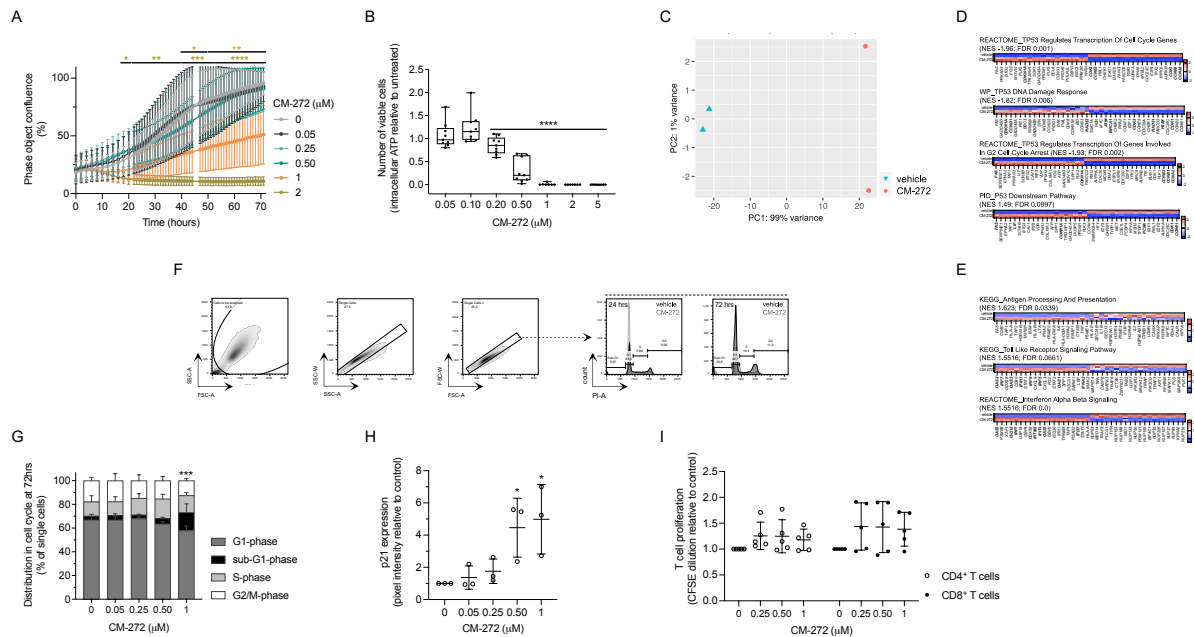
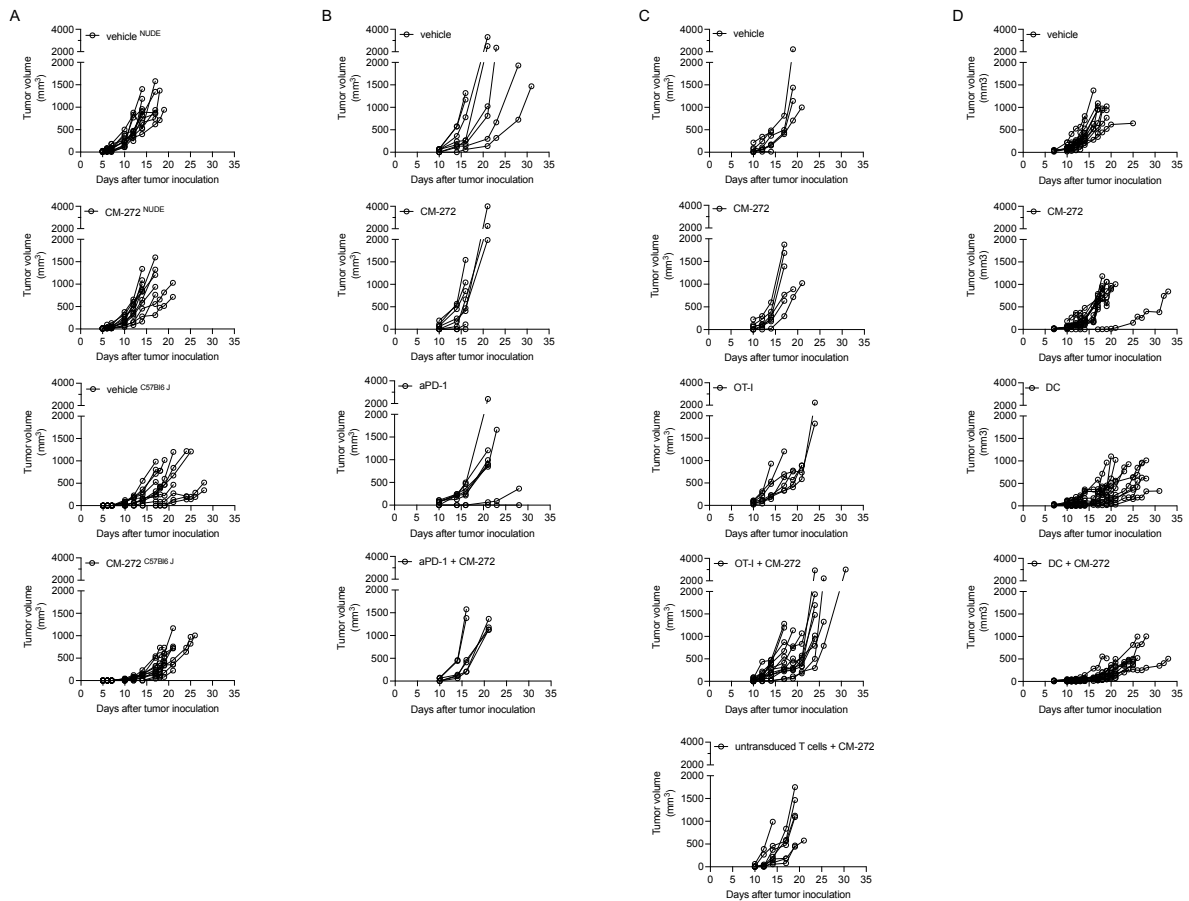


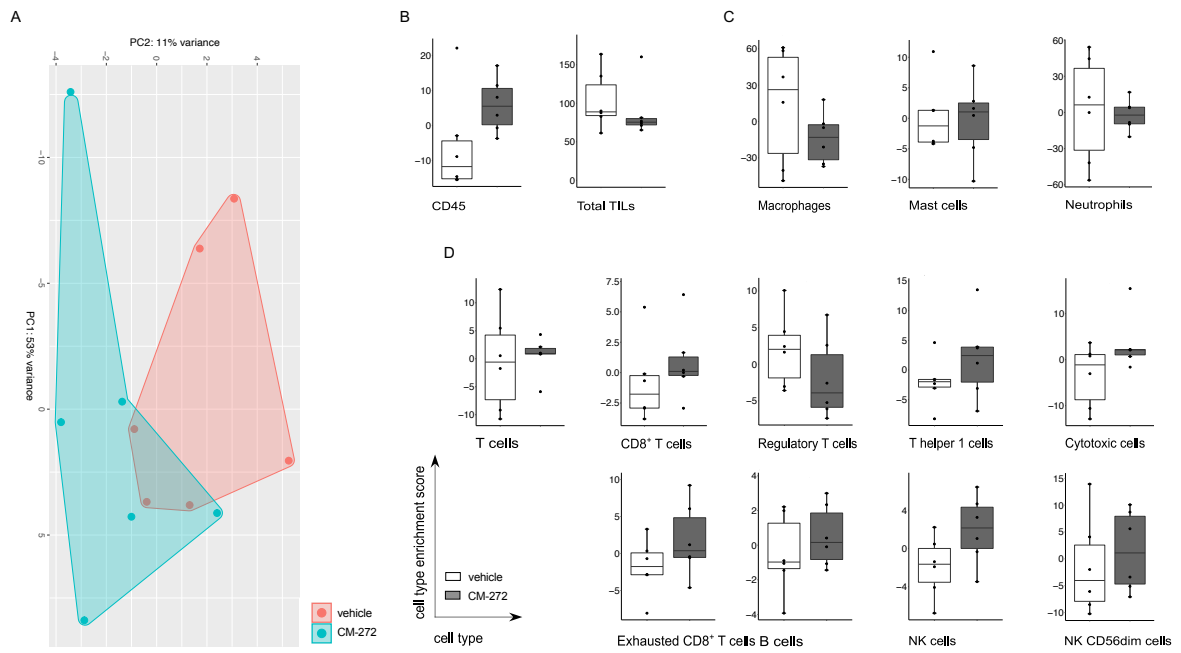
## SUPPLEMENTARY FIGURES



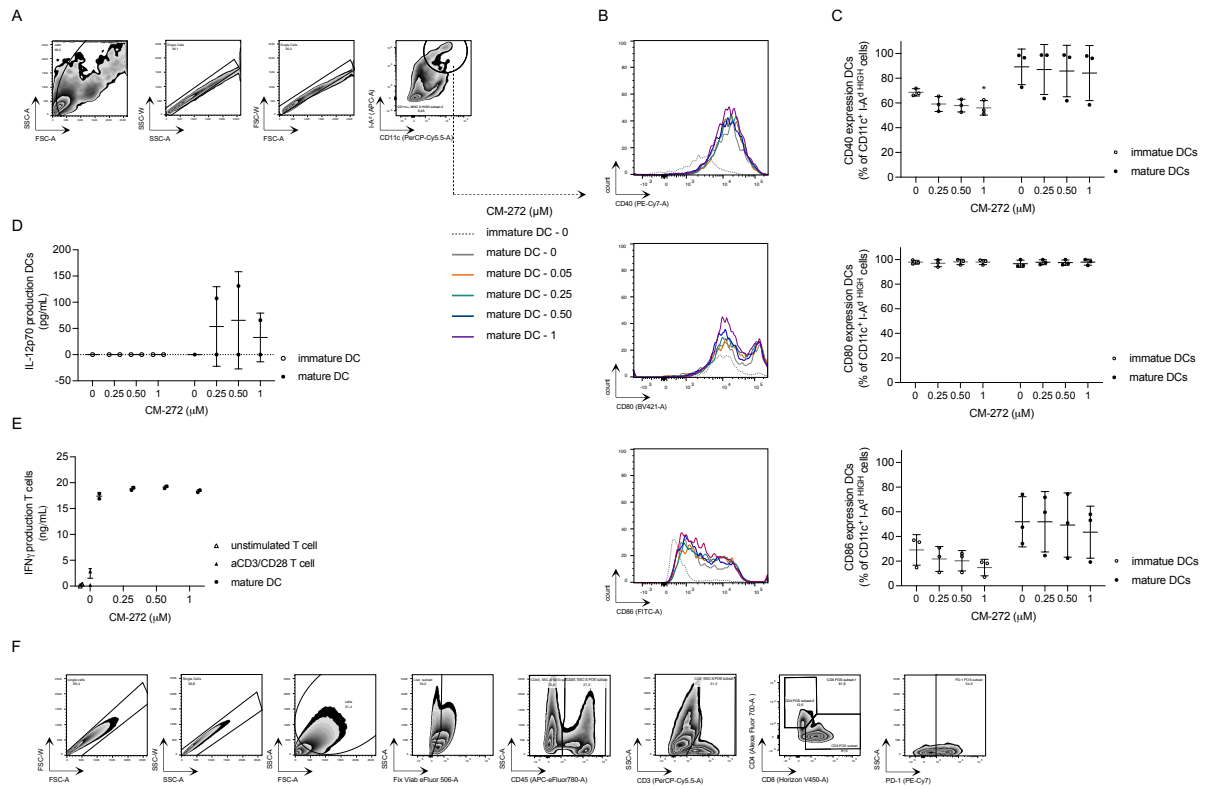
**Figure S1 - Validation of CM-272 effect on MO4 cells and T cells in vitro. (A-H)** CM-272 effect on MO4 cells. **(A)** Cell confluence in time (mean±SD;  $n=3$ ). **(B)** Number of viable cells at 72 hours, relative to untreated (mean±SD;  $n=8$ ). **(C-E)** RNA sequencing analysis. **(C)** Principal component analysis plot comparing vehicle and CM-272 samples. **(D-E)** Representative gene sets affected by CM-272, involved in **(D)** TP53 regulation of cell cycle progression, and of G1 phase arrest and apoptosis signaling, **(E)** Immune responses. Heatmaps list row-normalized gene expression of top-20 up/down-ranked genes. **(F)** Flow cytometry gating strategy of