

Immune checkpoint humanized KI models

For IO *in vivo* studies



WuXi AppTec Research Service Division, Oncology & Immunology Unit



2020.01

- Overview of WuXi IO *in vivo* models
- Immune-checkpoint humanized mouse models
 - Overview
 - Humanized PD-1 KI model
 - hPD-1 KI model-B6 mouse and MC38
 - hPD-1 KI model-BALB/c mouse and CT26
 - Humanized CTLA-4 mouse model
 - hCTLA4 KI model-B6 mouse and MC38
 - hCTLA4 KI model-BALB/c mouse and CT26
 - Humanized OX-40 mouse model
 - Humanized hTIM-3 mouse model
 - Humanized hGITR mouse model
 - Humanized PD-1/PD-L1 mouse model
 - Humanized PD-1/hGITR mouse model
 - Humanized hPD-1/hCTLA4 mouse model
 - Humanized hLag3 mouse model

WuXi IO *in vivo* models

Available mouse models to study IO modulators

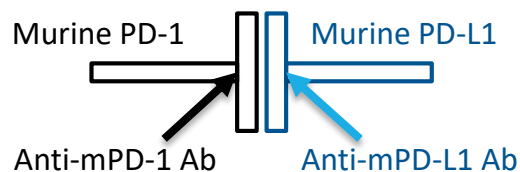
Model	Application	WuXi Highlight
Syngeneic murine models	Antitumor efficacy and MOA; proof of concept	Largest collection (55+) in Asia; Baseline of murine TIL analysis and drug response; Well validated tool antibodies to facilitate preclinical combo and MOA studies
Immune-checkpoint humanized models	Selection of antihuman checkpoint blockers	Available PD-1/CTLA-4/TIM-3/OX-40/hGITR KI models; Mature CRISPR platform for customized KO/KI GEMMs
Immune-Avatar humanized models	Antitumor efficacy and MOA	Large PDX/CDX collection to facilitate development of humanized models; Integrated/customized solution
Patient-derived xenograft models	ImmunePDX; Humanized PDX models	1000+ PDX models (700+ well annotated with oncogene amplification, mutation & fusion); Well established capability on human TIL analysis
Cell line-derived xenograft models	Humanized CDX models	170+ CDX models covering 22 tumor types; Luciferase labeled cells for orthotopic & metastatic models

WuXi IO checkpoint humanized models

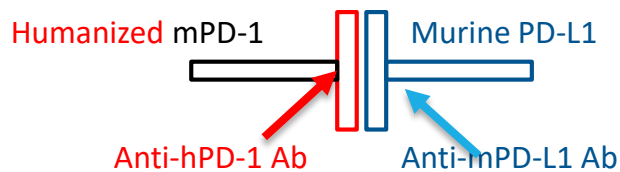
Modified syngeneic model

KI (knock-in) mice offer the possibility to study clinical grade checkpoint blockers targeting human checkpoints in the context of a fully functional immune system.

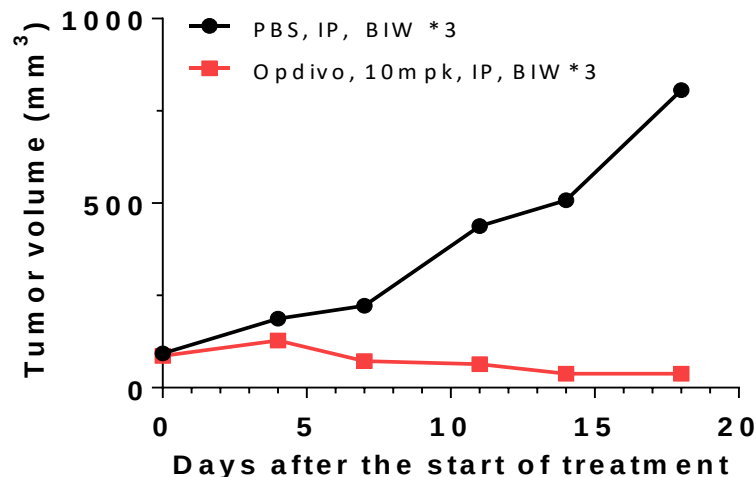
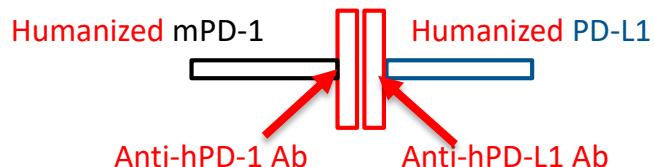
Existing syngeneic model



Modified syngeneic model



Modified syngeneic model and modified cell



Target	Background	Status
hPD-1	C57BL/6J, BALB/c	Available
hCTLA4	C57BL/6J, BALB/c	Available
hPD-1/hPD-L1	C57BL/6J	Available
hPD-1/hCTLA4	C57BL/6J	Available
hTIM3	C57BL/6J	Available
hPD-1/hTIM3	C57BL/6J	Available
hLAG3	C57BL/6J	Available
hOX40	C57BL/6J, BALB/c	Available
hGITR	C57BL/6J	Available
hPD-1/hGITR	BALB/c	Available
h4-1BB	C57BL/6J	Mid 2019
hICOS/hPD-1	C57BL/6J	Mid 2019
hTIGIT	C57BL/6J	Mid 2019
hCD40	C57BL/6J	Mid 2019
hPD-1/hCD47	C57BL/6J, BALB/c	Mid 2019
hSIRPA	C57BL/6J	Late, 2019
hPD-1/hSIRPA	C57BL/6J, BALB/c	Late 2019
hVISTA	C57BL/6J	Late 2019
hPD1/hCD28	C57BL/6J, BALB/c	Mid 2019
hPD-1/hPD-L1/h4-1BB	BALB/c	Late 2019
hPD-1/hPD-L1/hCTLA4	BALB/c	Late 2019
hPD-1/hPD-L1/hTIGIT	C57BL/6J	Late 2019
hPD-1/hPD-L1/hOX40	C57BL/6J	Late 2019
hPD-1/hVISTA	C57BL/6J	2020
hPD-1/hPD-L1/hCD73	C57BL/6J	2020
hPD-1/hPD-L1/hLAG3	C57BL/6J	2020

hPD-1 KI mouse model in C57BL/6 mice

Background



Name: hPD-1 KI mice

Strain type: Cas9-KI

Hair color: Black

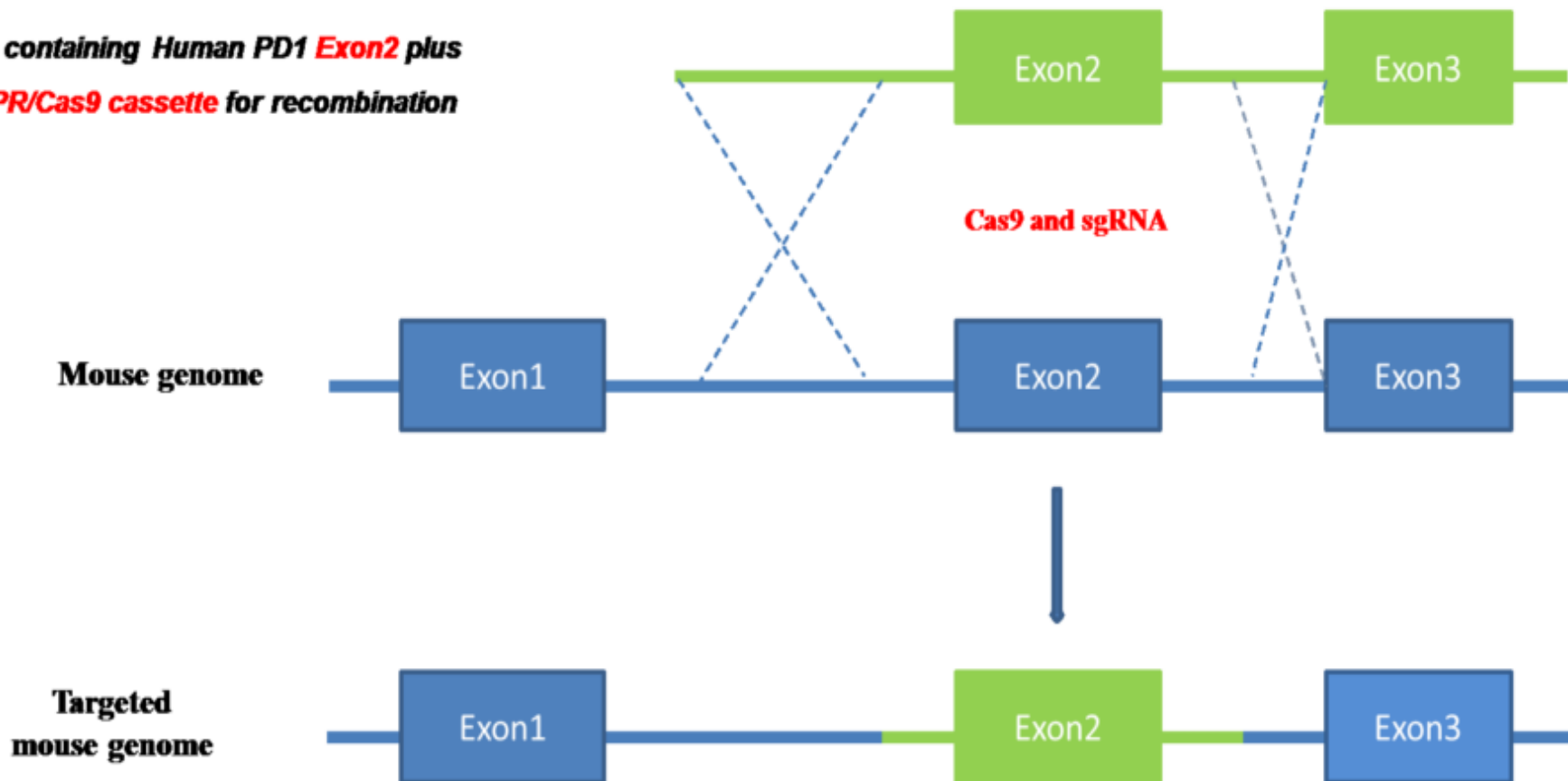
Background: C57BL/6J

Validated with hPD-1 antibodies (Opdivo, analog of Keytruda and Opdivo) on MC38 and huPD-L1-MC38 syngeneic model; validated with hPD-L1 (Analog of Tecentriq) on hPD-L1-MC38 model.

hPD-1 KI mouse model in C57BL/6 mice

Strategy

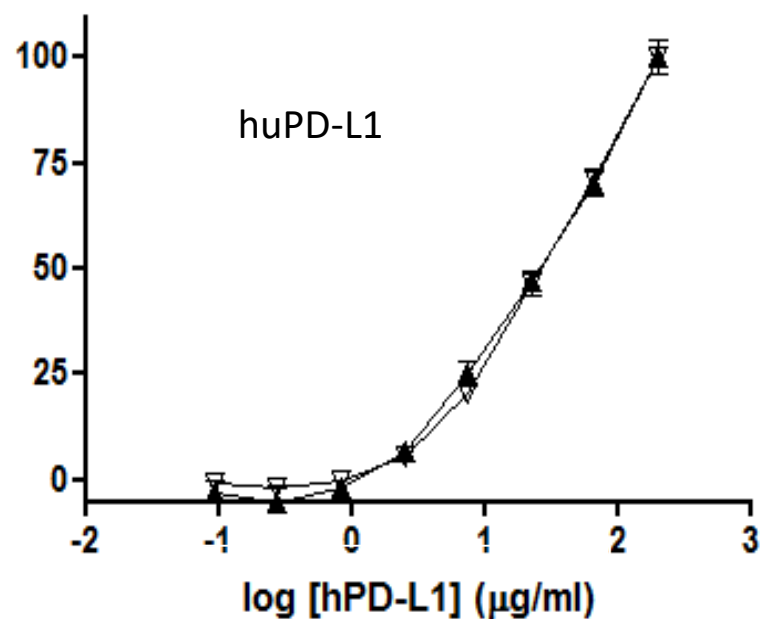
**Donor containing Human PD1 *Exon2* plus
CRISPR/Cas9 cassette for recombination**



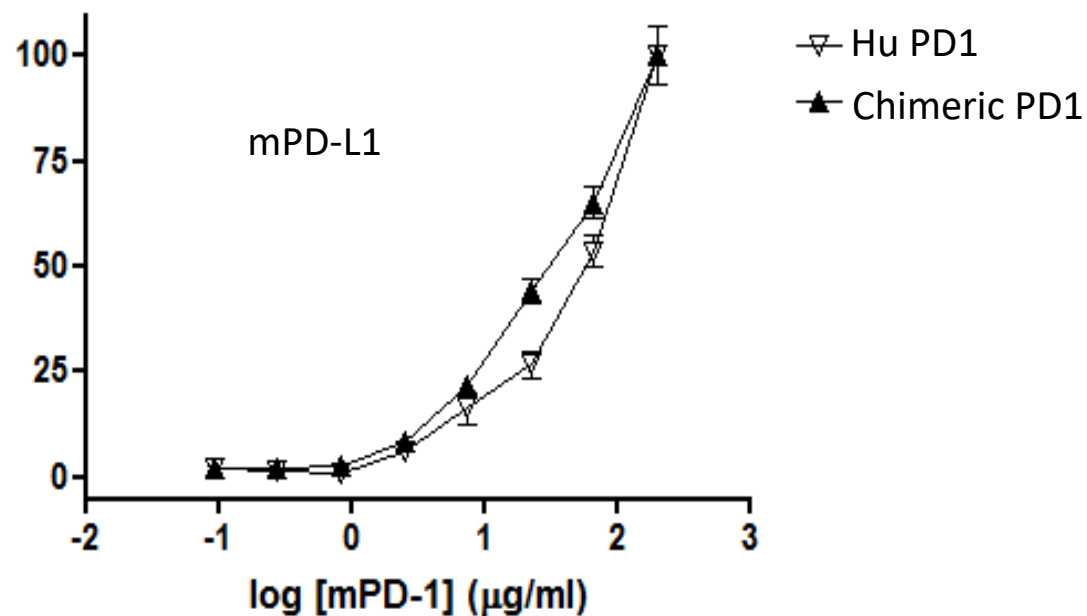
hPD-1 KI mouse model in C57BL/6 mice

Functional assay: Binding assay-ELISA

% of Control



% of Control

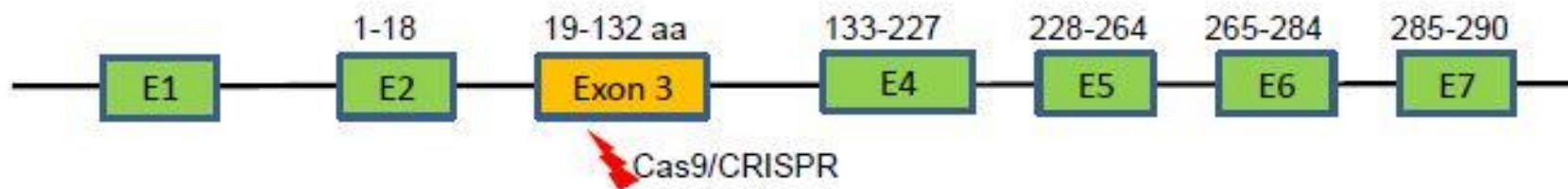


hPD-1 KI mouse model in C57BL/6 mice

Generating human PD-L1 KI in MC38 cells

Step 1

Mouse PD-L1 knockout by CRISPR/Cas9



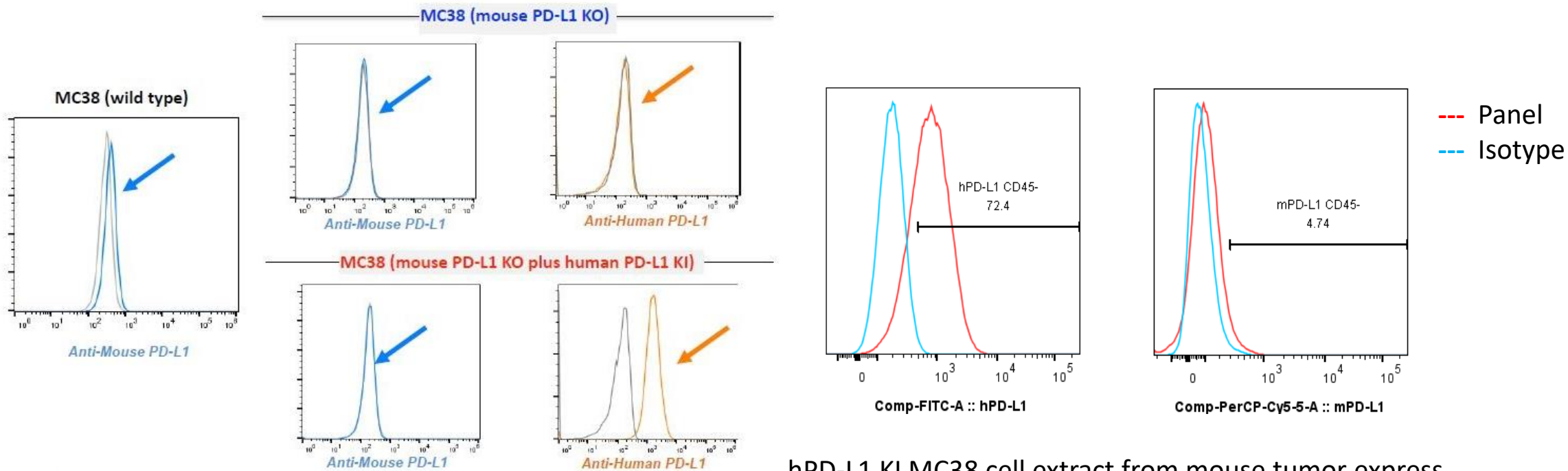
Step 2

Human PD-L1 knock-in



hPD-1 KI mouse model in C57BL/6 mice

FCM to detect hPD-L1 expression in cell line and tumor tissue



hPD-L1 KI MC38 cell line express hPD-L1

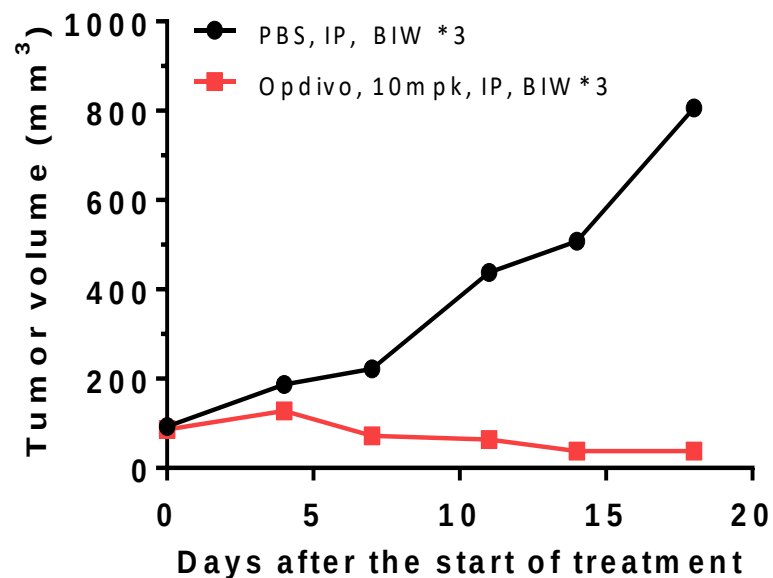
[Nanjing Galaxy Data](#)

hPD-L1 KI MC38 cell extract from mouse tumor express hPD-L1

[WuXi AppTec Data](#)

