

Supplementary Materials

Figure S1

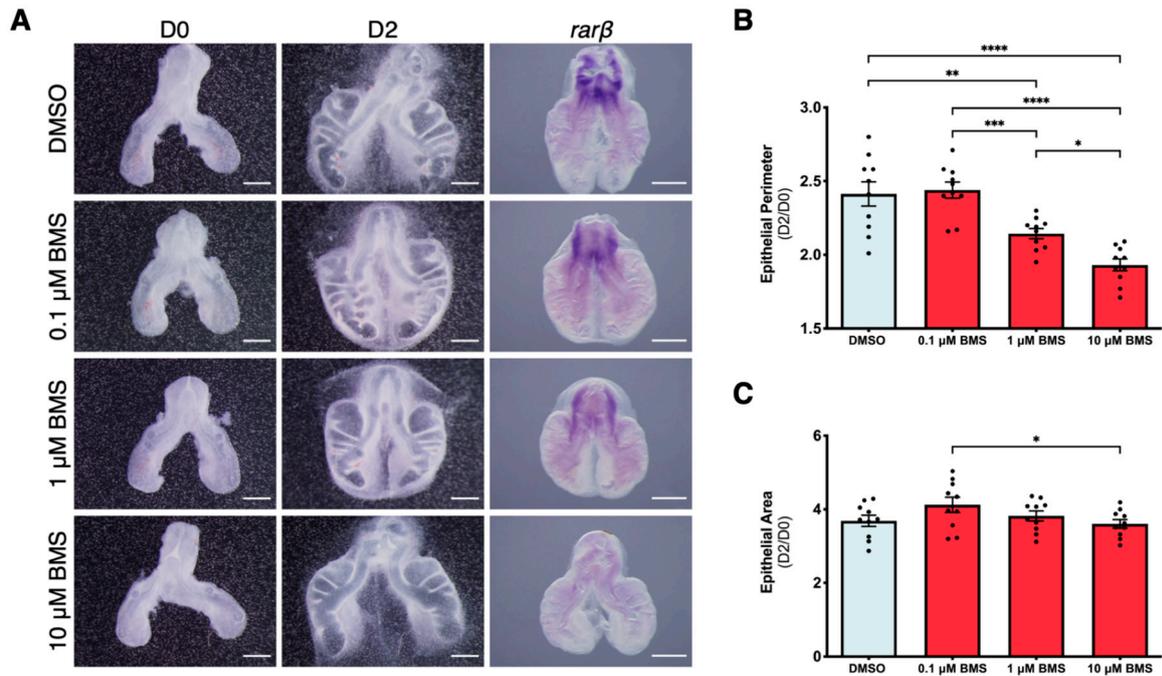


Figure S1. Dose-dependent effect of BMS treatment on RA signaling pathway and lung branching morphogenesis. (A) Representative examples of b2 lung explant culture at D0 (0 hours) and D2 (48 hours), treated with DMSO, 0.1 μM of BMS, 1 μM of BMS, and 10 μM of BMS; D2 lungs were probed for *rarβ*, a recognized target of the RA signaling pathway; $n = 5/\text{condition}$). scale bar: 500 μm . There is a dose-dependent decrease in *rarβ* expression, suggesting a gradual downregulation of the RA signaling pathway. Morphometric analysis of lung explants: (B) epithelial perimeter and (C) epithelial area. Results are expressed as D2/D0 ratio and represented as mean \pm SEM ($n = 10/\text{condition}$). One-Way ANOVA and Fisher's LSD test were performed. Significantly different results are indicated as: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. There is a progressive decrease in the epithelial perimeter, and no major alterations were detected in the epithelial area.

