

## Anti-CD8 alpha/Cd8a Antibody Picoband®

Catalog Number: A02236-1

### About Cd8a

The CD8 antigen is a cell surface glycoprotein found on most cytotoxic T lymphocytes that mediates efficient cell-cell interactions within the immune system. It is mapped to 2p11.2. The CD8 antigen, acting as a coreceptor, and the T-cell receptor on the T lymphocyte recognize antigen displayed by an antigen presenting cell (APC) in the context of class I MHC molecules. The functional coreceptor is either a homodimer composed of two alpha chains, or a heterodimer composed of one alpha and one beta chain. Both alpha and beta chains share significant homology to immunoglobulin variable light chains. This gene also encodes the CD8 alpha chain isoforms.

### Overview

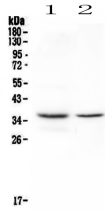
Product Name	Anti-CD8 alpha/Cd8a Antibody Picoband®
Reactive Species	Mouse, Rat
Description	Boster Bio Anti-CD8 alpha/Cd8a Antibody Picoband® catalog # A02236-1. Tested in ELISA, Flow Cytometry, IHC, WB applications. This antibody reacts with Mouse, Rat. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	ELISA, Flow Cytometry, IHC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na <sub>2</sub> HPO <sub>4</sub> , 0.05mg NaN <sub>3</sub> .
Storage Instructions	Store at -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	P01731

### Technical Details

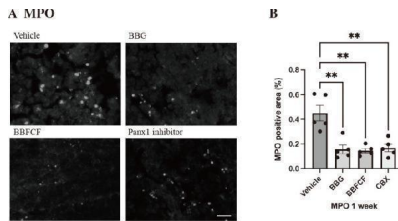
Immunogen	E. coli-derived mouse CD8 alpha recombinant protein (Position: K28-Y196).
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P).
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.

Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.1-0.5ug/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml Flow Cytometry (Fixed), 1-3ug/1x10 <sup>6</sup> cells ELISA, 0.1-0.5ug/ml

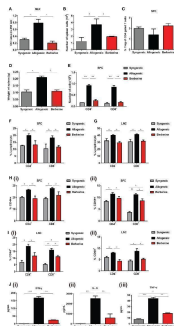
## Anti-CD8 alpha/Cd8a Antibody Picoband® (A02236-1) Images



Western blot analysis of CD8 alpha using anti-CD8 alpha antibody (A02236-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions. Lane 1: rat spleen tissue lysate, Lane 2: rat thymus tissue lysate. After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-CD8 alpha antigen affinity purified polyclonal antibody (Catalog # A02236-1) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for CD8 alpha at approximately 36KD. The expected band size for CD8 alpha is at 26KD.

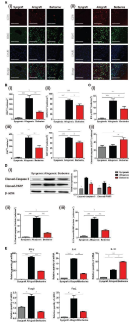


Immunohistochemical analysis of the CD8a-positive area in the injured spinal region 7 days after SCI. Bar: 40 μm. Index in PubMed under a CC BY license. PMID: 39355370

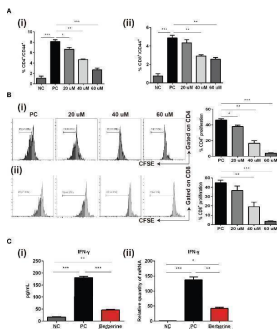


Effect of berberine treatment on the T cell immune responses to cardiac allografts. (A) MLR responses. Recipient SPCs were isolated at POD 7 (responders) and mitomycin C-treated naïve BALB/c SPCs (stimulators) were co-cultured for 3 days (n = 3 mice/group). Total SPCs were isolated at PDO 7, and the absolute numbers of (B) SPCs were determined by flow cytometry. (C) The percentage of CD4 + Foxp3 + Treg cells was determined by flow cytometry. Syngeneic recipients are shown for comparison (n = 3 mice/group). (D) The spleens of recipient mice were harvested and weighed at POD 7 (n = 5 mice/group). (E) The absolute numbers of CD4 + and CD8 + T cells SPCs (n = 3 mice/group). (F) SPCs and (G) LNCs were isolated at POD 7, and the percentage of CD4 + and CD8 + T cells was determined by flow cytometry. Syngeneic recipients are shown for comparison (n = 3 mice/group). (H) CD44 + CD69 + T cell activation assays. SPCs were isolated at POD 7, and the percentages of (i) CD4 + CD44 + CD69 + and (ii) CD8 + CD44 + CD69 + T cells were determined by flow cytometry (n = 3 mice/group). (I) LNCs were isolated at POD 7, and the percentages of (i) CD4 + CD44 + CD69 + and (ii) CD8 + CD44 + CD69 + T cells were determined by flow cytometry (n = 3 mice/group). (J) Serum plasma levels of proinflammatory cytokines. Peripheral blood was collected at POD 7, and (i) IFN-gamma, (ii) IL-6, and (iii) TNF-alpha plasma levels were measured by ELISA (n = 3 mice/group). SPCs, spleen cells; LNCs, lymph

