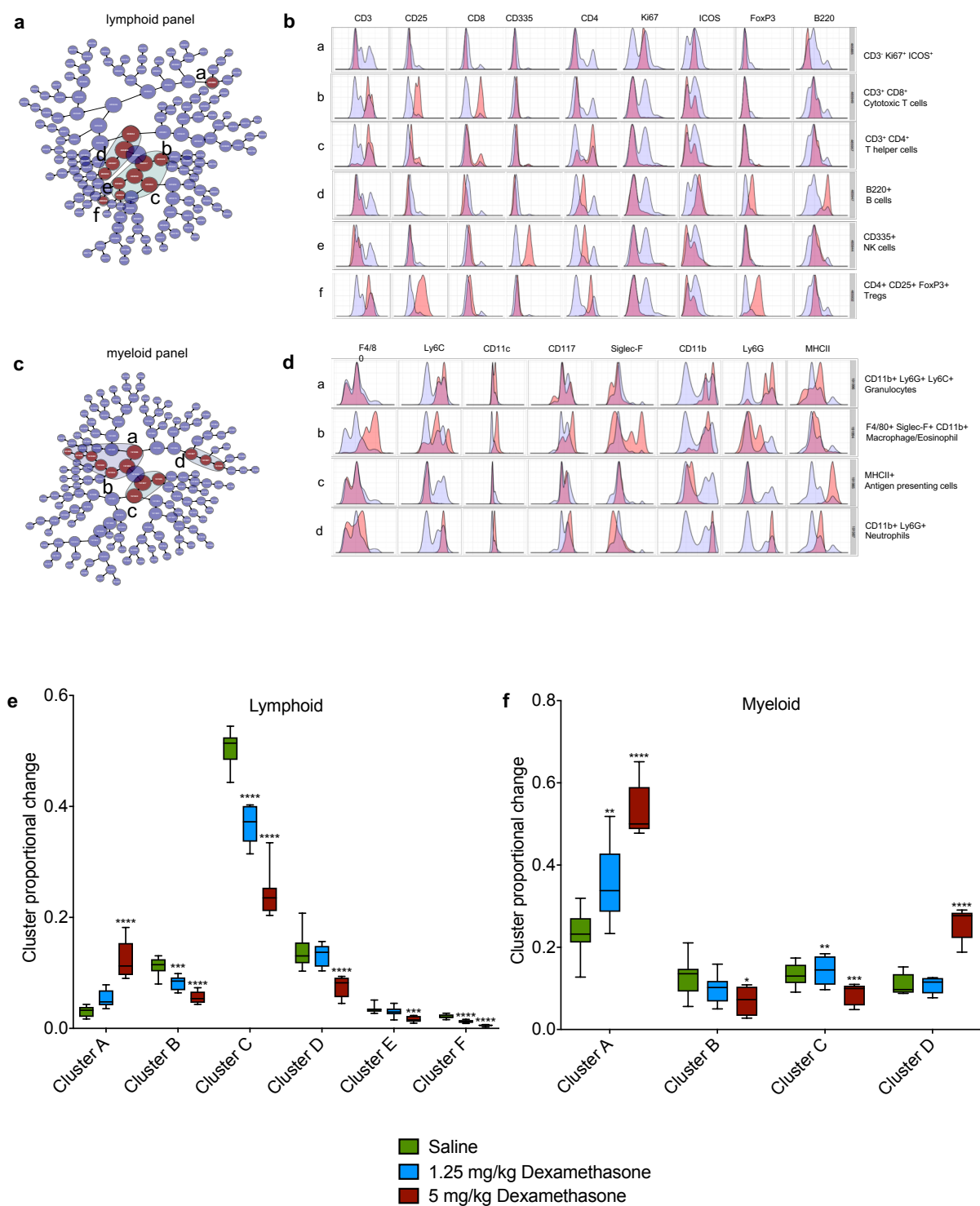


Antigen	Fluor	Clone	Isotype	Supplier	Catalog #	Antibody Registry	Panel	Dilution
CD45	FITC	REA737	hu IgG1	Miltenyi Biotec	130-110-796	AB_2658216	1	1/200
CD3	BUV395	145-2C11	ha IgG1	BD Bioscience	563565	AB_2738278	1, 3	1/500
CD4	BV786	GK1.5	rat IgG2b	BD Bioscience	563331	AB_2738140	1, 3	1/200
CD8	BV480	53-6.7	rat IgG2a	BD Bioscience	566096	AB_2739500	1	1/200
CD25	BV421	PC61	rat IgG1	Biolegend	102043	AB_2562611	1	1/200
FoxP3	AF647	150D	ms IgG1	Biolegend	320013	AB_439749	1	1/100
ICOS	PE-Cy7	C398.4A	ha IgG	Biolegend	313519	AB_10641839	1	1/200
Ki67	PE	B56	ms IgG1	BD Bioscience	556027	AB_2266296	1	1/100
CD335	BV711	29A1.4	rat IgG2a	Biolegend	137621	AB_2563289	1	1/200
B220	APCVio770	REA755	hu IgG1	Miltenyi Biotec	130-110-849	AB_2658286	1	1/100
CD45	BUV395	30-F11	rat IgG2b	BD Bioscience	564279	AB_2651134	2	1/250
F4/80	BV421	BM8	rat IgG2a	Biolegend	123131	AB_10901171	2	1/200
Ly6C	BV510	HK1.4	rat IgG2c	Biolegend	128033	AB_2562351	2	1/400
CD11c	eV605	N418	ha IgG	Biolegend	117333	AB_11204262	2	1/200
CD117	BV711	2B8	rat IgG2b	Biolegend	105835	AB_2565956	2	1/500
