Research Article

Porcine antiviral activity is increased by CRISPRa-SAM system

Jinhe Jiang1, Yumei Sun1, Rong Xiao2, Kai Wai1, Muhammad Jamil Ahmad1, Faheem Ahmed Khan3, Hongbo Zhou2, Zhiyong Li4, Yong Zhang5, Ao Zhou6,7 and Shujun Zhang1

1Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction Ministry of Education, Huazhong Agricultural University, Wuhan 430070, Hubei, People’s Republic of China; 2State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, Hubei, People’s Republic of China; 3The Center for Biomedical Research, Key Laboratory of Organ Transplantation, Ministry of Education, Ministry of Health, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430070, Hubei, People’s Republic of China; 4State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu, People’s Republic of China; 5Department of Veterinary Medicine, Gansu Agricultural University, Lanzhou, People’s Republic of China; 6Department of Medical Cell Biology and Genetics, Southwest Medical University, Luzhou, Sichuan, People’s Republic of China; 7Wellcome Genome Campus, Wellcome Sanger Institute, Hinxton, Cambridgeshire, United Kingdom

Correspondence: Ao Zhou (zhouao2008@aliyun.com) or Shunjun Zhang (sjxiaozhang@mail.hzau.edu.cn)

Clustered Regularly Interspaced Short Palindromic Repeat activation-synergistic activation mediator system (CRISPRa-SAM) has been efficiently used to up-regulate the targeted genes in human and mouse. But it is not known whether the CRISPRa-SAM system can be used against porcine disease because its two important transcriptional activation domains (P65 and heat shock transcription factor 1 (HSF1)) are from mouse and human, respectively. Pig is one of the most important meat sources, porcine viral infectious diseases cause massive economic losses to the swine industry and threaten the public health. We aimed to investigate whether the CRISPRa-SAM system could increase porcine antiviral activity by mediating two pig-specific target genes (Mx2 and β1,4 N-acetylgalactosaminyltransferase (B4galnt2)). First, we constructed PK-15 and IPEC-J2 cell lines expressing nuclease-deficient Cas9 (dCas9)-vp64 and MS2-P65-HSF1 stably. Next, in these two cell models, we activated Mx2 and B4galnt2 expression through CRISPRa-SAM system. Antiviral activity to PRV or H9N2 was improved in PK-15 cells where Mx2 or B4galnt2 was activated. Altogether, our results demonstrated the potential of CRISPRa-SAM system as a powerful tool for activating pig genes and improving porcine antiviral activity.

Introduction

Pig is one of the most important meat producing animals. Porcine viral infectious diseases cause massive economic losses to the swine industry. Enhancing the antiviral ability of pigs has been a keen interest for researchers. Zhao et al. (2017) reported to reduce the fat deposition, and increase lean meat production in porcine, through reconstitution of UCP1 using CRISPR/Cas9 [1]. Burkard et al. (2017) used CRISPR/Cas9 to produce pigs completely resistant to porcine reproductive and respiratory syndrome virus (PRRSV) by removing the CD163 subdomain 5 [2]. Nonetheless the Clustered Regularly Interspaced Short Palindromic Repeat activation-synergistic activation mediator system (CRISPRa-SAM) system needs to be explored for pig antiviral breeding. This system is an extension of the conventional CRISPR knockout system. Instead of wild-type Cas9, designed variants of a nuclease-deficient Cas9 (dCas9) carrying the D10A and H840A mutations are used in the CRISPRa-SAM system. The dCas9 is fused to herpes simplex virus (HSV) viral protein 16 (VP16) activation domains (known as VP64) and the system includes some synergistic activation mediators. These mediators comprise fusion proteins (MS2-guide RNA (gRNA), dCas9-VP64 and the MS2-VP65-HSF1), MS2 bacteriophage coat protein fused to the NF-κB (P65) and heat shock transcription factor 1 (HSF1).
Table 1 gRNA sequences of two pig genes (Mx2, B4galnt2)

| Target gene | Number | Guide sequence       | TSS distance |
|-------------|--------|----------------------|--------------|
| Mx2         | 1      | CTGGAAGGGAGGTACACCA   | −61          |
|             | 2      | TCGGGAAGGGAGCACATTCC  | +170         |
|             | 3      | GACGAGCCATAGTGGGTGCC  | −1           |
|             | 4      | ATTTTGATGGTCTTCT      | −112         |
| B4galnt2    | 1      | CGTGGGGGGGACATTGTC    | −87          |
|             | 2      | CAGTCCCGGCTTACGGC     | −202         |
|             | 3      | GGAGAGGCCGAACCGC      | −137         |
|             | 4      | GCGTCCGAGTTGGAACG     | −428         |

