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CRISPRoff and CRISPRon Epigenetic Editing: Molecular Mechanisms and Therapeutic Applications

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Abstract

Epigenetic modification plays a key role in gene regulation. CRISPRoff and CRISPRon, based on Nuclease-dead Cas9 (dCas9), can achieve precise, reversible regulation of gene expression without changing the DNA sequence, which is better than traditional cutting technology. This article systematically compares the two sets of systems. At the molecular level, the differences in composition and mechanism between the two are analyzed. CRISPRoff achieves long-lasting silencing through the coordinated activity of its effector domains writing trimethylation of histone H3 at lysine 9 (H3K9me3) and DNA methylation; CRISPRon achieves reversible activation through demethylation and collaborative transcription activation. On this basis, this article reviews the application of the two in disease treatment, including CRISPRoff targeting Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) for the treatment of familial hypercholesterolemia (FH), CRISPRon targeting the Forkhead Box P3 (FOXP3) Regulatory T-Cell-Specific Demethylated Region (TSDR) demethylation to stabilize regulatory T cells (Tregs) and exploring the synergistic prospects of the two in chimeric antigen receptor T-cell (CAR-T cell) remodeling. This article clarifies the applicable boundaries and complementary relationship between the two and provides a theoretical direction for tool selection and collaborative strategies. However, there are still problems to be solved in this field, such as in vivo collaborative application and delivery efficiency. Future research should focus on the development of efficient delivery systems and verify the feasibility of collaborative strategies in in vivo models to promote clinical transformation.

Keywords

CRISPRoff, CRISPRon, Epigenetic editing, Gene regulation, Therapeutic applications

1. Introduction

Epigenetic modification (DNA methylation, histone modification) plays a central role in gene regulation. Its dynamic changes deeply affect the determination of cell fate, development process and disease occurrence. Traditional gene editing techniques (such as CRISPR-Cas9) achieve gene function knockout by cutting DNA double strands. Although the effect is long-lasting, it will introduce irreversible genome permanent changes, and there are safety hazards in therapeutic applications.

In recent years, the epigenetic editor developed based on dCas9 can achieve accurate and reversible regulation of gene expression by directionally rewriting the epimodification state of specific gene sites without changing the DNA sequence. Among them, CRISPRoff and CRISPRon are two sets of representative tools,

which achieve long-lasting silencing and reversible activation of target genes respectively. Compared with the traditional CRISPR-Cas9 gene knockout, these two systems avoid the risk of toxicity caused by DNA double-strand breakage, and can not only establish lasting silence through CRISPRoff, but also restore gene expression by erasing methylation markers by CRISPRon, for gene function research and disease intervention, providing more flexible operating tools [1].

At the molecular mechanism level, CRISPRoff establishes H3K9me3 and DNA methylation double inhibition markers at the target site through dCas9- Krüppel-associated box (KRAB)-DNA methyltransferase 3A (Dnmt3A)-DNA methyltransferase 3-like protein (Dnmt3L) fusion protein to achieve from instantaneous inhibition to lasting silence [1,2]; CRISPRon realizes the coupling of active demethylation and transcription activation through the synergy of dCas9 fused to the catalytic domain of Ten-Eleven Translocation 1 (dCas9-TET1) and MS2 Coat Protein - VP64-p65-Rta fusion protein (MS2-VPR) system [3,4]. The two systems share the dCas9 target platform but have opposite functions [1,2]. At the application level, the *PCSK9* can cause an increase in low-density lipoprotein cholesterol (LDL-C) levels, thus causing FH [5]. Therefore, CRISPRoff treats the disease by silencing *PCSK9* [6]; CRISPRon through *FOXP3* TSDR demethylation stabilizes Tregs [7]; both in CAR-T cells has a synergistic prospect in remodeling [1,7].

Based on the differences in the above molecular mechanisms and disease applications, this article systematically compares the similarities and differences of CRISPRoff and CRISPRon and specifically discusses their differentiated applicable scenarios and joint intervention strategies, in order to provide a theoretical basis for researchers to choose suitable epigenetic editing tools and design joint treatment strategies.

2. CRISPRoff-mediated Persistent Gene Silencing of DNA Methylation and H3K9me3

2.1 Protein Structure and Function Analysis

Figure 1: Structural diagram of CRISPRoff protein [2]

CRISPRoff is formed by the fusion of dCas9 with three effector domains (KRAB, Dnmt3A, and Dnmt3L) (Figure 1) [2]. The dCas9 targeting module completely eliminates the endonuclease activity of the Cas9 protein by introducing the inactivated nuclease obtained by D10A and H840A double-point mutations, and only retains the precise targeting ability mediated by sgRNA, thus guiding the complex to the target promoter region [8].

