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Supplemental information

Visualization of direct and diffusion-assisted RAD51 nucleation by full-length human BRCA2 protein

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Supplementary Figures

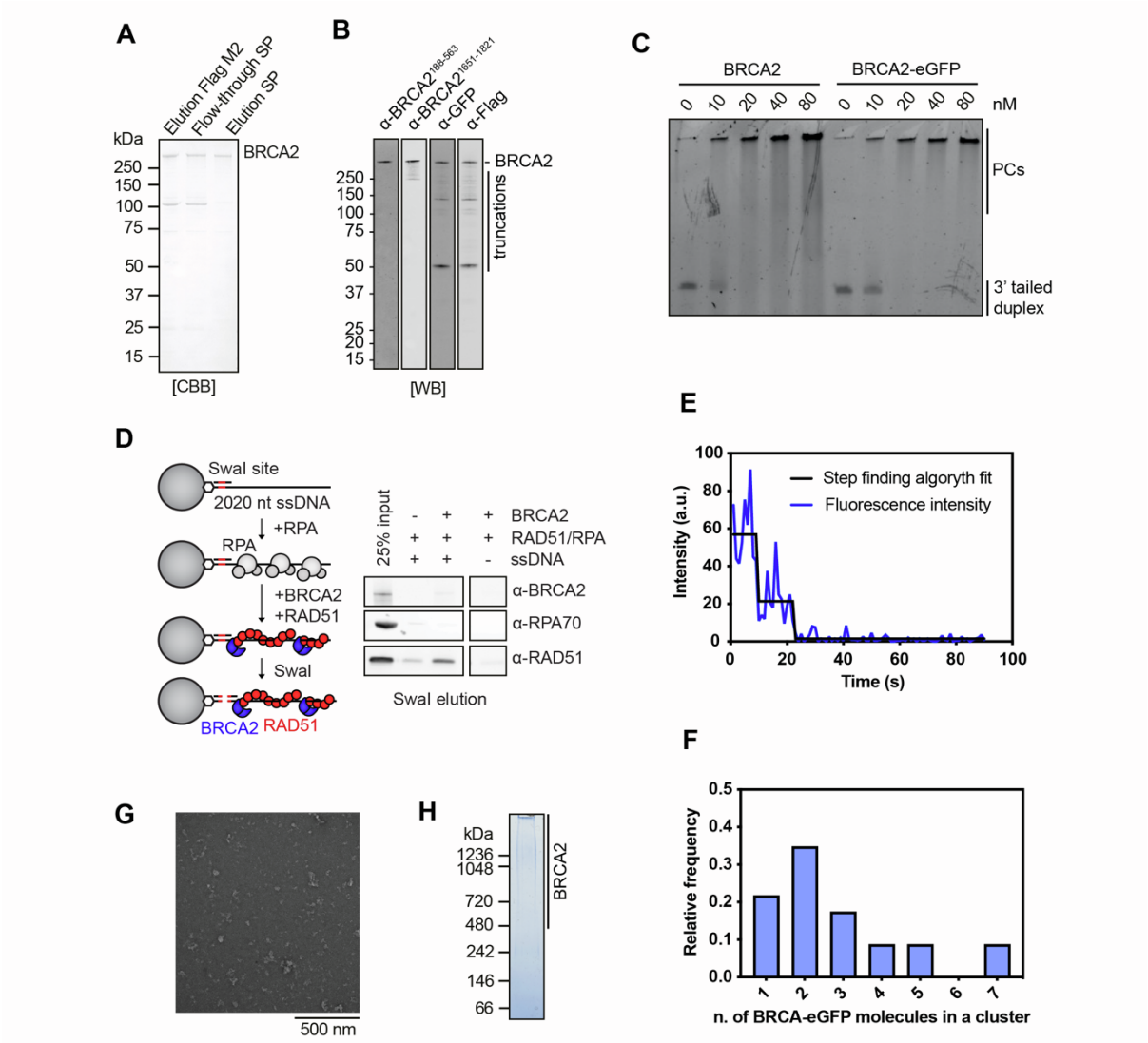

Figure S1: BRCA2 characterization. Related to Fig. 1. (A) Purification of full-length human BRCA2. **(B)** Western blot of purified BRCA2-eGFP performed using antibodies targeting indicated epitopes (N-terminal and central region of BRCA2; eGFP; FLAG). **(C)** Electrophoretic mobility shift assay (EMSA) performed with 167mer 3' tailed DNA and BRCA2 or BRCA2-eGFP. PCs - protein-DNA complexes. **(D)** DNA capture assay. Biotinylated RPA-coated 2020 nt-long ssDNA immobilized on streptavidin-coated beads was incubated with RAD51 and BRCA2, washed and proteins were eluted via SwaI cleavage of the DNA. Samples were analysed by SDS-PAGE. Quantitative analysis was performed following western-blotting and fluorescent detection using Licor system. **(E)** Examples of a fluorescence intensity trace of the BRCA2-eGFP cluster during continuous