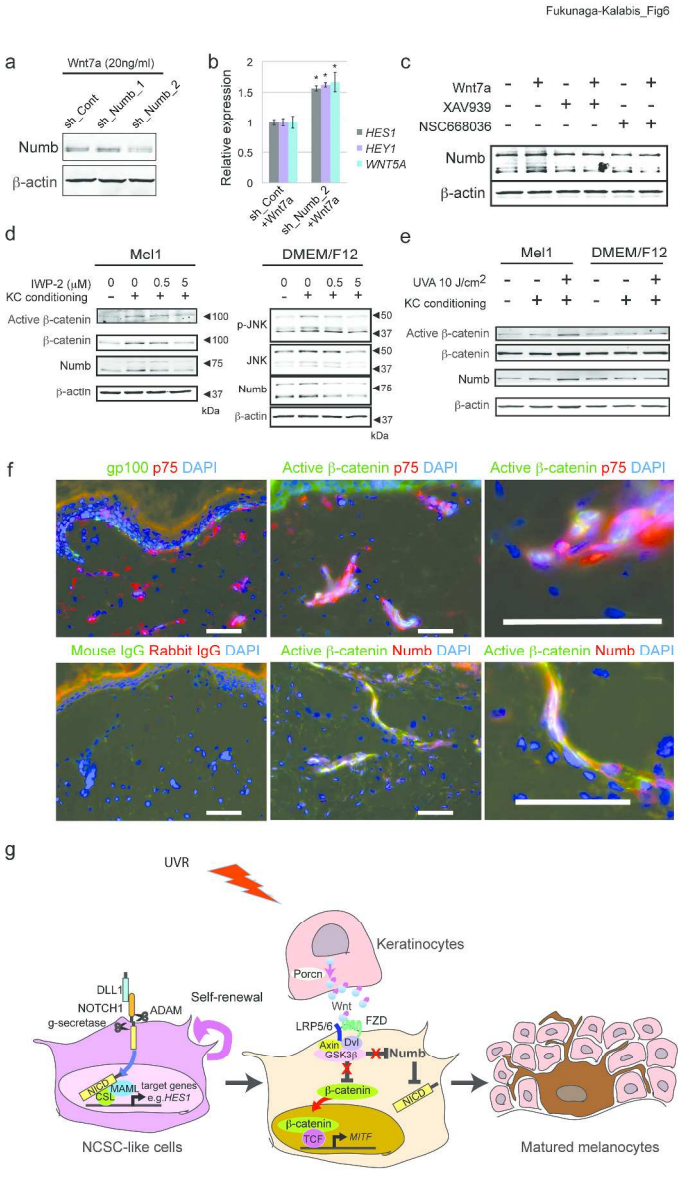
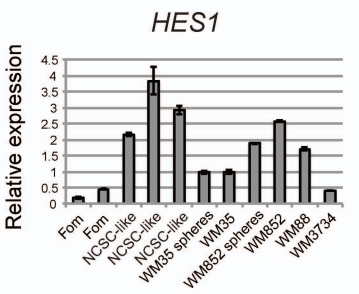
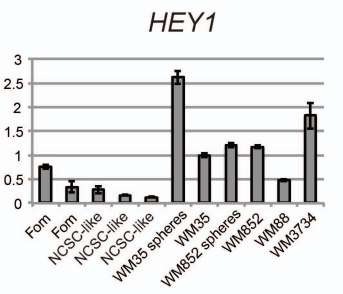
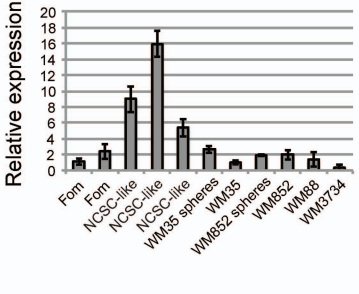
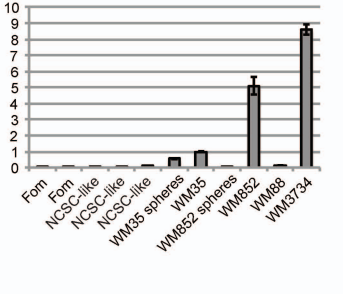


