Supplemental Figure 1. CpG-expanded CD8+ T cells regress large, neoantigen-expressing melanomas

A

Mice with B16KVP melanoma

Pmel + hgp100 culture

Vehicle expand w/ IL-2

CpG expand w/ IL-2

D0

D7

ACT

B

B16KVP

Tumor area (mm²)

Day Post-ACT

% Survival

0 25 50 75

0 100 200 300 400

NT

Veh Pmel

CpG Pmel

**Supplemental Figure 2.** The CpG-signature phenotype is preserved in mouse and human TIL

**A**

Day 7 phenotype *in vitro* (first gate on live CD8+VB13+).

- CD28
- CD27
- PD-1
- LAG-3
- TIM-3
- TIGIT
- OX-40
- SLAMF6
- KLRG1
- CD69
- CD26
- CD73
- CD38
- CXCR3
- CCR7
- CCR6
- IL-23R
- IL-7R
- CXCR5
- CD44
- CD62L

**B**

Day 4 post ACT TIL phenotype *in vivo* (first gate on live pmel-1 donor cells).

- ICOS
- CD39
- GRNZMB
- MFI
- PD-1
- LAG-3
- TIM-3
- MFI

**C**

- Veh
- CpG
- MitoTracker
- TMRM
- % of pmel-1

**D**

- IFN-γ
- IL-6
- TNF-α
- GM-CSF
- pg/mL plasma

**E**

Human CD4+ICOS+ TIL

- Veh
- CpG

- Human CD8+ICOS+ TIL

- Veh
- CpG

- CD39
- IL-2Rα
- 29
- 55
- CD39
- IL-2Rα
- 42
- 64
Supplemental Figure 3. Mouse T cells express nominal TLR9

A

TLR9 Expression Value Normalized by DESeq2

B

Expression Value Range

Intracellular TLR9

TLR9

T cell

B cell

DC

Mac

1.08%

10.5%

31.2%

52.1%

0

20

40

60

% TLR9+
Supplemental Figure 4. CpG does not confer purified T cells with an altered phenotype or enhanced antitumor ability

A

B

C

D

Supplemental material

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Supplemental Figure 5. CpG-induced soluble factors do not confer the IL-2Rα^{high} ICOS^{high} CD39^{low} phenotype on CD8^{+} T cells

A

B

C

D

E

Supplemental material placed on this supplemental material which has been supplied by the author(s)
Supplemental Figure 6. Blocking CD40L does not abrogate the CpG-mediated phenotype or antitumor immunity

A

B

C

Supplemental material
Supplemental Figure 7. Direct interaction between APCs and CD8+ T cells is critical for improved antitumor immunity with CpG

A. IL-2Rα, ICOS, CD39

B. Peptide vs. Plate-bound αCD3/αCD28

C. Peptide vs. Plate-bound αCD3
Supplemental Figure 8. The signature CpG phenotype and enhanced antitumor efficacy do not rely on CD4+ T cells, NK cells, or the combination of Macrophages and DCs.
Supplemental Figure 9. Co-cultured B and T cells induce the signature phenotype with CpG

A

IL-2Rα

***

**** ns

ICOS

***

** ns

CD39

***

* ns

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