CAL-101, a potent selective inhibitor of the p110δ isoform of Phosphatidylinositol 3-kinase, attenuates PI3K signaling and inhibits proliferation and survival of Acute Lymphoblastic Leukemia in addition to a range of other hematological malignancies

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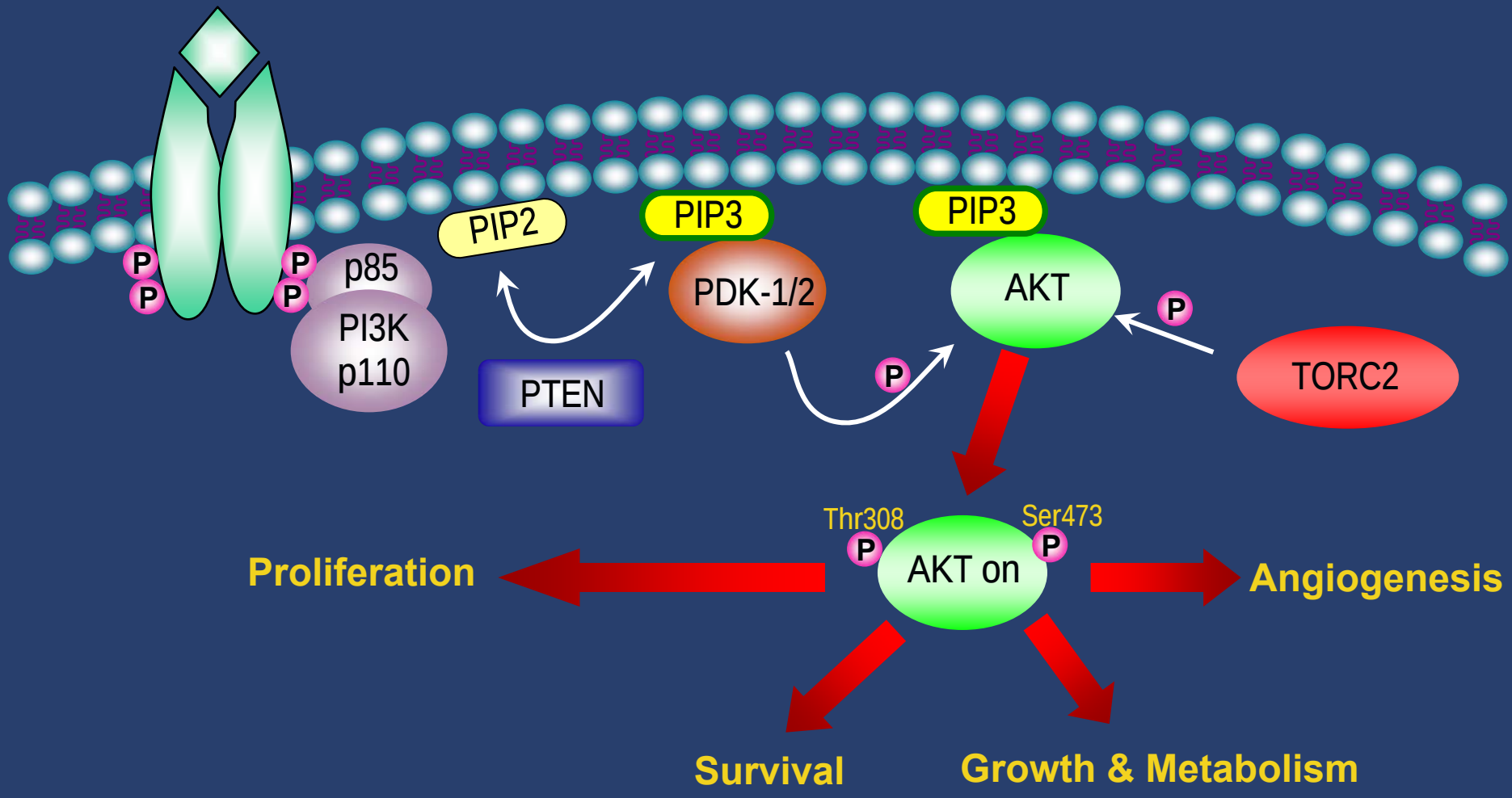
PI3K Signaling



