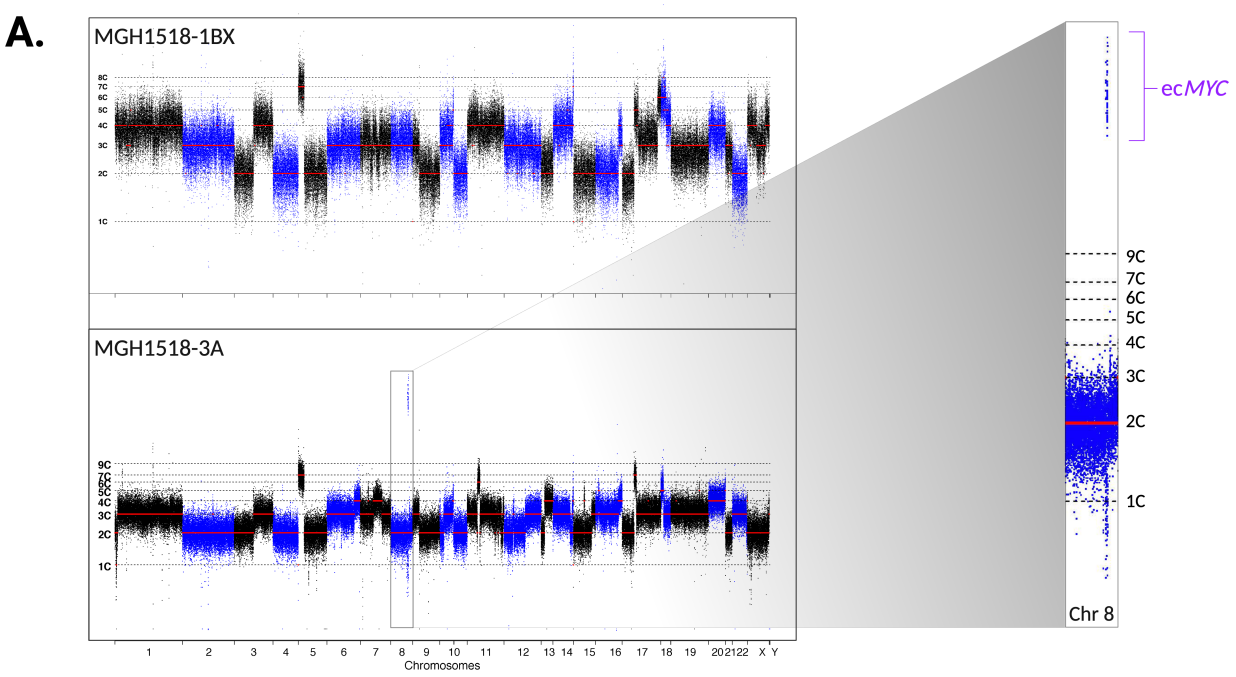
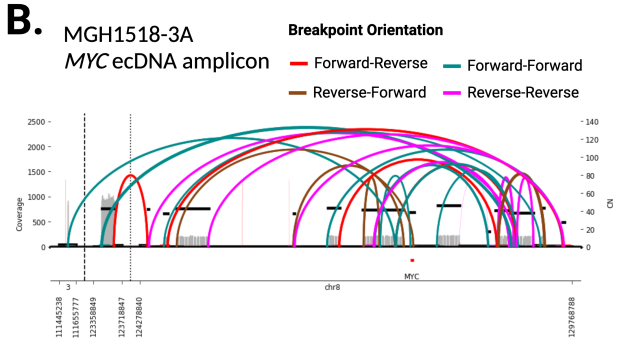
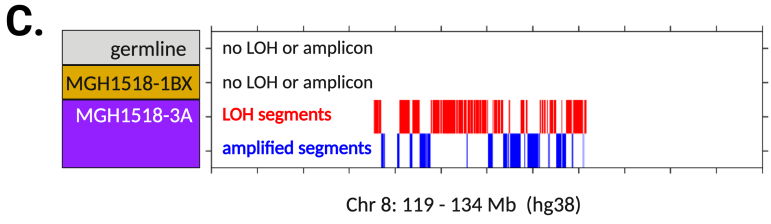
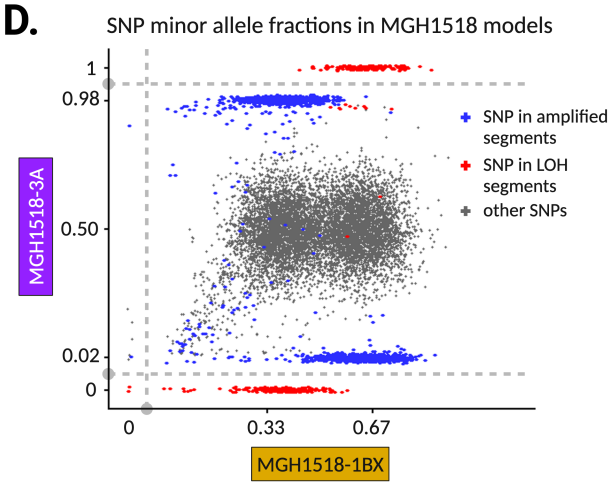
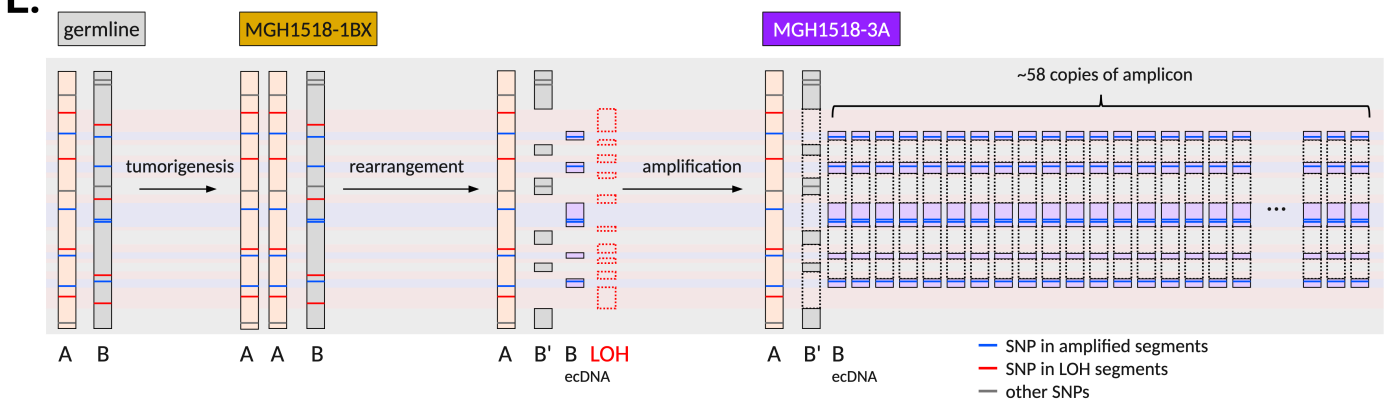
Supplementary Figure S3











Supplementary Figure S3. Genomic analysis of chromosome 8 focal amplification in MGH1518-3A. (**A**) Copy number variation across whole genomes of MGH1518-1BX and MGH1518-3A. Of note, 3 copies of chromosome 8 (chr 8) were present in MGH1518-1BX, whereas 2 were present in MGH1518-3A. Inset: chr 8 with region of ec*MYC* amplification indicated. (**B**) AmpliconArchitect reconstruction of the rearrangements that formed ec*MYC* in MGH1518-3A. (**C**) Fine map of amplified segments and loss of heterozygosity (LOH) in a 15 Mb region that contains the ec*MYC* amplification. Map derived from read counts at 14,055 positions with germline heterozygosity that can be analyzed as single nucleotide polymorphisms (SNPs). Maps for MGH1518 germline, MGH1518-1BX and MGH1518-3A are shown. In this region of chr 8, there was retention of heterozygosity (ROH) overall, and there was no evidence of LOH within the amplified segments. However, there was complete LOH in the un-amplified segments that surround the amplified segments. (**D**) Allelic ratios of SNPs on chr 8 in the MGH1518 models. The presence of 3 copies of chr 8 in MGH1518-1BX created an allelic imbalance that allowed complete phasing of germline alleles “A” and “B”, which are present in either a 1:2 ratio or a 2:1 ratio. These ratios are expressed as the minor allelic fraction (AF), with values of either ~0.33 or ~0.67 in MGH1518-1BX (x-axis). These SNP AFs changed in MGH1518-3A (y-axis) in a coordinated and location-dependent manner, depending on whether they were located within the ec*MYC*-amplified segments (blue), LOH segments (red) or neither (gray). SNPs