José L Pérez Gracia
Bruno Sangro
Salvador Martin -Algarra
JM Lopez Picazo
Alfonso Gúrpide
Javier Rodriguez
Jesús San Miguel
Jesús Prieto
Alberto Benito
Ivan Peñuelas
Alvaro Gonzalez
Mercedes Iñarrairaegui

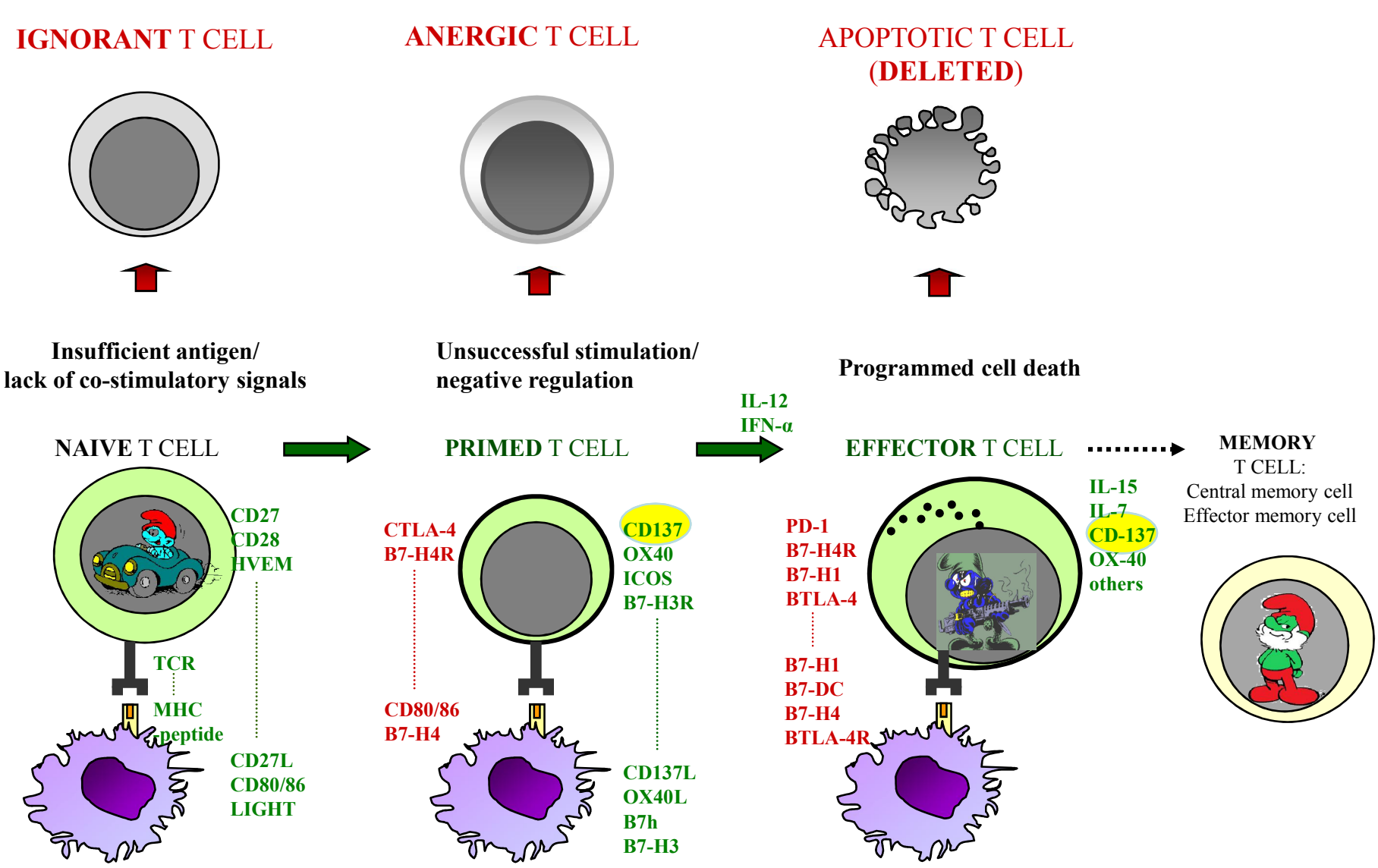
Miguel F Sanmamed
María Rodriguez
Carlos Alfaro
Carmen Oñate
Inmaculada Rodriguez

Aizea Morales
Arantza Azpilikueta
Elixabet Bolaños
Inmaculada Rodriguez
Sara Labiano
Eneko Elizalde
Alfonso Rodriguez
Angela Aznar
Maria Rodriguez-Ruiz
Pedro Berraondo
Luna Carneiro

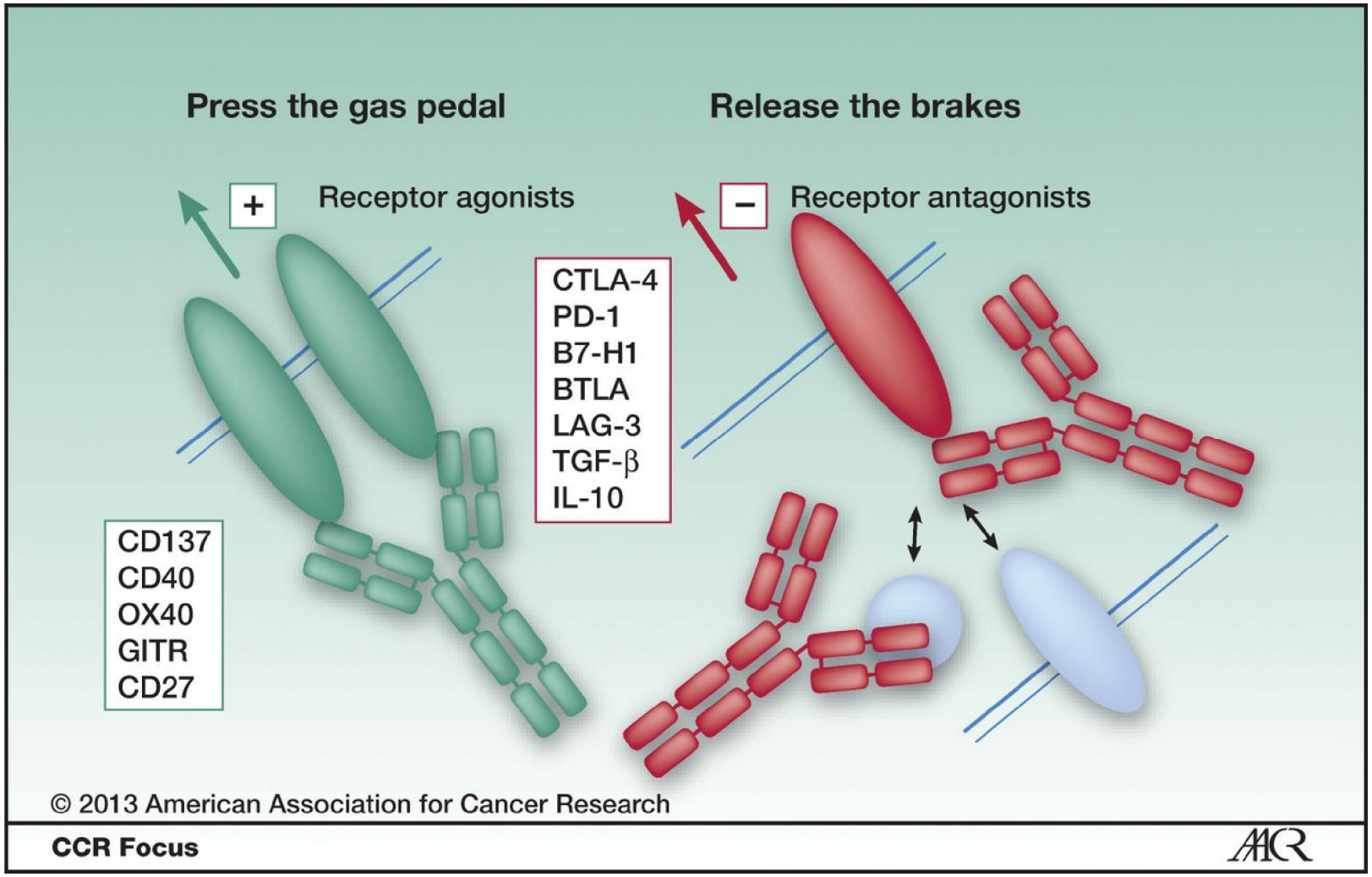
Bristol Myers Squibb
Roche/Genentech
Pfizer
AstraZeneca
Boehringer Ingelheim

Ines Guetgeman
Juan M Zapata
Manuel Rodriguez
Valerie Lang

Bettina Weigelin
Peter Friedl


IGNORANT T CELL
ANERGIC T CELL
APOPTOTIC T CELL
(DELETED)
Insufficient antigen/
lack of co-stimulatory signals
Unsuccessful stimulation/
negative regulation
Programmed cell death
IL-12
IFN-α
NAIVE T CELL
PRIMED T CELL
EFFECTOR T CELL
MEMORY
T CELL:
Central memory cell
Effector memory cell
CD27
CD28
HVEM
TCR
MHC
peptide
CD27L
CD80/86
LIGHT
CTLA-4
B7-H4R
CD80/86
B7-H4
CD137
OX40
ICOS
B7-H3R
CD137L
OX40L
B7h
B7-H3
PD-1
B7-H4R
B7-H1
BTLA-4
B7-H1
B7-DC
B7-H4
BTLA-4R
IL-15
IL-7
CD-137
OX-40
others

Schematic representation of the concept of immunostimulatory mAbs.



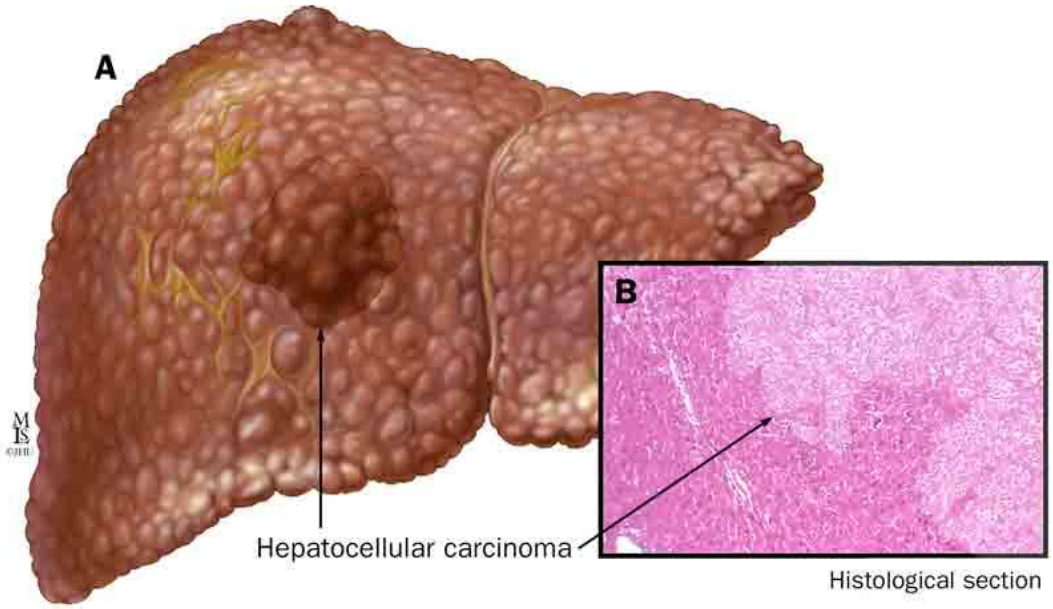
Melero I et al. Clin Cancer Res 2013;19:997-1008

©2013 by American Association for Cancer Research

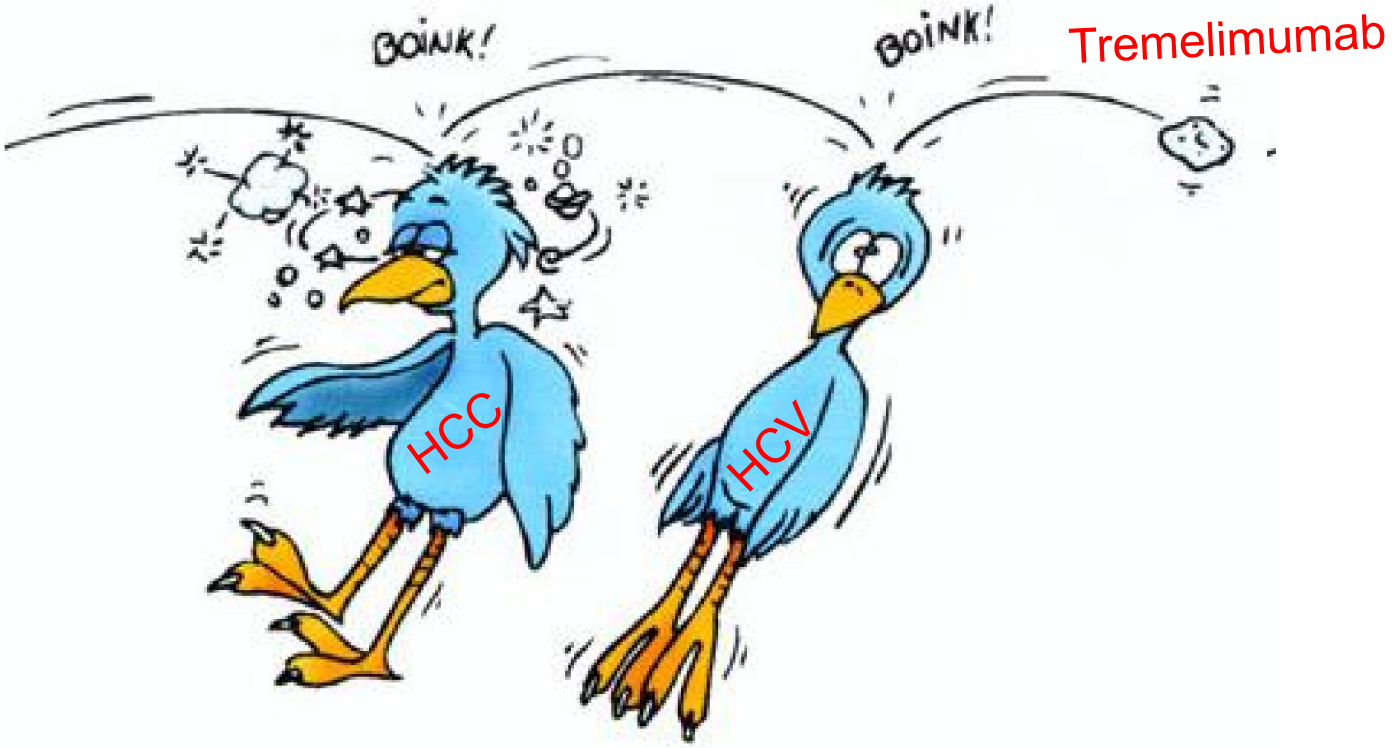
Immune Checkpoint Blockade against Malignancy

Hepatocellular carcinoma

A major target for immunotherapy with checkpoint inhibitors.



Killing two birds with one stone



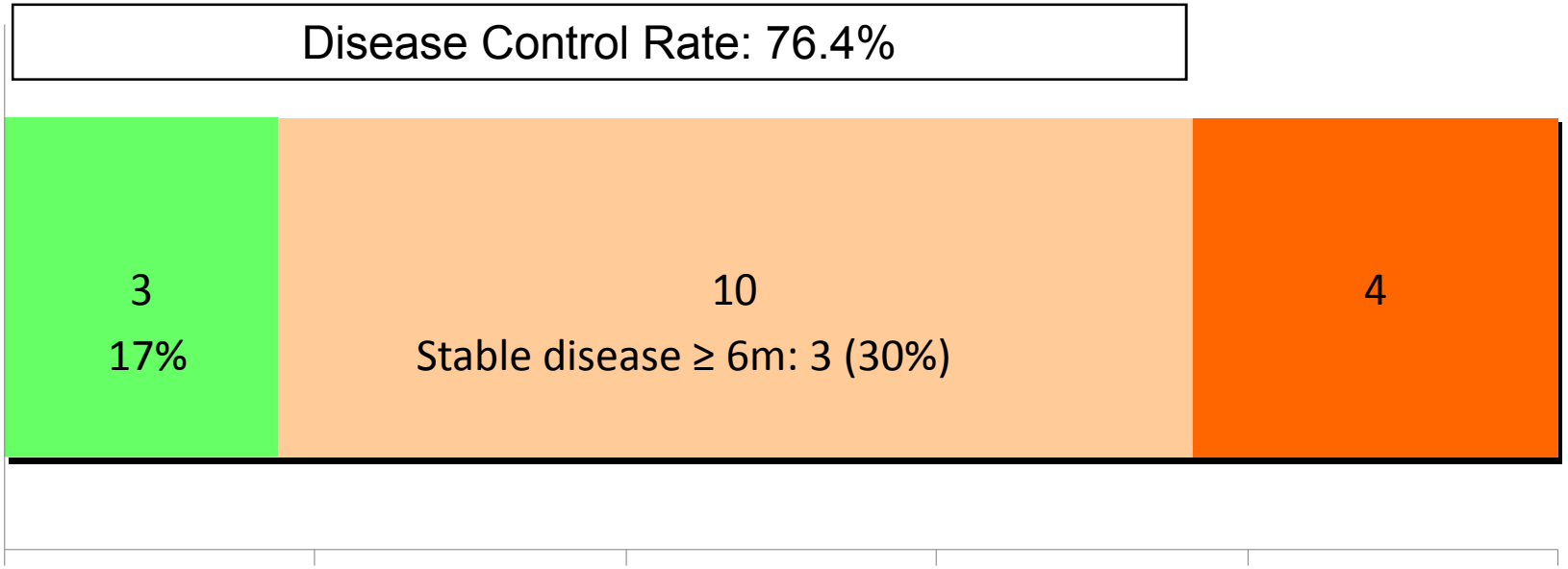
Phase II Trial of Anti-CTLA-4 Human Monoclonal Antibody CP-675,206 in Patients With Advanced HCC

Protocol code	CT-2007-01
EudraCT no.	2008-001177-15
NCT Identifier	NCT01008358
Sponsor:	University of Navarra, Pamplona, Spain

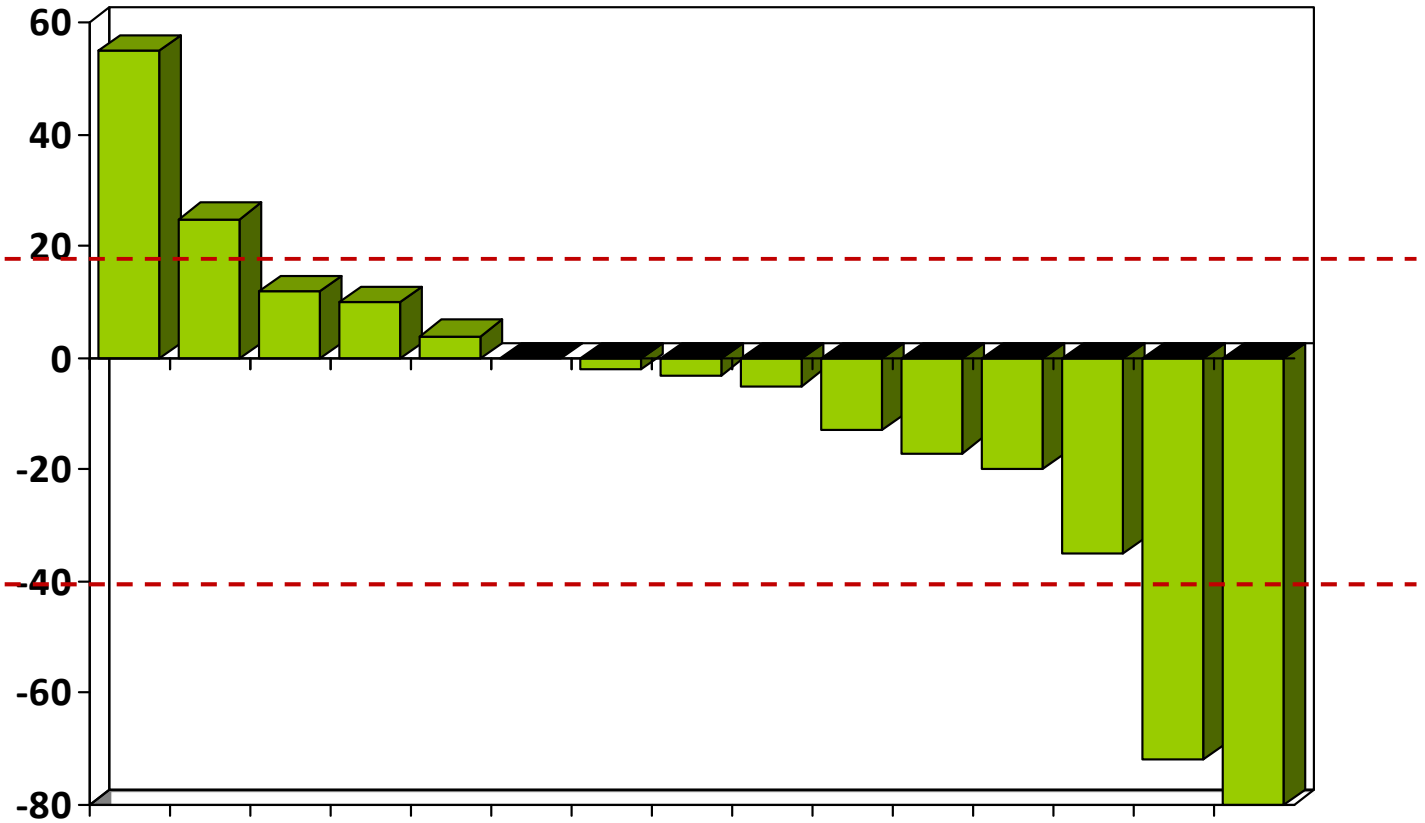
- **Tremelimumab** (CP 675,206) is a fully humanized IgG2 mAb that antagonizes binding of CTLA-4 to B7 ligands.
  - Antitumor activity has been demonstrated in a variety of murine tumor models
  - It has shown efficacy on melanoma patients after single or multiple doses. Similar drug Ipilimumab already approved for metastatic melanoma.
  - It had not been tested against hepatocellular carcinoma
  - Its antiviral effect had not been tested whatsoever

Tumor Response
(Efficacy Population)

- Median number of courses: 2 (range: 1-4)
  - At least 2 courses: 13 (76%)
  - At least 4 courses: 6 (35%)

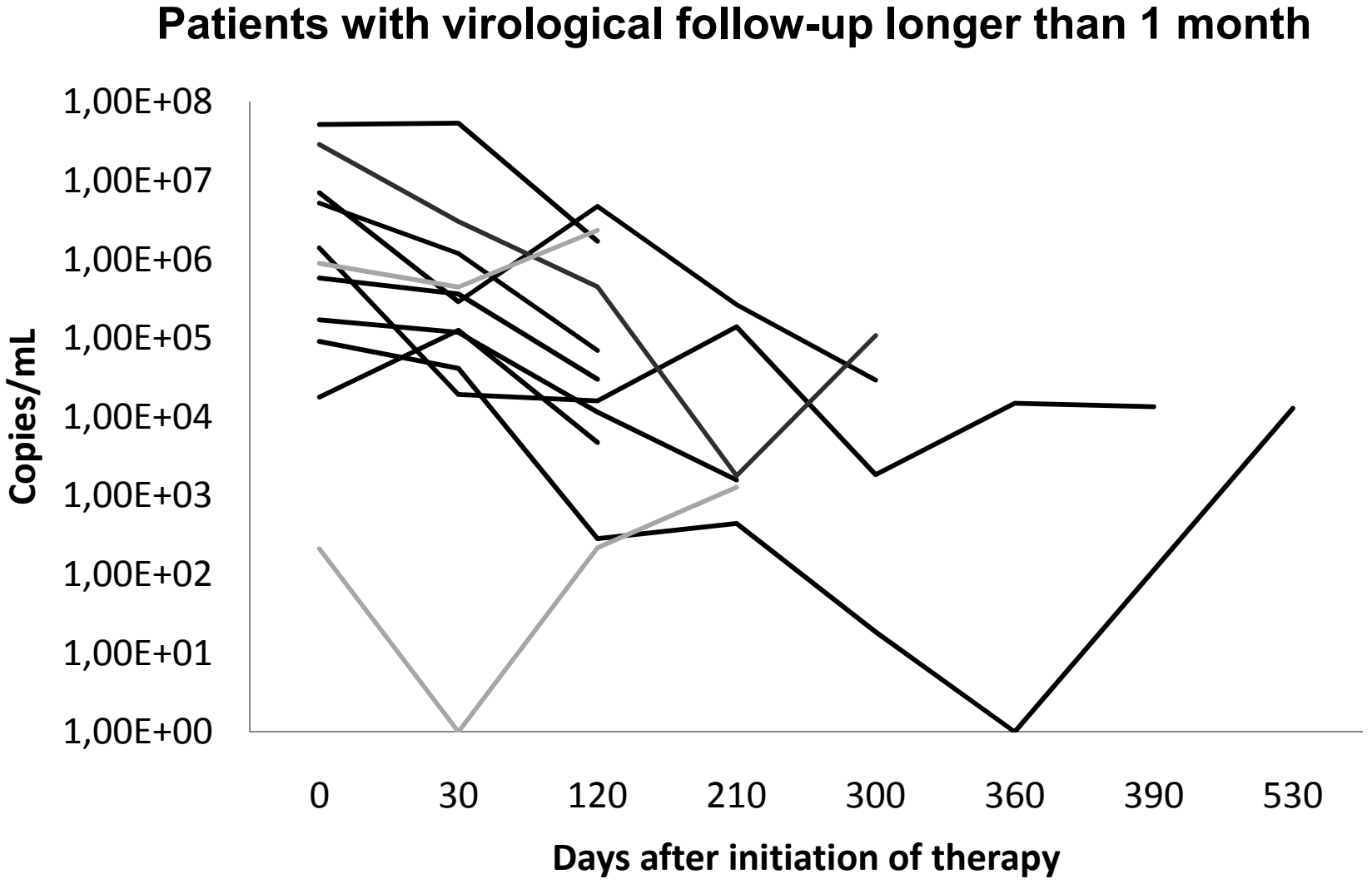


Tumor Response on Target Lesions

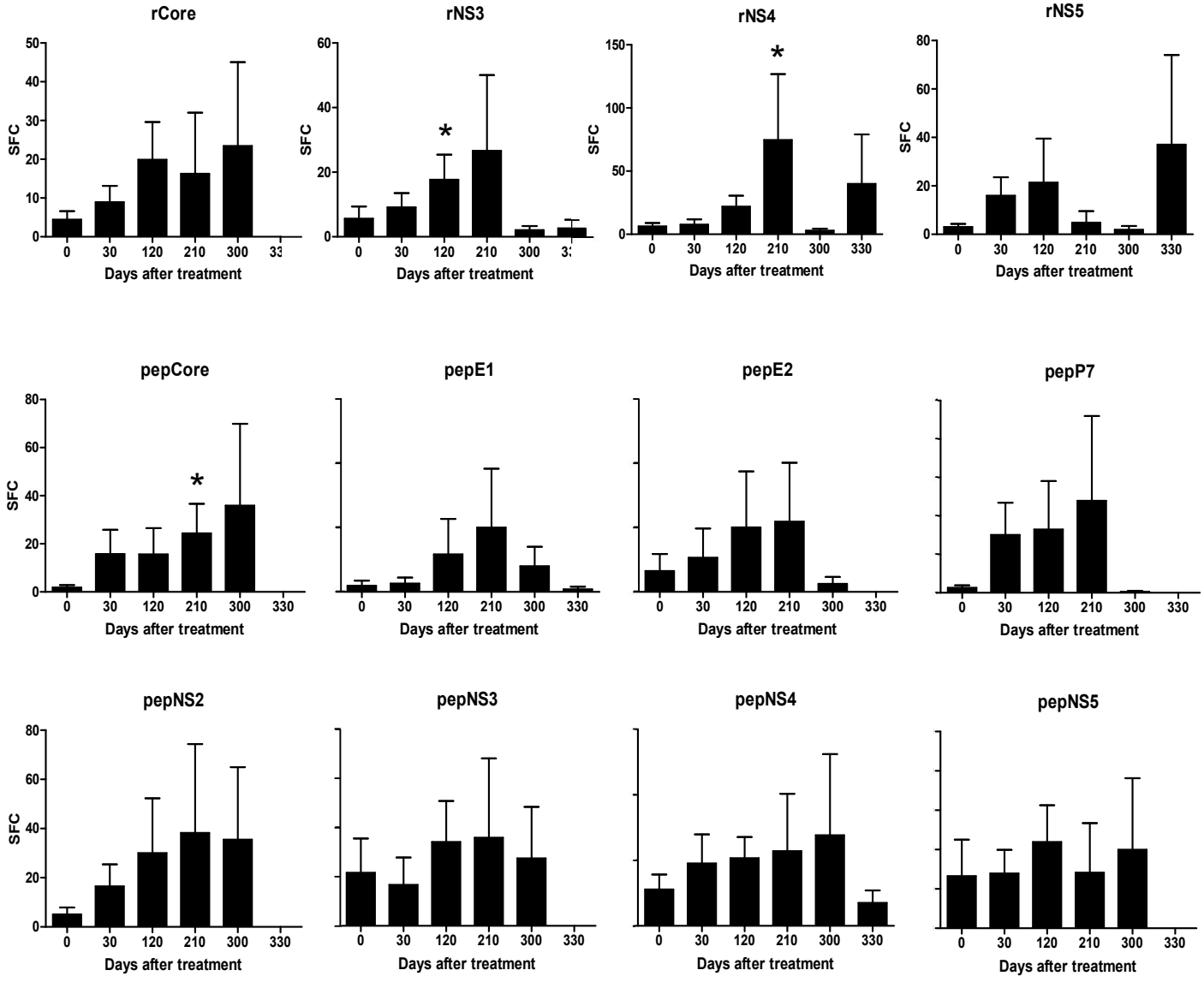


Antiviral Efficacy

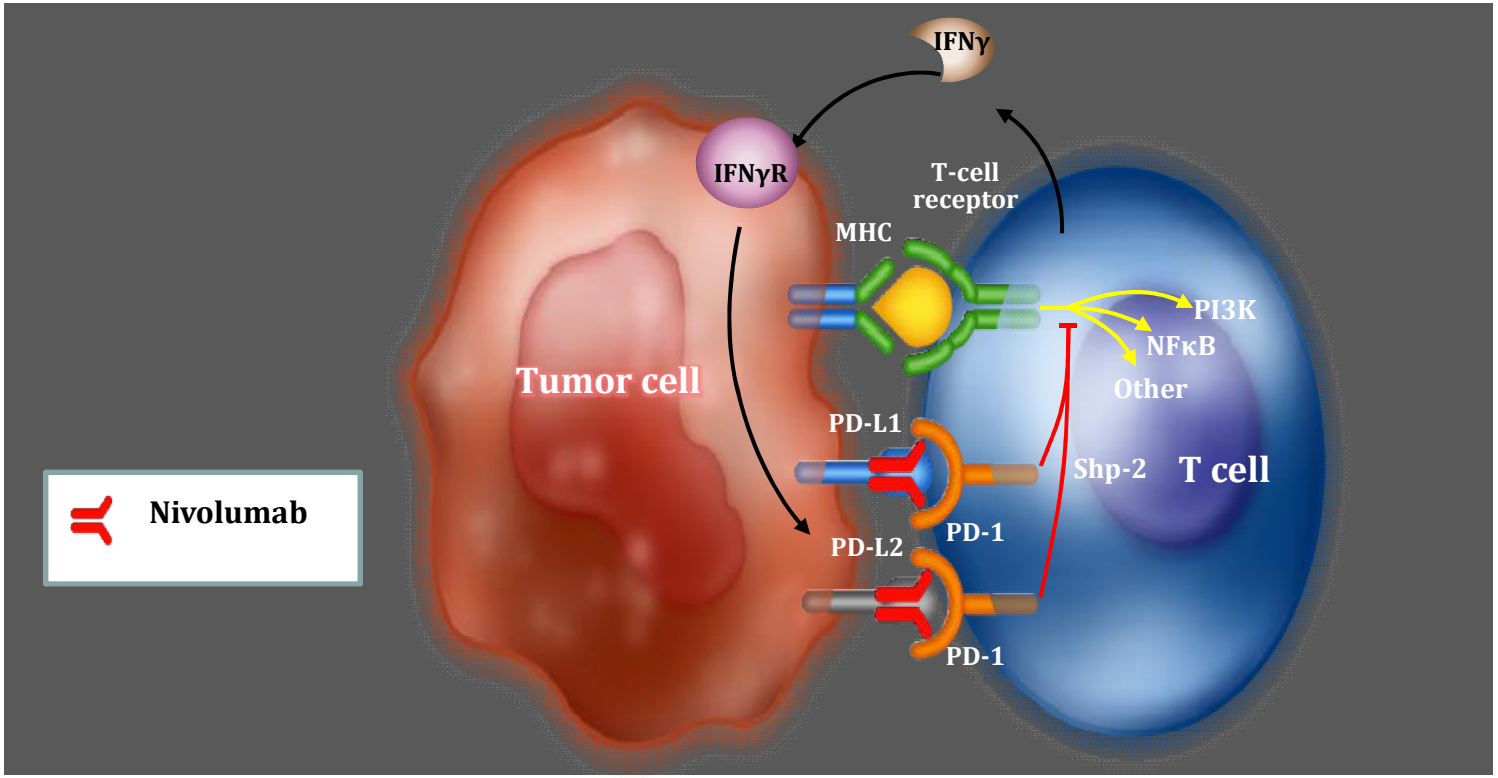
Serum HCV Viral Load



Immunological Outcomes
Global Changes in Anti-HCV Immune Response



Nivolumab and Immune Checkpoint Inhibition



- Nivolumab is a fully human IgG4 anti-PD-1 monoclonal antibody that selectively blocks the interaction between PD-1 and PD-L1/PD-L2,[1] restoring T-cell immune activity directed against the tumor cell

