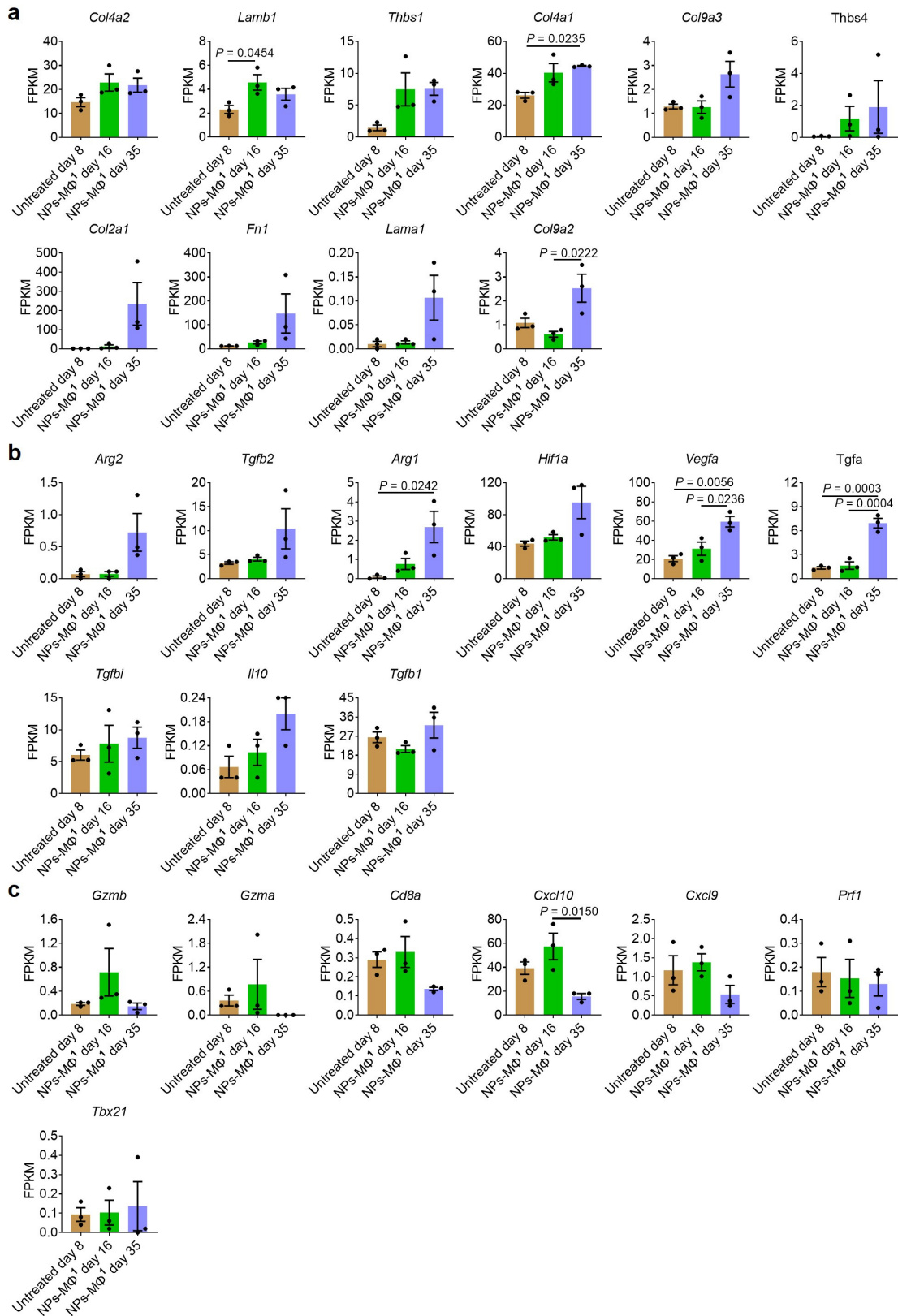


Supplementary Information

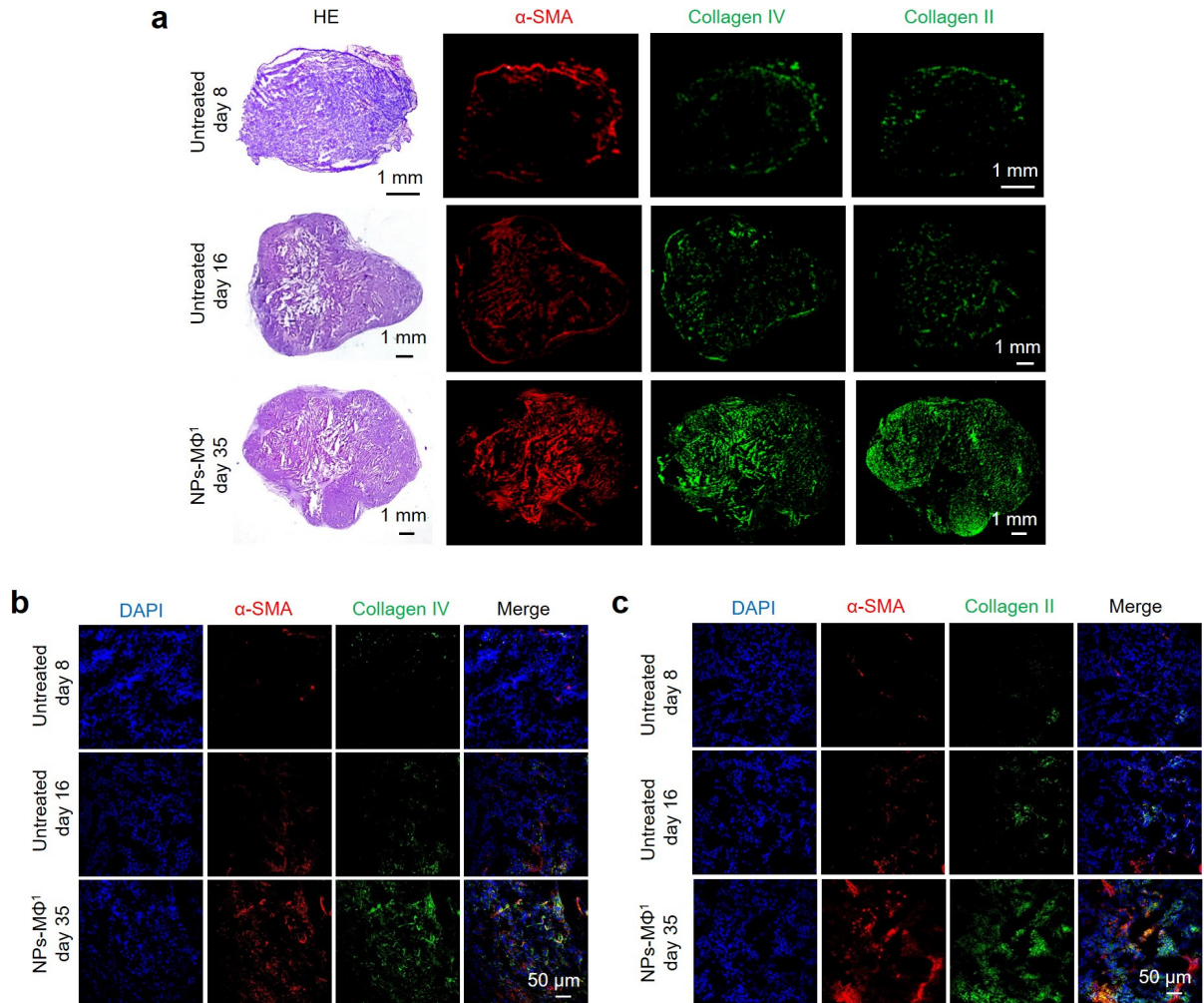
CD133⁺PD-L1⁺ cancer cells confer resistance to adoptively transferred engineered macrophage-based therapy in melanoma

Xu, J. et. al.

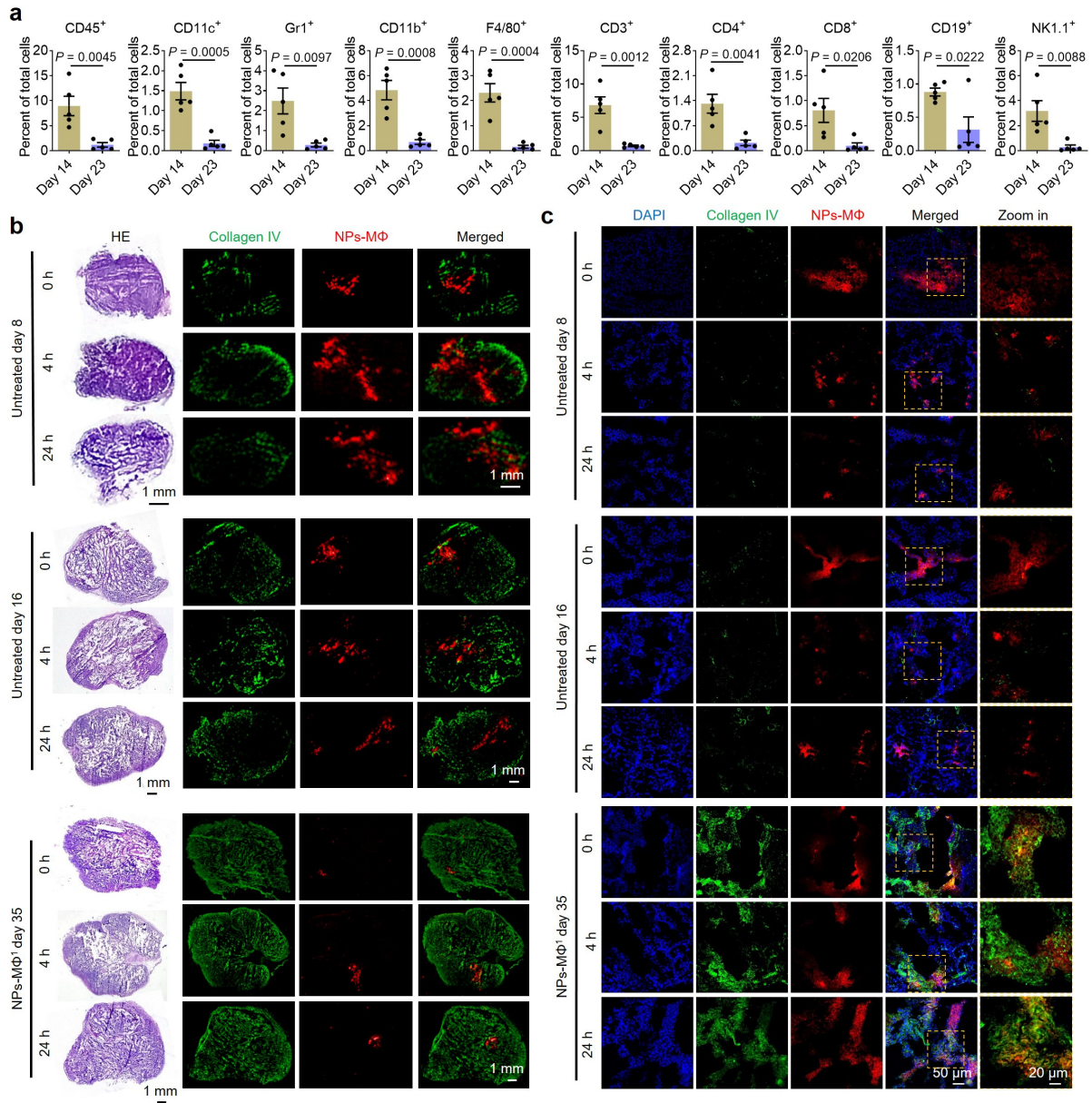
Supplementary Figures



Supplementary Figure 1. Quantitative analysis of the gene expression of the cluster (Fig. 1c) associated with the ECM (**a**), the key immunosuppressive molecules (**b**), or the CD8⁺ T effector cells (**c**) in the B16F10 tumor of C57BL/6 mice before the treatment at day 8, or after 1 cycle-treatment (NPs-MΦ¹) at days 16 or 35. FPKM, fragments per kilobase of transcript per million mapped reads. One-way ANOVA with Tukey's post-test ($n = 3$ mice). Data are expressed as mean $\pm$ s.e.m. Source data are provided as a Source Data file.

