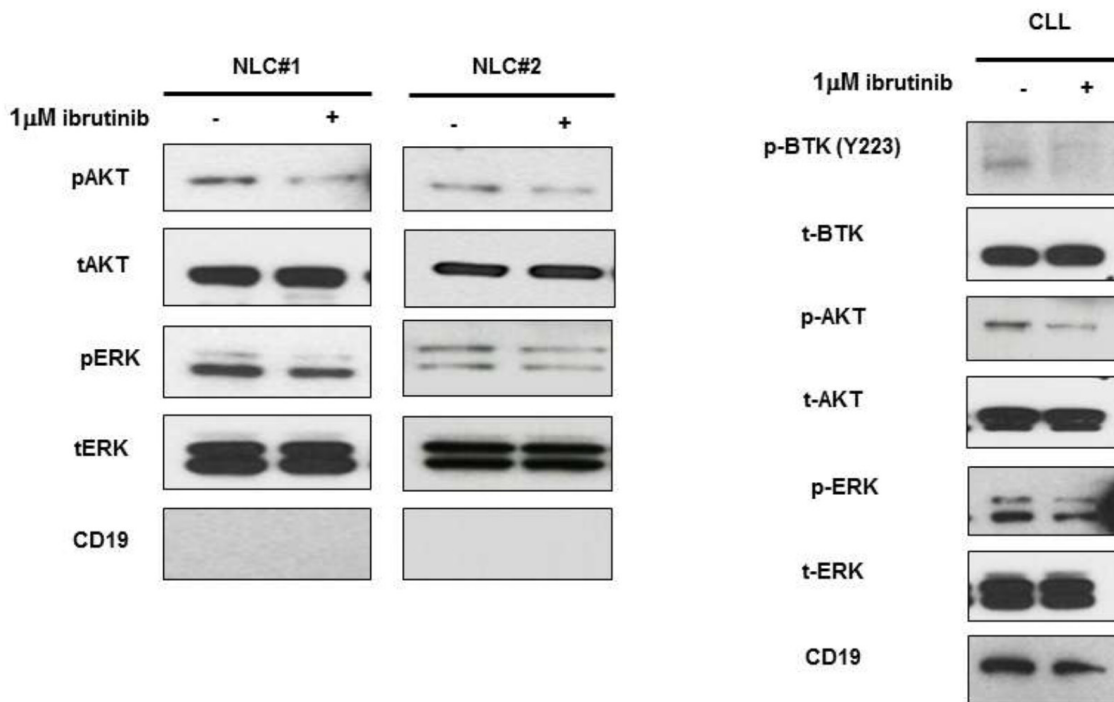
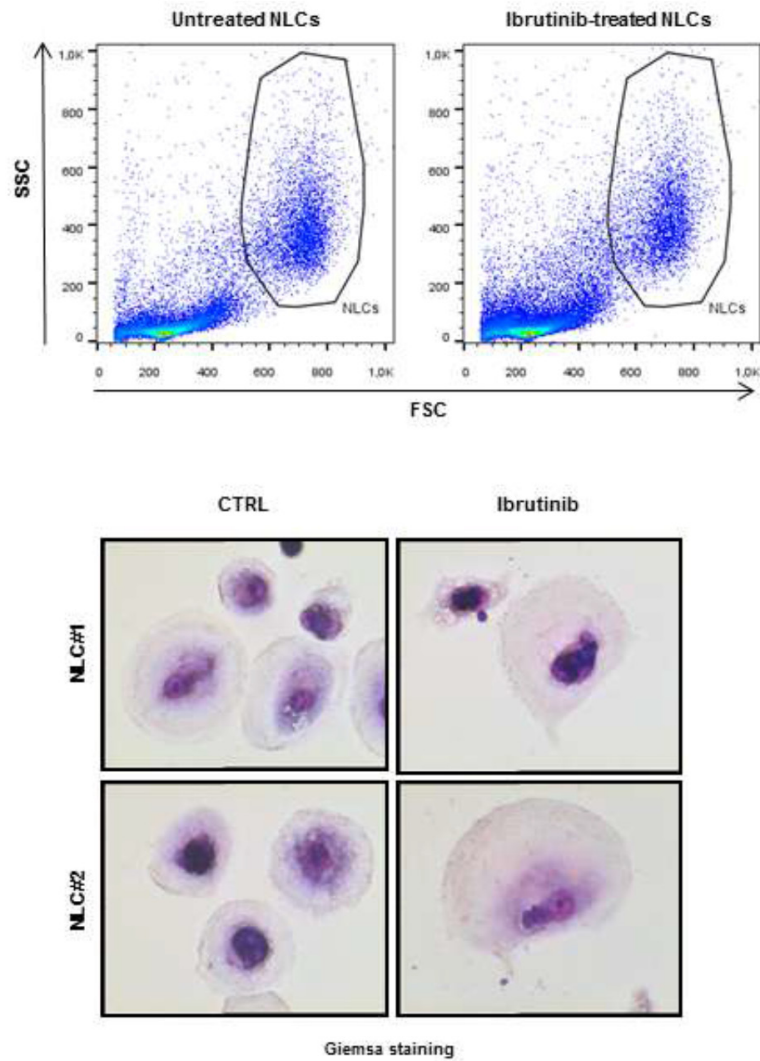


