Supplementary Figure Legends

Figure S1 Quantitation of TYR mRNA and protein levels in WT and 402Q/Q melanocyte strains.

(A) Q-RT-PCR analysis of TYR mRNA levels in three WT (402R/R) and three 402Q/Q melanocyte strains. (B) Endogenous protein levels of TYR in WT (lanes 1-3) and 402Q/Q (lanes 4-6).

Figure S2 Temperature sensitive assay of TYRP1 glycosylation in WT and 402Q/Q melanocyte strains.

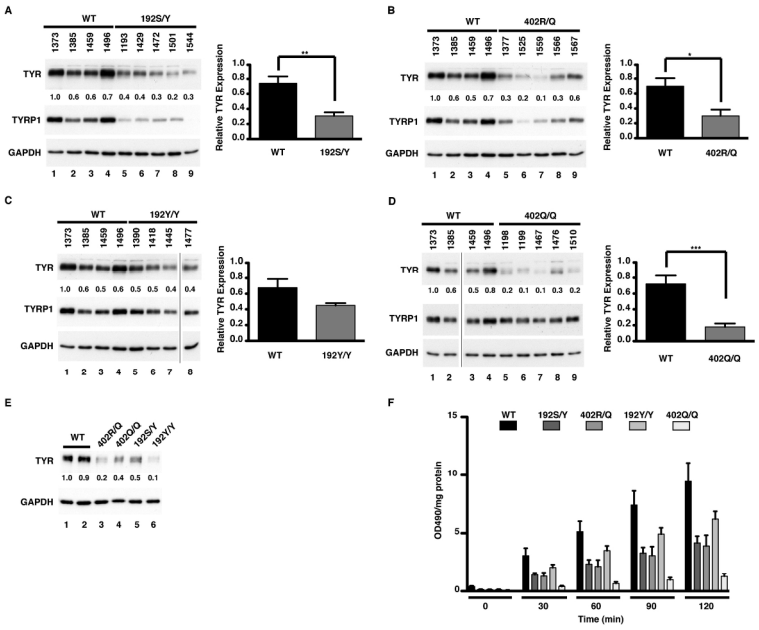
Glycosylation analysis of endogenous TYRP1 in primary melanocytes incubated at 37˚C and 31˚C for a 24hr time period.

Figure S3 Assay of TYR and TYRP1 glycosylation in 402R/Q and 192 Y/Y melanocyte strains.

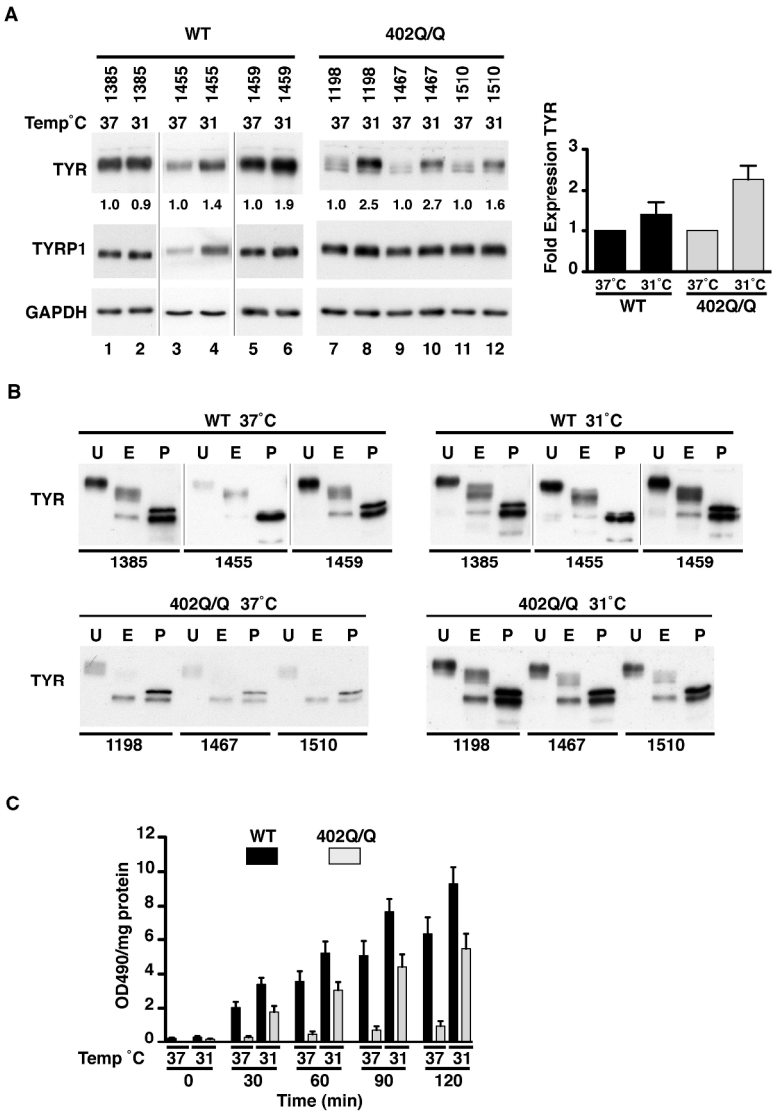
Glycosylation analysis of endogenous TYR and TYRP1 in primary melanocytes incubated at 37˚.