[3–5]. It is widely used to up-regulate genes in human cells [6–8], rats [9,10] and fruit flies [11]. The CRISPRa-SAM system's activation domains P65 and HSF1 are from mouse and human, so it is required to reveal the role of CRISPRa-SAM as a gene regulator in pig cells for porcine antiviral activity.

Myxovirus resistance proteins (Mx) are a family of dynamin-like GTPases, and can inhibit influenza A virus. In mammals, Mx gene has two isoforms, Mx1 and Mx2. Mx1 can cause hindrance in replication of RNA and DNA viruses [12]. Mx2 has a tendency to affect the anti-human immunodeficiency virus type 1 (HIV-1) activity of type I IFN [13,14]. Meanwhile, Mx2 can inhibit PRRSV infection in pig [15] and herpes virus infection [16]. Glycosylation is one of the most important post-translational modifications of proteins in eukaryotic cells. In the Golgi, terminal glycosylation reactions generate a huge panel of glycan that confer a variety of structural and functional roles to the glycoproteins exposed at the cell surface. β1,4 N-acetylgalactosaminyltransferase (B4GALNT2) catalyzes the last step in the biosynthesis of the human Sd(a) antigen through the addition of an N-acetylgalactosamine residue via a β-1,4 linkage to a subterminal galactose residue substituted with an α-2,3-linked sialic acid [17,18]. Recently, a study suggested that B4GALNT2 overexpression can prevent the infection of every avian influenza virus strain [19]. So we chose these two pig genes for the present study.

In the present study, we evaluated the ability of the CRISPRa-SAM activation systems to activate Mx2 and B4galnt2 gene expression in two kinds of porcine cell lines (PK-15, IPEC-J2). We observed PK-15 cells had more antiviral activity to PRV or H9N2 when Mx2 or B4galnt2 expression was activated. The present study highlights that biotechnology has great potential to manipulate pig breeding for improving porcine antiviral activity.

**Materials and methods**

**Plasmids construction**

The location of the transcription start site (TSS) of sgRNA is very important to cause activation of genes. The design of the sgRNA was based on the principle around the upstream of the target gene TSS followed the GN (19) NGG. Four sgRNAs of each gene were designed and synthesized, named as sgRNA1, sgRNA2, sgRNA3 and sgRNA4, respectively (Figure 2). The sgRNAs used in the present study were designed by http://crispr.mit.edu/, and are listed in Table 1. Oligomers were synthesized for all sgRNA sequences, annealed and cloned into lenti-sgRNA (MS2)-pure backbone (#73795) using BbsI digestion. Sequencing for all plasmids was done before use.

**Cell culture**

PK-15 cells (pig kidney cell line), IPEC-J2 cells (pig intestinal epithelial cell line) and HEK293T cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) and were cultured in an incubator at 37°C with 5% CO2.

**Generation of PK-15/J2-CRISPRa-SAM stable cell line and transfection**

HEK293T cells were used for generating lentiviruses expressing dCas9-VP64 and MS2-P65-HSF1 by co-transfection of the packaging plasmids psPAX.2, pMD2.G and lentidcas9-VP64 blast (#61425) or lentiMPHv2 (#89308) using Neofect transfection reagent (Neofect Biotech, China). Supernatant containing virus was harvested at 48 and 72 h post transfection. PK-15 or IPEC-J2 cells were exposed to virus with polybrene (8 μg/ml; Sigma–Aldrich) and selection was done using blasticidin (6 μg/ml; InvivoGen, Thermo Fisher) and hygromycin (300 μg/ml; Sangon Biotech). Expression of SAM components was tested using RT-qPCR. The cell lines successfully expressing the SAM components were named ‘PK15-CRISPRa-SAM and J2-CRISPRa-SAM’, respectively.

PK15-CRISPRa-SAM cells or J2-CRISPRa-SAM cells were seeded into 12-well plates for transfection. The cells were exposed to lentiviruses loaded Mx2 or B4galnt2 sgRNAs with polybrene (8 μg/ml; Sigma–Aldrich). After 48
h of infection, the cells were selected using puromycin (3 μg/ml; Gibco, Thermo Fisher) for at least 14 days while replacing the puromycin every 3 days.

