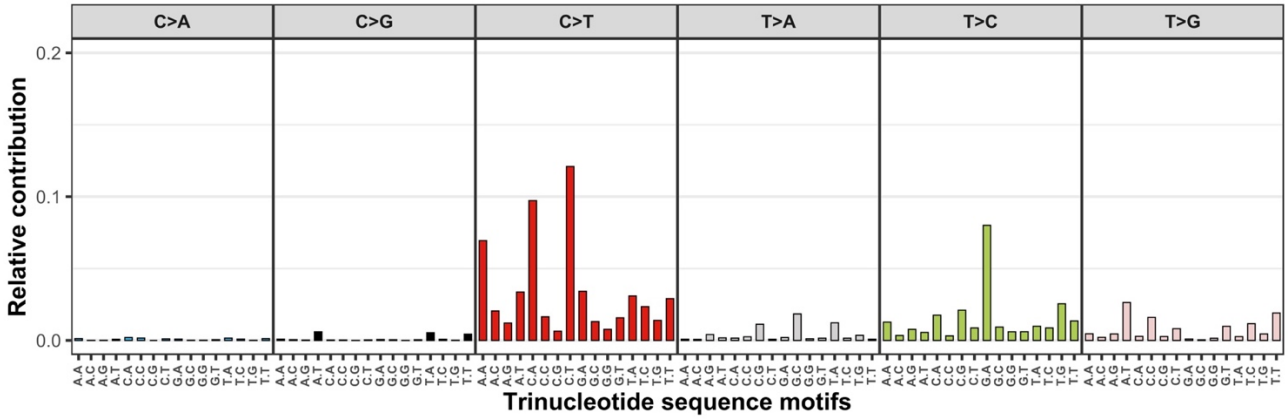
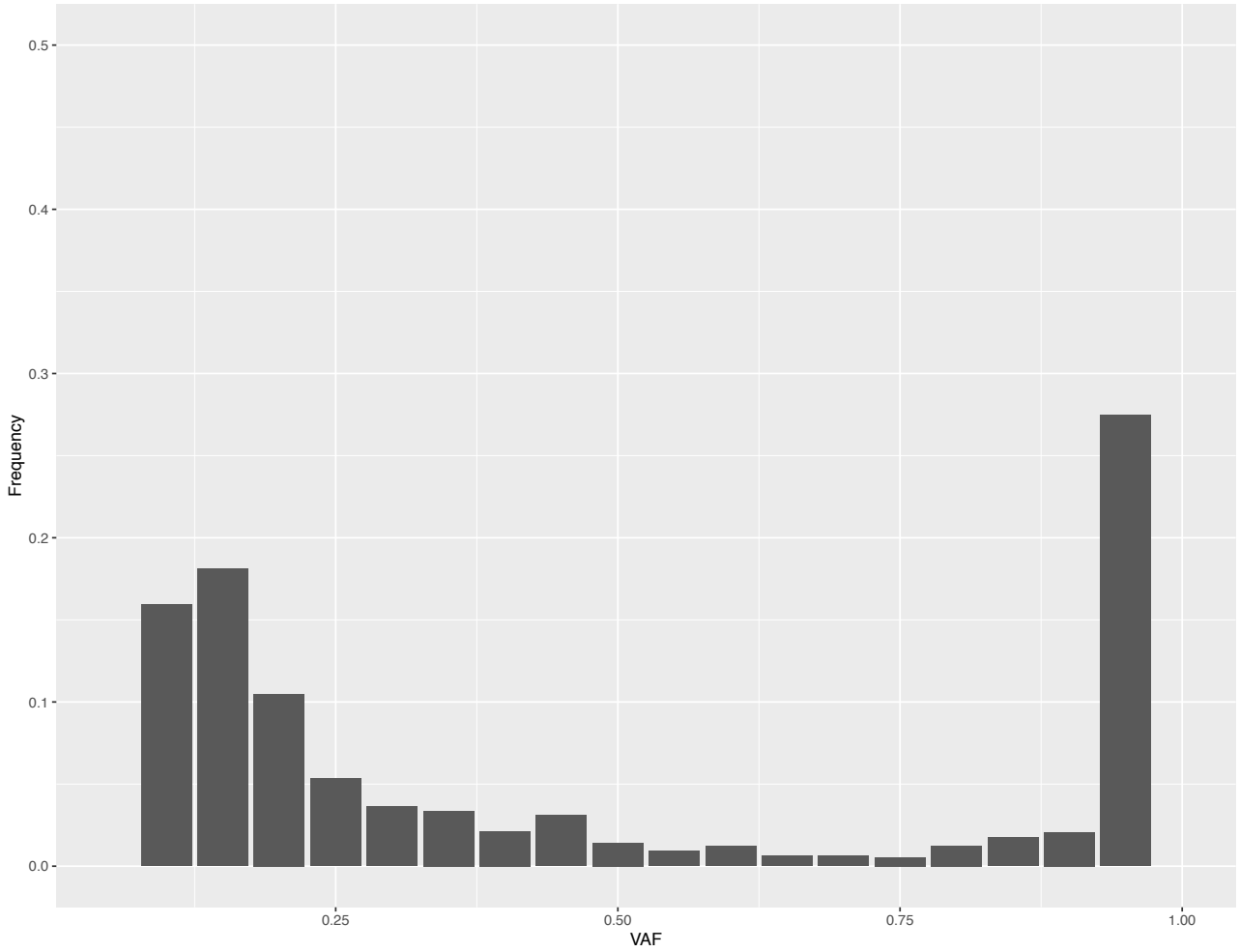
**Supplementary Information**

