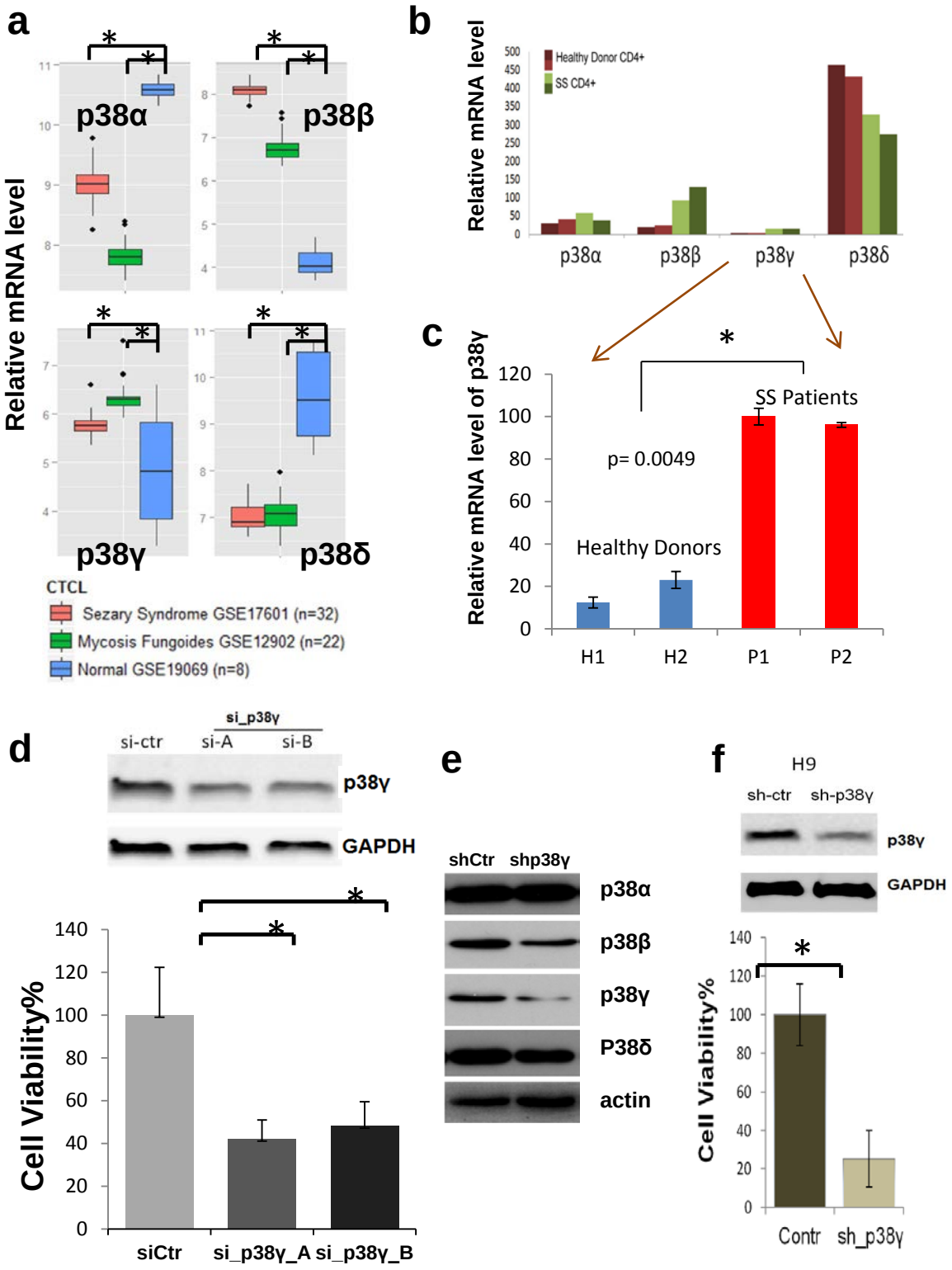
Suppl. Table S1. Primers used for the real time qRT-PCR experiments

