Expanded View Figures

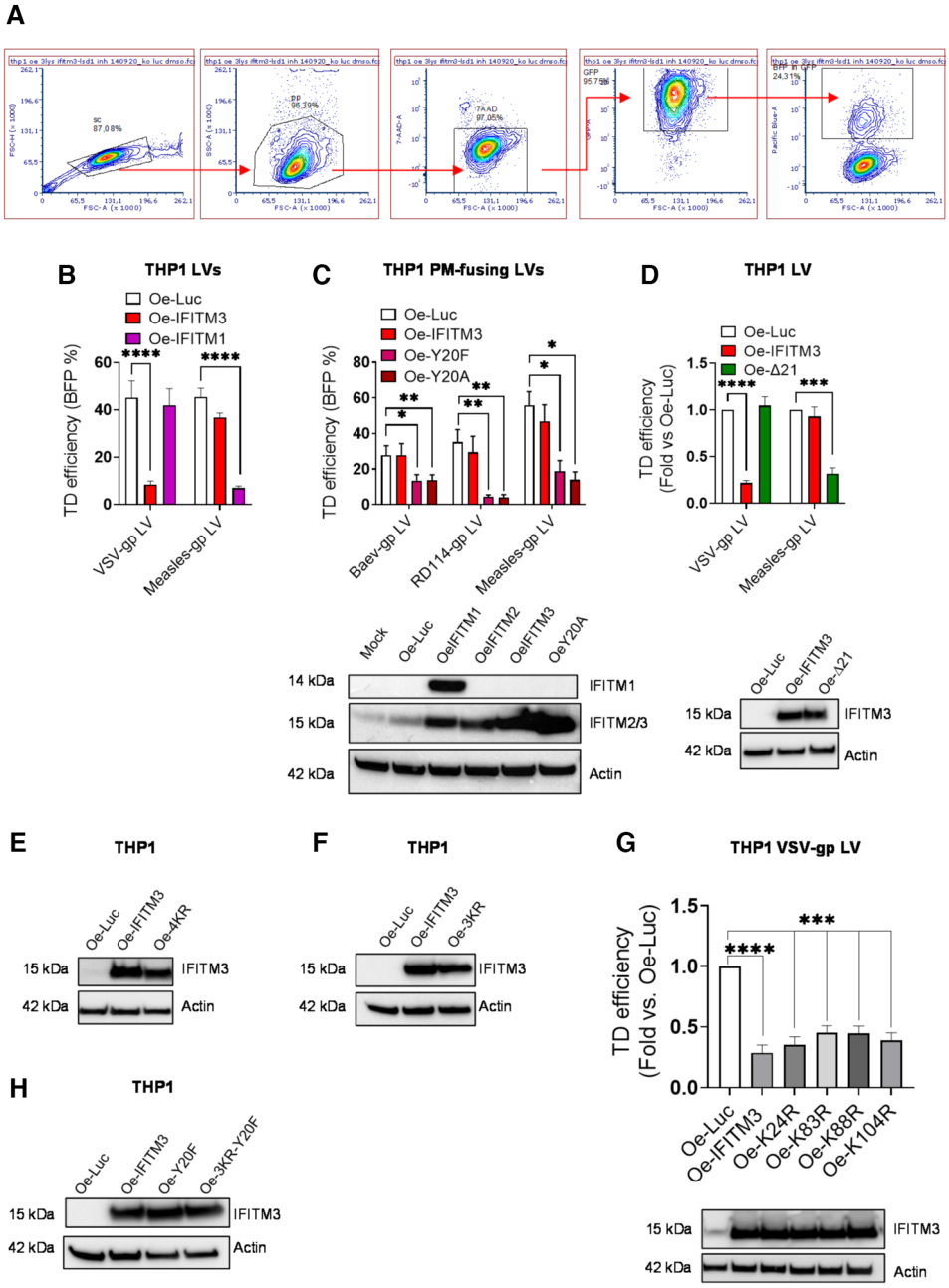

Figure EV1.

Figure EV1. IFITM1, IFITM3, and single lysine IFITM3 target different viral entry routes depending on their cellular localization.

