Supplementary Information

Wang et al.

The Aryl hydrocarbon receptor mediates tobacco-induced PD-L1 expression and is associated with response to immunotherapy

Supplementary Table 1. The 422 genes sequenced in tumor samples harvested before the patients received pembrolizumab.

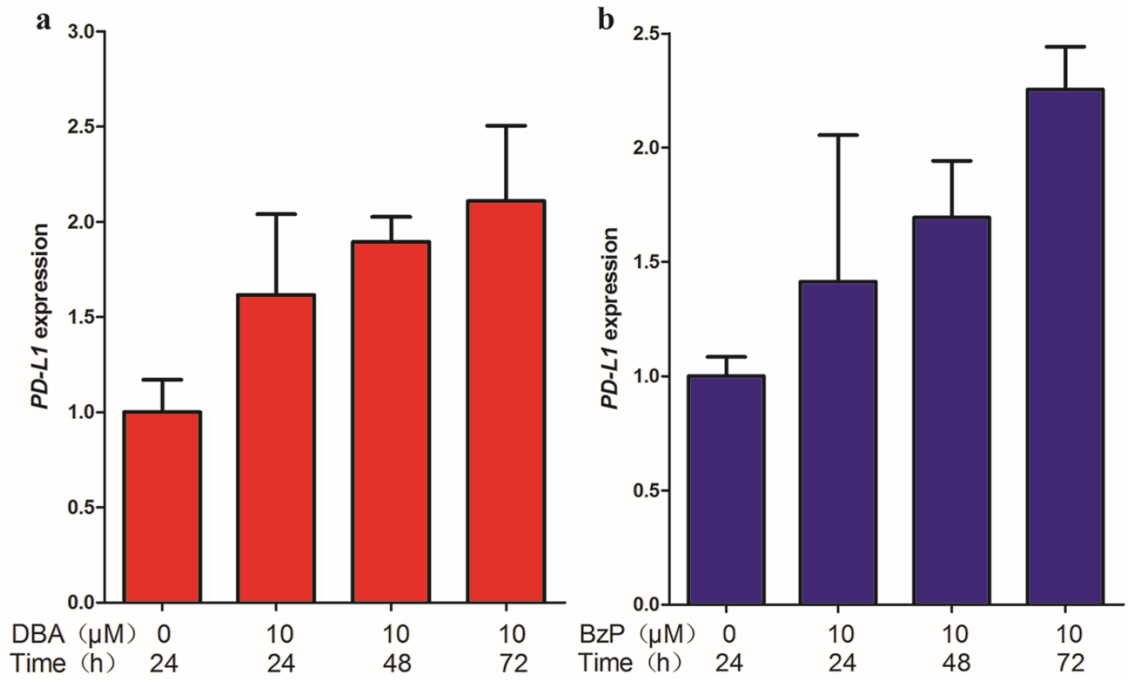
ABCB1(MDR1)	*CDK10*	*ERCC1*	*IDH2*	*MYC*	*PRKAR1A*	*SPOP*
ABCB4	*CDK12*	*ERCC2*	*IGF1R*	*MYCL*	*PRKCI*	*SPRY4*
ABCC2(MRP2)	*CDK4*	*ERCC3*	*IGF2*	*MYCN*	*PRKDC*	*SRC*
ADH1A	*CDK6*	*ERCC4*	*IKBKE*	*MYD88*	*PRSS1*	*SRY*
ADH1B	*CDK8*	*ERCC5*	*IKZF1*	*MYH9*	*PRSS3*	*STAG2*
ADH1C	*CDKN1A*	*ESR1*	*IL7R*	*NAT1*	*PTCH1*	*STAT3*
AIP	*CDKN1B*	*ETV1*	*INPP4B*	*NAT2*	*PTEN*	*STK11*
AKT1	*CDKN1C*	*ETV4*	*IRF2*	*NBN*	*PTK2*	*STMN1*
AKT2	*CDKN2A*	*EWSR1*	*JAK1*	*NCOR1*	*PTPN11*	*STT3A*
AKT3	*CDKN2B*	*EXT1*	*JAK2*	*NF1*	*PTPN13*	*SUFU*
ALDH2	*CDKN2C*	*EXT2*	*JAK3*	*NF2*	*PTPRD*	*TEK*
ALK	*CEBPA*	*EZH2*	*JARID2*	*NFE2L2*	*QKI*	*TEKT4*
AMER1	*CEBPB*	*FANCA*	*JUN*	*NFKBIA*	*RAC1*	*TERC*
APC	*CEBPD*	*FANCC*	*KDM5A*	*NKX2-1*	*RAC3*	*TERT*
AR	*CEP57*	*FANCD2*	*KDM6A*	*NKX2-2*	*RAD50*	*TET2*
