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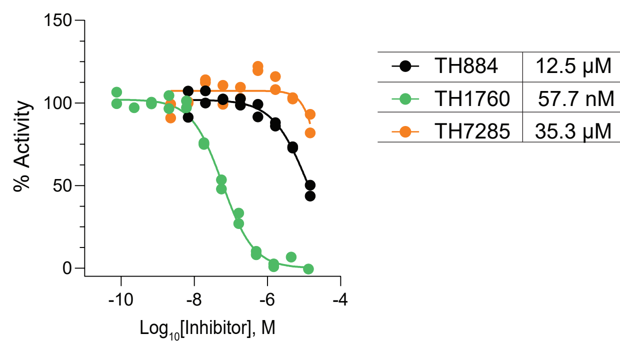
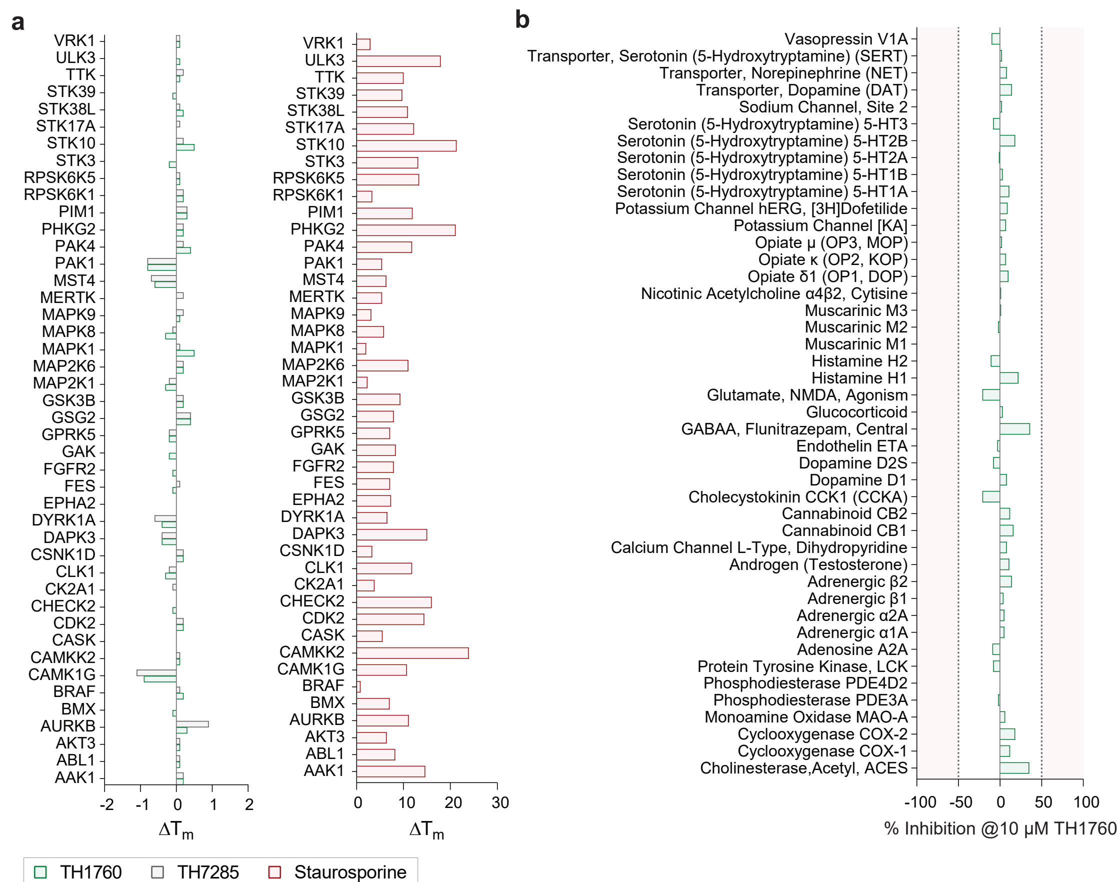


