

Figure S1

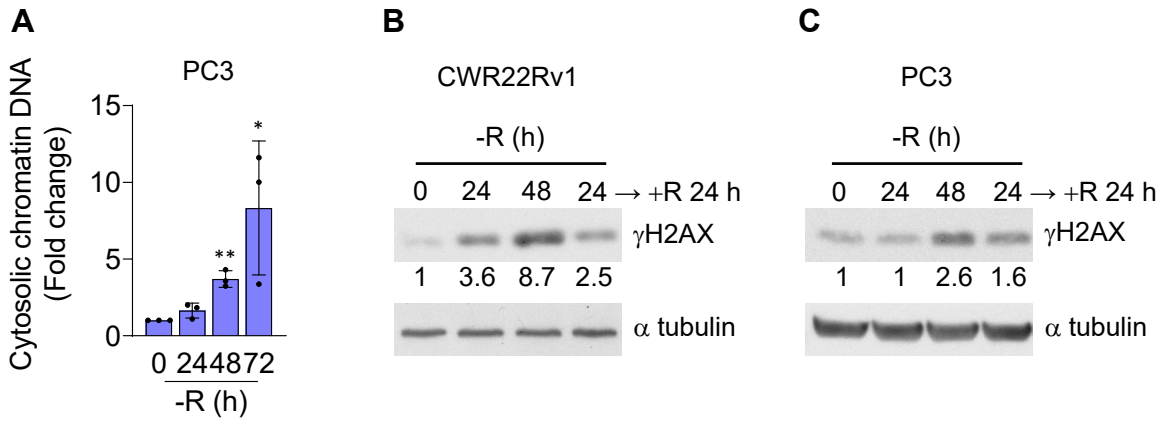


Figure S1. Arginine starvation induced DNA damage and chromatin leakage in prostate cancer cells.

- (A) PC3 cells were starved for indicated timepoints, and cytosolic chromatin DNA was measured with ELISA assay. (n = 3, *p < 0.05, **p < 0.01)
- (B) Immunoblot analysis of γ H2AX in CWR22Rv1 cells deprived of arginine. Fold change is listed below the blot.
- (C) Immunoblot analysis of γ H2AX in PC3 cells deprived of arginine. Fold change is listed below the blot.

Figure S2

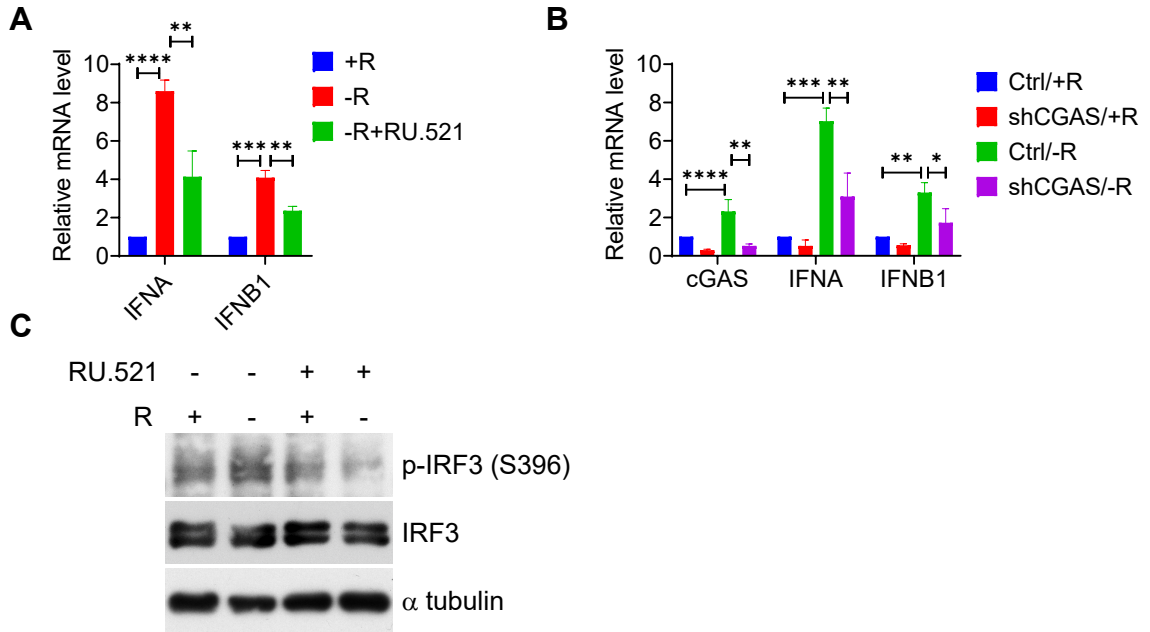


Figure S2. Inhibition of cGAS reduced the type I IFNs response.

