Senescence and Cancer

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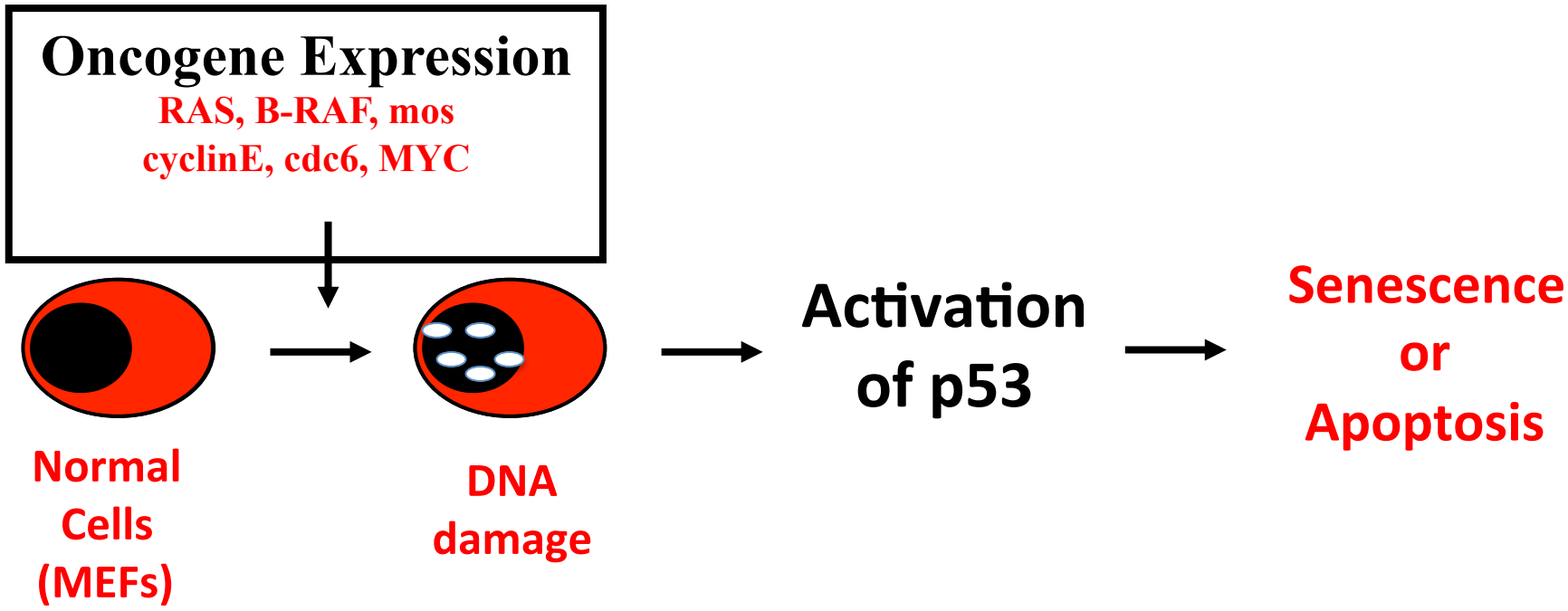
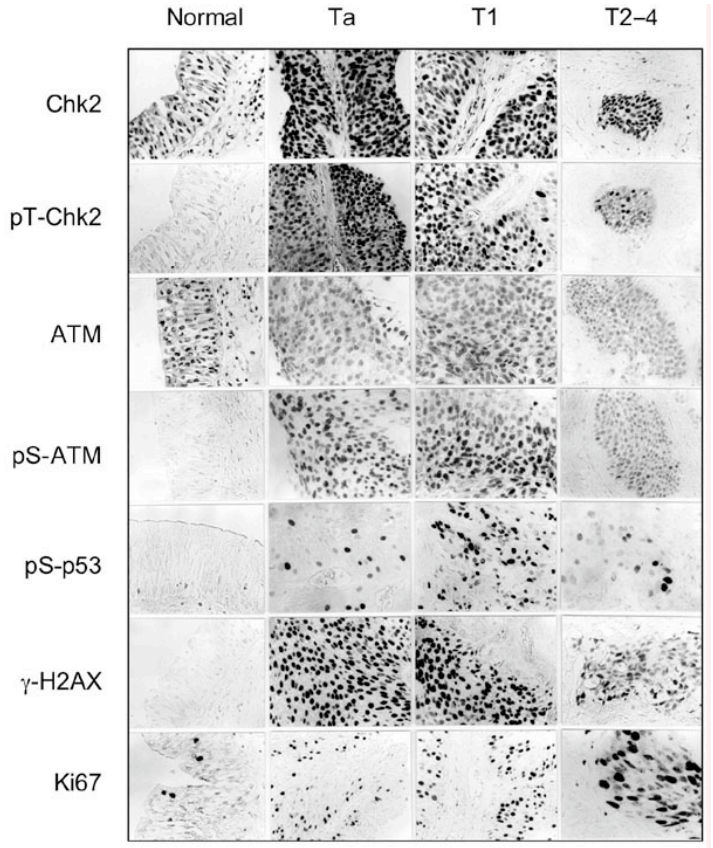
Expression of activated oncogenes in normal cells induces DNA damage and activates a p53-dependent Checkpoint-response leading to Senescence or Apoptosis

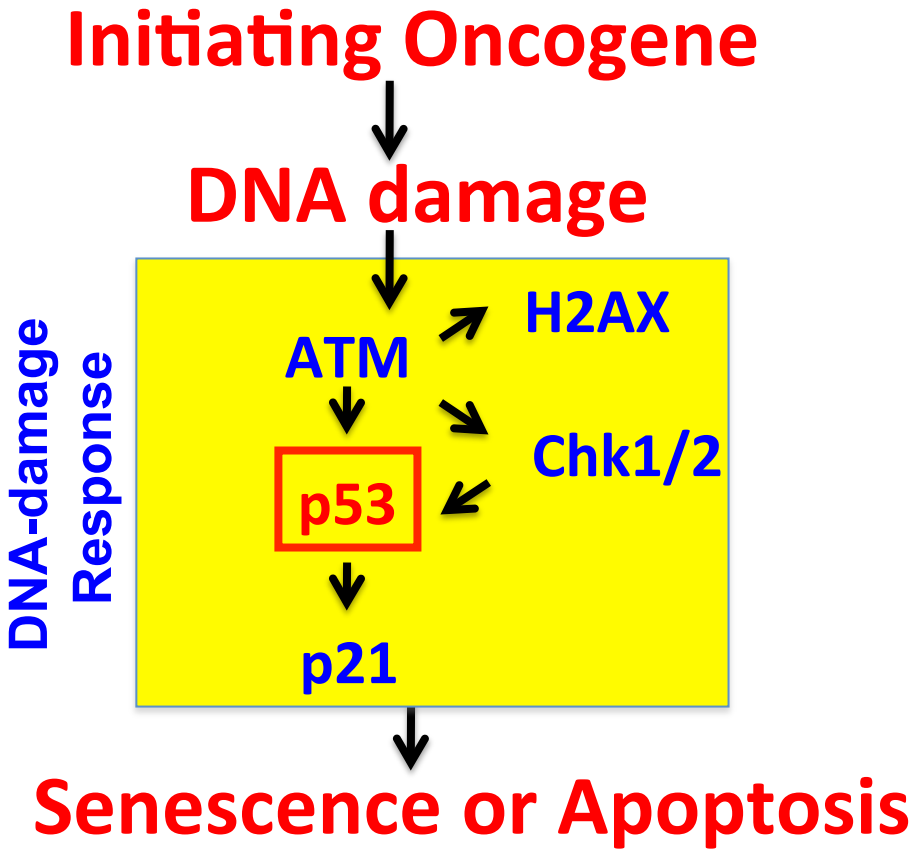


In the absence of p53, oncogene expression induces transformation

In pre-tumoral lesions ***(lung, colon, prostate, bladder; melanomas, lymphomas)*** **:**

Oncogene expression correlates with accumulation of DNA damage and activation of the p53-checkpoint response

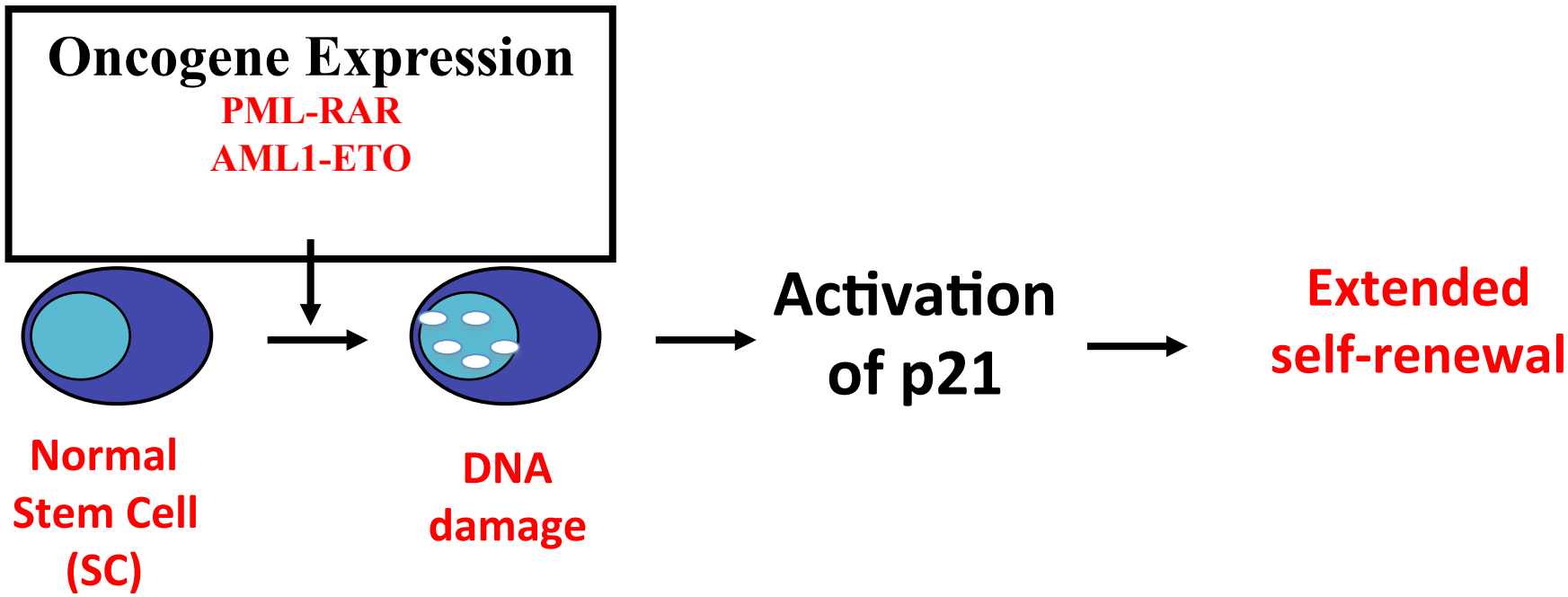




In model systems:

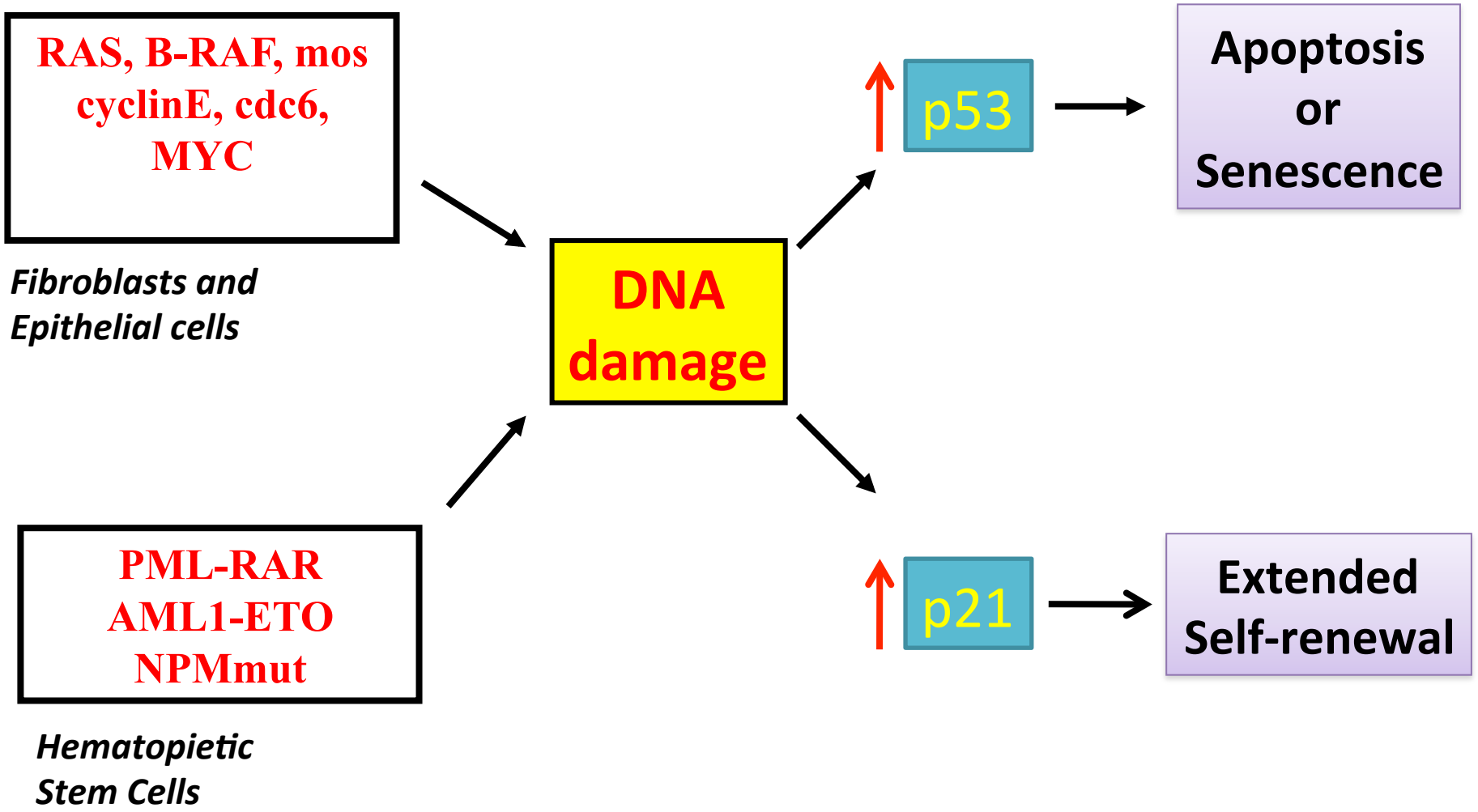
Activation of the p53 checkpoint-response limits tumor progression

Oncogene expression in normal Hematopietic Stem Cells Induces DNA damage and a p21-dependent response that extends their replicative potential

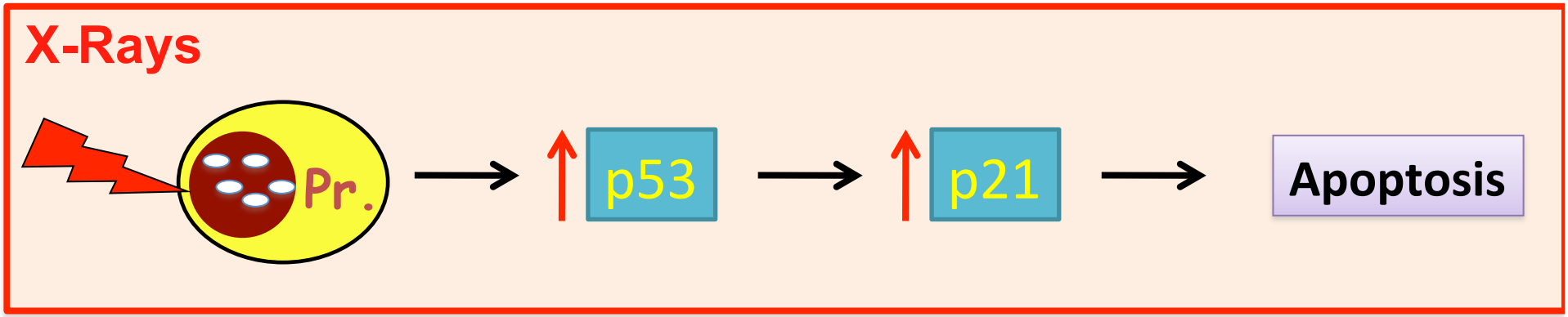


Viale et al., Nature 2010

Why different oncogene-responses in different cells?



Hematopoietic progenitors:
X-Rays induce p53-dependent apoptosis



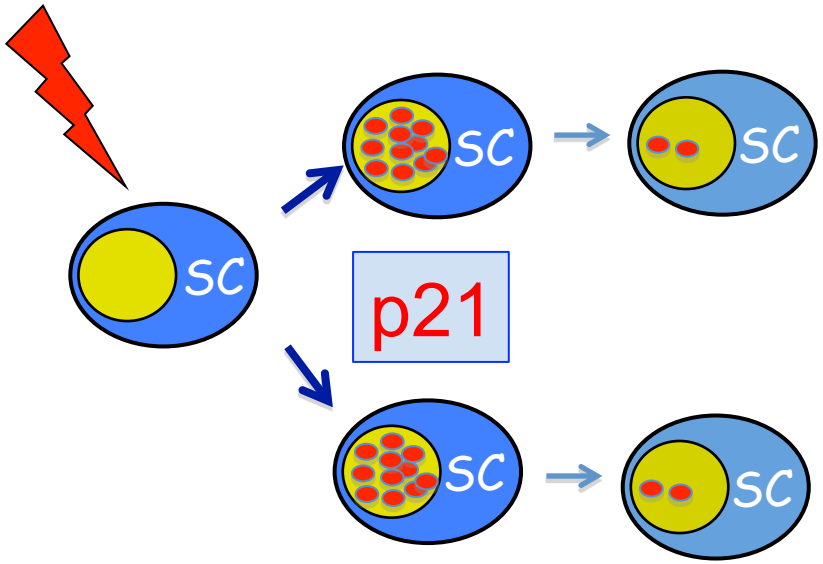
PNAS, Insinga et al. 2013

Normal Hematopoietic Stem Cells

Effects of X-ray treatment:

- Does not induce p53-dep apoptosis/senesc.
- Induces one round of symmetric division
- Activates DNA repair
- Dependent on p21 expression

