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Supplementary Materials for

Pharmacological modulators of epithelial immunity uncovered by synthetic genetic tracing of SARS-CoV-2 infection responses

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

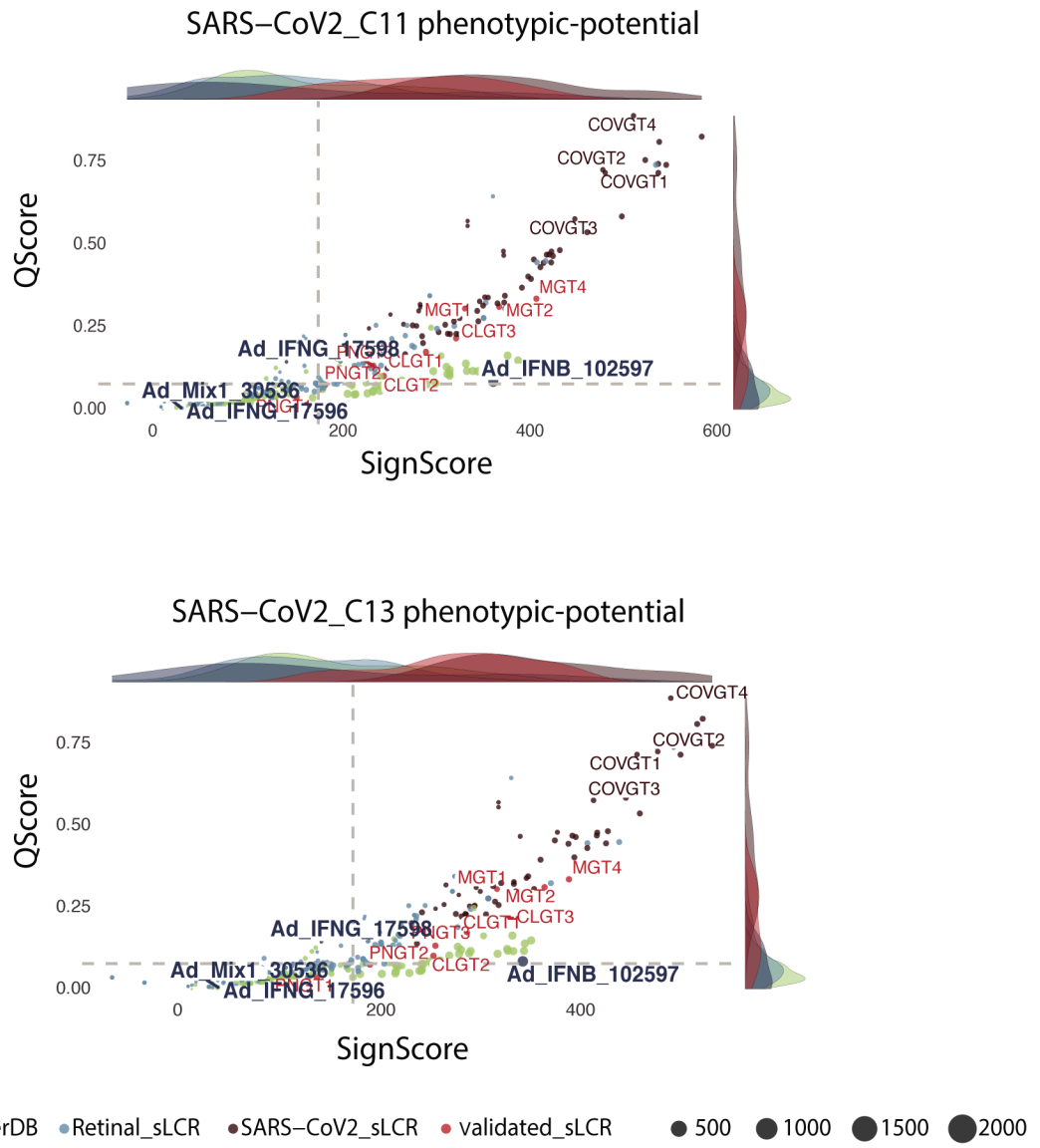
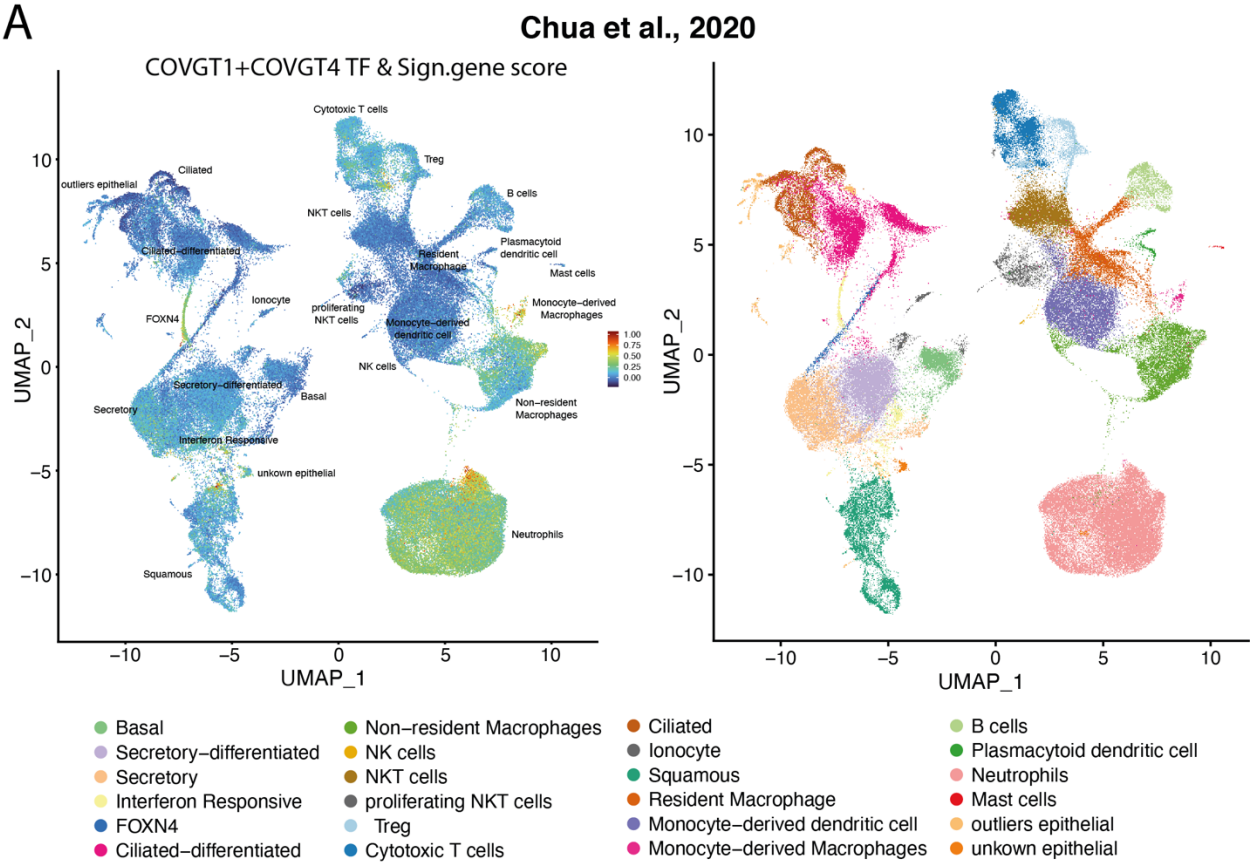


