Mechanism of Caspase Independent Activation Induced Cell Death of Human Primary T Lymphocytes: Implications for Cancer Immunotherapy

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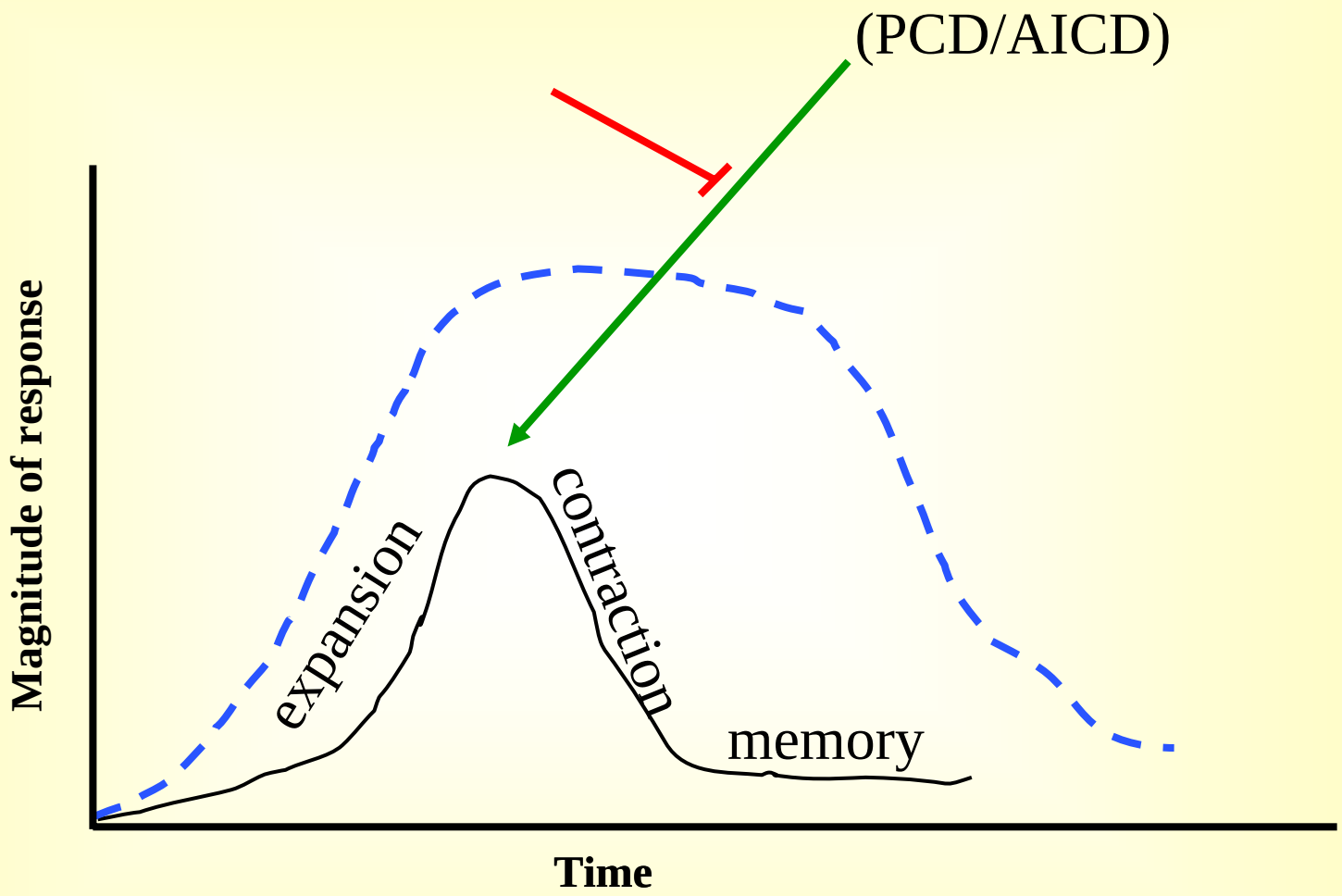
- *The efficacy of ``cancer vaccines`` and adoptive T cell therapy can be improved by preventing AICD*

- *It is widely believed that AICD in T cells is a death receptor dependent and caspase-mediated process*

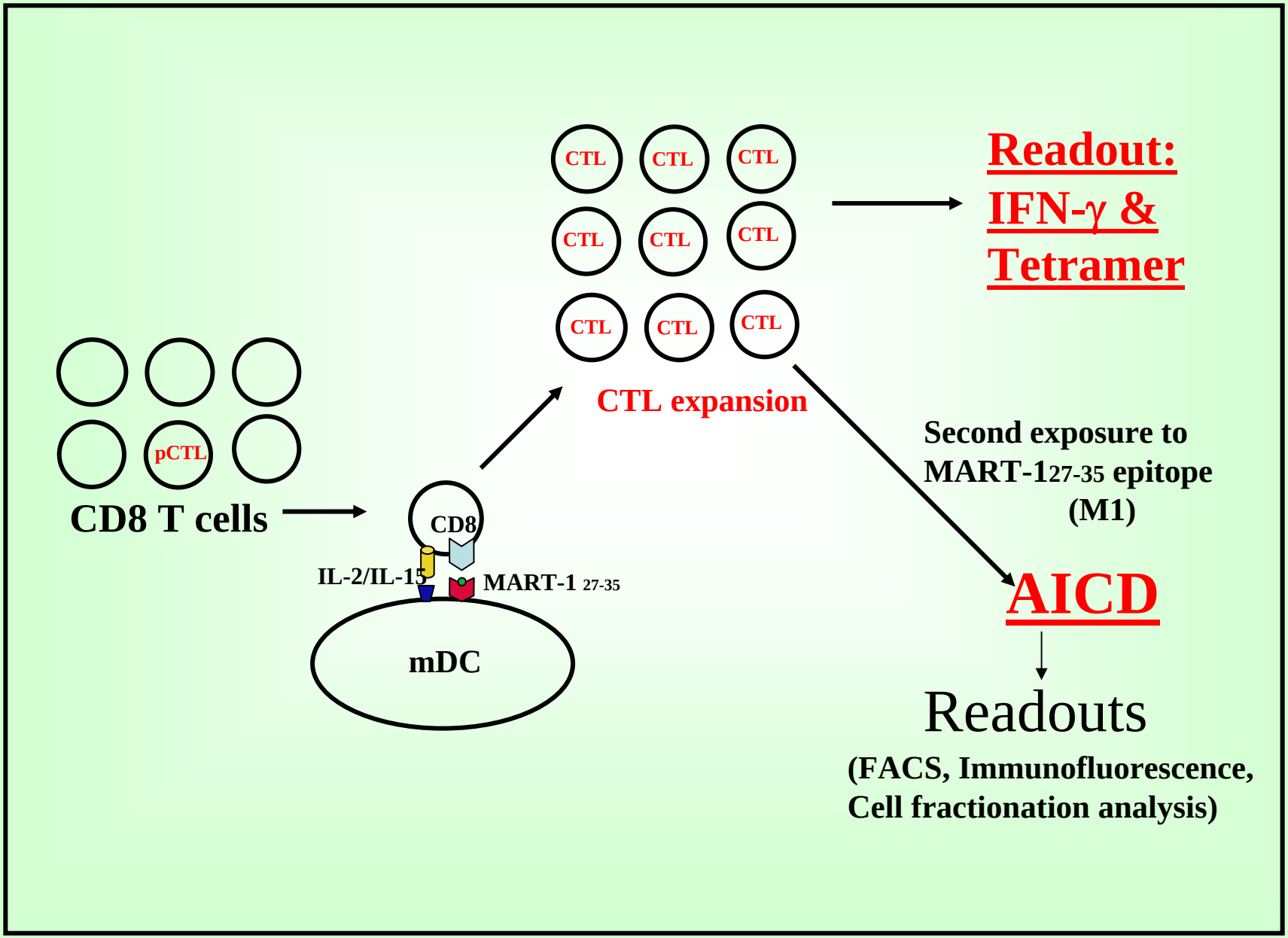
- *Recent reports have shown that T cells can die in DR-independent and caspase-independent manner, but the mechanism of caspase-independent death of T cells has not been examined carefully*

