[C]sgRNA系统调控 CRISPR 活性

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Optimization of Cas9 activity through the addition of cytosine extensions to single-guide RNAs

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Check for updates

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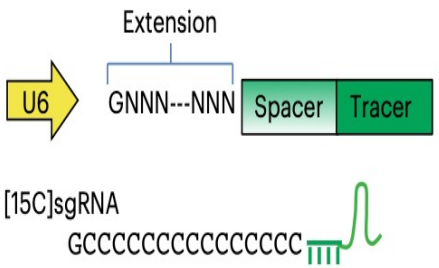
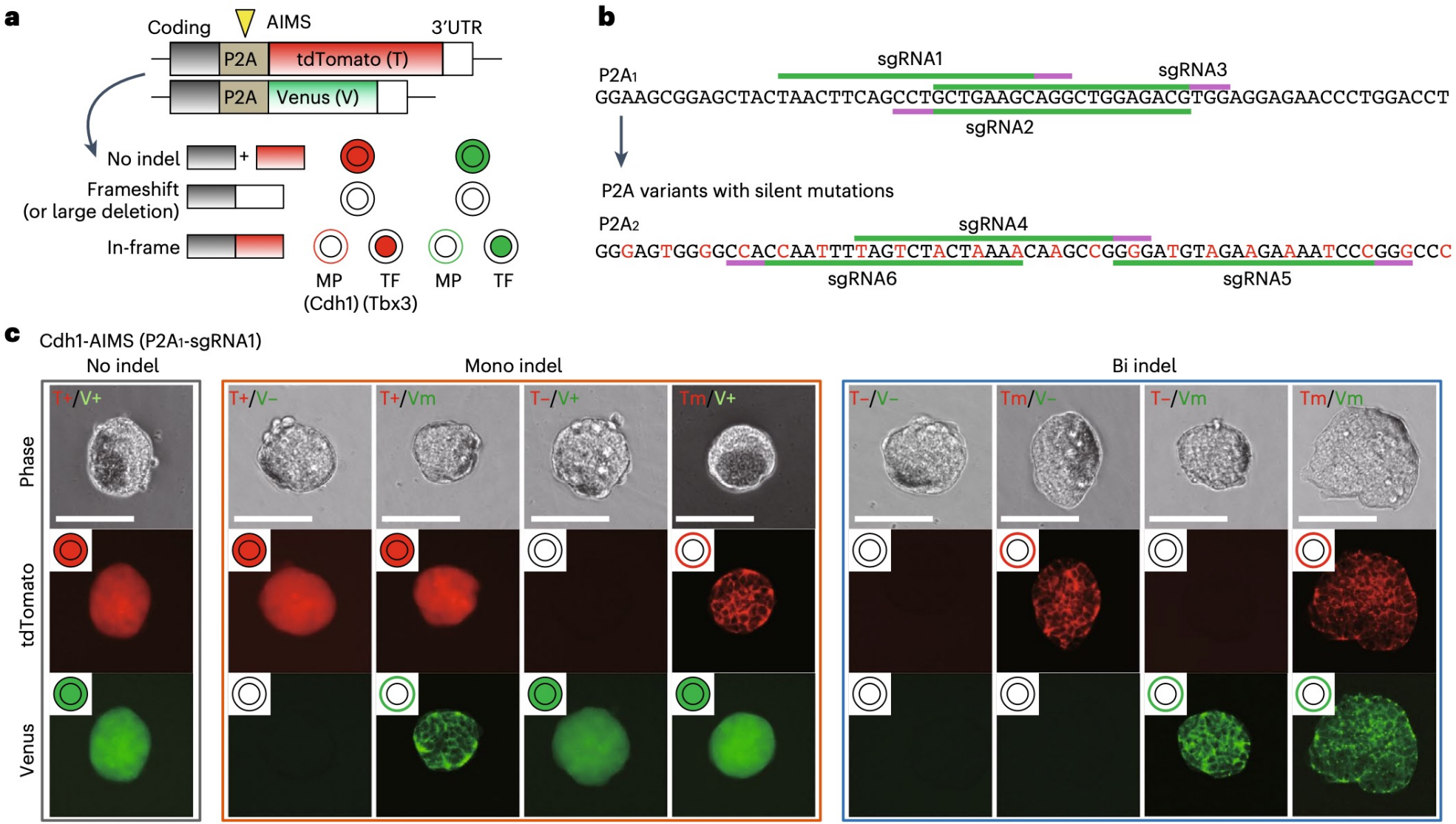
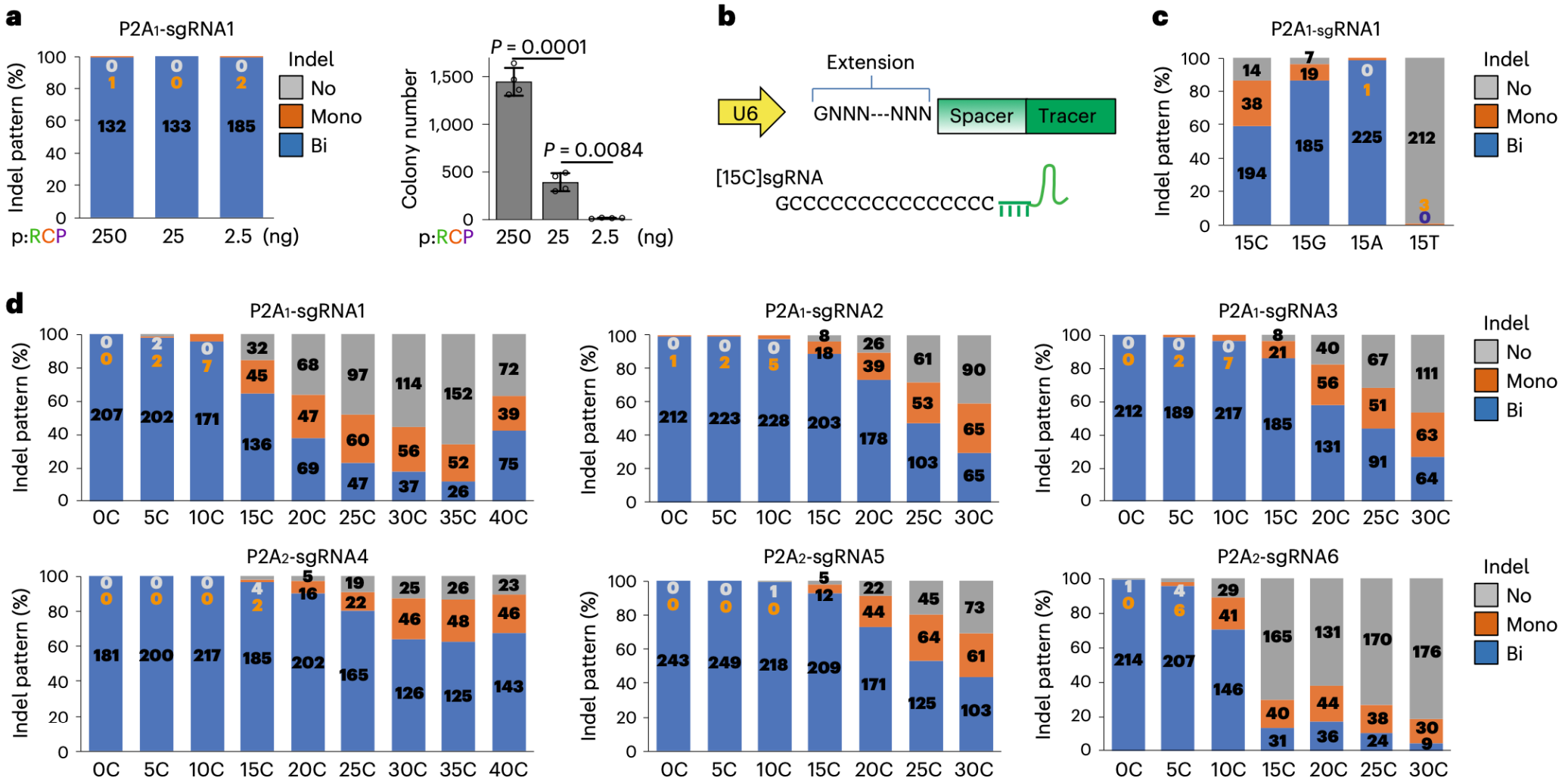


