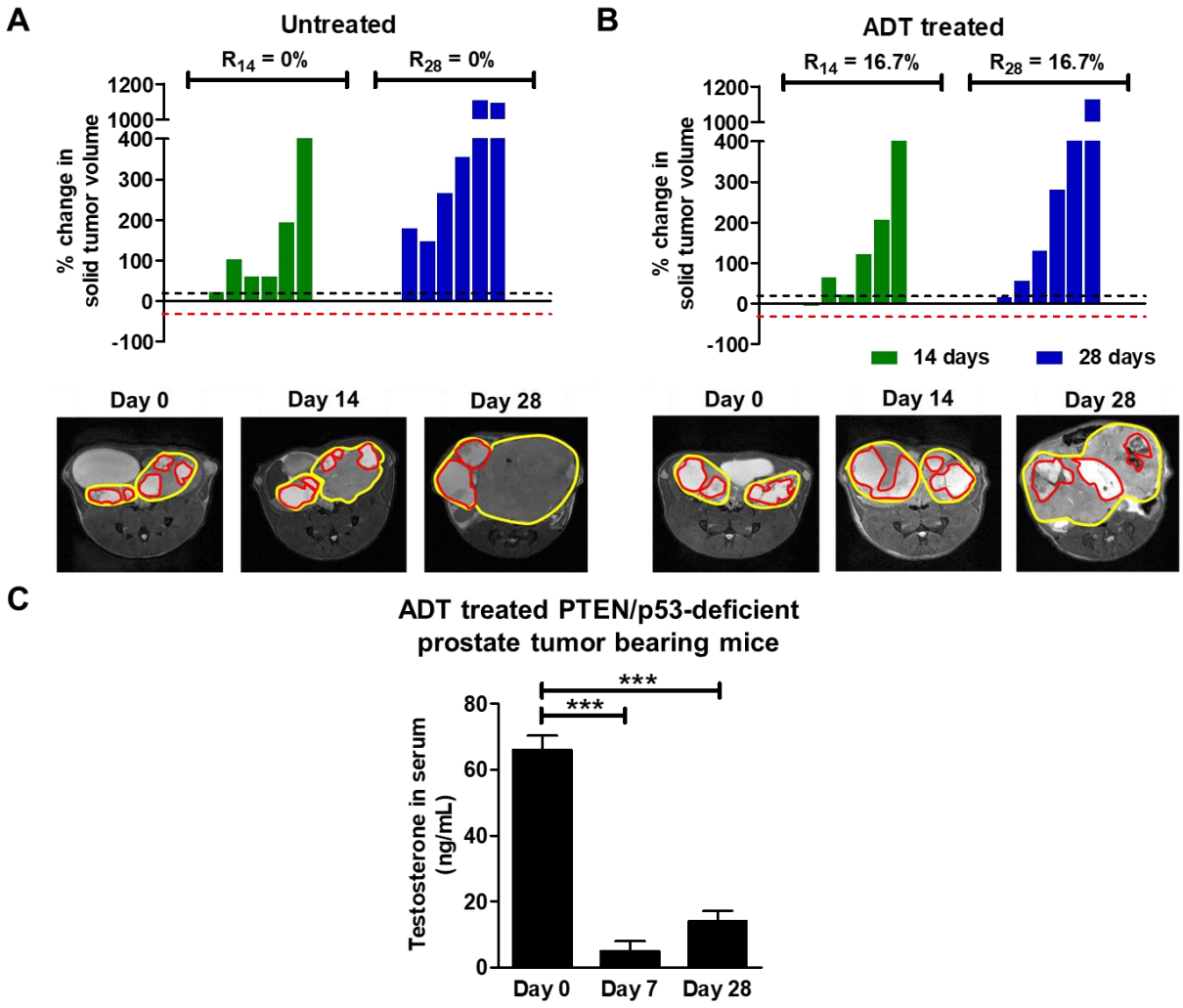
Supplementary figures:

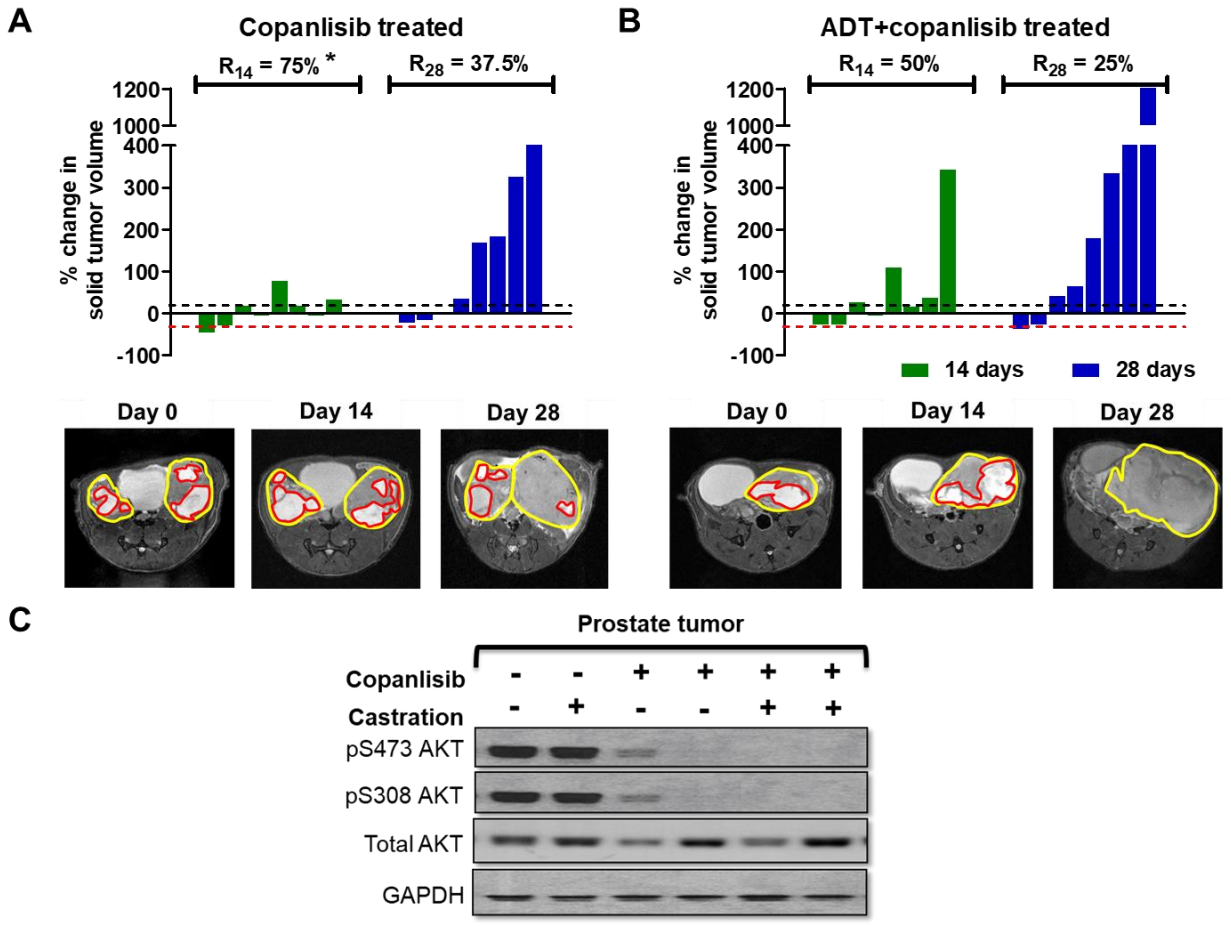
Supplementary Figure S1.



Supplementary Figure S1. The majority of Pb-Cre; PTEN$^{fl/fl}$ Trp53$^{fl/fl}$ mice are *de novo* resistant to ADT. (A-B) Pb-Cre; PTEN$^{fl/fl}$ Trp53$^{fl/fl}$ mice with established prostate tumors were randomized to untreated or degarelix (ADT, 0.625 mg, single dose) for 4 weeks. Tumor volumes were non-invasively monitored by MRI, and % ORR at days 14 (R_{14}) and 28 (R_{28}) were determined as described in Methods. The % change in solid tumor volume is represented by waterfall plots for untreated **(A)** and ADT-treated groups **(B)**. **(C)** Sera were collected from mice at 7 and 28 days post-treatment, and analyzed for testosterone levels by ELISA. n=6-8 mice per group.

Significances/p-values were calculated by Chi-square test (panel A and B, relative to untreated), one-way ANOVA (panel C) and indicates as follows, ***$p<0.001$.

