

## Identification of DAXX As A Restriction Factor Of SARS-CoV-2 Through A CRISPR/Cas9 Screen

Alice Mac Kain<sup>1,2</sup>, Ghizlane Maarifi<sup>3</sup>, Sophie-Marie Aicher<sup>2,4</sup>, Nathalie Arhel<sup>3</sup>, Artem Baidaliuk<sup>5</sup>, Thomas Vallet<sup>1</sup>, Quang Dinh Tran<sup>1,2</sup>, Alexandra Hardy<sup>1</sup>, Maxime Chazal<sup>4</sup>, Françoise Porrot<sup>6</sup>, Molly OhAinle<sup>7</sup>, Jared Carlson-Stevermer<sup>8</sup>, Jennifer Oki<sup>8</sup>, Kevin Holden<sup>8</sup>, Etienne Simon-Lorière<sup>5</sup>, Timothée Bruel<sup>6</sup>, Olivier Schwartz<sup>6</sup>, Nolwenn Jouvenet<sup>4</sup>, Sébastien Nisole<sup>3</sup>, Marco Vignuzzi<sup>§1</sup>, Ferdinand Roesch<sup>\*1,9</sup>

### Author Affiliations:

- 1 Viral populations and pathogenesis, Institut Pasteur, CNRS UMR 3569, F-75015 Paris, France
- 2 Université de Paris, F-75013 Paris, France
- 3 Institut de Recherche en Infectiologie de Montpellier (IRIM), Université de Montpellier, CNRS, 34090 Montpellier, France
- 4 Virus sensing and signaling, Institut Pasteur, CNRS UMR 3569, F-75015 Paris, France
- 5 Evolutionary genomics of RNA viruses, Institut Pasteur, F-75015 Paris, France
- 6 Virus and Immunity, Institut Pasteur, CNRS UMR 3569, F-75015 Paris, France
- 7 Divisions of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, United States
- 8 Synthego Corporation, 3565 Haven Avenue, Menlo Park, CA 94025
- 9 UMR 1282 ISP, INRAE Centre Val de Loire, Nouzilly, France

### \* Corresponding author:

Ferdinand Roesch.

ORCID: 0000-0003-0702-7778

Email: [ferdinand.roesch@inrae.fr](mailto:ferdinand.roesch@inrae.fr)

Phone: +33 2 47 42 79 15

Current address: Equipe 3IMo ; UMR 1282 ISP - Infectiologie et Santé Publique

Centre de recherche INRAE Val de Loire

Route de Crotelles 37380 Nouzilly, France

### \$ Co-corresponding author:

Marco Vignuzzi

ORCID: 0000-0002-4400-771X

Email: [marco.vignuzzi@pasteur.fr](mailto:marco.vignuzzi@pasteur.fr)

Phone: 33 1 4568 8242

1 **Abstract:**

2  
3 While interferon restricts SARS-CoV-2 replication in cell culture, only a handful of Interferon  
4 Stimulated Genes with antiviral activity against SARS-CoV-2 have been identified. Here, we describe  
5 a functional CRISPR/Cas9 screen aiming at identifying SARS-CoV-2 restriction factors. We identified  
6 DAXX, a scaffold protein residing in PML nuclear bodies known to limit the replication of DNA viruses  
7 and retroviruses, as a potent inhibitor of SARS-CoV-2 replication in human cells. Basal expression of  
8 DAXX was sufficient to limit the replication of the virus, and DAXX over-expression further restricted  
9 infection. In contrast with most of its previously described antiviral activities, DAXX-mediated  
10 restriction of SARS-CoV-2 was independent of the SUMOylation pathway. SARS-CoV-2 infection  
11 triggered the re-localization of DAXX to cytoplasmic sites of viral replication and led to its degradation.  
12 Together, these results demonstrate that DAXX is a potent restriction factor for SARS-CoV-2 and that  
13 the virus has evolved a mechanism to counteract its action.

14  
15 **Introduction.** Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the causative  
16 agent of COVID-19 and the third coronavirus to cause severe disease in humans after the emergence  
17 of SARS-CoV in 2002 and Middle East Respiratory Syndrome-related Coronavirus (MERS-CoV) in  
18 2012. Since the beginning of the pandemic, SARS-CoV-2 has infected more than 140 million people  
19 and claimed 3 million lives. While the majority of infected individuals experience mild (or no)  
20 symptoms, severe forms of COVID-19 are associated with respiratory failure, shock and pneumonia.  
21 Innate immune responses play a key role in COVID-19 pathogenesis: immune exhaustion (1) and  
22 reduced levels of type-I and type-III interferon (IFN) have been observed in the plasma of severe  
23 COVID-19 patients (2,3). Imbalanced immune responses to SARS-CoV-2, with a low and delayed IFN  
24 response coupled to early and elevated levels of inflammation, have been proposed to be a major  
25 driver of COVID-19 (4,5). Neutralizing auto-antibodies against type-I IFN (6) and genetic alterations in  
26 several IFN pathway genes (7) have also been detected in critically ill COVID-19 patients. These  
27 studies highlight the crucial need to characterize the molecular mechanisms by which IFN pathway  
28 effectors may succeed, or fail, to control SARS-CoV-2 infection.

29 Although SARS-CoV-2 has been described to antagonize the IFN pathway by different  
30 mechanisms involving the viral proteins ORF3b, ORF9b ORF6, and nsp15 (8), detection of SARS-  
31 CoV-2 by the innate immune sensor Mda5 (9,10) leads to the synthesis of IFN and expression of IFN  
32 Stimulated Genes (ISGs) in human airway epithelial cells (4). IFN strongly inhibits SARS-CoV-2  
33 replication when added in cell culture prior to infection (11,12) or when administered intranasally in  
34 hamsters (13), suggesting that some ISGs might have antiviral activity (14). However, relatively few  
35 ISGs with antiviral activity against SARS-CoV-2 have been identified so far. For instance, spike-  
36 mediated viral entry and fusion is restricted by LY6E (15,16) and IFITMs (17,18). Mucins have also  
37 been suggested in a recent pre-print to restrict viral entry (19). ZAP, which targets CpG dinucleotides  
38 in RNA viruses, also restricts SARS-CoV-2, albeit moderately (20). A recent overexpression screen  
39 identified 65 ISGs as potential inhibitors of SARS-CoV-2 (21), and found that BST-2/Tetherin is able to  
40 restrict viral budding, although this activity is counteracted by the viral protein ORF7a. The RNA  
41 helicase DDX42 was also shown to restrict several RNA viruses, including SARS-CoV-2 (22). We  
42 hypothesize that additional ISGs with antiviral activity against SARS-CoV-2 remain to be discovered.  
43 Other antiviral factors that are not induced by IFN may also inhibit SARS-CoV-2. While several whole-  
44 genome CRISPR/Cas9 screens identified host factors required for SARS-CoV-2 replication (23–28),  
45 none focused on antiviral genes.

46 Here, we performed a CRISPR/Cas9 screen designed to identify restriction factors for SARS-  
47 CoV-2, assessing the ability of 1905 ISGs to modulate SARS-CoV-2 replication in human epithelial  
48 lung cells. We report that the Death domain-associated protein 6 (DAXX), a scaffold protein residing in  
49 PML nuclear bodies (29) and restricting DNA viruses (30) and retroviruses (31,32), is a potent inhibitor  
50 of SARS-CoV-2 replication. SARS-CoV-2 restriction by DAXX is largely independent of the action of  
51 IFN, and unlike most of its other known activities, of the SUMOylation pathway. Within hours of  
52 infection, DAXX re-localizes to sites of viral replication in the cytoplasm, likely targeting viral

transcription. We also show that during the course of SARS-CoV-2 infection, DAXX is degraded, suggesting that SARS-CoV-2 developed a mechanism to counteract DAXX restriction.