1. Topalian SL, et al. *N Engl J Med*. 2012;366:2443-2454

HCC and Programmed Death-1 (PD-1)

- HCC is typically an inflammation-associated cancer and can be immunogenic[1]
- Association of hepatitis C and hepatitis B infection with upregulation of PD-1[2,3]
- Upregulation of PD-1 and the PD-1 immune checkpoint ligand, PD-L1, in HCC is associated with poor outcomes[4]
- Blockade of PD-1 with monoclonal antibodies combined with immunostimulatory monoclonal antibodies extended survival in a mouse model of HCC[5,6]
- Immune checkpoint inhibition (anti-CTLA-4) has shown encouraging activity in an early clinical trial in HCC[7]
- PD-1 blockade with nivolumab may boost host immunity against HCC and improve clinical outcomes

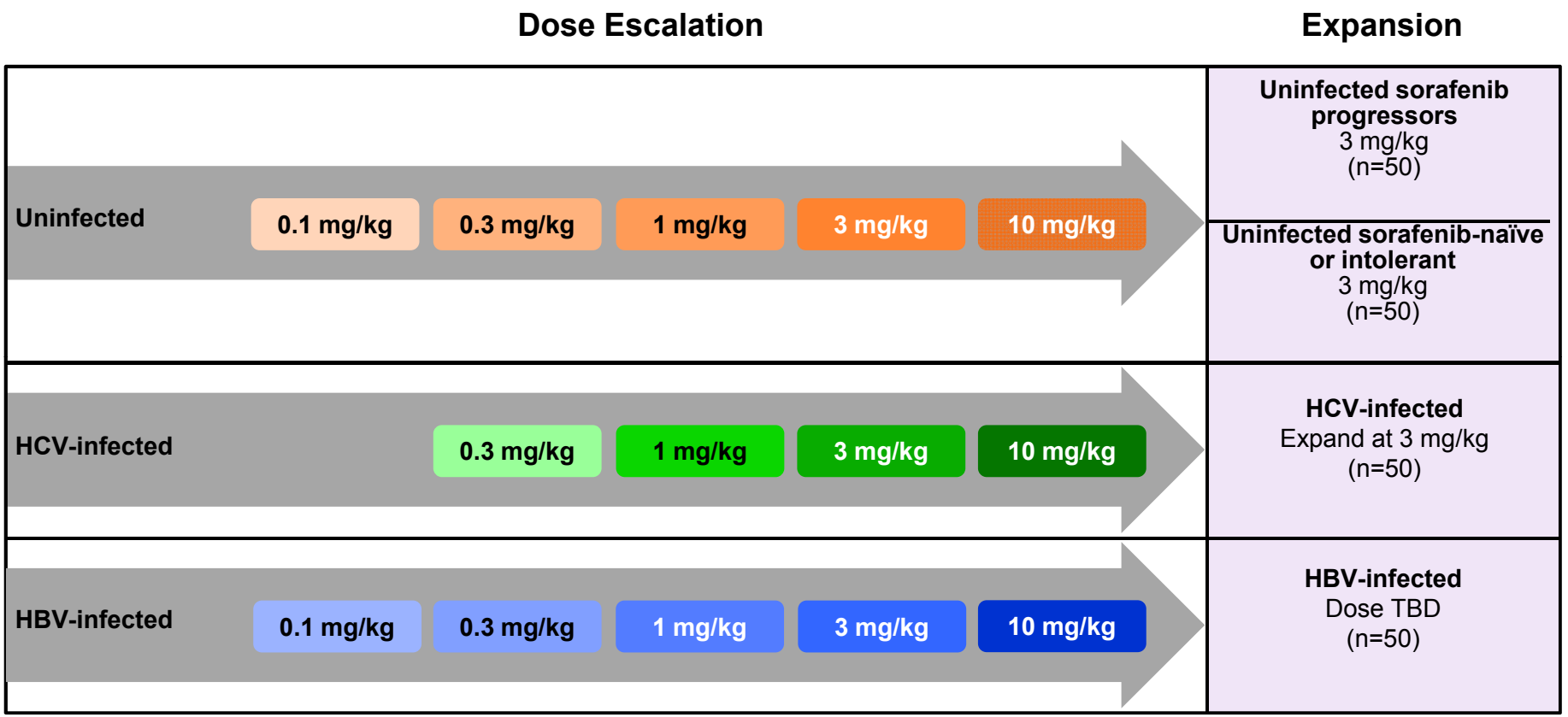
1. Hato T, et al. *Hepatol*. 2014;60:1776-1782
2. Xu P, et al. *Gut Liver*. 2014;8:186-195
3. Barathan M, et al. *Apoptosis*. 2015;20:466-480
4. Zeng Z, et al. *PLoS One*. 2011;6:e23621
5. Chen Y, et al. *Hepatology*. 2015;61:1591-1602
6. Morales-Kastresana A, et al. *Clin Cancer Res*. 2013;19:6151-6162
7. Sangro B, et al. *J Hepatol*. 2013;59:81-88

Phase 1/2 Safety and Antitumor Activity of Nivolumab in Patients With Advanced Hepatocellular Carcinoma (HCC): CA209-040

Anthony B. El-Khoueiry,[1] Ignacio Melero,[2] Todd S. Crocenzi,[3] Theodore H. Welling III,[4] Thomas Yau,[5] Winnie Yeo,[5] Akhil Chopra,[6] Joseph F. Grosso,[7] Lixin Lang,[7] Jeffrey Anderson,[7] Christine de la Cruz,[7] Bruno Sangro[2]

[1]University of Southern California Norris Comprehensive Cancer Center, Los Angeles, CA, USA; [2]Clinica Universidad de Navarra and CIBERehd, Pamplona, Spain; [3]Providence Cancer Center, Portland, OR, USA; [4]University of Michigan, Ann Arbor, MI, USA; [5]University of Hong Kong, China; [6]Johns Hopkins Singapore International Medical Centre, Singapore; [7]Bristol-Myers Squibb, Princeton, NJ, USA

Study Design



- Patients received nivolumab Q2W for up to 2 years (maximum of 48 doses), depending on response
  - Imaging for disease assessment performed every 6 weeks
- A 3+3 design was used in the phase 1 dose escalation phase
- Here, we report interim results from the ongoing dose escalation phase and part of the expansion phase

Baseline Patient Characteristics

	Uninfected (n=24)	HCV (n=12)	HBV (n=11)	Total (N=47)
Age (years), median (range)	63.5 (22–79)	67 (55–83)	62 (41–68)	64 (22–83)
Male, n (%)	18 (75)	7 (58)	9 (82)	34 (72)
Race, n (%)				
White	20 (83)	10 (83)	1 (9)	31 (66)
Asian	2 (8)	2 (16)	10 (91)	14 (30)
Black	2 (8)	0	0	2 (4)
Extrahepatic metastases, n (%)	17 (71)	7 (58)	9 (82)	33 (70)
Vascular invasion, n (%)	3 (13)	2 (17)	1 (9)	6 (13)
Child-Pugh Score, n (%)				
5	21 (88)	9 (75)	11 (100)	41 (87)
6	3 (13)	2 (17)	0	5 (11)
7	0	1 (8)	0	1 (2)
AFP >200 µg/L, n (%)*	8 (33)	2 (17)	7 (64)	17 (36)

*Baseline AFP values were missing for 5 patients

Prior Treatment History

Treatment Type, n (%)	Uninfected (n=24)	HCV (n=12)	HBV (n=11)	Total (N=47)
Prior surgical resection	14 (58)	8 (67)	9 (82)	31 (66)
Prior radiotherapy	6 (25)	1 (8)	1 (9)	8 (17)
Local treatment for HCC	12 (50)	8 (67)	9 (82)	29 (62)
TACE, TAE, or radioembolization	11 (46)	8 (67)	7 (64)	26 (55)
RFA or PEI	5 (21)	4 (33)	4 (36)	13 (28)
Other	1 (4)	0	1 (9)	2 (4)
Prior systemic therapy	17 (71)	7 (58)	11 (100)	35 (75)
Prior sorafenib	15 (63)	6 (50)	11 (100)	32 (68)

TACE, transcatheter arterial chemoembolization; TAE, transcatheter arterial embolization; RFA, radiofrequency ablation; PEI, percutaneous ethanol injection

Investigator-Assessed Best Overall Response

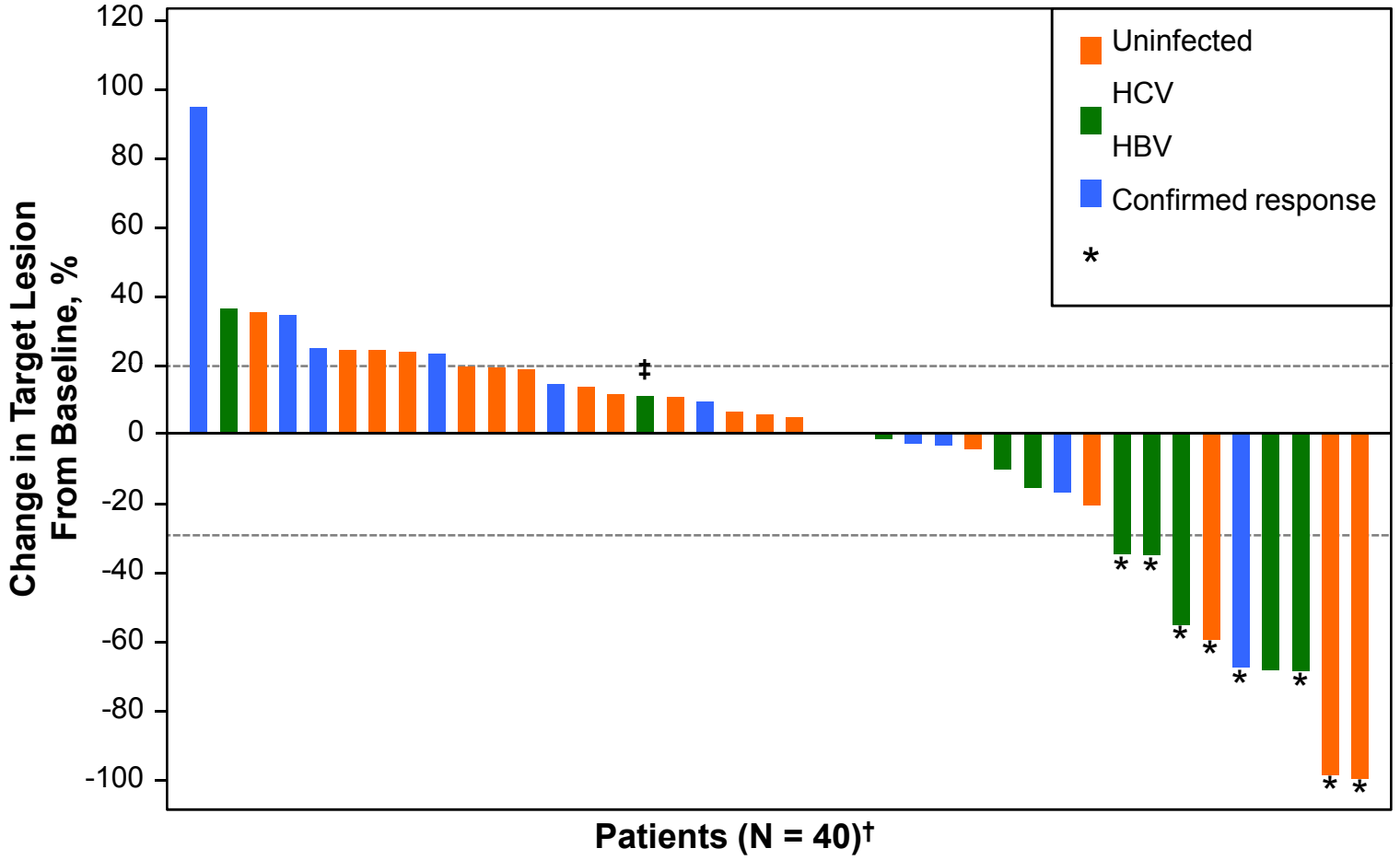
	Uninfected (n=21)	HCV (n=11)	HBV (n=10)	Total Evaluable * (n=42)
Objective response, n (%)	3 (14)	4 (36)	1 (10)	8 (19)
Complete response	2 (10)	0	0	2 (5)
Partial response	1 (5)	4 (36)	1 (10)	6 (14)
Stable disease	10 (48)	5 (45)†	5 (50)	20 (48)
Progressive disease	8 (38)	2 (18)	4 (40)	14 (33)
Ongoing response, n (%)	3/3 (100)	3/4 (75)	0	6/8 (75)

Responses assessed by RECIST 1.1

*5 patients not evaluable: first disease assessment not yet performed in 4 patients, 1 patient died from clinical progression before disease assessment

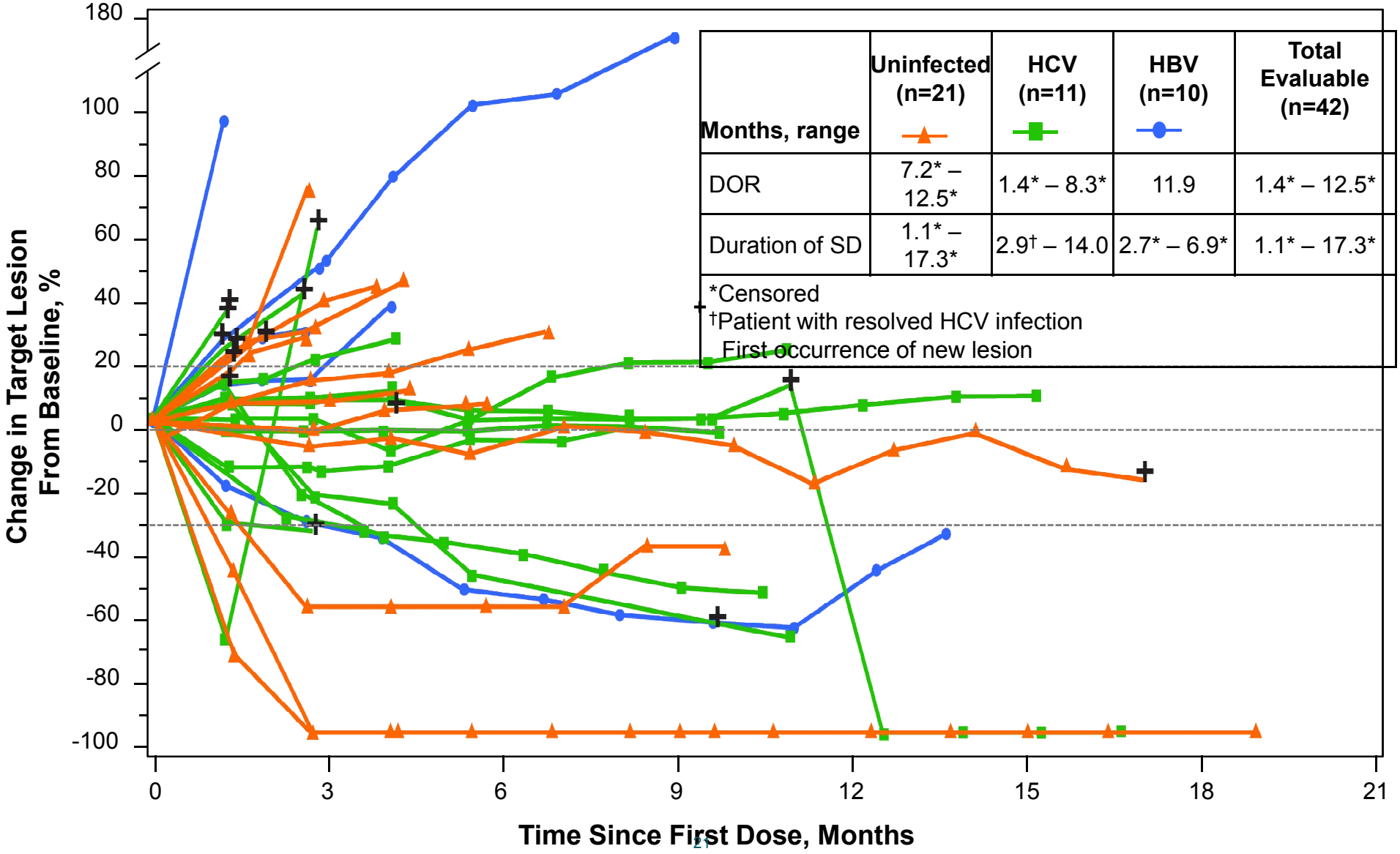
†Patient with resolved HCV infection

Maximal Change in Target Lesions From Baseline



†2 uninfected patients not shown: 1 had disease progression before the first assessment; 1 had a maximal change of +23%
‡Patient with resolved HCV infection

Response Kinetics

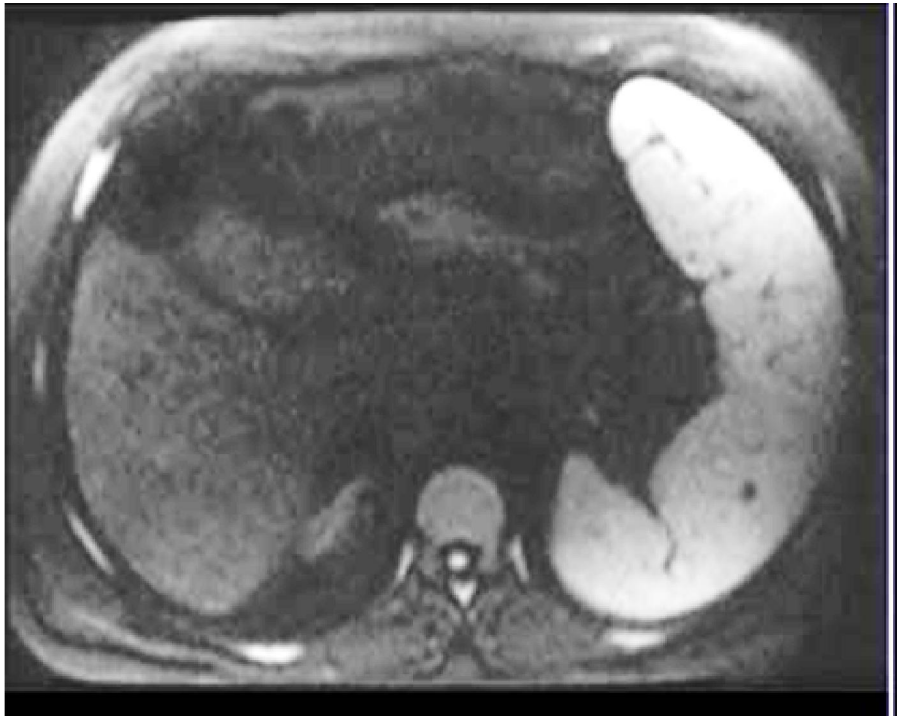


Months, range	Uninfected (n=21)	HCV (n=11)	HBV (n=10)	Total Evaluable (n=42)
DOR	7.2* – 12.5*	1.4* – 8.3*	11.9	1.4* – 12.5*
Duration of SD	1.1* – 17.3*	2.9† – 14.0	2.7* – 6.9*	1.1* – 17.3*

*Censored
†Patient with resolved HCV infection
+ First occurrence of new lesion

Partial Response to Nivolumab

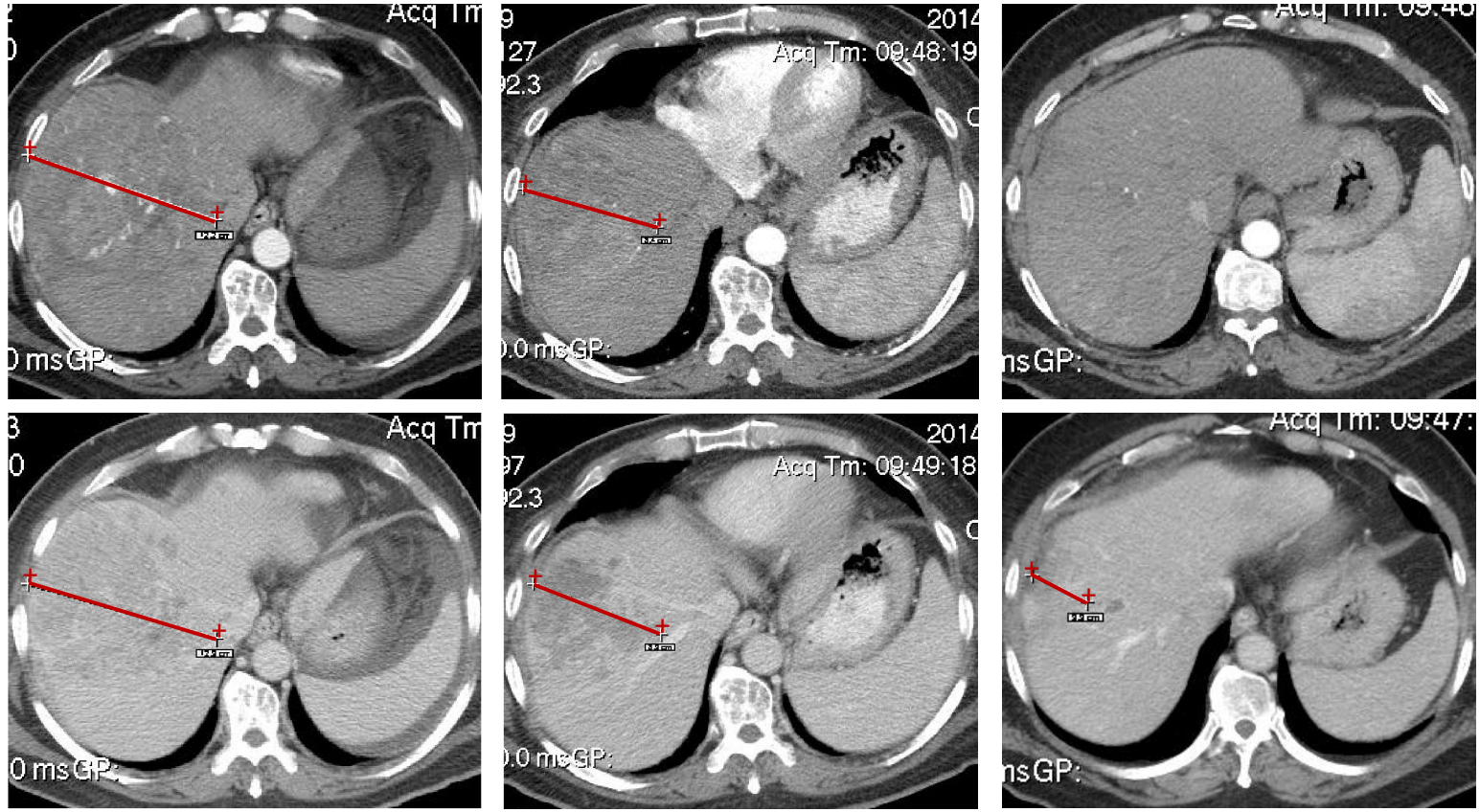
Baseline
AFP: 21,000 IU/mL



Week 6
AFP: 283 IU/mL

- 63 year-old male, uninfected HCC, Child-Pugh score A5
- No prior sorafenib or other treatment for HCC

Durable Partial Response to Nivolumab

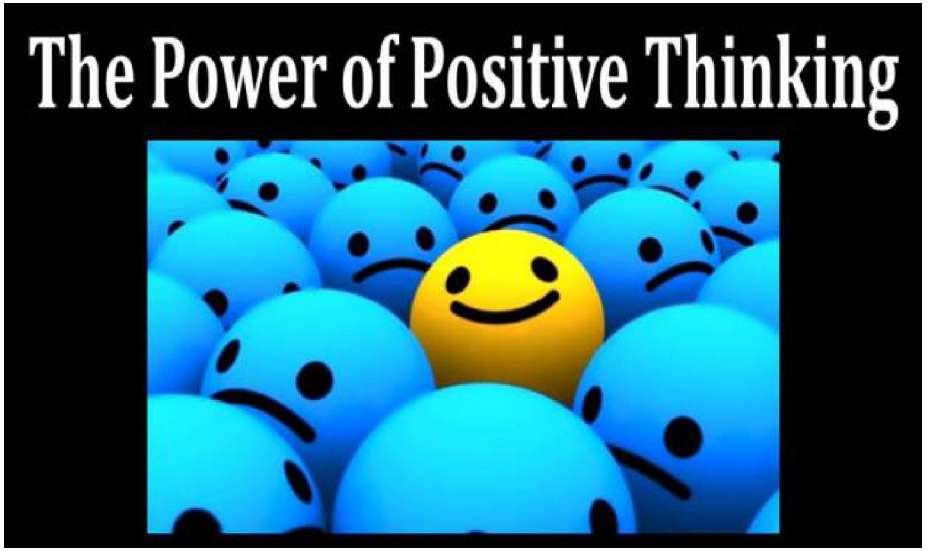


- 58-year-old white male with HCV-infected HCC, ECOG 0, Child-Pugh A5
- Progressed on sorafenib

Remove inhibitions

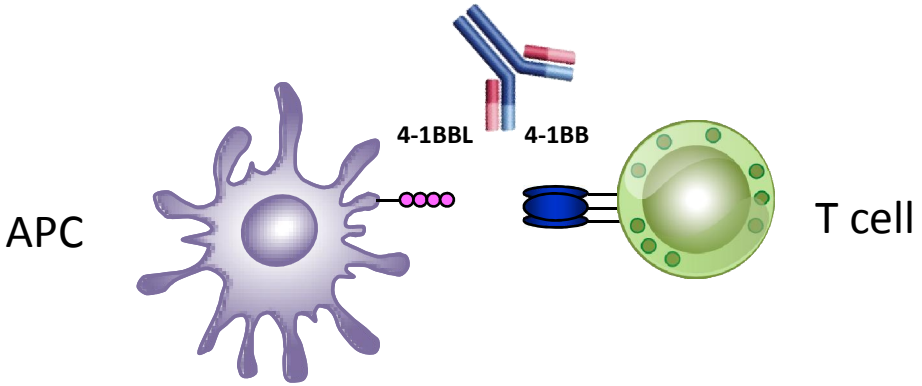
Release the brakes

Enforce costimulation



Press the gas pedal to the metal

CD137 (4-1BB, TNFRSF9)



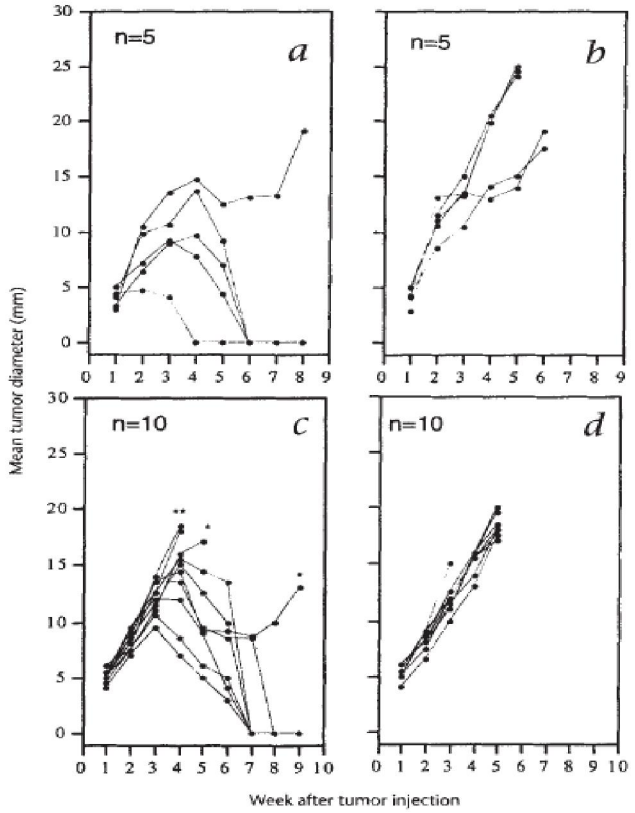
- CD137, also known as 4-1BB, is a surface glycoprotein involved in T cell costimulation
- Its cognate ligand is CD137L, which is expressed on APCs
- Functions: T cell proliferation, inhibition of apoptosis, enhances cytotoxic activity, cytokine production
- Therapeutic target: treatment with agonist anti-CD137 monoclonal antibodies can overcome tumour antigen tolerance
- Anti-human CD137 agonist monoclonal antibodies are undergoing phase I/II clinical trials

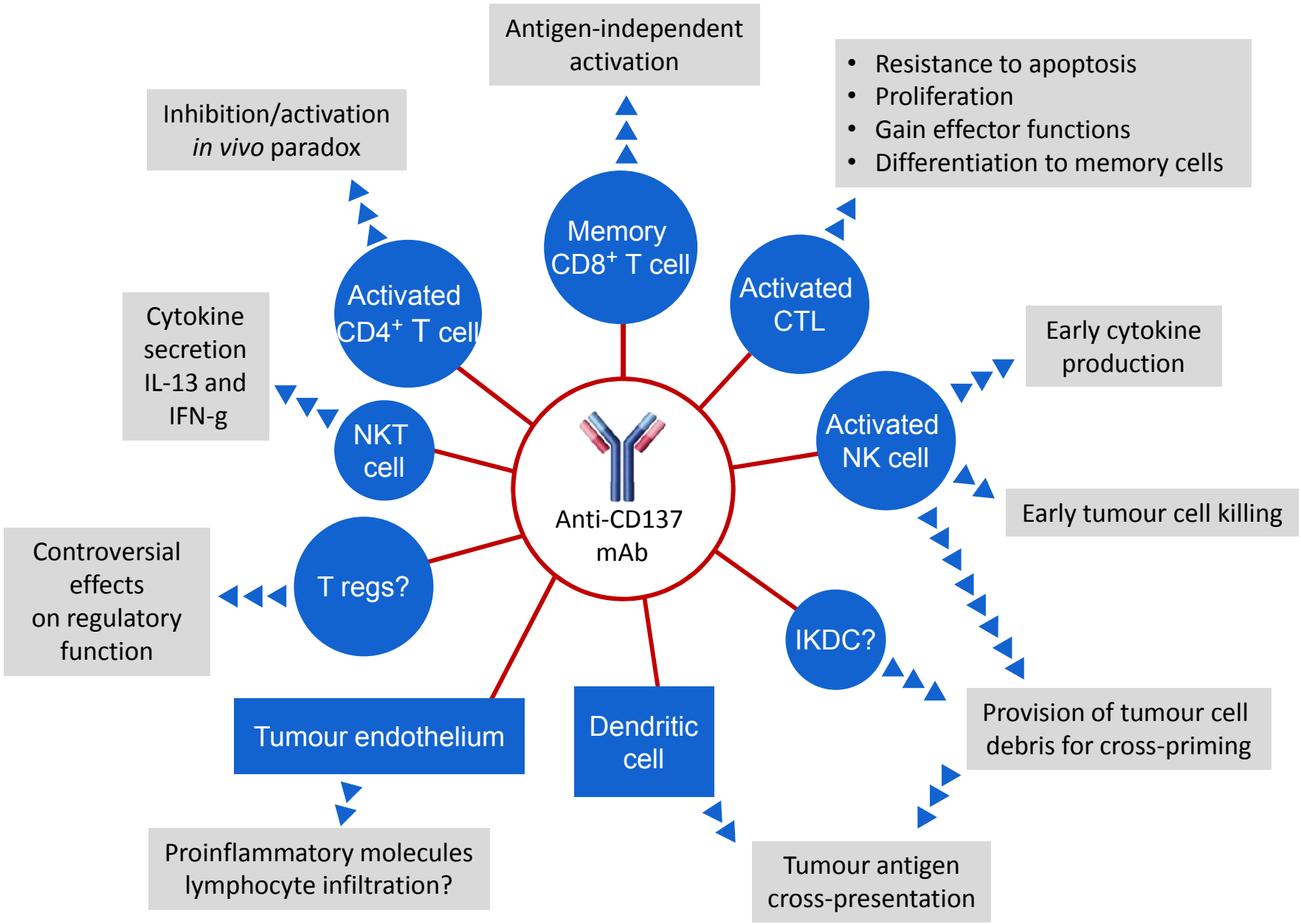
Melero, et al. Trends Pharmacol Sci 2008

Monoclonal antibodies against the 4-1BB T-cell activation molecule eradicate established tumors

IGNACIO MELERO, WALTER W. SHUFORD, STEPHANIE ASHE NEWBY, ALEJANDRO ARUFFO, JEFFREY A. LEDBETTER, KARL ERIK HELLSTRÖM, ROBERT S. MITTLER & LIEPING CHEN