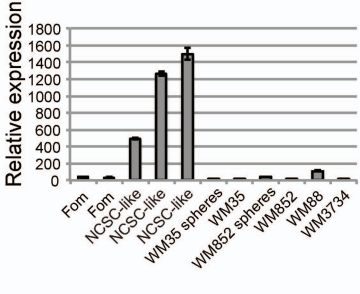
169x279mm (600 x 600 DPI)

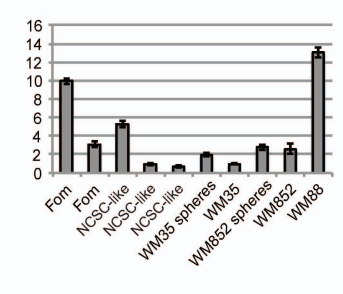

HES1
Relative expression
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Fom
Fom
NCSC-like
NCSC-like
NCSC-like
WM35 spheres
WM35
WM852 spheres
WM852
WM88
WM3734


HEY1
3
2.5
2
1.5
1
0.5
0
Fom
Fom
NCSC-like
NCSC-like
NCSC-like
WM35 spheres
WM35
WM852 spheres
WM852
WM88
WM3734

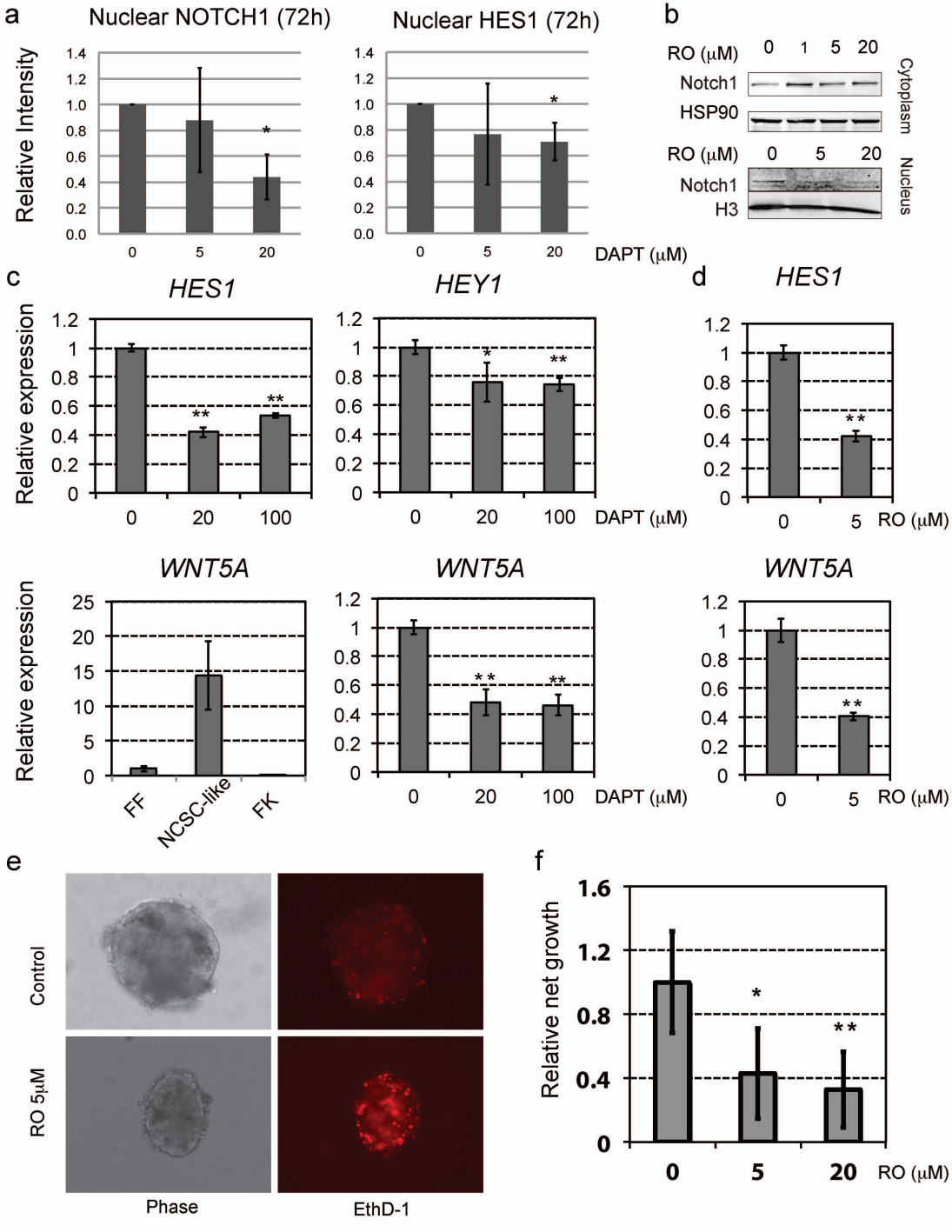

HES2
Relative expression
20
18
16
14
12
10
8
6
4
2
0
Fom
Fom
NCSC-like
NCSC-like
NCSC-like
WM35 spheres
WM35
WM852 spheres
WM852
WM88
WM3734


HEY2
10
9
8
7
6
5
4
3
