# SUPPLEMENTARY MATERIALS

## 1. SUPPLEMENTARY METHODS

### 1.1 Animal studies

To assess the immunogenicity of the selected peptides in a murine model, six-to eight-week-old female FVB mice (n = 4 per group) were obtained from Koatech (Gyeonggi, Korea). The mice were immunized via subcutaneously injection with 100 μg of each HSP90 peptides or phosphate buffered saline (PBS) mixed with complete Freund's adjuvant (CFA; F5881, Sigma-Aldrich, MO, USA) or incomplete Freund's adjuvant (IFA; F5506, Sigma-Aldrich). Three immunizations were administered 10 days apart. Ten days after the third vaccination, the spleen and serum were harvested from each mouse.

For the tumorigenesis experiments of the selected peptides in a murine model, six to eight-week-old female MMTV*neu*-transgenic mice (n = 5 per group) were purchased from Jackson Laboratory. The mice were immunized subcutaneously with 100 μg of each HSP90 peptides or PBS mixed with CFA/IFA. Three immunizations were administered 10 days apart. Seven days after the third vaccination, MMC cells ($5 \times 10^5$) were transplanted with subcutaneously into MMTV*neu*-transgenic mice. Three weeks after tumor implantation, the spleen and tumor tissues were harvested from each mouse.

For the depletion of $CD4^+$ or $CD8^+$ T cells in HSP90 peptides in a murine model, mice (n = 5 per group) were intraperitoneal (i. p.) injected with 200 μg of anti-CD4 (BE0003-1, Bioxcell, NH, USA) or anti-CD8 (BE0061, Bioxcell) mAbs 3, 2 and 1 days before immunization. Three immunizations were administered 10 days apart. After the first immunization, anti-CD4 or anti-CD8 mAbs were injected twice a week until the end of the experiment. Seven days after the third vaccination, MMC cells ($5 \times 10^5$) were implanted subcutaneously in MMTV*neu*-transgenic mice. Tumor and spleen were harvested five weeks after tumor implantation.

To evaluate the effects of combination therapy with HSP90 peptides and STING agonist (tlrl-dmx, InvivoGen, CA, USA), MMC cells ($5 \times 10^5$) were subcutaneously implanted in MMTV*neu*-transgenic mice (n = 3 per group). When the tumor reached a size greater than 100 $mm^3$, the mice were immunized via subcutaneously injection with 100 μg of each HSP90 peptides or PBS mixed with CFA/IFA, and i. p. injected with 100 μg of STING agonist. Three immunizations were administered 10 days apart. Spleens and tumors were harvested seven days after the third vaccination. To analyze survival due to the combined effects of HSP90 peptides and STING agonist, mice (n = 6 per group) were monitored twice week for 90 days following tumor implantation. When the



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tumor reached a size greater than 2,000 $mm^3$, the mice were considered dead.

To investigate the effects of combination therapeutic with HSP90 peptides/STING agonist/anti-CTLA-4 Ab (BP0032, Bioxcell), MMC cells ($5 \times 10^5$) were subcutaneously injected into MMTV*neu*-transgenic mice (n = 4 per group). When the tumor reached a size greater than 100 $mm^3$, the mice were immunized via subcutaneously injection with 100 μg of each HSP90 peptides or PBS mixed with CFA/IFA, and i. p. injected with 100 μg of STING agonist. After the first immunization, 150 μg of anti-CTLA-4 Ab was injected intraperitoneally twice a week until the end of the study. Three immunizations were administered 10 days apart. Two weeks after the third immunization, the spleen, tumor, and serum were harvested. The tumor diameters of all experimental mice were measured using calipers twice a week until the end of the experimental. Euthanasia of all mice were performed when humane welfare limits related to tumor volume (typically 1500-2000 $mm^3$) or tumor ulceration (ulcers in the skin above the tumor) and termination of the experiment. Tumor volume was calculated using the following formula: Volume ($mm^3$) = $width^2$ x length / 2. All experimental procedures involving mice were performed according to the guidelines approved by the Institutional Animal Care and Use Committee of Korea University (IACUC, Approval number: KOREA-2016-128). All methods were performed in accordance with the relevant guidelines and regulations.

