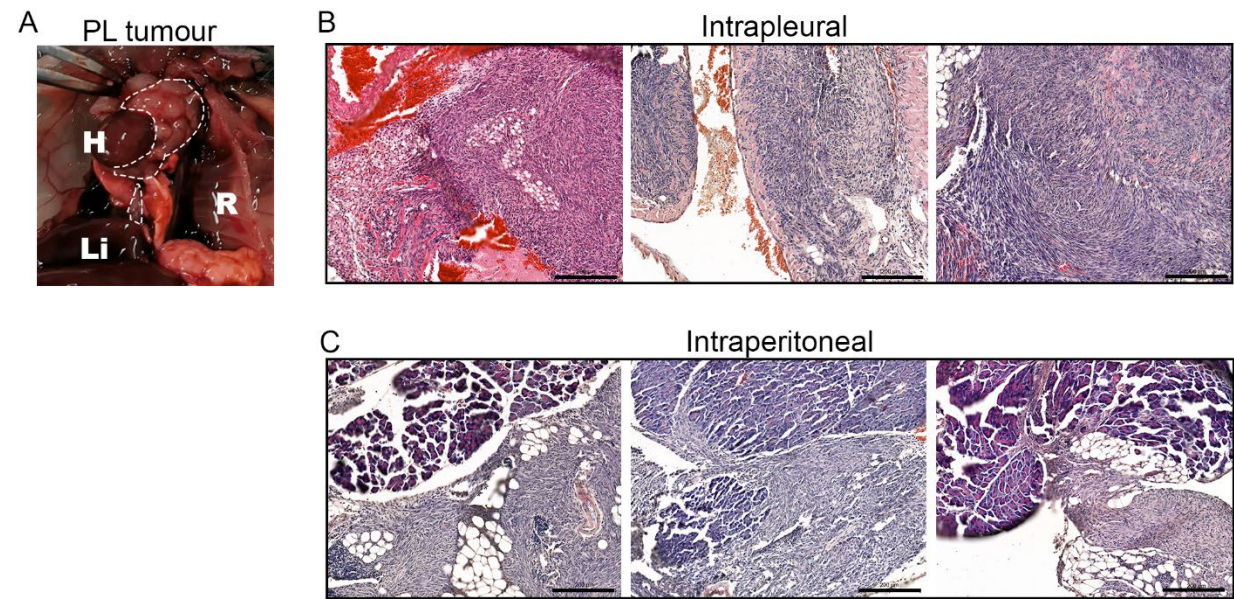
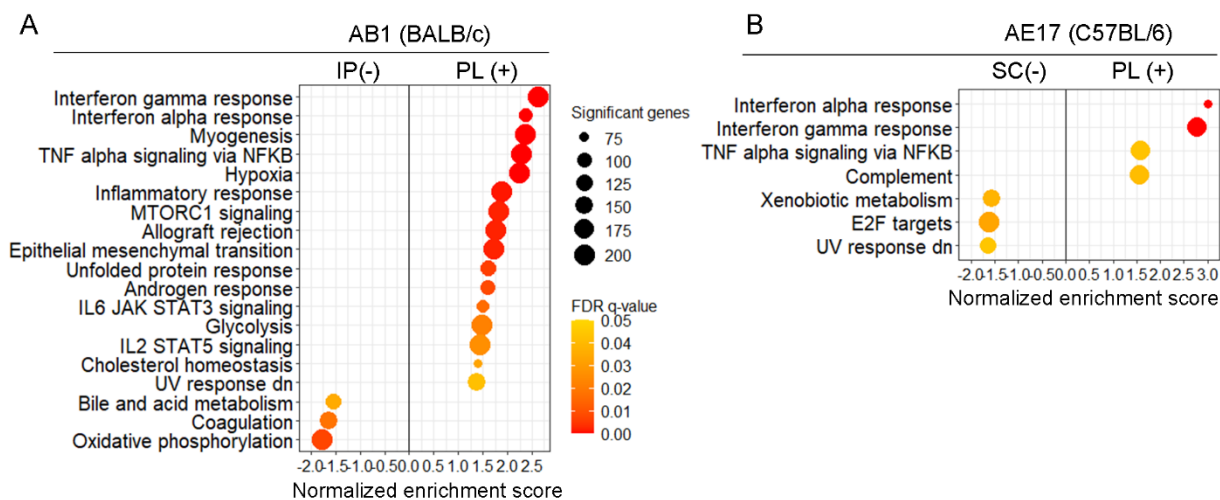


