Transient knockdown of p110δ expression
CLL cells were transiently transfected with siRNA/p110δ or nonspecific control siRNA (Ambion, Austin, TX) using Cell Line Nucleofector kit V (Amaxa Biosystems, Gaitherburg, MD). Immediately after transfection, cells were transferred to Hybridoma SFM culture media. After 36 hours cells were treated with 1 mg/mL CD40L for 2 hours prior to analysis.

Flow cytometry analysis of PI3K signaling
Cell lines and primary patient cells were quiesced for 2 hrs 37°C in 1 ml RPMI supplemented with 0.1% FBS and then incubated with CAL-101 for an additional 60 min. Cells were stimulated for 10 min with the addition of CXCL13 (250 ng/mL; PeproTech, Rocky Hill, NJ), CXCL12 (250 ng/mL; PeproTech), BAFF (50 ng/mL; PerproTech) or shCD40L (3 ug/mL; R&D Systems, Minneapolis, MN), washed with PBS, resuspended in 1 mL cold FACS buffer (PBS supplemented with 0.1% FBS), fixed with 1 mL Cytofix (Becton Dickinson, San Jose, CA) and incubated at 37°C for 10 min. Following incubation cells were washed twice to remove fixative and resuspended in 100 µL Permsolution II (Beckman Coulter). Approximately 0.5×10^6 cells were stained on ice for 30 min with either phospho-Akt (T308)-Alexa Fluor 488, phospho-Akt (Ser473) Alexa Fluor 488, or an isotype-matched control antibody (mouse IgG_1-Alexa Fluor 488 conjugate). For primary ALL, CLL, and MCL samples, cells were stained on ice for 30 min with either anti–CD19-FITC or anti–CD5-FITC and either anti–phospho-Akt T308 (Alexa Fluor 488), anti–phospho-Akt Ser473 (Alexa Fluor 488), or an isotype-matched control antibody (mouse IgG_1-Alexa Fluor 488 conjugate). The gates were set using anti-CD5 (FITC) and isotype-FITC. Stained cells were washed twice in FACS buffer and fluorescence was analyzed using the Beckman Coulter Cytomics FC 500MPL cytometer and data were collected and analyzed using CXP software (Beckman Coulter).

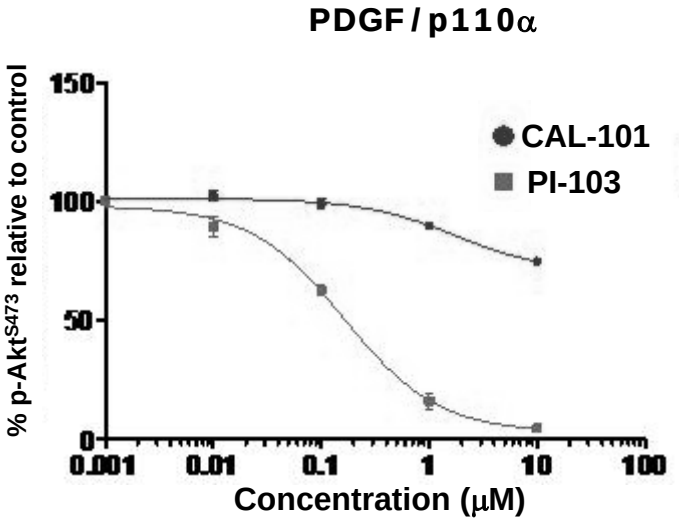
Figure S1. The activity of CAL-101 in PI3K isoform specific cell-based assays
The effect of CAL-101 on (A) PDGF induced Akt^{S473} phosphorylation in primary murine embryonic fibroblasts (p110α assay), (B) LPA-induced Akt^{S473} phosphorylation in primary murine embryonic fibroblasts (p110β assay). Data are presented mean ± S.D of percent reduction of Akt phosphorylation, and are representative of 4 independent experiments run in duplicate. (C) anti-FcεRI and fMLP-induced basophil activation in PBMCs measuring CD63 upregulation as described in the Materials and Methods section. Data are presented as the percent of CD63 positive basophils normalized to DMSO vehicle control. Data are mean ± SD and are representative of one to four independent experiments.