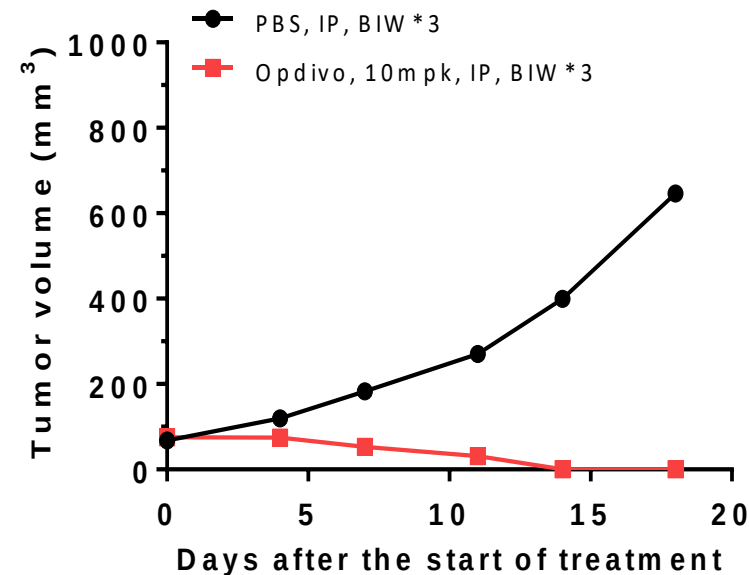
hPD-1 KI mouse model in C57BL/6 mice

In vivo validation with MC38 and hPD-L1 MC38 models



huPD-L1 KI MC38 cell

huPD-1 KI C57BL/6 mice

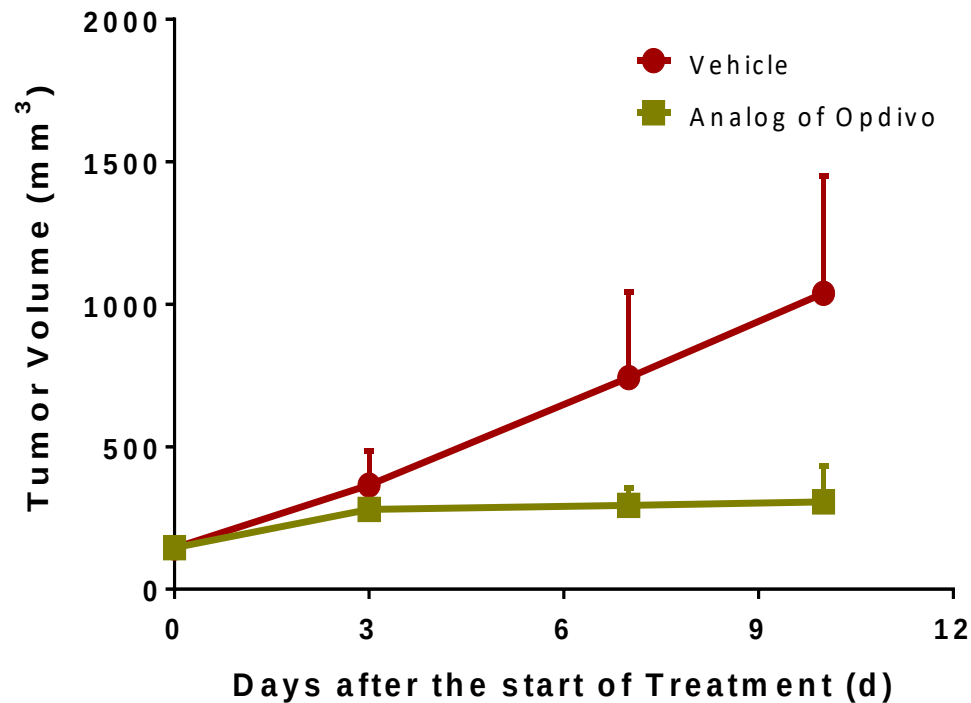
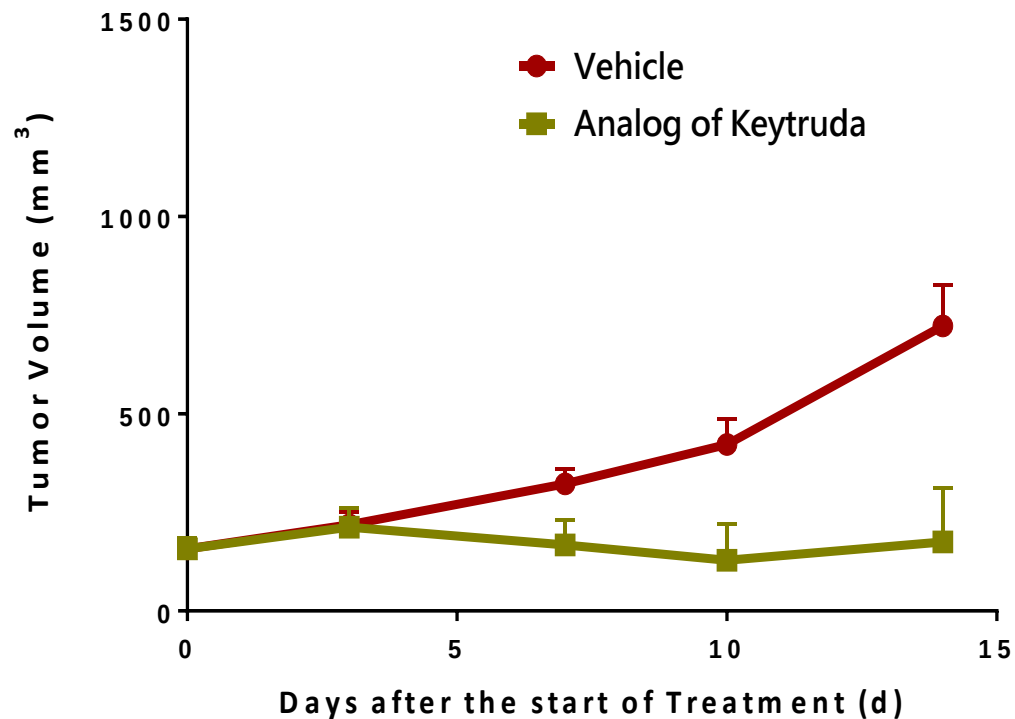


Wild type MC38 cell

huPD-1 KI C57BL/6 mice

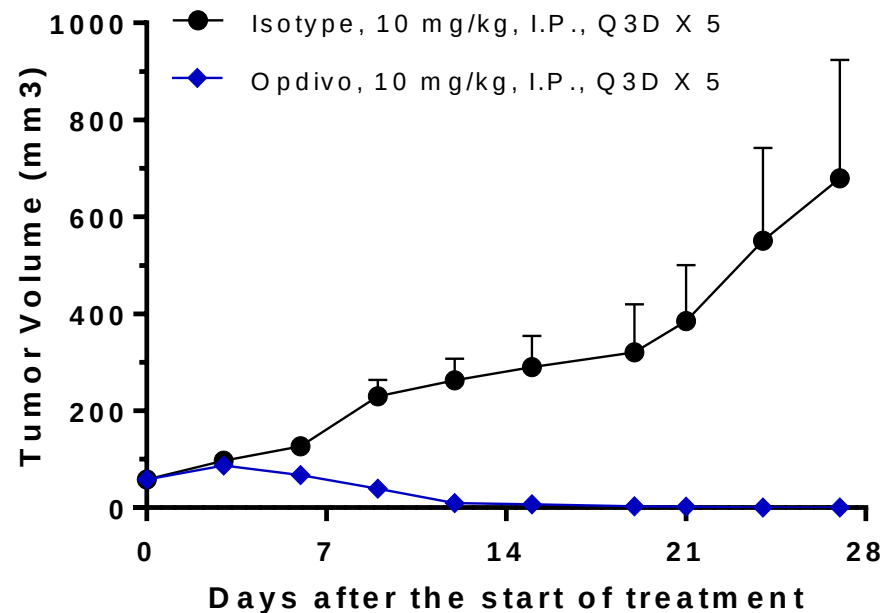
hPD-1 KI mouse model in C57BL/6 mice

Validated with Analog of Keytruda and Opdivo



hPD-1 KI mouse model in C57BL/6 mice

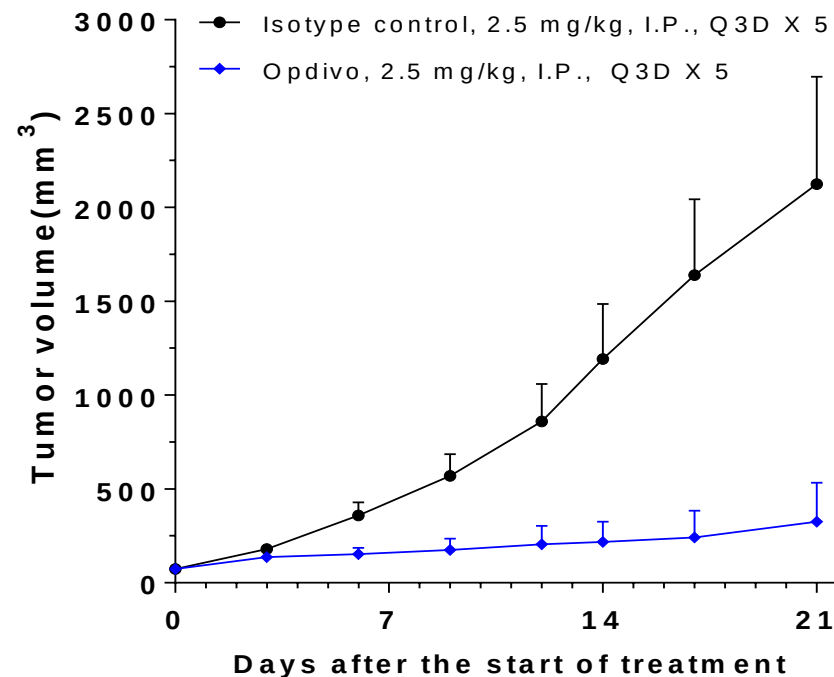
MC38-huPD-L1 model in hPD-1 KI mice



2017/04/10

Animal: **Female** hPD-1 KI mice

Cell: MC38-huPD-L1



2017/06/16

Animal: **male** hPD-1 KI mice

Cell: MC38-huPD-L1

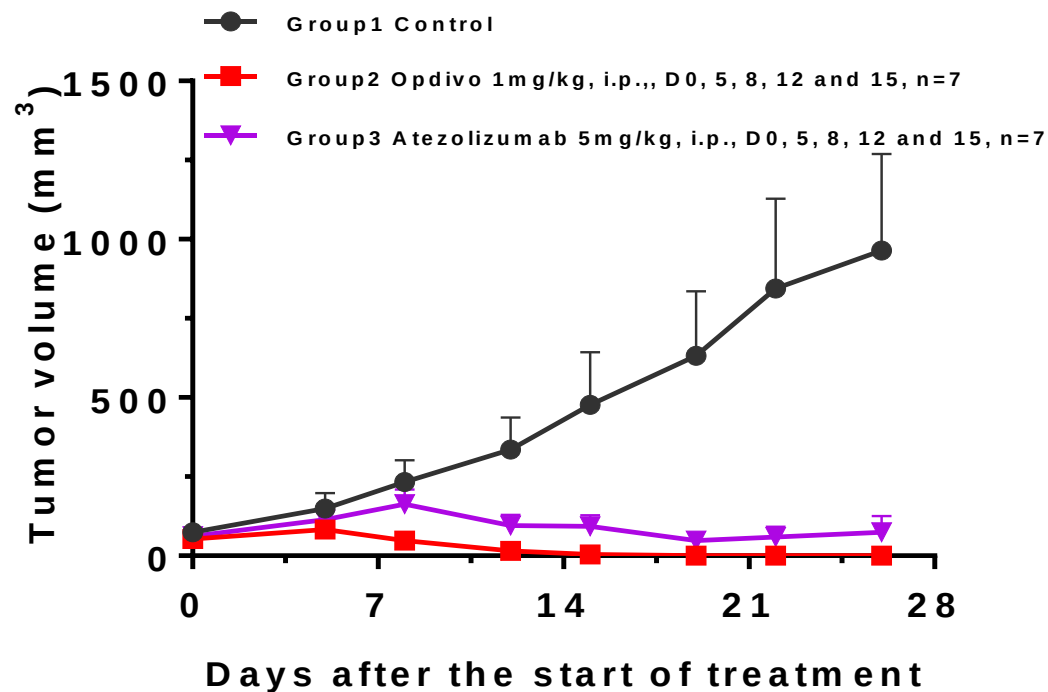
hPD-1 KI mouse model in C57BL/6 mice

MC38-huPD-L1 model in hPD-1 KI mice

2019/01/03

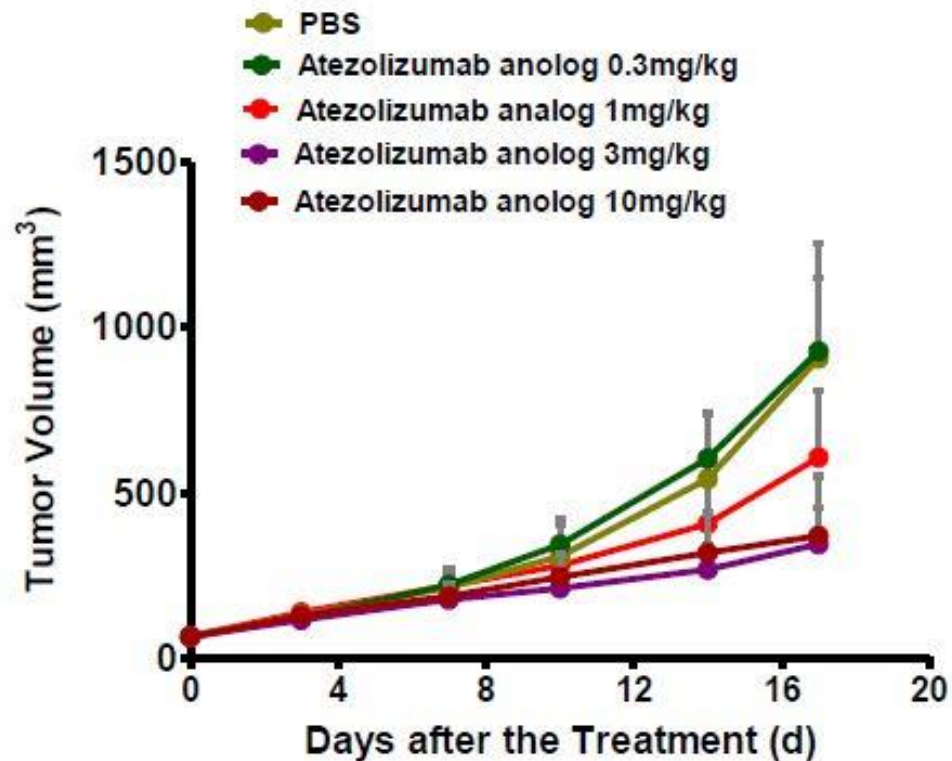
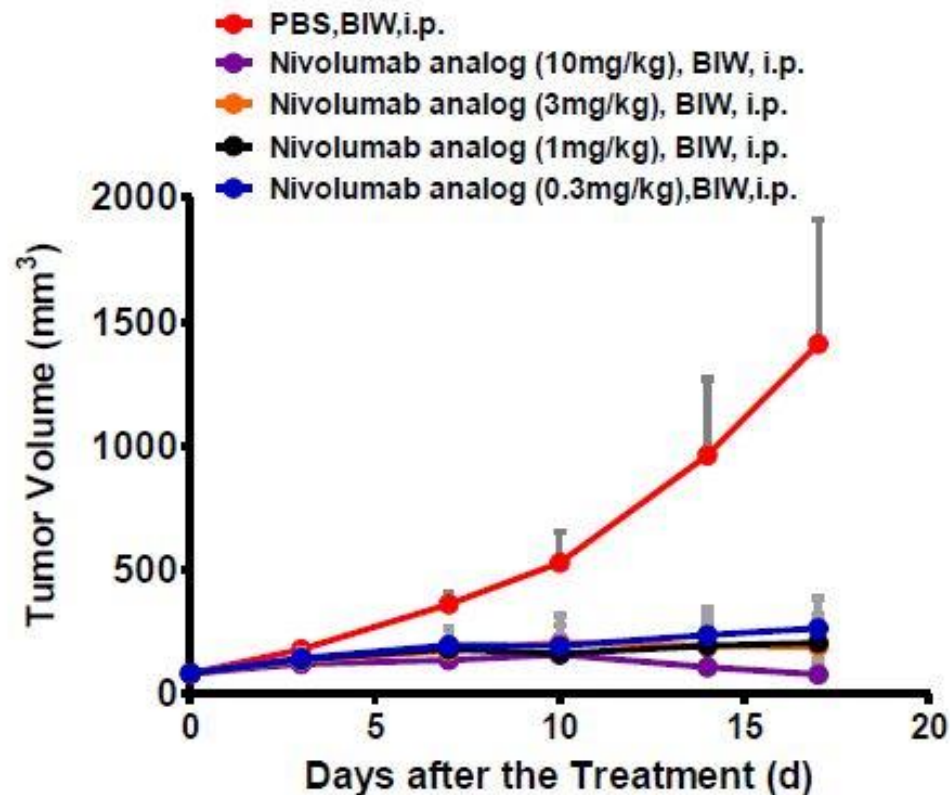
Animal: **Female** hPD-1 KI mice

