Supplemental Methods

Sample Preparation

Peripheral blood mononuclear cells (PBMCs) from before venetoclax initiation and upon documented clinical development of venetoclax resistance were enriched with Ficoll-Hypaque gradient centrifugation and cryopreserved until use. Prior to DNA preparation, B lymphocytes were purified by negative selection using the EasySep™ Human B Cell Enrichment Kit (Stemcell Technologies) following the manufacturers' recommendation. Genomic DNA was extracted using the QIAmp DNA Mini Kit (QIAGEN). Genomic DNA was fragmented using a Covaris Focused-ultrasonicator (Covaris).

Targeted MiSeq deep sequencing of *BTK, PLCG2* and *BCL2* genes

Illumina-compatible sequencing libraries were prepared using the KAPA HyperPrep Kit (Roche) and custom adapters using dual-unique barcodes and inline unique molecular indices (UMIs). Target Enrichment for the entire coding sequences of *BCL2*, *BTK*, and *PLCG2* was performed with xGen Lockdown Probes (IDT). Libraries were sequenced using Illumina MiSeq (Illumina, San Diego, CA). Results for *BTK, PLCG2*, and *BCL2* genes were confirmed using the Ion S5 system (A27212) as previously described.[1] DNA library preparations, target enrichment, and sequencing were performed according to manufacturers' instructions. Due to the small target space, a double-capture approach was used for maximal enrichment on the Illumina libraries.[2] Ampliseq HD technology was used on Ion Torrent. Sequenced reads were aligned to the *hg19* genome build using the Burrows-Wheeler Aligner.[3] Picard Tools was used to perform UMI-consensus calling on the aligned reads. The Genome Analysis Toolkit (GATK)[4] was used to realign insertions and deletions in the aligned reads and to perform base quality score recalibration for

those realigned regions. GATK's MuTect2[5] was used to perform variant calling. After variant calling, variants were annotated using SnpEff[6] and vcfanno[7] along with the dbSNP, COSMIC, 1000 genomes, and 6500 exomes variant databases. Mucor[8] was used for integrative mutation assessment. All called variants underwent visual inspection of the aligned reads using the Integrative Genomics Viewer. Variants in regions of high discrepancy, low quality, or mononucleotide runs were excluded. Common SNPs were excluded.

References

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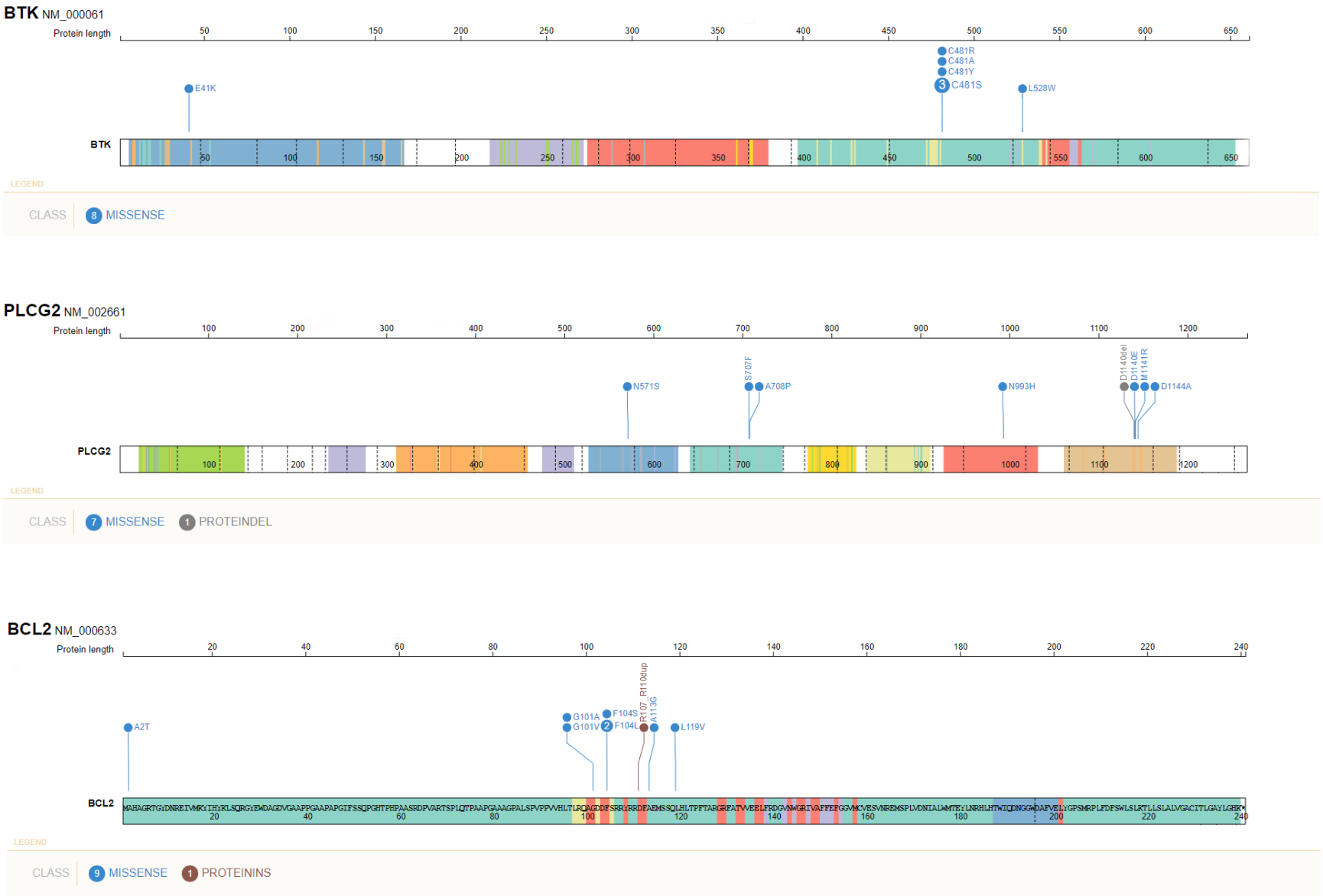
Suppl. Table 1: Sequencing results *BTK*, *PLCG2*, and *BCL2*



Supplemental Figure 1. Maps of (top to bottom) BTK, PLCG2, and BCL2 proteins demonstrating missense, insertion, and deletion mutations.