Figure S2. CAL-101 inhibits Akt phosphorylation at S473 that is mediated by CXCR5, CXCR4, BCR, and BAFFR in malignant B cell lines and primary tumor cells
Cells incubated in RPMI were pretreated with CAL-101 or vehicle for one hr and then stimulated with 250 ng/mL CXCL13 (CXCR5 activation), 200 ng/mL CXCL12 (CXCR4 activation), 10µg/mL anti-IgM (BCR activation), or 50 ng/mL BAFF (BAFFR activation) for 10 min prior to fixation and staining with anti–phospho-Akt^{S473} Alexa Fluor 488 or isotype matched Alexa Fluor 488 antibody. Cells analyzed by flow cytometry to quantify intracellular p-Akt^{T308} levels using the Beckman Coulter Cytomics FC 500MPL FACS machine employing CXP software. Bar graphs represent the percent difference in mean fluorescence intensity (MFI) values between isotype-matched control Ig and phospho-Akt^{S473}. Each bar-histogram and is an average of four independent experiments.

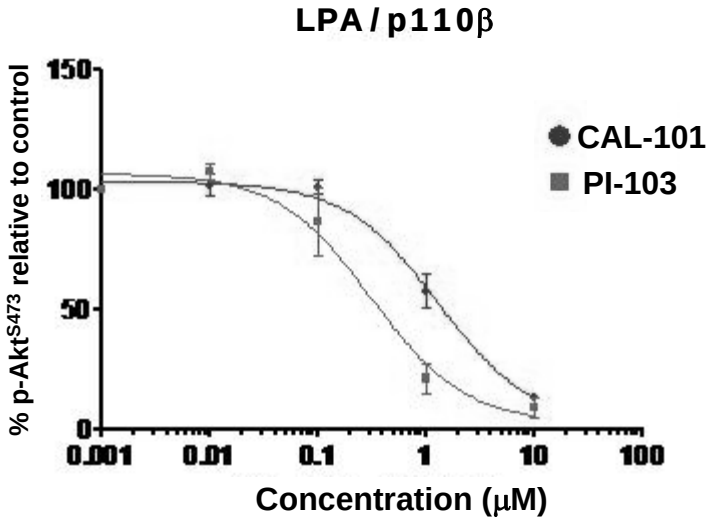
Figure S3. Knockdown of p110δ reduces Akt phosphorylation mediated by CD40 in malignant B cell lines

$CD19^+$ cells from CLL patients (N=3) were mock transfected or transfected with 50nM of p110δ or negative control siRNA. After 36 hours cells were treated with 1 mg/mL CD40L for 2 hours. Akt phosphorylation at S473, GSK3β phosphorylation at S9 and Mcl-1 expression was assessed by immunoblot. Quantification was done using the Alpha Innotech FluorChemQ MultiImage III system. Results show are representative of one of three experiments.

A)



B)



C)

