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PRAME Expression in Uveal Melanoma: prognostic factor and target for Immunotherapy.

SUPPLEMENTARY TABLES:

Table S1: panel of 15 genes used for gene expression profiling (Onken, Worley et al. 2010)

Table S2. Proportion of solid cancers with PRAME expression and prognostic significance of PRAME expression.

Table S3. Proportion of haematological malignancies expressing PRAME and prognostic significance of PRAME expression.

Table S4 Distribution of clinical, histopathological, and genetic features in PRAME-positive and PRAME-negative UM (D3 UM patients from the LUMC cohort, total 24).

Table S5. Distribution of clinical, histopathological, and genetic features in PRAME-positive and PRAME-negative UM (M3 UM patients from the LUMC cohort, total 40).

Table S6. Distribution of clinical, histopathological, and genetic features in UM with low PRAME and high PRAME(D3 UM patients from the TCGA study, total 38).

Table S7. Distribution of clinical, histopathological, and genetic features in UM with low PRAME and high PRAME (M3 UM patients from the TCGA study, total 42).

Table S8: Distribution of BAP1, SF3B1 and EIF1AX mutation in UM with respect to PRAME expression in the TCGA cohort

Table S9: Distribution of EMT markers in PRAME positive and PRAME negative tumours in a cohort of 64 UM patients, Mann Whitney U test.

Table S10: Distribution of infiltrate markers in PRAME positive and PRAME negative tumours in a 24 D3 and 40 M3 UM patients, Mann Whitney U test.

Table S11: Top 20 differentially expressed genes in PRAME-positive UM vs PRAME-negative UM. Table S11a. Most upregulated genes in 29 PRAME-positive UM vs 35 PRAME-negative UM. Table S11b. Most downregulated genes in 29 PRAME-positive UM vs 35 PRAME-negative UM.

Table S1: panel of 15 genes used for gene expression profiling (Onken 2010)

	Symbol	Gene name
Differentially expressed	CDH1	E-cadherin
	ECM1	Extracellular matrix protein 1
	HTR2B	5-Hydroxytryptamine receptor 2B
	RAB31	RAB31 (RAS oncogene family)
	EIF1B	Eukaryotic translation initiation factor 1B
	FXR1	Fragile X mental retardation, autosomal homolog 1
	ID2	Inhibitor od DNA binding 2
	LMCD1	LIM and cysteine-rich domains 1
	LTA4H	Leukotriene A4 hydrolase
	MTUS1	Microtubule-associated tumor suppressor 1
	ROBO1	Roundabout, axon guidance receptor, 1
	SATB1	SATB homeobox 1
Control genes	MRPS21	Mitochondrial ribosomal protein S21
	RBM23	RNA-binding motif protein 23
	SAP130	Sin3A-associated protein , 130 kDa

Table S2. Proportion of solid cancers with PRAME expression and prognostic significance of PRAME expression.					
Tumour	Reference	Method	Nr of samples	Expression	Prognosis
Cutaneous melanoma	(Ikeda, Lethé et al. 1997)	RT-PCR	49	88%	
	(Lezcano, Jungbluth et al. 2018)	IHC	155 primary 100 metastatic	83% in primary tumour (90% excluding desmoplastic) 92% in metastatic	
NSCLC	(Ikeda, Lethé et al. 1997)	RT-PCR	26 adenocarcinomas 65 squamous	46% adenocarcinomas 78% squamous	
	(Bankovic, Stojsic et al. 2010)	AP-PCR	30	73%	
	(Thongprasert, Yang et al. 2016)	qRT-PCR	349: 195 adenocarcinoma 116 squamous	49%: 36% adenocarcinomas 80% squamous	
	(Pan, Su et al. 2017)	qRT-PCR	192: 121 adenocarcinoma 71 squamous	 31% in adenocarcinomas 59% in squamous	No association with OS
Head and neck SCC	(Figueiredo, Mamede et al. 2006)	RT-PCR	33 tumours 15 lymph node tissue	42% of tumours 4/5 lymph node metastases	
	(Cuffel, Rivals et al. 2011)	RT-PCR	57	49%	No impact on survival
Salivary duct carcinoma	(Xu, Jungbluth et al. 2019)	IHC	40	82% (mostly cytoplasmic staining)	No impact on metastasis
Renal cell carcinoma	(Neumann, Engelsberg et al. 1998)	RT-PCR	39 (37 primary tumour, 2 metastases)	40% (predominantly clear cell carcinoma)	No correlation with TNM or tumour grade
Breast cancer	(Ikeda, Lethé et al. 1997)	RT-PCR	169	27%	
	(Epping, Hart et al. 2008)	Microarray	295	High PRAME in 33%: correlated with low differentiation and ER negative status	High PRAME expression correlated with shorter OS and shorter MFS
	(Doolan, Clynes et al. 2008)	RT-PCR	103	53%	PRAME + tumours have shorter DFS and OS; in the adjuvant chemotherapy group: shorter RFS

