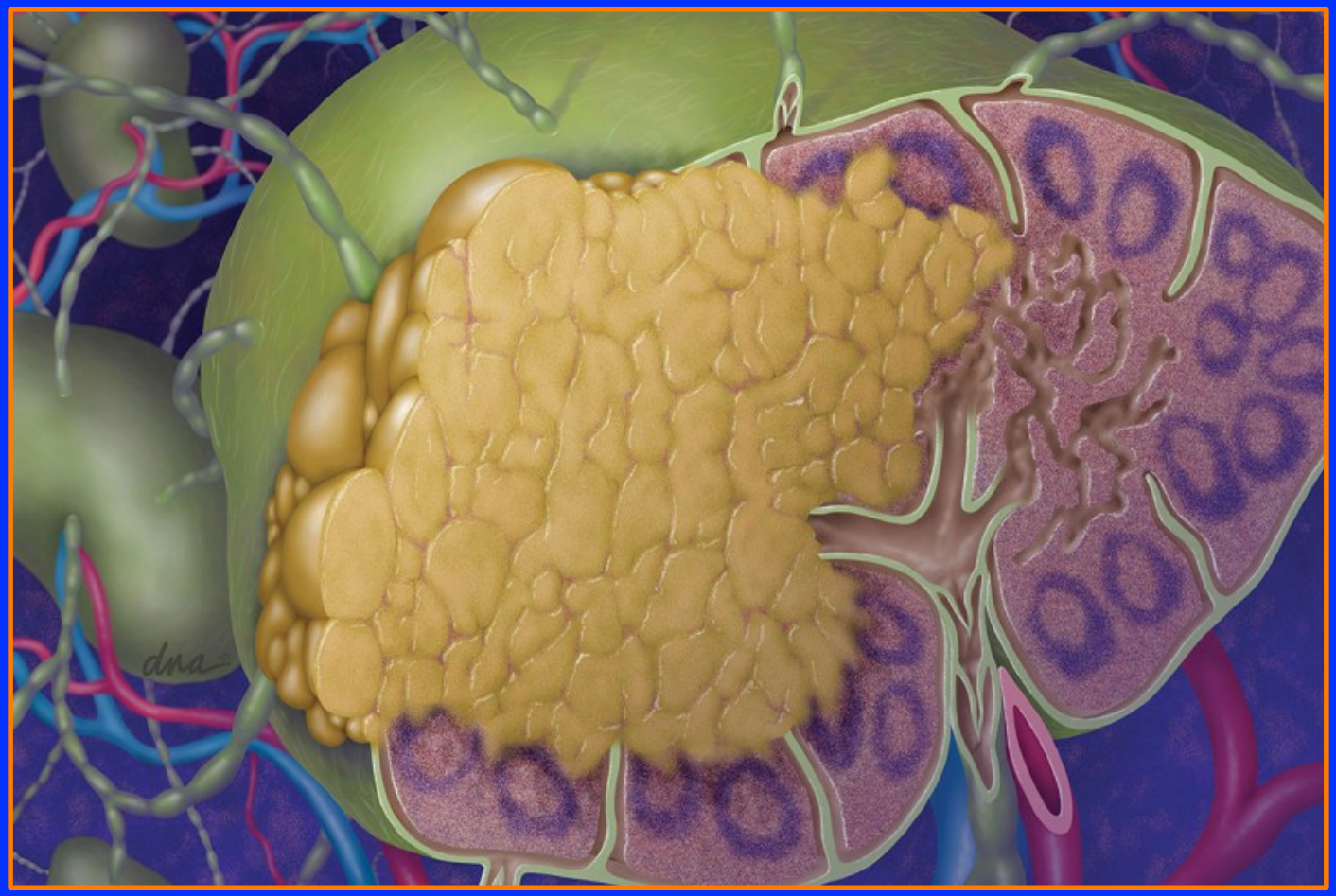
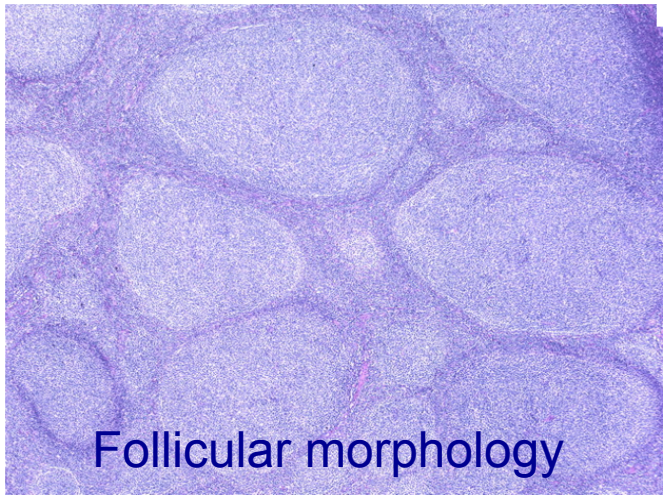
Targeting immune receptors in lymphoma

Hans-Guido Wendel, MSKCC

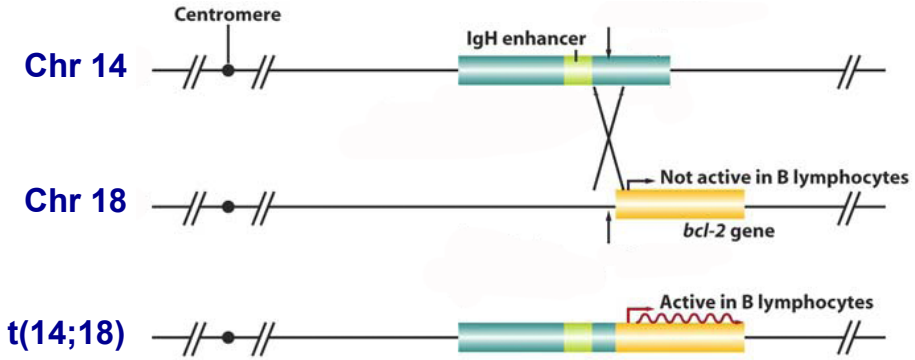


Follicular Lymphoma (FL)

- Typical follicular appearance
- *Slow* growth & relentless relapses
- Treatment chemotherapy and BMT



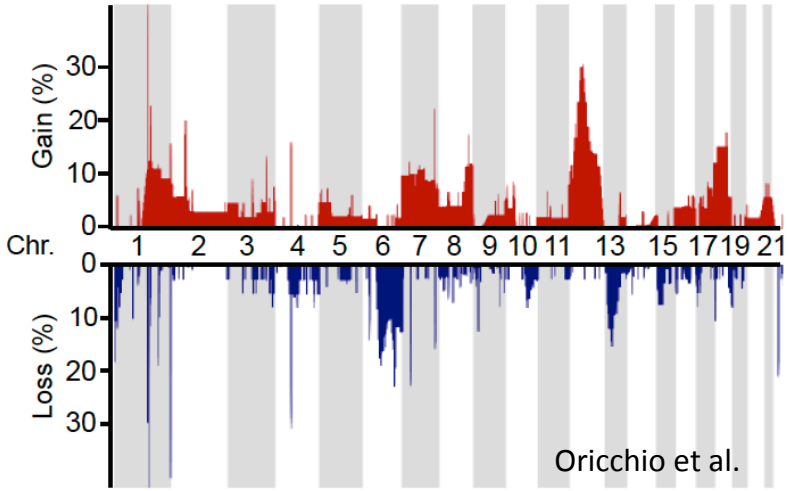
Genetic hallmark:
Translocation between chromosomes 14 and 18: t(14;18)



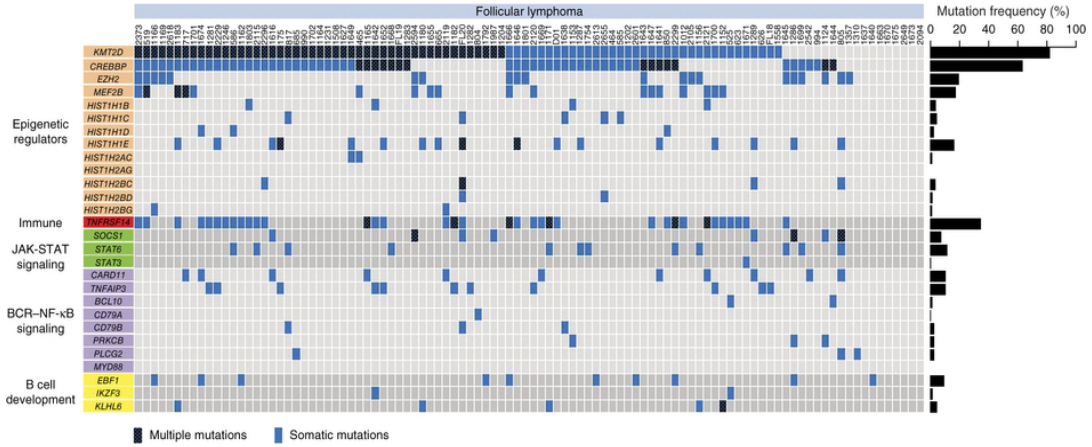
This fusion is found in ~50% of healthy adults…..

…. What else drives the disease?

Interpreting the follicular lymphoma genome



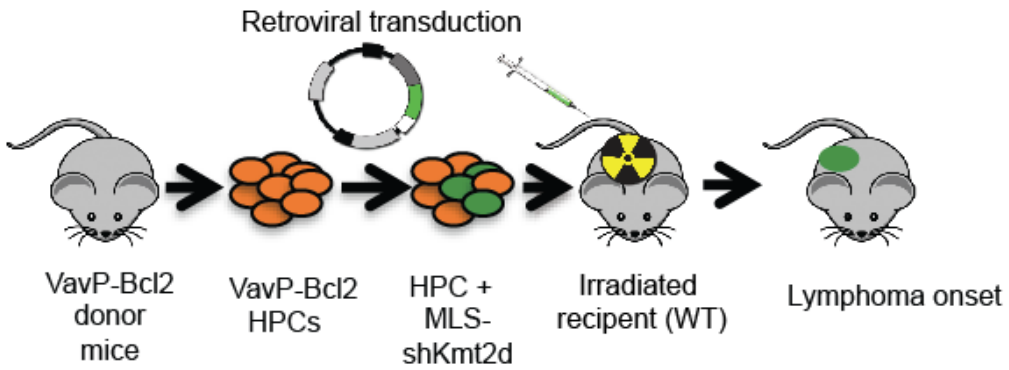
Oricchio et al.



Okosun et al.

Can these lesions lead us to new therapeutic opportunities?

A mouse model to explore the biology of FL



GC origin of lymphomas

Genetics and pathology resembles human FL

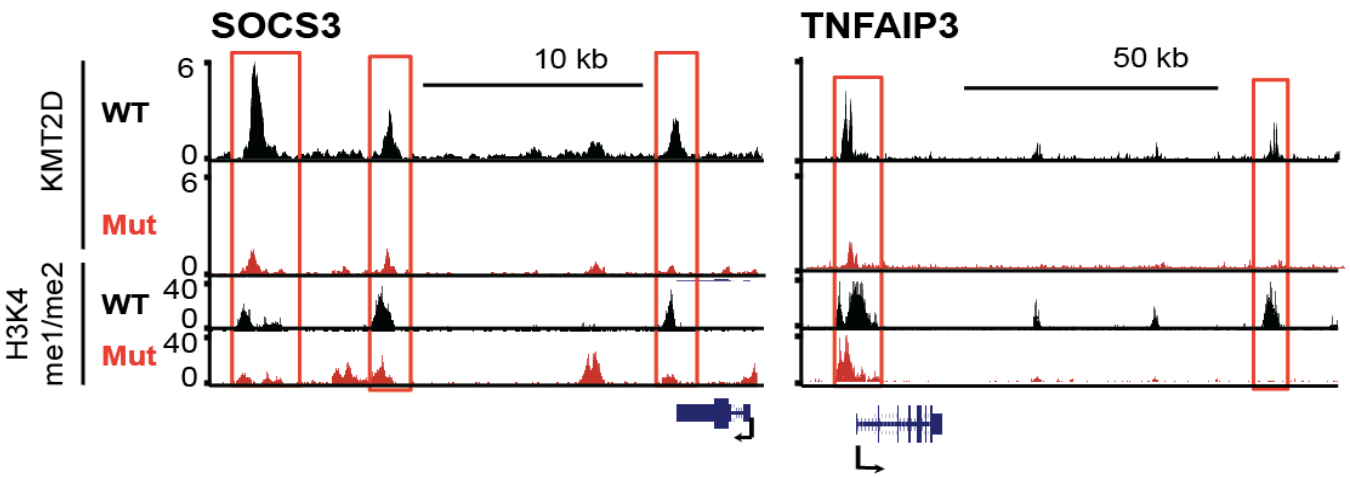
Accessible to genetic tools (Crispr, ShRNA etc)

The model is highly versatile and we can rapidly model FL genetics in vivo:

Del6q and Epha7 – Oricchio, CELL 2011
PIM and mTOR – Schatz, JEM 2011
CyclinD1, CDK4, Rb – Oricchio, JEM 2012
EZH2 and Sestrin, Oricchio Sci Transl. med. 2017

MLL2 – Ortega Molina, Nat. med. 2015
TNFRSF14/HVEM – Boice, Salloum, CELL 2016
CREBBP/EP300 – Yang, Cancer Discovery 2017

MLL2 mutations drive an aberrant gene expression program



MLL2 (KMT2D) is the most frequently mutated gene in FL.

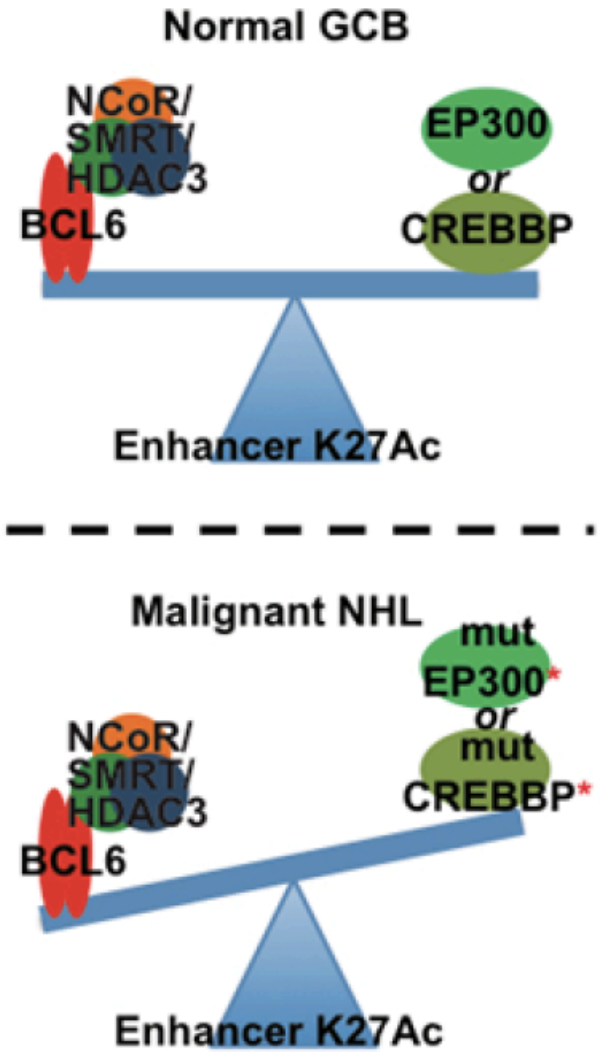
We identified direct MLL2 targets genes in FL: Tumor suppressors e.g. A20 and regulators of growth signals.

MLL2 loss disturbs B cell immune response e.g. Kabuki Syndrome

MLL2 loss changes response to epigenetic and kinase inhibitor therapies.

Ortega Nat. med. 2015

CREBBP and EP300 mutations cause dependence on HDAC3



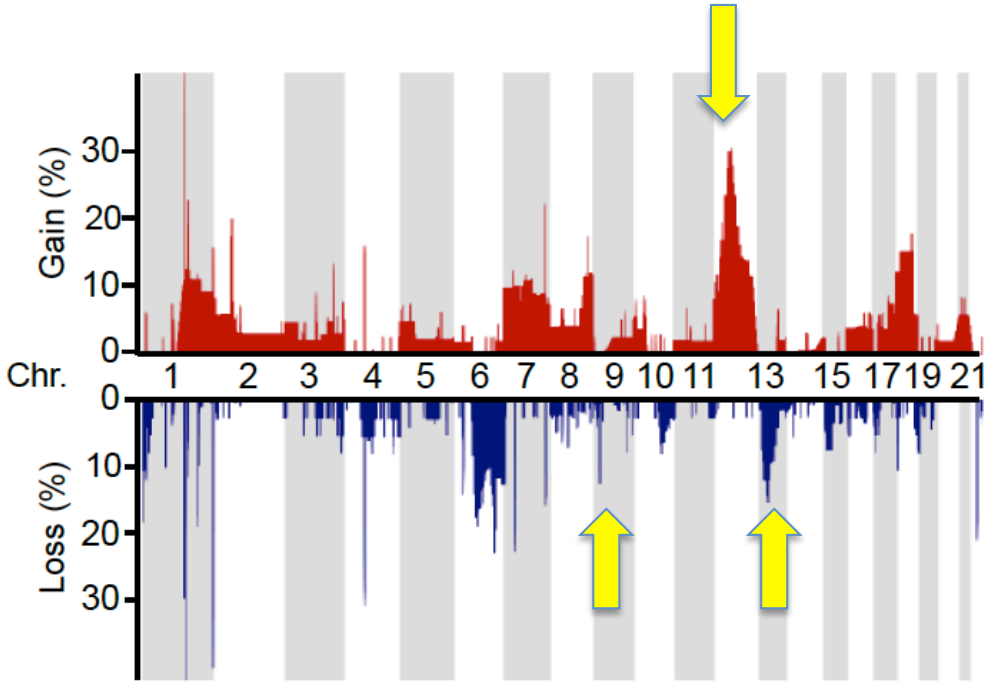
CREBBP and EP300 mutations shift enhancer control towards gene silencing by BCL6/ HDAC3.

This affects regulators of **BCR signaling** and the **immune response (MHCII)**.

CREBBP/EP300 defective tumor cells show **increased sensitivity to HDAC3 inhibition.**

Jiang, Ortega, Cancer Disc. 2016

Cell cycle genes are altered in in 50% of indolent stage FLs



Mutual exclusive lesions disrupt **cell cycle checkpoints in 50% of FL**: Rb, p16, **CDK4**, CCND3.

These lesions occur in the **indolent stage**.

RB phosphorylation (not Ki67) identifies tumors with **increased CDK4 dependency**.