Ki67	Vio515	REA183	hu IgG1	Miltenyi Biotec	130-108-856	AB_2652560	2	1/100
Siglec-F	PE	REA798	hu IgG1	Miltenyi Biotec	130-112-332	AB_2653439	2	1/400
CD11b	APC	REA592	hu IgG1	Miltenyi Biotec	130-109-364	AB_2654646	2	1/400
Ly6G	AF700	1A8	rat IgG2a	Biolegend	127621	AB_10640452	2	1/400
MHCII	APC-ef780	M5/114.15.2	rat IgG2b	BD Bioscience	47-5321-80	AB_1548792	2	1/200
CD8	AF700	53-6.7	rat IgG2a	BD Bioscience	557959	AB_396959	3	1/200
CD25	BV510	PC61	rat IgG1	Biolegend	102041	AB_2562269	3	1/200
FoxP3	FITC	FJK-16s	rat IgG2a	eBioscience	11-5773-82	AB_465243	3	1/100
GITR	BV711	DTA-1	rat IgG2b	BD Bioscience	563390	AB_2738176	3	1/400
OX40	APC	OX-86	rat IgG1	Biolegend	119413	AB_2561723	3	1/200
TIM3	PE	B8.2C12	rat IgG1	Biolegend	134004	AB_1626177	3	1/200
PD-1	PECF594	J43	ha IgG2	BD Bioscience	562523	AB_2737634	3	1/400
Viability	Zombie UV	-	-	Biolegend	423108	-	1, 2, 3	1/1000

Supplementary Table 1: Flow cytometry panels

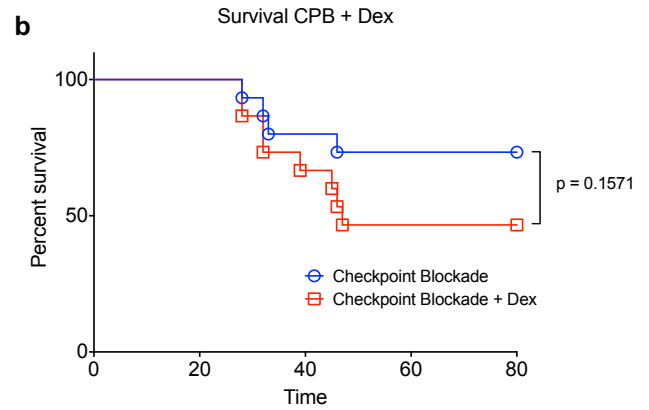
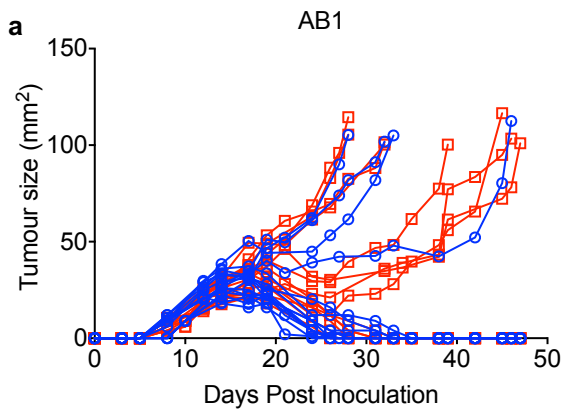
Phenotypic markers and associated fluorophores for the 3 flow cytometry panels used.

Abbreviations: ms = mouse, ha = hamster, hu = human.



