## SUPPLEMENTAL METHODS

### ELISA cytokine quantification

CCL22 and VEGF-C were determined using specific Raybiotech (Norcross, GA, USA) ELISA Kits and VEGF-A was evaluated by Mini TMB ELISA Development from Peprotech (London, UK). Cytokines levels were assessed in duplicates in supernatants harvested from FL primary cells ($2x10^6$ cells/mL) co-cultures. A standard curve was generated for each cytokine, and mean absorbance for each set duplicate were interpolated and transformed into concentrations. The optical density at 450nM was analyzed in a spectrophotometer (Synergy Bio-tek Instrument, Winooski, VT, USA)

### T cell migration assays

PBMCs obtained either from fresh tonsils or peripheral blood were enriched in T cells by depletion of B cells and monocytes with CD19 and CD14 magnetic beads, respectively (Miltenyi). Migration of the different T cell subpopulations was evaluated in 24-well chemotaxis chambers containing 5 µM pore size inserts (Corning, Life Science, Tewksbury, MA, USA). In brief, $2x10^5$ cells/transwell were allowed to migrate for 3h towards FL/FDC co-culture supernatants treated w/wo Idelalisib and the T regulatory cells ($T_{reg}$; $CD4^+/CD25^+$/FOXP3+) that migrated to the lower chamber were enumerated by flow cytometer. In the assays performed with PBMC from tonsils we distinguished two populations: T follicular helper (TFH CD4+/CXCR5+/CD25-) and T follicular regulatory ($T_{fr}$; CD4+, CXCR5+, FOXP3+) cells. Cell viability was assessed using Live/Dead Fixable Aqua Dead Cell Stain kit (Thermo Fisher Scientific). Net migration was determined for each T cell population by calculating the ratio between the number of cells migrated and the cells loaded.

### HUVEC tube formation assay

Human umbilical vein endothelial cells (HUVEC) were kindly provided by Dr Maria C. Cid and were cultured as previously described.[1] Supernatants from FL primary cells ($2x10^6$ cells/mL) were harvested after 48 hours of incubation with/without (w/wo) idelalisib in co-culture with FDC or in monoculture. 24-well plates were coated with Matrigel (BD Biosciences, San Jose, CA, USA) and allowed to polymerize for 45 minutes at 37ºC. Afterwards, the supernatants of interest were mixed (1:1) with HUVEC cells ($4x10^4$ cells) in HUVEC medium (RPMI 1640 medium that contains 25% of Bovine Calf Serum (HyClone, Thermo Fisher Scientific, Waltham, MA, USA), 150 µg/ml medium endothelial cell growth supplement

(ECGS) (BD Bioscience), and 4U/ml medium of sodium heparin salt (AppliChem, Darmstadt, Germany) and incubated for 24 hours. Pictures were taken at x40 magnification in a DMIL LED Leica microscope coupled to a DFC295 camera and analyzed with Suite v 3.7 software (Leica, Wetzlar, Germany). Then, the number of nodes and junctions was quantified in 5 randomly chosen fields using the Image J software (angiogenesis analyzer plugin).

**Adhesion assay to HUVEC cells**

HUVEC cells were plated in 96 wells plate at final concentration of $1x10^5$cells/well. in HUVEC medium and incubated overnight (ON) with TNF-α (10ng/ml) (R&D Systems, Minneapolis, MN, USA). Otherwise, FL co-cultured with FDC w/wo idelalisib were recovered after 48 hours of incubation and were counted and labeled with 1 μM Calcein, AM (Thermo Fisher Scientific) for 30 minutes at 37ºC. After washing twice with PBS, $1x10^5$ cells/well were seeded in a plate containing activated HUVEC cells for 3 hours with RPMI 1640 medium at 37ºC. Then, the plate was washed extensively with RPMI 1640 to remove non-adhered cells. Adhered cells were lysed with 1% Triton X-100, supernatant was transferred into black plates (Thermo Fisher Scientific) and fluorescence was measured in a spectrophotometer (Synergy Bio-Tek Instrument) (excitation filter: 485 ± 20 nm; band-pass filter: 530 ± 20 nm). Data were expressed as relative fluorescent units (RFU) after subtraction of non-specific adhesion (empty well).

**Transendothelial migration**

HUVEC cells ($2x10^5$ cells/well) were seeded on gelatin 0.1% coated transwells (Corning, Life Science, Tewksbury, MA, USA.) and incubated ON with TNF-α (10ng/ml). The next day, $5x10^5$ FL cells coming from 48 hours of FDC co-culture w/wo idelalisib were seeded into transwells with the endothelial monolayer, in RPMI medium with 10% FBS, and were allow to migrate for 6 hours in a gradient of FBS (RPMI medium + 20% FBS). $CD20^+$ cells crossing HUVEC barrier were counted by flow cytometer (Attune Acoustic Focusing Cytometer, Thermo Fisher Scientific).

**Flow cytometry**

Cells were seeded in co-culture or monoculture w/wo idelalisib and venetoclax (Selleck Chemicals, Houston TX, USA) as single agents or in combination for 72 h. Drugs cytotoxicity was evaluated by staining FL cells with CD19-PE (BD Bioscience), including Annexin V-FITC (Thermo Fisher Scientific) and 7-Aminoactinomycin D (7AAD) (Sigma-Aldrich Darmstadt,

Germany). Finally, cells were acquired and analyzed by flow cytometry. FL viable cells were identified as $CD19^+$, Annexin V-$FITC^-$ and $7AAD^-$. CD80 (Thermo Fisher Scientific), ICAM1, CD80 and SLAMF1 (BD Biosciences) surface expression was analyzed by flow cytometry.

Phosphoflow was performed to determine pS6 ribosomal protein. In brief, FL cells stimulated with anti-IgM (10ug/ml) for 30 minutes were recovered in ice, fixed and permeabilized using Phosflow Perm buffer III (BD Biosciences). Anti-human cytoplasmic CD20-FITC (BioLegend) was used to select B cell population and Phospho-S6 ribosomal protein (Ser235/236) antibody (eBiosciences) to measure S6 phosphorylation.

**Simple Western Methods (Peggy Sue)**

FL cells were harvested in lysis buffer (Cell Signaling Technology) containing: Protease Inhibitor Cocktail (Roche Diagnostics Corp), and phosphatase inhibitor sets 1 and 2 (EMD Millipore). Following 30 minutes on ice, cell lysates were cleared by centrifugation at 12,500 RPM for 10 minutes at 4°C. Lysates were analyzed by Simple Western using Peggy Sue™ or Sally Sue™ (ProteinSimple, San Jose, CA) according to manufacturer's standard protocol. Data was processed using Compass software (ProteinSimple). The following antibodies were purchased from Cell Signaling Technology: p-AKT (S473), pBAD (S112), pBAD (S136), BAD and β-actin.

**Gene expression profiling (GEP) and data analysis**

FL cells were purified using CD20 magnetic beads (Miltenyi, Bergisch Gladbach, Germany). Then, total RNA was obtained by using the TRIzol reagent (Thermo Fisher Scientific) followed by a cleaning step with the RNAeasy kit (Thermo Fisher Scientific). RNA integrity was examined with the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara CA, USA). Only RNAs with sufficient quality (RIN values between 7,2 and 9) were then retrotranscribed to cDNA and hybridized on HGU219 microarray. All samples were simultaneously run in a GeneTITAN platform (Thermo Fisher Scientific). Data was normalized to RMA using Expression Console software (Thermo Fisher Scientific). The microarray data has been deposited in the NCBI´s Gene Expression Omnibus (GEO) and it is accessible through the accession number GSE130918.