	(Naik, Thomas et al. 2021)	RNASeq	1066 (TCGA)		High PRAME expression associated with shorter OS (also in subgroup with immune-unfavourable tumours)
Synovial sarcoma	(Iura, Maekawa et al. 2017)	IHC qRT-PCR	108 for IHC 20 for qRT-PCR	IHC: 86% qRT-PCR: 100%	No correlation with survival
	(Luk, van der Steen et al. 2018)	mRNA microarray PRAME specific mRNA FISH	45	mRNA: 100% FISH: 100% (85% homogeneous)	
Myxoid and round cell liposarcoma	(Iura, Kohashi et al. 2015)	IHC Western blot qRT-PCR	93 for IHC 19 for WB 20 for qRT-PCR	IHC: 90% WB: 10/19 qRT-PCR: 100%	Tumours with high PRAME expression have shorter survival
	(Hemminger, Toland et al. 2014)	IHC qPCR	37 for IHC 8 for qPCR	IHC: 100% qPRC: 8/8 (6 high, 2 low)	
Osteosarcoma	(Toledo, Zago et al. 2011)	RT-PCR	29	68%	
	(Tan, Zou et al. 2012)	IHC RT-PCR	69 for IHC 36 for RT-PCR	IHC: 73% RT-PCR: higher PRAME expression in tumour than muscle tissue	Strong PRAME expression correlated to poor prognosis and predicted lung metastases
	(Zou, Shen et al. 2012)	qRT-PCR	28	96%	
Neuroblastoma	(Oberthuer, Hero et al. 2004)	RT-PCR Northern blot qPCR	94	RT-PCR: 93% NB: higher score in advanced stage qPCR: 100%, higher in advanced stage	RT-PCR and NB: PRAME expression correlated with worse survival (not significant) qPCR: tumours with higher PRAME have worse EFS
Medulloblastoma	(Orlando, Miele et al. 2018)	qRT-PCR IHC	60 for RT-PCR 40 for IHC	RT-PCR: 82% expression higher than normal tissue IHC: high PRAME expression	Tumours with high PRAME have worse OS
Epithelial ovarian cancer	(Partheen, Levan et al. 2006)	Microarray qPCR	54 for microarray* 20 for qPCR*		Lower PRAME expression in survivors than in deceased cases
	(Partheen, Levan et al. 2008)	qPCR Western blot IHC	19 for qPCR* 43 for WB* 43 for IHC*		qPCR and WB: higher PRAME in deceased cases than in survivors