Cell: MC38-huPD-L1



hPD-1 KI mouse model in C57BL/6 mice

Dose escalation studies with human PD-1 and PD-L1 antibodies



hPD-1 KI mouse model in Balb/c mice

Background



Name: hPD1 KI mice

Strain type: Cas9-KI

Hair color: White

Background: BALB/c

hPD1 KI mice express human PD1 in T cells

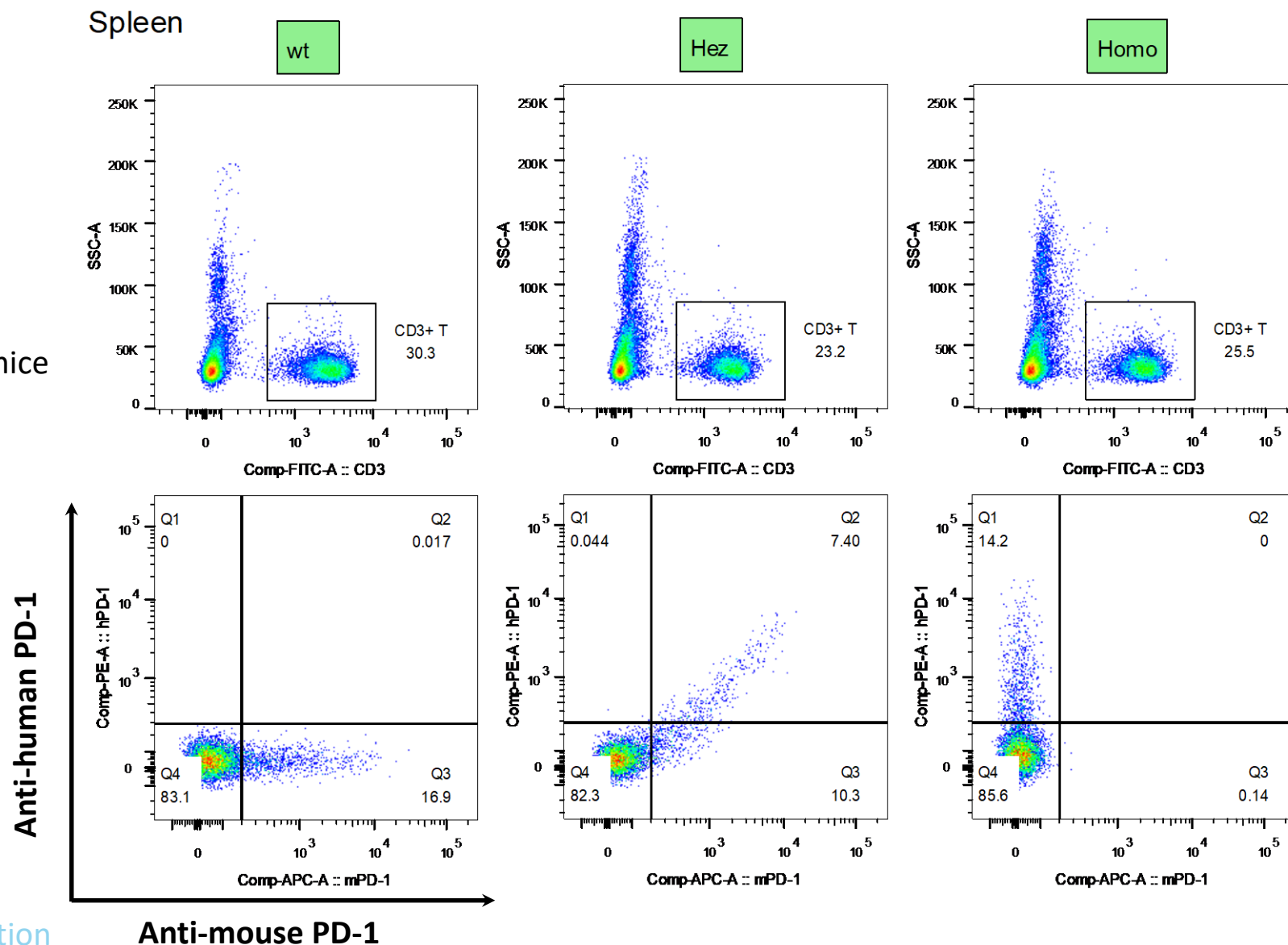
Validated with hPD1 antibody (Keytruda and Opdivo) on CT26 syngeneic model

hPD-1 KI mouse model in Balb/c mice

In vitro validation

Expressing validation-FACS

hPD-1 is expressed at comparable level in homo mice as mPD-1 expressing in the wildtype mice.

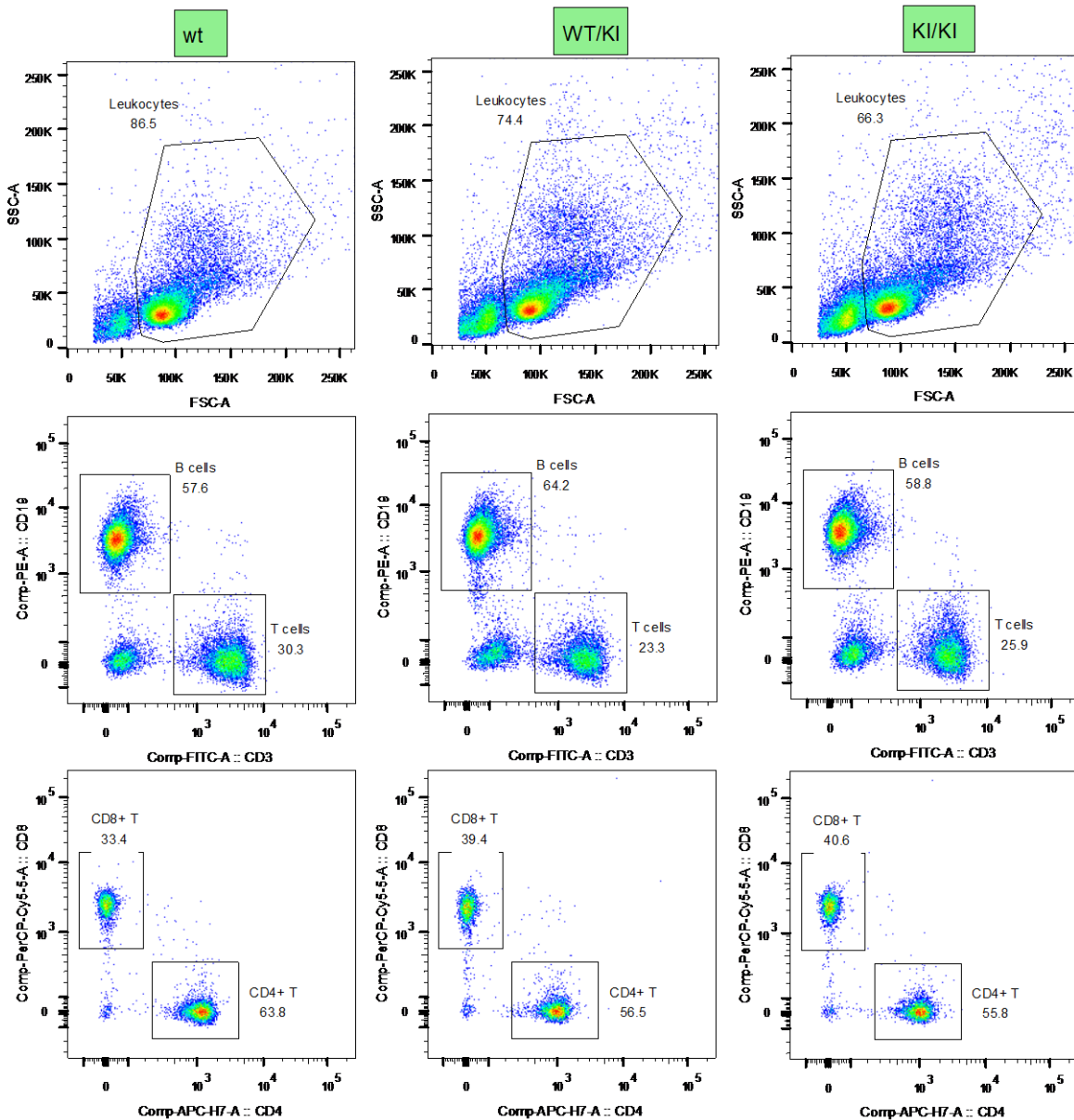


hPD-1 KI mouse model in Balb/c mice

In vitro validation

T/B background check-FACS

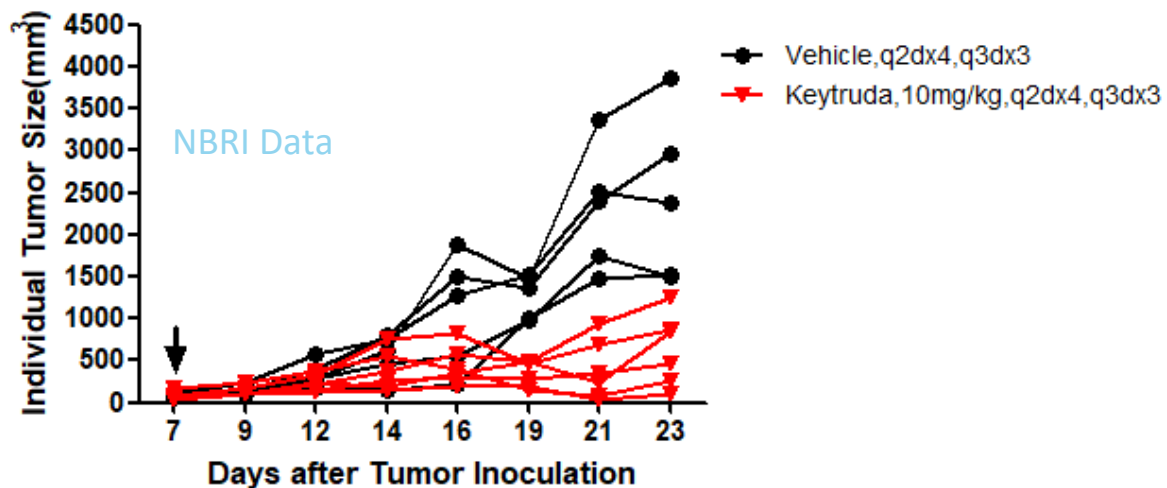
No obvious difference observed
between PD-1 humanized and
wildtype mice.



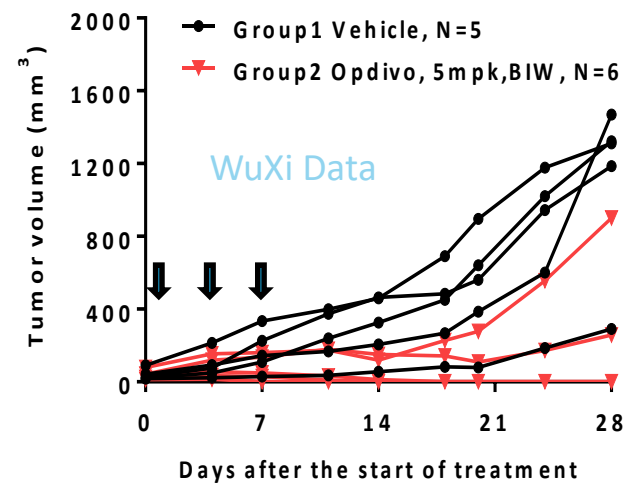
hPD-1 KI mouse model in Balb/c mice

Validation of CT26 model in hPD-1 KI mice—Keytruda and Opdivo

BALB/c-hPD1 Anti-tumor Efficacy Test



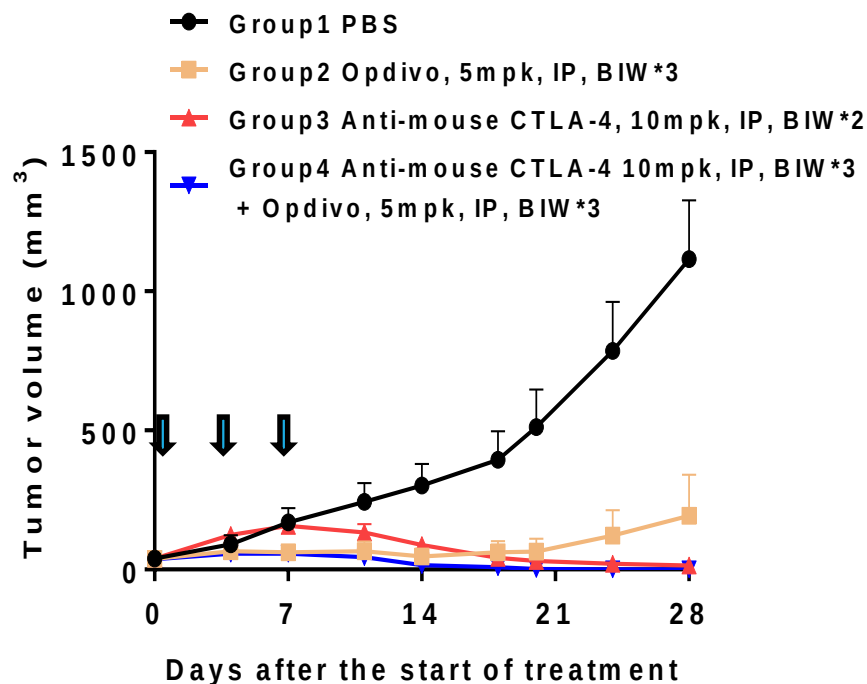
BALB/c-hPD1 Anti-tumor Efficacy Test



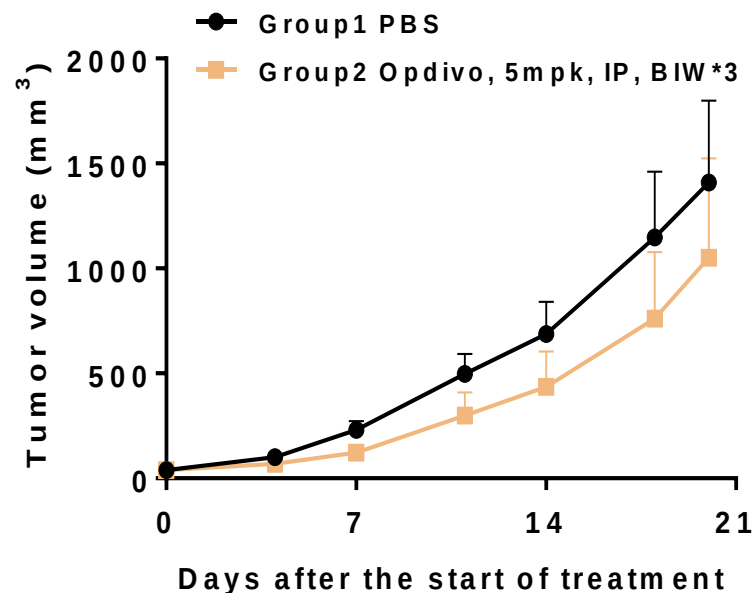
Significant tumor growth inhibition observed in hPD-1 homo mice treated with both Keytruda and Opdivo.

Combo of hPD-1 and mCTLA-4 in hPD-1 KI mouse model

In vivo validation with CT26 model



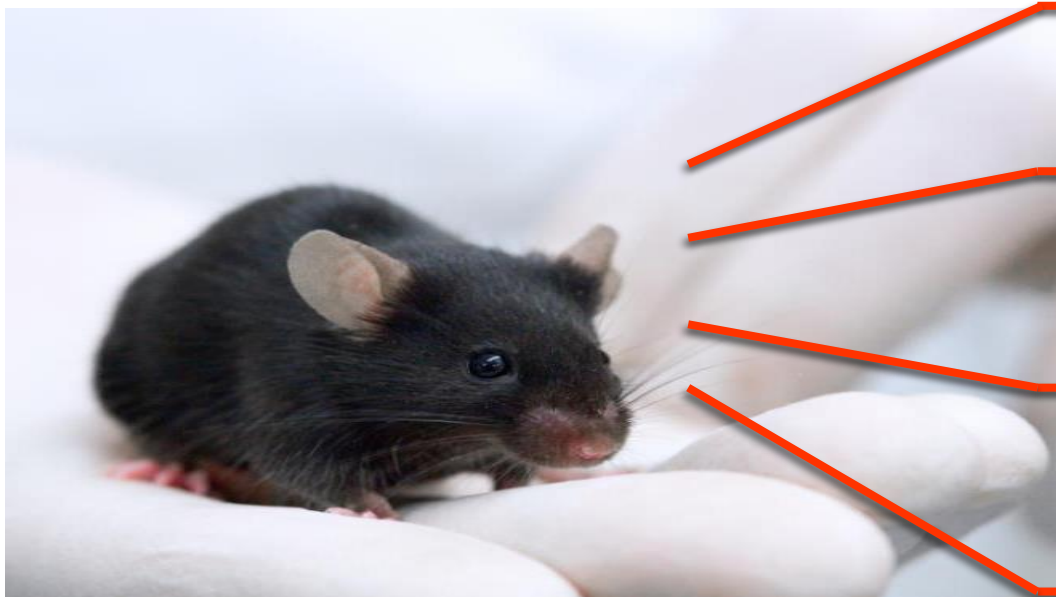
huPD-1 KI Balb/c



Wild type Balb/c

hCTLA-4 KI mouse model in C57BL/6 mice

Background



Name: hCTLA-4 KI mice

Strain type: Cas9-KI

Hair color: Black

Background: C57BL/6J

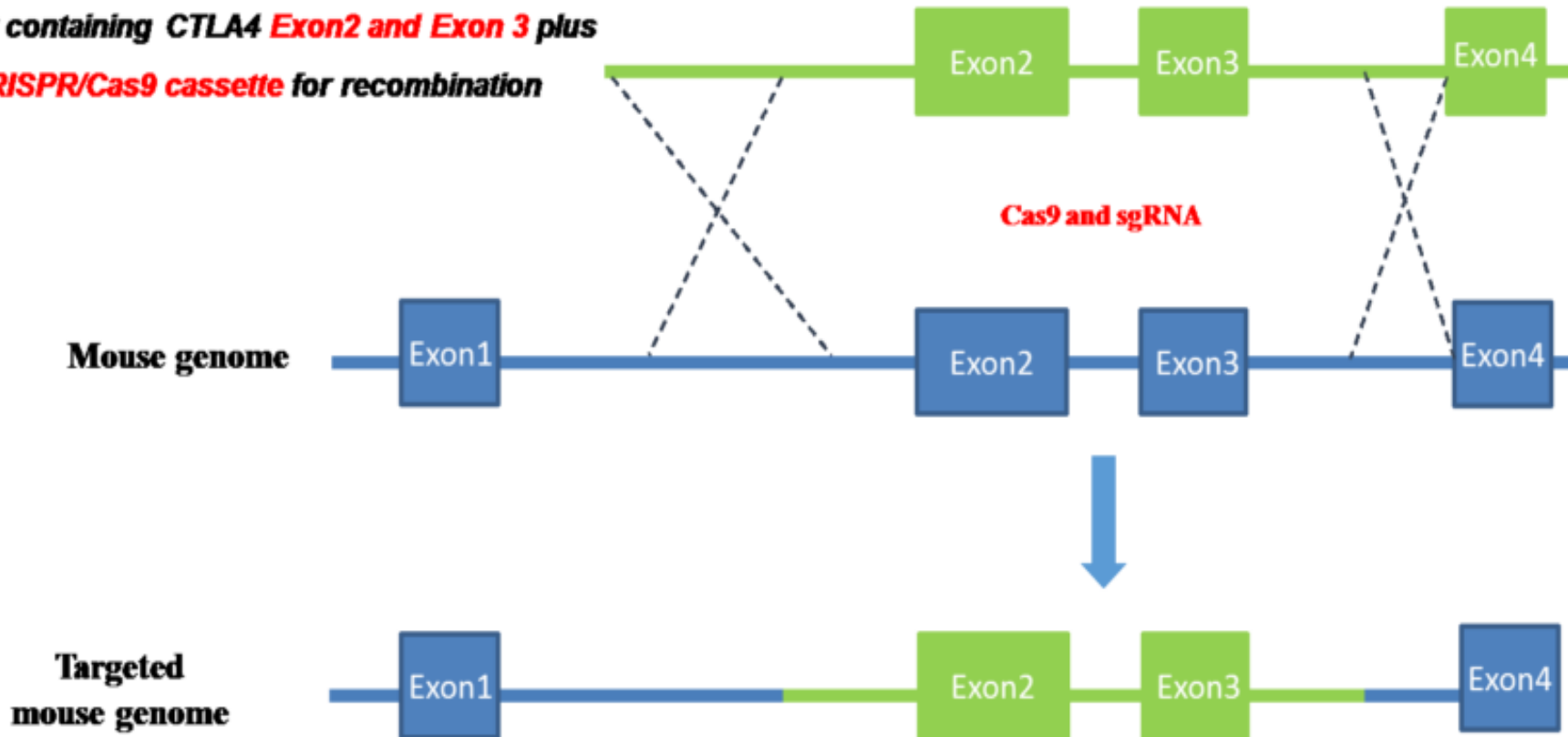
hCTLA-4 KI mice express human CTLA-4 in T cells

Validated with hCTLA-4 antibody (Yervoy) on MC38 syngeneic model

hCTLA-4 KI mouse model in C57BL/6 mice

Strategy

**Donor containing CTLA4 Exon2 and Exon 3 plus
CRISPR/Cas9 cassette for recombination**



The similar strategy was used in ES cell targeting to create CTLA4 chimeric knock-in mice.

