Supplementary Information

Substrate sequence selectivity of APOBEC3A implicates intra-DNA interactions

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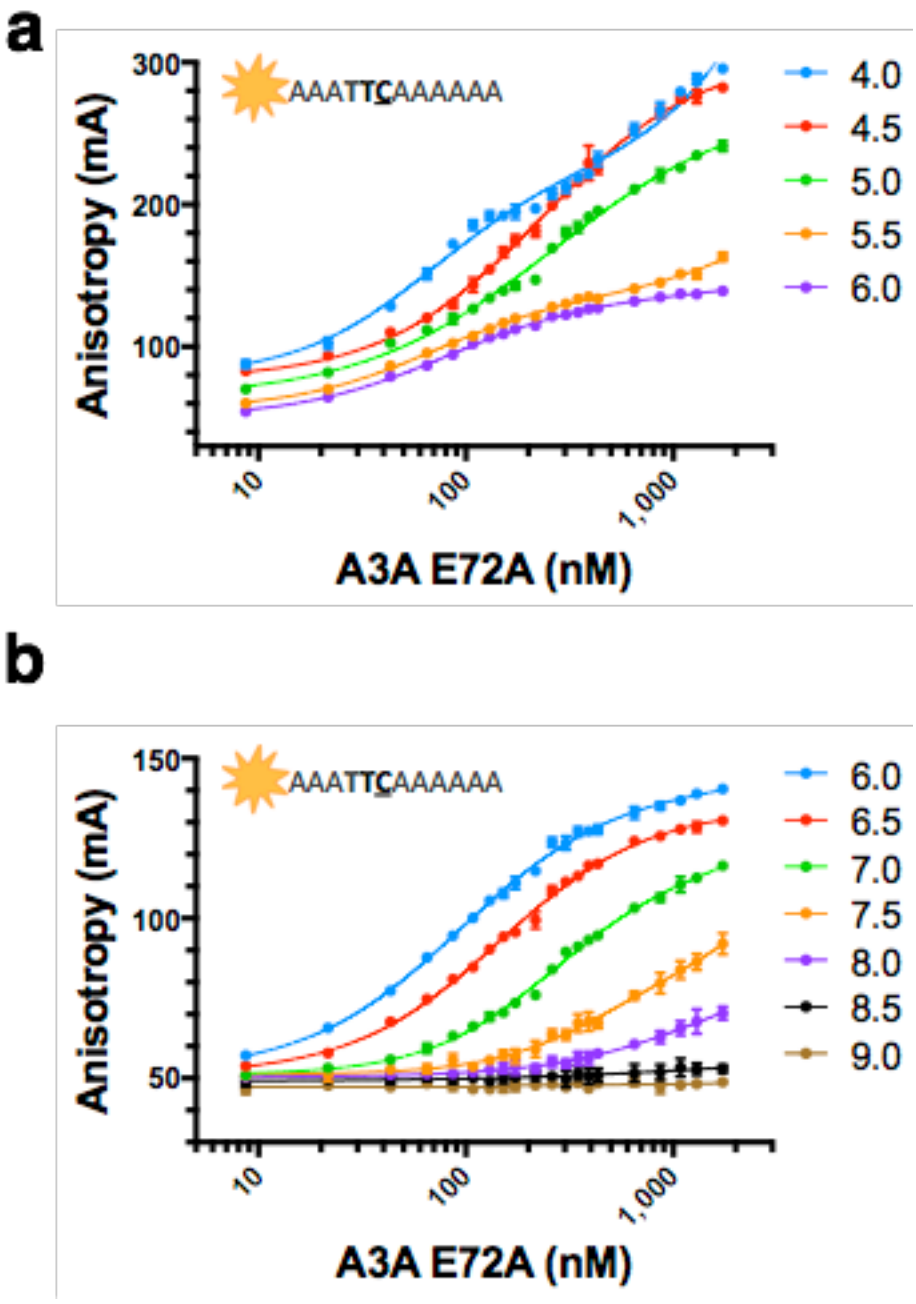
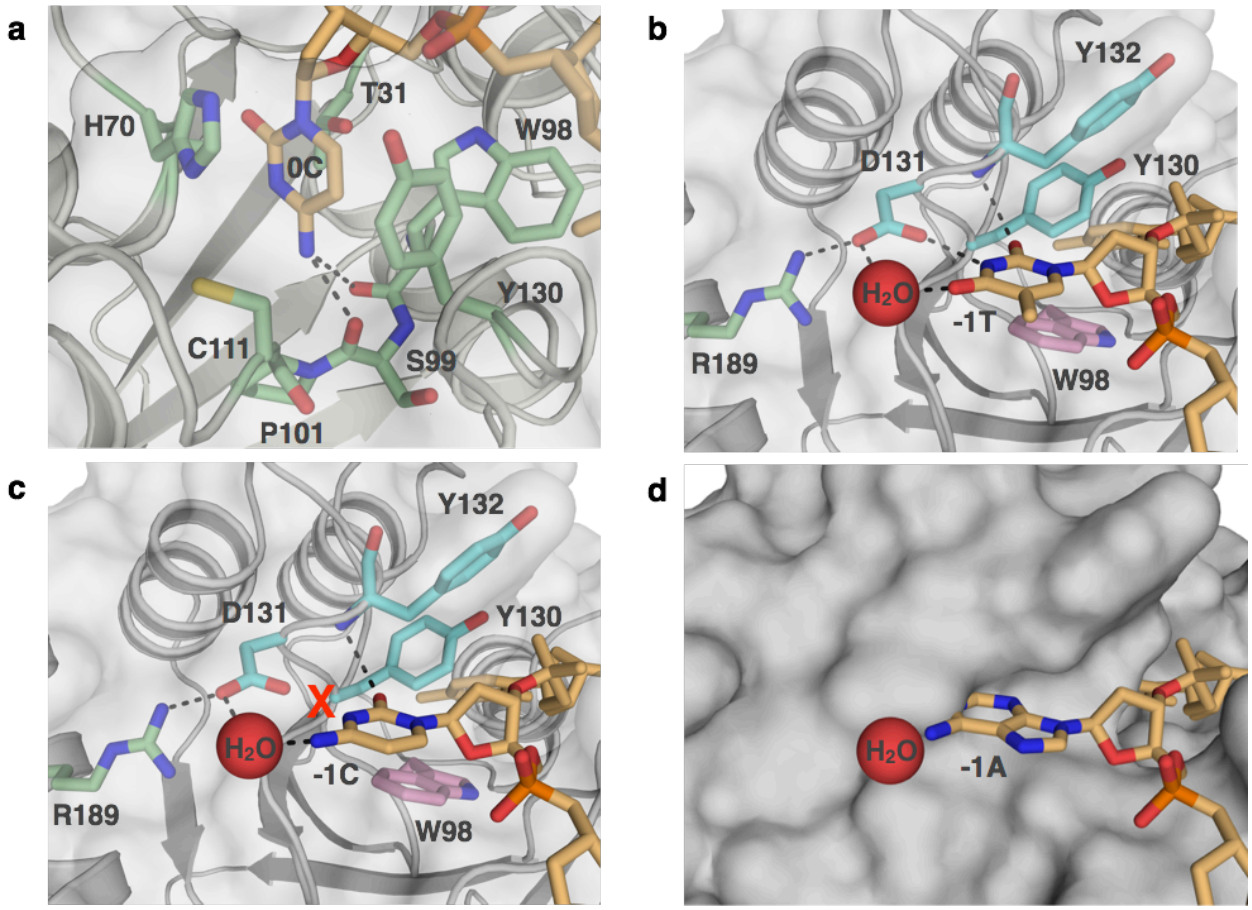


