

Mouse Dissection and Tumor Cell Isolation

The disease development in the mice was followed by circulating tumor cells, body weight loss and clinical signs of disease and pain. Circulating tumor cells were weekly detected on flow cytometry on the peripheral blood. The weekly body weight change was measured. Clinical signs of disease and pain were observed daily. The mice were sacrificed at humane endpoint.

The mouse was dissected and the internal organs were gently collected. Spleen size and weight were measured. Pieces of each organ were transferred to formaldehyde for histopathological analysis. The spleen was mechanically dissociated to collect the tumor cells. Liver, kidney and lung tissues were enzymatically and mechanically dissociated. The organs were treated with PBS containing 500 mg/l Collagenase IV (Sigma Aldrich, USA), 50 µg/l DNase I (Thermo Fisher Scientific, USA), 2% Fetal Bovine Serum (FBS)(Thermo Fisher Scientific, USA), and 0,6% Bovine Serum Albumin (BSA) (Thermo Fisher Scientific, USA) at 37° and the cell suspension was collected after mechanical dissection. The skin from cutaneous lesions of the mice was recovered after depilation and the subdermal fat was removed. The skin tissue was treated with RPMI containing 250 mg/l Liberase™ (Sigma Aldrich, USA) and DNase I addition (20 mg/l, Sigma Aldrich, USA) at 37° C.

Isolated tumor cells and tissues were cryopreserved into freezing medium including 10% DMSO, 50% FBS and 40% RPMI and stored in -150°C.

Immunohistochemistry

	<i>Antibody</i>	<i>Clone</i>	<i>Dilution</i>	<i>Manufacturer</i>
B cell	CD20	L26	1 :500	Dako France Sas
	CD30	Ber-H2	1 :70	Dako France Sas
T cell	CD3	F7.2.38	1 :50	Dako France Sas
	CD8	4B12	1 :100	Leica Biosystems
TFH cell	CD4	C8/144B	1 :200	Dako France Sas
	ICOS	SP98	1 :100	Abcam
	PD1	NAT105	1 :200	Abcam
	BCL6	LN22	1 :50	Leica Biosystems
	CXCL13	53610	1 :50	R&D Systems
	IDH2R172K	R172K	1 :200	Eweast Biosciences
	EBNA2	PE2	1 :50	Abcam
EBV	LMP	CS 1-4	1 :400	Dako France Sas
Apoptosis Proliferation	p21	EA10	1 :50	Calbiochem
	p53	D70	1 :20	Dako France Sas
	Cleaved-Caspase 3	Polyclonal	1 :100	Sigma Aldrich
	MIB1	30-9	Prediluted 1 :1000	Roche

In Situ Hybridization

Deparaffinized tissue sections were stained with a fluorescein isothiocyanate-coupled specific peptidic nucleic acid probe to detect Epstein-Barr encoded RNA transcripts 1 and 2 (EBERs). The tissue sections were heat denatured, digested with proteinase K and incubated overnight. Antifluorescein isothiocyanate monoclonal mouse antibodies, rabbit antimouse antibodies followed by alkaline phosphatase and antialkaline phosphatase monoclonal mouse antibodies were used for the detection of heterodimers. 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium were used for the observation of the staining, as previously described (1),(2).

Flow Cytometry

Isotype controls, compensation beads (Biolegend) and rainbow calibration particles (Biolegend) were used for the optimal analyses.

<i>Antibody</i>	<i>Clone</i>	<i>Fluorochrome</i>	<i>Isotype</i>	<i>Manufacturer</i>
<i>CD45</i>	HI30	FITC	Mouse IgG1, κ	Biolegend
<i>CD19</i>	HIB19	APC.Cy7	Mouse IgG1, κ	Biolegend
<i>CD3</i>	SK7	BV510	Mouse IgG1, κ	Biolegend
<i>CD4</i>	RPA-T4	BV605	Mouse IgG1, κ	Biolegend
<i>CD8</i>	RPA-T8	BV711	Mouse IgG1, κ	Biolegend
<i>ICOS</i>	C398.4A	BV421	Armenian Hamster IgG	Biolegend
<i>PD1</i>	A17188B	PE.Cy7	Mouse IgG2b, κ	Biolegend

Nucleic Acid Extraction

Genomic DNA (gDNA) or RNA was extracted from FFPE tissue or fresh cells using the Maxwell FFPE Plus DNA Kit (Promega, USA) or AllPrep DNA/RNA Mini/Micro Kits (Qiagen, CA) according to the manufacturers' recommendations, respectively. Nucleic acids were quantified via Qubit 3.0 using the Qubit dsDNA/RNA HS (Thermo Fisher, USA) assay kits.

Mutational Profiling by Next Generation Sequencing

Mutation analysis was performed on gDNA samples after amplification using 15 ng of tumoral gDNA as template and utilizing an Ampliseq custom panel (Thermo Fisher Scientific, USA). This includes a multiplex PCR-based library preparation method that targeted various coding regions (70–150 bp), involved in T cell lymphoma. Subsequently, the amplicons underwent digestion, barcoding, and amplification using the Iom Ampliseq Kit for Chef DL8 (Thermo

Fisher Scientific, USA), following the provided instructions. Following DNA library preparation and quantification, each library, at a concentration of 25 pM, underwent multiplexing and clonal amplification on ion-sphere particles (ISP) through emulsion PCR performed on The Ion Gene Studio S5 (ion, Thermo Fisher, USA) supported by the Genomic Platform of Mondor Biomedical Research Institute. The ISP templates were loaded onto an Ion-510, 520 or 530 chip and subjected to sequencing on an S5 sequencer with the Ion 510™ & Ion 520™ & Ion 530™ Kit–Chef, following the manufacturer’s instructions. The minimum sequencing depth of the samples was 1000x. Run performance was evaluated, and data analysis was conducted using the TORRENT SUITE Software versions between v.5.10 and v.5.16 (Thermo Fisher Scientific, USA). Sequencing data were processed through SeqPilot (JSI Medical Systems, Germany). Visualization of single-nucleotide variants (SNV) was accomplished using the Integrative Genomics Viewer, employing low stringency settings (threshold: 1%). The specific classes of variants detected included extronic (nonsynonymous SNV, stop gain, stop loss, frameshift deletion or insertion, non-frameshift deletion or insertion), and intronic (only splicing, only +/- 1-2 bp before/after exon) variants. The overage of the panel was 3001,4x [616-6398], the minimum coverage of the target region was 500 and minimum VAF was determined as 1%.