Figure S4 Temperature change has no effect on melanogenesis in melanocyte strains.

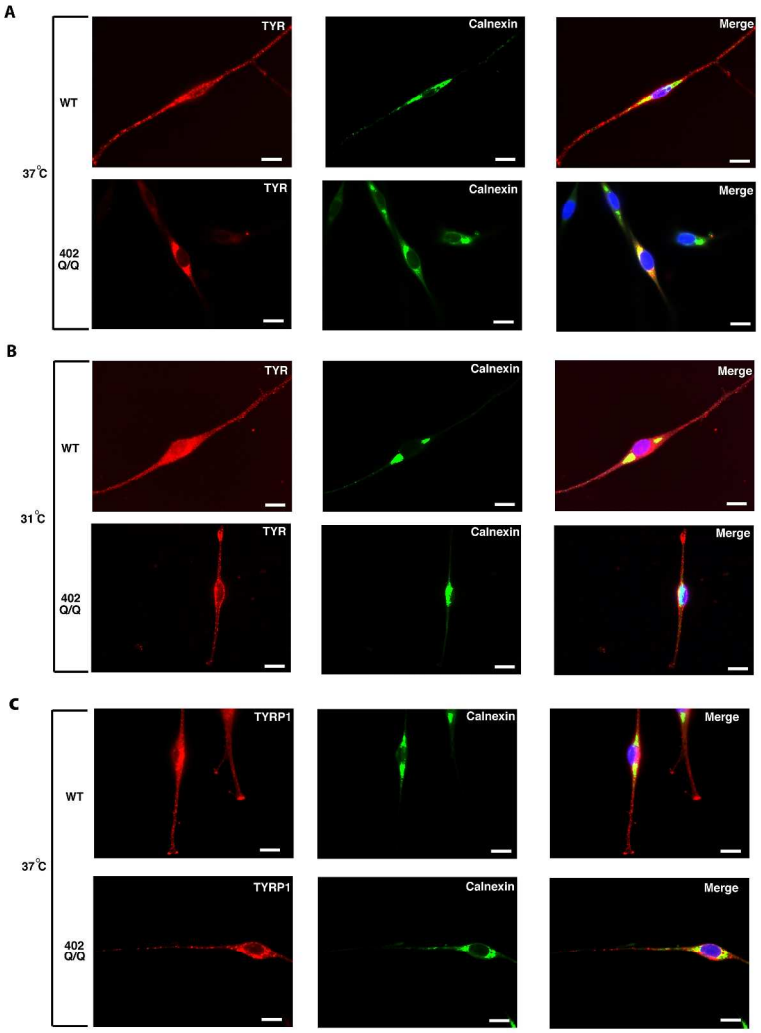
A. Melanin content in genotyped primary melanocytes incubated at 37˚C and 31˚C for a 24hr time period. **B.** Transmissive electron microscopy of melanocytes incubated at 37˚C and 31˚C for a 24hr time period.