## Results.

**A restriction factor-focused CRISPR/Cas9 screen identifies genes potentially involved in SARS-CoV-2 inhibition.** To identify restriction factors limiting SARS-CoV-2 replication, we generated a pool of A549-ACE2 cells knocked-out (KO) for 1905 potential ISGs, using the sgRNA library we previously developed to screen HIV-1 restriction factors (33). This library includes more ISGs than most published libraries, as the inclusion criteria was less stringent (fold-change in gene expression in THP1 cells, primary CD4<sup>+</sup> T cells or PBMCs  $\geq 2$ ). Transduced cells were selected by puromycin treatment, treated with IFN $\alpha$  and infected with SARS-CoV-2. Infected cells were immuno-labelled with a spike (S)-specific antibody and analyzed by flow cytometry. As expected (11,12), IFN $\alpha$  inhibited infection by 7-fold (**Fig. S1**). Infected cells were sorted based on S expression (**Fig. 1a**), and DNA was extracted from infected and non-infected control cells. Integrated sgRNA sequences in each cell fraction were amplified by PCR and sequenced by NGS. Statistical analyses using the MAGeCK package (34) led to the identification of sgRNAs significantly enriched or depleted in infected cells representing antiviral and proviral factors, respectively (**Fig. 1b**). Although our screen was not designed to explicitly study proviral factors, we did successfully identify the well-described SARS-CoV-2 co-factor cathepsin L (CTSL) (35), validating our approach. USP18, a negative regulator of the IFN signaling pathway (36), and ISG15, which favors Hepatitis C Virus replication (37), were also identified as proviral ISGs. In contrast, core IFN pathway genes such as the IFN receptor (IFNAR1), STAT1, and STAT2, were detected as antiviral factors, further validating our screening strategy. LY6E, a previously described inhibitor of SARS-CoV-2 entry (15,16), was also a significant hit. Moreover, our screen identified APOL6, IFI6, DAXX and HERC5, genes that are known to encode proteins with antiviral activity against other viruses (38–41), but had not previously been identified in the context of SARS-CoV-2 infection. For all these genes except APOL6, individual sgRNAs were consistently enriched (for antiviral factors) or depleted (for proviral factors) in the sorted population of infected cells, while non-targeting sgRNAs were not (**Fig. 1c**).

**LY6E and DAXX display antiviral activity against SARS-CoV-2.** To validate the ability of the identified hits to modulate SARS-CoV-2 replication in human cells, we generated pools of A549-ACE2 knocked-out (KO) cells for different genes of interest by electroporating a mix of 3 sgRNA/Cas9 ribonucleoprotein (RNP) complexes per gene target. Levels of gene editing were above 80% in all of the A549-ACE2 KO cell lines, as assessed by sequencing of the edited *loci* (**Table 1**). As controls, we used cells KO for IFNAR1, for the proviral factor CTSL or for the antiviral factor LY6E, as well as cells electroporated with non-targeting (NTC) sgRNAs/Cas9 RNPs. These different cell lines were then treated with IFN $\alpha$  and infected with SARS-CoV-2. Viral replication was assessed by measuring the levels of viral RNA in the supernatant of infected cells using RT-qPCR (**Fig. 2a**). In parallel, we titrated the levels of infectious viral particles released into the supernatant of infected cells (**Fig. 2b**). As expected, infection was significantly reduced in CTSL KO cells, confirming the proviral effect of this gene (35). Among the selected antiviral candidate genes, only 2 had a significant impact on SARS-CoV-2 replication: LY6E, and to an even greater degree, DAXX. Both genes restricted replication in absence of IFN $\alpha$ , an effect which was detectable at the level of viral RNA (8-fold and 42-fold reduction of infection, respectively, **Fig. 2a**) and of infectious virus (15-fold and 62-fold reduction, **Fig. 2b**). Based on available single-cell RNAseq datasets (42), DAXX is expected to be expressed in cell types relevant for SARS-CoV-2 such as lung epithelial cells, macrophages and T cells (**Fig. S3**).

In IFN $\alpha$ -treated cells. DAXX and LY6E KO led to a modest, but significant rescue of viral replication, which was particularly visible when measuring the levels of infectious virus by plaque assay titration (**Fig. 2b**), while the antiviral effect of IFN $\alpha$  treatment was completely abrogated in IFNAR1 KO cells, as expected (**Fig. 2c**). However, IFN $\alpha$  still had a strong antiviral effect on SARS-CoV-2 replication in both DAXX KO and LY6E KO cells (**Fig. 2c**). While DAXX and LY6E contribute to

105 the IFN-mediated restriction, this suggests that there are likely other ISGs contributing to this effect.  
106 Although DAXX is sometimes referred to as an ISG, its expression is only weakly induced by IFN in  
107 some human cell types (31,43). Consistent with this, we found little to no increase in DAXX expression  
108 in IFN $\alpha$ -treated A549-ACE2 cells (**Fig. S2**). In addition, we tested the antiviral effect of DAXX on  
109 several SARS-CoV-2 variants that have been suggested in a recent report to be partially resistant to  
110 the antiviral effect of IFN (44). In these experiments, the 20I/501Y.V1 (UK), together with the  
111 20J/501Y.V3 (Brazil) variant, were indeed less sensitive to IFN. DAXX, however, restricted all variants  
112 to a similar level than the historical strain of SARS-CoV-2 (**Fig. 2d**). This suggest that while some  
113 variants may have evolved towards IFN-resistance, they are still efficiently restricted by DAXX.

114 To further validate the antiviral activity of DAXX against SARS-CoV-2, we quantified the levels  
115 of several viral transcripts in WT and DAXX KO cells (**Fig. 2e**). The levels of all the transcripts tested  
116 strongly increased in DAXX KO cells (20 to 30-fold across all experiments). This further confirmed that  
117 DAXX strongly interferes with SARS-CoV-2 replication and suggests that it may target viral  
118 transcription, or an earlier step of the viral life cycle.

119  
120 **DAXX restriction is SUMO-independent.** DAXX is a small scaffold protein that acts by recruiting  
121 other SUMOylated proteins in nuclear bodies through its C-terminal SUMO-Interacting Motif (SIM)  
122 domain (45). The recruitment of these factors is required for the effect of DAXX on various cellular  
123 processes such as transcription and apoptosis, and on its antiviral activities (31,46–48). DAXX can  
124 also be SUMOylated itself (49), which may be important for some of its functions. To investigate the  
125 role of SUMOylation in DAXX-mediated SARS-CoV-2 restriction, we used overexpression assays to  
126 compare the antiviral activity of DAXX WT with two previously described DAXX mutants (50). First, we  
127 used a version of DAXX in which 15 lysine residues have been mutated to arginine (DAXX 15KR),  
128 which is unable to be SUMOylated; and second, a truncated version of DAXX that is missing its C-  
129 terminal SIM domain (DAXX $\Delta$ SIM) (47) and is unable to interact with its SUMOylated partners. A549-  
130 ACE2 were refractory to SARS-CoV-2 infection upon transfection with any plasmid, precluding us from  
131 using this cell line. Instead, we transfected 293T-ACE2 cells, another SARS-CoV-2 permissive cell line  
132 (18). Western blot (**Fig. S4a**) and flow cytometry (**Fig. S4b**) analyses showed that DAXX WT and  
133 mutants were expressed to similar levels, with a transfection efficiency of 40 to 50% for all three  
134 constructs.

135 We examined the effect of DAXX WT overexpression on the replication of SARS-CoV-2-  
136 mNeonGreen (51) by microscopy. DAXX overexpression starkly reduced the number of infected cells  
137 (**Fig. 3a**), revealing that DAXX-mediated restriction is not specific to A549-ACE2 cells. Using double  
138 staining for HA-tagged DAXX and SARS-CoV-2, we found that most of the DAXX-transfected cells  
139 were negative for infection, and conversely, that most of the infected cells did not express transfected  
140 DAXX (**Fig. 3a**), indicating that DAXX imposes a major block to SARS-CoV-2 infection.

141 In order to quantify the antiviral effect of overexpressed DAXX WT and mutants, we assessed  
142 the number of cells positive for the S protein (among transfected cells) by flow cytometry (**Fig. 3c-d**)  
143 and the abundance of viral transcripts by qRT-PCR (**Fig. S4c**). DAXX WT, 15KR and  $\Delta$ SIM all  
144 efficiently restricted SARS-CoV-2 replication. Indeed, at 24 hours p.i., the proportion of infected cells  
145 (among HA-positive cells) was reduced by 2 to 3-fold as compared to control transfected cells for all 3  
146 constructs (**Fig. 3c**). This effect was less pronounced but still significant at 48 hours p.i. (**Fig. 3d**).  
147 Moreover, DAXX overexpression led to a significant reduction of the levels of two different viral  
148 transcripts (**Fig. S4c**), in line with our earlier results showing that DAXX targets viral transcription (**Fig.**  
149 **2e**). Together, these results show that DAXX overexpression restricts SARS-CoV-2 transcription in a  
150 SUMOylation-independent mechanism.

