Figure S1

A.

Gene Symbol	Gene Name		Sequence 5' ->3'	Location	Amplicon Size	Gene Accession Number
TBP	TATA-box-binding protein	Forward	CCCGAAACGCCGAATATAATCC	560-581	80	NM_003194
		Reverse	AATCAGTGCCGTGGTTCGTG	639-620		
PIK3CD	Phosphoinositide-3-kinase, catalytic, delta polypeptide	Forward	TCAACTCACAGATCAGCCTCC	338-358	84	NM_005026
		Reverse	CGCGAAAGTCGTTCACTTCT	421-402		
PIK3CG	Phosphoinositide-3-kinase, catalytic, gamma polypeptide	Forward	GGCTACCATGAGCAGCTTACC	1000-1020	142	NM_002649
		Reverse	CTGTGAGGTCGGTGTTCCG	1141-1123		
PIK3R5	Phosphoinositide-3-kinase, regulatory subunit 5	Forward	CTTCCACGCTACGTGTTGTG	383-402	124	NM_001251851
		Reverse	TGAAGTTTGAAGAACCGTGTGAG	506-484		
PIK3R6	Phosphoinositide-3-kinase, regulatory subunit 6	Forward	CACAAGAAGGTCGAGCGAGAT	118-138	137	NM_001010855
		Reverse	GTGAGCACGTACATTACAGTGT	254-233		

B.

acaaccggtccgctctagagatccgacgcgccatctctaggcccgcgccggccccctcgcacggacttgtgggagaagctcggctactcccctgccccggttaatttgcatataat
atttcctagtaactatagaggcttaatgtgcgataaaagacagataatctgttctttttaatactagctacattttacatgataggcttggatttctataacttcgtatagcatacatta
tacgaagttataaacagcacaaaaggaaactcaccctaactgtaaagtaattgtgtgttttgagactataagtatcccttggagaaccaccttgttg**NNNNNNNNNNNNN
NNNNNNN**gtttaagagctaagctggaaacagcatagcaagtttaaataaggctagtccgttatcaacttgaaaaagtggcaccgagtcggtgctttttttctcgagtactagg
atccattaggtacctac

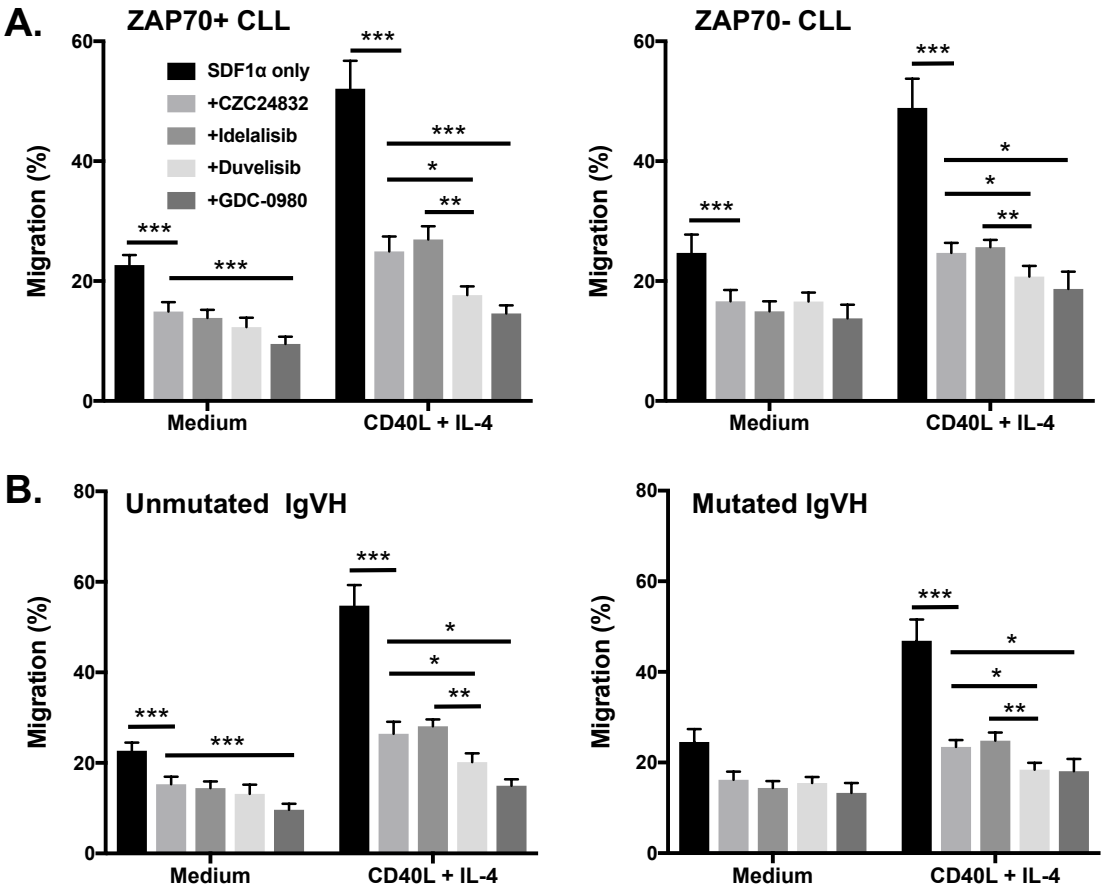
p110γ Crispr-i targeting sequence #1 - **GTTCCGGCAATAGTTTTGC**