NGS T cell lymphoma panel expanded through 3 different versions: V.1.1, V.1.2 and V.1.3. The first panel V.1.1 included the genes encoding CD28, DNMT3A, IDH2, PLCG1, RHOA, SETD2, STAT3, STAT5B and TET2. In the second version V.1.2, the JAK1 and JAK3 genes were added to the panel together with an improved coverage quality of genes of V.1.1. Finally, V.1.3 had an improved coverage quality on V1.2 genes and addition of the MSC, TNFRSF1B, TP53 and VAV1 genes.

NGS B cell lymphoma panel included the genes encoding ARID1A, B2M, BCL2, BRAF, BTK, CARD11, CCND3, CD58, CD79A, CD79B, CDKN2A, CDKN2B, CIITA, CREBBP, CXCR4, EP300, EZH2, FOXO1, GNA13, ID3, IRF4, MEF2B, MYC, MYD88, NOTCH1, NOTCH2, PIM1, PLCG2, PRDM1, SOCS1, STAT6, TCF3, TNFAIP3, TNFRSF14, TP53, and XPO1.

TCR Clonality Assay via DGGE-PCR technique

For T-cell clonality studies, DNA was analyzed by DNA amplification of the TCR-gamma VJ gene using consensus primers and separation of the amplicons on denaturing sequencing gel (polymerase chain reaction-denaturing gradient gel electrophoresis), as previously described

(3). This technique, using GC-clamp primers, provides tumor-specific imprints in as much as the procedure characterizes N sequence polymorphism at the VJ junctions.

Hybrid Capture T Cell Receptor Next Generation Sequencing

DNA extracted from the FFPE biopsies of TFHL patients or PDX mice was used for studying T-cell receptor gene rearrangements by PCR. A T cell receptor capture panel including all V(D)J segments of alpha beta gamma delta chain genes was used. The capture panel consisted of 1322 agilent Sureselect biotinylated baits targeting the 3' 100 bp of all TCR V gene regions, and 837 biotinylated baits targeting the 5' 100 bp of all TCR J gene regions as annotated by IMGT data base

<https://clicktime.symantec.com/3ZHTCSiunN8mg1iRCXthZX6H2?u=www.imgt.org>.

The dominant T-cell clone identified in patient's biopsies was tracked. Briefly, DNA was fragmented (180-250 bp) by enzymatic digestion and Agilent DNA libraries were generated from 10-200 ng of fragmented DNA using Sureselect XTBS2 target enrichment kit. Libraries were sequenced on an Illumina NextSeq2000 instrument, and FASQ were analyzed using Vidjil, a Web Platform for Analysis of High-Throughput Repertoire Sequencing. The dominant T-cell clone identified in patient's biopsies was tracked (4).

DNA Methylation Analysis

DNA from PDX and the corresponding patient tumor samples was bisulfite-converted for DNA methylation analysis using the EZ DNA Methylation kit (Zymo Research, USA) according to manufacturer's instructions. DNA methylation was interrogated by the Infinium® MethylationEPIC (EPIC) BeadChips (Illumina Inc., USA) following the manufacturer's guidelines. Raw intensity files (idat) were imported into the R programming (v4.3.1) using the minfi package. Data was normalized to controls using the preprocess Illumina function without background correction. Beta values were calculated representing the percentage of DNA methylation at a certain cytosine base. For downstream analysis, loci on gonosomes were excluded. Based on variance between all samples, the 10,000 most variable CpGs were selected and displayed as unsupervised analysis. Principal component analysis (PCA) was performed using the prcomp function and heatmaps were generated with the pheatmap package.

RNA-sequencing

RNA sequencing libraries were prepared with the Illumina Stranded mRNA Prep, Ligation (Illumina, USA) kit following manufacturer's recommendations. The protocol consisted of PolyA mRNA capture with oligo dT magnetic beads (25-1000 ng total RNA), purification and fragmentation of mRNA to approximately 300-400 pb, synthesis of first strand DNA (cDNA) using reverse transcriptase and random primers, replacement of dUTP to dTTP for the synthesis of second cDNA strand, addition of adenine and thymine bases to fragment ends, and ligation of IDT for Illumina TruSeq UD Indexes (Illumina, USA) adaptors for amplification of the library by PCR for sequencing. The library sequencing was performed by NextSeq 500 sequencer (Illumina, USA) with 21 millions reads in 75 bp paired-end mode following Mid Output Kit v2.5 1x150 cycles/phiX kit protocol.

Transcriptomic and Functional Analysis

RNA obtained from PDX cells was used for general transcriptomics, Human Endogenous Retrovirus (HERV) and antiviral response gene expression analysis which were quantified using a custom pipeline, as previously described (5). Briefly, RNA-seq reads were aligned to the GRCh38.p14 reference genome using bowtie2 (v2.5.3) with custom parameters to retain multimaps (--no-unal --score-min L,0,1.6 -k 100 --very-sensitive-local). SAM outputs were converted to BAM files using SAMtools (v1.19). HERV was quantified using Telescope (6) (v1.0.3, options --reassign_mode exclude) and featureCounts (v2.0.6, options -Q 10 -s 2) respectively.

