

Supplementary Results

Association of MITF E318K with body site and Breslow thickness

When we analysed the available data on body site for Australian cases, there was some indication of differences by body site for cases that carried the *MITF* variant compared to those without the *MITF* variant. Specifically, there was a higher proportion of melanomas on the head and neck and a lower proportion on the legs ($P = 0.03$). However no clear differences by body site were observed for the UK cases ($P = 0.46$), nor when the UK and Australian data were combined ($P = 0.16$). Similar to our findings by body site, when we analysed the available data on Breslow thickness for Australian cases, those who carried the *MITF* variant had thinner primary tumours (median 0.38 mm) than those who did not (median 0.50 mm, $P = 0.003$). Once again, however, no clear differences by Breslow thickness were observed for the UK cases ($P = 0.80$) nor when the UK and Australian data were combined (Breslow thickness for cases with variant = 0.80 mm versus without variant = 0.85 mm; $P = 0.18$).

MITF E318K in melanoma cell lines

Screening of a panel of 205 cutaneous melanoma cell lines identified seven that carried the *MITF* E318K variant; one was homozygous (the only such sample in the entire study). The carrier frequency (0.034) was higher than in any of the case or control populations from Australia or the UK, thus providing further support for an association with melanoma and suggesting that *MITF* E318K may provide an advantage to the establishment of melanoma cell lines in culture.

Tumour-associated functions of select genes differentially regulated by MITF E318K

Several of the non-pigmentation genes that were differentially regulated between wild-type MITF and MITF E318K in melanoma cells (Supplementary Figure 1b) have been associated with melanoma or other cancer types. *SPINK1* (*serine peptidase inhibitor, Kazal type I*) expression was activated to a greater degree by MITF E318K than wild-type MITF, and is associated with poor survival in ovarian, kidney, colorectal, bladder and prostate cancer^{1,2} and references therein. *SPINK1* also acts as an oncogene to regulate proliferation, invasion and intravasation in prostate cancer². We also observed more pronounced down-regulation of the anti-angiogenic gene (*THBS1*)³ (validated by qRT-PCR, Fig. 2eiii) and cysteine-rich protein-61 (*CYR61*) with expression of E318K. *THBS1* and *CYR61* have recently been identified as tumour suppressors in melanoma⁴, Bonazzi et al., submitted.

Supplementary Tables

Supplementary Table 1. Age of onset, multiple primary melanoma status of probands and *MITF*

E318K cosegregation data in melanoma families.

Family ID	MM cases in family	MM cases with E318K	MM cases with unknown genotype	Multiple primary MM in proband	Age at onset of cases with E318K	Age at onset of other cases in family*
FAM1	8	3	1	Y	35, 40, 55	22, 46, 65, 67, 73
FAM2	7	5	1	Y	40, 50, 50, 54, <65	17 [~] , <69
FAM3	5	3	1	Y	45, 59, 65	59, <36
FAM4	4	1	3	N	67	20, 27, 50
FAM5	4	1	2	Y	34	42, 44, 72
FAM6	4	1	2	Y	53	31, 79, 51
FAM7	3	3	0	N	30, 32, 47	
FAM8	3	3	0	N	35, 62, 68	
FAM9	3	3	0	N	11, 49, 55	
FAM10	3	3 [~]	0	Y	45, 58 [~] , 76	
FAM11	3	3	0	N	20, 53, 57	
FAM12	3	2	0	Y	33, 45	79
FAM13	3	2	1	N	58, 61	15
FAM14	3	2	1	N	65, 68	61
FAM15	3	1	2	N	57	79, <79
FAM16	3	2	1	Y	60, 72	88
FAM17	3	1	1	N	65	67, 61
FAM18	3	1	0	N	13	51, 46
FAM19	2	2	0	Y	18, 32	
FAM20	2	2	0	Y	17, 51	
FAM21	2	2	0	N	24, 43	
FAM22	2	2	0	Y	24, 63	
FAM23	2	1	1	Y	?	?
FAM24	2	1	1	N	64	46
FAM25	2	1	1	Y	54	53
FAM26	2	1	1	N	15	<64 [^]
FAM27	2	1	1	Y	38	72
FAM28	2	1	1	N	62	42
FAM29	2	1	1	N	35	49
FAM30	2	1	0	Y	52	59
FAM31	2	1	0	N	71	?

