

Supplementary Figure S1. Eight single clones with biallelic mutations in the TLR4 gene were obtained. (A) A total of thirty-two single colonies were picked after BxPC-3 cells were transfected with TLR4 sgRNA1, sgRNA2, or sgRNA3, which have either non-mutation, or mixed mutations, or biallelic mutations. (B) Sanger sequencing results of eight single clones with biallelic mutations. Dash line indicates 3 bp upstream of protospacer adjacent motif (PAM) site. LoF = loss-of-function; del = deletion; ins = insertion.

★ Stop Codon

sgRNA1_c1, c2, and c3

Allele 1: (A) del						Allele 2: (A) ins					
1	MMSASRLAGT	LIPAMAFISC	VRPEAGSPAW	RWFLILLINA	WS*ISTKSPT	1	MMSASRLAGT	LIPAMAFISC	VRPEKLGALR	GCGS*YYLSM	HGAEFQONPR
51	TSFSPQRIWT	*ALIP*GI*A	AIASSVQNC	RCWYFGVKS	RQLKMGHIRA	51	QPFLLNQEPG	FEL*SPFAFR	QL*LLQFPRT	AGAGFIQV*N	PDN*RWGISE
101	*ATSLP*Y*Q	ETPSRV*PWE	PFLDYQVYRS	WNLWROI*HL	*RTSPLODISK	101	KPFPYLYNID	RKHPHEFSPG	SLFWTIKFTF	AGCGGDKSSI	SRELPHWTSQ
151	L*KNMLWLT	LSNLSNYLSI	FLI*PI*STW	TFPAIRFKVF	IAQTGCGFIK	151	NFERT*CGSQ	SYPIFQIT*V	FF*SDQSRA	GPFQQQDSKY	LLHRLAGSTS
201	CPYSISL*TC	P*TL*TLNQ	VHLKKGFI	*L*EILIV*	M**KLVPKVV	201	NAFTQSLFRP	VPEPELYPT	RCI*RN*AS*	ADFKK*F**F	KCNENLYSRS
251	LV*KSIVWFV	ENLEKETWK	SLTNLL*AC	AI*PLKNSD*	HT*TTTSMIL	251	GWFRSPSPGS	GRI*K*RKLG	KV*QICSRG	VQFDH*RIPI	SILRLLR*Y
301	LYLIV*QMF	LHFF*V*LL	KG*KTFLIIS	DGNI*N*LTV	NLDSFPH*NS	301	Y*LI*LFDKC	FFIFPGECDY	*KGRFL*F	RMATFRIS*L	*IWTVSHIET
351	NLSKGLLSLP	TKVGMFLQKL	IYQALSF*IS	VEMA*VSKVA	VLKVLGQPA	351	QISQKAYFHF	QORWECFFRS	*STKP*VSRS	*QKWFQRL	LFSK*FWDNQ
401	*SI*I*ASMV	LLP*VQTSWA	*NN*NIWISS	IPI*NK*VSF	QYSYHSETSF	401	PKVFRSELQW	CYHEFKLLG	LRTTRISGFF	AFQFETNE*V	FSIPITQKPH
451	TLTFLILTPF	LLSMASMAC	PVSKS*KWLA	ILSRKTSFOI	SSQS*ET*PS	451	LP*HFSYSHQ	SCFQWHLQWL	VQSRSLENGW	QFFPGKLPSP	YLHRAEKLDL
501	WTSLSVNWSS	CLQQLHTSP	VERY*I*ATT	TSFHWIRFLI	SV*TPSRFLI	501	PGPLSVSTGA	VVNSI*LT	QSSGTYKFP	QLLFGYVSL	*VSELPPGS*
551	TVSIT*LPK	NRNYSIFQV	*LS*ILLRMT	LLVLNTRVS	CNGSRTRGSS	551	LQSQSHNDFO	KTGTATFSK*	SSFLKSYSE*	LCLYL*TPF	PAMDQGEAA
601	WWKLNENWVQ	HLQISRACLC	*V*ISPVRI	RPSLVCSSV	CL*YLL*QW	601	LQGS*INGMC	NTR*AGHAC	AEFEYHLSDE	*DHHWCVPQ	CACISCCSSS
651	SISSIFT*CF	LLAA*SMVEV	KTSMMLLST	QARMRTG*GM	S**RI*KKGC	651	GL*VLFSPDA	SCWLHKVW*R	*KHL*CLCYL	LKPG*GLGKE	*ASKEFRRRG
701	LHFFSAFTTE	TLFPVWPLP	TSSMKVSIKA	ER*LLWCPST	SSRAAGVSLN	701	ASISALPSLQ	RLYSRCGHCC	QHHP*RFK	PKGDCCGVPA	LHPEPLVYL*
751	MRLLRPGSF*	AVVLVSSSL	CRWRRCPSG	SRWCTAFSA	GTLTWSGRTV	751	I*DCSDLAS	EQSCWYHLC	PAEGGEDPAQ	AAGGAVPSPQ	QEHLPVGGQ
801	SWGTTSSGDD	SEKPCWMVNH	GIQKEQWVDQ	AIGRKQHL		801	CPGAHLLT	TQKSPAGW*I	MESRRNSGYR	MLAGSNITL	