Genes	Sequences
MAPK11	ForwardCCCGGACATATATCCAGTCC
	ReverseTCACTGCTCAATCTCCAGG
MAPK12	ForwardGCCCATCCCTACTTCGAGTC
	ReverseCTTCACAGAGGCGTCTCCTT
MAPK13	ForwardGGCAGTTTAACGTGGCCTGTTA
	ReverseACAGTGGATGAATGGAAGCAGC
MAPK14	ForwardGCCGAGCTGTTGACTGGAAG
	ReverseGGAGGTCCCTGCTTTCAAAGG
GAPDH	ForwardCCCGGACATATATCCAGTCC
	ReverseTCACTGCTCAATCTCCAGG

Supplemental Figure S1



Supplemental Figure S1

Figure S1. p38γ gene expression is limited in human tissues, and is important for viability in CTCL.

a) Publically available microarray databases were analyzed for mRNA expression of p38 isoforms in CTCL (GSE17601, n=32 for SS, pink; GSE12902, n=22 for MF, green) and healthy donors/primary $CD4^+$ T cell lines (GSE19069, n=8, blue). *$p<0.001$.

b) and c) QRT-PCR analysis was used to determine mRNA expression of indicated p38 isoforms (b) and p38γ (c), relative to their internal control GAPDH, in CD4+ T cells isolated from healthy donors (n=2) or SS patients (n=2).

d) Hut78 cells were treated with p38γ or control Cell viability presented as a percent of control-treated cells. Two siRNA sequences that targeting p38γ are indicated in the materials and methods. Three replicates performed for each sample, *$p<0.05$e.

e) Western blot was used to visualize protein expression of indicated p38 isoforms or actin (loading control) in Hut78 cells transduced with p38γ shRNA or scrambled control.

f) Cell viability assay in H9 cells transduced with lentiviral particles with scrambled shRNA control or p38γ shRNA. Data are an average of 3 replicates. *$p<0.05$.

Supplemental Table S2. IC_{50} (μM) of p38γ inhibitors F7, A10, and A11 in NCI60 cell line panel.

Cells	F7/PIK75	A10	A11	Disease	Cells	F7/PIK75	A10	A11	Disease