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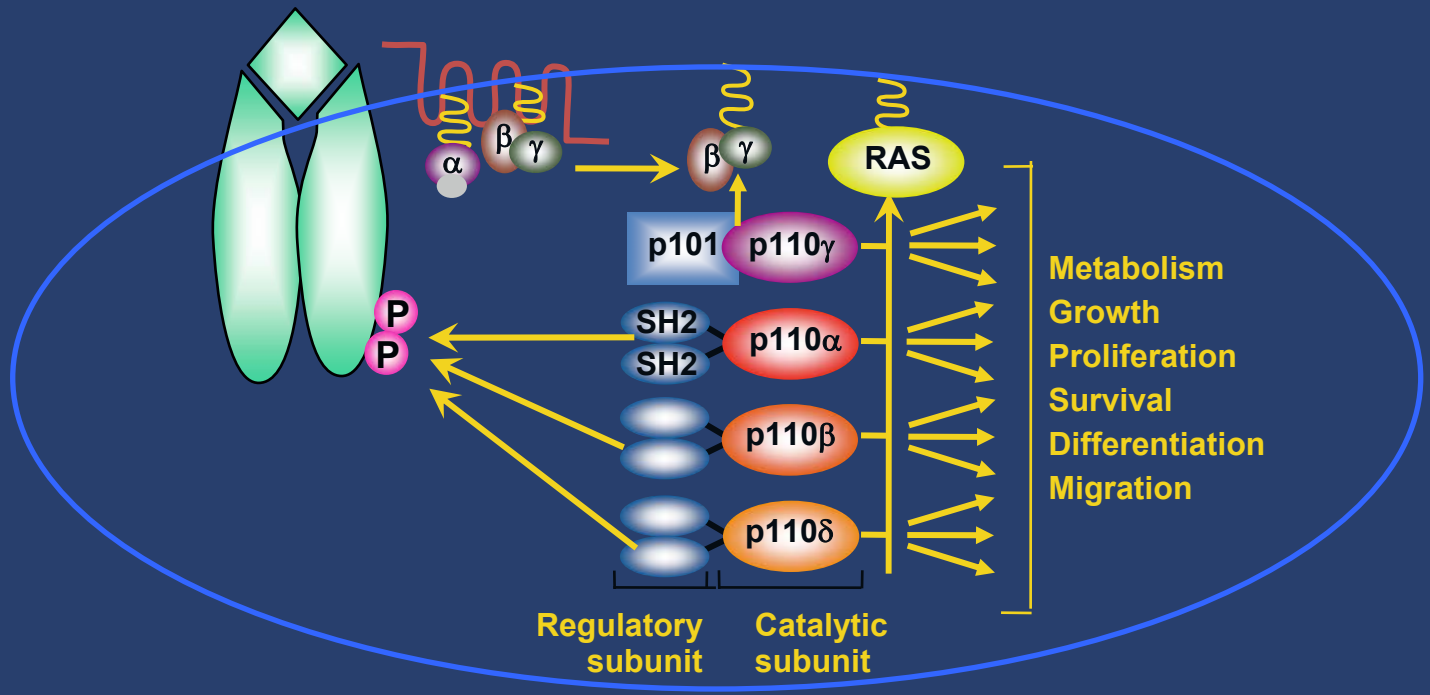
Class I Phosphoinositide 3-Kinases

Expression Profile	ubiquitous	ubiquitous	Leukocytes	Leukocytes
Class I PI3K Isoforms	α	β	δ	γ



Expression of p110δ is predominantly restricted to leukocytes in normal tissues

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Genetic Ablation of p110δ Results in Mild Phenotype Restricted to Immune System

- Fertile with no gross anatomical or behavioral abnormalities
- Peripheral blood cell counts normal
- T cell development in thymus appears normal
- Reduced number of B220+IgM+ B cell progenitors in bone marrow
- Reduced level of serum immunoglobulin
- B cell function most affected
  - Proliferation, differentiation and apoptosis
  - Response to B cell survival factors (BCR, CD40, IL-4, chemokines)

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CAL-101 is Highly Selective for PI3K p110δ

IC_{50} (nM)	Fold-Selectivity								
	Class I PI3Ks			Class II PI3K	Class III PI3K	Class IV PI3K		Other Phosphoinositide kinases	
p110δ	p110α	p110β	p110γ	CIIbeta	hVPS34	DNA-PK	mTOR	PIP5K α	PIP5K β
2.5	>300X	>200X	>40X	>400X	>400X	>3000X	>4000X	>400X	>400X

*Invitrogen's SelectScreen Kinase Profiling Service
ATP concentrations used at Km values

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CAL-101 is Highly Selective for PI3K p110δ

PI3Kα EC_{50} (nM)	PI3Kγ EC_{50} (nM)	PI3Kδ EC_{50} (nM)	PI3Kγ EC_{50} (nM)
Fibroblast Cell Line	Monocyte Cell Line	Whole Blood (Basophil)	Whole Blood (Basophil)
PDGF induced pAKT	C5a induced pAKT	Anti-FcεRI induced CD63 Expression	fMLP induced CD63 Expression
>20,000 (n=12)	3,894 (n=11)	65 (N=11)	3,190 (N=9)

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CAL-101 Has No Off Target Activity in Kinome-Wide Protein Kinase Screen

No activity against >300 protein kinases

Examples of Relevant Kinases in Screen

ABL	FGFR1	JAK1	P38MAPK	S6K
AKT	VEGFR1	JAK2	PDGFR	SLK
ALK	FLT3	JNK1	PIM	SRC
BLK	FRK	KIT	PKA	SYK
BRAF	FYN	LCK	PKC	TAK
BTK	HCK	LYN	PLK	TIE
CDK	HER2	MAPK	RAF	TRK
CSF1R	ICK	MEK	RET	TYK
EGFR	IGF1-R	MET	ROCK	YES
EPH	ITK	MLK	ROS	ZAP70