The precise regulation of the activity of Cas9 is crucial for safe and efficient editing. Here we show that the genome-editing activity of Cas9 can be constrained by the addition of cytosine stretches to the 5′-end of conventional single-guide RNAs (sgRNAs). Such a 'safeguard sgRNA' strategy, which is compatible with Cas12a and with systems for gene activation and interference via CRISPR (clustered regularly interspaced short palindromic repeats), leads to the length-dependent inhibition of the formation of functional Cas9 complexes. Short cytosine extensions reduced p53 activation and cytotoxicity in human pluripotent stem cells, and enhanced homology-directed repair while maintaining bi-allelic editing. Longer extensions further decreased on-target activity yet improved the specificity and precision of mono-allelic editing. By monitoring indels through a fluorescence-based allele-specific system and computational simulations, we identified optimal windows of Cas9 activity for a number of genome-editing applications, including bi-allelic and mono-allelic editing, and the generation and correction of disease-associated single-nucleotide substitutions via homology-directed repair. The safeguard-sgRNA strategy may improve the safety and applicability of genome editing.

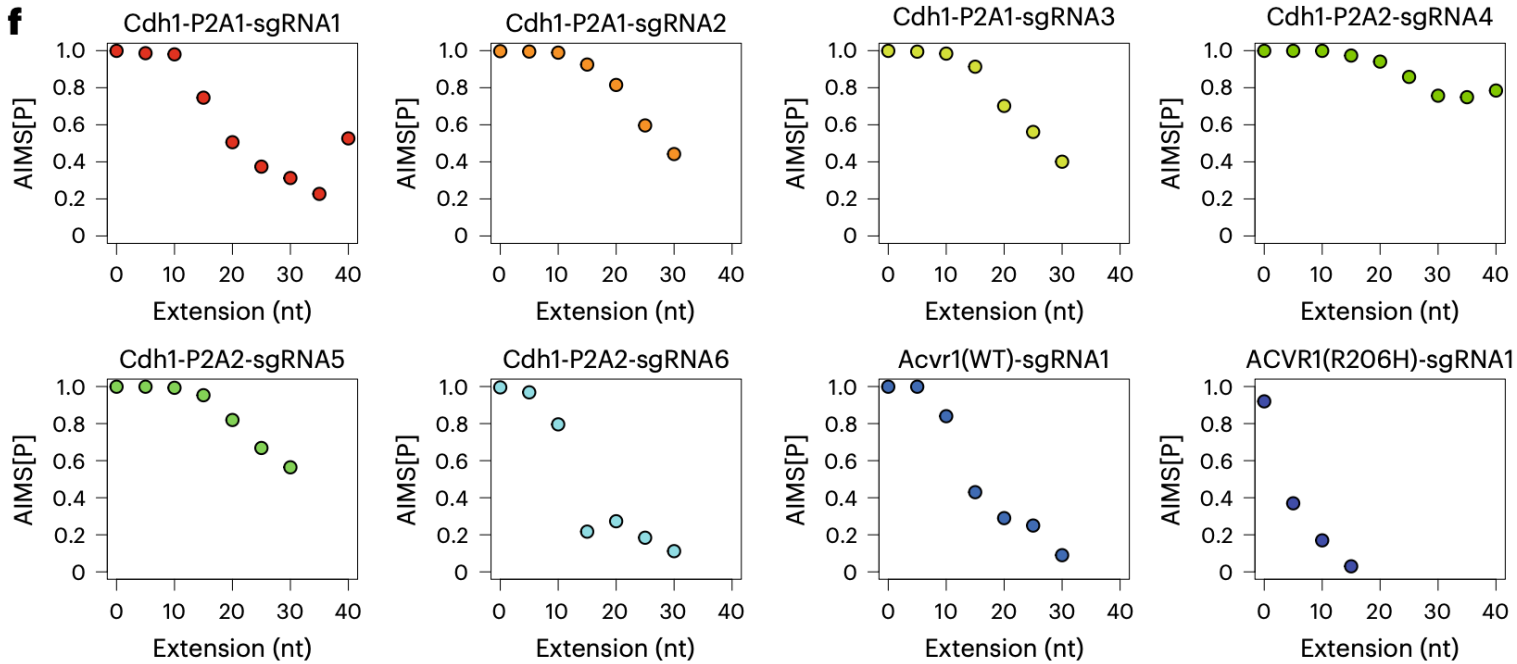
AISM: Allele-specific Indel Monitor System for the rapid and real-time quantitation of various editing patterns of a pair of alleles

