

Scientists use CRISPR activation method to reveal 'Rosetta Stone' of immune cell function

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Zachary Steinhart (left) and Ralf Schmidt (right), scientists at the Gladstone-UCSF Institute of Genomic Immunology led by Alex Marson, engineered a new CRISPR-based pipeline to test every gene in the genome and rapidly discover genes that can be "turned on" in immune cells to enhance their functions. Credit: Michael Short/Gladstone Institutes

CRISPR genome editing has served as a powerful tool for deleting or

altering DNA sequences and studying the resulting effect. Now, researchers at Gladstone Institutes and UC San Francisco (UCSF) have co-opted the CRISPR-Cas9 system to forcibly activate genes—rather than edit them—in human immune cells. The method, known as CRISPRa, let them discover genes that play a role in immune cell biology more thoroughly and rapidly than previously possible.

"This is an exciting breakthrough that will accelerate immunotherapy research," says Alex Marson, MD, Ph.D., director of the Gladstone-UCSF Institute of Genomic Immunology and senior author of the new study. "These CRISPRa experiments create a Rosetta Stone for understanding which genes are important for every function of [immune cells](#). In turn, this will give us new insight into how to genetically alter immune cells so they can become treatments for cancer and autoimmune diseases."

The study, published in the journal *Science*, is the first to successfully use CRISPRa at a large scale in primary human cells, which are cells isolated directly from a person.

The scientists activated each gene in the genome in different cells, enabling them to test almost 20,000 genes in parallel. This allowed them to quickly learn the rules about which genes provide the most powerful levers to reprogram cell functions in ways that could eventually lead to more powerful immunotherapies.

A New Kind of CRISPR

The CRISPR-Cas9 genome-editing system typically relies on Cas9 proteins, often described as "molecular scissors," to cut DNA at desired locations along the genome.

In recent years, Marson and his colleagues have used CRISPR's targeted

scissors to selectively remove (or "knock out") genes from various types of [human immune cells](#), including regulatory T cells and monocytes. Their results have begun to illuminate how immune cells can be engineered to be more effective against infections, inflammation, or cancer. But his team knew they were still missing part of the story.

"Knocking out genes is great for understanding the basics of how immune cells function, but a knock-out-only approach can miss pinpointing some really critical genes," says Zachary Steinhardt, Ph.D., a postdoctoral scholar in the Marson Lab and co-first author of the new paper.

In particular, knocking out a gene does not tell you what would happen if you instead made the gene more active.

So the researchers turned to CRISPRa, short for CRISPR activation. In CRISPRa, the Cas9 protein is altered so that it can no longer cut DNA. Rather, scientists can attach an activator—a molecular "on" switch—to Cas9, so that when it binds to a gene, it activates it. Alternatively, they can attach a repressor—an "off" switch—to Cas9 to turn genes off, achieving a result similar to a typical knockout approach (called CRISPRi for CRISPR interference).

Mapping T Cell Genes

T cells, a type of white blood cell, are one of the key mediators of immunity in the human body; they not only target invading pathogens, but also direct other immune cells to increase or decrease their responses to intruders or cancer cells. This messaging is achieved through the production of signaling molecules known as cytokines. Different types of T cells produce different repertoires of cytokines, and different cytokines or [cytokine](#) cocktails have different effects on the immune response.

Controlling T cell cytokines, Marson says, would offer new opportunities to reshape entire immune responses in a wide range of different disease contexts. But researchers have an incomplete understanding of exactly which genes control which cytokines.

In the new work, Marson, Steinhart, and co-first author Ralf Schmidt, MD, worked with their colleagues to adapt CRISPRa and CRISPRi to work at high efficiency in primary T cells—something never before done.

"This improved efficiency in delivering the CRISPRa or CRISPRi machinery into the cells was critical to enable genome-wide experiments and accelerate discoveries," says Marson.

Then, the research team used these approaches to activate or inactivate nearly 20,000 genes in human T cells isolated directly from multiple healthy volunteers. They screened the resulting cells for changes to cytokine production and homed in on hundreds of genes that serve as key cytokine regulators, including some never before identified in knock-out screens.

"Our work demonstrates the precision and scalability of this technology in human T cells," says Schmidt. "And we quickly learned the rules of which genes you could turn on to dial the levels of certain cytokines."

Better T Cell Therapies

To treat some cancer types, clinicians are increasingly using CAR-T cell therapy, in which T cells are removed from a patient's body, engineered in a lab to target cancer cells, and then reinfused. Boosting the ability of T cells to fight cancer—by altering their cytokine production, for instance—could make CAR-T cell therapy even more powerful.

"Our new data give us this incredibly rich instruction manual for T cells," says Marson. "Now we have a basic molecular language we can use to engineer a T cell to have very precise properties."

Marson's lab is now studying some of the individual genes identified in their screen, as well as working to further leverage CRISPRa and CRISPRi to discover [genes](#) that control other critical traits in human immune [cells](#).

"Working with the Gladstone-UCSF Institute of Genomic Immunology, the Innovative Genomics Institute, and the UCSF Living Therapeutics Initiative, our team now hopes to use our new instruction manual to create synthetic gene programs that can be CRISPR-engineered into the next generation cellular immunotherapies to treat a wide-range of diseases," says Marson.

More information: Ralf Schmidt et al, CRISPR activation and interference screens decode stimulation responses in primary human T cells, *Science* (2022). [DOI: 10.1126/science.abj4008](https://doi.org/10.1126/science.abj4008).
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Provided by Gladstone Institutes

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