* includes typed & untyped cases

[^] age at death from melanoma but age of onset unknown

[~] includes 1 presumed obligate carrier

[~]~ presumed wt

? denotes age unknown

E318K segregates

E318K may segregate

Supplementary Table 2. Nevus association with *MITF* E318K.

Sample	OR	se(ln(OR))	P	OR lower CI	OR upper CI
Q-MEGA	3.72	0.49	0.005	1.34	11.88
AMFS	1.69	0.60	0.574	0.47	7.55
UK	2.29	0.48	0.081	0.90	5.81
META	2.54	0.30	0.002	1.42	4.55

Meta-analysis was done on the basis of ORs and their standard errors, not on raw data, since there were differences in definition of "high"/"low" mole count

Supplementary Table 3. Eye colour association with *MITF* E318K.

Sample	Blue Eyes			Non-Blue Eyes			P	OR	95% CI
	Variant Carriers	Non-		Variant Carriers	Non-				
		Variant Carriers	Carrier Frequency		Variant Carriers	Carrier Frequency			
Aus (Q-MEGA+AMFS)	7	1265	0.0055	27	1721	0.0154	0.013	2.83	1.20-7.73
UK	9	681	0.0130	19	1069	0.0175	0.560	1.34	0.58-3.40
TOTAL	16	1946	0.0082	46	2790	0.0162	0.018	2.01	1.11-3.81

Supplementary Table 4. Lack of hair colour, skin colour and freckling associations with *MITF* E318K.

Sample	Freckling		Skin Colour		Hair Colour	
	OR	<i>P</i>	OR	<i>P</i>	OR	<i>P</i>
Aus-QIMR	1.59	0.326	1.77	0.360	0.87	1.000
Aus-AMFS	1.58	0.636	1.58	0.705	1.25	0.753
UK	1.31	0.228	1.10	0.484	0.98	0.771

Supplementary Table 5. The majority of gene products commonly regulated by wild-type and E318K mutant MITF have been previously reported to be MITF target genes.

		<i>(Strub et al. 2011)</i>		<i>(Hoek et al. 2008)</i>	
		<i>Gene Symbol</i>	<i>Direct MITF Target (MITF ChIP-Seq)</i>	<i>Indirect MITF Target</i>	<i>Direct or Indirect MITF Target</i>
Gene products with >1.5-fold difference in expression compared to uninduced parental cell line AND <1.25-fold difference in expression between wild-type and E318K mutant MITF	<i>Increased with induction of MITF</i>	<i>SILV</i>	Yes	No	Yes
		<i>CAPN3</i>	Yes	No	Yes
		<i>HSPA2</i>	No	No	No
		<i>SPRY1</i>	No	No	No
		<i>SPATS1</i>	No	Yes	Yes
		<i>LGALS3</i>	Yes	No	Yes
		<i>LOC730525</i>	No	No	No
		<i>MBP</i>	Yes	No	Yes
		<i>GYG2</i>	Yes	No	Yes
		<i>LAMA1</i>	Yes	No	Yes
		<i>ENO2</i>	Yes	No	Yes
Gene products with >1.5-fold difference in expression compared to uninduced parental cell line AND ≥1.25-fold difference in expression between wild-type and E318K mutant MITF	<i>Decreased with MITF induction</i>	<i>KRT81</i>	No	Yes	N.D.
		<i>ASNS</i>	No	Yes	N.D.
		<i>FLRT3</i>	No	Yes	N.D.
		<i>COL1A2</i>	No	No	Yes
		<i>KRT80</i>	No	No	N.D.
		<i>NPTX2</i>	No	No	Yes
		<i>JUN</i>	Yes	Yes	N.D.
		<i>PRKCDBP</i>	No	No	Yes
		<i>FST</i>	No	No	N.D.
		<i>PCLO</i>	No	No	Yes
		<i>TNFRSF12A</i>	No	No	Yes
		<i>CTGF</i>	No	Yes	Yes
Gene products with >1.5-fold difference in expression compared to uninduced parental cell line AND ≥1.25-fold difference in expression between wild-type and E318K mutant MITF	<i>Increased with induction of MITF</i>	<i>SPINK1</i>	No	No	Yes
		<i>PHACTR1</i>	Yes	No	Yes
		<i>TNFRSF14</i>	Yes	No	Yes
		<i>DCT</i>	Yes	No	Yes
		<i>CAPN3</i>	Yes	No	Yes
		<i>HSPA2</i>	No	No	N.D.
		<i>TSPAN10</i>	Yes	No	Yes
		<i>C18orf51</i>	No	No	Yes
		<i>TBC1D7</i>	Yes	No	Yes
		<i>Hs.406790</i>	No	No	No
		<i>PI15</i>	Yes	No	Yes
		<i>NDRG1</i>	No	No	No
		<i>MLANA</i>	Yes	No	Yes
	<i>Decreased with MITF induction</i>	<i>TMEM16A</i>	No	No	Yes
		<i>CYR61</i>	Yes	No	Yes
		<i>FST</i>	No	No	N.D.
		<i>THBS1</i>	No	No	Yes

