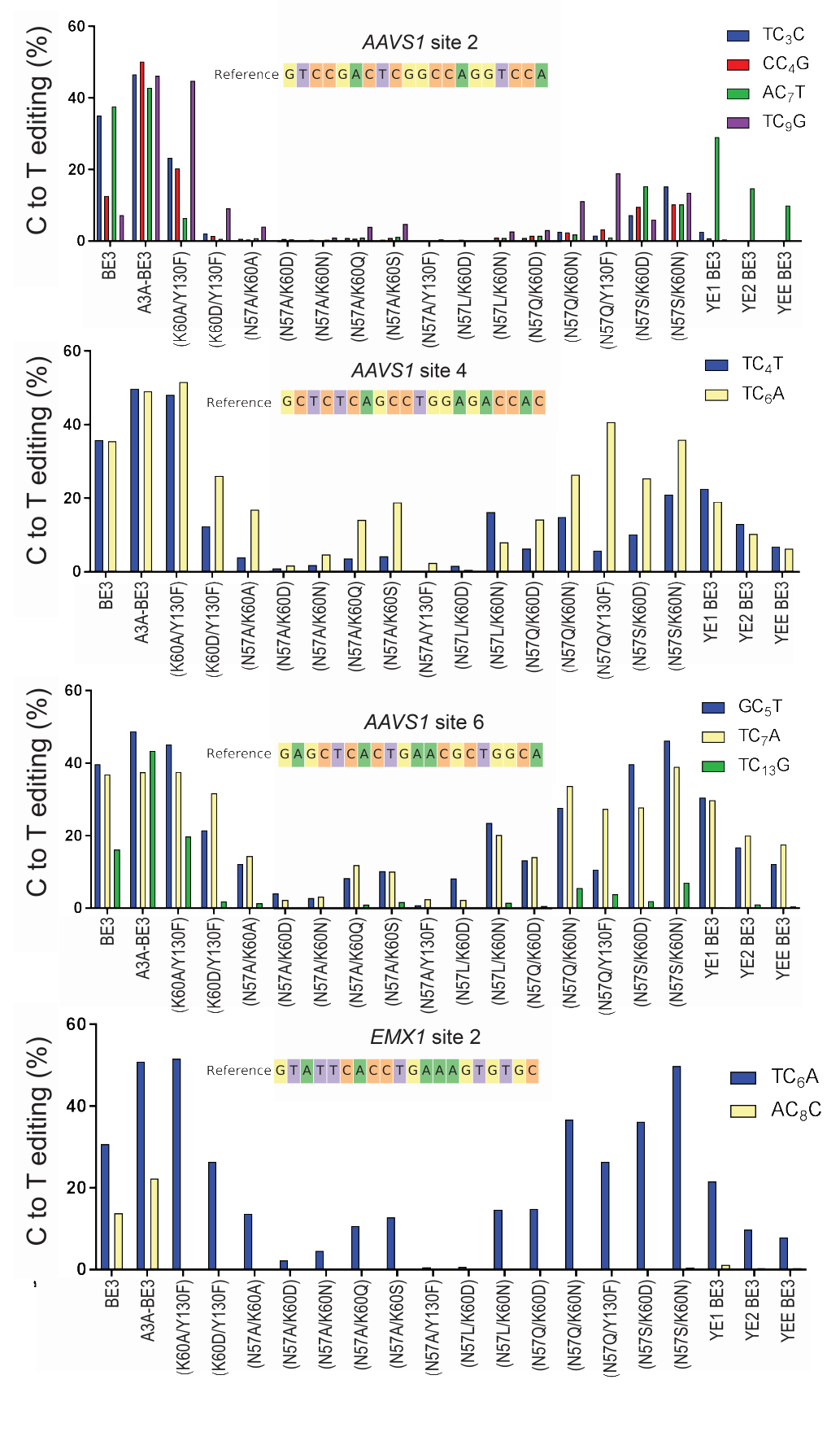
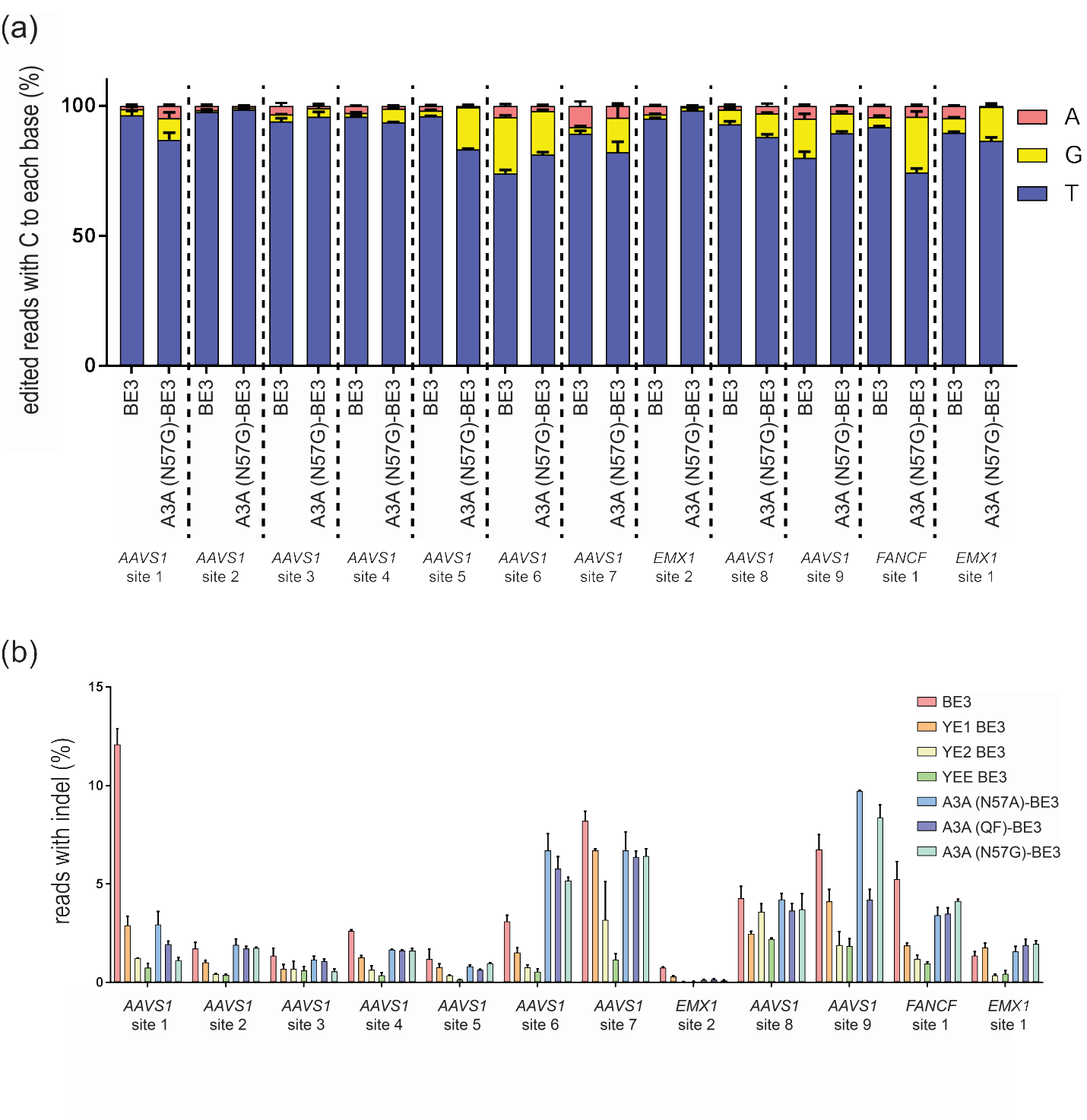
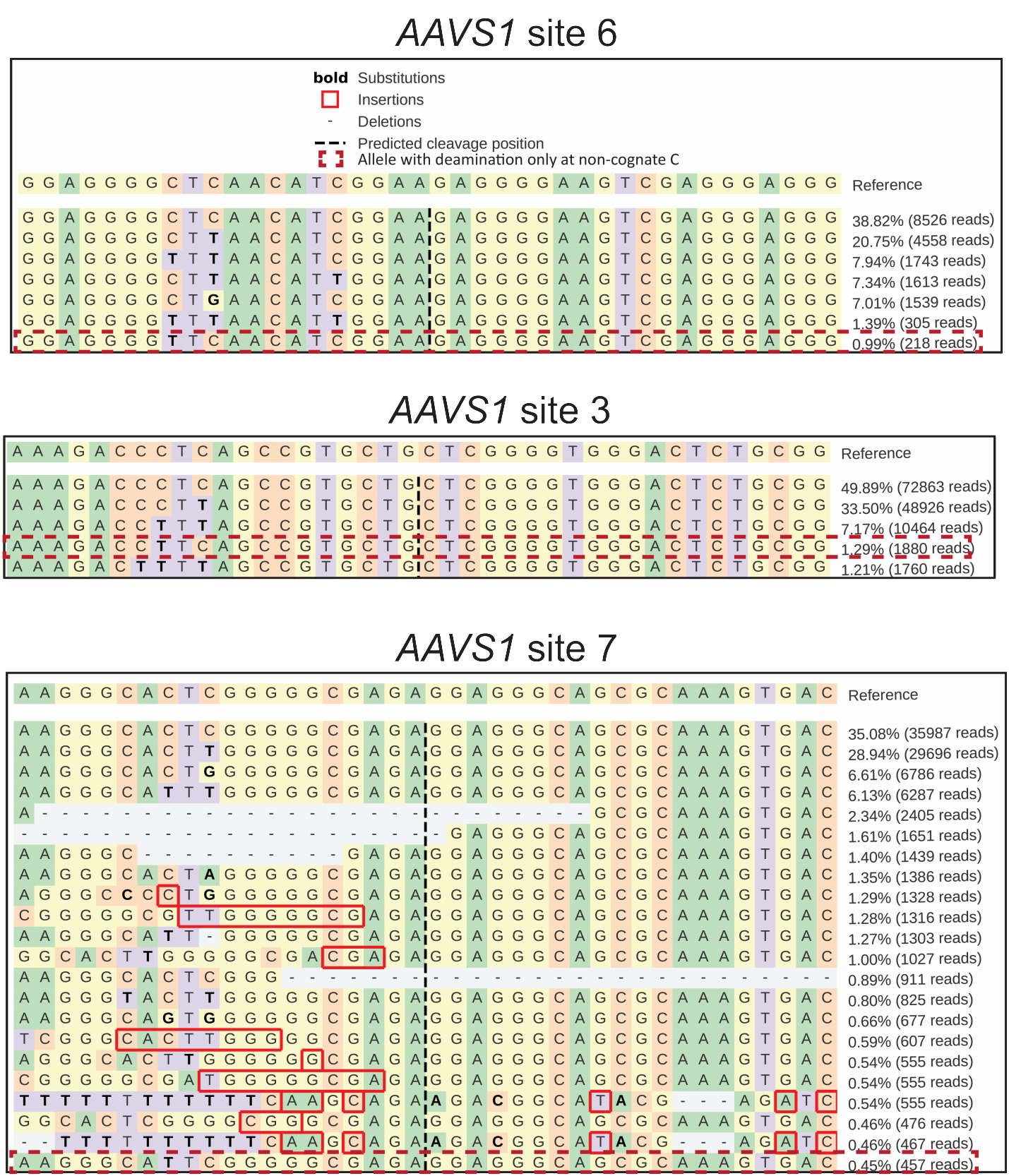
**SUPPLEMENTARY FIGURES**

