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Supplementary Methods

Isolation and flow cytometry of Peripheral blood mononuclear cells

The main exploratory objective of this study was to determined MWA-induced immune response 1 week after ablation, so a volume of 6 mL peripheral blood was withdrawn from the patients on the day before MWA or surgery and 1 week after the treatments. The peripheral blood was also examined 4 weeks after MWA to determine whether immune responses still exist after surgical resection of the ablated tumor in patients with enhanced CD4+ T cells 1 week after MWA. Then, PBMCs were isolated by Ficoll discontinuous density gradient centrifugation and cryopreservation. PBMC were thawed and stained using a Zombie Aqua™ Fixable Viability Kit (BioLegend, San Diego, USA). Cells were incubated with Human TruStain FcX™ (Fc Receptor Blocking Solution, BioLegend) to block the Fc receptors. Then, PBMCs were stained with a cocktail of antibodies (BioLegend) against the following surface markers: Alexa Fluor® 700 anti-human CD3 (clone OKT3), FITC anti-human CD4 (clone PRA-T4), APC/Cyanine7 anti-human CD8a (clone RPA-T8), PE/Cy7 anti-human/mouse/rat CD278 (ICOS) (clone C398.4A), PE anti-human CD152 (CTLA-4) (clone L3D10), APC anti-human CD279 (PD-1) (clone EH), Brilliant Violet 421™ anti-human TIGIT (VSTM3) (clone A15153G), Brilliant Violet 785™ anti-human CD366 (Tim-3) (clone F38-2E2), PE anti-human CD223 (LAG-3) (clone 11C3C65), Brilliant Violet 421™ anti-human CD45RA (clone HI100), and PE anti-human CD197 (CCR7) (clone G043H7). Flow cytometric analysis was performed using the BD FACS Aria II cytometer (BD Biosciences), and data were analyzed using FlowJo 10.4 software.

Analysis of cytokines and soluble immune checkpoint molecules

A volume of 4 mL peripheral blood was withdrawn from patients on the day before and 1 week after

the treatments. The serum was stored at -80 °C until further analysis. The plasma fraction was isolated from the whole blood by centrifugation at 10,000 ×g for 10 min. The concentrations of Tumor necrosis factor-α (TNF-α), Interferon-γ (IFN-γ), Interleukin-2(IL-2), Interleukin-4(IL-4), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-10 (IL-10), Interleukin-12p70 (IL-12p70), and Interleukin-18 (IL-18) in the plasma were measured using Human ELISA Kit (eBiosciences) according to the manufacturer's instructions. The concentrations of soluble PD-L1 (sPD-L1), PD-L2 (sPD-L2), PD-1 (sPD-1), TIM-3 (sTIM-3), LAG-3 (sLAG-3), and Galectin-9 (sGalectin-9) in the plasma were detected using LEGENDplex™ HU Immune Checkpoint Panel 1-TC (12-plex) w/VbP (BioLegend), according to the manufacturer's instructions.

TCRβ amplification and sequencing

Total RNA was extracted from PBMCs using the RNeasy Mini kit (Qiagen, Hilden, Germany) and cDNA was synthesized using a PrimeScript RT Master Mix (TaKaRa Shuzo Co, Shiga, Japan) according to the manufacturer's instructions. RNA concentration and integrity were measured by Agilent 2100 Bioanalyzer. The cDNA sample was used to construct a library for TCRβ chain sequencing using Multiplex PCR. Survey sequencing of TCRβ was performed by BGI Tech (Shenzhen, China) using the Illumina HiSeq4000 platform. Raw sequencing data were analyzed using IMonitor. Clean sequences were aligned to V, D and J germline alleles (IMGT database, www.imgt.org) by the BLAST, and V, D and J gene segments were assigned for each clone. Meanwhile, sequences with poor-quality control and those including stop codons or frameshift mutations were filtered out. Productive TCRβ CDR3 sequences are the object of this study. Of these 35 MWA cases, 5 paired pre- and post-ablation blood samples were available after quality control and the experiments of T cell response by flow cytometry.