Fig. S1. Activation potential of synthetic reporters in Calu-3 single-cell clusters C11 and C13. Scatter plots showing signature (x-axis) and affinity (y-axis; methods) phenotypic scores for the C11 (top) and C13 (bottom) from Wyler et al.. Color codes separate reporters and sLCRs based on target phenotype. Dot size indicates reporters' size (in base pairs). Interferon reporters available through Addgene are specifically named.

A


Chua et al., 2020
COVGT1+COVGT4 TF & Sign.gene score
Cytotoxic T cells
Treg
B cells
Ciliated
outliers epithelial
NKT cells
Plasmacytoid dendritic cell
Ciliated−differentiated
Resident Macrophage
Mast cells
Ionocyte
proliferating NKT cells
Monocyte−derived Macrophages
FOXN4
Monocyte−derived dendritic cell
NK cells
Secretory−differentiated
Basal
Non−resident Macrophages
Secretory
Interferon Responsive
unkown epithelial
Neutrophils
Squamous
UMAP_1
UMAP_2
Basal
Secretory−differentiated
Secretory
Interferon Responsive
FOXN4
Ciliated−differentiated
Non−resident Macrophages
NK cells
NKT cells
proliferating NKT cells
Treg
Cytotoxic T cells
Ciliated
Ionocyte
Squamous
Resident Macrophage
Monocyte−derived dendritic cell
Monocyte−derived Macrophages
B cells
Plasmacytoid dendritic cell
Neutrophils
Mast cells
outliers epithelial
unkown epithelial

B

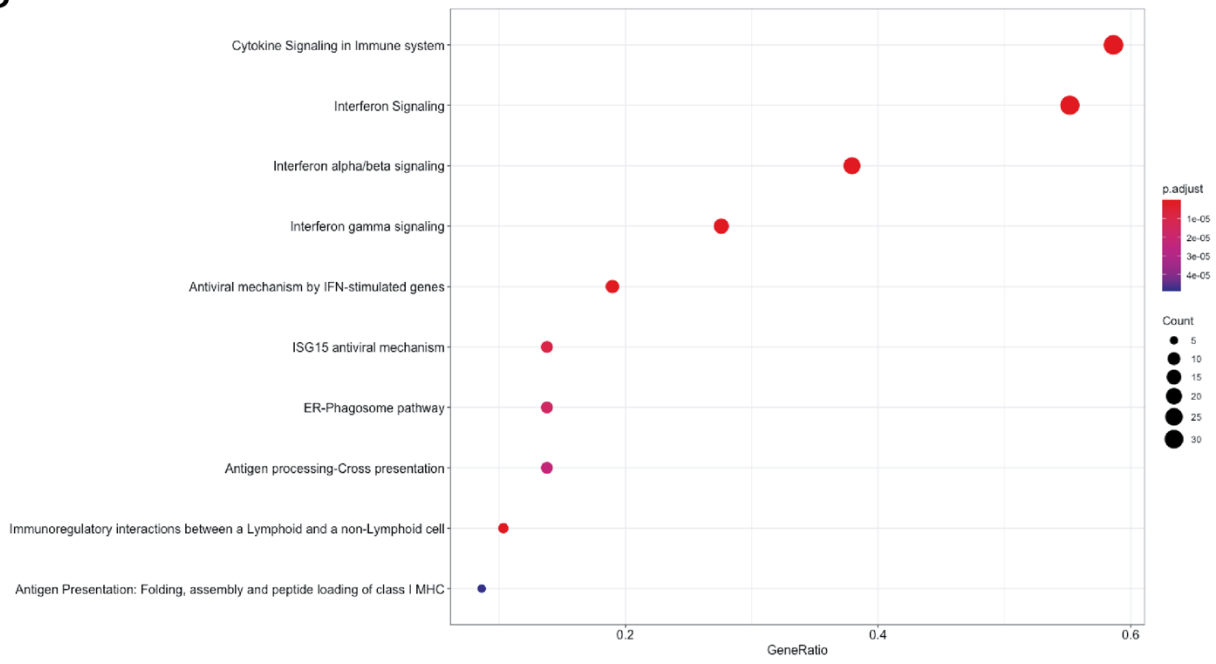

GSEA of overlap COVGT1+COVGT4 with Interferon-Resp. Epithelial Cells (Chua et al., 2020) signature genes
Cytokine Signaling in Immune system
Interferon Signaling
Interferon alpha/beta signaling
Interferon gamma signaling
Antiviral mechanism by IFN-stimulated genes
ISG15 antiviral mechanism
ER-Phagosome pathway
Antigen processing-Cross presentation
Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell
Antigen Presentation: Folding, assembly and peptide loading of class I MHC
GeneRatio
p.adjust
Count

