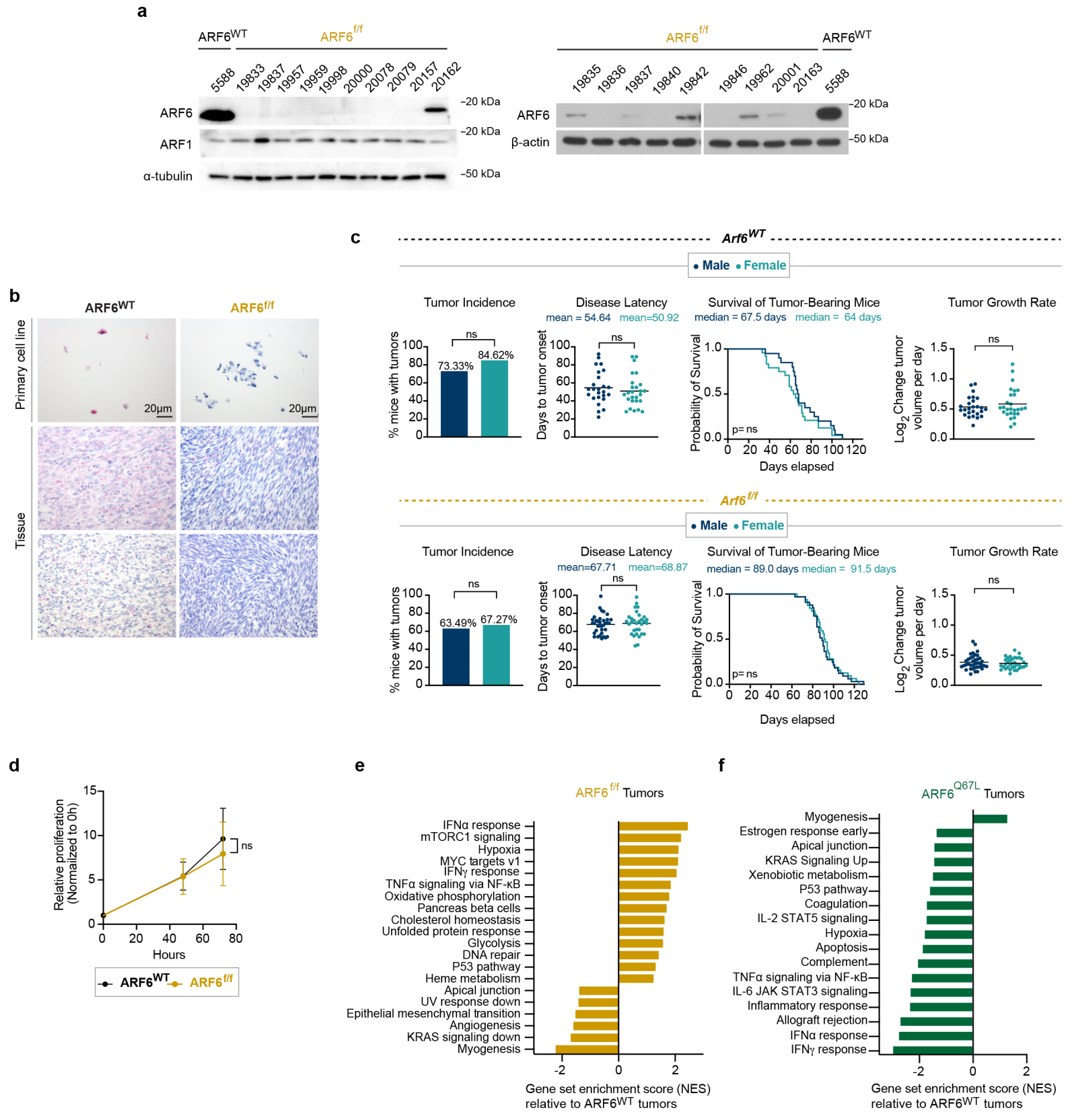
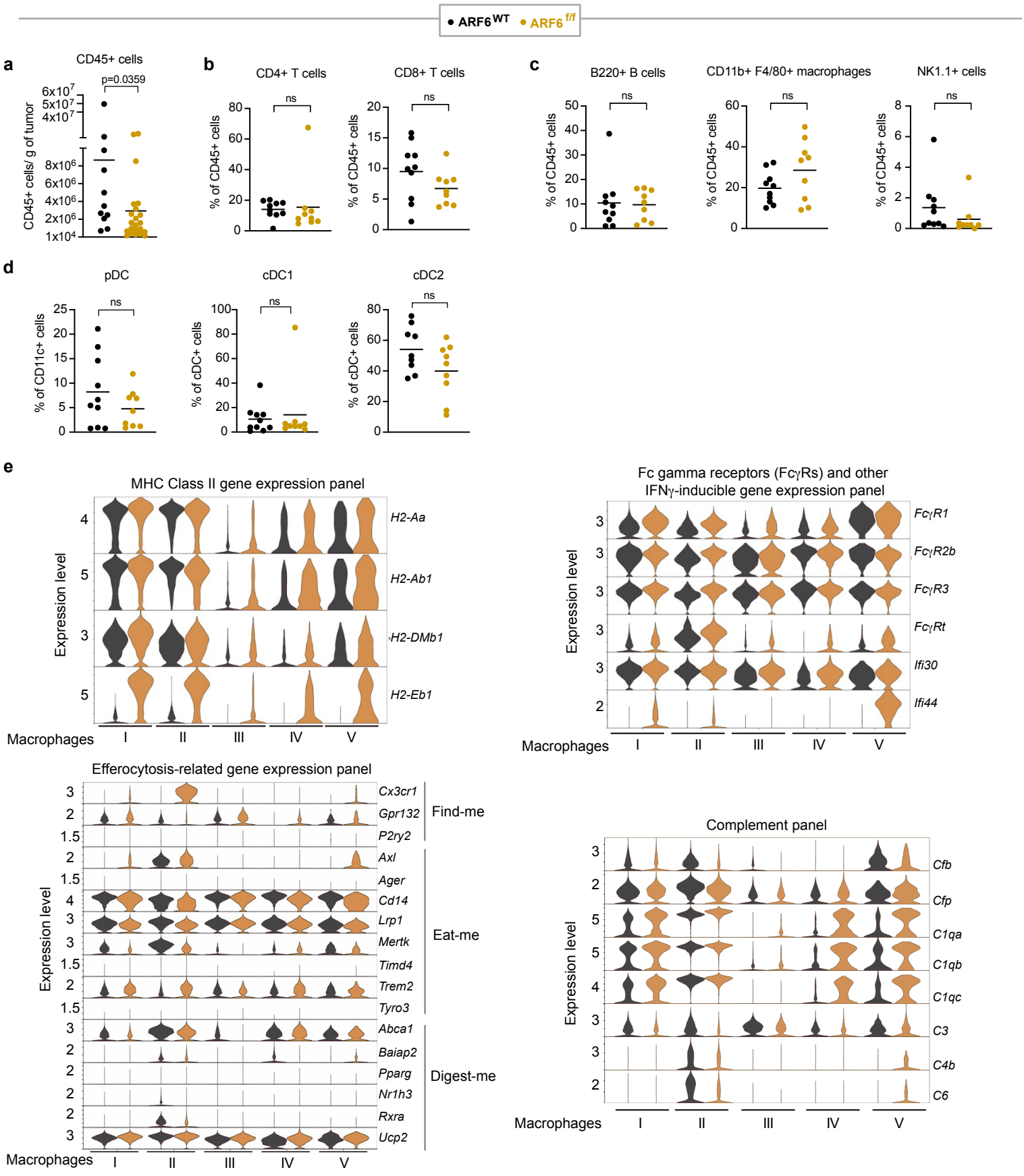
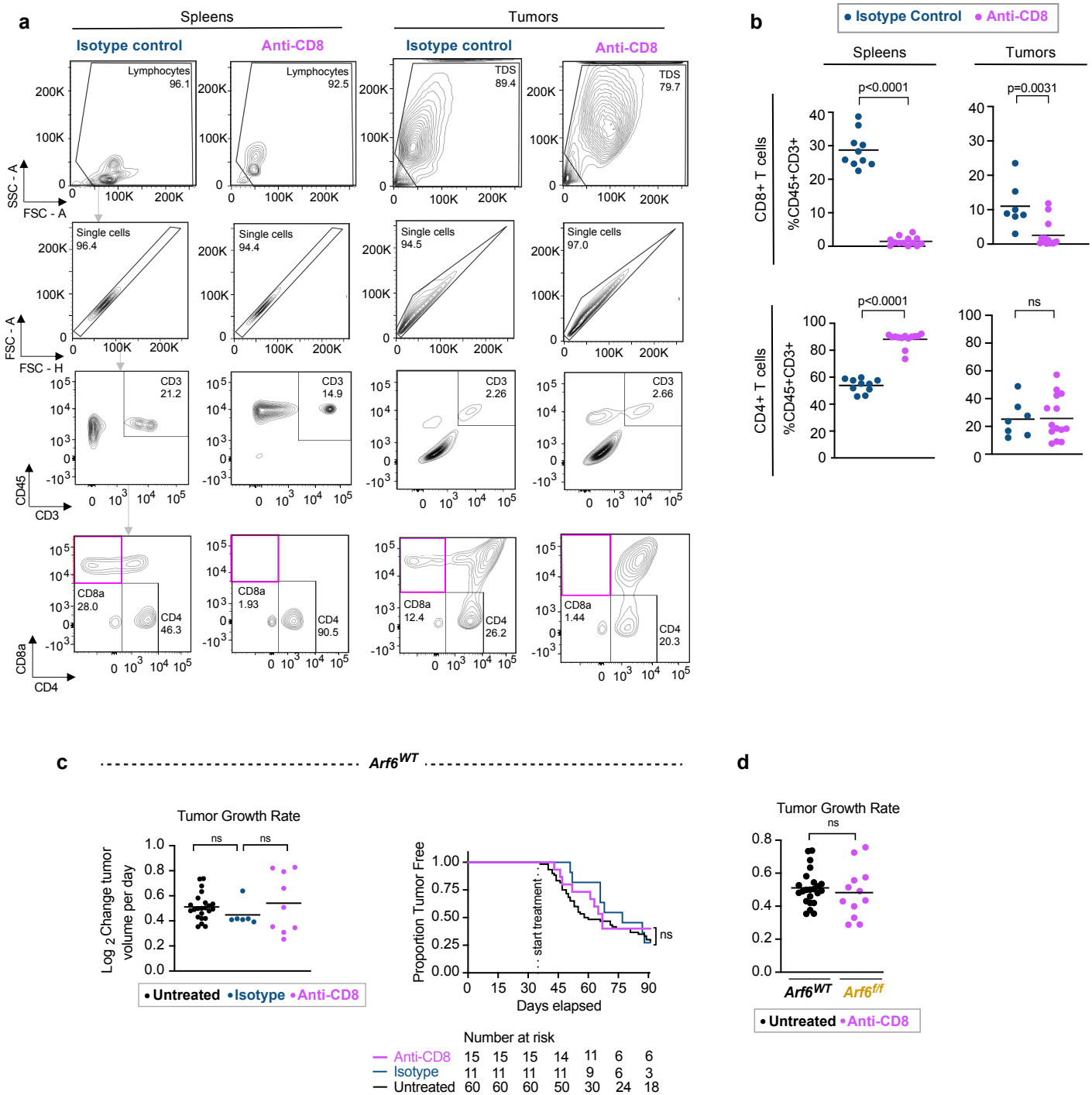