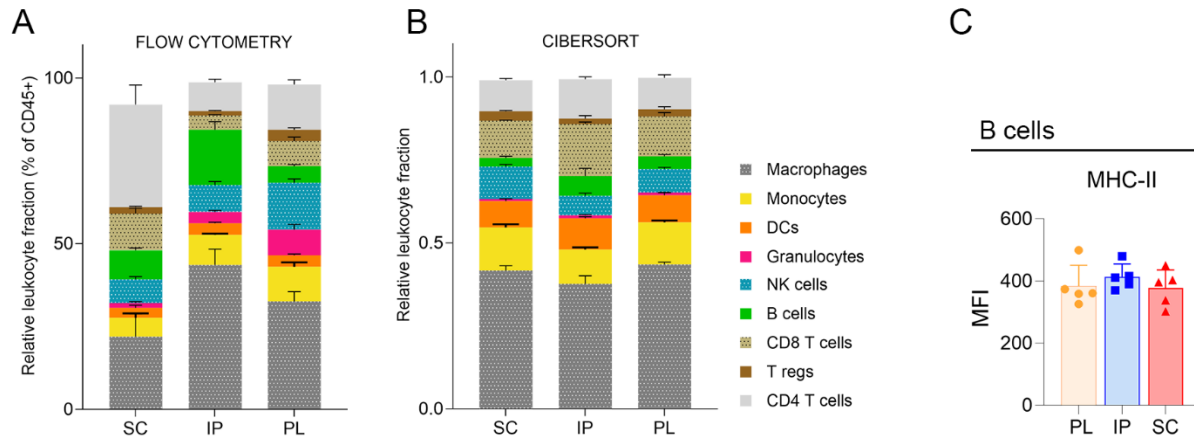
SUPPLEMENTARY DATA



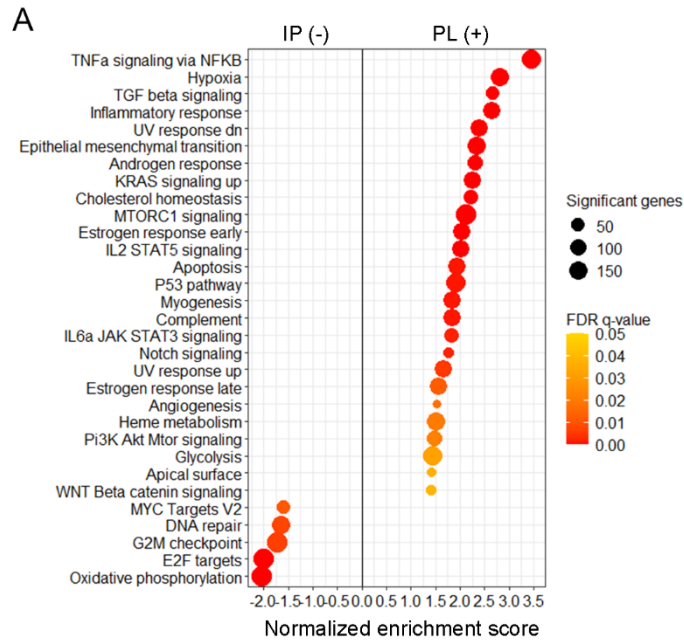
Supplementary Figure S1 (Related to Figure 1). Orthotopic intrapleural and intraperitoneal models mimic the human disease (A) Macroscopic image of AE17 mesothelioma PL tumour in the thoracic cavity. While dotted line indicates tumour location. White letters indicate: H; heart, R; rib cage, and Li; liver. (B and C) Hematoxylin and eosin staining for (B) PL, and (I) IP AE17 tumours in C57BL/6 mice. Scale bar = 200 µm (20x).



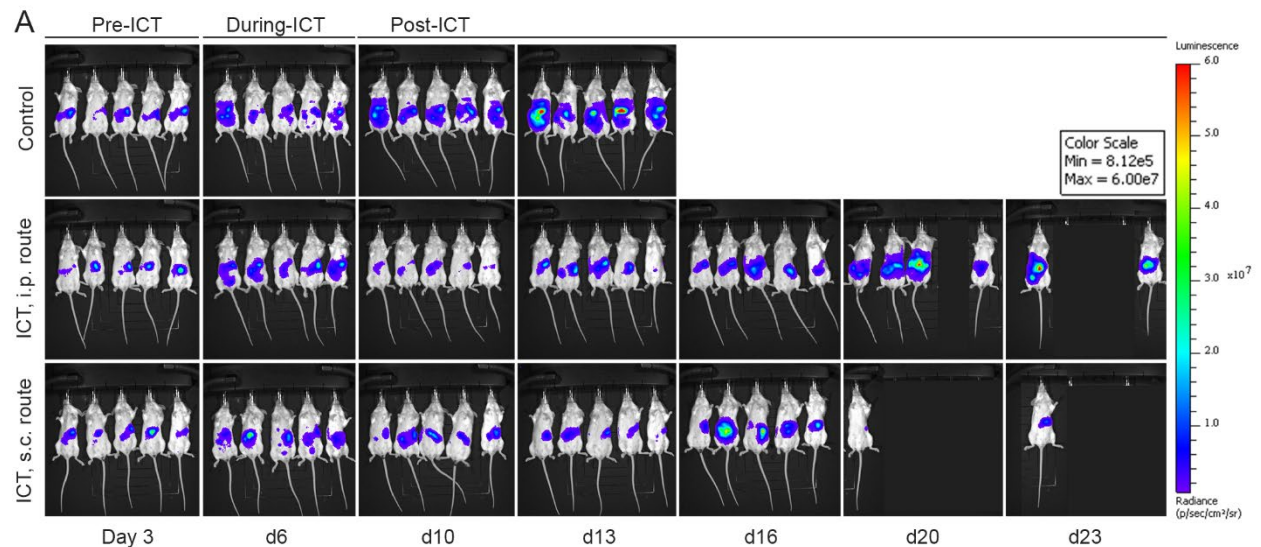
Supplementary Figure S2 (Related to Figure 2). GSEA comparison for AB1 PL tumours versus IP, and AE17 PL tumours versus SC (A and B) Significant GSEA hallmark gene sets in (A) PL (+NES) versus IP (-NES) in AB1 tumours, (n = 6) PL tumours, (n = 5) IP tumours, and (B) PL (+NES) and SC (-NES) in AE17 tumours, (n = 8) PL tumours, (n = 8) SC tumours. Filtered by adjusted p value < 0.05 and FDR q-value < 0.05. FDR q-value is represented in colour (yellow to red). Number of significant genes per pathway is represented in dot size.



