Development Of A Human Inducible Suicide Gene

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Need For A Suicide Gene

- Increasing use of Cell Therapies
  - T-cell therapies
    - Genetically modified T cells
      - Toxicity of transgene
      - Retroviral integration-associated toxicity
    - Tumor specific T cells
      - Autoimmunity
    - Allogeneic Donor lymphocytes post HSCT
      - Graft versus host disease

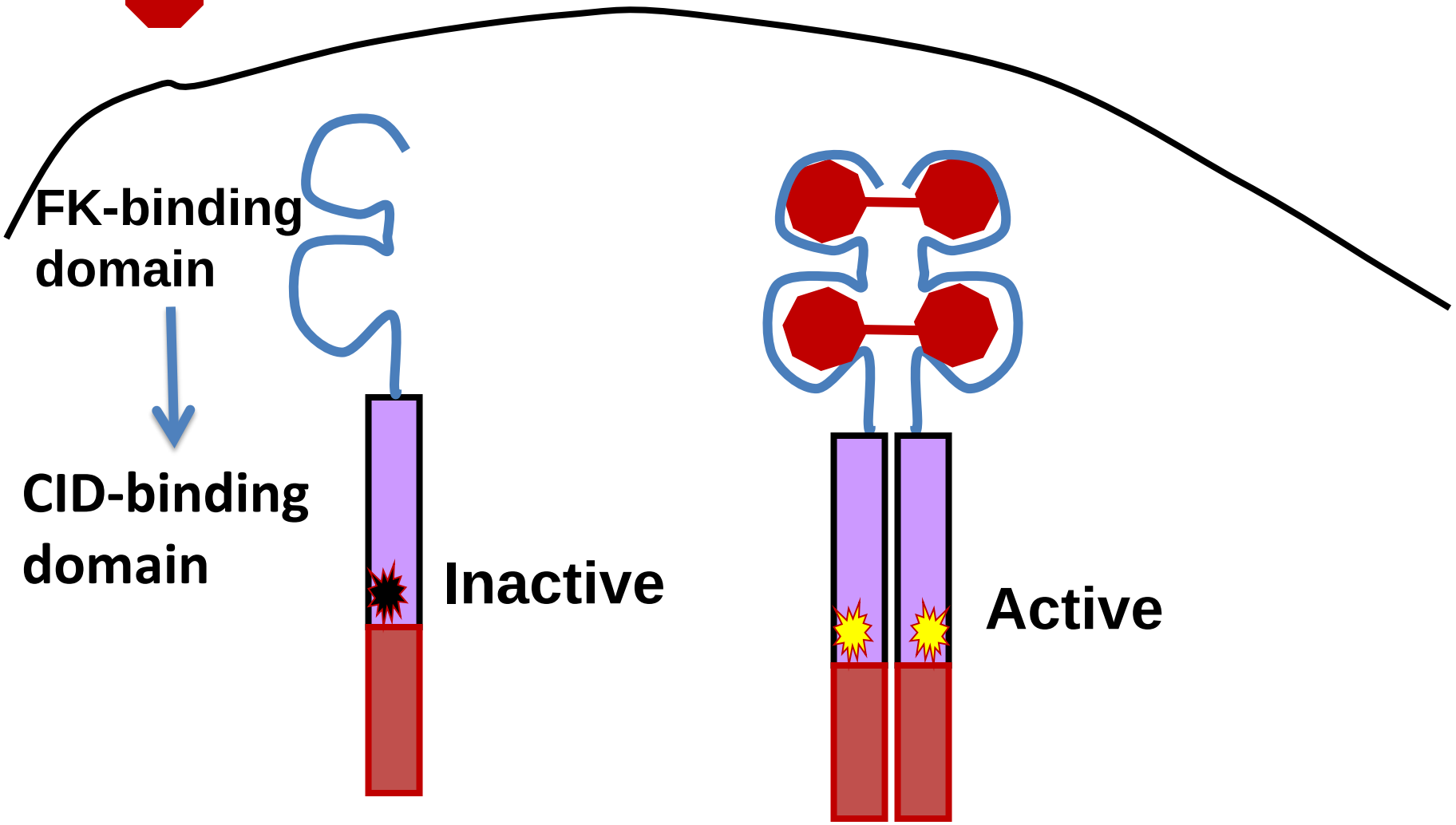
Requirements for Suicide Gene

- Must
  - Be expressed in all cells with potential toxicity
    - Co-expressed with therapeutic transgene
      - Include in vector
    - Or with selectable marker
  - Have low basal toxicity
  - High specific activity
  - Be non-immunogenic

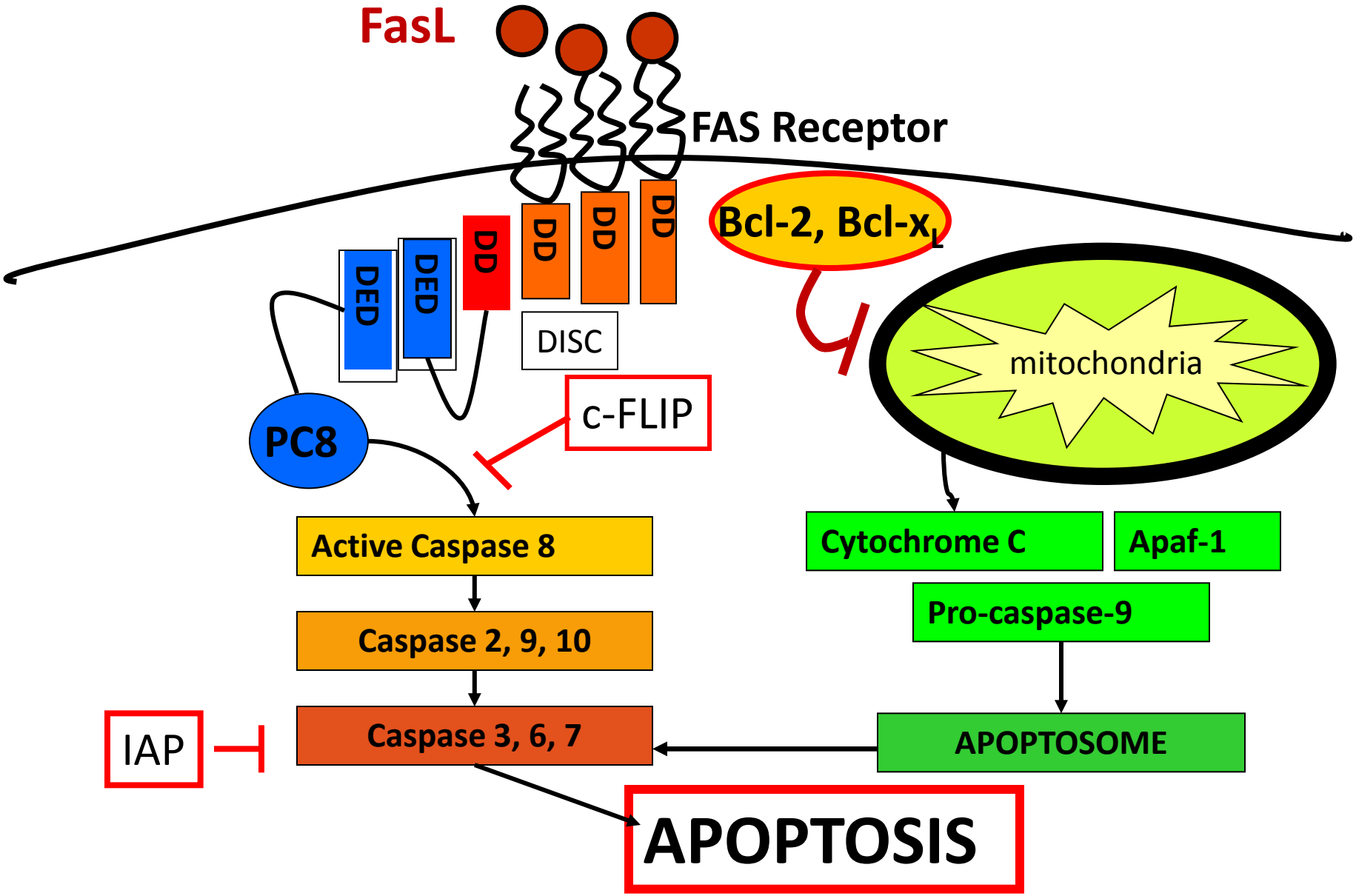
Problems with Available Suicide Genes Circa 2000

