

Fig.S1

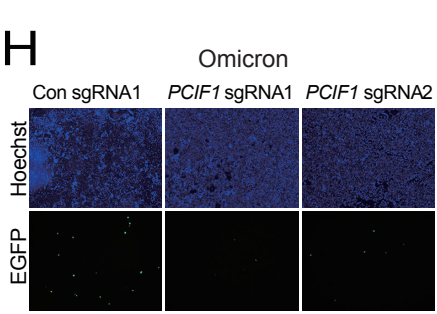
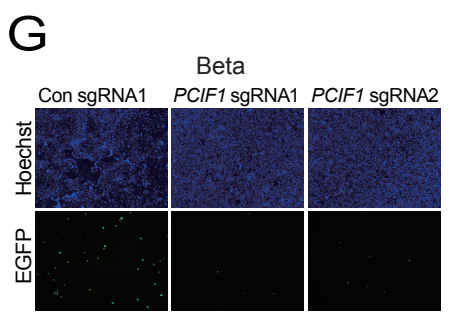
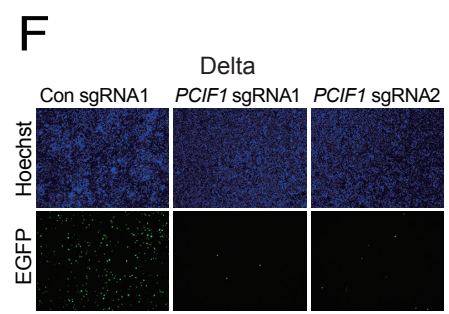
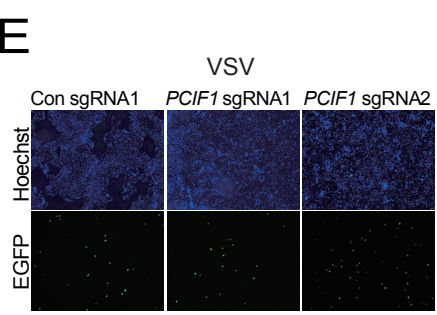
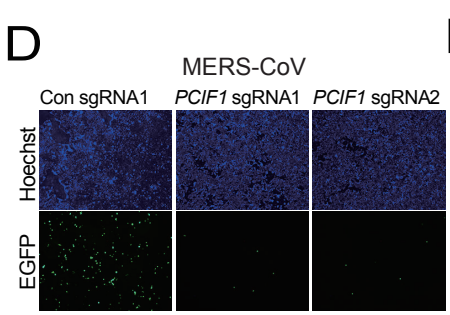
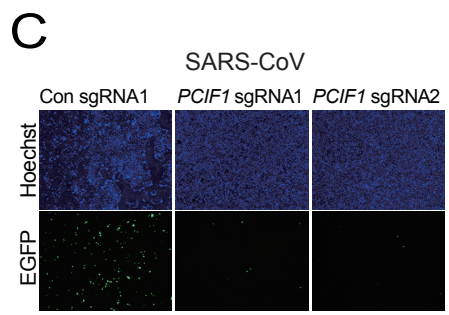
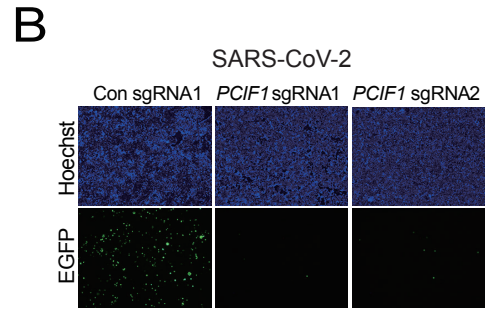
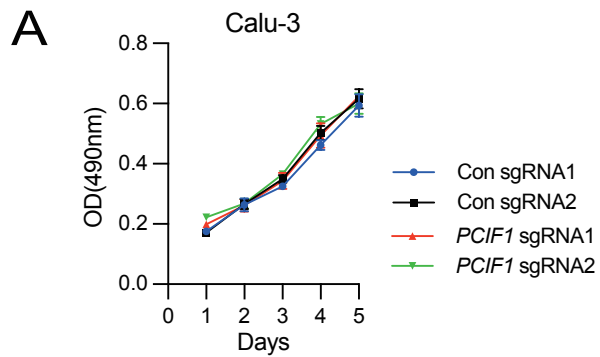


Fig. S1. PCIF1 knockout blocks viral entry in Calu-3 cells.