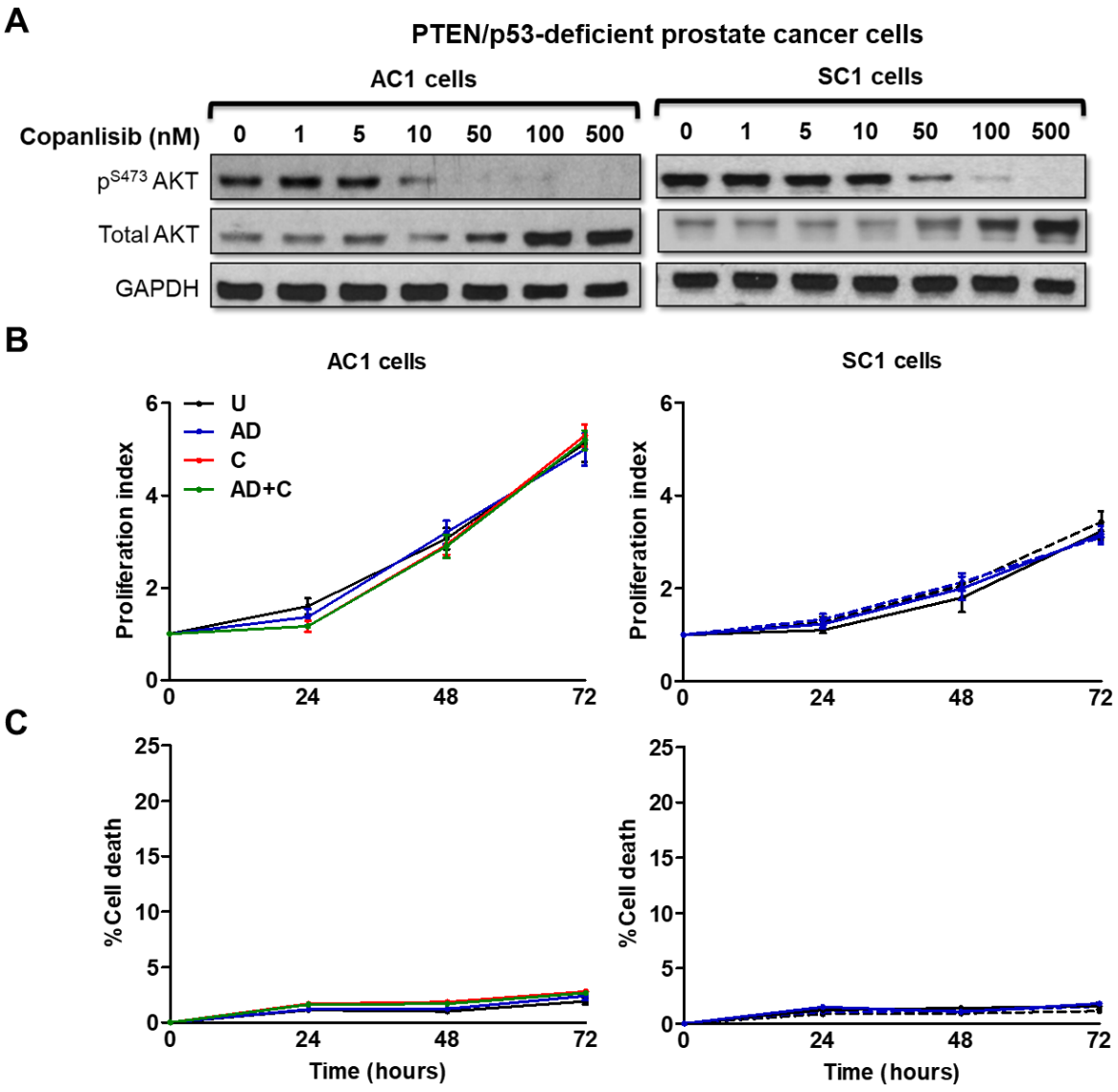
Supplementary Figure S2.



Supplementary Figure S2. ADT/PI3Ki combination therapy halts prostate tumor growth up to 14 days, followed by development of resistance in majority of Pb-Cre; PTEN$^{fl/fl}$ Trp53$^{fl/fl}$ mice. (A-B) Pb-Cre; PTEN$^{fl/fl}$ Trp53$^{fl/fl}$ mice bearing established tumors were treated with copanlisib (14 mg/kg, *iv*, every alternate day), singly or in combination with ADT (degarelix, 0.625 mg, single dose). Tumor volumes were non-invasively monitored by MRI and % ORR at days 14 (R_{14}) and 28 (R_{28}) were determined as described in Methods. The % change in solid tumor volume is represented by waterfall plots for copanlisib **(A)** and ADT + copanlisib treated groups **(B)**. n=6-8 mice per group. Statistical analysis was performed using Chi-square test. *p<0.05 vs untreated. **(C)** Tumor extracts were harvested following 4 weeks of treatment with the indicated drug(s), and

analyzed by western blotting for PI3K activation status. n=6-8 mice per group. Significances/p-values were calculated by Chi-square test (panel A and B, relative to untreated) and indicates as follows, *$p<0.05$.

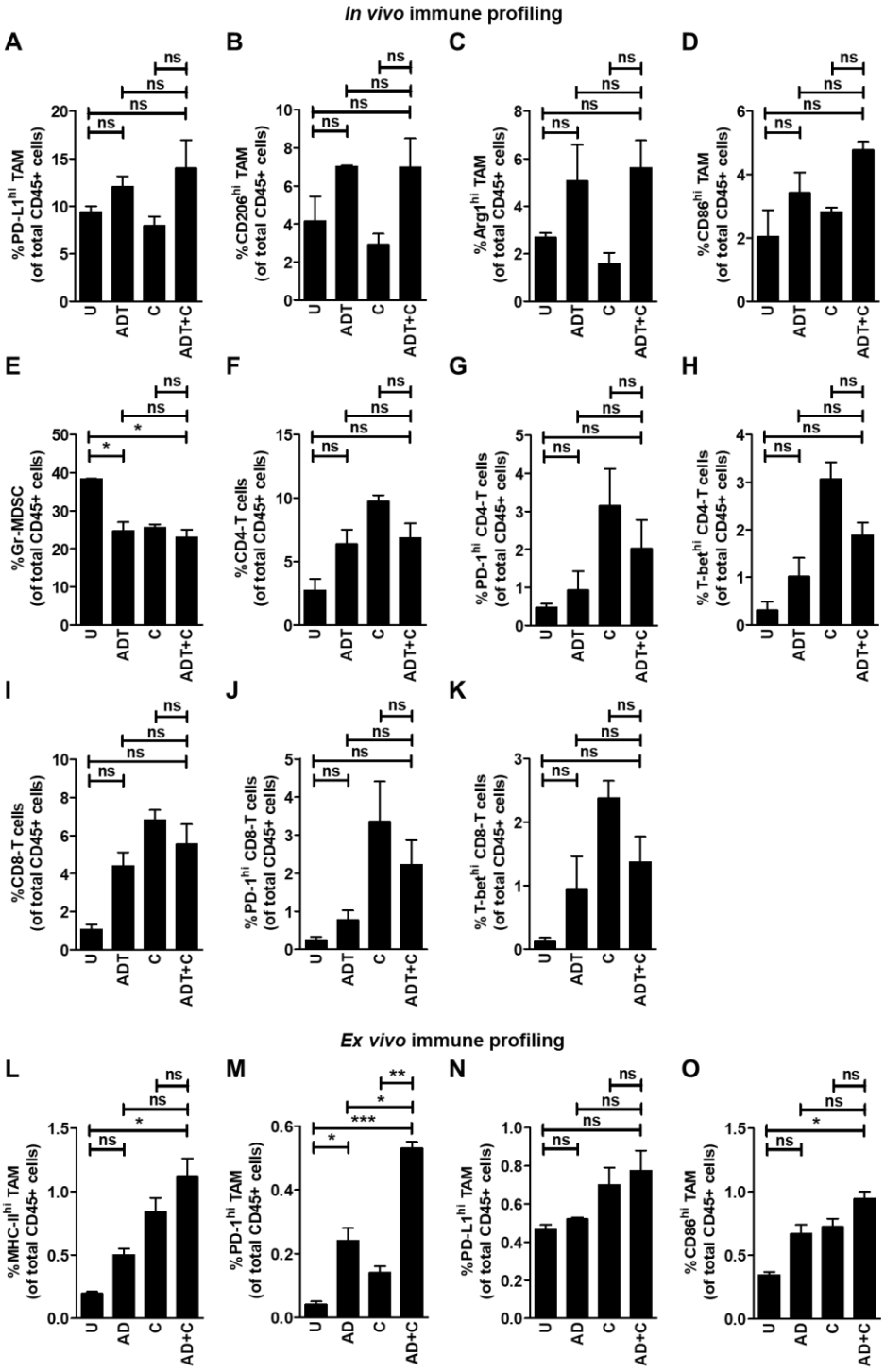
Supplementary Figure S3.



Supplementary Figure S3. PI3Ki treatment with concurrent androgen depletion does not alter proliferation and survival of PTEN/p53-deficient murine PC cells *in vitro*. (A) PTEN/p53-deficient GEMM tumor-derived PC cell lines (AC1/SC1) were treated with copanlisib (C, 0-500 nM, 24 hours) in a dose-dependent manner and western blot analyses were performed for indicated proteins to determine IC_{90}. **(B-C)** AC1/SC1 cells were treated with copanlisib (IC_{90}=100 nM, for 24, 48 and 72 hours) in a time-dependent manner in normal and AD conditions.