N.D., not determined as Hoek et al., 2008 did not include MITF downregulated genes.

Supplementary Table 6. Gene products uniquely regulated by either (a) E318K mutant MITF or (b) wild-type MITF in melanoma cell lines.

A. Gene products uniquely regulated by E318K mutant MITF and not wild-type MITF in both C-32 and HT144 melanoma cell lines as determined by microarray analysis.

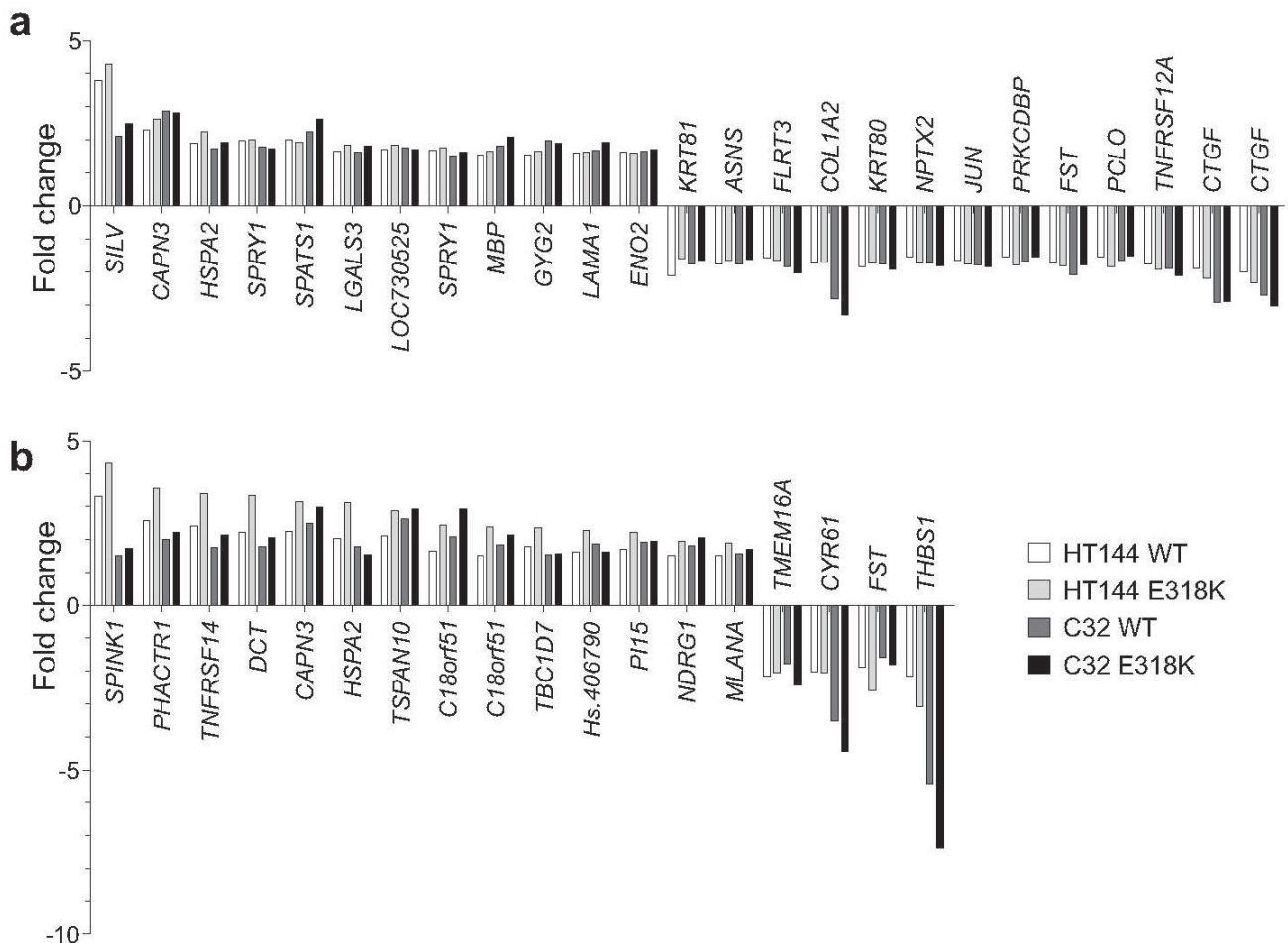
Gene Symbol	Fold change in gene expression with induction of E318K mutant MITF in HT144 cells	Fold change in gene expression with induction of E318K mutant MITF in C32 cells	(Strub et al. 2011)		(Hoek et al. 2008)
			Direct MITF Target (MITF ChIP-Seq)	Indirect MITF Target	Direct or Indirect MITF Target
NAGLU	1.85	1.58	Yes	No	Yes
FCGR2A	1.71	1.55	Yes	No	Yes
CASP1	1.68	1.50	No	No	Yes
TPD52L1	1.66	1.52	No	No	No
FAM69B	1.64	1.55	Yes	No	No
LASS4	1.59	1.70	Yes	No	No
CABC1	1.58	1.52	No	No	No
PRSS33	1.58	1.58	No	No	No
PNCK	1.52	1.55	No	No	No
CABLES1	1.51	1.68	Yes	No	Yes
FAM75A3	1.51	-1.52	No	No	No
ENC1	-1.70	-1.72	No	No	N.D.
KIF11	-1.63	-1.59	No	No	N.D.
LYPD1	-1.63	-1.51	No	No	Yes
PRNP	-1.58	-1.89	No	No	Yes
CAPN2	-1.54	-1.52	Yes	No	No

B. Gene products uniquely regulated by wild-type MITF and not E318K mutant MITF in both C-32 and HT144 melanoma cell lines as determined by microarray analysis.