The KRAB domain integrated into the dCas9 N-terminus is a transcription inhibition domain that exists in Zinc finger proteins. Its core function is to first recruit the KAP1 inhibitory protein as a scaffold, and then further recruit the SETDB1 histone methyltransferase to catalyze H3K9me3 modification at the target site [9]. H3K9me3 is the signature modification of heterochromatin, which can compress the chromatin structure, block the binding of transcription factors, and achieve transcription inhibition [9].

The Dnmt3A domain is the catalytic enzyme responsible for methylation from the beginning [10]. Through S-adenosyl-$_L$-methionine (AdoMet) as a methyl donor, it covalently attaches a methyl group to the carbon atom at the 5th position of the cytosine base to produce 5-methylcytosine (5mC), thus superimposing another layer of stable transcription inhibition markers at the DNA sequence level [10].

The Dnmt3L domain is a regulatory factor of Dnmt3A. It does not have catalytic activity itself, but it can stabilize the conformation of the Dnmt3A active center by directly interacting with Dnmt3A to form a butterfly tetramer, thus significantly enhancing the catalytic efficiency of Dnmt3A, which is the key to CRISPRoff to achieve efficient methylation [10].

2.2 Synergistic Mechanism Among the Three Domains

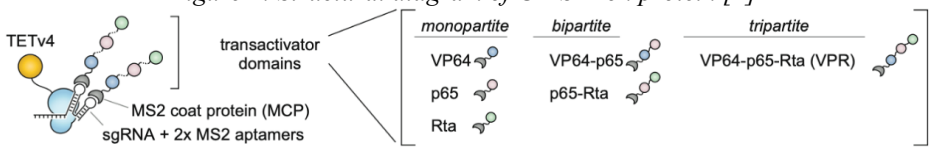
KRAB, Dnmt3A, and Dnmt3L work together to establish a stable inhibitory chromatin environment at the target site. KRAB alone can only achieve short-term inhibition; that is, the restoration of gene expression after H3K9me3 modification subsides [1]. In this process, the KAP1-SETDB1 complex recruited by KRAB can be

directly combined with the Plant Homeodomain (PHD) domain of Dnmt3A through SETDB1 [11], and Dnmt3A can be recruited to the Histone H3 lysine 4 unmethylated (H3K4me0) target site area [12]. Thus, the self-inhibition of the Dnmt3A N-terminal ATRX-DNMT3-DNMT3L (ADD) domain is relieved [13], so that Dnmt3A-3L can efficiently write DNA methylation. This methylation modification can be identified and maintained by endogenous DNMT1 during cell division, and then accurately replicated to progeny cells to realize the transmission of epigenetic regulation, so that the silencing effect stably maintains hundreds of division cycles [1]. In addition, Nuñez et al found that its sgRNA targeting window is significantly wider than CRISPRi (-500 to +500 bp from Transcription Start Site (TSS)) and it can target genes without CpG islands through whole-genome CRISPRoff screening, which greatly expands the scope of application of epigenetic editing [1].

3. CRISPRon Coupled TET1 Active Demethylation and VPR Synergistic Transcription Activation

CRISPRon is composed of two parts: a dCas9-TET1 fusion protein and an MS2-modified sgRNA-VPR system (Figure 2) [2]. The function can be divided into two levels: “demethylation writing” and “transcription activation collaboration”.

Figure 2: Structural diagram of CRISPRon protein [2]



3.1 Mechanism of Demethylation

At the level of demethylation, the TET1 catalytic domain is a Fe(II) and 2-oxoglutarate-dependent dioxygenases (2OG), which is located at the target under the guidance of dCas9 / sgRNA complex, catalyzing the oxidation of 5mC into 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) [3]. Among them, 5fC and 5caC can be identified and removed by thymine DNA glycosylase (TDG), and restored to unmodified cytosine through base excision repair (BER) pathway, thus completing active demethylation [3]. The optimized TETv4 design (TET1 moved to the N-terminus of dCas9 and added XTEN80-linked peptide) significantly improves the demethylation efficiency [2].

