Memory $CD8^+$ T cells induce precocious effector differentiation of naïve $CD8^+$ T cells in a FasL-Fas dependent manner

Youth corrupted.

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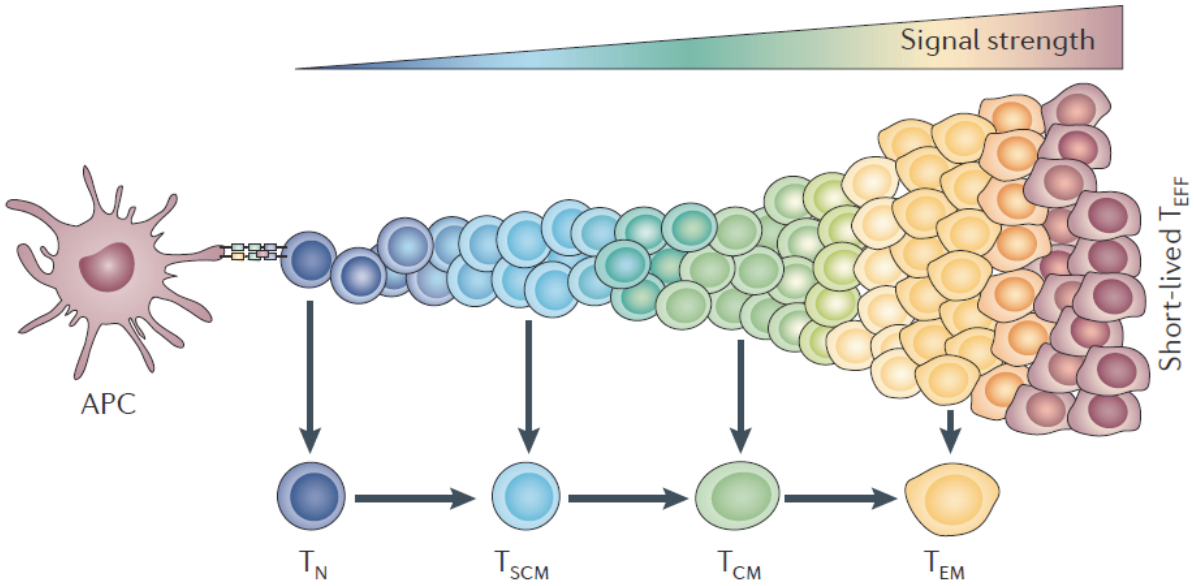
SITC 27th Annual Meeting, October 26th, 2012.

Disclosures

- U.S. Patent Application No. 61/623,733:

  Exposing T cells to Fas Ligand (FasL)-Fas Receptor (FasR) Antagonists Withholds Differentiation and Increases Expansion Making T cells More Suitable for Use in Cancer Immunotherapy

CD8⁺ T cells move through progressive stages of differentiation



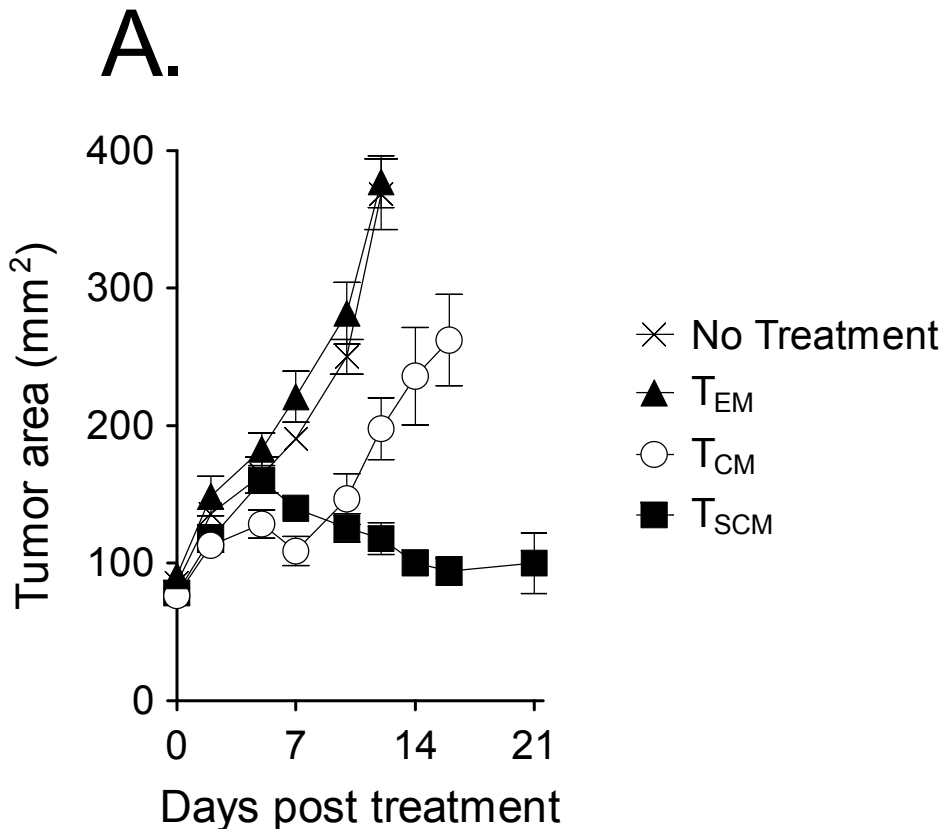
Phenotype	T_N	T_{SCM}	T_{CM}	T_{EM}
CCR7	+++	+++	++	–
CD62L	+++	+++	++	–
CD27	+++	+++	++	+/–
CD28	++	+++	+++	+/–
CD45RA	+	+	–	+/–
CD45RO	–	–	+	+
CD122	–	++	+++	+++
CD95	–	++	+++	+++
KLRG-1	–	–	+/–	++

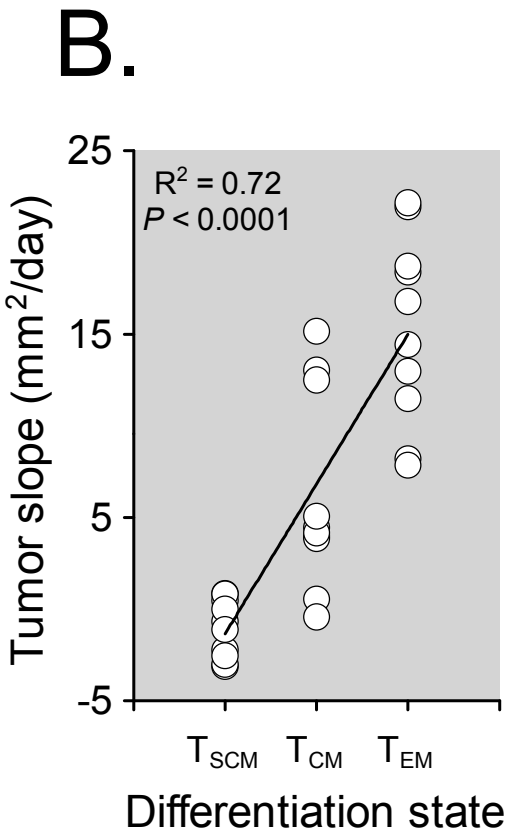
Fas =

Nature Reviews | Cancer

Gattinoni, Klebanoff and Restifo, *Nature Rev. Cancer*, 2012.

