Supplementary Figure Legends

Supplementary Figure 1. Cloning of Loop Variants and C-terminal truncated AID. A, shown is an alignment of the amino acid sequence of AID, against the C-terminal domains of APOBEC3G (201-384) and APOBEC3F (201-373). Residues are shaded according to degree of conservation. The loop of interest is highlighted by shading with a red box and limited secondary structure elements of APOBEC3G are shown. The cloning oligonucleotides are shown overlaid on the protein sequence denoting the corresponding portions of the AID gene that they align to (straight arrow) or the non-annealing portions that encode the loop variations or cloning restriction sites (bent portions of arrows). B, Generation of loop variant expression plasmids started with the plasmid for full-length MBP-AID-pET41, generated as in experimental methods. The first round of PCR generates two fragments where the PCR fragment 1-1 contains the N-terminal portion of AID and a gene encoding the loop from APOBEC3G or APOBEC3F on its 3' end. PCR fragment 1-2 contained a 5' sequence encoding the loop from APOBEC3G or APOBEC3F and the C-terminal portion of AID. After gel purification, the two PCR fragments were joined together through a PCR reaction. Oligonucleotides amplified from the 5' end of 1-1 to the 3' end of 1-2. Annealing of the region encoding the loop variants led to a single full-length product encoding AID with the loop alterations. The PCR product from PCR Round 2 was introduced between an EcoRI and XhoI site in the original MBP-AID-pET41 plasmid. The C-terminal deleted versions of the proteins were generated by amplifying from the start of the MBP gene with primer MBP-For through to AID-ΔC-Rev, which introduces a stop codon after amino acid 181. The PCR product was cloned back into pET41 between NdeI and XhoI sites to generate the final expression plasmids. All clones were verified by sequencing.

Supplementary Figure 2. C-terminal truncation increases deamination activity. Using assay conditions previously described, variable concentrations of a FAM labeled S27-AGC substrate were reacted with purified full-length MBP-AID (1.5 µg) or C-terminal truncated MBP-AID-ΔC (0.5 µg) for 12 hrs at 30 ºC. The products were quantified by taking the ratio of fluorescence of the substrate to the product. Shown here is the end concentration of product per µg of enzyme for each of the constructs. The results demonstrate that the C-terminal deletion (AID-WT) has increased catalytic activity over the full-length enzyme.

Supplementary Figure 3. Expression of loop graft variants. 1 mL cultures AID-WT, AID-3FL and AID-3GL co-expression with the chaperone trigger factor (TF) were separated into soluble (S) and insoluble (IS) fractions, showing that loop grafting does not compromise the expression of soluble protein in this system. The eluted proteins from the amylose affinity resin are shown (E), demonstrating comparable purity to AID-WT with protein yield listed. MBP is also denoted and is the major contaminant protein as confirmed by western blotting.

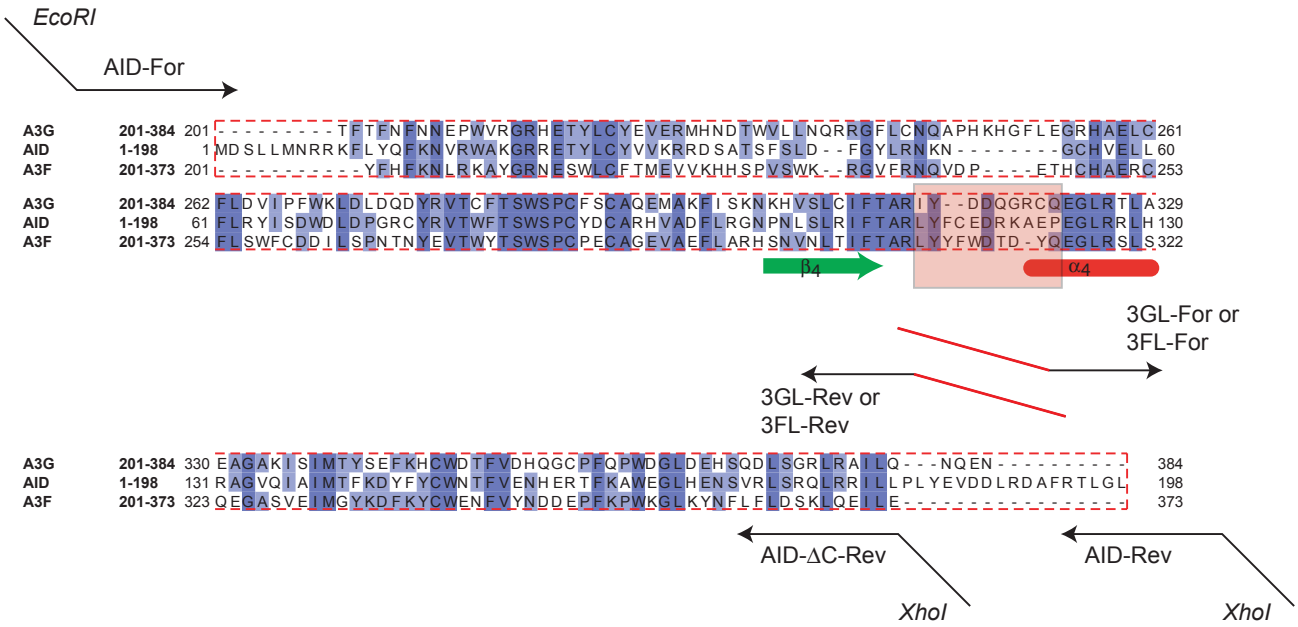
Supplementary Figure 4. Loop graft variants of AID alter sequence preferences for deamination. Under substrate limiting conditions for some substrates, 1 µM of S60-XXC substrate was subjected to 3 µg of enzyme for 12 hrs. The products after APE-mediated cleavage of abasic sites generated by UNG are shown and product formation was quantified. Product formation was broadly classified for good (66-100%, red), average (33-66%, light red) or poor substrates (0-33%, light blue), demonstrating that AID-3FL and AID-3GL shift their sequence preferences relative to AID-WT.