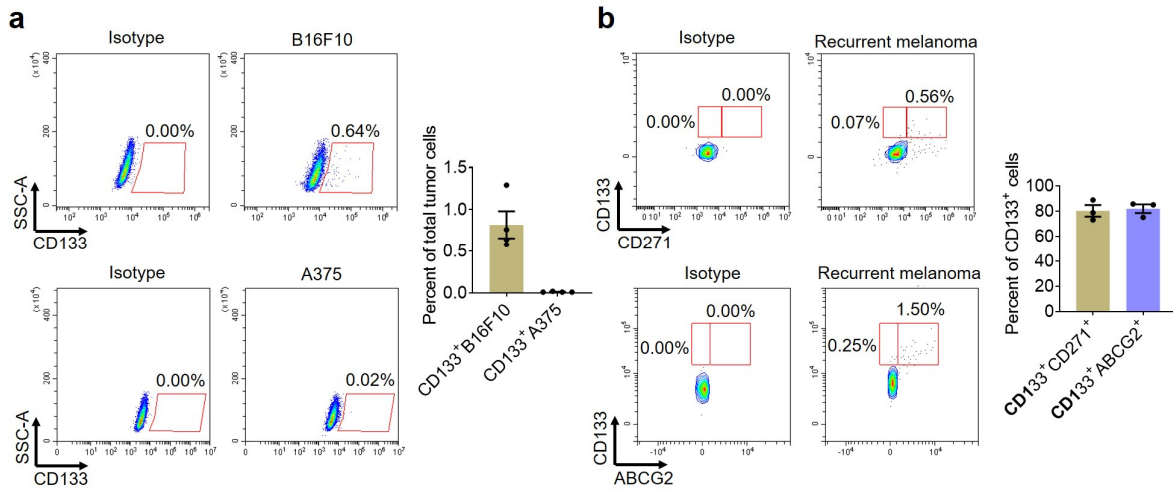


Supplementary Figure 2. Increased ECM and CAFs of the relapsed B16F10 melanoma following the adoptive macrophage therapy. **a** Micrographs of H&E staining and immunofluorescence staining with α -SMA (red), collagen IV (green) or collagen II (green) of tumor tissues following the NPs-M Φ^1 -treatment at day 35. Mice before treatment at day 8 and without treatment at day 16 were used as control. Of note, in the mice without treatment at day 16, the tumor volume reached the similar size ($\sim 500 \text{ mm}^3$) to that of mice following the NPs-M Φ^1 -treatment at day 35. Image shown is representative of $n = 3$ independent replicates of experiments with similar results. **b, c** Representative confocal microscopic images of α -SMA (red) and collagen IV (green, b) or collagen II (green, c) in the tumor of (a). Nuclei were counterstained with DAPI (blue). Image shown is representative of $n = 3$ independent replicates of experiments with similar results.



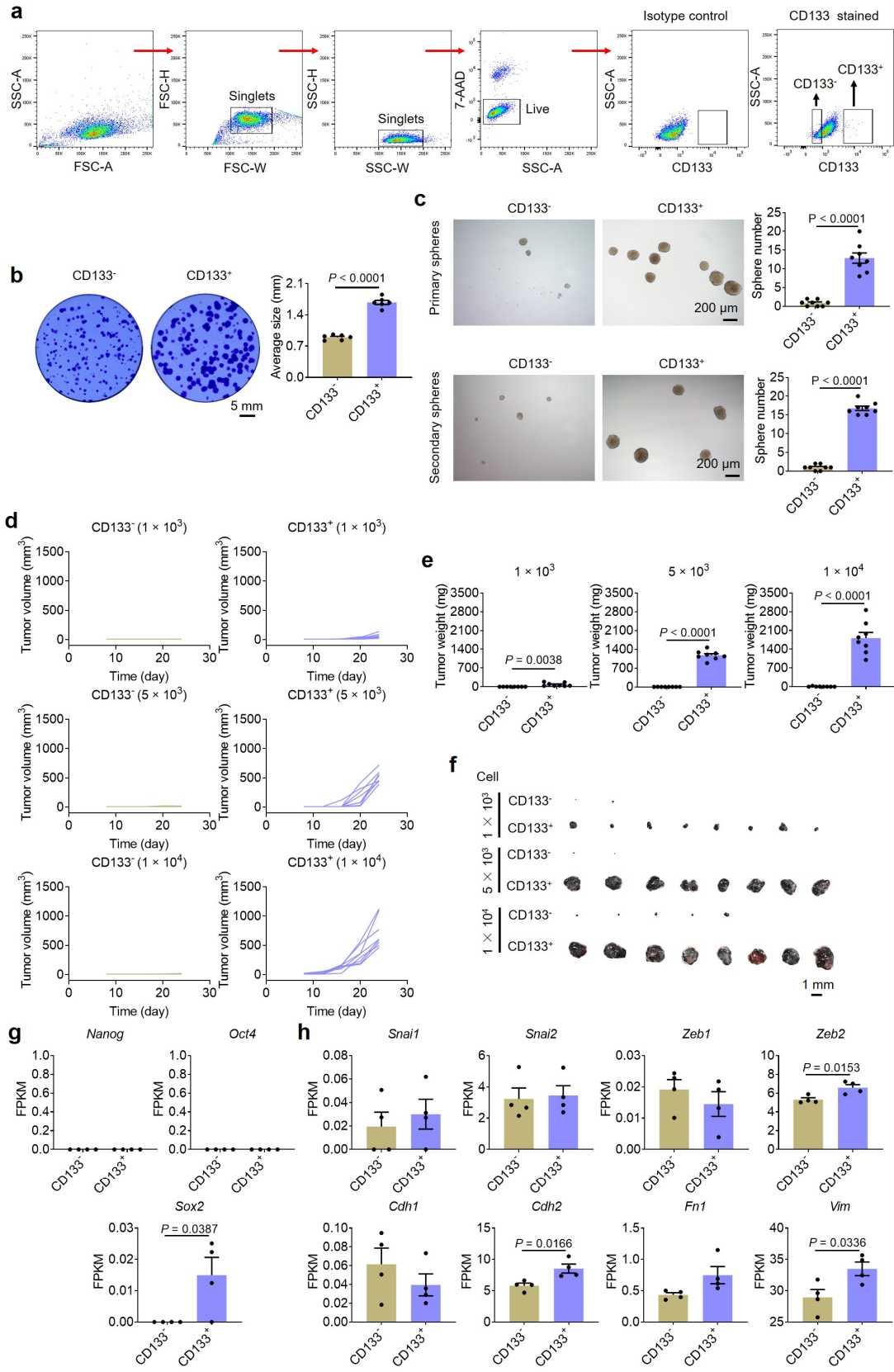
Supplementary Figure 3. Restricted tumor trafficking of the transferred macrophages and infiltration of various immune cells in the relapsed B16F10 melanoma. **a** Flow cytometric analysis of leukocyte populations at day 14 and day 23 after i.t. injected with 2×10^6 of NPs-MΦ per mouse at day 8 and day 11, respectively. Unpaired two-tailed *t*-test ($n = 5$ mice). **b, c** The tumor-bearing mice without NPs-MΦ¹ treatment at day 8 or day 16, or receiving the NPs-MΦ¹-treatment at day 35 were i.t. injected with the DiD-labeled NPs-MΦ at a dose of 10×10^4 in 20 μl of PBS per mouse. The tumor samples were collected

at 0 h, 4 h and 24 h post-administration, respectively. Images of H&E staining and the corresponding fluorescence images of the injected DiD-labeled NPs-M Φ (red) and the immuno-stained collagen IV (green) in the tumor sections (b). Representative confocal microscopic images of (b) of collagen IV (green) and the DiD-labeled NPs-M Φ (red) in the tumor (c). The nuclei were counterstained with DAPI (blue). Insets of yellow boxes are enlarged to view the merged signals of collagen IV and NPs-M Φ . Image shown is representative of $n = 3$ independent replicates of experiments with similar results. Data are expressed as mean $\pm$ s.e.m. Source data are provided as a Source Data file.

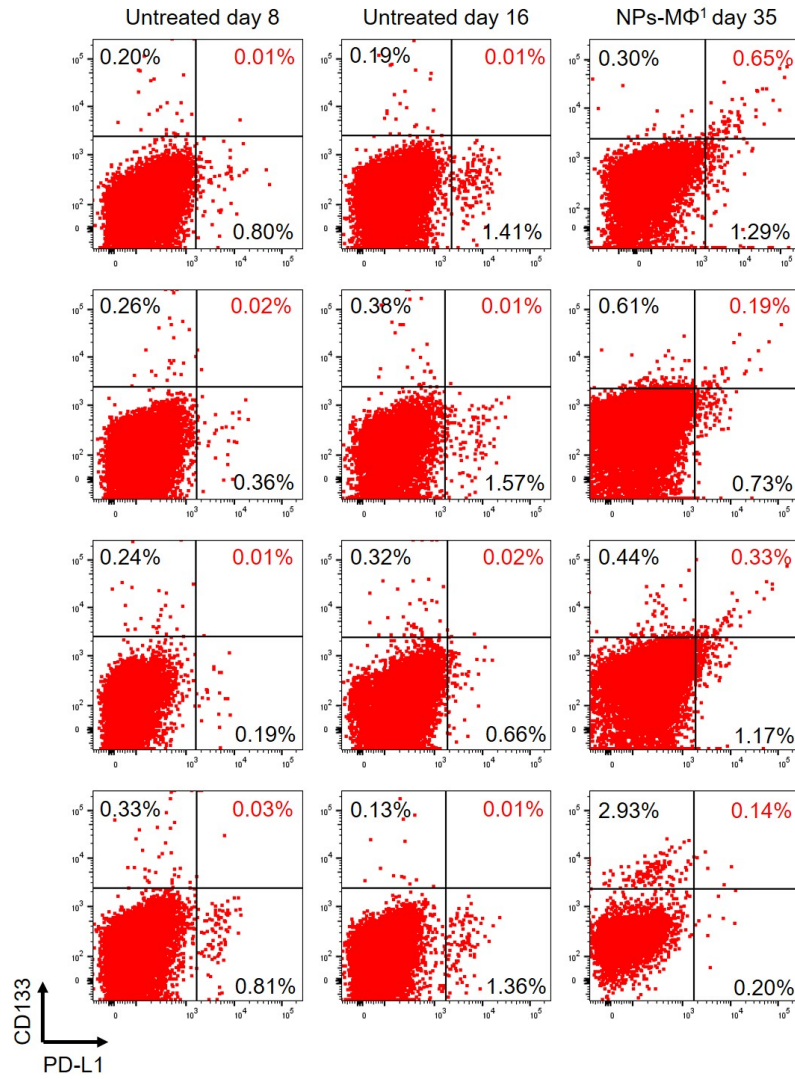


Supplementary Figure 4. Identification of CD133 and other stemness markers in melanoma cells. a

