

Fig. S1 Effect of ATO in TCR-T cell function in vitro. (A) Staining of AFP TCR-T cells or nontransduced T cells with anti-mouse TCR β -chain antibody and CD8 antibody. (B) HepG2 cells were incubated with different doses of ATO or vehicle 24h at 37°C. DMSO was used as a vehicle. The cells incubated with a 10% Triton X-100 solution was used as a positive control of cytotoxicity. (C) ATO induced HepG2 cells apoptosis in a concentration-dependent manner for 48h. (D) Inhibition of HepG2 cell proliferation was examined after 4-day culture with ATO. (E) ATO

could not induced TCR-T cells apoptosis for 48h. (F) After ATO or vehicle treated TCR-T cells for 9 days, Cell viability was detected by flow cytometry with 7-AAD staining. (G) Cleaved caspase-3 expression was detected by flow cytometry after HepG2 treated with TCR-T or combined with different concentrations of ATO for 6h (E : T=1 : 1). (H) Representative fluorescence microscopy images (10×) of (G). Each experiment was performed in triplicate. The experiment was repeated twice with similar data. Data are presented as mean \pm SD. $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$.

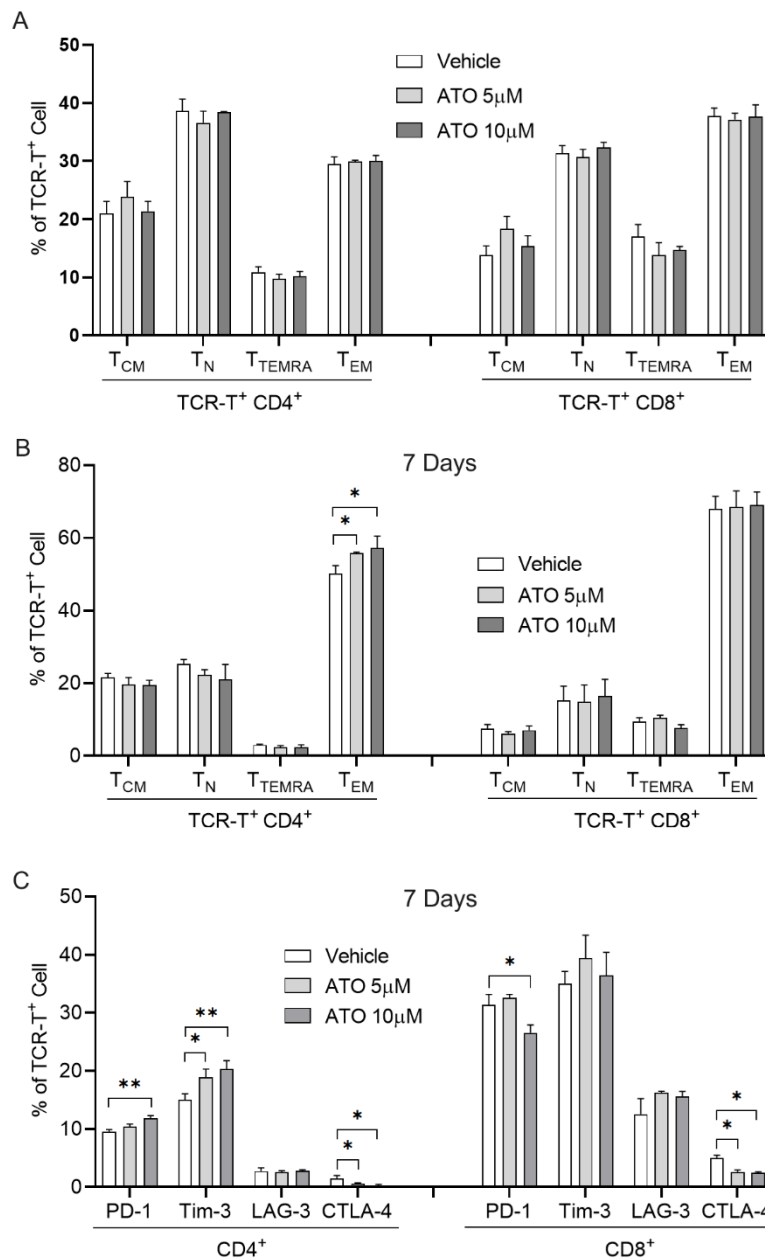


