Supporting Information

Supplementary Materials and Methods

Immunohistochemistry

Clinical specimens were examined using immunohistochemistry staining. After formalin fix and paraffin embedment, tissue sections were deparaffinized in dimethylbenzene, and rehydrated in alcohol and water. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide. Antigen retrieval was performed by heat treatment. Nonspecific binding was blocked with 10% BSA. Primary antibodies were incubated with the sections overnight at 4 ℃, followed by incubated with horseradish peroxidase-conjugated secondary antibody for 30 min at 37 ℃. Immunocomplexes in sections were stained with 3,3-diaminobenzidine to form brown reaction products. After counterstaining with hematoxylin, sections were dehydrated and mounted for microscopic examination. Immunohistochemical evaluation was performed with Image-Pro Plus 6.0 or Image J software. Three visual fields were randomly selected for each histological tablet, and the average value was determined as the result of the histological tablet. For the result judgment of each visual field, we used the software to calculate the mean optical density (MOD) [MOD = integral optical density (IOD) / positive area] to semi-quantify the expression of the target protein. And MOD ≥ 4 was regarded as high expression, MOD < 4 as low expression.

Cell lines, cell transfection and reagents

Human hepatoblastoma cell line HepG2 (HB-8065, ATCC, Manassas, VA), hepatoma cell lines Huh7 (JCRB0403, Japan) and mouse hepatoma cell line Hepa1-6 (Shanghai Institute of Cell Biology, Chinese Academy of Sciences) were all cultured in antibiotics-free Dulbecco's modified Eagle medium (DMEM, Gibco, Carlsbad, CA) with high glucose supplemented with 12% foetal bovine serum (FBS, Gibco) at 37°C with 5% CO_2. HepG2 and Huh7 cells were STR-authenticated by Shanghai Biowing Applied Biotechnology Co. LTD, Shanghai, China (STR Profiling Report). All cells were routinely screened and found to be free of mycoplasma.

The cells were seeded at a density of 5×10^5 cells/well, and DNA transfection was performed in a six-well plate

using Lipofectamine 2000 (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. The concentration of pcDNA3.1-HBx used in transient transfection was 1 μg/ml. The reagents used in this experiment included DMC (Sigma-Aldrich, St. Louis, MO), Atezolizumab (HY-P9904, MedChemExpress, NJ), Compound C (GC17243, GLPBIO, Montclair, CA) and tunicamycin (Solarbio, Beijing, China).

Western blot assay and ubiquitination detection

Thirty micrograms of protein was applied to 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretically transferred to a polyvinylidene fluoride (PVDF) membrane (Merck Millipore, Darmstadt, Germany). The membranes were blocked in Tris-buffered saline with Tween-20 containing 10% non-fat milk for 3 hours at room temperature, and subsequently incubated with a panel of specific antibodies overnight at 4 ℃. Secondary antibodies were labeled with horseradish peroxidase for 1 hour at room temperature. Immunoreactivity was detected using a BeyoECL Plus (P0018M, Beyotime, Shanghai, China) according to the instructions of manufacture in the Bio-RAD ChemiDoc Imaging System. The amount of each protein was semi-quantitatively determined as the ratio of GAPDH indicated on each gel. For ubiquitin detection, the cell lysate was immunoprecipitated with PD-L1 antibody to get the denatured protein solution, and the ubiquitin level of PD-L1 protein was detected by Western blot with Ubiquitin antibody. MG132 (Beyotime) was used to block the catalytic activity of proteasome in advance.

Construction of Hepa1-6 with stable overexpression of HBx（Hepa1-6/HBx）

Lentivirus expression vector was pLV-EF1α-MCS-IRES-BSD (puro). The DNA of the target gene HBx was amplified by using the PCR primers (HBx F': 5'-AGAGAATTCGGATCCatggctgctaggctgtgct-3'; HBx R': 5'-TGGCTCGAGCCCGGGttaggcagaggtgaaaaagttg-3'). *BamH*I and *Sma*I were used for digestion and *Exo*III was used for connection. Finally, the HBx lentivirus vector was sequenced. HBx lentivirus vector and lentivirus packaging mixture were co-transfected into 293T cells to obtain HBx lentivirus supernatant. HBx lentivirus was transfected into Hepa1-6 mouse hepatoma cells, and Hepa1-6 cells stably expressing HBx (Hepa1-6/HBx) were

obtained after puromycin screening.

