Figure E1.
Panel A controls for Figure 1: (A-B) Immunofluorescent staining for c-Kit (green), chymase (green), and DAPI (blue) in mouse skin showing the negativity of isotype control antibody staining; **(C-F)** qPCR analysis of the controls of non-toluidine blue-positive cells collected by laser capture microdissection.
Panel B controls for Figure 2: (G) Immunofluorescent staining for LTA (red) and DAPI (blue) in mouse skin showing the negativity of isotype control antibody staining; **(H-J)** Primary normal human keratinocytes (NHEK) immunostained with anti-LTA monoclonal antibody (red) and DAPI (blue). Keratinocytes were treated with isotype control (H), PBS (I) or LTA at 10 μg/ml for 24 h (J).
Panel C controls for Figure 3: (K) Immunofluorescent staining for TLR2 (red) and DAPI (blue) in mouse skin showing the negativity of isotype control antibody staining **(L)** qPCR analysis of *Tlr2* expression in MCs collected by laser capture microdissection based on toluidine blue staining of skin from SPF and GF mice.

Figure E2. TLR2 expression in MCs co-cultured with connective tissue components and TLR2 (LTA) and TLR4 (LPS) ligands.
FACS analysis of TLR2 expression in mouse bone marrow-derived MCs after 7 days of co-culture with different components of skin connective tissue and TLR2 (LTA) and TLR4 (LPS) ligands. Murine MCs were cultured in normal media with added PBS **(A)**, hyaluronic acid 10 μg/ml **(B)**, LPS 100 ng/ml **(C)**, LTA 10 μg/ml **(D)**, or they were cultured in plates coated with collagen type I **(E)**, fibronectin **(F)**, or gelatin **(G)**. Isotype control is shown in **(H)**.
(I) *Tlr2* expression in mouse keratinocytes: qPCR analysis of *Tlr2* expression in primary isolated mouse skin keratinocytes before and after stimulation with LTA at 10 μg/ml. (***$p<0.001$)

Figure E3. SCF expression in keratinocytes and fibroblasts.
(A) qPCR analysis of *SCF* in primary human keratinocytes after stimulation with LTA at 10μg/ml; **(B)** ELISA quantification of SCF in primary human keratinocytes after stimulation with LTA at 10μg/ml; **(C)** ELISA quantification of SCF in primary isolated mouse keratinocytes and fibroblasts after stimulation with LTA at 10μg/ml; **(D)** ELISA quantification of SCF in mouse fibroblasts isolated from the skins of K14-cre and K14-cre $Scf^{fl/fl}$ mice; **(E-F)** SCF (green) and DAPI (blue) immunostaining of the skins of K14-cre (E) and K14-cre $Scf^{fl/fl}$ mice (F). The inset shows a magnification of the squared area in the image; **(G-H)** SCF (green) and DAPI (blue) immunostaining in dermal fibroblasts isolated from K14-cre (G) and K14-cre $Scf^{fl/fl}$ mice (H). The inset shows that these mice have normal fibroblast morphology. (*$p<0.05$, **$p<0.01$, ***$p<0.001$)

Figure E4. Melanocytes in K14-cre $Scf^{fl/fl}$ mice.
(A-E) Melanocyte-marker HMB45 (red) and DAPI (blue) immunofluorescent staining reveals normal numbers of melanocytes in the skins of K14-cre adults and newborn pups (A and C), while K14-cre $Scf^{fl/fl}$ adults and newborn pups lack normal numbers of melanocytes in the skin (B and D). **(E)** Isotype control for anti-HMB45 antibody plus secondary antibody. **(F)** Intradermal injection (i.d.) of SCF (500 ng/mouse) every 2 days for 12 days induced hyperpigmentation in K14-cre mice, but failed to recover pigmentation in K14-cre $Scf^{fl/fl}$ mice.

Figure E5. SCF expression in different tissues of K14-cre $Scf^{fl/fl}$ mice.
(A-D) Immunofluorescent staining of SCF (green) or chymase (green) and DAPI (blue) reveals normal levels of SCF and MCs in the small intestine of K14-cre (A and B) and K14-cre $Scf^{fl/fl}$ mice (C and D); **(E)** *Scf* expression was assayed by qPCR from bone marrow cells extracted from the femurs of mice; **(F-G)** SCF (green) and DAPI (blue) immunofluorescent staining in the skins of K14-cre $Scf^{fl/fl}$ (F) and K14-cre (G) newborn pups; **(H-I)** Chymase (green) and DAPI (blue) immunofluorescent staining in the skins of K14-cre $Scf^{fl/fl}$ (H) and K14-cre (I) newborn pups.

