CRISPR-Cas12a nucleases function with structurally engineered crRNAs – SynThetic trAcrRNA

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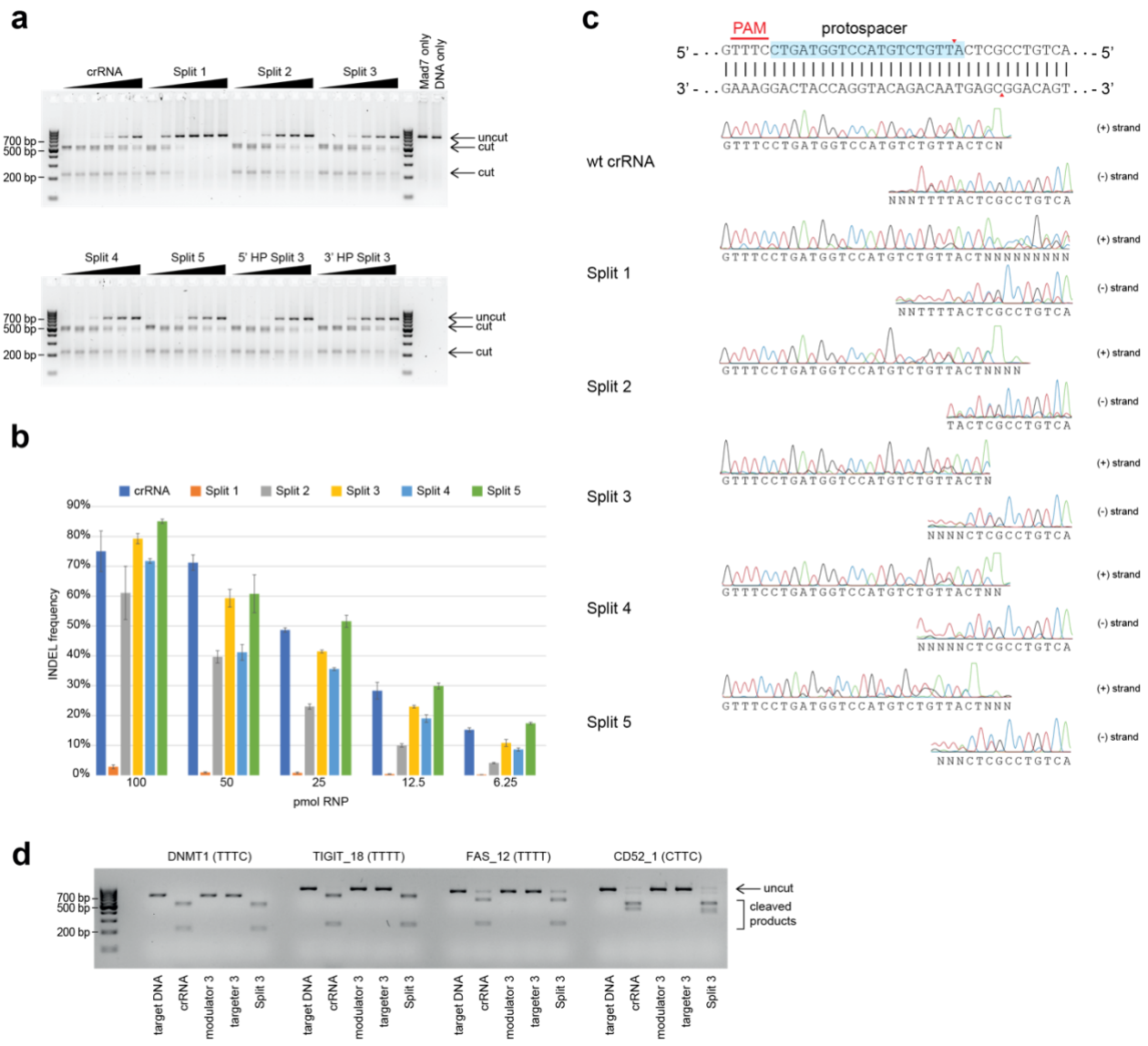
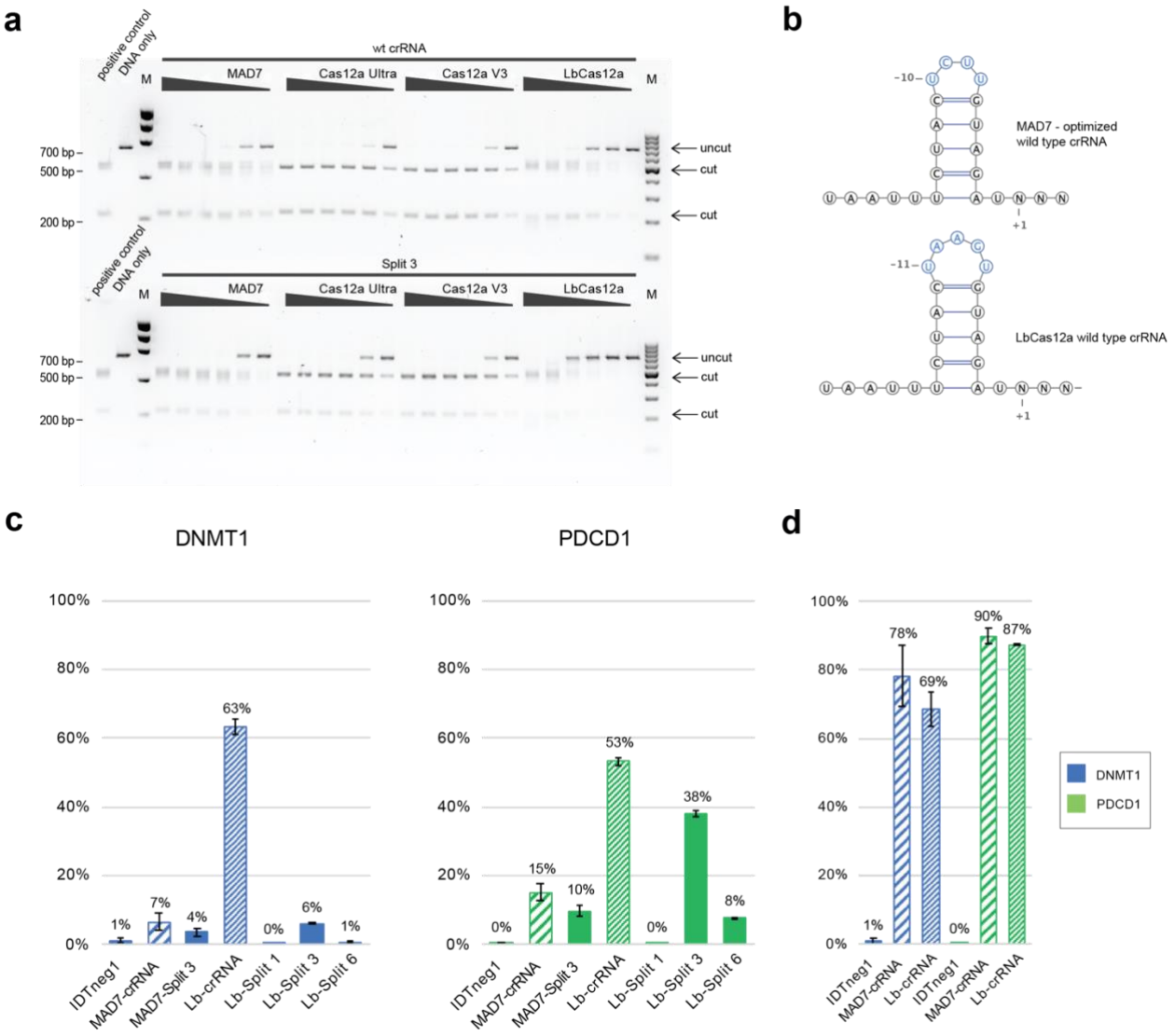
Supplementary Figure 1