Flow cytometry analysis

The samples were treated into single cell suspension and filtered into EP tube. After repeated washing, the final volume was adjusted to 100 μl, and then the blocking solution of Fc was added [the blocking solution of mouse cells was CD16/32 (14-0161-81, Ebioscience, San Diego, CA); and the blocking solution of human cells was Human TruStain FcX (422301, Biolegend, San Diego, CA)]. Then the cells were incubated with flow antibody at 4 ℃ and hidden from light for 30 minutes. After washing with precooled PBS, the cells were detected by the BD Accuri C6 PlusTM platform.

Cell viability assay

Cell viability was assessed using the CCK-8 Assay Kit (Dojindo Laboratories, Kumamoto, Japan) and operated according to the instructions. WST-8 was measured spectrophotometrically at 450 nm using a SpectraMax M5 (Molecular Devices, Sunnyvale, CA).

Construction of co-culture system

Hepatoma cells (HepG2 or Huh7) were inoculated in 6-well plates and transfected with HBx plasmid (1 μg/ml) for 24 hours. After changing to fresh medium, pre-extracted peripheral blood mononuclear cells (PBMCs) was added for co-culture. Meanwhile, DMC was added in the "HBx+DMC" group. After 24 hours, hepatoma cells and PBMCs were collected and detected by flow cytometry.

Microarray chip analysis

Microarray chip analysis was completed by Shanghai OE Biotech Co Ltd (OE Biotech, Shanghai, China). Total RNA were quantified by the NanoDrop ND-2000 (Thermo Scientific) and the RNA integrity was assessed using Agilent Bioanalyzer 2100 (Agilent Technologies).The sample labeling, microarray hybridization and washing were performed based on the manufacturer’s standard protocols. Briefly, total RNA were transcribed to double strand cDNA, then synthesized into cRNA and labeled with Cyanine-3-CTP. The labeled cRNAs were hybridized

onto the microarray. After washing, the arrays were scanned by the Agilent Scanner G2505C (Agilent Technologies). Feature Extraction software (version10.7.1.1, Agilent Technologies) was used to analyze array images to get raw data. Genespring (version13.1, Agilent Technologies) were employed to finish the basic analysis with the raw data. To begin with, the raw data was normalized with the quantile algorithm. The probes that at least 100% of the values in any 1 out of all conditions have flags in "Detected" were chosen for further data analysis. Differentially expressed genes were then identified through fold change as well as *P* value calculated with t-test. The threshold set for up- and down-regulated genes was a fold change ≥ 2.0 and a *P* value ≤ 0.05. Afterwards, GO analysis and KEGG analysis were applied to determine the roles of these differentially expressed mRNAs. Finally, Hierarchical Clustering was performed to display the distinguishable genes' expression pattern among samples.

Co-immunoprecipitation (Co-IP)

For co-immunoprecipitation, the cells were treated with lysis buffer (P0013, Beyotime) with PMSF and phosphatase inhibitor, and lysates were obtained by centrifugation. Non-specific antibodies were removed by using control IgG from the same source and protein A/G Plus-agarose (sc-2003, Santa Cruz, CA). Then lysates were mixed with protein A/G Plus-agarose and AMPKα antibody or PD-L1 antibody, and incubated overnight at 4 ℃. The immune complex were resuspended in SDT lysate and boiled at 100 ℃ for 5 minutes. The obtained sample can be used for Western blot or MS analysis. The secondary antibody of Mouse Anti-rabbit IgG (L27A9) mAb was used in Western blot for avoiding the interference of light and heavy chain IgG.

Immunofluorescence

For immunofluorescence staining, cells were first fixed with 4% paraformaldehyde, then permeabilised and blocked with blocking buffer. The cells were incubated with PD-L1 and GRP94 antibody overnight at 4 ℃, and then incubated with fluorescent secondary goat anti-rabbit IgG FITC antibody and goat anti-mouse IgG (Alexa Fluor 555 Conjugate) for 1 h at 37 ℃ in the dark. Cells were counterstained with 40,6-diamidino-2-phenylindole

(DAPI) and visualised using a Leica SP8 confocal microscope (Leica, Germany).

