doi:10.1038/nature10630

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 cancer[2]. 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 melanoma[4, 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 unknwown
~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.

	Blue Eyes			Non-Blue Eyes					
Sample	Variant Carriers	Non-Variant Carriers	Carrier Frequency	Variant Carriers	Non-Variant Carriers	Carrier Frequency	*P*	OR	95% CI
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.

	Freckling		Skin Colour		Hair Colour	
Sample	**OR**	***P***	**OR**	***P***	**OR**	***P***
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.

			(Strub et al. 2011)		*(Hoek et al. 2008)*
		Gene Symbol	*Direct MITF Target (MITF ChIP-Seq)*	*Indirect MITF Target*	*Direct or Indirect MITF Target*
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	***Increased with induction of MITF***	*SILV*	Yes	No	Yes
		CAPN3	Yes	No	Yes
		HSPA2	No	No	No
		SPRY1	No	No	No
		SPATS1	No	Yes	Yes
		LGALS3	Yes	No	Yes
		LOC730525	No	No	No
		MBP	Yes	No	Yes
		GYG2	Yes	No	Yes
		LAMA1	Yes	No	Yes
		ENO2	Yes	No	Yes
	Decreased with MITF induction	*KRT81*	No	Yes	N.D.
		ASNS	No	Yes	N.D.
		FLRT3	No	Yes	N.D.
		COL1A2	No	No	Yes
		KRT80	No	No	N.D.
		NPTX2	No	No	Yes
		JUN	Yes	Yes	N.D.
		PRKCDBP	No	No	Yes
		FST	No	No	N.D.
		PCLO	No	No	Yes
		TNFRSF12A	No	No	Yes
		CTGF	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	***Increased with induction of MITF***	*SPINK1*	No	No	Yes
		PHACTR1	Yes	No	Yes
		TNFRSF14	Yes	No	Yes
		DCT	Yes	No	Yes
		CAPN3	Yes	No	Yes
		HSPA2	No	No	N.D.
		TSPAN10	Yes	No	Yes
		C18orf51	No	No	Yes
		TBC1D7	Yes	No	Yes
		Hs.406790	No	No	No
		PI15	Yes	No	Yes
		NDRG1	No	No	No
		MLANA	Yes	No	Yes
	Decreased with MITF induction	*TMEM16A*	No	No	Yes
		CYR61	Yes	No	Yes
		FST	No	No	N.D.
		THBS1	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.