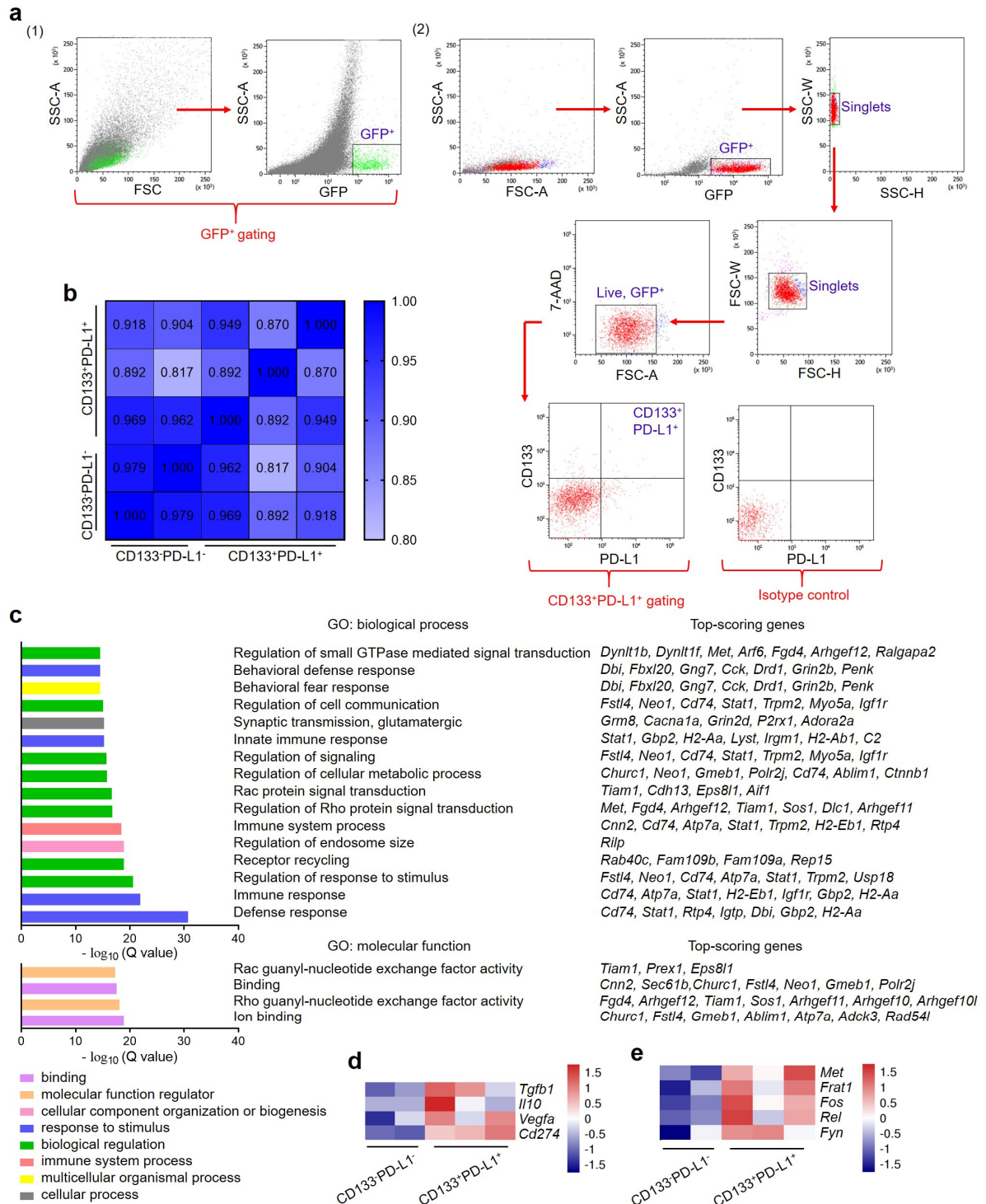
The CD133 expression in mouse B16F10 and the human A375 cell lines, respectively. Left, representative dot plots of the flow cytometric analysis. Image shown is representative of $n = 4$ independent replicates of experiments with similar results. Right, the corresponding percentage of CD133⁺ gated in total tumor cells ($n = 4$ biologically independent samples). **b** The co-expression of CD133 with CD271 and ABCG2 in the recurrent B16F10 melanoma samples, respectively. Left, the representative dot plots of the flow cytometric analysis. Image shown is representative of $n = 3$ independent replicates of experiments with similar results. Right, the corresponding percentage of CD133⁺CD271⁺ and CD133⁺ABCG2⁺ cancer cells gated in CD133⁺ cancer cells, respectively ($n = 3$ mice). Data are expressed as mean $\pm$ s.e.m. Source data are provided as a Source Data file.



Supplementary Figure 5. CD133⁺ B16F10 melanoma cancer cells exhibit some stem-like features. **a** FACS gating strategy for sorting of CD133⁺ and CD133⁻ fractions. SSC-A, side scatter area; FSC-A, forward scatter area; FSC-H, forward scatter height; FSC-W, forward scatter width; SSC-H, side scatter height; SSC-W, side scatter width. **b** Clonogenic assay. The sorted CD133⁺ and CD133⁻ cancer cells were seeded in 6-well plates at a density of 200 cells per well for 8 days, respectively. The average size of colonies was quantified by Nano Measurer software. Unpaired two-tailed *t*-test ($n = 6$ biologically independent samples). Image shown is representative of $n = 6$ independent replicates of experiments with similar results. **c** Sphere formation assays. The sorted CD133⁺ and CD133⁻ cancer cells were cultured in 24-well ultra-low-adherent plates at a density of 500 cells per well for 10 days, respectively. Spheres with a diameter over 70 μm were counted. For secondary tumorsphere formation, the primary tumorspheres were dissociated into single cells, following by replated in 24-well ultra-low-adherent plates at 500 cells per well. Unpaired two-tailed *t*-test ($n = 8$ biologically independent samples). Image shown is representative of $n = 8$ independent replicates of experiments with similar results. **d-f** In vivo tumorigenicity of CD133⁺ B16F10 cancer cells. (d) Individual tumor growth curves of mice s.c. injected with the sorted CD133⁺ or CD133⁻ cancer cells at the dose of 1×10^3 , 5×10^3 or 1×10^4 cells. (e, f) The weights (e) and photographs (f) of the dissected tumors in (d) at day 24 post injection. Unpaired two-tailed *t*-test ($n = 8$ mice). **g, h** Quantitative analysis of the gene expression profiles associated with the CSC features (g) or EMT signatures (h). Unpaired two-tailed *t*-test ($n = 4$ biologically independent samples). Data are expressed as mean $\pm$ s.e.m. Source data are provided as a Source Data file.



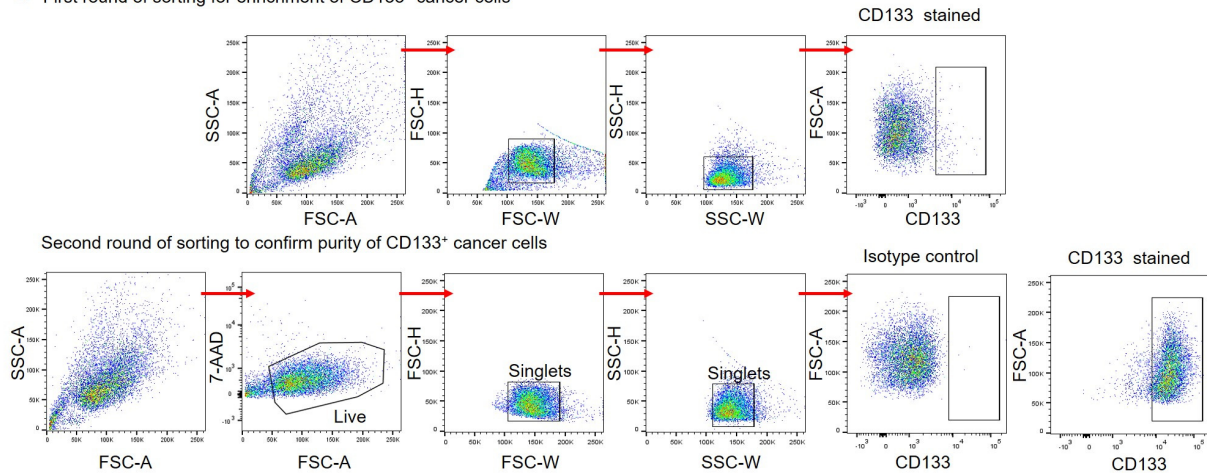
Supplementary Figure 6. Individual dot plots of the other four biologically independent samples of each group by the flow cytometric analysis in Fig. 2c.



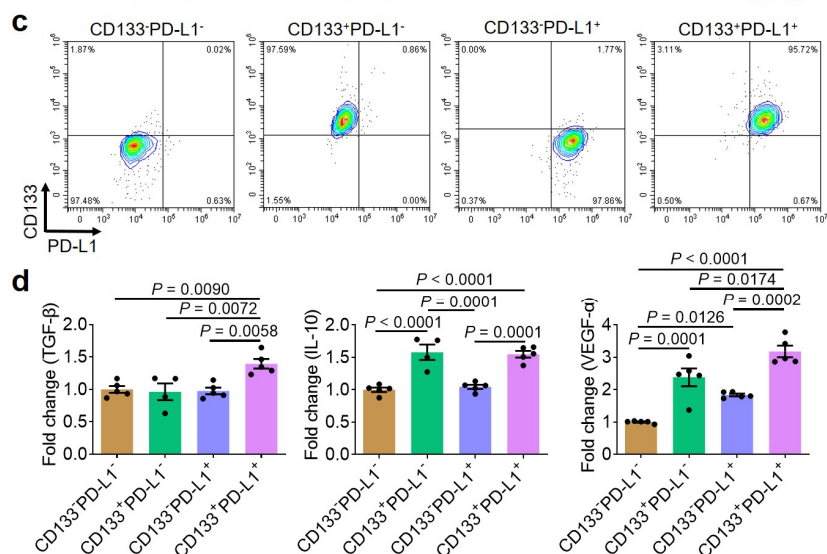
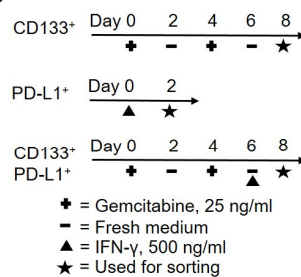
Supplementary Figure 7. Characterization of CD133⁺PDL1⁺ cancer cells in mouse melanoma following the adoptive macrophage therapy. **a** FACS gating strategy for CD133⁺PD-L1⁺ from B16-GFP

cells in the tumor at day 35 following NPs-M Φ ¹-treatment. The whole tumor cell suspensions were subjected to the selection by the GFP fluorescence to enrich the GFP⁺ cancer cells (1), and a second round of GFP selection followed by gating on the singlets (FSC-H versus FSC-W and SSC-H versus SSC-W) to ensure the individual cells. 7-Aminoactinomycin D (7-AAD) was used to exclude non-viable cells (2). The two subpopulation GFP⁺7-AAD⁻CD133⁺PD-L1⁺ and GFP⁺7-AAD⁻CD133⁻PD-L1⁻ cancer cells were sorted for the bulk RNA-seq analysis, respectively. **b** Correlation analysis between the two groups of the sorted cells, i.e. CD133⁺PD-L1⁺ ($n = 3$ mice) and CD133⁻PD-L1⁻ cancer cells ($n = 2$ mice). **c** Top 20 enriched gene ontology (GO) terms were significantly correlated with CD133⁺PD-L1⁺ cancer cells ranked by Q value. Only the top 7 genes per set are shown in each category. The x axis indicates the $-1 \times \log_{10}$ of the Q value. **d, e** Heatmaps of the cluster of genes associated with key immunosuppressive molecules (d) and proto-oncogenes (e) of the in vivo sorted CD133⁺PD-L1⁺ ($n = 3$ mice) or CD133⁻PD-L1⁻ ($n = 2$ mice) cancer cells. Source data are provided as a Source Data file.