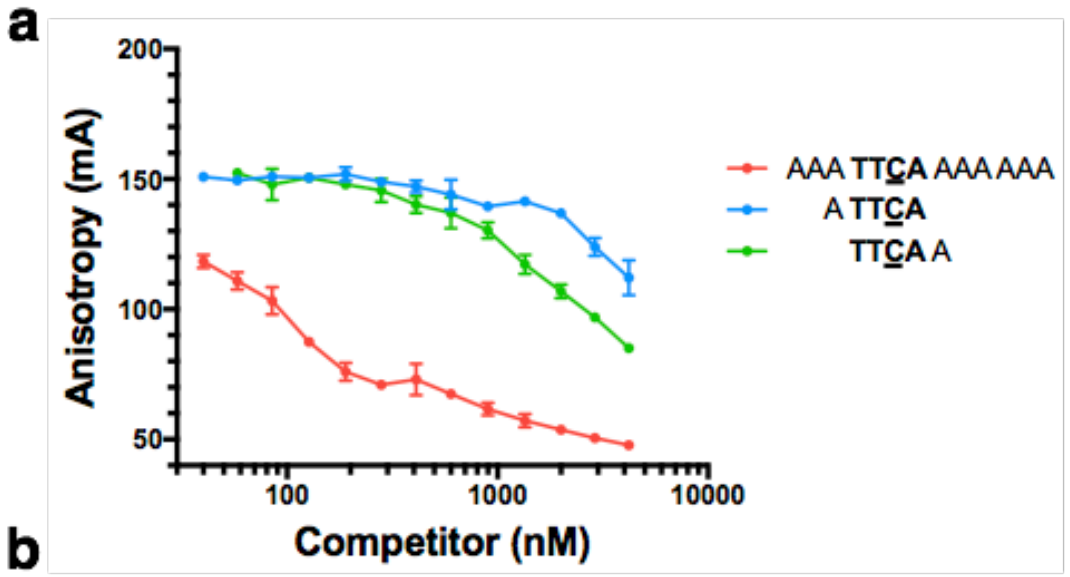
Supplementary Figure 1. A3A affinity to ssDNA at different pHs. Fluorescence anisotropy of TAMRA-labeled ssDNA 4A-TTC-6A binding to A3A(E72A). **a)** Binding of A3A to ssDNA at pH 4.0 (blue), 4.5 (red), 5.0 (green), 5.5 (orange), and 6.0 (purple). **b)** Binding of A3A to ssDNA at pH 6.0 (blue), 6.5 (red), 7.0 (green), 7.5 (orange), 8.0 (purple), 8.5 (black), 9.0 (brown).