## Ibrutinib modifies the function of monocyte/macrophage population in chronic lymphocytic leukemia

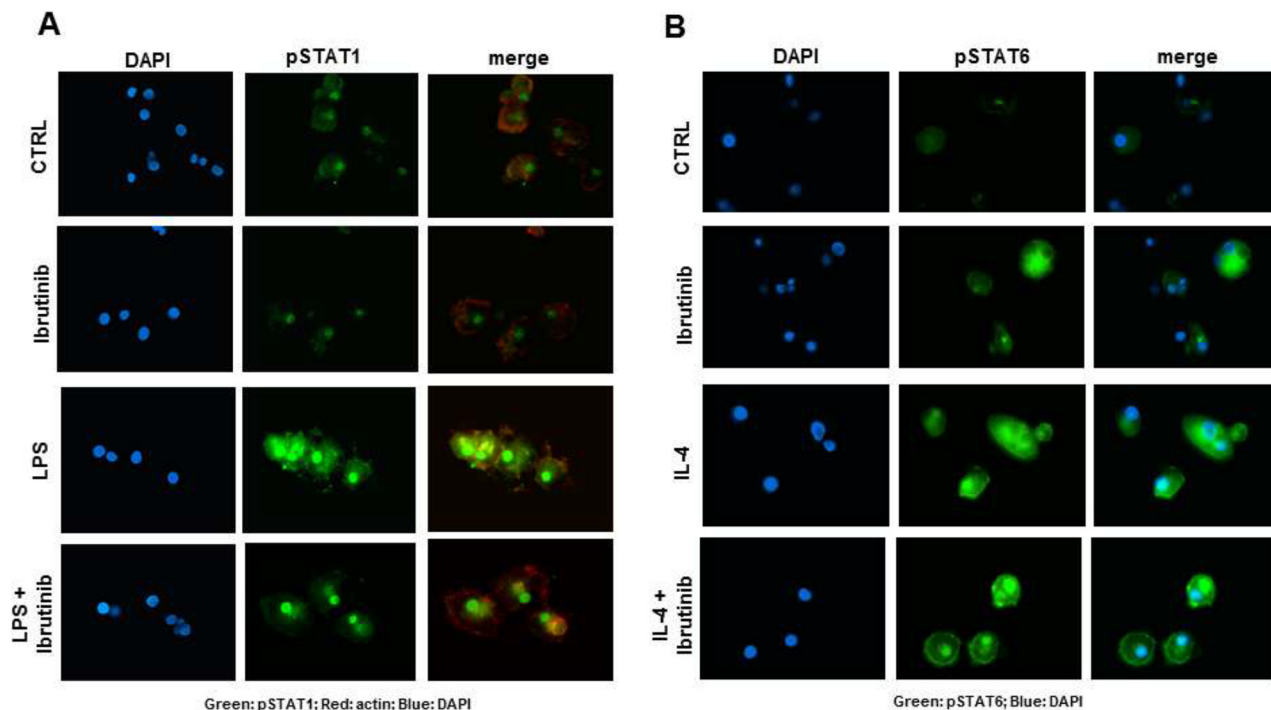
### SUPPLEMENTARY FIGURE AND TABLES



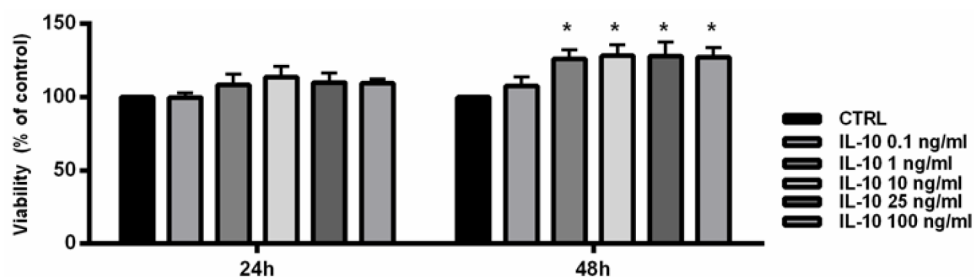
**Supplementary Figure S1: Ibrutinib impairs the activation of BTK-downstream signaling pathways.** Western Blot analysis of AKT and ERK phosphorylation and total levels was performed after 1 hour of treatment with ibrutinib 1 μM. CD19 was used as a marker for CLL cells. On the right, western blot shows a representative CLL sample blotted for BTK, AKT, ERK and CD19.



**Supplementary Figure S2: Ibrutinib does not affect NLCs formation.** Dot plots show the preserved morphology of NLCs after treatment with ibrutinib for 24 hours analyzed by flow-cytometric FCS and SSC plots. On the right, May-Grunwald Giemsa staining documented the morphology of NLCs in two representative samples.



**Supplementary Figure S3: Ibrutinib stimulates M2 signaling pathways affecting M1 polarization in NLCs.** **A.** Immunofluorescence images show pSTAT1 expression after stimulation with LPS for 3 hours either after exposure or not with ibrutinib. NLCs were stained for pSTAT1, DAPI and phalloidin. **B.** Images show pSTAT6 expression after stimulation with IL-4 for 30 minutes either after exposure or not with ibrutinib. NLCs were stained for pSTAT6 and DAPI.



**Supplementary Figure S4: IL-10 mediates pro-survival signals in CD19+ CLL cells.** CD19+ CLL cells (n=4) were cultured for 2 days with a dose escalation of IL-10. Bar diagrams show CLL viability measured by Annexin V-PI staining (\*P<0.05).

**Supplementary Table S1: Primers used in real-time PCR**

Gene	Forward primer	Reverse primer