(B) Proliferation index was calculated by counting cell number at the end of treatment, relative to their baseline seeding density. **(C)** Cells were stained with annexin V antibody/propidium iodide and % cell death was determined via flow cytometry. U=untreated group. n=3 independent experiments. Significances/p-values were calculated by one-way ANOVA.

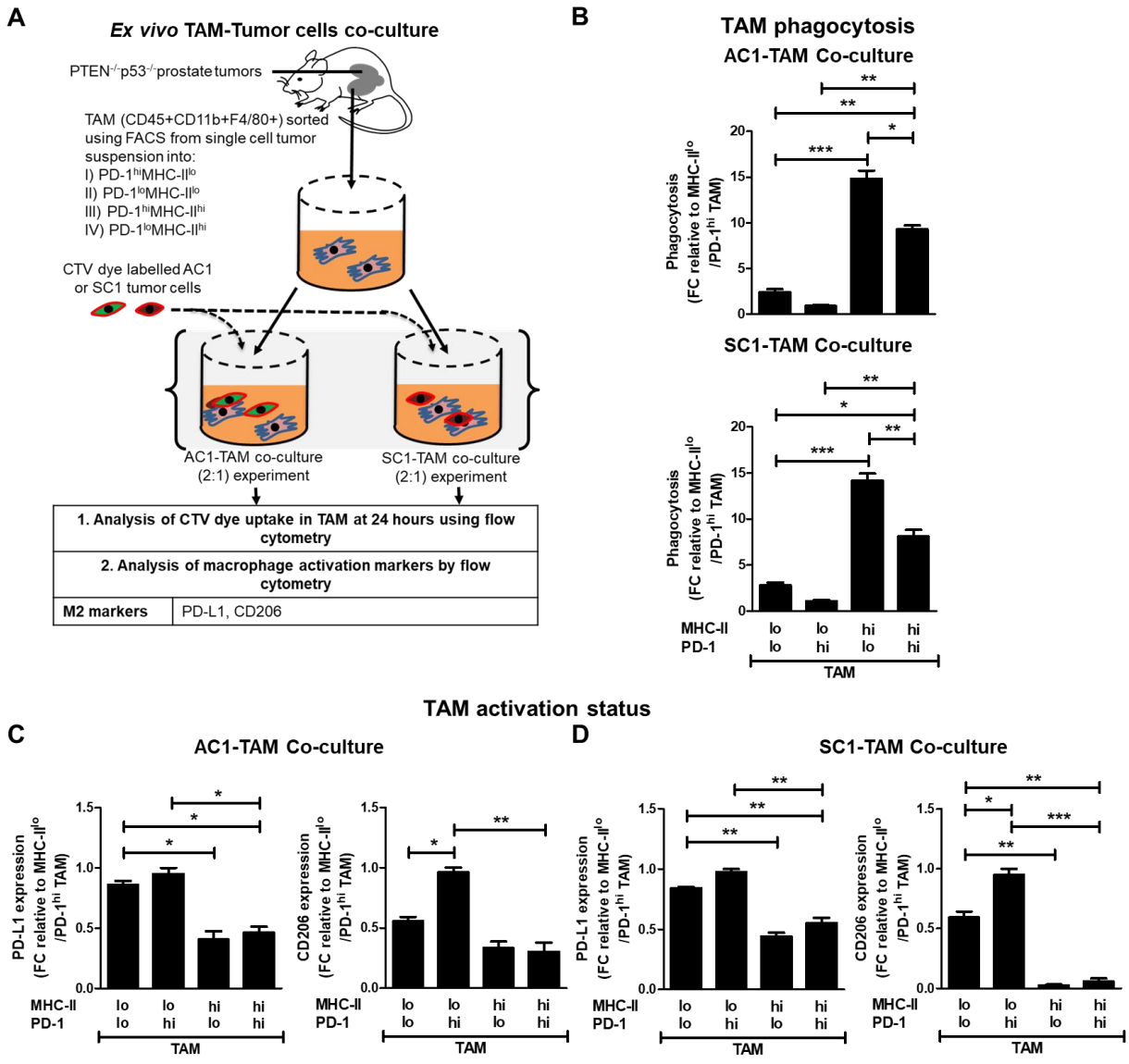
Supplementary Figure S4.



Supplementary Figure S4. ADT/PI3K inhibitor combination increases MHC-II and PD-1 expression on TAM within the TME of PTEN/p53-deficient murine PC. (A-K) Pb-Cre;

PTEN$^{fl/fl}$ Trp53$^{fl/fl}$ mice with established tumors were treated with degarelix (0.625 mg, single dose, ADT), copanlisib (14 mg/kg, *iv*, every alternate day, C) or their combination for 7 days. Tumor cell suspensions were collected and analyzed using flow cytometry. Bar graph demonstrates % frequency of TAM (suppressive **(A-C)**, and activated **(D)**), Gr-MDSC ($CD45^+CD11b^+F4/80^-Ly6g^+$) cells **(E)**), CD4-T cells ($CD45^+CD3^+CD4^+$ cells, total **(F)**, exhausted **(G)**, and activated **(H)**) and CD8-T cells ($CD45^+CD3^+CD8^+$ cells, total **(I)**, exhausted **(J)**, and activated **(K)**). **(L-O)** Single cell suspensions were prepared from established PTEN/p53-deficient prostate tumors and treated *ex vivo* with either DMSO (negative control) or copanlisib (C, 100 nM) under normal and AD conditions for 24 hours. Bar graph demonstrates % frequency of MHC-II **(L)**, PD-1 **(M)**, PD-L1 **(N)** and CD86 **(O)** expressing TAM. U=untreated group. For *in vivo* experiment, n=2 mice per group and for *ex vivo* study, n=3 independent experiments. Significances/p-values were calculated by one-way ANOVA and indicates as follows, *$p<0.05$, **$p<0.01$ and ***$p<0.001$; ns = not statistically significant.

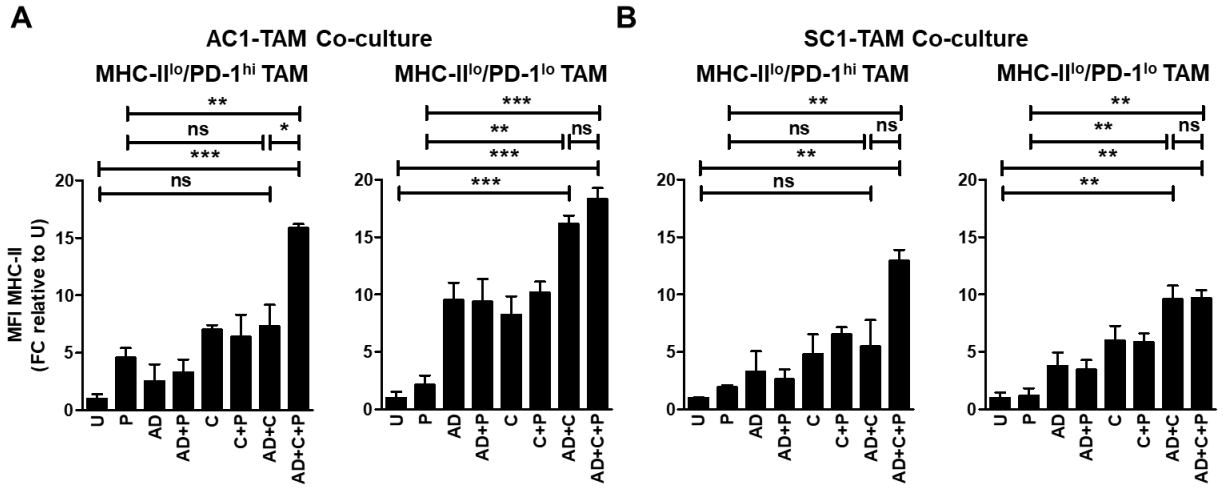
Supplementary Figure S5.