Supplementary Table 1: Oligonucleotides used for cloning or assays

Cloning	
MBP-For	gcc cgc cat atg aaa atc gaa gaa ggt aaa ctg
AID-For	cga ggg aag gat ttc aga att cga aaa cct gta
AID-Rev	tat act cga gtt aca ggc cca ggg tac
AID-ΔC-Rev	atg gtg ctc gag gat atc cta cag cag aat acg acg cag ctg acg
3FL-For	TAT TTT TGG GAC ACT GAC TAC CAG gaa ggc ctg cgt cgt ctg cat
3FL-Rev	CTG GTA GTC AGT GTC CCA AAA ATA ata cag acg cgc ggt aaa aat acg
3GL-For	ATT TAC GAC GAC CAA GGG AGG TGC CAG gaa ggc ctg cgt cgt ctg cat
3GL-Rev	CTG GCA CCT CCC TTG GTC GTC GTA AAT acg cgc ggt aaa aat acg acg cag
Assay	
S60-XXC	gtg gtg tgg ttt gat ggt atg XXC ttg gtg tgg ttg ata gtt gtg atg aat tgt ttt att
S60-AGU	gtg gtg tgg ttt gat ggt atg AGU ttg gtg tgg ttg ata gtt gtg atg aat tgt ttt att
S27-AGC	aga att aag tta AGC tag tta agt tat-6-carboxyfluorescein(FAM)

Supplementary Figure 1

A



B

PCR Round 1:

Template	MBP-AID-pET41		MBP-AID-pET41		
Primers	AID-For	3GL-Rev or 3FL-Rev	3GL-For or 3FL-For		AID-Rev
Products	3FL-1 or 3GL-1		3FL-2 or 3GL-2		

PCR Round 2:

Template	3FL-1 + 3FL-2	or	3GL-1 + 3GL-2	
Primers	AID-For		AID-Rev	

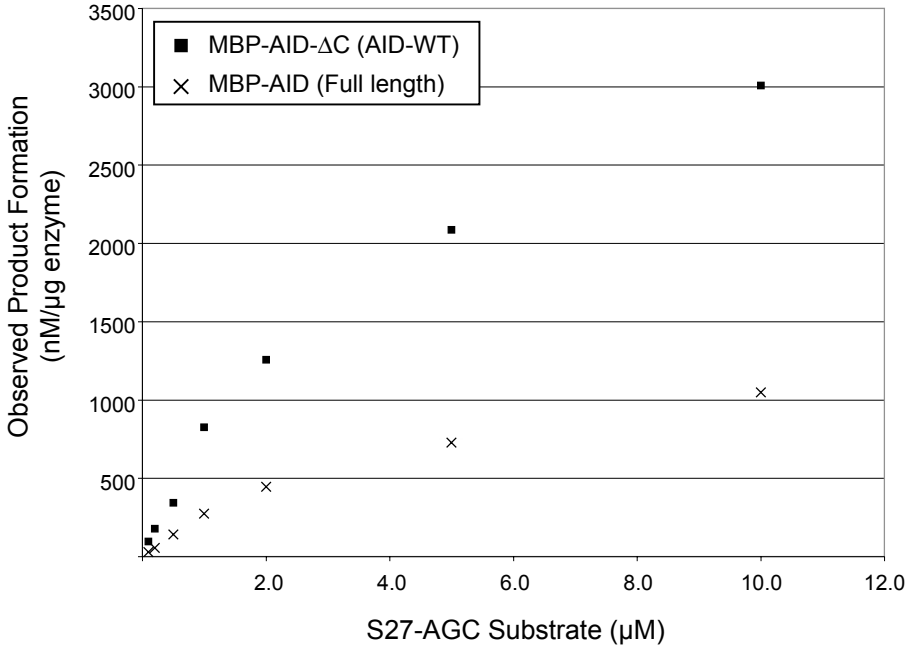
EcoRI/XhoI Digest
Subclone into EcoRI/XhoI digested MBP-AID-pET41

MBP-AID-3GL-pET41 or
MBP-AID-3FL-pET41

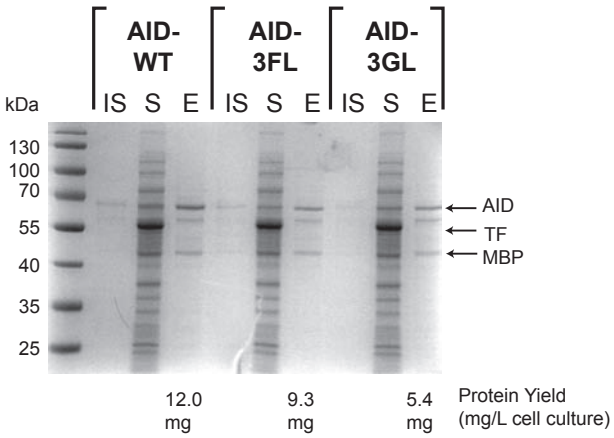
PCR Amplify with MBP-For and AID-ΔC-Rev
NdeI/XhoI Digest
Subclone into NdeI/XhoI digested pET41

MBP-AID-3GL-ΔC-pET41 or
MBP-AID-3FL-ΔC-pET41

Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4