## Supplementary Methods

### PBMC Isolation

Peripheral blood samples (25 mL) were drawn into Lithium heparin-containing vacutainer tubes and pooled together in a 50 mL conical tube, before being diluted with an equal volume (1:1) of balanced salt solution. Diluted blood was then layered carefully by transfer pipette over a 15 mL gradient of Ficoll–Paque PLUS (1.077 g/mL, GE Healthcare) and centrifuged (800 x g) for 20 min at 20˚C, without break. PBMCs (lymphocytes, monocytes and thrombocytes) which concentrate at the interface as a cloudy like buffy layer were gently transferred to a new 50 mL conical tube and washed with sterile autoMACS® running buffer (Miltenyi Biotech) followed by centrifugation (800 x g) at 4˚C for 10 min. The resulting supernatant was removed and the remaining cell pellet resuspended in autoMACS® buffer to be counted using a CELL-DYN Sapphire™ hematology analyser (CD Sapphire). Based on total cell number, PBMCs were then split for downstream DNA, protein and cytospin processing.

### MACS Isolation of Tumor Cells from Bone Marrow

Mononuclear cells were isolated from aspirate samples (5 mL) using a Ficoll–Paque PLUS (GE Healthcare) based density centrifugation procedure, as described above. Dissociated cells ($10^7$) collected from the buffy layer were then centrifuged (300 x g) at 4˚C for 10 min and tumor cells negatively selected for using a immunomagnetic cell sorting system (MACS), according to manufacturer's instructions. In brief, cells were resuspended in 70 µL autoMACS® running buffer (Miltenyi Biotech) and subsequently incubated for 10 min at 4˚C with 20 µL fcR blocking reagent (Miltenyi Biotech) and a 10 µL mix containing various biotinylated antibodies (Supplementary Table S6) whose targets are not expressed on the tumor cells of interest. Next, cells were washed, centrifuged and resuspended in 60 µl buffer, before being magnetically labelled with 20 µl of Anti-Biotin MicroBeads (Miltenyi Biotech) for 15 min at 4°C. To remove excess beads from solution, cells were once again washed and centrifuged (300 x g) at 4˚C for 10 min. Following disposal of the wash solution, cell pellets were resuspended in 500 µL buffer, and the resulting suspension added to a prepped MACS® LS column fitted in the magnetic field of a MACS Separator (Miltenyi Biotec). Once flow through was collected, the column was washed a further three times with 3 mL buffer to ensure complete removal of unlabelled tumor cells. Normal cell populations retained on the column, were then eluted in 3 mL buffer. Lastly, both cell fractions were counted using a CELL-DYN Sapphire™ hematology analyser (CD Sapphire) and then split for downstream DNA, protein and cytospin processing.

**Cytospin Preparation**

PBMCs, MACS sorted leukocytes and cultured cells were fixed in freshly prepared 2% paraformaldehyde (Electron Microscopy Sciences) solution for 5 min at room temperature and then washed two times with phosphate-buffered saline (PBS). Following fixation, cells were spun onto pre-charged SuperFrost® Plus slides (Menzel Gläser) using a double cytology funnel (to allow side-by-side placement of pre-treatment (baseline) sample with each treatment sample on the same slide) (Biomedical Polymers Inc.) in a Shandon Cytospin 3 centrifuge operated at 200xg for 5 min. Lastly, slides were air dried for 1 min and stored at -20˚C, until ready to process for fluorescent in situ hybridization (FISH) analysis.

**Tumor Biopsy Processing and Histology**

Tissue specimens (skin, lymph nodes and liver) collected via ultrasound guided biopsy were fixed in 10% neutrally buffered formalin for 24 hr and then dehydrated and embedded in paraffin using standardized and automated procedures. Trephine biopsies of the bone marrow were fixed in B5 for 2 hr and then decalcified in OsteoSoft for 24 hr before being processed with the other specimens to paraffin-wax embedding. For conventional histology or FISH analysis, 4-5 μm thickness sections were cut from formalin-fixed paraffin-embedded (FFPE) blocks and adhered onto pre-charged SuperFrost® Plus slides (Menzel Gläser). All histology processing outlined above was performed by the department of Anatomical Pathology at the Peter MacCallum Cancer Centre.

**Protein Immunoblotting**

Harvested biopsy tissue was snap-frozen in liquid nitrogen and processed for protein extraction using a ceramic mortar and pestle in the presence of SDS lysis buffer (0.5 mM EDTA, 20 mM HEPES, 2% SDS pH 7.9). Following quantitation using the Bio-Rad DC Protein Assay kit, equal amounts of protein were resolved by SDS-PAGE, electrophoretically transferred to an Immobilon-P polyvinylidene fluoride (PVDF) membrane (Millipore) and then blocked for 1 hr in 5% non-fat dry milk containing Tris-buffered saline (20 mM Tris and 150 mM NaCl) with 0.1% Tween-20. Membranes were subsequently blotted with primary antibodies overnight at 4˚C and then detected using horseradish peroxidase–conjugated secondary antibodies (Supplementary Table S7), followed by exposure to enhanced chemiluminescence (Perkin Elmer) and X-ray film (Fujifilm SuperRX) using an Agfa CP1000 developer.

