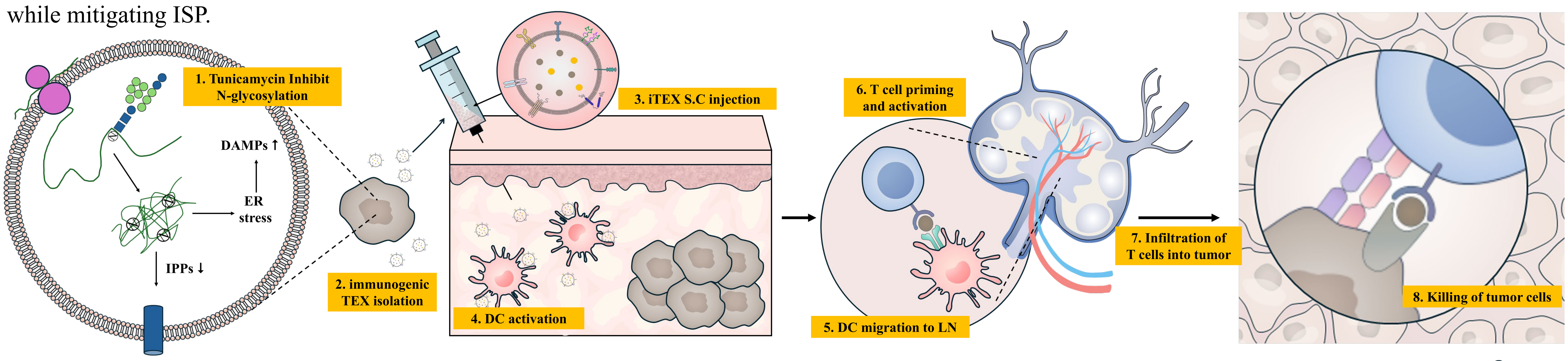
# Endoplasmic Reticulum Stress-Based Approach for Reprogramming Tumor-Derived Exosome

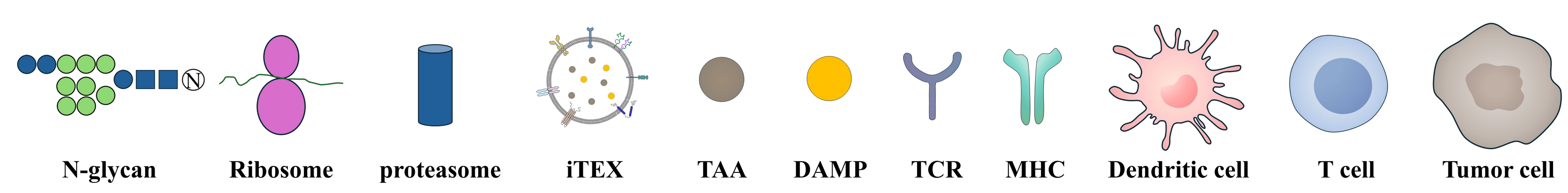
**Chang Hyun Lee**[a], Chan Ho Kim[a], Kyung Hee Han[a], Jae Hyung Park[a,b,c*]

[a] School of Chemical Engineering, College of Engineering, Sungkyunkwan University, Suwon 440-746, Republic of Korea
[b] Department of MetaBioHealth, ICS, Sungkyunkwan University, Suwon 440-746, Republic of Korea
[c] Department of Health Sciences and Technology, SAIHST, Sungkyunkwan University, Suwon 440-746, Republic of Korea

## Introduction

Despite their potential of Tumor Associated Antigens (TAA)-based cancer vaccine, most TAAs require additional adjuvants. Tumor-derived exosome (TEX) posses unique characteristics, containing TAA and damage associated molecular patterns (DAMP) that can function as adjuvants. However, TEX presents a double-edge due to their inherent immunosuppressive proteins (ISP). So, we developed a novel approach by pre-conditioning cancer cells using endoplasmic reticulum stressor for the purpose of generating immunogenic exosome (iTEX). The designed iTEX can effectively deliver TAAs and DAMPs to dendritic cells while mitigating ISP.