**Quantitative RT-PCR analysis**

Total RNA kit I (Omega, U.S.A.) was used for total RNA collection following the manufacturer's instructions. Total RNA in equal volumes were reverse transcribed for cDNA using RevertAid First-Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Baltic, U.S.A.). Real-time PCR was performed (CFX96 Real-Time System; Bio-Rad) for all the samples in duplicate and data were normalized to β-actin. Thermal cycling conditions were 10 min at 95°C and 40 cycles of 10 s at 95°C, 30 s at 60°C and 10 s at 72°C. Mx2 forward primer is 5-CCGAGAAAGTTGTCCTGAATGTG-3, reverse primer is 5-TGCGGATGCGAGTGAAAGAAT-3. B4galnt2 forward primer is 5-GCGACTCCAAAGAATTGGCTTC-3, reverse primer is 5-TGGTGACCTATGATCACGTGTG-3. β-actin forward primer is 5- TGGCACAACCTTCTACA-3, reverse primer is 5-ATCTTCTCAGGGTGCTTGTG-3.

**Virus titration**

PK15-CRISPRa-SAM cells and PK-15 cells with activated (ten-fold) B4galnt2 gene were infected with A/chicken/Shanghai/SC197/2013 (SH13, H9N2) at an MOI (0.001 or 0.01). A/chicken/Shanghai/SC197/2013 (SH13, H9N2) was gifted by Professor Chenjun Li from Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences. Viral supernatants were harvested post-infection at the indicated time points for hemagglutination assay to titrate virus titer as described previously [20].

**Virus infection**

PK15-CRISPRa-SAM cells and PK-15 cells with activated (600-fold) Mx2 gene were infected with PRV BAC (pBecker2) at an MOI (100 or 10) for 24 h. pBecker2 was gifted by Professor Hanzhong Wang from Wuhan institute of Virology, Chinese Academy of Sciences. Fluorescence microscopy (Carl Zeiss, LSM 800) was used to observe under a 20× objective.

**Statistical analysis**

All the experiments were run three times, and results were documented as means ± SEM. The qPCR results were analyzed by two-way ANOVA in GraphPad Prism 6.01, while other results were analyzed by Student’s t test in GraphPad Prism 6.01. Statistical significance was defined by a P-value of less than 0.05.

**Results**

The transcriptional activation domains of pig P65 and HSF1 genes were highly homologous with that of P65, HSF1 of the CRISPRa-SAM system

First, we checked whether the transcriptional activation domains of pig (P65, HSF1) are homologous to the activation domains (P65, HSF1) of the CRISPRa-SAM system. Results computed by NCBI blast showed pig P65 (FN999988.1) and HSF1 (XM_005655310.3) were highly homologous to that of CRISPRa-SAM system. The identity and gap for HSF1 in pig were 89.37% (311/348) and 6.45% (24/372), respectively (Figure 1), while those of P65 were 71.03% (385/542) and 2.34% (13/555), respectively (Figure 1). It is worth mentioning that the efficacy of CRISPRa-SAM system has not been validated in pigs before.

The important components of CRISPRa-SAM system efficiently expressed in two pig cell lines

We checked the expression till day 14 post selection, for important components of CRISPRa-SAM system by infecting PK-15 and IPEC-J2 cells with lentiviruses expressing dCas9-VP64 and MS2-P65-HSF1, respectively. The result showed that dCas9-VP64 and MS2-P65-HSF1 were stably and effectively expressed in two pig cell lines. Ct values of dCas9-VP64 and MS2-P65-HSF1 were 22.14 + 0.79 and 21.65 + 0.11, respectively in the PK15-CRISPRa-SAM cell line, meanwhile Ct values of dCas9-VP64 and MS2-P65-HSF1 were 21.44 + 0.01 and 21.24 + 0.05 in the J2-CRISPRa-SAM cell line, respectively (Tables 2 and 3).
Figure 1. Comparison of transcriptional activation domains sequences in CRISPRa-SAM system and pig gene sequences (A) Comparison of p65 sequences in CRISPRa-SAM system and pig p65 gene. (B) Comparison of HSF1 sequences in CRISPRa-SAM system and pig HSF1 gene. The lower sequences were from pig HSF1 and p65, and the upper sequences were from human HSF1 and murine p65 as transcriptional activation domains in CRISPRa-SAM system.

