Supplementary information

"TCF/Lef1 activity controls establishment of diverse stem and progenitor compartments in mouse epidermis"

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Supplementary methods

Isolation of primary keratinocytes and FACS-analyses

Primary keratinocytes were isolated as described previously (Niemann et al, 2002; Morris et al, 2004 ; Blanpain et al, 2004; Jensen et al, 2009). Single cell suspensions were incubated in 5% FCS/PBS containing antibodies for 45 minutes at 4°C. Cell viability was assessed by either 7AAD (BD Bioscience) or propidium iodide (PI, Sigma-Aldrich, Germany) labelling.

The Itga6^{+high} cell population were defined on a plot of (PE) Itga6 versus (APC) CD34 that show two distinct, a Itga6^{+high}/ and a Itga6^{+low}/CD34^{+} cell population. For subsequent analysis the Itga6^{+high} cell population (~77% of the living cell population) were gated for endogenous YFP expression and for expression of the surface marker CD34, Lrig1 or Plet1/MTS24 antigen. The positive cell populations for each of these hair follicle markers were defined by comparing the expression level of the labelled cells with cells labelled with the isotype-control, the secondary antibodies only or cells isolated from control mice without Cre activation. However, the gates for Lrig1 positive cells were set differently for samples isolated at 2 days and 6 days following Tam pulse due to a shift of the entire cell population.

Subsequent analysis was carried out using a FACSCanto^{TM}II Cytometer (BD Bioscience) equipped with a BD FACSDiva Software. Cell sorting for RNA isolation and expression analysis were performed on a FACSVantage SE System (BD Bioscience).

Isolation of genomic DNA and semi-quantitative PCR analyses

FAC sorted keratinocytes were lysed and a phenol/chloroform/isoamylalcohol mixture (Roth, Germany) was added. After a centrifugation step, genomic DNA was precipitated with isopropylalcohol and dissolved in pure water. An amount of 20ng of gDNA was employed for subsequent semi-quantitative PCR-reaction using HotStarTaqPlus DNA Polymerase (Qiagen, Germany). Recombination events within the stop-EYFP cassette was analysed using the following primer YFPfor 5'-ggttgaggacaaactcttcgcggt-3' and YFPrev primer as listed in Supplementary Table 1 and (Srinivas et al, 2001). To validate for equal amounts of template DNA, GapDH primer were used (Supplementary Table 1).

Supplementary Figures

Supplementary Figure 1. Labelling of HF bulge stem cells and their progeny in K15CreER(G)T2/R26RLacZ mice

(A-D) Analyses of tail skin whole-mounts of C_K15CreER^{high}/R26RLacZ (A,B) and A_K15CreER^{low}/R26RLacZ (C,D) mice for β-Galactosidase (A,D) and nuclear Cre recombinase (B,C) 3 days following Tam administration. Note that no specific LacZ signal and only diffuse cytoplasmic Cre enzyme is detected in mice treated with vehicle as control (right panels in A,B,D). With increasing tracing time labelled bulge cells are observed at the upper isthmus (UI) and the SG ducts (D), subsequently resulting in LacZ+ HF bulge progeny at the lower tip of the SG and the entire SG at 7 days of tracing (D). No specific LacZ signal is observed in vehicle controls at day 7 of chasing (ctrl). Scale bars 50μm.

(E) Statistical analysis of LacZ+ and YFP+ cells within the interfollicular epidermis (IFE) at 3 days following Cre activation in A_K15CreER^{low}/R26RLacZ and A_K15CreER^{low}/R26RYFP mice.

Supplementary Figure 2. Sorting and PCR analysis of HF SCs and progenitors by flow cytometry

(A-H) Kerationcytes were isolated from back skin of A_K15CreER^{low}/R26RYFP 8 weeks old mice (telogen) 2 days (A,E) and 6 days (B,F) after Cre activation by Tam and from control mice (oil ctrl, C,G). Single cell suspensions from each of these mice were sorted for Itga6^{+}/CD34^{+} (A-C) and MTS24^{+} keratinocytes (E-G). Dead cells (7AAD positive) and differentiated cells (high forward and sideward scatter) were gated out. The positive cell populations were defined by comparing the expression level of labelled cells with the coresponding isotype-control or secondary antibody respectively (D,H).