K-562	0.416	> 10	> 10	CML	UACC257	2.32	> 10	> 10	Melanoma
RPMI-8226	0.077	9	> 10	MM	LOX IMVI	0.073	4.4	3.58	Melanoma
SR	< 0.001	0.372	0.286	Leukemia	SK-MEL-5	> 10	> 10	> 10	Melanoma
HL60	0.005	1.14	0.247	AML	MALME-3M	0.116	> 10	3.05	Melanoma
MOLT-4	0.527	5.2	6.98	ALL	UACC-62	0.362	> 10	6.12	Melanoma
CCRF-CEM	3.72	2.5	2.5	Leukemia	SK-MEL-2	0.317	> 10	> 10	Melanoma
NCI-H226	8.39	> 10	> 10	Lung	M14	0.195	10	3.33	Melanoma
NCI-H460	0.01	> 10	10	Lung	MDA-MB-435	> 10	6.6	> 10	Melanoma
HOP-92	0.094	6.04	6.4	Lung	SK-MEL-28	1.69	> 10	9.6	Melanoma
NCI-H522	> 10	> 10	> 10	Lung	T-47D	> 10	6.19	> 10	Breast
NCI-H322M	1.5	> 10	> 10	Lung	BT-549	< 0.001	4.78	0.705	Breast
NCI-H23	> 10	> 10	> 10	Lung	MDA-MB-231	0.466	> 10	> 10	Breast
HOP-62	0.008	> 10	9.94	Lung	MCF7	0.238	10	3.77	Breast
EKVX	1.22	> 10	> 10	Lung	HS 578T	0.586	5.83	> 10	Breast
A549	2.66	> 10	> 10	Lung	MDA-MB-468	0.103	6.97	1.77	Breast
HCT-15	0.008	3.84	2.54	Colon	U251	0.253	1.92	4.57	CNS
KM12	0.006	> 10	2.28	Colon	SF-539	0.177	2.55	3.68	CNS
HCT-116	0.001	3.2	1.26	Colon	SF-268	2.23	> 10	> 10	CNS
HCC-2998	> 10	> 10	> 10	Colon	SF-295	0.141	> 10	7.64	CNS
Colo 205	2.18	> 10	> 10	Colon	SNB-19	0.385	> 10	> 10	CNS
SW-620	0.074	> 10	3.93	Colon	SNB-75	0.435	> 10	6.14	CNS
HT29	0.087	> 10	5.04	Colon	UO31	0.115	> 10	4.03	Renal
NCI/ADR-RES	0.024	3.67	1.44	Ovarian	786-0	0.008	7.77	9.3	Renal
OVCAR-3	0.071	> 10	2.52	Ovarian	RXF 393	0.08	> 10	3.47	Renal
SKOV3	0.291	> 10	> 10	Ovarian	ACHN	0.092	4.46	> 10	Renal
IGR-OV1	0.251	> 10	> 10	Ovarian	A498	0.027	> 10	> 10	Renal
OVCAR-8	0.059	> 10	3.6	Ovarian	CAKI-1	0.35	> 10	> 10	Renal
OVCAR-4	0.403	> 10	> 10	Ovarian	SN12C	0.468	> 10	10	Renal
OVCAR-5	0.04	> 10	5.31	Ovarian	TK-10	0.805	> 10	> 10	Renal
PC-3	0.02	> 10	9.18	Prostate	DU-145	0.001	1.38	2.26	Prostate

