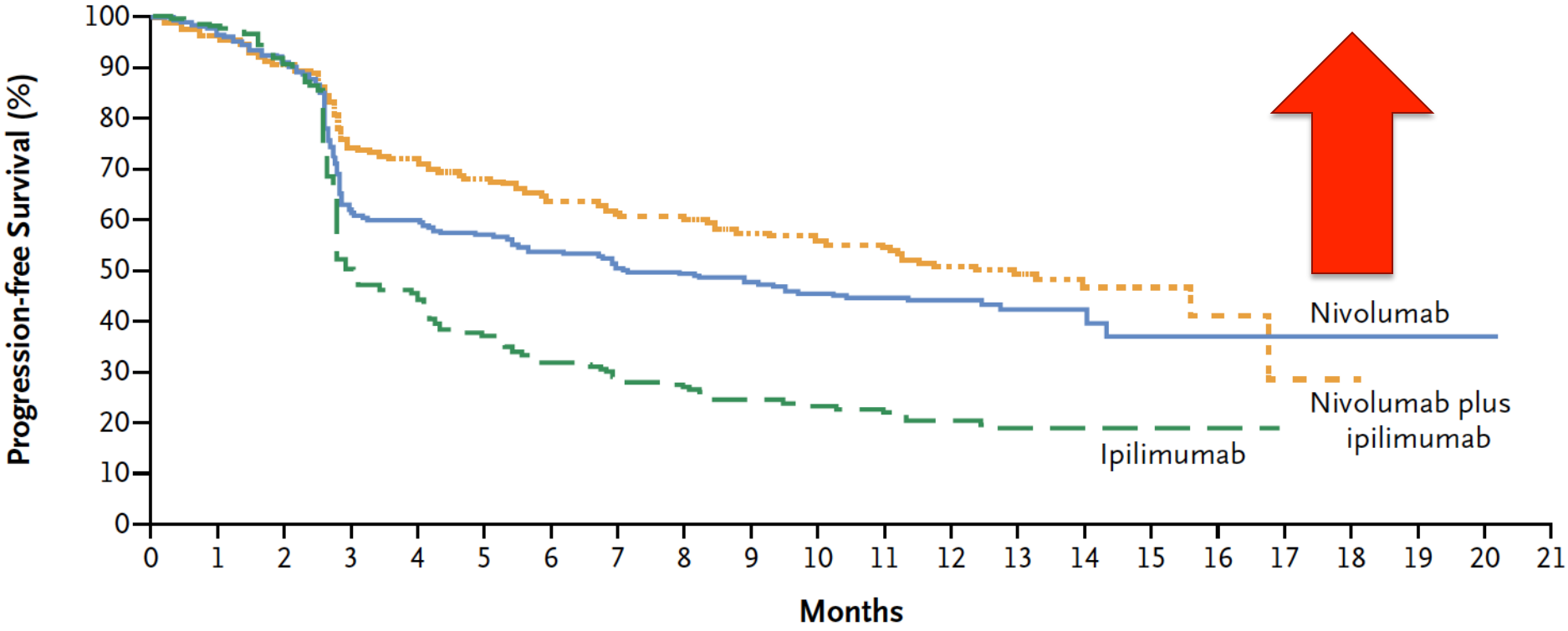
The role of the T cell-inflamed and non-T cell-inflamed tumor microenvironment for immunotherapy

Cancer Crosslinks, Oslo – 01/14/2016

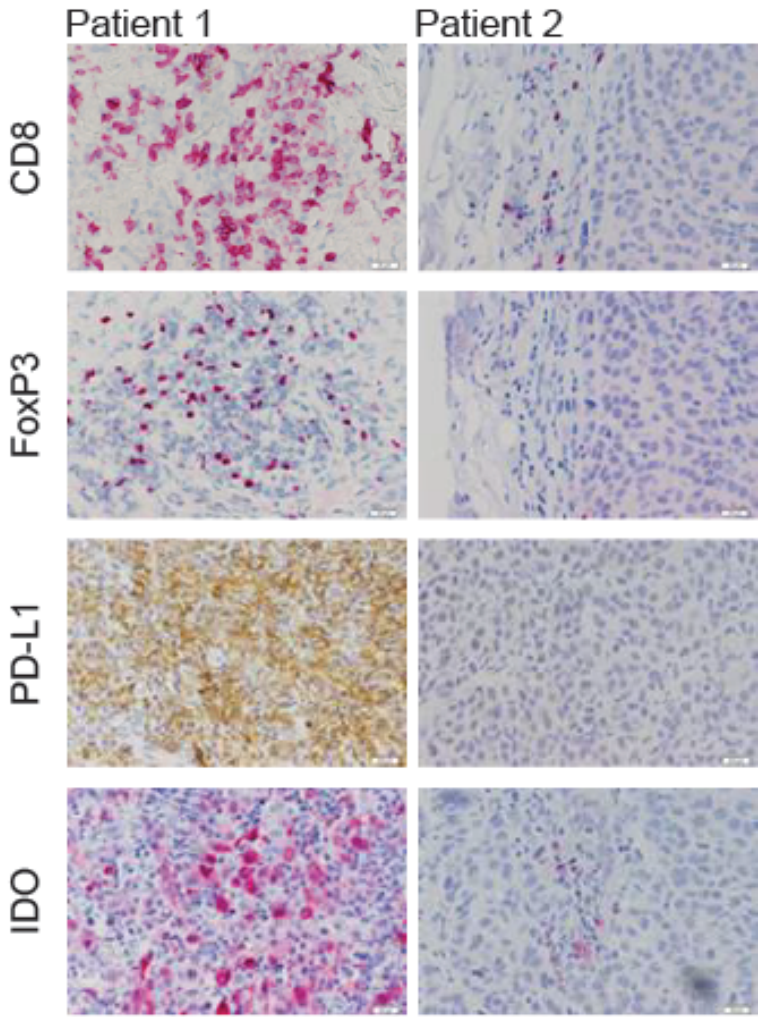
Malignant melanoma and the path towards immunotherapy

- Malignant melanoma (stage IV) had a median survival of 6-9 months
- Melanoma became the “model cancer” for immunotherapy development
- Immunotherapy/Checkpoint blockade has changed the course of disease with a predicted 5-year survival of approximately 40%



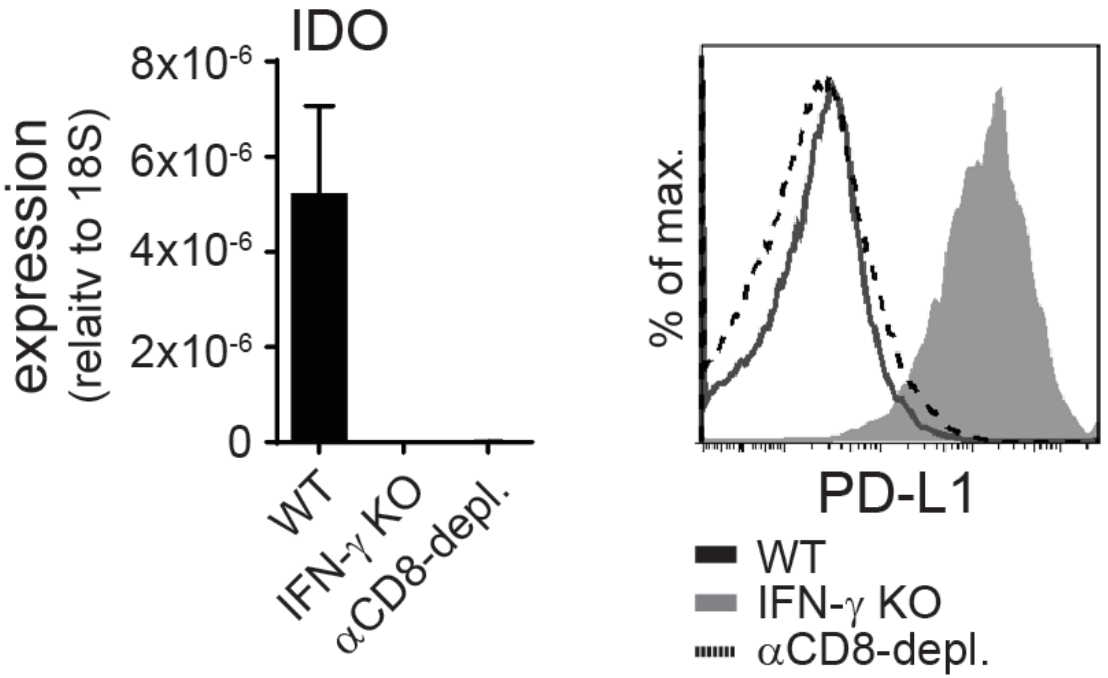
Larkin et al. , NEJM 2015

$CD8^+$ T cell-inflamed melanoma shows signs of increased immune suppressive mechanisms



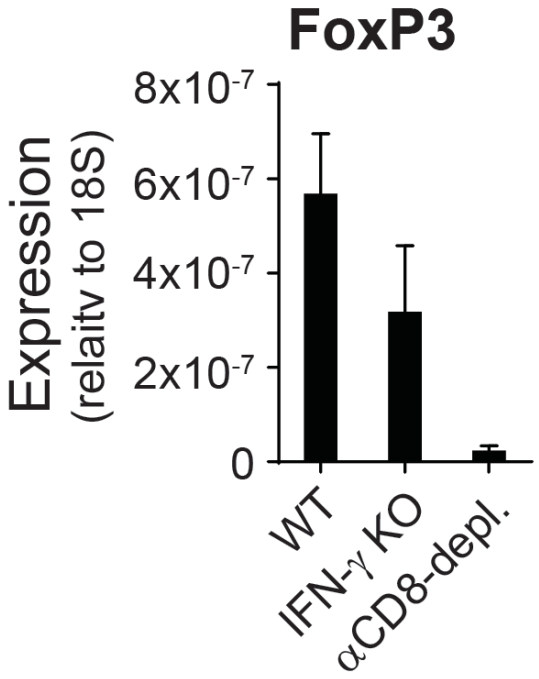
Spranger et al., STM 2013

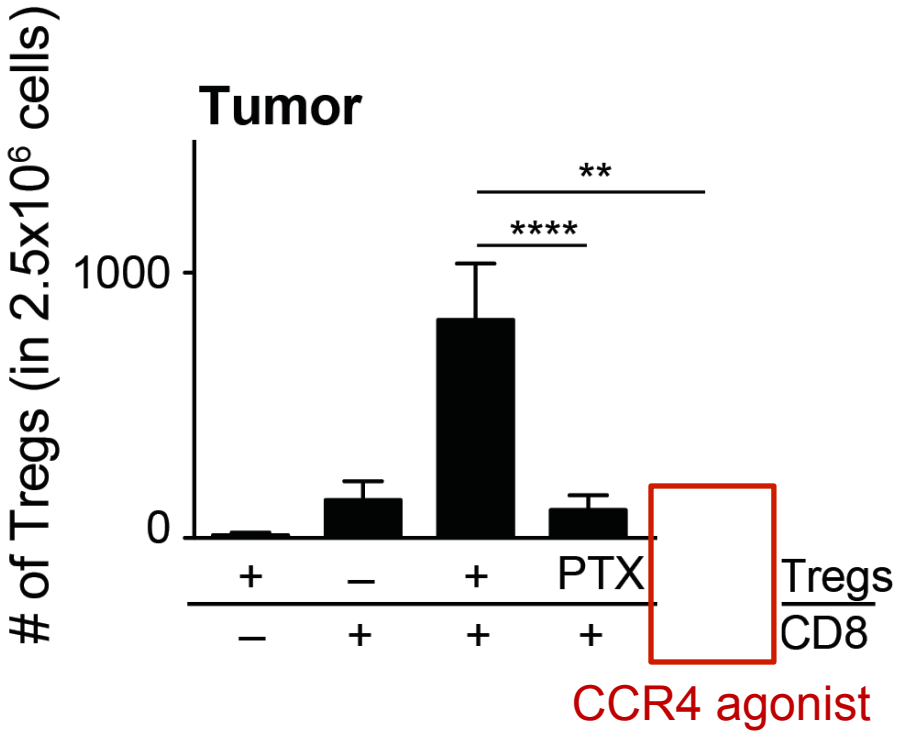
IFN-γ produced by CD8+ tumor infiltrating T cells mediated upregulation of IDO and PD-L1



Spranger et al., STM 2013

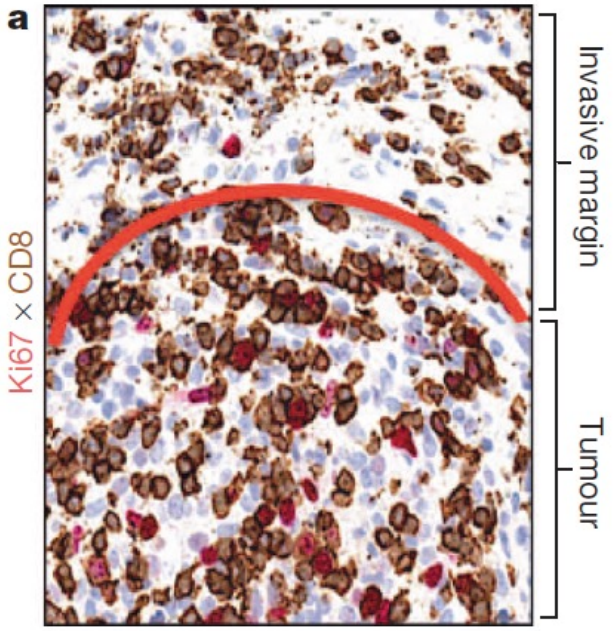
Recruitment of regulatory T cells is mediated through CCL22 produced by CD8$^+$ TIL

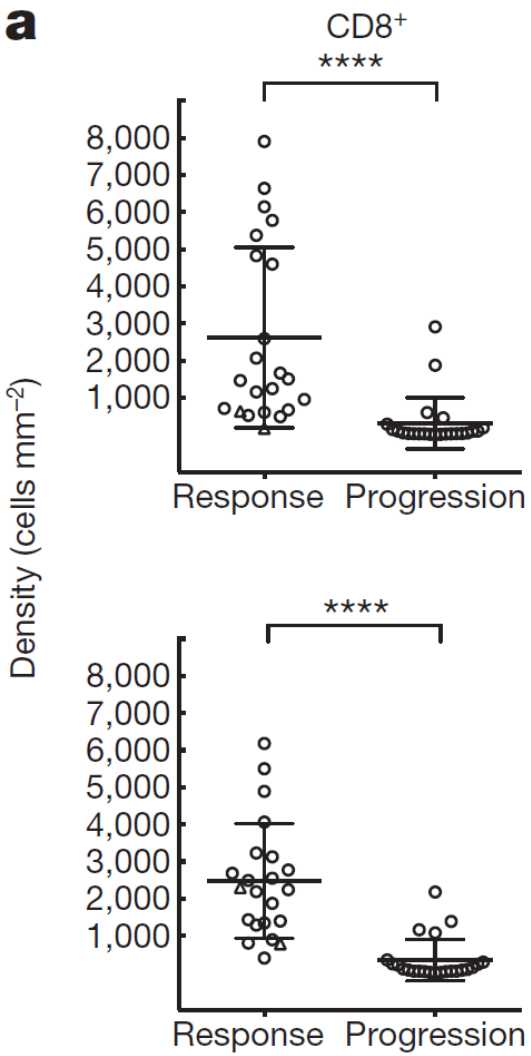




Spranger et al., STM 2013

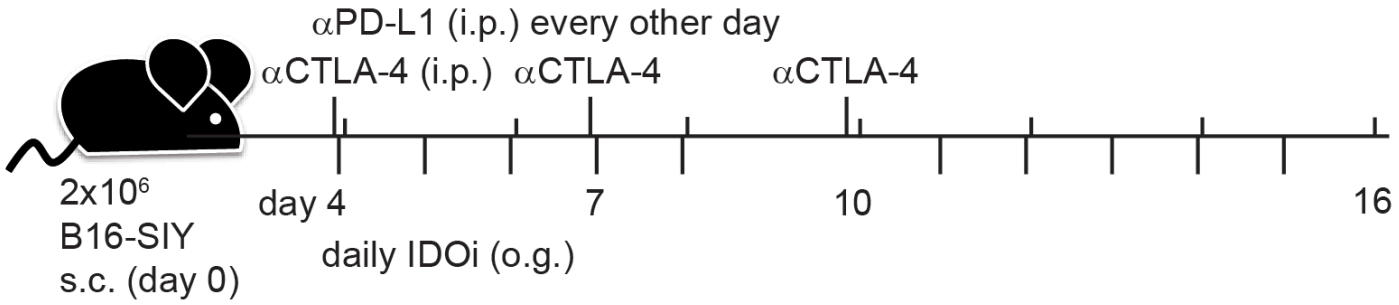
Anti-PD-1 therapy appears to be preferentially effective in T cell-inflamed tumors

