reverse transcribed with Superscript^{TM} II reverse transcriptase (Invitrogen, Carlsbad, CA) using an oligo-dT primer. A region of the cDNA (947-1175 of the ADAT1 gene, 2050-2324 of hADAR2 gene) was amplified by quantitative real-time PCR using a Roche Light Cycler with SYBR-Green fluorescent NTPs and gene specific primers. RT-PCR product amounts were normalized to that of a housekeeping gene (GAPDH) for quantitation.

Editing assays

hADAR2 concentrations in extracts prepared from BCY123 or BCY-*ipk1*Δ were quantified by western blotting. The blot was probed with a penta-His primary antibody (Qiagen) and alkaline phosphatase conjugated anti-mouse secondary antibody (Sigma-Aldrich, St. Louis, MO). Protein bands were detected using a fluorescent alkaline phosphatase ECF substrate (Amersham, Piscataway, NJ). The blot was scanned on a PhosphorImager, and the hADAR2 bands were quantified after generating a standard concentration curve of known amounts of a purified protein (the histidine tagged R_2D truncation of hADAR2, *S1*). Editing of the R/G site RNA by hADAR2 in vitro was performed as previously described (*S1*). Briefly, 5 nM RNA was reacted with increasing concentrations of hADAR2 present in yeast extracts (final concentrations of hADAR2 are indicated in Fig. 4A) for 1 hour at 30°C.

Yeast extracts for editing tRNA in vitro were prepared as described above and quantified by Bradford assay. 5 nM of A34 or A37 labeled $tRNA^{ala}$ was reacted with 10^{-3}, 10^{-2}, 0.1, 2.9, 5.8 μg/μl total protein for 1 hour at 30°C. IP_6 or IS_6 (concentrations noted in the legend to Fig. 4A; Sigma-Aldrich) were incubated with 0.1 μg/μl extract for