Supplementary files for

Targeting Tumor Vasculature to Improve Anti-Tumor Activity of T Cells Armed Ex vivo with T Cell Engaging Bispecific Antibody

Supplementary table S1. Cross interactions between human and murine VEGF ligands and receptors.

Ligand	Receptor	Interaction	Ref	BVZ Effect expected	DC101 Effect expected
hVEGF	hVEGFR	strong	[31]	Yes	No
hVEGF	mVEGFR	weak/intermediate	[30, 32, 33]	Yes	Yes
mVEGF	mVEGFR	strong	[30]	No	Yes
mVEGF	hVEGFR	weak	[32]	No	No

Supplementary table S2. Bioluminescence intensities (BLIs) of tumor infiltrating lymphocytes (TILs) analyzed by area under curves (AUCs).

Reference group	Treatment group	Fold increase in BLI (AUC-BLI)	*P*-value	Tumor model	Figure
HER2-EATs	Unarmed T cells	0.04	0.02	osteosarcoma PDX	Fig. 2A
HER2-EATs	HER2-EATs + BVZ	7.5	0.05	osteosarcoma PDX	Fig. 2A
HER2-EATs	HER2-EATs + DC101	2.4	0.02	osteosarcoma PDX	Fig. 2A
GD2-EATs	Unarmed T cells	0.004	0.01	neuroblastoma PDX	Fig. 2B
GD2-EATs	GD2-EATs + BVZ	2.8	0.01	neuroblastoma PDX	Fig. 2B
GD2-EATs	GD2-EATs + DC101	8.1	0.05	neuroblastoma PDX	Fig. 2B

Supplementary fig. S1. VEGF expression on tumor cells was positively correlated with seeding density, and anti-VEGF antibody reduced serum VEGF levels.

(A) Flow cytometry analysis of VEGF expression on a variety of tumor cell lines with increasing seeding density. M14Luc, HCC1954, NCI-N87, and HL60 cell lines were harvested after 48 hours of incubation for analysis of surface VEGF expression. (B) Mouse serum hVEGF levels were analyzed after treatment with unarmed T cells or HER2-EATs with or without VEGF blockade using bevacizumab (BVZ) or DC101 in the HGSOC1 osteosarcoma PDX model. Asterisks denote significant differences as indicated, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Supplementary fig. S2. The effect of VEGF blockade on TH1 cell cytokine levels after EAT therapy.

Anti-VEGF (BVZ) or anti-VEGFR2 (DC101) antibody was given one day before each EAT injection. (A) Mouse serum human TH1 cell cytokine levels were measured 2 hours after the first EAT injection and compared among groups. (B) Serum TH1 cell cytokine levels were analyzed over time. Asterisks denote significant differences as indicated, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Supplementary fig. S3. (A) Neuroblastoma PDXs were treated with combination of GD2-EATs and BVZ or DC101. Luciferase-transduced T cells [Luc(+) T cells] were used for GD2-EATs or unarmed T cell injection on day 0 of treatment. Non-luciferase-transduced T cells or GD2-EATs were used for following injection on day 4 post-treatment. Bioluminescence of Luc(+) unarmed T cells or Luc(+) GD2-EATs in tumors were monitored over time.

Supplementary fig. S4. VEGF blockade enhanced BsAb-driven T cell infiltration into solid tumors.

(A) 143B osteosarcoma CDXs were treated with a combination of unarmed T cells or GD2-EATs and BVZ or DC101, and the tumors were harvested and analyzed by flow cytometry on day 60 post-treatment. (B) The tumors were also analyzed by immunohistochemical (IHC) staining of human CD3, and the CD3(+) T cells were counted with Q-path 0.1.2 and compared among groups. G1, unarmed T cells; G2, unarmed T cells plus BVZ; G3, unarmed T cells plus DC101; G4, GD2-EATs; G5, GD2-EATs plus BVZ; G6, GD2-EATs plus CD101. Asterisks denote significant differences as indicated, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Supplementary fig. S5. The effect of VEGF blockade on complete blood counts and tumor infiltrating myeloid cells in a neuroblastoma PDX model.

