**RNA preparation**

For the in vitro editing assays, RNA substrates were prepared using the splint ligation method of Moore and Sharp (*S13*). The R/G 27-mer RNA used for the hADAR2 editing assays was prepared as described (*S14*). The tRNAs were chemically synthesized in two halves. For labeling A34, the two RNAs spanned residues 1-33 (the 5' half) and 34-76 (the 3' half); for labeling A37, the two RNAs spanned residues 1-36 (the 5' half) and 37-76 (the 3' half). The 3' halves were phosphorylated (at either A34 or A37) with $^{32}$P using polynucleotide kinase, and annealed, along with their respective 5' partner, to a DNA "splint" of complementary sequence to the 76-nucleotide tRNA$^{ala}$. The RNAs were then ligated using T4 DNA ligase, the DNA splint was digested with RQ1 DNase (Promega, Madison, WI), and the RNA purified on a 12% denaturing polyacrylamide gel.

To assay editing in vivo, total RNA was prepared from the S100 extract by extraction with Trizol$^{TM}$ reagent (Invitrogen) as per the manufacturers instructions. The RNA was precipitated with isopropanol and treated with the restriction enzyme Cac8I (New England BioLabs, Ipswich, MA), followed by RQ1 DNase (Promega) to digest any remaining DNA. The RNA was extracted with phenol, precipitated with ethanol, and quantified. tRNA$^{ala}$ was amplified by RT-PCR, using primers specific for the tRNA$^{ala}$ sequence and the PCR product was sequenced (see below).

RT-PCR was used to measure amounts of the ADAT1 and hADAR2 mRNAs expressed in the *ipk1*Δ strains relative to those messages produced in the wild-type strain. Total RNA was prepared as described above, except after RQ1 DNase treatment, the restriction digest step was omitted and the RNA was further purified using an RNeasy$^{TM}$ kit (Qiagen, Valencia, CA) according to the manufacturers instructions. The RNA was