3.2 Mechanism of Transcription Activation Collaboration

At the level of transcription activation, the CRISPRon system adopts a step-by-step recruitment strategy. First of all, the MS2 RNA adapter can specifically bind to the MS2 coat protein (MCP), and this interaction enables the MCP-fused effector to be recruited to the MS2 stem-loops engineered into the sgRNA [14]. The structure then recruits VPR triple complexes to the target site, so that a single RNA recruits multiple VPR complexes at the same time [2]. The VPR complex is a powerful transcription-activating factor, which can efficiently initiate transcription at the target gene [4]. Compared with the strategy of directly integrating only a single VPR with dCas9, this strategy significantly extends the duration of the gene activation effect.

4. Comparison of the Molecular Mechanism of CRISPRoff and CRISPRon

Although CRISPRoff and CRISPRon are based on the dCas9 target platform, they are diametrically opposed in terms of effector composition, direction of action and regulation mechanism, forming a complete reversible epigenetic regulation system. As show as in Table 1, there are significant differences between two systems in multi-dimensionalities such as effector composition and regulation mechanism.

Table 1: Comparison of core characteristics of CRISPRoff and CRISPRon

Comparison Dimensions	CRISPRoff	CRISPRon
Effector	KRAB+Dnmt3A+Dnmt3L [2]	TET1 catalytic domain+MS2-VPR system [2]
Direction of Action	DNA methylation+ H3K9me3 [9,10]	Oxidative demethylation + transcription activation [3,4]

Comparison Dimensions	CRISPRoff	CRISPRon
KRAB Function	Recruiting KAP1→SETDB1→H3K9me3 [9]	/
Dnmt3A Function	Catalyzing DNA methylation (5mC) [10]	/
Dnmt3L Function	Enhancing the catalytic efficiency of Dnmt3A [10]	/
TET1 Function	/	5mC→5hmC→5fC→5caC, starting BER, active demethylation [3]
VPR Function	/	Collaborative enhancement of gene transcription activation [4]
Endurance	Long-lasting (450 cell divisions, >15 months) [2]	Long-lasting (maintained for 28 days in the primary T cells) [7]
Specificity	High (methylation modification concentrated at the target site) [2]	Methylation changes mainly limited to the target site [7]
Targeted Window	Wide (-500~+500 bp from TSS) (including no CpG island gene) [2]	Promoter and enhancer regions [7]
Representative Data	CLTA silencing maintained for 15 months, 38/39 clones [2]	TETv4 increased reactivation efficiency from 20% to >70% [2]; FOXP3 activation maintained for 28 days [7]

5. Application Examples and Collaboration Strategies of CRISPRoff and CRISPRon

The effector function of CRISPRoff and CRISPRon determines their applicable scenarios in different disease models: CRISPRoff is suitable for persistent gene silencing scenarios, and CRISPRon is suitable for reversible gene activation scenarios. The two can be applied independently to different scenarios, or can be used together in the same strategy.

5.1 CRISPRoff Targeted PCSK9 for Persistent Gene Silencing of FH

In FH, the PCSK9 gene increases the level of LDL-C by degrading Low-Density Lipoprotein Receptor (LDLR), which is the key pathogenic gene of the disease [5]. The existing PCSK9 inhibitors (monoclonal antibody (mAbs) and small interfering RNAs (siRNAs)) need to be given repeatedly for a long time, and compliance in patients is poor [15].

Based on the above problems, Tremblay et al based on the core structure of CRISPRoff (dCas9-KRAB-Dnmt3A/3L), designed an epigenetic editor (PCSK-EE) targeting *PCSK9* promoters, and the final gRNA editing combination was confirmed through gRNA screening and *in vivo* experiments. The difficulty of this design is that hepatocytes are actively proliferating, and mitosis will continue to dilute the established epigenetic modification. If silencing cannot be transmitted with division, the clinical goal of long-term treatment cannot be achieved [6].

For this reason, the internal logic designed by the researchers is: the KRAB domain first recruits KAP1-SETDB1 to establish H3K9me3 to achieve the instantaneous stagnation of PCSK9 transcription, but this modification can only provide instantaneous suppression [1]; Dnmt3A-3L domain is responsible for writing into DNA methylation in CpG island of *PCSK9* promoter, and at the same time, the modification can be accurately transmitted to the daughter strand by endogenous DNMT1 in a semi-conservative manner during DNA replication, thus transforming instantaneous inhibition into persistent silence [1,6]. At the targeting level, by screening 240 gRNAs covering the *PCSK9* promoter region, it was determined that the most effective target is concentrated near TSS, and finally two gRNAs (gRNA41+gRNA49) are selected for joint use to ensure that the promoter CpG island is high-density methylation coverage [6].

