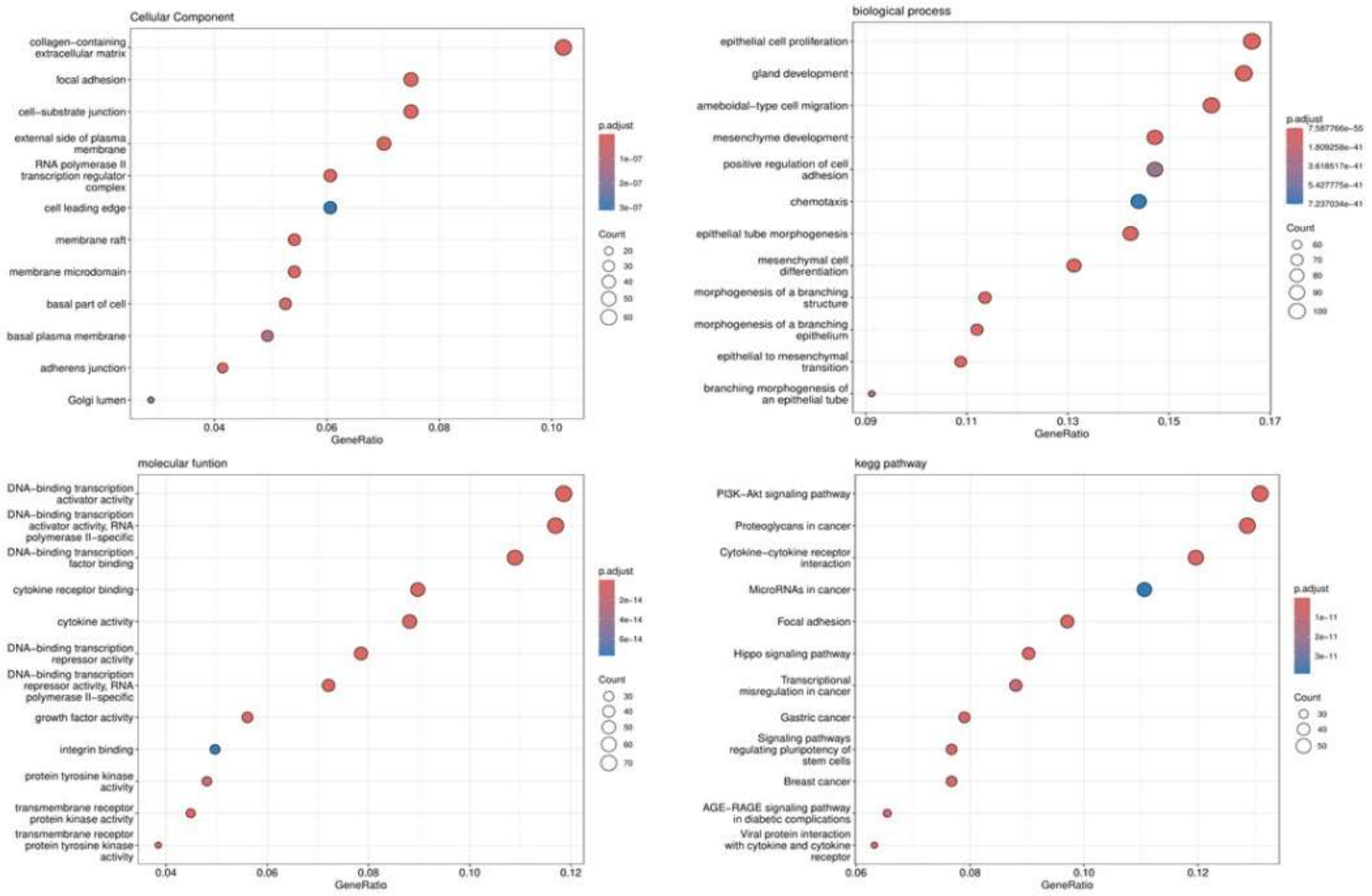


Supplementary Figure 1. DEG genes Enrichment:
The dot plot for the Gene Ontology (GO) associated with the DEG genes includes biological processes (BP), cellular components (CC), and molecular functions (MF). The top 10 enriched KEGG pathway terms for deg genes.