(I) PCR analysis of genomic DNA for excision of stop cassette upstream of YFP of different cell pools isolated from A_K15CreER^{low}/Rosa26RYFP mice. CD34+ and MTS24+ cells were isolated 2 days and 6 days following Cre activation. Control animals were treated with vehicle alone and possess no recombination. GapDH PCR demonstrates input of equal amount of gDNA for the recombination PCR.

(J,K) Statistical analysis of YFP+ (J) and LacZ+ (K) keratinocytes localised in the bulge (B) or SG in whole-mounts of A_K15CreER^{low}/R26RYFP and A_K15CreER^{low}/R26RLacZ mice at different time points following administration of oil vehicle.

Supplementary Figure 3. Continuous regeneration of SG by HF bulge cells occurs independently of the hair cycle

(A) Distinct phases and keratinocyte populations of the synchronized hair cycle.

(B-D) Cre activation by Tam pulse in telogen to anagen transition as shown in (B) resulted in YFP+ bulge progeny migrating down for HF regeneration and up for SG renewal during early anagen 3 days (C) and 6 days after Cre activation (D).

(E-G) Labelling of bulge stem cells in telogen to anagen transition (E) demonstrated contribution of YFP+ keratinocytes to HF lineages (F) and SG renewal (G) 4 weeks after Cre activation during anagen of the following hair cycle.

(H-L) Tam treatment of A_K15CreER^{low}/R26RLacZ (I,J,L) and A_K15CreER^{low}/R26RYFP (K) at the beginning of anagen (H) revealed contribution of bulge progeny to renewal of SGs during anagen (J,K). Control mice were negative for LacZ+ cells (L). Note that bulge stem cell progeny does not migrate downward the ORS for HF regeneration when cells were labelled after telogen to anagen transition. Scale bars 50μm.

Supplementary Figure 4. Commitment of HF SC progeny to all hair lineages

(A-D) Contribution of YFP+ HF bulge progeny to all cell lineages of the HF after Cre activation at telogen to anagen transition. Note YFP+ cells are found in hair germ (A), outer root sheath (ORS, B), matrix cells (Mx, C), inner root sheath (IRS, C) and precortex of the hair bulb (Bb, D) 6 days after Tam administration.

(E-G) Three-dimensional projections of whole-mount reconstructions illustrating localisation and morphology of YFP+ cell clones expanding from the bulge towards hair bulb and SG.

(H,I) Bulge progeny differentiates into all hair lineages in long-term studies when A_K15CreER^{low}/R26RLacZ mice were analyzed 6 months after Cre activation. Scale bars 50µm.

(J) Statistical analysis of number of labelled cells within the SG at 3, 8 and 180 days following Cre administration at telogen to anagen transition.

(K,L) qRT-PCR of sorted cells populations comparing expression levels for YFP (K) and CD34 and MTS24/Plet1 (L) at 2 and 6 days following Tam administration.

Supplementary Figure 5. Localisation and proliferation of HF SC progeny

(A-C) Single stacks of 2.5µm size are displayed as xy planes to show co-localisation of YFP+ bulge progeny with Lrig1 (A) and SCD1(C). For YFP/SCD1 double staining (C) also xz and yz planes are shown. (B) High magnification of YFP and MTS24 antigen.

(D) Time-lapse studies on whole-mount explant cultures from A_K15CreER^{low}/Rosa26RYFP 6 days following Tam administration in vivo. Individual labelled bulge progeny was monitored for 18h and cell division was detected at the JZ/periphery at the SG with migration of one daughter cell towards the lower tip of the gland. The red bar marks the distance covered by the cells. Scale bars 50µm (A-C) and 70µm (D).

(E,F) Statistical analysis of number of labelled cells at the junctional zone (JZ, E) and the SG duct (F) at 3, 8 and 180 days following Tam administration.

Supplementary Figure 6. Establishment of new progenitor compartments in K14ΔNLef1 transgenic mice