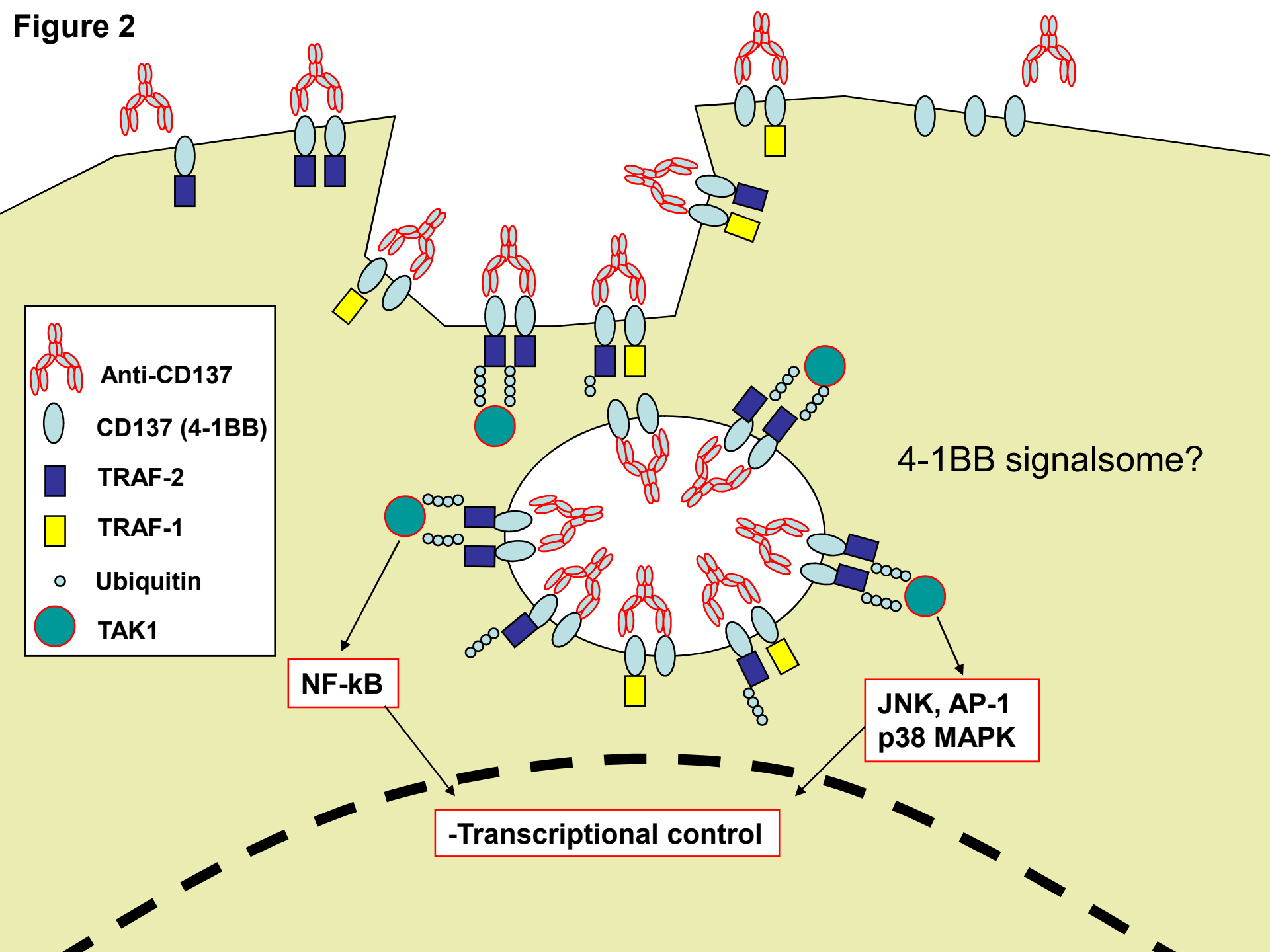
NATURE MEDICINE • VOLUME 3 • NUMBER 6 • JUNE 1997

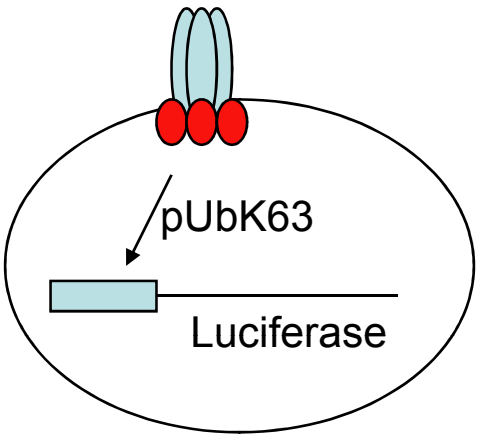


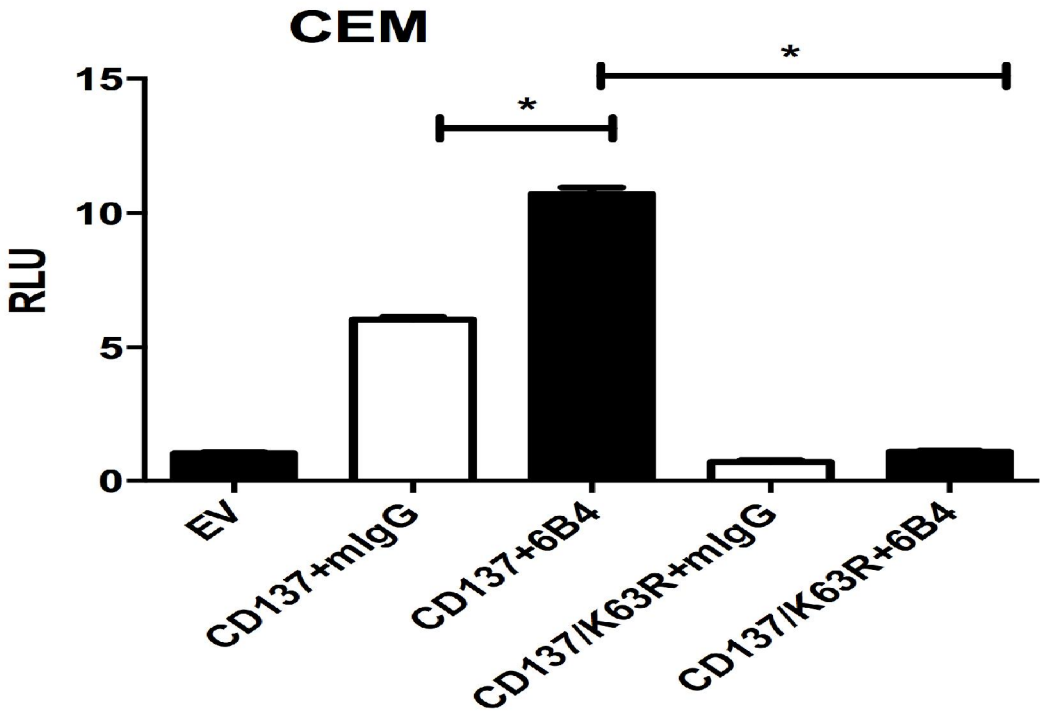


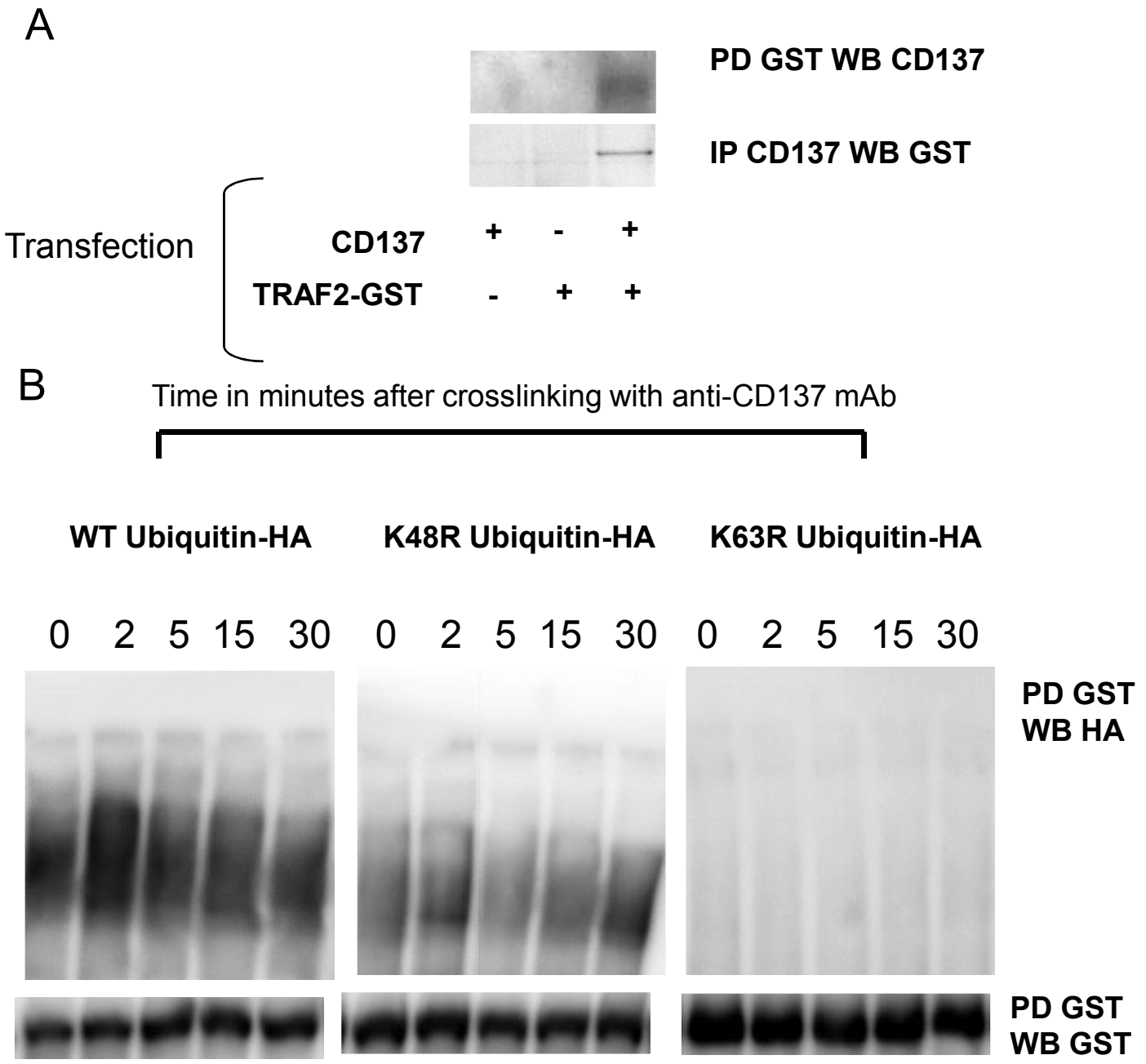
Melero, et al. Trends Pharmacol Sci.2008

Figure 2

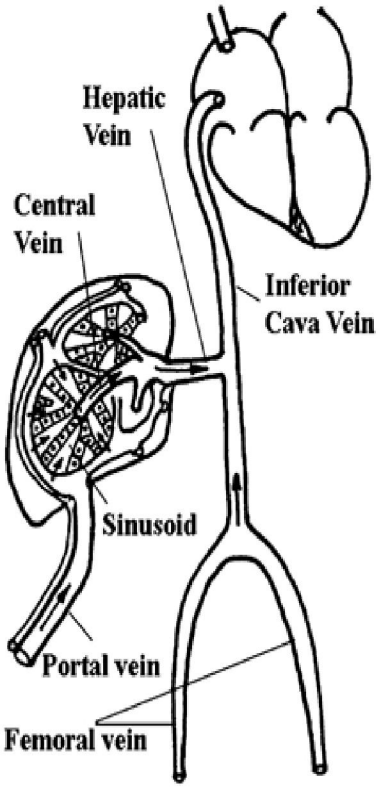

Anti-CD137
CD137 (4-1BB)
TRAF-2
TRAF-1
Ubiquitin
TAK1
4-1BB signalsome?
NF-kB
JNK, AP-1
p38 MAPK
-Transcriptional control

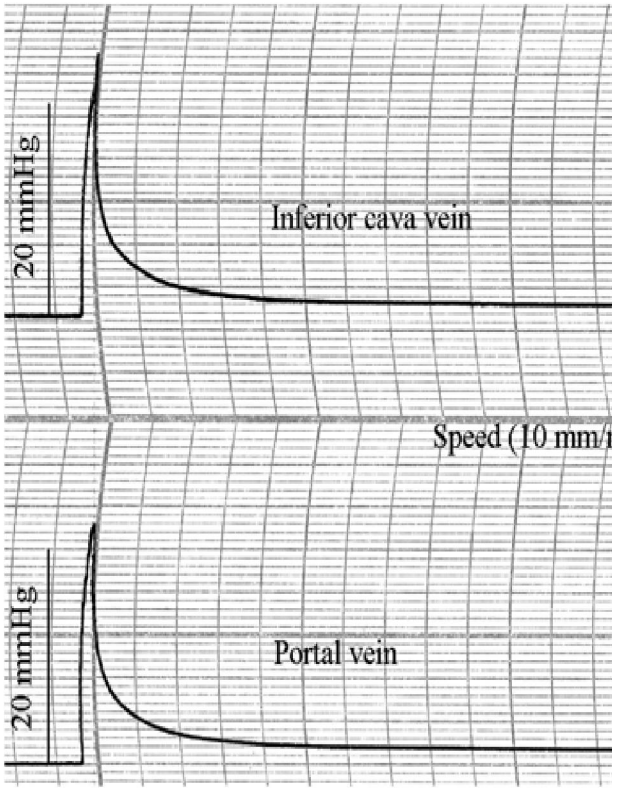

pUbK63
Luciferase

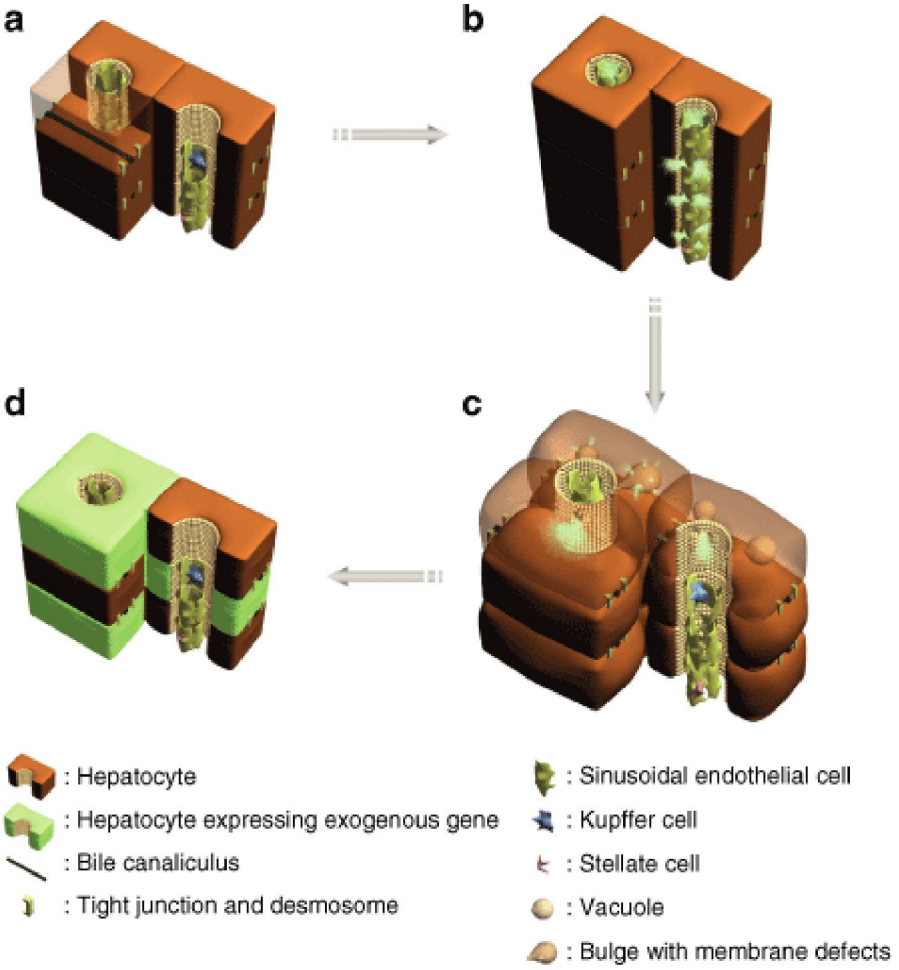

CEM
RLU
15
10
5
0
*
*
EV
CD137+mIgG
CD137+6B4
CD137/K63R+mIgG
CD137/K63R+6B4


A
PD GST WB CD137
IP CD137 WB GST
Transfection
CD137 + - +
TRAF2-GST - + +
B
Time in minutes after crosslinking with anti-CD137 mAb
WT Ubiquitin-HA
K48R Ubiquitin-HA
K63R Ubiquitin-HA
0 2 5 15 30 0 2 5 15 30 0 2 5 15 30
PD GST WB HA
PD GST WB GST

Hydrodinamic injection of expression plasmids

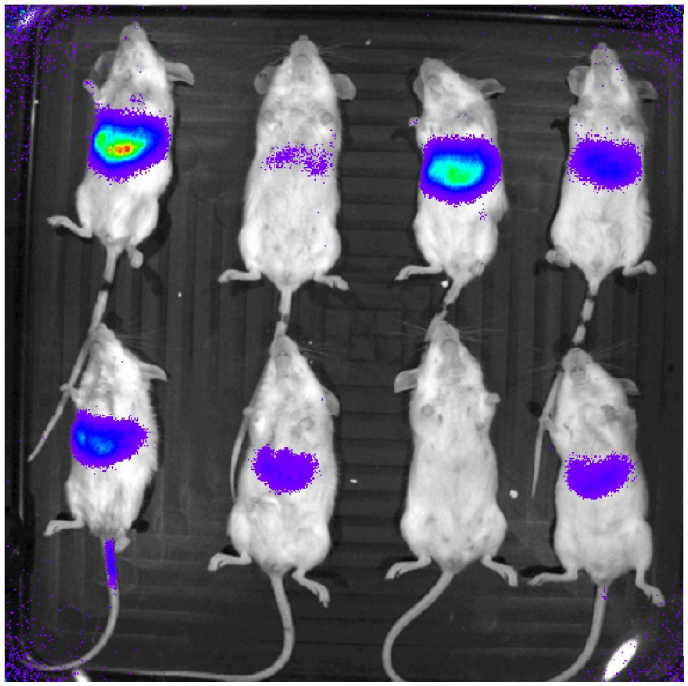


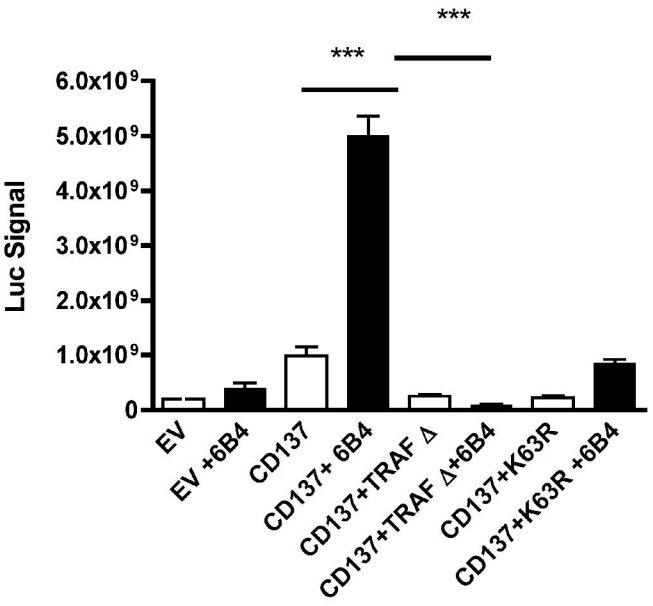




Anti-CD137

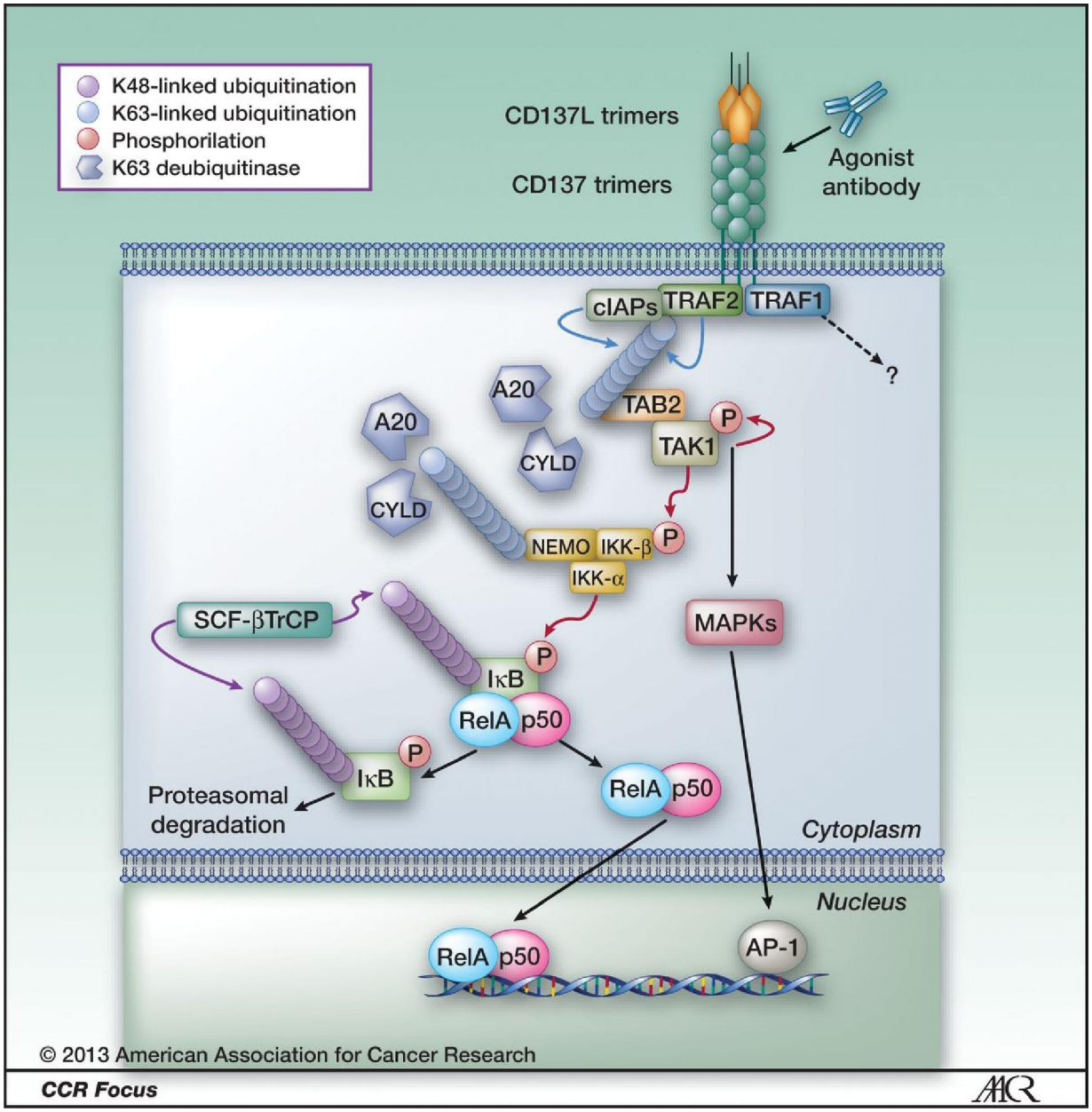
Control Ab





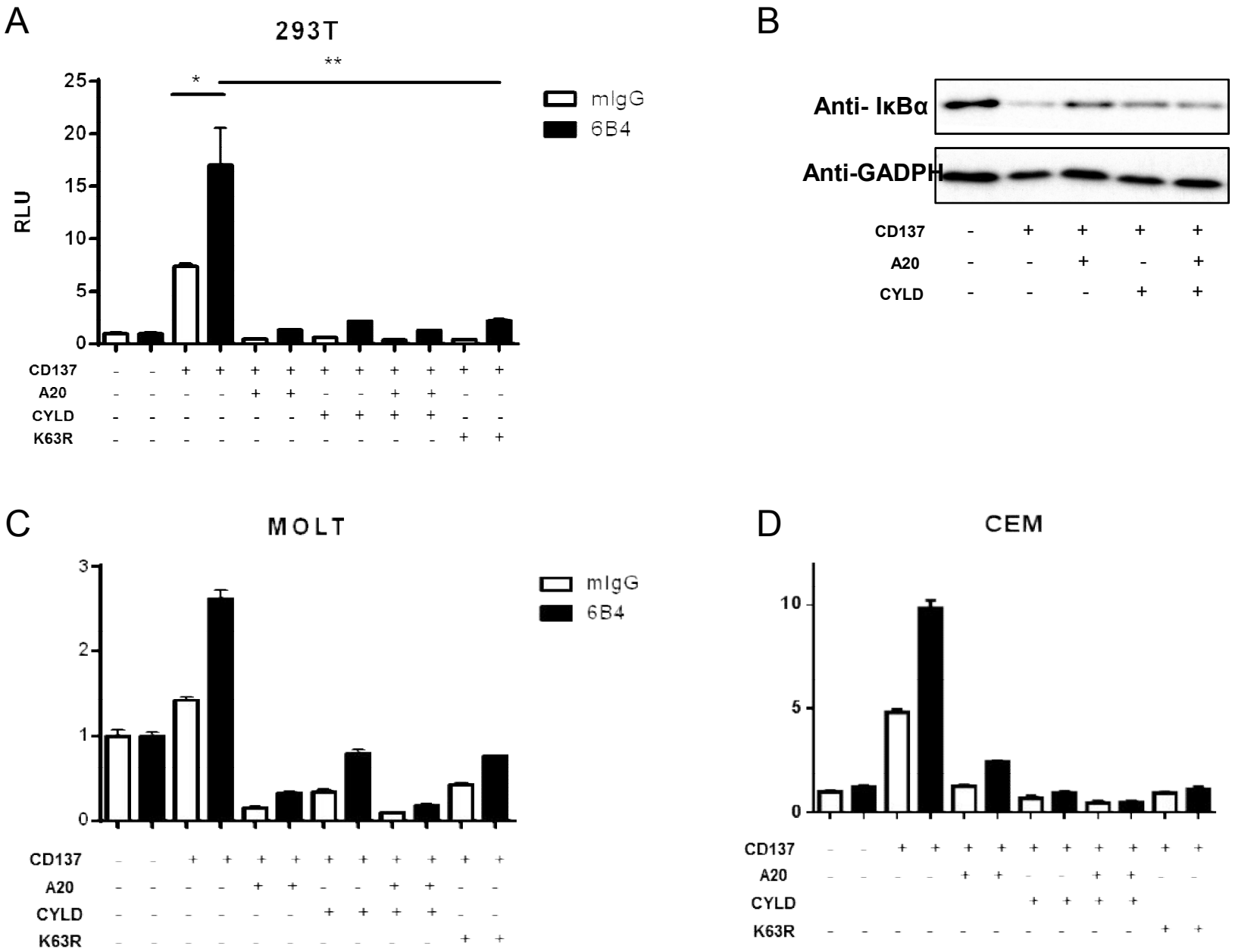
CD137	+	+	+	-
TRAFΔ	-	-	+	-
K63R	-	+	-	-

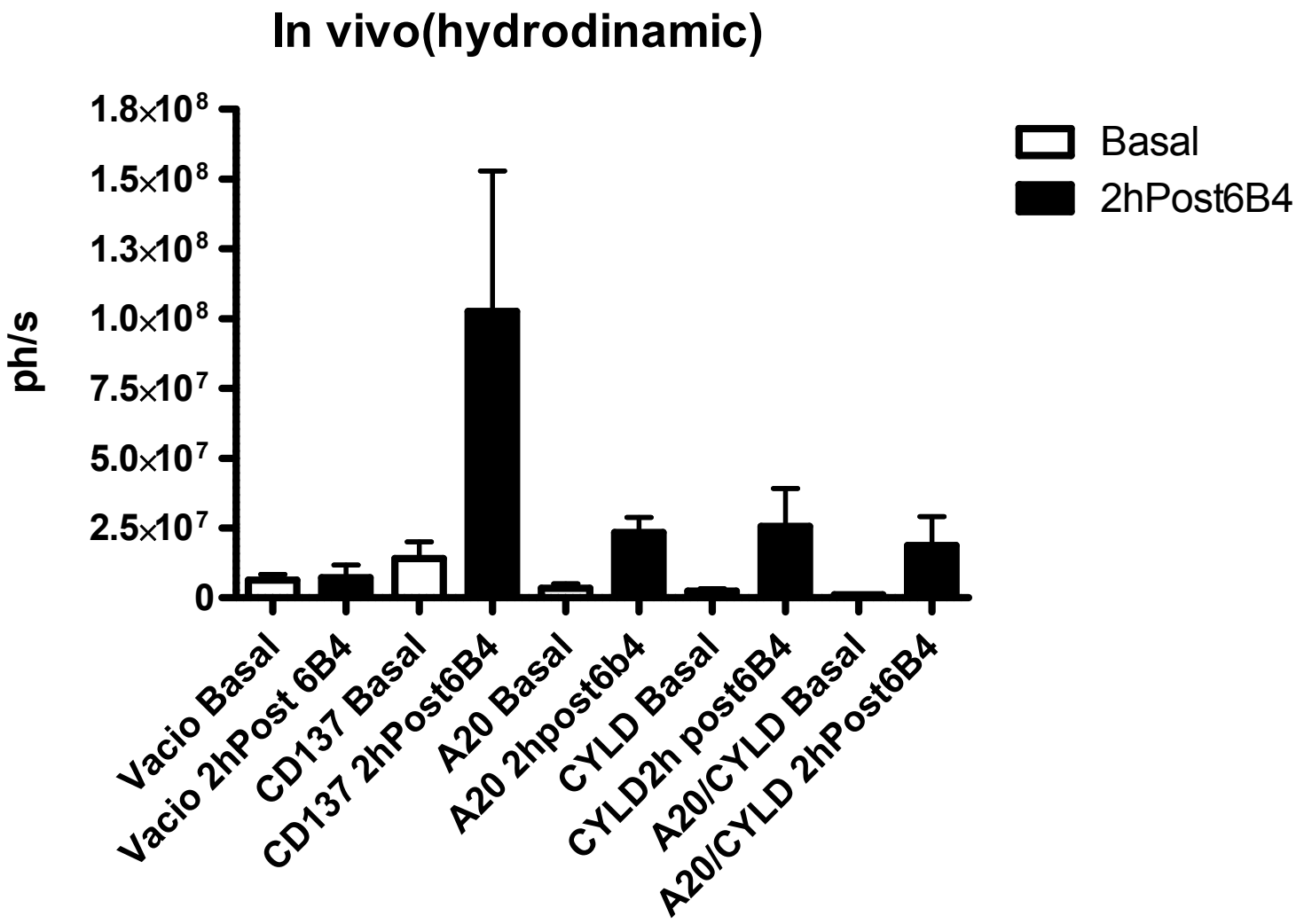
How is it regulated?