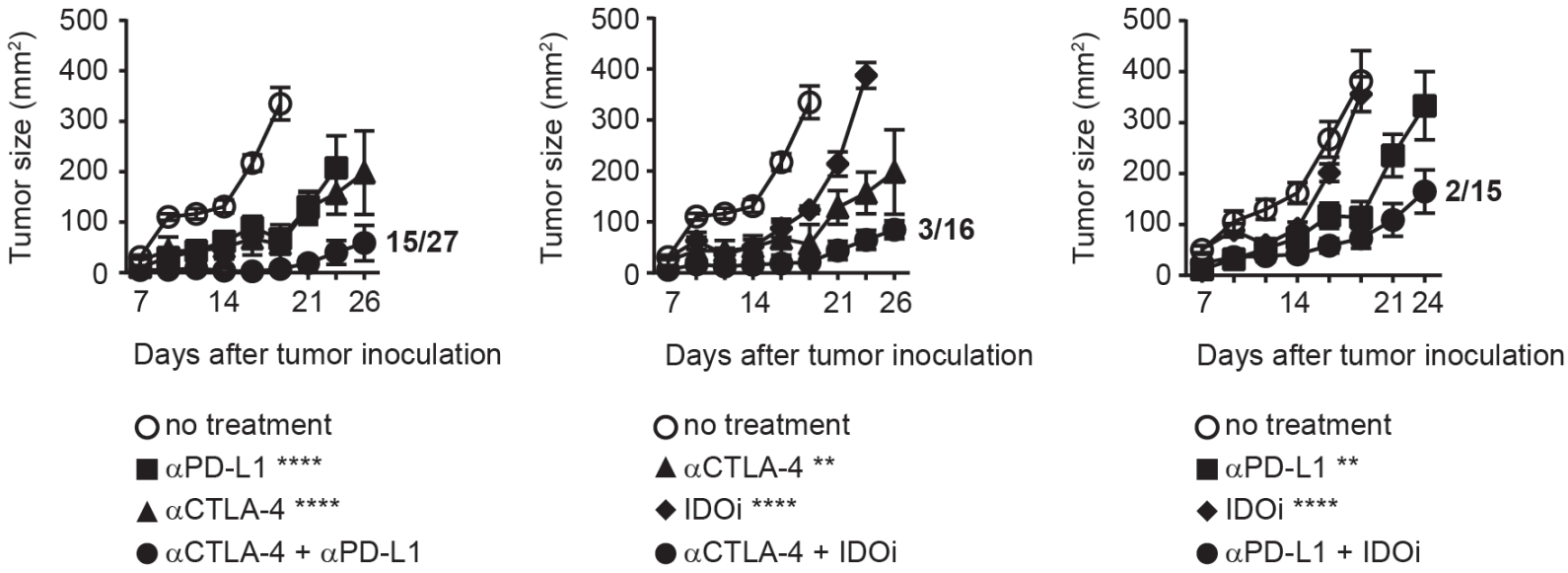




Tumeh et al., Nature 2014

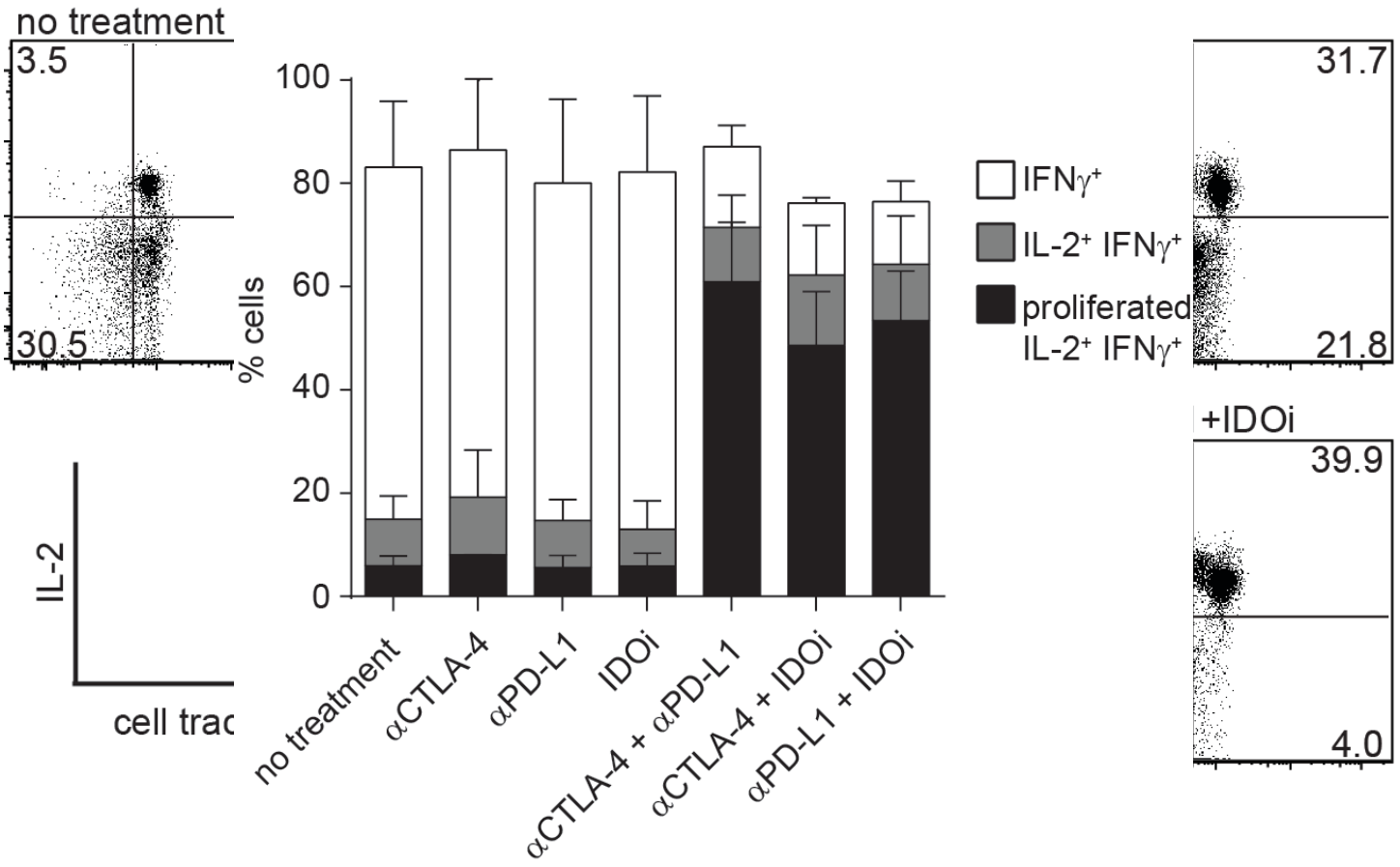
Combination of checkpoint blockade results in increased tumor control compared to single treatments





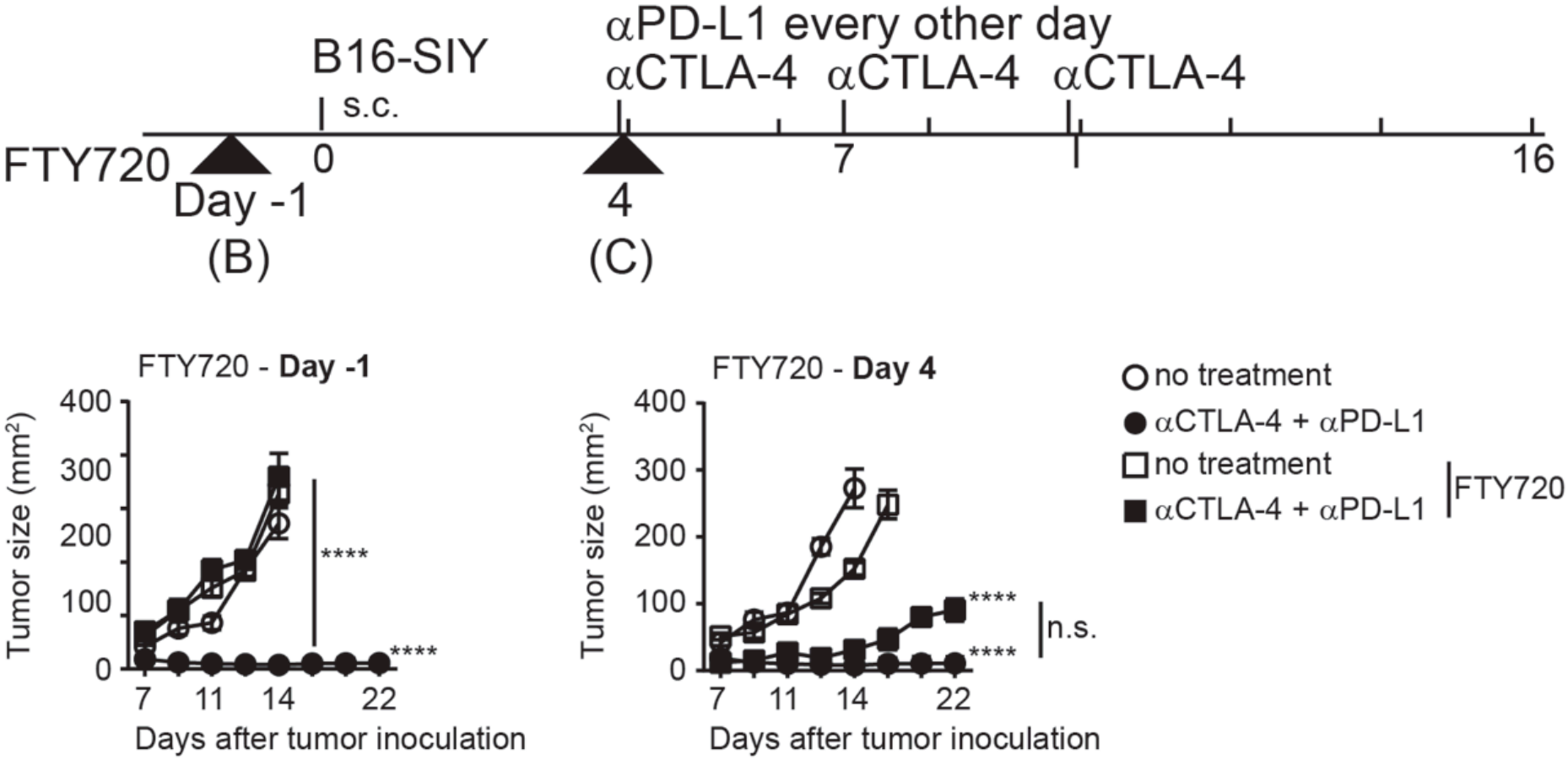
Spranger et al., JITC 2014

Checkpoint blockade is re-activating CD8+ T cells within the tumor microenvironment