LC-MS/MS analysis

The denatured protein solution can be obtained according to the step of co-immunoprecipitation and sent to the mass spectrometer for analysis. LC-MS/MS was performed by Shanghai Applied Protein Technology Co. Ltd. (Shanghai, China). Briefly, each fraction was injected for nanoLC-MS/MS analysis. The peptide mixture was loaded onto a reverse phase trap column (Thermo Scientific Acclaim PepMap100, 100 μm×2 cm, nanoViper C18) connected to the C18-reversed phase analytical column (Thermo Scientific Easy Column, 10 cm long, 75 μm inner diameter, 3 μm resin) in buffer A (0.1% Formic acid) and separated with a linear gradient of buffer B (84% acetonitrile and 0.1% Formic acid) at a flow rate of 300 nl/min controlled by IntelliFlow technology. LC-MS/MS analysis was performed on a Q Exactive mass spectrometer (Thermo Scientific) that was coupled to Easy nLC (Proxeon Biosystems, Thermo Fisher Scientific). The mass spectrometer was operated in positive ion mode. MS data was acquired using a data-dependent top10 method dynamically choosing the most abundant precursor ions from the survey scan (300–1800 m/z) for HCD fragmentation. Automatic gain control (AGC) target was set to 3e6, and maximum inject time to 10 ms. Dynamic exclusion duration was 40.0 s. Survey scans were acquired at a resolution of 70,000 at m/z 200 and resolution for HCD spectra was set to 17,500 at m/z 200, and isolation width was 2 m/z. Normalized collision energy was 30 eV and the underfill ratio, which specifies the minimum percentage of the target value likely to be reached at maximum fill time, was defined as 0.1%. The instrument was run with peptide recognition mode enabled.

RNA interference

All small interfering RNAs (siRNAs) of RBX1 (RBX1 siRNA1: CUGGGAUAUUGUGGUUGAUTT; RBX1 siRNA2: GAAGCGCUUUGAAGUGAAATT), including NC with no homology to known human genes, were chemically synthesised by GenePharma (Shanghai, China). One hundred picomoles of RBX1 siRNA or NC were used for transfection according to the manufacturer's instructions.

Statistic analysis

Chi-square test, Pearson, analysis of variance (ANOVA) and t-test statistical analysis were performed using GraphPad Prism7 software (San Diego, CA). Pearson used for analyzing the correlation between CD163 and PD-L1 expression. All values were expressed as mean ± standard deviation (SD) of replicates. $P<0.05$ indicates statistical significance.

Antibodies:

Name	Catalog number	Source
HBx	ab39716	Abcam, Cambridge, UK
human CD8	ab93278	Abcam
mouse CD8	ab209775	Abcam
human PD-L1	ab213524	Abcam
mouse PD-L1	#64988	Cell Signaling Technology, Beverly, MA
CD163	ab182422	Abcam
Ubiquitin	#3936	Cell Signaling Technology
PD-L1	ab213524	Abcam
CD163	ab182422	Abcam
AMPKα	#5831	Cell Signaling Technology
Thr172-pAMPKα	#2535	Cell Signaling Technology
GAPDH	#5174	Cell Signaling Technology
HSPH1	ab109624	Abcam
RBX1	ab133565	Abcam
UBE2K	ab52930	Abcam
anti-mouse CD45 (FITC)	103108	Biolegend
anti-mouse CD8a (PerCP)	100731	Biolegend
anti-PD-L1 antibody	12-5983-42	Invitrogen, Carlsbad, CA
anti-PD-L1 isotype control	12-4714-81	Invitrogen
anti-human CD3 antibody	317305	Biolegend
anti-human CD8 antibody	344707	Biolegend
Mouse Anti-rabbit IgG (L27A9) mAb	#5127	Cell Signaling Technology
GRP94	60012-1	Proteintech, Chicago, IL
goat anti-rabbit IgG FITC antibody	ab6717	Abcam
goat anti-mouse IgG (Alexa Fluor 555 Conjugate)	#4409	Cell Signaling Technology

Supplementary Figure Legends

Fig. S1. The difference of PD-L1 expression in immune cells between HBx positive and negative HCC tissues (200×). The red arrow indicated the positive expression of PD-L1 in immune cells.The bottom left corner of each histochemical picture was a magnification representative field (400×).

Fig. S2. Tumor data obtained from GEPIA database were used to analyze the correlation between CD274 and CD163 gene levels. The results showed that there was a positive correlation between CD274 and CD163 gene expression, which was statistically significant.