Melero I et al. Clin Cancer Res 2013;19:1044-1053

©2013 by American Association for Cancer Research


A
293T
**
*
25
20
15
10
5
0
RLU
mIgG
6B4
CD137 - - + + + + + + + + + +
A20 - - - - + + - - + + - -
CYLD - - - - - - + + + + - -
K63R - - - - - - - - - - + +
B
Anti- IκBα
Anti-GADPH
CD137 - + + + +
A20 - - + - +
CYLD - - - + +
C
MOLT
3
2
1
0
mIgG
6B4
CD137 - - + + + + + + + + + +
A20 - - - - + + - - + + - -
CYLD - - - - - - + + + + - -
K63R - - - - - - - - - - + +
D
CEM
10
5
0
CD137 - - + + + + + + + + + +
A20 - - - - + + - - + + - -
CYLD - - - - - - + + + + - -
K63R - - - - - - - - - - + +

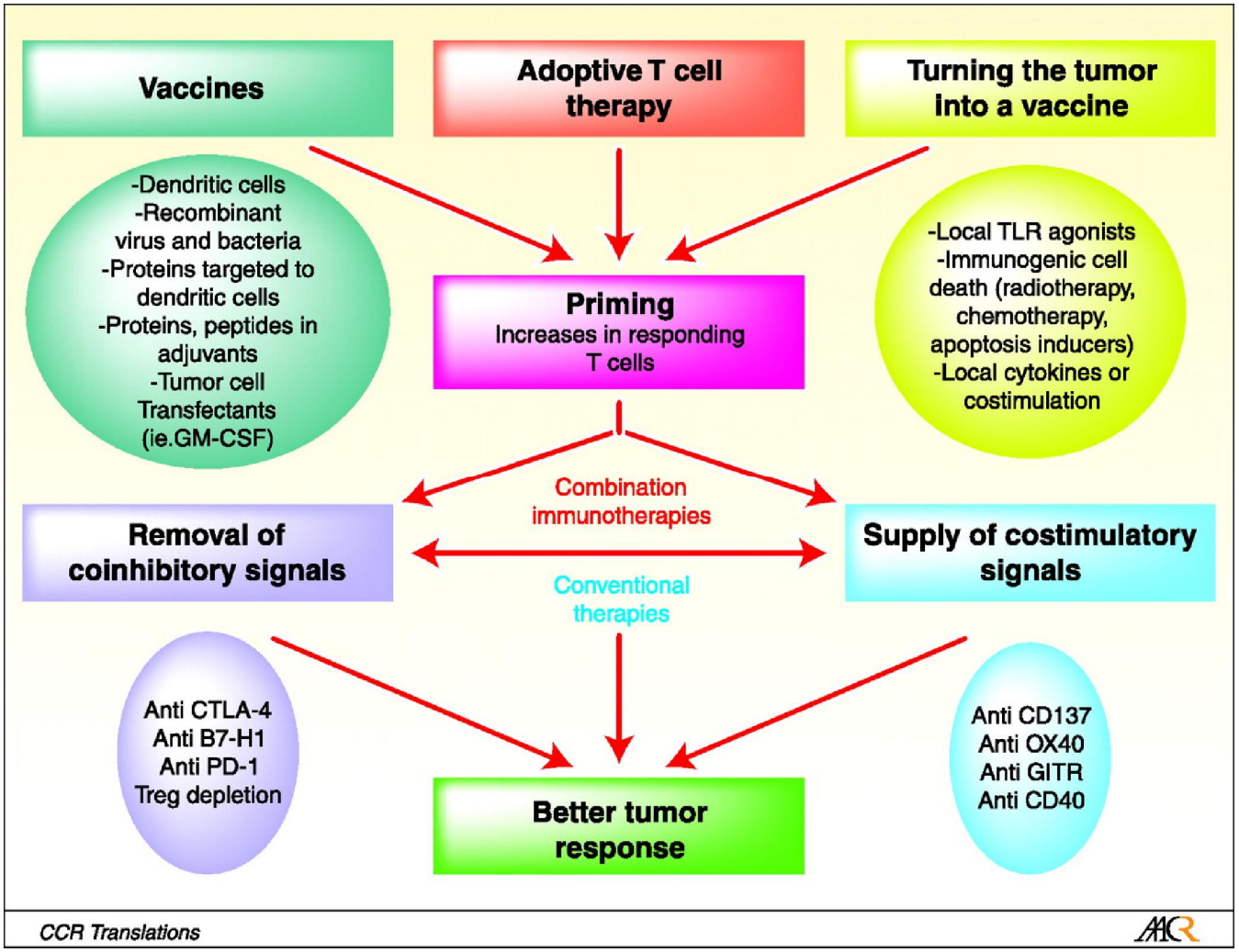


Pool 6 mice /group

SYNERGY

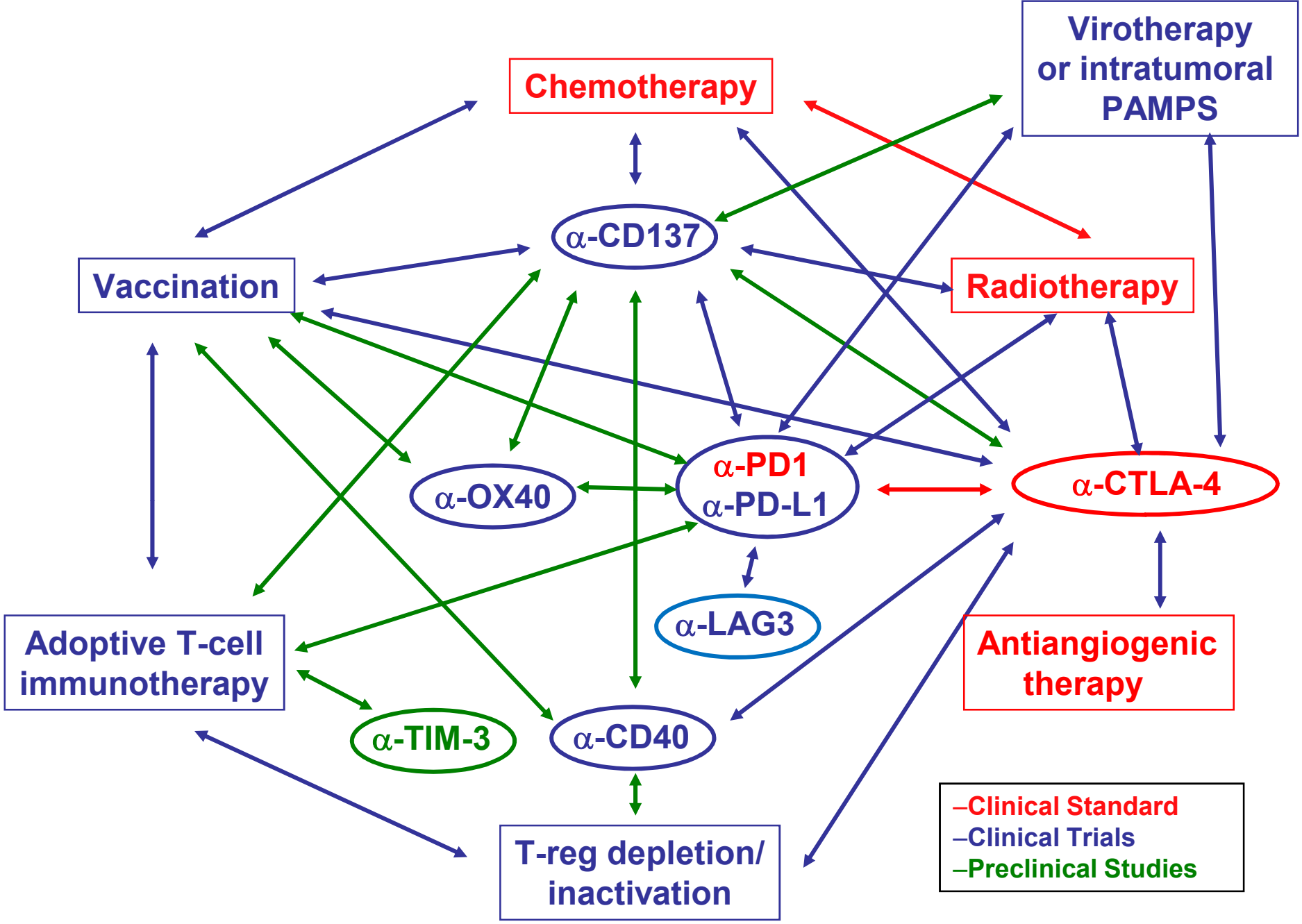
***syn-ergos*, *συνεργός*, meaning 'working together'.**

Strategies for immunotherapy combinations.



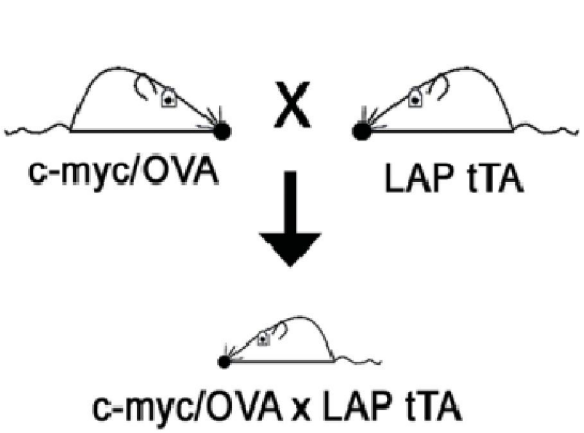
Melero I et al. Clin Cancer Res 2009;15:1507-1509

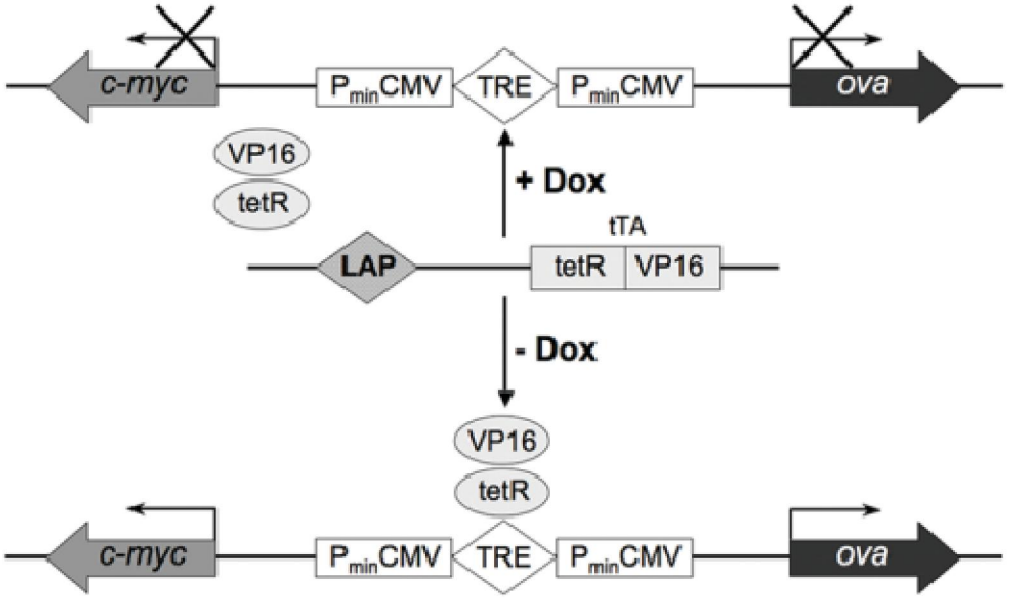
©2009 by American Association for Cancer Research


Virotherapy or intratumoral PAMPS
Chemotherapy
α-CD137
Vaccination
Radiotherapy
α-PD1
α-PD-L1
α-OX40
α-CTLA-4
α-LAG3
Adoptive T-cell immunotherapy
Antiangiogenic therapy
α-TIM-3
α-CD40
T-reg depletion/ inactivation
–Clinical Standard
–Clinical Trials
–Preclinical Studies

Spontaneous HCC

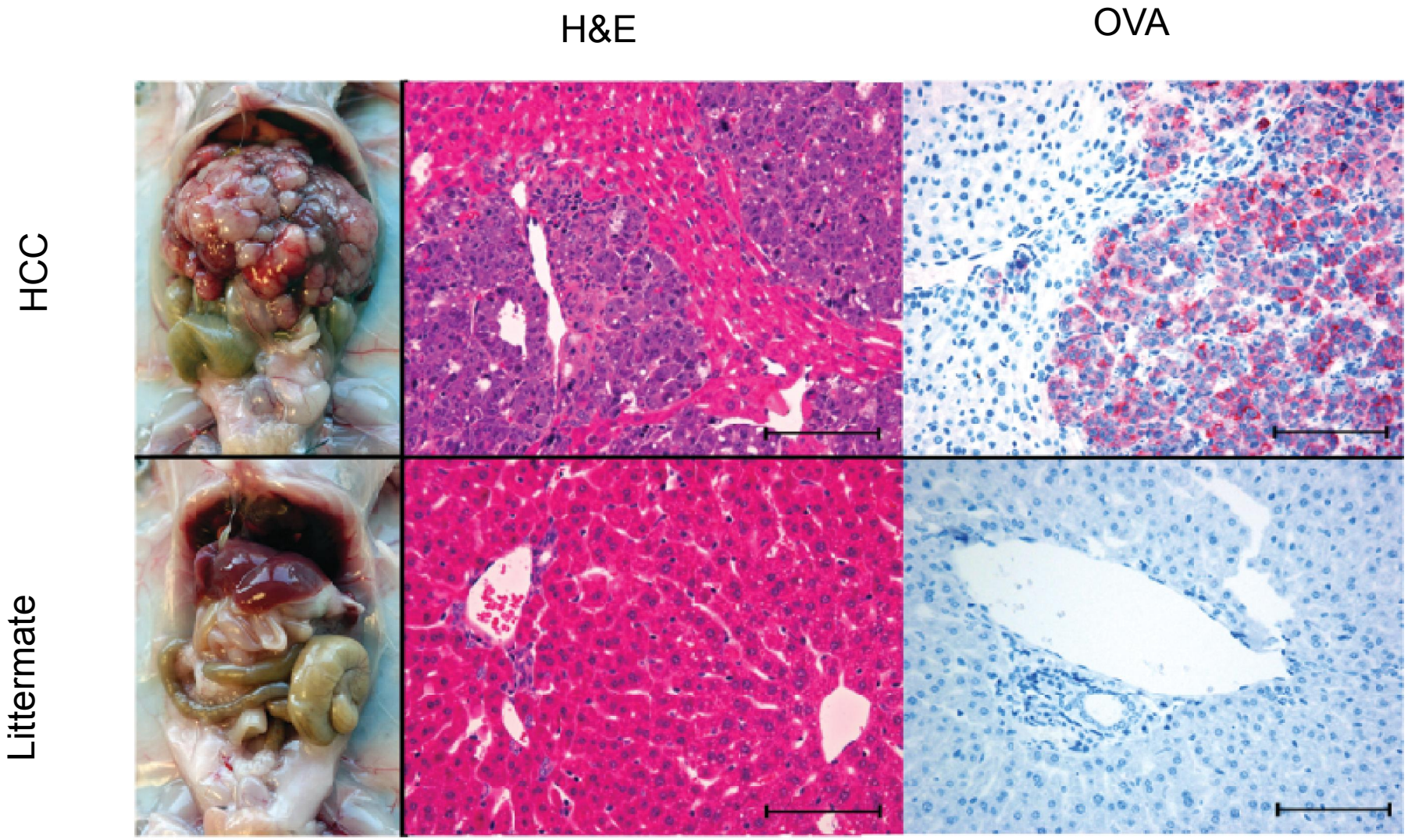
(c-mycOVA75/tTALAP)



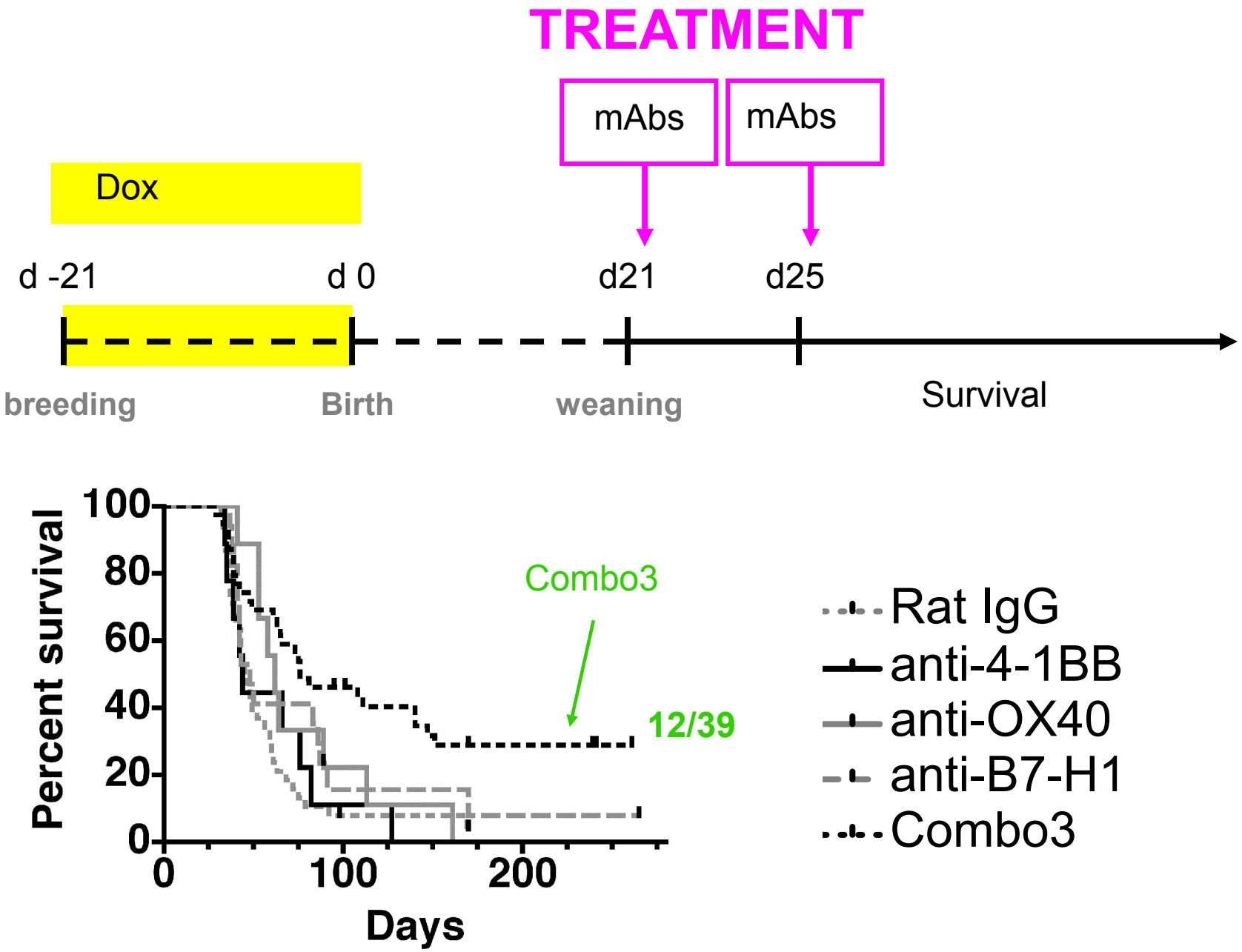


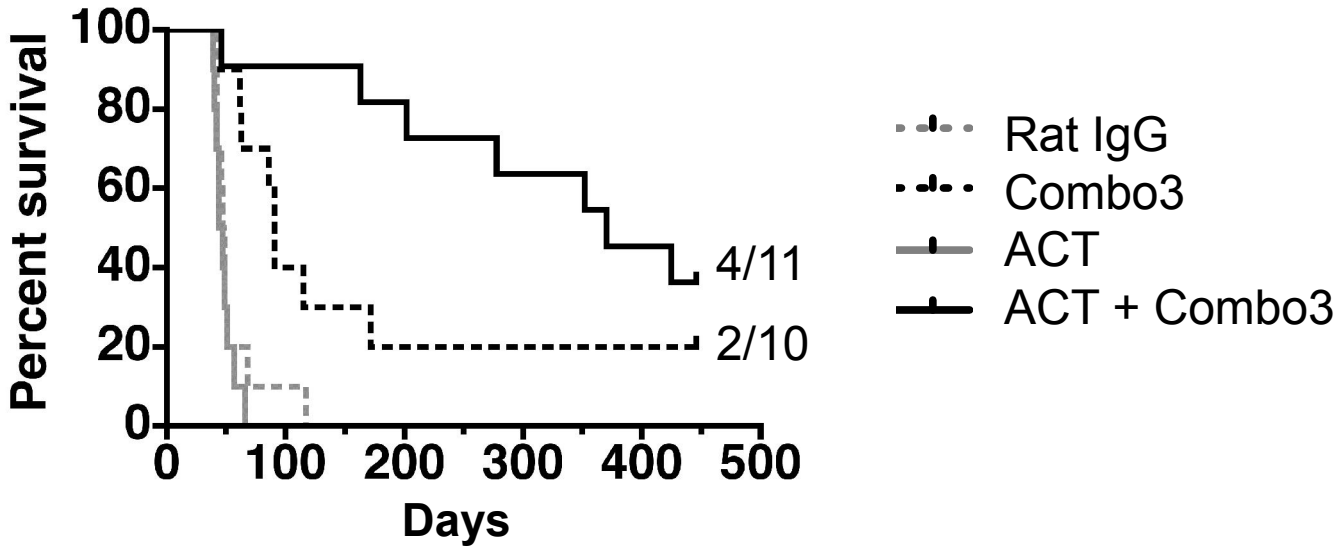
Ney et al, Hepatology 2009

Week 5



Anti-CD137 + anti-PD-1 + anti-OX40


TREATMENT
mAbs
mAbs
Dox
d -21
d 0
d21
d25
breeding
Birth
weaning
Survival
Percent survival
100
80
60
40
20
0
0
100
200
Days
Combo3
12/39
Rat IgG
anti-4-1BB
anti-OX40
anti-B7-H1
Combo3



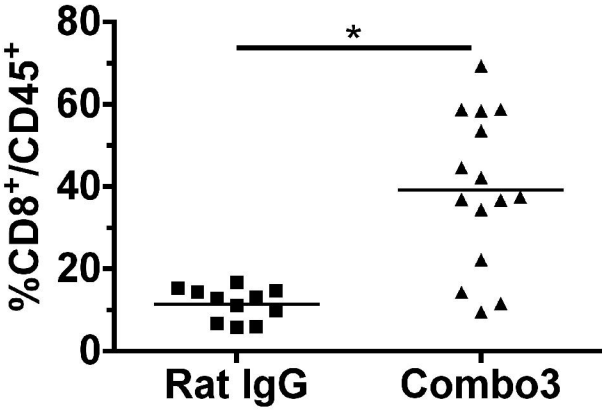
Rat IgG | Combo3 | ACT + Combo3

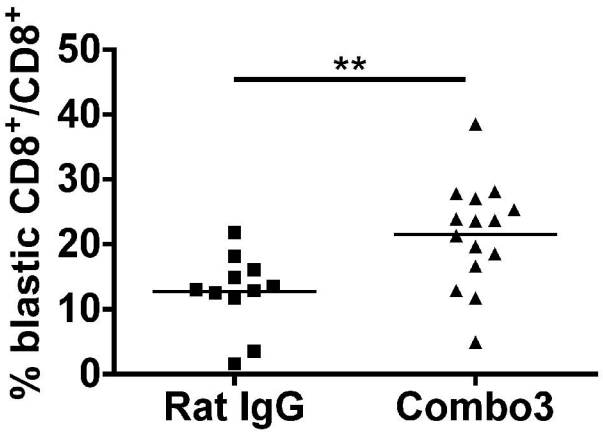
Week 6

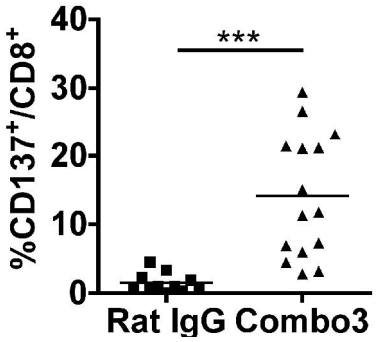
42,42 mm^2 | 11,18 mm^2 | No tumor

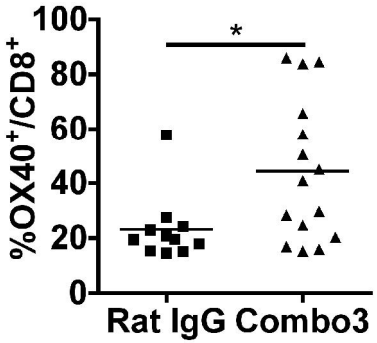
TILs

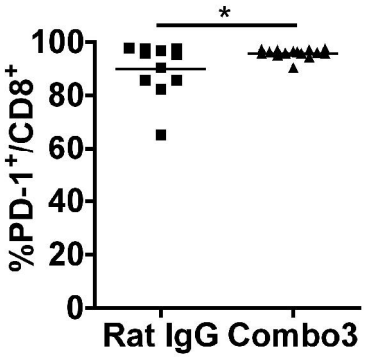
$CD8^+$

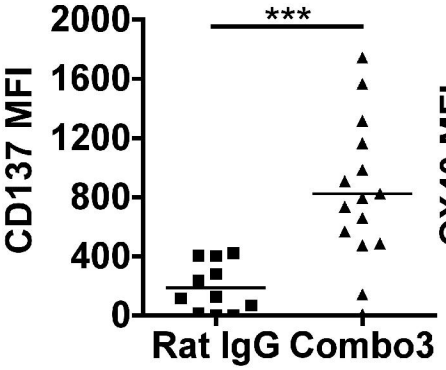

%CD8+/CD45+
80
60
40
20
0
*
Rat IgG
Combo3

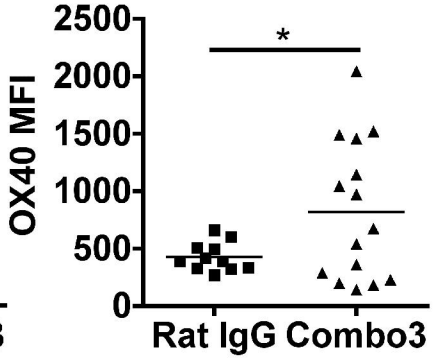

% blastic CD8+/CD8+
50
40
30
20
10
0
**
Rat IgG
Combo3

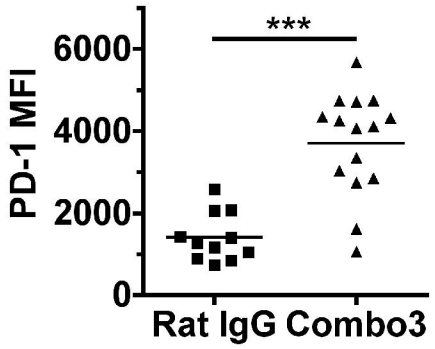

%CD137+/CD8+
40
30
20
10
0
***
Rat IgG Combo3


%OX40+/CD8+
100
80
60
40
20
0
*
Rat IgG Combo3


%PD-1+/CD8+
100
80
60
40
20
0
*
Rat IgG Combo3

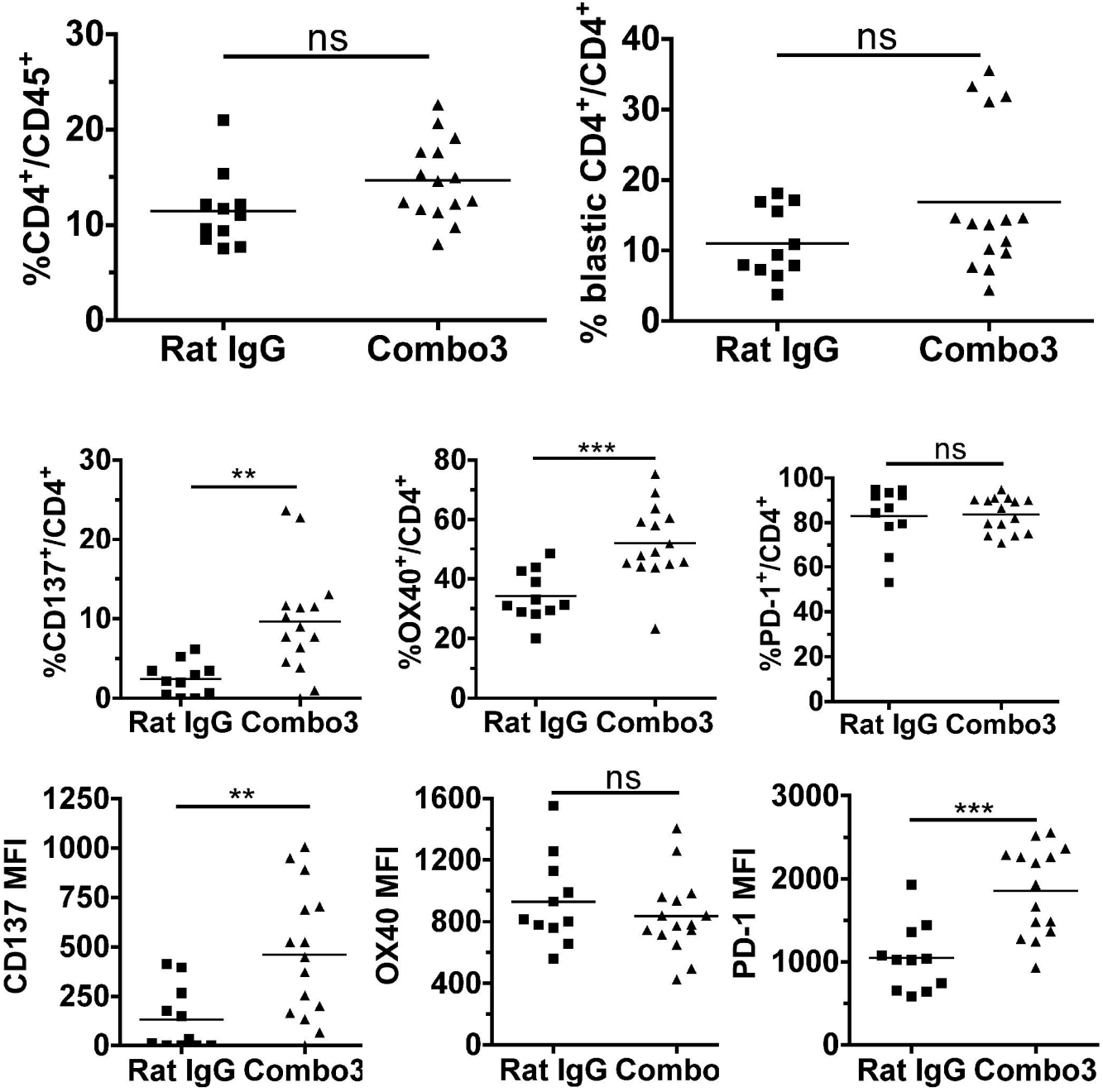

CD137 MFI
2000
1600
1200
800
400
0
***
Rat IgG Combo3


OX40 MFI
2500
2000
1500
1000
500
0
*
Rat IgG Combo3

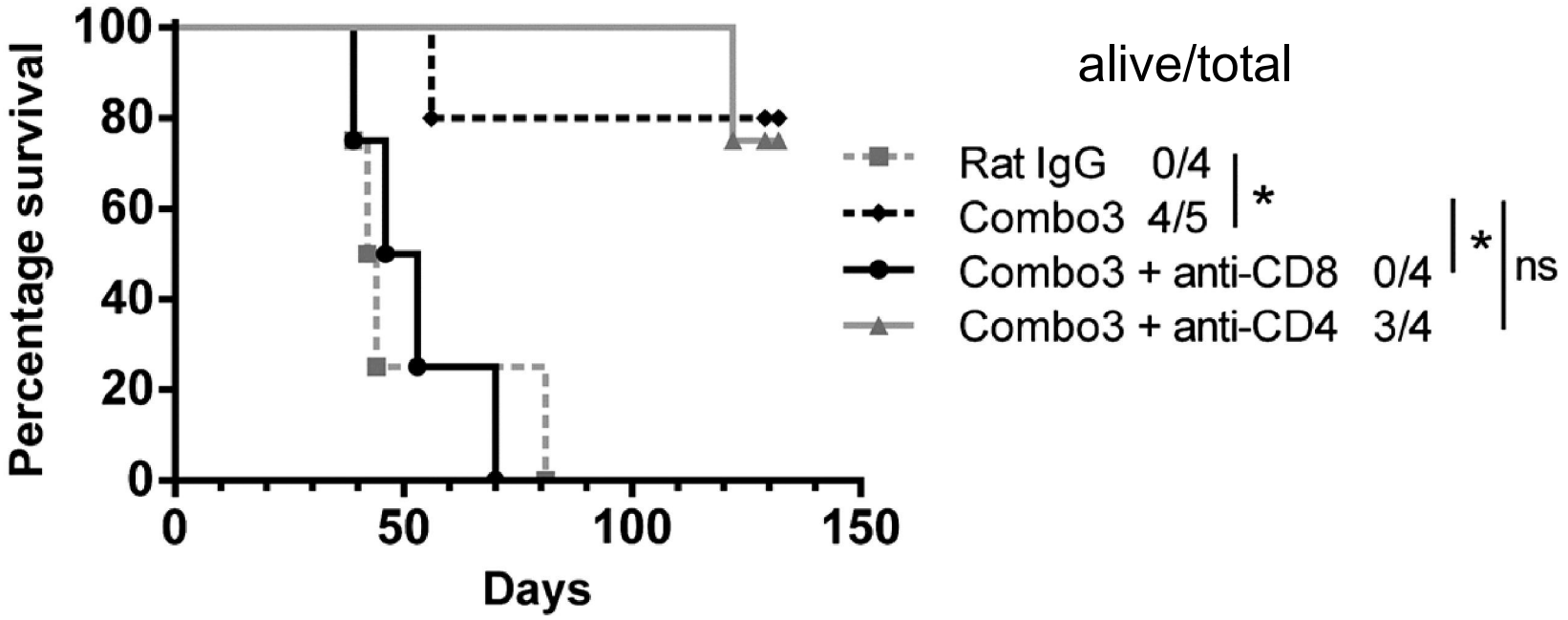

PD-1 MFI
6000
4000
2000
0
***
Rat IgG Combo3

TILs

$CD4^+$

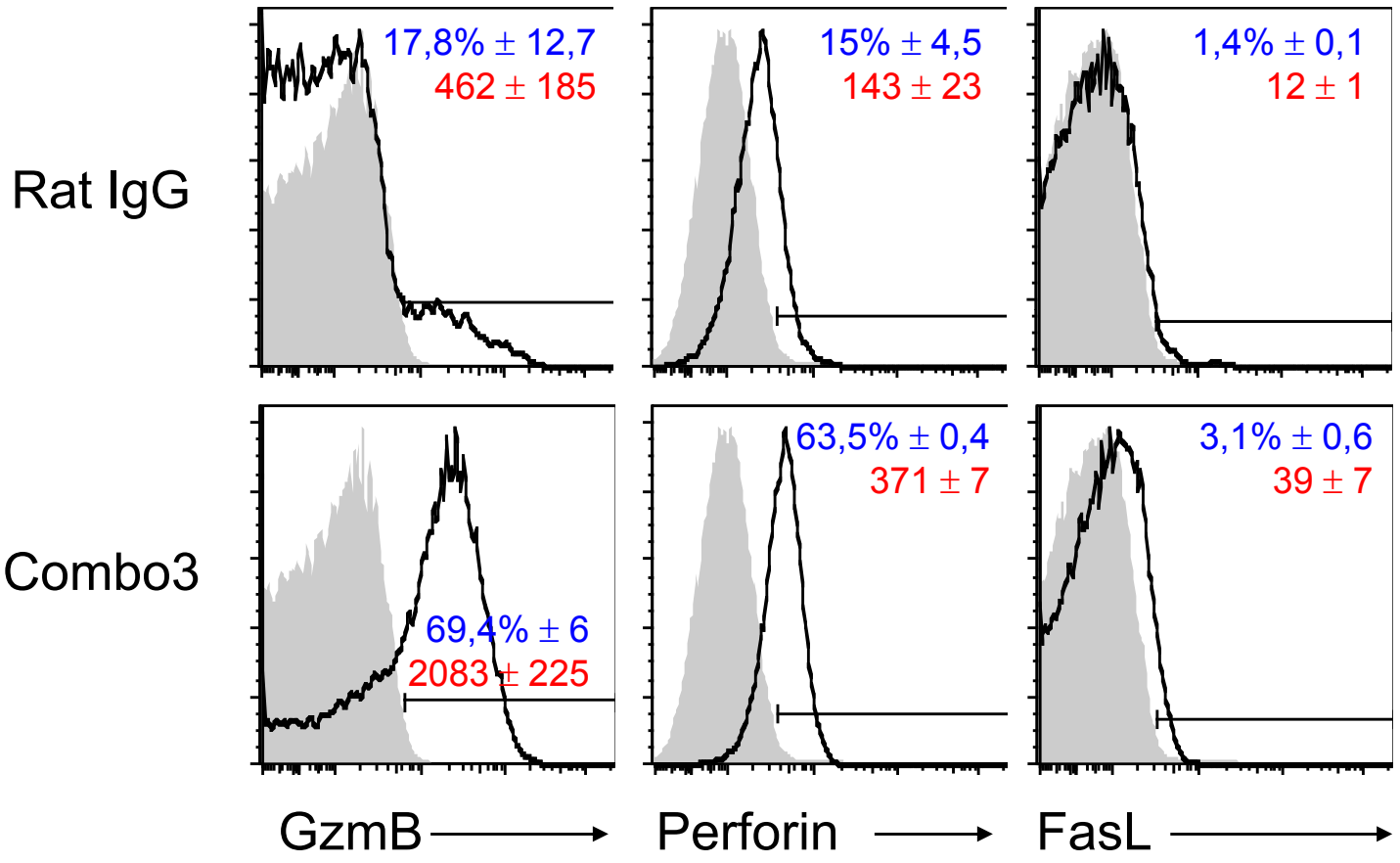

%CD4+/CD45+
ns
Rat IgG
Combo3
% blastic CD4+/CD4+
ns
Rat IgG
Combo3
%CD137+/CD4+
**
Rat IgG Combo3
%OX40+/CD4+
***
Rat IgG Combo3
%PD-1+/CD4+
ns
Rat IgG Combo3
CD137 MFI
**
Rat IgG Combo3
OX40 MFI
ns
Rat IgG Combo
PD-1 MFI
***
Rat IgG Combo3