151  
152 **SARS-CoV-2 infection triggers DAXX re-localization and degradation.** DAXX mostly localizes in  
153 nuclear bodies (29), whereas SARS-CoV-2 replication occurs in the cytoplasm. We reasoned that  
154 DAXX localization may be altered during the course of infection in order for the restriction factor to  
155 exert its antiviral effect. To test this hypothesis, we infected 293T-ACE2 cells with SARS-CoV-2 and  
156 used high-resolution confocal microscopy to study the localization of endogenous DAXX (**Fig. 4**). As  
157 expected (29), DAXX mostly localizes in the nuclei of non-infected cells, forming discrete *foci*. 6h after

158 SARS-CoV-2 infection, DAXX begins to re-localize to the cytoplasm, although nuclear *foci* can still be  
159 detected. At 24h post-infection, however, DAXX is completely depleted from nuclear bodies, and is  
160 found almost exclusively in the cytoplasm of infected cells, in close association with SARS-CoV-2  
161 dsRNA. Western blot analysis revealed that SARS-CoV-2 infection induces a marked decrease of total  
162 DAXX expression in infected cells (**Fig. 5a**). This effect is visible at MOI 0.1, and almost complete  
163 DAXX degradation can be observed at MOI 1. These results suggest that DAXX may be actively  
164 targeted by SARS-CoV-2 for degradation during the course of infection. SARS-CoV-2 papain-like  
165 protease (PLpro) is a possible candidate for this function, as it cleaves ISG15 from Mda5 (52) and  
166 IRF3 (53). It was also shown that foot-and-mouth disease virus (FDMV) PLpro degrades DAXX (54).  
167 We treated cells with GRL-0617, an inhibitor of SARS-CoV-2 PLpro (53). Strikingly, GRL-0617  
168 treatment partially restores DAXX expression (**Fig. 5a**) and subcellular localization to nuclear bodies in  
169 infected cells at 24h p.i. at MOI 0.1 (**Fig. 5b**). Although we cannot exclude that GRL-0617 treatment  
170 may have an indirect effect on DAXX levels by inhibiting SARS-CoV-2 replication itself, this is unlikely  
171 to be a major mitigating effect at 24h post-infection, particularly since imaging analysis reveals a  
172 restoration of DAXX specifically in SARS-CoV-2 infected cells (**Fig. 5b**). Further work will be required  
173 to uncover whether PLPro or a proteolytic product of the viral polyprotein chain degrades DAXX.

## 174 175 **Discussion.**

176  
177 **Comparison with other screens.** The whole-genome CRISPR/Cas9 screens conducted to date on  
178 SARS-CoV-2 infected cells mostly identified host factors necessary for viral replication (23–28) and  
179 did not focus on antiviral genes, as did our screen. Two overexpression screens, however, identified  
180 ISGs with antiviral activity against SARS-CoV-2 (16,21). In the first one, Pfaender *et al.* screened 386  
181 ISGs for their antiviral activity against the endemic human coronavirus 229E, and identified LY6E as a  
182 restriction factor inhibiting both 229E and SARS-CoV-2. Our screen also identified LY6E as a top hit  
183 (**Fig.1**), further validating the findings of both studies. Four additional genes had significant p-values in  
184 both Pfaender *et al.* and our work: IFI6, HERC5, OAS2 and SPSB1 (**Table S5-S6**). We showed that  
185 knocking-out LY6E and DAXX only partially rescued SARS-CoV-2 replication in IFN-treated cells (**Fig.**  
186 **2**), suggesting that they contribute modestly to IFN-mediated restriction and that other IFN effectors  
187 active against SARS-CoV-2 remain to be identified. For instance, other ISGs, such as IFITMs, inhibit  
188 SARS-CoV-2 viral entry (17–19). In the second screen, Martin Sancho *et al.* tested 399 ISGs against  
189 SARS-CoV-2. Among the 65 antiviral ISGs identified, they focused on BST-2/Tetherin, that targets  
190 viral budding. BST-2/Tetherin was not a significant hit in our screen (**Table S5-6**). This discrepancy  
191 can be easily explained by the fact that our screen relies on the sorting of S-positive cells, and is  
192 therefore unable to detect late-acting antiviral factors. Of note, DAXX was absent from the ISG  
193 libraries used by both overexpression screens, which explains why it was not previously identified as  
194 an antiviral ISG for SARS-CoV-2. In contrast, our sgRNA library, by including 1905 genes, targeted a  
195 wider set of ISGs and “ISG-like” genes, including genes like DAXX that are not (or only weakly)  
196 induced by IFN in some cell types (31,43). Interestingly, IFN has a stronger effect on DAXX  
197 expression levels in other mammals, including in some bat species (55). Future studies may  
198 investigate whether DAXX orthologs of different species are also able to restrict SARS-CoV-2 and  
199 whether DAXX participates in IFN-mediated viral restriction in these hosts.

200  
201 **DAXX is a restriction factor for SARS-CoV-2.** Our CRISPR/Cas9 screen identifies DAXX as a  
202 potent antiviral factor restricting the replication of SARS-CoV-2, acting independently of IFN and likely  
203 targeting an early step of the viral life cycle such as transcription. DAXX fulfills all of the criteria  
204 defining a *bona fide* SARS-CoV-2 restriction factor: knocking-out endogenous DAXX leads to an  
205 enhanced viral replication (**Fig. 2**), while over-expression of DAXX restricts infection (**Fig. 3**). DAXX  
206 co-localizes with viral dsRNA (**Fig. 4**) and SARS-CoV-2 antagonizes DAXX to some degree, as  
207 evidenced by the degradation of DAXX induced by viral replication (**Fig. 5**). Although DAXX  
208 expression is not upregulated by IFN (**Fig. S2**), basal levels of expression are sufficient for its antiviral  
209 activity, as has been shown for other potent restriction factors. Single-cell RNAseq analyses (**Fig. S3**)

210 indicated that DAXX is expressed in cell types targeted by the virus in patients, such as lung epithelial  
211 cells and macrophages.  
212

213 **Mechanism of DAXX-mediated restriction.** DAXX is mostly known for its antiviral activity against  
214 DNA viruses replicating in the nucleus, such as adenovirus 5 (AdV5) (56) and human papillomavirus  
215 (HPV) (57). Most of these viruses antagonize PML and/or DAXX, which interacts with PML in nuclear  
216 bodies (29). We show here that DAXX is also able to restrict a positive sense RNA virus that replicates  
217 in the cytoplasm, which may represent a first step into establishing DAXX as a broad-spectrum  
218 restriction factor. Recent studies have shown that DAXX inhibits the reverse transcription of HIV-1 in  
219 the cytoplasm (31,32). Within hours of infection, DAXX subcellular localization was altered, with DAXX  
220 accumulating in the cytoplasm and colocalizing with incoming HIV-1 capsids (32). Here, we observed  
221 a similar phenomenon, with a rapid re-localization of DAXX from the nucleus to viral replication sites  
222 (**Fig. 4**), where it likely exerts its antiviral effect. Early events in the replication cycle of both HIV-1 and  
223 SARS-CoV-2, such as viral fusion or virus-induced stress, may thus trigger DAXX re-localization to the  
224 cytoplasm. DAXX seems to inhibit SARS-CoV-2, however, by a distinct mechanism: whereas the  
225 recruitment of SUMOylated partners through the SIM-domain is required for the effect of DAXX on  
226 HIV-1 reverse transcription (31), it was not the case in the context of SARS-CoV-2 restriction. This  
227 result was surprising, since DAXX has no enzymatic activity and rather acts as a scaffold protein  
228 recruiting SUMOylated partners through its SIM domain (50). Some DAXX functions, such as  
229 interaction with the chromatin remodeler ATRX (29), are however SIM-independent. Future work  
230 should determine which DAXX domains and residues are required for its antiviral activity.  
231

232 **Antagonism of DAXX by SARS-CoV-2.** SARS-CoV-2 replication triggers DAXX degradation (**Fig. 5**),  
233 which likely represents an efficient antagonism strategy. Other viruses are also able to degrade DAXX:  
234 for instance, the AdV5 viral protein E1B-55K targets DAXX for proteasomal degradation (56), and  
235 FDMV PLpro directly degrades DAXX (54). We speculate that the SARS-CoV-2 proteases PLpro or  
236 3C-like proteinase might be involved. Treatment of cells with GRL-0617, an inhibitor of PLpro, partially  
237 prevented virus-induced DAXX degradation and restored DAXX localization to the nucleus. However,  
238 this effect could be indirect, since GRL-0617 also blocks SARS-CoV-2 replication by preventing  
239 polyprotein cleavage. Future work will be necessary to formally demonstrate the direct degradation of  
240 DAXX by PLpro and to determine whether other viral strategies promote evasion from DAXX  
241 restriction.  
242