					Kaplan-Meier: tumours with high PRAME have lower survival
	(Partheen, Levan et al. 2009)	qPCR Western blot	30 for qPCR† 98 for WB†		No difference in PRAME between survivors and deceased cases
	(Zhang, Barger et al. 2016)	qRT-PCR TCGA data‡: microarray, RNAseq	119	qRT-PCR: 60% PRAME overexpression compared to normal tissue	TCGA: tumours with high PRAME (microarray) have better OS and PFS
Gastric cancer	(Baba, Kanda et al. 2020)	qRT-PCR	300	65% (correlated to differentiated phenotype)	Higher PRAME in stage IV tumours with liver metastases. High PRAME expression predictive of liver recurrence. Tumours with high PRAME have lower OS and DFS.
Urothelial carcinoma	(Dyrskjot, Zieger et al. 2012)	qRT-PCR	350	20% (correlated to high stage and grade)	No influence on survival. Tumours with PRAME expression have worse response to chemotherapy
Hepatocellular carcinoma	(Zhu, Wang et al. 2018)	Western blot IHC qRT-PCR	96	PRAME upregulated in cancer compared to normal tissue (correlated to tumour size, stage and metastases)	Tumours with high PRAME have worse survival
	(Oyama, Kanki et al. 2017)	qRT-PCR	100	27% (correlated with higher stage and number of tumours)	Cases with PRAME + non-tumour tissue have shorter OS and DFS

NSCLC = Non small cell lung cancer, SCC = squamous cell carcinoma, ER = estrogen receptor, GEP = gene expression profiling, OS = overall survival, MFS = metastasis-free survival, DFS = disease-free survival, RFS = relapse-free survival, EFS = event-free survival

*= stage III serous papillary adenocarcinoma

†= stage III-IV serous papillary adenocarcinoma

‡= only high grade serous cancer

Table S3. Proportion of haematological malignancies expressing PRAME and prognostic significance of PRAME expression.					
Tumour	Paper	Method	Samples	Expression	Prognosis
Acute myelogenous leukaemia	(Van Baren, Chambost et al. 1998)	RT-PCR	108	35% (associated with t(8;21)*, t(15;17)*)	
	(Greiner, Ringhoffer et al. 2000)	RT-PCR	34	47% high, 12% low, 41% negative	
	(Matsushita, Ikeda et al. 2001)	Semiquantitative RT-PCR	35	49%	
	(Greiner, Schmitt et al. 2006)	cDNA microarray RT-PCR	116	Higher PRAME in t(8;21)* and t(15;17)*	Cases with high PRAME expression have better survival (not significant)
Childhood acute myelogenous leukaemia	(Steinbach, Hermann et al. 2002)	RT-PCR	50	62% (higher in t(8;21))*	Cases with high PRAME have higher OS and DFS, lower WBC count and blast percentage
Acute lymphoblastic leukaemia	(Van Baren, Chambost et al. 1998)	RT-PCR	90	15%	
	(Watari, Tojo et al. 2000)	RT-PCR		PRAME positivity in Ph-positive cases†	
	(Matsushita, Ikeda et al. 2001)	RT-PCR	14	64%	
Childhood acute lymphoblastic leukaemia	(Steinbach, Viehmann et al. 2002)	RT-PCR	50	42% overexpression	Cases with high PRAME have better survival (not significant), lower WBC count (not significant)
Chronic myelogenous leukaemia	(Watari, Tojo et al. 2000)	RT-PCR		PRAME positivity in blastic crisis	
	(Matsushita, Ikeda et al. 2001)	Semiquantitative RT-PCR	32	42%	
Chronic lymphoblastic leukaemia	(Proto-Siqueira, Falcão et al. 2003)	Semiquantitative RT-PCR	38	16%	
Mantle cell lymphoma	(Proto-Siqueira, Falcão et al. 2003)	Semiquantitative RT-PCR	16	44%	

Non-Hodgkin lymphoma	(Van Baren, Chambost et al. 1998)	RT-PCR	20	3/20	
	(Matsushita, Ikeda et al. 2001)	Semiquantitative RT-PCR	13	23%	
	(Mitsuhashi, Masuda et al. 2014)	IHC qRT-PCR	160 for IHC‡ 20 for qRT-PCR‡	IHC§: 68% 0, 11% 1+, 8% 2+, 13% 3+ qRT-PCR: correlated with IHC	Cases with 3+ staining have worse OS and PFS
	(Takata, Chong et al. 2022)	IHC RNA-Seq	347 for IHC‡ 322 for RNA-Seq‡	IHC: 30%	Cases with high PRAME mRNA expression have longer DSS
Hodgkin lymphoma	(Ercolak, Paydas et al. 2015)	IHC Real time PCR	82	IHC: 18% PCR: 10%	Cases with high PRAME by PCR have shorter DFS and OS
Multiple myeloma	(Van Baren, Chambost et al. 1998)	RT-PCR	9	2/9	
	(Pellat-Deceunynck, Mellerin et al. 2000)	RT-PCR	21	52%	
Myelodysplastic syndromes	(Huang, Wang et al. 2019)	RQ-PCR	1110 ||	67% overexpression	Cases with overexpression have more blasts, worse cytogenetics, higher risk of AML evolution, worse OS and PFS