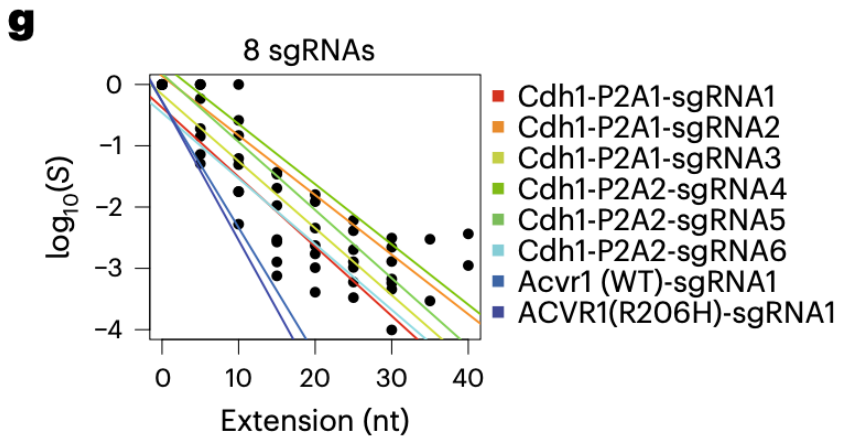


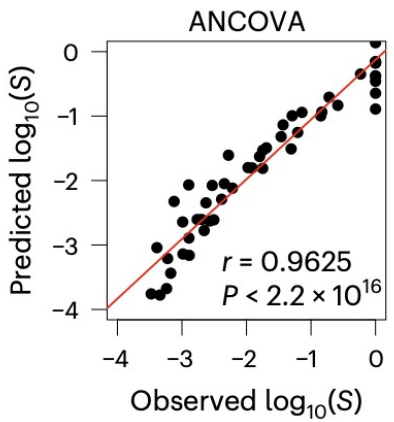
Fine-tuning of Cas9 activity with cytosine extension on sgRNAs



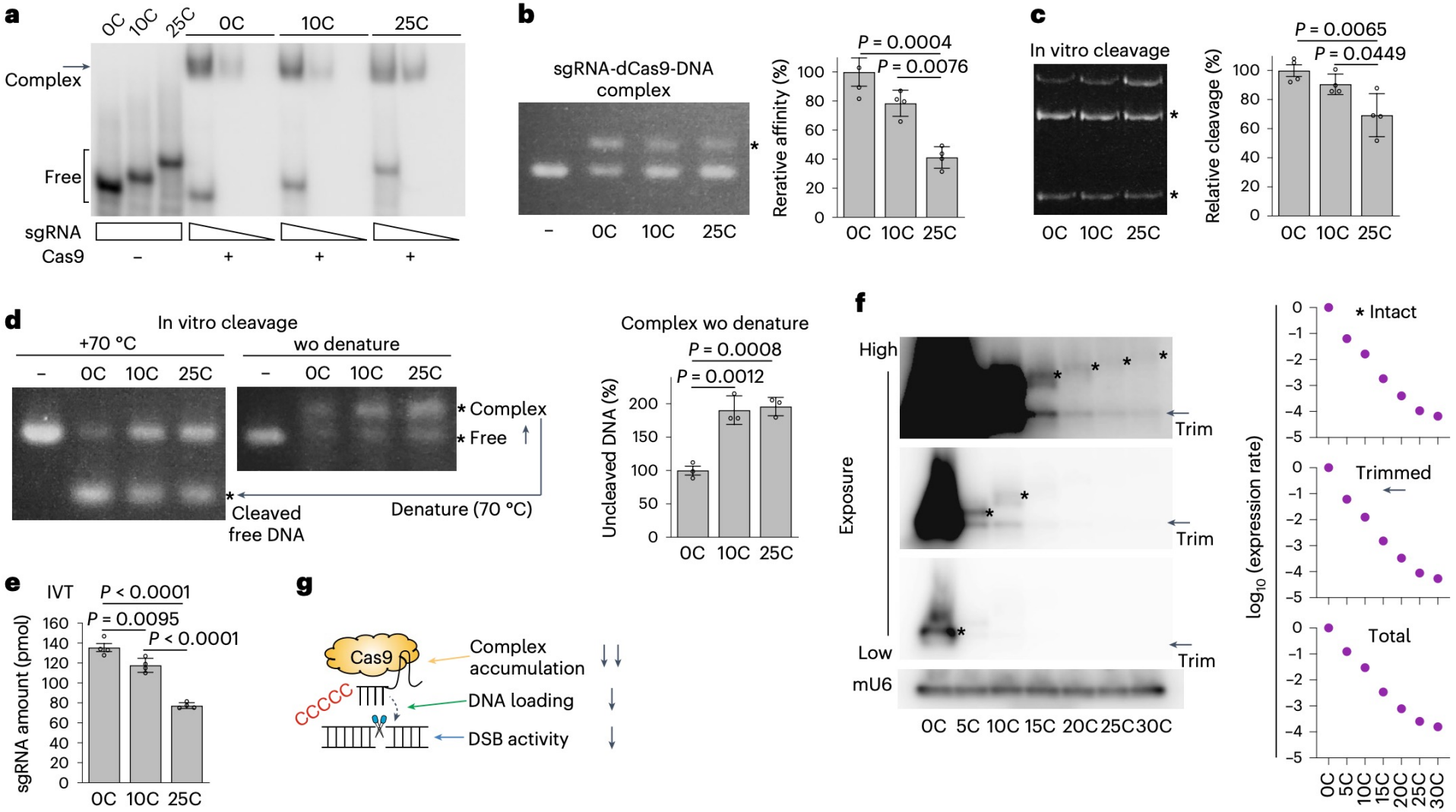
[C]sgRNA system work in a length-dependent manner, irrespective of the sgRNA sequence





