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Supplementary Materials

Ascorbate in Pharmacological Concentrations Potentiates the Anti-Tumor Activity of NK and CD8$^+$ T Cells and Synergizes with Chemotherapy for Enhanced Anti-Tumor Immune Response against 3D Breast Cancer Spheroid Models

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Table S1. Experimental groups for CD8$^+$ T cell and spheroid co-culture.

Group	CD8$^+$ T Cell Pre-Treatment (30 min)	CD8$^+$ T Cell Treatment (6 h)	Target Spheroid	Control Group
1	No CAT	Media only	MDA-MB-231 or MCF-7	Yes (CD8$^+$ T Cell Control)
2	No CAT	250 µM Vit-C	MDA-MB-231 or MCF-7	
3	No CAT	500 µM Vit-C	MDA-MB-231 or MCF-7	
4	No CAT	1 mM Vit-C	MDA-MB-231 or MCF-7	
5	With CAT (300 U/mL)	CAT only	MDA-MB-231 or MCF-7	Yes (CD8$^+$ T Cell +CAT) Control
6	With CAT (300 U/mL)	CAT + 250 µM Vit-C	MDA-MB-231 or MCF-7	
7	With CAT (300 U/mL)	CAT + 500 µM Vit-C	MDA-MB-231 or MCF-7	
8	With CAT (300 U/mL)	CAT + 1 mM Vit-C	MDA-MB-231 or MCF-7	

CAT: Catalase; Vit-C: Vitamin C. All CD8$^+$ T cells were pre-activated with CD3/CD28 Dynabeads for 24 h before the catalase/Vit-C treatments. All co-cultures were performed for 24 h. For NK cells alone or CD8$^+$ T cells alone, the effector-to-target (E:T) ratio was 2:1. For mixed NK + CD8$^+$ T cells, the ratio was 1:1:1 (NK:CD8$^+$ T:spheroid). Media: 50:50 mix of complete DMEM and complete RPMI-1640.

Table S2. Experimental groups for NK cell and spheroid co-culture.

Group	NK Cell Pre-Treatment (30 min)	NK Cell Treatment (6 h)	Target Spheroid	Control Group
1	No CAT	Media only	MDA-MB-231 or MCF-7	Yes (NK Control)
2	No CAT	250 μM Vit-C	MDA-MB-231 or MCF-7	
3	No CAT	500 μM Vit-C	MDA-MB-231 or MCF-7	
4	No CAT	1 mM Vit-C	MDA-MB-231 or MCF-7	
5	With CAT (300 U/mL)	CAT only	MDA-MB-231 or MCF-7	Yes (NK+CAT) Control
6	With CAT (300 U/mL)	CAT + 250 μM Vit-C	MDA-MB-231 or MCF-7	
7	With CAT (300 U/mL)	CAT + 500 μM Vit-C	MDA-MB-231 or MCF-7	
8	With CAT (300 U/mL)	CAT + 1 mM Vit-C	MDA-MB-231 or MCF-7	

CAT: Catalase; Vit-C: Vitamin C. NK cells received catalase pre-treatment as indicated in Groups 5–8; Groups 1–4 received no catalase. All co-cultures were performed for 24 h. E:T ratio: 2:1 for NK cells alone; 1:1:1 when mixed with $CD8^+$ T cells. Media: 50:50 mix of complete DMEM and complete RPMI-1640.

Table S3. Experimental Groupings with Untreated MDA-MB-231 and MCF-7 Spheroids.

Immune Effector(s) in Co-Culture	Spheroid Pre-Treatment	Immune Cell Treatment
NK Cells Only	Untreated	CAT only (Control)
NK Cells Only	Untreated	CAT + 1 mM Vit-C
CD8+ T Cells Only	Untreated	CAT only (Control)
CD8+ T Cells Only	Untreated	CAT + 1 mM Vit-C
NK Cells + CD8+ T Cells	Untreated	CAT only (Control)
NK Cells + CD8+ T Cells	Untreated	CAT + 1 mM Vit-C

Table S4. Experimental Groupings for cytokine and Granzyme B production with MDA-MB-231 and MCF-7 Spheroids.

Immune Effector(s) in Co-Culture	Spheroid Pre-Treatment (24 h)
NK Cells Only (Control: CAT only)	Untreated (Vehicle Control: ddH_2O)
	Vit-C (1 mM) DOX (0.5 μM) DOCE (0.5 μM) Vit-C + DOX + DOCE
CD8+ T Cells Only (Control: CAT only)	Untreated (Vehicle Control: ddH_2O)
	Vit-C (1 mM) DOX (0.5 μM) DOCE (0.5 μM) Vit-C + DOX + DOCE
NK Cells + CD8+ T Cells (Control: both cell types, CAT only)	Untreated (Vehicle Control: ddH_2O)
	Vit-C (1 mM) DOX (0.5 μM) DOCE (0.5 μM) Vit-C + DOX + DOCE

All immune cells were pre-treated for 30 min with catalase (300 U/mL) followed by 6 h with or without 1 mM Vit-C as indicated. $CD8^+$ T cells were pre-activated with CD3/CD28 Dynabeads for 24 h before catalase/Vit-C treatment. Co-culture was performed for 24 h at an E:T ratio of 2:1 for NK cells alone or $CD8^+$ T cells alone, or 1:1:1 for mixed NK+$CD8^+$ T cells. Spheroid pre-treatment was for 24 h; after treatment, spheroids were washed twice with PBS before co-culture. Control spheroids received ddH_2O (vehicle).