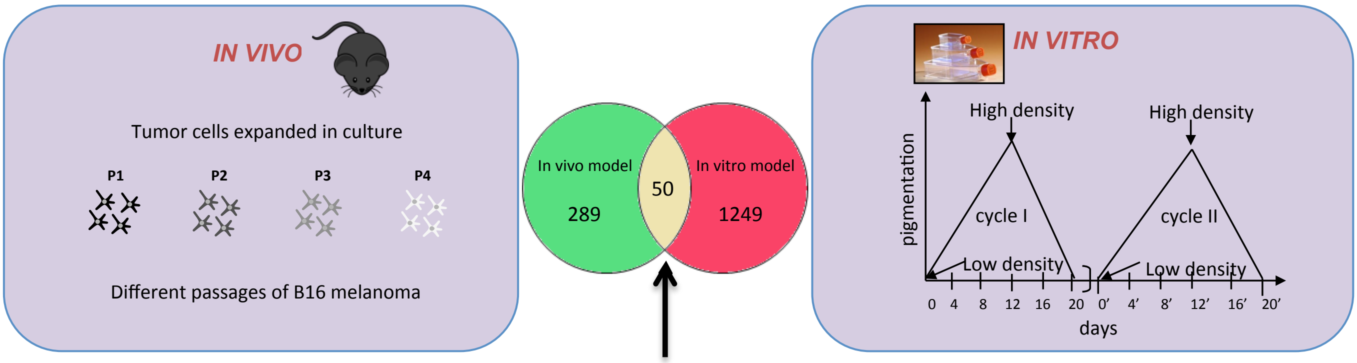
Appendix Figure S1



Potential regulators of melanocyte differentiation

0610011F06Rik	5031439G07Rik	9930108O06Rik	A830073O21Rik	Bcl6	Bfsp2	Bglap-rs1	Bglap2	C130078N17Rik
H2afz	Ifitm3	Irf1	Kdelr3	Kif1b	LOC380706	Mcc	Mcoln3	Mlana
D10Ertd610e	D11Lgp2e	D14Ertd668e	Daam1	Dlgap4	Gadd45a	Gsta1	Gsta2	Gsta3
Plxnd1	Rab11fip5	Rab27a	Rapsn	Rrm2	Si	Socs3	Syt9	Tgfb3
Ca14	Catnb	Cdk2	Nap1l1	Ndrg2	Nos3	H2-T23	Aph1a	Tyrp1
