Supplemental Methods

CRISPR Knockdown

For the inducible sgRNA constructs, FgH1tUTG (Addgene #70183)[1] plasmid was used. To clone individual sgRNAs, 24-bp oligonucleotides containing the sgRNA sequences were synthesized (Sigma-Aldrich). They included a 4-bp overhang for the forward (TCCC) and complementary reverse (AAAC) oligos to enable cloning into the BsmBI site of the lentiviral construct. SgRNAs were cloned by annealing two DNA oligonucleotides and ligating into a BsmBI-digested FgH1tUTG vector. The sequences of sgRNA are listed in supplemental Table 7. For CRISPR knockdown, cells with stable Cas9 expression were sorted after transduction with the Cas9 vector (Addgene # 99154). These cells were then transduced with the inducible sgRNA vector containing the designed sequences. For treatment of cell lines to induce expression of the sgRNA, doxycycline hyclate was dissolved in sterile water at a stock concentration of 1 mg/ml and added to tissue culture media for a final concentration of 1 μg/ml.

Overexpression Experiment

The human *ZFP91* open reading frame was subcloned from cDNA of SU-DHL-1 cells into pLVX-EF1a-AcGFP1-N1 (a gift from Steen Hansen laboratory, Boston Children's Hospital, USA). The human *CSNK2B* open reading frame was subcloned from pDONR223-CSNK2B (addgene plasmid #23359) into pLVX-EF1a-AcGFP1-N1.

The sequences of primer pairs used for PCR cloning are:

ZFP91 forward 5'− TATATATGGATCCGCCACCATGCCGGGGGGAGACGGA −3'
ZFP91 reverse 5'− TTAATTGCTAGCCTAAGGTCCGGCAGAGTC −3'
CSNK2B forward 5'− AATTTCGCGGATCCGCCACCATGAGCAGCTCAGAGGAGGT −3'
CSNK2B reverse 5'− AAGGAATTAAGCGGCCGCTCAGCGAATCGTCTTGACTGG −3'

Site-Directed Mutagenesis

ZFP91 G406A was generated using the QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent, #210518) according to the manufacturer's instructions. Briefly, 100 ng of pLVX-EF1a-ZFP91-WT vector DNA and 125 ng of the mutagenesis primers were mixed with 10 × reaction buffer, dNTP mix, and QuickSolution reagent. PCR cycling parameters were as follows: initial denaturing at 95°C for 5 min, followed by 18 cycles of 20 s denaturing (95°C), 10 s annealing (60°C) and 6 min elongation (68°C). A final elongation step lasted for 10 min (68°C). The G406A mutation was verified by Sanger sequencing.

The sequences of primer pair used for SDM is:

Forward 5′-CAATGTGAGATCTGTGCATTTACTTGTCGACAAAAGG-3′
Reverse 5′-CCTTTTGTCGACAAGTAAATGCACAGATCTCACATTG-3′

Western Blot Analysis

Sample preparation of whole cell lysates, SDS-PAGE, membrane transfer and blotting were performed according to standard protocols. Briefly, cells were lysed in Cell Lysis Buffer (Cell Signaling Technologies, #9803) supplemented with anti-protease and anti-phosphatase cocktails (Thermo Fisher, #78442). Protein concentration was determined using BCA Protein Assay Kit (Pierce, #23225). 5~15 μg of protein was resolved by SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were blocked and incubated overnight at 4°C with gentle agitation with primary antibodies. Then primary antibodies were conjugated to secondary HRP-conjugated antibodies (Bio-Rad #170-6515, Invitrogen #62-6520) and the signal was detected using ECL Kit (Sigma, GERPN2232) and acquired on Chemidoc MP System (Bio-Rad). Antibodies to IKZF1 (#14859), IKZF3 (#15103), IL-10 (#12163), Erk (#4695), phospho-Erk (#4370), c-Jun (#9165), and GAPDH (#5174) were purchased from Cell Signaling Technologies. Antibodies to ZFP91 (A303-245A) and CK2β (A301-984A) were purchased from Bethyl

Laboratories. Antibody to CRBN (NBP1-91810) was purchased from Novus Biologicals. Antibody to phospho-Ser249 (pS249) c-Jun (PA5-64506) was purchased from Thermo Fisher. Antibody to RAB28 (PA5-48813) was purchased from Life Technologies. Antibody to α-Tubulin (T9026) was purchased from Sigma. Antibodies were used at 1:1000 dilution, or at 1:3000 dilution (for GAPDH), or 1:5000 dilution (for α-Tubulin).

Quantitative PCR

Total RNA was extracted from cells using Qiagen RNAeasy kit (Stock# 74134) per manufacture's instruction. 1µg of RNA was transcribed into cDNA using the iScript cDNA Synthesis Kit (Bio-Rad, #1708891). Quantitative real-time PCR was performed on a CFX96 Real-Time system (Bio-Rad) using iTaq Universal SYBR Green Supermix (Bio-Rad, #1725121). Target gene expression was normalized to the mean Ct values of the housekeeping gene *RPS18*. The sequences of primer pairs used for quantitative real-time PCR are listed in supplemental Table 7.

Luciferase Reporter Assay

293T cells with wild-type *ZFP91* or *ZFP91* knockout were grown in a 6-well plate and co-transfected with 2 µg of firefly luciferase reporter (pGL4.23) and 0.2 µg of pRL-TK (Promega). Fourteen hours after transfection, media containing transfection mixture were removed and fresh media with or without 1 µM pomalidomide were added to cells. Forty-eight hours after the last transfection, growth media were removed. Cells were washed once with 1× PBS and lysis with Passive Lysis Buffer for 15 mins at room temperature. Cell lysate were harvested and subjected to measurement of firefly luciferase and *Renilla* luciferase activity using Dual-Luciferase Reporter Assay Kit (Promega, E1910).

Fluorescence In Situ Hybridization

Fluorescent in situ hybridization (FISH) experiments were performed by CytoGenomics Core Laboratory at Brigham and Women's Hospital (HMS, USA). Briefly, a direct preparation (DP) was made for FISH by treating cells with hypotonic (0.075 M) potassium chloride at 37°C for 20 min and then fixed in 3:1 methanol:acetic acid. DPs were stored at -20°C until use for FISH. A commercial two-color FISH probe set was purchased from Empire Genomics: CEP6 (CHR06-10-GR) probe at 6p11.1-q11.1 and served as an internal chromosome 6 copy number probe, and *CSNK2B* (CSNK2B-20-OR) mapped to 6p21.33 chromosome region and served as the amplicon copy number probe. Each probe was diluted in hybridization buffer according to manufacturer's instructions. The probe set was then hybridized to SU-DHL-1 parental, SU-DHL-1 lenalidomide-regrown cells, as well as normal T lymphocytes as a control. After hybridization overnight at 37°C, slides were washed for 2 min at 72°C in 0.4X SSC/0.3% NP-40, 2 min at room temperature in 2X SSC/0.1% NP-40, air-dried, and mounted in antifade solution with DAPI counterstain. Hybridized slides were examined on an Olympus BX-51 microscope equipped with appropriate filters. One hundred nuclei were scored for each sample except for the SUDHL1 parental cells for which 500 nuclei were scored. Images were captured with CytoVysion (Leica) imaging software.

Copy Number and Survival Analysis

Using previously published genomic copy number data, we extracted the copy number status of 6p21.33 from 73 PTCL cases with corresponding clinical outcome data. The 73 cases represented molecularly classified cases of AITL (n=23), PTCL-NOS (n=39), and PTCL with T follicular helper phenotype (n=11). The molecular and immunohistochemical classifications of these cases was described previously.[2,3] The copy number status was determined as previously described using Nexus Copy Number (BioDiscovery). The 5-year overall survival was estimated using the Kaplan-Meier method, and the differences were assessed using the Log-Rank test. Statistical analyses were performed in GraphPad Prism. Differences among groups were considered significant at p-values below 0.05.

Sample Preparation TMT LC-MS3 Mass Spectrometry and Analysis

Cells were treated with DMSO (biological triplicate) or IMiD drugs at indicated dose and time and cells were harvested by centrifugation. Cell lysis and Tandem Mass Tagged (TMT) tryptic peptides were prepared for LC-MS analysis following procedures published.[4]

Data were collected using an Orbitrap Fusion Lumos mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) coupled with a Proxeon EASY-nLC 1200 LC pump (Thermo Fisher Scientific). Peptides were separated on a 100 µm inner diameter microcapillary column packed with ~50 cm of Accucore C18 resin (2.6 mM, 100 Å, Thermo Fisher Scientific). Peptides were separated using a 190 min gradient of 6 - 27% acetonitrile in 1.0% formic acid with a flow rate of 350 nL/min.

Each analysis used a MS3-based TMT method as described previously (McAlister et al., 2014). The data were acquired using a mass range of m/z 340 – 1350, resolution 120,000, AGC target 5×10^5, maximum injection time 100 ms, dynamic exclusion of 120 s for the peptide measurements in the Orbitrap. Data dependent MS2 spectra were acquired in the ion trap with a normalized collision energy (NCE) set at 35%, AGC target set to 1.8×10^4 and a maximum injection time of 120 ms. MS3 scans were acquired in the Orbitrap with HCD collision energy set to 55%, AGC target set to 2×10^5, maximum injection time of 150 ms, resolution at 50,000 and with a maximum synchronous precursor selection (SPS) precursor set to 10.