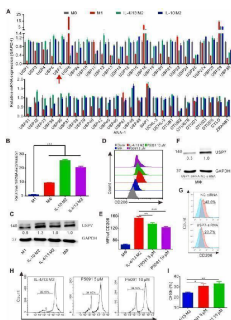
node cells; MLR, mixed lymphocyte reaction; POD, post-operative day. \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001 compared to the normal saline-treated group. Index in PubMed under a CC BY license. PMID: 33732240



Phenotypic and functional characteristics of allograft-infiltrating CD4 + or CD8 + T cells. Allografts were recovered at POD 7, and POD 100 syngeneic grafts are shown for comparison. (A) (i) Immunofluorescent staining of CD4 (red), KI67 (green), and 4',6-diamidino-2-phenylindole (DAPI, blue) in grafts. (ii) Immunofluorescent staining of CD8 (red), KI67 (green), and DAPI in grafts (Scale bar = 200 um; original magnification: ×200). (B) Proportion and absolute number of graft-infiltrating (i) CD4 + T cells and their expression of (ii) KI67, and proportion and absolute number of graft-infiltrating (iii) CD8 + T cells and their expression of (iv) KI67 (n = 3 mice/group). (C) Proportion of (i) IFN-gamma and (ii) cleaved-caspase-3 in graft-infiltrating CD3 + T cells (n = 3 mice/group). (D) (i) Cleaved-caspase-3 and cleaved-PARP protein expression in grafts. Myocardial cell apoptosis co-immunofluorescence staining and expression of (ii) cleaved-caspase-3 and (iii) cleaved-PARP (n = 3 mice/group). (E) Relative mRNA expression of IFN-gamma , IL-6, IL-10 , Foxp3 , and FasL in grafts measured by qPCR (n = 3 mice/group). SPCs, spleen cells; LNCs, lymph node cells; POD, post-operative day. \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001 compared to the normal saline-treated group. Index in PubMed under a CC BY license. PMID: 33732240

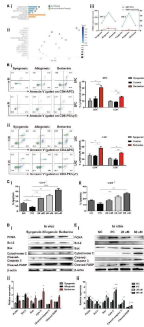


The effects of berberine on CD4 + and CD8 + T cell apoptosis, activation, and proliferation in vitro . (A) T cell activation assay. The percentages of (i) CD4 + CD44 + and (ii) CD8 + CD44 + T cells were determined by flow cytometry. (B) T cell proliferation assay. T cells from naïve C57BL/6 mice were labeled with CFSE and then co-stimulated with anti-CD3/CD28 Abs in the absence or presence of berberine. After 3 days of co-culture, (i) CD4 + and (ii) CD8 + T cell division was determined by flow cytometry. (C) (i) Supernatant levels of IFN-gamma measured by ELISA, and (ii) mRNA expression of IFN-gamma in T cells measured by qPCR. \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001 compared to the (Positive Control) PC group. Index in PubMed under a CC BY license. PMID: 33732240

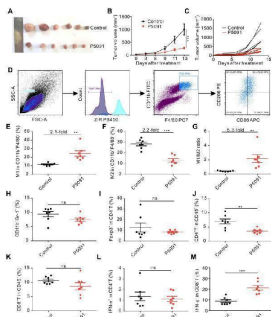


Targeting USP7 inhibits murine M2 phenotype and function in vitro . (A) The mRNA expression of common genes related to DUBs was detected by RT-PCR in M0, M1 (LPS/IFN-gamma M1), and M2 (IL-4/13 M2 and IL-10 M2) induced from ANA-1. ( B ) The mRNA expression of USP7 in MΦs, M1, and M2 induced from BMDMs was detected by RT-PCR. ( C ) Western blotting showing the expression of USP7 in MΦ, M1, and M2 induced from BMDMs. ( D-E ) Flow cytometry analyses of the expression of CD206 in IL-4/13-induced BMDM M2 cells, which had been treated with P5091 (5 uM or 10 uM) for 24 h. Data are presented as the mean ± SEM (n = 3). ( F ) Detection of the expression of USP7 in BMDMs, which were transfected with either NC- or USP7-siRNA by western

