Epigenetic control of CAR T cell anti-tumor activity and persistance

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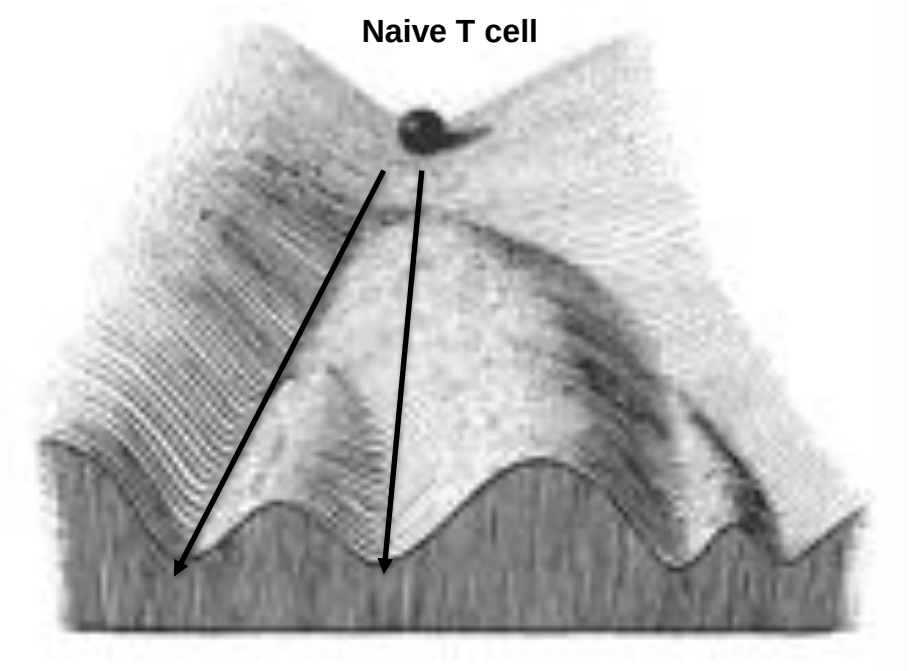
Disclosures

Scientific founder, share holder, Mnemo Therapeutics

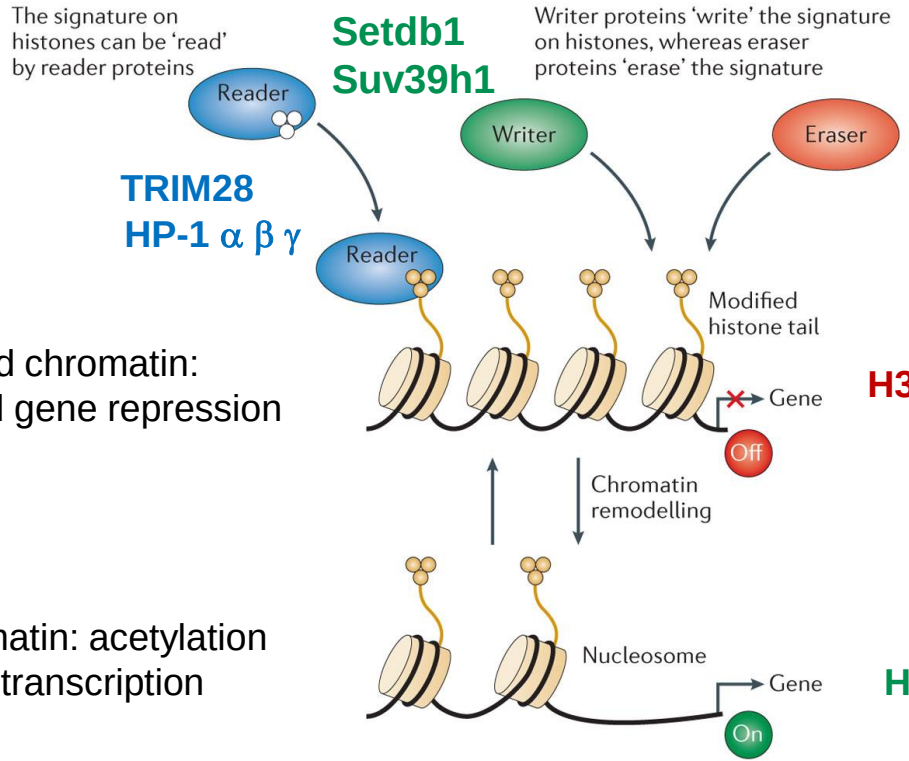
SAB member 2021 :

- Stimmunity
- Biomunex
- Innate Pharma
- Neovacs

Is heterochromatin involved in lineage comitment in CD4+ and CD8+ T cell responses ?



Heterochromatin is a hallmark of silent chromatin: constitutive and facultative heterochromatin



Compacted chromatin: methylation and gene repression

H3K9me3

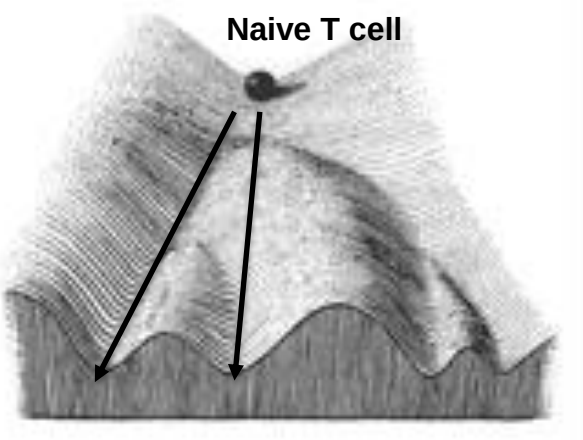
Suv39h1 KO

Relaxed chromatin: acetylation and active transcription

H3K9ac

Adapted from Nature Reviews | Drug Discovery

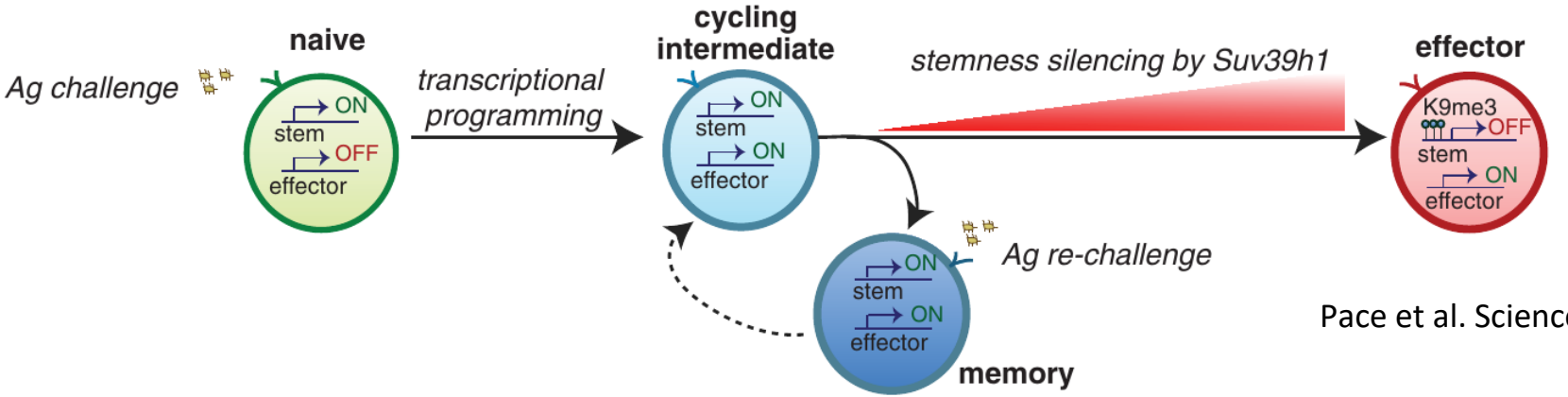
Heterochromatin is involved in lineage comitment in CD4+ and CD8+ T cell responses



Suv39h1 is a histone methyltransferase that controls plasticity of lineage commitment in T cell differentiation

CD4+ T cells	**Th1**	**Th2**	**Th1 lineage suppression in Th2 (**Allan et al, Nature 2012)
CD8+ T cells	**Memory**	**Effector**	**CD8+ T stemness silencing in effectors** (Pace et al, Science 2018)