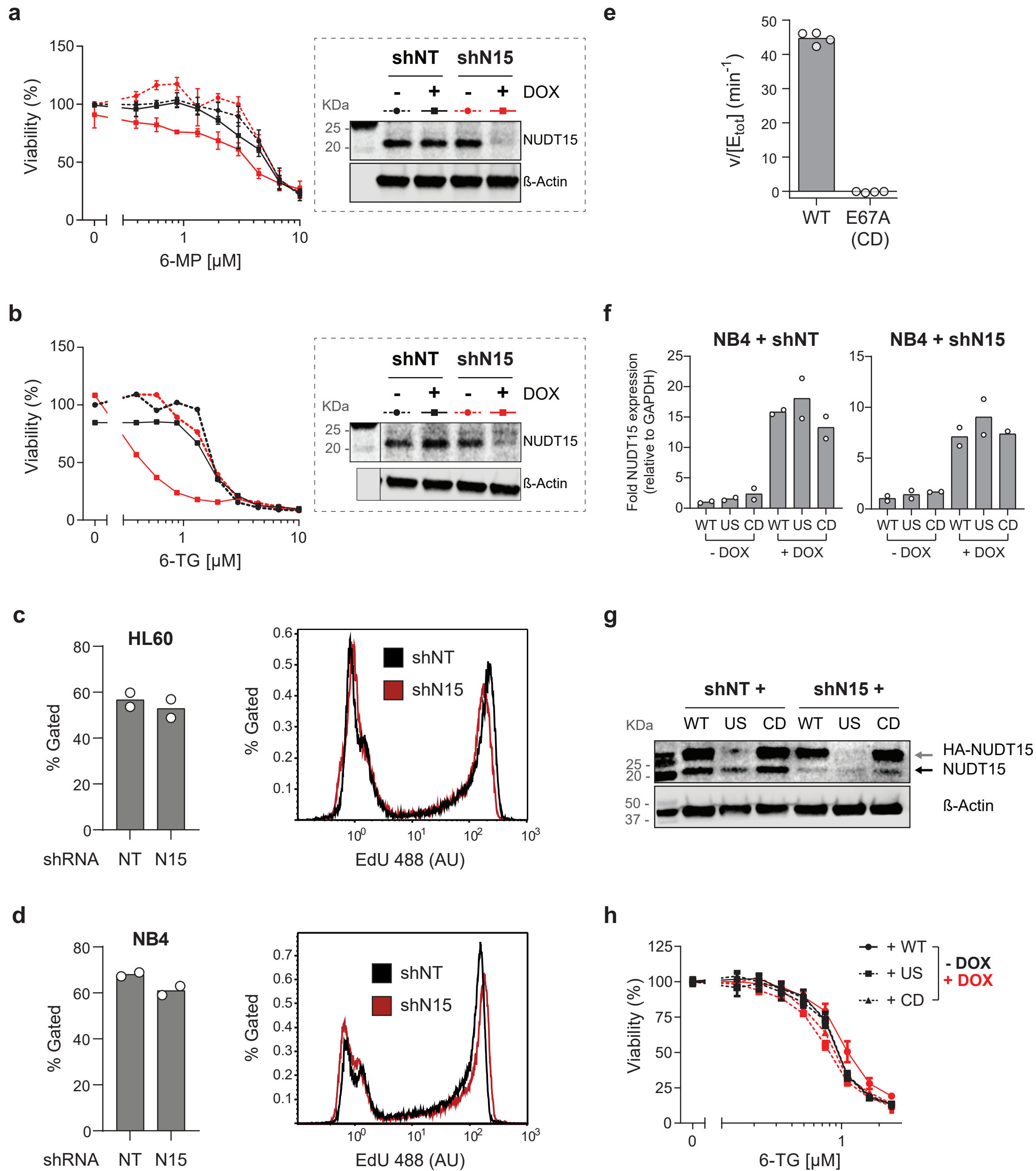
Extended Data Fig. 1 TH1760 potently inhibited the 6-thio-dGTPae activity of NUDT15

TH1760 had over 200-fold potency improvement as compared to TH884, shown using PPiLight inorganic pyrophosphate assay (Lonza, #LT07-610). Individual data of n=2 independent experiments performed in duplicates shown with estimated IC_{50} values.