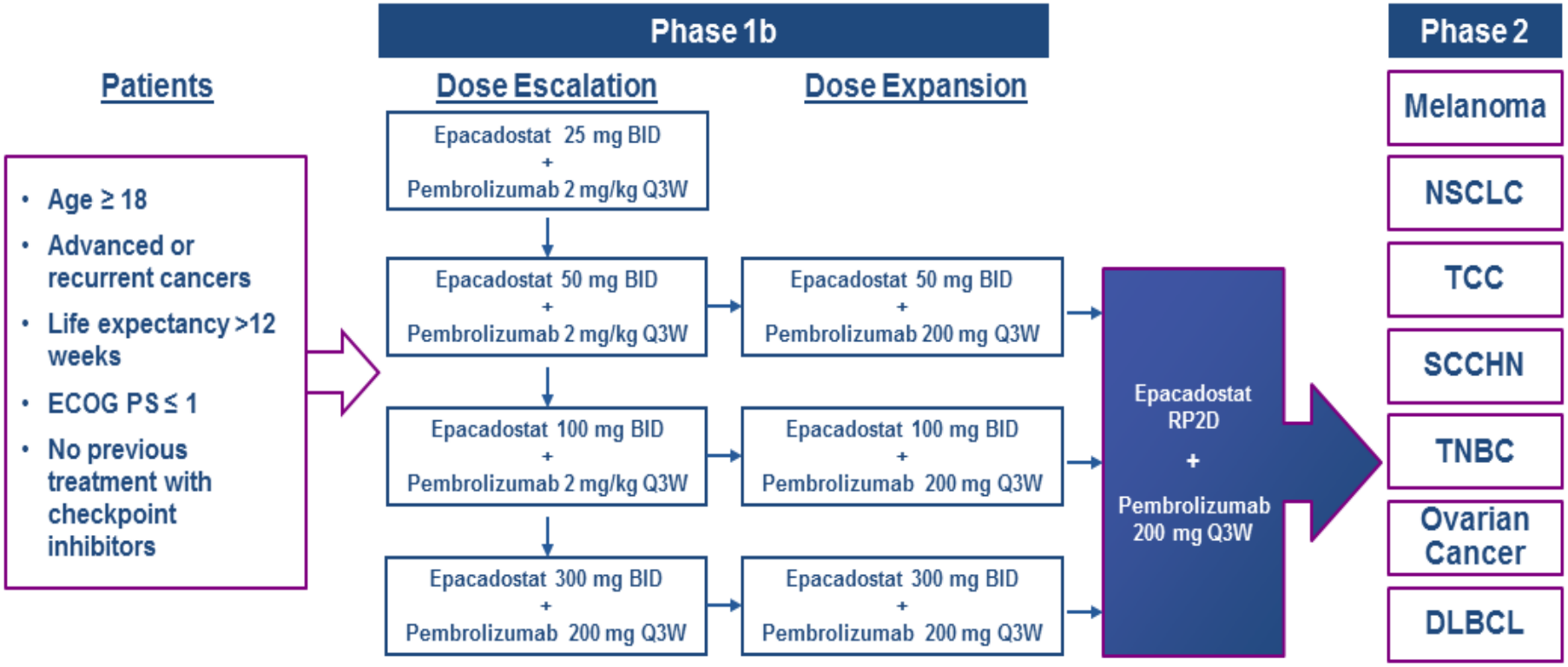
Spranger et al., JITC 2014

Therapeutic efficacy requires T cells already in the tumor microenvironment



Spranger et al., JITC 2014

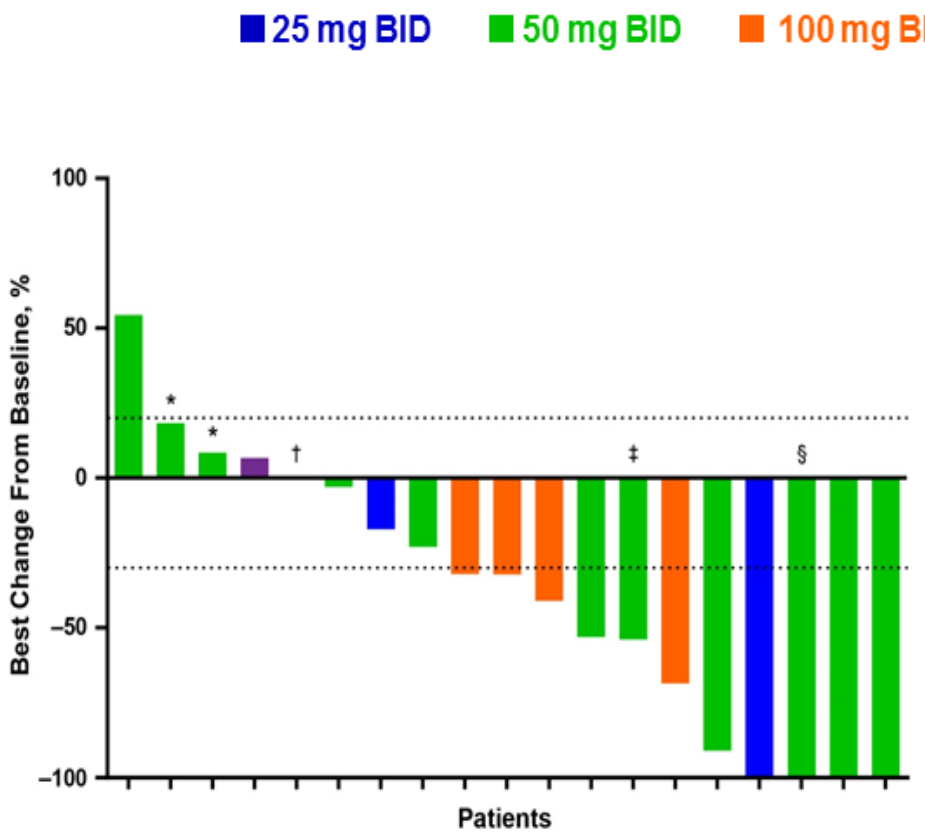
Study Design

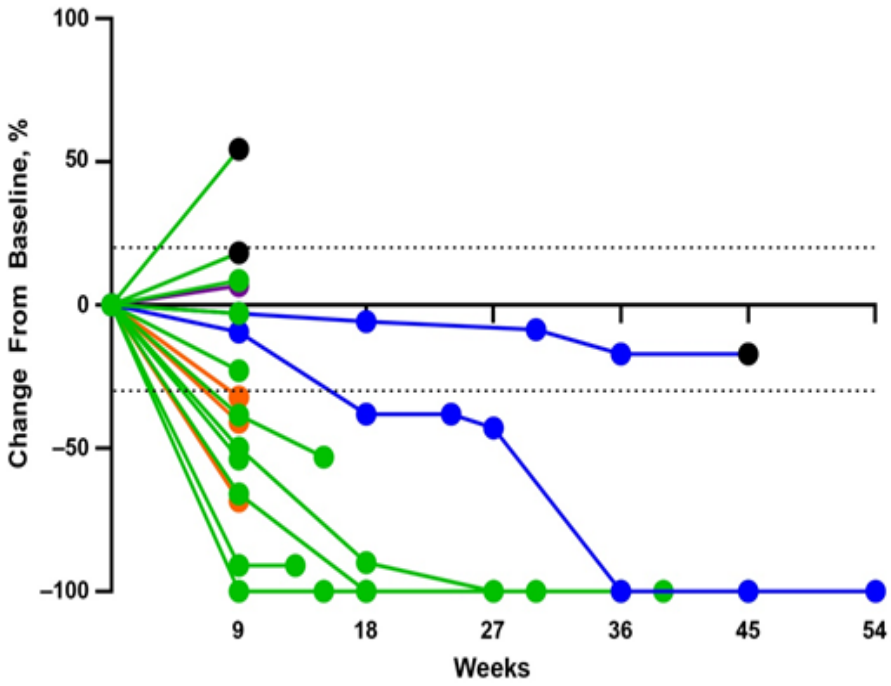


- Response assessment every 9 weeks per RECIST 1.1

Clinicaltrials.gov: NCT02178722

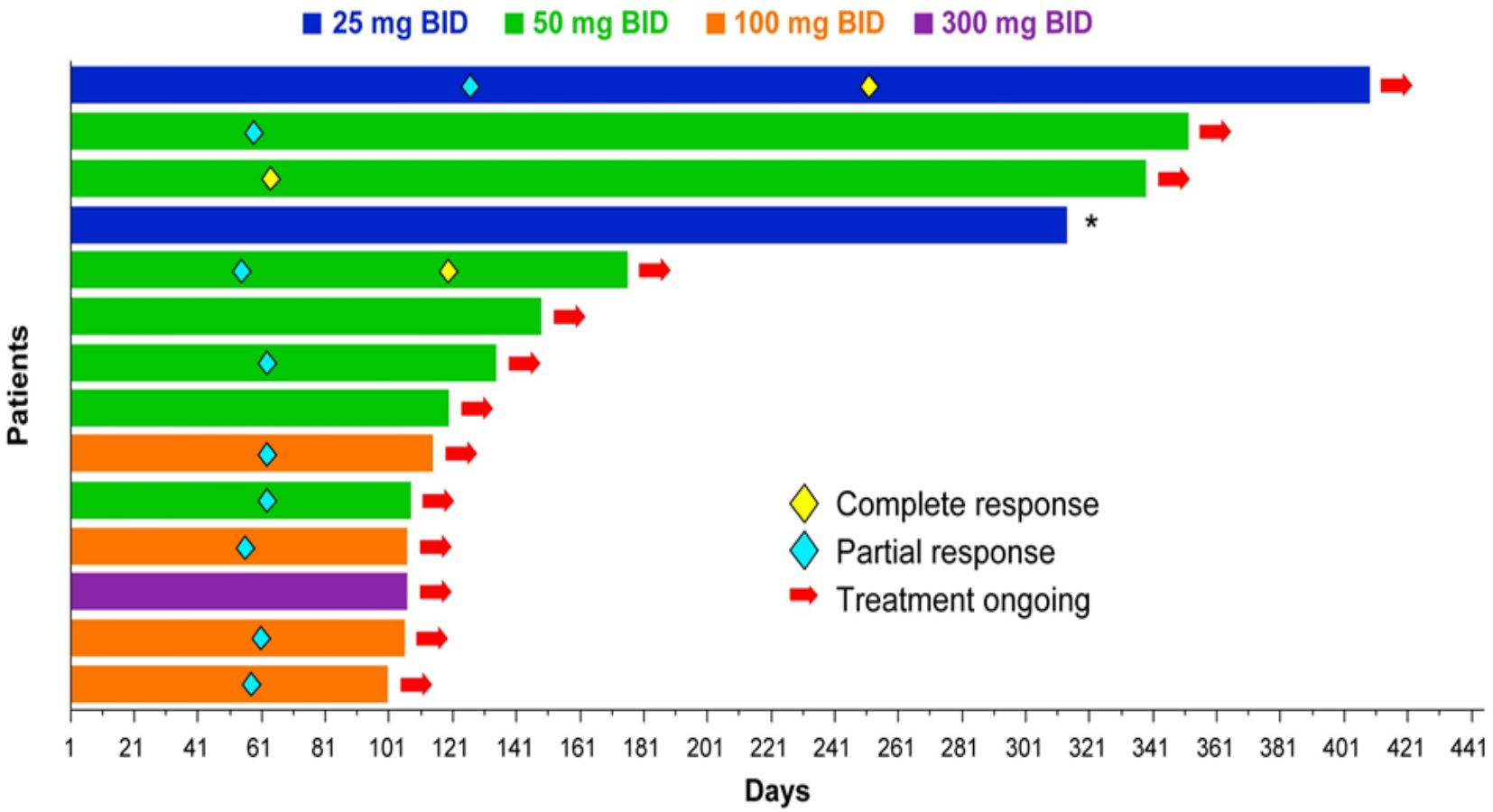
Combination treatment of PD-1 and IDOi mediates strong tumor shrinkage in melanoma patients





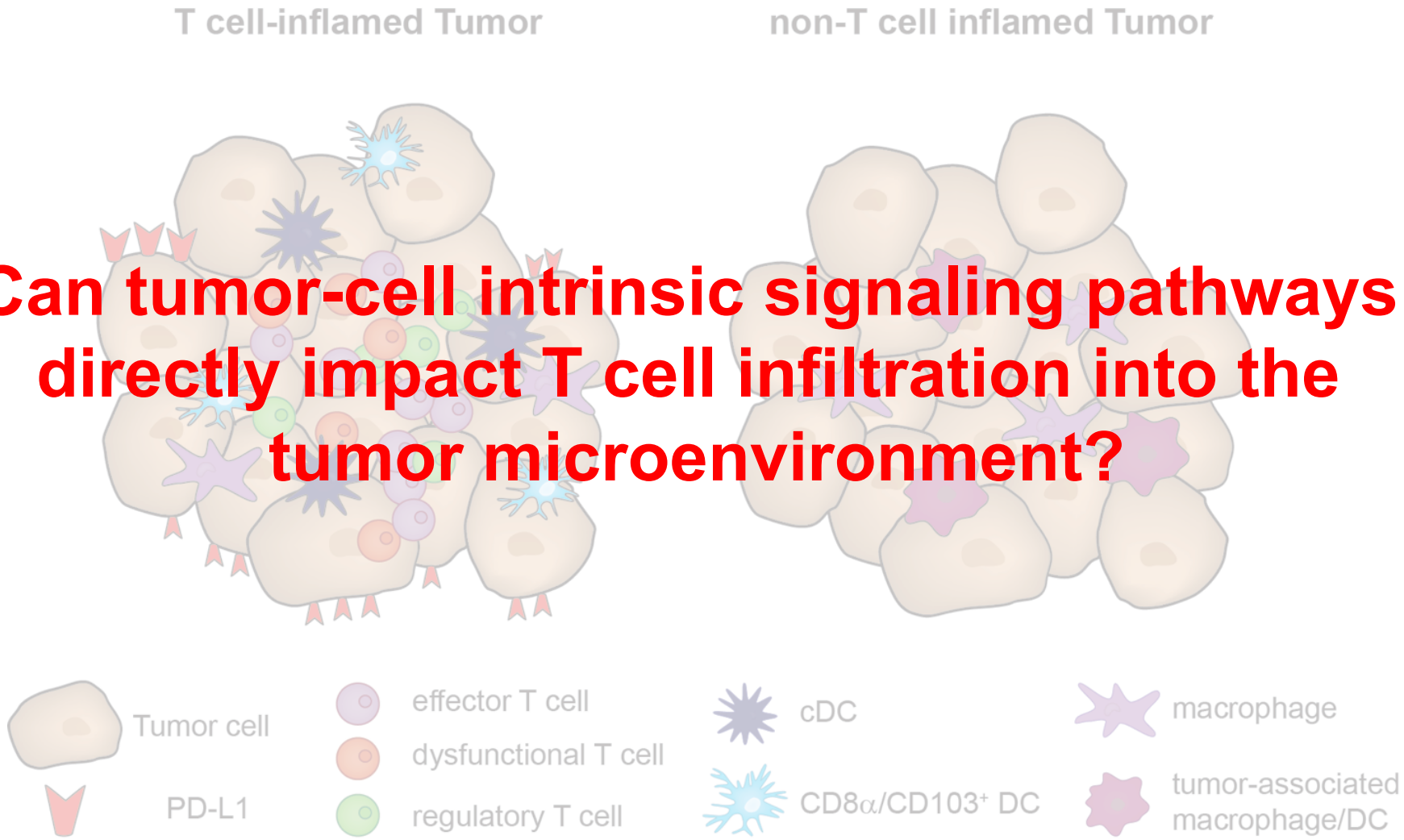
*Overall response is PD (SD for target lesions; PD for non-target lesions).
†Overall response is PD (target lesions not assessed; PD per new lesions).
‡Overall response is PD (PR for target lesions; PD per new lesions).
§Overall response is PR (CR for target lesions; non CR/non PD for non-target lesions).

Melanoma: Duration of Treatment in Patients With SD, PR, or CR



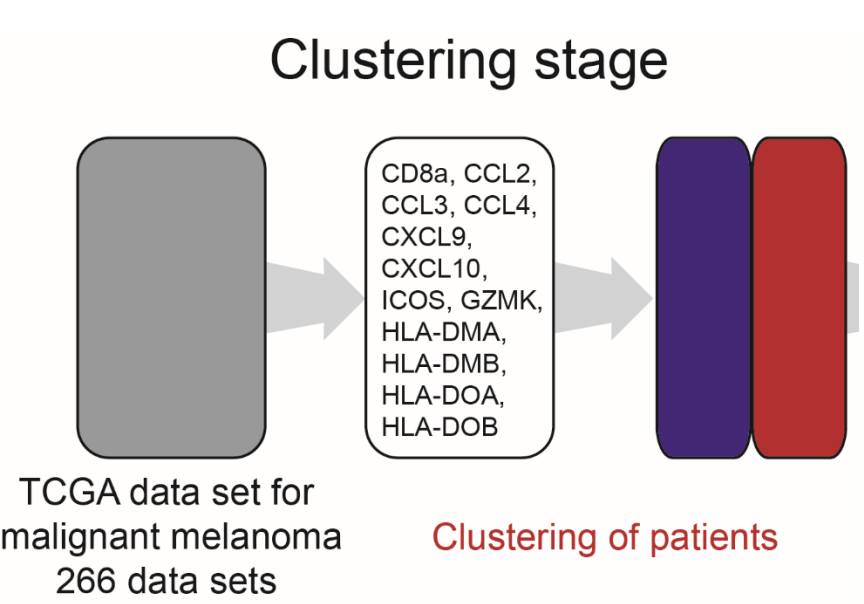
*Patient discontinued for PD.

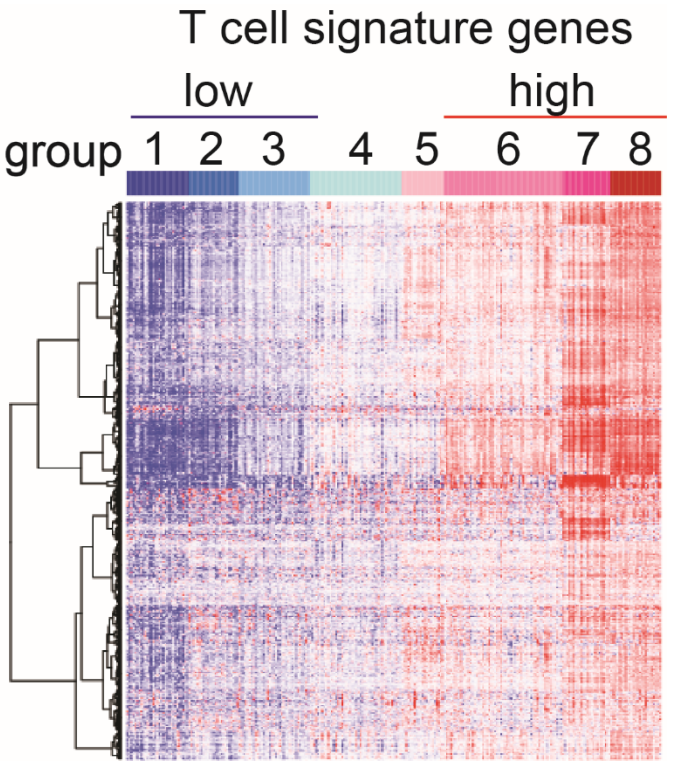
What tumor cell-intrinsic mechanisms cause the non-T cell-inflamed tumor phenotype?



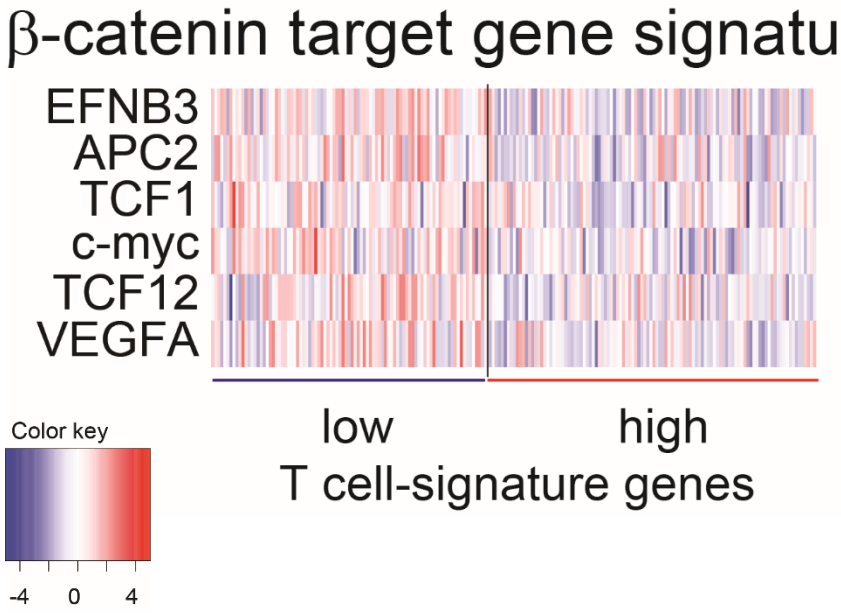
Can tumor-cell intrinsic signaling pathways directly impact T cell infiltration into the tumor microenvironment?

Workflow to identify oncogenic pathways differentially activated between T cell-inflamed and non-T cell-inflamed patients

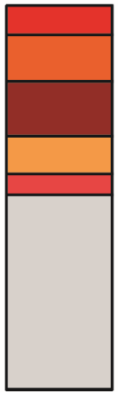




49% of non-T cell-inflamed tumors were linked with active β-catenin signaling



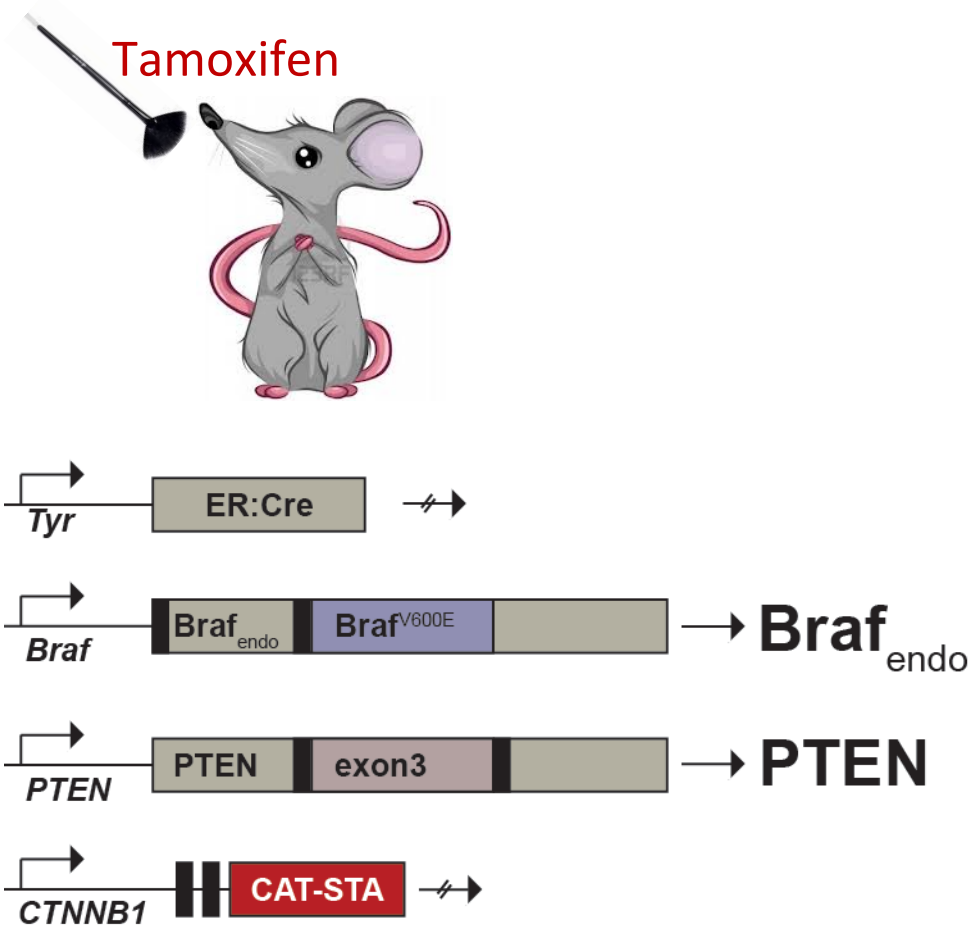
Molecular mechanism
for activated β-catenin signaling
T cell-signature low patients



8% CTNNB1 GOF mutations
12% LOF mutations in CTNNB1 inhibitors
14% overexpression of Wnt7b
10% overexpression of Fzd3
5% overexpression of CTNNB1