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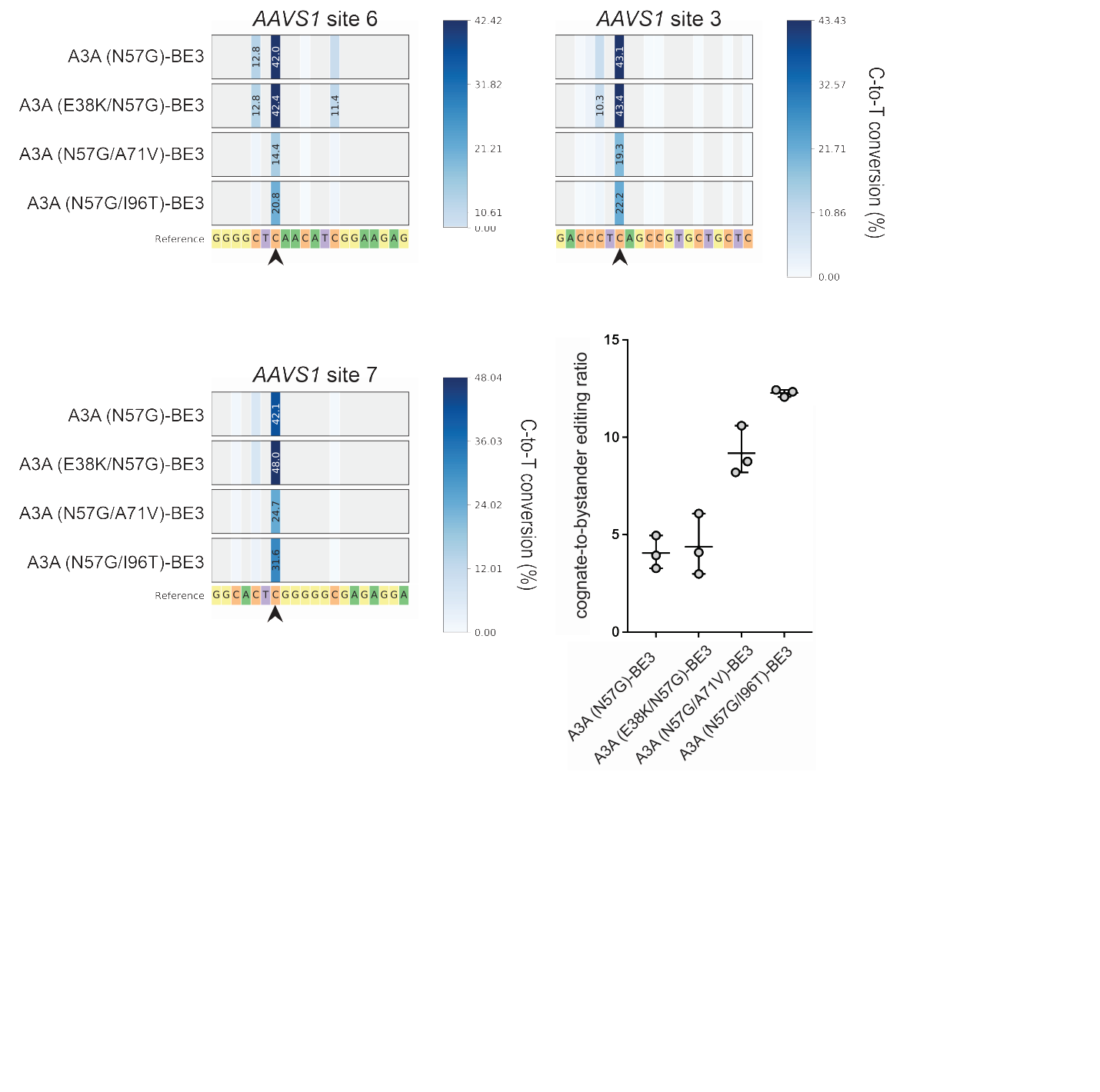
**Supplementary Figure 1: Base editing activities of engineered A3A-BE3 variants with mutations designed to disrupt non-specific interactions with substrate ssDNA.** Graphs illustrating the frequencies of C to T editing by a series of A3A-BE3 variants containing various pairs of mutations in A3A on bystander and cognate cytidines at four endogenous human gene target sites. The reference sequence of each target site is shown at the top of each graph.

