoMac1 cells were co-treated with  $\alpha$ -tocopherol at the indicated concentrations and EDK-87 or -88 at 10  $\mu$ M for 48h. Cell viability was assessed by flow cytometry. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$  in Two-way ANOVA (A) or One-way ANOVA (C).

**Supplementary Figure S5. EDK-87 and EDK-88 induce AML differentiation through a TFEB-dependent mechanism. A.** KG1 and MonoMac1 cells were loaded with Fura2 probe and treated with 10  $\mu$ M EDK-87 or EDK-88, 50  $\mu$ M chloroquine (negative control) or 1  $\mu$ M ionomycin (positive

control). Fluorescence was acquired at indicated time points. The ratio (340/508)/(380/508) is represented. **B.** HL60 or MonoMac1 cells were treated at indicated times with EDK-87 or -88 at 10  $\mu$ M, and gene expression of *TFEB* and *MYC* was assessed by Real Time PCR and normalized to time 0. **C.** KG1 and MonoMac1 cells were treated with EDK-87 or EDK-88 at 2.5 and 5  $\mu$ M for 72h, and surface CD11b expression was analyzed by flow cytometry **D.** HL60, KG1 and MonoMac1 cells were treated for 72h with EDK-87 or EDK-88 at 5  $\mu$ M. Morphology analysis was performed upon May-Grünwald-Giemsa staining. The figure shows representative images (scale bars = 25  $\mu$ m). \* $p$ <0.05; \*\* $p$ <0.01; \*\*\* $p$ <0.001; \*\*\*\* $p$ <0.0001 in one-way ANOVAs, except calcium mobilization, Mixed-effects two-way ANOVA with Geisser Greenhouse correction.

**Supplementary Figure S6. EDK compounds induce mitochondrial damage and apoptosis. A.** MonoMac1 cells were treated for 24h with EDK-87 or EDK-88 at 5 and 10  $\mu$ M, and basal Oxygen Consumption Rate (OCR) was analyzed using Seahorse. **B.** KG1 and MonoMac1 cells were treated for 24h with EDK-87 or EDK-88 at 10  $\mu$ M, stained with the mitochondrial ROS probe MitoSOX and acquired in a flow cytometer. **C.** KG1 and MonoMac1 cells were treated with 10  $\mu$ M EDK-87, EDK-88 or etoposide (positive control) for 24h, and effector caspase activation was measured by the luminescence-based CaspaseGlo protocol. KG1 and MonoMac1 cells were treated for 24h with 10  $\mu$ M EDK-87 or EDK-88. Apoptotic cells were detected by flow cytometry according to 7AAD and annexin-V staining (early apoptotic, AnnV+ 7AAD-; late apoptotic, AnnV+ 7AAD+). The figure displays **D.** representative flow cytometry plots and **E.** frequency of apoptotic cells. HL60, KG1 and MonoMac1 cells were treated for 48h with 10  $\mu$ M EDK-87 alone or in combination with the ROS scavenger NAC, and **F.** viability or **G.** mitochondrial ROS were assessed by flow cytometry. **H.** MonoMac1 cells were treated with 10  $\mu$ M EDK-87 or EDK-88 for 48 h in high glucose-, no glucose- or galactose-containing media. Viability was assessed by flow cytometry. \* $p$ <0.05; \*\* $p$ <0.01; \*\*\* $p$ <0.001; \*\*\*\* $p$ <0.0001 in One-way ANOVAs, except glucose depletion (Two-way ANOVA).

**Supplementary Figure S7. EDK-87 and EDK-88 synergize with ATO in AML cells but not in healthy samples. A.** HL60 and MonoMac1 cells were treated with combinations of EDK-87 and ATO, cell viability was analyzed after 48h by flow cytometry (frequency of live cells) and synergistic values were calculated (Bliss scores). **B.** AML patient samples (n=3, left) and two healthy donor blood samples (n=2, right, BC stands for buffy coat) were treated with combinations of EDK-87 and ATO and analyzed as in A. **C.** Cytotoxicity effect of the combination of 10  $\mu$ M EDK-87 and different concentrations of ATO, in AML (red) vs. healthy (green) samples in order to visualize the synergic therapeutic window and the therapeutic index (viability of healthy/AML). **D.** Cytotoxic effects of 10  $\mu$ M EDK-87, 2  $\mu$ M ATO or the combination (combo) in AML samples and healthy blood cells (BC). **E.** CFU assay of HL60 and MonoMac1 cells treated for 24h with 3  $\mu$ M EDK-87 or EDK-88, 1  $\mu$ M ATO or in combination (combo). Bars show the number of colonies normalized to control. Written numbers represent the Bliss score of the combos **F.** HL60 and MonoMac1 cells were treated for 24h with 5  $\mu$ M EDK-87, 1  $\mu$ M ATO or in combination (combo). OCR was measured by the Seahorse technique. \* $p$ <0.05; \*\* $p$ <0.01; \*\*\* $p$ <0.001; \*\*\*\* $p$ <0.0001 in One-way ANOVA comparing inside families (D, E) or Welch t-test (F).

**Supplementary Figure S8. Pharmacological profile of EDK compounds.** **A.** ADMET properties of EDK-87 and EDK-88 **B.** In vitro hERG inhibition capacity of EDK compounds. **C.** Lipinski's rule of five properties of selected compounds. **D.** Pharmacokinetic parameters in mice for EDK-87 and EDK-88 after oral or subcutaneous administration, calculated from plasma concentration levels at indicated time points as observed in **E.**