Table 1. Characteristics of patients: Clinical and demographic information

Age at diagnosis	59 ± (16)	TNM staging		Gender		Race	
Unknown	8	Not Reported	14 (3.2%)	Female	180 (38%)	Asian	12 (2.6%)
		Stage 0	7 (1.6%)	Male	290 (62%)	Black or African American	1 (0.2%)
		Stage I	77 (18%)			Not reported	10 (2.1%)
		Stage II	140 (32%)			White	447 (95%)
		Stage III	171 (40%)				
		Stage IV	23 (5.3%)				
		Unknown	38				

N = 470, Mean ± (SD); n (%)

Table 2. List of genes for each module in this study

Blue	Brown	Green	Grey	Turquoise	Turquoise	Yellow
CREBBP	LASP1	KDM1A	HIPK2	MRC2	CDH2	ITGA3
CX3CL1	PLXND1	DVL2	EPHA8	IGF1	HOXB9	PROM1
PKD1	SCIN	NOX1	SREBF1	WWTR1	HAS2	CNTN1
UBE3C	PLAUR	JARID2	PAX2	HGF	PDGFD	CDH1
BTBD7	RUNX3	BRCA1	PEBP1	FHL1	HS6ST2	ROS1
VIM	RNH1	TACC3	HNF4A	EHD2	ALK	LIMA1
CD44	NR1H3	SNAI2	MET	VCAN	COL8A2	PRSS8
IFT88	FAS	HMGB3	SKP1	HSPA5	EIF2AK3	LAMC2
GAB2	GRN	NEDD4L	HRG	LTBP1	CERS6	NTN1
TIMP2	TNC	MTA3	RBP2	DKK3	CYP7B1	FGFR2
CUL3	HDAC9	MTHFD2	PLA2G4A	ALX4	LEP	TP63
MAGEC2	HIPK2	YBX1	AMHR2	FOXC1	PCSK1	HOXA9
LETMD1	PRKCQ	PFN2	ALDOB	CDK14	FOXC2	EDN1
FOXN3	RHOA	EPHA8	CDKL2	PDE4A	BDNF	CEACAM1
FOXJ2	RASSF1	MBD3	EGF	CRMP1	HIC1	GRHL2
ELAVL1	NUCB2	SREBF1	SIRT3	ST6GAL1	CAVIN1	WNT11
KLF6	CDC42	TRPC5	NR1I2	GLI2	CD151	CEACAM6
TP53BP1	PTGS2	ENO1	CBR1	NOTCH3	MAGED1	PTHLH
ROCK1	EED	SRI	LMNA	TEAD2	CDH5	PITPNM3
HDAC4	TP73	RASAL2	SPZ1	MGLL	FZD2	HNF4A
TGFBR3	TCF7	PAG1	CDX2	NTN4	ADIPOQ	BMP7
LRP6	EPB41L3	SENP1	APBB1	NUAK1	F2R	FERMT1
MAP4K4	CD82	GSK3B	GJB1	FSCN1	SOX2	GATA1
TCF3	ABCB1	EIF3I	FSHR	PAX2	EPHB3	KLF8
SMC1A	ESR1	UIMC1	ALK	MCAM	NOG	KLF5
KDM5A	EZR	AURKA	GRIN1	FGFR1	PCDH9	MSLN
FERMT2	IL11	DNMT3B	F2	FBLN1	SLIT3	MAPK3
ITCH	FKBP5	PEBP1	TFDP3	TNS1	WT1	ESRP2
HSP90AA1	JAK2	BIRC5	TLE1	MMP2	ROR1	ESRP1
MAP3K4	OSM	GEMIN2	MUC2	ITGA6	NR2F2	BBC3
XRN2	LGALS1	CBY1	MICA	TGFB2	NANOS3	CEACAM5
YPEL3	XBP1	TXN2	ANG	NRP1	TCF4	DNAJB6
TYRO3	HMOX1	MYBL2	CPEB1	MMP11	FAM3C	HSPB1
HDAC6	NFKBIA	SALL4	HOXB7	CARD10	NOTCH4	CYP3A5
SIRT1	MMP9	E2F1		TIMP3	TUG1	AGR2