CD3+ T cells sorting

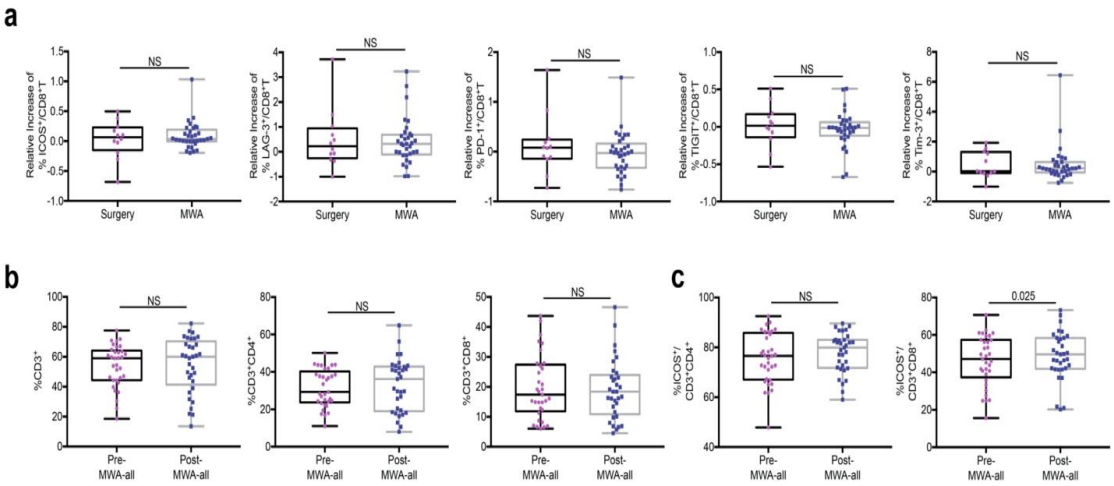
CD3+ T cells were sorted from PBMCs with Alexa Fluor® 700 anti-human CD3 antibody by FACS Aria II flow cytometer, and the purity of the CD3+ T cells was ≥ 95%. These cells were then processed for RNA extraction and resuspended in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 1% streptomycin, and 1% penicillin.

RNA isolation and real-time quantitative PCR

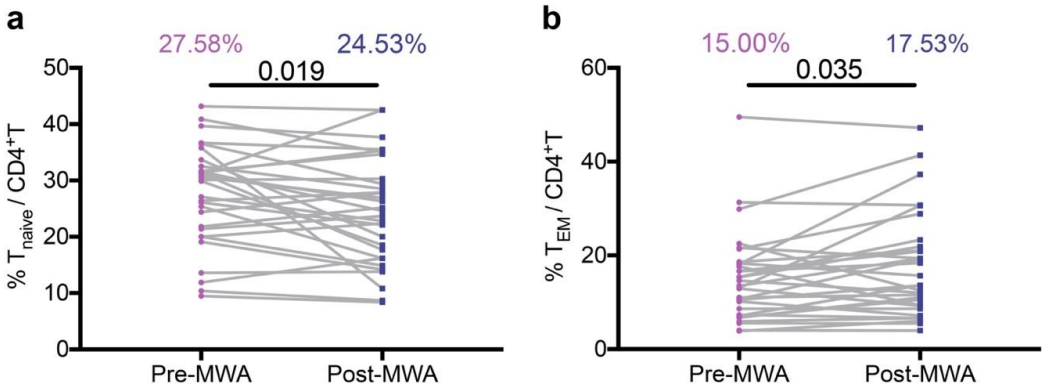
Total RNA was extracted from purified $CD3^+$ T cells using the RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Absorbance was measured at 260/280 nm to assess the purity of mRNA at a ratio > 1.86. cDNA was synthesized using a PrimeScript RT Master Mix (TaKaRa Shuzo Co., Shiga, Japan). Specific primers from Invitrogen (Shanghai, China) were used for the quantitation of the transcripts. All quantitative real-time PCR reactions were performed using SYBR Premix Ex Taq II (TaKaRa) on StepOne Plus Real-Time PCR system (Applied Biosystems, USA) in 96-well plates. The expression of the target genes was analyzed by the $2^{-\Delta CT}$ method. The primer sequences of mouse genes were as follows: *GAPDH*: forward 5'-AGAAGGCTGGGGCTCATTTG-3', reverse 5'-AGGGGCCATCCACAGTCTTC-3'; *ICOS*: forward 5'- CAGGAGAAATCAATGGTTCTGCC-3', reverse 5'-CCTTTTGTCTTAGTGAGATCGCA-3'; *IFN-γ:* forward 5'-TCGGTAACTGACTTGAATGTCCA-3', reverse 5'- TCGCTTCCCTGTTTTAGCTGC-3'; *CD4*: forward 5'-TGCCTCAGTATGCTGGCTCT-3', reverse 5'- GAGACCTTTGCCTCCTTGTTC-3'; *CD8*: forward 5'- ATGGCCTTACCAGTGACCG-3', reverse 5'- AGGTTCCAGGTCCGATCCAG-3'; *PIK3R1*: forward 5'-ACCACTACCGGAATGAATCTCT-3', reverse 5'- GGGATGTGCGGGTATATTCTTC-3'; *ERK*: forward 5'- TCACACAGGGTTCCTGACAGA-3', reverse 5'-