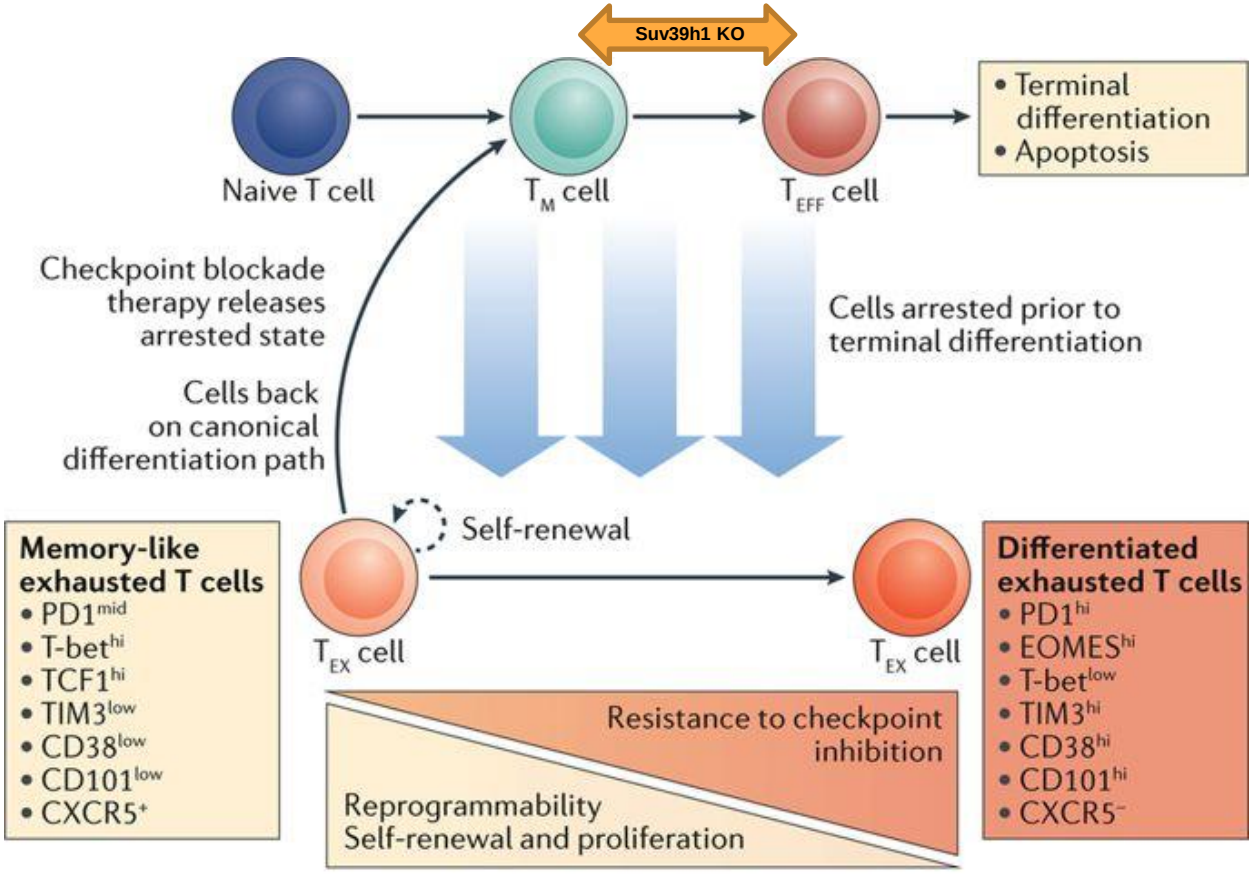
SUV39h1 silence stem/memory genes during CD8+ T effector terminal differentiation.



Pace et al. Science, 2018

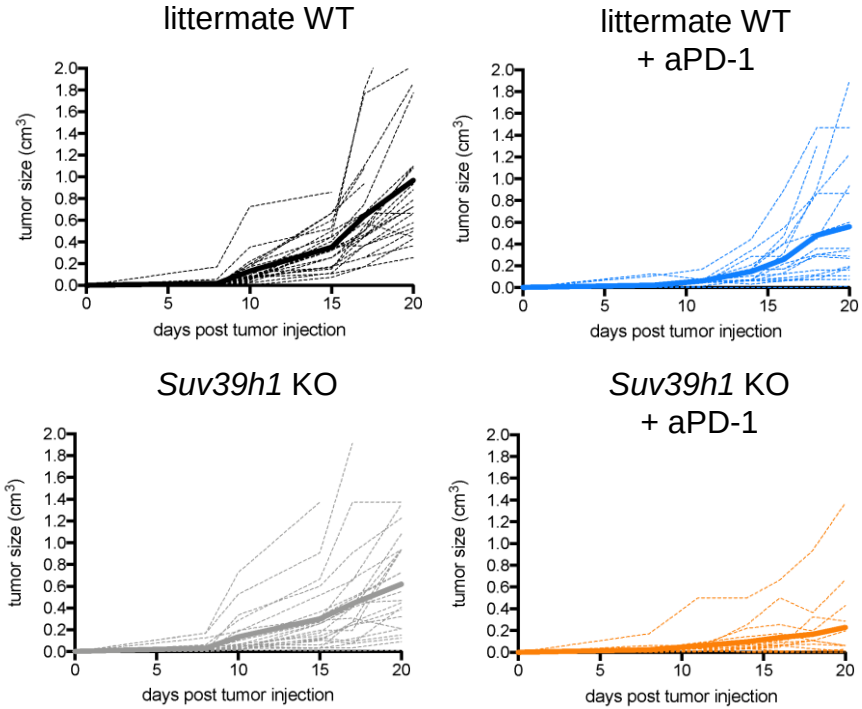
- Suv39h1 silences stem/memory gene expression during murine CD8+ T effector terminal differentiation in a *Listeria monocytogenes* infection model.
- Suv39h1 KO CD8+ T cells have increased plasticity between memory and effector phenotypes.

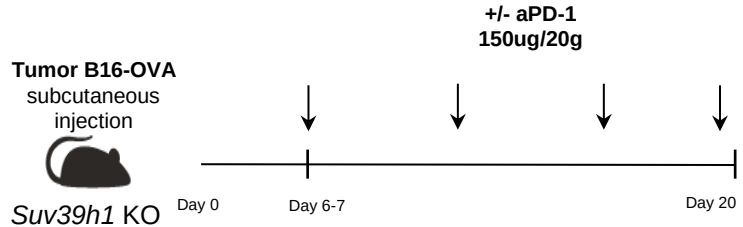
SUV39h1 silence stem/memory genes during exhausted CD8+ T terminal differentiation

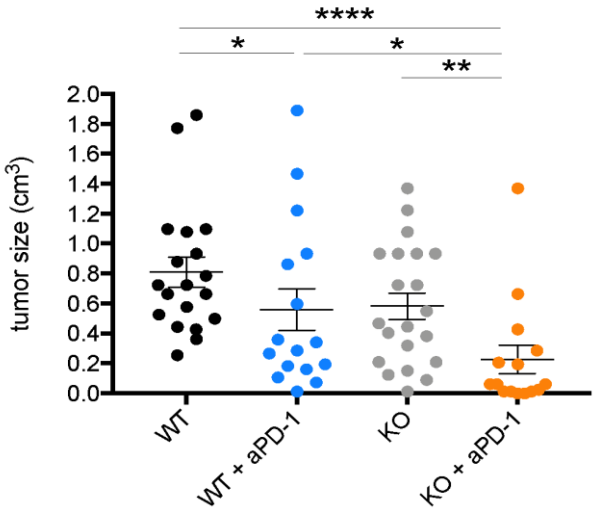


Nature Reviews | Immunology

SUV39h1 deficient mice and B16.F10-OVA tumor growth

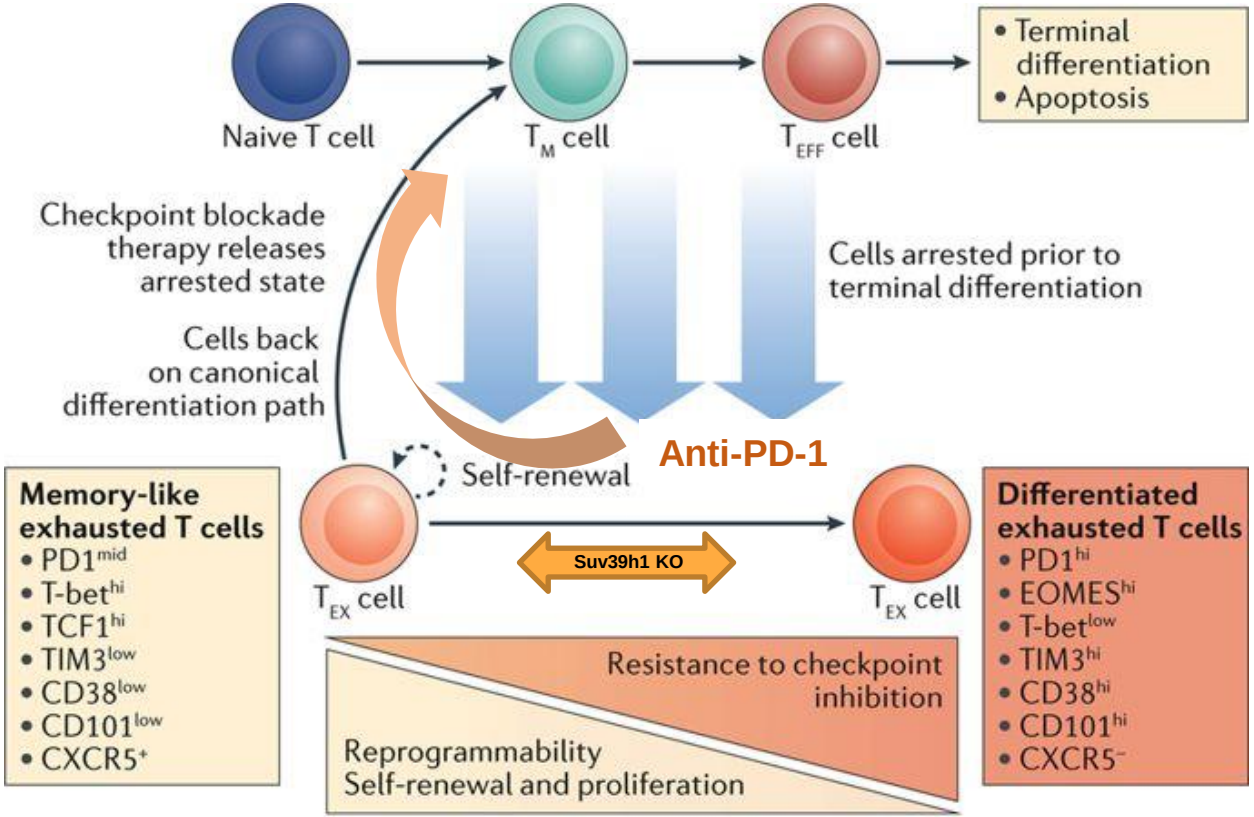






Niborski et al, Nat Comm, 2022

SUV39h1 silence stem/memory genes during exhausted CD8+ T terminal differentiation