Table 2 Expression of SAM components in the PK15-CRISPRa-SAM cell line

| Gene       | PK-15 C\textsubscript{T} values | PK15-CRISPRa-SAM C\textsubscript{T} values | P-value |
|------------|---------------------------------|--------------------------------------------|---------|
| dCas9-vp64 | 28.94 ± 0.63                    | 22.14 ± 0.79                               | >0.0001 |
| Ms2-p65-HSF1 | +35                             | 21.65 ± 0.11                               | >0.0001 |
| β-actin    | 18.67 ± 0.18                    | 19.37 ± 0.11                               | 0.04    |

Table 3 Expression of SAM components in the J2-CRISPRa-SAM cell line

| Gene       | IPEC-J2 C\textsubscript{T} values | J2-CRISPRa-SAM C\textsubscript{T} values | P-value |
|------------|---------------------------------|--------------------------------------------|---------|
| dCas9-vp64 | 28.55 ± 0.49                    | 21.44 ± 0.01                               | >0.0001 |
| Ms2-p65-HSF1 | +35                             | 21.24 ± 0.05                               | >0.0001 |
| β-actin    | 18.31 ± 0.19                    | 18.12 ± 0.00                               | 0.14    |

Figure 2. The strategy of design the sgRNA of target pig gene

Single-gRNA (sgRNA) scaffold was under the control of a U6 promoter. The sgRNAs (sgRNA1, sgRNA2, sgRNA3 and sgRNA4) were located to the upstream of TSS of the target gene.

CRISPRa-SAM system could effectively activate transcription in different pig cell lines with different efficiencies

We designed the gRNAs of Mx2 and B4galnt2 through the website http://crispr.mit.edu/. These gRNAs are listed in Table 1. After the lentivirus expressing gRNAs of Mx2 or B4galnt2 infected PK15-CRISPRa-SAM cell line or J2-CRISPRa-SAM cell line, we checked the activation of pig genes (Mx2 and B4galnt2) expression using CRISPRa-SAM system in two different pig cell lines (PK-15 and IPEC-J2). The results showed up-regulation of Mx2 and B4galnt2 genes in both (PK-15 and IPEC-J2) pig cell lines (Figures 3 and 4). Up-regulation of activated Mx2 and B4galnt2 genes were validated by qPCR (Figure 4).
Figure 3. Activation of target pig gene in pig PK15 cells by CRISPRa-SAM system
(A) Related mRNA expression of pig B4galnt2. (B) Related mRNA expression of pig Mx2. Lentivirus expressing gRNA of target pig gene infected PK15-CRISPRa-SAM cells, the cells were selected with 3 μg/ml puromycin for 14 days. Related mRNA expression of target genes were detected by RT-qPCR. Lentivirus expressing gRNA of no target gene was used to as the control, PK15-CRISPRa-SAM cells were mock. The data were represented as the mean ± SEM (n=3). Statistically significant differences were determined by two-way ANOVA, ns > 0.05, *P<0.05, **P<0.01.

Figure 4. Activation of target pig gene in pig IPEC-J2 cells by CRISPRa-SAM system
(A) Related mRNA expression of pig B4galnt2. (B) Related mRNA expression of pig Mx2. Lentivirus expressing gRNA of target pig gene infected IPEC-J2-CRISPRa-SAM cells, the cells were selected with 3 μg/ml puromycin for 14 days. Related mRNA expression of target genes were detected by RT-qPCR. Lentivirus expressing gRNA of no target gene was used as the control, IPEC-J2-CRISPRa-SAM cells were mock. The data were represented as the mean ± SEM (n=3). Statistically significant differences were determined by two-way ANOVA, ns > 0.05, *P<0.05, **P<0.01.

was 600- and 10-fold in pig cells PK-15 and IPEC-J2, respectively, while activated B4galnt2 gene was up-regulated by 10-fold in PK-15 cell line and 7-fold in IPEC-J2 cell line (Figures 3 and 4).