p110γ Crispr-i targeting sequence #2 - **GCTTCCTTCTATTTTTTGCTCTTTG**

Figure S1: DNA sequences of RT qPCR primers and CRISPRi gRNA inserts .

(A) Table providing qPCR primer sequences along with gene names and symbols, gene sequence accession number, location of primers and length of amplicon. **(B)** The sequence of the gRNA expression cassette. XbaI and XhoI cloning enzyme sites are underlined. The location of specific p110γ CRISPRi targeting sequences within the expression cassette are shown by bolded NNNs and are shown below.

Figure S2



<u>Figure S2:</u> Impact of PI3Kγ inhibition on CLL patient samples, stratified by ZAP-70 status and IgVH mutation.

CLL samples were treated and assessed for migration as described in Figure 1. Results are grouped according to patient ZAP-70 status **(A) or** IgH mutation status **(B)**.

Figure S3

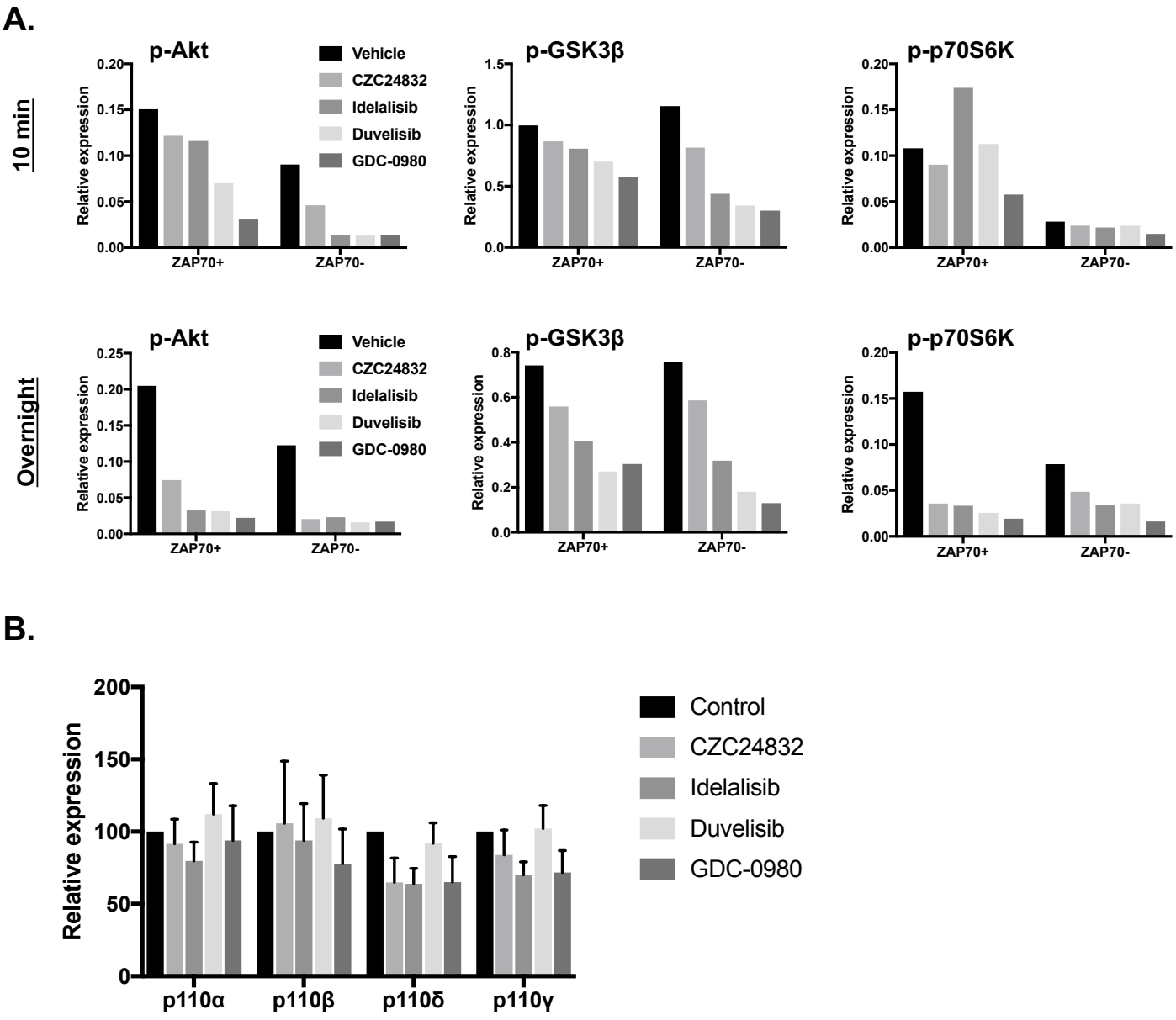
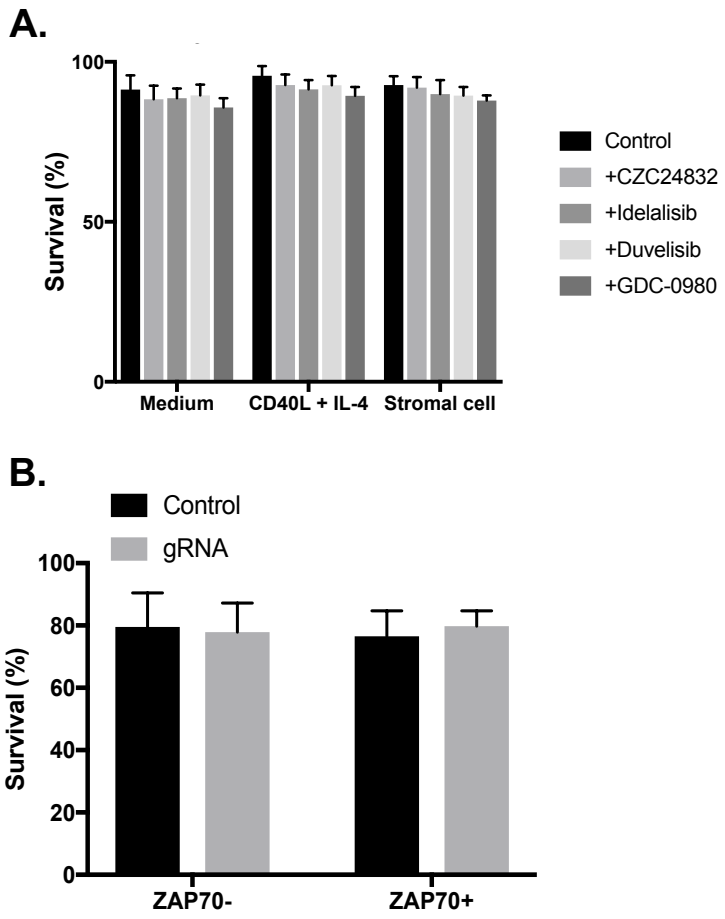


Figure S3: Impact of different PI3K inhibitors on Akt pathway activity and PI3K isoform expression in CLL cells.