- HSV-TK
  - Foreign (immunogenic)
  - Dependent on ganciclovir for activation
    - Removes drug as a therapeutic
- E. coli cytosine deaminase
  - Foreign (immunogenic)

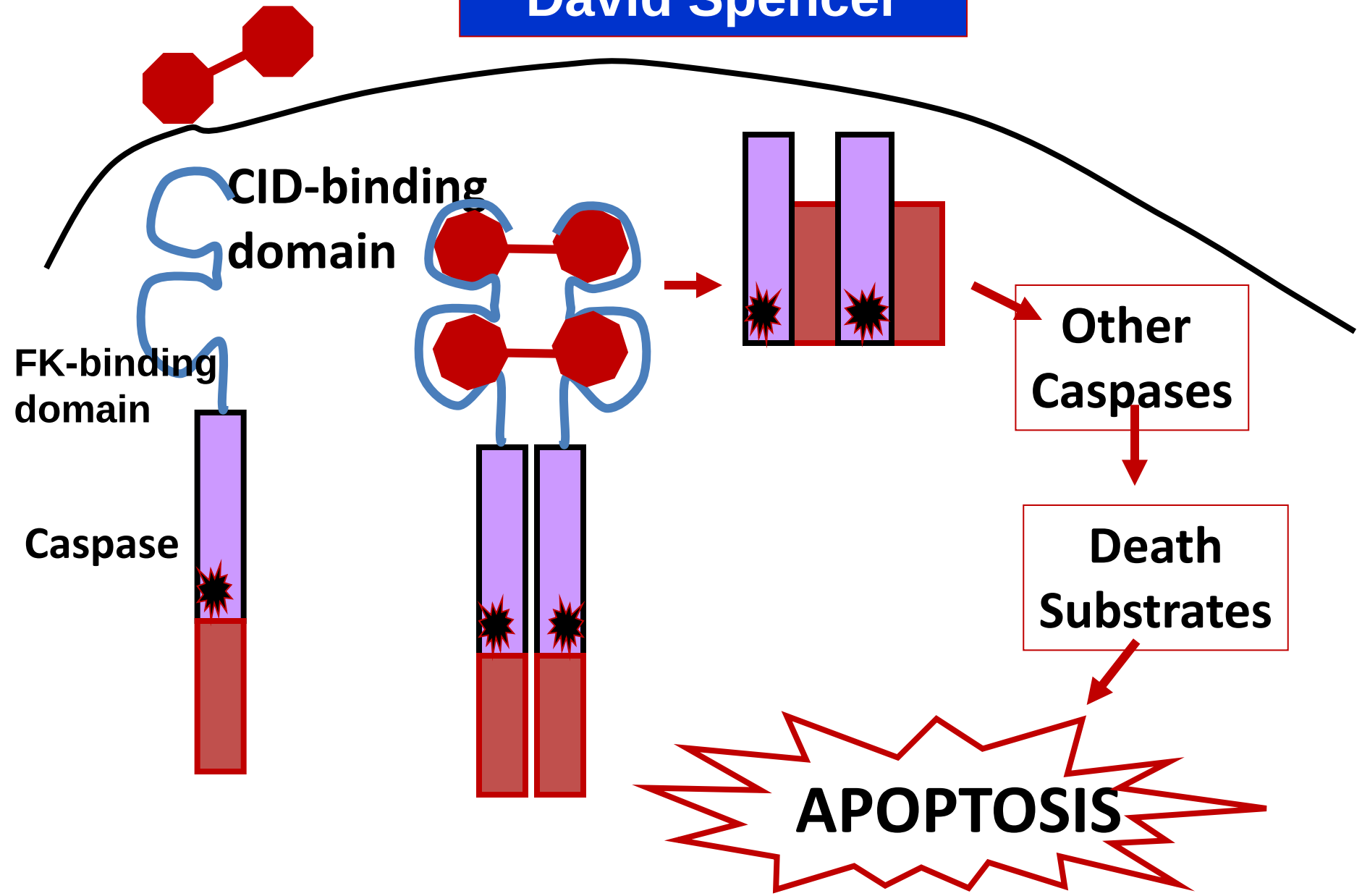
Activation by Dimerization
Chemical Inducer of Dimerization (CID) (AP1903)
David Spencer



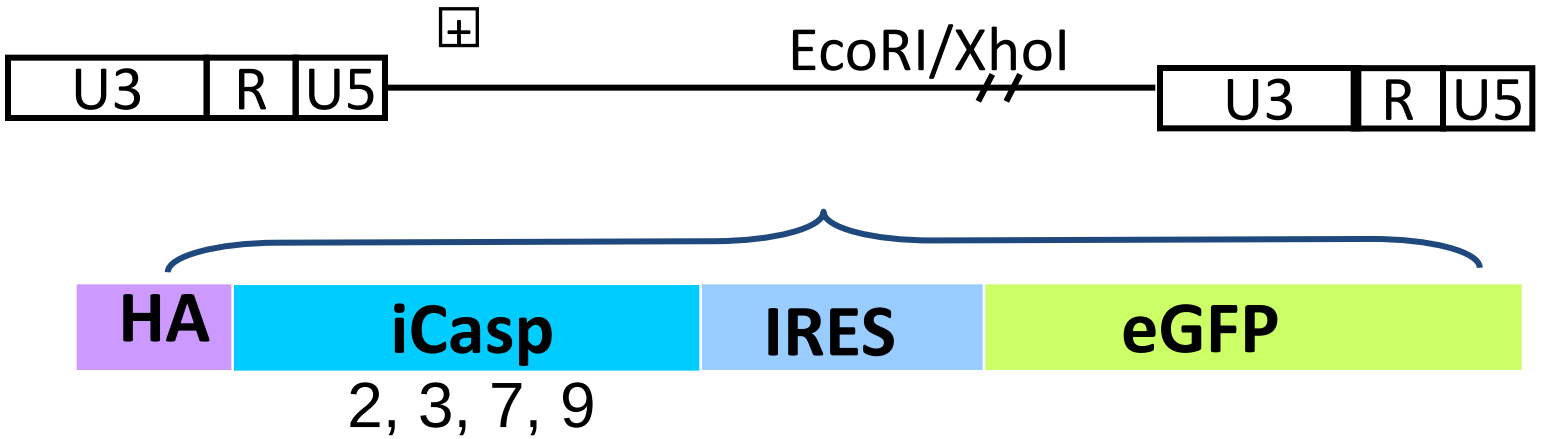
Caspase Pathway of Cell Death



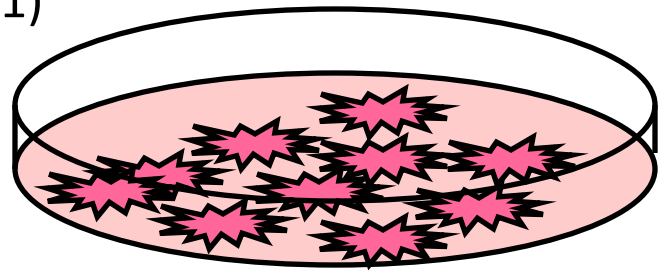
Inducible Caspase
David Spencer



Retrovirus expression system for iCaspase and eGFP



Haemaglutinnin Tag (HA.11)