photobleaching. Black line represents stepping fit of the trace. **(F)** Histogram of BRCA2 cluster size in the presence of RAD51 (N = 23 molecules). **(G)** Electron micrograph of recombinant BRCA2 negatively stained with uranyl acetate. The sample is polydisperse owing to the range of oligomeric states observed. **(H)** 3-12% Native PAGE of recombinant BRCA2, stained with Coomassie.

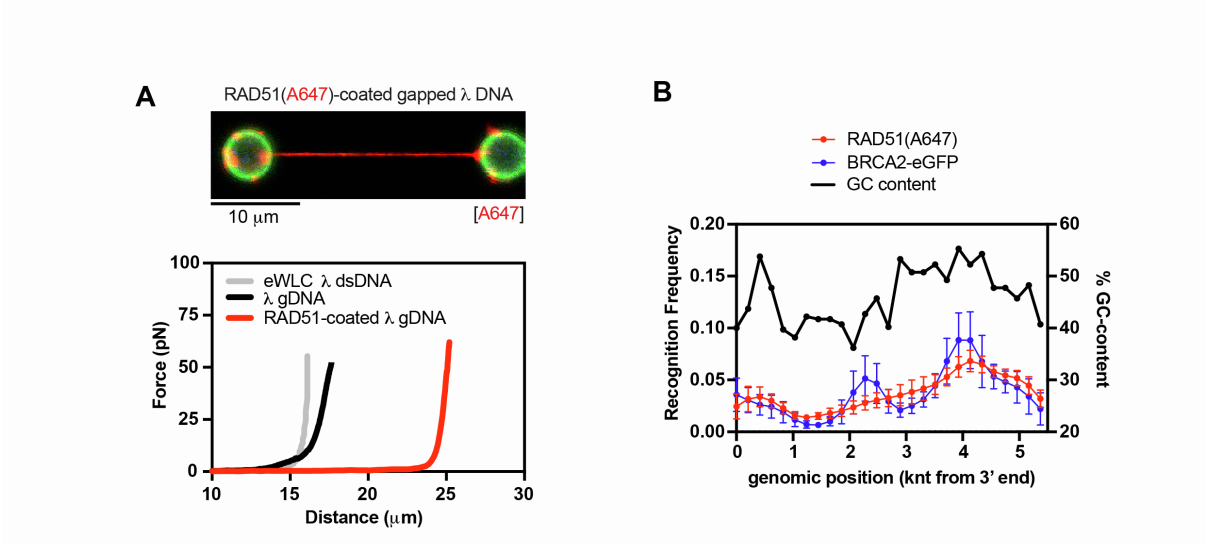

Figure S2: RAD51-gDNA characterization and further nucleation position-sequence analysis. Related to Fig. 2 and Fig. 3. (A) Top: representative image of RAD51(A647)-coated λ gDNA held at ~5 pN force. Bottom: force extension curves of λ gDNA (with 5.374 knt-long ssDNA gap, black), force extension curves of λ gDNA coated with RAD51(A647) (red). Gray line represents extensible worm-like chain model (eWLC) for 48.5 kb λ dsDNA as a reference. **(B)** Position analysis of RAD51(A647) (red) or BRCA2-eGFP (blue) binding along the length of 5.374 knt ssDNA gap in the absence of RPA. N = 6 molecules. Error bars represent S.E.M. 200 nt bins. GC-content of the DNA sequence within each bin is indicated in black.

