SUPPLEMENTAL METHODS

CLL patient samples:

Peripheral blood samples were collected from CLL patients at Mayo Clinic, in compliance with the Declaration of Helsinki and the Mayo Clinic Institutional Review Board. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque (GE Healthcare) gradient centrifugation, cryopreserved, and stored in liquid nitrogen.

Plating single CLL cells:

Cryopreserved PBMC from CLL patients were thawed, and stained with CD5-APC and CD19-FITC (BD Bioscience), for FACS sorting. Pure sorted CLL cells were then plated into 96 well PCR plates at the cell density of 20 cells/100 µl PBS/plate (0.2 cells/1.0 µl PBS/well). Plates were used for PCR immediately or stored at -20°C and analyzed at a later date.

PCR amplification of *SF3B1* sequences in single cells:

Single CLL cells in 1.0 µl PBS were lysed in an additional 5 µl of initial PCR mix, which contains 3 µl of 2x HotstarTaq PCR mix (Qiagen), 2 pmol of each primer PF1 (5'-TGGTCTGGCTACTATGATCTC-3') and PR1 (5'-AAAAGGTAATTGGTGGATTTAC-3'), and 0.2% Triton X-100 in 2.0 µl water. Two microliters of initial PCR mix was then re-amplified in a 25 µl PCR volume using primer set PF1 and PR2 (5'-TCCATAAAGGCTTTAACACAG). The final PCR products were resolved on 1% agarose gels, and SF3B1 amplicons were purified and subjected to Sanger sequencing.

For single cell RT-PCR, single CLL cells in 1.0 µl PBS were lysed in an additional 2 µl cell lysis solution consisting of 0.15% Triton X-100 and 1 unit of RNaseOUT™ (Invitrogen) for 30 mins on ice. The cell lysates were then used for one-step RT-PCR (Qiagen) using primer set PF1 and

PR2 in a total volume of 6 µl, followed by allele (mutation)-specific qPCR and/or nested PCR using primer set PF1 and PR3 (5'-CATAAGGAGTTGCTGCTTCAG-3') for Sanger sequencing.

shRNA SF3B1 knockdown and clonogenic assay.

HEK293 cells were infected with recombinant lentivirus (Sigma) encoding control or SF3B1 shRNA, and puromycin selection began the next day. After two days of selection, the viable cells were then seeded in clonogenic assays at 200 cells/petri dish (100 mm). During the 10-day clonogenic assay, puromycin was continuously present. Clones were finally fixed with 2% formaldehyde and stained with Coomassie blue.

Analysis of Truncation Mutations

For truncation mutation analysis, we verified and tallied all deposited mutations of all 35 top cancer genes, and calculated the percentage of mutations that are truncation mutations including frameshifting insertions and deletions, and nonsense mutations. We began this analysis using the COSMIC v59 release and continued to update our analysis as later updated version (v63 release) became available.