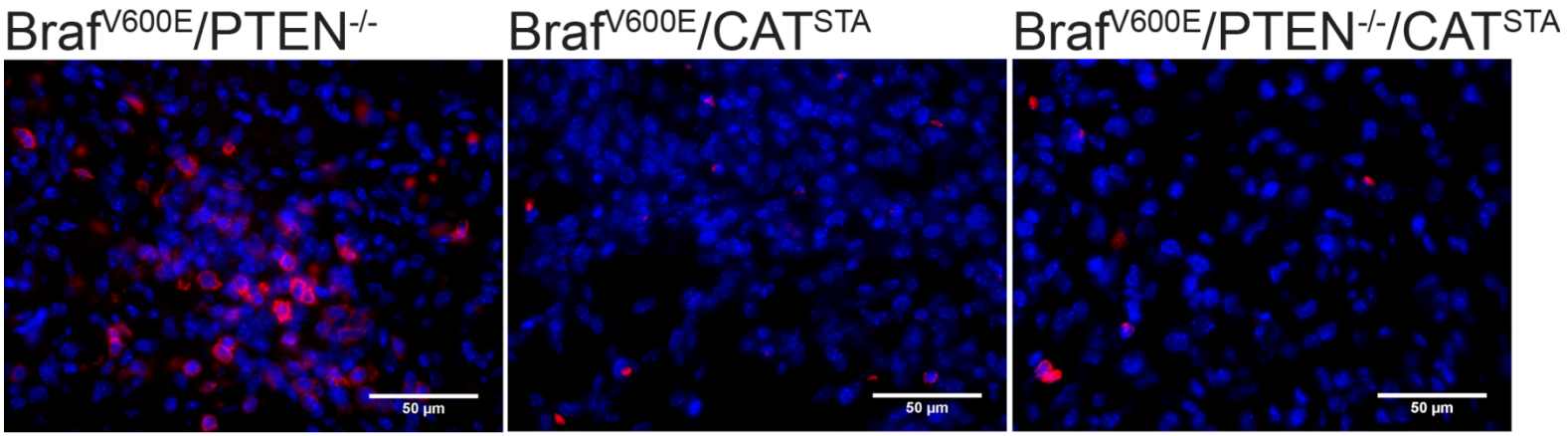
51% no alterations in CTNNB1 pathway

Experimental model system



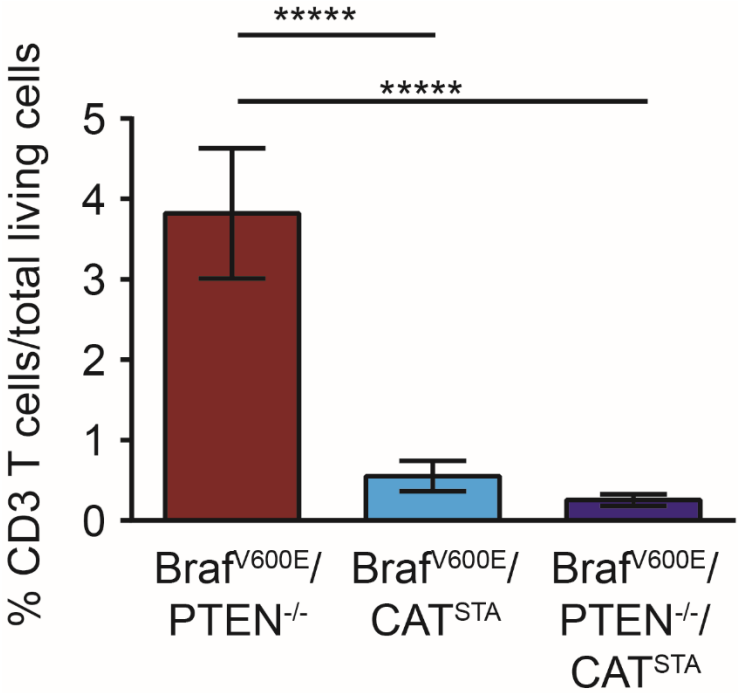
Genetically engineered mouse tumors with active β-catenin lack T cell infiltration

IF on tumor



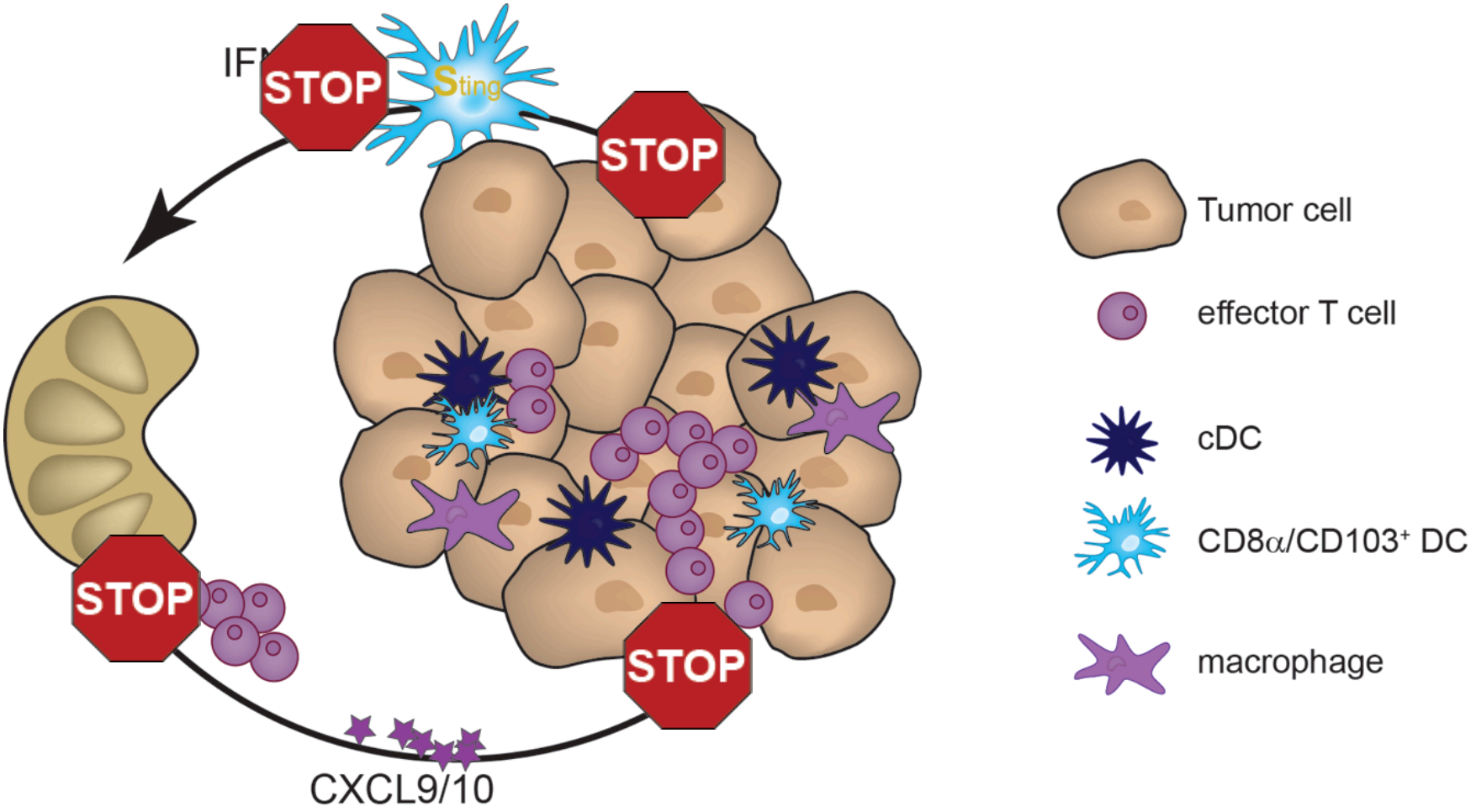
Red: T cells
Blue: nuclei

Flow cytometry

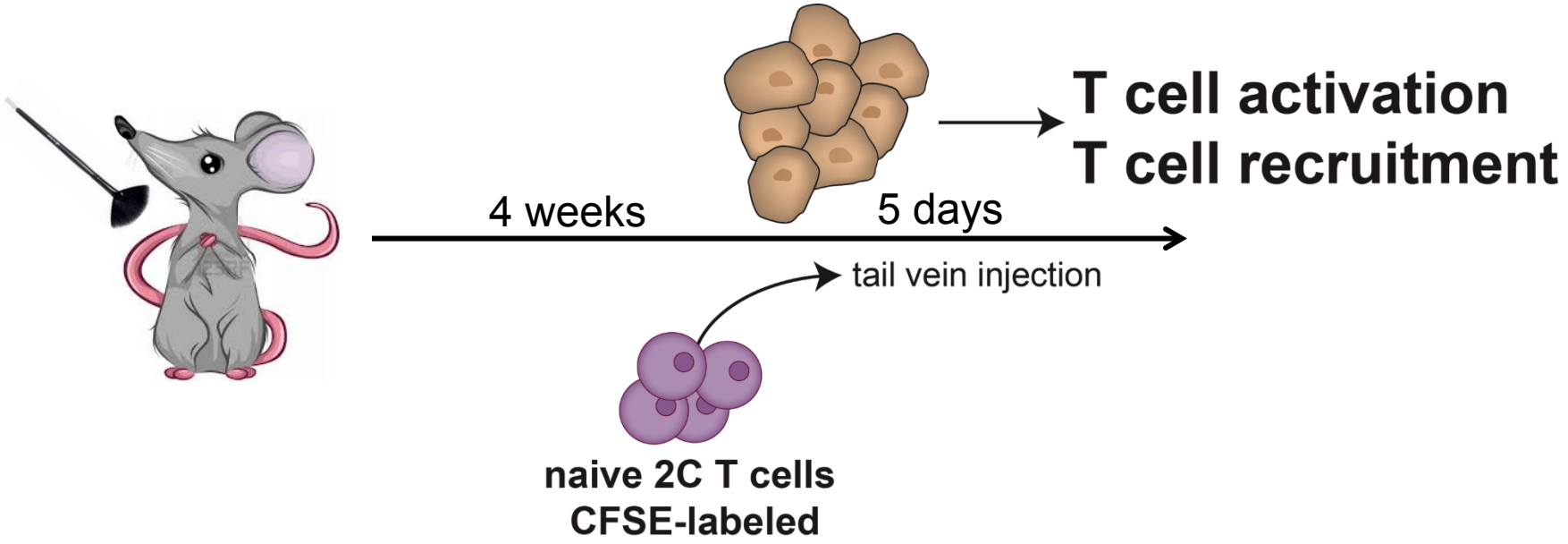


Spranger et al., Nature 2015

How might tumor-cell intrinsic β-catenin signaling mediate T cell exclusion?



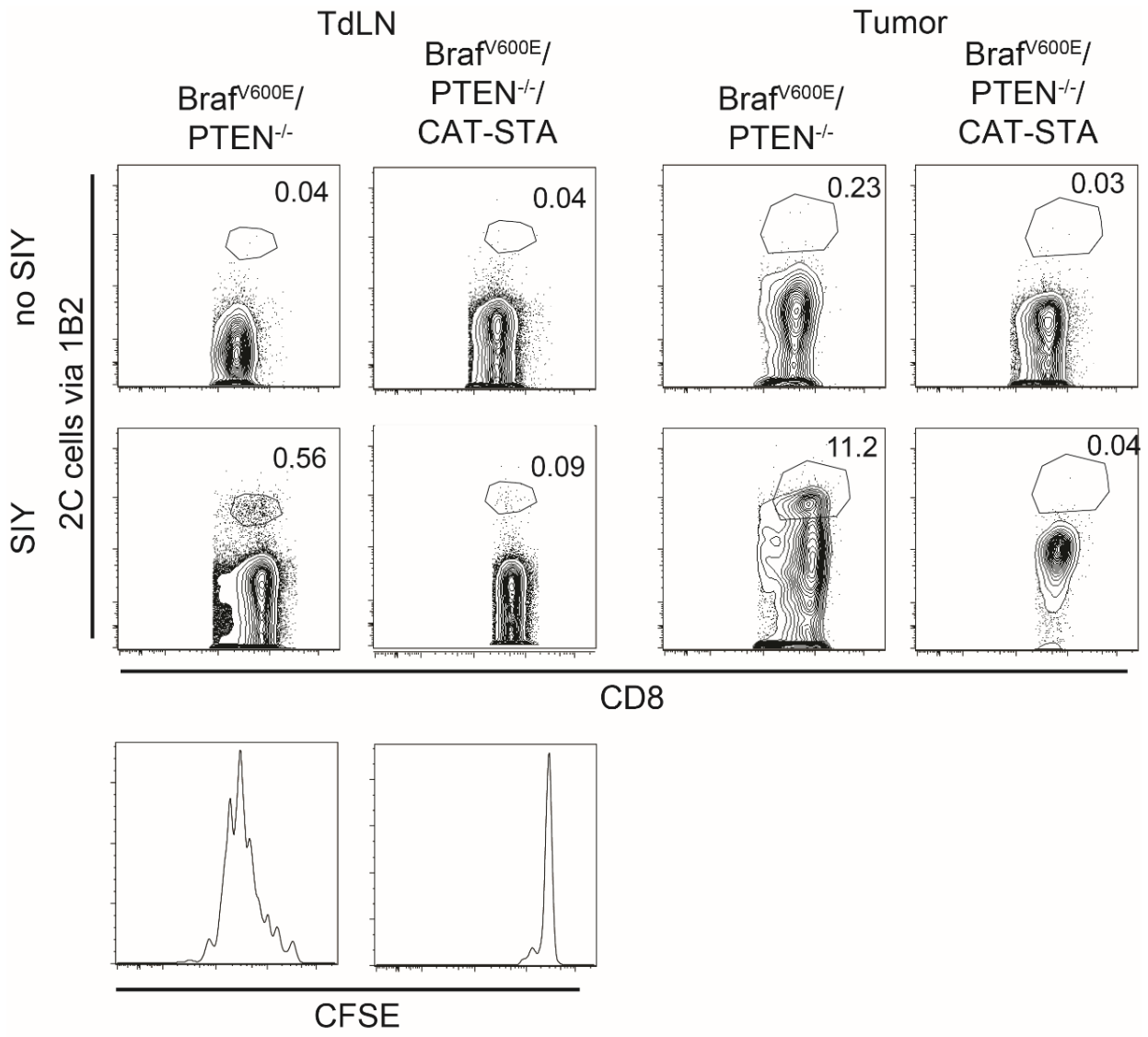
Is the lack of T cell infiltration caused by a lack of initial T cell priming?

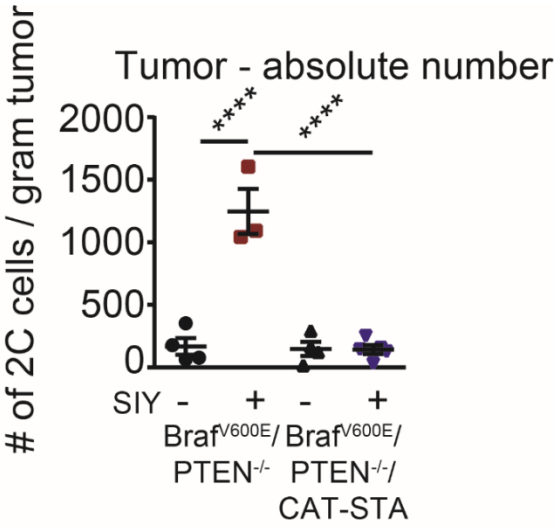


$Braf^{V600E}/PTEN^{-/-}$
$Braf^{V600E}/PTEN^{-/-}$ /CAT-STA

+/- LoxP-Stop-LoxP SIY

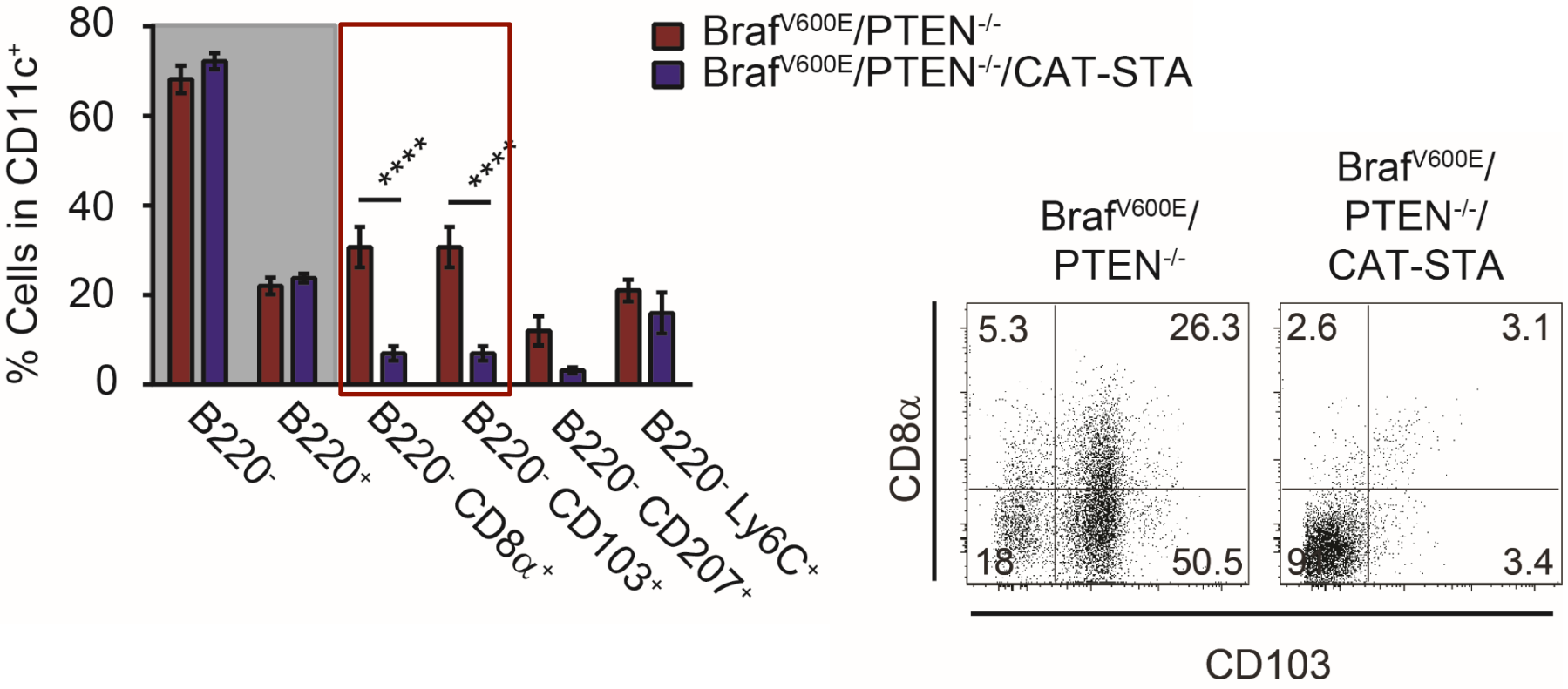
β-catenin-expressing tumors fail to prime 2C TCR-transgenic T cells





