# Supplementary Data

## RNA-Seq Analysis Identifies A Novel Set of Editing Substrates for Human ADAR2 Present in *Saccharomyces cerevisiae*

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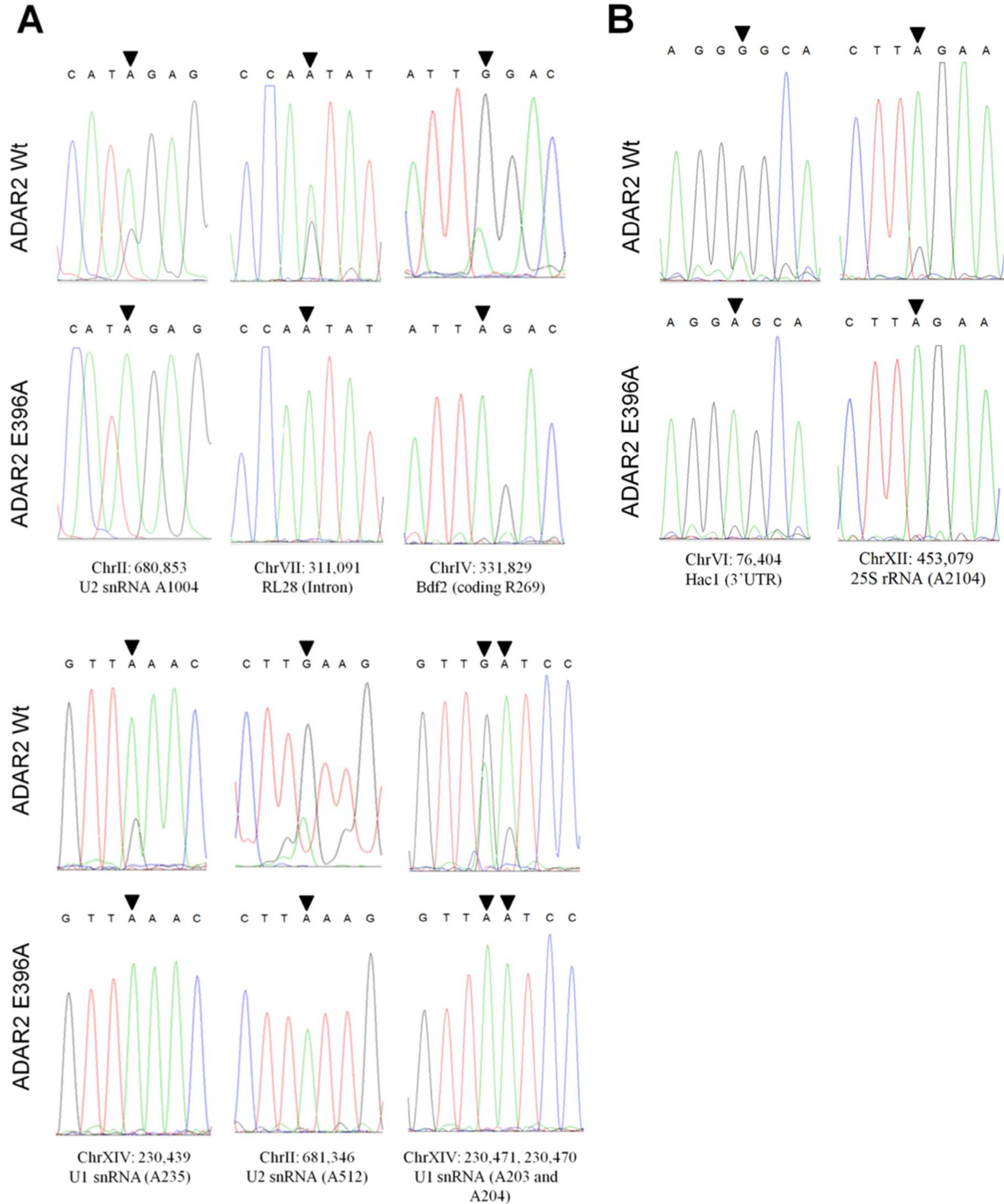
**Table S1**. For A positions in the reference genome present in the RNA transcript: Candidate sites exhibiting 50 or more A reads for inactive ADAR2 sample and 50 or more G reads in active ADAR2 sample with the %G for inactive ADAR2 is <2% and the %G for active ADAR2 is >10%.