Combo3 efficacy is dependent on CD8^{+} T cells



Effector machinery is enhanced upon Combo3 Tx

Gated on CD8 TILs



Synergy with adoptive T cell therapy

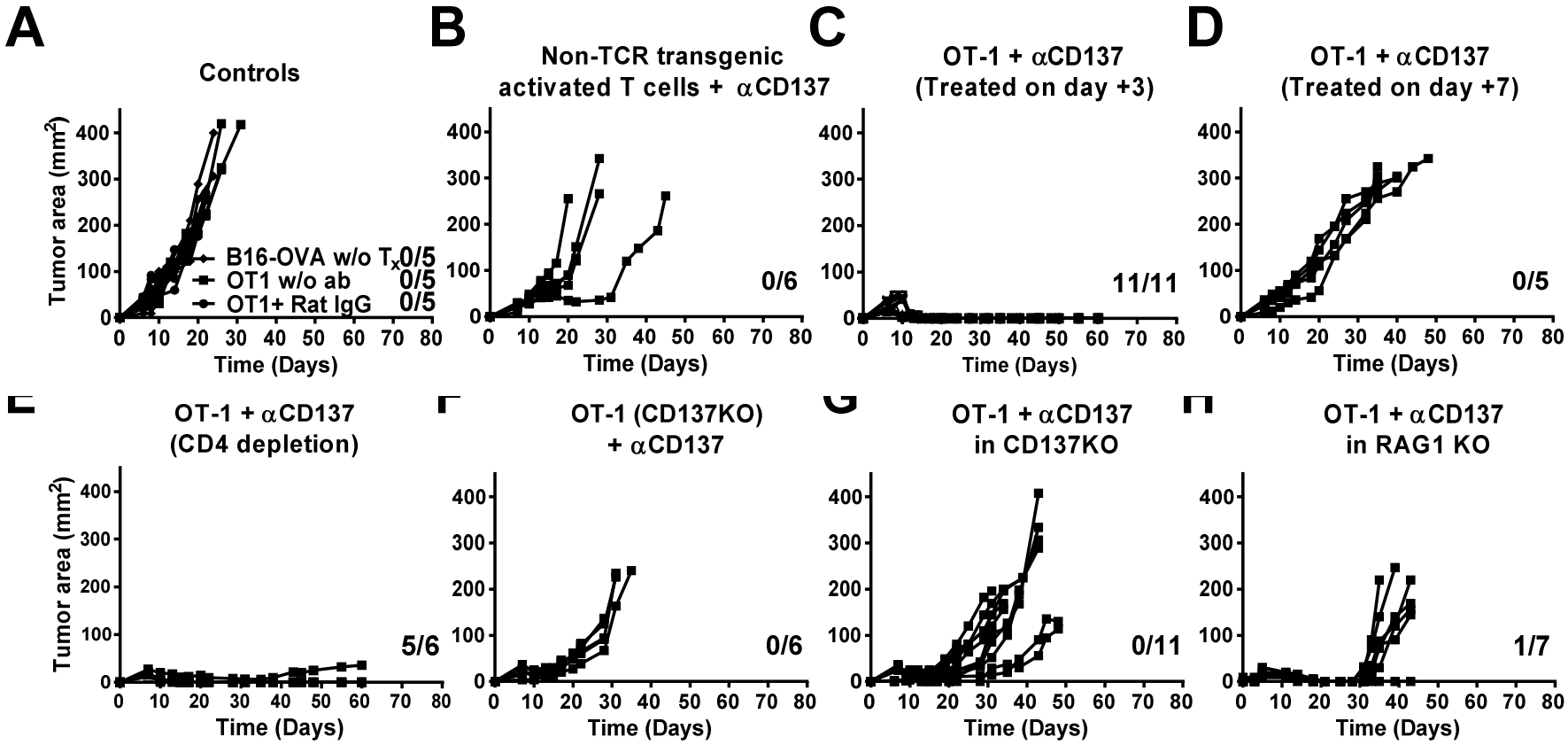

A
Controls
B16-OVA w/o Tx 0/5
OT1 w/o ab 0/5
OT1+ Rat IgG 0/5
B
Non-TCR transgenic
activated T cells + αCD137
0/6
C
OT-1 + αCD137
(Treated on day +3)
11/11
D
OT-1 + αCD137
(Treated on day +7)
0/5
E
OT-1 + αCD137
(CD4 depletion)
5/6
F
OT-1 (CD137KO)
+ αCD137
0/6
G
OT-1 + αCD137
in CD137KO
0/11
H
OT-1 + αCD137
in RAG1 KO
1/7
Tumor area (mm2)
Time (Days)

Figure 2.

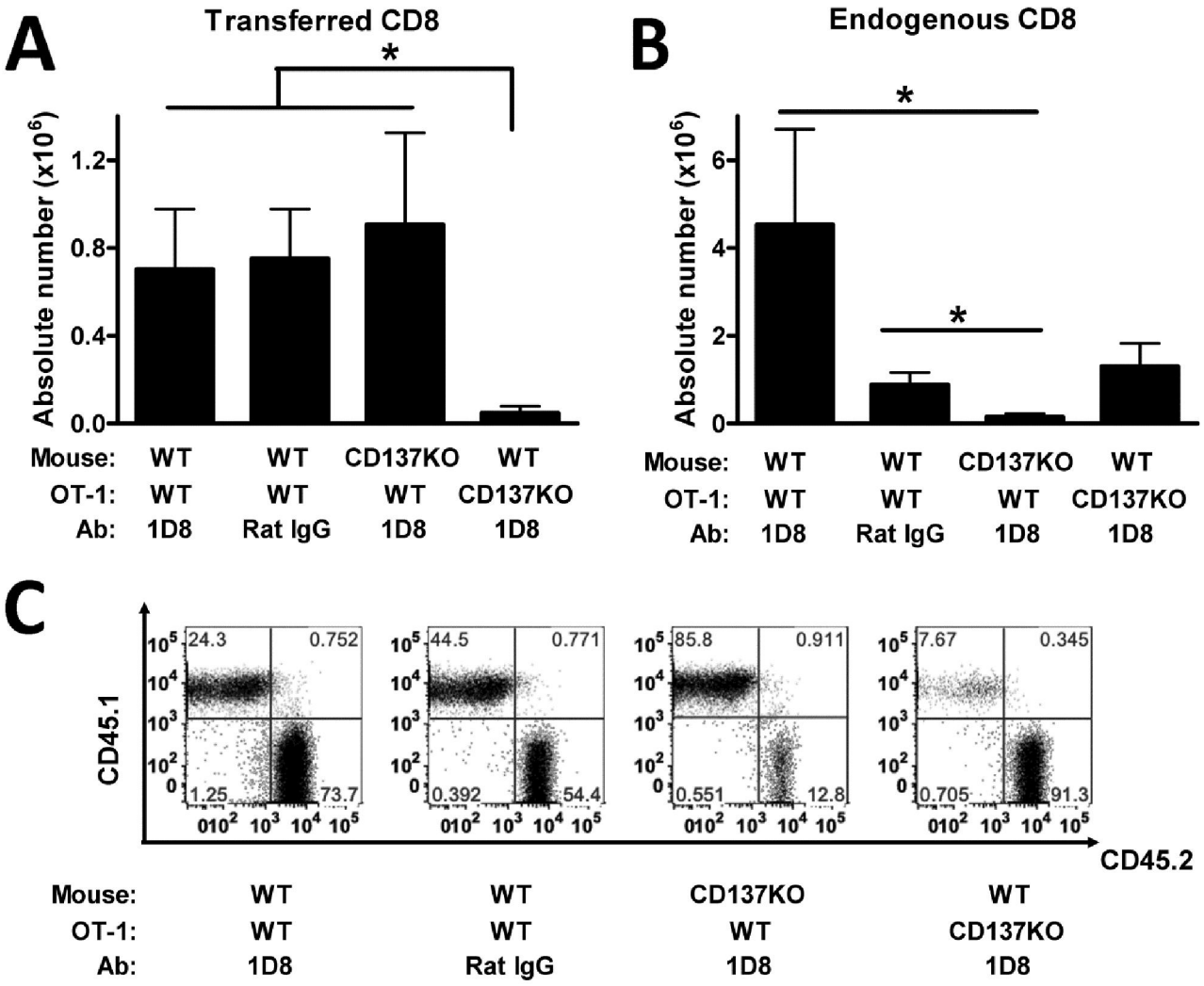
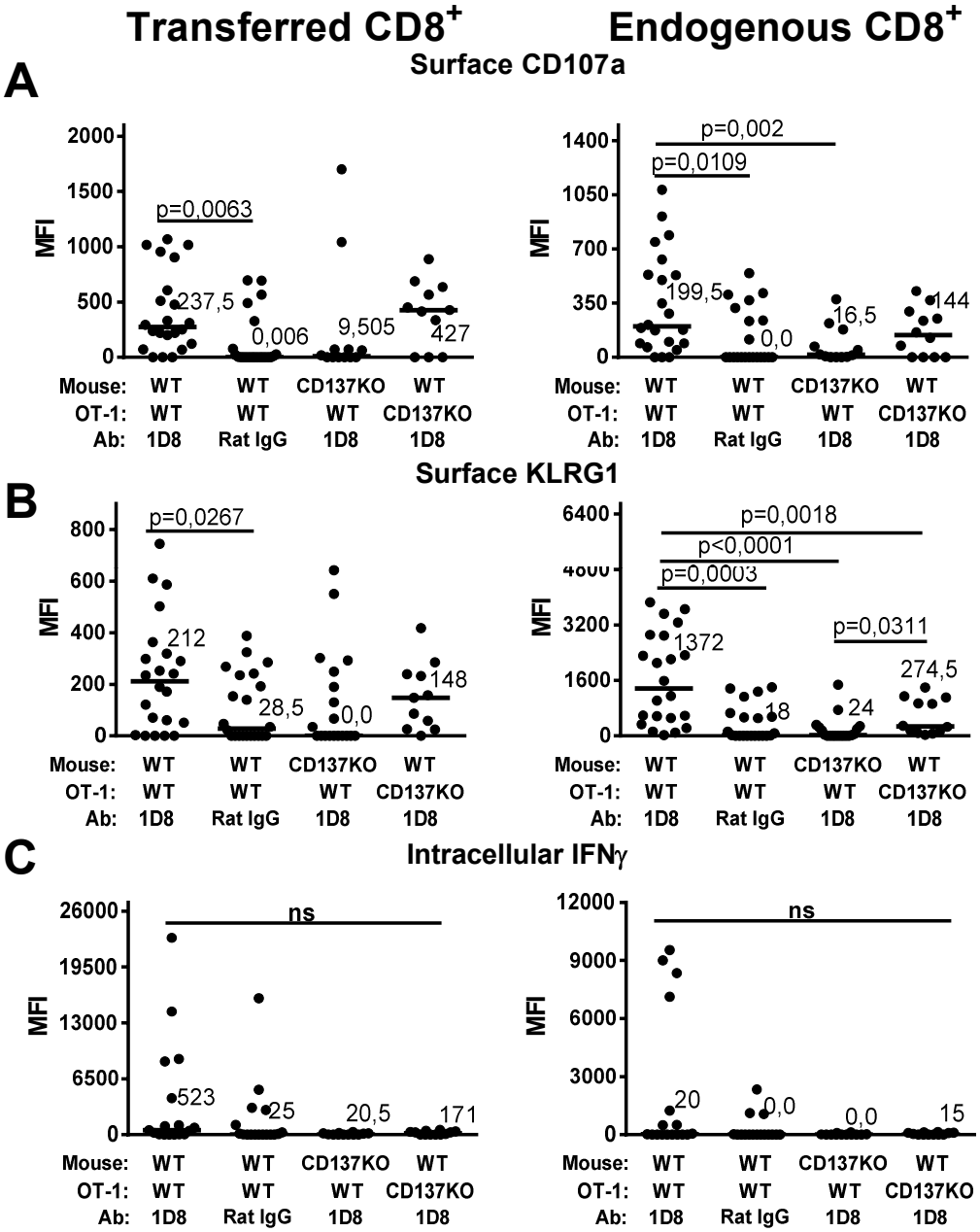
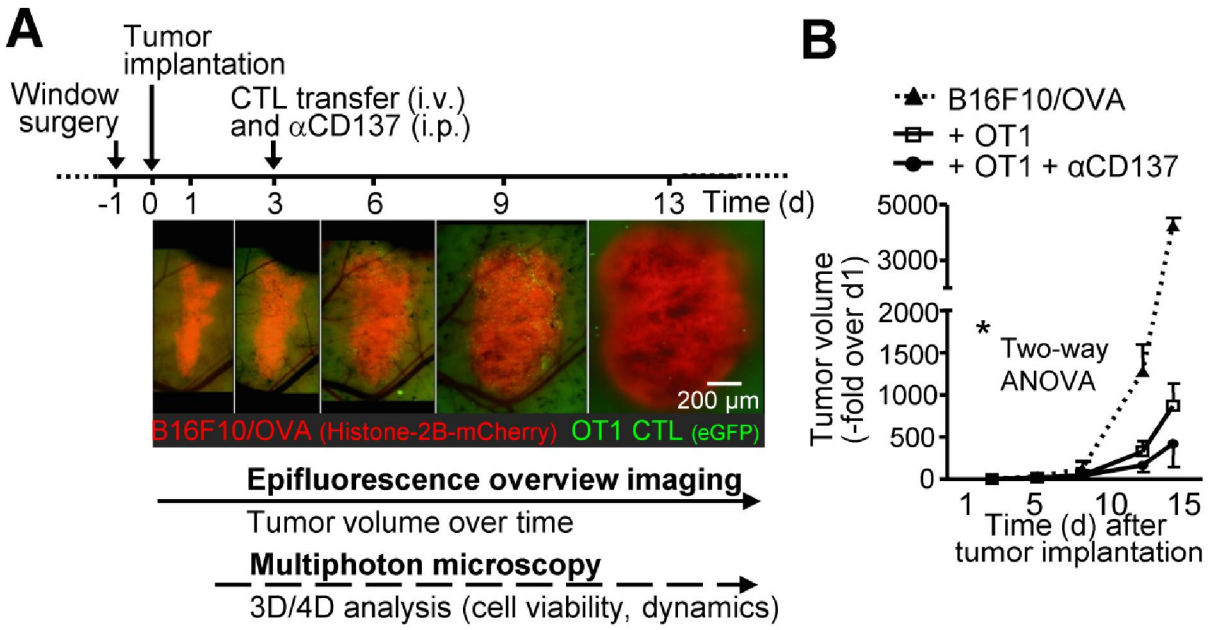
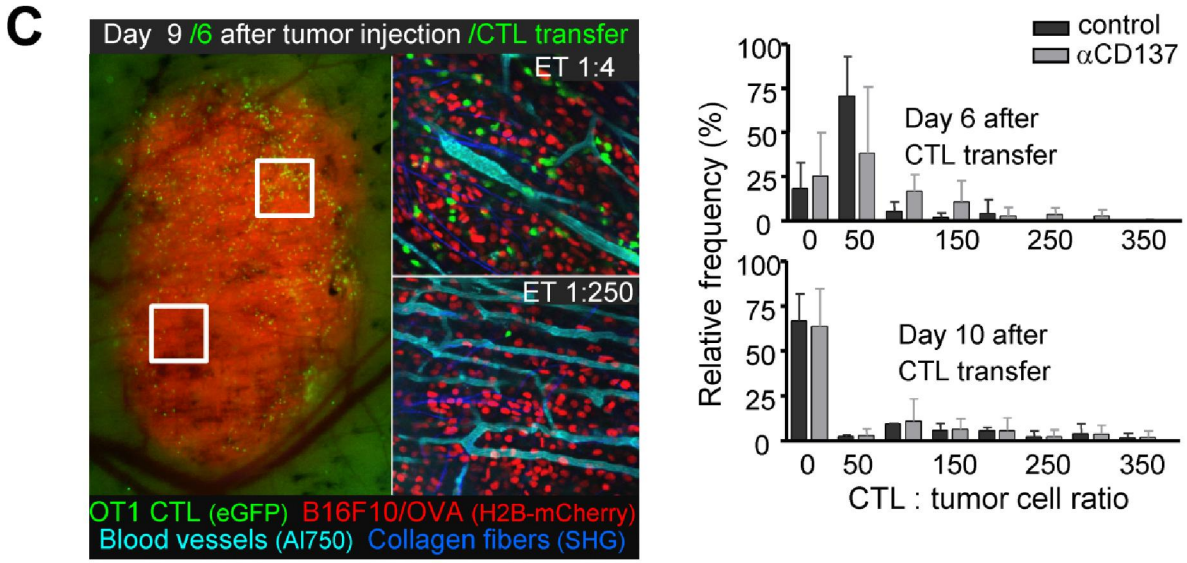
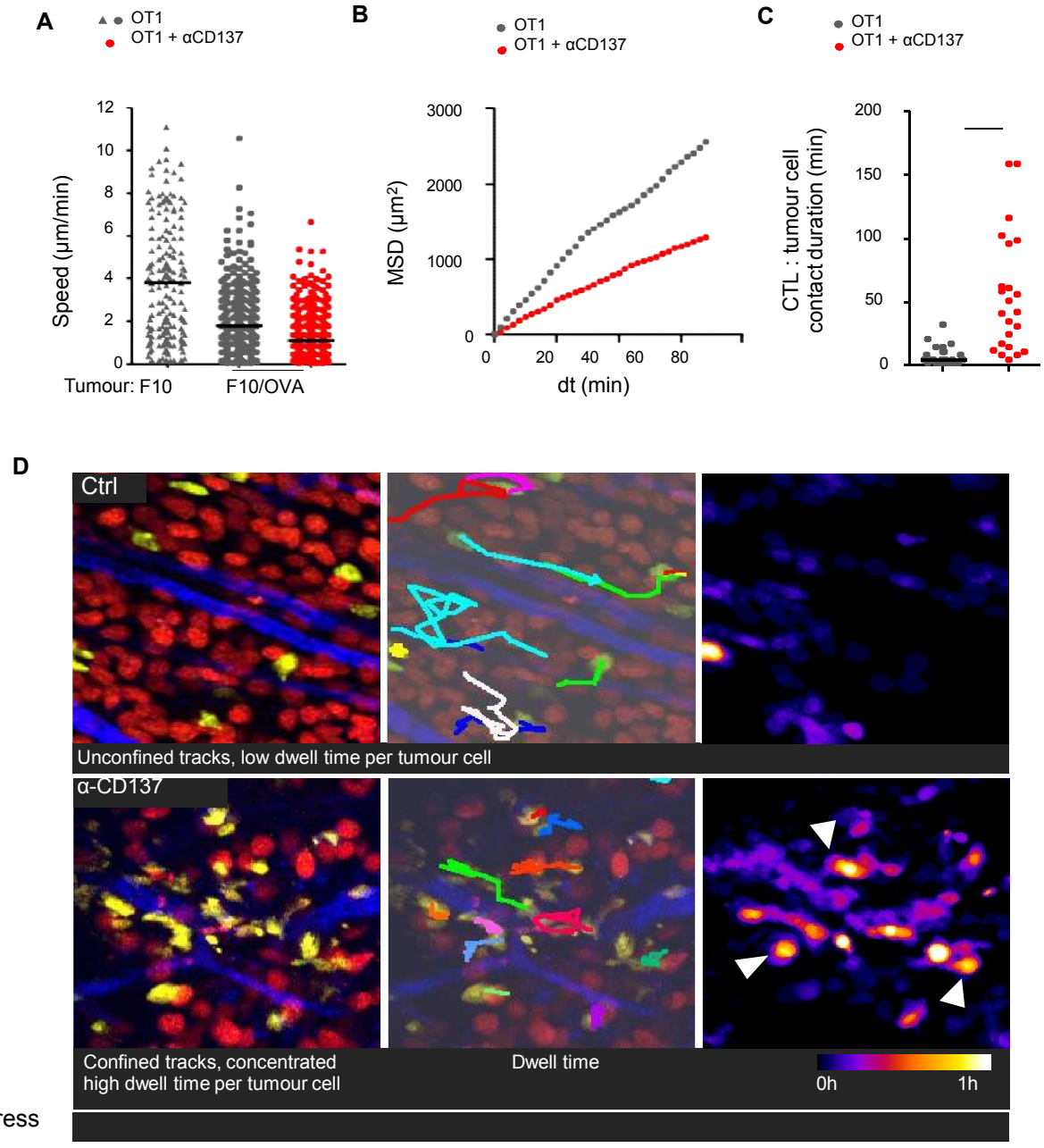

A
Transferred CD8
*
Absolute number (x10^6)
1.2
0.8
0.4
0.0
Mouse: WT WT CD137KO WT
OT-1: WT WT WT CD137KO
Ab: 1D8 Rat IgG 1D8 1D8
B
Endogenous CD8
*
*
Absolute number (x10^6)
6
4
2
0
Mouse: WT WT CD137KO WT
OT-1: WT WT WT CD137KO
Ab: 1D8 Rat IgG 1D8 1D8
C
CD45.1
CD45.2
24.3
0.752
1.25
73.7
44.5
0.771
0.392
54.4
85.8
0.911
0.551
12.8
7.67
0.345
0.705
91.3
Mouse: WT WT CD137KO WT
OT-1: WT WT WT CD137KO
Ab: 1D8 Rat IgG 1D8 1D8

Figure 3.

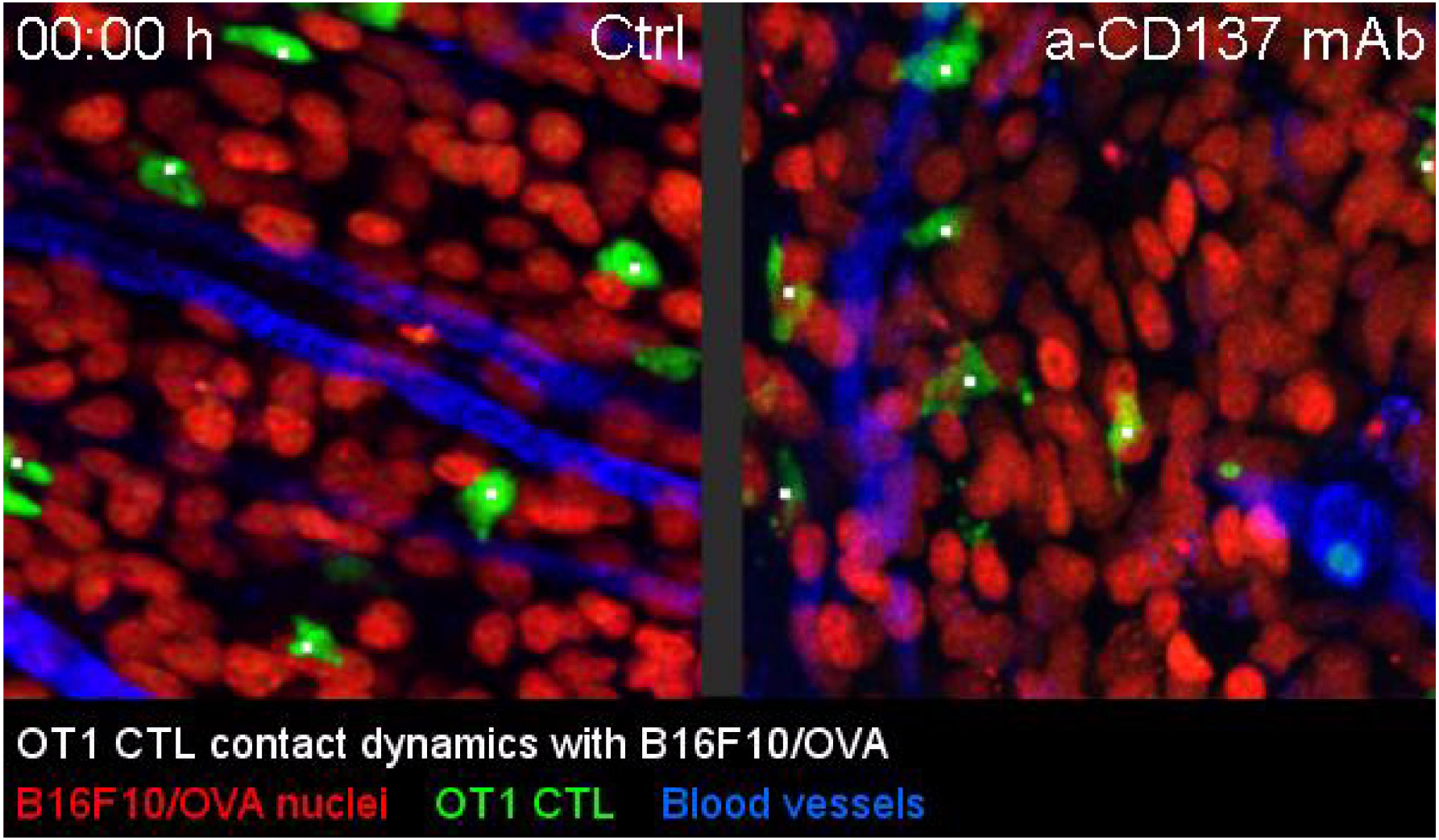

Transferred CD8+
Endogenous CD8+
A
Surface CD107a
MFI
p=0,0063
237,5
0,006
9,505
427
p=0,002
p=0,0109
199,5
0,0
16,5
144
B
Surface KLRG1
p=0,0267
212
28,5
0,0
148
p=0,0018
p<0,0001
p=0,0003
1372
18
p=0,0311
24
274,5
C
Intracellular IFNγ
ns
523
25
20,5
171
20
0,0
0,0
15
Mouse: WT WT CD137KO WT
OT-1: WT WT WT CD137KO
Ab: 1D8 Rat IgG 1D8 1D8

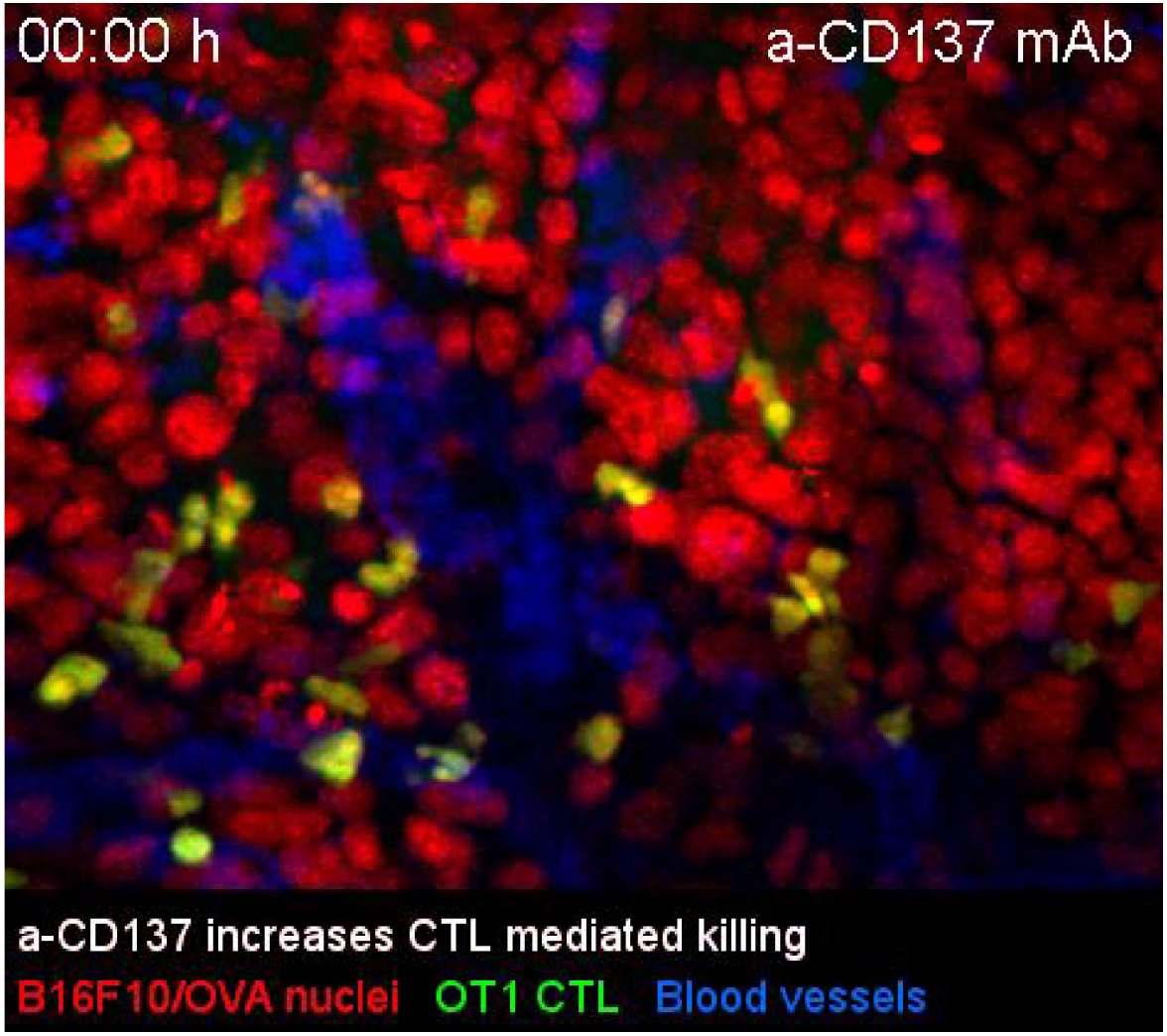

A
Tumor implantation
Window surgery
CTL transfer (i.v.) and αCD137 (i.p.)
-1 0 1 3 6 9 13 Time (d)
200 µm
B16F10/OVA (Histone-2B-mCherry) OT1 CTL (eGFP)
Epifluorescence overview imaging
Tumor volume over time
Multiphoton microscopy
3D/4D analysis (cell viability, dynamics)
B
B16F10/OVA
+ OT1
+ OT1 + αCD137
Tumor volume (-fold over d1)
5000
3000
2000
1500
1000
500
0
*
Two-way ANOVA
1 5 10 15
Time (d) after tumor implantation


C
Day 9 /6 after tumor injection /CTL transfer
ET 1:4
ET 1:250
OT1 CTL (eGFP) B16F10/OVA (H2B-mCherry)
Blood vessels (AI750) Collagen fibers (SHG)
control
αCD137
Relative frequency (%)
100
75
50
25
0
Day 6 after CTL transfer
0 50 150 250 350
Day 10 after CTL transfer
CTL : tumor cell ratio