Lute KD, May KF Jr, Lu P, et al. Human CTLA4 knock-in mice unravel the quantitative link between tumor immunity and autoimmunity induced by anti-CTLA-4 antibodies. Blood 2005;106:3127-313

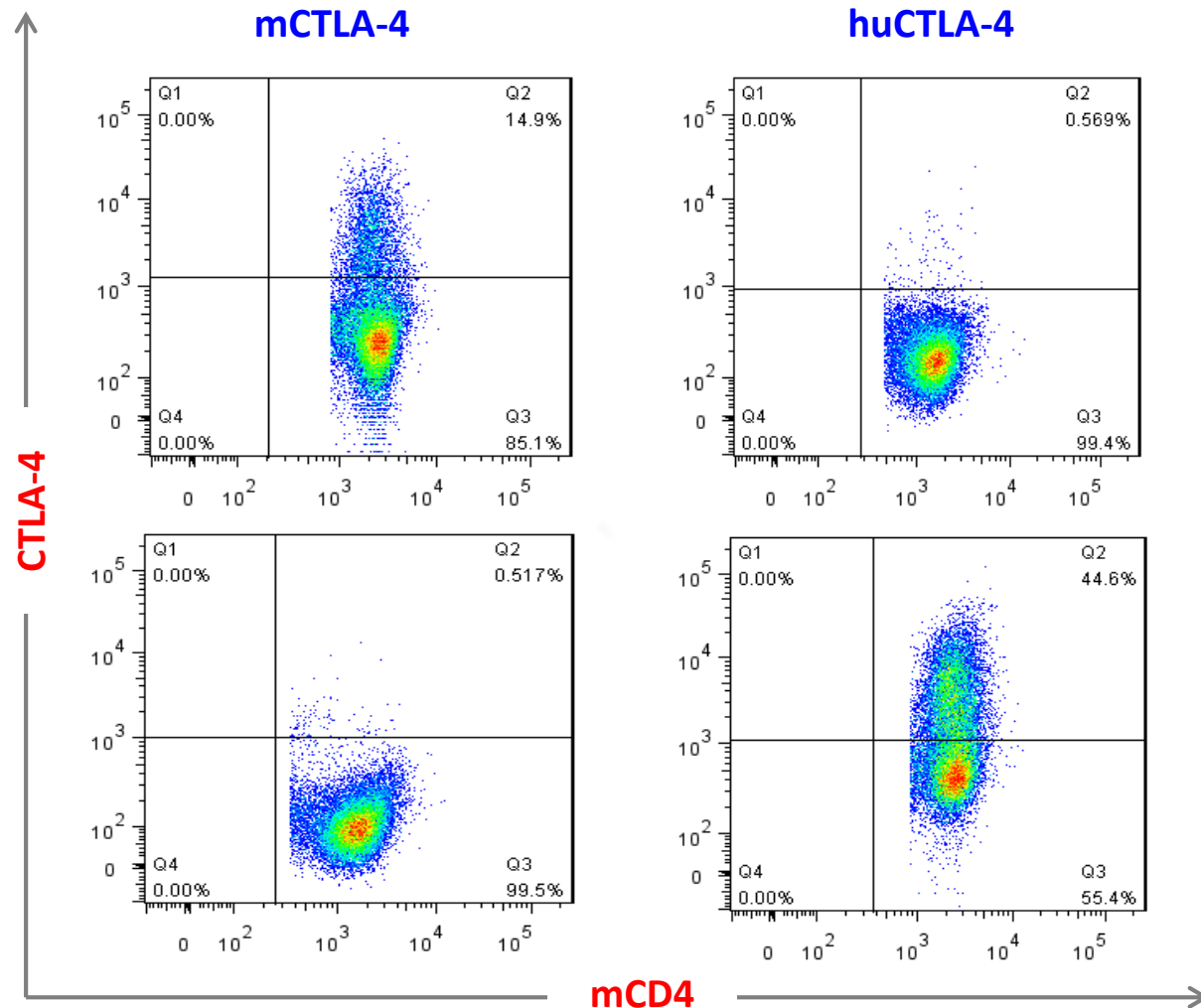
hCTLA-4 KI mouse model in C57BL/6 mice

In vitro validation

WT mice

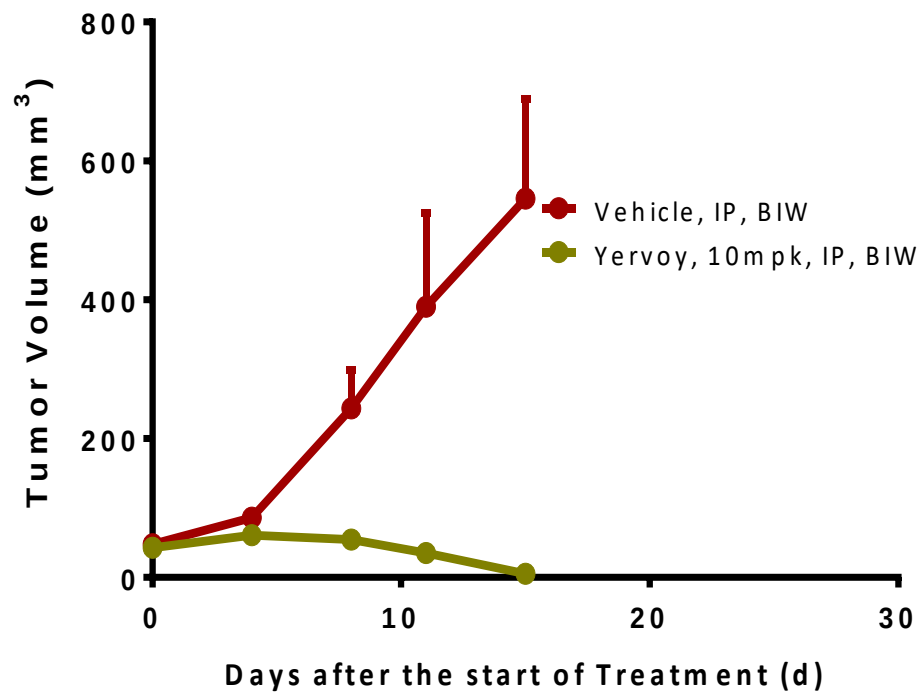
hCTLA-4 KI mice

CTLA-4 KI mice express human
CTLA-4 in T cells

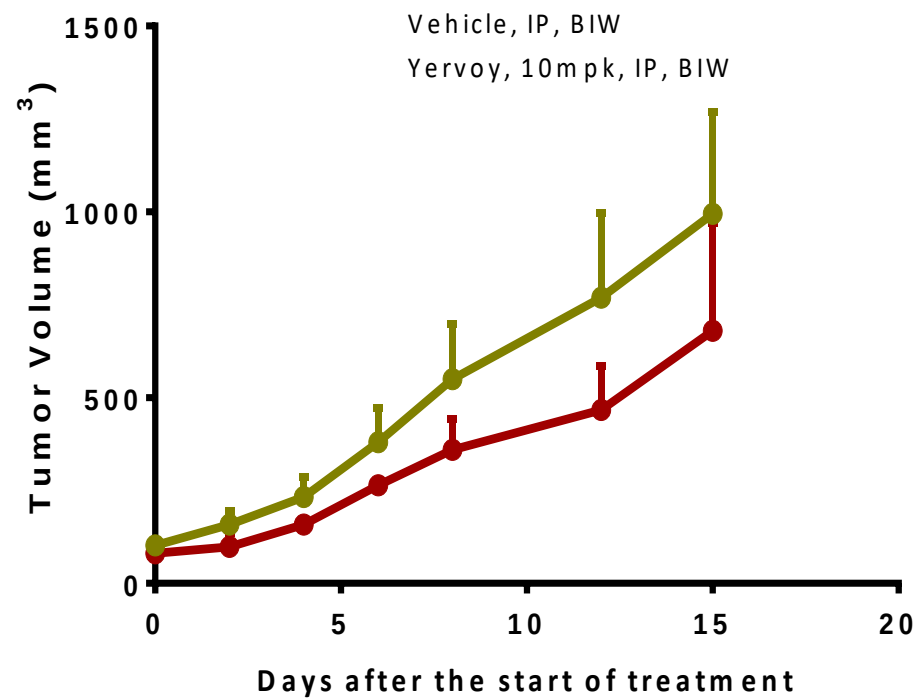


hCTLA-4 KI mouse model in C57BL/6 mice

In vivo validation with MC38 model



huCTLA-4 KI C57BL/6



Wild type C57BL/6

hCTLA4 KI mouse model in Balb/c mice

Background



Name: hCTLA4 KI mice

Strain type: Cas9-KI

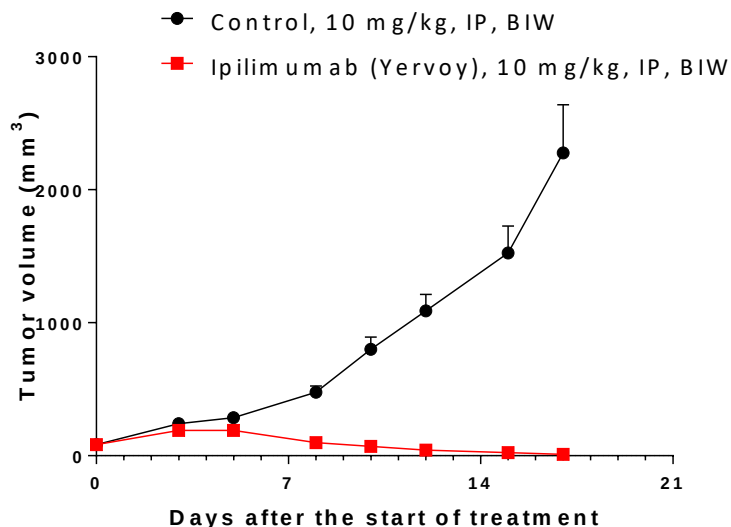
Hair color: White

Background: BALB/c

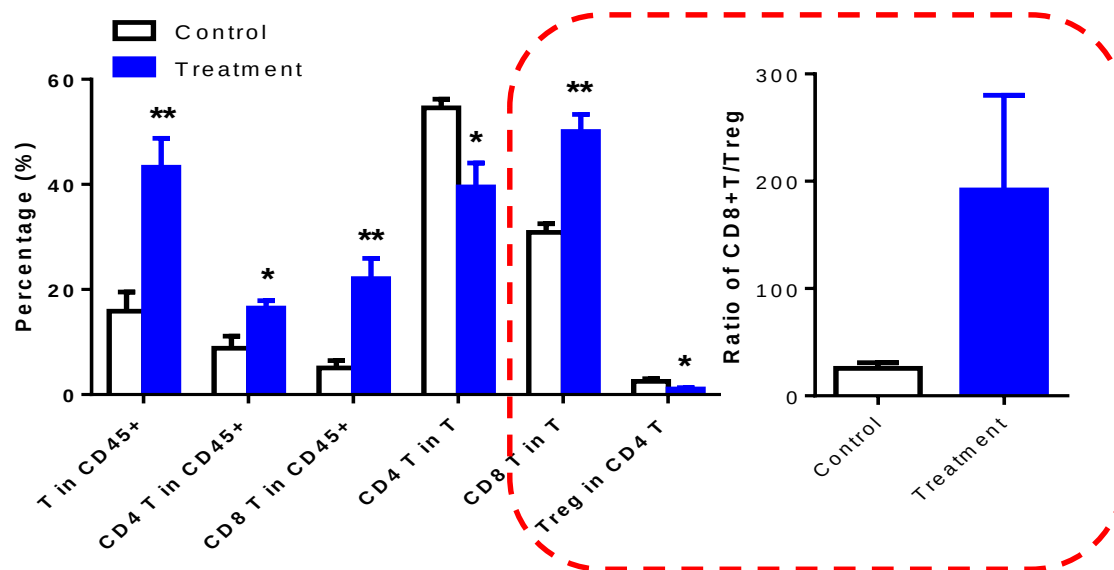
hCTLA-4 KI mice express human CTLA-4 in T cells

Validated with hCTLA-4 antibody (Yervoy) on MC38 syngeneic model

Efficacy of anti-CTLA-4 (Yervoy)



TIL analysis in satellite mice at day 5



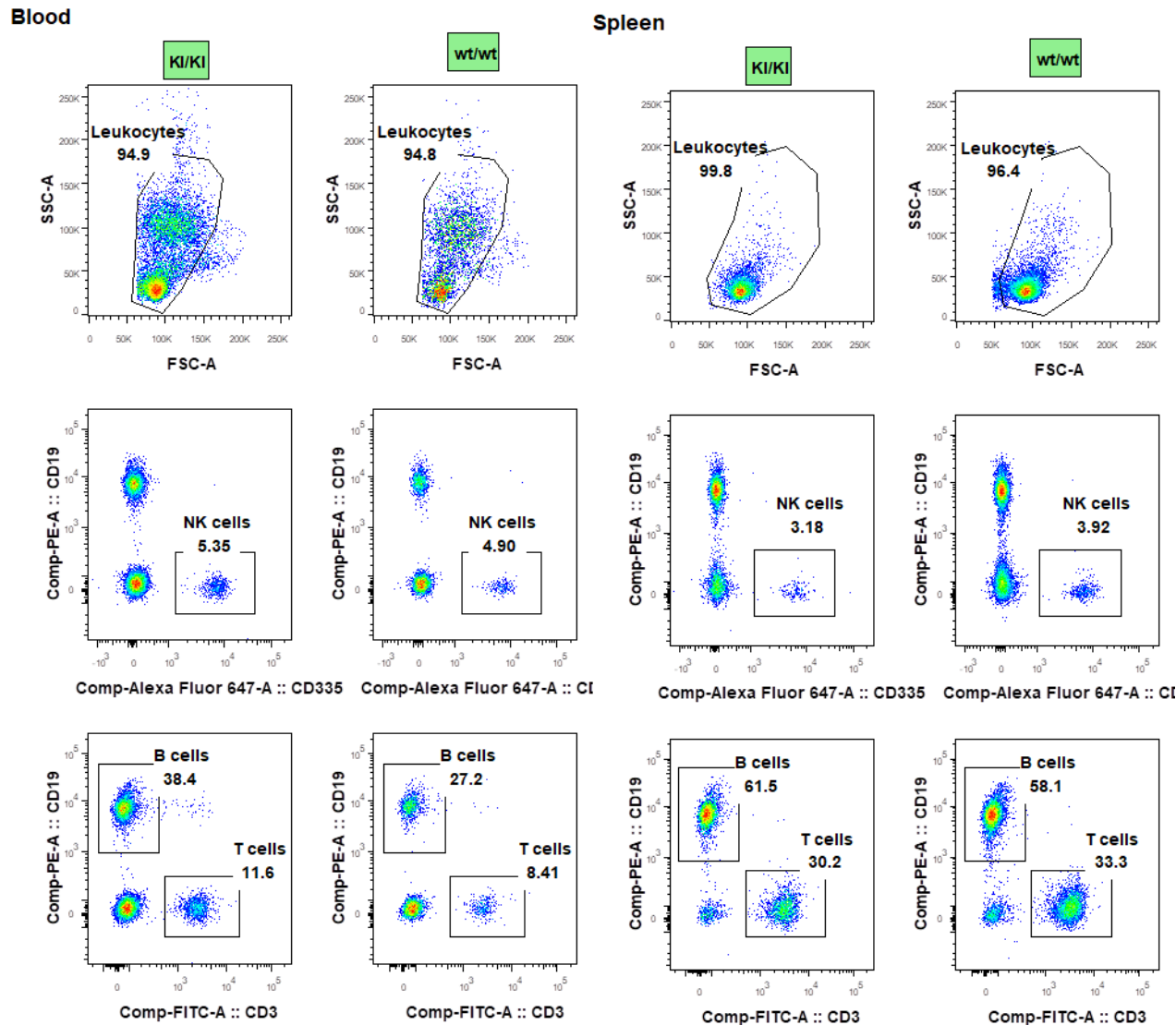
- MC38 model was established in human gene KI mice. Mice were treated with Isotype or anti-CTLA-4. Tumors for TIL analysis were collected on Day 5 post treatment. **Increased CD8+ T and reduced Treg infiltration** were observed in the early stage.

hCTLA-4 KI mouse model in Balb/c mice

In vitro validation

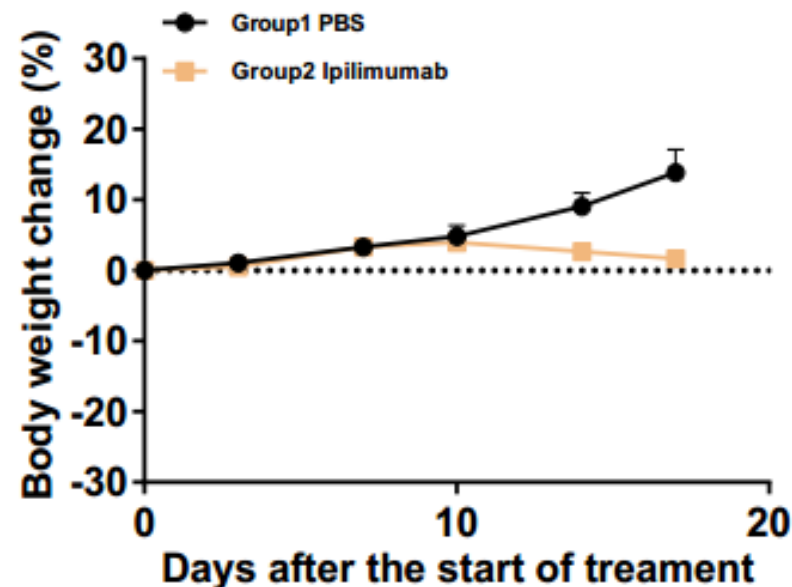
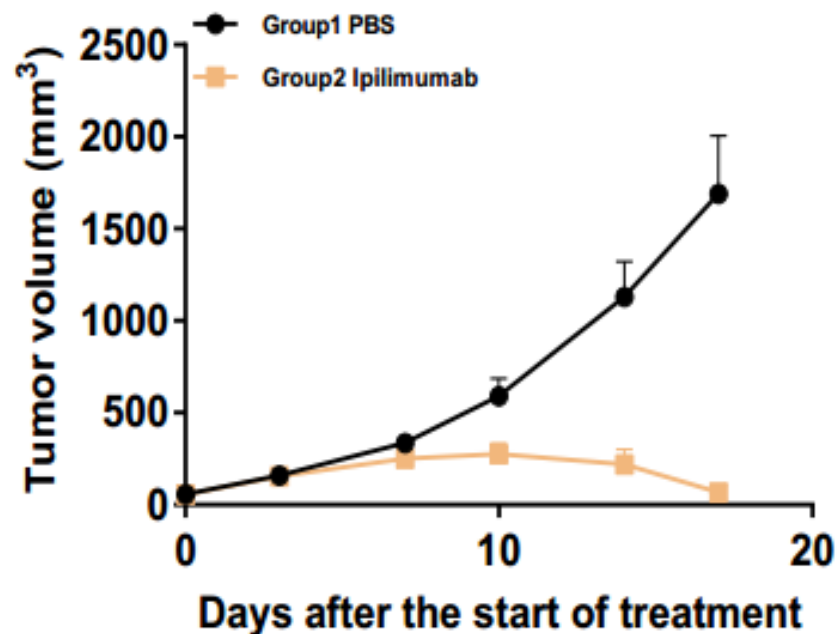
T/B/NK check