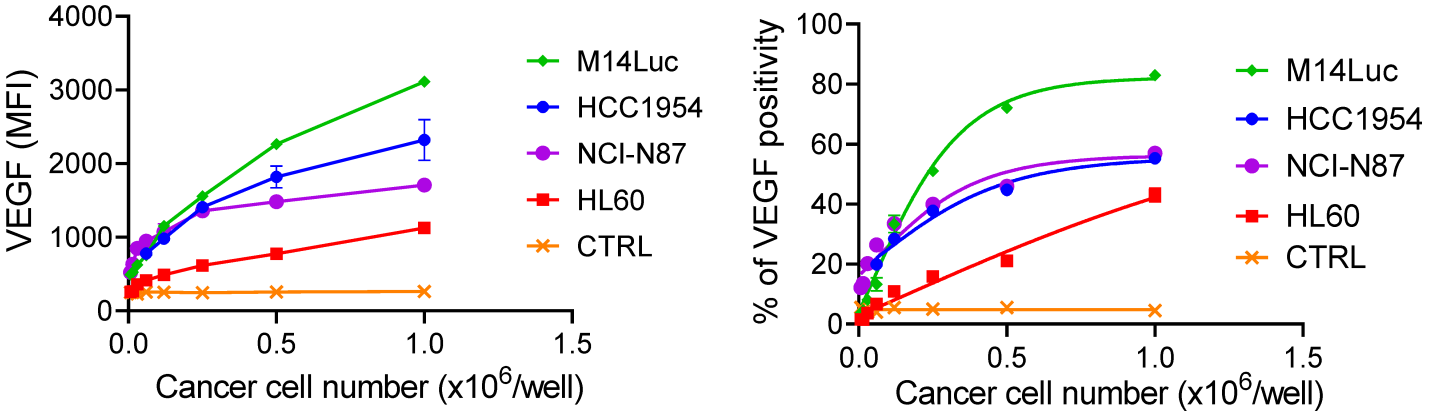
(A) GD2-EATs with or without VEGF blockade were administered to treat neuroblastoma PDXs, and complete blood counts (CBC) were analyzed on day 5 post-EAT treatment. (B) Tumors were harvested on day 10 post-EAT treatment, and tumor infiltrating myeloid cells (TIMs) were analyzed by flow cytometry and compared among groups. Asterisks denote significant differences as indicated, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Supplementary fig. S6. The effect of VEGF blockade on complete blood counts (CBC) and tumor infiltrating myeloid cells (TIMs) in an osteosarcoma CDX model.

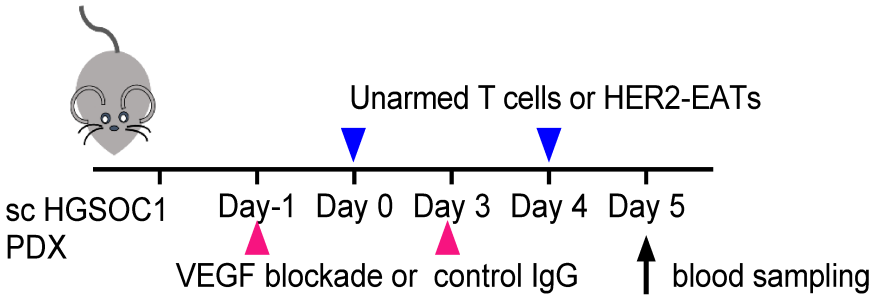
(A) Unarmed T cells or GD2-EATs with or without VEGF blockade were administered to 143B osteosarcoma bearing mice, and CBC was analyzed on day 5 post-EAT treatment. (B) Tumors were harvested on day 60 post-treatment, and tumor infiltrating myeloid cells (TIMs) including PMN-MDSC, M-MDSC, and TAMs were analyzed by flow cytometry and compared among groups.

(A)