Proteome Discoverer 2.2 (Thermo Fisher Scientific) was used for .RAW file processing and controlling peptide and protein level false discovery rates, assembling proteins from peptides, and protein quantification from peptides. MS/MS spectra were searched against a Uniprot human database (September 2016) with both the forward and reverse sequences as well as known contaminants such as human keratins. Database search criteria were as follows: tryptic with two missed cleavages, a precursor mass tolerance of 20 ppm, fragment ion mass tolerance of 0.6 Da, static alkylation of cysteine (57.02146 Da), static TMT labeling of lysine residues and N-termini of peptides (229.16293 Da), and variable oxidation of methionine (15.99491 Da).

RNA Sequencing and Analysis

3'DGE-seq was performed on SU-DHL-1 parental (n=3) and lenalidomide-regrown cells (n=3) treated with DMSO or lenalidomide for 18 hours. Cells were washed in cold PBS and total RNA was extracted using Qiagen RNAeasy kit (#74134). Total RNA was submitted to MIT BioMicroCenter (Cambridge, MA) for cDNA libraries construction and sequencing.

mRNA-seq was performed on dox-induced *ZFP91* knockout cells (including both SU-DHL-1 parental and lenalidomide-regrown cells) and on *CSNK2B* overexpression cells (including both SU-DHL-1 and KI-JK cells). Total RNA was extracted using Qiagen RNAeasy kit and submitted to Novogene (Sacramento, CA) for cDNA libraries construction and sequencing. Sequencing libraries were generated using NEBNext Ultra RNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer's recommendations and index codes were added to attribute sequences to each sample. RNA was sequenced using the Illumina NovaSeq 6000 platform.

For 3'DGE-seq, FastQC (http://www.bioinformatics.babraham.ac.uk/) was used to perform a basic quality control on the resulting reads. Reads were aligned to the human genome hg38 with BOWTIE (v2.2.3). Differential expression between experimental criteria was determined using raw counts and normalization procedures within the DESeq2 package in R based on a negative binomial distribution. Prior to testing rows that had no counts or only a single count across all samples were removed. Unsupervised clustering was performed using the Euclidean distance with complete linkage method in the R package pheatmap. PCA analysis was performed on the top 1000 most variable genes as determined using the rlog Transformation 'regularized-log' (rlog) transformation function within the DESeq2 package to stabilize the variance. All heatmaps used the rlog transformed count data. Sequencing depth correction is performed automatically for both variance stabilizing transformation (VST) and rlog transformations. The false discovery rate (FDR)

by Benjamini and Hochberg method was used to adjust for multiple comparisons. The ordered lists determined by the differential expression test statistics obtained from DESeq2 were then used in Gene Set Enrichment Analysis (GSEA, Broad Institute) and the FDR q-values.

For RNA-seq, raw reads were cleaned up by removing adaptor contamination, uncertain nucleotides (N > 10%), and low-quality nucleotides (Base Quality less than 5). Clean RNA-seq reads were then aligned to the human reference genome hg38 using STAR (v2.4.0.1). FPKM (Fragments Per Kilobase of transcript sequence per Millions base pairs mapped reads) value was used to measure gene expression. Differentially expressed genes were assessed using DESeq2 package. ClusterProfiler software was used for enrichment analysis, including GO Enrichment, KEGG, Reactome Enrichment and DisGeNET database Enrichment.

Differentially expressed genes between SU-DHL-1 parental (n=3) and lenalidomide regrown (n=3) cells, and between lenalidomide treatment (n=3) and DMSO control (n=3) were determined by 3'DGE-seq at thresholds of fold change (FC) > 1.5 or FC < 0.67 and p value < 0.05. Differentially expressed genes between ZFP91 knockout (KO) (n=3) and ZFP91 wild type (WT) control (n=3) in both SU-DHL-1 parental and lenalidomide regrown cells were measured by RNA-seq at thresholds of fold change (FC) > 1.5 or FC < 0.67 and adjusted p value < 0.05.

Precision Run-On Sequencing and Analysis

SUDHL1 parental (n=3) or SUDHL1 lenalidomide-regrown (n=3) cells were cultured in RPMI 1640, 10% fetal bovine serum, and 1% penicillin/streptomycin. After harvest, cells were kept cold (on ice or at 4°C) unless otherwise specified. Cells were washed once in ice-cold 1x phosphate buffered saline and resuspended in Wash Buffer (10mM Tris-HCl pH 8, 10mM KCl, 250mM Sucrose, 5mM $MgCl_2$, 1mM EDTA, 0.5mM DTT, 10% Glycerol) at a density of 5×10^6 cells/mL. Cell suspensions were strained through 35 µm nylon mesh (Falcon #352235) to ensure single-cell suspension and 9 volumes of Permeabilization Buffer (10mM Tris-HCl pH 8, 10 mM KCl, 250 mM Sucrose, 5 mM $MgCl_2$, 1 mM EDTA, 0.5 mM DTT, 10% Glycerol, 0.05% Igepal CA-630) was gently added. The cells were incubated 3 min, pelleted and washed once with in 1mL of Freezing Buffer (50mM Tris-HCl pH 8.3, 40% Glycerol, 5 mM $MgCl_2$. 0.5 mM DTT, 2 µL/mL RNase Inhibitor (SUPERaseIN, Ambion)). Permeabilized cells were resuspended in Freezing Buffer at a density of 1×10^6 cells/100µL, flash frozen in liquid nitrogen, and stored at -80°C.

Aliquots of frozen (-80°C) permeabilized cells were thawed on ice and pipetted gently to fully resuspend. Aliquots were removed and permeabilized cells were counted using a Luna II, Logos Biosystems instrument. For each sample, 1 million permeabilized cells were used for nuclear run-on, with 50,000 permeabilized *Drosophila* S2 cells added to each sample for normalization. Nuclear run-on assays and library preparation were performed essentially as described in Reimer et al[5] with modifications noted: 2X nuclear run-on buffer consisted of (10 mM Tris (pH 8), 10 mM MgCl2, 1 mM DTT, 300mM KCl, 40uM/ea biotin-11-NTPs (Perkin Elmer), 0.8U/uL SuperaseIN (Thermo), 1% sarkosyl). Run-on reactions were performed at 37°C. Adenylated 3' adapter was prepared using the 5' DNA adenylation kit (NEB) and ligated using T4 RNA ligase 2, truncated KQ (NEB, per manufacturer's instructions with 15% PEG-8000 final) and incubated at 16°C overnight. 180uL of betaine blocking buffer (1.42g of betaine brought to 10mL with binding buffer supplemented to 0.6 uM blocking oligo (TCCGACGATCCCACGTTCCCGTGG/3InvdT/)) was mixed with ligations and incubated 5 min at 65°C and 2 min on ice prior to addition of streptavidin beads. After T4 polynucleotide kinase (NEB) treatment, beads were washed once each with high salt, low salt, and blocking oligo wash (0.25X T4 RNA ligase buffer (NEB), 0.3uM blocking oligo) solutions and resuspended in 5' adapter mix (10 pmol 5' adapter, 30 pmol blocking oligo, water). 5' adapter ligation was per Reimer but with 15% PEG-8000 final. Eluted cDNA was amplified 5-cycles (NEBNext Ultra II Q5 master mix (NEB) with Illumina TruSeq PCR primers RP-1 and RPI-X) following the manufacturer's suggested cycling protocol for library construction. A portion of

preCR was serially diluted and for test amplification to determine optimal amplification of final libraries. Pooled libraries were sequenced using the Illumina NovaSeq platform.

All custom scripts described herein are available on the AdelmanLab Github (https://github.com/AdelmanLab). Using a custom script (trim_and_filter_PE.pl), FASTQ read pairs were trimmed to 41bp per mate, and read pairs with a minimum average base quality score of 20 retained. Read pairs were further trimmed using cutadapt 1.14 to remove adapter sequences and low-quality 3' bases (--match-read-wildcards -m 20 -q 10). R1 reads, corresponding to RNA 3' ends, were then aligned to the spiked in Drosophila genome index (dm3) using Bowtie 1.2.2 (-v 2 -p 6 --best --un), with those reads not mapping to the spike genome serving as input to the primary genome alignment step (using Bowtie 1.2.2 options -v 2 --best). Reads mapping to the hg38 reference genome were then sorted, via samtools 1.3.1 (-n), and subsequently converted to bedGraph format using a custom script (bowtie2stdBedGraph.pl). Because R1 in PRO-seq reveals the position of the RNA 3' end, the "+" and "-" strands were swapped to generate bedGraphs representing 3' end position at single nucleotide resolution.

Statistics, including raw read counts, mappable read counts to the spike in and reference genomes, and Spearman's correlation coefficient between promoter reads in replicate samples are below. For promoter reads, annotated transcription start sites were obtained from human (GRCh38.99) GTF from Ensembl. After removing transcripts with (immunoglobulin, Mt_tRNA, Mt_rRNA) biotypes, PRO-seq signal in each sample was calculated in the window from the annotated TSS to +150 nt downstream, using a custom script, make_heatmap. Given good agreement between replicates and similar return of spike-in reads, bedGraphs were merged and depth-normalized within conditions, to generate bigWig files binned at 10bp.

