Supplementary materials

α-Viniferin Improves Facial Hyperpigmentation via Accelerating Feedback Termination of cAMP/PKA-Signaled Phosphorylation Circuit in Facultative Melanogenesis

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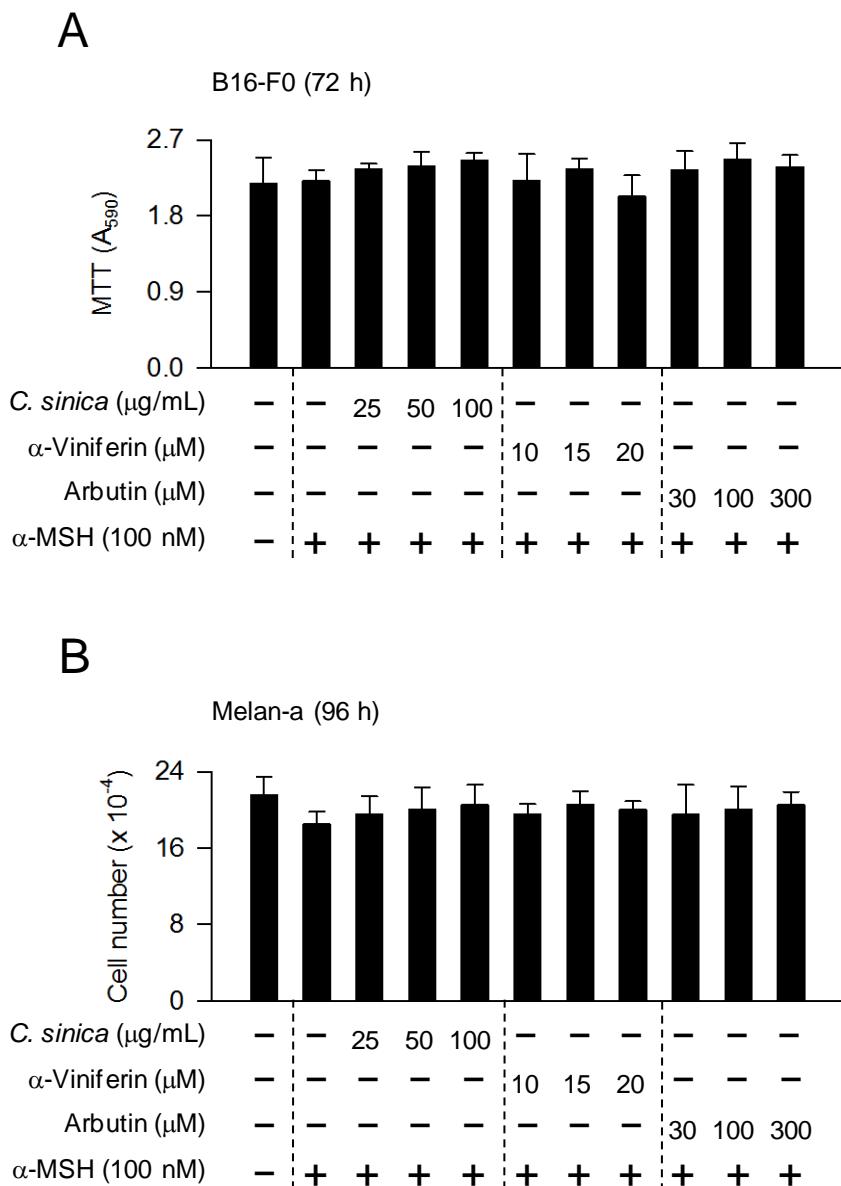
Figure S1. Effect of *C. sinica* or α-viniferin on cell viability. (A) B16-F0 cells were incubated with *C. sinica* or α-viniferin for 72 h in the presence of α-MSH, and reacted with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 50 µg/mL) for another 3 h. Formazan crystals were dissolved in 50% dimethyl sulfoxide and measured absorbance values at 590 nm (A_{590}). (B) Melan-a cells were incubated with *C. sinica* or α-viniferin for 96 h in the presence of α-MSH. Cell numbers were counted after exclusion with trypan blue. Data are mean ± SEM.

(Figure S2)

Patient (age) / treatment		0 time	After 4 weeks	After 6 weeks	After 8 weeks
W-1 (45)	Right (R) (Vehicle)	S01-V1-R	S01-V2-R	S01-V3-R	S01-V4-R
	Left (L) (C. sinica)	S01-V1-L	S01-V2-L	S01-V3-L	S01-V4-L
W-2 (48)	R (Vehicle)	S02-V1-R	S02-V2-R	S02-V3-R	S02-V4-R
	L (C. sinica)	S02-V1-L	S02-V2-L	S02-V3-L	S02-V4-L
W-3 (39)	R (C. sinica)	S03-V1-R	S03-V2-R	S03-V3-R	S03-V4-R
	L (Vehicle)	S03-V1-L	S03-V2-L	S03-V3-L	S03-V4-L
W-4 (41)	R (Vehicle)	S04-V1-R	S04-V2-R	S04-V3-R	S04-V4-R
	L (C. sinica)	S04-V1-L	S04-V2-L	S04-V3-L	S04-V4-L

(Figure S2 continued)