fig. S1

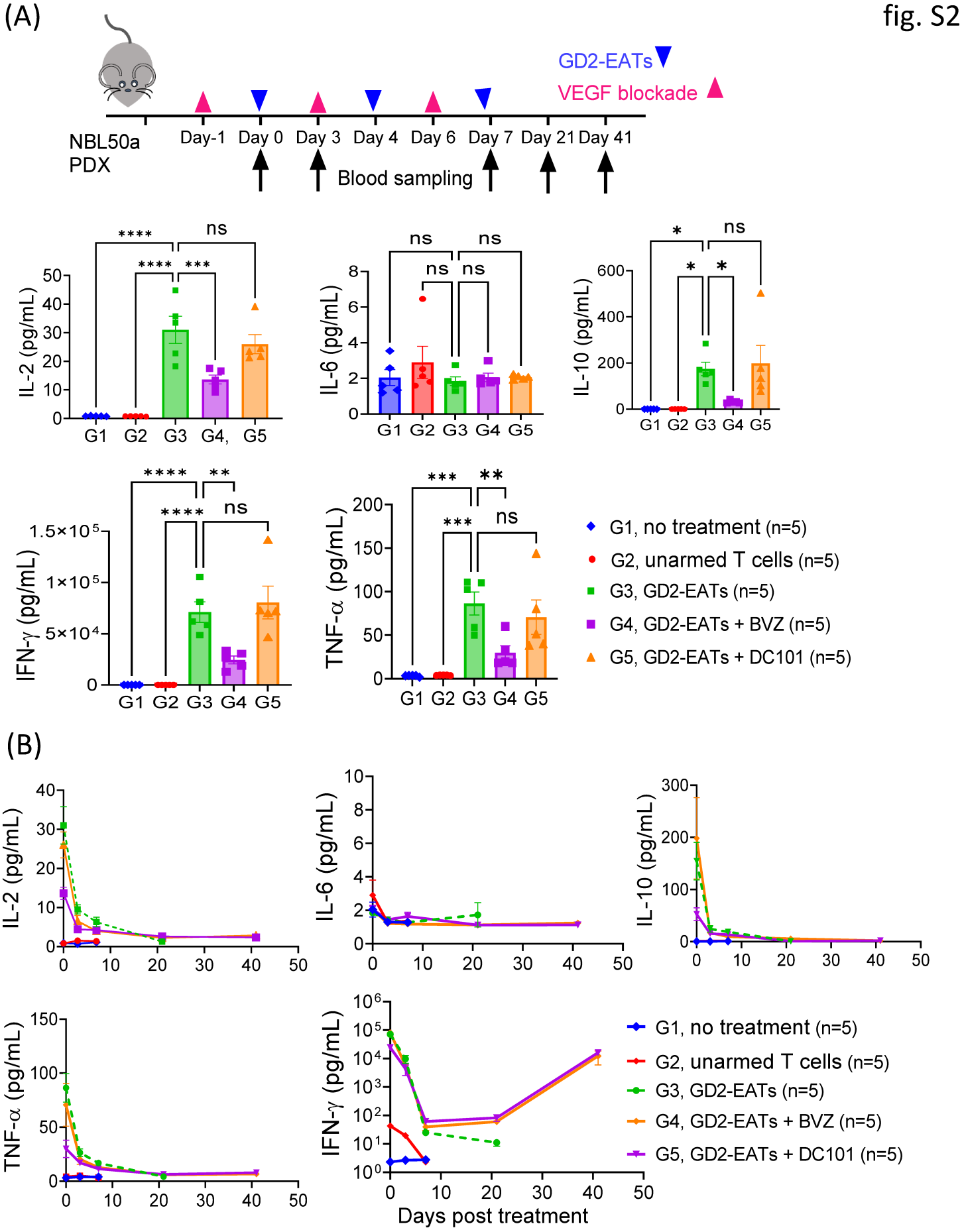

VEGF (MFI)
4000
3000
2000
1000
0
0.0
0.5
1.0
1.5
Cancer cell number (x10^6/well)
M14Luc
HCC1954
NCI-N87
HL60
CTRL
% of VEGF positivity
100
80
60
40
20
0
0.0
0.5
1.0
1.5
Cancer cell number (x10^6/well)
M14Luc
HCC1954
NCI-N87
HL60
CTRL

(B)


Unarmed T cells or HER2-EATs
sc HGSOC1 PDX
Day-1
Day 0
Day 3
Day 4
Day 5
VEGF blockade or
control IgG
blood sampling

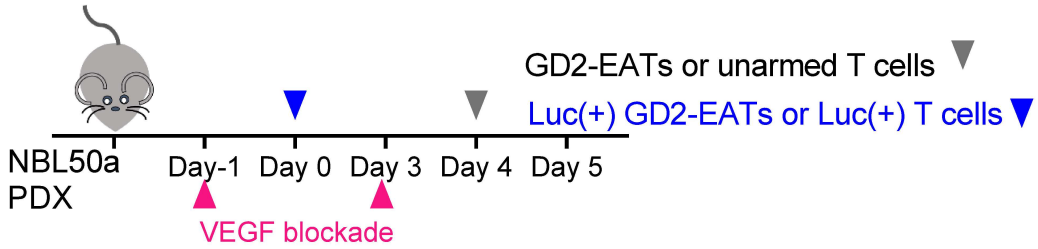

ns
ns
****
****
hVEGF (pg/mL)
100
80
60
40
20
0
unarmed T cells+ control IgG
unarmed T cells + BVZ
unarmed T cells + DC101
HER2-EATs+ control IgG
HER2-EATs+ BVZ
HER2-EATs+ DC101

fig. S2


(A)
GD2-EATs
VEGF blockade
NBL50a PDX
Day-1 Day 0 Day 3 Day 4 Day 6 Day 7 Day 21 Day 41
Blood sampling
IL-2 (pg/mL)
IL-6 (pg/mL)
IL-10 (pg/mL)
IFN-γ (pg/mL)
TNF-α (pg/mL)
G1 G2 G3 G4 G5
G1, no treatment (n=5)
G2, unarmed T cells (n=5)
G3, GD2-EATs (n=5)
G4, GD2-EATs + BVZ (n=5)
G5, GD2-EATs + DC101 (n=5)
(B)
Days post treatment