ARAF	*CHD4*	*FANCE*	*KDR(VEGFR2)*	*NKX2-4*	*RAD51*	*TGFBR2*
ARID1A	*CHEK1*	*FANCF*	*KEAP1*	*NOTCH1*	*RAD51C*	*THADA*
ARID1B	*CHEK2*	*FANCG*	*KIF1B*	*NOTCH2*	*RAD51D*	*TMEM127*
ARID2	*CLEC2D*	*FANCL*	*KIF5B*	*NOTCH3*	*RAF1*	*TMPRSS2*
ARID5B	*CREBBP*	*FANCM*	*KIT*	*NPM1*	*RARA*	*TNFAIP3*
ASCL4	*CRKL*	*FAT1*	*KITLG*	*NQO1*	*RARG*	*TNFRSF11A*
ASXL1	*CSF1R*	*FBXW7*	*KLLN*	*NRAS*	*RASGEF1A*	*TNFRSF14*
ATF1	*CTCF*	*FGF19*	*KMT2A(MLL)*	*NRG1*	*RB1*	*TNFRSF19*
ATIC	*CTLA4*	*FGFR1*	*KMT2B*	*NSD1*	*RECQL4*	*TNFSF11*
ATM	*CTNNB1*	*FGFR2*	*KMT2C*	*NTRK1*	*RELN*	*TOP1*
ATR	*CUL3*	*FGFR3*	*KMT2D(MLL2)*	*NTRK3*	*RET*	*TOP2A*
ATRX	*CUX1*	*FGFR4*	*KRAS*	*PAK3*	*RHOA*	*TP53*
AURKA	*CXCR4*	*FH*	*LHCGR*	*PALB2*	*RICTOR*	*TP63*
AURKB	*CYLD*	*FLCN*	*LMO1*	*PALLD*	*RNF43*	*TPMT*
AXIN2	*CYP19A1*	*FLT1(VEGFR1)*	*LRP1B*	*PARK2*	*ROS1*	*TSC1*
AXL	*CYP2A13*	*FLT3*	*LYN*	*PARP1*	*RPTOR*	*TSC2*
BAI3	*CYP2A6*	*FLT4*	*LZTR1*	*PARP2*	*RRM1*	*TSHR*
BAK1	*CYP2A7*	*FOXA1*	*MAP2K1(MEK1)*	*PAX5*	*RUNX1*	*TTF1*
BAP1	*CYP2B6*6*	*FOXP1*	*MAP2K2(MEK2)*	*PBRM1*	*RUNX1T1*	*TUBB*