## Methods

To obtain iTEX containing DAMPs, B16F10 cells were treated with tunicamycin, followed by exosome isolation. The isolated exosomes were characterized using TEM and Nanoparticle Tracking Analysis. Western blot analysis confirmed the presence of exosomal markers, Alix and CD9. Additionally, ISPs CD47 and S100A9, as well as HMGB1 and HSP70, were evaluated. iTEX was administered subcutaneously to tumor-bearing mice. Subsequently, dendritic cells were isolated from tumor-draining lymph nodes and assessed for maturation and activation status via analyzing CD80/86 and CCR7 expression. Flow cytometry was employed to analyze changes in tumor-infiltrating lymphocyte populations. The systemic anti-tumor immunity capacity of iTEX was further validated using a post-surgical model.

## Result

### Characterization of iTEX

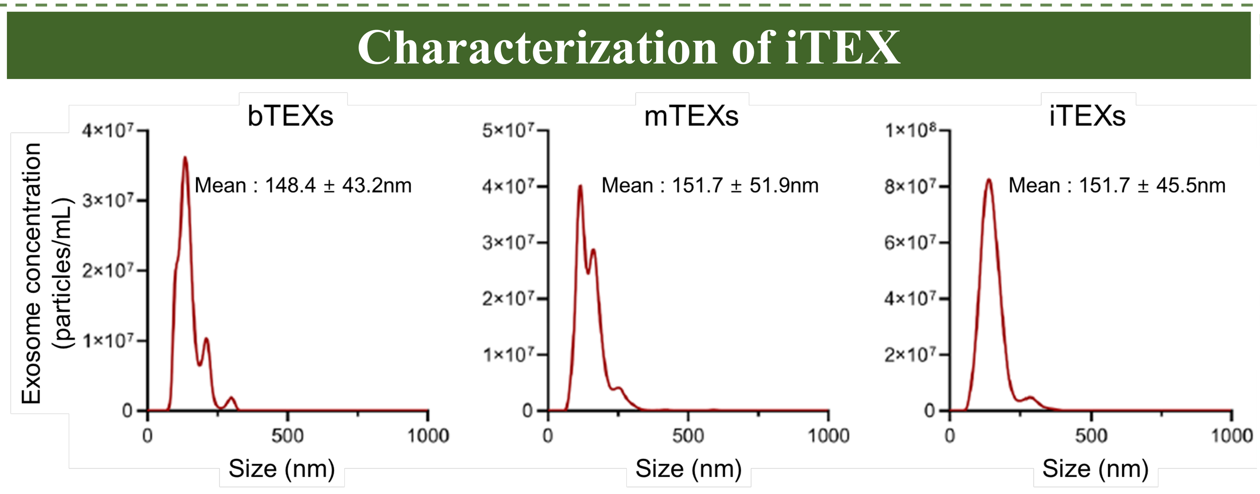



**Fig 1**. Size distribution and mean size value (±SD) measured by a nanoparticle tracking analysis system.

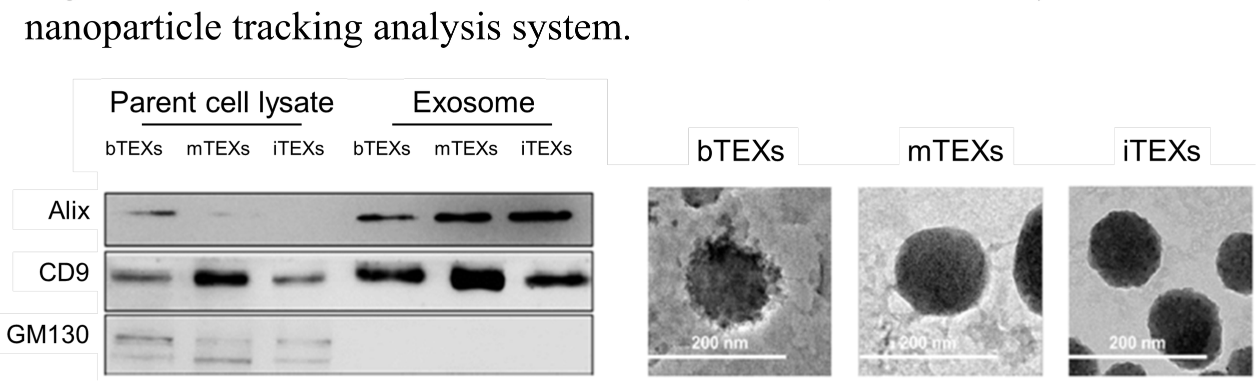



**Fig 2**. Western blotting analysis of exosome protein markers in TEX samples and their parent cell lysate. TEM images of TEX samples

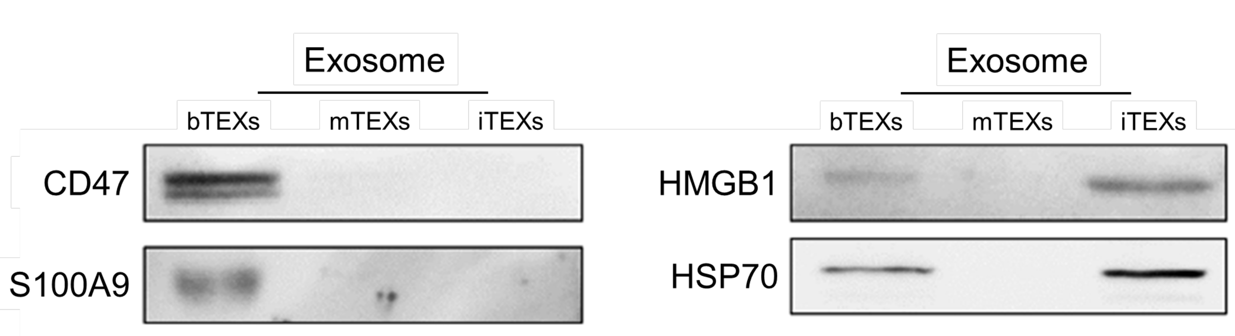



**Fig 3**. Western blotting analysis of immune-suppressive proteins and DAMPs.

### Immune-boosting function of iTEX at dendritic cell

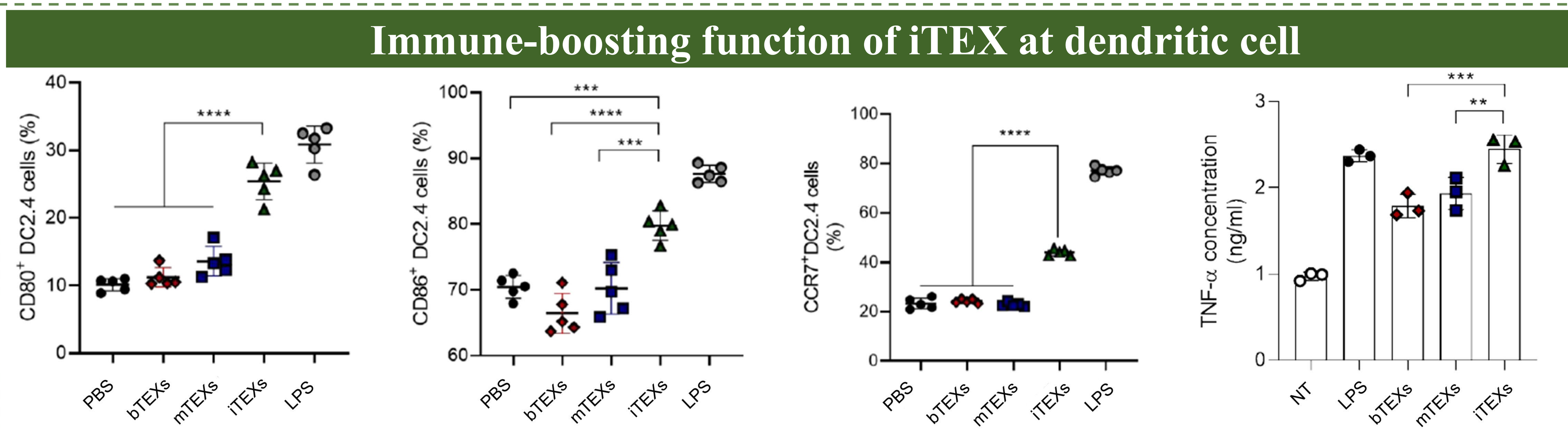



**Fig 4**. Quantification of the expression level of CD80, CD86 and CCR7 in DC2.4 cells by measuring the mean fluorescence intensity (n = 5) and The secretion of cytokines from DC2.4