# Mutations in the HPV16 genome induced by APOBEC3 are associated with viral clearance

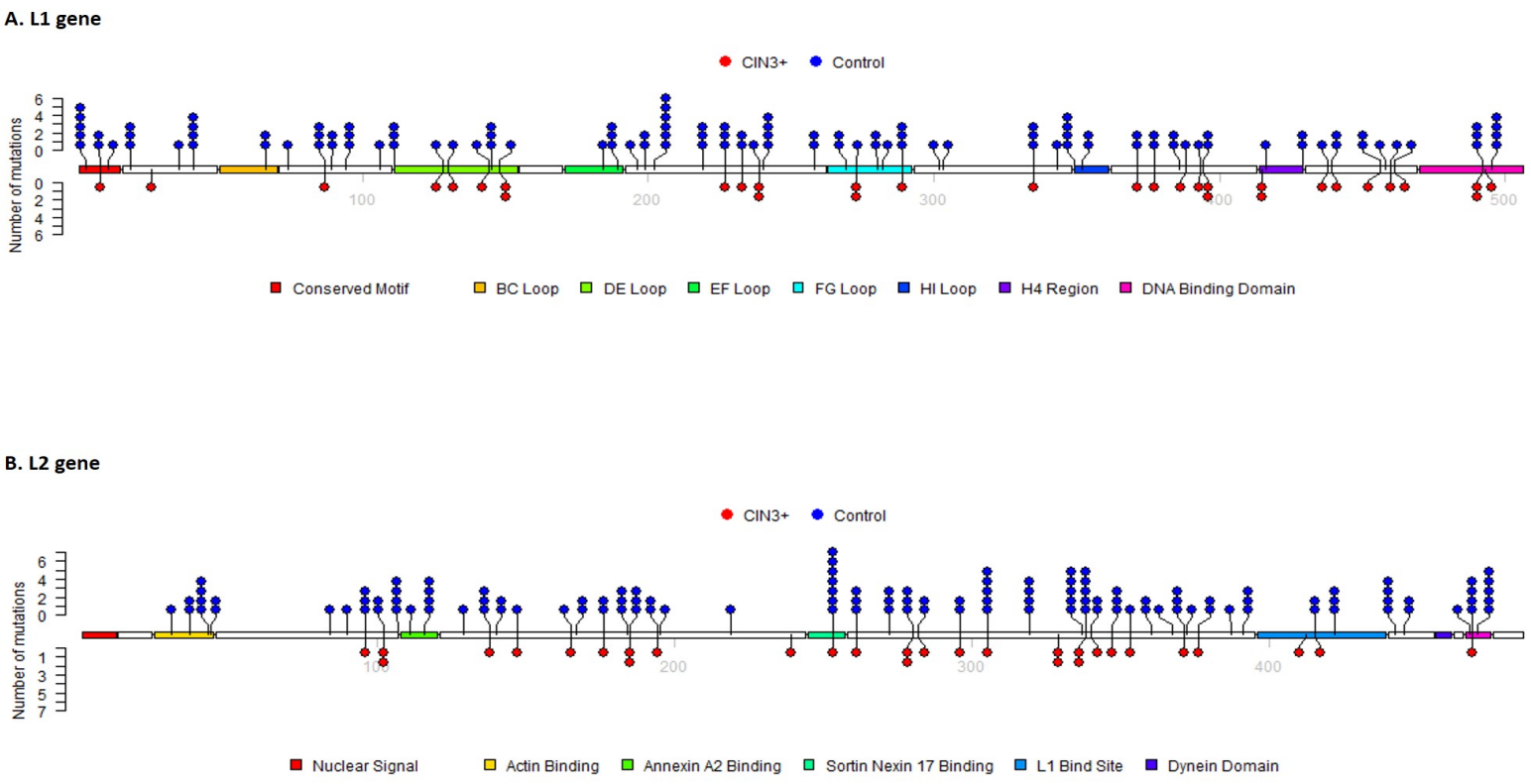
**Zhu et al.**



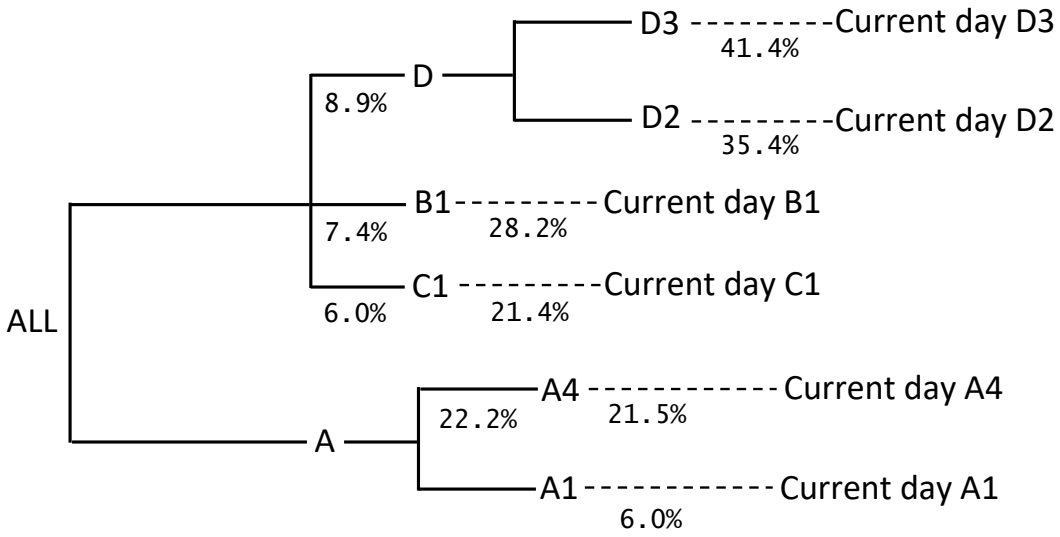

**Supplementary Figure 1:** Frequency of the 96 trinucleotide mutation types for variants across the HPV16 genome in women from the PaP cohort. The x-axis indicates the 5' and 3' nucleotides for each of the top panel substitutions for the 3 base-pair motifs.



