Supporting Material

Materials and Methods

Construction, expression and purification of hADAR2-D

The expression vector encoding hADAR2-D was constructed by PCR amplification from a hADAR2 cDNA template and ligated into the vector YEpTOP2PGAL1 as described previously (*S1*). This construct encodes an N-terminal 10-histidine tag, a TEV protease recognition sequence, and residues 299-701 of hADAR2. The protein was expressed in *S. cerevisiae* and purified using nickel chelating, heparin ion exchange, and gel filtration chromatography, as described for other N-terminal truncations of hADAR2 (*S1*). The purified protein was dialyzed into buffer (20 mM Tris-HCl pH 8.0, 100 mM NaCl, 1 mM 2-mercaptoethanol, and 5% glycerol) and concentrated to 10 mg/ml.

RNA preparation and deamination assays

Duplex RNA encoding sense and antisense sequences of the chloramphenicol acetyl transferase gene (CAT duplex) was internally labeled during *in vitro* transcription with α-^{32}P ATP, and gel purified as previously described (*S2*). The *in vitro* editing assay was performed by incubating purified protein and CAT duplex RNA under conditions described previously (*S1*), with concentrations described in the legend to Figure S1.

Crystallization and Data Collection

hADAR2-D was crystallized by vapor diffusion at 21°C by mixing 2 μl of protein solution with 2 μl of well solution (0.1 M Bis-Tris pH 6.8, 0.2 M $(NH_4)_2SO_4$, 24% PEG 3350, and 7.5% glycerol). After 10 days, crystals were mounted in nylon loops and