(A)

fig. S3


GD2-EATs or unarmed T cells
Luc(+) GD2-EATs or Luc(+) T cells
NBL50a PDX
Day-1
Day 0
Day 3
Day 4
Day 5
VEGF blockade

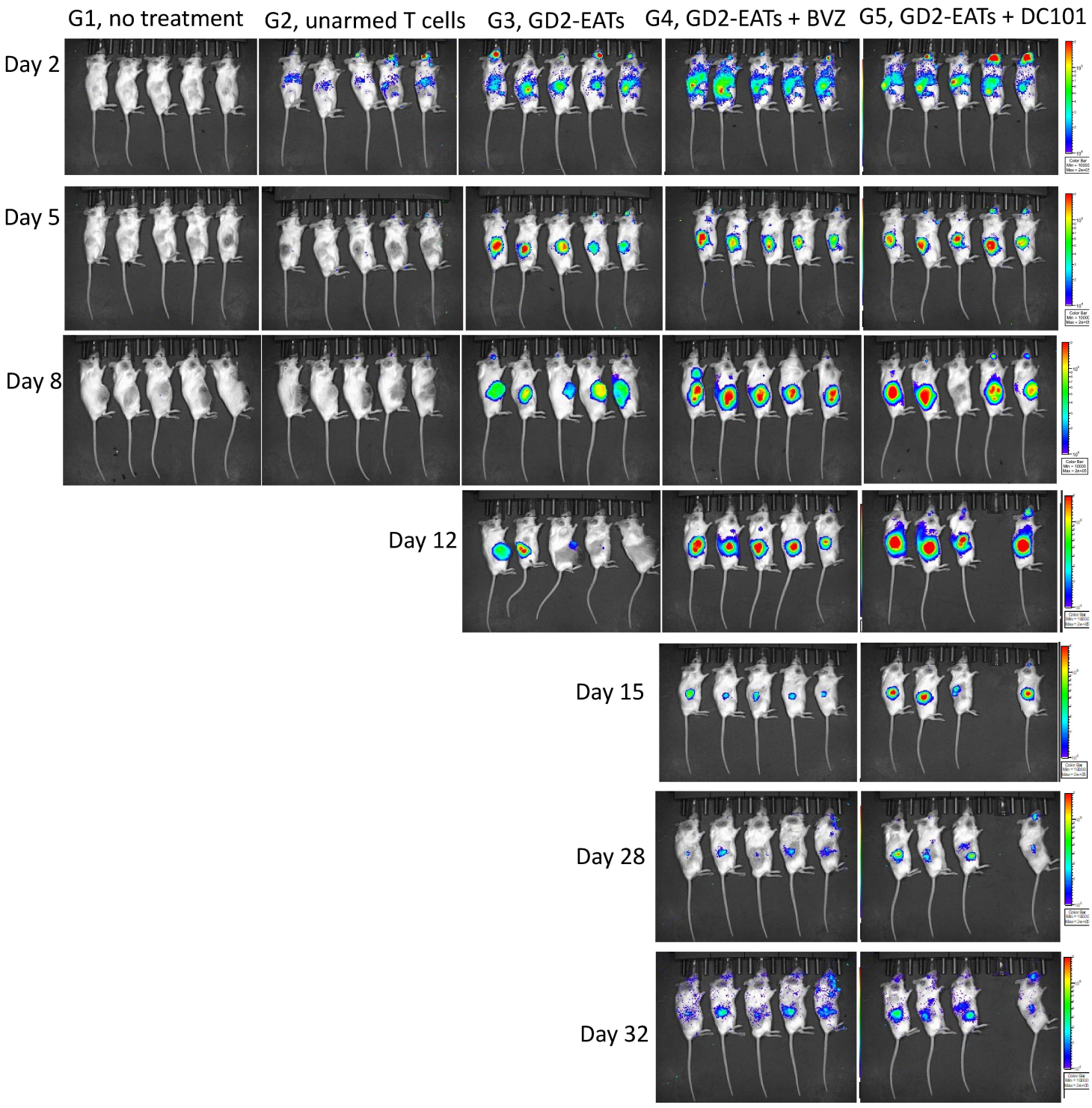
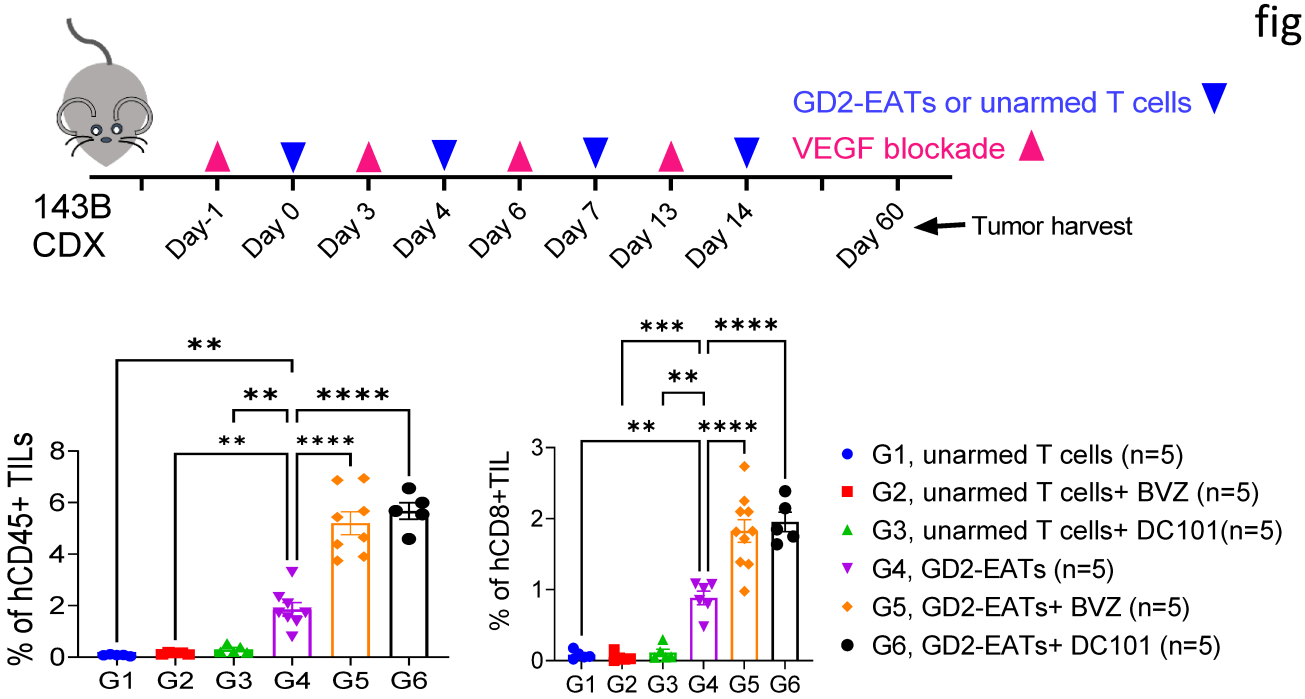