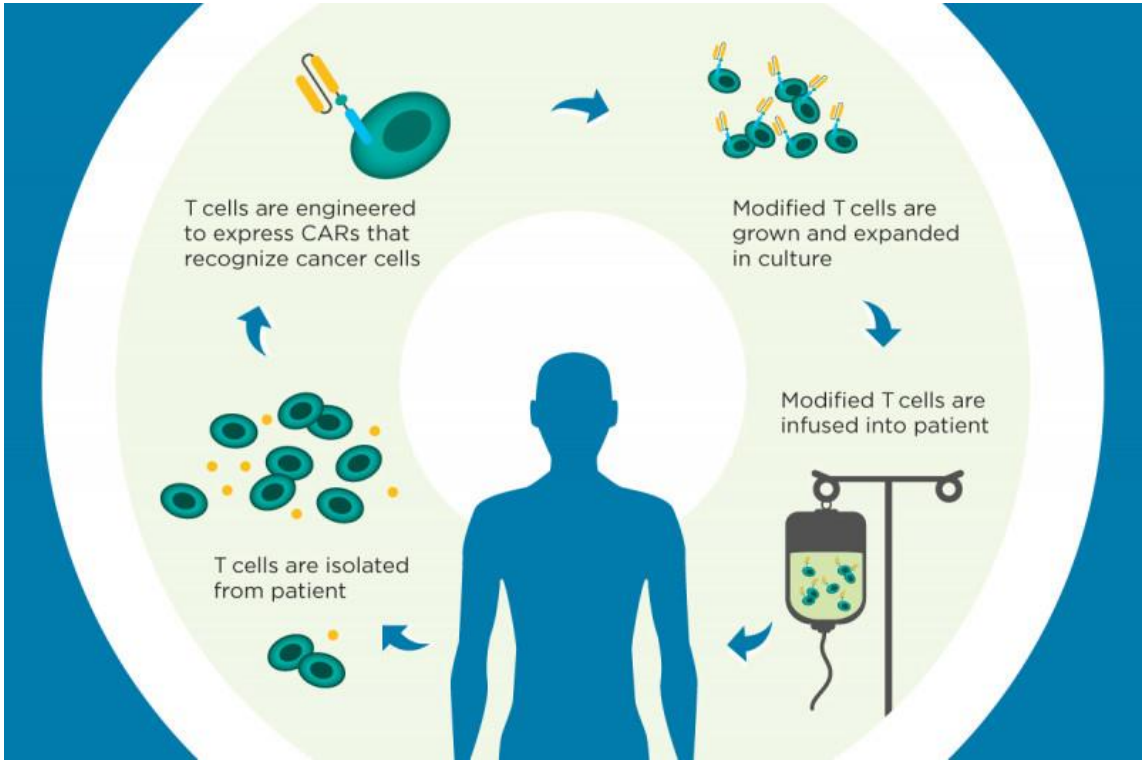


Silencing of stem genes imposes an epigenetic barrier that limits reprograming of terminally differentiated exhausted cells

Suv39h1 contributes to silencing stem-related genes during progression from early memory-like to terminally differentiated exhausted cells

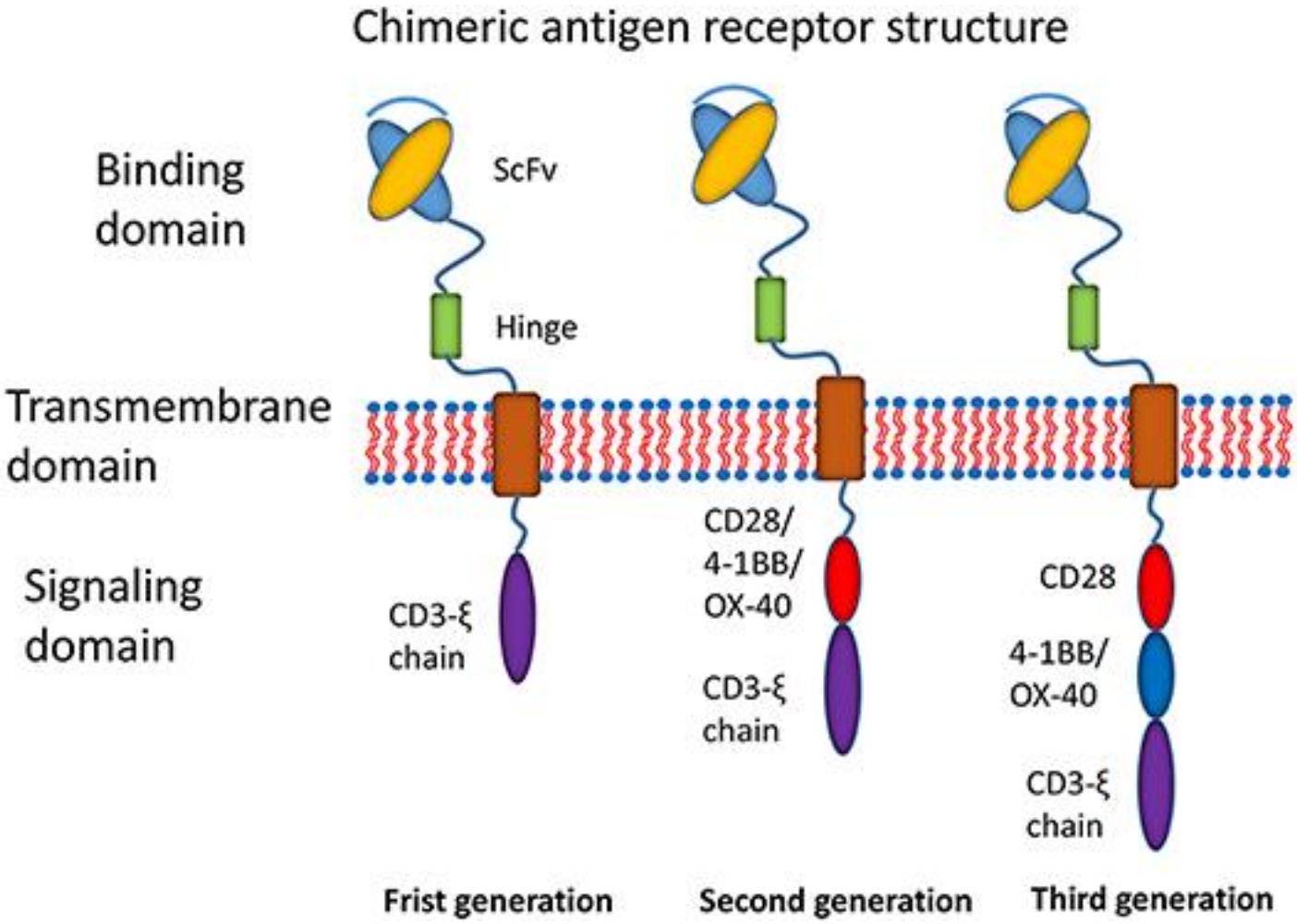
Suv39h1 KO T cells become more sensitive to re-programing by anti-PD-1

CAR T cells



- 80-90% complete responses in different types of B cell malignancies (anti-CD19 CAR-T)
- But… 40-50% relapses after 2 years
- Low responses, high relapses, in solid tumors

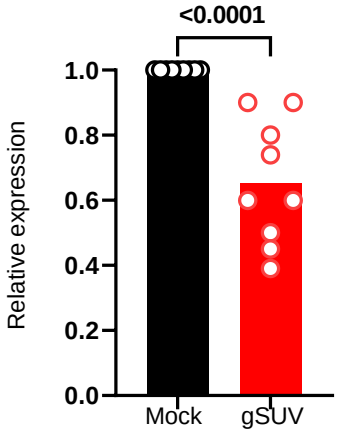
and… stem/memory CAR T cells are more effective than effector CAR T cells


Chimeric antigen receptor structure
Binding domain
Transmembrane domain
Signaling domain
ScFv
Hinge
CD3-ξ chain
CD28/ 4-1BB/ OX-40
CD3-ξ chain
CD28
4-1BB/ OX-40
CD3-ξ chain
Frist generation
Second generation
Third generation