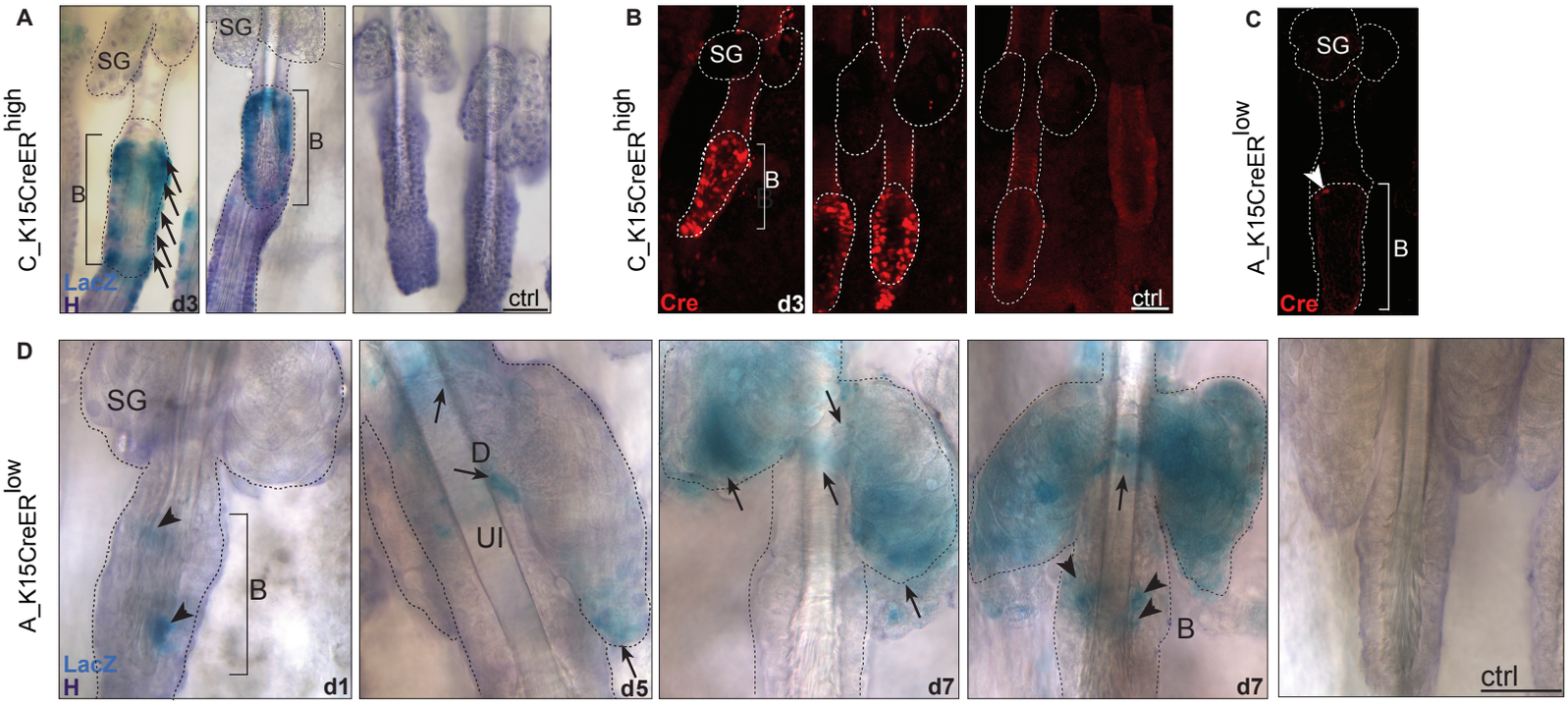
(A-D) Whole-mounts of K14ΔNLef1 mice of 3 weeks (A,C) and 3 months of age (B,D) were analyzed for expression of progenitor marker Lrig1 (red, A,B) and Plet1/MTS24 antigen (red, C,D). Note that Lrig1 and Plet1 are detected at the periphery of de novo SGs (nSG) at an early time point of development (A,C) and are highly elevated in older mice (B,D). The basal cell compartment of whole-mounts is stained with antibodies against K14 (green). Scale bar 50µm

(E-I) FACS analysis of YFP+ keratinocytes (YFP/Itga6+) with bulge and isthmus markers in AK15CreER/K14□NLef1/YFP mice at 2 and 6 days of chase.

(K) FACS analysis of YFP+ cells in AK15CreER/R26RYFP and AK15CreER/K14ΔNLef1/R26RYFP mice following 2 days of Tam administration. The percentage of YFP+ cells in vehicle control treated genotype matched animals ranged between 0.2% and 0.6%.

(L) High magnification of SCD1/YFP+ cells in SG and nSG of K14ΔNLef1 mice. Images are shown as xy projections of single stacks (2.5µm).

