



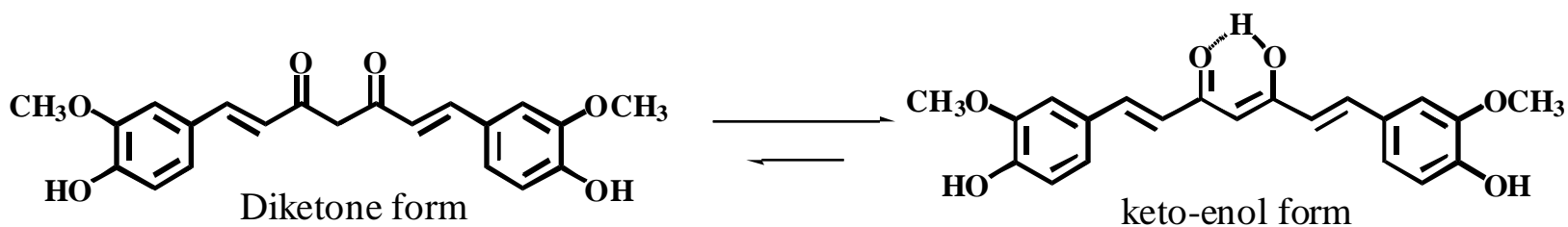
Small Molecule Curcumin Analogs Induce Apoptosis In Human Melanoma Cells Via Stat3 Inhibition But Do Not Alter The Cellular Response To Immunotherapeutic Cytokines

Gregory B. Lesinski, Matthew A. Bill, James R. Fuchs,
Chenglong Li, Courtney Bakan, Don M. Benson, Jr.,
Eric B. Schwartz, Jiayuh Lin, Dale G. Hoyt,
Stacey L. Fossey, Gregory S. Young,
William E. Carson, III, Pui-Kai Li

Presenter Disclosures

- Gregory B. Lesinski, Ph.D.
- I have no relationships to disclose.

Curcumin as a Lead Compound



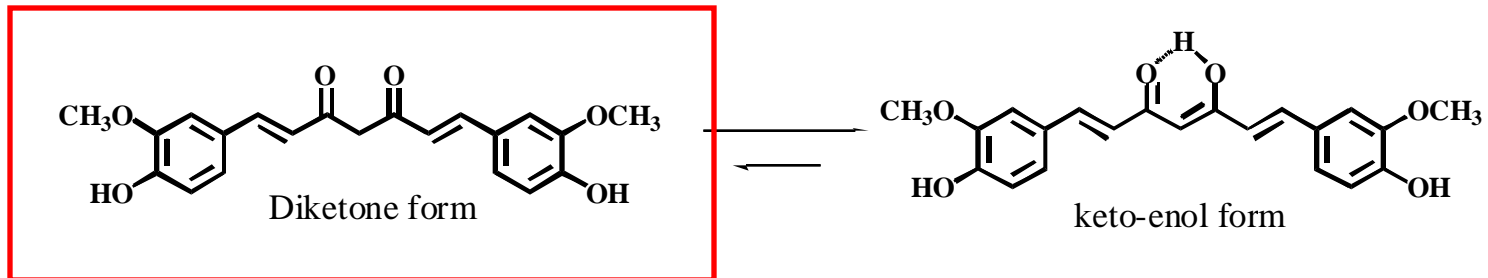
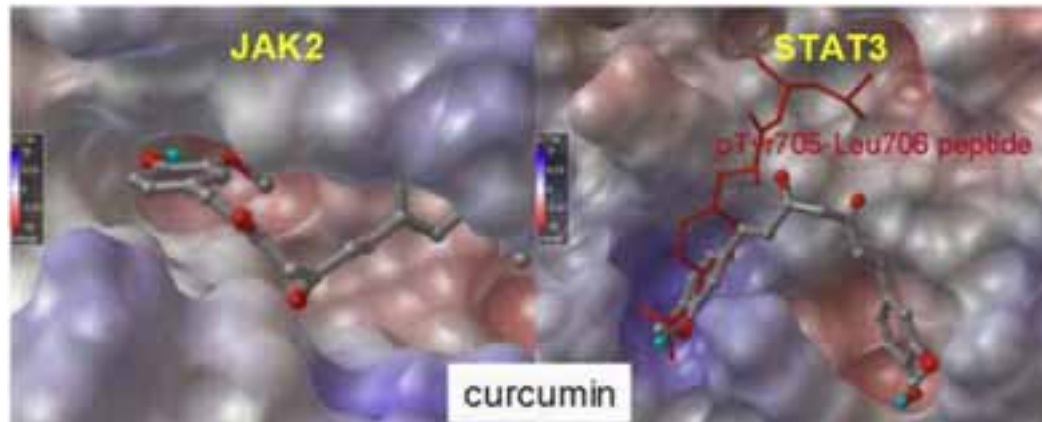
Has powerful chemopreventative and chemotherapeutic properties in animal models.

However, its lack of specificity can antagonize the action of clinically relevant cytokines.

(Bill *et al.* Mol Cancer Ther, 2009)

Experimental Approach

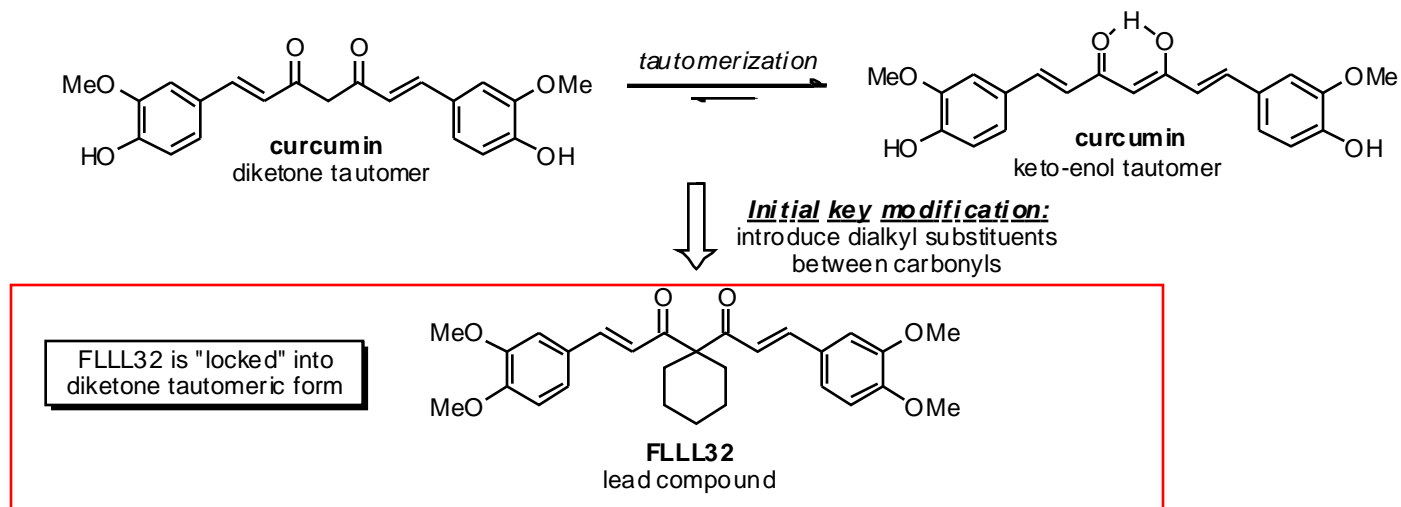
- Computational Docking Studies:
Studied the binding of curcumin to Jak2 and STAT3 proteins.