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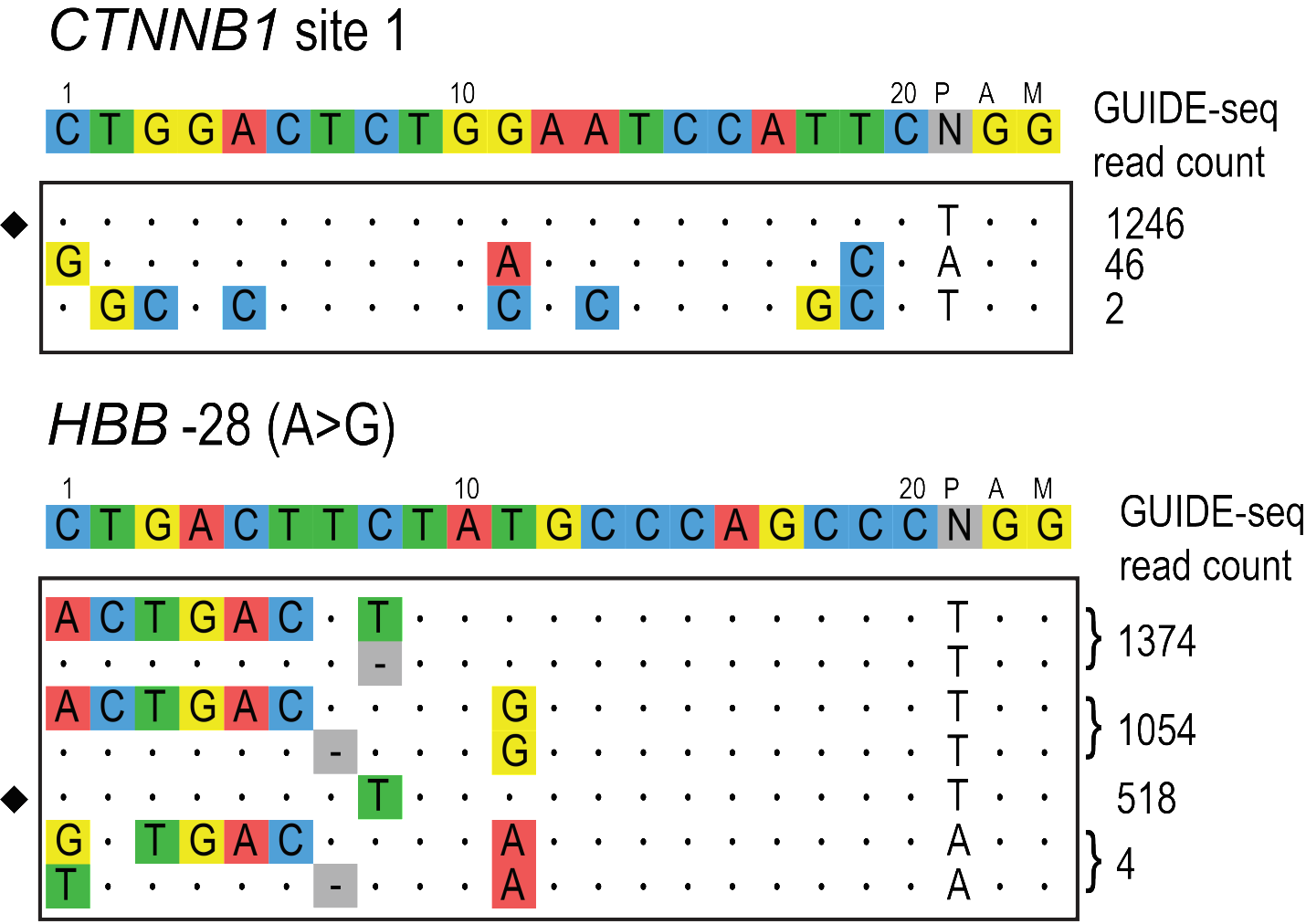
**Supplementary Figure 2: Comparison of product purities and indel mutation frequencies for BE3 and eA3A-BE3 on endogenous human gene target sites.** (a) Graph showing normalized frequencies of cognate Cs edited to A, T, or G for twelve endogenous human gene target sites from Fig. 1d when targeting with BE3 or A3A (N57G)-BE3. (b) Graph showing indel mutation frequencies for BE3, the YE BE3s, and different engineered A3A-BE3 variants at the same 12 sites shown in (a). All data shown represent the mean of three biological replicates with error bars representing SEMs.