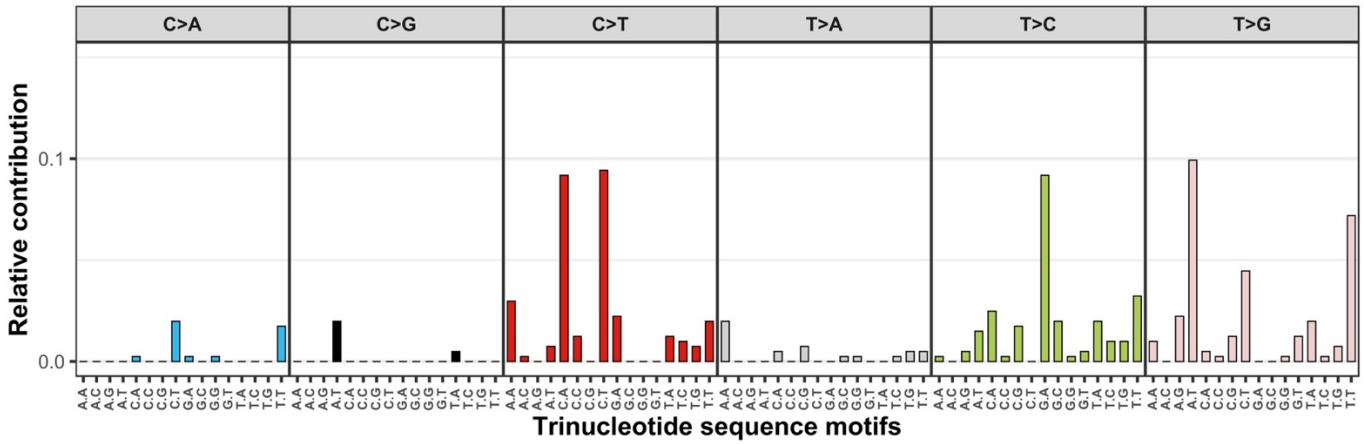

**Supplementary Figure 2:** Frequency distribution of APOBEC3-induced mutations across the HPV16 genome by variant allele fraction (VAF) for women in the PaP cohort. The APOBEC3-induced mutations are categorized into 18 groups based on their VAFs and each bar shows the proportion of APOBEC3-induced mutations belonging to the VAF group.




**Supplementary Figure 3:** Somatic low VAF APOBEC3-induced mutations observed in the (A) L1 and (B) L2 ORF in the CIN3+ cases (red lollipops) and controls (blue lollipops) in women from the PaP cohort. The domains of L1 and L2 are colored, see legend. Cases are cervical intraepithelial neoplasia grade 3 and cancer cases (CIN3+). The number of circles shown with each variant indicate the number of individuals with that variant.




**Supplementary Figure 4:** APOBEC3-induced mutations contributed to the evolution of viral lineages. HPV16 phylogenetic tree illustrating each main sublineage (A1, A4, C1, B1, D2, D3) and the percentage of the lineage-defining sites that were potentially induced by APOBEC3 compared with the ancestral sequence at each node of the tree.




**Supplementary Figure 5:** Frequency of the 96 trinucleotide mutation types for variants across 45 HPV16 genomes sequenced by Hirose et al., J Virol 2018:92;e00017-00018. HPV16 genome sequence data retrieved from GenBank, accession numbers: LC368952 to LC368996. The common evolutionary-derived HPV16 lineage-defining variants are removed.

**Supplementary Table 1.** Number of women with and without APOBEC3-induced mutations stratified by variant allele fraction (VAF) in CIN2+ cases and controls of the NCI-Kaiser PaP cohort.

| VAF | Status | No APOBEC3 mutation | APOBEC3 mutation | % | OR | 95% CI | *P*-value[1] |
|---|---|---|---|---|---|---|---|
| **Low**[2] | CIN2+ | 2023 | 291 | 12.6% | 0.48 | (0.40, 0.57) | $5.8 \times 10^{-16}$ |
| | Control | 971 | 294 | 23.2% | ref | | |
| **High**[3] | CIN2+ | 1840 | 474 | 20.5% | 1.03 | (0.87, 1.23) | 0.76 |
| | Control | 1012 | 253 | 20.0% | ref | | |

CIN2+, cervical intraepithelial neoplasia grade 2 and grade 3, and cancer cases; OR, odds ratio; CI, confidence intervals; ref, referent group;

[1] Fisher's exact test, two-sided, comparing the number of women with at least one APOBEC3-induced mutation and those without APOBEC3-induced mutations in the CIN2+ cases to controls;

[2] Low VAF is defined as VAF >10% and <=60%;

[3] High VAF is defined as VAF >60%.

**Supplementary Table 2.** Burden of APOBEC3-induced mutations in CIN3+ cases vs. controls and nonsynonymous vs. synonymous mutations by variant allele fraction (VAF) in the NCI-Kaiser PaP cohort.