Table E1.
Blood analysis: White Blood Cell (WBC) and Red Blood Cell (RBC) counts and differentials of K14-cre $Scf^{fl/fl}$, K14-cre, and wild-type C57 mice. There is no significant difference in hematology between K14-cre $Scf^{fl/fl}$ mice and control mice.

Table E1

Species		**K14-cre *Scf*$^{fl/fl}$**			**K14-cre**			**Wild type C57**		
Sample ID		1	2	3	1	2	3	1	2	3
WBC	K/µL	6.58	6.94	6.86	7.7	7.76	9.24	9.18	9.64	9.88
NE#	K/µL	0.33	1.11	1.02	0.68	0.66	0.6	0.6	1.06	1.07
LY#	K/µL	5.77	5.13	5.24	6.32	6.48	8.15	8.15	7.9	8.14
MO#	K/µL	0.44	0.55	0.46	0.68	0.61	0.44	0.4	0.64	0.62
EO#	K/µL	0.03	0.11	0.11	0.01	0.01	0.03	0.02	0.03	0.04
BA#	K/µL	0.01	0.03	0.03	0.01	0	0.01	0.02	0	0.01
NE%	%	4.99	16.03	14.89	8.88	8.46	6.54	6.5	10.97	10.84
LY%	%	87.68	73.97	76.33	82.11	83.46	88.25	88.74	81.98	82.34
MO%	%	6.75	7.9	6.72	8.82	7.89	4.74	4.33	6.67	6.32
EO%	%	0.45	1.65	1.59	0.13	0.15	0.36	0.26	0.36	0.44
BA%	%	0.12	0.45	0.46	0.07	0.04	0.1	0.17	0.02	0.07
RBC	M/µL	9.26	11.46	11.79	9.74	9.36	9.98	9.75	9.29	9.45
HB	g/dL	12.9	14.9	15.4	14.2	14.1	13.7	13.5	12.8	13
HCT	%	39.7	45.7	45.3	41.4	41	38.1	38.1	36.9	37.7
MCV	fL	42.9	39.9	38.4	42.5	43.8	38.2	39.1	39.7	39.9
MCH	Pg	13.9	13	13.1	14.6	15.1	13.7	13.8	13.8	13.8
MCHC	g/dL	32.5	32.6	34	34.3	34.4	36	35.4	34.7	34.5
RDW	%	16.9	21.1	20.2	18.6	19.2	17.6	18.5	19.8	19.8
PLT	K/µL	411	644	653	925	952	881	900	1061	1158
MPV	fL	6	5.1	5	4.6	4.6	5.1	5.1	4.4	4.4