OS = overall survival, DFS = disease-free survival, PFS = progression-free survival, DSS = disease-specific survival, WBC = white blood cell, Ph = Philadelphia chromosome
*= subtype with good prognosis
†= subtype with bad prognosis
‡= diffuse large B cell lymphoma
§= IHC score: 0 if <25% positive cells, 1+ if 25-50% positive cells, 2+ if 50-75% positive cells, 3+ if >75% positive cells
|| = myelodysplastic syndromes with thrombocytopenia

Table S4. Distribution of clinical, histopathological, and genetic features in PRAME-positive and PRAME-negative UM (D3 UM patients from the LUMC cohort, total 24).

Feature	Patients, No. (%)§||		*p* value
	PRAME negative n=16 (67%)	PRAME positive n=8 (33%)	
Sex			
Male	10 (62%)	6 (75%)	0.67*
Female	6 (37%)	2 (25%)	
Age at enucleation	57.6 (13-82)	51.7 (34-73)	0.27‡
Median Follow up (months)	154.5 (10-219)	98 (17-176)	0.21‡
Largest Basal Diameter	11.3 (9-17)	15.1 (13-18)	0.001‡
Thickness	6.9 (2-11)	7.8 (2-12)	0.4‡
Mitotic count	6.1 (1-23)	5.6 (1-11)	0.93‡
Cell type			
Spindle	7 (44%)	6 (75%)	0.21*
Epithelioid-mixed	9 (56%)	2 (25%)	
Ciliary body involvement			
No	13 (81%)	6 (75%)	1.0*
Yes	3 (19%)	2 (25%)	
Extrascleral extension			
None/superficial	10 (62%)	2 (25%)	0.19*
Deep/total	6 (37%)	6 (75%)	
TNM stage			
I-IIB	14 (87%)	4 (50%)	0.13*
IIIA-IIIC	2 (12%)	4 (50%)	
Tumour pigmentation			
Light	15 (94%)	4 (50%)	0.028*
Dark	1 (6%)	4 (50%)	
8q status			
Normal	9 (60%)	1 (12%)	0.04†
Gain	5 (33%)	4 (50%)	
Amplification	1 (7%)	3 (37%)	
6p status			
Normal	6 (37%)	0 (0%)	0.07*
Gain	10 (62%)	8 (100%)	
BAP1 IHC expression			
Positive	12 (80%)	7 (100%)	0.52*
Negative	3 (20%)	0 (0%)	

*: Fisher's exact test
†: Likelihood ratio
‡: Mann Whitney U test
§: Percentages are rounded and may not total 100
||: Percentages were calculated excluding missing data

Table S5. Distribution of clinical, histopathological, and genetic features in PRAME-positive and PRAME-negative UM (M3 UM patients from the LUMC cohort, total 40).

