GNAQ-regulated ZO-1 and ZO-2 Act as Tumor Suppressors by Inhibiting EMT Potential and Tumor-repressive Microenvironment in Lung Cancer

Supplementary Materials

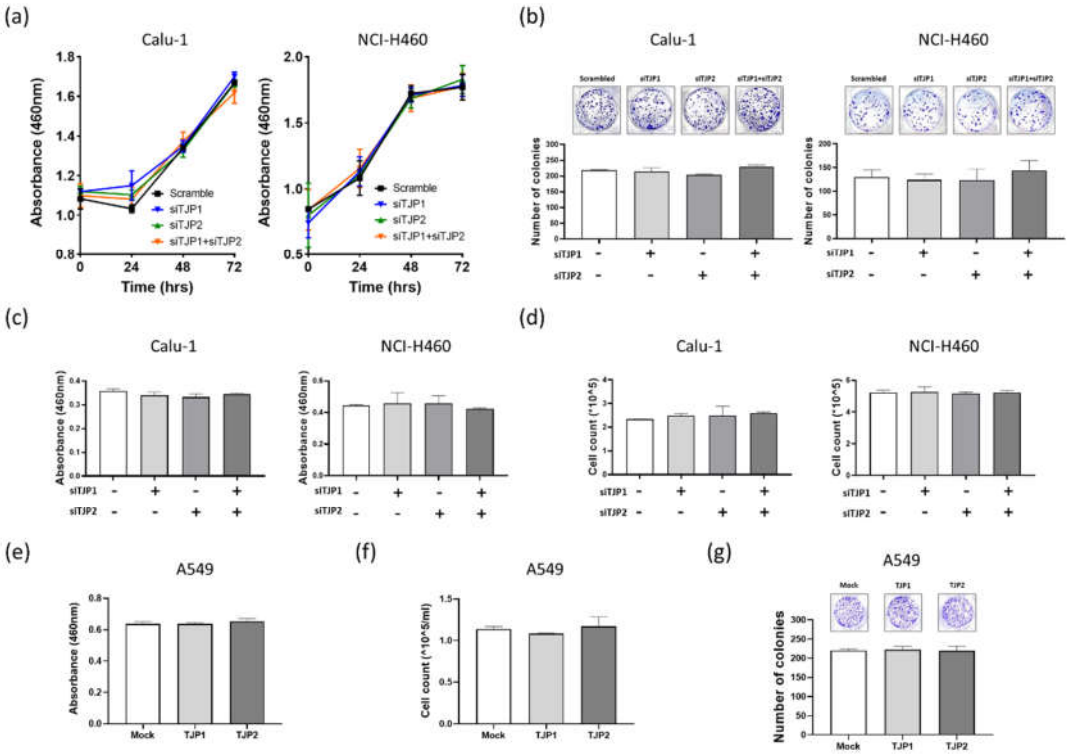
Supplementary Table S1. List of siRNAs used in transfection.

siRNA/miRNA	Strand	Nucleotide sequence
Control siRNA	Sense	5'-CCUACGCCACCAAUUUCGU-3'
	Antisense	5'-ACGAAAUUGGUGGCGUAGG-3'
TJP1 siRNA	Sense	5'-GGAUAGAAGUGCAAGUAGA-3'
	Antisense	5'-UCUACUUGCACUUCUAUCC-3'
TJP2 siRNA	Sense	5'-GACAAGGUGUCAAAACCAU-3'
	Antisense	5'-AUGGUUUUGACACCUUGUC-3'
GNAQ siRNA	Sense	5'-AGUGUACCAGUUUACAGAU-3'
	Antisense	5'-AUCUGUAAACUGGUACACU-3'

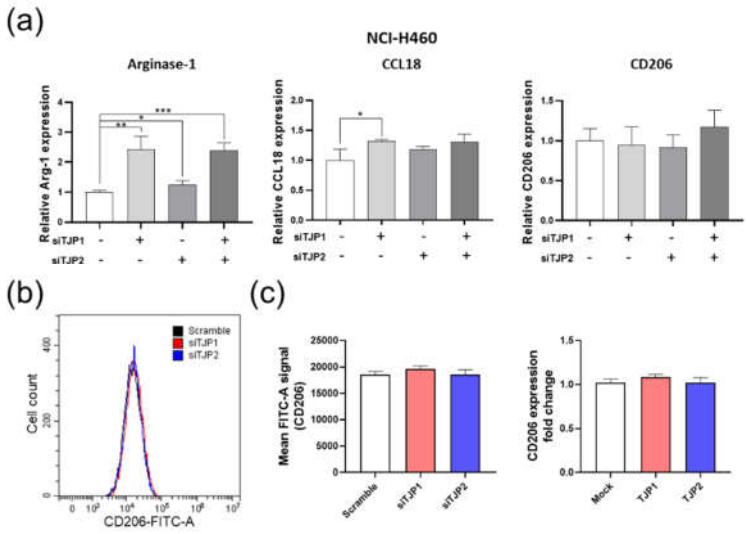
Supplementary Table S2. List of primer sequences used in qRT-PCR experiments.

Gene	Primer	Nucleotide sequence
TJP1	Forward	5'-CCAGAAATACCTGACGGTGC-3'
	Reverse	5'-TGTCTGTGCTCATAGCCTTG-3'
TJP2	Forward	5'-CAAAACCATTCTCACAGCCCC-3'
	Reverse	5'-CCGGACACTGCAATTCCAAA-3'
Arg-1	Forward	5'-CTTGGCAAAAGACTTATCCTTAG-3'
	Reverse	5'-ATGACATGGACACATAGTACCTTTC-3'
CCL18	Forward	5'-TGGCAGATTCCACAAAAGTTCA-3'
	Reverse	5'-GGATGACACCTGGCTTGGG-3'
CD206	Forward	5'-TGAATTGTACTGGTCTGTCCT-3'
	Reverse	5'-CTGTGGTGCTGTGCATTTATCT-3'
GNAQ	Forward	5'-TGGAGTCCATCATGGCGTG-3'
	Reverse	5'-CACTCTCTCCTGTCCCGAGC-3'
β-actin	Forward	5'-GCCAACAGAGAGAAGATGACAC-3'
	Reverse	5'-GTAACACCATCACCAGAGTCCA-3'

Supplementary Figures



Supplementary Figure S1. ZO-1 and ZO-2 do not affect the cell proliferation of lung cancer cells (a) The cell growth was examined at different time points measured by Cell Counting Kit-8 assay at the absorbance of 450 nm after knockdown of ZO-1 or ZO-2. (b) The growth of lung cancer cells was determined by clonogenic assay at 72 h. (c) The lung cancer cells' viability was examined using Cell Counting Kit-8 at 72 h. The absorbance at 450 nm is presented. (d) Cell viability was assessed by counting the viable cells at 72 h. (e,f) The cell proliferation or viability of ZO-1 or ZO-2 overexpressing A549 cells was determined by Cell Counting Kit-8 or a cell counter at 72 h. (g) Clonogenic assay and quantification of ZO-1 or ZO-2 overexpressing A549 cells, cultured over 2 weeks, followed by crystal violet staining.



Supplementary Figure S2. M2-like phenotypic polarization of THP-1 was not affected by NCI-H460 cells (a) M2 phenotype markers were assessed by qRT-PCR after co-culture with ZO-1 or ZO-2 repressed NCI-H460. (b) Histogram of CD206 expression analyzed by flow cytometry. (c) Raw peak signals of CD206-FITC (left) and their fold changes (right) are shown in the bar graphs.