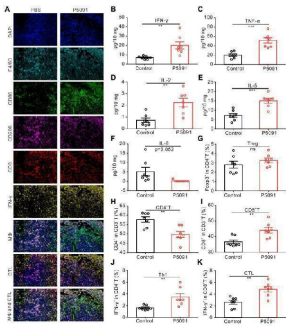
blotting. ( G ) The expression of CD206 in BMDMs of NC-siRNA and USP7-siRNA group was detected by flow cytometry. ( H-I ) Flow cytometry analyses of CFSE expression on the surface of CD8 + T cells in the presence of conditioned medium from either DMSO- or P5091- (5 uM or 10 uM) treated IL-4/13-induced BMDM M2 cells. Data are presented as the mean  $\pm$  SEM (n = 3). Index in PubMed under a CC BY license. PMID: 32802195



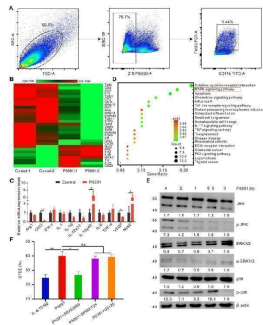
Berberine induces T cell apoptosis via the mitochondrial apoptosis pathway. (A) (i) KEGG functional categories of differentially expressed genes following berberine and saline treatment. The Y-axis represents the KEGG functional categories. (ii) KEGG analysis of the significantly altered signaling pathways in cell growth- and death-associated genes. The X-axis represents the rich ratio of the number of differentially expressed genes and the Y-axis represents the KEGG pathways. (iii) qPCR analysis of Bcl-2 and TNF-alpha mRNA expression in SPCs collected from heart transplant recipients treated with berberine or not. (B) T cell apoptosis assays in vivo . (i) SPCs and (ii) LNCs were collected at POD 7. The percentages of apoptotic CD4 + and CD8 + T cells were determined by flow cytometry (n = 3 mice/group). (C) T cell apoptosis assay in vitro . T cells from naïve C57BL/6 mice were co-stimulated with anti-CD3/CD28 Abs in the absence or presence of berberine. The percentages of apoptotic (i) CD4 + and (ii) CD8 + T cells were determined by flow cytometry. (D) Berberine activates the mitochondrial apoptosis pathway in vivo . (i) Relative protein expression of Bcl-2, Bax, cytochrome c, cleaved-caspase-3, and cleaved-PARP in SPC. (ii) beta-actin was used as a loading control (n = 3 mice/group), and OD values (relative to beta-actin) are presented as means  $\pm$  SEMs. \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001 compared to the normal saline-treated group. (E) Berberine activates the mitochondrial apoptosis pathway in vitro . Relative protein expression of Bcl-2, Bax, cytochrome c, cleaved-caspase-3, and cleaved-PARP expression in CD3 + T cells. (ii) beta-actin was used as a loading control; OD values (relative to beta-actin) are presented as means  $\pm$  SEMs. SPCs, spleen cells; LNCs, lymph node cells; POD, post-operative day. \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001 compared to the PC group. Index in PubMed under a CC BY license. PMID: 33732240



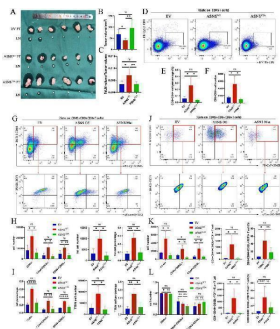
Targeting USP7 inhibits tumor growth and induces local anti-tumor immunity in vivo . ( A ) Harvested and photographed tumors in the P5091 and the Control group. ( B ) Lewis tumor growth following P5091 or Vehicle treatment in vivo. Data are presented as the mean  $\pm$  SEM (n = 6 per group). ( C ) Spider diagram of the tumor volume growth in each mouse from the P5091 group and the Control group. ( D ) Gating strategy for detection of the TAMs by flow cytometry. ( E-G ) Proportions of M1 ( E ) and M2 ( F ), and M1/M2 ratio ( G ) in the TME of the P5091 group and the Control group. ( H-M ) Percentages of MDSC ( H ), Treg ( I ), CD4 + T ( J ), CD8 + T ( K ), Th1 ( L ), and CTLs ( M ) within the TME in each group. Data are presented as the mean  $\pm$  SEM (n = 7) for ( E-M ). Index in PubMed under a CC BY license. PMID: 32802195



