

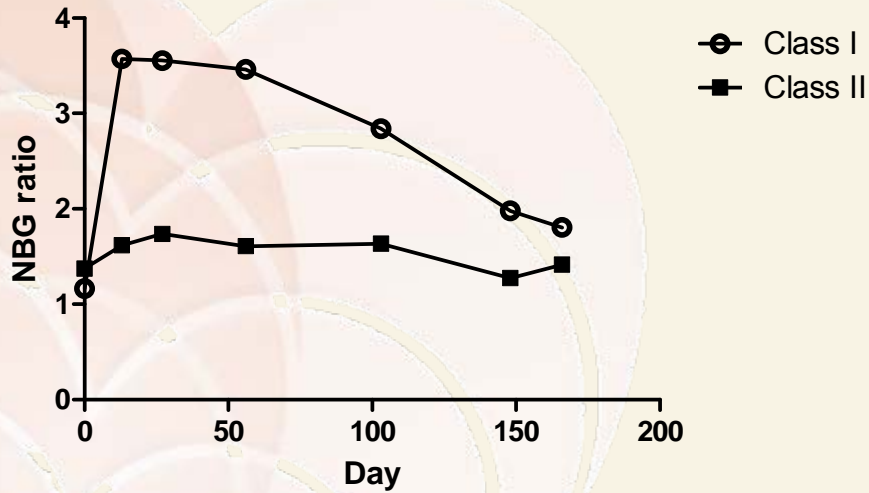
Pathogen reduction effects on non-infectious transfusion outcome

Philip Norris

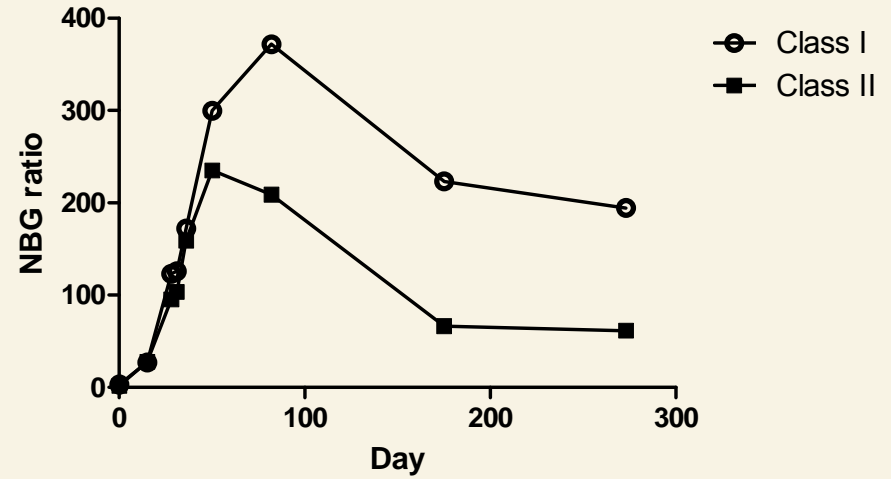


Is HLA alloimmunization a concern in recipients of leukoreduced blood?

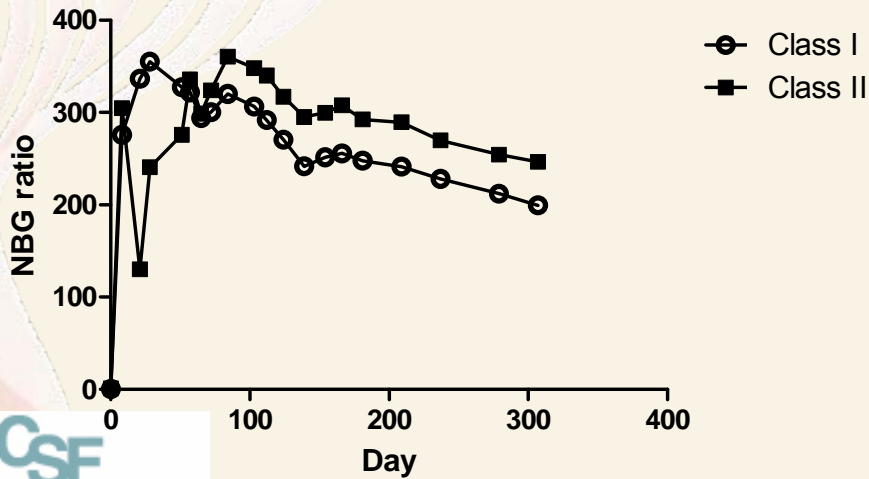
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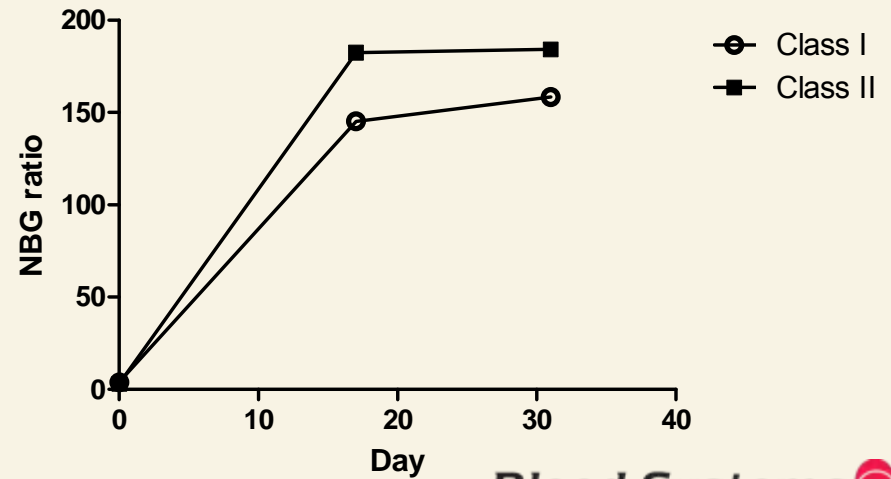
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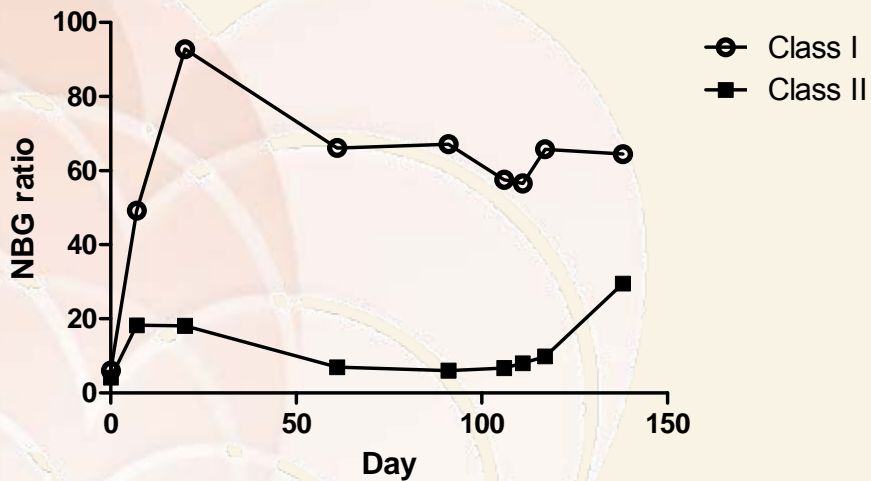
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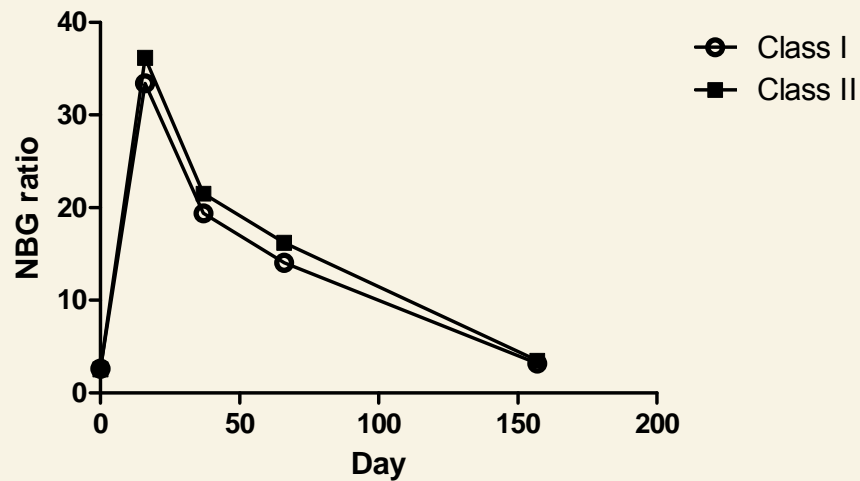
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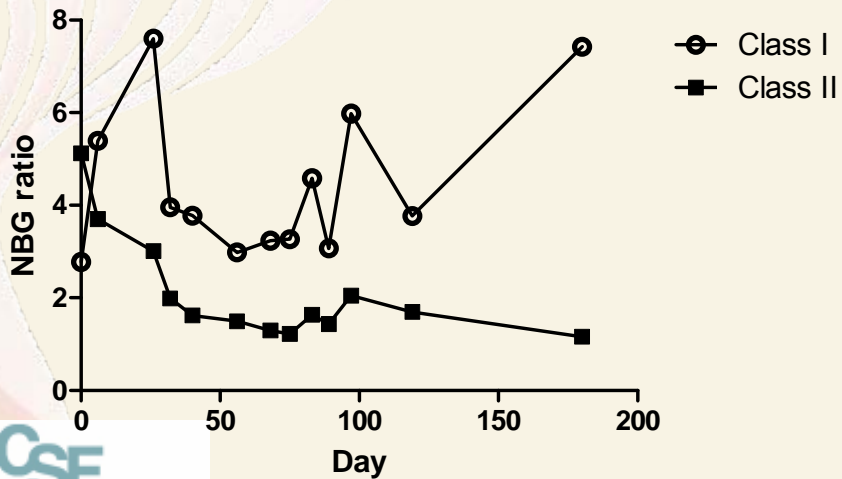
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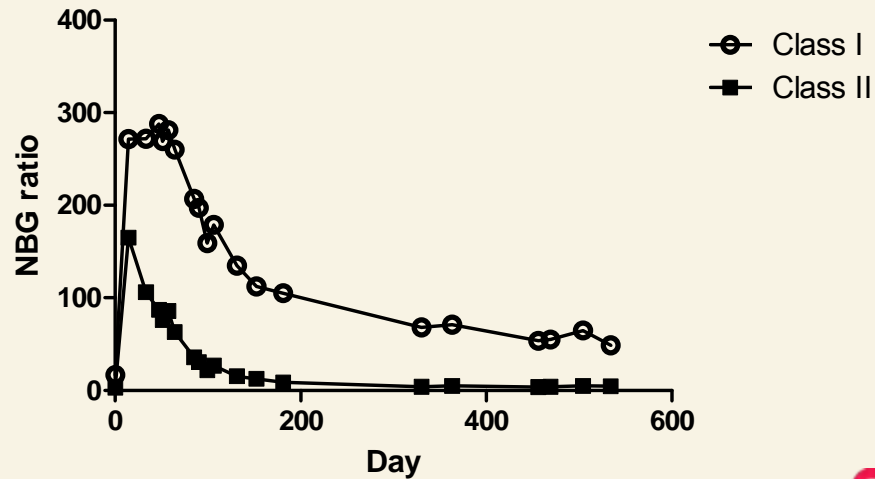
03-0225



03-0720



03-0534



Frequency of alloimmunization

	NBG ratio	Pre-existing Abs			New Ab		
		Class I	Class II	MICA	Class I	Class II	MICA
TTVS	2.2	19 (66%)	8 (28%)	9 (31%)	10 (100%)	13 (62%)	6 (30%)
	10.8/6.9	5 (17%)	3 (10%)	0	16 (67%)	13 (50%)	1 (3%)
TRIPS	2.2	7 (35%)	6 (30%)	3 (15%)	4 (31%)	0	0
	10.8/6.9	3 (15%)	2 (10%)	0	3 (18%)	2 (11%)	0