Ambit KINOME*scan*™

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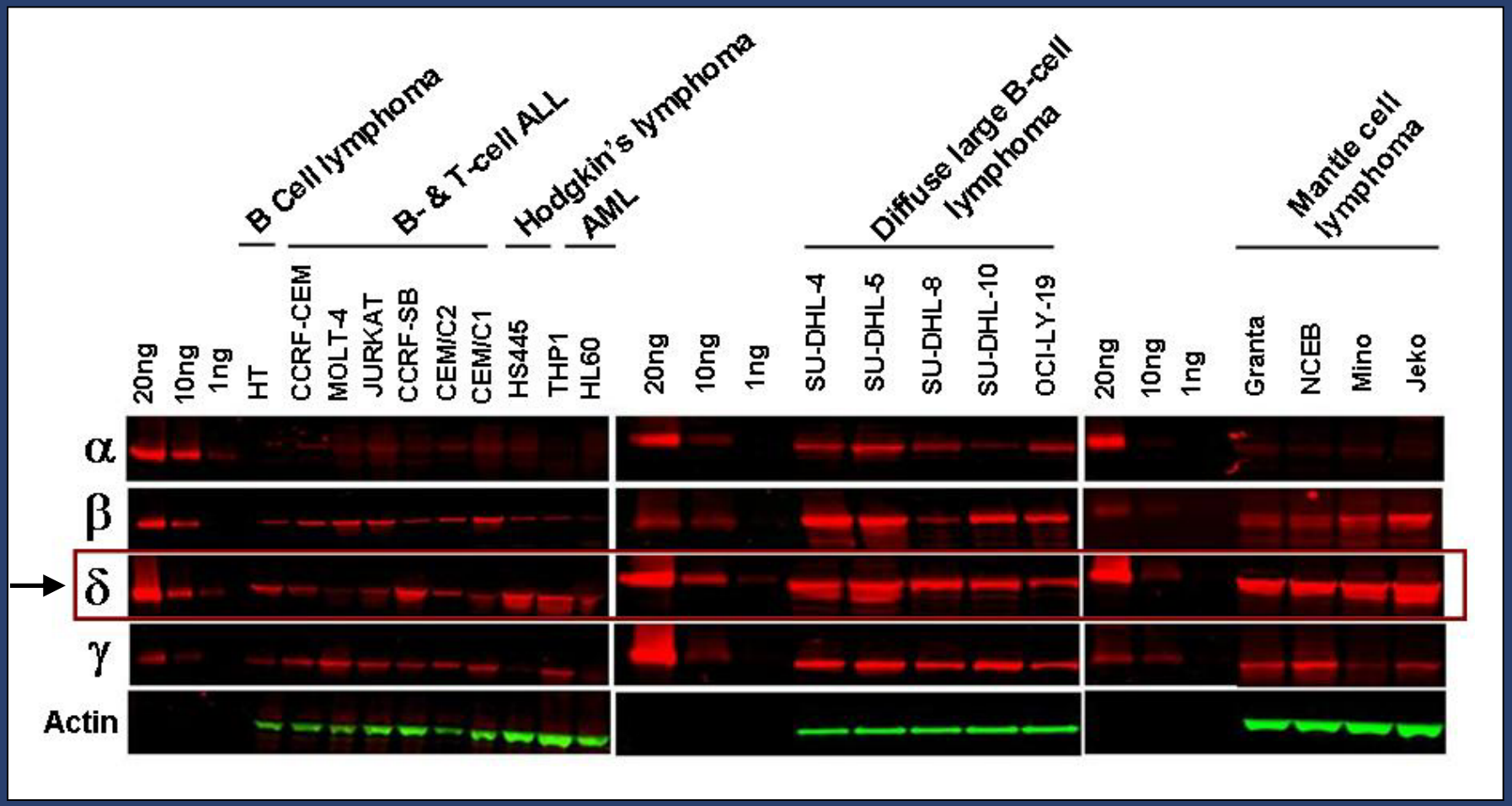
PI3K p110δ Highly Expressed in a Broad Range of Leukemia and Lymphoma Cell Lines