Fig. S2. COVGT5 enrichment score in single-cell samples from COVID-19 patients

(**A**) UMAP representation of COVGT5 gene (i.e. COVGT1+4) cumulative expression in SARS-CoV-2 patient samples. Gene set enrichment scores for COVGT5 signature genes and transcription factors on annotated cell clusters of Chua et al. 2020 are represented (left). Color code indicates marked cell populations (right).

(**B**) Dot plot showing enrichment for signaling pathways of COVGT5 (i.e. COVGT1+4). Signature genes common between epithelial cells (Chua et al., 2020) and COVGT1+4 sLCRs were assigned to respective signaling pathways and sorted by gene ratio (X-axis value) and the number of genes (dot size).

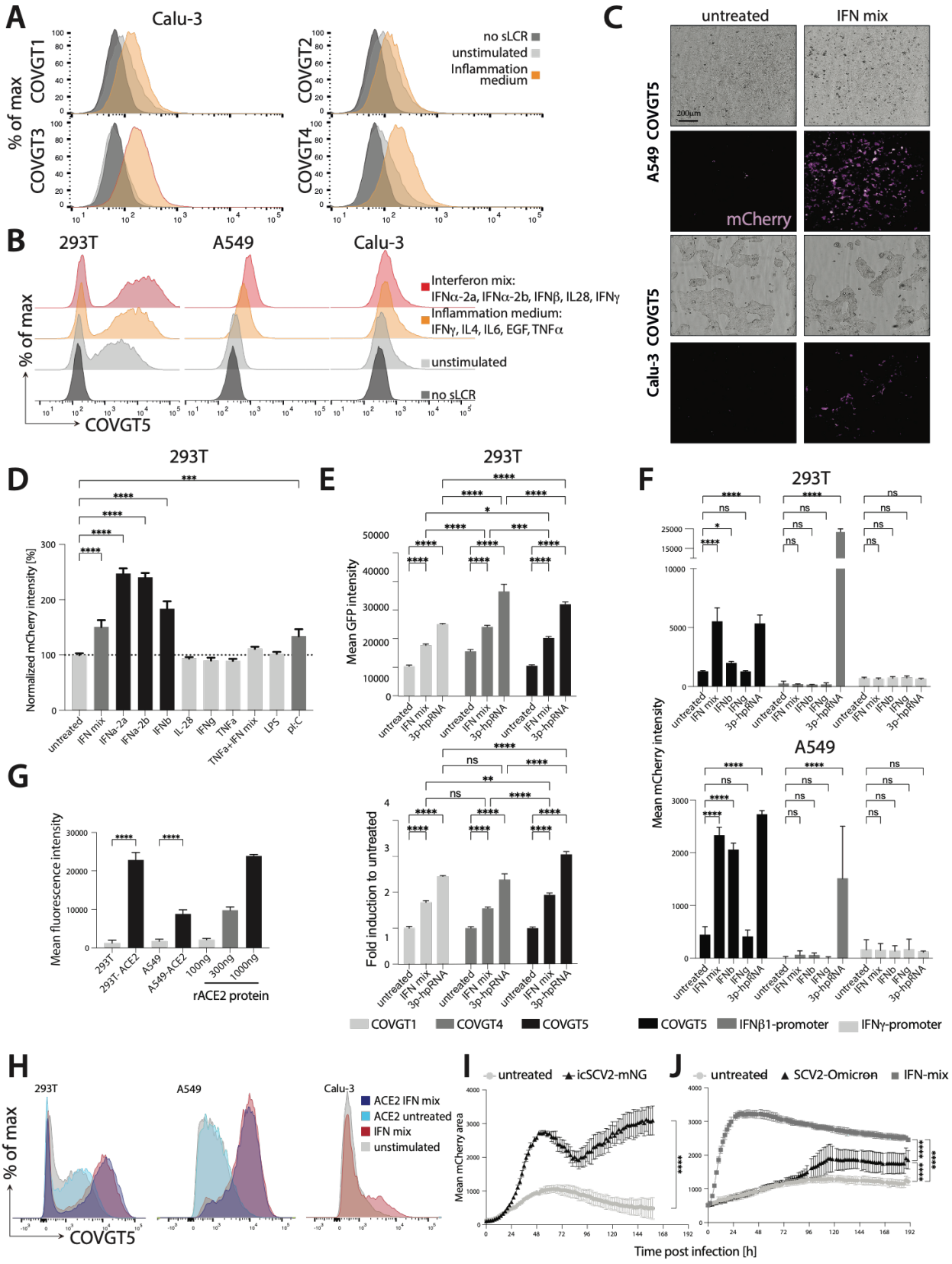

A
Calu-3
% of max
COVGT1
COVGT2
COVGT3
COVGT4
no sLCR
unstimulated
Inflammation medium
B
293T
A549
Calu-3
% of max
COVGT5
Interferon mix:
IFNα-2a, IFNα-2b, IFNβ, IL28, IFNγ
Inflammation medium:
IFNγ, IL4, IL6, EGF, TNFα
unstimulated
no sLCR
C
untreated
IFN mix
A549 COVGT5
Calu-3 COVGT5
200µm
mCherry
D
293T
Normalized mCherry intensity [%]
untreated
IFN mix
IFNa-2a
IFNa-2b
IFNb
IL-28
IFNg
TNFa
TNFa+IFN mix
LPS
pI:C
E
293T
Mean GFP intensity
Fold induction to untreated
untreated
IFN mix
3p-hpRNA
F
293T
A549
Mean mCherry intensity
untreated
IFN mix
IFNb
IFNg
3p-hpRNA
G
Mean fluorescence intensity
293T
293T-ACE2
A549
A549-ACE2
100ng
300ng
1000ng
rACE2 protein
COVGT1
COVGT4
COVGT5
COVGT5
IFNβ1-promoter
IFNγ-promoter
H
293T
A549
Calu-3
% of max
COVGT5
ACE2 IFN mix
ACE2 untreated
IFN mix
unstimulated
I
untreated
icSCV2-mNG
Mean mCherry area
Time post infection [h]
J
untreated
SCV2-Omicron
IFN-mix
Time post infection [h]