Patient (age) / treatment		0 time	After 4 weeks	After 6 weeks	After 8 weeks
W-5 (35)	R (Vehicle)	S05-V1-R	S05-V2-R	S05-V3-R	S05-V4-R
	L (C. sinica)	S05-V1-L	S05-V2-L	S05-V3-L	S05-V4-L
W-6 (53)	R (C. sinica)	S06-V1-R	S06-V2-R	S06-V3-R	S06-V4-R
	L (Vehicle)	S06-V1-L	S06-V2-L	S06-V3-L	S06-V4-L
W-7 (42)	R (Vehicle)	S07-V1-R	S07-V2-R	S07-V3-R	S07-V4-R
	L (C. sinica)	S07-V1-L	S07-V2-L	S07-V3-L	S07-V4-L
W-8 (48)	R (C. sinica)	S08-V1-R	S08-V2-R	S08-V3-R	S08-V4-R
	L (Vehicle)	S08-V1-L	S08-V2-L	S08-V3-L	S08-V4-L

Patient (age) / treatment		0 time	After 4 weeks	After 6 weeks	After 8 weeks
W-9 (46)	R (Vehicle)				
	L (*C. sinica*)				
W-10 (42)	R (Vehicle)				
	L (*C. sinica*)				
W-11 (41)	R (Vehicle)				
	L (*C. sinica*)				
W-12 (41)	R (Vehicle)				
	L (*C. sinica*)				

Patient (age) / treatment		0 time	After 4 weeks	After 6 weeks	After 8 weeks
W-13 (47)	R (*C. sinica*)				
	L (Vehicle)				
W-14 (36)	R (Vehicle)				
	L (*C. sinica*)				
W-15 (54)	R (*C. sinica*)				
	L (Vehicle)				
W-16 (55)	R (*C. sinica*)				
	L (Vehicle)				
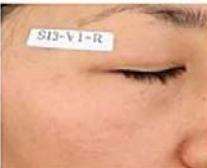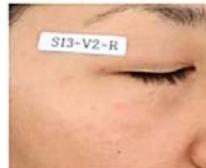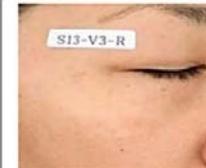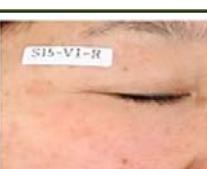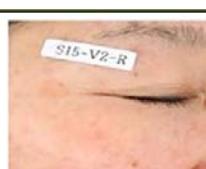
Patient (age) / treatment		0 time	After 4 weeks	After 6 weeks	After 8 weeks
W-17 (45)	R (C. sinica)				
	L (Vehicle)				
W-18 (43)	R (Vehicle)				
	L (C. sinica)				
W-19 (38)	R (C. sinica)				
	L (Vehicle)				
W-20 (49)	R (Vehicle)				
	L (C. sinica)				
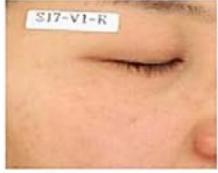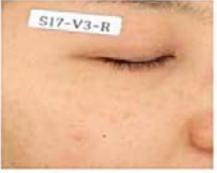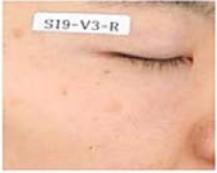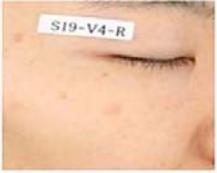
(Figure S2 continued)