Transient DNA-damage

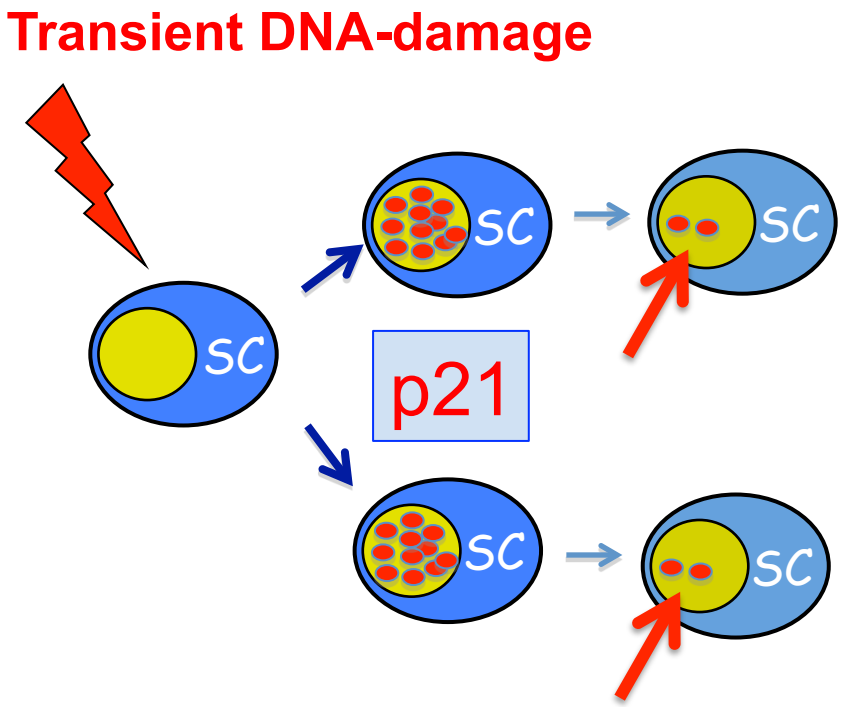


Expansion of a pool of functional Stem Cells

Adaptive response to tissue damage

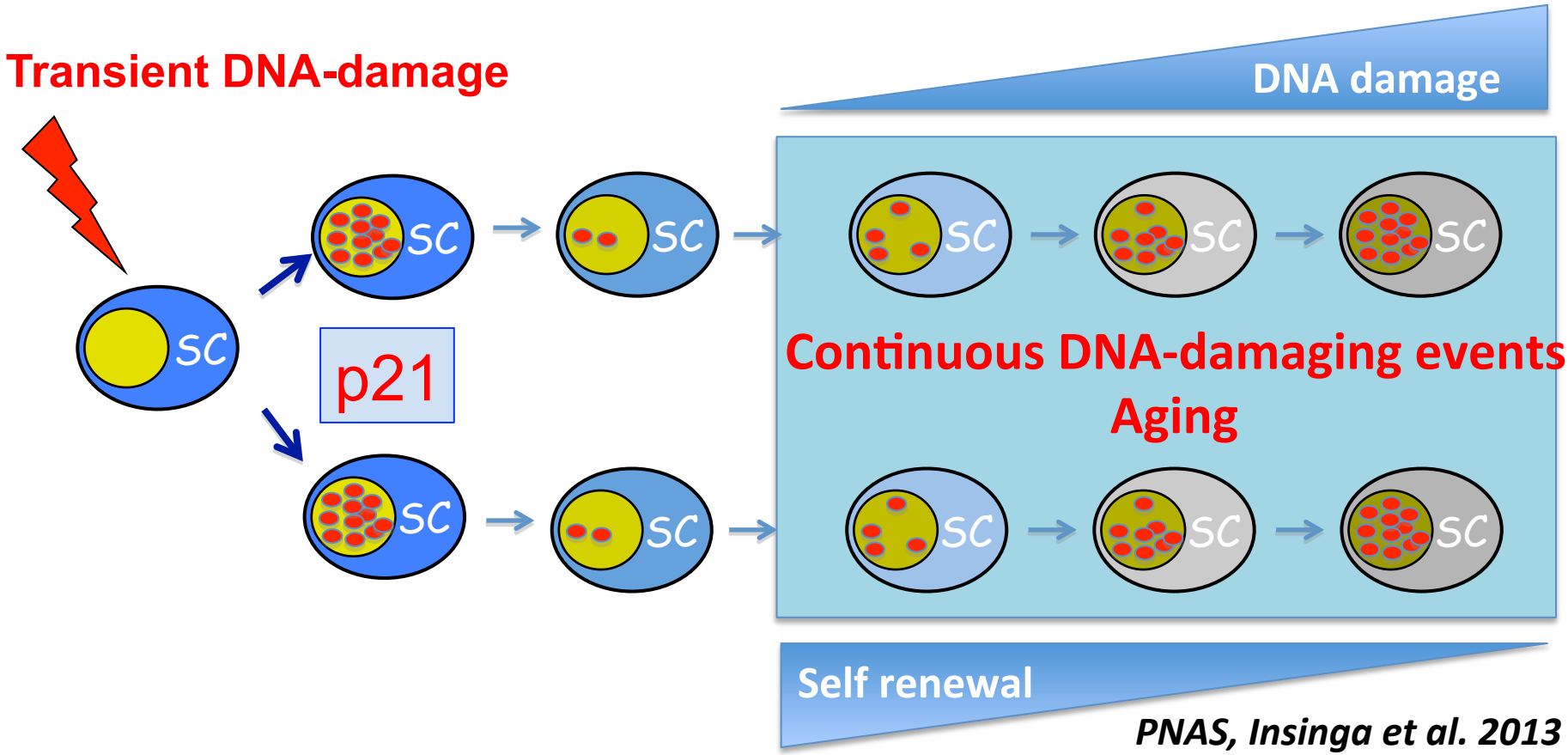
PNAS, Insinga et al. 2013

- **DNA repair is never complete**



PNAS, Insinga et al. 2013

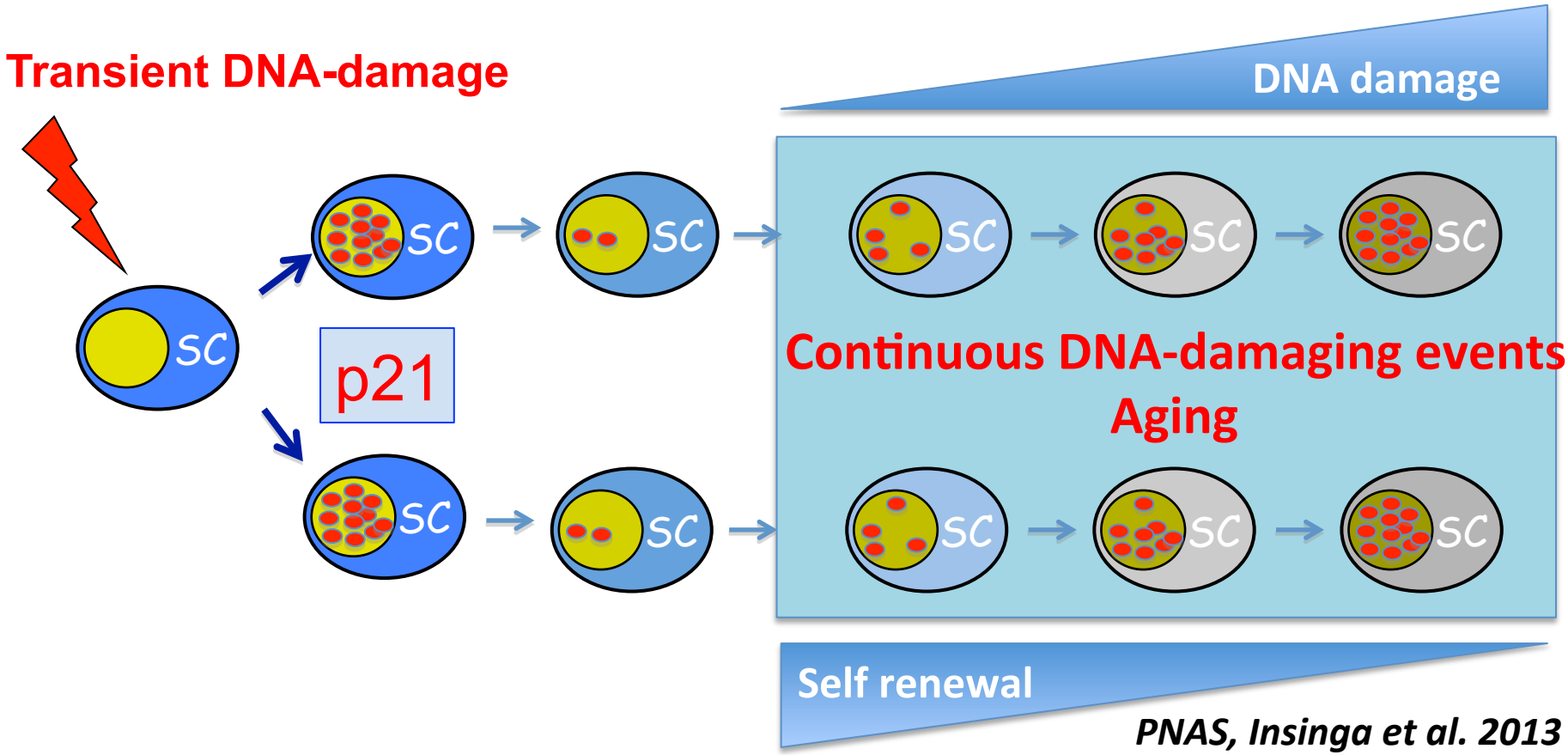
- DNA repair is never complete
- After multiple DNA-damaging events or during aging: progressive accumulation of persistent DNA damage and loss of self-renewal (tumor suppression)



PNAS, Insinga et al. 2013

Normal Hematopoietic Stem Cells

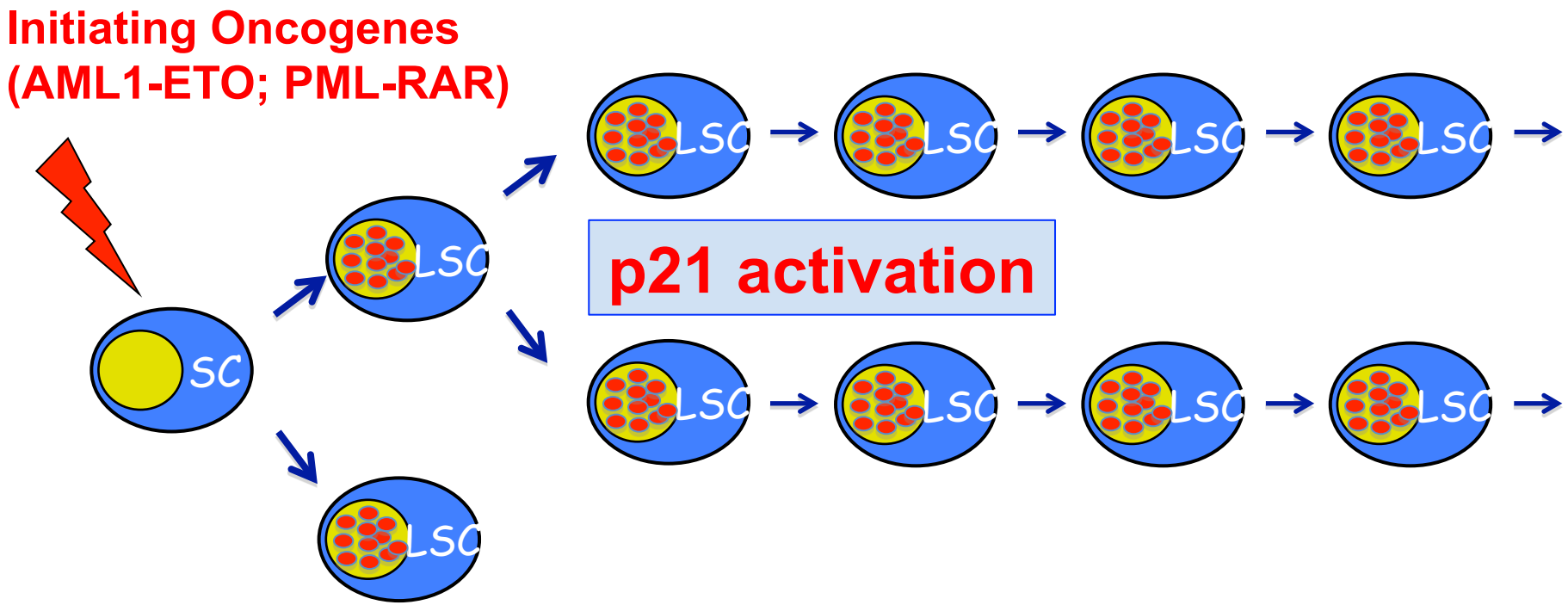
have evolved a p21-dependent response to DNA damage
that leads to their immediate expansion
and limits their long-term survival
(tumor suppression mechanism?)



PNAS, Insinga et al. 2013

Leukemia SCs: Effects of oncogene expression

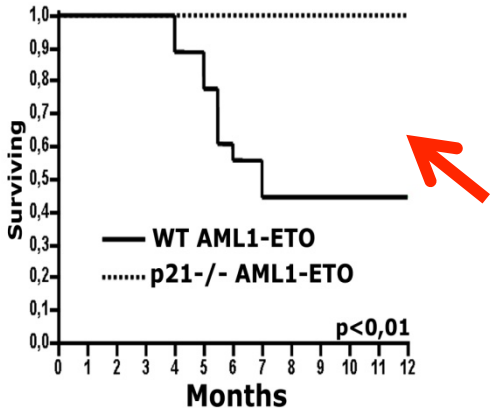
- DNA-damage
- p21 constitutive activation
- Active DNA repair
- Extended self-renewal



Nature, Viale et al. 2010

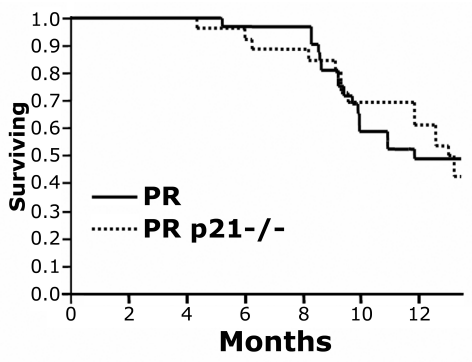
In the absence of p21, leukemogenesis does not proceed

AML1-ETO in $p21^{-/-}$ mice

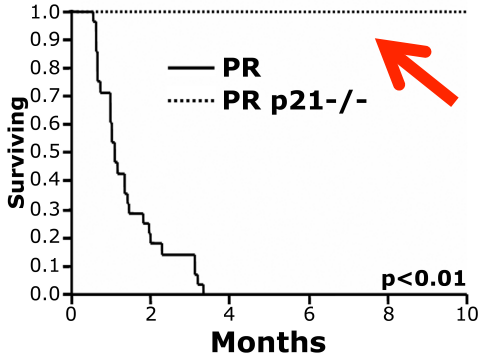


No leukemia

PML-RAR in $p21^{-/-}$ mice

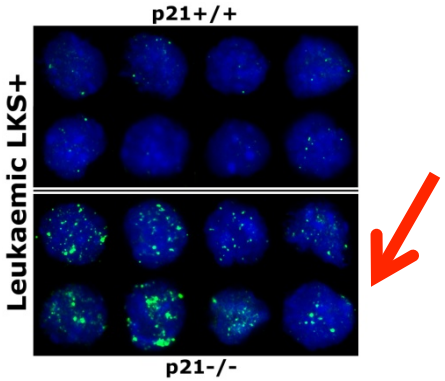


Leukemia → **Not transplantable**

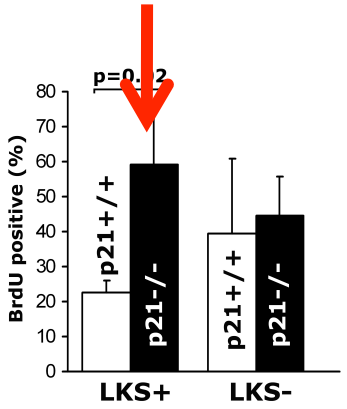


Nature, Viale et al. 2010

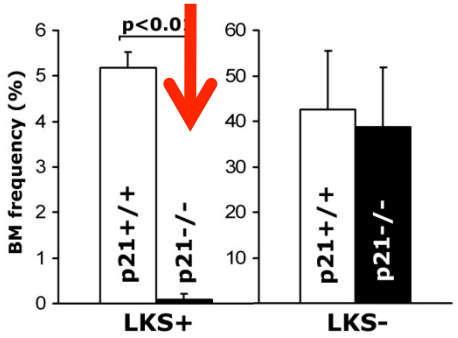
In the p21$^{-/-}$ APLs, LSCs accumulate massive DNA-damage, hyperproliferate and are reduced in numbers



Accumulate massive DNA damage



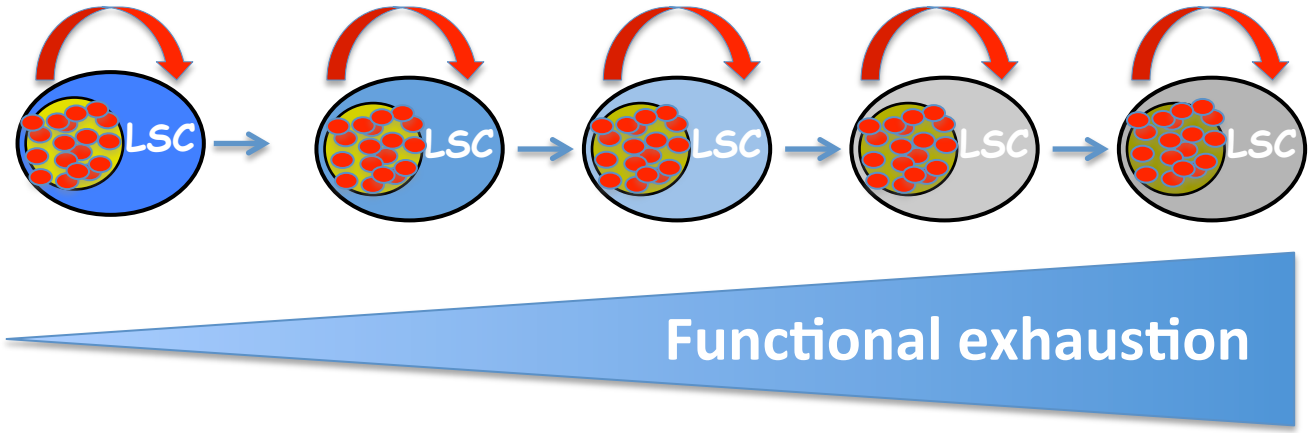
Hyper-proliferate



Are markedly reduced in numbers

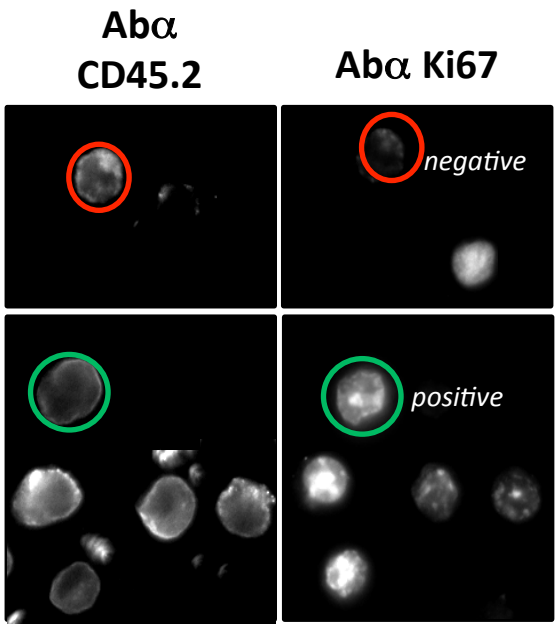
Nature, Viale et al. 2010

In the absence of p21, LSCs hyperproliferate and progressively lose self-renewal

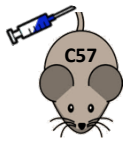


Nature, Viale et al. 2010

In the healthy mice transplanted with p21$^{-/-}$ APLs, rare blasts are found in the PB, BM and spleen, which hyper-proliferate and do not show increased apoptosis



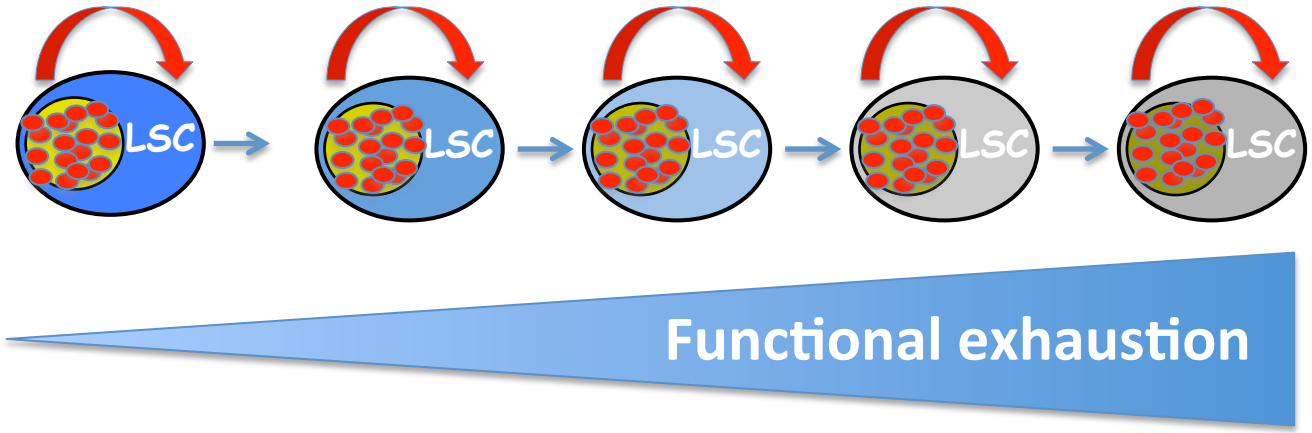
	% 5.2+ Ki67 G1	% 5.2+ Ki67 G2/M	% 5.2+ Casp3+
p21$^{-/-}$ PR ki	64.5	27.3	2.7
PR ki	18.3	10.8	1.6



No leukemia after transplantation (0/5)

unpublished

In the absence of p21, LSCs hyperproliferate and progressively lose self-renewal



- Why the $p21^{-/-}$ LSCs do not expand in vivo?
- Do they senesce?
- How are cleared in vivo?

Are cell-extrinsic mechanisms involved?