Fig. S3. Extended characterization of COVGT sLCRs response to triggers of epithelial immune response and SARS-CoV-2

(**A**) FACS analysis of Calu-3 with COVGT1-4 stimulated with inflammation medium for 75h.

(**B**) FACS analysis comparison between 293T, A549, and Calu-3 COVGT5 reporter cell lines. Cells were stimulated with 'IFN' or 'inflammation' mix for 48h.

(**C**) Representative images of COVGT5-driven mCherry expression (magenta) in A549 and Calu-3. Images were acquired by Operetta at 48h after IFN mix treatment. The scale indicates 200µm.

(**D**) Bar plot quantification comparing induction of 293T-COVGT5 by individual cytokines, LPS, and pI:C. Reporter induction was assessed by normalization against untreated reporter cells 48h post-treatment. Mean mCherry intensity was calculated from technical triplicates (n=3). Statistical significance was determined by 1-way ANOVA.

(**E**) Bar plot quantification comparing inducibility of COVGT1, COVGT4 and COVGT5. 293T cells were induced with IFN mix or 1µg/ml 3p-hpRNA. Reporter induction was assessed after 48h by FACS. Mean GFP intensity (top) and fold induction to untreated cells (bottom) were calculated from technical replicates (n=4). Statistical significance was determined by 1-way ANOVA.

(**F**) Bar plot quantification comparing inducibility of IFNb1-promotor, IFNg-promotor and COVGT5 in 293T (top) and A549 (bottom) reporter cells. Cells were treated with IFN mix, 20ng/ml IFNb, 20ng/ml IFNg or 1µg/ml 3p-hpRNA. Reporter induction was assessed after 48h by FACS. Mean mCherry intensity was calculated from technical replicates (n=4). Statistical significance was determined by 1-way ANOVA.

(**G**) ACE2 enzymatic activity of 293T and A549 ACE2 cells. Mean fluorescence was determined using an MCA-based peptide substrate assay comparing it to the activity of cell-free recombinant ACE2 protein. Fluorescence was quantified using the Tecan Spark in technical replicates (n=4). Statistical significance was determined by 1-way ANOVA.

(**H**) FACS analysis comparing the inducibility of 293T and A549 reporter cells with their ACE2-expressing variants. Cells were treated with the IFN mix and analyzed after 48h.

(**I-J**) Longitudinal measurement of 293T-COVGT5 upon SCV2 infection. Cells were treated with (**I**) 6600pfu/ml icSCV2-mNG or (**J**) 88pfu/ml SCV2-Omicron and IFN mix. Measurement was performed using the Incucyte. Each data point/error bar represents a single measurement. The relative mean fluorescent area was calculated from technical replicates (n=4). Statistical significance was determined by 1-way ANOVA.