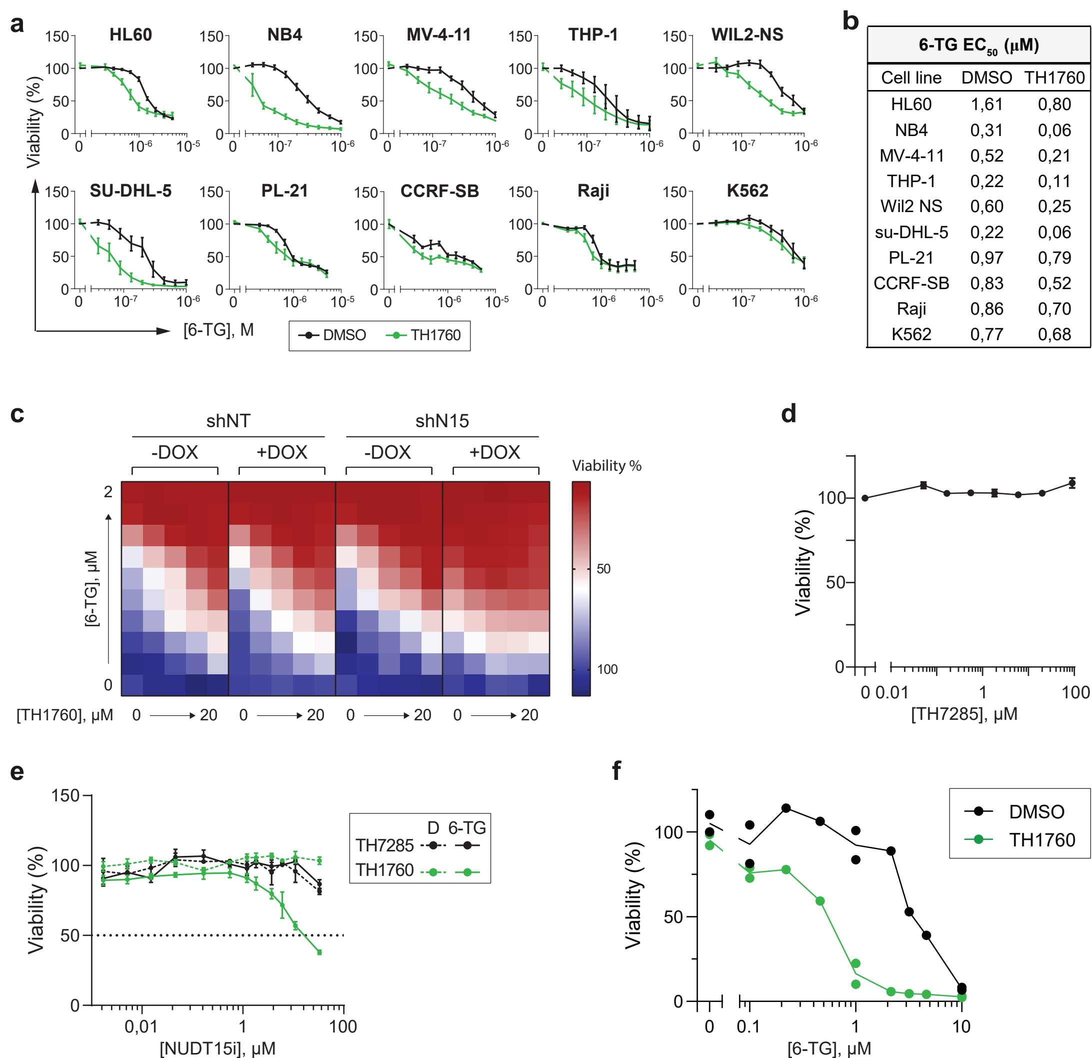
Extended Data Fig. 2 TH1760 demonstrated impressive selectivity

a. TH1760 and TH7285 selectivity at 12 μM against a curated library of 44 kinases, tested using DSF with staurosporine as the positive control compound. Mean change in protein T_m (ΔT_m) of one experiment performed in triplicates shown. **b**. TH1760 selectivity at 10 μM against the SafetyScreen44^{TM} panel from Eurofins Cerep Panlabs. Mean % inhibition of an experiment performed in duplicates shown.