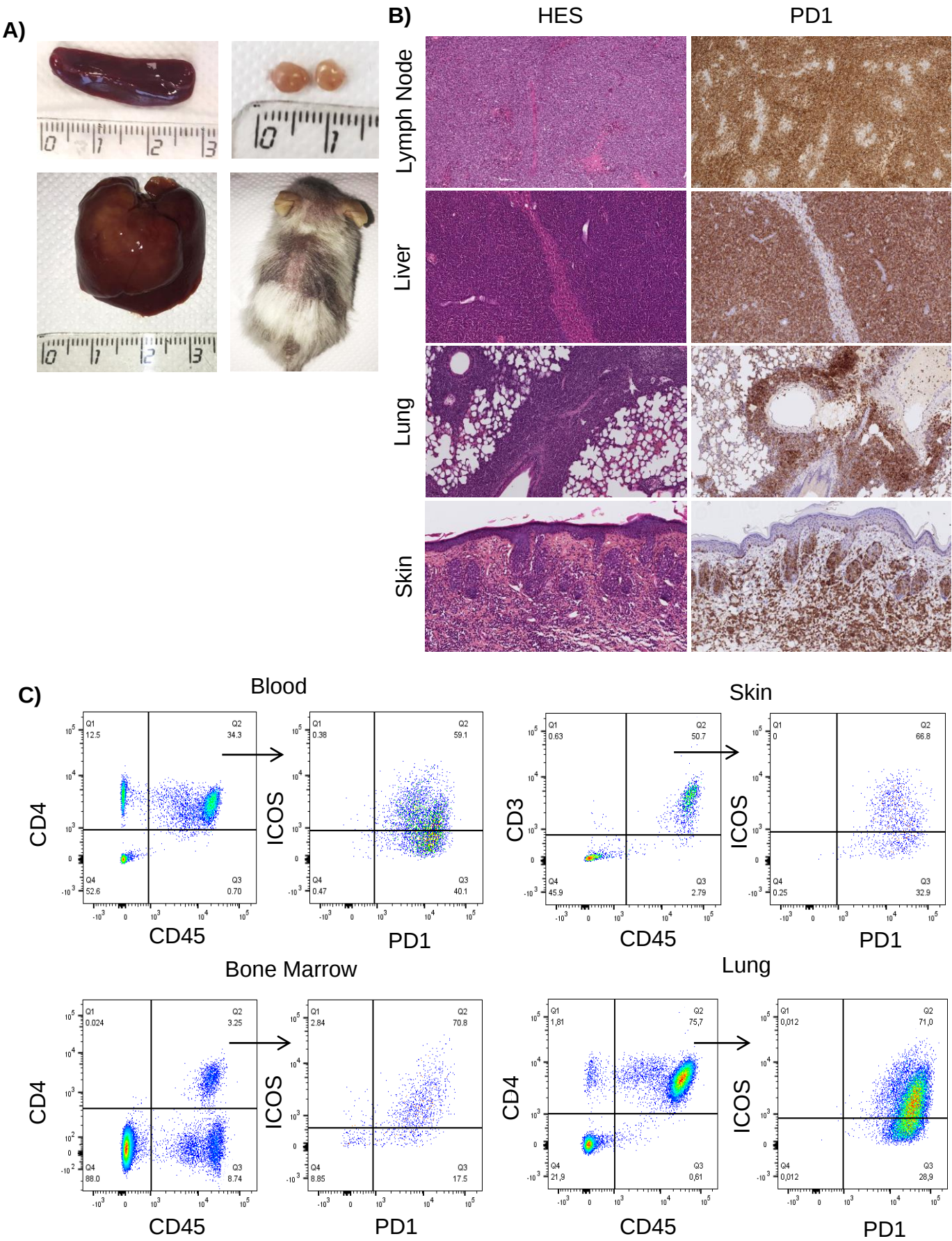
For HERVs, a custom transcriptome with 14 968 HERV transcriptional units compiled from RepeatMasker annotations was used as reference. For antiviral genes, the GENCODE v45 reference transcriptome was used. Raw counts were concatenated and normalized independently for each PDX using DESeq2 (v1.42.0) with variance stabilizing transformation (VST). Differential expression analysis was performed using DESeq2 (v1.42.0).

Differentially expressed human genes (DEG) were subjected to gene set enrichment analysis. DEG, functional gene set enrichment analyses were performed with clusterProfiler (v4.10.1) and fgsea (v1.24.0) (7). Immune gene sets obtained from the Reactome database and a custom viral mimicry gene set manually curated from relevant papers (8–11). For visualization of the results through volcano plots and heatmaps, several packages as enhancedVolcano (1.20.0), ComplexHeatmap (v2.18.0) and pheatmap (v1.0.12) in R program were utilized.

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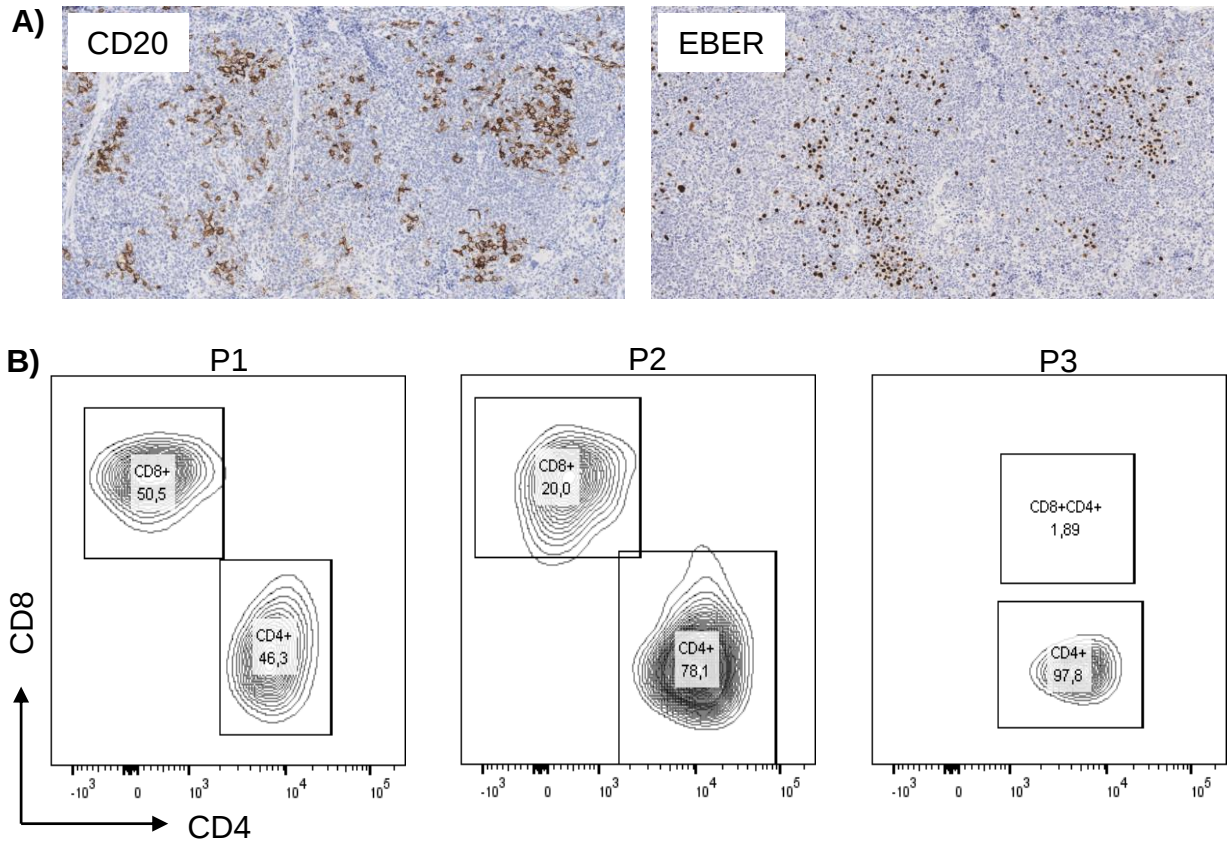
Supplementary Figure 1 – Pathophysiology of TFHL-PDX models