Endogenous protein expression and activity of TYR in primary melanocytes of defined genotype.
209x148mm (300 x 300 DPI)



Temperature sensitive recovery of TYR activity in melanocyte strains.
297x420mm (300 x 300 DPI)



Localization of TYR in 402Q/Q melanocytic cells is temperature dependent.
423x496mm (300 x 300 DPI)

Table S1. Genotypes of QF melanocyte strains used in this study

QF Strain	*TYR-192*[1]	*TYR-402*[1]	*MATP-374*[1]	*NCKX5-111*[1]	*OCA2*[2]	*MC1R*[3]
QF1373	S/S	R/R	F/F	T/T	C/C	+/+
QF1385	S/S	R/R	F/F	T/T	C/C	+/+
QF1455	S/S	R/R	F/F	T/T	C/C	+/+
QF1459	S/S	R/R	F/F	T/T	C/C	+/+
QF1496	S/S	R/R	F/F	T/T	C/C	+/r
QF1198	S/S	Q/Q	F/F	T/T	C/C	+/+
QF1199	S/S	Q/Q	F/F	T/T	C/C	+/+
QF1467	S/S	Q/Q	F/F	T/T	C/C	+/+
QF1476	S/S	Q/Q	F/F	T/T	C/C	+/+
QF1510	S/S	Q/Q	F/F	T/T	C/C	+/r
QF1377	S/S	R/Q	F/F	T/T	C/C	+/+
QF1525	S/S	R/Q	F/F	T/T	C/C	+/+
QF1559	S/S	R/Q	F/F	T/T	C/C	+/+
QF1566	S/S	R/Q	F/F	T/T	C/C	+/+
QF1567	S/S	R/Q	F/F	T/T	C/C	+/+
QF1390	Y/Y	R/R	F/F	T/T	C/C	r/r
QF1418	Y/Y	R/R	F/F	T/T	C/C	+/+
QF1445	Y/Y	R/R	F/F	T/T	C/C	+/+
QF1448	Y/Y	R/R	F/F	T/T	C/C	+/+
QF1477	Y/Y	R/R	F/F	T/T	C/C	r/R
QF1193	S/Y	R/R	F/F	T/T	C/C	+/+
QF1429	S/Y	R/R	F/F	T/T	C/C	+/+
QF1472	S/Y	R/R	F/F	T/T	C/C	+/+
QF1501	S/Y	R/R	F/F	T/T	C/C	+/+
QF1544	S/Y	R/R	F/F	T/T	C/C	+/+

[1] Amino acid encoded by SNPs for indicated position is given for TYR rs1042602 and rs1126809, MATP rs16891982 and NCKX5 rs1426654

[2] Genotype according to SNP assay for rs12913832

[3] For MC1R, either Consensus +/+ genotype with or variant alleles shown as R or r as defined previously

Table S2. Genotypes of additional QF melanocyte strains for quantitation of TYR mRNA and protein levels

QF Strain	*TYR-192*[1]	*TYR-402*[1]	*MATP-374*[1]	*NCKX5-111*[1]	*OCA2*[2]	*MC1R*[3]
QF1554	S/Y	R/R	F/L	A/T	T/T	+/r
QF1575	S/S	R/R	F/L	T/T	C/T	+/r
QF1579	S/Y	R/R	F/F	A/T	C/C	+/+
QF1517	S/S	Q/Q	F/F	T/T	T/T	+/r

[1] Amino acid encoded by SNPs for indicated position is given for TYR rs1042602 and rs1126809, MATP rs16891982 and NCKX5 rs1426654

[2] Genotype according to SNP assay for rs12913832

[3] For MC1R, either Consensus +/+ genotype with or variant alleles shown as R or r as defined previously

Table S3. Expected TYR genotype frequencies (%) and proposed strength based on an additive penentrance model

TYR phase [a] **(% frequency**[b]**)**		1-1 (35.0)	2-1 (32.0)	1-2 (30.0)	2-2 (1.9)
	TYR protein[c] **= Strength**[d]	S-R = 3	Y-R = 2	S-Q = 1	Y-Q = 0
1-1 (35.0)	S-R = 3	**A** = 6 (12.25)			
2-1 (32.0)	Y-R = 2	**B** = 5 (22.4)	**E** = 4 (10.24)		
1-2 (30.0)	S-Q = 1	**C** = 4 (21.0)	**F** = 3 (19.2)	**H** = 2 (9.0)	
2-2 (1.9)	Y-Q = 0	**D** = 3 (1.33)	**G** = 2 (1.216)	**I** = 1 (1.14)	**J** = 0 (0.0361)

