

Supporting Information

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Constructs and Antibodies. *MITF-M*, *MITF-A*, and *MITF-H* promoter driven luciferase reporter constructs were a generous gift from Dr. Shigeki Shibahara (Tohoku University, Miyagi, Japan). pGL3-basic was from Promega. The *TYRO3* retroviral expression construct was from the GNF cDNA retroviral collection. *BRAF(V600E)* retroviral expression construct was from Dr. Nanxin Li of GNF. Mission shRNA lentiviral constructs targeting *TYRO3* were from Sigma. Kinase-dead *TYRO3* mutant (K550A) was generated using QuikChange Site-Directed Mutagenesis Kit (Stratagene) following the manufacturer's instructions.

Goat anti-TYRO3 antibody was purchased from Santa Cruz. Mouse anti-MITF (C5+D5) and mouse anti-SOX10 were from Abcam. Mouse anti-Flag and mouse anti- γ -tubulin were from Sigma. Goat anti-PAX3 and goat anti-CREB were from R&D. Rabbit anti cleaved Caspase 3 antibody was from Cell Signaling.

Immunofluorescent Staining. Cells were fixed with 10% formalin solution at room temperature for 10 min, permeabilized with 0.5% Triton X-100 (Sigma) in PBS (PBS) solution for 5 min, then blocked with PBS containing 0.1% Triton X-100, 10% horse serum, and 1% BSA at room temperature for 1 h. The cells were incubated with primary antibodies in PBS solution containing 0.1% Triton X-100 and 0.1% BSA at 4 °C overnight, then rinsed with PBS three times, and incubated with fluorescently-labeled secondary antibodies in PBS solution containing 0.1% Triton X-100, 0.1% BSA, and DAPI at room temperature for 1 h. Cells were rinsed with PBS containing 0.1% Triton X-100 at least three times and subjected to microscopic analysis.

Nuclear Fraction Extraction. Cells were washed with ice-cold PBS, covered with cell lysis buffer (20 mM HEPES, pH7.9, 10 mM NaCl, 3 mM MgCl₂, 0.1% Nonidet P-40, 10% glycerol, 0.2 mM EDTA, 1 mM DTT, and protease inhibitor mixture) and incubated on ice for 15 min. The cells were scraped off the dishes mechanically, and pipetted up and down gently to break the cell debris. The cell lysate was centrifuged at 2,000 rpm at 4 °C for 5 min and the supernatants were saved as the cytosolic fraction. The pellets were re-suspended in wash buffer (20 mM HEPES, pH 7.9, 20% glycerol, 0.2 mM EDTA, 1 mM DTT, and protease inhibitor mixture) and centrifuged at 2,000 rpm for 5 min. The supernatants were discarded, the pellets were re-suspended in nuclear extraction buffer (20 mM HEPES, pH 7.9, 400 mM NaCl, 20% glycerol, 0.2 mM EDTA, 1 mM DTT, and protease inhibitors mixture) and then incubated on ice for approximately 45 min. The mixtures were centrifuged at 13,000 rpm for 15 min at 4 °C, and the supernatants were saved as nuclear fractions.

Retroviral and Lentiviral Particle Production and Cell Infection. Retroviral particles carrying *BRAF(V600E)* and *TYRO3* genes were

generated in *Gag-Pol*-expressing 293T cells (GP2s). Briefly, *BRAF(V600E)* and *TYRO3* retroviral vectors were co-transfected with *VSV-G* vector into GP2s. Viral supernatants were collected 48 h and 72 h post-transfection and concentrated using Amicon filters (Millipore). Primary melanocytes were infected with the appropriate retroviral supernatants in the presence of 5 μ g/mL PolyBrene (Millipore). ShRNA lentiviral vectors targeting human *TYRO3* (Sigma) were used to produce lentiviral transduction particles according to the manufacturer's protocol. Stable clones of melanoma cell lines expressing scrambled or *TYRO3* shRNA were selected in the presence of puromycin and subjected to further analysis.