CD8+ T cell differentiation status is highly correlated with anti-tumor efficacy in mice





Klebanoff C.A. *et al*, *Clin Cancer Res* 2011.
Gattinoni L. *et al*, *Nat Med* 2009.

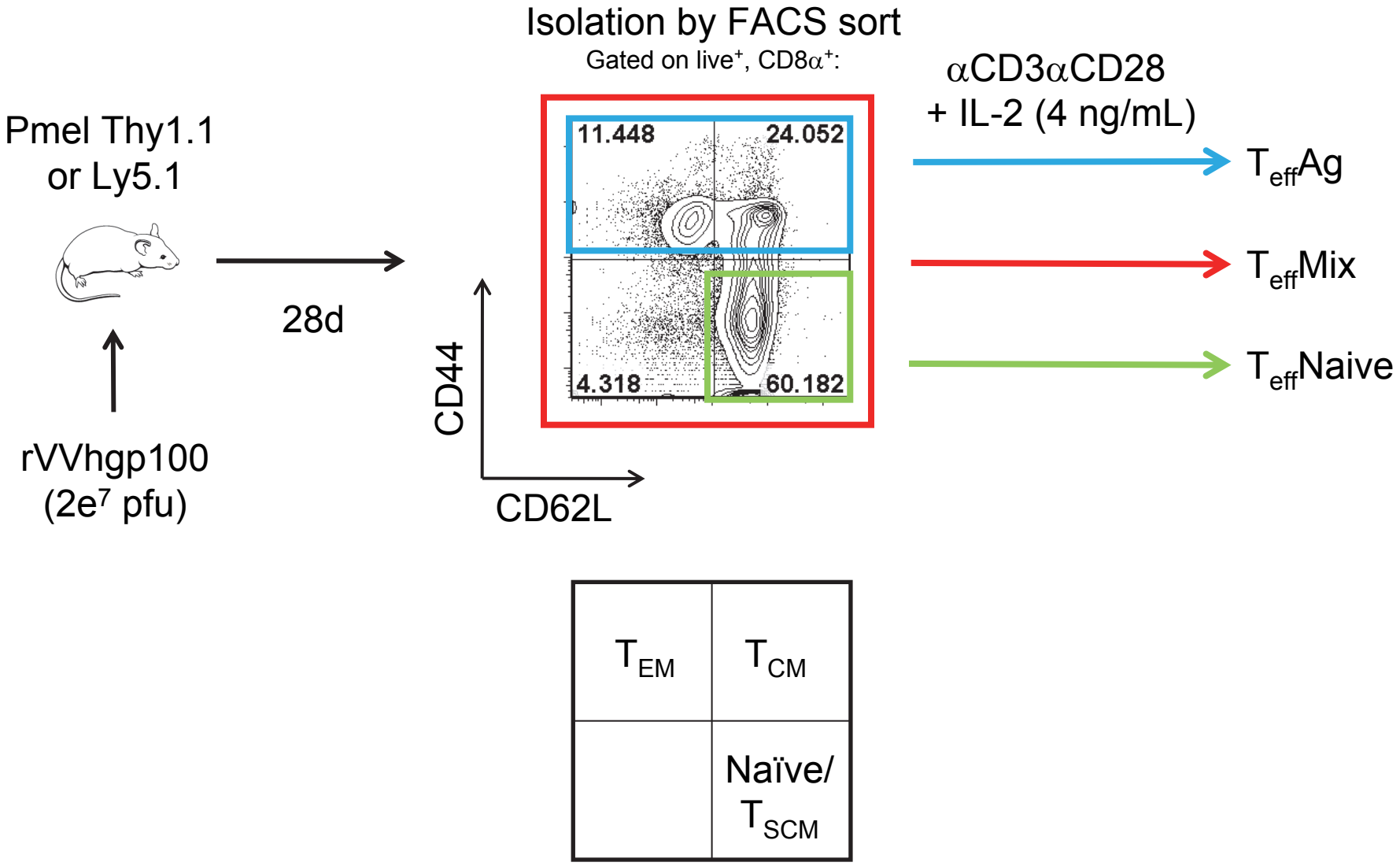
“At present, these is no evidence that the presence of “older” cells with limited potential for clonal expansion within cell grafts containing “younger” cells is detrimental."

-Linnemann, Schumacher, and Bendle, J Invest Derm 2011.

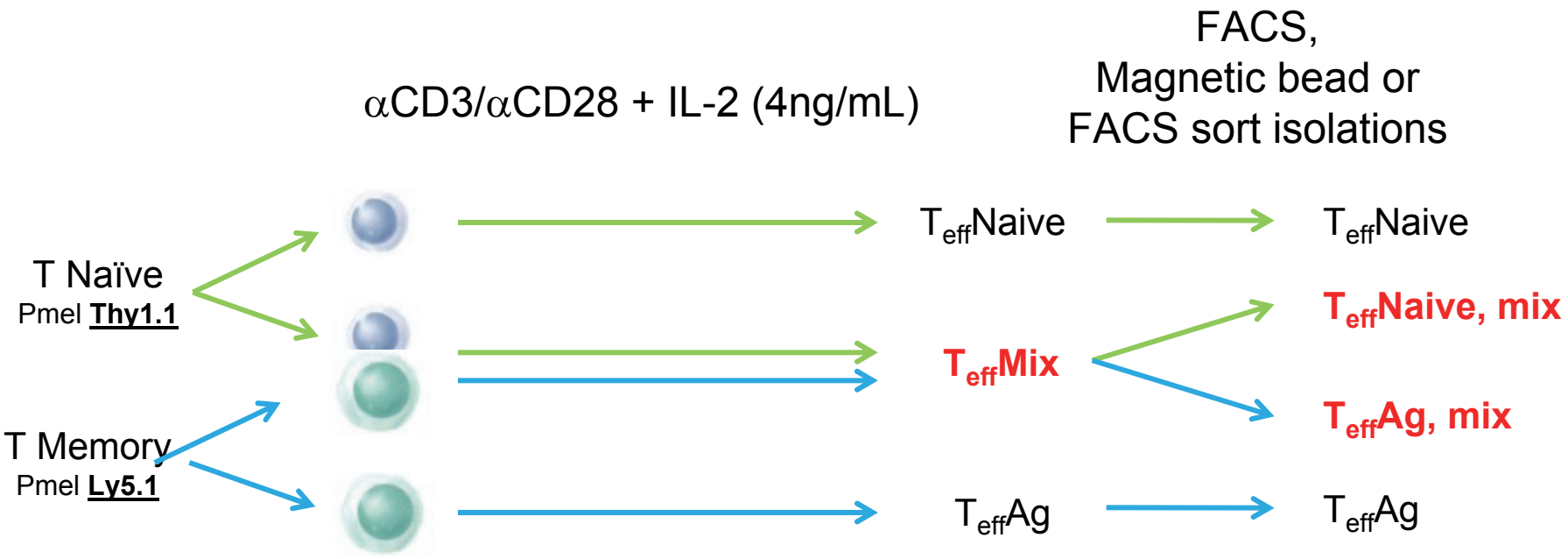
Central experimental question:

- Is it strictly necessary to physically isolate naïve $CD8^+$ T cells from other T cell subsets, or is their presence sufficient to convey optimal *in vivo* expansion, persistence, and anti-tumor function?

Generation, isolation, and expansion of *in vivo* generated tumor-reactive CD8$^+$ T cell subsets for ACT

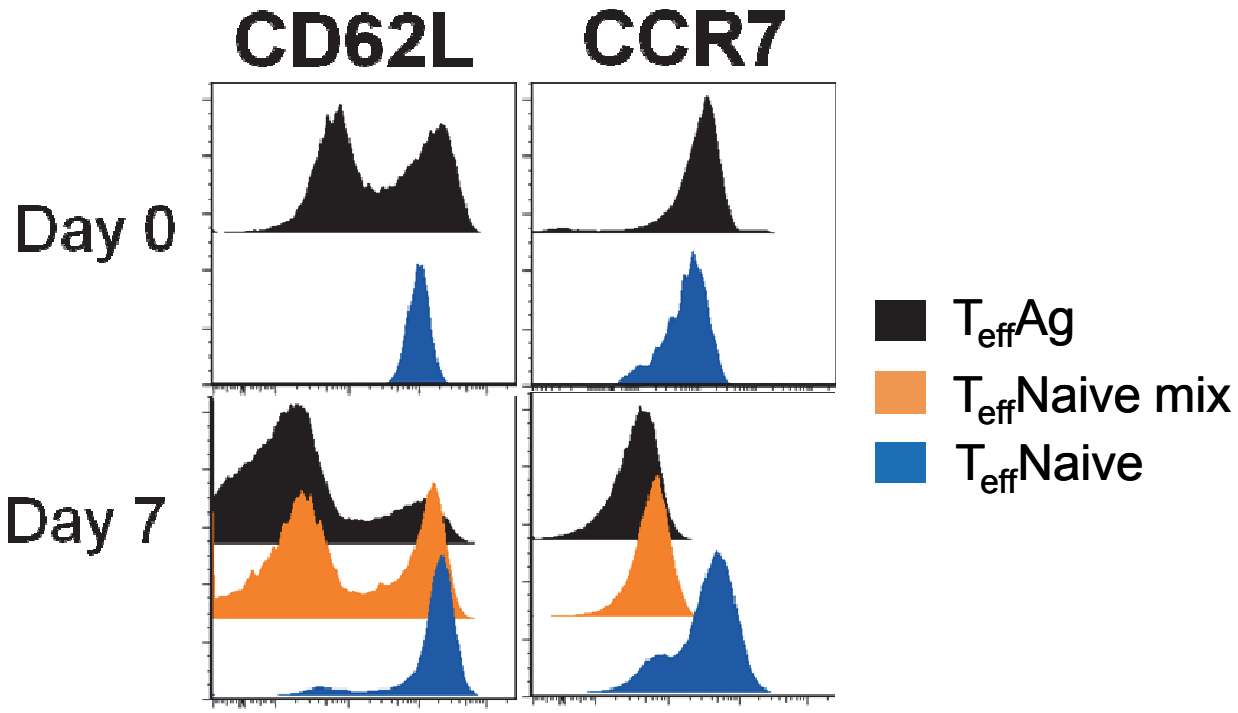


Indelible fate tracking of CD8⁺ T cells subsets using congenic markers

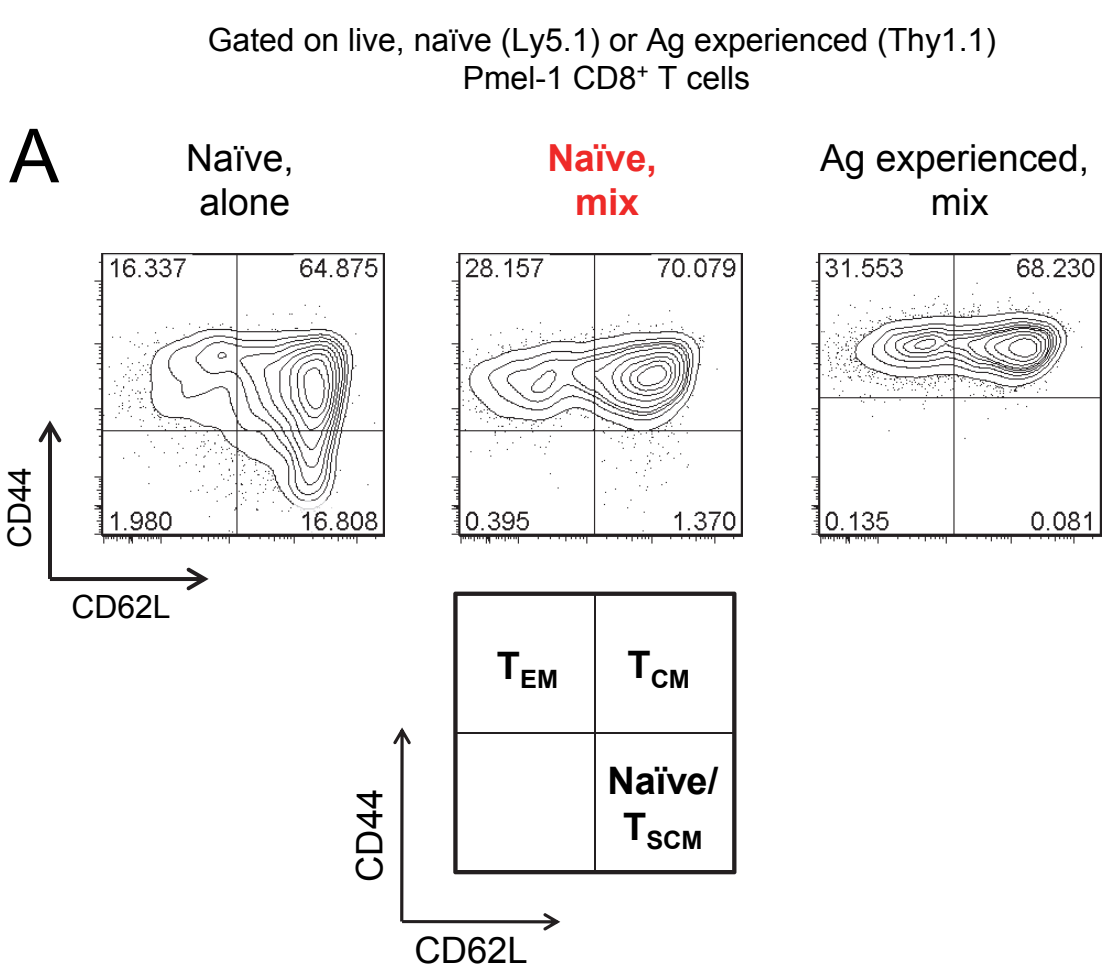