Spranger et al., Nature 2015

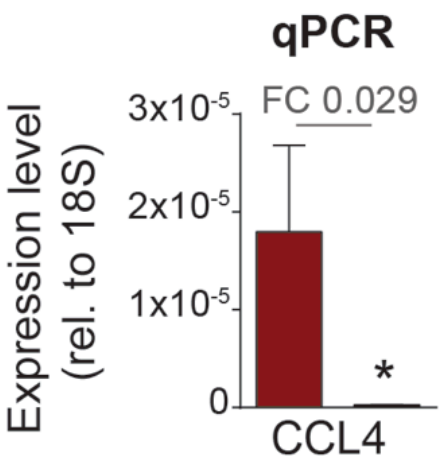
β-catenin-expressing tumors show reduced numbers of CD8α+ and CD103+ dendritic cells

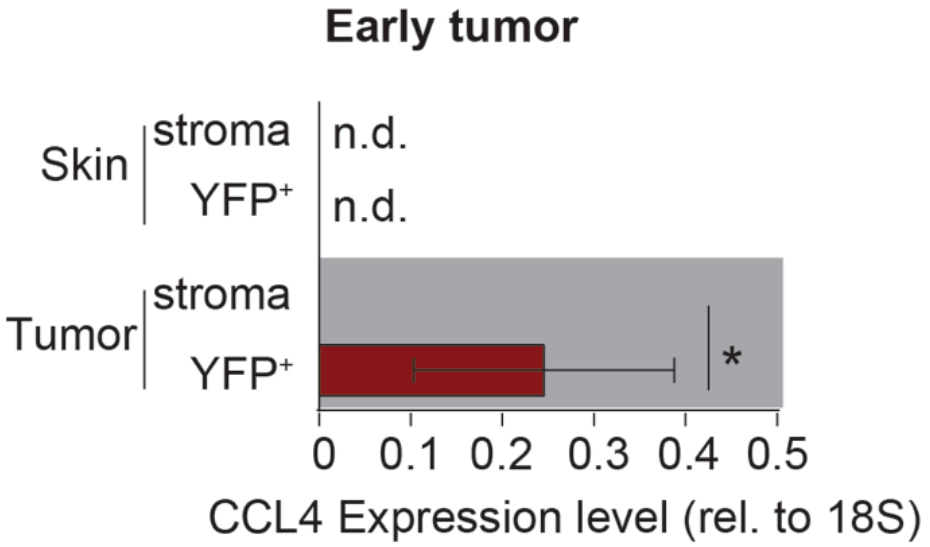


Spranger et al., Nature 2015

Which signal is required for $CD103^{+}$ dendritic cells to infiltrate into the tumor?

CCL4 expression is lost in β-catenin^{+} tumors



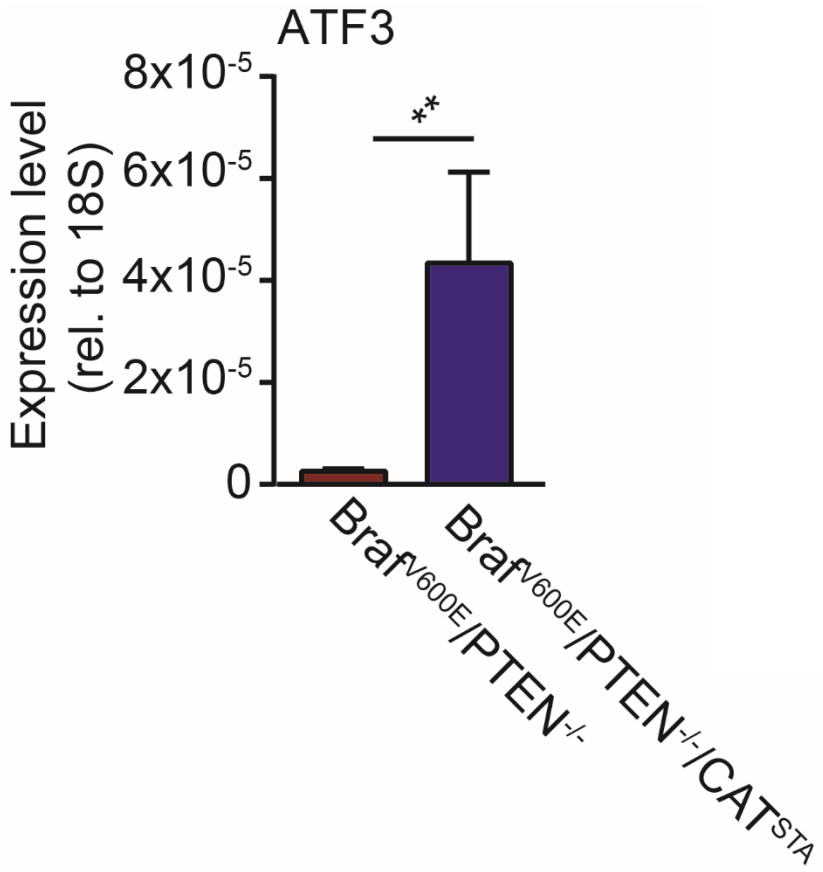


$Braf^{V600E}/PTEN^{-/-}$

$Braf^{V600E}/PTEN^{-/-}/CAT\text{-}STA$

Spranger et al., Nature 2015

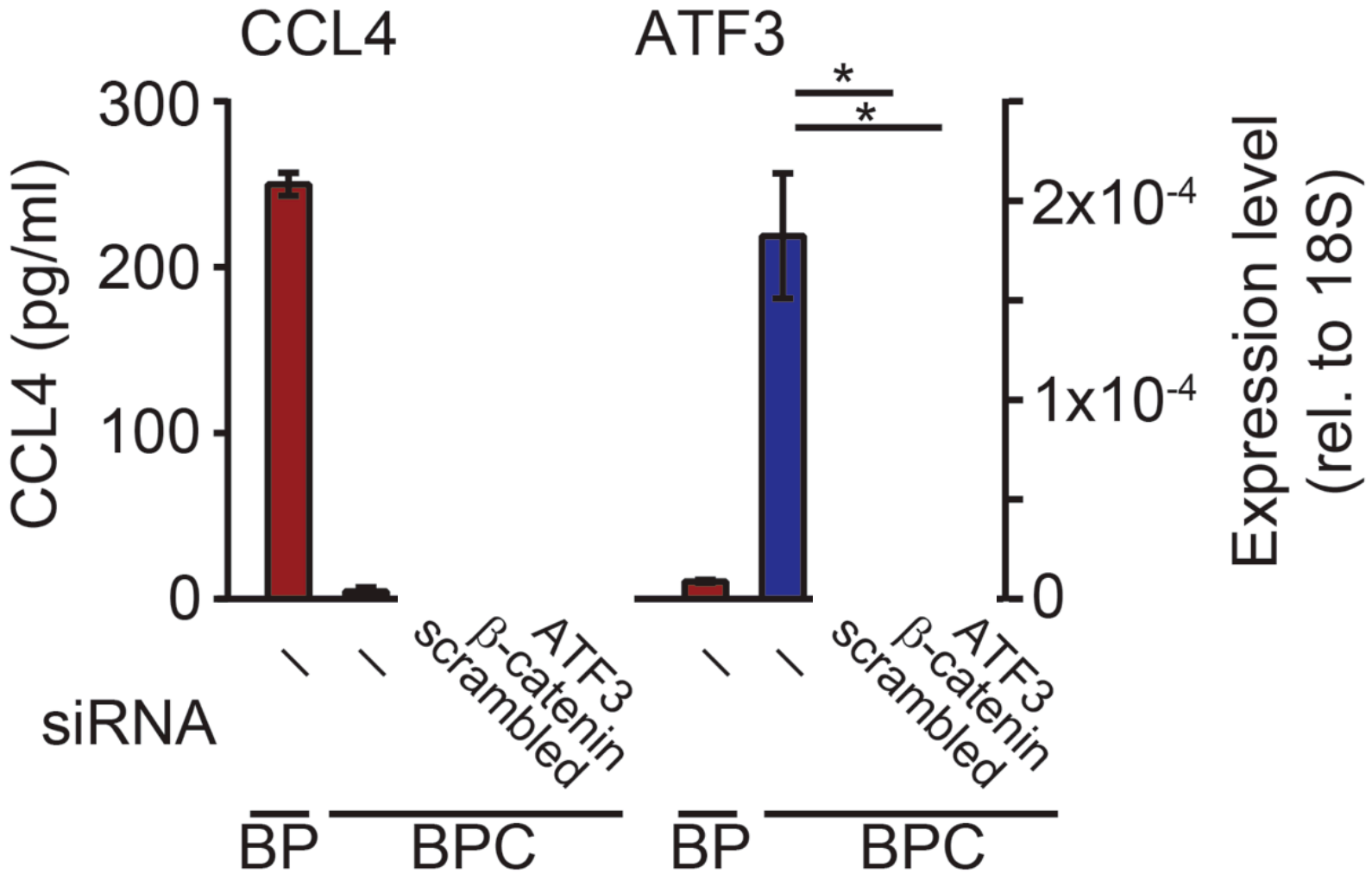
Transcriptional repressor ATF3 is downstream of β-catenin and blocks CCL4 expression



BP cell line from Braf^{V600E}/PTEN$^{-/-}$; BPC cell line from Braf^{V600E}/PTEN$^{-/-}$/CAT-STA

Spranger et al., Nature 2015

Knockdown of ATF3 restores CCL4 expression in β-catenin^{+} tumor cells

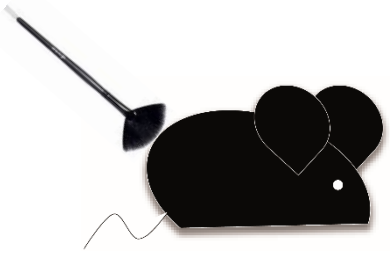


BP cell line from Braf^{V600E}/PTEN$^{-/-}$; BPC cell line from Braf^{V600E}/PTEN$^{-/-}$/CAT-STA

Spranger et al., Nature 2015

Does β-catenin-mediated lack of T cell infiltration facilitate resistance towards checkpoint inhibition?

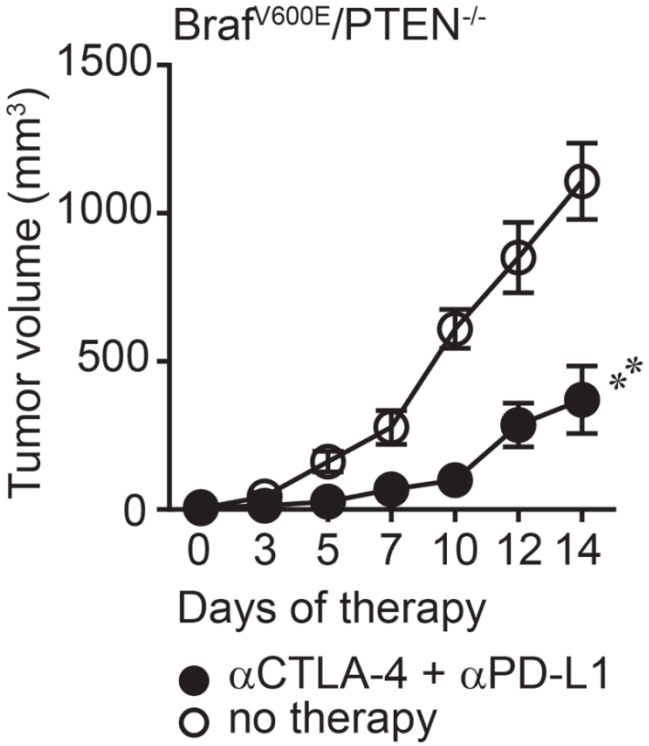
Checkpoint blockade fails to control β-catenin-expressing tumors

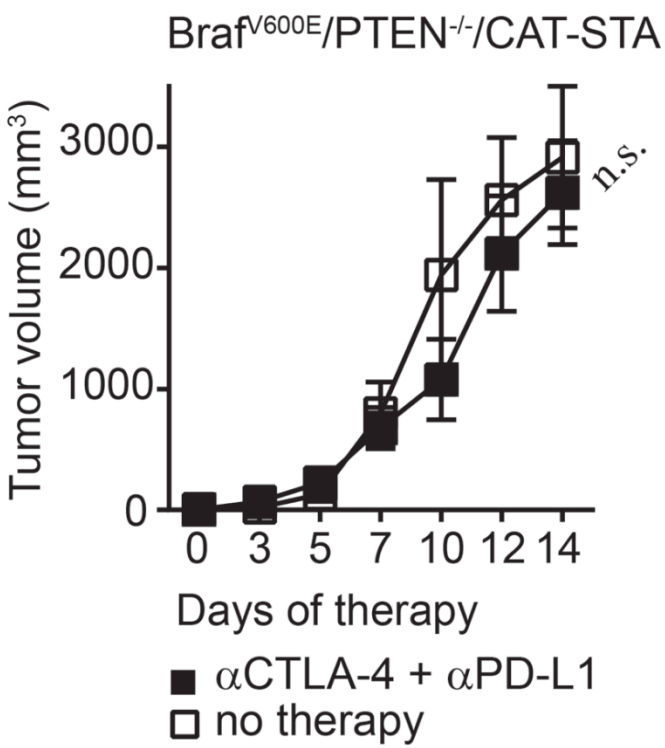


4 weeks every other day

Analysis of tumor growth and T cell infiltration

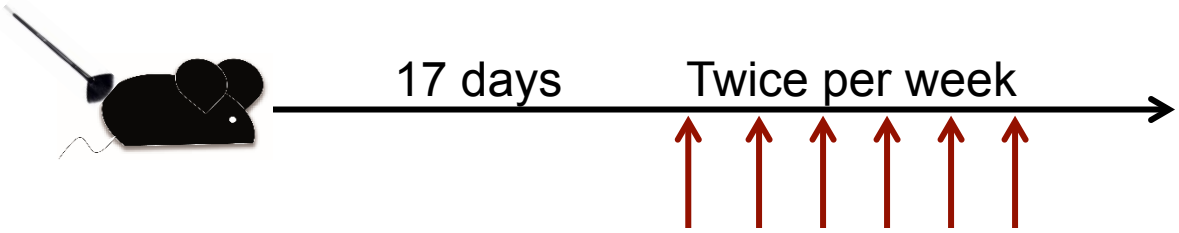
Combination therapy of αCTLA-4 and αPD-L1

