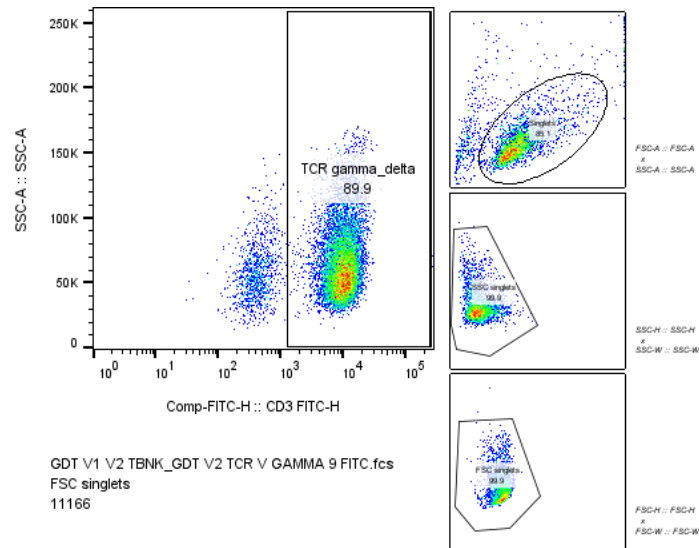


## Supplementary

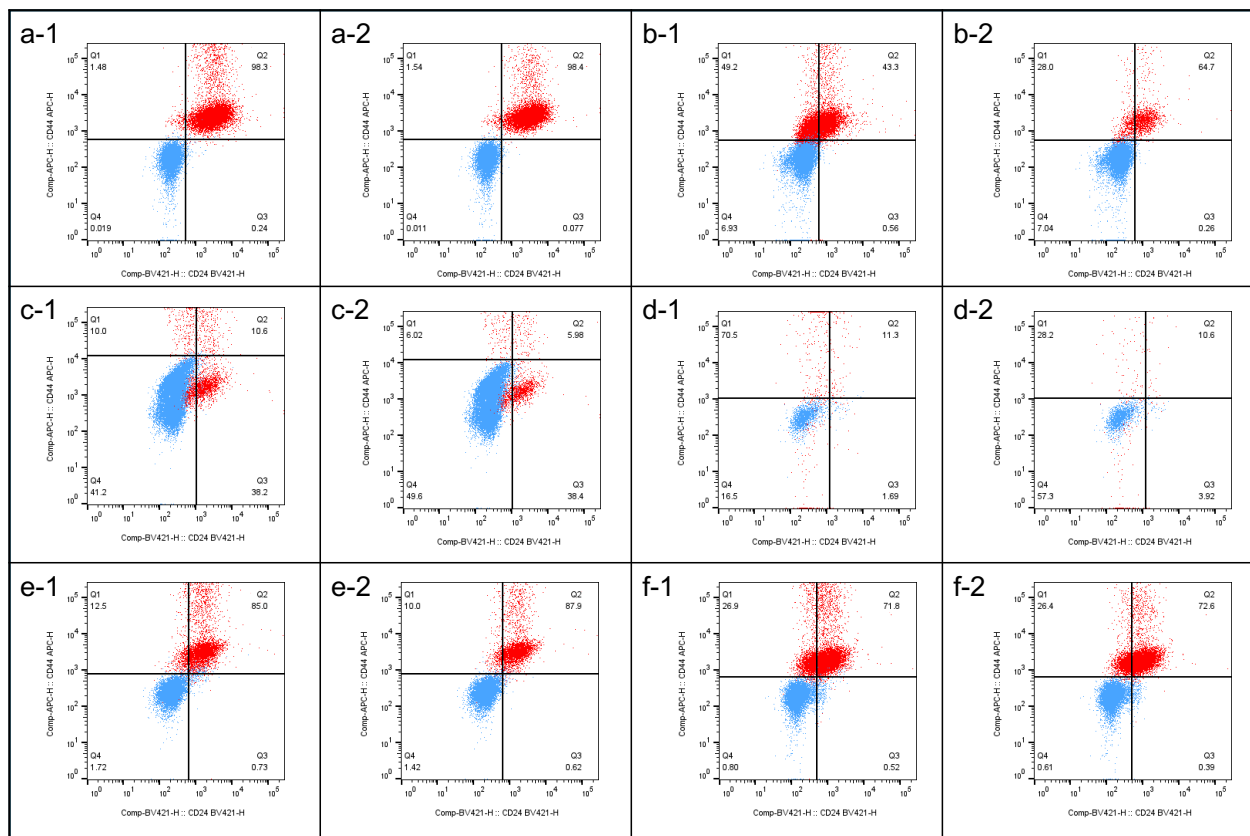
Chau Truong, N., Thao Huynh, N., Thanh Do, N., Thi-hong Tran, D., & Van Pham, P. (2026). Metformin enhances, whereas acetaminophen attenuates,  $\gamma\delta$ T cell antitumor efficacy and shapes stem-like escape in breast cancer. *Biomedical Research and Therapy*, 13(5), 8544-8557.