| VAF | Parameter | Interpretation | Viral gene | Mutation burden[1] | 95% CI | FDR adjusted *P*-value[2] |
|---|---|---|---|---|---|---|
| **Low[3]** | $r_{nonsyn}$ | Enrichment of nonsynonymous mutations in cases vs. controls by gene region | *E6* | 1.13 | (0.70, 1.81) | 0.70 |
| | | | *E7* | 0.55 | (0.27, 1.11) | 0.16 |
| | | | *E1* | 0.72 | (0.54, 0.96) | 0.06 |
| | | | *E2* | 1.07 | (0.79, 1.45) | 0.71 |
| | | | *E4* | 1.01 | (0.49, 2.09) | 0.99 |
| | | | *E5* | 0.35 | (0.08, 1.58) | 0.22 |
| | | | *L2* | 0.46 | (0.31, 0.68) | $6.7 \times 10^{-4}$ |
| | | | *L1* | 0.54 | (0.36, 0.80) | 0.01 |
| **High[4]** | $r_{nonsyn}$ | Enrichment of nonsynonymous mutations in cases vs. controls by gene region | *E6* | 0.64 | (0.37, 1.09) | 0.17 |
| | | | *E7* | 0.22 | (0.05, 1.05) | 0.12 |
| | | | *E1* | 0.94 | (0.58, 1.54) | 0.82 |
| | | | *E2* | 1.14 | (0.76, 1.71) | 0.63 |
| | | | *E4* | 7.99 | (1.85, 34.43) | 0.01 |
| | | | *E5* | 1.15 | (0.51, 2.63) | 0.82 |
| | | | *L2* | 0.75 | (0.55, 1.03) | 0.14 |
| | | | *L1* | 0.65 | (0.33, 1.30) | 0.30 |

CIN3+, cervical intraepithelial neoplasia grade 3 and cancer cases; CI, confidence intervals;

[1] Mutation burden ratio of APOBEC3-induced mutations was calculated using a Poisson regression model to compare the mutation burden or enrichment of APOBEC3-induced mutations per virus between cases and controls for nonsynonymous mutations ($r$) by viral gene region;

[2] False discovery rate (FDR) adjusted P-values; P-values are generated by a Wald test of the Poisson regression model;

[3] Low VAF is defined as VAF >10% and <=60%;

[4] High VAF is defined as VAF >60%.

**Supplementary Table 3.** Number of women with and without APOBEC3-induced mutations stratified by variant allele fraction (VAF) in SUCCEED CIN3+ cases and IARC cancer cases.

| VAF | Study | Status | No APOBEC3 mutation | APOBEC3 mutation | % | OR[4] | 95% CI | *P*-value |
|---|---|---|---|---|---|---|---|---|
| **Low[2]** | SUCCEED | CIN3+ | 389 | 55 | 12.4% | 0.47 | (0.34, 0.64) | $5.1x10^{-7}$ |
| | IARC | Cancers | 1142 | 163 | 12.5% | 0.47 | (0.38, 0.58) | $1.1x10^{-12}$ |
| **High[3]** | SUCCEED | CIN3+ | 333 | 111 | 25.0% | 1.33 | (1.02, 1.73) | 0.03 |
| | IARC | Cancers | 992 | 313 | 24.0% | 1.26 | (1.04, 1.53) | 0.02 |

CIN3+, cervical intraepithelial neoplasia grade 3 and cancer cases; OR, odds ratio; CI, confidence intervals;
[1] Fisher's exact test, two-sided, comparing the number of women with at least one APOBEC3-induced mutation and those without APOBEC3-induced mutations in the cases to controls;
[2] Low VAF is defined as VAF >10% and <=60%;
[3] High VAF is defined as VAF >60%;
[4] Odd ratios, 95% CI and *P*-values, estimated using logistic regression comparing SUCCEED or IARC cases to the PaP controls (referent group).

**Supplementary Table 4**. Number of women with and without the specified signature-induced mutation at a low variant allele fraction in CIN3+ cases and controls in the NCI-Kaiser PaP cohort.

| Mutational signature | Status | No signature mutation | Signature mutation | % | OR | 95% CI | *P*-value[4] |
|---|---|---|---|---|---|---|---|
| **Signature B[1]** | CIN3+ | 819 | 463 | 36.1% | 0.74 | (0.63, 0.87) | 0.0003 |
| | Control | 718 | 547 | 43.2% | ref | | |
| **Signature C[2]** | CIN3+ | 1030 | 252 | 18.5% | 0.50 | (0.41, 0.60) | $3.1 \times 10^{-14}$ |
| | Control | 849 | 416 | 32.9% | ref | | |
| **Signature D[3]** | Case | 257 | 1025 | 20.0% | 0.60 | (0.50, 0.72) | $3.4 \times 10^{-8}$ |
| | Control | 373 | 892 | 29.5% | ref | | |

CIN3+, cervical intraepithelial neoplasia grade 3 and cancer cases; OR, odds ratio; CI, confidence intervals;
[1] Signature B is defined as C>T mutations outside the TCW motif (excluding C>T mutations in signature A);
[2] Signature C is defined as all T>C mutations;
[3] Signature D is defined as all other mutations not in signatures A, B or C;
[4] Fisher's exact test, two-sided.