ATGCAGCCTACAGACCAAATATC-3'; *JNK*: forward 5'- TGTGTGGAATCAAGCACCTTC-3', reverse 5'- AGGCGTCATCATAAAACTCGTTC-3'; *T-bet*: forward 5'- GTCCAACAATGTGACCCAGAT-3', reverse 5'- ACCTCAACGATATGCAGCCG-3'. All the experiments were performed in triplicate, and the expression of the target genes was estimated and normalized to that of the endogenous control *GAPDH* gene.

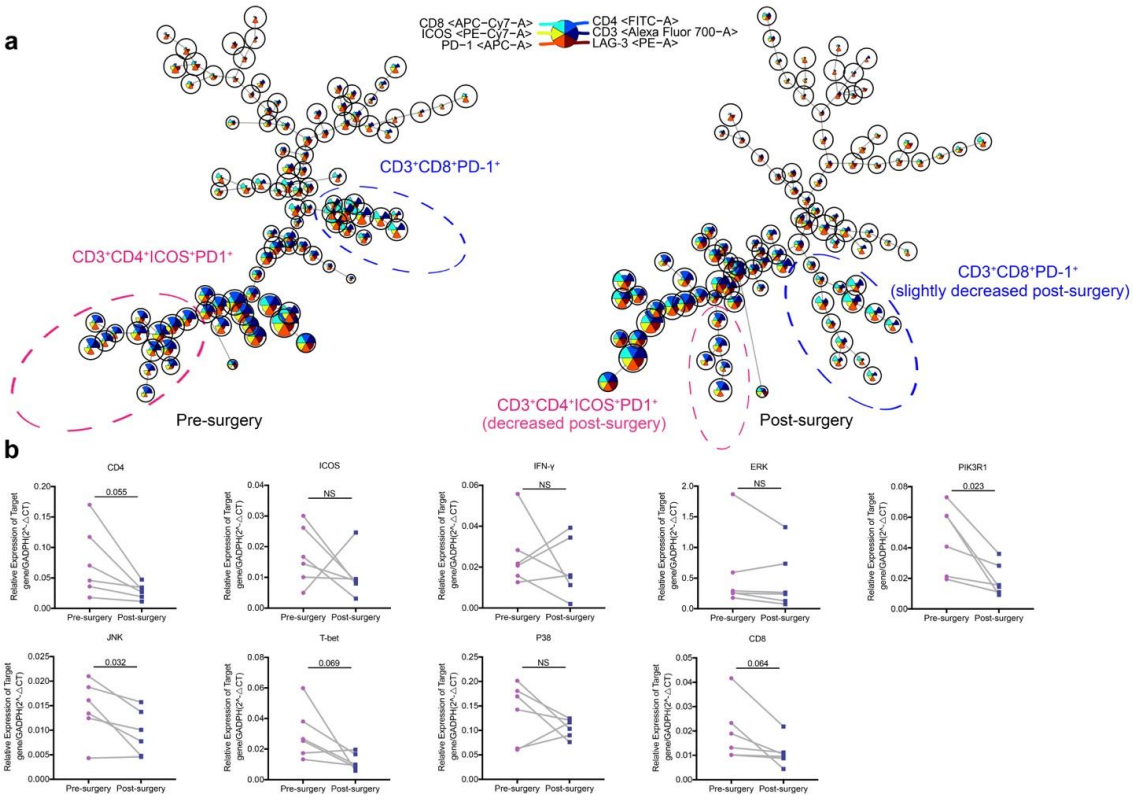
Supplementary Figures and Tables



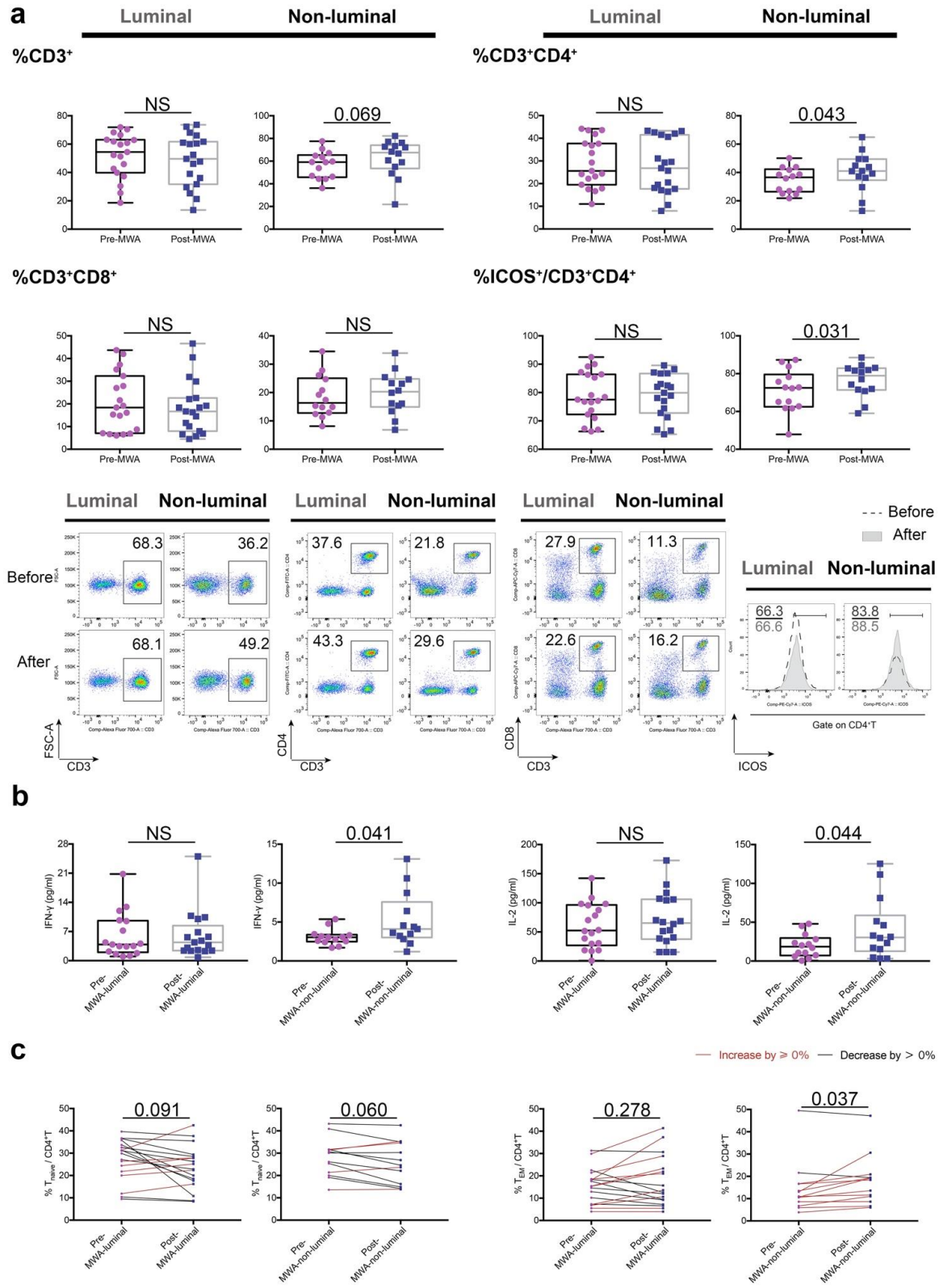
Supplementary Fig. 1. The frequency of peripheral T cell subsets before and 1 week after MWA (n=33). **a.** Scatter plots showing the changes of the activated (ICOS) and exhausted (LAG-3, TIGIT, TIM-3 and PD-1) CD8+ T cells. **b.** Peripheral T cells, CD4+ T, and CD8+ T cells did not significantly increase after MWA. **c.** The activated ICOS+CD4+ T cells increased with a frequency of 2.29% without significant differences and the frequency of activated CD8+ T cells significantly increased after MWA. NS, not significant. Data are presented as mean ± SD. Source data are provided as a Source Data File.