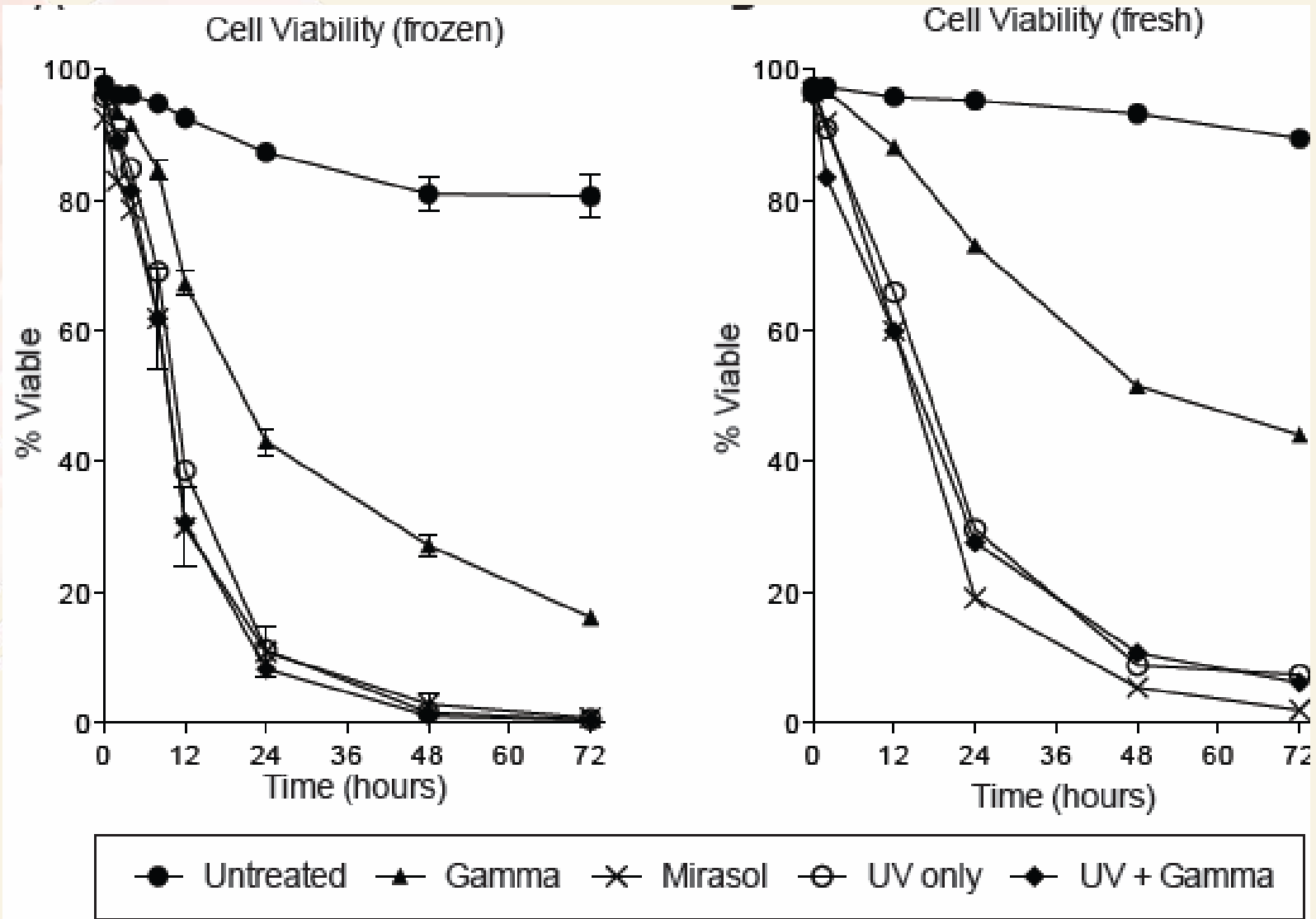
Mirasol Treatment: Pathogen Reduction

- Riboflavin molecules form complexes with nucleic acids of pathogens
- Exposure to UV light activates the riboflavin, causing irreversible damage to DNA, making pathogens unable to replicate

Pathogen reduction: Donor Cell Inactivation

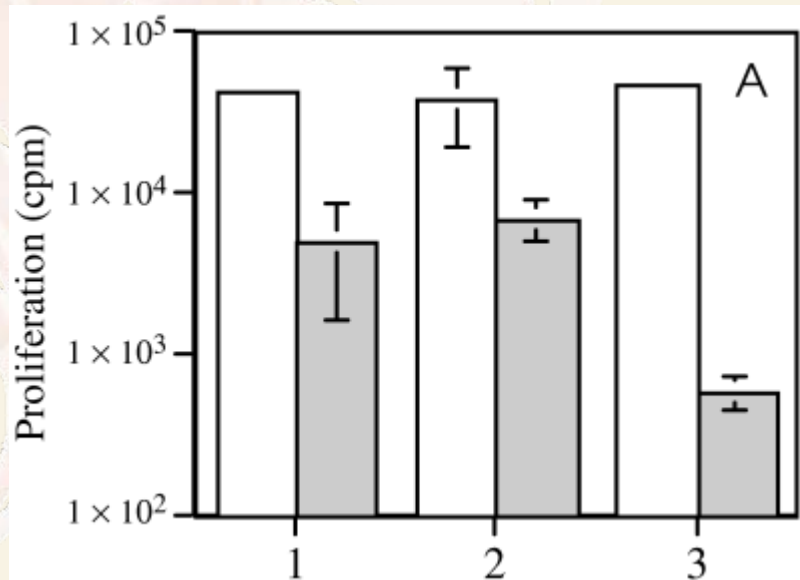
- Nucleated donor cells as well as pathogens are effected by this process
- Unable to divide or survive
- Lose immunogenicity : can no longer stimulate allogeneic response

PR and UV treatment accelerate cell death

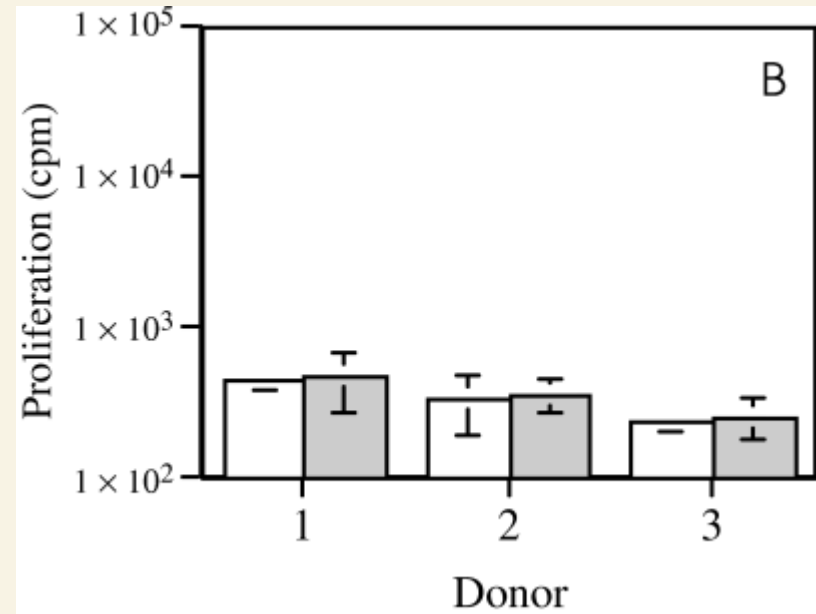




PR Treatment: Loss of Allogeneic response

Untreated



PRT Treated



 Responders + Stimulators
 Responders Alone

Mixed Lymphocyte Reaction

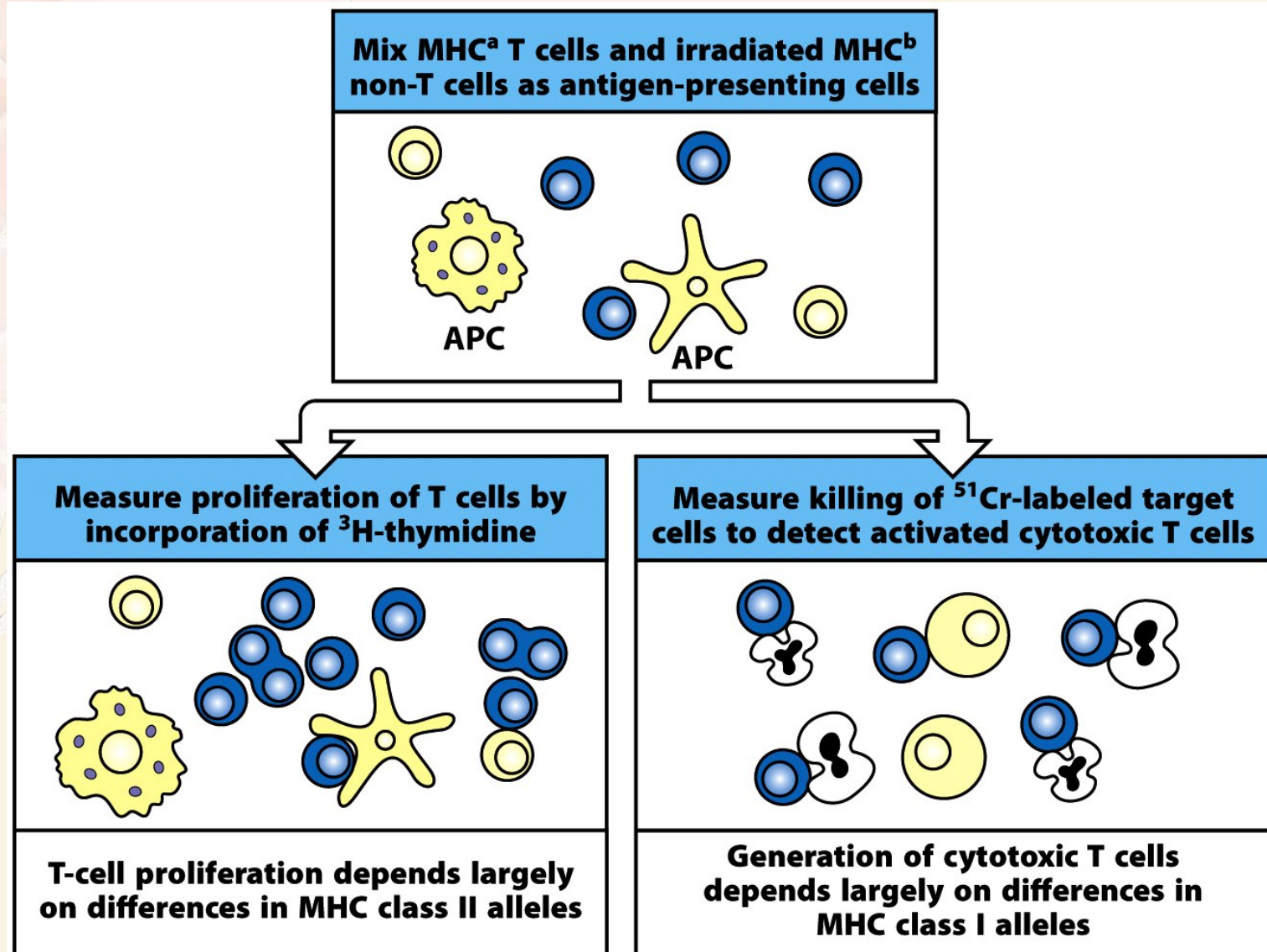
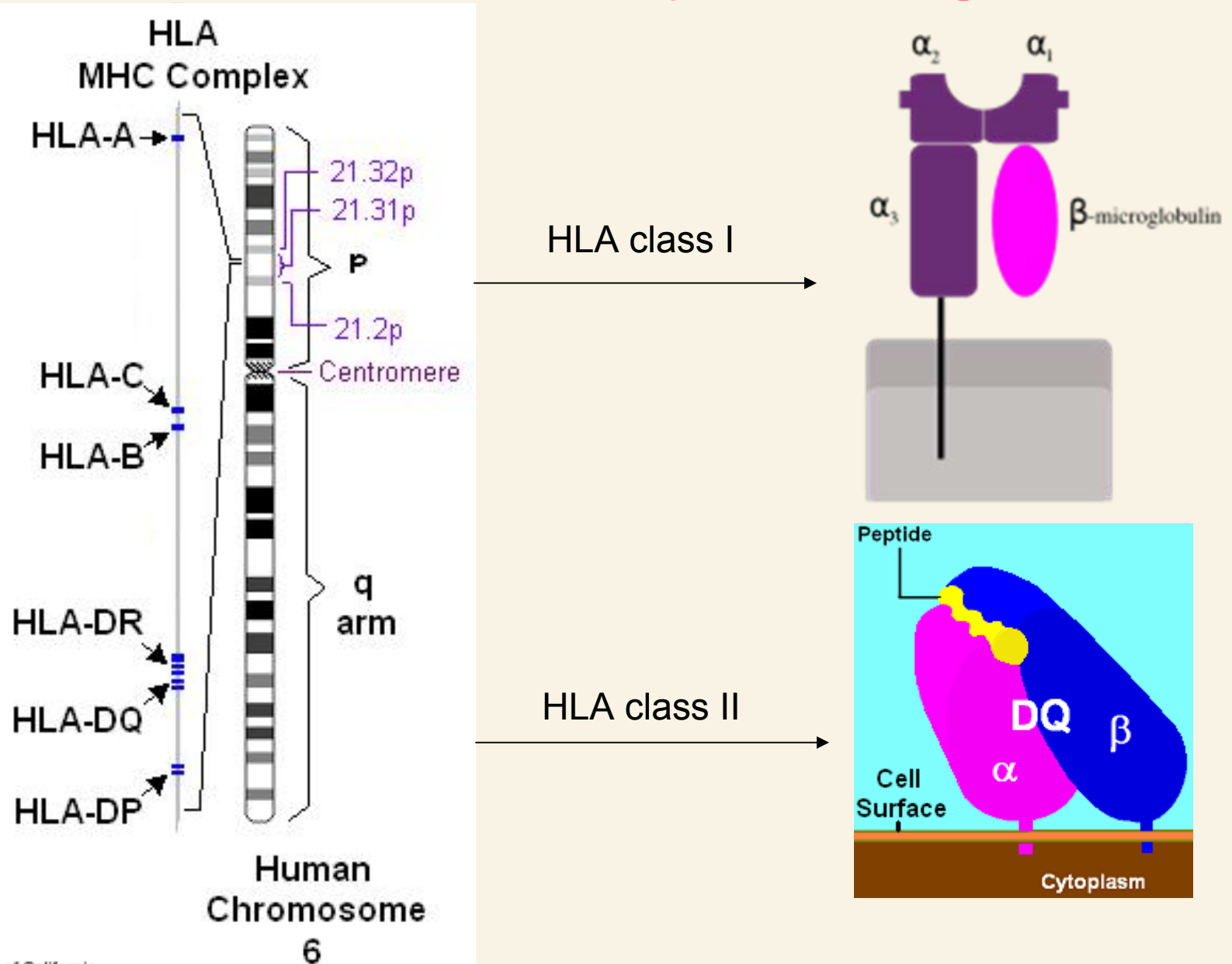