MZF1	CTSZ	LAMA1	PDGFB	CUX1	CA9
CIRBP	PIM2	AXIN1	HIF1A	ITGB3	VTN
CRKL	CCL22	GPI	PAK5	HOXB7	FOXN1
MAPK1	PARD6A	AKT2	JAG1	ETV1	IL17A
TAB1	AQP9	ERF	SMAD7	KRT8	VEGFA
EP300	CSK	GSK3A	TIMP1		SKP1
SNW1	KCNN4	MET	FGF9		STC2
DICER1	JAK3	EZH2	FLT1		HRG
YY1	NAMPT	INPP4B	RGCC		RBP2
ZMYND8	SERPINE1	PPARGC1A	SALL1		HES1
PGRMC1	GATA3	CCND1	CEMIP		ITGB6
PLS3	CXCL12	FOXM1	TJP1		FHL2
FMR1	CCL2	TIMELESS	BRF2		SDC1
TAZ	ABCC3	GAPDH	NDRG1		EPHA4
TSC2	SLC9A3R1	MAPK14	TRPS1		SLC2A1
KAT8	NFKB1	RNF8	TGFB1		F3
UBR5	EHD1	GMNN	PTN		IRF6
EEF1D	IL23A	SRF	CAV1		MMP8
TGFBR1	WNT5B	LMNB1	HOXA13		UCHL3
MAPK8	MYL2	ARMC8	PTPRZ1		EPCAM
SUFU	CDKN1B	ECT2	AHR		MSX2
SMC3	VDR	EIF4G1	LHX2		LYPD3
TRIM37	PTPN6	NCL	TLE4		SPDEF
RPS6KB1	ST8SIA1	HDAC1	ENG		CDKN1A
DDX5	OPRM1	TRIM62	ACTA2		SOX9
DLX4	SOD2	MEF2D	DKK1		FOXA2
HDAC5	CCR6	MAD2L2	NKX3-2		OVOL2
SMURF2	IL4	KDM5B	CRYAB		ID1
MMD	FGF1	PPP1R8	MDK		IL22
FBXW7	BCL6	ONECUT2	KRT18		KRT17
STIM2	CBLB	TBX2	GLI1		ACKR4
CEP164	CCL20	KHDRBS1	BVES		FOXA1
BIRC2	POMC	CKS2	HBEGF		EGLN3
PITPNM1	FN1	GLO1	LOX		EPO
ELK3	STAT1	CAPNS1	SPARC		GDF15
ING4	ID2	PRMT1	HAND1		RAF1
NANOG	CAPZA1	HTN1	PDGFRB		ITGB4
QKI	NR5A2	TRAP1	WNT5A		CRP
ERBIN	PRDX1	ELK1	ACVR1		PEBP4
GOLPH3	PIK3R3	PIN1	LOXL3		BHLHE40
KAT2B	MYB	PAK4	EFEMP1		HOOK1
EPB41L5	ABCG2	TRIM28	WNT6		ELF5
GLS	CCND2	DNMT1	DLX2		KRT7
KDM3A	CD274	PSME3	EPAS1		MDM2
STK11	TNFSF11	LIN28A	PRRX1		SCEL
NRP2	EGR1	EIF5A	ERRFI1		RAC1
CREB1	CLU	EIF2S1	ARHGEF2		ALDOB

MED28	SPOP	CD63	PLA2G4A	TMPRSS4
FOXO3	CCR2	FHOD1	PROX1	MMP7
ESRRB	TNFSF10	ACTL6A	TCF21	MARVELD3
GNA13	CXCR4	TXN	SPP1	ERBB2
PCMT1	GLIPR2	MYC	SATB2	EMP3
PIK3CA	NEUROG3	OLA1	PTPA	TUFT1
AGO2	NR4A1	CDKL2	PGF	S100A8
USP22	NMI	SLC39A6	TGFB3	MUC4
USP9X	NCOA3	BRD4	SMAD9	CXCL14
SOX4	ATXN1	SIRT3	TWIST1	EGFR
LRRFIP1	HS3ST3B1	HDGF	MMP19	LCN2
PLAGL2	IL1B	PRUNE1	RAB22A	ST14
KDM4B	CCR7	SETDB1	SNAI1	MMP3
AJUBA	STAT5A	KCNH1	RUNX2	BAG3
ART1	DYRK2	TP53BP2	EFNB2	WNT3A
ACTN4	ZFP36	PARP1	BMP4	KIT
LATS1	LIF	EML4	BMP2	ETS2
EXOC4	ELL3	CCNA2	SIX1	F11R
PRKAA1	CCL25	SKP2	HIP1	CBR1
KDM6B	LGALS3	G3BP1	KDR	CYB5R1
DCLK1	PPARG	HDAC8	MGAT3	HK2
KRAS	FLOT2	NUBPL	FOXP2	LMNA
VHL	EPHB2	TRIM11	HOXD9	AQP5
NOTCH2	STK26	MMP14	MTUS1	VSNL1
ROCK2	ETS1	IGSF8	STK33	S100A9
SOX5	CTSL	NCSTN	LAMA5	CLDN1
MAP3K7	HAVCR2	PBXIP1	SERPINF1	S100P
GLS2	AMHR2	CIP2A	NES	MST1R
SPRY2	IL6	PRKCI	WASF3	HPGD
SKIL	KLF4	EIF5A2	POSTN	FOXQ1
ARRB1	TLR4	SENP2	KL	SHH
YAP1	TLN1	ANXA5	MICAL2	NFIL3
RDX	CCL21	TERT	NUMB	AQP3
ADAM10	HINT2	CDK5	LOXL2	GJB2
FBXO11	IDH1	CTNNB1	MYCN	CDX2
TET1	SEMA7A	ELSPBP1	FST	NDRG2
CCNG2	CXCL9	GJB1	NREP	MAPK7
PHLDA1	LEF1	PCBP1	ANXA1	KLK6
RB1	EGF	PROKR1	GOLM1	SFTPC
CUL4A	ASCL1	FASN	TBX3	PDCD6IP
IGF1R	ESR2	HSPA4	CD36	LRG1
FOXK2	PSTPIP1	FSHR	TFCP2	KRT19
SMAD4	IRF8	BSG	DAB2IP	HDAC3
AKT1	RORC	ESRRA	MMP13	VANGL1
MCL1	RHOB	FBXO45	GIPC2	BRMS1
IL17RD	PLAC8	ETV4	CYP1B1	HRAS
PIK3R1	MSN	TYMS	FGF2	SCUBE2