Supplementary Figure S5. PD-1 upregulation suppresses phagocytic capacity of activated TAM. (A) Experimental schema for phagocytosis experiment, in which sorted TAM from PTEN/p53-deficient prostate GEMM tumors are co-cultured with CTV dye stained-AC1/SC1 cells for 24 hours. **(B)** Bar graphs demonstrate fold change (FC) in phagocytosis of AC1 (upper panel) and SC1 (lower panel) cells by MHC-II$^{hi/lo}$/ PD-1$^{hi/lo}$ expressing TAM, relative to untreated group. PD-L1 and CD206 expression were analyzed in the TAM subsets following co-culture with AC1

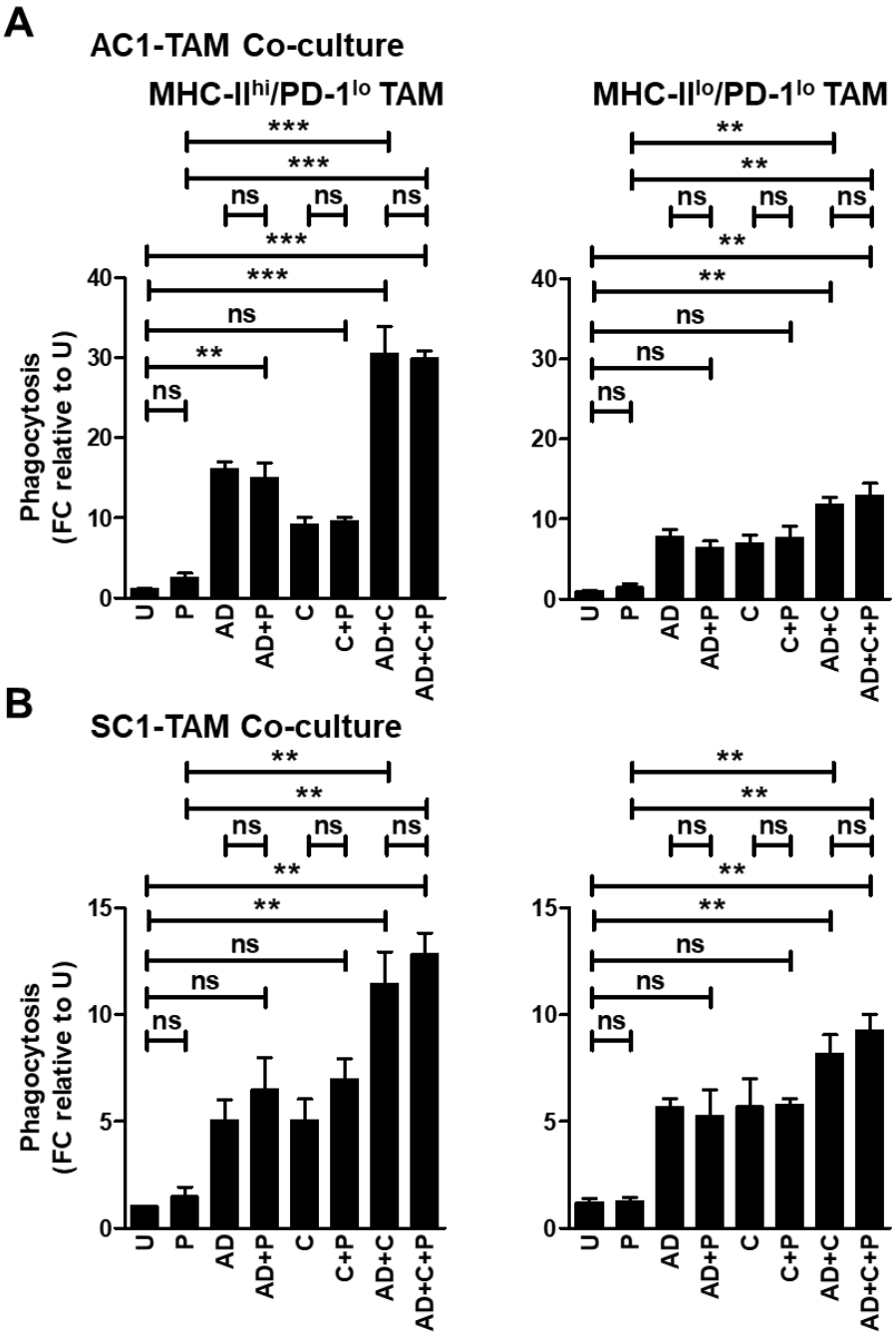
(C) and SC1 **(D)** cells. n=2 independent experiments. Significances/p-values were calculated by one-way ANOVA and indicates as follows, *$p<0.05$, **$p<0.01$ and ***$p<0.001$.

Supplementary Figure S6.



Supplementary Figure S6. ***Ex vivo*** **AD + PI3Ki + PD-1 antibody treatment activates MHC-II^{lo} TAM when co-cultured with PTEN/p53-deficient murine prostate tumor cells. (A-B)** TAM subsets (MHC-II^{lo}/PD-1$^{hi/lo}$) were sorted from PTEN/p53-deficient prostate tumors using flow cytometry, and co-cultured with PTEN/p53-deficient prostate tumor cells under normal and AD conditions. These co-cultures were treated with copanlisib (C, 100 nM), PD-1 antibody (P, 10 μg/mL) or their combination for 24 hours. Bar graphs demonstrate MHC-II expression on MHC-II^{lo}/PD-1$^{hi/lo}$ TAM following co-culture with AC1 **(A)** and SC1 **(B)** cells in the presence of indicated treatments, relative to untreated (U). FC=fold change. n=2 independent experiments. Significances/p-values were calculated by one-way ANOVA and indicates as follows, *p<0.05, **p<0.01 and ***p<0.001; ns = not statistically significant.

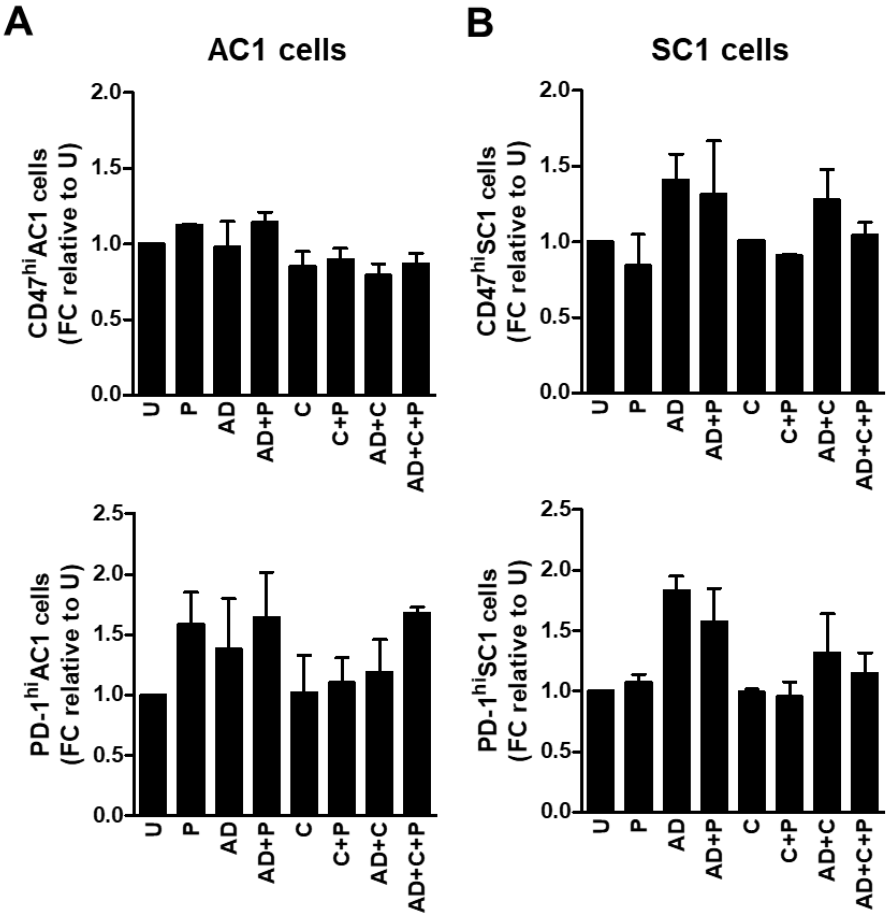
Supplementary Figure S7.



Supplementary Figure S7. The addition of PD-1 blockade to androgen depletion/PI3Ki therapy does not alter phagocytic capacity of PD-1^{lo} macrophages. (A) TAM subsets (MHC-II$^{hi/lo}$/PD-1^{lo}) sorted from PTEN/p53-deficient prostate tumors using flow cytometry were co-