BARD1	*CYP2C19*2*	*FRG1*	*MAP2K4*	*PDCD1(PD1)*	*RUNX3*	*TUBB2A*
BCL	*CYP2C9*3*	*GATA1*	*MAP3K1*	*PDCD1LG2(PD-L2)*	*SBDS*	*TUBB2B*
BCL2L11(BIM)	*CYP2D6*	*GATA2*	*MAP3K4*	*PDE11A*	*SDC4*	*TUBB3*
BCR	*CYP3A4*4*	*GATA3*	*MAP4K3*	*PDGFRA*	*SDHA*	*TUBB4A*
BIRC3	*CYP3A5*	*GATA4*	*MAX*	*PDGFRB*	*SDHB*	*TUBB4B*
BLM	*DAXX*	*GATA6*	*MCL1*	*PDK1*	*SDHC*	*TUBB6*
BMPR1A	*DDR2*	*GNA11*	*MDM2*	*PGR*	*SDHD*	*TYMS*
BRA	*DENND1A*	*GNA15*	*MDM4*	*PHOX2B*	*SEPT9*	*U2AF1*
BRCA1	*DHFR*	*GNAQ*	*MECOM*	*PIK3C3*	*SETBP1*	*UGT1A1*
BRCA2	*DHFRL1*	*GNAS*	*MED12*	*PIK3CA*	*SETD2*	*VEGFA*
BRD4	*DICER1*	*GRIN2A*	*MEF2B*	*PIK3R1*	*SF3B1*	*VHL*
BRIP1	*DNMT3A*	*GRM3*	*MEN1*	*PIK3R2*	*SGK1*	*WAS*
BTG2	*DPYD*	*GRM8*	*MET*	*PKHD1*	*SLC34A2*	*WISP3*
BT	*DUSP2*	*GSTM1*	*MGMT*	*PLAG1*	*SLC7A8*	*WRN*
BUB1B	*EGFR*	*GSTM4*	*MIT*	*PLK1*	*SMAD2*	*WT1*
c11orf30	*EML4*	*GSTM5*	*MLH1*	*PMS1*	*SMAD3*	*XPA*
CASP8	*EP300*	*GSTP1*	*MLH3*	*PMS2*	*SMAD4*	*XPC*
CB	*EPAS1*	*GSTT1*	*MLLT1*	*POLD1*	*SMAD7*	*XRCC1*
CBL	*EPCAM*	*HDAC2*	*MLLT3*	*POLD3*	*SMARCA*	*YAP1*
CC2D2B	*EPHA2*	*HDAC9*	*MLLT4*	*POLE*	*SMARCB*	*ZNF2*
CCND1	*EPHA3*	*HG*	*MPL*	*POLH*	*SMO*	*ZNF217*
CCNE1	*EPHA5*	*HLA-A*	*MRE11A*	*POT1*	*SOS1*	*ZNF703*
CD274(PD-L1)	*EPHB2*	*HNF1A*	*MSH2*	*PPP2R1A*	*SOX1*	
CD74	*ERBB2(HER2*	*HNF1B*	*MSH6*	*PRDM1*	*SOX14*	
CDA	*ERBB2IP*	*HRAS*	*MTHFR*	*PRF1*	*SOX2*	
CDC73	*ERBB3*	*HSD3B1*	*MTOR*	*PRKACA*	*SOX21*	
CDH1	*ERBB4*	*IDH1*	*MUTYH*	*PRKACG*	*SOX3*	