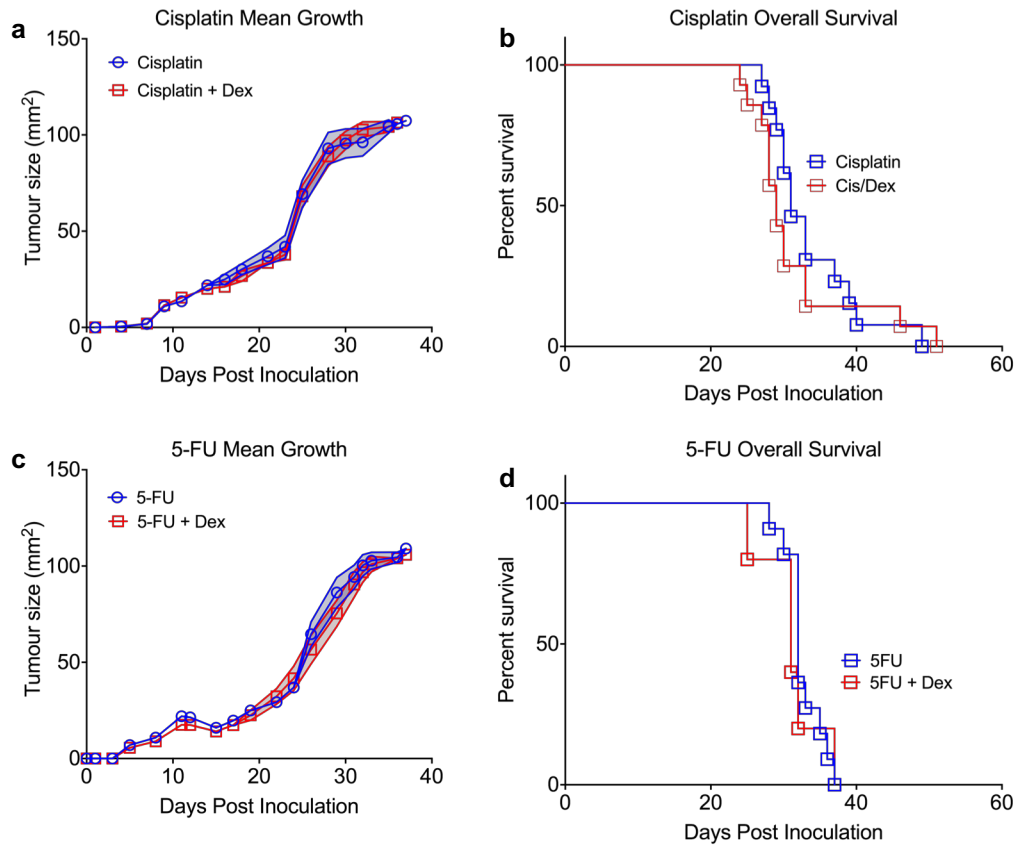
Supplementary Fig 1: CITRUS analysis identifies distinct differences in immune cell clusters in tumour after dexamethasone administration.

An unsupervised CITRUS analysis was performed to determine if there are differences between immune subsets within solid tumours after receiving dexamethasone. **A**, Hierarchy plots of stratifying features and **B**, corresponding markers of a lymphoid panel and **C**, hierarchy plots and **D**, markers for a myeloid FACS panel. Subsets that are differentially present in dexamethasone-treated mice are depicted in red. Proportional changes of **E**, lymphoid and **F**, myeloid clusters are shown. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



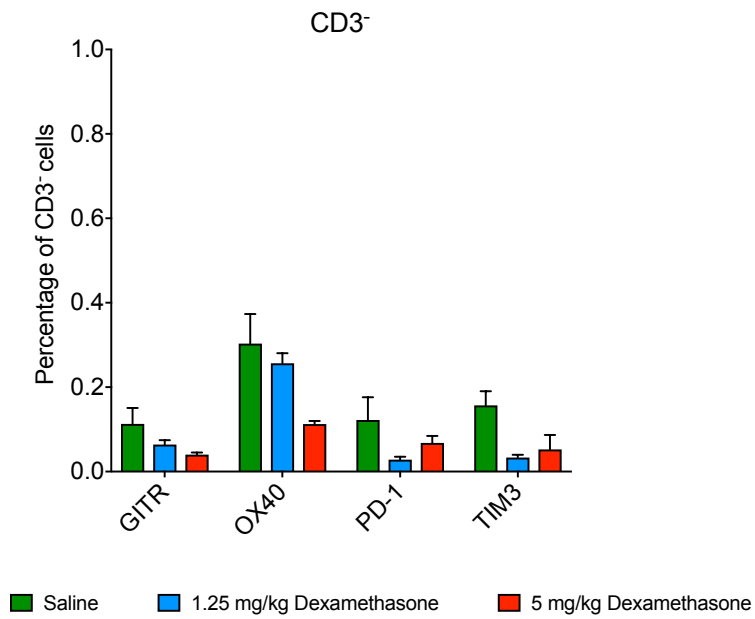
Supplementary Fig 2: Dexamethasone has an inhibitory effect on the efficacy of checkpoint blockade.

A, Individual growth curves and **B**, survival plot of anti-CTLA4/anti-PD-L1, with or without dexamethasone in s.c. AB1 mesothelioma-bearing BALB/c mice. Mixed model analysis of variance performed on tumour growth and log rank (Mantel-Cox) analysis performed on survival curve.



Supplementary Fig 3: Dexamethasone does not effect response to chemotherapy.

A, Individual growth curves and **B**, survival plot of cisplatin and **C**, Individual growth curves and **D**, survival plot of 5-FU, with or without dexamethasone in s.c. AB1 mesothelioma-bearing BALB/c mice. Mixed model analysis of variance performed on tumour growth and log rank (Mantel-Cox) analysis performed on survival curve.



Supplementary Fig 4: CD3⁻ cells do not express significant levels of immune checkpoint molecules.

Expression of select immune checkpoint molecules on CD3⁻ intratumoural cells.