| | | Wt ADAR2 strain Read Counts | | ADAR2 E396A strain Read Counts | | Wt ADAR2 | ADAR2 E396A |
|---|---|---|---|---|---|---|---|
| Chr# | Position | A | G | A | G | %G | %G |
| chrII | 278998 | 2492 | 437 | 3127 | 1 | 14.9% | 0.03% |
| chrII | 278999 | 2608 | 318 | 3112 | 2 | 10.9% | 0.06% |
| chrIV | 331829 | 30 | 51 | 90 | 0 | 62.9% | 0.00% |
| chrVI | 75228 | 699 | 129 | 558 | 4 | 15.6% | 0.71% |
| chrVI | 75995 | 267 | 61 | 236 | 0 | 18.6% | 0.00% |
| chrVI | 76061 | 363 | 55 | 255 | 0 | 13.2% | 0.00% |
| chrVI | 76276 | 583 | 94 | 250 | 0 | 13.9% | 0.00% |
| chrVI | 76404 | 483 | 143 | 225 | 1 | 22.8% | 0.44% |
| chrVII | 311091 | 85 | 71 | 148 | 0 | 45.5% | 0.00% |
| chrXIII | 634284 | 499 | 57 | 789 | 1 | 10.3% | 0.13% |

**Table S2**. For T positions in the reference genome complementary to the RNA transcript: Candidate sites exhibiting 50 or more T reads for inactive ADAR2 sample and 50 or more C reads in active ADAR2 sampe with the %C for inactive ADAR2 is <2% and the %C for active ADAR2 is >10%.