Chromatin Immunoprecipitation Sequencing and Analysis

Chromatin immunoprecipitation (ChIP) was performed on SU-DHL-1 parental, SU-DHL-1 lenalidomide-regrown, SU-DHL-1 with JUN-KO, Karpas 299, SMZ-1, and SUP-M2 cells (20×10^6). The ChIP assay was performed with EZ-ChIP kit (Cell Signaling Technology, #9003) following the manufacturer's instruction. Briefly, cells were fixed in 1% formaldehyde at room temperature for 10 min, quenched with 0.125 M glycine for 5 min at room temperature, washed with ice-cold PBS. The cells were lysed in ice-cold 1 x Buffer A + DTT + Protease Inhibitor Cocktail (PIC) for 10 min and resuspend in 2 ml 1 x Buffer B + DTT. The chromatin was digested by 2 µl of Micrococcal Nuclease for 20 min at 37°C with frequent mixing. The digestion was stopped by adding 20 µl 0.5M EDTA. Nuclei were lysed by sonication (Qsonica) in 1 x ChIP Buffer + PIC with 3 sets of 20% power of pulses for 15 sec and pause for 15 sec in between. The lysates were then centrifuged for 10 min at 4 °C, supernatants collected and diluted with 4 volume of 1 x ChIP Buffer + PIC. A small portion (2%) of the digested chromatin was set aside as input sample and stored at -20°C. For immunoprecipitation (IP) reaction, 10 µg of ChIP-Grade antibody was added to IP samples and incubate end over end overnight at 4°C. The following day, ChIP-Grade Protein G Magnetic Beads were added to IP reaction and incubated end over end at 4°C for 2 h. Beads were washed with low-salt buffer, high-salt buffer, and LiCl buffer. DNA was then eluted in elution buffer. The crosslinking was reversed overnight at 65 °C. Input and ChIPed DNA was cleaned up and submitted to Novogene (Sacramento, CA) for libraries construction and sequencing.

Immunoprecipitated DNA sample quality was assessed by DNA Tapestation (Agilent Technologies Inc., California, USA) and quantified by Qubit 2.0 DNA HS assay (ThermoFisher, Massachusetts, USA). The KAPA HyperPrep kit (Roche, Indianapolis, USA) was used for library preparation and indexing was performed using Illumina 8-nt dual-indices. Final libraries were quantified by Qubit 2.0 (ThermoFisher, Massachusetts, USA) and run on TapeStation D1000 ScreenTape (Agilent Technologies Inc., California, USA) to evaluate the quality of the purified libraries. Final library size was approximately 300 bp. Equimolar pooling of libraries was performed based on QC values and sequenced on an Illumina NovaSeq S4 (Illumina, California,

USA) with a read length configuration of 150 PE for 80M PE reads per sample (40M in each direction). Antibodies used for ChIP-seq were as follows: anti-ZFP91 (Bethyl Laboratories, A303-245A), anti-IKZF1 (Abcam, Ab229275), anti-H3K4me3 (Cell Signaling Technology, #9751), anti-H3K27ac (Cell Signaling Technology, #8173), and control IgG (Cell Signaling Technology, #2729).

For all ChIP-seq data sets, paired-end reads were mapped to the hg38 reference genome using Bowtie v1.2.2 (-v 2 --maxins 1000 --allow-contain --best). For ZFP91 ChIP-seq data, HOMER v4.10.3 was used to generate tag directories via makeTagDirectory and call peaks via findPeaks (-style factor). Common and unique peaks across conditions were identified by way of the intersect sub-command of bedtools v2.27.1 (common: -wo, unique: -v). Common peaks that did not share the exact same midpoint were deduplicated by retaining the one with the highest HOMER findPeaks Score for downstream analysis. Using ChIP-seq from both parental and lenalidomide regrown cells, 1419 ZFP91 peaks were defined.

The distance from each peak center to the nearest active gene TSS was determined and peaks were considered proximal if they were within 1kb of an active TSS (N=494) or distal if the peak was >1kb of an active TSS (N=925). For analysis of gene activity at genes nearest ZFP91 peaks, all peaks within 50kb of a gene TSS were included. If two ZFP91 peaks were nearest the same gene TSS, the peak that was closest to the gene promoter was retained (this removed 237 ZFP91 peaks). For each of the remaining ZFP91 peak-promoter pairs (N=757), we investigated the PRO-seq and RNA-seq signal in parental and lenalidomide regrown cells, using windows from the TSS to transcription termination site for PRO-seq, and all transcript models consistent with these windows for RNA-seq. Motif analysis was performed using MEME-ChIP . IGV was used to display the genomic tracks.

ChIP-seq and PRO-seq Data Combined Analysis

ChIP-seq data were depth normalized for comparison between conditions. Using the normalized ZFP91 ChIP-seq datasets (N=3 per condition) the read counts were summed in the region +/-150 bp around each peak center and compared between parental and resistant cells. Peaks with higher read counts in Parental (>20 read difference) were considered Enriched in Parental whereas peaks with higher read counts in lenalidomide regrown cells (>20 reads) were considered Enriched in Lenalidomide Regrown cells. Matrices were made of normalized ChIP-seq data for ZFP91, H3K27ac and H3K4me3, aligned around ZFP91 peak centers or active TSSs, using make_heatmap and 50 bp bins. These matrices were used to generate heatmaps using Partek Genomics Suite. For metagene analyses, average read counts per bin were graphed with each read being counted once and placed at the read center.

Normalized PRO-seq data are shown as the single nucleotide position of the RNA 3' end. Matrices for heatmaps and metagene plots were made for PRO-seq data as described for ChIP-seq but used reads only from the sense strand.

RNA-seq and ChIP-seq Data Combined Analysis

Paired-end reads from RNA-seq were mapped to the hg38 reference genome via HISAT2 v2.2.1 (--known-splicesite-infile). To select gene-level features for differential expression analysis, as well as for pairing with our ChIP- and PRO-seq data, we assigned a single, dominant TSS to each active gene. This was accomplished using a custom script, get_gene_annotations.sh (available at https://github.com/AdelmanLab/GeneAnnotationScripts), which uses RNA-seq abundances and PRO-seq R2 reads (RNA 5' ends) to identify dominant TSSs. Exon- and transcript-level features for these 17,792 dominant TSSs were selected from an hg38 reference GTF and RNA-seq reads were quantified across these features using featureCounts (minMQS=10, countChimericFragments=FALSE, strandSpecific=0) within the Rsubread v2.0.1 package.

Whole Exome Sequencing and Analysis

Whole exome sequencing was performed on SU-DHL-1 parental and three lenalidomide-regrown lines. Genomic DNA was extracted using DNeasy Blood & Tissue Kit (QIAGEN, #69506) according to the manufacturer's protocol. DNA samples were submitted to Novogene (Sacramento, CA) for libraries construction and sequencing. Briefly, genomic DNA was fragmented (Covaris sonication) to size of 180-280bp fragments and further purified using Agentcourt AMPure XP beads. Size-selected DNA was then ligated to specific adapters during library preparation. Each library was made with sample-specific barcodes. Libraries were pooled and sequenced over Illumina Novaseq 6000.

All the raw sequencing data was processed using CCLE variant calling pipeline. Mutation analysis for single nucleotide variants (SNVs) was performed using MuTect (v1.1.6) in single sample mode with default parameters. Short indels were detected using Strelka (v2.9.4) in single sample mode with default parameters. To ensure high quality variant calls, we required minimum coverage of 4 reads with minimum two reads supporting the alternate allele. Moreover, variants with low allelic fraction (AF < 0.1) and variants outside protein coding region were excluded. To remove germline-like variants, any variant with allelic frequency greater than 1E-5 in Exome Aggregation Consortium (ExAC) project were excluded with the exception of any cancer recurrent variant defined by minimum TCGA frequency of 3 or COSMIC frequency of 10. Somatic copy number variation (CNV) was detected using Control-FREEC (v11.4).

In vivo experiments

All in vivo experiments were conducted under Dana-Farber Cancer Institute Animal Care and Use Committee protocol #13-034. A full description of each PDX model is available online at the Public Repository of Xenografts (www.PRoXe.org), including clinical history and genomic data.

For PDX models, viably frozen PDX cells were thawed and washed in 1 × PBS before tail-vein injection at 1×10^6 cells per mouse. Tumor burden was monitored periodically by flow cytometry of peripheral blood. Blood was processed with Red Blood Cell Lysis Buffer (Qiagen) before staining with antibodies against human CD45 (BD Biosciences #566026) and human CD2 (Invitrogen #17-9459) in 1 × PBS with 1 mM EDTA plus 1% fetal bovine serum. Data were acquired on a BD LSRFortessa flow cytometer and analyzed with FlowJo Software. For SUPM2 model, cells were washed in 1 × PBS before subcutaneous injection at 5×10^6 cells per mouse. Tumor size was measured in 2 perpendicular dimensions, and volume was calculated as [(longest dimension × perpendicular dimension^2)/2].

Upon engraftment, mice were randomized to vehicle or treatment arms. Pomalidomide (3 mg/kg) and CC-92480 (1 mg/kg) were dissolved in 2% DMSO + 30% PEG300 + 2% Tween 80 + ddH$_2$O and given orally by gavage once per day. The vehicle was given orally by gavage on the same schedule as the drugs. Both drugs and vehicle were dosed in 10 mL/kg volume based on body weight. Mice were monitored daily for clinical signs of disease and humanely euthanized when they reached a clinical end point or a subcutaneous tumor reached 2 cm in the longest dimension. Cells from spleen and bone marrow were collected to determine cell apoptosis of tumor cells using Annexin V Apoptosis Detection Kit. Mouse cells were then depleted by using EasySep Mouse/Human Chimera Isolation Kit (Stem Cell Technologies, #19849). Human tumor cell numbers and viability were assessed with Trypan blue staining. Tumor burden is reported as the number of viable tumor cells.