sgRNA3_c3, c4, and c10

Allele 1: (A) ins										Allele 2: (AGCATT) del									
1	MMSASRLAGT	LIPAMAFISC	VRPEWEPVCV	EVVFNITYQC	MELNFYKIPD					1	MMSASRLAGT	LIPAMAFISC	VRPEWEPVCV	EVVFNITYQC	MELNFYKIPD				
51	NLPFSTKNLD	LSFNPLKAFR	QL*LLQFPRT	AGAGFIQV*N	PDN*RWGISE					51	NLPFSTKNLD	LSFNPLAAI	AIASSVQNCRC	WYIPGVKSRQ	LKMGHIRA*A				
101	KPFPYLYNID	RKPHPEFSPG	SLFWTIKFTF	AGCGGDKSSI	SRELPHWTSQ					101	TSPL*Y*QET	PSRV*PWEFF	LDYQVYRSWW	LWROI*HL*R	TSPLDISK*				
151	NFERT*CGSQ	SYPIFQIT*V	FF*SDQSRA	GPFQQQDSKY	LLHRLAGSTS					151	KNLMLWLTIS	LSNLSNYLSIF	I*PI*STWTF	PATRFKRVFIA	QTGCGFIKCP				
201	NAFTQSLFRP	VPEPELYPT	RCI*RN*AS*	ADFKK*F**F	KCNENLYSRS					201	YISL*TCF*TL	TL*TLNQVH	LKKGFI*LS	*EILIV*M*	*KLVPKVVLL				
251	GWFRSPSPGS	GRI*K*RKLG	KV*QICSRG	VQFDH*RIPI	SILRLLR*Y					251	*KSIVMFWN	ENLEKMTKWSL	TNLL*ACAI	*PLKNSD*H	*TTTSMIL				
301	Y*LI*LFDKC	FFIFPGECDY	*KGRFL*F	RMATFRIS*L	*IWTVSHIET					301	LYLIV*QMF	FLP*V*LLKG	*KTLFISLD	N*H*LTVNL	DSFFH*NSNL				
351	QISQKAYFHF	QORWECFFRS	*STKE*VSRS	*QKWFQRL	LFSK*FWDNQ					351	SKGLLSLPTK	VGMLFQKLIY	QALSF*ISVE	MA*VSKVAVL	KVLGQPA*S				
401	PKVFRSELQW	CYHEFKLLG	LRTTRISGFF	AFQETNE*V	FSIPITQKPH					401	I*I*ASVLL	P*VQTSWA*N	*NN*NIWISS	I*KN*VSFOY	SYHSETSFTL				
451	LP*HFSYSHQ	SCFQWHLQWL	VQSRSLENGW	QFFPGKLPSP	YLHRAEKL					451	TLTFLITPELL	SSMASSMACPV	SKS*KWLAIL	SKRTSFOISS	QS*ET*PSWT				
501	PGPLSVSTGA	VVNSI*LT	QSSGTYKFP	QLLFGYVSL	*VSELPPGS*					501	SLSVNWSSCL	QQLHTSPVF	RY*I*ATTIS	FWIRFLISV	*TPSRFLITV				
551	LQSQSHNDFO	KTGTAFSK*	SSFLKSYSE*	LCLYL*TPF	PAMDQGEAA					551	SIT**LPKNR	NYISIFQV*V	S*ILLRMTL	VLNTRVSCN	GSRTGSSWW				
601	LQGS*INGMC	NTR*AGHAC	AEFEYHLSDE	*DHHWCVPQ	CACISCCSSS					601	KLNENWVQHL	QISRACLC*	*ISPVRI	SLVCRSSVCL	*YLL*QWFSI				
651	GL*VLFSPDA	SCWLHKVW*R	*KHL*CLCYL	LKPG*GLGKE	*ASKEFRRRG					651	SISSIFT*CFLL	AA*SMVEVKT	SMMPLLSQAR	RMRTG*GMS*	*RI*KKGCLH				
701	ASISALPSLQ	RLYSRCGHCC	QHHP*RFK	PKGDCCGVPA	LHPEPLVYL*					701	FSSAFTTETL	FLPVWPLP	TSMASSMAC	*LLWCPST	SSRAAGVSLN				
751	I*DCSDLA	EQSCWYHLC	PAEGGEDPAQ	AAGGAVPSPQ	QEHLPVGGQ					751	LLRPGSF*AV	VLVSSSLSCR	RRWRRCPSGR	WSCTAFSAGT	LWTSGRVTSW				
801	CPGAHLLT	TQKSPAGW*I	MESRRNSGYR	MLAGVNSITL						801	GGTSSGDDSE	KPCQWVNHGI	QKEQWQDAI	GRKQHL					