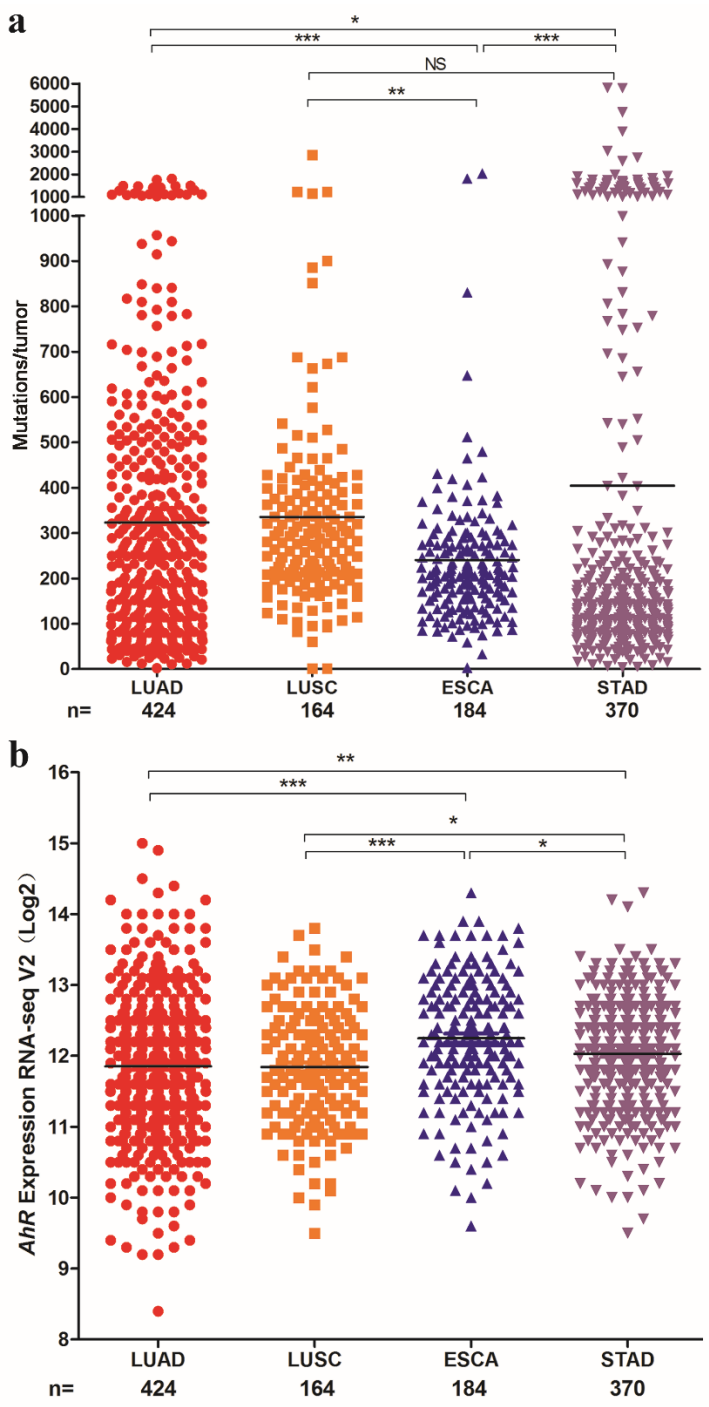
Supplementary Table 2. Primers used in the study.