Feature	Patients, No. (%)||#		*p* value
	PRAME negative n=19 (47.5%)	PRAME positive n=21 (52.5%)	
Sex			
Male	9 (47%)	8 (38%)	0.55*
Female	10 (53%)	13 (62%)	
Age at enucleation	63.1 (27-85)	64 (36-88)	0.95§
Median Follow up (months)	52 (9-178)	30 (2-145)	0.20§
Largest Basal Diameter	13.3 (8-20)	14.7 (9-30)	0.35§
Thickness	7.95 (3-12)	8.2 (2-12)	0.74§
Mitotic count	6.9 (1-33)	6.6 (0-20)	0.71§
Cell type			
Spindle	7 (37%)	2 (9%)	0.06†
Epithelioid-mixed	12 (63%)	19 (90%)	
Ciliary body involvement			
No	13 (68%)	7 (33%)	0.03*
Yes	6 (32%)	14 (67%)	
Extrascleral extension			
None/superficial	11 (58%)	12 (58%)	0.96*
Deep/total	8 (42%)	9 (42%)	
TNM stage			
I-IIB	11 (65%)	7 (33%)	0.05*
IIIA-IIIC	6 (35%)	14 (67%)	
Tumour pigmentation			
Light	13 (68%)	11 (55%)	0.39*
Dark	6 (32%)	9 (45%)	
8q status			
Normal	1 (5%)	2 (9%)	0.01‡
Gain	11 (58%)	3 (14%)	
Amplification	7 (37%)	16 (76%)	
6p status			
Normal	17 (89%)	20 (95%)	0.57†
Gain	2 (10%)	1 (5%)	
BAP1 IHC expression			
BAP1 positive	12 (80%)	7 (100%)	0.68†
BAP1 negative	3 (20%)	0 (0%)	

*: Pearson's χ^2 test

†: Fisher's exact test

‡: Likelihood ratio

§: Mann Whitney U test

||: Percentages are rounded and may not total 100

#: Percentages were calculated excluding missing data

Table S6. Distribution of clinical, histopathological, and genetic features in UM with low PRAME and high PRAME (D3 UM patients from the TCGA study, total 38).

Feature	Patients, No. (%)||#		p value
	Low PRAME n=21 (55%)	High PRAME n=17 (45%)	
Sex			
Male	12 (57%)	9 (53%)	0.80*
Female	9 (43%)	8 (47%)	
Age at enucleation	62.57 (39-79)	52.82 (22-78)	0.05§
Largest Basal Diameter	15.18 (8-25)	16.59 (11-21)	0.27§
Thickness	10.21 (6-16)	10.40 (6-13)	0.72§
Mitotic count			
0-5	20 (95%)	14 (82%)	0.045‡
>5-10	0 (0%)	3 (18%)	
>11	1 (5%)	0 (0%)	
Cell type			
Spindle cell	18 (86%)	13 (76%)	0.68†
Epithelioid-mixed cell	3 (14%)	4 (24%)	
Ciliary body involvement			
No	18 (86%)	13 (76%)	0.68†
Yes	3 (14%)	4 (23%)	
Extrascleral extension			
None	21 (100%)	16 (94%)	0.48‡
<5 mm	0 (0%)	1 (6%)	
>= 5 mm	0 (0%)	0 (0%)	
TNM stage			
I-IIB	14 (67%)	9 (53%)	0.39*
IIIA-IIIC	7 (33%)	8 (47%)	
Tumour pigmentation			
Light	16 (76%)	7 (76%)	0.080‡
Mixed	3 (14%)	7 (41%)	
Heavy	2 (10%)	3 (18%)	
8q status			
Normal	16 (76%)	2 (12%)	<0.001‡
Gain	2 (9%)	11 (65%)	
Amplification	3 (14%)	4 (23%)	
6p status			
Normal	5 (24%)	0 (0%)	0.05†
Gain	16 (76%)	17 (100%)	
BAP1 expression			
BAP1 high	20 (95%)	17 (100%)	1.00†
BAP1 low	1 (5%)	0 (0%)	

*: Pearson's χ^2 test

†: Fisher's exact test

‡: Likelihood ratio

§: Mann Whitney U test

||: Percentages are rounded and may not total 100

#: Percentages were calculated excluding missing data

Table S7. Distribution of clinical, histopathological, and genetic features in UM with low PRAME and high PRAME (M3 UM patients from the TCGA study, total 42).

