Expanded View Figures

Figure EV1. Selection and characterization of *ADPGK-AS1*.

A Schematic overview of criteria for choosing appropriate deregulated lncRNA candidates based on RNA-seq data for M1-like and M2-like macrophages.

B Exon/intron structure of the three annotated transcript variants of *ADPGK-AS1* (lower panel) with RNA sequencing coverage (sashimi plot) from our sequencing of M2 macrophages of the same genomic location (upper panel), split by read per strand (m2_f=plus strand, m2_r=minus strand).

C 5′ and 3′ sequences of *ADPGK-AS1* identified by 5′/3′ RACE are compared with the sequence of *ADPGK-AS1* transcript variant 1.

D Coding probability and coding potential score using the cDNA-sequence of *ADPGK-AS1*, with β-actin as an example of a protein-coding sequence and MALAT1 as an example of a noncoding transcript.

E Transcription and subsequent translation of *ADPGK-AS1* to exclude the coding function of proteins > 10 kDa. The asterisk denotes the encoded protein in the positive control (PC) and negative control (NC).

F RNA expression analysis of *ADPGK-AS1* in different cell lines and macrophage phenotypes. $n = 6$. Data was represented as mean ± SEM, $^{**}P \leq 0.01$ compared to healthy cell line B2B.

G RNA expression of *ADPGK-AS1* in an Illumina Seq dataset (Source: UCSC genome browser, July 2021).

H, I RNA expression analysis of *ADPGK-AS1* in PBMC-derived (left panel) and THP1-derived macrophages (right panel), either left untreated (M0) or activated to the M1-like phenotype by stimulation with IFNɣ and LPS or to the M2-like phenotype by stimulation with IL-4. $n = 4$. (PBMC-derived macrophages) and $n = 6$ (THP1-derived macrophages) independent experiments, mean ± SEM, student's unpaired *t*-test. $^{*}P \leq 0.05$, $^{***}P \leq 0.001$ compared with M0.

J RNA-pulldown validation of *ADPGK-AS1* using RNA isolated from streptavidin beads of *ADPGK-AS1* or control samples. $n = 4$ independent experiments.

K Representative immunoblot of MRPL15, MRPL35 localization after subcellular fractionation of macrophages into cytoplasmic and mitochondrial fractions. β-actin served as the cytoplasmic control, succinate dehydrogenase (SDHA) as the mitochondrial control, ERp72 and protein disulfide isomerase (PDI) as endoplasmic reticulum (ER) controls, and Lamin A/C as the nuclear control. $n = 3$ independent experiments.

Source data are available online for this figure.