For the identification of differentially expressed genes, Limma was applied using a paired analysis with False Discovery Rate (FDR) ≤ 0.05. Gene Set enrichment Analysis (GSEA) v2.0 (Broad Institute, Cambridge, MA, USA) was used to interrogate C2, C3, GO and Hallmark 0.5 gene sets from the Molecular Signature Database v2.5, and experimentally derived custom

gene sets. A two-classes analysis with 1000 permutations of gene sets and a weighted metric was used. Benjamini-Hochberg correction for multiple testing was applied and only gene sets with FDR ≤ 0.05 and a normalized enrichment score (NES) of ≥1.5 were considered significant. The list of enriched gene-sets is detailed in Table S2. The leading-edge genes were displayed using Morpheus (Broad Institute).

**Multiplex Fluidigm**

RNA was extracted using RNeasy kit of Qiagen. Then, cDNA was prepared with Fluidigm Reverse Transcription Master Mix, and a preamplification reaction was performed with specific TaqMan Assays (PreAmp Master Mix, Fluidigm). High-throughput quantitative PCR was performed on the 48.48 IFC Dynamic Chip and Biomark system (Fluidigm). Cycling threshold ($C_T$) values were calculated with BioMark system software for each assay. *GUSB, ACTB* and *B2M* houskeeping genes were used for normalization

**Targeted Next Gene Sequencing (NGS)**

We performed NGS of 10 genes (*TNFRSF14, CREBBP, TP53, MEF2B, RRAGC, EP300, KMT2D, EPHA7, TNFAIP3 and EZH*2), targeting all exons and their flanking regions. Libraries were generated using HaloPlex HS target enrichment system (Agilent technologies, Santa Clara, CA) following the manufacturer's protocol with an input of 60 ng of genomic DNA. Libraries were sequenced in a MiSeq instrument (Illumina, San Diego, CA) in a paired-end run of 150 bp. The variant calling was performed using an updated version of our in-house pipeline.[2] Briefly, quality control and trimming of the raw sequencing reads was done using the FastQC (v0.11.5) and Surecall Trimmer (v4.0.1) algorithms, respectively. Trimmed reads were aligned to the GRCh37/hg19 human reference genome using the Burrows-Wheeler Aligner-MEM algorithm (v0.7.17)[3]. Base quality score recalibration and indel realignment steps were subsequently performed according to the GATK Best Practices (GATK, v3.8)[4,5]. The mean coverage obtained was 2585x, with 96% of the target region covered at >100x (Samtools (v1.6)[6,] custom scripts). The variant calling was done in parallel using VarScan2 (v2.4.3)[7] Mutect2 (GATK, v4), VarDict (v1.4)[8], and deepSNV-shearwater (v1.24.0)[9]. The post processin was used to filter the mutations detected by VarScan2. All variants detected by any of the variant callers were combined and annotated using SnpEff and SnpSift (v4.3)[10,11]. Finally, a custom script was applied to filter out recurrent artifacts, low quality, low variant allele frequency (VAF <2%), intronic and synonymous variants. Polymorphisms described in the Single Nucleotide Polymorphism Database (dbSNP149) with a European population

frequency higher than 1% (1000 Genomes Project database) or in our in-house CLL database[12] were also excluded. All programs were executed following the authors' recommendations.

**CD40L stimulation with YK6-CD40L cells**

YK6-TPC cells, an immortalized cell line derived from FDC, or YK6 engineered to express CD40L, were generated and kindly provided by Dr. Daniel J. Hodson. YK6 cells were plated in a 12-well plate at $2x10^5$ cells/well and treated the next day with Mitomycin C (10µg/ml for 1hour) (Sigma). FL cells were then labeled with CFSE (0.5µM) and co-cultured together with YK6 +/- CD40L cells in a ratio 1:1, during 5 days, in RPMI medium supplemented with 10ng/ml of human IL4 (Peprotech). At day 5, cells were analyzed by flow cytometry in the Attune Acoustic Focusing Cytometer (Thermo Fisher Scientific). Alive B cells (LIVE/DEAD $AQUA^-$ $CD19^+$) were examined for CFSE fluorescence. The percentage of cells with low expression of CFSE referred to expression at day 0 was used as a read-out of proliferation [13].

**Statistical analysis**

Unpaired and paired T-tests were used to assess differences between two groups using GraphPad Prism software 7.0. Standard deviation (SD) was calculated among experimental replicates in each case. The statistical significance convention used along the manuscript was as follows: * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

**Identification of sensitive FL samples by qPCR**

The microarray data showed that the expression pattern of different patients was modified in different degree by the Idelalisib treatment. In base of these results, we performed additional gene expression analyses to verify and refine a gene expression signature affected by Idelalisib. Specifically, we first used the microarray data to select 39 candidate genes whose expression was affected by the treatment. Then, we obtained cells from 25 new FL patients and treated these cells with three different conditions: untreated, FL-FDC co-culture, and FL-FDC co-culture with Idelalisib. The expression of the selected genes in the treated cells was then measured by qPCR. Finally, to identify which of the 25 patients were affected by the Idelalisib treatment we performed, for each one, a paired t-test between the log2 expression of the co-culture without and with Idelalisib treatment. The obtained *p*-values were then adjusted with the Benjamini-Hochberg method (*q*-values) and the 9 patients that

showed a significant downregulation (*q*-value<0.05) were classified as sensitive, whereas the other (with no change or up-regulation) were categorized as resistant.

**Reduction of the idelalisib gene signature**

The initial idelalisib gene signature selected from the microarray data included 39 genes. To define a minimal gene signature able to detect sensitive patients, we used the qPCR data of the 25-patient cohort. First, we ordered the genes according to the variance of the difference in log2 expression between the co-culture without and with idelalisib treatment. We then performed a paired t-test between the two conditions using from 3 to 39 genes. When 18 genes were used, only 1 of the 25 patients had a different test result when compared to using all 39 genes, corresponding to a patient whose expression was minimally modified.

**SUPPLEMENTAL REFERENCES**


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## SUPPLEMENTAL FIGURES

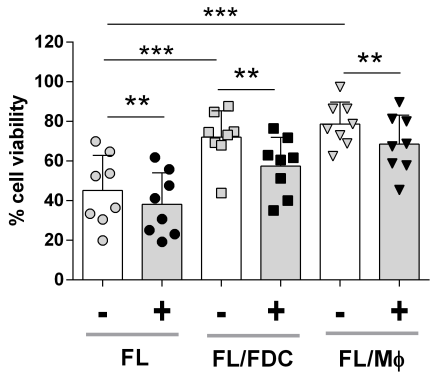


**Figure S1. Idelalisib induces moderate cytotoxicity in FL monocultures and FL-FDC co-cultures.** Cell viability (Annexin V-/7AAD-) was assessed in FL cells from monocultures and FL-FDC co-cultures w/wo idelalisib (500nM, 72 hrs)

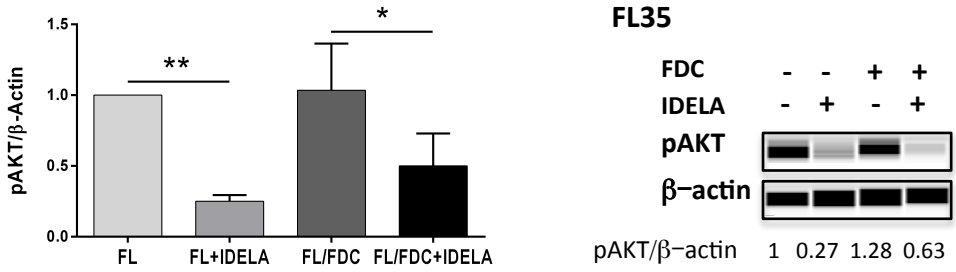


**Figure S2. Idelalisib blocks PI3K/AKT pathway in FL monocultures and FL-FDC co-cultures.** FL patient samples (n=3) were treated for 3h with idelalisib (IDELA, 500nM) and phosphorylation of AKT at Ser 473 was assessed by Peggy Sue simple western and quantified by densitometry. Images from a representative case are shown (FL35).