Fig. S3. The volcano plot of differential genes in microarray results. According to the screening rules, the blue dot on the left and the red dot on the right meet the selection range of differential genes (screening criteria: up-regulation or down-regulation multiple change$\geqslant$2.0 and $P\leqslant$0.05).

Fig. S4. (A-B) The base peak diagram of MS.

Fig. S5. The protein enrichment pathway was analyzed by MS data software, and the red box in the picture was the first three enrichment pathway.

Fig. S6. The number of protein types detected in the two different treatment groups DMC- (control group) and DMC+ (test group). In the middle "710" was the common protein type of the two groups, and the left and right were the respective protein types.

Fig. S7. The second-order mass spectra of HSPH1 (A), RBX1 (B) and UBE2K (C) protein sequences, which reflect the mass-charge ratio distribution of the actual detected secondary ions (b, y ions matching map).

Fig. S1

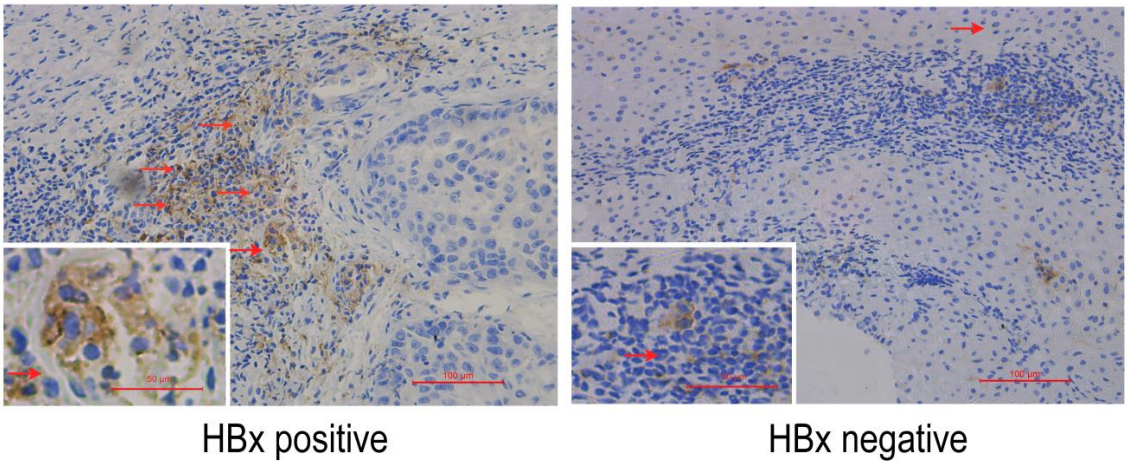

HBx positive
HBx negative

Fig. S2

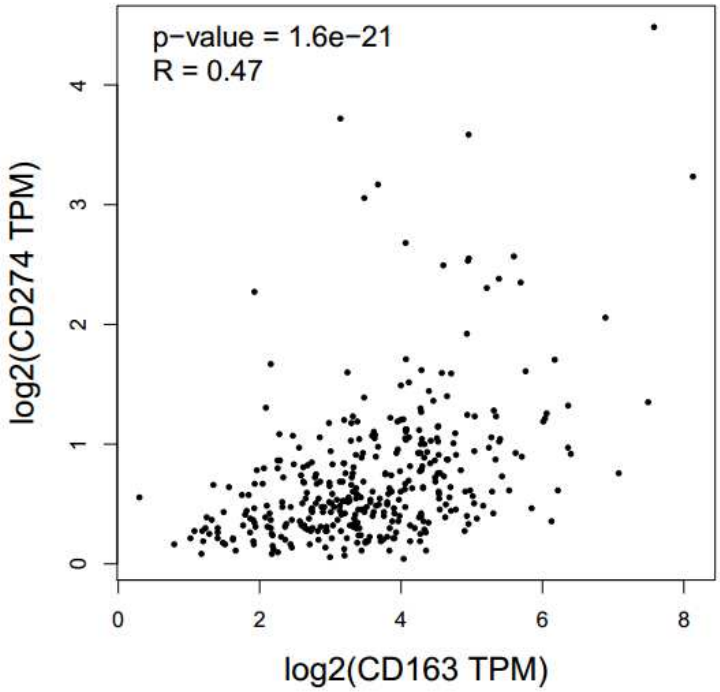

p−value = 1.6e−21
R = 0.47
log2(CD274 TPM)
log2(CD163 TPM)
0
1
2
3
4
0
2
4
6
8

Fig. S3

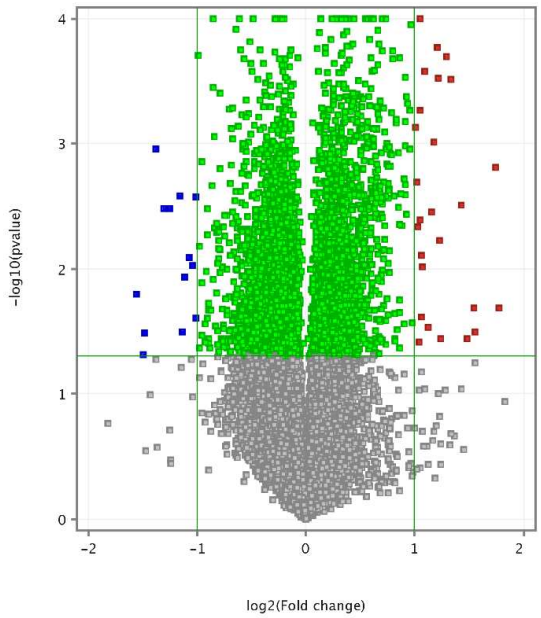

-log10(pvalue)
log2(Fold change)

Fig. S4

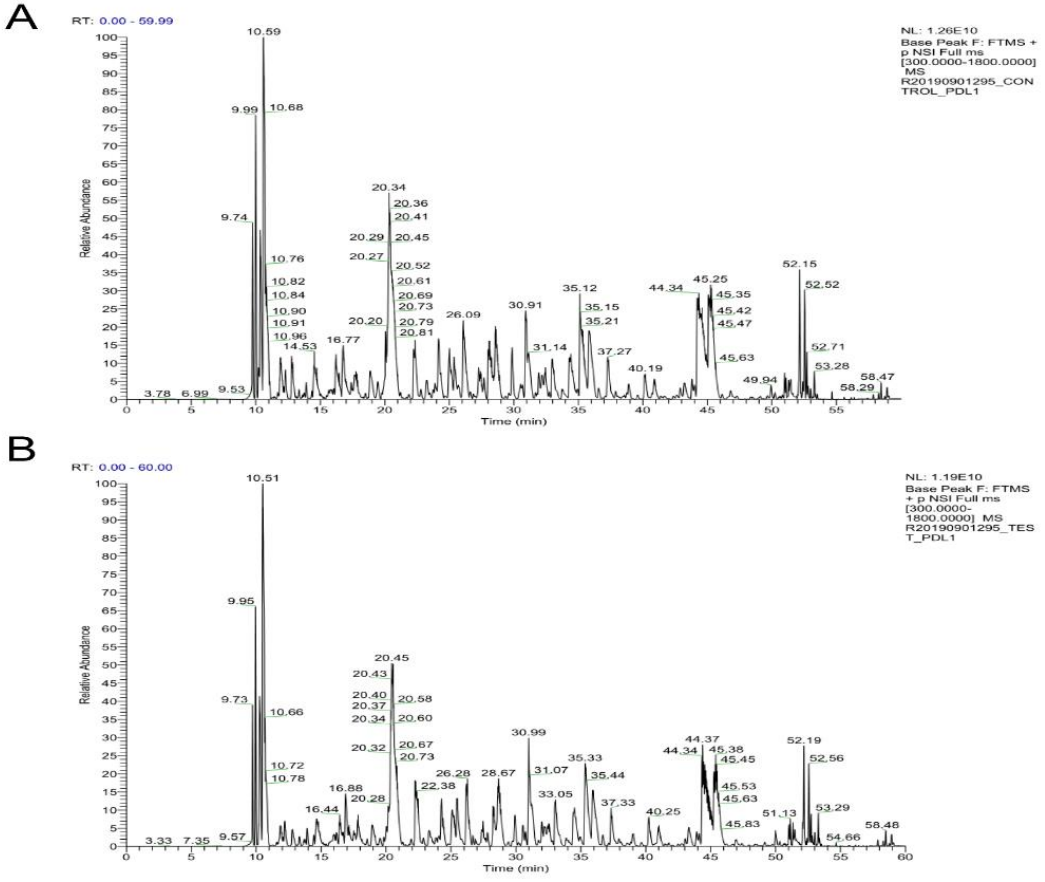

A
RT: 0.00 - 59.99
NL: 1.26E10
Base Peak F: FTMS + p NSI Full ms [300.0000-1800.0000] MS R20190901295_CONTROL_PDL1
Relative Abundance
Time (min)
B
RT: 0.00 - 60.00
NL: 1.19E10
Base Peak F: FTMS + p NSI Full ms [300.0000-1800.0000] MS R20190901295_TEST_PDL1
Relative Abundance
Time (min)

