Our team developed a universal toolkit, termed uniCAS, that enables customizable gene regulation in mammalian cells. Therefore, we engineered the recently understood and highly promising Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system. The CRISPR-associated catalytically inactive dCas9 is the heart of our toolkit: A protein that enables multiple and sequence-specific DNA binding. The key components for binding dCas9 to its targets are two small, non-coding RNAs: The CRISPR-RNA (crRNA) and the transactivating crRNA (tracrRNA). The RNA complex guides dCas9 to the DNA sequences of interest. By fusing several effector domains to dCas9, we constructed novel engineered proteins for gene regulation, even controllable by light stimulus.

**Activation**

We fused dCas9 to the transactivation domain of VP16. This fusion protein was successfully used to activate gene expression of the secreted embryonic alkaline phosphatase (SEAP) reporter in mammalian cells in presence of appropriate crRNAs. We achieved over 25-fold upregulation of SEAP expression (see Figure 1 C).

**Histone Modification**

Specific chromatin modification was achieved by fusing the histone methyltransferase G9a to dCas9 and thereby resulting in an epigenetic BioBrick. G9a primarily methylates histone 3. Different endogenous loci in the vascular endothelial growth factor (VEGF) gene were targeted in mammalian cells. This resulted in an up to 50% repression (*, p<0.05) (see Figure 2 C).

**Repression**

The transcriptional repressor domain Krüppel-associated box (KRAB) was fused to dCas9 resulting in a fusion protein with the ability to repress gene expression. The device was tested in mammalian cells by targeting different sites in the endogenous VEGF locus. Up to 50% repression was achieved (Figure 3 C).

**uniBAss**

We developed a novel and innovative ELISA-based method to quantify the binding efficiencies of our dCas9 fusion proteins: The uniCAS Binding Assay (uniBAss). For this purpose, biotinylated oligos were coated on 96-well ELISA plates via the interaction with streptavidin.

**Multiple Targeting**

The greatest advantage of the CRISPR/Cas9 system is that only one protein is required for targeting various DNA sequences. The only component which needs to be replaced is the CRISPR-RNA (crRNA). We therefore designed an RNA plasmid termed the RNAimer. It provides the backbone for easy insertion with the crRNA sequences.

**Light Switch**

We aimed to control our system with light thereby allowing for gene regulation with high spatiotemporal resolution. We engineered a system for induction by red, UVB and blue light. The blue light system is based on the light-activated interaction of CRY2 and CIB1. CRY2 was fused to dCas9 which can upon light-stimulus recruit the CIB1-VP16 fusion protein to any DNA sequence of interest.

**Conclusion & Outlook**

Gene regulation was achieved based on a modified CRISPR/Cas9 system. We engineered a toolkit which offers efficient and customizable gene activation and repression via effector domains fused to the dCas9 protein. Combining uniCAS with multiple targeting, light induction and the uniBAss we generated a universally applicable toolkit which allows regulation of any gene of interest. Our toolbox of standardized parts of the CRISPR/Cas9 system offers broad application in research fields such as tissue engineering, stem cell reprogramming and fundamental research.

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**References**

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