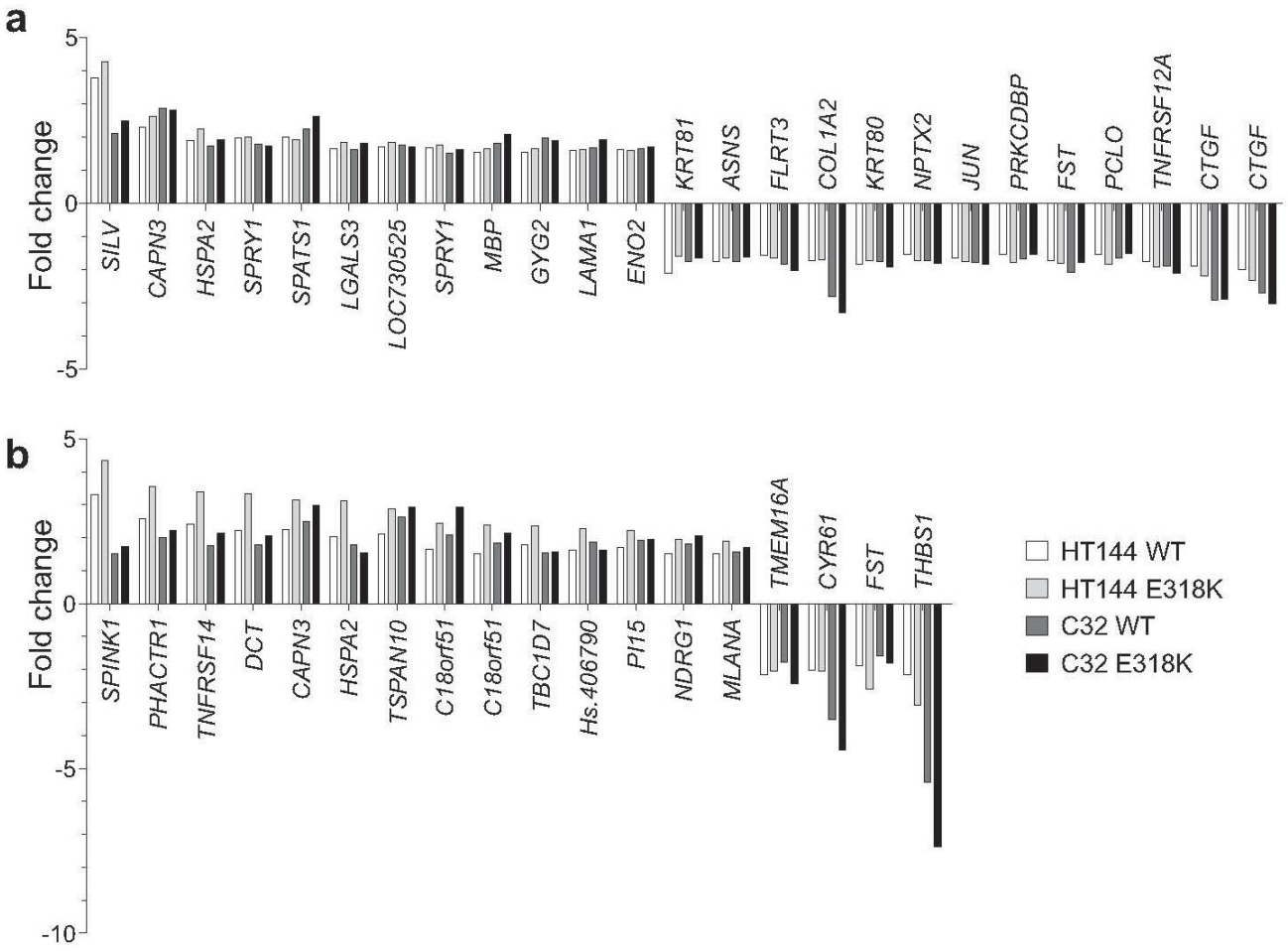
			(Strub et al. 2011)		*(Hoek et al. 2008)*
Gene Symbol	*Fold change in gene expression with induction of E318K mutant MITF in HT144 cells*	*Fold change in gene expression with induction of E318K mutant MITF in C32 cells*	*Direct MITF Target (MITF ChIP-Seq)*	*Indirect MITF Target*	*Direct or Indirect MITF 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.