See supplemental Methods for a description of CRISPR knockdown, overexpression, site-directed mutagenesis, western blot, luciferase reporter assay, fluorescence in situ hybridization, RNA-seq, PRO-seq, ChIP-seq, and whole exome sequencing data generation and analysis, and In vivo experiments.

Supplemental Tables

Supplemental Table 1. STR profiling and WES in len-regrown TCLs

Supplemental Table 2. DEG between *ZFP91* knockout and WT cells

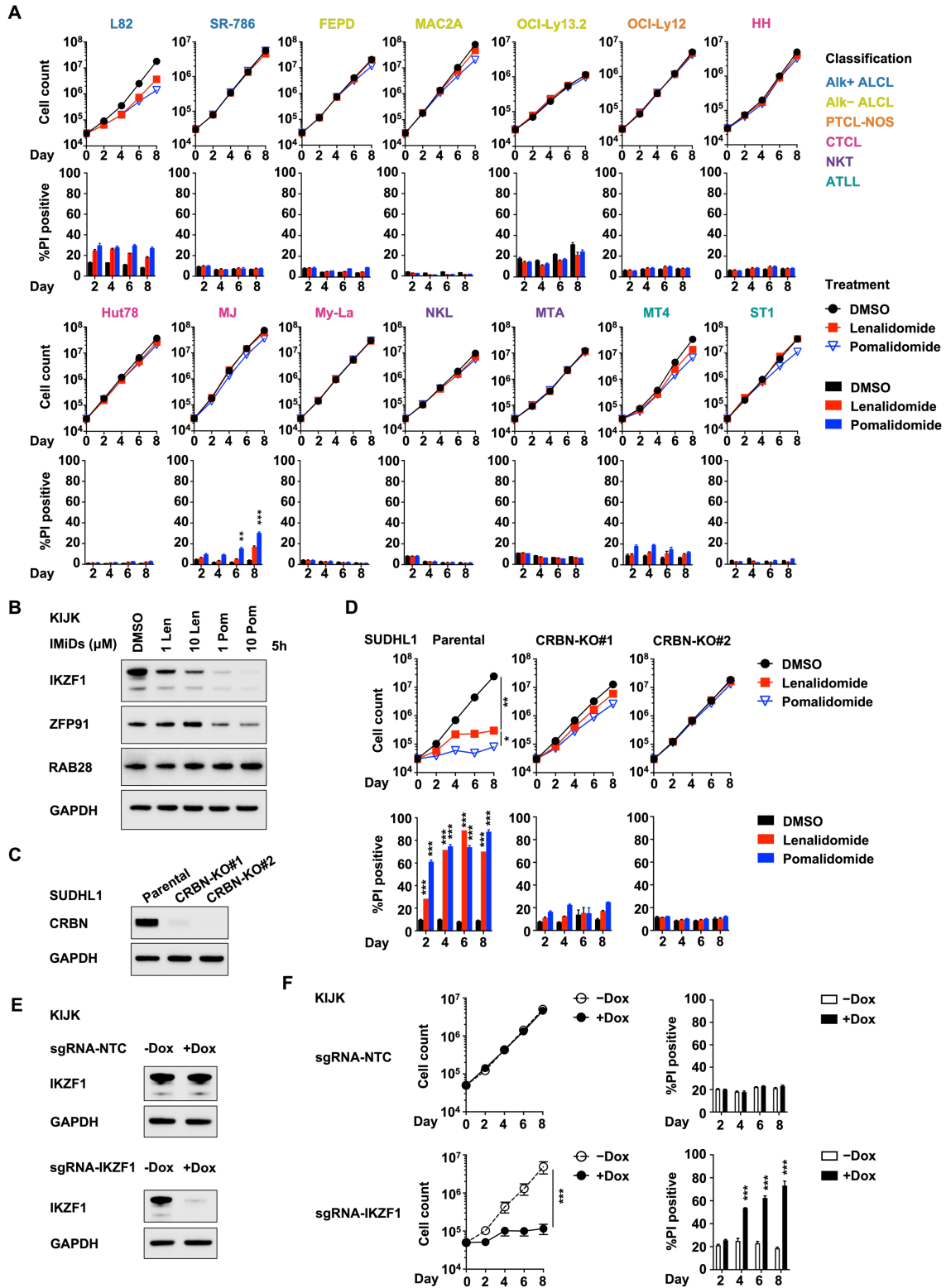**Supplemental Table 3.** ZFP91 ChIP-seq peaks

Supplemental Table 4. Transcriptional levels of AP-1 TFs

Supplemental Table 5. DEG between len-regrown and parental cells

**Supplemental Table 6.** Summary of FISH signal patterns

Supplemental Table 7. Sequence information of sgRNAs and primers

Supplemental Figure 1. *IKZF1* **is a vulnerability and key drug target in lenalidomide-sensitive TCL cells.** (A) Cell count and percent propidium iodide (PI) positive in T-cell lymphoma (TCL) lines treated with
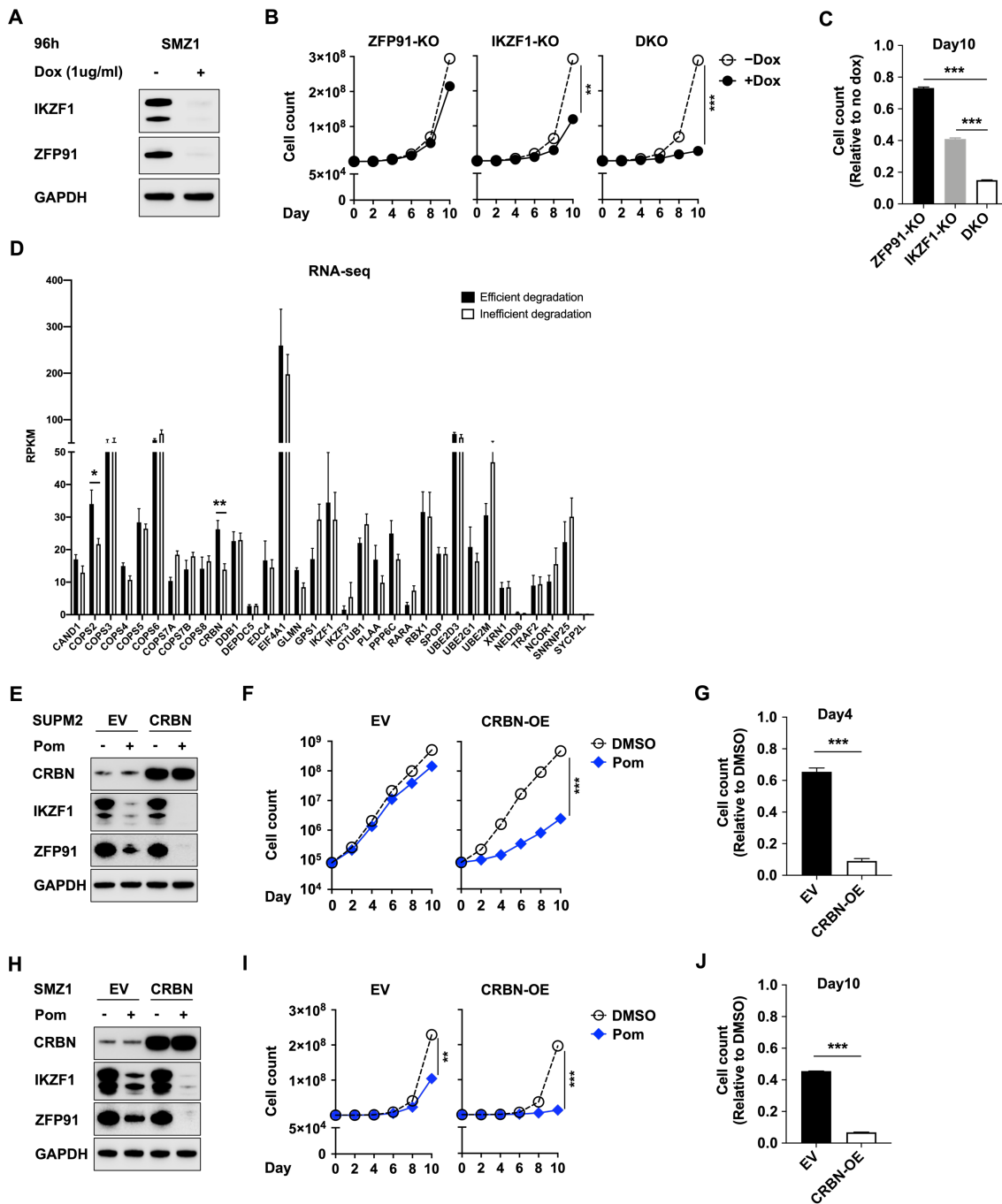
IMiDs compared to DMSO. The experiment was performed in triplicate and replicated twice. Data are presented as mean ± SD. Comparisons are by 2-way ANOVA with Bonferroni correction for multiple comparisons. (B) Western blot analysis of IKZF1, ZFP91, and RAB28 expression in KI-JK cells upon exposure to lenalidomide (Len) or pomalidomide (Pom) at indicated doses for 5 hours. (C and D) Western blot analysis (C), cell count and percent PI positive (D) in SU-DHL-1 cells with *CRBN* knockout (KO) using two independent sgRNAs. The experiment was performed in triplicate and replicated twice. (E and F) Western blot analysis (E), cell count and percent PI positive (F) in KI-JK cells with doxycycline (dox)-induced expression of sgRNA targeting *IKZF1* or nontargeting control (NTC). The experiment was performed in triplicate and replicated twice. Data are presented as mean ± SD. Comparisons are by 2-way ANOVA with Bonferroni correction for multiple comparisons. *p<0.05; **p<0.01; ***p<0.001.
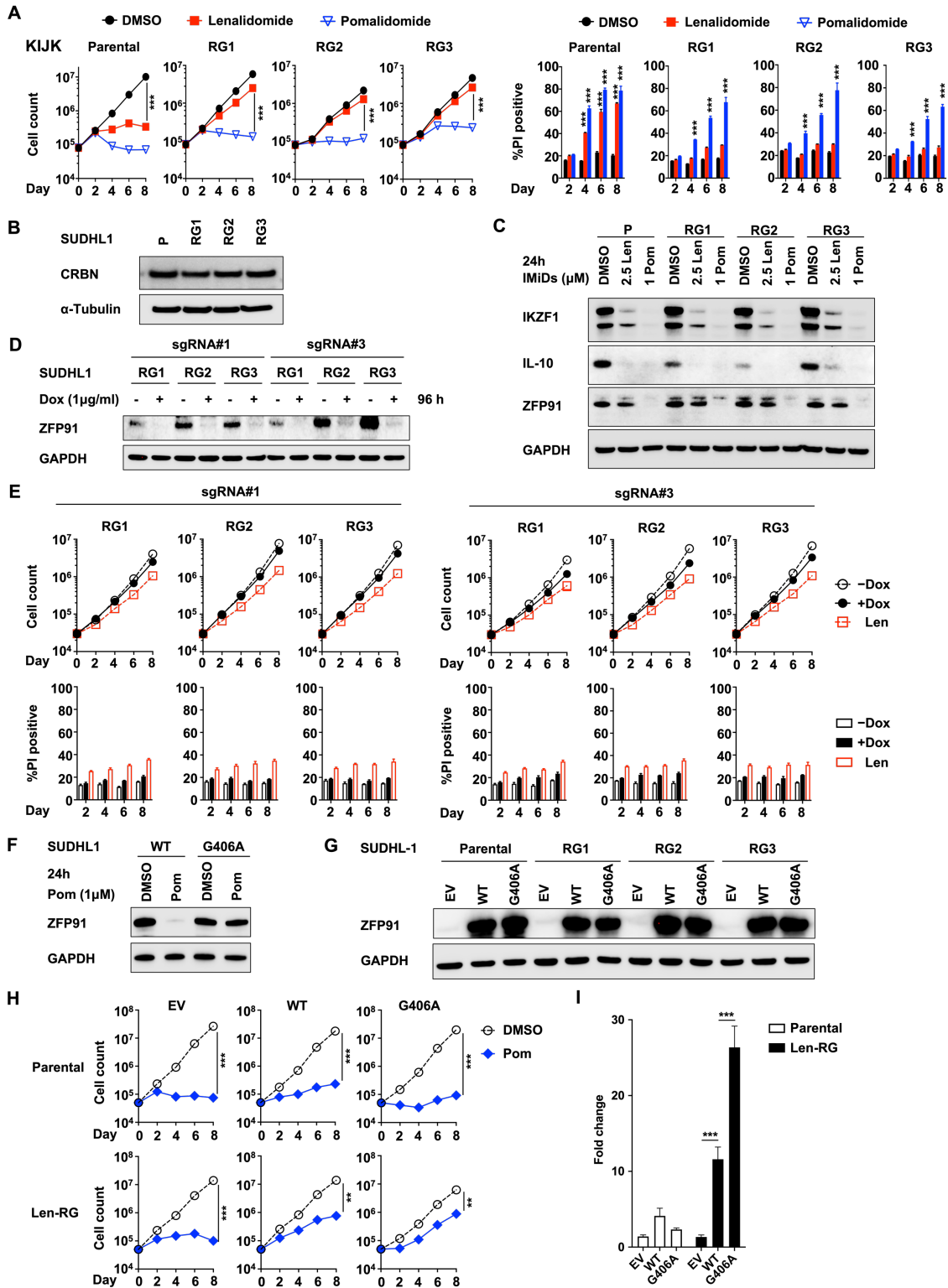
**Supplemental Figure 2. ZFP91 contributes to the sensitivity of len-regrown cells to pomalidomide.**
(A) Cell count and percent PI positive in KI-JK parental and len-regrown (RG) cells treated with 1μM lenalidomide, pomalidomide or DMSO. The experiment was performed in triplicate and replicated twice.