## 243 **Material & Methods.**

244  
245 **Cells, viruses & plasmids.** HEK 293T (ATCC #CRL-11268) were cultured in MEM (Gibco #11095080)  
246 complemented with 10% FBS (Gibco #A3160801) and 2 mM L-Glutamine (Gibco # 25030081). VeroE6 (ATCC  
247 #CRL-1586), A549 (ATCC #CCL-185) and HEK 293T, both overexpressing the ACE2 receptor (A549-ACE2 and  
248 HEK 293T-ACE2, respectively), were grown in DMEM (Gibco #31966021) supplemented with 10% FBS (Gibco  
249 #A3160801), and penicillin/streptomycin (100 U/mL and 100 µg/mL, Gibco # 15140122). Blastidicin (10 µg/mL,  
250 Sigma-Aldrich #SBR00022-10ML) was added for selection of A549-ACE2 and HEK 293T-ACE2. All cells were  
251 maintained at 37°C in a 5% CO<sub>2</sub> atmosphere. Universal Type I Interferon Alpha (PBL Assay Science #11200-2)  
252 was diluted in sterile-filtered PBS 1% BSA according to the activity reported by the manufacturer. The strains  
253 BetaCoV/France/IDF0372/2020 (historical); hCoV-19/France/IDF-IPP11324/2020 (20I/501Y.V1 or UK); and  
254 hCoV-19/France/PDL-IPP01065/2021 (20H/501Y.V2 or SA) were supplied by the National Reference Centre for  
255 Respiratory Viruses hosted by Institut Pasteur and headed by Pr. Sylvie van der Werf. The human samples from  
256 which the historical, UK and SA strains were isolated were provided by Dr. X. Lescure and Pr. Y. Yazdanpanah  
257 from the Bichat Hospital, Paris, France; Dr. Besson J., Bioliance Laboratory, saint-Herblain France; Dr. Vincent  
258 Foissaud, HIA Percy, Clamart, France, respectively. These strains were supplied through the European Virus  
259 Archive goes Global (Evag) platform, a project that has received funding from the European Union's Horizon  
260 2020 research and innovation programme under grant agreement #653316. The hCoV-19/Japan/TY7-501/2021  
261 strain (20J/501Y.V3 or Brazil) was kindly provided by Jessica Vanhomwegen (Cellule d'Intervention Biologique  
262 d'Urgence; Institut Pasteur). The mNeonGreen reporter SARS-CoV-2 was provided by Pei-Yong Shi (51). Viral  
263 stocks were generated by infecting VeroE6 cells (MOI 0.01, harvesting at 3 dpi) using DMEM supplemented with  
264 2% FBS and 1 µg/mL TPCK-trypsin (Sigma-Aldrich #1426-100MG). The Human Interferon-Stimulated Gene

265 CRISPR Knockout Library was a gift from Michael Emerman and is available on Addgene (Pooled Library  
266 #125753). The plentiCRISPRv.2 backbone was ordered through Addgene (Plasmid #52961). pMD2.G and  
267 psPAX2 were gifts from Didier Trono (Addgene #12259; #12260). pcDNA3.1 was purchased from Invitrogen.  
268 Plasmids constructs expressing WT and mutant HA-tagged DAXX constructs were kindly provided by Hsiu-Ming  
269 Shih (50).

270  
271 **Antibodies.** For Western Blot, we used mouse anti-DAXX (diluted 1:1000, Abnova #7A11), rat anti-HA clone  
272 3F10 (diluted 1:3000, Roche #11867423001) and mouse anti-GAPDH clone 6C5 (diluted 1:3000, Millipore  
273 #FCMAB252F). Secondary antibodies were goat anti-mouse and anti-rabbit HRP-conjugates (diluted 1:5000,  
274 GE Healthcare #NA931V and #NA934V). For immunofluorescence, we used rabbit anti-DAXX (diluted 1:50,  
275 Proteintech #20489-1-AP) and mouse anti-dsRNA J2 (diluted 1:50, Scicons #10010200). Secondary antibodies  
276 were goat anti-rabbit AF555 and anti-mouse AF488 (diluted 1:1000, ThermoFisher #A-21428 and #A-28175).  
277 For flow sorting of infected cells, we used the anti-S2 H2 162 antibody (diluted 1:150), a kind gift from Dr. Hugo  
278 Mouquet, (Institut Pasteur, Paris, France). Secondary antibody was donkey anti-mouse AF647 (diluted 1:1000,  
279 Invitrogen #A31571). For FACS analysis, we used rat anti-HA clone 3F10 (diluted 1:100, Sigma #2158167001)  
280 and mouse anti-dsRNA J2 (diluted 1:500, Scicons #10010200). Secondary antibodies were goat anti-rat AF647  
281 and anti-mouse AF488 (diluted 1:1000, ThermoFisher #A-21247 #A-28175).

282  
283 **Generation of CRISPR/Cas9 library cells.** HEK 293T cells were transfected with the sgRNA library plasmid  
284 together with plasmids coding for Gag/Pol (R8.2) and for the VSVg envelope (pVSVg) using a ratio of 5:5:1 and  
285 calcium phosphate transfection. Supernatants were harvested at 36h and 48h, concentrated 80-fold by  
286 ultracentrifugation (22,000 g, 4°C for 1h) and pooled. To generate ISG KO library cells,  $36 \times 10^6$  A549-ACE2 cells  
287 were seeded in 6 well plates ( $10^6$  cells per well) 24h before transduction. For each well, 100  $\mu$ L of concentrated  
288 lentivector was diluted in 500  $\mu$ L of serum-free DMEM, supplemented with 10  $\mu$ g/mL of DEAE dextran (Sigma  
289 #D9885). After 48h, transduced cells were selected by puromycin treatment for 20 days (1  $\mu$ g/mL; Sigma  
290 #P8833).

291  
292 **CRISPR/Cas9 screen.**  $4 \times 10^7$  A549-ACE2 cells were treated with IFN $\alpha$  (200U/mL). 16h later, cells were infected  
293 at a MOI of 1 in serum-free media complemented with TPCK-trypsin and IFN $\alpha$  (200 U/mL). After 90 min, the viral  
294 inoculum was removed, and cells were maintained in DMEM containing 5% FBS and IFN $\alpha$  (200 U/mL). After  
295 24h, cells were harvested and fixed for 15 min in Formalin 1%. Fixed cells were washed in cold FACS buffer  
296 containing PBS, 2% Bovine Serum Albumin (Sigma-Aldrich #A2153-100G), 2 mM EDTA (Invitrogen #15575-  
297 038) and 0.1% Saponin (Sigma-Aldrich #S7900-100G). Cells were incubated for 30 min at 4°C under rotation  
298 with primary antibody diluted in FACS buffer. Incubation with the secondary antibody was performed during 30  
299 min at 4°C under rotation. Stained cells were resuspended in cold sorting buffer containing PBS, 2% FBS, 25  
300 mM Hepes (Sigma-Aldrich #H0887-100ML) and 5 mM EDTA. Infected cells were sorted on a BD FACS Aria  
301 Fusion. Sorted and control (non-infected, not IFN-treated) cells were centrifugated (20 min, 2,000g) and  
302 resuspended in lysis buffer (NaCl 300 mM, SDS 0.1%, EDTA 10 mM, EGTA 20 mM, Tris 10 mM) supplemented  
303 with 1% Proteinase K (Qiagen #19133) and 1% RNase A/T1 (ThermoFisher #EN0551) and incubated overnight  
304 at 65°C. Two consecutive phenol-chloroform (Sigma #P3803-100ML) extractions were performed and DNA was  
305 recovered by ethanol precipitation. Nested PCR was performed using the Herculase II Fusion DNA Polymerase  
306 (Agilent, #600679) and the DNA oligos indicated in **Table S1**. PCR1 products were purified using QIAquick PCR  
307 Purification kit (Qiagen #28104). PCR2 products were purified using Agencourt AMPure XP Beads (Beckman  
308 Coulter Life Sciences #A63880). DNA concentration was determined using Qubit dsDNA HS Assay Kit (Thermo  
309 Fisher #Q32854) and adjusted to 2 nM prior to sequencing. NGS was performed using the NextSeq 500/550  
310 High Output Kit v2.5 75 cycles (Illumina #20024906).