- *Almost all the studies aimed at T cell AICD were done in hybridomas or cell lines. Human primary T cells have not been studied for mechanistic purposes*

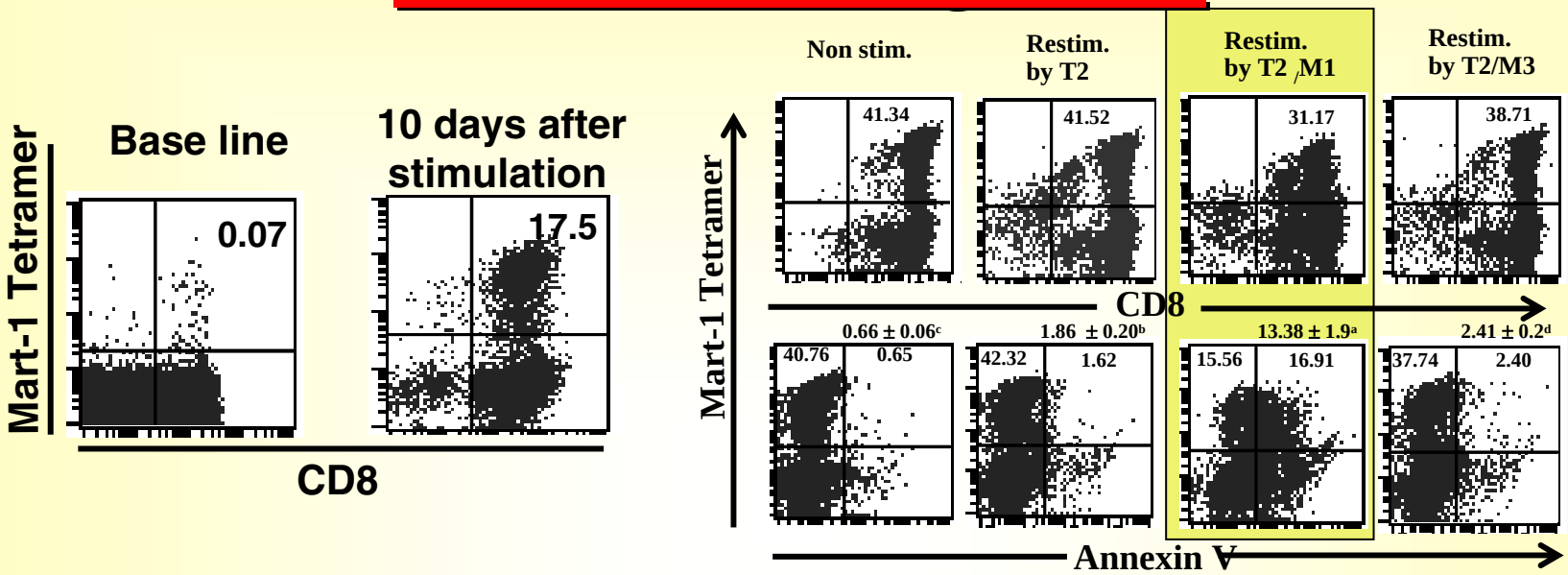
Customizing anti-tumor CTL response

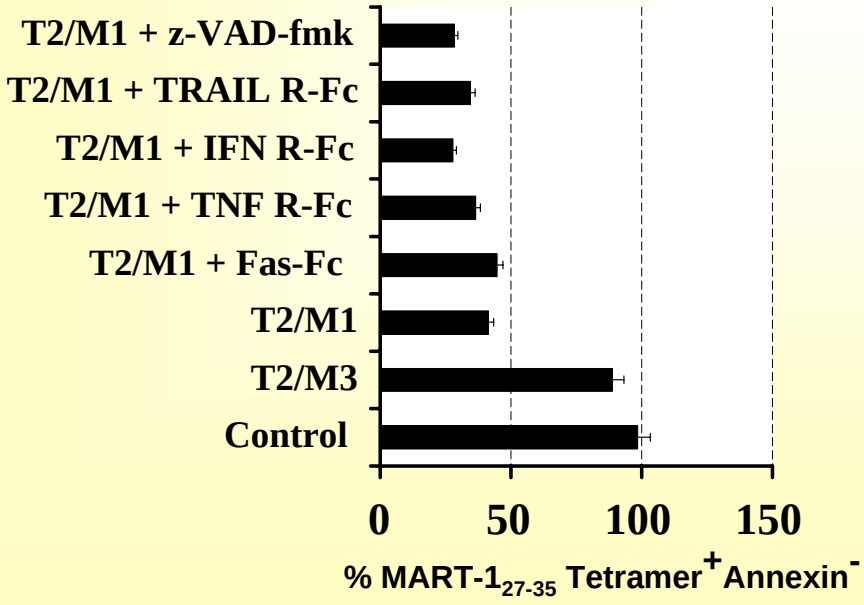


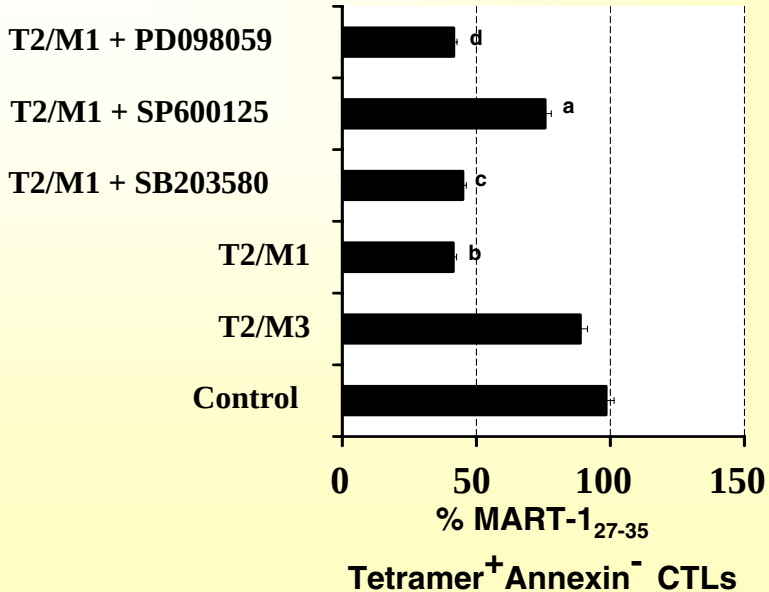
Basic Protocols



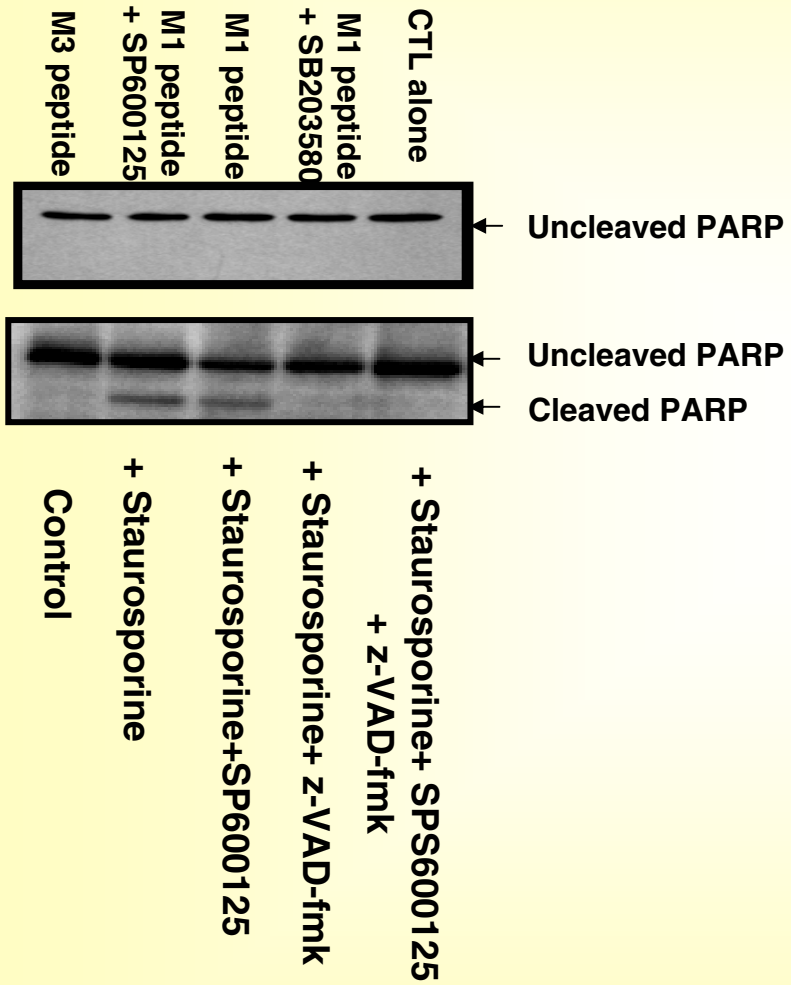
How do the TAA epitope specific human primary CTLs die during AICD