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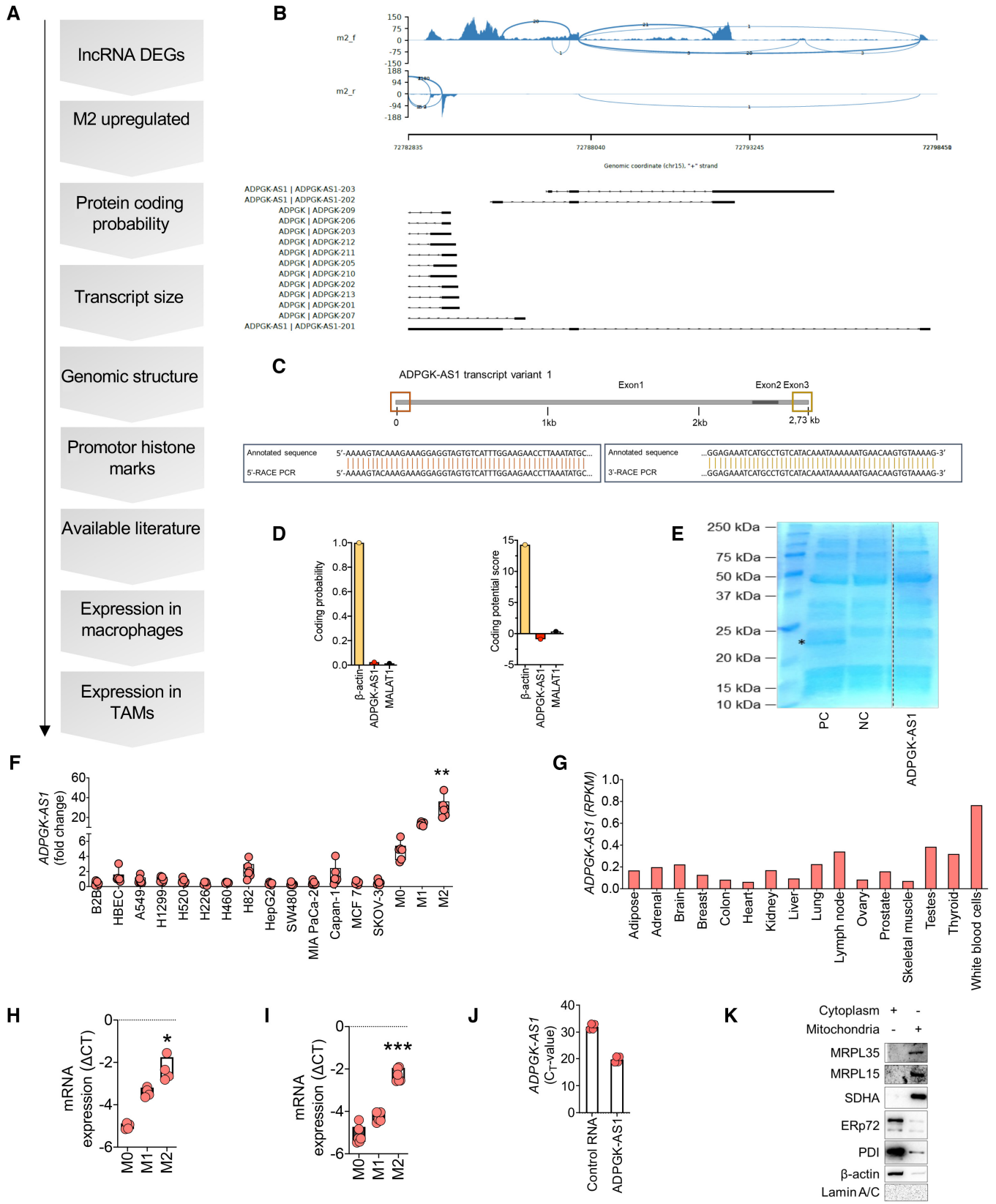

Figure EV1.