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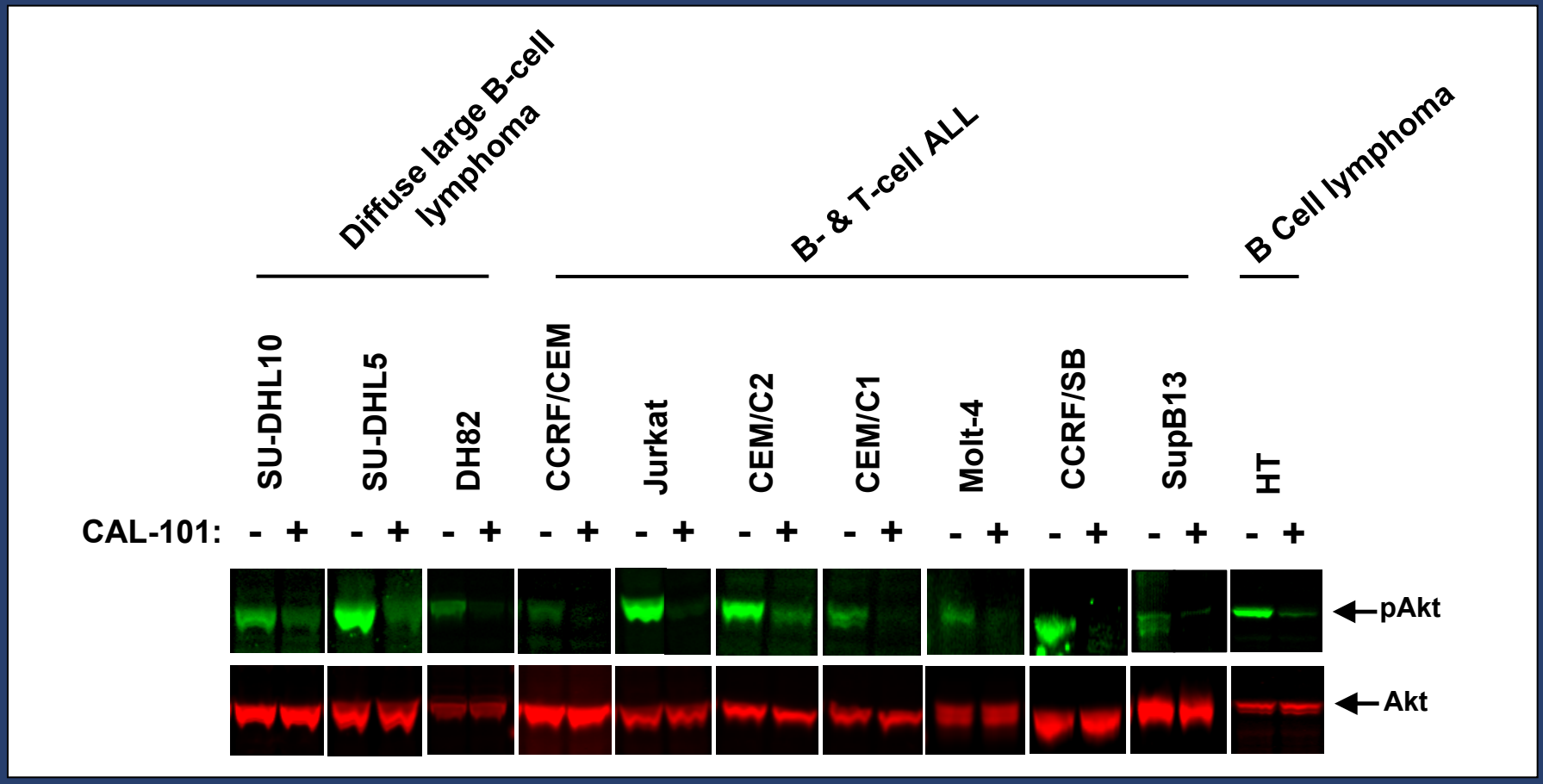
CAL-101 Inhibition of p110δ Blocks PI3K Signaling in Leukemia and Lymphoma Cell Lines



PI3Kδ Implicated in Many Hematological Malignancies

Indication	CAL-101 Preclinical POC
AML	**Primary patient cells** •Blocks PI3K signaling •Inhibits proliferation
Non-Hodgkins Lymphomas (MCL, DLBCL, FL)	**Cell lines** •Blocks PI3K signaling •Induces apoptosis
MM *Myeloma-Pathophysiology & Preclinical studies Sunday **(Poster II-84)**	**Primary patient cells** • P110δ overexpressed in 24/24 samples • Induces apoptosis
CLL *Chronic Lymphocytic Leukemia – therapy Monday **(Poster II-247)**	**Primary patient cells** •Induces apoptosis •Blocks survival factors
ALL	**Cell lines** •Blocks PI3K signaling •Induces apoptosis •G_1 arrest