G1, no treatment
G2, unarmed T cells
G3, GD2-EATs
G4, GD2-EATs + BVZ
G5, GD2-EATs + DC101
Day 2
Day 5
Day 8
Day 12
Day 15
Day 28
Day 32

fig. S4

(A)


GD2-EATs or unarmed T cells
VEGF blockade
143B CDX
Day-1
Day 0
Day 3
Day 4
Day 6
Day 7
Day 13
Day 14
Day 60
Tumor harvest
% of hCD45+ TILs
% of hCD8+TIL
G1
G2
G3
G4
G5
G6
G1, unarmed T cells (n=5)
G2, unarmed T cells+ BVZ (n=5)
G3, unarmed T cells+ DC101(n=5)
G4, GD2-EATs (n=5)
G5, GD2-EATs+ BVZ (n=5)
G6, GD2-EATs+ DC101 (n=5)

(B)

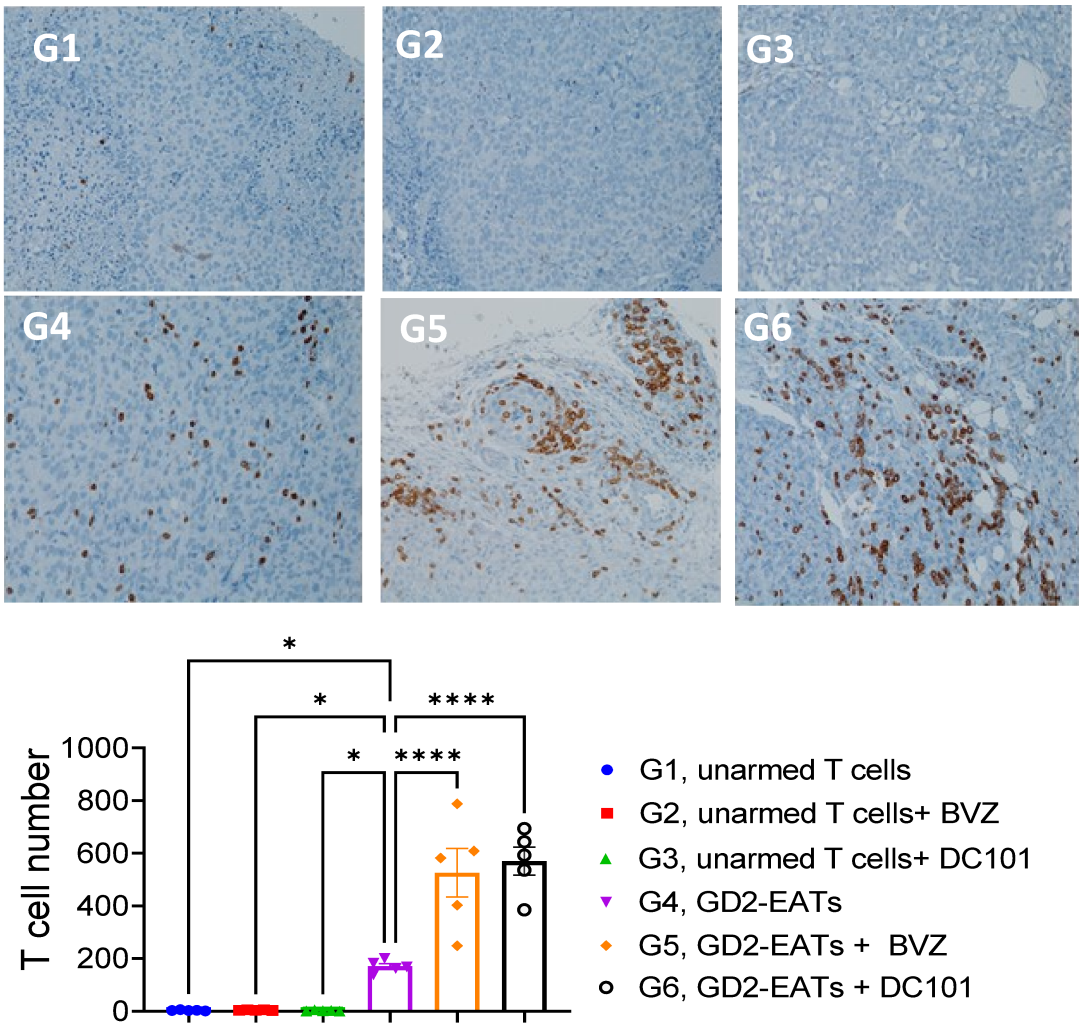

G1
G2
G3
G4
G5
G6
T cell number
G1, unarmed T cells
G2, unarmed T cells+ BVZ
G3, unarmed T cells+ DC101
G4, GD2-EATs
G5, GD2-EATs + BVZ
G6, GD2-EATs + DC101