a First round of sorting for enrichment of CD133⁺ cancer cells



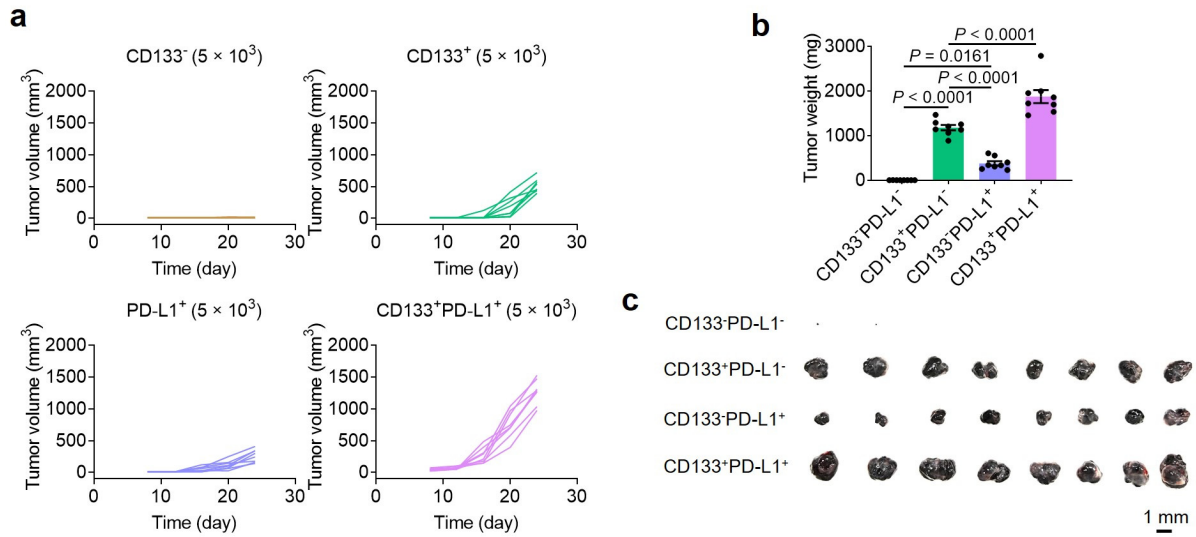
b



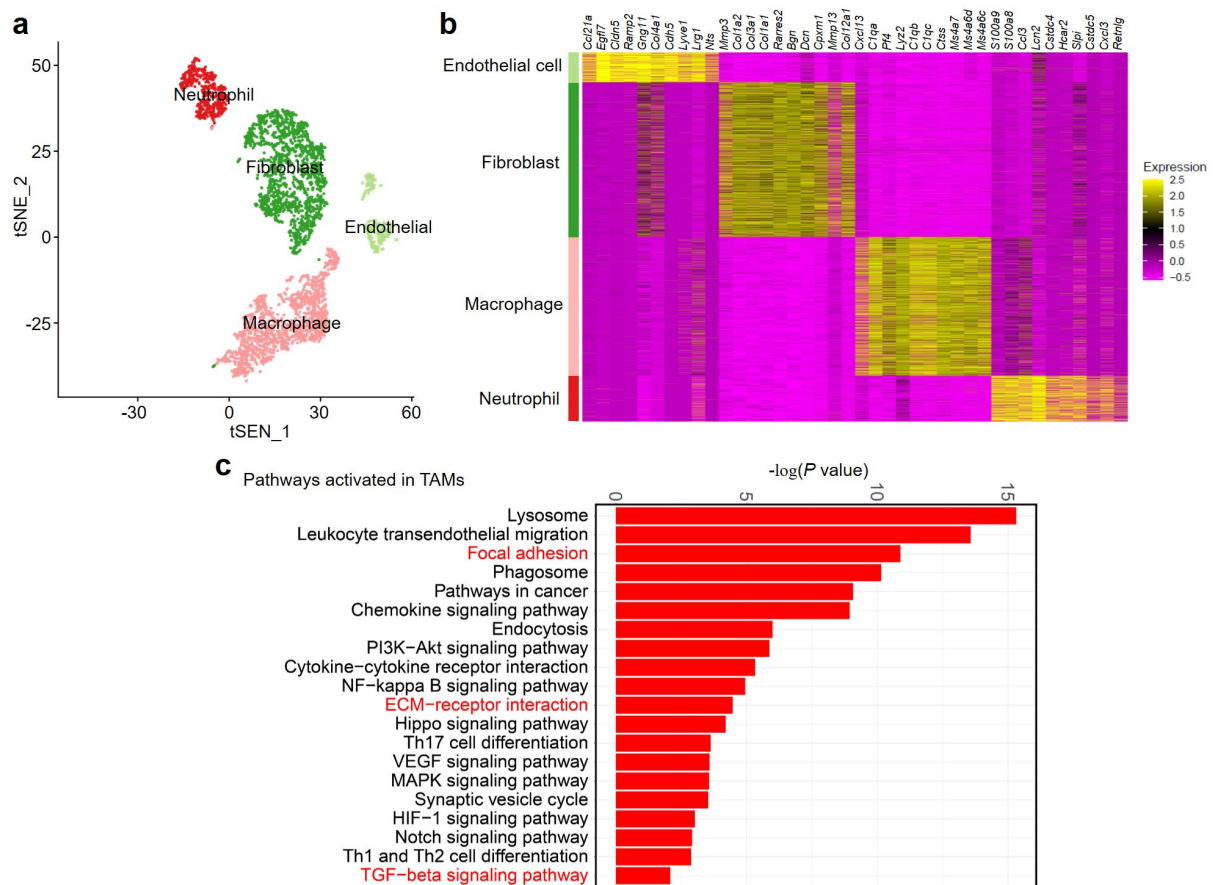
Supplementary Figure 8. Induction and characterization of the CD133⁺PD-L1⁺ cancer cells in vitro.

a FACS gating strategy in the first round of sorting for enrichment of the CD133⁺ B16F10 cancer cells (Upper) and second round of sorting to confirm the purity of CD133⁺ B16F10 cancer cells (Lower). Doublets were excluded on the basis of FSC and SSC. The 7-AAD⁻ live cancer cells were selected. The CD133⁺ cells were first sorted by flow cytometry following the CD133 immunostaining. These sorted CD133⁺ cells were then subjected to a second round of sorting to confirm their purity, showing approximately 100% of the CD133 positivity rate. **b** Scheme for in vitro induction of CD133⁺PD-L1⁻, CD133⁺PD-L1⁺ and CD133⁺PD-L1⁺ B16F10 cancer cells, respectively. **c** The

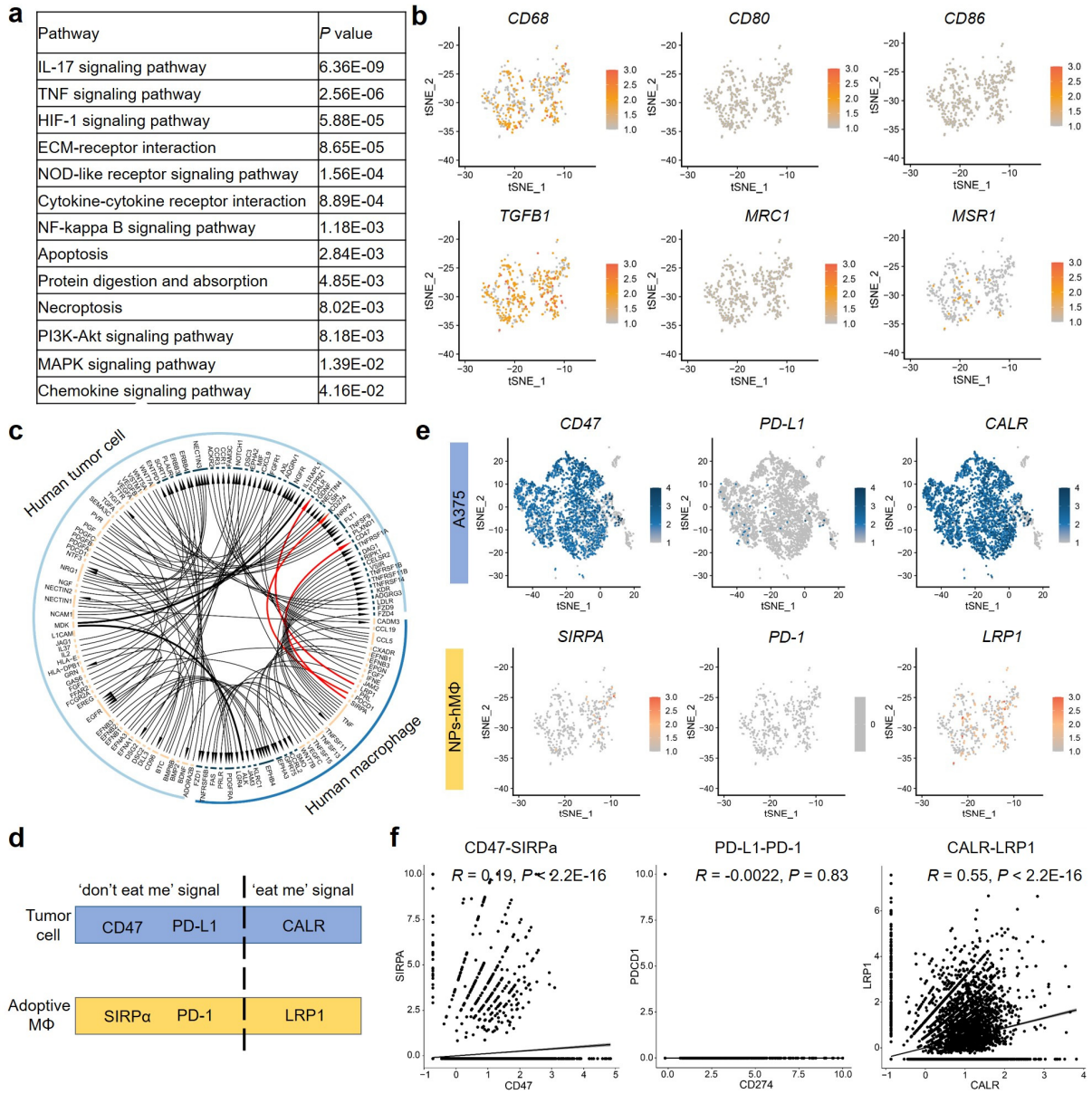
corresponding phenotypes confirmed by flow cytometry. Image shown is representative of $n = 3$ independent replicates of experiments with similar results. **d** Flow cytometric determination of the level of TGF- β , IL-10 and VEGF- α in CD133⁻PD-L1⁻, CD133⁺PD-L1⁻, CD133⁻PD-L1⁺ and CD133⁺PD-L1⁺ cancer cells, respectively. The expression of TGF- β , IL-10 or VEGF- α were normalized with the corresponding CD133⁺PD-L1⁻ cancer cells. One-way ANOVA with Tukey's post-test ($n = 4$ biologically independent samples of CD133⁺PD-L1⁻ cancer cells, and $n = 5$ biologically independent samples of CD133⁻PD-L1⁻, CD133⁻PD-L1⁺ or CD133⁺PD-L1⁺ cancer cells for TGF- β or IL-10; $n = 5$ biologically independent samples of each group of cancer cells for VEGF- α). Data are expressed as mean $\pm$ s.e.m. Source data are provided as a Source Data file.