RASA1	UHRF2	TUFM	RGS3	SMAD2
KDM6A	CDKN2A	ZNF746	LGR5	P2RY2
MTDH	ADM	SIAH2	FBLN5	FOSL1
ALAD	PAK1	IDH2	NTRK3	GRIN1
PARD3	IL18	TFDP3	FURIN	PAWR
ATM	DAB2	MITF	TGFB1I1	CTNNBIP1
ITGB1	VSIG4	FOXR2	CDH11	THBD
PDCD4	CXCL13	TLE1	CDH13	F2
EIF4E	PADI4	HNRNPAB	GATA6	CXCR2
EPS8	FCN2	L1CAM	TP53	PHLDA2
ADAM17	IL6R	EHMT2	TNFRSF11A	CLDN7
PTPN14	CCR5	CSNK2B	NFIC	ANXA2
MSI2	SQSTM1	MICA	EPHA2	GKN2
NODAL	CXCL16	POU5F1	VASH2	PKP3
ZFYVE9	PDPN	REPIN1	TRPM8	MUC1
RNF111	SLC30A7	CPEB1	VGLL4	WWOX
BRAF	NLRP3	NME1	LRIG1	CYP4Z1
SKI	TNFAIP8L2	RBM8A	COL8A1	CLDN4
SON	PAQR3	UHRF1	NR1I2	AJAP1
CTBP1	IGFBP7		AGTR1	MMP1
POGLUT1	TGFBR2		SETD7	S100A2
RPL22L1	SERPINI1		SFRP2	OCLN
YWHAZ	CXCL5		SCUBE3	S100A6
TSC1	SPZ1		TPBG	CTNND1
BTRC	CSF2		IGFBP3	MUC2
APBB1	MICALL2		GPC3	DDR1
ZNF143	TP53INP1		NTRK2	SERPINB4
SMAD3	ALDH1A1		GSN	TRIM16
AKTIP	FBP1		NOTCH1	TWIST2
GOLGA2	VCP		ZEB1	SPRR2A
CRK	FRAT1		ADAM12	HOXA10
RBFOX3	BRD7		HMGA2	CD24
VWCE	ILK		FOXO1	CFTR
BMI1	GREM1		CRIM1	
SEMA4C	CCNDBP1		EDNRA	
MAP2K1	STIM1		SPOCK1	
IRS1	ANGPTL4		BMP6	
SIN3A	BATF2		PRKCA	
PTK2	STAT3		MARVELD1	
ZEB2	LGALS9		RHOC	
LIMS1	CXCL8		FZD7	
KIF5B	CD14		TIAM1	
PPM1D	JUNB		ERG	
PRKCE	BCL2L1		HOXB13	
BCL2	MLLT3		RUNX1	
KLF17	PTEN		SIM2	
ZNF217	CEBPB		ZYX	