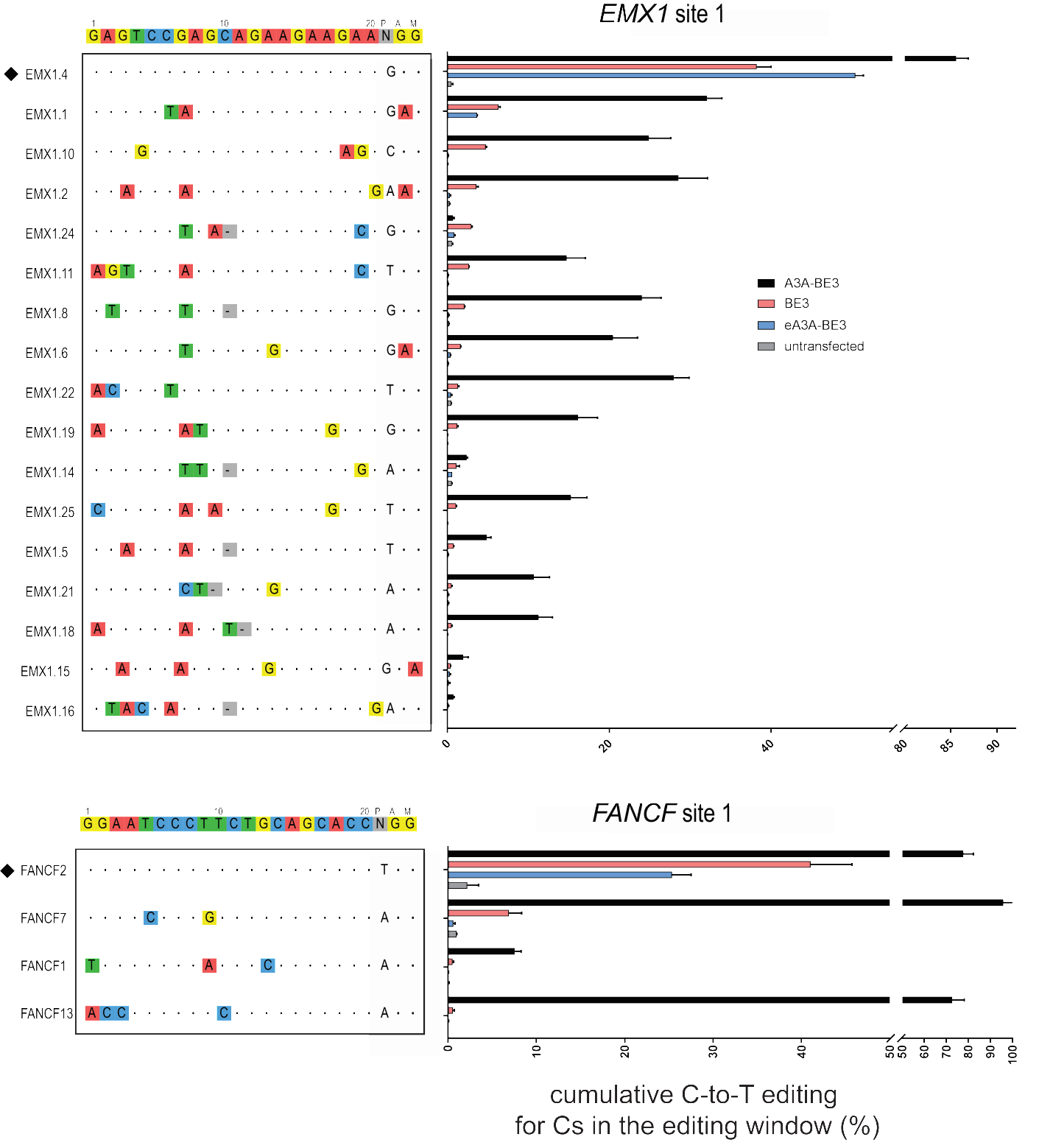
**Supplementary Figure 3: eA3A-BE3-mediated editing of bystander cytidines typically occurs on the same allele as editing of cognate cytidines.** Allele frequency tables for three sites on which eA3A-BE3 exhibits cognate-to-bystander editing ratios less than five.

