

What determines the CD4:CD8 T cell ratio in the immune system ?

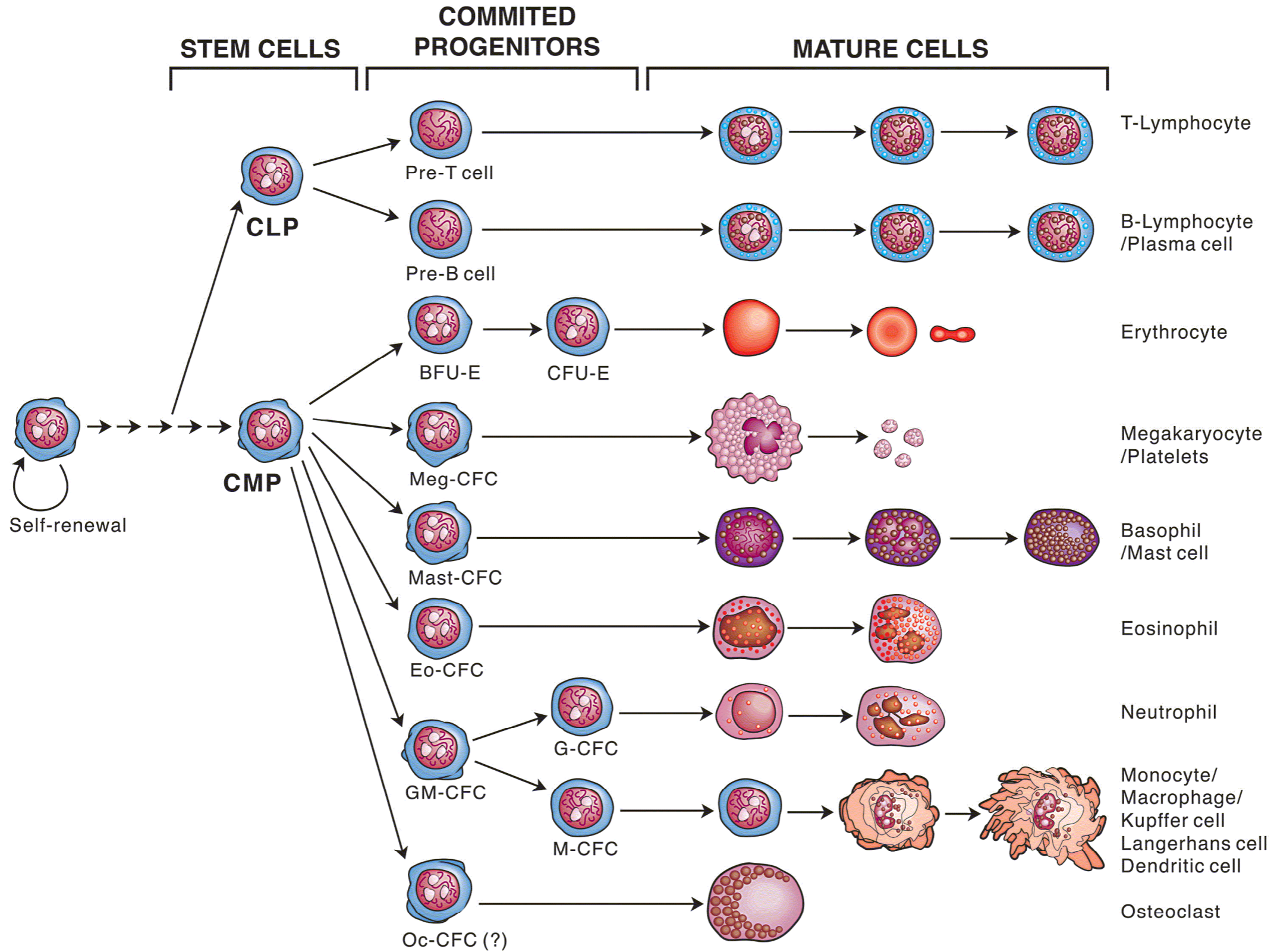
Insights from genetic and mathematical modelling of thymocyte development

Benedict Seddon

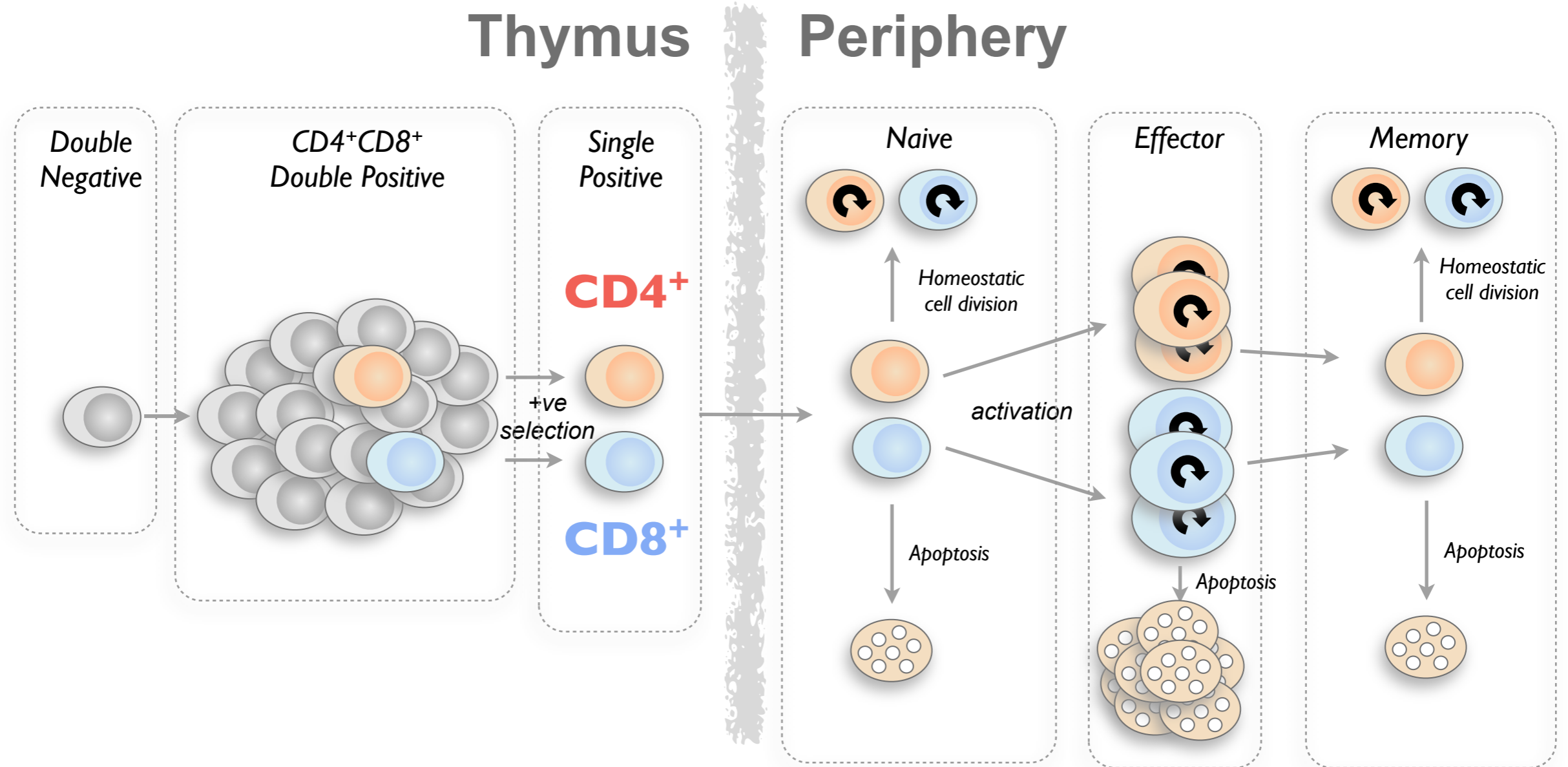


MRC | National Institute
for Medical Research

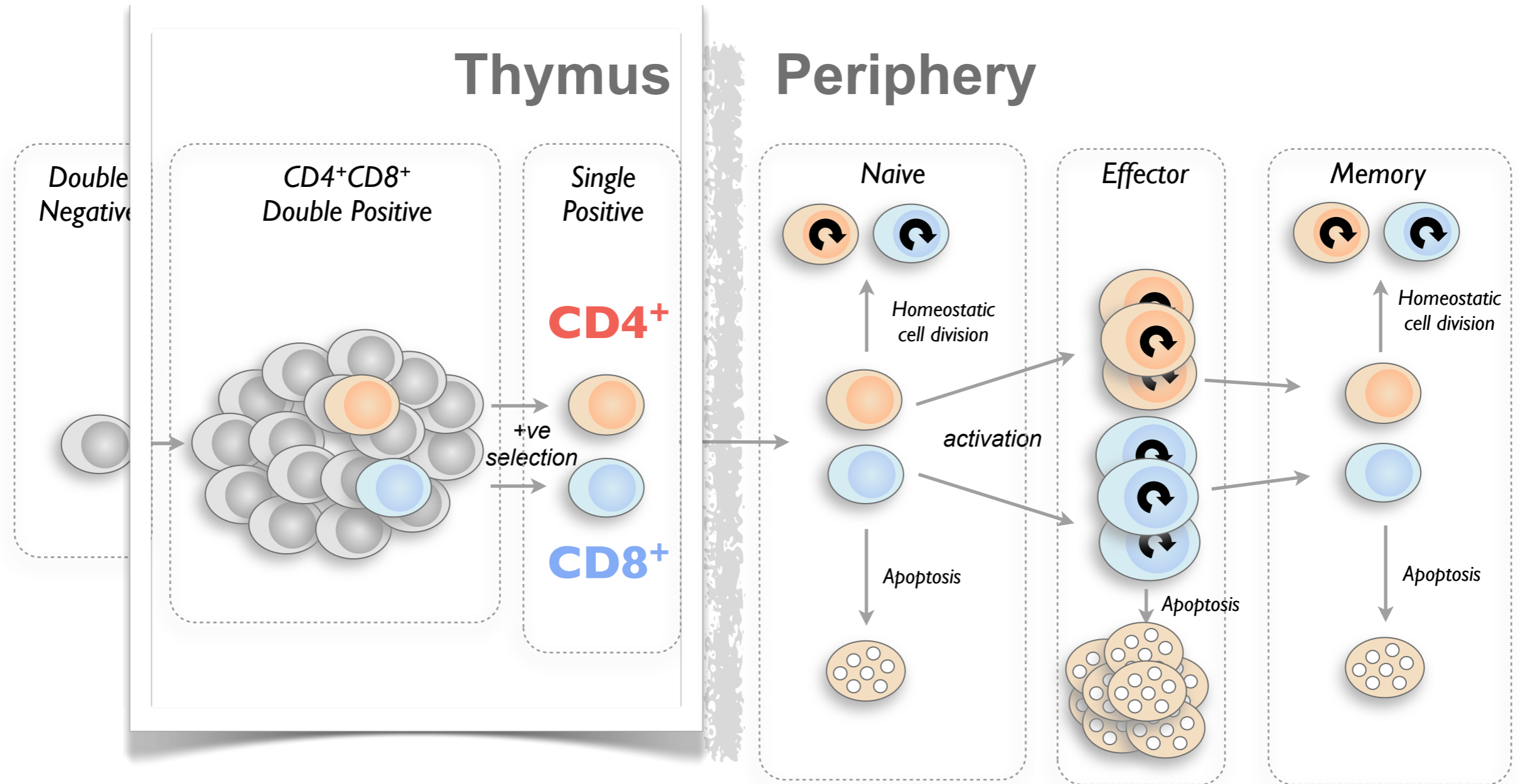
Cell of the immune system



Maintaining homeostasis of the T cell compartment

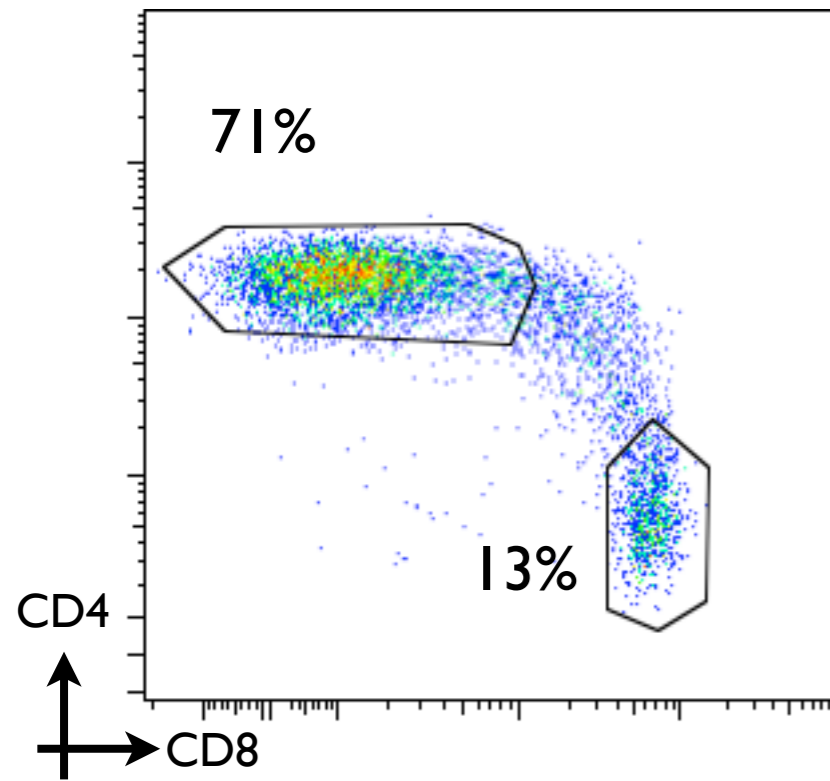


Maintaining homeostasis of the T cell compartment



How and why does the thymus make more CD4 than CD8 cells ?

?



+

Andy Yates



+

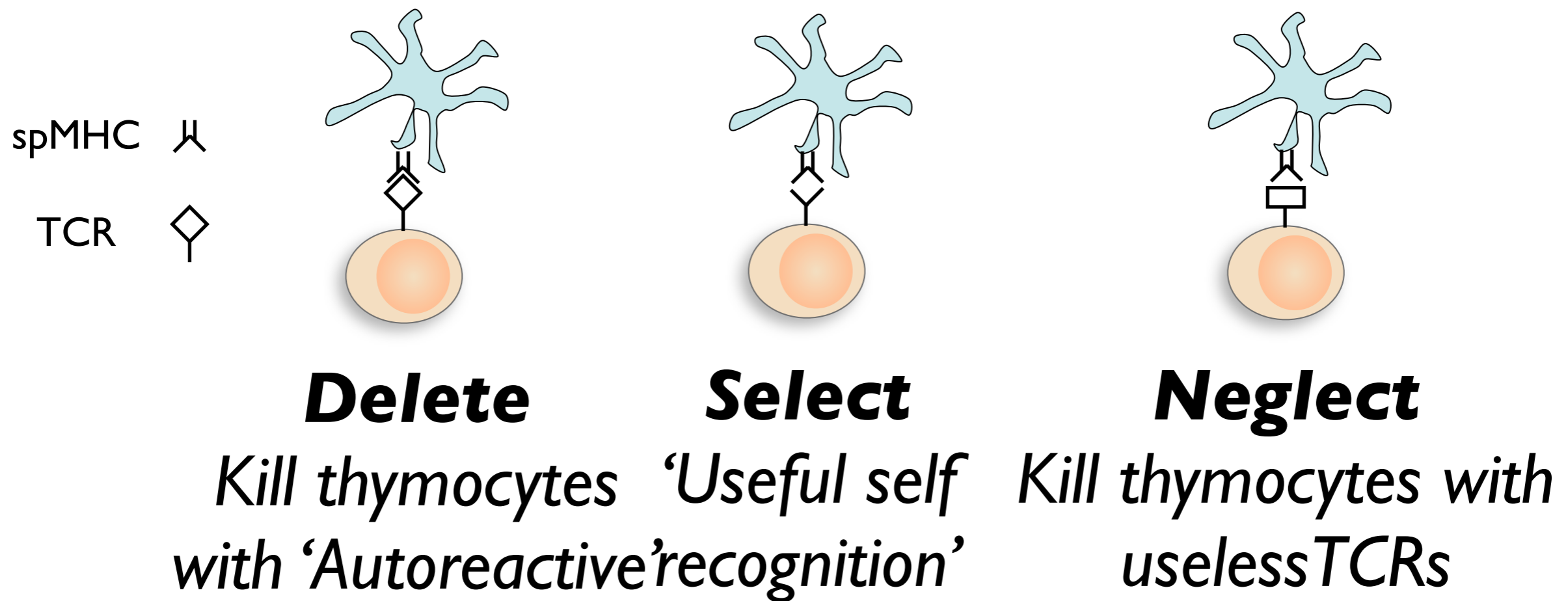


Characteristic 4:1 ratio -
Conserved across
species
Why ?



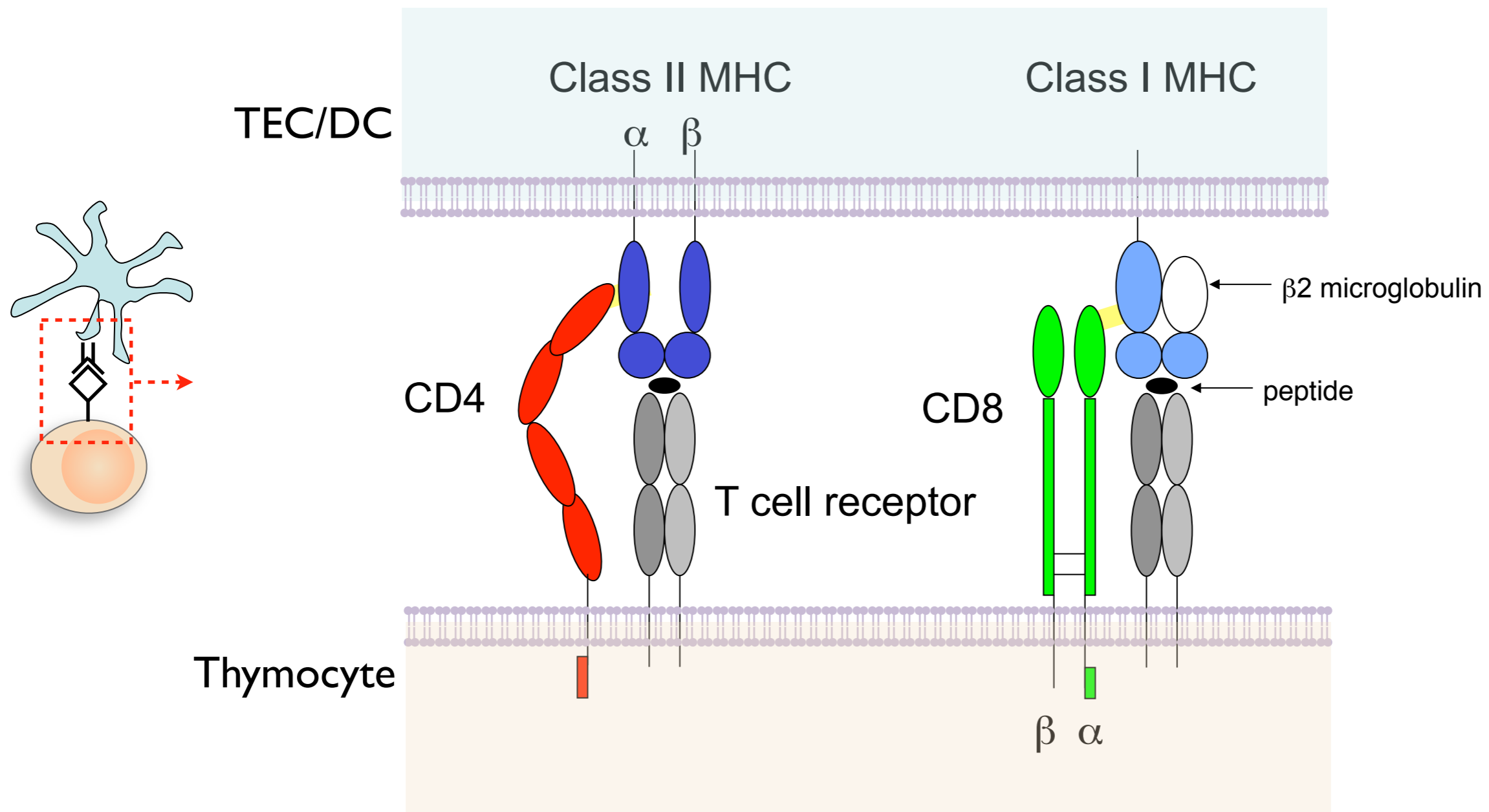
Thymocyte selection - two purposes

I. Tolerance - delete autoreactive T cells

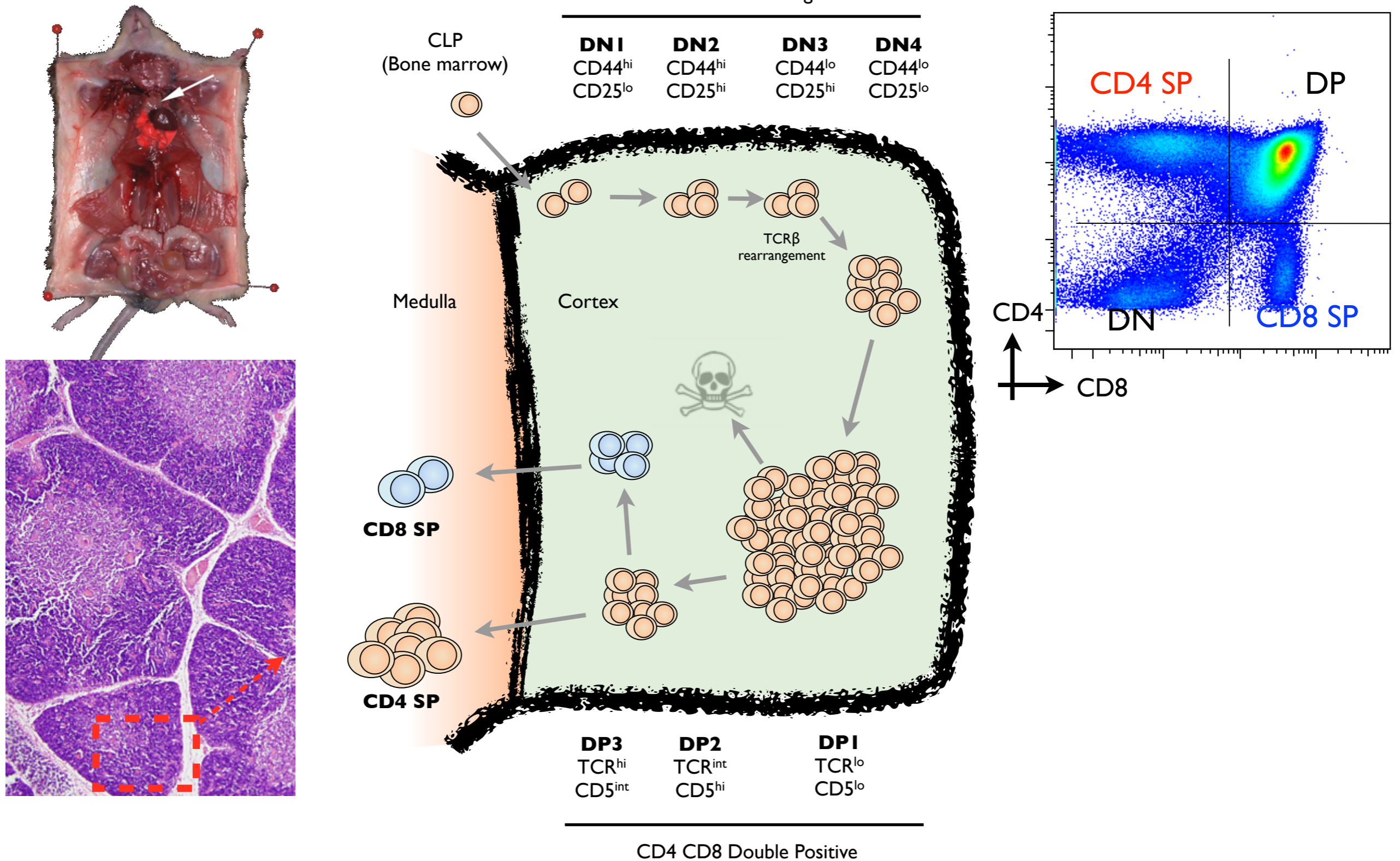


Thymocyte selection - two purposes

2. CD4 vs CD8 lineage specification ensuring TCR-MHC restriction and lineage correlate

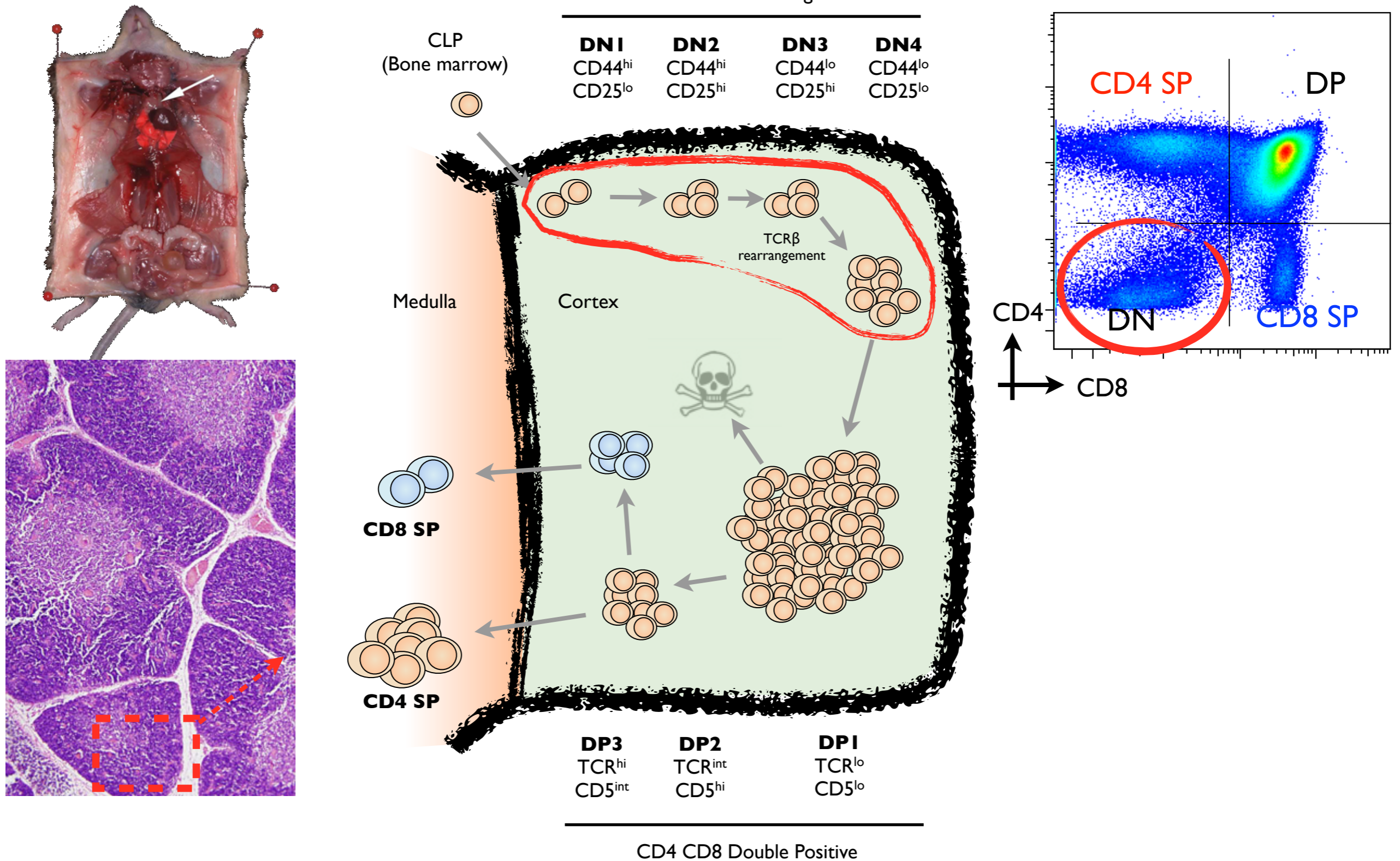


Thymic development - what happens



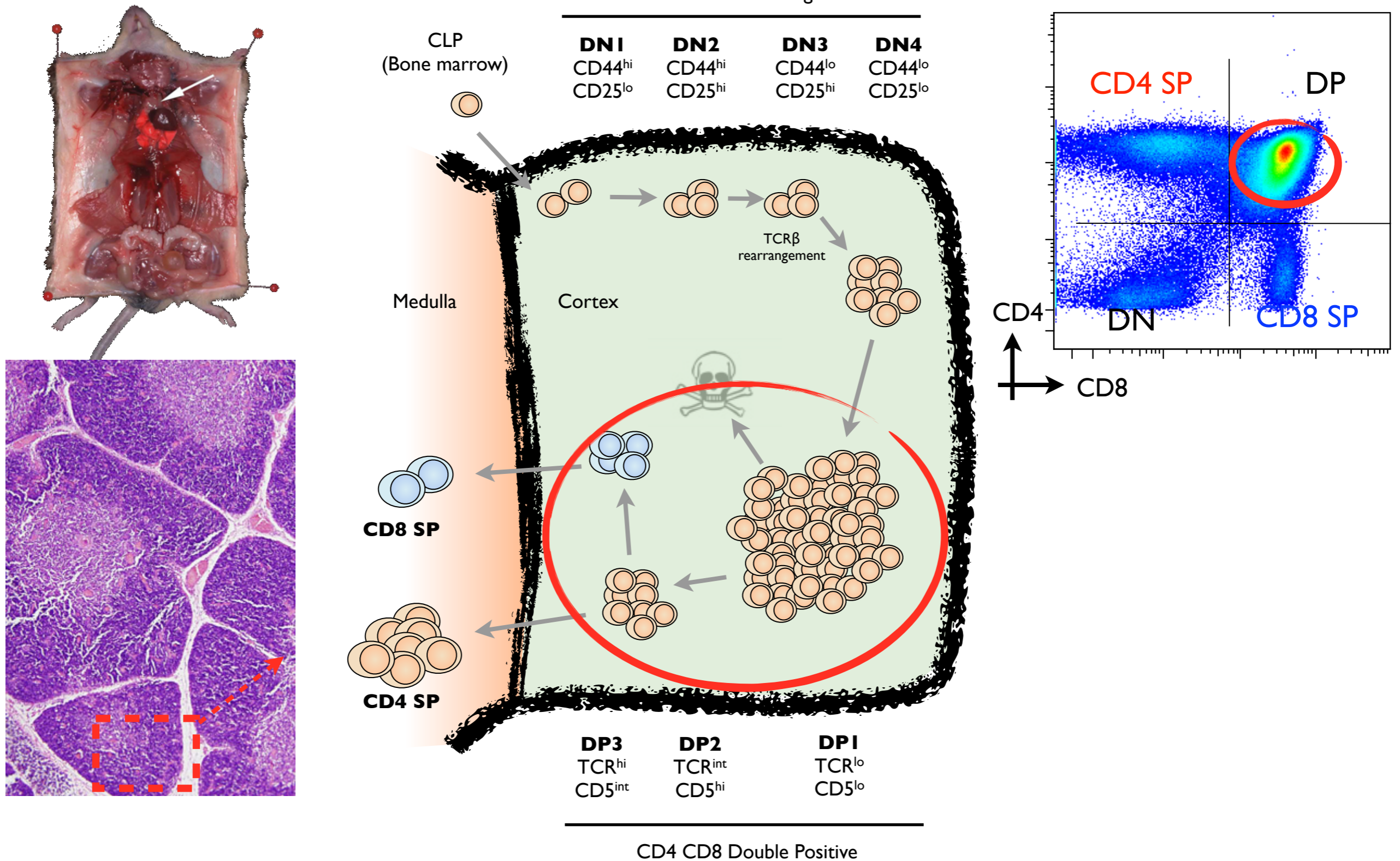
Saini, M. et al. Regulation of Zap70 expression during thymocyte development enables temporal separation of CD4 and CD8 repertoire selection at different signaling thresholds. *Sci Signal* **3**, ra23 (2010).