Antiviral activity was increased in PK-15 cells
Mx2 gene has broad-spectrum antiviral effect, hence the overexpression of Mx2 could inhibit DNA and RNA viruses. To check this hypothesis, up-regulation of pig genes mediated by the CRISPRa-SAM system in pig cells could improve their normal functions, we tested the Mx2 expression level with relation to its antiviral activity against Pseudorabies virus (PRV) (MOI = 100 or 10) by infecting PK-15 cells activated Mx2 approximately 600-fold higher by CRISPRa-SAM system and PK-15 cells. Results shown in Figure 5A illustrate that PRV replication was inhibited in PK15 cells after overactivation of Mx2.

For further investigations, antiviral activity of B4galnt2 against pan-avian influenza virus infection [19] was checked for any increase by infecting the PK-15 cells activated B4galnt2 approximately ten-fold higher by the CRISPRa-SAM system and PK-15 cells with H9N2 virus. The H9N2 virus is widely circulating in the world, causing rasorial infections and serving as a gene donor for H5N1, H7N9 and H10N8 viruses [21]. The results showed that the virus titer of the H9N2 virus produced by PK-15 cells activated B4galnt2 was lower than the control (without
Activated Mx2 and B4galnt2 improved their antivirus functions

(A) PK15-CRISPrα-SAM cells (control) or PK-15 cells in which Mx2 gene was activated with 600-fold were infected with PRV-GFP at an MOI of 100 or 10, 24 h after infection, fluorescence microscopy microscopy was used to observe them (200 ×). (B) PK15-CRISPRα-SAM cells (Control) or PK-15 cells in which B4galnt2 gene was activated by ten-fold were infected with H9N2 virus at an MOI of 0.01 and 0.001. Viral supernatants were harvested at the indicated time points post infection and hemagglutination assay to titrate virus titer. Statistically significant differences were determined by Student’s t test, *P < 0.05.

Discussion

There are many advantages of the CRISPRα-SAM system over traditional overexpression gene technology. Exogenous expression vectors are used to clone cDNA sequences for traditional gene overexpression that is hard to achieve when the target gene has a long sequence or is rich in GC contents.

The CRISPRα-SAM system is capable of activating the target gene with only an sgRNA regardless the size of target gene. The CRISPRα system has been shown to activate target genes in many species [6,9,22]. Gain-of-function screening using a pool of sgRNA libraries, has been a powerful and effective tool for target gene screening [3,5,19,23–25] while cDNA library overexpression still retains some problems [3,5,19,23–25].

In the present study, we investigated the ability of the CRISPRα-SAM system to activate endogenous pig gene expression for porcine antiviral activity. Our results suggested that the CRISPRα-SAM system can effectively activate the pig endogenous gene transcriptional expression in both pig PK-15 and IPEC-J2 cell lines with increased porcine antiviral activity. We concluded that the CRISPRα-SAM system could effectively activate transcription in different pig cell lines with different efficiencies. The effect of CRISPRα-SAM was higher in PK-15 cells with 600 fold up-regulation of sgRNA1 for Mx2, compared with ten-fold in the IPEC-J2 cell line (Figures 3 and 4), while B4galnt2 gene up-regulation was ten-fold in PK-15 cells vs seven-fold in IPEC-J2 cells. In a pool of four sgRNAs, the effect of sgRNA1 of Mx2 gene had significantly higher efficiency than three others in PK-15 cells and IPEC-J2 cells and
the effect of sgRNA2 and sgRNA1 of B4galnt2 were significantly higher than others in both the cells. The porcine antiviral activity to PRV or H9N2 was improved in PK-15 cells where Mx2 or B4galnt2 was activated.

Similarly, in human cell lines, the efficiency of CRISPRa system to activate different target genes varied with different sgRNA. Konermann et al. (2015) [3] reported fold activation of 12 different genes (VEGFA, HBGI, TERT, IL-1B, IL-1R2, ZFP42, MYC, LIN28A, SOX2, NANOG, KLF4, POU5F1) mediated by the CRISPRa-SAM system and plotted against the sgRNA location in 293FT cells. In another study, a panel of validated sgRNAs was used to target the promoters of both coding (TTN, RHOXY2, ASCL1, HBG1) and non-coding (MIAT, TUNA) genes in HEK293 cell, MOLM14 cell, K562 cell and HIIL60 cell, and found sgRNAs gave the highest activation in MOLM14 cell [24]. Conclusively, the efficiency of CRISPRa-SAM system is highly dependent on the cell type, target gene basal expression level and the location of gRNA. Porcine Mx2 and B4galnt2 were activated by CRISPRa-SAM system and porcine antiviral capacity was increased because target genes were activated in pig cells. CRISPRa-SAM system is a powerful tool to activate the expression of pig endogenous genes. The development of these pig cell models and results are not only valuable for the genome-wide screening of antiviral pig genes but also are the foundation for pig antiviral breeding with the CRISPRa-SAM system.

