Supplemental Materials

Animals. IL-12p35 knockout mice were originally purchased from the Jackson Laboratory and were bred at Geisel School of Medicine at Dartmouth. Female mice (6-8 week old) were used for experiments.

NK depletion. Depleting anti-NK (clone PK136) antibodies were produced as bioreactor supernatant. 250 ml, equivalent to 250 mg of the antibody, was administered intraperitoneally 2 days prior to hyperthermia, on the day of hyperthermia and 5 days later. Greater than 95% depletion of NK cells was confirmed by flow cytometry**.**

MHC class I expression on B16. B16F10 cells were plated on 4 of T25 flasks. Next day, 2 flasks were transferred to an incubator set at 43°C and incubated for 30min. 24 or 48h after heating, cells were harvested from heated and unheated flasks. $2x10^5$ cells were blocked with anti-CD16/32 (eBioscience, CA), stained with anti-mouse H-$2K^b$ (clone AF6-88.5) or its isotype antibody (BioLegend, CA), and analyzed by 7-Color MACSQuant (Miltenyi Biotec, CA).

***In vitro* heating of tumor-infiltrating leukocytes.** Established B16 tumors were minced and filtered through 70μm strainers to obtain single cell suspension. Pooled cells were resuspended in 80% Percoll (GE Healthcare, WI) and placed in 50mL tubes. 40% Percoll was carefully added on the top and the tubes were spun at 410g for 25min at 18°C with no brake. Tumor-infiltrating leukocytes were harvested from the buffy layer, heated *in vitro* at 43°C 30min, and incubated at 37°C 14h. Cells were then stained with CD45, CD3e, CD8a, CD44 and CD69 and analyzed by 7-Color MACSQuant (Miltenyi Biotec, CA).

Vaccination study. Eppendorf tubes containing $6x10^6$ CT26 cells in 600 ml plain RPMI medium were g-irradiated (10,000 rads) and heated on a heat block at 37, 40, 43, or 46°C for 30 min and were rested at 37°C for 2h. On day -14 and -7, mice were ID vaccinated with 50ml of the heated cell solution (equivalent to $5x10^5$ CT26 cells) on the right flank. On day 0, mice were challenged ID with $1x10^5$ CT26 cells on the right (vaccinated side) and left flanks (contralateral side).

Supplemental Figure Legends

Supplemental Figure 1. Intratumoral heat difference is minimal. **(A)** Two temperature probes were placed approximately 3 mm apart within a tumor, and the tumor was heated as described in Method. **(A)** Readings from two temperature probes. Examples from two mice, showing the maximal temperature differences are both smaller than ± 0.4°C, the accuracy of our system.

Supplemental Figure 2. IONPs only or an AMF only does not affect the growth kinetics of distal tumors. **(A)** Experimental design to test the effect of IONPs only or an AMF only in CT26 model. **(B)** Growth kinetics of distal CT26 tumors. **(C)** Experimental design to test the effect of IONP only or an AMF only in B16 model. **(D)** Growth kinetics of distal B16 tumors.

Supplemental Figure 3. IONPs only or an AMF only does not affect the growth kinetics of secondary B16 tumors. **(A)** Experimental design to test the effect of IONPs only or an AMF only. **(B)** Growth kinetics of secondary tumors.

Supplemental Figure 4. NK cells are not required for the treatment efficacy. **(A)** Experimental design to test if NK cells are required for the treatment efficacy. **(B)** Histogram of splenocytes from mice with or without NK depleting antibody treatment, showing successful NK cell depletion. **(C)** Growth kinetics of secondary tumors.

Supplemental Figure 5. IL-12 is not required for the treatment efficacy. **(A)** Experimental design to test if IL-12 is required for the treatment efficacy. **(B)** Growth kinetics of secondary tumors.

Supplemental Figure 6. 45°C 20 min increases but does not activate $CD8^+$ T cells in dLN. **(A)** Experimental design to test if 45°C 20 min increases and activates $CD4^+$ and $CD8^+$ T cells. **(B)** % $CD4^+$ T cells and % $CD8^+$ T cells among leukocytes ($CD45^+$ cells) in dLN (top) and % $CD44^+$ $CD69^+$ cells among $CD4^+$ T cells and $CD8^+$ T cells in dLN (bottom).

Supplemental Figure 7. CD8 depleting antibody treatment successfully depletes $CD8^+$ T cells without affecting other major immune populations. **(A)** Experimental design to test how CD8 depleting antibodies affect major immune populations. **(B)** Number of $CD8^+$ T cells, $CD4^+$ T cells, $B220^+$ cells, $CD11b^+$ cells, $CD11c^+$ cells, and $NK1.1^+$ cells in the spleen from mice with or without CD8 depleting antibody treatment.

Supplemental Figure 8. Heating B16 cells *in vitro* at 43°C 30min induces upregulation of surface MHC class I. **(A)** Experimental design to test if heating B16 cells *in vitro* at 43°C 30min induces upregulation of surface MHC class I. **(B)** Representative histograms for unheated cells (top) and heated cells (middle), comparing isotype and MHC class I stainings, and histograms for MHCI class I stainings, comparing unheated cells and heated cells (bottom).

Supplemental Figure 9. Direct heating of tumor-infiltrating leukocytes at 43°C 30min does not activate T cells. **(A)** Experimental design to test if heating tumor-infiltrating leukocytes *in vitro* at 43°C 30min activates $CD4^+$ or $CD8^+$ T cells. **(B)** % $CD44^+$ $CD69^+$ cells among $CD4^+$ T cells and $CD8^+$ T cells after *in vitro* heating.

Supplemental Figure 10. Heating tumor cells for prophylactic vaccination *in vitro* before vaccination at 43°C or above dampens the vaccination effect. **(A)** Experimental design to test if heating tumor cells for prophylactic vaccination *in vitro* before vaccination affects the vaccination effect. **(B)** Growth kinetics of tumors given after vaccinations on the vaccinated side (left) and contralateral side (right).