Feature	Patients, No. (%)||#		p value
	Low PRAME n=19 (45%)	High PRAME n=23 (56%)	
Sex			
Male	10 (53%)	14 (61%)	0.59*
Female	9 (47%)	9 (39%)	
Age at enucleation	64.89 (41-86)	64.65 (46-86)	0.96§
Largest Basal Diameter	16.17 (12-25)	18.20 (12-25)	0.06§
Thickness	11.14 (7-15)	11.29 (4-16)	0.71‡
Mitotic count			
0-5	12 (63%)	17 (74%)	0.71†
>5-10	4 (21%)	4 (17%)	
>11	3 (16%)	2 (9%)	
Cell type			
Spindle cell	8 (42%)	4 (17%)	0.08*
Epithelioid-mixed cell	11 (58%)	19 (83%)	
Ciliary body involvement			
No	10 (53%)	13 (54%)	0.80*
Yes	9 (47%)	10 (45%)	
Extrascleral extension			
None	19 (100%)	18 (77%)	0.04‡
<5 mm	0 (0%)	2 (9%)	
>= 5 mm	0 (0%)	3 (14%)	
TNM stage			
I-IIB	7 (37%)	6 (23%)	0.45*
IIIA-IV	12 (63%)	17 (77%)	
Tumour pigmentation			
Light	12 (63%)	4 (17%)	0.007‡
Mixed	4 (21%)	13 (57%)	
Heavy	3 (16%)	6 (26%)	
8q status			
Normal	3 (16%)	0 (0%)	0.007‡
Gain	9 (47%)	5 (18%)	
Amplification	7 (37%)	18 (82%)	
6p status			
Normal	16 (84%)	14 (59%)	0.10*
Gain	3 (16%)	9 (41%)	
BAP1 expression			
BAP1 high	1 (5%)	2 (9%)	1.00†
BAP1 low	18 (95%)	21 (92%)	

*: Pearson's χ^2 test

†: Fisher's exact test

‡: Likelihood ratio

§: Mann Whitney U test

||: Percentages are rounded and may not total 100

#: Percentages were calculated excluding missing data

Table S8: Distribution of BAP1, SF3B1 and EIF1AX mutation in UM with respect to PRAME expression in the TCGA cohort

	Total TCGA			TCGA – D3			TCGA – M3		
	PRAME-low	PRAME-high	p value	PRAME-low	PRAME-high	p value	PRAME-low	PRAME-high	p value
BSE mutation									
EIF1AX	8 (31%)	1 (3%)	0.002‡	8 (89%)	1 (7%)	< 0.001†	/	/	/
SF3B1	3 (12%)	13 (27%)		1 (11%)	13 (93%)		2 (12%)	0 (0%)	0.20†
BAP1	15 (58%)	20 (58%)		/	/	/	15 (88%)	20 (100%	

‡Likelihood ratio
†Fisher's exact test

Table S9: Distribution of EMT markers in PRAME positive and PRAME negative tumors in a cohort of 64 UM patients, Mann Whitney U test.

	PRAME negative	PRAME positive	*p* value
SNAI2 probe 1	8.25 (±0.6)	8.07 (±0.6)	0.36
SNAI2 probe 2	9.55 (±0.7)	9.41 (±0.6)	0.50
STAT3 probe 1	8.02 (±0.4)	8.02 (±0.4)	0.90
STAT3 probe 2	9.07 (±0.5)	9.03 (±0.5)	0.86
ZEB2	7.73 (±0.3)	7.59 (±0.2)	0.02
SOX10	11.95 (±0.4)	11.97 (±0.4)	0.89

Table S10: Distribution of infiltrate markers in PRAME positive and PRAME negative tumors in a 24 D3 and 40 M3 UM patients, Mann Whitney U test.

