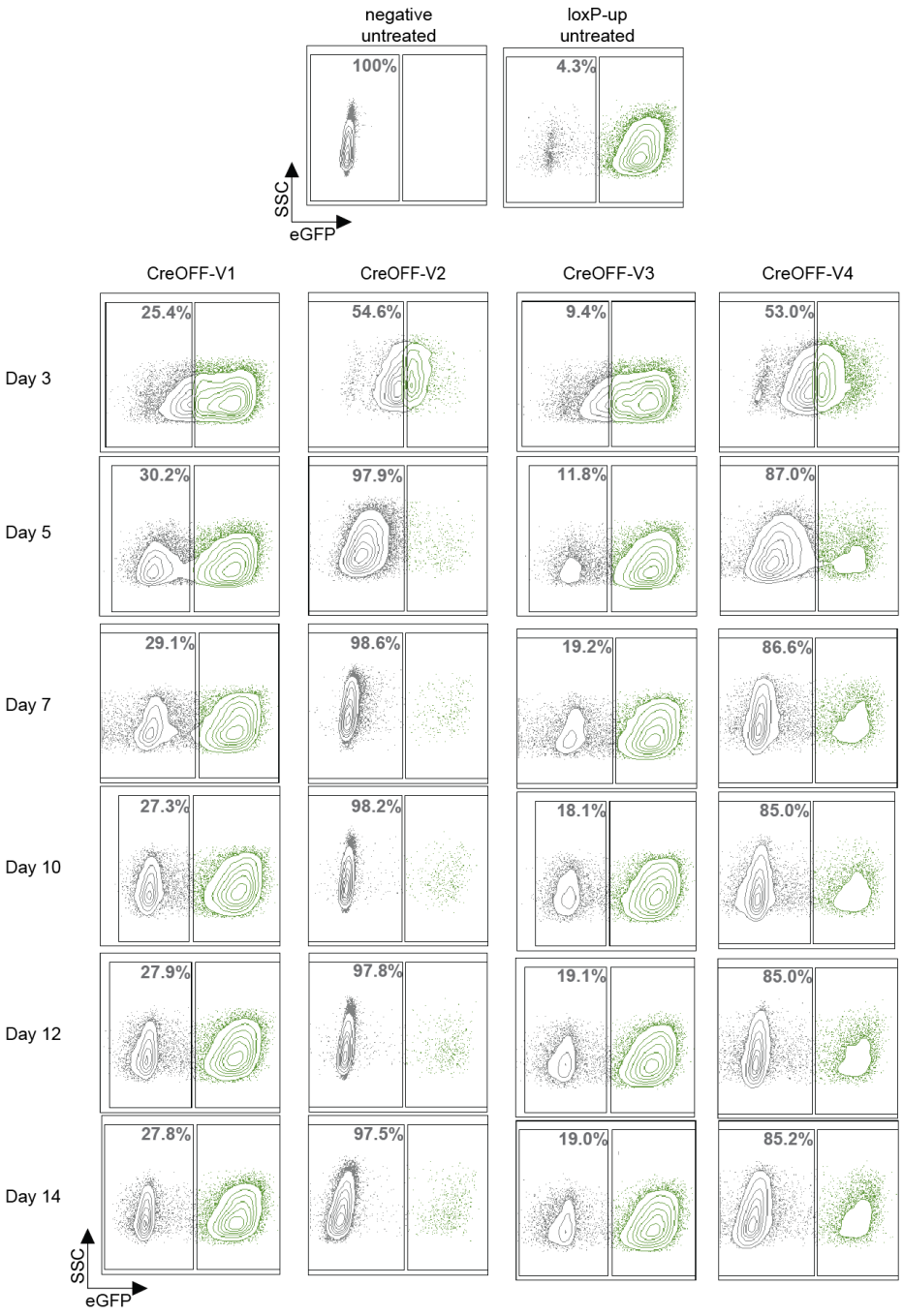


Figure S1: Influence of CreOFF domain arrangements on silencing efficiency. Representative flow cytometry plots showing eGFP-negative HEK293T cells, untreated loxP-up cells, and unselected loxP-up cells following CreOFF IVT-mRNA nucleofection with each variant on all time points. Grey numbers indicate the percentage of eGFP-negative cells.