Oricchio JEM 2012

BCL2 delays cell cycle entry and CDK4 activation is needed for growth

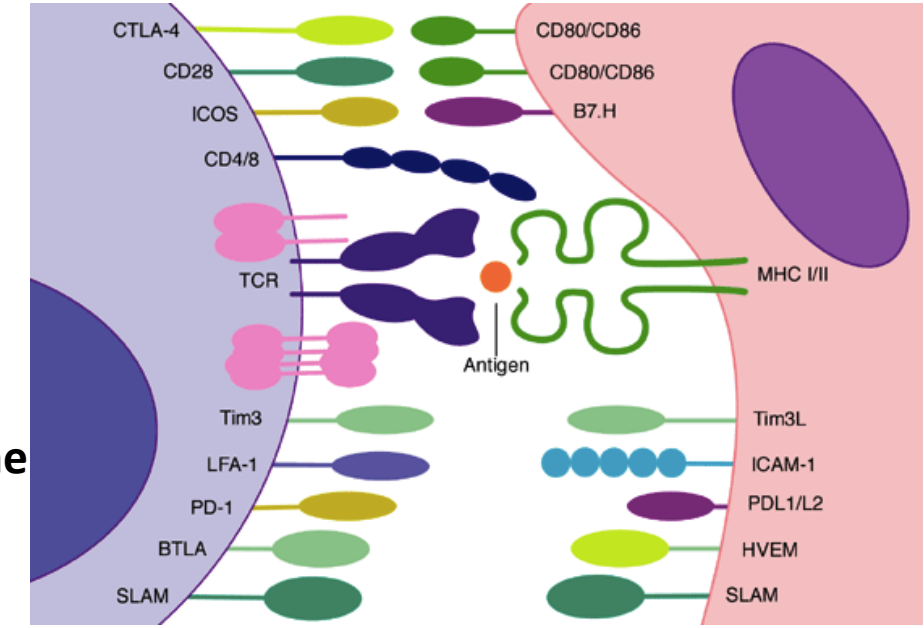
Immune receptors in lymphoma biology and therapy

A large number of activating and inhibitory receptors control lymphocyte biology and some are targets of genomic lesions in lymphoma, indicating a pathogenic role.

Receptors interact with soluble ligands or engage in cell-cell interactions.

Expressed surface receptors are directly accessible for inhibition/activation.

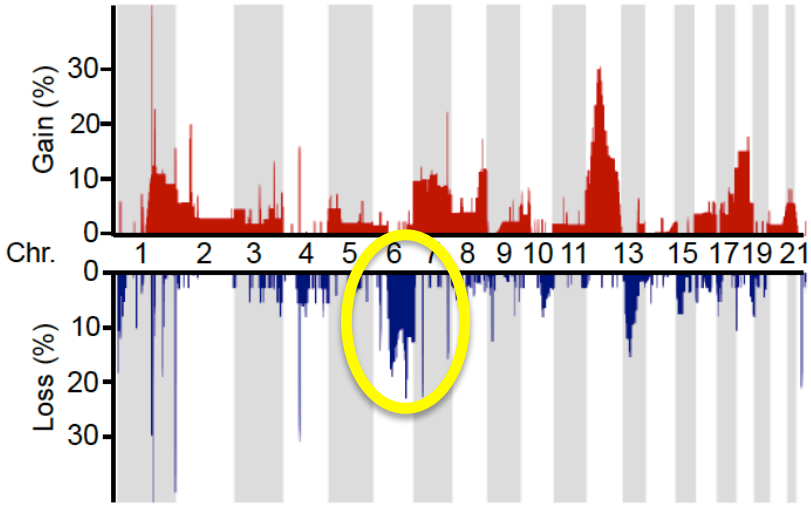
If a receptor/ligand is lost we can still engage the receptor on the target cells.



(nicked from somewhere)

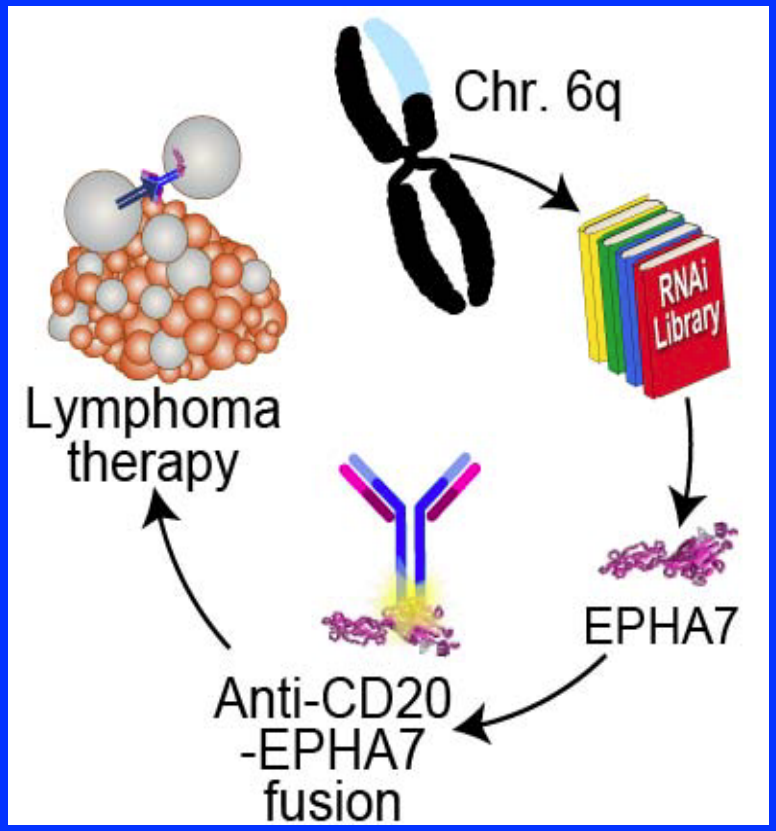
Through B cell receptors we can target lymphoma cells and their microenvironment.

Example 1: Chr. 6q deletions targets several genes including the EPHA7 receptor



Del 6q occurs in **~30%** and is associated with **shorter survival**

Restoring extrinsic tumor suppressors: The EPHA7 receptor protein



A genetic screen identified tumor suppressors at Chr. 6q deletion.

EPHA7 is lost in 72% of FLs and enables B cell growth.

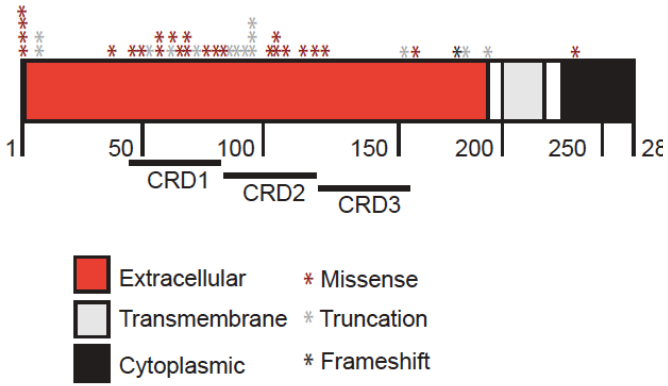
Soluble EPHA7 protein kills lymphoma cells.

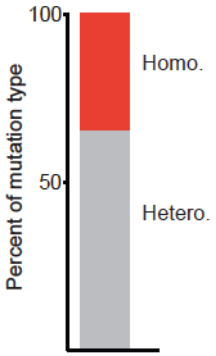
Oricchio, Cell 2011

We can engineer bi-functional antibodies to deliver receptor ligands to lymphomas in vivo

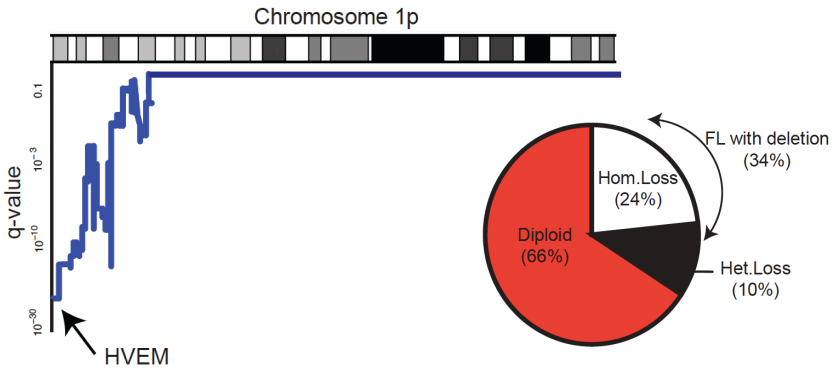
Example 2: The HVEM/TNFRSF14 receptor is mutated in FL/DLBCL

HVEM mutations in ~38%





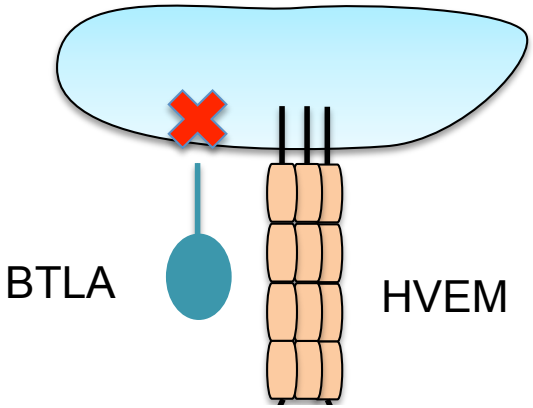
HVEM deletions in ~34%



HVEM (TNFRSF14) is a major mutational target in FL

Boice et al., CELL 2016

The HVEM/TFRSF14 receptor engages in cell-cell interactions



Studies in T cells have shown that HVEM interacts with:

Activating receptors (LIGHT, CD160)

Inhibitory receptor (BTLA)

For B cells we know:

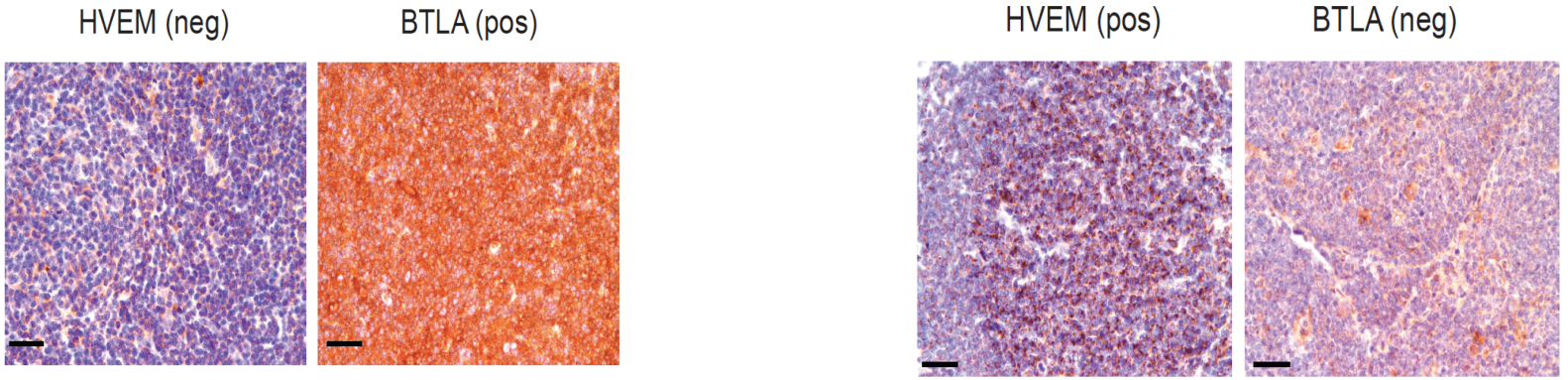
Only HVEM and BTLA are expressed

HVEM-BTLA can interact in *cis* (same cell)

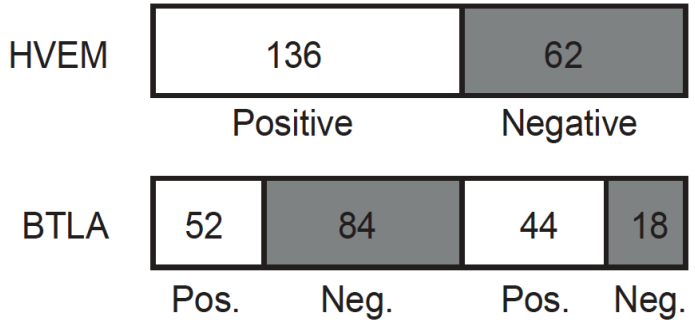
BTLA can bind and block the B cell receptor

What is the relation between HVEM and BTLA in human FLs?

(A. Mottock, R Gascoyne)

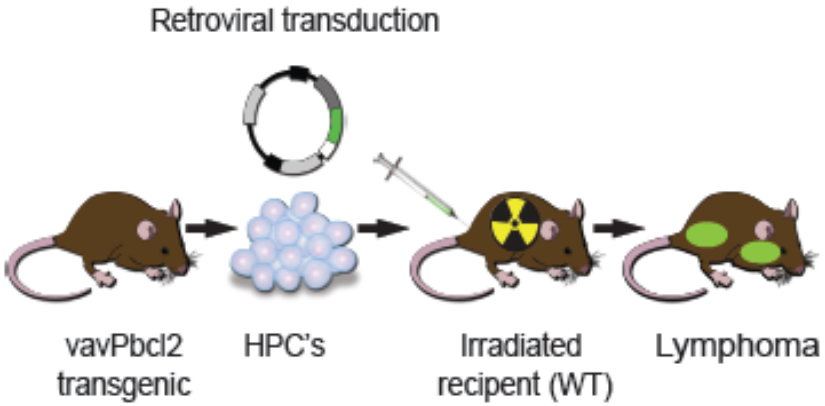


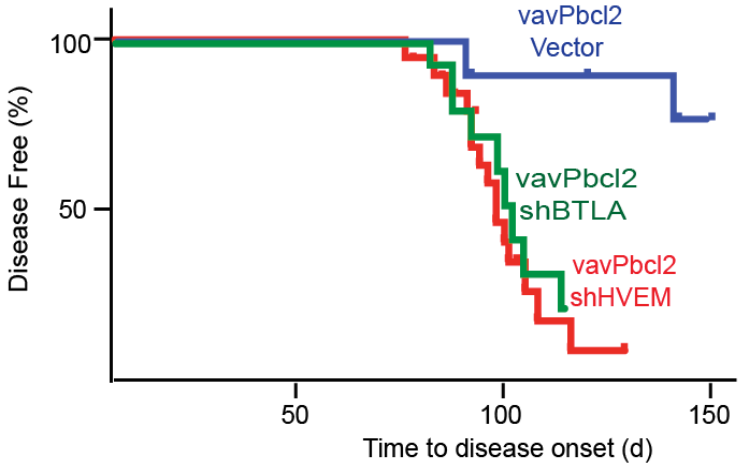
Tissue array (n = 198; p = 0.001)



The HVEM – BTLA interaction is lost in ~75% of FLs.

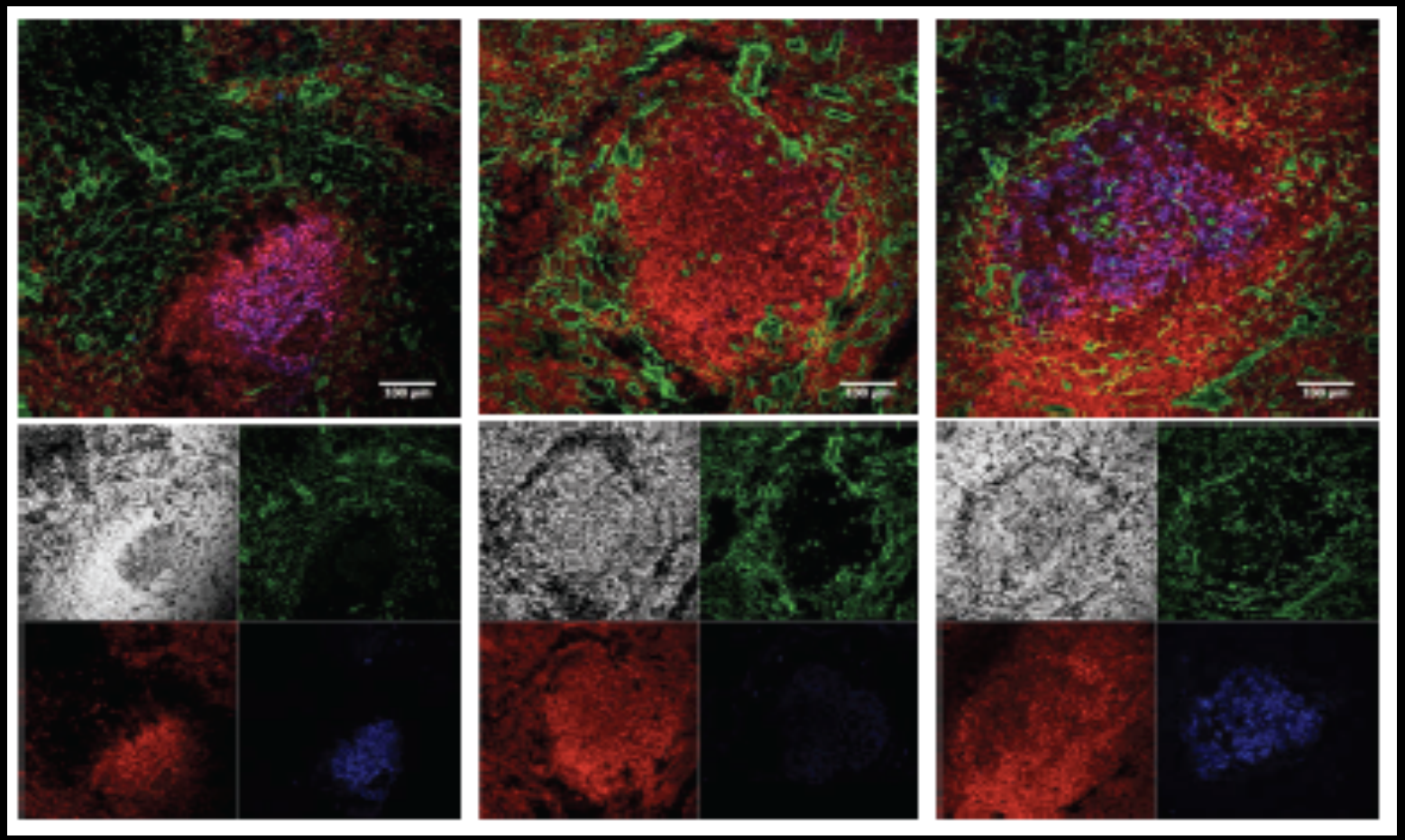
Loss of HVEM or BTLA causes lymphomas in vivo