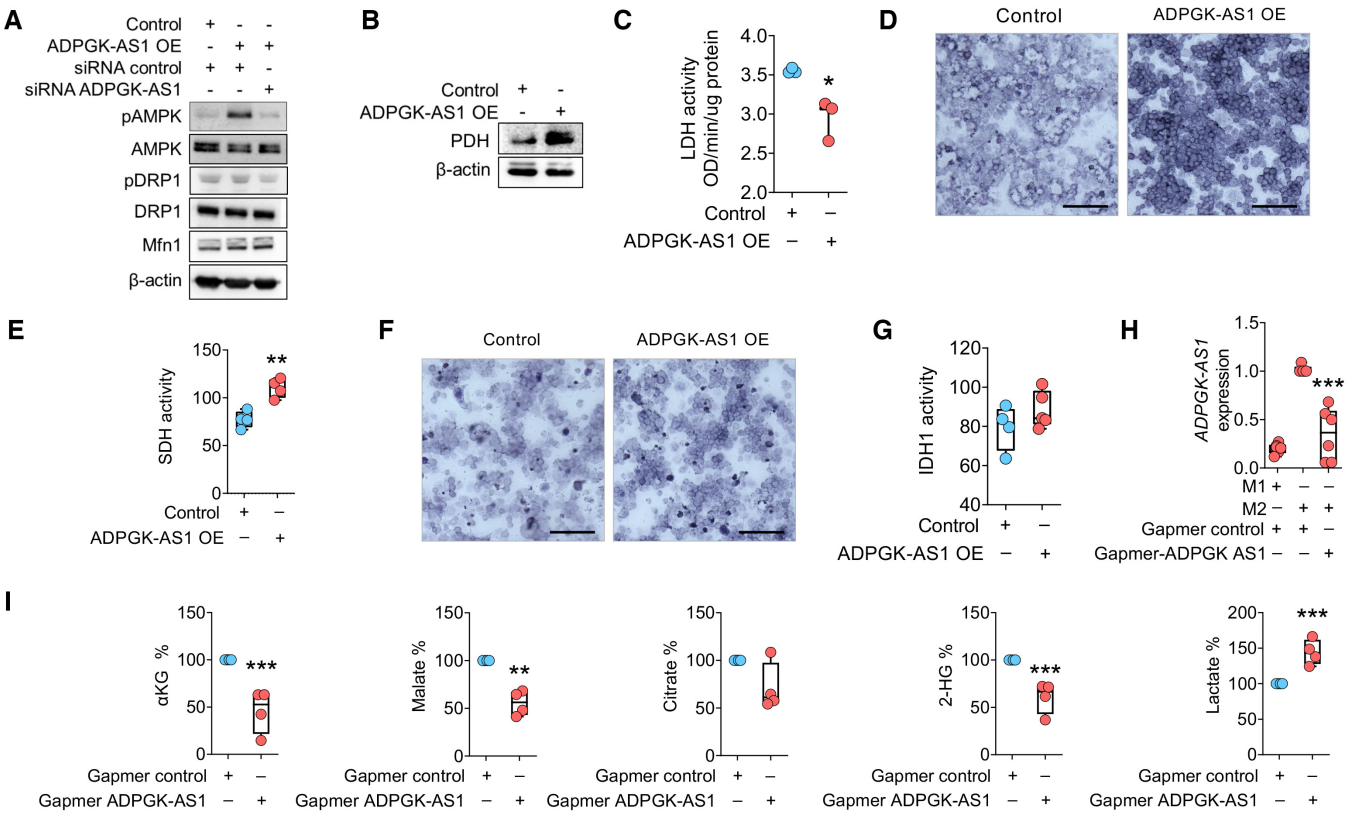

Figure EV2. ***ADPGK-AS1 enhances the mitochondrial respiratory chain and stimulates the activities of TCA cycle enzymes.***

A Representative immunoblot of mitochondrial fission (DRP1, pDRP1, AMPK, pAMPK) and fusion (Mfn1) marker markers and β-actin in THP1 control and *ADPGK-AS1* OE macrophages transfected with a siRNA against *ADPGK-AS1* or a negative control. $n = 3$ independent experiments.

B Representative immunoblot of pyruvate dehydrogenase (PDH) and β-actin in THP1 control and *ADPGK-AS1* OE macrophages. $n = 3$ independent experiments.

C LDH enzyme activity in *ADPGK-AS1* OE macrophages compared to control cells. $n = 3$ independent experiments, mean ± SEM, student's unpaired *t*-test, $^{*}P \leq 0.05$, compared with control.

D–G Enzymatic activity by staining and quantification of succinate dehydrogenase (SDH) and isocitrate dehydrogenase (IDH1) in THP1 control and *ADPGK-AS1* OE macrophages. scale bar = 100 μm $n = 4$ independent experiments, mean ± SEM, student's unpaired *t*-test, $^{**}P \leq 0.01$, compared with control.

H *ADPGK-AS1* expression in PBMC-derived macrophages polarized to the M1-like (M1, +LPS/IFNɣ for 24 h), M2-like (M2, +IL-4 for 24 h) or M0 (unstimulated) state and then transfected with antisense LNA GapmeRs against *ADPGK-AS1* or a negative control. $n = 6$ biological replicates, mean ± SEM, one-way ANOVA, $^{***}P \leq 0.001$, compared with M2 control.

I LC–MS/MS analysis of deregulated metabolites of PBMC-derived macrophages polarized to the M2-like state (M2, +IL-4 for 24 h) and then transfected with antisense LNA GapmeRs against *ADPGK-AS1* or a negative control. Results represent the percentage of the ratio of lysate to supernatant. $n = 4$ biological replicates, mean ± SEM, student's unpaired *t*-test, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ compared with the GapmeR control.

Source data are available online for this figure.