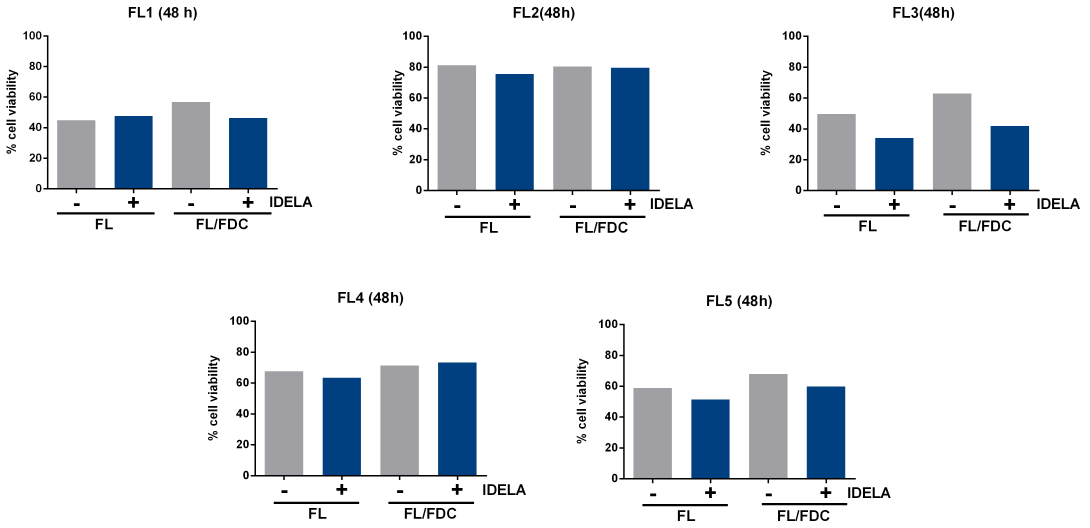


**Figure S3. Cell viability of FL samples subjected to microarrays analysis.** Viability was assessed by flow cytometry in FL primary cells from monocultures or FL-FDC co-cultures, treated w/wo idelalisib (500nM, 48h). Cells were labeled with CD20, Annexin V and 7AAD. The percentages of $CD20^{+}$/Annexin $V^{-}$/ $7AAD^{-}$ cells are plotted.

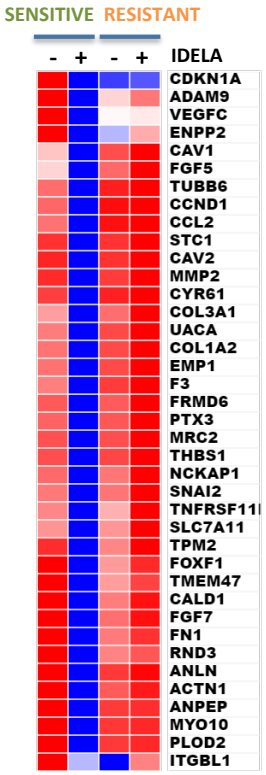


**Figure S4. A 39-gene signature segregates idelalisib sensitive and resistant FL cases.** FL primary cells were isolated from FL-FDC (n=25) co-cultures treated w/wo idelalisib (500nM, 48h), RNA was extracted and subjected to a multiplex RT-PCR by Fluidigm to characterize 39 selected genes as in figure 1B. Heatmap displays the mean expression of these genes in either sensitive (n=9) or resistant (n=16) patients classified as in figure 1B. The -ΔCt obtained by Fluidigm were first normalized using 3 houskeeping genes, and they were then transformed to fold change referred to the corresponding baseline control without co-culture.

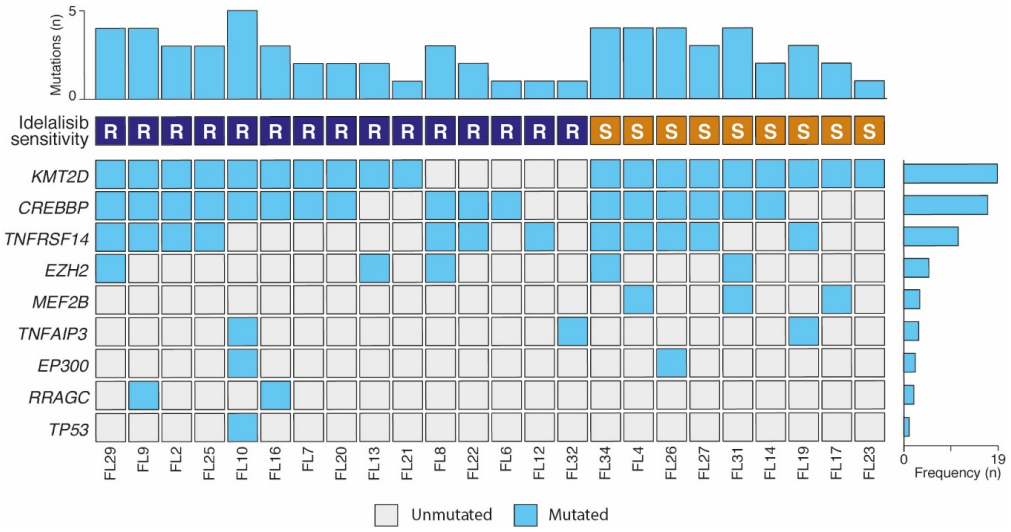


**Figure S5. Somatic mutations and idelalisib sensitivity in FL-FDC co-cultures.** DNAs from 24 FL patients were analyzed by targeted next-generation sequencing (Haloplex) for the presence of somatic mutations in 10 recurrently mutated genes in FL. No mutations were identified in *EPHA7*. Cases (in columns) carrying a mutation in each specific gene are depicted in blue. R (resistant to idelalisib); S (sensitive to idelalisib)

