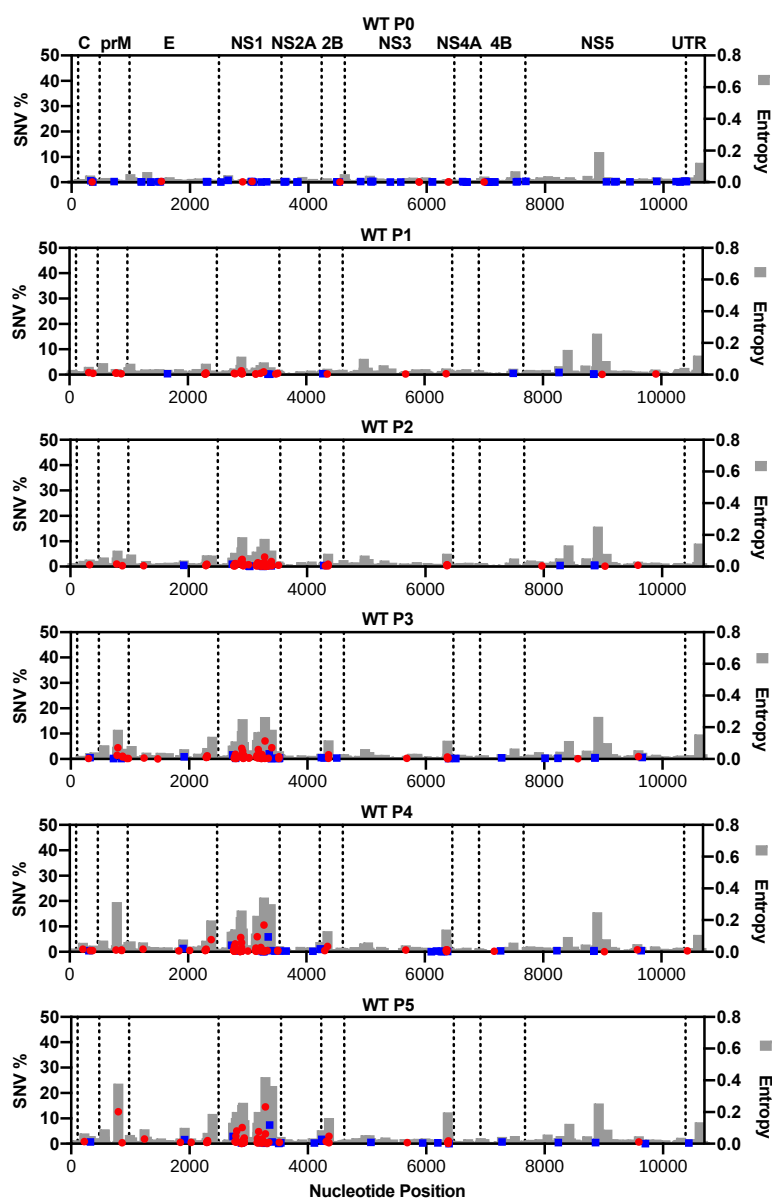
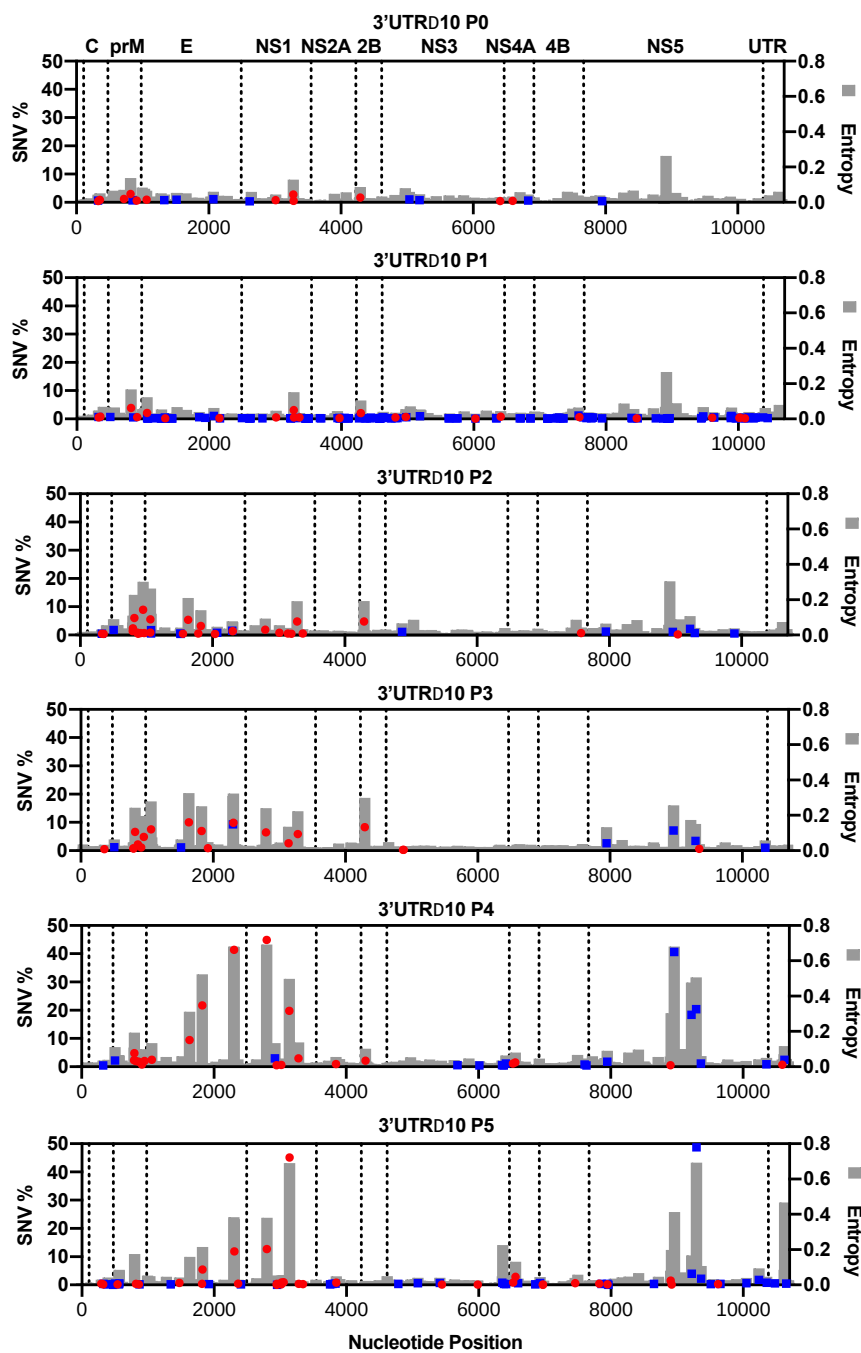