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Author Contribution
J.J. designed the experiment, did the expression and activation of pig genes. Y.S. performed the PRV infection part. R.X. performed the H9N2 infection part. K.W., M.J.A., F.A.K., A.Z. and S.Z. helped in the write-up and editing of manuscript. All authors statistically analyzed, discussed, critically revised the contents and approved the final manuscript.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Abbreviations
B4GALNT2, β1,4 N-acetylgalactosaminyltransferase; CRISPRa-SAM, Clustered Regularly Interspaced Short Palindromic Repeat activation-synergistic activation mediator system; dCas9, nuclease-deficient Cas9; gRNA, guide RNA; HSF1, heat shock transcription factor 1; IFN, interferon; MOI, multiplicity of infection; PRRSV, porcine reproductive and respiratory syndrome virus; TSS, transcription start site.

References
1 Zheng, Q., Lin, J., Huang, J., Zhang, H., Zhang, R., Zhang, X. et al. (2017) Reconstitution of UCP1 using CRISPR/Cas9 in the white adipose tissue of pigs decreases fat deposition and improves thermogenic capacity. Proc. Natl. Acad. Sci. U.S.A. 114, E9474–E9482, https://doi.org/10.1073/pnas.1707853114
2 Burkard, C., Lillico, S.G., Reid, E., Jackson, B., Mileham, A.J., Alt-All, T. et al. (2017) Precision engineering for PRRSV resistance in pigs: macrophages from genome edited pigs lacking CD163 SGR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function. PLoS Pathog. 13, e1006206, https://doi.org/10.1371/journal.ppat.1006206
3 Konermann, S., Brigham, M.D., Trevino, A.E., Joung, J., Abudayyeh, O.O., Barcena, C. et al. (2015) Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. Nature 517, 583–588, https://doi.org/10.1038/nature14136
4 Kampmann, M. (2018) CRISPRi and CRISPRa screens in mammalian cells for precision biology and medicine. ACS Chem. Biol. 13, 406–416, https://doi.org/10.1021/acschembio.7b00657
5 Joung, J., Konermann, S., Gootenberg, J.S., Abudayyeh, O.O., Platt, R.J., Brigham, M.D. et al. (2017) Genome-scale CRISPR-Cas9 knockdown and transcriptional activation screening. Nat. Protoc. 12, 828–863, https://doi.org/10.1038/nprot.2017.016
6 Maeder, M.L., Linder, S.J., Cascio, V.M., Fu, Y., Ho, H.O. and Joung, J.K. (2013) CRISPR RNA-guided activation of endogenous human genes. Nat. Methods 10, 977–979, https://doi.org/10.1038/nmeth.2598
7 Hilton, L.B., D’Apolito, A.M., Vockley, C.M., Thakore, P.I., Crawford, G.E., Reddy, T.E. et al. (2015) Epigenome editing by a CRISPRa-Cas9-based acetyltransferase activates genes from promoters and enhancers. Nat. Biotechnol. 33, 510–517, https://doi.org/10.1038/nbt.3199
8 Xiong, K., Zhou, Y., Blichfeld, K.A., Hyttel, P., Bolund, L., Freude, K.K. et al. (2017) RNA-guided activation of pluripotency genes in human fibroblasts. Cell. Reprogram. 19, 189–198, https://doi.org/10.1089/cell.2017.0006
9 Chavez, A., Tuttle, M., Pruitt, B.W., Ewen-Campen, B., Chari, R., Ter-Ovanesyan, D. et al. (2016) Comparison of Cas9 activators in multiple species. Nat. Methods 13, 563–567, https://doi.org/10.1038/nmeth.3871

10 Lundh, M., Plucinska, K., Taidor, M.S., Petersen, P.S.S. and Emanuelli, B. (2017) Bidirectional manipulation of gene expression in adipocytes using CRISPRa and siRNA. Mol. Metab. 6, 1313–1320, https://doi.org/10.1016/j.molmet.2017.07.001