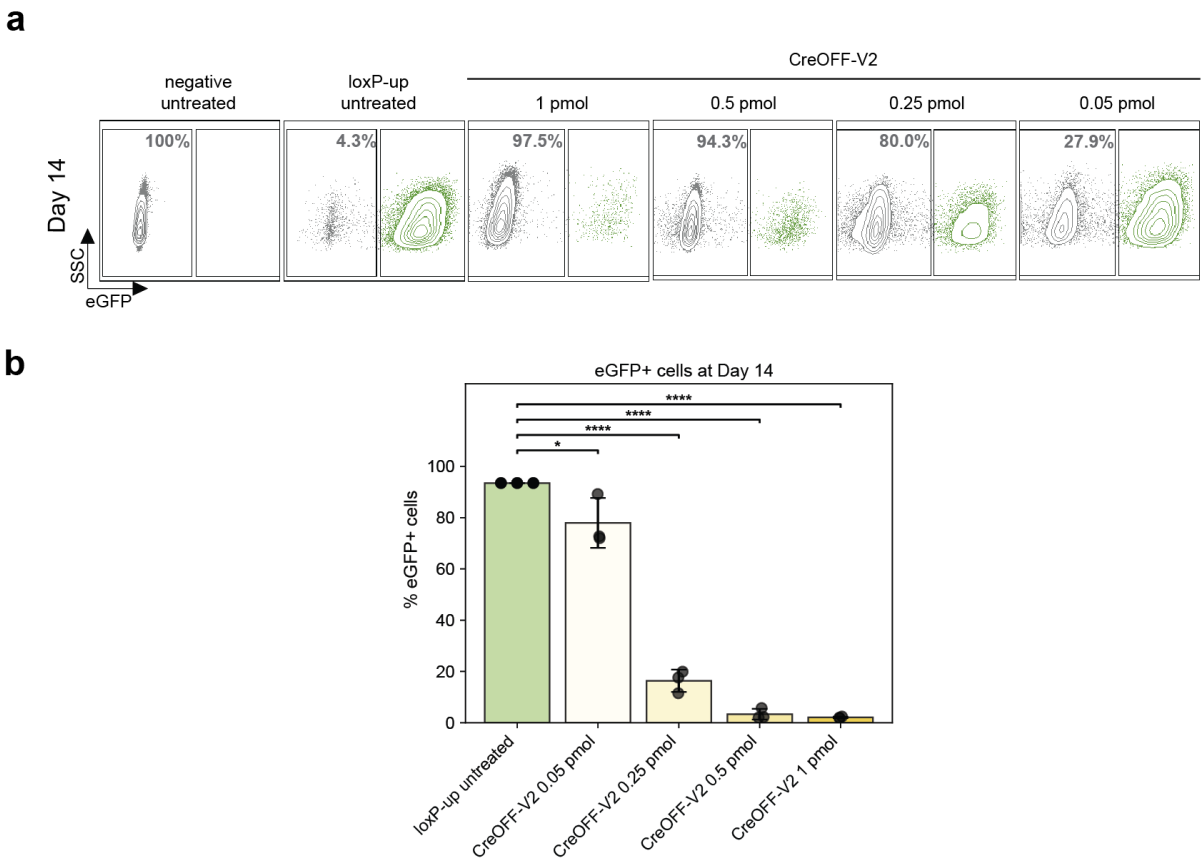


Figure S2: Dose-dependent efficiency of CreOFF-mediated silencing via IVT-mRNA nucleofection. **(a)** Representative flow cytometry plots depicting eGFP-negative HEK293T cells, untreated loxP-up cells, and unselected loxP-up cells nucleofected with CreOFF-V2 IVT-mRNA (with various amounts indicated in pmol) at the experimental endpoint (Day 14). Grey numbers indicate the percentage of eGFP-negative cells. **(b)** Bar plot showing the percentage of eGFP-positive cells at Day 14 (n = 3). Individual dots represent biological replicates, error bars indicate standard deviation, and asterisks denote statistical significance (* - $P < 0.05$, **** - $P < 0.0001$, one-way ANOVA).