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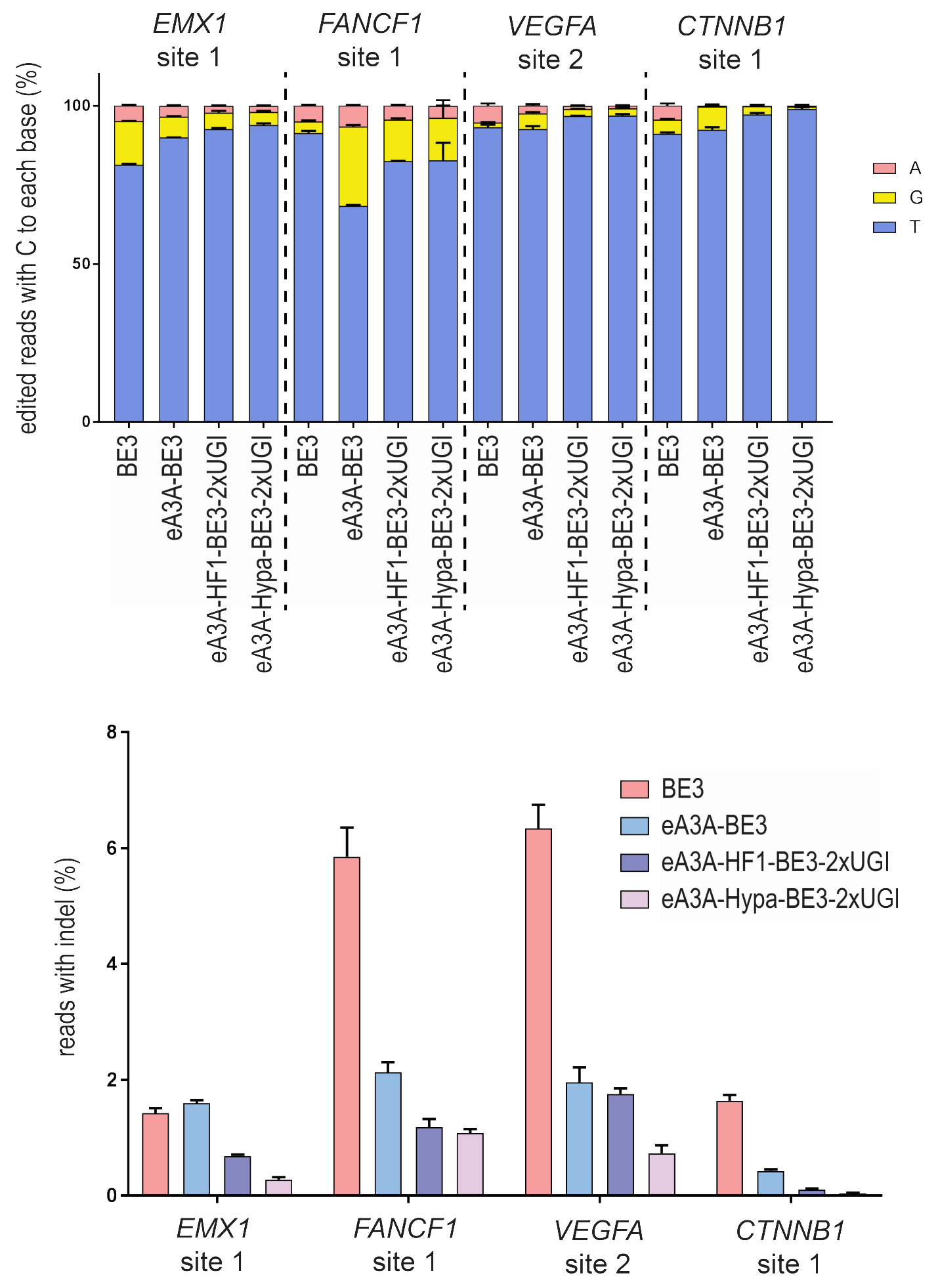
**Supplementary Figure 4: Mutations designed to decrease the catalytic rate of eA3A can increase the cognate-to-bystander editing ratio of eA3A-BE3.** Heat maps showing C-to-T editing efficiencies for eA3A-BE3 and three eA3A-BE3 variants bearing the indicated mutations at three endogenous human gene target sites, each bearing a cognate cytidine preceded by a 5’ T and one or more bystander cytidines within the editing window. Editing efficiencies shown represent the mean of three biological replicates. Graph showing cognate-to-bystander editing ratios for all three target sites with eA3A-BE3 and each of the three eA3A-BE3 variants. Data points shown represent ratios of the means of the three biological replicates performed for each base editor. Mean values are indicated by a line and error bars represent the SEM.