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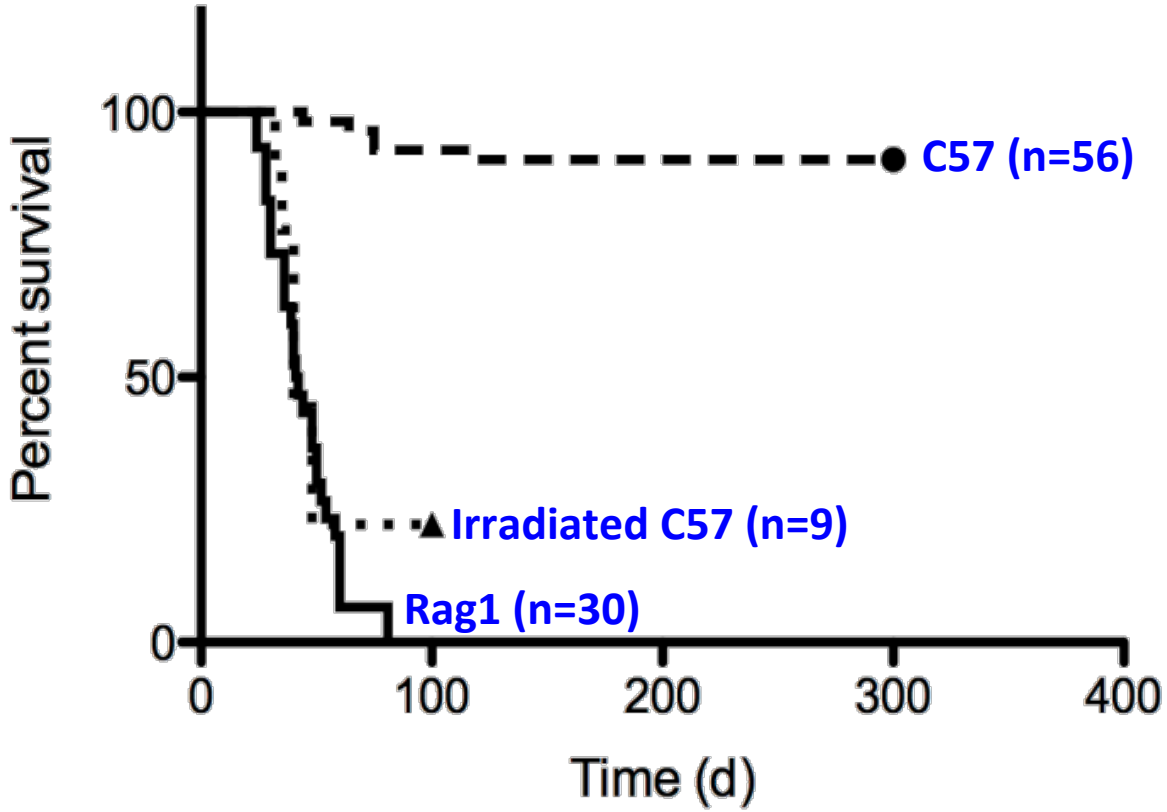
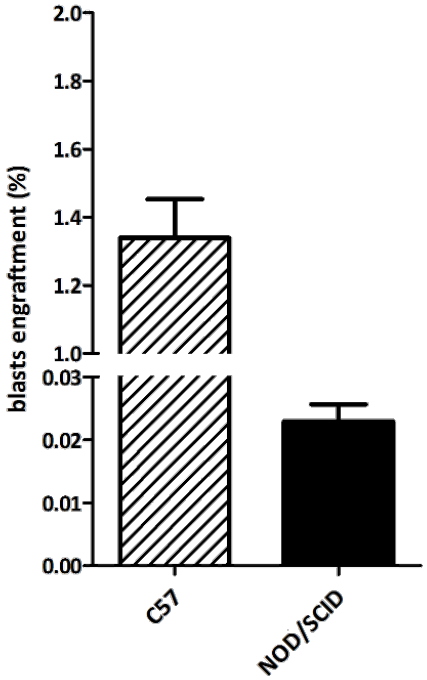
Barbara Gallo

Manuscript in preparation

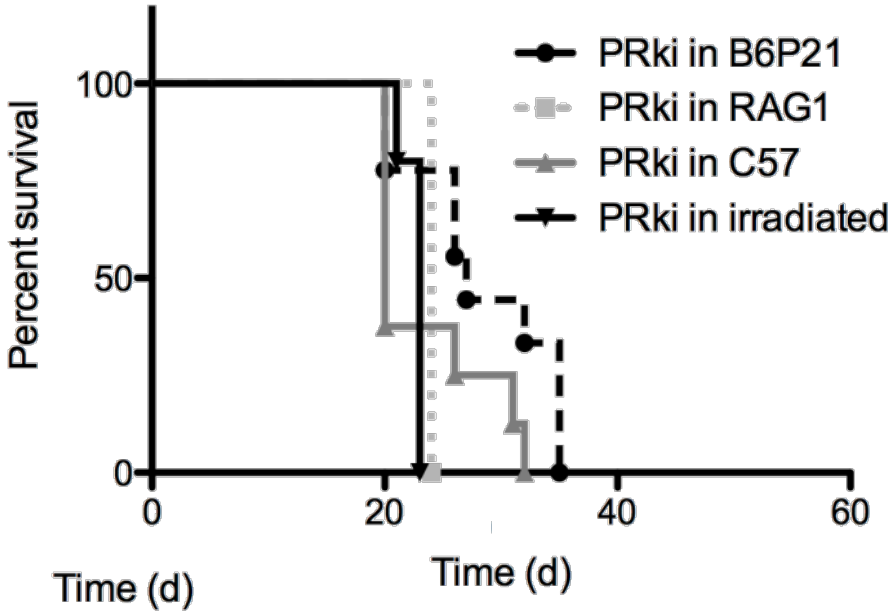
$p21^{-/-}$ APLs "re-acquire" the ability to initiate leukemogenesis when transplanted into immunodeficient mice or into syngenic mice after γ-irradiation



Transplantation of p21$^{-/-}$ APLs in immunodeficient mice is NOT due to facilitated homing or different growth potential in immunodeficient *vs* syngenic mice



Homing of p21$^{-/-}$ APLs in syngenic and immunodeficient mice



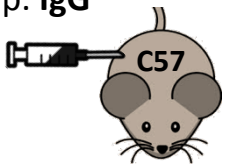
Growth of APL in RAG1, C57, irradiated (6Gy) C57 and C57 p21-/-

p21-/- APL growth depends on the immunological status of the recipient

Macrophages and Monocytes of recipient mice are not involved in the immune-mediated clearance of p21-/- APLs

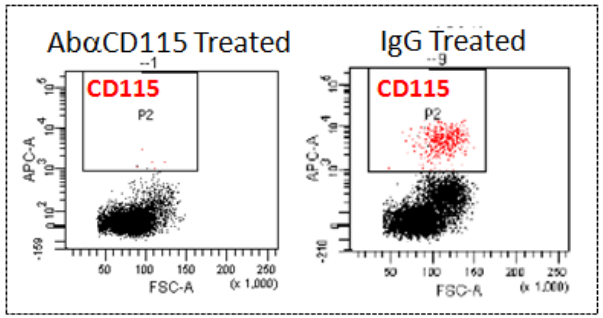
- **Neutralizing antibody against CD115**
- **Rat IgG as a control**

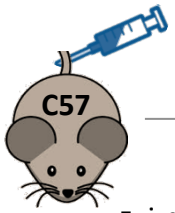
i.p. **IgG**



YES macrophages/monocytes

5 days

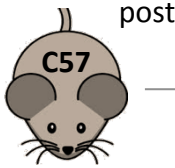




p21$^{-/-}$ APL

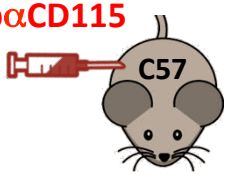
0/3 Leukemia

- i.p IgG every 3-4 weeks
- Experiment stopped 90 days post leukemia transplantation



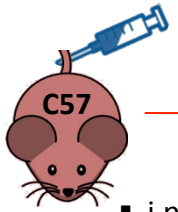
0/3 Leukemia

i.p.
AbαCD115



NO macrophages/monocytes

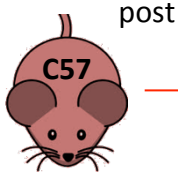
5 days



p21$^{-/-}$ APL

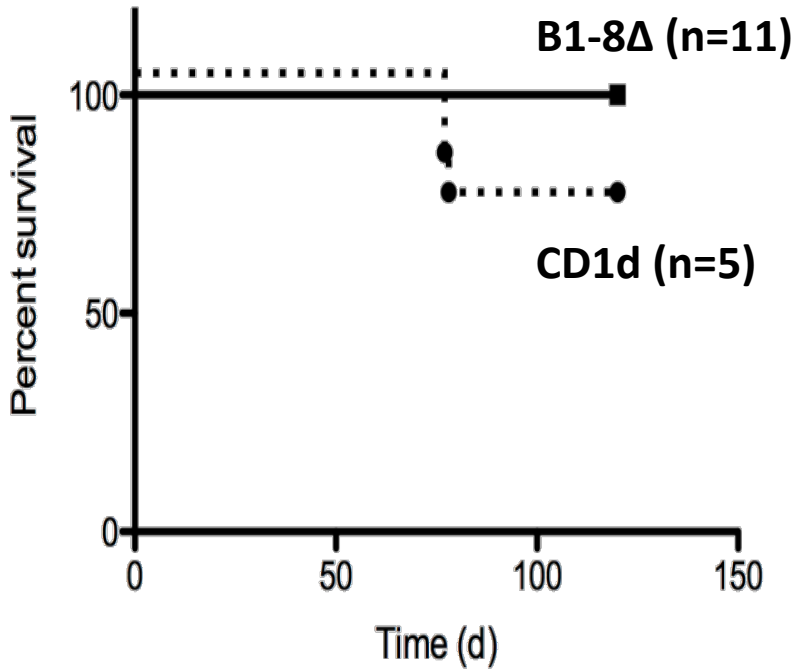
0/5 Leukemia

- i.p AbαCD115 every 3-4 weeks
- Experiment stopped 90 days post leukemia transplantation



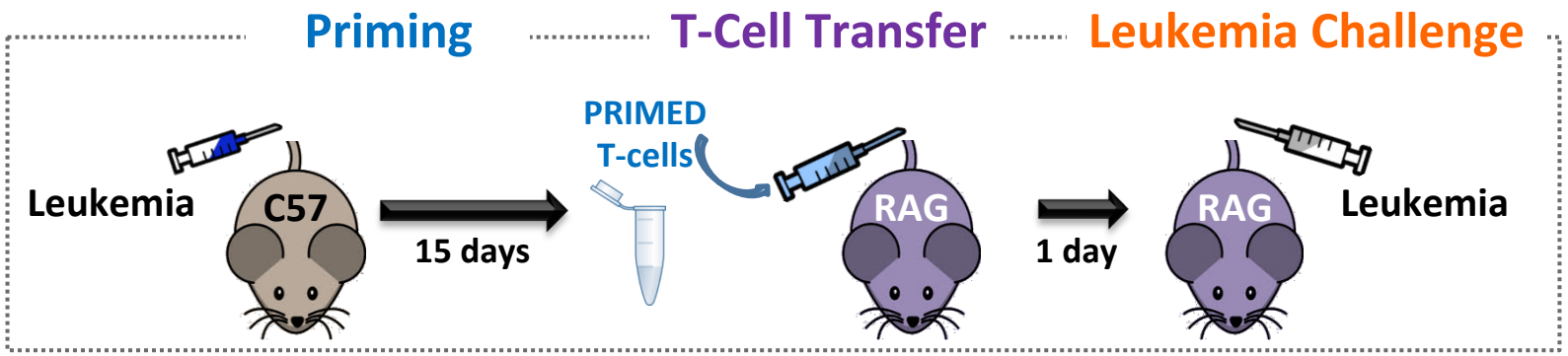
0/3 Leukemia

B and T-NK cells of recipient mice are not involved in the immune-mediated clearance of $p21^{-/-}$ APLs



B1-8Δ (B-deficient) and CD1d (T-NK deficient) recipients injected with $p21^{-/-}$ APLs

Are T-cells Involved in the clearance of $p21^{-/-}$ APLs *in vivo*?

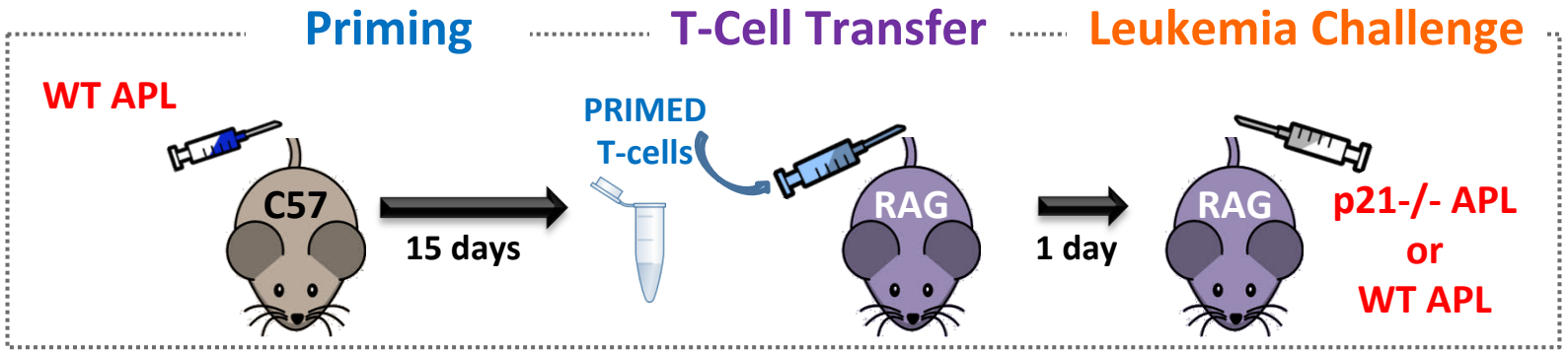


- **Priming:** Immunocompetent C57 mice were exposed to leukemic blasts for 15 days

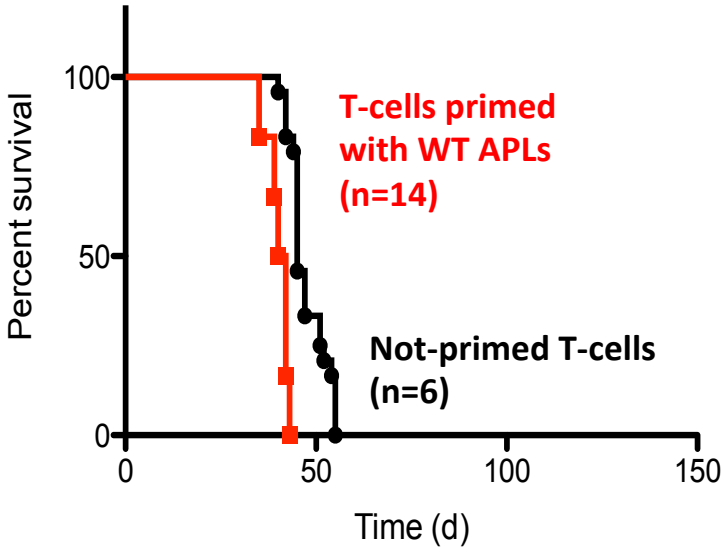
- **T-Cell Transfer:** T-cells were purified from spleens of primed mice and transferred into immunodeficient mice

- **Challenge:** T-cell transferred immunodeficient mice were injected with leukemia cells

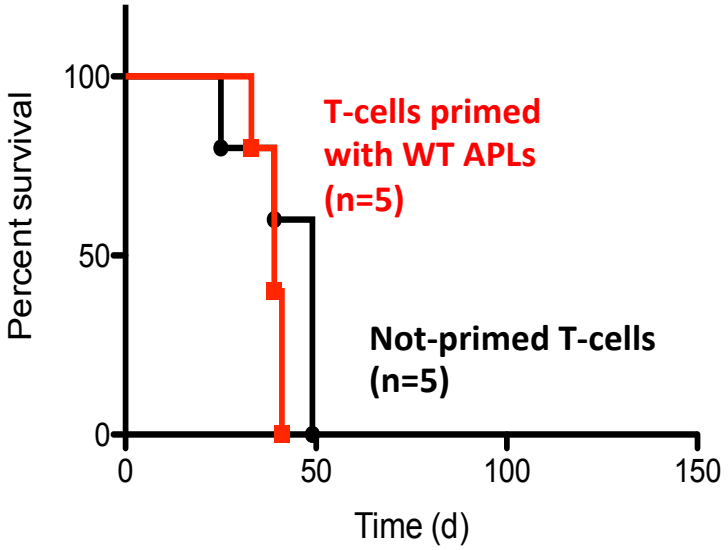
T-cells primed with “WT APL” do not protect against p21$^{-/-}$ or “WT APLs”