Supplementary Table 1. Primer Sequences used for qRT-PCR analysis of distinct stem and progenitor cell populations



E

IFE	number of LacZ+ cells		
	1	2	>2
n=2 total 500HF	21	2	0

IFE	number of YFP+ cells		
	1	2	>2
n=4 total 1738HF	4	2	2

Suppl.Fig. S1
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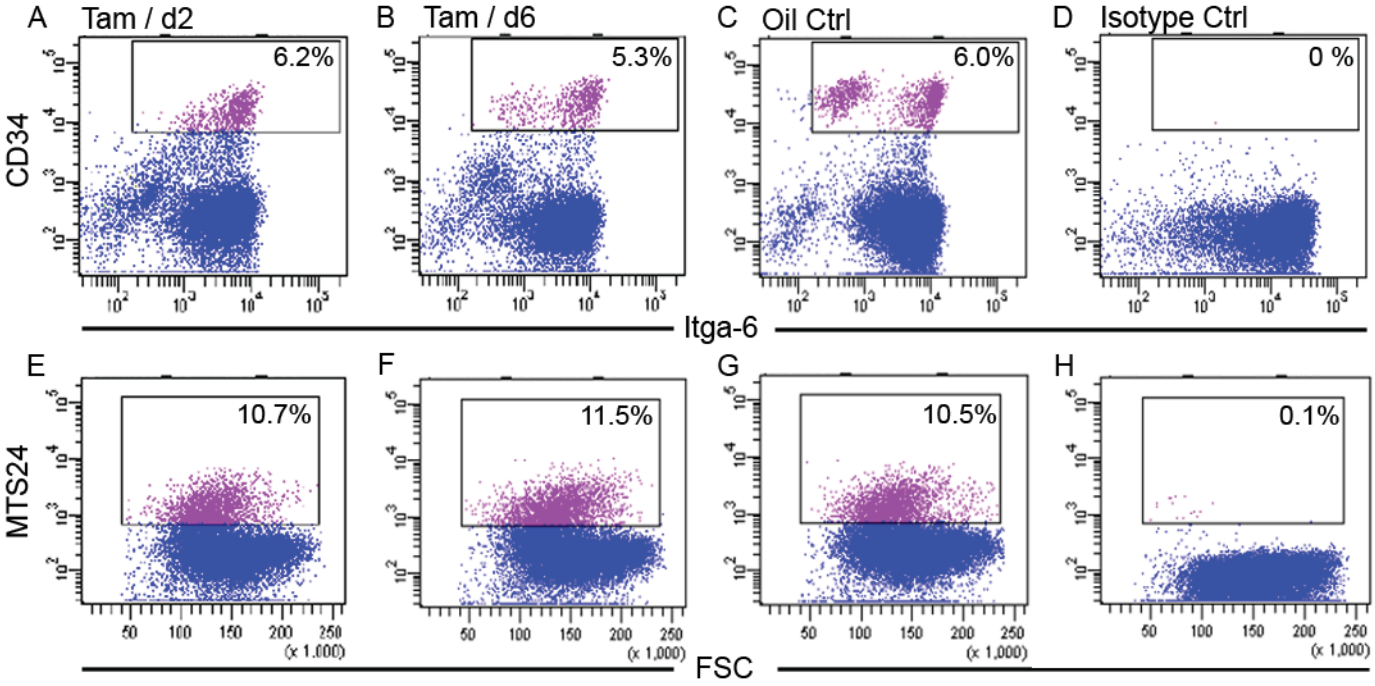

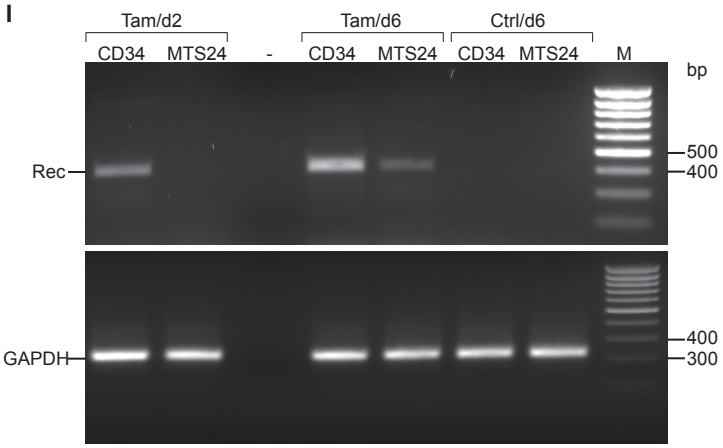

J

	scored HF	number of YFP+ cells B	SG
d3 n=3	145	1	0
d5 n=1	48	1	0
d11 n=1	103	1	0
d63 n=1	90	0	0