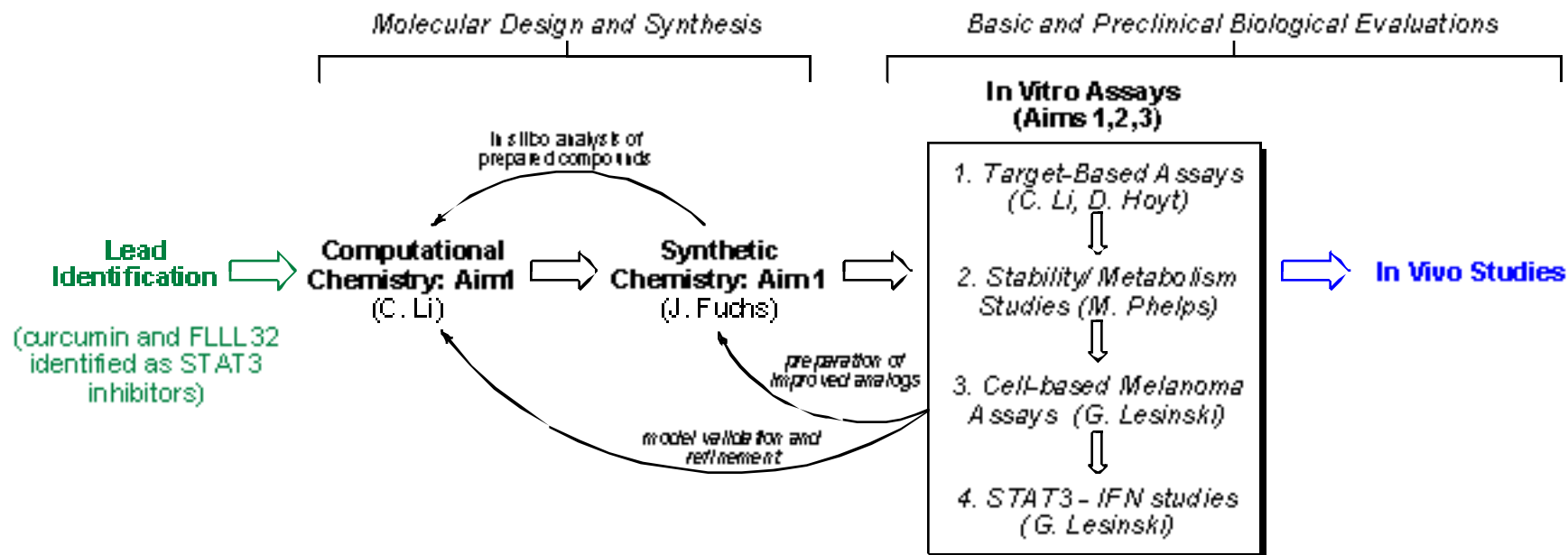
Hypothesis

We hypothesized the diketone tautomer form of curcumin can be manipulated to inhibit STAT3 phosphorylation, dimerization and induce apoptosis in melanoma cells.

FLLL32: A curcumin analog with STAT3 Inhibitory Activity.