[a]Designated for rs1042602*C/A = 1/2 - rs1126809*G/A = 1/2
[b]Haplotype frequencies as % based on Table 2
[c]Protein phases designated at S192Y-R402Q as 192S = 1, 192Y = 2; 402R = 1, 402Q = 2
[d]Based on a four point scale S-R = 3, Y-R =2, S-Q =1, Y-Q = 0
[e]Expected genotype frequency shown as a % based on Hardy-Weinberg equilibrium

Figure S1

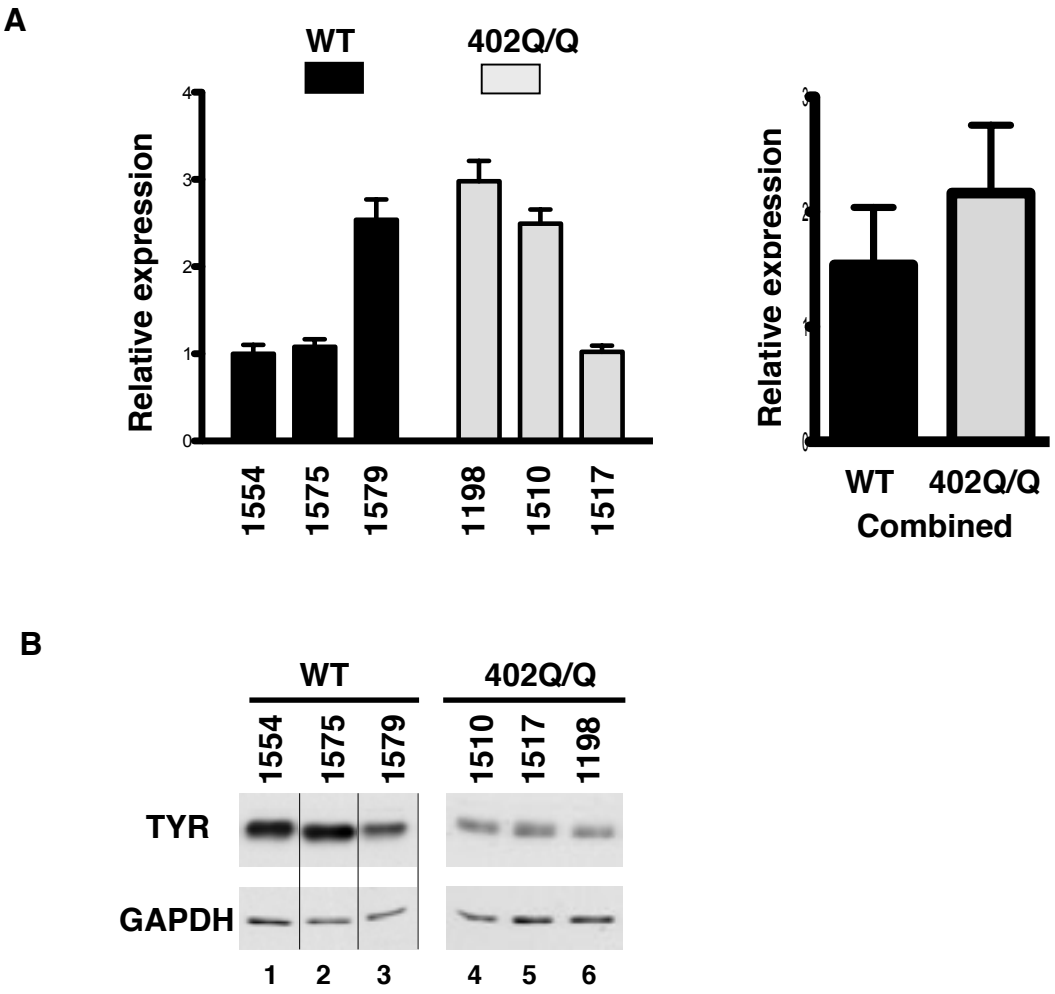


Figure S1 Quantitation of TYR mRNA and protein levels in WT and 402Q/Q melanocyte strains.

(A) Q-RT-PCR analysis of TYR mRNA levels in three WT (402R/R) and three 402Q/Q melanocytes strains are plotted separately in the left panel as mean + SD normalised to 18S ribosomal RNA. In the right panel these are presented as the mean + SEM of relative expression of combined WT or 402Q/Q assays. (B) Endogenous protein levels of TYR in WT (lanes 1-3) and 402Q/Q (lanes 4-6) GAPDH was used to determine protein loading.