Reverse Transcription-PCR (RT-PCR) and Real-Time Quantitative PCR (qPCR). RNA was isolated using the RNeasy kit (Invitrogen) according to the manufacturer's protocol. Three micrograms of RNA was used for each reverse transcription reaction (RT-PCR kit, Invitrogen). The melanoma cDNA tissue array was from Origene. Gene specific primers were used for PCR analysis of *MITF-M* and β -*Actin* expression. Primers used for RT-PCR: mouse *Mitf-M*, forward: atgctggaatgctagaatacagt; reverse: atcatccatctgcatgcac; mouse β -*Actin*, forward: cctaaggcaaccgtgaaaag; reverse: tcttcattggtctaggagcca. For real-time quantitative PCR, gene specific assays for mRNA levels for *TYRO3*, *AXL*, *MER*, *MITF-M*, β -*Actin* and *GAPDH* were obtained from Applied Biosystems, and the reactions were set up following the manufacturer's instructions.

Adhesion-Independent Colony Formation Assay. Anchorage-independent colony formation was performed in soft agar. Cells (2×10^3 cells/well, 6-well plate) were suspended in 0.3% agar in DMEM containing 10% FBS and plated on solidified agar (0.5%). Cells were incubated for 3 weeks at 37 °C in 5% CO₂, and the number of colonies was counted under a microscope.

Subcutaneous Tumor Formation Assay. Cells were trypsinized and resuspended in serum free DMEM. One million cells in a volume of 50 μ L were injected into each nude mouse s.c. Tumor sizes were measured once every 3 days, the volume was calculated as length \times width \times height.

Tumor formation of melanoma cells with *TYRO3* knockdown was significantly repressed in the s.c. model as well (Fig. S6). At day 30, small tumors were detectable on mice injected with *TYRO3* knockdown cells (≈ 10 mm³ for shRNA1 expressing cells and ≈ 3 mm³ for shRNA2 expressing cells, respectively) comparing to large tumors formed by the non-targeting shRNA expressing control cells (≈ 400 mm³). We then isolated the cells from the tumors, and found that those tumor cells do not have *Tyro3* shRNA construct incorporated, therefore, those tumors likely arose from cells without *Tyro3* shRNA construct.

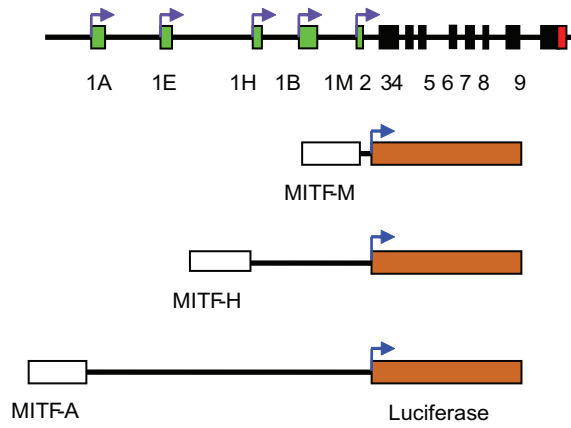


Fig. S1. The promoter structure of *MITF* and luciferase reporter genes used in the screen. Green boxes represent isoform-specific exon 1; black boxes represent common exons; the red box represents the stop codon; open boxes represent promoter regions; brown boxes represent firefly luciferase-coding sequence.

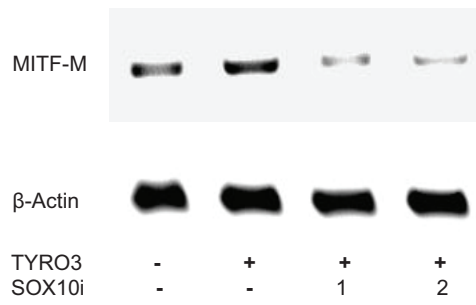


Fig. S3. Semiquantitative RT-PCR for *Mitf-M* in B16-F0 cells upon *Sox10* knockdown and *TYRO3* overexpression. The numbers 1 and 2 designate two siRNA pieces targeting *Sox10* mRNA at different sequences.