2
1
0
Fom
Fom
NCSC-like
NCSC-like
NCSC-like
WM35 spheres
WM35
WM852 spheres
WM852
WM88
WM3734

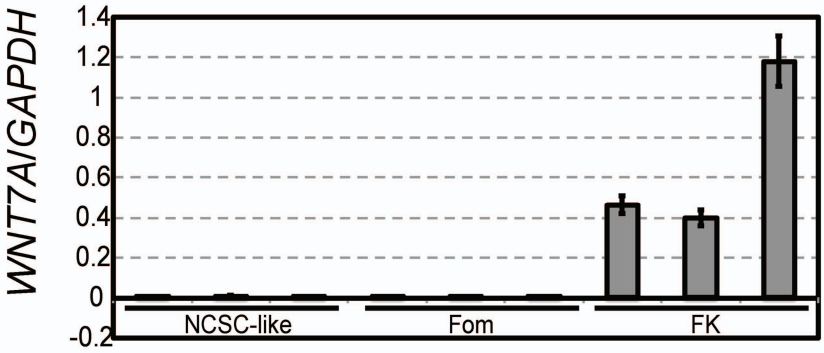

DLL1
Relative expression
1800
1600
1400
1200
1000
800
600
400
200
0
Fom
Fom
NCSC-like
NCSC-like
NCSC-like
WM35 spheres
WM35
WM852 spheres
WM852
WM88
WM3734


JAG1
16
14
12
10
8
6
4
2
0
Fom
Fom
NCSC-like
NCSC-like
NCSC-like
WM35 spheres
WM35
WM852 spheres
WM852
WM88

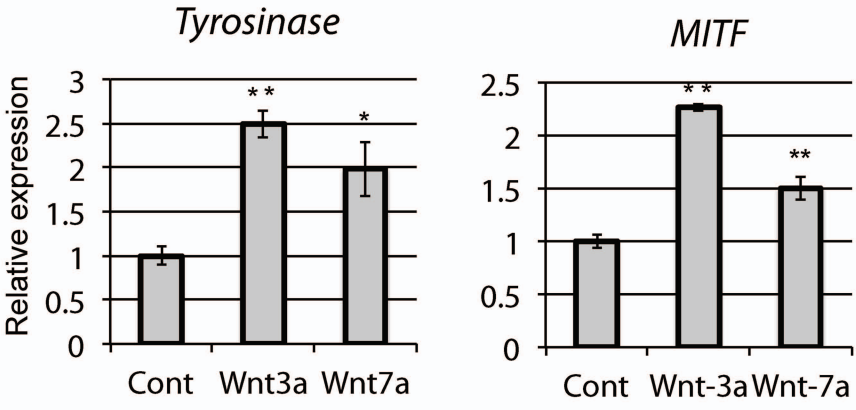
Fukunaga-Kalabis_Fig S2



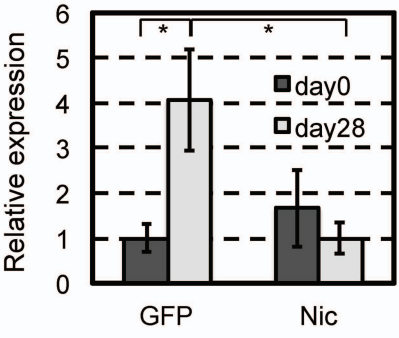
a


WNT7A/GAPDH
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
NCSC-like
Fom
FK