Figure S2

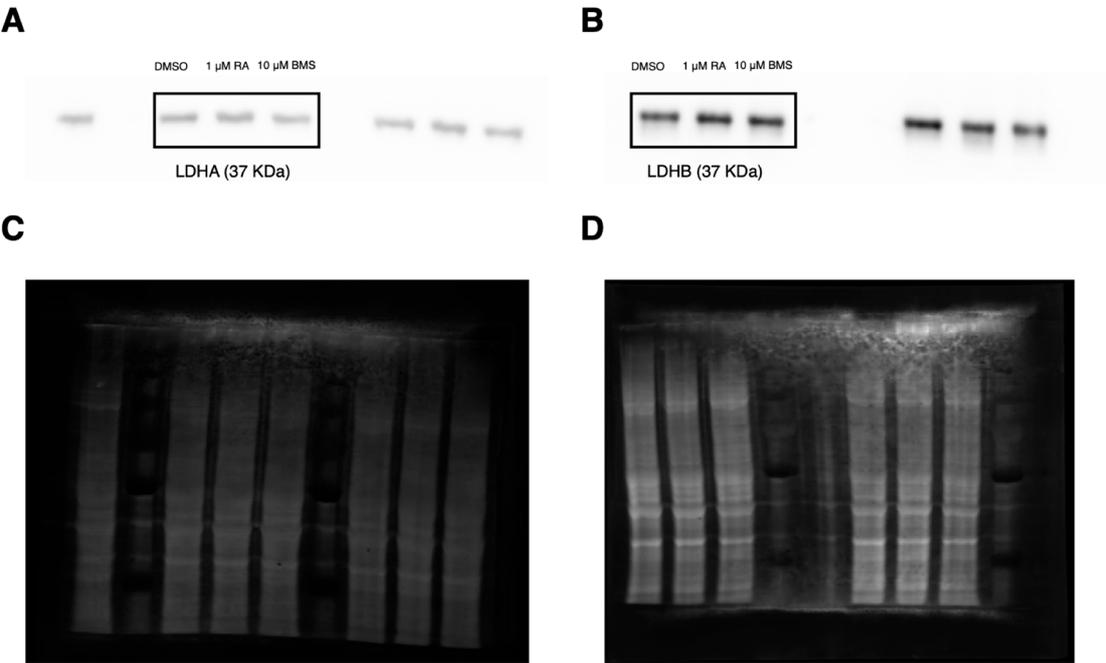


Figure S2. LDHA and LDHB full-length blots and total protein. (A) LDHA immunoblot (37 KDa). **(B)** LDHB immunoblot (37 KDa). **(C)** LDHA membrane total protein staining. **(D)** LDHB membrane total protein staining. Quantitative fluorescent total protein stain was performed using AzureRed technology.

