Supplemental information

T-cell protein tyrosine phosphatase (TCPTP) is irreversibly inhibited by etoposide-quinone, a reactive metabolite of the chemotherapy drug etoposide

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METHODS

RT-qPCR of STAT1 regulated genes from Jurkat cells

Jurkat cells ($5x10^6$) were washed three times with PBS and then treated with DMSO (Control) or 50 µM EQ for 1h in PBS at 37 °C (5% CO_2). Cells were washed and further incubated for 30 min in fresh medium supplemented with 10 ng/ml of human IFNγ. Cells were finally rinsed three times with fresh medium and maintained at 37 °C (5% CO_2) for 3 h. Total RNA from the cells was extracted using Trizol reagent (Invitrogen Carlsbad, CA, USA). 500 ng of the extracted RNA was converted into cDNA using superscript II cDNA kit (Invitrogen Carlsbad, CA, USA). The expression of three well-known STAT1 regulated genes (*IRF1*, *GBP1* and *APOL1*) (Hartman *et al*., Genes & Dev., 2005; Reardon and McKay**,** J. Immunol., 2007) were studied using SYBR green method (Applied biosystems, Thermo Fischer Scientific, Rockford, IL, USA) and was run in a 7500 Fast Real-time PCR System (Applied Bio-systems, Carlsbad, CA). The Ct values of the target genes were normalized with *RPL19* (house keeping gene) and the fold differences were calculated using $2^{-\Delta\Delta Ct}$ method. The primer sequences of the genes used in the experiments were :

IRF1 (For: CAACAGATGAGGATGAGGAAGGGAA; Rev: CCATAGACAGAGGTGGGCTGG), *GBP1* (For: ACAGGGTCCAGTTGCTGAAAGA; Rev: TTGGTTAGGGGTGACAGGAAGG), *APOL1* (For: GCTGCTGCTGAACTGCCC; Rev: TCTGTACTGCTGGCCTTTATCGT) and *RPL19* (For: GGCTCGCCTCTAGTGTCCTC; Rev: CAAGGTGTTTTTCCGGCATC).

Effects of etoposide and etoposide quinone on PTP1B activity

Human PTP1B (catalytic domain residues 1-321) was expressed and purified from *E. coli* as described previously (Krishnan et al., 2018). Recombinant PTP1B (1 µM) was incubated with ETOP or different concentrations of EQ in 100 mM sodium acetate, pH 6 for 30 minutes at 37 °C (total volume of 50 µl). Samples were diluted 10 times with acetate buffer then assayed for residual phosphatase activity using pNPP as described previously (Montalibet et al., 2005).

LEGENDS

Supplementary Figure 1: Determination of EQ IC_{50} for Human TCPTP inhibition

EQ data from figure 1 were analyzed by nonlinear regression (using Qtiplot) in order to determine IC_{50} and fitted to the Hill's equation as described under Methods.

Supplementary Figure 2: ETOP activated to EQ by myeloperoxidase inhibits TCPTP activity

(A) Effect of ETOP activation to EQ by peroxidase/H_2O_2 on TCPTP activity measured by pNPP dephosphorylation. Following the procedure described in Materials and Methods, 5 units of active or boiled peroxidase were incubated with 100 µM ETOP combined with 100 µM H_2O_2 in parallel to the following different control conditions: Ctrl (untreated control); 100 µM ETOP; 40 µM EQ. Results are the mean of three independent experiments, error bars indicate S.D. * $p<0.05$ determined using ANOVA followed by Dunnett's post-hoc analysis. NBT (B) and IAF (C) labeling experiments were carried out and analyzed as described in Methods. Membranes shown here are representative of three independent experiments.

Supplementary Figure 3: ETOP activated to EQ by myeloperoxidase inhibits TCPTP activity

Jurkat cells were treated with EQ (50 µM) for 1 h, then incubated or not with IFNγ for 30 min. Relative gene expression of *APOL1*, *GBP1* and *IRF1* were analyzed by RT-qPCR. Error bars indicate S.D. *$p<0.05$ compared with control (Ctrl).

Supplementary Figure 4: Effect of ETOP and EQ on PTP1B activity

1 µM PTP1B was incubated with 100 µM ETOP and 5 to 40 µM EQ for 30 min at 37°C and diluted 10-fold prior to measurement of residual activity in the presence of 5 mM pNPP as described in the Methods section. Results are the mean of three independent experiments, error bars indicate S.D.