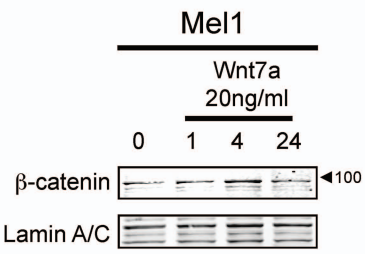
b


Tyrosinase
Relative expression
3
2.5
2
1.5
1
0.5
0
**
*
Cont
Wnt3a
Wnt7a
MITF
2.5
2
1.5
1
0.5
0
**
**
Cont
Wnt-3a
Wnt-7a

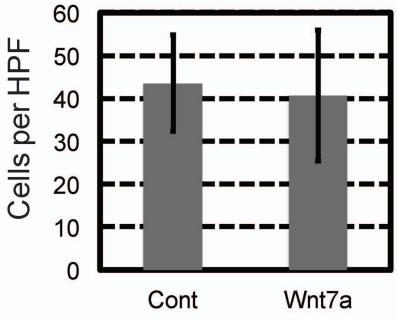
c

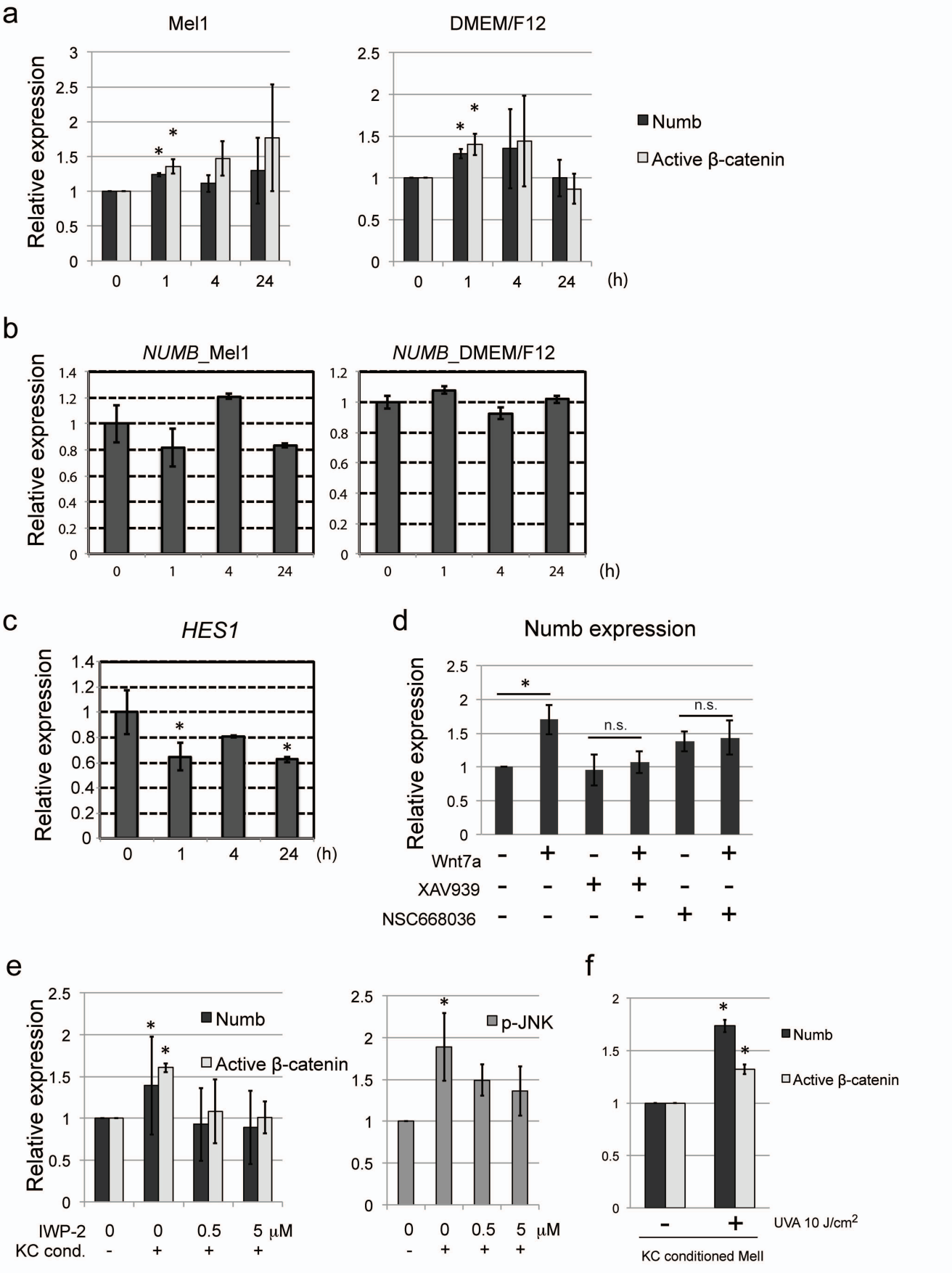

Tyrosinase
Relative expression
6
5
4
3
2
1
0
*
*
day0
day28
GFP
Nic

d

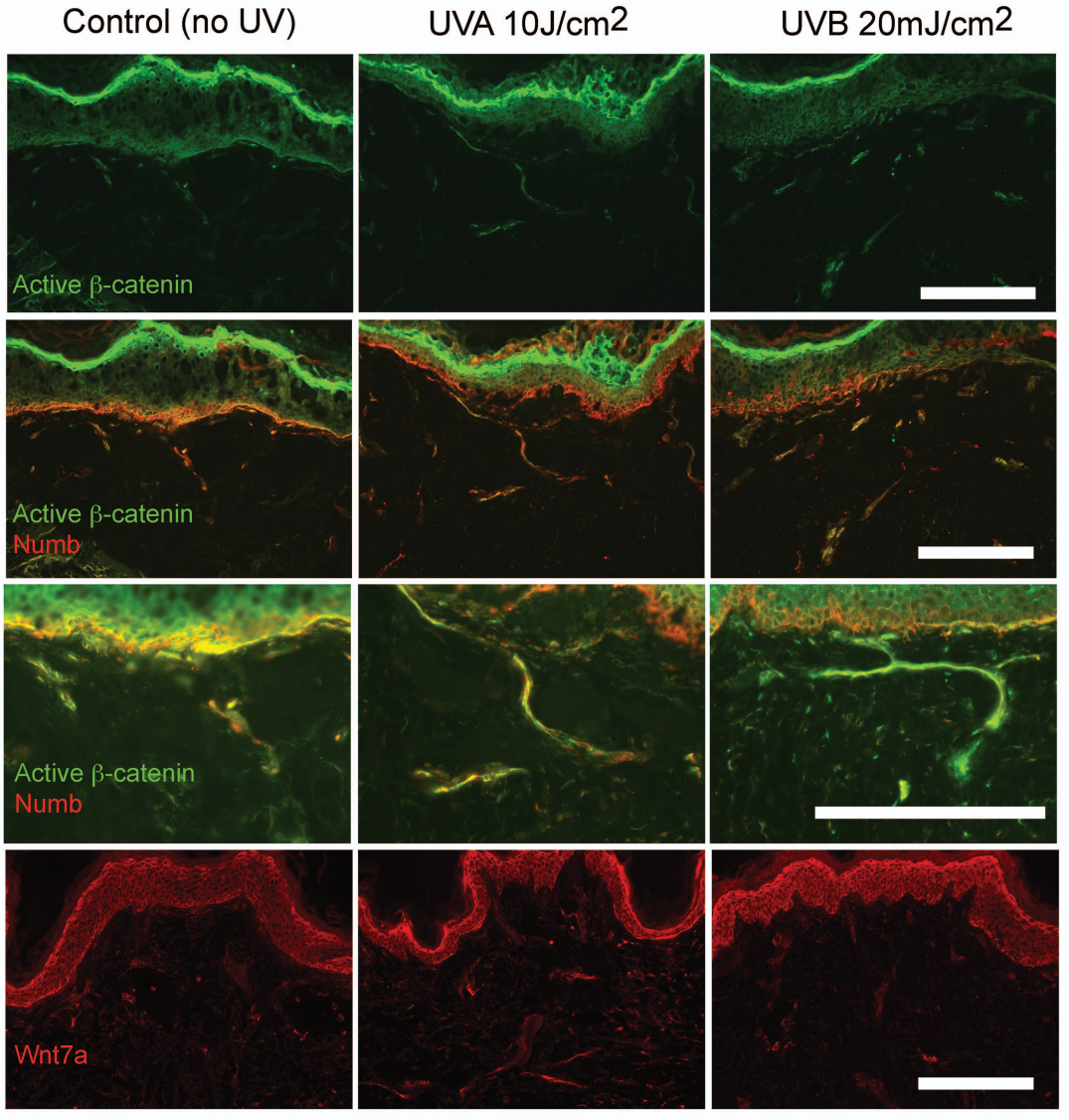