No obvious difference
observed between CTLA4
humanized and wildtype
mice.



hCTLA4 KI mouse model in Balb/c mice

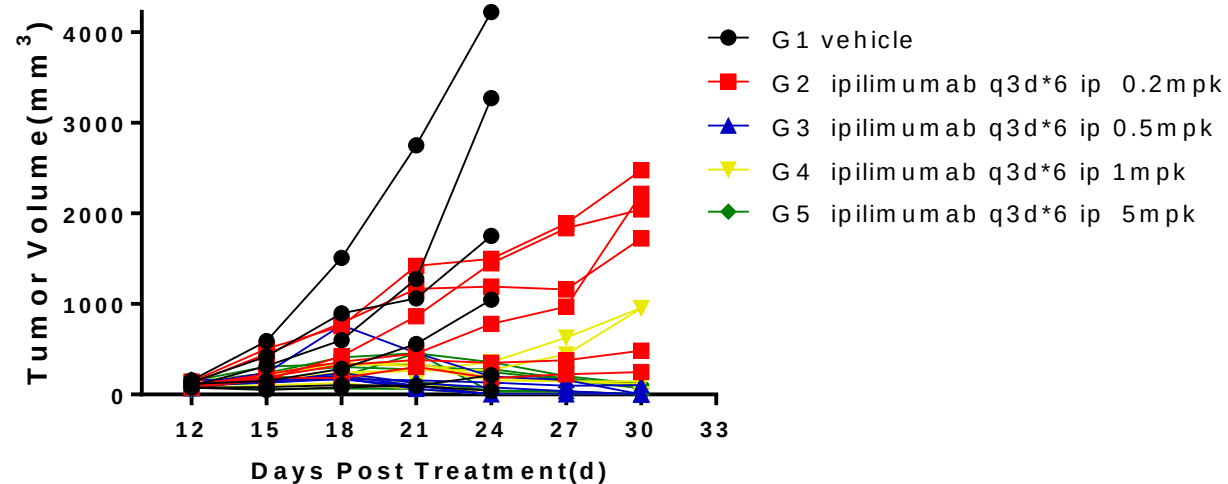
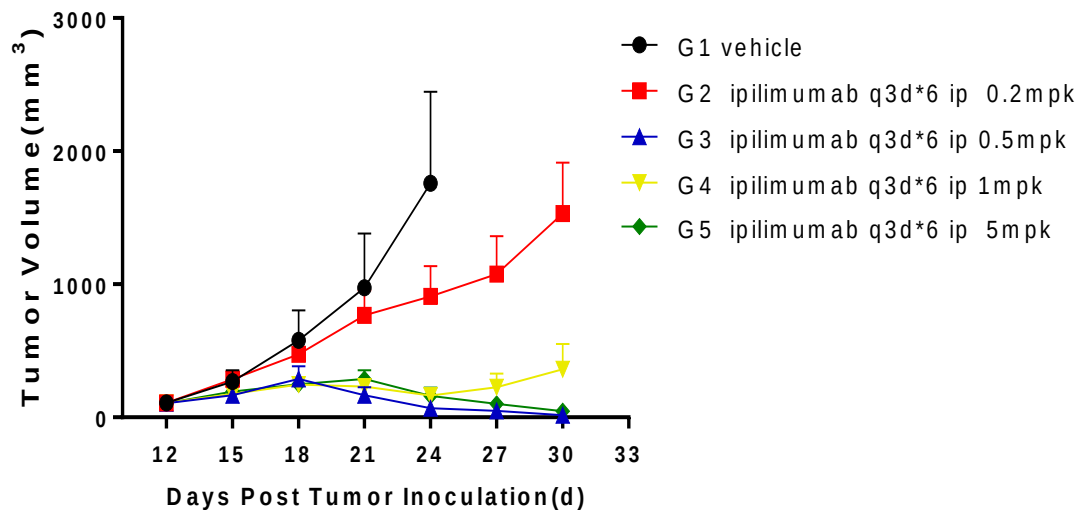
Validation of CT26 model in hCTLA4 KI mice—Ipilimumab



N=7, Ipilimumab, 10mpk, i.p., 2wks

hCTLA4 KI mouse model in Balb/c mice

Validation of CT26 model in hCTLA4 KI mice—Ipilimumab



hOX-40 KI mouse model in C57BL/6 mice

Background



Name: hOX40 KI mice

Strain type: Cas9-KI

Hair color: Black

Background: C57BL/6

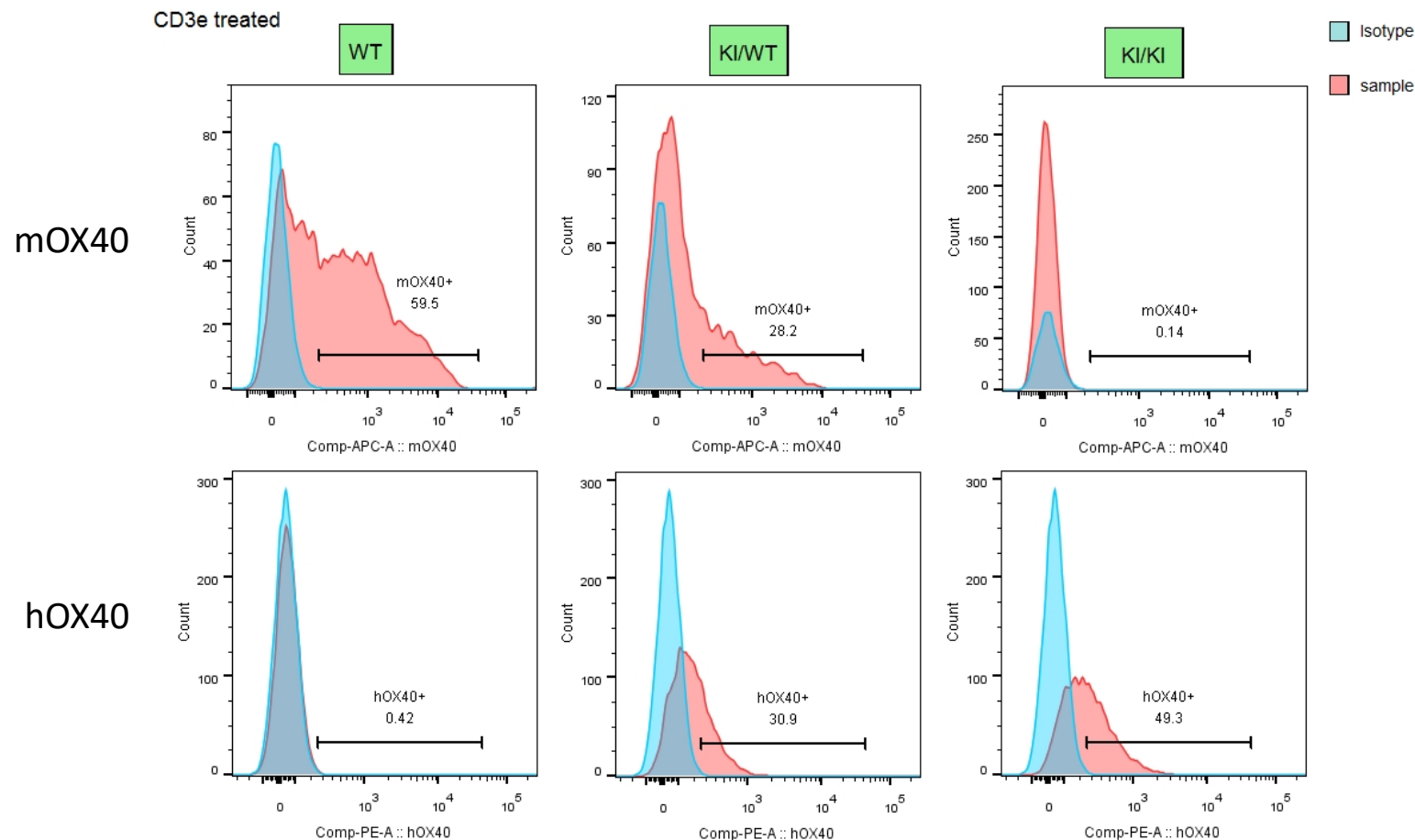
hOX40 KI mice express human OX40 in T cells
Validated with MOXR0916 analog on MC38 syngeneic model

hOX-40 KI mouse model in C57BL/6 mice

In vitro validation

hOX40 Expressing
validation-FACS

hOX40 is expressed at
comparable level in homo as
mOX40 expressing in wildtype
mice after treated with anti-Cd3e
antibody.

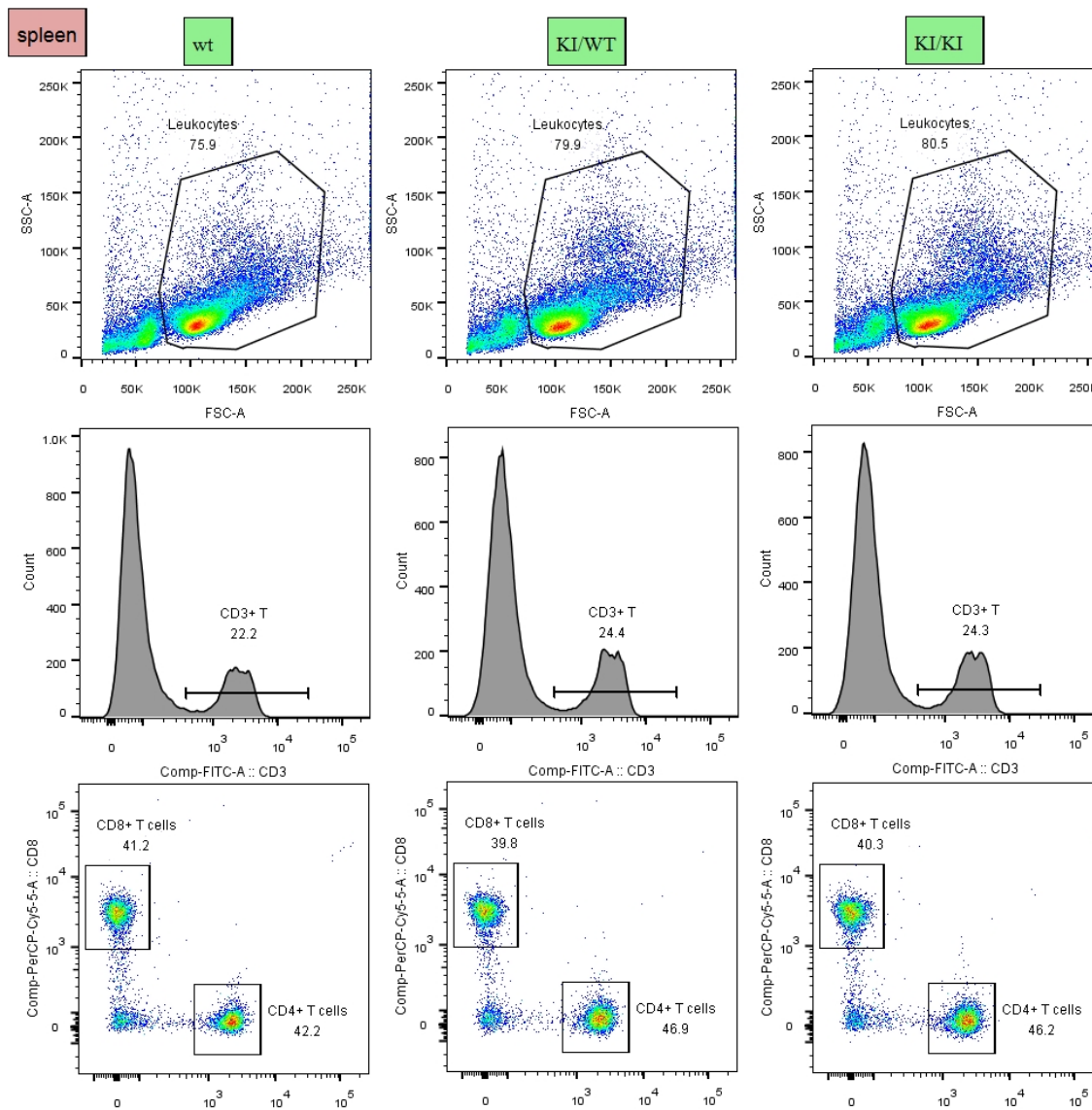


hOX-40 KI mouse model in C57BL/6 mice

In vitro validation

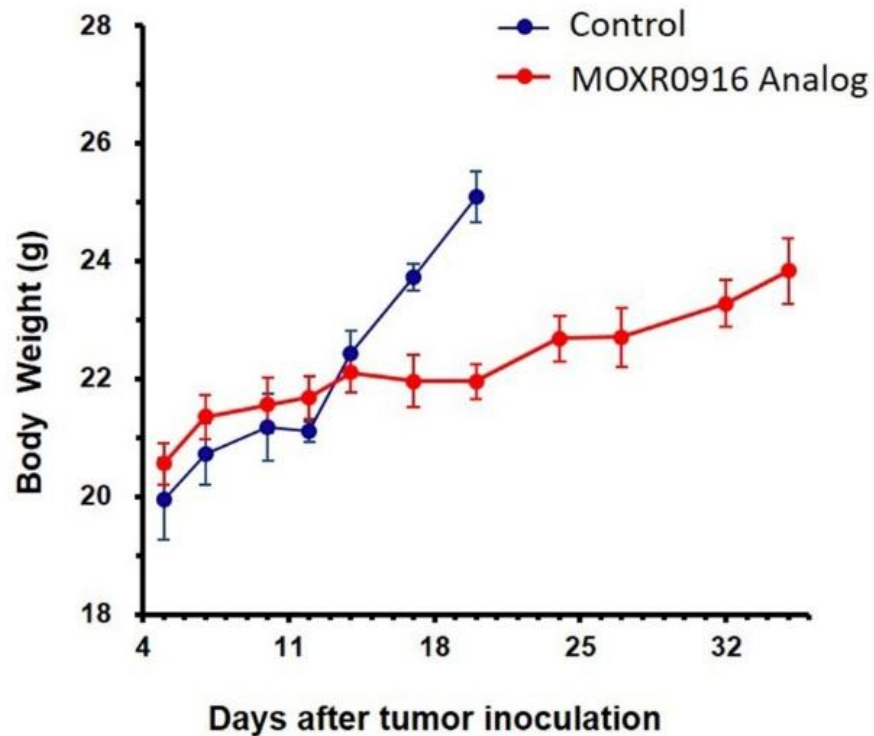
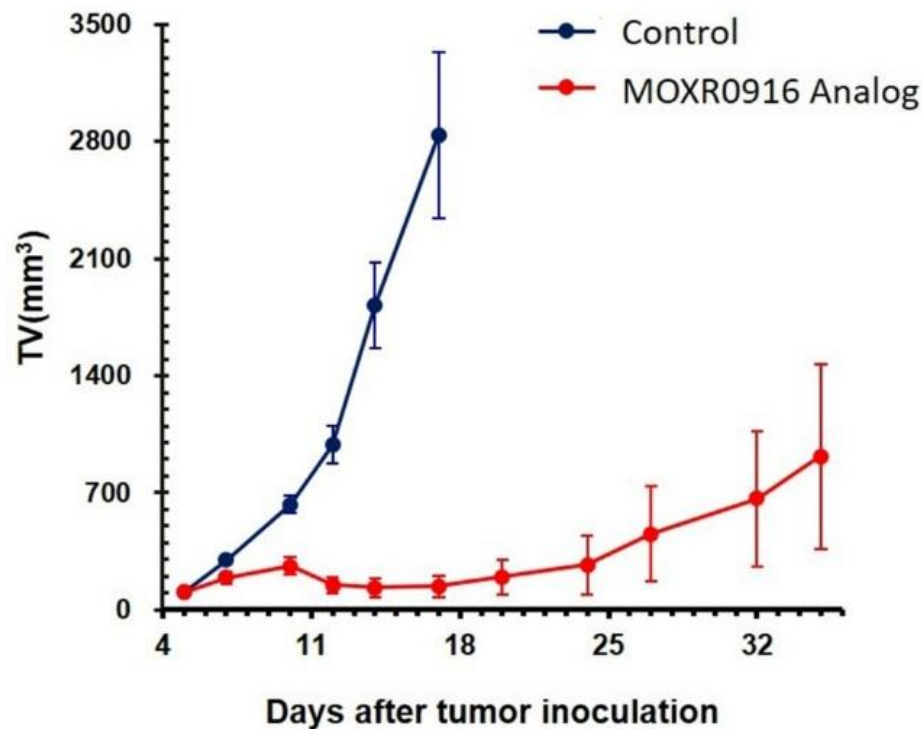
T/B background check-FACS

No obvious difference observed
between OX40 humanized and
wildtype mice.



hOX-40 KI mouse model in C57BL/6 mice

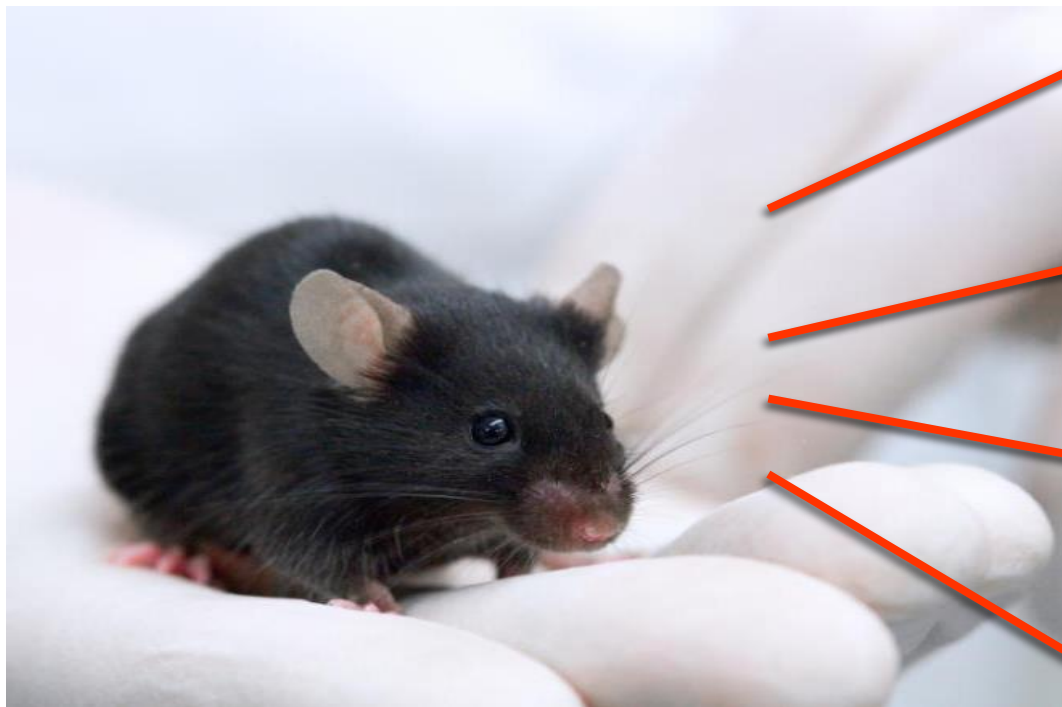
Validation of MC38 model in hOX40 KI mice