Camk2d	Mvp	H2-Q5	Tyr	Vcl				

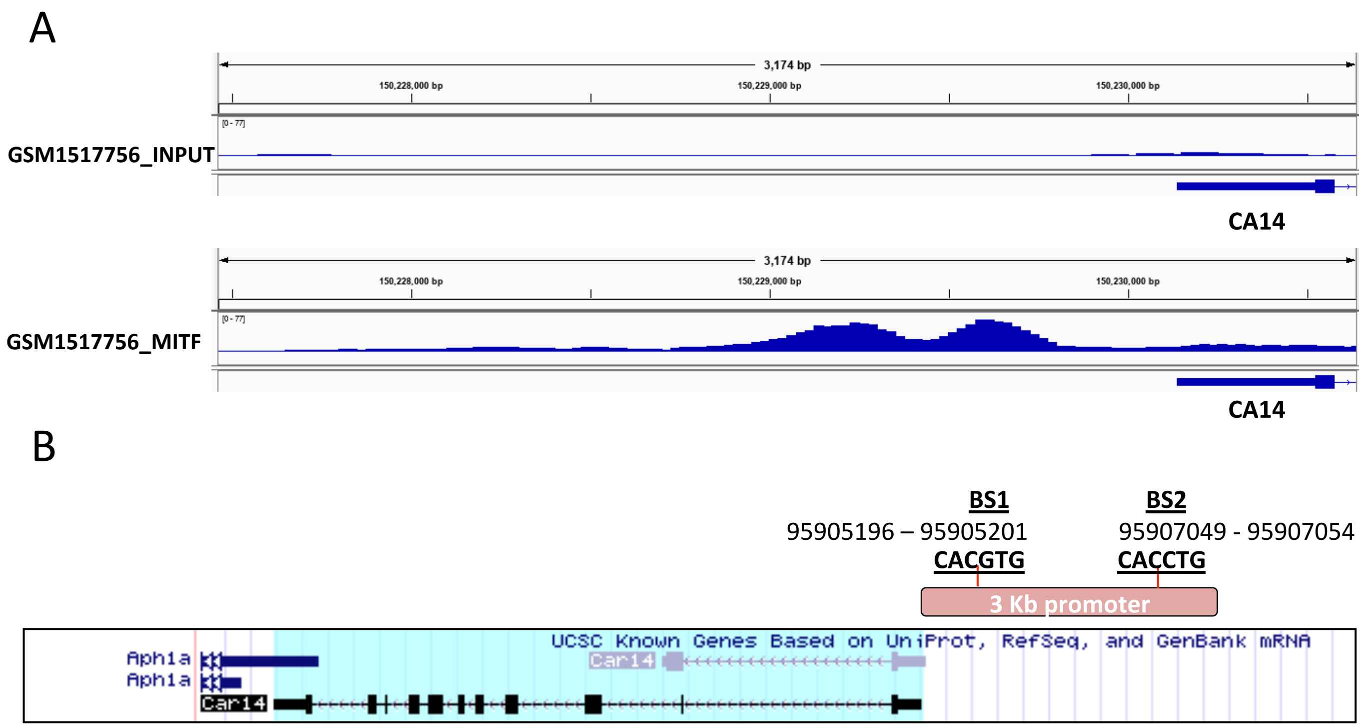
Progressive depigmentation model (*in vivo*):
B16 cells were grown as tumor in C57BL/6 mice. Tumor was dispersed and was grown in vitro as adherent culture. The cells were harvested at different passages (P1-P4) and noted to be progressively depigmented and devoid of melanin by P4. When these cells were injected back into the mice and grown as tumor, the cells regained pigmentation.