Fig. S2 Effect of ATO on TCR-T subsets in response to cancer stimulation. (A) TCR-T cells memory subsets were analyzed by flow cytometry after treated with ATO for 9 days. Medium was changed every two days and maintain the appropriate ATO concentration. (B) TCR-T cell memory subsets and (C) inhibitory receptor expression (PD-1, LAG-3, TIM-3 and CTLA-4) were analyzed by flow cytometry after TCR-T single stimulation with HepG2 cell line for 3 rounds of repeated stimulation over 7 days in the presence of different concentrations of ATO. Each experiment was performed in triplicate. The experiment was repeated twice with similar data, Data are presented as mean \pm SD. $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$.

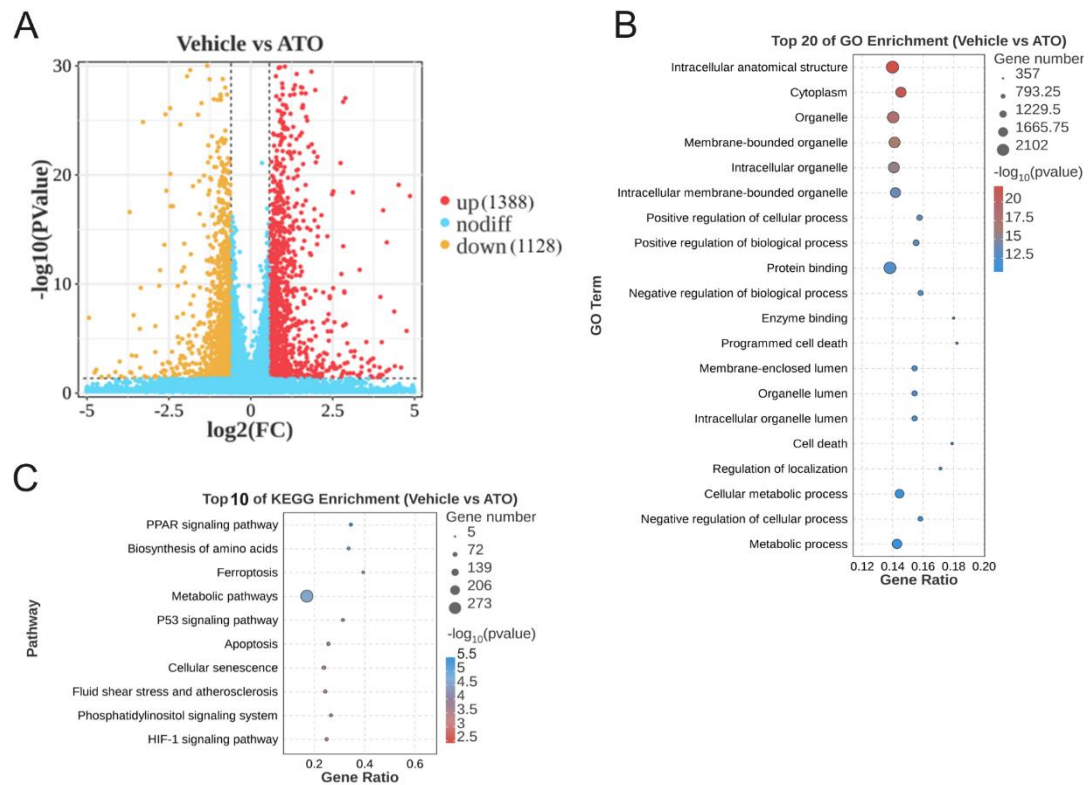


Fig. S3 Transcriptomic analysis of the effects of ATO combined with AFP TCR-T. (A) DEGs ($|\log_2FC| > 0.58$, $p < 0.05$) containing upregulated genes (red) and downregulated genes (yellow) were visualized by volcano plot based on gene expression difference (\log_2 fold change, x axis) and statistical significance ($-\log_{10}$ pvalue, y axis). (B) GO analysis and (C) KEGG pathway enrichment analysis of DEGs.