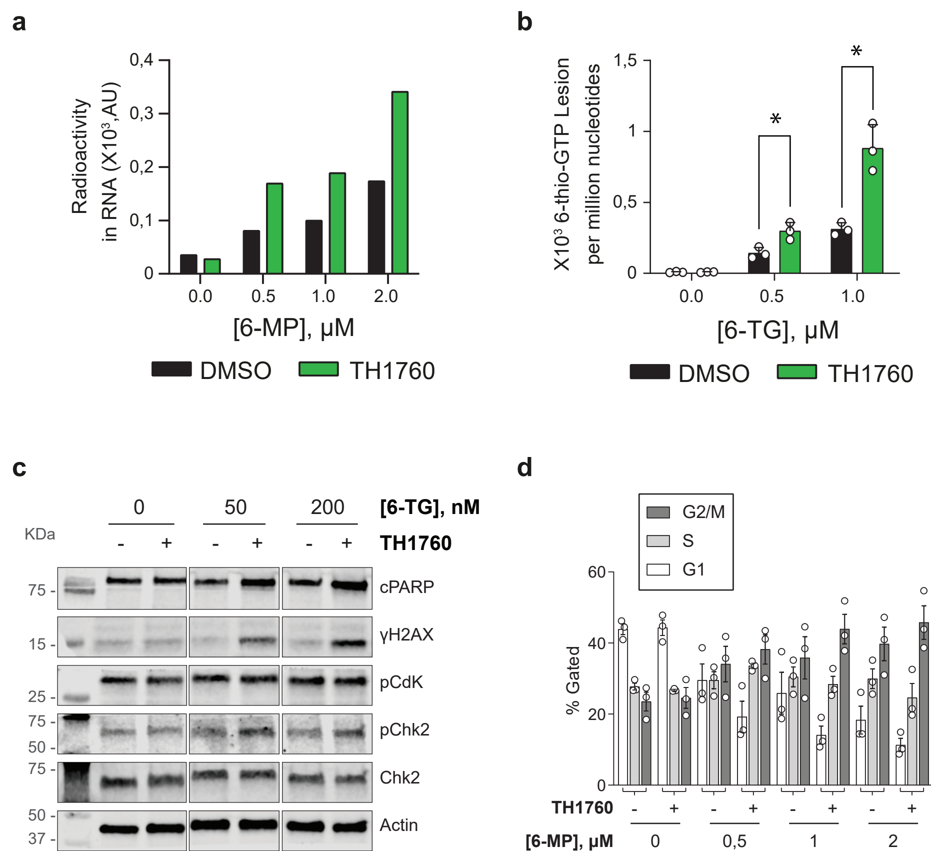
Extended Data Fig. 3 Depletion of NUDT15 in HL-60 and NB4 cells potentiated thiopurine efficacy

a. b. NUDT15 depletion sensitized NB4 cells to 6-MP (a), and HL-60 cells to 6-TG (b). Cell viabilities assessed using resazurin viability assay after 96 (a) or 72 (b) h of treatment and calculated by normalizing to no DOX, DMSO-treated controls. Mean ± SEM of n=3 (a) or mean of n=2 (b)experiments performed in triplicates shown. Left panels: resazurin viability curve; right panels: Western blot demonstrating DOX-induced NUDT15 knockdown. **c. d.** Depletion of NUDT15 in HL-60 (c) or NB4 (d) did not affect DNA replication, evidenced by EdU incorporation. Cells expressing DOX-inducible NUDT15-specific (N15) or non-targeting (NT) shRNA were treated with DOX for 48 h, before EdU labelling. Left panels: Mean EdU+ve population% of n=2 experiments shown. Right panels: representative FACS histogram showing EdU signal intensity. **e.** E67A variant of NUDT15, compared to the wildtype (WT) construct, is catalytically inactive against 6-thio-dGTP (tested at 50µM), shown using enzyme-coupled MG assay. Mean activity of a representative experiment shown with individual repeat values. **f.** RT-qPCR analysis of NUDT15 mRNA levels in NB4 cells co-expressing DOX-inducible shRNA, and shRNA-resistant, HA-tagged NUDT15 constructs (wildtype, WT; unstable, US; or catalytically dead, CD), with GAPHD as the house keeping gene. NUDT15 mRNA levels were normalized to cells expressing WT NUDT15 construct. Mean of n=2 experiments performed in triplicate shown. **g.** Doxycycline treatment induced the co-expression of shRNA (shNT and shN15) and shN15-resistant, HA-tagged NUDT15 overexpression constructs (WT, CD, or US) in NB4 cells. **h.** NB4 cells co-expressing DOX-inducible shNT shRNA and shRNA-resistant, HA-tagged NUDT15 overexpression constructs (WT, CD, or US) were assayed for viabilities under 6-TG treatment. Overexpression of WT NUDT15 conferred marginal resistance to 6-TG Mean ± SEM of n=3 independent experiments performed in duplicates shown.