**Fluorescent *In Situ* Hybridization Targeting Ribonucleic Acid Molecules (RNA-FISH)**

Prior to undergoing hybridization, cytospin slides were permeabilized with 100% methanol for 10 min at −20˚C and then pre-washed two times (5 min each) with diethylpyrocarbonate treated water (DEPC-$H_2O$) containing 10% deionised formamide (Life Technologies) and 2x saline-sodium citrate (SSC, Ambion®). FFPE tissue sections were dewaxed in xylene and rehydrated in decreasing concentrations of ice cold ethanol (100%, 85% and 70%), before being pre-treated with 10 mM sodium citrate (pH 6) for 2 min in a pressure cooker at 120˚C. Sections were then washed in DEPC-$H_2O$ and heated in a water bath for 10 min with 1 µg/ml Proteinase K (*Pichia pastoris*) solution (Roche Applied Science) at 37˚C, followed by immediate fixation in 4% paraformaldehyde/PBS for 20 min at room temperature. To confirm on-target activity, a single oligonucleotide probe conjugated to Cyanine3, was custom designed by Stellaris LGC Biosearch technologies to detect the 5'ETS region of ribosomal RNA 5'UGAGAGCACGACGUCACCACAUCGAUCGAAGAGC'3. To perform hybridization, probe was diluted and applied to all samples at 10 µg/mL in a hybridization buffer containing, 10% formamide, 2x SSC, 10% dextran sulfate (Sigma-Aldrich), 50 µg/mL BSA (Sigma-Aldrich), 0.5 µg/mL yeast tRNA (Sigma-Aldrich), 2 mM ribonucleoside vanadyl complex (New England Biolabs) and DEPC-$H_2O$. Each slide was cover slipped, sealed with Fixogum rubber cement (Marabu), and incubated for 3 hr at 37˚C in a dark humidified chamber. Following hybridization, rubber cement was removed and the slides washed two times in 10% formamide-containing 2x SSC buffer (5 min each), allowing coverslips to wash off in solution. After rinsing, slides were washed 3 times with 0.1x SSC at 37˚C for 5 min in a water bath. Slides were then soaked in a final wash of PBS for 5 min and mounted with fluorescent mounting medium containing 4',6-diamidino-2-phenylindole (DAPI) (Vector Laboratories). FISH Images were captured at 60x magnification using an Olympus BX-61 wide field fluorescence microscope (Olympus America Inc.), equipped with a SPOT RT3 digital camera and SPOT Advanced™ software, version 5.0 (Diagnostic Instruments Inc.). Signal intensity was quantitated by configuring an analysis solution in Definiens Tissue Studio®, version 3.6 (Definiens AG, Germany). To identify cells of interest, a nucleus detection tool based on the local contrast of DAPI stain and the typical nucleus size was initially applied by the software. Regions of hybridization were then identified using a spot detection tool, by gating above a user-defined 5'ETS stain threshold. For quantification of total 5'ETS signal, the local background intensity of each nucleus (excluding nucleoli spots) was subtracted from the average fluorescent intensity of all nucleoli spots within each respective nucleus. All images were finally batch processed using the same manually set parameters and thresholds. Data values were plotted using GraphPad Prism software (Version 6.0f) and overlays

of FISH images were adjusted equally for brightness and contrast using ImageJ (1.47v, NIH) or Adobe Photoshop CS4 (Version 11.0.2).

**Immunohistochemical Analysis**

p53 immunostaining was performed on FFPE skin sections by the department of Anatomical Pathology at the Peter MacCallum Cancer Centre, using standard automated procedures. Slides were then scanned at 20x on the ScanScope® XT system (Aperio Technologies).

**Patient Tumor Mutation Status**

Genomic DNA was isolated from MACS sorted leukocytes or snap frozen tumor tissue by the department of Molecular Pathology at the Peter Mac Callum Cancer Centre using the DNeasy Blood & Tissue Kit (Qiagen) according to manufactures instructions. All 13 available patient DNA samples were assayed using a custom targeted hybridisation-based next generation sequencing panel as described previously (1) to identify sequence variants in genes associated with the CX-5461 response including *TP53*, *ATM*, *ATR*, and *CHEK2* (for the complete list of 79 genes see Supplementary Table S4) and copy number variations in *MYC* and *MDM2* (all data shown in Supplementary Table S5).

**Supplementary References**

1. Ryland GL, Jones K, Chin M, Markham J, Aydogan E, Kankanige Y, et al. Novel genomic findings in multiple myeloma identified through routine diagnostic sequencing. J Clin Pathol. 2018;71:895–99.