Figure E1

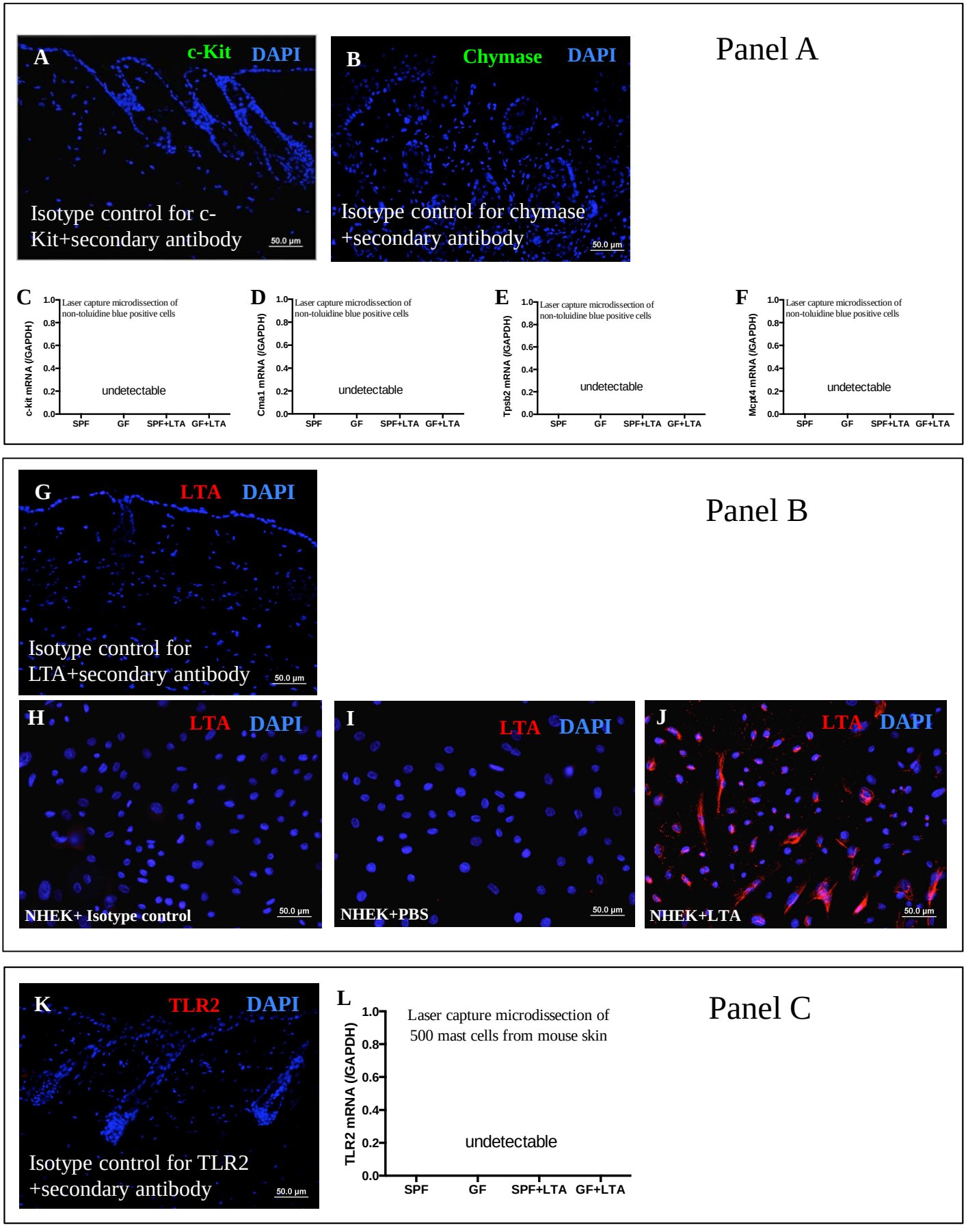
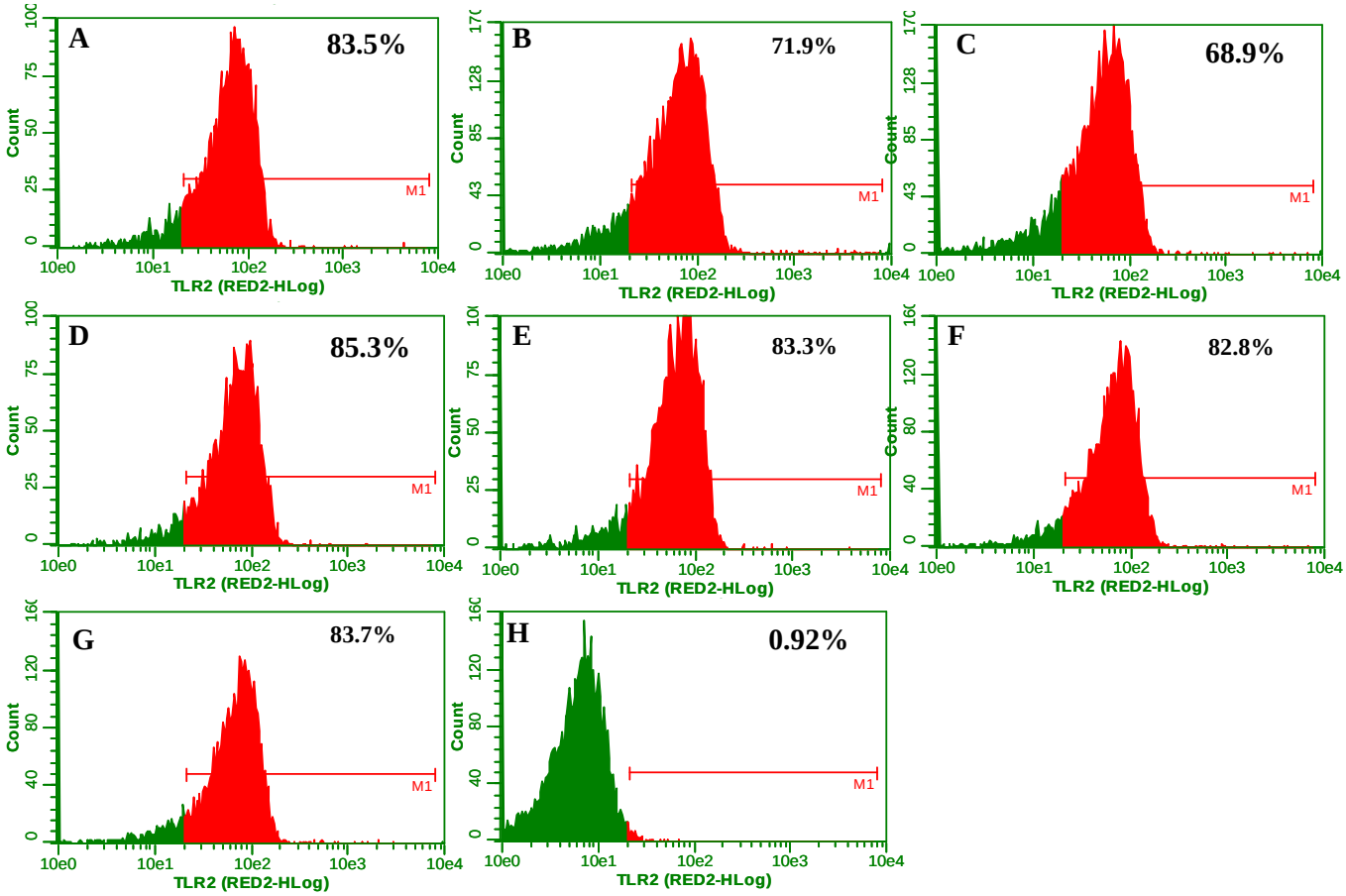