Lymphoid stroma activation in HVEM deficient tumors

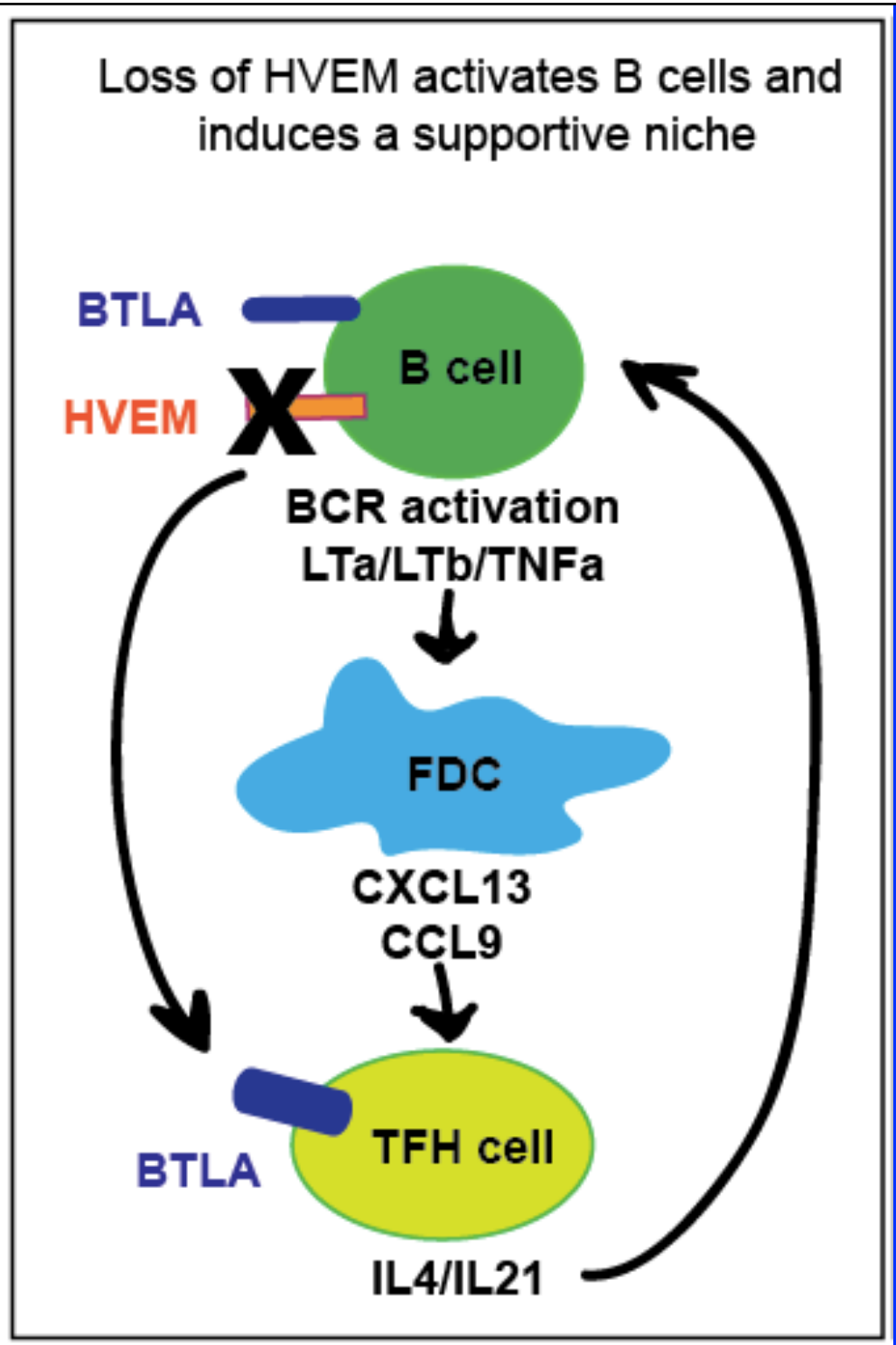
Reactive GC | Lymphoma | HVEM Lymphoma



B cells

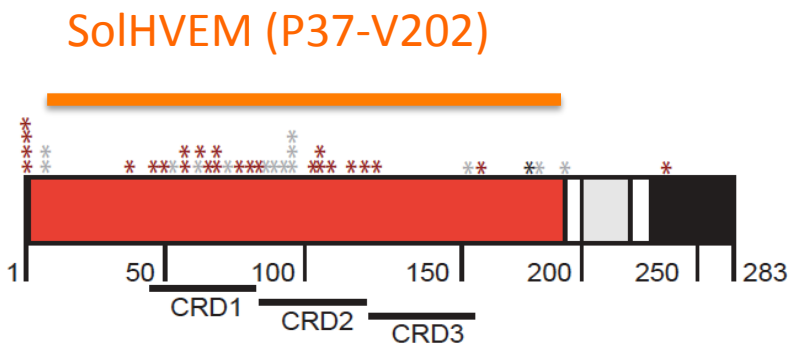
FRCs

FDCs



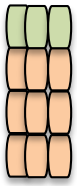
Boice et al., CELL 2016

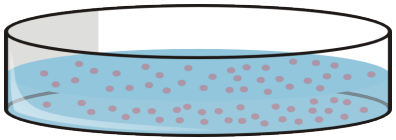
Can we use the HVEM protein for therapy?



Soluble HVEM is the ectodomain that binds HVEM receptors BTLA and LIGHT

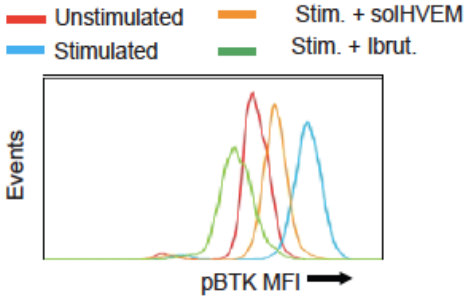
The SolHVEM protein has biological activity against lymphoma cells

Stimulation +/-  solHVEM

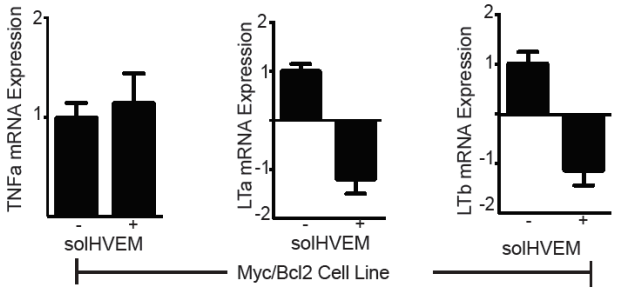


SolHVEM $^{(P37\text{-}V202)}$: HVEM ectodomain

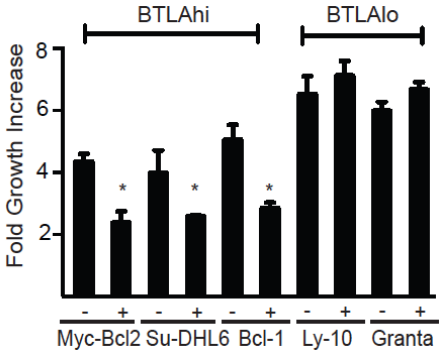
Inhibition of mitogenic signals



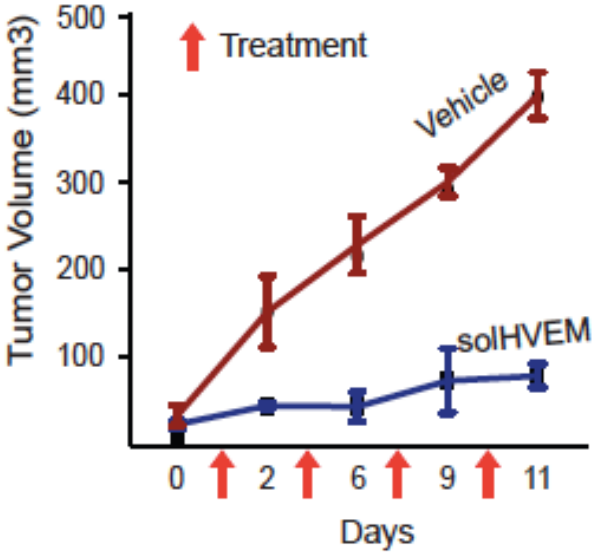
Partial reversal of cytokine effects



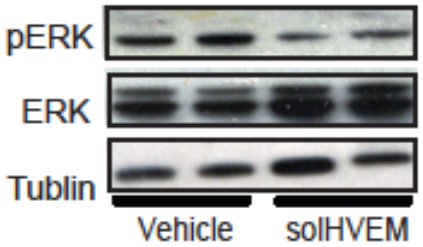
Growth inhibition

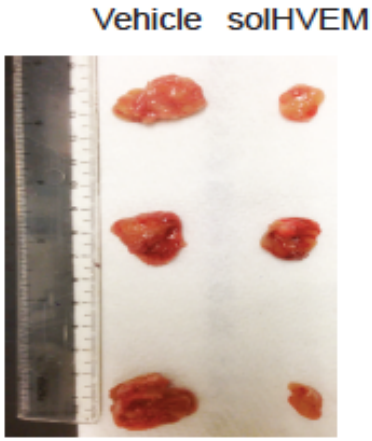


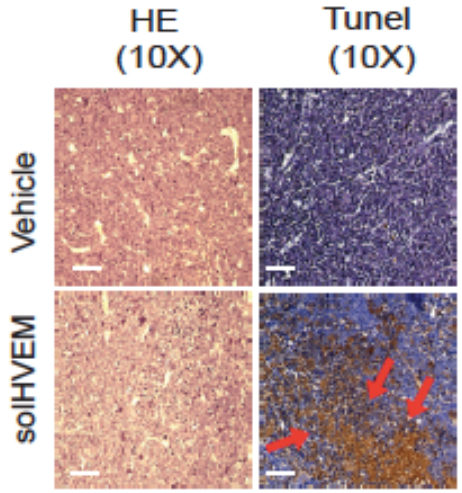
SolHVEM suppresses lymphoma growth *in vivo*



MYC/BCL2 mouse lymphomas; s.c. inject; 20μg

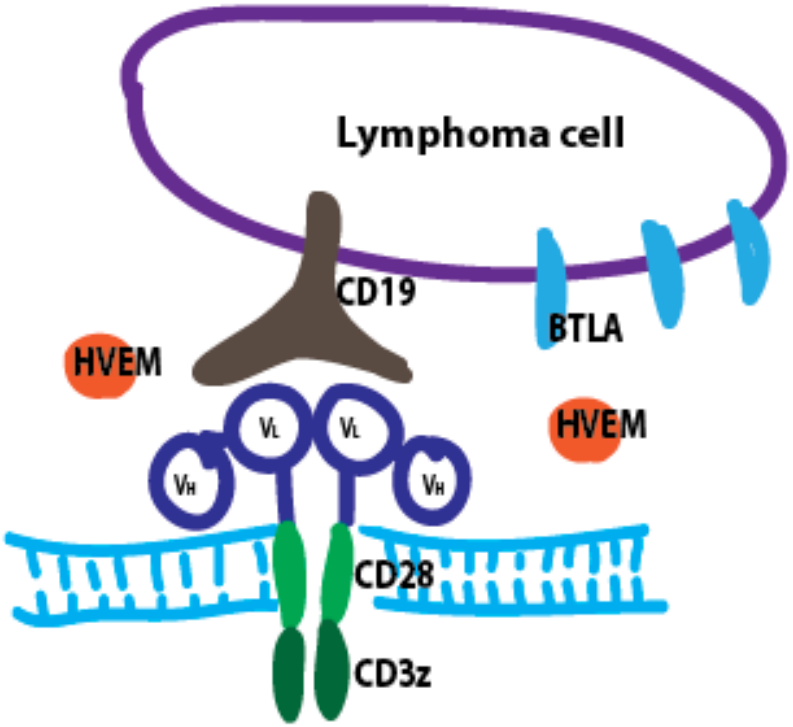


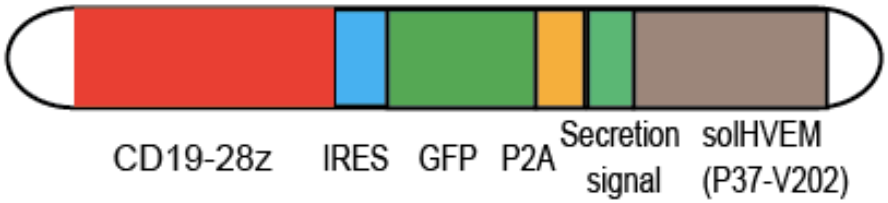


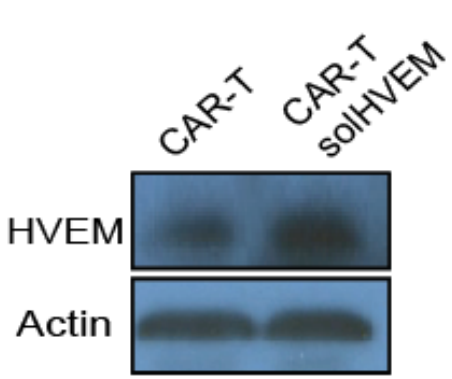


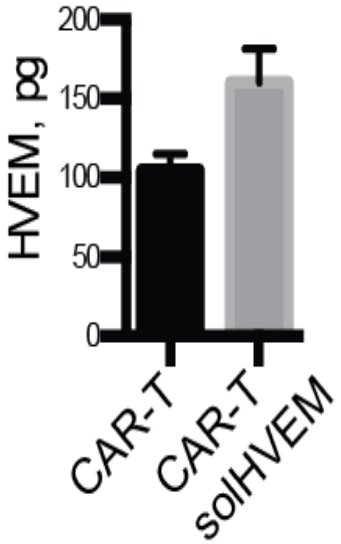
How can we deliver solHVEM to lymphomas in vivo?

Engineering CAR-T to secrete solHVEM *locally and continuously*



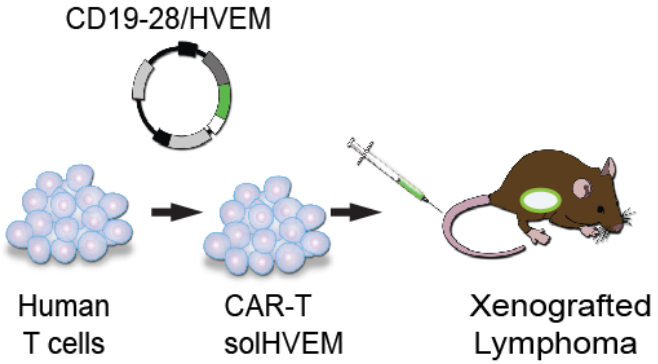


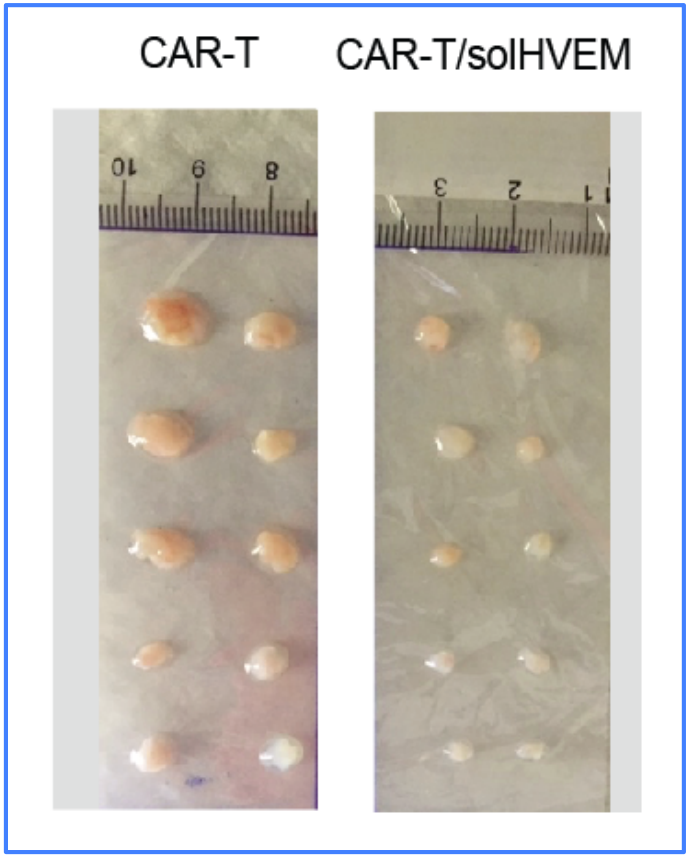


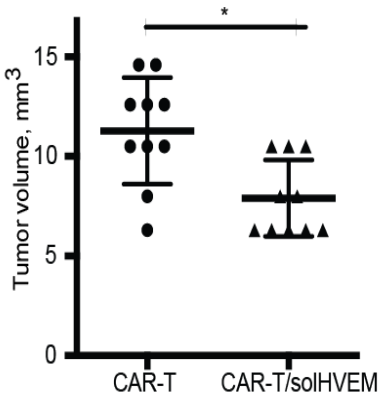


CAR-T cells as HVEM producing 'micro-pharmacies'

CAR-T/solHVEM have enhanced anti-lymphoma activity

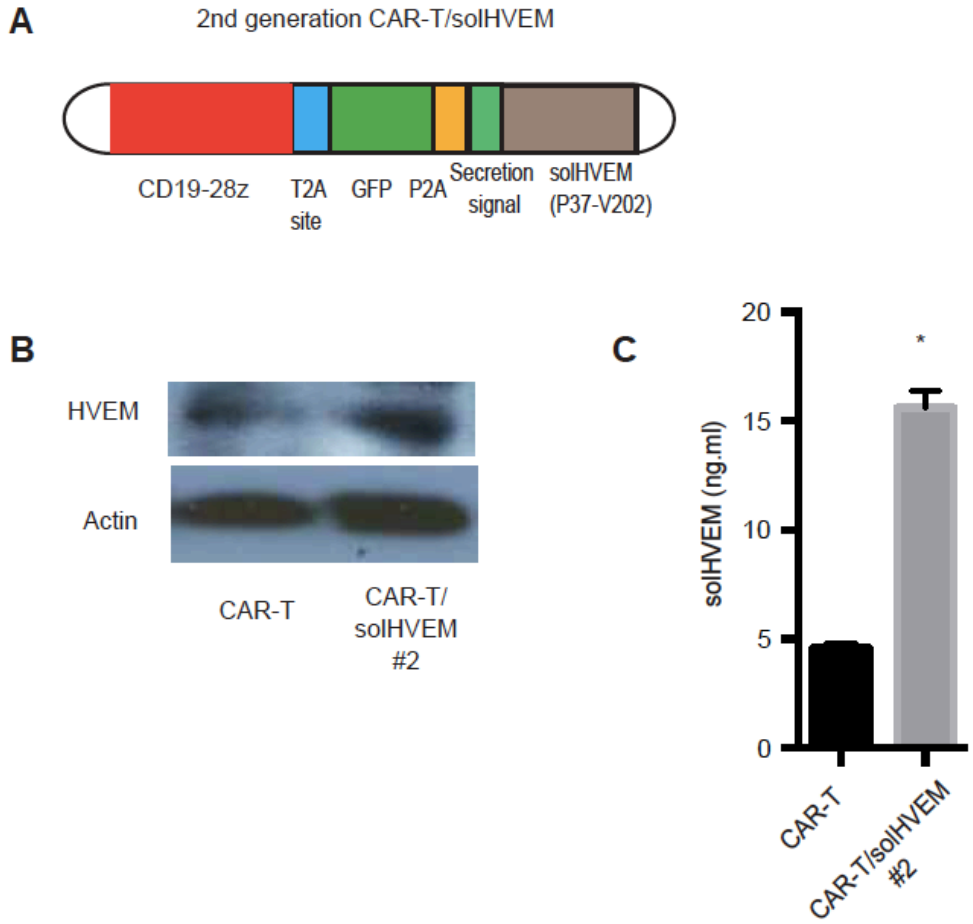






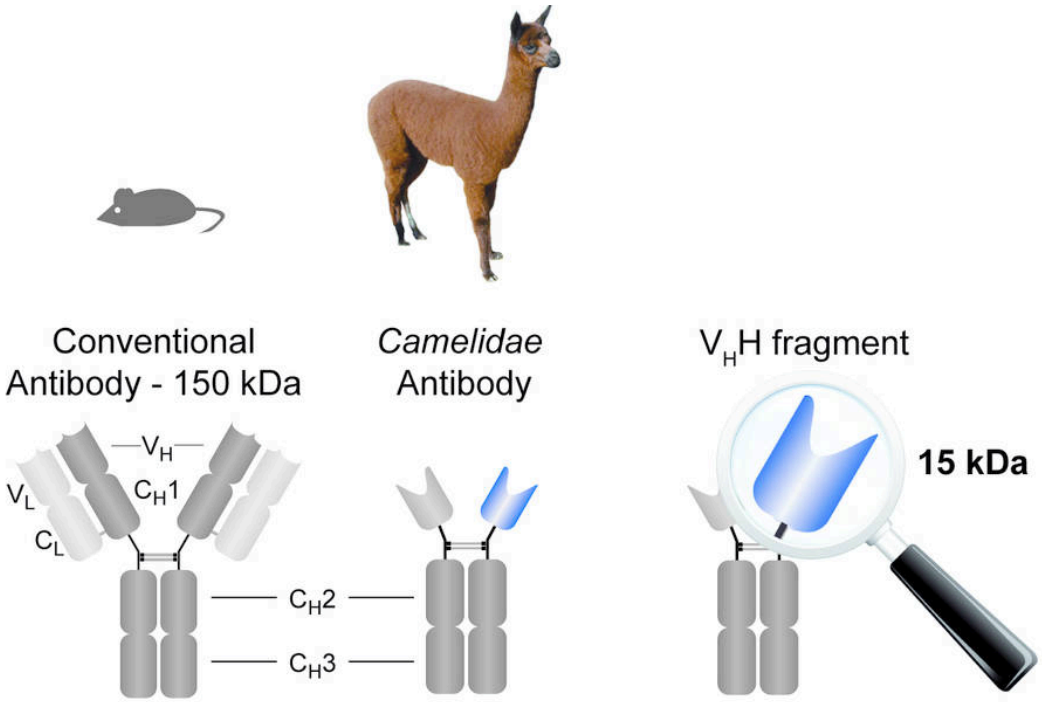
CAR-T cells can produce and secrete anti-tumor proteins locally and function as "Micro-pharmacies".