Target	**Forward primer (5'→3')**	**Reverse primer (5'→3')**
HUMAN		
GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC
Pd-l1	AGTGGTAAGACCACCACCACCAAT	TCATTTGGAGGATGTGCCAGAGGT
MOUSE		
GAPDH	AGTATGACTCCACTCACGGCAA	TCTCGCTCCTGGAAGATGGT
Pd-l1	GCTCCAAAGGACTTGTACGTG	TGATCTGAAGGGCAGCATTTC
TNFa	CCTCCACTTGGTGGTTTGCTA	CAGACCCTCACACTCAGATCATCT
IFNγ	TCAAGTGGCATAGATGTGGAAGAA	TGGCTCTGCAGGATTTTCATG
ChIP		
Pd-l1 (region 1)	CTGAAAGCTTCCGCCGATT	CTACCTGCAGGCGGACAGA
Pd-l1 (region 2)	CCCATTCACTAACCCAAAGCT	AAAAGAACTTCCCATCCCGA
siRNA	**Sequence 1 (5'→3')**	**Sequence 2 (5'→3')**
siAhR	GGAUUAAAUUAGUUUGUGAdTdT	UCACAAACUAAUUUAAUCCdAdA

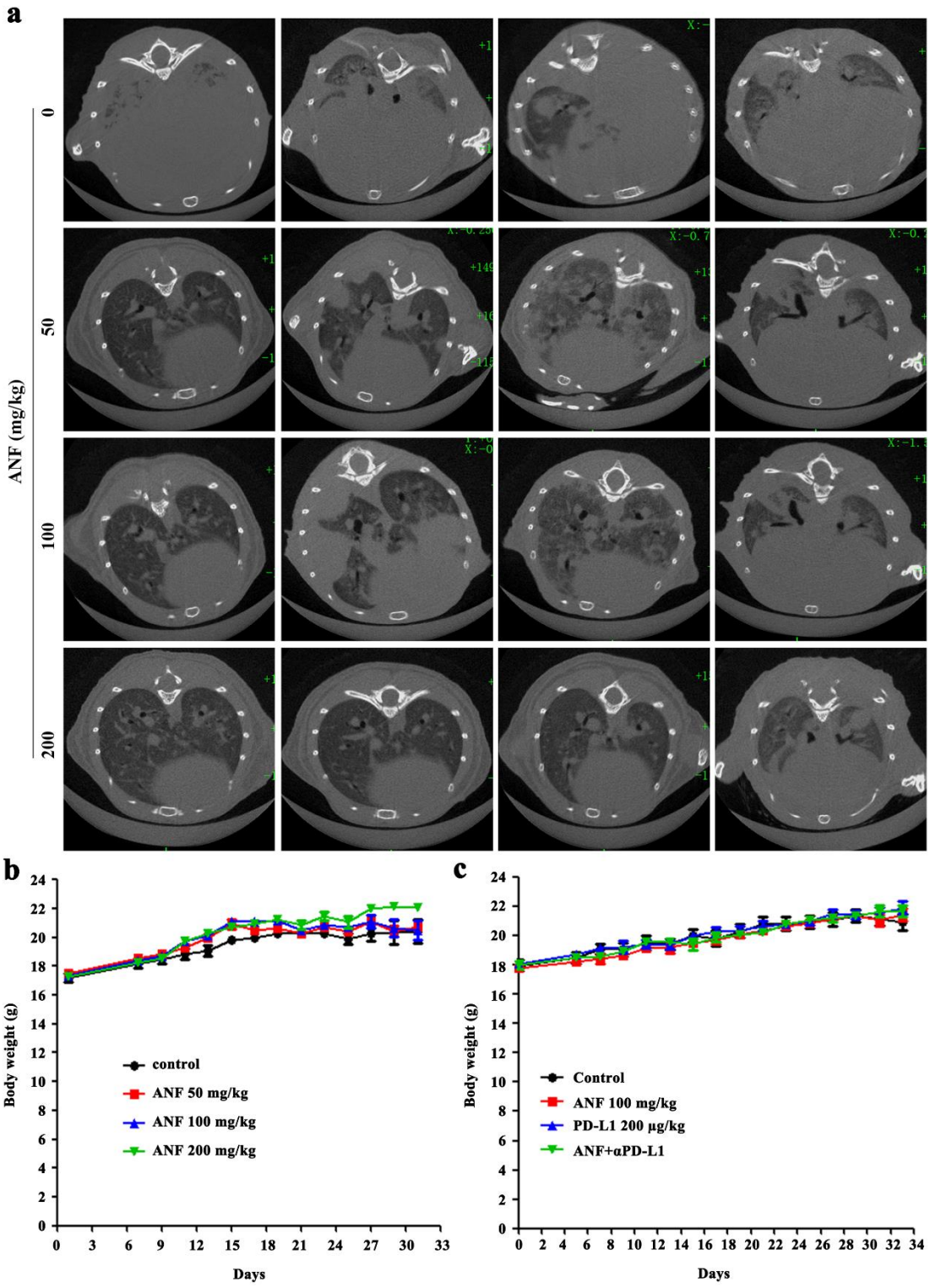
Supplementary figures and legends



Supplementary Figure 1. The effects of DBA (a) and BzP (b) on the expression *PD-L1* in 16HBE cells.