Figure S2

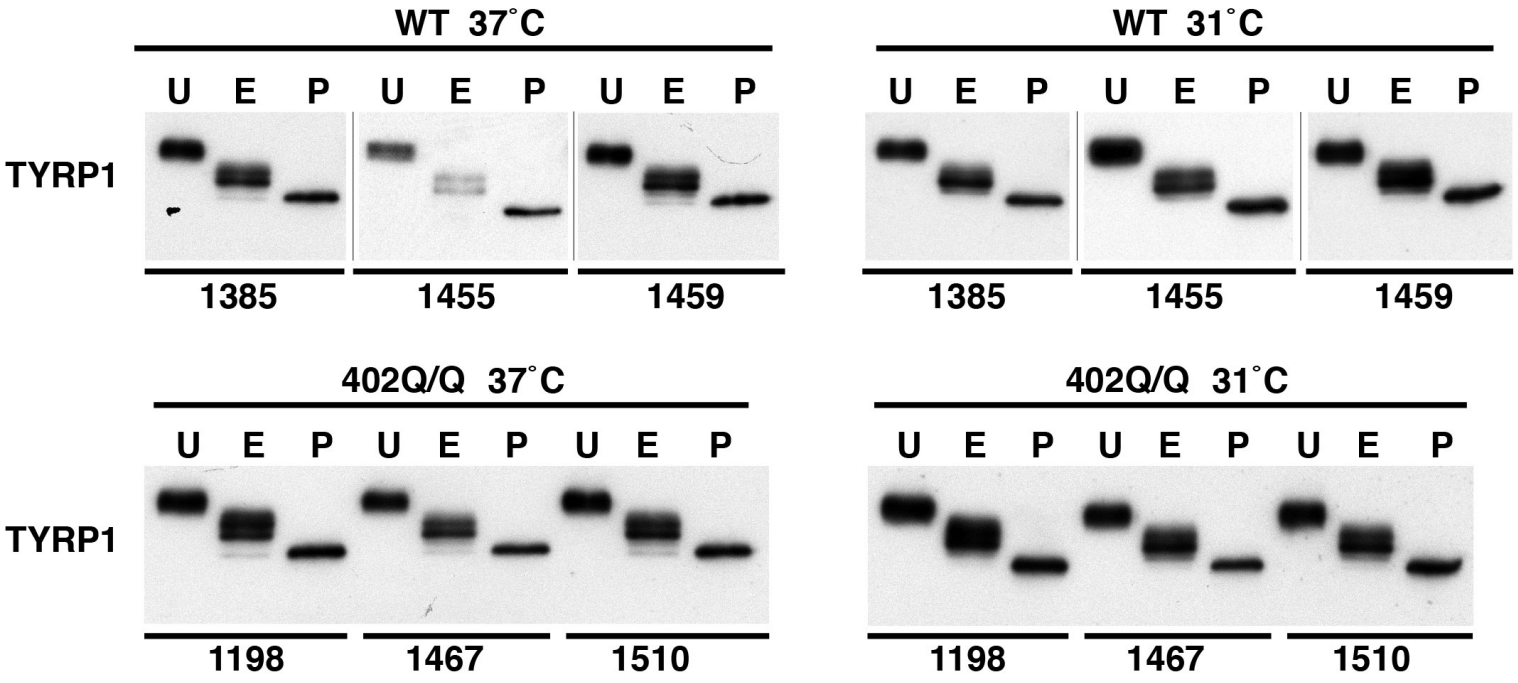


Figure S2 Temperature sensitive assay of TYRP1 glycosylation in WT and 402Q/Q melanocyte strains.

Glycosylation analysis of endogenous TYRP1 in primary melanocytes incubated at 37˚C and 31˚C for a 24hr time period. Protein cell lysates of genotyped primary melanocytes, WT (n=3) and 402Q/Q (n=3), were untreated (U) or digested with the glycosidase EndoH (E) or PNGaseF (P). Samples were immunoblotted and probed with anti-TYRP1 antibody to determine the extent of digestion. Vertical lines indicate sample (1455) was run on a different gel.