***Pigmentation Oscillator* (*in vitro*):**
B16 cells were maintained at sub-confluence with 5% CO$_2$ levels in DMEM supplemented with 10% Fetal bovine serum. The pigmentation-depigmentation model was set up by plating ~100 cells/cm^2, and cells were harvested at days 4, 8, and 12. For depigmentation, cells were re-plated at 10,000 cells/cm^2 on day 12 and harvested after 4 and 8 d to complete the 20-d cycle. The second cycle was initiated from the depigmented cells obtained after a gap of 4 d to allow for all changes to stabilize.

Differentially regulated genes were determined by microarray analysis and a common set of 50 genes were sorted (provided in the table above).

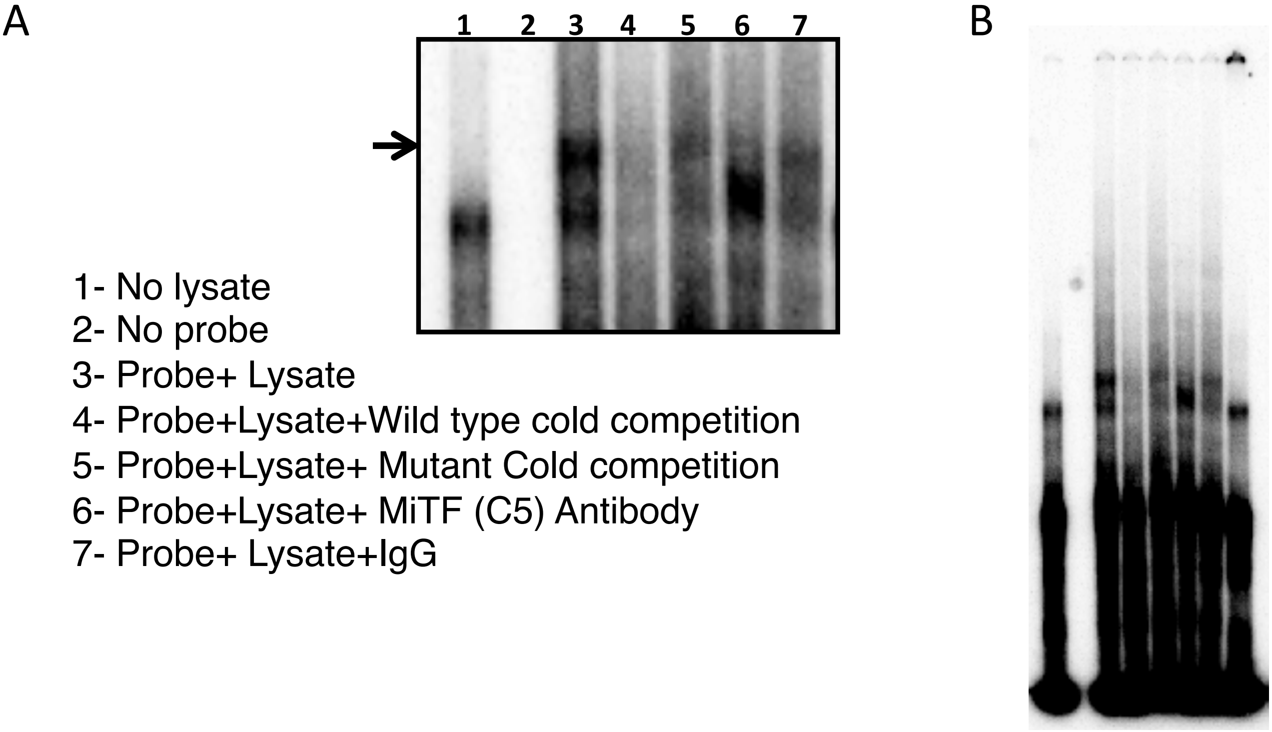
This model is used in this study to synchronize the pigmentation process and elucidate the mechanism of melanocyte maturation.

