

Supplementary Appendix B

Table S1: Characteristics of patients with melanoma brain metastases that underwent transcriptome sequencing

Patient No.	Age	Sex	Primary Melanoma Type	Stage at Start of Systemic Therapy	Mutation	OS (Months)	Overall Survival Follow up (Months)	First Line Systemic Treatment	Best Intracranial Response to First Line Treatment	Time to Progression First Line	Second Line Systemic Treatment	Time to Excision From Initiation of Systemic Treatment (Months)	Prior Radiation to Excised Metastasis	Extracranial Metastasis
1	60	M	Cutaneous	M1D	V600E	41.66		D-T	CR	24.15	None	31.34 [#]	SRS	Inguinal node
2	54	M	Unknown	M1D	V600K		50.33	D-T	PD	3.45	I-N	19.19	No	None
3	46	F	Unknown	M1D	V600K		55.43	D-T	CR	12.65	V-C	30.32	No	None
4	52	M	Cutaneous	M1D	V600E		96.16	Dabrafenib	PD	0.23	Ipi	N/A	No	Inguinal node (n=2)
5	62	F	Cutaneous	M1D	V600E	10.15		D-T	PD	1.74	I-N	N/A	No	Gall bladder, inguinal node
6	64	M	Cutaneous	M1D	V600K	44.98		D-T	PD	11.66	E-C	N/A	No	None
7	51	F	Cutaneous	M1D	V600K	3.78		D-T	PD	3.02	I-N	N/A	No	None
8	45	M	Unknown	M1D	V600E		32.43	D-T	N/A		I-N	N/A	No	None
9	53	M	Cutaneous	M1D	V600E	10.32		D-T	PR	4.60	Pembro	N/A	No	None
10	46	M	Cutaneous	M1D	V600E	5.82		Dabrafenib	Unknown	5.82	None	N/A	No	None
11	33	M	Cutaneous	M1D	V600E	29.27		D-T	PD	2.69	Ipi	N/A	No	None
12	69	M	Cutaneous	III	V600K	5.88		Dabrafenib	N/A	3.42	None	N/A	No	None
13	36	F	Cutaneous	M1D	V600E	4.11		D-T	PD	3.29	None	N/A	No	None
14	45	M	Cutaneous	M1D	V600E		45.90	D-T	PR	10.94	I-N	N/A	No	None
15	67	M	Unknown	M1D	V600K	7.82		I-N	PD	2.89	D-T	N/A	No	None
16	56	M	Cutaneous	M1D	V600K		38.93	V-C	PD	12.91	D-T	N/A	No	None
17	50	M	Cutaneous	M1D	V600E	1.97		I-N	PD	0.43	None	N/A	No	None

18	37	F	Unknown	M1D	V600E		76.35	D-T	PR	13.57	Pembro	N/A	No	None
19	66	M	Cutaneous	M1D	V600E	4.93		Dabrafenib	PD	3.91	None	N/A	No	None
20	50	M	Cutaneous	M1A	V600E		79.15	D-T	N/A	19.22	I-N	33.05	SRS	Lung and extra-peritoneal nodule
21	50	F	Cutaneous	M1C	V600E	34.69		D-T	N/A	15.67	I-N	31.51	SRS	Axillary node
22	77	M	Cutaneous	M1A	V600E	50.86		D-T	N/A	49.15	None	49.54	No	Abdominal wall
23	93	M	Cutaneous	M1D	V600K	27.79		D-T	PR	9.30	Pembro	9.46	No	Intransit
24	50	M	Cutaneous	M1C	V600E	26.78		D-T	N/A	15.28	D-T	15.57	No	Inguinal node
25	66	M	Cutaneous	M1D	V600E	10.32		Vemurafenib	PD	1.81	None	2.92	No	Inguinal node
27	49	M	Cutaneous	M1D	V600E	15.28		D-T*	PR	5.48	V-C	6.97	SRS	Pelvic lymph node
28	39	F	Cutaneous	M1D	V600E	32.82		D-T	SD	11.47	Pembro	26.41	No	Inguinal node
29	50	M	Cutaneous	M1D	V600K		17.71	D-T	SD	6.93	I-N	11.04	No	None
30	31	F	Cutaneous	M1C	V600E		108.32	V-C	N/A	5.82	Temozolamide	14.62	SRS	None