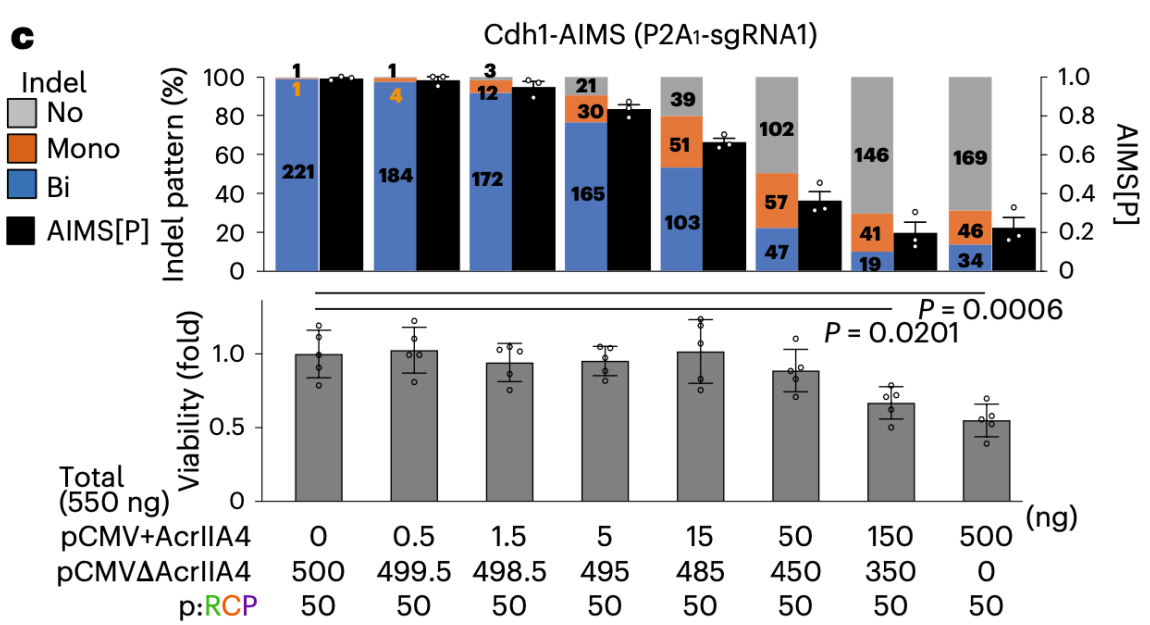


Mechanisms of Cas9 activity reduction via [C]sgRNAs system

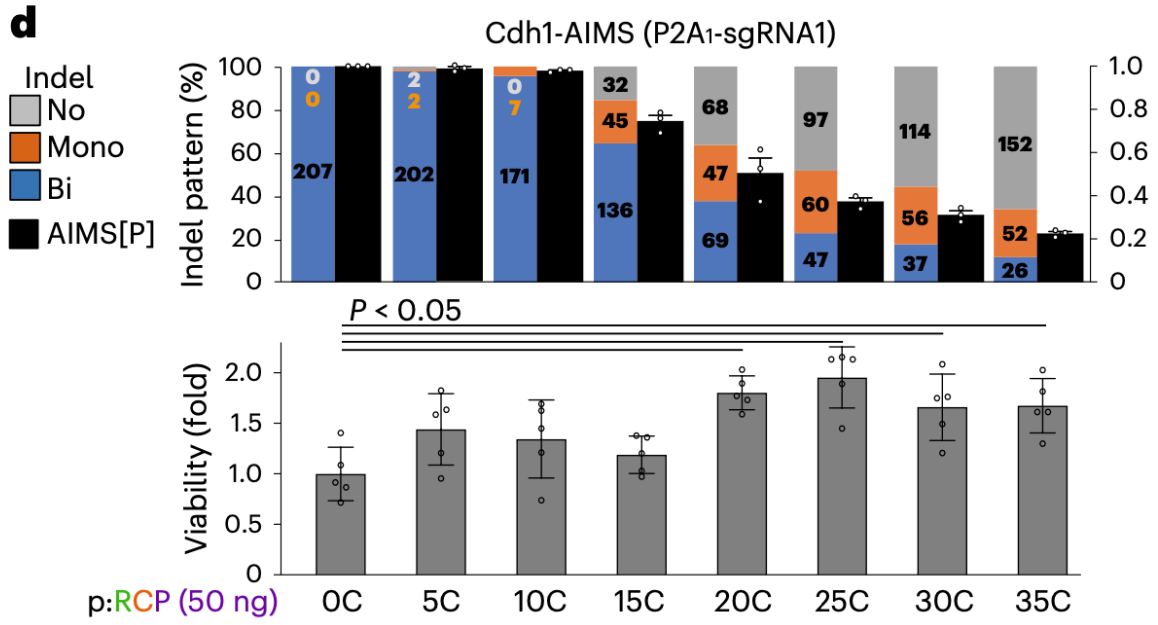


Comparisons between [C]sgRNA system and Cas9 inhibition using anti-CRISPR protein AcrIIA4