Patient (age) / treatment		0 time	After 4 weeks	After 6 weeks	After 8 weeks
W-21 (50)	R (C. sinica)	S21-V1-R	S21-V2-R	S21-V3-R	S21-V4-R
	L (Vehicle)	S21-V1-L	S21-V2-L	S21-V3-L	S21-V4-L
W-22 (24)	R (C. sinica)	S22-V1-R	S22-V2-R	S22-V3-R	S22-V4-R
	L (Vehicle)	S22-V1-L	S22-V2-L	S22-V3-L	S22-V4-L
W-23 (50)	R (C. sinica)	S23-V1-R	S23-V2-R	S23-V3-R	S23-V4-R
	L (Vehicle)	S23-V1-L	S23-V2-L	S23-V3-L	S23-V4-L
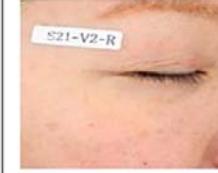
Figure S2. Facial digital photographs of individual patients. Clinical investigation was designed as a randomized, double-blind, vehicle-controlled, split-face trial. Twenty-three patients (Korean women, W-1 to W-23), who had melasma and freckles on both sides of the face, were topically applied with *C. sinica* cream on one half of the face and vehicle cream on the other half in a twice-daily regimen for consecutive 8 wk. Facial photographs with high resolution were taken at baseline (0 time), 4 wk, 6 wk or 8 wk using Visia-CR.
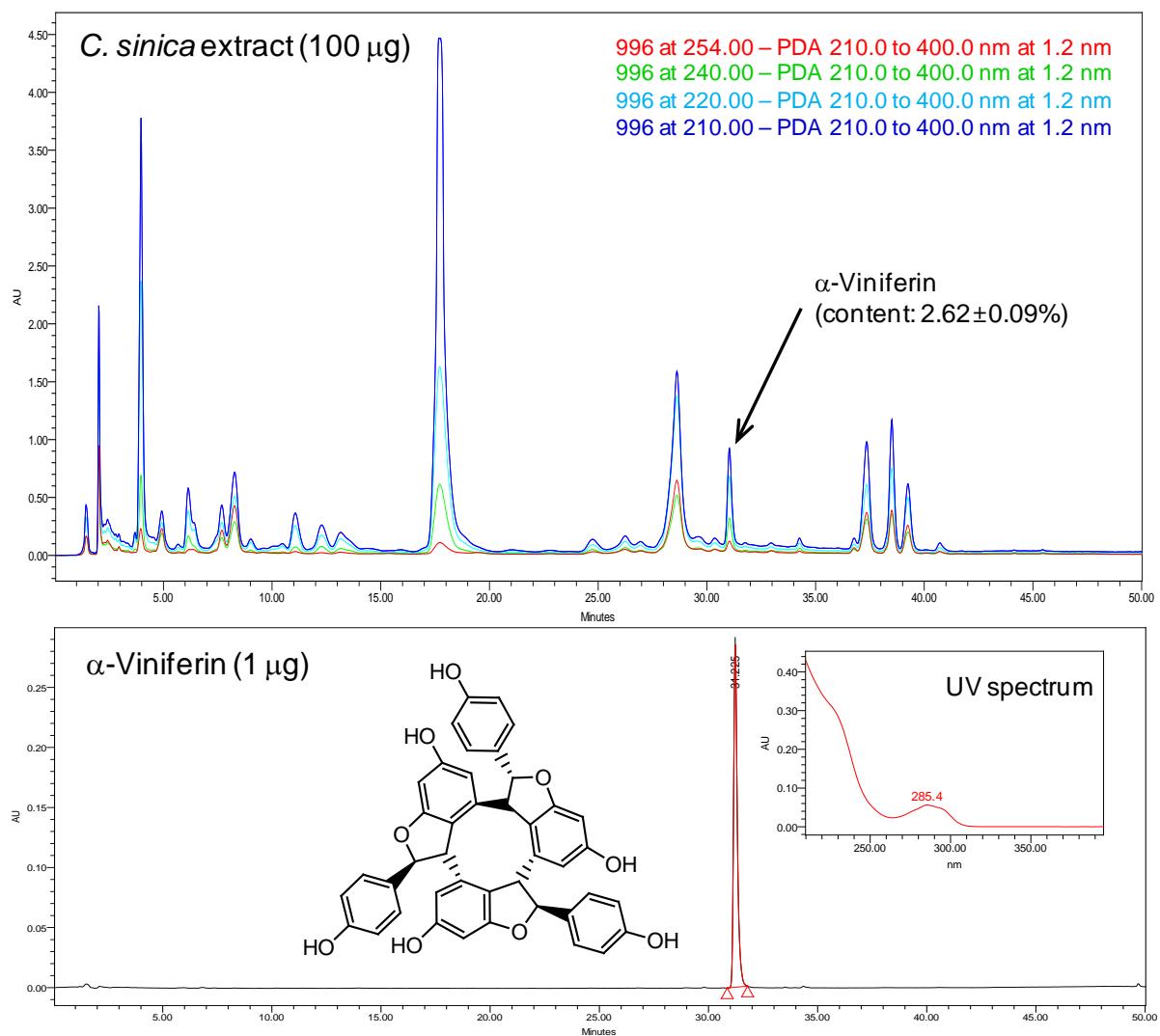
Figure S3. HPLC-DAD chromatogram of *C. sinica* extract. The roots of *C. sinica* were extracted with 95% ethyl alcohol and evaporated under reduced pressure. To determine the contents of α-viniferin, *C. sinica* extracts were subjected to HPLC analysis using Waters system (two 515 pumps with a 996 photodiode array detector), YMC J'sphere ODS-H80 column (4 mm, 150 x 20 mm i.d.), and a mixed solvent system of CH_3CN-H_2O (25:75, 0 time-20 min; 25:75 to 50:50, 20-50 min) at a flow rate of 1 mL per min. A chromatogram of α-viniferin is also presented as a standard.
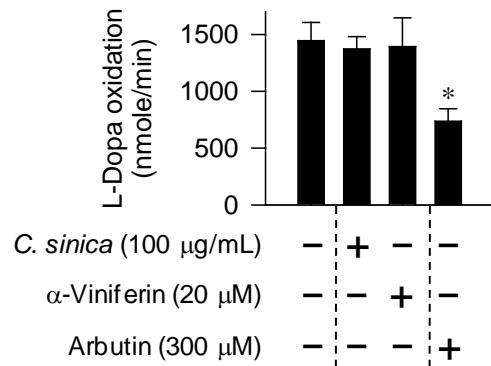
Figure S4. Effect of *C. sinica* or α-viniferin on catalytic activity of cell-free Tyro. B16-F0 cells were stimulated with α-MSH alone for 48 h. Cell lysates were prepared with phosphate buffer, treated with *C. sinica* or α-viniferin for 5 min, and measured the velocity increasing absorbance values at 475 nm upon adding 1 mM L-dopa. Tyro activity is represented as the initial velocity of L-dopa oxidation (nmole/min). Data are mean ± SEM (n = 5). *$p < 0.05$ *vs.* cell lysate alone-containing group.
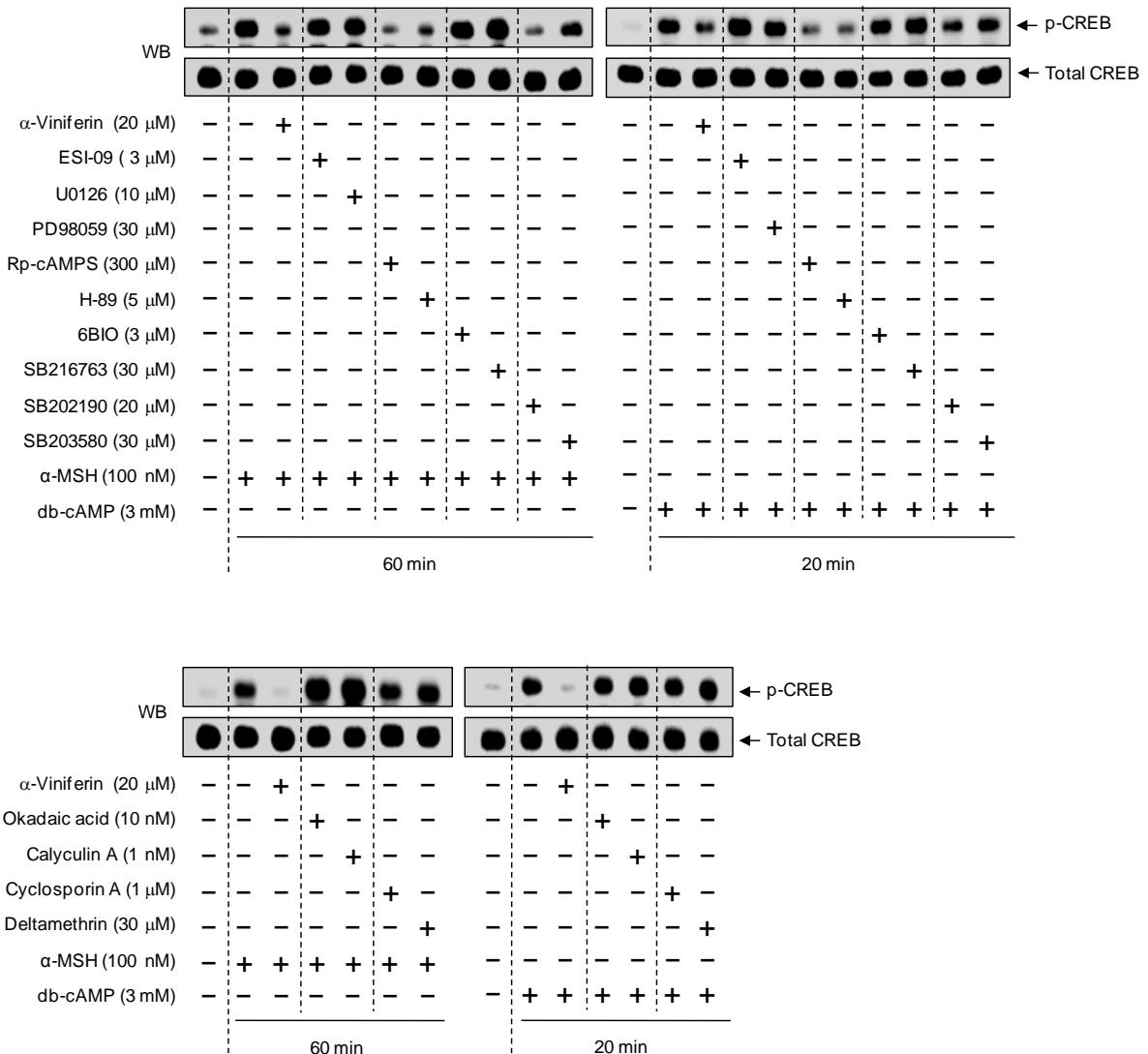
Figure S5. Effects of α-viniferin, EPAC inhibitor, protein kinase inhibitors or PP inhibitors on CREB phosphorylation. B16-F0 cells were pretreated with α-viniferin, EPAC inhibitor (ESI-09), MEK1/2 inhibitors (U016, PD98059), PKA inhibitors (Rp-cAMPS, H-89), GSK-3α/β inhibitors (6BIO, SB216763), p38 MAPK inhibitors (SB202190, SB203580), dual inhibitors of PP1 and PP2A (okadaic acid, calyculin A) or PP2B inhibitors (cyclosporin A, deltamethrin) for 2 h and stimulated with α-MSH for 1 h or db-cAMP for 20 min in the presence of α-viniferin or each of the inhibitors. Cell extracts were subjected to Western blot (WB) analysis with anti-p-CREB or anti-CREB antibody.