fig. S5

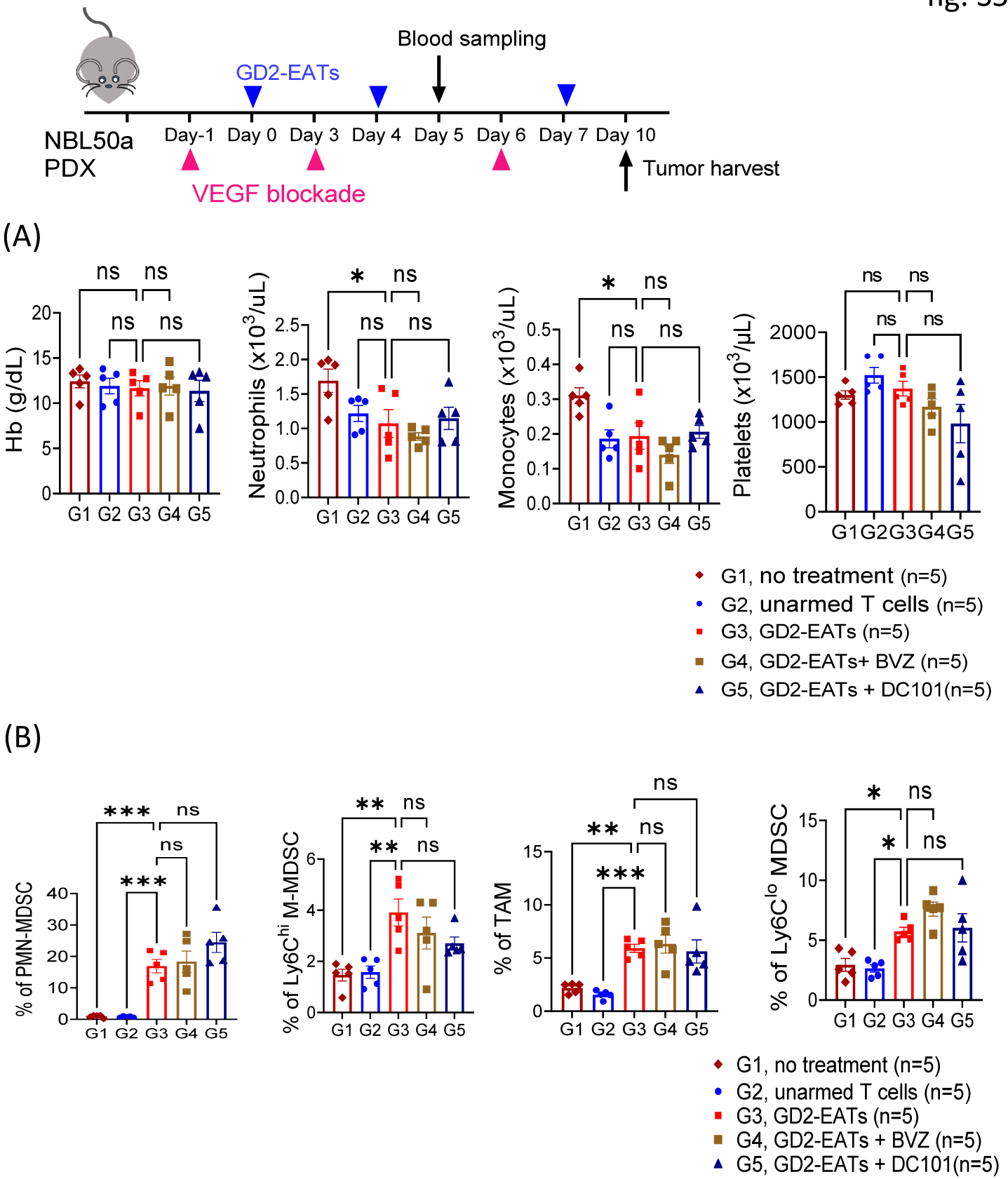