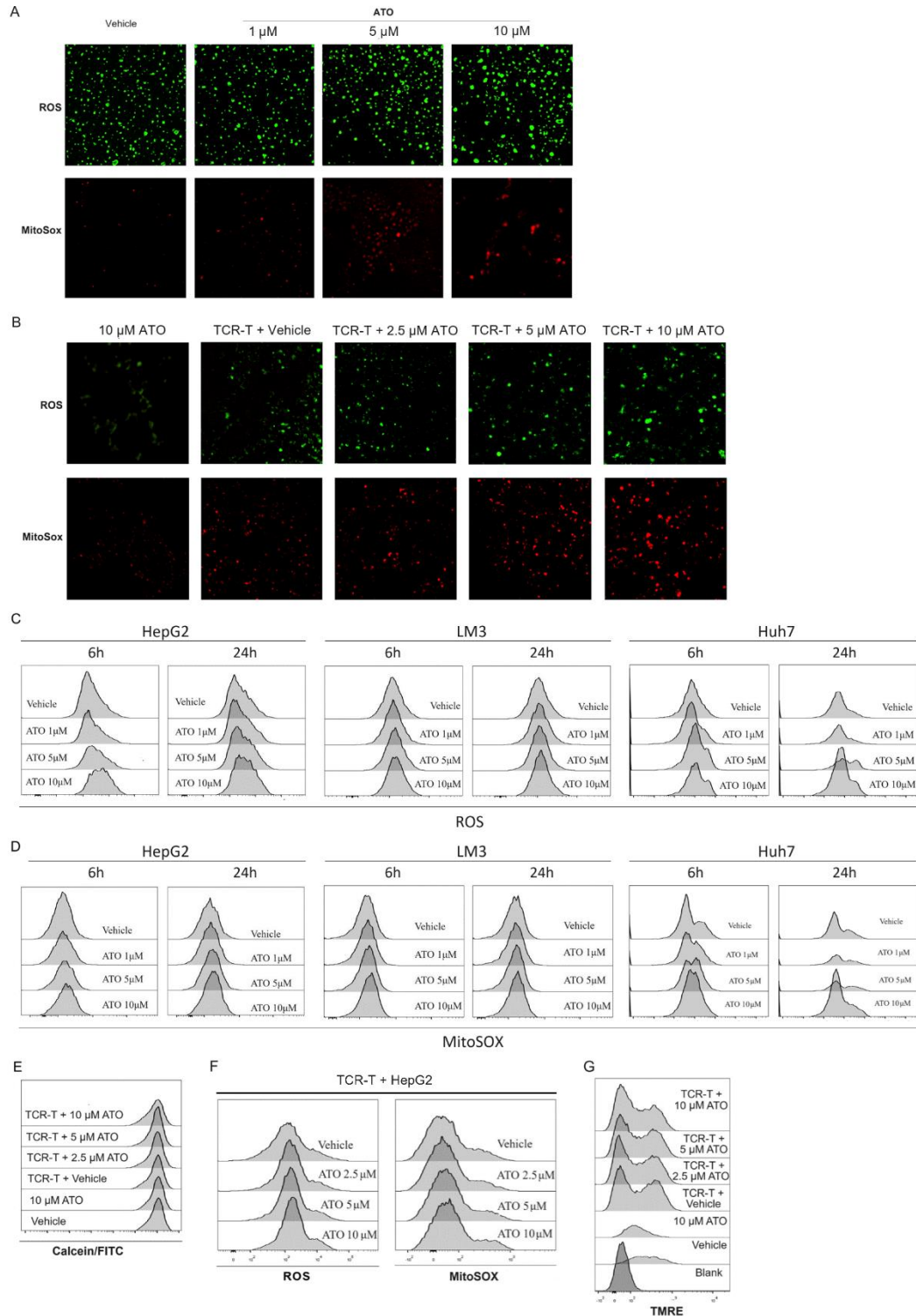


Fig. S4 ATO combined treatment with TCR-T enhanced ferroptosis of HCC through excessive ROS accumulation. (A) and (B) Representative fluorescence microscopy images (20 \times) of HepG2 stained with fluorescent dyes. Green color indicates HepG2 cells stained with DCFH-DA for intracellular ROS and red color indicates HepG2 cells stained with MitoSOX red indicator for mitochondrial ROS. Effect of different concentrations of ATO alone (A) or combined with TCR-T cells (E : T=1 : 2) (B) on HepG2 cells after 6h treatment. (C-G) Representative flow cytometry histograms of Fig. 5A-E.