	Disomy 3 (24)			Monosomy 3 (40)		
	PRAME negative	PRAME positive	p	PRAME negative	PRAME positive	P
CD3	6.85 (±1.3)	6.86 (±0.4)	0.13	6.90 (±0.8)	7.64 (±1.2)	0.01
CD4	6.54 (±0.4)	6.73 (±0.3)	0.01	6.60 (±0.2)	6.77 (±0.3)	0.10
CD8A	6.95 (±1.6)	6.90 (±0.6)	0.22	6.98 (±1.0)	7.88 (±1.3)	0.02
CD68	10.06 (±0.9)	11.18 (±1.0)	0.01	10.80 (±0.6)	11.32 (±0.8)	0.04
HLA-A pr 1	10.62 (±0.7)	11.02 (±0.8)	0.14	11.74 (±0.7)	12.05 (±0.7)	0.005
HLA-A pr 2	13.22 (±0.8)	13.31 (±0.7)	0.33	13.97 (±0.6)	14.40 (±0.4)	0.006
HLA-B	10.31 (±1.7)	9.88 (±1.2)	0.58	11.53 (±1.4)	12.49 (±1.2)	0.02

Table S11a. Most upregulated genes in 29 PRAME-positive UM vs 35 PRAME-negative UM										
Symbol	**logFC**	**AveExpr**	**t**	**P.Value**	**adj.P.Val**	**B**	**Gene name**	**Location**	**Role in cancer**	**Role in UM**
PUF60	0.625558	11.582971	5.719239	3.006E-07	0.00097149	6.424361	Binding Splicing Factor 60	8q24.3		
DGAT1	0.637533	8.1651707	5.255655	1.791E-06	0.00289424	4.822221	Diacylglycerol O-Acyltransferase 1	8q24.3	High expression correlated with poor survival in gastric cancer; high expression protects cancer cells from oxidative damage and improves FA storage (Cheng 2020, He 2021, Wilcock 2022)	
NAPRT1	0.786418	9.5010336	5.162251	2.551E-06	0.00374742	4.504817	Nicotinate Phosphoribosyltransferase	8q24.3	Deficiency makes cancer cells more susceptible to treatment with NAPMT inhibitors with nicotinic acid (Watson 2009, Cerna 2012, Shames 2013)	
SLC52A2	0.748486	9.3376813	5.049801	3.894E-06	0.00479878	4.125463	Solute Carrier Family 52 Member 2	8q24.3	Upregulated, linked to a worse survival and increase in T cell exhaustion markers and M2 macrophages (Zhang 2022)	Upregulated, linked to a worse survival and increase in T cell exhaustion markers and M2 macrophages (Zhang 2022)
CEBPB	0.65662	10.522645	5.032253	4.158E-06	0.00479878	4.066551	CCAAT Enhancer Binding Protein Beta	20q13.13	Upregulated in breast cancer compared to normal tissue (Matherne 2023)	
OPLAH	0.6464	8.5489751	4.815561	9.289E-06	0.00819323	3.345868	5-Oxoprolinase, ATP-Hydrolysing	8q24.3		
EPHX1	0.638304	8.0740934	4.798138	9.903E-06	0.00819323	3.288493	Epoxide Hydrolase 1	1q42.12	Associated with ncreased recurrence rate and a worse prognosis in AML (Cheng 2019).	
DNASE1L3	0.706244	7.5942977	4.384255	4.401E-05	0.01687724	1.953754	Deoxyribonuclease 1 Like 3	3p14.3	Down-regulated in several types of cancer,	