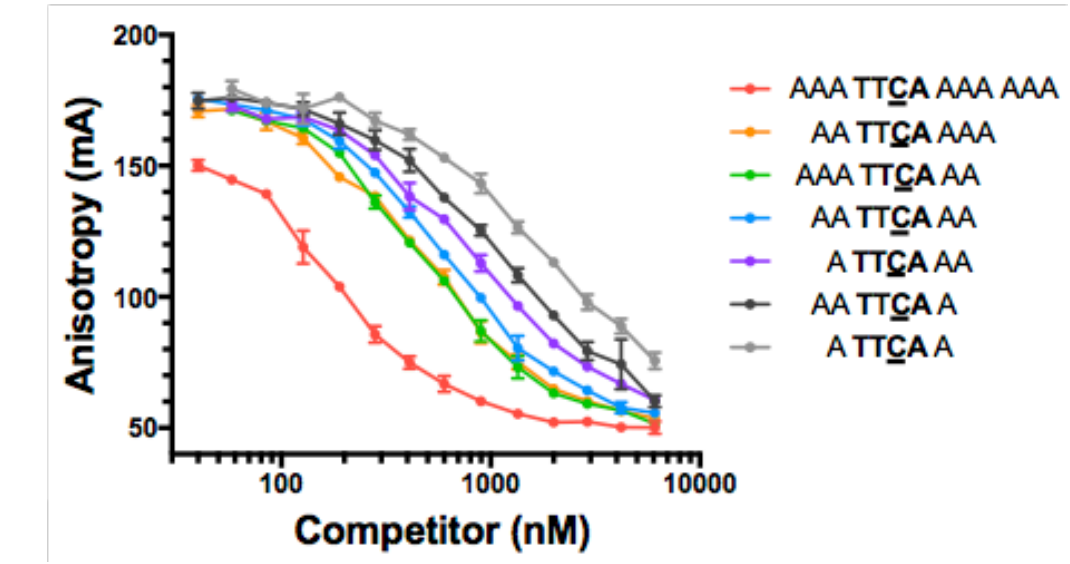
Supplementary Table 1. A3A affinity (K_d in nM) for ssDNA Poly A -TTC in a range of pHs.

pH	KD
4.0	Could not calculate
4.5	Could not calculate
5.0	Could not calculate
5.5	68 ± 3
6.0	90 ± 1
6.5	146 ± 8
7.0	260 ± 21
7.5	> 4,500
8.0	> 5,000
8.5	no detectable binding
9.0	no detectable binding