A, MTS cellular proliferation assay of control and PCIF1-depleted Calu-3 cells. Data are mean \pm SD of n=3. **B-H**, Fluorescence microscopy images of PCIF1-KO and control Calu-3 cells infected with EGFP-expressing SARS-CoV-2 (**B**), SARS-CoV (**C**), MERS-CoV (**D**), VSV (**E**), Delta (**F**), Beta (**G**) and Omicron (**H**) pseudoviruses. Nuclei were stained with Hoechst 33342.

Fig.S2

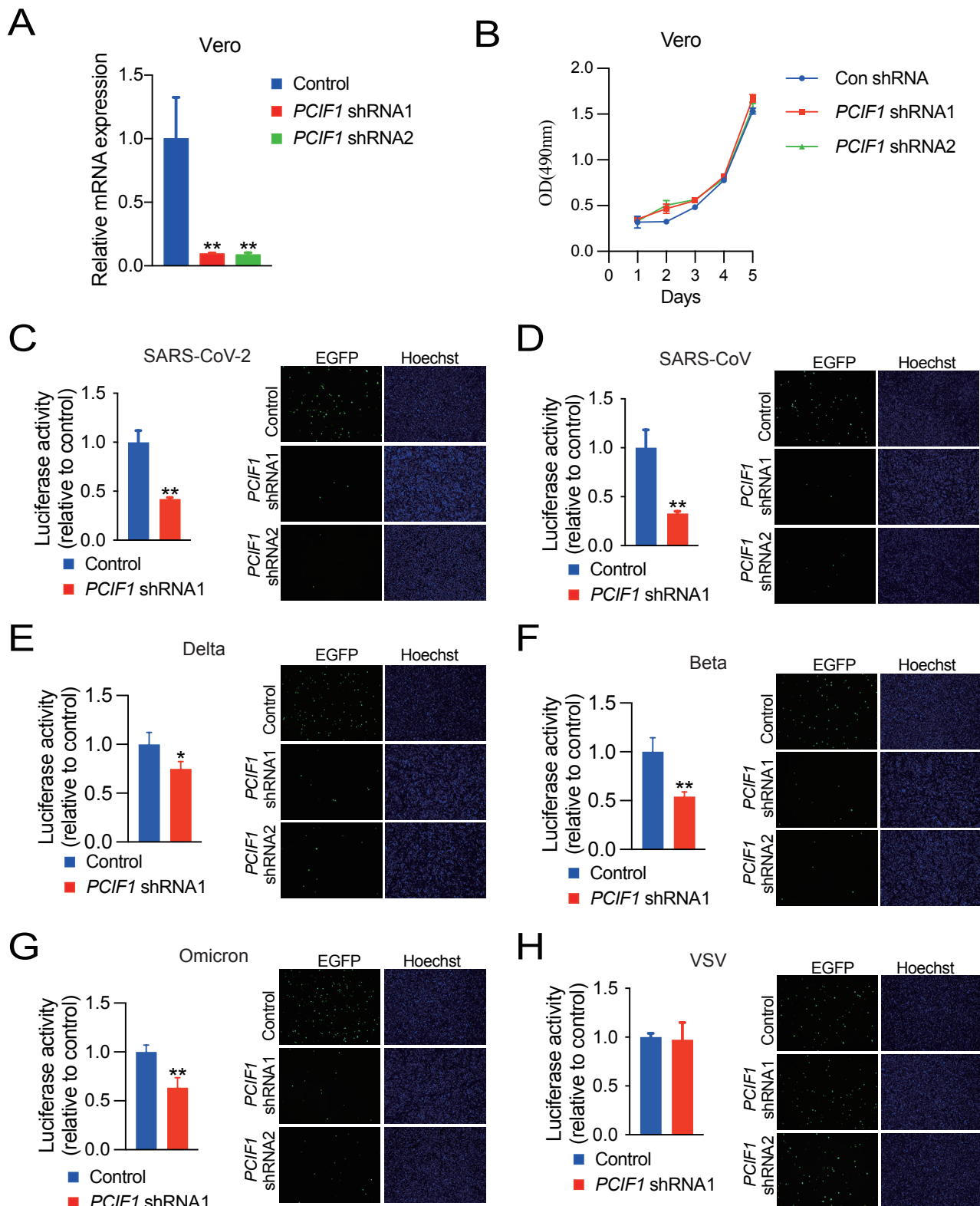


Fig. S2. Depletion of PCIF1 inhibits pseudovirus infection in Vero cells.