311  
312 **Screen analysis.** Reads were demultiplexed using bcl2fastq Conversion Software v2.20 (Illumina) and  
313 fastx\_toolkit v0.0.13. Sequencing adapters were removed using cutadapt v1.9.1 (58). The reference library was  
314 built using bowtie2 v2.2.9 (59). Read mapping was performed with bowtie2 allowing 1 seed mismatch in --local  
315 mode and samtools v1.9 (60). Mapping analysis and gene selection were performed using MAGeCK v0.5.6,  
316 normalizing the data with default parameters. sgRNA and gene enrichment analyses are available in **Table S5-  
317 S6**, respectively and full MAGeCK output at [https://github.com/Simon-LorriereLab/crispr\\_isg\\_sarscov2](https://github.com/Simon-LorriereLab/crispr_isg_sarscov2).

318  
319 **Generation of multi-guide gene knockout cells.** 3 sgRNAs per gene were designed (**Table S2**). 10 pmol of  
320 NLS-Sp.Cas9-NLS (SpCas9) nuclease (Aldevron #9212) was combined with 30 pmol total synthetic sgRNA (10  
321 pmol for each sgRNA) (Synthego) to form RNPs in 20  $\mu$ L total volume with SE Buffer (Lonza #V5SC-1002). The  
322 reaction was incubated at room temperature for 10 min.  $2 \times 10^5$  cells per condition were pelleted by centrifugation

323 at 100g for 3 min, resuspended in SE buffer and diluted to  $2 \times 10^4$  cells/ $\mu$ L. 5  $\mu$ L of cell solution was added to the  
324 pre-formed RNP solution and gently mixed. Nucleofections were performed on a Lonza HT 384-well nucleofector  
325 system (Lonza #AAU-1001) using program CM-120. Immediately following nucleofection, each reaction was  
326 transferred to a 96-well plate containing 200  $\mu$ L of DMEM 10% FBS ( $5 \times 10^4$  cells per well). Two days post-  
327 nucleofection, DNA was extracted using DNA QuickExtract (Lucigen #QE09050). Cells were lysed in 50  $\mu$ L of  
328 QuickExtract solution and incubated at 68°C for 15 min followed by 95°C for 10 min. Amplicons were generated  
329 by PCR amplification using NEBNext polymerase (NEB #M0541) or AmpliTaq Gold 360 polymerase  
330 (ThermoFisher #4398881) and the primers indicated in **Table S3**. PCR products were cleaned-up and analyzed  
331 by Sanger sequencing. Sanger data files and sgRNA target sequences were input into Inference of CRISPR  
332 Edits (ICE) analysis <https://ice.synthego.com/#/> to determine editing efficiency and to quantify generated indels  
333 (61). Percentage of alleles edited is expressed as an ice-d score.

334  
335 **SARS-CoV-2 infection assays.** A549-ACE2 cells were infected by incubating the virus for 1h with the cells  
336 maintained in DMEM supplemented with 1  $\mu$ g/ml TPCK-trypsin (Sigma #4370285). The viral input was then  
337 removed and cells were kept in DMEM supplemented with 2% FBS. For 293T-ACE2 cells, infections were  
338 performed without TPCK-trypsin. All experiments involving infectious material were performed in Biosafety Level  
339 3 facilities in compliance with Institut Pasteur's guidelines and procedures.

340  
341 **Hit validation.**  $2.5 \times 10^4$  A549-ACE2 KO cells were seeded in 96-well plates 18h before the experiment. Cells  
342 were treated with IFN $\alpha$  and infected as described above. At 72h post-infection, supernatants and cellular  
343 monolayers were harvested in order to perform qRT-PCR and plaque assay titration. Infectious supernatants  
344 were heat-inactivated at 80°C for 10 min. For intracellular RNA, cells were lysed in a mixture of Trizol Reagent  
345 (Invitrogen #15596018) and PBS at a ratio of 3:1. Total RNA was extracted using the Direct-zol 96 RNA kit  
346 (Zymo Research #R2056) or the Direct-zol RNA Miniprep kit (Zymo Research #R2050). qRT-PCR was  
347 performed either directly on the inactivated supernatants or on extracted RNA using the Luna Universal One-  
348 Step RT-qPCR Kit (NEB #E3005E) in a QuantStudio 6 thermocycler (Applied Biosystems) or in a StepOne Plus  
349 thermocycler (Applied Biosystems). Primers used are described in **Table S4**. Cycling conditions were the  
350 following: 10 min at 55°C, 1 min at 95°C and 40 cycles of 95°C for 10s and 60°C for 1 min. Results are  
351 expressed as PFU equivalents/mL as the standard curve was performed by diluting RNA extracted from a viral  
352 stock with a known titer. For plaque assay titration, VeroE6 cells were seeded in 24-well plates ( $10^5$  cells per  
353 well) and infected with serial dilutions of infectious supernatant diluted in DMEM during 1h at 37°C. After  
354 infection, 0.1% agarose semi-solid overlays were added. At 72h post-infection, cells were fixed with Formalin 4%  
355 (Sigma #HT501128-4L) and plaques were visualized using crystal violet coloration.

356  
357 **Overexpression assay.**  $2 \times 10^5$  293T-ACE2 cells were seeded in a 24-well plate 18h before experiment. Cells  
358 were transfected with 500 ng of plasmids expressing HA-DAXX WT, HA-DAXX 15KR and HA-DAXX $\Delta$ SIM  
359 plasmids, using Fugene 6 (Promega # E2691), following the manufacturer's instructions. HA-NRB1 was used as  
360 negative control. After 24h cells were infected at the indicated MOI in DMEM 2% FBS. When indicated, cells  
361 were treated with 10 mM of remdesivir (MedChemExpress #HY-104077) at the time of infection. For flow  
362 cytometry analysis, cells were fixed with 4% formaldehyde and permeabilized in a PBS 1% BSA 0.025% saponin  
363 solution for 30 min prior to staining with corresponding antibodies for 1h at 4°C diluted in the permeabilization  
364 solution. Samples were acquired on a BD LSR Fortessa and analyzed using FlowJo. Total RNA was extracted  
365 using a RNeasy Mini kit and submitted to DNase treatment (Qiagen). RNA concentration and purity were  
366 evaluated by spectrophotometry (NanoDrop 2000c, ThermoFisher). In addition, 500 ng of RNA were reverse  
367 transcribed with both oligo dT and random primers, using a PrimeScript RT Reagent Kit (Takara Bio) in a 10 mL  
368 reaction. Real-time PCR reactions were performed in duplicate using Takyon ROX SYBR MasterMix blue dTTP  
369 (Eurogentec) on an Applied Biosystems QuantStudio 5 (ThermoFisher). Transcripts were quantified using the  
370 following program: 3 min at 95°C followed by 35 cycles of 15s at 95°C, 20s at 60°C, and 20s at 72°C. Values for  
371 each transcript were normalized to expression levels of RPL13A. The primers used are indicated in **Table S4**.