The 2nd generation CAR-T/HVEM cells produce >10fold more HVEM



CAR-T/HVEM enhance the lymphoma activity of CAR-T cells

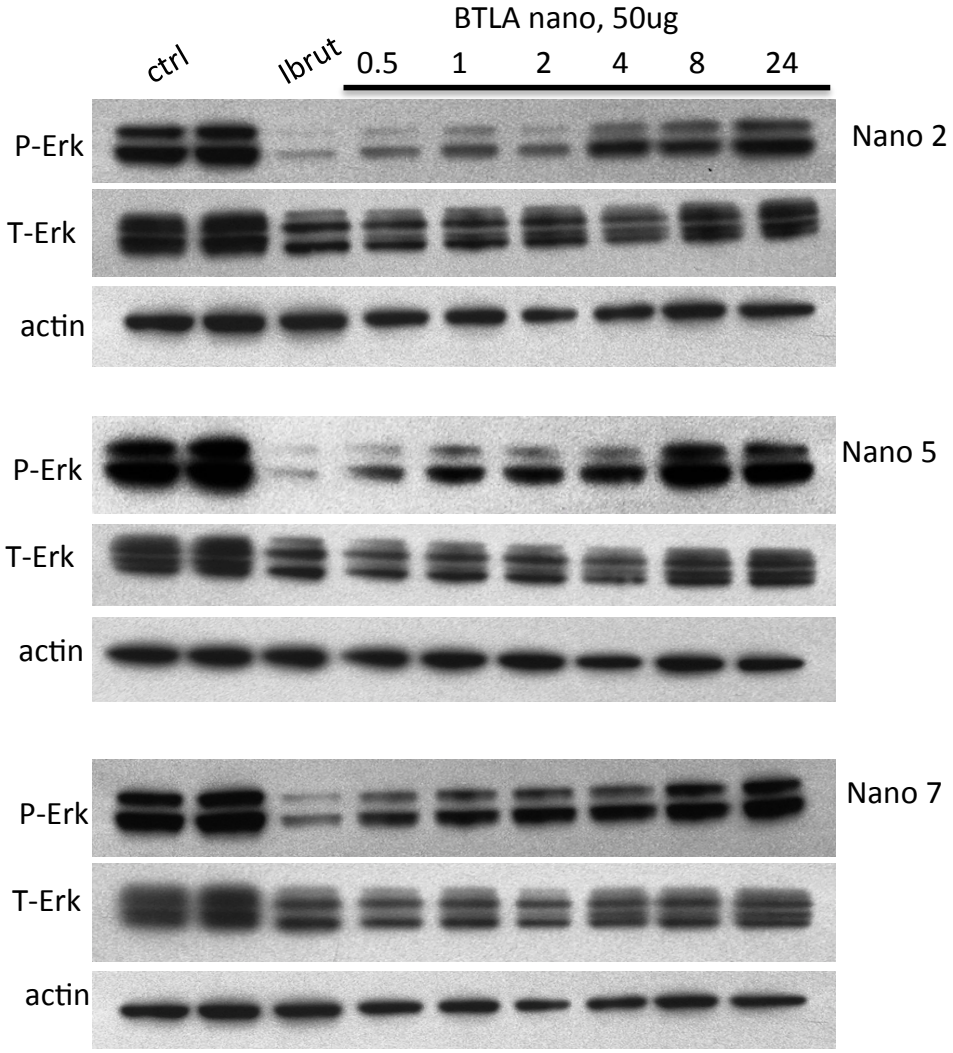
Production BTLA engaging Lama nanobodies



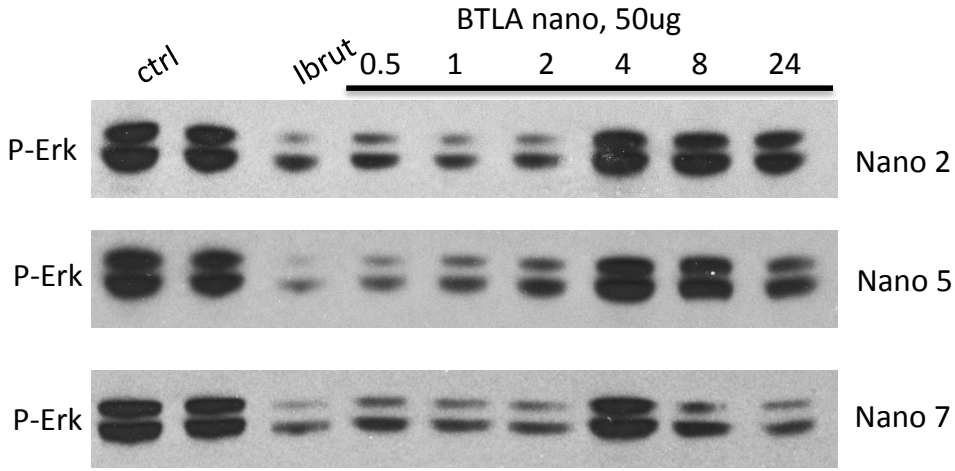
Nanobodies are selected for enhanced BTLA binding
and they are ideal antibody engineering or secretion from CAR-T cells

Production BTLA engaging nanobodies

Raji

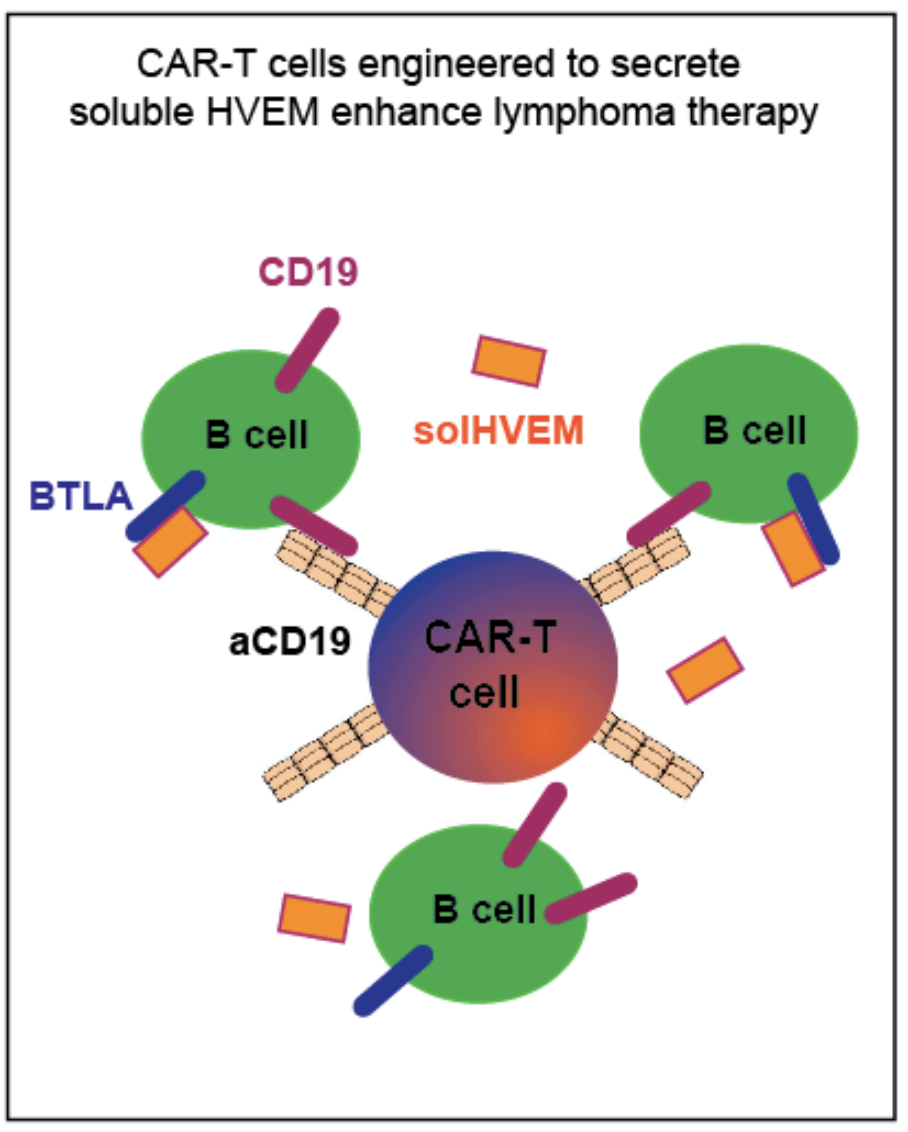


DOHH2



Some nanobodies show improved BTLA engagement and ERK blockade

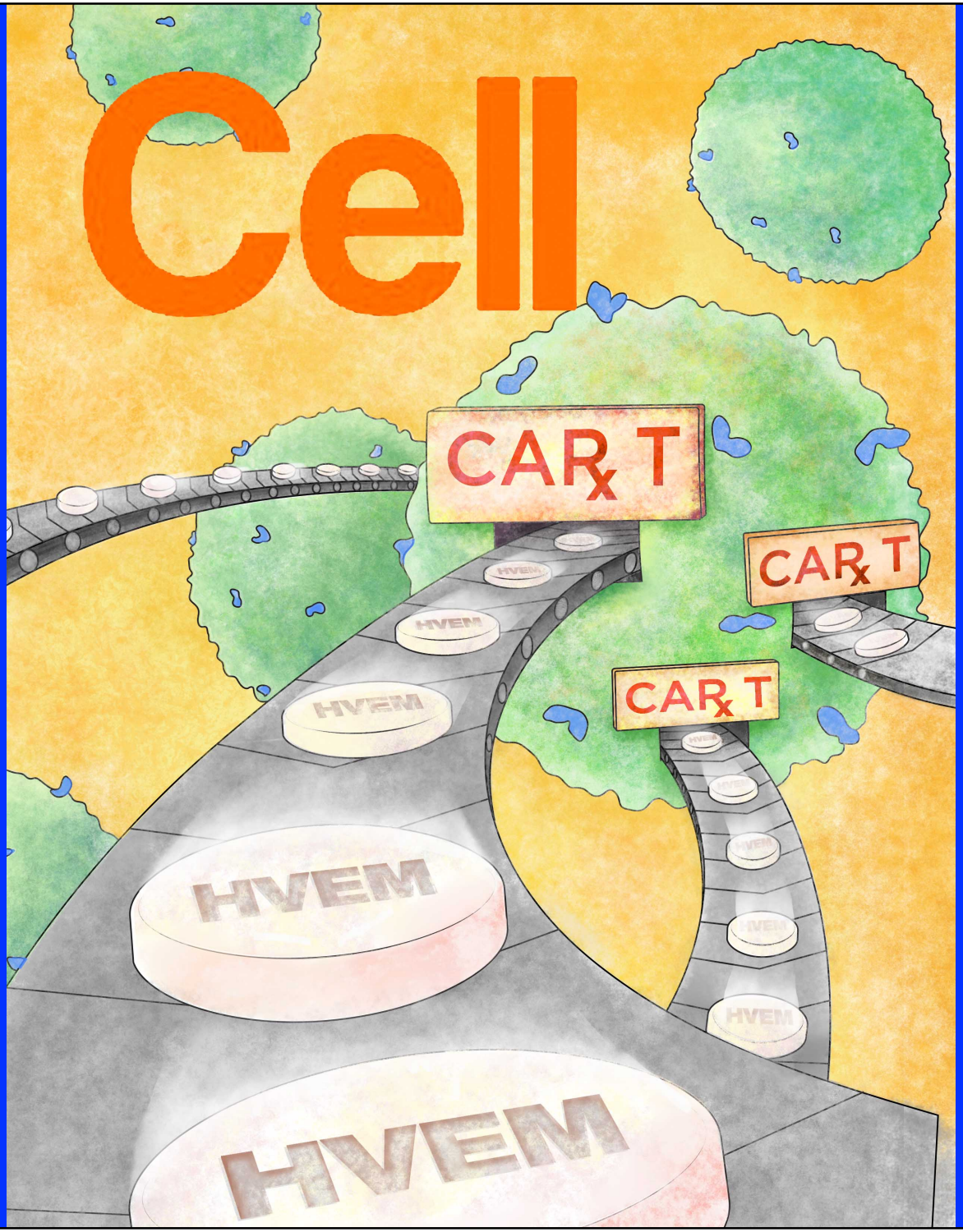
CAR-T cell ‘micro-pharmacies’ can deliver an anti-tumor protein



- solHVEM blocks lymphoma growth.
- Modified CAR-T cells produce HVEM locally.
- CAR-T/HVEM show better activity.

What are other uses for CAR-T cell ‘micro-pharamacies’?

Salloum, Boice et al., CELL 2016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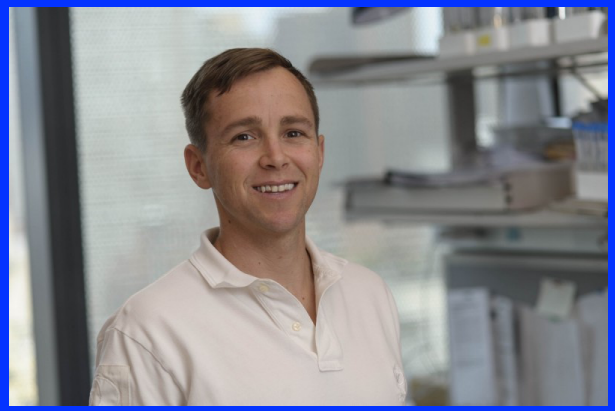

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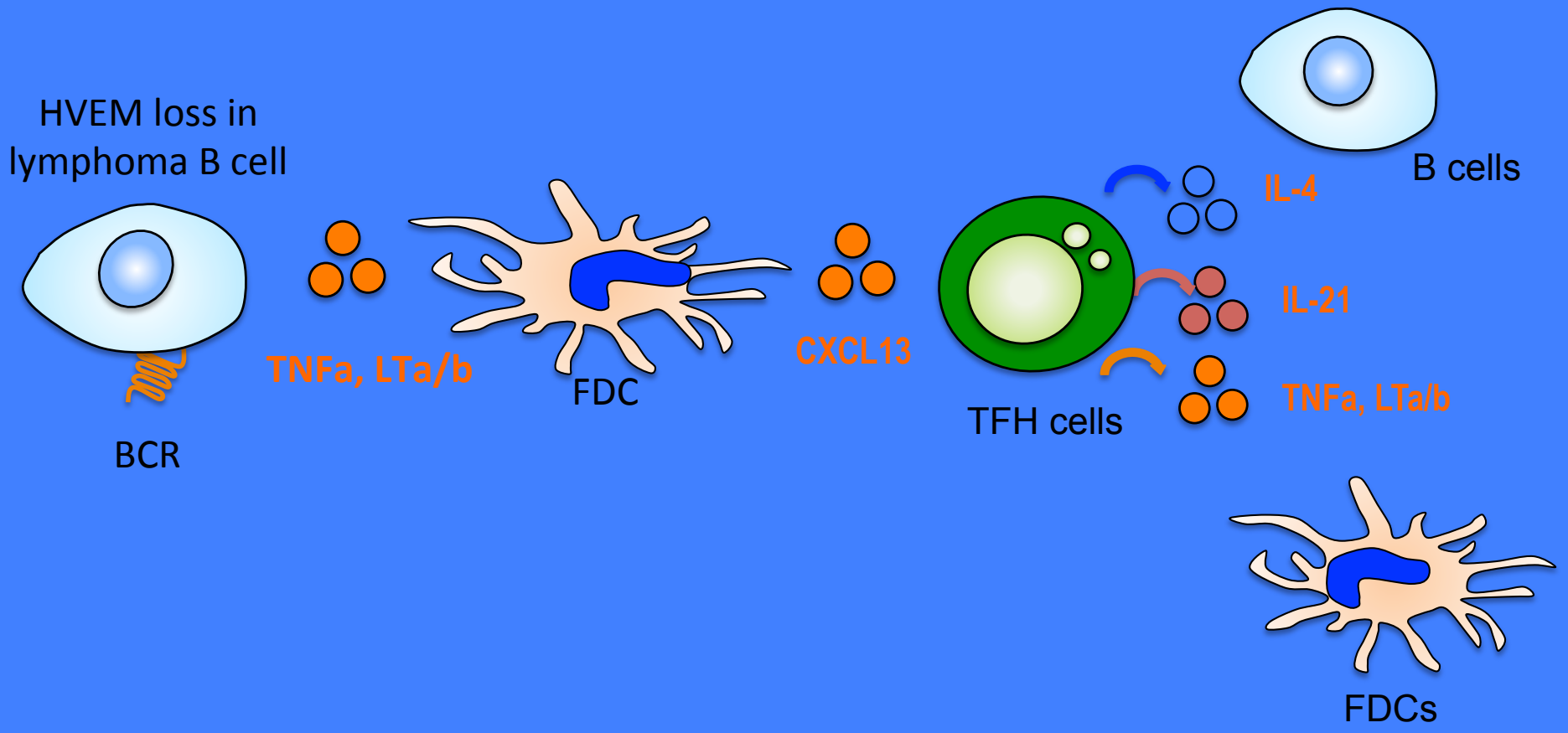
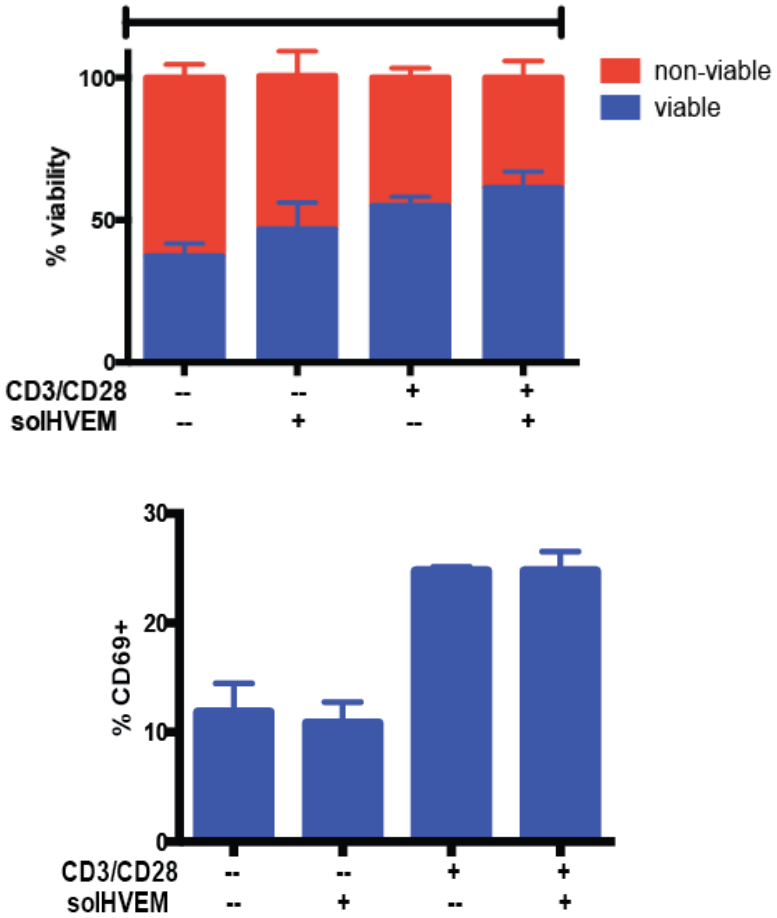
Supported by: NIH, LLS, LRF.