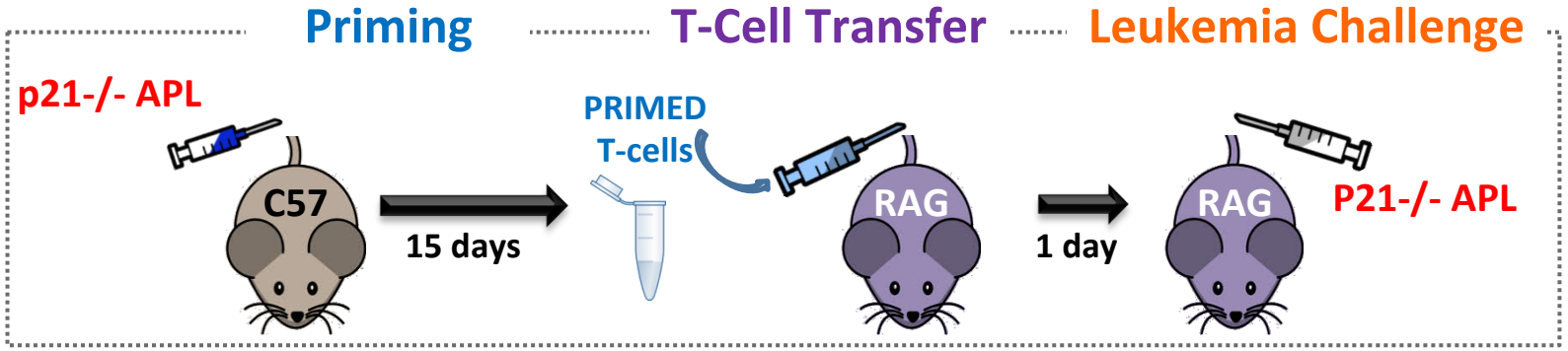
Challenge with p21-/- APLs

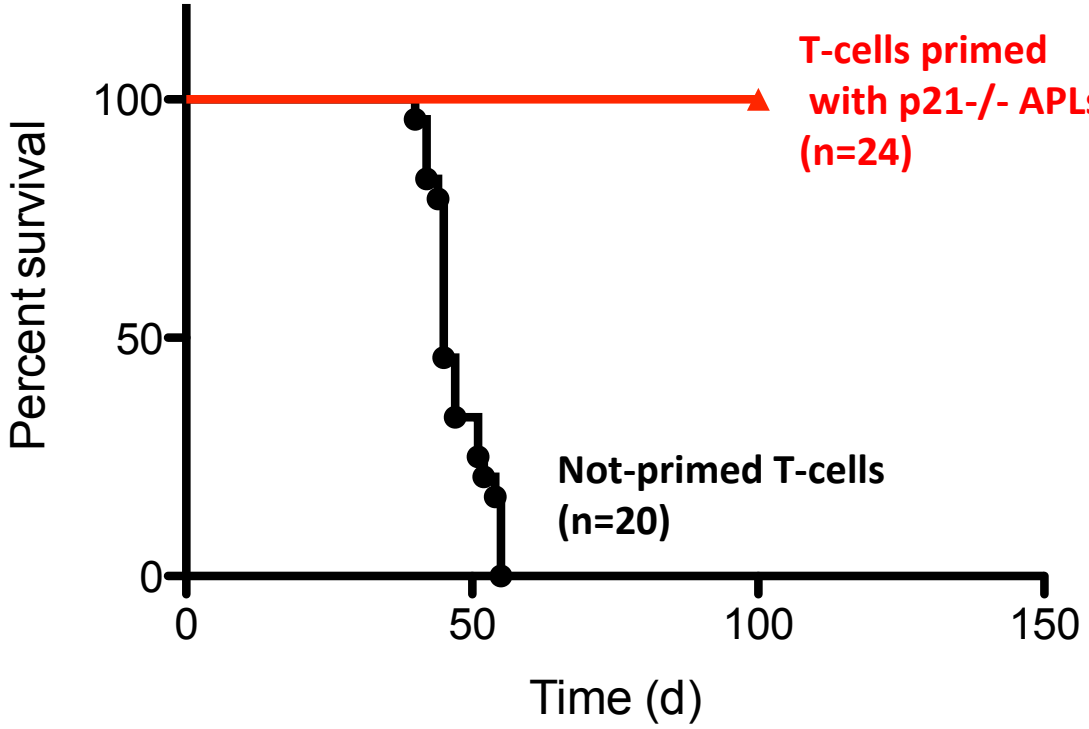


Challenge with WT APLs

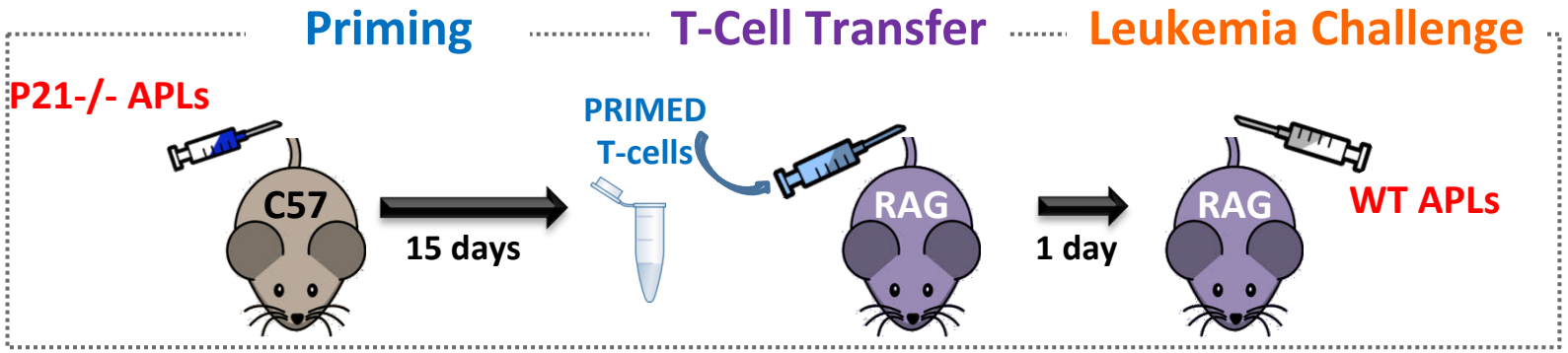


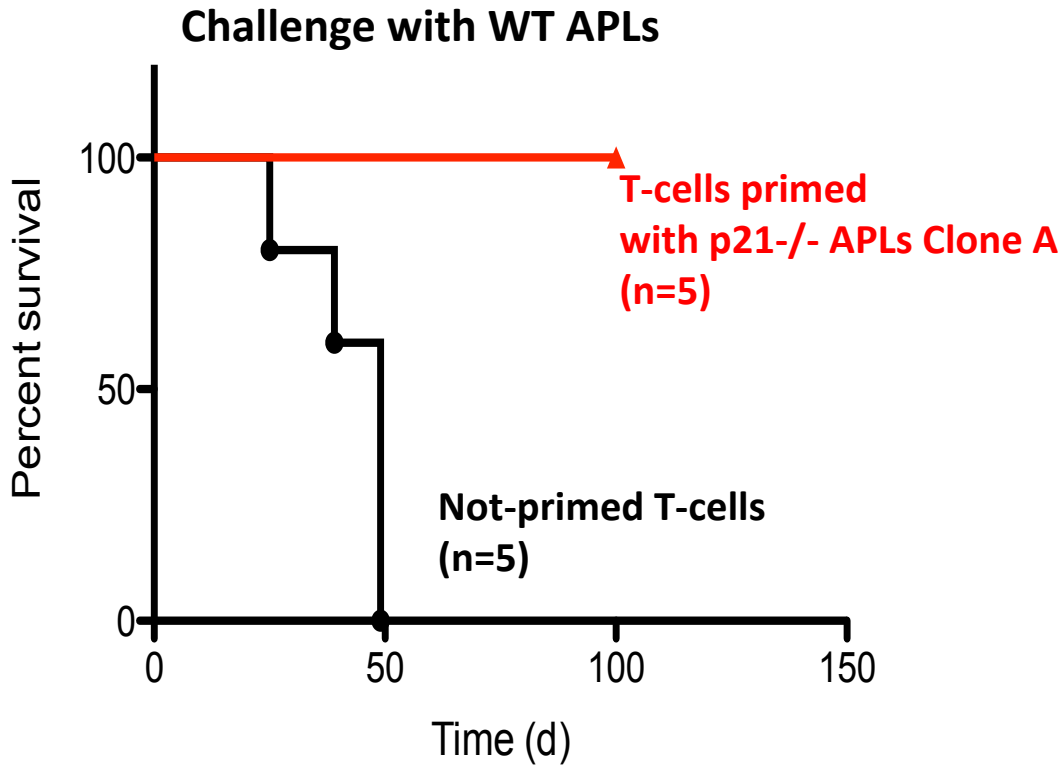
T-cells primed with p21$^{-/-}$ APLs protect against p21$^{-/-}$ APLs



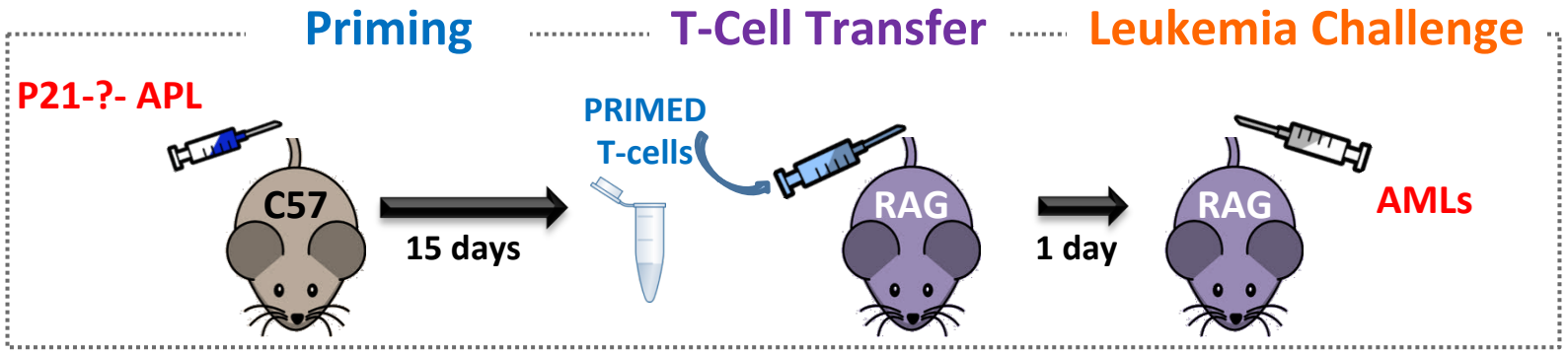


T cells primed with p21$^{-/-}$ APLs protect against WT APLs

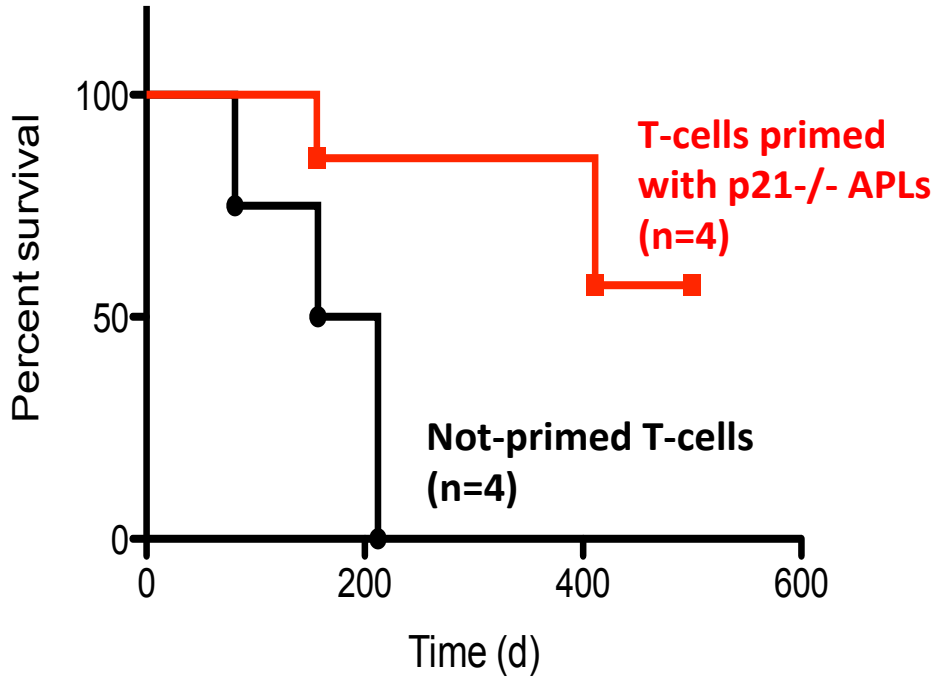




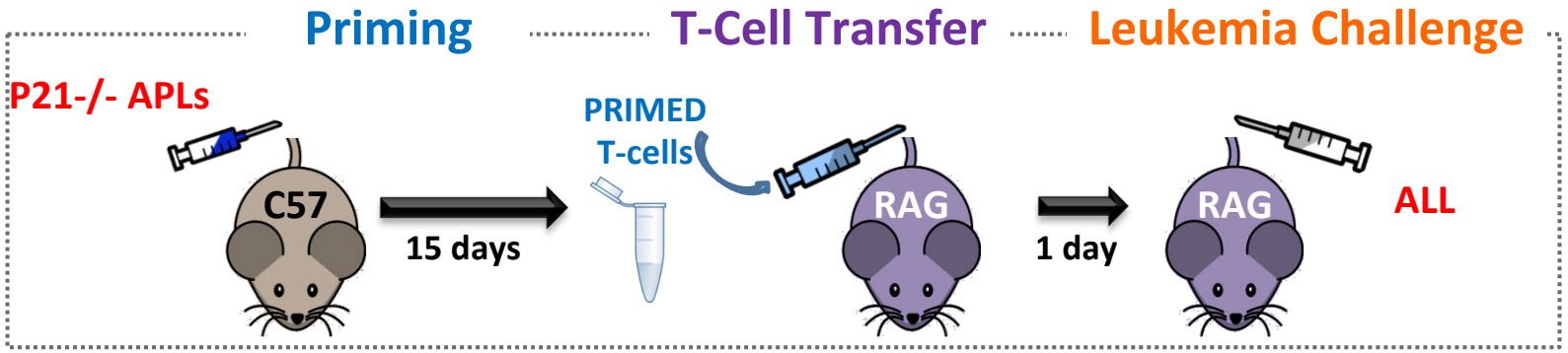
T cells primed with p21$^{-/-}$ leukemia
Protect against other AMLs (NPMc; FLT3ITD)

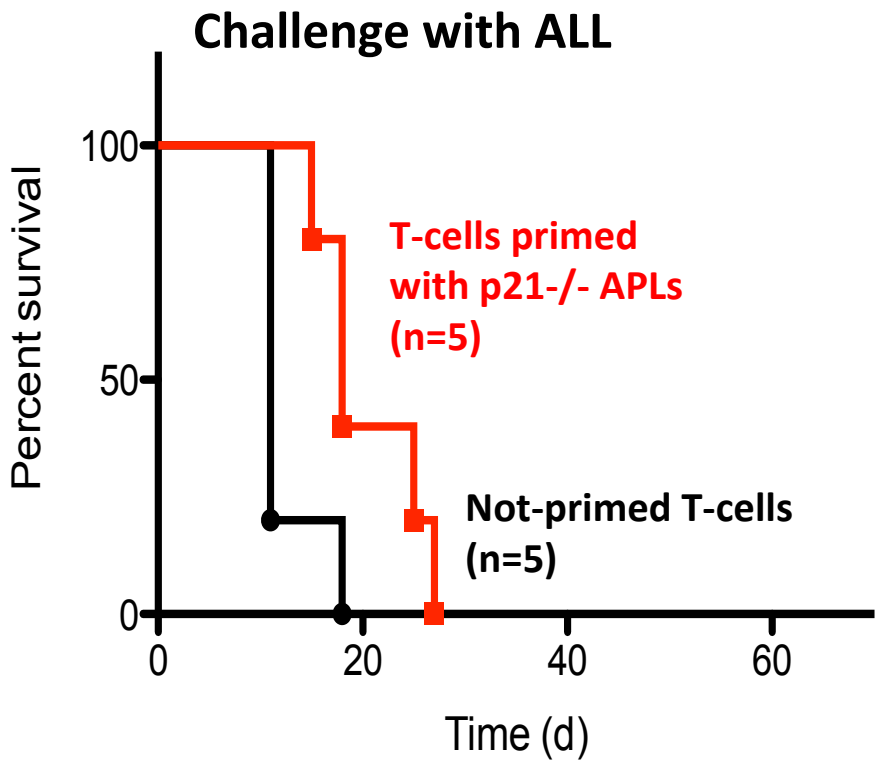


Challenge with NPM-AMLs (n=2) or FLT3-AMLs (n=2)

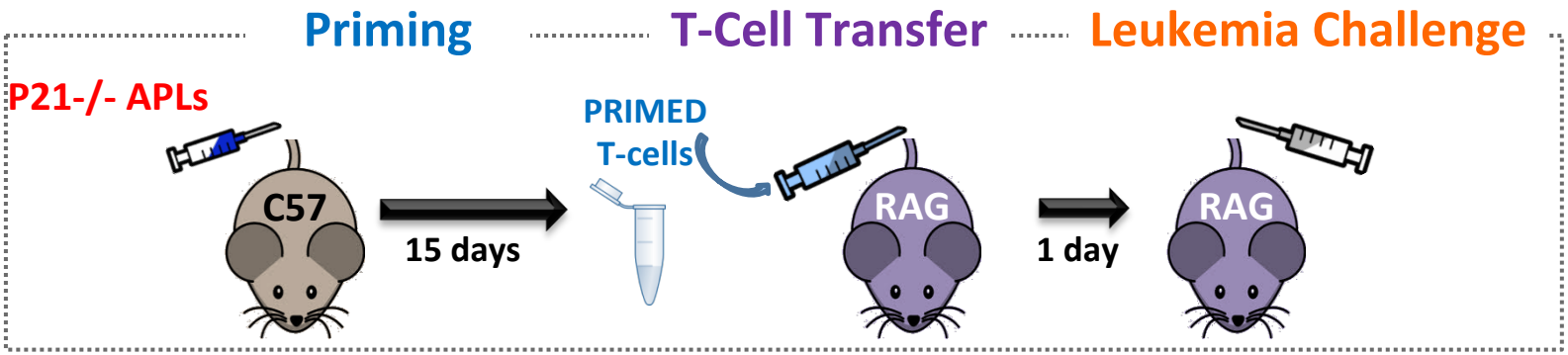


T cells primed with p21$^{-/-}$ APLs do not protect against ALLs



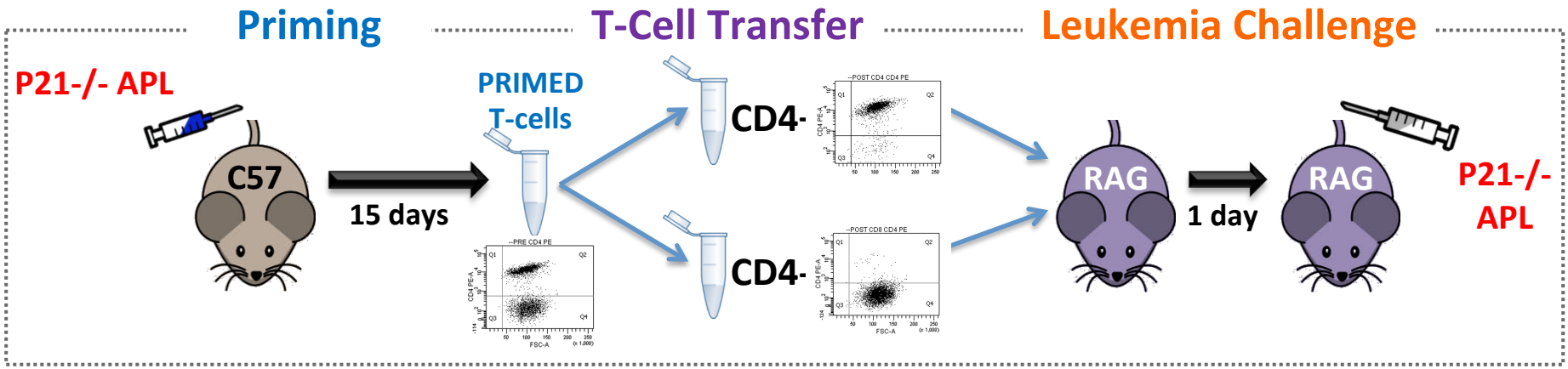


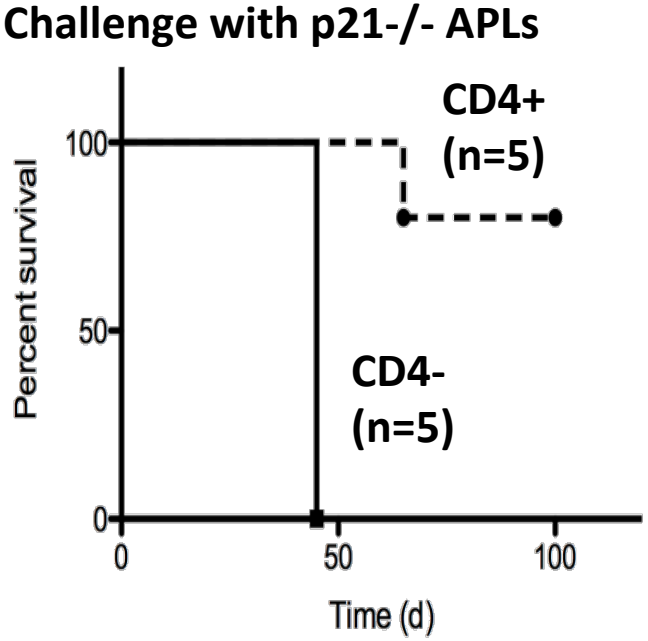
Summary



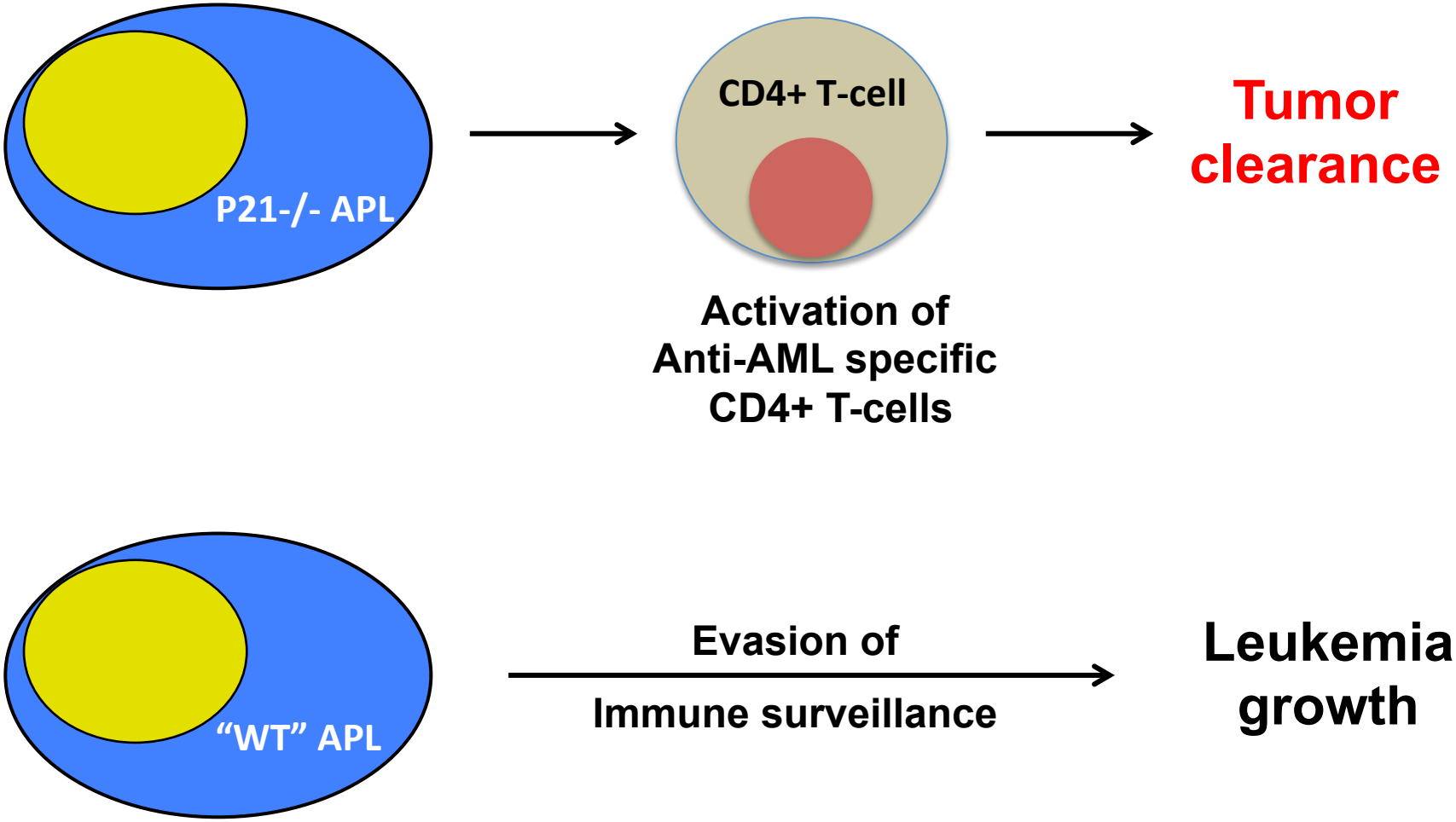
Priming with p21-/- APLs generates T-cells that protect against wtAPLs and other AMLs (do not against ALLs)

The effector T-cells are CD4^{+}

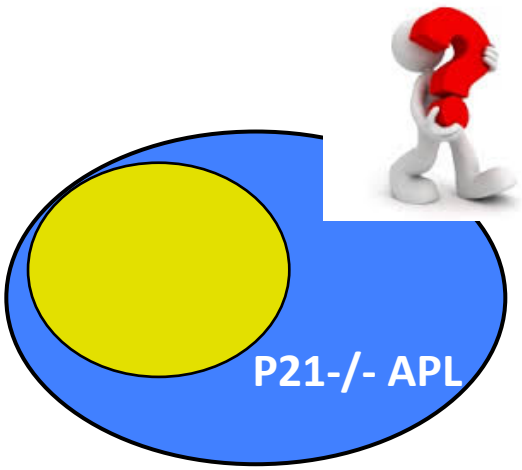




P21-/- APLs activate a population of anti-leukemia CD4+ lymphocytes



Do p21-/- blasts express surface proteins that activates a CD4+ specific anti myeloid-leukemia response?