Figure 14-46 Immunobiology, 7ed. (© Garland Science 2008)

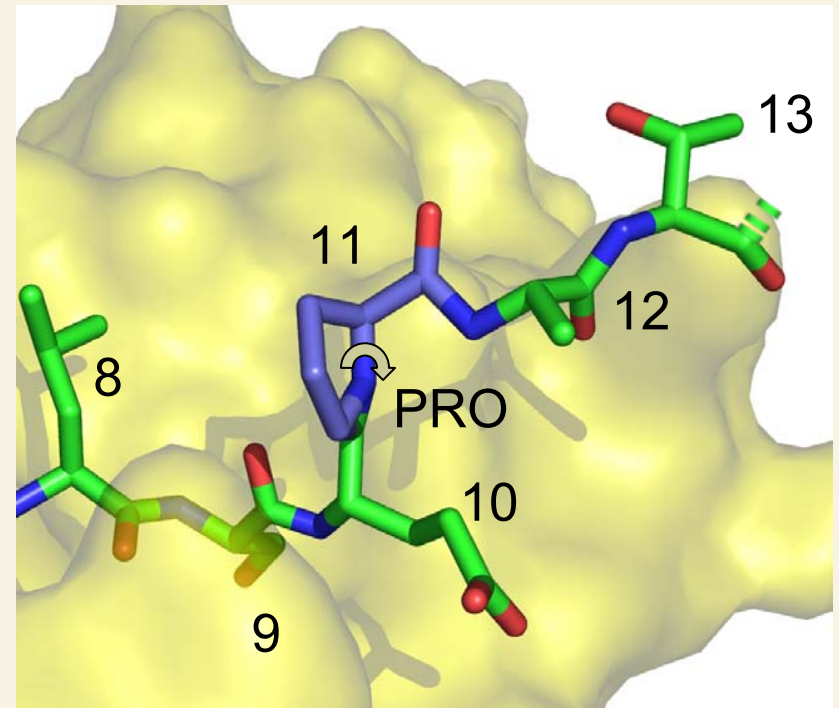
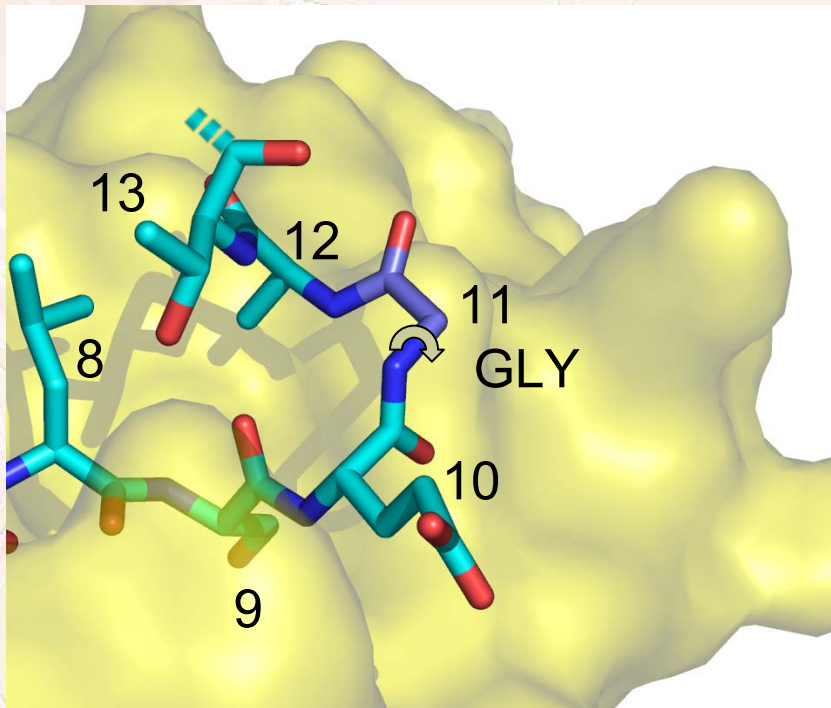


Why are allo-immune responses so common?