(A) Primary CLL cells were pre-incubated with the inhibitors (1hr) and then cultured in medium containing CD40L + IL4 (50 ng/ml) plus inhibitors for the indicated time points. Whole cell lysates were harvested for simultaneous analysis of phospho-Akt (Ser473), phospho-GSK3β-(Ser9) and phospho-p70S6K (Ser240/244) using Meso Scale Diagnostics Akt Signalling Panel II (Cat#K15177D-1). Results are expressed as relative phospho/total protein ratios. **(B)** CLL cells were treated overnight with the indicated inhibitors and the relative expression of p110 isoforms was measured by qPCR.

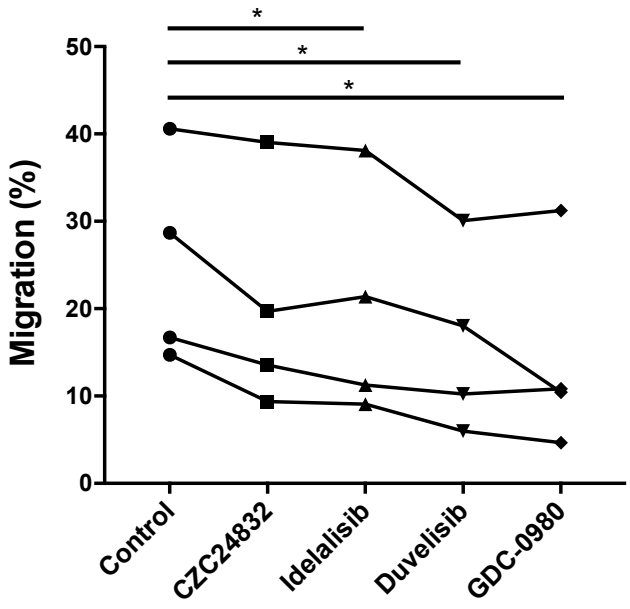
Figure S4



<u>Figure S4:</u> Survival analysis of CLL cells and malignant B cells for the various experiments conducted in the study.

(A) Survival of CLL cells after PI3K inhibitor treatment. Cells were cultured under the indicated conditions used for migration or adhesion assays and **(B)** Survival of CLL cells following PI3Kγ Crispr-I knockdown.