A Gating strategy for acquisition of transduction efficiencies in the different cell lines.

B THP1 overexpressing (Oe) IFITM3, IFITM1, or control were transduced with VSV-gp or measles-gp-pseudotyped LVs at MOI 1 (mean ± SEM, $n > 6$ biological replicates run in technical duplicate), transduction efficiency was measured by flow cytometry 5-day post-TD as % of BFP^+ cells. *P*-values are for Mann–Whitney test, ****for $P < 0.0001$.

C THP1 overexpressing IFITM3 wild-type, IFITM3 PM-confined mutants or control were transduced at MOI 1 with PM-fusing LVs (mean ± SEM, $n > 5$ biological replicates run in technical duplicate), *P*-values are for Mann–Whitney test, * for $P < 0.05$, **for $P < 0.01$. IFITM1, IFITM2, IFITM3, Y20F, and Y20A overexpression was confirmed by WB in THP1.

D THP1 overexpressing the putative IFITM3 isoform (Δ21) encoded by the SNP rs-1225-C or control were transduced with either VSV-gp or measles-gp-pseudotyped LVs. Transduction efficiency was measured 5 days later (mean ± SEM, $n = 5$ biological replicates run in technical duplicate), *P*-values are for one-sample *t*-test, *** for $P < 0.001$, **** for $P < 0.0001$. IFITM3-WT and IFITM3-Δ21 overexpression was confirmed by WB in THP1.

E, F IFITM3 protein expression was evaluated by WB at time of TD in THP1 overexpressing IFITM3, IFITM3-4KR, or IFITM3-3KR.

G THP1 overexpressing single lysine mutants IFITM3 or control were transduced with VSV-gp-pseudotyped LV. Transduction efficiency was measured by flow cytometry 5-day post-TD (mean ± SEM, $n = 6$ biological replicates run in technical duplicate). Overexpression was confirmed by WB at the time of TD. *P*-values are for one-sample *t*-test versus Oe-Luc = 1. *** for $P < 0.001$, **** for $P < 0.0001$.

H THP1 overexpressing IFITM3, IFITM3-Y20F, IFITM3-Y20F-3KR, or control were validated by WB.

Source data are available online for this figure.