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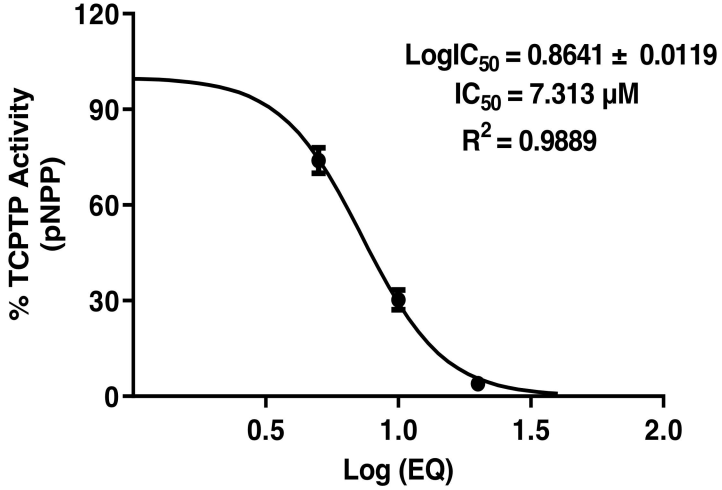
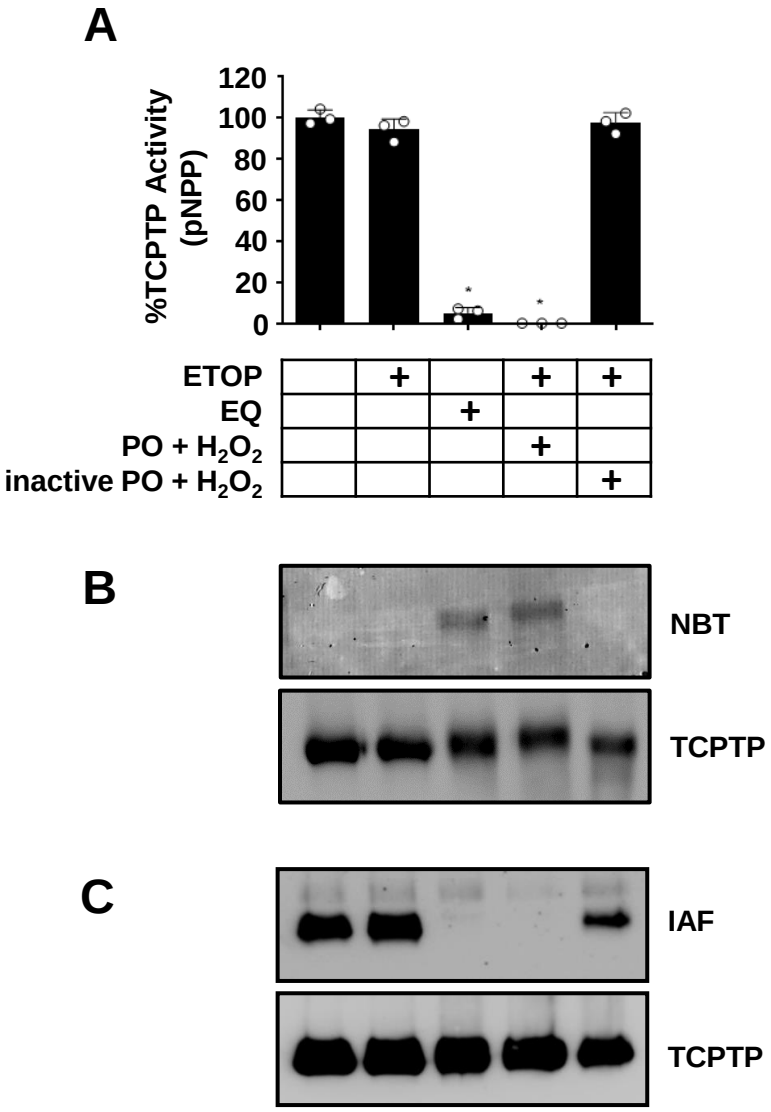
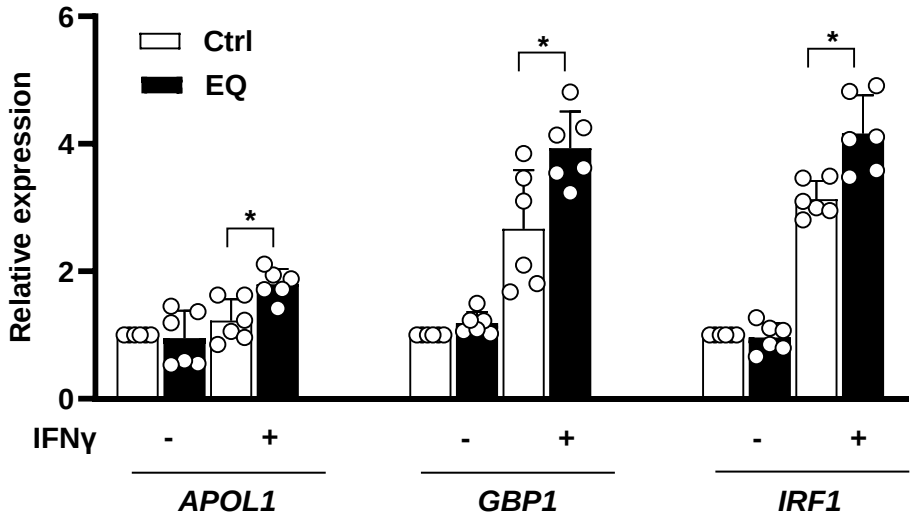


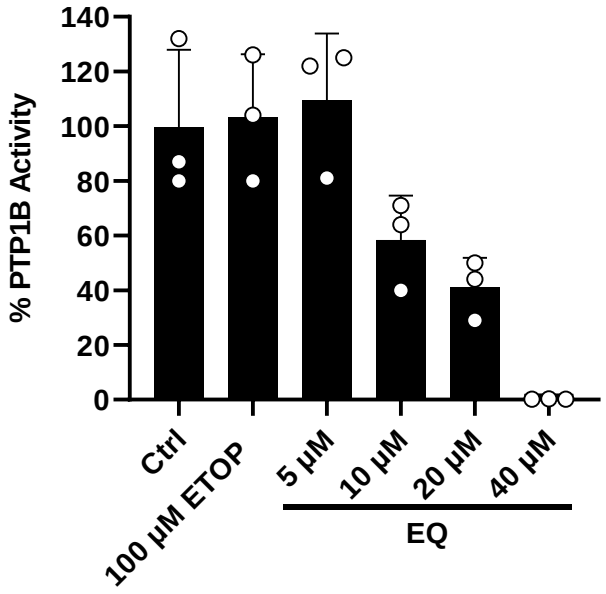
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4