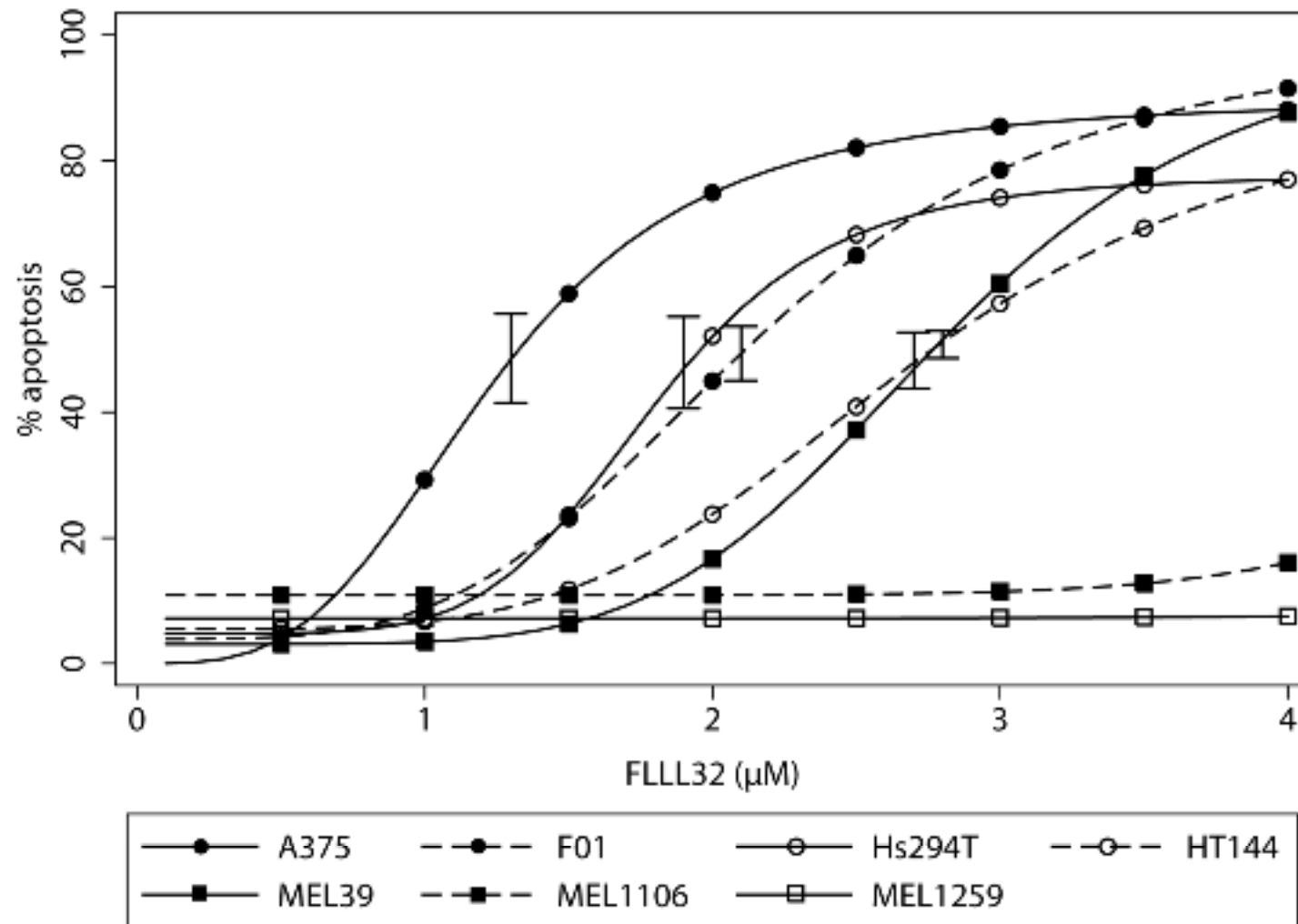
The Molecular Target Team (MTT) at OSU



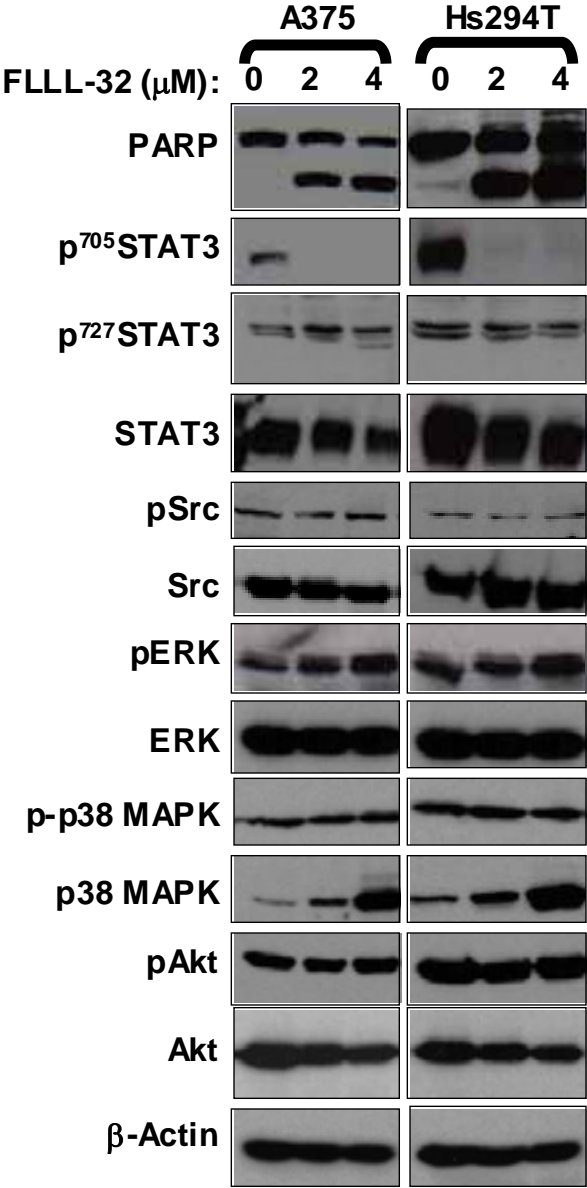
Main Focus of The MTT:

Use of natural products as lead compounds for anti-cancer drug development.

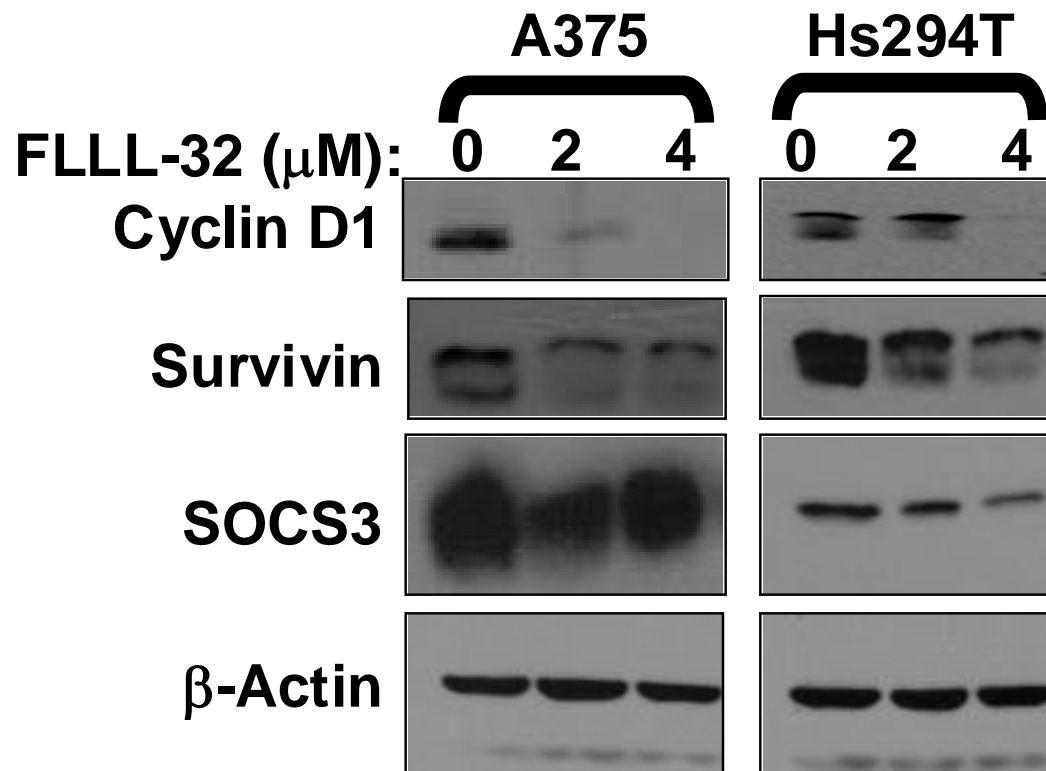
FLLL32 Induces Apoptosis in Melanoma Cells.