Figure S3

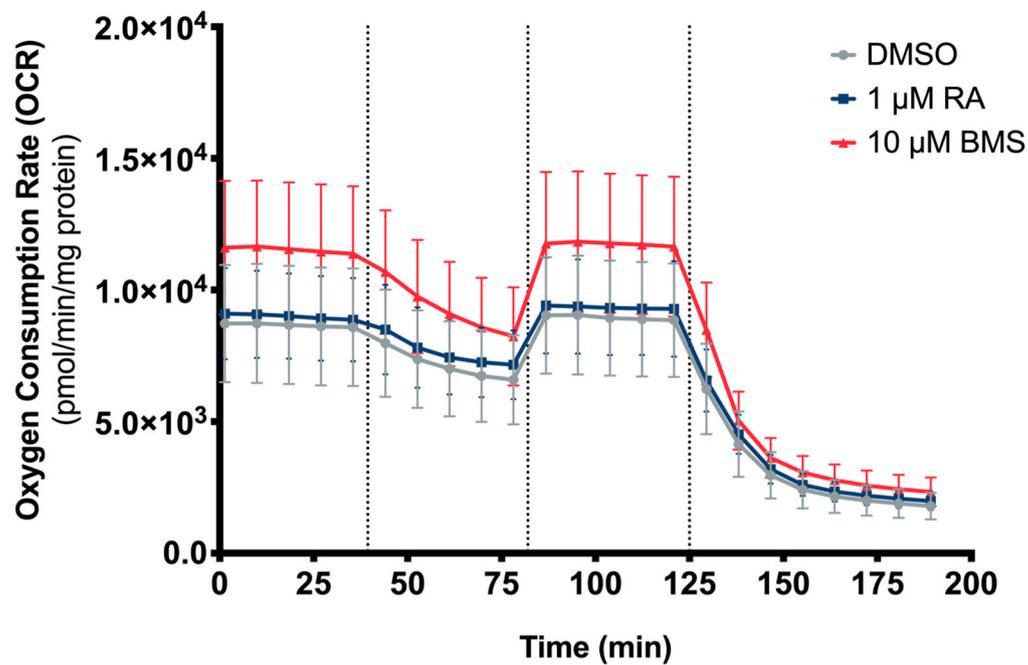


Figure S3. Seahorse OCR profile. Real-time measurement of oxygen consumption rate (OCR) of D2 lung explants exposed to DMSO, 1 μM of RA, and 10 μM of BMS. Time of measurements and moment of injections: 1st injection composed of Oligomycin (inhibition of complex V), 2nd injection composed of FCCP (mitochondrial oxidative phosphorylation uncoupler), and 3rd injection composed of Rotenone and Antimycin A (inhibition of complex I and III, respectively). Results are represented in pmol/min/mg protein. Results are expressed as mean ± SEM ($n \geq 13$ /condition).

Figure S4

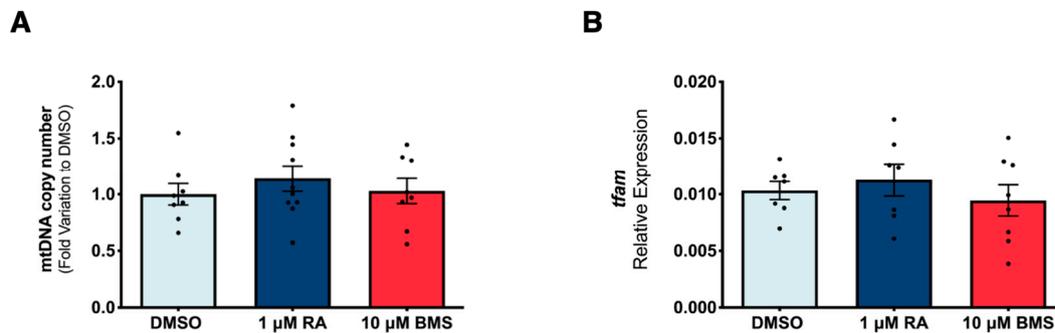


Figure S4. Mitochondrial biogenesis in lung branching morphogenesis. Mitochondrial biogenesis analysis of lung explants after 48 hours in culture supplemented with DMSO, 1 μM of RA, and 10 μM of BMS. **(A)** mtDNA copy number ($n \geq 8$ /condition). Results are represented in fold variation to DMSO. **(B)** *tfam* relative expression levels ($n \geq 7$ /condition). Results are expressed as mean \pm SEM. One-Way ANOVA and Fisher's LSD test were performed. No significantly different results.