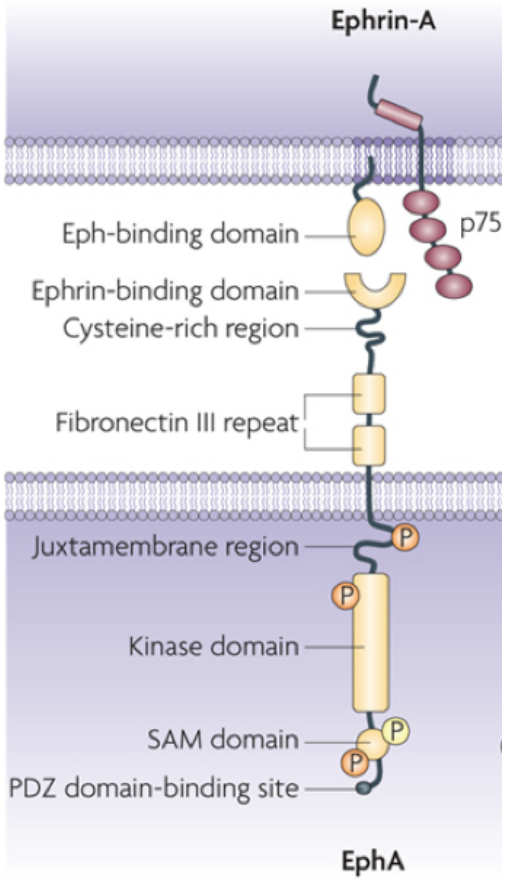
HVEM loss activates B cells and creates a supportive niche



HVEM does not affect T cell activation/viability



Ephrin receptors engage in cell-cell interactions



EPH receptors bind to ephrins

EPH receptors are inhibitory RTKs and signal into MAPK/AKT pathways

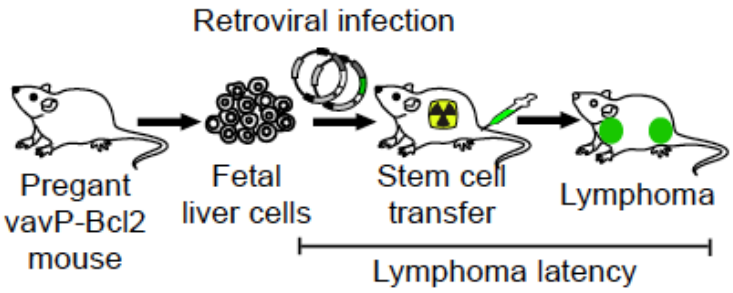
Expressed in various tissues incl. lymphocytes

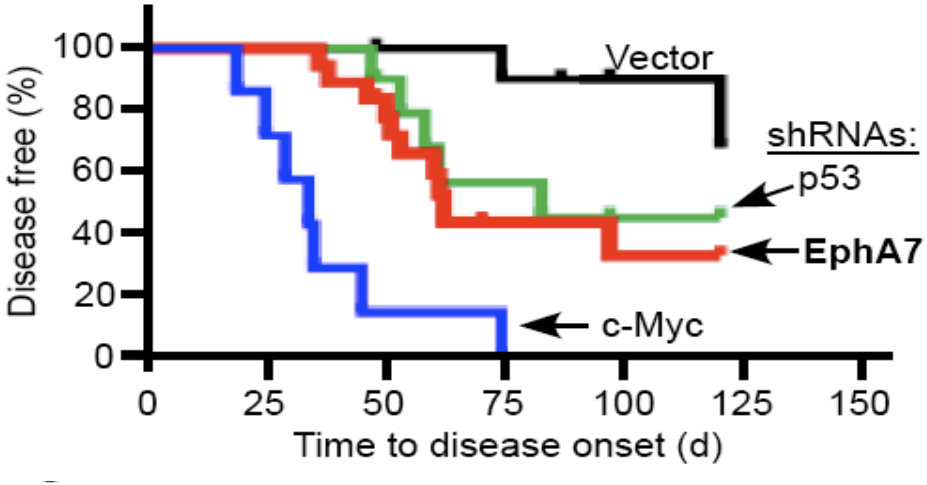
Mutations observed in several solid tumors (melanoma, SCLC, breastCA etc).

Roles in cell density/repulsion/adhesion (best studies in axon guidance)

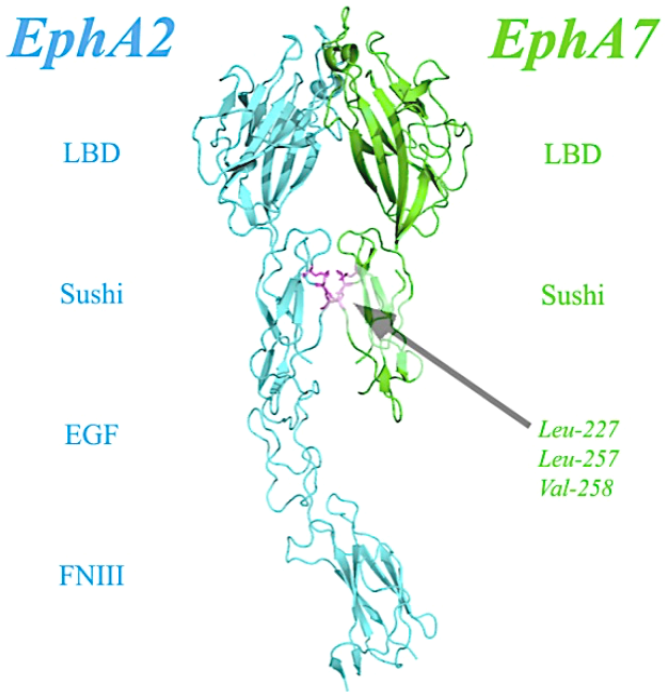
A role in lymphocyte biology is new and surprising

EPHA7 acts as a tumor suppressor in a mouse FL model





A soluble EPHA7^{TR} protein blocks other EPHA receptors



B-cell express only the soluble EPHA7 ectodomain

The EPHA7 ectodomain acts as a inhibitor of EPHA2/3 receptors

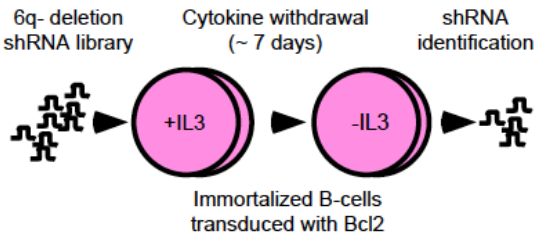
In lymphoma EPHA7 is deleted or silenced in >50%.

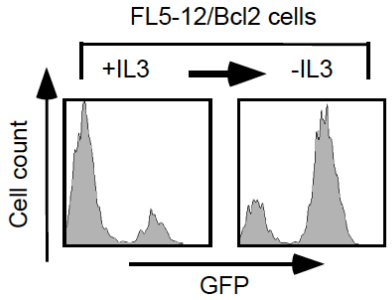
Loss of EPHA7 leads to activation of ERK, SRC kinases.

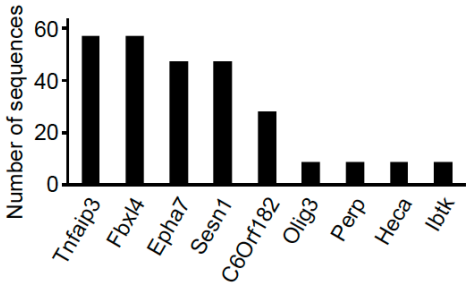
This suggests that EPHA7^{TR} should be able to block EPHA2/3 signaling in lymphoma

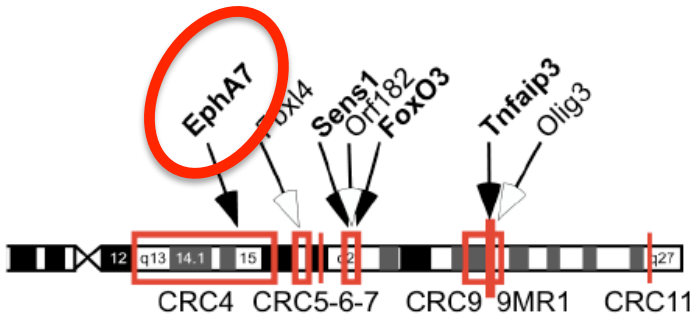
A genetic screen pinpoints candidates genes within Del6q

Surrogate screen **for cooperation with Bcl2 in lymphocytes** in vitro







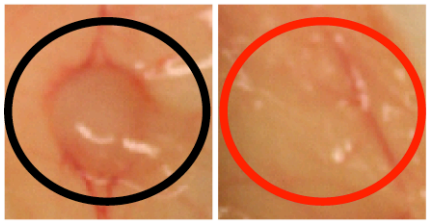


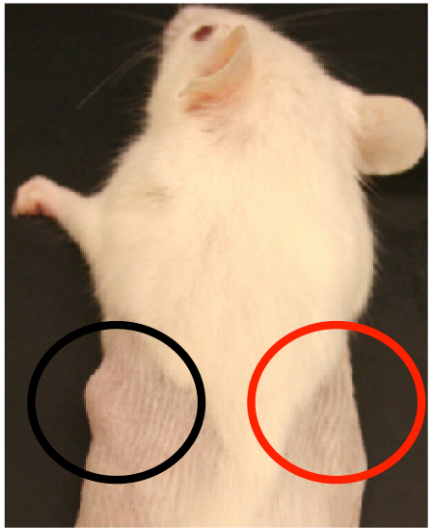
Candidate 6q tumor suppressors: Epha7, Sestrin1, Foxo3, Tnfaip3, Prdm etc.

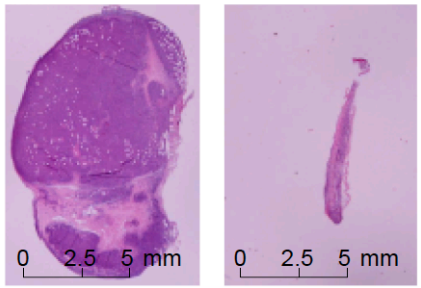
The EphA7^{TR} protein has anti-tumor activity

Local injection

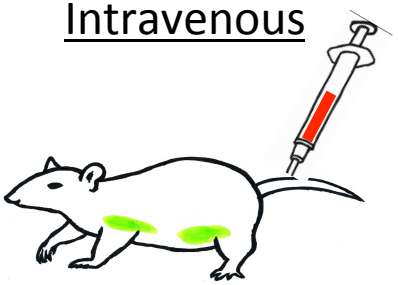
Vehicle EPHA7^{FC}

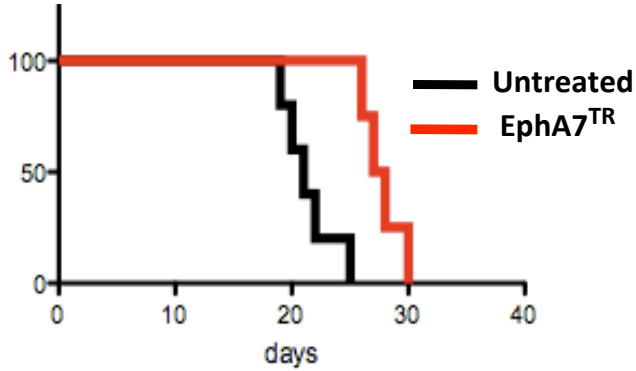






Intravenous

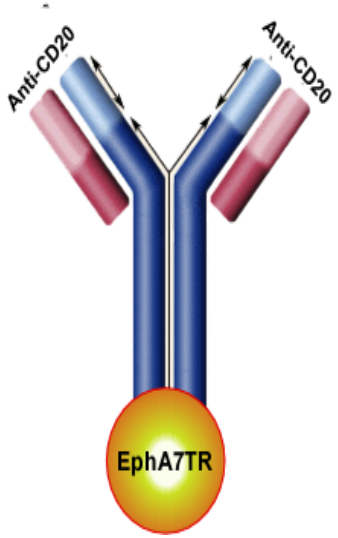


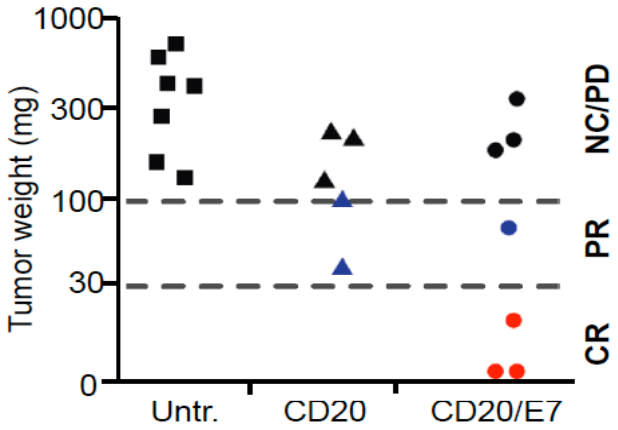


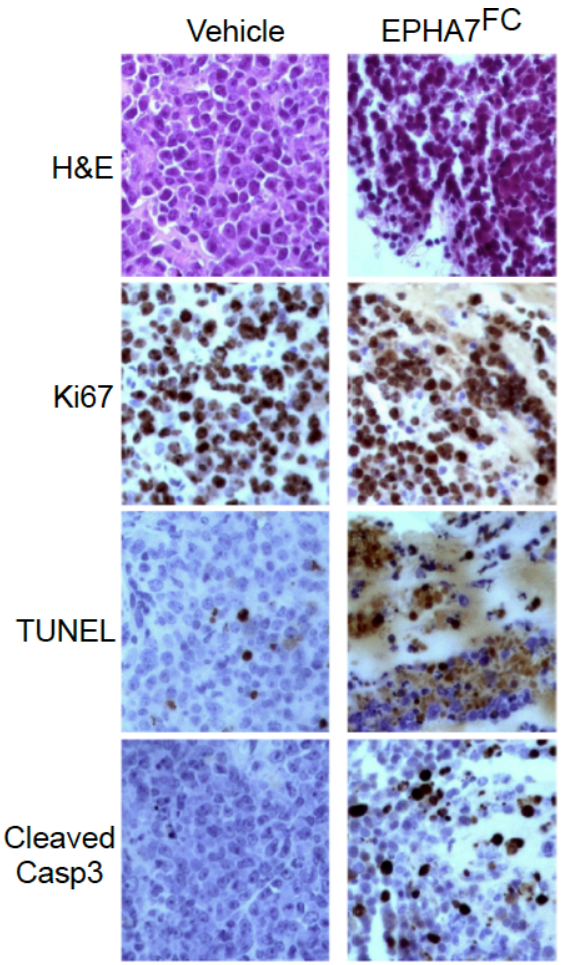
EPHA7^{TR} has biological activity in lymphoma.

Systemic delivery is suboptimal.

CD20-EphA7 fusion antibody delivers Epha7 to lymphomas

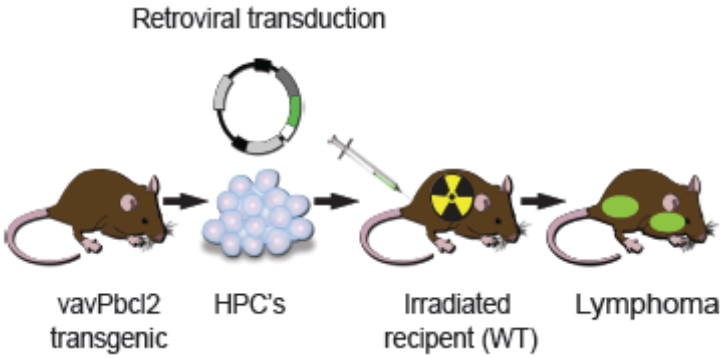


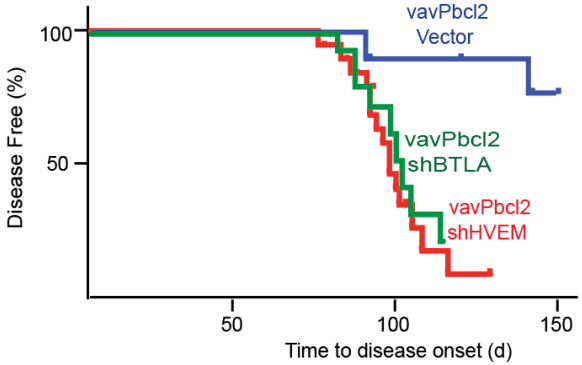


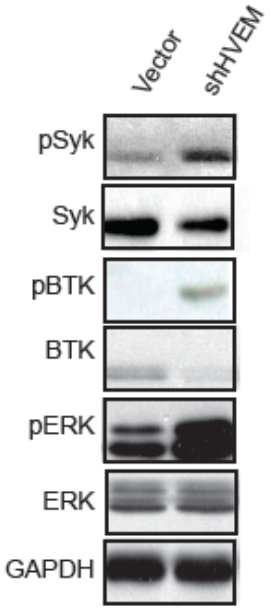


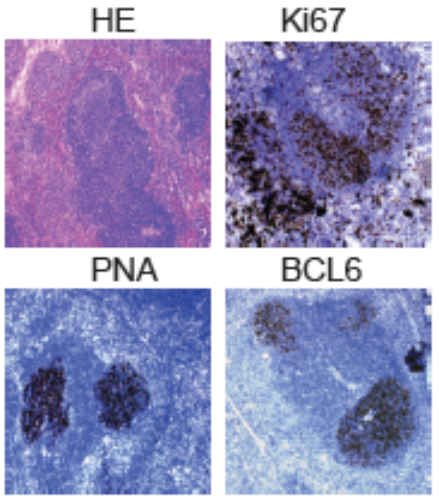
The fusion antibody binds CD20 and restores EPHA7 activity in vivo

HVEM and BTLA act as a tumor suppressor genes *in vivo*





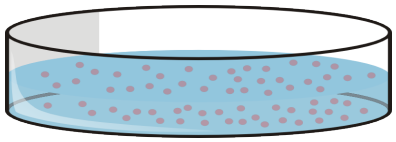




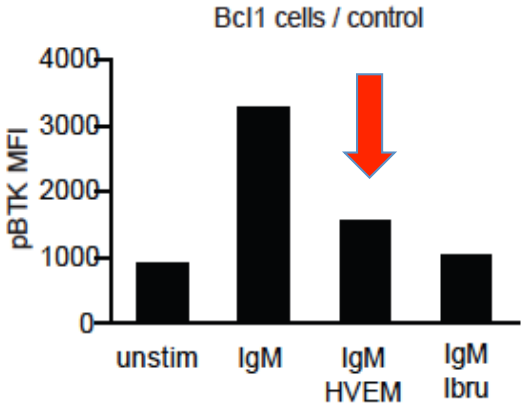
HVEM defective lymphomas show BCR signal activation

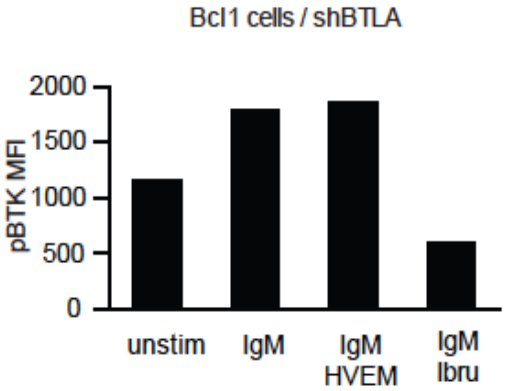
Does HVEM introduction reverse BCR activation?