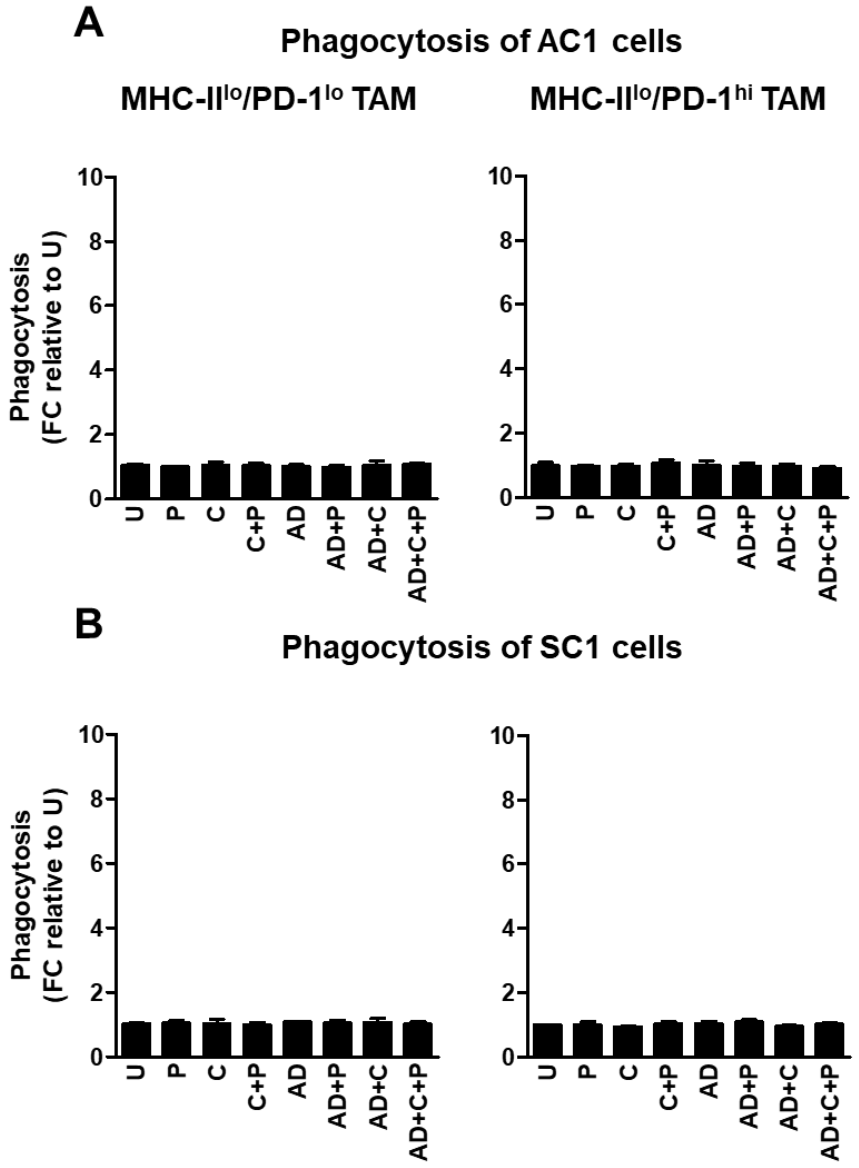
cultured with CTV dye stained-AC1/SC1 cells under normal and AD conditions. These co-cultures were treated with copanlisib (C, 100 nM), PD-1 antibody (P, 10 μg/mL) or their combination for 24 hours. Bar graphs demonstrate fold change (FC) in phagocytic activity of MHC-II$^{hi/lo}$/PD-1^{lo} expressing TAM relative to untreated group (U) in AC1 **(B)** and SC1 **(C)** cells. n=2 independent experiments. Significances/p-values were calculated by one-way ANOVA and indicates as follows, **p<0.01 and ***p<0.001; ns = not statistically significant.

Supplementary Figure S8.



Supplementary Figure S8. The combination of androgen depletion, PI3Ki and aPD-1 blockade does not alter phagocytic checkpoint expression on PTEN/p53-deficient prostate tumor cells. (A-B) AC1/SC1 cells were treated with copanlisib (C, 100 nM), PD-1 antibody (P, 10 μg/mL) or their combination under normal and AD conditions for 24 hours. Bar graphs demonstrate phagocytosis inhibitory checkpoints (CD47 and PD-1) expression on **(A)** AC1 and **(B)** SC1 cells following indicated treatments. U=untreated group. FC=fold change. n=2 independent experiments. Significances/p-values were calculated by one-way ANOVA.

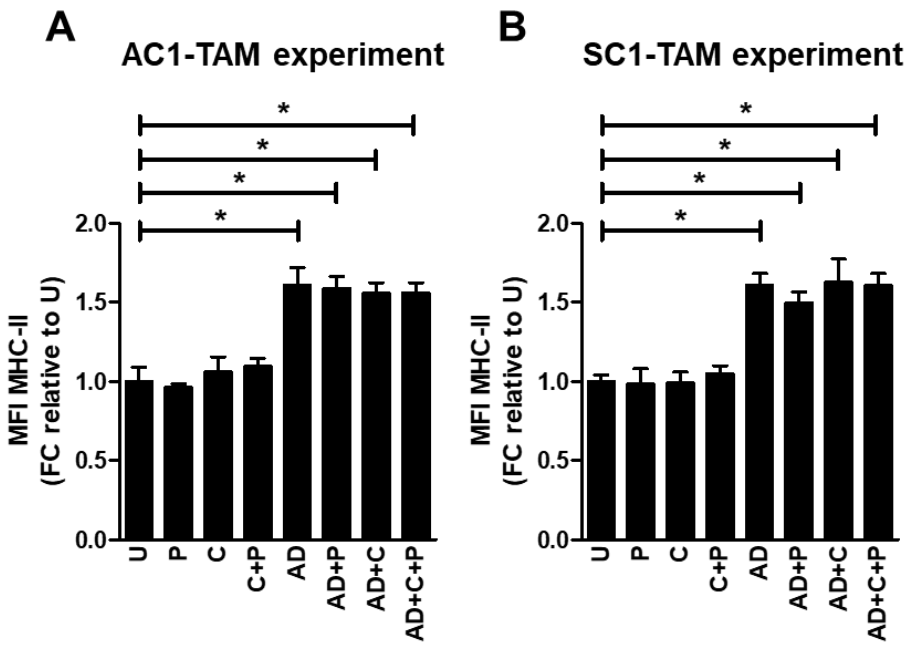
Supplementary Figure S9.



Supplementary Figure S9. Androgen depletion, singly and in combination with aPD-1, did not alter phagocytosis activity of inactivated MHC-II^{lo}/PD-1^{lo} and MHC-II^{lo}/PD-1^{hi} TAM subsets. (A-B) Pre-treatment of FACS-sorted TAM subsets from PTEN/p53-deficient prostate tumors with copanlisib (C, 100 nM), PD-1 antibody (P, 10 μg/mL) or their combinations under normal and AD conditions for 24 hours. After PBS wash, treated TAM were co-cultured with CTV dye stained-AC1/SC1 cells for 2 hours. Bar graphs demonstrate fold change (FC) in phagocytosis

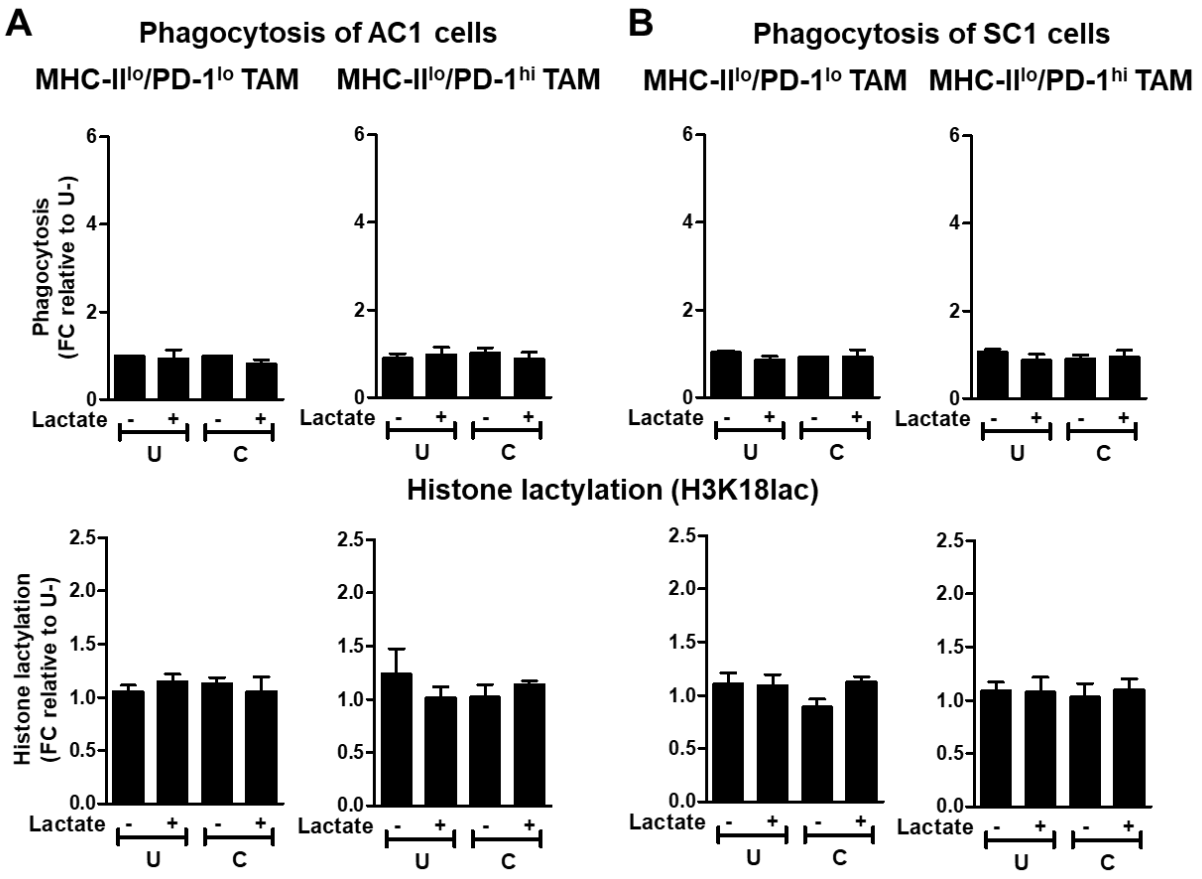
of AC1 **(A)** and SC1 **(B)** cells by MHC-II^{lo}/PD-1$^{hi/lo}$ expressing TAM, relative to untreated group (U). n=2 independent experiments. Significances/p-values were calculated by one-way ANOVA.