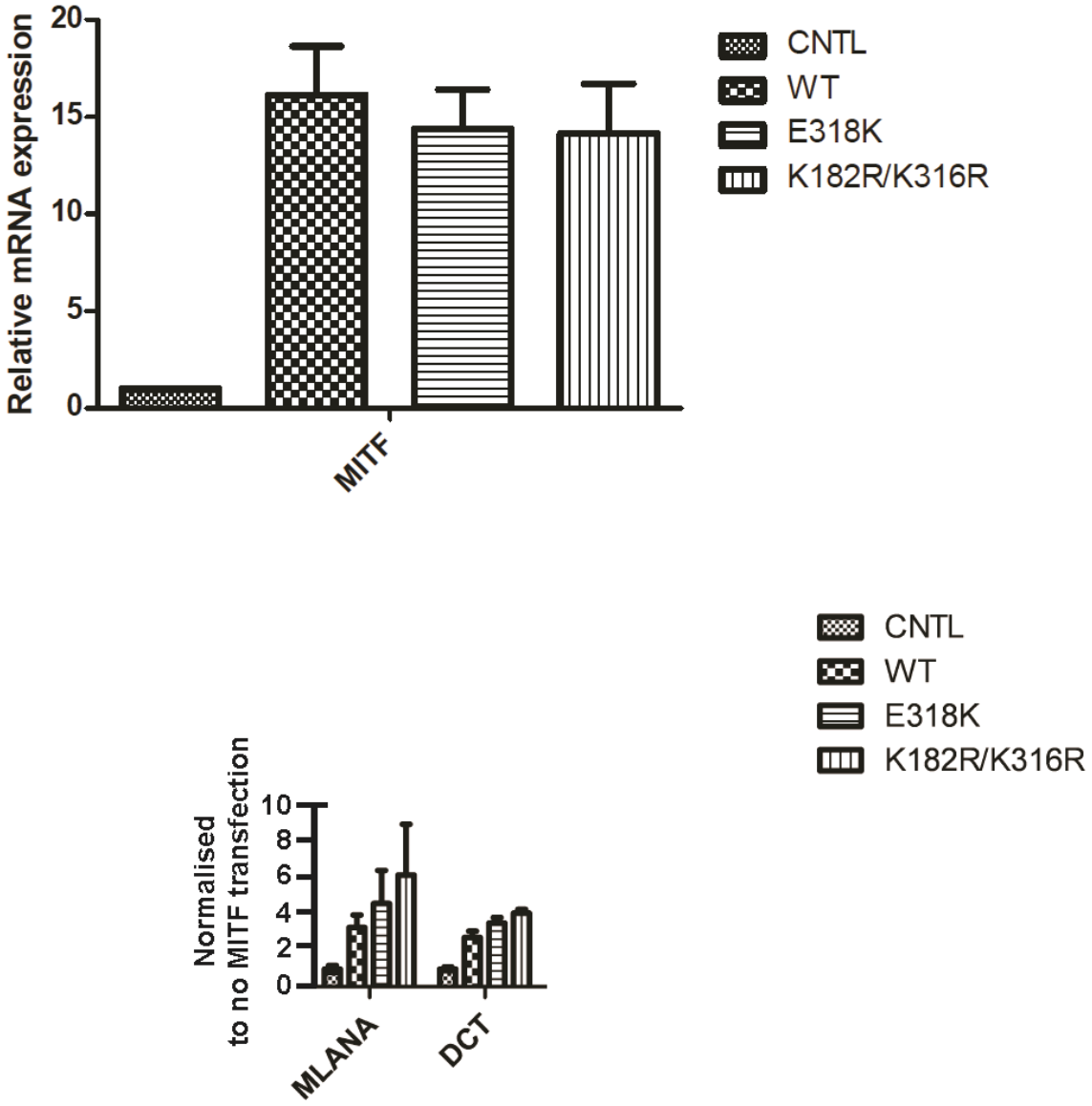
			(Strub et al. 2011)		*(Hoek et al. 2008)*
Gene Symbol	*Fold change in gene expression with induction of wildtype MITF in HT144 cells*	*Fold change in gene expression with induction of wildtype MITF in C32 cells*	*Direct MITF Target (MITF ChIP-Seq)*	*Indirect MITF Target*	*Direct or Indirect MITF Target*
TBC1D14	1.54	1.50	Yes	No	Yes
FXYD3	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