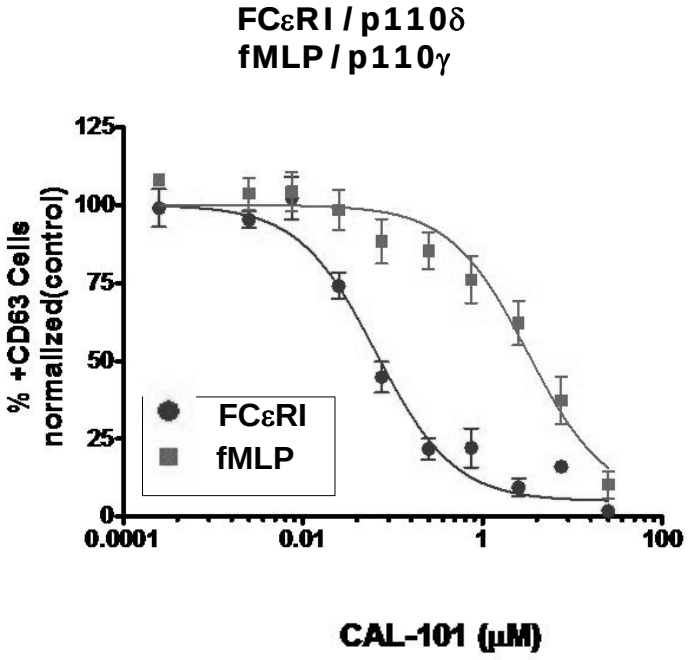


Figure S1

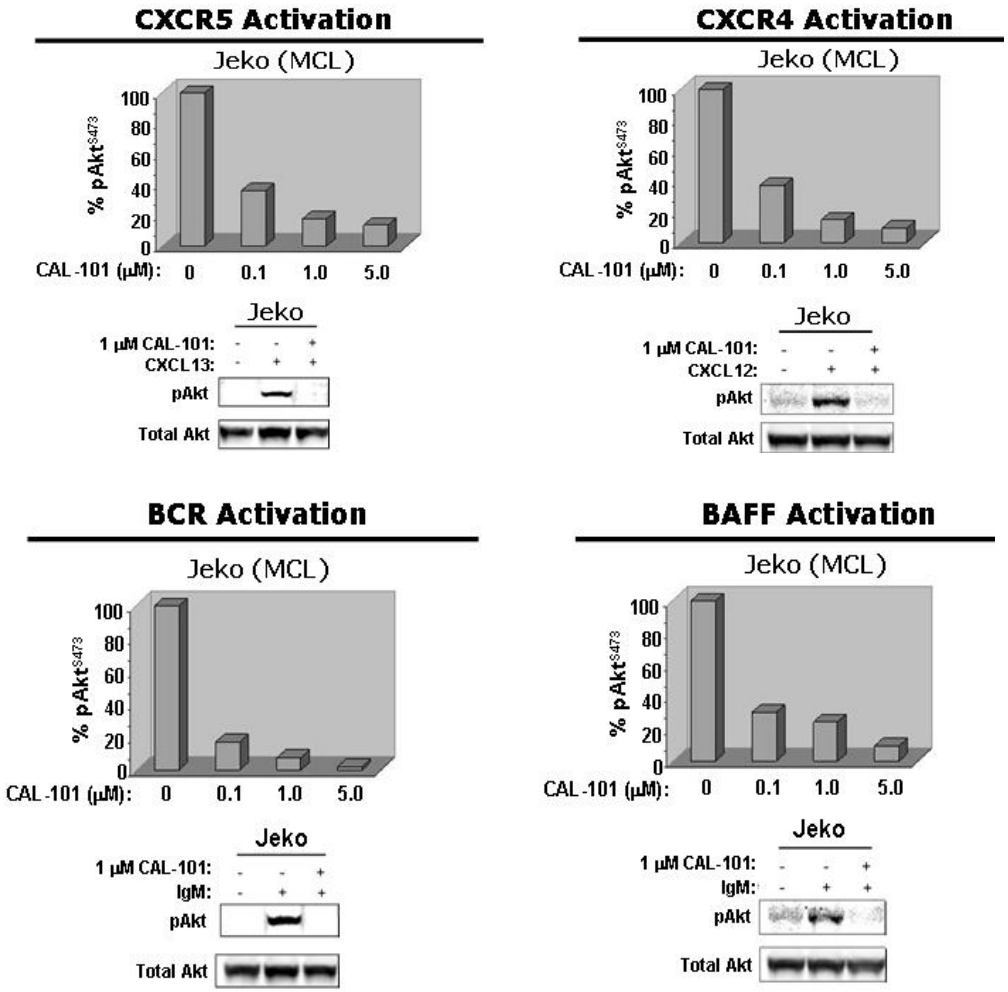

CXCR5 Activation
Jeko (MCL)
% pAkt S473
100
80
60
40
20
0
CAL-101 (μM): 0 0.1 1.0 5.0
Jeko
1 μM CAL-101: - - +
CXCL13: - + +
pAkt
Total Akt
CXCR4 Activation
Jeko (MCL)
% pAkt S473
100
80
60
40
20
0
CAL-101 (μM): 0 0.1 1.0 5.0
Jeko
1 μM CAL-101: - - +
CXCL12: - + +
pAkt
Total Akt
BCR Activation
Jeko (MCL)
% pAkt S473
100
80
60
40
20
0
CAL-101 (μM): 0 0.1 1.0 5.0
Jeko
1 μM CAL-101: - - +
IgM: - + +
pAkt
Total Akt
BAFF Activation
Jeko (MCL)
% pAkt S473
100
80
60
40
20
0
CAL-101 (μM): 0 0.1 1.0 5.0
Jeko
1 μM CAL-101: - - +
IgM: - + +
pAkt
Total Akt