Thymic development - what happens



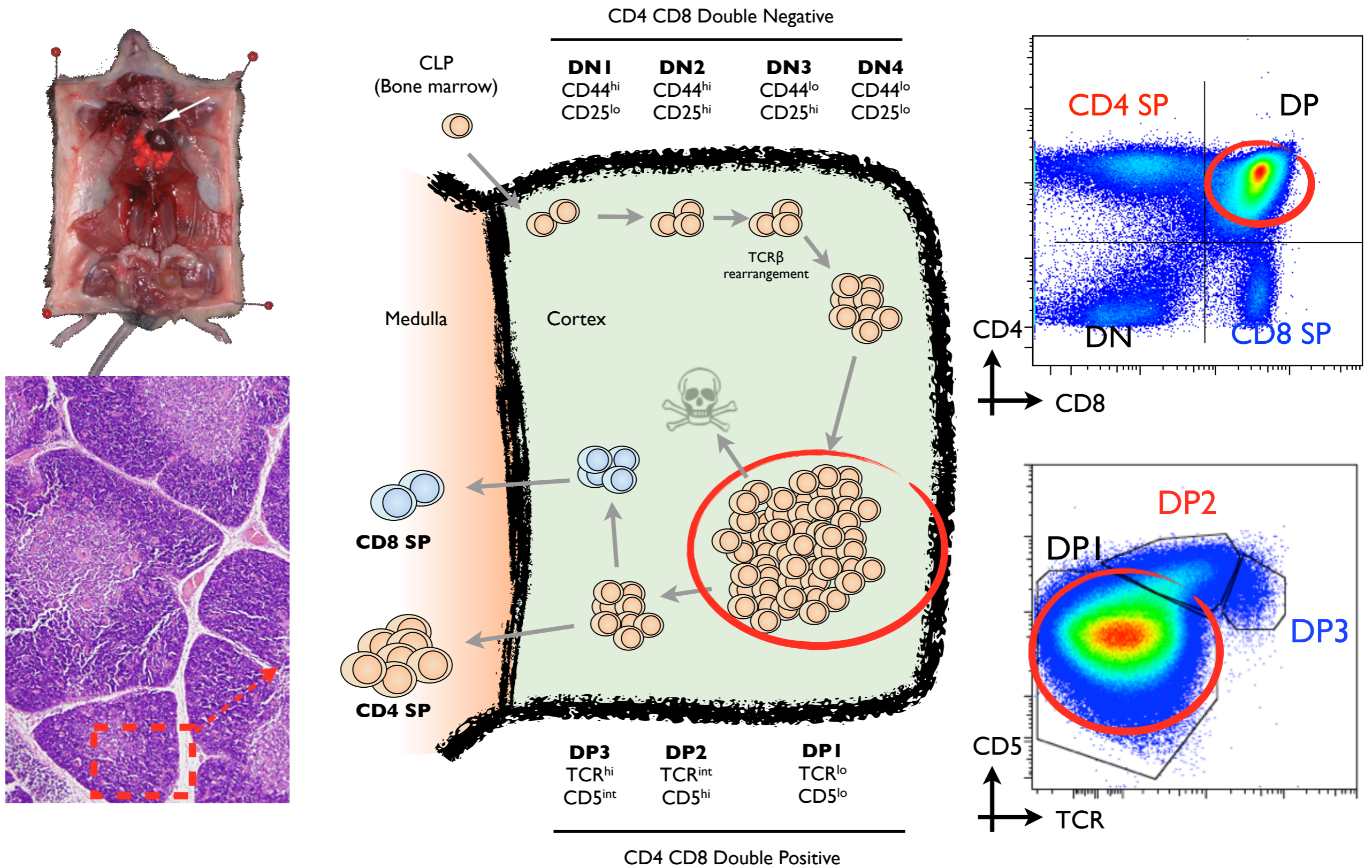
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Thymic development - what happens



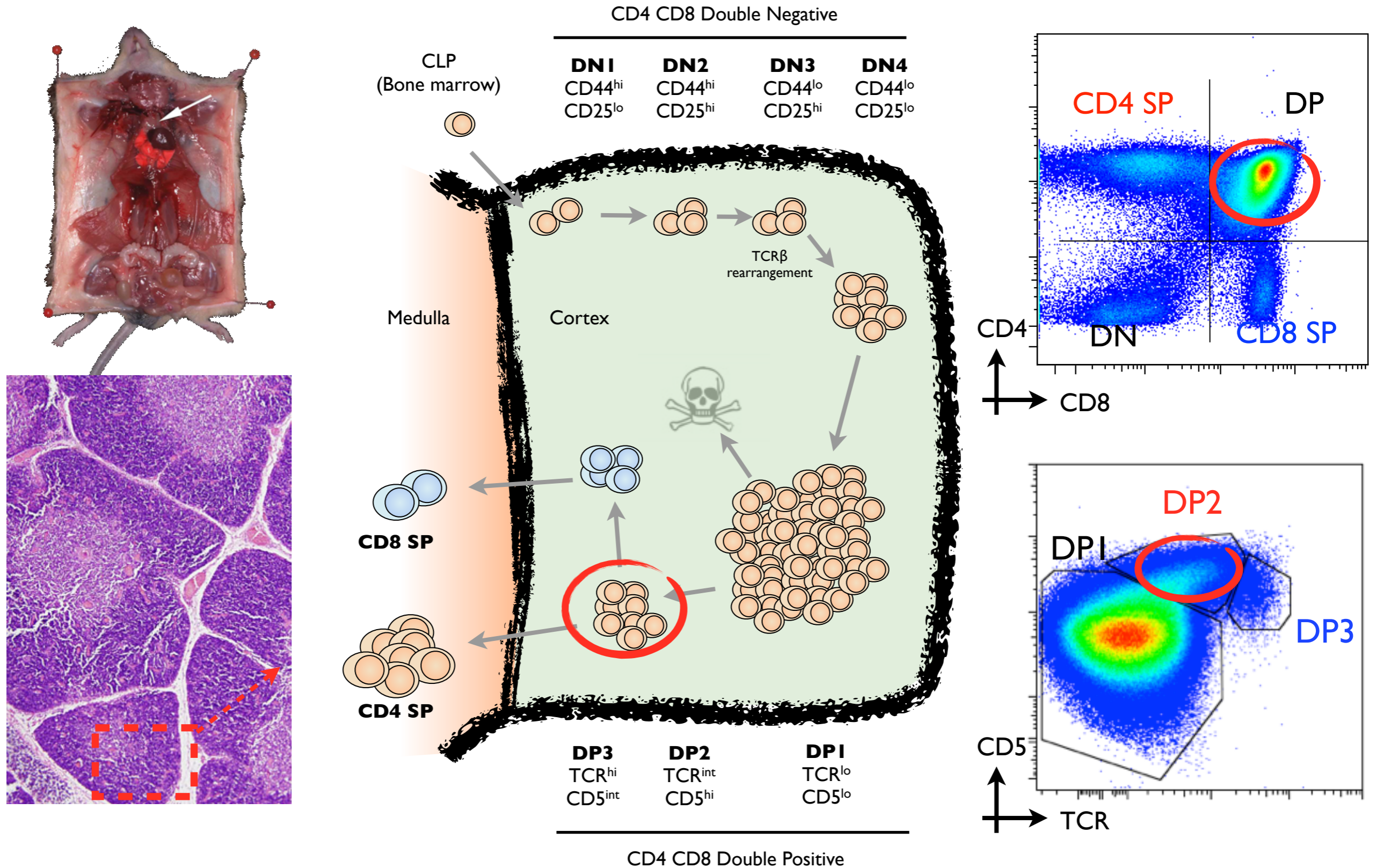
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Thymic development - what happens



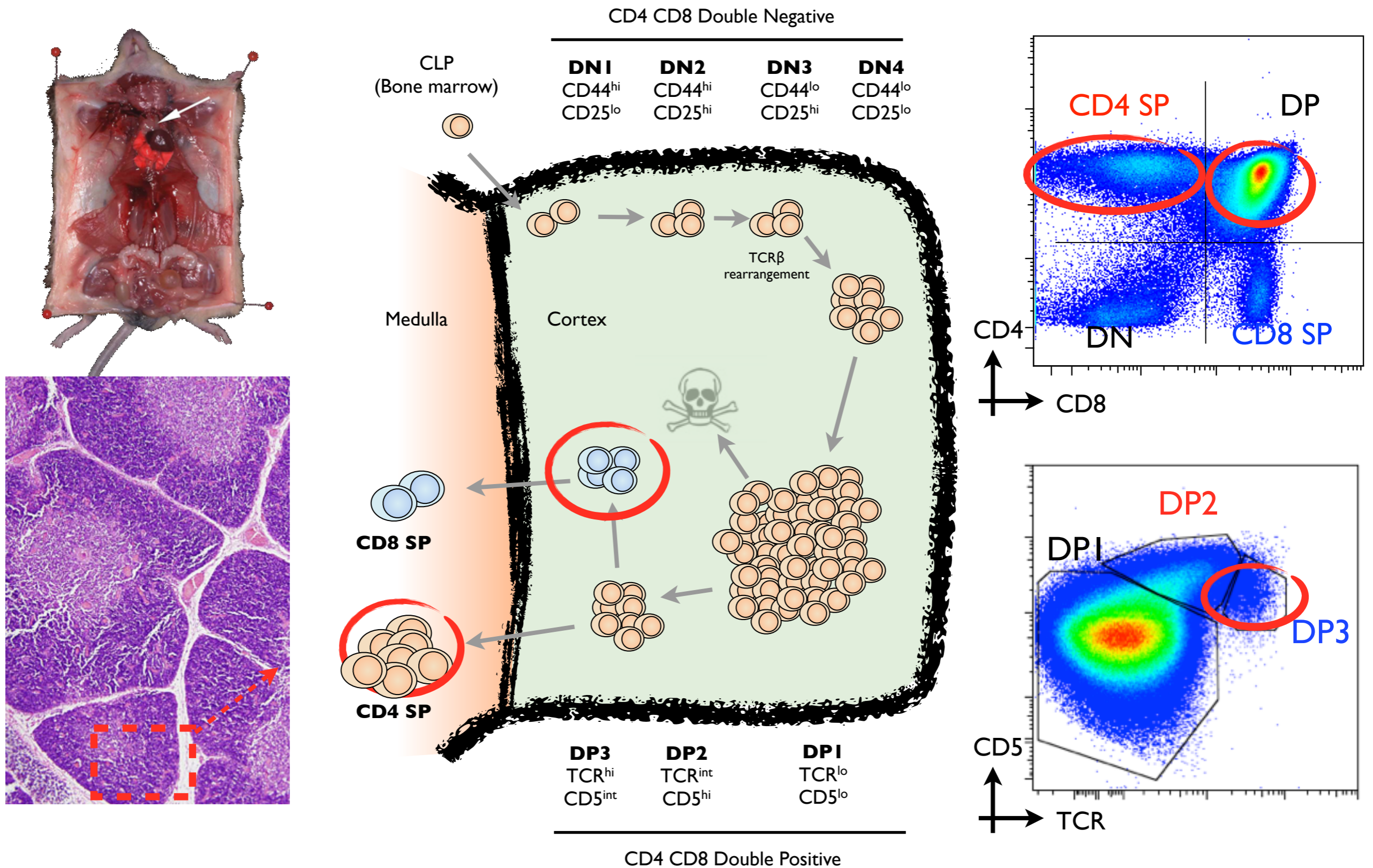
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Thymic development - what happens



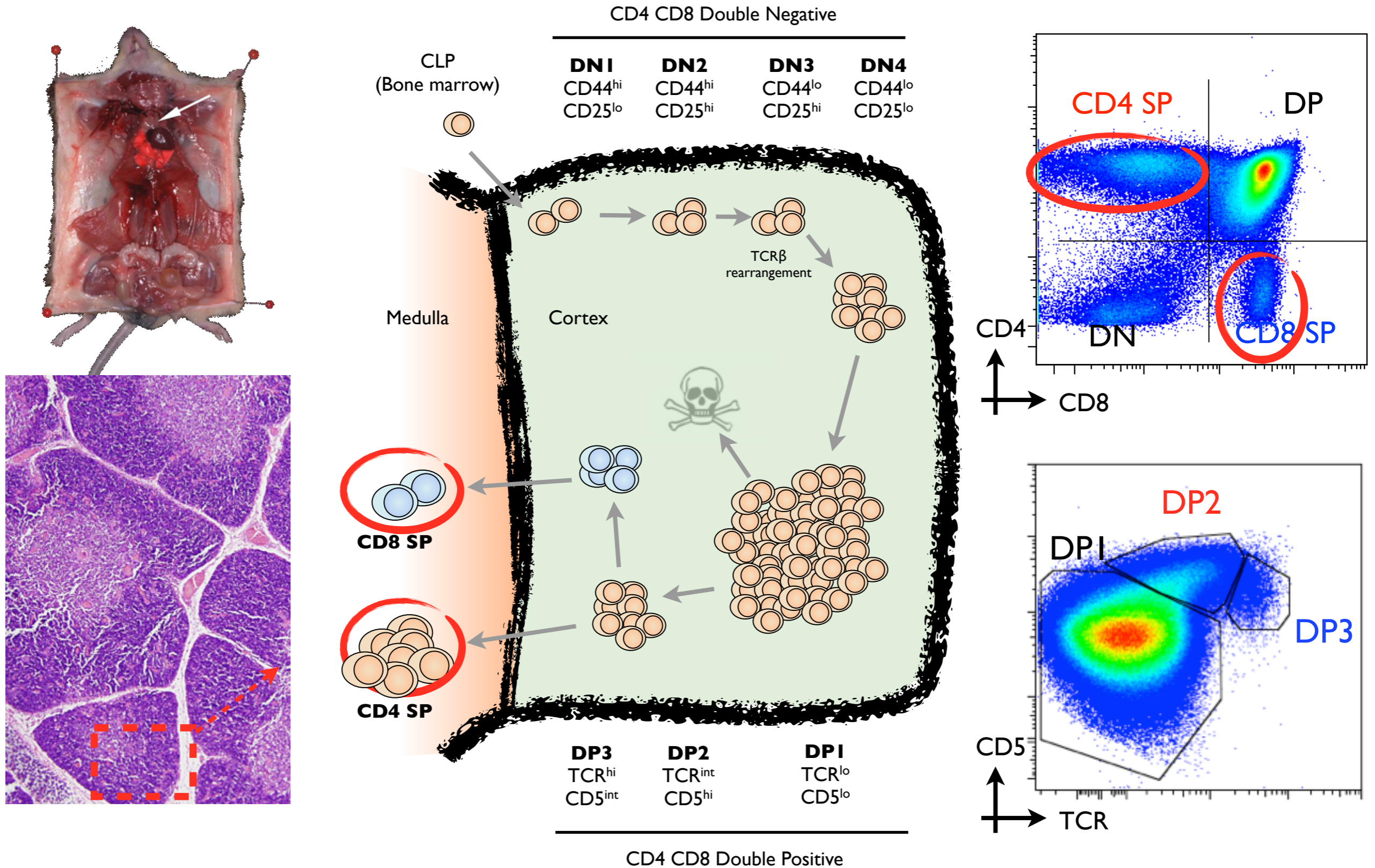
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Thymic development - what happens



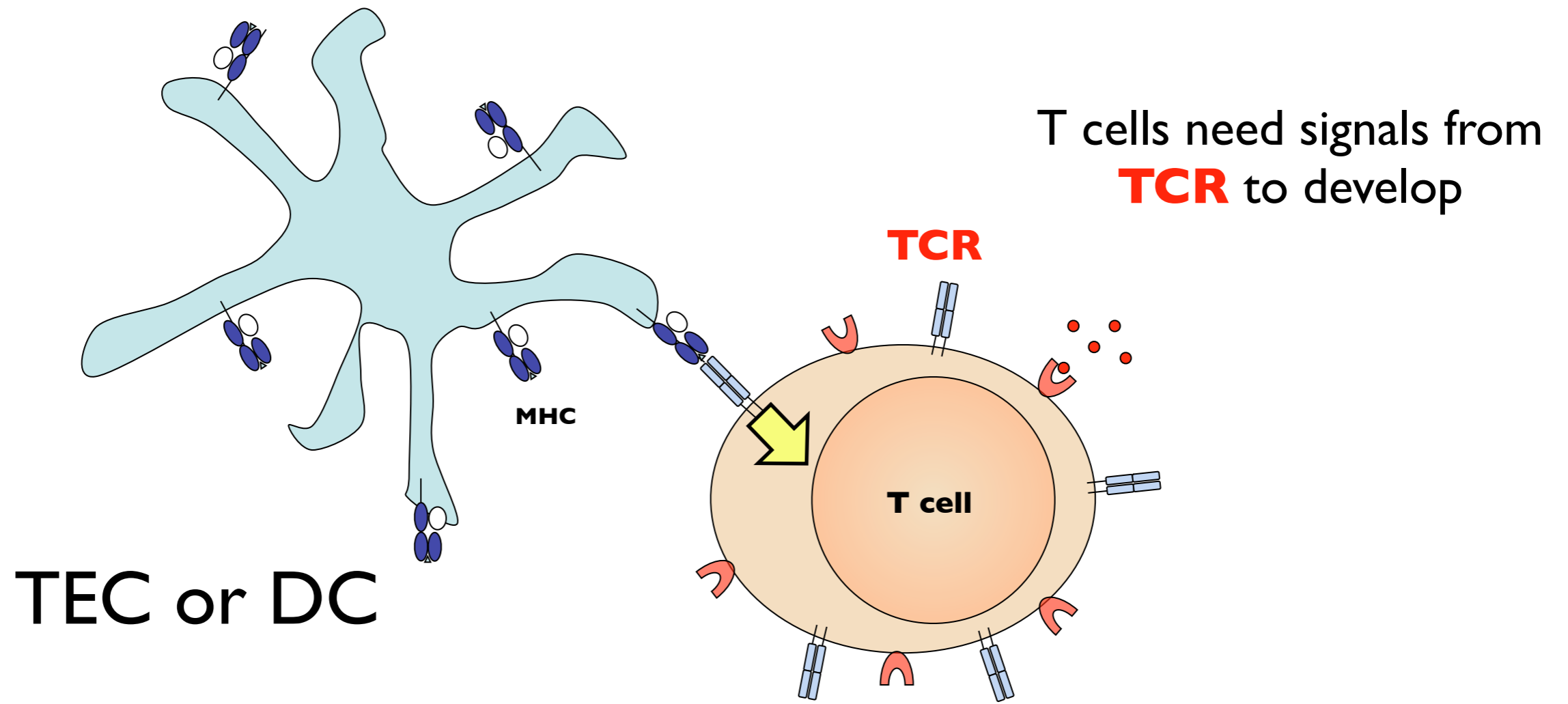
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Thymic development - what happens

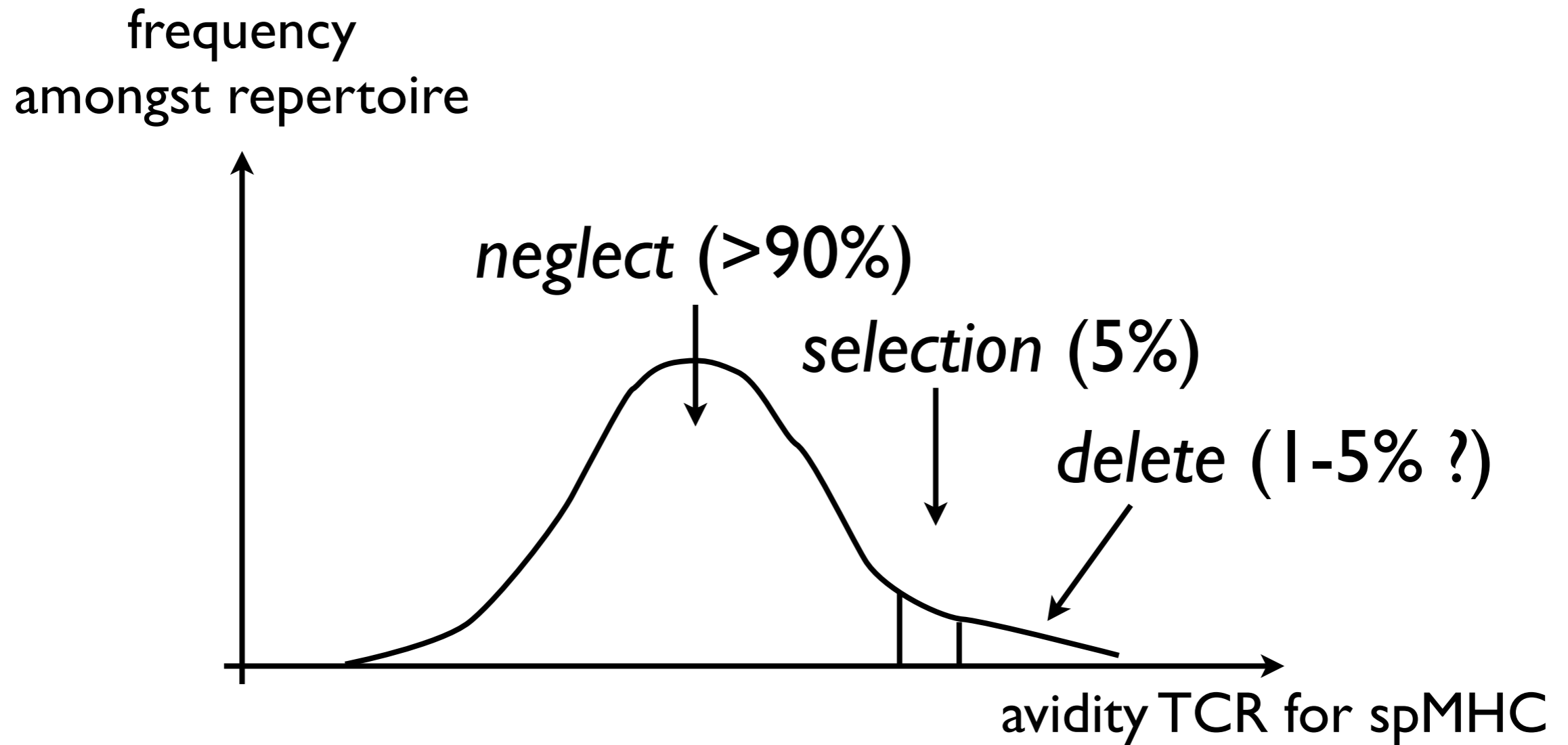


Saini, M. et al. Regulation of Zap70 expression during thymocyte development enables temporal separation of CD4 and CD8 repertoire selection at different signaling thresholds. *Sci Signal* **3**, ra23 (2010).