Supplementary Figure S3 (Related to Figure 3). Flow cytometry and CIBERSORT comparison of collapsed immune cell populations from the three different models (A) Flow cytometry of dissociated tumours from PL, IP, and SC, n = 5 tumours. Collapsed cell populations in relative leukocyte fraction (% of CD45⁺). (B) CIBERSORT analysis from PL (n = 6), IP (n = 5), and SC (n = 5) tumours. Collapsed cell populations in relative leukocyte fraction. (C) MFI of MHC-II in B cells. Data are depicted as means ± SD values. Two-way ANOVA with Tukey's multiple comparison test.

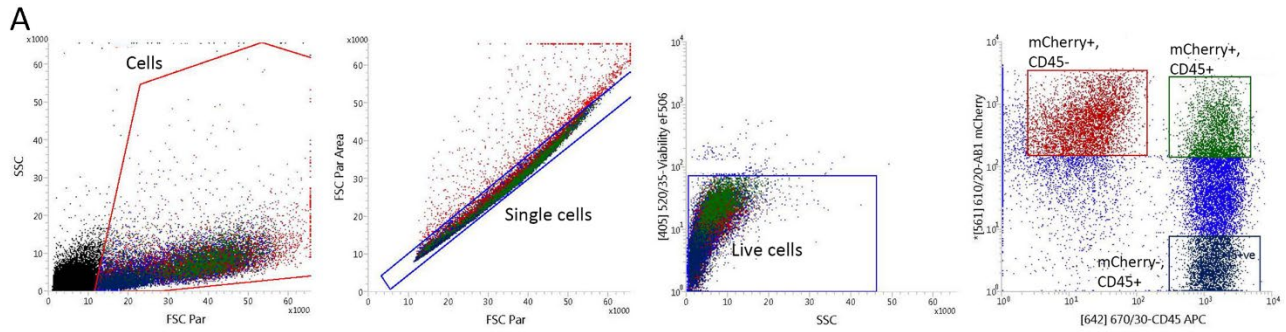


Supplementary Figure S4 (Related to Figure 4). GSEA comparison for AE17 PL tumours versus IP (A) Significant GSEA hallmark gene sets in PL (+NES) versus IP (-NES) in tumours, (n = 6) PL tumours, (n = 5) IP tumours. Filtered by adjusted p value < 0.05 and FDR q-value < 0.05. FDR q-value is represented in colour (yellow to red). Number of significant genes per pathway is represented in dot size.