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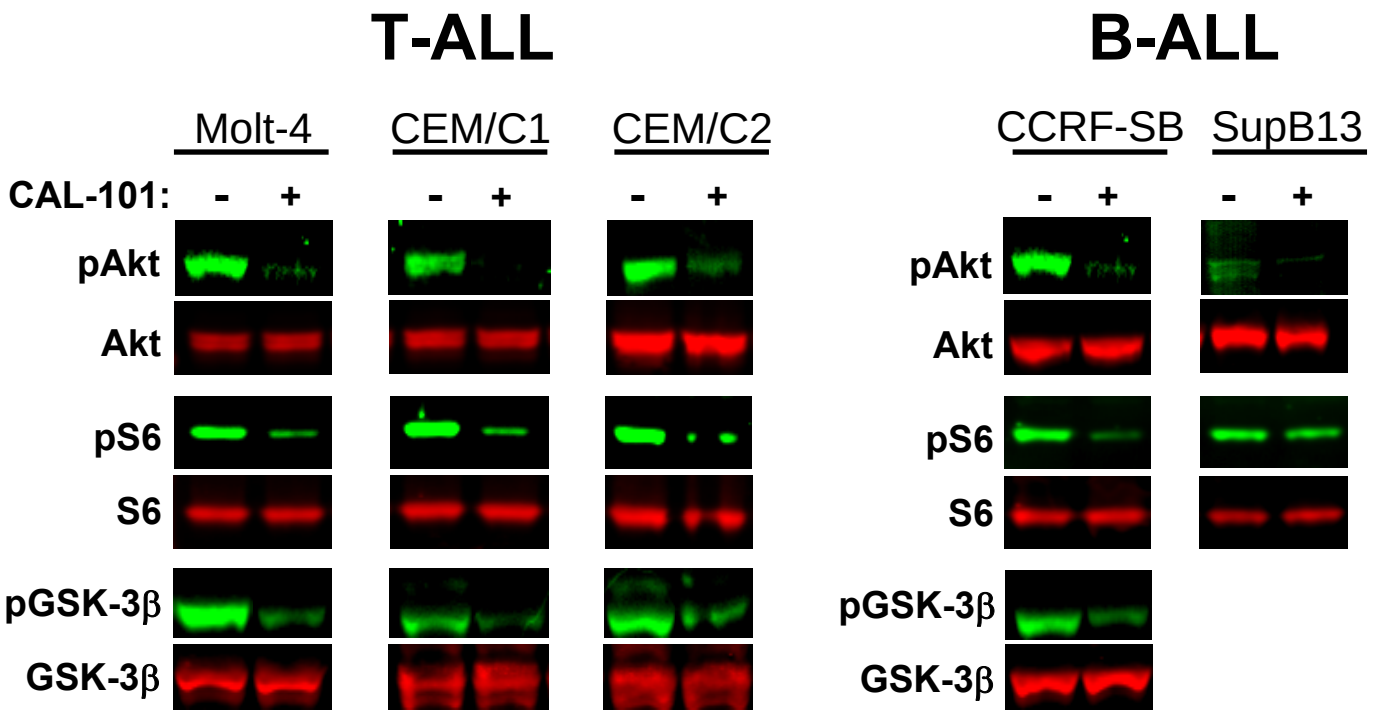
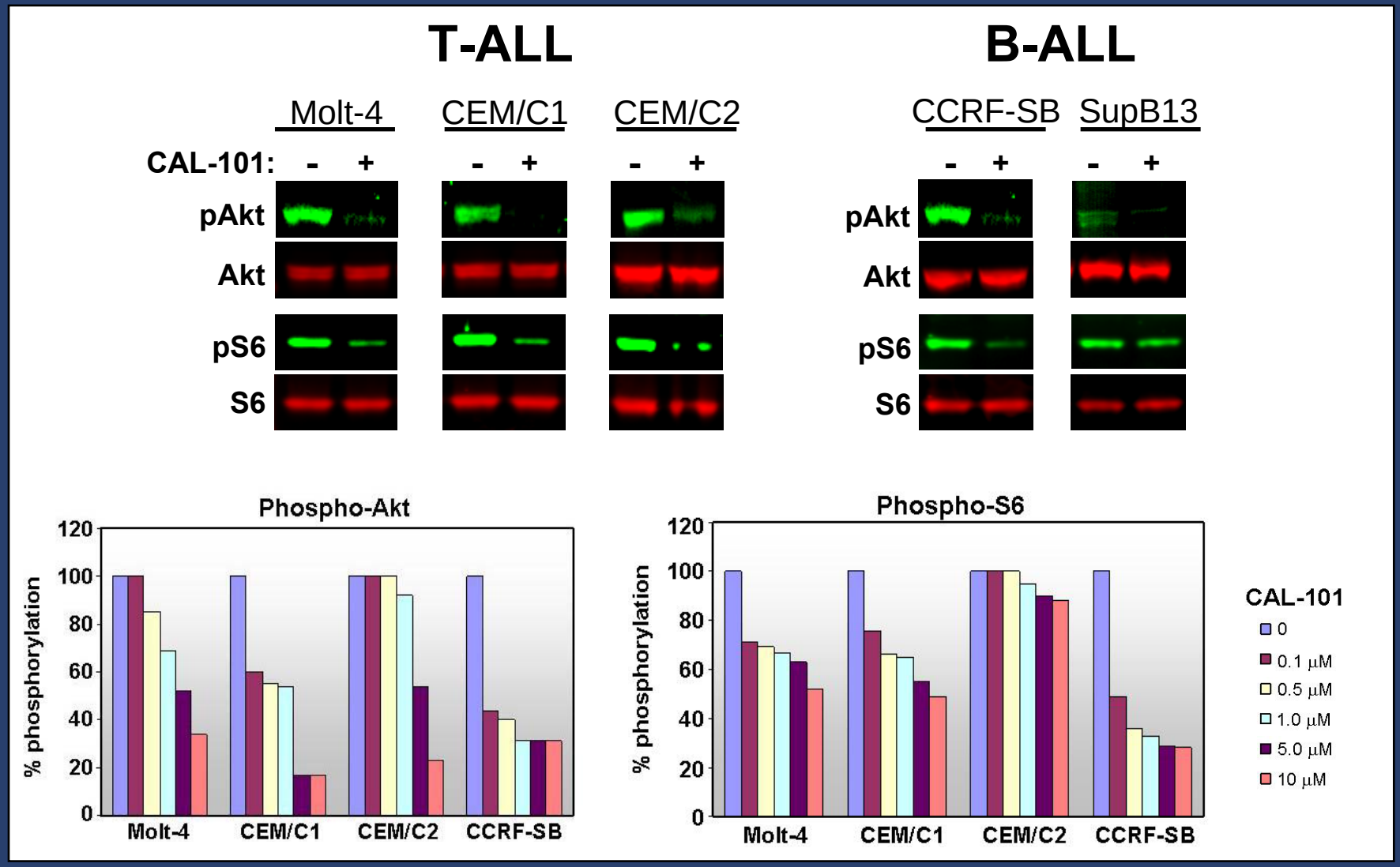
CAL-101 Inhibition of p110δ Blocks PI3K signaling in ALL Cell Lines