Significant tumor growth inhibition observed in hOX40 homo mice treated with MOXR0916 analog.

hTIM-3 KI mouse model in C57BL/6 mice

Background



Name: hTIM3 KI mice

Strain type: Homologous recombination-promoted KI

Hair color: Black

Background: C57BL/6N

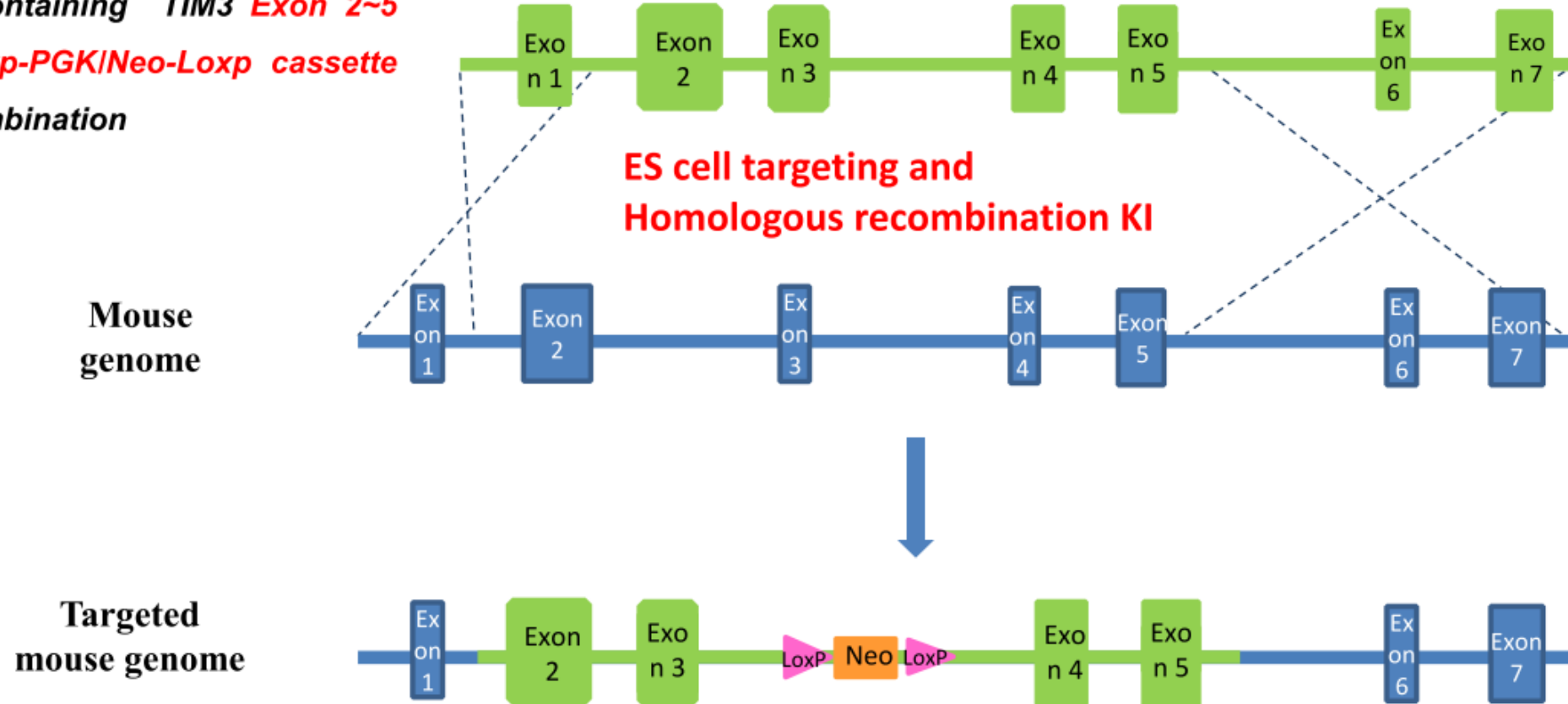
TIM3 KI mice express human TIM3 in PBMC cells

Validated with hTIM-3 and mPD-1 antibody combination on MC38 syngeneic model

hTIM-3 KI mouse model in C57BL/6 mice

Strategy

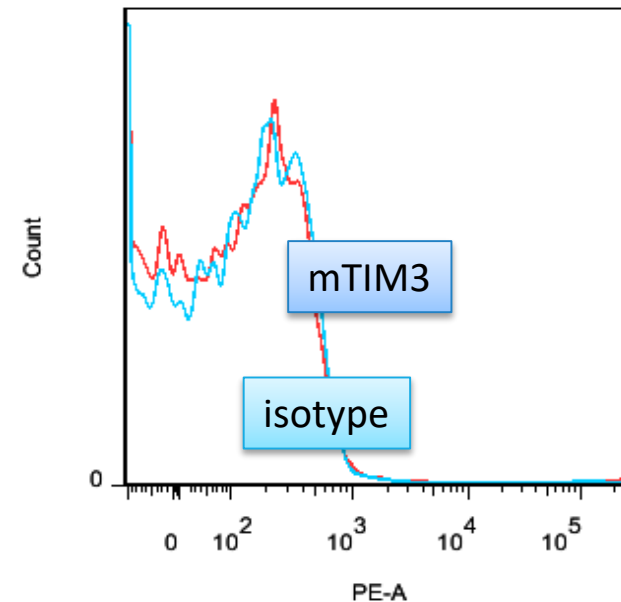
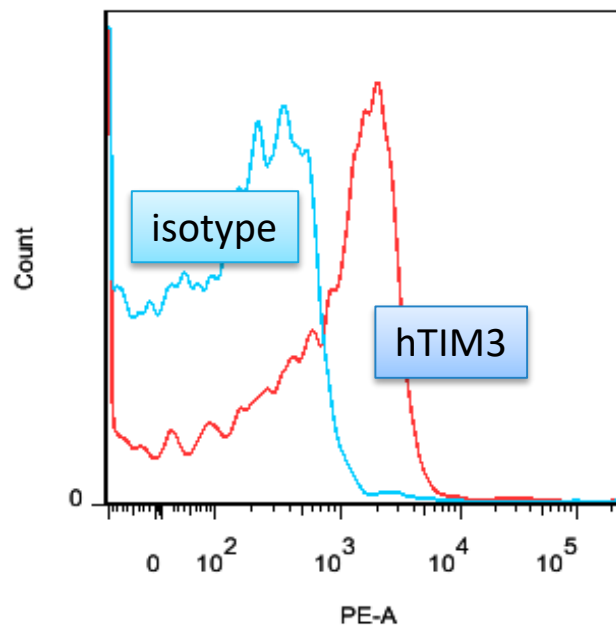
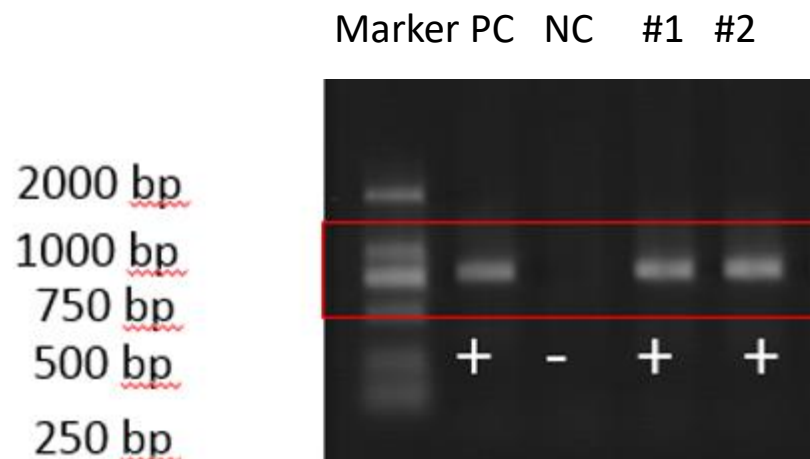
Donor containing **TIM3 Exon 2~5**
plus **Loxp-PGK/Neo-Loxp** cassette
for recombination



hTIM-3 KI mouse model in C57BL/6 mice

Genotyping and *in vitro* validation

Genotyping: PCR to detect hTIM-3



TIM3 KI mice only express human TIM3 in PBMC cells

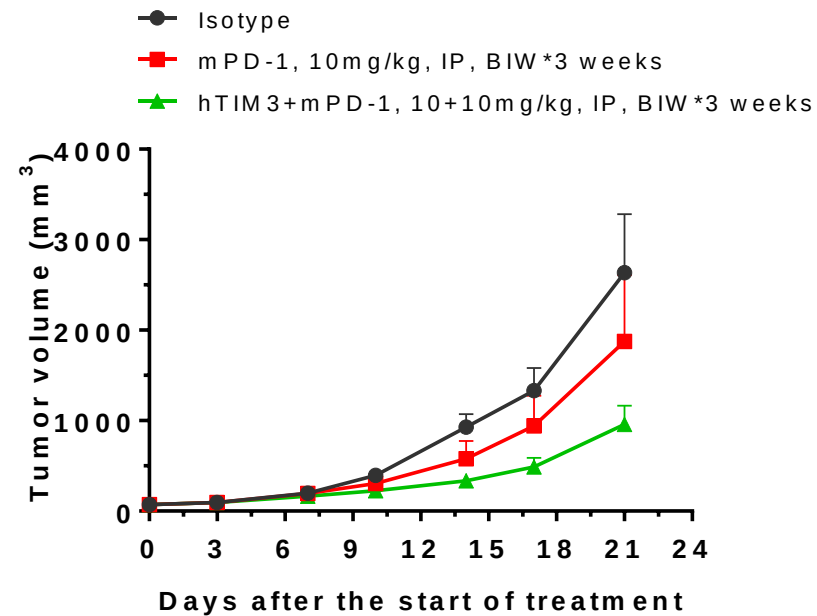
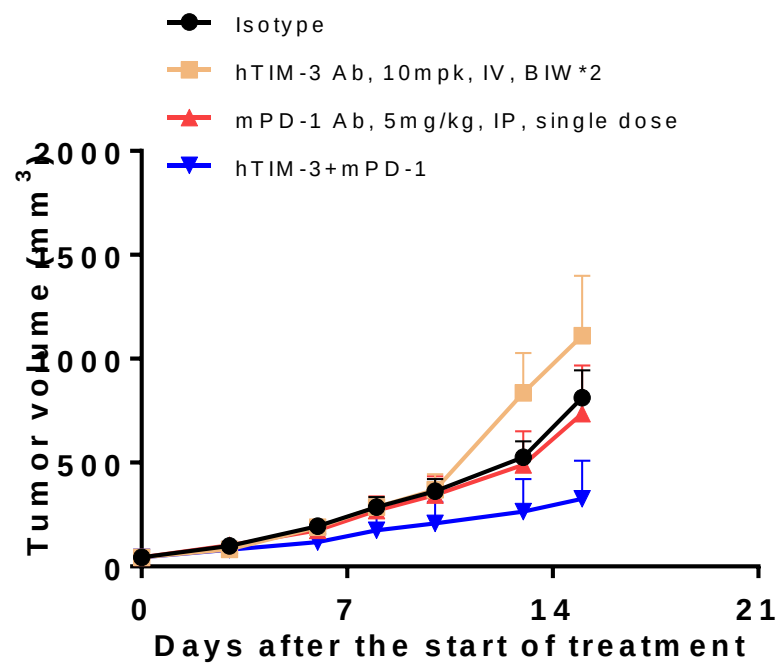
hTIM-3 KI mouse model in C57BL/6 mice

In vivo validation

Host: hTIM-3 KI B6

Tumor cell: murine colon cell MC38

Test Abs: hTIM3 and mPD-1



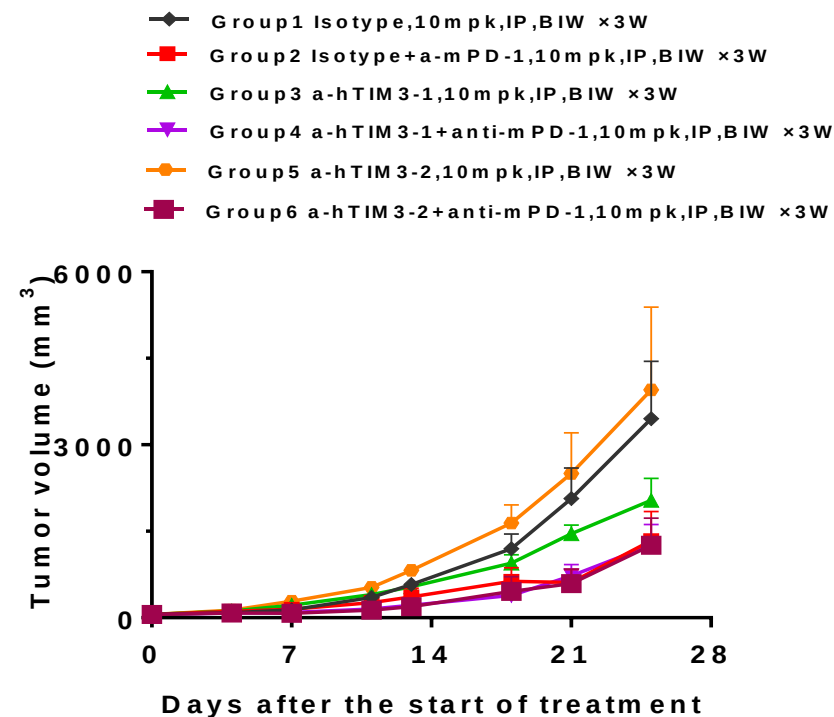
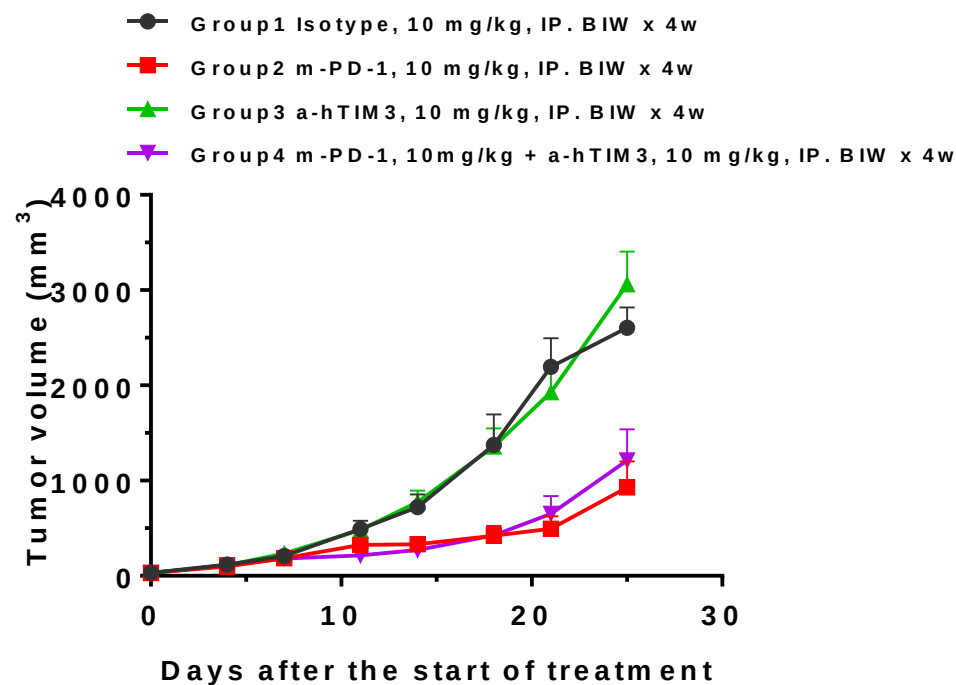
hTIM-3 KI mouse model in C57BL/6 mice

Validation of MC38 model in hTIM-3 KI mice

Host: hTIM-3 KI B6

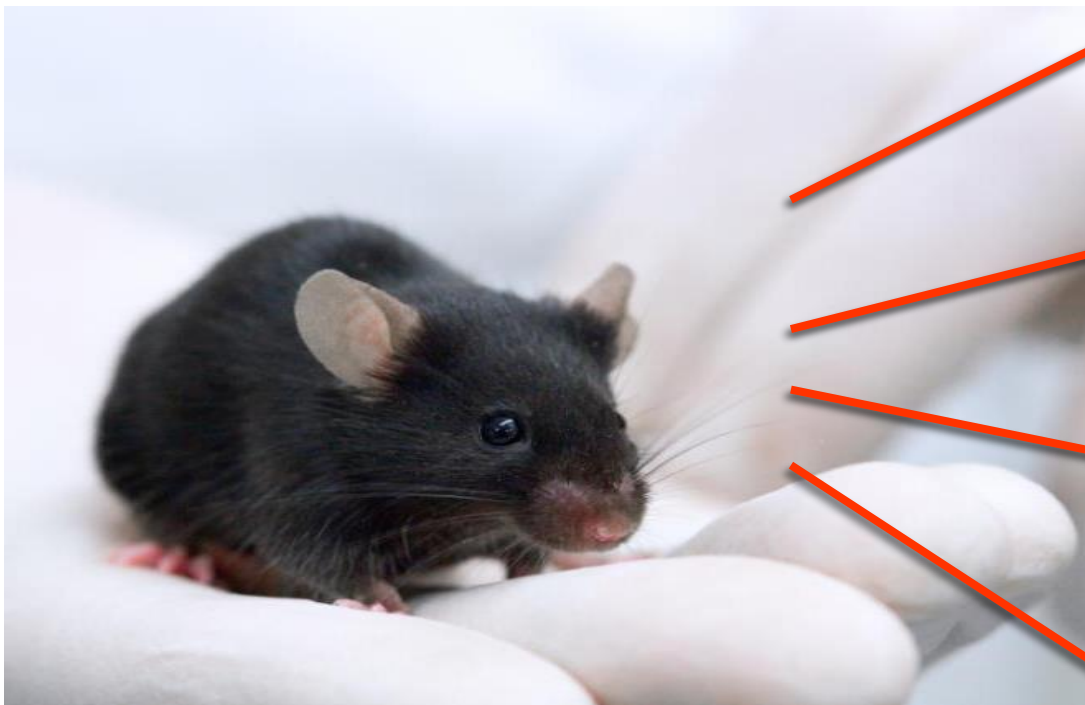
Tumor cell: murine colon cell MC38

Test Abs: hTIM3 and mPD-1



hGITR KI mouse model in C57BL/6 mice

Background



Name: hGITR KI mice

Strain type: Cas9-KI

Hair color: Black

Background: C57BL/6J

hGITR KI mice express human hGITR in PBMC cells
Validated with hGITR antibody on MC38 syngeneic model