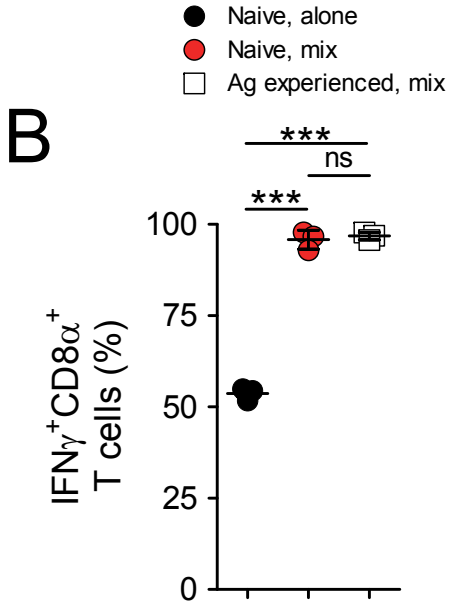
Naïve CD8⁺ T cells differentially lose CD62L and CCR7 when primed with Ag experienced CD8⁺ T cells

Gated on live, naïve (Ly5.1) or Ag experienced (Thy1.1)
Pmel-1 CD8⁺ T cells



Naïve CD8⁺ T cells acquire greater effector functions when primed with Ag experienced T cells

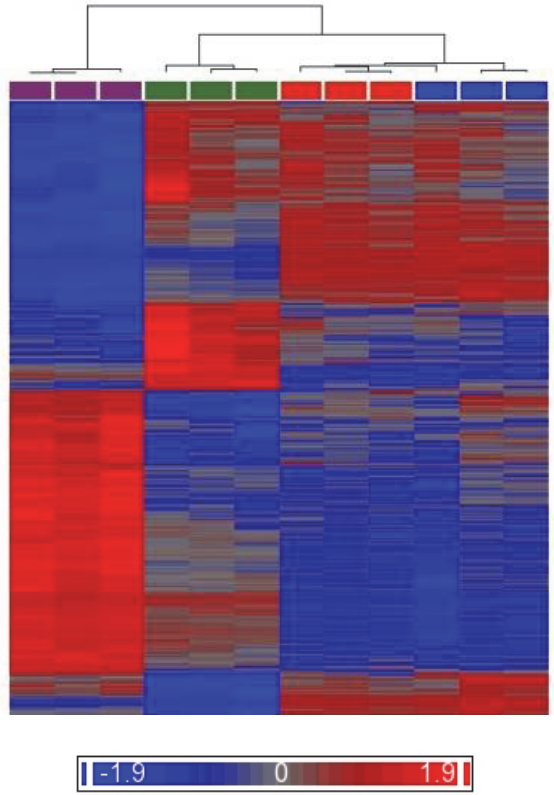




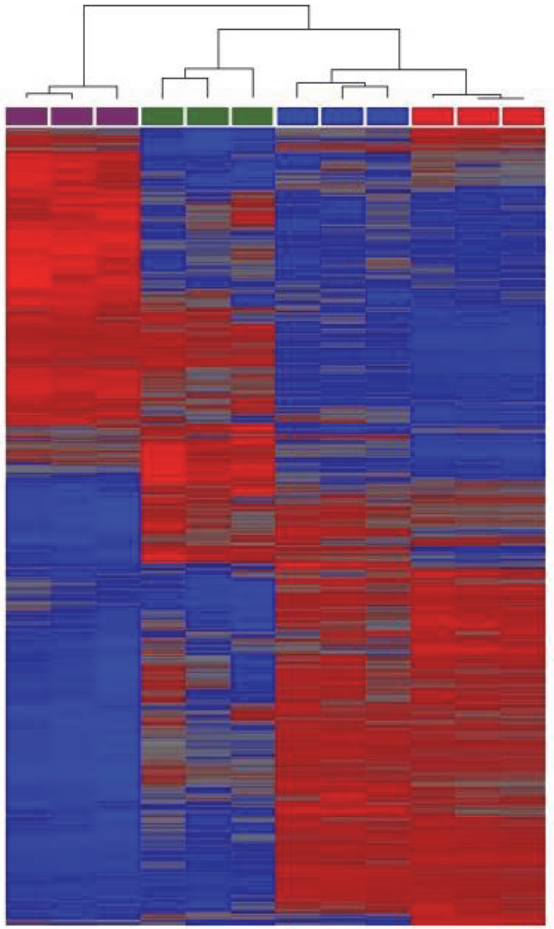
Naïve-derived CD8$^+$ T cells cluster genetically with Ag experienced T cells when expanded together