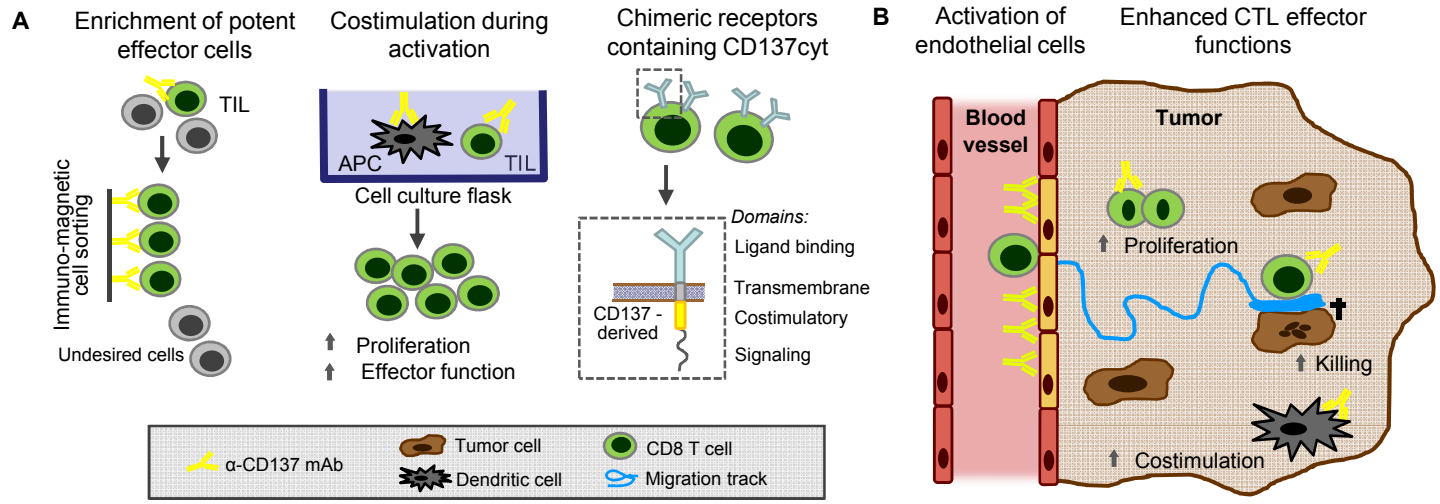


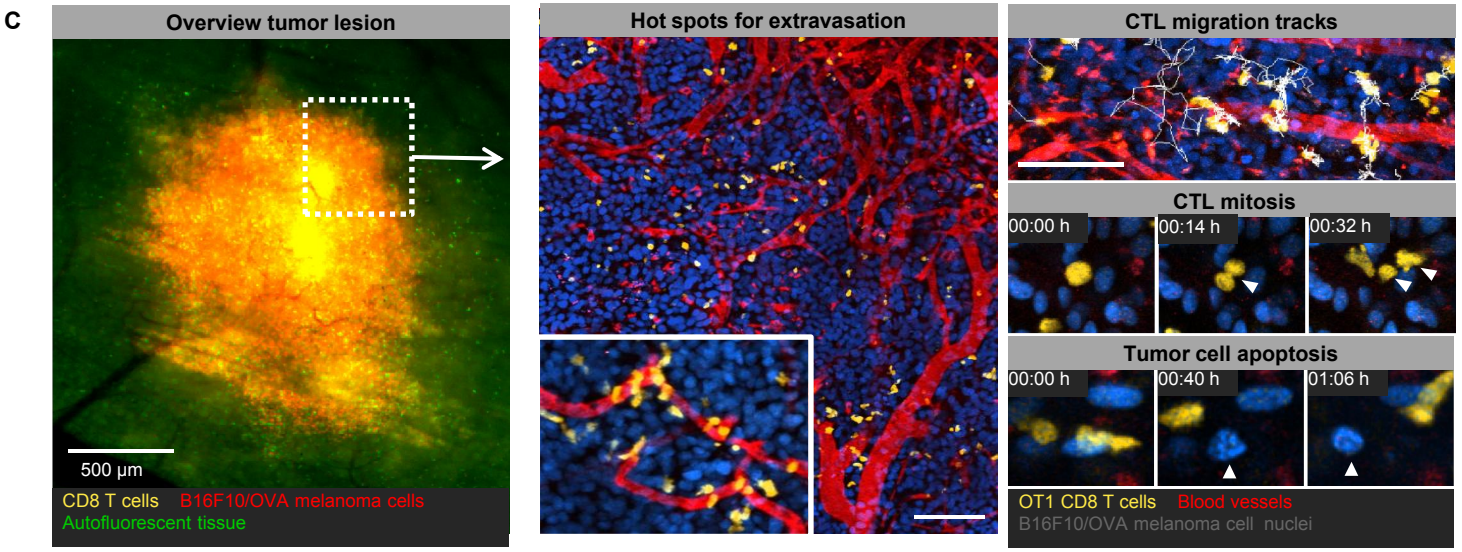
Weigelin, et al. PNAS in press

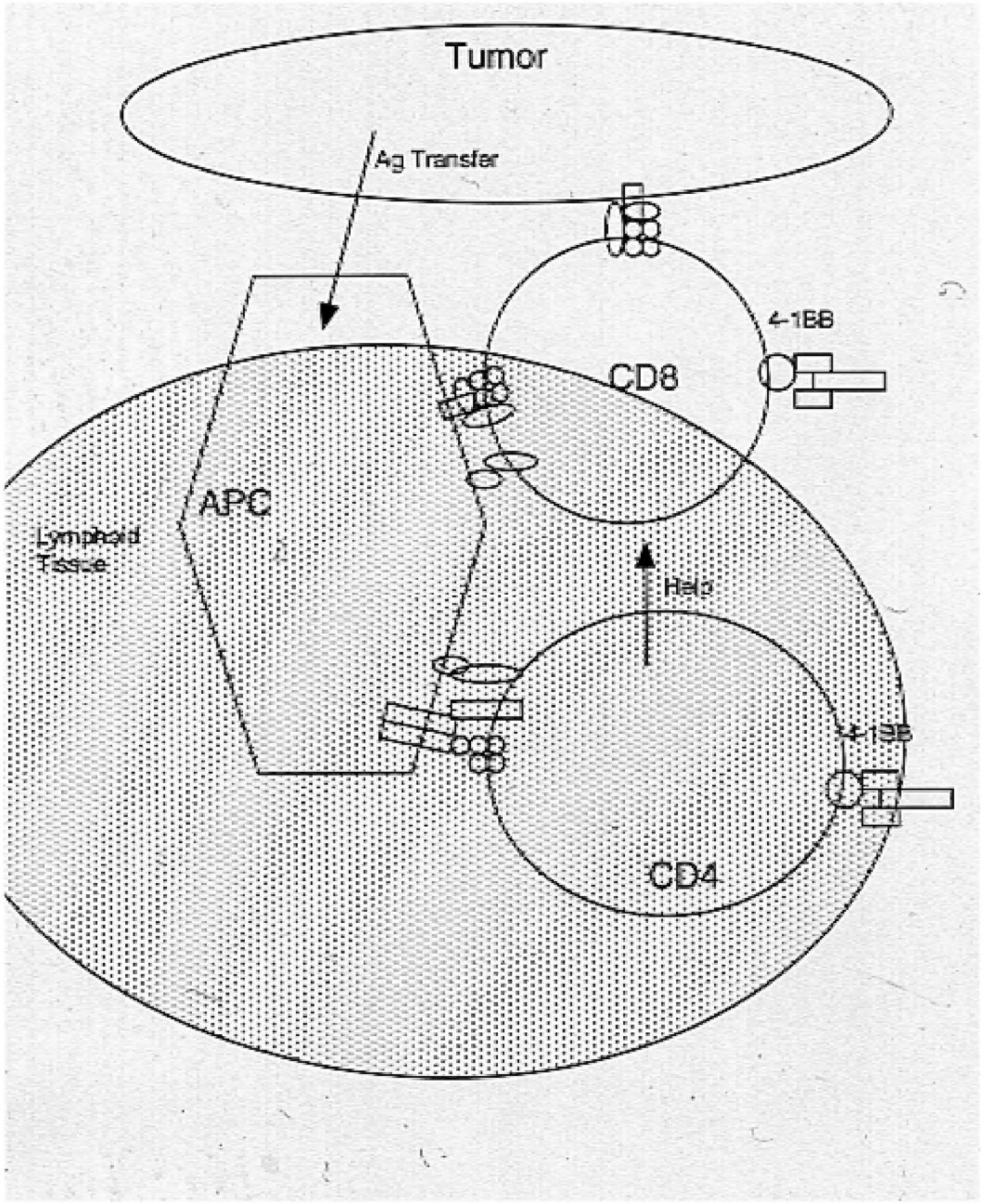

00:00 h
Ctrl
a-CD137 mAb
OT1 CTL contact dynamics with B16F10/OVA
B16F10/OVA nuclei
OT1 CTL
Blood vessels


00:00 h
a-CD137 mAb
a-CD137 increases CTL mediated killing
B16F10/OVA nuclei
OT1 CTL
Blood vessels

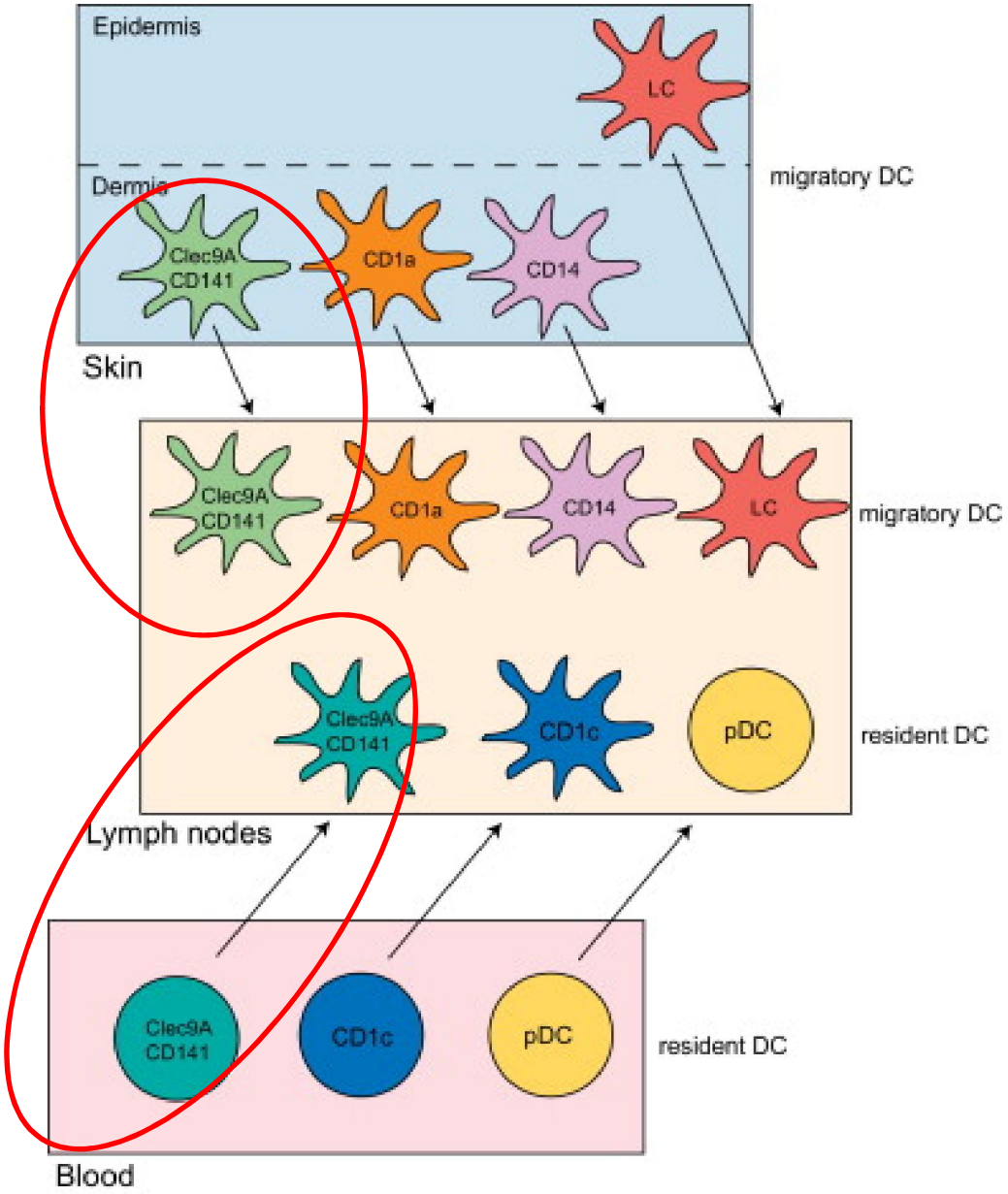
CD137 and adoptive T cell transfer. A perfect marriage?

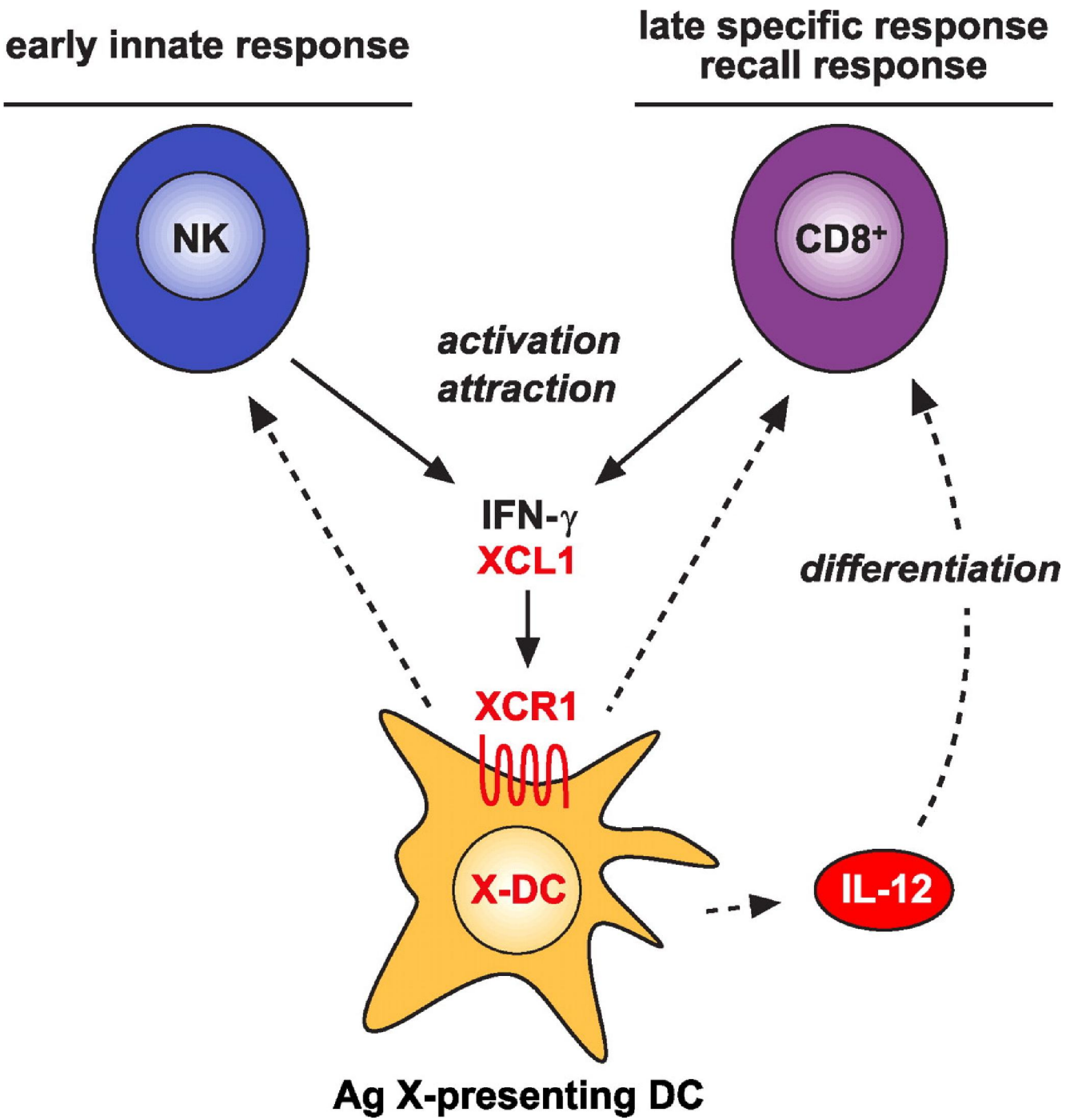




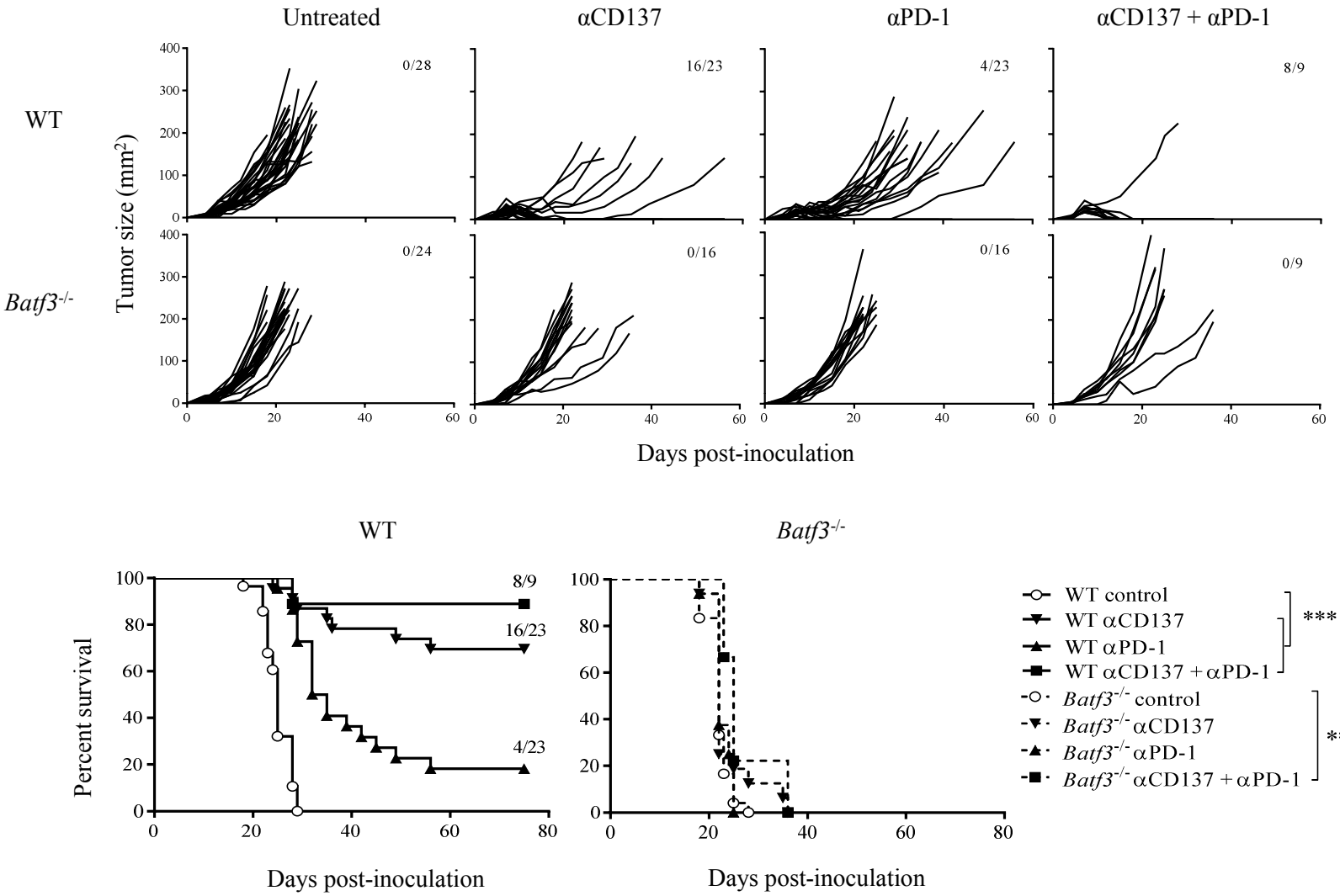

Tumor
Ag Transfer
4-1BB
CD8
APC
Lymphoid
Tissue
Help
4-1BB
CD4

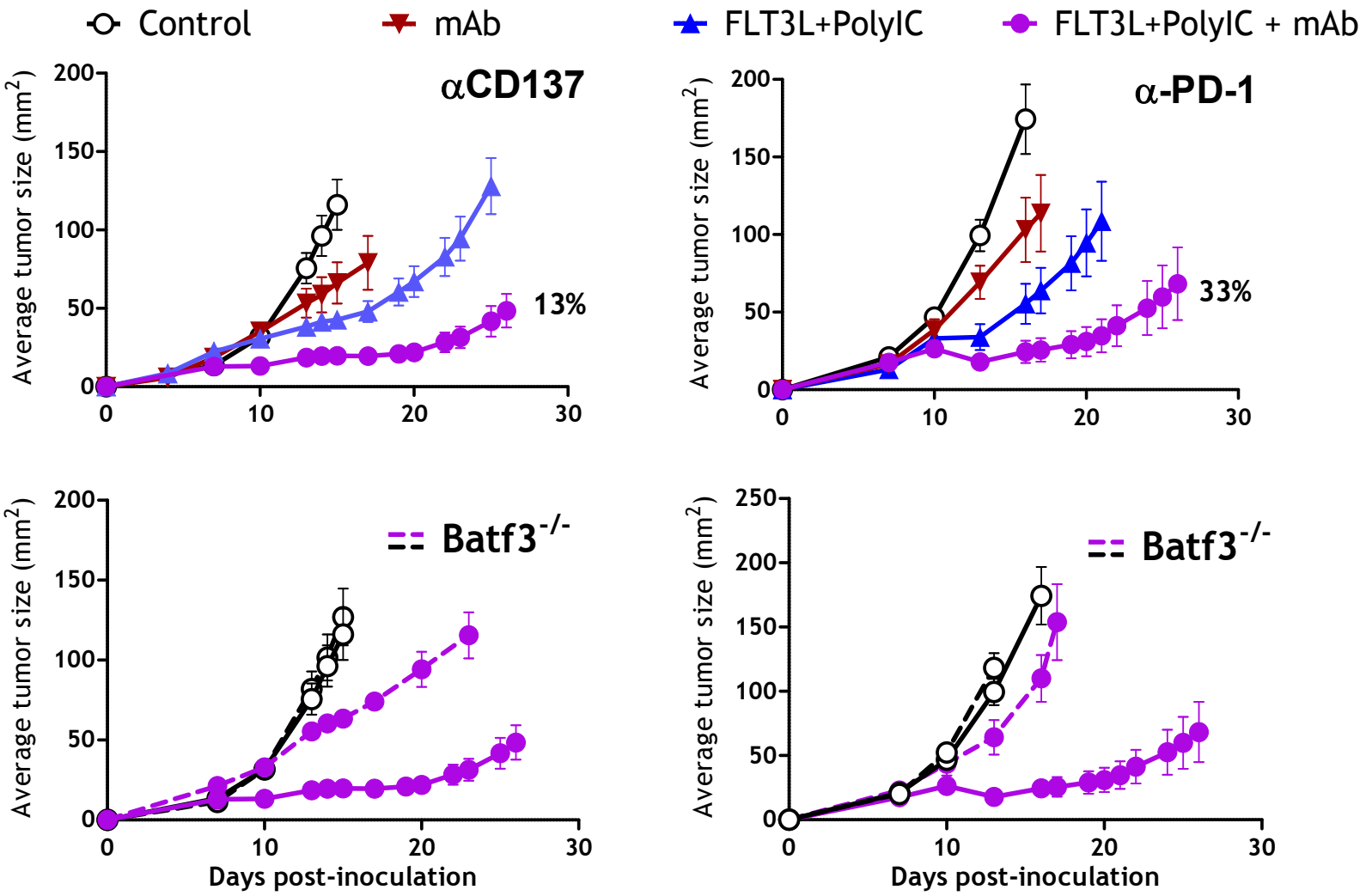
PROFESSIONAL
CROSSPRIMING
DENDRITIC CELLS




early innate response
late specific response
recall response
NK
CD8+
activation
attraction
IFN-γ
XCL1
differentiation
XCR1
X-DC
IL-12
Ag X-presenting DC

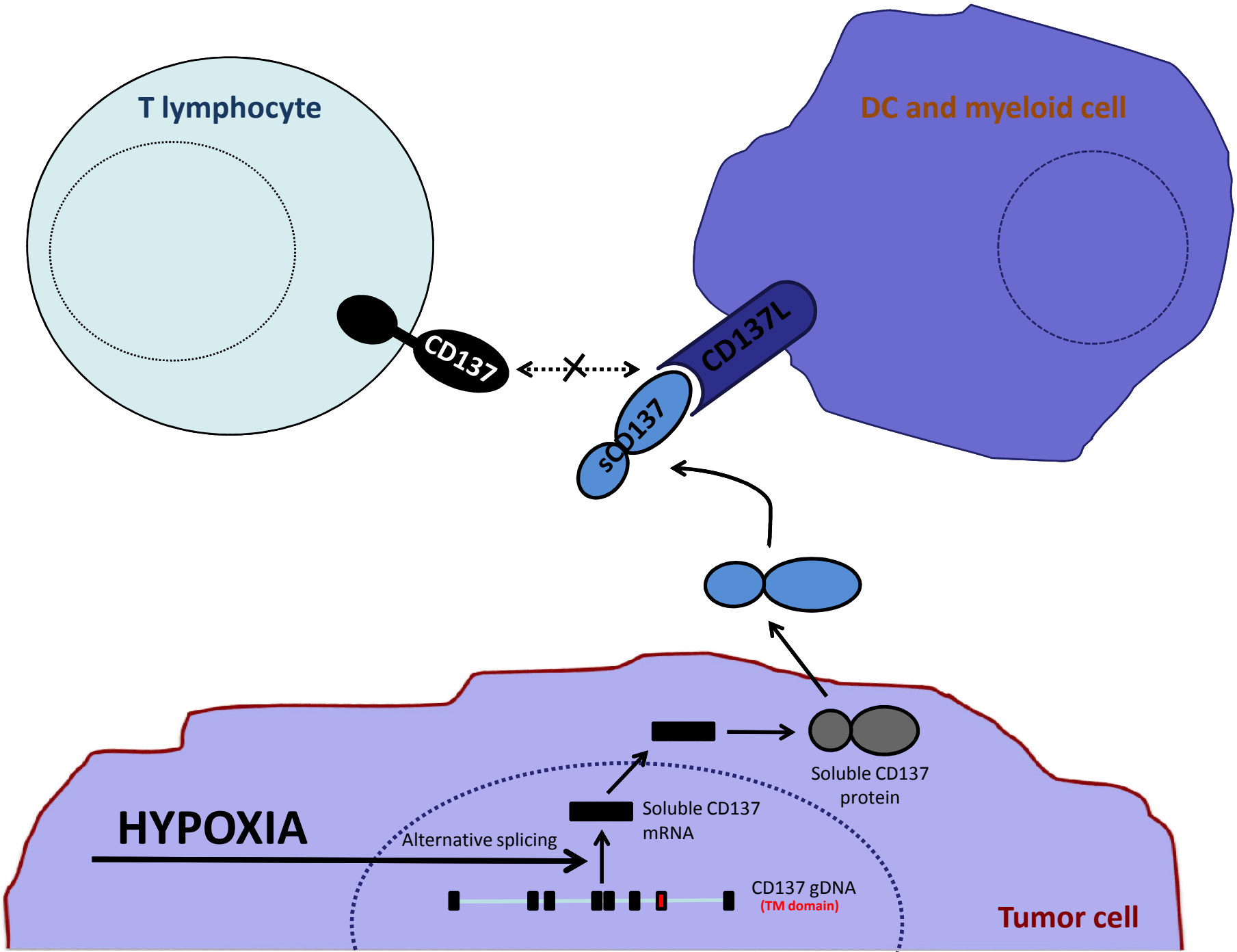
Crosspriming DC absolutely needed!



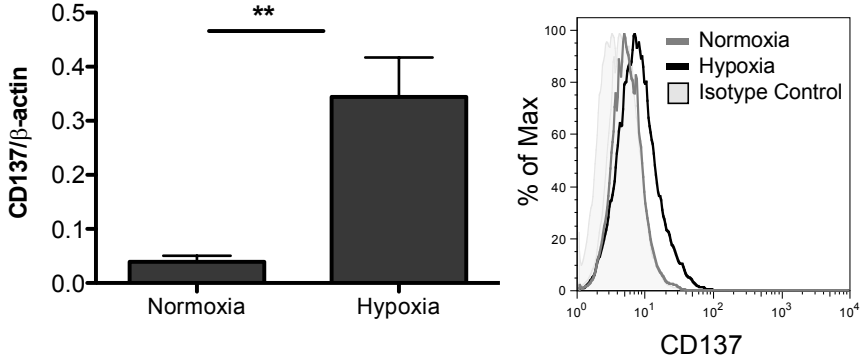

Control
mAb
FLT3L+PolyIC
FLT3L+PolyIC + mAb
αCD137
α-PD-1
Batf3-/-
Batf3-/-
13%
33%
Average tumor size (mm2)
Days post-inoculation
0
10
20
30
50
100
150
200
250

Cancer Immunotherapy

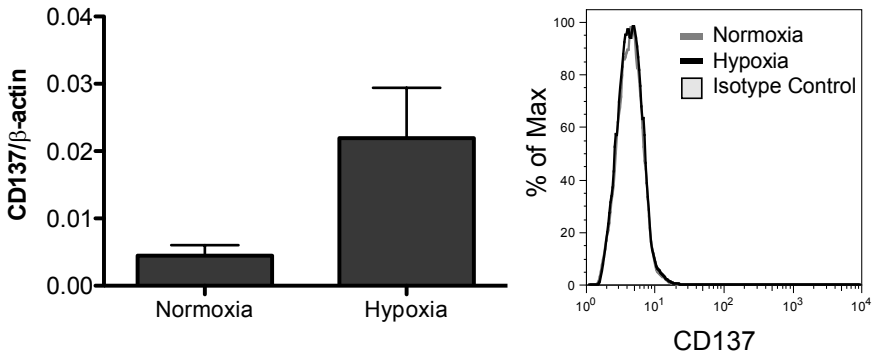
“Never understimate the power of the dark side”


T lymphocyte
DC and myeloid cell
CD137
CD137L
sCD137
HYPOXIA
Alternative splicing
Soluble CD137 mRNA
Soluble CD137 protein
CD137 gDNA
(TM domain)
Tumor cell

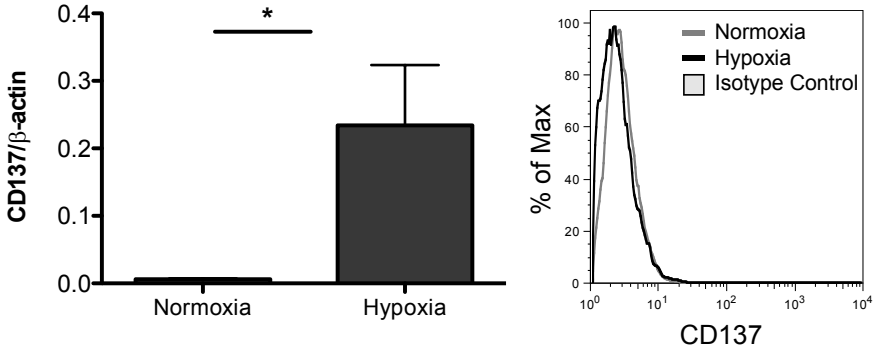
CT26 (Colon carcinoma)



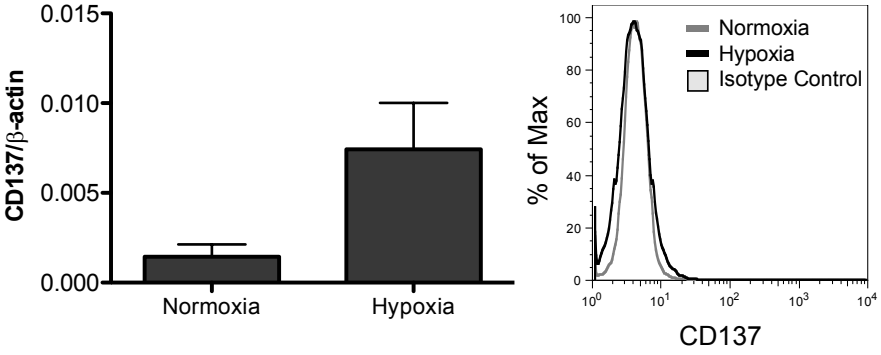
EL-4 (Thymoma)