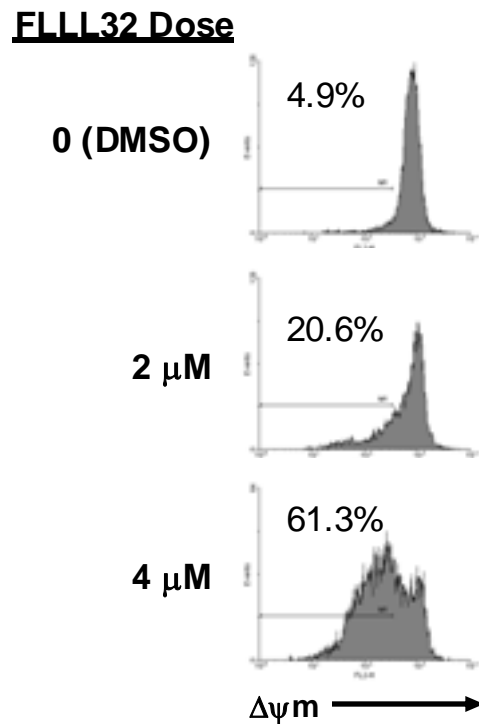
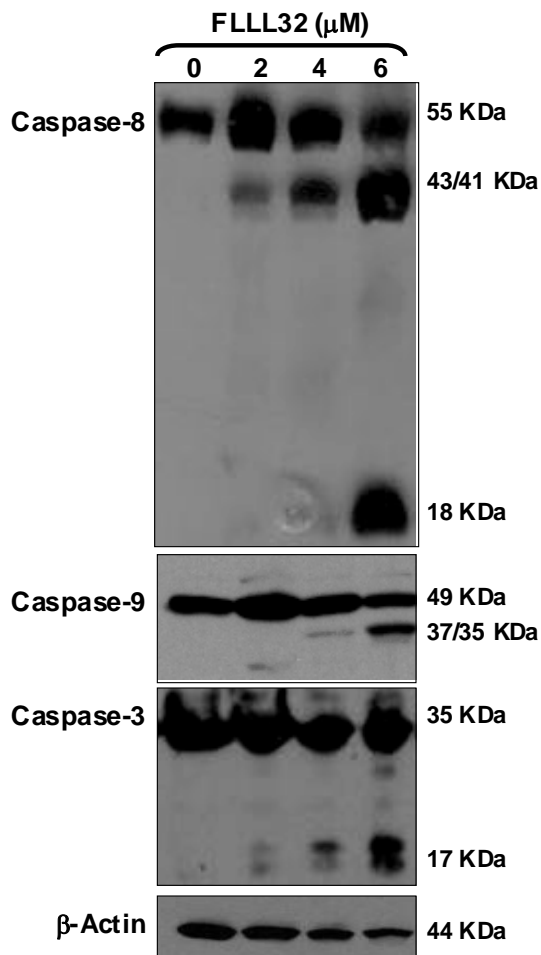
FLLL32 Induces Apoptosis in Melanoma Cells.



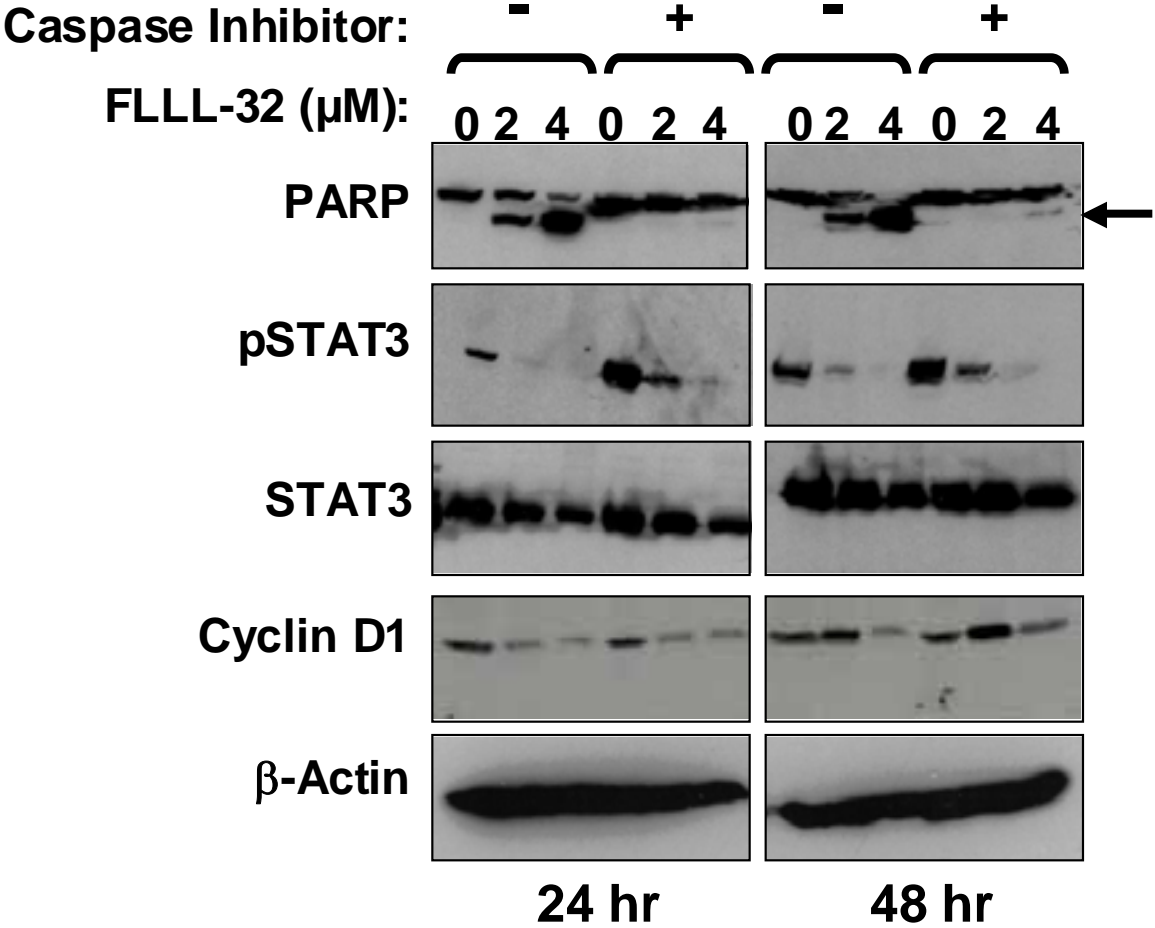
FLLL32 Inhibits STAT3 Regulated Gene Expression



FLLL32 Induces Processing of Caspase Proteins and Loss of Mitochondrial Membrane Permeability



FLLL32 Induces Caspase-Dependent Apoptosis

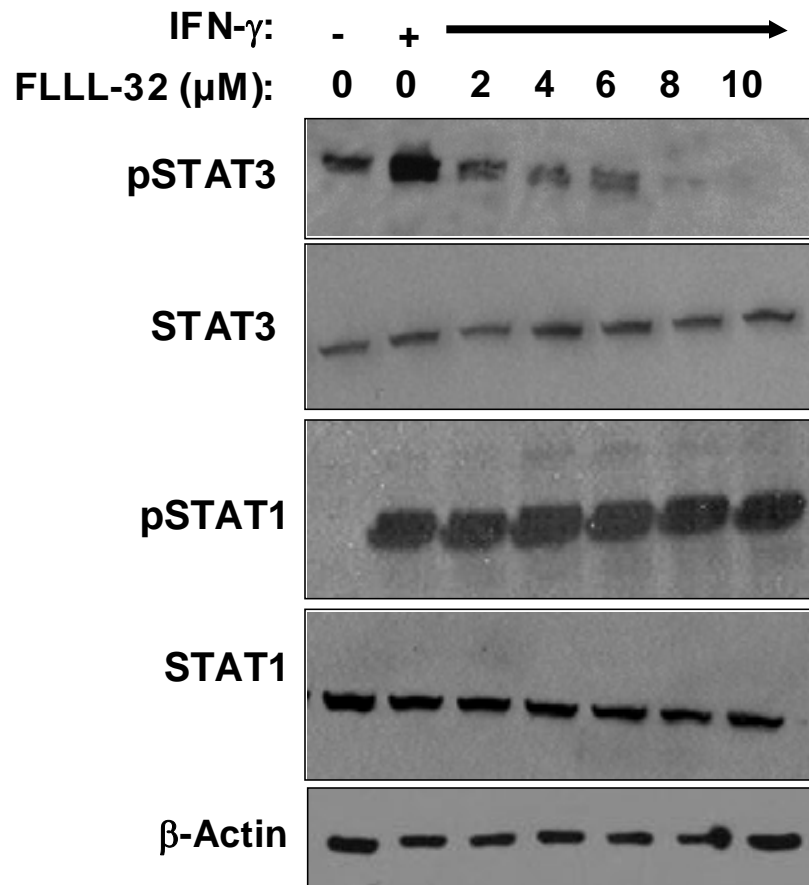


Specificity of FLLL32 for STAT3 vs. Other STAT Proteins.