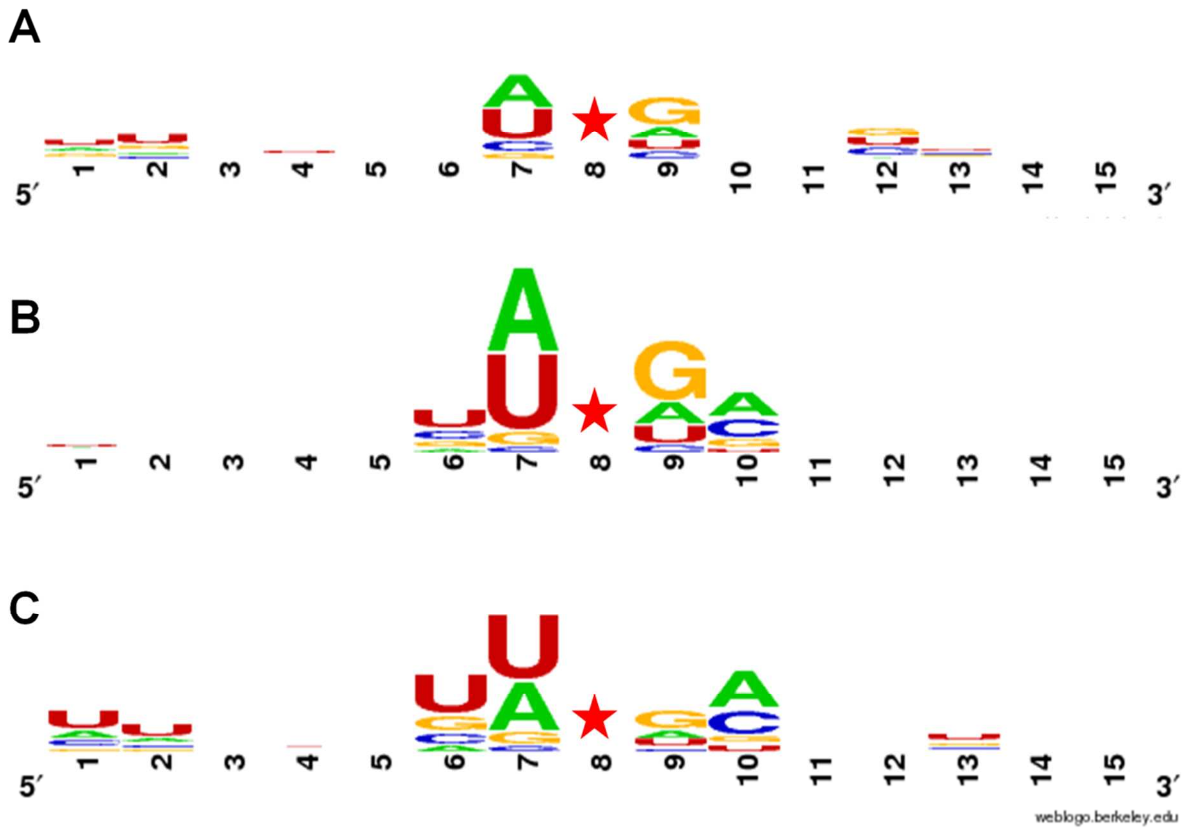
| | | Wt ADAR2 strain Read Counts | | ADAR2 E396A strain Read Counts | | Wt ADAR2 | ADAR2 E396A |
|---|---|---|---|---|---|---|---|
| Chr# | Position | C | T | C | T | %C | %C |
| chrII | 680804 | 51 | 54 | 1 | 73 | 48.6% | 1.35% |
| chrII | 680826 | 112 | 33 | 1 | 66 | 77.2% | 1.49% |
| chrII | 680853 | 164 | 34 | 0 | 128 | 82.8% | 0.00% |
| chrII | 681346 | 183 | 25 | 0 | 96 | 88.0% | 0.00% |
| chrXII | 453079 | 220 | 1215 | 0 | 646 | 15.3% | 0.00% |
| chrXIV | 230439 | 212 | 150 | 0 | 60 | 58.6% | 0.00% |
| chrXIV | 230607 | 74 | 137 | 0 | 59 | 35.1% | 0.00% |

**Supplementary Figure 1.** Confirmation of predicted editing sites in *S. cerevisiae* transcriptome confirmed via Sanger sequencing of cDNA derived from RT-PCR of yeast total RNA. Edited sites are indicated by a black triangle. **A** Sequence traces incorporating predicted editing sites derived from total RNA isolated from *S. cerevisiae* strain INVSc1 expressing ADAR2 wt or inactive mutant ADAR2 E396A. **B** Sequence traces of cDNA incorporating predicted editing sites derived from total RNA isolated from *S. cerevisiae* strain INVSc1 that was incubated in micromolar concentrations of ADAR2 wt or ADAR2 E396A for 4 hours at 30 °C.