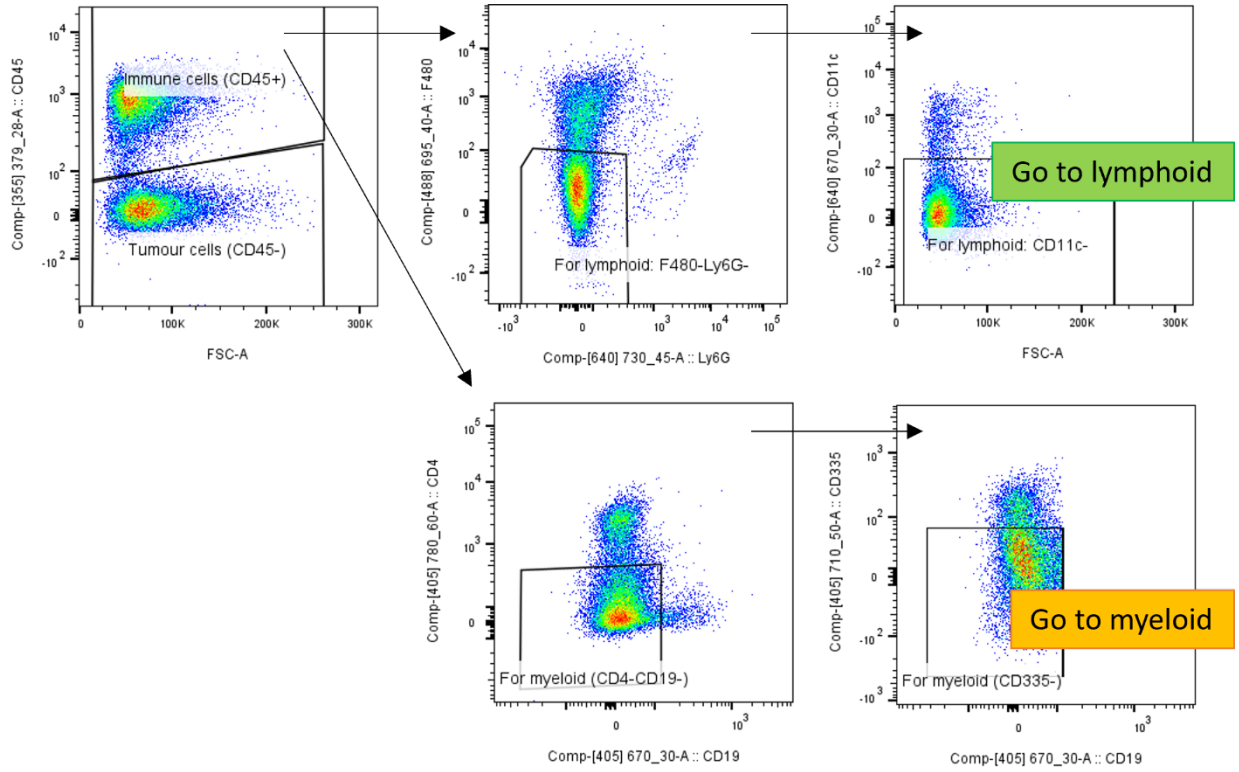
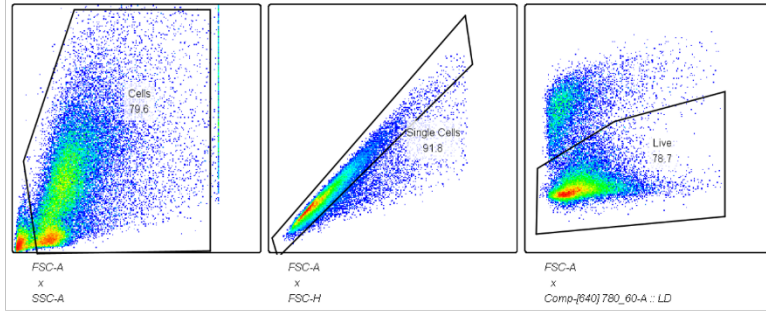
Supplementary Figure S5 (Related to Figure 5). Representative bioluminescence images from IP model experiment (A) Bioluminescence images AB1-Luc IP bearing BALB/c mice. n = 5 mice per group per repeat experiment. All mice in the control, ICT via intraperitoneal (i.p.) injection, and subcutaneous (scruff, s.c.) injection groups were imaged on day 3 (pre-

ICT treatment), day 6, (during ICT treatment), day 10 and day 13 (post ICT treatment). Mice in the treatment groups were further imaged on day 16, 20 and 23 of the tumours were smaller than the previous imaging session. Luminescence is calculated in radiance (p/sec/cm²/sr) over time.

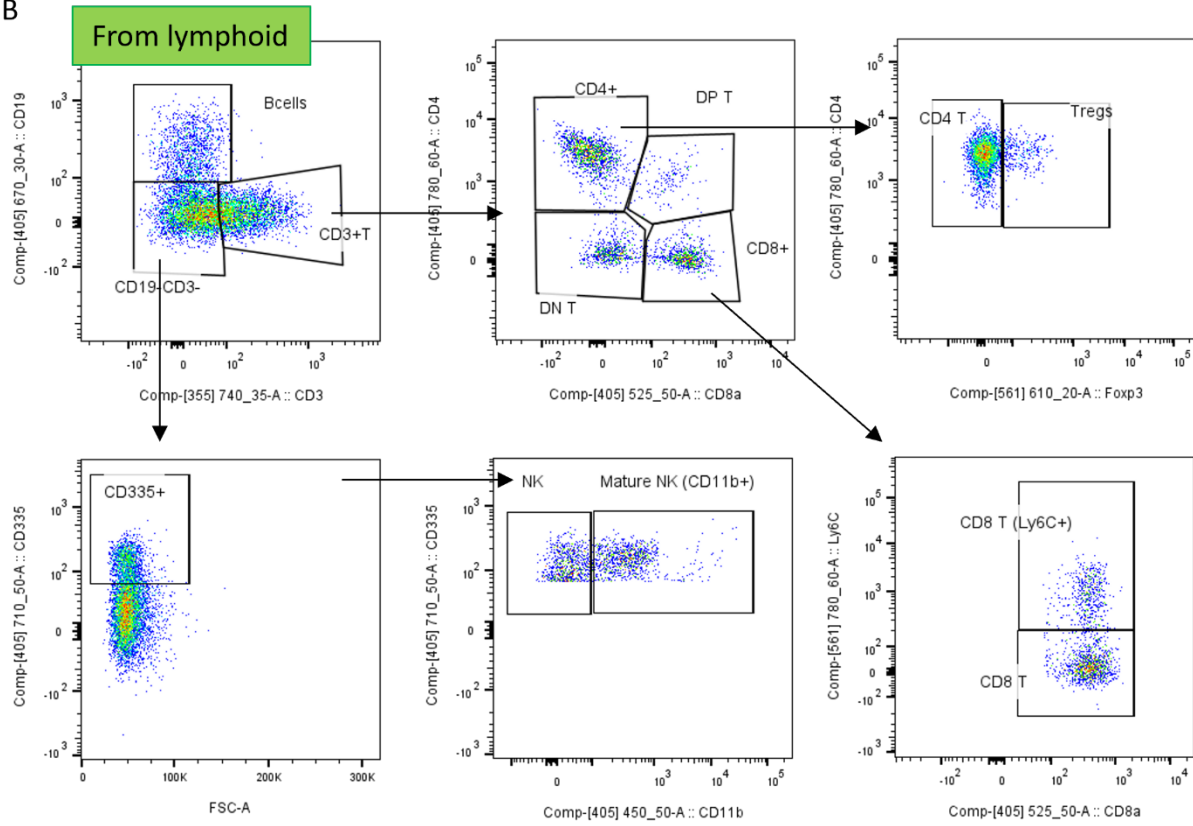


Supplementary Figure S6 (Related to Methods). FACS gating strategy. (A) Gating strategy for cells, singlets, live cells, and sorting cells within mCherry and CD45 populations.