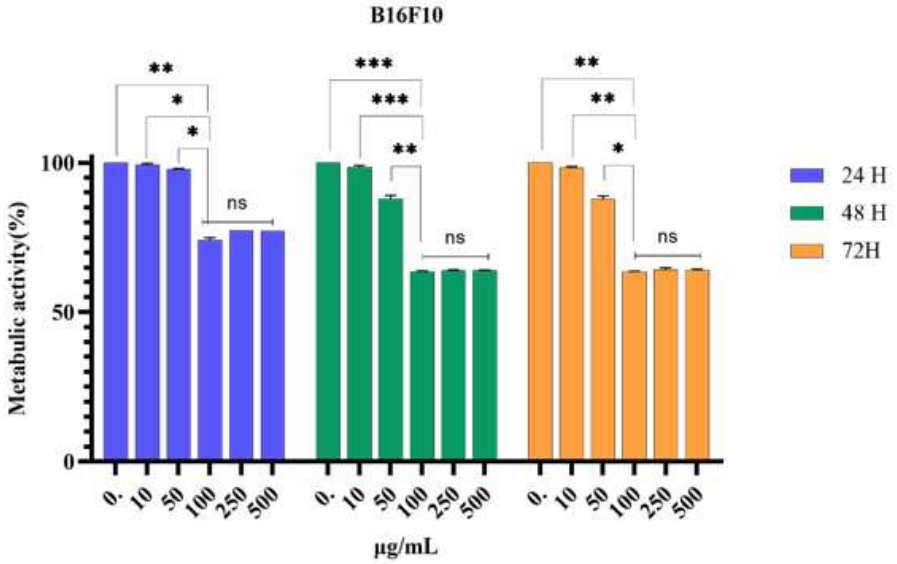
EGFL7	CCL19	SHC1
RELA	RAB43	ITGA5
PEAK1	MYD88	PRKAA2
CSPG4	HPSE	DDR2
NPPA	GLRX	ACTG2
CLK2	CCR9	TGFA
NR2C2	STAT5B	FSTL1
ZBTB33	UCP2	NKX6-1
TBL1XR1	SPHK1	PTX3
IMP3	RHOG	SPRY1
SUZ12	JUN	ITGA2
PTPN11	ERN1	F2RL2
SCRIB	MUC16	TBX20
EXOC7	TNFSF15	SOX17
MTA1	SATB1	CTHRC1
KMT5A	CAMK1D	ANPEP
FOXO4	ASCL2	PBX3
PTP4A3	TBK1	PRRX2
JAG2	MACC1	AXL
HSF1	PTP4A2	TNXB
IRS2	SOCS3	AXIN2
FSCN2	SP1	ROR2
TEAD1	ISG15	AR
TET3	PDCD1	AFAP1L2
HMGB1	S100A4	SDC2
SIAH1	SEMA4A	YWHAG
HDAC2	FUT4	HSPB2
NF1	IL27	CMTM8
DAPK1	C5AR1	SEMA3E
ARHGEF12	TGM2	KRT8
FLNA	ANG	CDH2
SRC	TNF	HOXB9
TRIM33	IRGM	HAS2
MDM4	TNFSF12	PDGFD
MTOR	TDGF1	HS6ST2
MAP3K3	CEBPA	ALK
AGER	LYN	COL8A2
RACK1	TXNIP	EIF2AK3
LGR4	CCL5	CERS6
MCRIP1	CCL18	CYP7B1
PEG10	CCL3	LEP
LEFTY1		PCSK1
CDK3		FOXC2
NANOGP8		BDNF
GH1		HIC1
BOP1		CAVIN1
IKBKG		CD151