P21-/- APL

CD4+ T-cell

Activation of
Anti-AML specific
CD4+ T-cells

Tumor clearance

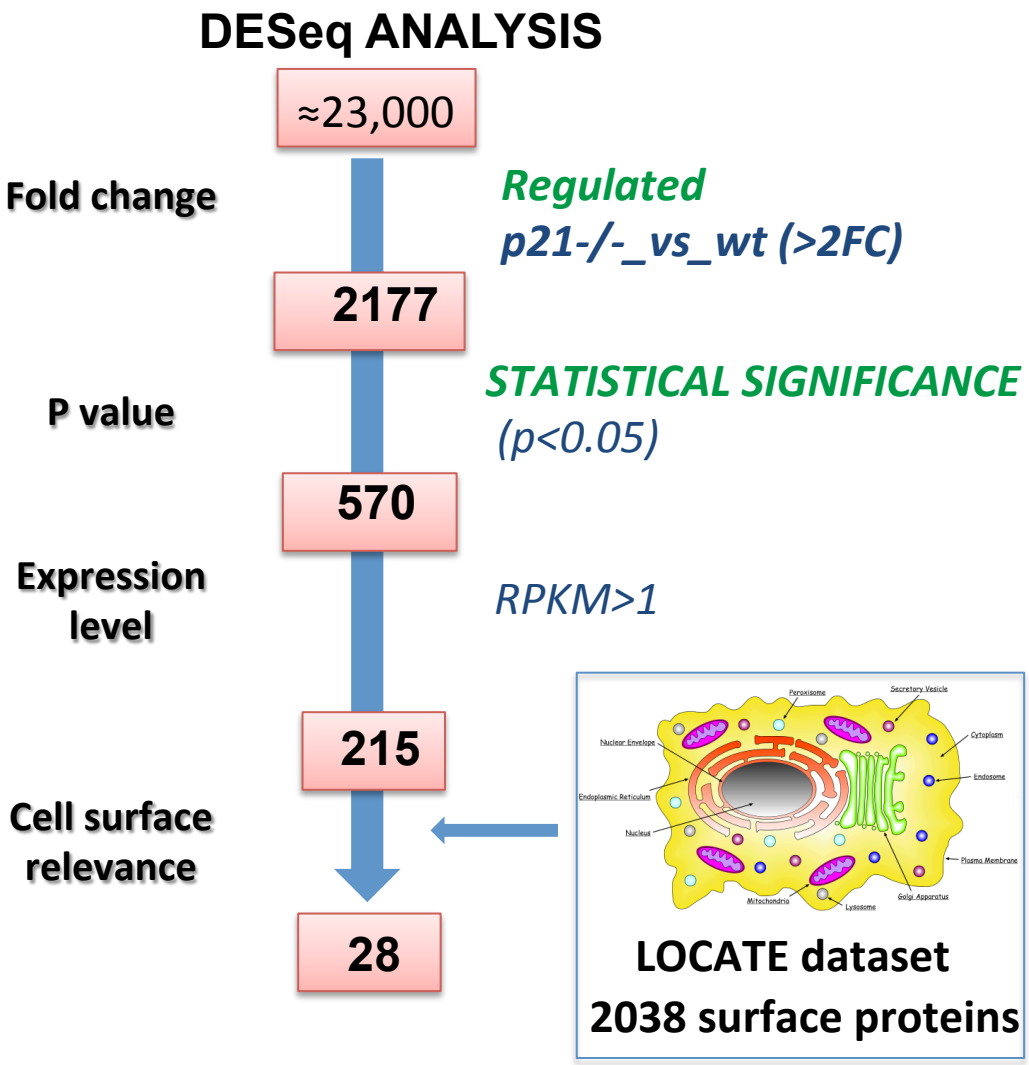
"WT" APL

Evasion of
Immune surveillance

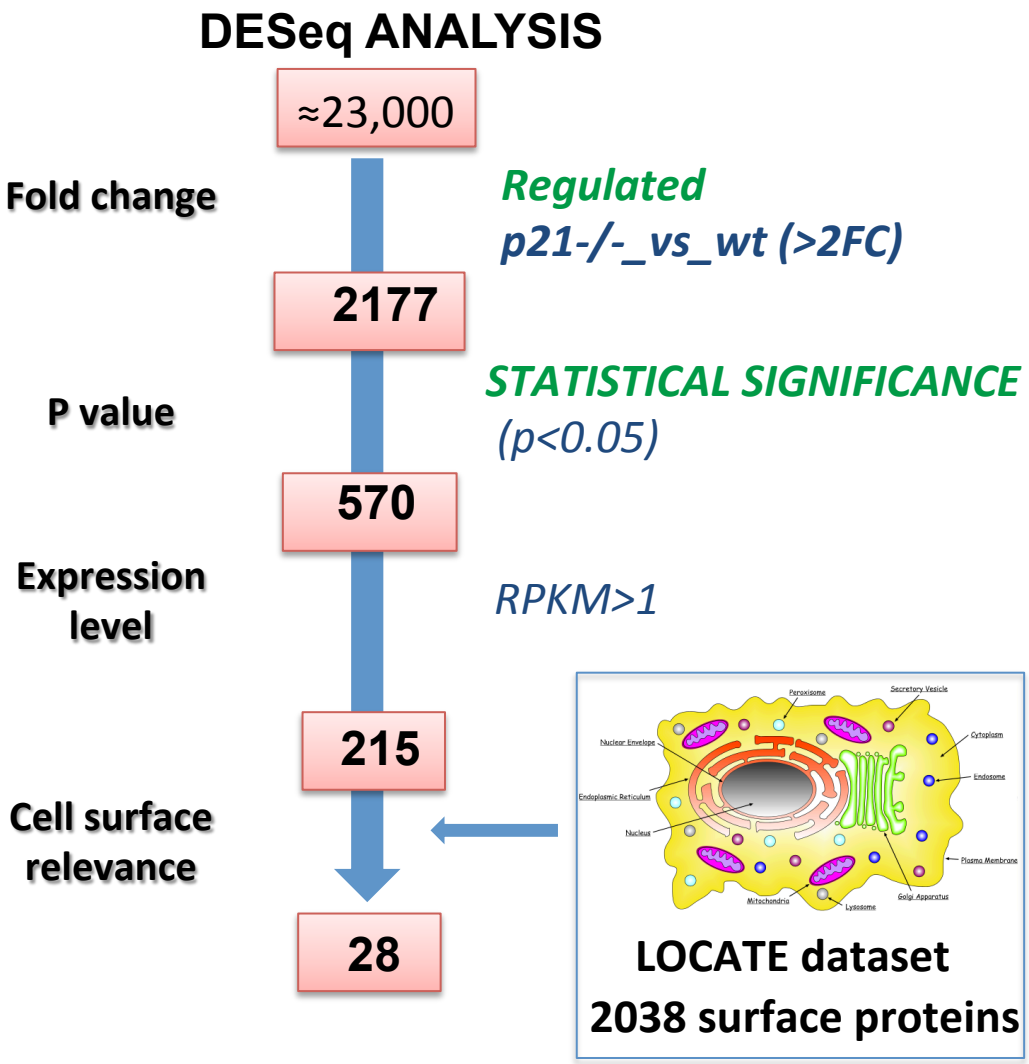
Leukemia growth

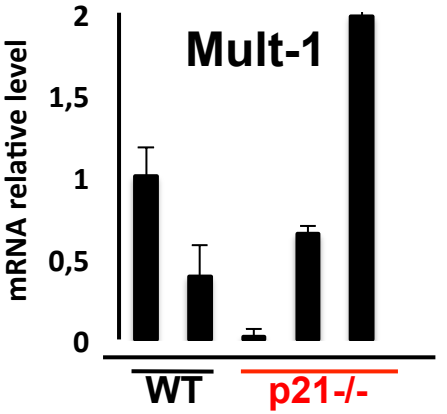
Experimental approach:

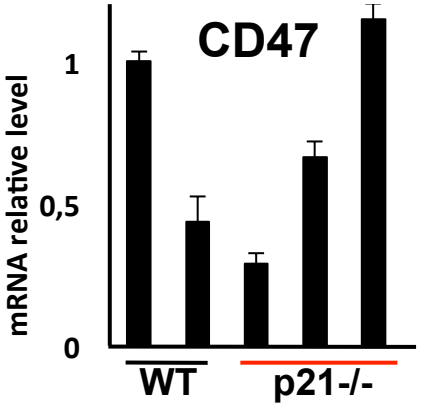
RNAseq of primary p21-/- vs WT APL blasts (n=12)

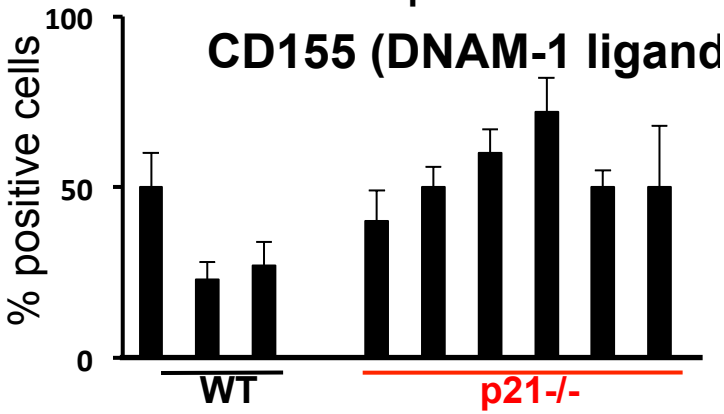


High expression-variability among APL samples of several gene-candidates

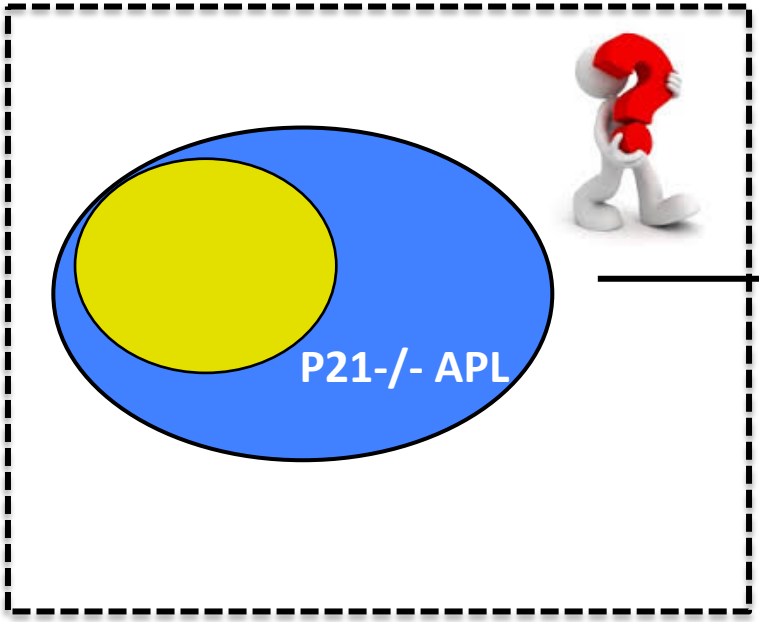


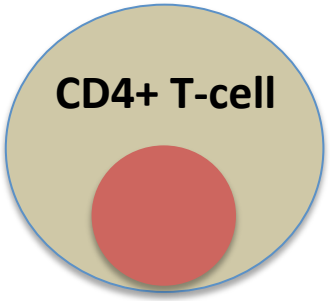






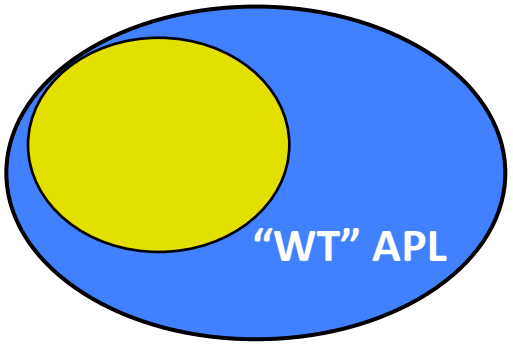
Does the p21-/- "micro-environment" activate a CD4+ specific anti myeloid-leukemia response?





Tumor clearance

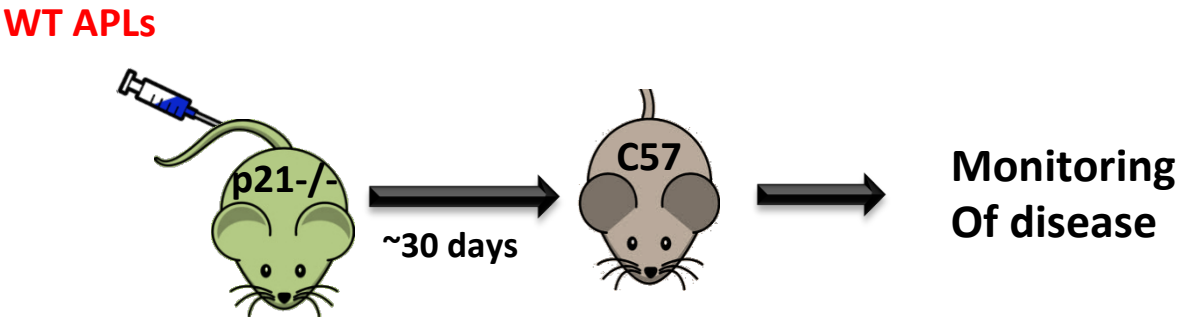
Activation of Anti-AML specific CD4+ T-cells

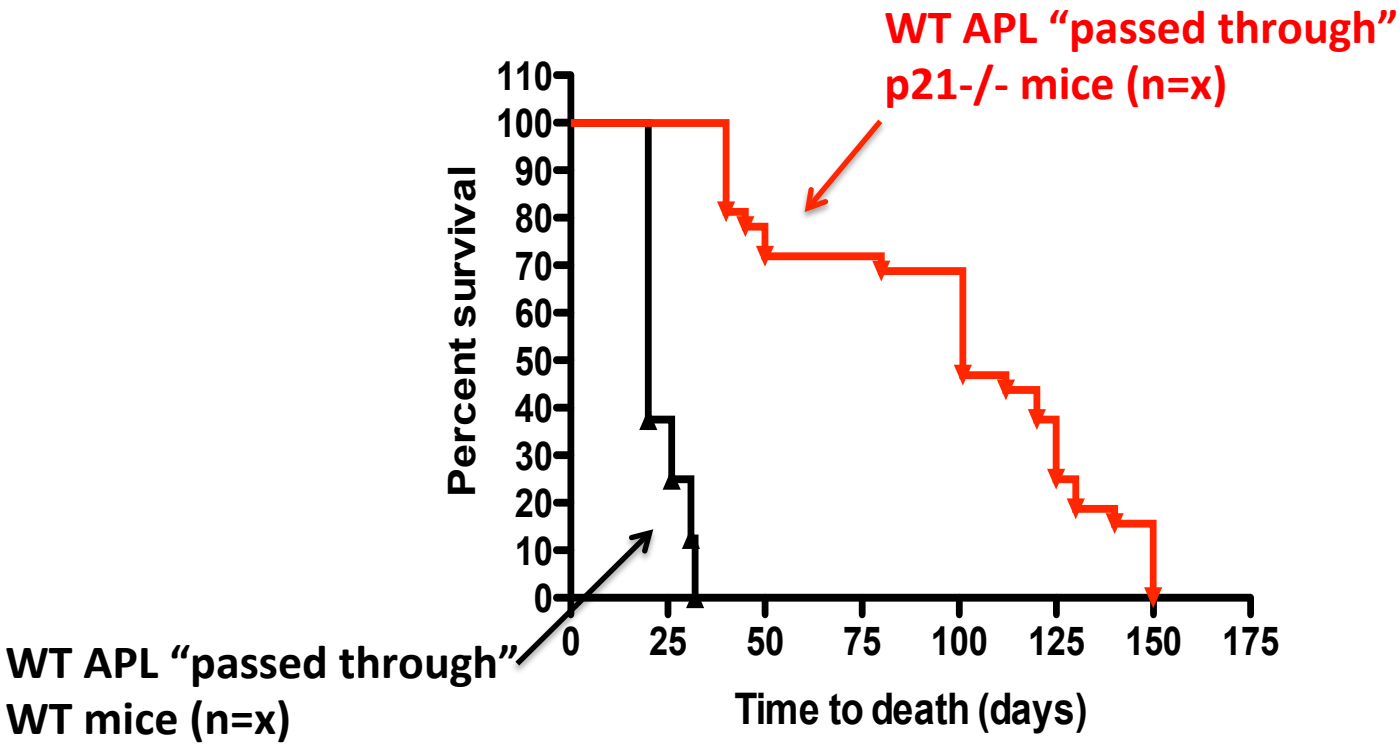


Evasion of Immune surveillance

Leukemia growth

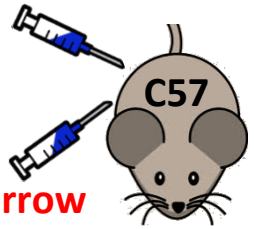
Exposure of wt APLs to the p21-/- "micro-environment" protects from leukemia development





A cellular component of the p21-/- micro-environment (spleen or bone marrow) is sufficient to protect mice from leukemia development

WT APLs

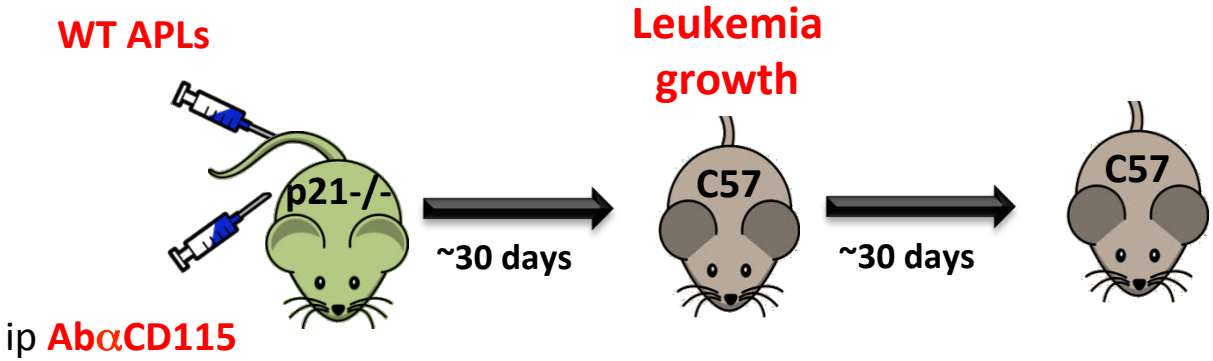


P21-/- Bone Marrow
Or p21-/- Spleen

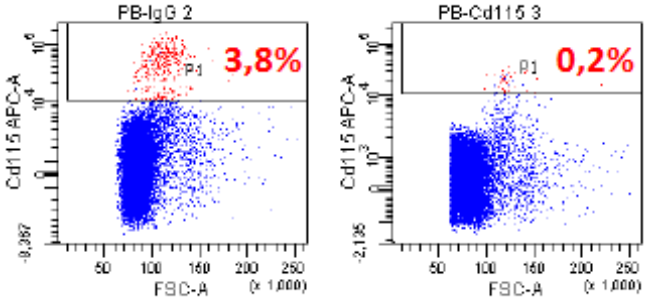
→ Monitoring of leukemia growth and survival

	Leukemia
P21-/- APL	8/8
P21-/- APL + 5x10^6 p21-/- Splenocytes	**0/7**
P21-/- APL + 5x10^6 p21-/- BM cells	**0/7**
P21-/- APL + 5x10^6 p21-/- Splenocytes	2/2
P21-/- APL + 5x10^6 p21-/- BM cells	2/2

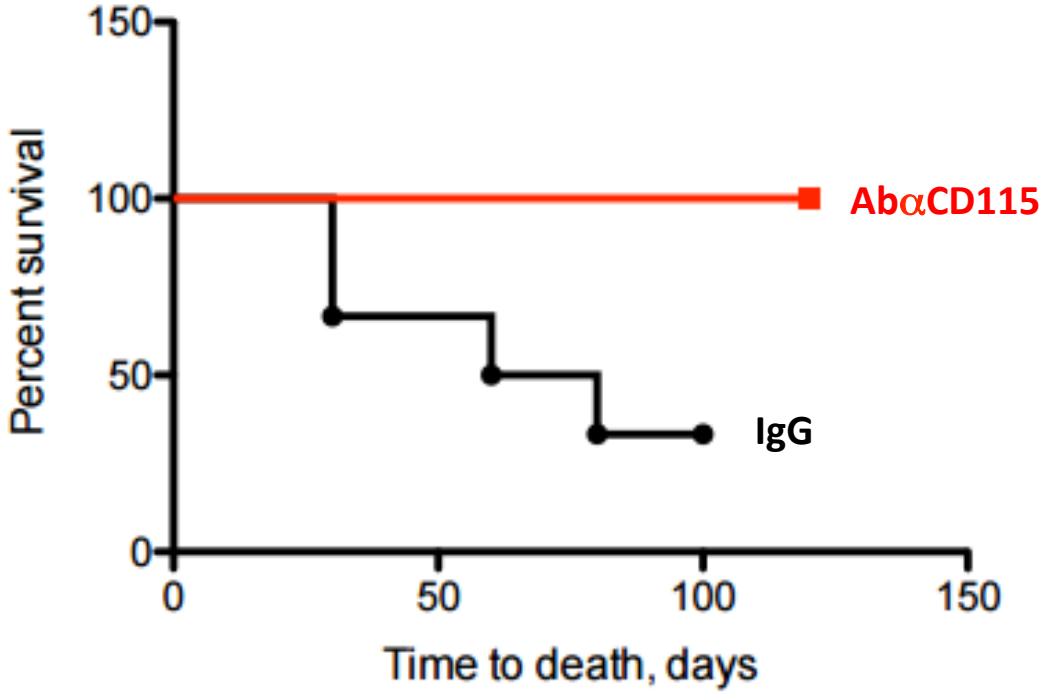
Depletion of macrophages from the p21-/- micro-environment rescues the growth potential of WT APLs



Spleen

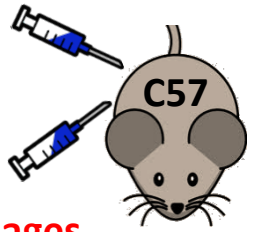


Ctrl IgG AbαCD115



Addition of purified p21-/- macrophages (from the bone marrow) protect from leukemia development

WT APLs



P21-/- Macrophages

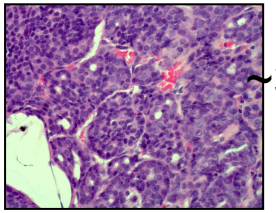
Monitoring of leukemia growth and survival

	Leukemia
WT APL	2/2
WT APL + WT Mac	4/5
WT APL + p21-/- Mac	0/5

Preparation of Macrophages form the Bone marrow: 6 days culture in adherent conditions of Ly6g^{neg} and CD11b^{pos} BM cells

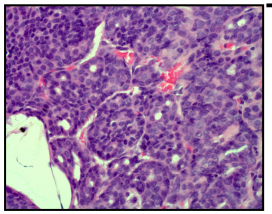
BREAST CANCER:
A role for p21 in the immune-mediated clearance of breast cancer?