Thymic development - how it happens

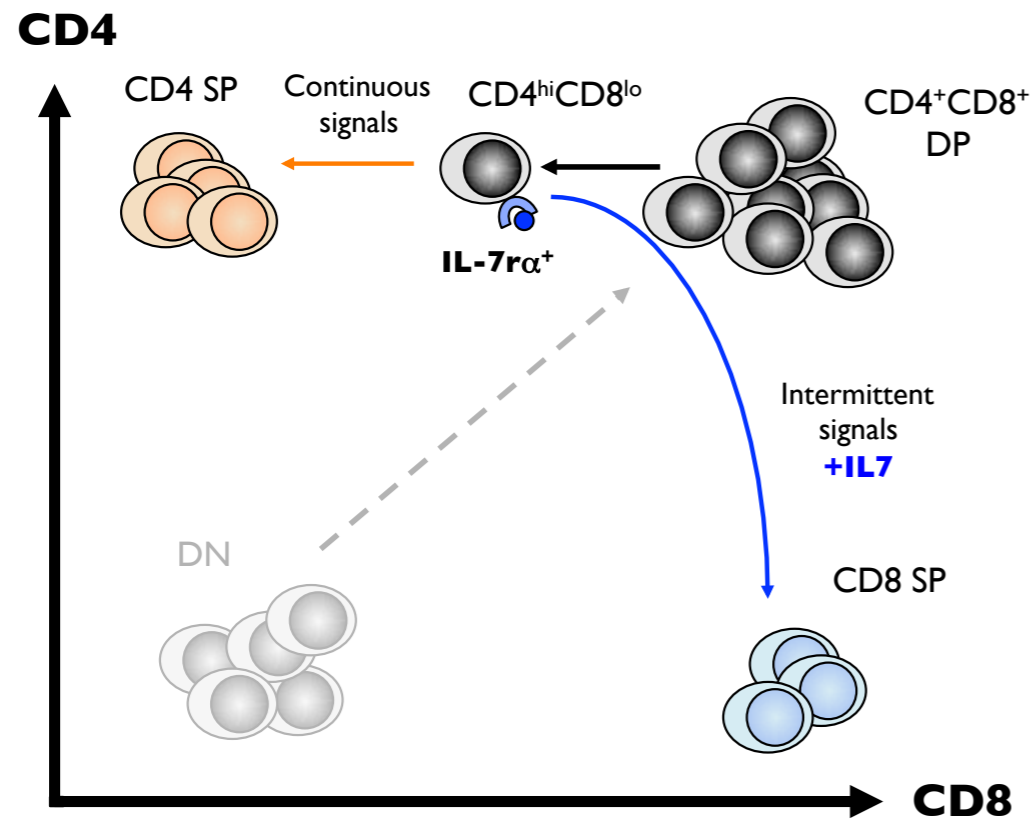


Positive and negative selection - goldilox models

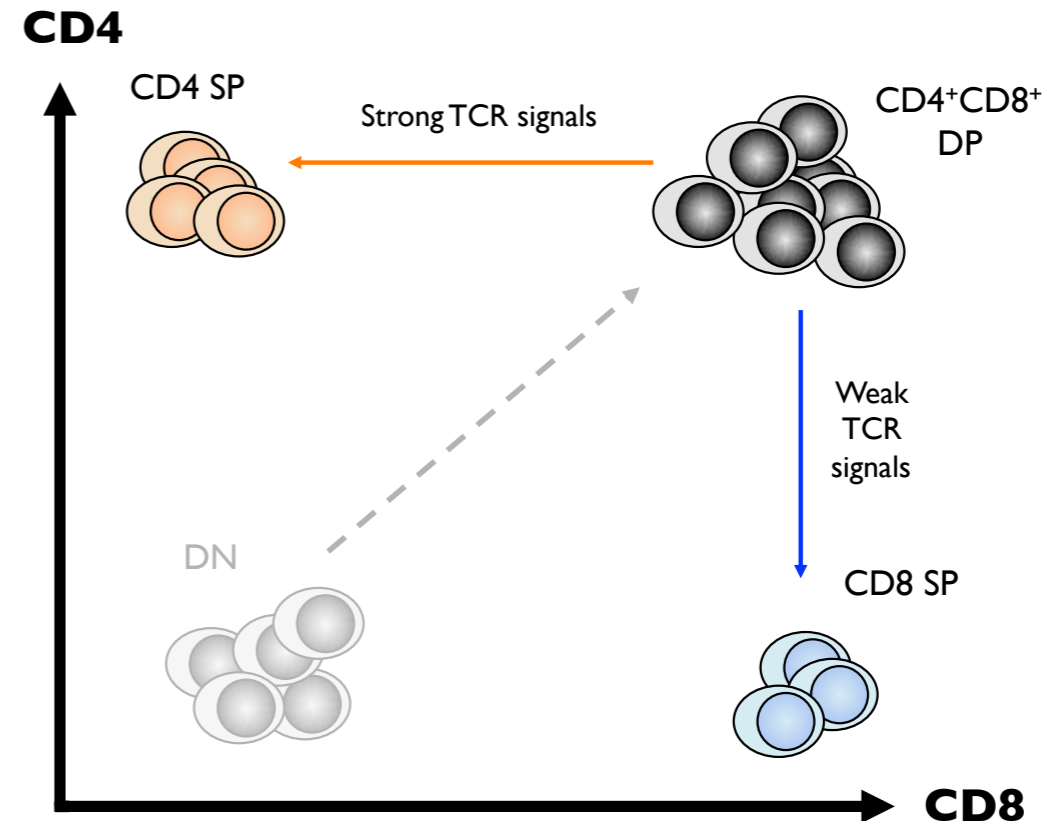


Lineage commitment - signals instruct fate

Kinetic



Quantitative instructive



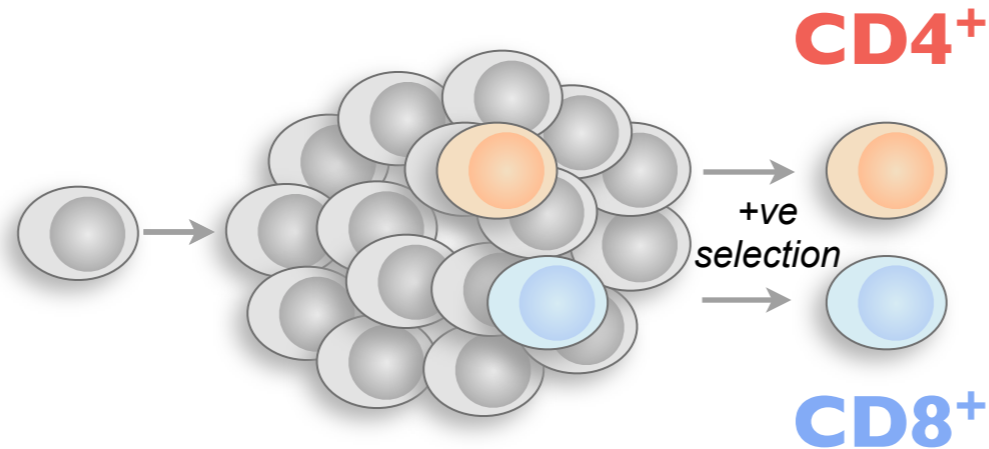
Origin of CD4 biased selection

Thymus

Double
Negative

CD4⁺CD8⁺
Double Positive

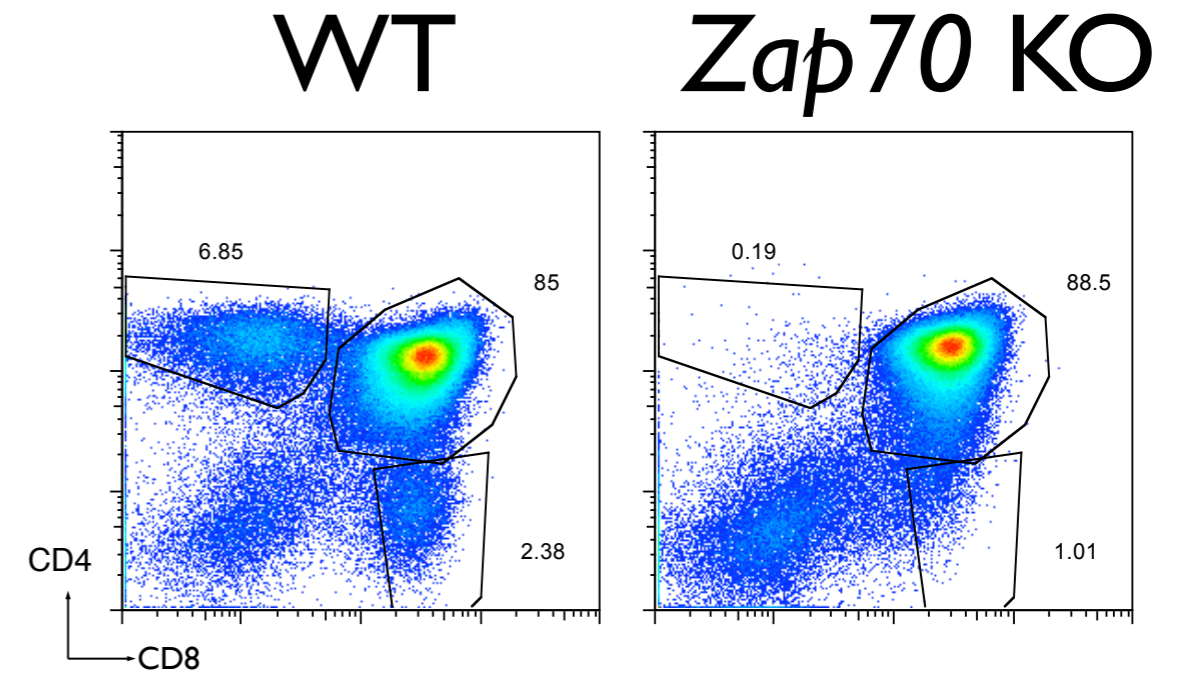
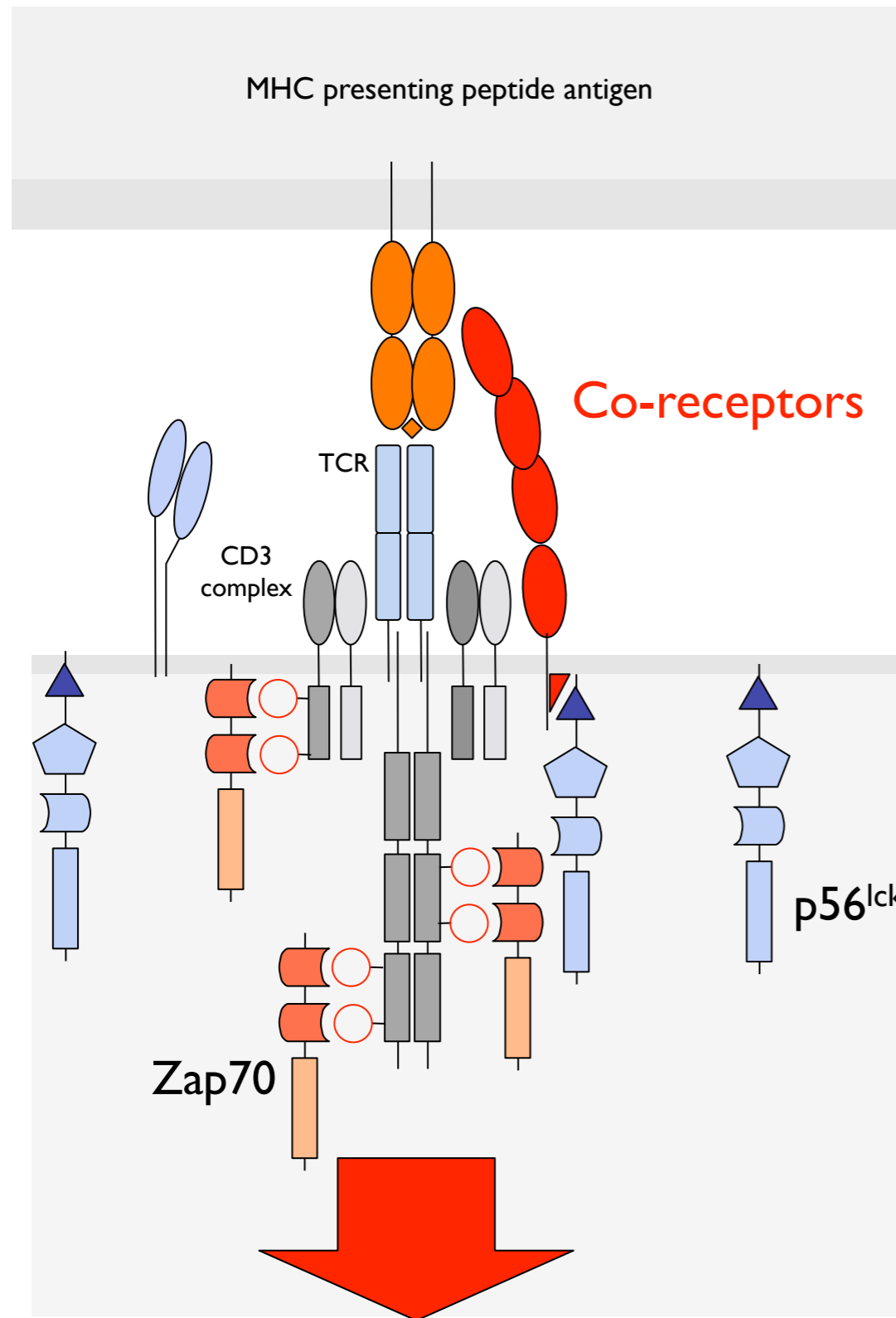
Single
Positive



1. Intrinsic differences in Class I and Class II restricted precursors due to TCR repertoire generation

2. Differences in 'efficiency' of selection between CD4 and CD8 lineage cells

Zap70 deficient mice blocked at double positive stage

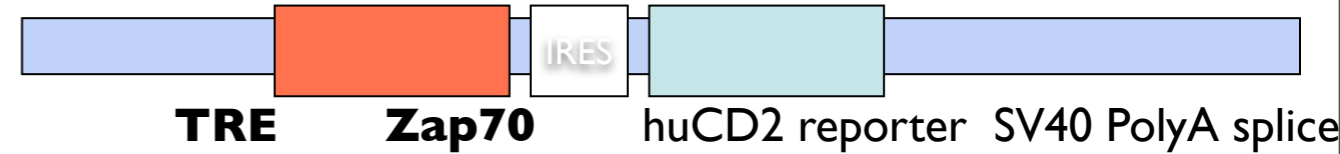
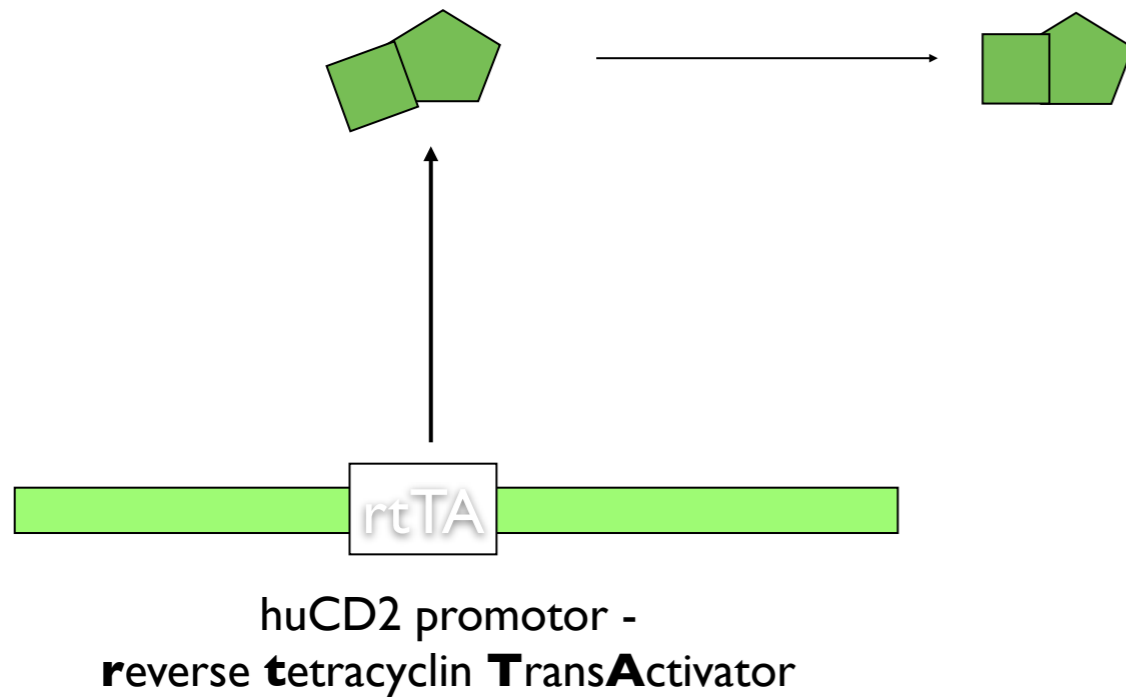


★ Role of Zap70 in positive selection :

◆ Conditionally express Zap70

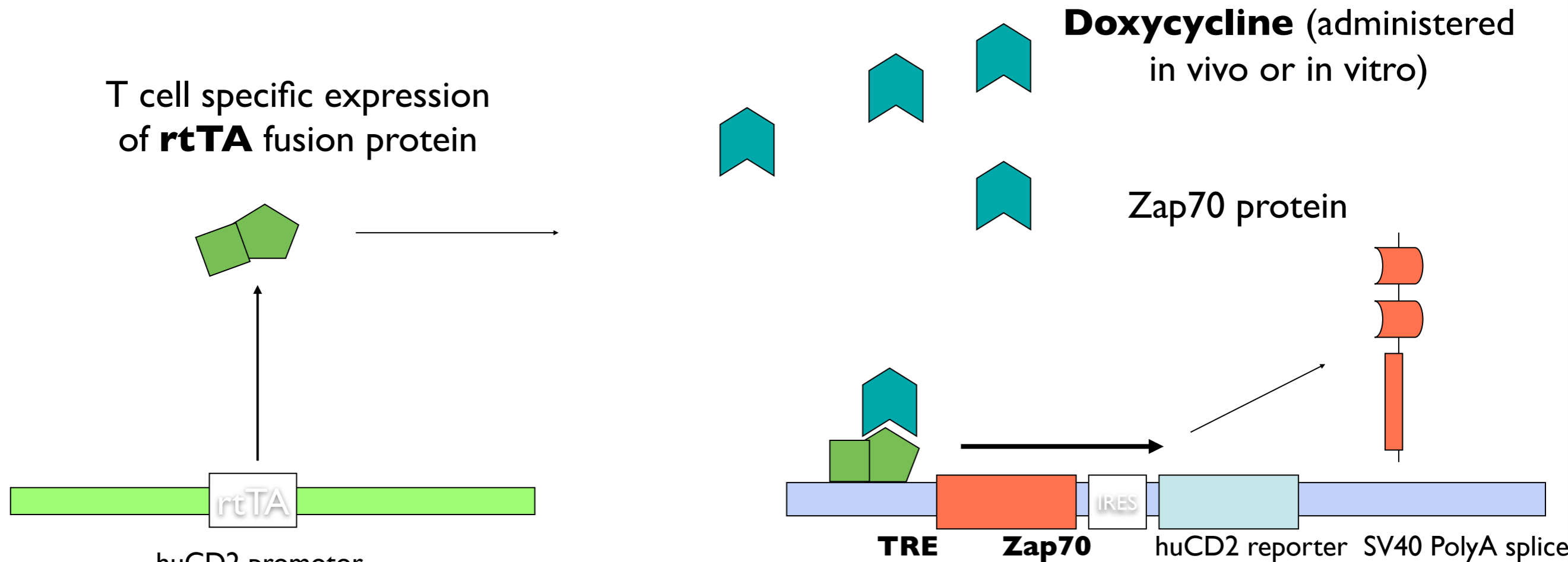
Conditional Zap70 expression mouse model

T cell specific expression
of **rtTA** fusion protein

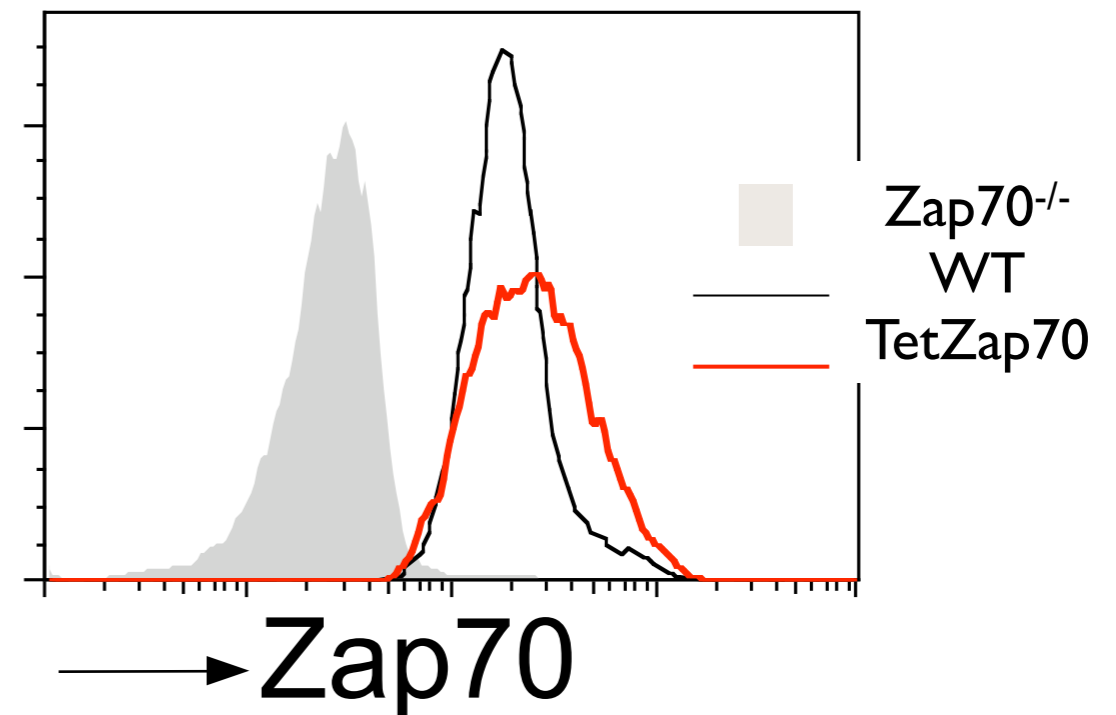


Zap70^{Tre} rtTA.C^{huCD2} Zap70^{-/-}
Zap70^{Tet} mice

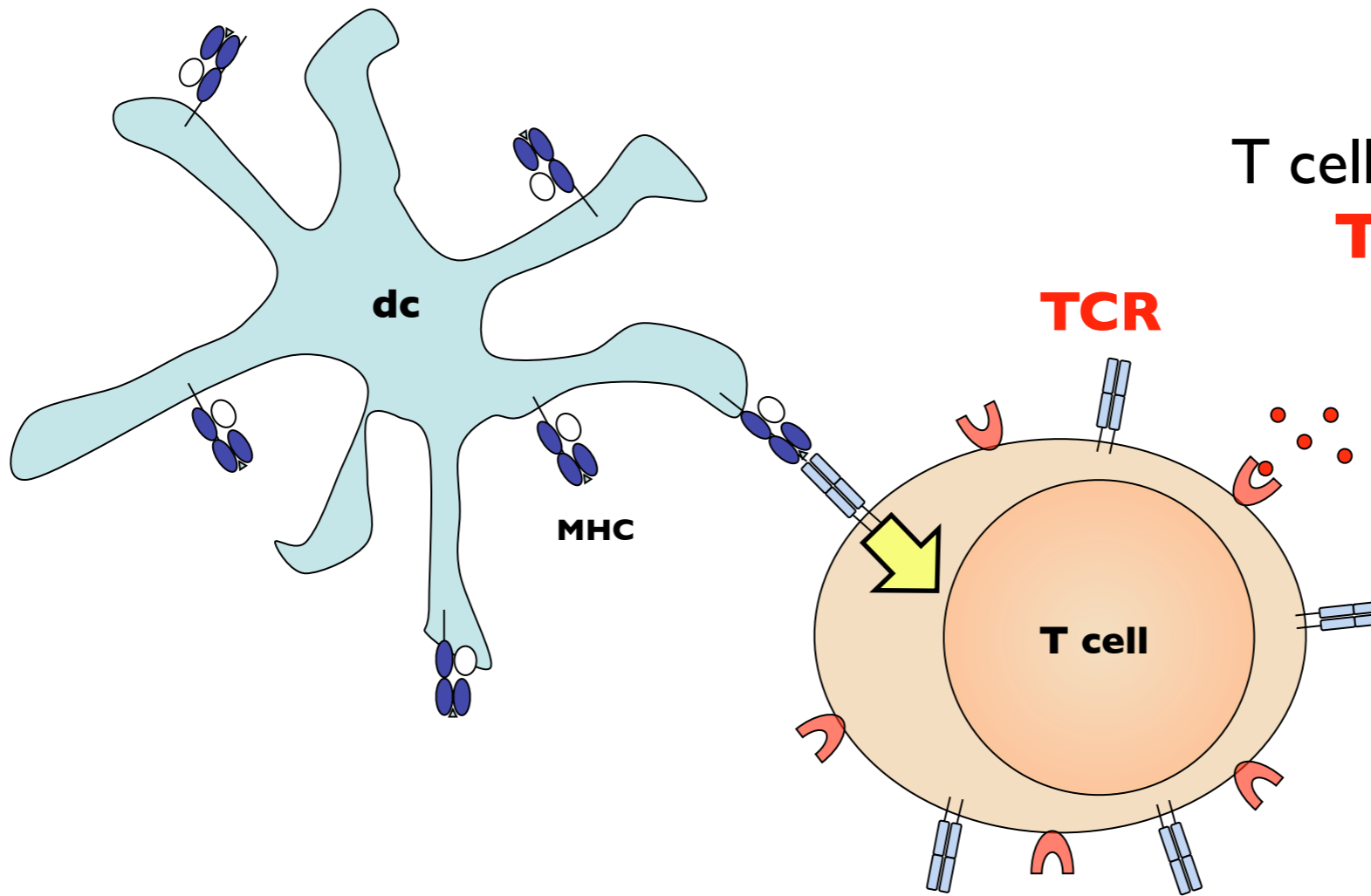
Conditional Zap70 expression mouse model



Zap70^{Tre} rtTA.C^{huCD2} Zap70^{-/-}
Zap70^{Tet} mice



Inducible T cell development



T cells need signals from **TCR** to develop

Genetic mutant with inducible TCR signalling



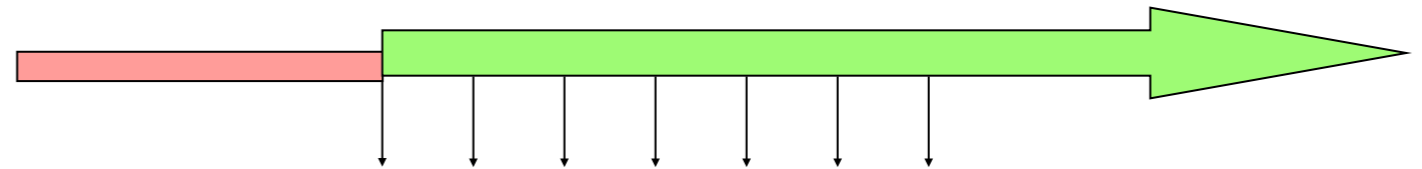
TetZap70

TreZap^{OFF}

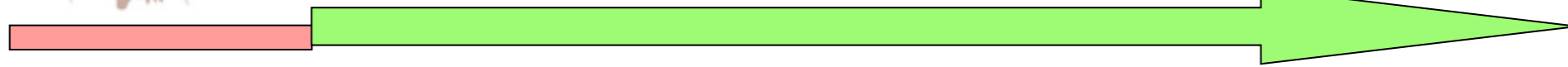
TreZap^{ON}

6-8wks

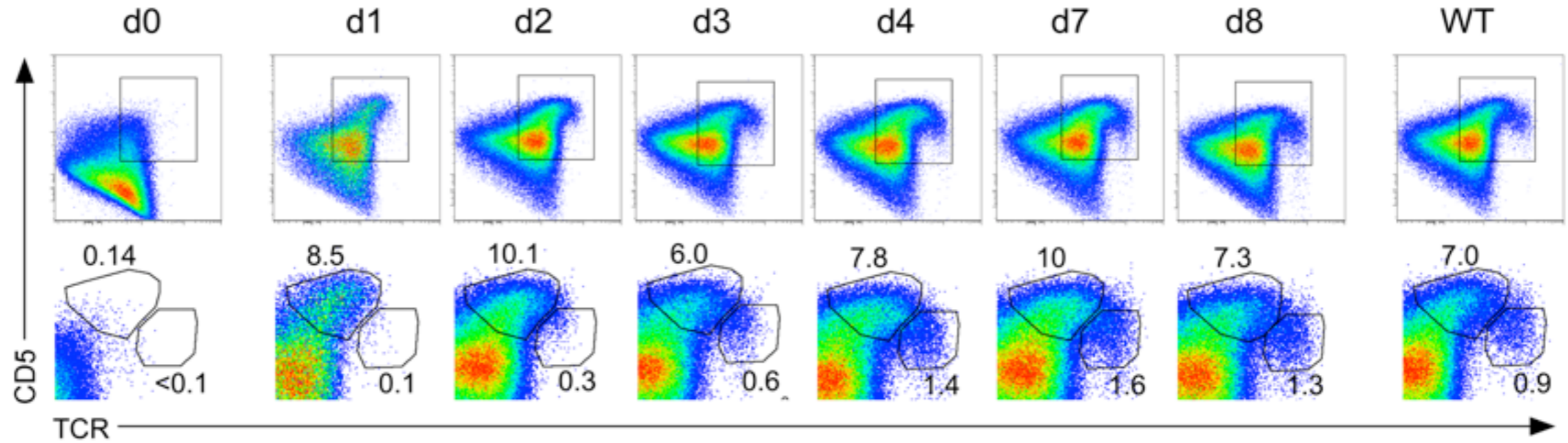
+ Dox food



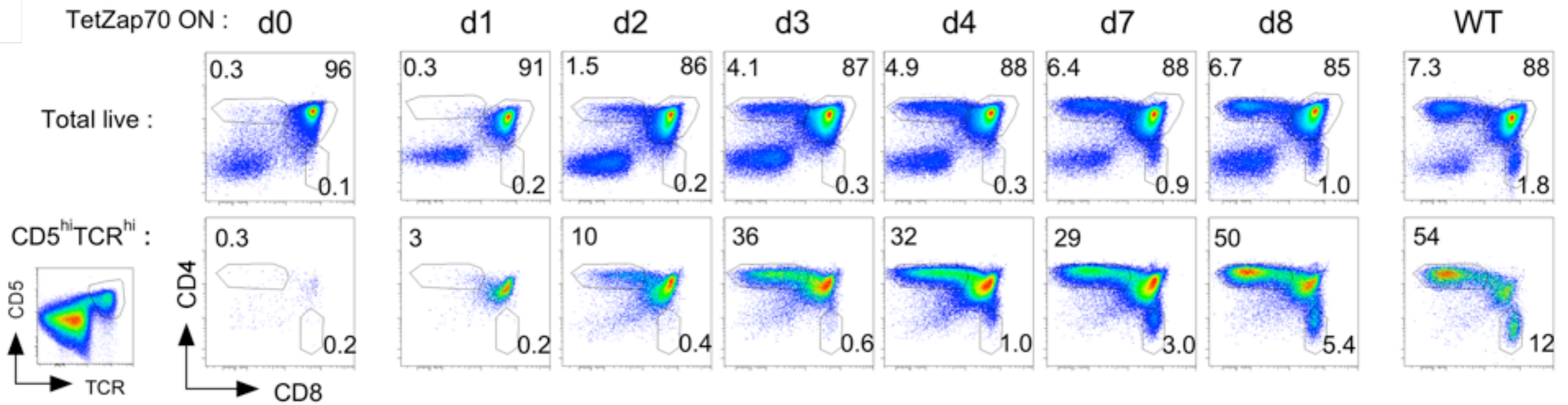
Inducible T cell development



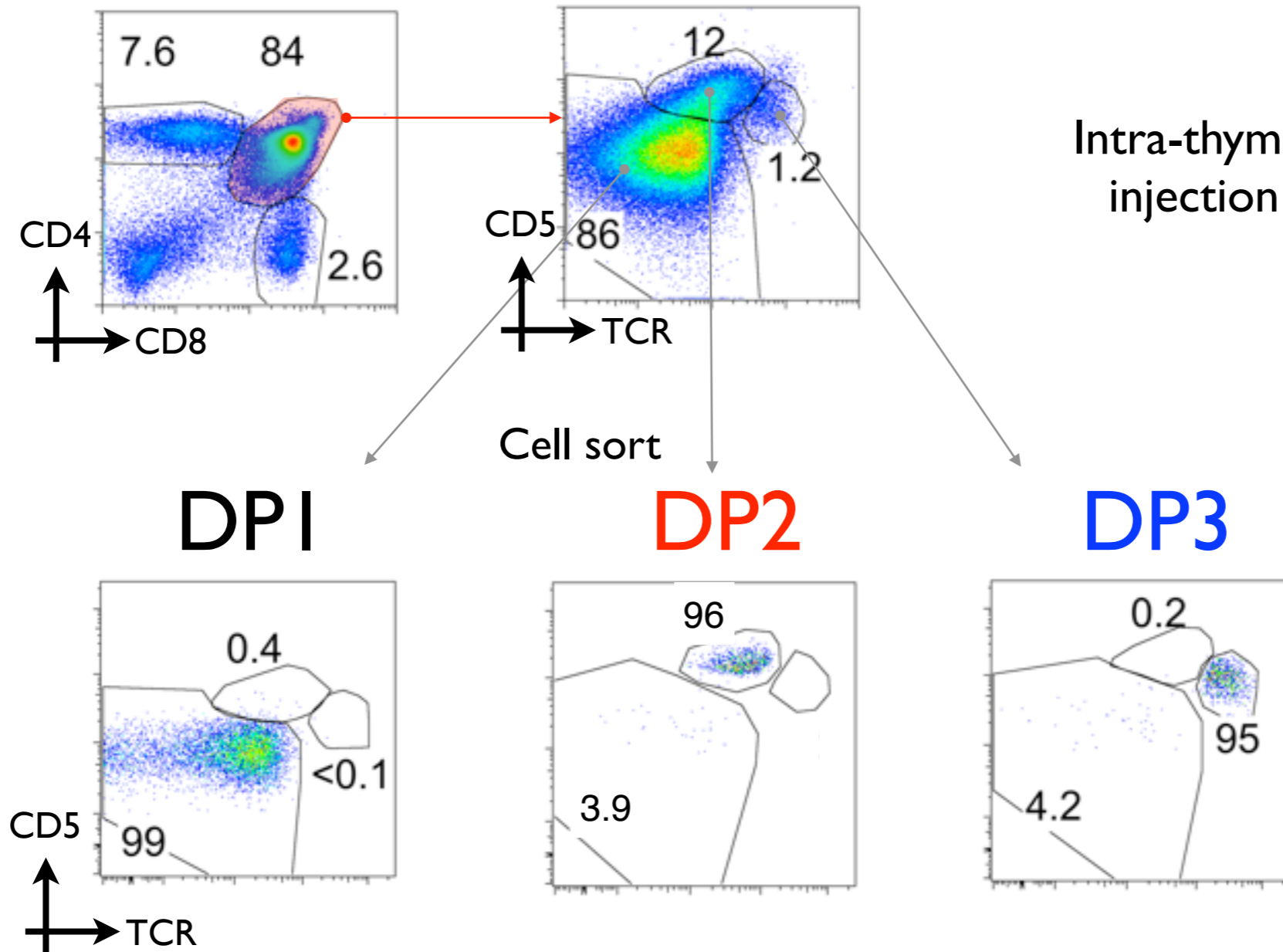
DP thymocytes :



SP thymocytes :