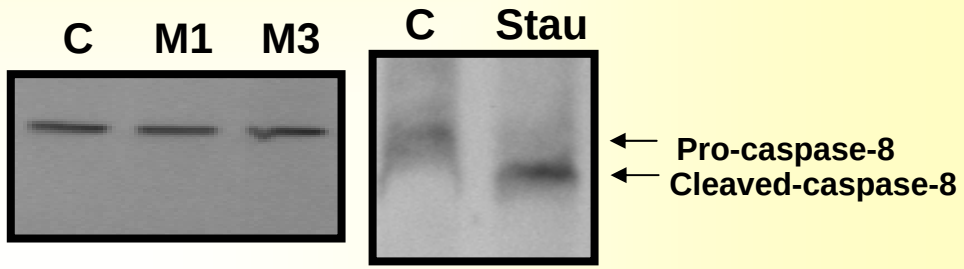


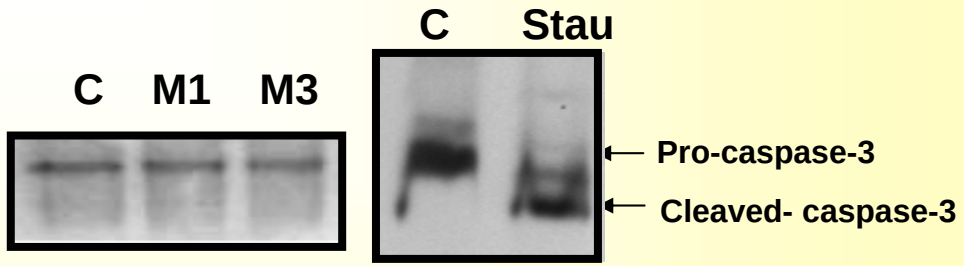




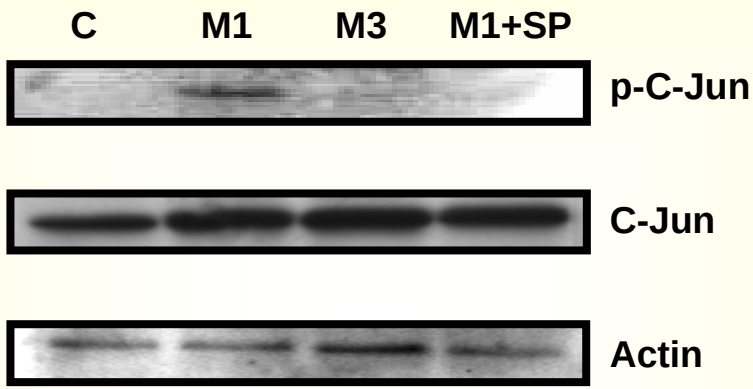
Caspase independent death of the CTLs







Activation of c-jun in CTL upon epitope encounter and the effect of SP600125



Intrinsic Death Signal

↓

JNK

↓

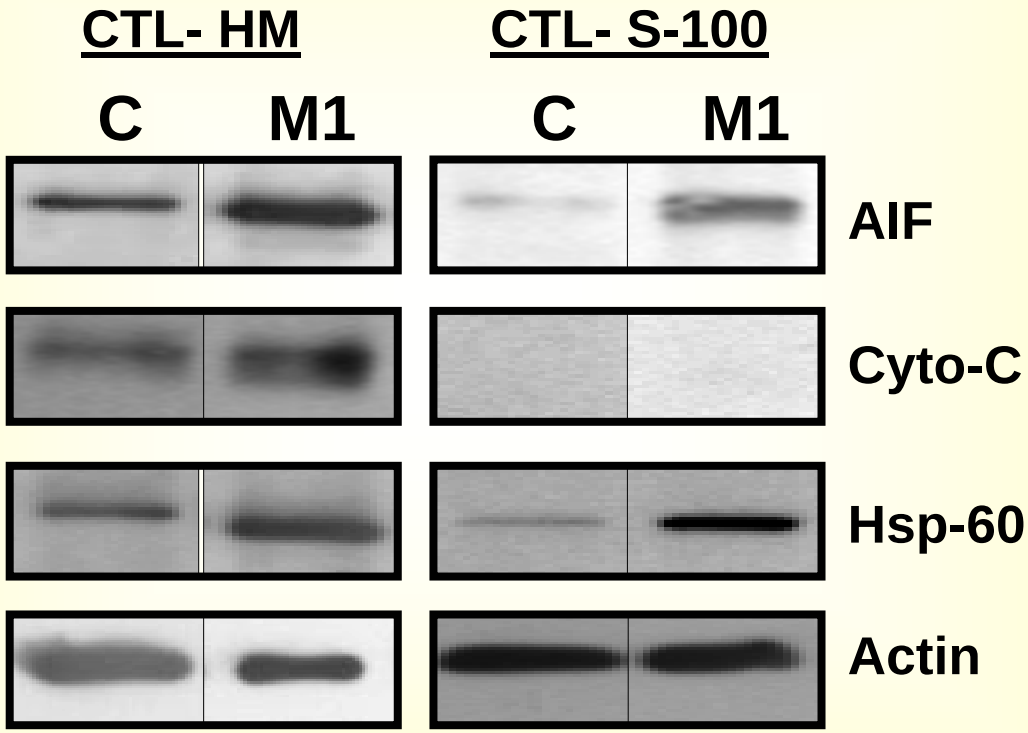
Modulation of Bcl- family proteins

↓

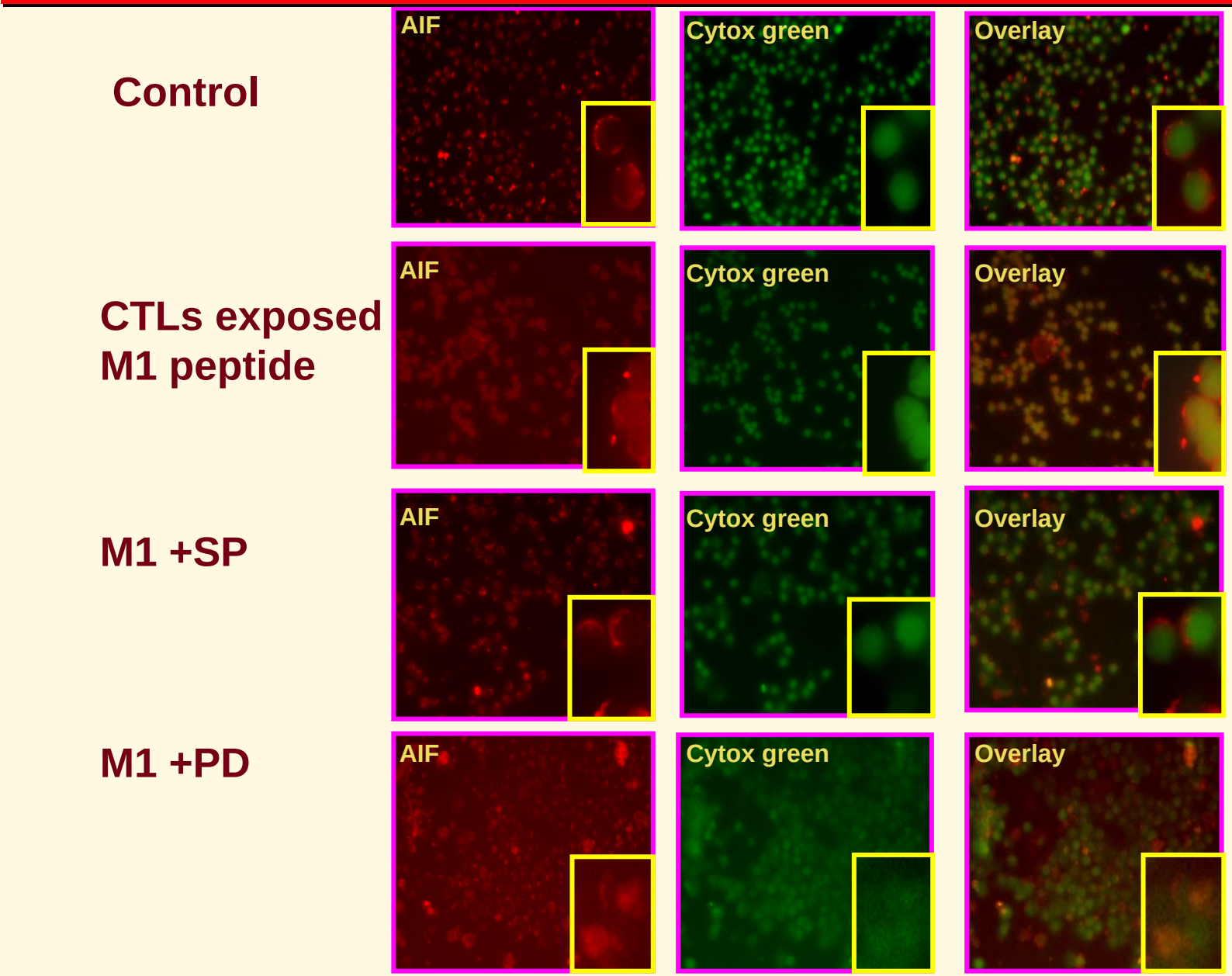
Caspase dependent death (Cytochrome-C)