Figure S2

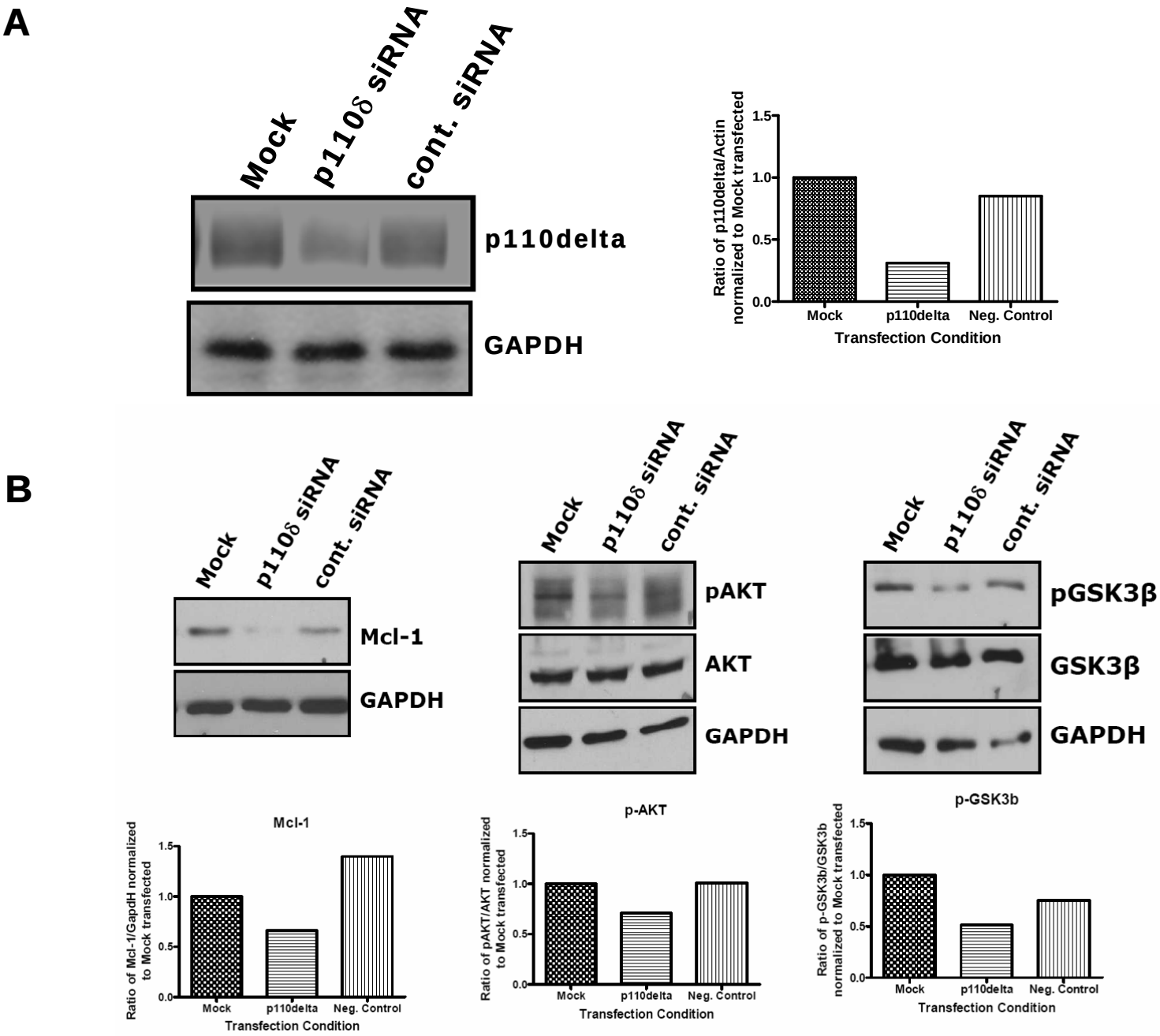

A
Mock
p110δ siRNA
cont. siRNA
p110delta
GAPDH
Ratio of p110delta/Actin normalized to Mock transfected
1.5
1.0
0.5
0.0
Mock
p110delta
Neg. Control
Transfection Condition
B
Mock
p110δ siRNA
cont. siRNA
Mcl-1
GAPDH
pAKT
AKT
GAPDH
pGSK3β
GSK3β
GAPDH
Mcl-1
Ratio of Mcl-1/GapdH normalized to Mock transfected
p-AKT
Ratio of pAKT/AKT normalized to Mock transfected
p-GSK3b
Ratio of p-GSK3b/GSK3b normalized to Mock transfected
Transfection Condition

Figure S3