FVB-MMTV-ErbB2  **FVB p21-/-** 



~3 months latency (as in wt mice)

$p21^{-/-}$ breast cancer cells do not transplant in syngeneic mice

FVB-MMTV-ErbB2  FVB p21-/- →  Tumor formation after ~3 months latency (as in wt mice)

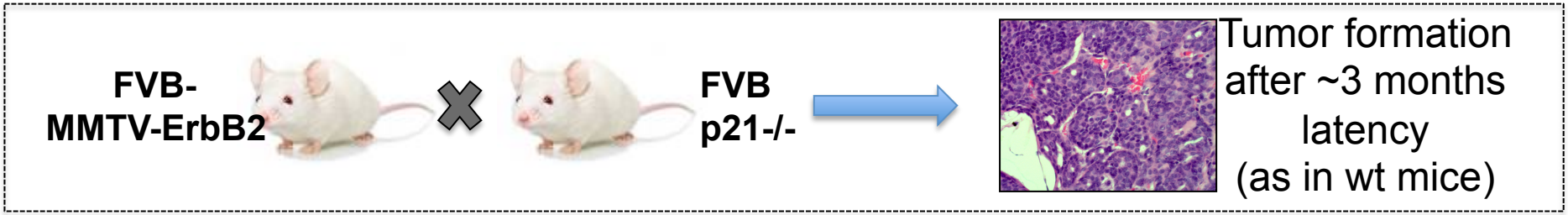
$p21^{-/-}$ ErbB2-tumors

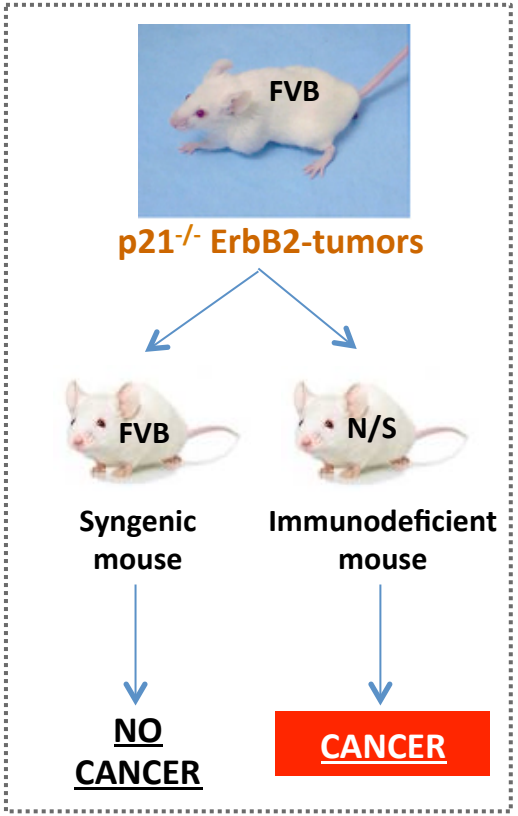
Syngenic mouse

NO CANCER

RECIPIENT	BREAST CANCER	ENGRAFTMENT
FVB *Syngenic*	NO	0/20

$p21^{-/-}$ breast cancer cells re-acquire the ability to initiate tumorigenesis when transplanted in the mammary gland of immunodeficient mice

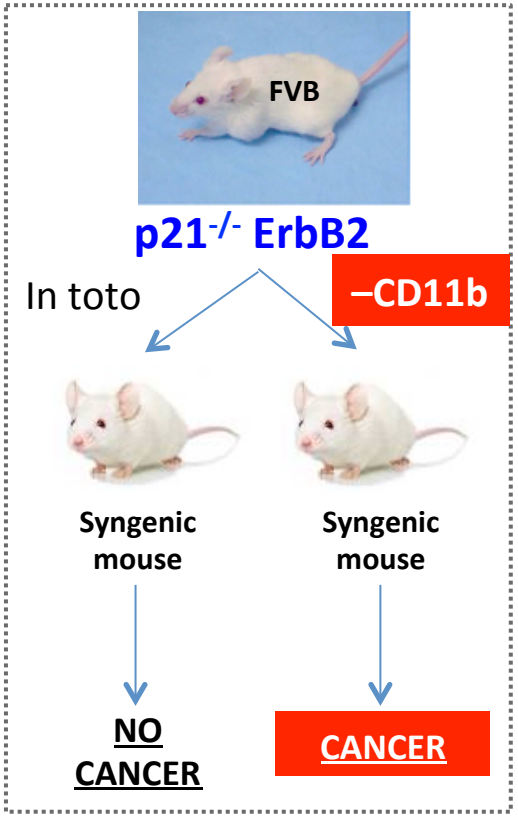




RECIPIENT	BREAST CANCER	ENGRAFTMENT
FVB *Syngenic*	NO	0/20
NOD/SCID	YES	12/12

In vivo role of macrophages in mammary tumor growth

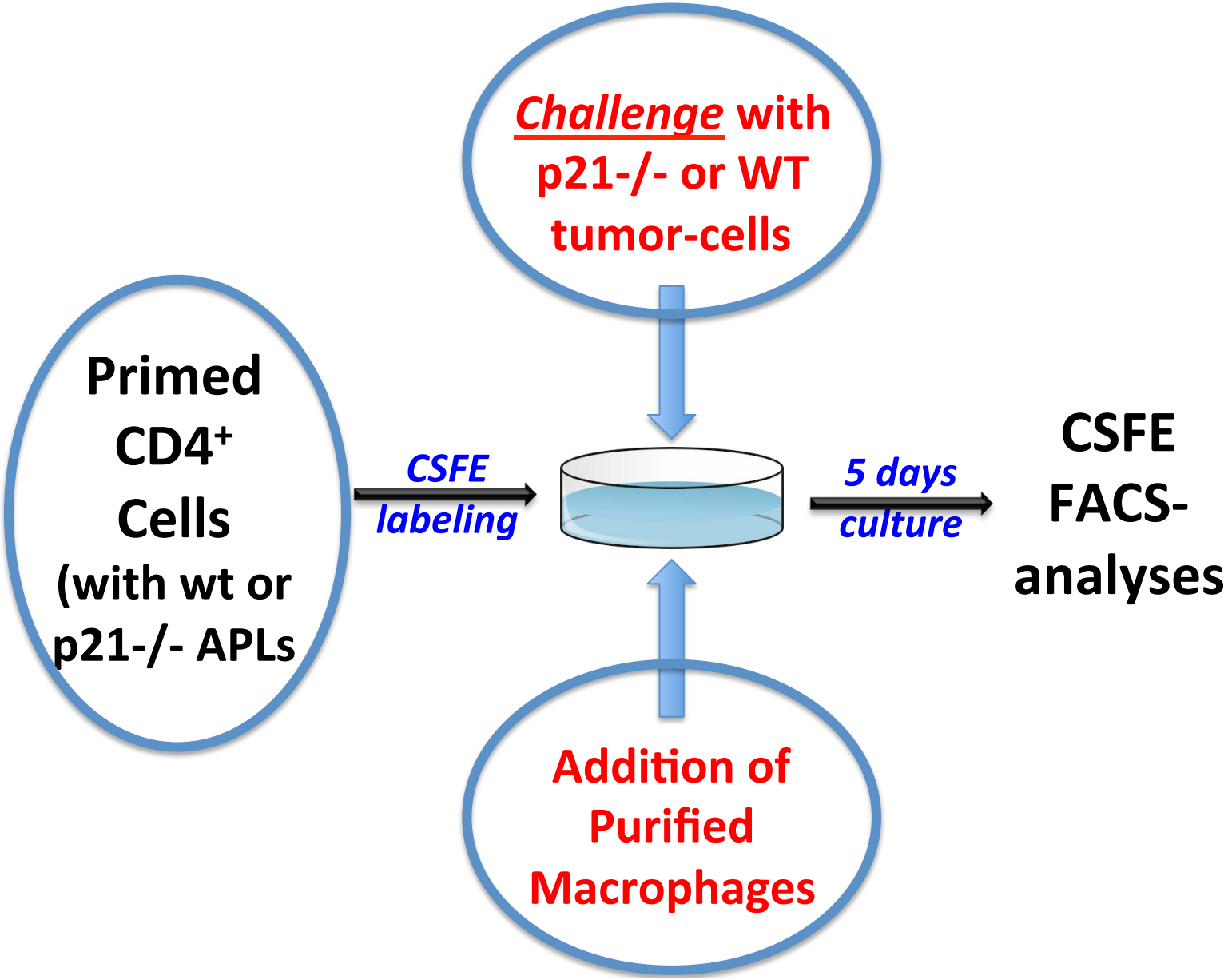
1. Depletion of macrophages restores p21-/- breast cancer transplantability



P21-/-

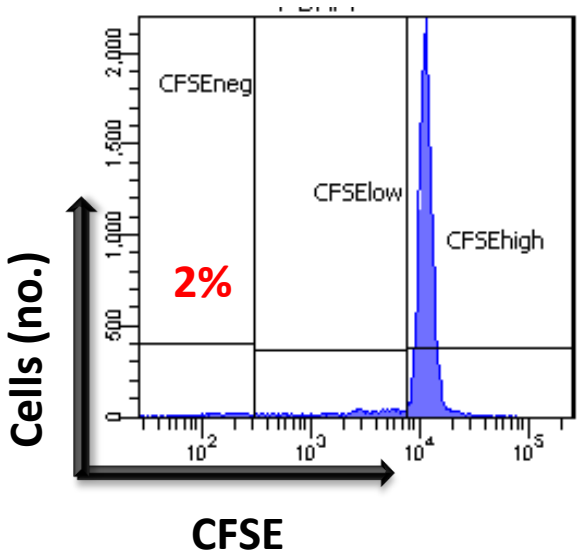
RECIPIENT	BREAST CANCER CELLS	ENGRAFTMENT
FVB *Syngenic*	IN TOTO	0/5
FVB *Syngenic*	–CD11b	4/5

In vitro reconstitution of the anti-cancer effect of p21-/- Macrophages

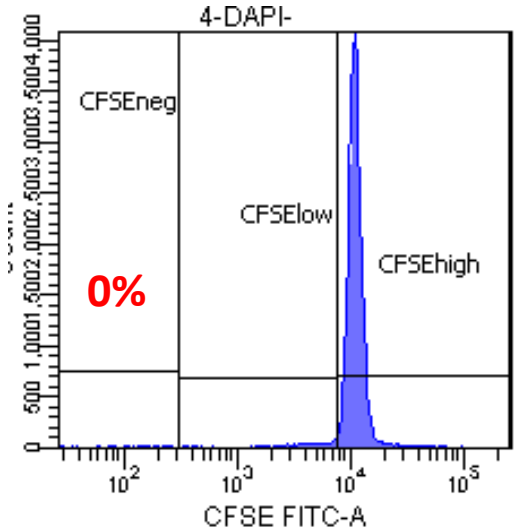


CD4+ T-Cells primed with WT tumor-cells do not proliferate after in vitro challenging with WT tumor-cells

(WT-ErbB2) Primed CD4+

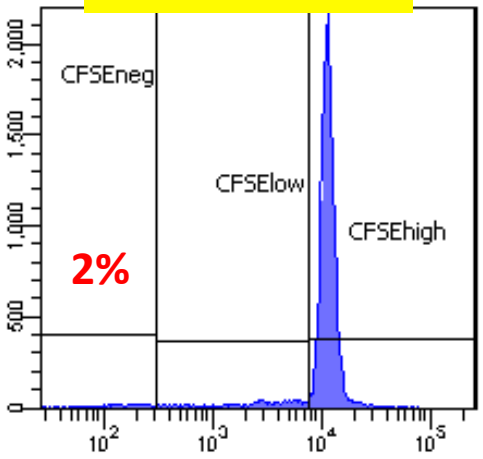


+ WT ErbB2

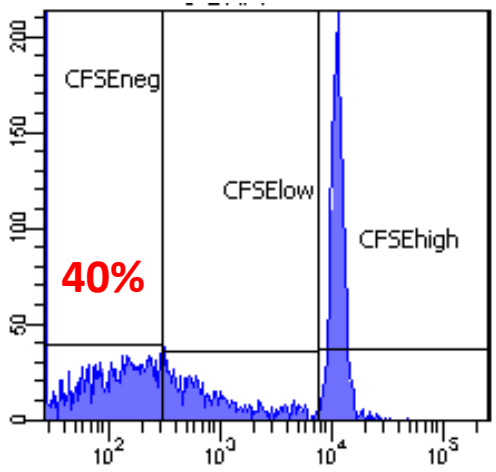


The presence of p21-/- tumors-cells (either as priming or challenging cells) induces CD4+ proliferation