Treatment	60 min											20 min										
α-Viniferin (20 μM)	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
Okadaic acid (10 nM)	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
Calyculin A (1 nM)	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
Cyclosporin A (1 μM)	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
Deltamethrin (30 μM)	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Rp-cAMPS (300 μM)	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−
H-89 (5 μM)	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−
SB202190 (20 μM)	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−
SB203580 (30 μM)	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+
α-MSH (100 nM)	−	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
db-cAMP (3 mM)	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
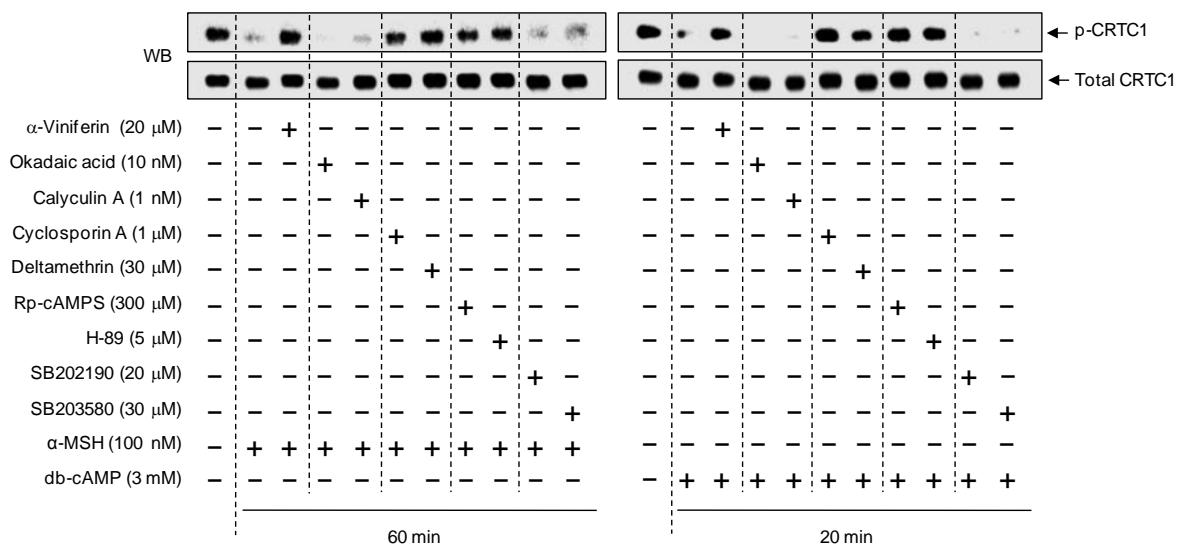
Figure S6. Effects of α-viniferin, PP inhibitors or protein kinase inhibitors on CRTC1 dephosphorylation. B16-F0 cells were pretreated with α-viniferin, dual inhibitors of PP1 and PP2A (okadaic acid, calyculin A), PP2B inhibitors (cyclosporin A, deltamethrin), PKA inhibitors (Rp-cAMPS, H-89) or p38 MAPK inhibitors (SB202190, SB203580) for 2 h and stimulated with α-MSH for 1 h or db-cAMP for 20 min in the presence of α-viniferin or each of the inhibitors. Cell extracts were subjected to Western blot (WB) analysis with anti-p-CRTC1 or anti-CRTC1 antibody.