Panel A
A
c-Kit DAPI
Isotype control for c-Kit+secondary antibody
50.0 μm
B
Chymase DAPI
Isotype control for chymase +secondary antibody
50.0 μm
C
Laser capture microdissection of non-toluidine blue positive cells
c-kit mRNA (/GAPDH)
undetectable
SPF GF SPF+LTA GF+LTA
D
Laser capture microdissection of non-toluidine blue positive cells
Cma1 mRNA (/GAPDH)
undetectable
SPF GF SPF+LTA GF+LTA
E
Laser capture microdissection of non-toluidine blue positive cells
Tpsb2 mRNA (/GAPDH)
undetectable
SPF GF SPF+LTA GF+LTA
F
Laser capture microdissection of non-toluidine blue positive cells
Mcpt4 mRNA (/GAPDH)
undetectable
SPF GF SPF+LTA GF+LTA
Panel B
G
LTA DAPI
Isotype control for LTA+secondary antibody
50.0 μm
H
LTA DAPI
NHEK+ Isotype control
50.0 μm
I
LTA DAPI
NHEK+PBS
50.0 μm
J
LTA DAPI
NHEK+LTA
50.0 μm
Panel C
K
TLR2 DAPI
Isotype control for TLR2 +secondary antibody
50.0 μm
L
Laser capture microdissection of 500 mast cells from mouse skin
TLR2 mRNA (/GAPDH)
undetectable
SPF GF SPF+LTA GF+LTA

Figure E2


A
83.5%
B
71.9%
C
68.9%
D
85.3%
E
83.3%
F
82.8%
G
83.7%
H
0.92%
M1
Count
TLR2 (RED2-HLog)
10e0
10e1
10e2
10e3
10e4