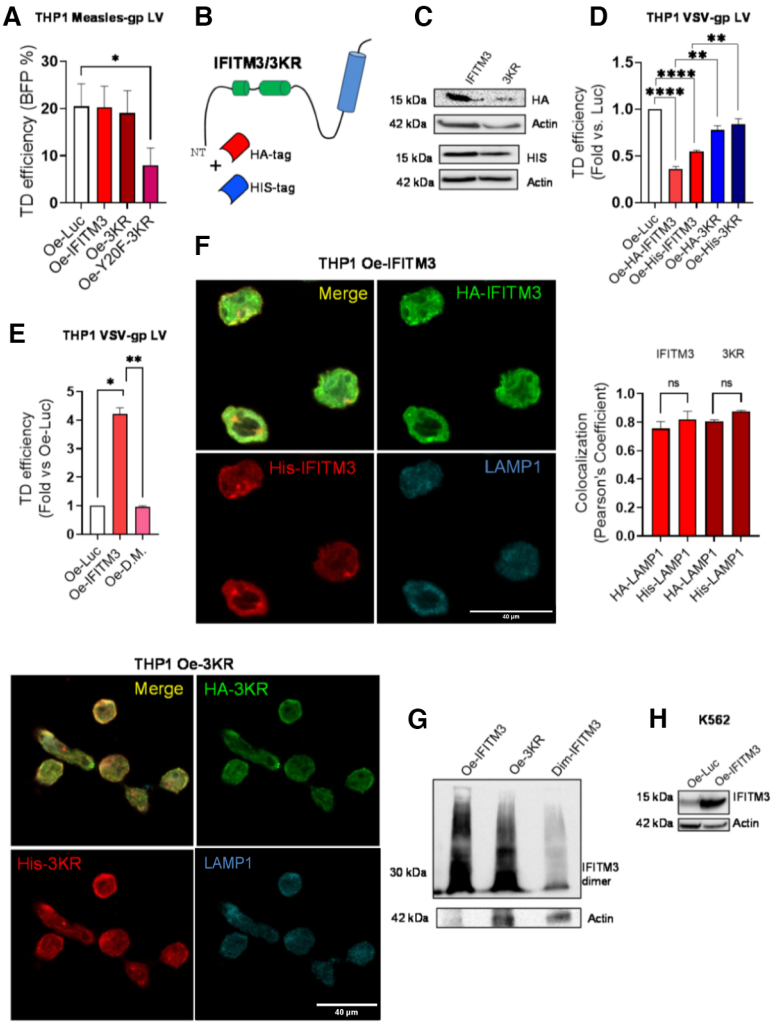

Figure EV2. IFITM3 loss of antiviral activity is localization and dimerization independent.

A THP1 overexpressing IFITM3, IFITM3-3KR, IFITM3-Y20F-3KR, or control were transduced with measles-gp-pseudotyped LV. Transduction efficiency was evaluated 5-day post-TD (mean ± SEM, $n = 3$ biological replicates run in technical duplicate). *P*-values are for one paired *t*-test. * for $P < 0.05$.

B Schematic representation of IFITM3 wild-type or 3KR mutant tagged with hemagglutinin (HA) or Histidine at the N terminus (NT).

C Overexpression of IFITM3-tagged constructs was validated in THP1 by WB.

D THP1 overexpressing tagged IFITM3, tagged IFITM3-3KR or control were transduced with VSV-gp-pseudotyped LV. Transduction was evaluated 5-day post-TD (mean ± SEM, $n = 6$ biological replicates run in technical duplicate). *P*-values are for one-sample *t*-test versus Oe-Luc = 1 for comparisons with tagged IFITM3 or Mann–Whitney test for comparisons between tagged IFITM3 and tagged IFITM3-3KR. ** for $P < 0.01$, **** for $P < 0.0001$.

E THP1 overexpressing tagged IFITM3, tagged IFITM3-dimer mutant or control were transduced with VSV-gp-pseudotyped LV. Transduction was evaluated 5-day post-TD (mean ± SEM, $n = 6$ biological replicates run in technical duplicate). *P*-values are for one-sample *t*-test versus Oe-Luc = 1 for comparisons with tagged IFITM3 or Mann–Whitney test for comparisons between tagged IFITM3 and tagged IFITM3-dimer mutant. * for $P < 0.05$, ** for $P < 0.01$.

F HA-IFITM3, His-IFITM3, HA-3KR, His-3KR, and Lamp1 levels were measured by immunofluorescence (mean ± SEM, $n = 3$ biological replicates; zoomed images are shown, scale bar 40 μm). The Pearson's correlation coefficient of HA, His, and Lamp1 colocalization of three independent biological experiments was calculated. *P*-values are for Mann–Whitney test. ns for not significant.

G IFITM3 dimerization was assessed by native PAGE followed by WB analysis in THP1 ($n = 4$ biological replicates).

H IFITM3 overexpression was confirmed in K562 at the time of TD.

Source data are available online for this figure.