- (A) CWR22Rv1 cells were deprived of arginine with or without RU.521, and type I IFNs expression were analyzed by RT-qPCR. (n = 3, **p < 0.01, ***p < 0.001, ****p < 0.0001)
- (B) CGAS was knockdown by shRNA, and cGAS and type I IFNs expression were analyzed by RT-qPCR. (n = 3, **p < 0.01, ***p < 0.001, ****p < 0.0001)
- (C) IRF3 phosphorylation was analyzed by western blots.

Figure S4

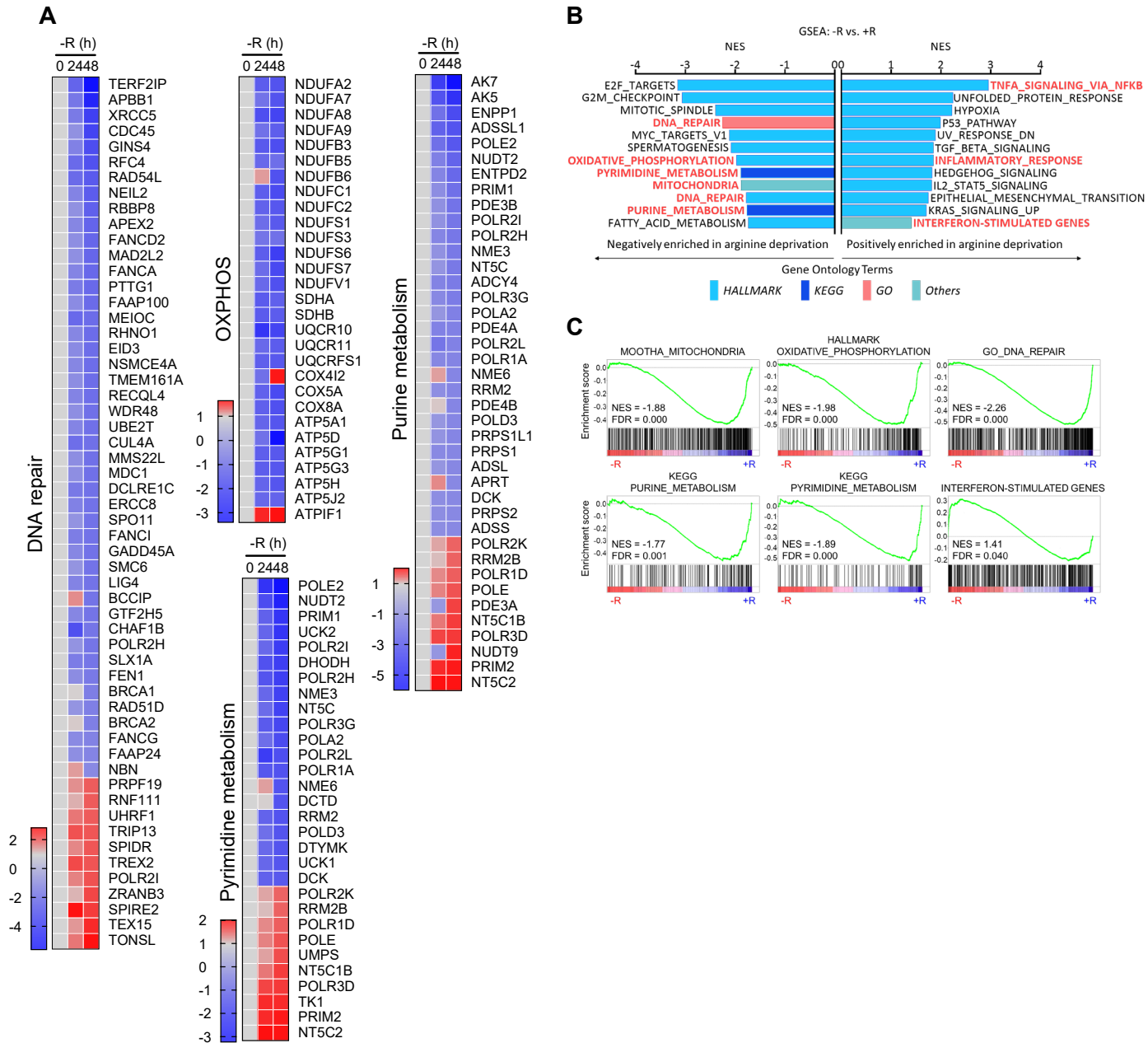


Figure S4. Arginine starvation downregulated OXPHOS and DNA-repair genes.

- (A) Gene expression of metabolic and DNA repair related genes was analyzed by microarray.
- (B) PC3 cells were deprived of arginine for 48 h and subjected to GSEA analysis. All enrichment results met the statistical criteria of $p < 0.05$ and $FDR < 25\%$.
- (C) Enrichment score plots of selected gene sets in PC3 cells.