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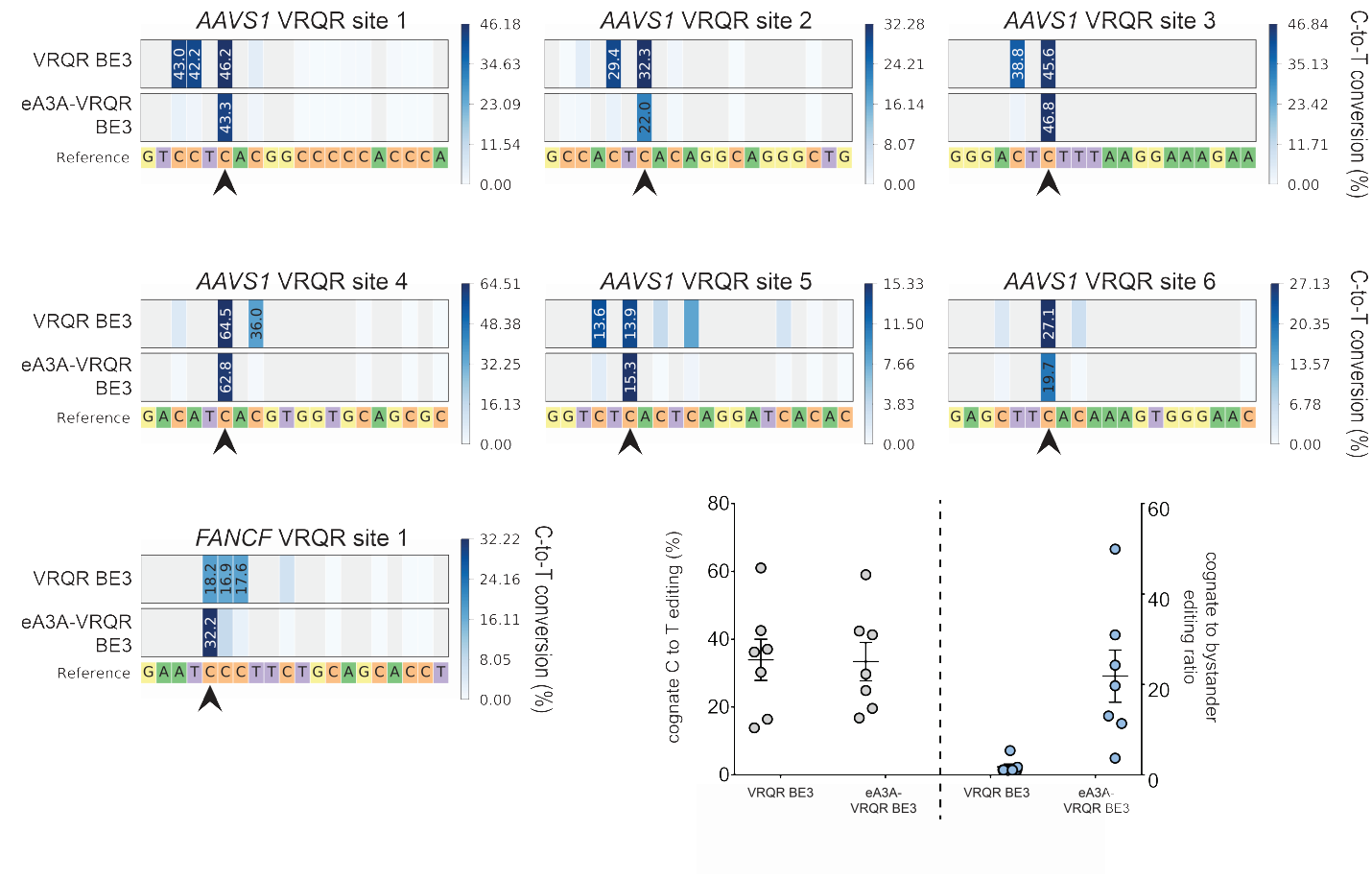
**Supplementary Figure 5: SpCas9 nuclease off-targets discovered by GUIDE-seq for the *CTNNB1* and *HBB* -28 (A>G) gRNAs.** GUIDE-seq plots depicting the target site for both gRNAs (top of each figure panel) and off-target sites discovered by GUIDE-seq shown below with base positions containing mismatches to the on-target site highlighted with a colored box and RNA bulges indicated with a dash. Sites with more than one potential alignment (i.e. where either an RNA bulge or additional mismatches are both plausible target sites) are shown with brackets. GUIDE-seq read counts for each site discovered are shown in the right column. The on-target site for the *CTNNB1* gRNA and the single mismatched on-target site for the *HBB* -28 (A>G) gRNA are indicated with a small black square to the left.

