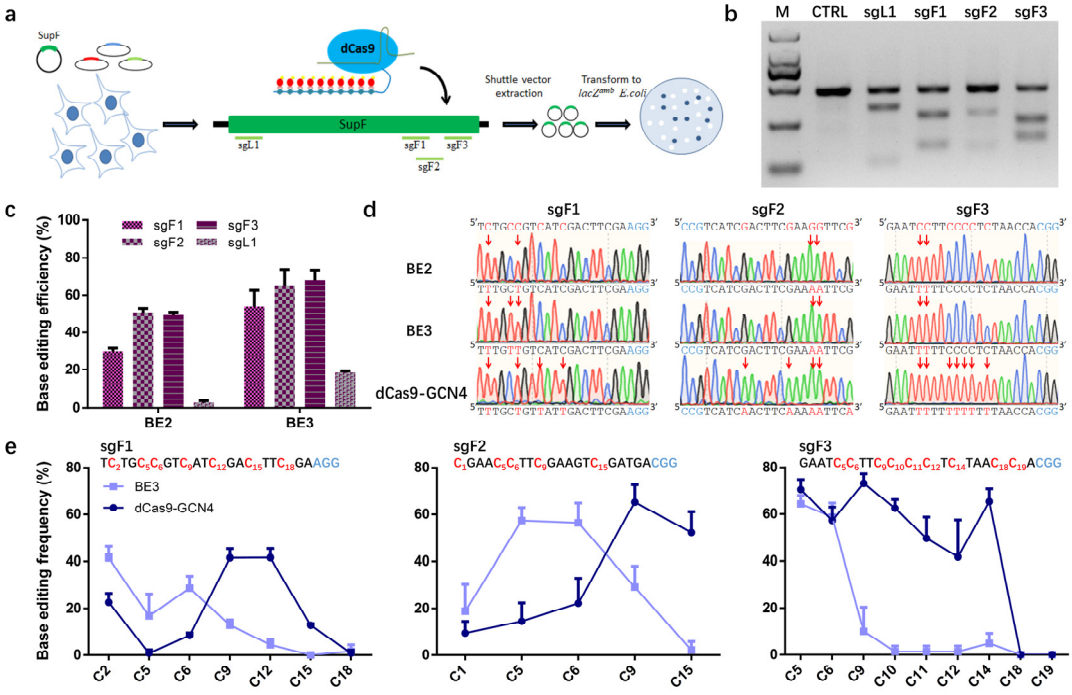
a
SupF
dCas9
SupF
sgL1
sgF1
sgF3
sgF2
Shuttle vector extraction
Transform to lacZ^amb E.coli
b
M
CTRL
sgL1
sgF1
sgF2
sgF3
c
Base editing efficiency (%)
sgF1
sgF3
sgF2
sgL1
BE2
BE3
d
sgF1
sgF2
sgF3
BE2
BE3
dCas9-GCN4
e
sgF1
TC2TGC5C6GTC9ATC12GAC15TTC18GAAGG
BE3
dCas9-GCN4
Base editing frequency (%)
C2
C5
C6
C9
C12
C15
C18
sgF2
C1GAAC5C6TTC9GAAGTC15GATGACGG
C1
C5
C6
C9
C15
sgF3
GAATC5C6TTC9C10C11C12TC14TAAC18C19ACGG
C5
C6
C9
C10
C11
C12
C14
C18
C19

Figure S1 Activity assay of dCas9-GCN4 in reporter system.

a. Schematic illustration of blue/white colony screening. The plasmids expressing dCas9-GCN4 (blue), scFv-APOBEC-UGI-GB1 (red) and sgRNA (Kelly green) together with the pSP189 vector (green) were co-transfected into HEK293FT cells. Forty-eight hours after transfection, the shuttle vector pSP189 was extracted and transformed into *lacZ*^{amb}*E. coli*. White and blue colonies were formed by edited and non-edited vectors, respectively.

b. Detection of Cas9-mediated on-target cleavage by T7EN1 cleavage assay. Regions targeted by sgRNAs L1, F1, F2 and F3 were amplified by PCR. The PCR products were subjected to T7EN1 cleavage assay. The primers used for PCR amplification are listed (Supplementary information, Table 2S). CTRL, wild type control. M: DL2 000 marker (Takara, 3427A).

c. Base editing efficiency of the sgRNAs L1, F1, F2 and F3 was evaluated by blue/white colony screening. The plasmids expressing sgRNAs L1, F1, F2 and F3 were transiently transfected into HEK293FT cells together with BE2 or BE3. Base conversion frequency was calculated as the number of white colonies among total number of colonies. Error bars (±) indicate the standard deviations of 3 replicates.

d. Sanger sequencing results of white colonies edited by BE2, BE3 or dCas9-GCN4 are shown together with sgRNAs F1, F2 and F3. Sequencing profiles of white colonies are aligned to wild-type genomic sequences (top), including the protospacer (Cs are shown in red; others are shown in black) and PAM (blue). Red arrows indicate substituted nucleotides. Different base editors are labelled on the left. The data shows a representative experiment from three independent experiments.

e. Analysis of base editing window. The C-to-T conversion rates at all possible positions within the protospacer of each sgRNA are shown. The protospacer (Cs are shown in red; others are shown in black) and PAM (blue) are indicated in the sequences above each chart. Error bars (±) indicate the standard deviations of 3 replicates.