Figure S3

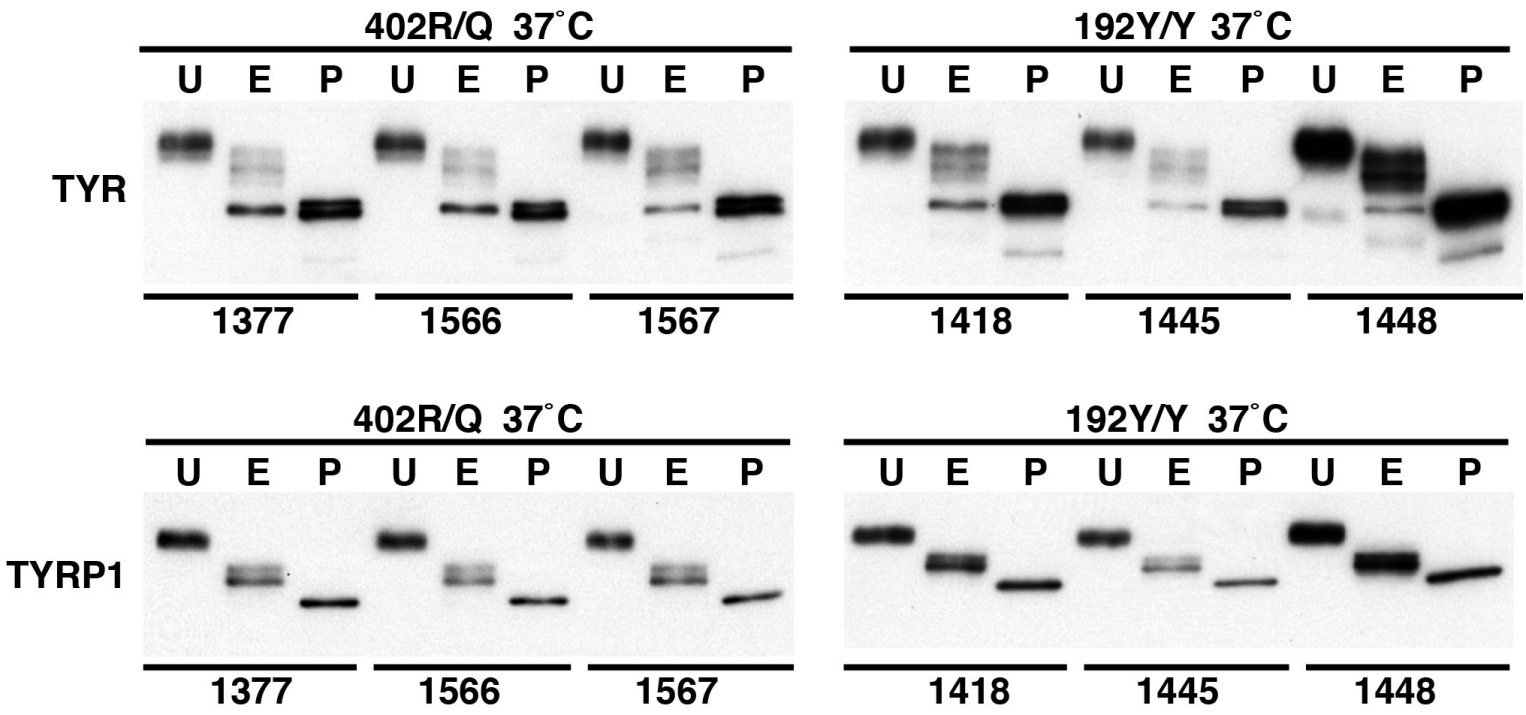


Figure S3 Assay of TYR and TYRP1 glycosylation in 402R/Q and 192 Y/Y melanocyte strains.

Glycosylation analysis of endogenous TYR and TYRP1 in primary melanocytes incubated at 37˚. Protein cell lysates of genotyped primary melanocytes, 402R/Q (n=3) and 192Y/Y (n=3) were untreated (U) or digested with the glycosidase EndoH (E) or PNGaseF (P). Samples were immunoblotted and probed with anti-TYR and anti-TYRP1 antibodies to determine the extent of digestion.