Supplementary Figure 1: ARF6 expression and ARF6-dependent gene expression pathways in murine tumours, related to Figure 1 and 2.
(a) Western blot detection of indicated proteins in early-passage primary tumour cell lines. One independent experiment includes n=1 tumour cell line derived from one *Arf6* ^{WT} mouse (see also Figure 4c, 4f, Supplementary Figure 5c for ARF6 expression from additional tumour cell lines derived from other *Arf6* ^{WT} mice), n=18 tumour cell lines derived from eighteen *Arf6* $^{f/f}$ mice. **(b)** In situ hybridization detection of *Arf6* mRNA (pink). Left panels show expected diffuse signal. Right panels show expected loss of signal. Right middle panel shows representative low-level heterogenous Arf6 signal in murine tumour 19835, consistent with the low level of ARF6 detected by Western blot for the 19835 primary tumour cell line (see Supplementary Figure 1a). **(c)** Sex-based analysis of Figure 1d-g for *Arf6*^{WT} and *Arf6*$^{f/f}$ mice. Tumour incidence (*Arf6* ^{WT}: Females n=45, Males n=39; *Arf6* $^{f/f}$: Females n=55, Males n=63) two-sided Fisher's exact test; disease latency (*Arf6* ^{WT}: Females n=26, Males n=25; *Arf6* $^{f/f}$: Females n=31, Males n=35) two-tailed t-test with Welch's correction; tumour growth rate (*Arf6* ^{WT}: Females n=27, Males n=26; *Arf6* $^{f/f}$: Females n=35, Males n=38) two-tailed t-test with Welch's correction, and survival (*Arf6* ^{WT}: Females n=27, Males n=26; *Arf6* $^{f/f}$: Females n=35, Males n=38) Log-rank (Mantle-Cox) test. **(d)** *In vitro* proliferation of murine melanoma, mean values shown for n=5 different cell lines per genotype. Two-way ANOVA test. **(e and f)** Bulk tumour transcriptomes (RNAseq) with significantly enriched gene sets (MSigDB Hallmark) in **(e)** ARF6$^{f/f}$ (n=6) *versus* ARF6^{WT} (n=6) and **(f)** ARF6^{Q67L} (n=6) *versus* ARF6^{WT} (n=4) tumours. **(c)** Solid line within data points= mean. **(d)** Error bars= SD. Source data are provided as a Source Data file.

