Supporting Information

Amplified detection of nucleic acids and proteins using an isothermal proximity CRISPR Cas12a assay

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Content

Table S1. DNA sequences and modifications.

| Name	Sequence (5'→3')
Nucleic Acid Detection	
P2 (Template)	GCT TGT GGC CG TTTA CGT CGC CGT CCA GCT CGA CCTCAGC CGTAGA TT GAC TCT GGC TTT-InvT
P1 (Primer)	ATC TCT CTG AAG TT TCTACG
Blocking DNA	TTT TTT CGTAGAᶜ
Synthetic Target	AAA AGA TAA CAA GAA AGAC AAA GCC AGA GTC CTT CAG AGA GA TAC AGA AAC TCT AAT TCA
CRISPR-Cas12a	
crRNA	UAA UUU CUA CUA AGU GUA GAU CGU CGC CGU CCA GCU CGA CC
Signal Reporter	FAM-TTATT-Quencher

Supporting Figures
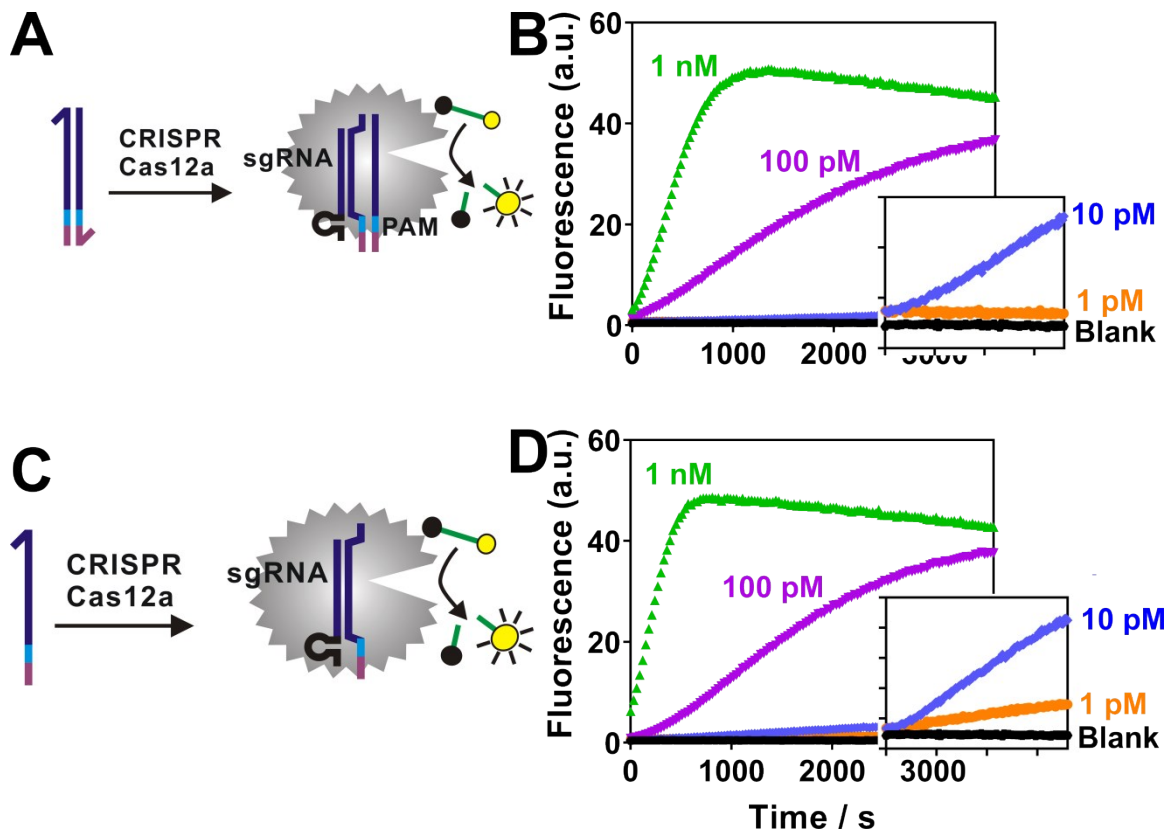