Supplemental Figure S2

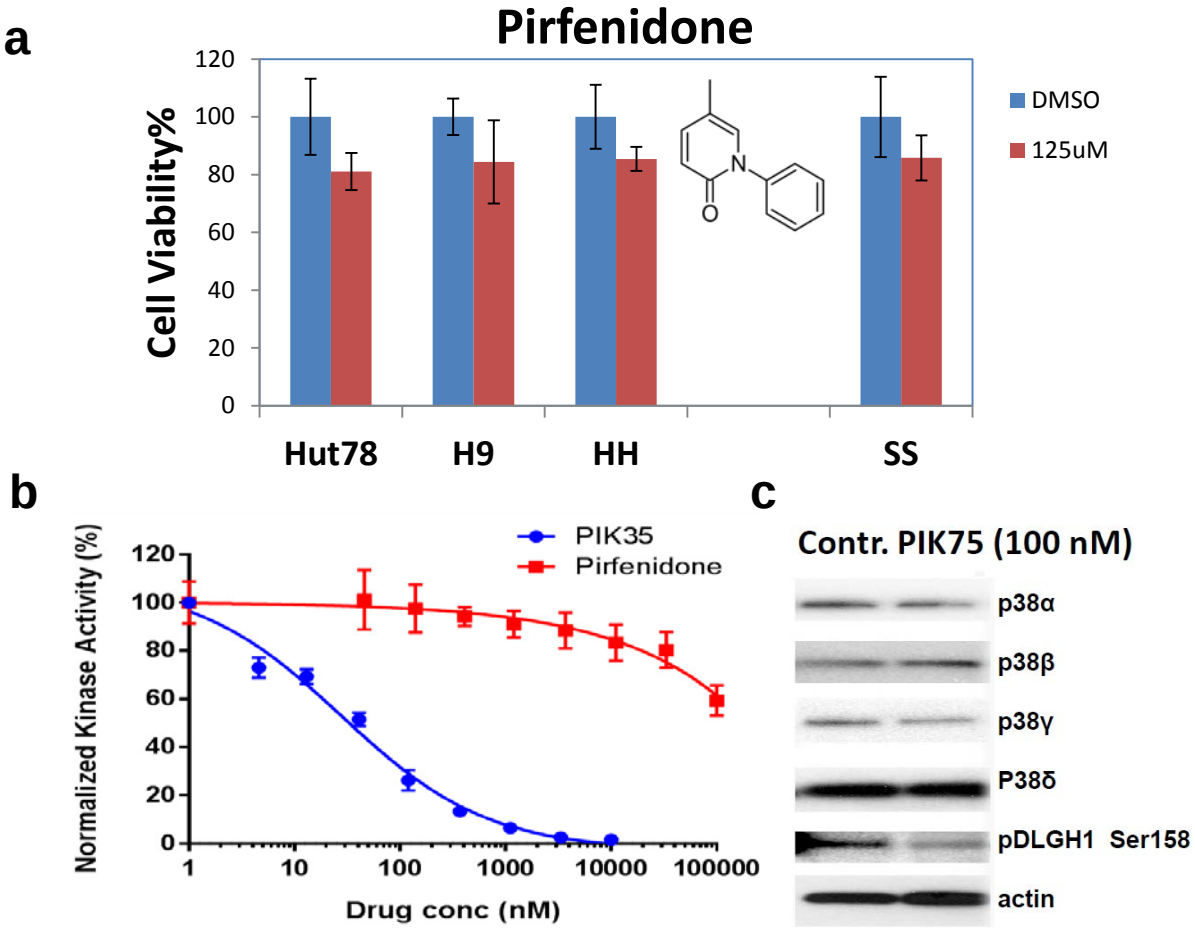


Figure S2. p38γ inhibitors F7/PIK75 and pirfenidone

a) IC_{50} determination in CTCL cells by pirfenidone. CellTiterGlo assay was used to measure viability in Hut78, H9, or HH CTCL cell lines and one SS patient sample treated with DMSO (vehicle control) or Pirfenidone (125 μM). Data are an average of 3 replicates.

b) p38γ inhibitor Pirfenidone effects on p38γ kinase activity. ADP-Glo *in vitro* kinase assay was used to measure p38γ kinase activity in with varying concentrations of F7 (PIK75) or Pirfenidone, normalized to DMSO control in cell-free-based assays. Data are an average of 3 replicates.

c) PIK75 interferes p38γ kinase activities. Western blot was used to visualize protein expression of indicated p38 isoforms, phosphorylated DLGH1 Ser158, and actin (loading control) in CD4+ T cells from healthy donors (n=2) or SS patients (n= 2) treated with F7/PIK75 (100nM) or DMSO control for 24 h.

Supplemental Table S3. F7/PIK75 caused apoptosis in an SS patient sample and Hut78 cells.

PBMC	Percentage	Live cells	AnnexV_PI	PI Stained
SS	Control	87.3	4.26	4.78
	F7 (200nM)	42.797	17.8	35.2
Hut78	Control	82.9	4.81	5.38
	F7 (200nM)	44.48	25.5	29.53