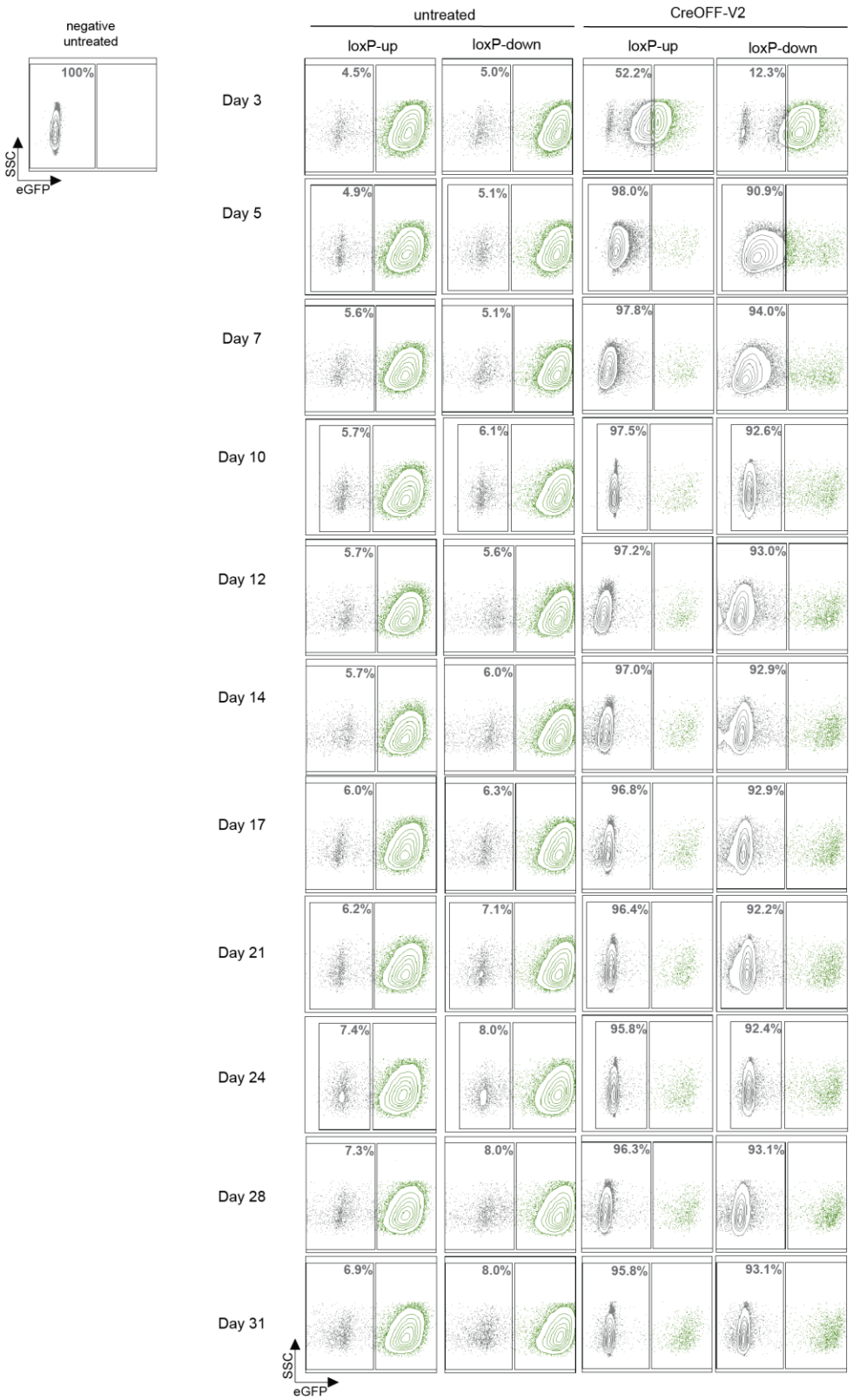


Figure S3: Long-term stability of CreOFF-mediated silencing at different binding site positions. Representative flow cytometry plots showing eGFP-negative HEK293T cells, untreated loxP-up and loxP-down cells, and unselected loxP-up and loxP-down cells treated with CreOFF IVT-mRNA at all time points.

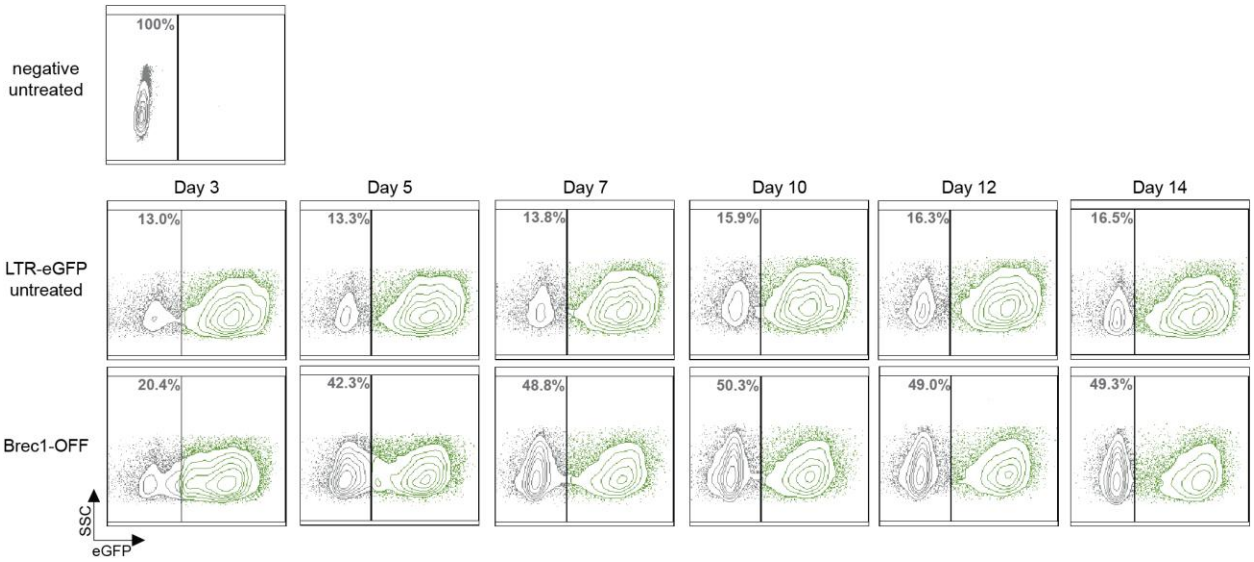


Figure S4: Brec1-OFF mediates silencing in LTR-eGFP reporter system. Representative flow cytometry profiles showing eGFP-negative HEK293T cells, untreated LTR-eGFP cells, and unselected LTR-eGFP cells treated with 4 pmol Brec1-OFF IVT-mRNA at representative time points.

Table S1: Primer sequences for targeted bisulfite sequencing

Forward primer (5’ – 3’)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGnnnnnGATTTTATTTGTAGGTTTGGTAAGTTAG
Reverse primer (5’ – 3’)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGnnnnnCATTTCAAATCCTTAAAAAAACCTAAAA