The effectiveness of this design was verified by the partial hepatectomy (PHx) model: after 2/3 of the hepatectomy of the mice that had been silent for 35 days, the ability of methylation markers to maintain under continuous cell division can be tested [6]. The results showed that the level of PCSK9 promoter methylation after regeneration (Day 28: 47%±4%; Day 365: 43%±8%) is almost the same as before resection, and the cycle of PCSK9 protein inhibition >98% can last for at least 1 year, confirming that DNA methylation is transmitted with division through replication mechanism of DNMT1 [6].

This mechanism allows *PCSK9* to be in a long-term silent state after a single administration, providing FH with a potential treatment strategy that distinguishes it from the traditional long-term administration scheme [6].

5.2 CRISPRon Targeted *FOXP3* TSDR to Restore the Functional Stability of Tregs

In Treg therapy, the *FOXP3* TSDR region amplified *in vitro* shows incomplete demethylation, and this epigenetic instability makes it prone to functional degeneration under inflammatory conditions in the body, resulting in transient efficacy [16]. The stable expression of *FOXP3* requires complete demethylation of the key CpG sites in the TSDR region, and partial demethylation will produce short and unstable transcription [7]. Based on this, Goudy et al based on the core structure of CRISPRon (dCas9-TET1-VPR), designed an epigenetic activator targeting the *FOXP3* TSDR to achieve instantaneous expression through the whole RNA delivery system [7].

At the effector level, the researchers adopted the optimized TETv4 design to improve the demethylation efficiency of TET1 in the special environment of the primary T cells and satisfy the requirement for complete demethylation of multiple sites within the TSDR [2,7]; at the same time, MS2-modified sgRNA in the CRISPRon system can recruit multiple VPR triple complexes to the target site and jointly activate *FOXP3* transcription on the basis of demethylation, making the gene activation effect more durable [2,7]. At the target level, based on the characteristic that TSDR is the key regulatory element for the stable expression of *FOXP3*, sgRNA is designed to target the TSDR region instead of the TSS, and through preliminary screening and combination optimization (selecting top three from the candidate sgRNAs, using a pool-of-three strategy), they ensured that demethylation modification covers the entire TSDR regulatory region [7].

The design has been verified by a primary T cell editing experiment: after editing, *FOXP3* expression is maintained under natural conditions for at least 28 days, and the epigenetic editing strategy avoids safety risks such as chromosomal translocation and cytotoxicity caused by Cas9 [7]. This allows CRISPRon to restore the stability of Tregs while retaining the integrity of the genome, providing a feasible path to solve the dilemma that functional persistence and safety cannot be achieved at the same time in Treg therapy.

5.3 CRISPRoff and CRISPRon Jointly Edited and Reshaped CAR-T Cells

In CAR-T cell therapy, the upregulation of inhibitory receptors such as RASA2 (RAS p21 protein activator 2) is one of the key factors leading to T cell exhaustion [17]; at the same time, effector molecules such as IFNG (interferon gamma) and GZMB (granzyme B) limit its anti-tumor activity [1]. It is difficult for a single tool to achieve the dual purposes of “silence” and “activation”, while the combined use of CRISPRoff and CRISPRon has the theoretical synergy advantage.

The key challenge of the combined use of two systems is the potential interference between methyltransferase and demethylase in the same T cell, which needs to be coordinated from target site dimension. Therefore, at the target level, CRISPRoff targets the CpG islands of the RASA2 gene promoter region (Chromosome 3) to establish DNA methylation; CRISPRon targets IFNG or GZMB gene enhancer region (Chromosome 12) for demethylation activation, thus targeting completely independent genome site and operating in parallel in the same nucleus without antagonism [1,7]. At the effector level, Dnmt3A-3L module of CRISPRoff achieves long-lasting silence through the replication mechanism of endogenous DNMT1 after writing DNA methylation; TET1 module of CRISPRon achieves reversible demethylation activation through continuous oxidation. Both functions form a reversible mechanism [1,3].

At present, Goudy et al have confirmed that the silencing of CRISPRoff to RASA2 can be combined with the knock-in of the CAR gene, which significantly prolongs the survival of Nalm6-bearing mice *in vivo* experiments ($P=2.2\times10^{-16}$) [7]; Yashi et al have also systematically discussed the potential application direction of using CRISPRon to activate T cell effector molecules (IFNG, GZMB) to enhance the function of $CD8^{+}$T cells in a review [1]. Although there has been no experimental report on the collaborative editing of CRISPRoff and CRISPRon in the same T cell, the design logic of the joint strategy has a sufficient theoretical foundation, which is expected to optimize the function of CAR-T cells from two dimensions of “persistent silence” and “reversible activation”.