PCGF2	MAGED1
	PBX3
	PRRX2
	AXL
	TNXB
	AXIN2
	ROR2
	AR
	AFAP1L2
	SDC2
	YWHAG
	HSPB2
	CMTM8
	SEMA3E

Supplementary results

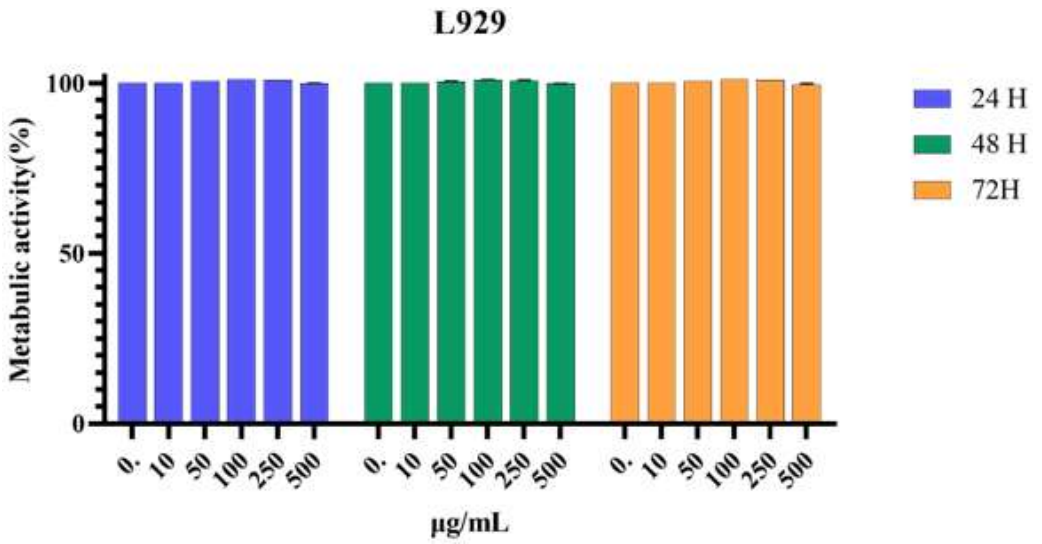
MTT assay and optimization of anti-PD-1 and CAP treatment conditions

To determine the optimal concentration and exposure time of Anti-PD-1, an MTT assay was performed over a range of concentrations and time points in B16F10 melanoma cells. The results demonstrated that the maximal reduction in metabolic activity of B16F10 cells occurred at an Anti-PD-1 concentration of approximately 100 μg/mL. Increasing the concentration beyond this level did not lead to a further decrease in metabolic activity. Time-course analysis indicated that the strongest effect of Anti-PD-1 was observed at 48 hr post-treatment, with no significant difference between 48 and 72 hr (Supplementary Figure 2).



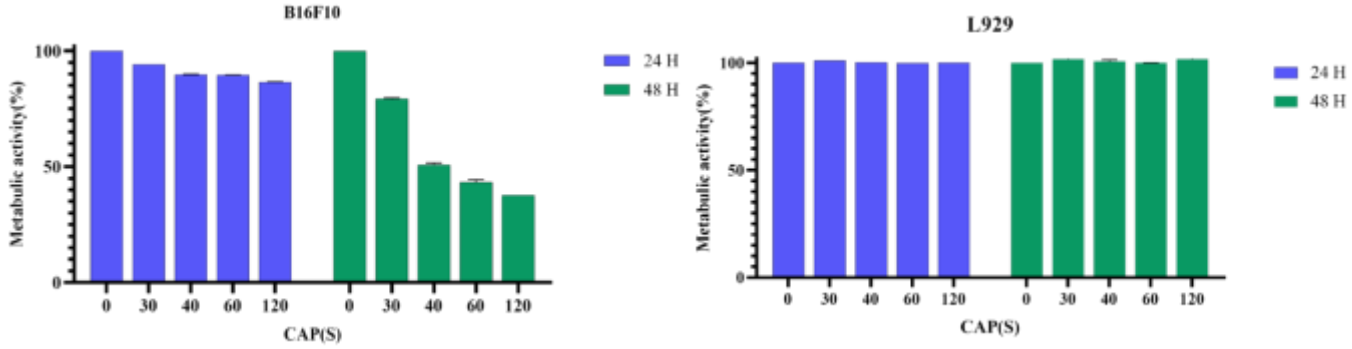
Supplementary Figure 2. MTT assay results for different concentrations and time points of Anti-PD-1 in B16F10 cells

In contrast, treatment of normal L929 fibroblast cells with various concentrations of Anti-PD-1 at different time points did not result in a significant change in metabolic activity (Supplementary Figure 3).



Supplementary Figure 3. MTT assay results for different concentrations and time points of Anti-PD-1 in L929 cells

The effect of cold atmospheric plasma (CAP) on B16F10 cells was evaluated at 48 hr post-treatment. CAP exposure for approximately 40 s in a 96-well plate resulted in an approximately 50% reduction in metabolic activity of B16F10 cells. In contrast, MTT assay results for L929 cells at 24 and 48 hr following different CAP exposure times showed no significant changes in metabolic activity. These results indicate a selective cytotoxic effect of CAP on cancer cells without detectable toxicity to normal cells (Supplementary Figure 4).



Supplementary Figure 4. MTT assay results for CAP treatment in B16F10 and L929 cells