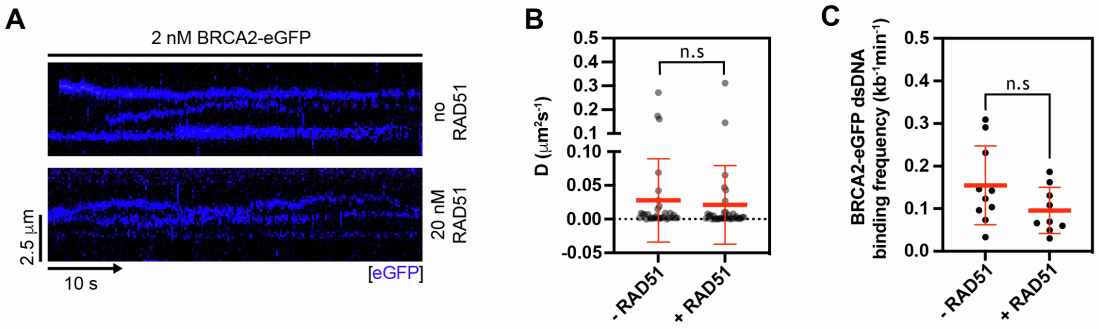

Figure S3: BRCA2 diffusion on dsDNA in the absence of RAD51. Related to Fig. 4. (A) Representative kymographs demonstrating diffusion of BRCA2-eGFP (blue) along λ dsDNA at 10 pN in the presence of 50 mM NaCl, 2 mM $MgCl_2$ and 2 mM ATP in the presence or absence of 20 nM RAD51 (unlabelled). **(B)** Diffusion coefficient (D) calculated from MSD analysis for BRCA2-eGFP complexes moving on dsDNA in the presence or absence of RAD51. Lines represent mean. Error bars represent S.D. P values by Mann-Whitney test. (n.s.) $P > 0.05$; (*) $P \leq 0.05$; (**) $P \leq 0.01$; (***) $P \leq 0.001$; (****) $P \leq 0.0001$. **(C)** dsDNA binding frequency of BRCA2-eGFP in the presence or absence of 20 nM RAD51, 50 mM NaCl, 2 mM $MgCl_2$, 2 mM ATP on dsDNA held at 10 pN force. Lines represent mean. Error bars represent S.D. P values by student t test.