6. Conclusion

This article systematically compares two sets of epigenetic regulation tools, CRISPRoff and CRISPRon. CRISPRoff collaboratively writes into H3K9me3 and DNA methylation through KRAB, Dnmt3A and Dnmt3L domains to achieve persistent gene silencing; CRISPRon achieves target gene reverse activation through TET1 demethylation and VPR transcriptional activation. The two share the dCas9 targeting platform, but there are significant differences in the direction of action and regulation mechanism and other dimensions, which determines their differentiated application: CRISPRoff is suitable for long-term gene silencing therapy (such as *PCSK9*-targeted therapy for FH); CRISPRon is suitable for reversible gene activation therapy (such as *FOXP3* TSDR demethylation to stabilize Treg therapy). In addition, the two have a synergistic prospect in CAR-T cell remodeling.

Based on the above content, this article clarifies the applicable boundaries and complementary relationship between the two regulatory models, which not only provides a more flexible and safe basis for the selection of tools for different clinical disease models, but also puts forward the prospect of combined reversible regulation of the two sets of tools and provides the theoretical direction for epigenetic therapy and multi-target synergistic strategies for polygenic complex diseases. This direction goes beyond the regulation method of traditional gene editing with knockout as the only strategy. However, the collaborative utilization, delivery efficiency and long-term safety of the two sets of tools still need to be evaluated in depth. Future research should focus on developing a more efficient *in vivo* delivery system and verifying the feasibility of the two in an *in vivo* model to promote CRISPRoff and CRISPRon to the clinical stage and provide new treatment strategies for medicine.

References

[1] Yahsi, B., Palaz, F., & Dincer, P. (2024). Applications of CRISPR Epigenome Editors in Tumor Immunology and Autoimmunity. *ACS synthetic biology*, *13*(2), 413–427. https://doi.org/10.1021/acssynbio.3c00524

[2] Nuñez, J. K., Chen, J., Pommier, G. C., Cogan, J. Z., Replogle, J. M., Adriaens, C., Ramadoss, G. N., Shi, Q., Hung, K. L., Samelson, A. J., Pogson, A. N., Kim, J. Y. S., Chung, A., Leonetti, M. D., Chang, H. Y., Kampmann, M., Bernstein, B. E., Hovestadt, V., Gilbert, L. A., & Weissman, J. S. (2021). Genome-wide programmable transcriptional memory by CRISPR-based epigenome editing. *Cell*, *184*(9), 2503–2519.e17. https://doi.org/10.1016/j.cell.2021.03.025

[3] Wu, X., & Zhang, Y. (2017). TET-mediated active DNA demethylation: mechanism, function and beyond. *Nature reviews. Genetics*, *18*(9), 517–534. https://doi.org/10.1038/nrg.2017.33

[4] Chavez, A., Scheiman, J., Vora, S., Pruitt, B. W., Tuttle, M., P R Iyer, E., Lin, S., Kiani, S., Guzman, C. D., Wiegand, D. J., Ter-Ovanesyan, D., Braff, J. L., Davidsohn, N., Housden, B. E., Perrimon, N., Weiss, R., Aach, J., Collins, J. J., & Church, G. M. (2015). Highly efficient Cas9-mediated transcriptional programming. *Nature methods*, *12*(4), 326–328. https://doi.org/10.1038/nmeth.3312

[5] Mansfield, B. S., Bene-Alhasan, Y., Ballantyne, C. M., & Raal, F. J. (2026). The evolving therapeutic landscape of PCSK9 inhibition. *Atherosclerosis*, *414*, 120670. https://doi.org/10.1016/j.atherosclerosis.2026.120670

[6] Tremblay, F., Xiong, Q., Shah, S. S., Ko, C. W., Kelly, K., Morrison, M. S., Giancarlo, C., Ramirez, R. N., Hildebrand, E. M., Voytek, S. B., El Sebae, G. K., Wright, S. H., Lofgren, L., Clarkson, S., Waters, C., Linder, S. J., Liu, S., Eom, T., Parikh, S., Weber, Y., … Jaffe, A. B. (2025). A potent epigenetic editor targeting human PCSK9 for durable reduction of low-density lipoprotein cholesterol levels. *Nature medicine*, *31*(4), 1329–1338. https://doi.org/10.1038/s41591-025-03508-x