Figure S5

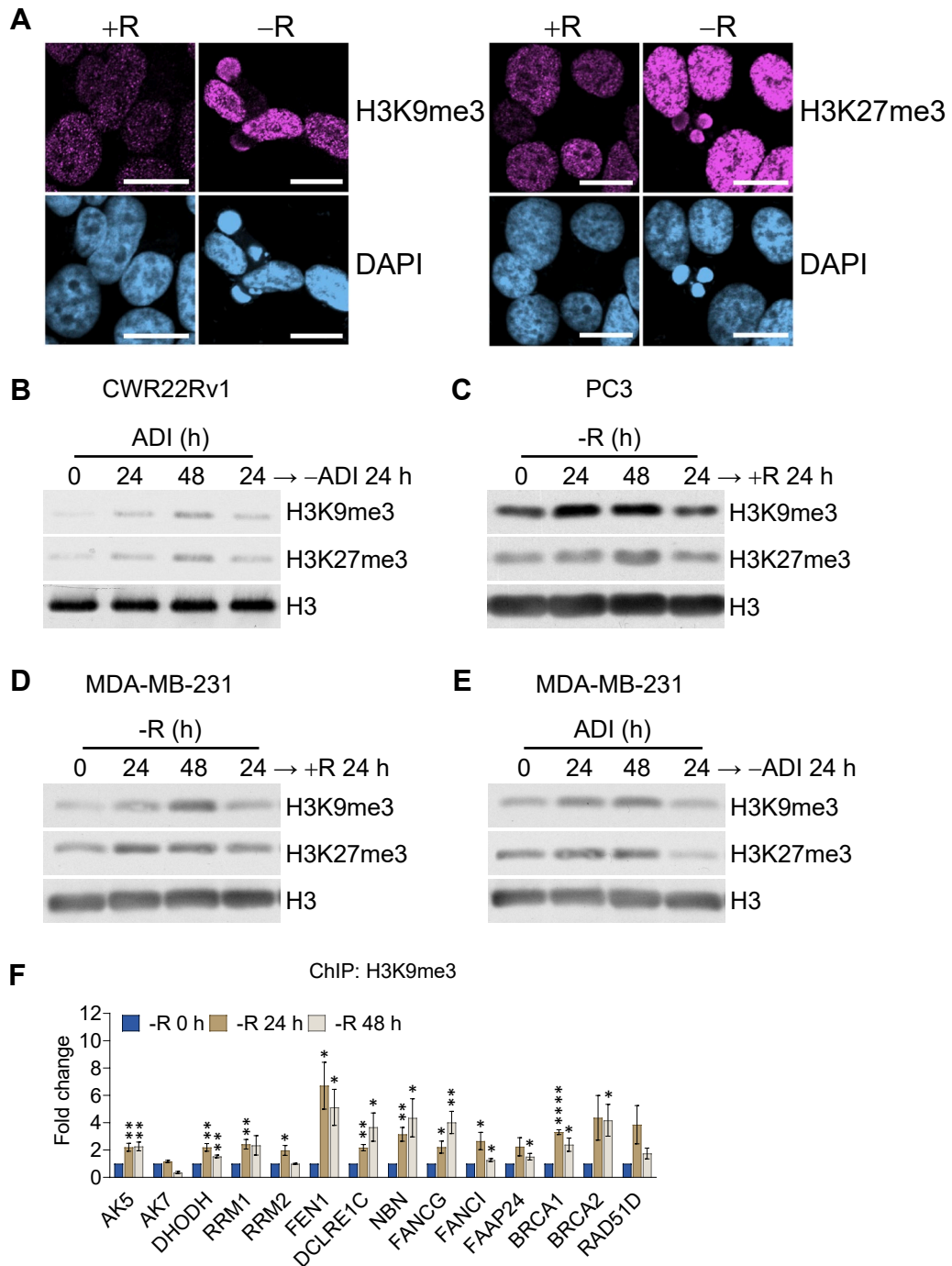


Figure S5. H3K9me3 and H3K27me3 were upregulated by arginine starvation in ASS1-low cells.

- (A) H3K9me3 and H3K27me3 expressions in CWR22Rv1 cells were analyzed with immunostaining. Scale bars, 10 μ m.
- (B) Immunoblot analysis of H3K9me3 and H3K27me3 in CWR22Rv1 cells treated with ADI-PEG20.
- (C) H3K9me3 and H3K27me3 expression in arginine-deprived PC3.
- (D) H3K9me3 and H3K27me3 expression in arginine-deprived MDA-MB-231.
- (E) H3K9me3 and H3K27me3 expression in ADI-PEG20-treated MDA-MB-231.
- (F) H3K9me3 recruitment to the promoter regions of DNA-repair genes was analyzed by ChIP-qPCR. (n = 3, *p < 0.05, **p < 0.01, ****p < 0.0001)