I

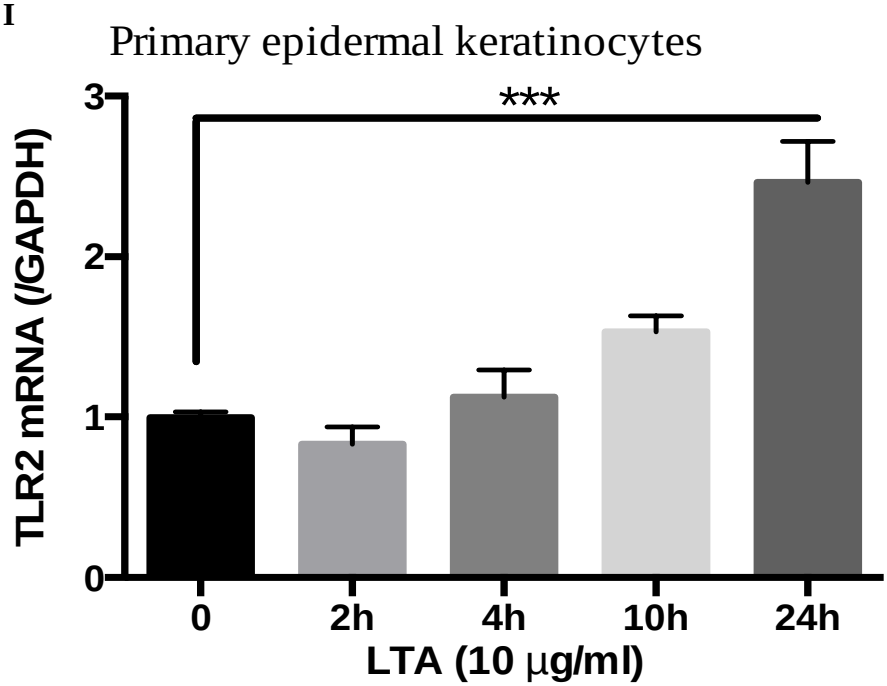

Primary epidermal keratinocytes
***
TLR2 mRNA (/GAPDH)
0
1
2
3
0
2h
4h
10h
24h
LTA (10 μg/ml)