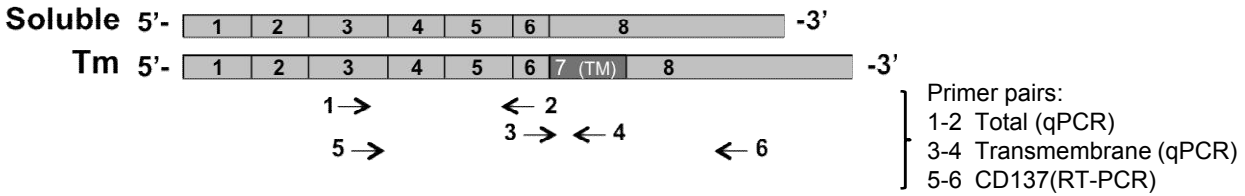


RENCA (Renal cell carcinoma)

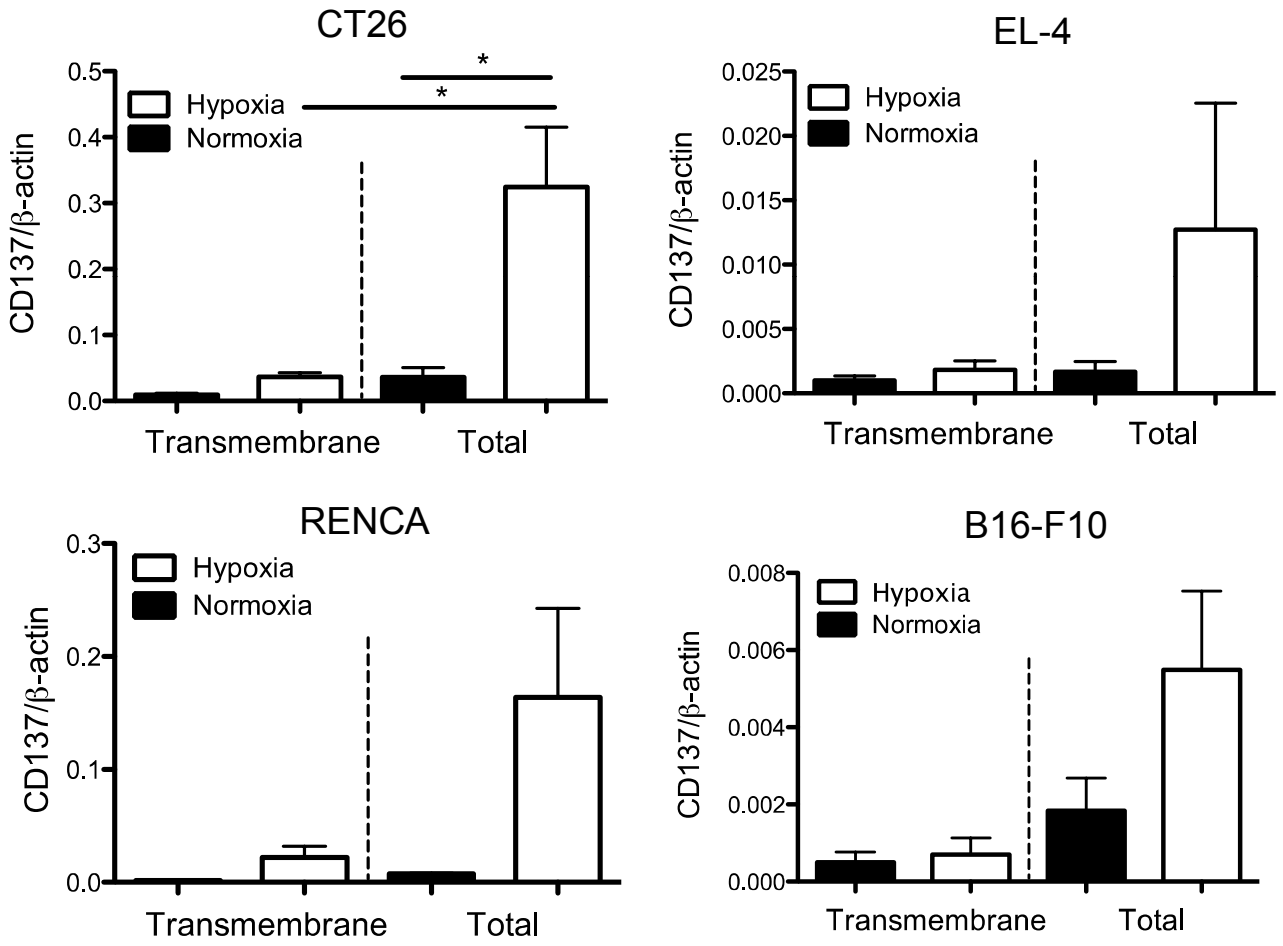


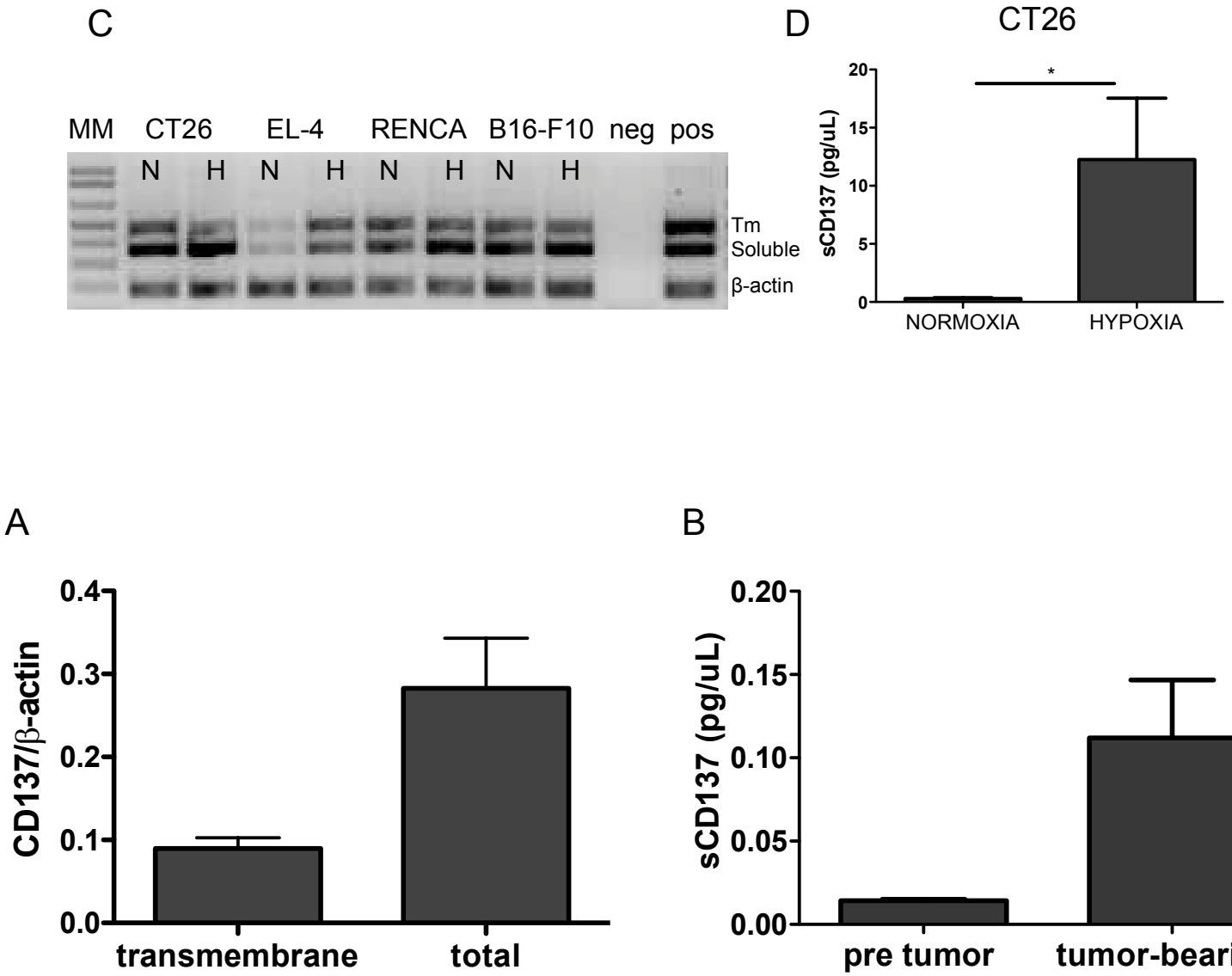
B16-F10 (Melanoma)

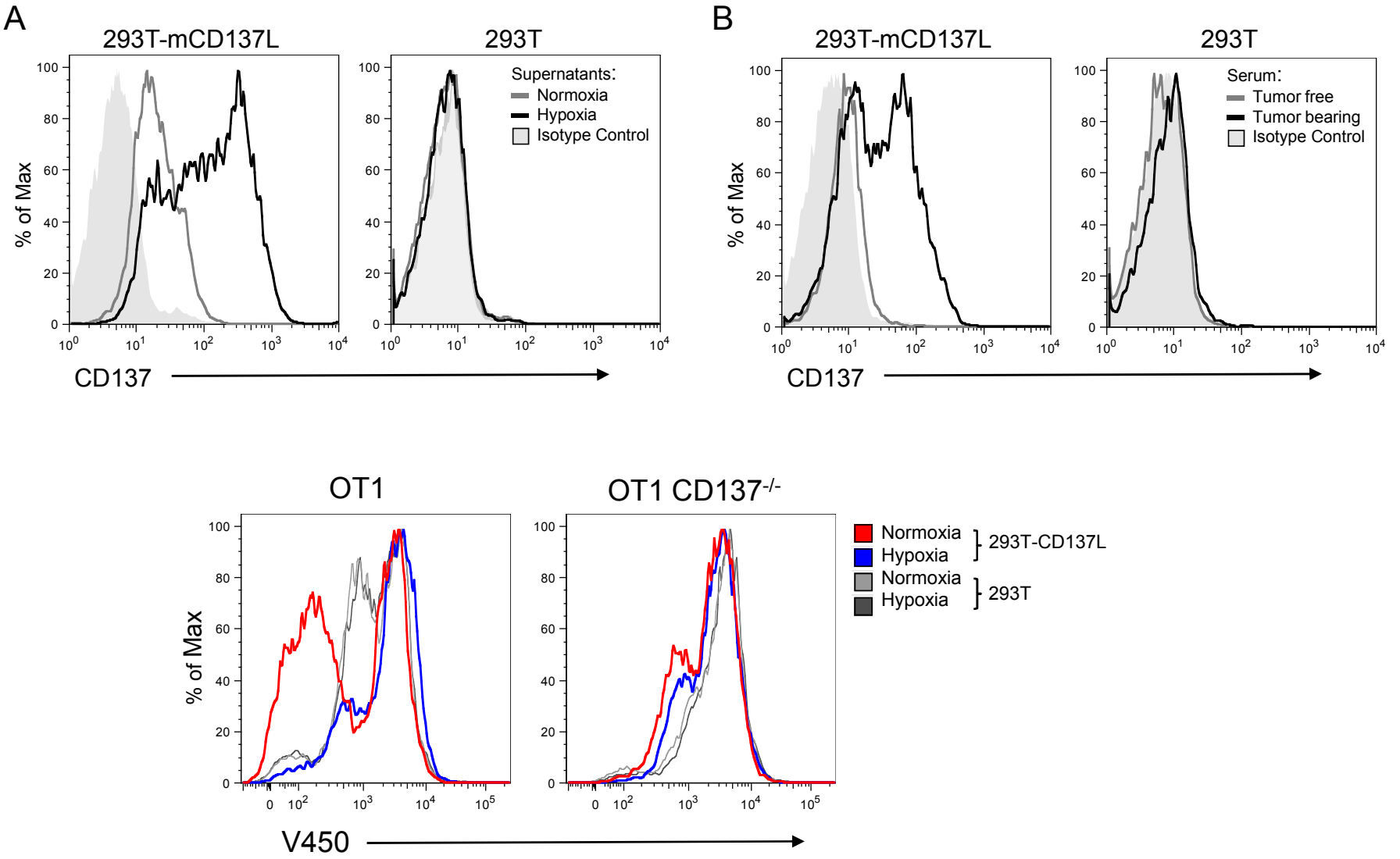


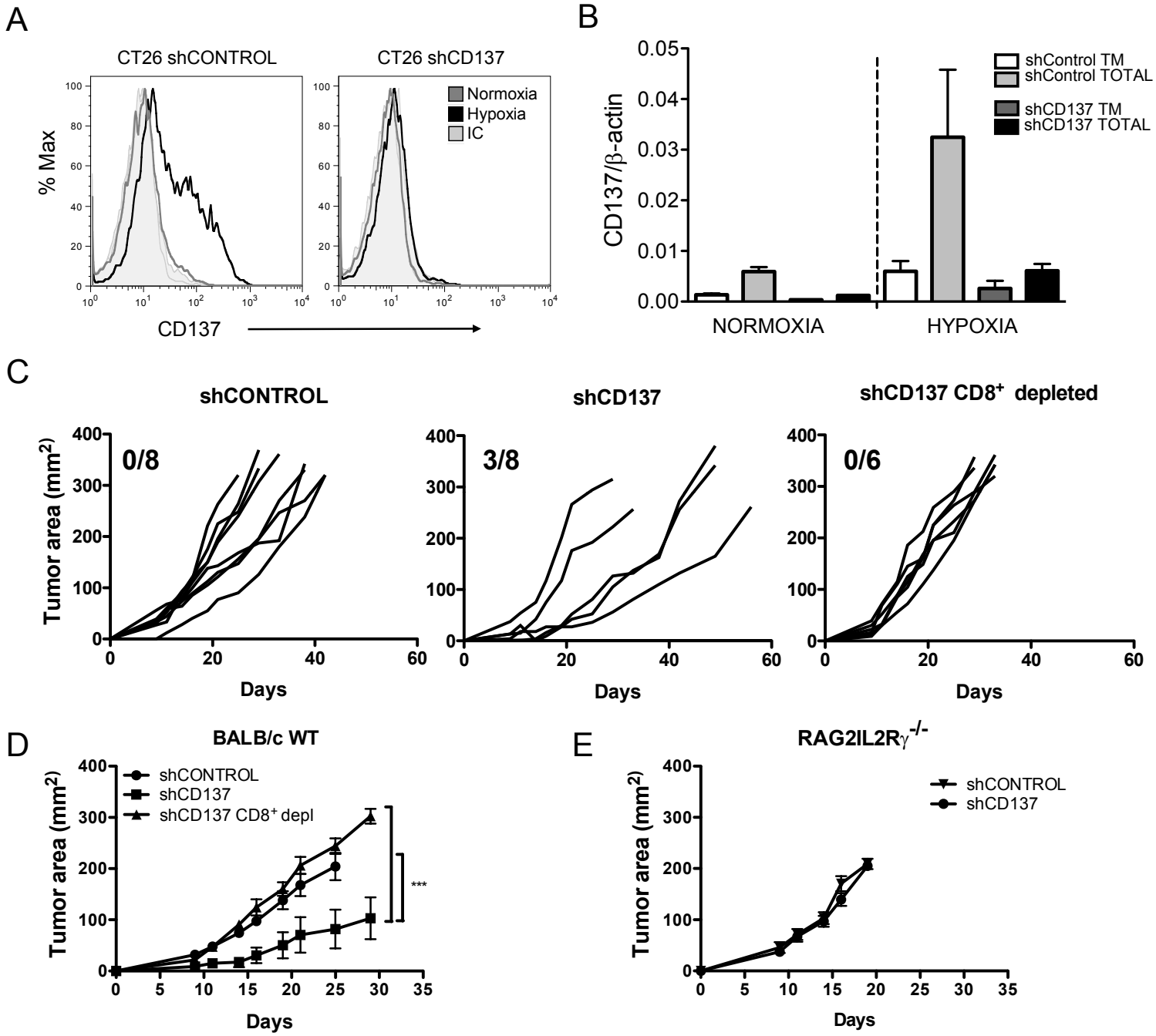

Soluble 5'-
1
2
3
4
5
6
8
-3'
Tm 5'-
1
2
3
4
5
6
7 (TM)
8
-3'
1
2
3
4
5
6
Primer pairs:
1-2 Total (qPCR)
3-4 Transmembrane (qPCR)
5-6 CD137(RT-PCR)

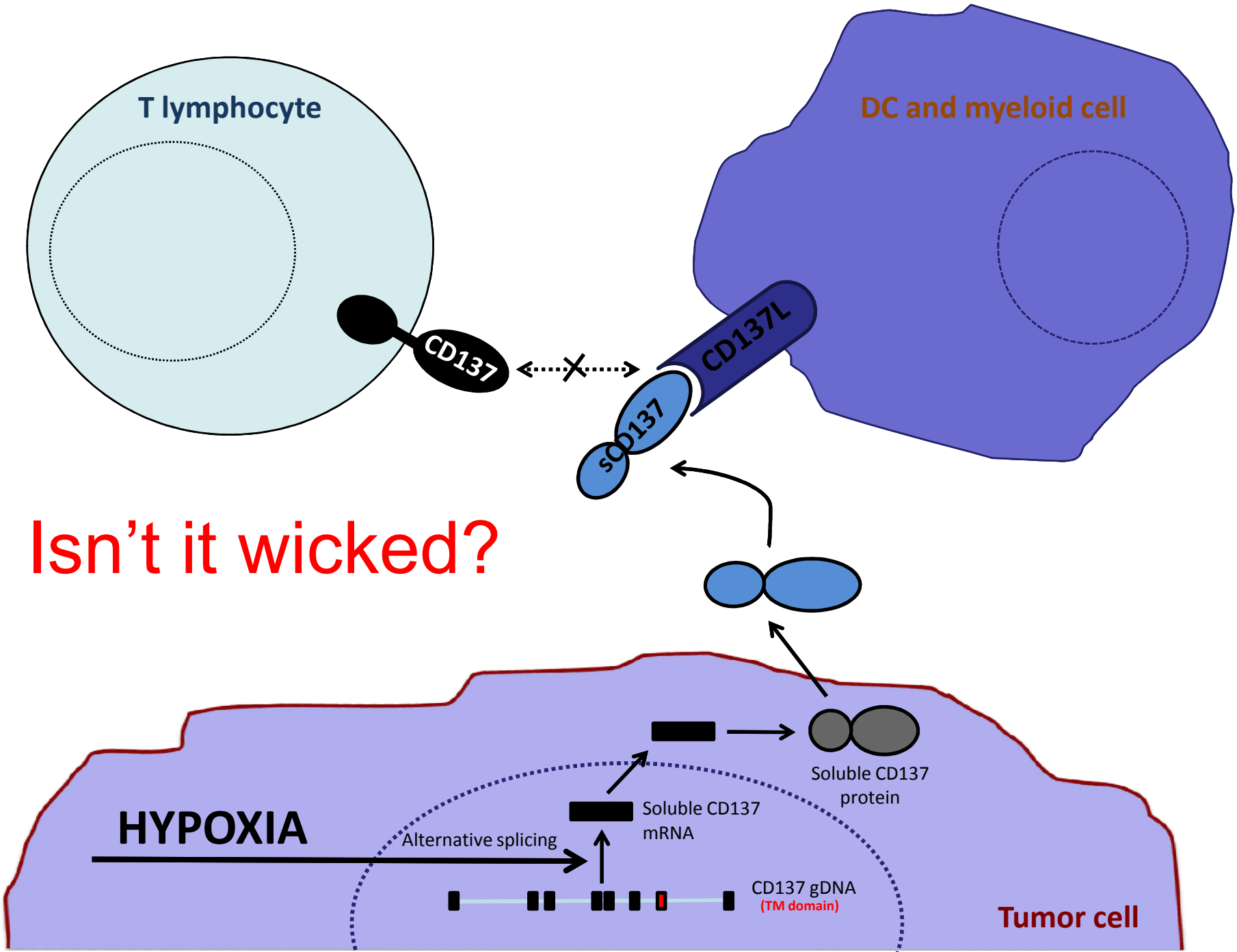
B


CT26
EL-4
RENCA
B16-F10
Hypoxia
Normoxia
CD137/β-actin
Transmembrane
Total
*
*
0.5
0.4
0.3
0.2
0.1
0.0
0.025
0.020
0.015
0.010
0.005
0.000
0.008
0.006
0.004
0.002


C
MM
CT26
EL-4
RENCA
B16-F10
neg
pos
N
H
N
H
N
H
N
H
Tm
Soluble
β-actin
D
CT26
sCD137 (pg/uL)
20
15
10
5
0
*
NORMOXIA
HYPOXIA
A
CD137/β-actin
0.4
0.3
0.2
0.1
0.0
transmembrane
total
B
sCD137 (pg/uL)
0.20
0.15
0.10
0.05
0.00
pre tumor
tumor-bearing


A
293T-mCD137L
293T
Supernatants:
Normoxia
Hypoxia
Isotype Control
% of Max
CD137
B
293T-mCD137L
293T
Serum:
Tumor free
Tumor bearing
Isotype Control
% of Max
CD137
OT1
OT1 CD137-/-
Normoxia
Hypoxia
293T-CD137L
Normoxia
Hypoxia
293T
% of Max
V450


A
CT26 shCONTROL
CT26 shCD137
Normoxia
Hypoxia
IC
% Max
CD137
B
CD137/β-actin
shControl TM
shControl TOTAL
shCD137 TM
shCD137 TOTAL
NORMOXIA
HYPOXIA
C
shCONTROL
shCD137
shCD137 CD8+ depleted
0/8
3/8
0/6
Tumor area (mm2)
Days
D
BALB/c WT
shCONTROL
shCD137
shCD137 CD8+ depl
***
Tumor area (mm2)
Days
E
RAG2IL2Rγ-/-
shCONTROL
shCD137
Tumor area (mm2)
Days

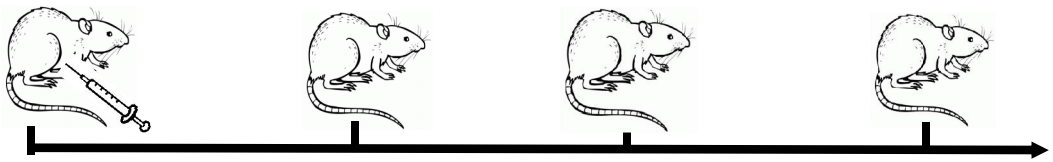

T lymphocyte
DC and myeloid cell
CD137
CD137L
sCD137
Isn't it wicked?
HYPOXIA
Alternative splicing
Soluble CD137 mRNA
Soluble CD137 protein
CD137 gDNA
(TM domain)
Tumor cell

IMMUNOAVATAR

MICE PROJECTS

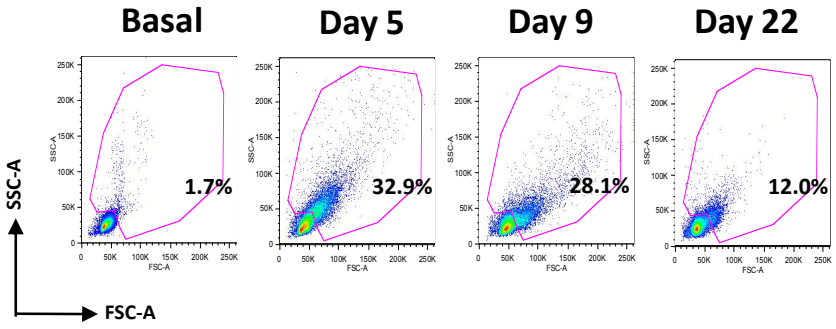
Adoptive transfer of human T-cells

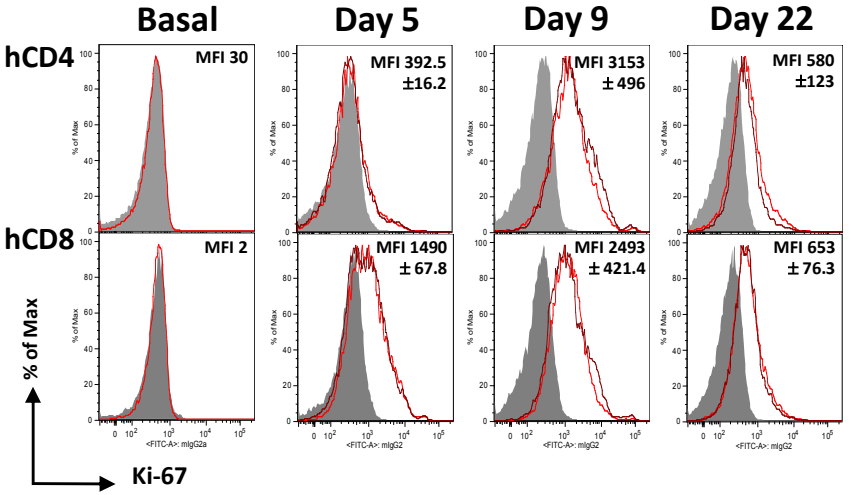
10^7 PBMCs (i.p.)

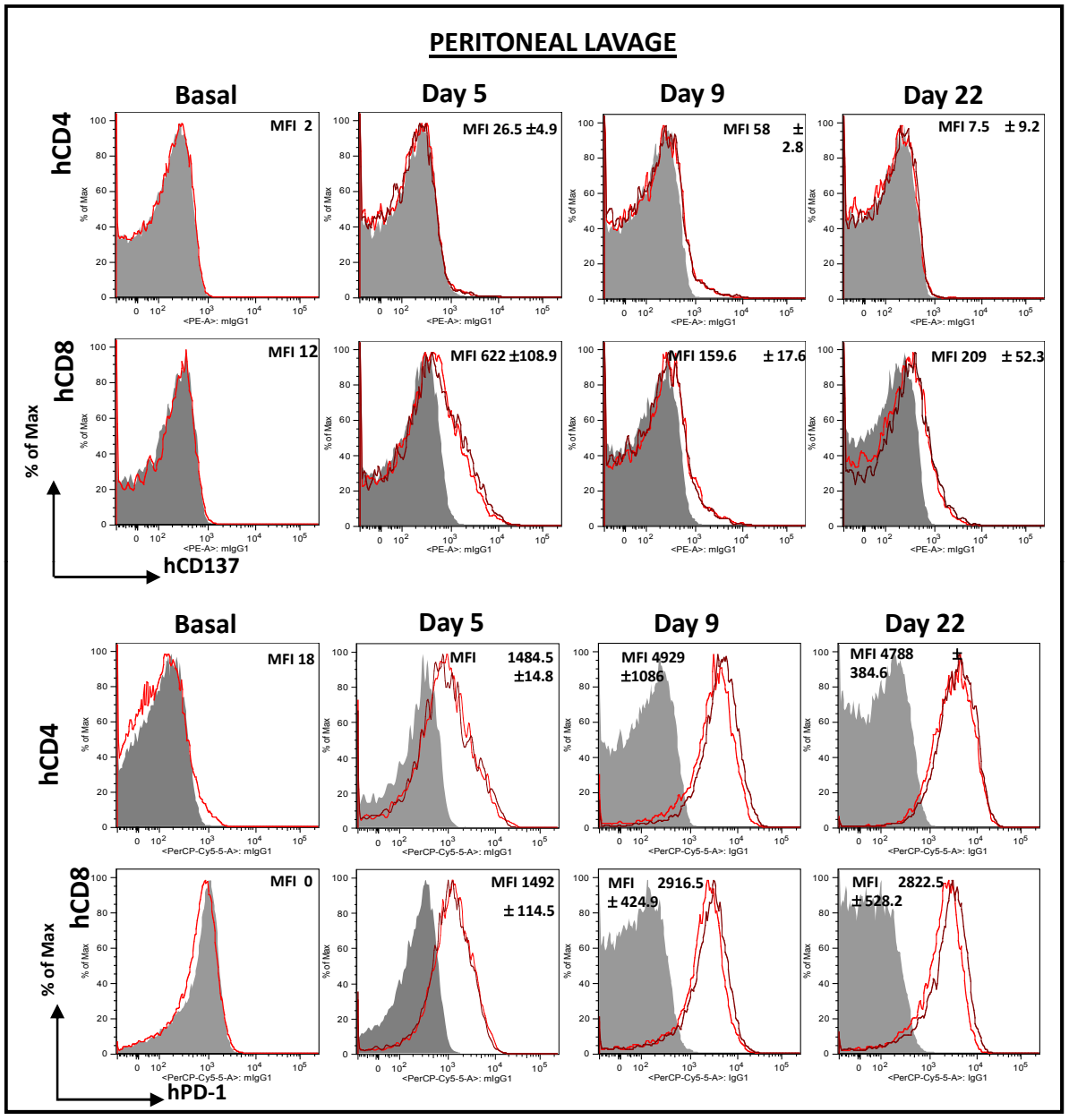


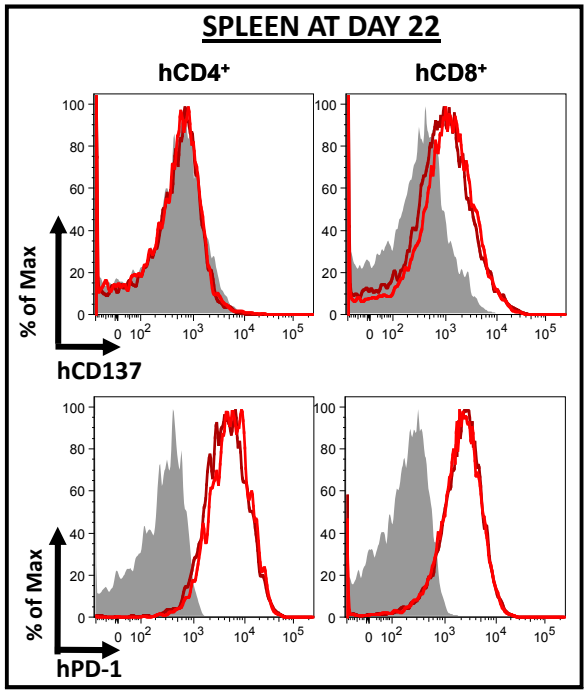
Peritoneal Lavage

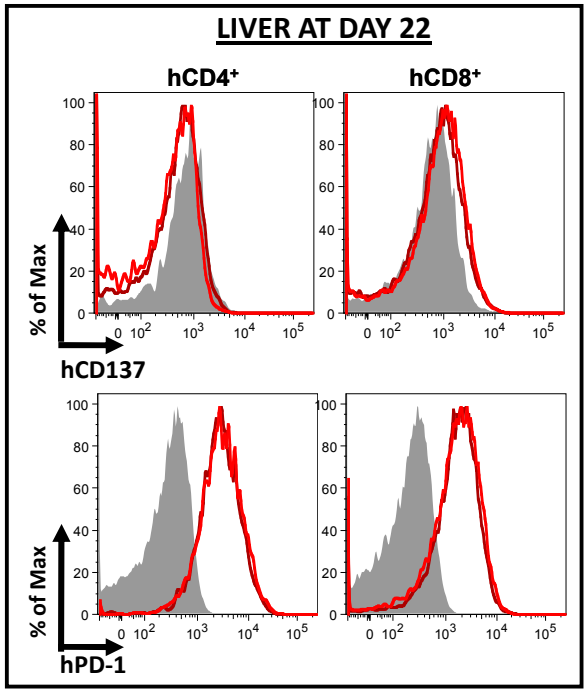
Facs Analyses



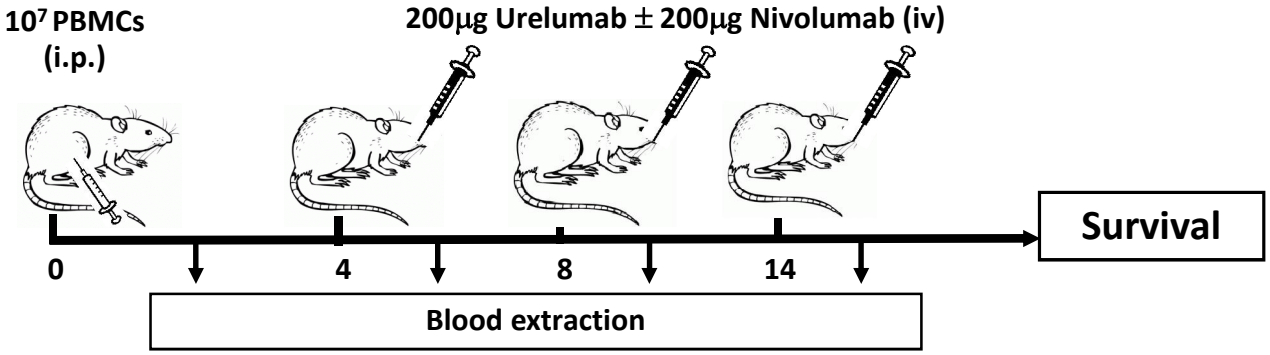


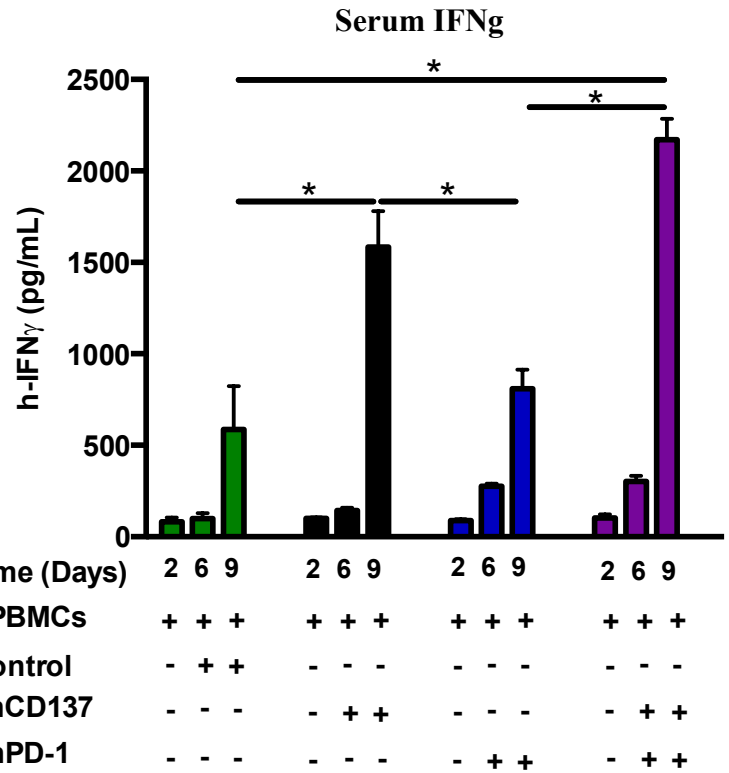

PERITONEAL LAVAGE
Basal
Day 5
Day 9
Day 22
hCD4
hCD8
MFI 2
MFI 26.5 ±4.9
MFI 58 ± 2.8
MFI 7.5 ± 9.2
MFI 12
MFI 622 ±108.9
MFI 159.6 ± 17.6
MFI 209 ± 52.3
% of Max
hCD137
MFI 18
MFI 1484.5 ±14.8
MFI 4929 ±1086
MFI 4788 ± 384.6
MFI 0
MFI 1492 ± 114.5
MFI 2916.5 ± 424.9
MFI 2822.5 ± 528.2
hPD-1