Supplementary Fig. 2. The frequencies of peripheral naïve CD4+ T cells (**a**) and CD4+ effector memory T cells (**b**) before and 1 week after MWA (n=31). Data are presented as mean ± SD. Source data are provided as a Source Data File.

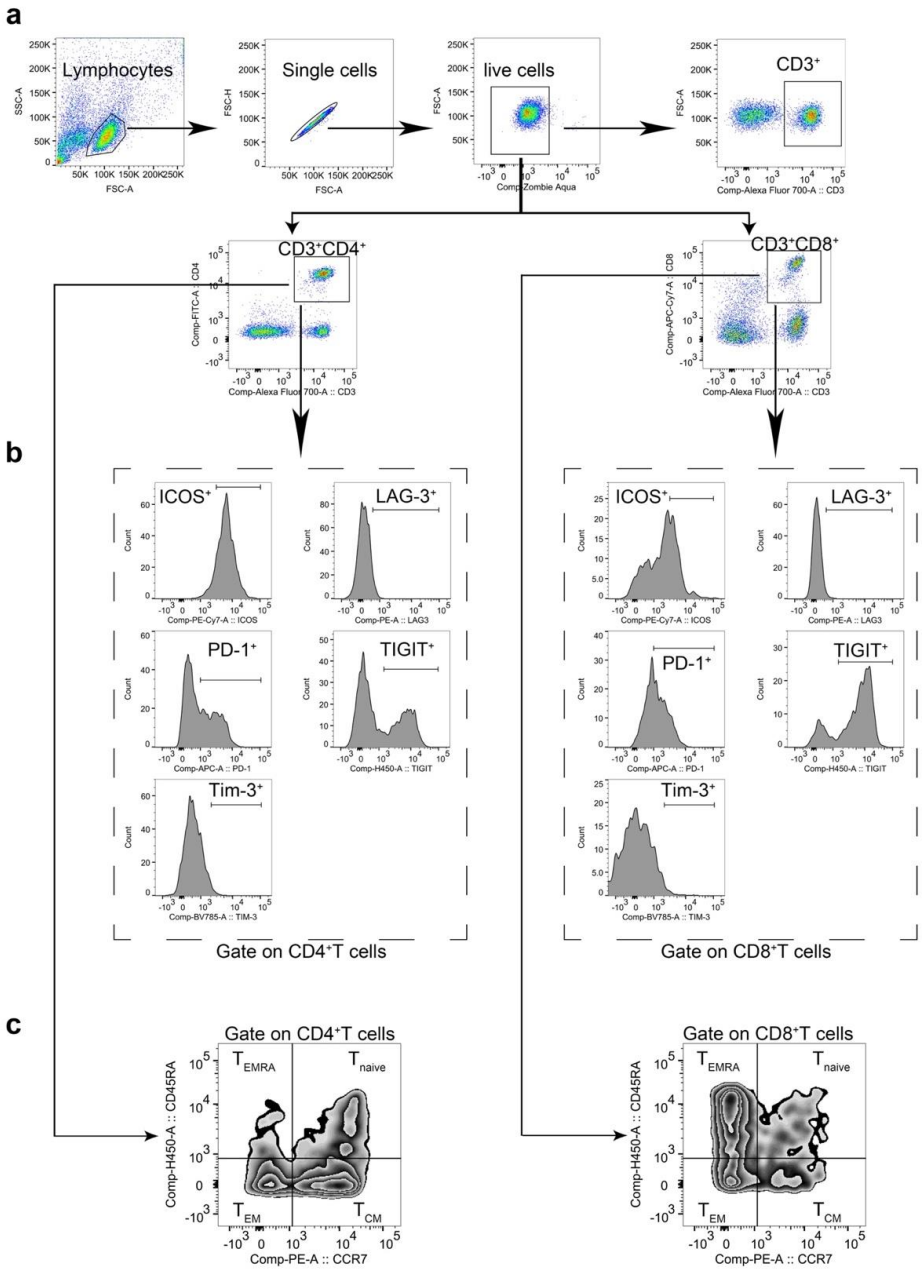


Supplementary Fig. 3. ICOS pathway of T cells wasn't activated by surgery of breast cancers. **a.** Unsupervised clustering of T cells in early stage breast cancer patients pre- and post-surgery. Large cell populations are highlighted. **b.** The relative mRNA expressions of ICOS pathway in peripheral T cells before and after surgery(n=6). NS, not significant. Data are presented as mean ± SD. Source data are provided as a Source Data File.



Supplementary Fig. 4 MWA-induced Th1-type immune response in non-luminal and luminal breast

cancers. a. The changes of peripheral T cells, CD4+ T cells, CD8+ T cells and ICOS+CD4+T cells after MWA treatment in non-luminal (n=14) and luminal (n=19) breast cancers. b. Serum IFN-γ and IL-2 significantly increased after MWA treatment in non-luminal (n=13 IFN-γ, n=14 IL-2) breast cancers, but not in luminal (n=17 IFN-γ, n=18 IL-2) breast cancers. c. The percentage of peripheral naïve CD4+ T cells decreased after MWA in luminal (n=18) and non-luminal (n=13) breast cancers with borderline significant differences. The percentage of peripheral CD4+ effector memory T cells increased mainly in non-luminal breast cancers not luminal breast cancers. NS, not significant. Data are presented as mean ± SD. Source data are provided as a Source Data File.