293Tcells

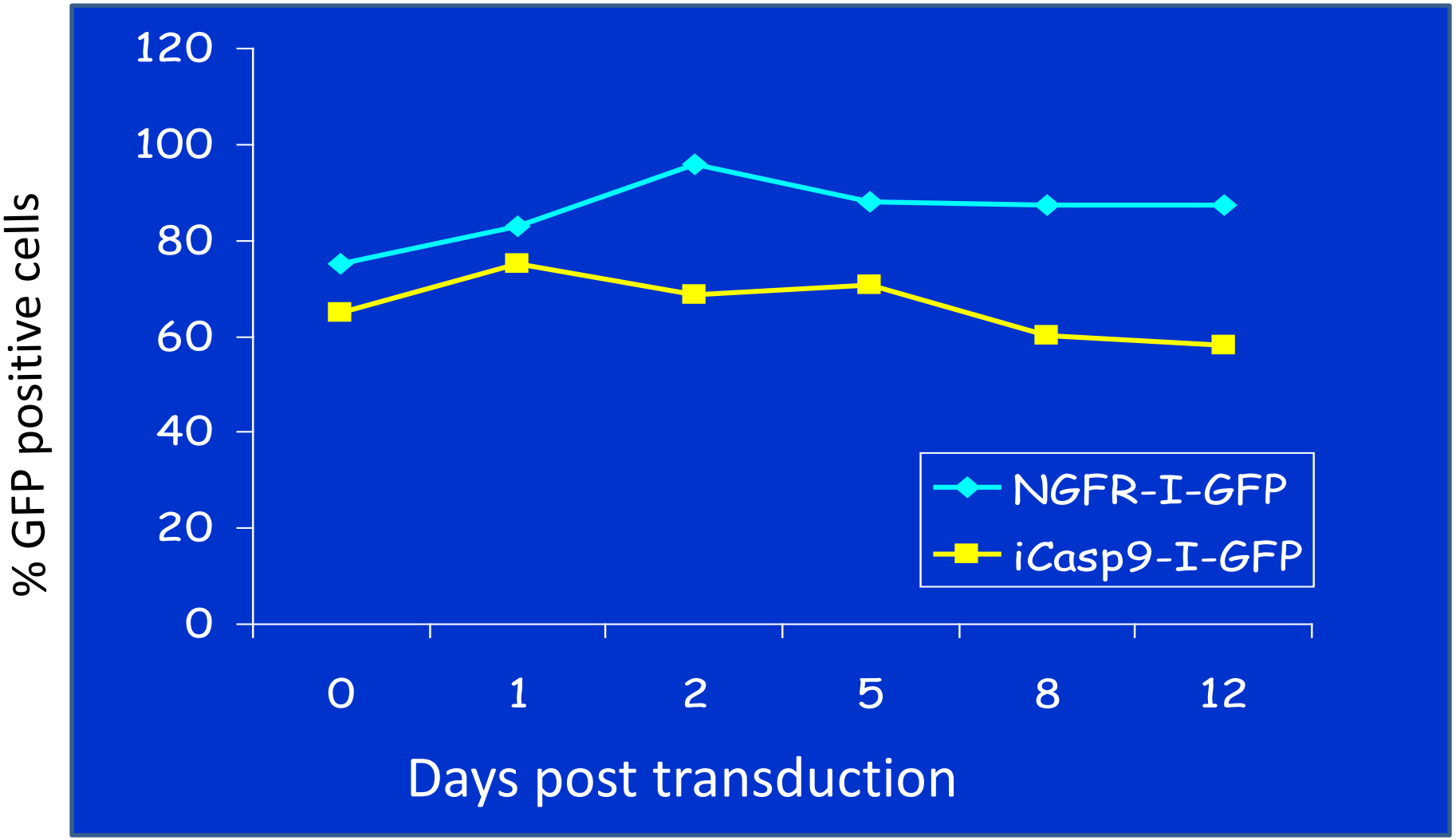
Co-transfection:

1) MSCV/iCaspase.I.GFP

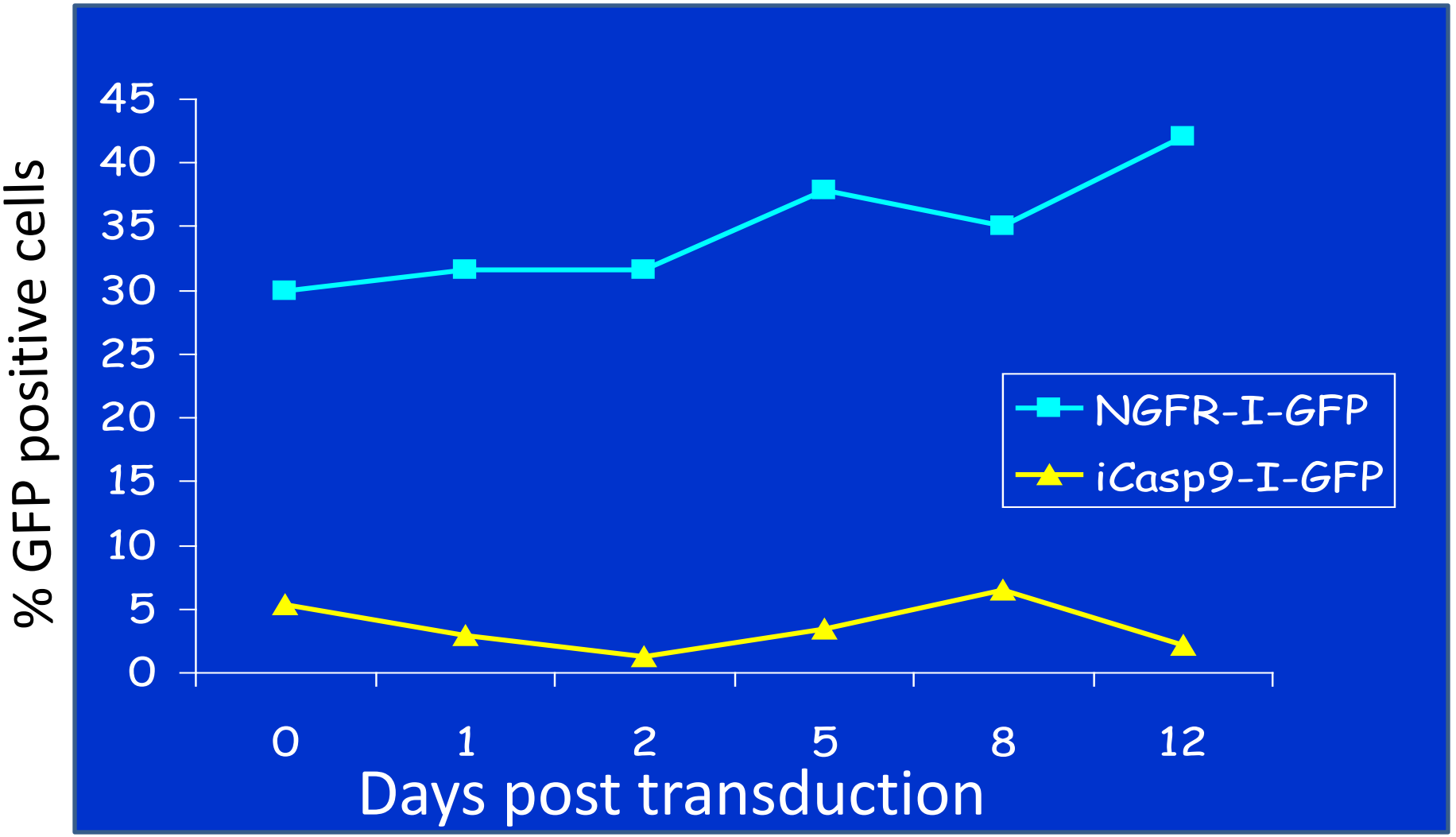
2) Gag-pol

3) RD114 env

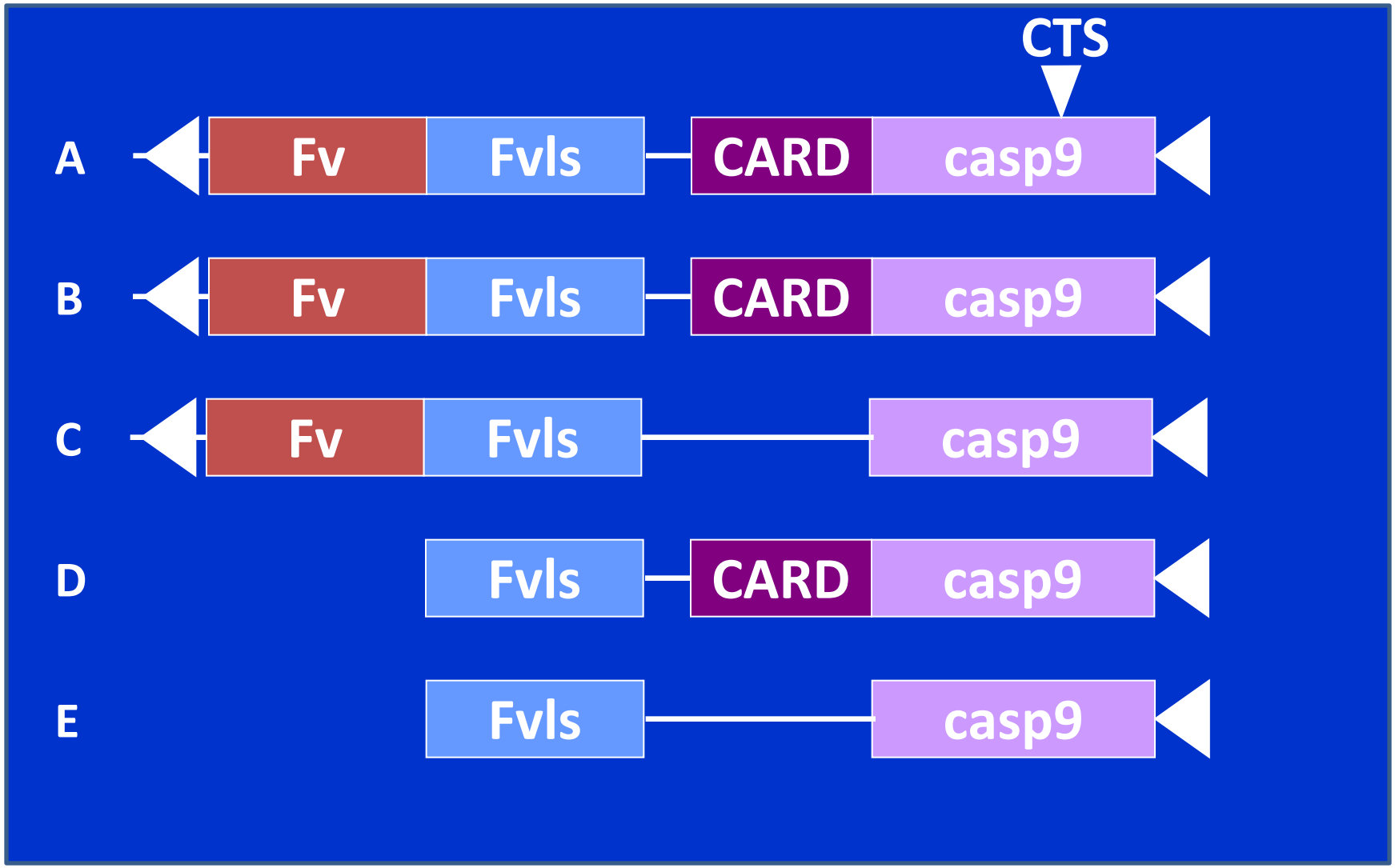
Stable expression of iCaspase 9 in 293 cells