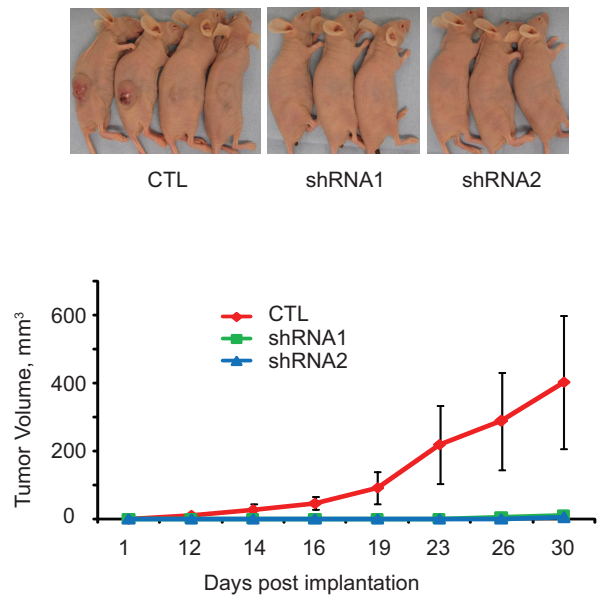


Fig. S6. Subcutaneous tumor formation assay of A2058 melanoma cells with *TYRO3* knockdown. Thirty days post-injection of 10^6 cells per mouse.

Table S1. List of hits from *MITF-M* reporter screen

Number	Symbol	Description	GenBank Accession
1*	Ndufs4	NADH dehydrogenase (ubiquinone) Fe-S protein 4	BC004618
2*	Rbm3	RNA binding motif protein 3	BC006580
3	Ppp1cc	protein phosphatase 1, catalytic subunit, gamma isoform	BC010613
4	Dlst	dihydrolipoamide S-succinyltransferase	BC006702
5	DHRS9	dehydrogenase/reductase (SDR family) member 9, transcript variant 2	BC058883
6*	Mfap1	microfibrillar-associated protein 1	BC005728
7	Rnmt	RNA (guanine-7-) methyltransferase	BC021794
8*	Tbl3	transducin (beta)-like 3	BC019504
9*	Car10	carbonic anhydrase 10	BC017606
10	STOML3	stomatin (EPB72)-like 3	BC025760
11	Tde1	tumor differentially expressed 1	BC029026
12	AZGP1	alpha-2-glycoprotein 1, zinc	BC033830
13*	Rara	retinoic acid receptor, alpha	BC010216
14*	A1429612	expressed sequence A1429612	BC046457
15*	Eif5a	eukaryotic translation initiation factor 5A	BC008093
16*	PIK3C3	phosphoinositide-3-kinase, class 3	BC053651
17*	TYRO3	TYRO3 protein tyrosine kinase	BC051756
18*	EHF	ets homologous factor	BC038995
19	SEC10L1	SEC10-like 1	BC041126
20*	GPR114	G protein-coupled receptor 114	BC032401
21*	RNASET2	ribonuclease T2	BC039713
22*	FCRH1	Fc receptor-like 1	BC033690
23*	DDB1	damage-specific DNA binding protein 1, 127kDa	BC011686

Asterisks indicate these genes increase reporter activity significantly compared to empty vector.