Supplementary Figure S10.



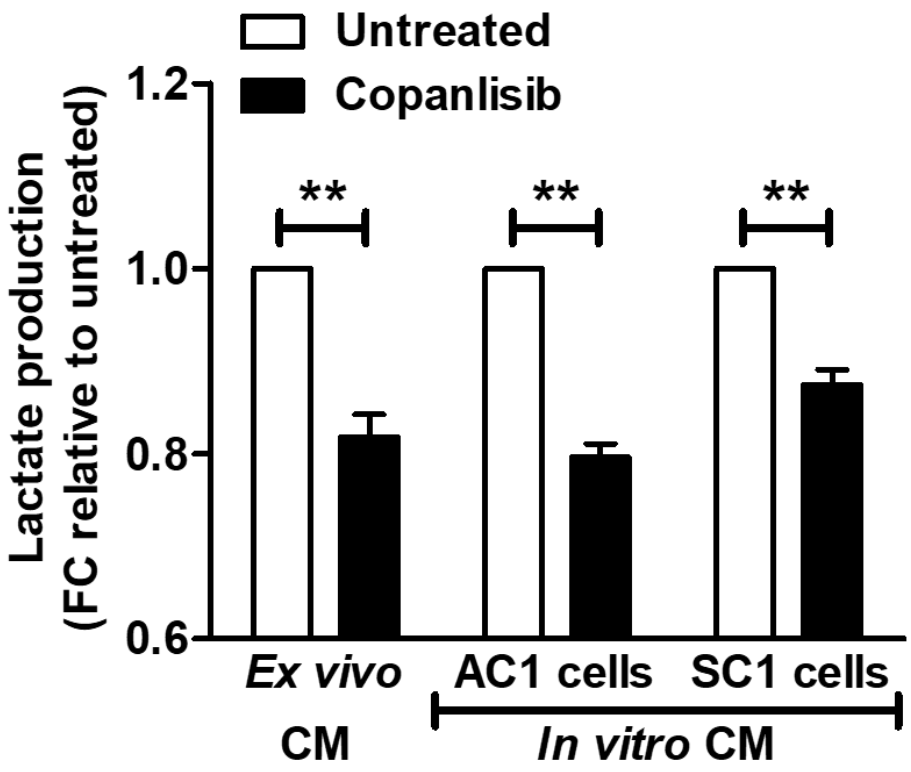
Supplementary Figure S10. Androgen depletion, not PI3Ki or aPD1, directly enhances TAM activation within the TME of PTEN/p53-deficient PC. (A-B) FACS-sorted TAM from PTEN/p53-deficient prostate tumors were directly treated with copanlisib (C, 100 nM), PD-1 antibody (P, 10 μg/mL) or their combinations under normal and AD conditions for 24 hours. Bar graphs demonstrate MHC-II expression on TAM following co-culture with AC1 **(A)** and SC1 **(B)** cells for 2 hours, in the presence of indicated treatments. U=untreated group. FC=fold change. n=2 independent experiments. Significances/p-values were calculated by one-way ANOVA and indicates as follows, *$p<0.05$.

Supplementary Figure S11.



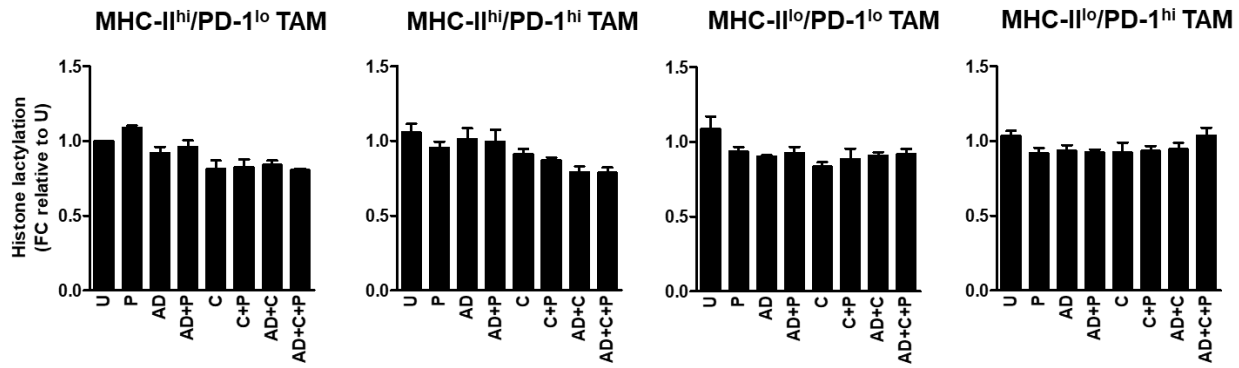
Supplementary Figure S11. PI3Ki does not alter phagocytosis/histone lactylation status of MHC-II^{lo}/PD-1^{lo} TAM and MHC-II^{lo}/PD-1^{hi} TAM. (A-B) Single cell suspension of PTEN/p53-deficient prostate GEMM tumors were treated with copanlisib (C, 100 nM) and CM was collected 24 hours following treatment. FACS-sorted TAM from PTEN/p53-deficient prostate tumors were cultured *ex vivo* with CM for 24 hours in presence or absence of lactate (100 nmol/μL). After PBS wash, TAM were co-cultured with CTV dye stained-AC1/SC1 cells for 2 hours. Bar graphs demonstrate fold change (FC) in phagocytic activity and histone lactylation status of inactivated MHC-II^{lo}/PD-1^{lo} and MHC-II^{lo}/PD-1^{hi} TAM following incubation with AC1 **(A)** and SC1 **(B)** cells, relative to untreated group. n=2 independent experiments. Significances/p-values were calculated by one-way ANOVA.

Supplementary Figure S12.



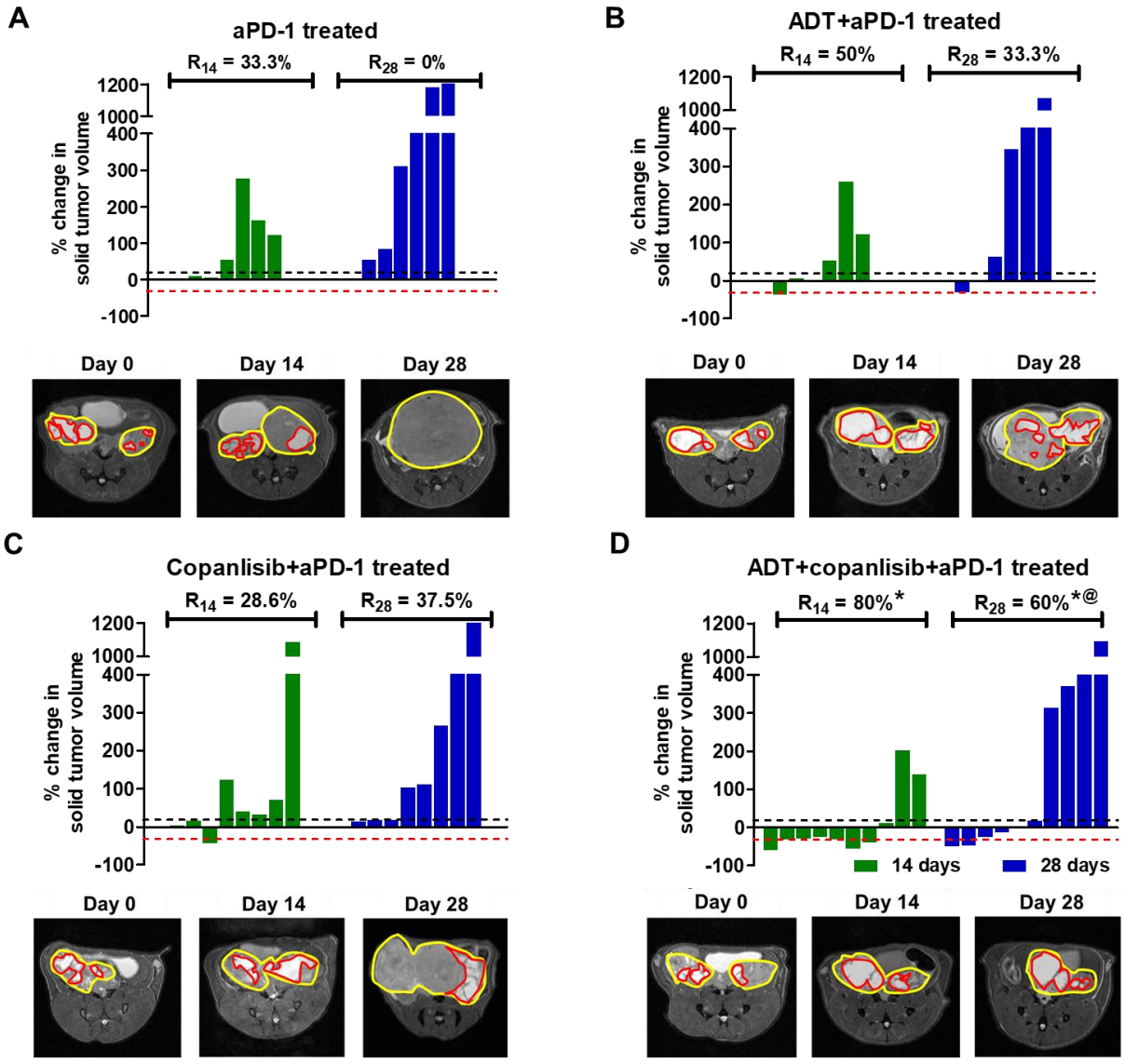
Supplementary Figure S12. PI3Ki inhibits lactate secretion from PTEN/p53-deficient prostate tumor cells within TME. Single cell suspensions of PTEN/p53-deficient prostate GEMM tumors or tumor-derived AC1/SC1 cells were treated with copanlisib (100 nM) for 24 hours. *Ex vivo* or *in vitro* CM were collected from these groups and analyzed for lactate content using colorimetry kits. n=3 independent experiments. Significances/p-values were calculated by one-way ANOVA and indicates as follows, **$p<0.01$.