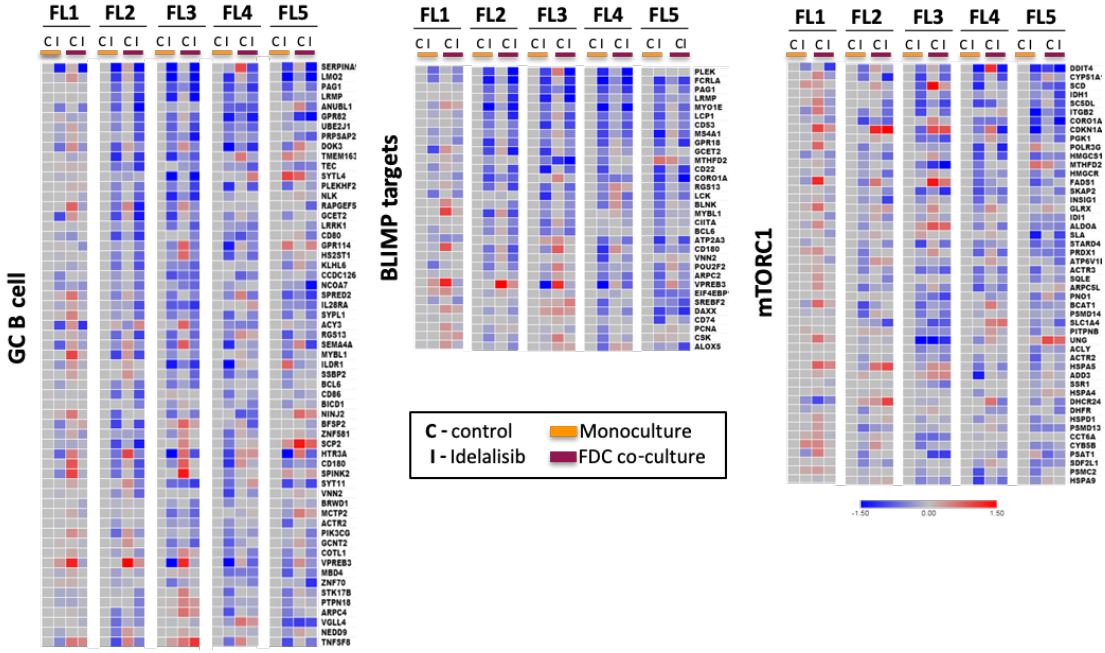


**Figure S6. Idelalisib inhibits gene sets related to GC program and mTORC1.** FL cells (n=5) were cultured for 48h with idelalisib (500nM), and B cells were purified and subjected to GEP. Gene sets regulated by idelalisib in the presence and absence of FDC co-culture were identified by Gene Set Enrichment Analysis (GSEA) using custom genes set (http://lymphochip.nih.gov/signaturedb/index.html). Heatmaps of the corresponding leading edge of selected gene sets are shown including the relative gene expression of FL cells cultured w/wo idelalisib referred to the untreated control.

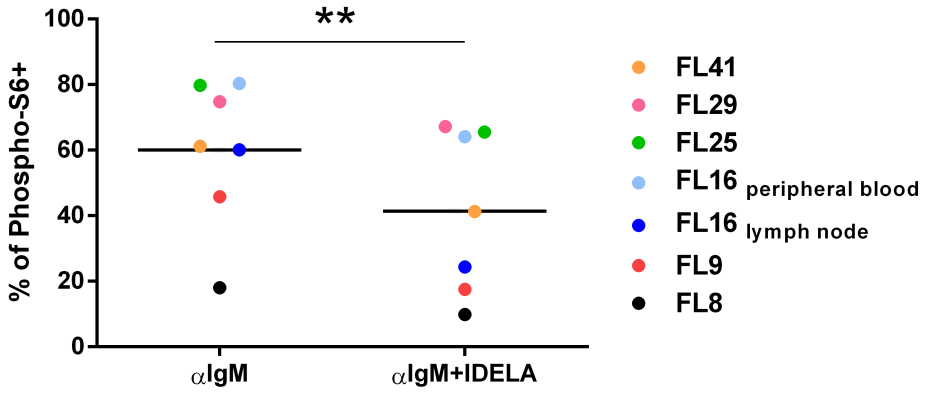


**Figure S7. Idelalisib inhibits the phosphorylation of the downstream mTOR target pS6.** FL cells (n=7) were treated w/wo Idelalisib (500nM, 1h) and stimulated with anti-IgM (10μg/ml) for additional 30 minutes. Afterwards, cells were collected in ice, fixed with 4% paraformaldehyde and permeabilized with Perm Buffer III (BD Phosflow). FL cells were labeled with anti-CD20-FITC (Biolegend) and anti-PS6-PE (S235, S236 (eBioscience)) and analyzed in a Fortessa 4L flow cytometer.

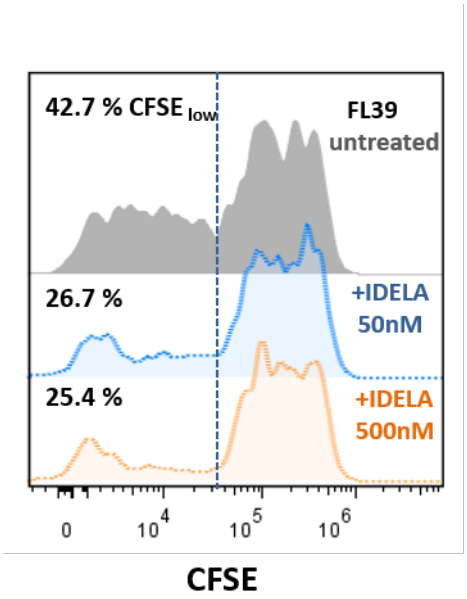


**Figure S8. Idelalisib reduces CD40L-induced FL cells proliferation.** FL cells (shown FL39) were labeled with CFSE (0.5μM) and co-cultured for 5 days w/wo 500nM idelalisib on pre-established layers of FDCs engineered for CD40L expression. The percentage of viable CD19+ cells with low CFSE fluorescence was used as a read-out of proliferation.

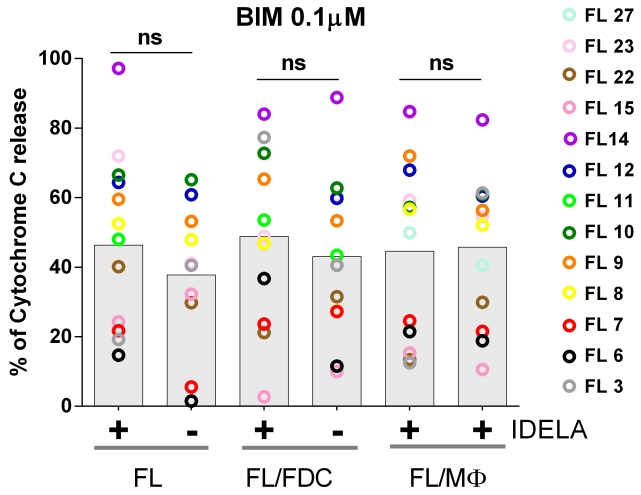


**Figure S9. Idelalisib does not modify BIM induced priming.** FL cells (n=13) either in monoculture or in co-culture with FDC or Mϕ, were treated with idelalisib 500nM for 24 hour. Then cells were permeabilized and incubated for 1 hr with 0.1µM BIM peptide fixed/stained for intracellular Cytochrome C and evaluated by flow cytometry as a read-out of apoptosis priming

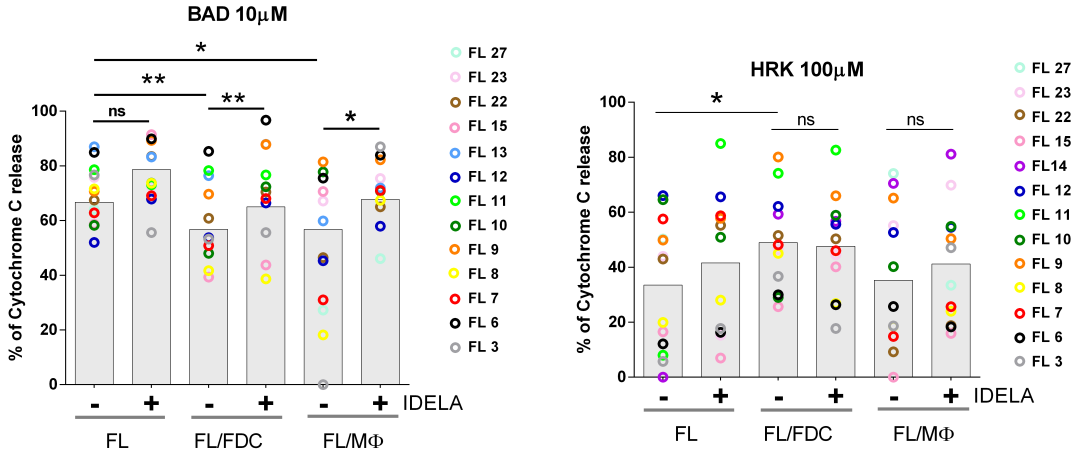


**Figure S10. Effect of Idelalisib on BAD and HRK mediated cell priming.** FL cells (n=13) either in monoculture or in co-culture with FDC or Mϕ, were treated with idelalisib 500nM for 24 hour. Then cells were permeabilized and incubated for 1 hr with 10µM BAD or 100µM HRK, fixed/stained for intracellular Cytochrome C and finally evaluated by flow cytometry as a read-out of apoptosis priming.