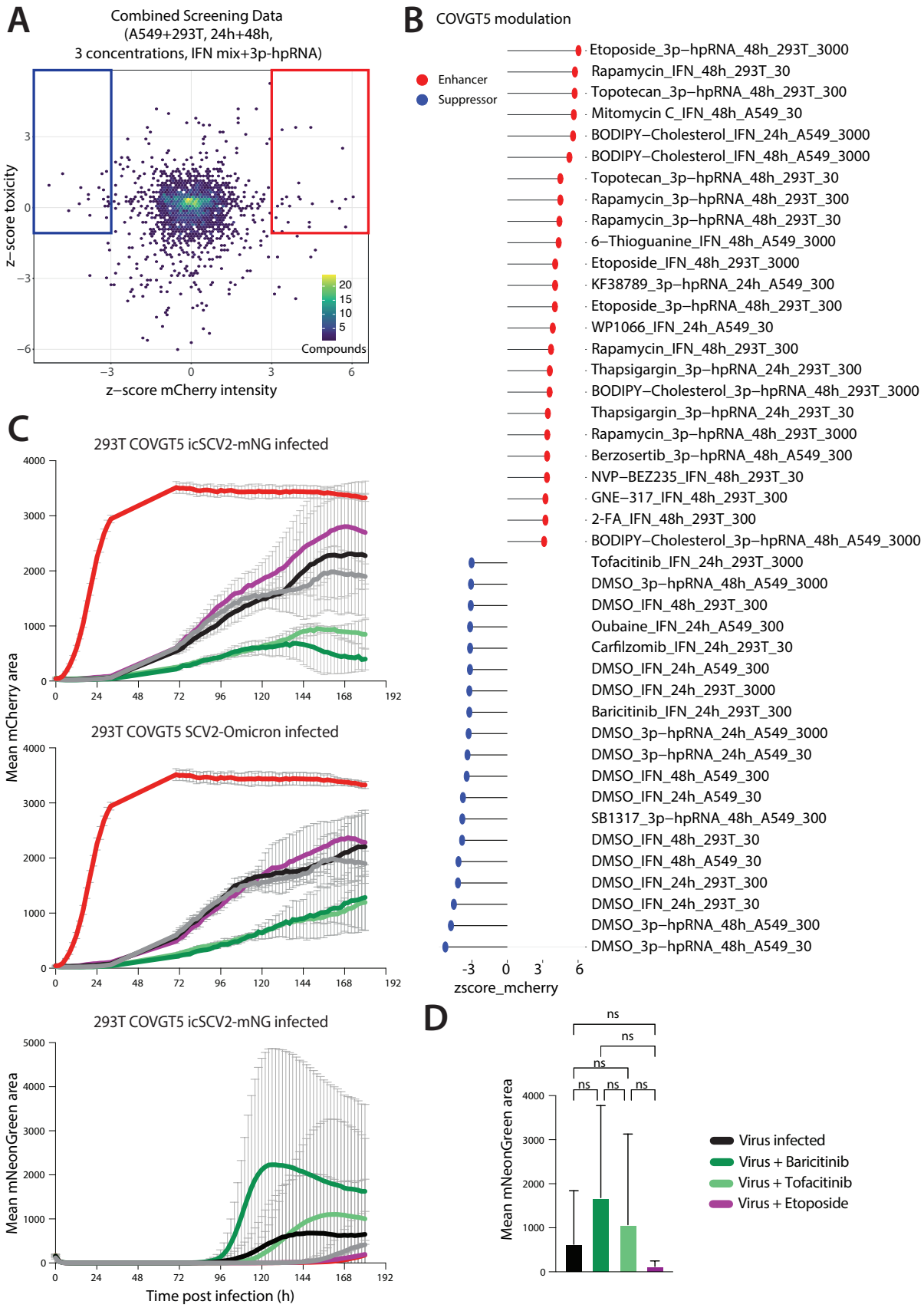

A
Combined Screening Data
(A549+293T, 24h+48h,
3 concentrations, IFN mix+3p-hpRNA)
z−score toxicity
z−score mCherry intensity
Compounds
B
COVGT5 modulation
Enhancer
Suppressor
Etoposide_3p−hpRNA_48h_293T_3000
Rapamycin_IFN_48h_293T_30
Topotecan_3p−hpRNA_48h_293T_300
Mitomycin C_IFN_48h_A549_30
BODIPY−Cholesterol_IFN_24h_A549_3000
BODIPY−Cholesterol_IFN_48h_A549_3000
Topotecan_3p−hpRNA_48h_293T_30
Rapamycin_3p−hpRNA_48h_293T_300
Rapamycin_3p−hpRNA_48h_293T_30
6−Thioguanine_IFN_48h_A549_3000
Etoposide_IFN_48h_293T_3000
KF38789_3p−hpRNA_24h_A549_300
Etoposide_3p−hpRNA_48h_293T_300
WP1066_IFN_24h_A549_30
Rapamycin_IFN_48h_293T_300
Thapsigargin_3p−hpRNA_24h_293T_300
BODIPY−Cholesterol_3p−hpRNA_48h_293T_3000
Thapsigargin_3p−hpRNA_24h_293T_30
Rapamycin_3p−hpRNA_48h_293T_3000
Berzosertib_3p−hpRNA_48h_A549_300
NVP−BEZ235_IFN_48h_293T_30
GNE−317_IFN_48h_293T_300
2-FA_IFN_48h_293T_300
BODIPY−Cholesterol_3p−hpRNA_48h_A549_3000
Tofacitinib_IFN_24h_293T_3000
DMSO_3p−hpRNA_48h_A549_3000
DMSO_IFN_48h_293T_300
Oubaine_IFN_24h_A549_300
Carfilzomib_IFN_24h_293T_30
DMSO_IFN_24h_A549_300
DMSO_IFN_24h_293T_3000
Baricitinib_IFN_24h_293T_300
DMSO_3p−hpRNA_24h_A549_3000
DMSO_3p−hpRNA_24h_A549_30
DMSO_IFN_48h_A549_300
DMSO_IFN_24h_A549_30
SB1317_3p−hpRNA_48h_A549_300
DMSO_IFN_48h_293T_30
DMSO_IFN_48h_A549_30
DMSO_IFN_24h_293T_300
DMSO_IFN_24h_293T_30
DMSO_3p−hpRNA_48h_A549_300
DMSO_3p−hpRNA_48h_A549_30
zscore_mcherry
C
293T COVGT5 icSCV2-mNG infected
Mean mCherry area
293T COVGT5 SCV2-Omicron infected
Mean mCherry area
293T COVGT5 icSCV2-mNG infected
Mean mNeonGreen area
Time post infection (h)
D
ns
Mean mNeonGreen area
Virus infected
Virus + Baricitinib
Virus + Tofacitinib
Virus + Etoposide

Fig. S4. Alternative representation of COVGT5 screening results and SARS-CoV-2 validation experiments.
(A) Combined representation of 293T and A549 screening results. Z-scores for Toxicity (y-axis) and mCherry intensity (x-axis) are plotted against each other. Marked areas indicate drugs with no negative effect on cell viability and amplifying (red) or inhibitory (blue) effect on COVGT5 induction. The color scale indicates the number of compounds in each hexagon.
(B) Lollipop plot showing marked data points from a) ranked based on COVGT5 z-scores.
(C) Longitudinal measurement of 293T-COVGT5 upon SCV2 infection. 6600 pfu/ml SCV2-Omicron (left) or icSCV2-mNG (right) were used. Longitudinal measurement of icSCV2-mNG replication is shown below, and 172 hpi data are shown in bar plot to the right. P-value is non-significant by 1-way ANOVA. Measurement was performed by Incucyte. The relative mean fluorescent area was determined from technical replicates (n=4).
(D) Independent bar plot quantifications of data from (C). The relative mean fluorescent area was determined from technical replicates (n=4). P-values denote significance by 1-way ANOVA.