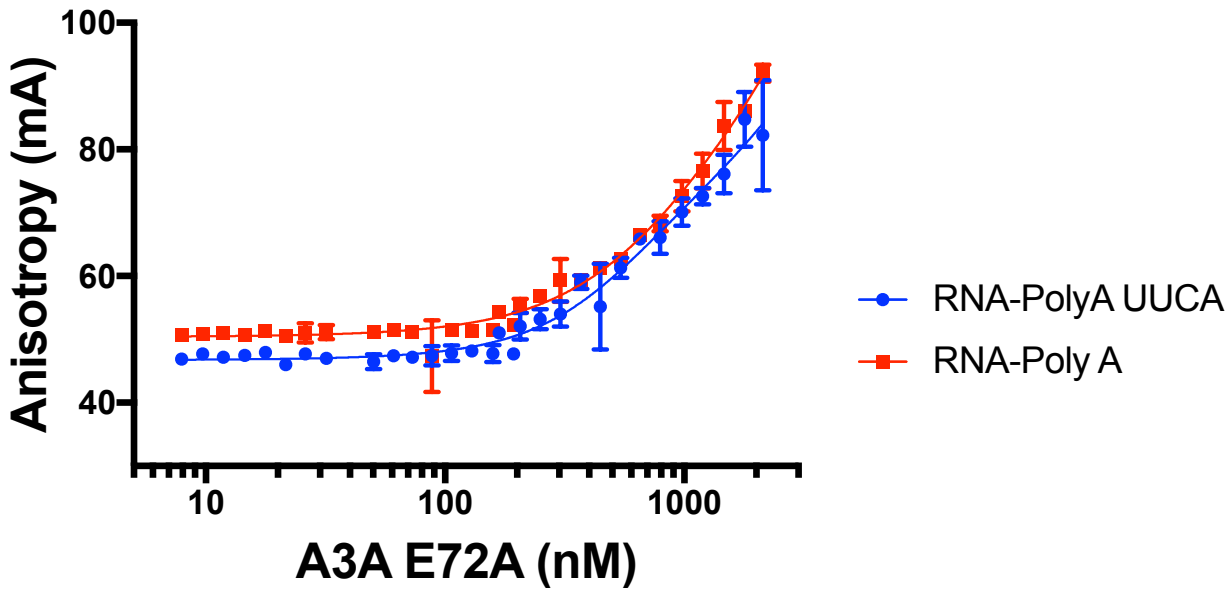


Supplementary Figure 2. A3A recognition of substrate cytidine and pyrimidines at -1. Crystal structure of A3A(E72A/C171A) shown in surface view (gray) bound to Poly T-1C ssDNA sequence represented as sticks (PDB ID: 5KEG). **a)** substrate cytidine (orange sticks) is buried in active site of A3A. Residues interacting with cytidine are shown in green sticks. **b)** -1 nucleotide thymidine (orange sticks) surrounded by Y130, D131 and Y132 of loop 7 (light blue sticks), W98 of loop 5 (pink sticks), and R189 (green sticks). **c)** Cytidine modeled into -1 position (orange sticks). N3 atom lacks proton to hydrogen bond with D131 indicate with a red X. **d)** Adenosine modeled into -1 position (orange sticks) shows severe van der Waal clashes if occupying the same site as the pyrimidines. Other nucleotides are shown as orange sticks. Hydrogen bond and a salt bridges shown in dashes black lines. Water shown as red spheres. Nitrogen and oxygen of residues and nucleic acids are in blue and red respectively.





Supplementary Figure 3. A3A affinity to ssDNA of varied lengths. Fluorescence anisotropy of TAMRA-labeled ssDNA 3A-TTCA-6A to A3A(E72A) competing with unlabeled ssDNA of different lengths. **a)** Binding of A3A to labeled ssDNA preincubated with unlabeled 3A-TTCA-6A (red), 1A-TTCA (blue), and TTCA-1A (green). **b)** Binding of A3A to labeled ssDNA preincubated with unlabeled 3A-TTCA-6A (red), 2A-TTCA-3A (blue), 3A-TTCA-2A (green), 2A-TTCA-2A (blue), 1A-TTCA-2A (purple), 2A-TTCA-1A (black), and 1A-TTCA-1A (gray).



Supplementary Figure 4. A3A affinity to ssRNA
Fluorescence anisotropy of TAMRA-labeled ssRNA sequences to A3A(E72A). Binding of A3A to ssRNA with PolyA UUCA (blue) and ssRNA Poly A (red).