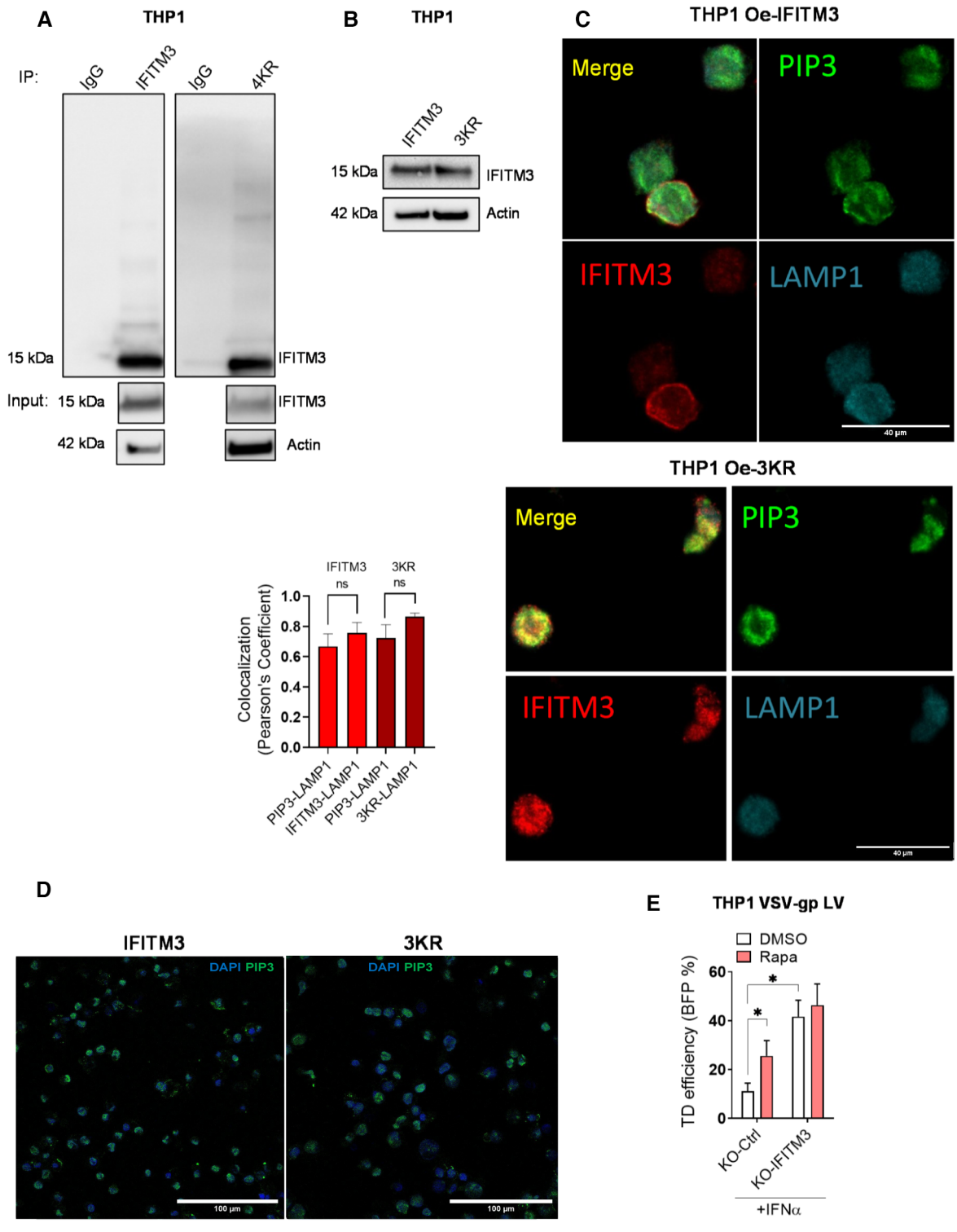
A
THP1
IP:
IgG
IFITM3
IgG
4KR
15 kDa
IFITM3
Input: 15 kDa
IFITM3
42 kDa
Actin
B
THP1
IFITM3
3KR
15 kDa
IFITM3
42 kDa
Actin
C
THP1 Oe-IFITM3
Merge
PIP3
IFITM3
LAMP1
40 µm
THP1 Oe-3KR
Merge
PIP3
IFITM3
LAMP1
40 µm
IFITM3
3KR
ns
ns
Colocalization (Pearson's Coefficient)
1.0
0.8
0.6
0.4
0.2
0.0
PIP3-LAMP1
IFITM3-LAMP1
PIP3-LAMP1
3KR-LAMP1
D
IFITM3
3KR
DAPI PIP3
DAPI PIP3
100 µm
100 µm
E
THP1 VSV-gp LV
DMSO
Rapa
TD efficiency (BFP %)
60
40
20
0
*
*
KO-Ctrl
KO-IFITM3
+IFNα

Figure EV3.

Figure EV3. IFITM3 requires interaction with PIP3 for its antiviral activity.

A IFITM3 wild-type or IFITM3-4KR immunoprecipitations were confirmed by WB.

B IFITM3 wild-type or IFITM3-3KR expression levels were measured in THP1 KO for endogenous IFITM3.