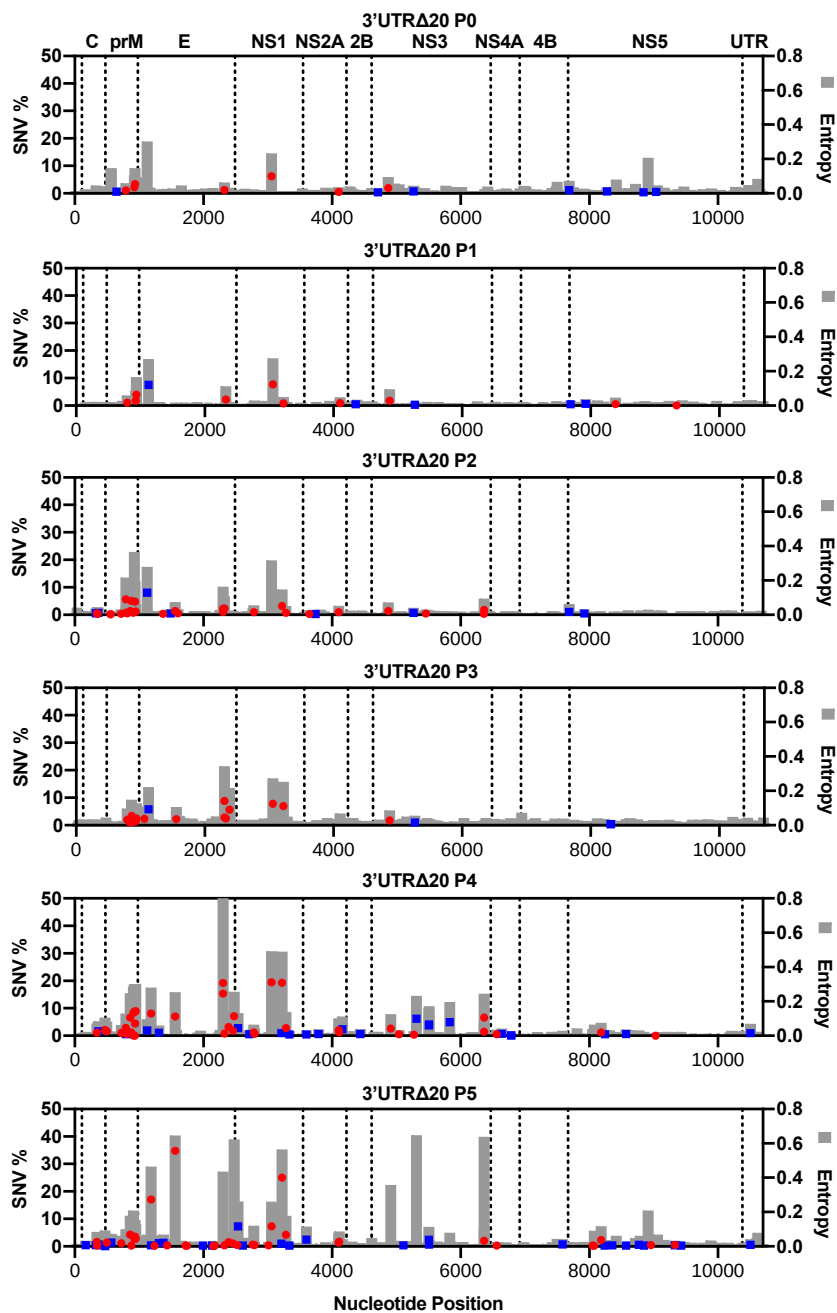
# Supplementary