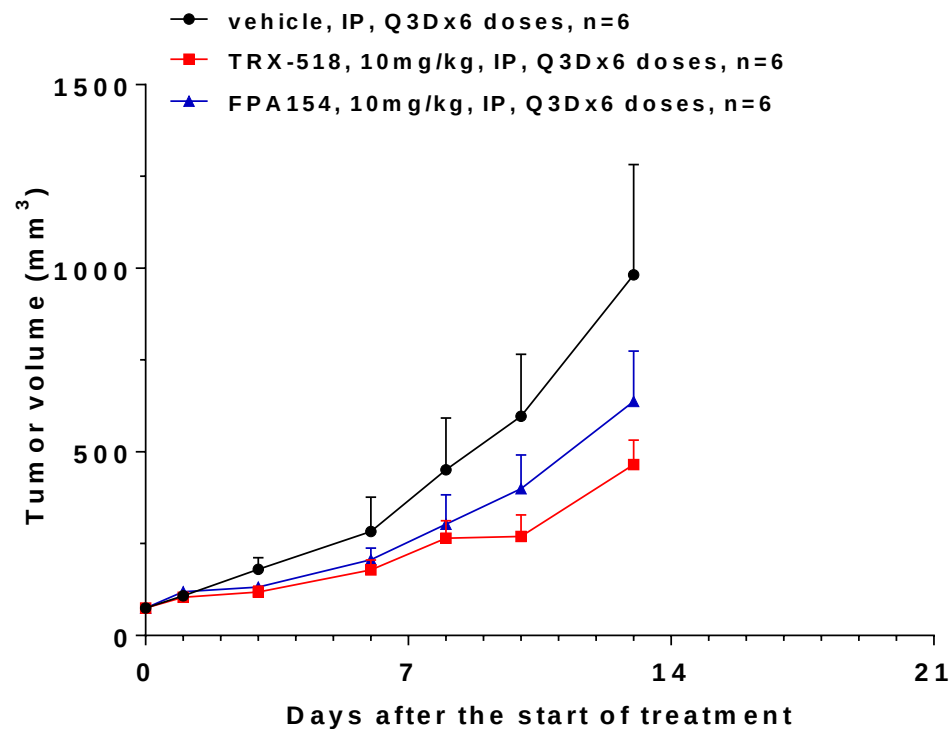
hGITR KI mouse model in C57BL/6 mice

Validation of MC38 model in hGITR KI mice

Host: hGITR KI B6

Tumor cell: murine colon cell MC38

Test Abs: TRX-518 and FPA-154



hPD-1/hPD-L1 KI mouse model in C57BL/6 mice

Background



Name: hPD-1/hPD-L1 KI mice

Strain type: Cas9-KI

Hair color: Black

Background: C57BL/6J

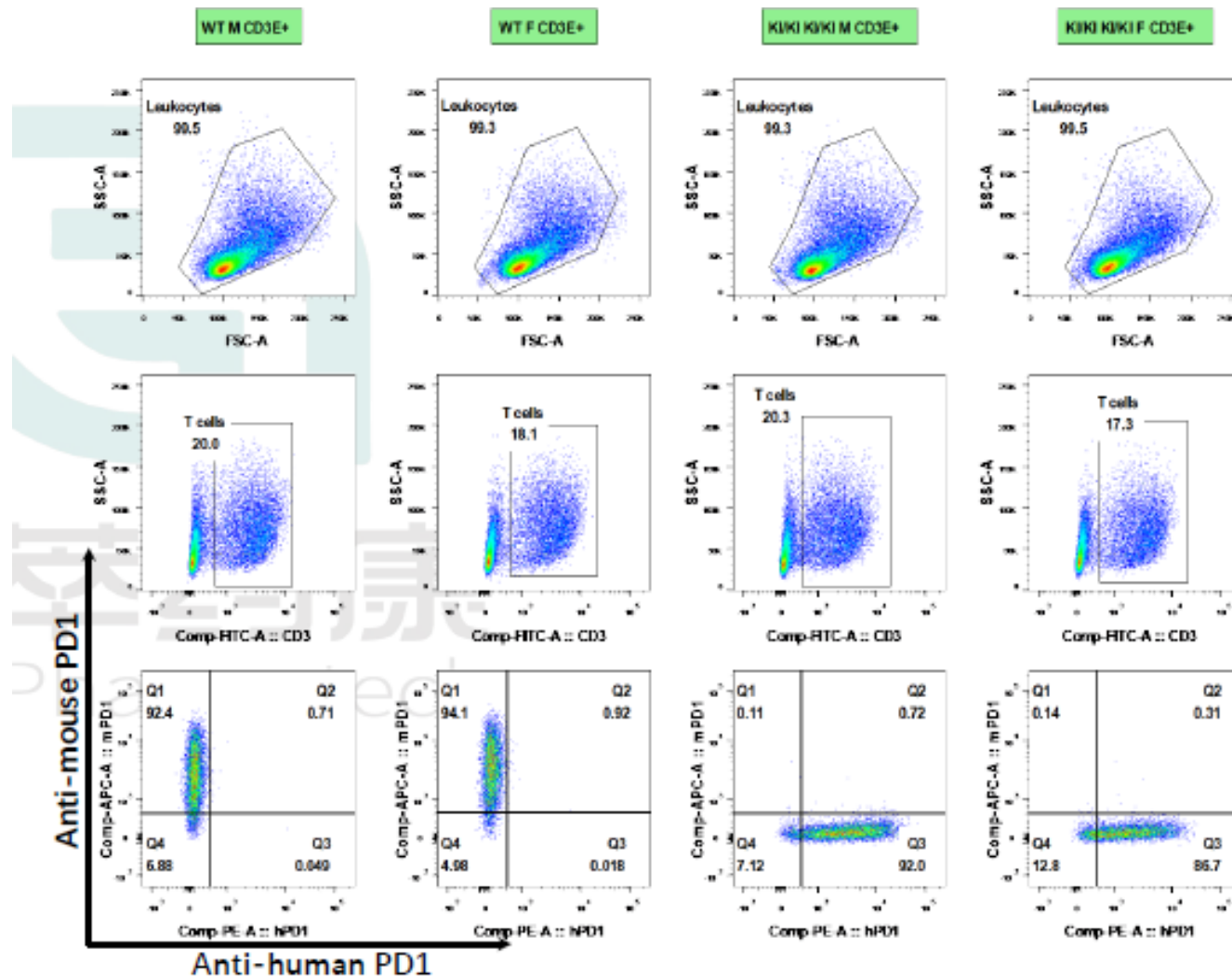
Validated with hPD-1 and hPD-L1 antibodies on huPD-L1-MC38 syngeneic model

hPD-1/hPD-L1 KI mouse model in C57BL/6 mice

FCM to validate hPD-1/hPD-L1 expression in KI mice

hPD-1 expressing validation

hPD1 is expressed at comparable level in homo as mPD1 expressing the wildtype mice

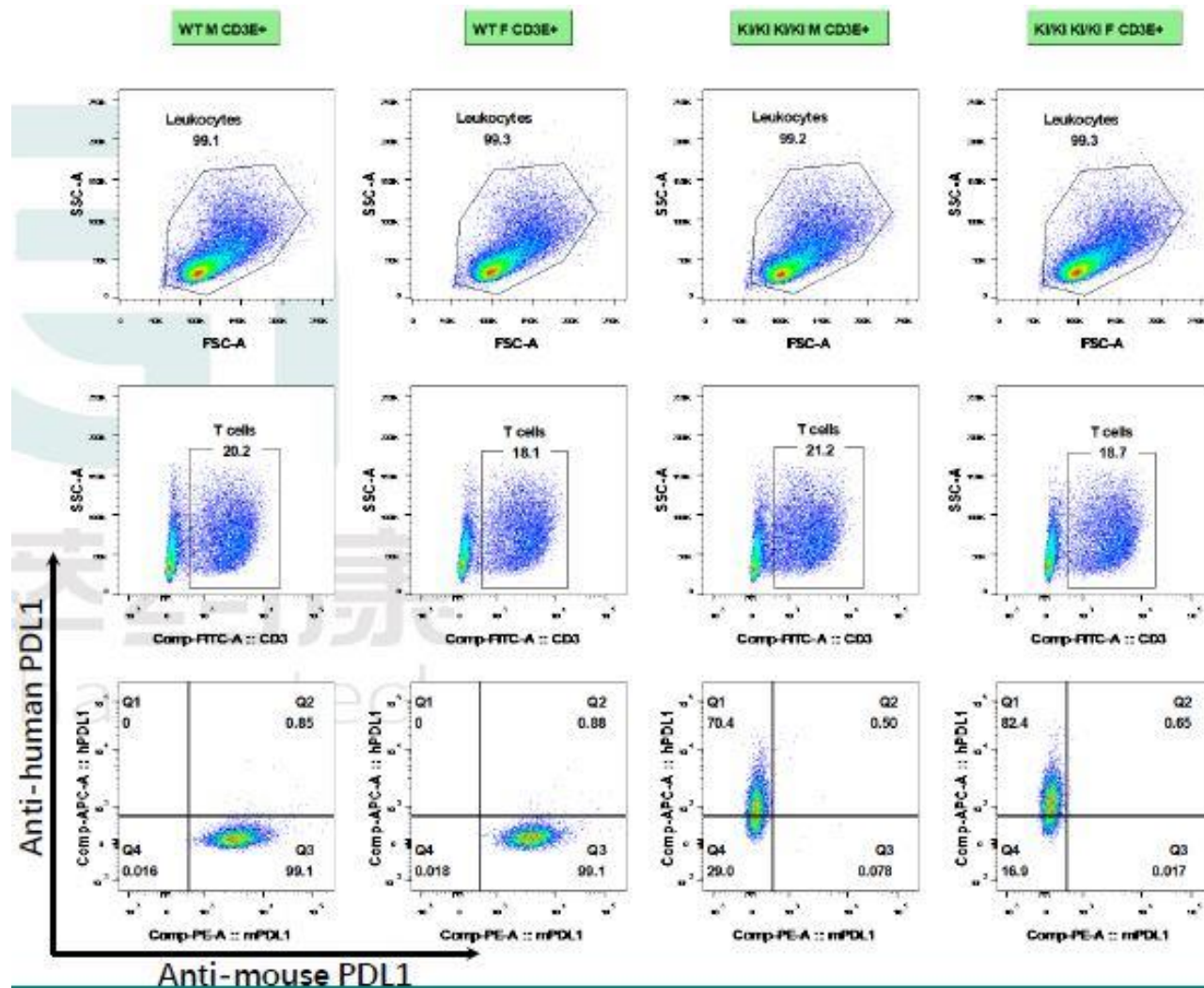


hPD-1/hPD-L1 KI mouse model in C57BL/6 mice

FCM to validate hPD-1/hPD-L1 expression in KI mice

hPD-L1 expressing validation

hPD-L1 is expressed at comparable level in homo as mPD1 expressing the wildtype mice

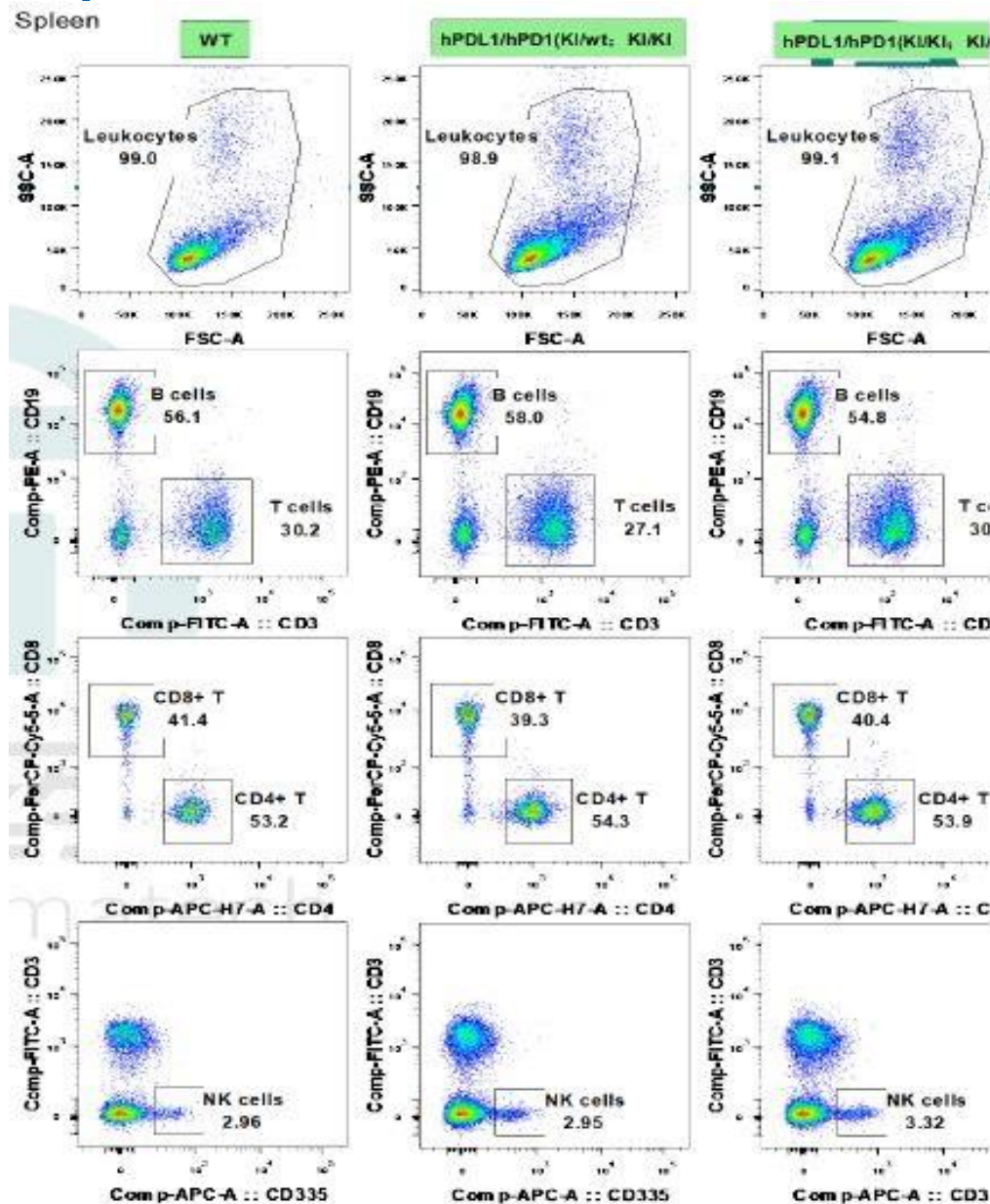


hPD-1/hPD-L1 KI mouse model in C57BL/6 mice

FCM to validate T/B/NK cell expression in KI mice

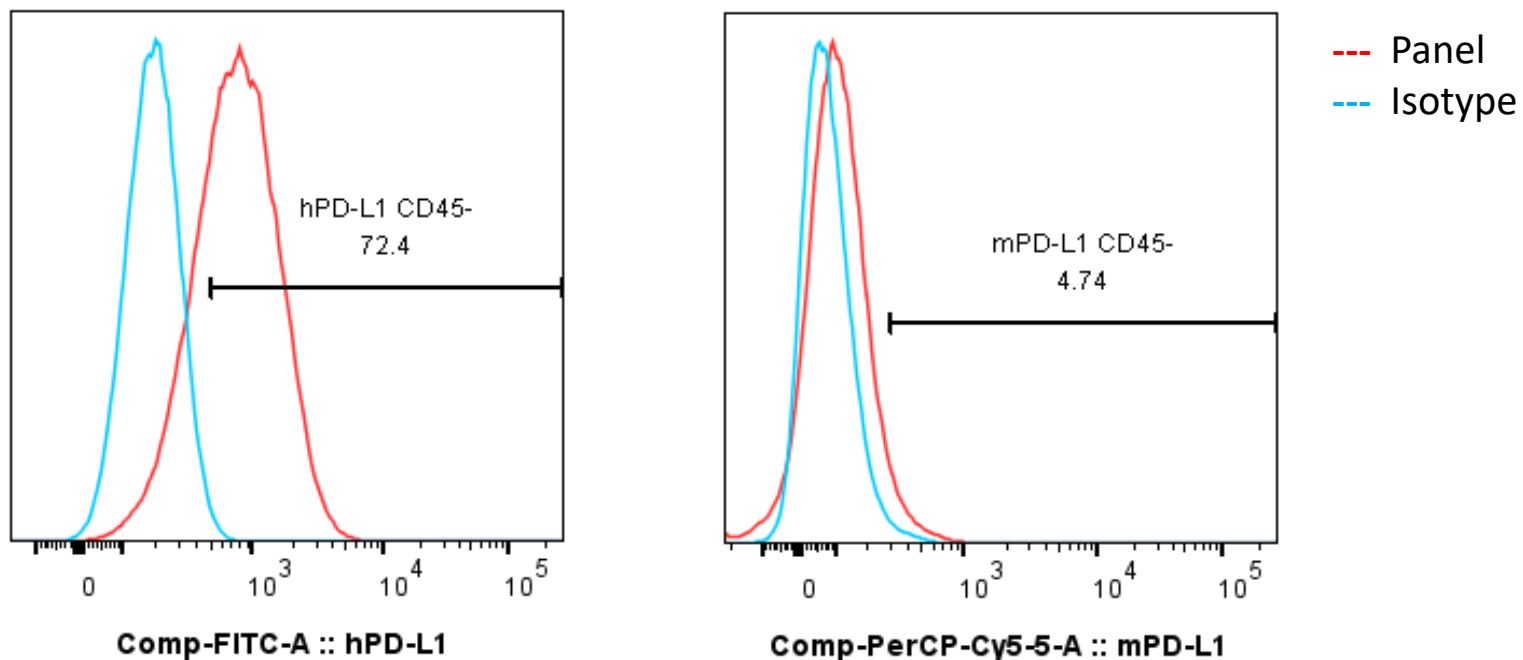
T/B/NK cell population check

No significant difference
observed between hPD-1/hPD-
L1 KI mice and wildtype
C57BL/6 mice



hPD-L1 KI MC38 cell line

FCM to detect hPD-L1 expression in cell line and tumor tissue



hPD-L1 KI MC38 cell extract from mouse tumor express hPD-L1

hPD-1/hPD-L1 KI mouse model in C57BL/6 mice

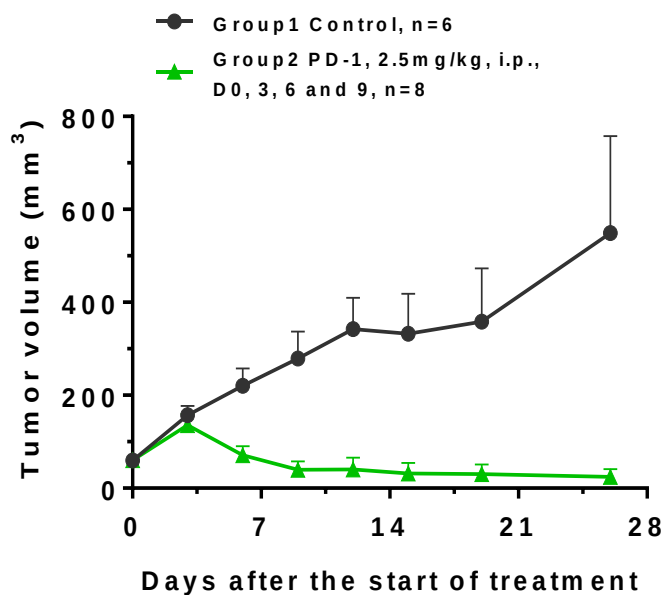
In vivo validation with hPD-L1 MC38 model