Table S2. List of signaling pathways whose component gene expression is affected upon *TYRO3* knockdown

Number in Fig. S5B	Signaling Pathway
1	Ceramide Signaling
2	Regulation of Actin-Based Motility by Rho
3	Integrin Signaling
4	PI3K/AKT Signaling
5	CNTF Signaling
6	Actin Cytoskeleton Signaling
7	Cell Cycle: G1/S Checkpoint Regulation
8	IL-15 Signaling
9	Wnt/ β -Catenin Signaling
10	Ephrin Receptor Signaling
11	ERK/MAPK Signaling
12	Protein Ubiquitination Pathway
13	SPAK/JNK Signaling
14	PTEN Signaling
15	Natural Killer Cell Signaling
16	Insulin Receptor Signaling
17	IL-9 Signaling
18	NF- κ B Signaling
19	Tight Junction Signaling
20	EGF Signaling
21	IL-8 Signaling
22	NRF2-mediated Oxidative Stress Response
23	IL-2 Signaling
24	JAK/Stat Signaling
25	Neurotrophin/TRK Signaling
26	IL-3 Signaling
27	PDGF Signaling
28	IL-17 Signaling
29	LPS-stimulated MAPK Signaling
30	TGF- β Signaling
31	FGF Signaling
32	TR/RXR Activation
33	VEGF Signaling
34	IGF-1 Signaling
35	p53 Signaling
36	CDK5 Signaling
37	HIF1 α Signaling
38	IL-12 Signaling and Production in Macrophages
39	Estrogen Receptor Signaling
40	Relaxin Signaling
41	CXCR4 Signaling
42	RAR Activation
43	Acute Phase Response Signaling
44	G-Protein Coupled Receptor Signaling

Category	Function	Function Annotation	Molecules
Cancer	cell death	cell death of tumor cell lines	ADI1, ATP2A2, CDK6, CTBP1, CYLD, EPHA2, HOXA1, ID1, PPP2R1B, SFRP1, TNFAIP3, TUBA1A
Cancer	renal-cell carcinoma	renal-cell carcinoma	CDK6, HDAC7, TUBA1A
Cancer	bone tumor	bone tumor	CDK6, EPHA2, HDAC7
Cancer	angiogenesis	angiogenesis of tumor	COL4A1, EPHA2
Cancer	metastatic tumor	metastatic tumor	HDAC7, TUBA1A
Cancer	colony formation	colony formation of glioma cells	SFRP1
Cancer	senescence	senescence of breast cancer cell lines	ID1
Cancer	lung tumor	lung tumor	CDK6, DICER1, EPHA2, HDAC7, TUBA1A
Cancer	developmental process	developmental process of tumor cell lines	CDK6, DLG5, EPHA2, ID1, ID3, IL11, IL17RD, PIK3CD, SOX2, TXNIP
Cancer	epithelial ovarian cancer	epithelial ovarian cancer	HDAC7, TUBA1A
Cancer	activation	activation of chronic lymphocytic leukemia B cells	IL11
Cancer	bronchiolo-alveolar adenocarcinoma	bronchiolo-alveolar adenocarcinoma	DICER1
Cancer	cell movement	cell movement of glioma cells	SFRP1
Cancer	differentiation	differentiation of tumor cell lines	CDK6, ID1, ID3, IL11, IL17RD
Cancer	apoptosis	apoptosis of breast cancer cell lines	CYLD, EPHA2, HOXA1, SFRP1
Cancer	breast cancer	breast cancer	CDK6, EPHA2, HDAC7, PPP2R1B, SOX2, TUBA1A
Cancer	non-small-cell lung carcinoma	non-small-cell lung carcinoma	EPHA2, HDAC7, TUBA1A
Cancer	lung carcinoma	lung carcinoma	DICER1, EPHA2, HDAC7, TUBA1A
Cancer	ovarian tumor	ovarian tumor	HDAC7, TBC1D2B, TUBA1A, TXNIP
Cancer	chondrosarcoma	chondrosarcoma	EPHA2
Cancer	development	development of T-cell non-hodgkin lymphoma	ID1
Cancer	neoplasia	neoplasia of skin	EPHA2
Cancer	uterine tumor	uterine tumor	ABI2, CDK6, CTSC, SFRP1, TUBA1A
Cancer	endometrial cancer	endometrial cancer	CDK6, TUBA1A
Cellular Growth and Proliferation	proliferation	proliferation of cells	CDK6, COL4A1, CTBP1, CYLD, DICER1, DLG5, EPHA2, FOXP1, HDAC7, HOXA1, ID1, ID3, IL11, NRP2, PIK3CD, PTP4A1, SFRP1, SOX2, TXNIP, WIPF1
Cellular Growth and Proliferation	hypertrophy	hypertrophy of heart cells	CTSC, IL11, RAB2A, TNFAIP3
Cellular Growth and Proliferation	formation	formation of bone marrow cells, osteoclasts	IL11, LGMN
Cellular Growth and Proliferation	stimulation	stimulation of lymphocytes, megakaryocyte, mast cells, colony-forming erythroid cells and multipotential hemopoietic progenitor cells	EPHA2, IL11
Cellular Growth and Proliferation	colony formation	colony formation of multipotential hemopoietic progenitor cells, glioma cells and granulocyte-macrophage progenitor cells	IL11, SFRP1
Cellular Growth and Proliferation	production	production of mononuclear cells	IL11
Cellular Growth and Proliferation	growth	arrest in growth of vascular smooth muscle cells	ID3
Cellular Growth and Proliferation	growth	growth of stomach cancer cell lines	TXNIP
Cellular Growth and Proliferation	quantity	quantity of erythroid cell lines	IL11
Gene Expression	binding	binding of GATA-4 binding site, Nkx2.5 binding site, E box motif, serum response element	ID1, ID3
Gene Expression	transactivation	transactivation of E box motif	ID1, ID3
Gene Expression	transactivation	transactivation of HNF3 binding site	IL11
Gene Expression	transactivation	transactivation of Ttf1 binding site	IL11
Gene Expression	transcription	transcription of E box motif	ID1, ID3
Gene Expression	transcription	transcription of TCF binding site	SFRP1
Gene Expression	stabilization	stabilization of RNA	ADAR