Figure S6

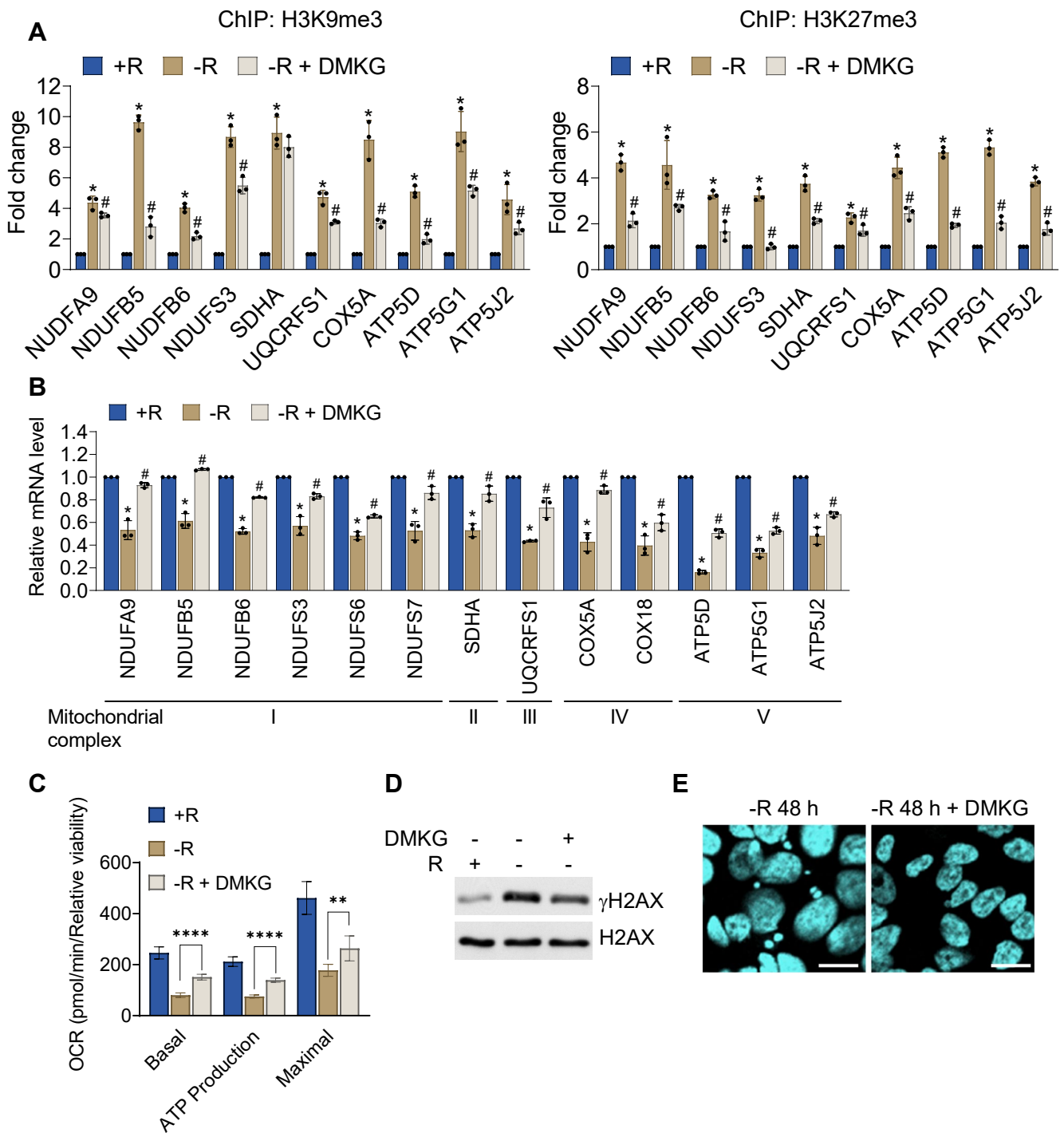


Figure S6. Supplement of α KG partially restored OXPHOS gene expression and alleviated DNA damage.

- (A) H3K9me3 and H3K27me3 distribution on the promoter regions of OXPHOS genes in arginine-deprived CWR22Rv1 with or without DMKG. (n = 3, * represents -R vs +R, and # represents -R + DMKG vs -R. n = 3, *, #p < 0.05)
- (B) OXPHOS gene expression in arginine-deprived CWR22Rv1 with or without DMKG. (* represents -R vs +R, and # represents -R + DMKG vs -R. n = 3, *, #p < 0.05)
- (C) Basal respiration, ATP production and maximal respiration in arginine-deprived CWR22Rv1 with or without DMKG. (n = 5, **p < 0.01, ****p < 0.0001)
- (D) DMKG reduced arginine starvation-induced γ H2AX in CWR22Rv1.
- (E) Representative images of chromatin leakage in the presence of DMKG. Scale bars, 10 μ m.