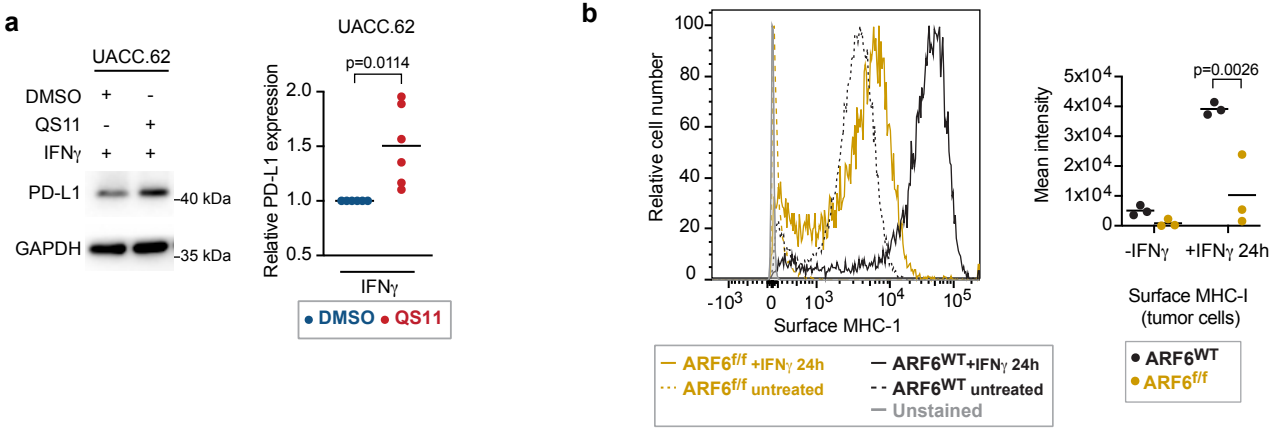


Supplementary Figure 2: Immune profiling of tumour microenvironment, related to Figure 3.
(a) The absolute numbers of CD45+ cells per gram of tumour.ARF6WT: n=11 tumours, ARF6 f/f: n=22 tumours **(b)** Fractions of CD4+ and CD8+ T cells in CD45+cells. **(c)** Fractions of B220+ B cells, CD11b+F4/80+ macrophages, and NK1.1+ cells in CD45 +cells.**(d)** Fractions of plasmacytoid dendritic cells (pDC) and conventional dendritic cell subsets (cDC1 and cDC2). **(b-d)** $ARF6^{WT}$: n=10 tumours, $ARF6^{f/f}$: n=9 tumours. **(a-d)** Solid line within data points= mean. Two-tailed Mann-Whitney t-test. **(e)** Expression of IFNγ-inducible genes related to antigen presentation (MHC Class II), phagocytosis (FcγR and other genes), efferocytosis-related genes and complement genes, across different subtypes of macrophages. Macrophages I: n= 2, 812 cells from $ARF6^{WT}$, n= 3,695 cells from $ARF6^{f/f}$ tumours; Macrophages II: n= 2, 069 cells from $ARF6^{WT}$, n= 4, 152 cells from $ARF6^{f/f}$ tumours; Macrophages III: n= 1, 525 cells from $ARF6^{WT}$, n= 1, 422 cells from $ARF6^{f/f}$ tumours; Macrophages IV: n= 899 cells from $ARF6^{WT}$, n= 777 cells from $ARF6^{f/f}$ tumours; Macrophages V: n= 225 cells from $ARF6^{WT}$, n= 286 cells from ARF6f/f tumours; from n=3 tumours of each genotype. A comprehensive list of adjusted p-values, obtained from Two-sided Seurat's Wilcoxon Rank Sum test for differentially expressed genes, is provided in Supplementary Table 2. Source data are provided as a Source Data file.