Supplementary Figure 1. Pathophysiology of TFHL PDX models

- A) Pathology of TFHL PDX models splenomegaly enlarged lymph nodules, hepatomegaly cutaneous lesions
- B) IHC phenotype of different organs lymph node, liver lung and skin HES (10X), PD1 (10X)
- C) FACS phenotype of different organs (blood, bone marrow, skin, and lung)

Supplementary Figure 2 – Tumor microenvironment

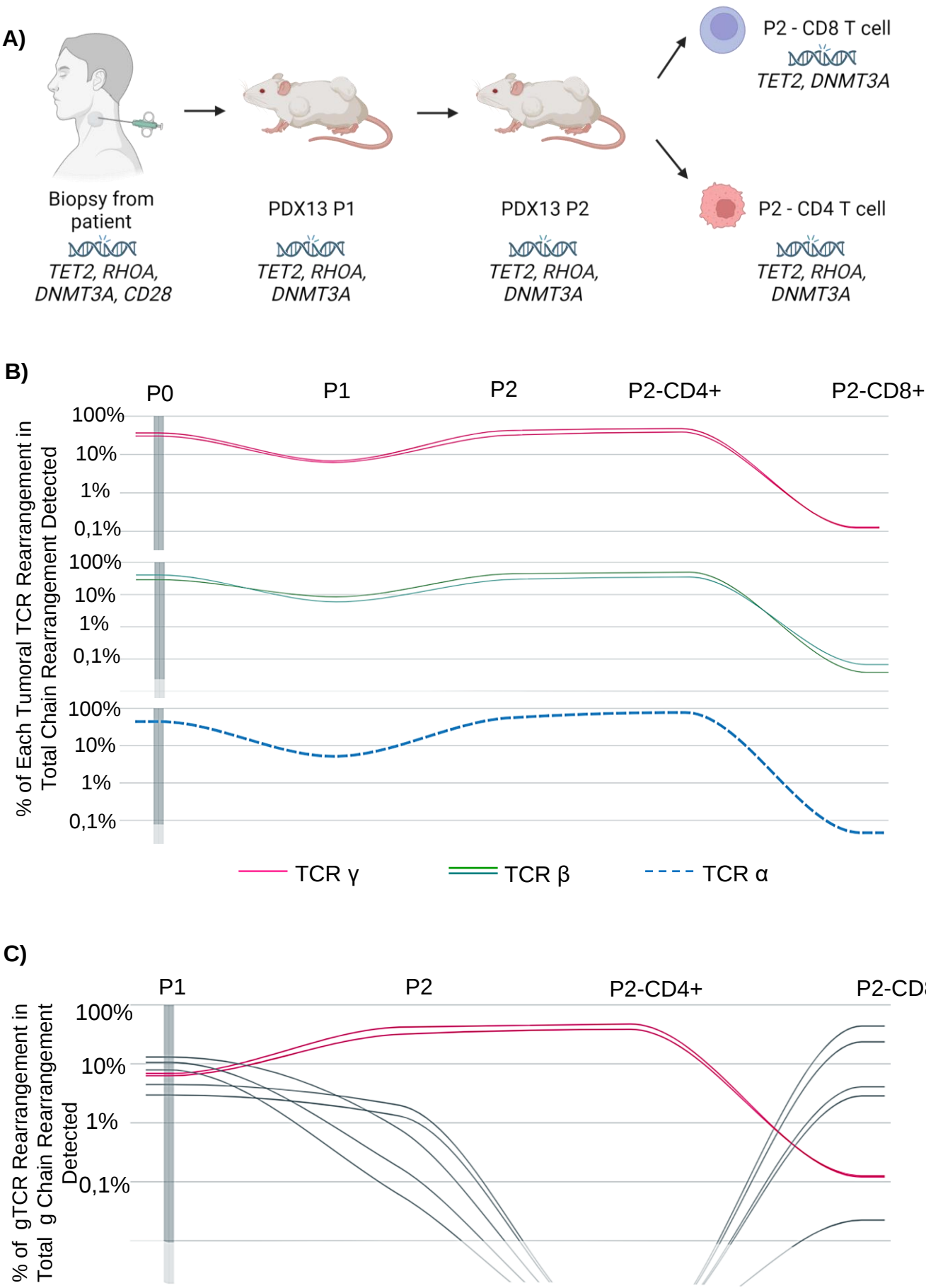


A) Phenotype of spleen for EBV infected B blast in tumor microenvironment. IHC; CD20 (10X), FISH EBER (10X)

B) FACS phenotype of splenocytes through passages. Decrease on CD 8 population was shown