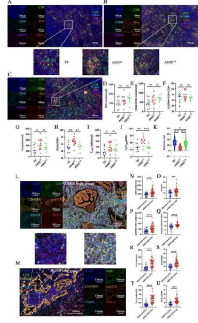
Targeting USP7 activates local and systemic anti-tumor immunity. ( A ) Multicolor immunofluorescence detection of TAMs and CTLs in the TME of the P5091 group and the Control group. ( B-F ) Cytokines IFN-gamma ( B ), TNF-alpha ( C ), IL-2 ( D ), IL-5 ( E ), and IL-6 ( F ) in the TME of each group were detected by Mul-Analyte Flow Assay Kit. ( G-K ) Proportion of Treg ( G ), CD4 + T ( H ), CD8 + T ( I ), Th1 ( J ), and CTLs ( K ) in the spleen of each group. Data are presented as the mean  $\pm$  SEM ( n = 7 ) for ( B-K ). Index in PubMed under a CC BY license. PMID: 32802195



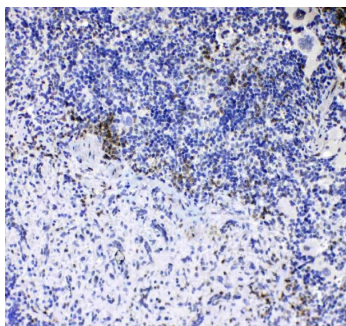
Targeting USP7 can activate the p38 MAPK pathway to reprogram TAMs. ( A ) The strategy for sorting TAMs in TME by flow cytometry. ( B ) Heat maps illustrating the differentially expressed M1- and M2-related genes in TAMs between the P5091 group and the Control group based on the results of RNA sequencing. ( C ) RT-PCR further verifying the differentially expressed genes of sorted TAMs in each group. Data are presented as the mean  $\pm$  SEM ( n = 3 ). ( D ) KEGG analysis identifying 20 most obviously enriched pathways based on the differentially expressed genes of the two groups. ( E ) Western blotting detection of the expression of JNK, p-JNK, ERK1/2, p-ERK1/2, p38, p-p38, and beta-actin in IL-4/13-BMDM M2 cells treated with P5091 ( 10  $\mu$ M ) at the indicated time points. ( F ) Flow cytometry analysis of CFSE expression on the surface of CD8 + T cells in the presence of conditioned medium of IL-4/13-induced BMDM M2 cells from various indicated treatments. Treatments indicated: DMSO stimulation, P5091 ( 10  $\mu$ M ) stimulation, P5091 ( 10  $\mu$ M ) stimulation in the presence of inhibitors of p38 ( SB203580, 10  $\mu$ M ), JNK ( SP600125, 10  $\mu$ M ), Erk1/2 ( U0126-EtOH, 10  $\mu$ M ). Data are presented as the mean  $\pm$  SEM ( n = 4 ). Index in PubMed under a CC BY license. PMID: 32802195



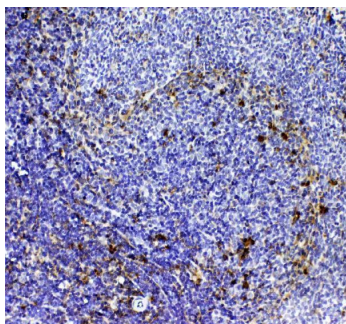
ASNS shapes the immune landscapes in metastatic TdLN and primary tumor site. Figure 5A-C. LN metastasis model was conducted on C57BL/6 mice with LLC-ASNS WT ( n = 6 ), ASNSC2A overexpression cells ( n = 6 ) and control group ( n = 6 ), primary tumor and popliteal lymph nodes were isolated at the end of the experiment, and primary tumor volume ( B ) and TdLN volume/tumor volume ( C ) was measured and analyzed. 5D-F. ( D ) Representative FACS profiles of CD8 + T cells are shown. The percentage ( E ) and number ( F ) of CD8 + subset in TIL cells isolated from primary tumor is shown. 5G-I. ( G ) Representative FACS profiles of the co-expression pattern of CD44 and CD62L, or the co-expression pattern of TCF-1 and TOX in CD8 + T cells are shown. The number ( H ) and percentage ( I ) of CD44 + , CD44 + CD62L + , CD44 + CD62L - , Tsl and TTSM subset in CD8 + T cells isolated from TdLN is shown. 5J-L. ( J ) Representative FACS profiles of the co-expression pattern of CD44 and CD62L, or the co-expression pattern of TCF-1 and TOX in CD8 + T cells are shown. The number ( K ) and percentage ( L ) of CD44 + , CD44 + CD62L + , CD44 + CD62L - , Tsl and TTSM subset in CD8 + T cells isolated from primary tumor is shown. Index in PubMed under a CC BY license. PMID: 41208878