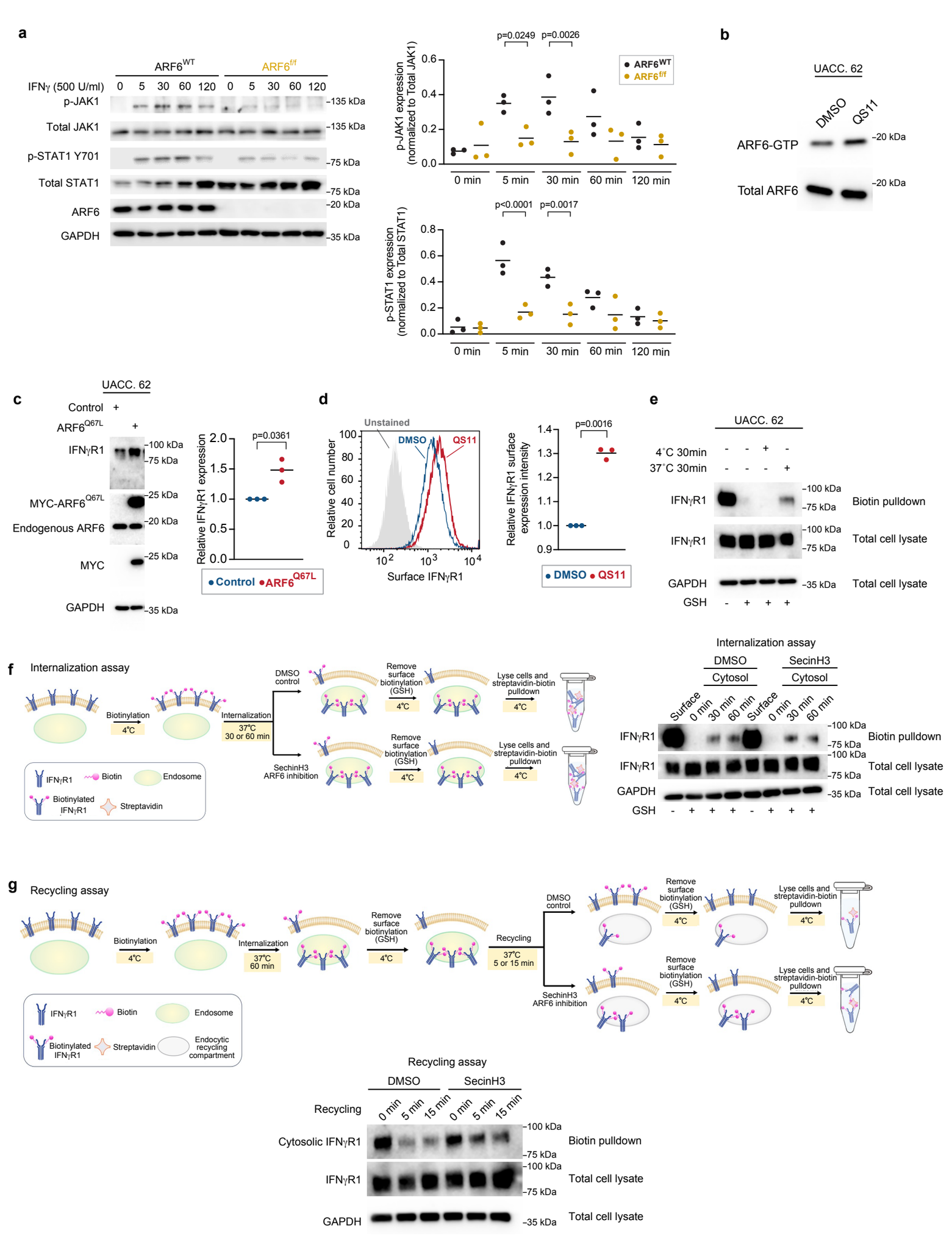


Supplementary Figure 3: Efficiency of CD8 T cell depletion, related to Figure 3.
(a and b) Quantitation of T cells by flow cytometry **(a)** and graph representing the mean **(b)** in spleens and tumours of *Arf6* $^{f/f}$ mice treated with isotype control (IgG2b) or anti-CD8 antibody. Two-tailed Mann-Whitney t-test. **(c)** Tumour-free survival (Kaplan-Meier log-rank test) and rate of tumour growth (untreated n=23, isotype n=6, anti-CD8 n=9), Two-tailed Welch's t-test, *Arf6* ^{WT} mice without or with CD8 T cell depletion. Antibody treatments were initiated when mice were 5-week-old and continued for 8 weeks. (d) Rate of tumour growth of untreated *Arf6*^{WT} mice (n=23) compared to CD8 T cell depleted *Arf6* $^{f/f}$ mice (n=12), Two-tailed Welch's t-test. (b, c, d) Solid line within data points= mean. Source data are provided as a Source Data file.