CAL-101 Inhibition of p110δ Blocks PI3K signaling in ALL Cell Lines



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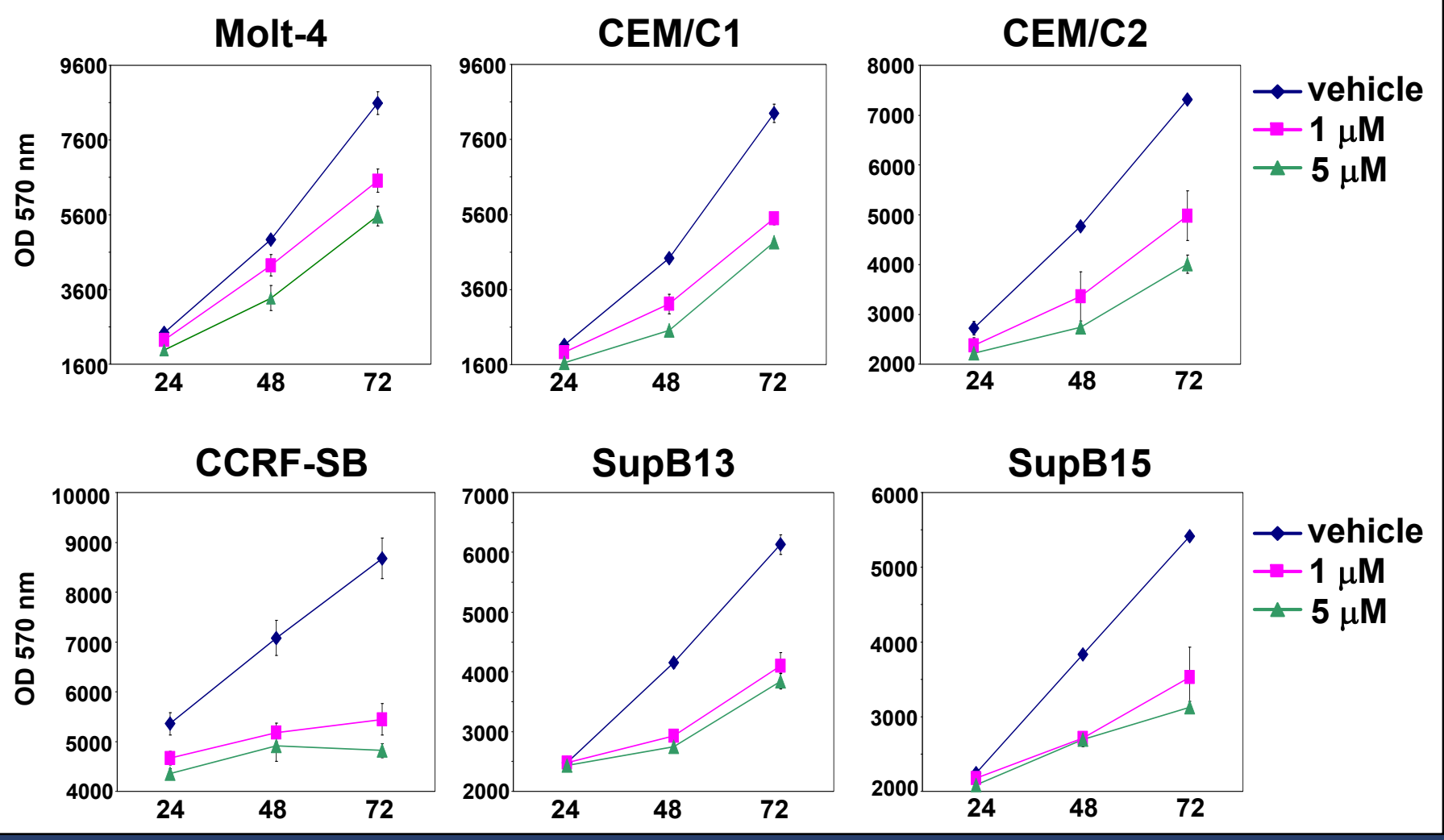
CAL-101 Inhibits Proliferation in Both T-ALL and B-ALL Cell Lines