Supplementary Fig. 5. Gating strategy for T cell phenotyping. **a.** Human lymphocytes were identified within single cells. T cells were identified as CD3+ lymphocytes. CD4+T cells and CD8+T cells were identified as CD3+CD4+ lymphocytes or CD3+CD4+ lymphocytes, respectively. **b.** Each subset was assessed for expression of ICOS, PD-1, TIGIT, Tim-3, CTLA-4 and LAG-3. **c.** CD45RA and CCR7 were used to identify the functional subpopulation of CD4+T cells and CD8+T cells.

Supplementary Table 1. Baseline clinical characteristics of the study

Gender	Age (years)	Tumor size (mm)	Ablation time (minute)	ER (%+)*	PR (%+)*	HER2 status	Ki-67 (%+)*	Treatments	Nodal stage#	TCR sequencing	PCR
Female	46	14	/	90	90	Negative	15	Surgery	pN0		
Female	53	27	/	90	50	Negative	40	Surgery	pN1		
Female	40	18	/	90	40	Negative	40	Surgery	pN1		
Female	64	14	/	95	10	Negative	25	Surgery	pN0		
Female	56	11	/	95	40	Negative	20	Surgery	pN1		
Female	56	21	/	0	0	Positive	60	Surgery	pN2		
Female	38	21	/	90	80	Negative	40	Surgery	pN1		
Female	32	20	/	0	0	Negative	90	Surgery	pN0		
Female	44	37	/	60	0	Negative	80	Surgery	pN2		
Female	41	27	/	90	70	Negative	60	Surgery	pN2		
Female	45	23	/	0	0	Negative	70	Surgery	pN1		
Female	59	19.5	/	80	2	Positive	60	Surgery	pN0		
Female	64	34	/	0	0	Negative	70	Surgery	pN2		
Female	64	17	3	90	90	Negative	30	MWA	cN0		
Female	74	15	2.5	90	70	Negative	5	MWA	cN0		
Female	50	17	3	90	90	Negative	20	MWA	cN0		
Female	51	12	2	0	0	Positive	50	MWA	cN0		
Female	80	17	2.5	90	0	Negative	20	MWA	cN0	Yes	
Female	86	23	2.25	90	90	Negative	10	MWA	cN0		
Female	87	15	2	90	50	Negative	10	MWA	cN0		
Female	86	23	3	90	5	Negative	15	MWA	cN0	Yes	
Female	70	12	2.5	90	20	Negative	15	MWA	cN0		
Female	53	15	2	90	90	Negative	15	MWA	cN0		Yes
Female	85	24	2	80	80	Negative	50	MWA	cN0		Yes