Stimulation +/- solHVEM



SolHVEM $^{(P37\text{-}V202)}$: HVEM ectodomain

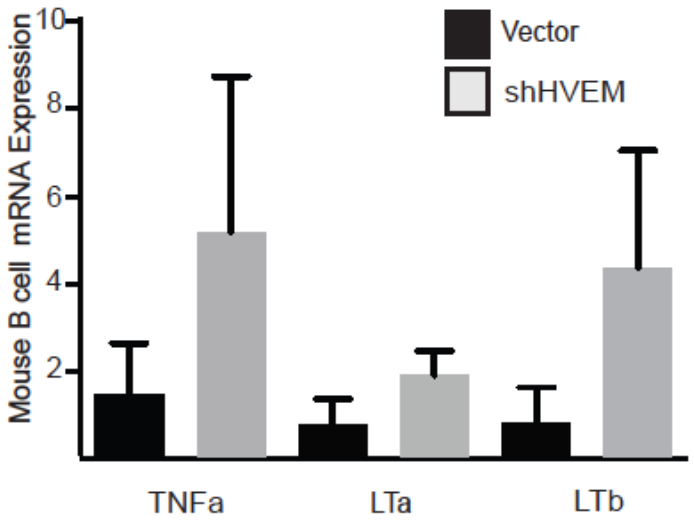




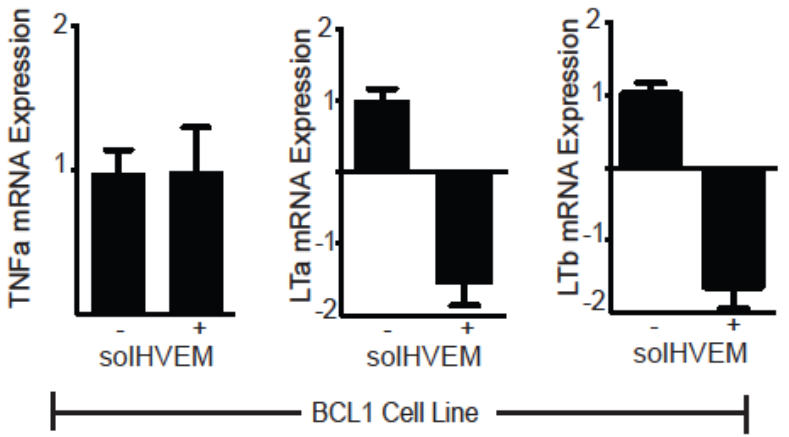
HVEM blocks BCR activation in a cell autonomous manner that requires BTLA

Aberrant cytokine production in HVEM deficient lymphomas

Sorted lymphoma B cells



solHVEM

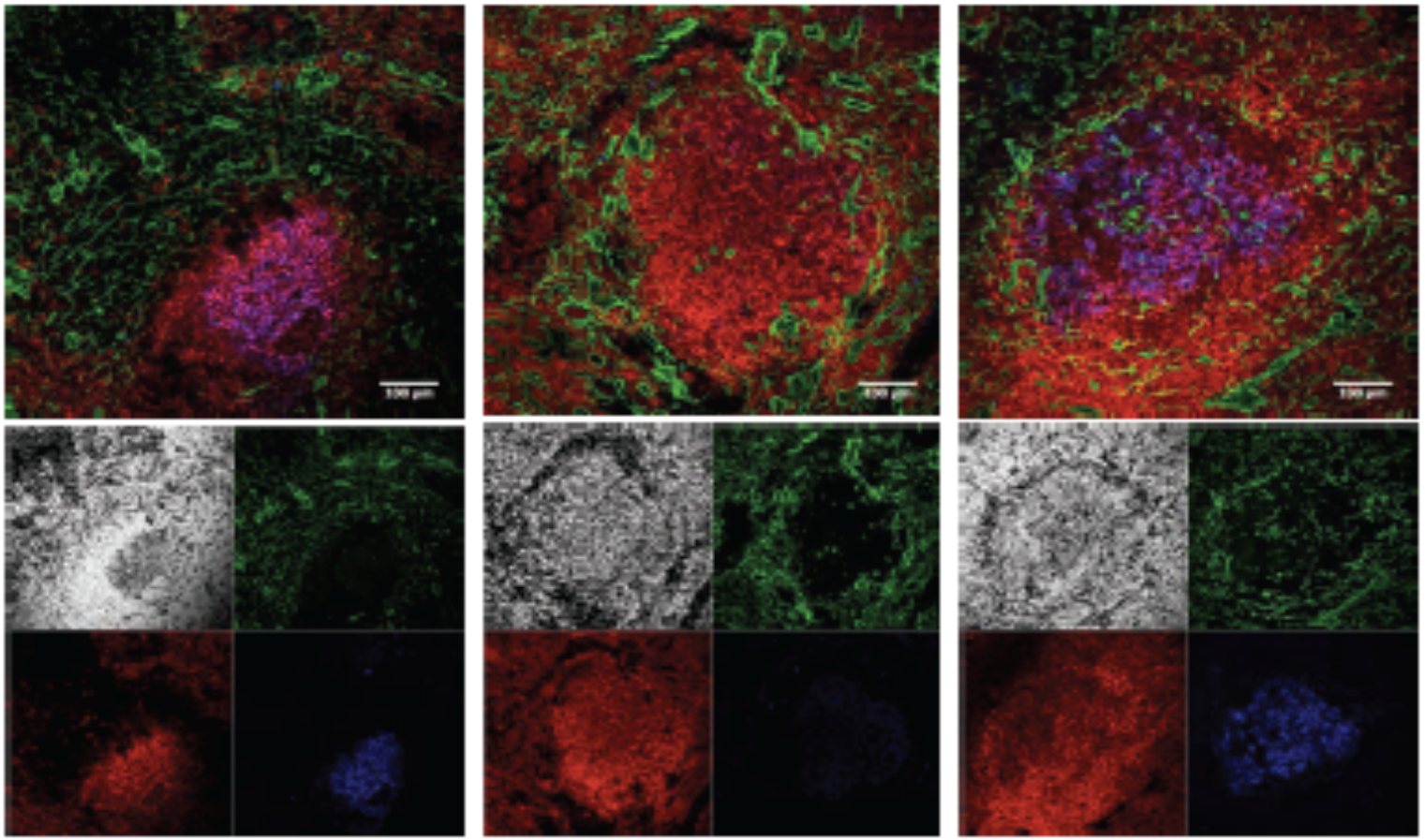


HVEM deficient B cells produce excess amounts of cytokines that activate the lymphoid stroma

Lymphoid stroma activation in HVEM deficient tumors

Karin Tarte

Reactive GC | Lymphoma | HVEM Lymphoma



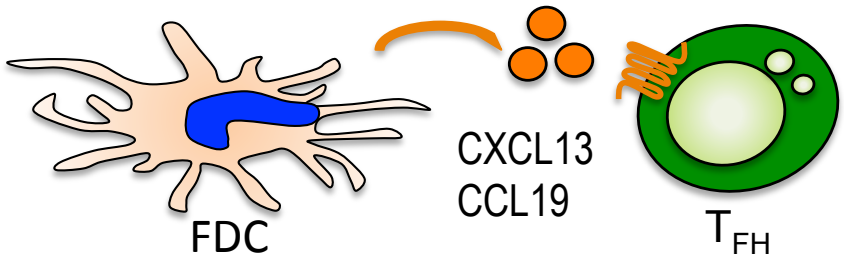
B cells

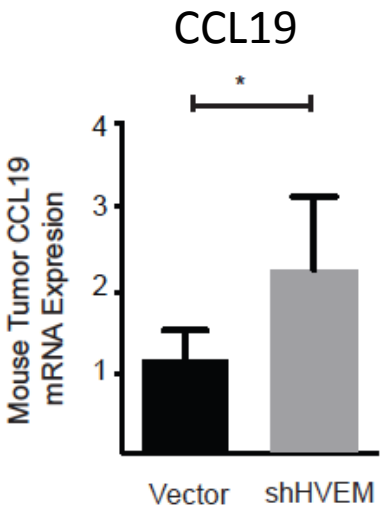
FRCs

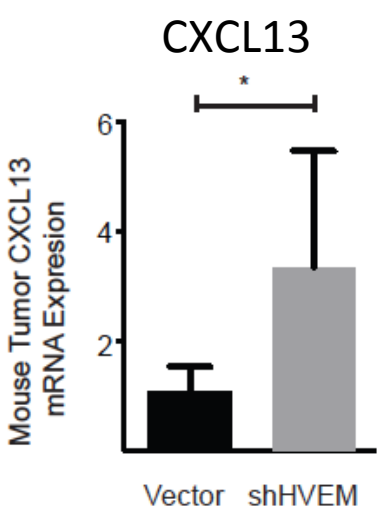
FDCs

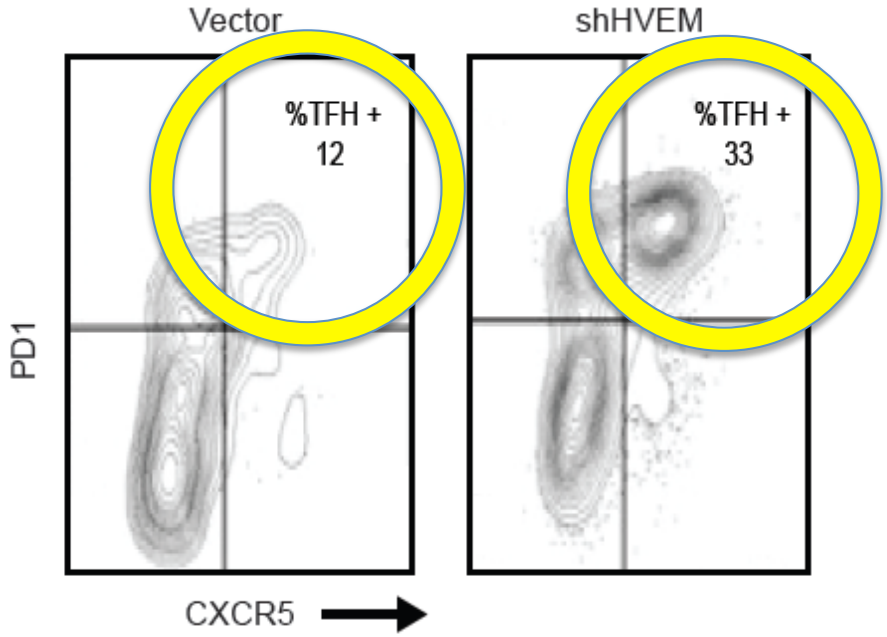
What does an activated stroma do for lymphomas?

The lymphoid stroma produces chemo-attractants for TFH cells



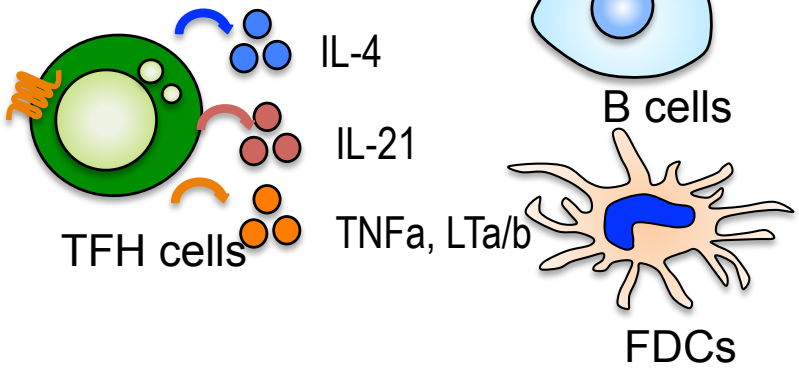




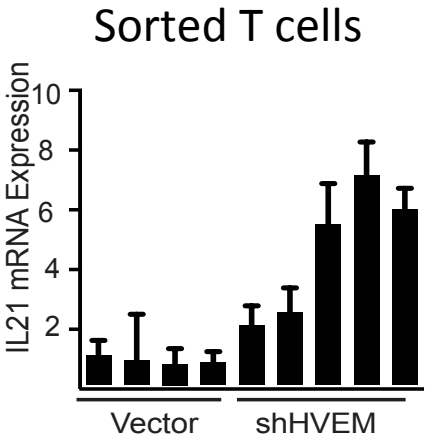


HVEM deficient lymphomas recruit ~3x more TFH cells

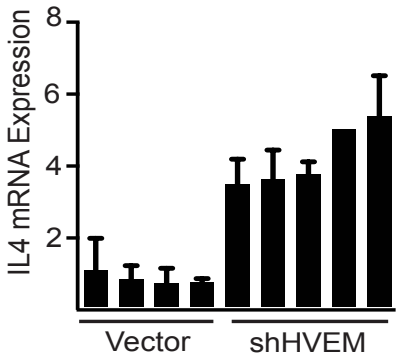
T_{FH} cells support B cell growth by producing IL4 and IL21



IL21



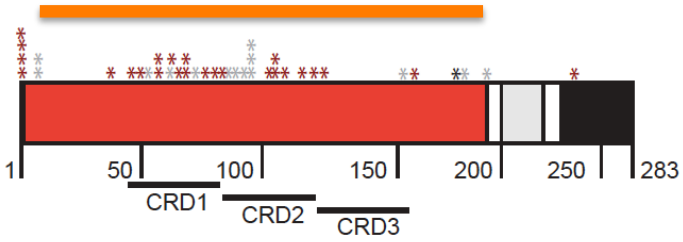
IL4



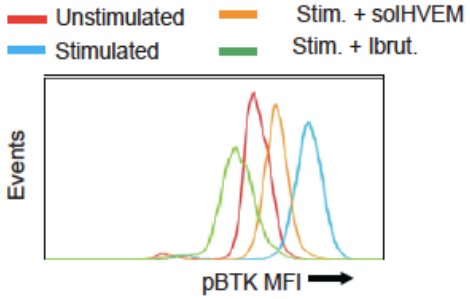
TFH cytokines (IL4/IL21 and LTa/b) contribute to a supportive niche

The SolHVEM protein reverses some effects of HVEM loss

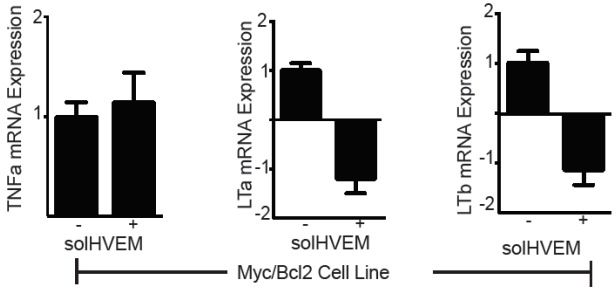
SolHVEM (P37-V202)



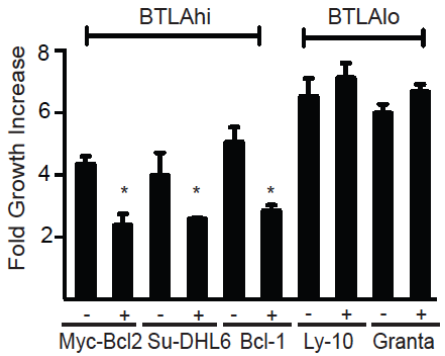
Inhibition of mitogenic signals



Partial reversal of cytokine effects

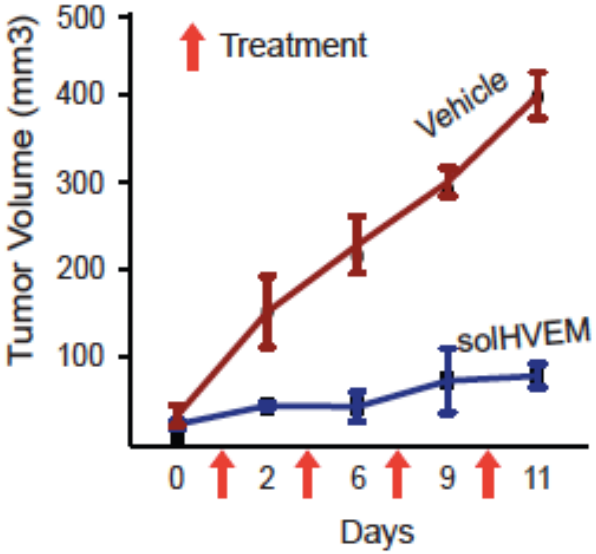


Growth inhibition

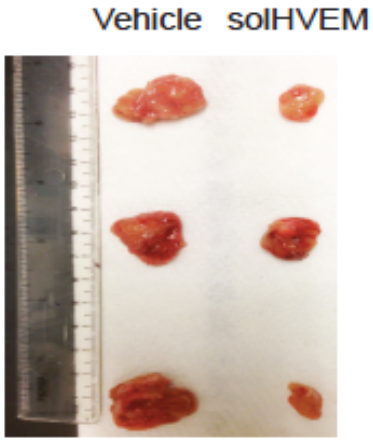


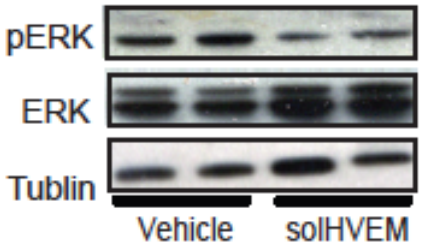
Can we use the solHVEM protein to treat lymphomas?