Data are presented as mean ± SD. Comparisons are by 2-way ANOVA and Bonferroni correction for multiple comparisons. (B) Western blot analysis for CRBN protein in SU-DHL-1 parental and len-regrown cells. (C) Western blot analysis for IKZF1, IL-10, and ZFP91 proteins in SU-DHL-1 parental and len-regrown cells treated with lenalidomide (len), pomalidomide (pom) or DMSO for 24 h. (D and E) Western blot analysis (D), cell count and percent PI positive (E) for SU-DHL-1 len-regrown cells with doxycycline (dox)-induced expression of two independent sgRNAs targeting *ZFP91*. The experiment was performed in triplicate and replicated twice. Data are presented as mean ± SD. Comparisons are by 2-way ANOVA with Bonferroni correction for multiple comparisons. (F) Western blot analysis for ZFP91 protein in SU-DHL-1 cells with overexpression of either wild-type (WT) ZFP91 or ZFP91 G406A and treated with 1μM pomalidomide or DMSO control. (G) Western blot analysis for ZFP91 protein in SU-DHL-1 parental and len-regrown cells with overexpression of either WT ZFP91 or ZFP91 G406A. (H and I) Cell count of SU-DHL-1 parental and len-regrown cells with overexpression of either WT ZFP91 or ZFP91 G406A and treated with 1μM pomalidomide or DMSO. Shown in (I), comparison of the fold change of cell number relative to empty vector (EV) control at day 8. The experiment was performed in triplicate and repeated twice. Data are presented as mean ± SEM. **p<0.01; ***p<0.001.