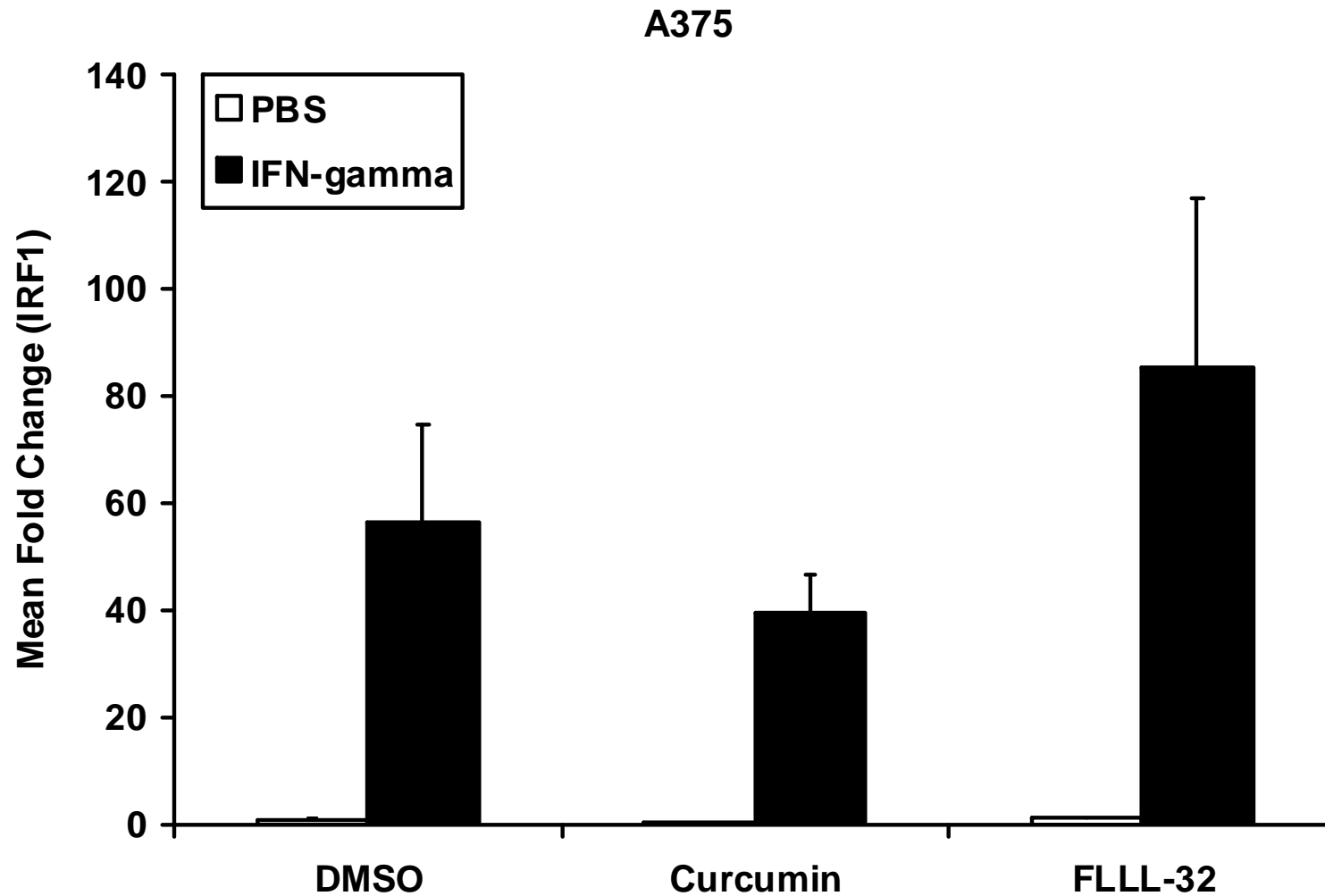
- STAT3 is highly homologous to other STAT proteins that play a role in responsiveness to cytokines that promote anti-tumor immunity.
- STAT3 inhibition represents an attractive approach to enhancing immunotherapy of cancer.
- Key Concerns:
 - IFN-gamma signals via STAT1-STAT1 homodimers.
 - Interleukin-2 signals via STAT5.
 - Jak2 inhibition may compromise IL-2 induced signaling.

FLLL32 Does Not Inhibit STAT1 Activation by IFN-gamma.

