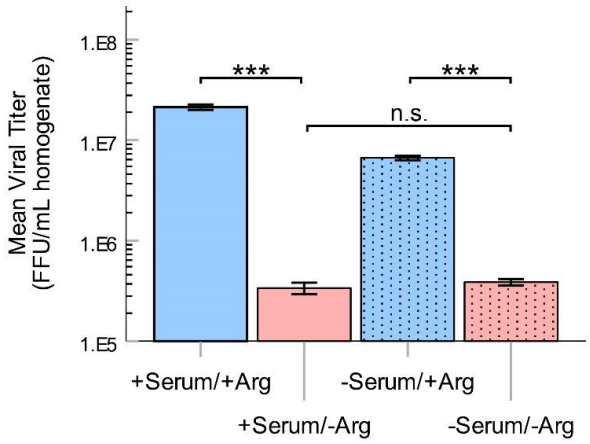
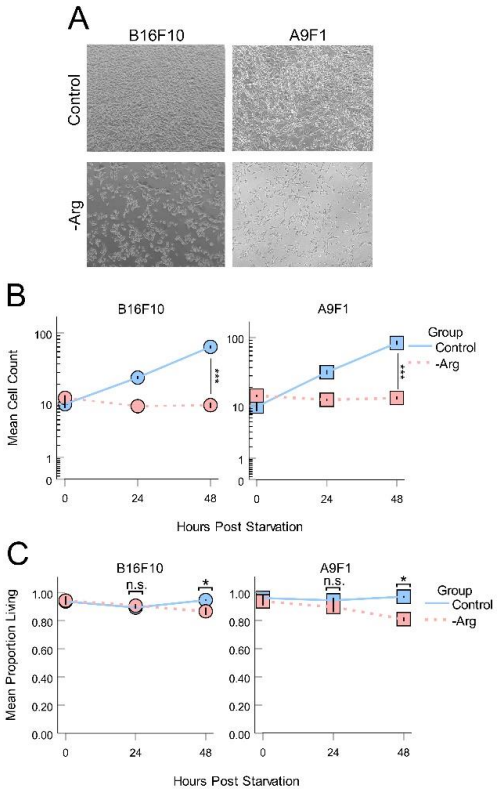
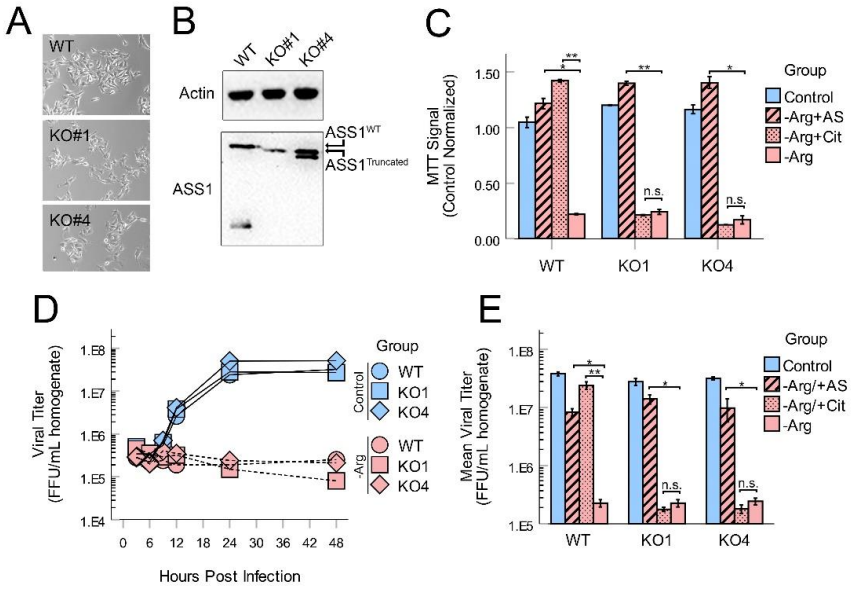


Supplemental Figure S1 – Serum does not contain functional amounts of Arg or Citrulline: $ASS1^{+}$ B16F10 cells were incubated in either complete media or media lacking Arg or FBS as indicated for 24 hours. Cells were then infected with MyxGFP at an MOI=5. 24 hours post infection, cells were harvested and the amount of infectious virus present quantified using viral titration assays. The lack of rescue of viral titer in media lacking Arg but containing FBS (compared to media lacking both Arg and FBS) indicates that the serum used does not contain levels of either Arg or citrulline that can rescue viral replication. Statistical significance was determined using ANOVA with Tukey's HSD post-hoc test (*** $p < 0.001$, n.s. = no significance). n = 3 per group.