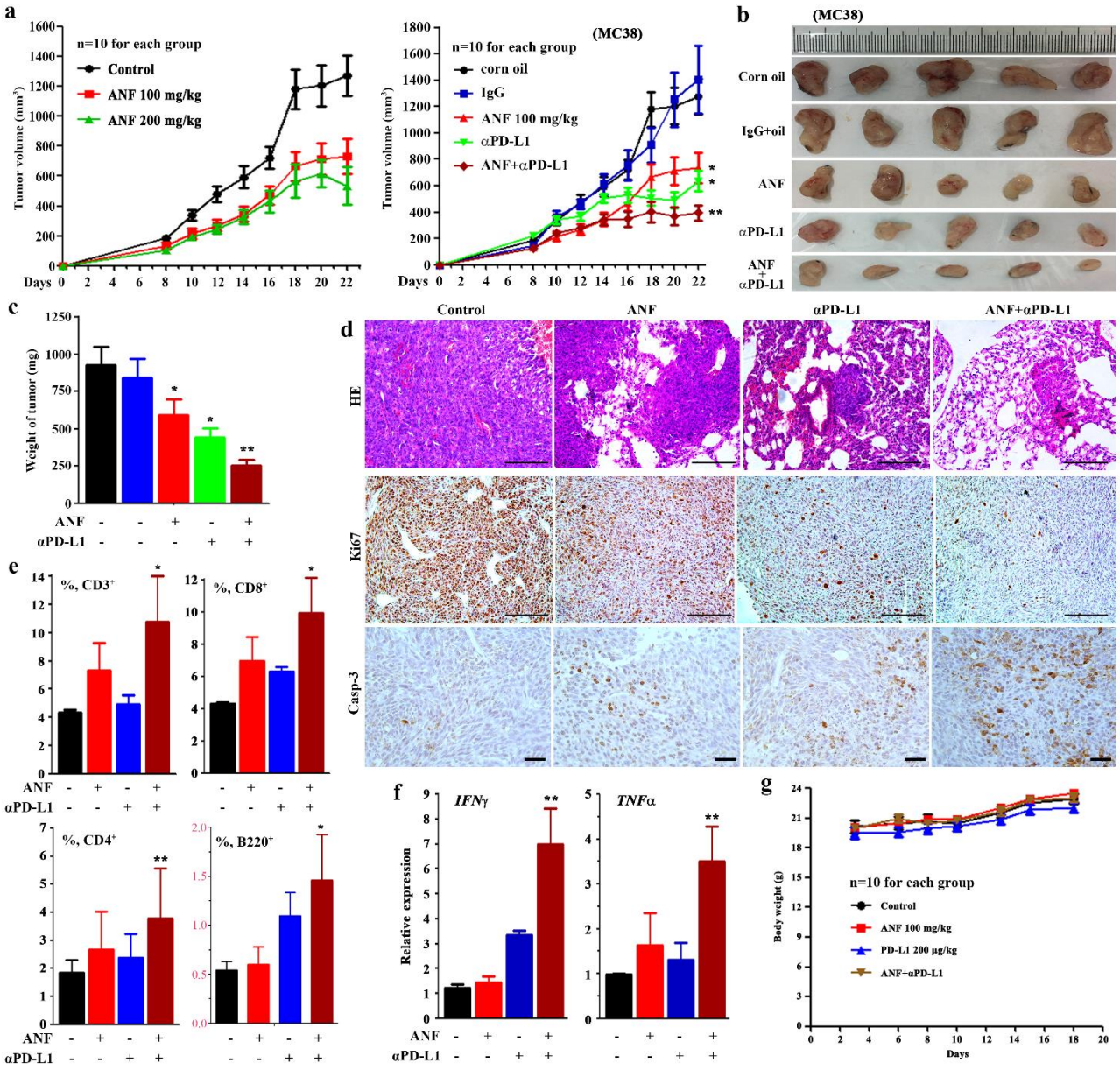


Supplementary Figure 2. AhR expression levels are not associated with mutation loads of the patients. Data are from TCGA datasets. (a) Mutation loads of patients with LUAD, LUSC, esophageal carcinoma (ESCA), and stomach adenocarcinoma (STAD). (b) *AhR* expression levels of the patients.



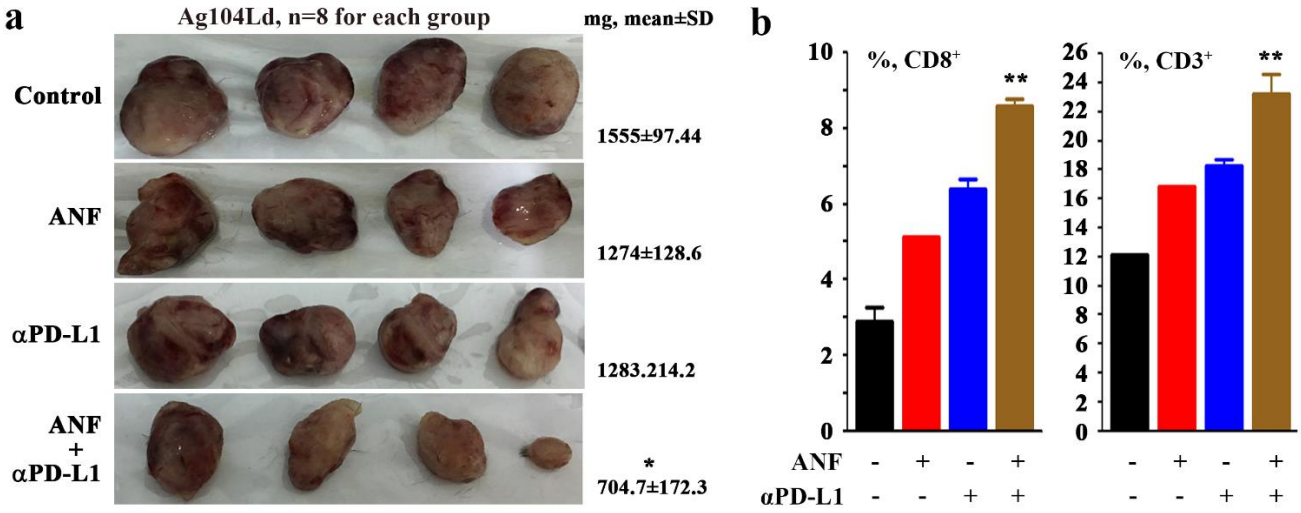
Supplementary Figure 3. AhR inhibitor exhibits anti-lung cancer activity and low toxicity in C57BL/6. mice. (a) The C57BL/6 mice were intravenously injected with LLC cells, and 3 days later randomized to receive vehicle or ANF treatment. Micro-CT scanning images of the mice are shown. (b) Treatment of ANF at 50 to 200 mg/kg did not affect the body weight of

C57BL/6 mice. (c) Treatment of ANF and/or anti-PD-L1 antibody did not significantly affect the body weight of the mice.