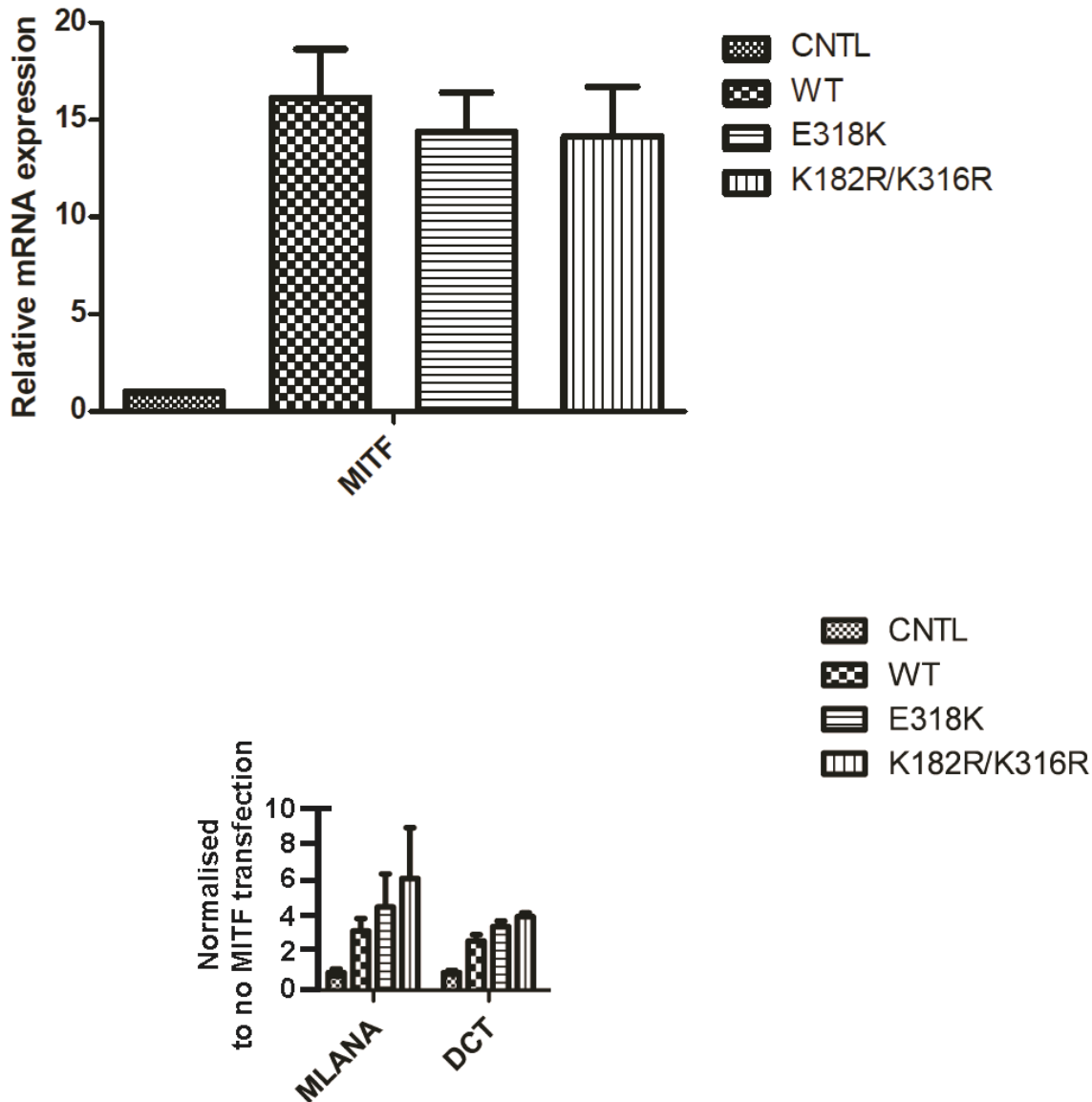
Gene Symbol	Fold change in gene expression with induction of wildtype MITF in HT144 cells	Fold change in gene expression with induction of wildtype MITF in C32 cells	(Strub et al. 2011)		(Hoek et al. 2008)
			Direct MITF Target (MITF ChIP-Seq)	Indirect MITF Target	Direct or Indirect MITF Target
TBC1D14	1.54	1.50	Yes	No	Yes
FXD3	1.65	1.76	Yes	No	Yes

N.D., not determined as Hoek et al. (2008) did not include MITF downregulated genes.