372  
373 **Western blot.** Cell lysates were prepared using RIPA lysis and extraction buffer (ThermoFisher #89901). Protein  
374 concentration was determined using Bradford quantification. Proteins were denaturated using 4X Bolt LDS  
375 Sample Buffer (Invitrogen) and 10X Bolt Sample Reducing Agent (Invitrogen). 40  $\mu$ g of proteins were separated  
376 on Bolt 4-12% Bis-Tris Mini Protein Gels (Invitrogen) and transferred on membranes using the iBlot Transfer  
377 Stack PVDF mini (Invitrogen) and an iBlot Dry Blotting System (Invitrogen). Membranes were blocked with 5%  
378 BSA in PBS (blocking buffer) and incubated with primary antibodies diluted in blocking buffer. Membranes were  
379 washed and incubated with secondary antibodies diluted in blocking buffer. SuperSignal West Pico PLUS



380 Chemiluminescent Substrate (ThermoFisher #34579) was added on the membranes and pictures were taken on  
381 a myECL Imager (ThermoFisher).  
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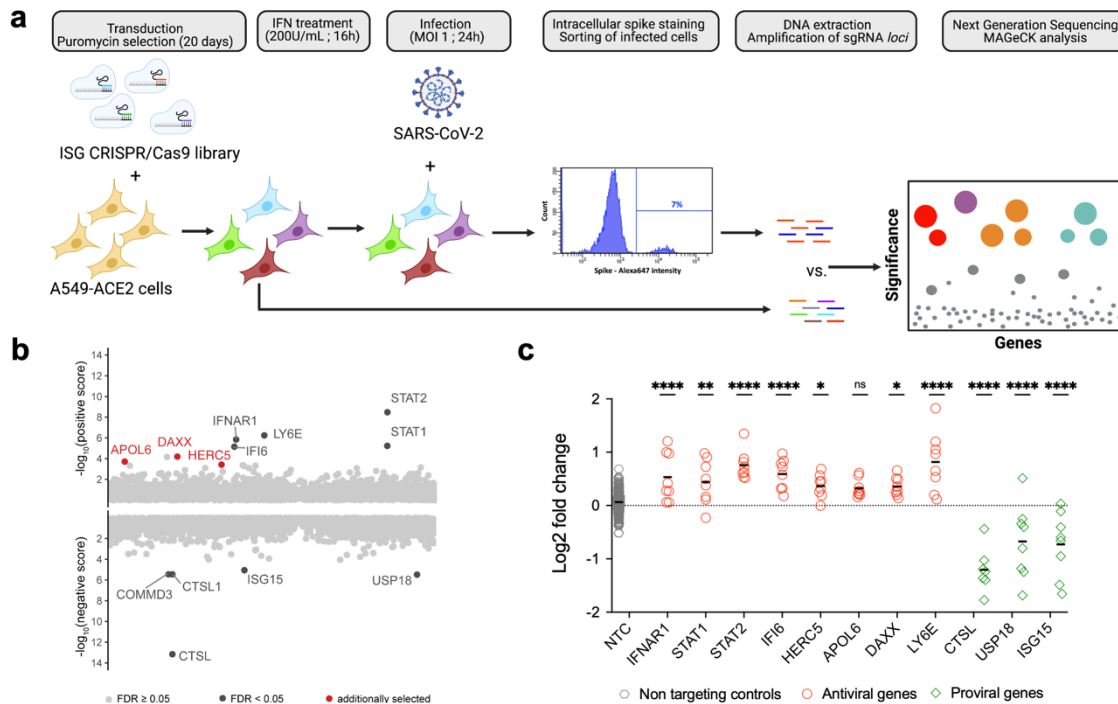
383 **Microscopy Immunolabeling and Imaging.** 293T-ACE2 cells were cultured and infected with SARS-CoV-2 as  
384 described above. When indicated, cells were treated with 50 mg/mL of GRL-0617 (MedChemExpress #HY-  
385 117043), a specific inhibitor of SARS-CoV-2 PLpro (53), at the time of infection.  
386 Cultures were rinsed with PBS and fixed with 4% paraformaldehyde (electronic microscopy grade; Alfa Aesar) in  
387 PBS for 10 min at room temperature, treated with 50 mM NH<sub>4</sub>Cl for 10 min, permeabilized with 0.5% Triton X-  
388 100 for 15 min, and blocked with 0.3% BSA for 10 min. Cells were incubated with primary and secondary  
389 antibodies for 1h and 30 min, respectively, in a moist chamber. Nuclei were labeled with Hoechst dye (Molecular  
390 Probes). Images were acquired using a LSM700 (Zeiss) confocal microscope equipped with a 63X objective or  
391 by Airyscan LSM800 (Zeiss). Image analysis was performed using ImageJ.  
392

393 **Single-cell RNAseq analysis.** Single cell RNAseq analysis were performed in the BioTuring Browser Software  
394 (v2.8.42) developed by BioTuring, using a dataset made available by Liao *et al.* (42) (ID: GSE145926). All  
395 processing steps were done by BioTuring Browser (62). Cells with less than 200 genes and mitochondrial genes  
396 higher than 10% were excluded from the analysis.  
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398 **Statistical analysis.** GraphPad Prism was used for statistical analyses. Linear models were computed using  
399 Rstudio.  
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## Figures.



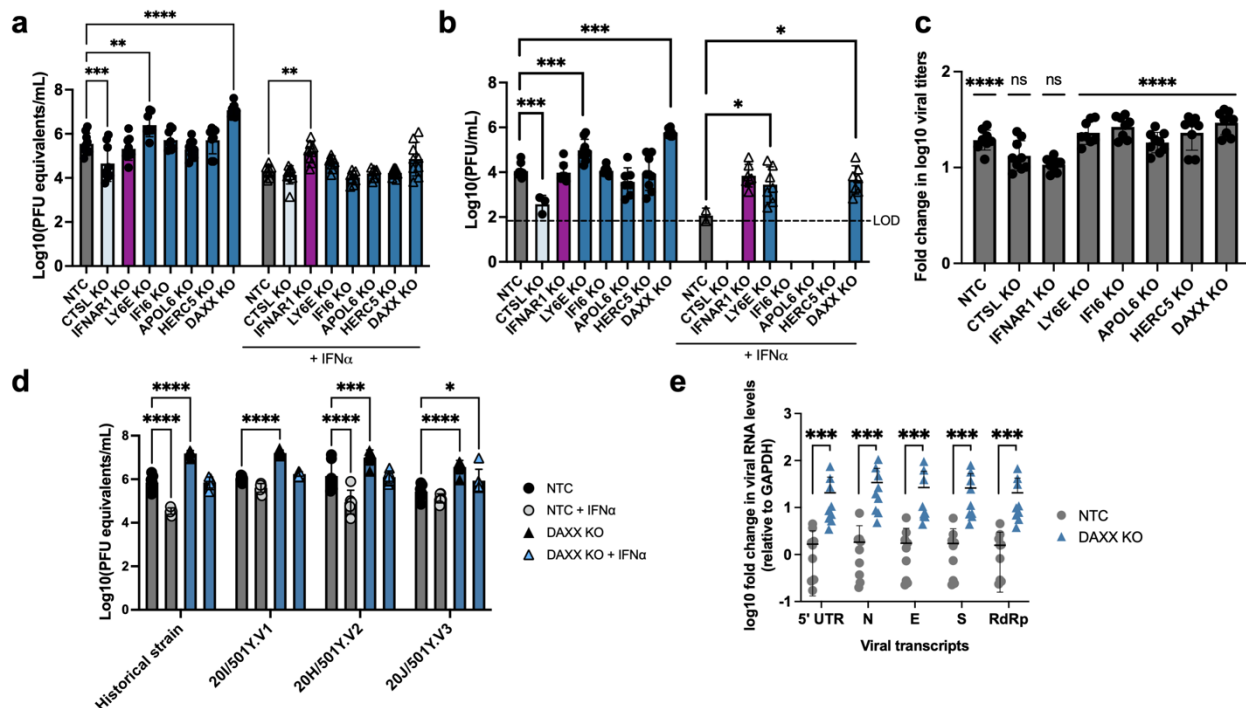
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405 **Figure 1: ISG-focused CRISPR/Cas9 screening approach to identify restriction factors for**  
 406 **SARS-CoV-2. a: CRISPR/Cas9 screen outline.** A549-ACE2 cells were transduced with lentivectors  
 407 encoding the ISG CRISPR/Cas9 library and selected by puromycin treatment for 20 days. Library cells  
 408 were then pre-treated with 200 U/mL of IFN $\alpha$  for 16 hours, and infection with SARS-CoV-2 at MOI 1.  
 409 24 hours post infection, infected cells were fixed with formalin treatment, permeabilized by saponin  
 410 treatment and stained with a monoclonal anti-spike antibody. After secondary staining, infected cells  
 411 were sorted and harvested. Non-infected, non-IFN $\alpha$  treated cells were harvested as a control. DNA  
 412 was extracted from both cellular fractions and sgRNA *loci* amplification was carried out by PCR.  
 413 Following NGS, bio-informatic analysis using the MAGeCK package was conducted. **b: Screen**  
 414 **results.** By taking into account the enrichment ratios of each of the 8 different sgRNAs for every gene,  
 415 the MAGeCK analysis provides a modified robust rank aggregation ( $\alpha$ -RRA) score, with further one-  
 416 sided significance testing. A positive score is assigned to KOs enriched in infected cells (*i.e.* restriction  
 417 factor, represented in the top fraction of the graph) and a negative score is assigned to KOs depleted  
 418 in infected cells (*i.e.* proviral factors, represented in the bottom portion of the graph). Gene with an  
 419 FDR < 0.05 are represented in black. 3 genes with a FDR > 0.05, but with a p-value < 0.005 were  
 420 additionally selected and are represented in red. **c: Individual sgRNA enrichment.** For the indicated  
 421 genes, the enrichment ratio of the 8 sgRNAs present in the library was calculated as the MAGeCK  
 422 normalized read counts in infected cells divided by those in the original pool of cells and is  
 423 represented in log<sub>2</sub> fold change. As a control, the enrichment ratios of the 200 non-targeting control  
 424 sgRNAs (NTCs) are also represented, merged together in one NTC for visualization purposes only.  
 425 Statistics: one-way ANOVA, ns = p-value > 0.05, \* = p-value < 0.05, \*\* = p-value < 0.01, \*\*\*\* = p-value  
 426 < 0.0001.  
 427