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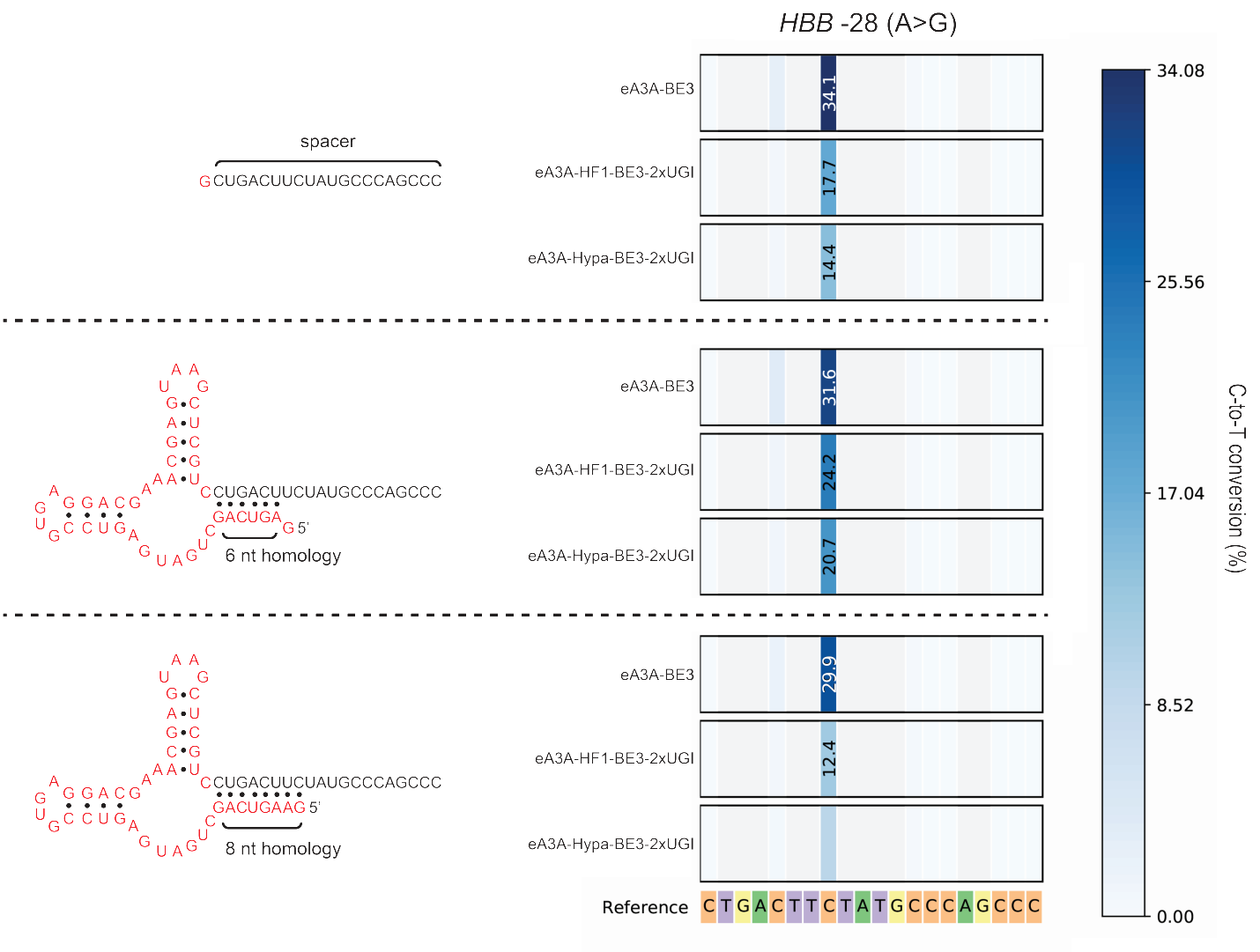
**Supplementary Figure 6: The N57G mutation is important for decreased off-target activity of eA3A-BE3.** Editing frequencies for *EMX1* site 1 and *FANCF* site 1 gRNAs with BE3, eA3A-BE3, and untransfected cells (from **Figs. 2a – 2b**) are re-plotted with editing frequencies with these same gRNAs observed with A3A-BE3 from a separate experiment. Sequences of the on- and off-target sites are shown to the left of the bar plot below the target sequence with mismatches relative to the on-target site highlighted with colored boxes and bulges with a grey highlighted dash. The on-target sites for the *EMX1* site 1 and *FANCF* site 2gRNAs are indicated with a small black square to the left.