									but correlated with immune infiltration (Deng 2021).	
MGAT1	0.620343	10.553904	4.359655	4.8E-05	0.01723484	1.876258	Alpha-1,3-Mannosyl-Glycoprotein 2-Beta-N-Acetylglucosaminyl-transferase	5q35.3	increases progression and invasiveness of cervical cancer, prostate cancer and hepatocellular carcinoma cell lines (Zavareh 2012, Akiva 2018).	
APOC1	1.403344	10.779708	4.298809	5.944E-05	0.0188526	1.68553	Apolipoprotein C1	19q13.32	Highly expressed in several types of cancers (e.g. colorectal cancer, gastric cancer and renal cell carcinoma), associated with a worse prognosis (Ren 2019, Yi 2019, Cui 2020).	
LY6E	0.683731	11.853244	4.112825	0.0001132	0.02257851	1.111359	Lymphocyte Antigen 6 Family Member E	8q24.3	Overexpressed, linked to a worse prpognosis in several cancer types, acts through TGF-β and PTEN-PI3K/AKT pathways (Upadhyay 2019, AlHossiny 2016, Yeom 2016)	
PARP10	0.691261	8.8007201	4.031011	0.0001496	0.02402675	0.863195	Poly(ADP-Ribose) Polymerase Family Member 10	8q24.3	Promotes cell proliferation *in vitro* and tumour formation *in vivo* from HeLa cells (Schleicher 2018)	Increased in UM samples; PARP inhibitor increases the efficacy of dacarbazin treatment on UM PDX and cell lines (Koning 2019).
CD68	0.827099	10.836711	3.963273	0.0001881	0.0260977	0.659866	CD68 Molecule	17p13.1	High CD68 correlated with an adverse prognosis in glioblastoma, kidney renal clear cell carcinoma, lower-grade glioma, liver hepatocellular	Higher number of CD68+ macrophages correlated with worse prognosis and higher in M3 than D3 UM (Makitie 2001, Bronkhorst 2011)

									carcinoma, lung squamous cell carcinoma, thyroid carcinoma, and thymoma and a favorable prognosis in kidney chromophobe. (Zhang 2022)	
PTP4A3	0.818955	8.4462143	3.953231	0.0001945	0.02641256	0.62989	Protein Tyrosine Phosphatase 4A3	8q24.3	Upregulated in several cancer types, correlated to prognosis in ccRCC, papillary renal cancer and breast cancer (Den Hollander 2016, Song 2021)	Increased in UM cases with early metastases; it increased cell migration of UM cell lines *in vitro* and invasiveness *in vivo* (Laurent 2011). It interacts with MMP-14 and CRPM2 (Maacha 2016, Duciel 2019).

Table S11b. Most downregulated genes in 29 PRAME-positive UM vs 35 PRAME-negative UM										
Symbol	**logFC**	**AveExpr**	**t**	**P.Value**	**adj.P.Val**	**B**	**Gene name**	**Location**	**Role in cancer**	**Role in UM**
RPL13P5	-0.96596	9.942009	-6.14222	5.695E-08	0.00046004	7.916899	Ribosomal Protein L13 Pseudogene 5	12p13.31	Negatively correlated with HER2 in a comparison of HER2 breast cancer with normal tissue (Yang 2016).	
CSNK2A2	-0.64941	9.0176943	-4.79164	1.014E-05	0.00819323	3.267104	Casein Kinase 2 Alpha 2	16q21	Different expression patterns and different roles in different cancers (Strum 2022)	Downregulated in UM cells compared to normal uveal melanocytes; lower in class 2 UM than in class 1 UM (Onken 2006, Bergeron 2012)
ID2	-0.74846	8.5848253	-4.25107	7.021E-05	0.0199025	1.536875	Inhibitor Of DNA Binding 2	2p25.1	Overexpressed in colon and pancreatic	Downregulated in class 2 UM compared to

									cancer; its downregulation promotes aggressiveness and invasion in breast, prostate and bladder cancer (Roschger 2017, Mao 2021)	class 1 UM; its downregulation can simulate a class 2 phenotype in UM cell lines *in vitro* and *in vivo* (Onken 2006)
SLCO4A1-AS1	-0.95988	10.055974	-4.1953	8.521E-05	0.02117138	1.3643	SLCO4A1 Antisense RNA 1	20q13.33	Promotes tumour progression in colorectal cancer and pancreatic cancer (Zhang 2022, Zhang 2022, Wu 2021)	Downregulated in UM with a high inflammatory infiltrate (Zhou 2022).
RPL21P28	-0.6364	11.044889	-4.03589	0.0001472	0.02402675	0.877925	Ribosomal Protein L21 Pseudogene 28	1q32.3		
EFCAB1	-0.8422	6.9796114	-3.89938	0.000233	0.02851422	0.469917	Calaxin	8q11.21	Downregulated in lung adenocarcinoma compared to normal lung tissue (Yang 2022)	Lower in metastatic UM compared to non-metastatic UM (Fagone 2017).

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