Supplemental Figure 3. Overexpression of *CRBN* increases target protein degradation and results in greater sensitivity to pomalidomide in IMiD-resistant TCL cells. (A) Western blot analysis for IKZF1 and ZFP91 proteins in SMZ1 cells with doxycycline (dox)-induced double knockout (DKO) of *IKZF1* and *ZFP91*. (B and C) Cell count in SMZ1 cells with dox-induced *IKZF1* and *ZFP91* single or double-knockout. Shown in (C), comparison of cell number decreases relative to no-dox control at day 10. The experiment was performed in triplicate and replicated twice. Data are presented as mean ± SD. Comparisons are by
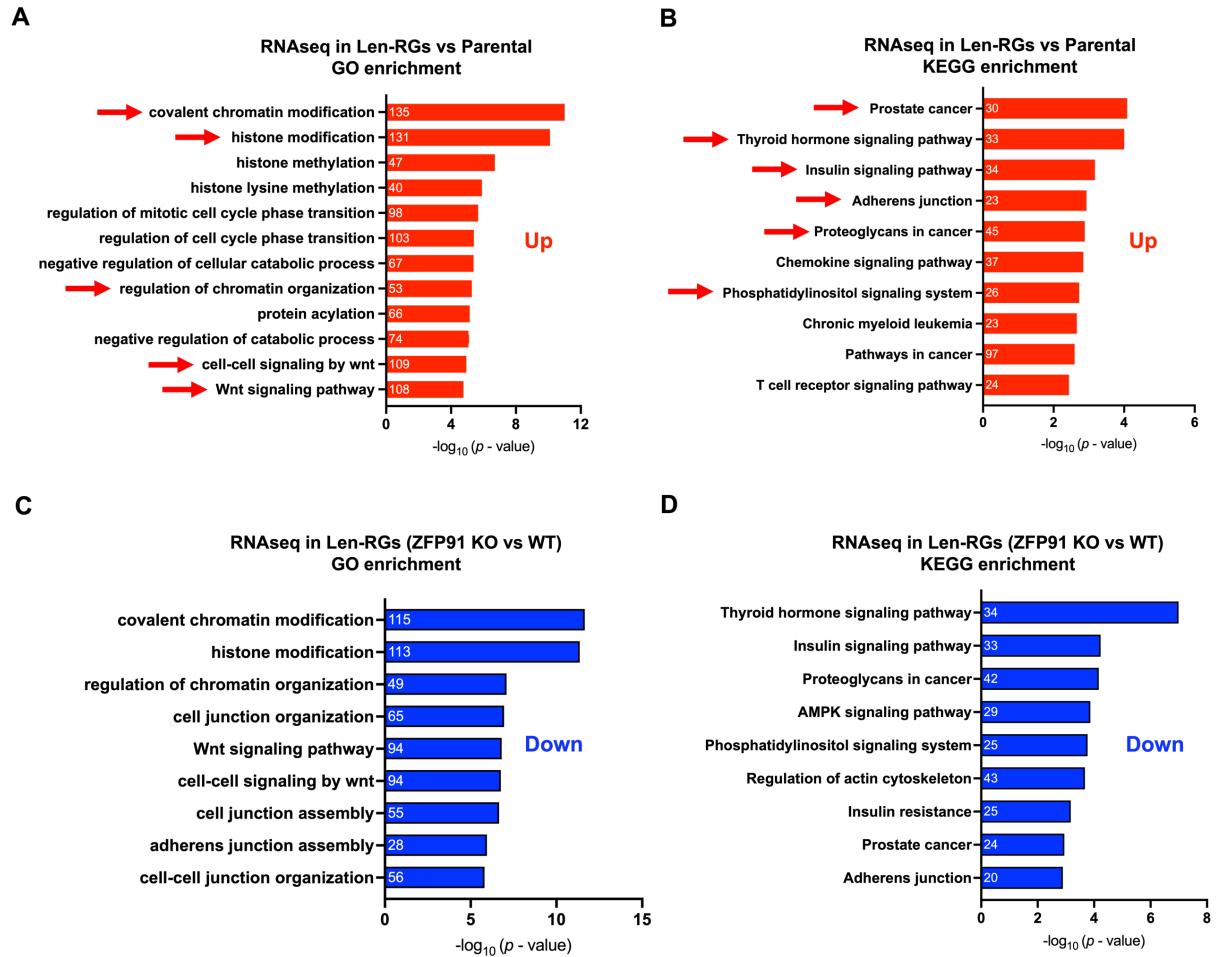
two-tailed Student's t test. (D) Gene expression of 33 known regulators of IMiD activity in two groups of TCL lines divided based on IKZF1 degradation activity. (E) Western blot analysis for CRBN, IKZF1 and ZFP91 proteins in SUP-M2 cells with CRBN overexpression compared to empty vector control upon exposure to 1µM pomalidomide (Pom) or DMSO for 24 hours. (F and G) Cell count of SUP-M2 cells with *CRBN* overexpression compared to empty vector control in the setting of 1µM pomalidomide or DMSO. Shown in (G), comparison of cell number decreases relative to DMSO control at day 4. (H) Western blot analysis for CRBN, IKZF1 and ZFP91 proteins in SMZ1 cells with CRBN overexpression compared to empty vector control upon exposure to 1µM pomalidomide or DMSO for 24 hours. (I and J) Cell count of SMZ1 cells with CRBN overexpression compared to empty vector control in the setting of 1µM pomalidomide or DMSO. Shown in (J), comparison of cell number decreases relative to DMSO control at day 10. The experiment was performed in triplicate and replicated twice. Data are presented as mean ± SD. Comparisons are by two-tailed Student's t test. *p<0.05; **p<0.01; ***p<0.001.
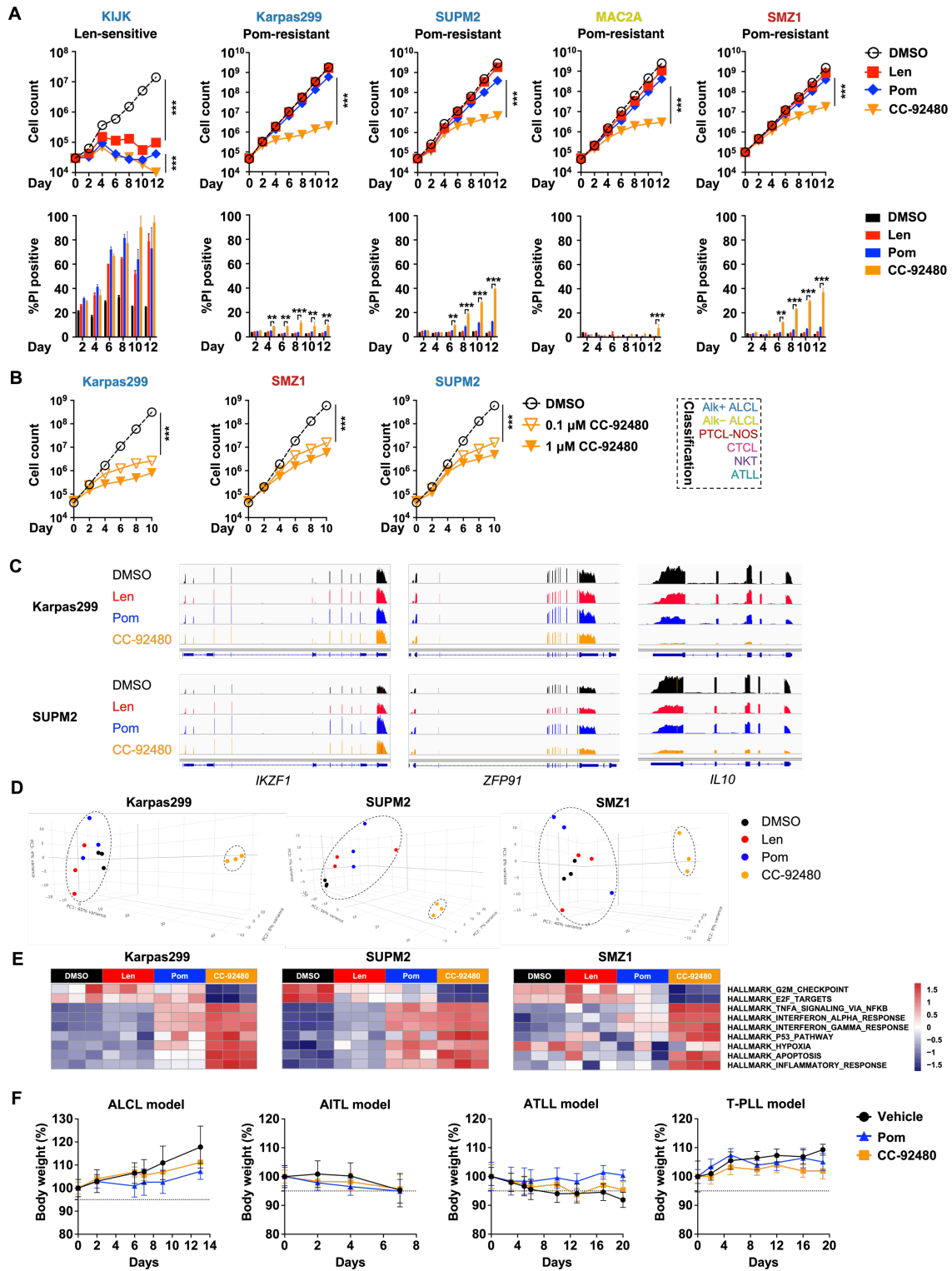
Supplemental Figure 4. CC-92480 is highly active in TCL models and induces transcriptional signatures associated with apoptosis and cell cycle arrest. (A) Cell count and percent PI positive of TCL cell lines treated with 1 µM IMiDs or CC-92480 compared to DMSO. The experiment was performed

in triplicate and replicated twice. Data are presented as mean ± SD. Comparisons are by 2-way ANOVA with Bonferroni correction for multiple comparisons. (B) Cell count of three IMiD-resistant TCL lines treated with CC-92480 compared to DMSO. (C) IGV tracks show *IKZF1*, *ZFP91*, and *IL10* transcriptional signals in Karpas-299 and SUP-M2 cells treated with 1 µM lenalidomide, pomalidomide or CC-92480 for 24 hours compared to DMSO. Note that tracks are unchanged by drug treatment at *IKZF1* and *ZFP91* but decrease at *IL10*. (D and E) 3D-principal component analysis (PCA) (D) and Gene set variation analysis (GSVA) (E) in three IMiD-resistant TCL lines treated with 1 µM lenalidomide, pomalidomide or CC-92480 for 24 hours compared to DMSO. (F) Tracking of mouse body weight in four different in vivo models under treatment with 3mg/kg pomalidomide, 1mg/kg CC-92480 or vehicle control. **p<0.01; ***p<0.001.

A

RNAseq in Len-RGs vs Parental
GO enrichment

- → covalent chromatin modification — 135
- → histone modification — 131
- histone methylation — 47
- histone lysine methylation — 40
- regulation of mitotic cell cycle phase transition — 98
- regulation of cell cycle phase transition — 103
- negative regulation of cellular catabolic process — 67
- → regulation of chromatin organization — 53
- protein acylation — 56
- negative regulation of catabolic process — 74
- → cell-cell signaling by wnt — 109
- → Wnt signaling pathway — 108

Up

$-\log_{10} (p - \text{value})$

B

RNAseq in Len-RGs vs Parental
KEGG enrichment

- → Prostate cancer — 30
- → Thyroid hormone signaling pathway — 33
- → Insulin signaling pathway — 34
- → Adherens junction — 23
- → Proteoglycans in cancer — 45
- Chemokine signaling pathway — 37
- → Phosphatidylinositol signaling system — 26
- Chronic myeloid leukemia — 23
- Pathways in cancer — 97
- T cell receptor signaling pathway — 24

Up

$-\log_{10} (p - \text{value})$

C

RNAseq in Len-RGs (ZFP91 KO vs WT)
GO enrichment

- covalent chromatin modification — 115
- histone modification — 113
- regulation of chromatin organization — 49
- cell junction organization — 65
- Wnt signaling pathway — 94
- cell-cell signaling by wnt — 94
- cell junction assembly — 55
- adherens junction assembly — 28
- cell-cell junction organization — 56

Down

$-\log_{10} (p - \text{value})$

D

RNAseq in Len-RGs (ZFP91 KO vs WT)
KEGG enrichment

- Thyroid hormone signaling pathway — 34
- Insulin signaling pathway — 33
- Proteoglycans in cancer — 42
- AMPK signaling pathway — 29
- Phosphatidylinositol signaling system — 25
- Regulation of actin cytoskeleton — 43
- Insulin resistance — 25
- Prostate cancer — 24
- Adherens junction — 20