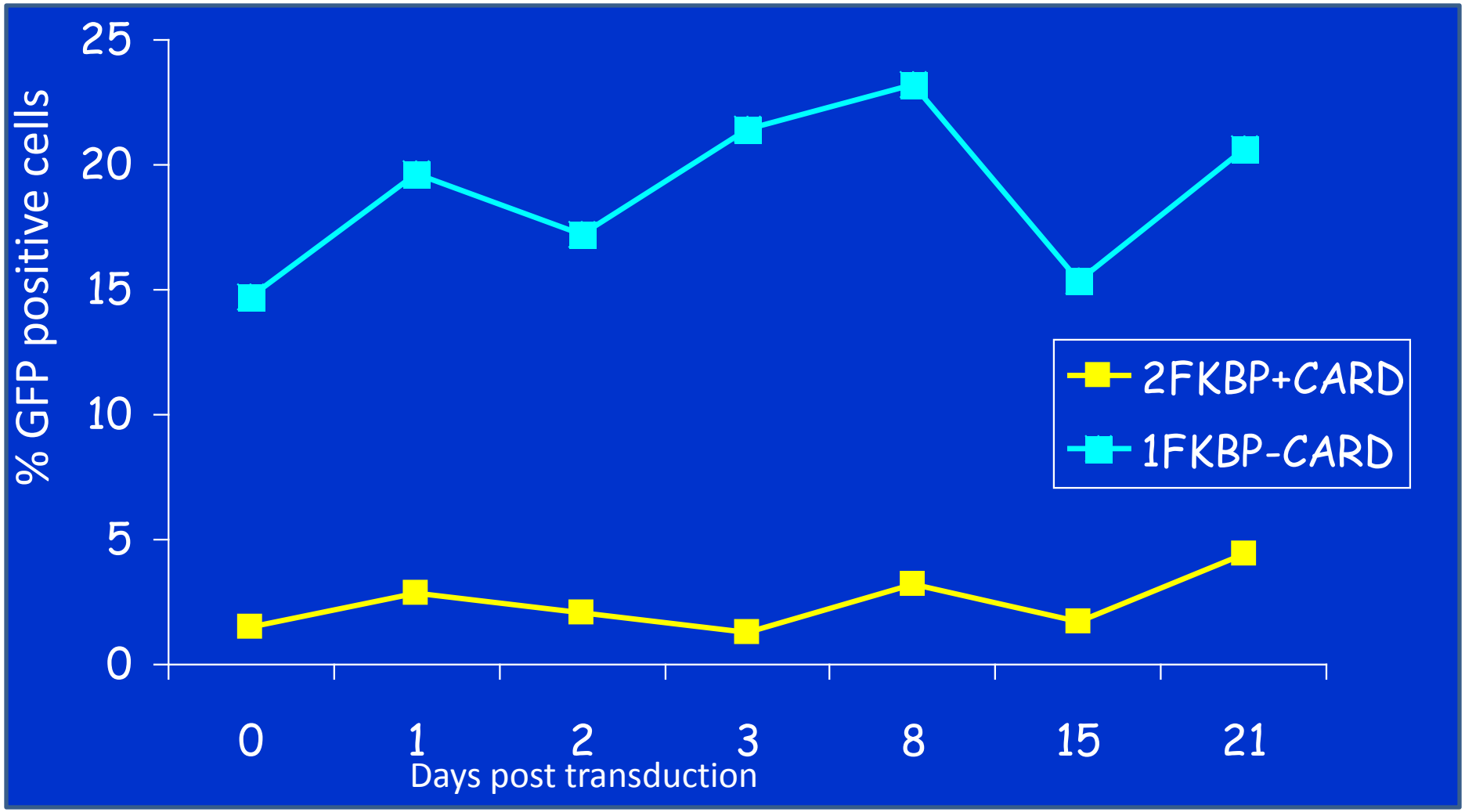
Low expression of iCaspase 9 in T-Cells



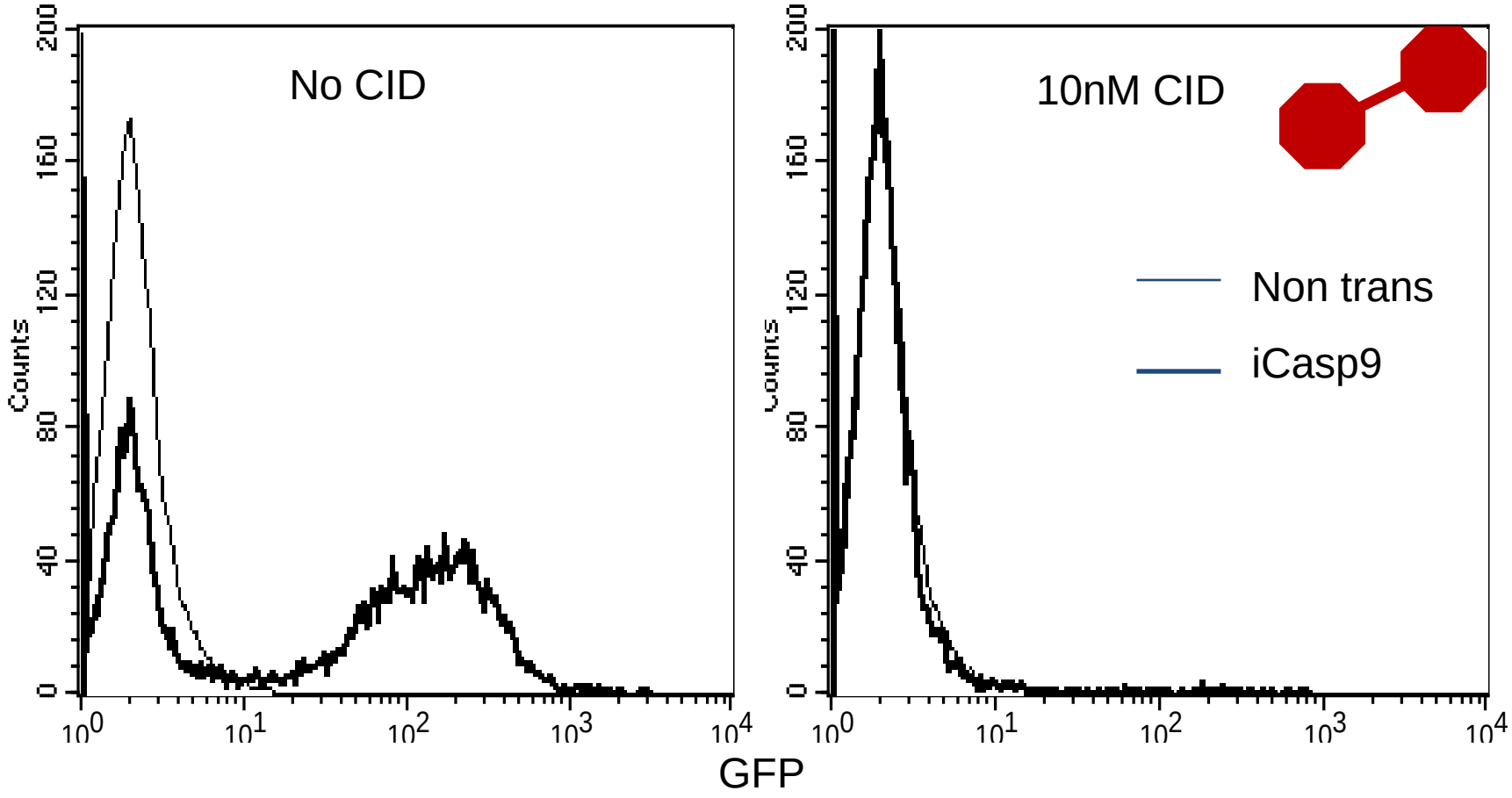
How To Enhance Expression While Maintaining Function?



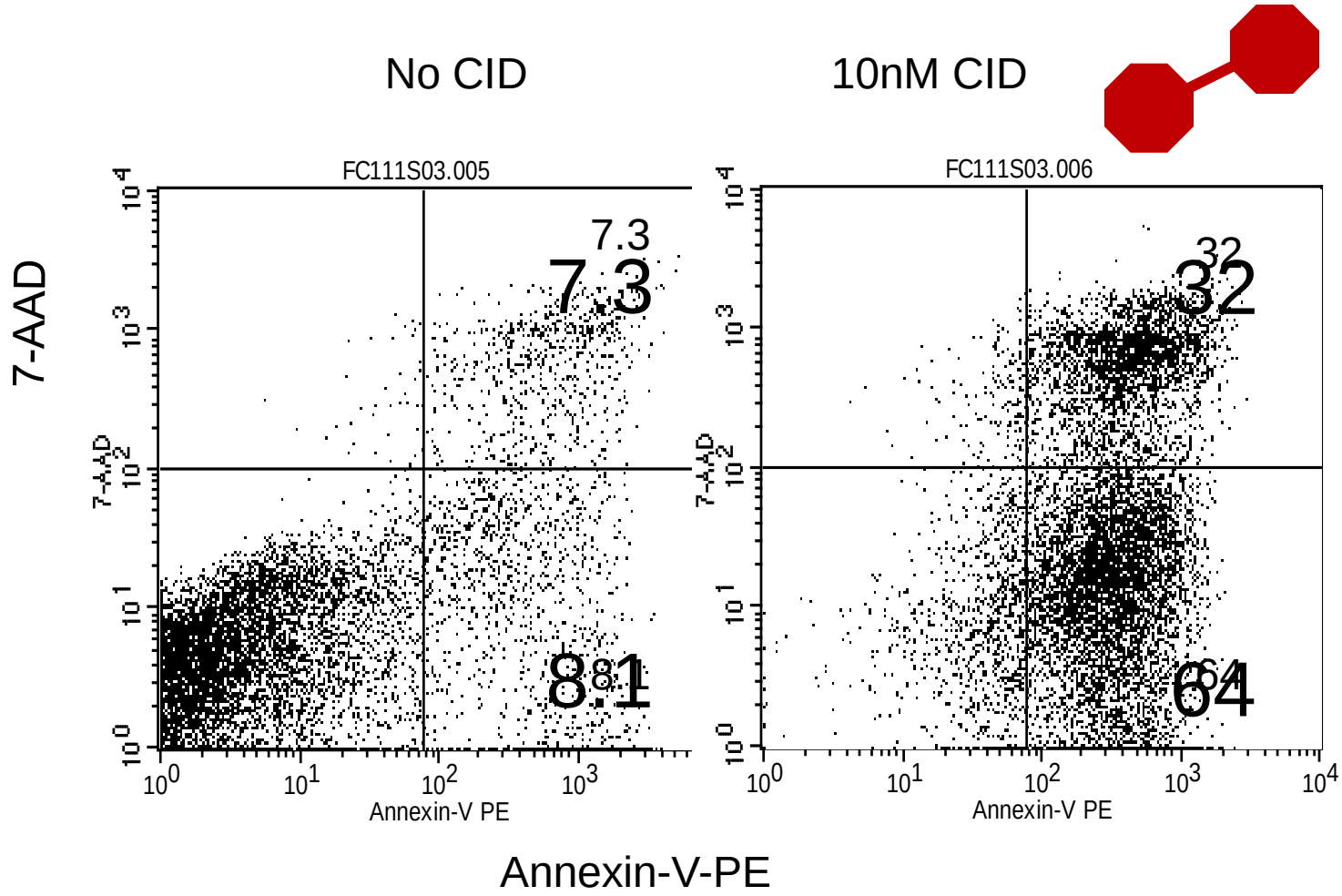
Removal of FKBP and CARD increased stability of iCasp 9 expression in CTL