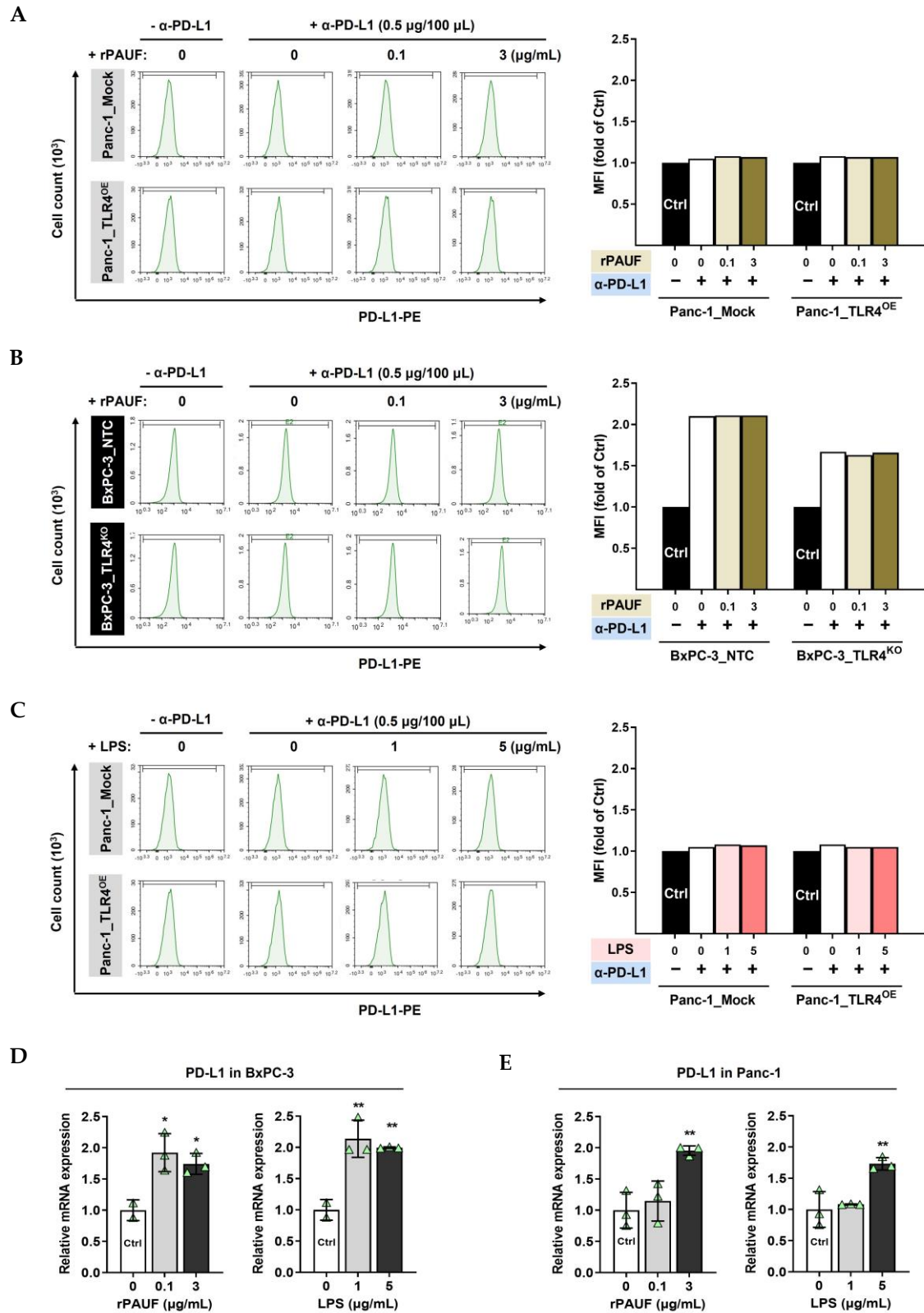
sgRNA3_c6

Allele 1: (AGG) del						Allele 2: (AA) ins + (AGG) del					
1	MMSASRLAGT	LIPAMAFISC	VRPESWPCV	EVVFNITYQC	MELNFYKIPD	1	MMSASRLAGT	LIPAMAFISC	VRPESWPCV	EVVFNITYQC	MELNFYKIPD
51	NLPFSTKNLD	LSFNPLHLGS	YSFSSFPFELQ	VLDSRCEIQ	TIEGAYQSL	51	NLPFSTKNLD	LSFNPL*AAI	AIASSVQNCRC	WYIPGVKSRQ	LKMGHIRA
101	SHLSTLITLG	NPQSLALGA	FSGLSLQKL	VAVETNLASL	ENFPIGHLKT	101	*ATSLP*Y*Q	ETPSRV*PWE	PFLDYQVYRS	WNLWROI*HL	*RTSPLODISK
151	LKELNVAHNL	IQSKFLPEYF	SNLTNLEHLD	LSNKSQISYI	CTDLRVLHQM	151	L*KNLMLWLT	LSNLSNYLSI	FLI*PI*STW	TFPAIRFKVF	IAQTGCGFIK
201	PLNLSLSDLS	LNPMWFIQPG	AFKEIRLHKL	TLRNNFDSLN	VMKTCIQGLA	201	CPYSISL*TC	P*TL*TLNQ	VHLKKGFI	*L*EILIV*	M**KLVPKVV
251	GLEVHRLVGL	EFNRGNLEK	FQSALEGLC	NLTIEEFRIA	YLDYVLDIOI	251	LV*KSIVWFV	ENLEKETWK	SLTNLL*AC	AI*PLKNSD*	HT*TTTSMIL
301	DLFNCLTNVS	SFSLVSYTIE	RVKDFSYNFG	WQHLVLNCK	FGQFFTLKLL	301	LYTLVL*QMF	LHFF*V*LL	KG*KTFLIIS	DGNI*N*LTV	NLDSFPH*NS
351	SKRLTLFTSN	KGNAFSEVD	LPSLELDLS	RNGLSFKGCG	SQSDGTITSL	351	NLSKGLLSLP	TKVGMFLQKL	IYQALSF*IS	VEMA*VSKVA	VLKVLGQPA