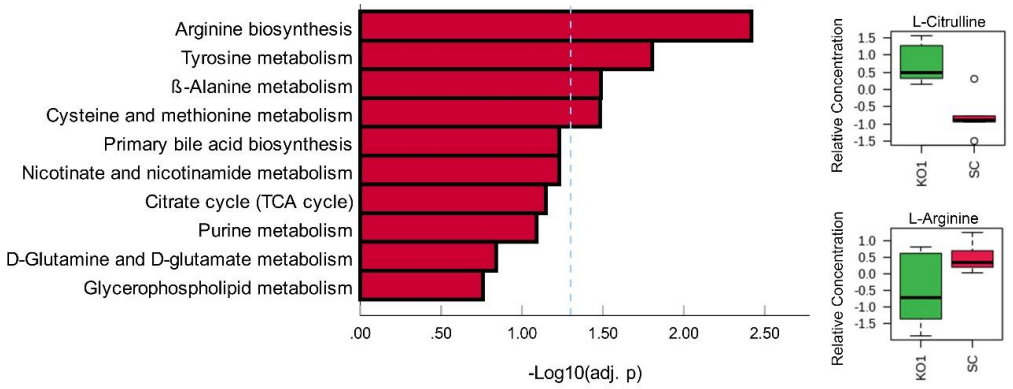


Supplemental Figure S2 – Arg starvation induces cytostasis but not loss of viability over 48 hours: The indicated cell types were incubated in either complete media or media lacking Arg for 48 hours. **(A)** Pictures showing the overall cell morphology of cells after 48 hours of Arg starvation. **(B)** At 0, 24, or 48 hours cells were harvested and the numbers of cells physically counted using a hemocytometer. **(C)** At 0, 24, or 48 hours cells were harvested and their membrane integrity analyzed using trypan blue exclusion. Taken together, the results show that Arg starvation reduces the total number of cells in a culture but does not cause an acute loss of membrane integrity suggesting that it induces cytostasis. Statistical significance in B determined by an unpaired Student's t-test (*** $p < 0.001$), n = 3 per group. Statistical significance in C determined by ANOVA with Tukey's HSD post-hoc test (* $p < 0.05$, n.s. = no significance), n = 3 per group.

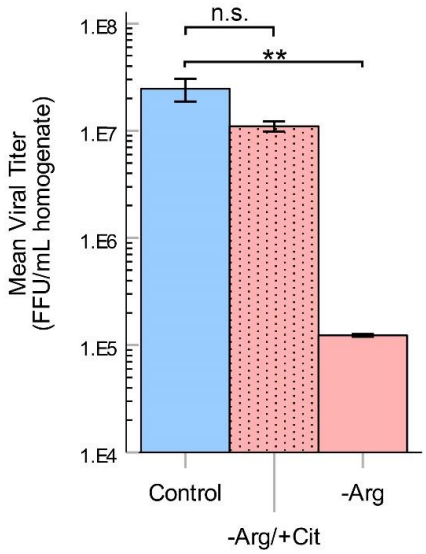


Supplemental Figure S3 – Validation of ASS1^{KO} B16F10 cell lines: B16F10 cells obtained from ATCC were treated with a CRISPR/Cas9 system targeting murine ASS1 (gRNA sequence: TCAGGCCAACATTGGCCAGA; plasmid: PX459, Genscript, Piscataway NJ) as described in the methods section. Following CRISPR/Cas9 treatment, two distinct clonal ASS1^{KO} lines were isolated (KO#1 and KO#4). Control B16F10 cells treated with a scrambled gRNA (referred to as WT cells and described in the methods section) were used as a control. **(A)** Light microscopy images showing the overall morphology of WT and KO#1/#4 cells. **(B)** Western blot showing the expression and size of actin and ASS1 from WT and KO#1/#4 cells. **(C)** Growth of WT and KO cells in various medias. WT and KO#1/#4 cells were incubated in the indicated media for 48 hours. The number of viable cells in each culture was then determined using MTT assay. **(D)** Single-step growth curve analysis of MyxGFP in WT, KO#1, and KO#4 cell lines. Cells were incubated in either control media or media lacking Arg for 24 hours and then infected with MyxGFP at an MOI=5. At the indicated time points, cells were harvested and the amount of infectious virus present quantified using viral titration assays. **(E)** WT and KO#1/#4 cells were incubated in the indicated medias for 24 hours and then infected with MyxGFP at an MOI=5. 24 hours post infection, the amount of infectious virus present

quantified using viral titration assays. Statistical significance in C and E were determined by ANOVA with Tukey's HSD post-hoc (* $p < 0.05$, ** $p < 0.01$, n.s. = no significance), n = 3 per group.



Supplemental Figure S4 – Loss of ASS1 alters B16F10 metabolite profiles. Either WT or KO#1 tumors were established in C57Bl/6J mice. 11 days after implantation, tumors were harvested and immediately snap frozen in liquid nitrogen. Whole tumor homogenates were then used to quantify a set of 226-metabolite species via LC-MS as described in the methods section. n = 5 of each tumor type. **(A)** Metabolite Set Enrichment Analysis of LC-MS results. **(B)** Specific results for citrulline and Arg.



Supplemental Figure S5 – Replication of vPD1/IL12 is also Arg dependent. (A) ASS1^{WT} B16F10 cells were incubated as above and then infected with MyxPD1/IL12 at an MOI=5. 24 hours post-infection the amount of infectious virus present was quantified using viral titration assays. Statistical significance was determined by ANOVA with Tukey's HSD post-hoc test (** $p < 0.01$, n.s. = no significance). n = 3 per condition.