Down

$-\log_{10} (p - \text{value})$

Supplemental Figure 5. Loss of ZFP91 reverses the transcriptional differences between len-regrown and parental cells. (A and B) Enrichment of upregulated pathways from Gene Ontology (GO) term enrichment (A) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (B) analysis in SU-DHL-1 len-regrown (RG) versus parental cells. (C and D) Enrichment of downregulated pathways from GO (C) and KEGG (D) analysis in ZFP91 knockout (KO) versus wild-type (WT) SU-DHL-1 len-regrown cells. Downregulated pathways that are also enriched upon ZFP91 knockout (C and D) in Len-regrown cells are marked with red arrows in (A and B).
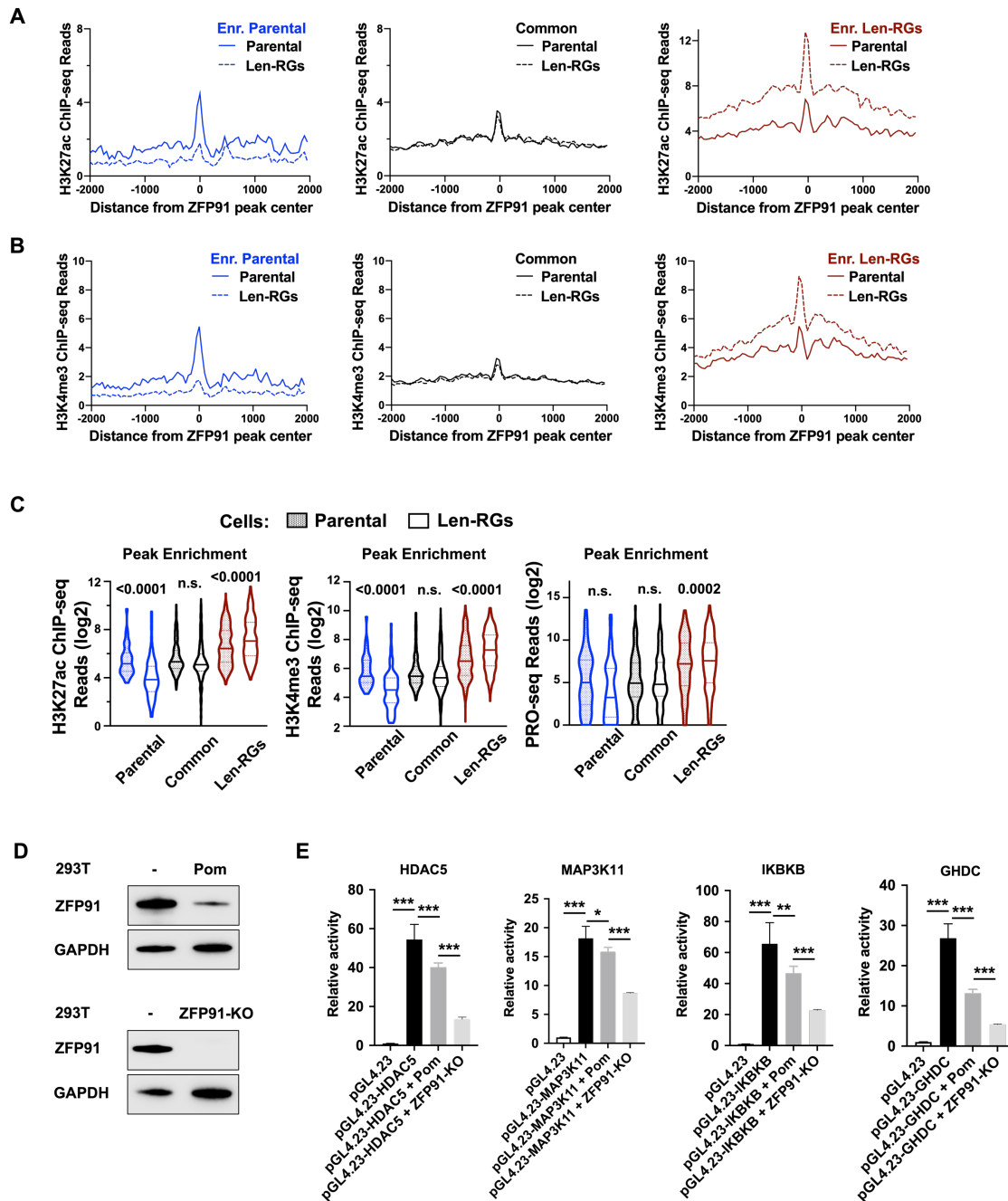
Supplemental Figure 6. ZFP91 functions as a transcriptional activator. (A and B) Metagene plots show the distribution of H3K27ac (A) and H3K4me3 (B) ChIP-seq signals centered on each group (defined in Figure 5D) of ZFP91 distal peaks (>1kb from transcription start site (TSS)) in SU-DHL-1 parental and len-regrown (Len-RG) cells. (C) Violin plots depict enrichment of ChIP-seq reads of H3K27ac (left) and H3K4me3 (middle), or PRO-seq reads (right) around each group (defined in Figure 5D) of ZFP91 distal peaks in SU-DHL-1 parental and len-regrown cells. P-values from paired, two-tailed t-tests. (D) Western blot analysis of ZFP91 expression in 293T cells treated with 1μM pomalidomide (Pom) for 24 hours or control (upper), or in 293T cells with or without ZFP91 knockout (KO) (bottom). (E) Luciferase reporter

assay analysis of ZFP91 target sequences. pGL4.23-HDAC5, pGL4.23-MAP3K11, pGL4.23-IKBKB, or pGL4.23-GHDC (pGL4.23 plasmid containing ZFP91 binding DNA regions) was co-transfected with a renilla luciferase expression construct (pRL-TK) into 293T cells with or without ZFP91 knockout and treated with 1μM pomalidomide or control. Luciferase activity was normalized to pGL4.23 empty vector control after dividing by Renilla luciferase activity. The experiment was performed in triplicate and replicated twice. Data are presented as mean ± SD. *$p<0.05$; **$p<0.01$; ***$p<0.001$.
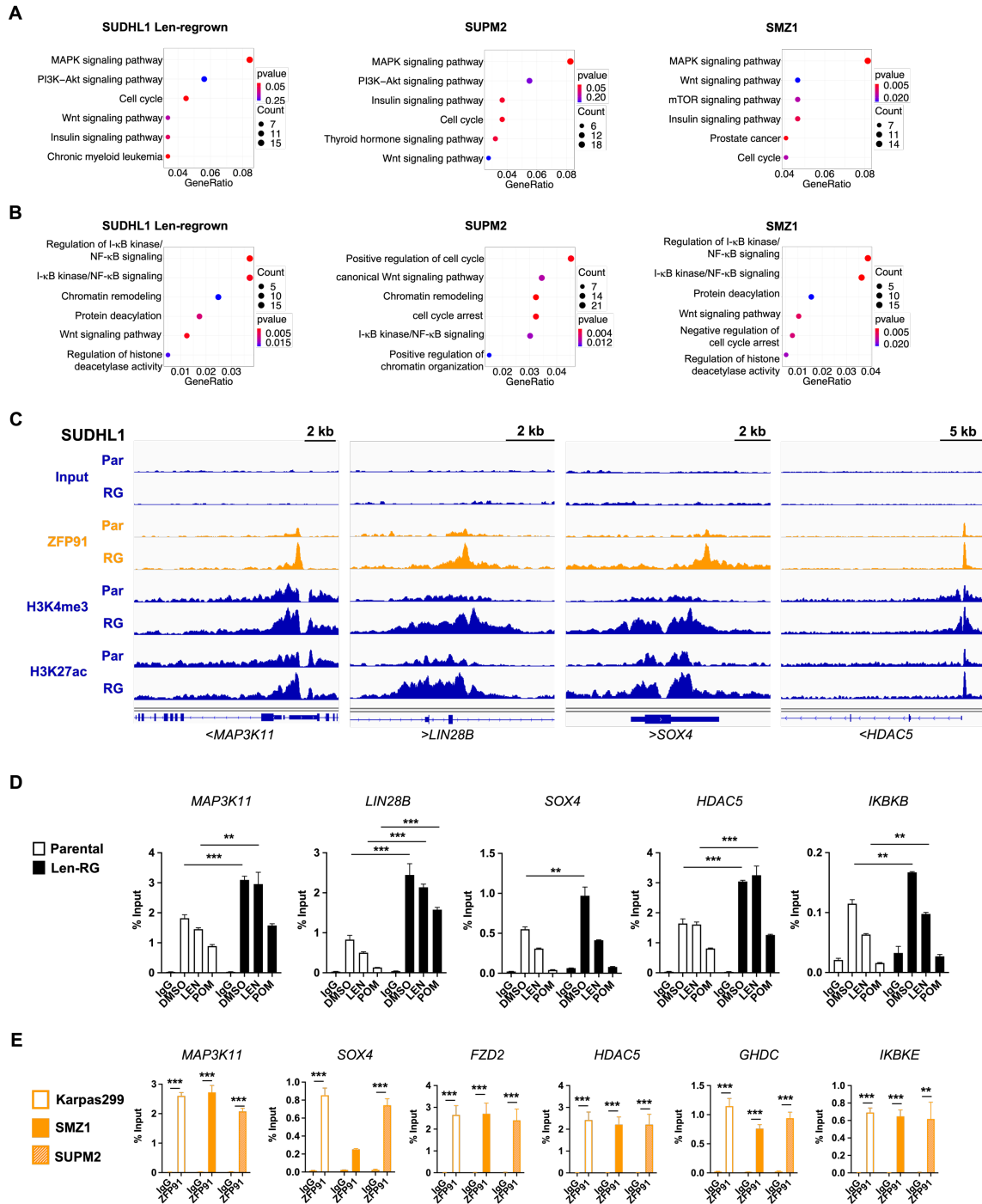
Supplemental Figure 7. ZFP91 regulates genes from histone modification, WNT, NF-kB, and MAP kinase signaling in TCL cells. (A and B) Enrichment of ZFP91 ChIP-seq target genes from KEGG (A) and GO (B) analysis in SU-DHL-1 len-regrown and two IMiD-resistant TCL lines. (C) IGV tracks show ZFP91 binding at *MAP3K11*, *LIN28B*, *SOX4*, and *HDAC5* with H3K4me3 and H3K27ac signals in SU-DHL-1 parental (Par) and len-regrown (RG) cells. (D) ChIP experiments were performed in SU-DHL-1 parental and len-regrown cells treated with 1 μM lenalidomide, pomalidomide, or DMSO control using IgG or anti-