Supplementary Figure 1: **a**, *In-vitro* cleavage assay with dilution series. The amplicon containing the DNMT1 locus target sequence is cleaved with crRNA and STAR-crRNA variants at nuclease : gRNA : target DNA in 20 : 60 : 1, 10 : 30 : 1, 5 :10 : 1, 2.5 : 7.5 : 1, 1.25 : 3.75 : 1, and 0.625 : 1.875 : 1 molar ratio. Cropped image from iBright FL 1000, size marker: GeneRuler 100 bp DNA Ladder (ThermoFisher Scientific). Full-length gel image is presented in Expanded Supplementary Figure S1a. **b**, Jurkat titration with RNPs assayed by INDEL frequency (%) of MAD7 with wild-type crRNA and Split STAR-crRNAs targeting the DNMT1 locus, measured by amplicon sequencing (error bars: mean ± SD for n = 3). **c**, MAD7 cut site investigation with wild-type crRNA and Split STAR-crRNAs variants, oligonucleotide containing DNMT1 target site was cleaved with RNPs, resulting Sanger sequencing traces are shown. **d**, *in-vitro* cleavage assay on the amplicons containing DNMT1, TIGIT, FAS, and CD52 loci target sites. Cropped image from iBright FL 1000, size marker: GeneRuler 100 bp DNA Ladder (ThermoFisher Scientific). Full-length gel image is presented in Expanded Supplementary Figure S1d.

Supplementary Figure 2



Supplementary Figure 2: **a**, *In-vitro* cleavage assay with dilution series. The amplicon containing the DNMT1 locus target sequence is cleaved with divergent Cas12a nucleases: MAD7, Cas12a Ultra, Cas12a V3, and LbCas12a using wild-type crRNA and Split 3 STAR-crRNA at nuclease : gRNA : target DNA in 20 : 60 : 1, 10 : 30 : 1, 5 :10 : 1, 2.5 : 7.5 : 1, 1.25 : 3.75 : 1, and 0.625 : 1.875 : 1 molar ratio. Cropped image from iBright FL 1000, size marker: GeneRuler 100 bp DNA Ladder (ThermoFisher Scientific). Full-length gel image is presented in Expanded Supplementary Figure S2a. **b**, Structure of MAD7-optimized crRNA and native LbCas12a crRNA. **c**, INDEL frequency (%) of LbCas12a with MAD7-optimized wild-type crRNA (MAD7-crRNA), MAD7-Split 3 STAR-crRNAs, and native Lb-crRNA and Lb-Split 3 STAR-crRNA targeting the DNMT1 and PDCD1 locus, measured by amplicon sequencing (error bars: mean ± SD for n ≥ 3). **d**, INDEL frequency (%) MAD7 with wild-type crRNA (MAD7-crRNA) and native Lb-crRNA targeting the DNMT1 and PDCD1 locus, measured by amplicon sequencing (error bars: mean ± SD for n ≥ 3).