cell cycle distribution. **(G)** Percentage of MO4 cells in cell cycle phases at 72 hours (mean±SD;  $n=4$ ). Asterisks represent sub-G1-phase. **(H)** p21 protein expression after 24 hours of treatment (mean±SD;  $n=3$ ). **(I)** CFSE dilution upon 72 hours of polyclonal stimulation of CD4<sup>+</sup> or CD8<sup>+</sup> T cells (mean±SD;  $n=4$ ). Vehicle and CM-272 conditions were compared using REML modeling and post-hoc Sidak multiple comparison test (A); Ordinary one-way Anova and post-hoc Dunnett's multiple comparison test (B,G,H,I). Asterisks indicate statistical significance: \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; \*\*\*  $p \leq 0.001$ ; \*\*\*\*  $p \leq 0.0001$ .



**Figure S2 - Follow-up of in vivo MO4 tumor volume evolution in time.** (A-D) Evolution of tumor volume in time of each individual mouse, presented per experimental group. (A) CM-272 in immune-deficient (NUDE) and immune-competent (C57Bl6 J) setting. (B) Single or combined CM-272 and PD-1 blockade (aPD-1) treatment. (C) Single or combined CM-272 and adoptive T-cell treatment. (D) Single or combined CM-272 and DC vaccination treatment.



**Figure S3 - Gene expression-based TIL-scoring on ex vivo tumor tissue.** Tumors of MO4 tumor-bearing mice subjected to the CM-272 treatment regimen were resected at  $706.9 \pm 193.8$  mm<sup>3</sup> tumor volume ( $n=1, 12$  m.p.c.) and subsequently subjected to multiplex analysis of 770 genes included in the Nanostring nCounter PanCancer Mouse Immune Profiling Panel. **(A)** Principal component analysis plot comparing vehicle and CM-272 samples. **(B-D)** Cell score of **(B)** total TILs and CD45<sup>+</sup> immune cells, **(C)** immune cells of the myeloid lineage, and **(D)** immune cells of the lymphoid lineage. Vehicle and CM-272 condition was compared using the Wilcoxon rank sum test (B-D).



**Figure S4 - Sensitivity of DCs to CM-272 exposure and flow cytometry gating strategy of ex vivo tumor T cell infiltrate.** (A-C) Bone marrow-derived DCs were subjected to CM-272 for 24 hours before effect was determined on maturation marker (CD40, CD80, and CD86) expression of I-A<sup>d</sup> HIGH/CD11c<sup>+</sup> DCs. (A) Flow cytometry gating strategy. (B-C) Maturation marker expression (representative histograms [b]/ mean±SD; n=3 [c]). (D) IL-12p70 production by DCs (mean±SD; n=2). (E) IFN-γ production signifying T-cell activation by DCs (mean±SD; n=2). (F) Flow cytometry gating strategy of T-cell infiltration *in vivo*. Vehicle and CM-272 conditions were compared using ordinary one-way Anova and post-hoc Dunnett's multiple comparison test (C).