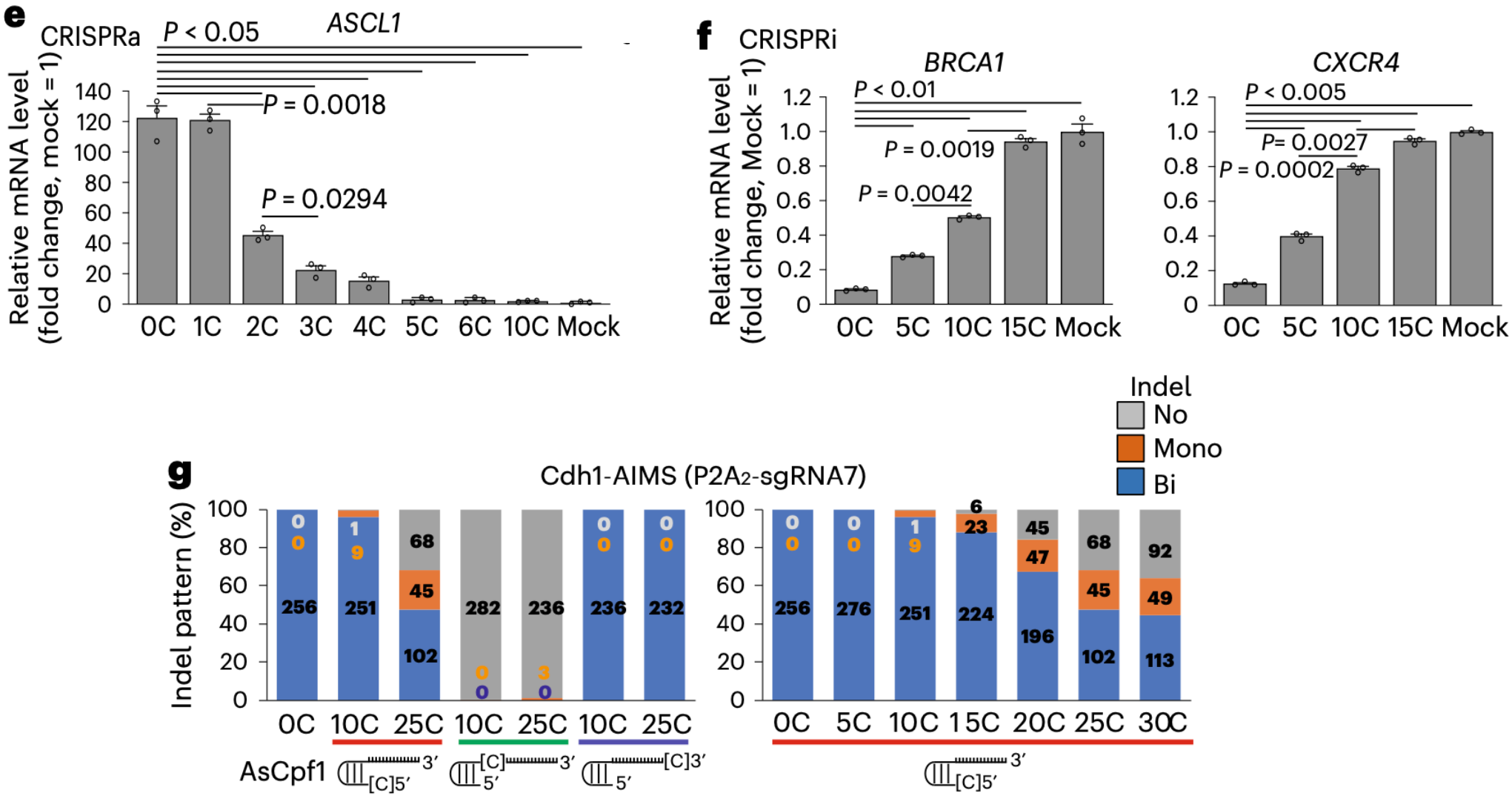
Cas9 inhibition through AcrIIA4



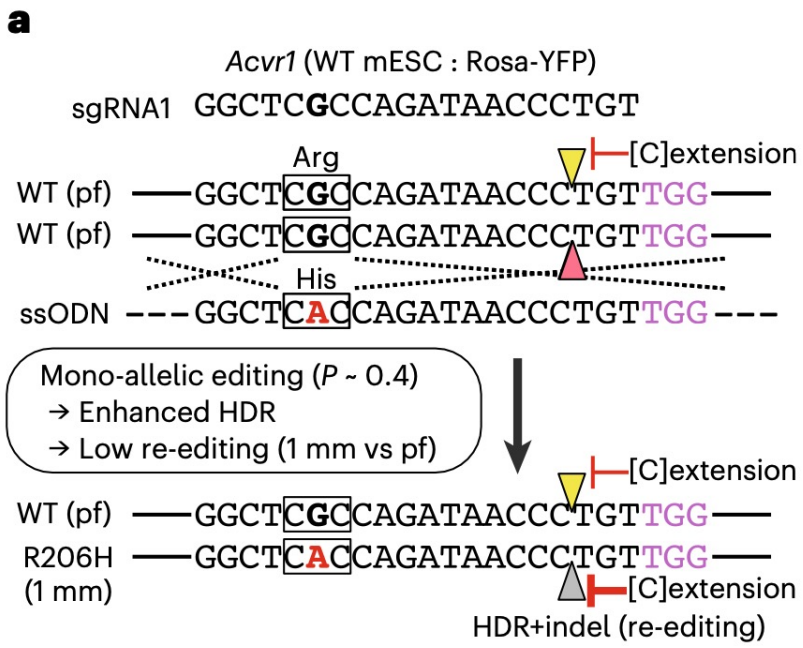
Cas9 inhibition through C-extension

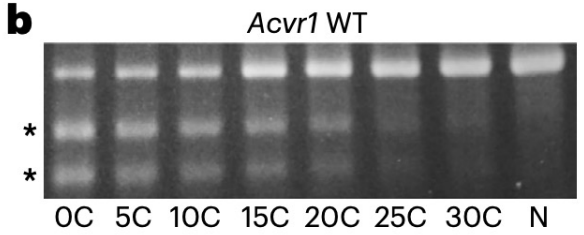


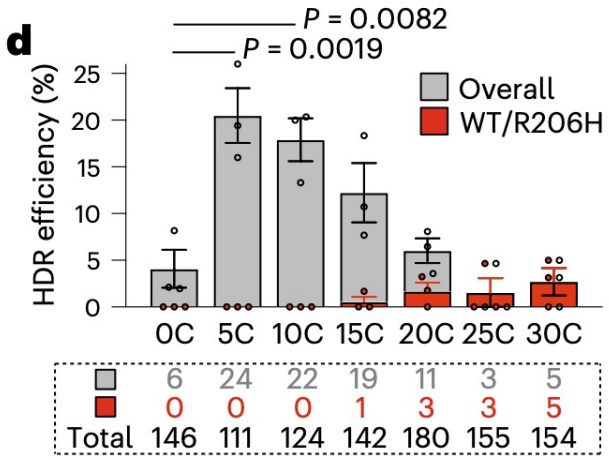
[C]sgRNA system is applicable to different CRISPR-based applications