T_{eff}Naive T_{eff}Naive mix T_{eff}Ag mix T_{eff}Ag

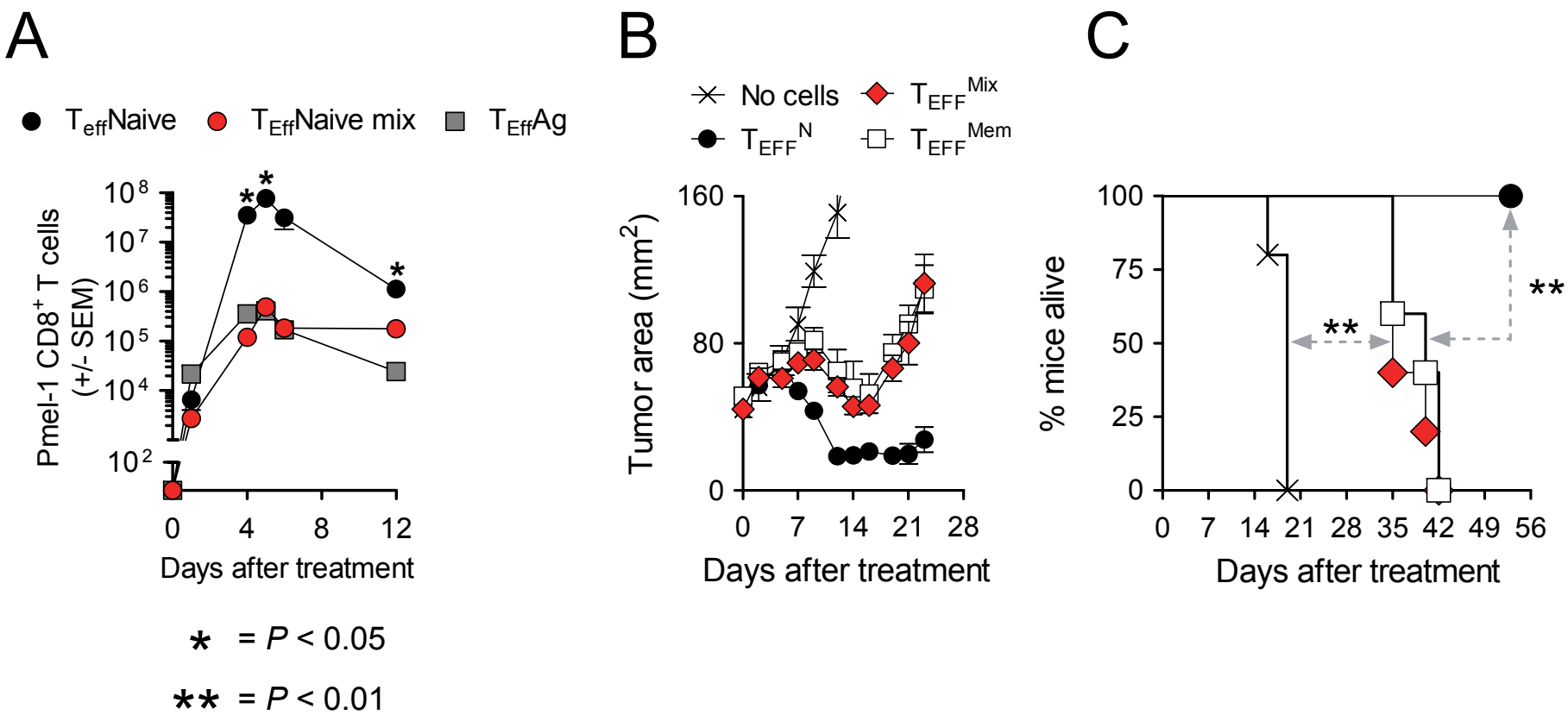
18h



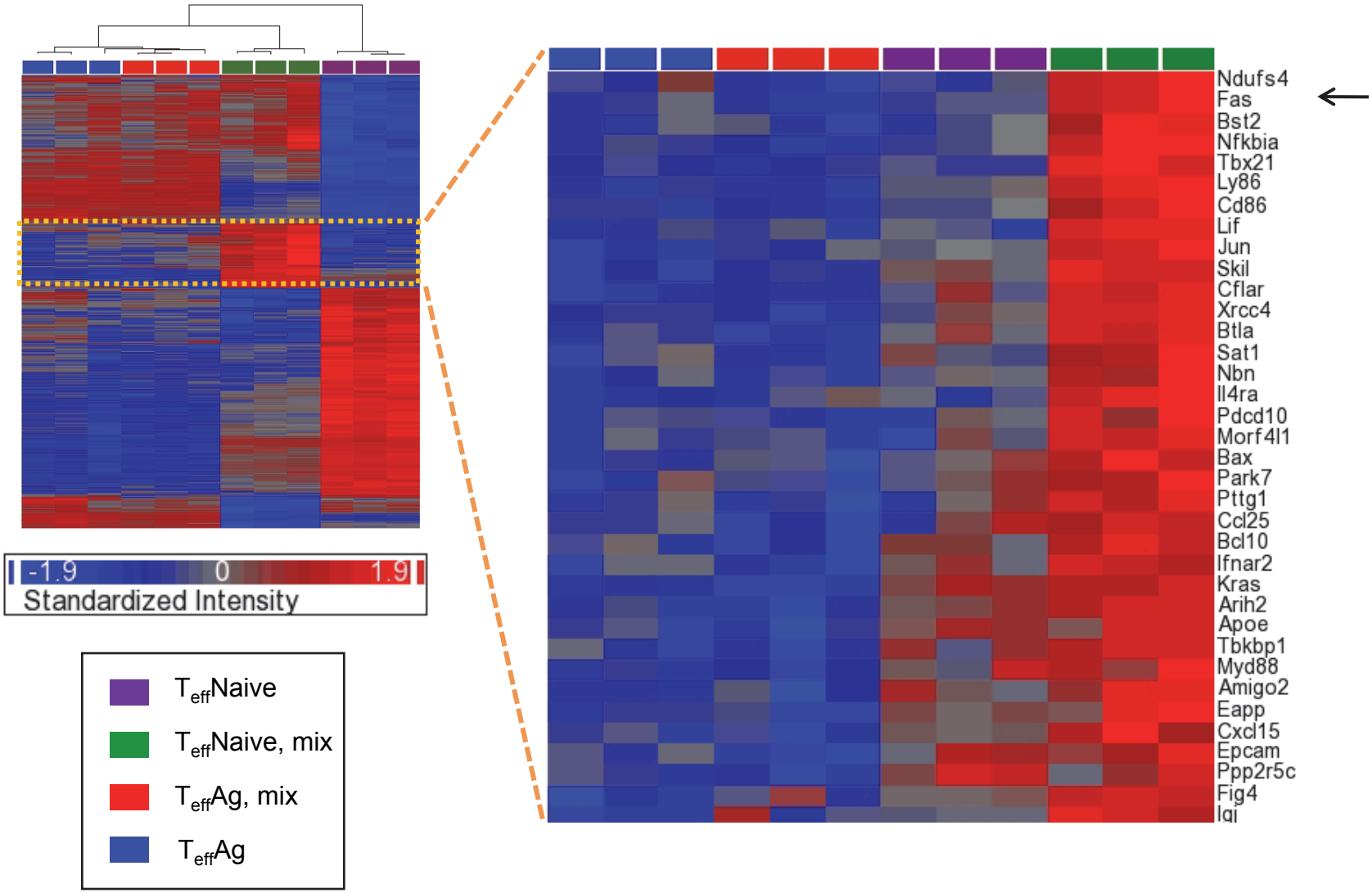
96h



Naïve CD8$^+$ T cells primed *in vitro* with Ag experienced T cells have impaired expansion and anti-tumor efficacy