Establishing precursor-product relationships



Intra-thymic injection

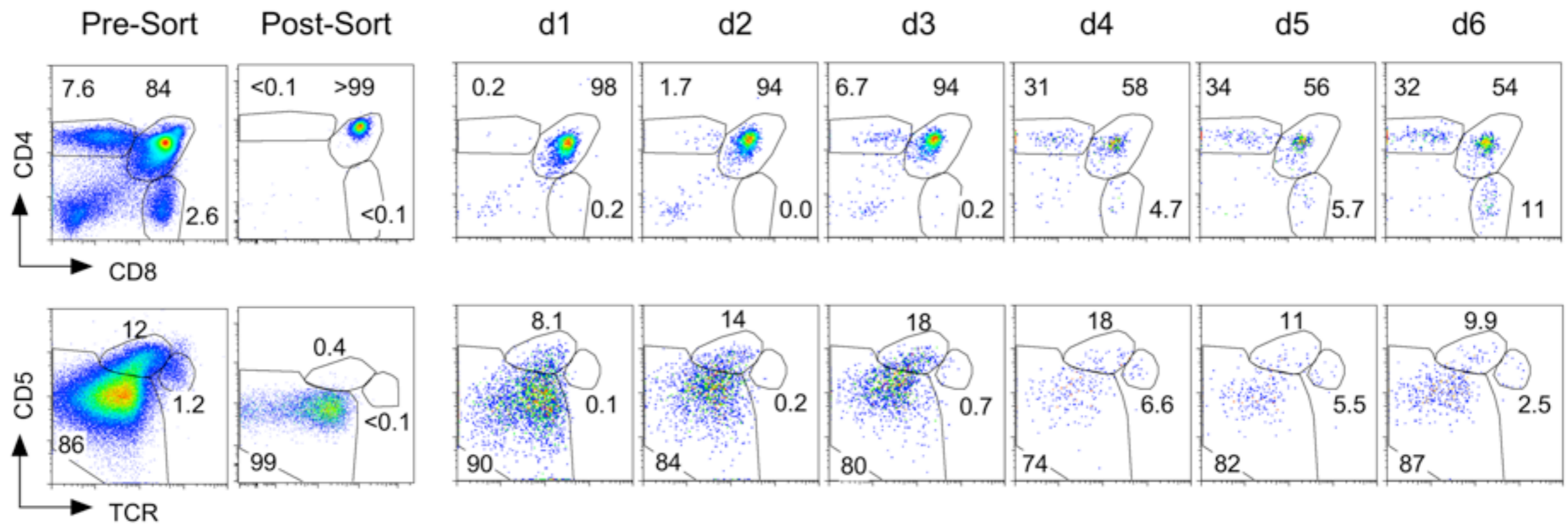


d1-6

Measure relative recovery

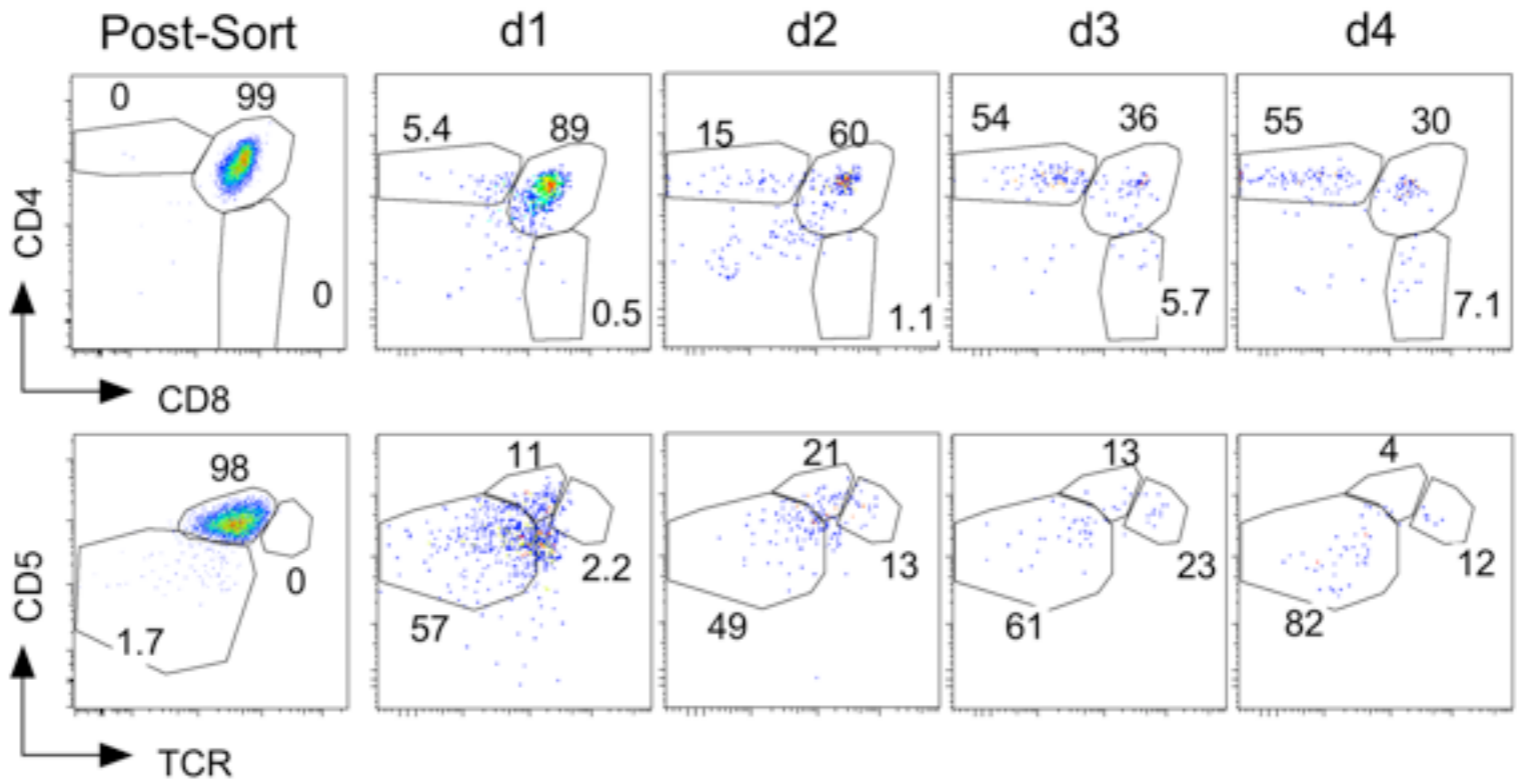
Establishing precursor-product relationships - I

DPI

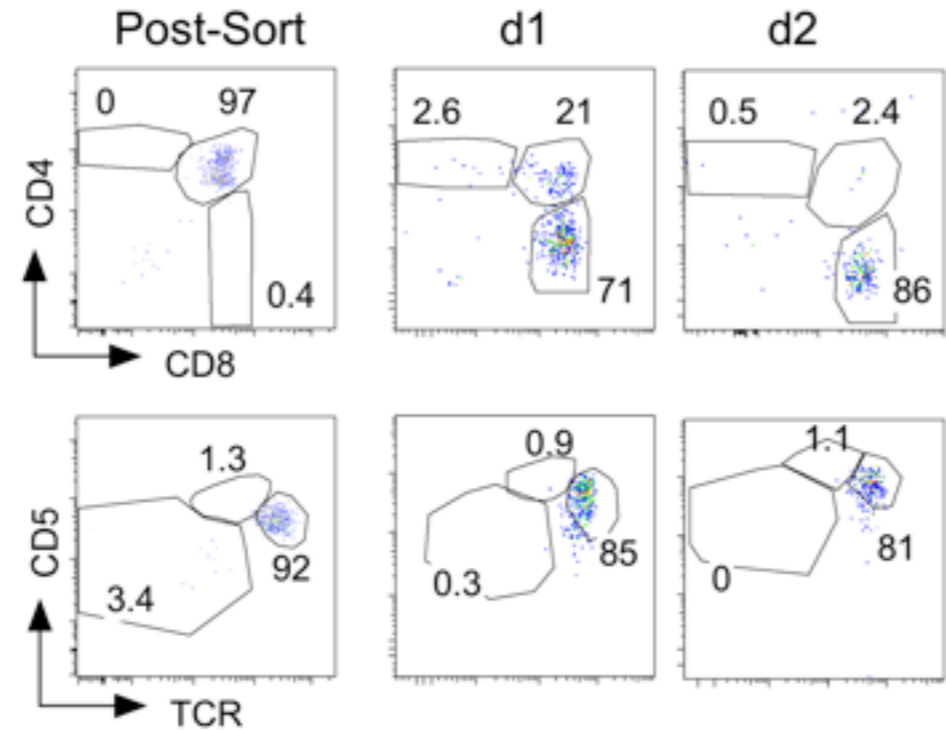


Establishing precursor-product relationships - II

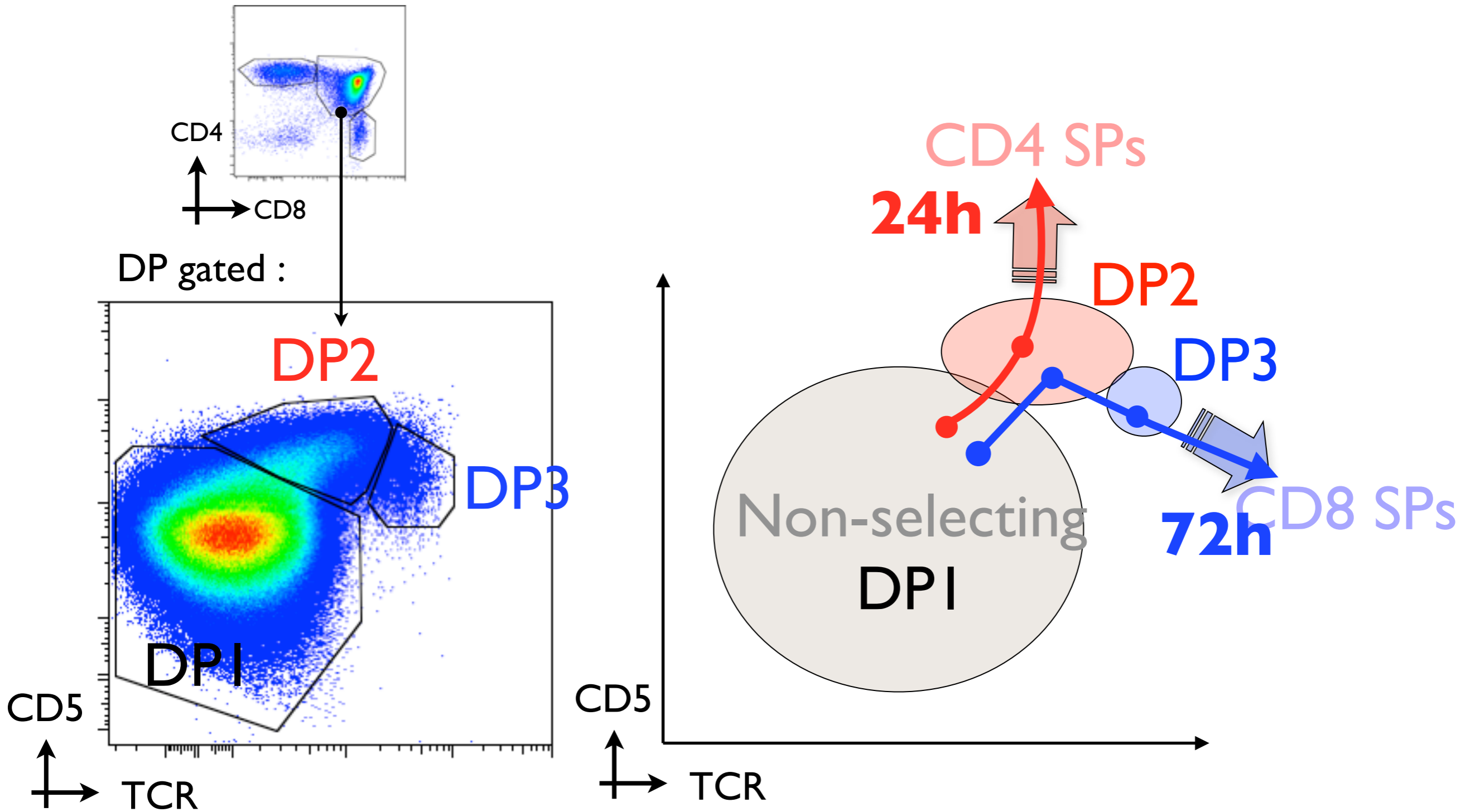
DP2



DP3

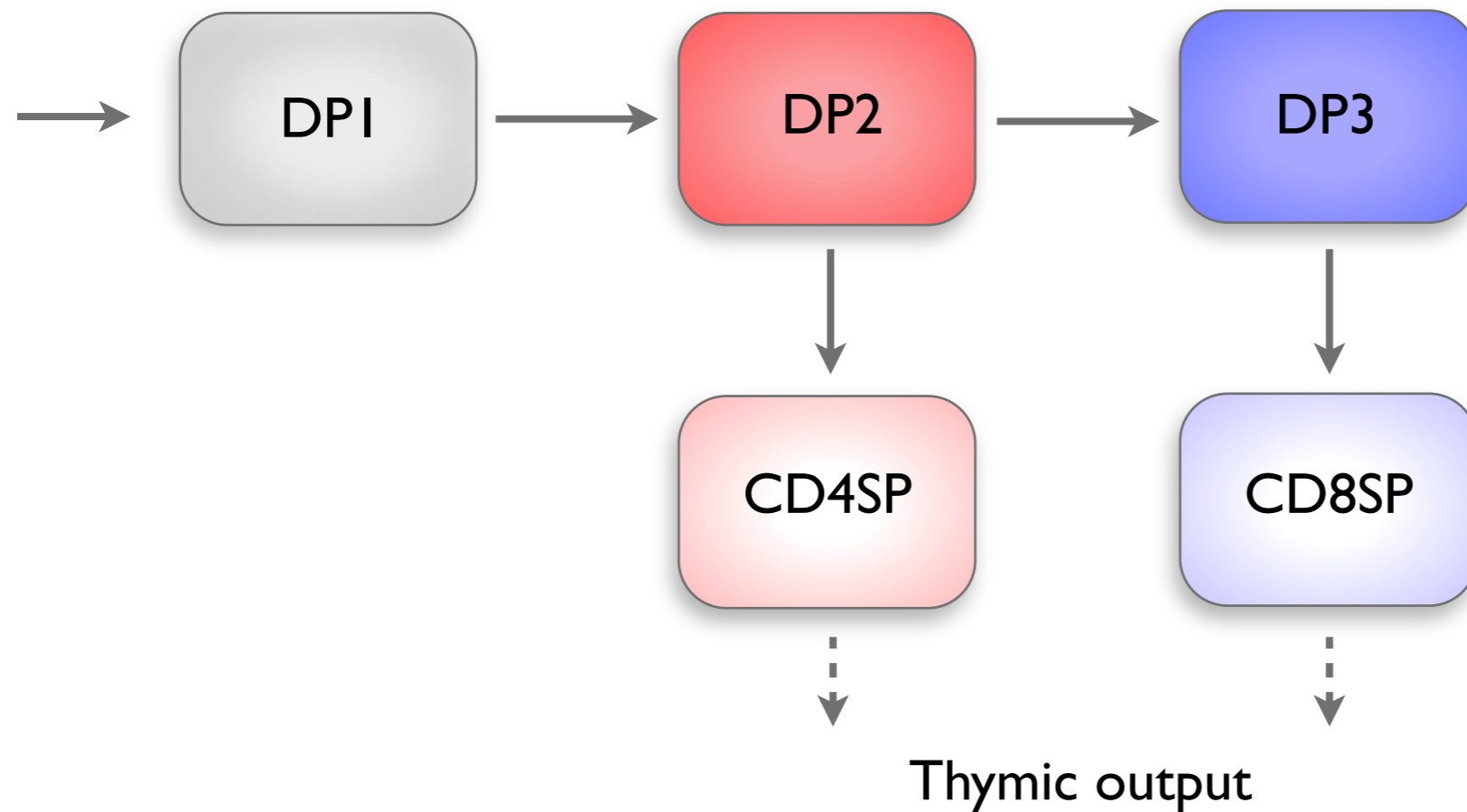
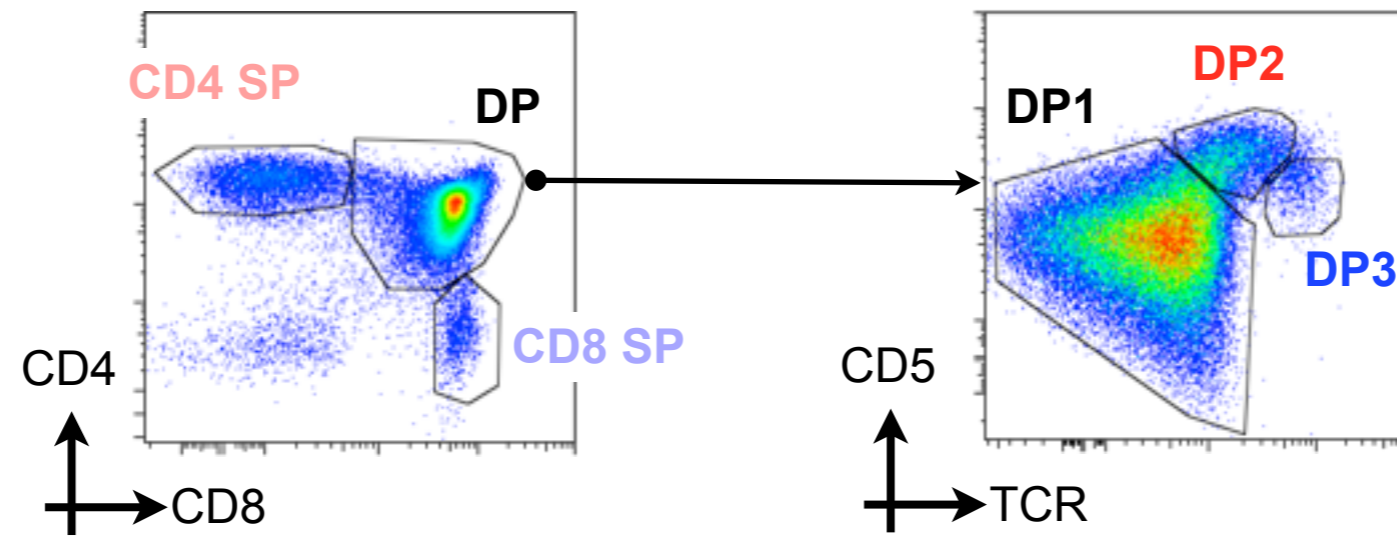


Temporal dynamics of positive selection

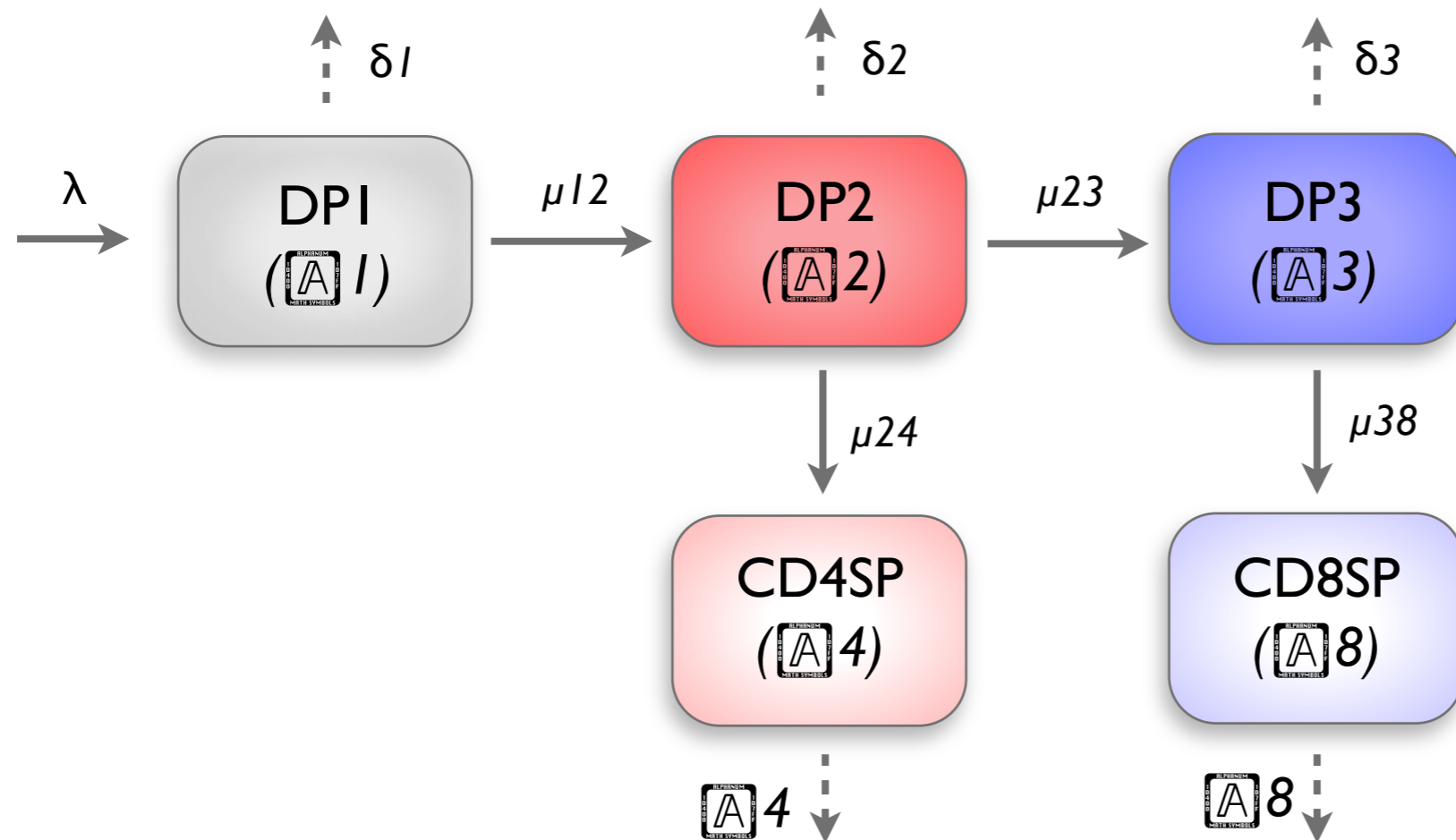
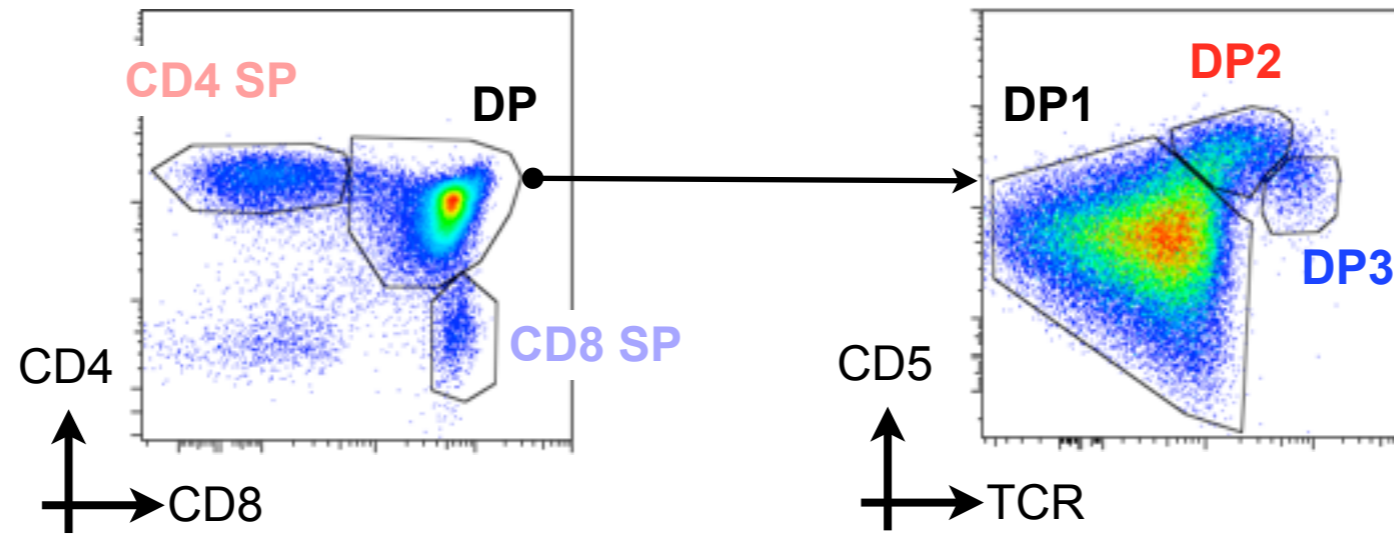


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Temporal regulation of thymic selection



Mathematical description of development

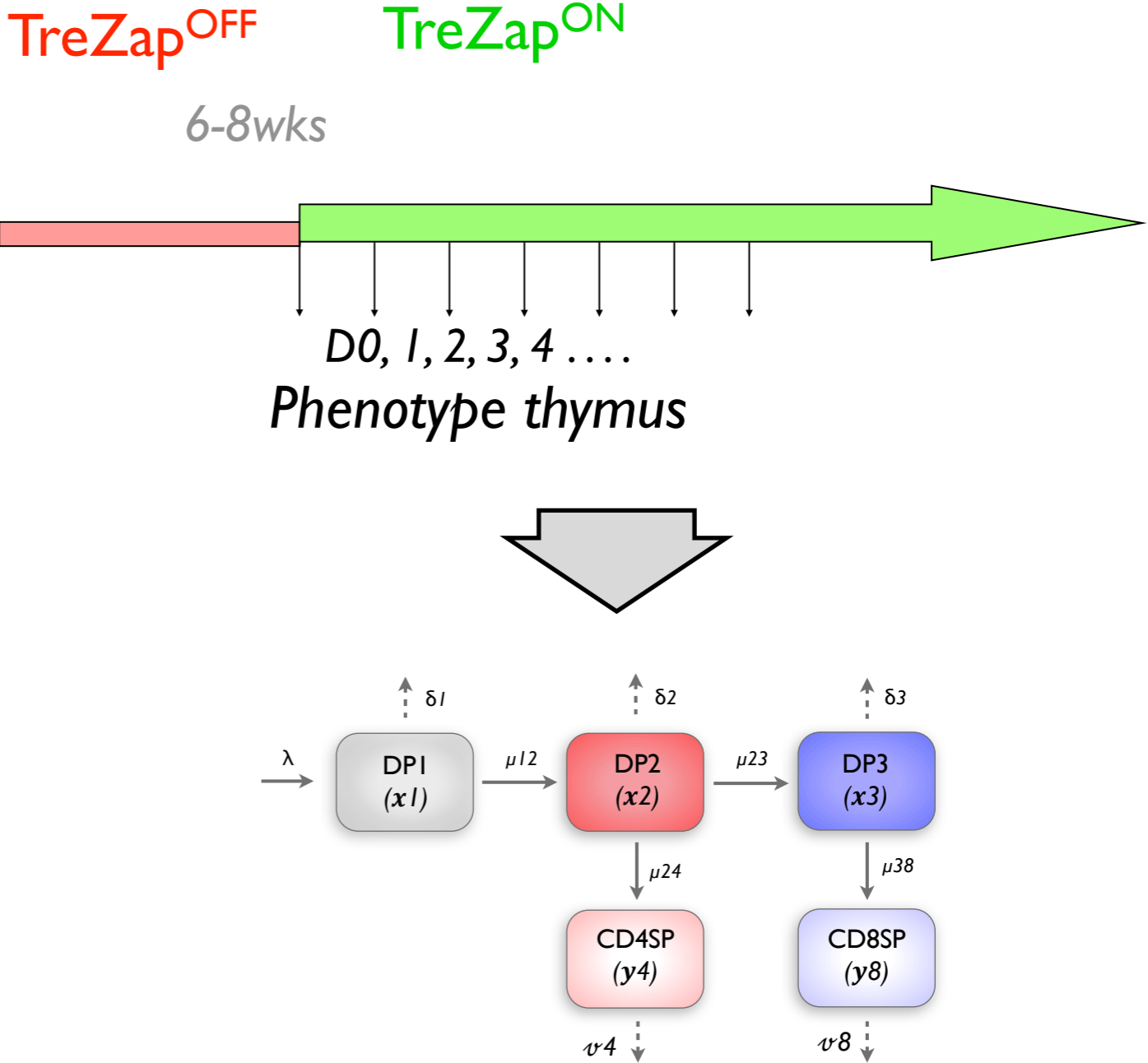
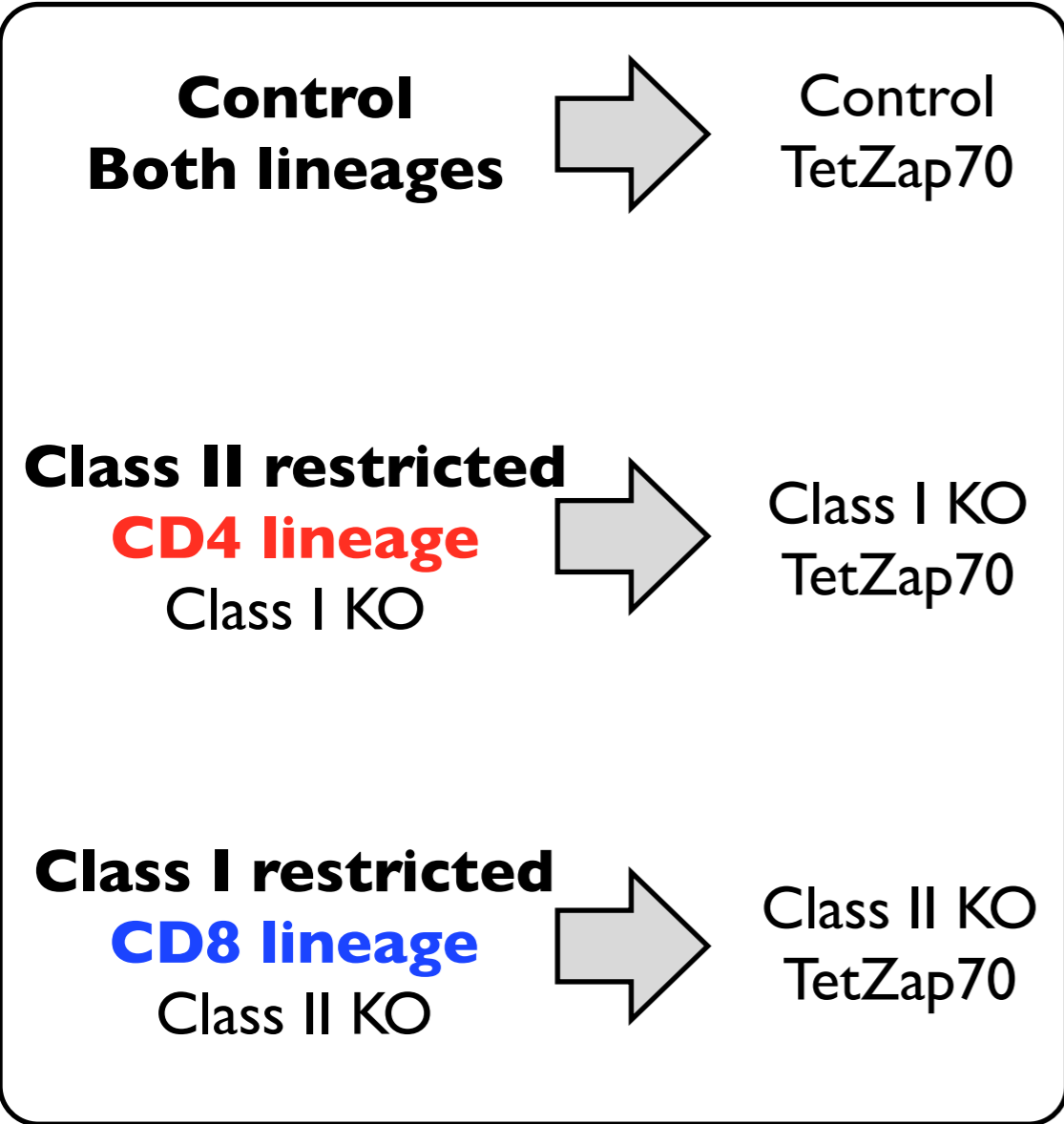