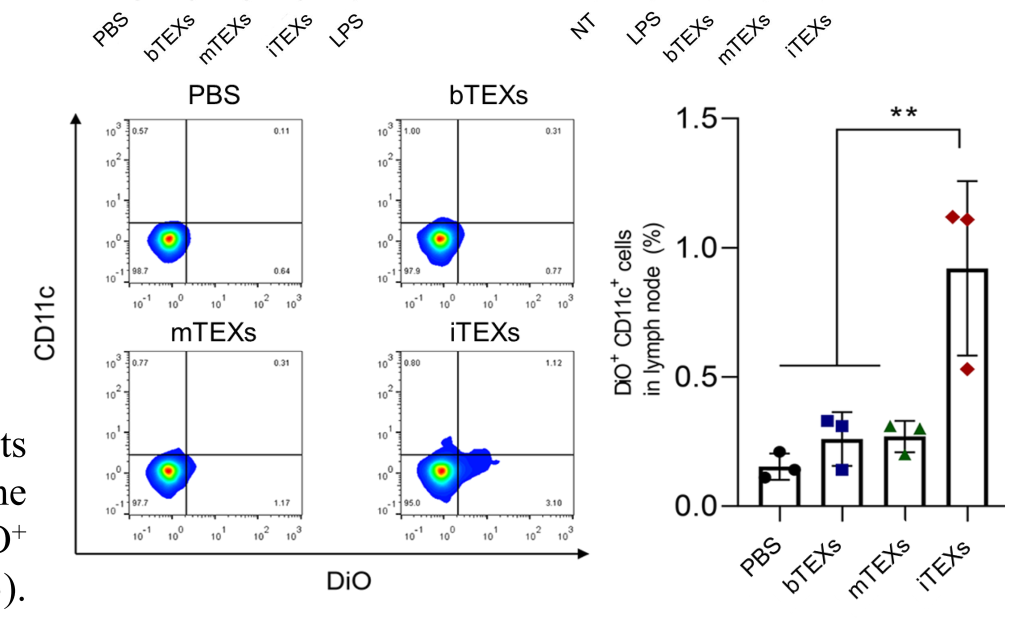



**Fig 5**. Representative flow cytometry plots showing CD11c and DiO dye expression in the lymph node. The proportion of CD11c+ DiO+ cells in a lymph node ($n$ = 3).