Generation of a heterozygous single-nucleotide polymorphism (SNP) disease model through optimized mono-allelic editing

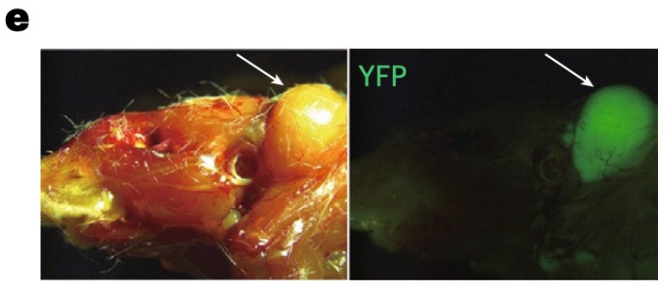




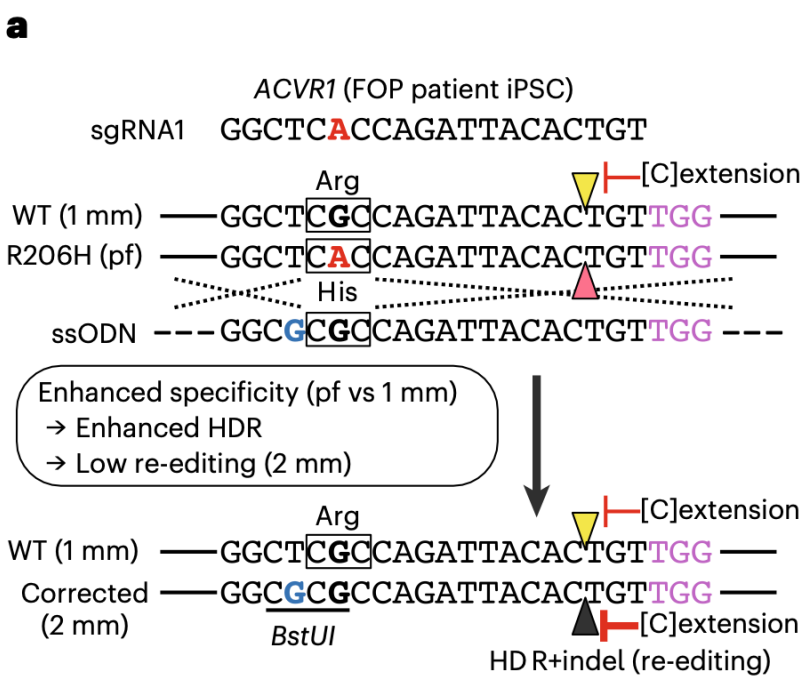


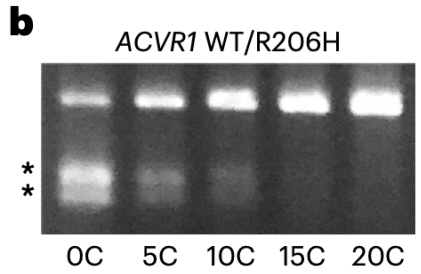
c

Bac[P]	0C	5C	10C	15C	20C	25C	30C
WT (pf)	1.00	1.00	0.84	0.43	0.30	0.27	0.09
R206H (1 mm)	1.00	1.00	0.78	0.38	0.13	0.12	0.04



p53-activation-free systematic precise gene correction in human iPSCs





c Bac[P]	0C	5C	10C	15C
R206H (pf)	0.93	0.38	0.20	0.03
WT (1 mm)	0.77	0.17	0.06	0.04
Correct (2 mm)	0.52	0	0	0