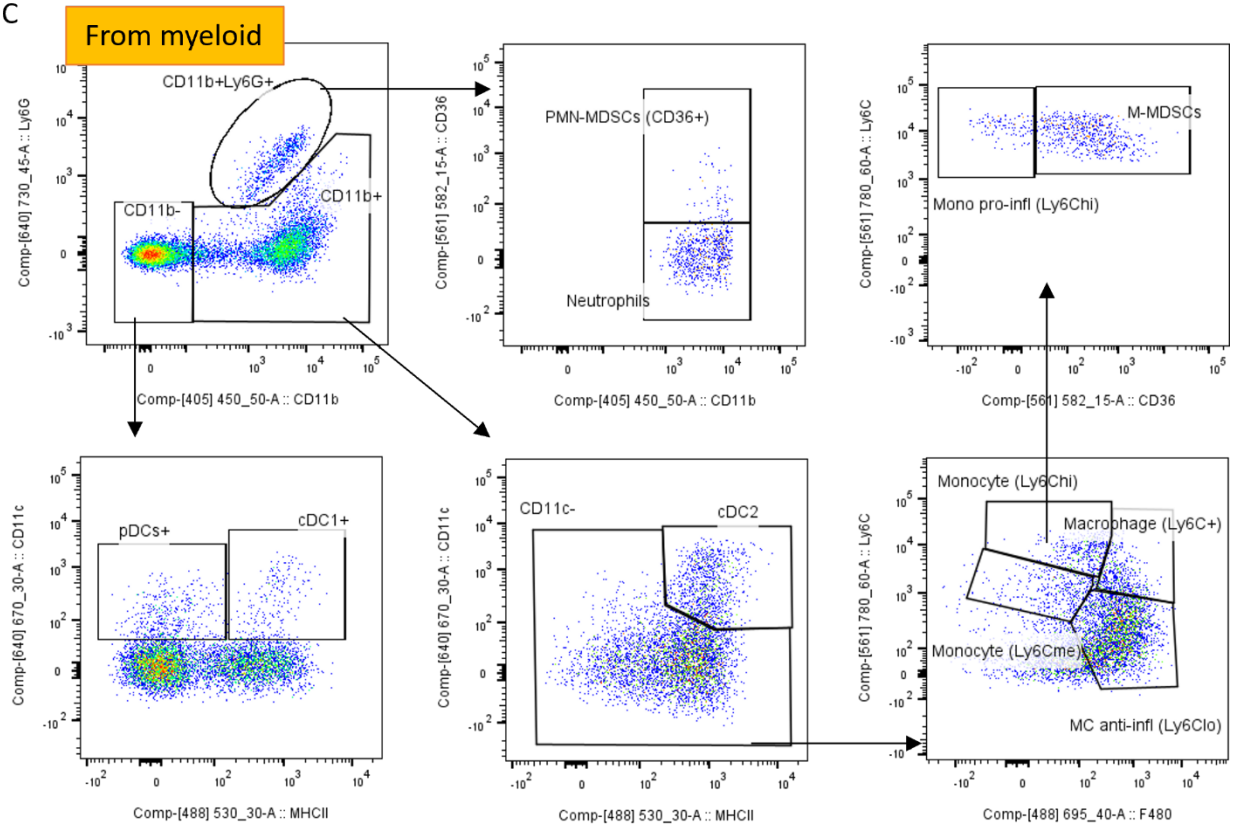
A



B



C



Supplementary Figure S7 (Related to methods). Flow cytometry gating strategy (A) Gating strategy for cells, singlets, live cells and gating out markers from lymphoid or myeloid panel. (B) Gating strategy for lymphoid cells. (C) Gating strategy for myeloid cells. Used antibodies are shown in Table S5.

Supplementary Table S1 (Related to Figure 2). Transcriptional regulators from upstream regulator analysis on whole tumour for the three models.