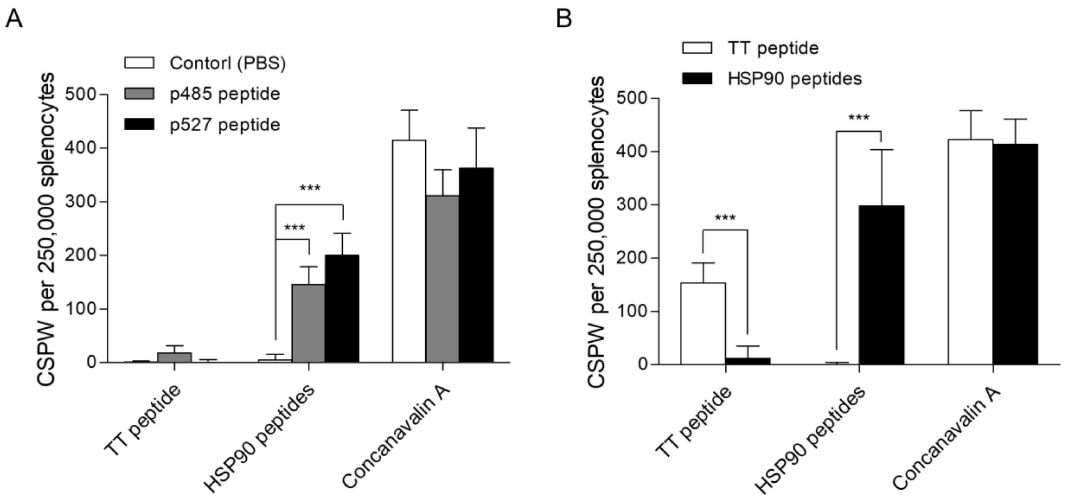
## 2. SUPPLEMENTARY FIGURES

**Supplemental table S1. Clinico-pathologic characteristics of the patients with HER2-positive breast cancer**

| **Characteristics** | | **Number (%)** |
|---|---|---|
| **HER2 status** | | |
| HER2+ | | 32 (100) |
| **Estrogenreceptor (ER)/progesterone receptor (ER)** | | |
| ER positive/PR positive | | 7 (21.9) |
| ER positive/PR negative | | 0 (0) |
| ER negative/PR positive | | 3 (9.4) |
| ER negative/PR negative | | 22 (68.8) |
| **TNM state** | | |
| T | 0-1 | 10 (31.3) |
| | 2 | 12 (37.5) |
| | 3-4 | 10 (31.3) |
| N | 0 | 7 (21.9) |
| | 1-3 | 22 (68.8) |
| M | 0 | 4 (12.5) |
| | 1 | 5 (15.6) |
| **Stage** | | |
| I | | 7 (21.9) |
| II | | 4 (12.5) |
| III | | 12 (37.5) |
| IV | | 9 (28.1) |
| **Grade** | | |
| NA | | 3 (9.4) |
| G1 | | 0 (0) |
| G2 | | 7 (21.9) |
| G3 | | 22 (68.8) |

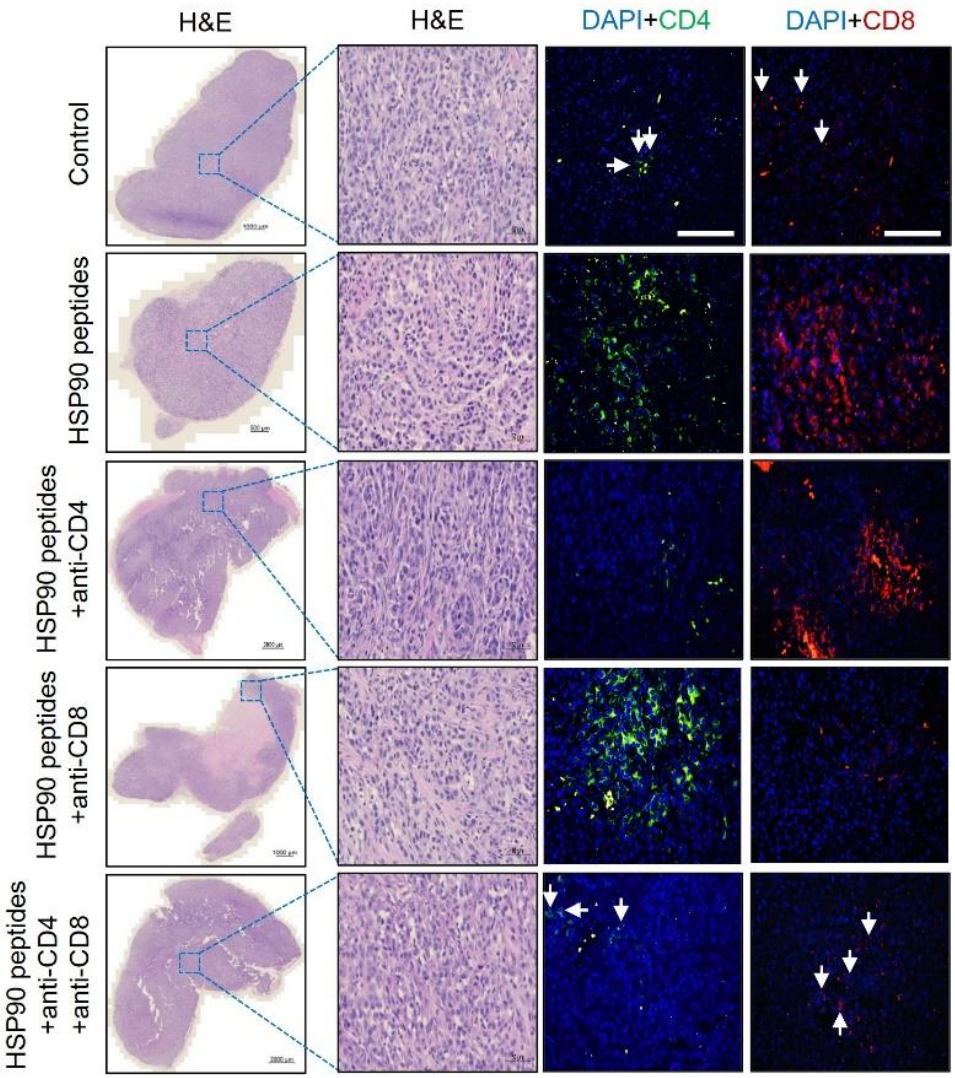
**Supplemental figure S1. Immunogenicity of multiple HSP90 peptides shows potent antigen-specific T cells responses in FVB mouse model.**