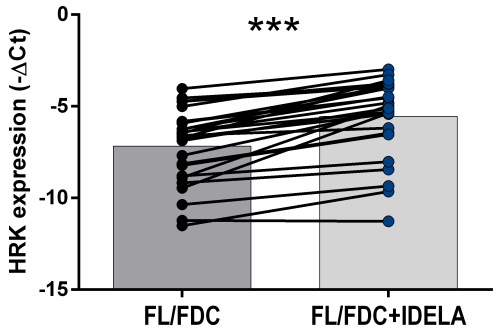


**Figure S11. Idelalisib upregulates the expression of *HRK* in FL-FDC co-cultures.** FL-FDC co-cultures (n=26) were treated w/wo idelalisib (500nM, 48h), RNA extracted and *HRK* gene expression was measured by real-time PCR in primary FL cells. *GUSB, ACTB* and *B2M* houskeeping genes were used for normalization.

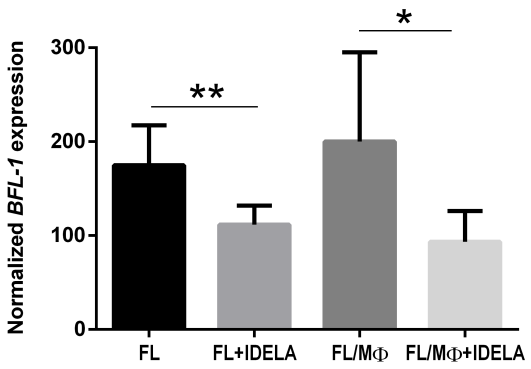


**Figure S12. Idelalisib downregulates the expression of *BFL-1* in FL-Mϕ co-cultures.** FL primary cells were isolated from FL-Mϕ (n=5) co-cultures treated w/wo idelalisib (500nM, 48h), RNA was extracted and subjected to GEP in HG-U219 microarrays as indicated in materials and methods.

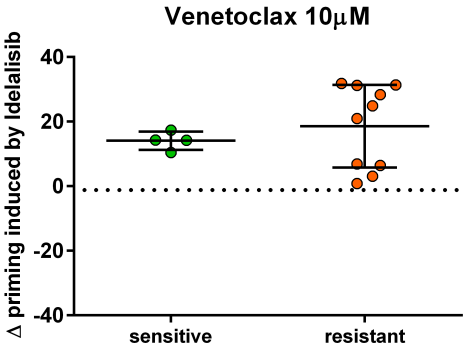


**Figure S13. Idelalisib improves Venetoclax response both in sensitive (n=4) and resistant (n=9) patients.** FL-FDC co-cultures were treated w/wo idelalsib (500nM, 24h), then cells were permeabilized and incubated for 1 hr with 10μM venetoclax, fixed/stained for intracellular Cytochrome C and evaluated by flow cytometry as a read-out of apoptosis priming. The increase in priming (Δ priming = (% cyt C release FL/FDC+IDELA) – ((% cyt C release FL/FDC))

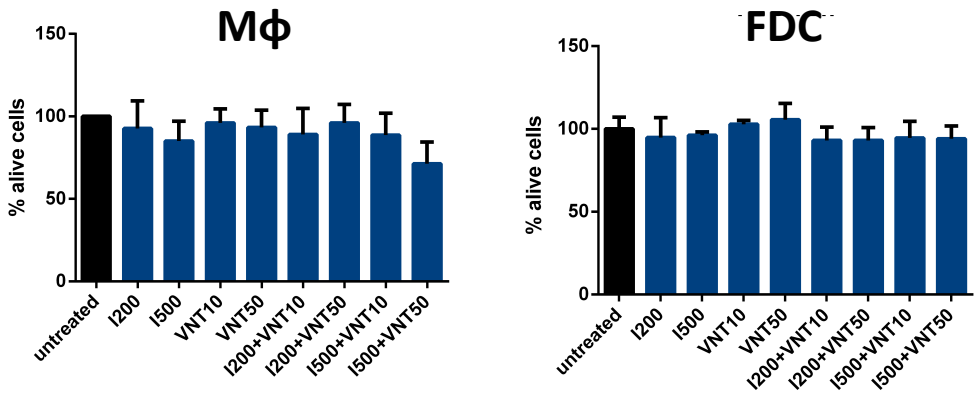


**Figure S14. Idelalisib and venetoclax do no affect Mϕ or FDC viability.** Macrophages (derived from peripheral blood and differentiated with M-CSF (100ng/ml) for 7 days) and HK cells (FDC from tonsil) were plated in triplicates at the same density used for co-cultures and treated with Idelalisib (I200nM, I500nM), Venetoclax (VEN10nM, VEN50nM) or their respective combinations. After 72h of treatment, Tetrazolium salt (MTT) was added to cultures and the subsequent formation of formazan crystals for metabolically active cells (meaning alive cells) was assessed by spectrophotometry. Results were plotted as percentage referred to the untreated control.