Mel1
Wnt7a
20ng/ml
0
1
4
24
β-catenin
100
Lamin A/C

e

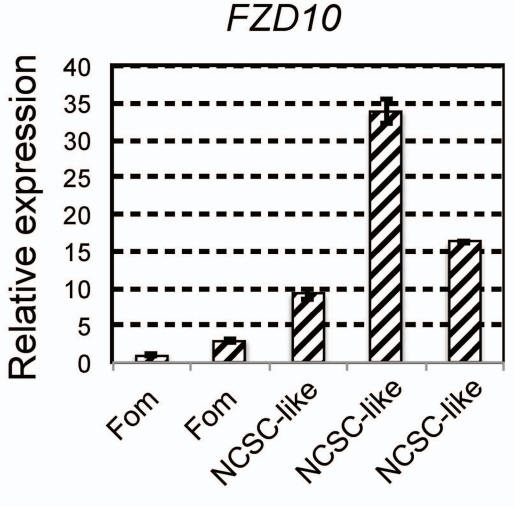

Cells per HPF
60
50
40
30
20
10
0
Cont
Wnt7a


a
Mel1
DMEM/F12
Relative expression
Numb
Active β-catenin
(h)
b
NUMB_Mel1
NUMB_DMEM/F12
Relative expression
(h)
c
HES1
Relative expression
(h)
d
Numb expression
Relative expression
n.s.
Wnt7a
XAV939
NSC668036
e
Relative expression
Numb
Active β-catenin
p-JNK
IWP-2
KC cond.
μM
f
Numb
Active β-catenin
UVA 10 J/cm2
KC conditioned Mel1

a


Control (no UV)
UVA 10J/cm2
UVB 20mJ/cm2
Active β-catenin
Active β-catenin
Numb
Active β-catenin
Numb
Wnt7a

b


FZD10
Relative expression
40
35
30
25
20
15
10
5
0
Fom
Fom
NCSC-like
NCSC-like
NCSC-like