Figure S5

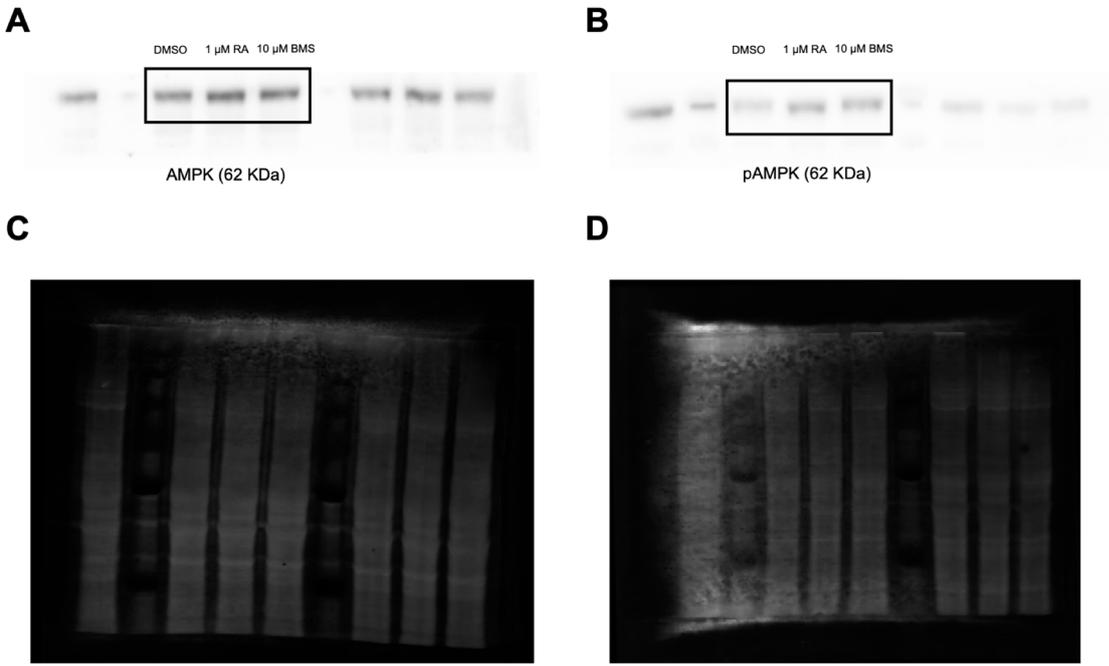


Figure S5. AMPK and pAMPK full-length blots and total protein. (A) AMPK immunoblot (62 KDa). (B) pAMPK immunoblot (62 KDa). (C) AMPK membrane total protein staining. (D) pAMPK total protein staining. Quantitative fluorescent total protein stain was performed using AzureRed technology.

Table S1

Table S1. Primers and qPCR conditions. Primer sequences forward (Fw) and reverse (Rv), corresponding PCR product size, annealing temperature and the number of cycles.

Gene	Sequence 5'-3'	Size (bp)	Annealing T (°C)	Cycles
<i>actin-β</i>	Fw - CTTCTAAACCGGACTGTTACCA	100	58	30
	Rv - AAACAAATAAAGCCATGCCAATCT			
<i>18s</i>	Fw - TCTTTCTCGATTCCGTGGGT	157	58	30
	Rv - AACGCCACTTGTCCTCTAC			
<i>pfk1</i>	Fw - CGTGGGAGGAGCTTTGAGAA	236	56	40
	Rv - CAGCCCACCTCACGTATCTG			
<i>g6pd</i>	Fw - CTGGGGCAGTACGTGGGTAA	232	62	40
	Rv - CCGAAATATCGCCCGGAACC			
<i>pgd</i>	Fw - AATAAATTAGTGCCGTTGTTGGA	174	60	40
	Rv - TGGCATGAGTGAAGGACCAT			
<i>tfam</i>	Fw - GGAGAAAACGGCTGGCAAAA	211	60	40
	Rv - AGCTGAAGGTATGGCTGCTT			
<i>sreb1</i>	Fw - GCTCTCGGCTTCGACGAT	145	60	40
	Rv - CGAACAGCCCTGAGAAGTCAT			
<i>fasn</i>	Fw - CGGATCTCTCCCACTCTGGA	143	60	40
	Rv - CCGTGCAATGCCATCTTAGC			
<i>cpt1</i>	Fw - TCATTGCGGGGAAAACCTC	158	64	40
	Rv - CCCACGGCCTTTATTGCTC			
<i>nd1</i>	Fw - TGTAGAATATGCCGCCGGAC	214	62	40
	Rv - GTCATAGCGGAACCGTGGAT			
<i>agrt1</i>	Fw - TGGCCATAGTCATCCAGTG	119	62	40
	Rv - ACGATGAATGATGACGGGCA			