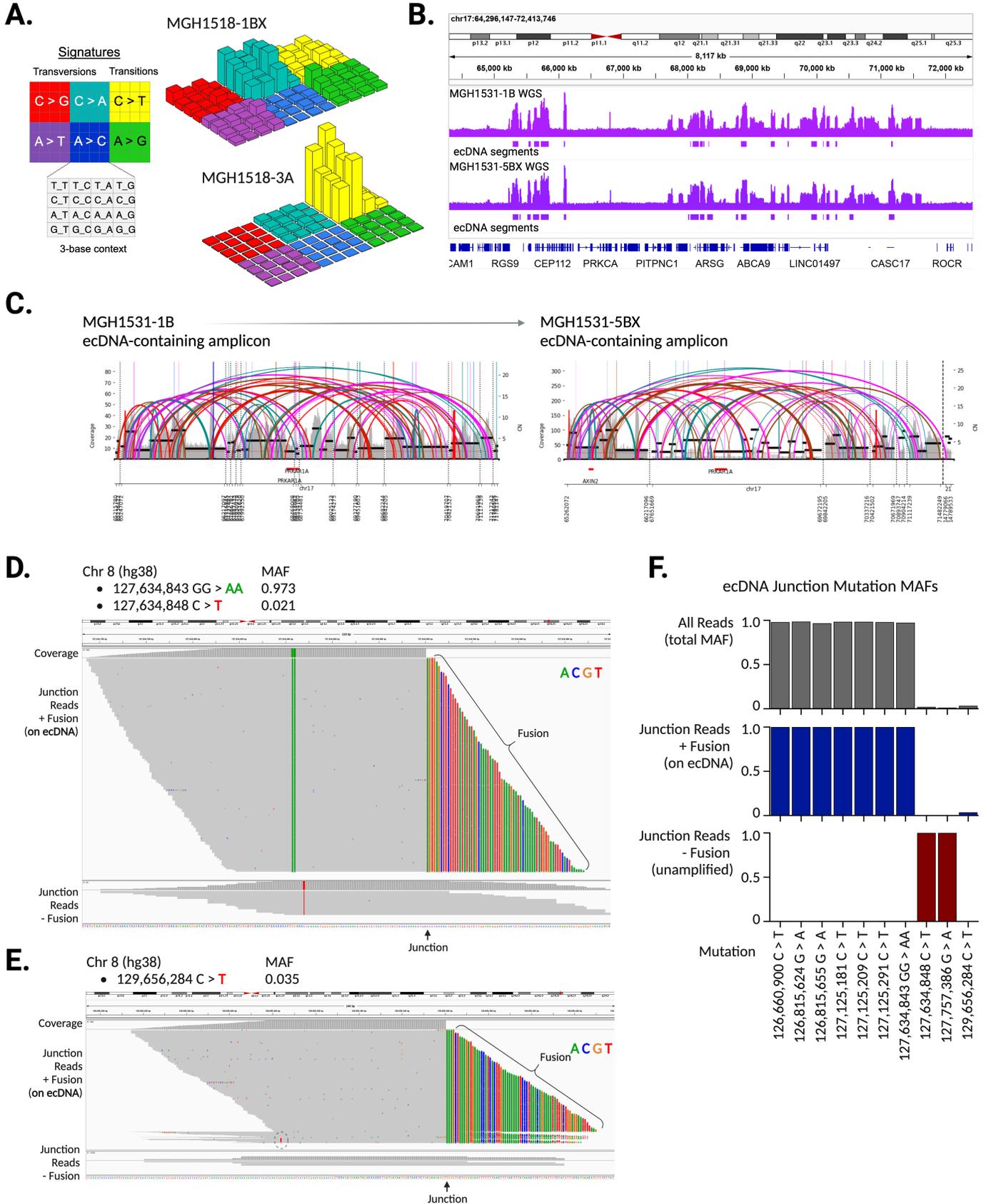


# Supplementary Figure S4



**Supplementary Figure S4. Genomic analysis of chromosome 17 focal amplification in MGH1531-5BX and analysis of ecDNA junction mutations in MGH1518-3A.** (A) Three-dimensional bar plots (also called “Lego plots”) representing mutational signatures in a three-base context for each model. (B) Copy number variation on chromosome 17 demonstrated focal amplification of discontinuous segments in both MGH1531-1B and MGH1531-5BX. Peak heights = counts per million mapped reads (CPM). (C) AmpliconArchitect reconstruction of the rearrangements that formed the shared ecDNA in the MGH1531 models. (D-E) TMZ-induced mutations that occur near *ecMYC* junctions, viewed on Integrative Genomics Viewer (IGV) with reads sorted by the presence (top) or absence (bottom) of an ecDNA fusion sequence (49). (D) Two TMZ-induced mutations near the same ecDNA junction, one with a high MAF that is present only on reads that contain a fusion sequence, and one with a low MAF that is present only on reads that lack a fusion. Both are detected on all reads within their fusion categories, and none in the opposite category. (E) A single sub-clonal TMZ-induced mutation was detected in a small proportion of fusion-containing junctional reads. (F) 10 TMZ-induced mutations were close enough to *ecMYC* junctions that their frequencies on reads that either did or did not contain a fusion sequence could be scored, as well as total MAF on all reads. (created with BioRender.com)