Host: hPD-1/hPD-L1 KI B6

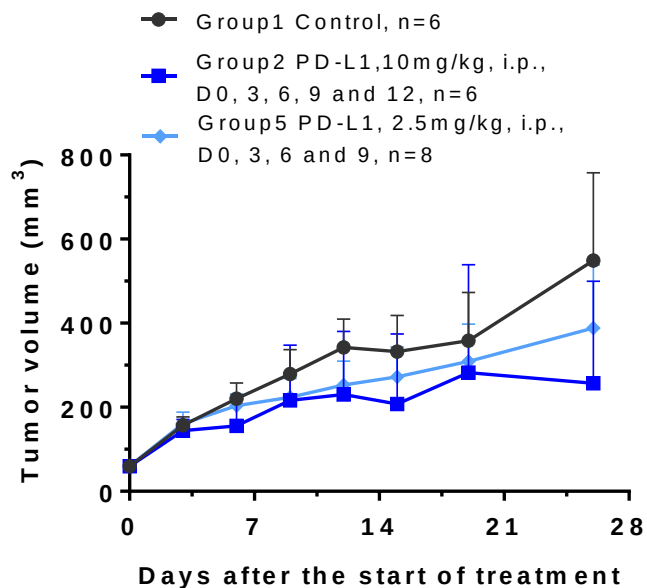
Tumor cell: hPD-L1 MC38 cell

Test Abs: a-hPD-1 and a-hPD-L1

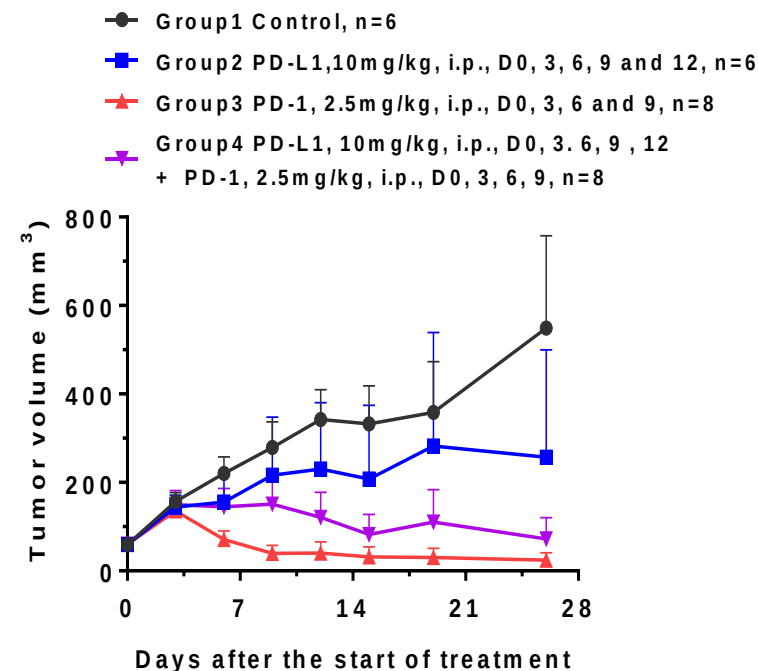
PD-1 treatment



PD-L1 treatment (high and low dose)



PD-1 and PD-L1 combination



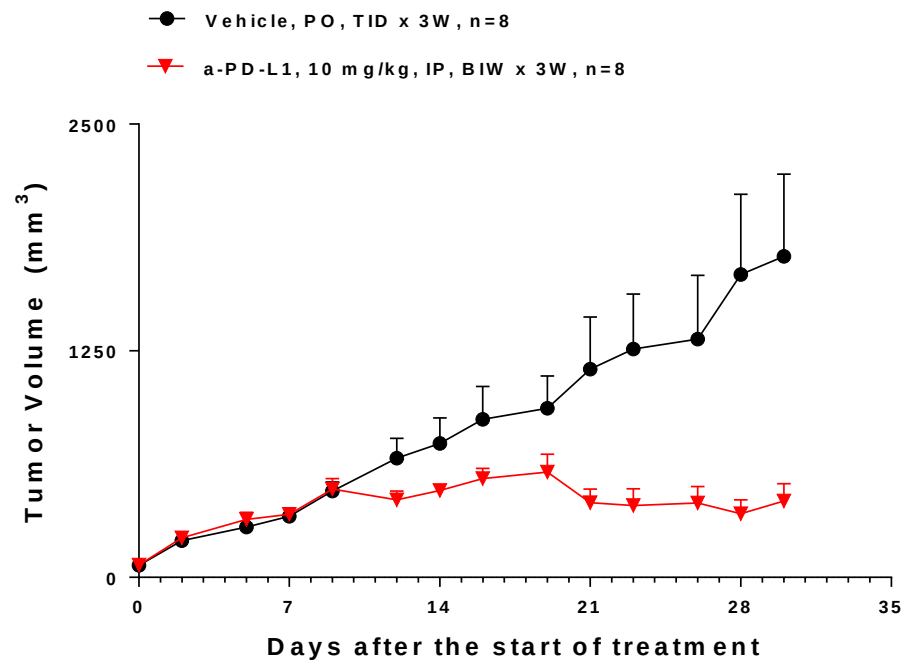
hPD-1/hPD-L1 KI mouse model in C57BL/6 mice

In vivo validation with hPD-L1 MC38 model

Host: hPD-1/hPD-L1 KI B6

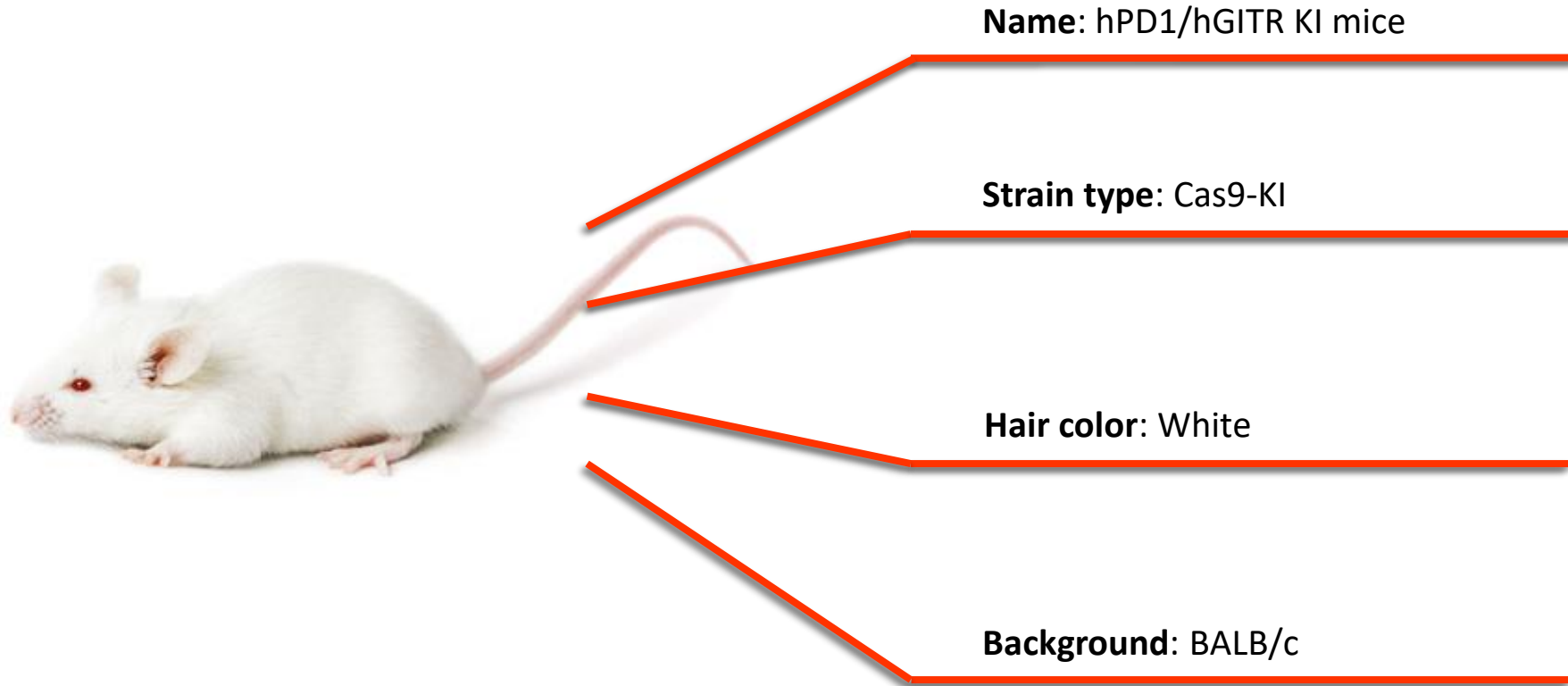
Tumor cell: hPD-L1 MC38 cell

Test Abs: a-PD-L1



hPD-1/hGITR KI mouse model in Balb/c mice

Background



hGITR KI mice express human hGITR in PBMC cells

Validated with hGITR and hPD-1-cross mouse antibody on Colon26 syngeneic model

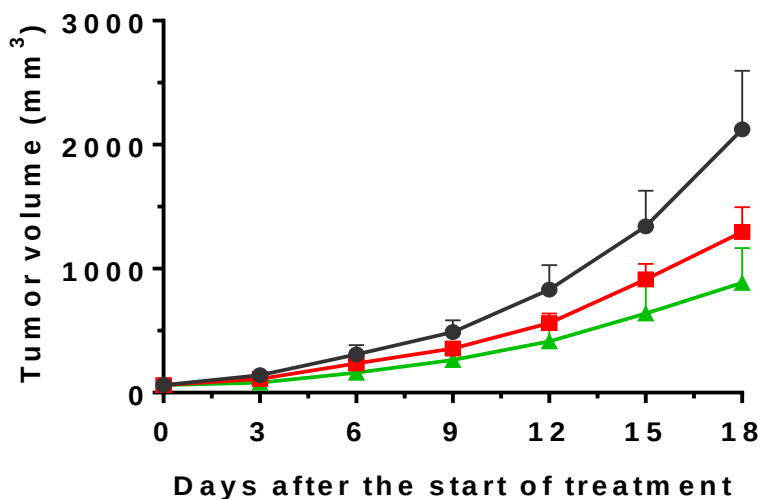
Efficacy study in hPD1/hGITR KI mouse model

Validation of Colon26 model in hPD1/hGITR KI mouse model in Balb/c mice

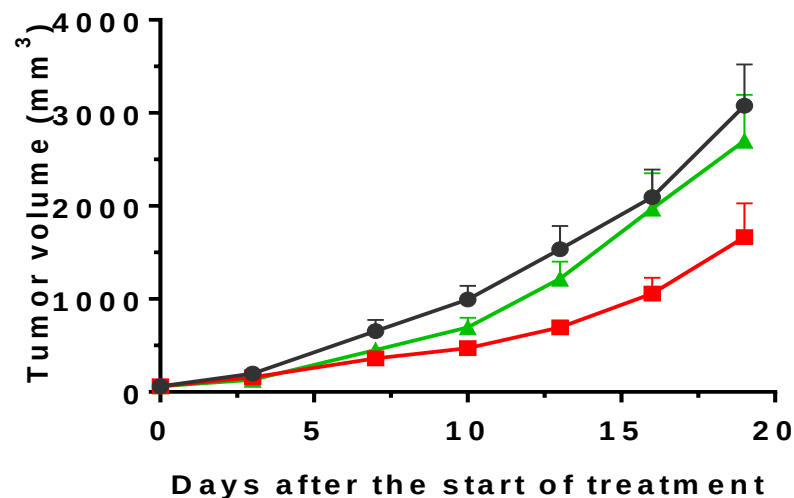
Host: hPD1/hGITR KI Balb/c
Tumor cell: murine colon 26
Test Abs: anti-hPD 1, anti-hGITR for human

Host: WT Balb/c
Tumor cell: murine colon 26
Test Abs: anti-hGITR for human

- Group1 PBS IP. Q3D x 6Doses
- Group2 anti-PD-1-cross-mouse 10 mg/kg IP. Q3D x 6 Doses
- ▲ Group3 anti-GITR for human 10 mg/kg IP. Q3D x 6 Doses



- Group1 PBS IP. Q3D x 6Doses
- Group2 anti-PD-1-cross-mouse 10 mg/kg IP. Q3D x 6 Doses
- ▲ Group3 anti-GITR for human 10 mg/kg IP. Q3D x 6 Doses



hPD-1/hCTLA4 KI mouse model in C57BL/6 mice

Background



Name: hPD-1/hCTLA4 KI mice

Strain type: Cas9-KI

Hair color: Black

Background: C57BL/6J

Validated with hPD-1 and hCTLA4 antibodies on MC38 syngeneic model

hPD-1/hCTLA4 KI mouse model in C57BL/6 mice

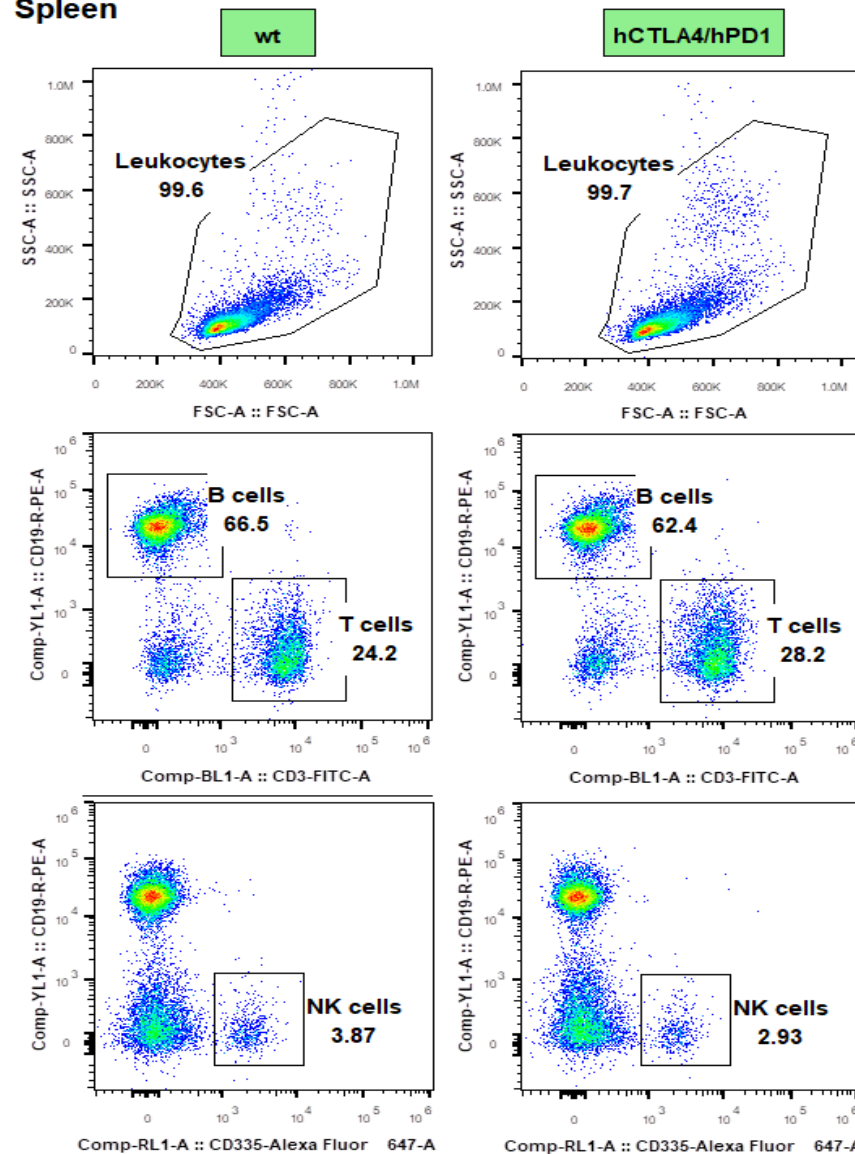
In vitro validation

T/B/NK check

No obvious difference was observed between hPD1/hCTLA4 humanized and wildtype mice.

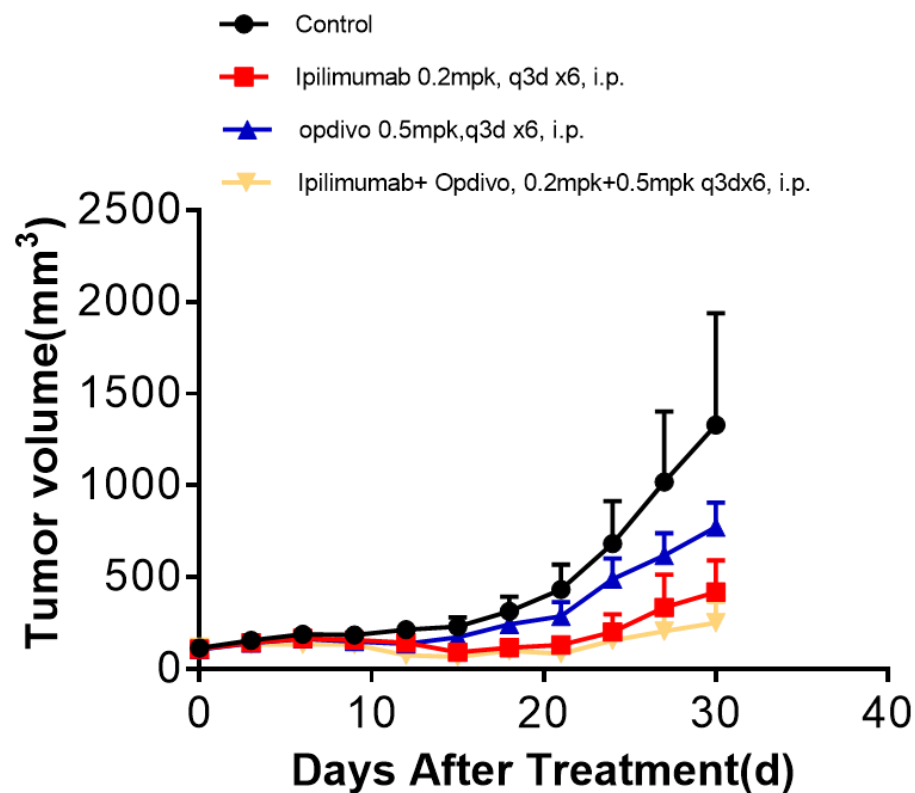
GemPharmatech Data

Spleen



hPD-1/hCTLA4 KI mouse model in C57BL/6 mice

Validation of MC38 model in hPD-1/hCTLA KI mice—Ipillimumab and Opdivo



Yervoy (TGI=68.4%)

Opdivo (TGI=41.9%)

Yervoy+ Opdivo (TGI=81.0%)



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