11 Jia, Y., Xu, R.G., Ren, X., Ewen-Campen, B., Rajakumar, R., Zirin, J. et al. (2018) Next-generation CRISPR/Cas9 transcriptional activation in Drosophila using flySAM. PNAS 115, 4719–4724, https://doi.org/10.1073/pnas.1800677115

12 Busnadiego, I., Kane, M., Rihn, S.J., Preugschas, H.F., Hughes, J., Blanco-Melo, D. et al. (2014) Host and viral determinants of Mx2 antiretroviral activity. J. Virol. 88, 7738–7752, https://doi.org/10.1128/JVI.00214-14

13 Goujon, C., Moncorge, O., Bauby, H., Doyle, T., Ward, C.C., Schaller, T. et al. (2013) Human MX2 is an interferon-induced post-entry inhibitor of HIV-1 infection. Nature 502, 559–562, https://doi.org/10.1038/nature12542

14 Kane, M., Yadav, S.S., Bitzegeio, J., Kutluay, S.B., Zang, T., Wilson, S.J. et al. (2013) MX2 is an interferon-induced inhibitor of HIV-1 infection. Nature 502, 563–566, https://doi.org/10.1038/nature12653

15 Wang, H., Bai, J., Fan, B., Li, Y., Zhang, Q. and Jiang, P. (2016) The interferon-induced Mx2 inhibits porcine reproductive and respiratory syndrome virus replication. J. Interferon Cytokine Res. 36, 129–139

16 Schilling, M., Bulli, L., Weigang, S., Graf, L., Naumann, S., Patzina, C. et al. (2018) Human MxB protein is a pan-herpesvirus restriction factor. J. Virol. 92, e01056–18, https://doi.org/10.1128/JVI.01056-18

17 Byrne, G.W., Du, Z., Stalboerger, P., Kogelberg, H. and McGregor, C.G. (2014) Cloning and expression of porcine beta1,4 N-acetylgalactosaminyl transferase encoding a new xenoreactive antigen. Xenotransplantation 21, 543–554, https://doi.org/10.1111/xen.12124

18 Groux-Degroote, S., Schulz, C., Cogezi, V., Noel, M., Portier, L., Vicogne, D. et al. (2018) The extended cytoplasmic tail of the human B4GALNT2 is critical for its Golgi targeting and post-Golgi sorting. FEBS J. 285, 3442–3463, https://doi.org/10.1111/febs.14621

19 Heaton, B.E., Kennedy, E.M., Dumm, R.E., Harding, A.T., Sacco, M.T., Sachs, et al. (2017) A CRISPR activation screen identifies a pan-avian influenza virus inhibitory host factor. Cell Rep. 20, 1503–1512, https://doi.org/10.1016/j.celrep.2017.07.060

20 Esfeld, A.J., Neumann, G. and Kawooka, Y. (2014) Influenza A virus isolation, culture and identification. Nat. Protoc. 9, 2663–2681, https://doi.org/10.1038/nprot.2014.180

21 Sun, Y. and Liu, J. (2015) H9N2 influenza virus in China: a cause of concern. Protein Cell 6, 18–25

22 Wang, X.G., Ma, S.Y., Chang, J.S., Shi, R., Wang, R.L., Zhao, P. et al. (2019) Programmable activation of Bombyx gene expression using CRISPR/dCas9 fusion systems. Insect Sci. 26, 983–990, https://doi.org/10.1111/1744-7917

23 Jung, J., Engreitz, J.M., Konermann, S., Abudayyeh, O.O., Verdine, V.K., Aguet, F. et al. (2017) Genome-scale activation screen identifies a lncRNA locus regulating a gene neighbourhood. Nature 549, 343–346, https://doi.org/10.1038/nature23451

24 Bester, A.C., Lee, J.D., Chavez, A., Lee, Y.R., Nachmani, D., Vora, S. et al. (2018) An integrated genome-wide CRISPRa approach to functionalize IncRNAs in drug resistance. Cell 173, 649–664.e620, https://doi.org/10.1016/j.cell.2018.03.052

25 Liu, Y., Yu, C., Daley, T.P., Wang, F., Cao, W.S., Bhat, S. et al. (2018) CRISPR activation screens systematically identify factors that drive neuronal fate and reprogramming. Cell Stem Cell 23, 758–771.e758, https://doi.org/10.1016/j.stem.2018.09.003