Appendix Figure S2



(A) Integrative genomics viewer (IGV) tracks displaying ChIP enrichment peaks for MITF (bottom)as compared to INPUT (top). Derived from dataset GSM1517756 – 501Mel human melanoma cells. **(B)** Image depicting the Mitf binding sites in mouse Car14 gene promoter (marked by BS1 and BS2 which are present in proximal 1kb and distal 1kb from TSS respectively)

Appendix Figure S3



1- No lysate
2- No probe
3- Probe+ Lysate
4- Probe+Lysate+Wild type cold competition
5- Probe+Lysate+ Mutant Cold competition
6- Probe+Lysate+ MiTF (C5) Antibody
7- Probe+ Lysate+IgG

(A) Electrophoretic Mobility Shift Assay (EMSA) performed on B16 nuclear lysate using labeled wildtype (BS1) probe encompassing E/M box sequence in *Ca14* promoter. When indicated reactions were carried out in presence of an excess (10-fold) of unlabelled homologous or mutated probe. Arrow indicates the bands specific to the shift. Lane 6 contains 2ug C5 monoclonal antibody to MITF ad Lane 7 contain 2ug normal mouse IgG. Shift ablation is observed in lane 6 **(B)** Full length EMSA gel pertaining to A

Appendix Figure S4

A

	Phenotype percentage
Control MO	8 ± 1
Ca14 MO	75 ± 5

B

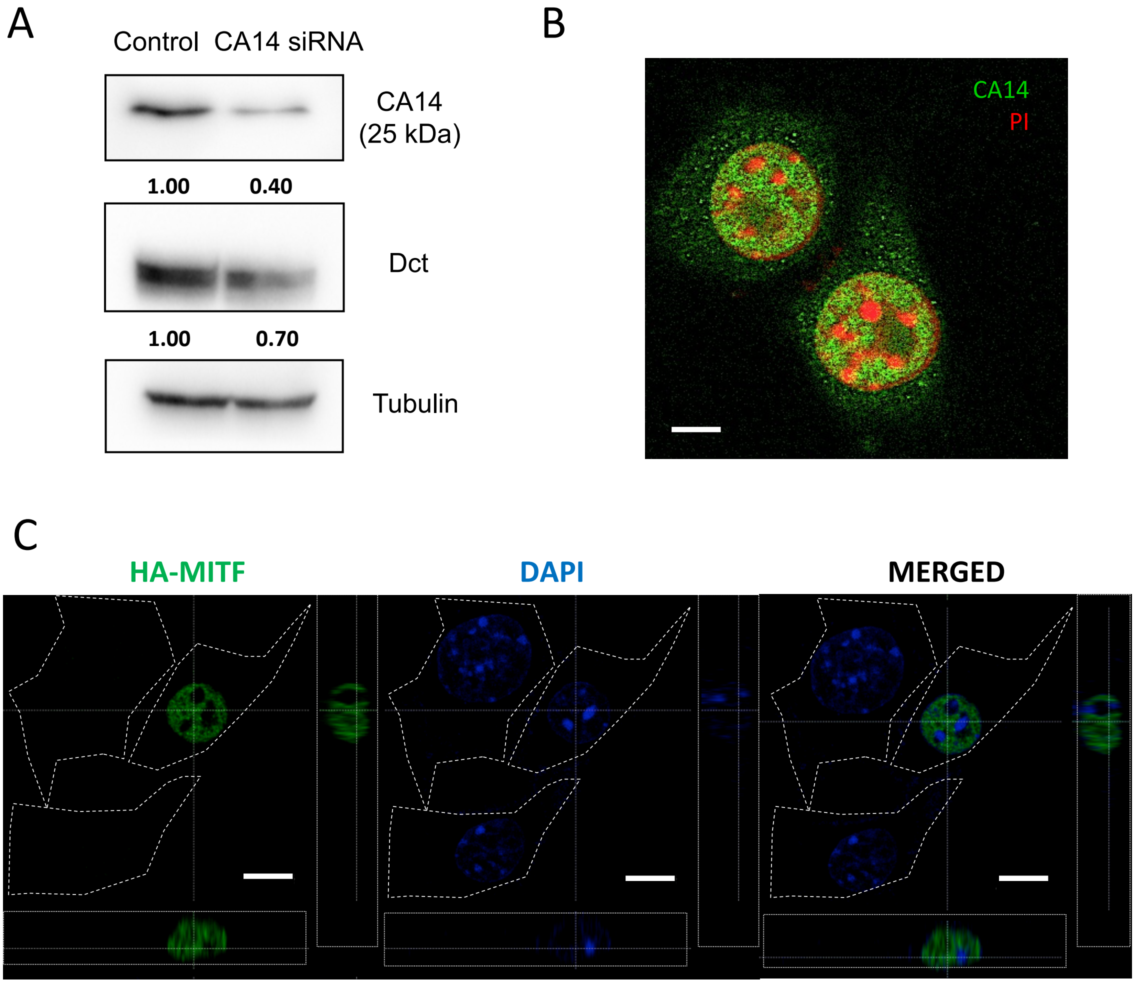
	Survival percentage
Control MO	92 ± 2
Ca14 MO	78 ± 6

C

	Cell Viability
Control shRNA	95 ± 2
Ca14 shRNA	90 ± 5
DMSO	98 ± 3
C646 treated	87.5 ± 8

Tables representing (A) percentage phenotype (B) survival percentage for control morpholino and ca14 morpholino injected embryos. (C) Percent cell viability of B16 cells transfected with control non silencing shRNA or shRNA targeting Ca14, DMSO treatment and 10uM C646 treatment after 48 hours of intervention.

Appendix Figure S5



A. Western blot analysis Ca14 and Dct normalized to tubulin. Numbers represent tubulin normalized fold changes corresponding to control siRNA.

B. Immunofluorescence staining of CA14 (in green) in B16 cells and counterstaining by propidium iodide (in red) Scale bar = 5µm.