### Immune-boosting function of iTEX

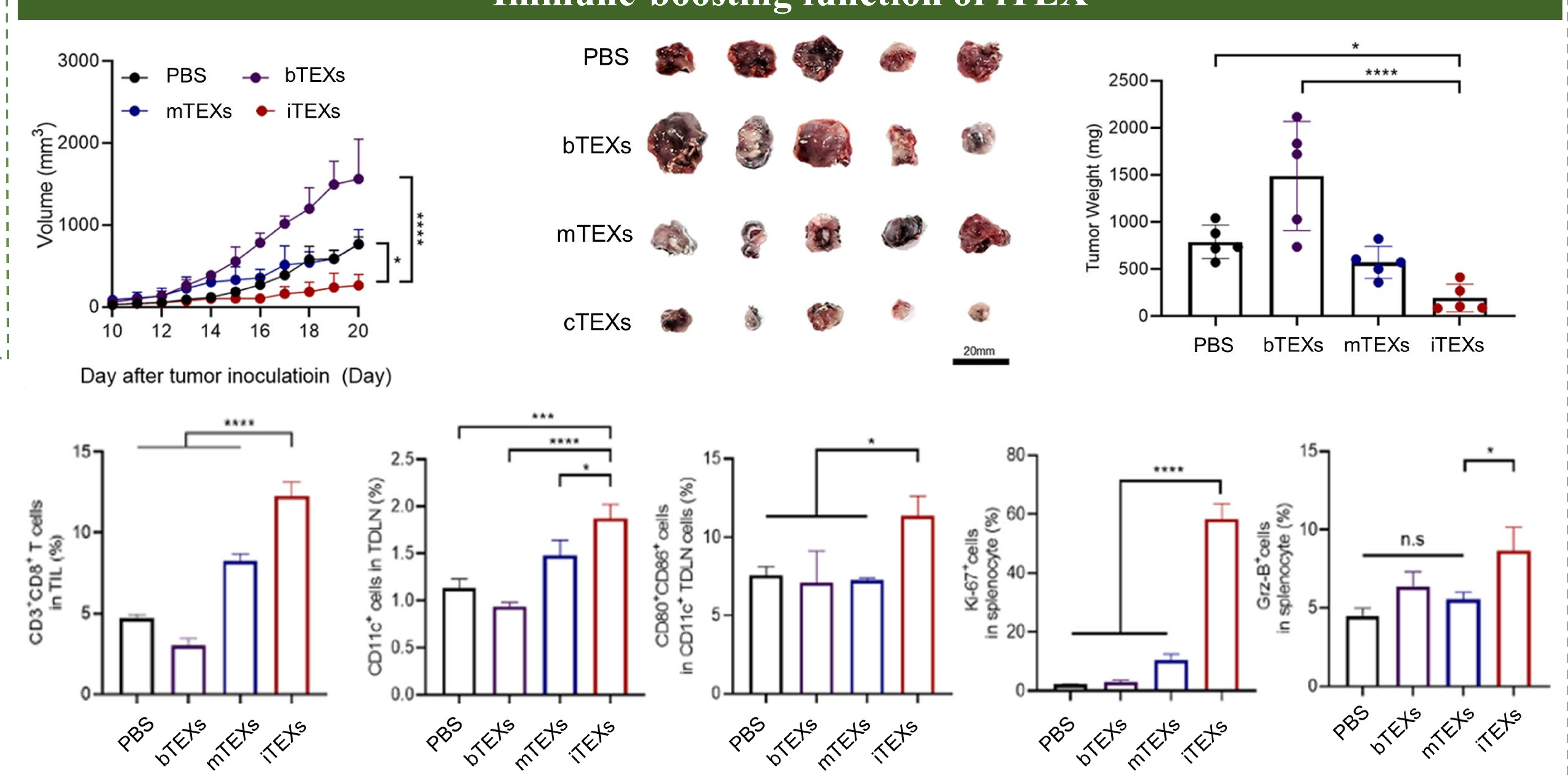



**Fig 6**. Tumor volumes measured from day 10 postinoculation (n = 5). Individual tumor growth data for each treatment group. The proportion of CD3+CD8+ T cells in CD45+ TILs (n = 3). The proportion of CD11c+ cells in TDLN (n = 3). The proportion of CD80+CD86+ cells in CD11c+ cells from TDLN (n = 3). The proportion of Ki-67+ cells and Granzyme-B+ cells in splenocytes after treatment with tumor lysate, respectively (n = 3). Error bars represent the SD. Statistical significances were calculated via one-way ANOVA (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$).

## Conclusion

iTEX cancer vaccine demonstrates significant potential in modulating the tumor microenvironment (TME) by regulating the expression of immunosuppressive proteins and damage-associated patterns. iTEX exhibits remarkable anticancer effects through enhancing the maturation and activation of dendritic cells and substantially increasing the fraction of cytotoxic T cells within the TME.

## Reference / Funding

Kim, H. Y.; Kwon, S.; Um, W.; Shin, S.; Kim, C. H.; Park, J. H.; Kim, B. S. Functional Extracellular Vesicles for Regenerative Medicine. Small 2022, 18, 2106569.

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