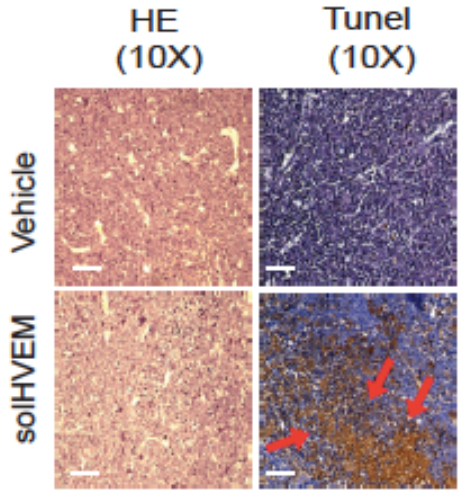
SolHVEM has anti-lymphoma effects in vivo



MYC/BCL2 mouse lymphomas; s.c. inject; 20μg

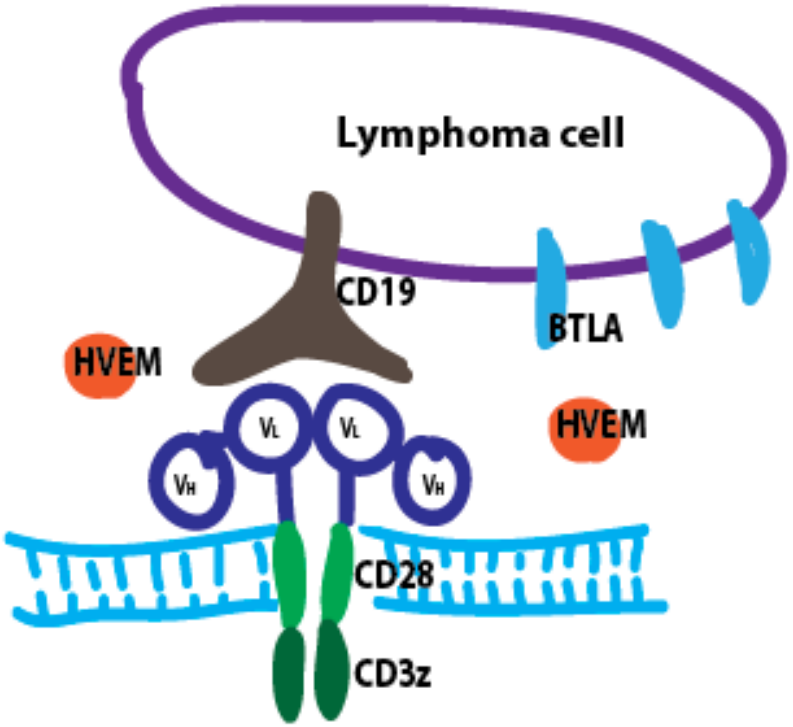


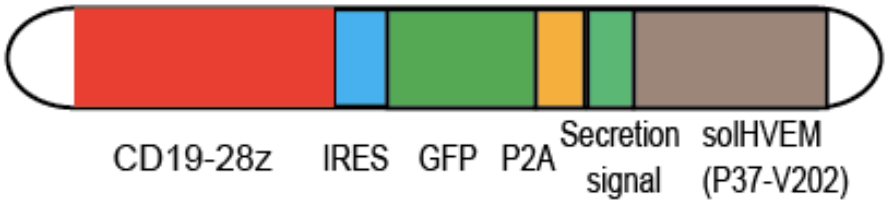


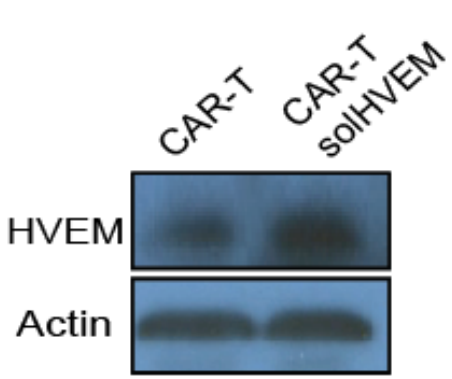


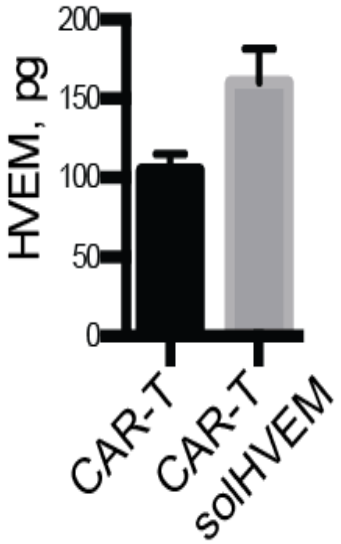
How can we deliver solHVEM to lymphomas in vivo?

Engineering CAR-T to secrete solHVEM *locally and continuously*



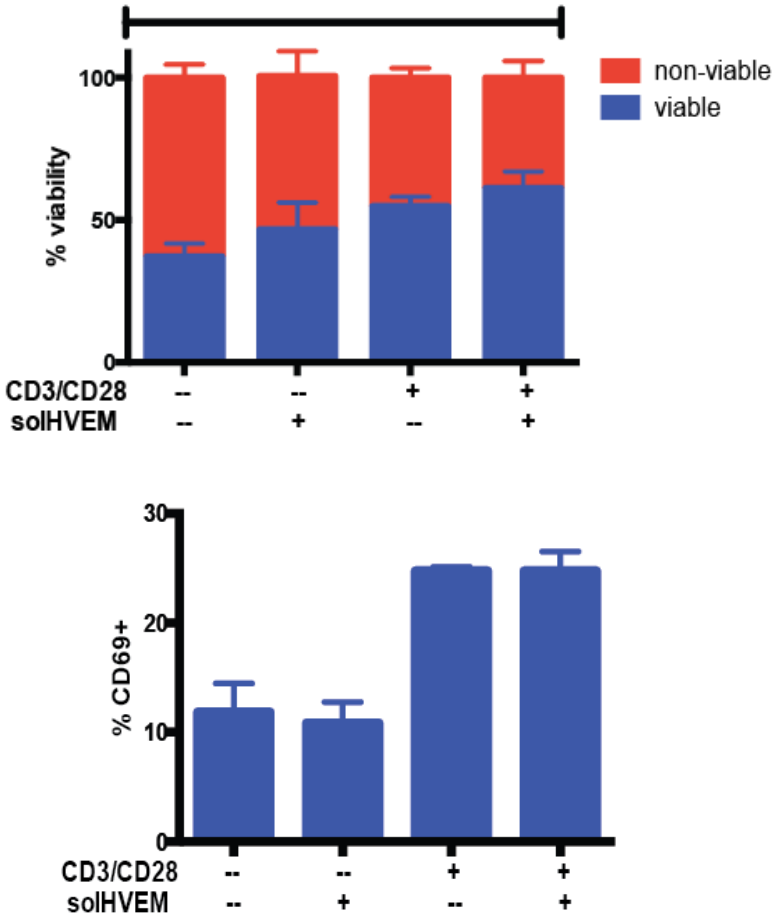




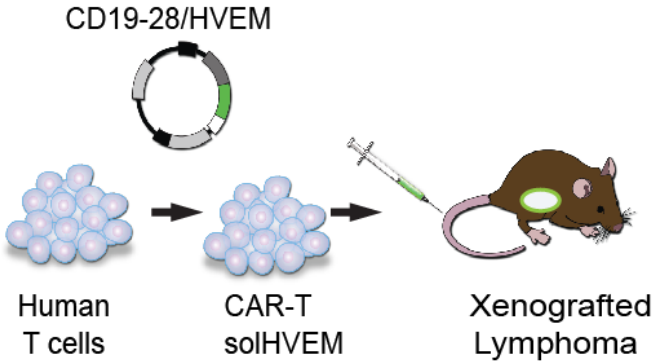


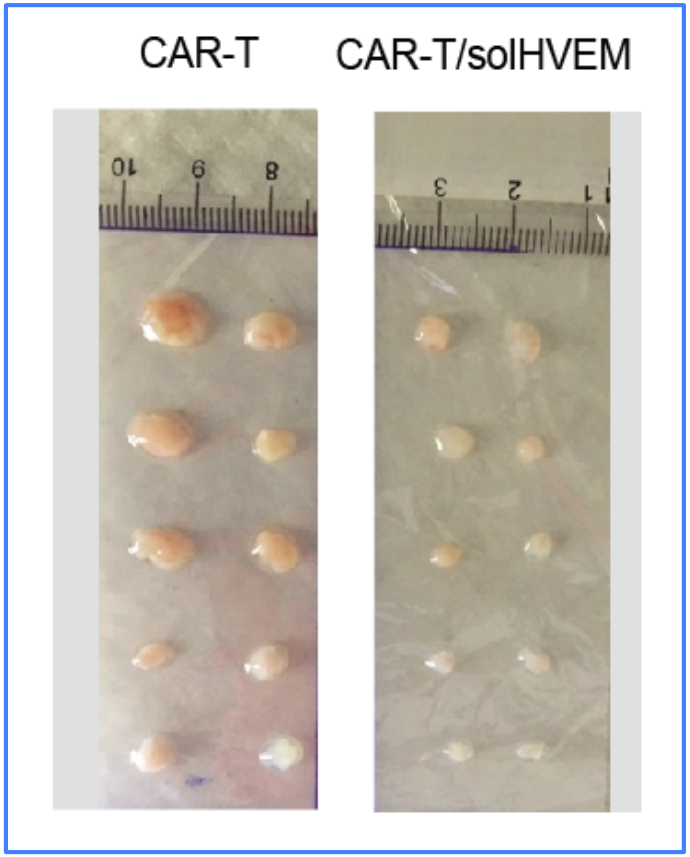
CAR-T cells as HVEM producing 'micro-pharmacies'

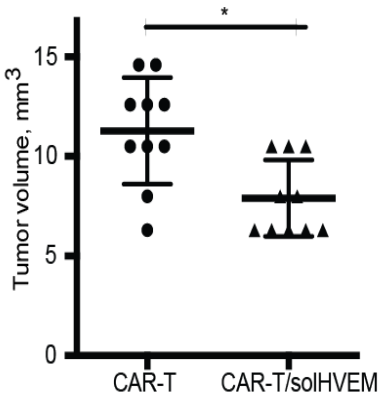
HVEM does not affect T cell activation/viability



Do CAR-T/solHVEM have therapeutic activity in vivo?

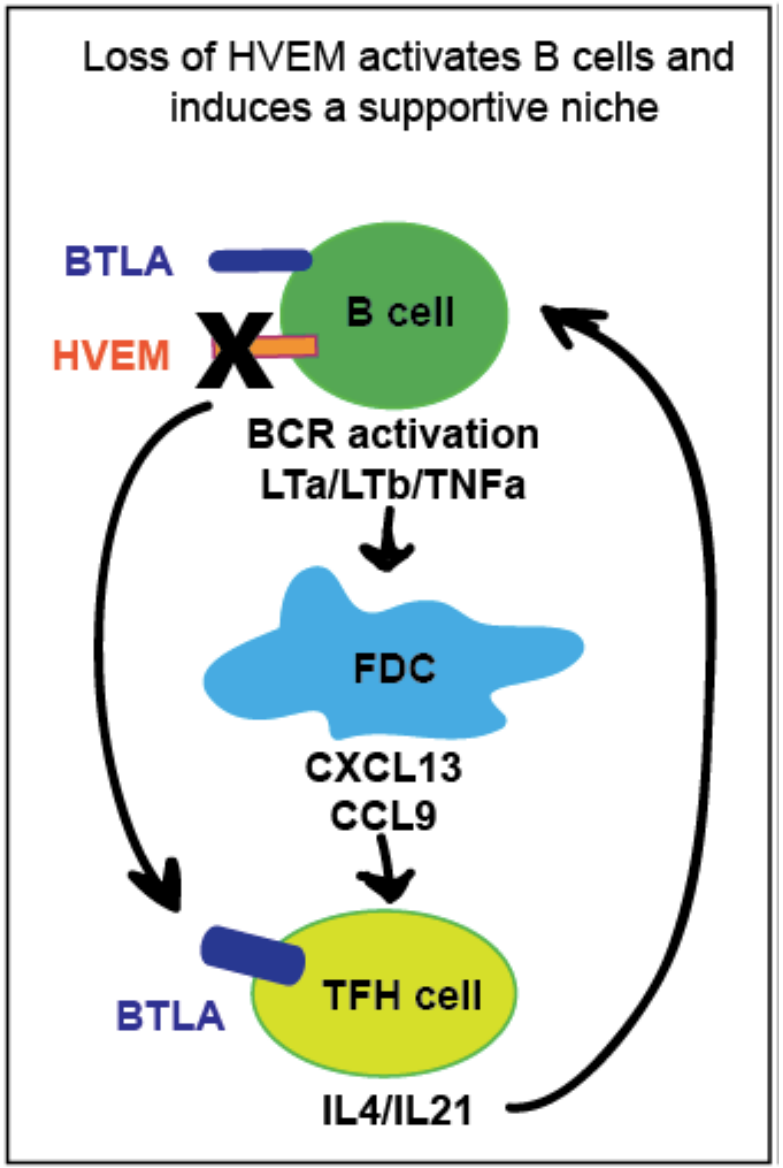






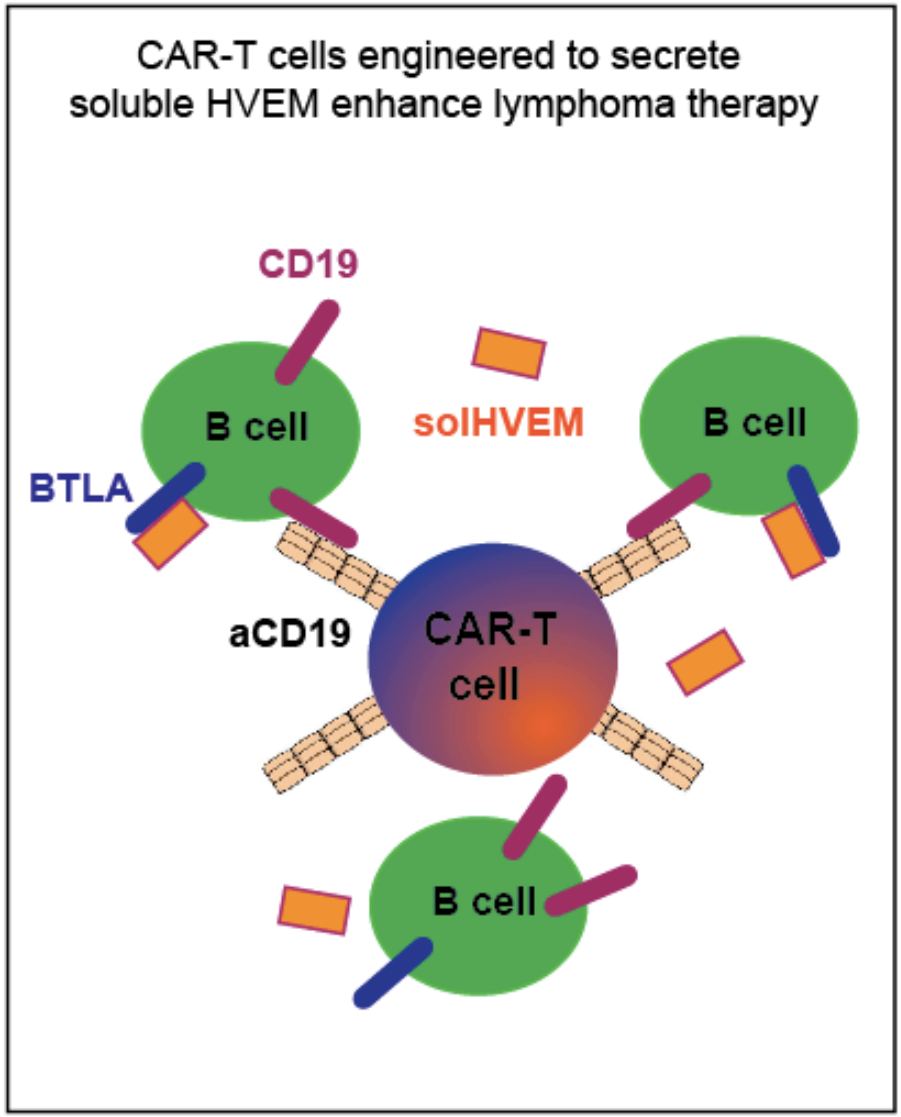
We can deliver solHVEM using CAR-Ts and improve responses

The HVEM-BTLA interaction is lost in most FLs



- Activation of BCR signaling
- Aberrant cytokines (LTa/b, TNFa)
- Lymphoid stroma activation
- TFH recruitment and activation

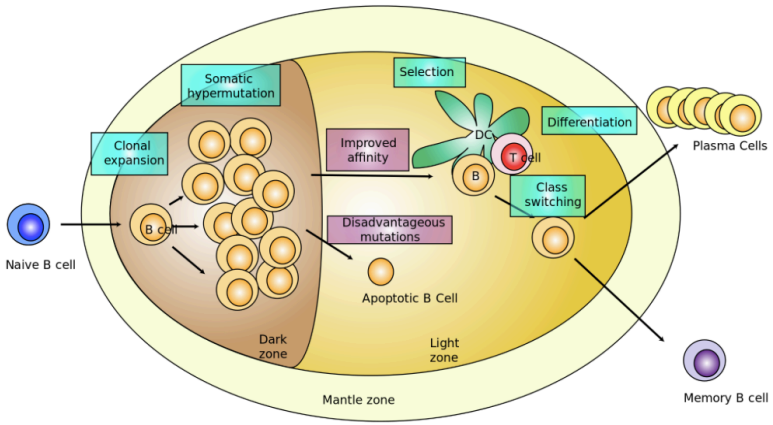
CAR-T cell ‘micro-pharmacies’ can deliver an anti-tumor protein



- solHVEM blocks lymphoma growth.
- Modified CAR-T cells produce HVEM locally.
- CAR-T/HVEM show better activity.

There are likely other uses for CAR-T cell ‘micro-pharamacies’

What about the germinal center micro-environment?



Simplified view of GC

GCs are site of B cell maturation into plasma and memory cells.

GC B cells undergo:

- somatic hypermutation
- genomic rearrangements
- PLUS: explosive growth

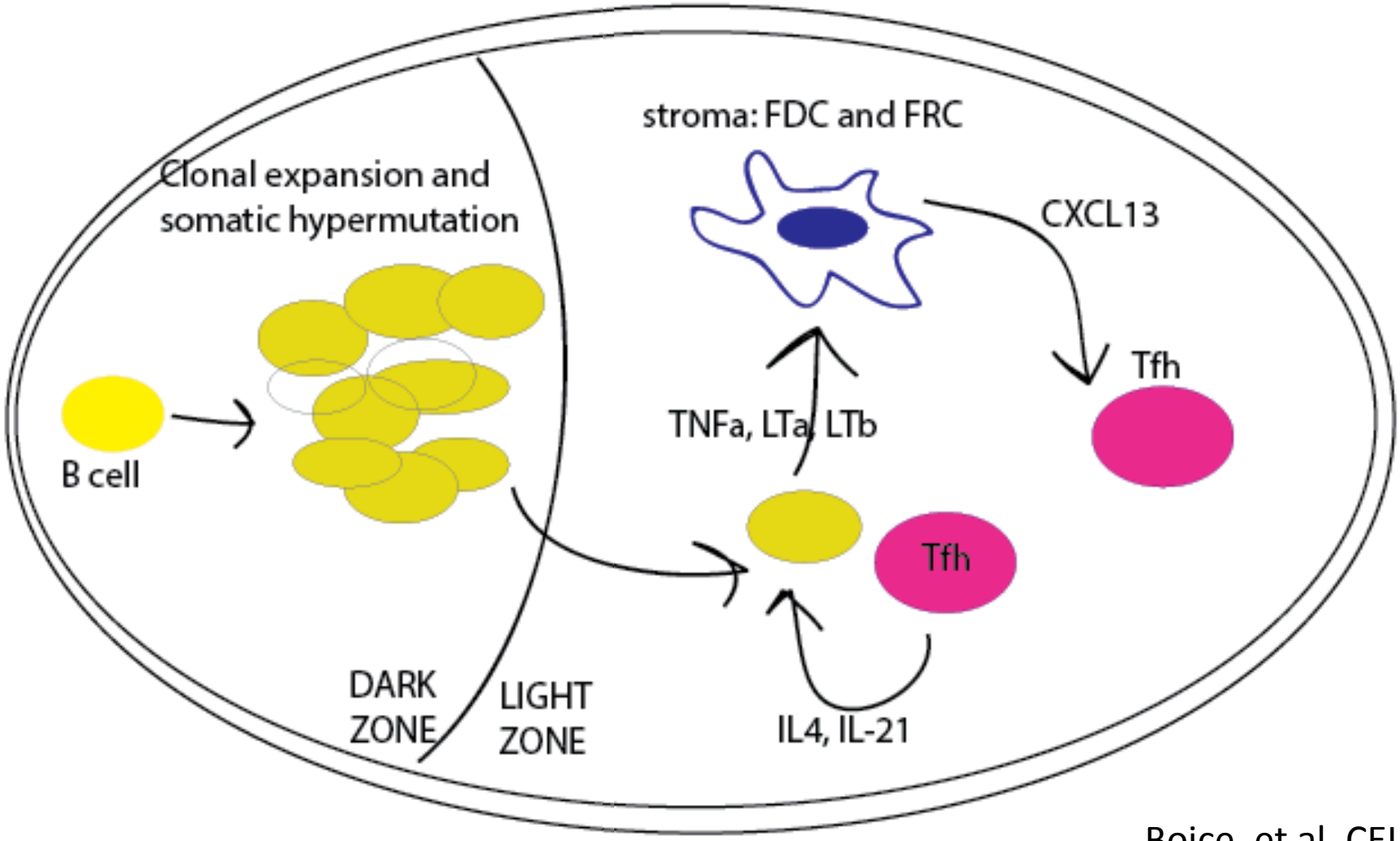
➔ Risky place!