428 **Table 1: Gene editing efficiency.**  
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<b>Gene</b>	<b>% of alleles edited</b>
<b>LY6E</b>	97
<b>DAXX</b>	82
<b>APOL6</b>	99
<b>HERC5</b>	97
<b>CTSL</b>	87
<b>IFI6</b>	83
<b>IFNAR1</b>	79

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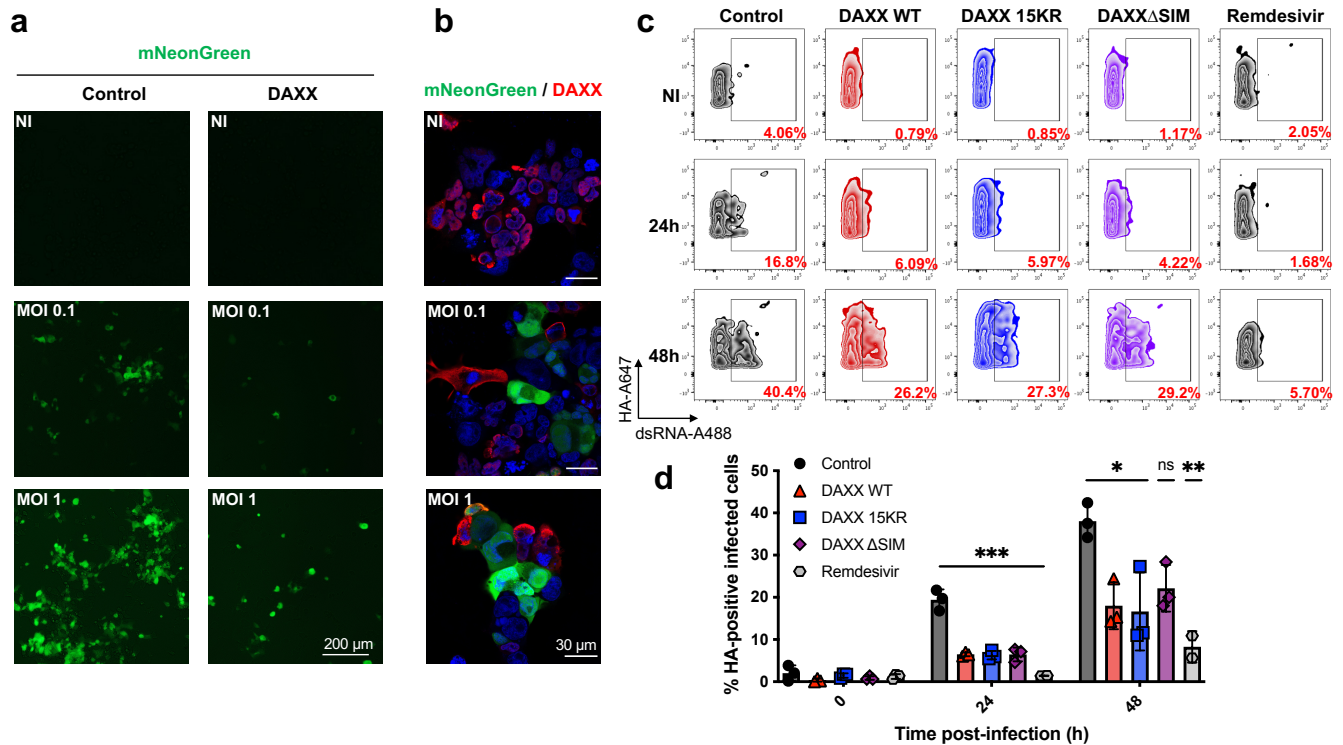
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**Figure 2: DAXX is a restriction factor for SARS-CoV-2. A-C: Antiviral activity of ISGs against SARS-CoV-2.** A549-ACE2 knocked-out for the indicated genes were generated using a multi-guide approach, leading to pools of KO cells with a high frequency of indels. KO cells were pre-treated with 0 (circles) or 200 (triangles) U/mL of IFN $\alpha$  24h prior to triplicate infection with SARS-CoV-2 (MOI 0.1). Supernatants were harvested at 72h post infection. The mean of three independent experiments, with infections carried out in triplicate, is shown. **a:** For the titration of RNA levels, supernatants were heat inactivated prior to quantification by qRT-PCR. Serial dilutions of a stock of known infectious titer was used as a standard (PFU equivalents/mL). Statistics: 2-way ANOVA, \* = p-value < 0.05, \*\* = p-value < 0.01, \*\*\* = p-value < 0.001, \*\*\*\* = p-value < 0.0001. **b:** For the titration of infectious virus levels by plaque assay, supernatants were serially diluted and used to infect VeroE6 cells. Plaques formed after 3 days of infection were quantified using crystal violet coloration. Statistics: Dunnett's test on a linear model, \* p-value < 0.05, \*\* p-value < 0.01, \*\*\* p-value < 0.001. **c:** For each of the indicated KO, the data shown in A is represented as fold change in log<sub>10</sub> titers (*i.e.* the triplicate log<sub>10</sub> titers of the non-treated condition divided by the mean of the triplicate log<sub>10</sub> titers IFN $\alpha$ -treated condition, n=3). Statistics: 2-way ANOVA, ns = p-value > 0.05, \*\*\*\* = p-value < 0.001. **d:** A549-ACE2 WT or DAXX KO cells were infected in triplicates at an MOI of 0.1 with the following SARS-CoV-2 strains: BetaCoV/France/IDF0372/2020 (historical strain) ; hCoV-19/France/IDF-IPP11324/2020 (20I/501Y.V1, sometimes referred to as United Kingdom or B.1.1.7) ; hCoV-19/France/PDL-IPP01065/2021 (20H/501Y.V2, sometimes referred to as South Africa or B.1.351) ; hCoV-19/Japan/TY7-501/2021 (20J/501Y.V3, sometimes referred to as Brazil or P.1). Supernatants were harvested at 72h post infection. Supernatants were heat inactivated prior to quantification by qRT-PCR. Serial dilutions of a stock of known infectious titer was used as a standard (PFU equivalents/mL). The mean of two independent experiments, with infections carried out in triplicate, is shown. Statistics: 2-way ANOVA, \* = p-value < 0.05, \*\*\* = p-value < 0.001, \*\*\*\* = p-value < 0.0001. **e:** A549-ACE2 WT or DAXX KO were infected in triplicates with SARS-CoV-2 at a MOI of 0.1. After 72h of infection, cell monolayers were harvested and cellular RNAs were extracted. The levels of each of the indicated viral transcripts were quantified by qRT-PCR and normalized to GAPDH levels. Fold change in DAXX KO cells compared to the average of control cells is represented. 3 independent experiments are shown and taken into account as fixed effects in a linear model. Statistics: Dunnett's test on a linear model, \* p-value < 0.05, \*\* p-value < 0.01, \*\*\* p-value < 0.001.