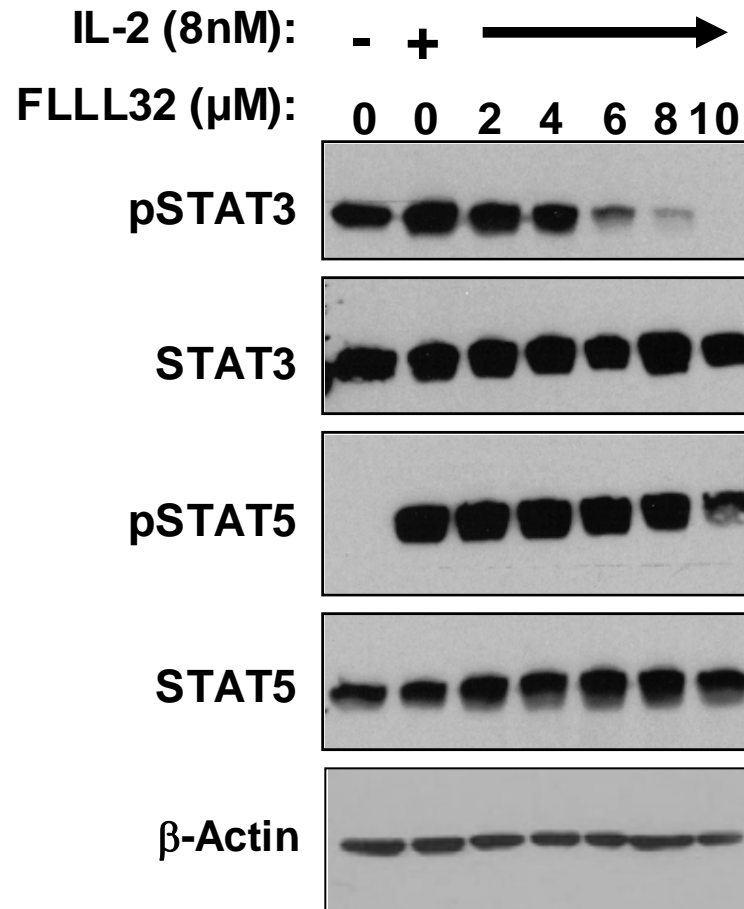
A375 Human
Melanoma
Cells



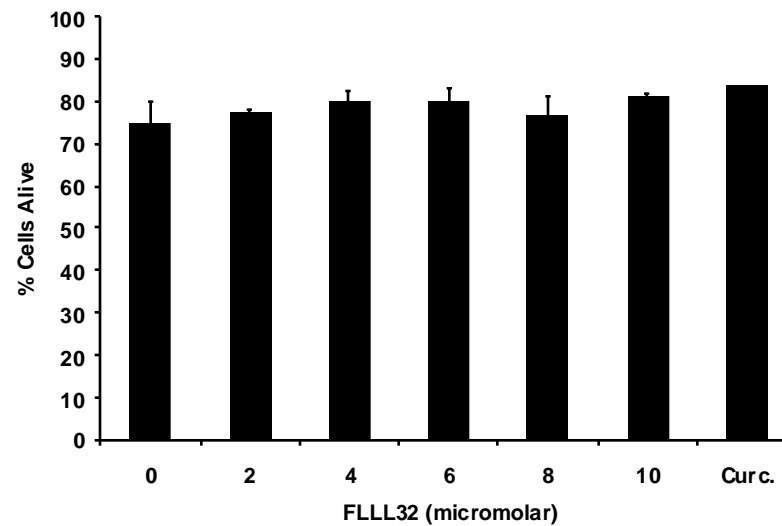
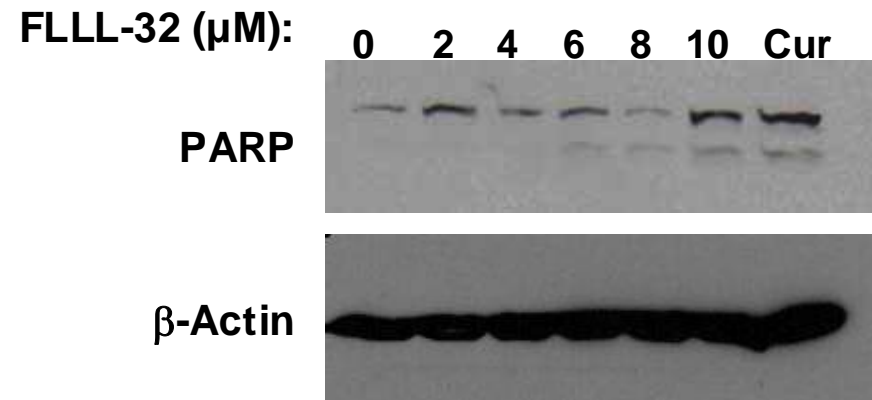
FLLL32 Does Not Inhibit IFN-gamma Induced Gene Expression.



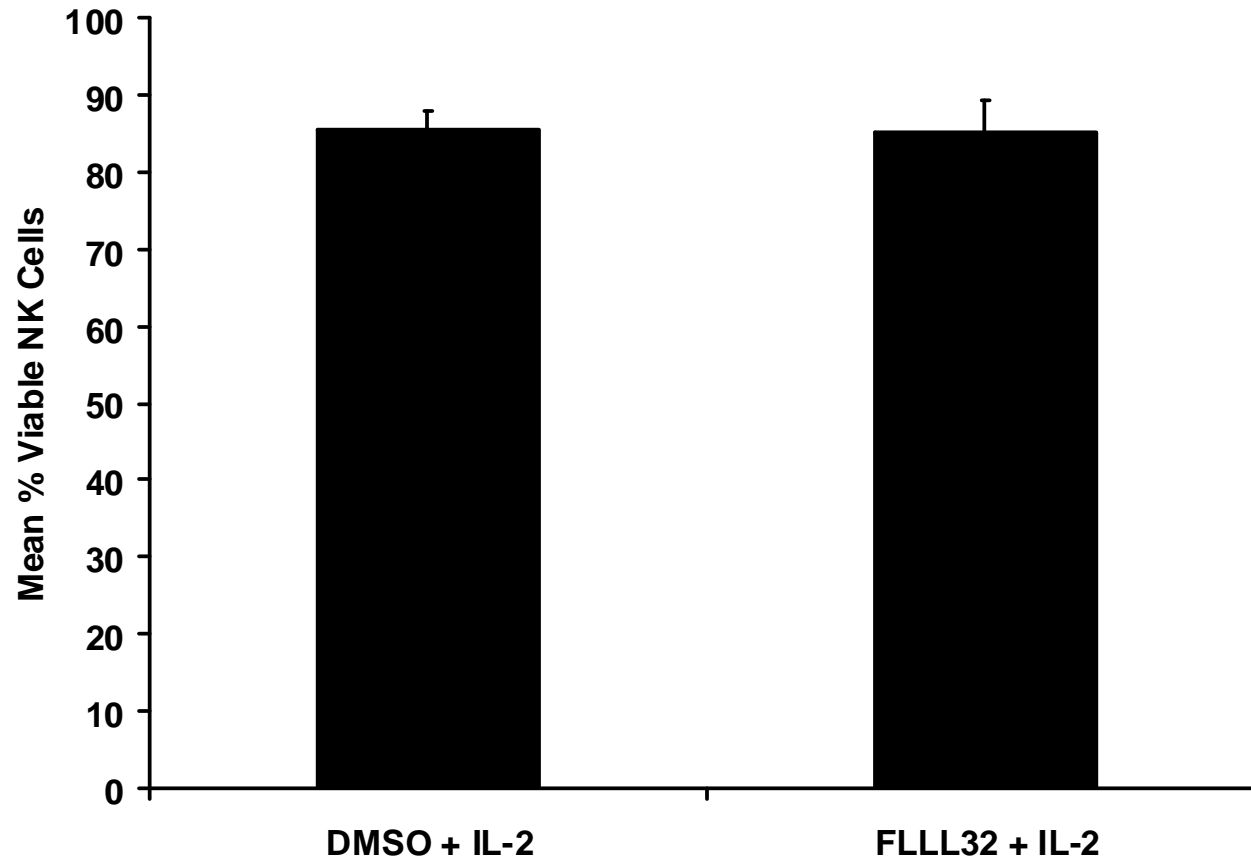
PBMCs: FLLL32 Does Not Inhibit STAT5 Activation by IL-2.