Figure S1. Direct detection of double-stranded DNA (dsDNA) (**A**, **B**) or single-stranded DNA (ssDNA) (**C**, **D**) using direct CRISPR RNA (crRNA) recognition and Cas12a cleavage. The limit of detection (LOD) was determined to be 10 pM for dsDNA (**B**) and 1 pM for ssDNA (**D**).
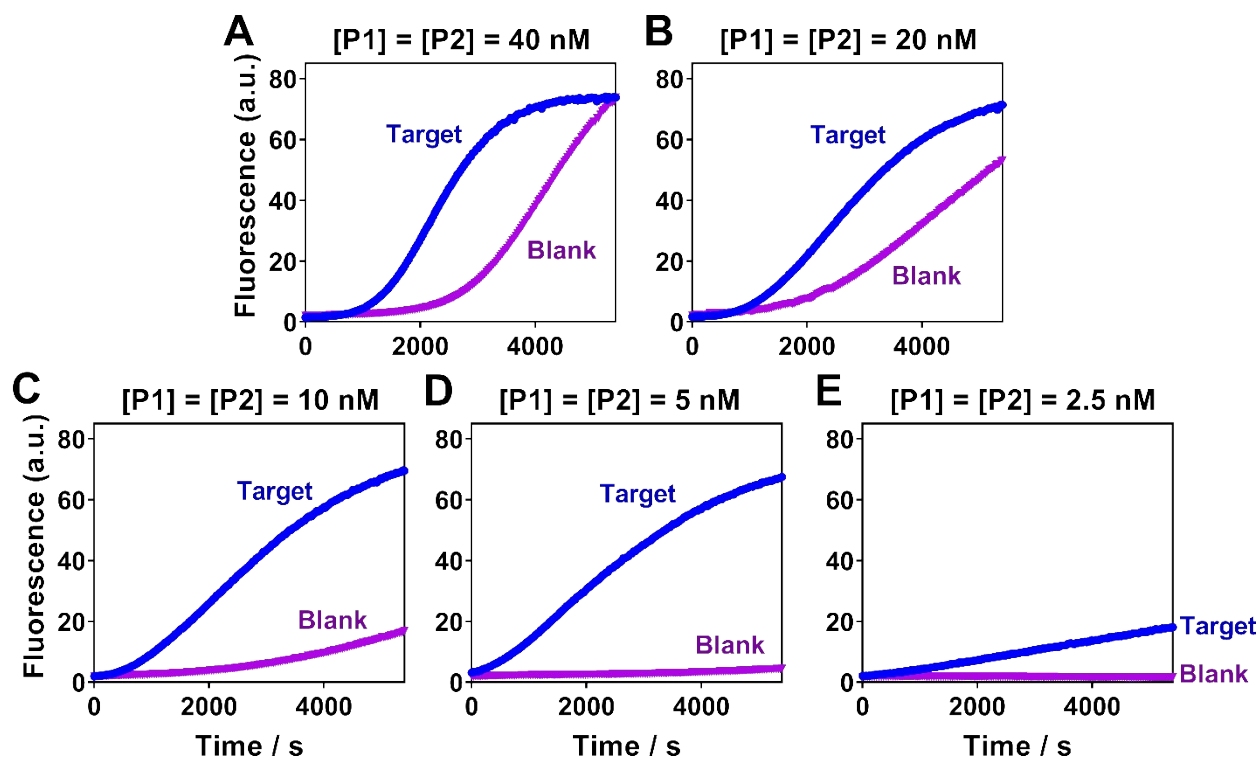
Figure S2. Optimization of the concentrations of proximity probes P1 and P2 for iPCCA. Binding-induced primer extension using varying concentrations of P1 and P2 from 40 nM to 2.5 nM. The optimal concentration of P1 and P2 is 5 nM as it maximizes the target-dependent fluorescence signal and minimizes background signal.
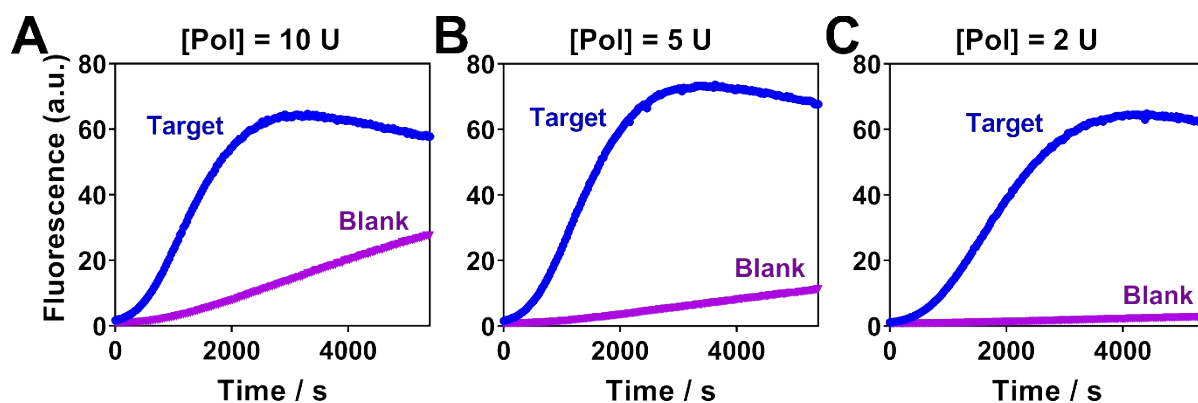