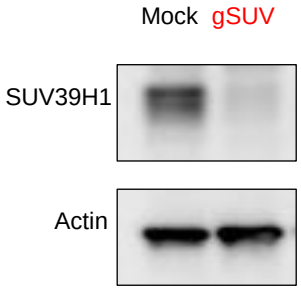
Generation of SUV39H1 deficient human CAR T cells

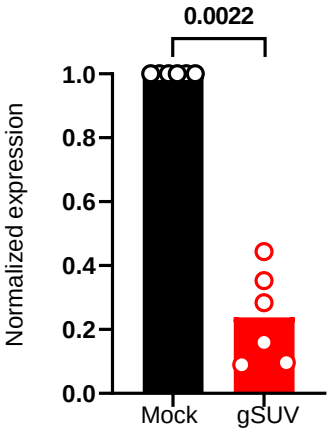
CD19 / 4-1BB / CD3z CAR (19BBz CAR-T), lentivirus

RT-qPCR

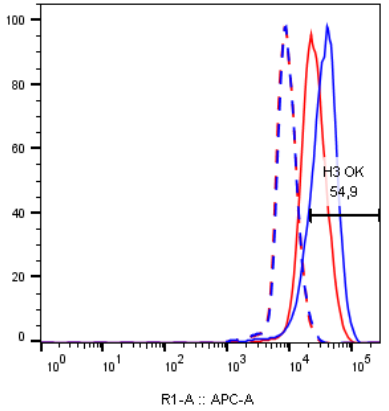


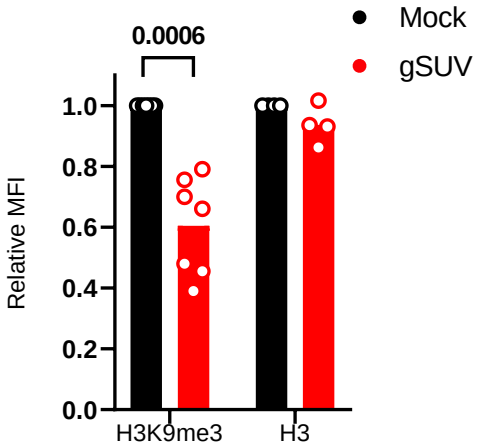
Western blot



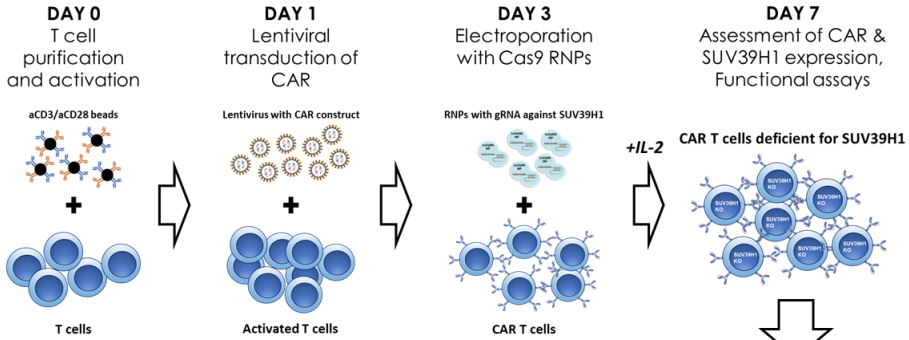


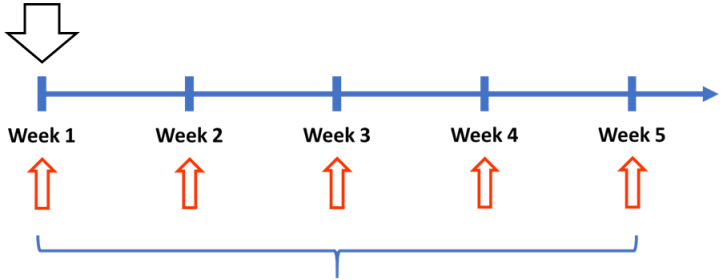
Flow cytometry for H3K9me3





SUV39H1 inhibition increases metabolic fitness of CAR T cells





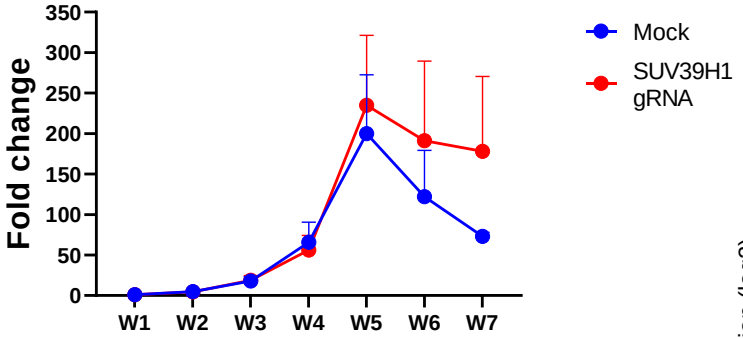
Conclusions:

- Increased memory phenotype
- Increased expansion after 3 re-stimulations
- Increased metabolic fitness (both glycolysis and oxidative respirations are up)

Effect of SUV39H1 inhibition on CAR-T in vitro

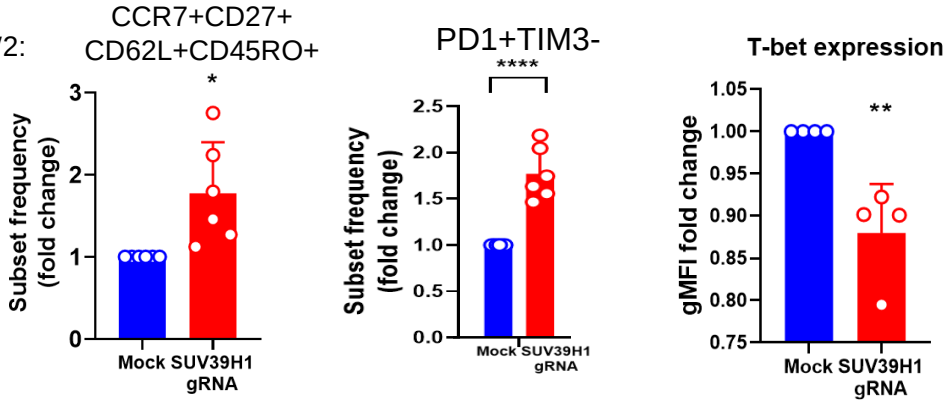
- **Increased expansion**
- **Increased memory**

CD3+ CAR T cells

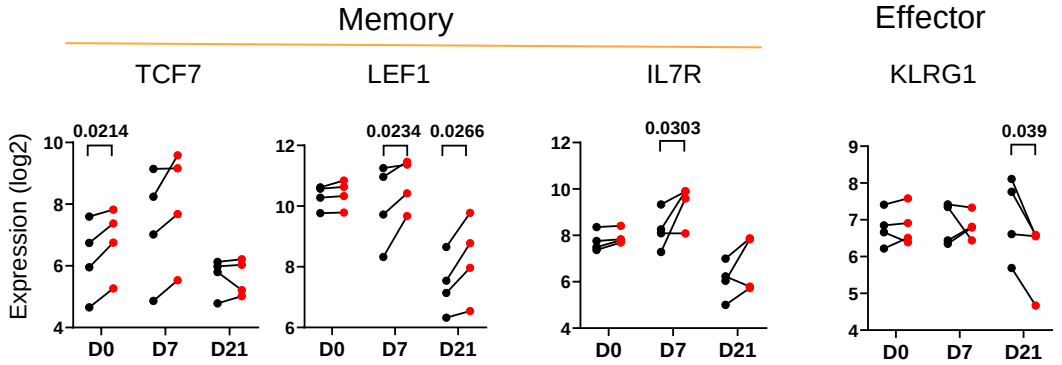


N= 5

W2:



Unpaired t-test, **** p<0.0001, **p<0.01, * p<0.05

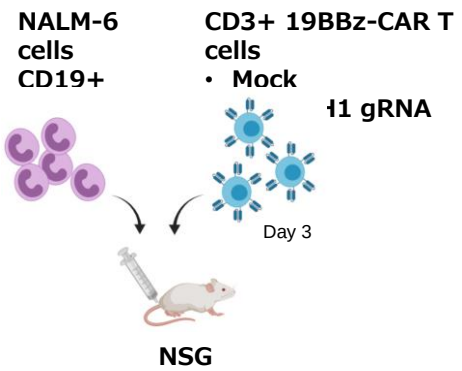


Same cytotoxicity
Same cytokine production

(Nanostring)