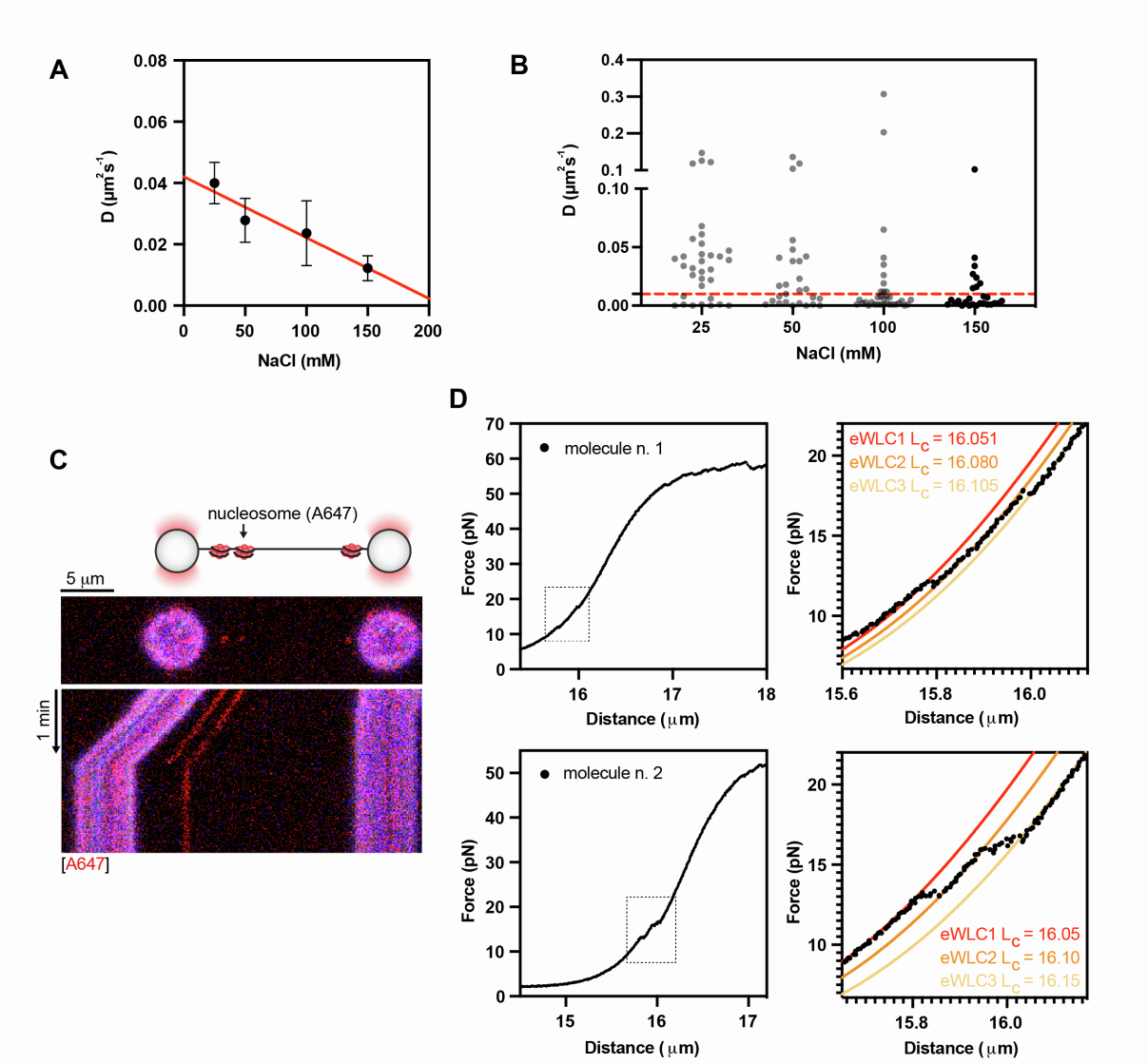

Figure S4: Characterization of chromatinized λ DNA. Related to Fig. 5. (A) Diffusion coefficient (N = 27-34) calculated for BRCA2-RAD51 complexes as a function of salt concentration. Error bars represent S.E.M**. (B)** Diffusion coefficient distribution calculated for BRCA2-RAD51 complexes at different salt concentrations. Red dashed line represents static fraction cut-off. **(C)** Representative kymograph showing stretching of sparsely chromatinized λ DNA. Alexa Fluor 647-labelled nucleosomes are shown in red. **(D)** Two examples of force-distance curve of sparsely chromatinized λ DNA. Individual unwrapping events characterized by sudden distance increase under the same force are highlighted in a dashed line box. Lines correspond to extensible worm-like chain (eWLC) models of dsDNA aligned with the corresponding force-extension curve segments. L_c corresponds to calculated contour length of DNA from the fit. L_c difference between adjacent eWLCs corresponds to length of DNA released during a nucleosome unwrapping event.