Category	Function	Function Annotation	Molecules
Gene Expression	repression	repression of synthetic promoter	CTBP1, HDAC7
Gene Expression	repression	repression of AML1 binding site	CTBP1
Gene Expression	induction	induction of synthetic promoter	IL17RD
Gene Expression	recruitment	recruitment of mRNA	EIF3A
Tumor Morphology	proliferation	proliferation of adenocarcinoma cells	ID1, ID3
Tumor Morphology	adhesion	adhesion of prostate cancer cells	EPHA2
Tumor Morphology	invasion	invasion of skin tumor	EPHA2
Tumor Morphology	growth	growth of skin tumor	EPHA2
Tumor Morphology	angiogenesis	angiogenesis of tumor	COL4A1, EPHA2
Tumor Morphology	colony formation	colony formation of glioma cells	SFRP1
Tumor Morphology	development	development of T-cell non-hodgkin lymphoma	ID1
Cell Cycle	cell cycle progression	re-entry into cell cycle progression of fibroblast cell lines	ID1, ID3
Cell Cycle	cell stage	cell stage	CDC23 (includes EG:8697), CDC34 (includes EG:997), CDK6, CYLD, ID1, ID3, RPS6KA2, SAC3D1, SOX2, TXNIP
Cell Cycle	cell division process	cell division process of kidney cell lines	CDK6, RPS6KA2, TXNIP
Cell Cycle	cell division process	exit from cell division process of cells	CDK6, CYLD
Cell Cycle	polyploidization	polyploidization of embryonic stem cells	SOX2
Cell Cycle	sub-G1 phase	arrest in sub-G1 phase of embryonic cell lines	TXNIP
Cell Cycle	interphase	interphase of kidney cell lines	CDK6, TXNIP
Cell Cycle	S phase	initiation of S phase of kidney cell lines	CDK6
Cell Cycle	S phase	S phase of breast cell lines	ID1
Cell Cycle	metaphase	arrest in metaphase of embryonic cell lines	RPS6KA2
Cell Cycle	mitosis	exit from mitosis of cervical cancer cell lines	CYLD
Cell Cycle	senescence	senescence of fibroblast cell lines	DICER1, ID1
Cell Cycle	formation	formation of PML nuclear bodies	HDAC7
Cell Cycle	metaphase/anaphase transition	metaphase/anaphase transition	CDC23 (includes EG:8697)
Cellular Function and Maintenance	maintenance	maintenance of stem cells	DICER1, SOX2
Cellular Function and Maintenance	exocytosis	exocytosis of dense-core vesicles	PIP5K1C
Cellular Function and Maintenance	autophagy	autophagy of embryonic cell lines	GOPC
Cellular Function and Maintenance	cytostasis	cytostasis of smooth muscle cells	ID1
Cellular Function and Maintenance	incorporation	incorporation of cells	EPHA2
Cellular Function and Maintenance	function	function of cytoskeleton	WIPF1
Cellular Function and Maintenance	positive selection	positive selection of thymocytes	ID3
Cellular Function and Maintenance	movement	movement of organelle	KLC2, WIPF1
Cell Death	survival	survival of cervical cancer cell lines	CASK, CDK6, CERK, PPP2R1B, SBF1
Cell Death	survival	survival of heart cell lines	TXNIP
Cell Death	survival	survival of glioma cells	SFRP1
Cell Death	survival	survival of dorsal root ganglion cells	IL11
Cell Death	cell death	cell death of eukaryotic cells	ADI1, ATP2A2, CDK6, CTBP1, CYLD, DICER1, EPHA2, FOXP1, HOXA1, ID1, ID3, IL11, IL17RD, PIK3CD, PPP2R1B, RPS6KA2, SFRP1, TNFAIP3, TUBA1A, TXNIP, TYRO3
Cell Death	anoikis	anoikis of pancreatic cancer cell lines	EPHA2
Cell Death	necrosis	necrosis of mucosa cells	IL11
Cell Death	self-renewal	self-renewal of normal cells	ID1, IL11