A, qRT-PCR analysis of Vero cells transfected with control and *PCIF1*-targeting shRNAs. Data are presented as the mean \pm S.D. (n=3). **P < 0.01 by Student's t-test. **B**, MTS cellular proliferation assay of control and PCIF1 knockdown Vero cells. Data are mean \pm SD of n=3. **C-H**, Luciferase quantification (left panels) and fluorescence microscopy imaging (right panels) of infection of PCIF1-KD and control Vero cells with luciferase- or EGFP-expressing SARS-CoV-2 (C), SARS-CoV (D), Delta (E), Beta (F), Omicron (G), or VSV (H) pseudoviruses. Nuclei were stained with Hoechst 33342. Data are presented as the mean \pm S.D. (n=3). *P < 0.05, **P < 0.01 by Student's t-test.

Fig.S3

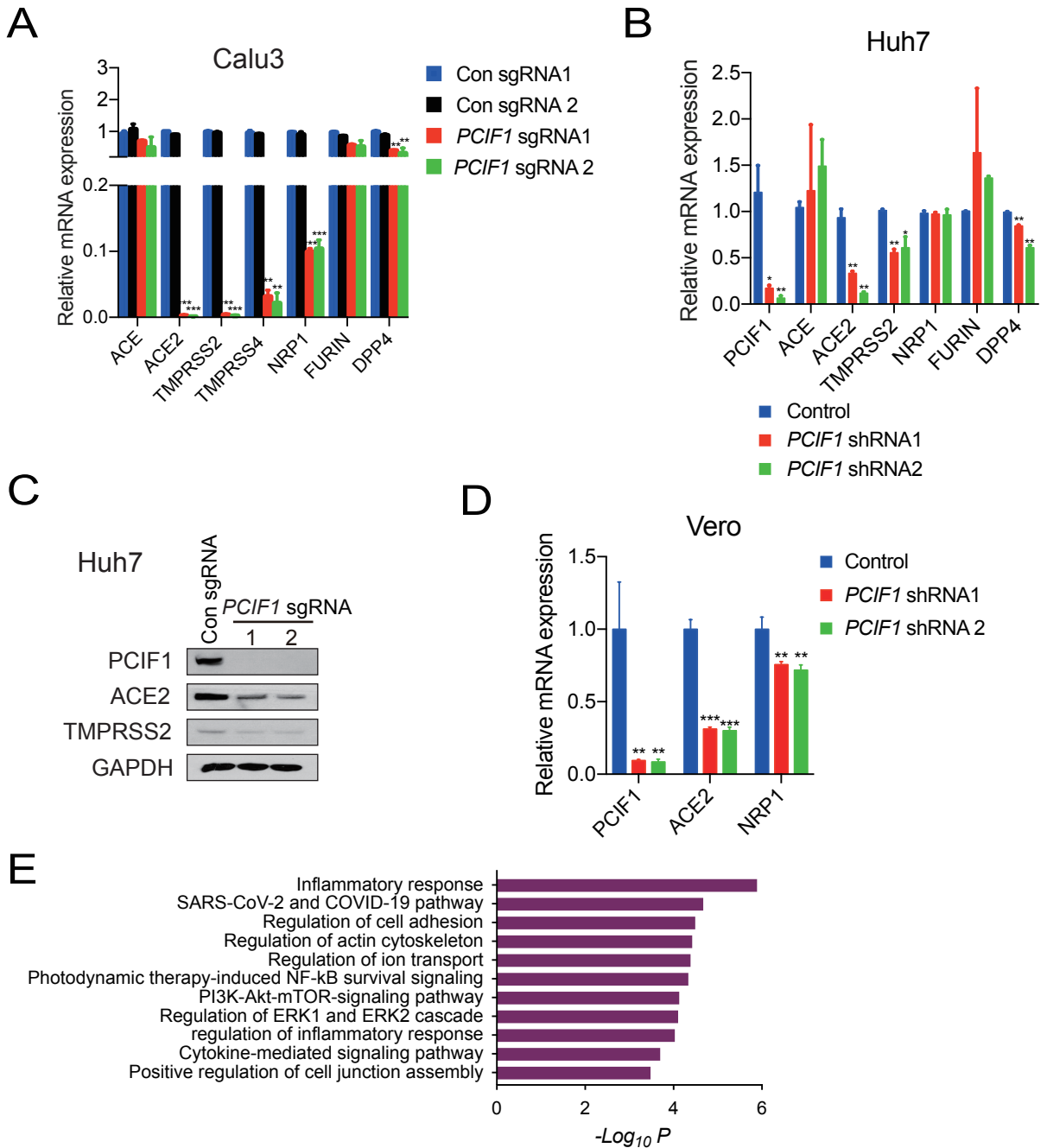


Fig. S3. Validation of altered gene expression upon PCIF1 depletion in multiple cell lines.

A, B, qRT-PCR analysis of the indicated genes identified from the RNA-seq data. Data are presented as the fold change in mRNA levels in PCIF1-KO Calu-3 cells (a) or PCIF1-KD Huh7 cells (b) relative to the corresponding control cells. Data are presented as the mean \pm S.D. (n=3). *P < 0.05, **P < 0.01, ***P < 0.001 by Student's t-test. **C**, Western blot analysis of PCIF1, ACE2, and TMPRSS2 protein levels in