Supplemental Figure S3

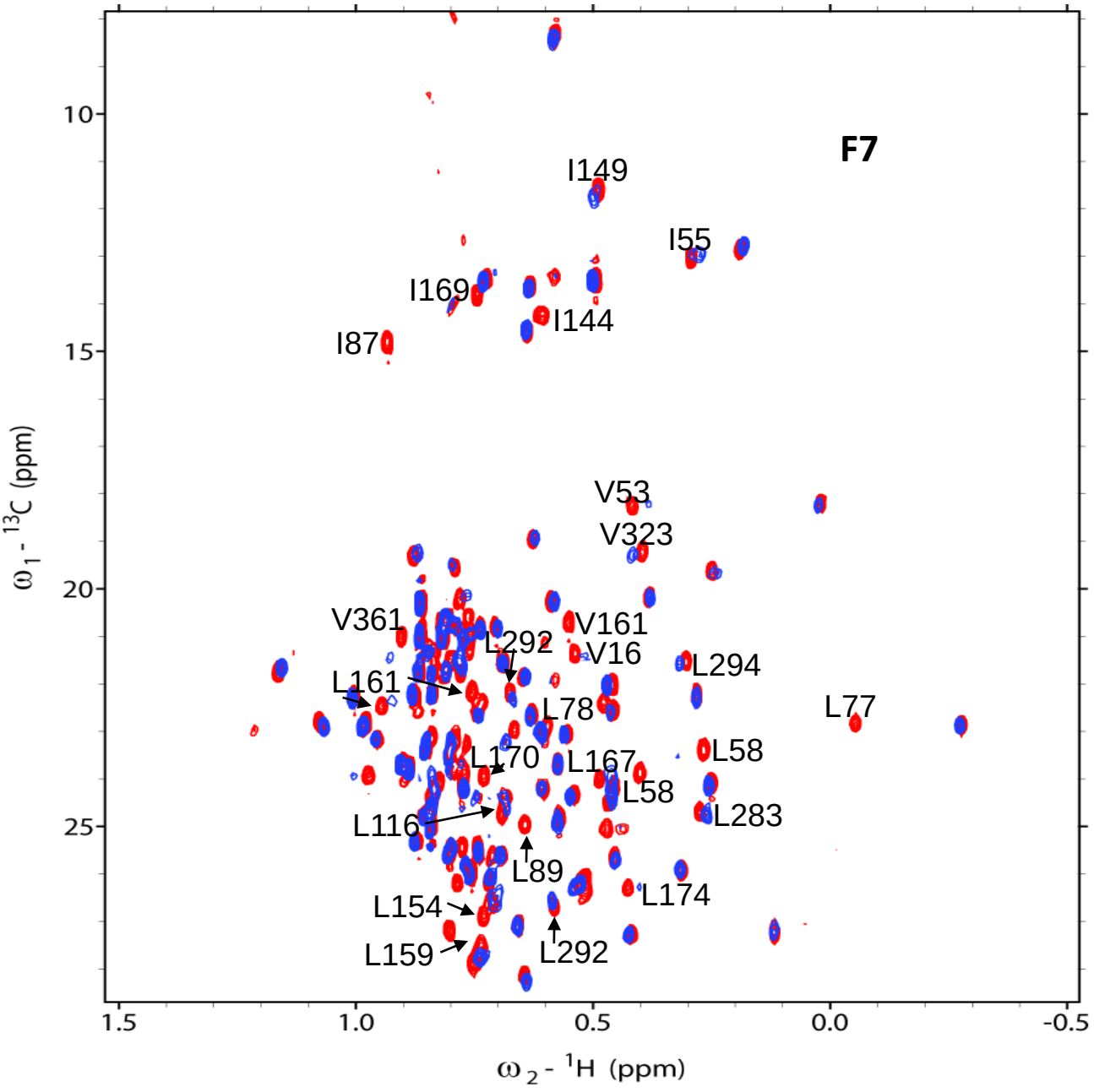


Figure S3. Assignments of F7/PIK75 to p38γ residues by NMR experiment.
Overlay of the ^{1}H-^{13}C HMQC spectra in the methyl region for p38γ, free (red), and in complex with F7/PIK75 (blue). The peaks that undergo large chemical shift changes (CSP > 0.05 ppm) or line broadening are labeled with their corresponding residue number.

Supplemental Table S4. Effect of compound F7/PIK75 on p38 isoforms.

p38	%Inhib. 50nM	%Inhib. 200nM
p38 α	- 1.92%	- 0.48685%
p38 β	- 21.26%	- 24.845%
p38 δ	5.35%	34.91%
p38 γ	22.45%	58.98%

Supplemental Table S5.

NanoString RNA and IPA data analysis revealed top 9 pathways in Hut78 cells altered by F7/PIK75 treatment (50 nM for 10 h) including 2 upregulated pathways and 7 downregulated pathways.