Clinical characteristics of melanoma brain metastases that underwent transcriptome sequencing. A total of 29 patients with 18 brain metastases naïve to treatment with 14 excised with prior progression of BRAF-MEKi treatment were sequenced and underwent analysis. Additionally, 14 patient matched extracranial metastases were also sequenced that were sampled prior to initiation of systemic therapy. Patients 1 and 2 had matched naïve and progression tumours sequenced. Naïve brain metastases were analysed from patients 1-19 inclusive except patient 3. Progressive brain metastases were analysed from patients 1-3 and 20-30 inclusive apart from 26 (not shown) which was excluded from analysis due to technical factors.

Patient 1 had two progressive tumours excised at 31.34 and 36.76 months which were included in the analysis

* Patient 27 swapped from dabrafenib-trametinib to vemurafenib-cobimetinib due to poor tolerability.

Unknown primary – denotes primary melanoma that could not be located. D-T: Dabrafenib-trametinib, E-C: Encorafenib-binimetinib, V-C: Vemurafenib cobimetinib, I-N: Ipilimumab-nivolumab, SRS: stereotactic radiosurgery, SD: stable disease, PD: progressive disease, PR: partial response,

Supplementary Appendix B**Table S2: RNA sequencing and mapping statistics**

	Total Input Reads	Uniquely Mapped Reads	Exonic Reads	Intergenic Reads	Intronic Reads
Median – no. reads x 10 ⁶ (%)	85.61	57.83 (70.69)	30.28 (26.43)	14.51 (11.60)	71.13 (59.30)
Average – no. reads x 10 ⁶ (%)	88.28	58.37 (67.68)	32.85 (27.31)	14.96 (13.56)	71.96 (59.13)
St Dev – no. reads x 10 ⁶ (%)	22.45	22.42 (12.91)	14.56 (6.08)	4.74 (5.75)	30.21 (7.36)
Minimum – no. reads x 10 ⁶ (%)	39.96	16.31 (32.73)	10.94 (16.71)	5.25 (7.45)	13.66 (40.11)
Maximum – no. reads x 10 ⁶ (%)	137.40	115.38 (84.37)	73.21 (44.48)	27.27 (38.90)	140.85 (69.14)

All samples were sequenced as a single batch at the Australian Genomics Research Facility using the Agilent Novoseq 6000. Counts reflect paired reads. A total of 29 patients with 18 brain metastases naïve to treatment with 14 excised with prior progression on BRAF-MEKi treatment were sequenced and underwent analysis. Additionally, 14 patient matched extracranial metastases were also sequenced that were sampled prior to initiation of systemic therapy.

