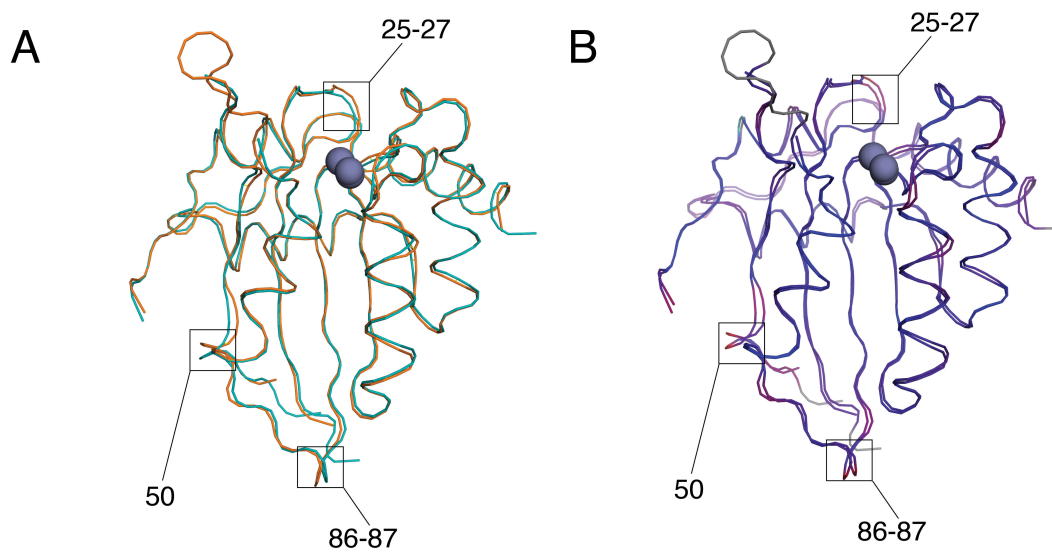
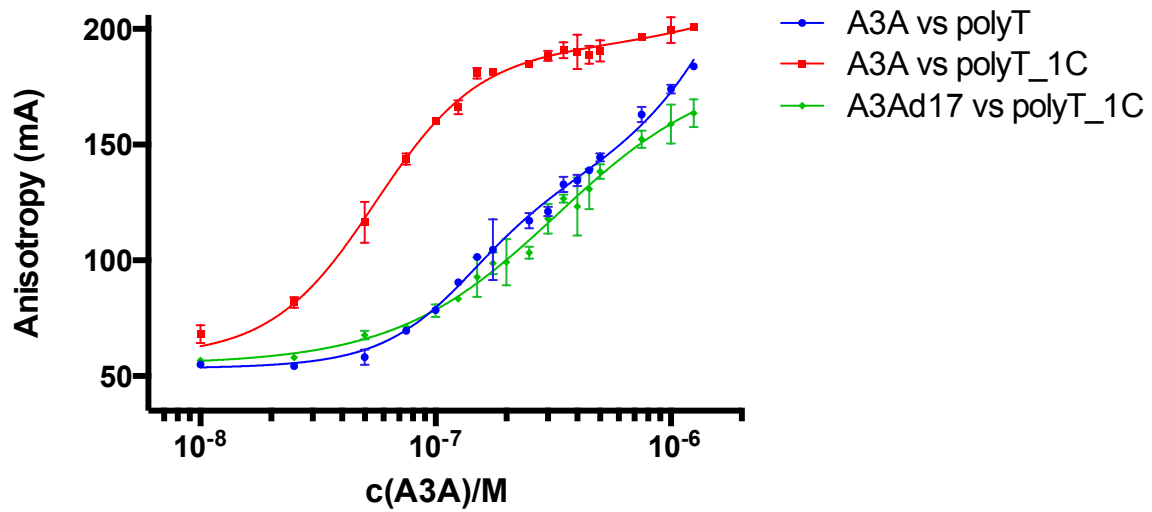


**Supplemental Table 1, related to Table 1.** Nucleotide sequences for ssDNA oligomers used in APOBEC3A binding experiments.

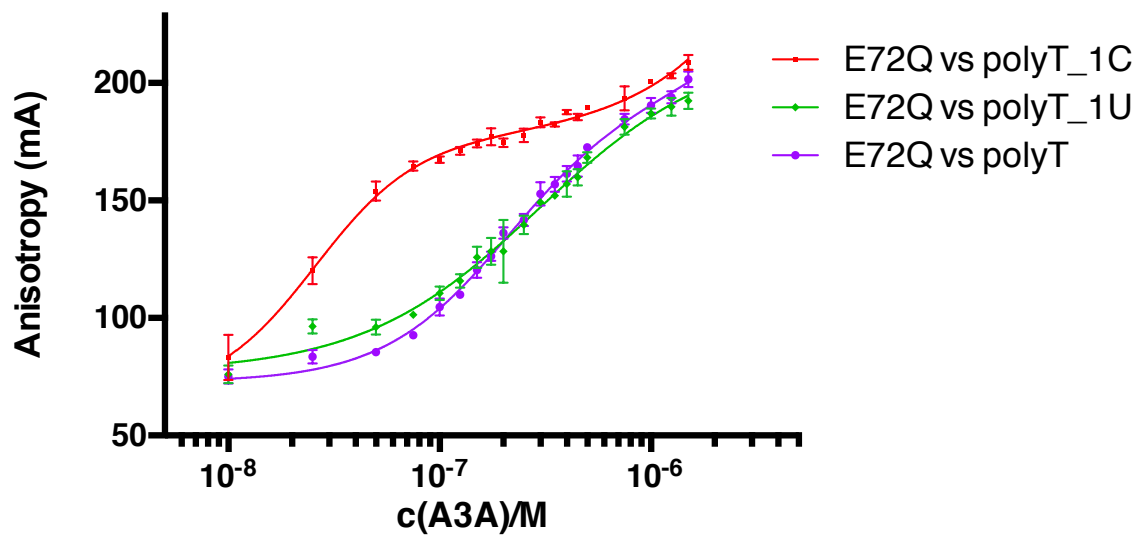
| <b>Oligomer name</b> | <b>Oligomer sequence</b> |
|----------------------|--------------------------|
| polyA                | AAAAAAAAAAAAAAAAA        |
| TTC                  | AAAAAATTCAAAAAA          |
| TCC                  | AAAAAATCCAAAAAA          |
| CCC                  | AAAAAACCCAAAAAA          |
| polyT_1C             | TTTTTTTTCTTTTTT          |
| polyT_2C             | TTTTTTTTCCTTTTT          |
| polyT_3C             | TTTTTTTTCCCTTTT          |
| polyT_4C             | TTTTTTTTCCCCTTT          |
| polyT                | TTTTTTTTTTTTTTTT         |
| polyT_1U             | TTTTTTTTTUTTTTTT         |