C. Immunofluorescence of HA tagged MITF (in green) using HA antibody counterstained by DAPI (in blue) Scale bar = 10µm.

Appendix Table S1: List of primers utilized in this study

CRISPR primers	
zfca14 crispr (Target region)	TAATACGACTCACTATAGGGTAAAATCATGGATCATATCGTTTTAGAGCTAGAAATAGC
sgRNA core seq	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC
zfca14 crispr targeting amplicon Forward Primer	TGCAACTTTTCAGTGACCAGCA
zfca14 crispr targeting amplicon Reverse Primer	GCACTCCACACACTTCAAAACAA
Zebrafish RNA primer	
mca14 with modified kozak for mRNA injection Forward primer	TAATACGACTCACTATAGGGAGAGCAAACATGTTGTTCTTCGCTCTCCTGT
mca14 with modified kozak for mRNA injection Reverse primer	TCACGCCTCTGTGGTAGCC
Cloning primers	
ca14 3kb promoter fp	**GGTACC**CCACAAAATTTATCTGCCCTTTCTC
ca14 3kb pomoter rp	**AAGCTT**GGGAGAGAGAGGAGTTTCTGGC
ca14 mouse CDS FP (for overexpression cloning)	AAGCTTATGTTGTTCTTCGCTCTCCTGT
ca14 mouse CDS RP (for overexpression cloning)	CCATGGTCACGCCTCTGTGGTAGCC
ca14 proximal 1kb promoter Forward primer	GAGAGAGGAGTTTCTGGCGT
ca14 proximal 1kb promoter Reverse primer	TGGAGTCCGCCTTTTTCTCA
ca14 middle 1kb promoter Forward primer	CATGTGACCAGGTGGGTGAG
ca14 middle 1kb promoter Reverse primer	CCCCAAACTCTCAGCACCAA
ca14 distal 1kb promoter Forward primer	AGTCTTAGTCCCGAGTCAACA
ca14 distal 1kb promoter Reverse primer	TGCATCTATTGAATCGGAAGGCT
Site directed mutatgenesis primers	
mouse ca14 CDS thr to Ile mutation	CTACAACGGCTCACTCATAACTCCCCCCTGCTAC
mouse ca14 CDS thr to Ile mutation complement	GTAGCAGGGGGGAGTTATGAGTGAGCCGTTGTAG
Ca14 1kb binding site mutation	TGGTGAGGCTTAGGTCGAAGCGCCTGTGTAGCCTGGGG
Ca14 1kb binding site mutation complement	CCCCAGGCTACACAGGCGCTTCGACCTAAGCCTCACCA
Ca14 3kb binding site mutation	CCCTCTTCTCCCCTTGGAACCGGCCCAAGGACCACCAA
Ca14 3kb binding site mutation complement	TTGGTGGTCCTTGGGCCGGTTCCAAGGGGAGAAGAGGG
EMSA primers	
Ca14 1Kb binding site FP BS1	TGGTGAGGCTTAGGTCCACGTGCCTGTGTAGCCTGGGG
Ca14 1Kb binding site RP BS1	CCCCAGGCTACACAGGCACGTGGACCTAAGCCTCACCA
Ca14 3Kb binding site FP BS2	CCCTCTTCTCCCCTTGCACCTGGCCCAAGGACCACCAA
Ca14 3Kb binding site RP BS2	TTGGTGGTCCTTGGGCCAGGTGCAAGGGGAGAAGAGGG
Chromatin immunoprecipitation q-RT primers	
ca14 mitf binding site proximal Forward primer	GCCATCCTGCATGCCTAATC
ca14 mitf binding site proximal Reverse primer	TCGTAATCTCCACACCACTTACTG
ca14 mitf binding site middle Forward primer	GAACGCCTGAAGGATTCCCA
ca14 mitf binding site middle Reverse primer	TGTAGAAACCAGGCATGGGG
ca14 mitf binding site distal Forward primer	CCTAGCGTTTTGGCCAGAGATA
ca14 mitf binding site distal Reverse primer	GGGCCTAGTCTATGTTGAGGG
ca14 mitf binding site intronic Forward primer	GAACGCCTGAAGGATTCCCA
ca14 mitf binding site intronic Reverse primer	TGTAGAAACCAGGCATGGGG
cdk2 mitf binding Forward primer	TTGAGCTTGAACATTGTGGATGA
cdk2 mitf binding Reverse primer	AGGGCATGACCAAGTCTACAA
tyrp1 mitf binding Forward primer	GAC TTT AAC CAT CAC AAG GAA ACC A
tyrp1 mitf binding Reverse primer	CTC ACT AAT CCC TTC TCA CAC CAG
tyr promoter upstream to TSS Forward primer	GGGAGGAAAGGGTGCTTGAG
tyr promoter upstream to TSS reverse primer	AGGCTTGGGTTGTAATGCCA
dct promoter upstream to TSS Forward primer	GGGAGCTTTCGTCTTGCTCT
dct promoter upstream to TSS reverse primer	TCCATTAAGGGCGCATAGCC
tyrp1 promoter upstream to TSS Forward primer	CCAGTGTGAGGAATCTGGCTTG
tyrp1 promoter upstream to TSS reverse primer	TGCCAGCTGTTAATTGCCCG
gp100 promoter upstream to TSS Forward primer	GCCCTAGAAGGTGAGTGTGG
gp100 promoter upstream to TSS reverse primer	AACCCAGAGCTGGTGTTCAA
mitf promoter upstream to TSS Forward primer	TTCTGGTCCAAGTCCCAAGC
mitf promoter upstream to TSS reverse primer	ACTTCGAAATCCGCCACGAT