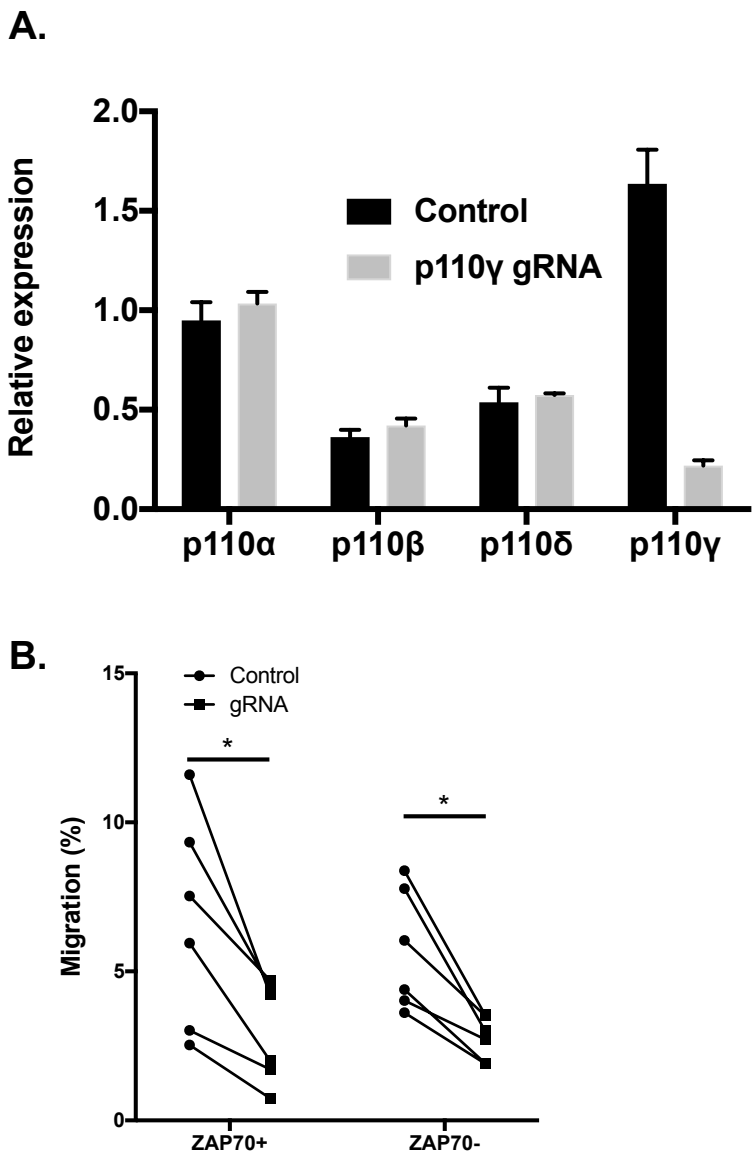
Figure S5



<u>Figure S5:</u> Impact of PI3K inhibitors on normal human B cell migration.

Normal B cells were purified from blood, treated with the respective inhibitor as previously described and subjected to a transwell migration assay. Individual subject migration responses are connected by lines.