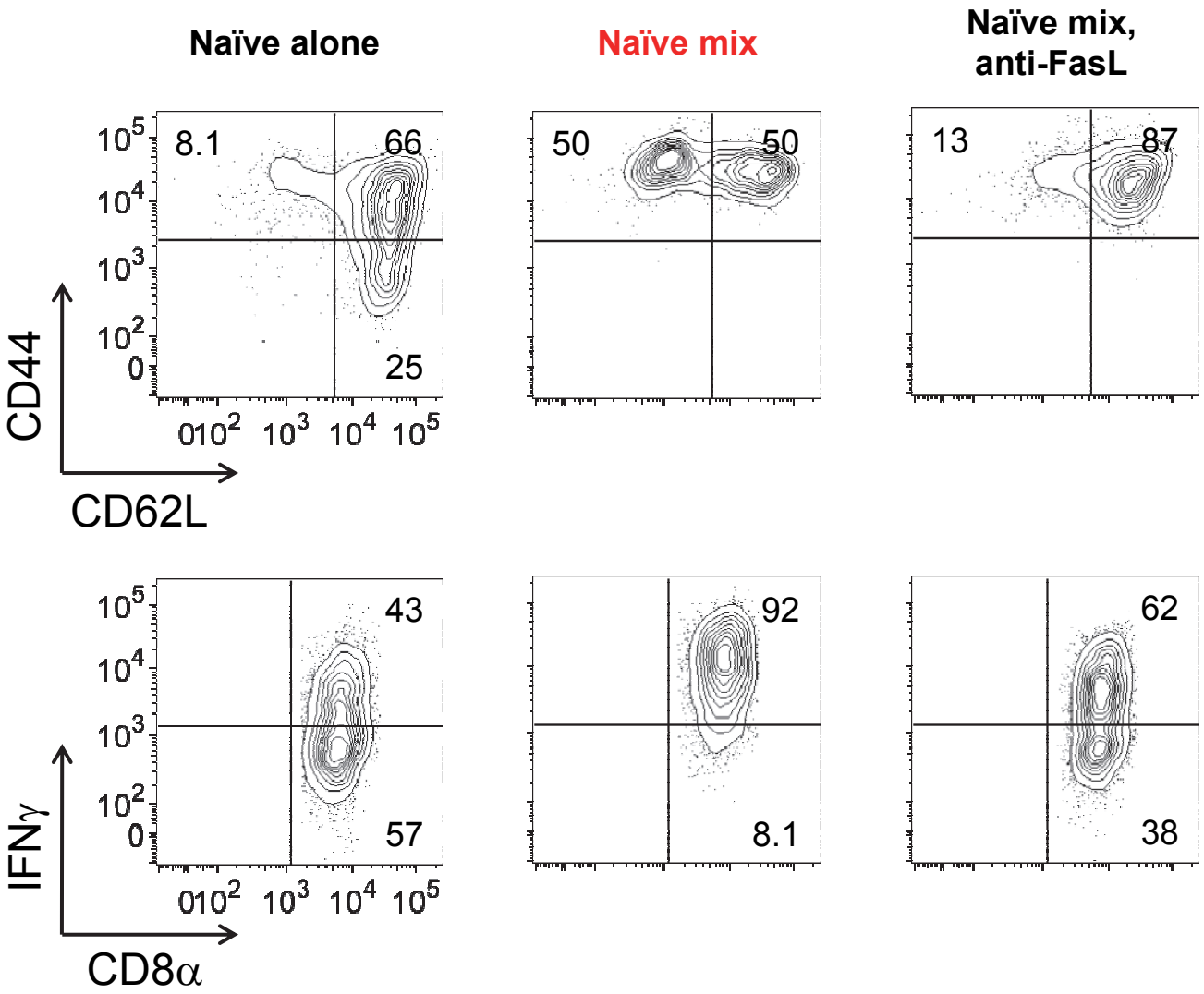


What factor(s) are responsible for inducing precocious differentiation?

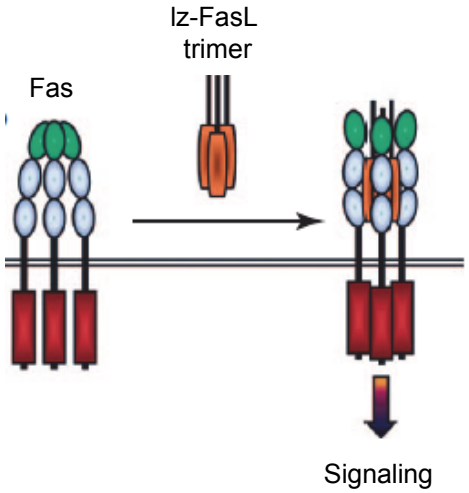


Precocious effector differentiation of naïve-derived effector cells can be retarded by blockade of FasL

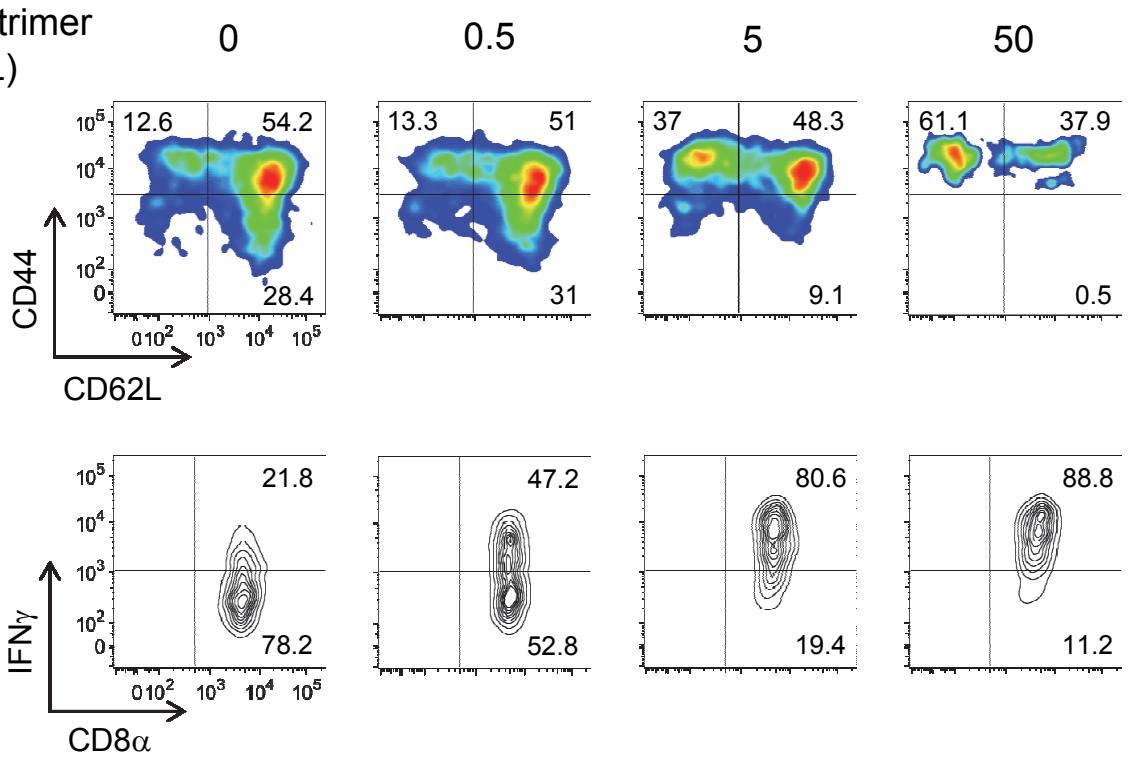
Gated on live$^+$, CD8α^+, naïve-derived cells:



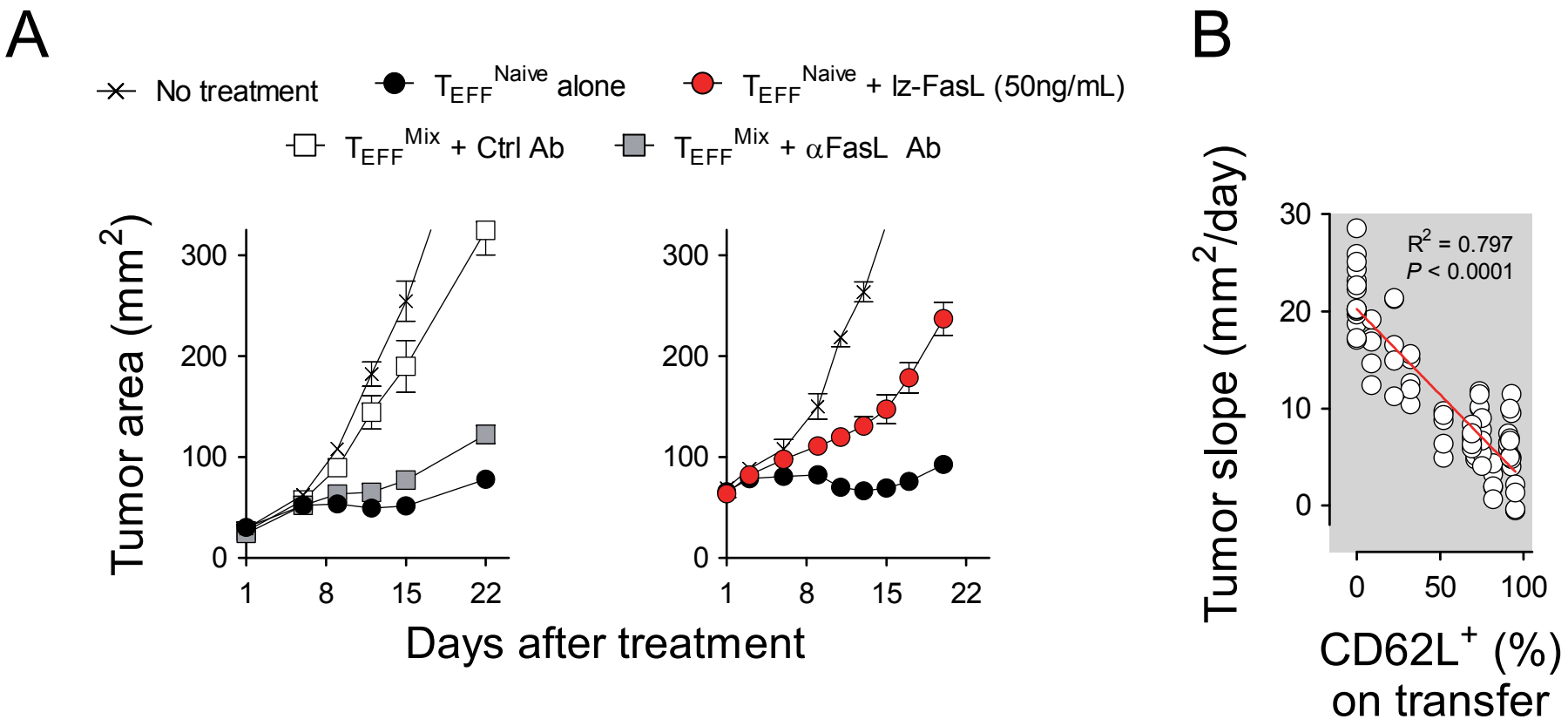
FasL trimer can induce precocious differentiation of naïve CD8⁺ T cells independently of Ag experienced T cells



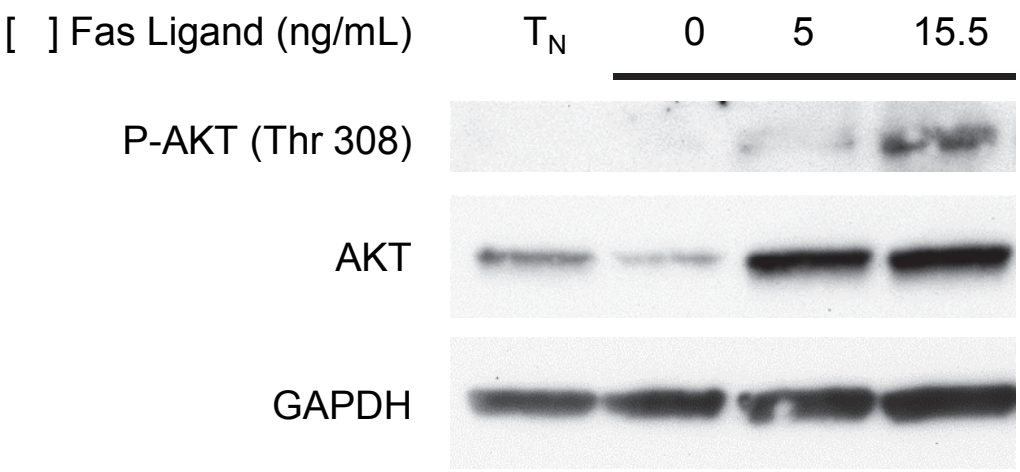
Lobito AA *et al*, Trends Mol Med, 2011.