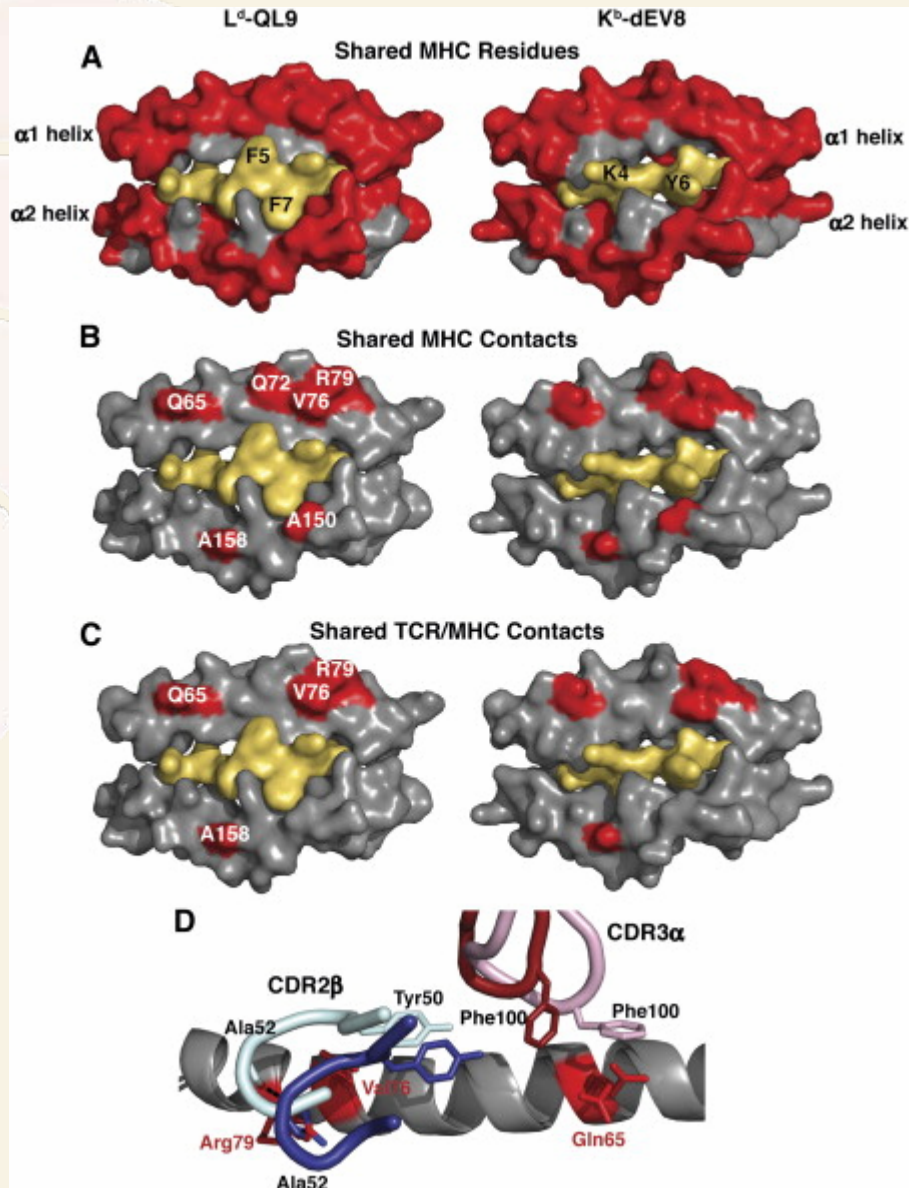
Human leukocyte antigen



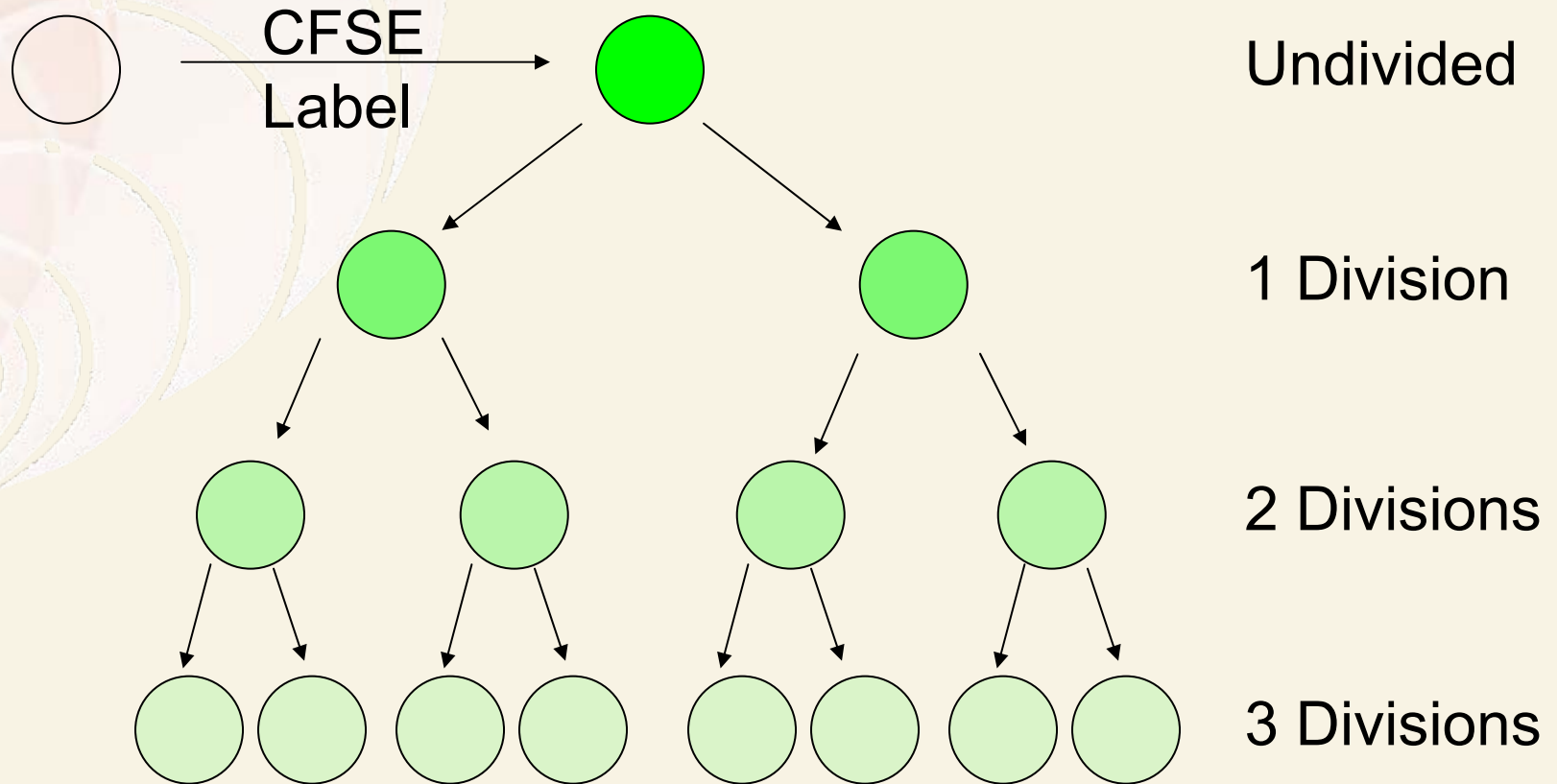
Peptide sits in the binding groove



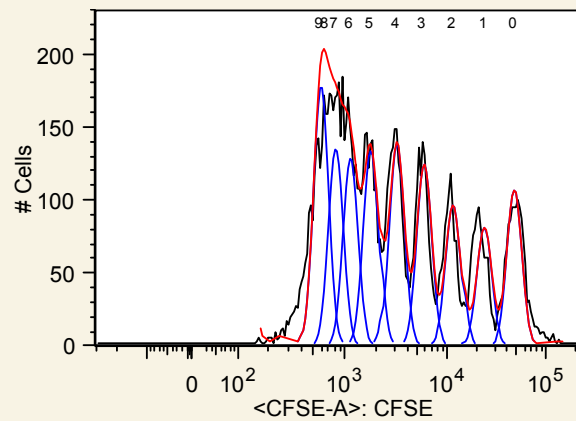
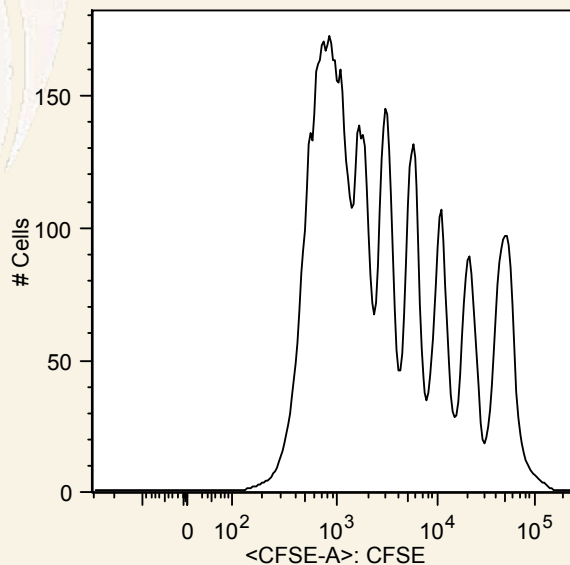
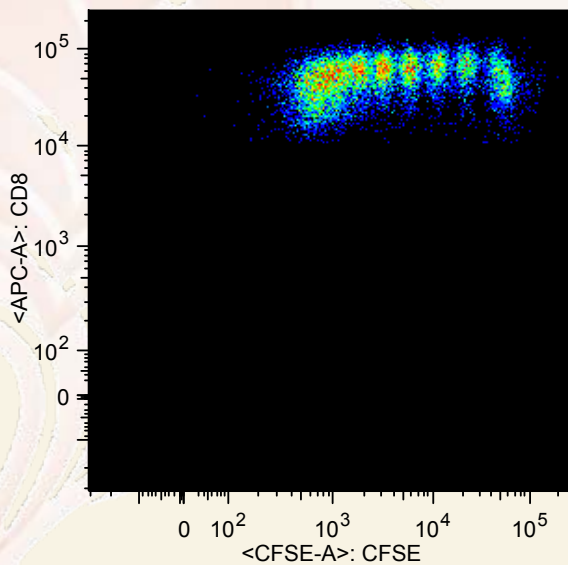
Structural basis for allo-responses



Measuring Proliferation with CFSE dilution



Measuring Proliferation with CFSE dilution



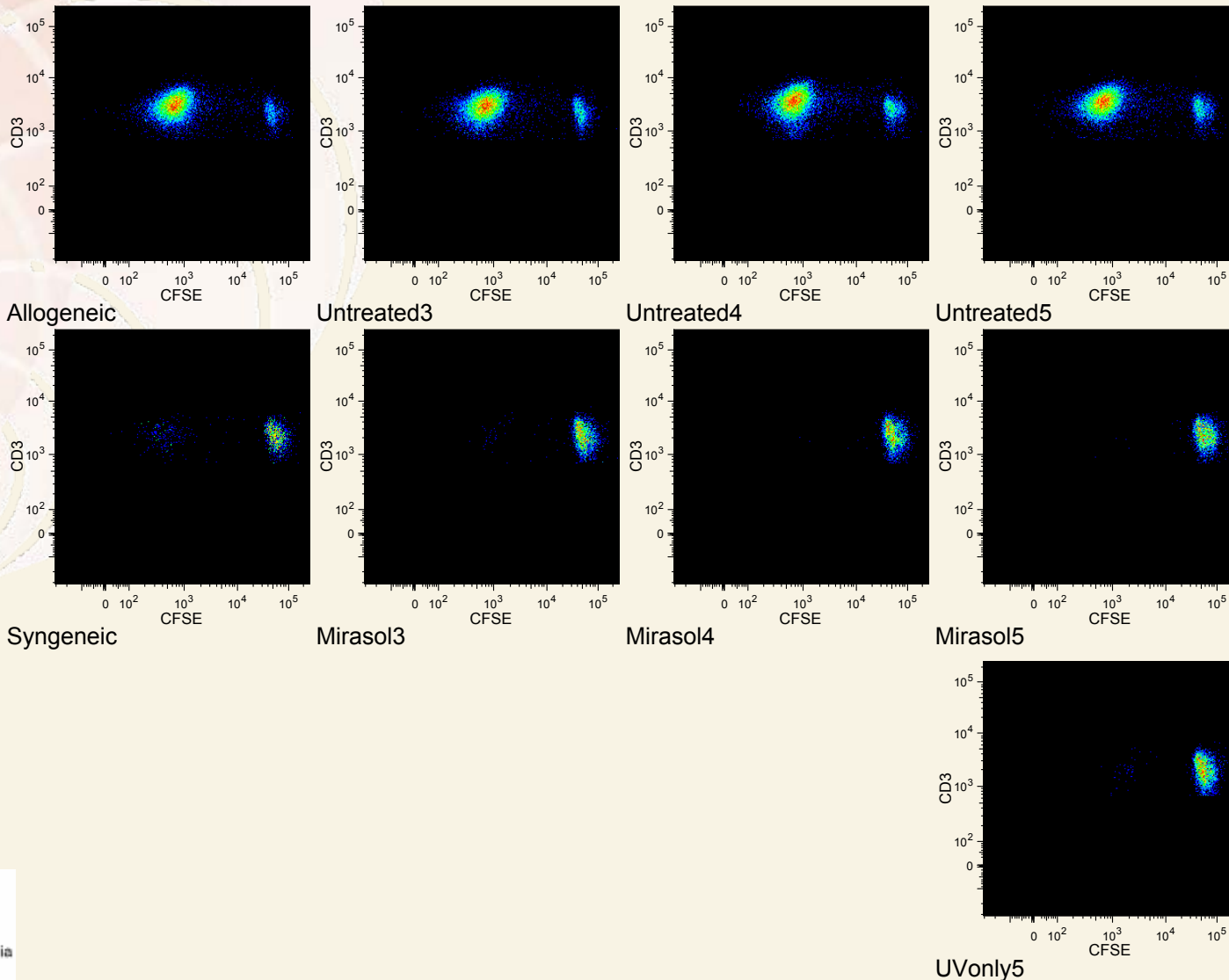
%Divided = 47.5
Div. Index = 1.05
Prol. Index = 2.21.

Measuring Proliferation with CFSE dilution

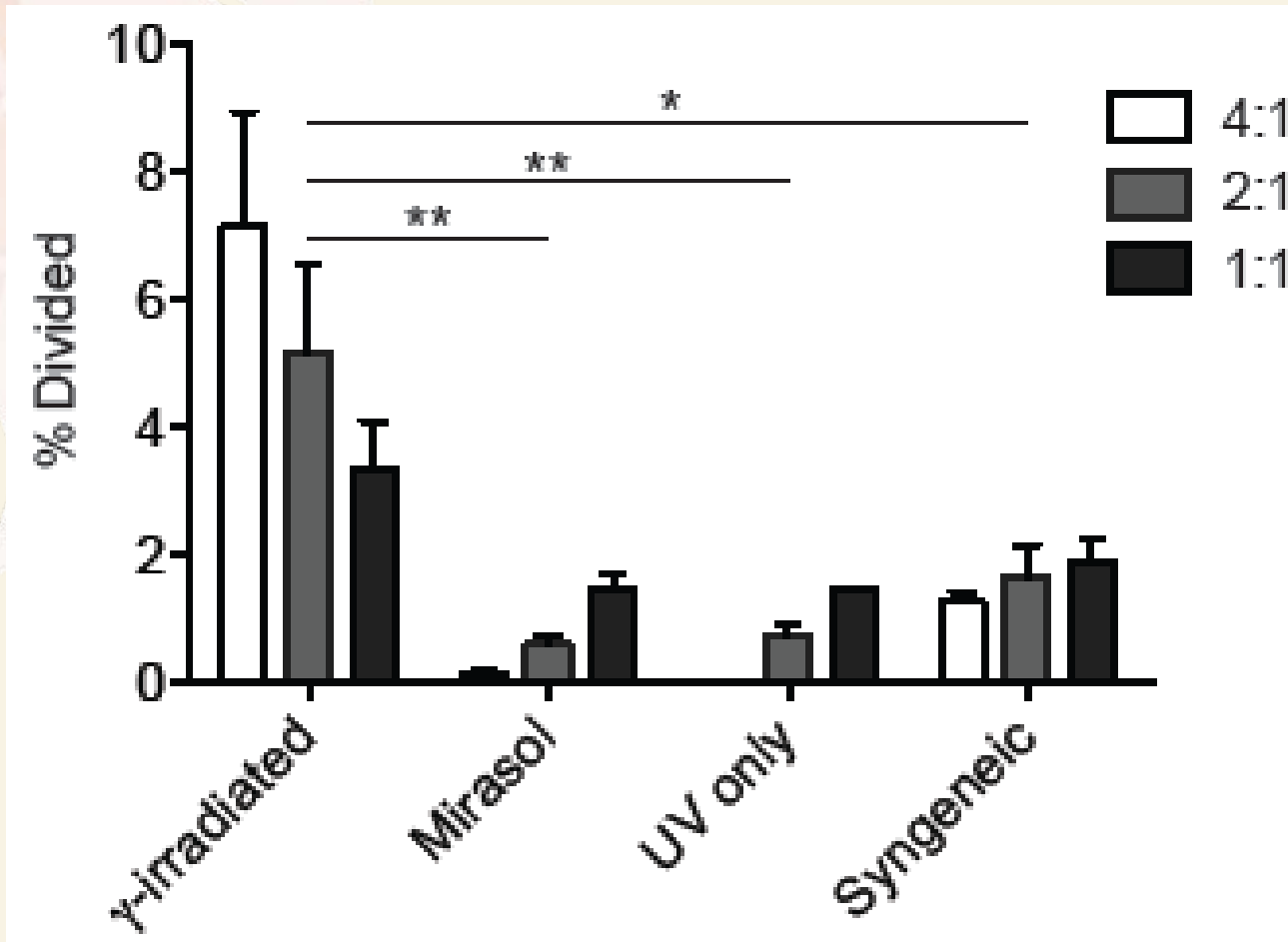
Advantages over ^3H -thymidine incorporation:

- More precise measurement of proliferation
 - Proportion of cells responding
 - Number of divisions
- Analysis of additional phenotypic markers

PR and UV treated cells fail to stimulate allogeneic proliferation



UV treated cells fail to stimulate allogeneic proliferation



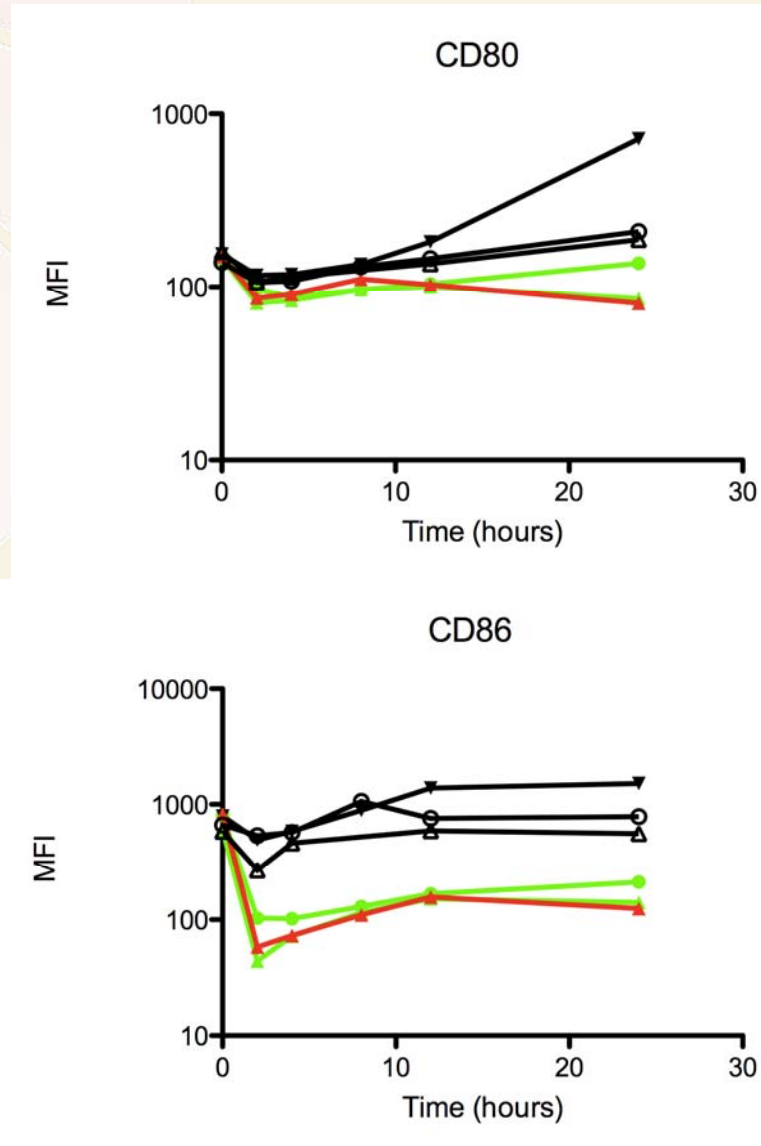
Why no response?

Weak signal?

Inhibitory or tolerizing signal?

Failure to bind?

Reduced surface expression of costimulatory molecules on UVB treated APCs



- Mirasol 3
- ▲ Mirasol 5
- ▼ Normal
- ▲ UV only 5
- Untreated 3
- ▲ Untreated 5

Why no response?

Weak signal?

Maybe

Inhibitory or tolerizing signal?

Failure to bind?

Can PR Treated cells inhibit an allo response?

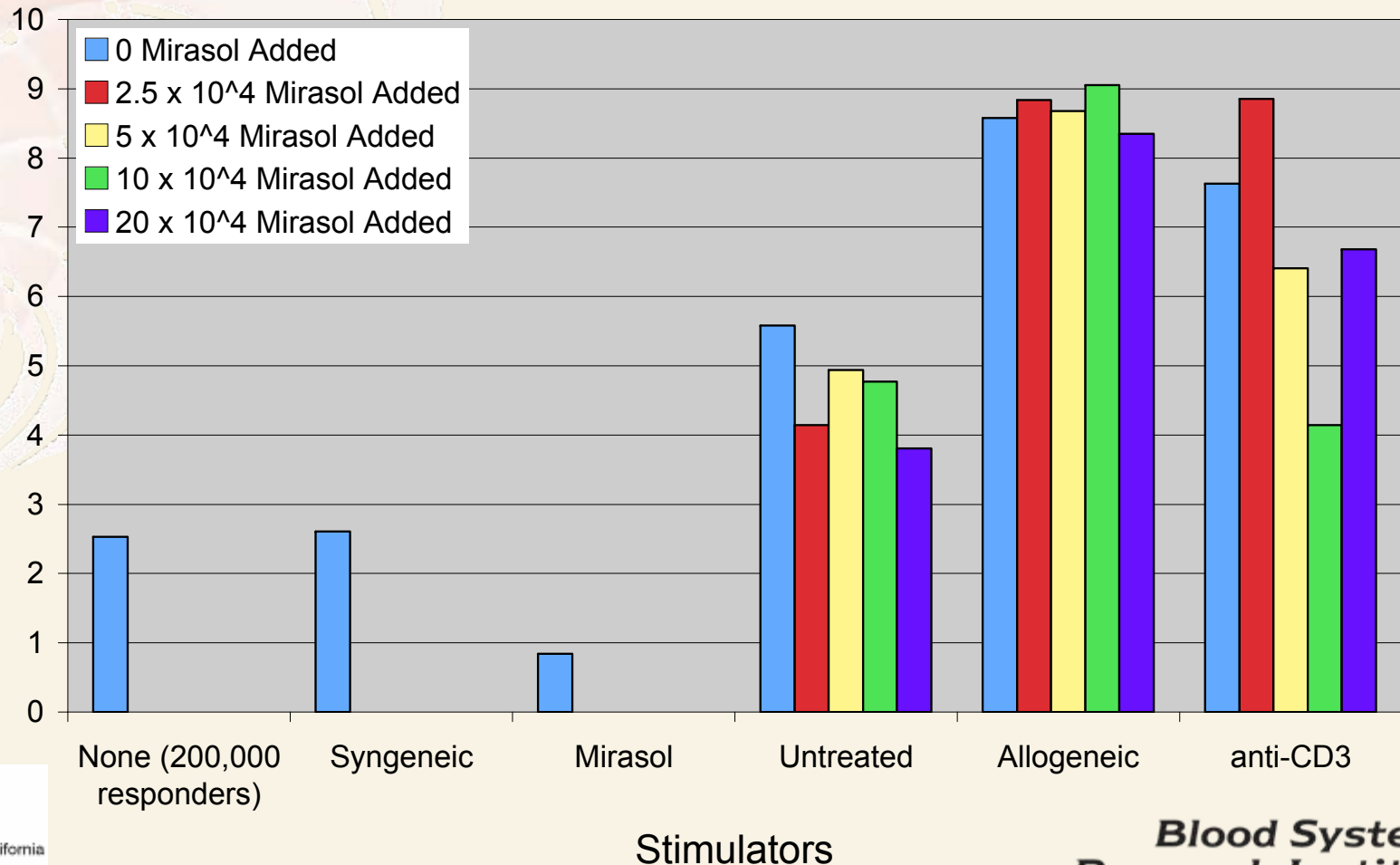
- Add PR treated cells to MLRs with in varying ratios with stimulator cells
(PR treated:stimulator = 1:8, 1:4, 1:2, 1:1, 0:1)
- Look at effect on allogeneic MLR and anti-CD3 responses
- Look at effect on allogeneic cells from the same or different donor as the PR treated cells

Can PR Treated cells induce tolerance?

- Prime normal PBMCs in MLR with PR treated or untreated allogeneic cells
- Challenge with same PR treated cells, same untreated cells, new allogeneic cells, syngeneic cells, or anti-CD3
- Look for failed response to untreated cells in responders that have been primed with PR treated cells from the same or different donor

PR treated cells do not inhibit allo response to untreated cells

% Divided total T cells (100,000 responders)



Why no response?

Weak signal?

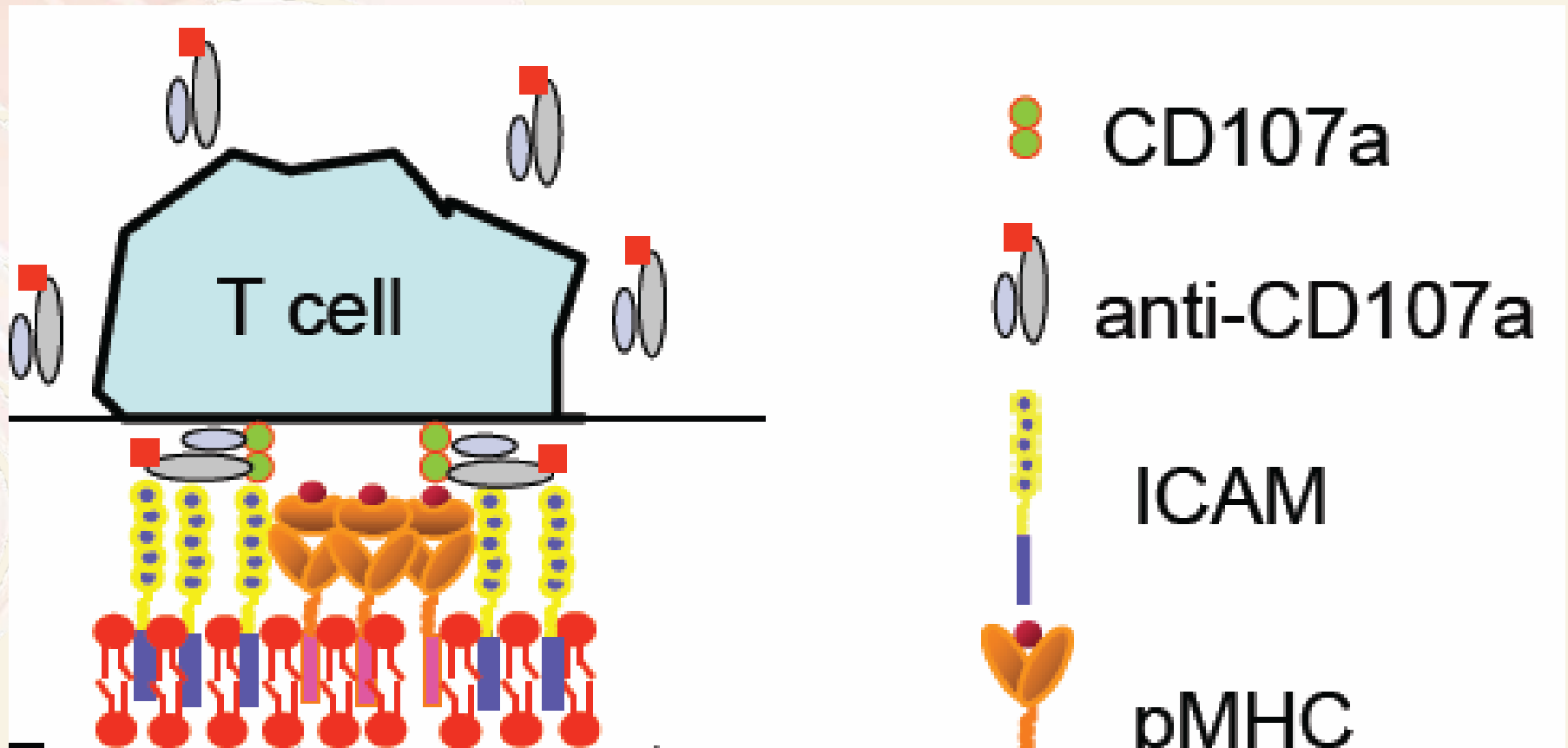
Maybe

Inhibitory or tolerizing signal?

No

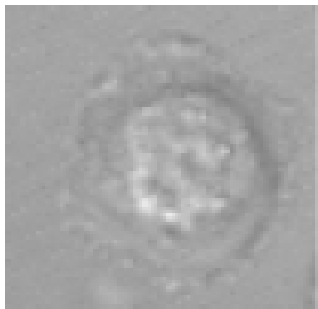
Failure to bind?