Caspase independent death (AIF, EndoG)

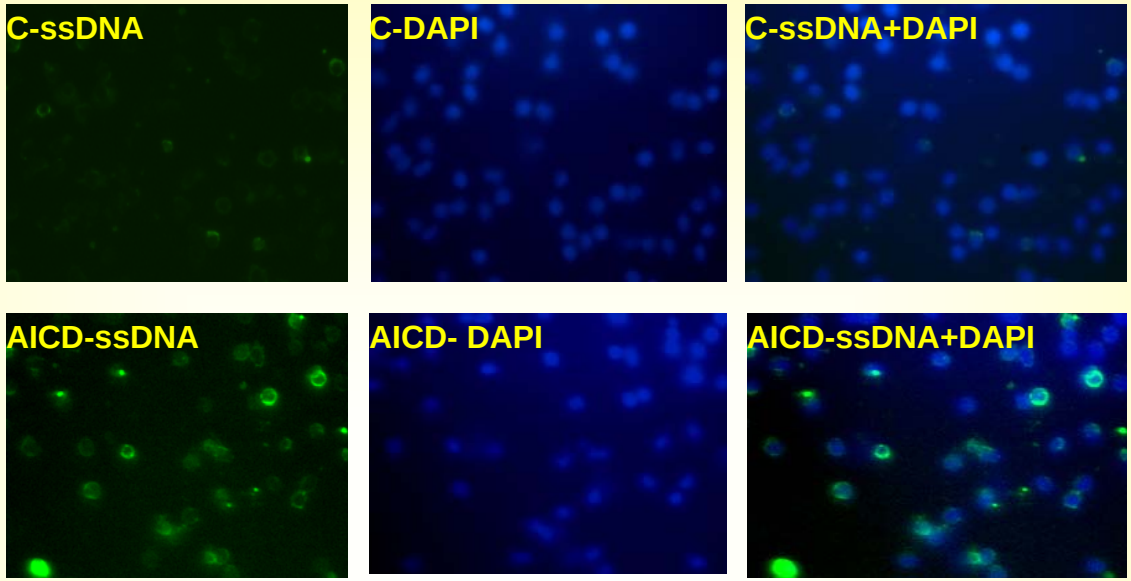
Cell Fractionation analysis of CTL for role of caspase-independent death executioners in AICD

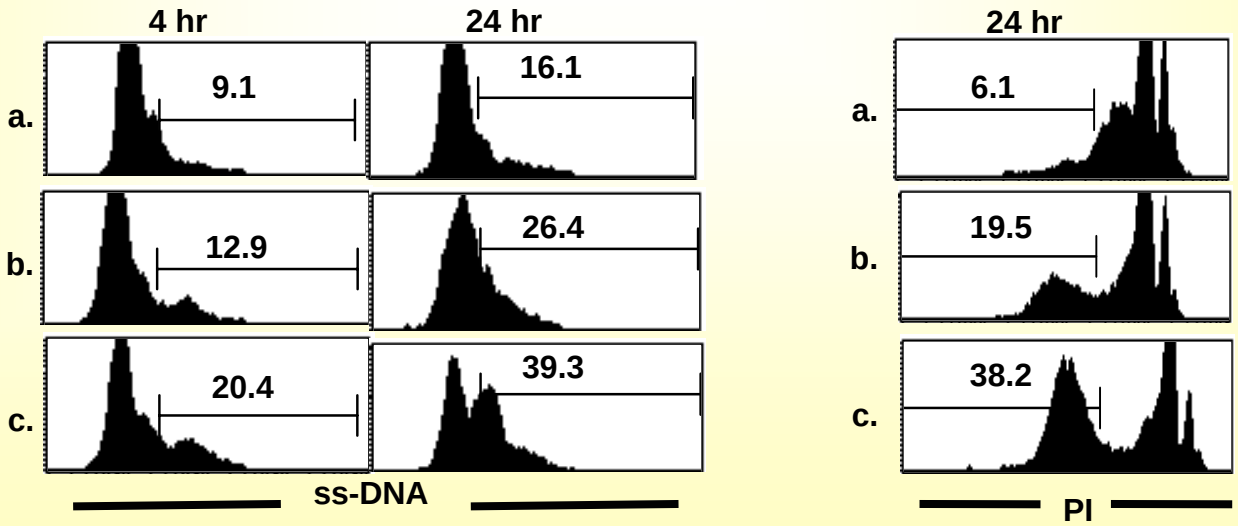


Mitochondrio-nuclear Translocation of AIF during AICD and blockade of this translocation by SP600125

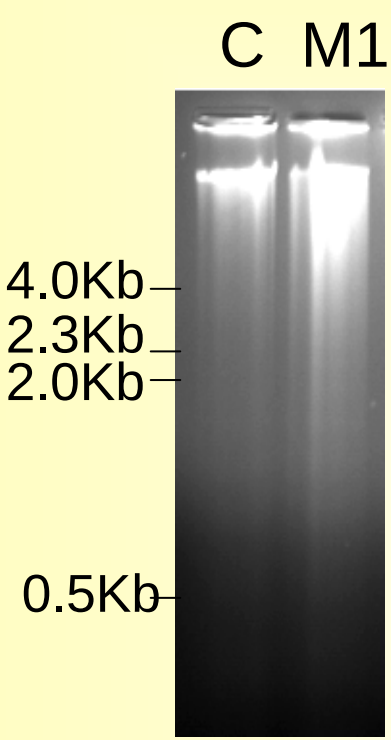


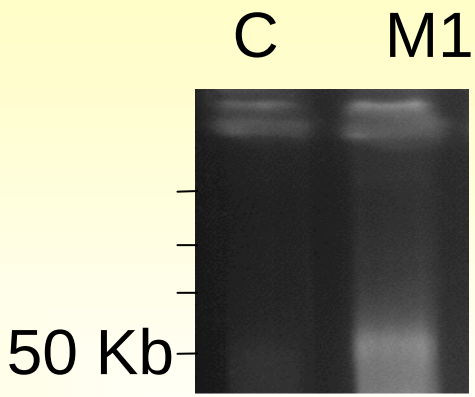
Analysis of Single Strand DNA Break Generation in AICD