Figure S7

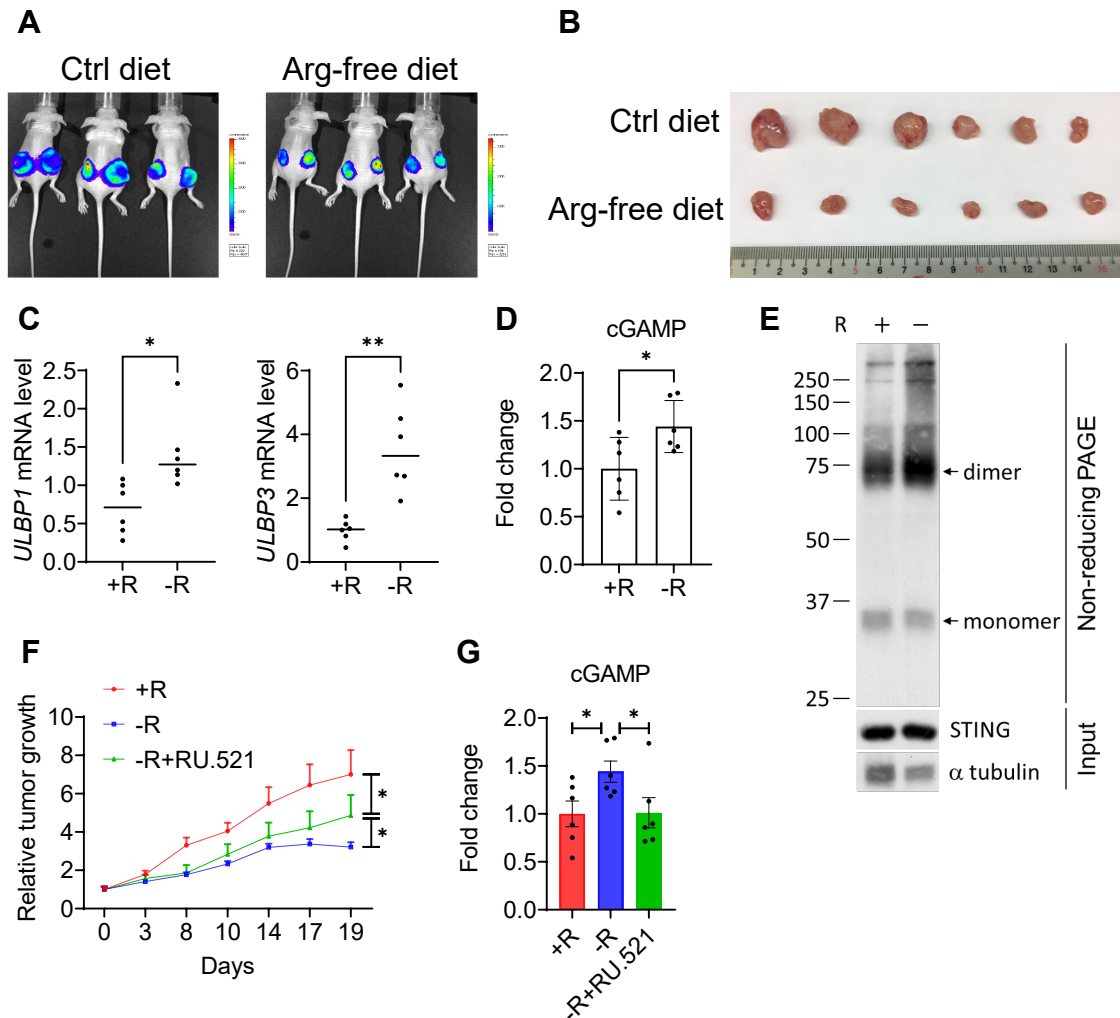


Figure S7. Arginine starvation regulated expression of NK cell ligands.

- (A) IVIS images of CWR22Rv1 xenografts.
- (B) Tumor images of isolated xenografts.
- (C) Expression of NK cell ligands, ULBP1 and ULBP3 were analyzed with RT-qPCR. (n = 6, *p < 0.05, **p < 0.01)
- (D) CWR22Rv1 xenografts were lysed with IP lysis buffer, and cGAMP level was determined by ELISA assay. (n = 6, *p < 0.05)
- (E) Cell lysates from the xenografts were separated in non-reducing SDS-PAGE for detecting dimerized STING.
- (F) Treatment of RU.521 partially rescued the tumor growth inhibition in an arginine-free diet mouse xenograft model.
- (G) ELISA assay for measurement of cGAMP levels in the tumors of (F).

Figure S8