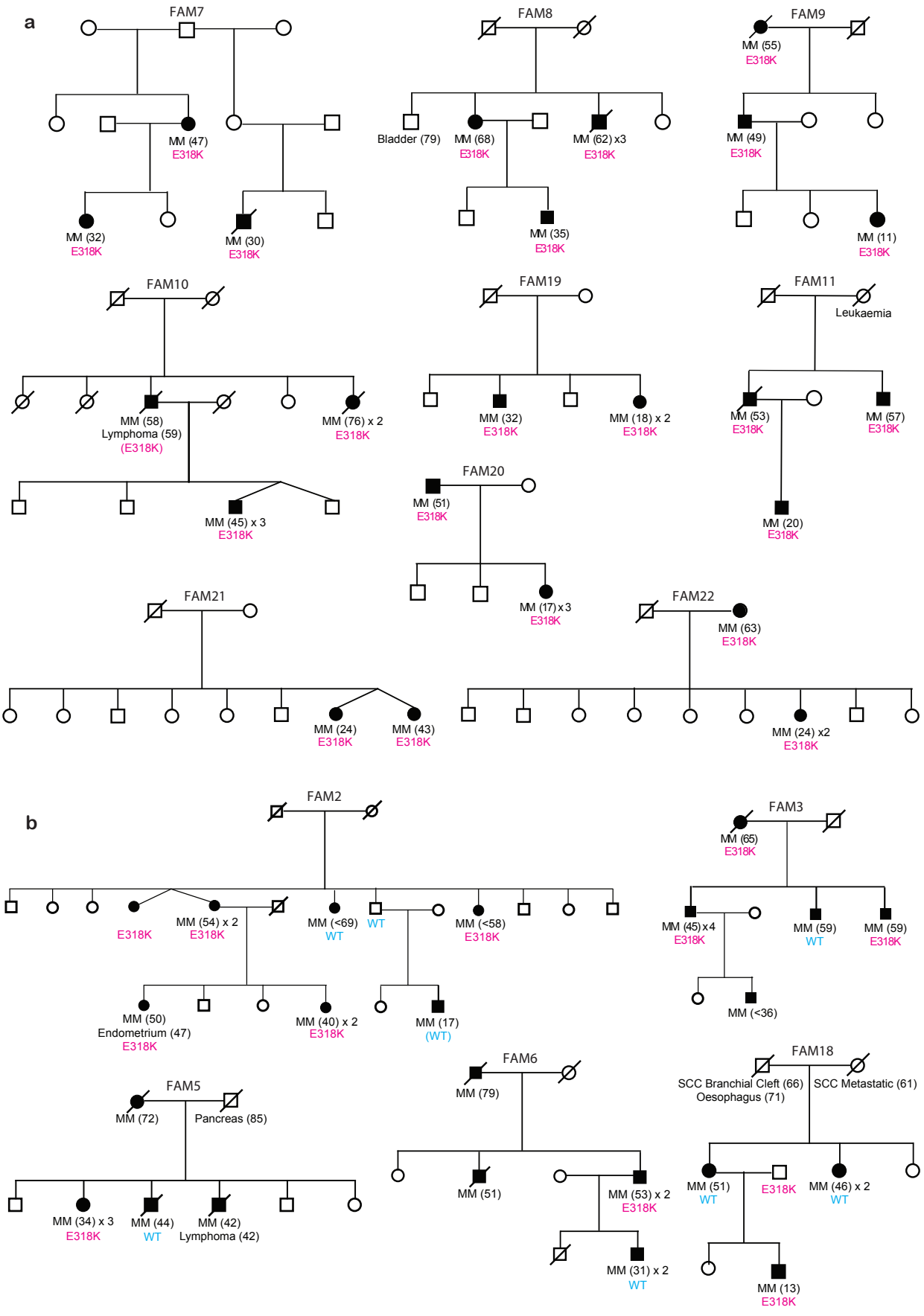


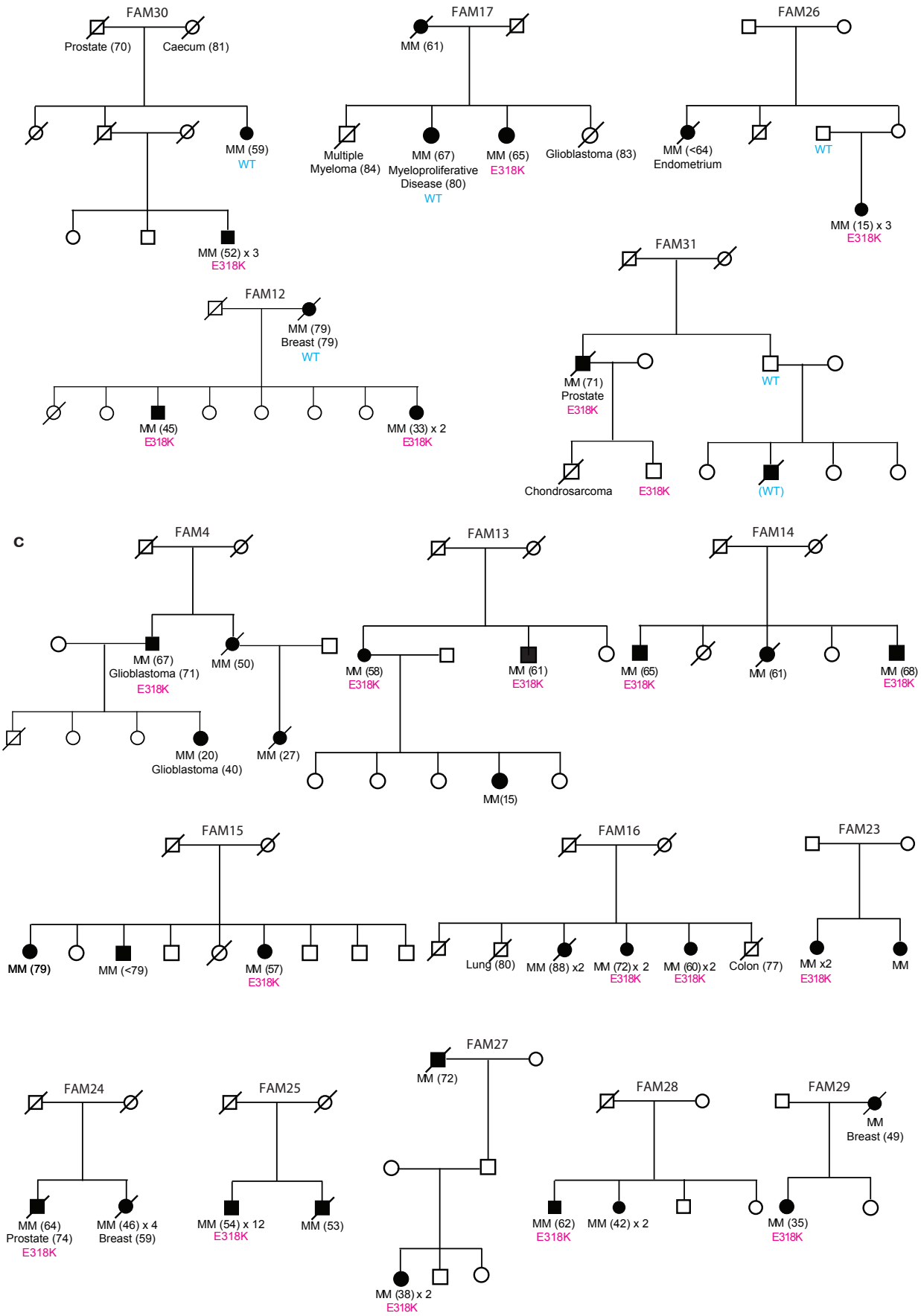
Supplementary Figure 1. Gene products commonly regulated by wild-type and E318K mutant MITF. Expression of either wild-type or E318K mutant MITF was induced by treatment with tetracycline for 48 hours prior to RNA extraction and whole genome expression profiling. A total of 43 entities representing 37 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 ± 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.