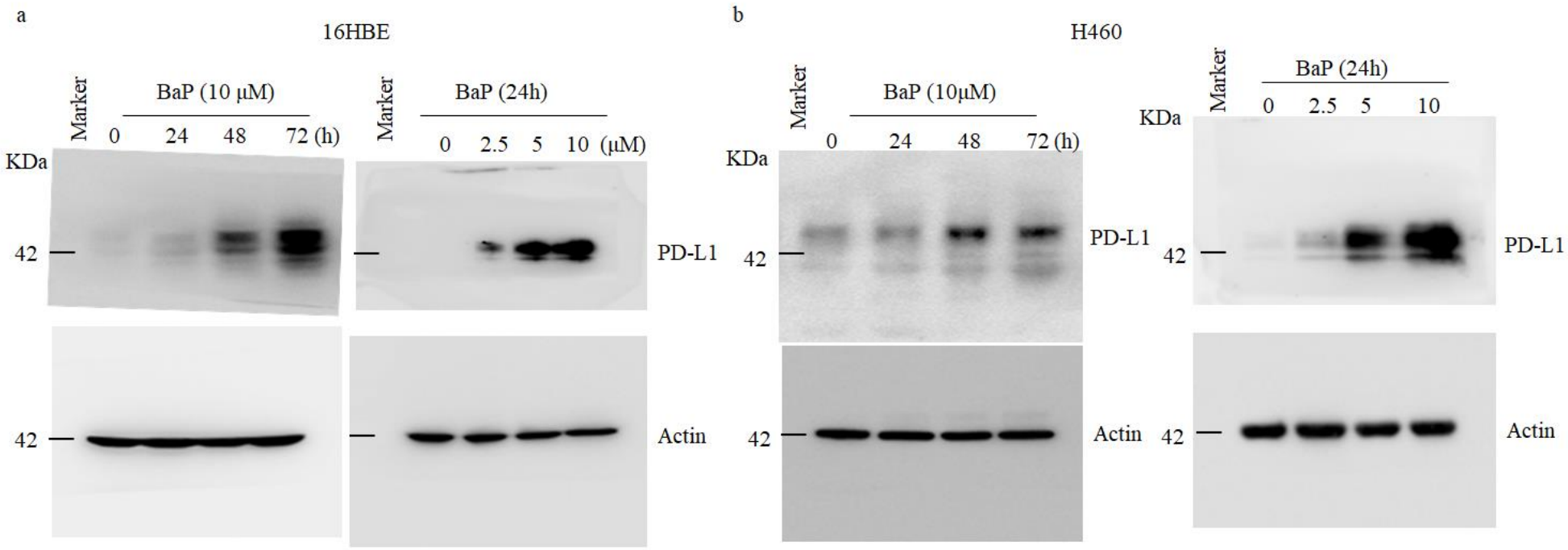


Supplementary Figure 4. Effects of AhR inhibitor on colon cancer murine model. (a) C57BL/6 mice were subcutaneously inoculated with 5×10^5 MC38 cells, and then treated with ANF and/or anti-PD-L1 antibody. Tumor volume was estimated every two days. (b) Images of xenograft tumors obtained from the mice. (c) Weight of xenograft tumors obtained from the mice. (d) HE staining and IHC analyses of lung sections of the mice. (e) Flow cytometry