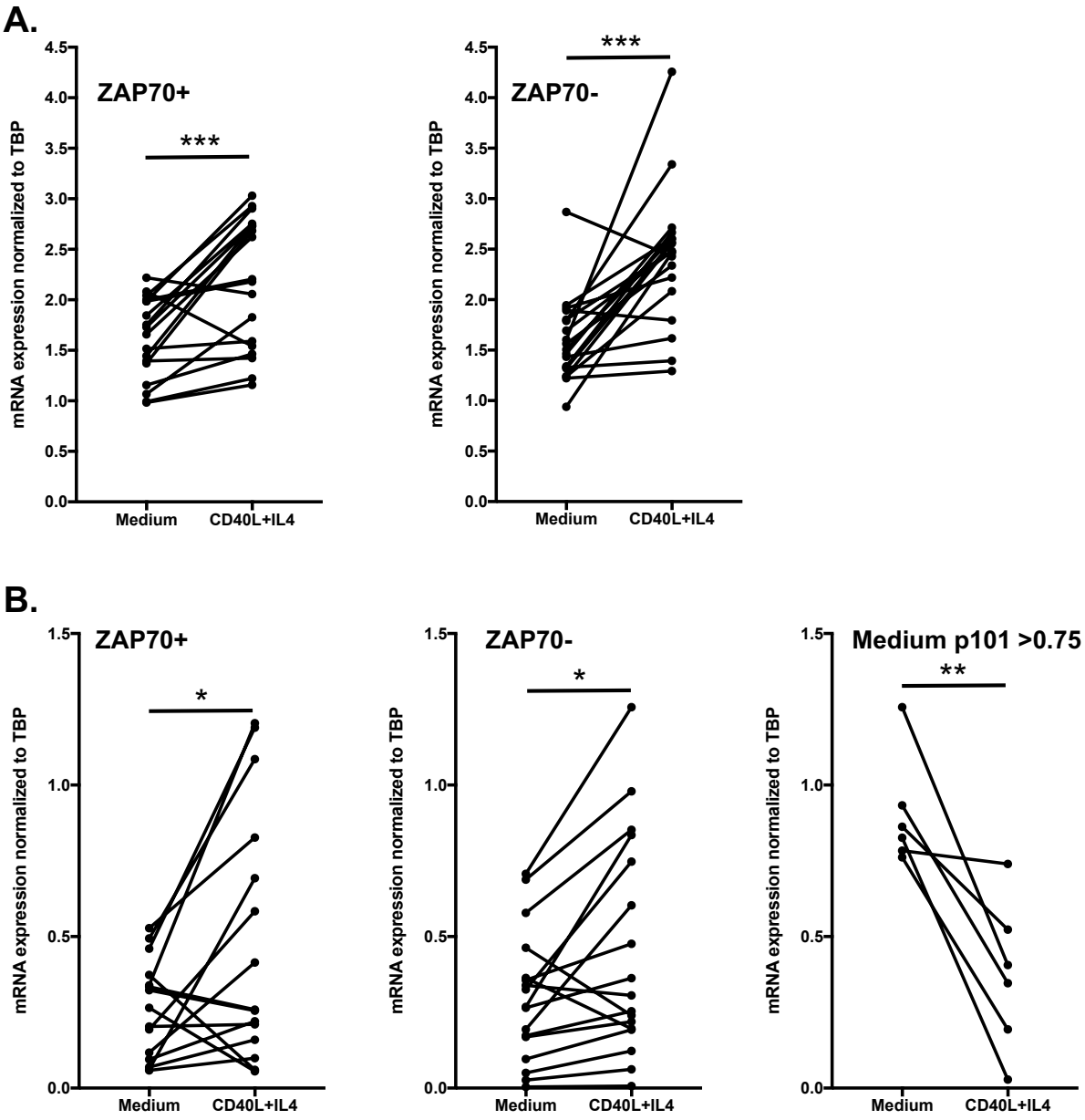
Figure S6



<u>Figure S6:</u> Effect of Crispr-I knock-down of p110γ on p110 isoform expression and CLL migration.

(A) Ramos cells were transfected with dCas9-GFP plasmid alone or dCas9-GFP plus p110γ gRNA plasmid and p110 subunit expression was measured by qPCR. **(B)** CLL cells transfected with dCas9-GFP plasmid alone or dCas9-GFP plus p110γ gRNA were assessed for migration capacity in transwell assay. Patients are grouped according to ZAP70 status.