## SUPPLEMENTAL TABLES

### Table S1. Patient clinical data

| Study label | Sex/Age | Histologic Grade[1] | Ann Arbor stage | FLIPI[2] | Disease status[3] | % T cells[4] | Genomic alterations[5] | First line therapy[6] | DFS[7] (months) | Number of relapses |
|---|---|---|---|---|---|---|---|---|---|---|
| **FL1** | M/71 | 2 | IV | I | D | 21.2 | NA | R | 86 | 0 |
| **FL2** | M/77 | NA | NA | NA | NA | 30.8 | 1,2,3 | NA | NA | NA |
| **FL3** | F/83 | 2 | III | I | D | 18 | 1,2,3 | R | 60 | 0 |
| **FL4** | M/65 | 2 | I | L | D | 22 | 1,2,3,5 | R | 67 | 0 |
| **FL5** | F/73 | 2 | IV | H | D | 12.8 | NA | R-L | 34 | 1 |
| **FL6** | M/80 | 2 | IV | I | D | 9.4 | 1 | R-B | 25 | 0 |
| **FL7** | M/73 | 2 | IV | H | D | 13.5 | 1,3 | R-B | 1 | 2 |
| **FL8** | M/75 | 2 | I | L | D | 14.4 | 1,2,3,6 | CP | 26 | 0 |
| **FL9** | M/69 | 2 | IV | H | D | 14.6 | 1,2,3,9 | R-CHOP | 25 | 0 |
| **FL10** | F/55 | 3a | IV | H | D | NA | 1,3,4,7,8 | R-CHOP | 1 | 2 |
| **FL11** | M/50 | 2 | II | L | D | 30.7 | 1,3,5,8 | R-CHOP | 23 | 1 |
| **FL12** | F/73 | 3a | III | I | D | 18.3 | 2 | R-CHOP | 31 | 1 |
| **FL13** | F/60 | 2 | IV | I | D | 37.8 | 3,6 | R-FCM | 168 | 0 |
| **FL14** | F/70 | 2 | I | L | R | 23.5 | 1,3 | RTx | 32 | 1 |
| **FL15** | F/54 | 2 | IV | L | D | 28.1 | 1,4 | R-CHOP | 11 | 0 |
| **FL16** | M/54 | 2 | IV | H | D | 13.7 | 1,3,7,9 | FCM | 47 | 1 |
| **FL17** | F/69 | 2 | IV | I | D | 35.7 | 3,5 | R-CHOP | 60 | 0 |
| **FL18** | M/58 | 3a | IV | L | R | 59.2 | 1 | Chl | 106 | 1 |
| **FL19** | M760 | 2 | IV | L | D | 30.2 | 2,3,7 | R | 13 | 1 |
| **FL20** | F/66 | 1 | IV | I | R | 40.05 | 1,3 | CHOP | 38 | 2 |
| **FL21** | F/50 | 2 | IV | L | D | 38.4 | 3 | FCM | 190 | 0 |
| **FL22** | M/70 | 2/3 | IV | I | R | 13.9 | 1,2,3 | RTx | 33 | 1 |
| **FL23** | M/50 | 1 | I | L | R | 33.3 | 3 | CHOP | 64 | 1 |
| **FL24** | F/80 | 2 | III | H | D | 29.8 | 1,2,3,4,6 | R-CHOP | 152 | 0 |
| **FL25** | F/32 | 2 | IV | I | D | 23.17 | 1,2,3 | R-CHOP | 16 | 0 |
| **FL26** | M/53 | 3a | IV | I | R | 34.4 | 2,3,4 | R-CHOP | 37 | 2 |
| **FL27** | F/59 | 2 | IV | I | R | 30.43 | 1,2,3 | R-CHOP | 57 | 2 |
| **FL28** | F/59 | 2/3 | IV | H | D | 14.7 | NA | R-CHOP | 162 | 0 |
| **FL29** | M/56 | 2 | IV | I | R | 11.3 | 1,2,3,6 | R-CHOP | 4 | 2 |
| **FL30** | F/66 | 1 | IV | H | D | 29.8 | NA | R-CHOP | 13 | 0 |
| **FL31** | M/60 | 1/2 | III | I | D | 36.3 | 1,3,5,6,7 | R-CHOP | 15 | 0 |
| **FL32** | F/77 | 3 | IV | H | D | 15.8 | 7 | R-CHOP | 22 | 0 |
| **FL33** | M/57 | 1 | IV | I | R | 34.3 | NA | R-CHOP | 16 | 1 |
| **FL34** | F/ | 2 | NA | NA | NA | 47.7 | 1,2,3,6 | NA | NA | NA |
| **FL35** | M/59 | 2 | IV | I | D | 13.13 | NA | none | 35 | 0 |
| **FL36** | F/73 | 2 | IV | H | D | 12.7 | NA | R-L | 34 | 1 |
| **FL37** | F/83 | 1 | III | I | D | 13.94 | NA | R-COP | 52 | 0 |
| **FL38** | F/35 | 2 | IV | H | D | 36.3 | NA | R-CHOP | 42 | 0 |
| **FL39** | F/47 | 2 | IV | I | D | 4.5 | NA | R-Lena | 93 | 0 |
| **FL40** | M/52 | 2 | IV | H | D | 19.8 | NA | R-Lena | 20 | 1 |
| **FL41** | M/23 | 1 | I | L | D | 45 | NA | CHOP | NA | NA |
| **FL42** | F/53 | 1 | IV | L | D | NA | NA | FCM | 2 | 2 |

[1]Grade was evaluated by two different pathologists; [2]FLIPI: Follicular Lymphoma International Prognostic Index: >3 high-risk (H), 2 intermediate risk (I), and 0-1 low-risk (L); [3]Samples were obtained at D: diagnosis, or R: relapse; [4]Percentage of T cells in the FL sample assessed by flow cytometry [5]Somatic mutations identified by NGS: (1) CREBBP, (2) TNFRSF14, (3) KMT2D, (4) EP300, (5) MEF2B, (6) EZH2, (7) TNFAIP3, (8) TP53 and (9) RRAGC. [6] First line therapy consisted of R: Rituximab; L: Lenalidomide; B: Bendamustine; C: Cyclophosphamide; P:Prednisone; CHOP: Chemotherapy combination of Cyclophosphamide, Hydroxydaunorubicin, Oncovin and Prednisone; FCM: Fludarabine, Cyclophosphamide and Mitoxantrone; RTx: Radiotherapy; or Chl: Chlorambucil. [7]DFS (Disease free survival) is referred to time passed from initial therapy to first relapse. NA: not available

**Table S2. Recurrent somatic mutations in FL**

| Study label | *CREBBP* | *TNFRSF14* | *KMT2D* | *EP300* | *MEF2B* | *EZH2* | *TNFAIP3* | *TP53* | *RRAGC* |
|---|---|---|---|---|---|---|---|---|---|
| **FL2** | p.Phe1484del | p.Cys42Tyr | p.Gln4609*<br>p.Pro868fs | | | | | | |
| **FL3** | p.Asp1037fs | p.Arg62Gly | p.Trp339* | | | | | | |
| **FL4** | p.Gln1209fs | p.Cys54Ser | p.Cys1474Tyr | | p.Thr70Arg | | | | |
| **FL6** | p.Pro1948Leu<br>p.Ser1436Arg<br>p.Cys1219* | | | | | | | | |
| **FL7** | p.Tyr1503Asn<br>p.Gln338fs | | p.Gln2819* | | | | | | |
| **FL8** | p.Tyr1450Cys | p.His134fs | | | | p.Tyr646Asn | | | |
| **FL9** | p.Arg1446Leu | p.Trp201* | p.Glu1588*<br>p.Pro1460fs | | | | | | p.Trp115Arg |
| **FL10** | p.Arg1498* | | p.Ile1208fs | p.Trp1436Arg | | | p.His195fs | p.Ser240Arg | |
| **FL11** | p.Tyr1503Ser | | p.Val5155fs<br>p.His3588fs<br>p.Glu902fs | | p.Pro340Leu | | | p.Arg248Trp | |
| **FL12** | | p.Pro55Ser | | | | | | | |
| **FL13** | | | p.Tyr2199fs<br>p.Cys276fs, | | | p.Tyr646Phe | | | |
| **FL14** | p.Arg1446Cys, | | p.Tyr1495fs<br>p.Ile982fs<br>p.Tyr343Cys | | | | | | |
| **FL15** | p.Gln442* | | | p.Glu1246* | | | | | |
| **FL16** | p.Tyr1450Ser | | p.Gln3737*<br>p.Arg3452* | | | | | | p.Pro87Leu |
| **FL17** | | | p.Ser2488fs | | p.Thr70Lys | | | | |
| **FL18** | p.Ser1680del | | | | | | | | |
| **FL19** | | p.Gln158* | p.Thr3384fs | | | | p.Gln370* | | |
| **FL20** | p.Gln1491Lys | | p.Ser4456fs,<br>p.Gln4412* | | | | | | |
| **FL21** | | | p.Gln4347fs<br>p.Val2551fs | | | | | | |
| **FL22** | p.Leu1454Arg | p.Cys127Tyr | | | | | | | |
| **FL23** | | | p.Pro2931fs<br>p.Arg1388Leu | | | | | | |
| **FL24** | p.His1487Tyr | p.Ser112Pro | p.Arg5154Gln<br>p.Cys5117*<br>p.Arg348Cys | | | p.Tyr646Phe | | | |
| **FL25** | p.Ser1680del | p.Cys93* | p.Gln2445*<br>p.Arg1709fs | | | | | | |
| **FL26** | | p.Lys17fs | p.Val4642fs<br>p.Leu3245* | p.Gln1720His | | | | | |
| **FL27** | p.Arg1446His | p.Gly8fs | p.Arg4198*<br>p.Gln3265fs | | | | | | |
| **FL29** | p.Ser1680del<br>p.Tyr1503Asp | p.Cys138Tyr | p.Arg3321*<br>p.Gln425* | | | p.Ala692Val | | | |
| **FL31** | p.Tyr1503His | | p.His5114Tyr | | p.Arg24Gln | p.Ala682Gly | | | |
| **FL32** | | | | | | | p.Gln350fs<br>p.His351Tyr | | |