Supplementary Figure 9. Tumorigenesis of CD133⁺PD-L1⁺ B16F10 cells in C57BL/6 mice. **a** Individual tumor growth curves of mice following s.c. injection of 5×10^3 of CD133⁻PD-L1⁻, CD133⁺PD-L1⁻, CD133⁻PD-L1⁺ or CD133⁺PD-L1⁺ B16F10 cancer cells into the left flank of mice ($n = 8$ mice). **b**, **c** The weights (b) and photographs (c) of the dissected tumors in (a). One-way ANOVA with Tukey's post-test ($n = 8$ mice). Data are expressed as mean $\pm$ s.e.m. Source data are provided as a Source Data file.

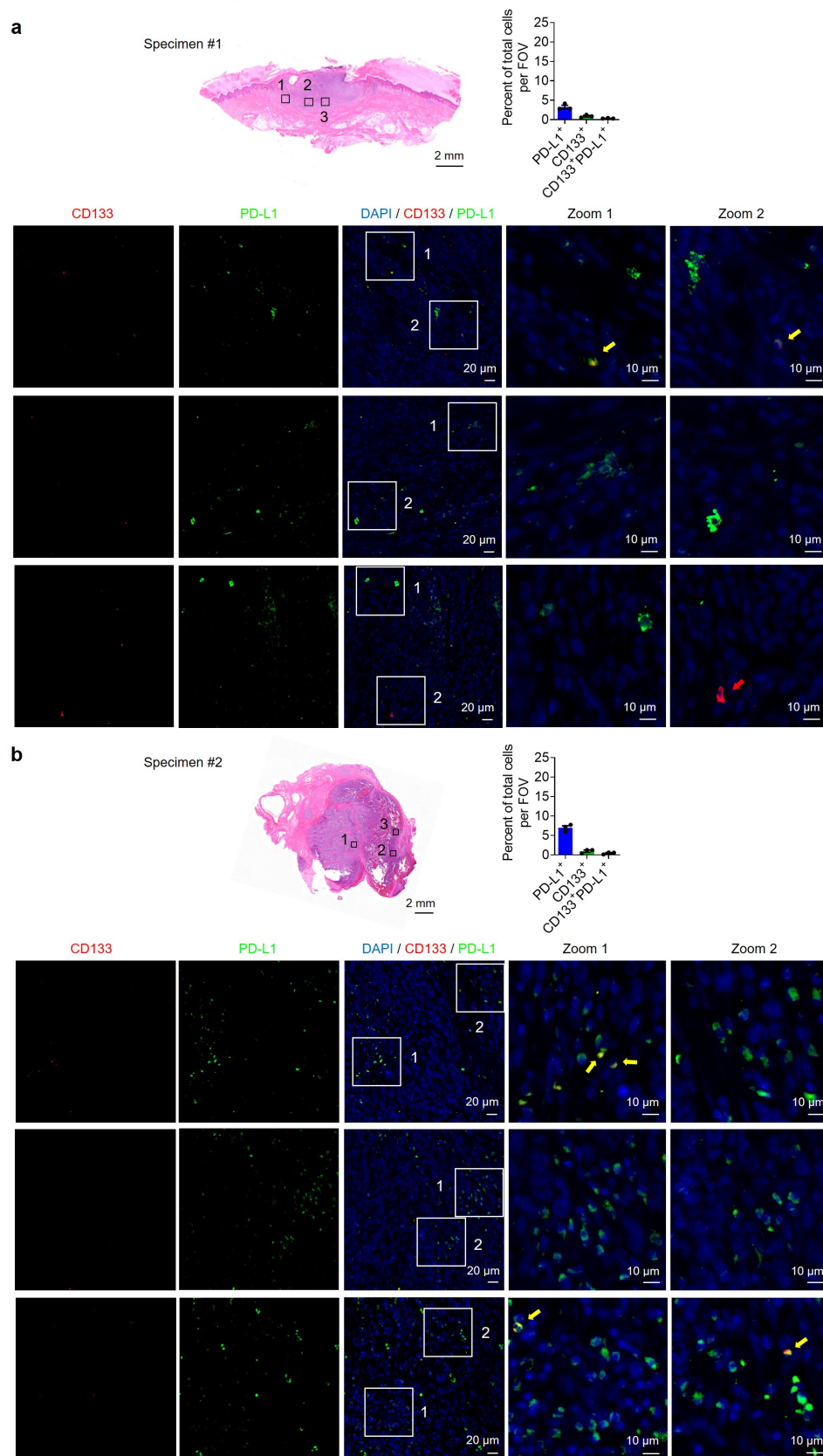


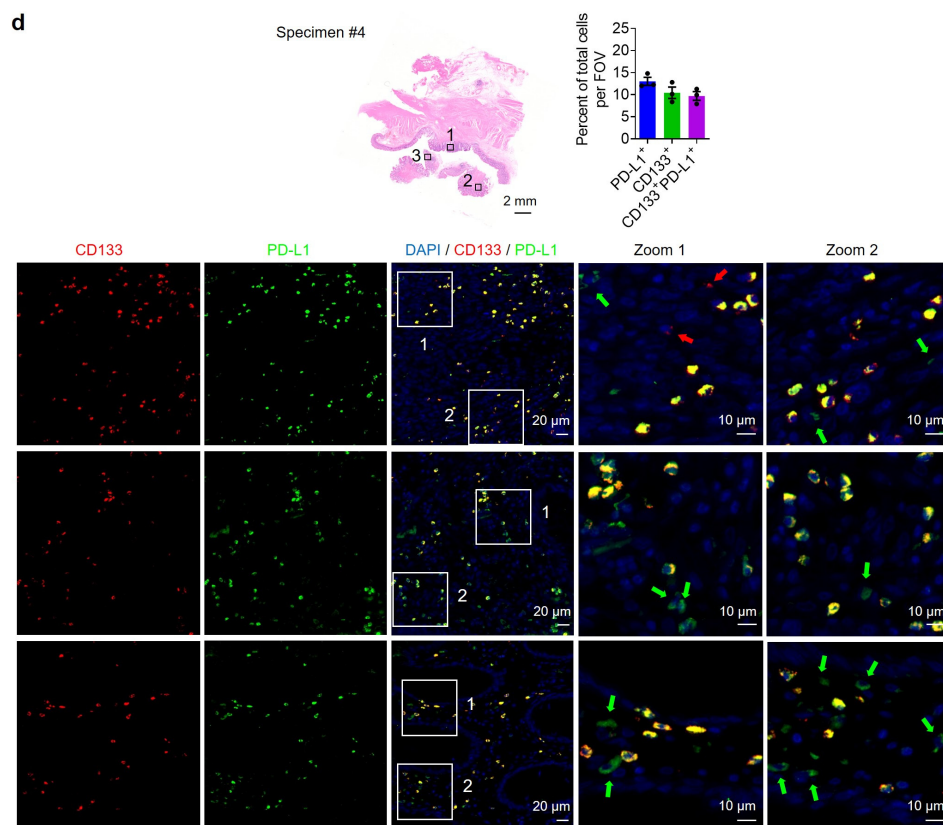
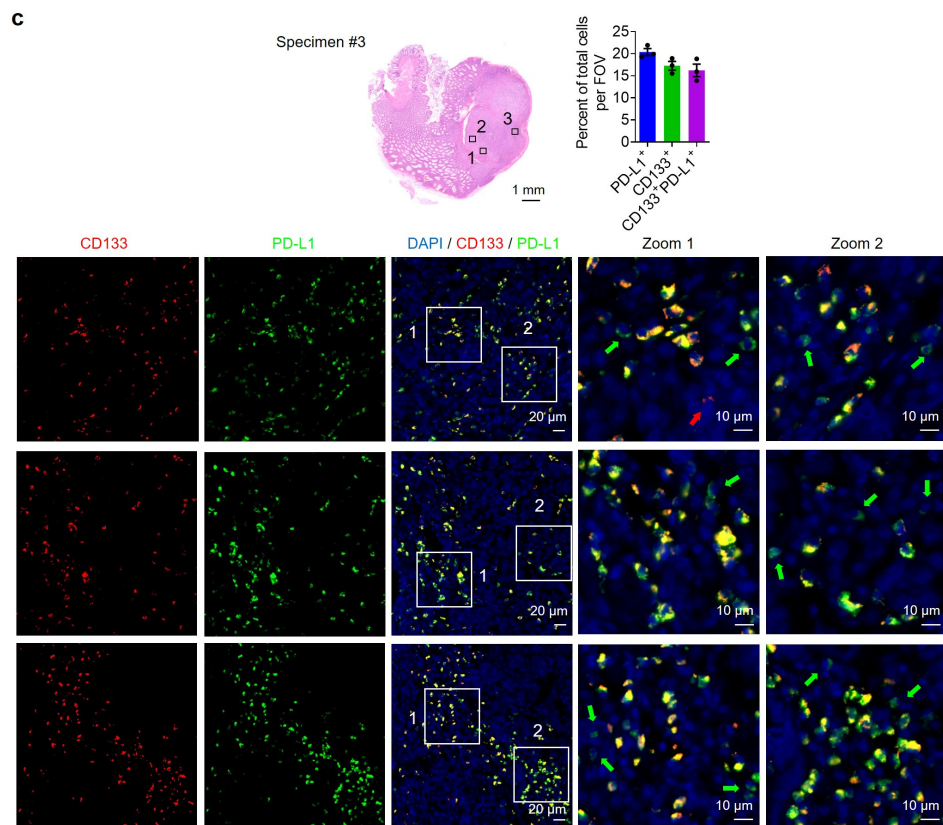
Supplementary Figure 10. The TME of the relapsed A375 melanoma characterized by the pro-tumoral TAMs. Tumor samples from the A375-bearing B-NDG mice at day 50 following the adoptive transfer of NPs-hMΦ were used for scRNA-seq. **a** tSNE plots showed the cell components in the TME including neutrophils, endothelial cells, CAFs and tumor associated macrophages (TAMs). $n = 579$ of neutrophils, $n = 392$ of endothelial cells, $n = 1955$ of CAFs and $n = 1754$ of TAMs. **b** Heatmaps showed the marker genes representative for the identified cells in the TME. **c** KEGG enrichment showed that the significantly activated pathways promoted the TAMs adopted a pro-tumoral polarization state. Comparison was performed between TAMs ($n = 1754$ cells) and other mouse-derived cell types ($n = 2926$ cells) in the tumor.