Formation of T cell synapses

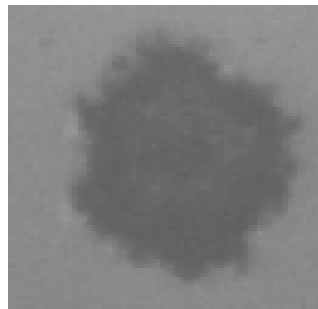


Visualization of the T cell synapse

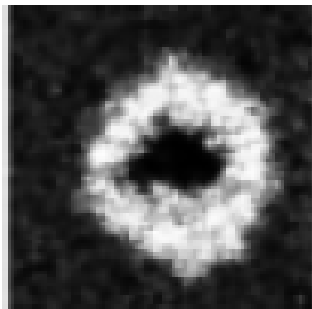
Bright field



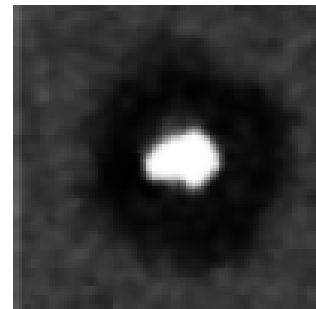
IRM



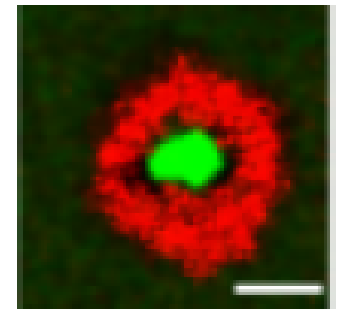
ICAM-1



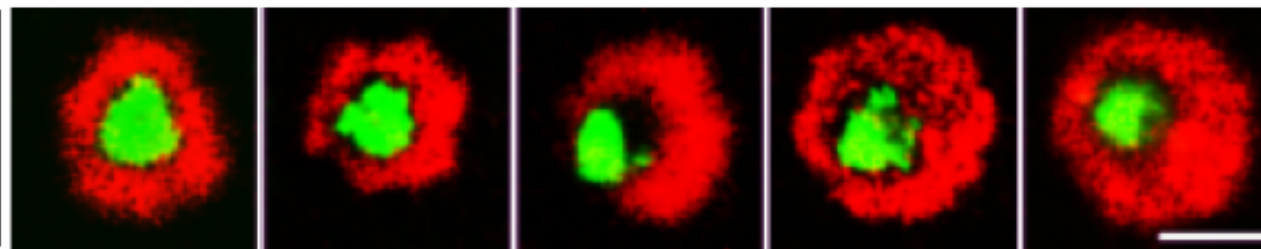
pMHC



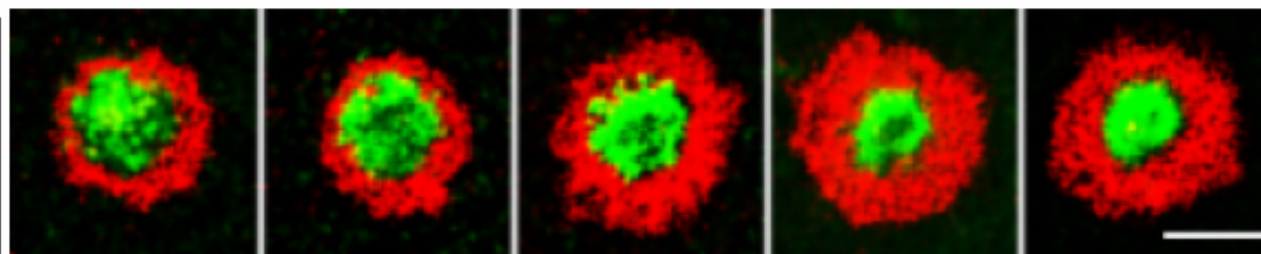
Merge



CD4⁺ AC-25
500 pMHC/ μm^2



CD8⁺ CER43
500 pMHC/ μm^2



5

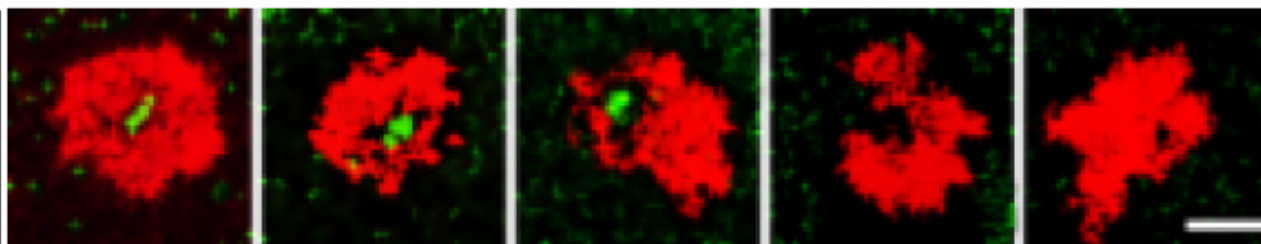
10

20

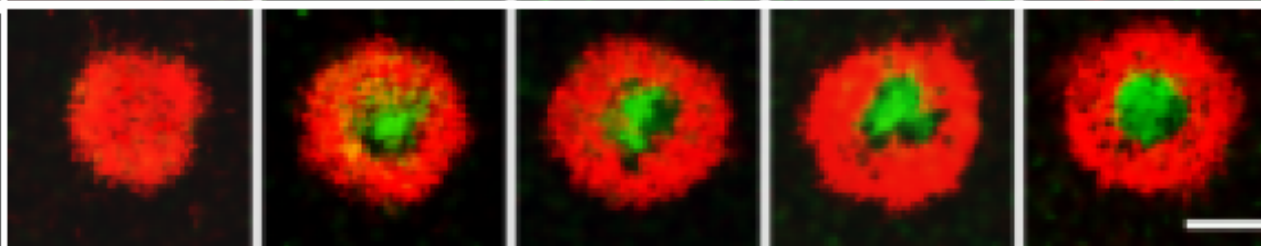
40

60

CD4⁺ AC-25
25 pMHC/ μm^2



CD8⁺ CER43
25 pMHC/ μm^2



5

10

20

30

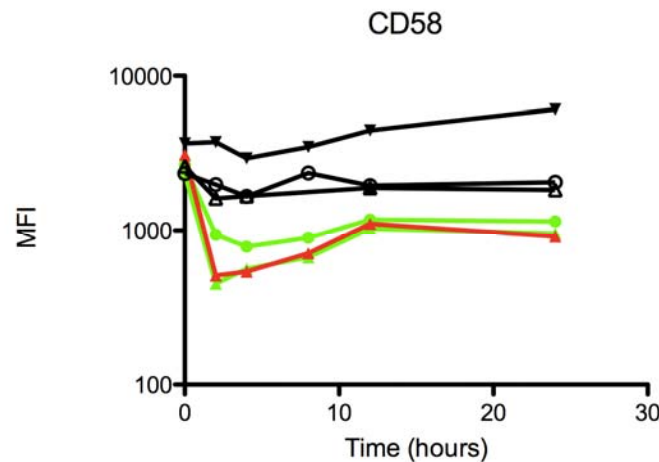
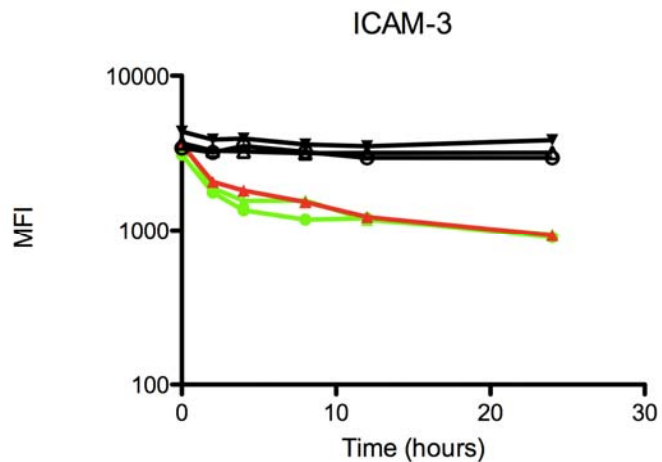
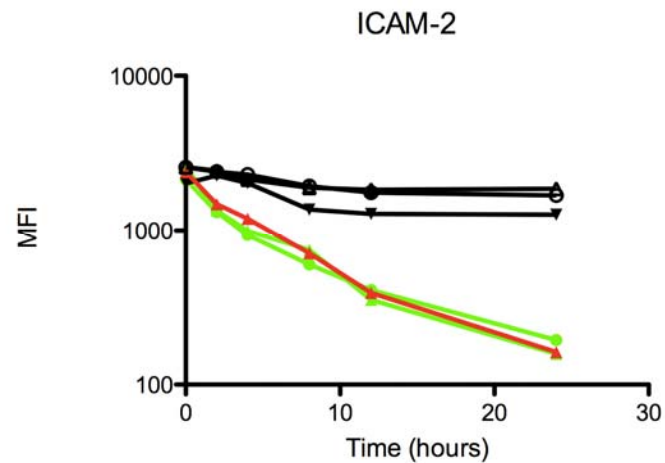
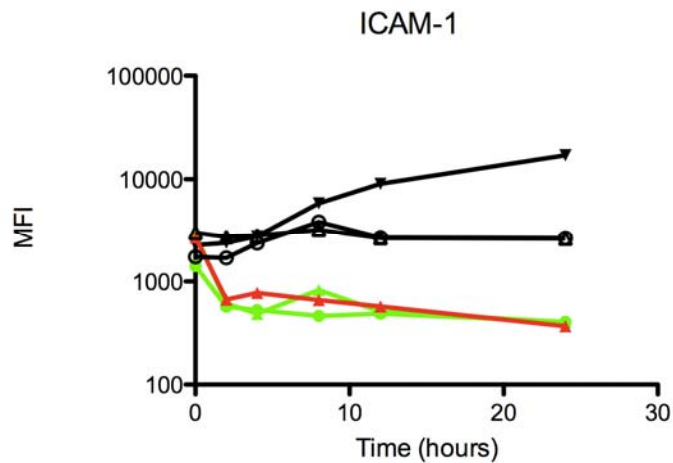
60

Time (min)

Does PR treatment impact expression of adhesion molecules?