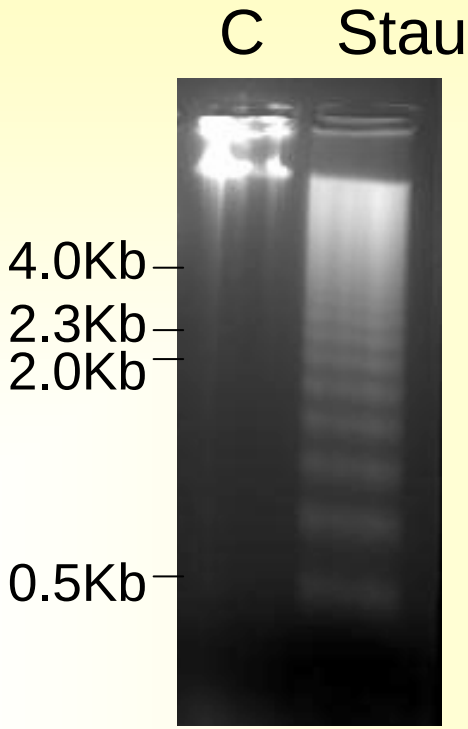


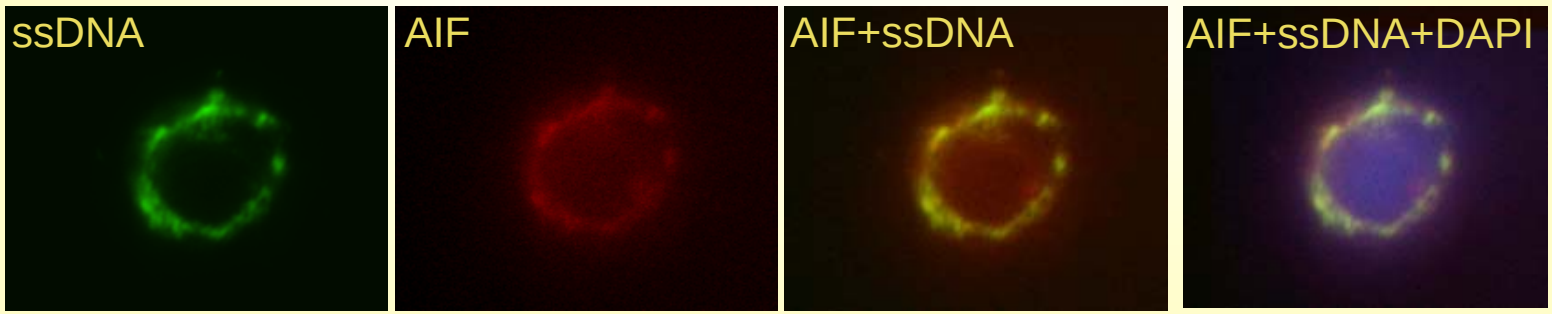
AICD is Associated With Larger DNA Fragments





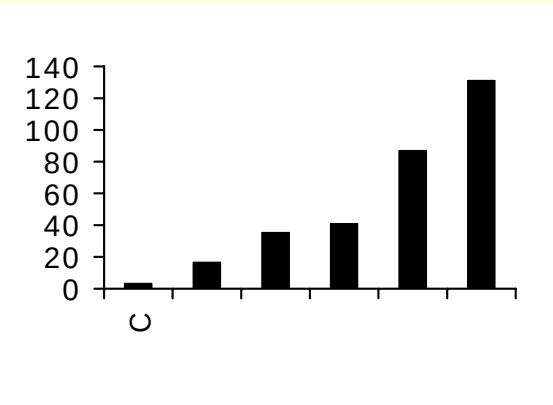
PFGE

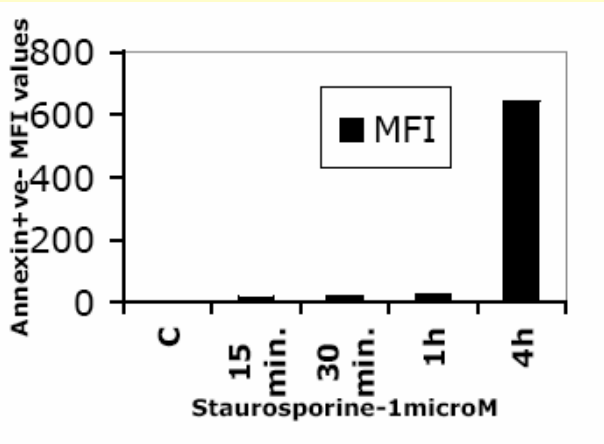


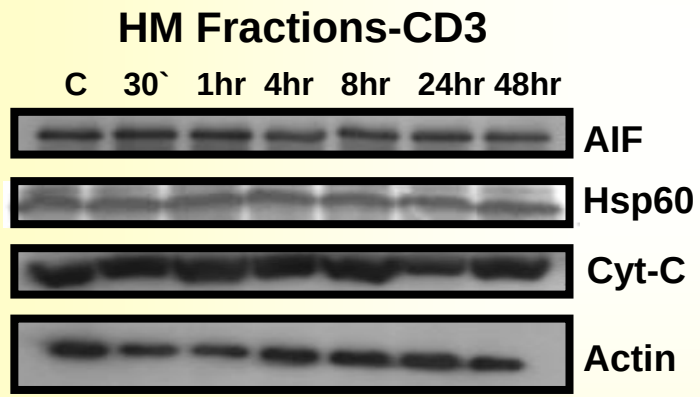


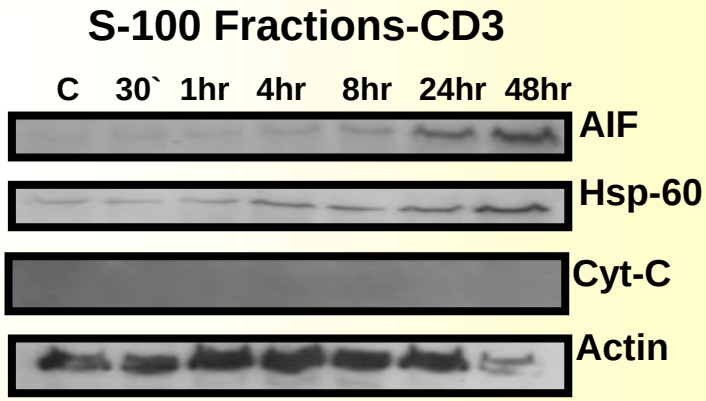
Colocalization of AIF and ssDNA breaks in AICD

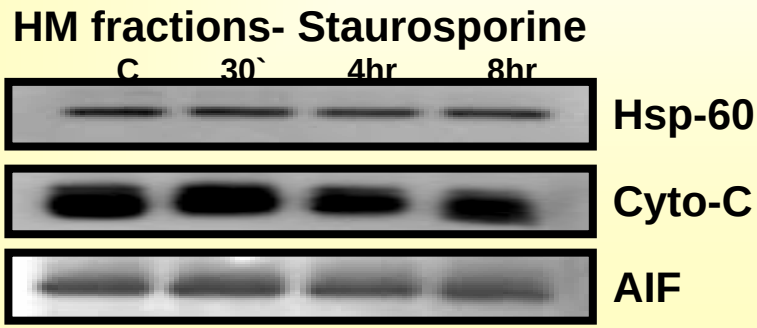
AIF release in TCR driven apoptosis of Jurkat cells