P1: Passage 1, P2: Passage 2, P3: Passage 3

Supplementary Figure 3 – TCR Clonality of TFHL-PDX

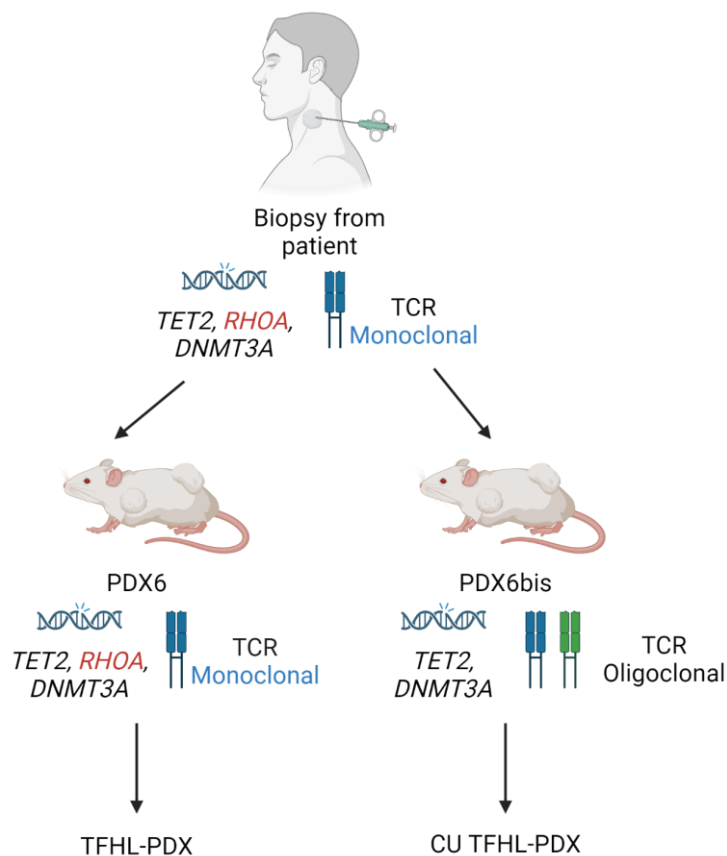


Supplementary Figure 3 – TCR Clonality of TFHL-PDX

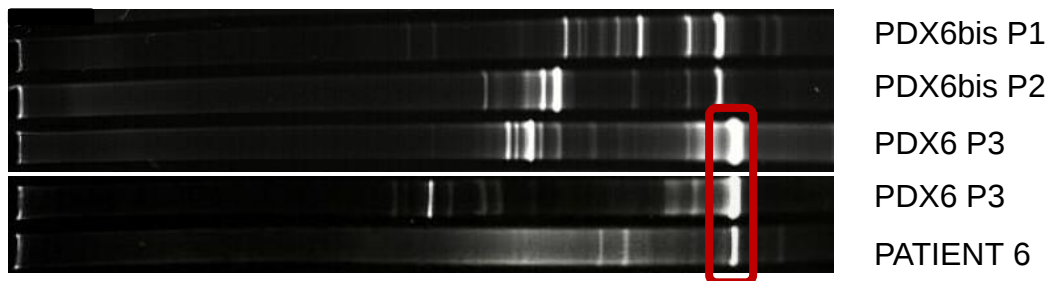
- A) Schematic representation of generation of PDX13 and molecular analysis. CD4 T cells and CD8 T cells from Passage 2 (P2) were sorted for further analysis.
 - B) The clonal evolution of PDX13 model. Tumoral clone was characterised by 2 gamma (pink lines), 2 alpha (green lines) and 1 beta (blue line) chain gene rearrangements. Specific tumoral rearrangements were then tracked in passage 1, passage 2 PDX mice, Clone was mostly detected in the CD4+ lymphocytes fraction selected from P2 PDX mouse.
 - C) Clonality assay of PDX13 through passages. During Passage 1 numerous non tumoral cells expanded corresponding to CD8 T cells as assessed by their presence in the CD 8 sorted cells from passage 2
- P0: Patient P1: Passage 1, P2: Passage 2, P3: Passage 3

Supplementary Figure 4 – Clonally Unrelated TFHL PDX6bis model

A)



B)