Figure S4

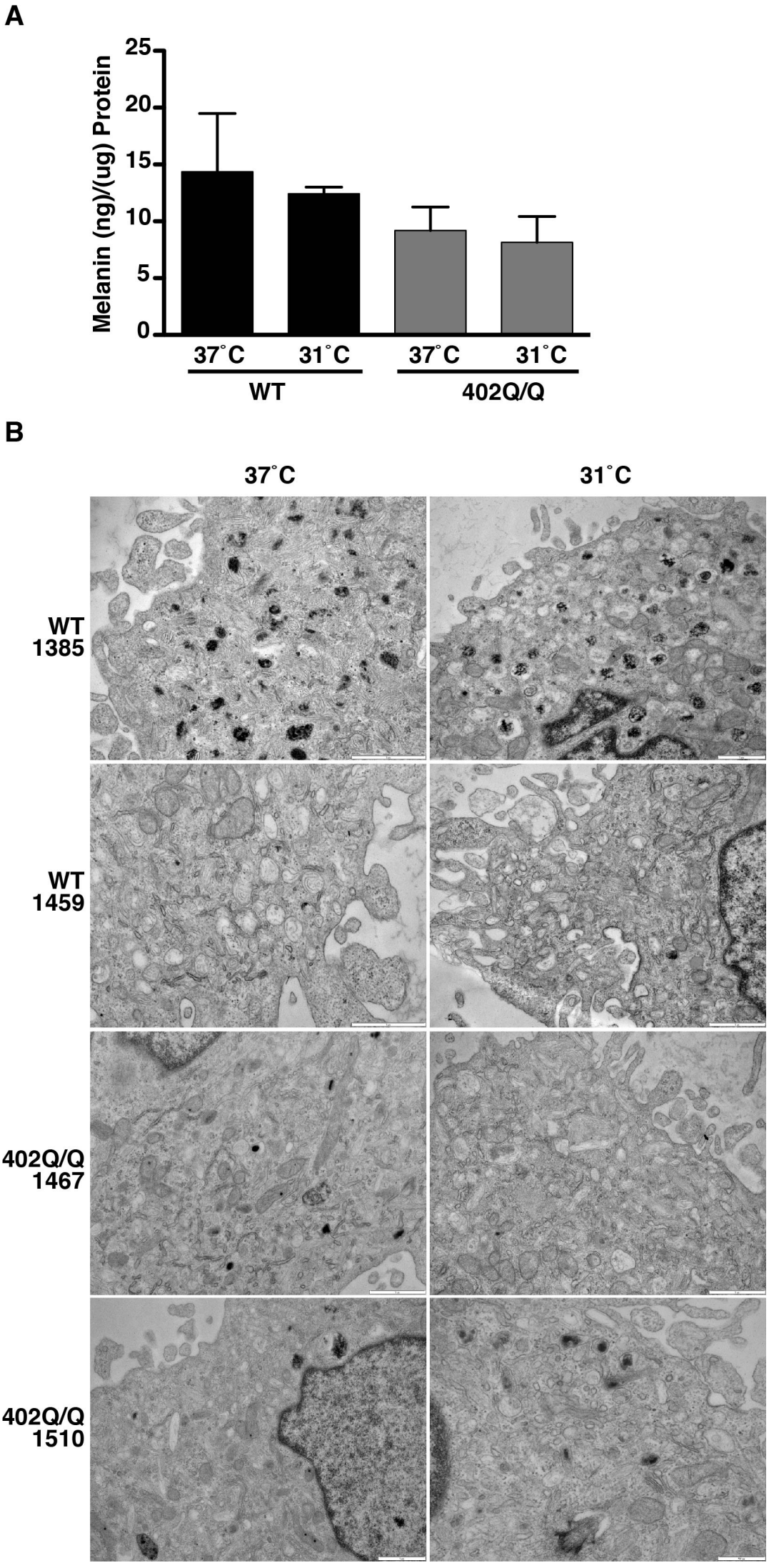

A
Melanin (ng)/(ug) Protein
25
20
15
10
5
0
37˚C
31˚C
37˚C
31˚C
WT
402Q/Q
B
37˚C
31˚C
WT
1385
WT
1459
402Q/Q
1467
402Q/Q
1510

Figure S4 Temperature change has no effect on melanogenesis in melanocyte strains.

A. Melanin content in genotyped primary melanocytes incubated at 37˚C and 31˚C for a 24hr time period. Values represent the mean + SEM (n=3) of 3 different melanocyte strains of each genotype normalised to total protein. **B.** Transmissive electron microscopy of melanocytes incubated at 37˚C and 31˚C for a 24hr time period. Micrographs are representative of genotyped primary melanocytes, WT (n=2) and 402Q/Q (n=2). Scale bar = 1µm.