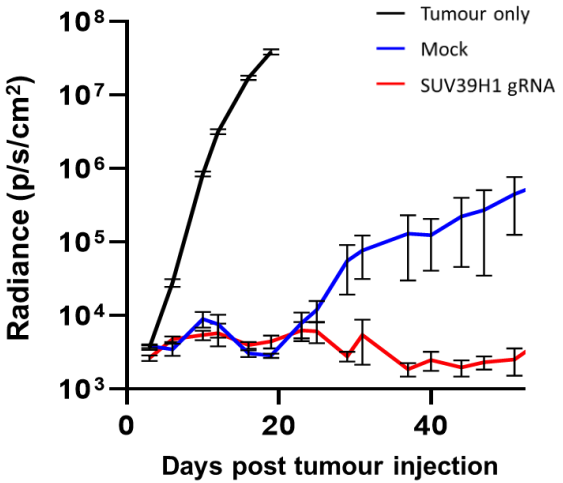
SUV39H1 KO in 19BBz CAR-T increases NALM6 rejection and promotes prolonged survival (hematological tumor, BM)

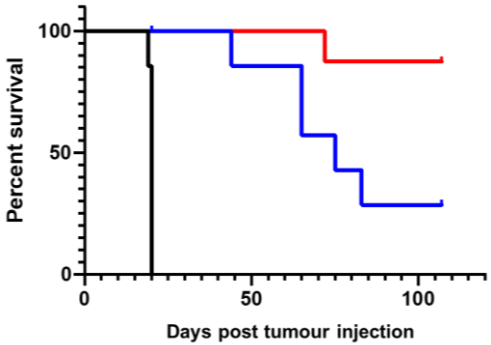
Leukemia model



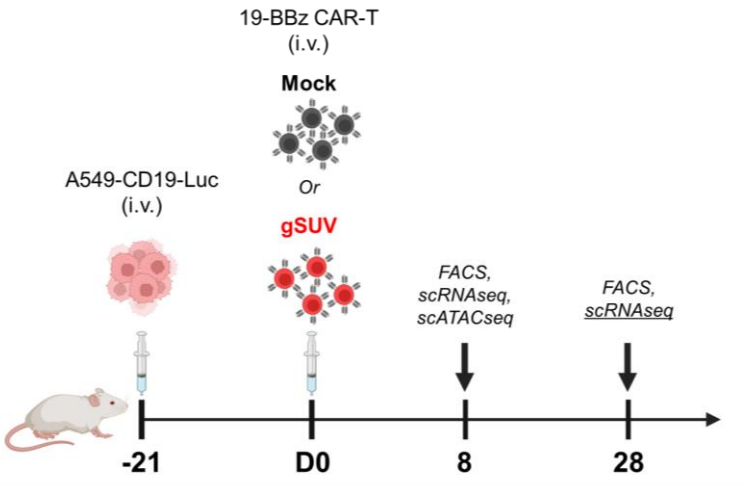
CAR infusion at day 3-4

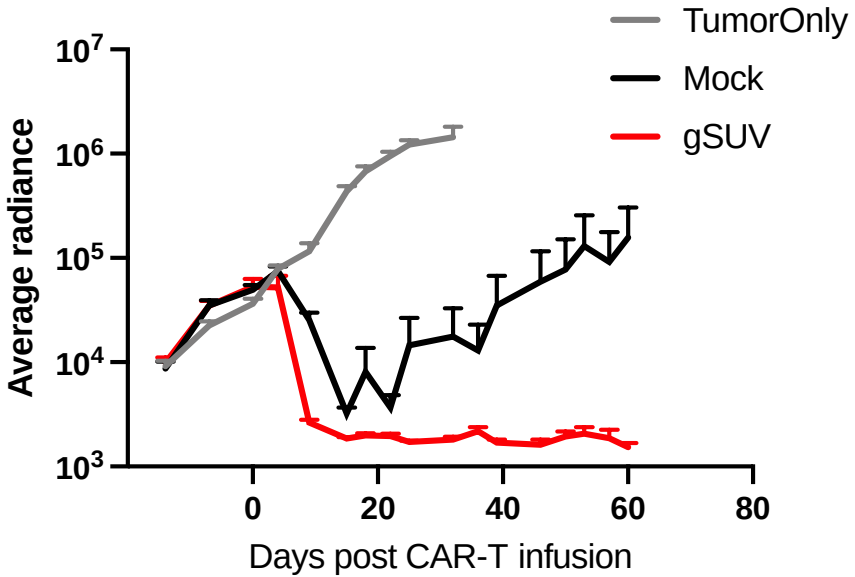
($5x10^5$)



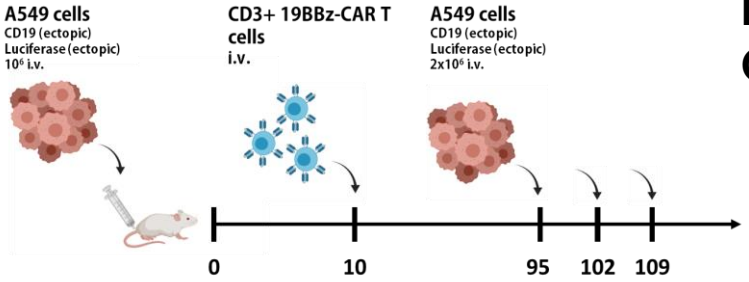


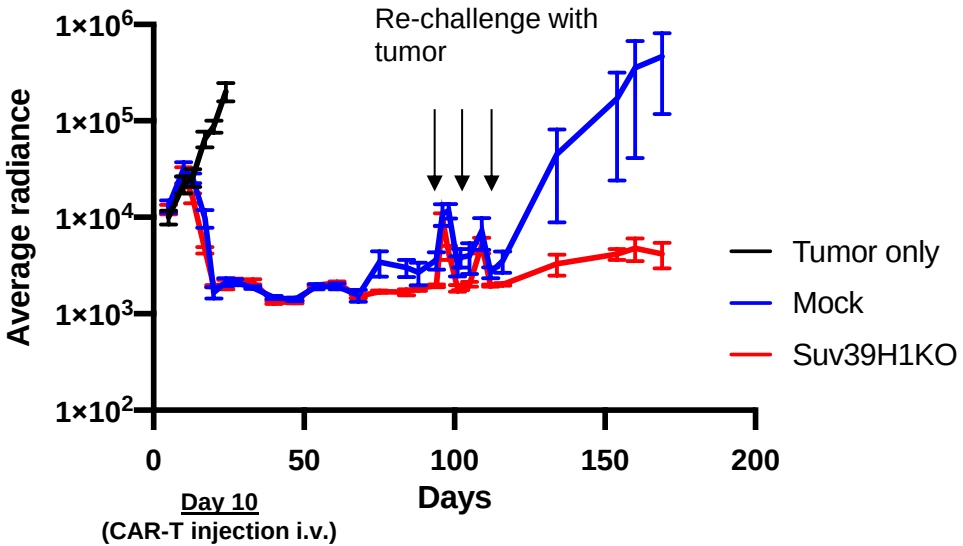
SUV39H1 KO in 19BBz CAR-T increases A549 rejection and promotes prolonged survival (solid tumor, lung)