C IFITM3 and PIP3 colocalization with LAMP1 was evaluated by immunofluorescence. Representative zoomed images are shown (mean ± SEM, $n = 3$ biological replicates), scale bar 40 μm. The Pearson's correlation coefficient of HA, His, and Lamp1 colocalization of three independent biological experiments was calculated. ns for not significant.

D Total PIP3 was quantified by IF in THP1 cells overexpressing the double-tagged His/HA IFITM3 wild-type or IFITM3-3KR. Representative images are shown.

E THP1 KO for IFITM3 or control were stimulated with IFNα for 24 h and then transduced with VSV-gp-pseudotyped LV in the presence or absence of Rapamycin. Transduction levels were measured 5 days after TD (mean ± SEM, $n = 6$ biological replicates run in technical duplicate). *P*-values are for Wilcoxon test, * for $P < 0.05$.

Source data are available online for this figure.

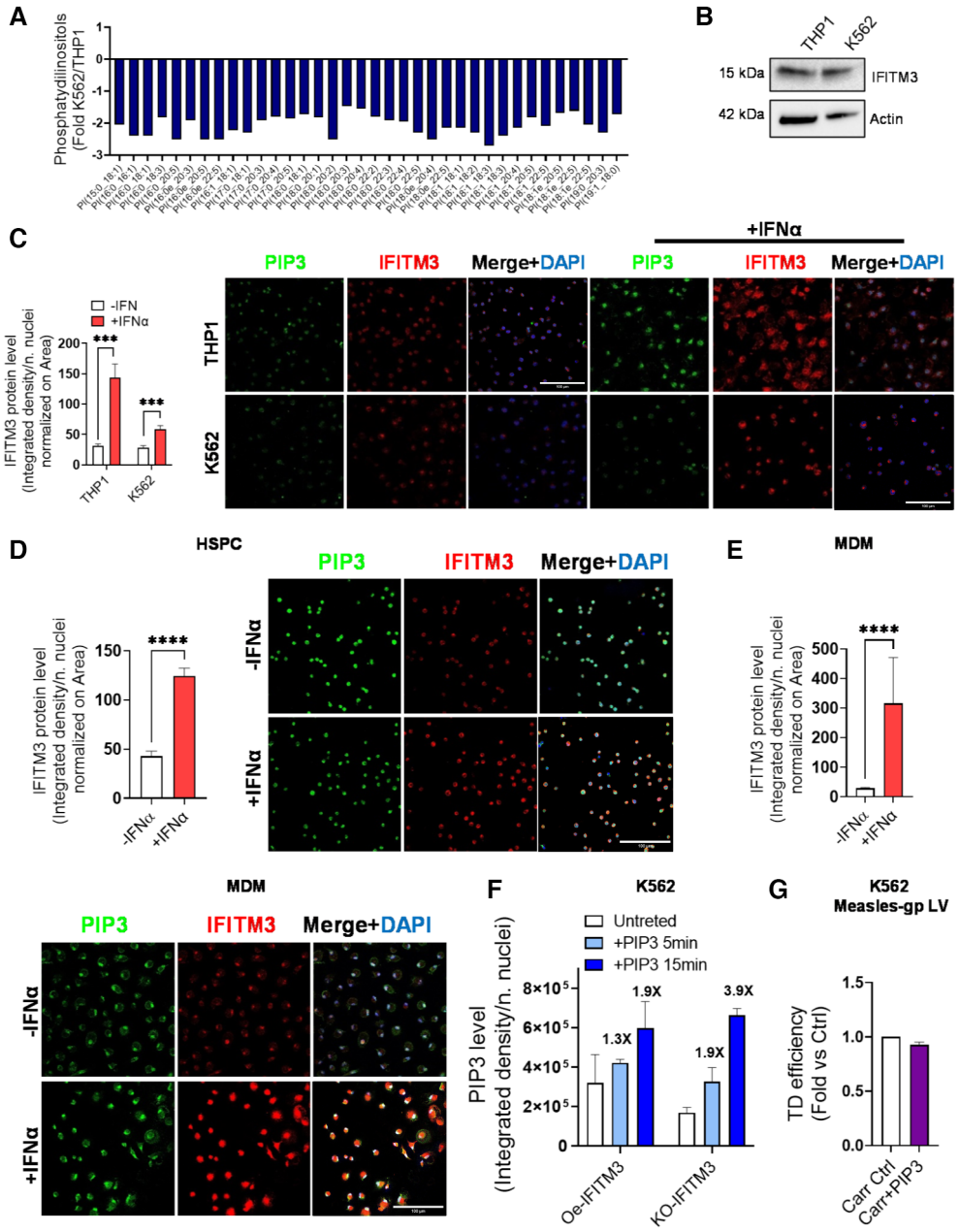

Figure EV4.

Figure EV4. PIP3 is an interferon-inducible phospholipid.

A All Phosphatidylinositol (PI) species were measured in K562 and THP1. Log plots of PI ratio in K562 versus THP1 are shown.

B IFITM3 expression was evaluated by WB in THP1 and K562 cells KO for endogenous IFITM3 and overexpressing IFITM3.

C–E PIP3 levels were measured by immunofluorescence in THP1 and K562 (C), HSPC (D) and MDM (E) prestimulated with IFNα. (mean ± SEM, $n = 3$ biological replicates). *P*-values are for Mann–Whitney test, * for $P < 0.05$. Representative images are shown, scale bar 100 µm.

F K562 were provided of exogenous PIP3 or Carr Ctrl alone. Successful PIP3 intake was verified by IF 5 and 15-min postdelivery by IF ($n = 8$ acquired from two independent biological experiments).

G K562 were provided of exogenous PIP3 or Carr Ctrl alone and then transduced with measles-gp-pseudotyped LV. Transduction was measured 5 days later (mean ± SEM, $n = 4$ biological replicates run in duplicate).

Source data are available online for this figure.