[7] Goudy, L., Ha, A., Borah, A. A., Umhoefer, J. M., Chow, L., Tran, C., Winters, A., Talbot, A., Hernandez, R., Li, Z., Subramanya, S., Arab, A., Kale, N., Lee, J. H. J., Muldoon, J. J., Liu, C., Schmidt, R., Santangelo, P., Carnevale, J., Eyquem, J., … Gilbert, L. A. (2025). Integrated epigenetic and genetic programming of primary human T cells. *Nature biotechnology*, 10.1038/s41587-025-02856-w. Advance online publication. https://doi.org/10.1038/s41587-025-02856-w

[8] Qi, L. S., Larson, M. H., Gilbert, L. A., Doudna, J. A., Weissman, J. S., Arkin, A. P., & Lim, W. A. (2013). Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell*, *152*(5), 1173–1183. https://doi.org/10.1016/j.cell.2013.02.022

[9] Sobocińska, J., Molenda, S., Machnik, M., & Oleksiewicz, U. (2021). KRAB-ZFP Transcriptional Regulators Acting as Oncogenes and Tumor Suppressors: An Overview. *International journal of molecular sciences*, *22*(4), 2212. https://doi.org/10.3390/ijms22042212

[10] Jia, D., Jurkowska, R. Z., Zhang, X., Jeltsch, A., & Cheng, X. (2007). Structure of Dnmt3a bound to Dnmt3L suggests a model for de novo DNA methylation. *Nature*, *449*(7159), 248–251. https://doi.org/10.1038/nature06146

[11] Yuan, L., Sun, B., Xu, L., Chen, L., & Ou, W. (2021). The Updating of Biological Functions of Methyltransferase SETDB1 and Its Relevance in Lung Cancer and Mesothelioma. *International journal of molecular sciences*, *22*(14), 7416. https://doi.org/10.3390/ijms22147416

[12] Binda, O., LeRoy, G., Bua, D. J., Garcia, B. A., Gozani, O., & Richard, S. (2010). Trimethylation of histone H3 lysine 4 impairs methylation of histone H3 lysine 9: regulation of lysine methyltransferases by physical interaction with their substrates. *Epigenetics*, *5*(8), 767–775. https://doi.org/10.4161/epi.5.8.13278

[13] Guo, X., Wang, L., Li, J., Ding, Z., Xiao, J., Yin, X., He, S., Shi, P., Dong, L., Li, G., Tian, C., Wang, J., Cong, Y., & Xu, Y. (2015). Structural insight into autoinhibition and histone H3-induced activation of DNMT3A. *Nature*, *517*(7536), 640–644. https://doi.org/10.1038/nature13899

[14] Konermann, S., Brigham, M. D., Trevino, A. E., Joung, J., Abudayyeh, O. O., Barcena, C., Hsu, P. D., Habib, N., Gootenberg, J. S., Nishimasu, H., Nureki, O., & Zhang, F. (2015). Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. *Nature*, *517*(7536), 583–588. https://doi.org/10.1038/nature14136

[15] Blais, J. E., Zhong, W., Li, C. Y. V., Kongkaew, C., & Chan, A. H. Y. (2026). Adherence to PCSK9 Inhibitors in Clinical Practice: Systematic Review and Meta-Analysis of Observational Studies. *JACC. Advances*, *5*(5), 102733. https://doi.org/10.1016/j.jacadv.2026.102733

[16] Chen, K. Y., Kibayashi, T., Giguelay, A., Hata, M., Nakajima, S., Mikami, N., Takeshima, Y., Ichiyama, K., Omiya, R., Ludwig, L. S., Hattori, K., & Sakaguchi, S. (2025). Genome-wide CRISPR screen in human T cells reveals regulators of FOXP3. *Nature*, *642*(8066), 191–200. https://doi.org/10.1038/s41586-025-08795-5

[17] Carnevale, J., Shifrut, E., Kale, N., Nyberg, W. A., Blaeschke, F., Chen, Y. Y., Li, Z., Bapat, S. P., Diolaiti, M. E., O'Leary, P., Vedova, S., Belk, J., Daniel, B., Roth, T. L., Bachl, S., Anido, A. A., Prinzing, B., Ibañez-Vega, J., Lange, S., Haydar, D., … Marson, A. (2022). RASA2 ablation in T cells boosts antigen sensitivity and long-term function. *Nature*, *609*(7925), 174–182. https://doi.org/10.1038/s41586-022-05126-w

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