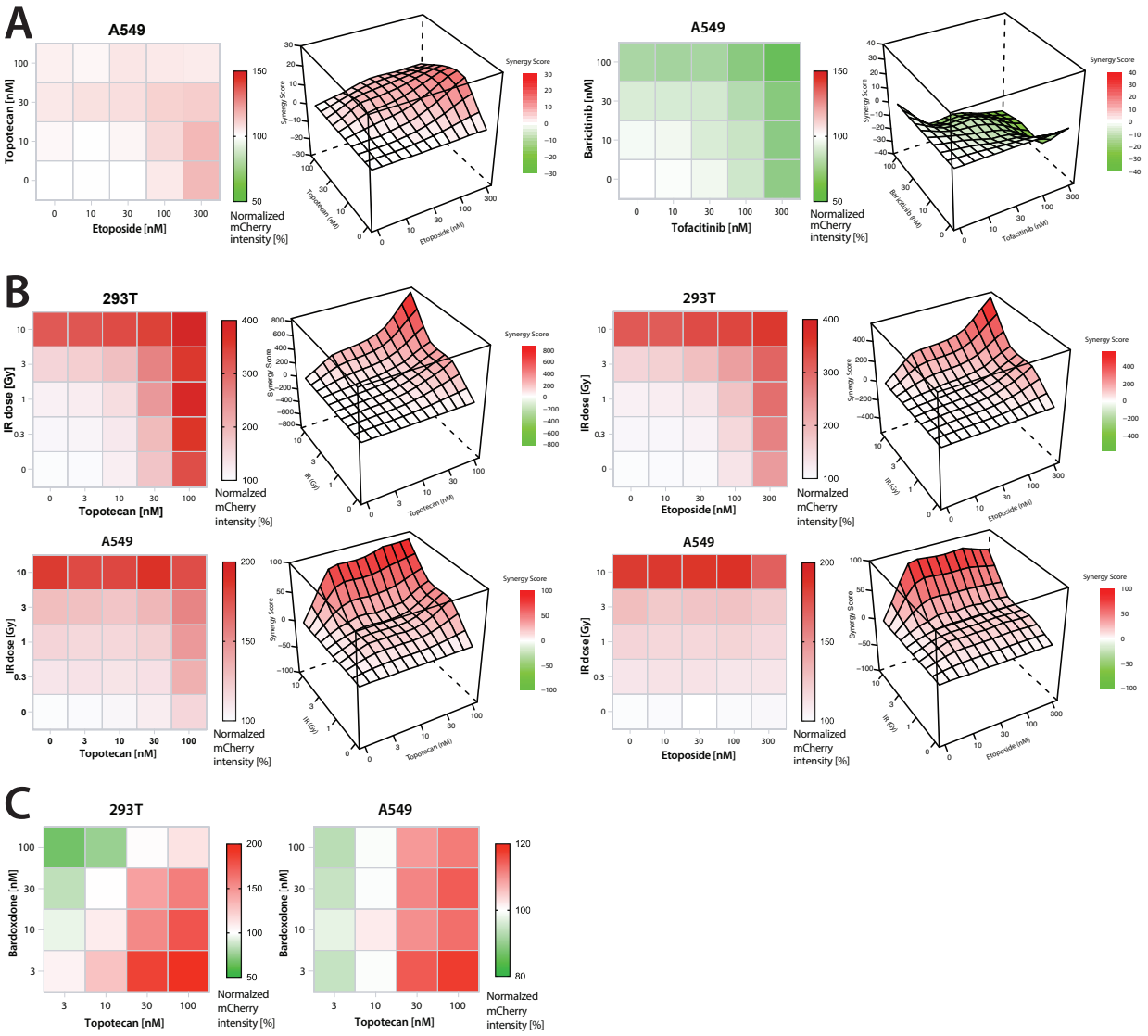


Fig. S5. Extended data on synergistic regulation of COVGT5.

COVGT5 reporter induction and mCherry quantifications were acquired using high throughput FACS by measuring mean mCherry intensity of technical triplicates (n=3) and normalized to non-drug-treated cells.

(A) Dose-escalation of Topotecan against Etoposide (left) and Baricitinib against Tofacitinib (right). A549-COVGT5 received IFN mix induction followed by combinatorial drug treatment. Bliss synergy scores inform on synergism between Topotecan + Etoposide (left) and Baricitinib + Tofacitinib (right) in modulating COVGT5 response.

(B) Dose-escalation of IR against Topotecan (left) and against Etoposide (right). 293T (top) and A549 (bottom) reporter cells received 0.1 ug/ml 3p-hpRNA transfection followed by IR and drug treatment. Bliss synergy scores inform on synergism between IR + Topotecan (left) and IR + Etoposide (right) in modulating COVGT5 response.

(C) Dose-escalation of Bardoxolone against Topotecan in 293T (left) and A549 (right) reporter cells.