<i>Whole tumour: SC vs PL</i>					
<i>SUBCUTANEOUS</i>			<i>INTRAPLEURAL</i>		
<i>Transcription regulator</i>	<i>Adjusted p value</i>	<i>z-score</i>	<i>Transcription regulator</i>	<i>Adjusted p value</i>	<i>z-score</i>
SPI1	7.4E-09	2.69	PPARGC1A	1.694E-06	3.07
ZEB2	8.3E-09	2.11	FOXO4	1.868E-06	3.36
CEBPA	1.2E-07	2.29	Tcf7	5.52E-06	2.33
TP53	1.9E-06	3.25	SP3	6.532E-06	2.16
MRTFA	3.0E-06	2.38	GFI1	1.811E-05	2.38
ETV5	2.0E-05	2.38	ARNT	6.101E-05	2.17
SOX9	9.0E-05	2.39	GATA6	0.0024722	2.22
JUND	1.8E-04	2.19	IKZF2	0.0037366	2.32
JUNB	6.5E-04	2.38	BCL6	0.0065352	2.40
COMMD3-BMI1	7.0E-04	2.58			
HOXA7	1.8E-03	2.43			
ELF3	5.0E-03	2.16			
SOX7	6.4E-03	2.12			
Esrra	6.9E-03	2.72			
PAX7	9.6E-03	2.24			
HOXA9	1.1E-02	2.03			
<i>Whole tumour: SC vs IP</i>					
<i>SUBCUTANEOUS</i>			<i>INTRAPERITONEAL</i>		
<i>Transcription regulator</i>	<i>Adjusted p value</i>	<i>z-score</i>	<i>Transcription regulator</i>	<i>Adjusted p value</i>	<i>z-score</i>
STAT3	5.409E-22	2.54	SIX1	1.88E-13	3.33
MEF2C	1.812E-14	2.21	CITED2	2.666E-09	3.30
STAT1	2.467E-14	2.81	PPARGC1A	7.513E-08	2.52
SMARCA4	6.098E-14	3.33	BCL6	3.848E-07	3.12
SP1	2.103E-12	2.09	IKZF1	4.182E-07	2.38
MRTFB	5.966E-12	2.75	IKZF2	9.024E-06	3.16
SMAD3	7.149E-12	2.39	TAF4	2.933E-05	2.14
JUN	7.149E-12	2.46	GFI1	0.0001106	2.82

CEBPA	1.739E-11	2.51	GPS2	0.0001358	2.06
ETS1	1.868E-11	2.38	KLF11	0.0001459	2.03
JUNB	1.486E-10	2.38	NOSTRIN	0.000557	2.23
MRTFA	2.754E-10	2.25	KDM5A	0.002771	4.00
SPIB	3.62E-10	3.16	ZBTB18	0.0040844	2.19
BHLHE40	8.577E-10	2.45	NFIA	0.0265155	2.24
SPI1	9.43E-10	3.06			
TBX21	2.422E-09	2.98			
ZEB2	8.311E-08	2.27			
HOXD3	1.406E-07	2.35			
HIF1A	1.433E-07	2.45			
RELA	2.267E-07	2.56			
<i>Whole tumour: IP vs PL</i>					
<i>INTRAPLEURAL</i>			<i>INTRAPERITONEAL</i>		
<i>Transcription regulator</i>	<i>Adjusted p value</i>	<i>z-score</i>	<i>Transcription regulator</i>	<i>Adjusted p value</i>	<i>z-score</i>
MEF2C	8.266E-07	2.95	SIX1	3.225E-08	2.71
MYOD1	2.362E-06	2.25	KDM5A	5.8E-05	2.83
HAND2	0.0001013	2.22			
GATA4	0.0001073	2.77			
TEAD3	0.0003433	2.00			
TEAD1	0.0007843	2.20			
NPM1	0.0293211	2.00			

Supplementary Table S2 (Related to Figure 2). GSEA hallmark gene sets in orthotopic models compared with the SC model from AB1 immune checkpoint blockade response-associated tumour microenvironment signature. Significant pathways are depicted in grey.

Hallmark signalling pathway	Significant genes (SIZE)	Normalized Enrichment Score (NES)	P. value
Upregulated in orthotopic models when compared to SC tumours			
HYPOXIA	39	2.2	0.000
MTORC1_SIGNALING	32	1.56	0.054
ADIPOGENESIS	18	1.47	0.075
HEME_METABOLISM	22	1.33	0.122
GLYCOLYSIS	33	1.21	0.220
ESTROGEN_RESPONSE_EARLY	21	1.13	0.320
G2M_CHECKPOINT	17	1.09	0.341
E2F_TARGETS	22	0.85	0.651
DNA_REPAIR	19	0.74	0.778

XENOBIOTIC_METABOLISM	20	0.67	0.874
UV_RESPONSE_UP	15	0.62	0.908
Downregulated in orthotopic models when compared to SC tumours			
ALLOGRAFT_REJECTION	43	-2.3	0.000
EPITHELIAL_MESENCHYMAL_TRANSITION	37	-2.3	0.000
IL2_STAT5_SIGNALING	27	-1.8	0.004
TNFA_SIGNALING_VIA_NFKB	36	-1.76	0.003
KRAS_SIGNALING_UP	39	-1.74	0.004
INFLAMMATORY_RESPONSE	41	-1.64	0.016
COAGULATION	21	-1.6	0.033
P53_PATHWAY	28	-1.46	0.078
COMPLEMENT	33	-1.42	0.083
INTERFERON_GAMMA_RESPONSE	22	-1.34	0.133
IL6_JAK_STAT3_SIGNALING	21	-1.27	0.204
APOPTOSIS	22	-1.27	0.202
APICAL_JUNCTION	27	-1.06	0.382
MYOGENESIS	20	-1.04	0.430
ESTROGEN_RESPONSE_LATE	22	-0.86	0.657
MYC_TARGETS_V1	21	-0.86	0.633
UV_RESPONSE_DN	16	-0.77	0.749

Supplementary Table S3 (Related to Figure 3). nCounter® PanCancer Immune Profiling Panel genes allocated to cellular populations.