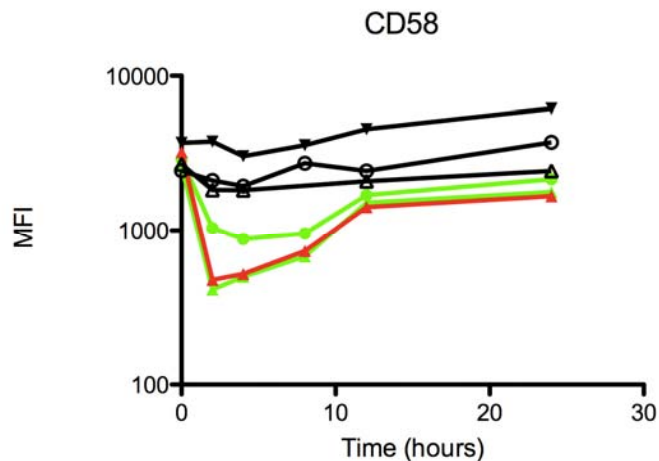
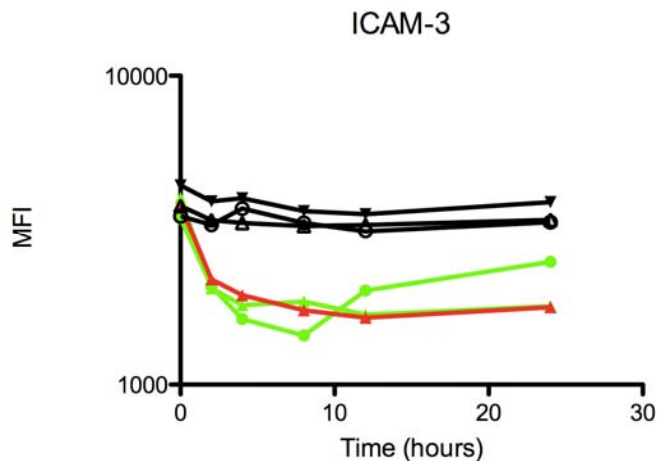
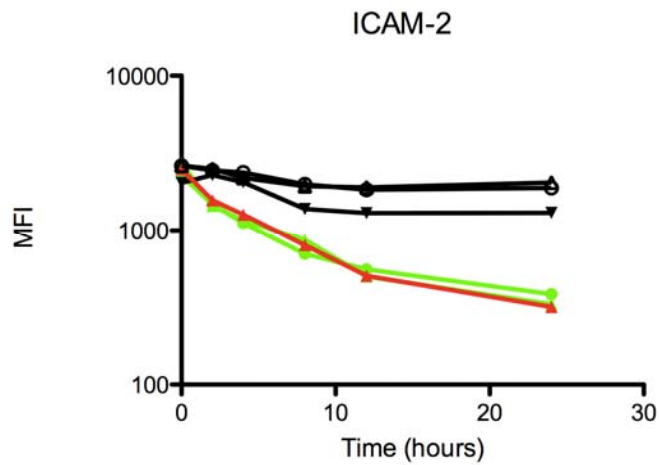
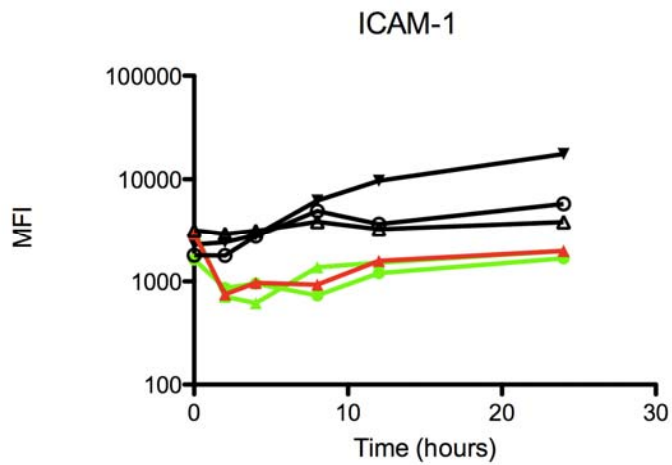
- The surface expression of some adhesion molecules has been shown to be effected by UV exposure
- Culture treated and untreated cells and look at surface expression of adhesion molecules over time

Surface adhesion molecules down-regulated on UV treated APCs



- Mirasol 3
- Mirasol 5
- Normal
- UV only 5
- Untreated 3
- Untreated 5

Down regulation of ICAM-1 and CD58 linked with death



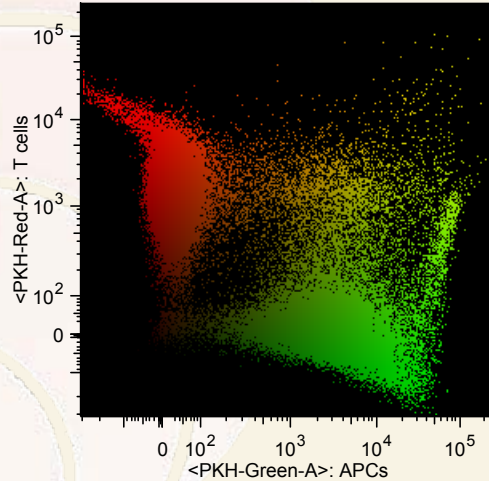
- Mirasol 3
- Mirasol 5
- Normal
- UV only 5
- Untreated 3
- Untreated 5

Do treated cells form antigen specific conjugates?

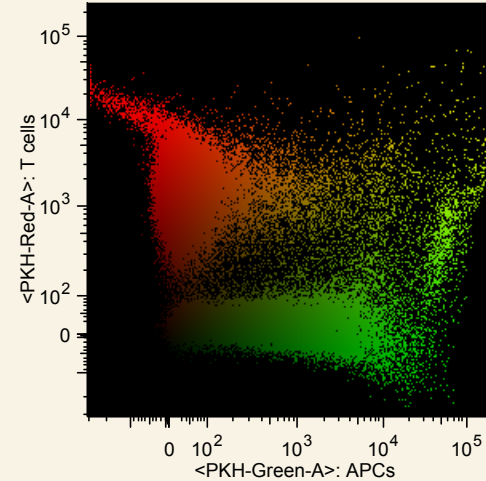
- Enrich for T cells in the responder population, and deplete T cells in stimulator population
- Label responders and stimulators with two different colored dyes
- Culture cells together for 24 hours, and then run on cytometer looking for double positive doublets

PR treated cells may have conjugation defect

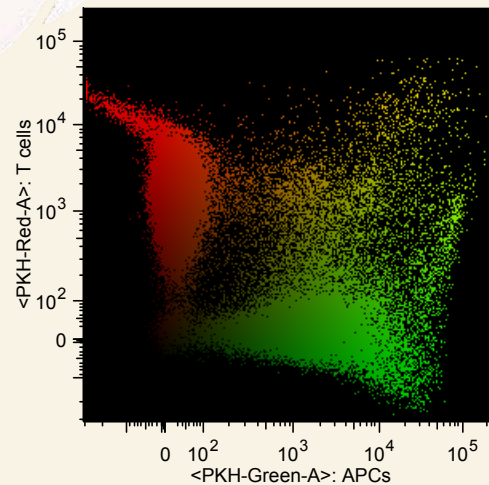
Untreated
APCs



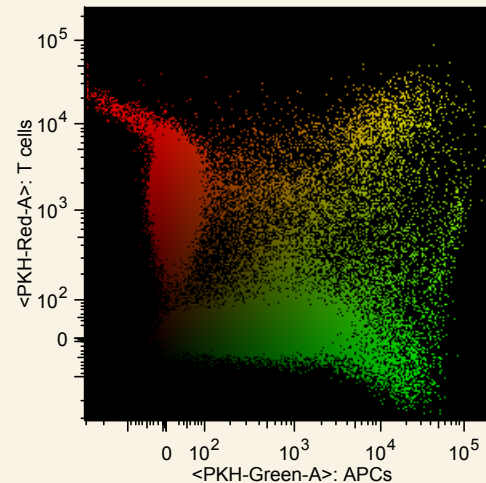
Mirasol
Treated
APCs



Normal
Allogeneic
APCs

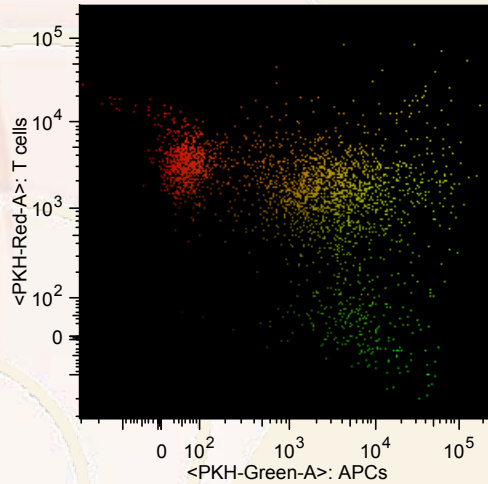


Normal
Syngeneic
APCs

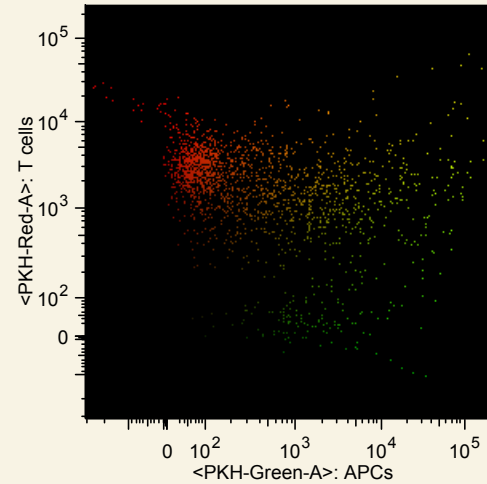


PR treated cells may have conjugation defect

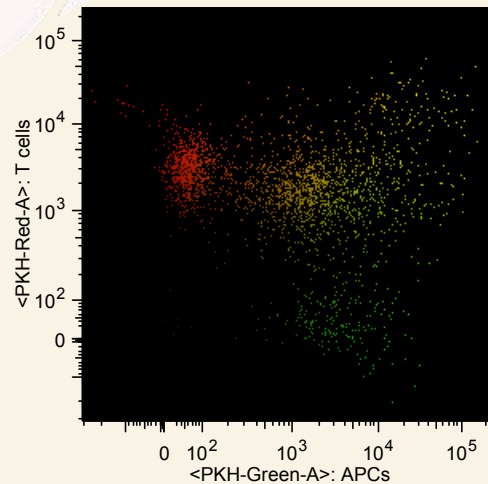
Untreated
APCs



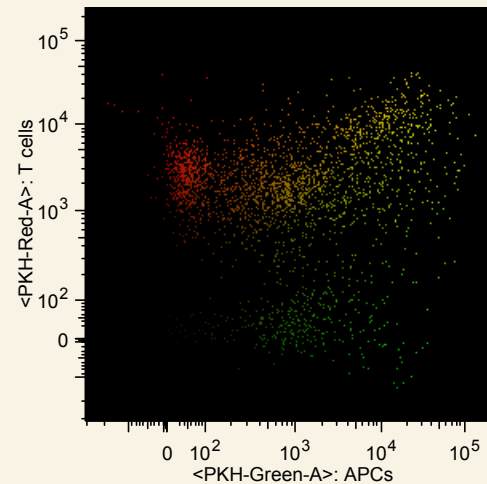
Mirasol
Treated
APCs



Normal
Allogeneic
APCs



Normal
Syngeneic
APCs

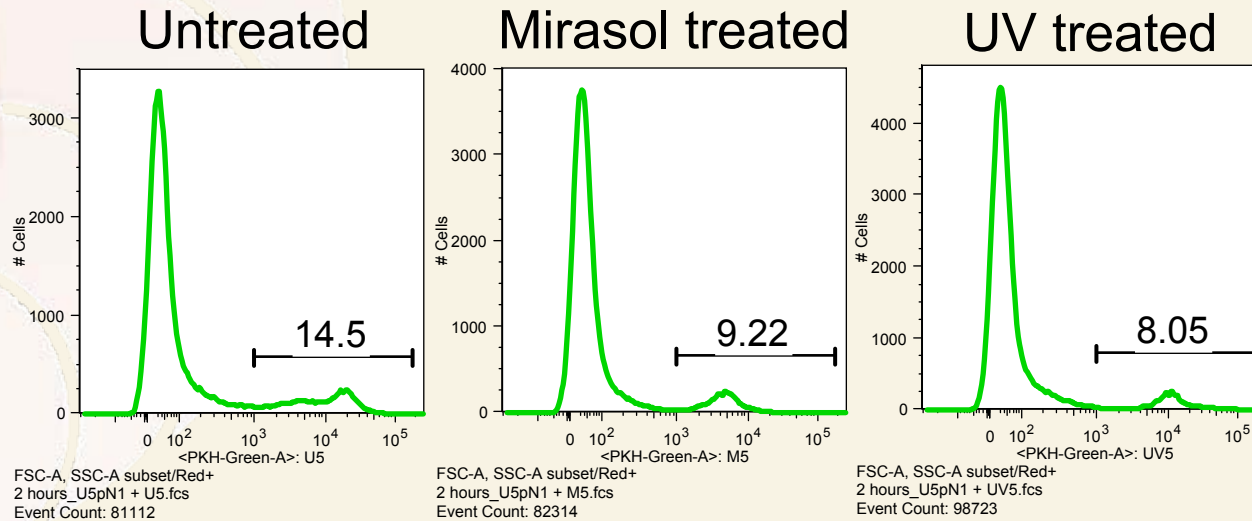


Do treated cells form antigen specific conjugates?