Melanin pigments

Condition																			
α-Viniferin (20 μM)	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
ESI-09 (3 μM)	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
U0126 (10 μM)	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
PD98059 (30 μM)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
Rp-cAMPS (300 μM)	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−
H-89 (5 μM)	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−
6BIO (3 μM)	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−
SB216763 (30 μM)	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−
SB202190 (20 μM)	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
SB203580 (30 μM)	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
α-MSH (100 nM)	−	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
db-cAMP (3 mM)	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+

Melanin pigments

Condition														
α-Viniferin (20 μM)	−	−	+	−	−	−	−	−	−	+	−	−	−	−
Okadaic acid (10 nM)	−	−	−	+	−	−	−	−	−	−	+	−	−	−
Calyculin A (1 nM)	−	−	−	−	+	−	−	−	−	−	−	+	−	−
Cyclosporin A (1 μM)	−	−	−	−	−	+	−	−	−	−	−	−	+	−
Deltamethrin (30 μM)	−	−	−	−	−	−	+	−	−	−	−	−	−	+
α-MSH (100 nM)	−	+	+	+	+	+	+	−	−	−	−	−	−	−
db-cAMP (3 mM)	−	−	−	−	−	−	−	−	+	+	+	+	+	+
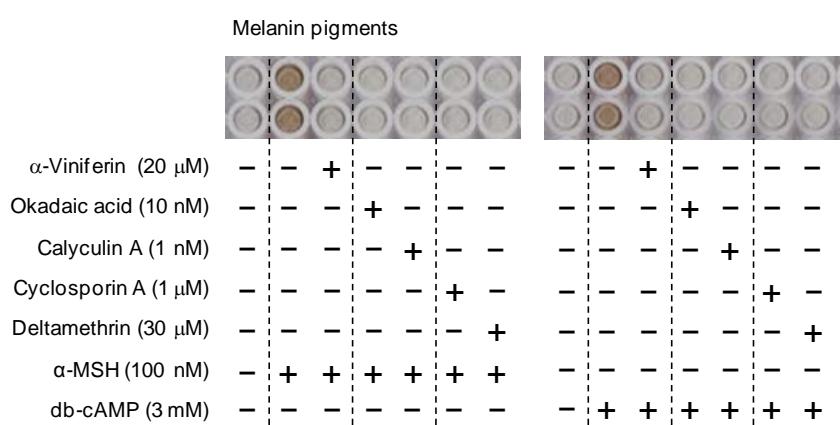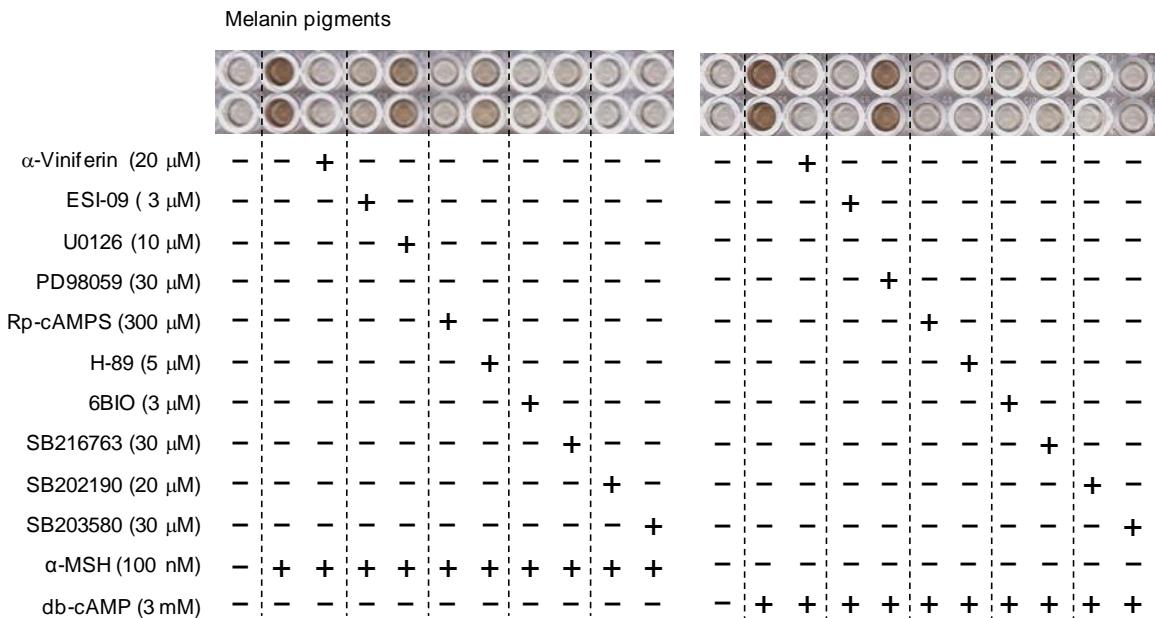
Figure S7. Effects of α-viniferin, EPAC inhibitor, protein kinase inhibitors or PP inhibitors on melanin production. B16-F0 cells were pretreated with α-viniferin, EPAC inhibitor (ESI-09), MEK1/2 inhibitors (U016, PD98059), PKA inhibitors (Rp-cAMPS, H-89), GSK-3α/β inhibitors (6BIO, SB216763), p38 MAPK inhibitors (SB202190, SB203580), dual inhibitors of PP1 and PP2A (okadaic acid, calyculin A) or PP2B inhibitors (cyclosporin A, deltamethrin) for 2 h and stimulated with α-MSH or db-cAMP for 72 h in the presence of α-viniferin or each of the inhibitors. Melanin pigmentation is presented as an experimental photograph.