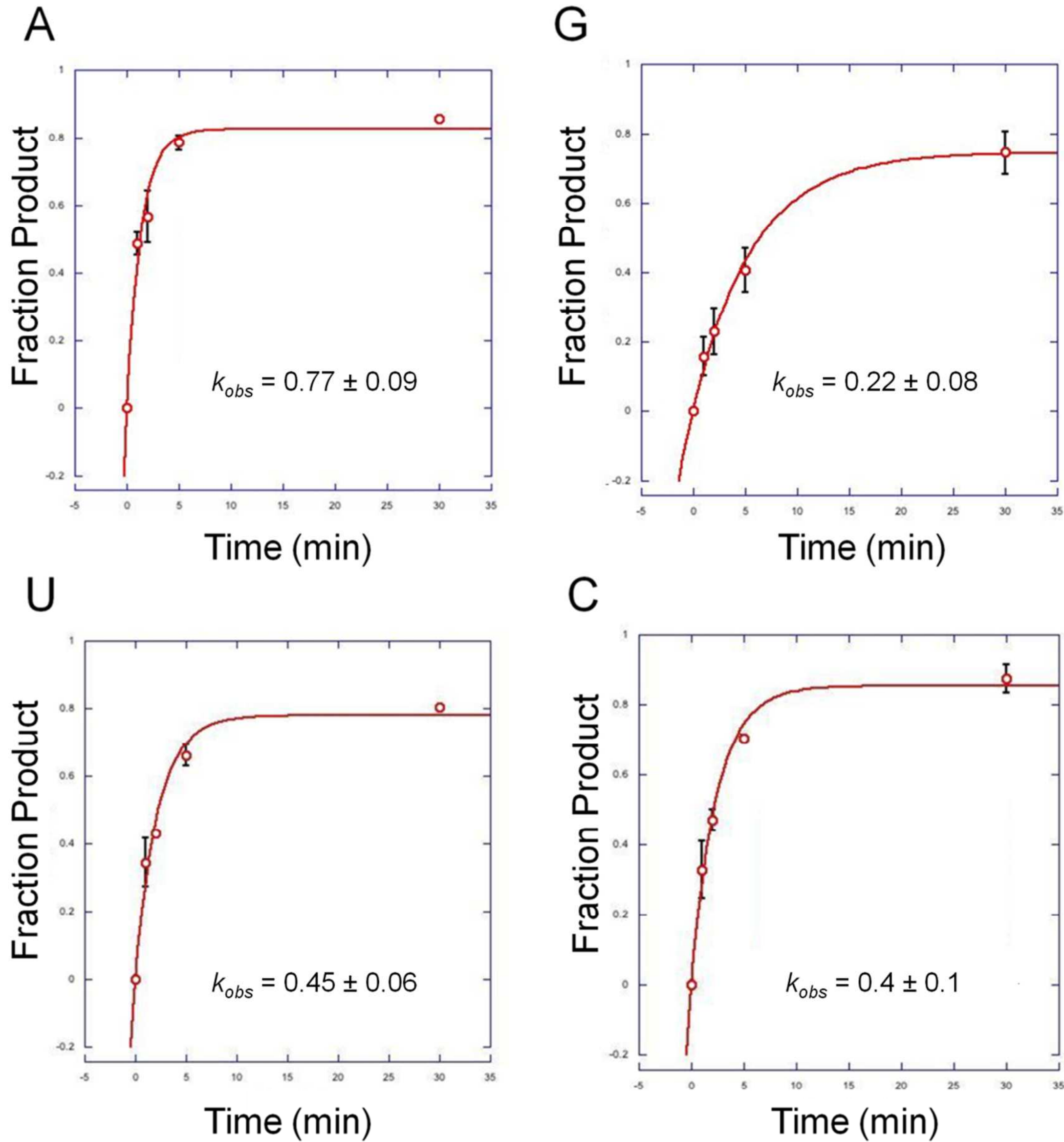


**Supplementary Figure 2.** Predicted secondary structures of RNA containing adenosines targeted for editing by ADAR2 in *S. cerevisiae*. Structures generated using mFold (http://mfold.bioinfo.rpi.edu/). Edited nucleotides are colored green.