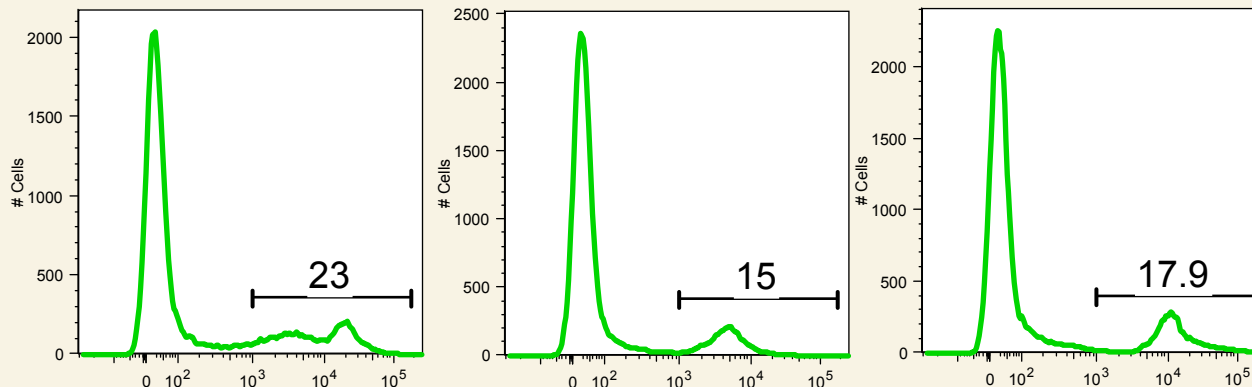
- Too much background with low signal
- Boost antigen specific population by priming with untreated cells first

Reduction, but not loss of conjugation following PR treatment

Exp #1



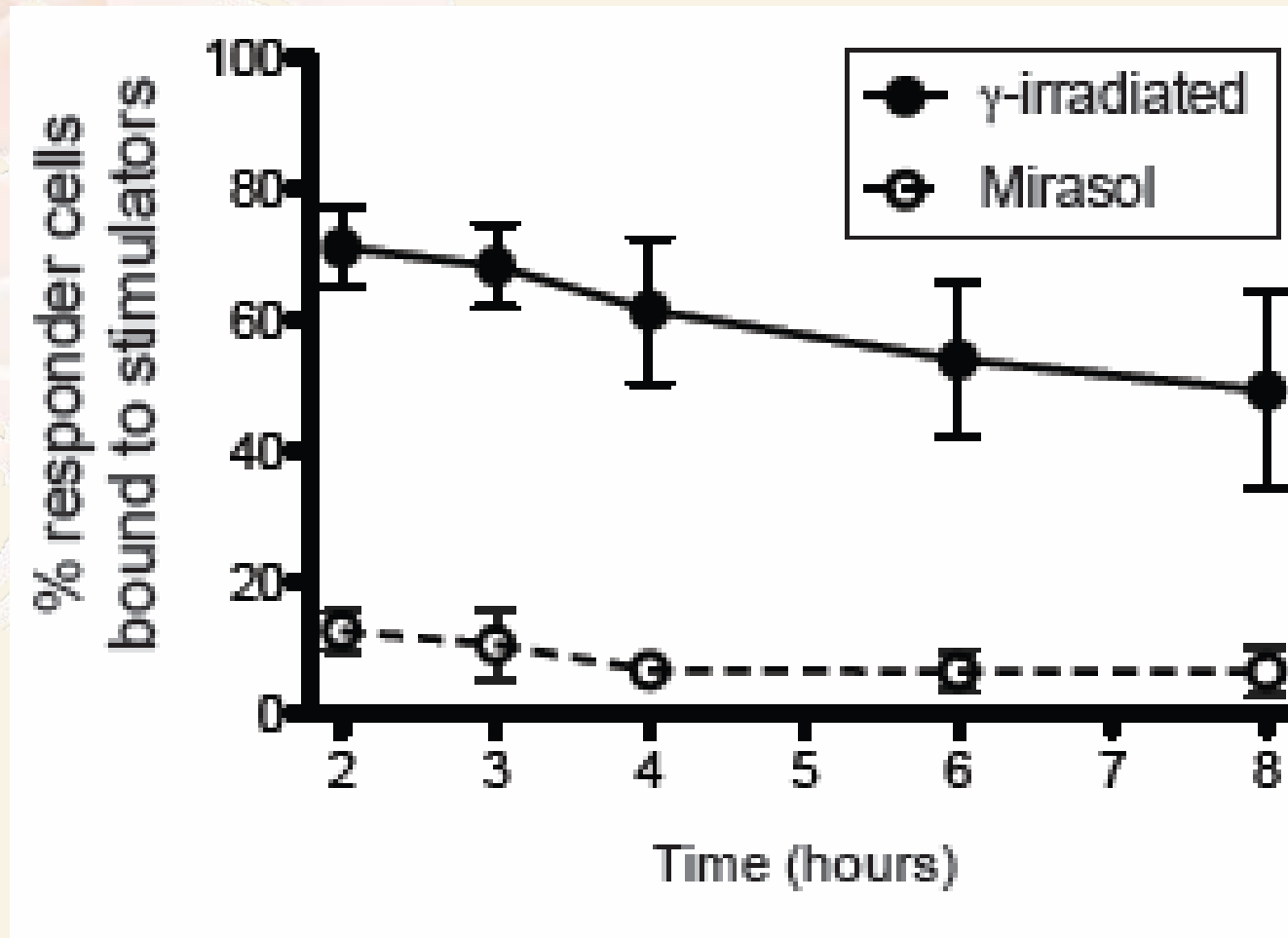
Exp #2



Are conjugates long lasting?

- This assay just shows a snapshot in time
- Check conjugation level at hourly intervals

Conjugates between Mirasol treated and responder cells are short-lived



Why no response?

Weak signal?

Maybe

Inhibitory or tolerizing signal?

No

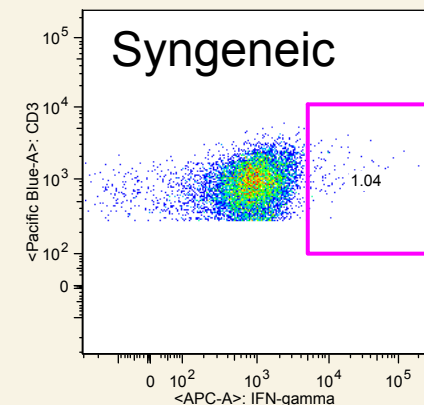
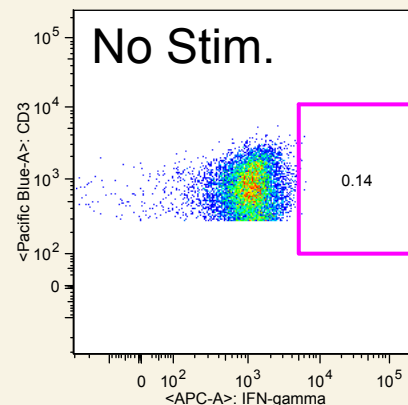
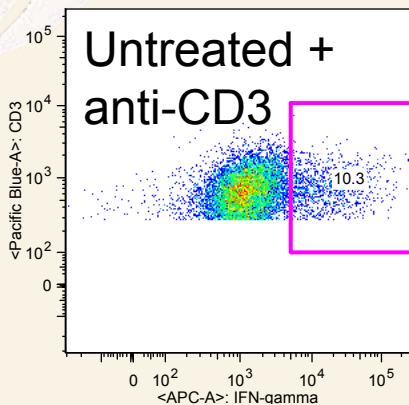
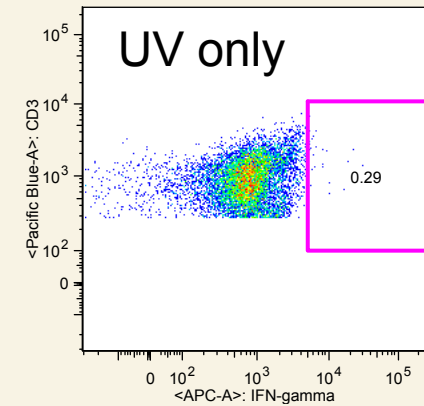
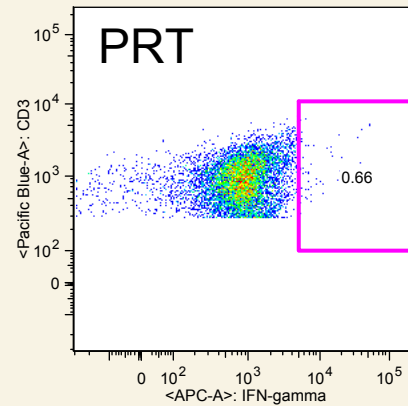
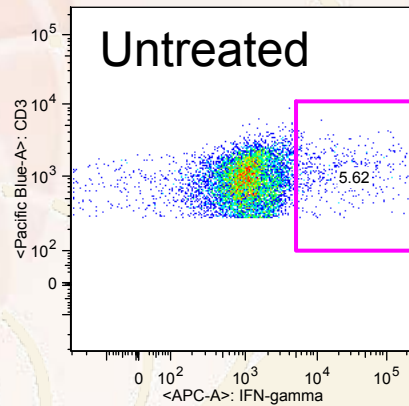
Failure to bind?

**Defect in ability to form lasting
conjugates**

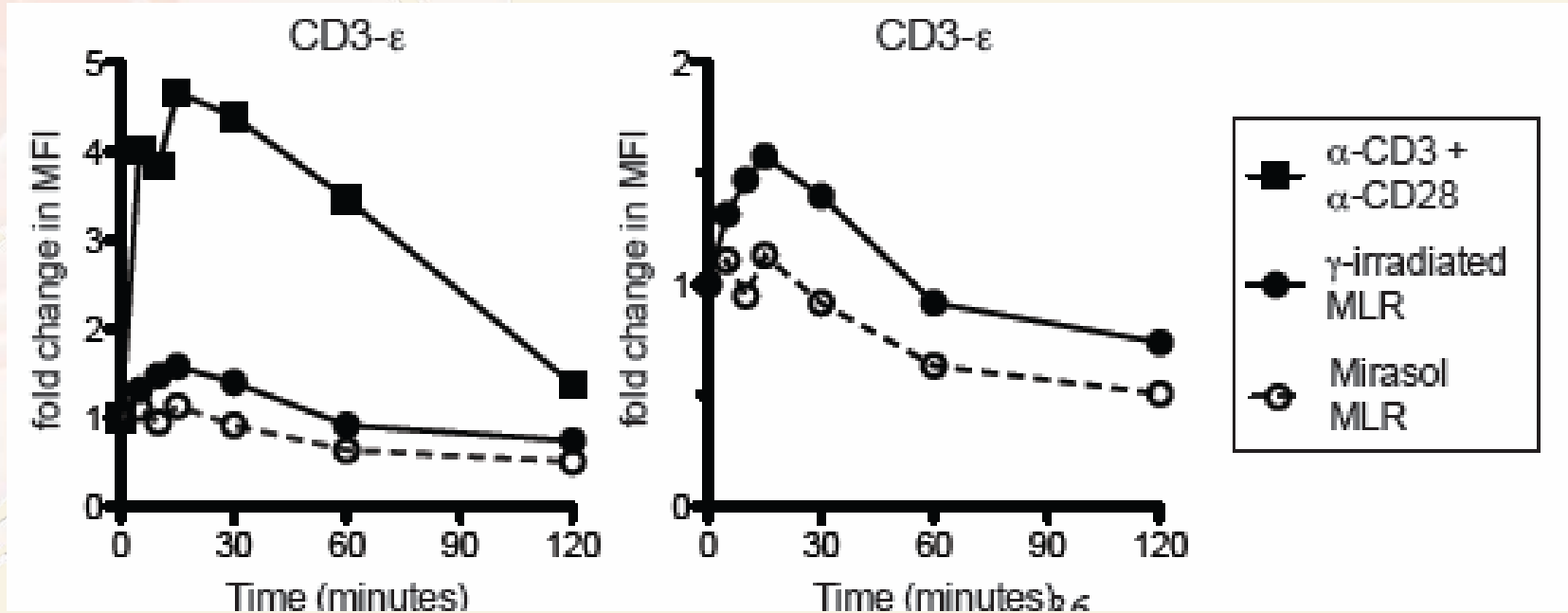
Is there any sign of activation?

- Look for the production of cytokines
intracellular cytokine staining
- Look for changes in phosphorylation of
signaling molecules

PR treated cells stimulate minimal IFN- γ production



PR treated cells defective in stimulating the TCR?



Pathogen reduction effects

- Rapidly kills treated WBCs
- Reduces surface expression of co-stimulatory molecules moderately and adhesion molecules markedly
- Abrogates the ability to induce proliferative responses in allogeneic cells

Pathogen reduction effects

- Only low-level cell-cell conjugation occurs after treatment
- IFN- γ secretion is reduced to background levels
- No intracellular STAT-3, STAT-5, or CD3- ϵ phosphorylation seen when using Mirasol treated cells as stimulators

Acknowledgments

BSRI

- Dale Hirsch Korn
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- Kirk Weimer

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