Fig. S5

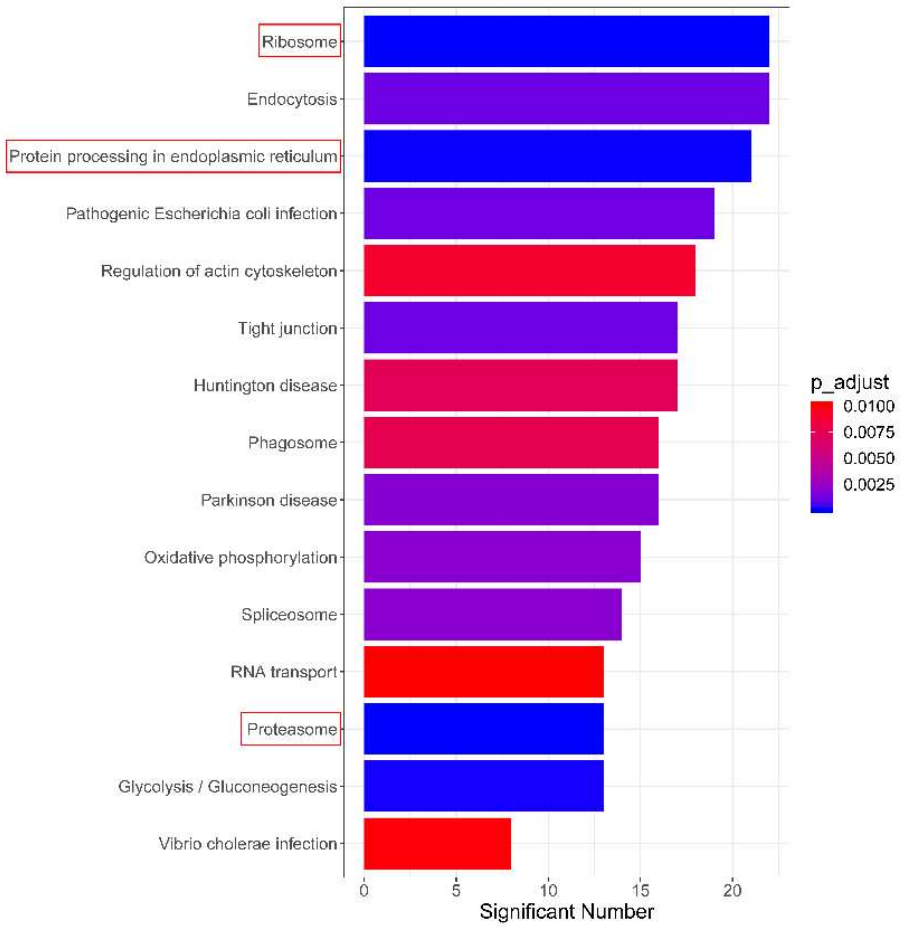

Ribosome
Endocytosis
Protein processing in endoplasmic reticulum
Pathogenic Escherichia coli infection
Regulation of actin cytoskeleton
Tight junction
Huntington disease
Phagosome
Parkinson disease
Oxidative phosphorylation
Spliceosome
RNA transport
Proteasome
Glycolysis / Gluconeogenesis
Vibrio cholerae infection
0
5
10
15
20
Significant Number
p_adjust
0.0100
0.0075
0.0050
0.0025