**Supplemental Figure 1, related to Figure 2.** Superposition of the two monomers in the crystallographic dimer colored by (A) the two monomers (orange and cyan) and (B) the alpha-Carbon deviations between the two monomers increasing from dark to lighter shades of purple. Regions displaying the highest deviations are indicated by residue numbers.

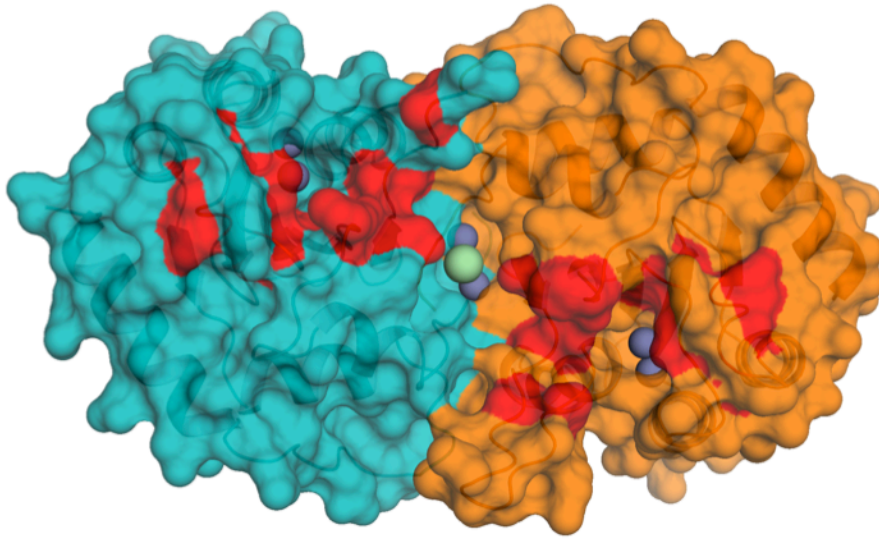


**Supplemental Figure 2, related to Figure 1.** Fluorescence anisotropy measurements show that deleting the N-terminus (A3Ad17) impairs specific, high-affinity binding of APOBEC3A to polyT-1C (ideal substrate). Higher concentrations of protein used in Figure 1 were not possible due to instability of the A3Ad17 protein, making measurement of accurate  $K_d$  or hill coefficient impossible.

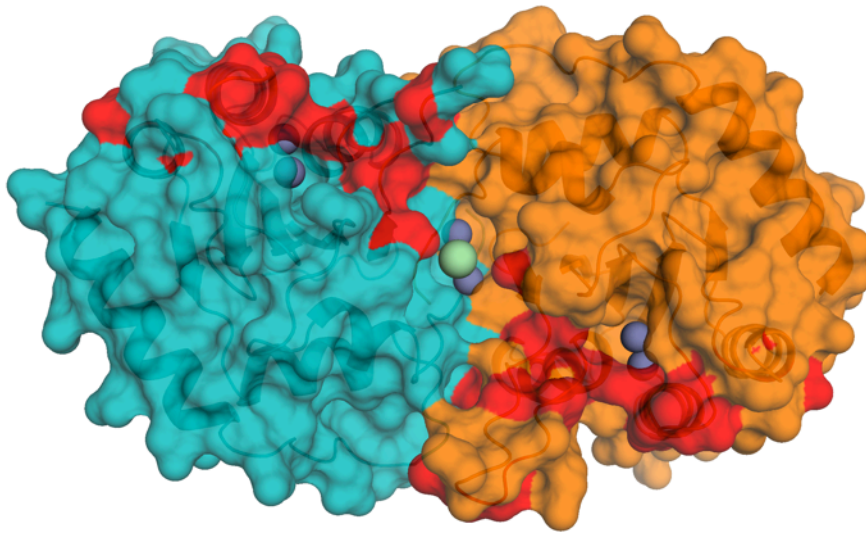


**Supplemental Figure 3, related to Figure 1.** Fluorescence anisotropy measurements of the catalytically inactive variant E72Q show the same kind of substrate dependent cooperativity as observed for E72A.

(A)



(B)



**Supplemental Figure 4, related to Figure 4.** Residues identified to be involved in DNA binding by (A) Didier Trono's (Bulliard et al., 2011) and (B) Angela Gronenborn's and Judith Levin's group (Mitra et al., 2014) are colored in red on the APOBEC3A dimeric structure. The two monomers are in cyan and orange, and the metal ions are indicated as spheres as in Figures 2-4.