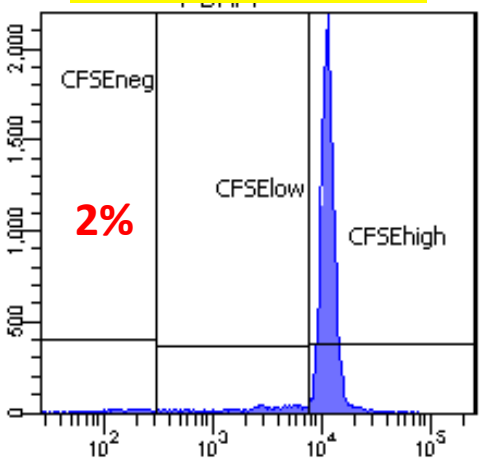
(WT-ErbB2) Primed CD4+



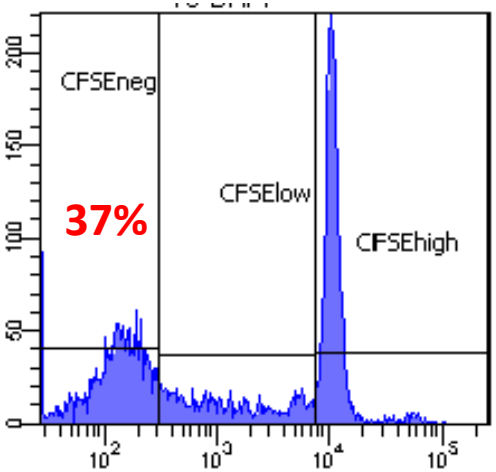
+ p21-/- ErbB2



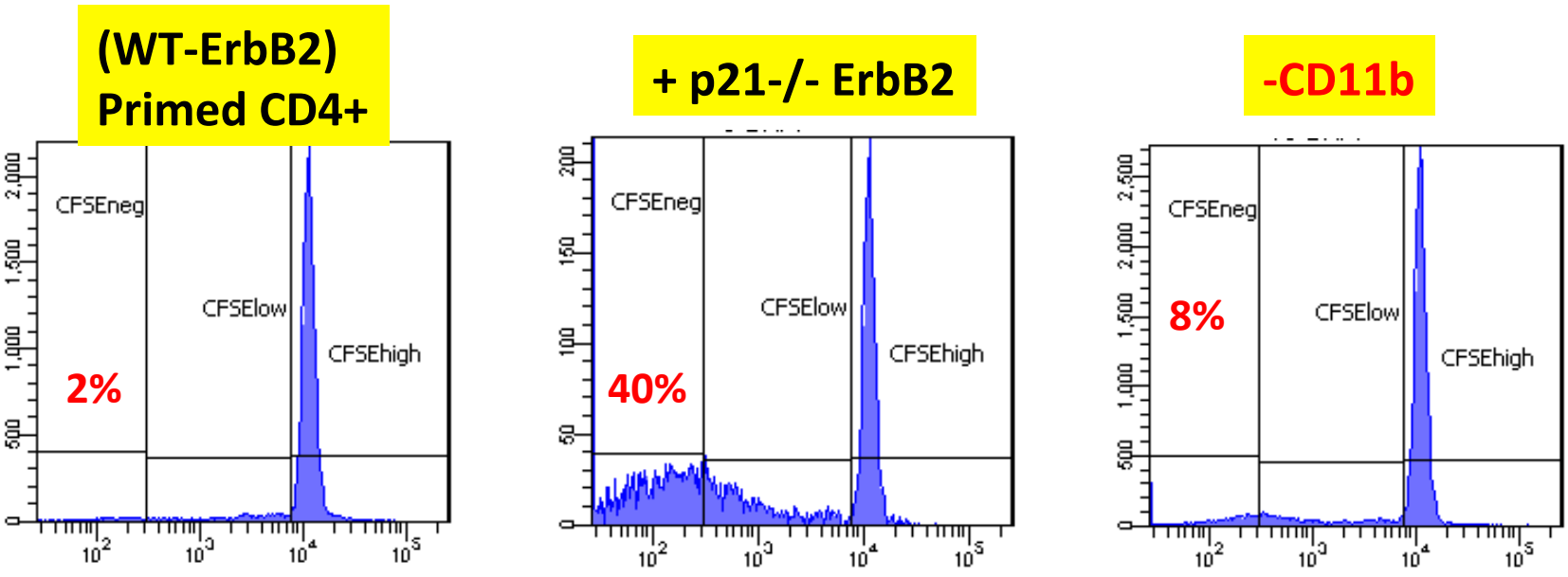
(p21-/- ErbB2) Primed CD4+



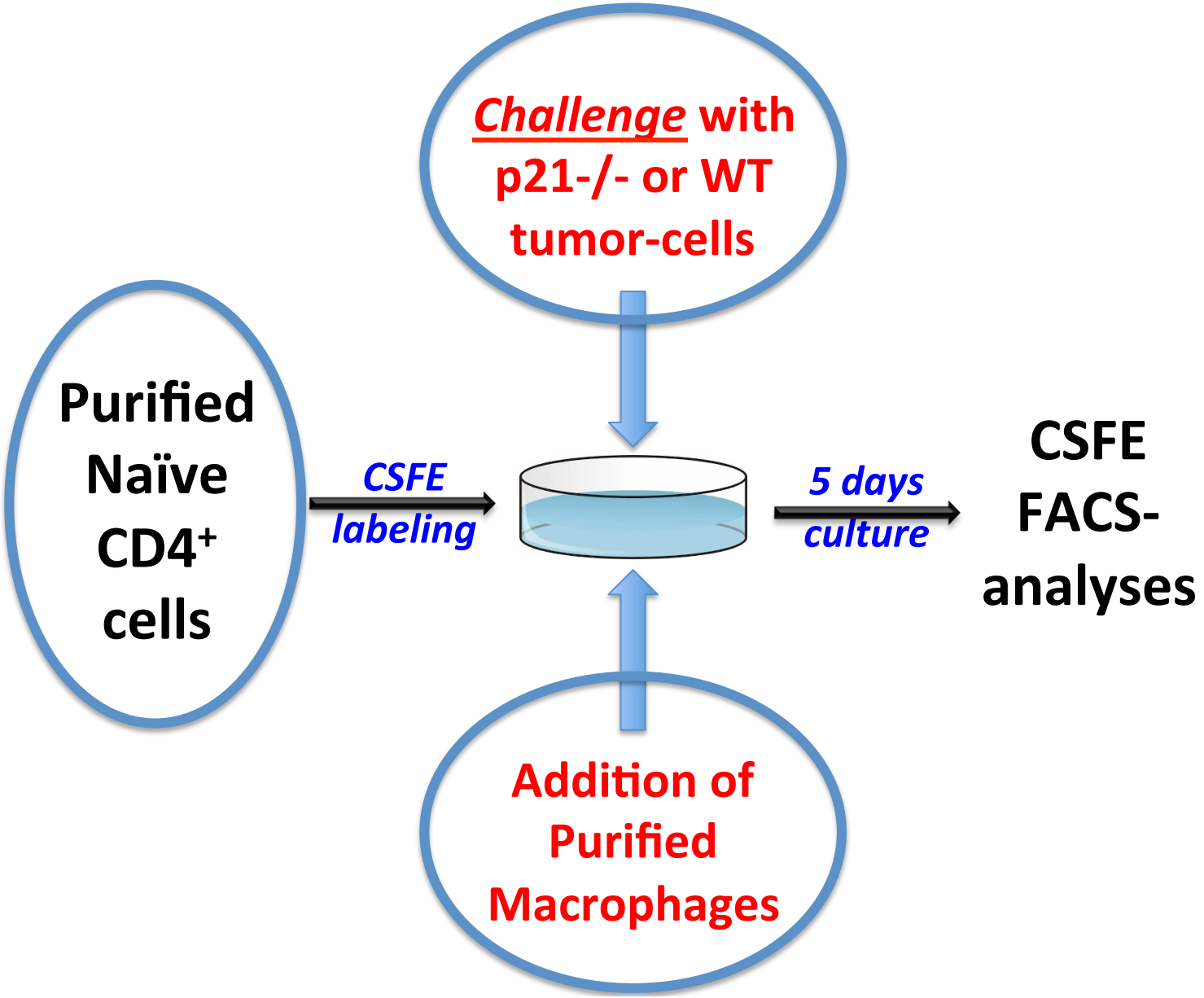
+ WT ErbB2



Depletion of Macrophages inhibits CD4+ T-Cell proliferation

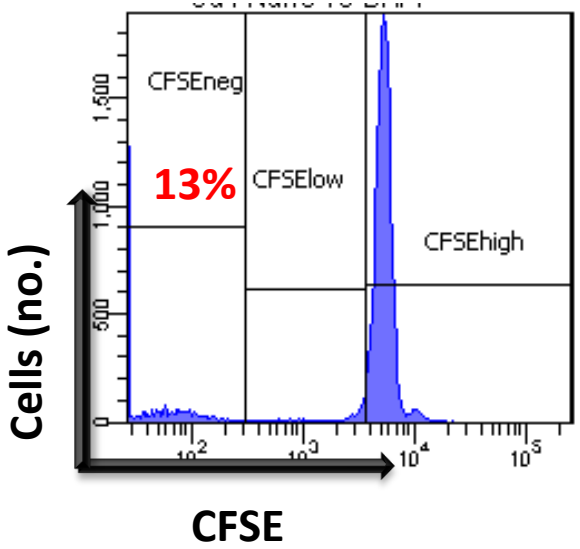


In vitro reconstitution of the anti-cancer effect of p21-/- Macrophages

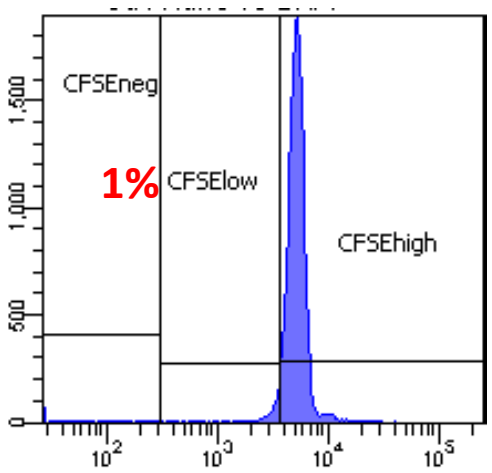


NAÏVE CD4+ T-Cells do NOT proliferate *in vitro* after challenging with WT ErbB2 cells

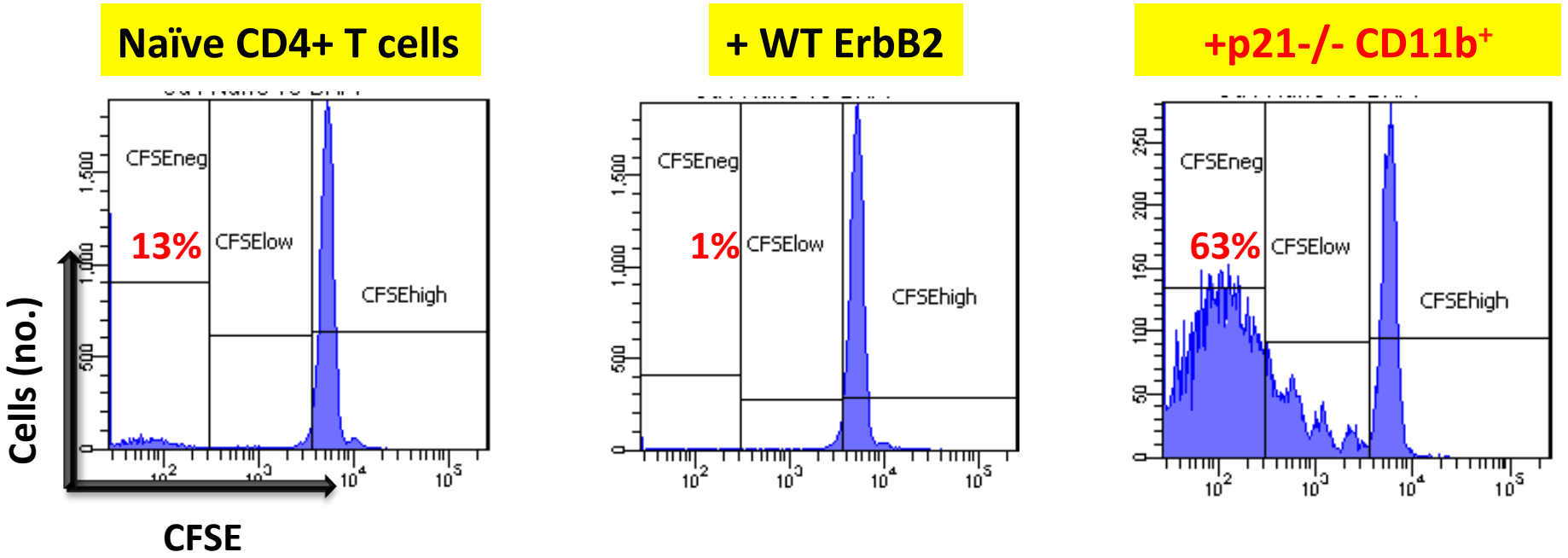
Naïve CD4+ T cells



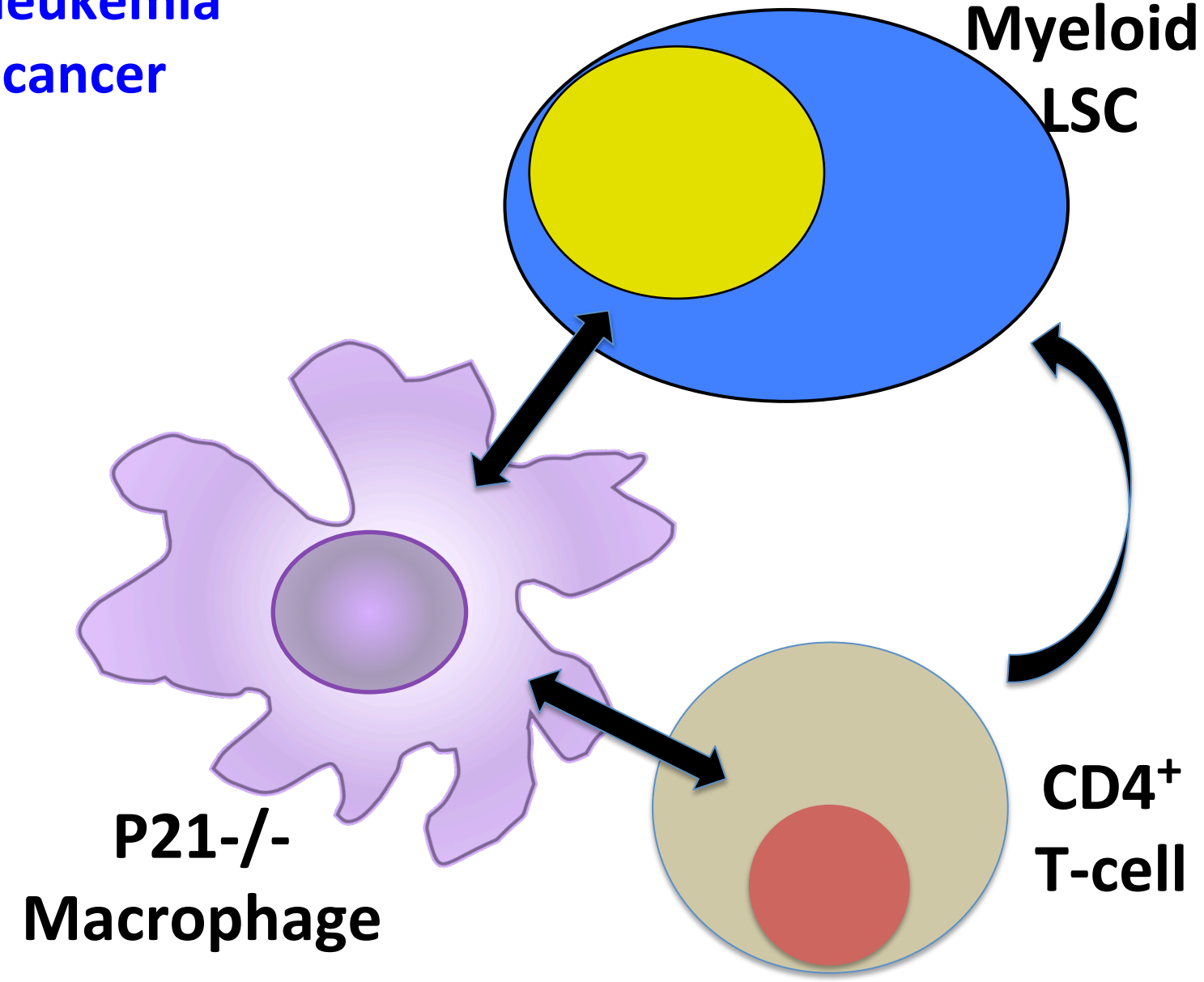
+ WT ErbB2



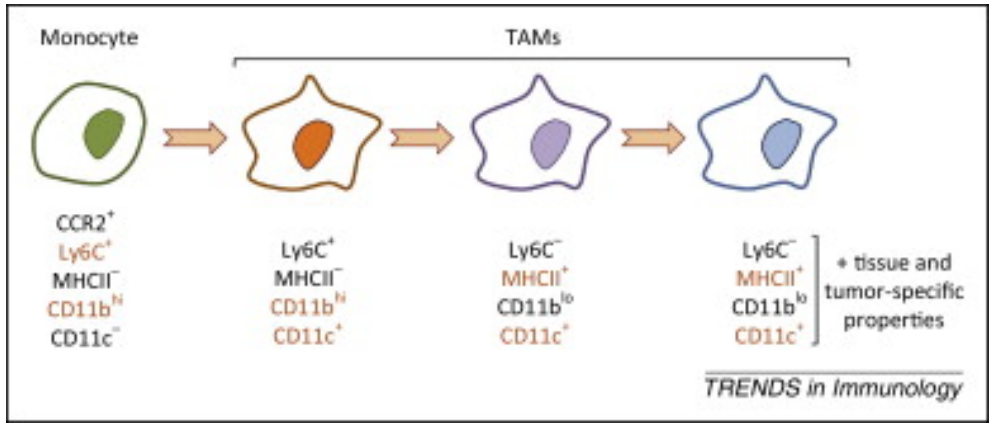
NAÏVE CD4+ T-Cells proliferate *in vitro* after challenging with WT ErbB2 cells and addition of p21-/- Macrophages



p21-/- macrophages activate a CD4+ specific anti myeloid-leukemia or mammary-cancer response



Increased “activation” of p21-/- macrophages under steady-state conditions (Bl6 mice)



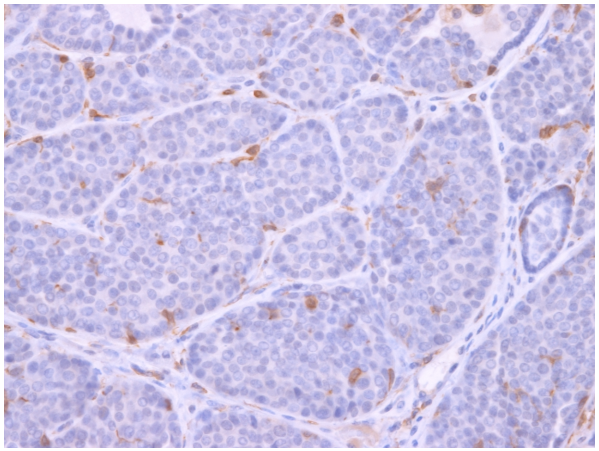
Natoli et al.

	MHC II		Ly6c	
	%	MF	%	MF
WT	59.46	1.96	22.90	3.47
p21-/-	88.90	2.11	4.39	1.74

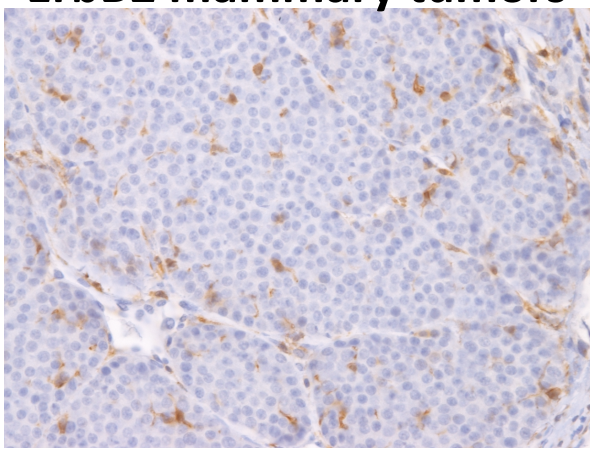
Higher numbers of Macrophages in p21-/- ErbB2 breast cancers

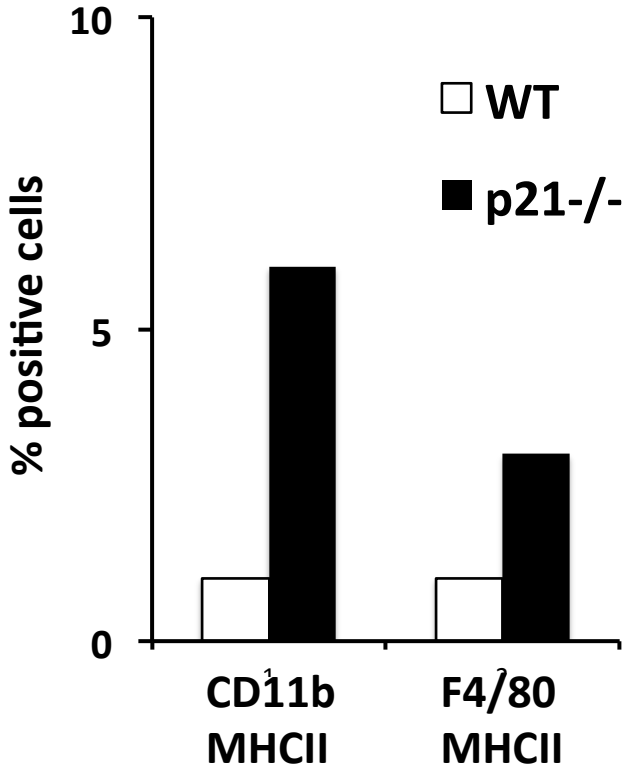
Anti-Iba-1 staining on paraffin sections (20X)

WT
ErbB2 mammary tumors



P21-/-
ErbB2 mammary tumors





FACS analysis of wt and p21-/- ErbB2 tumors (-organoids)

Journal of Clinical Investigation

p21 mediates macrophage reprogramming through regulation of p50-p50 NF-κB and IFN-β

Gorjana Rackov,1 Enrique Hernández-Jiménez,2 Rahman Shokri,1 Lorena Carmona-Rodríguez,1 Santos Mañes,1 Melchor Álvarez-Mon,3 Eduardo López-Collazo,2 Carlos Martínez-A,1 and Dimitrios Balomenos1

First published July 18, 2016 -

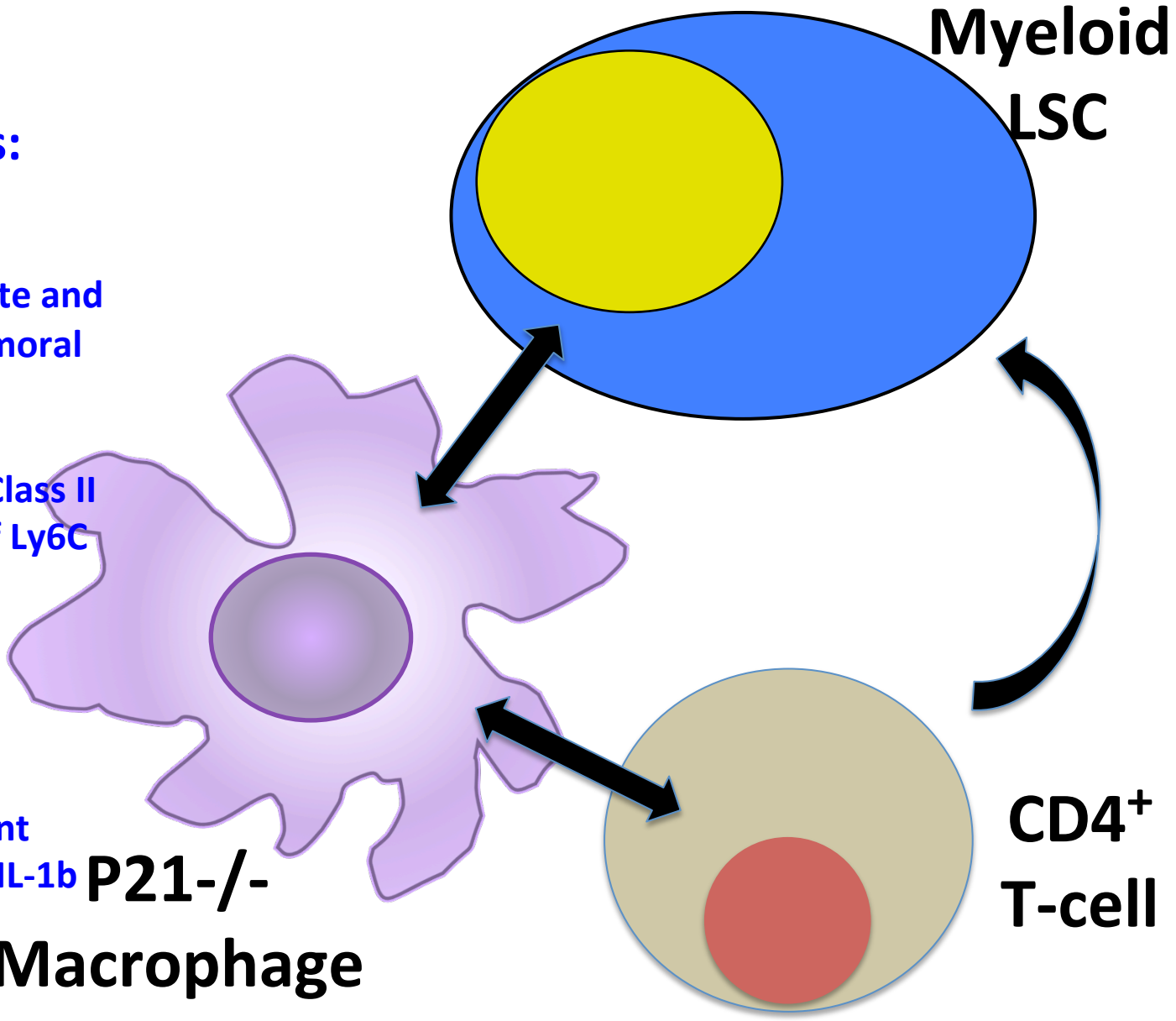
p21 is a negative regulator of macrophage activation

p21-/- macrophages:

- Increased numbers and activation at steady-state and after challenge with tumoral cells
- Up-regulation of MHC Class II and down-regulation of Ly6C
- increased phagocytosis (apoptotic cell)

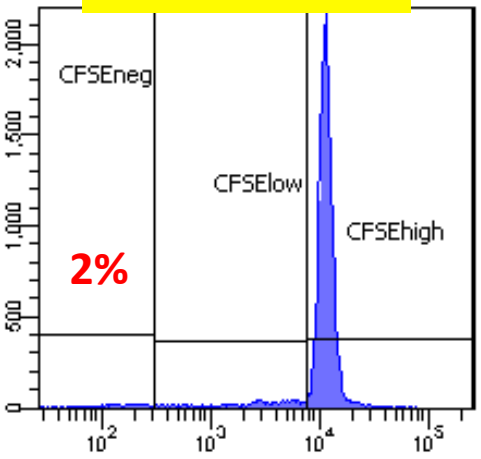
  (Circulation. 2004;110:3830)
- increased LPS-dependent induction of TNF-a and IL-1b