Failsafe mechanism:

- cellular tumor suppressors
- interactions with other GC cells

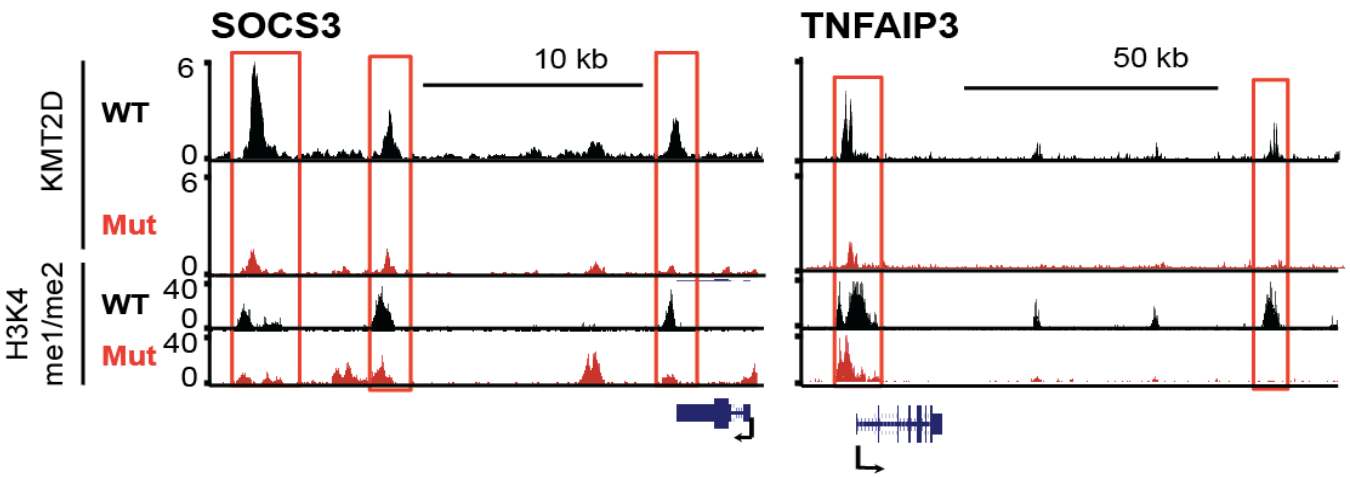
Introducing the cast of the story

How does the malignant process interact with the GC environment?



Boice, et al. CELL 2016

Epigenetic lesions drive aberrant gene expression programs



MLL2 (KMT2D) is the most frequently mutated gene in FL.

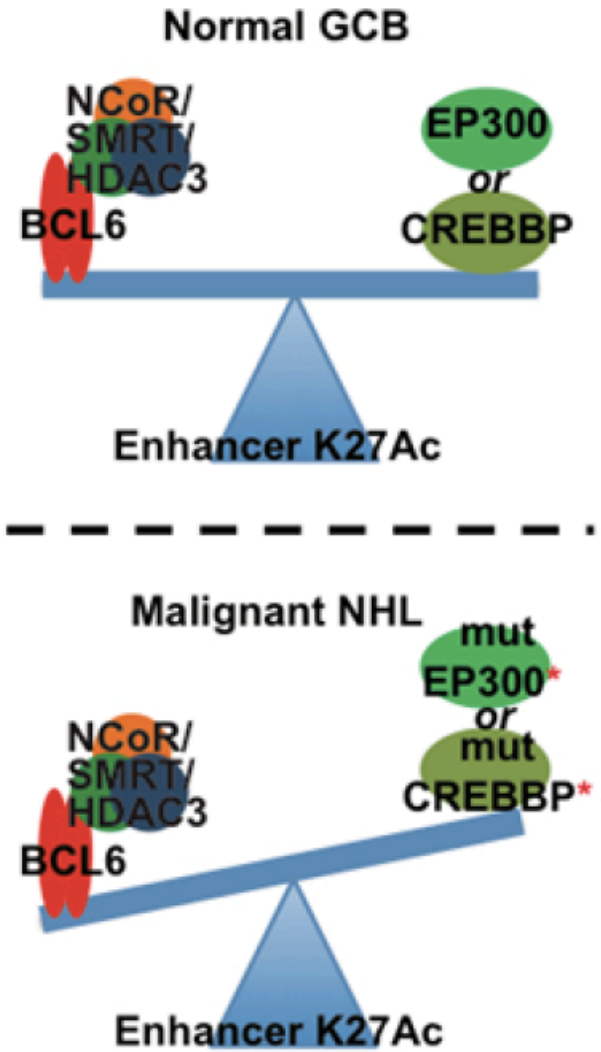
We identified direct MLL2 targets genes in FL: Tumor suppressors e.g. A20 and regulators of growth signals.

MLL2 loss disturbs humoral immunity e.g. Kabuki Syndrome

MLL2 loss may impact epigenetic and kinase inhibitor therapies.

Ortega Nat. med. 2015

Disrupting the histone acetylation balance



CREBBP and EP300 mutations shift enhancer control towards gene silencing by BCL6/ HDAC3.

This affects regulators of BCR signaling and the immune response (MHCII).

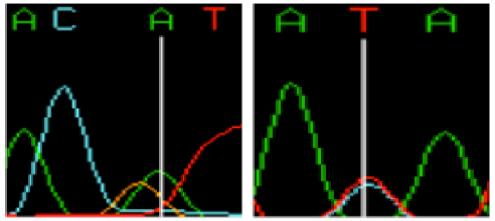
CREBBP/EP300 defective tumor cells show increased sensitivity to HDAC3 inhibition.

Jiang, Ortega, Cancer Disc. 2016

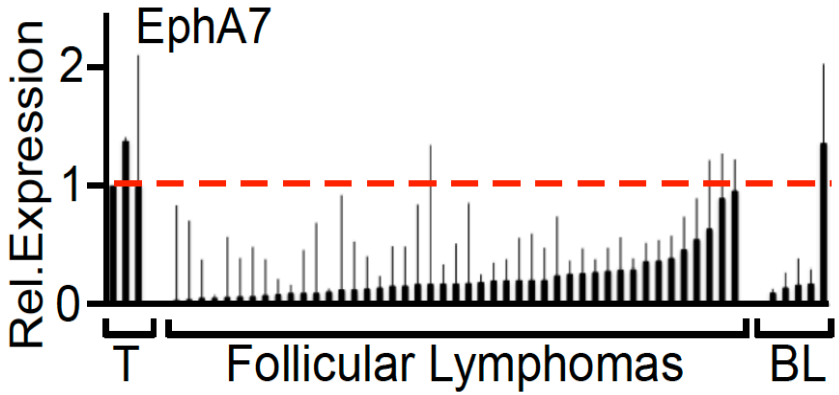
Do this gene behave as classical TSGs?

EphA7 (SNPs)

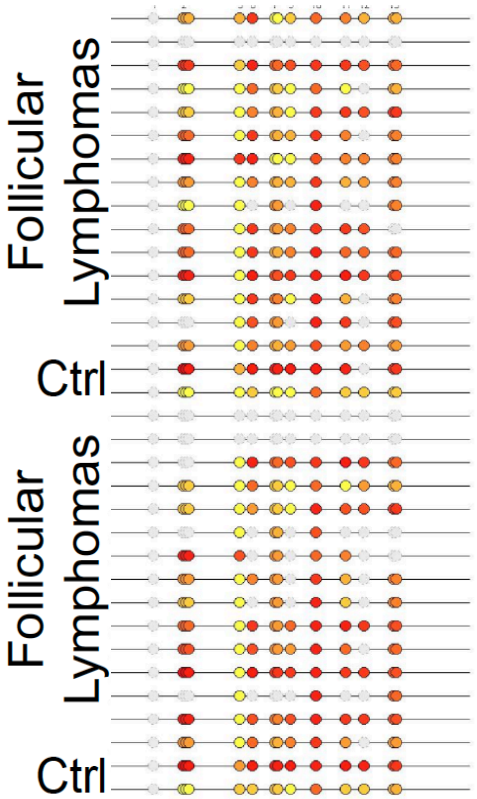
P278S I138V



No mutations

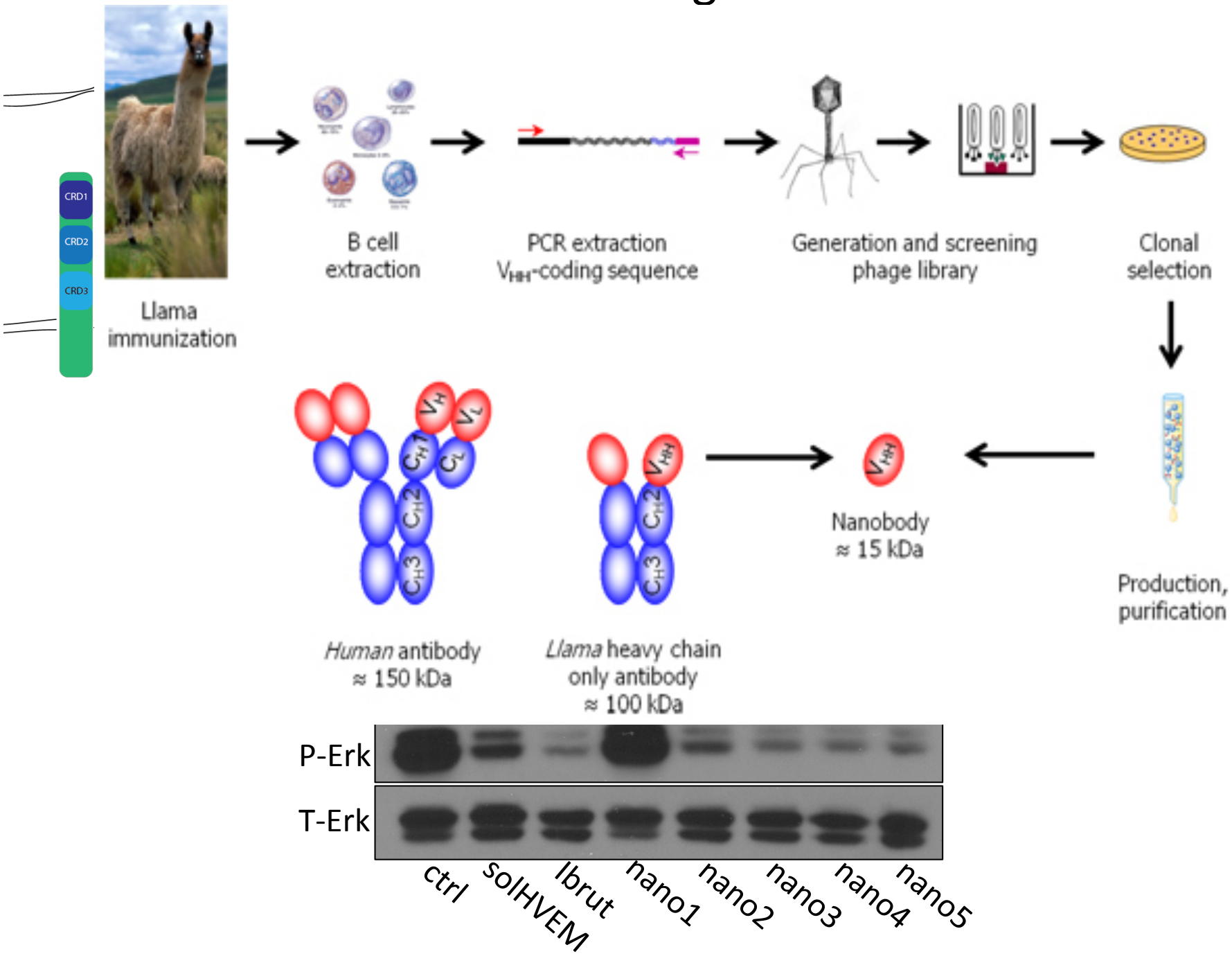


Promoter methylation

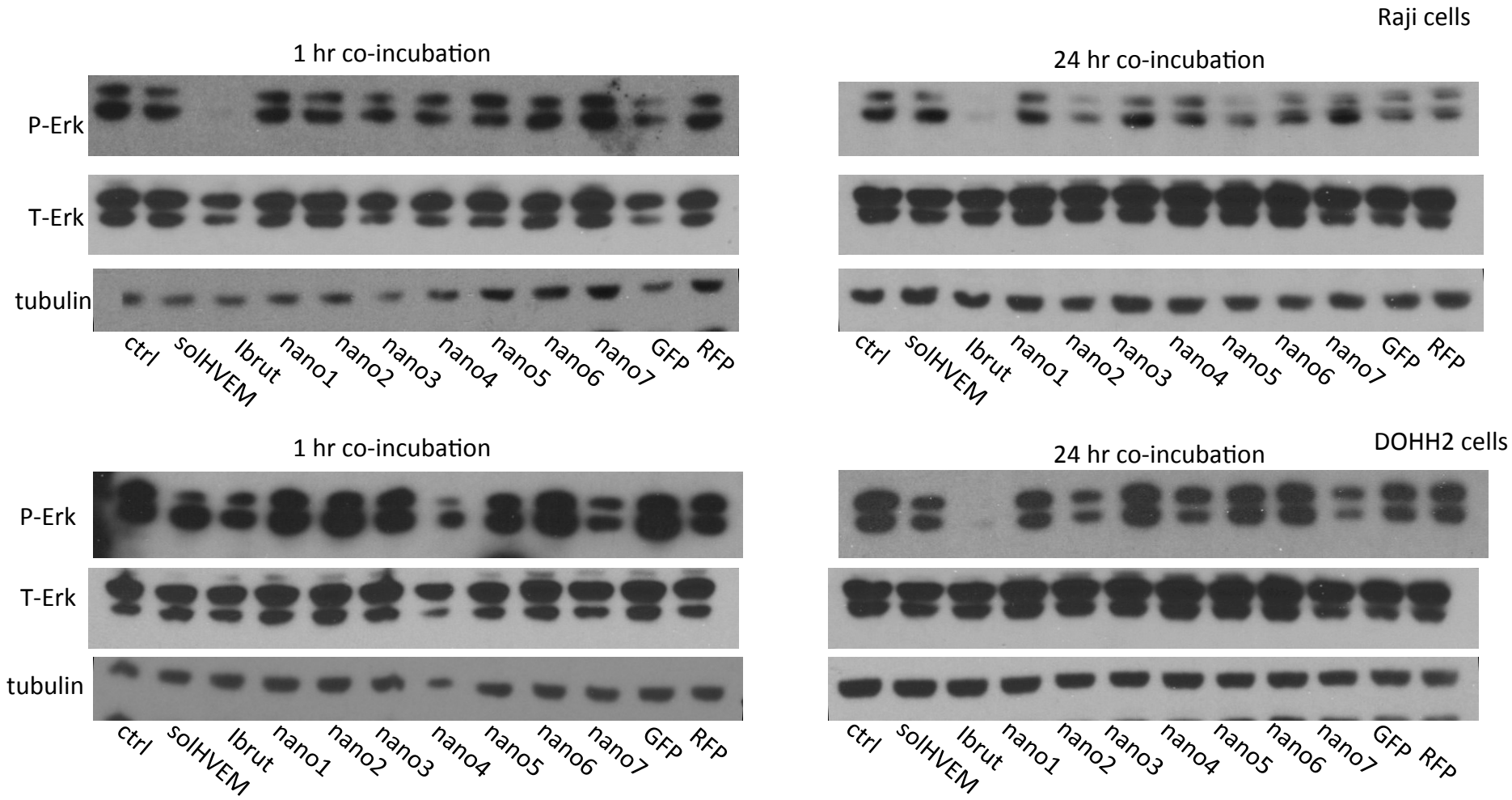


The EphA7 is a new candidate tumor suppressor gene

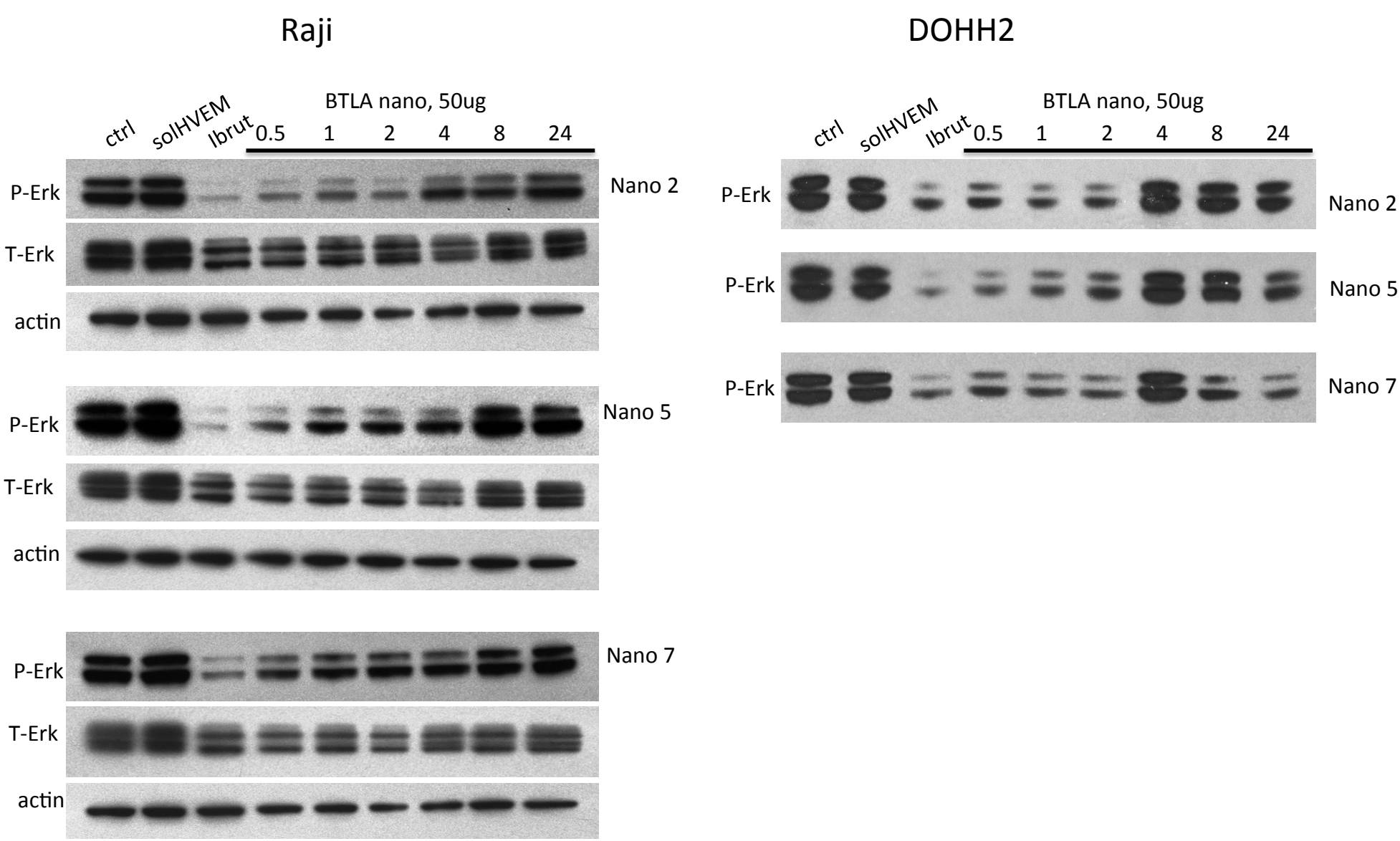
Nanobodies against BTLA



BTLA nanobodies



BTLA nanobodies, time course



BTLA nanobodies, effect on PBMCs