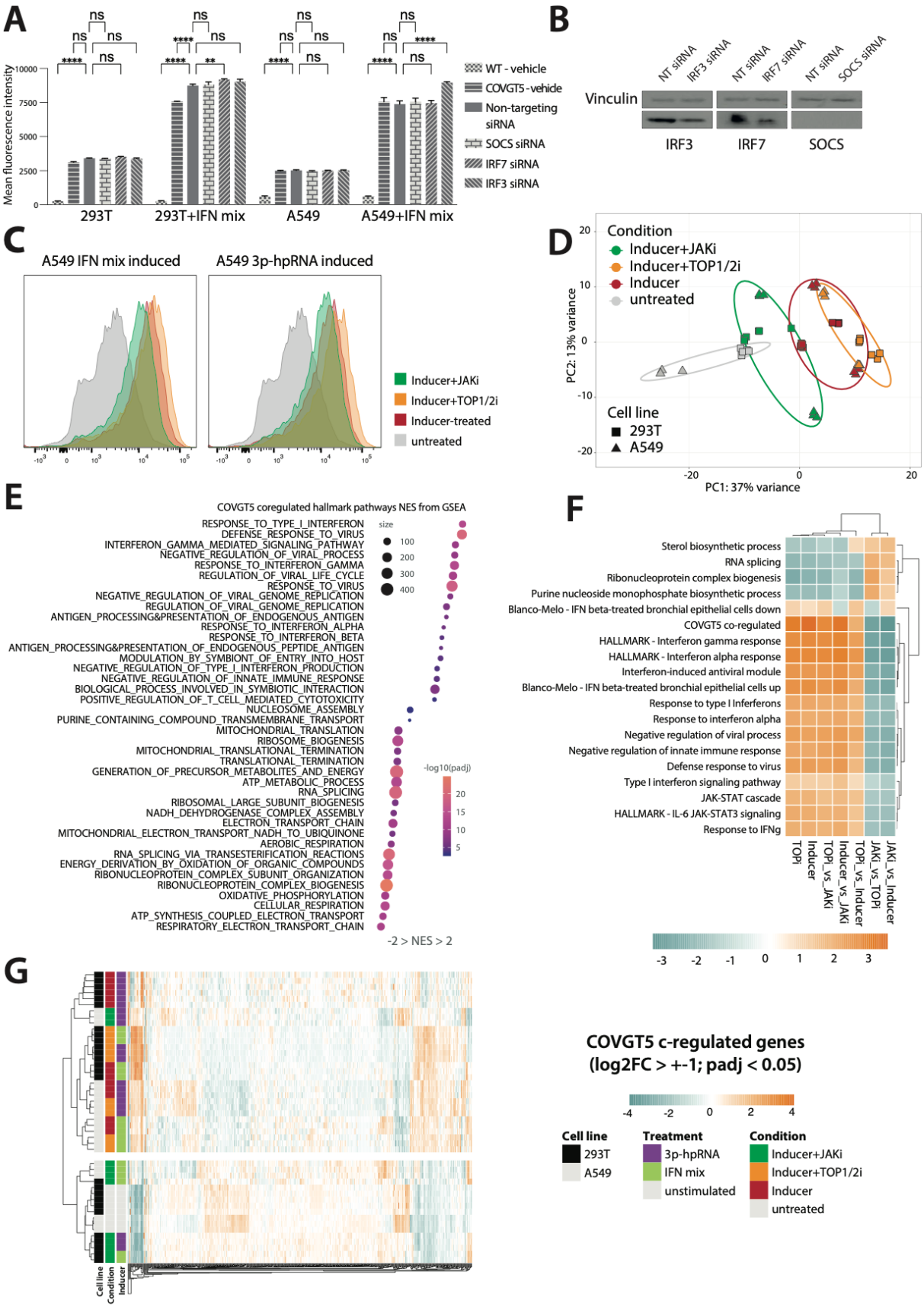

A
Mean fluorescence intensity
293T
293T+IFN mix
A549
A549+IFN mix
WT - vehicle
COVGT5 - vehicle
Non-targeting siRNA
SOCS siRNA
IRF7 siRNA
IRF3 siRNA
B
NT siRNA
IRF3 siRNA
IRF7 siRNA
SOCS siRNA
Vinculin
IRF3
IRF7
SOCS
C
A549 IFN mix induced
A549 3p-hpRNA induced
Inducer+JAKi
Inducer+TOP1/2i
Inducer-treated
untreated
D
Condition
Inducer+JAKi
Inducer+TOP1/2i
Inducer
untreated
Cell line
293T
A549
PC2: 13% variance
PC1: 37% variance
E
COVGT5 coregulated hallmark pathways NES from GSEA
size
-log10(padj)
-2 > NES > 2
F
Sterol biosynthetic process
RNA splicing
Ribonucleoprotein complex biogenesis
Purine nucleoside monophosphate biosynthetic process
Blanco-Melo - IFN beta-treated bronchial epithelial cells down
COVGT5 co-regulated
HALLMARK - Interferon gamma response
HALLMARK - Interferon alpha response
Interferon-induced antiviral module
Blanco-Melo - IFN beta-treated bronchial epithelial cells up
Response to type I Inferferons
Response to interferon alpha
Negative regulation of viral process
Negative regulation of innate immune response
Defense response to virus
Type I interferon signaling pathway
JAK-STAT cascade
HALLMARK - IL-6 JAK-STAT3 signaling
Response to IFNg
TOPi
Inducer
TOPi_vs_JAKi
Inducer_vs_JAKi
TOPi_vs_Inducer
JAKi_vs_TOPi
JAKi_vs_Inducer
G
Cell line
Condition
Inducer
COVGT5 c-regulated genes
(log2FC > +-1; padj < 0.05)
Cell line
293T
A549
Treatment
3p-hpRNA
IFN mix
unstimulated
Condition
Inducer+JAKi
Inducer+TOP1/2i
Inducer
untreated

Fig. S6. Extended data on endogenous genes coregulated with COVGT5.
(**A**) Bar plot quantification of COVGT5 expression in 293T and A549 cells by FACS. Cells were transfected with siRNA targeting SOCS, IRF7, IRF3 or non-targeting siRNA control, induced with IFN mix or non-induced and harvested 42h after induction. Mean mCherry intensity was determined by FACS and calculated from technical triplicates (n=3). Statistical significance was determined by 2-way ANOVA.
(**B**) Immunoblot of A549 representative of siRNA knockdown efficiency. Note the SOCS1 is not detectable by standard immunoblot.
(**C**) FACS plot representative of A549 COVGT5 reporter cells treated with IFN mix (left) or 3p-hpRNA (right) and drug combinations. 30nM Topotecan, 100nM Etoposide, 100nM Baricitinib and 300nM Tofacitinib concentrations were determined to be synergistic in Fig. 5b-c. A549 and 293T cells were harvested and sorted for RNA-seq based on COVGT5 levels 48h after treatment.
(**D**) PCA plot using batch-corrected variance stabilizing transformation values. Each cell line is represented by a different shape, each treatment is represented by a different colour, and IFN mix or 3p-hpRNA stimulation is summarized as 'inducer' plus or minus additional TOP1/2i or JAKi addition (i.e. condition). Ellipses were drawn on the 95% confidence interval of the multivariate t-distribution and adjusted to highlight treatments.
(**E**) Dot plot showing top 10 up- and downregulated independent biological pathways from GSEA of COVGT5-coregulated gene set based on normalized enrichment score (NES).
(**F**) Heatmap showing the GSEA normalized enrichment scores (NES) for selected biological pathways and the COVGT5-coregulated gene set for the differentially expressed genes of the given comparisons or against the untreated control, if not otherwise stated. Inducer denotes treatment with IFN mix or 3p-hpRNA.
(**G**) Heatmap showing the expression levels of COVGT5 co-regulated (n = 454) and anti-regulated genes (n = 104; log2 FC± 1, padj < 0.05). Color code annotations are indicated above and below.