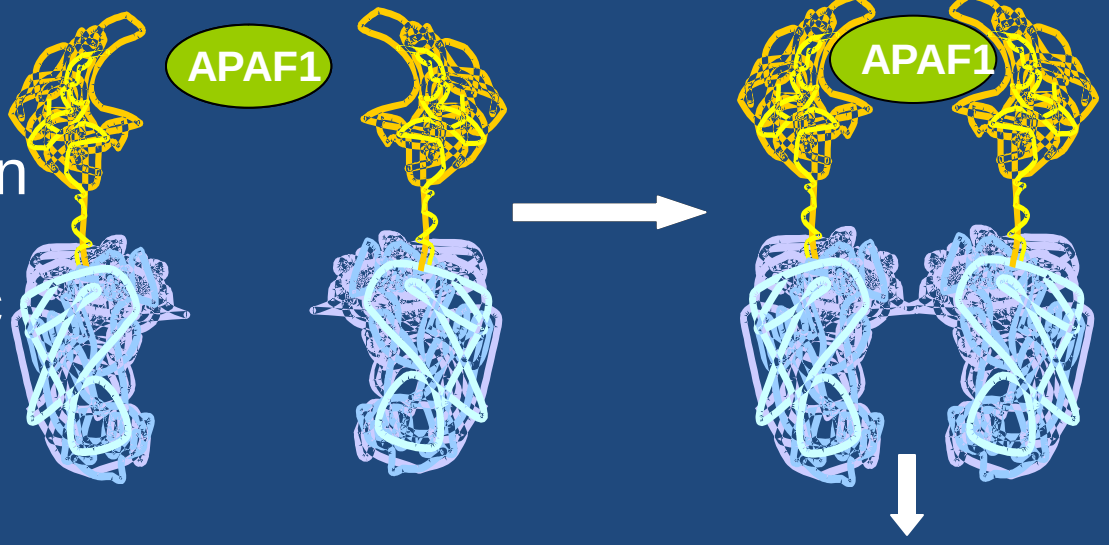
Stable expression and function of iCasp9 in CTL