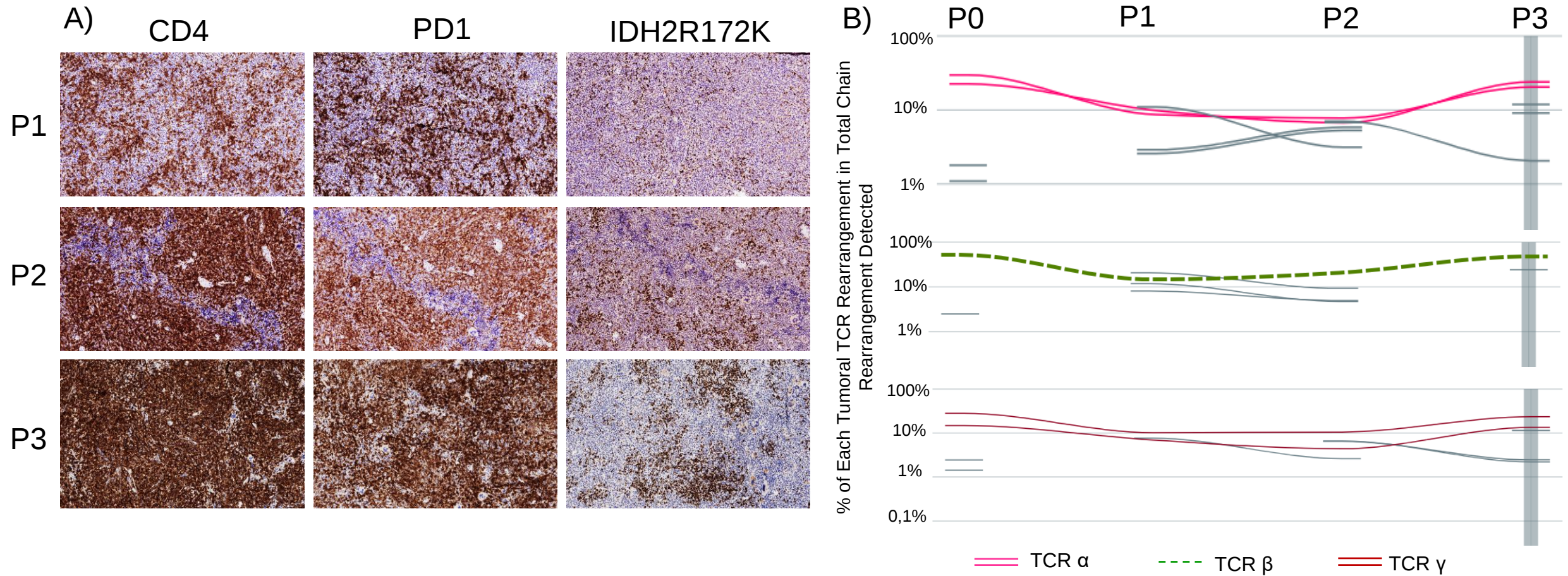


A) Schematic representation of PDX6 and PDX6bis generation from Patient 6

B) TCR clonality assay of PDX6 and PDX6bis via DEGG-PCR technique

P1: Passage 1, P2: Passage 2, P3: Passage 3

Supplementary Figure 5 – IDH2 mutated TFHL-PDX

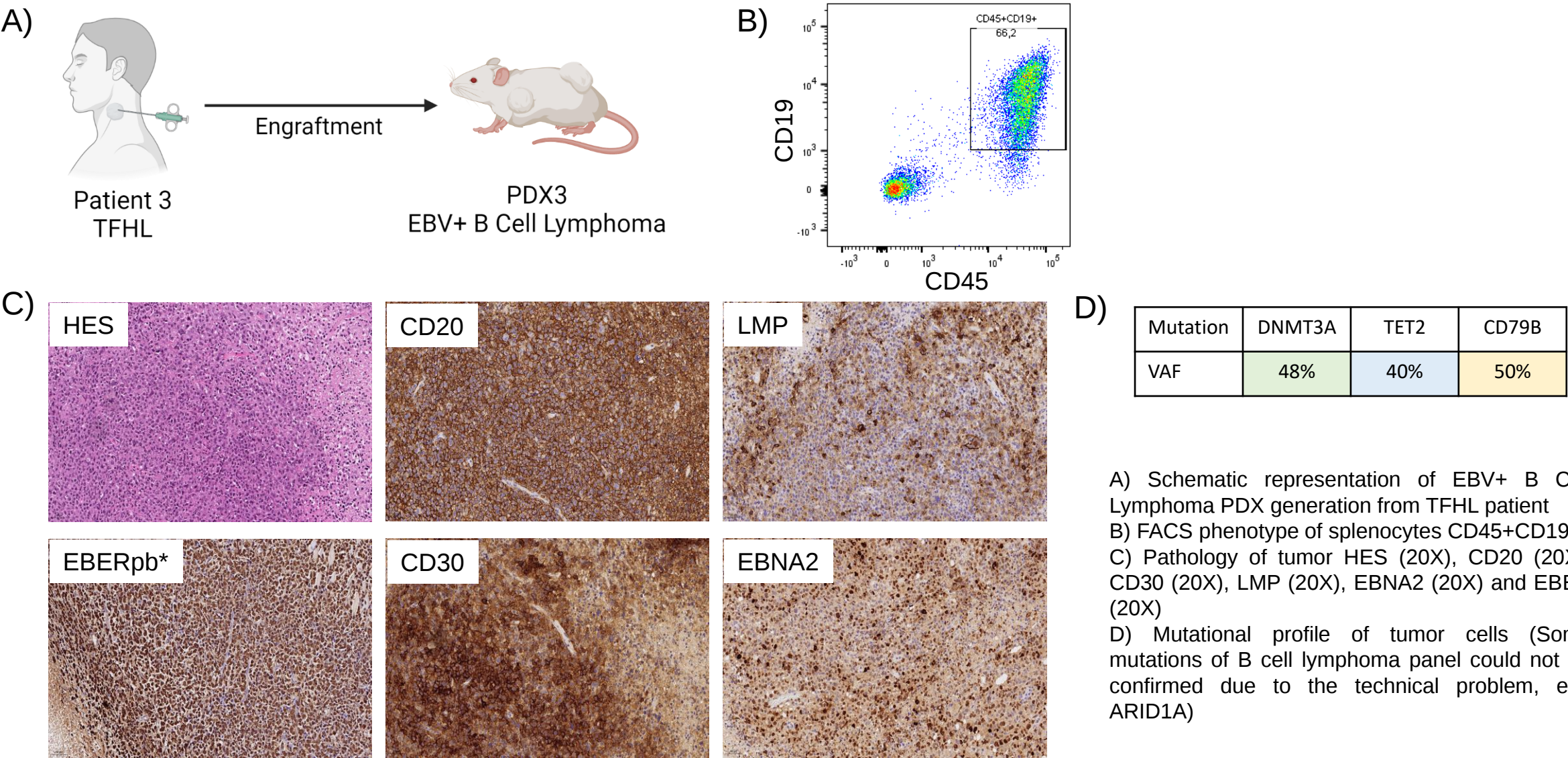


A) Pathology of spleen tissue from PD26 mice CD3 (20X), PD1 (20X), and IDH2 R172K (20X)

B) The clonal evolution of PDX26 model. Tumoral clone was characterised by 2 alpha (pink lines), 1 beta (green line) and 2 gamma beta (dark red lines) chain gene rearrangements. Specific tumoral rearrangements were then tracked in passage 1, passage 2 and passage 3 PDX mice. Gray lines represents different clones.

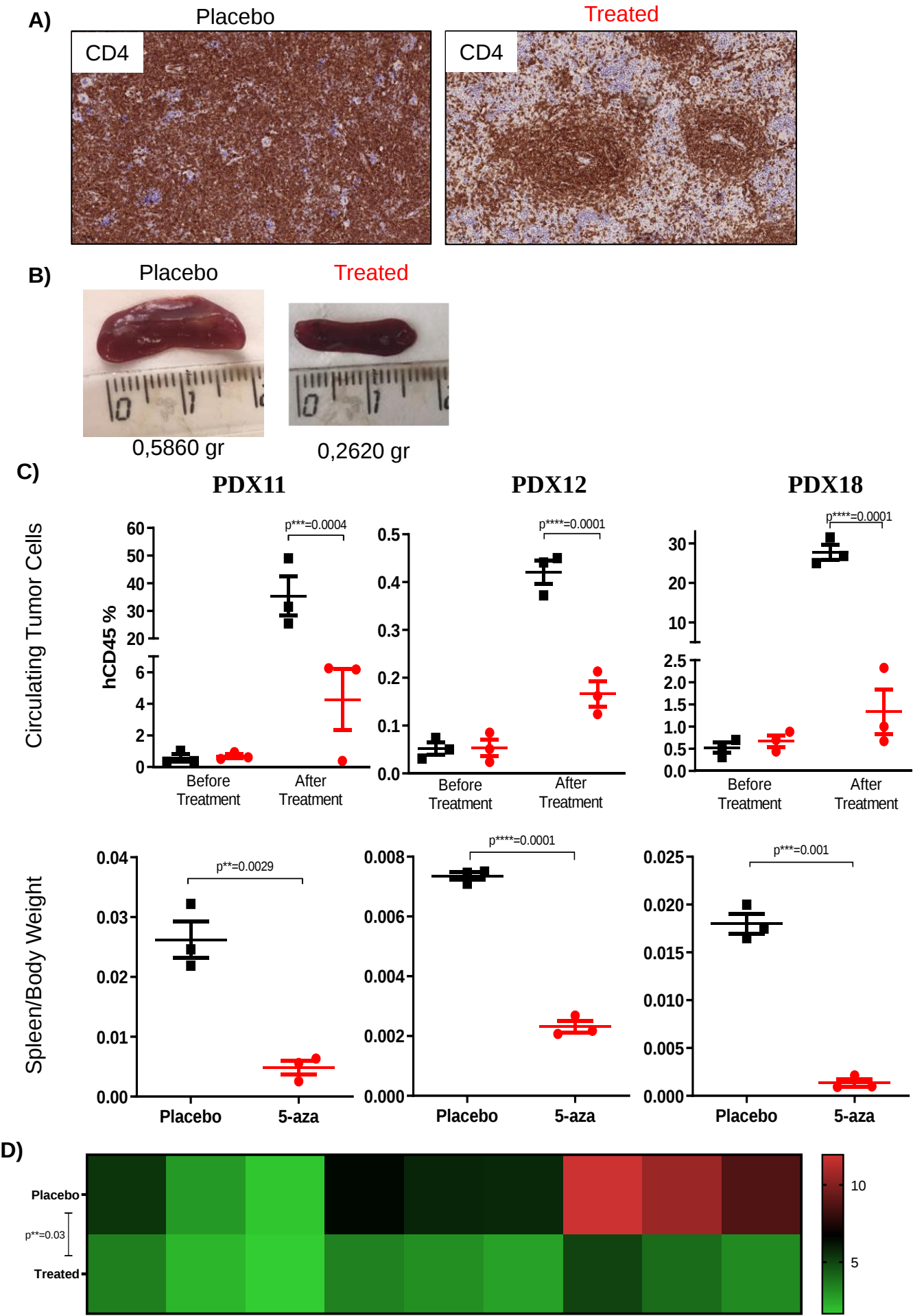
P0: Patient P1: Passage 1, P2: Passage 2, P3: Passage 3