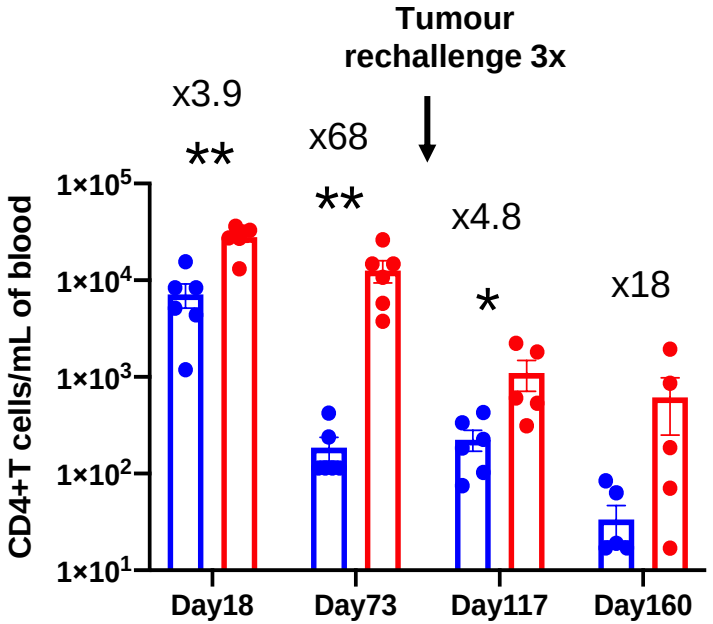




Increased persistence of SUV39H1 KO 19BBz-CAR T cells during tumour rejection

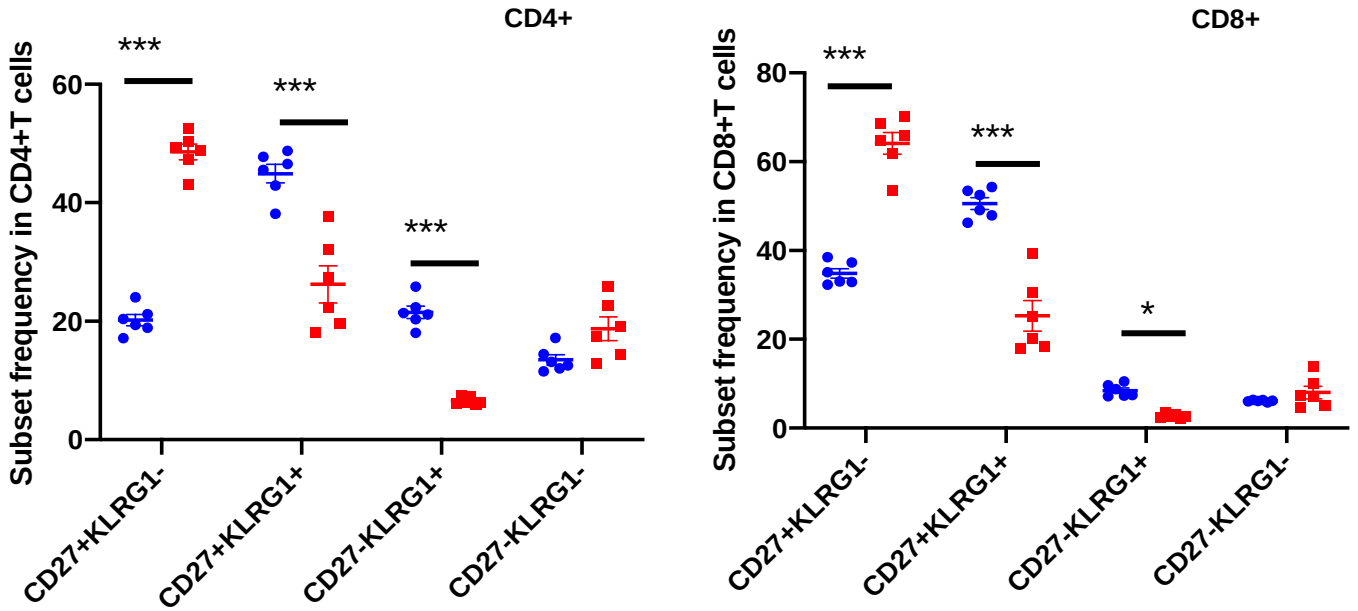




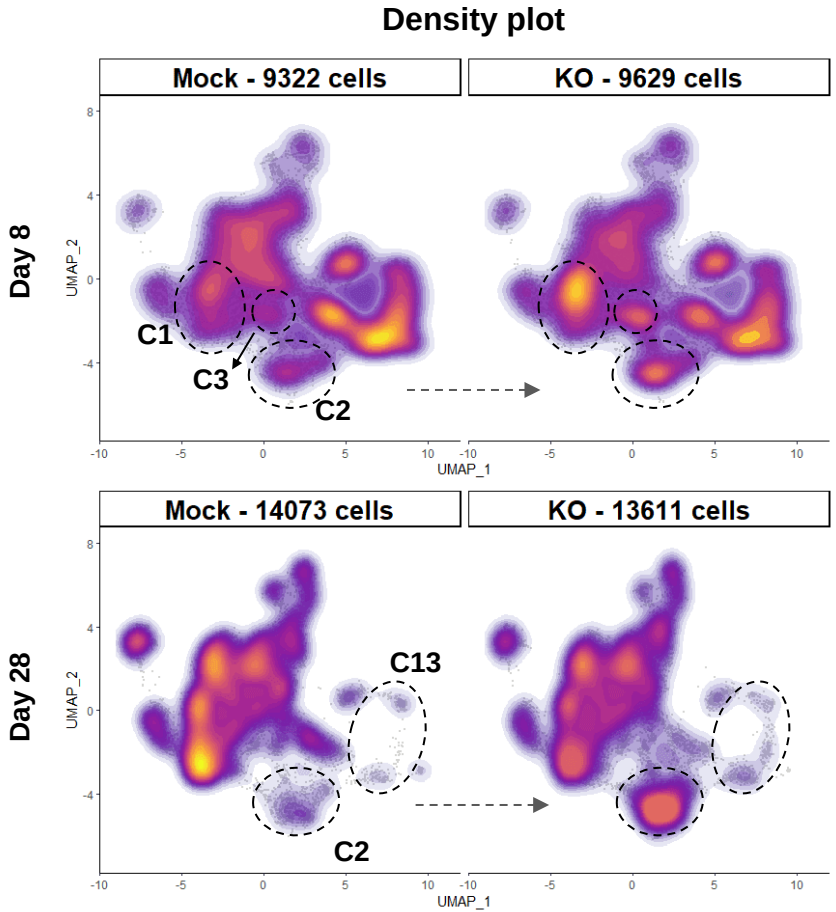


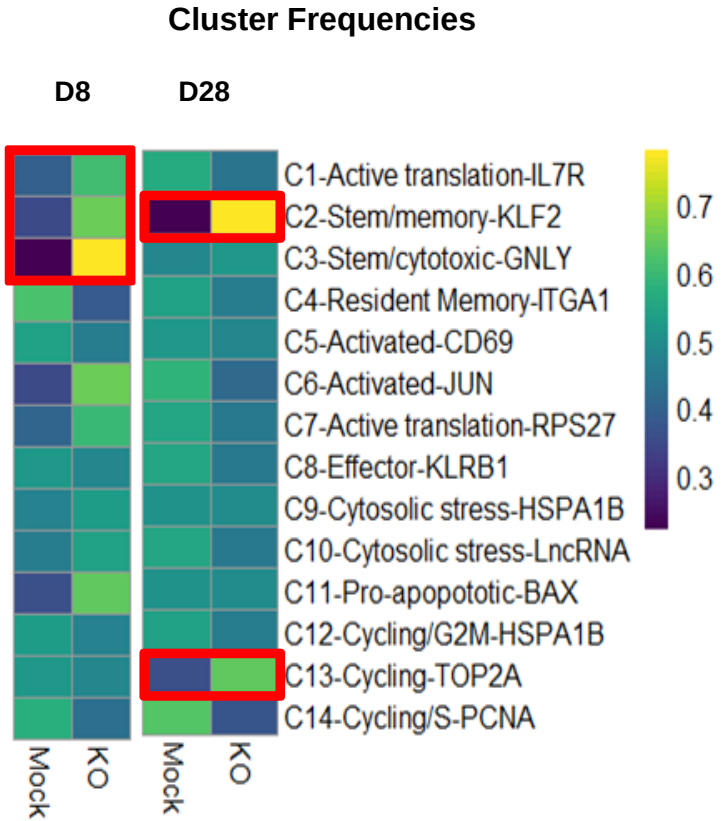
Increased memory phenotype (CD27+ KLRG1-) of SUV39H1 KO 19BBz-CAR T cells during tumour rejection

