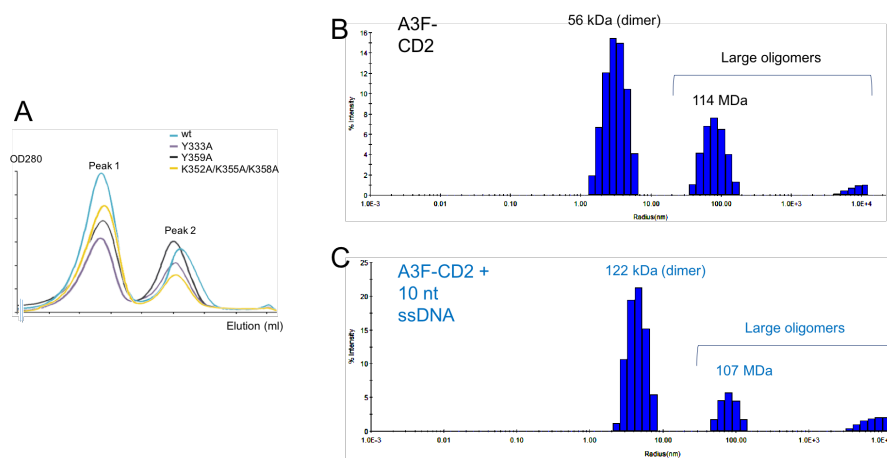
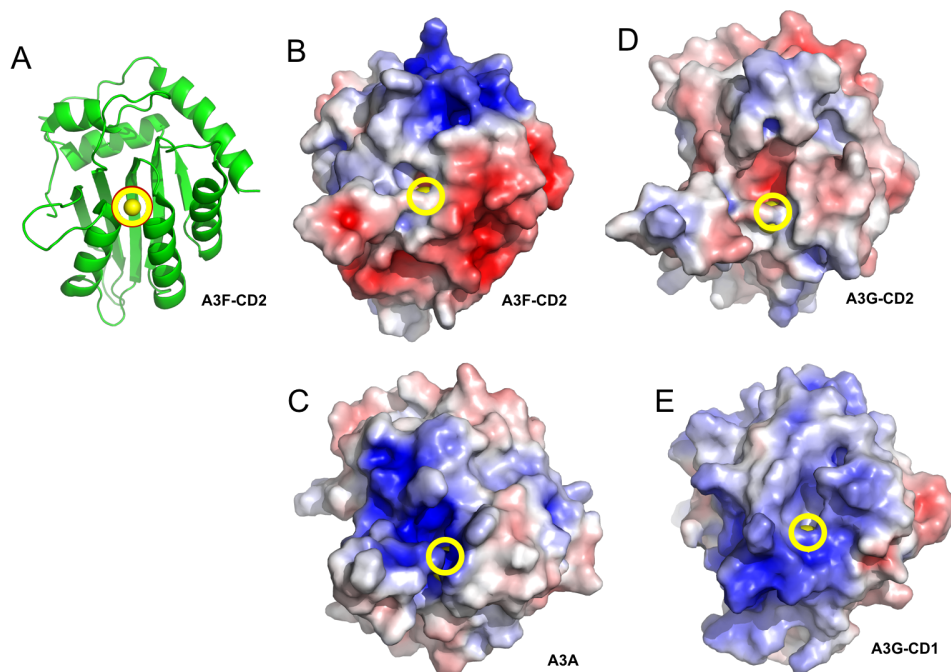


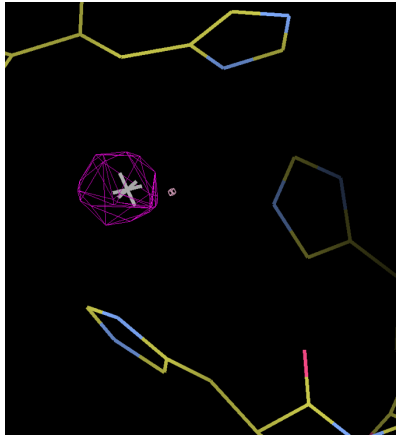
## Supplementary Figures



**Supplement Figure 1.** The size exclusion chromatography (SEC) elution profiles of A3F-CD2 constructs and the dynamic light scattering (DLS) characterization of A3F-CD2 in the presence or absence of 10 nt dT ssDNA. **(A)** Overlap of SEC profiles of the indicated A3F-CD2 constructs performed at 4°C. All constructs showed two peaks. Peak 1 was large oligomers in the void volume, and peak 2 had an estimated molecular weight of approximately 29 kD, slightly larger than the calculated molecular weight (MWt) of a monomer (21 kD) but smaller than a theoretical dimer (42 kD). **(B, C)** DLS analysis of the WT A3F-CD2 construct, which was used for the DNA co-crystal structure determination, in the absence (B) or presence (C) of 10 nt dT ssDNA. The DLS was performed with the sample chamber temperature adjusted to 8°C. The protein alone (B) showed the presence of dimer and larger oligomers (114 MDa or larger). When mixed with DNA, the dimer peak disappeared and a tetramer appeared, together with some larger oligomers.



**Supplement Figure 2.** Comparison of the charged surface features around the Zn-active center of four APOBEC domains. The location of Zn at the active center are indicated by a yellow circle in all panels. **(A)** The ribbon representation of A3F-CD2, showing the orientation of all the charged surface representation from panels B-E. **(B-E)** The charged surface of A3F-CD2, A3A, A3F-CD2 and A3G-CD1. The differences in charge features around the Zn center is obvious. A3A and A3G-CD1 are currently the only two wild-type domains solved with ssDNA bound to the Zn-active center in co-crystal structures, and both have strong positive charge right around the active site. A3F-CD2 has a positively charged patch away from the Zn-center, which is the location of the observed ssDNA binding in our structure. The charge around the Zn-center of A3F-CD2 is about neutral, which may suggest a weaker interaction for the ssDNA substrate with the Zn-center, and may explain the relatively low deaminase activity. A3G-CD2 has very low deaminase activity and undetectable ssDNA binding, which is consistent with it having largely negatively charge around the Zn-center.



**Supplement Figure 3.** The difference Fourier map, showing a strong peak at 5.0 sigma level, suggesting an electron rich atom similar to that of a Zn. This peak is within bonding range of three histidine residues around it, H247 and H249 of one protein monomer, and H228 of another. This peak position is about 5 Å away from the canonical Zn-active center of A3F-CD2.