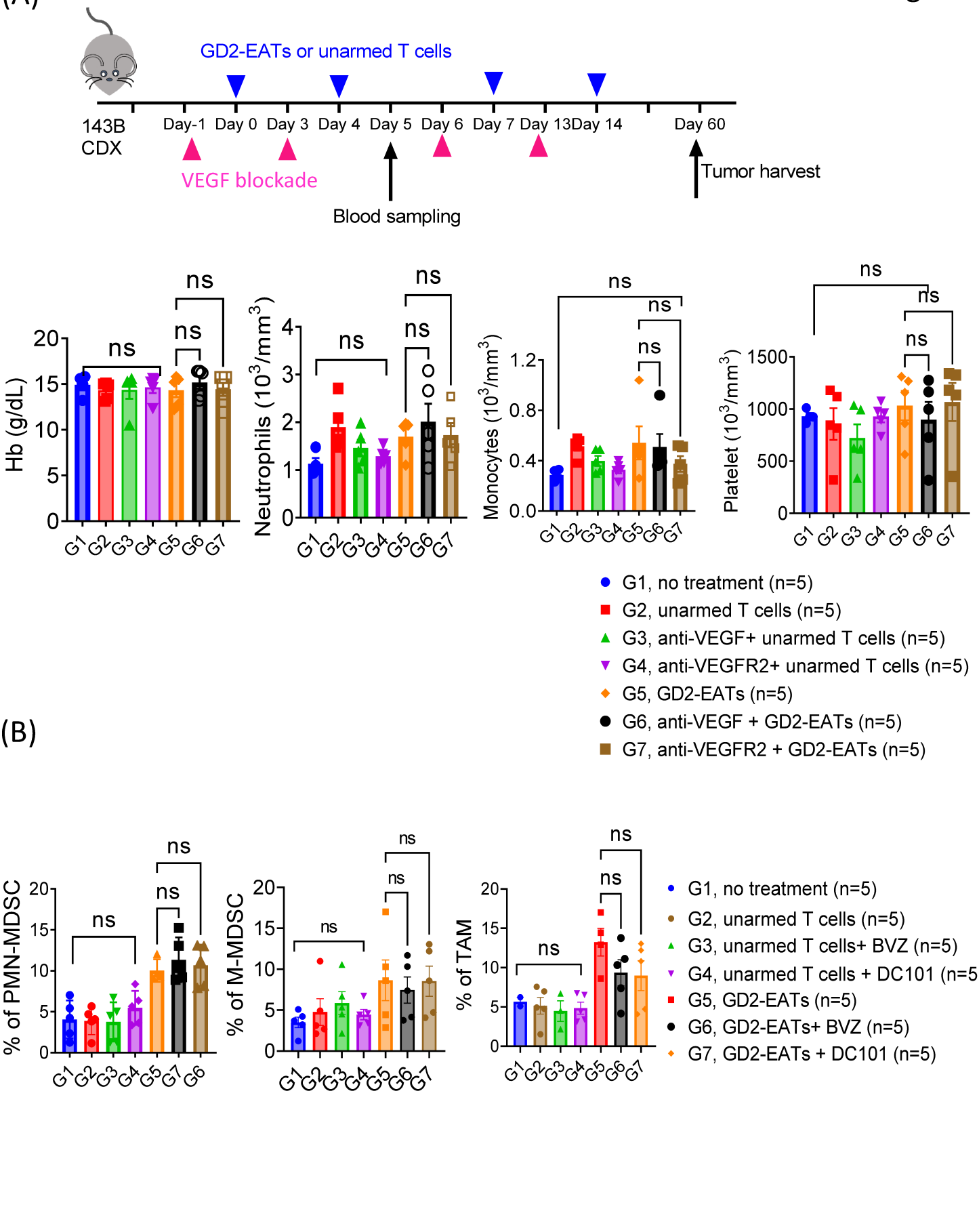