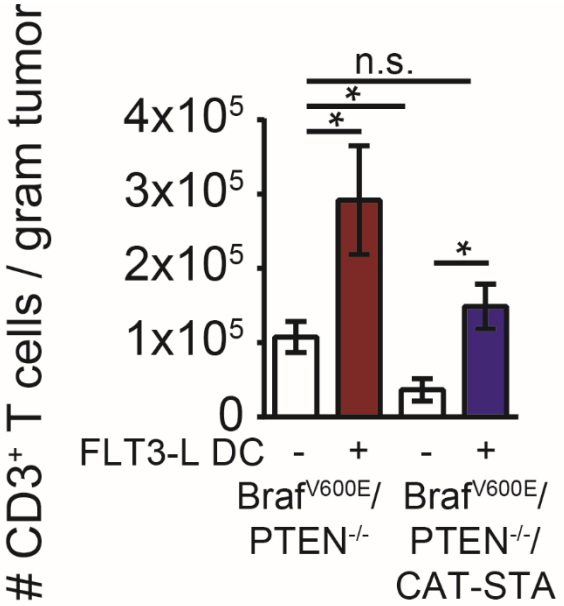


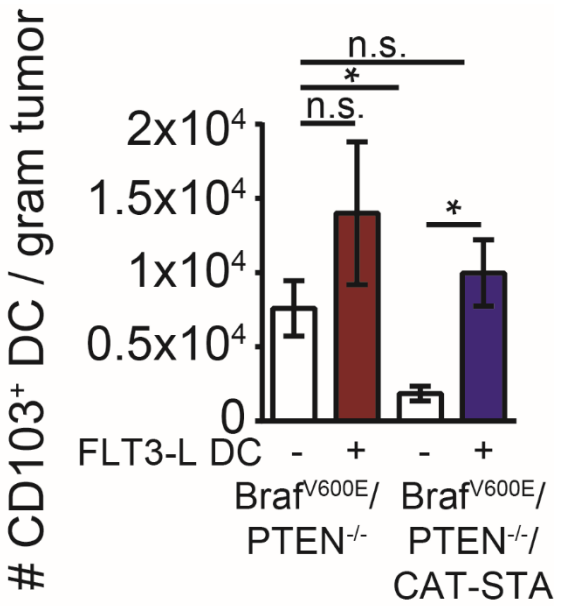


Spranger et al., Nature 2015

Injection of Flt-3L BMDCs can “reverse” the non-inflamed phenotype

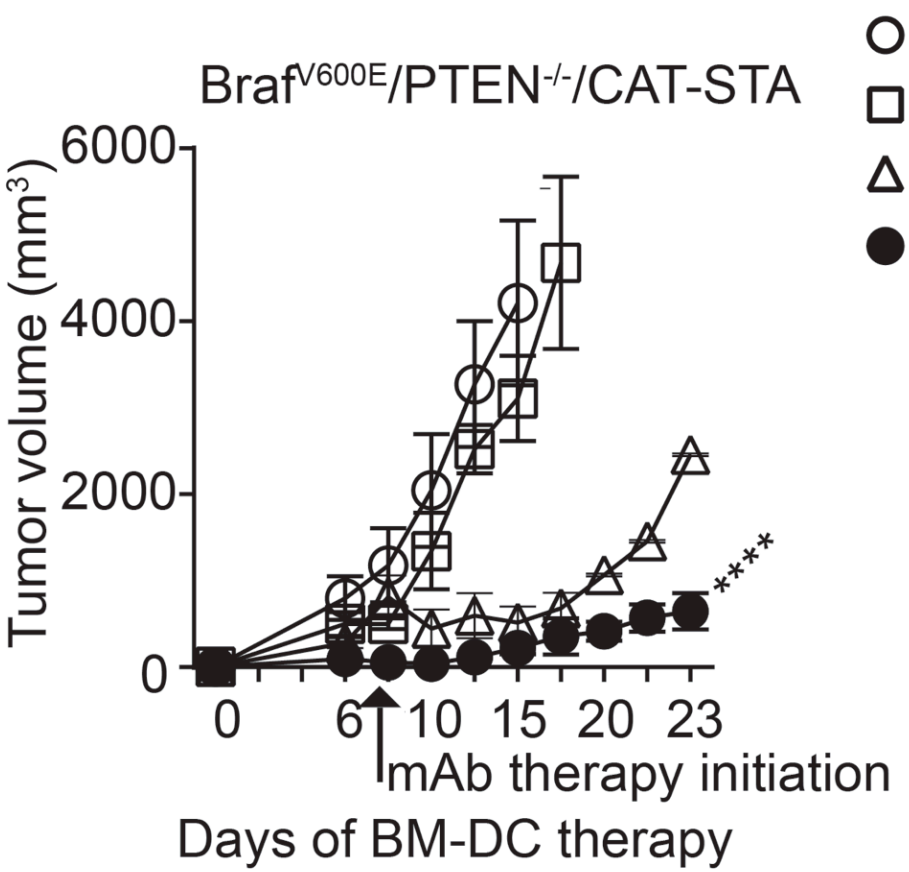


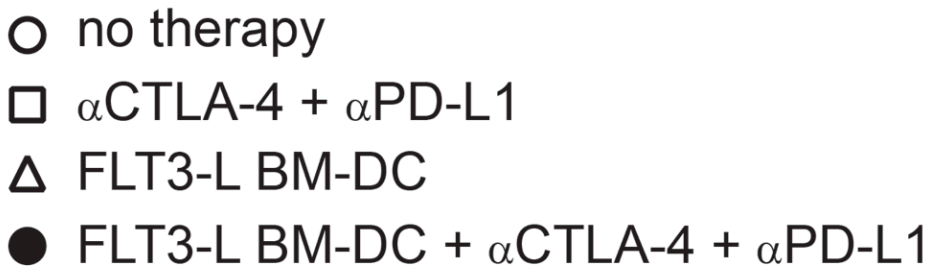




Spranger et al., Nature 2015

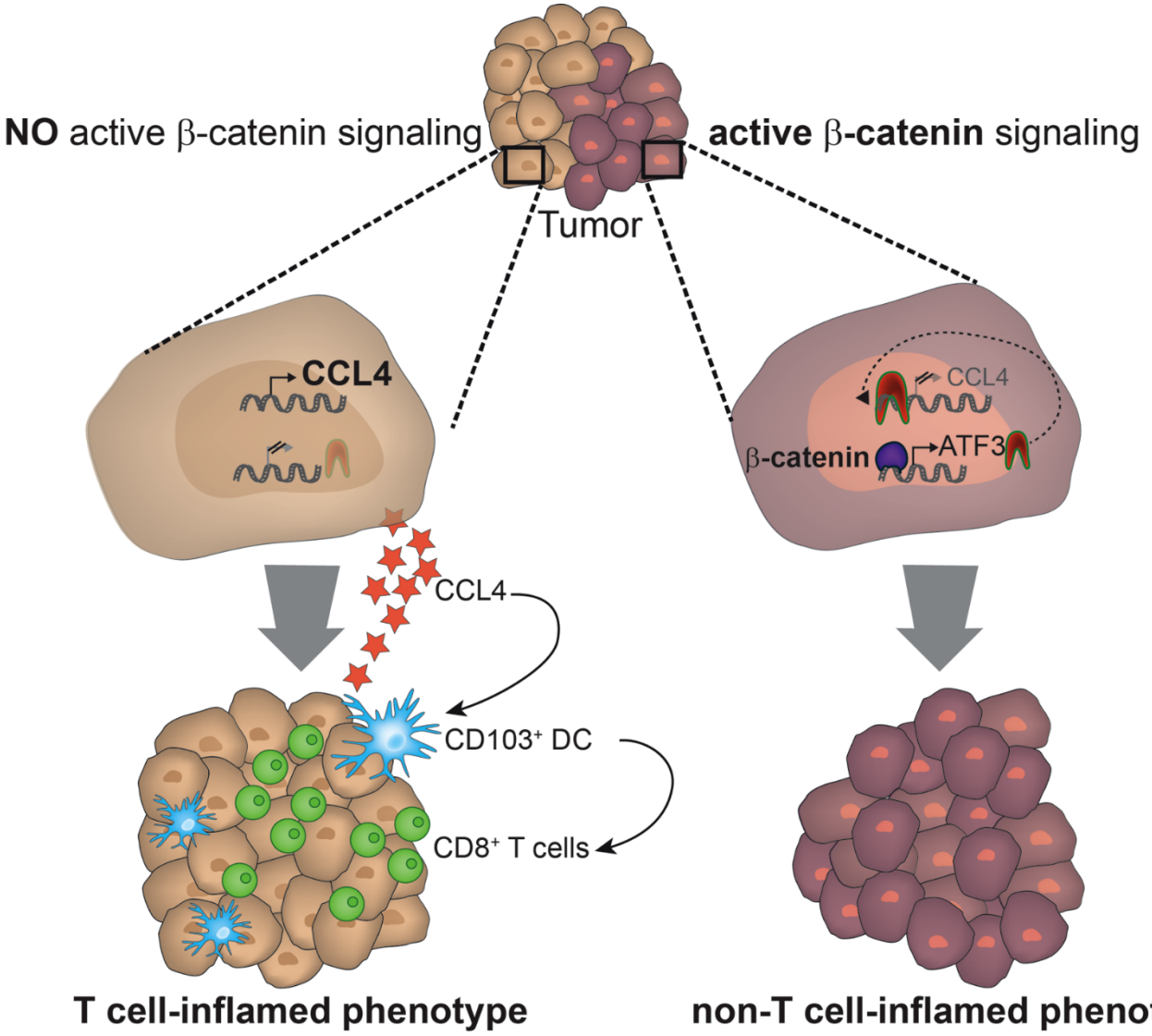
DC-mediated T cell infiltration restores responsive towards checkpoint-blockade in β-catenin^{+} tumors





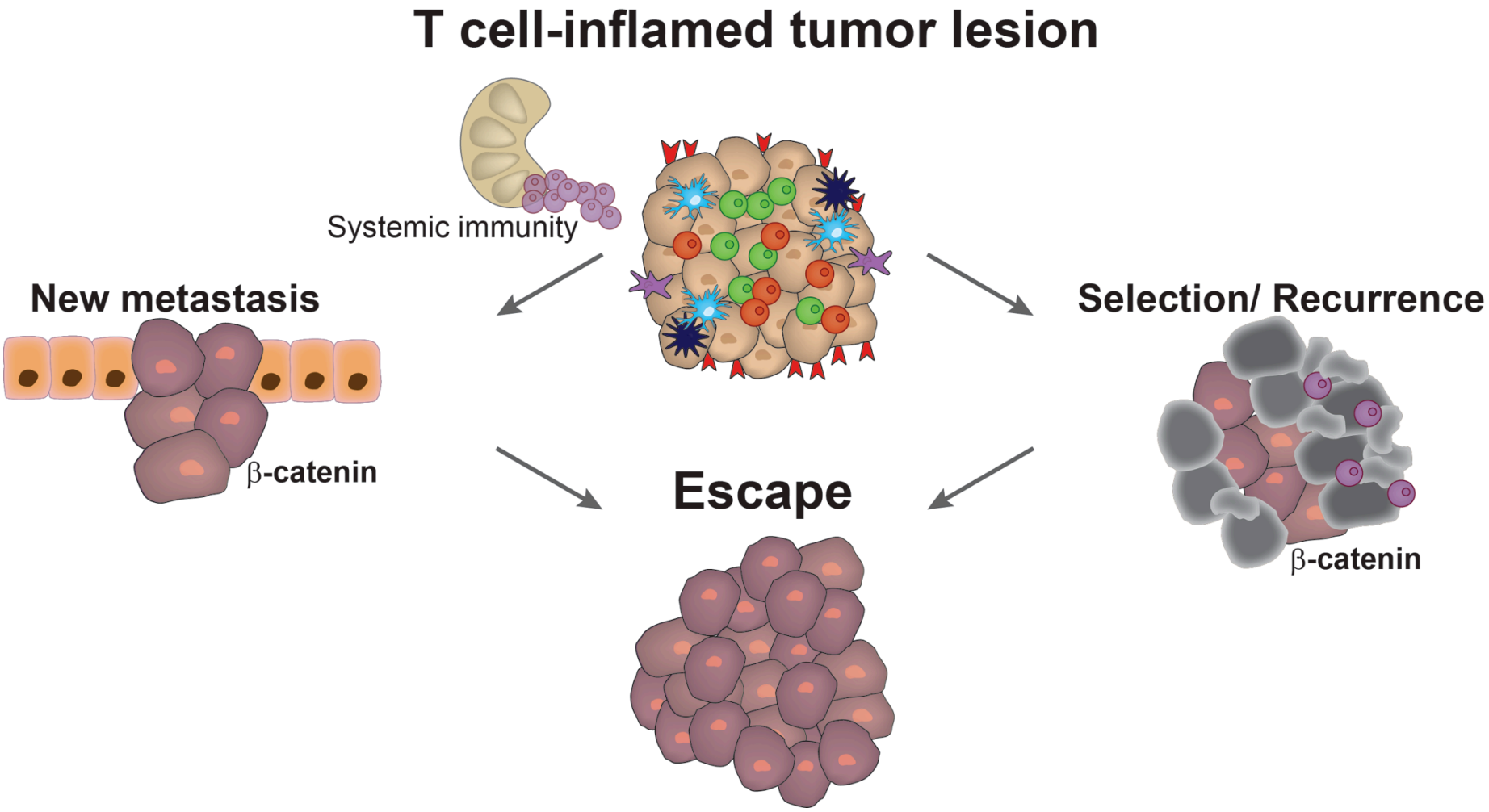
Spranger et al., Nature 2015

Tumor-intrinsic β-catenin signaling mediates lack of T cell infiltration and resistance towards checkpoint inhibition

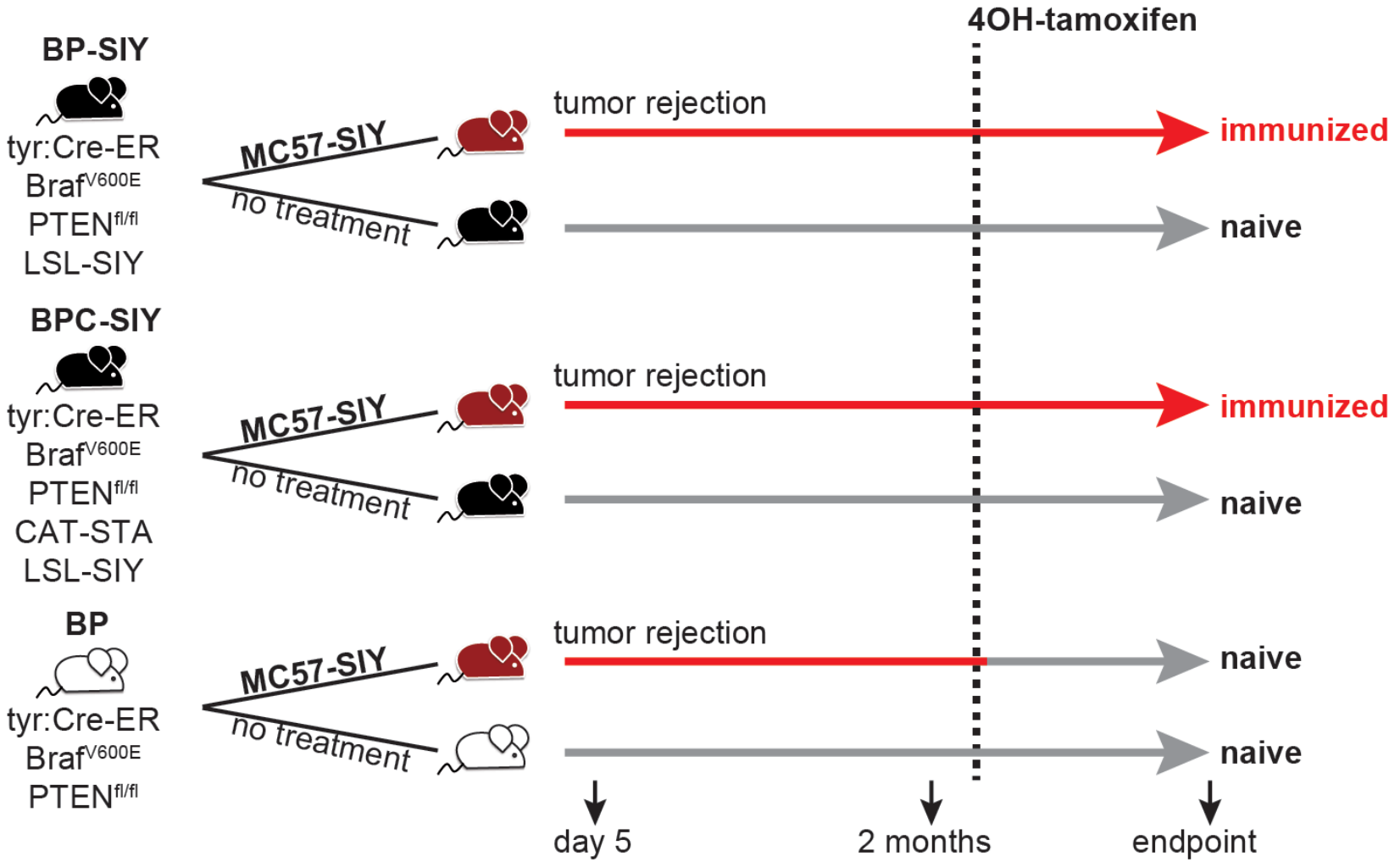


Spranger et al., Nature 2015

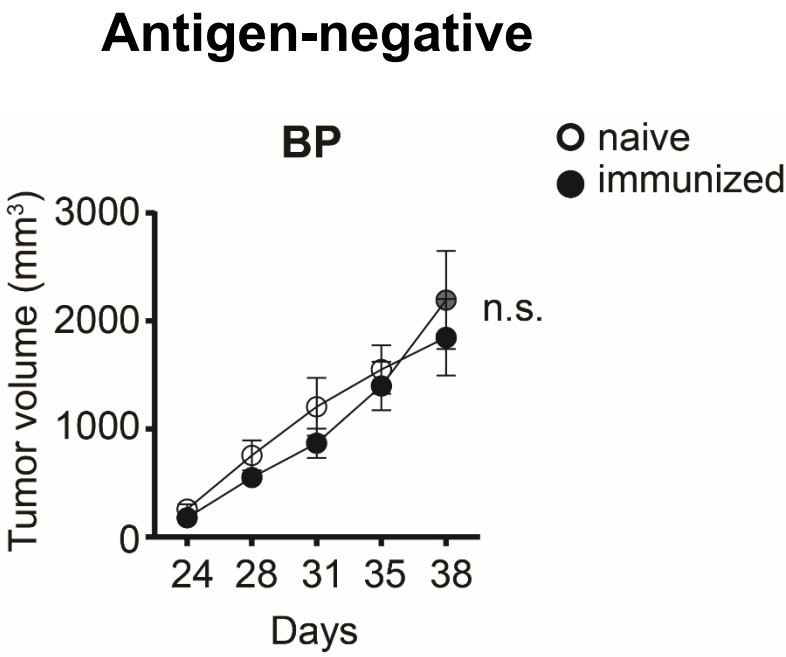
Does tumor-intrinsic β-catenin also mediate T cell exclusion against an existing anti-tumor immune response?