control and PCIF1-KO Huh7 cells. GAPDH served as a loading control. **D**, qRT-PCR analysis of *PCIF1*, *ACE2*, and *NRP1* mRNA levels in PCIF1-KD Vero cells relative to control cells. Data are presented as the mean \pm S.D. (n=3). **P < 0.01, ***P < 0.001 by Student's t-test. **E**, Summary of the meta-enrichment analysis for the 172 m⁶A_m-modified genes downregulated by PCIF1-KO in Calu-3 cells (shown in Figure 2d).

Table S1: qPCR Primers

Genes Name	5'-3'
<i>PCIF1</i>	GGAGAATCGTCCCTACTACTT/ GCTTTCTGGGCTTGTCT
<i>GAPDH</i>	GTGGACCTGACCTGCCGTCT/ GGAGGAGTGGGTGTCGCTGT
<i>ACE</i>	CCGAAATACGTGGAACATCAA/ CACGAGTCCCCTGCATCTACA
<i>ACE2</i>	TGGGACTCTGCCATTTACTTAC/ CCCAACTATCTCTCGCTTCATC
<i>TMPRSS2</i>	GGAGTGTACGGGAATGTGATG/ GGACGAAGACCATGTGGATTAG
<i>TMPRSS4</i>	CCATCTGTCTGCCCTTCTTT/ GTCAGACATCTTCCCTCCATTC
<i>NRP1</i>	GTCTGCCCTGGAGAACTATAAC/ CCTTCATGCCTCCGAATAAGTA
<i>FURIN</i>	GACGGCTACACCAACAGTATC/ GTCACGATCTGCTTCTCATTCT
<i>DPP4</i>	TGTGAGCTGAATCCGGAAAG/ CACGCTGCTGTGTAGAGTATAG
<i>Nucleocapsid</i>	CACATTGGCACCCGCAATC/ GAGGAACGAGAAGAGGCTTG
<i>ACE2</i> m ⁶ Am	CATACACTCTGGCAATGAGGACACT/ GGGATCACAACAACATAGAATTCAAAGAG
<i>TMPRSS2</i> m ⁶ Am	GGCGCGAGCTAAGCAGGA/ TCCAGGCGGCGCTCCCCG
<i>FURIN</i> m ⁶ Am	GATAGGAGCCTGACTGTTGCAG/ CTCCAGAGCACCTGGGATTCA