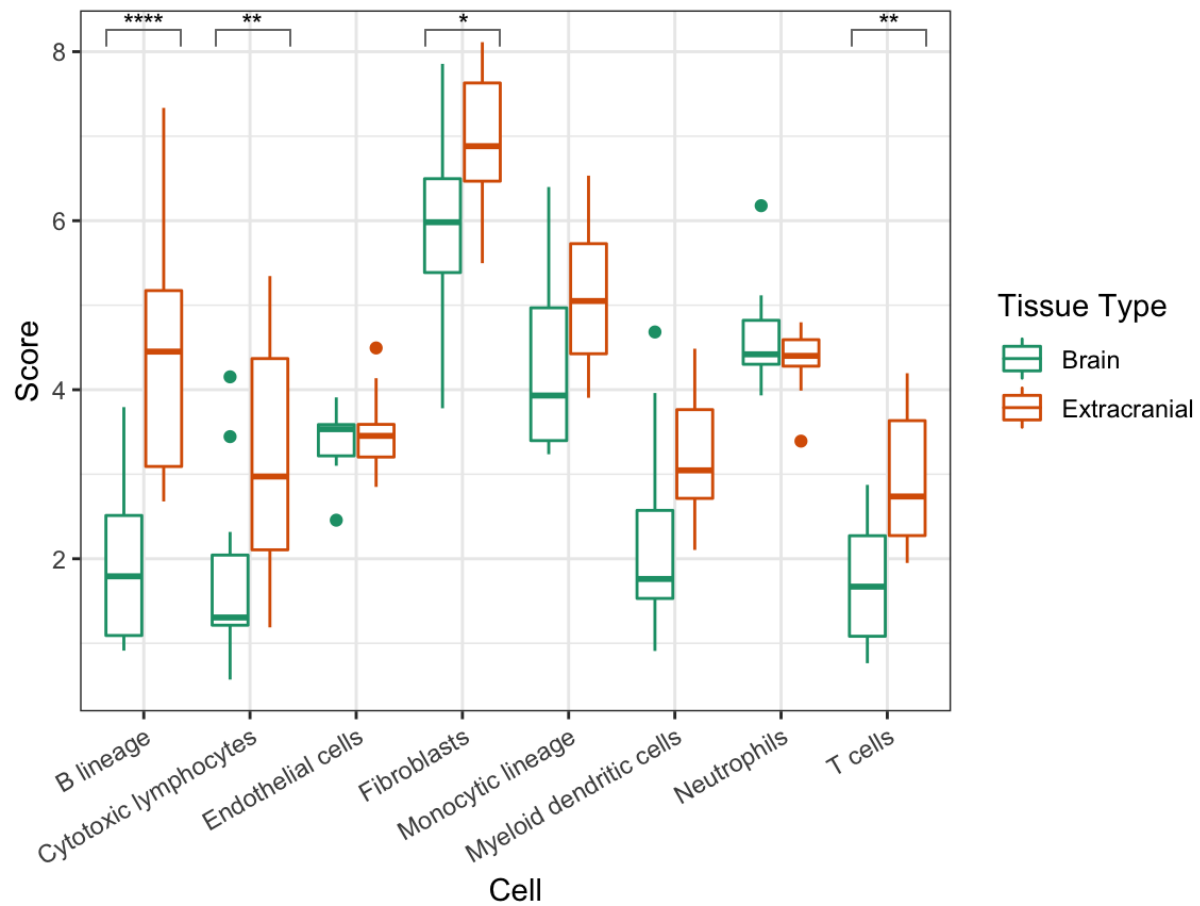
Supplementary Appendix B**Table S3: Gene set enrichment analysis of patient matched brain and extracranial metastases**

Hallmark Pathway	No. Genes	Direction	P Value	FDR
Hallmark Glycolysis	169	Up	0.00138	0.0606
Hallmark Adipogenesis	177	Up	0.00268	0.0606
Hallmark UV Response Up	133	Up	0.00476	0.0606
Hallmark Allograft Rejection	153	Down	0.00485	0.0606
Hallmark Heme Metabolism	162	Up	0.00629	0.0629
Hallmark TGF Beta Signaling	52	Down	0.0102	0.084
Hallmark KRAS Signaling Up	151	Down	0.0127	0.084
Hallmark Unfolded Protein Response	105	Up	0.0147	0.084
Hallmark Reactive Oxygen Species Pathway	45	Up	0.0171	0.084
Hallmark IL2 Stat5 Signaling	174	Down	0.0193	0.084
Hallmark DNA Repair	142	Up	0.02	0.084
Hallmark Interferon Gamma Response	184	Down	0.0214	0.084
Hallmark Hypoxia	168	Up	0.0219	0.084
Hallmark UV Response Dn	136	Down	0.0254	0.0908
Hallmark Fatty Acid Metabolism	132	Up	0.0363	0.118
Hallmark Epithelial Mesenchymal Transition	178	Down	0.0377	0.118
Hallmark Angiogenesis	29	Down	0.04	0.118
Hallmark Oxidative Phosphorylation	183	Up	0.047	0.121
Hallmark Xenobiotic Metabolism	142	Up	0.048	0.121
Hallmark Inflammatory Response	154	Down	0.0483	0.121