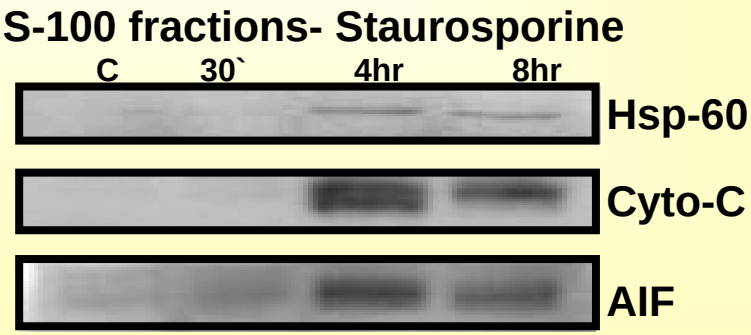










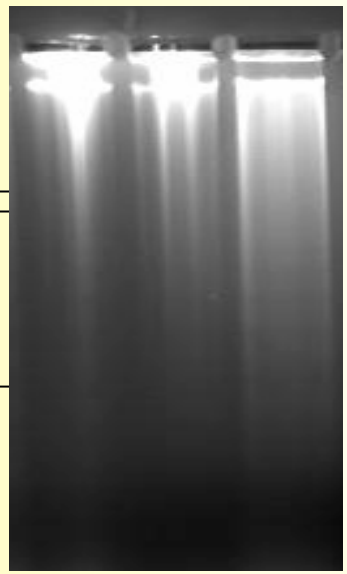


Generation of Large Scale DNA Fragmentation in TCR Driven Apoptosis of Jurkat Cells

CD3

C 4h 8h

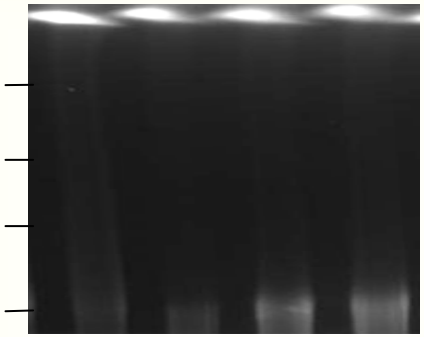
2.3Kb
2.0Kb
0.5Kb



Pulse Field Gel/CD3

C 8h 24h 48h

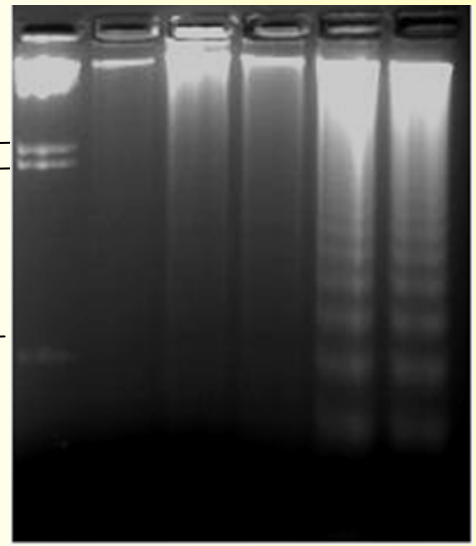
50 Kb



Staurosporine

C 30` 1h 4h 6h

2.3Kb
2.0Kb
0.5Kb

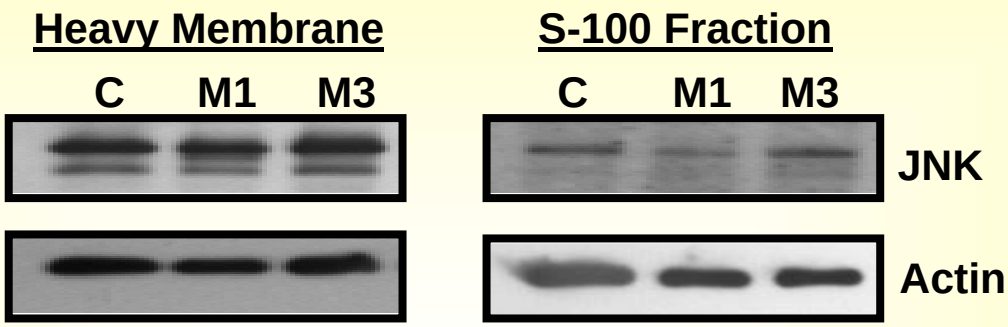


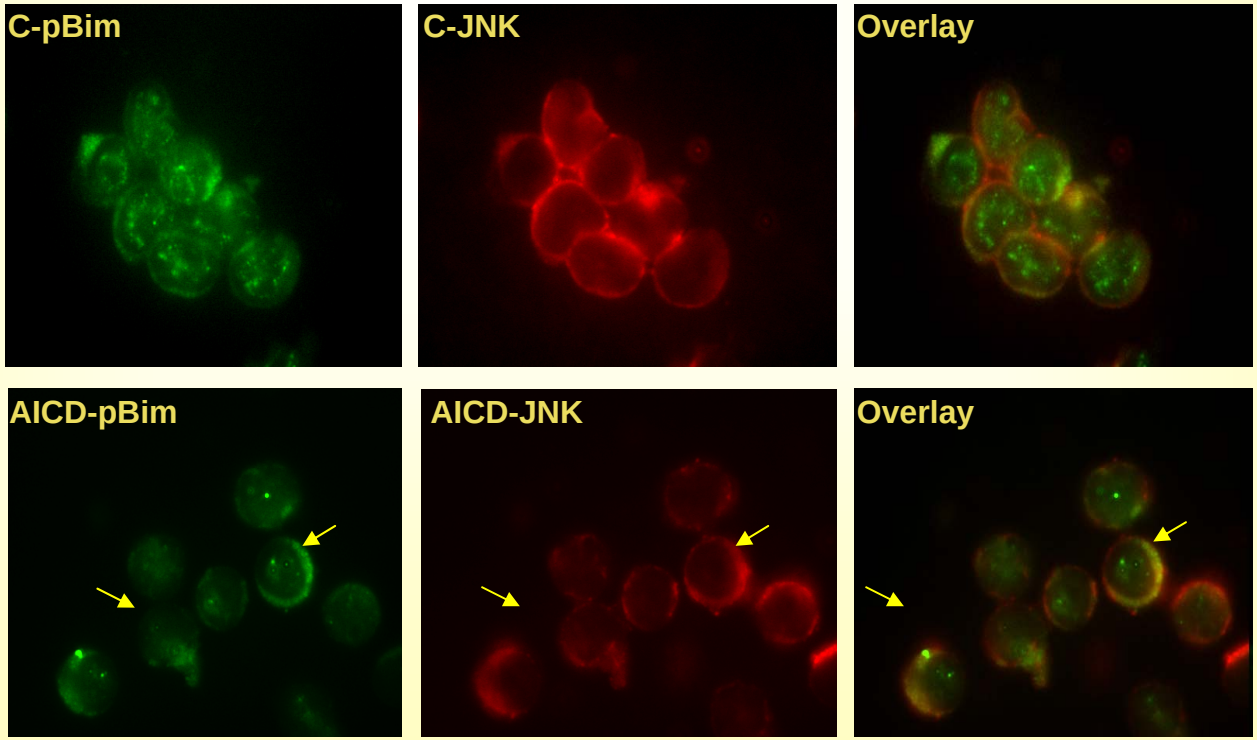
Conclusion

- **AICD of CTL is DR-independent process**
- **Caspase-8 and caspase-3 are not involved in AICD**
- **AICD is accompanied by JNK activation followed by mitochondrio-nuclear translocation of AIF**
- **Cytochrome-c is not released during AICD of primary CTL**
- **DNA fragmentation during AICD is not oligosomal (characterstic of caspase mediated cell death) but comprised of larger DNA fragments (~50Kb), characterstic of AIF mediated death**