ZFP91 antibodies. The binding of ZFP91 at potential gene loci was quantified by qPCR. Data are presented as mean ± SEM. (E) ChIP-qPCR analysis of ZFP91 binding at *MAP3K11*, *LIN28B*, *SOX4*, *HDAC5*, *IKBKB*, and *IKBKE* in three IMiD-resistant TCL lines. The experiment was performed in triplicate and replicated twice. Data are presented as mean ± SEM. **p<0.01; ***p<0.001.
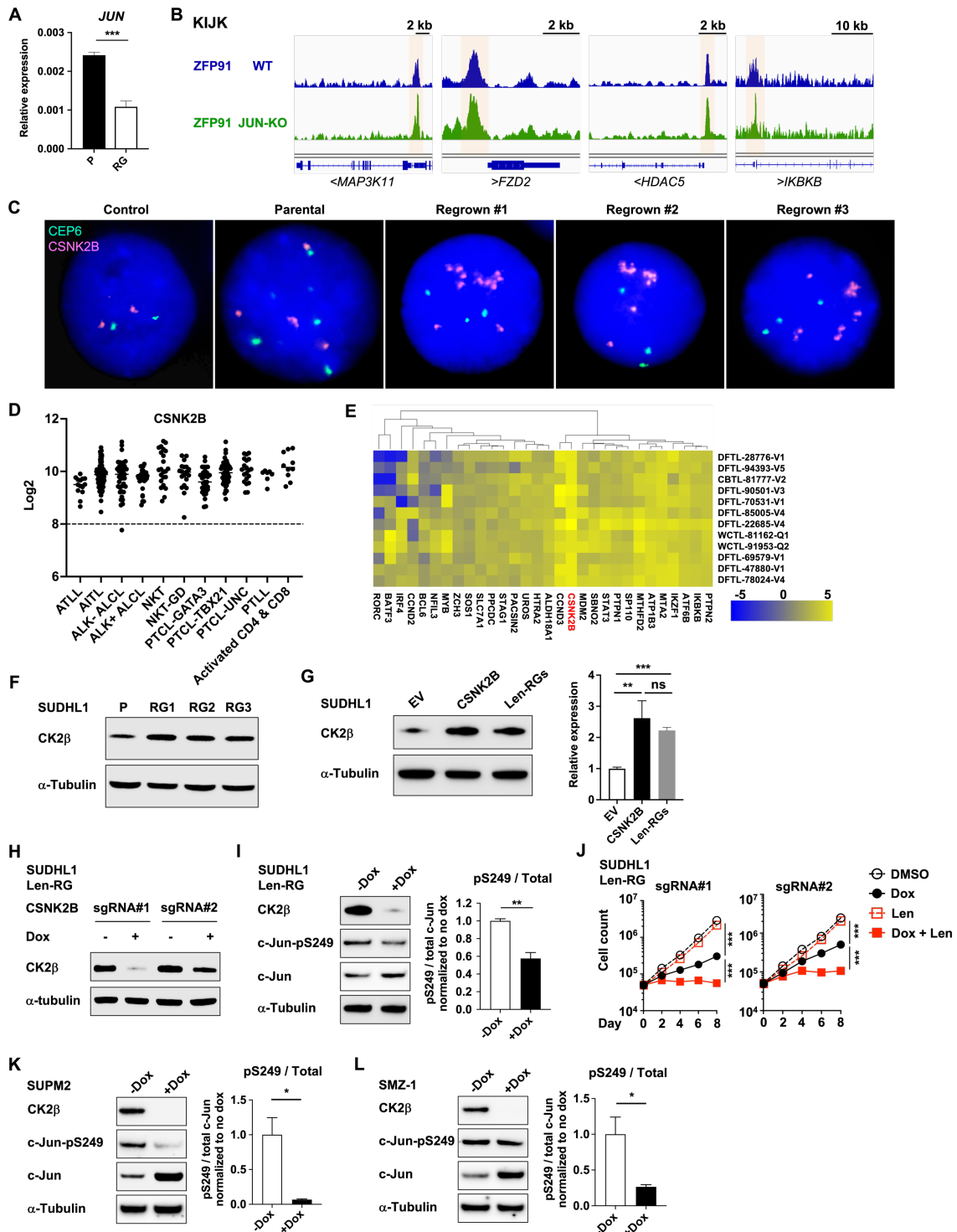
Supplemental Figure 8. *CSNK2B* **negatively regulates c-Jun via phosphorylation at serine 249.** (A) qPCR analysis of *JUN* (n = 3). Gene expression is normalized to RPS18 and presented as mean ± SEM. (B) IGV tracks represent ZFP91 binding at the *MAP3K11*, *FZD2*, *HDAC5*, and *IKBKB* gene loci in KI-JK

JUN-knockout (KO) cells (Green) compared to wild-type (WT) control (blue). (C) Representative images of FISH signals show centromere region of chromosome 6 (CEP6, green) and 6p21.33 region (indicated by *CSNK2B* probe, pink) in SU-DHL-1 parental and three len-regrown pools as well as normal T lymphocyte control. (D) Microarray analysis of *CSNK2B* transcript expression among PTCL entities. Mean of log2 intensity ≥8 is considered expression (indicated by the dashed line). (E) Heatmap shows normalized expression of 30 genes across 12 PTCL patient-derived xenografts (PDX). The 30 genes were identified as the top vulnerabilities in a previous CRISPR screen of PTCL lines[6]. (F) Western blot analysis of CSNK2B protein (CK2b) expression in SU-DHL-1 parental (P) and len-regrown (RG) cells. (G) Western blot analysis after *CSNK2B* overexpression in parental SU-DHL-1 cells and compared to untransfected len-regrown cells. Relative expression levels of CK2b protein were normalized to empty vector (EV) control (right). (H) Western blot analysis of CK2b expression in SU-DHL-1 len-regrown cells with doxycycline (dox)-induced expression of two independent sgRNAs targeting *CSNK2B*. (I) Representative Western blot (left) and quantitative analysis (right) of pS249 and total c-Jun expression in SU-DHL-1 len-regrown cells with doxycycline (dox)-induced *CSNK2B* knockout compared to control. Data are presented as mean ± SEM. Comparisons are by two-tailed Student's t test. (J) Cell count of SU-DHL-1 len-regrown cells with dox-induced *CSNK2B* knockout treated with 1μM lenalidomide or DMSO. The experiment was performed in triplicate and replicated twice. Data are presented as mean ± SD. Comparisons are by 2-way ANOVA and Bonferroni correction for multiple comparisons. (K and L) Representative Western blot (left) and quantitative analysis (right) of c-Jun pS249 and total c-Jun in SUP-M2 (K) and SMZ-1 (L) cells with doxycycline-induced *CSNK2B* knockout compared to no dox control. Data are presented as mean ± SEM. Comparisons are by two-tailed Student's t test. *$p<0.05$; **$p<0.01$; ***$p<0.001$.

References

1.   Aubrey BJ, Kelly GL, Kueh AJ, et al. An inducible lentiviral guide RNA platform enables the identification of tumor-essential genes and tumor-promoting mutations in vivo. *Cell Rep*. 2015;10(8):1422-1432.
2.   Heavican TB, Bouska A, Yu J, et al. Genetic drivers of oncogenic pathways in molecular subgroups of peripheral T-cell lymphoma. *Blood*. 2019;133(15):1664-1676.
3.   Dobay MP, Lemonnier F, Missiaglia E, et al. Integrative clinicopathological and molecular analyses of angioimmunoblastic T-cell lymphoma and other nodal lymphomas of follicular helper T-cell origin. *Haematologica*. 2017;102(4):e148-e151.
4.   Donovan KA, An J, Nowak RP, et al. Thalidomide promotes degradation of SALL4, a transcription factor implicated in Duane Radial Ray syndrome. *Elife*. 2018;7.
5.   Reimer KA, Mimoso CA, Adelman K, Neugebauer KM. Co-transcriptional splicing regulates 3' end cleavage during mammalian erythropoiesis. *Mol Cell*. 2021;81(5):998-1012 e1017.
6.   Ng SY, Yoshida N, Christie AL, et al. Targetable vulnerabilities in T- and NK-cell lymphomas identified through preclinical models. *Nat Commun*. 2018;9(1):2024.