Table S1. Melanin index or lightening index of individual patients.

Clinical investigation was designed as a randomized, double-blind, vehicle-controlled, split-face trial. Twenty-three patients (Korean women, W-1 to W-23) aged 24-55 years, who had melasma and freckles on both sides of the face, were topically applied with *C. sinica* cream on one half of the face (n = 23) and vehicle cream on the other half (n = 23) in a twice-daily regimen for consecutive 8 wk. Melanin index on the face was measured at baseline (0 time), 4 wk, 6 wk or 8 wk using mexameter (A), and lightening index using chromameter (B). Data were analyzed with ANOVA followed by the Student's *t*-test.

Table S1A. Melanin index (M.I.) of individual patients.

[*C. sinica* cream-treated group]

Patient (age)	Treatment	0 time M.I.	4 weeks M.I.	△ (%)	6 weeks M.I.	△ (%)	8 weeks M.I.	△ (%)
W-1 (45)	Left (L)	182.667	135.000	-26.095	144.000	-21.168	149.333	-18.249
W-2 (48)	L	179.667	172.667	-3.896	167.000	-7.050	159.667	-11.132
W-3 (39)	Right (R)	87.333	111.333	27.481	84.333	-3.435	107.333	22.901
W-4 (41)	L	158.000	148.333	-6.118	152.333	-3.587	153.000	-3.165
W-5 (35)	L	100.000	104.000	4.000	96.333	-3.667	97.667	-2.333
W-6 (53)	R	100.000	90.333	-9.667	89.667	-10.333	85.333	-14.667
W-7 (42)	L	89.333	77.667	-13.059	84.667	-5.223	85.000	-4.850
W-8 (48)	R	107.667	99.667	-7.430	99.667	-7.430	100.000	-7.121
W-9 (46)	L	136.333	144.667	6.113	134.000	-1.711	140.667	3.179
W-10 (42)	L	124.667	116.667	-6.417	108.000	-13.369	118.000	-5.348
W-11 (41)	L	162.333	148.333	-8.624	149.333	-8.008	149.667	-7.802
W-12 (41)	L	91.667	86.000	-6.182	87.333	-4.728	86.667	-5.455
W-13 (47)	R	123.667	130.000	5.121	121.667	-1.617	122.333	-1.079
W-14 (36)	L	87.000	87.333	0.383	86.333	-0.767	84.667	-2.682
W-15 (54)	R	88.333	73.000	-17.358	76.000	-13.962	84.000	-4.905
W-16 (55)	R	104.000	94.333	-9.295	93.667	-9.936	97.000	-6.731
W-17 (45)	R	90.333	101.667	12.547	87.667	-2.951	87.333	-3.321
W-18 (43)	L	93.333	88.000	-5.714	85.667	-8.214	85.000	-8.928
W-19 (38)	R	111.667	108.333	-2.986	100.333	-10.150	104.667	-6.269
W-20 (49)	L	73.333	81.000	10.455	71.667	-2.272	72.667	-0.908
W-21 (50)	R	103.333	97.667	-5.483	96.667	-6.451	101.000	-2.258
W-22 (24)	R	119.333	113.667	-4.748	111.000	-6.983	117.000	-1.955
W-23 (50)	R	100.000	95.333	-4.667	92.000	-8.000	90.667	-9.333
Average		113.652	108.913	-3.115	105.188	-7.001	107.768	-4.453
SEM		6.400	5.475	2.262	5.550	0.996	5.457	1.581

Δ (%), change (%) of M.I. compared with 0 time (baseline)

[Vehicle cream alone-applied group]