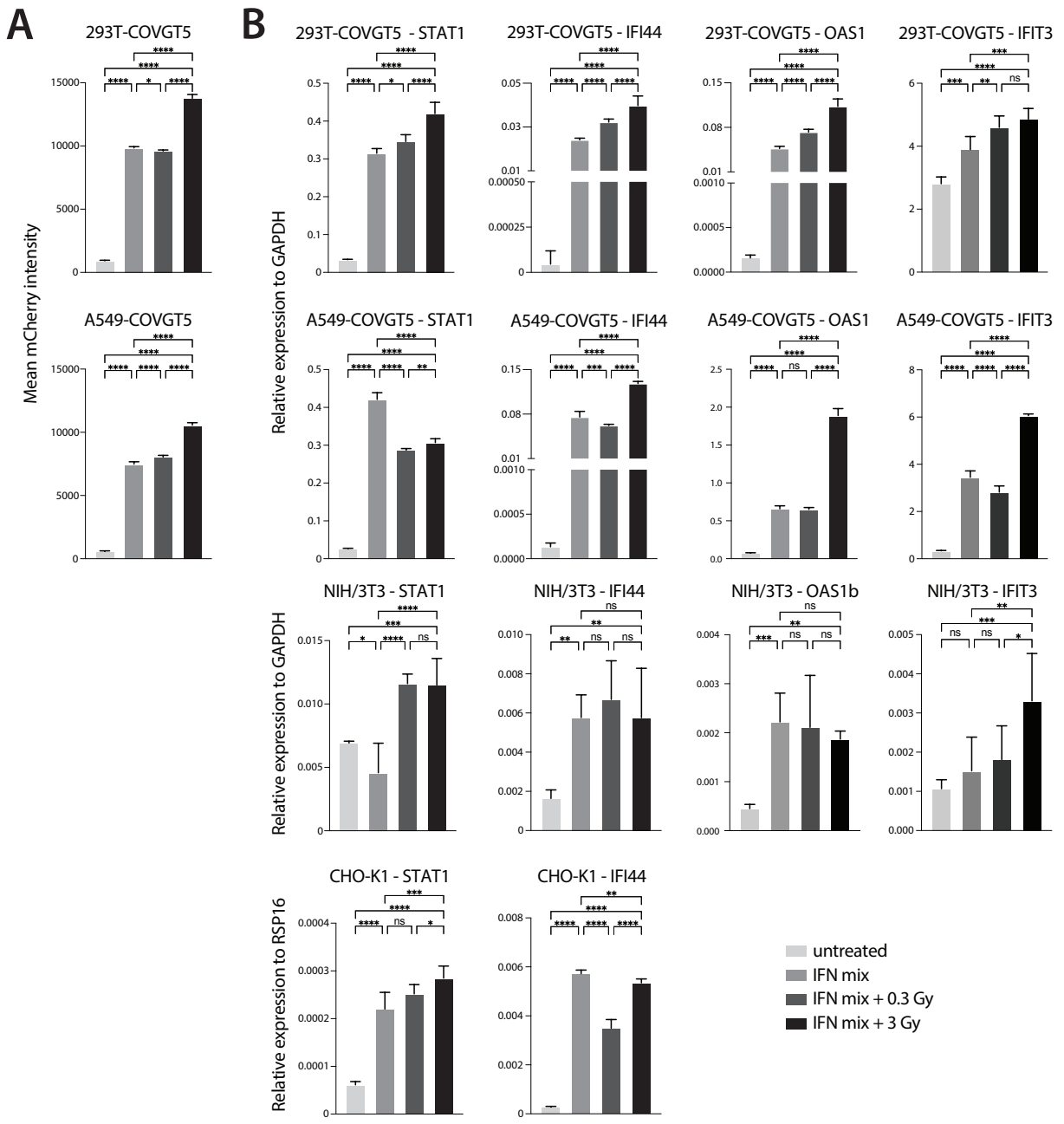


Fig. S7. Validation of COVGT5 co-regulated genes by qRT-PCR.

(A) Bar plot showing FACS quantification of reporter induction by treatment with IFN mix alone or combined with IR doses in 293T and A549 COVGT5 reporter cells.

(**B**) Bar plot showing RT-qPCR expression of *STAT1*, *IFI44* and *OAS1* in human 293T and A549 COVGT5 reporter expressing cells (top right) and rodent NIH/3T3 and CHO-K1 cell lines (bottom) upon treatment. Gene expression levels were normalized to *GAPDH* or *Rsp16*.

Mean values were calculated from technical triplicates (n=3). Statistical significance was determined by 1-way ANOVA.

Table S1.
Extended data on COVGT sLCRs design

Table S1 is provided as a separate file.

Table S2.
Source data for COVG5 phenotypic screen

Table S2 is provided as a separate file.

Table S3.
Extended data analysis for COVGT5 coregulated gene expression program

Table S3 is provided as a separate file.

Table S4.
RT-qPCR oligos used in this study

Table S4 is provided as a separate file.