**Table S3. Complete list of gene sets regulated by idelalisib treatment in FL-FDC co-cultures in sensitive patients**

| Custom gene sets | | | | |
|---|---|---|---|---|
| **Biological process** | **Gene set name** | **Size** | **NES** | **FDR q-val** |
| Human angiogenesis | | | | |
| | HUMAN ANGIOGENESIS MERGED | 120 | 2.63 | 0.00000 |
| IRF4 pathway | | | | |
| | MYELOMA IRF4 | 243 | 1.98 | 0.00455 |
| Cell cycle regulation | | | | |
| | CELL CYCLE PATHWAY | 31 | 1.98 | 0.00390 |
| | G1 PATHWAY | 24 | 1.77 | 0.02410 |
| Integrin pathway | | | | |
| | INTEGRIN PATHWAY | 35 | 1.92 | 0.00676 |
| Serum response | | | | |
| | SERUM_RESPONSE_FB_UP | 158 | 1.85 | 0.01314 |
| **Canonical Pathways (C2CP)** | | | | |
| **Biological process** | **Gene set name** | **Size** | **NES** | **FDR q-val** |
| Focal adhesion-Integrins | | | | |
| | KEGG_FOCAL_ADHESION | 179 | 2.80 | 0.00000 |
| | PID_INTEGRIN1_PATHWAY | 61 | 2.71 | 0.00000 |
| | PID_INTEGRIN3_PATHWAY | 41 | 2.55 | 0.00000 |
| | PID_AVB3_INTEGRIN_PATHWAY | 70 | 2.46 | 0.00000 |
| | REACTOME_INTEGRIN_CELL_SURFACE_INTERACTIONS | 71 | 2.39 | 0.00000 |
| | ST_INTEGRIN_SIGNALING_PATHWAY | 78 | 2.14 | 0.00016 |
| | PID_INTEGRIN_A9B1_PATHWAY | 24 | 2.10 | 0.00046 |
| | PID_FAK_PATHWAY | 55 | 2.07 | 0.00113 |
| | PID_INTEGRIN_A4B1_PATHWAY | 30 | 2.00 | 0.00299 |
| | PID_A6B1_A6B4_INTEGRIN_PATHWAY | 42 | 2.00 | 0.00306 |
| | BIOCARTA_INTEGRIN_PATHWAY | 36 | 1.92 | 0.00925 |
| | PID_INTEGRIN2_PATHWAY | 27 | 1.77 | 0.03477 |
| Extracellular matrix formation | | | | |
| | NABA_CORE_MATRISOME | 243 | 2.76 | 0.00000 |
| | NABA_ECM_GLYCOPROTEINS | 173 | 2.67 | 0.00000 |
| | KEGG_ECM_RECEPTOR_INTERACTION | 72 | 2.61 | 0.00000 |
| | REACTOME_EXTRACELLULAR_MATRIX_ORGANIZATION | 80 | 2.51 | 0.00000 |
| | REACTOME_COLLAGEN_FORMATION | 54 | 2.40 | 0.00000 |
| | NABA_COLLAGENS | 37 | 2.26 | 0.00000 |
| | NABA_ECM_REGULATORS | 214 | 2.14 | 0.00015 |
| Angiogenesis (VEGF/PDGF pathways) | | | | |
| | REACTOME_SIGNALING_BY_PDGF | 113 | 2.29 | 0.00000 |
| | PID_LYMPH_ANGIOGENESIS_PATHWAY | 25 | 2.29 | 0.00000 |
| | PID_VEGFR1_PATHWAY | 24 | 2.03 | 0.00183 |
| | PID_VEGFR1_2_PATHWAY | 66 | 1.86 | 0.01682 |
| | PID_PDGFRA_PATHWAY | 22 | 1.80 | 0.02621 |
| Cell adherent junctions –ECadherin | | | | |
| | PID_ECADHERIN_STABILIZATION_PATHWAY | 40 | 2.08 | 0.00096 |
| | REACTOME_CELL_JUNCTION_ORGANIZATION | 74 | 2.07 | 0.00113 |
| | KEGG_ADHERENS_JUNCTION | 70 | 2.04 | 0.00187 |
| | PID_ECADHERIN_NASCENT_AJ_PATHWAY | 38 | 2.04 | 0.00188 |
| | PID_NCADHERIN_PATHWAY | 32 | 1.83 | 0.02121 |
| Cell cycle G1-M | | | | |
| | REACTOME_MITOTIC_PROMETAPHASE | 77 | 2.01 | 0.00272 |
| | SA_G1_AND_S_PHASES | 15 | 1.97 | 0.00461 |
| | BIOCARTA_CELLCYCLE_PATHWAY | 22 | 1.91 | 0.01036 |
| | BIOCARTA_G1_PATHWAY | 27 | 1.84 | 0.02107 |
| | REACTOME_MITOTIC_M_M_G1_PHASES | 150 | 1.82 | 0.02304 |
| | REACTOME_G1_PHASE | 32 | 1.80 | 0.02632 |
| | KEGG_CELL_CYCLE | 113 | 1.74 | 0.04301 |
| Transendothelial cell migration | | | | |
| | KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION | 107 | 1.90 | 0.01151 |
| | REACTOME_SEMA4D_INDUCED_CELL_MIGRATION_AND_GROWTH_CONE_COLLAPSE | 22 | 1.78 | 0.03143 |

| Motif gene sets (C3TFT) | | | | |
|---|---|---|---|---|
| **Biological process** | **Gene set name** | **Size** | **NES** | **FDR q-val** |
| Serum Response Factors (SRF) | | | | |
| | V$SRF_Q6 | 218 | 2.31 | 0.00000 |
| | V$SRF_C | 192 | 2.27 | 0.00000 |
| | CCAWWNAAGG_V$SRF_Q4 | 81 | 2.16 | 0.00000 |
| | V$SRF_Q5_01 | 199 | 2.16 | 0.00000 |
| | V$SRF_Q4 | 206 | 2.16 | 0.00000 |
| | V$SRF_01 | 43 | 2.11 | 0.00016 |
| Interferon Response Factors (SRF) | | | | |
| | STTTCRNTTT_V$IRF_Q6 | 162 | 1.85 | 0.00869 |
| | V$IRF_Q6 | 207 | 1.78 | 0.01853 |
| NFAT | | | | |
| | V$NFAT_Q6 | 221 | 1.73 | 0.02107 |
| NF-KB | | | | |
| | V$NFKB_Q6 | 230 | 1.64 | 0.04138 |