Pathway Name	Exp fold change	Activation z-score	p-value
NF-kB signaling	-0.633	-3.623	5.76 E-90
Th2 pathway	-0.687	-2.236	7.96 E-87
Th1 pathway	-0.719	-3.533	6.95 E -85
IL-6 Signaling	-0.717	-3.109	1.80E -79
Role of NFAT in regulation of the immune response	-0.53	-3.962	7.96 E -67
PI3K signaling	-0.586	-3.108	1.04E -55
PTEN	0.588	1.25	3.21 E _52
PI3KI/AKT signaling	-0.54	-0.63	1.11E -46
Wnt/B-catenin signaling	0.426	1.152	5.70E -41

Supplemental Figure S4

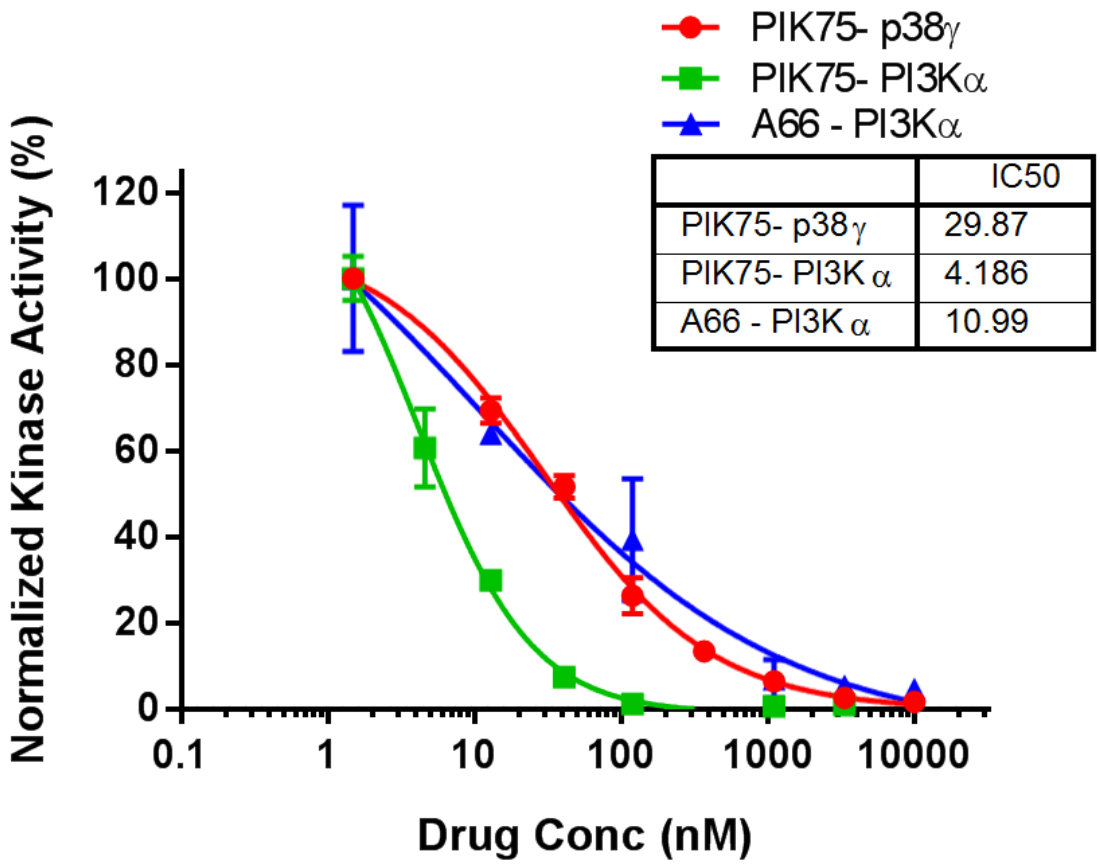


Figure S4. A66 effects on cell-based analysis**.** Western blot was used to visualize A66 effects on Hut78 cells, indicated by protein expression level of downstream targets of PI3Kp110α. GAPDH is a control for protein loading.