CID Eliminates >95% Of Selected iCasp9 Transduced CTL



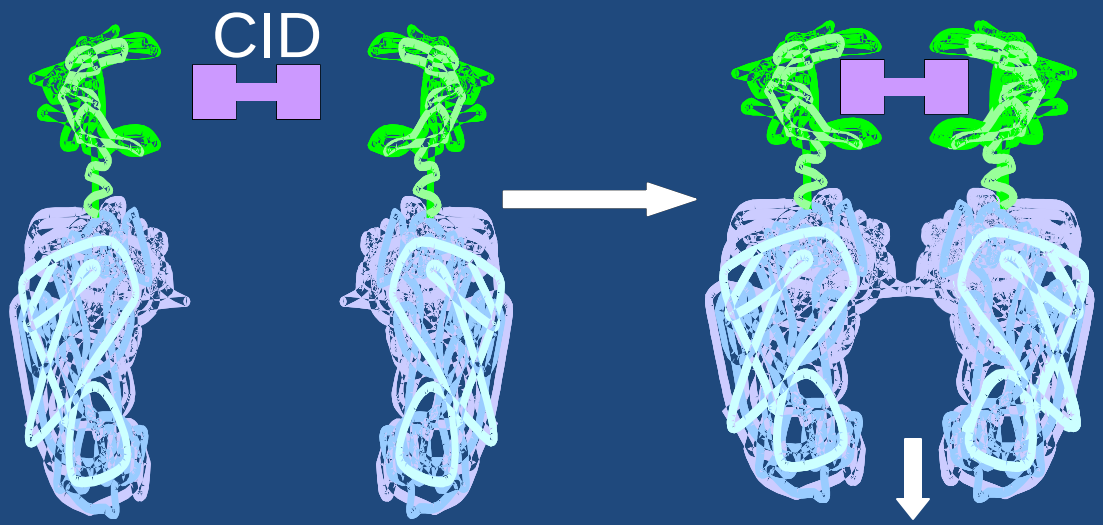
Native Caspase 9



active site
formed by
homodimer

apoptosis

Inducible Caspase 9

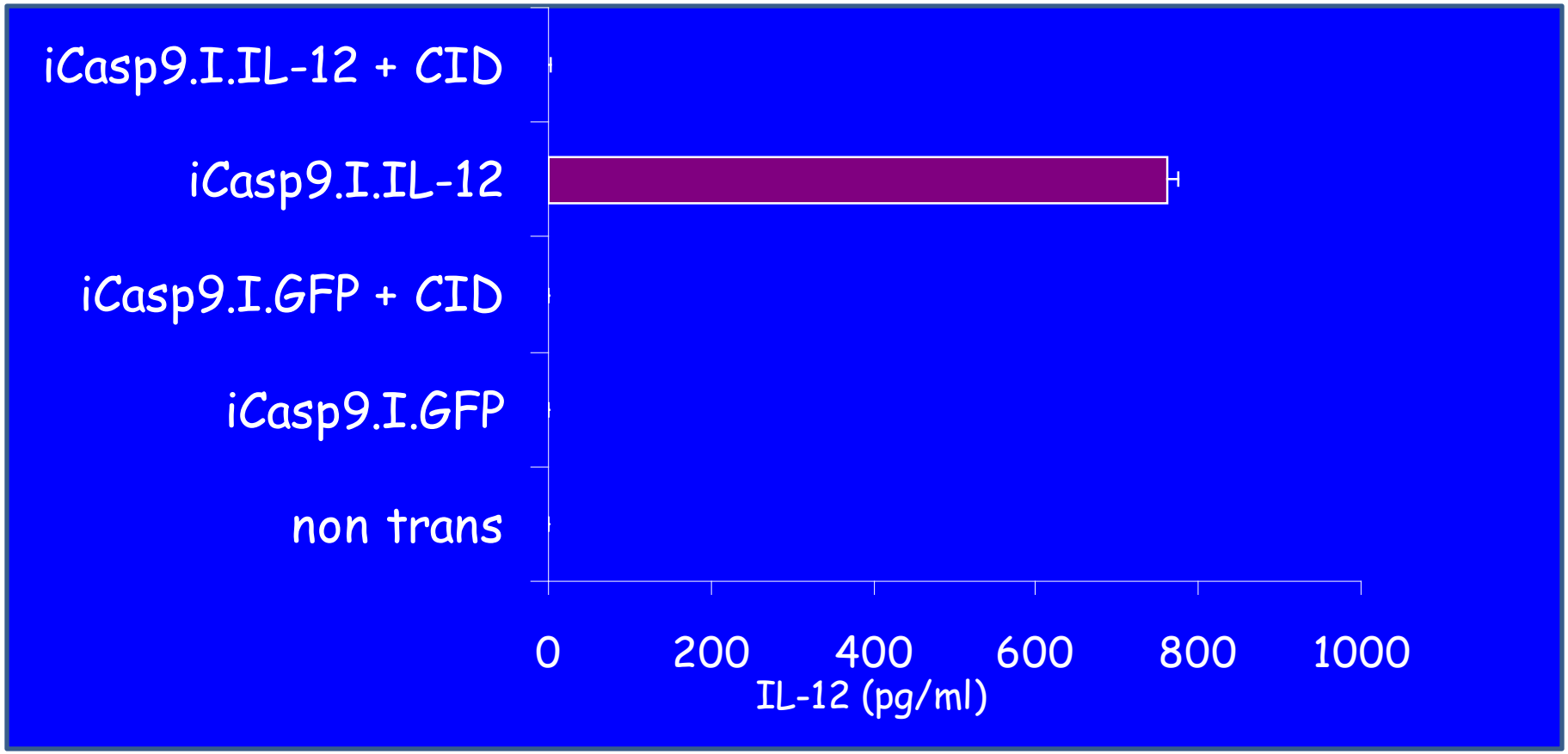


active site
formed by
homodimer

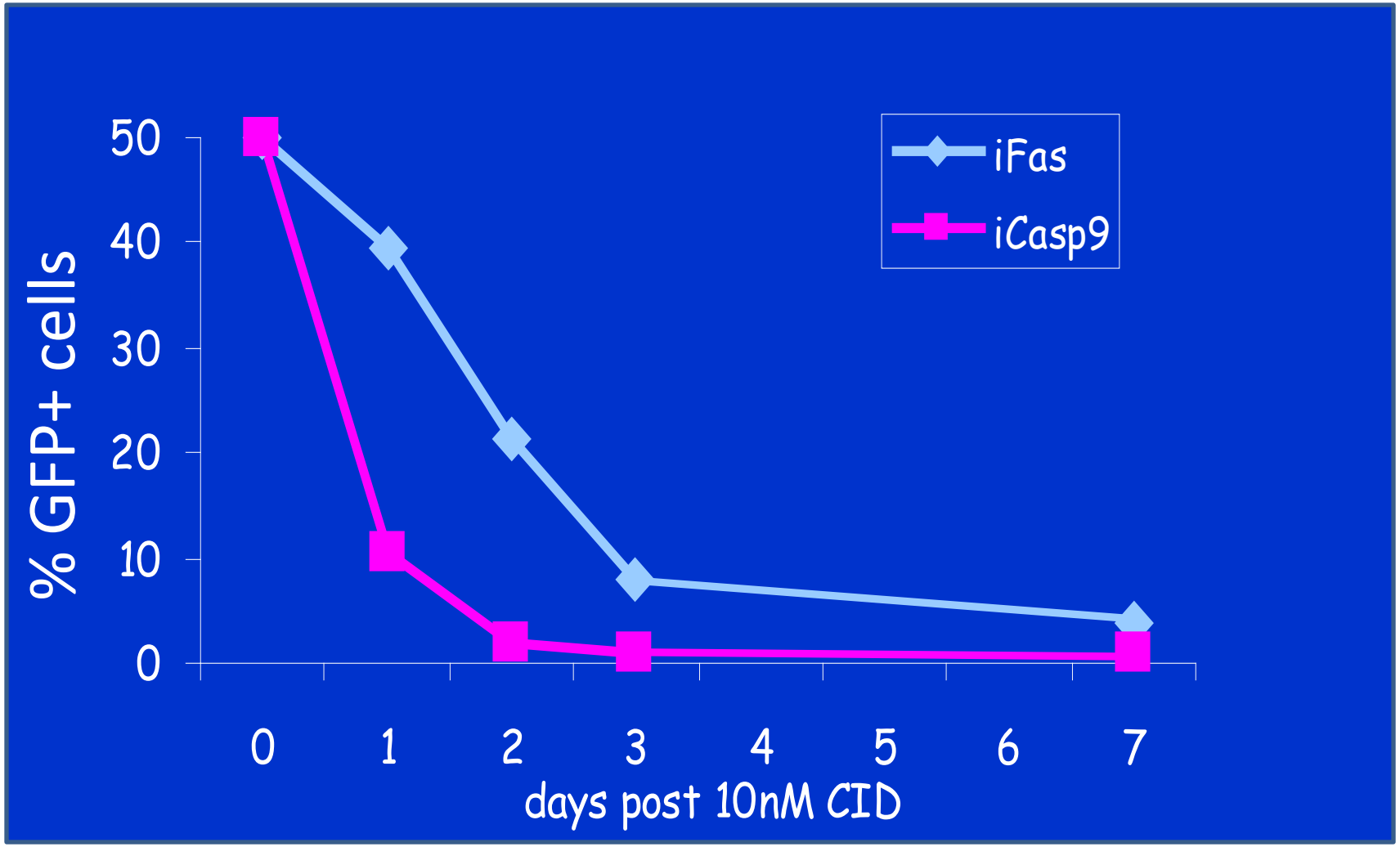
apoptosis

Straathof and Spencer

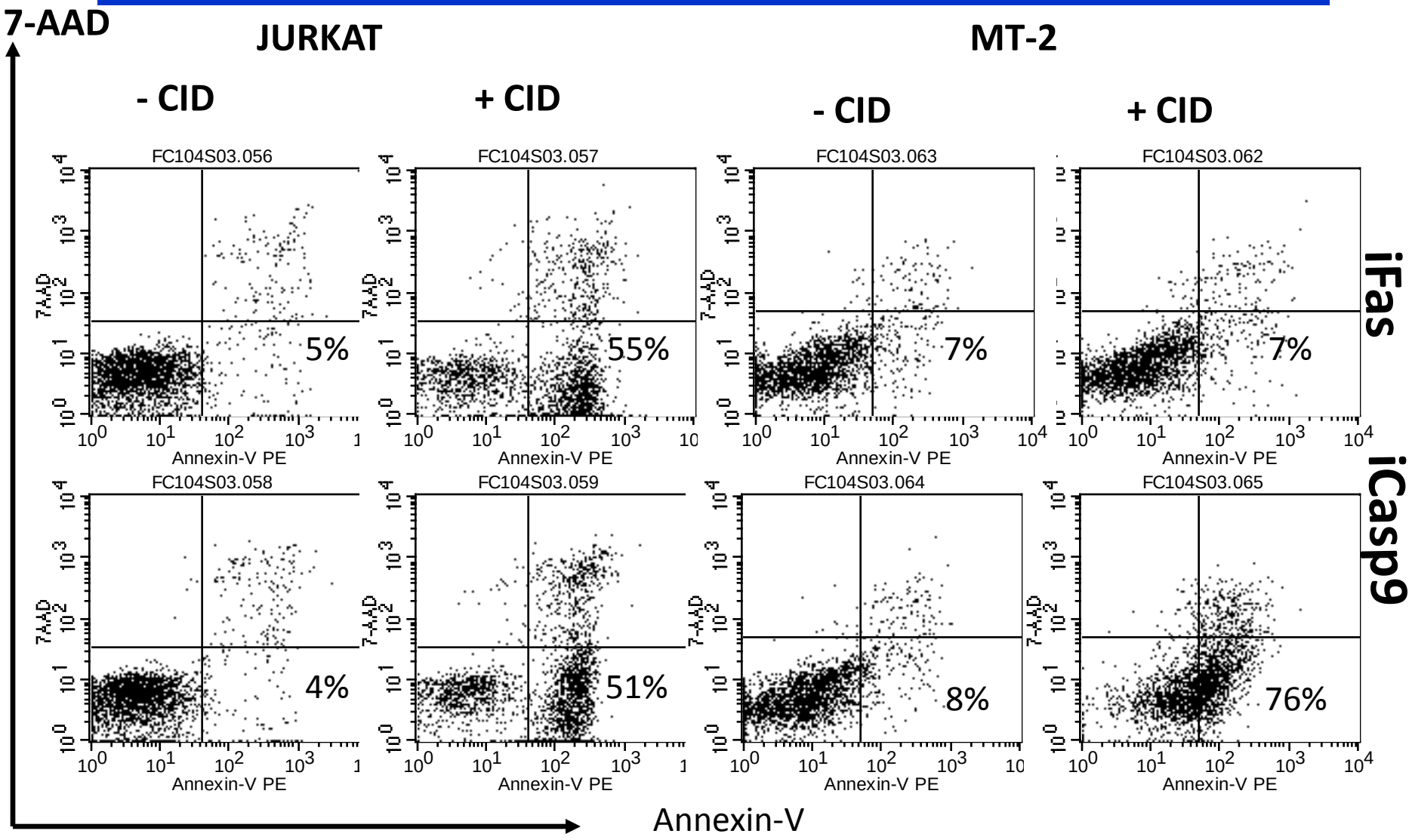
CID Eliminates All IL-12 Secreting CTLs



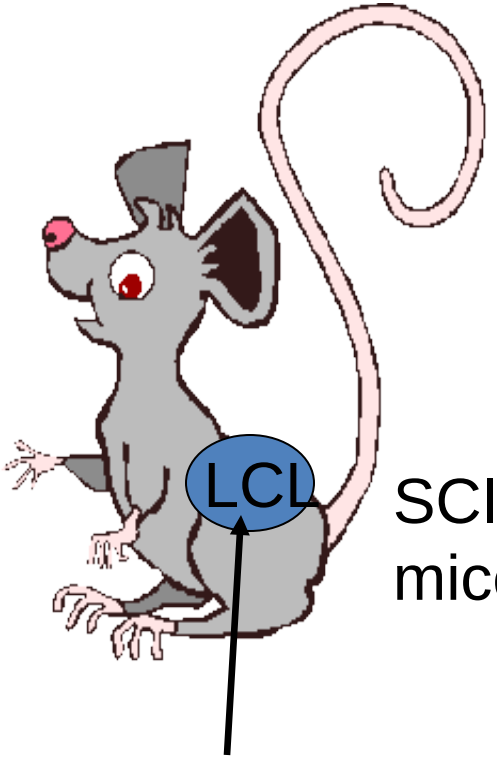
iCaspase 9 more sensitive to CID than iFas?



iCaspase 9 Functions in MT2 Cells That Overexpress Bcl-x_L

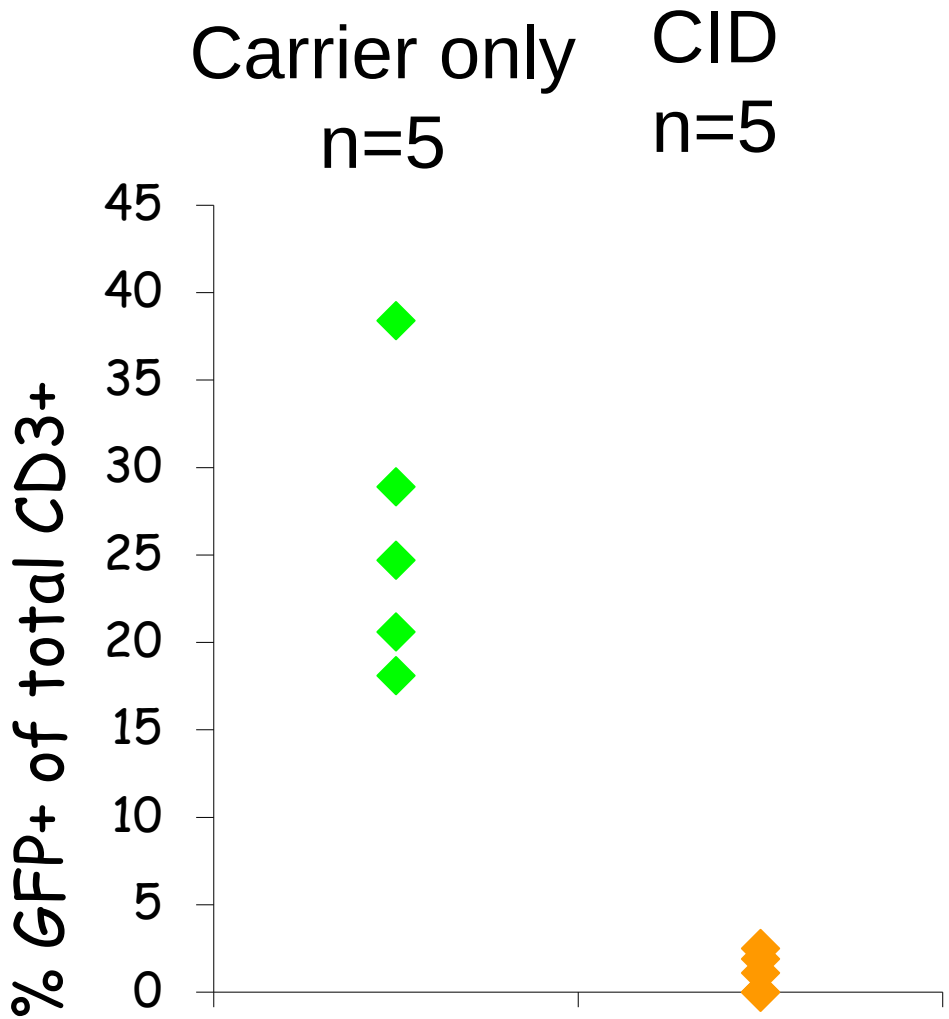


CID Eliminates iCaspase 9 Transduced T cells in Vivo



SCID mice

CTL: 50% non trans
50% iCasp9.IRES.GFP



Preclinical Safety of iCaspase 9

- No change in T cell phenotype
- No change in T cell specificity
- No clonal outgrowth
- Continued dependence on antigen and growth factors

Preclinical Summary

- Expression of iCaspase 9 does not alter phenotype and function of CTL
- Activation of modified iCaspase 9 eliminates up to 90% of total and 100% of functional transgene expressing CTL
- In contrast to iFas, iCasp9 is still functional in bcl-x_L over expressing tumor T cells