**Online methods**

**Protein modeling**. Prediction of the protein structure of PmCDA1 was done by I-TASSER1 based on the homology with human AID 2 (PDB: 5W1C).

**Plasmid construction**. JM109 chemically competent E. coli were used for cloning and preparation of the plasmids by using FastGene Plasmid Mini Kit or NucleoSpin® Plasmid Transfection-grade for transfection. For the yeast experiments, Target-AID variant constructs were made by modifying the original Target-AID vector pRS315e\_pGal-nCas9(D10A)-PmCDA1 (Addgene #79617)3 (Supplementary sequence 1). Plasmids for gRNA expression were made from p426-SNR52p-gRNA.CAN1.Y-SUP4t (Addgene #43803)4 by replacing the target sequence (Supplementary table 1). For mammalian transfection, plasmid constructs were generated based on YE1-BE4max (Addgene #138155)5 (Supplementary sequence 2). SaCas9-AID versions were made from pX601-AAV-CMV::NLS-SaCas9-NLS-3xHA-bGHpA;U6::BsaI-sgRNA(Addgene #61591)6. ScpI promoter7 and ploy-A tail8 (Supplementary sequence 3) were synthesized by Eurofin. For R-loop assay, nickase SaCas9(D10A) vector was developed from dead SaCas9 (Addgene #138162)5 by replacing the promoter with ScpI. The U6 promoter gRNA plasmid was constructed based on pU6-Sp-pegRNA-RNF2 (Addgene # 135957). DNA was PCR-amplified by PrimerSTARMax polymerase (TaKaRa) followed by gel extraction (FastGene Gel/PCR Extraction Kit). Gibson assembly follows the reported protocol9 and Ligation high Ver.2 (TOYOBO) was used for ligation reactions.

**Yeast experiments**. *Saccharomyces cerevisiae* BY4741 cells were transformed by the lithium acetate method and grown in the galactose-induction conditions as described previously3. CAN1 on-target mutants and LYP1 off-target mutants were selected by canavanine (60 ug/ml) and thialysine (S-Aminoethyl-l-cysteine) (100 ug/ml), respectively. Mutation frequencies were calculated by colony formation of the serial dilutions on the selection media. The plate images were acquired using an Image Quant LAS 4000 (GE Healthcare Japan, Tokyo, Japan).

**Mammalian cell experiments**. HEK293T cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were incubated at 37˚C with 5% carbon dioxide and passaged every 3–4 days. For transfection, cells were seeded onto 48-well poly-lysine-coated plate (Corning®) at the density of 50,000 cells/well with 250 µl of DMEM and incubated for about 24 hours. For both on-target and R-loop assay, cells were transfected with 300 ng of base-editor plasmid, 300 ng of nSaCas9 plasmid, 200 ng of SpCas9 guide RNA plasmid, and 200 ng of SaCas9 guide RNA plasmid by FuGENE® HD Transfection Reagent (Promega) following the manufacturer’s instructions. For transfection controls, GFP expression plasmid was introduced, while for R-loop assay controls, pUC19 DNA was co-transfected with Sp and Sa guide RNA plasmid together to keep the total quantity of transfected DNA at 1,000 ng. The transfected cells were incubated for 24 hours and the medium was exchanged with 250 µl of fresh DMEM. The cells were harvested 72 hours after transfection and the genomic DNA was extracted by using Kaneka Easy DNA Extraction Kit (Version 2).

**Fluorescence-activated cell sorting (iRFP670)**. To normalize the transfection efficiency, cell sorting was performed for all-in one SaCas9-AID versions. HEK293T cells were seeded in a collagen-I-coated 24-well plate (IWAKI) at a density of 100,000 cells/well with 500 µl DMEM. Transfection proceeded after ~24 hours incubation and a total of 1,000 ng plasmid was applied along with 2 µl of FuGENE® HD Transfection Reagent. The transfected cells were incubated for 24 hours and the medium was exchanged with fresh 500 µl DMEM. The cells were harvested 72 hours after transfection and washed with 500 µl PBS solution. The cells were trypsinized and resuspended with DMEM media and centrifuged at 1,000 rpm for 2 mins to collect the cells. After removing the solution and wash with 800 µl PBS solution, cells were resuspended into fresh 800 µl PBS solution and filtered through cell strainer caps (Falcon®). Finally, cell sorting was executed by SH800S Cell Sorter (Sony Biotechnology Inc., Japan) with the standard iRFP670 expression plasmid setting following the manufacture’s instruction.

**Next generation sequencing (NGS)**. The targets and primers used in this study are listed in Supplementary table 2 and 3. The amplicon samples were prepared as previously described3, except for the amplicon length of 220 bp and analyzed by Miniseq system (Illumina, CA, USA) to obtain paired 2 × 150 bp read length and more than 24,000 reads per sample. Obtained Fastq data were processed and analyzed by CLC genomics workbench version 12 as previously described and Crispresso210. Note, all analyzed data were obtained with paired end reads except for highly redundant regions (gRNA-dependent off-target VEGFA site 1, VEGFA site 3, and VEGFA site 4), for which only reverse end reads were used as the forward reads and did not pass the quality criteria due to high redundancy. The Fastq sequence data were deposited to SRA…

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