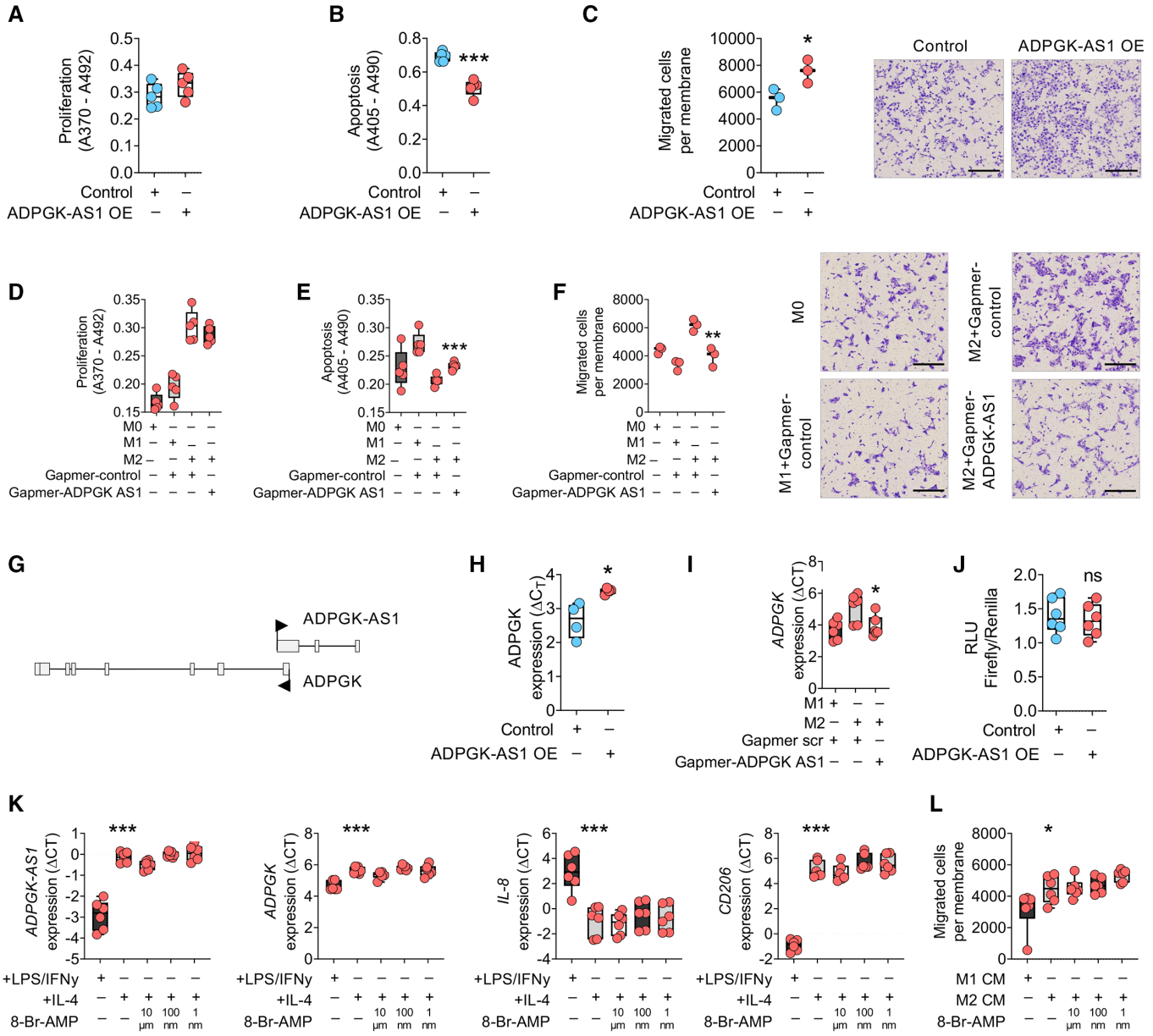

Figure EV3. ***ADPGK-AS1 has antitumor activity in vitro, and its function is not regulated via ADPGK.***

A–C Proliferation (n = 5), apoptosis (n = 5), and migration (n = 3) (left panel) with representative membrane images (right panel) of H1650 cells treated with CM from THP1 control or *ADPGK-AS1* OE macrophages. The n values represent independent experiments, mean ± SEM, student's unpaired t-test, $^*P \leq 0.05$, $^{***}P \leq 0.001$, compared with control. Scale bar = 400 µm.

