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Tae Seok Moon

Austin G. Rottinghaus

Aura Ferreira

Skye R.S. Fishbein

Gautam Dantas

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GENETICALLY STABLE CRISPR-BASED KILL SWITCHES FOR ENGINEERED MICROBES

Tae Seok Moon, Washington University in St. Louis, United States

tsmoon@wustl.edu

Austin G. Rottinghaus, Washington University in St. Louis, United States

Aura Ferreiro, Washington University in St. Louis, United States

Skye R.S. Fishbein, Washington University School of Medicine, United States

Gautam Dantas, Washington University School of Medicine, United States

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Microbial biocontainment is an essential goal for engineering safe, next-generation living therapeutics [1, 2]. However, the genetic stability of biocontainment circuits, including kill switches, is a challenge that must be addressed. Kill switches are among the most difficult circuits to maintain due to the strong selection pressure they impart, leading to high potential for evolution of escape mutant populations. We engineered two CRISPR-based kill switches in the probiotic *Escherichia coli* Nissle 1917, a single-input chemical-responsive switch and a 2-input chemical- and temperature-responsive switch. We employed parallel strategies to address kill switch stability, including functional redundancy within the circuit, modulation of the SOS response, antibiotic-independent plasmid maintenance, and provision of intra-niche competition by a closely related strain. We demonstrate that strains harboring either kill switch can be selectively and efficiently killed inside the murine gut, while strains harboring the 2-input switch are additionally killed upon excretion. Leveraging redundant strategies, we demonstrate robust biocontainment of our kill switch strains and provide a template for future kill switch development [3].

1. Amroffell, Rottinghaus, and Moon, *Curr Opin Biotechnol*, 2020. 66: p. 11-17.
2. Rottinghaus, Amroffell, and Moon, *Biotechnology Journal*, 2020. 15: p. 1900319.
3. Rottinghaus et al. *Nature Communications*, 2022.

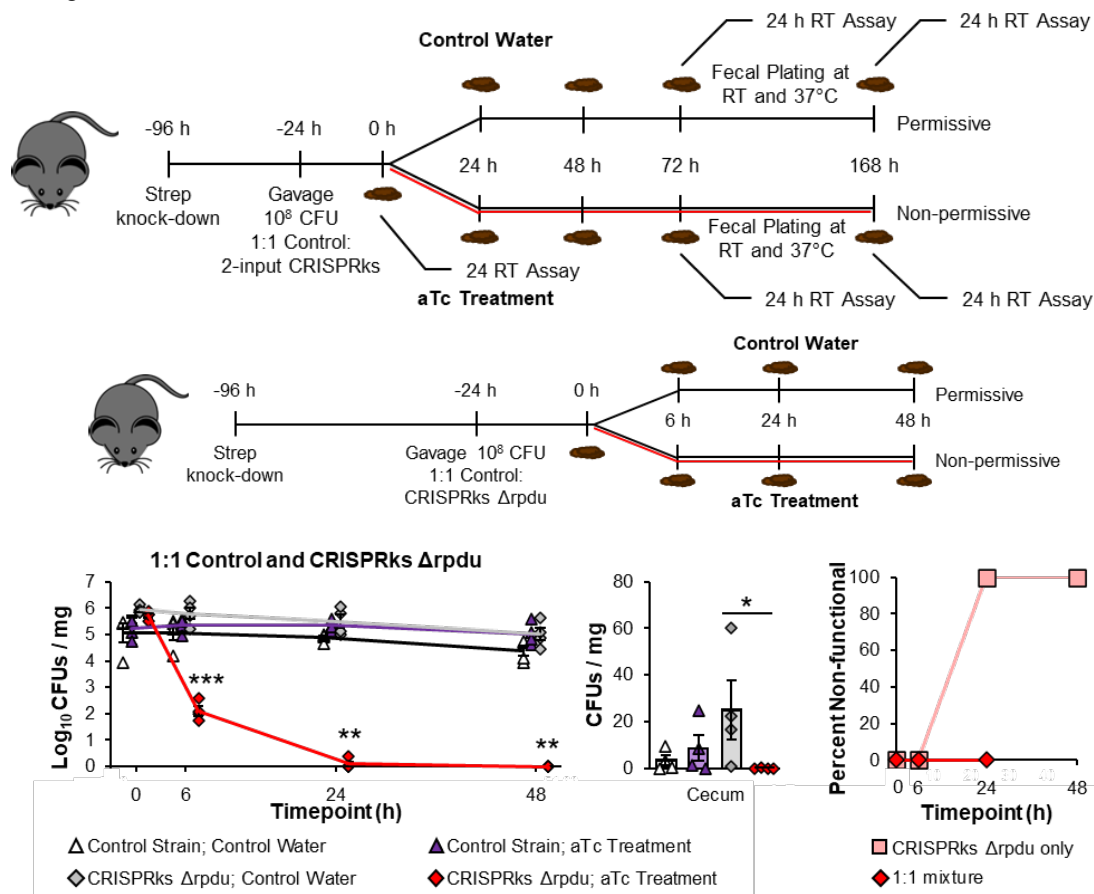


Figure. Complete killing achieved by the optimized CRISPR kill-switch in vivo.