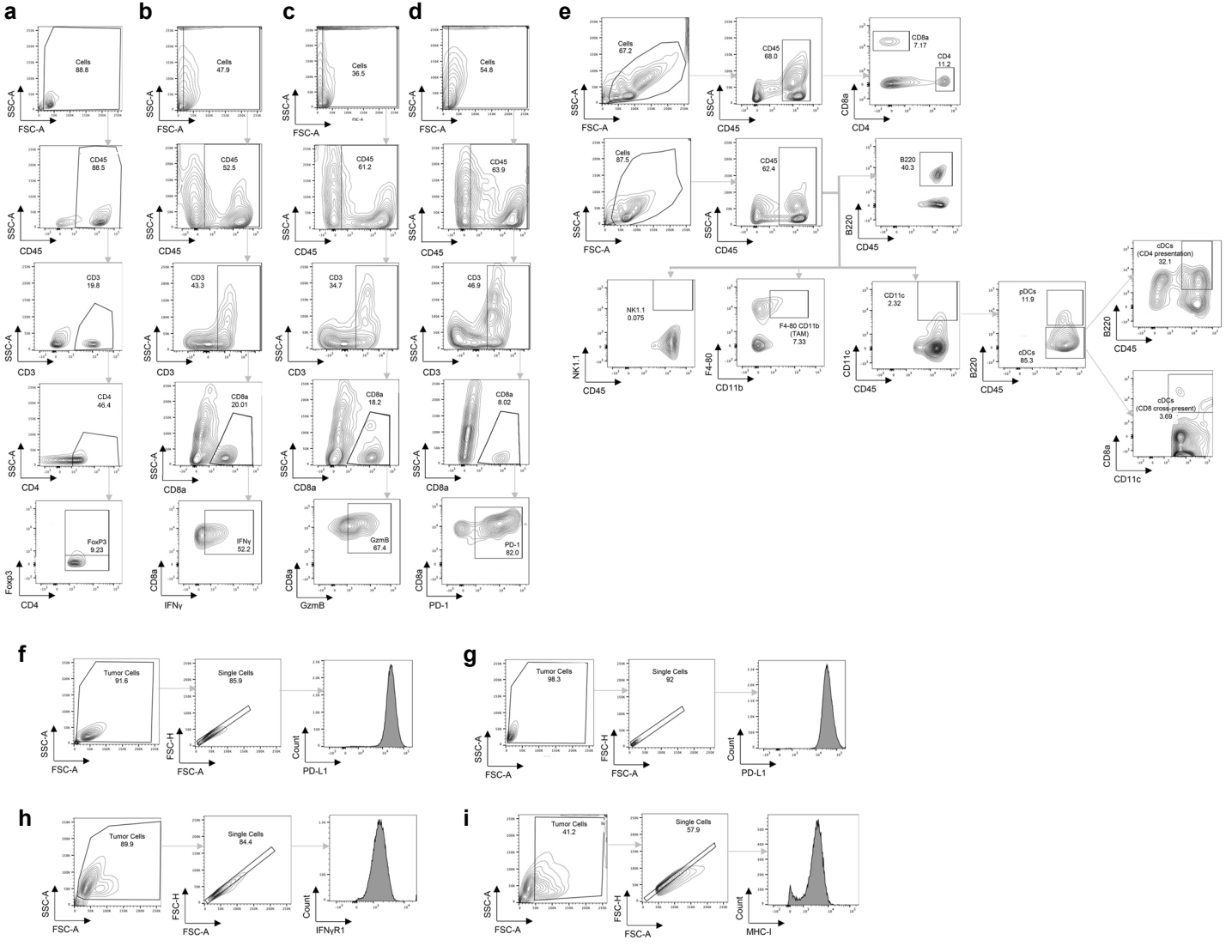
Supplementary Figure 4: ICB treatment outcomes, related to Figure 4.
(a) Systemic anti-PD-1 treatment initiated in *Arf6* ^{WT}and *Arf6* $^{f/f}$ mice with established tumours (up to 5mm in greatest dimension, 27-72mm^3). *Arf6* ^{WT}(untreated controls n=24, anti-PD-1 n=32) and *Arf6* $^{f/f}$ (untreated controls n=38, anti-PD-1 n=22). Rate of tumour growth measured from initiation of treatment, Two-tailed Welch's t-test. Survival (primary tumour reached 2cm) from initiation of treatment, Log-rank (Mantle-Cox) test. **(b)** T cell effector function, anti-PD-1 treated mice, measured by flow cytometric detection of IFNγ and granzyme B (GmzB) in tumour-infiltrating CD8+ T cells. Quantification of IFNγ (*Arf6* ^{WT}: untreated n=10, Anti-PD-1 treated n=10; *Arf6* $^{f/f}$: untreated n=17, Anti-PD-1 treated n=9); granzyme B (GmzB) (*Arf6* ^{WT}: untreated n=10, Anti-PD-1 treated n=9; *Arf6* $^{f/f}$: untreated n=16, Anti-PD-1 treated n=6). **(c)** Association of ICB treatment outcome in melanoma patients with mRNA levels of *CYTH1*. **(d)** Total ARF6 and ARF6 GTP pulldown in UACC.62 human melanoma cells with or without knockdown of *CYTH1*. n=1 biologically independent experiment. **(e-l)** Association of ICB treatment outcome in melanoma patients with mRNA levels of *CYTH4* **(e)** *CD8A* (T cells) **(f)** *CD4* (T cells) **(g)** *ITGAX* (*CD11c*) (dendritic cells) **(h)** *ITGAM* (*CD11b*), *CD14*, *FCGR3A*, *CD68* (macrophages) **(i)**, *ARF1* **(j)** and *ACAP1* **(k)** in transcriptomes of pretreatment melanoma biopsies (Cancer-Immu expression analysis). **(l)** Lack of association of *ARF6* and *CYTH1* expression (tumour) with survival of non-ICB treated melanoma patients with stage III and stage IV (TCGA, Q1 n=41 vs. Q4 n=41). p value= log-rank test comparison. LR= likelihood ratio. **(m-n)** Association of ICB treatment outcome in melanoma patients with mRNA levels of *CD274* **(m)**, *IDO1* **(n)** in transcriptomes of pretreatment melanoma biopsies, Cancer-Immu expression analysis, queried melanoma clinical studies, adjusted p-values, Benjamini and Hochberg procedure, LR= likelihood ratio (df=1) aggregated data from n=13. **(c,e,j,k)** PFS n=140, OS n=160 in each high and low cohort. **(f,g,h,i,m,n)** PFS n=155, OS n=160 in each high and low cohort **(a-b)** Solid line within data points= mean. **(a-b,d)** Source data are provided as a Source Data file.



Supplementary Figure 5: Expression of PD-L1, MHC-1 and LAG3 ligands, related to Figure 5.
(a) Western blot detection of PD-L1. 2μM QS11 for 4h. 500U/ml IFNγ. n=6 biologically independent experiments. Two-tailed Ratio paired t-test **(b)** Flow cytometric detection of tumour cell surface MHC-I expression, n=3 biologically independent cell lines of each genotype. Two-way ANOVA test. **(c)** Western blot detection of Galectin3 (Gal3) and LSECtin in murine melanoma, n=3 biologically independent cell lines of each genotype. **(d)** Western blot detection of Gal3 in UACC.62 cells with or without ARF6 knockdown, n=3 biologically independent experiments. **(a,b)** Solid line within data points= mean. Source data are provided as a Source Data file.



Supplementary Figure 6: Tumour-intrinsic ARF6-dependent IFNγ signaling, related to Figure 6.
(a) IFNγ- induced JAK-STAT signaling detection in early-passage murine melanoma cell lines. n=3 biologically independent cell lines of each genotype. Two-way ANOVA with Tukey's multiple comparisons test. **(b)** Total ARF6 and ARF6 GTP pulldown in UACC.62 cells without or with 2mM QS11 treatment for 1hr. n=1 biologically independent experiment. **(c)** Western blot for indicated proteins in UACC.62 cells with or without adenoviral-mediated ectopic expression of constitutively active ARF6 (ARF6^{Q67L}), control= empty vector, n=3 biologically independent experiments. Two-tailed Ratio paired t-test. **(d)** Flow cytometric detection of surface IFNγR1 expression. 2μM QS11 treated for 6h prior to flow cytometry, n=3 biologically independent experiments. Two-tailed Ratio paired t-test. **(e)** Ligand-independent endocytosis of IFNgR1 induced at 37˚C, followed by Biotin pulldown. GSH= glutathione stripping buffer. n=1 biologically independent experiment. **(f)** Internalization assay of IFNγR1, top panel: Schematic representation of internalization assay of IFNγR1 in human melanoma (UACC.62) treated with DMSO (control) or ARF6 inhibitor SecinH3 (30μM). bottom panel: Western Blot of internalized IFNγR1. n=1 biologically independent experiment. **(g)** Recycling assay of IFNγR1, top panel: Schematic representation of recycling assay of IFNγR1 in human melanoma (UACC.62) treated with DMSO (control) or ARF6 inhibitor SecinH3 (30μM). bottom panel: Western Blot of remaining cytosolic IFNγR1. n=1 biologically independent experiment. **(c-d)** Solid line within data points= mean. Source data are provided as a Source Data file.