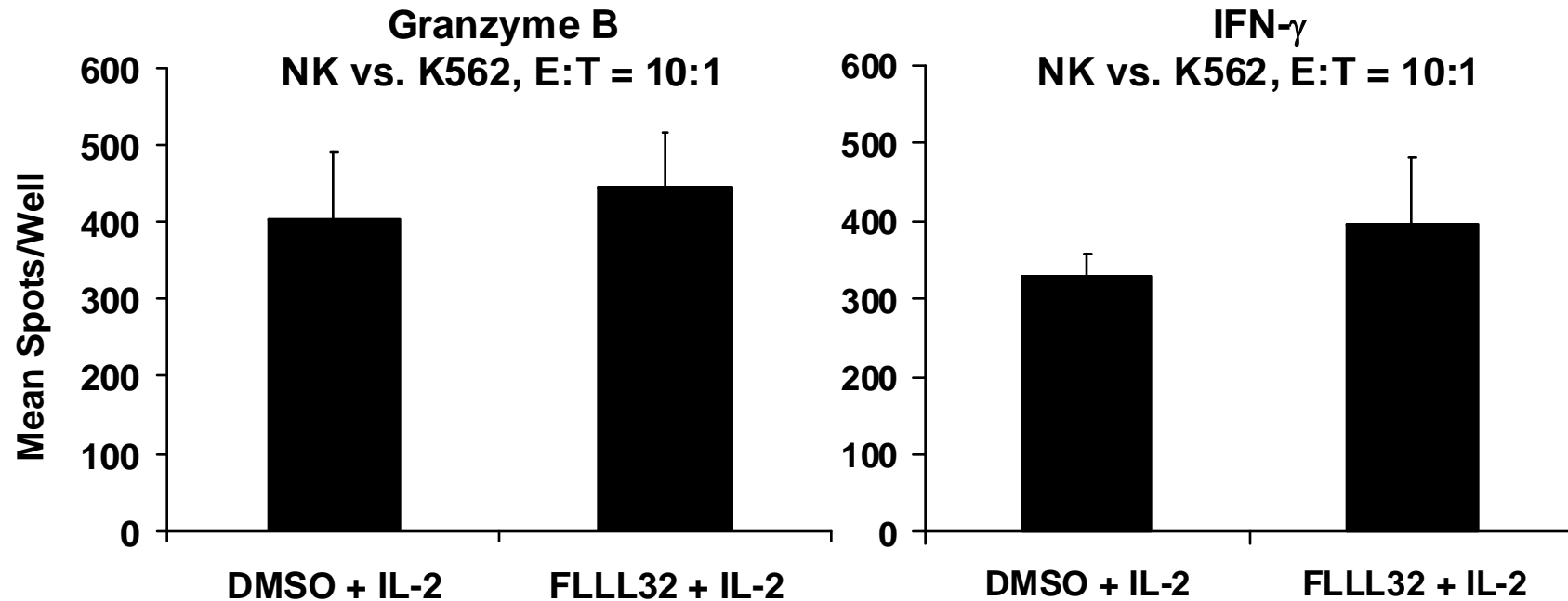
FLLL32 Does Not Induce Cell Death in PBMCs.



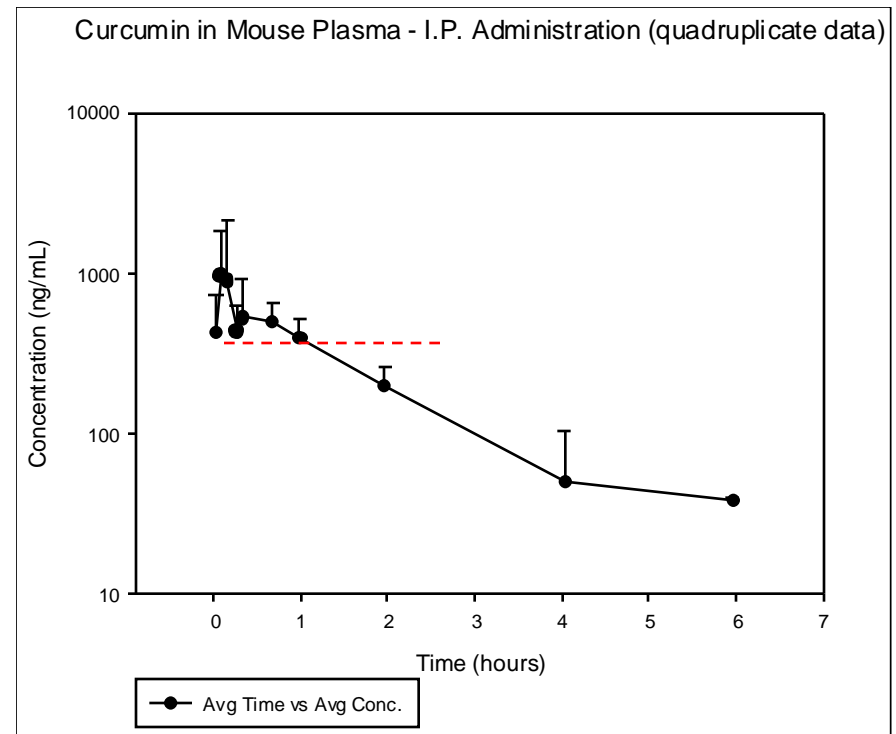
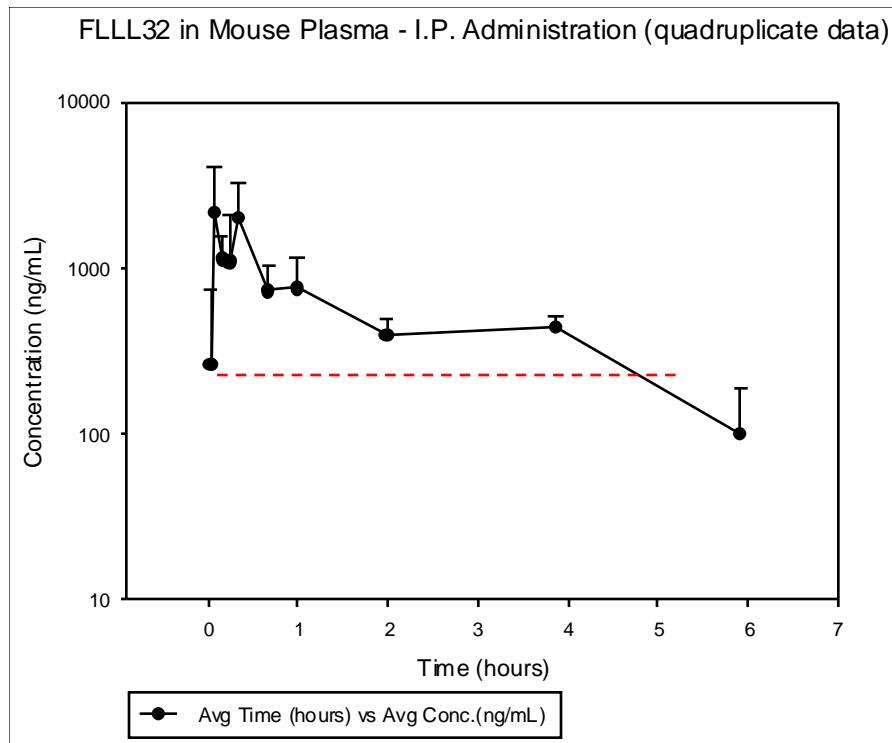
FLLL32 Effects on NK Cell Viability



FLLL32 Effects on NK Cell Cytotoxicity



FLLL32 Pharmacokinetics In Vivo.