Patient (age)	Treatment	0 time M.I.	4 weeks M.I.	△ (%)	6 weeks M.I.	△ (%)	8 weeks M.I.	△ (%)
W-1 (45)	Right (R)	212.333	156.667	-26.216	204.333	-3.768	207.333	-2.355
W-2 (48)	R	169.667	170.000	0.196	174.333	2.750	164.000	-3.340
W-3 (39)	Left (L)	106.333	113.667	6.897	107.333	0.940	101.000	-5.015
W-4 (41)	R	142.333	163.333	14.754	150.667	5.855	153.667	7.963
W-5 (35)	R	109.333	127.000	16.159	109.667	0.305	108.000	-1.219
W-6 (53)	L	96.333	95.667	-0.691	92.333	-4.152	99.000	2.769
W-7 (42)	R	89.000	86.333	-2.997	90.000	1.124	81.667	-8.239
W-8 (48)	L	91.000	90.667	-0.366	88.333	-2.931	88.000	-3.297
W-9 (46)	R	126.000	133.000	5.556	131.667	4.498	129.333	2.645
W-10 (42)	R	143.000	145.333	1.631	143.667	0.466	143.000	0.000
W-11 (41)	R	156.667	159.000	1.489	156.333	-0.213	156.000	-0.426
W-12 (41)	R	95.333	93.667	-1.748	96.667	1.399	95.000	-0.349
W-13 (47)	L	137.000	151.000	10.219	143.333	4.623	143.667	4.866
W-14 (36)	R	105.667	104.333	-1.262	108.000	2.208	107.333	1.577
W-15 (54)	L	78.333	84.333	7.660	80.667	2.980	76.333	-2.553
W-16 (55)	L	102.000	97.333	-4.575	100.000	-1.961	101.000	-0.980
W-17 (45)	L	87.000	97.333	11.877	85.000	-2.299	90.000	3.448
W-18 (43)	R	98.000	101.667	3.742	94.000	-4.082	101.333	3.401
W-19 (38)	L	106.667	104.000	-2.500	100.333	-5.938	103.333	-3.126
W-20 (49)	R	86.667	92.000	6.153	89.333	3.076	84.000	-3.077
W-21 (50)	L	114.000	111.333	-2.339	114.000	0.000	106.333	-6.725
W-22 (24)	L	149.000	142.667	-4.250	133.667	-10.291	144.333	-3.132
W-23 (50)	L	107.333	104.667	-2.484	102.667	-4.347	108.333	0.932
Average		117.783	118.478	1.605	117.232	-0.424	117.043	-0.706
SEM		6.744	5.809	1.788	6.700	0.811	6.771	0.797

Δ (%), change (%) of M.I. compared with 0 time (baseline)

p value (M.I.) within *C. sinica* group
- between 4 week and baseline : 0.5765137878
- between 6 week and baseline : 0.3232219265
- between 8 week and baseline : 0.4878610305

p value (M.I.) within vehicle group
- between 4 week and baseline : 0.9380546737
- between 6 week and baseline : 0.9540654728
- between 8 week and baseline : 0.9386966930

p value (△ M.I.) between *C. sinica* and vehicle
- 4 week between G41 group and vehicle group : 0.1087908622
- 6 week between test group and vehicle group : 0.0000064668
- 8 week between test group and vehicle group : 0.0399757516

Table S1B. Lightening index (L.I.) of individual patients.

[*C. sinica* cream-treated group]

Patient (age)	Treatment	0 time L.I.	4 weeks L.I.	4 weeks △ (%)	6 weeks L.I.	6 weeks △ (%)	8 weeks L.I.	8 weeks △ (%)
W-1 (45)	Left (L)	68.390	68.500	0.161	68.800	0.600	69.587	1.750
W-2 (48)	L	57.320	59.850	4.414	58.767	2.524	59.527	3.850
W-3 (39)	Right (R)	60.703	63.573	4.728	63.000	3.784	64.867	6.860
W-4 (41)	L	60.463	60.917	0.751	61.117	1.082	62.247	2.951
W-5 (35)	L	66.110	66.543	0.655	67.650	2.329	68.157	3.096
W-6 (53)	R	63.500	63.950	0.709	63.683	0.288	64.923	2.241
W-7 (42)	L	63.557	65.170	2.538	65.910	3.702	65.360	2.837
W-8 (48)	R	62.773	63.910	1.811	63.443	1.067	64.767	3.177
W-9 (46)	L	57.930	58.687	1.307	60.207	3.931	61.010	5.317
W-10 (42)	L	63.377	65.993	4.128	63.067	-0.489	65.303	3.039
W-11 (41)	L	62.670	61.257	-2.255	62.500	-0.271	63.317	1.032
W-12 (41)	L	63.800	63.717	-0.130	64.013	0.334	64.953	1.807
W-13 (47)	R	59.703	61.210	2.524	61.773	3.467	62.300	4.350
W-14 (36)	L	62.010	61.557	-0.731	63.223	1.956	62.757	1.205
W-15 (54)	R	59.637	59.620	-0.029	58.733	-1.516	62.767	5.248
W-16 (55)	R	58.043	61.077	5.227	59.427	2.384	60.267	3.832
W-17 (45)	R	62.187	63.423	1.988	64.347	3.473	64.190	3.221
W-18 (43)	L	66.157	65.827	-0.499	67.283	1.702	66.413	0.387
W-19 (38)	R	66.447	66.620	0.260	66.857	0.617	66.320	-0.191
W-20 (49)	L	66.310	66.427	0.176	64.803	-2.273	66.773	0.698
W-21 (50)	R	61.100	61.057	-0.070	61.200	0.164	62.747	2.696
W-22 (24)	R	66.817	65.423	-2.086	65.763	-1.577	65.483	-1.996
W-23 (50)	R	60.037	59.713	-0.540	59.607	-0.716	60.247	0.350
Average		62.567	63.218	1.089	63.268	1.155	64.099	2.511
SEM		0.650	0.576	0.428	0.607	0.385	0.534	0.418