Gene	Cellular Population
Cd6	T cell
Cd3g	T cell
Cd3e	T cell
Cd2	T cell
Klrd1	Cytotoxic cell
Gzma	Cytotoxic cell
Prf1	CD8 T cell
Gzmm	CD8 T cell
Flt3l	CD8 T cell
Cd8b1	CD8 T cell
Cd8a	CD8 T cell
Nup107	T helper cell
Icos	T helper cell
Cd28	T helper cell
Batf	T helper cell
Anp32b	T helper cell

Tbx21	Th1 cell
Stat4	Th1 cell
Lta	Th1 cell
Il12rb2	Th1 cell
Ifng	Th1 cell
Ctla4	Th1 cell
Csf2	Th1 cell
Cd38	Th1 cell
Stat6	Th2 cell
Smad2	Th2 cell
Pmch	Th2 cell
Gata3	Th2 cell
Cxcr6	Th2 cell
Rorc	Th17 cell
Il17ra	Th17 cell
Il17a	Th17 cell
Fez1	T $\gamma\delta$ cell
Cd160	T $\gamma\delta$ cell
Pdcd1	T follicular helper cell
Bcl6	T follicular helper cell
Nfatc4	T effector memory cell
Ltk	T effector memory cell
Ewsr1	T effector memory cell
Ccr2	T effector memory cell
Akt3	T effector memory cell
Reps1	T central memory cell
Nefl	T central memory cell
Dock9	T central memory cell
Atm	T central memory cell
Foxp3	T regulatory cell
Zfp13	NK cell
Ncr1	NK cell
Bcl2	NK cell
Rrad	NK cell
Pla2g6	NK cell
Mpped1	NK cell
Foxj1	NK cell
Il21r	NK cell
Gzmb	NK cell
Gtf3c1	NK cell
Msr1	Macrophage

Marco	Macrophage
Cxcl5	Macrophage
Chit1	Macrophage
CD68	Macrophage
Ccl7	Macrophage
Apoe	Macrophage
Mme	Neutrophil
Fpr2	Neutrophil
Csf3r	Neutrophil
Tpsab1	Mast cell
Prg2	Mast cell
Ms4a2	Mast cell
Kit	Mast cell
Ctsg	Mast cell
Cma1	Mast cell
Thbs1	Eosinophil
Smpd3	Eosinophil
Ptgdr2	Eosinophil
Il5ra	Eosinophil
Ccr3	Eosinophil
Tnfrsf17	B cell
Ms4a1	B cell
Cr2	B cell
Cd19	B cell
Blk	B cell
Hsd11b1	Dendritic cell
Cd209e	Dendritic cell
Ccl22	Dendritic cell
Ccl17	Dendritic cell
Il3ra	Plasmacytoid dendritic cell
Syt17	Immature dendritic cell
F13a1	Immature dendritic cell
Cd1d2	Immature dendritic cell
Oas3	Activated dendritic cell
Lamp3	Activated dendritic cell
Ido1	Activated dendritic cell
Ebi3	Activated dendritic cell
Ccl1	Activated dendritic cell

Supplementary Table S4 (Related to Figure 4). Transcriptional regulators from upstream regulator analysis on tumour cells from three models.

<i>Tumour cells: SC vs PL</i>					
<i>SUBCUTANEOUS</i>			<i>INTRAPLEURAL</i>		
<i>Transcription regulator</i>	<i>Adjusted p value</i>	<i>z-score</i>	<i>Transcription regulator</i>	<i>Adjusted p value</i>	<i>z-score</i>
FOS	6.981E-14	2.32	PPARGC1A	1.452E-15	4.05
SP1	3.248E-12	4.47	SMAD7	4.948E-09	2.09
CEBPA	3.355E-12	2.53	SCX	1.941E-06	2.00
SMARCA4	4.91E-12	5.00	IKZF1	5.364E-06	2.81
CEBPB	2.102E-11	2.41	NOSTRIN	6.208E-06	2.97
GATA2	3.878E-11	3.74	STAT5B	1.829E-05	2.34
TP53	5.227E-11	3.41	RUNX3	7.387E-05	2.89
JUN	5.612E-11	3.26	SMARCA5	8.847E-05	3.00
SMAD3	7.338E-11	2.00	HOXC9	0.000312	2.24
MRTFB	5.104E-10	2.39	CBX5	0.0010102	2.33
STAT1	7.427E-10	3.42	TRIM24	0.0020838	3.15
GLI1	4.297E-09	3.49	MEOX2	0.004641	2.37
CTNNB1	4.443E-09	3.64	GFI1	0.0070493	2.11
SOX4	4.557E-08	3.18			
SPI1	6.537E-08	3.73			
SPIB	4.58E-07	2.38			
TEAD2	1.385E-06	2.31			
ARNT2	1.674E-06	3.00			
HTT	1.784E-06	3.19			
SS18	2.185E-06	2.53			
<i>Tumour cells: SC vs IP</i>					
<i>SUBCUTANEOUS</i>			<i>INTRAPERITONEAL</i>		
<i>Transcription regulator</i>	<i>Adjusted p value</i>	<i>z-score</i>	<i>Transcription regulator</i>	<i>Adjusted p value</i>	<i>z-score</i>
STAT3	1.43E-40	4.43	KLF2	4.5E-22	2.04
STAT1	3.28E-28	7.33	PPARGC1A	5.92E-15	3.71
TP53	6.66E-25	5.97	HOXA10	5.7E-13	2.36
FOS	2.43E-23	3.88	TRIM24	1.31E-12	4.27
NFKBIA	1.46E-21	2.88	SIRT1	2.58E-11	4.58
CREB1	4.86E-21	4.06	SMAD7	5.66E-10	2.95
SMAD3	4.52E-20	4.34	RUNX3	1.39E-09	3.97
JUN	1.76E-19	4.62	GPS2	2.48E-09	3.71
SP1	4.99E-18	6.19	GFI1	4.19E-09	3.83
SMARCA4	5.23E-18	8.50	NOSTRIN	4.37E-09	3.69