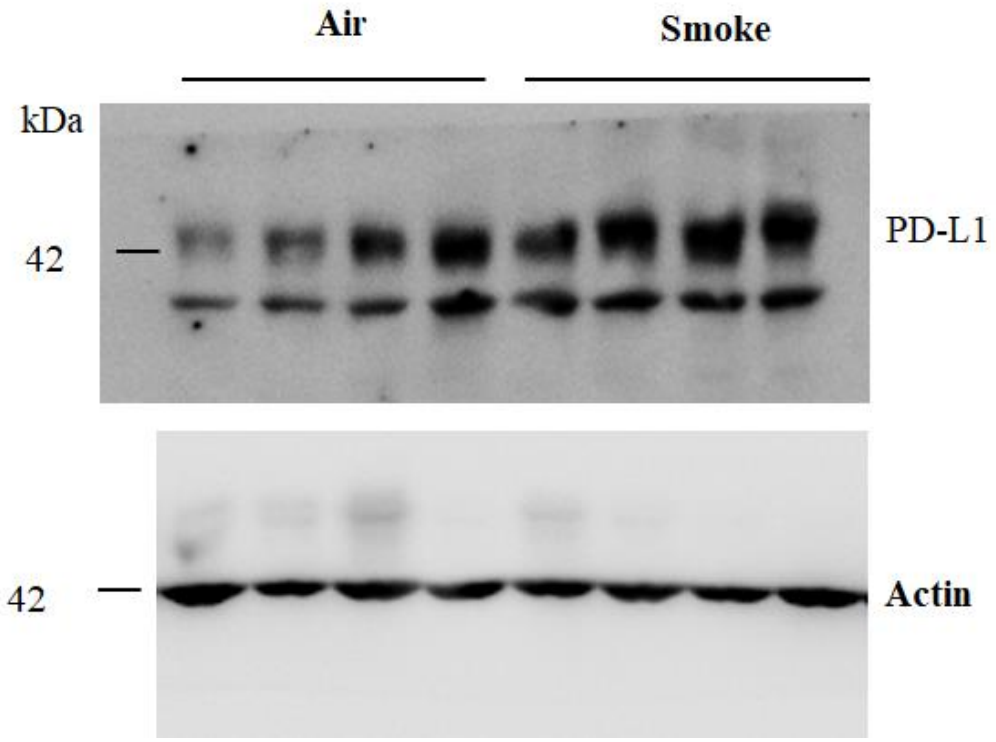
analysis of $CD45^+CD3^+$, $CD8^+$, $CD4^+$, and $B220^+$ cells in the lung tissues. (f) The expression of *IFNγ* and *TNFα* in the lung tissues was detected by real-time PCR. (g) Body weight of C57BL/6 mice treated with ANF and/or anti-PD-L1 antibody. Error bars, sd.



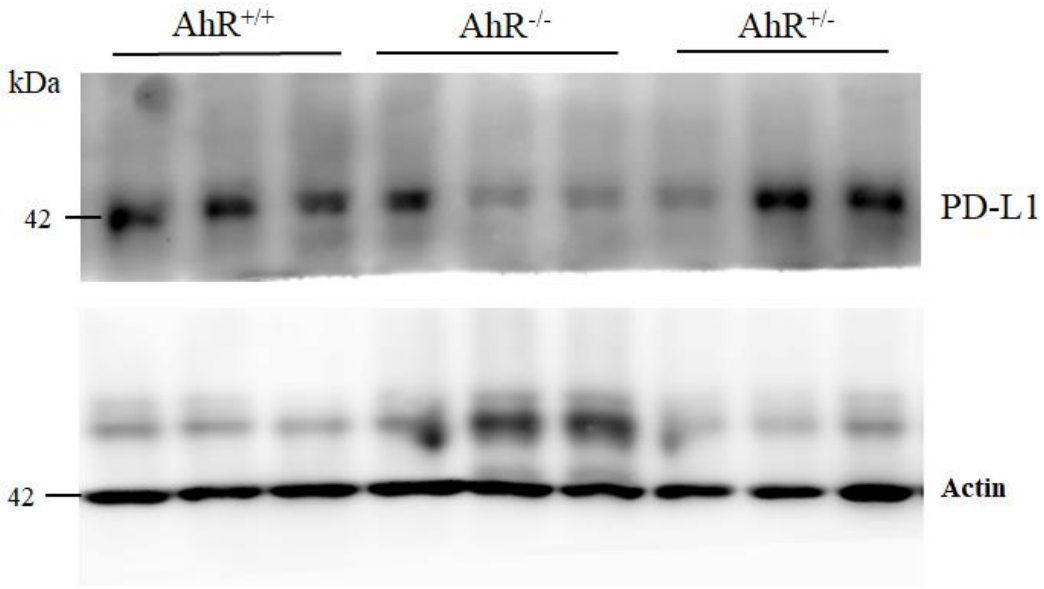
Supplementary Figure 5. The therapeutic efficacy of AhR inhibition in fibrosarcoma murine model. (a) B6C3F1 mice were subcutaneously injected with Ag104Ld (5×10^5) cells and treated with ANF and/or an anti-PD-L1 antibody. Images showed xenograft tumors obtained from the mice. (b) Flow cytometry analysis of $CD8^+$ and $CD3^+$ cells in the lung tissues. Error bars, sd.



Supplementary Fig. 6. Uncropped and unprocessed scans of Fig. 1h.



Supplementary Fig. 7. Uncropped and unprocessed scans of Fig. 2e.



Supplementary Fig. 8. Uncropped and unprocessed scans of Fig. 3l.