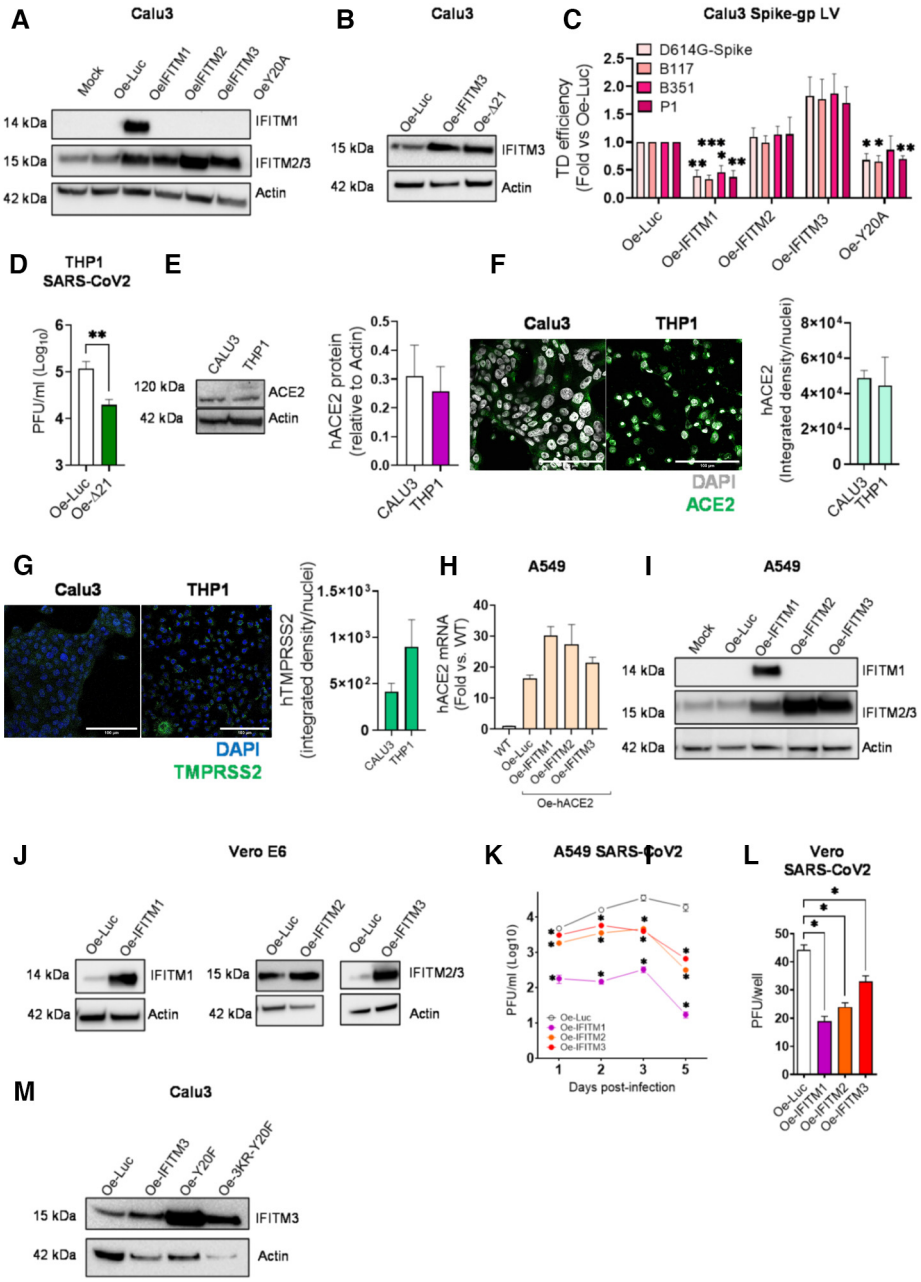

Figure EV5.

Figure EV5. IFITM1 blocks entry of SARS-CoV2 in Calu3 and THP1.

A Overexpression of IFITM1, IFITM2, IFITM3, and IFITM3-Y20A was verified by WB in Calu3.

B IFITM3-WT and IFITM3-Δ21 overexpression was confirmed by WB in Calu3.

C Calu3 overexpressing IFITM1, IFITM2, IFITM3, IFITM3-Y20A mutant, and control were transduced with LV-pseudotyped with different Spike variants. Error bars are presented as mean ± SEM ($n = 6$ biological replicates run in duplicate); *P*-values are for one-sample *t*-test, * for $P < 0.05$, **for $P < 0.01$, ***for $P < 0.001$.

D THP1 overexpressing Δ21-IFITM3 or control were infected with SARS CoV2 for 3 days. Viral titer was assessed by plaque assay in Vero E6 cells (mean ± SEM, $n = 4$ biological replicates run in technical triplicate). *P*-values are for Mann–Whitney test, ** for $P < 0.01$.

E ACE2 protein level was measured in Calu2 and THP1. Quantification was performed by ImageJ over the normalizer (mean ± SEM, $n = 5$ biological replicates).