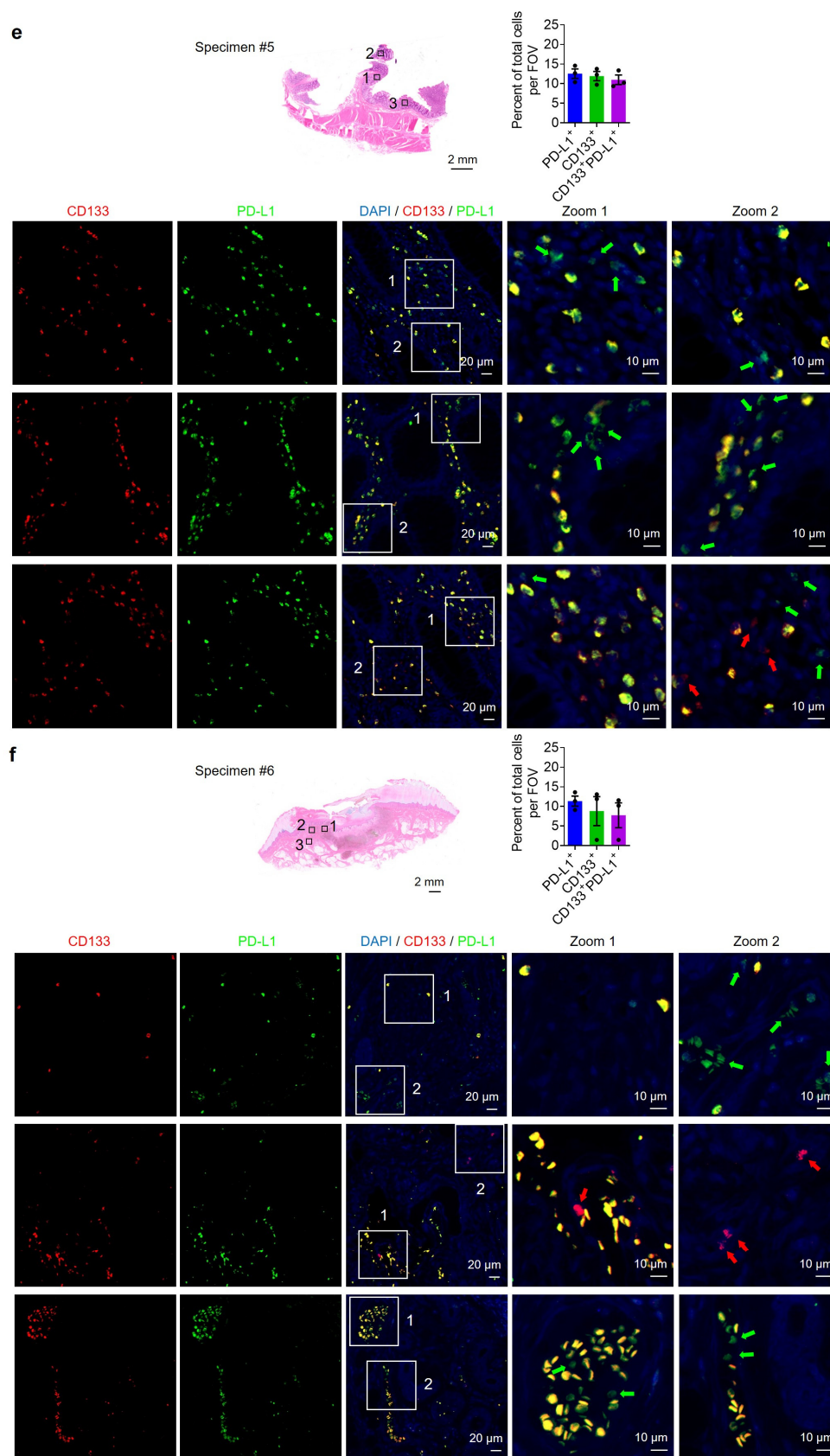


Supplementary Figure 11. Impaired antitumor capability of the remaining transferred human macrophages in the relapsed A375 melanoma. Tumor samples from the A375-bearing B-NDG mice following the adoptive transfer of NPs-hMΦ at day 50 were used for scRNA-seq. **a** Pathways activated in the remaining NPs-hMΦ. **b** tSNE plots of M1 and M2 marker genes in the remaining NPs-hMΦ. $n = 485$ of the transferred NPs-hMΦ. **c** Circos plots showed the whole ligand-receptor interactions between the NPs-hMΦ and the A375 cancer cells. Arrows, directions from ligands to receptors. $n = 4485$ of A375 cancer cells. n

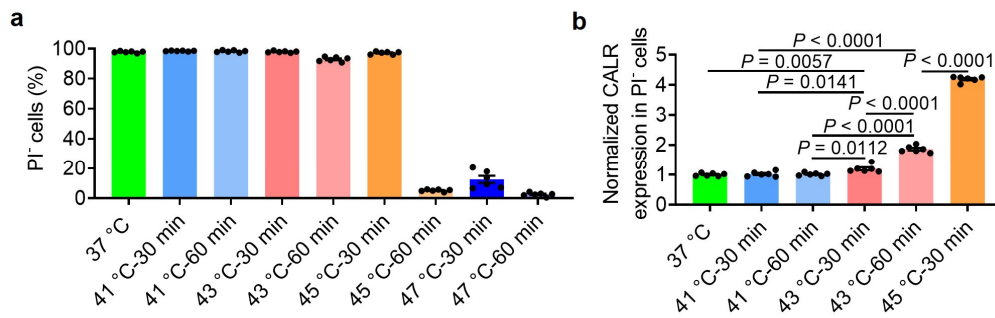
= 485 of the transferred NPs-hMΦ. **d** Scheme of the indicated ‘don’t eat me’ signals and ‘eat me’ signals between the cancer cells and the macrophages. **e** tSNE plots of the expression of selected genes associated with phagocytosis checkpoints, including CD47-SIRPα, PD-L1-PD-1, CALR-LRP1. *n* = 4485 of A375 cancer cells. *n* = 485 of the transferred NPs-hMΦ. **f** Correlation analysis of the phagocytosis related ligand-receptor pairs between the NPs-hMΦ and the A375 cancer cells. Two-tailed Pearson correlation test. *n* = 4485 of A375 cancer cells. *n* = 485 of the transferred NPs-hMΦ.





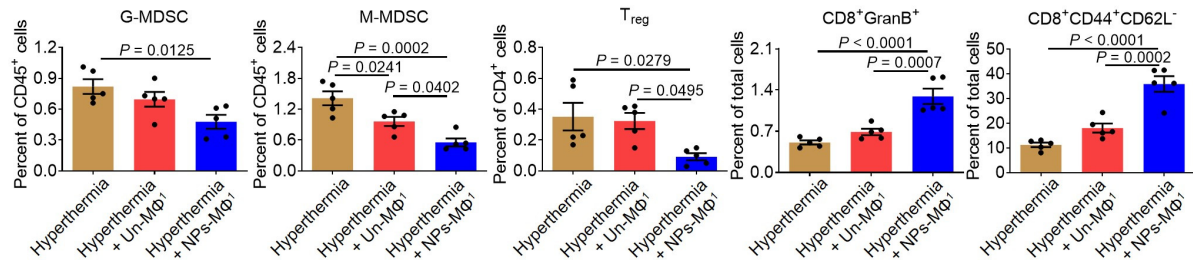


Supplementary Figure 12. Identification of the CD133⁺PD-L1⁺ cancer cells in human primary and metastatic melanoma specimens. Two specimens of primary melanoma (#1 and #2) **a, b** and four specimens of metastatic melanoma (#3-#6) **c-f** were stained with CD133 (red) and PD-L1 (green), respectively. The nuclei were counterstained with DAPI (blue). The number of CD133⁺, PD-L1⁺ or CD133⁺PD-L1⁺ cancer cells within the field of view (FOV) was analyzed by Fiji ImageJ software. Three FOVs from each sample were selected for the analysis. Data are expressed as mean $\pm$ s.e.m. Yellow arrow, CD133⁺PD-L1⁺ cells; green arrows, CD133⁻PD-L1⁺ cells; red arrows, CD133⁺PD-L1⁻ cells. The detailed information of the specimens was presented in Supplementary Table 3. Source data are provided as a Source Data file.

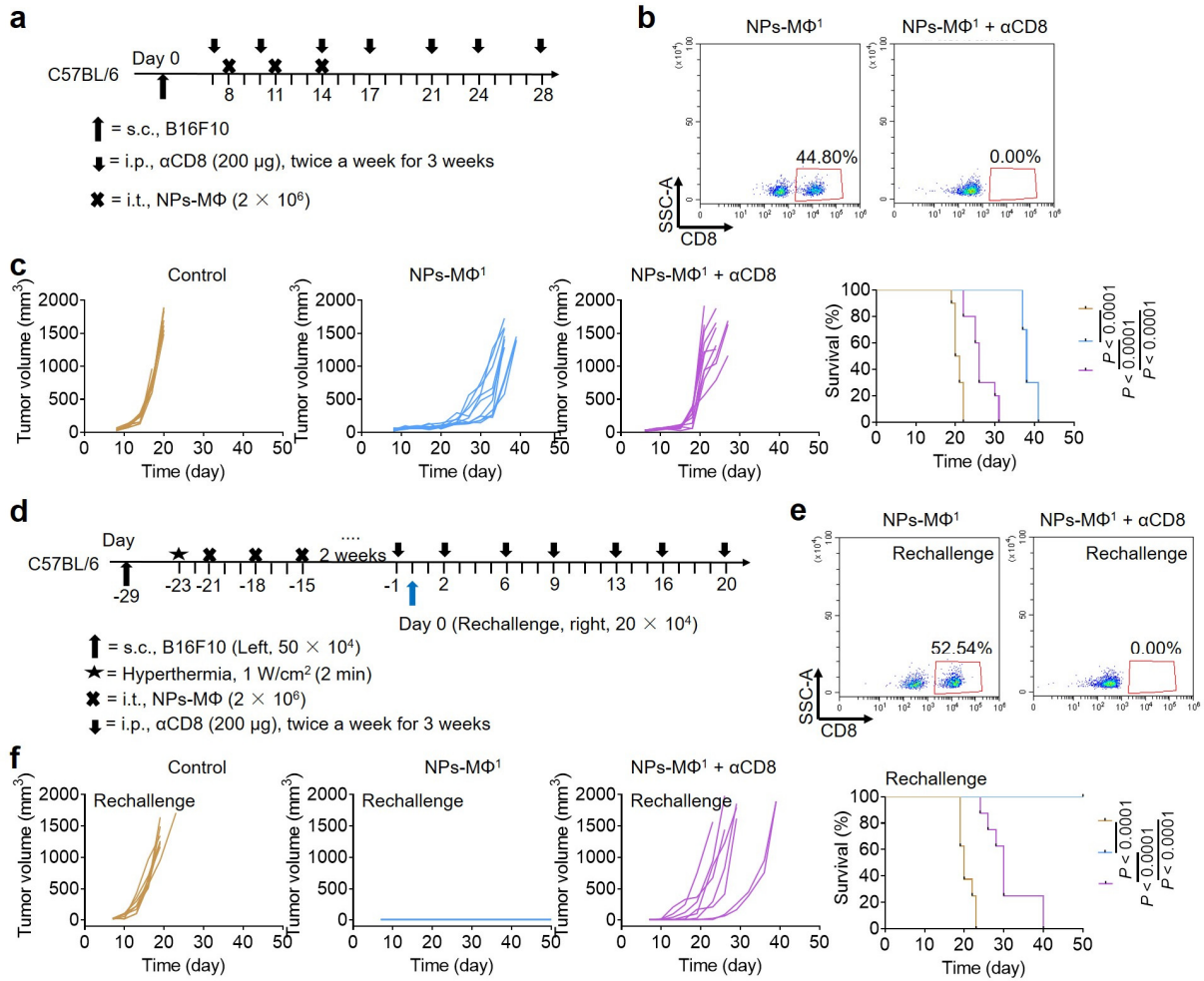


Supplementary Figure 14. CALR expression in the CD133⁺PD-L1⁺ cancer cells under hyperthermia.