(A) Immunogenicity of p485 and p527 peptide were evaluated antigen-specific T cells responses by IFN-γ ELISPOT assay. (B) HSP90 peptides was evaluated by comparing the antigen-specific IFN-γ secreting response to tetanus toxoid (TT) as control peptide. Concanavalin A, positive control. The error bars represent the standard deviation. ***$p < 0.001$ in Bonferroni pisttests of two-way ANOVA and in student's *t*-test.



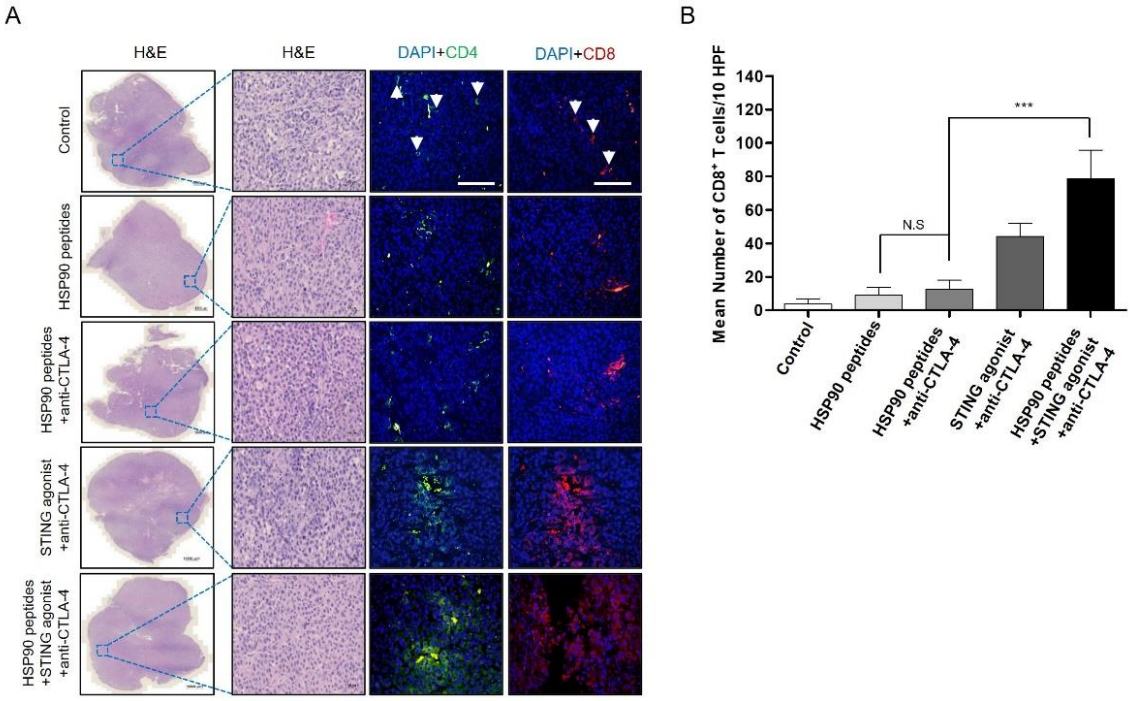
**Supplemental figure S2. *In vivo* CD4$^+$ or CD8$^+$ T cell depletion and tumor growth inhibiting efficacy of HSP90 peptides.**

Representative staining results from hematoxylin and eosin (H&E) and immunohistochemical staining for CD4$^+$ and CD8$^+$ T cells in tumor tissues isolated from MMTV*neu*-transgenic mice. Clusters of CD4$^+$ and CD8$^+$ T cells are marked by arrow. Scale bar = 100 μm.



**Supplemental figure S3. Combination therapy with HSP90 peptides / STING agonist / anti-CTLA-4 Ab enhances CD4$^+$ and CD8$^+$ T cells in tumor tissue.**

(A) Representative staining results from hematoxylin and eosin (H&E) and immunohistochemical staining for CD4 and CD8 T cells in tumor tissues isolated from MMTV*neu*-transgenic mice. (B) The graph bar show mean number of CD8$^+$ T cells per 10 high-power field (HPF) in tumor from each experiment mice. The error bars represent the standard deviation. Clusters of CD4 and CD8 T cells are marked by arrow. Scale bar = 100 μm. N.S, not significant, ***$p < 0.001$ in Tukey's multiple comparison test of one-way ANOVA.



**Supplemental figure S4. Multiple IHC results from tumor samples of individual animal in each group.**

Each histogram represents total number of each phenotype of T cells per area ($mm^2$) of tumor by multiple IHC on each tumor tissue slide in the group.