401	KYLDLSFNGV	ITMSNFIPLG	EQLEHLDFOH	SNLQMSFES	VFLSLRNLIY	401	*SI*I*ASMV	LLP*VQTSWA	*NN*NIWISS	IPI*PNK*VSF	QYSYHSETSF
451	LDISHTHTRV	AFNGFINGLS	SEVLKMGAN	SQGFENLFDI	FTELRLNFTL	451	TLTFLITLTPF	LLSMASMAC	PVSKS*KWLA	ILSRKTSFOI	SSQS*ET*PS
501	DLSQOGLQEL	SPTAFNSLSS	LQVLNMSHNN	FFSLDTFFPK	CLNLSQVLDY	501	WTSLSVNWSS	CLQQLHSP	VERY*I*ATT	TSFHWIRFLI	SV*TPSRFLI
551	SLNHMTSKK	QELQHFFSSL	AFNLNTONDF	ACTCEHQSLF	QWIKDQRL	551	TVSIT*LPK	NRNYSIFQV	*LS*ILLRMT	LLVLNTRVS	CNGSRTRGSS
601	VEVERMECAT	PSDKQGMVPL	SLNITCOMNK	TIIGVSVLVS	LVVSVAVVLV	601	WWKLNENWVQ	HLQISRACLC	*V*ISPVRI	RPSLVCSSV	CL*YLL*QW
651	YKFYFHLMLL	AGCIKYGRGE	NIYDAFVYS	SQDEDMVRNE	LVNLEEGVVP	651	SISSIFT*CF	LLAA*SMVEV	KTSMMLLST	QARMRTG*GM	S**RI*KKGC
701	FPOLCIHYRD	FIPGVAAIAN	IIHEGFHKS	KVIVVVSQHS	IQSRWCIFEY	701	LHFFSAATTE	TLFPVWPLP	TSSMKVSIKA	ER*LLWCPST	SSRAAGVSLN
751	EIAQTWQFLS	SRAGIITFVL	QVETKILLRQ	QVELYRLLSR	NTYLEWEDSV	751	MRLLRPGSF*	AVVLVSSSL	CRWRRCPSG	SRWCTAFSA	GTLTWSGRTV
801	LGRHIFWRL	RKALLDGKSW	NFEGTVGTGC	NWQEATSI*		801	SWGTTSSGDD	SEKPCWMVNH	GIQKEQWVDQ	AIGRKQHL	