Hallmark Gene set enrichment analysis of patient matched brain (n = 13) compared to extracranial metastases (n = 14). For this exploratory analysis, gene pathways with p value < 0.05 and False discovery rate < 0.15 were considered statistically relevant. Gene sets denoting metabolic pathways were enriched in brain compared to extracranial metastases such as glycolysis, adipogenesis, fatty acid metabolism and oxidative phosphorylation. Immune signalling gene sets such as TGF- β signalling, IL-2 Stat 5, IFN- γ response and angiogenesis were enriched in extracranial lesions in comparison.

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Figure S1: MCPcounter analysis of patient matched melanoma brain metastases vs extracranial metastases



MCPcounter Analysis of Patient Matched Melanoma Brain Metastases (n = 13) vs Extracranial Metastases (n = 14). Extracranial lesions exhibited higher B Cell lineage, cytotoxic lymphocytes, fibroblast and T cell scores compared to melanoma brain metastases.

Statistical significance assessed using two tailed Mann-whitney * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001

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Table S4: Differentially expressed genes of naïve and progressive melanoma brain metastases

Rank	Gene Name	Gene Biotype	logFC	logCPM	p value	FDR
1	ACAN	protein_coding	4.266	5.229	2.93E-07	9.61E-04
2	ARHGEF5	protein_coding	3.551	4.982	3.20E-07	9.61E-04
3	TSPAN12	protein_coding	3.200	3.313	1.92E-07	9.61E-04
4	ANPEP	protein_coding	2.998	4.526	3.43E-07	9.61E-04
5	EMILIN2	protein_coding	2.060	5.980	7.34E-08	9.61E-04
6	SNX22	protein_coding	2.402	3.393	8.24E-07	0.00165004
7	ACOT11	protein_coding	1.965	4.332	7.34E-07	0.00165004
8	CXCL2	protein_coding	2.227	1.867	2.53E-06	0.00443418
9	CLEC5A	protein_coding	2.178	3.229	3.47E-06	0.00540213
10	TREM1	protein_coding	2.788	3.107	1.03E-05	0.0102764
11	CHI3L1	protein_coding	2.837	6.892	1.14E-05	0.0102764
12	SCARA3	protein_coding	2.347	4.123	8.41E-06	0.0102764
13	STEAP3	protein_coding	1.986	3.756	4.12E-06	0.00577527
14	LUCAT1	lincRNA	2.660	3.794	1.32E-05	0.0102764
15	CA12	protein_coding	2.387	2.918	1.39E-05	0.0102764
16	G0S2	protein_coding	2.959	2.975	2.46E-05	0.01380234
17	TG	protein_coding	1.930	1.705	1.02E-05	0.0102764
18	CD36	protein_coding	2.021	4.738	1.39E-05	0.0102764
19	RPS6KA2	protein_coding	1.858	5.514	1.12E-05	0.0102764
20	CRMP1	protein_coding	2.222	3.062	1.94E-05	0.01254751
21	MROH7	protein_coding	1.901	1.717	1.33E-05	0.0102764
22	PDE1C	protein_coding	2.285	3.729	2.44E-05	0.01380234
23	CD300E	protein_coding	2.192	2.040	2.30E-05	0.01380234
24	SERPINA1	protein_coding	2.304	5.213	2.71E-05	0.01385224
25	CLMP	protein_coding	2.479	3.225	3.28E-05	0.0143534
26	IL8	protein_coding	2.718	5.352	4.22E-05	0.01518659