Figure E3

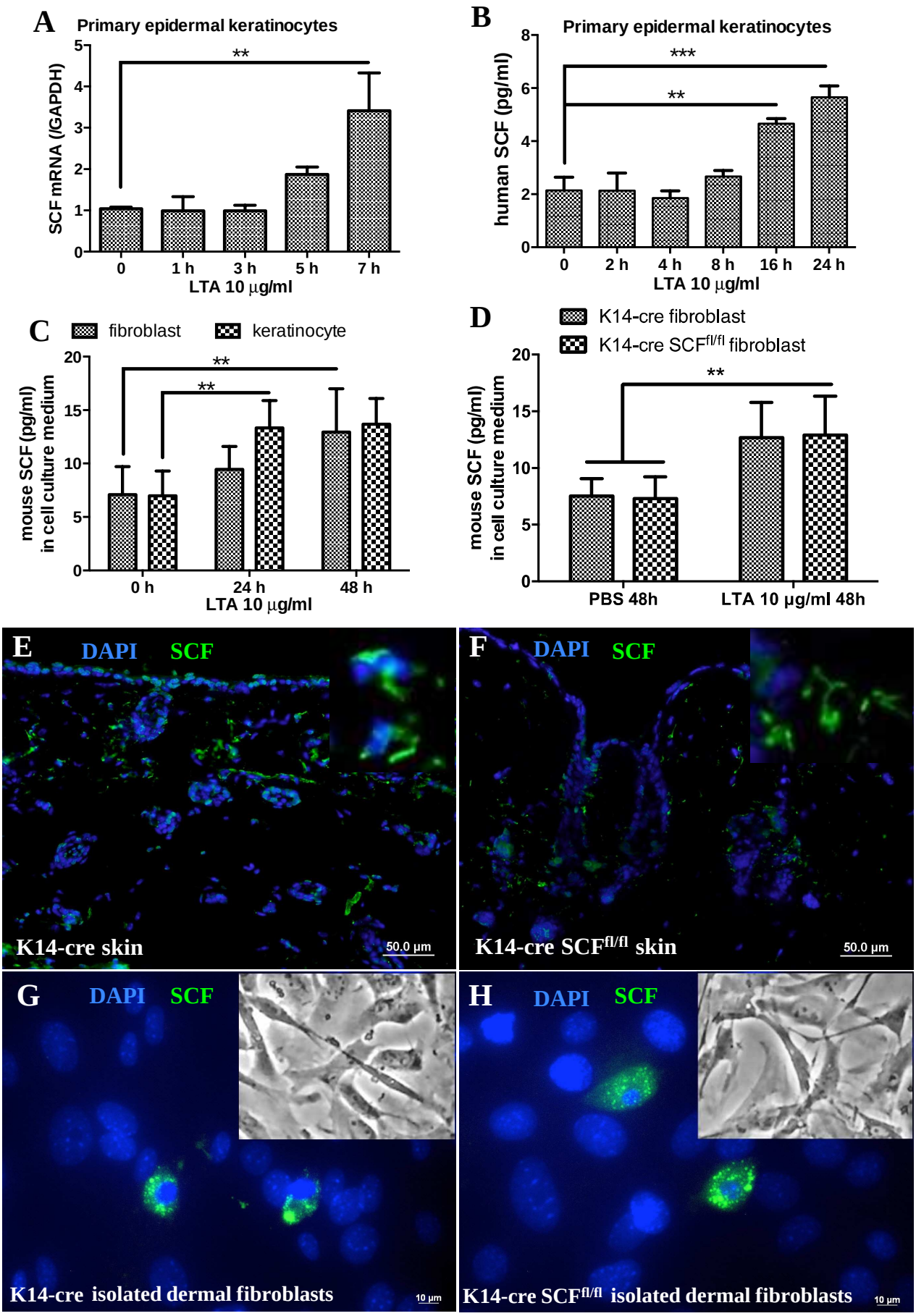
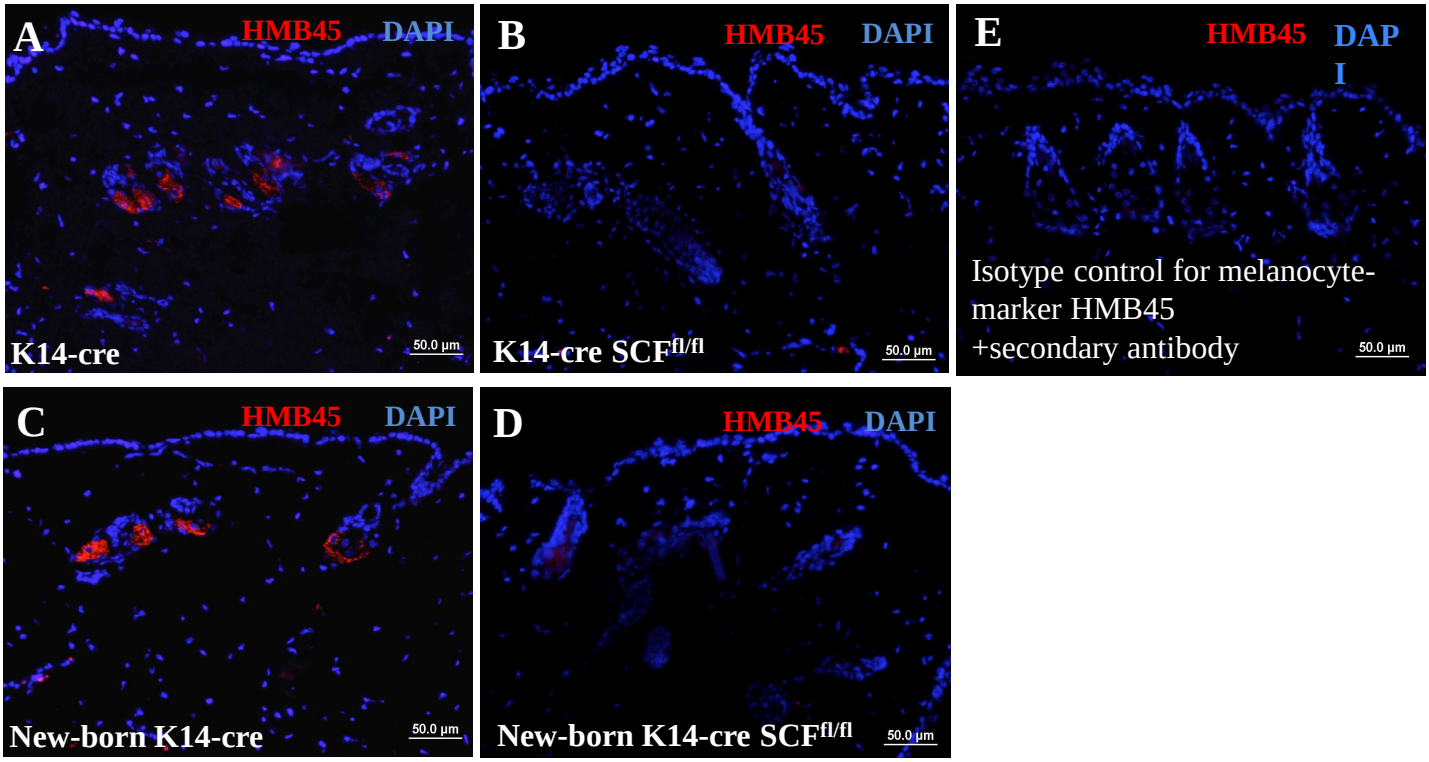

A
Primary epidermal keratinocytes
SCF mRNA (/GAPDH)
**
0
1 h
3 h
5 h
7 h
LTA 10 μg/ml
B
Primary epidermal keratinocytes
human SCF (pg/ml)
***
**
0
2 h
4 h
8 h
16 h
24 h
LTA 10 μg/ml
C
fibroblast
keratinocyte
mouse SCF (pg/ml) in cell culture medium
**
**
0 h
24 h
48 h
LTA 10 μg/ml
D
K14-cre fibroblast
K14-cre SCFfl/fl fibroblast
mouse SCF (pg/ml) in cell culture medium
**
PBS 48h
LTA 10 μg/ml 48h
E
DAPI
SCF
K14-cre skin
50.0 μm
F
DAPI
SCF
K14-cre SCFfl/fl skin
50.0 μm
G
DAPI
SCF
K14-cre isolated dermal fibroblasts
10 μm
H
DAPI
SCF
K14-cre SCFfl/fl isolated dermal fibroblasts
10 μm