sgRNA3_c9

Allele 1: (A) ins						Allele 2: (AG) del + (A) ins					
1	MMSASRLAGT	LIPAMAFISC	VRPEWEPVCV	EVVFNITYQC	MELNFYKIPD	1	MMSASRLAGT	LIPAMAFISC	VRPEWEPVCV	EVVFNITYQC	MELNFYKIPD
51	NLPFSTKNLD	LSFNPLKA	QL*LLQFPRT	AGAGFIQV*N	PDN*RWGISE	51	NLPFSTKNLD	LSFNPL*AAI	AIASSVQNCRC	WYIPGVKSRQ	LKMGHIRA*A
101	KPFPYLYNID	RKPHPEFSPG	SLFWTIKFTF	AGCGGDKSSI	SRELPHWTSQ	101	*ATSLP*Y*Q	ETPSRV*PWE	PFLDYQVYRS	WNLWROI*HL	*RTSPLODISK
151	NFERT*CGSQ	SYPIFQIT*V	FF*SDQSRA	GPFQQQDSKY	LLHRLAGSTS	151	L*KNLMLWLT	LSNLSNYLSI	FLI*PI*STW	TFPAIRFKVF	IAQTGCGFIK
201	NAFTQSLFRP	VPEPELYPT	RCI*RN*AS*	ADFKK*F**F	KCNENLYSRS	201	CPYSISL*TC	P*TL*TLNQ	VHLKKGFI	*L*EILIV*	M**KLVPKVV
251	GWFRSPSPGS	GRI*K*RKLG	KV*QICSRG	VQFDH*RIPI	SILRLLR*Y	251	LV*KSIVWFV	ENLEMETWK	SLTNLL*AC	AI*PLKNSD*	HT*TTTSMIL
301	Y*LI*LFDKC	FFIFPGECDY	*KGRFL*F	RMATFRIS*L	*IWTVSHIET	301	LYLIV*QMF	LHFF*V*LL	KG*KTFLIIS	DGNI*N*LTV	NLDSFPH*NS
351	QISQKAYFHF	QORWECFFRS	*STKP*VSRS	*QKWFQRL	LFSK*FWDNQ	351	NLSKGLLSLP	TKVGMLFQKL	IYQALSF*IS	VEMA*VSKVA	VLKVLGQPA
401	PKVFRSELQW	CYHEFKLLG	LRTTRISGFF	AFQFETNE*V	FSIPITQKPH	401	*SI*I*ASMV	LLP*VQTSWA	*NN*NIWISS	IPI*NK*VSF	QYSYHSETSF
451	LP*HFSYSHQ	SCFQWHLQWL	VQSRSLENGW	QFFPGKLPSP	YLHRAEKL	451	TLTFLITLTPF	LLSMASMAC	PVSKS*KWLA	ILSRKTSFOI	SSQS*ET*PS
501	PGPLSVSTGA	VVNSI*LT	QSSGTYKFP	QLLFGYVSL	*VSELPPGS*	501	WTSLSVNWSS	CLQQLTHSP	VERY*I*ATT	TSFHWIRFLI	SV*TPSRFLI
551	LQSQSHNDFO	KTGTAFSK*	SSFLKSYSE*	LCLYL*TPF	PAMDQGEAA	551	TVSIT*LPK	NRNYSIFQV	*LS*ILLRMT	LLVLNTRVS	CNGSRTRGSS
601	LQGS*INGMC	NTR*AGHAC	AEFEYHLSDE	*DHHWCVPQ	CACISCCSSS	601	WWKLNENWVQ	HLQISRACLC	*V*ISPVRI	RPSLVCSSV	CL*YLL*QW