**Supplementary Table 5**. Estimated number of APOBEC3 targetable sites for each HPV16 sublineage genome.

| HPV16 Sublineage | TCW motifs | Targetable sites[†] |
|---|---|---|
| A1 | 394 | 263 |
| A2 | 392 | 261 |
| A4 | 390 | 260 |
| B1 | 388 | 259 |
| C1 | 384 | 256 |
| D2 | 370 | 247 |
| D3 | 369 | 246 |

[†] the number of APOBEC3 targetable sites was estimated as two thirds of the total number of TCW motifs present in each genome since there are three possible changes at each nucleotide position (C>A, C>T and C>G) and APOBEC3 is associated with two changes, C>T and C>G.

**Supplementary Table 6.** Number of total mutations, stratified by nonsynonymous (nonsyn) and synonymous (syn), at a low variant allele fraction in CIN3+ cases vs. controls in the NCI-Kaiser PaP cohort.

| Status | No. of women | All† mutations | All, nonsyn | % | All, syn | APOBEC3‡ mutations | APOBEC3, nonsyn | % | APOBEC3, syn |
|---|---|---|---|---|---|---|---|---|---|
| **CIN3+** | 1282 | 2515 | 1772 | 70.5% | 743 | 238 | 227 | 95.4% | 11 |
| **Control** | 1265 | 5985 | 4284 | 71.6% | 1701 | 644 | 613 | 95.2% | 31 |
| **Total** | 2547 | 8500 | 6056 | 71.2% | 2444 | 882 | 840 | 95.2% | 42 |

CIN3+, cervical intraepithelial neoplasia grade 3 and cancer cases;
† includes all substitutions observed across the HPV16 genome;
‡ includes only the APOBEC3-induced mutations.

**Supplementary Table 7.** Number of women with the specified number of APOBEC3-induced nonsynonymous (nonsyn) and synonymous (syn) mutations at a low variant allele fraction in CIN3+ cases vs. controls in the NCI-Kaiser PaP cohort.

| Status | Number of APOBEC3-induced mutations | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4+ |
| **CIN3+, N=1,282** | | | | | |
| **Nonsyn** | 1140 | 100 | 20 | 10 | 12 |
| **Syn** | 1272 | 9 | 1 | 0 | 0 |
| **Control, N=1,265** | | | | | |
| **Nonsyn** | 985 | 133 | 64 | 40 | 43 |
| **Syn** | 1238 | 23 | 4 | 0 | 0 |

CIN3+, cervical intraepithelial neoplasia grade 3 and cancer cases.

**Supplementary Table 8.** Burden of APOBEC3-induced mutations on the positive vs. negative strand.

| VAF | Strand | Mutation burden | *P*-value[4] |
|---|---|---|---|
| **Low**[1] | Positive | 6.3/kb | 0.47 |
| | Negative | 6.9/kb | |
| **High**[2] | Positive | 5.0/kb | 0.18 |
| | Negative | 4.0/kb | |
| **All**[3] | Positive | 5.8/kb | 0.61 |
| | Negative | 6.2/kb | |

[1] Low variant allele fraction (VAF)is defined as VAF>10% and <=60%;
[2] High VAF is defined as VAF >60%;
[3] All VAF is defined as VAF>10%;
[4] Proportion test.

**Supplementary Table 9.** Burden of APOBEC3-induced mutations for APOBEC3A vs. APOBEC3B possible sites.

| VAF | APOBEC3 | Mutation burden | *P*-value[4] |
|---|---|---|---|
| **Low**[1] | APOBEC3A | 0.33/kb | 0.98 |
| | APOBEC3B | 0.32/kb | |
| **High**[2] | APOBEC3A | 0.22/kb | 0.10 |
| | APOBEC3B | 0.15/kb | |
| **All**[3] | APOBEC3A | 0.35/kb | 0.86 |
| | APOBEC3B | 0.34/kb | |

[1] Low variant allele fraction (VAF) is defined as VAF>10% and <=60%;
[2] High VAF is defined as VAF >60%;
[3] All VAF is defined as VAF>10%;
[4] Proportion test.