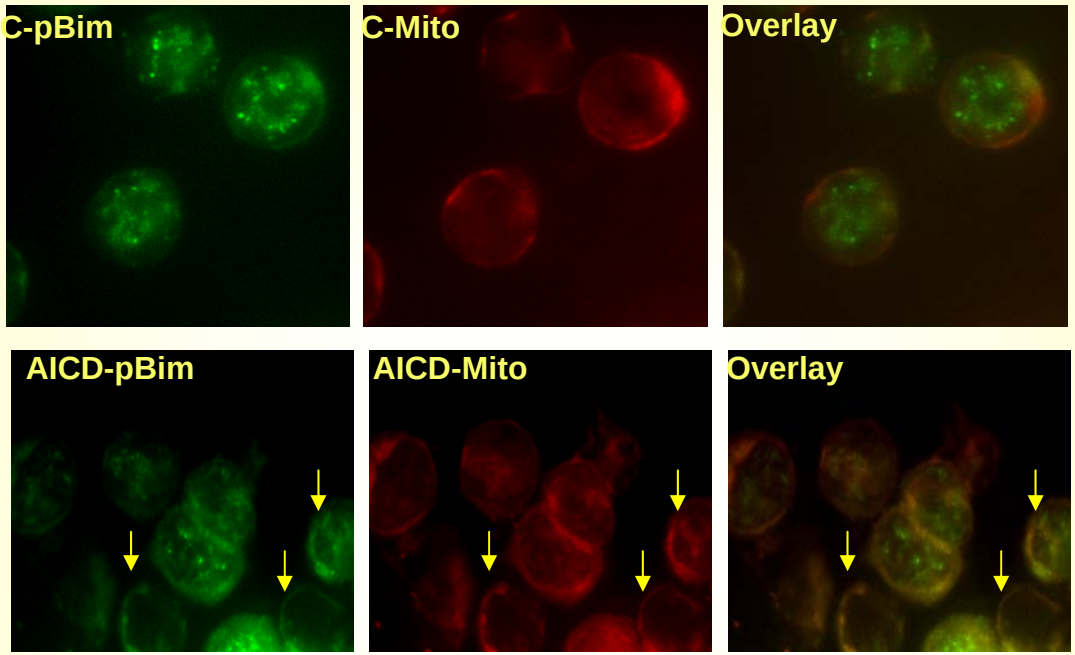
- **How Does JNK activation regulates selective release of AIF during AICD**

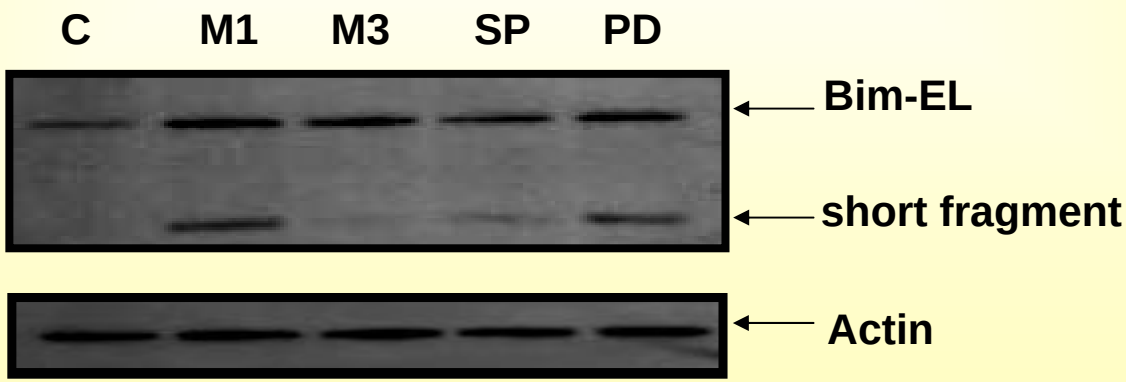
Co-localization of Mitochondrial JNK and phospho-Bim in CTL undergoing AICD





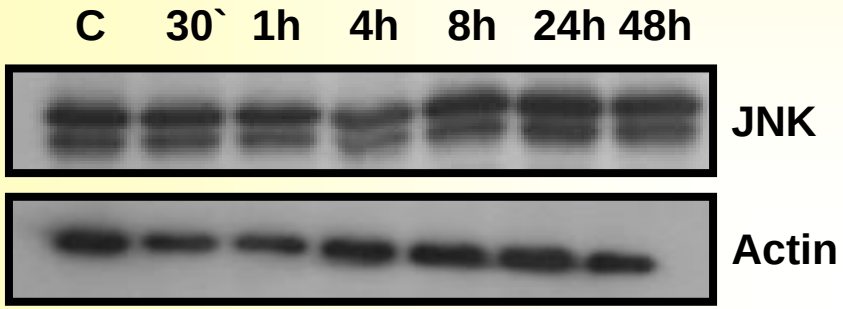
Mitochondrial Relocalization of Bim and Generation of a Small pBim Fragment During AICD

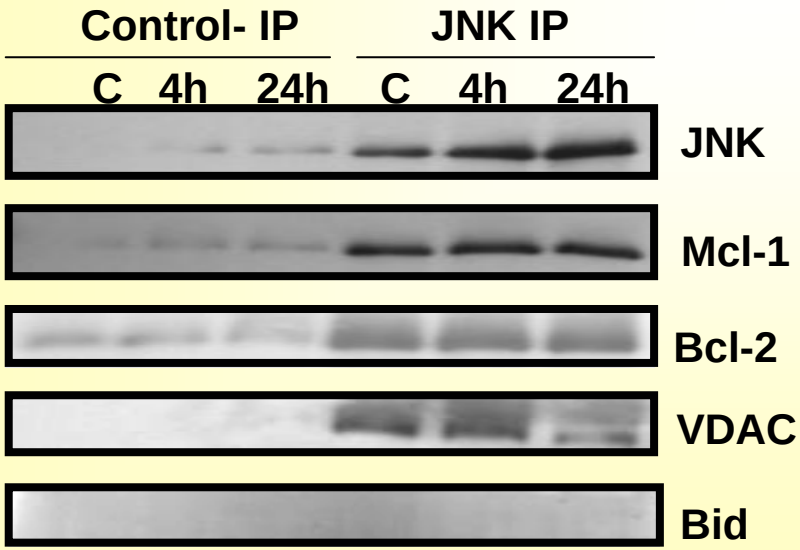




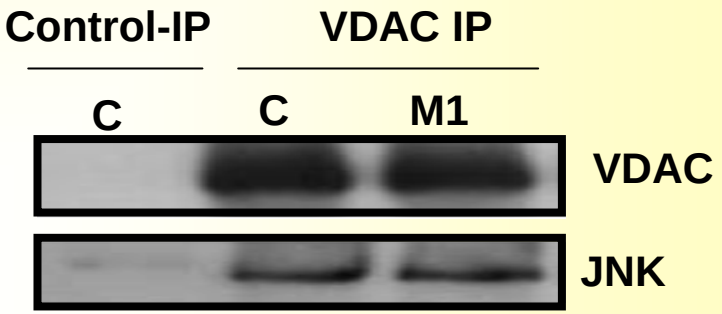
Interaction of Mitochondrial JNK with Bcl Family Proteins and VDAC in Jurkat and CTL