Supplementary Figure 7: Gating Strategies for Flow Cytometry
(a) Tregs (for Figure 3c) **(b)** CD8+ IFNγ+ Cells (for Figure 3a) **(c)** CD8+ GzmB+ Cells (for Figure 3a) **(d)** CD8+PD-1+ Cells (for Figure 3a) **(e)** Cell populations in Supplementary Figure 2a-d. **(f)** Surface PD-L1 (for Figure 5b). **(g)** Total PD-L1 (for Figure 5b). **(h)** Surface IFNγR1 (for Figure 6a). **(i)** Surface MHC-1 (for Supplementary Figure 5b).

Supplementary Table 1. ARF6 pathway genes.	
ARFs	*ARF1, ARF3, ARF4, ARF5, ARF6*
GEFs	*CYTH1, CYTH2, CYTH3, CYTH4, IQSEC1/GEP100, IQSEC2, IQSEC3, GBF1, ARFGEF1, ARFGEF2, PSD/EFA6*
GAPs	*ACAP1, ACAP2, ACAP3, ARAP1, ARAP2, ARAP3, ARFGAP1, ARFGAP2, ARFGAP3, ASAP1, ASAP2, ASAP3, GIT1, GIT2, SMAP1, SMAP2, ADAP1, ADAP2, AGAP1, AGAP2, AGAP3, AGAP4, AGFG1, AGFG2, PDCD6IP*

Supplementary Table 2. Adjusted p-values using Two-sided Seurat's Wilcoxon Rank Sum test for Supplementary Figure 2e.

Gene Function	Gene	Adjusted p-values				
		Macrophage I	Macrophage II	Macrophage III	Macrophage IV	Macrophage V
MHC Class II gene expression panel	*H2-Aa*	1.95E-75	1.00E+00	8.69E-21	0.504831556	1.00E+00
	H2-Ab1	5.20E-35	1.10E-22	1.07E-23	2.29E-15	1.00E+00
	H2-DMb1	6.71E-72	0.007224454	2.17E-27	1.78E-09	1.00E+00
	H2-Eb1	<2.225074e-308	<2.225074e-308	8.83E-139	4.31E-106	2.08E-32
Fc gamma receptors (FcγRs) and other IFNγ-inducible gene	*FcγR1*	2.91E-156	2.25E-159	7.50E-31	1.00E+00	1.00E+00
	FcγR2b	7.83E-26	4.23E-163	1.00E+00	1.00E+00	1.00E+00
	FcγR3	1.09E-39	1.94E-169	1.00E+00	1.00E+00	1.00E+00
	FcγRt	3.10E-31	3.11E-76	1.20E-11	5.00E-18	1.48E-05
	Ifi30	5.48E-24	1.00E+00	3.13E-23	2.04E-11	0.581995712
	Ifi44	<2.225074e-308	4.88E-161			1.39E-61
Complement	*Cfb*	3.50E-13	2.92E-137	5.43E-13		1.00E+00
	Cfp	1.00E+00	6.40E-91	1.00E+00	1.00E+00	1.00E+00
	C1qa	1.32E-74	1.91E-142	1.17E-07	8.11E-64	2.42E-13
	C1qb	1.50E-42	2.70E-08	0.400581784	1.92E-43	3.35E-09
	C1qc	6.96E-53	3.94E-12		1.52E-53	8.79E-13
	C3	6.51E-53	1.74E-143	5.44E-11	0.017446926	1.00E+00
	C4b		1.39E-18			0.000667637
	C6		3.22E-67			3.17E-09
Efferocytosis- Find-me	*Cx3cr1*	1.39E-145	<2.225074e-308			1.84E-09
	Gpr132	0.89241392	1.98E-22	6.51E-10	1.00E+00	1.00E+00
	P2ry2					
Efferocytosis-Eat-me	*Axl*	2.87E-86	0.000256594			3.20E-11
	Ager					
	Cd14	1.75E-51	2.69E-69	4.28E-06	2.30E-46	2.51E-11
	Lrp1	2.08E-42	7.13E-207	1.00E+00	1.00E+00	1.00E+00
	Mertk	1.22E-117	<2.225074e-308	2.83E-08	1.00E+00	1.00E+00
	Timd4					
	Trem2	3.30E-55	1.38E-153	1.00E+00	1.45E-07	1.00E+00
	Tyro3					
Efferocytosis-Digest-me	*Abca1*	1.18E-47	4.57E-29	9.16E-35	1.00E+00	1.00E+00
	Baiap2		1.00E+00		1.00E+00	1.00E+00
	Pparg					
	Nr1h3		1.00E+00			
	Rxra		1.95E-75			
	Ucp2	2.22E-30	1.00E+00	1.94E-28	4.42E-22	1.00E+00

Color	Description
	low gene expression
	gene expression of macrophages in ARF6$^{f/f}$ is greater than ARF6^{WT} tumors, and the difference is significant
	gene expression of macrophages in ARF6^{WT} is greater than ARF6$^{f/f}$ tumors, and the difference is significant
	gene expression of macrophages in ARF6$^{f/f}$ tumors is trending higher, but not significant
	gene expression of macrophages in ARF6^{WT} tumors is trending higher, but not significant

Supplementary Table 3. Cancer-Immu analysis of ARF6 pathway genes.