F, G Human Ace2 (F) and TMPRSS2 (G) expression at the cell surface was analyzed in Calu3 and THP1 cells by immunofluorescence. Protein quantification was performed using ImageJ software (mean ± SEM, $n = 4$–3 biological replicates); representative images are shown ($n = 12$–6 images), scale bar 100 μm.

H A549 overexpressing ACE2 was validated by RT–qPCR. (mean ± SEM, $n = 3$ biological replicates).

I, J Overexpression of IFITM1, IFITM2, or IFITM3 was measured in A549 (I) or Vero E6 cells (J).

K A549 cells overexpressing IFITM1, IFITM2, IFITM3, or control were infected with SARS-CoV2 at MOI 1. Viral supernatants were collected at different time points and tittered on Vero E6 cells. Data are presented as mean ± SEM ($n = 4$ biological replicates run in technical duplicate). *P*-values are for Mann–Whitney test, $*P < 0.05$.

L Vero E6 cells overexpressing IFITM1, IFITM2, IFITM3, or control were infected with SARS-CoV2 at MOI 1. Titers were calculated through direct plaque assay 72-h postinfection. ($n = 3$ biological replicates run in technical duplicate). *P*-values are for Mann–Whitney test, $*P < 0.05$.

M Calu3 overexpressing IFITM3, IFITM3-Y20F, IFITM3-Y20F-3KR, or control was validated by WB.

Source data are available online for this figure.