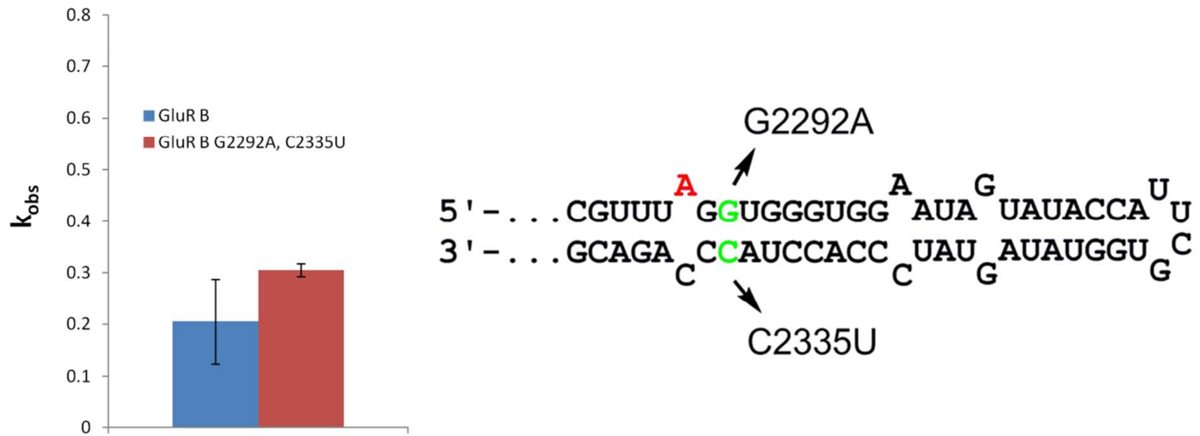
**Supplementary Figure 3.** Extended WebLogo sequence alignment analysis of predicted ADAR2 editing site regions in *S. cerevisiae*. Edited adenosine is indicated by a star. **A** WebLogo incorporating flanking regions of edited sites with a %G of at least 6.0 in strains expressing ADAR2 wt and a %G of less than 2.0 in strains expressing ADAR2 E396A, i.e., at least a three-fold difference in %G from ADAR2 wt to ADAR2 E396A (36 sites). **B** WebLogo incorporating flanking regions of edited sites with a %G of at least 8.0 in strains expressing ADAR2 wt and a %G of less than 2.0 in strains expressing ADAR2 E396A, i.e., at least a four-fold difference in %G from ADAR2 wt to ADAR2 E396A (25 sites). **C** WebLogo incorporating flanking regions of editing sites with a %G of at least 10.0 in strains expressing ADAR2 wt and a %G of less than 2.0 in strains expressing ADAR2 E396A, i.e., at least a five-fold difference in %G from ADAR2 wt to ADAR2 E396A (19 sites).



**Supplementary Figure 4.** *In vitro* deamination of Bdf2 RNA and 3' next nearest neighbor variants by ADAR2. Deamination expressed as fraction product over time with 0, 1, 2, 5 and 30 minute time points (n=3). Data were fitted to the equation $y = m1*(1-e^{(-m2*m0)})$, where m1 is the fitted reaction end point, m2 is the $k_{obs}$ or fitted rate constant ($min^{-1}$), and y is the fraction product at time m0. Graphs were generated using KaleidaGraph (Synergy Software).



**Supplementary Figure 5.** Comparison of *in vitro* deamination rates of ADAR substrates GluR B and GluR B G2292A, C2335U with hADAR2. Rates were determined as described above (**Supplementary Figure 2**). GluR B $k_{obs}$ 0.21 ± 0.08, GluR B G2292A, C2335U $k_{obs}$ 0.30 ± 0.01. Error represents standard error (n = 3, $P$ = 0.29). Edited adenosine is colored red and mutated nucleotides are colored green.



**Supplementary Figure 6.** Co-expression of Bdf2p suppresses hADAR2-induced slow growth phenotype. **A** Spotting assay of candidate transcripts co-expressed with hADAR2. INVSc1 transfected with ADAR2 wt or ADAR2 E396A, *BDF2*, or *HAC1*, cell count decreasing from right to left. **B** Western analysis of cell lysates from yeast transfected with ADAR2 wt + *BDF2* and ADAR2 wt alone. 1 – ADAR2 wt standard, 2 – yeast lysate ADAR2 wt + *BDF2*, 3 – yeast lysate ADAR2 wt.