Supplementary Table 1

Western blot	Antibodies	Vendor	Catalog Number	dilution
	Notch1 (D6F11) XP®	Cell signaling	4380	1000
	Notch2	abcam	ab8927	1000
	Notch3	abcam	ab23426	1000
	Notch4 (H-225)	Santa Cruz	sc-5594	200
	HES1	Millipore	AB5702	1000
	Active-β-Catenin, 8E7	Millipore	05-665	1000
	β-Catenin	BD Biosciences	610153	1000
	Phospho-SAPK/JNK (G9)	Cell signaling	9255	1000
	SAPK/JNK (56G8)	Cell signaling	9258	1000
	Numb	abcam	ab14140	1000
	β-Actin	Sigma-Aldrich	A5441	5000
	HSP90 (C45G5)	Cell signaling	4877	1000
	Lamin A/C	Cell signaling	2032	1000
	Wnt-7a	Novus Biologicals	23030002	2500
	Histone H3	Cell signaling	4499	1000
Immunostaining	Notch1	abcam	ab8925	
	HES1	Millipore	AB5702	
	gp100 (HMB45)	Dako	M0634	
	TYRP1 (Mel-5)	Covance	SIG-38150	
	p75 (EP1039Y)	abcam	ab52987	

qRTPCR primers	Target gene	Forward sequence	Reverse sequence	references
	HES1	GTG CAT GAA CG	GTA TTA ACG CCC TCG CAC GT	
	HES2	AGA ACT CCA AC	CGG TCA TTT CCA GGA CGT CT	
	HEY1	GCC GAG ATC CT	TGC CGT ATG CAG CAT TTT CA	
	HEY2	AGA TGC TTC AG	CAA GAG CGT GTG CGT CAA AG	
	DLL1	CAA CGT GGA CG	TTG GGA CCC CCG TAG CCT CG	
	JAG1	GTG GCT TGG AT	TTG GTG GTG TT	Borghese et al Stem Cells 2010 PMID: 20235098
	WNT7A	CAT AGG AGA AG	CGG CAA TGA TG	Nakatsu et al. Invest Ophthalmol Vis Sci. 2011 PMID: 21357396
	WNT3A	CAA GAT TGG CA	ATG AGC GTG TCA CTG CAA AG	
	WNT3	CGA GTC GGC CT	CGA GTC ACA GC	Nakatsu et al. Invest Ophthalmol Vis Sci. 2011 PMID: 21357396
	WNT11	CTG ACC TCA AG	CGA GTT CCG AG	Nakatsu et al. Invest Ophthalmol Vis Sci. 2011 PMID: 21357396
	Tyrosinase	CTA GAA GGA TT	CCT GTA CCT GGG ACA TTG TTC	
	TYRP1	GCT TTT CTC AC	GGC TCT TGC AAC ATT TCC G	
	NUMB	CCG GCA TGC TC	TCT GGC TAA GA	Jiao et al. Scand J Rheumatol. 2010 PMID: 20132067
	MITF	TCAGGTGCAGAC	GGCACCGGTGGCATGACATGAT	

Supplementary Figure 1: qRT-PCR showing that among Notch target genes, *HES1* and *HES2* are highly expressed by NCSC-like cells. Notch ligand delta-like 1 gene (*DLL1*) is highly expressed by NCSC-like stem cells, while another Notch ligand *JAG1* is expressed predominantly in melanocytes and in melanomas. mRNA levels of target genes were normalized to *GAPDH*.

Supplementary Figure 2: (a) Quantification of the Western blot analysis shown in Figure 1e. Intensities of protein bands derived from single membranes were quantified using Odyssey Image Studio software. Quantification was measured by taking the median signal, while the background was taken from the left/right side of each band. The amount of each protein was normalized to Lamin A/C. Data represent means ± SD, n=3, $*p \leq 0.05$. (b) Immunoblot analysis showing that treatment with the γ-secretase inhibitor RO4929097 blocks the nuclear translocation of Notch1 in NCSC-like cells. Blotting for HSP90 and Histone H3 serves as loading controls. (c) Top: qRT-PCR showing that 20 μM DAPT treatment significantly decreases the expression of the Notch targets *HES1* and *HEY1* in NCSC-like stem cells. mRNA levels of target genes were normalized to *GAPDH*. Bottom: qRT-PCR showing that NCSC-like stem cells express the *WNT5A* gene at higher levels compared to human fibroblasts (FF) or human keratinocytes (FK). DAPT treatment significantly decreases the expression of the *WNT5A* gene in NCSC-like stem cells. mRNA levels of target genes were normalized to *GAPDH*. Data represent means ± SD, n=4, $*p \leq 0.05$, $**p \leq 0.001$. (d) Top: qRT-PCR showing that 5 μM RO4929097 treatment significantly decreases the expression of the Notch targets *HES1* and *WNT5A* in NCSC-like stem cells. mRNA levels of target genes were normalized to *GAPDH*. Data represent means ± SD, n=4, $**p \leq 0.001$. (e) Representative images of NCSC-like cells from control DMSO-treated and from 5 μM RO4929097 treated cells on