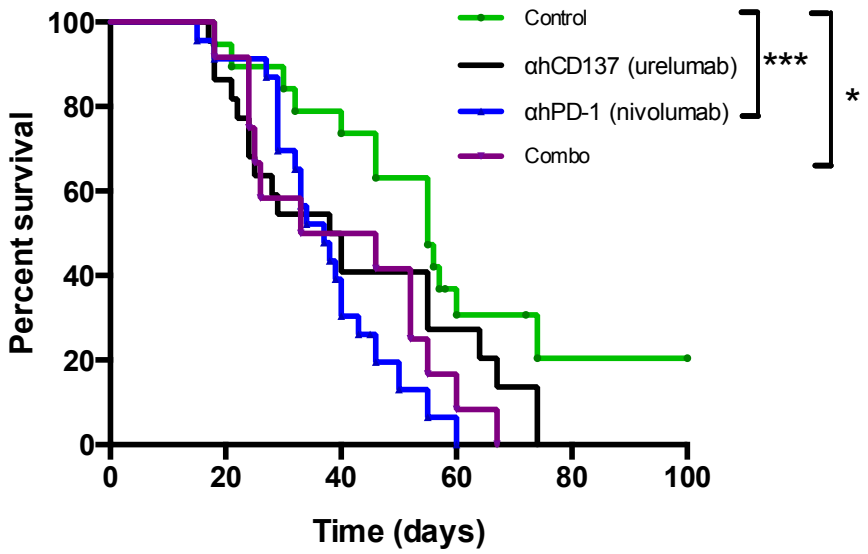

SPLEEN AT DAY 22
hCD4⁺
hCD8⁺
% of Max
hCD137
hPD-1


LIVER AT DAY 22
hCD4⁺
hCD8⁺
% of Max
hCD137
hPD-1

Xenograft versus host disease

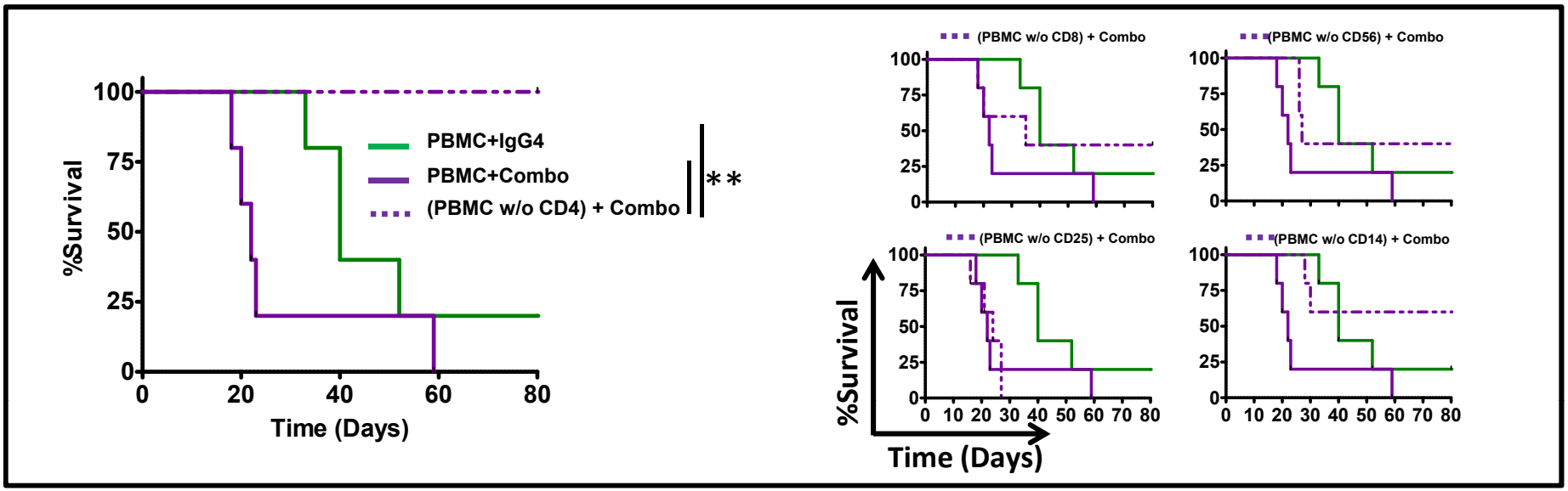


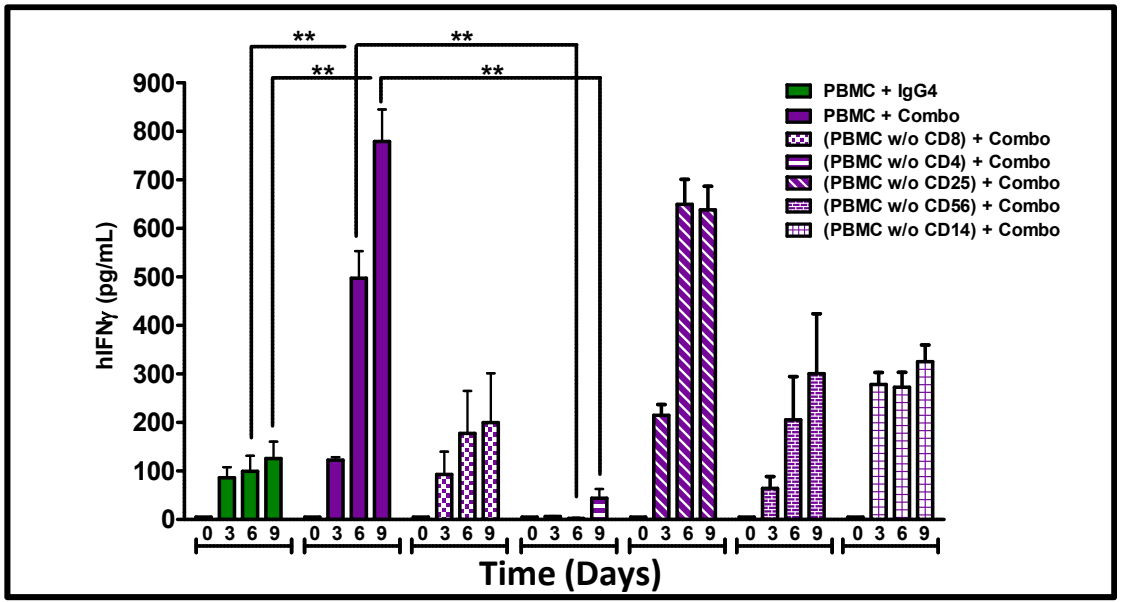




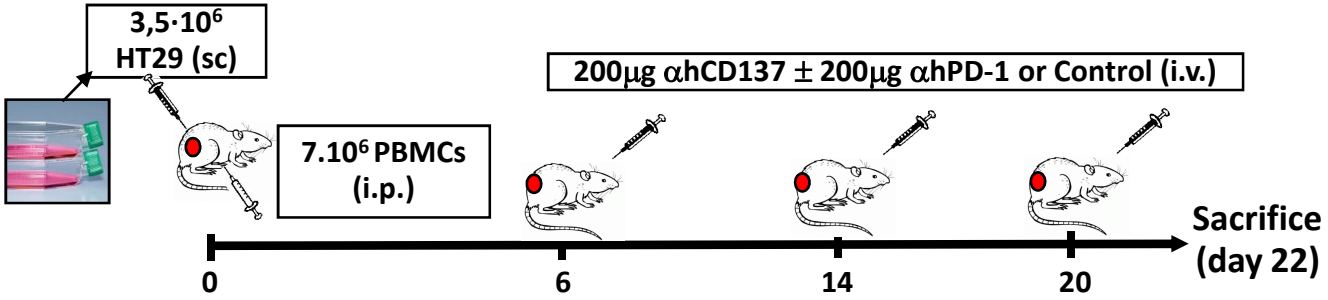
Xenograft versus host disease

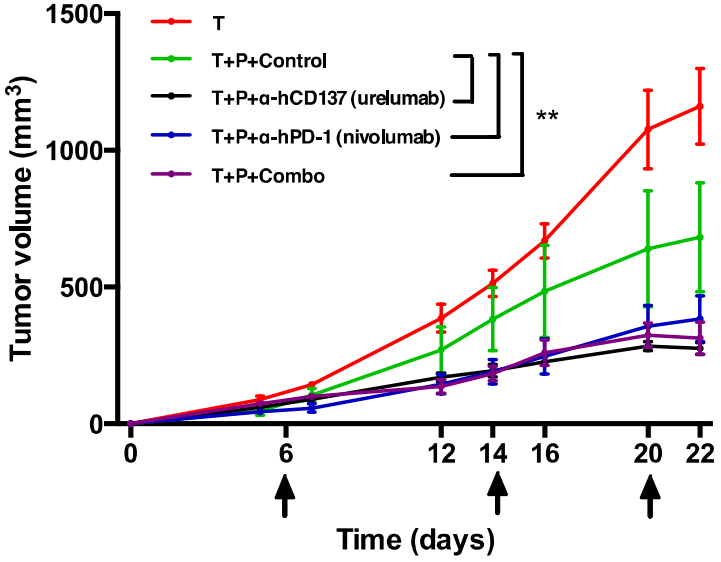
xGVHD and human IFN-γ elevation in plasma are dependent on human CD4 T cells

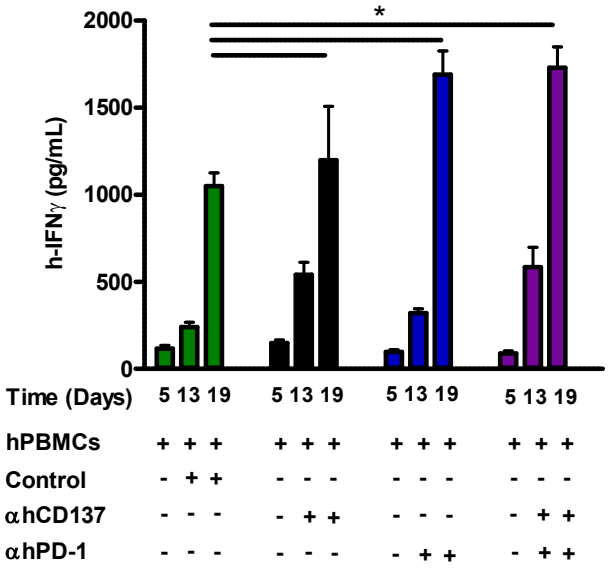


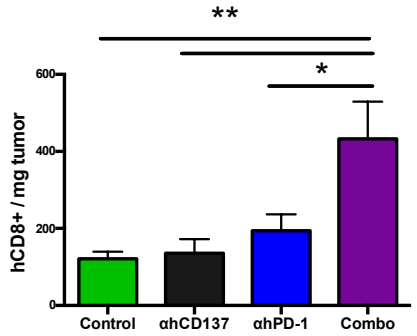


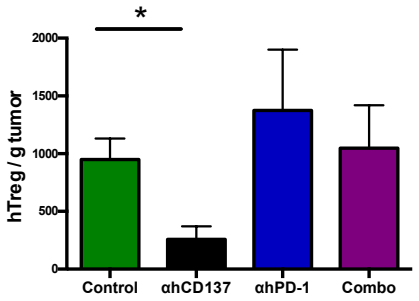
Xenografted colon cancer experiments

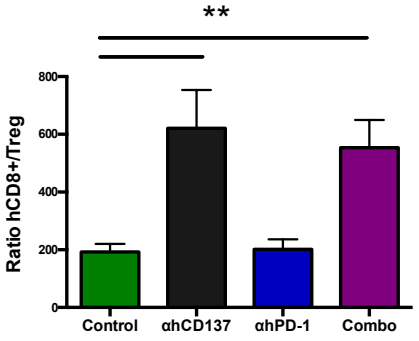




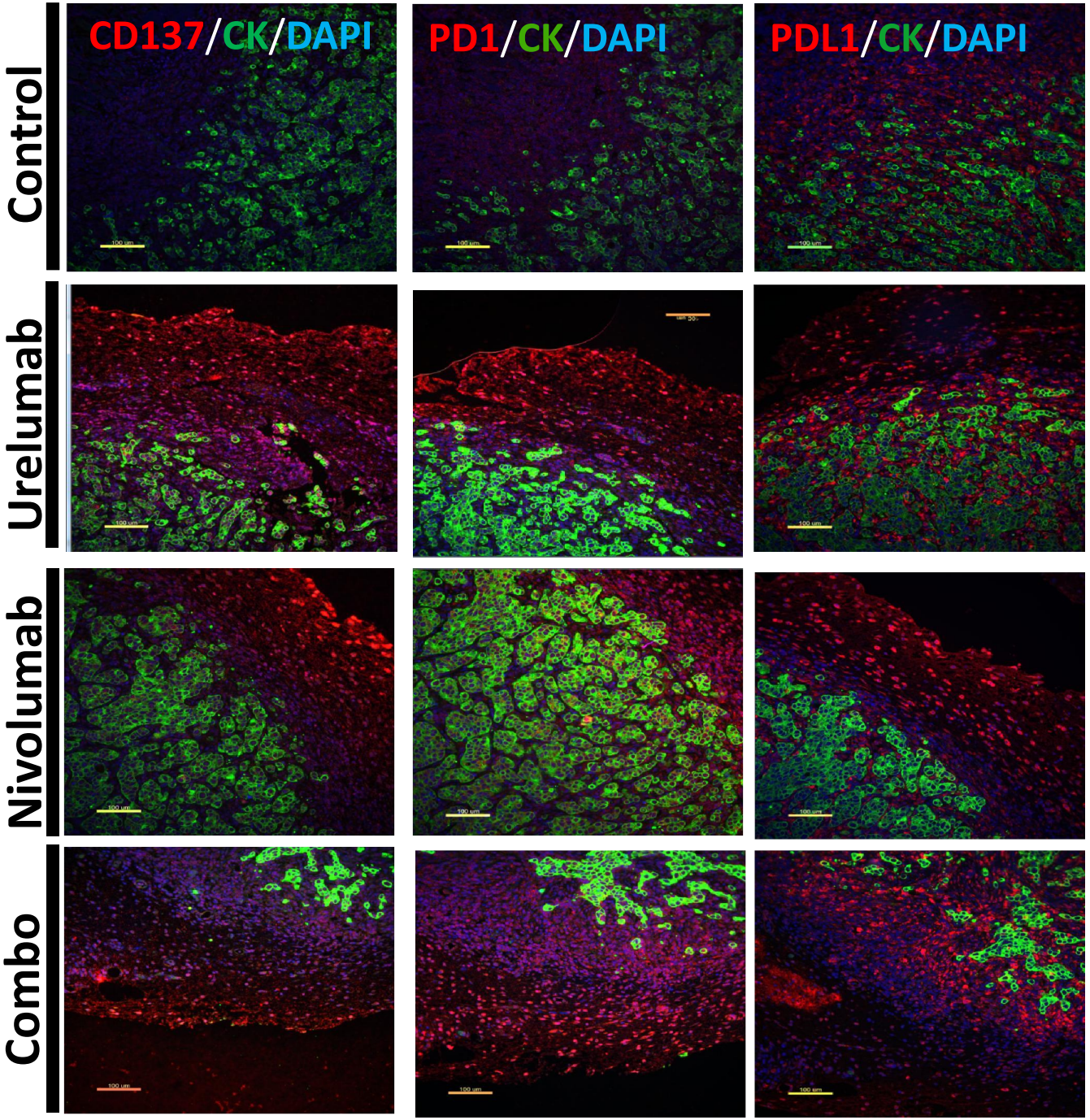


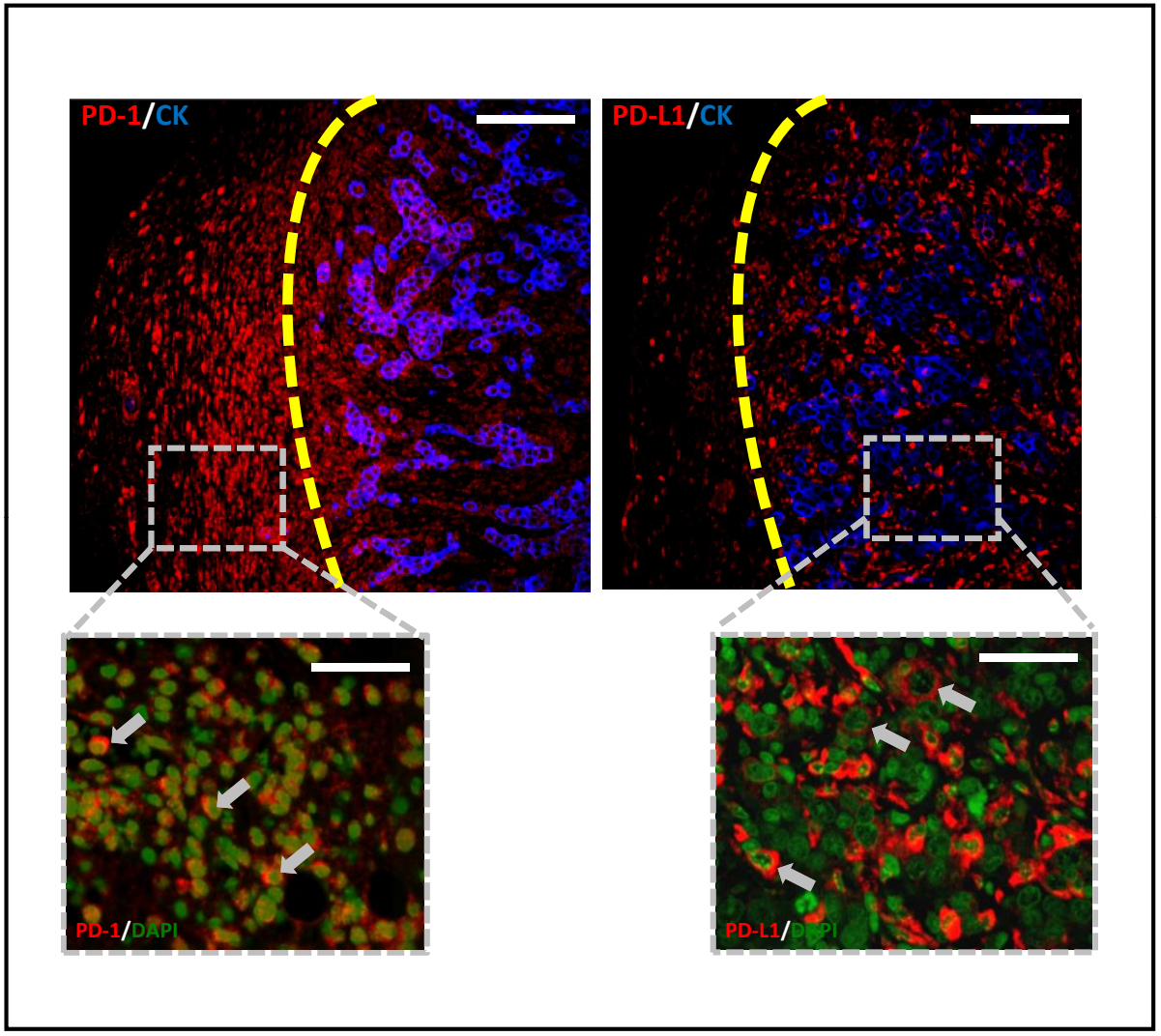


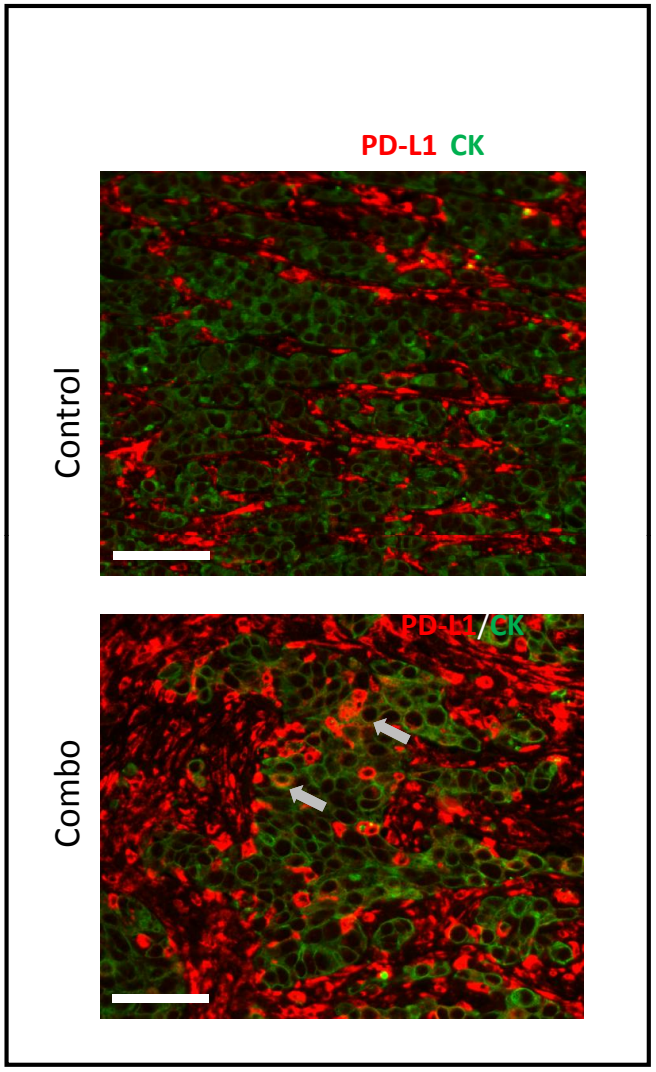




H&E
αhCD3
αhCD8
αhCD4
Control
Ureluma
b
Nivoluma
b
Combo
20x

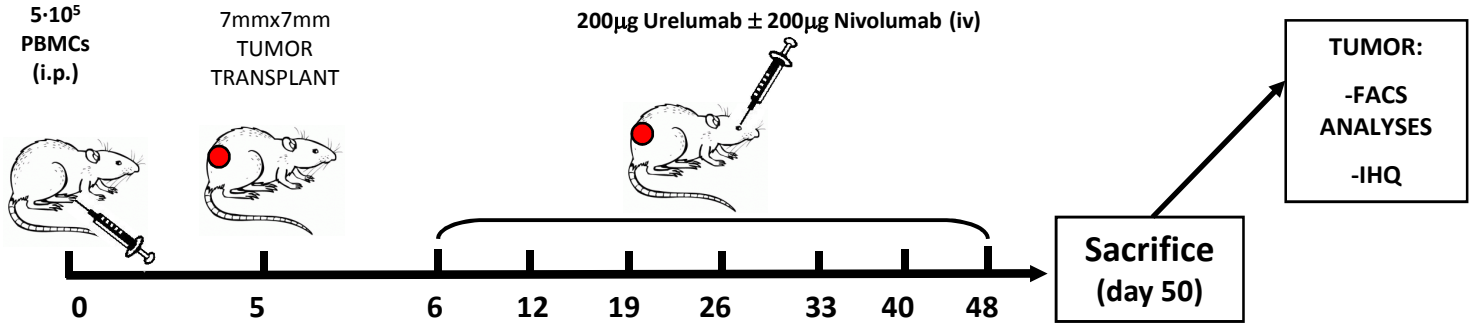

CD137/CK/DAPI
PD1/CK/DAPI
PDL1/CK/DAPI
Control
Urelumab
Nivolumab
Combo

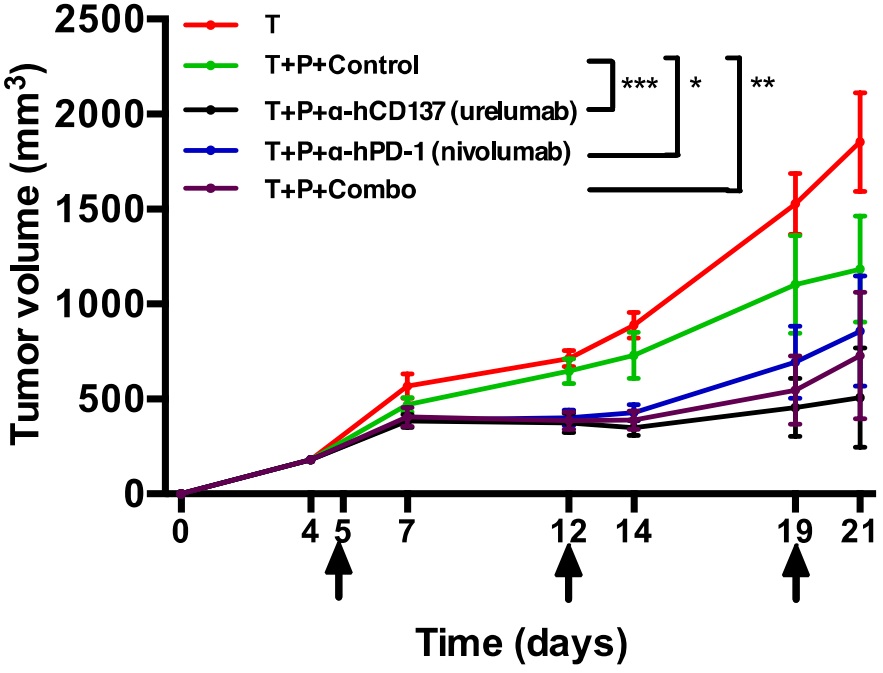

PD-1/CK
PD-L1/CK
PD-1/DAPI
PD-L1/DAPI

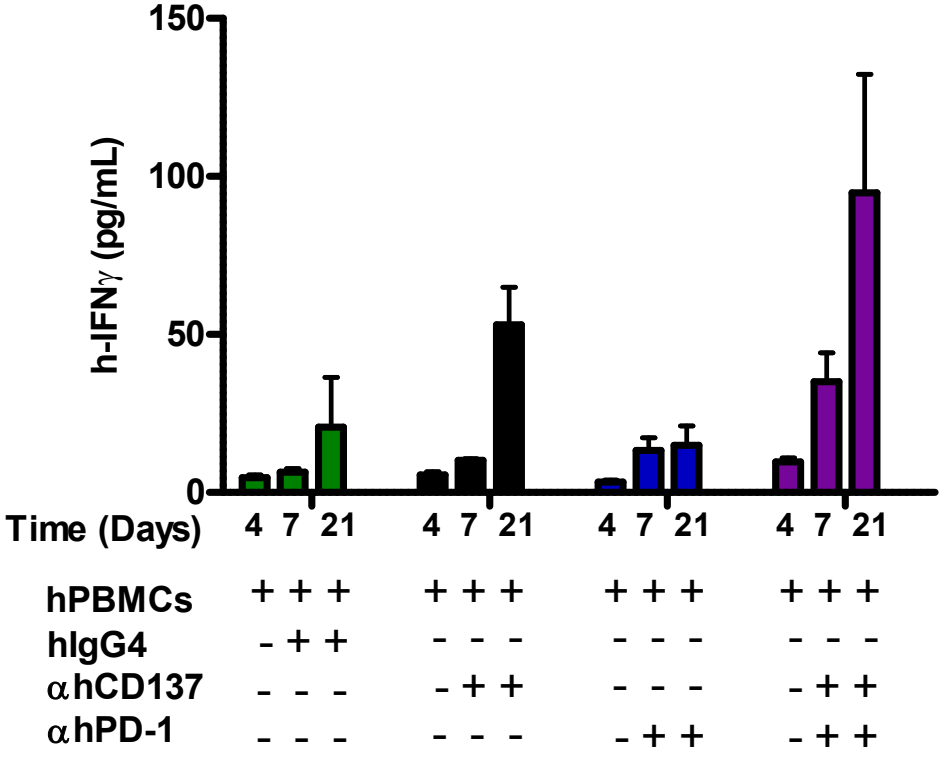

PD-L1 CK
Control
Combo
PD-L1/CK

Xenografted syngenic gastric cancer with autologous PBMCs experiments

Immunostimulatory monoclonal antibodies anti-hCD137 (urelumab) and anti-hPD-1 (nivolumab) alone or in combination show antitumor activity against a xenografted human colon cancer mediated by transferred allogenic human PBMC.

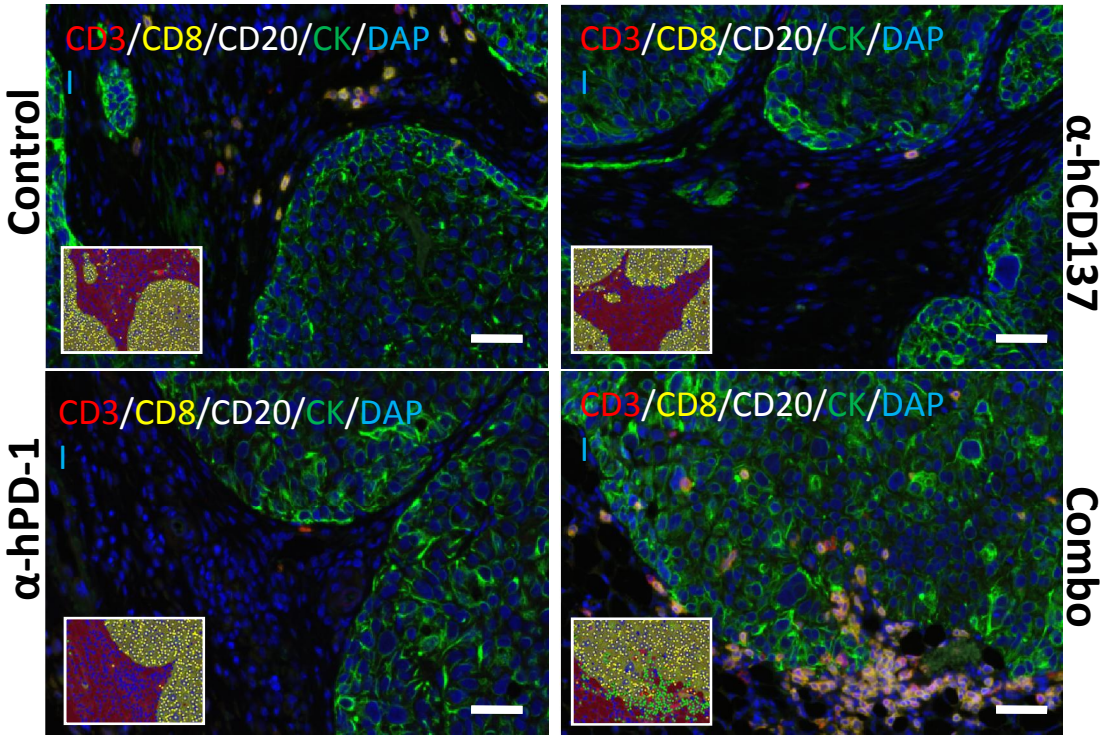


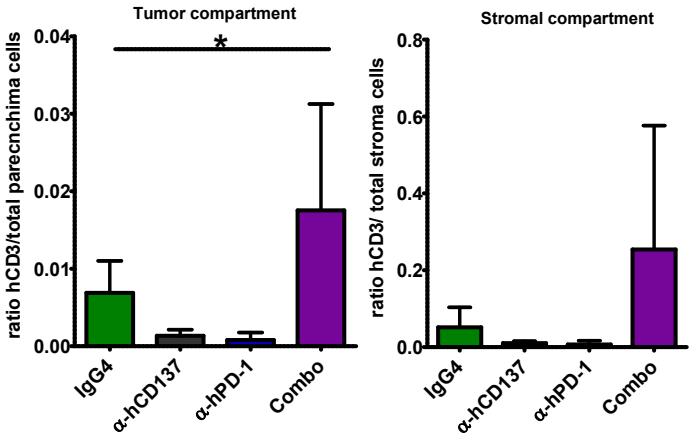




Xenografted syngenic gastric cancer with autologous PBMCs experiments

Multiplexed immunofluorescence microphotographs showing the architecture of tumor xenografts





OUR GRATITUDE GOES TO:

Patients and

their families

Central facilities at

CIMA and CUN

MINECO

Gob Navarra

EU FP7 & H2020

AECC

AICR

Fundación BBVA

Fundación CAN