Gene	Immunotherapy Response	Progression Free Survival		Overall Survival	
	p-value	Likelihood ratio	p-value	Likelihood ratio	p-value
***ARF*s**					
ARF1	0.8	0.013	0.91	0.17	0.68
ARF3	0.19	0.078	0.78	0.0013	0.97
ARF4	0.2	4.6	0.032	1.2	0.27
ARF5	0.76	0.0000022	1	1.8	0.18
ARF6	0.006	3.5	0.061	7.1	0.0078
***GEF*s**					
CYTH1	0.00073	11	0.00077	9.7	0.0018
CYTH2	0.32	0.025	0.88	1.5	0.21
CYTH3	0.4	0.0019	0.96	0.39	0.53
CYTH4	0.0085	6.3	0.012	7.6	0.0059
IQSEC1/GEP100	0.49	1.3	0.26	8.6	0.0033
IQSEC2	0.87	0.24	0.62	2.6	0.11
IQSEC3	0.71	1.1	0.29	5.1	0.023
GBF1	0.23	1.6	0.21	2.2	0.14
ARFGEF1	0.44	0.45	0.5	4.3	0.038
ARFGEF2	0.67	0.084	0.77	0.087	0.77
PSD/EFA6	0.0051	6.5	0.011	4.4	0.035
***GAP*s**					
ACAP1	0.00033	11	0.0011	5.6	0.018
ACAP2	0.63	1.2	0.27	0.25	0.61
ACAP3	0.62	0.77	0.38	2.5	0.11
ARAP1	0.51	1.1	0.3	6.4	0.011
ARAP2	0.0071	3	0.084	1.9	0.16
ARAP3	0.59	0.74	0.39	9.2	0.0025
ARFGAP1	0.32	0.99	0.32	0.79	0.37
ARFGAP2	0.13	1	0.32	3.8	0.052
ARFGAP3	0.6	0.028	0.87	0.048	0.83
ASAP1	0.14	0.88	0.35	0.73	0.39
ASAP2	0.51	0.16	0.69	1.4	0.23
ASAP3	0.41	0.37	0.54	1.5	0.22
GIT1	0.78	0.013	0.91	0.39	0.53
GIT2	0.24	0.098	0.75	0.63	0.43
SMAP1	0.14	0.28	0.6	5.8	0.016
SMAP2	0.021	3	0.081	4	0.045
ADAP1	0.38	0.062	0.8	0.51	0.48
ADAP2	0.18	1.2	0.27	0.091	0.76
AGAP1	0.2	0.56	0.45	0.24	0.62
AGAP2	0.23	2	0.16	1.3	0.26
AGAP3	0.92	1.1	0.29	0.038	0.85
AGAP4	0.88	0.89	0.35	0.85	0.36
AGFG1	0.82	0.011	0.92	0.25	0.61
AGFG2	0.49	0.16	0.69	0.12	0.73
PDCD6IP	0.82	0.034	0.85	0.00097	0.98

Cancer-Immu expression analysis, aggregated data from n=13 queried melanoma clinical studies, adjusted p-values, Benjamini and Hochberg procedure, LR=likelihood ratio with df=1. Related to Figure 4 and Supplementary Figure 4.

Supplementary Table 4. Key resource table

Antibodies

Target Antigen	Fluorophore	Clone	Vendor	Catalog#	Working Dilution
Antibodies (Flow Cytometry-Cell surface staining)					
B220	FITC	RA3-6B2	BioLegend	103206	1:400
CD3	Percp-cy5.5	17A2	BioLegend	100218	1:200
CD3	PE	17A2	BioLegend	100205	1:400
CD4	AF700	GK1.5	BioLegend	100430	1:400
CD45	BV711	30-F11	BioLegend	103147	1:1000
CD8a	PE/Cy7	53-6.7	BioLegend	100722	1:200
CD8a	APC	53-6.7	BioLegend	100712	1:200
CD11b	APC/Cy7	M1/70	BioLegend	101226	1:200
CD11c	PE	N418	BioLegend	117308	1:200
CD16/32	-	S17011E	BioLegend	156604	1:200
F4/80	APC	BM8	BioLegend	123130	1:400
IFNγR1(CD119)	PE	2E2	ThermoFisher	A16396	1:200
IFNγR1(CD119)	PE	GIR-94	BioLegend	308704	1:200
IFNγR1(CD119)	PE	GIR-208	BioLegend	308606	1:200
Ly6C	BV421	1A8	BioLegend	127628	1:200
Ly6G	BV605	HK1.4	BioLegend	128035	1:200
MHCII	BV510	M5/114.15.2	BioLegend	107636	1:1000
NK1.1	Percp	PK136	BioLegend	108725	1:200
PD-1	PE/Cy7	29F.1A12	BioLegend	135216	1:200
PD-L1(CD274)	PE	10F.9G2	BioLegend	124308	1:200
H-2Kd/H-2Dd	PE	34-1-2S	BioLegend	114708	1:100
IgG2a,k isotype Ctrl	PE	MOPC-173	BioLegend	400212	1:100
Human CD119	PE	GIR-208	BioLegend	308606	1:100
Antibodies (Flow Cytometry-Intracellular staining)					
Foxp3	FITC	FJK-16s	BioLegend	11-5773-82	1:200
Foxp3	eFluor506	FJK-16s	ThermoFisher	69-5773-82	1:200
GzmB	PE	NGZB	ThermoFisher	12-8898-82	1:200
GzmB	APC	NGZB	ThermoFisher	17-8898-82	1:200
IFNγ	FITC	XMG1.2	BioLegend	505806	1:100
IFNγR1(CD119)	PE	2E2	Abcam	ab95673	1:100
PD-L1(CD274)	PE	10F.9G2	BioLegend	124308	1:200
Antibodies (Western Blot-Primary antibodies)					
ARF6	-	D12G6	Cell Signaling Technology	5740s	1:1000
a-tubulin	-	DM1A	Cell Signaling Technology	3873s	1:1000
ARF1	-	-	Invitrogen	PA1-127	1:1000
GAPDH	-	D16H11	Cell Signaling Technology	5174	1:10000
IFNγR1	-	EPR24127-89	Abcam	ab280353	1:1000
IFNγR1	-	D-3	Santa Cruz Biotechnology	SC-28363	1:1000
IDO	-	D5J4E	Cell Signaling Technology	86630S	1:1000
IDO	-	D8W5E	Cell Signaling Technology	5185S	1:1000
CD80	-	E6J6N	Cell Signaling Technology	54521S	1:1000

JAK1	-	6G4	Cell Signaling Technology	3344S	1:1000
p-JAK1(Tyr1034/1035)	-	D7N4Z	Cell Signaling Technology	74129S	1:1000
Lamp1	-	D2D11	Cell Signaling Technology	9091S	1:1000
PD-L1	-	D4H1Z	Cell Signaling Technology	60475S	1:1000
PD-L1	-	E1L3N	Cell Signaling Technology	13684S	1:1000
STAT1	-	D1K9Y	Cell Signaling Technology	14994S	1:1000
p-STAT1(Tyr701)	-	D4A7	Cell Signaling Technology	7649S	1:1000
LSECtin	-	EPR13724	Abcam	ab181196	1:1000
Galectin-3/LGALS3	-	-	Cell Signaling Technology	12733S	1:1000
Galectin-3/LGALS3	-	D4I2R	Cell Signaling Technology	87985S	1:1000
CYTH1	-	2E11	ThermoFisher	MA1-060	1:1000
DYKDDDDK Tag	-	D6W5B	Cell Signaling Technology	14793S	1:1000
Antibodies (Western Blot-Secondary antibodies)					
IgG	-		Jackson ImmunoResearch	715-035-152	1:5000
IgG	-		Jackson ImmunoResearch	711-035-152	1:5000 (1:10000 only for GAPDH)