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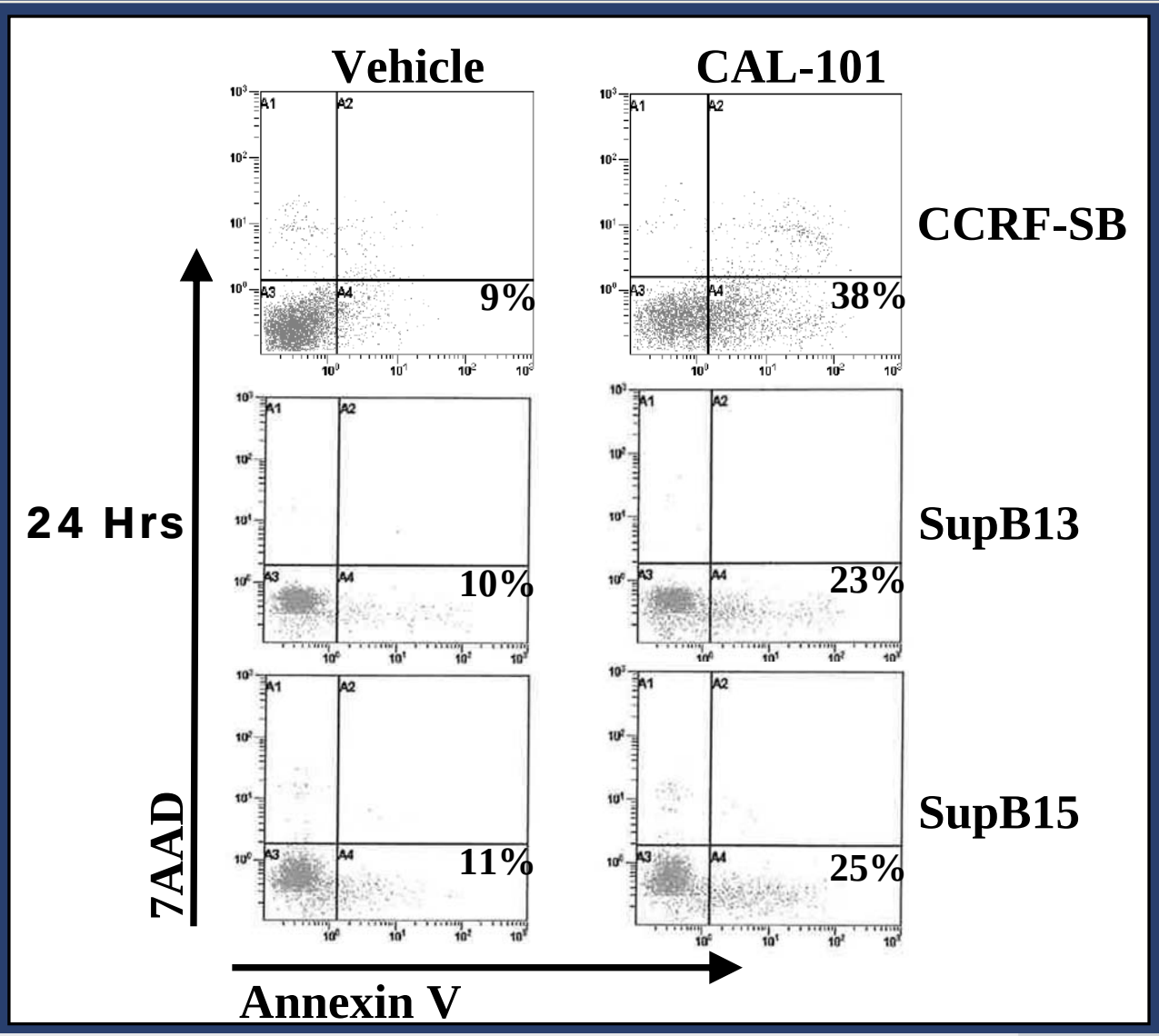
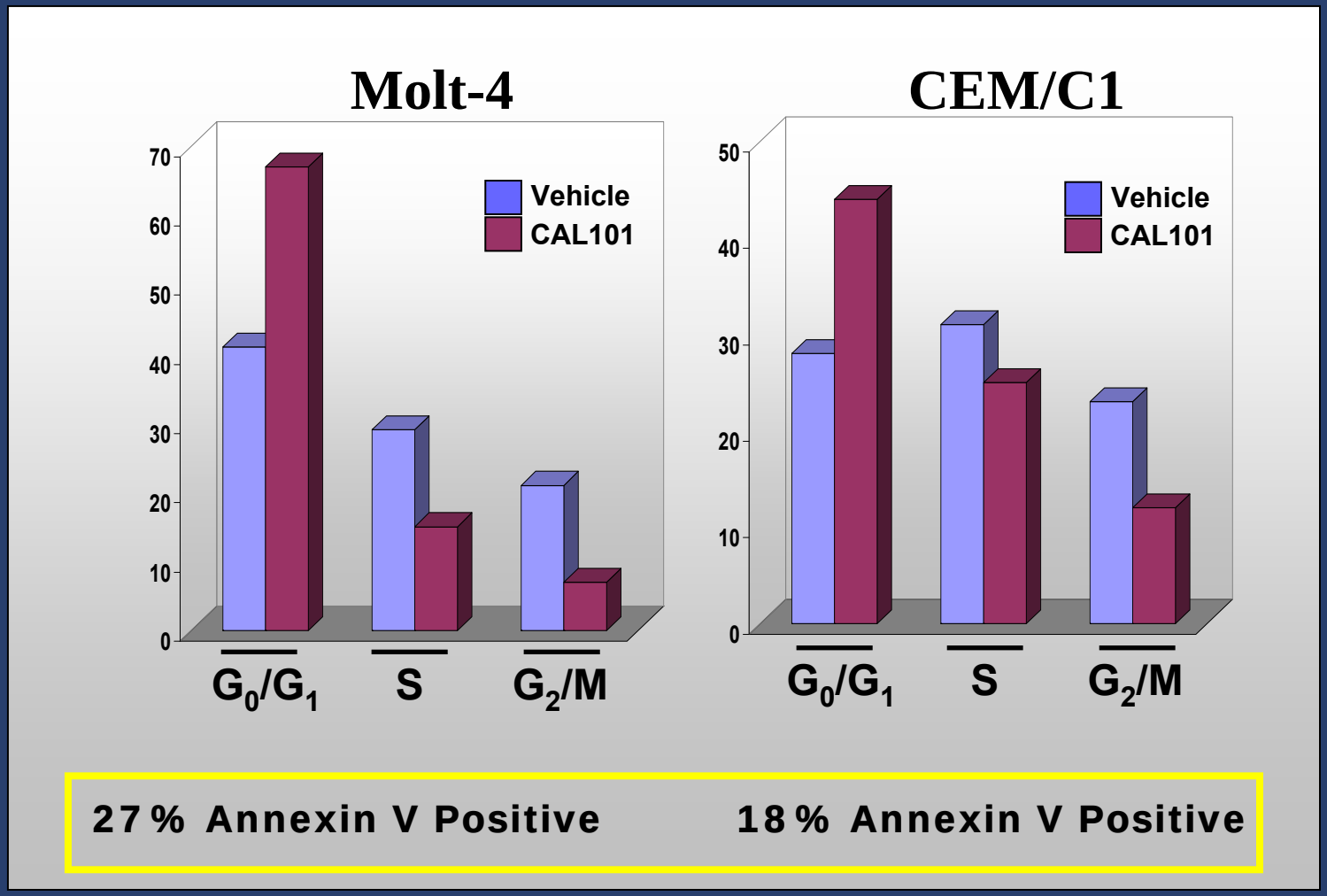
CAL-101 Induces Apoptosis in B-ALL Cell Lines