Supplementary Figure S13.



Supplementary Figure S13. Direct *ex vivo* treatment of TAM with PI3Ki, singly and in combination with PD-1 antibody and/or androgen depletion does not alter their histone lactylation profile. FACS-sorted TAM from PTEN/p53-deficient prostate tumors were directly treated with copanlisib (C, 100 nM), PD-1 antibody (P, 10 μg/mL) or their combinations under normal and AD conditions for 24 hours. Bar graphs demonstrate histone lactylation status of MHC-II$^{hi/lo}$/PD-1$^{hi/lo}$ expressing TAM in response to indicated treatments. U=untreated group. FC=fold change. n=2 independent experiments. Significances/p-values were calculated by one-way ANOVA.

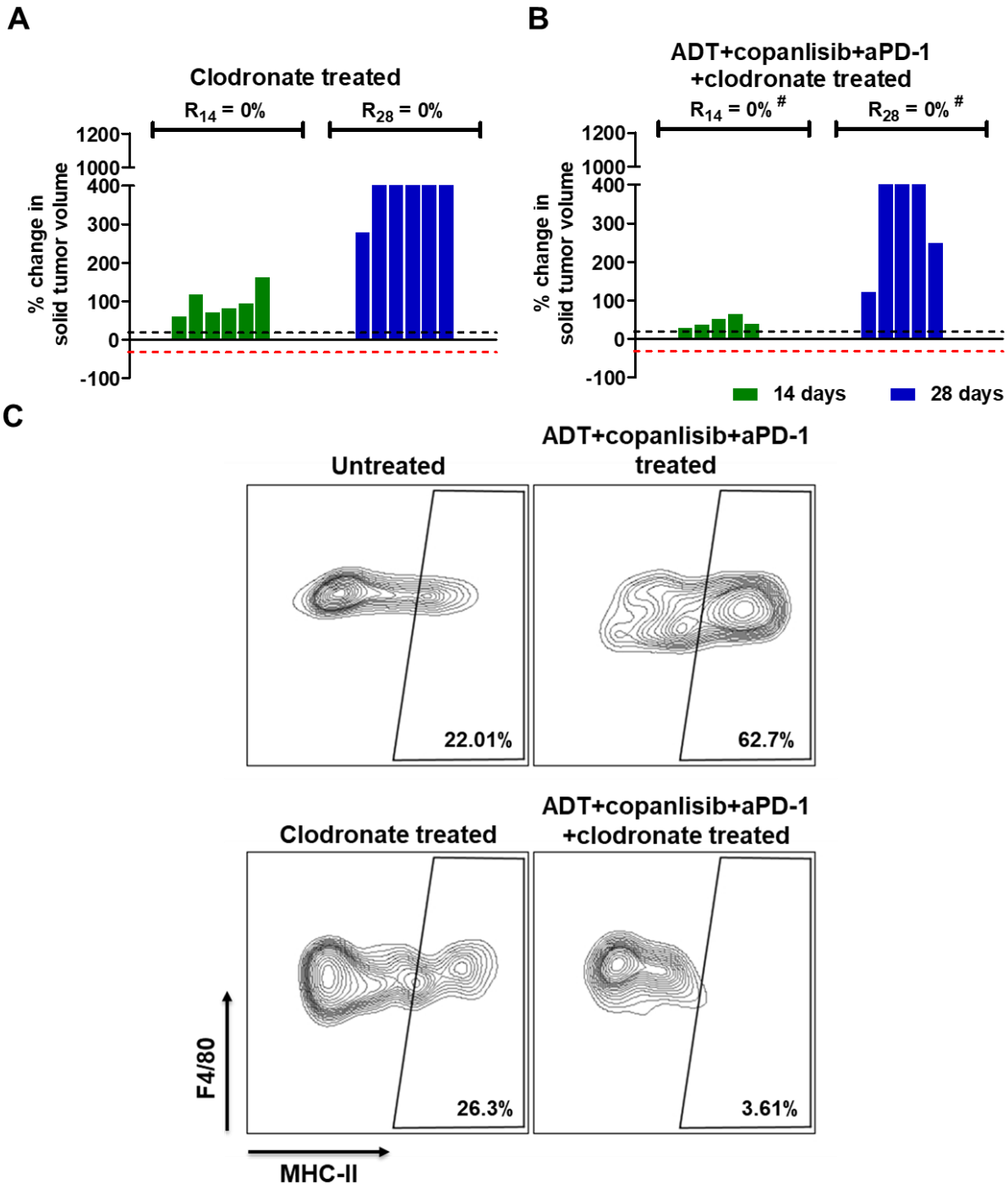
Supplementary Figure S14.



Supplementary Figure S14. ADT + PI3Ki + aPD-1 induces tumor control in 60% of Pb-Cre; PTEN$^{fl/fl}$ TP53$^{fl/fl}$ mice. (A-D) Pb-Cre; PTEN$^{fl/fl}$ Trp53$^{fl/fl}$ mice with established tumors were treated with PD-1 antibody (aPD-1, 100 µg/mouse, *ip*, every alternate day) alone or in combination with ADT (degarelix, 0.625mg, single dose) or copanlisib (14 mg/kg, *iv*, every alternate day) or ADT + copanlisib. Tumor volumes were non-invasively monitored by MRI and % ORR at days 14 (R_{14}) and 28 (R_{28}) were determined, as described in Methods. The % change in solid tumor

volume are represented by waterfall plot for the following treated groups: aPD-1 **(A)**, ADT + aPD-1 **(B)**, copanlisib + aPD-1 **(C)** and ADT + copanlisib + aPD-1 **(D)**. n=6-10 mice per group. Significances/p-values were calculated by Chi-square test and indicated as follows, *$p<0.05$ and $^{@}p<0.05$ (relative to untreated and aPD-1 treated groups, respectively).