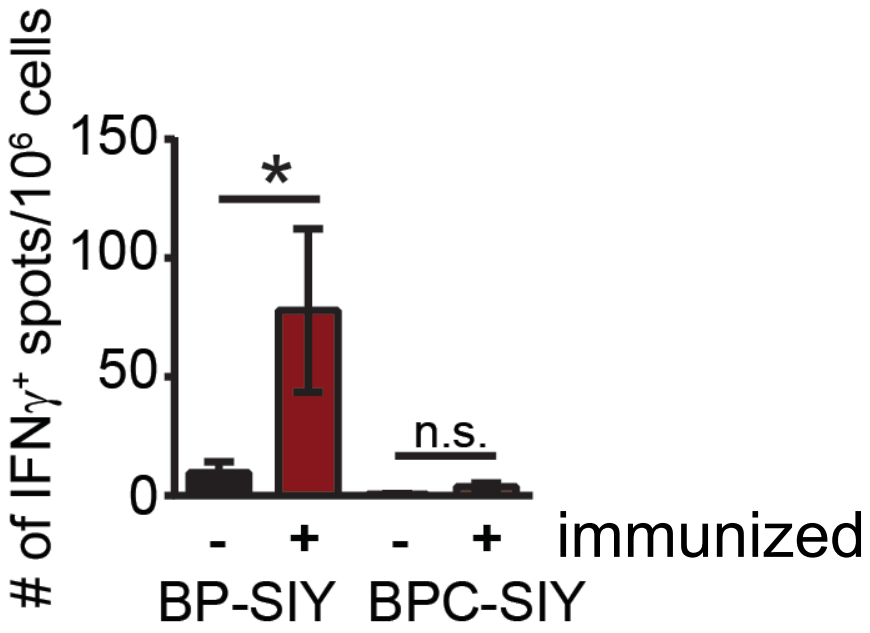
Experimental approach to evaluate impact of tumor-intrinsic β-catenin against an existing anti-tumor immune response



Existing antigen-specific T cell memory fails to control β−catenin^{+} tumors expressing the shared antigen

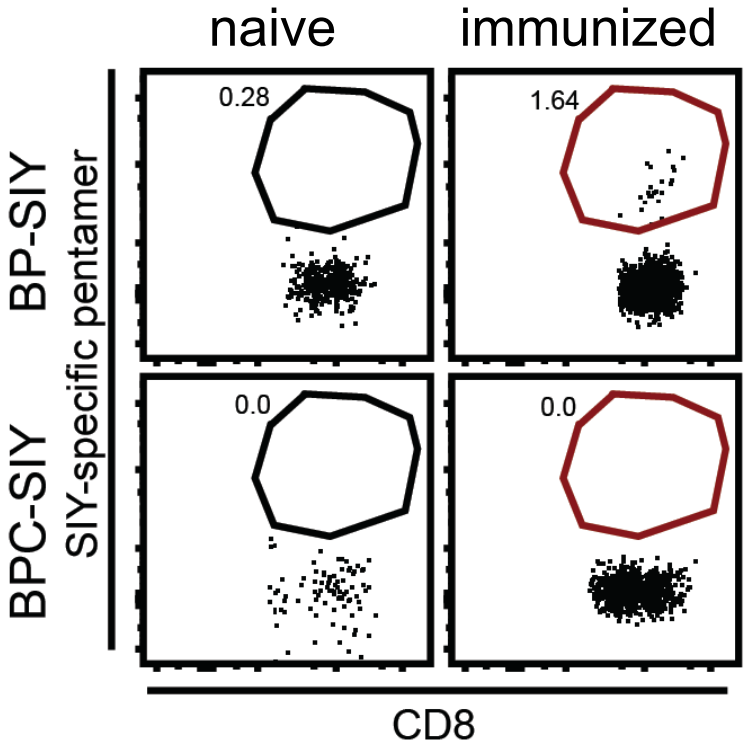


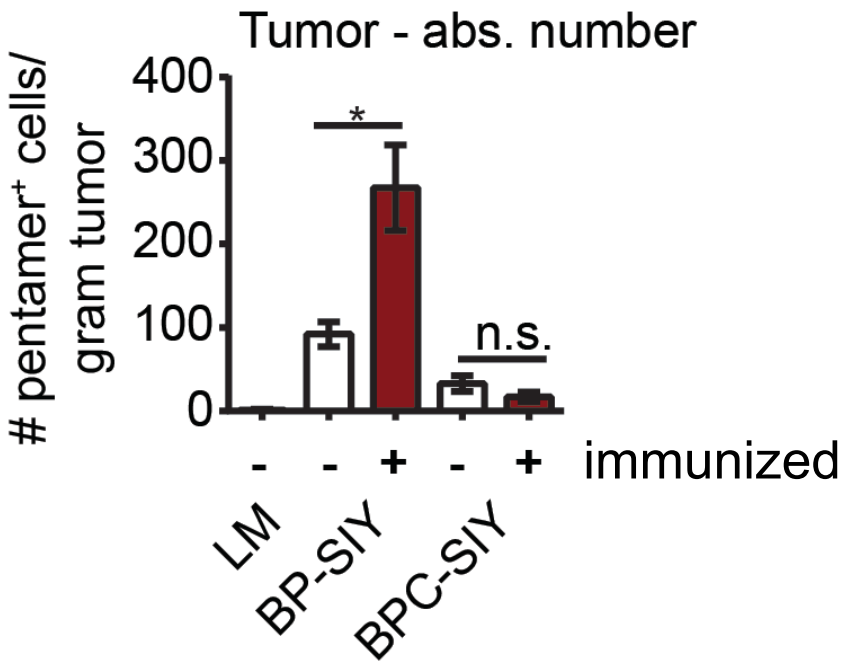
Increased tumor control is accompanied by increased peripheral and intra-tumoral immune response



Response to secondary tumor

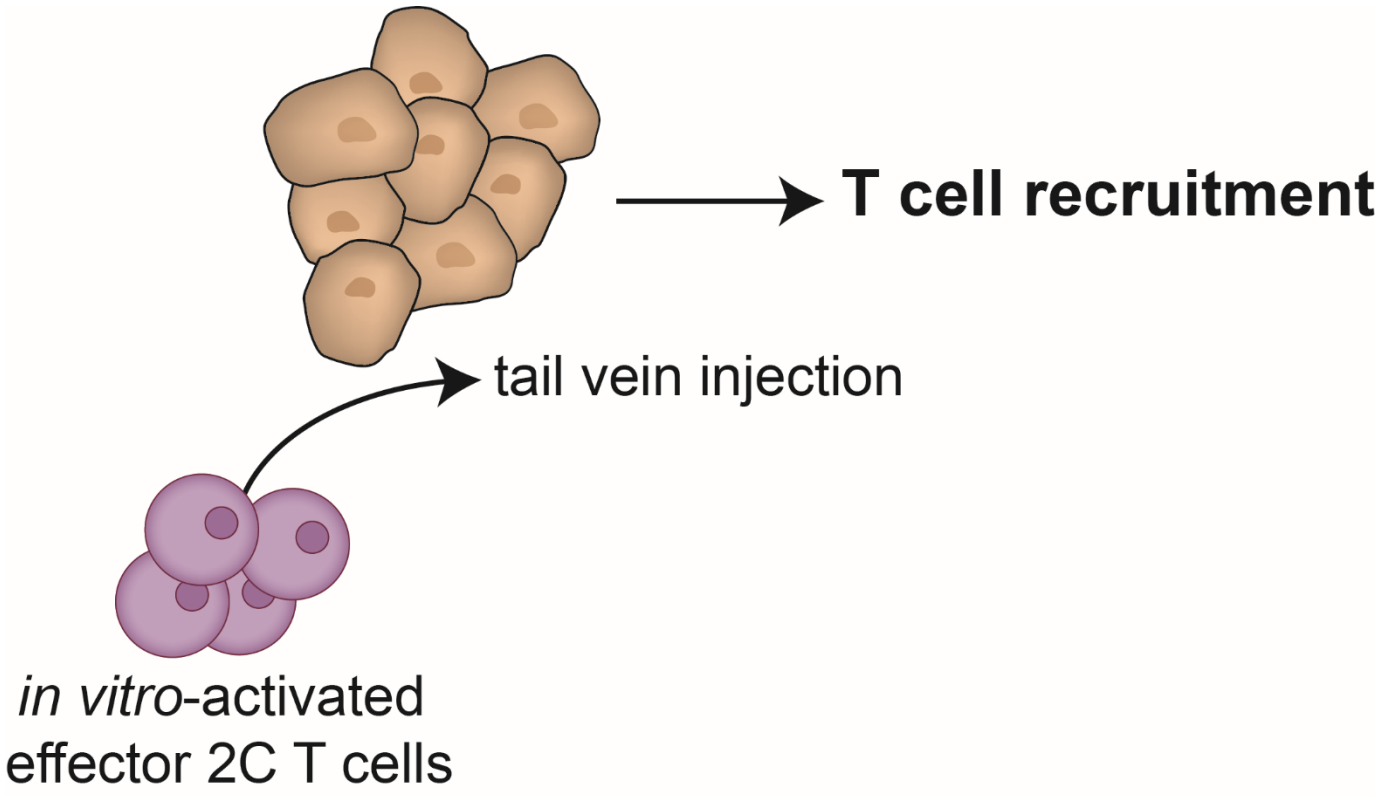
Increased tumor control is accompanied by increased peripheral and intra-tumoral immune response



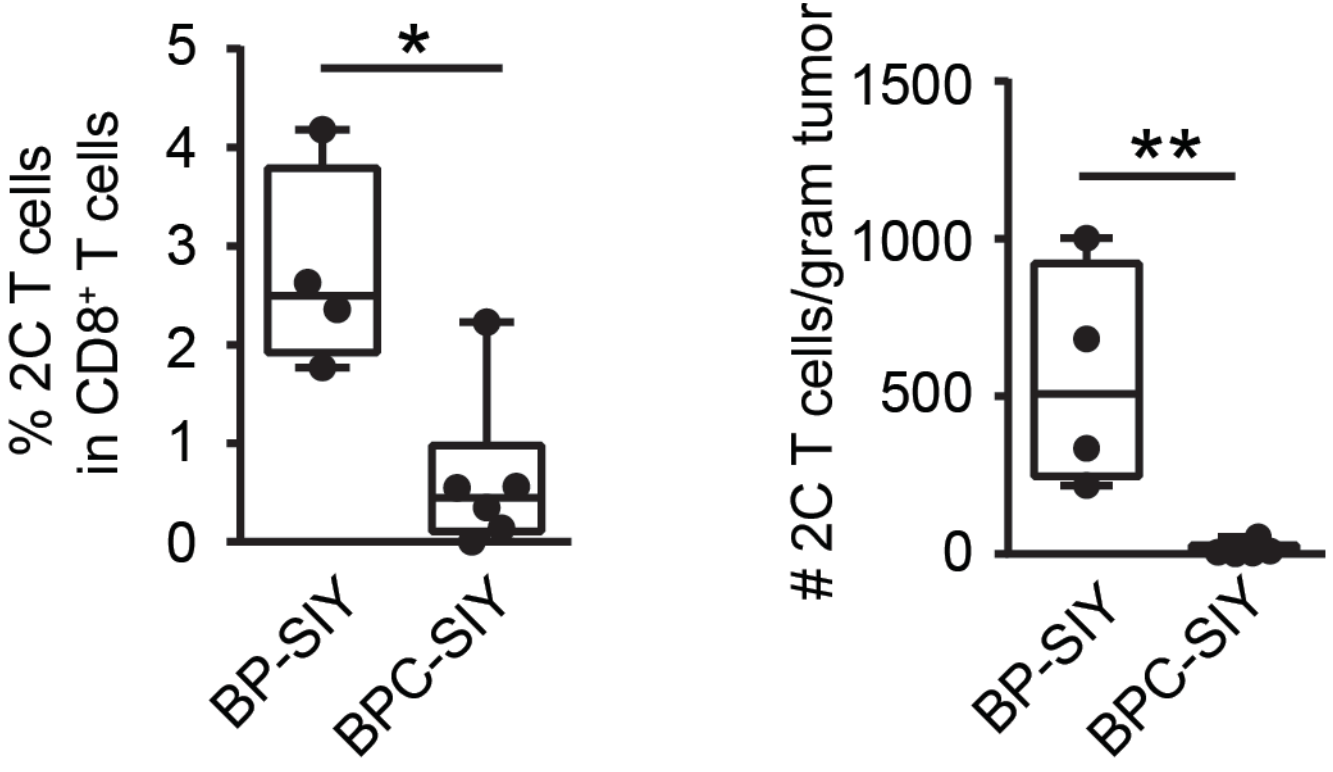


Response to secondary tumor

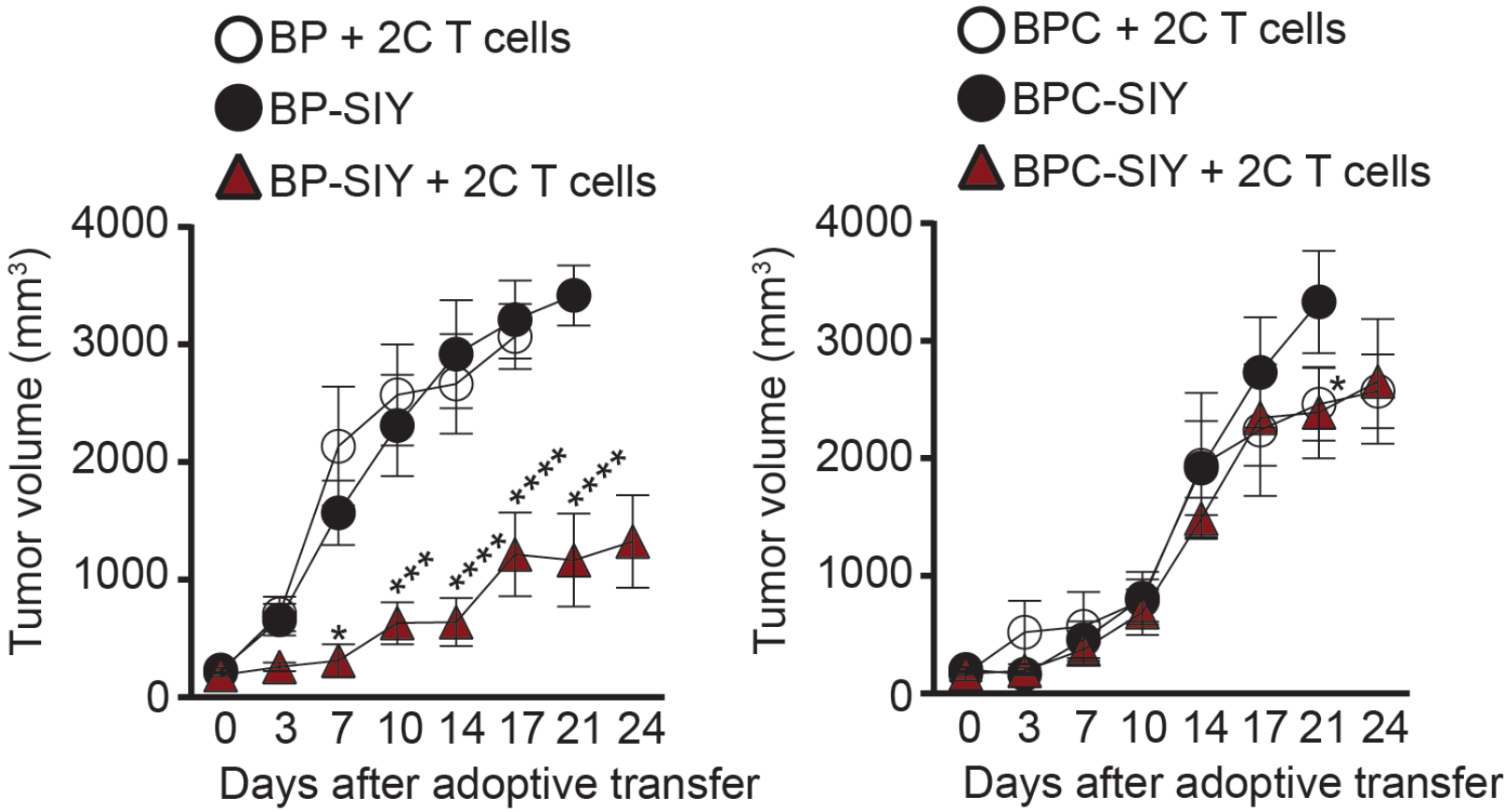
Is lack of effector T cell accumulation due to poor effector T cell recruitment into the tumor microenvironment?