Materials



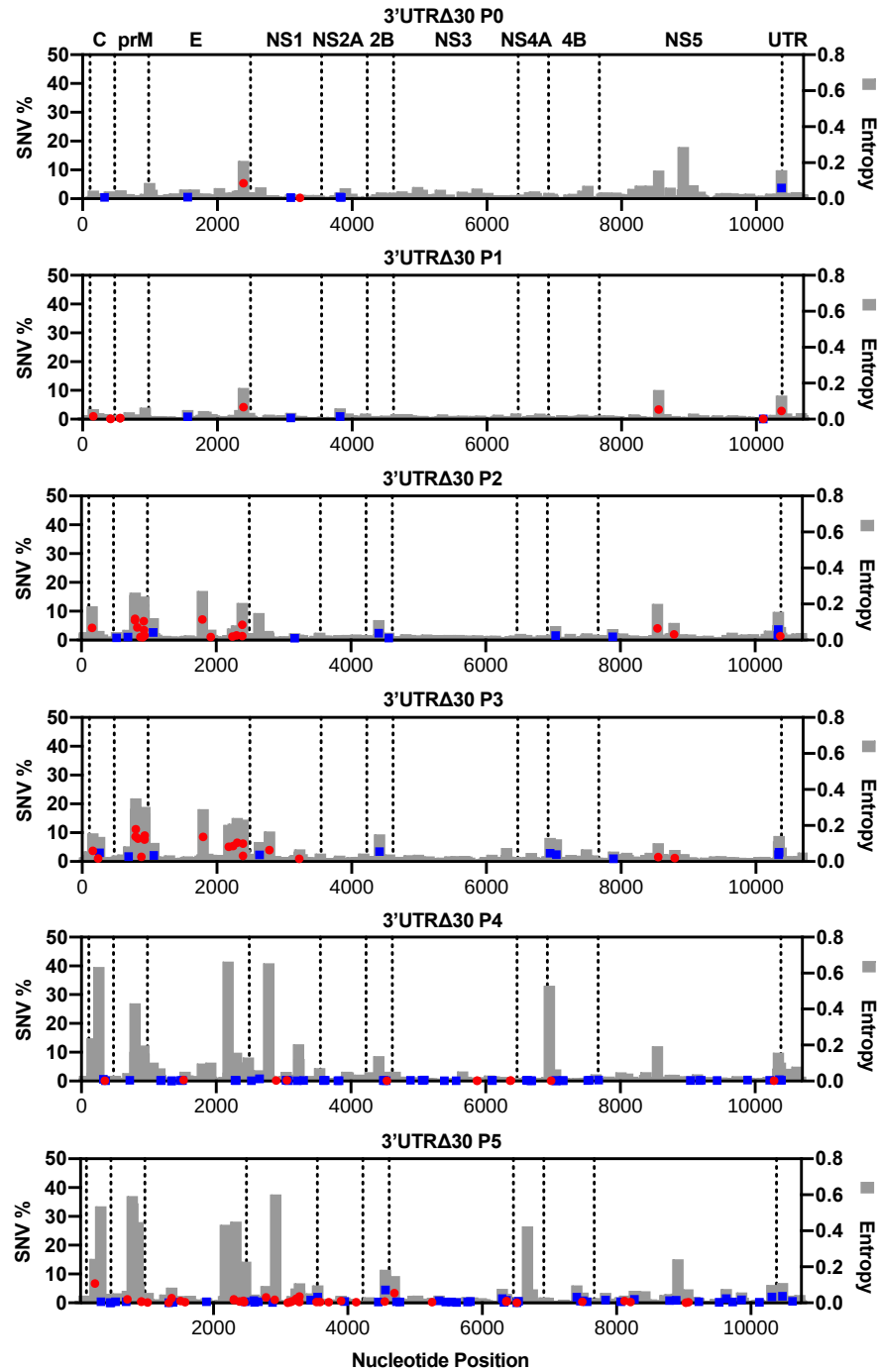
(a)