Blood sampling
GD2-EATs
NBL50a PDX
Day-1 Day 0 Day 3 Day 4 Day 5 Day 6 Day 7 Day 10
VEGF blockade
Tumor harvest
(A)
Hb (g/dL)
Neutrophils (x10^3/uL)
Monocytes (x10^3/uL)
Platelets (x10^3/μL)
G1 G2 G3 G4 G5
G1, no treatment (n=5)
G2, unarmed T cells (n=5)
G3, GD2-EATs (n=5)
G4, GD2-EATs+ BVZ (n=5)
G5, GD2-EATs + DC101(n=5)
(B)
% of PMN-MDSC
% of Ly6C^hi M-MDSC
% of TAM
% of Ly6C^lo MDSC
G1, no treatment (n=5)
G2, unarmed T cells (n=5)
G3, GD2-EATs (n=5)
G4, GD2-EATs + BVZ (n=5)
G5, GD2-EATs + DC101(n=5)

fig. S6

(A)


GD2-EATs or unarmed T cells
143B CDX
Day-1
Day 0
Day 3
Day 4
Day 5
Day 6
Day 7
Day 13
Day 14
Day 60
VEGF blockade
Blood sampling
Tumor harvest
Hb (g/dL)
Neutrophils (10^3/mm^3)
Monocytes (10^3/mm^3)
Platelet (10^3/mm^3)
ns
G1
G2
G3
G4
G5
G6
G7
G1, no treatment (n=5)
G2, unarmed T cells (n=5)
G3, anti-VEGF+ unarmed T cells (n=5)
G4, anti-VEGFR2+ unarmed T cells (n=5)
G5, GD2-EATs (n=5)
G6, anti-VEGF + GD2-EATs (n=5)
G7, anti-VEGFR2 + GD2-EATs (n=5)

(B)

% of PMN-MDSC
% of M-MDSC
% of TAM
ns
G1
G2
G3
G4
G5
G7
G6
G1, no treatment (n=5)
G2, unarmed T cells (n=5)
G3, unarmed T cells+ BVZ (n=5)
G4, unarmed T cells + DC101 (n=5)
G5, GD2-EATs (n=5)
G6, GD2-EATs+ BVZ (n=5)
G7, GD2-EATs + DC101 (n=5)