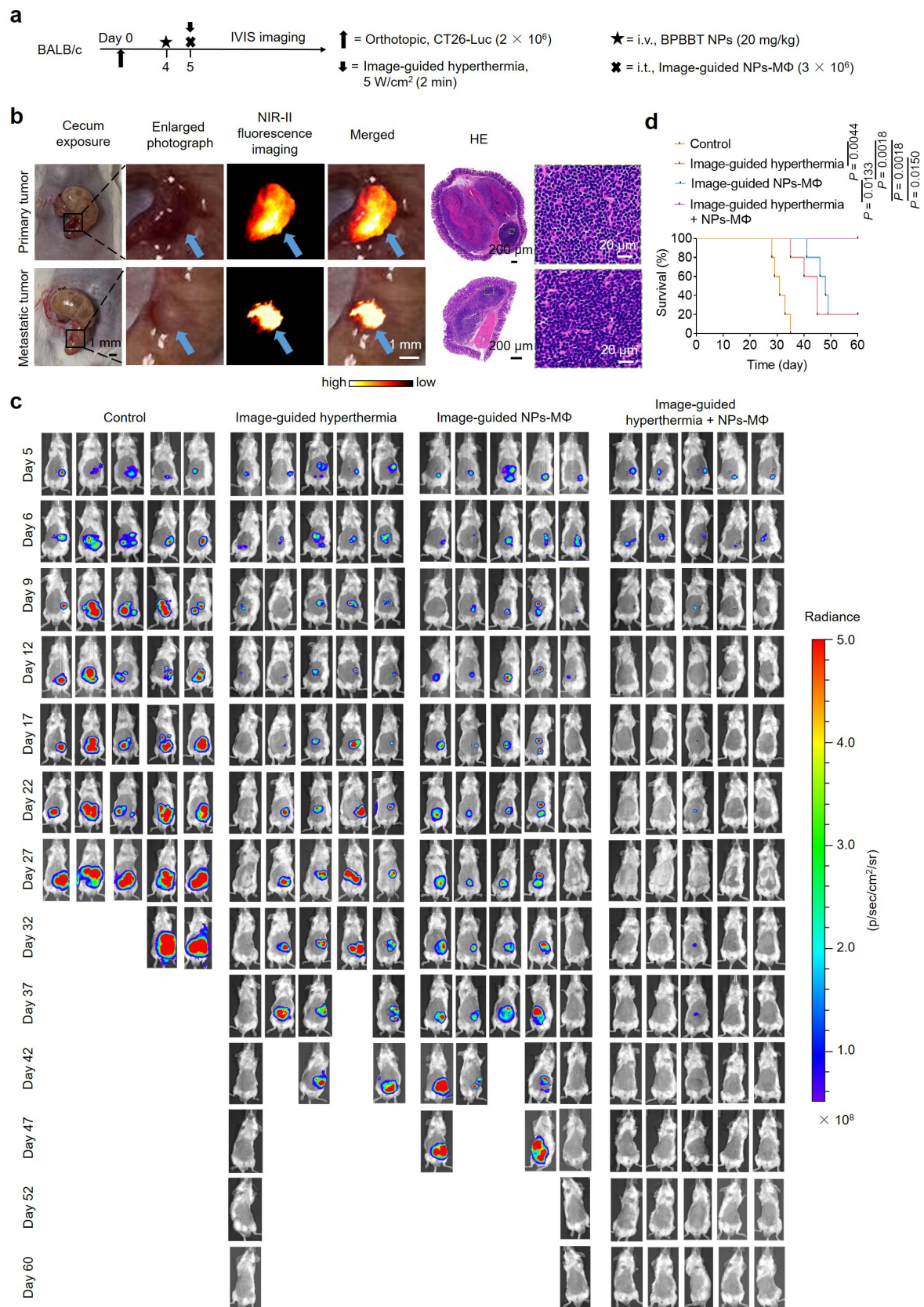
a The percentage of propidium iodide⁺ (PI⁺) cells under different hyperthermia treatment ($n = 6$ biologically independent samples). **b** The surface CALR expression of the PI⁺ cells was analyzed. The cells incubated at 37 °C were used as control. One-way ANOVA with Tukey's post-test ($n = 6$ biologically independent samples). Data are expressed as mean $\pm$ s.e.m. Source data are provided as a Source Data file.



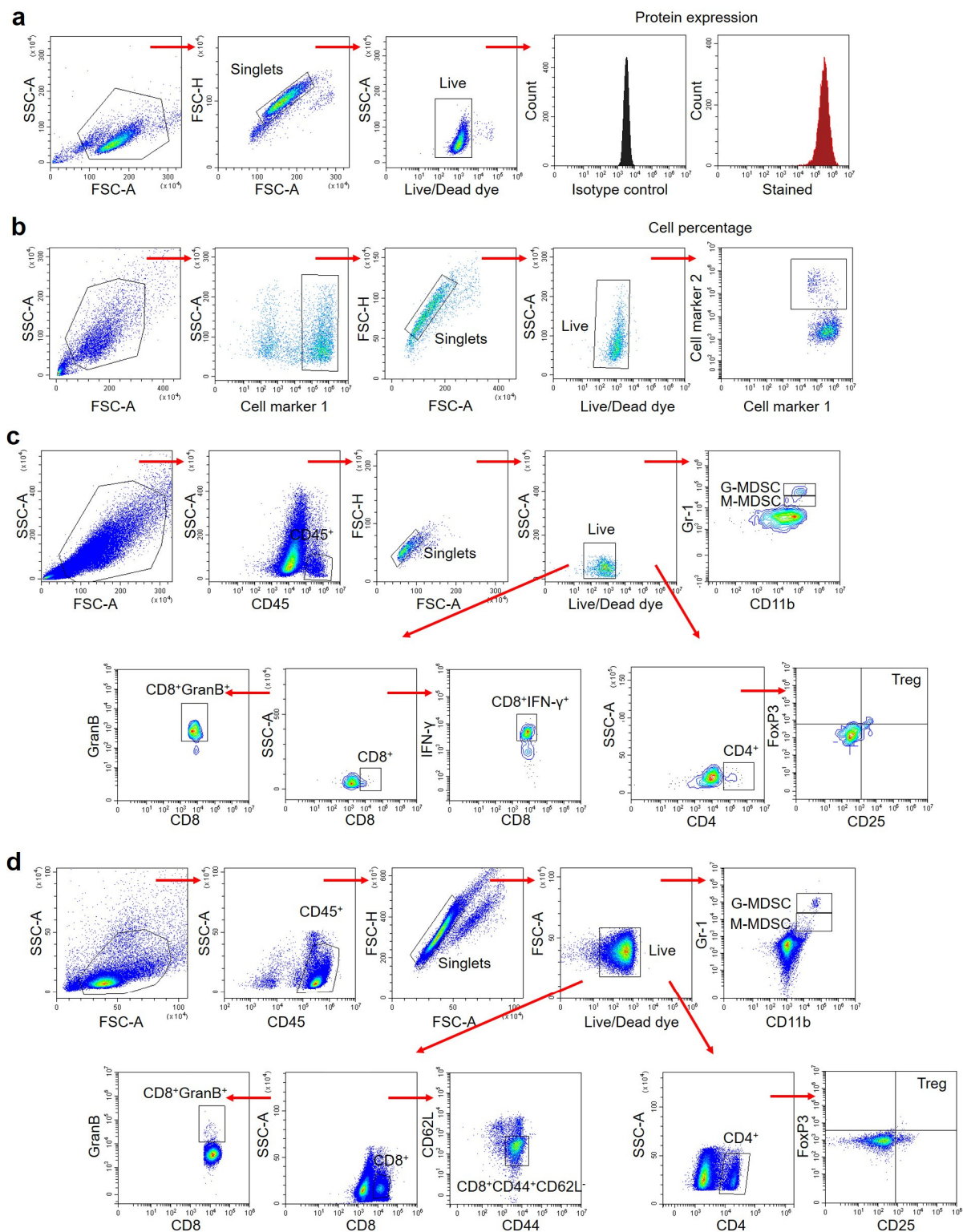
Supplementary Figure 15. The changes of immune cell populations in spleen after combinatorial hyperthermia and the NPs-MΦ¹ treatment in the B16F10 mouse model at day 21. CD11b⁺Gr-1⁺ MDSC subsets (CD11b⁺Gr-1^{high} granulocytic (G-MDSC) and CD11b⁺Gr-1^{int} monocytic (M-MDSC)), T_{reg} cells (CD4⁺CD25⁺ Foxp3⁺) as well as granzyme B-positive CD8⁺ T cells (CD8⁺GranB⁺) and effector memory T cells (CD8⁺CD44⁺CD62L⁻) were included. One-way ANOVA with Tukey's post-test ($n = 5$ mice). Source data are provided as a Source Data file.



Supplementary Figure 16. CD8 $^+$ T cells enhanced the antitumor efficacy of the NPs-M Φ transfer. a-c The combinatorial antitumor effect of CD8 $^+$ T cell depletion and adoptive NPs-M Φ^1 -treatment. Treatment regimens (a). The percentage of CD8 $^+$ T cells in blood following the in vivo depletion with anti-CD8 antibody (α CD8) (b). Image shown is representative of $n = 3$ mice with similar results. Individual tumor growth curves and the corresponding Kaplan-Meier survival curves, log-rank analysis ($n = 10$ mice) (c). **d-f** The combinatorial effect of CD8 $^+$ T cell depletion and adoptive NPs-M Φ^1 -treatment on tumor rejection. Treatment regimens (d). The percentage of CD8 $^+$ T cells in blood following the in vivo depletion with α CD8 (e). Image shown is representative of $n = 3$ mice with similar results. Individual tumor growth curves and the corresponding Kaplan-Meier survival curves, log-rank analysis ($n = 8$ mice) (f). Source data are provided as a Source Data file.



Supplementary Figure 17. Intraoperative fluorescence image-guided hyperthermia and adoptive transfer of NPs-M Φ for treatment of mice bearing orthotopic CT26-Luc colon cancer. **a** Treatment regimens. **b** Intraoperative NIR-II fluorescence imaging of tumors. Left four columns, intraoperative photograph and NIR-II fluorescence images of the exposed mouse cecum with primary or metastatic tumor. Arrows, tumor. Right two columns, H&E staining of the resected tumors. Image shown is representative of $n = 3$ independent replicates of experiments with similar results. **c** In vivo bioluminescence imaging of CT26-Luc tumors after treatment ($n = 5$ mice). **d** Kaplan-Meier survival curves, log-rank analysis ($n = 5$ mice). Source data are provided as a Source Data file.