Figure S3. Optimization of the concentrations of DNA polymerase (Klenow Fragment, unit) for iPCCA. The optimal amount of Klenow Fragment was found to be 5 units, as it maximizes detection signals and kinetics while maintains a reasonably low background.
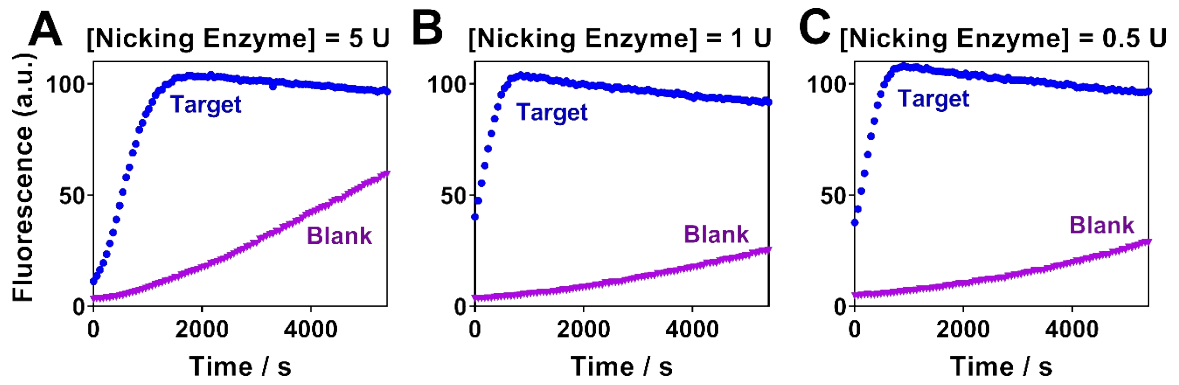
Figure S4. Optimization of nicking endonuclease for iPCCA. The optimal amount of nicking endonuclease was found to be 0.5 units, as it maximizes detection signals and minimizes the background.
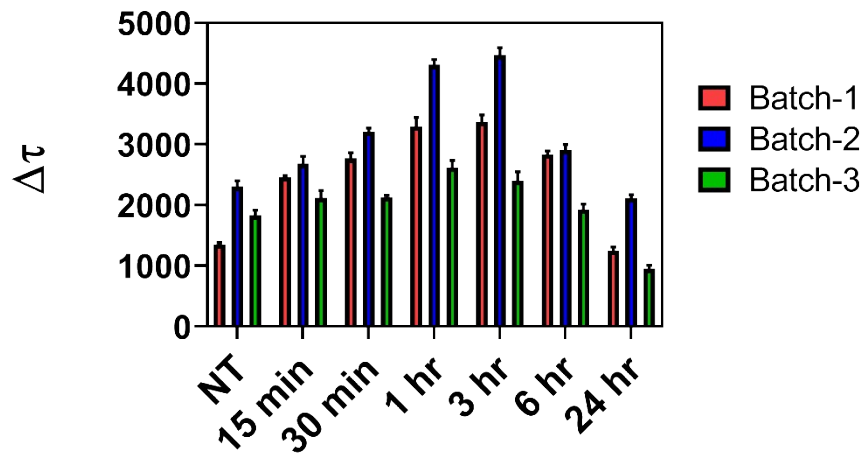

Figure S5. Quantitative profiling of allergen-mediated mast cell activation in three independent cultures of BMMCs. The same temporal profiles were obtained from each culture of mast cells sensitized using TNP-specific IgE and stimulated with 100 ng/ml TNP-BSA and SCF for the indicated time points.