Population dynamics

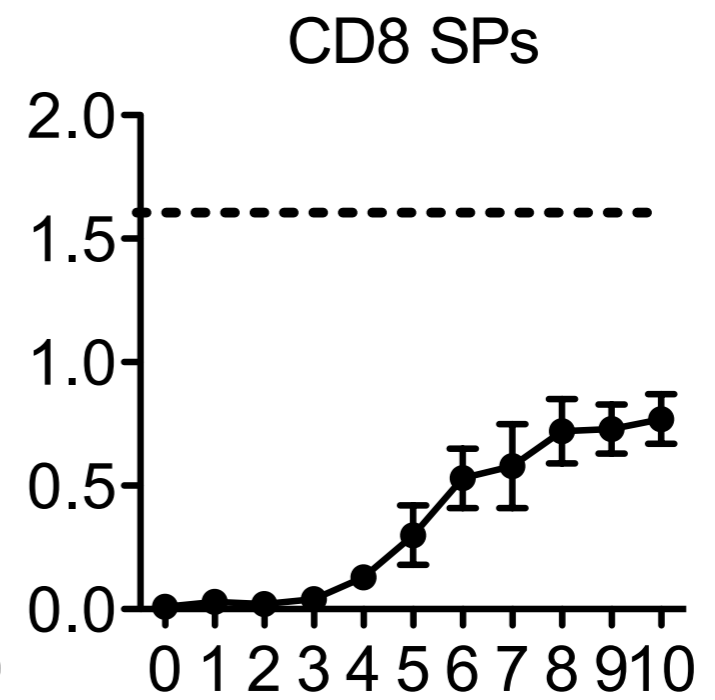
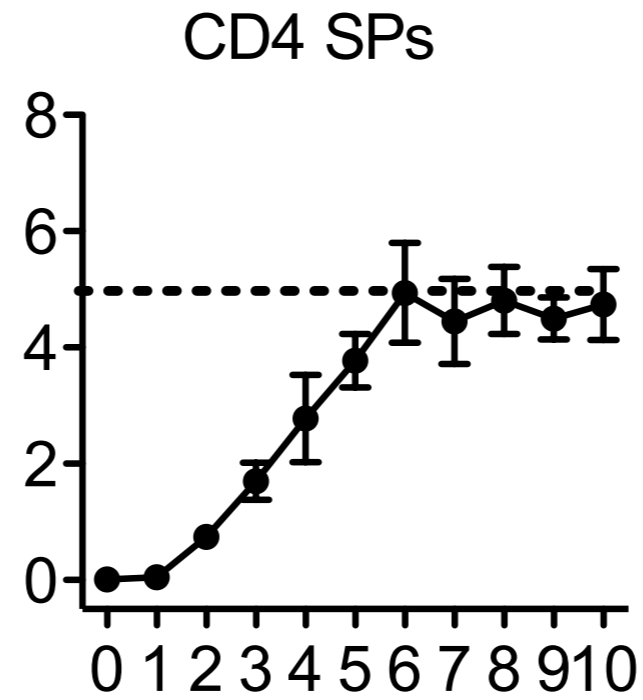
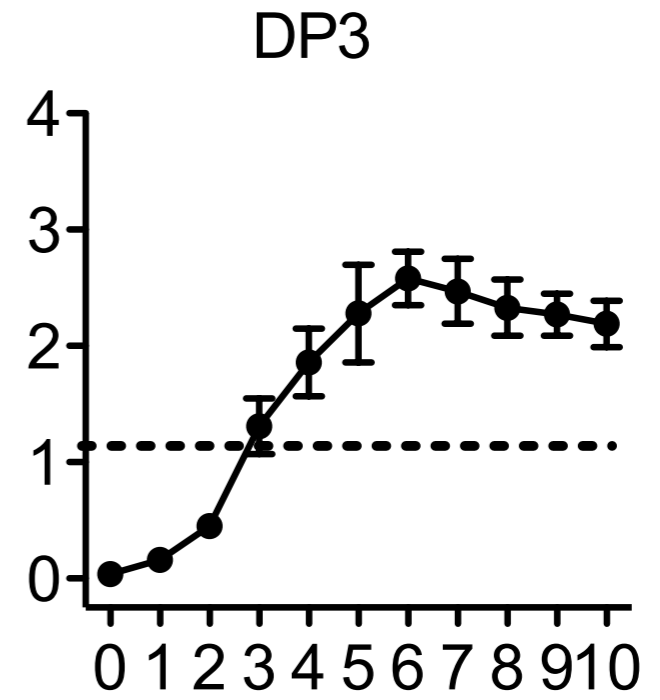
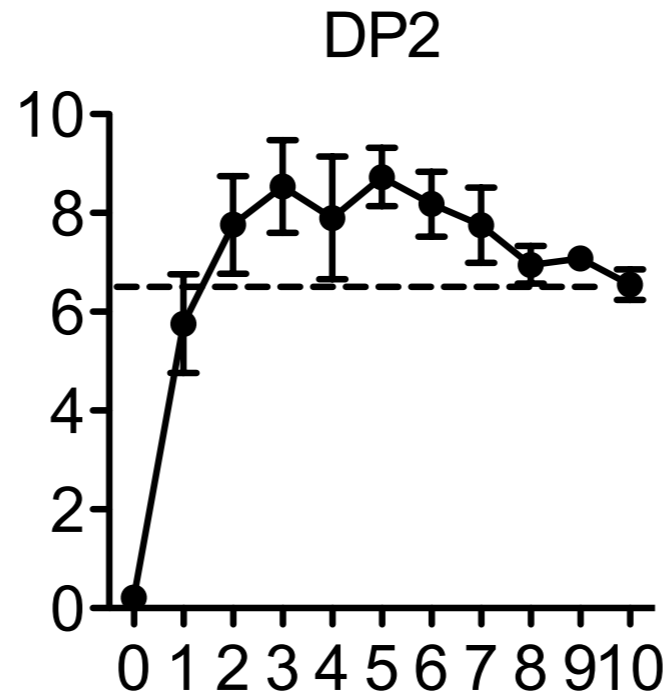
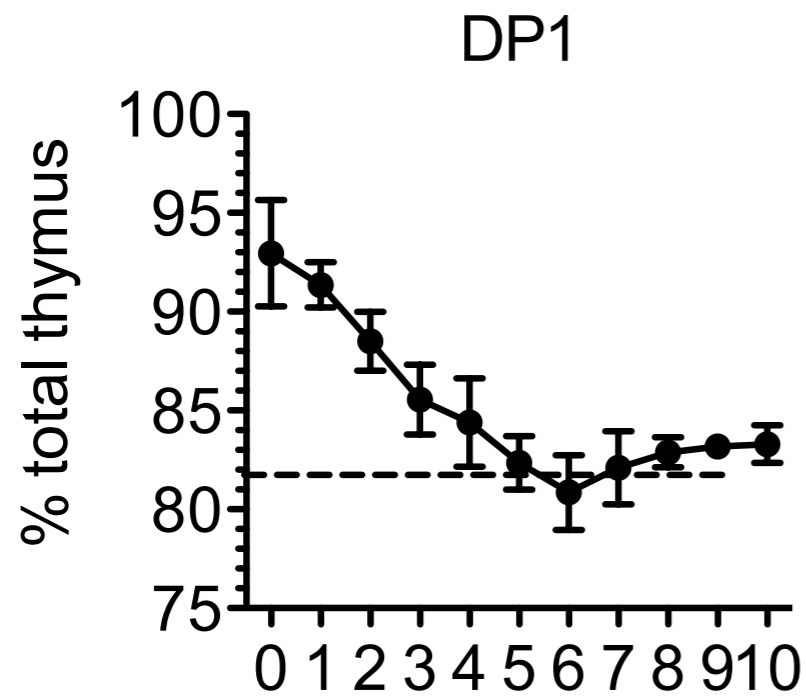
	$\frac{dx_1}{dt} = \lambda - (\delta_1 + \mu_{12})x_1[t]$
	$\frac{dx_2}{dt} = \mu_{12}x_1[t] - (\delta_2 + \mu_{23} + \mu_{24})x_2[t]$
	$\frac{dx_3}{dt} = \mu_{23}x_2[t] - (\delta_3 + \mu_{38})x_3[t]$
	$\frac{dy_4}{dt} = \mu_{24}x_2[t] - \nu_4 y_4[t]$
	$\frac{dy_8}{dt} = \mu_{38}x_3[t] - \nu_8 y_8[t]$

Thymic output

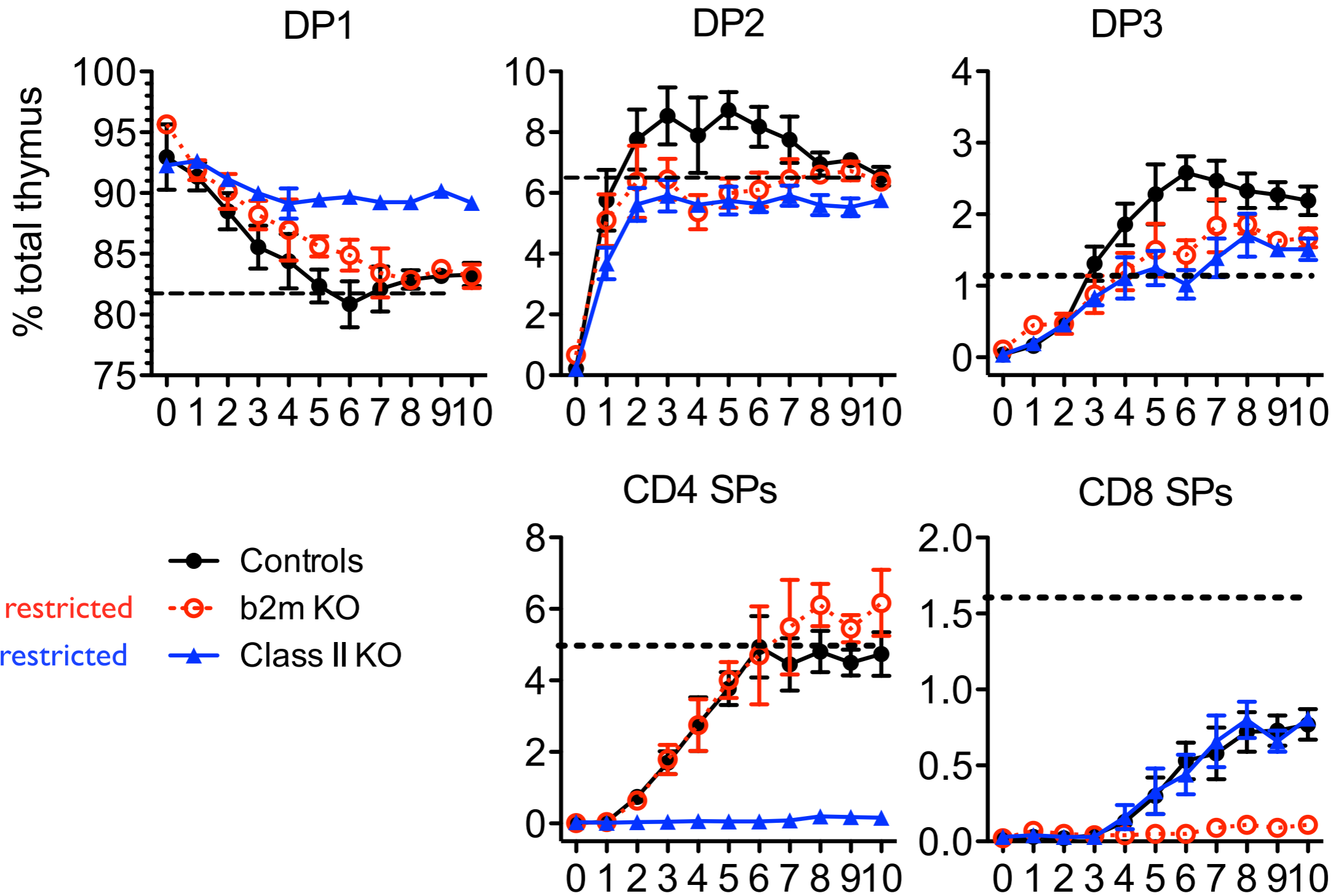
Generating timecourse data of Class I and Class II restricted T cell development



Control time course



Class I vs Class II restricted T cell development

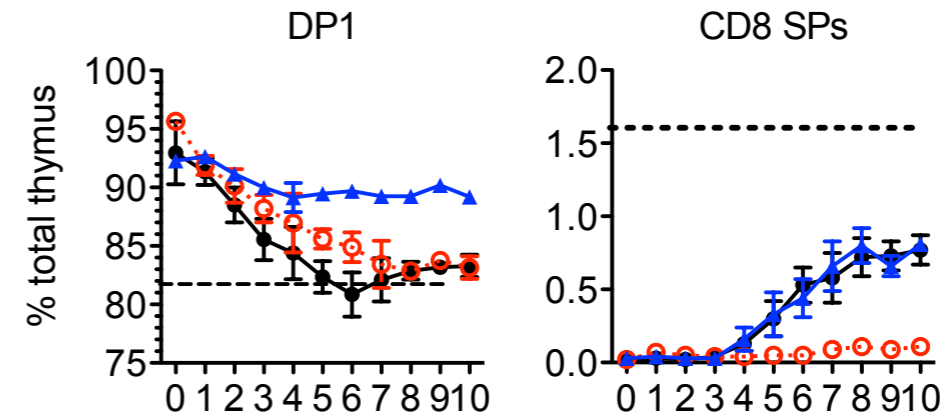


Use data to identify parameters of model

Log vs Lin transformed data

Assume SP plateau

Parameter estimations by
**minimising sum of
squares residuals
(Nelder-Mead algo in R)**



● Controls
○ b2m KO
▲ Class II KO

Parameter	Rag control		MHC I knockout		MHC II knockout	
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
λ	27.503		(25.095, 29.911)			
μ_{12}	0.091	(0.076, 0.106)	0.08	(0.055, 0.106)	0.061	(0.043, 0.08)
μ_{23}	0.37	(0.234, 0.506)	0.299	(0.116, 0.482)	0.48	(0.196, 0.763)
μ_{24}	0.484	(0.393, 0.575)	0.583	(0.473, 0.692)	0.012	(-0.019, 0.043)
μ_{38}	0.255	(-0.697, 1.207)	0.012	(-0.027, 0.051)	0.136	(-0.153, 0.424)
δ_1	0.249	(0.218, 0.281)	0.251	(0.214, 0.289)	0.251	(0.219, 0.283)
δ_2	0.653	(0.441, 0.866)	0.781	(0.365, 1.197)	0.792	(0.427, 1.158)
δ_3	0.143	(-0.884, 1.171)	0.317	(-0.034, 0.669)	0.378	(-0.392, 1.149)
ν_4	0.23	(0.132, 0.327)	0.142	(0.058, 0.225)	-	-
ν_8	1	(-3.289, 5.289)	-	-	0.1	(-0.72, 0.92)

		Rag-control	MHC I KO	MHC II KO
Mean time spent in DP1 (days)	$\frac{1}{\mu_{12} + \delta_1}$	2.94	3.02	3.2
Mean time spent in DP2	$\frac{1}{\mu_{23} + \mu_{24} + \delta_2}$	0.91	0.82	1.01
Mean time spent in DP3	$\frac{1}{\mu_{38} + \delta_3}$	3.24	3.72	3.03
Mean time spent in CD4SP	$\frac{1}{\nu_4}$	6.33	10.11	-
Fraction of DP1 that die	$\frac{\delta_1}{\mu_{12} + \delta_1}$	0.69	0.73	0.79
Fraction of DP1 recruited to DP2	$\frac{\mu_{12}}{\mu_{12} + \delta_1}$	0.31	0.27	0.21
Fraction of DP2 that die	$\frac{\delta_2}{\mu_{23} + \mu_{24} + \delta_2}$	0.78	0.79	0.9
Fraction of DP2 recruited to DP3	$\frac{\mu_{23}}{\mu_{23} + \mu_{24} + \delta_2}$	0.1	0.07	0.09
Fraction of DP2 recruited to CD4SP	$\frac{\mu_{24}}{\mu_{23} + \mu_{24} + \delta_2}$	0.13	0.14	0.003
Fraction of DP3 that die	$\frac{\delta_3}{\mu_{38} + \delta_3}$	0.78	0.96	0.61
Fraction of DP3 recruited to CD8SP	$\frac{\mu_{38}}{\mu_{38} + \delta_3}$	0.22	0.04	0.39

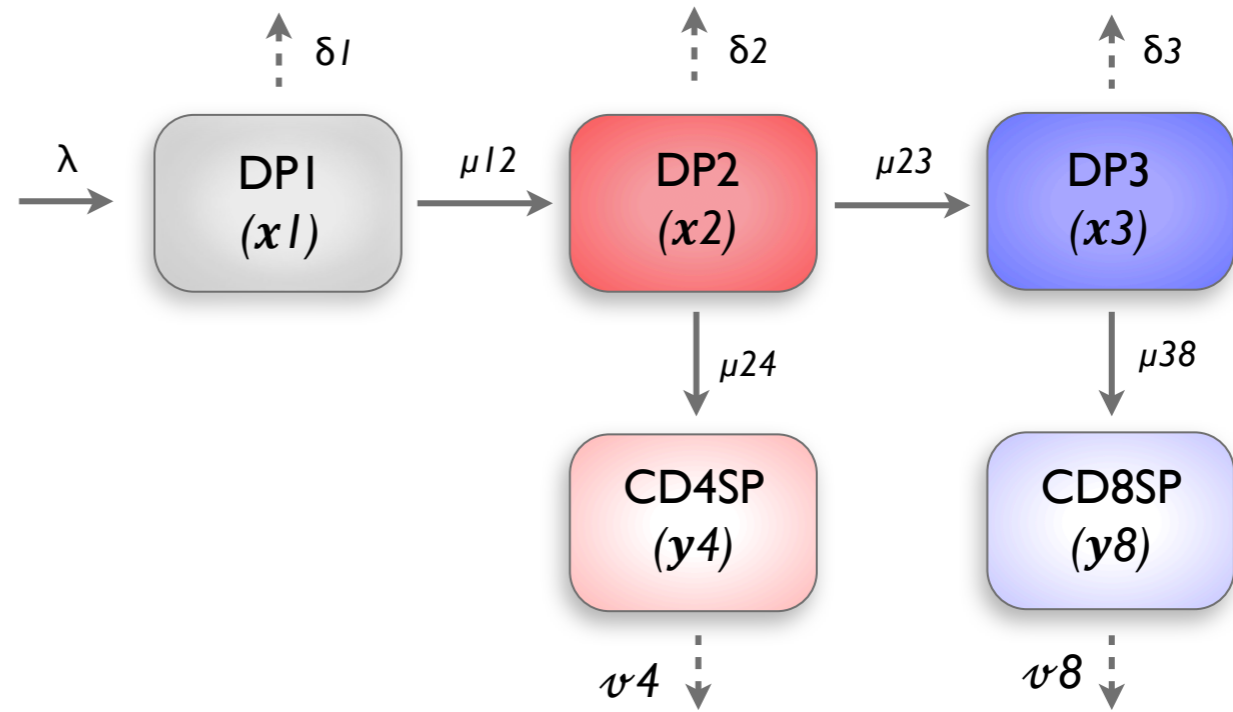
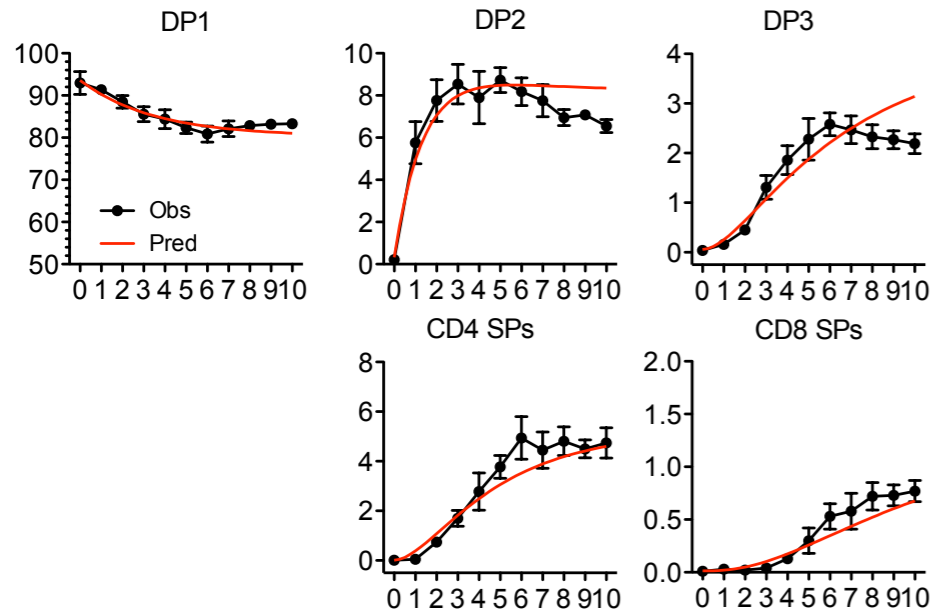
We assumed:

- (i) the rate of input into the DP1 compartment of cells (cells/day) (from DNs) was the same in the WT, MHC I KO and MHC II KO mice
- (ii) CD4 and CD8 cell numbers are at steady-state (or plateau) from day 7 onwards. We use this assumption to constrain our estimate for the rate of export from the SP compartments. All other parameters were completely free.

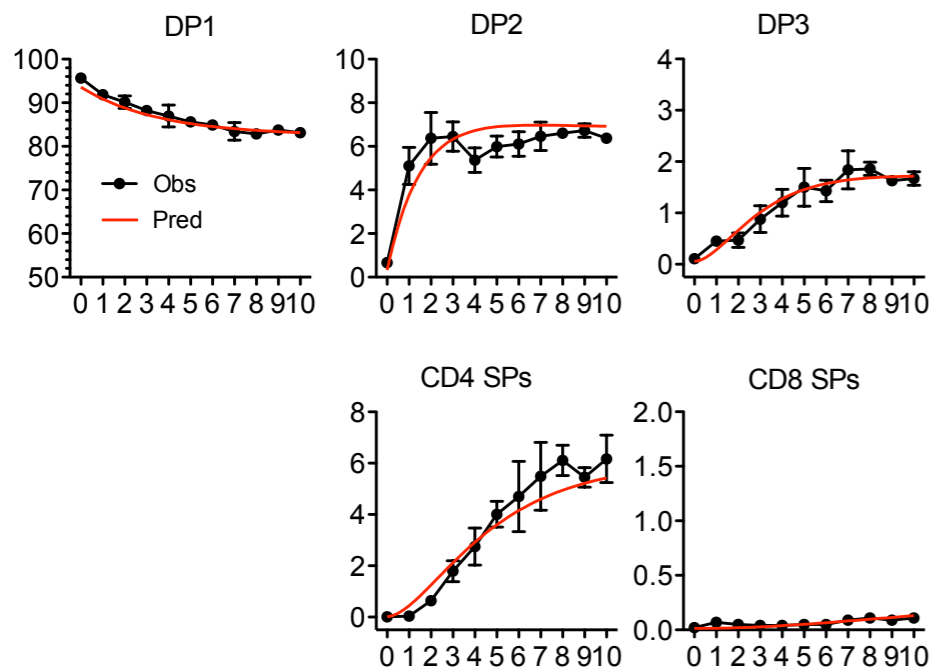
The 95% CI come from a bootstrap procedure: I create a new dataset by randomly re-sampling the observations (with replacement) and find new set of parameters to describe this resampled-dataset. I repeat this $n=10^4$ times. The 2.5 and 97.5 percentiles of the new parameters is used to determine the 95% confidence intervals.

Model fits to timecourses of T cell development

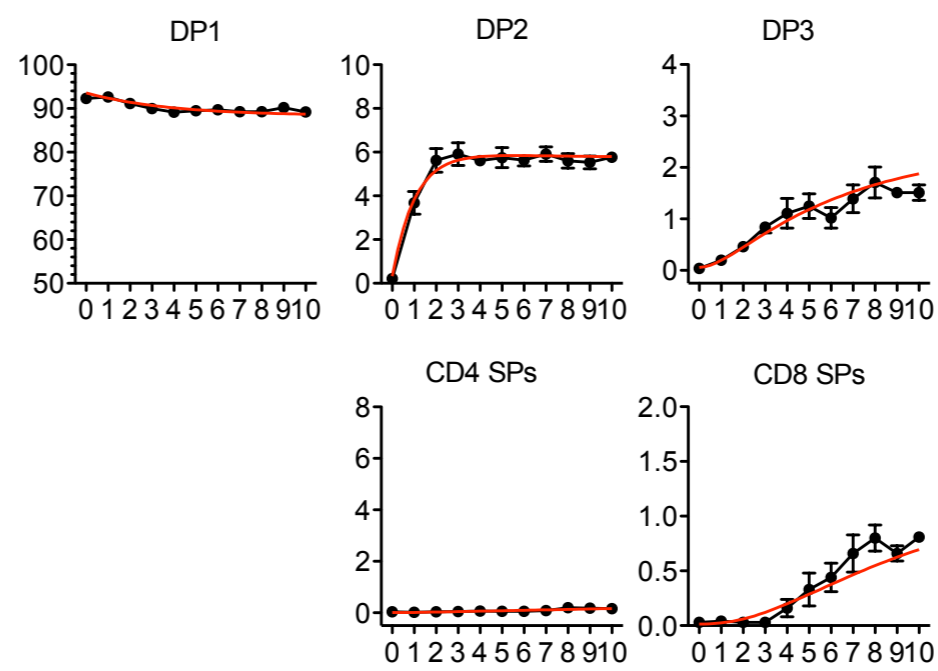
Class I + Class II restricted



Class II restricted



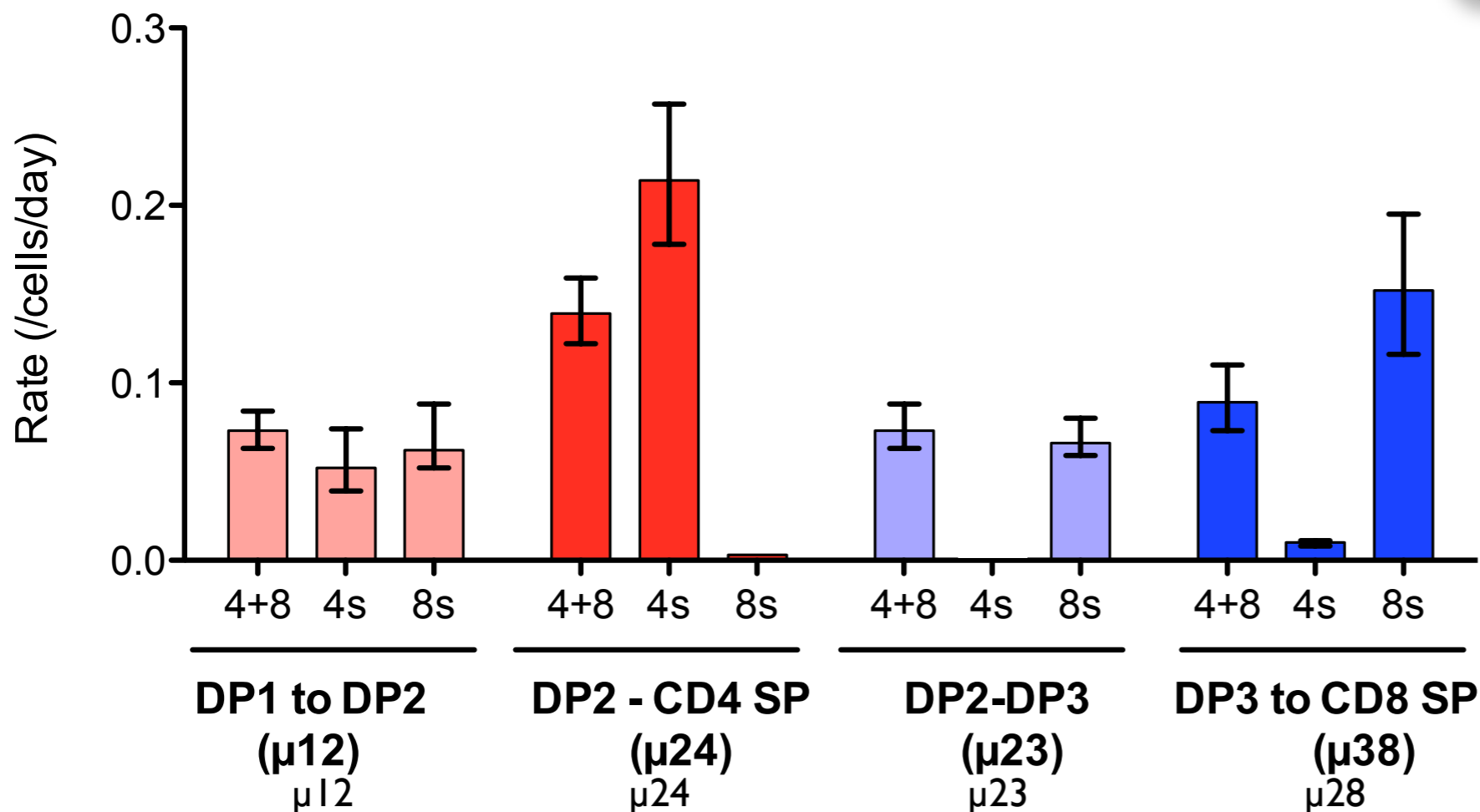
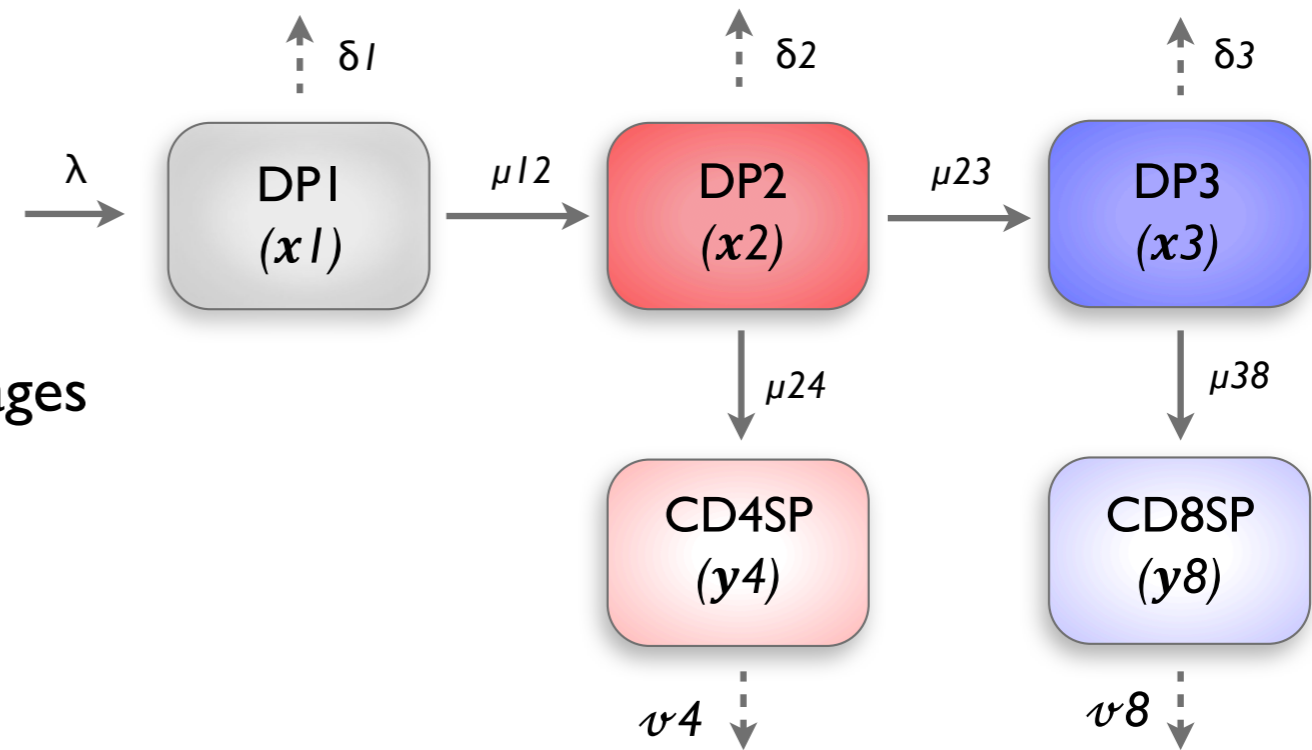
Class I restricted