27	SNORD116-19	snoRNA	-3.370	4.177	6.51E-05	0.02027677
28	MAP1B	protein_coding	1.675	9.550	1.79E-05	0.01252503
29	TGFBI	protein_coding	1.891	7.021	2.99E-05	0.01399727
30	CHI3L2	protein_coding	1.856	3.064	3.37E-05	0.0143534
31	HAP1	protein_coding	2.184	2.814	4.68E-05	0.01625422
32	KCNN3	protein_coding	1.583	2.720	2.77E-05	0.01385224
33	CCDC136	protein_coding	1.944	5.528	3.89E-05	0.0143534
34	LRRN4CL	protein_coding	-2.111	4.435	5.22E-05	0.01728583
35	DNAH7	protein_coding	2.224	2.998	5.42E-05	0.01728583
36	NFASC	protein_coding	1.756	5.636	3.81E-05	0.0143534
37	DKK3	protein_coding	1.597	5.361	3.55E-05	0.0143534
38	TACC2	protein_coding	1.564	3.906	3.58E-05	0.0143534
39	IRAK3	protein_coding	1.970	5.612	5.34E-05	0.01728583
40	SOD2	protein_coding	1.215	8.193	1.97E-05	0.01254751
41	EPHX1	protein_coding	1.167	5.178	3.77E-05	0.0143534
42	CCL2	protein_coding	1.680	4.257	6.83E-05	0.02036597
43	TPD52L1	protein_coding	1.753	3.790	7.36E-05	0.02107629
44	MUC1	protein_coding	1.306	2.143	4.75E-05	0.01625422
45	FAM20C	protein_coding	1.920	5.731	8.78E-05	0.02366727
46	FMOD	protein_coding	2.013	4.148	1.11E-04	0.02717091
47	HEG1	protein_coding	0.954	6.043	3.59E-05	0.0143534
48	GPD1L	protein_coding	-0.892	5.037	2.94E-05	0.01399727
49	SAMD14	protein_coding	1.348	1.656	7.03E-05	0.0205353
50	NHS	protein_coding	1.410	2.695	8.08E-05	0.02267148
51	SPHK1	protein_coding	1.444	3.050	9.35E-05	0.02473851
52	OSMR	protein_coding	1.605	5.328	1.12E-04	0.02717091
53	AQP9	protein_coding	2.375	3.427	1.54E-04	0.03217733
54	CD163L1	protein_coding	1.534	3.438	1.12E-04	0.02717091

55	SPEG	protein_coding	1.949	3.733	1.46E-04	0.03147874
56	UBTD2	protein_coding	-0.708	4.955	2.69E-05	0.01385224
57	ABI3BP	protein_coding	1.714	3.132	1.43E-04	0.03131073
58	C12orf76	protein_coding	-1.438	6.690	1.24E-04	0.02907412
59	ACE	protein_coding	1.453	2.617	1.31E-04	0.03007766
60	EEPD1	protein_coding	1.277	2.872	1.11E-04	0.02717091
61	BAI2	protein_coding	1.361	3.291	1.22E-04	0.0290733
62	CNTNAP1	protein_coding	1.067	4.452	8.45E-05	0.02323696
63	TIMP3	protein_coding	-1.225	8.438	1.36E-04	0.0306834
64	SLC5A4	protein_coding	3.443	5.329	2.18E-04	0.03967831
65	SNX30	protein_coding	-0.732	7.061	6.80E-05	0.02036597
66	MICAL2	protein_coding	1.241	5.474	1.51E-04	0.03215024
67	BCAT1	protein_coding	1.343	5.746	1.65E-04	0.03371056
68	PLXNA4	protein_coding	2.234	2.836	2.16E-04	0.03967831
69	ME3	protein_coding	1.868	2.070	2.06E-04	0.03850849
70	CD93	protein_coding	1.085	5.733	1.39E-04	0.03088344
71	MMP25	protein_coding	2.194	2.262	2.35E-04	0.04163492
72	DNER	protein_coding	2.410	3.427	2.45E-04	0.04179708
73	ITGA5	protein_coding	1.358	5.508	1.90E-04	0.03645266
74	OLFML2B	protein_coding	1.332	4.800	1.88E-04	0.03645266
75	NRP1	protein_coding	1.216	6.716	1.76E-04	0.03476106
76	BCKDHB	protein_coding	-0.821	4.616	1.11E-04	0.02717091
77	RNA5SP161	rRNA	-2.109	9.504	2.47E-04	0.04179708
78	CSMD2	protein_coding	1.963	2.458	2.48E-04	0.04179708
79	ADAMTS14	protein_coding	1.513	2.962	2.26E-04	0.04061663
80	CACNA2D4	protein_coding	1.848	2.052	2.53E-04	0.04179708
81	RAB31	protein_coding	1.030	5.863	1.73E-04	0.03461729
82	DUSP6	protein_coding	-0.976	7.458	1.66E-04	0.03371056