β-catenin-expressing SIY$^+$ tumors fail to recruit primed antigen-specific 2C T cells

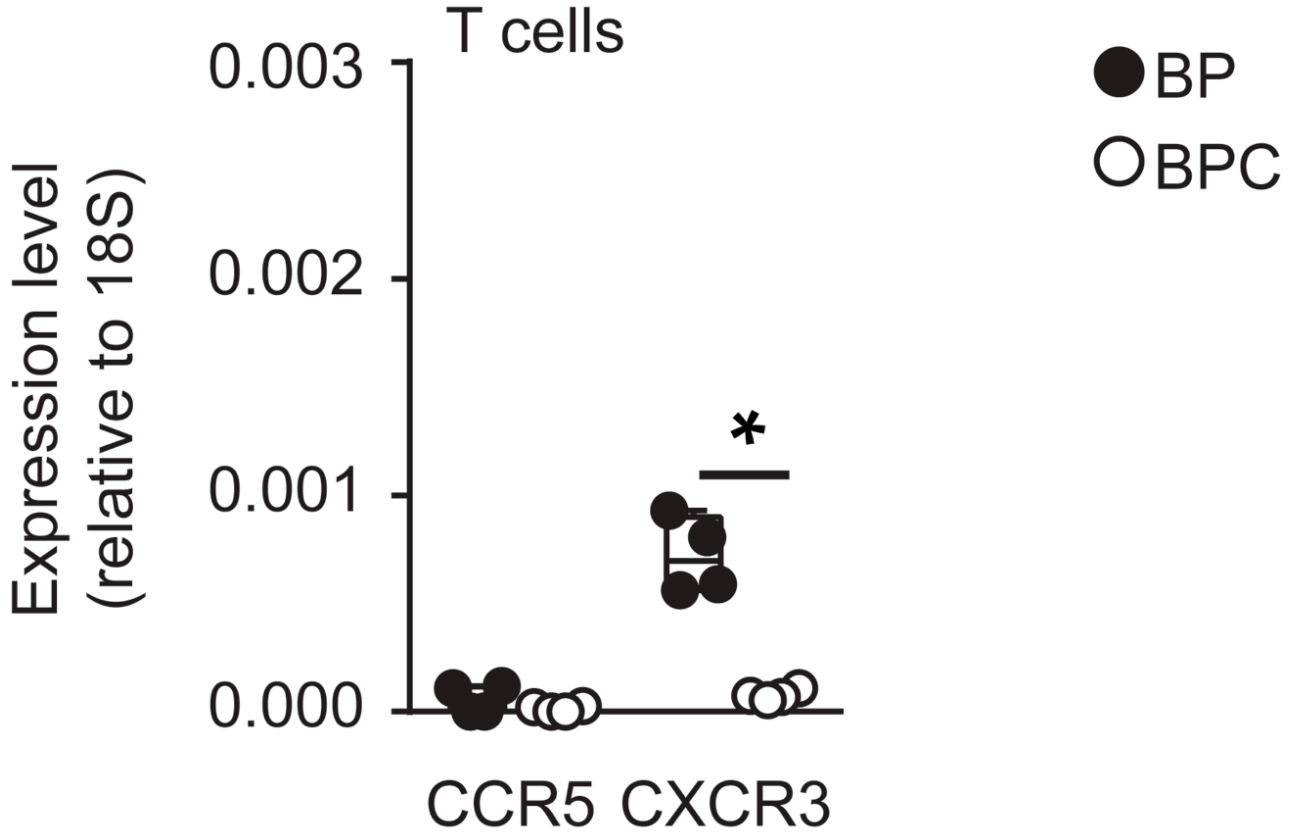


Adoptive transfer of effector 2C T cells fails to control β-catenin-expressing tumors

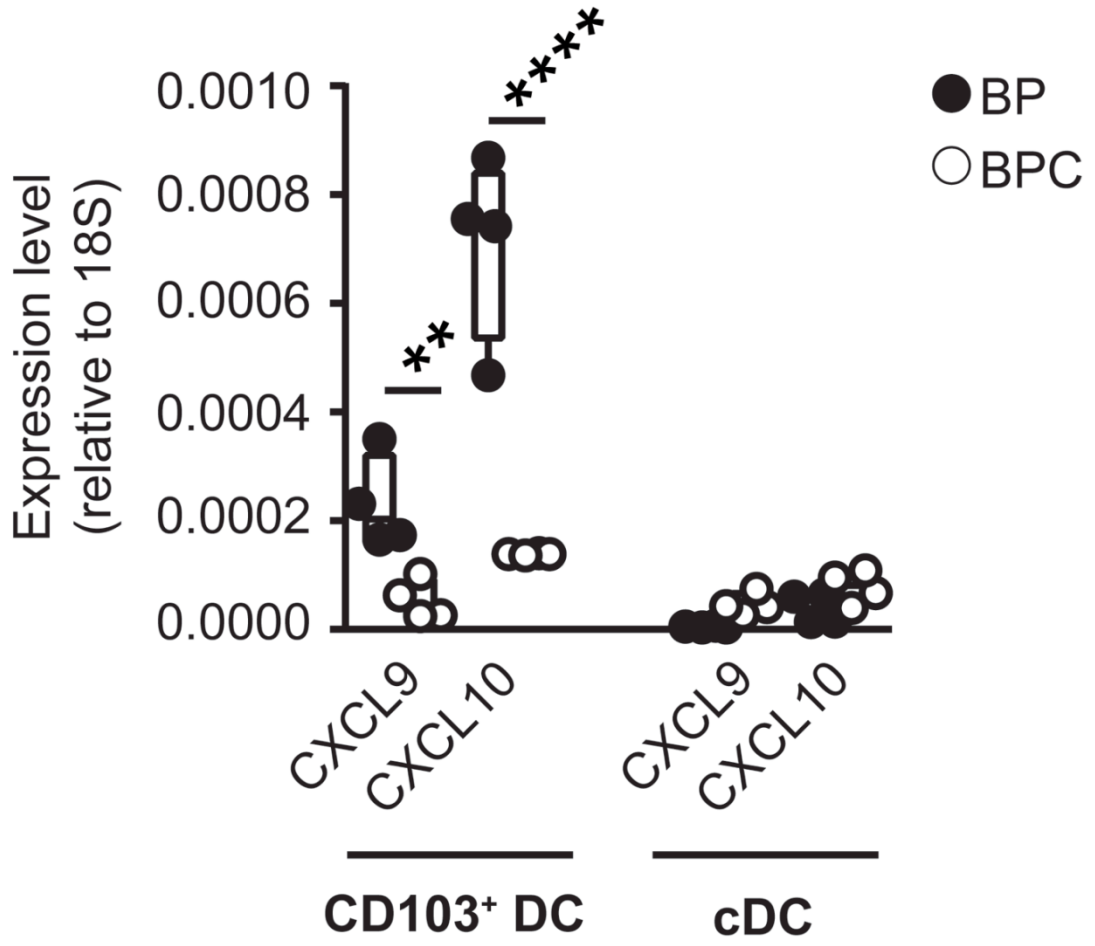


Which cell type within the tumor microenvironment is required for effector T cell recruitment?

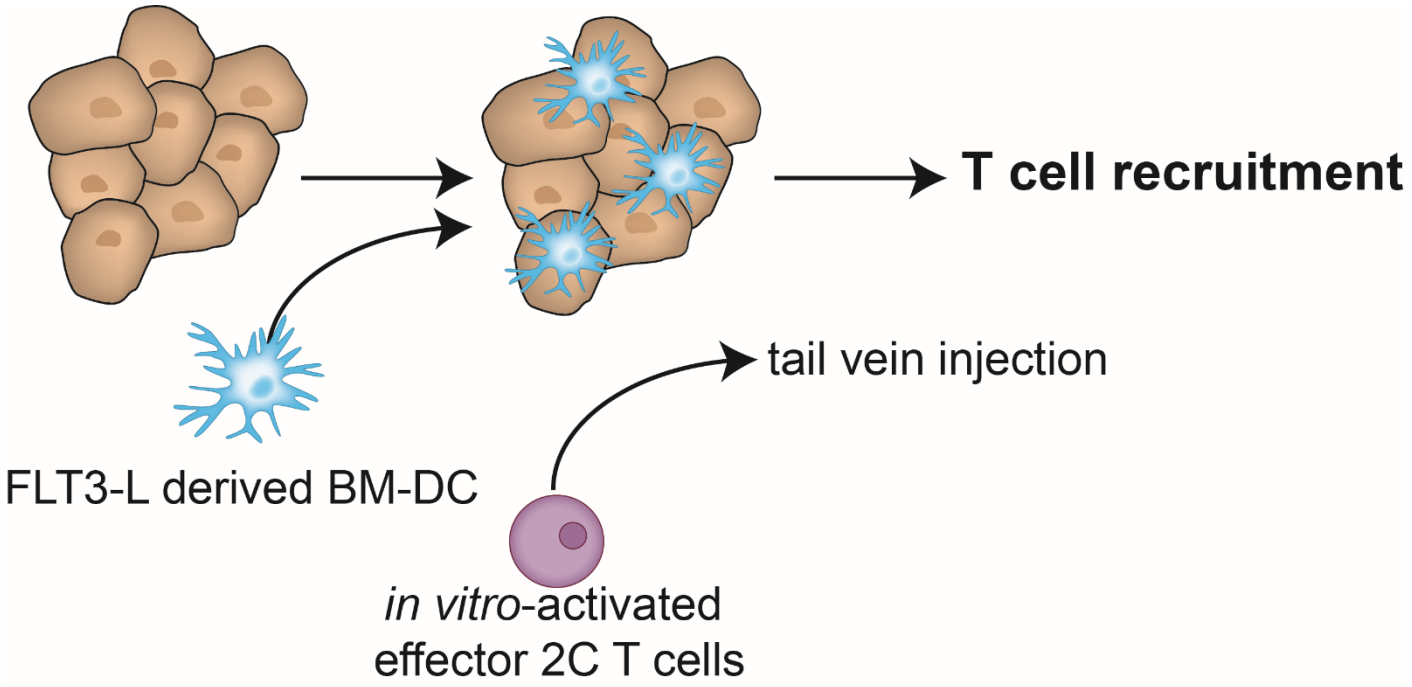
Effector T cells isolated from β-catenin^{+} tumors express reduced levels of CXCR3



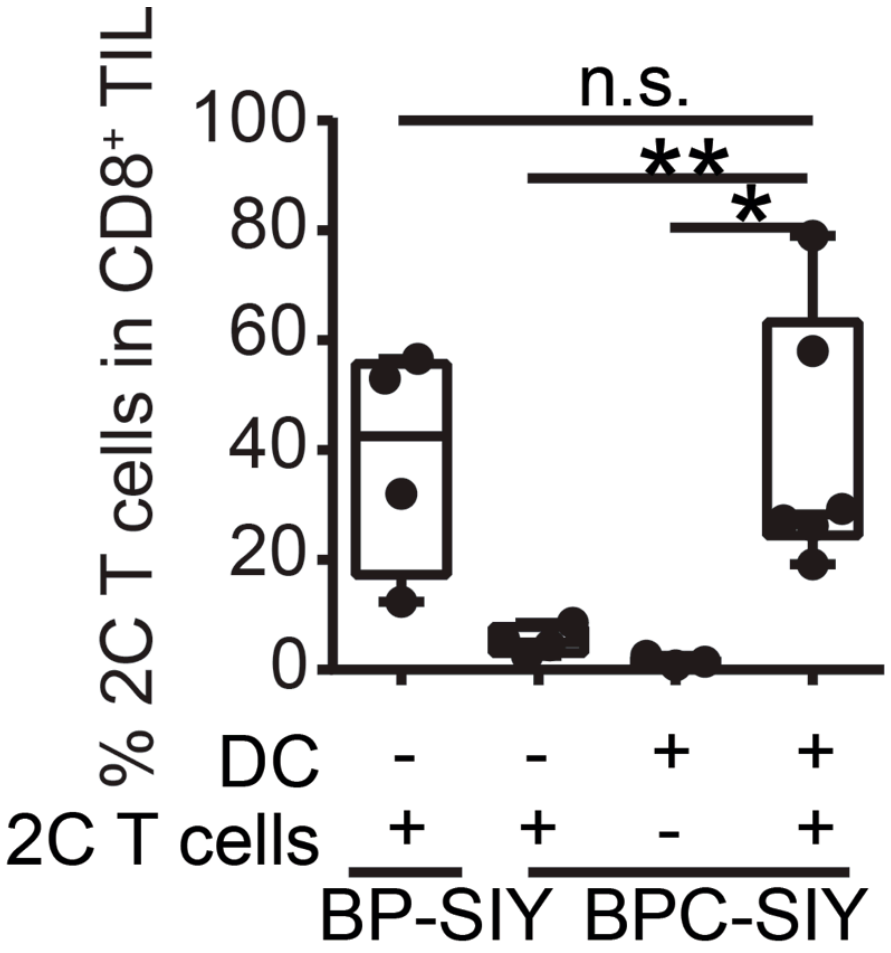
CD103+ dendritic cells are the predominat source of CXCR3 chemokine ligands



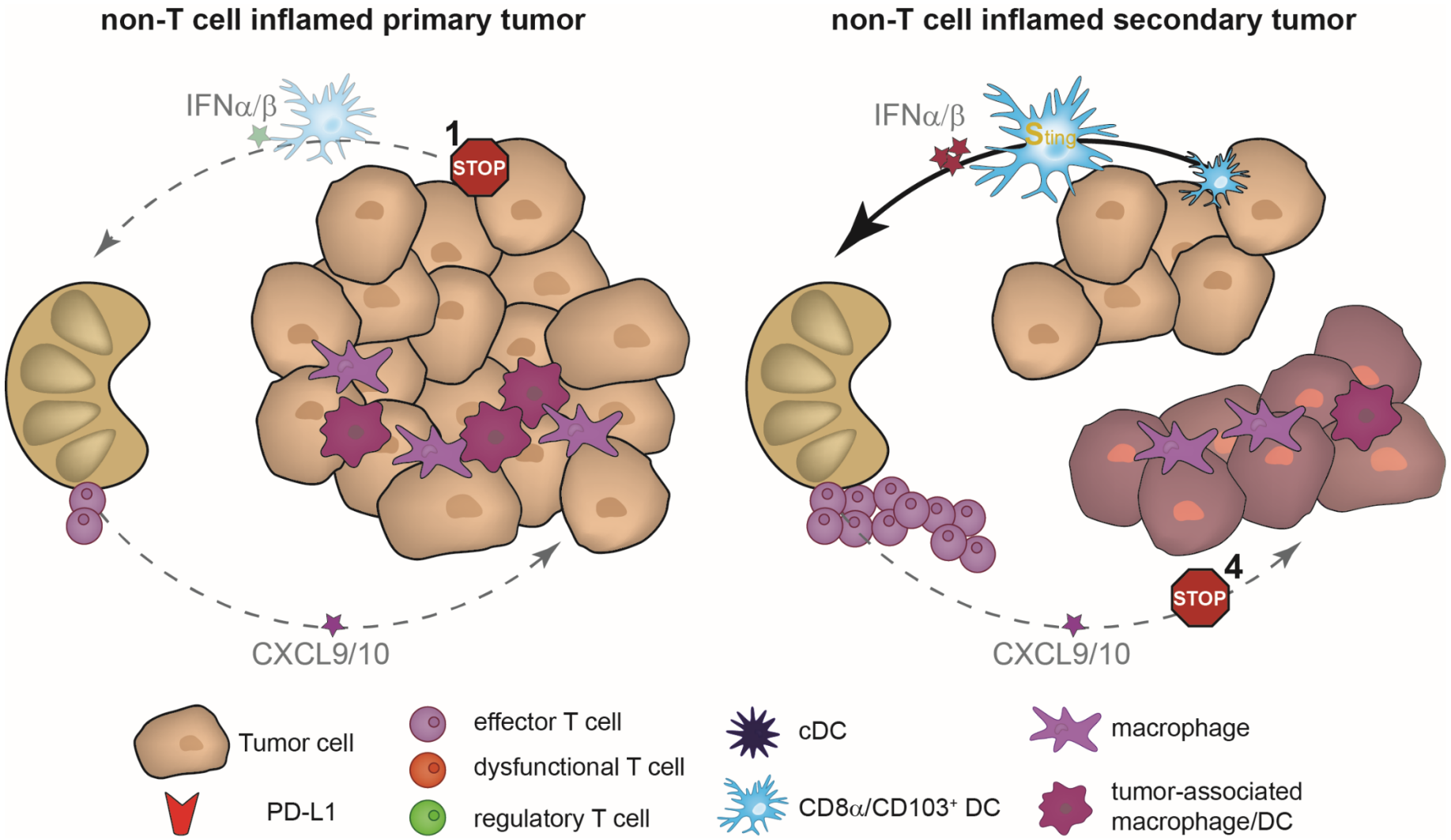
Are dendritic cells within the tumor microenvironment sufficient for the recruitment of effector T cells?



Reconstitution of β-catenin-expressing SIY$^+$ tumors with FLT3L-DCs restores effector T cell recruitment



Tumor cell-intrinsic β-catenin mediates immune exclusion through blocking T cell priming and blocking T cell recruitment



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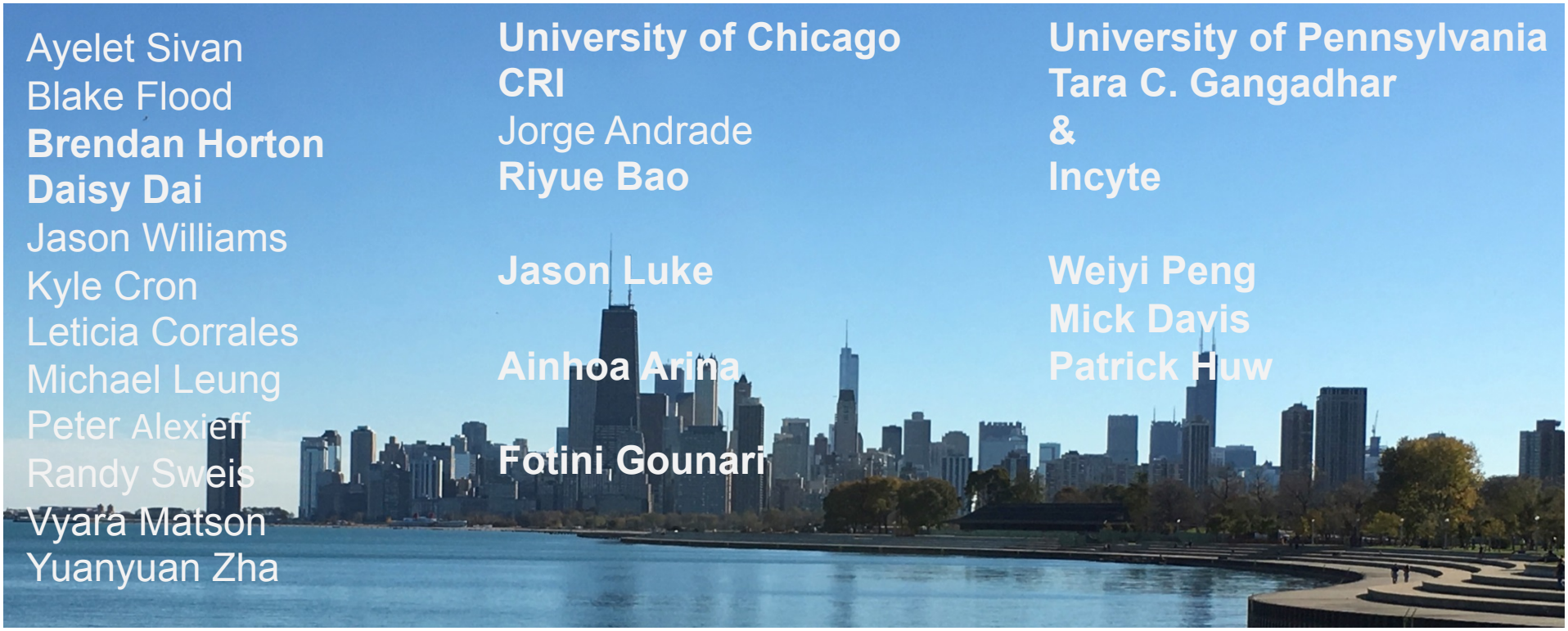

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