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**Supplementary Figure 7: Effects of adding a second UGI domain and HF1 or Hypa high-fidelity mutations eA3A-BE3 on base editing product purities and indel frequencies at four endogenous human gene target sites.** Graph in top panel shows normalized frequencies of cognate Cs edited to A, T, or G for the four endogenous human gene target sites of Figures 2a – 2d when targeting each site with BE3, eA3A-BE3, or eA3A-BE3 variants incorporating HF1 or Hypa mutations and a second UGI domain. Graph in bottom panel shows indel frequencies induced by the same base editors at the same sites. All data shown represent the mean of three biological replicates and error bars represent SEMs.

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**Supplementary Figure 8: eA3A-BE3 bearing VRQR mutations that alter PAM recognition specificity can can be used with the VRQR SpCas9 variant recognizing NGA PAMs for highly precise single nucleotide editing.** Heat maps showing C-to-T editing efficiencies for VRQR BE3 and eA3A-VRQR BE3 at seven endogenous human gene target sites bearing NGAN PAM sequences, each bearing a cognate cytidine preceded by a 5’ T and one or more bystander cytidines within the editing window. Editing efficiencies shown represent the mean of three biological replicates. Graph shows cognate-to-bystander C to T editing ratios for VRQR BE3 and eA3A-VRQR BE3. Data points shown represent ratios calculated from the mean values shown in the heat maps. Mean values are indicated by a line and error bars represent the SEM.

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**Supplementary Figure 9: Incorporating a self-cleaving hammerhead ribozyme on the 5’ end of the gRNA preserves perfect matching of the spacer and target site and rescues the activities of the eA3A-BE3 variants bearing HF1 and Hypa high-fidelity mutations.** Heat maps showing C-to-T editing efficiencies for eA3A variants incorporating HF1 or Hypa mutations targeting *HBB* -28 (A>G) using a gRNA with a 5’ mismatched guanine (top), a self-cleaving hammerhead ribozyme with 6 nucleotides of self-complementarity (middle), or a self-cleaving hammerhead ribozyme with 8 nucleotides of self-complementarity (bottom). The 5’ mismatched guanine or the ribozyme sequence is shown in red, while the spacer sequence is shown in black.Editing efficiencies shown represent the mean of three biological replicates.

**Supplementary Information**

Protein sequences used in this study

**A3A-BE3**

MEASPASGPRHLMDPHIFTSNFNNGIGRHKTYLCYEVERLDNGTSVKMDQHRGFLHNQAKNLLCGFYGRHAELRFLDLVPSLQLDPAQIYRVTWFISWSPCFSWGCAGEVRAFLQENTHVRLRIFAARIYDYDPLYKEALQMLRDAGAQVSIMTYDEFKHCWDTFVDHQGCPFQPWDGLDEHSQALSGRLRAILQNQGNSGSETPGTSESATPESDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDSGGSTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSDAPEYKPWALVIQDSNGENKIKMLSGGSPKKKRKV

**A3A (N57Q/Y130F)-BE3**

MEASPASGPRHLMDPHIFTSNFNNGIGRHKTYLCYEVERLDNGTSVKMDQHRGFLHQQAKNLLCGFYGRHAELRFLDLVPSLQLDPAQIYRVTWFISWSPCFSWGCAGEVRAFLQENTHVRLRIFAARIFDYDPLYKEALQMLRDAGAQVSIMTYDEFKHCWDTFVDHQGCPFQPWDGLDEHSQALSGRLRAILQNQGNSGSETPGTSESATPESDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDSGGSTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSDAPEYKPWALVIQDSNGENKIKMLSGGSPKKKRKV

**eA3A-BE3**

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**eA3A-BE3-2xUGI**

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