Supplementary Figure 6 – EBV+ B Cell Lymphoma



A) Schematic representation of EBV+ B Cell Lymphoma PDX generation from TFHL patient
B) FACS phenotype of splenocytes CD45+CD19+
C) Pathology of tumor HES (20X), CD20 (20X), CD30 (20X), LMP (20X), EBNA2 (20X) and EBER (20X)
D) Mutational profile of tumor cells (Some mutations of B cell lymphoma panel could not be confirmed due to the technical problem, e.g. ARID1A)

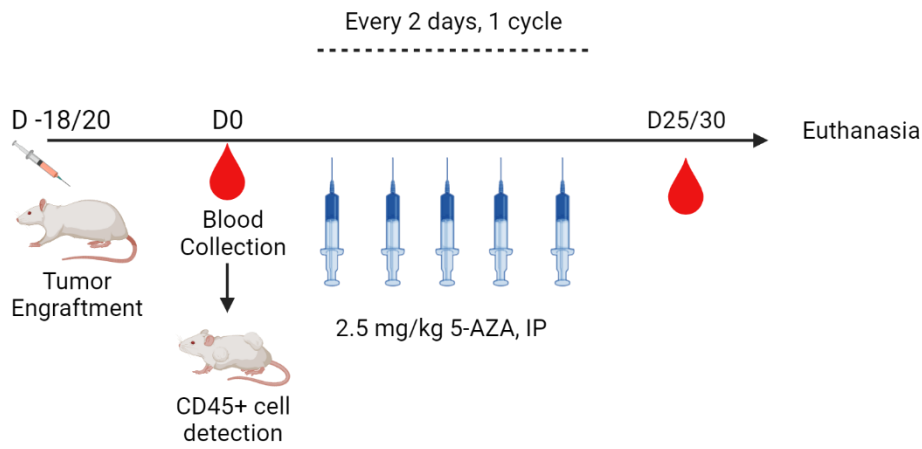
Supplementary Figure 7 – Azacitidine treatment response



Supplementary Figure 7 – Azacitidine treatment response

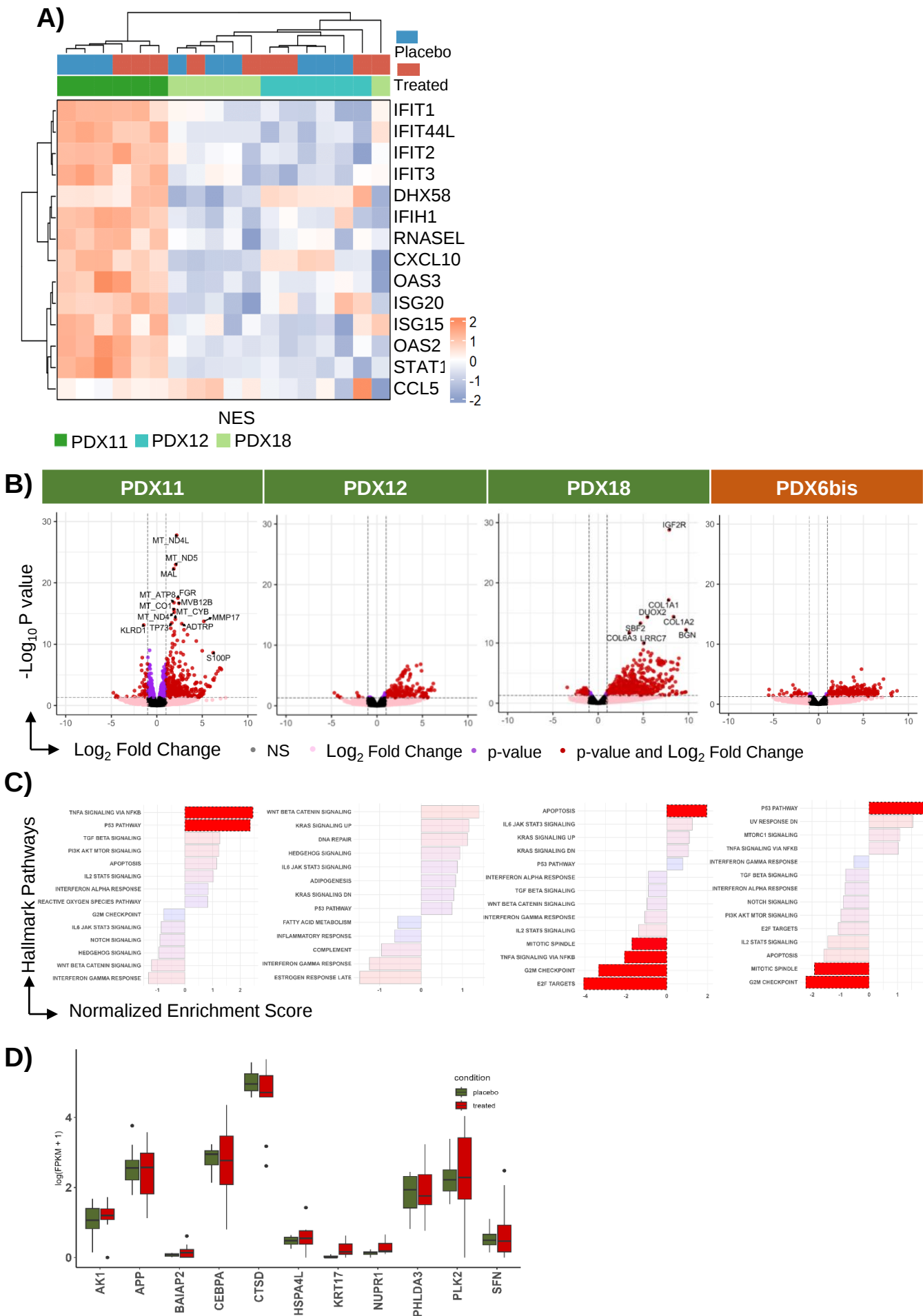
- A) IHC phenotype of splenocytes aza vs Placebo treated mice. CD4(20X) staining showed less tumor cells in azacitidine treated mice.
- B) Splenomegaly difference aza vs Placebo treated mice
- C) Treatment efficacy on PDX11, PDX12 and PDX18. Shown here with decreased circulating tumor cell and spleen/body weight ratio on azacitidine treated mice.
- D) Heapmap of ICOS expression. MFI of ICOS expression was normalized to healthy PBMC.