Category	Function	Function Annotation	Molecules
Cell Death	apoptosis	apoptosis of eukaryotic cells	ADI1, CDK6, CTBP1, CYLD, DICER1, EPHA2, FOXP1, HOXA1, ID1, ID3, IL11, IL17RD, PIK3CD, RPS6KA2, SFRP1, TNFAIP3, TXNIP
Cell Death	cytotoxicity	cytotoxicity of cell lines	TNFAIP3, WIPF1
Cell Death	cytolysis	cytolysis of endothelial cell lines	IL11
Cell Morphology	length	length of cortical neurons	DPYSL3
Cell Morphology	orientation	orientation of motor axons	HOXA1
Cell Morphology	orientation	orientation of stereocilia bundles	SFRP1
Cell Morphology	thickness	thickness of type I collagen fibrils	SERPINH1
Cell Morphology	transepithelial electrical resistance	transepithelial electrical resistance of breast cancer cell lines	ID1
Cell Morphology	transformation	transformation of cell lines	CDK6, EPHA2, PIK3CD, SBF1, SFRP1, TYRO3
Cell Morphology	branching	branching of cortical neurons	DPYSL3
Cell Morphology	permeability	permeability of kidney cell lines	EPHA2
Cell Morphology	shape change	shape change of mast cells	WIPF1
Cell Morphology	tubulation	tubulation of endothelial cell lines	ID1, ID3
Cell Morphology	remodeling	remodeling of axons	SFRP1
Cell Morphology	elongation	elongation of cardiomyocytes	ATP2A2
Cell Morphology	sprouting	sprouting of endothelial cell lines	DICER1