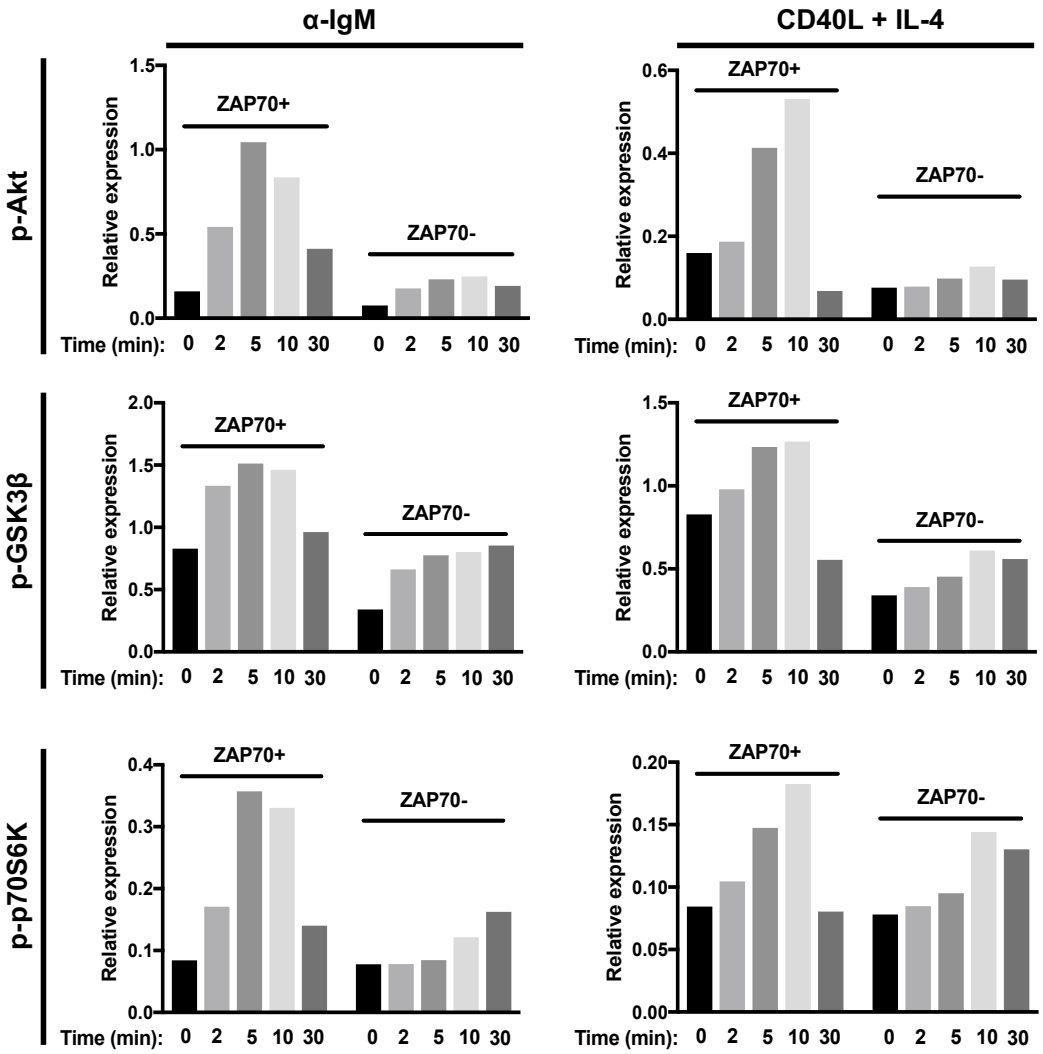
Figure S7



<u>Figure S7:</u> Paired analysis of CLL patient PI3Kγ subunit expression with or without CD40L+IL4.

qPCR data from Figure 3 are graphed with connecting lines linking the same patient cultured in medium versus CD40L+IL4. **(A)** p110γ expression in ZAP70+ or ZAP70- patients. **(B)** p101 expression in ZAP70+ or ZAP70- patients. Left and centre graphs illustrate that the majority of patients show