the low-attachment surface. RO4929097 induces cell death, indicated by EthD-1 staining (red). (f) Histogram depicting the relative growth of secondary NCSC-like spheres. Data represent means ± SD, n = 4. *p = 0.059, **p ≤ 0.05.

Supplementary Figure 3: (a) qRT-PCR showing that human keratinocytes (FK) express the *WNT7A* gene, whereas NCSC-like stem cells and human melanocytes barely express *WNT7A*. mRNA levels of target genes were normalized to *GAPDH*. (b) qRT-PCR showing that Wnt7a induces the expression of melanocyte-specific genes *Tyrosinase* and *MITF* in a similar manner to Mel1 media supplemented with Wnt3a. mRNA levels of target genes were normalized to *GAPDH*. Data represent means ± SD, n=3 *$p \leq 0.05$, **$p \leq 0.0001$. (c) qRT-PCR showing that the overexpression of the constitutively active form of Notch1 in NCSC-like cells suppresses the expression of *Tyrosinase* induced by Mel1 media supplemented with Wnt7a. mRNA levels of target genes were normalized to *GAPDH*. Data represent means ± SE, n=5 *$p \leq 0.05$. GFP: control vector transduced NCSC-like cells, Nic: active Notch1 transduced NCSC-like cells. (d) β-catenin is detected in the nuclear fractions of Wnt7a-treated NCSC-like stem cells, suggesting that Wnt7a activates the canonical Wnt pathway in NCSC-like stem cells. Blotting for Lamin A/C serves as a loading control. (e) Quantification of the number of NCSC-like cells that migrated after 6 h with and without Wnt7a. Error bars indicate 1 SD of the average number of cells in five high power fields per Transwell® membrane. n=3.

Supplementary Figure 4: (a) Quantification of the Western blot analysis shown in Figure 4a. Intensities of protein bands derived from single membranes were quantified using Odyssey Image Studio software. The amount of each protein was normalized to β-

actin. Data represent means ± SD, n=3, *$p \leq 0.05$. (b) qRT-PCR showing that 20 ng/ml Wnt7a treatment does not increase the expression of *NUMB* within 24 h. mRNA levels of target genes were normalized to *GAPDH*. Data represent means ± SD, n=4. (c) qRT-PCR showing that HES1 is down-regulated by Wnt7a treatment in NCSC-like stem cells. mRNA levels of target genes were normalized to GAPDH. Data represent means ± SD, n=4, *$p \leq 0.05$. (d) Quantification of the Western blot analysis shown in Figure 6c. Numb expression was normalized to β-actin. Data represent means ± SD, n=3, *$p \leq 0.05$. (e) Quantification of the Western blot analysis shown in Figure 6d. The amount of each protein was normalized to β-actin. Data represent means ± SD, n=3, *$p \leq 0.05$. (f) Quantification of the Western blot analysis shown in Figure 6e (NCSC-like cells treated with the Mel1 media conditioned from non-irradiated keratinocytes vs NCSC-like cells treated with the Mel1 media conditioned from UVA-irradiated keratinocytes). The amount of each protein was normalized to β-actin. Data represent means ± SD, n=3, *$p \leq 0.05$.

Supplementary Figure 5: (a) Immunofluorescent staining of human skin explants (from left, control without irradiation, 10 J/cm^2 of UVA, 20mJ/cm^2 of UVB). Skin explants were harvested 48 hours after UV irradiation. Active β-catenin positive cells (green) in the dermis co-expressed Numb (red), and their number increased in UV-irradiated samples. Scale bar = 200 μm. (b) qRT-PCR showing that FZD10 is highly expressed by NCSC-like stem cells. mRNA levels of target genes were normalized to *GAPDH*.