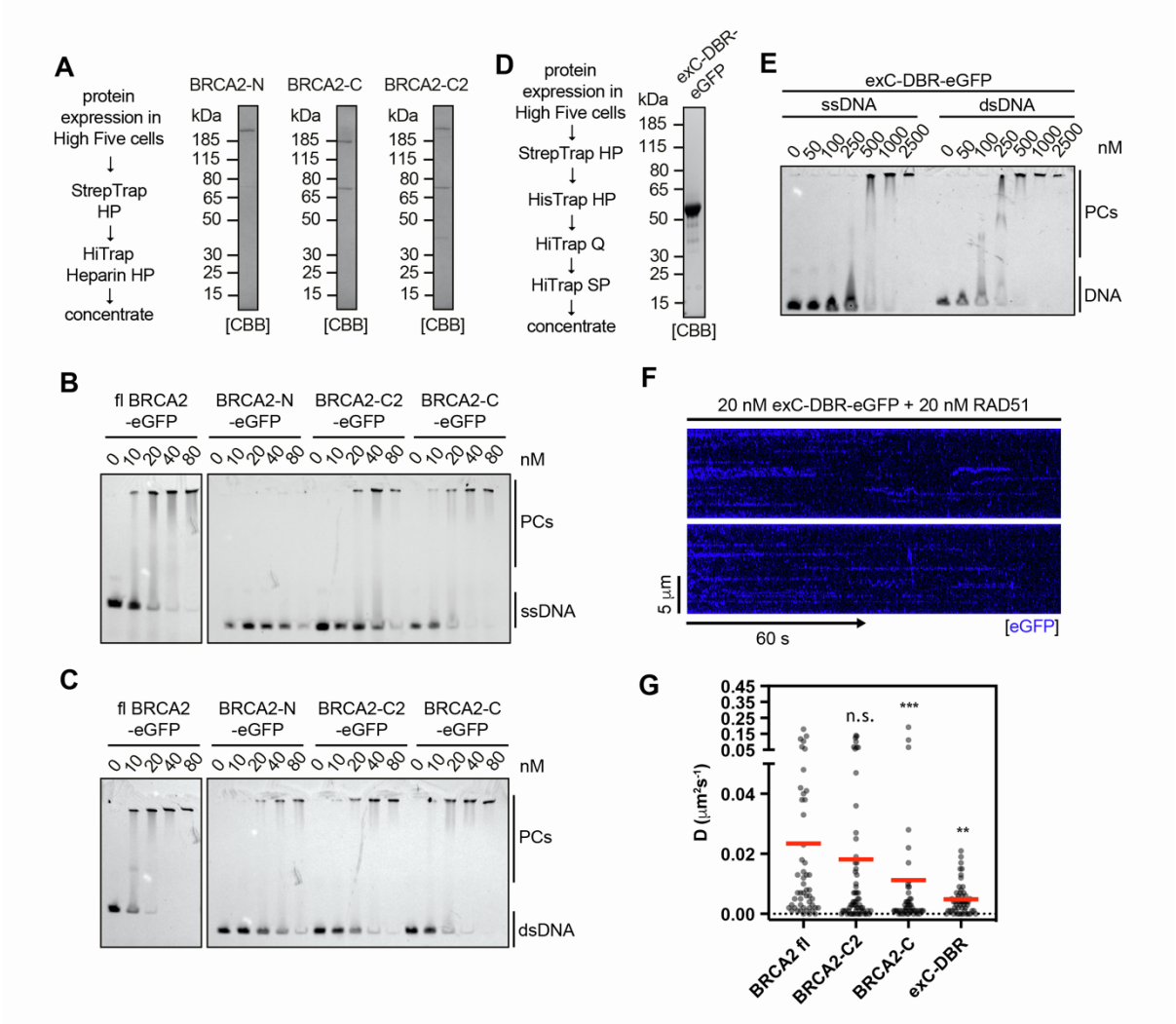

Figure S5: Further characterization of BRCA2 mutants. Related to Fig. 6. (A) Purification of BRCA2-N, BRCA2-C and BRCA2-C2 truncation mutants. Proteins were resolved by SDS-PAGE and stained by Coomasie Briliant Blue staining (CBB). **(B)** Electrophoretic mobility shift assay (EMSA) to assess affinity of BRCA2 and BRCA2 truncation mutants to 100mer ssDNA. **(C)** Electrophoretic mobility shift assay (EMSA) to assess affinity of BRCA2 and BRCA2 truncation mutants to 100mer dsDNA. PCs - protein-DNA complexes. **(D)** Purification of exC-DBR-eGFP fusion. Protein resolved by SDS-PAGE and stained by Coomasie Briliant Blue staining (CBB). **(E)** Electrophoretic mobility shift assay (EMSA) to assess affinity of exC-DBR-eGFP to 100mer ss- and dsDNA. PCs - protein-DNA complexes. **(F)** Two representative kymographs demonstrating limited diffusion of exC-DBR-eGFP (blue) along λ dsDNA at 10 pN in the presence of 50 mM NaCl, 2 mM $MgCl_2$, 2 mM ATP and 20 nM RAD51 (unlabelled). **(G)** Diffusion coefficient (D) calculated from MSD analysis for exC-DBR-eGFP molecules moving on dsDNA in the presence of RAD51. Lines represent mean. Data for BRCA2 fl, BRCA2-C and BRCA2-C2 are of the same dataset as in Fig. 6H. P values by Mann-Whitney test. (n.s.) $P > 0.05$; (*) $P \leq 0.05$; (**) $P \leq 0.01$; (***) $P \leq 0.001$; (****) $P \leq 0.0001$.