(b)



(c)



(d)

**Figure 1.** Entropy values and variant frequencies across the ZIKV genome. The frequency of synonymous (blue square symbols) and non-synonymous SNVs (in red circle symbols) is plotted according to nucleotide position over the entropy values (in gray bars) of each position across the genome for WT (a), 3'UTRΔ10 (b), 3'UTRΔ20 (c), 3'UTRΔ30 (d) infectious clone passages 0–5 in Vero cells. Only significant SNVs as determined by Vphaser2.0 are shown. Strand bias filter applied to SNVs:  $\alpha = 0.05$ .

**Table 1.** Significance comparison of positional entropy values. Kruskal Wallis with Dunnett's post-test p-values of mean positional entropy values.

| Passages | WT      | 3'UTRΔ10 | 3'UTRΔ20 | 3'UTRΔ30 |
|----------|---------|----------|----------|----------|
| 0 to 1   | 0.9375  | <0.0001  | <0.0001  | <0.0001  |
| 0 to 2   | <0.0001 | <0.0001  | <0.0001  | <0.0001  |
| 0 to 3   | <0.0001 | <0.0001  | <0.0001  | <0.0001  |
| 0 to 4   | <0.0001 | <0.0001  | <0.0001  | 0.0109   |
| 0 to 5   | <0.0001 | <0.0001  | <0.0001  | <0.0001  |
| 1 to 2   | <0.0001 | <0.0001  | >0.9999  | <0.0001  |
| 1 to 3   | <0.0001 | <0.0001  | <0.0001  | <0.0001  |
| 1 to 4   | <0.0001 | <0.0001  | 0.4621   | <0.0001  |
| 1 to 5   | <0.0001 | <0.0001  | <0.0001  | <0.0001  |
| 2 to 3   | 0.9002  | <0.0001  | <0.0001  | <0.0001  |
| 2 to 4   | <0.0001 | >0.9999  | 0.0023   | <0.0001  |
| 2 to 5   | 0.0154  | <0.0001  | <0.0001  | <0.0001  |
| 3 to 4   | 0.006   | <0.0001  | <0.0001  | <0.0001  |
| 3 to 5   | <0.0001 | <0.0001  | <0.0001  | <0.0001  |
| 4 to 5   | <0.0001 | <0.0001  | <0.0001  | <0.0001  |