Maturation rates in different hosts

$\lambda = 0.23$

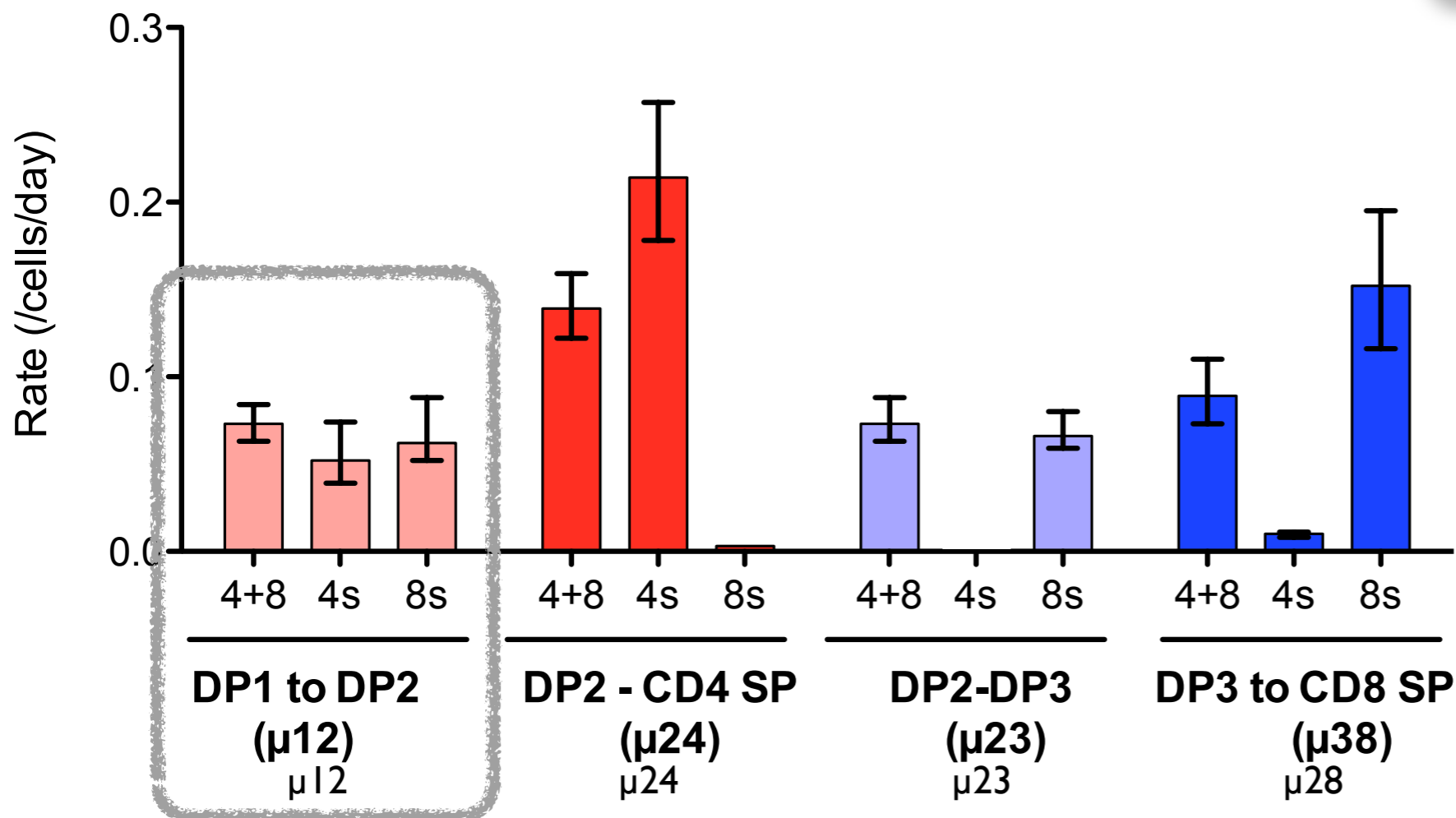
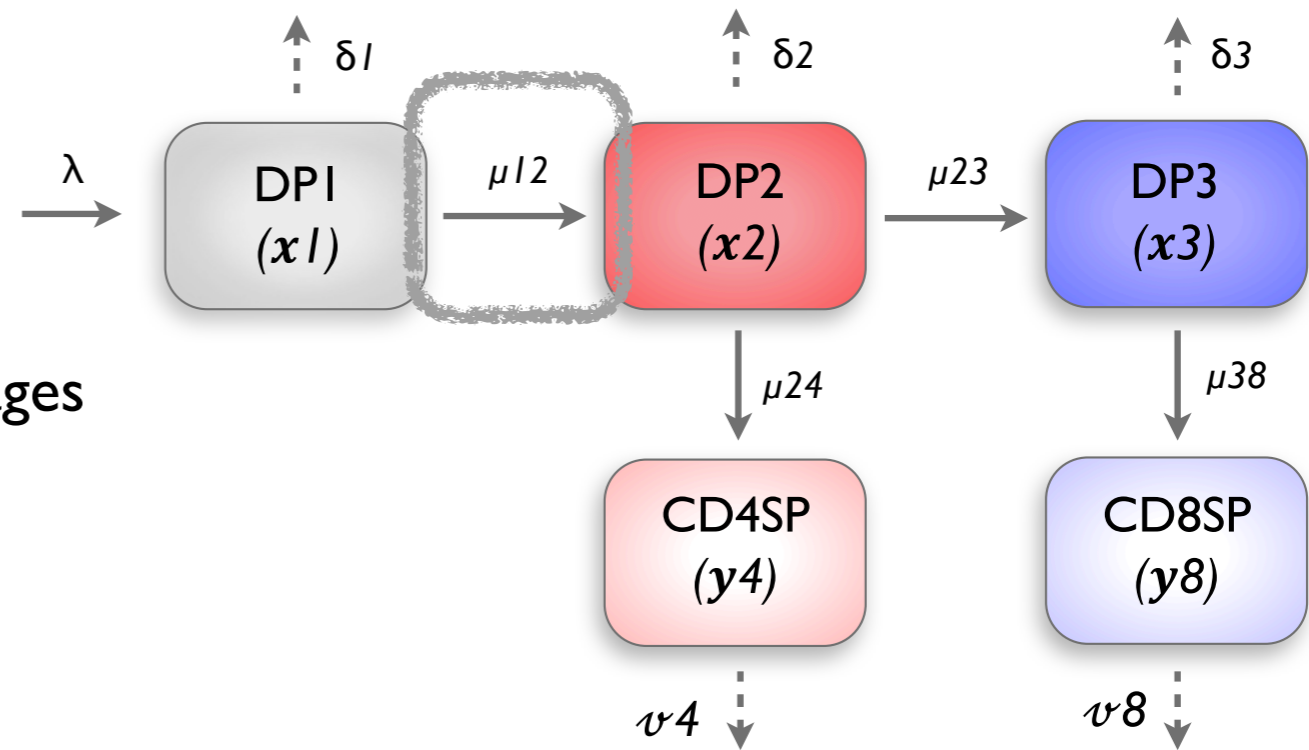
- 4+8** = control - both CD4 and CD8 lineages
- 4s** = CD4 lineage only (Class I KOs)
- 8s** = CD8 lineage only (Class II KOs)



Maturation rates in different hosts

$\lambda = 0.23$

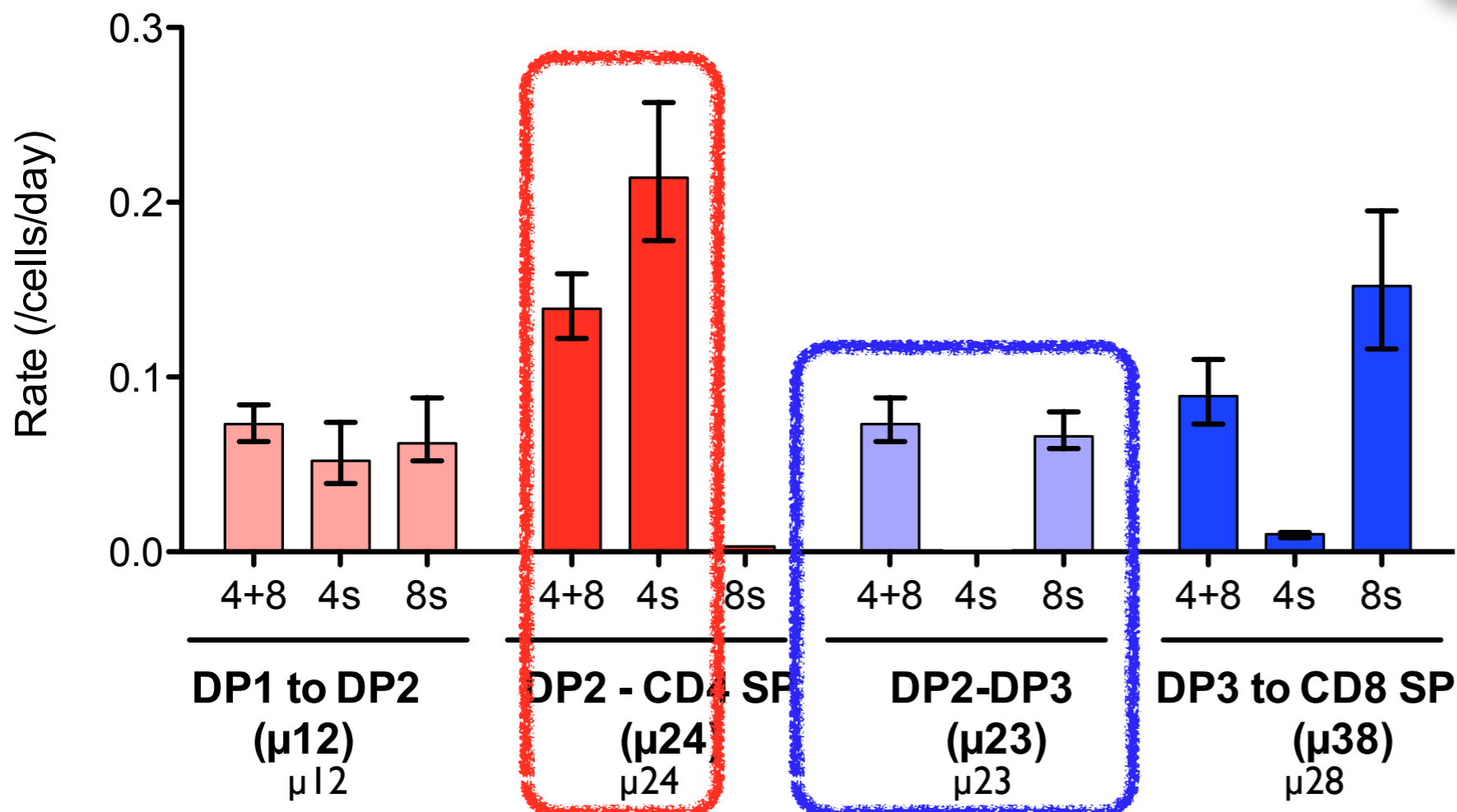
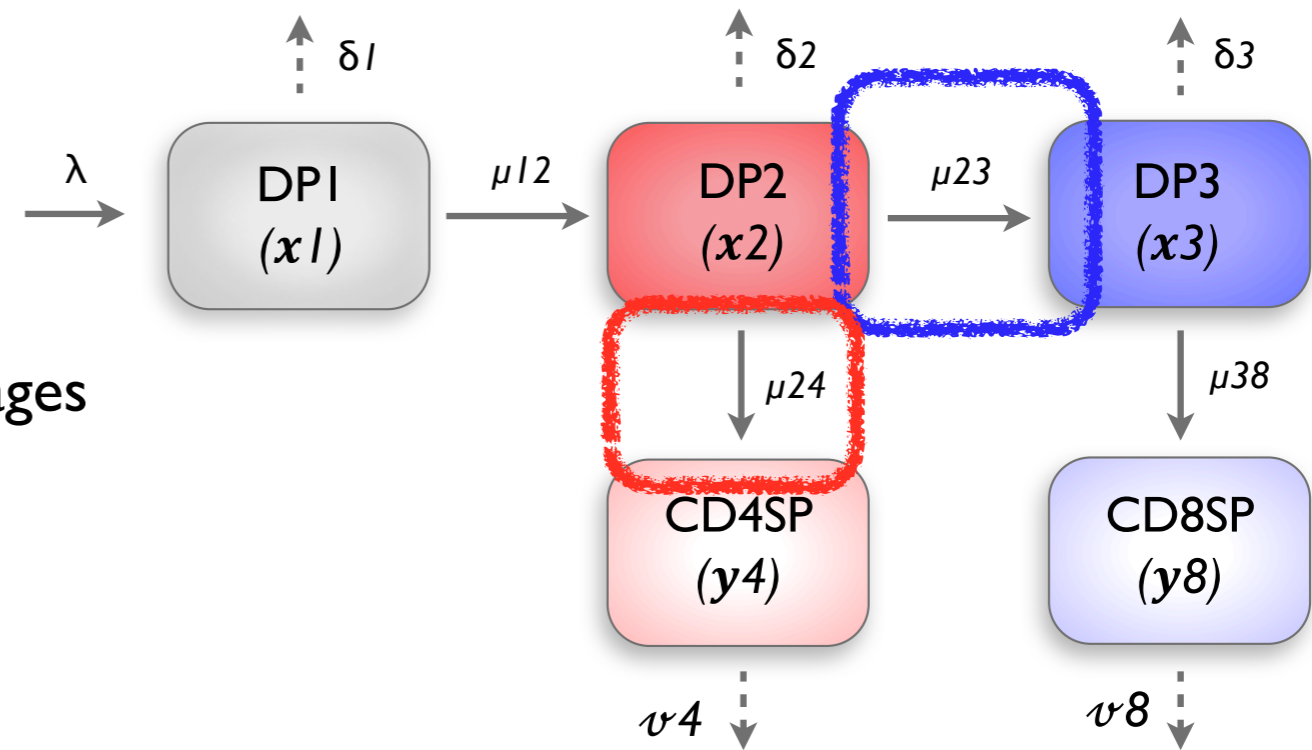
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Maturation rates in different hosts

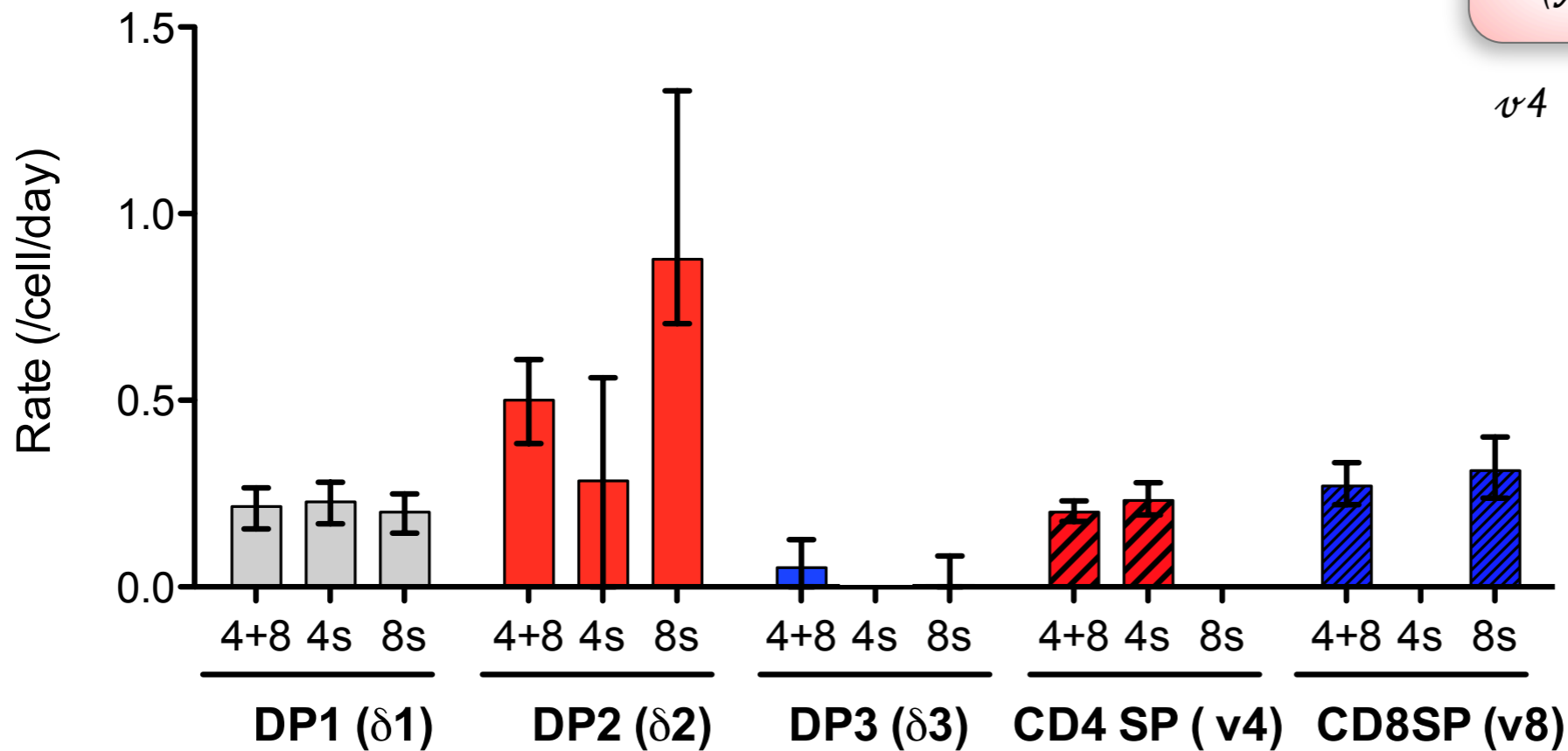
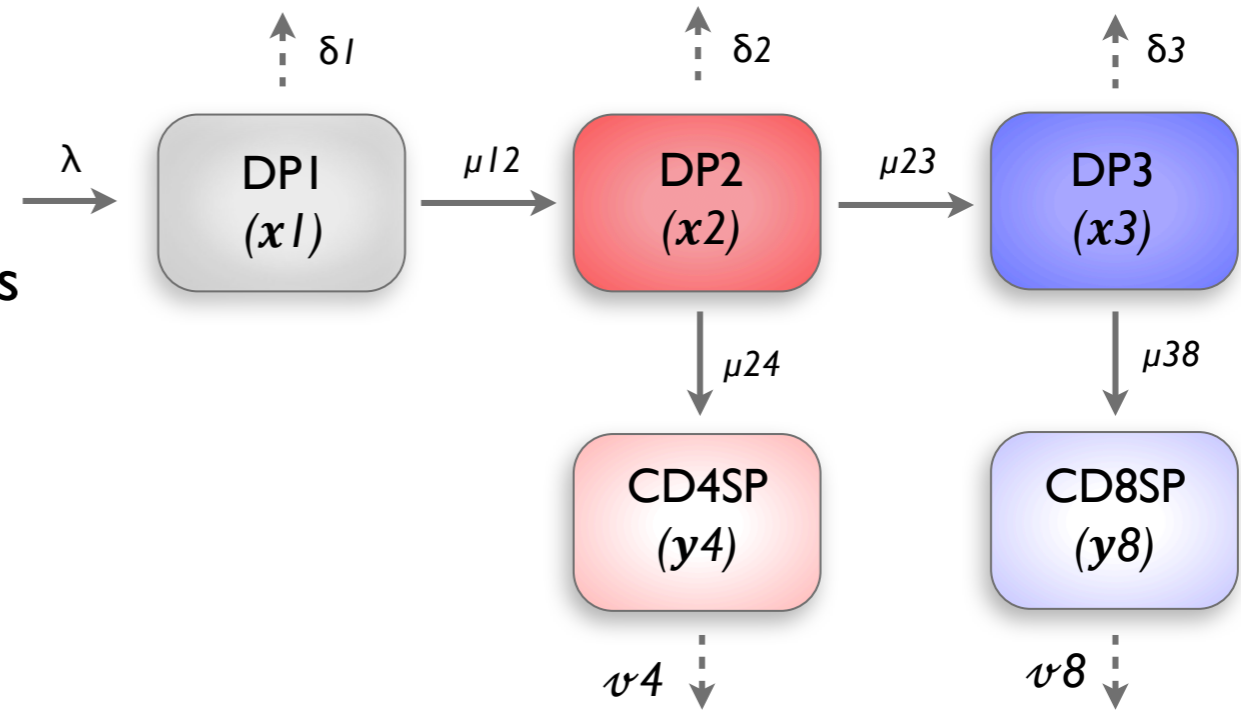
$$\lambda = 0.23$$

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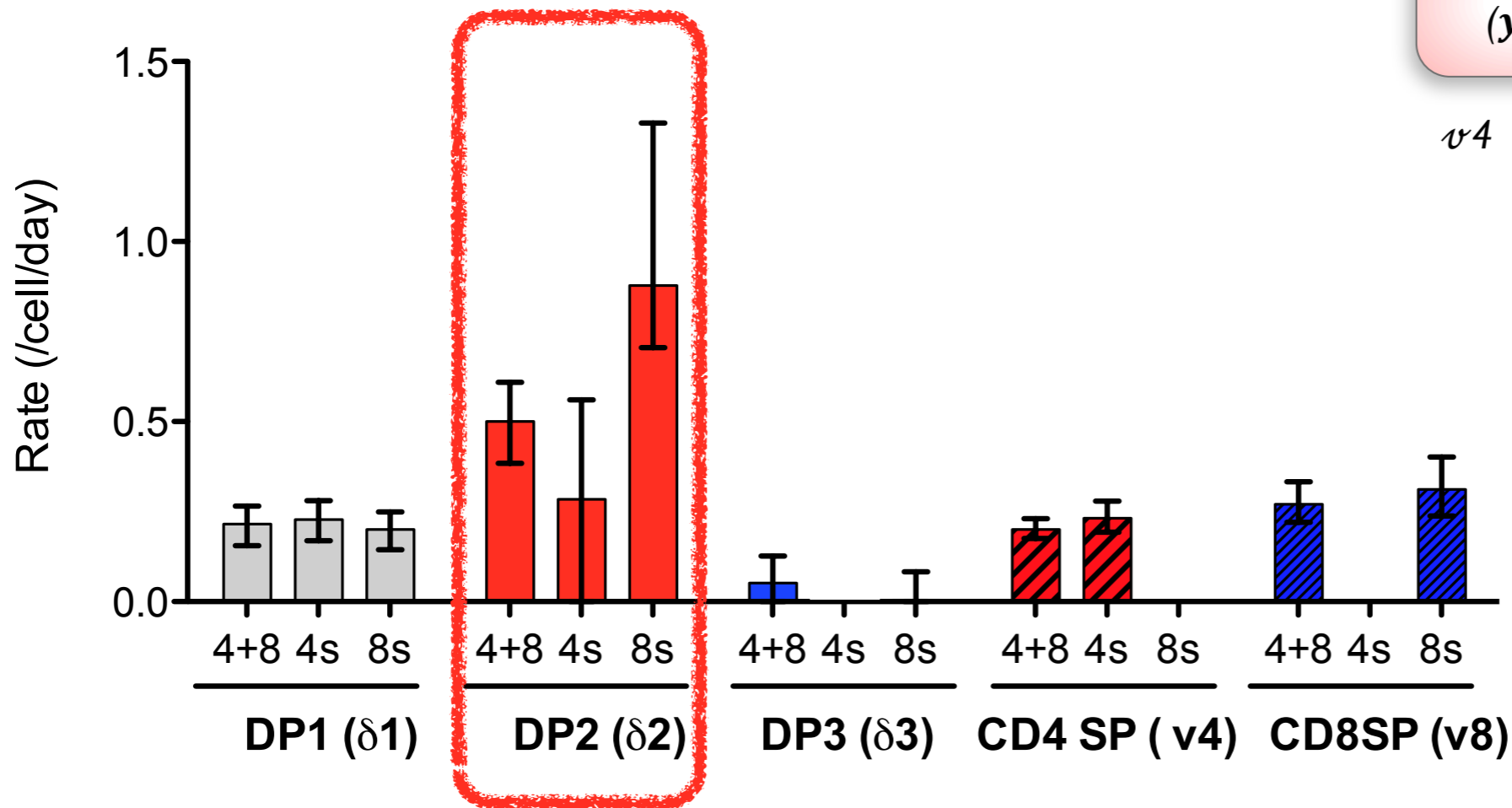
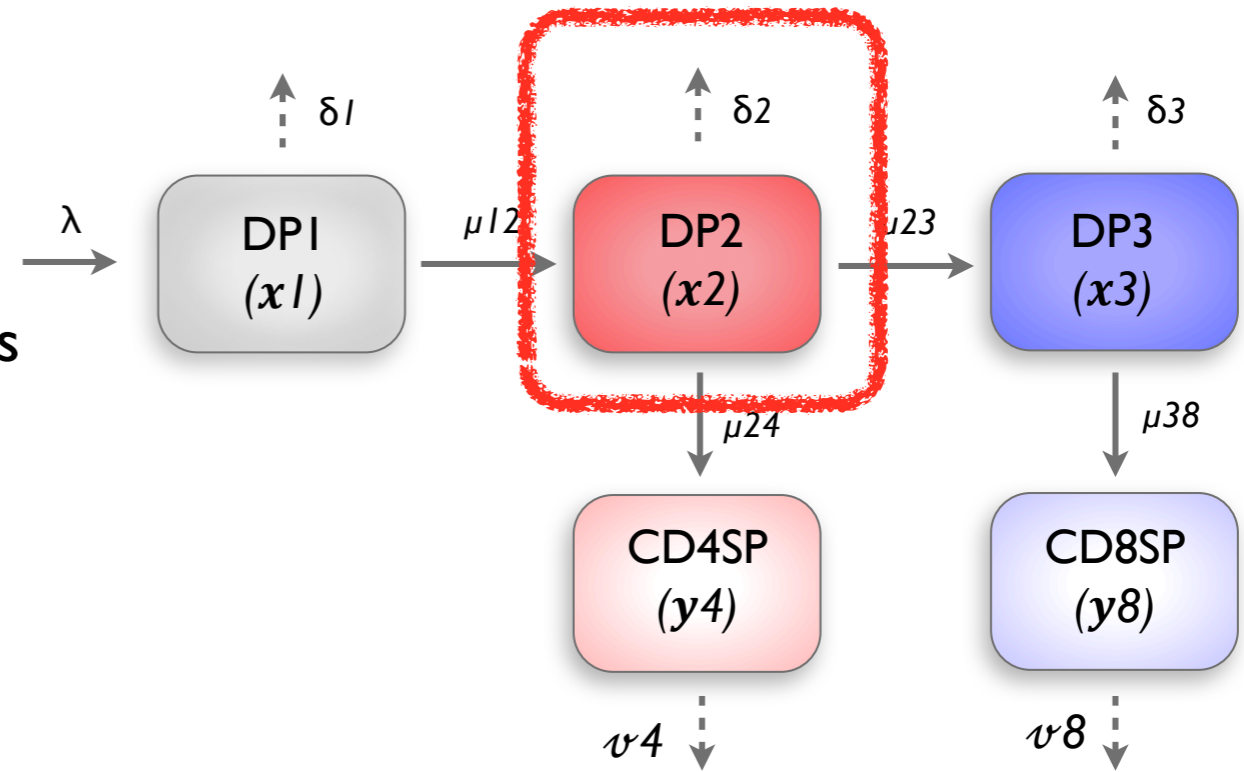
Death during selection

4+8 = control - both CD4 and CD8 lineages
4s = CD4 lineage only (Class I KOs)
8s = CD8 lineage only (Class II KOs)



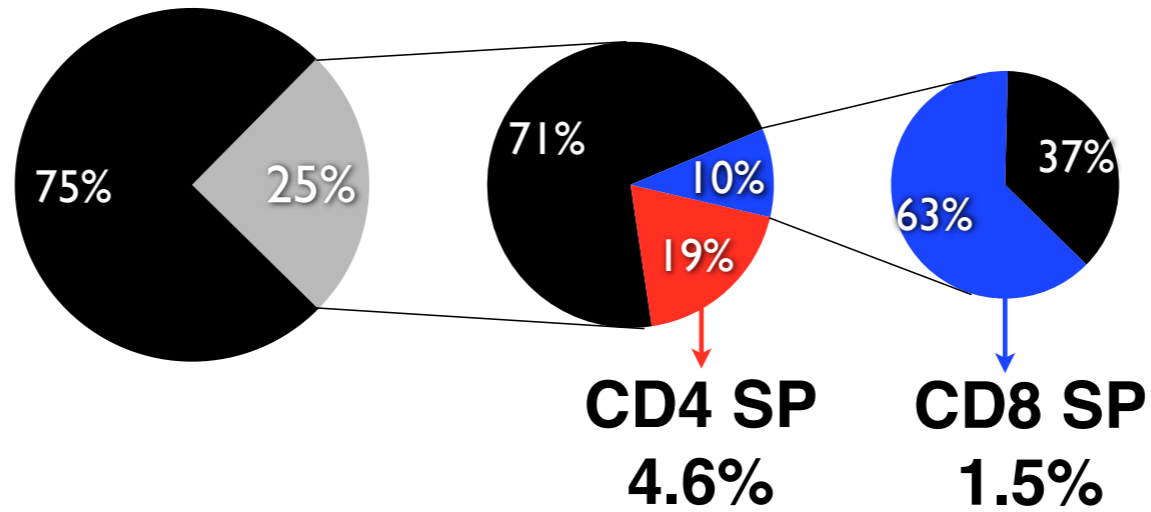
Death during selection

4+8 = control - both CD4 and CD8 lineages
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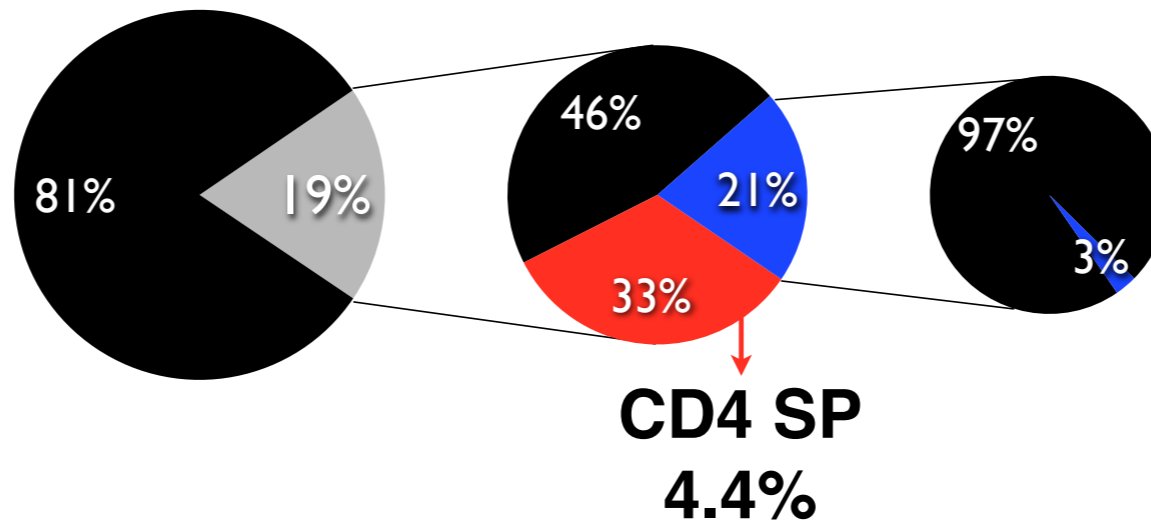