  (Eur. J. Immunol. 2009; 39: 676;
  Eur. J. Immunol. 2009; 39:683)

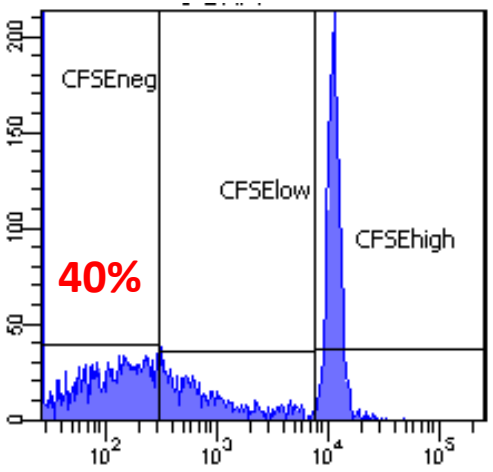


Addition of an αMHCII blocking-Ab inhibits CD4+ T-Cell proliferation

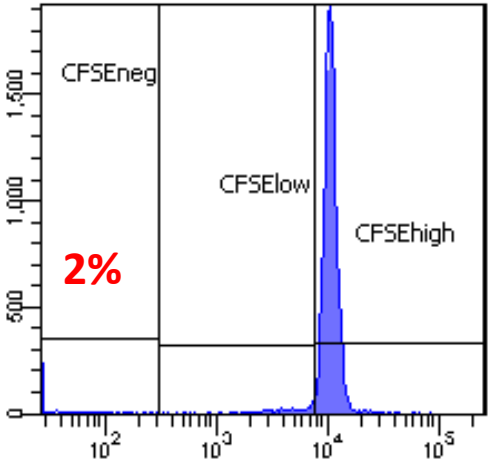
(WT-ErbB2) Primed CD4+



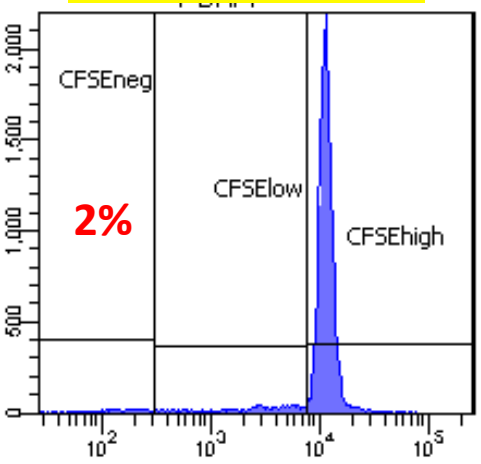
+ p21-/- ErbB2



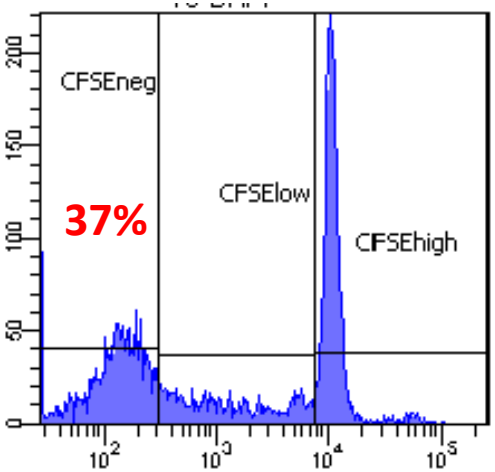
+aMHCII



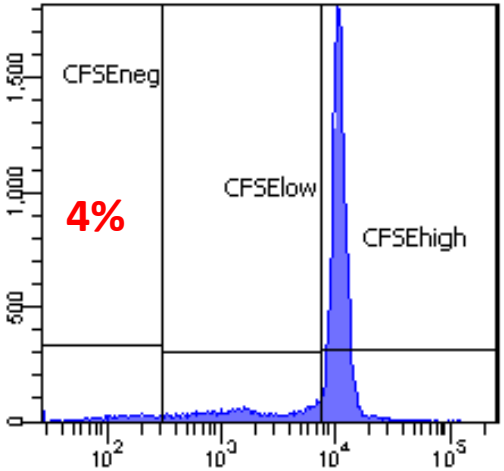
(p21-/- ErbB2) Primed CD4+



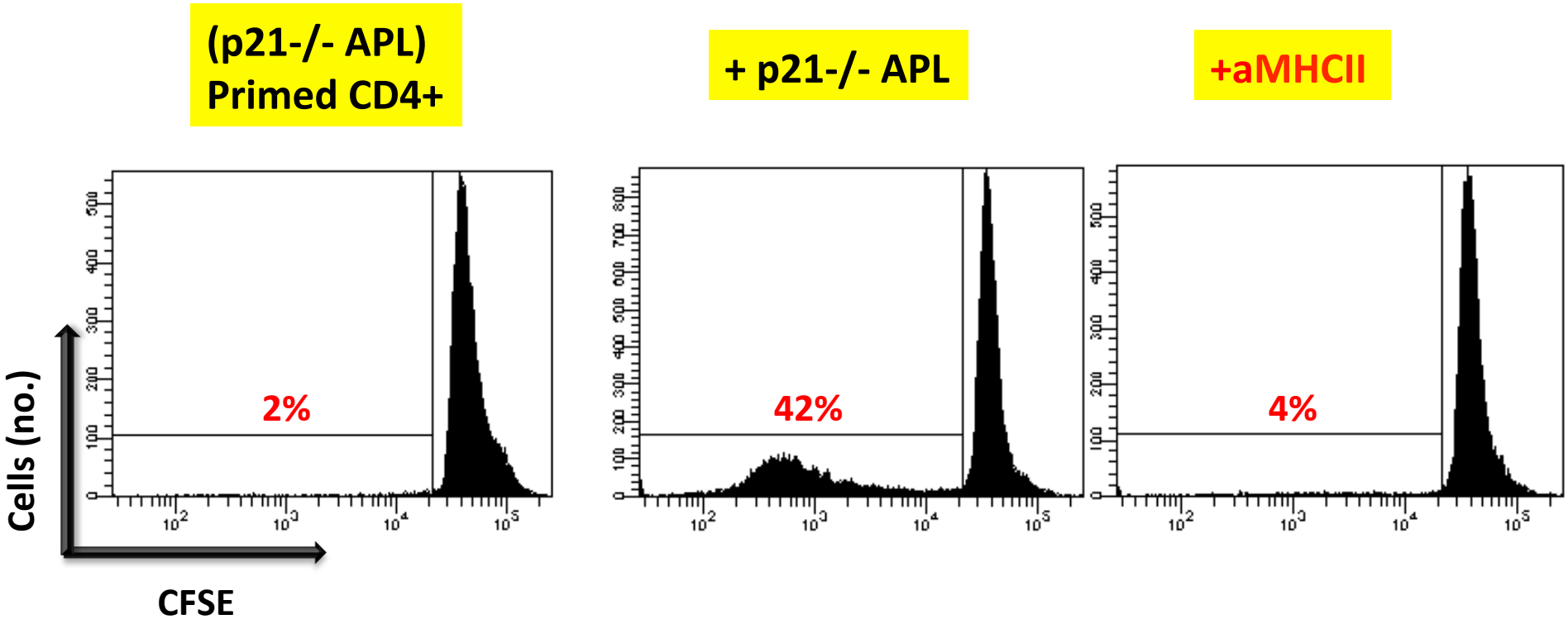
+ WT ErbB2



+aMHCII

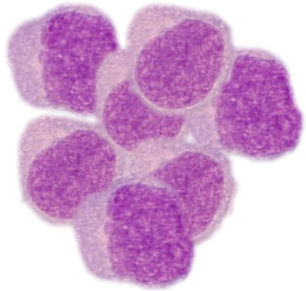


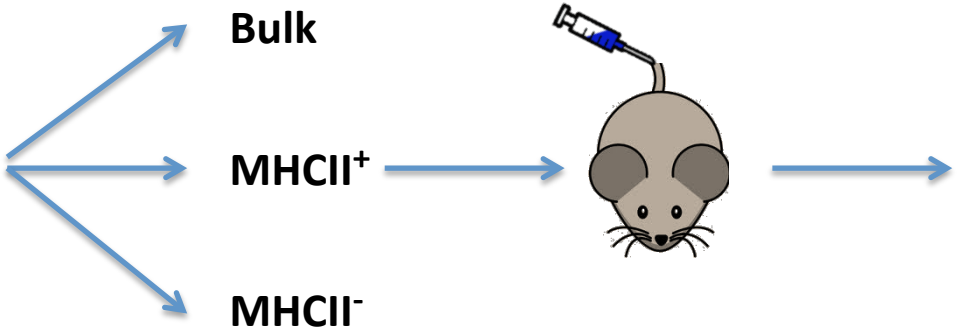
CD4+ T-Cells primed with p21-/- APL proliferate *in vitro* after challenging with APL cells and are inhibited by addition of an αMHCII blocking-Ab



The MHC Class-II subpopulation of p21-/- APL grows in syngeneic mice

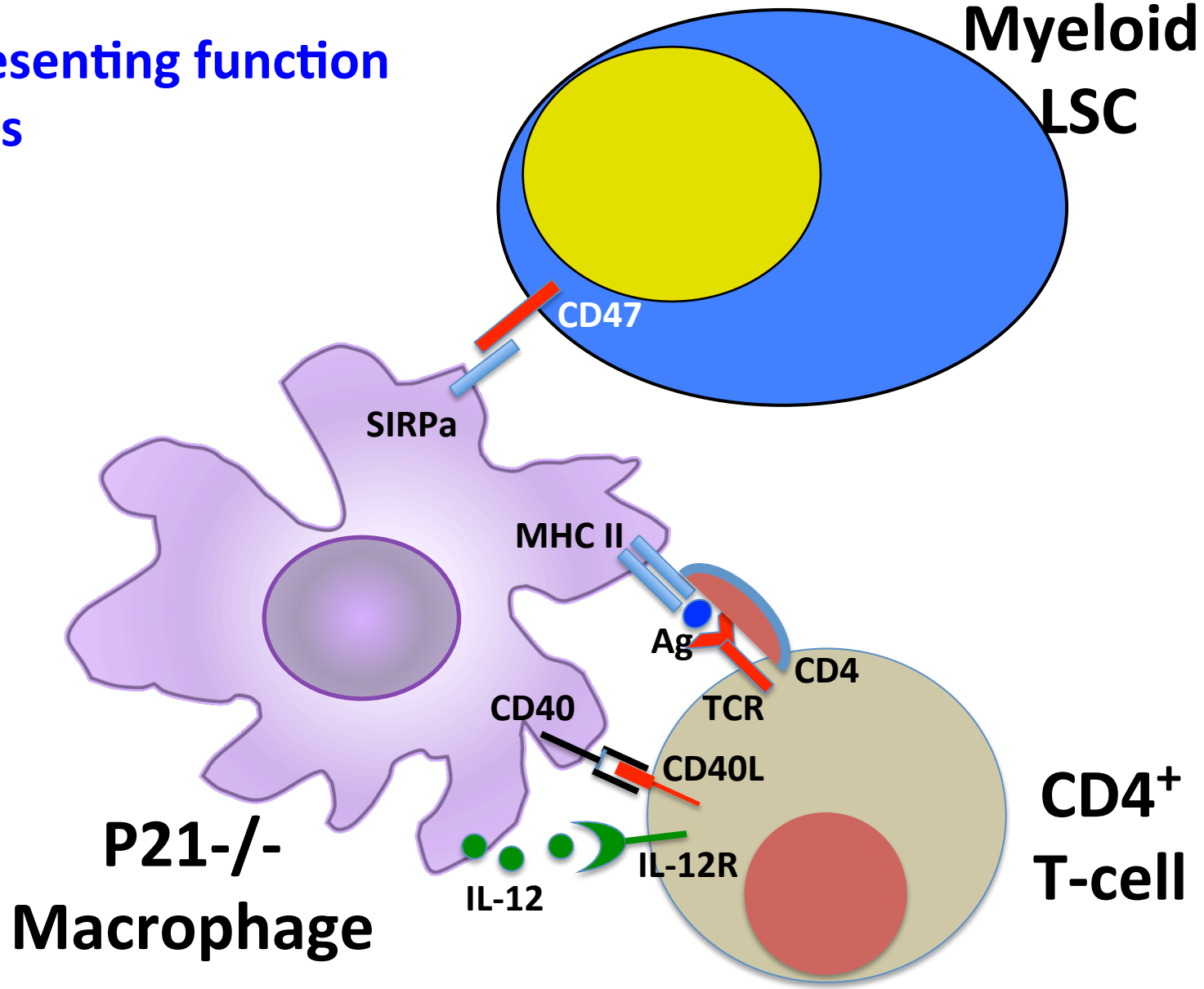
"WT" APL (Ly5.2)
Or
p21-/- APL (Ly5.2)



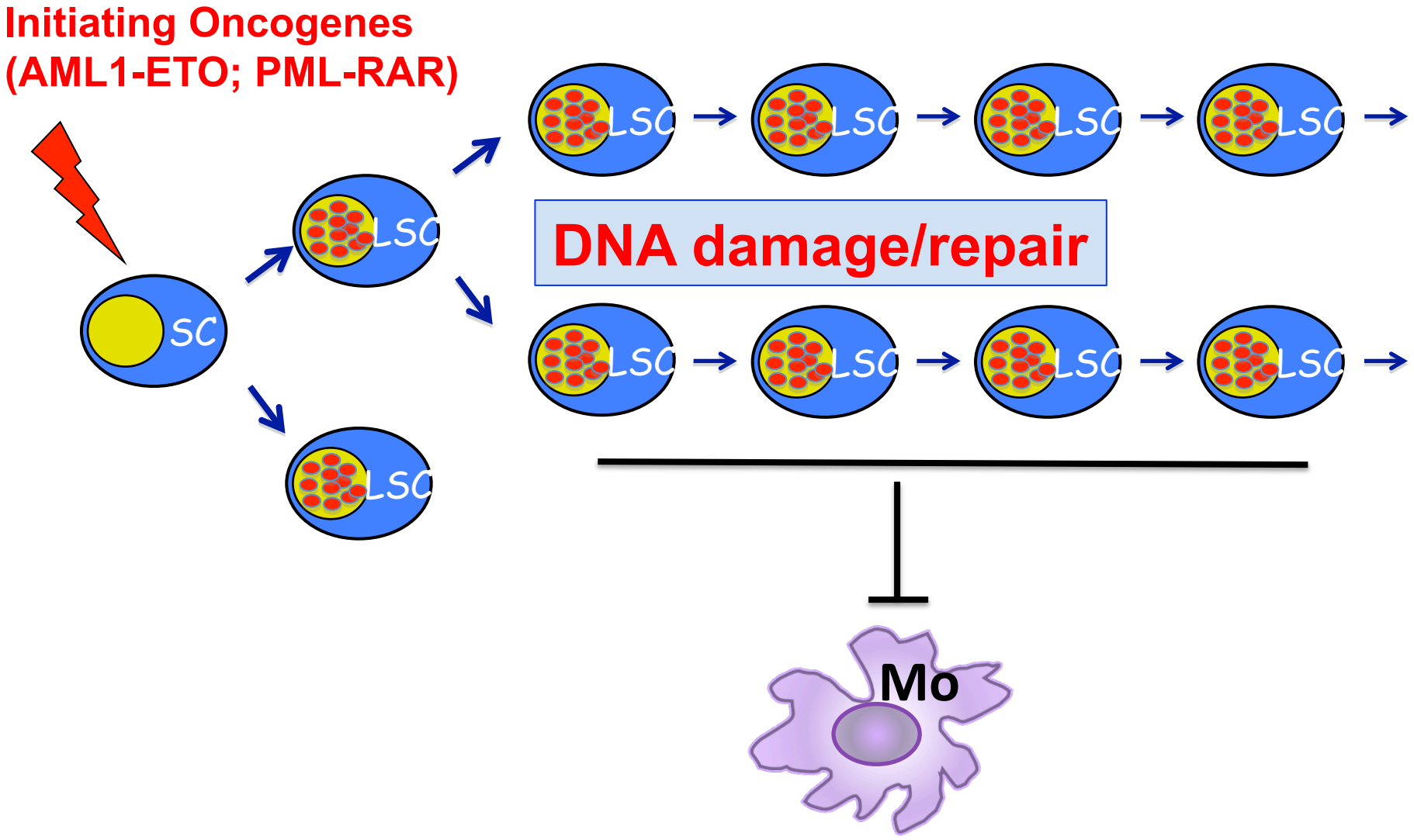


PML-RAR leukemia	Population	Leukaemia development in syngenic mice
P21-/-	Bulk	0/8
	MHCII+	0/2
	MHCII–	8/8
WT	Bulk	2/2
	MHCII+	2/2
	MHCII–	3/3

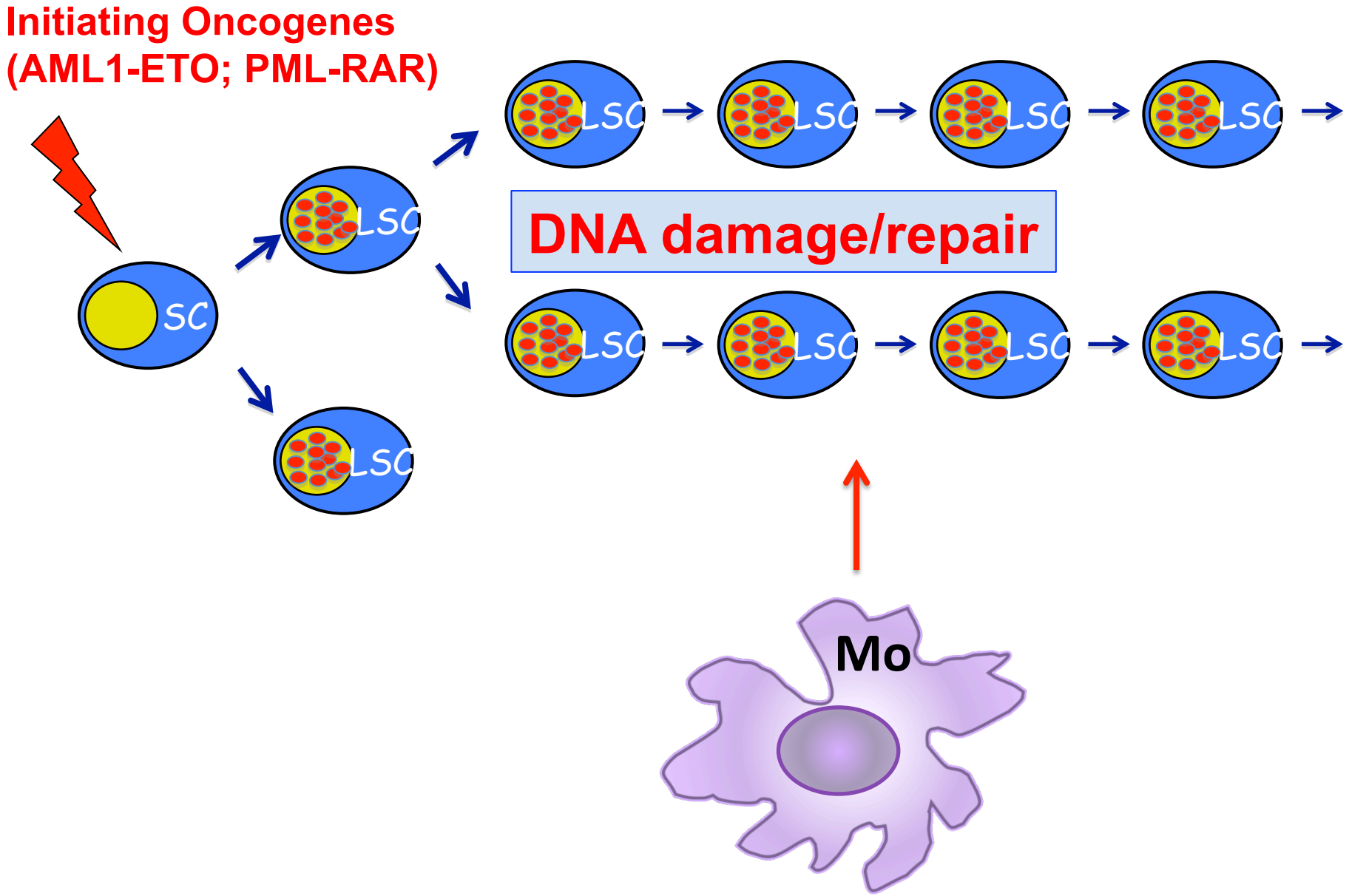
Working hypothesis:
p21 regulates
the antigen-presenting function
of Macrophages



Leukemia SCs must evade a macrophage-dependent Immune-surveillance mechanism



Macrophage activation (by p21 attenuation)
Activates an anti-tumor immune response



Do Macrophages mediate clearance Of damaged Hematopoietic Stem Cells?