D73

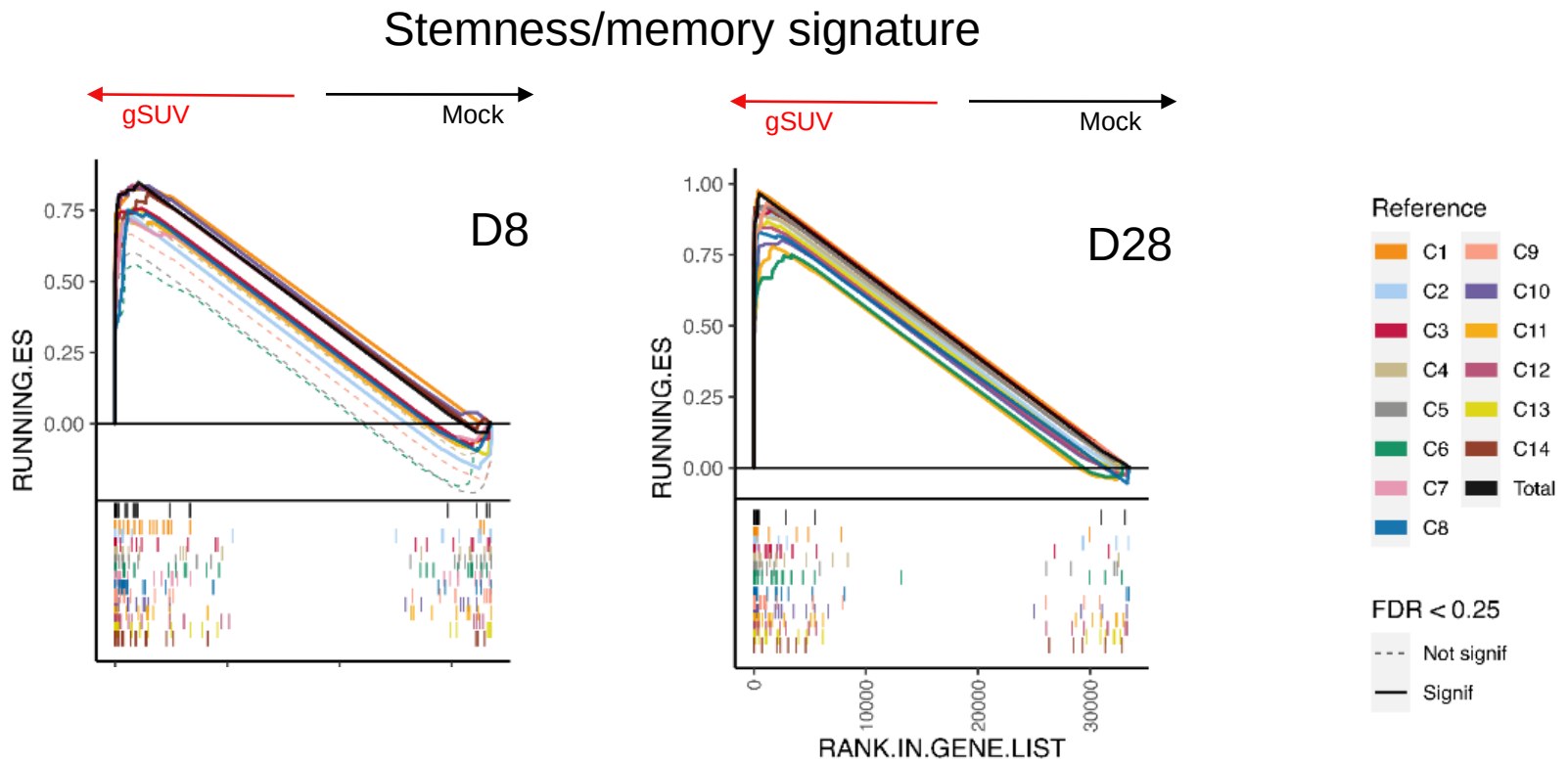


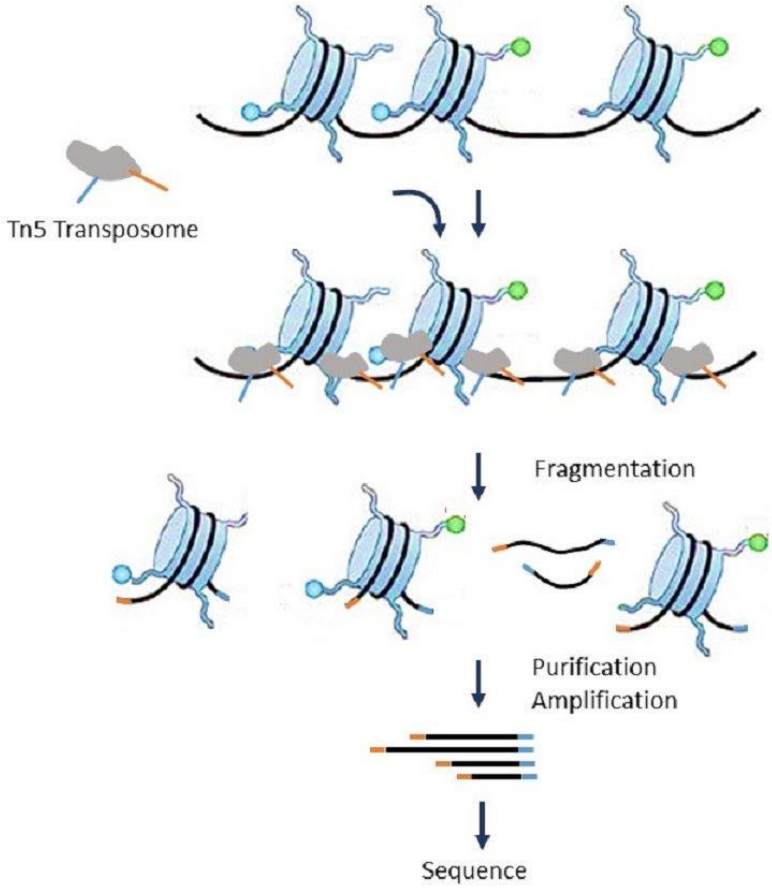
Stem/memory clusters are more enriched in SUV39H1 KO CAR T cells treated mice



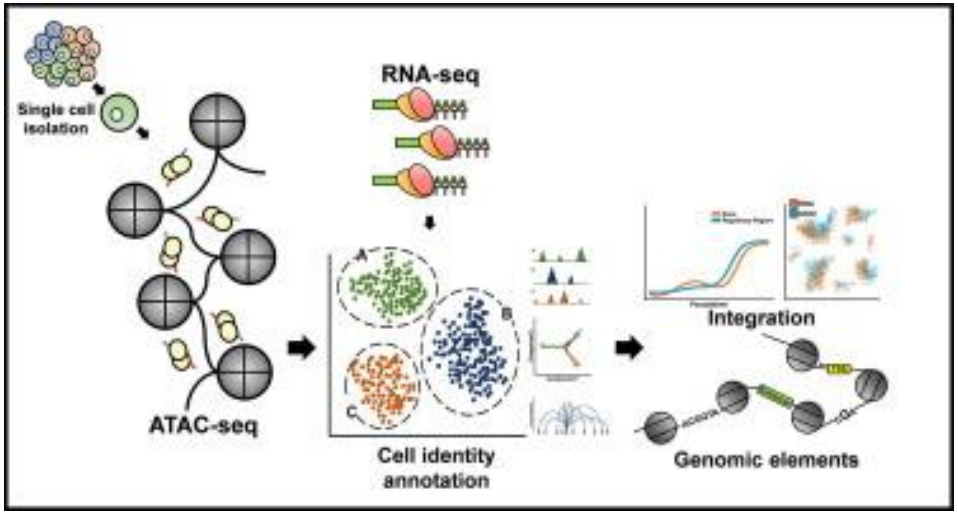


All clusters are enriched in the SUV39H1 KO CAR T are enriched for the stem/memory signature (GSEA)

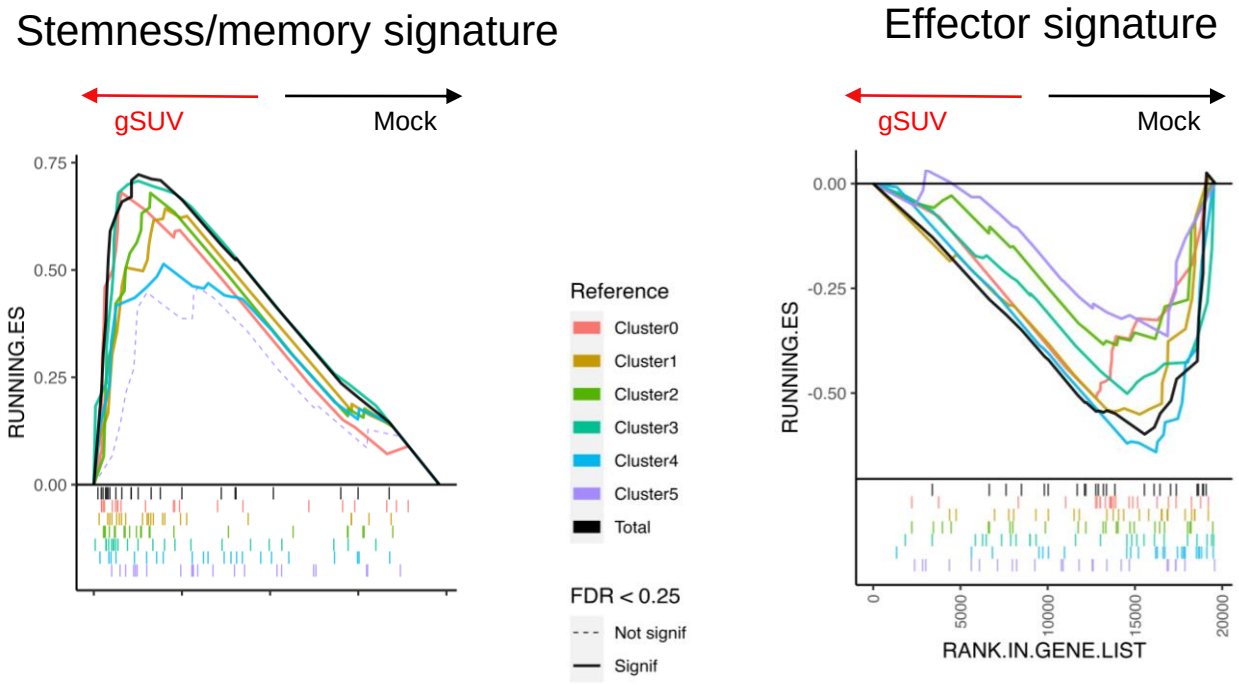




ATAC-seq (**A**ssay for **T**ransposase-**A**ccessible **C**hromatin using **seq**uencing)

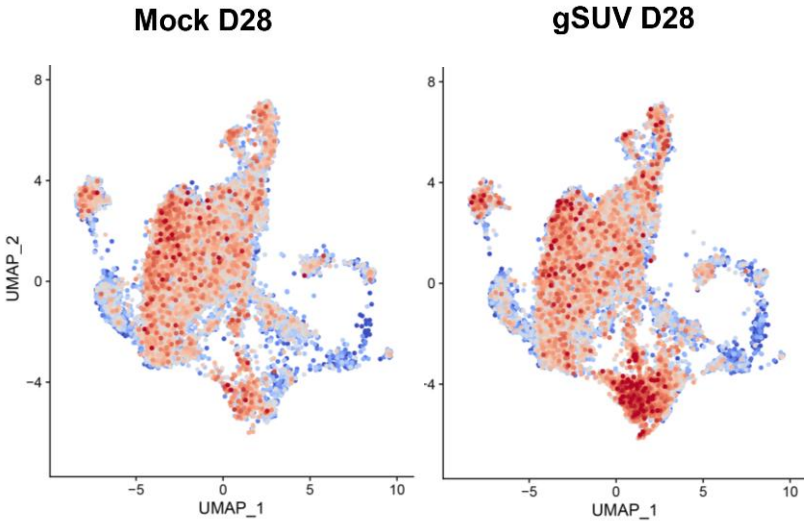


All clusters SUV39H1 KO CAR T clusters are imprinted for the stem/memory signature (GSEA) at Day 8