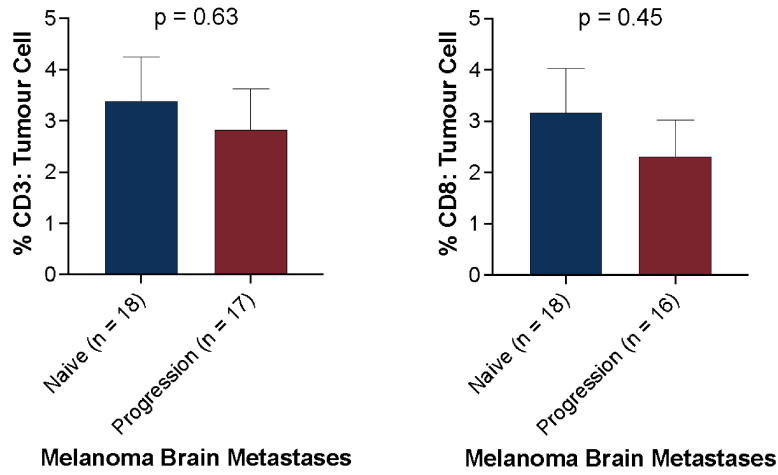
83	ABTB2	protein_coding	-1.131	4.872	2.02E-04	0.0383414
84	TFPI	protein_coding	1.410	5.344	2.56E-04	0.04179708
85	FAAH	protein_coding	1.291	1.035	2.62E-04	0.04227024
86	ACTN2	protein_coding	1.933	3.202	2.92E-04	0.04596313
87	RAPGEFL1	protein_coding	0.942	2.958	2.41E-04	0.04179708
88	CEBPB	protein_coding	1.051	5.929	2.56E-04	0.04179708
89	NCR3LG1	protein_coding	1.697	3.721	2.97E-04	0.04626502
90	ASTN2	protein_coding	1.098	3.424	2.79E-04	0.04438875
91	CCDC19	protein_coding	1.213	0.377	3.01E-04	0.0464555
92	TRABD2A	protein_coding	1.825	2.149	3.24E-04	0.04810431
93	GRIN2D	protein_coding	1.400	1.985	3.15E-04	0.04794728
94	ABCC3	protein_coding	1.896	4.286	3.26E-04	0.04810431
95	GPR162	protein_coding	1.576	1.135	3.23E-04	0.04810431
96	TMC7	protein_coding	1.559	1.925	3.33E-04	0.04870214
97	NOVA2	protein_coding	1.469	2.939	3.50E-04	0.05060804
98	MPZL2	protein_coding	1.643	3.968	3.58E-04	0.05128482
99	DPP4	protein_coding	2.006	2.602	3.64E-04	0.05158836
100	TMEM117	protein_coding	-0.982	5.121	3.73E-04	0.05197931
101	ST14	protein_coding	1.748	2.756	3.74E-04	0.05197931
102	TPRG1	protein_coding	1.433	2.870	3.88E-04	0.05228727
103	ALPK3	protein_coding	1.188	3.421	3.88E-04	0.05228727
104	EFEMP1	protein_coding	1.487	5.202	3.91E-04	0.05228727
105	FGFR3	protein_coding	3.375	5.345	3.91E-04	0.05228727
106	ITGA1	protein_coding	1.008	6.606	3.99E-04	0.05269083
107	DNAH6	protein_coding	1.758	2.049	4.02E-04	0.05269083
108	CREB3L1	protein_coding	1.757	2.386	4.14E-04	0.05348077
109	RP11-792D21.2	lincRNA	1.255	2.792	4.16E-04	0.05348077
110	SIAH1	protein_coding	-0.707	5.227	4.36E-04	0.05539379