Appendix Table S2: List of TaqMAN probes utilized in this study

Catalog No	Gene	Organism
Hs00201626_m1	CA14	human
Mm00488564_m1	car14	Mouse
Mm00434954_m1	mitf	mouse
Mm00495817_m1	tyr	mouse
Mm00453201_m1	tyrp1	mouse
Mm99999915_g1	gapdh	mouse
Mm01225584_m1	dct	mouse
Mm00607939_s1	actb	mouse
Mm0__8996_m1	gp100	mouse
4310893E	18s rRNA	Eukaryotes
Dr03439955_g1	rps11	zebrafish
Dr03439955_g1	ca14	zebrafish
Dr03093326_m1	dct	zebrafish
Dr03121366_m1	tyrp1b	zebrafish
Dr03138104_m1	tyr	zebrafish
Dr03124868_m1	mitfa	zebrafish

Appendix Table S3: List of siRNA and shRNA utilized in this study

	Gene	Catalog No	Organism	Company	Type
siRNA	Car14	L-048804-01-0005	Mouse	Dharmacon	Smartpool
	CA14	L-009918-01-0005	Human	Dharmacon	Smartpool
	Mitf	L-047441-00-0005	Mouse	Dharmacon	Smartpool
	MITF	L-008674-00-0005	Human	Dharmacon	Smartpool
GIPZ shRNA	Car14	RMM4431-200335793 (Clone ID V2LMM_35119) **(TATTGTGTAGATCCAAAGG)**	Mouse	Dharmacon	Single
GIPZ shRNA	Car14	RMM4431-200335793 (Clone ID V2LMM_28855) (**TCATATGTCCAGTGGTGAC)**	Mouse	Dharmacon	Single
GIPZ Non silencing shRNA		RHS4346		Dharmacon	Single

Appendix Table S4: List of antibodies utilized in this study

Antibody	Catalog number	Company	Dilution used in study
ca14	ab92575	Abcam	1;750
dct	ab74073	Abcam	1;1000
gp100	ab137078	Abcam	1;1000
mitf (C5)	ab12039	Abcam	1;1000
tubulin	ab21058	Abcam	1;10000
gapdh	ab9385	Abcam	1;10000
actin	ab20272	Abcam	1;10000
Ac H2A.Z	ab18262	Abcam	1;1000
H2A.Z	ab4174	Abcam	1;1000
H2AK5	Acetyl histone antibody sampler kit (9933)	Cell Signalling Technology	1;1000
H2A	Acetyl histone antibody sampler kit (9933)	Cell Signalling Technology	1;1000
H3K9	Acetyl histone antibody sampler kit (9933)	Cell Signalling Technology	1;1000
H3	Acetyl histone antibody sampler kit (9933)	Cell Signalling Technology	1;1000
H4K12	Acetyl histone antibody sampler kit (9933)	Cell Signalling Technology	1;1000
H4	Acetyl histone antibody sampler kit (9933)	Cell Signalling Technology	1;1000
H3K27	ab4729	Abcam	1;1000
Mitf C5 sera	Kind gift from Dr David Fisher		1;100

Appendix Table S5: Mean ± SEM along with p-values for Fig 5F

Gene	Sh NT	Sh Ca14	p-value
Tyr	1±0.04	0.49±0.086	0.032
Dct	1±0.014	0.67±0.068	0.041
Gp100	1±0.06	0.84±0.044	0.215
Ca14	1±0.09	0.88±0.10	0.212
Tyrp1	1±0.001	0.98±0.028	0.66
Mitf	1±0.001	0.99±0.003	0.95
Cdk2	1±0.011	0.98±0.012	0.92

Appendix Table S6: Mean ± SEM along with p-values for Fig 5G

Gene	IgG	P300	p-value
Tyr	0.005±0.002	0.016±0.001	0.022
Dct	0.013±0.007	0.03±0.002	0.032
Gp100	0.009±0.004	0.031±0.033	0.014
Ca14	0.005±0.016	0.064±0.005	0.71
Tyrp1	0.025±0.005	0.035±0.046	0.049
Mitf	0.034±0.001	0.067±0.012	0.056
Cdk2	0.046±0.016	0.07±0.005	0.078

Appendix Table S7: Mean ± SEM along with p-values for Fig 5H

Gene	DMSO	C646	p-value
Tyr	100±7.623	44.48±3.302	0.021
Dct	100±12.79	15.58±5.79	0.026
Gp100	100±5.23	87.05±4.05	0.189
Ca14	100±5.55	34.12±5.16	0.013
Tyrp1	100±18.95	59.22±23.44	0.308
Mitf	100±6.96	94.07±3.57	0.527
Cdk2	100±0.49	105.7±7.67	0.536