CAL-101 Induces Cell Cycle Arrest in T-ALL Cell Lines Followed by Apoptosis



27% Annexin V Positive **18% Annexin V Positive**

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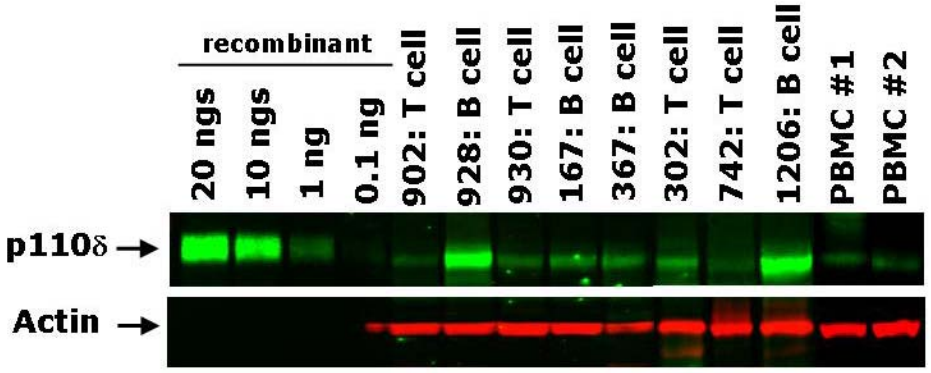
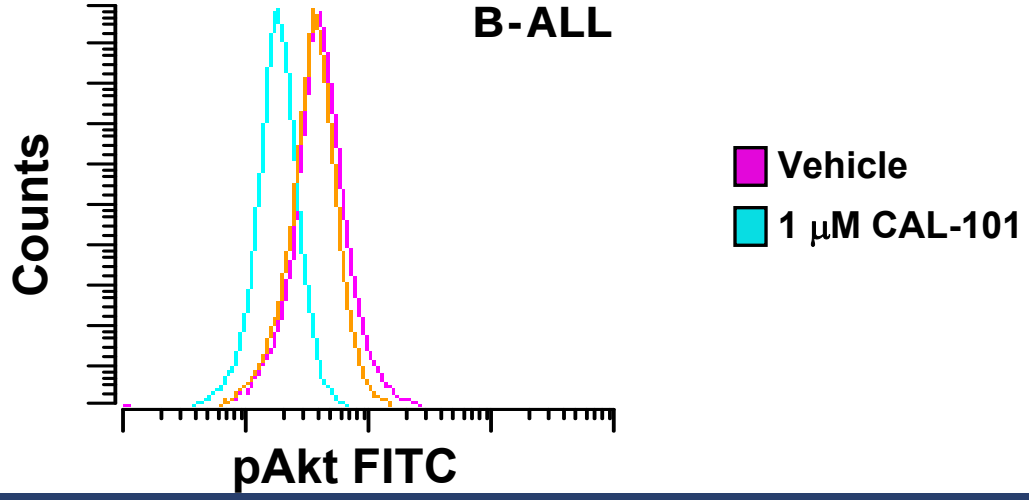
Primary ALL Patients Samples Express p110δ and have elevated levels of pAkt which are reduced with CAL-101 Treatment



Phospho-Flow Cytometric data



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Conclusions

- P110δ is highly expressed across a broad range of leukemia and lymphoma cell lines
- CAL-101 has been shown to be effective in a wide range of hematological malignancies
- Effects of CAL-101 on ALL cell lines
  - Inhibition of p110δ with CAL-101 treatment results in a reduction of Akt phosphorylation which correlates in a decrease in cellular proliferation
  - CAL-101 induced apoptosis in both T and B ALL
- Samples from ALL patients express p110δ and have elevated levels of pAkt which is inhibited in the presence of CAL-101
- CAL-101 is the first selective p110δ inhibitor currently in a phase 1 clinical trial

Acknowledgments

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