



Figure S3: A-to-I base editing efficiencies in HEK293T cells by various ABE mutants at alternative RNA off-target site 1'. Values and error bars reflect the mean and SD of four independent biological replicates performed on different days.

Experimentally tested sites:

RNA site 1 (DNAJB1) (chr: 14518195)

GCGCTACCACCCGACAAGAACAAGGAGCCCGGCCGAGGAGAAGTTCAAGGAGATCGCTGAGGCC**TACG**ACGTGCTCAGCGACCCGC
GCAAGCGGAGATCTTCGACCCTACGGGGAGGAAGGCCATAAGGGGAGTGGCCCCAGTGGCGGTAGCGGGTGGTGCCAATGGTACC
TCTTCAGCTACACATTCCATGGAGACCCTCATGCCATG

RNA site 2 (MTA2) (chr: 62594034)

TCTGGCTTCAGGGATTTCGTTCAAGCTCACAGCCAGCAGCCAAGCGTCAGAAACTAAACCCAGCTGATGCCCCAATCCTGTGGTGTGTTG
TGGCCACAAAGGATACCAGGGCC**TACG**GAAGGCTCTGACCCATCTGGAATGCGGCGAGCTGCTCGCCGACCCAACCTGCCCCGAAG
GTGAAGCCAACGCTGATTGCAGTGGGCCCCCTGTCCCTCTACCTGCACCCCTCACATC

RNA site 3 (PTBP2) (chr: 96813053)

AGATTTTGGTAATTCCCATTGCATCGTTTTAAGAAACCTGGATCCAAAAATTTTCAAACATTTTTCTCCTTCTGCCACCCTTCACC
TATCTAATATCCCTCCATCAGTAGCAGAAGAGGAT**TACG**AACACTGTTCGCTAACACTGGGGCACTGTGAAAGCATTTAAGTTTTTT
CAAAGAGATCACAAAATGGCTCTTCTTCAGATGGCAACAGTGAAGAAGCTATTCAGG

RNA site 4 (SAP30BP) (chr: 75703316)

CAGAACCCCTGGCAGATGTTCAAATCACTTGCAAGACAAGATCCAGAAGCTTTATGAACGAAAGATAAAGGAGGGAATGGATATGAAC
TACATTATCCAAAGGAAGAAAGAATTTTCGGAACCCTAGCAT**TACG**AGAAGCTGATCCAGTTCTGTGCCATTGACGAGCTTGGCACCAA
CTACCCAAAGGATATGTTTGATCCCCATGGCTGGTCTGAGGACTCCTACT

RNA site 5 (LCMT1) (chr: 25164711)

ATTGCCAACACTCCTGATAGCTGAATGTGTGCTGGTTTACATGACTCCAGAGCAGTCCGCAAACCTCCTGAAGTGGGCAGCCAACAGTT
TTGAGAGGCCATGTTCAATAA**TACG**AACAGGTGAACATGGGTGATCGGTTTGGGCAGATCATGATTGAAAACCTGCGGAGACGCCAG

TGTGACCTGGCGGGAGTGGAGACCTGCAAGTC

RNA site 6 (SCAP) (chr: 47420696)

CCATTGACATTCGCCGGATGGAGCTAGCAGACCTGAACAAGCGACTGCCCCCTGAGGCCTGCCTGCCCTCAGCCAAGCCAGTGGGACAG
CCAACGCGCTACGAGCGGCAGCTGGCTGTGAGGCCGTCCACACCCACACCATCACGTTGCAGCCGTCTTCTTCCGAAACCTGCGGCT
CCCCAAGAGGCTGCGTGTGTCTACTTC

Sequence used in the simulations:

UUGACUACGAUCAA

Figure S4: RNA sequences tested in the experimental analyses and simulation models. The amplicon sequences pertain to the cDNA used for sequencing analysis. Thus, every T should be considered a U in RNA. The consensus sequence for all sites tested is hence -UACG-, which is the same as the native RNA substrate sequence used in the simulation models.