Extended Data Fig. 4 TH1760 treatment sensitized cancer cell lines to thiopurine

a. TH1760 sensitized a panel hematological cell lines to 6-TG. Cells were treated with increasing concentrations of 6-TG alone or in combination with 10 µM TH1760 for 96 h, before viabilities were determined using resazurin viability assay. Viability % was calculated by normalizing to DMSO-treated controls and mean ± SEM of n = 3 experiments shown. **b.** 6-TG cytotoxic EC_{50} values in the cell lines shown in a, determined by curve-fitting cell viabilities *via* non-linear regression model (Graphpad prism, [Inhibitor] vs. response – variable slope model). **c.** TH1760 sensitized NB4 cells to 6-TG in a NUDT15-dependent manner. NB4 cells stably expressing shNT or shN15 shRNA were treated with a dose-response concentration matrix of 6-TG and TH1760 for 96 h, before viabilities determined by resazurin assay. Viability % was calculated by normalizing to DMSO-treated controls and mean viabilities of n = 2 experiments shown in heat map. **d.** TH7285 was not cytotoxic in HL-60 cells up to 100 µM. Viabilities of HL-60 cells treated with TH7285 for 96h were assessed by resazurin viability assay. Viability % was calculated by normalizing to DMSO-treated controls, and mean ± SEM of n=4 experiments performed in duplicates shown. **e.** TH7285 did not potentiate 6-TG in HL-60 cells. HL-60 cells were treated with 10 µM compounds alone or combined with 320 nM 6-TG (EC_{10}) for 96 h, before resazurin viability assay. Viability % was calculated by normalizing to DMSO-treated controls and mean ± SEM of n = 4 independent experiments shown. **f.** TH1760 (10 µM) substantially reduced the 6-TG EC_{50} in 697 cells by approximately 10-fold, upon co-treatment for 96h. Viabilities determined by resazurin assay and normalized to DMSO-treated control. Viabilities of n=2 experiments performed in duplicates shown.



Extended Data Fig. 5 TH1760 potentiated thiopurine-induced cytotoxicity through elevating the intracellular pool of thiopurine metabolites

a. b. TH1760 significantly enhanced the RNA incorporation of metabolites of 6-MP (a) or 6-TG (b). HL-60 cells were treated with increasing concentrations of thiopurines alone or in combination with 10 µM TH1760. Sixteen hours post-treatment, cellular RNA was isolated and incorporation of ^{14}C-labbeled 6-MP metabolites were determined *via* radioactive counts (a) and incorporation of 6-TG metabolites *via* mass spectrometry analysis (b). Mean of n=1 and mean± SEM of n=3 experiment(s) are shown for a and b, respectively. In b, DMSO Vs. TH1760 group: at 0.5µM 6-TG, *p = 0.02, t ratio=3.72, df=4; at 1µM 6-TG, *p = 0.0047, t ratio=5.699, df=4 (two-tailed multiple t-test, Holm-Sidak correction, Graphpad Prism). **c.d.** TH1760 potentiated 6-TG-induced cellular responses in NB4 (c) and HL-60 cells (d). NB4 cells treated with 6-TG alone or in combination with 10 µM TH1760 were assayed for DNA damage response and apoptotic marker *via* Western blot at 48 h post-treatment. HL-60 cells treated with 6-MP alone or in combination with 10 µM TH1760 were subject to propidium iodide staining followed by cell cycle analysis *via* flow cytometry at 72 h post-treatment. Mean % ± SEM of n=3 independent experiments shown.