| Hallmarks (H) | | | | |
|---|---|---|---|---|
| **Biological process** | **Gene set name** | **Size** | **NES** | **FDR q-val** |
| Epithelial mesenchymal transition | | | | |
| | HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION | 189 | 3.26 | 0.00000 |
| Angiogenesis | | | | |
| | HALLMARK_ANGIOGENESIS | 35 | 2.26 | 0.00000 |
| mTOR | | | | |
| | HALLMARK_MTORC1_SIGNALING | 182 | 1.96 | 0.00016 |
| Interferon α and γ_responses | | | | |
| | HALLMARK_INTERFERON_GAMMA_RESPONSE | 178 | 1.60 | 0.01498 |
| | HALLMARK_INTERFERON_ALPHA_RESPONSE | 87 | 1.51 | 0.03042 |

| GO genesets (C5) | | | | |
|---|---|---|---|---|
| **Biological process** | **Gene set name** | **Size** | **NES** | **FDR q-val** |
| Extracellular matrix formation | | | | |
| | GO_EXTRACELLULAR_MATRIX_COMPONENT | 109 | 2.92 | 0.00000 |
| | GO_EXTRACELLULAR_STRUCTURE_ORGANIZATION | 271 | 2.90 | 0.00000 |
| | GO_EXTRACELLULAR_MATRIX | 381 | 2.79 | 0.00000 |
| | GO_PROTEINACEOUS_EXTRACELLULAR_MATRIX | 322 | 2.72 | 0.00000 |
| | GO_EXTRACELLULAR_MATRIX_BINDING | 48 | 2.53 | 0.00000 |
| | GO_EXTRACELLULAR_MATRIX_STRUCTURAL_CONSTITUENT | 65 | 2.46 | 0.00000 |
| | GO_EXTRACELLULAR_MATRIX_DISASSEMBLY | 68 | 2.36 | 0.00000 |
| | GO_REGULATION_OF_EXTRACELLULAR_MATRIX_ORGANIZATION | 26 | 2.00 | 0.00264 |
| Adhesion-Integrins | | | | |
| | GO_CELL_ADHESION_MOLECULE_BINDING | 175 | 2.57 | 0.00000 |
| | GO_REGULATION_OF_CELL_SUBSTRATE_ADHESION | 159 | 2.46 | 0.00000 |
| | GO_CELL_SUBSTRATE_ADHESION | 151 | 2.41 | 0.00000 |
| | GO_CELL_MATRIX_ADHESION | 107 | 2.29 | 0.00000 |
| | GO_POSITIVE_REGULATION_OF_CELL_SUBSTRATE_ADHESION | 90 | 2.23 | 0.00004 |
| | GO_SUBSTRATE_ADHESION_DEPENDENT_CELL_SPREADING | 38 | 2.17 | 0.00018 |
| | GO_REGULATION_OF_CELL_MATRIX_ADHESION | 82 | 2.10 | 0.00064 |
| | GO_FOCAL_ADHESION_ASSEMBLY | 24 | 2.05 | 0.00161 |
| | GO_NEGATIVE_REGULATION_OF_CELL_MATRIX_ADHESION | 29 | 2.04 | 0.00165 |
| Vasculature-angiogenesis-Endothelial cell growth | | | | |
| | GO_VASCULATURE_DEVELOPMENT | 443 | 2.49 | 0.00000 |
| | GO_ANGIOGENESIS | 276 | 2.41 | 0.00000 |
| | GO_SPROUTING_ANGIOGENESIS | 43 | 2.38 | 0.00000 |
| | GO_REGULATION_OF_VASCULATURE_DEVELOPMENT | 218 | 2.27 | 0.00000 |
| | GO_POSITIVE_REGULATION_OF_ENDOTHELIAL_CELL_PROLIFERATION | 65 | 2.22 | 0.00005 |
| | GO_POSITIVE_REGULATION_OF_VASCULATURE_DEVELOPMENT | 124 | 2.21 | 0.00009 |
| | GO_POSITIVE_REGULATION_OF_BLOOD_VESSEL_ENDOTHELIAL_CELL_MIGRATION | 23 | 2.15 | 0.00022 |
| | GO_VASCULAR_ENDOTHELIAL_GROWTH_FACTOR_RECEPTOR_SIGNALING_PATHWAY | 65 | 2.11 | 0.00057 |
| | GO_BLOOD_VESSEL_ENDOTHELIAL_CELL_MIGRATION | 22 | 2.09 | 0.00075 |
| | GO_REGULATION_OF_ENDOTHELIAL_CELL_CHEMOTAXIS | 15 | 2.05 | 0.00161 |
| | GO_REGULATION_OF_BLOOD_VESSEL_ENDOTHELIAL_CELL_MIGRATION | 47 | 2.03 | 0.00204 |
| | GO_ENDOTHELIAL_CELL_DIFFERENTIATION | 69 | 2.01 | 0.00258 |
| | GO_ENDOTHELIAL_CELL_DEVELOPMENT | 43 | 1.91 | 0.00723 |
| | GO_CELLULAR_RESPONSE_TO_VASCULAR_ENDOTHELIAL_GROWTH_FACTOR_STIMULUS | 29 | 1.90 | 0.00810 |
| | GO_REGULATION_OF_SPROUTING_ANGIOGENESIS | 27 | 1.70 | 0.04004 |
| Cell cycle G1-S and G2-M | | | | |
| | GO_POSITIVE_REGULATION_OF_CELL_CYCLE_PHASE_TRANSITION | 64 | 2.09 | 0.00078 |
| | GO_REGULATION_OF_CELL_CYCLE_G2_M_PHASE_TRANSITION | 55 | 2.03 | 0.00203 |
| | GO_REGULATION_OF_CELL_CYCLE_G1_S_PHASE_TRANSITION | 134 | 1.97 | 0.00379 |
| | GO_POSITIVE_REGULATION_OF_CELL_CYCLE_G2_M_PHASE_TRANSITION | 16 | 1.87 | 0.01143 |
| | GO_REGULATION_OF_CELL_CYCLE_PHASE_TRANSITION | 291 | 1.86 | 0.01228 |
| | GO_CELL_CYCLE_G2_M_PHASE_TRANSITION | 122 | 1.72 | 0.03510 |

**Table S4. Common gene sets regulated by idelalisib in monoculture and in FL-FDC co-cultures**

| GSEA analysis | CONTROL vs IDELA | | FDC vs FDC IDELA | |
|---|---|---|---|---|
| **Gene sets** | **NES** | **FDR, q-value** | **NES** | **FDR, q-value** |
| BLIMP1 targets[1] | 2.51 | <0.0001 | 2.21 | <0.0001 |
| CD40 signaling during GC development[1] | 2.37 | <0.0001 | 2.20 | <0.0001 |
| GC B CELL[1] | 2.24 | <0.0001 | 2.46 | <0.0001 |
| mTORC1 pathway[2] | 2.26 | <0.0001 | 2.30 | <0.0001 |

Gene sets regulated by idelalisib (IDELA) were identified by Gene Set Enrichment Analysis (GSEA) using: [1]Experimentally derived custom genes sets (http://lymphochip.nih.gov/signaturedb/index.html); [2]Hallmark signatures from Molecular Signature database (v2.5). NES: Normalized Enriched Score; FDR: False Discovery Rate. Threshold FDR<0.05 and NES>1.5.