Fig. S6

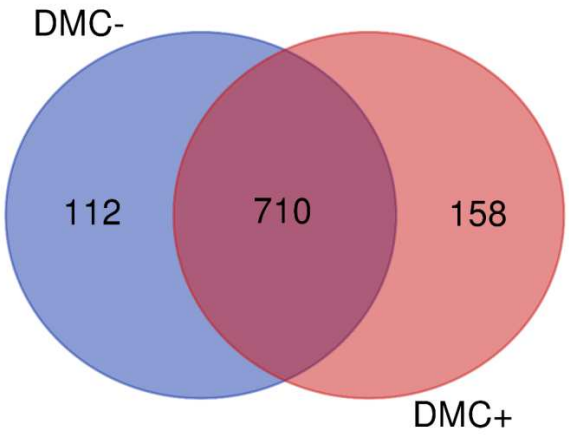
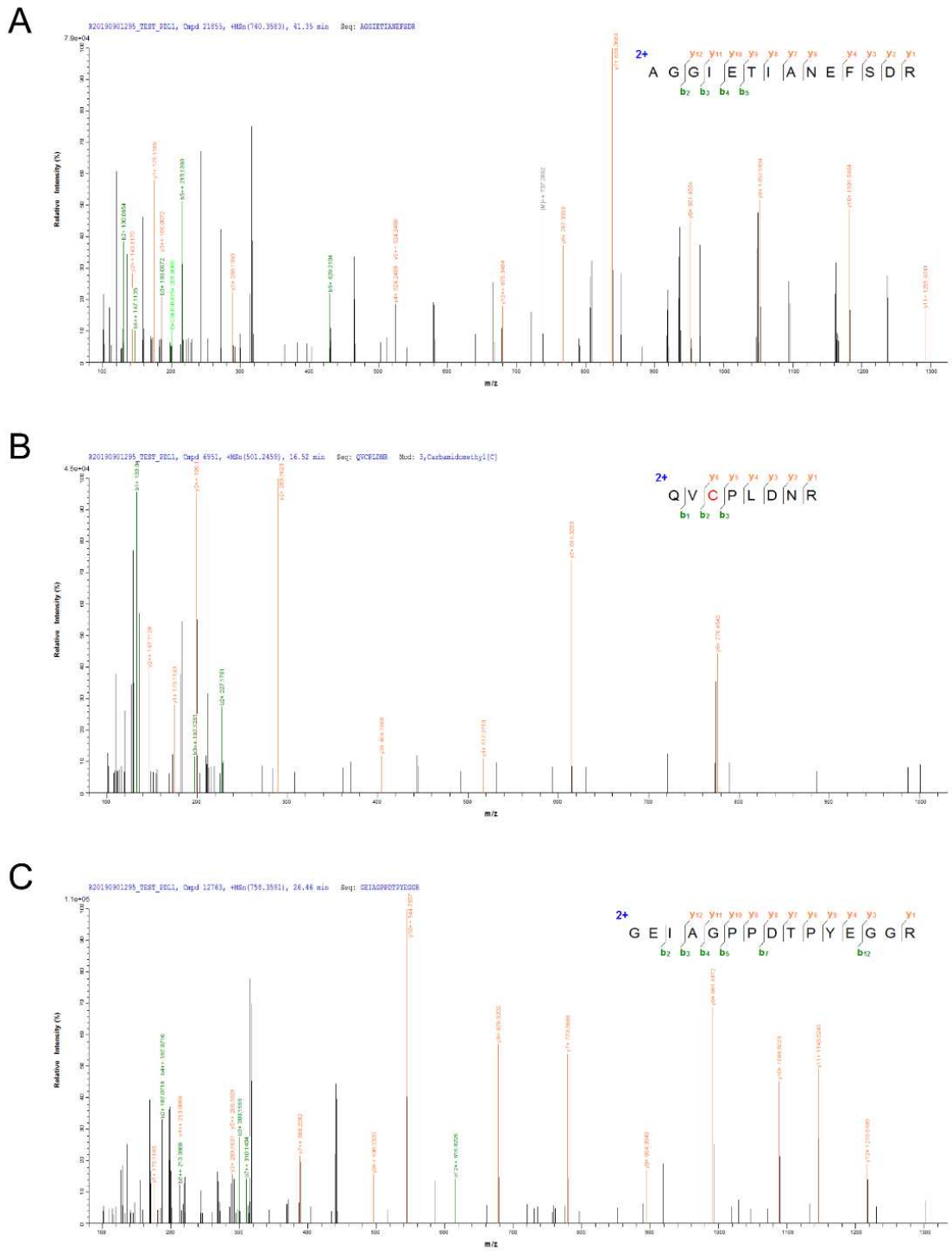

DMC-
112
710
158
DMC+

Fig. S7


A
B
C
Relative Intensity (%)
m/z
2+
A G G I E T I A N E F S D R
Q V C P L D N R
G E I A G P P D T P Y E G G R