Jurkat

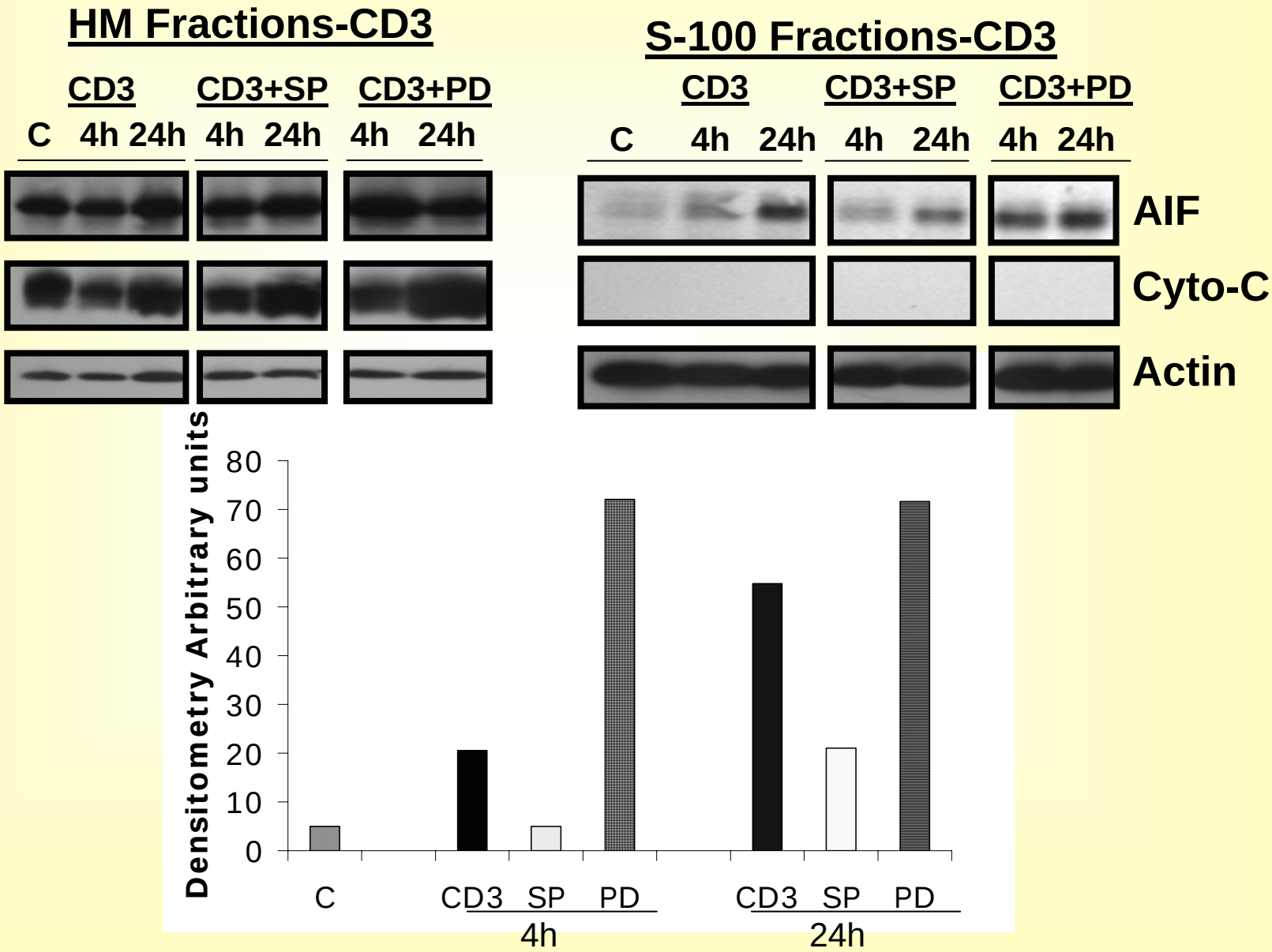


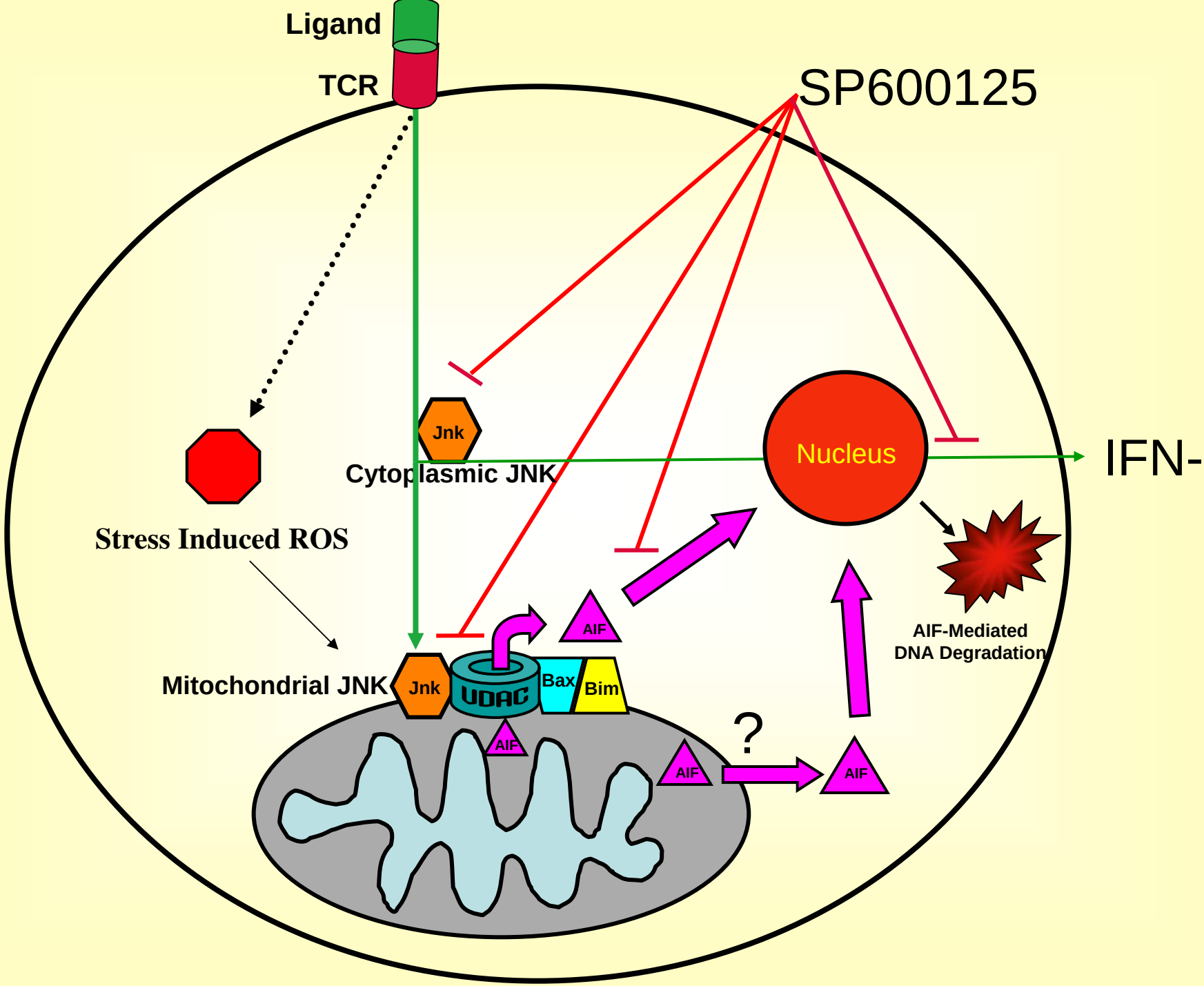


CTL



Effect of SP600125 on AIF Release in TCR Driven Death of Jurkat Cells




Ligand
TCR
SP600125
Jnk
Cytoplasmic JNK
Nucleus
IFN-
Stress Induced ROS
AIF
AIF-Mediated
DNA Degradation
Mitochondrial JNK
Jnk
UDAC
Bax
Bim
AIF
AIF
?
AIF

Summary

- Although it is widely believed that the signal for AICD in T cells originates from Fas and TNF family DRs and that the death is caspase-dependent, our results strongly suggest that AICD of CD8+ T cells is DR-independent caspase-8 and caspase-3 independent process.

- Our work strongly supports the involvement of mitochondria in AICD of these T cells.

- Our data reveals the existence of a novel mitochondrion-based and caspase-independent process (i.e., AIF release and no cytochrome c release) in AICD of primary CTL.

- Our results implicate JNK in orchestrating the selective AIF release and reveal large-scale DNA fragmentation(as opposed to short oligosomal DNA breaks``ladder``) as the mode of death in T cells.

- Our results reveal targets (JNK, AIF, Bim) and provide opportunities to interfere with the caspase independent death of antigen specific CTL for improved immunotherapy protocols

Future Directions

- To firmly establish the role of JNK and AIF in AICD by siRNA
- To understand the regulation of the selective release of AIF in the absence of cytochrome-c

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