Supplementary Figures and Legends

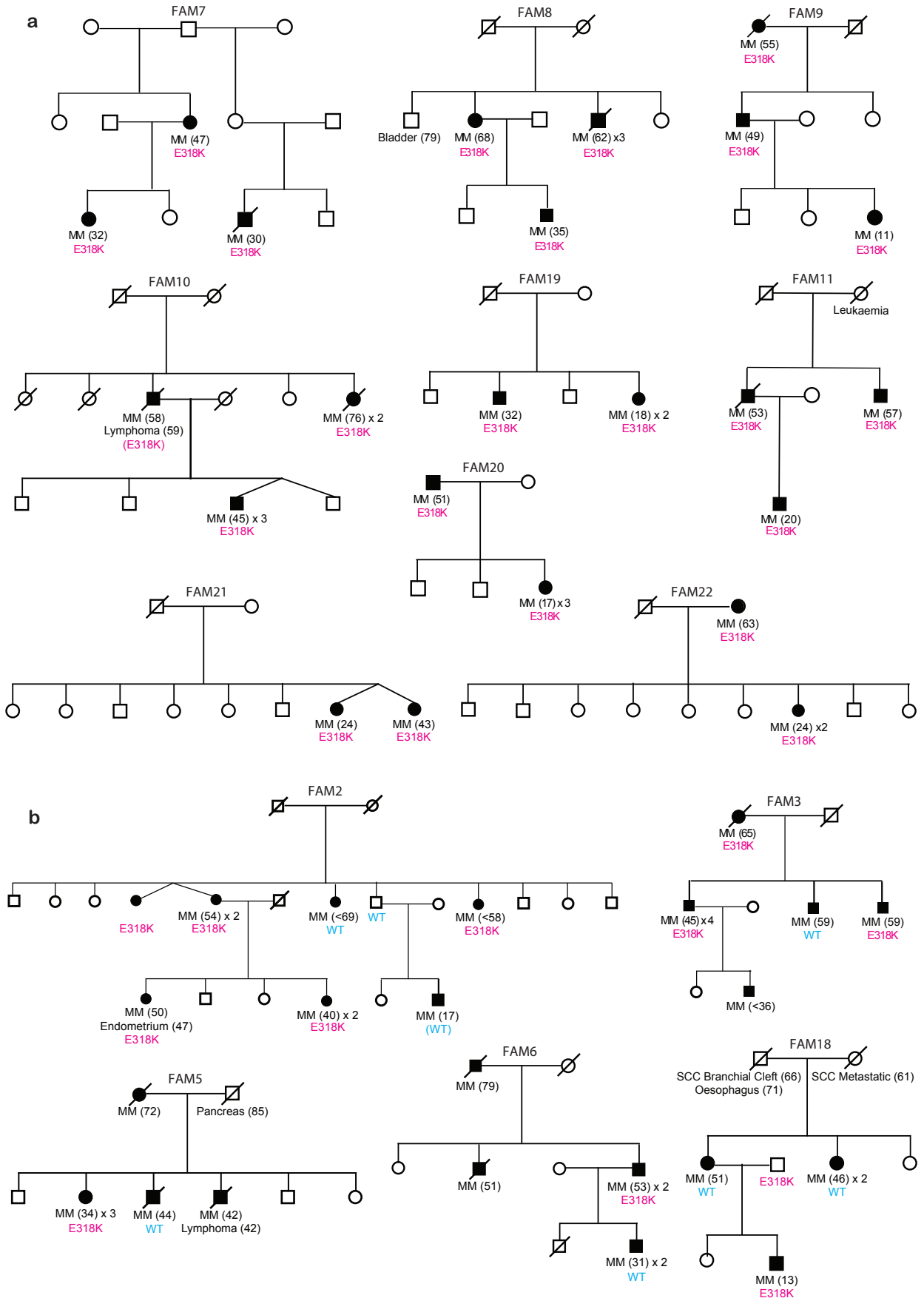


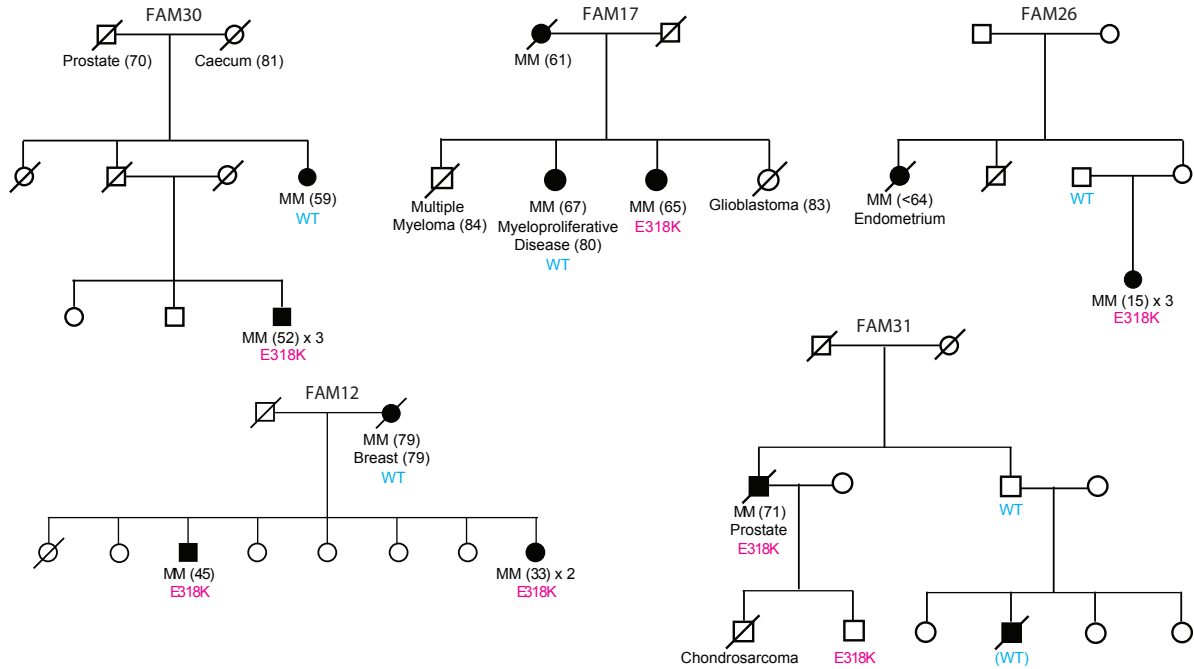
Supplementary Figure 1. Gene products commonly regulated by wild-type and E318K mutant MITF. Expression of either wild-type or E318K mutant MITF was induced by treatment with tetracycline for 48 hours prior to RNA extraction and whole genome expression profiling. A total of 43 entities representing 37 gene products were identified as being commonly regulated by wild-type and E318K mutant MITF in both of the cell lines. **a**, Gene products with less than 1.25-fold difference in expression between wild-type and E318K mutant MITF. **b**, Gene products with greater than 1.25-fold difference between E318K mutant and wild-type MITF in at least one of the two melanoma cell lines following induction. Fold changes are shown compared to the level of gene expression from un-induced control cells.