Female	86	23	2	90	90	Negative	15	MWA	cN0	Yes	
Female	50	23	2	90	90	Negative	30	MWA	cN0		
Female	76	25	4.5	90	80	Positive	10	MWA	cN0		
Female	80	21	3.5	90	30	Negative	15-20	MWA	cN0		
Female	71	19	2.5	90	50	Negative	6	MWA followed by surgery	pN0		
Female	38	30	2	40	10	Negative	80	MWA followed by surgery	pN0		
Female	59	9.8	2	90	30	Negative	70	MWA followed by surgery	pN0		
Female	47	22	2.5	10	0	Positive	50	MWA followed by surgery	pN0		
Female	47	25	3.5	0	0	Negative	80	MWA followed by surgery	pN1		
Female	73	25	3	90	50	Negative	15	MWA followed by surgery	pN2		
Female	68	17	3	0	0	Negative	70-80	MWA followed by surgery	pN0	Yes	
Female	57	18	4	0	0	Negative	90	MWA followed by surgery	pN0	Yes	
Female	70	13	2	90	90	Negative	15	MWA followed by surgery	pN0		
Female	62	16	2	90	5	Positive	30	MWA followed by surgery	pN1		
Female	47	20	2	0	0	Positive	30	MWA followed by surgery	pN1		
Female	40	6	2	0	0	Negative	40	MWA followed by surgery	pN0		
Female	39	29	3	0	0	Negative	75	MWA followed by surgery	pN1		Yes
Female	49	23	2.5	90	90	Negative	40	MWA followed by surgery	pN0		Yes
Female	52	22	2	0	0	Positive	60	MWA followed by surgery	pN0		Yes
Female	55	20	2.5	0	0	Negative	50	MWA followed by surgery	pN0		Yes
Female	47	17	2	0	0	Negative	15	MWA followed by surgery	pN2		
Female	41	19	2	95	50	Positive	80	MWA followed by surgery	pN0		
Female	58	22	3.5	95	80	Negative	30	MWA followed by surgery	pN0		
Female	62	19	2.25	0	0	Negative	25	MWA followed by surgery	pN0		

*percentage of positive cells

cN, clinical nodal stage; pN, pathological nodal stage

Supplementary Table 2. Therapies for the 15 patients who underwent MWA without local surgery.

Subtype (n)	Chemotherapy	Radiotherapy	Endocrine therapy	Anti-HER2
HR+/HER2- (13)	0	0	13	0
HR+/HER2+ (1)	0	0	1	0
HR-/HER2+ (1)	0	0	0	1

Supplementary Table 3. MWA-related complications of 35 patients after ablation[#]

Complications	N (%)
Ecchymosis	0
Hematomas	0
Skin burns	0
Swelling	35 (100%)
Nipple retraction	0
Infection	0

Two patients (5.7%) suffered moderate pain in the procedure of ablation, and the prescheduled ablation was completed after additional local anaesthesia.

Supplementary Table 4.TCRβ CDR3 sequencing results

No.	Pre-MWA		Post-MWA	
	Reads	Clonotypes	Reads	Clonotypes
case 3	10475722	677841	9688005	434780
case 4	3052282	325563	8433647	323504
case 7	5595142	550215	9590101	618280
case 17	9440539	639993	5583372	570627
case 21	3231695	365748	5860889	531590
	Total			
	Reads		Clonotypes	
mean	7095139		503814	
SD	2768841		132260	