Supplementary Figure 8– Azacitidine Mechanism of Action



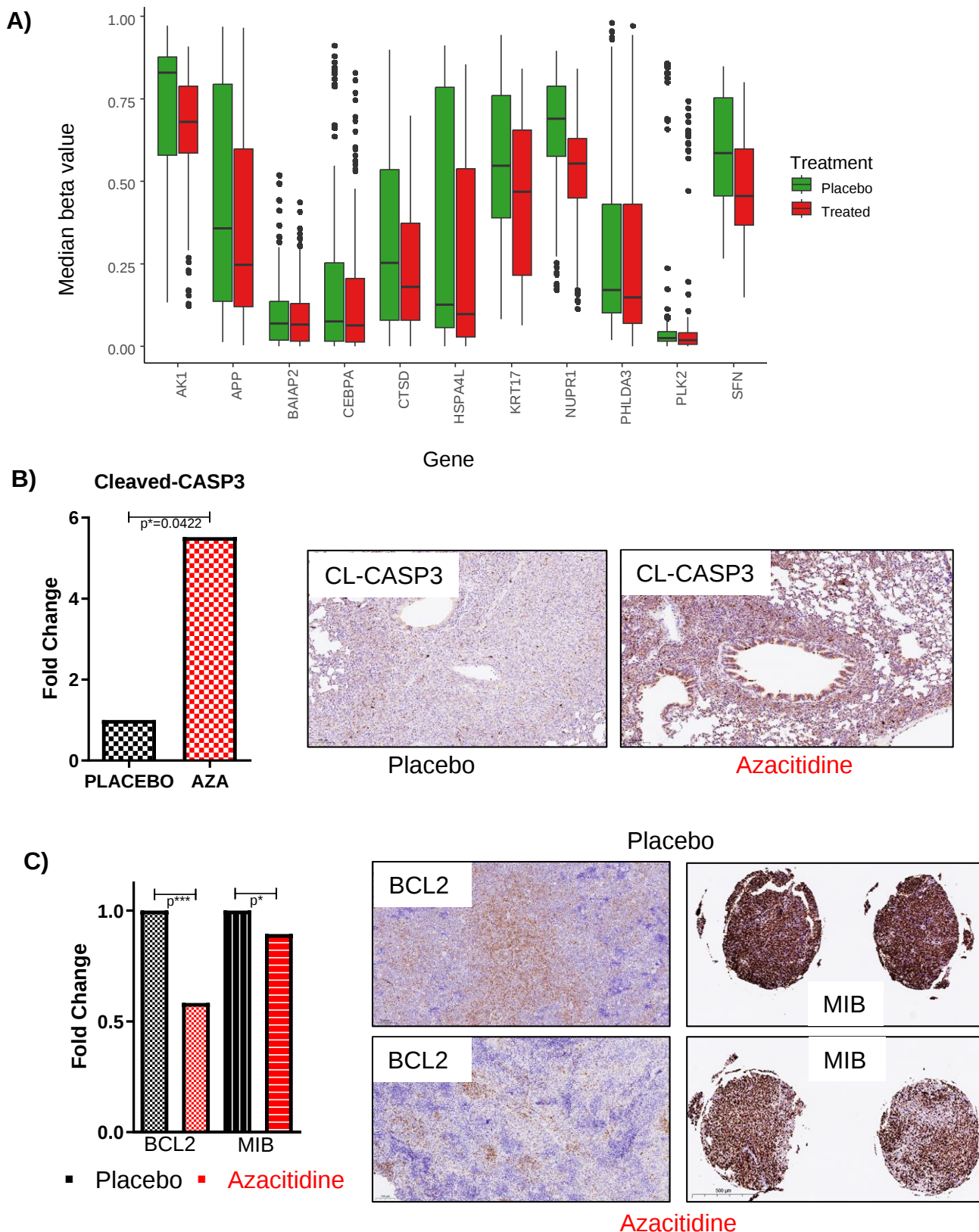
Schematic representation of the azacitidine mechanism of action experimental design

Supplementary Figure 9. Transcriptomic effects and functional analysis of azacitidine treatment



- A) The heatmaps of selected viral mimicry associated genes of responsive models
- B) The volcano plots of expression change of protein-coding genes in the models as compared to placebo after 5-aza treatment.
- C) Selected hallmark gene set enrichment of Differentially Expressed Genes (i.e. $|\log_2(\text{foldchange})| > 1$ and $p\text{-value} < 0.05$). Hallmarks with boxed filled red and in black dashed line showed significant changes.
- D) Comparison of the expression of genes found to be associated with hallmarks p53 pathway in DEGs set of sensitive models.

Supplementary Figure 10 – p53 mediated apoptosis effect of azacitidine



A) Median promoter DNA methylation of the p53-related differentially expressed genes for placebo and azacitidine treated sensitive models.

B) Azacitidine increases apoptosis shown here with cleaved caspase 3 staining

C) Azacitidine decreases anti-apoptotic protein BCL2 and proliferation shown here with MIB/Ki-67 staining

Positive cells were quantified via Quapath software and normalized to CD4+ cell count and placebo