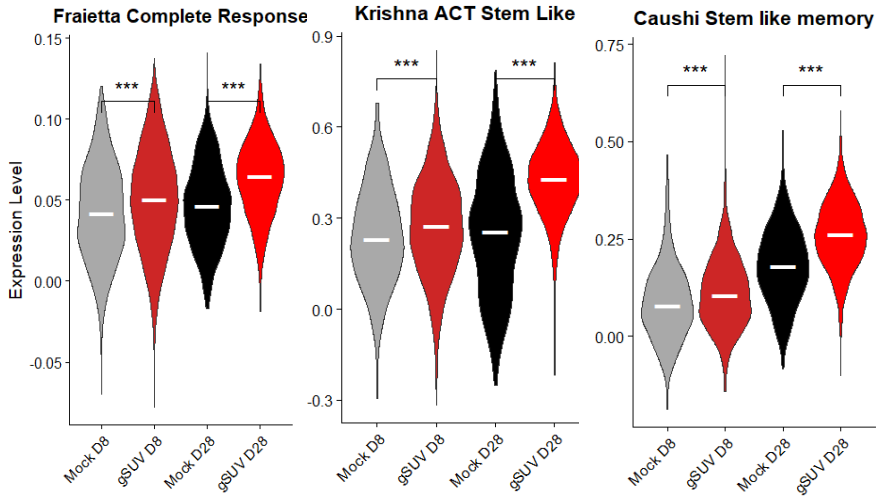


SUV39H1 KO cells express complete response and stem T cell signatures (Fraietta et al. 2018)

19-BBz CAR-T Complete Response
CLL Signature (Fraietta et al. 2018)

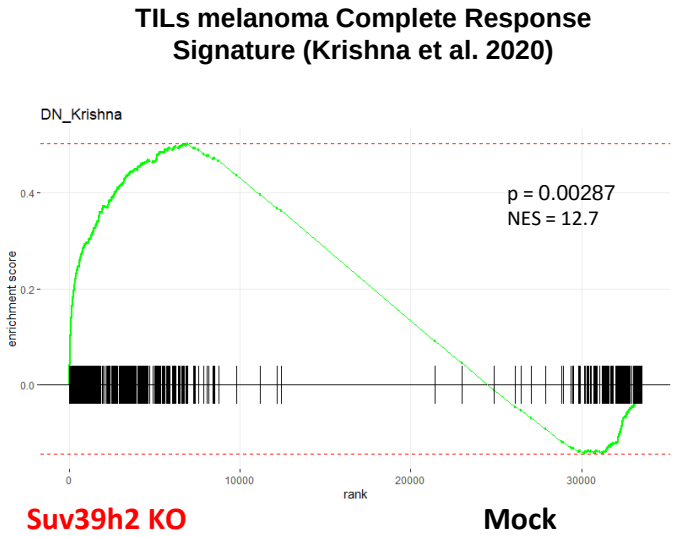


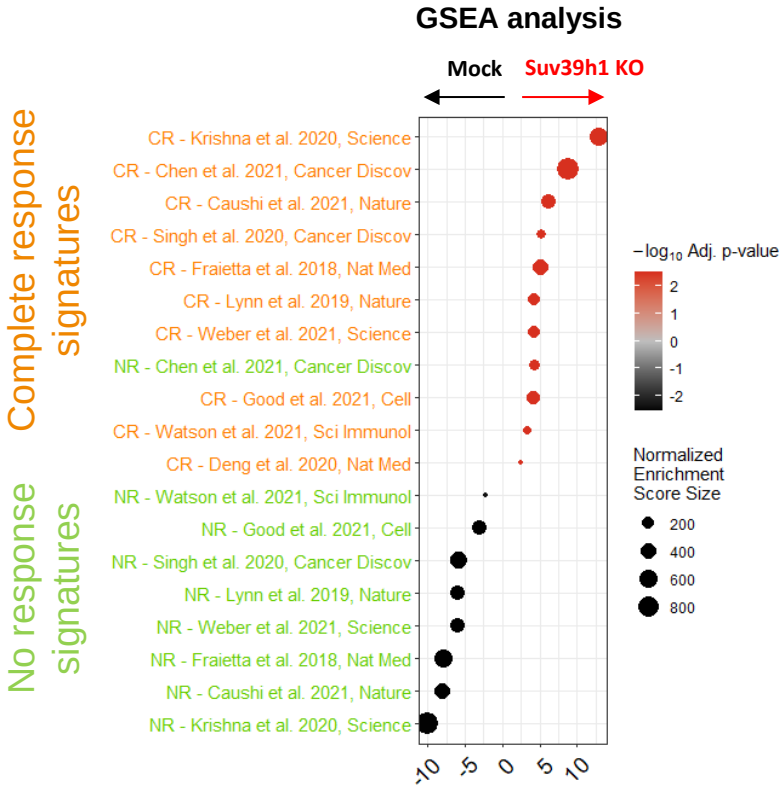
C2-KLF2-CD4



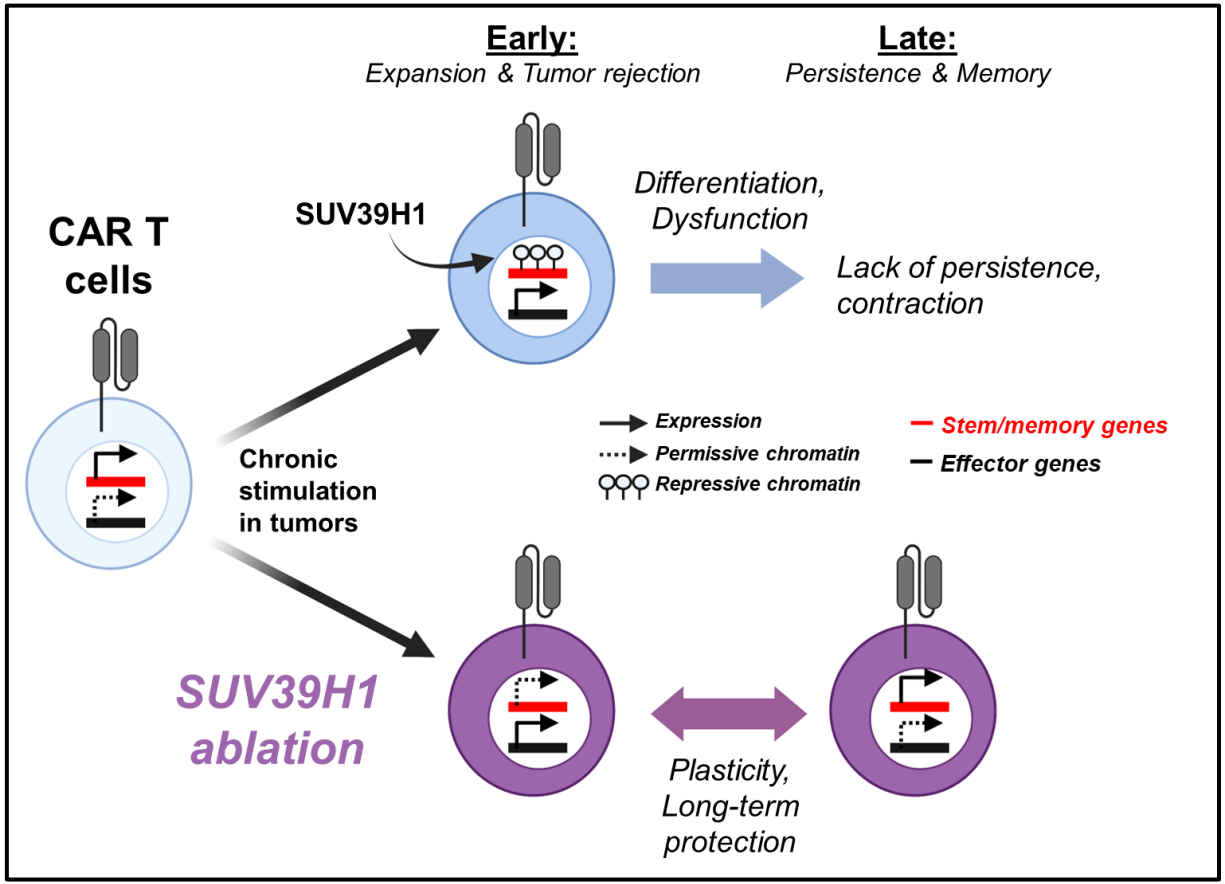
Complete response signature - CAR-T clinical trial in CLL

SUV39H1 KO cells correlate with complete response signatures from clinical studies





A working model for Suv39h1-defective CAR T cells



- Launched a cell therapy program in Curie Institute,
- Obtained funding for 2 clinical trials, one in solid and one in liquid cancers.

Acknowledgements

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Piaggio Team