that were undetectable (AF = 0) or present in all reads (AF = 1) are separated by dashed lines. (**E**) Model of ec*MYC* formation derived from the patterns of amplification, LOH, and SNP AF changes in the MGH1518 serial models. Most SNPs were not located within an amplified segment or LOH segment. These SNPs converged from allelic imbalance in MGH1518-1BX (AF ~0.33 or ~0.67) to allelic balance in MGH1518-3A (AF ~0.5), reflecting an overall change in chr 8 copy number from 3 to 2. In contrast, the SNPs within amplified segments and LOH segments diverged to greater allelic imbalance in MGH1518-3A, depending on their initial AF in MGH1518-1BX. Intra-amplicon SNPs with AF ~0.33 before rearrangement and amplification (MGH1518-1BX) rose to AF ~0.98 in MGH1518-3A. Conversely, intra-amplicon SNPs with an initial AF ~0.67 fell to AF ~0.02 in MGH1518-3A. These patterns suggest that the chr 8 with 1 copy (“B”) in MGH1518-1BX was rearranged and amplified to form ec*MYC*, whereas the chr 8 with 2 copies (“A”) remained intact in MGH1518-3A but 1 of the copies was lost. For the intra-amplicon SNPs with a pre-amplification AF ~0.67, the distinction between a post-amplification AF of ~0.02 and 0 is important, as it signifies dilution among amplified segments as opposed to LOH. For SNPs in the adjacent LOH segments, an inverse relationship was observed. Intra-LOH SNPs with an initial AF ~0.33 in MGH1518-1BX were lost after rearrangement and amplification (AF=0 in MGH1518-3A), but SNPs with an initial AF ~0.67 became homozygous after rearrangement (AF=1 in MGH1518-3A). These patterns suggest that the segments on copy “B” adjacent to the ecDNA segments were lost during rearrangement. Importantly, for SNPs within the segments that formed ec*MYC*, rearrangement and amplification resulted in a highly bimodal distribution of AFs. (created with BioRender.com)