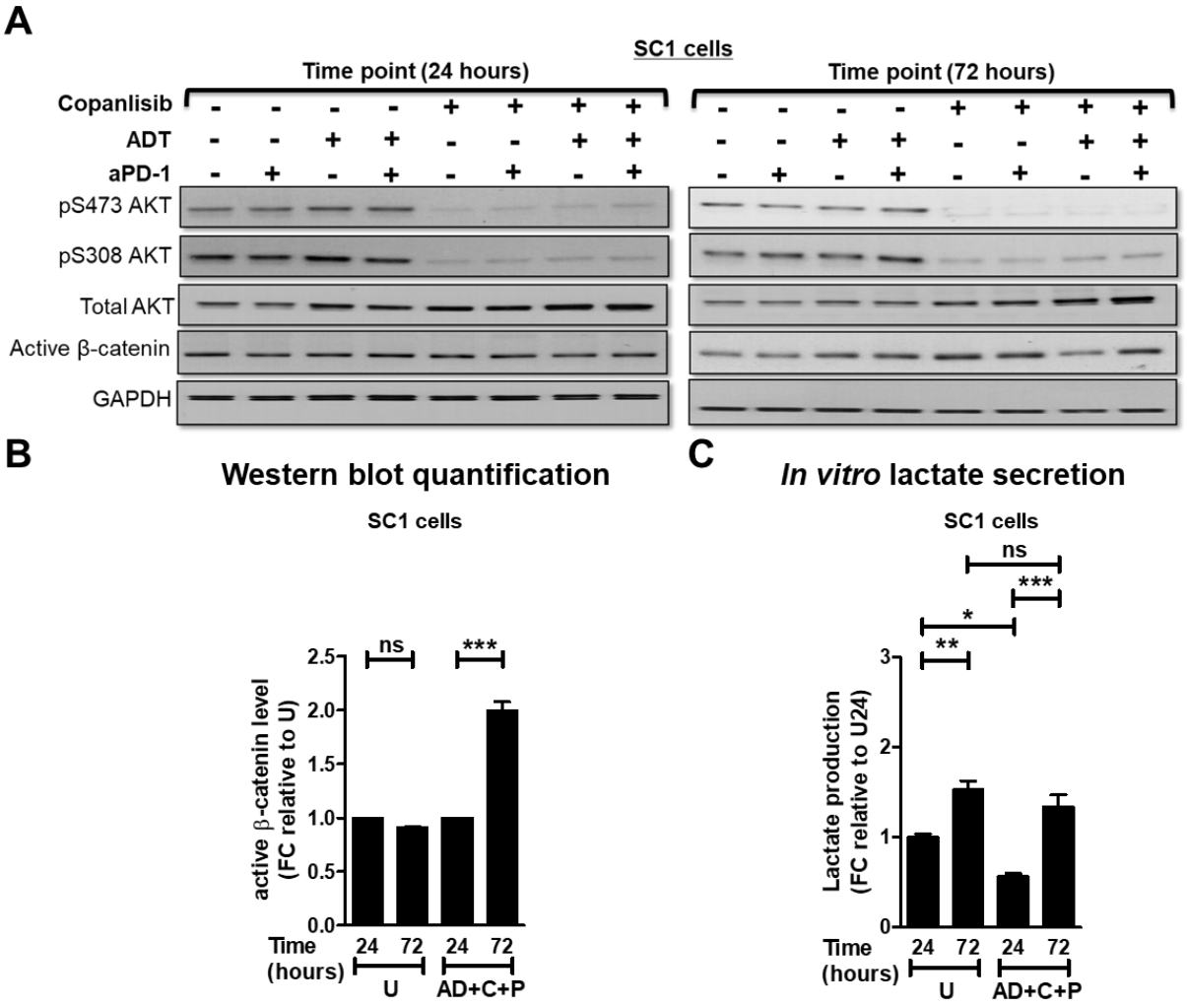
Supplementary Figure S15.



Supplementary Figure S15. Depletion of activated TAM abrogates anti-cancer response elicited by ADT + PI3Ki + PD-1 antibody treatment in the PTEN/p53-deficient murine

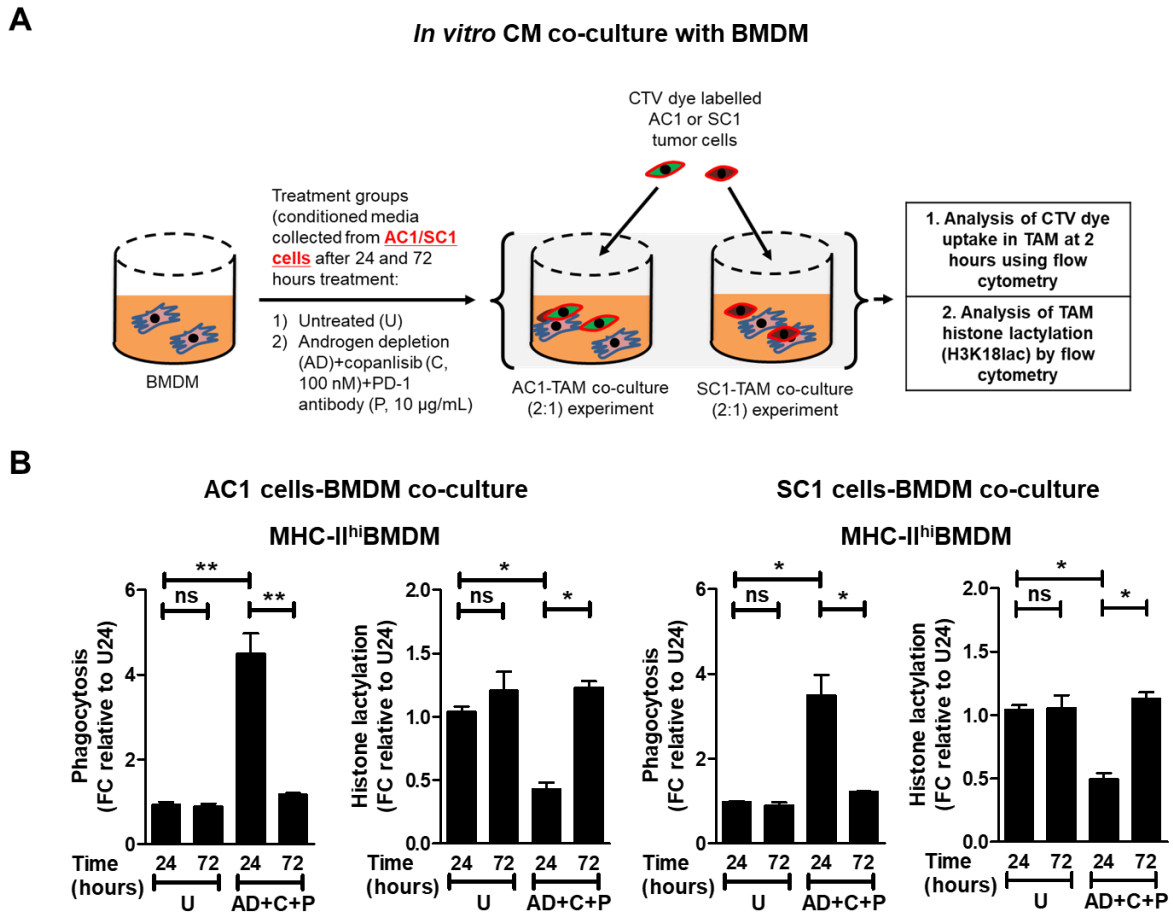
prostate GEMM tumors. (A-B) Pb-Cre; $PTEN^{fl/fl}$ $Trp53^{fl/fl}$ mice with established prostate tumors were treated with clodronate (which depletes activated macrophages, 200 μg/mouse, *ip*, every week) alone or in combination with ADT (degarelix, 0.625mg, single dose) + copanlisib (14 mg/kg, *iv*, every alternate day) + PD-1 antibody (aPD-1, 100 μg/mouse, *ip*, every alternate day). Tumor volumes were non-invasively monitored by MRI and % ORR at days 14 (R_{14}) and 28 (R_{28}) were determined as described in Methods. The % change in solid tumor volume are represented by waterfall plot for clodronate **(A)** and ADT + copanlisib + aPD-1 + clodronate **(B)** treated groups. **(C)** Flow cytometry plot represents frequency of MHC-II expressing TAM within GEMM tumors from mice treated with the indicated drugs, relative to untreated mice. n=5-6 mice per group. Significances/p-values were calculated by Chi-square test and indicates as follows, #$p<0.05$ (relative to ADT/copanlisib/aPD-1 combination treated group).

Supplementary Figure S16.



Supplementary Figure S16. Long-term treatment of ADT + PI3Ki + aPD-1 activates Wnt/β-catenin pathway in murine PTEN/p53-deficient GEMM-derived SC1 cells. (A-C) SC1 cells were treated with copanlisib (C, 100 nM) + PD-1 antibody (P, 10 μg/mL) under AD condition for 24 and 72 hours. Western blot analyses **(A)** were performed for indicated protein markers on SC1 cell lysates, quantified using Image J software **(B)**, and lactate levels **(C)** were determined by fluorimetry in the indicated SC1 supernatant groups. U=untreated group. n=3 independent experiments. Significances/p-values were calculated by one-way ANOVA and indicates as follows, *p<0.05, **p<0.01 and ***p<0.001; ns = not statistically significant.

Supplementary Figure S17.



Supplementary Figure S17. Feedback Wnt/β-catenin-pathway activation within murine PTEN/p53-deficient GEMM-derived PC cells following long-term ADT + copanlisib + aPD-1 treatment suppresses phagocytosis via increased histone lactylation within bone marrow derived macrophages (BMDM). (A) Schema illustrating phagocytosis experiment on *in vitro* CM. AC1/SC1 cells were treated with copanlisib (C, 100 nM) + PD-1 antibody (P, 10 µg/mL) under AD condition for 24 and 72 hours. *In vitro* CM were collected from these groups and co-cultured with BMDM for 24 hours. After PBS wash, these BMDM were co-cultured with CTV dye stained-AC1/SC1 cells for 2 hours. **(B)** Bar graphs demonstrate fold change (FC) in phagocytic activity and histone lactylation status of activated (MHC-II^{hi}) BMDM when co-cultured with

AC1/SC1 cells, relative to untreated group (U). n=3 independent experiments. Significances/p-values were calculated by one-way ANOVA and indicates as follows, *$p<0.05$ and **$p<0.01$; ns = not statistically significant.