651	GL*VLFSPDA	SCWLHKVW*R	*KHL*CLCYL	LKPG*GLGKE	*ASKEFRRRG	651	SISSIFT*CF	LLAA*SMVEV	KTSMMLLST	QARMRTG*GM	S**RI*KKGC
701	ASISALPSLQ	RLYSRCGHCC	QHHP*RFK	PKGDCCGVPA	LHPEPLVYL*	701	LHFFSAFTTE	TLFPVWPLP	TSSMKVSIKA	ER*LLWCPST	SSRAAGVSLN
751	I*DCSDLA	EQSCWYHLC	PAEGGEDPAQ	AAGGAVPSPQ	QEHLPVGGQ	751	MRLLRPGSF*	AVVLVSSSL	CRWRRCPSG	SRWCTAFSA	GTLTWSGRTV
801	CPGAHLLT	TQKSPAGW*I	MESRRNSGYR	MLAGSNITL		801	SWGTTSSGDD	SEKPCWMVNH	GIQKEQWVDQ	AIGRKQHL	

Supplementary Figure S2. Prediction of amino acid sequences of the eight single clones with biallelic mutations in the TLR4 gene using Vector NTI software. Highlighted parts show stop codon (denoted by asterisk *) caused by the mutations. In sgRNA3_c6, the AGG deletion in allele 1 resulted in one amino acid deletion of TLR4 rather than premature translation termination. del = deletion; ins = insertion.



Supplementary Figure S3. rPAUF does not impact cell surface PD-L1 expression, but increases PD-L1 expression in cytoplasm, of pancreatic cancer cells. And PD-L1 expression in cells with higher basal surface PD-L1 expression (BxPC-3>>Panc-1), is more sensitive to TLR4 activity change. Flow cytometry showed cell surface PD-L1 expression in (A) Panc-1_Mock and Panc-1_TLR4OE cells, and in (B) BxPC-3_NTC and BxPC-3_TLR4KO cells, after the cells were treated by 0, 0.1, or 3 μ g/mL of rPAUF for 24 hr, and in (C) Panc-1_Mock and Panc-1_TLR4OE cells after cells were treated by 0, 1, or 5 μ g/mL of LPS for 24 hr. mRNA expression of PD-L1 in (D) BxPC-3 and (E) Panc-1 cells, after the cells were treated by rPAUF (0, 0.1, or 3 μ g/mL) or LPS (0, 1, or 5 μ g/mL) for 6 hr.