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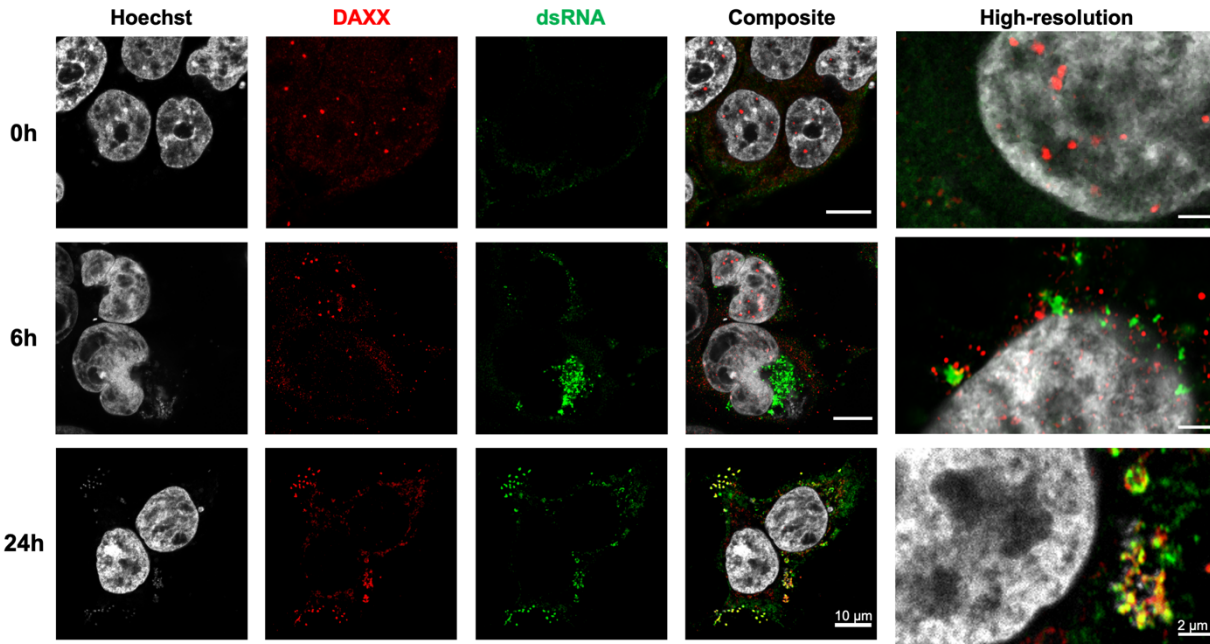
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**Figure 3: DAXX restriction of SARS-CoV-2 is SUMOylation independent. A-B: DAXX overexpression restricts SARS-CoV-2.** 293T-ACE2 cells were transfected with DAXX WT. 24h after transfection, cells were infected with the mNeonGreen fluorescent reporter SARS-CoV-2 at the indicated MOI. Cells were either visualized with an EVOS fluorescence microscope (a) or stained with an HA-antibody detecting DAXX and imaged by confocal microscopy (b). Scale bars correspond to 200  $\mu$ m (a) and 30  $\mu$ m (b). **c-d: DAXX mutants are still able to restrict SARS-CoV-2.** 293T-ACE2 cells were transfected with HA-DAXX WT ; H-ADAXX 15KR ; HA-DAXX $\Delta$ SIM ; or with HA-NRB1 as negative control plasmid. 24h after transfection, cells were infected with SARS-CoV-2 at an MOI of 0.1. When indicated, cells were treated with remdesivir at the time of infection. After 24 or 48h, infected cells were double-stained recognizing dsRNA (to read out infection) and HA (to read out transfection efficiency) and acquired by flow cytometry. The percentage of infected cells among HA-positive (transfected) cells for one representative experiments is shown in c, for the mean of 3 independent experiments in d. Statistics: one-way ANOVA Holm corrected, ns = p-value > 0.05, \* = p-value < 0.05, \*\* = p-value < 0.01, \*\*\* = p-value < 0.001.

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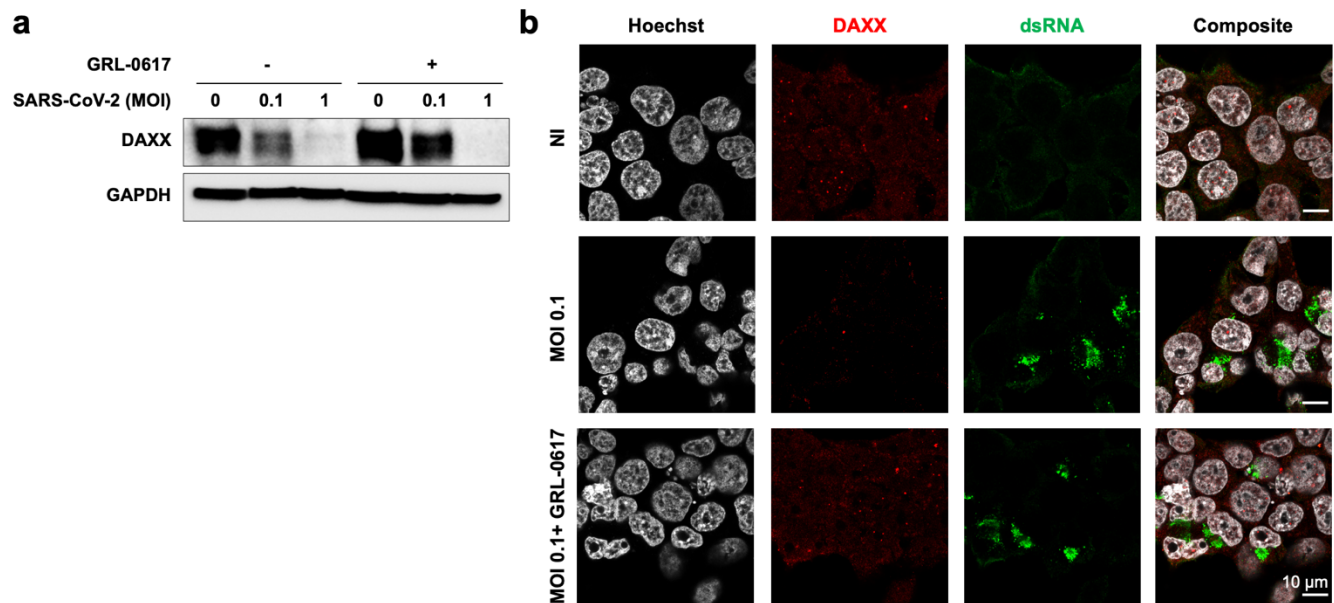
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**Figure 4: SARS-CoV-2 infection induces DAXX cytoplasmic re-localization to sites of viral replication.** 293T-ACE2 cells were infected with SARS-CoV-2 at the indicated MOI 1. 24h post-infection, cells were labelled with Hoescht and with antibodies against dsRNA (detecting viral RNA, in green) and HA (detecting DAXX, in red). When indicated, the high-resolution Airyscan mode was used. Scale bars correspond to 10 μm for confocal images, and 2 μm for the high-resolution images.



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**Figure 5: SARS-CoV-2 antagonizes DAXX restriction. a: DAXX degradation after infection.** 293T-ACE2 cells were infected with SARS-CoV-2 at the indicated MOI. After 24h, cells were harvested and levels of DAXX and GAPDH were analyzed by Western Blot. When indicated, cells were treated with the viral protease inhibitor GRL-0617 at the time of infection. **b: GRL-0617 treatment partially reverses DAXX re-localization and expression.** 293T-ACE2 cells were infected with SARS-CoV-2 at the indicated MOI 0.1. 24h post-infection, cells were labelled with Hoescht and with antibodies against dsRNA (detecting viral RNA, in green) and HA (detecting DAXX, in red). When indicated, cells were treated with the viral protease inhibitor GRL-0617 at the time of infection. Scale bars correspond to 10  $\mu$ m.

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508  
509 **Contributions.** F.R. designed the research project. F.R. and M.V. secured the funding for the study.  
510 A.M.K., S.M.A., A.H., N.A., S.N., G.M., D.Q.T., M.C., T.V. and F.R. performed and analyzed the *in*  
511 *vitro* experiments. F.P. produced the stocks of lentivirus. J.C.S., J.O. and K.H. generated and  
512 validated KO cell lines. T.B. performed the single-cell RNAseq data analysis. A.B. and E.S.L.  
513 performed the bio-informatic analyses of the CRISPR/Cas9 screen. M.O., T.B., O.S., N.J., S.N., and  
514 M.V. analyzed the data and supervised the project. A.M.K. and F.R. wrote the manuscript. All authors  
515 edited the manuscript.

516  
517 **Competing Interests:** J.C.S., J.O. and K.H. are employees and shareholders from Synthego  
518 Corporation.

519  
520 **Correspondence** and requests for materials should be addressed to M.V. or F.R.

521  
522 **Data availability:** Raw NGS data was deposited to the NCBI GEO portal and is accessible with the  
523 number GSE173418.

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