Figure E4


A
HMB45
DAPI
K14-cre
50.0 µm
B
HMB45
DAPI
K14-cre SCF fl/fl
50.0 µm
E
HMB45
DAPI
Isotype control for melanocyte-marker HMB45
+secondary antibody
50.0 µm
C
HMB45
DAPI
New-born K14-cre
50.0 µm
D
HMB45
DAPI
New-born K14-cre SCF fl/fl
50.0 µm

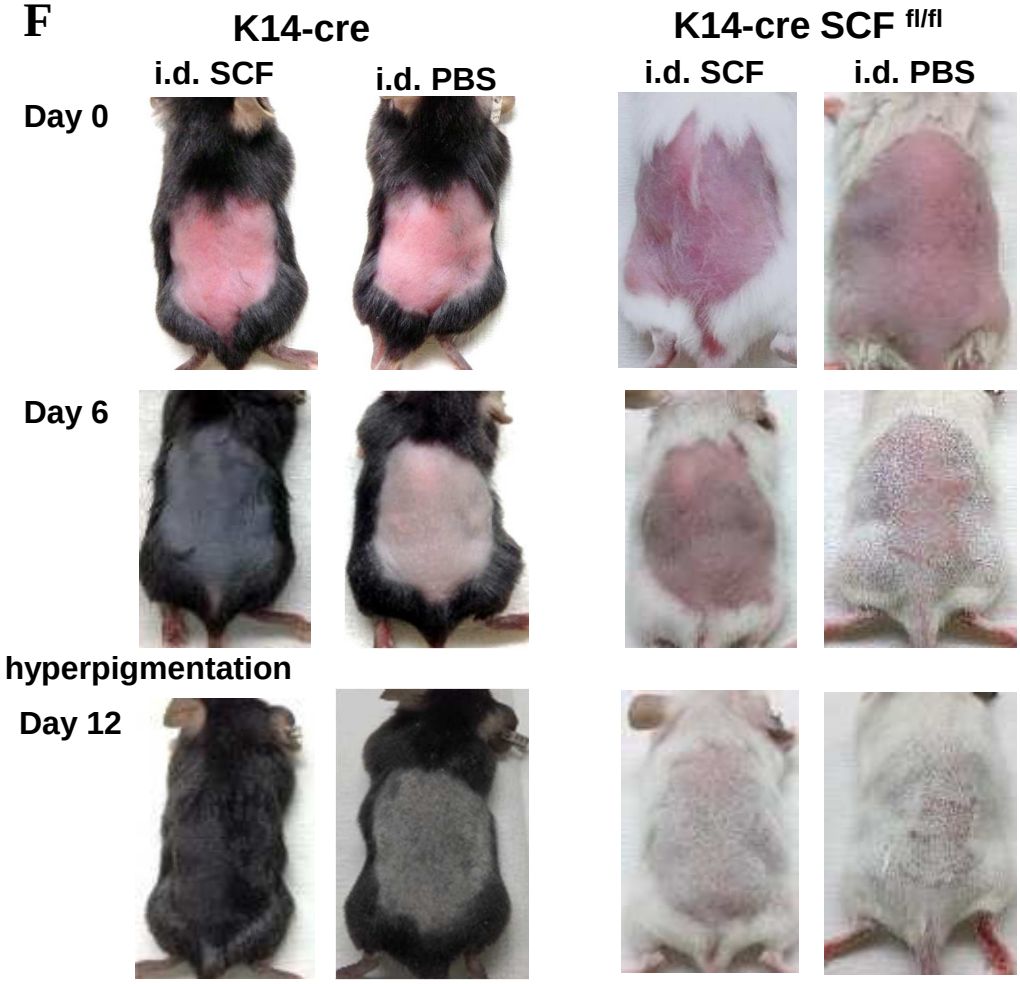
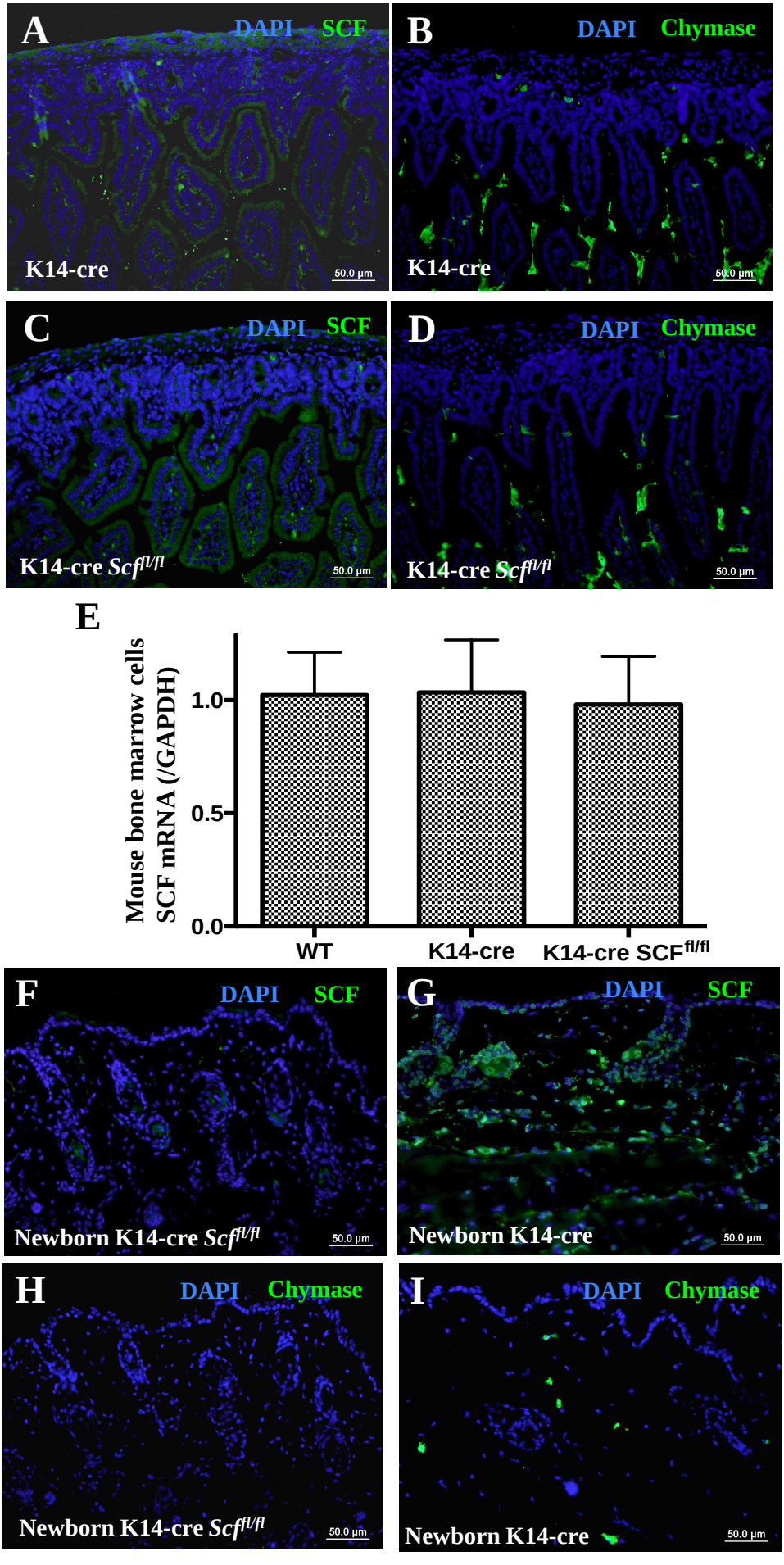

F
K14-cre
K14-cre SCF fl/fl
i.d. SCF
i.d. PBS
i.d. SCF
i.d. PBS
Day 0
Day 6
hyperpigmentation
Day 12

Figure E5


A
DAPI SCF
K14-cre
50.0 μm
B
DAPI Chymase
K14-cre
50.0 μm
C
DAPI SCF
K14-cre Scf fl/fl
50.0 μm
D
DAPI Chymase
K14-cre Scf fl/fl
50.0 μm
E
Mouse bone marrow cells
SCF mRNA (/GAPDH)
1.0
0.5
0.0
WT
K14-cre
K14-cre SCF fl/fl
F
DAPI SCF
Newborn K14-cre Scf fl/fl
50.0 μm
G
DAPI SCF
Newborn K14-cre
50.0 μm
H
DAPI Chymase
Newborn K14-cre Scf fl/fl
50.0 μm
I
DAPI Chymase
Newborn K14-cre
50.0 μm