D–F Proliferation (n = 5), apoptosis (n = 5), and migration (n = 3) (left panel) with representative membrane images (right panel) of H1650 cells treated with CM from primary M1-like (M1) or M2-like (M2) macrophages transfected with antisense LNA GapmeRs specific for *ADPGK-AS1* or a negative control. The n values represent biological replicates, mean ± SEM, one-way ANOVA, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, compared with M2 control. Scale bar = 400 µm.

G Schematic diagram of the genomic region of the ADPGK and *ADPGK-AS1* genes.

H mRNA expression analysis of *ADPGK* in THP1 control and *ADPGK-AS1* OE macrophages. n = 4 technical replicates, mean ± SEM, student's unpaired t-test, $^*P \leq 0.05$, compared with control.

I Expression of *ADPGK* mRNA in primary M1-like (M1) and M2-like (M2) macrophages transfected with antisense LNA GapmeRs specific for *ADPGK-AS1* or a negative control. n = 6 technical replicates, mean ± SEM, one-way ANOVA, $^*P \leq 0.05$, compared with M2 control.

J Luciferase-based assay for analyzing activation of the ADPGK promotor in THP1 control and *ADPGK-AS1* OE cells. n = 6 technical replicates, mean ± SEM, student's unpaired t-test.

K RNA expression of *ADPGK-AS1*, *ADPGK*, and macrophage activation markers *IL-8* and *CD206* in macrophages treated with different concentrations of ADPGK inhibitor 8-Br-AMP. n = 6 technical replicates, mean ± SEM, one-way ANOVA, $^*P \leq 0.05$, compared with M2 control.

L Migration of tumor cells (A549) treated with CM of M1-like or M2-like macrophages treated with 8-Br-AMP. n = 6 technical replicates, mean ± SEM, one-way ANOVA, $^*P \leq 0.05$, compared with M1.

Figure EV4. ***ADPGK-AS1 influences lung cancer cell proliferation and apoptosis ex vivo in normal lung PCLS.***

A Schematic overview and experimental design of precision-cut lung slices (PCLS) using healthy (nontumor) lung lobes and subsequent seeding with A549-GFP cells.

B Representative images of healthy PCLS (red, autofluorescence) with A549-GFP (green), apoptotic cells (TUNEL$^+$, magenta), and proliferative cells (EdU$^+$, cyan). Scale bar = 500 μm (brightfield images), 50 μm (fluorescence images on the right).

C, D Quantification of GFP$^+$ tumor cells (A549, $n = 6$), EdU$^+$ (proliferative, $n = 5$), and TUNEL$^+$ (apoptotic, $n = 4$) cells on healthy PCLS treated with CM (medium control, M0, M1, or M2 macrophages transfected with antisense LNA GapmeRs specific for *ADPGK-AS1*) or negative control, THP1 control (EV), and *ADPGK-AS1* overexpressing (OE) macrophages. The *n* values represent biological replicates, mean ± SEM, one-way ANOVA, $^*P \leq 0.05$, $^{***}P \leq 0.001$, compared with M2 GapmeR-control, $^{§§§}P \leq 0.001$ compared with EV control.