Cellular fate during development

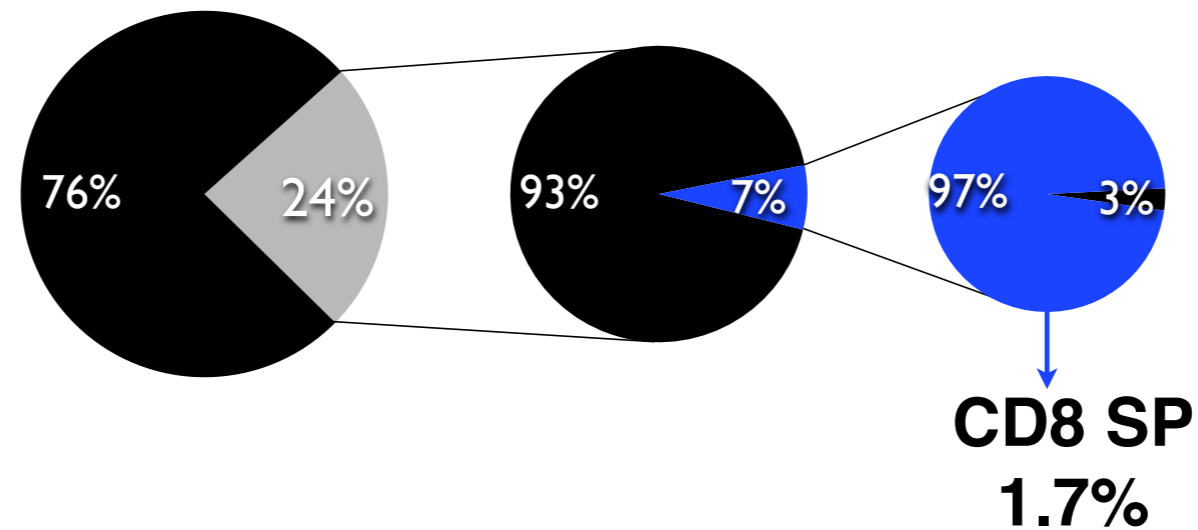
Both
(Control hosts)



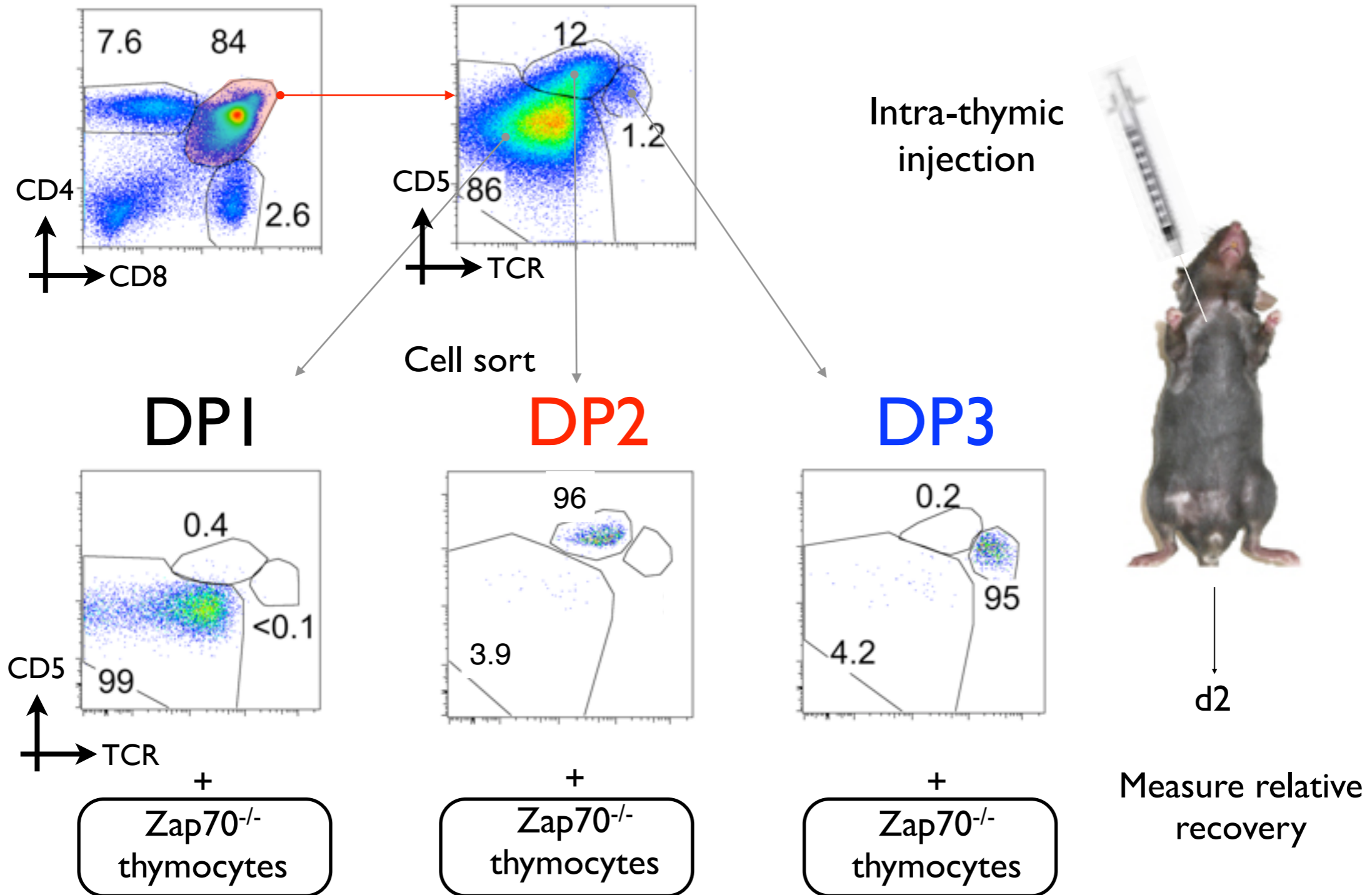
CD4 lineage only
(Class I KO)



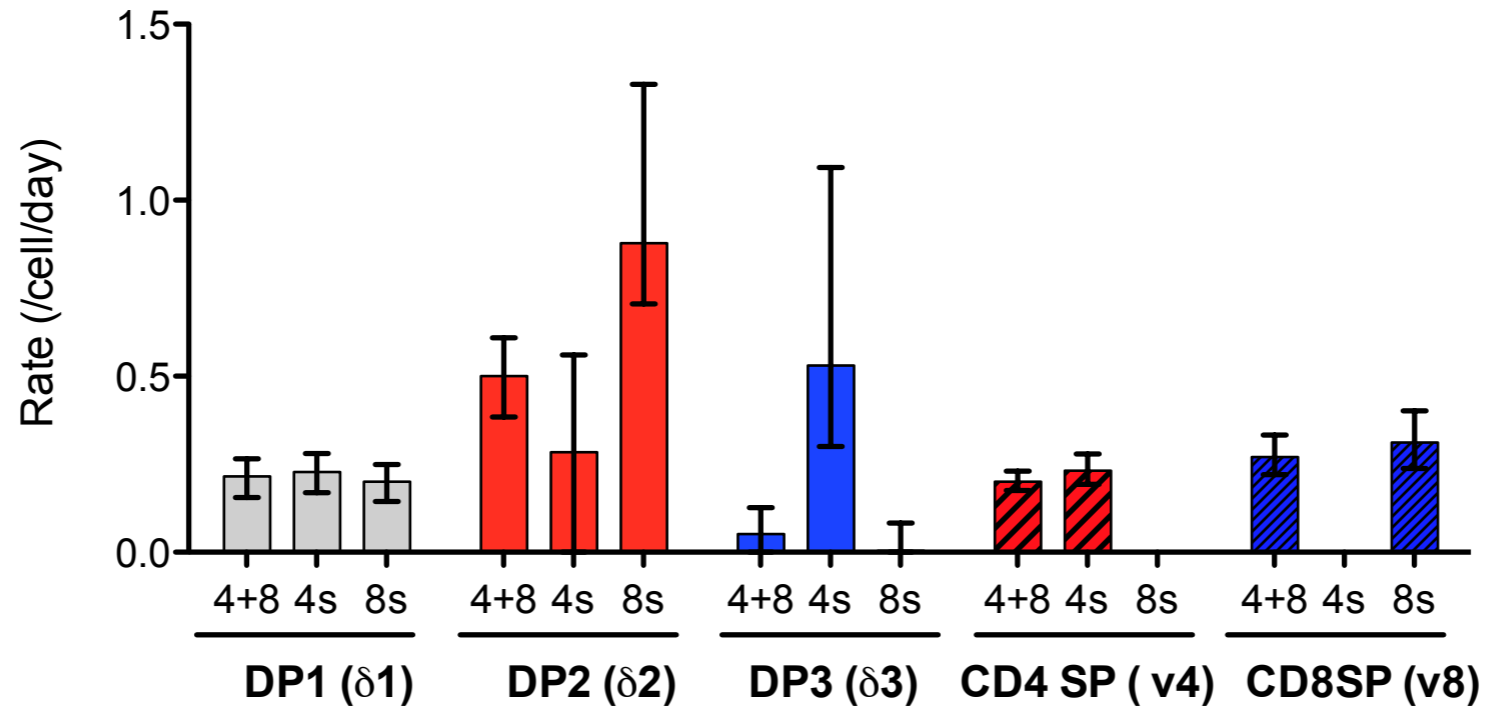
CD8 lineage only
(Class II KO)



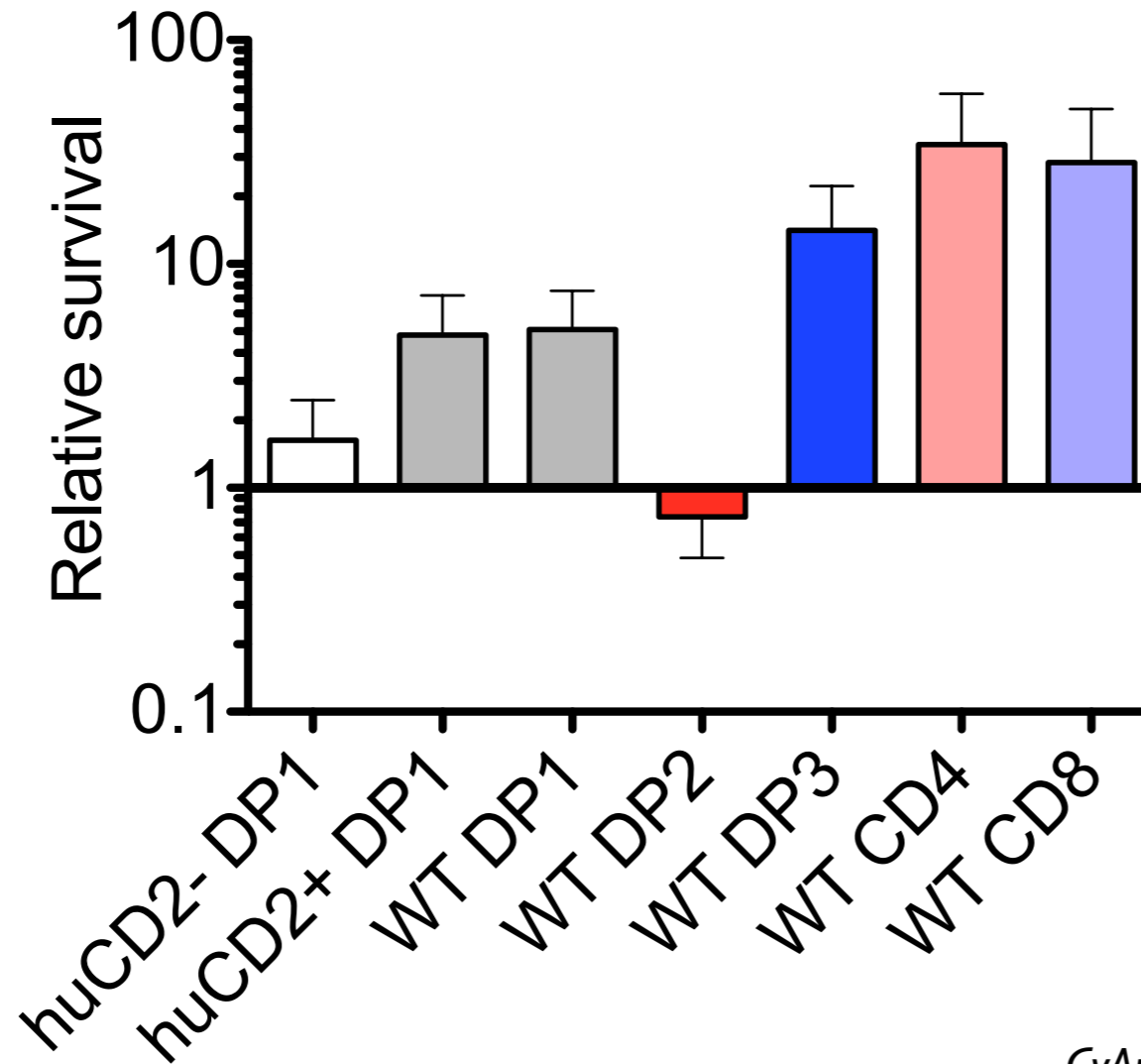
Testing for high death rate in DP2 thymocytes



Death rates



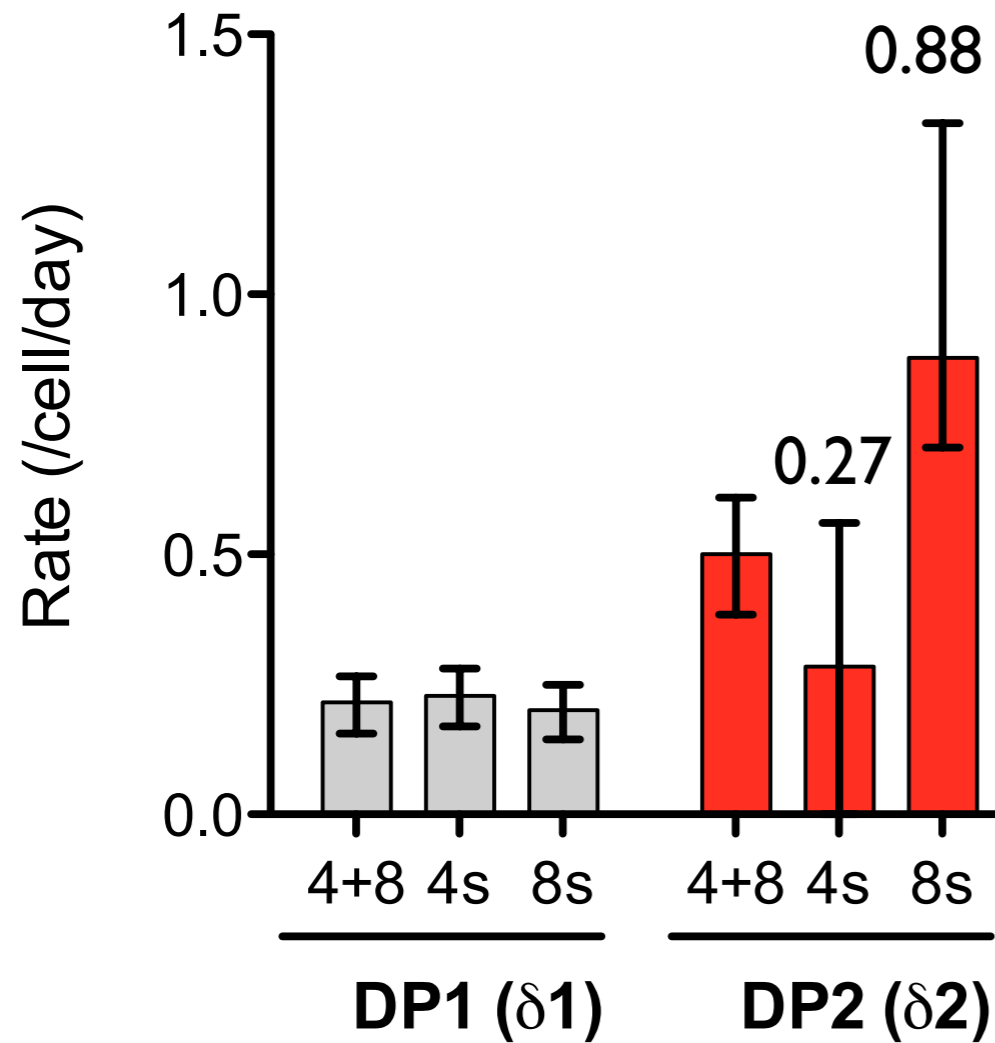
In vivo survival



CyAn ADP

Distinct death rates amongst Class I and Class II restricted DP2s

Model

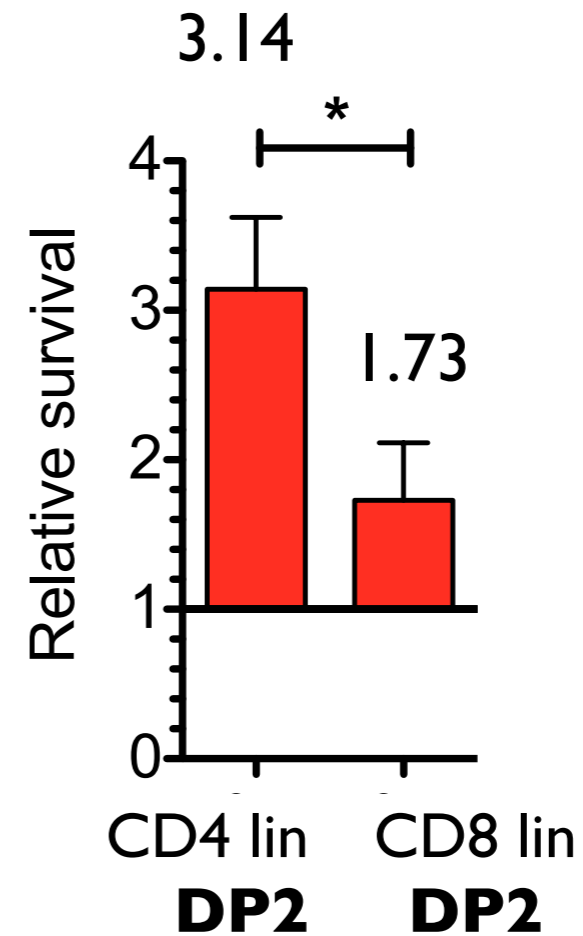


Death rate 8s = 0.88

Death rate 4s = 0.27

Relative survival : $e^{-0.88}/e^{-0.27} = \mathbf{0.54}$

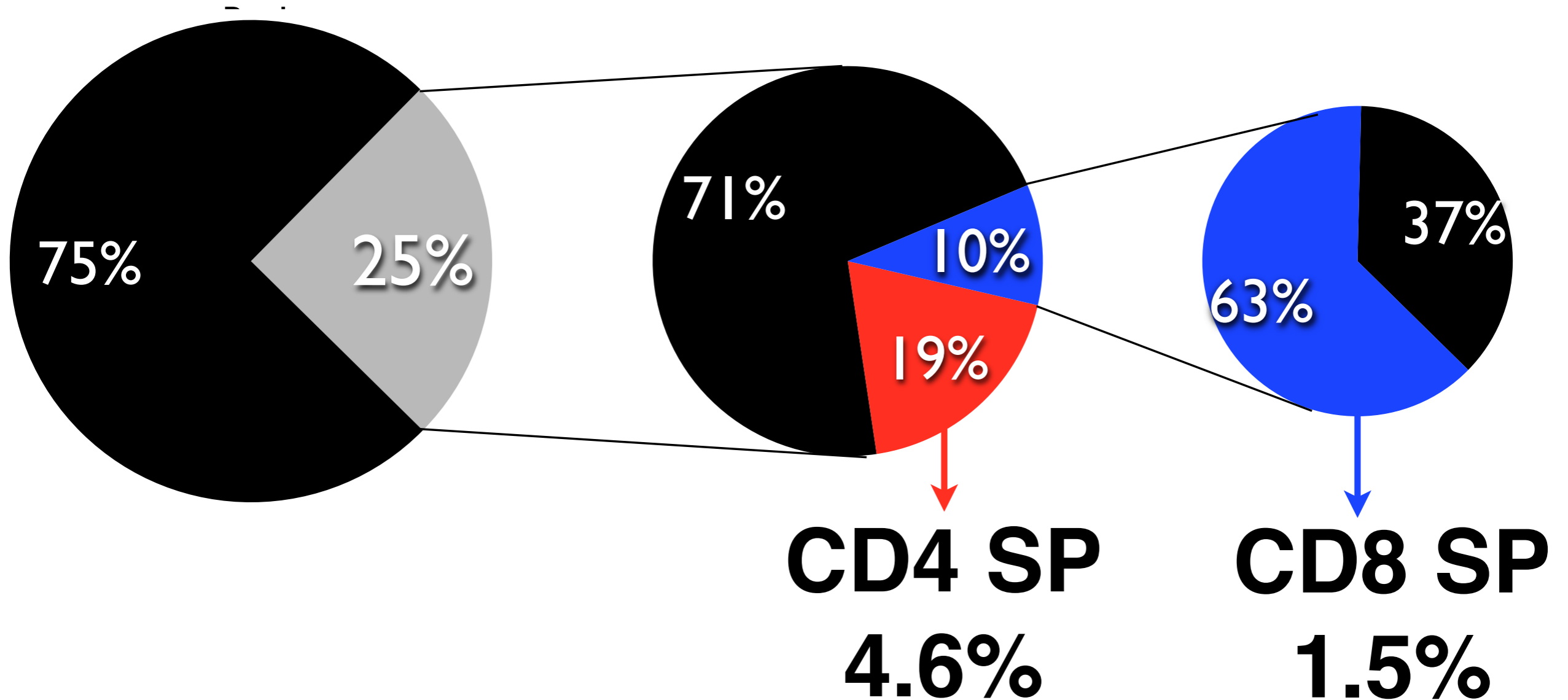
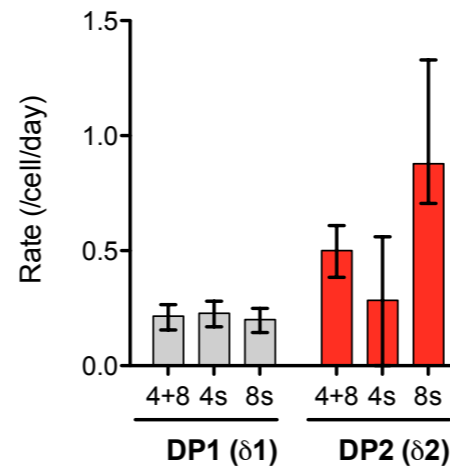
Observed



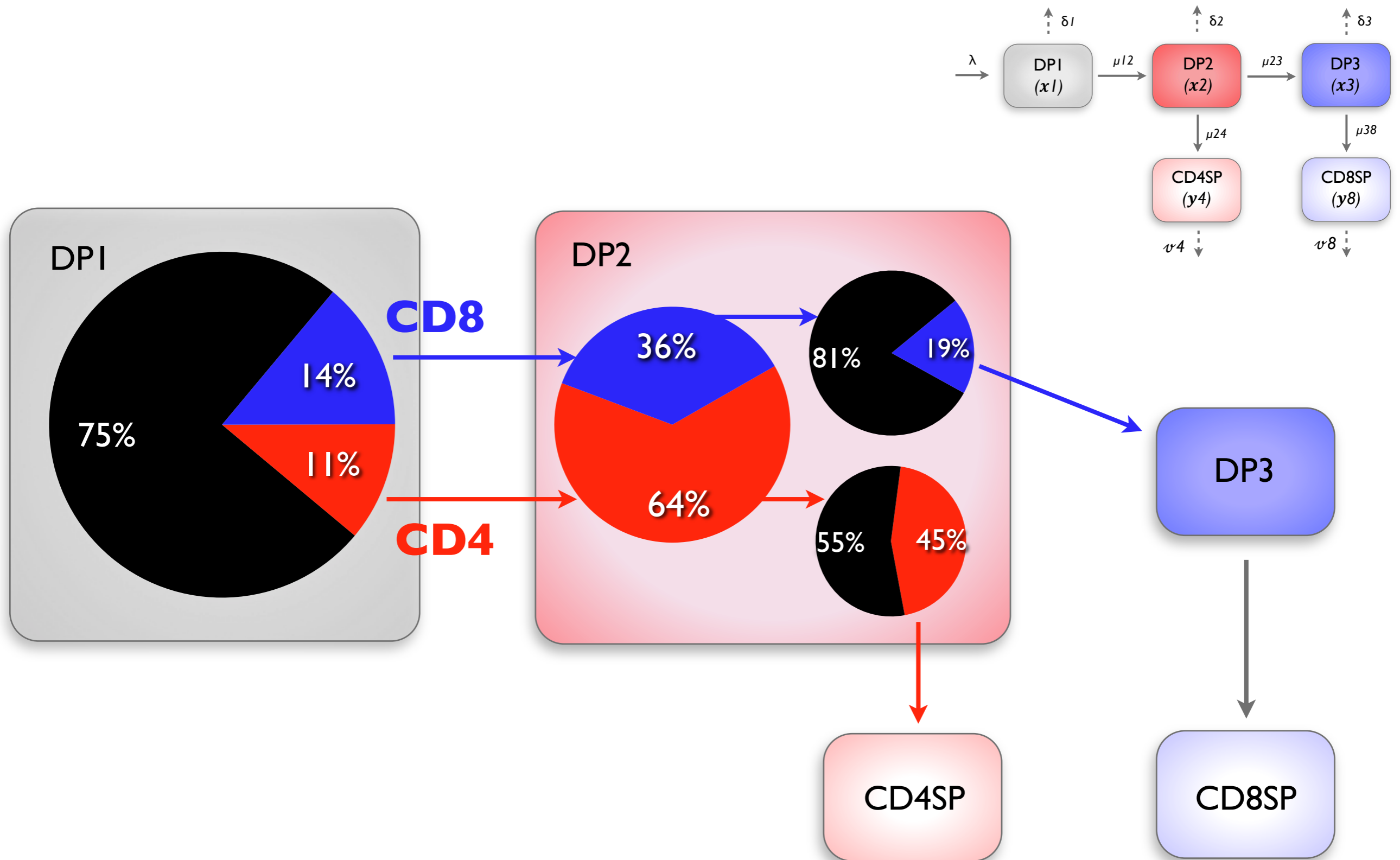
Survival 8s/survival 4s

$1.73/3.14 = \mathbf{0.55}$

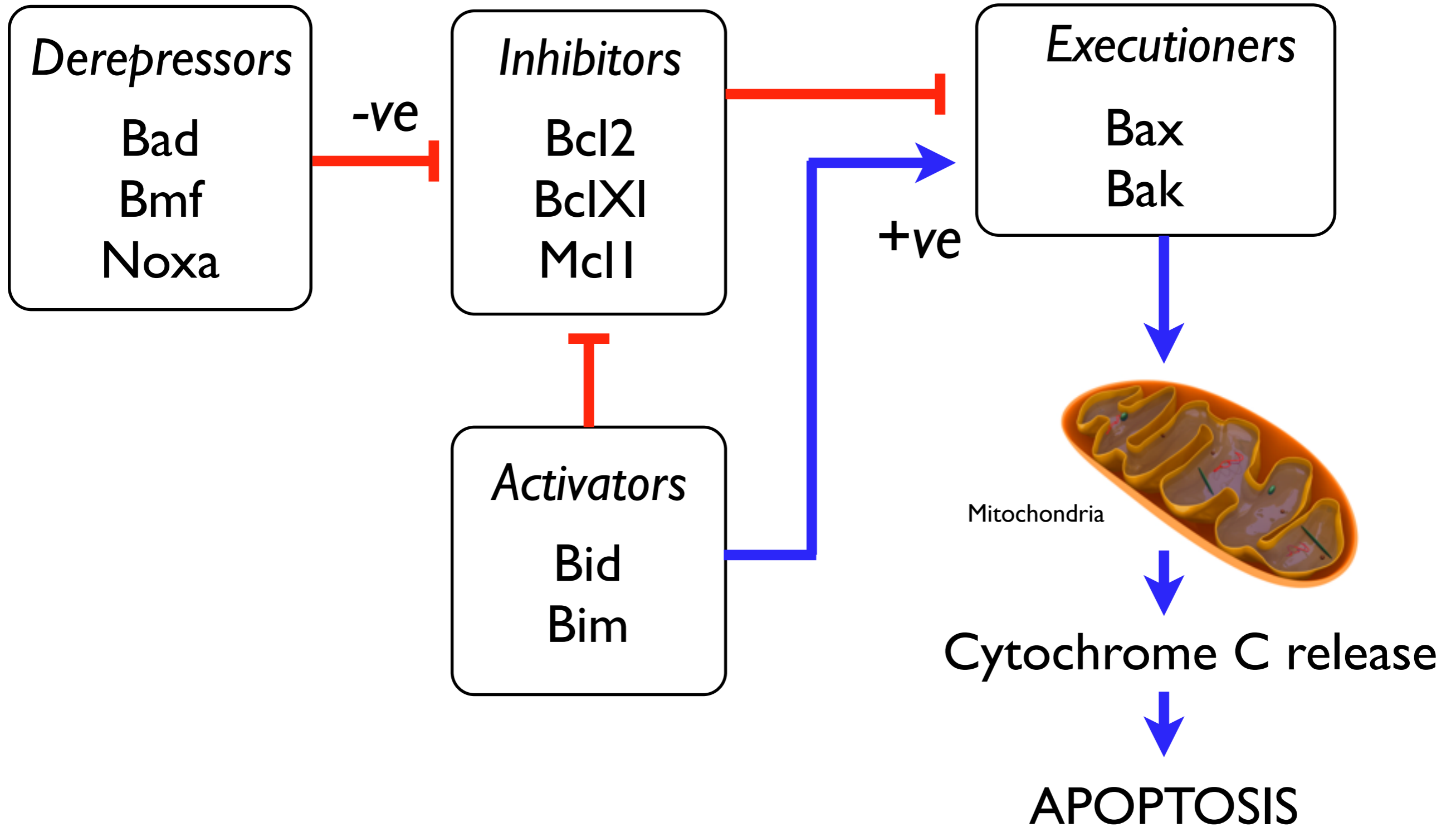
Using lineage specific death rates to quantify lineage efficiencies



Modeling reveals unexpectedly high CD8 lineage biased death in selection

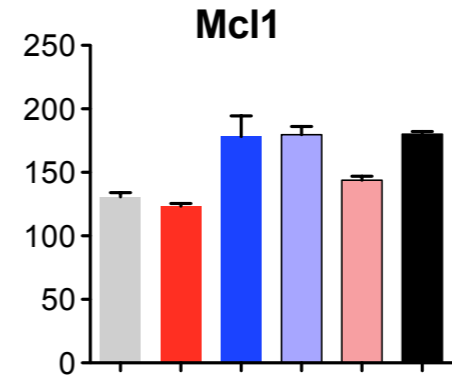
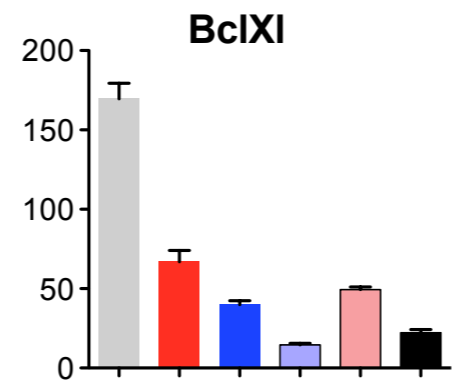
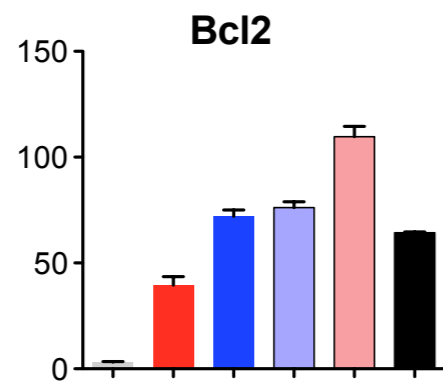