increased p101 expression after stimulation. Outliers showing high p101 without stimulation have been removed and are graphed separately in the right panel; interestingly all of these patients show significant reduction in p101 expression after stimulation. Statistical significance was determined by paired T-test.

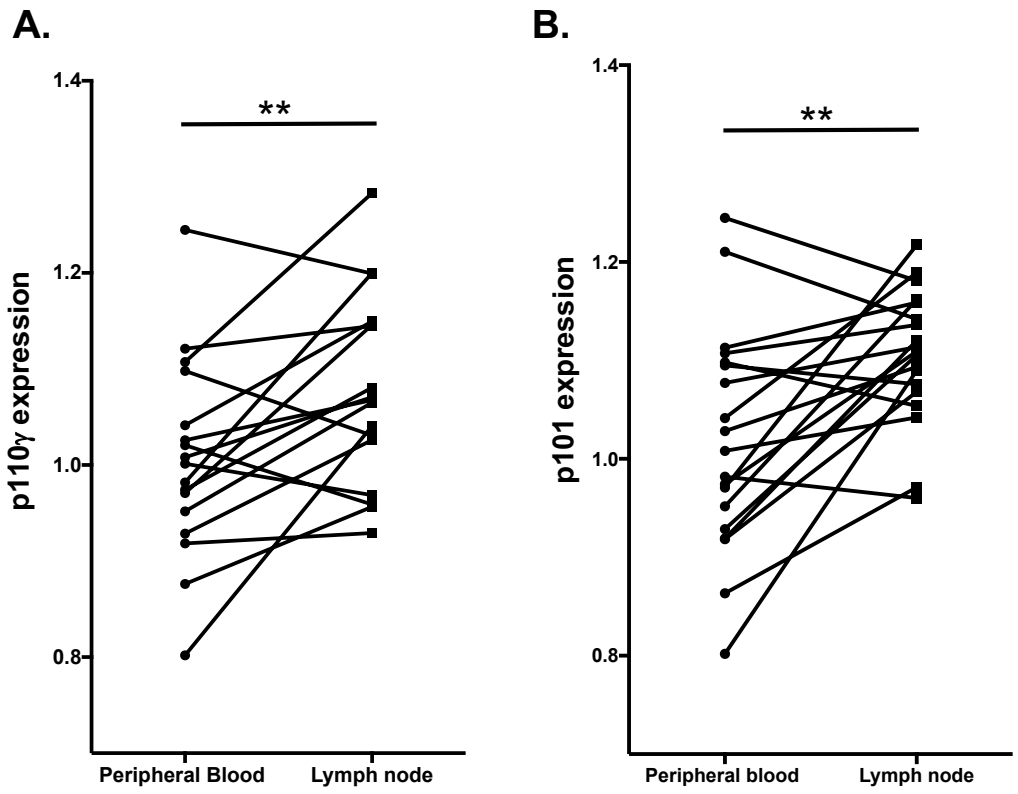
Figure S8



<u>Figure S8:</u> Confirmation of α-IgM and CD40L + IL4 stimulation effect on CLL cells.