Supplementary Figure 18. Flow cytometry gating strategy. The general gating strategy started with FSC-A/SSC-A to exclude cell debris, followed by FSC-A/FSC-H to confirm singlets. Live cells were selected by gating Live/Dead dye negative cells. **a** Gating strategy for analyzing the expression of the surface or intracellular markers (PD-L1, TGF- β , IL-10, VEGF- α , ROS, CD86, MHC II, CD163, SIRP α , CALR, PD-1) in Figures 2a, 2b, 2d, 3b, 4b, 4c, 4f, 7b, 8b, 8e, 8g, and Supplementary Figures 8d, 13b, 14b. **b** Gating strategy for analyzing the percentage of the marker cells (Phagocytosis, CD69, CD45, CD11c, CD133, CD271, ABCG2, PI) in Figures 3c, 4e, 4h, 8c, and Supplementary Figures 3a, 4, 14a. **c** Gating strategy for analyzing the immune cell populations in tumor (CD11b⁺Gr-1⁺ MDSC subsets, T_{reg} cells (CD4⁺CD25⁺ Foxp3⁺), granzyme B-positive CD8⁺ T cells (CD8⁺GranB⁺) and IFN- γ -positive CD8⁺ T cells (CD8⁺IFN- γ ⁺)) in Figure 7f. **d** Gating strategy for analyzing the immune cell populations in spleen (CD11b⁺Gr-1⁺ MDSC subsets, T_{reg} cells (CD4⁺CD25⁺ Foxp3⁺), granzyme B-positive CD8⁺ T cells (CD8⁺GranB⁺) and effector memory T cells (CD8⁺CD44⁺CD62L⁻)) in Supplementary Figure 15. Of note, the gating strategy for analyzing the percentage of GFP⁺CD133⁺PD-L1⁻, GFP⁺CD133⁻PD-L1⁺ and GFP⁺CD133⁺PD-L1⁺ cancer cells in tumor of Figures 2c, 3a, 8e, 8g is described in Supplementary Figure 7a.

Supplementary Table 1. A summary of median survival times of mice bearing B16F10 melanoma following different treatment regimens.

Treatment regime	Group	Median survival time
3 cycle-based macrophage transfer therapy (Fig. 1b)	Control	21 (<i>n</i> = 10 mice)
	Un-MΦ ³	30 (<i>n</i> = 10 mice)
	NPs-MΦ ³	42.5 (<i>n</i> = 10 mice)
Combinatorial αPD-L1 macrophage transfer therapy (Fig. 7a)	Control	21 (<i>n</i> = 8 mice)
	αPD-L1	26.5 (<i>n</i> = 8 mice)
	NPs-MΦ ¹	38 (<i>n</i> = 10 mice)
	αPD-L1 + NPs-MΦ ¹	25 (<i>n</i> = 8 mice)
Combinatorial αPD-1 macrophage transfer therapy (Supplementary Fig. 13a)	Control	20.5 (<i>n</i> = 10 mice)
	αPD-1	26 (<i>n</i> = 8 mice)
	NPs-MΦ ¹	38 (<i>n</i> = 10 mice)
	αPD-1 + NPs-MΦ ¹	49.5 (<i>n</i> = 8 mice)
Combinatorial TGF-β inhibitor macrophage transfer therapy (Fig. 7e)	Control	20.5 (<i>n</i> = 10 mice)
	TEW-7197	26 (<i>n</i> = 10 mice)
	NPs-MΦ ¹	38 (<i>n</i> = 10 mice)
	TEW-7197 + NPs-MΦ ¹	45 (<i>n</i> = 10 mice)
Combinatorial hyperthermia macrophage transfer therapy (Fig. 8d)	Hyperthermia	22.5 (<i>n</i> = 10 mice)
	Hyperthermia + Un-MΦ ¹	30.5 (<i>n</i> = 10 mice)
	Hyperthermia + NPs-MΦ ¹	N.A. (<i>n</i> = 10 mice) ^a

^aN.A., not available to calculate due to 90% survival at the terminal time point (210 days) of the experiment.

Fig. 7a, Supplementary Figure 13a and Fig. 7e shared the same data of the NP-MΦ¹ group. Supplementary Figure 13a and Fig. 7e shared the same data of the Control group.

Supplementary Table 2. A summary of median survival time of mice bearing human A375 melanoma following different treatment regimens.

Treatment regime	Group	Median survival time
1 cycle-based macrophage transfer therapy (Fig. 5a)	Control	27 (<i>n</i> = 6 mice)
	Un-hMΦ	49 (<i>n</i> = 6 mice)
	NPs-hMΦ	67 (<i>n</i> = 6 mice)
Combinatorial hyperthermia macrophage transfer therapy (Fig. 8f)	Hyperthermia	41 (<i>n</i> = 6 mice)
	Hyperthermia + Un-hMΦ	64.5 (<i>n</i> = 6 mice)
	Hyperthermia + NPs-hMΦ	96.5 (<i>n</i> = 6 mice)

Supplementary Table 3. A summary information of human melanoma specimens.

Specimen number	Pathological histology	Location	Tumor Type	Tumor Grade	Tumor Stage	Condition	Therapy-Naive
#1	Melanoma	Left plantar region	Primary	High-grade	Stage IIA Early-stage	Pre-relapse	Yes
	Treatment description: No treatment.						
#2	Melanoma	Vagina	Primary	High-grade	Stage IIB Early-stage	Pre-relapse	Yes
	Treatment description: No treatment.						
#3	Melanoma	Rectum	Metastatic	High-grade	Stage III Late-stage	Post-relapse	No
	Treatment description: Surgery was followed by treatment with the immunomodulator thymopentin. Specimens were collected at approximately 7 months after surgery.						
#4	Melanoma	Rectum	Metastatic	High-grade	Stage IV Late-stage	Post-relapse	No
	Treatment description: Surgery was followed by immunotherapy with camrelizumab. Specimens were collected at approximately 3 months after the last dose of camrelizumab.						
#5	Melanoma	Rectum	Metastatic	High-grade	Stage IIIB Late-stage	Post-relapse	No
	Treatment description: Surgery was followed by immunotherapy with camrelizumab. Specimens were collected at 14 days after the last dose of camrelizumab.						
#6	Melanoma	Left ankle joint	Metastatic	High-grade	Stage IV Late-Stage	Post-relapse	No
	Treatment description: Surgery. Specimens were collected at 10 days after surgery.						

Supplementary Table 4. A summary of antibodies used for flow cytometry.

Antibodies	Source	Identifier	Clone	Lot	Dilution
anti-mouse CD11c PE	Invitrogen	12-0114-82	N418	E01079-1633	1/80
anti-mouse Ly-6G/Ly-6C PE	Invitrogen	12-5931-81	RB6-8C5	4335548	1/600
anti-mouse CD11b FITC	Invitrogen	11-0112-82	M1/70	2172513	1/200
anti-mouse F4/80 FITC	Invitrogen	11-4801-85	BM8	4318484	1/200
anti-mouse CD3 PerCP-Cyanine5.5	Invitrogen	45-0031-80	145-2C11	4304568	1/40
anti-mouse CD4 PE	Invitrogen	12-0041-83	GK1.5	E01010-1636	1/300
anti-mouse CD8 FITC	Invitrogen	11-0081-85	53-6.7	4271604	1/200
anti-mouse CD19 PE	Invitrogen	12-0193-81	eBio1D3	1946584	1/300
anti-mouse NK1.1 FITC	Invitrogen	11-5941-82	PK136	E00742-1632	1/200
anti-mouse PD-L1 PE	Invitrogen	12-5982-82	MIH5	4276912	1/600
anti-mouse CD16/32	Invitrogen	14-0161-82	93	E03557-1634	1/100
anti-mouse IL-10 PerCP-Cy5.5	Invitrogen	45-7101-82	JES5-16E3	2791188	1/80
anti-mouse CD45 APC	Invitrogen	17-0451-82	30-F11	2504481	1/300
anti-mouse CD45 PE	Invitrogen	12-0451-82	30-F11	E01244-1635	1/1200
anti-mouse CD4 FITC	Invitrogen	11-0043-82	RM4-4	2407411	1/400
anti-mouse CD25 APC	Invitrogen	17-0251-81	PC61.5	E07105-1640	1/300
anti-mouse CD45 PE-Cyanine5	Invitrogen	15-0451-82	30-F11	2785857	1/600
anti-mouse CD11b PerCP-Cyanine5.5	Invitrogen	45-0112-82	M1/70	2563995	1/160
anti-mouse Gr-1 APC	Invitrogen	17-9668-82	1A8-Ly6g	4286053	1/80
anti-mouse CD44 PerCP-Cyanine5.5	Invitrogen	45-0441-82	IM7	1984139	1/160
anti-mouse CD62L APC	Invitrogen	17-0621-81	MEL-14	4300018	1/600
anti-mouse Granzyme B PE	Invitrogen	12-8898-80	NGZB	2295595	1/300
anti-mouse IFN- γ PerCP-Cyanine5.5	Invitrogen	45-7311-80	XMG1.2	4329723	1/160
anti-mouse Foxp3 PerCP-Cyanine5.5	Invitrogen	45-5773-82	FJK-16s	4295813	1/160
anti-human CD133 PE	Invitrogen	12-1338-42	TMP4	2633386	1/20
anti-human PD-L1 APC	Invitrogen	17-5983-42	MIH1	2764014	1/20
anti-human CD8 FITC	Invitrogen	11-0088-42	RPA-T8	2247551	1/20
anti-human CD69 PE	Invitrogen	12-0699-42	FN50	2264967	1/20
anti-mouse CD271 PE	Invitrogen	12-9400-42	ME20.4	2618940	1/20
anti-mouse CD133 APC	BioLegend	141207	315-2C11	B232568	1/80
anti-mouse CD133 PE	BioLegend	141203	315-2C11	B262124	1/20
anti-mouse PD-L1 PE-Cy7	BioLegend	124314	10F.9G2	B261299	1/80
anti-mouse ABCG2 APC	BioLegend	164303	S17006D	B393993	1/20
anti-human CD68 FITC	BioLegend	333806	Y1/82A	B281870	1/20
anti-human CD86 PE/Cyanine5	BioLegend	305408	IT2.2	B284508	1/20
anti-human CD163 APC	BioLegend	333610	GHI/61	B311341	1/20

anti-human HLA-DR PE	BioLegend	307606	L243	B340727	1/20
anti-human HLA-A,B,C FITC	BioLegend	311404	W6/32	B281620	1/20
anti-human SIRP α APC	BioLegend	372105	15-414	B316544	1/20
anti-human TGF- β PE- Cyanine7	BioLegend	300007	S20006A	B404652	1/20
rabbit anti-CRT	Abcam	ab92516	EPR3924	GR3252549-1	1/50
anti-mouse α SMA PE	Novus Biologicals	NBP2-34522PE	1A4/asm-1	59- 1PABX170905- 021519-PE	1/150
anti-mouse TGF- β BV421	BD Biosciences	565638	TW7-16B4	8085548	1/20
anti-mouse VEGF- α APC	Novus Biologicals	NB100-664APC	VG1	F-2-080218- APC	1/100