CD163	5'-GAAGATGCTGGCGTGACAT-3'	5'-GCTGCCTCCACCTCTAAGTC-3'
IL10	5'-CATAAATTAGAGGTCTCCAAAATCG-3'	5'-AAGGGGCTGGGTGAGCTAT-3'
MRC1	5'-CACCATCGAGGAATTGGACT-3'	5'-ACAATTCGTCATTTGGCTCA-3'
CCL18	5'-ATGGCCCTCTGCTCCTGT-3'	5'-AATCTGCCAGGAGGTATAGACG-3'
IL1	5'-GGTTGAGTTTAAGCCAATCCA-3'	5'-TGCTGACCTAGGCTTGATGA-3'
TNFA	5'-CAGCCTCTTCTCCTCCTGAT-3'	5'-GCCAGAGGGCTGATTAGAGA-3'

Commercially available primers (TaqMan Gene Expression Assays; Life Technologies) used were: Hs00237184\_m1 (NAMPT) and Hs01125301\_m1 (PD-L1).

Supplementary Table S2: Antibodies used in immunoblotting

Antibody	Manufacturer
Anti-phospho-BTK Tyr <sup>551</sup> rabbit	GeneTex, Irvine, CA
Anti-phospho-BTK Tyr <sup>223</sup> rabbit	Cell Signaling Tech, Beverly, MA
Anti-phospho-STAT1 mouse	ECM Biosciences, Versailles, KY
Anti-STAT1 mouse	Cell Signaling Tech, Beverly, MA
Anti-phospho-Erk 1/2 rabbit	Cell Signaling Tech, Beverly, MA
Anti-Erk 1/2 rabbit	Cell Signaling Tech, Beverly, MA
Anti-phospho-Akt rabbit	Cell Signaling Tech, Beverly, MA
Anti-Akt mouse	Cell Signaling Tech, Beverly, MA
Anti-phospho-I $\kappa$ B $\alpha$ mouse	Cell Signaling Tech, Beverly, MA
Anti-I $\kappa$ B $\alpha$ mouse	Cell Signaling Tech, Beverly, MA
Anti-phospho-STAT6 rabbit	Cell Signaling Tech, Beverly, MA
Anti-STAT6 rabbit	Cell Signaling Tech, Beverly, MA
Anti-SHIP1 rabbit	Cell Signaling Tech, Beverly, MA
Anti-phospho-STAT3 rabbit	Cell Signaling Tech, Beverly, MA
Anti-STAT3 mouse	Cell Signaling Tech, Beverly, MA
Anti- $\beta$ actin mouse	Abcam, Cambridge, UK