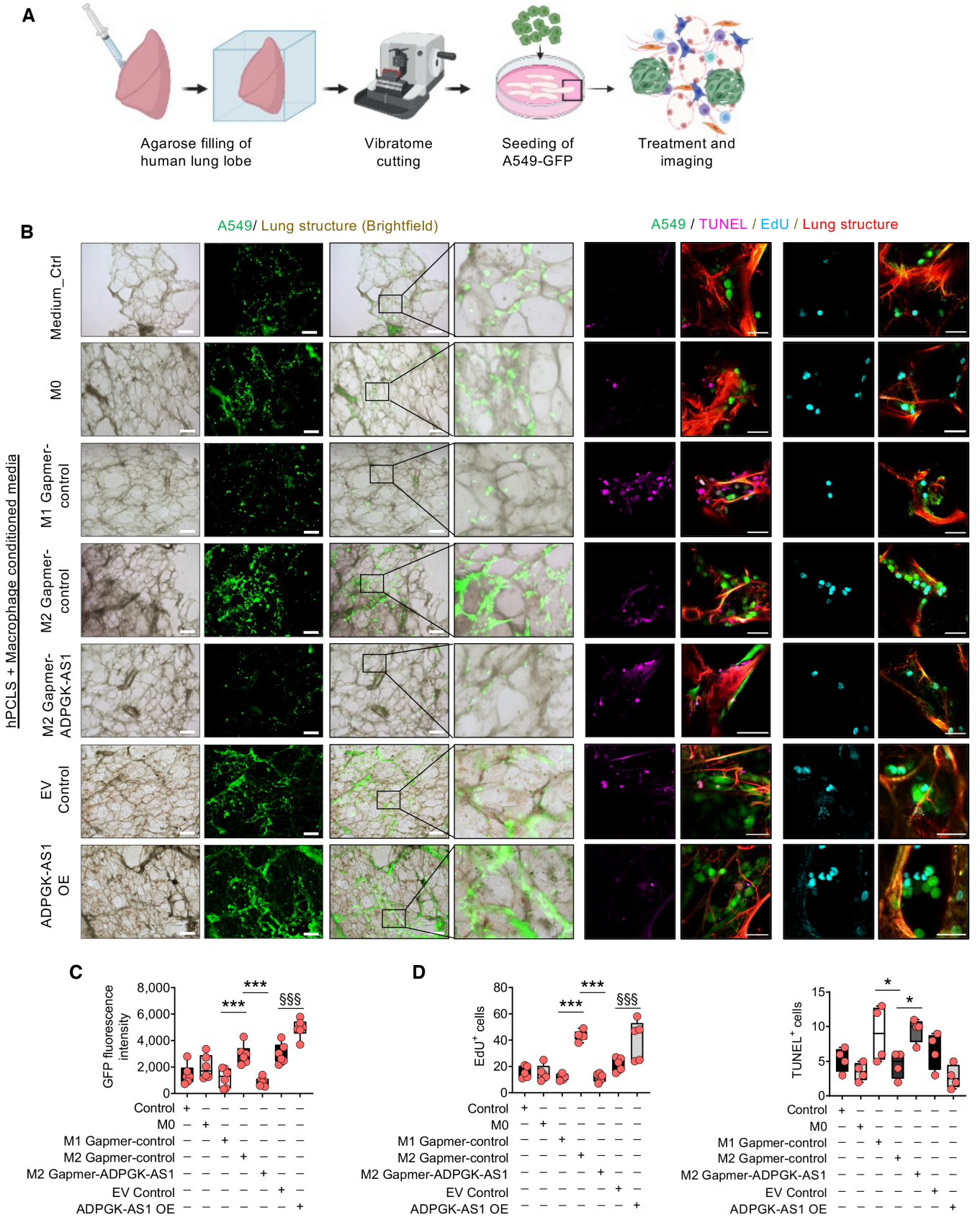

Figure EV4.