Chemical Compounds

Chemical compound	Vendor	Catalog#
Recombinant Murine IFNγ	PeproTech	315-05
Recombinant Human IFNγ	PeproTech	300-02
Bafilomycin A1	Sigma-Aldrich	SML1661
MG132	MedChemExpress	HY-13259
QS11	Tocris Bioscience	3324
SecinH3	MedChem Express	HY-100559

Oligonucleotides

Oligonucleotides (qRT-PCR)	
Ifnγr1 Forward sequence	5'-CTTGAACCCTGTCGTATGCTGG-3'
Ifnγr1 Reverse sequence	5'-TTGGTGCAGGAATCAGTCCAGG-3'
Gapdh Forward sequence	5'-AGGTCGGTGTGAACGGATTTG-3'

Gapdh Reverse sequence	5’- TGTAGACCATGTAGTTGAGGTCA-3’
Pd-l1(Cd274) Forward sequence	5’-GCTCCAAAGGACTTGTACGTG-3’
Pd-l1(Cd274) Reverse sequence	5’- TGATCTGAAGGGCAGCATTTC-3’
Ido1 Forward sequence	5’-GGGCTTCTTCCTCGTCTCTC-3’
Ido1 Reverse sequence	5’-TGGATACAGTGGGGATTGCT-3’
Oligonucleotides (PCR)	
Arf6 Forward sequence	5’- TGAGGCATACACCATTATTGCTCC -3’
Arf6 Reverse sequence	5’- GTAATAGCAGTGTAATGTTCCAGTTG -3’

Supplementary Table 5. Gene expression markers for distinguishing immune cell lineages.

Cell Type	Identity Genes	Exclusion Genes
B cell[1]	*Cd45, B220(Cd45r), Cd19, Cd38,* either *immunoglobulin (Ig)*	*IgD*
CD4+[2]	*Cd45, Cd3, Cd4*	*Cd8*
CD8+[2]	*Cd45, Cd3, Cd8*	*Cd4*
DC[3]	*Cd45, Itgax (CD11c), MhcII*	*Cd3*, *Adgre1(F4/80)*
Macrophage[4-7]	*Cd45, Itgam (Cd11b), Csf1r(Cd115), Adgre1(F4/80), Cd68*	
MDSC-PMN[8-9]	*Cd45, Ly6G, Cd11b, Cd84, Cd244, Cd36*	
Mono/Mac[4-7]	*Cd45, Itgam (Cd11b), Csf1r(Cd115), Adgre1(F4/80), Cd68, Ly6c, Cd62l*	
Monocyte[4-7]	*Cd45, Ly6c, Itgam (Cd11b), Csf1r(Cd115)*	
Naïve B cell[1]	*Cd45, Cd19, IgD*	
Neutrophil[8]	*Cd45, Ly6G, Cd11b(Itgam), Ly6c^{Low}*	*Csf1r(Cd115)*, *Adgre1(F4/80), Cd144*
NK[10]	*Cd45, NK1.1*	
Treg[2]	*Cd45, Cd3, Cd4, Foxp3*	*Cd8*
CD8_EarlyActive[2]	*Cd45, Cd3, Cd8, Pd-1$^{Low/Intermediate}$*	*Cd4*
CD8_EffectorMemory[2]	*Cd45, Cd3, Cd8, Gzm, Pd-1$^{Low/Intermediate}$*	*Cd4*
CD8_NaïveLike[2]	*Cd45, Cd3, Cd8, CD62L, CCR7, LFA-1*	*Cd44*
Tfh[2]	*Cd45, Cd3, Cd4, Cxcr5, Tox, Slamf6*	*Cd8*
Th1[2]	*Cd45, Cd3, Cd4, Ifngr1, Fasl*	*Cd8*
CD8_Tex[2]	*Cd45, Cd3, Cd8, Gzm^{Hi}, PD-1* either *Ctla4, Lag3, Tigit, Havcr2/Tim3*	*Cd4*
CD8_Tpex[2]	*Cd45, Cd3, Cd8, Gzm^{Low}, PD-1, Tcf7, Ctla4, Tox*	*Cd4*
CD4_Naive_Like[2]	*Cd45, Cd3, Cd4, Tcf7, Ccr7*	*Cd8, Pd-1 and Tnfrsf9/4-1bb*

Identity genes for validation in Single R and ProjecTIL data sets. The abbreviations used in the table are as follows: *B220* (*B-lymphocyte antigen 220*), *Cd* (*Cluster of Differentiation*), *Ig* (*Immunoglobulin*), DC (Dendritic Cell), *Itgax* (*Integrin alpha X*), *MhcII* (*Major Histocompatibility Complex Class II*), *Itgam* (*Integrin alpha M*), *Adgre1* (*Adhesion G protein-coupled receptor E1*), *Csf1r* (*Colony stimulating factor 1 receptor*), MDSC-PMN (Myeloid-Derived Suppressor Cell – Polymorphonuclear), Mono (Monocyte), Mac (Macrophage), *Foxp3* (*Forkhead box P3*), NK (Natural Killer), Treg (Regulatory T cell), Tfh (T follicular helper cell), Th1 (T helper 1 cell), *Pd-1* (*Programmed cell death protein 1*), *Gzm* (*Granzyme*), *Ccr7* (*C-C chemokine receptor type 7*), *LFA-1* (*Lymphocyte*

function-associated antigen 1), *Cxcr5* (*C-X-C chemokine receptor type 5*), *Tox* (*Thymocyte selection-associated high mobility group box protein*), *Slamf6* (*Signaling lymphocytic Activation Molecule Family Member 6*), *Ifngr1* (*Interferon gamma receptor 1*), *Fasl* (*Fas ligand*), *Ctla4* (*Cytotoxic T-lymphocyte-associated protein 4*), *Lag3* (*Lymphocyte-activation gene 3*), *Tigit* (*T cell immunoreceptor with Ig and Itim domains*), *Havcr2/Tim3* (*Hepatitis A virus cellular receptor 2*, also known as *T cell immunoglobulin and mucin domain-containing protein 3*), *Tcf7* (*Transcription factor 7*), and *Tnfrsf9/4-1bb* (*Tumor necrosis factor receptor superfamily member 9*).

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