111	PTPN14	protein_coding	-0.793	7.824	4.42E-04	0.05539379
112	SPOCD1	protein_coding	1.836	3.188	4.42E-04	0.05539379
113	SLC11A1	protein_coding	1.643	5.141	4.51E-04	0.05571458
114	SRPX	protein_coding	1.140	5.993	4.53E-04	0.05571458
115	LGI2	protein_coding	1.344	1.310	4.64E-04	0.05656141
116	MAP1A	protein_coding	1.634	6.551	4.76E-04	0.05749064
117	PCOLCE	protein_coding	2.014	5.726	4.83E-04	0.05790029
118	ELL2	protein_coding	1.081	6.189	4.89E-04	0.05809489
119	PCDH17	protein_coding	1.082	4.022	4.95E-04	0.05837914
120	CTD-2267D19.6	lincRNA	1.198	0.666	5.00E-04	0.05837914
121	PROCR	protein_coding	1.254	1.199	5.09E-04	0.05902383
122	COLEC12	protein_coding	1.139	5.394	5.15E-04	0.05918391
123	PLIN3	protein_coding	0.643	4.803	5.36E-04	0.0607334
124	MMP19	protein_coding	1.458	3.491	5.41E-04	0.0607334
125	PDE1A	protein_coding	1.449	2.586	5.41E-04	0.0607334
126	MAPK8IP1	protein_coding	1.449	3.465	5.58E-04	0.0620853
127	CACNB2	protein_coding	1.799	2.277	5.64E-04	0.06225968
128	CSF3R	protein_coding	1.828	5.217	5.77E-04	0.06323609
129	CTNND2	protein_coding	2.263	4.200	6.06E-04	0.06554697
130	SOCS3	protein_coding	1.153	5.043	6.08E-04	0.06554697
131	CLCN5	protein_coding	-0.869	6.454	6.15E-04	0.06581986
132	RPL10A	protein_coding	-0.781	7.475	6.21E-04	0.06596586
133	AGPAT4	protein_coding	1.498	3.525	6.44E-04	0.06790351
134	MAP3K8	protein_coding	1.246	4.479	6.52E-04	0.06822371
135	RNA5SP481	rRNA	-1.987	6.857	6.68E-04	0.06942797
136	ALDH1A2	protein_coding	1.463	2.308	6.75E-04	0.06960721
137	CLU	protein_coding	1.976	7.668	6.87E-04	0.07035545
138	FOSL2	protein_coding	1.048	6.376	6.99E-04	0.07099613