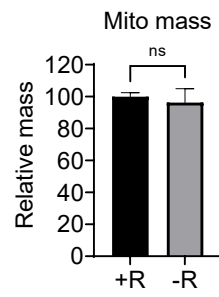


Figure S8. CWR22Rv1 cells were cultured in arginine-free medium for 72 h, and mitochondrial mass was determined using Mitotracker green. (n = 3)

Table S1

Table S1. Metabolite analysis in PC3 treated with ADI-PEG20

PC3/Metabolite	R(-)/R(+)
Quinolinic acid	2.123
L-Pyroglutamic acid	1.522
D-Glucose 6-P	1.015
D-Fructose 6-P	1.015
Tryptophan	0.993
Citrulline	0.942
Ribose 5-P	0.935
Glyceraldehyde-3-P	0.92
Phosphoenolpyruvate (PEP)	0.917
Glucose	0.912
L-Carnitine	0.899
Fructose-1,6-BP	0.859
3-Phosphoglycerate	0.853
NAD+	0.602
Glutamate	0.502
α-Ketoglutarate	0.402
2,3-dinor Thromboxane B2	0.18
Thromboxane B2	<0.1
Pyruvate	<0.1
Malate	<0.1
Succinate	<0.1
Lactate	<0.1
Prostaglandin D2	<0.1
11-dehydro-2,3-dinor Thromboxane B2	<0.1