<https://doi.org/10.15419/hmhsx229>



**Figure S1. Flow cytometric assessment of  $\gamma\delta$ T cells composition prior to co-culture.**

Flow cytometric analysis showed a high proportion of  $\gamma\delta$ T cells (TCR gamma/delta<sup>+</sup>, 89.9%) exceeding the  $\geq 50\%$  quality threshold for *in vitro*-expanded  $\gamma\delta$ T cell products.



**Figure S2. Representatives flow plots of CD44<sup>+</sup>CD24<sup>-</sup> cell proportion in MCF-7 cultures during co-culture with  $\gamma\delta$  cells, with or without APAP and MET. Analyses of the total cell population (7-AAD<sup>+</sup>): (a-1) control (MCF-7), (b-1) MCF-7+ $\gamma\delta$  T cell, (c-1) MCF-7+ $\gamma\delta$ T+APAP, (d-1) MCF-7+ $\gamma\delta$ T+MET, (e-1) APAP, (f-1) MET. Analyses of the viable cell population (7-AAD<sup>-</sup>): (a-2) control (MCF-7), (b-2) MCF-7+ $\gamma\delta$  T cell, (c-2) MCF-7+ $\gamma\delta$ T+APAP, (d-2) MCF-7+ $\gamma\delta$ T+MET, (e-2) APAP, (f-2) MET.**

**Table S1. List of primers and the primer sequences**

No.	Primer	Sequence	NCBI Ref. Sequence	Ref.
1	hB7-H3 (CD276)	Forward: 5'- GTGGGGCTGTCTGTCTGTCTCAT-3'	<a href="#">NM_001024736.2</a>	(1)
		Reverse: 5'- GCTGTCAGAGTGTTCAGAGGCT-3'		
2	hCD95 /Fas	Forward: 5'- TGAAGGACATGGCTTAGAAGTG-3'	<a href="#">NM_000043.6</a>	(2) (3)
		Reverse: 5'- GGTGCAAGGGTCACAGTGTT-3'		
3	hCD95L (Fas, CD178)	Forward: 5'- GGTTCTGGTTGCCTTGGTAGGA-3'	<a href="#">NM_000639.3</a>	(4)
		Reverse: 5'- CTGTGTGCATCTGGCTGGTAGA-3'		
4	hGAPDH	Forward: 5'- TGCTGGCGCTGAGTAGTACGTCG-3'	<a href="#">NM_001256799.3</a>	(5)
		Reverse: 5'- TGACCTTGGCCAGGGGTGCT-3'		

**Table S2. The absolute CD44<sup>+</sup>CD24<sup>-</sup> cancer stem cell numbers enriched in different culture conditions.**

	7-AAD+/-				7-AAD-			
	unstained sample		stained sample		unstained sample		stained sample	
	+	single event	+	single cell no.	+	single event	+	single cell no.
<b>Control (MCF-7)</b>	14	10.259	171	10.347	13	10.198	154	9.027
<b>MCF7+ <math>\gamma\delta</math>T</b>	36	10.960	6.111	11.660	30	10.855	597	1.814

<b>MCF7+ γδT+APAP</b>	8	13.763	218	2.260	1	13.694	80	1.456
<b>MCF7+ γδT+MET</b>	4	1.651	879	1.246	4	1.637	72	255
<b>MCF7 +APAP</b>	27	6.672	448	3.506	24	6.525	260	2.516
<b>MCF7 +MET</b>	12	10.465	2.777	10.401	11	10.367	2.076	7.872

The value +event is equivalent to the number of CD44+CD24- CSC events; Single cell no. corresponds to the total number of single cell events. 7-AAD+/- analyses are performed on the total number of cells regardless of whether they are alive or dead. The analyses in the 7-AAD- group, however, only analyze living cells. The result is the average value of the repetitions ( $n=3$ ).

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