139	DYNC111	protein_coding	1.217	3.999	7.08E-04	0.07145833
140	ARHGAP33	protein_coding	0.878	3.707	7.26E-04	0.07269674
141	OCIAD2	protein_coding	1.458	1.894	7.39E-04	0.07354148
142	TMBIM4	protein_coding	-1.995	6.160	7.62E-04	0.07527197
143	IL10	protein_coding	1.449	1.153	7.74E-04	0.07544209
144	POR	protein_coding	0.870	5.381	7.78E-04	0.07544209
145	SOX5	protein_coding	-1.124	6.880	7.80E-04	0.07544209
146	GLIPR1	protein_coding	1.239	4.923	7.91E-04	0.0754959
147	C22orf34	protein_coding	1.344	3.417	7.91E-04	0.0754959
148	CD163	protein_coding	1.589	8.908	7.99E-04	0.07568597
149	FABP7	protein_coding	1.726	3.839	8.32E-04	0.07829088
150	ADORA1	protein_coding	1.526	1.695	8.66E-04	0.08093207
151	GJA1	protein_coding	1.284	4.872	8.85E-04	0.08193437
152	HRH1	protein_coding	1.276	2.174	8.88E-04	0.08193437
153	EML6	protein_coding	1.312	2.562	9.13E-04	0.0834609
154	MFSD2A	protein_coding	1.516	2.404	9.16E-04	0.0834609
155	AP1B1	protein_coding	0.644	6.687	9.27E-04	0.08349724
156	NEB	protein_coding	1.172	4.467	9.29E-04	0.08349724
157	SLC1A3	protein_coding	1.213	6.849	9.45E-04	0.08400495
158	TBC1D8	protein_coding	0.881	4.628	9.46E-04	0.08400495
159	CXCL14	protein_coding	1.822	2.155	9.55E-04	0.08421171
160	IGF1R	protein_coding	-1.042	8.270	9.68E-04	0.08421171
161	MBOAT1	protein_coding	-0.951	5.304	9.77E-04	0.08421171
162	NTRK2	protein_coding	1.678	5.695	9.77E-04	0.08421171
163	CD209	protein_coding	1.661	3.495	9.79E-04	0.08421171
164	KBTD7	protein_coding	-0.827	4.129	0.00101707	0.08697215
165	ARHGAP42	protein_coding	-0.894	5.845	0.00103724	0.08815914
166	ZNF117	protein_coding	0.959	4.424	0.00104847	0.08833382

167	SLC45A3	protein_coding	1.330	1.878	0.00105189	0.08833382
168	SUSD1	protein_coding	0.939	4.027	0.001086	0.09065479
169	LOX	protein_coding	2.259	5.949	0.00111892	0.09285045
170	NUMBL	protein_coding	0.680	4.881	0.00112949	0.09317596
171	C2orf82	protein_coding	1.963	2.902	0.00114913	0.09424237
172	RP11-359E10.1	lincRNA	1.587	2.239	0.00116102	0.09466341
173	MAOB	protein_coding	1.999	4.137	0.00117243	0.09504125
174	TNFAIP3	protein_coding	1.205	5.954	0.00118914	0.09584204
175	STC1	protein_coding	1.984	5.675	0.00122774	0.09720429
176	ITGAM	protein_coding	1.173	4.539	0.0012284	0.09720429
177	GNG11	protein_coding	1.674	4.686	0.00123377	0.09720429
178	RGS16	protein_coding	1.623	2.513	0.00126123	0.09881267

Differentially expressed genes (n=178) with a false discovery rate < 0.1 and log₂ fold change of ≥ 0.58 or ≤ -0.58 included. A total of 155 out of 178 genes were upregulated with log₂ fold change ≥ 0.58. A total of 96 differentially expressed genes were found with a FDR < 0.05. LogFC denotes log₂ fold change. Log CPM denotes Log counts per million. FDR denotes false discovery rate

Supplementary Appendix B**Figure S2: CD3 and CD8 immunohistochemistry of naïve and progression melanoma brain metastases**

Immunohistochemistry of melanoma brain metastases of CD3 and CD8 as a percentage of tumour content. Statistical significance was determined using unpaired t test. Mean with error bars showing standard error of the mean are displayed.