Conclusions

- FLLL32 is a novel curcumin analog and lead compound that can act as an inhibitor of the STAT3 pathway.
- FLLL32 induces caspase-dependent apoptosis in melanoma cells.
- FLLL32 (unlike curcumin) does not inhibit IFN-gamma or IL-2 induced signal transduction in PBMCs.

Ongoing Projects

- Pharmacokinetic Studies in athymic mice.
- In vitro testing of more soluble FLLL32 derivatives.
 - Sulfamate and phosphate derivatives;
- In vivo efficacy studies against human xenografts.
- Can STAT3 inhibition be used to augment immunotherapy?

Acknowledgments

- Lesinski Laboratory

- Matthew Bill
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Mitch Phelps

William E. Carson, III

Gregory Young

Don Benson, Jr.

Courtney Bakan

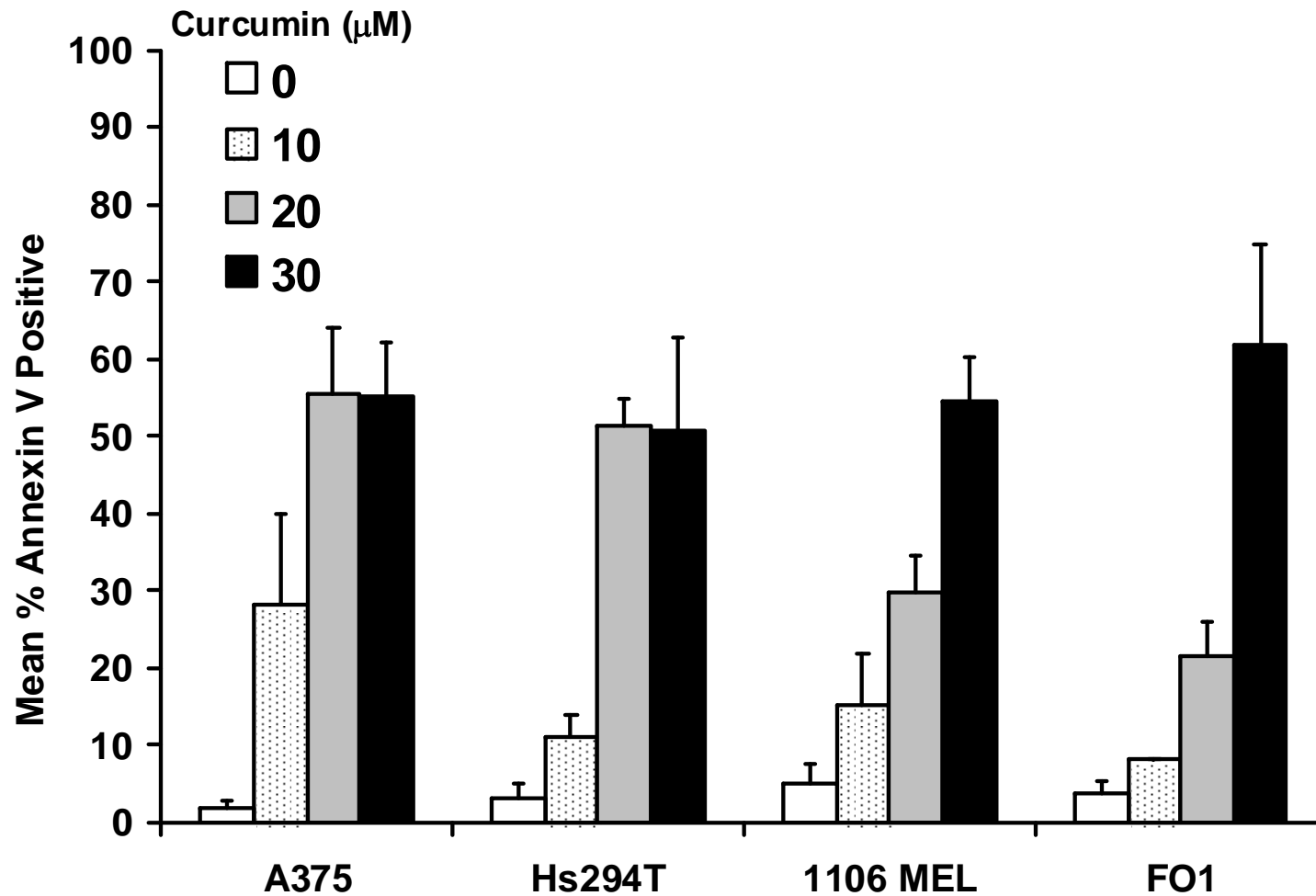
- The Molecular Target Team

- Pui-Kai (Tom) Li
- Chenglong Li
- James Fuchs
- Dale Hoyt
- Jiayuh Lin
- Samuel Kulp

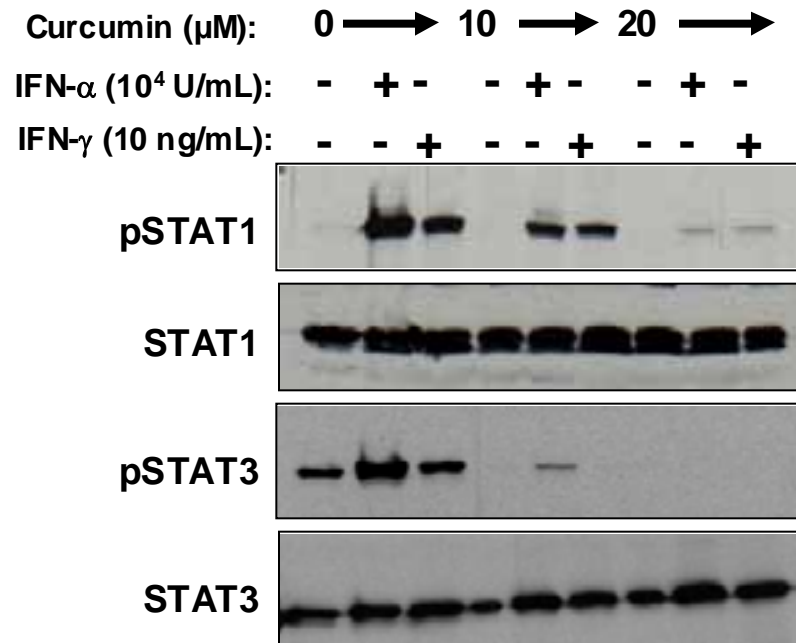
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- NCI K22 CA134551 (PI: Lesinski)

Curcumin Induces Apoptosis of Human Melanoma Cells



Curcumin Modulates IFN-responsiveness of Human Immune Cells



Curcumin Modulates IL-2 Responsiveness of Human Immune Cells