Oligonucleotides		
Oligonucleotide name and sequence	Source	Identifier
100mer ssDNA 5’-Cy5- CCAAGAAGCTGTTCAGAATCAGAATGAGCCGCAACTT CGGGATGAAAATGCTCACAATGACAAA- TCTGTCCACGGAGTGCTTAATCCAACTTACCAAGCT-3’	IDT, this study	N/A
100mer dsDNA bottom 5’- AGCTTGGTAAGTTGGATTAAGCACTCCGTGGACAGAT TTGTCATTGTGAGCATTTTCATCCCGAAGTTGCGGCTC ATTCTGATTCTGAACAGCTTCTTGG-3’	IDT, this study	N/A
3’tailed DNA top 5’-Cy5- CTGCTTTATCAAGATAATTTTTCGACTCATCAGAAATA TCCGTTTCCTATATTTATTCCTATTA- TGTTTTATTCATTTACTTATTCTTTATGTTCATTTTTTAT ATCCTTTACTTTATTTTCTCTGTTTATTCATTTACTTATT TTGTATTATCCTTATCTTATTTA-3’	IDT, this study	N/A
3’tailed DNA bottom 5’-CGGATATTTCTGATGAGTCGAAAAATTATCTTGA- TAAAGCAG-3’	IDT, this study	N/A
biot-SwaI-2023bp-f 5’-biotin- AACAAAATATTAACGCTTACAATTTAAATGGTGGCAC TTTTCG-3’	IDT, this study	N/A
2023bp-r 5’-TCAACGTGCAATCAAGTTAATGAATCGG-3’	IDT, this study	N/A
biot-SwaI-rc 5’- CGAAAAGTGCCACCATTTAAATTGTAAGCGTTAATAT TTTGTT-3’	IDT, this study	N/A
λ DNA end-cap 1 5’- AGGTCGCCGCCCGGAGTTGAACG(BT)(BT)T(BT)T(BT) ACGTTCAACTCC-3’	IDT, Belan et al, 2021	N/A
λ DNA end-cap 2 5’-GGGCGGCGA CCTCAAGTTGGACAA(BT)T(BT)T(BT)(BT)TGTCCAACT TG-3’	IDT, Belan et al, 2021	N/A
λ DNA handles oligo 1 5’-GGGCGGCGACCTGGACAA-3’	Sigma Aldrich, Belan et al, 2021	N/A
λ DNA handles oligo 2 5’- AGGTCGCCGCCCTTTTTTTT(BT)TT(BT)TT(BT)-3’	Sigma Aldrich, Belan et al, 2021	N/A

λ DNA handles oligo 3 5'- T(BT)TT(BT)TT(BT)TTTTTTTAGAGTACTGTACGATCTA GCATCAATCTTGTCC-3')	Sigma Aldrich, Belan et al, 2021	N/A
crRNA λ4 5'-CAGAUAUAGCCUGGUGGUUCGUUUUAGAGC UAUGCUGUUUUG-3'	IDT, Belan et al, 2021	N/A
crRNA λ5 5'- GGCAAUGCCGAUGGCGAUAGGUUUUAGAGCUAUG CUGUUUUG-3'	IDT, Belan et al, 2021	N/A
tracr RNA (trRNA) 5'-GGACAGCAUAGCAAGU UAAAAUAAGGCUAGUCCGUUAUCAACUUGAAA AAGUGGCACCGAGUCGGUGCUUUUU-3'	IDT, Belan et al, 2021	N/A

Table S1: Oligonucleotides used in the study. Related to KEY RESOURCE TABLE.