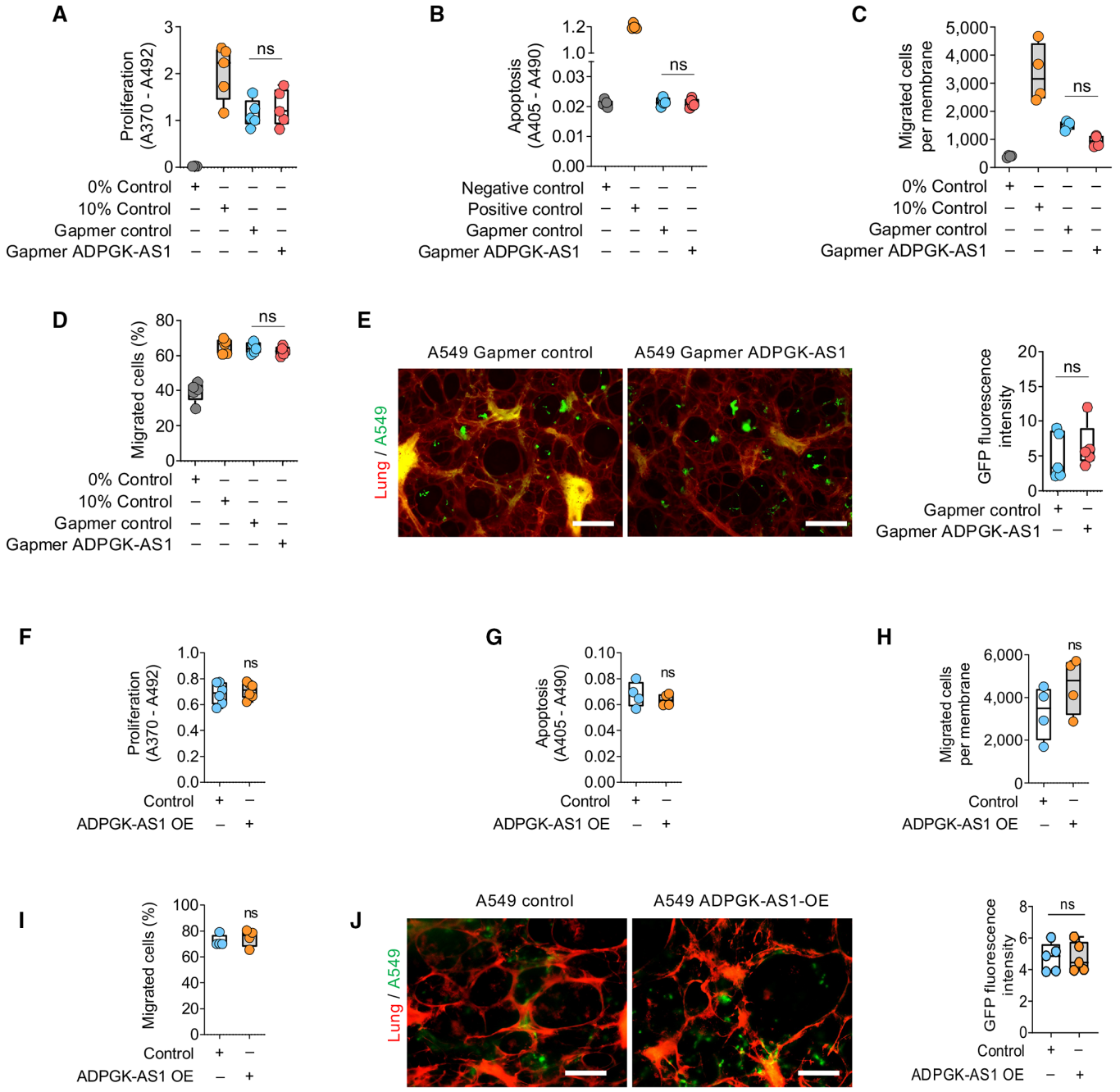

Figure EV5. ***ADPGK-AS1 knockdown has no effect on A549 cells.***

A–D Proliferation (n = 5), apoptosis (n = 5), migration (transwell, n = 4), and wound healing (n = 5) assays of tumor cells (A549) transfected with antisense LNA GapmeRs against *ADPGK-AS1* or a negative control. The n values represent independent experiments, mean ± SEM, one-way ANOVA. ns, nonsignificant.

E Human healthy PCLS seeded with A549-GFP cells transfected with antisense LNA GapmeRs against *ADPGK-AS1* or a negative control with quantification of A549-GFP-positive cells after 48 h. n = 5 independent experiments, mean ± SEM, student's unpaired t-test. ns, nonsignificant, scale bar = 500 µm.

F–I Proliferation (n = 6), apoptosis (n = 4), migration (transwell, n = 4), and wound healing (n = 4) assays of tumor cells (A549) control and *ADPGK-AS1* OE cells. The n values represent independent experiments, mean ± SEM, one-way ANOVA. ns, nonsignificant.

J Human healthy PCLS seeded with A549-GFP transfected with the *ADPGK-AS1* OE plasmid or an empty vector control with quantification of A549-GFP positive cells after 48 h. n = 5 independent experiments, mean ± SEM, student's unpaired t-test. ns, nonsignificant scale bar = 500 µm.