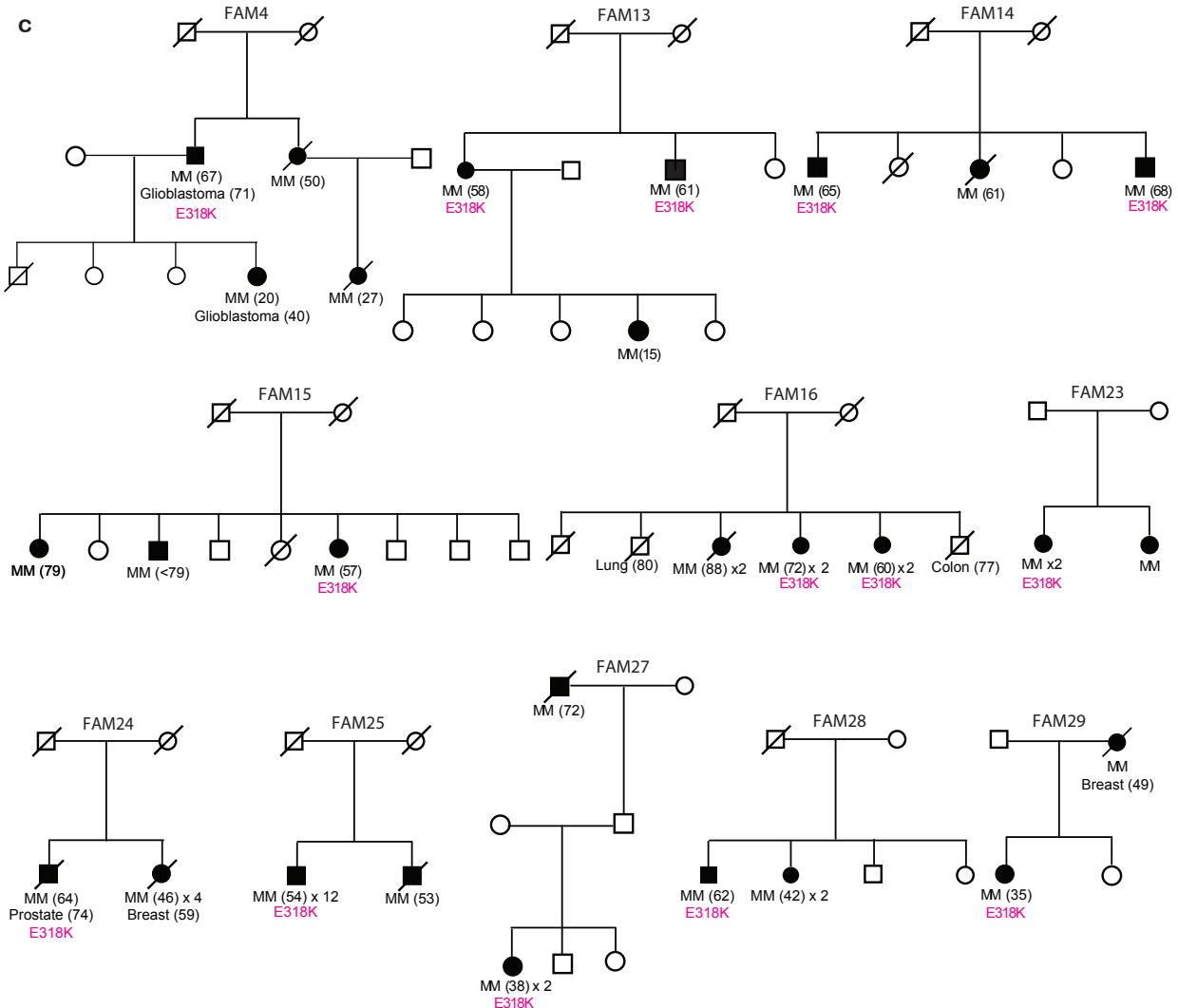
Supplementary Figure 2. Quantitative RT-PCR analysis of total RNA isolated from UACC62 human melanoma cells which were transfected with control vector, wild-type *MITF*, or the indicated mutants. Each target gene expression level was normalized to *ACTB* mRNA (Figure 2f shows data normalized to *MITF* expression). Fold induction is shown as the ratio to each mRNA expression with vector control. Data are mean \pm SD of at least three independent experiments.

Supplementary Figure 3. Cosegregation analysis of the *MITF* E318K variant in melanoma families. Legend as for Fig. 1. **a.** Families in which all melanoma cases carry the *MITF* E318K variant (Excluding FAM1). **b.** Families in which the *MITF* E318K variant does not segregate perfectly with melanoma. **c.** Families in which the genotype of some melanoma cases is not known.





c



Supplementary References

- 1 Gaber, A. *et al.* High expression of tumour-associated trypsin inhibitor correlates with liver metastasis and poor prognosis in colorectal cancer. *Br J Cancer* **100**, 1540-1548 (2009).
- 2 Ateeq, B. *et al.* Therapeutic targeting of SPINK1-positive prostate cancer. *Sci Transl Med* **3**, 72ra17 (2011).
- 3 Bornstein, P. Thrombospondins as matricellular modulators of cell function. *J Clin Invest* **107**, 929-934 (2001).
- 4 Dobroff, A. S. *et al.* Silencing cAMP-response element-binding protein (CREB) identifies CYR61 as a tumor suppressor gene in melanoma. *J Biol Chem* **284**, 26194-26206 (2009).