ASNS-high-expression metastases generated lymphocyte niches enriched with activated T cells, memory T cells, Tsl and TTSM. Figure 6A-C. Representative immunofluorescence staining images of metastatic TdLNs from LN metastasis model. 6D. The number of CD8+ T cells in the metastasis locations within TdLNs (ASNSWT, n=6, ASNSC2A, n=5, and EV, n=4). 6E-F. The number(E) and percentage(F) of CD44+CD8+T cells among all CD8 T cells in the metastasis locations within TdLNs (ASNSWT, n=6, ASNSC2A, n=5, and EV, n=4). 6G-H. The number(G) and percentage(H) of Tsl cells among all CD8 T cells in the metastasis locations within TdLNs (ASNSWT, n=6, ASNSC2A, n=5, and EV, n=4). 6I-J. The number(I) and percentage(J) of TTSM cells among all CD8 T cells in the metastasis locations within TdLNs (ASNSWT, n=5, ASNSC2A, n=4, and EV, n=3). 6K. Quantitative estimates of the distance from ova+ to CD8+CD44+CD62L+TCF+(TTSM) (ASNSWT, n=6, ASNSC2A, n=5, and EV, n=4). 6L-M. Representative immunofluorescence staining images of metastatic TdLNs from NSCLC patients. 6N-O. The number(C) and percentage(D) of CD8+ T cells in the metastasis locations within TdLNs(ASNS high group, n=7, and ASNS low group, n=6). 6P-Q. The number (E) and percentage(F) of CD45RO+CD8+ T cells in CD8+T cells in the metastasis locations within TdLNs(ASNS high group, n=7, and ASNS low group, n=6). 6R-S. The number (G) and percentage(H) of Tsl cells in CD8+T cells in the metastasis locations within TdLNs(ASNS high group, n=7, and ASNS low group, n=6). 6T-U. The number (I) and percentage(J) of TTSM cells in CD8+T cells in the metastasis locations within TdLNs(ASNS high group, n=7, and ASNS low group, n=6).Index in PubMed under a CC BY license. PMID: 41208878

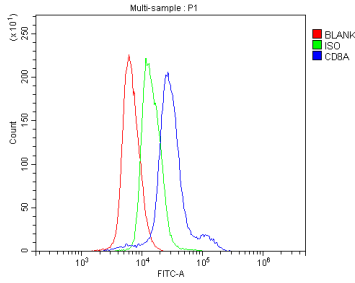


IHC analysis of CD8 alpha using anti-CD8 alpha antibody (A02236-1). CD8 alpha was detected in paraffin-embedded section of mouse spleen tissue . Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-CD8 alpha Antibody (A02236-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.



IHC analysis of CD8 alpha using anti-CD8 alpha antibody (A02236-1). CD8 alpha was detected in paraffin-embedded section of rat spleen tissue . Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-CD8 alpha Antibody (A02236-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the

chromogen.



Flow Cytometry analysis of mouse spleen tissues using anti-CD8 alpha antibody (A02236-1). Overlay histogram showing mouse spleen tissues stained with A02236-1 (Blue line). The tissues were fixed with 4% paraformaldehyde and blocked with 10% normal goat serum. And then incubated with rabbit anti-CD8 alpha Antibody (A02236-1, 1 ug/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight® 488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

## 11 Publications Citing This Product

1. PubMed ID: 10.3389/fimmu.2021.616074, Berberine Prolongs Mouse Heart Allograft Survival by Activating T Cell Apoptosis via the Mitochondrial Pathway
2. PubMed ID: 10.1177/09636897211054503, Leflunomide Inhibits rat-to-Mouse Cardiac Xenograft Rejection by Suppressing Adaptive Immune Cell Response and NF-kappaB Signaling Activation:
3. PubMed ID: 10.1039/D1BM00726B, NO/ROS/RNS Cascaded-Releasing Nano-Platform for Gas/PDT/PTT/Immunotherapy of Tumor

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