Δ (%), change (%) of L.I. compared with 0 time (baseline)

Vehicle cream alone-applied group

Patient (age)	Treatment	0 time L.I.	4 weeks L.I.	4 weeks △ (%)	6 weeks L.I.	6 weeks △ (%)	8 weeks L.I.	8 weeks △ (%)
W-1 (45)	Right (R)	66.827	68.023	1.790	67.087	0.389	66.770	-0.085
W-2 (48)	R	57.320	59.527	3.850	58.433	1.942	59.850	4.414
W-3 (39)	Left (L)	63.603	65.263	2.610	62.243	-2.138	62.657	-1.487
W-4 (41)	R	60.263	61.820	2.584	60.310	0.078	59.850	-0.685
W-5 (35)	R	65.227	66.583	2.079	64.903	-0.497	63.867	-2.085
W-6 (53)	L	64.393	64.527	0.208	61.937	-3.814	62.750	-2.552
W-7 (42)	R	63.547	63.620	0.115	64.253	1.111	64.360	1.279
W-8 (48)	L	64.730	65.700	1.499	62.517	-3.419	64.020	-1.097
W-9 (46)	R	58.880	60.977	3.561	60.557	2.848	60.327	2.458
W-10 (42)	R	60.117	60.200	0.138	58.907	-2.013	59.543	-0.955
W-11 (41)	R	62.387	62.407	0.032	61.247	-1.827	60.610	-2.848
W-12 (41)	R	64.043	64.730	1.073	63.810	-0.364	64.787	1.162
W-13 (47)	L	60.740	63.087	3.864	61.027	0.473	62.057	2.168
W-14 (36)	R	60.603	62.393	2.954	61.357	1.244	61.753	1.898
W-15 (54)	L	62.203	61.997	-0.331	58.553	-5.868	61.810	-0.632
W-16 (55)	L	61.983	61.327	-1.058	61.370	-0.989	60.317	-2.688
W-17 (45)	L	63.493	63.833	0.535	63.850	0.562	62.750	-1.170
W-18 (43)	R	64.627	64.453	-0.269	63.797	-1.284	62.430	-3.400
W-19 (38)	L	68.367	67.037	-1.945	65.150	-4.705	67.517	-1.243
W-20 (49)	L	66.657	64.973	-2.526	63.757	-4.351	64.013	-3.967
W-21 (50)	L	63.100	63.340	0.380	59.790	-5.246	61.117	-3.143
W-22 (24)	L	67.467	65.123	-3.474	66.557	-1.349	64.493	-4.408
W-23 (50)	L	61.057	60.563	-0.809	60.080	-1.600	60.007	-1.720
Average		63.115	63.544	0.733	62.239	-1.340	62.507	-0.904
SEM		0.584	0.472	0.417	0.511	0.492	0.462	0.470

Δ (%), change of L.I. compared with 0 time (baseline)

p value (L.I.) with *C. sinica* group
- between 4 week and baseline : 0.4571813244
- between 6 week and baseline : 0.4345515079
- between 8 week and baseline : 0.0752508280

p value (L.I.) in vehicle group
- between 4 week and baseline : 0.5708128698
- between 6 week and baseline : 0.2653211644
- between 8 week and baseline : 0.4188231143

p value (△ L.I.) between *C. sinica* and vehicle
- 4 week between test group and vehicle group : 0.5548901174
- 6 week between test group and vehicle group : 0.0002464817
- 8 week between test group and vehicle group : 0.0000023041

Table S2. Composition of vehicle or *C. sinica* cream.

Ingredients	Contents (w/w %)	
	Vehicle cream	*C. sinica* cream
Carbomer	0.10	0.10
Cetearyl alcohol	2.00	2.00
Cetearyl olivate	0.50	0.50
C. sinica extract	-	3.20
Dimethicone	3.00	3.00
Dipropylene glycol	6.00	6.00
EDTA disodium	0.02	0.02
Fragrance	0.10	0.10
Glycerin	4.00	4.00
Glyceryl stearate	2.00	2.00
Hydrogenated C-(6-14) olefin polymer	1.50	1.50
Olive oil	3.00	3.00
PEG-100 stearate	1.50	1.50
P. oleracea extract	2.00	2.00
Polysorbate 60	0.30	0.30
Stearic acid	1.00	1.00
Tocopheryl acetate	0.20	0.20
Tromethamine	0.08	0.08
Xanthan gum	0.15	0.15
H_2O (purified)	72.55	69.35
Total	100.00	100.00

Table S3. Nucleotide sequences of RT-PCR primers.

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Target	Nucleotide sequence		Amplicon (base pairs)
DCT	Forward	5'-ACAGACGACTGGCTTGGAGCAGCAA-3'	517
	Reverse	5'-ACATTCGGTTGTGACCAATGGGTGC-3'	
MITF-M	Forward	5'-TACAGTCACTACCAGGTGCAG-3'	397
	Reverse	5'-CCATCAAGCCCAAAATTTCTT-3'	
Tyro	Forward	5'-CATTTTTGATTTGAGTGTCT-3'	1,211
	Reverse	5'-TGTGGTAGTCGTCTTTGTCC-3'	
TYRP1	Forward	5'-GATATGGCGAAGCGCACAACTCACC-3'	536
	Reverse	5'-AGACGCTGCACTGCTGGTCTCCCTA-3'	
β-Actin	Forward	5'-TGGAATCCTGTGGCATCCATGAAAC-3'	349
	Reverse	5'-TAAAACGCAGCTCAGTAACAGTCCG-3'	

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