CLL cells were stimulated with α-IgM (10 μg/ml) or CD40L + IL4 (50 ng/ml) for the indicated time points. Whole cell lysates were harvested for simultaneous analysis of phospho-Akt (Ser473), phospho-GSK3β-(Ser9) and phospho-p70S6K (Ser240/244) using Meso Scale Diagnostics Akt Signalling Panel II (Cat#K15177D-1). Results are expressed as relative phospho/total protein ratios.

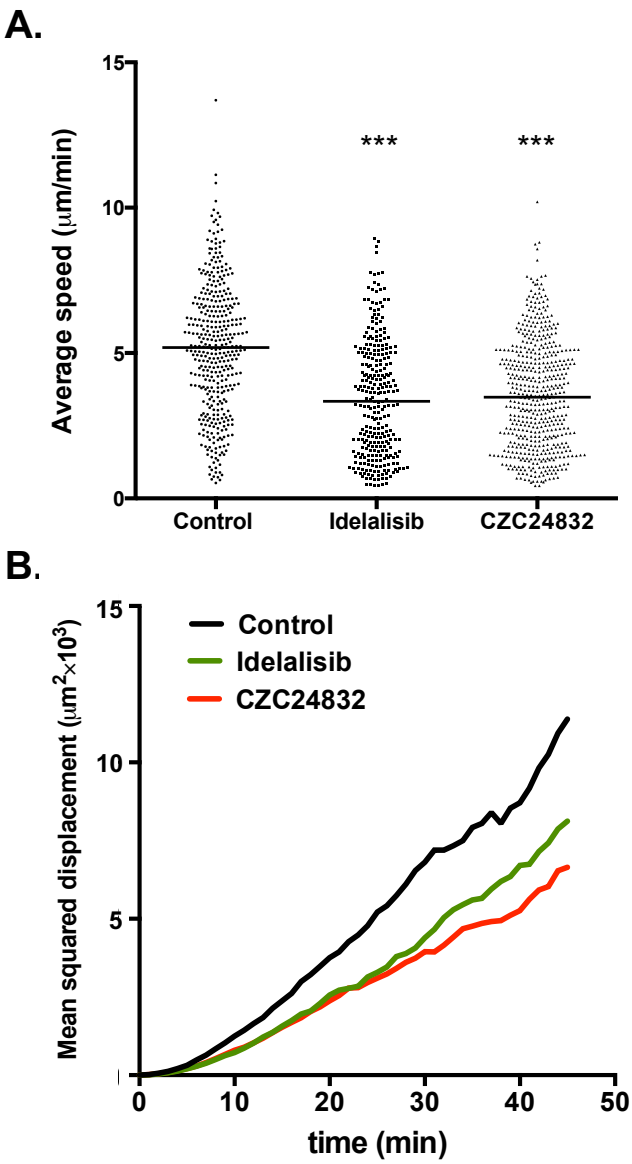
Figure S9



<u>Figure S9:</u> Microarray data analysis of p110γ and p101 expression in CLL patient blood versus lymph node from the GEO database.

Expression of (A) p110γ and (B) p101 in CLL isolated from lymph node or peripheral blood. Data were obtained from the GEO database (record # GDS4176), as reported by Herishanu et al (ref 40). Expression of p110γ (PIK3CG) or p101 (PIK3R5) were extracted from the dataset and graphed. Statistical significance was determined by paired T-test.

Figure S10



<u>Figure S10:</u> PI3Kγ and PI3Kδ inhibition impair Ramos cell migration speed and persistance on a VCAM-1-coated surface.

Ramos cells were seeded into VCAM-1 coated chamber slides then treated with the indicated inhibitors for 30 minutes. After 100 nM SDF1α stimulation, time-lapse videos of Ramos cell migration were taken and cell migration tracks analyzed using IMARIS 8.0 software. Migration speed **(A)** and mean squared displacement **(B)** were determined for each migrating cell. ***$p < 0.001$ by Mann-Whitney test comparing inhibitor treated to control group.