Regulation of thymocyte survival



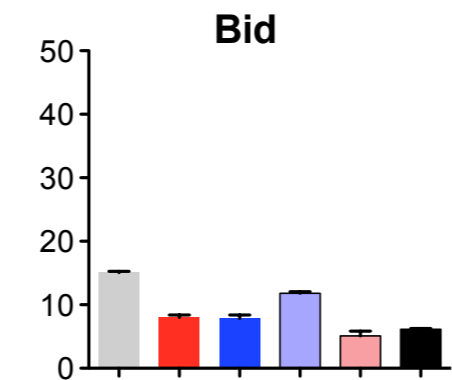
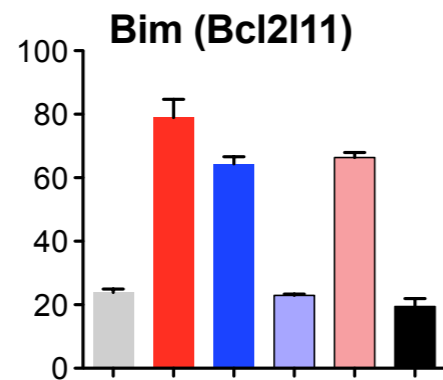
“Paralogous” switch between Bcl-2 and Bcl-XI sensitises DP thymocytes to negative selection

Inhibitors

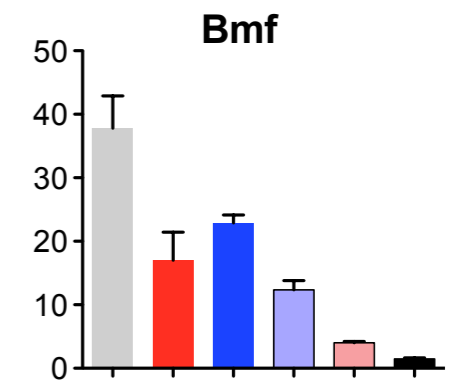
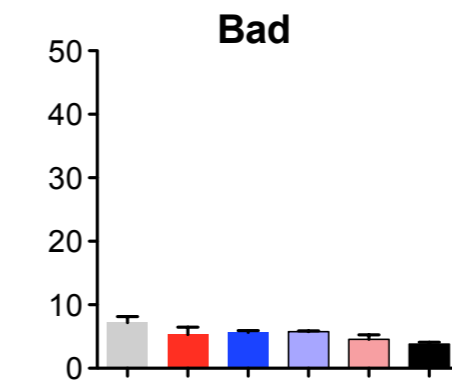


Expression (nRPKM)

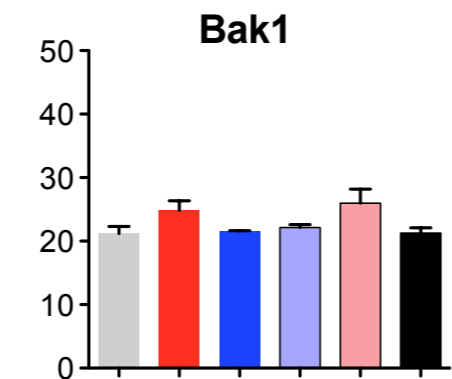
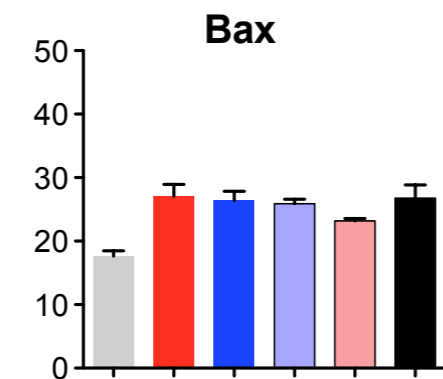
Activators



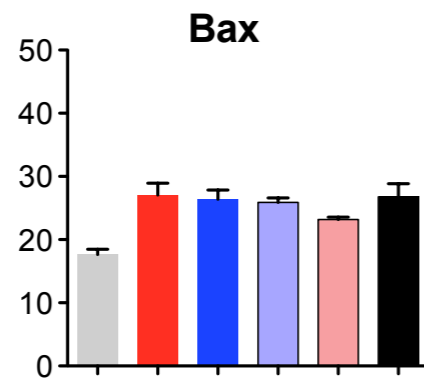
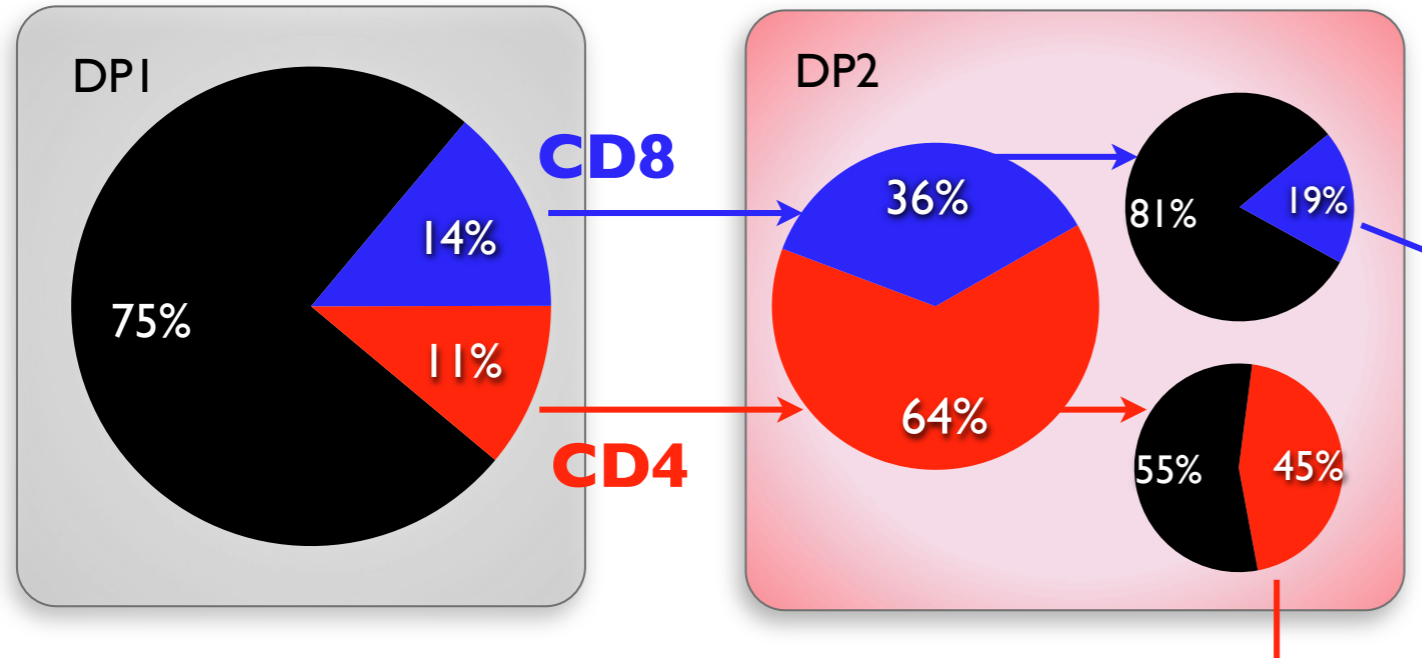
Derepressors



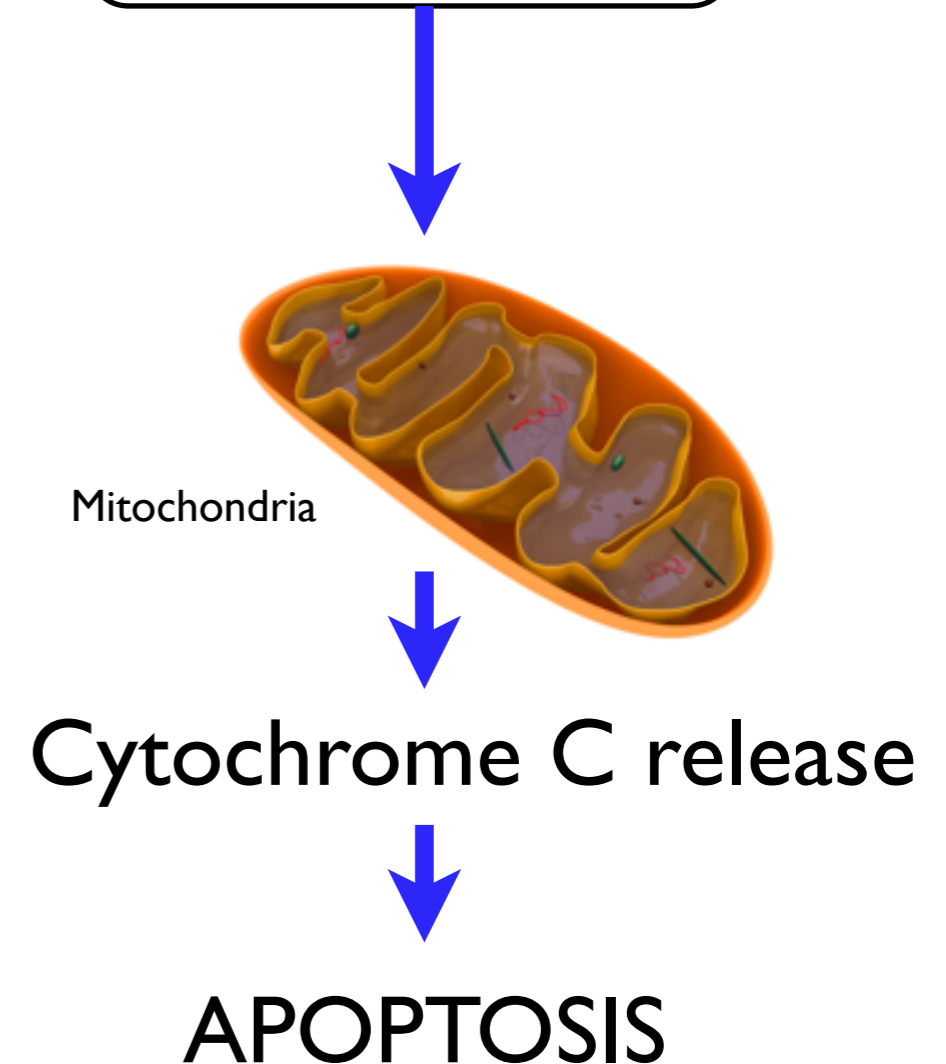
Executioners



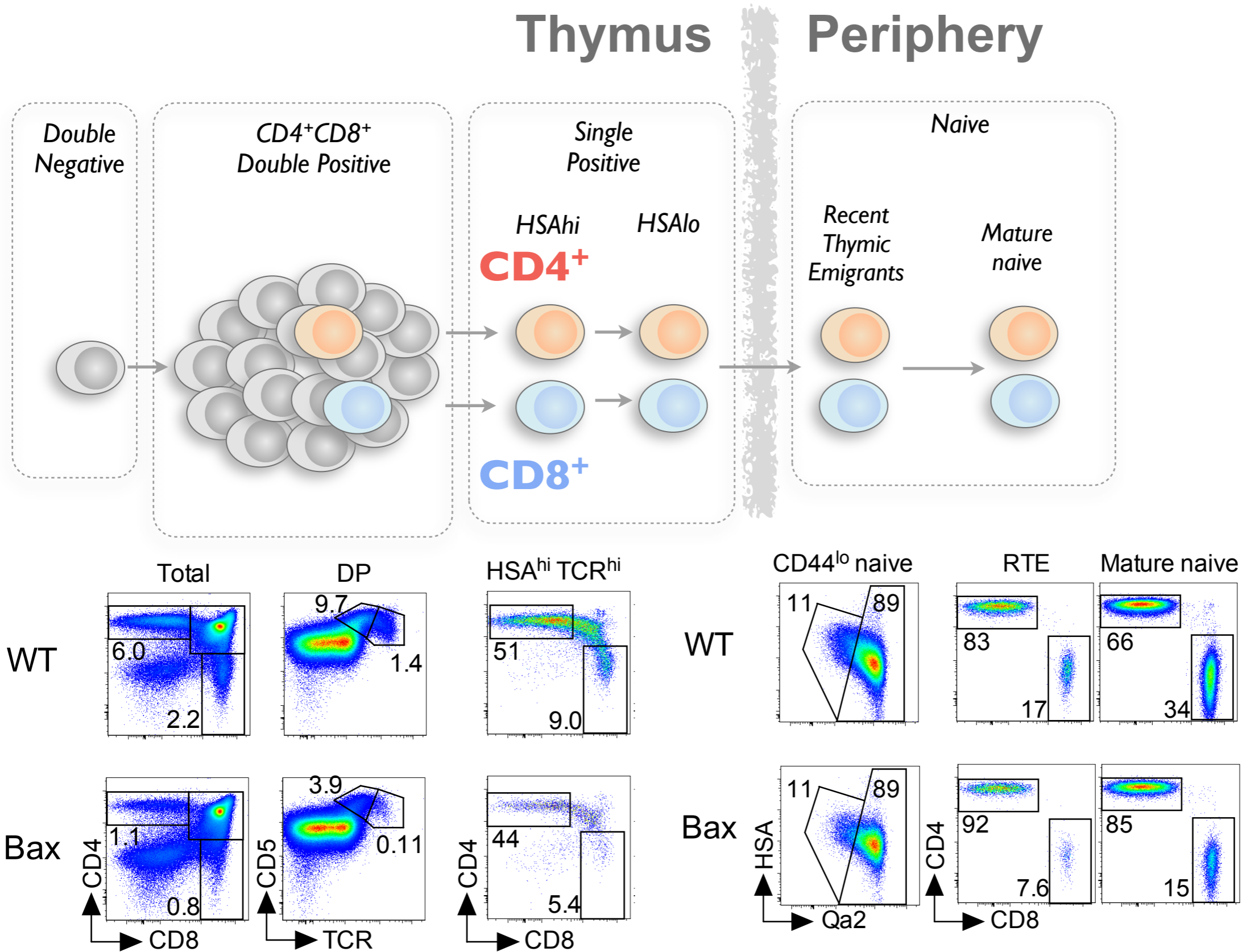
Test the impact of apoptosis - introduce apoptotic stress to thymocytes



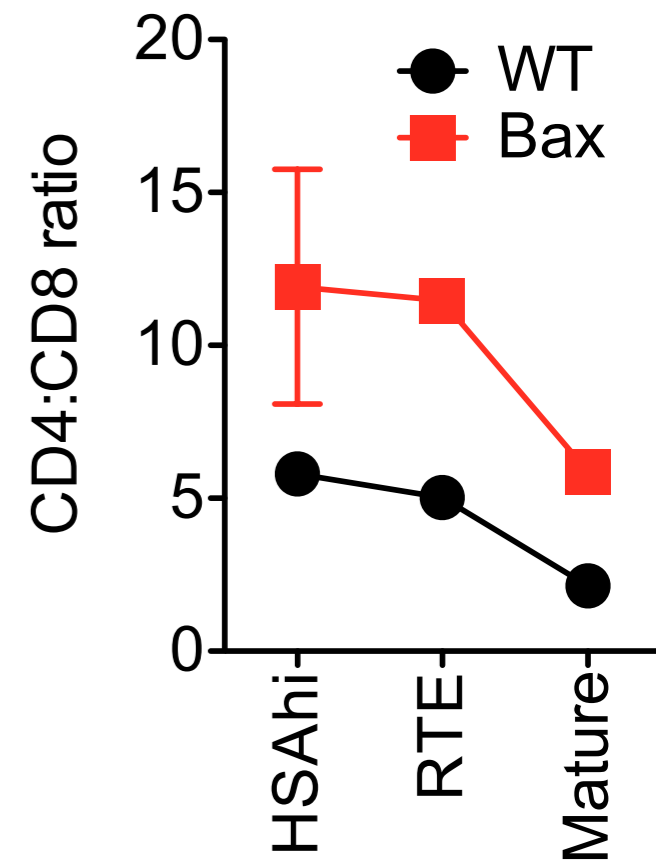
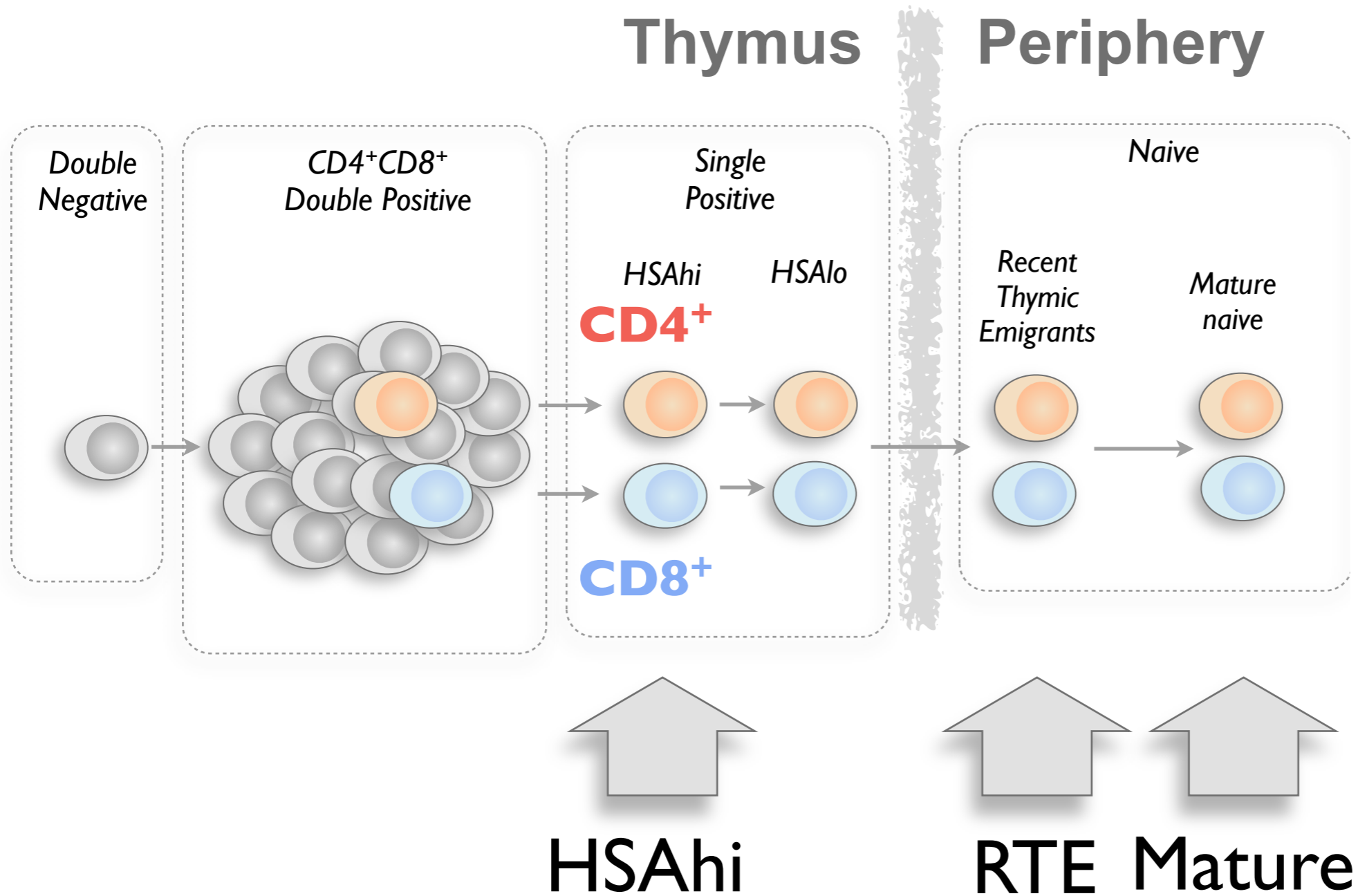
Bax^{huCD2}
Transgenic mice



Applying apoptotic stress preferentially kills CD8 lineage cells



CD4:CD8 ratio only affected by Bax during DP2 stage



Thanks to ...

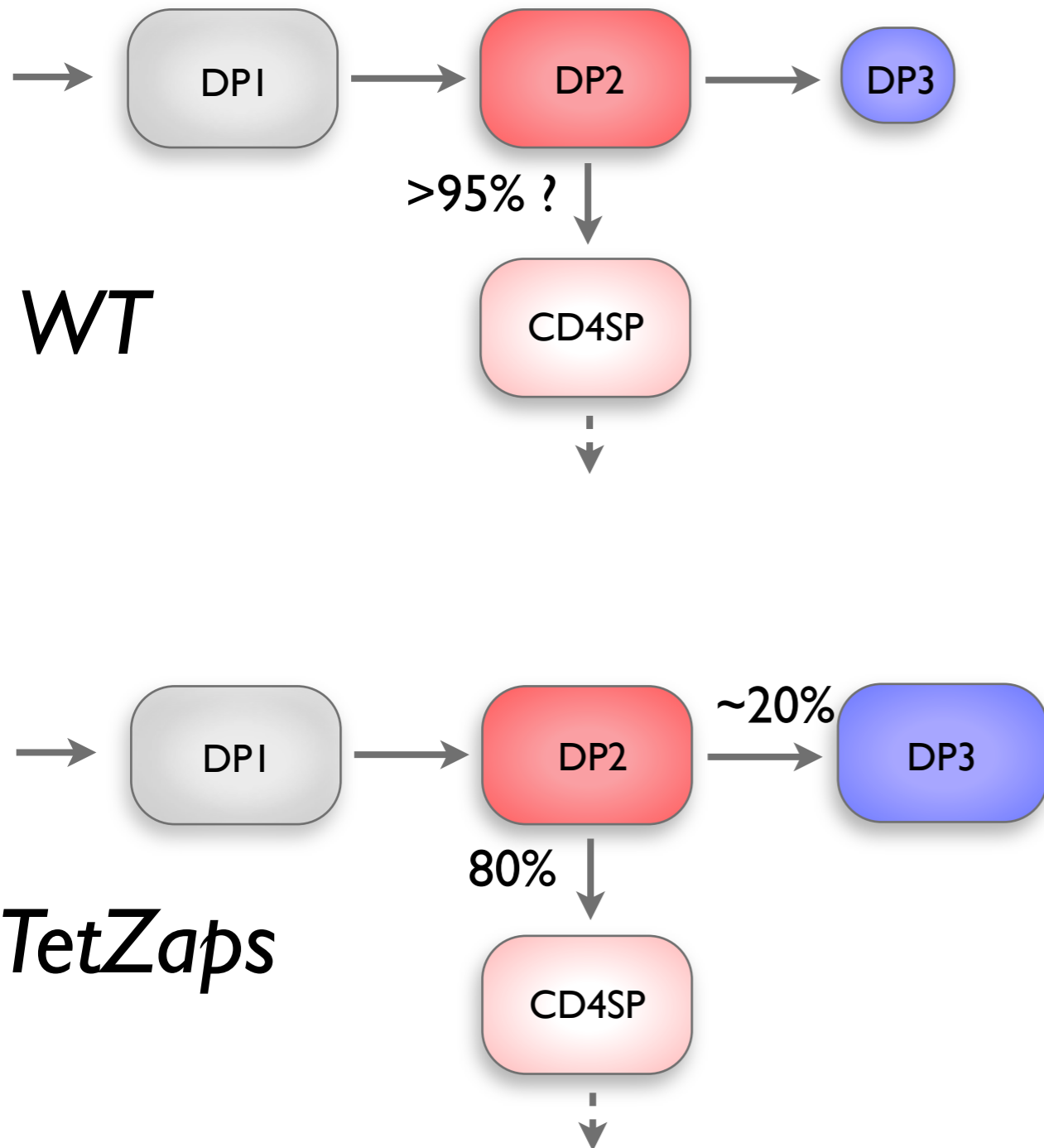
From the Lab
Charles Sinclair
Iren Bains

New York
Andy Yates,
Albert Einstein College of Medicine, NY, US

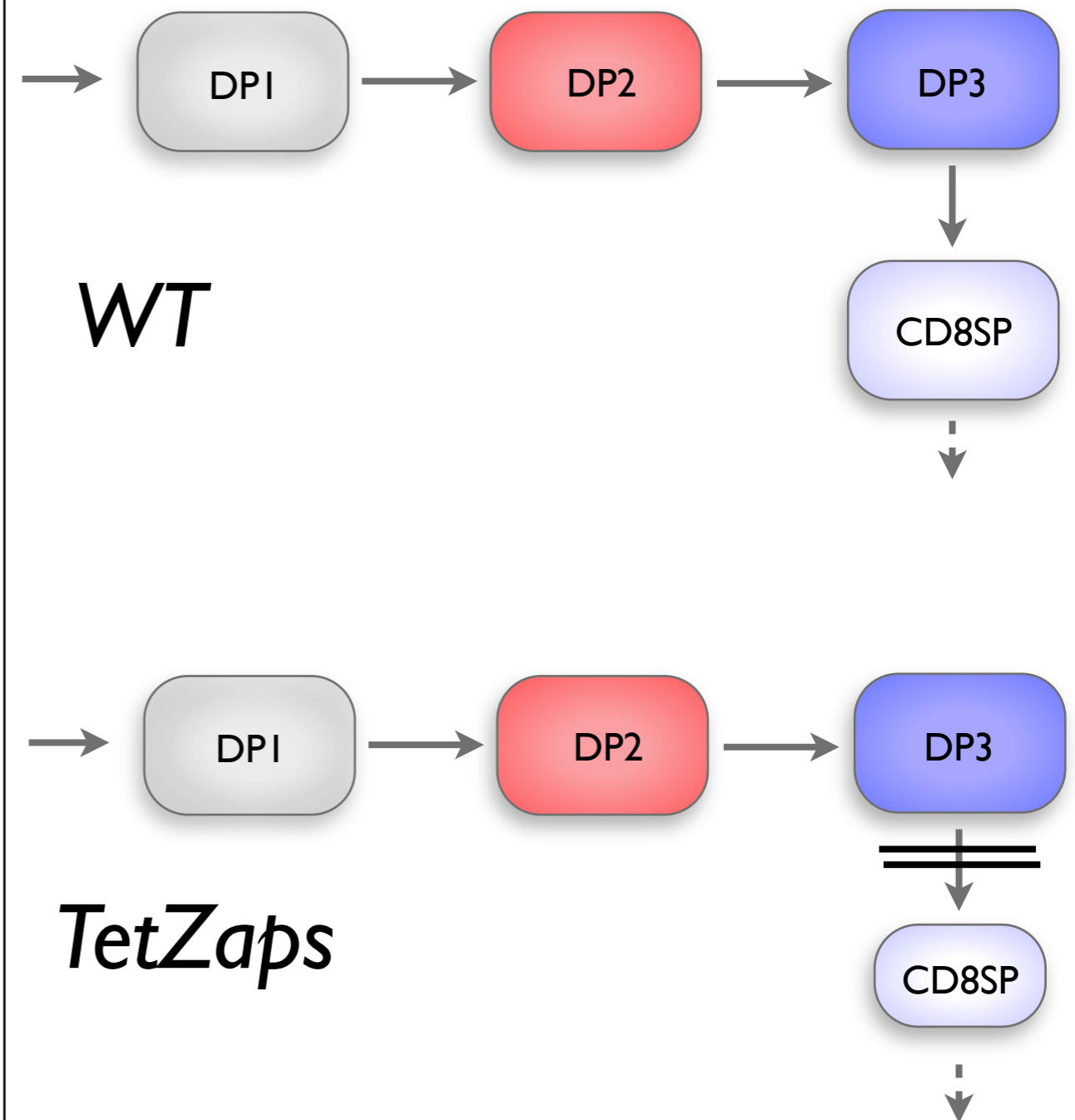


TetZap70 development vs WT

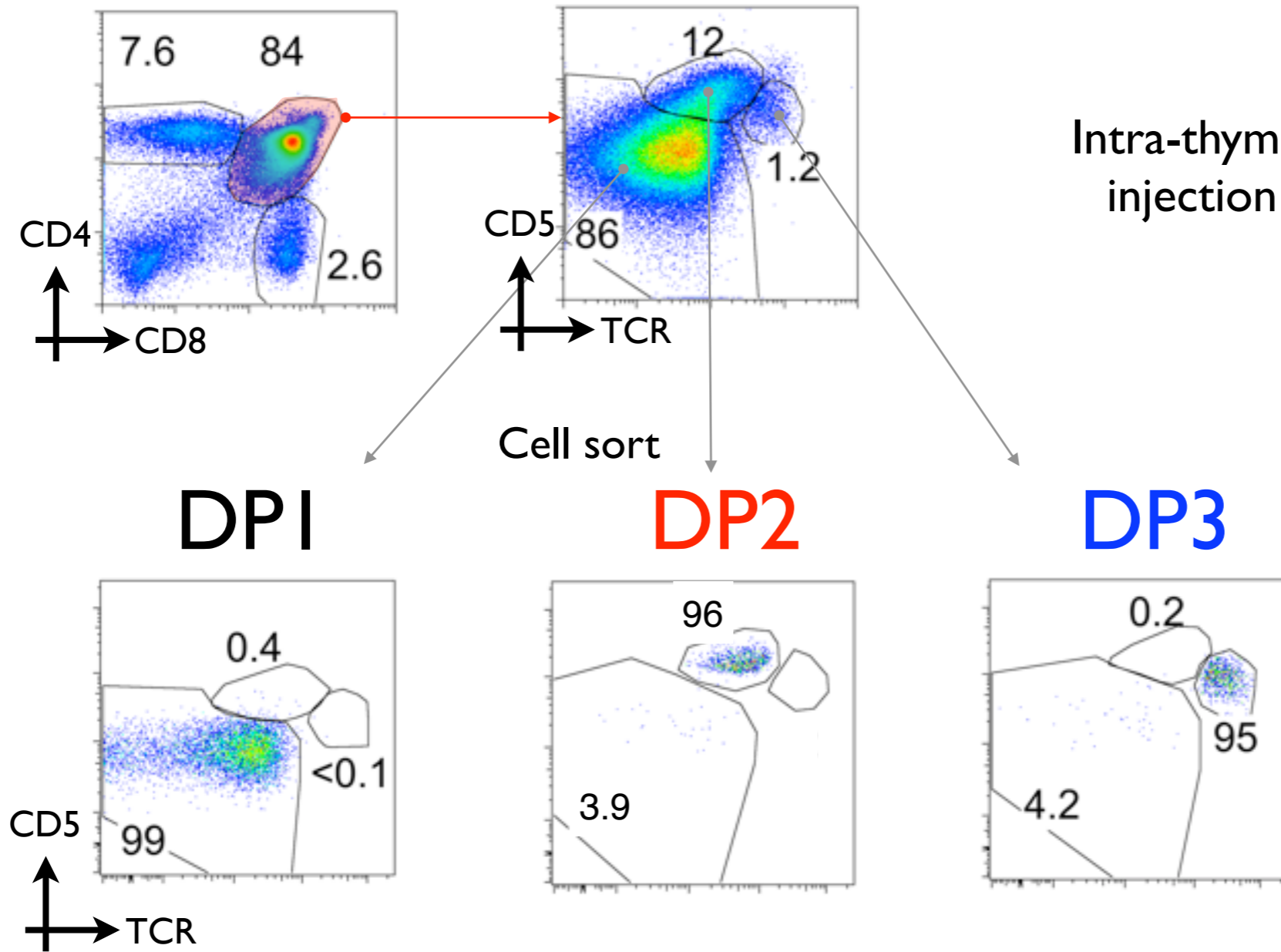
CD4 lineage



CD8 lineage



Establishing precursor-product relationships



Intra-thymic injection



d1-6

Measure relative recovery