CTNNB1	7.22E-18	6.11	IKZF1	1.81E-08	3.75
SPI1	3.38E-17	6.28	ZFP36	2.32E-08	2.65
EP300	5.35E-17	3.19	SMARCA5	2.55E-08	4.67
IRF3	1.27E-16	6.31	TAF4	6.52E-08	2.79
EGR1	2.69E-16	4.27	NEUROG1	8.55E-08	2.29
NFAT5	2.9E-16	5.39	BCL3	2.06E-07	2.98
FOXO1	3.22E-16	2.89	PRDM16	2.56E-07	2.39
CEBPB	1.88E-15	5.27	SIN3A	6.78E-07	2.33
KLF4	2.04E-15	3.13	MEOX2	9.01E-07	3.90
IRF7	2.22E-15	6.79	ATF3	1.94E-06	2.71
<i>Tumour cells: PL vs IP</i>					
<i>INTRAPLEURAL</i>			<i>INTRAPERITONEAL</i>		
<i>Transcription regulator</i>	<i>Adjusted p value</i>	<i>z-score</i>	<i>Transcription regulator</i>	<i>Adjusted p value</i>	<i>z-score</i>
TP53	7.25E-25	4.08	KLF2	1.15E-10	4.06
NUPR1	2.25E-22	5.86	ZFP36	1.50E-07	3.10
FOXO3	2.78E-18	2.76	TAF4	5.77E-05	2.58
CREB1	4.07E-17	4.93	MEOX2	1.11E-04	3.11
EP300	2.96E-15	2.74	GPS2	1.22E-04	2.56
HIF1A	1.14E-14	5.01	DACH1	1.35E-04	2.96
CREM	4.5E-14	4.13	RCAN1	1.46E-04	2.40
FOXO1	3.75E-13	3.44	ZBED6	1.83E-04	2.09
FOS	8.49E-13	2.78	HIC1	2.80E-04	2.27
STAT3	1.28E-11	6.35	BCL3	3.65E-04	2.91
CREBBP	1.3E-11	2.02	IKZF1	4.43E-04	3.59
ZBTB17	1.6E-10	2.18	CBL	4.81E-04	2.40
SMAD3	1.03E-09	3.86	MNT	8.01E-04	2.24
EPAS1	2.4E-09	3.45	SPDEF	2.35E-03	3.00
EGR1	4.61E-09	2.91	HNF4A	3.16E-03	2.57
CTNNB1	5.45E-09	5.60	SIN3A	3.28E-03	2.45
JUN	9.88E-09	3.50	SP110	3.57E-03	2.18
SMAD4	1.08E-08	3.41	CIC	4.53E-03	2.00
FOXL2	1.33E-08	4.20			
NFATC2	1.38E-08	3.36			

Supplementary Table S5 (Related to methods). Flow cytometry panel antibody information.

Fluorochrome	Antigen	Dilution	Clone	Company	Catalogue number	Cocktail
-	CD16/CD32 (Fc block)	1:100	93	eBioscience™	14-0161-82	Blocking
FVS780	Live/Dead	1:1000	-	BD Horizon™	565388	Viability
BUV395	CD45	1:300	30-F11	BD Horizon™	565967	Surface
BUV737	CD3	1:200	17A2	BD Horizon™	612803	
BV421	CD11b	1:500	M1/70	BD Horizon™	562605	
BV480	CD8a	1:200	53-6.7	BD Horizon™	566096	
BV650	CD19	1:500	1D3	BD Horizon™	563235	
BV711	CD335	1:200	29A1.4	Biolegend	137621	
BV786	CD4	1:200	GK1.5	BD Horizon™	5563331	
FITC	MCHII	1:500	2G9	BD Horizon™	562009	
BB700	F4/80	1:500	T45-2342	BD Horizon™	746070	
PE	CD36	1:300	CRF D-2712	BD Horizon™	562702	
PE-Cy7	Ly-6C	1:300	AL-21	BD Horizon™	560593	
APC	CD11c	1:200	HL3	BD Horizon™	56119	
AF700	Ly-6G	1:500	1A8	BD Horizon™	561236	
PE-eFluor 610	Foxp3	1:500	FJK-16s	eBioscience™	61-5773-82	Intracellular