a
FAM7
MM (47)
E318K
MM (32)
E318K
MM (30)
E318K
FAM8
Bladder (79)
MM (68)
E318K
MM (62) x3
E318K
MM (35)
E318K
FAM9
MM (55)
E318K
MM (49)
E318K
MM (11)
E318K
FAM10
MM (58)
Lymphoma (59)
(E318K)
MM (76) x 2
E318K
MM (45) x 3
E318K
FAM19
MM (32)
E318K
MM (18) x 2
E318K
FAM11
Leukaemia
MM (53)
E318K
MM (57)
E318K
MM (20)
E318K
FAM20
MM (51)
E318K
MM (17) x 3
E318K
FAM21
MM (24)
E318K
MM (43)
E318K
FAM22
MM (63)
E318K
MM (24) x2
E318K
b
FAM2
E318K
MM (54) x 2
E318K
MM (<69)
WT
WT
MM (<58)
E318K
MM (50)
Endometrium (47)
E318K
MM (40) x 2
E318K
MM (17)
(WT)
FAM3
MM (65)
E318K
MM (45) x4
E318K
MM (59)
WT
MM (59)
E318K
MM (<36)
FAM5
MM (72)
Pancreas (85)
MM (34) x 3
E318K
MM (44)
WT
MM (42)
Lymphoma (42)
FAM6
MM (79)
MM (51)
MM (53) x 2
E318K
MM (31) x 2
WT
FAM18
SCC Branchial Cleft (66)
Oesophagus (71)
SCC Metastatic (61)
MM (51)
WT
E318K
MM (46) x 2
WT
MM (13)
E318K


c
FAM30
Prostate (70)
Caecum (81)
MM (59)
WT
MM (52) x 3
E318K
FAM17
MM (61)
Multiple Myeloma (84)
MM (67) Myeloproliferative Disease (80)
WT
MM (65)
E318K
Glioblastoma (83)
FAM26
MM (<64) Endometrium
WT
MM (15) x 3
E318K
FAM12
MM (79)
Breast (79)
WT
MM (45)
E318K
MM (33) x 2
E318K
FAM31
MM (71)
Prostate
E318K
WT
Chondrosarcoma
E318K
(WT)
FAM4
MM (67)
Glioblastoma (71)
E318K
MM (50)
MM (20)
Glioblastoma (40)
MM (27)
FAM13
MM (58)
E318K
MM (61)
E318K
MM(15)
FAM14
MM (65)
E318K
MM (61)
MM (68)
E318K
FAM15
MM (79)
MM (<79)
MM (57)
E318K
FAM16
Lung (80)
MM (88) x2
MM (72) x 2
E318K
MM (60) x2
E318K
Colon (77)
FAM23
MM x2
E318K
MM
FAM24
MM (64)
Prostate (74)
E318K
MM (46) x 4
Breast (59)
FAM25
MM (54) x 12
E318K
MM (53)
FAM27
MM (72)
MM (38) x 2
E318K
FAM28
MM (62)
E318K
MM (42) x 2
FAM29
MM
Breast (49)
MM (35)
E318K

Supplementary References

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