Modulation of Fas-signaling causes dynamic changes in the phenotypic and functional qualities of CD8+ T cells

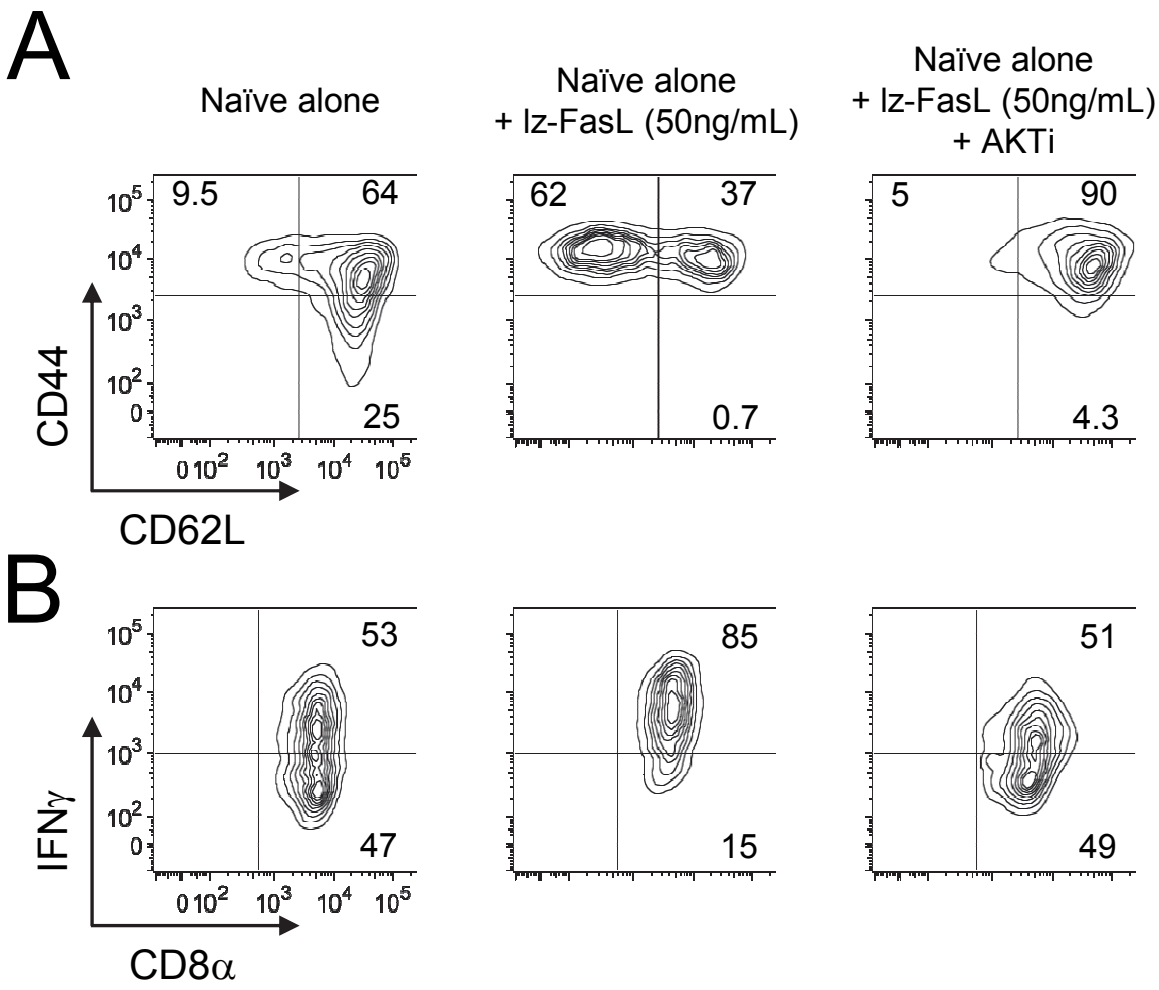


FasL induces dose-dependent activation of the pro-differentiation AKT pathway in naïve CD8$^+$ T cells



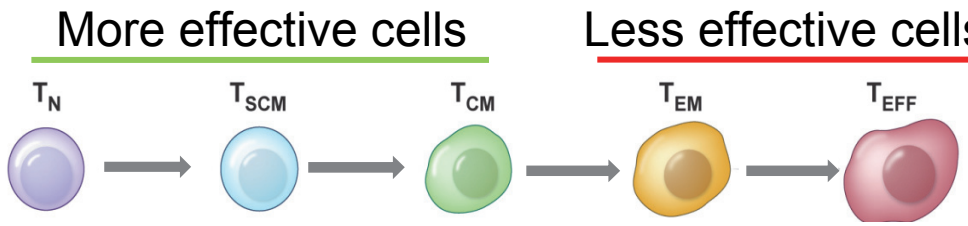
Naïve enriched Pmel-1 CD8$^+$ T cells
Expanded with αCD3/αCD28 x 24h +/- lz-FasL

Inhibition of AKT blocks precocious differentiation induced by signals delivered through FasL



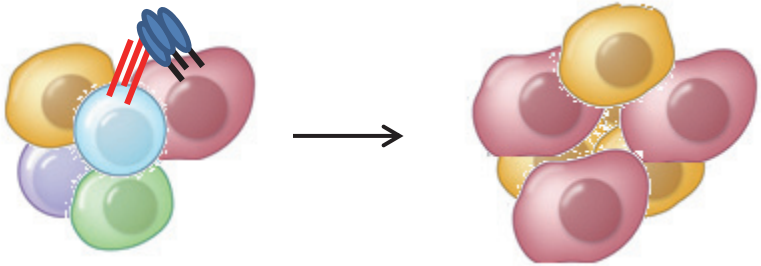
Summary and conclusions

Preservation of youth

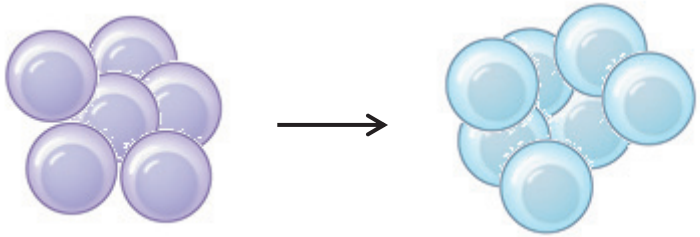


. . . 'younger' cells remain in contact with 'older,' more differentiated cells

Fas/FasL



. . . younger cells are physically separated away from 'older' cells



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