K

	scored HF	number of LacZ+ cells B	SG	IFE
d3 n=3	774	0	1	5
d8 n=3	1107	0	1	7

Suppl.Fig.S2
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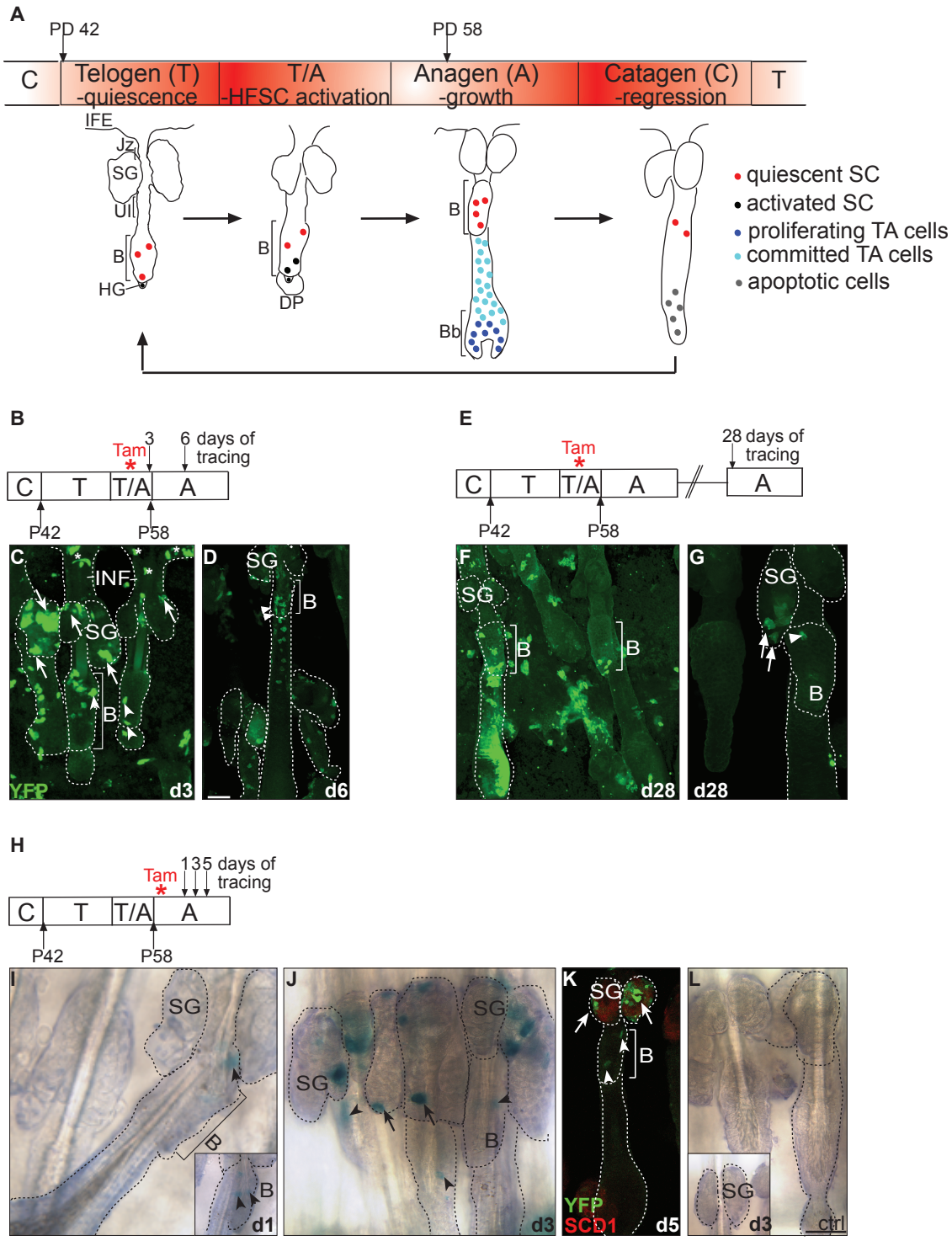

Suppl.Fig. S3
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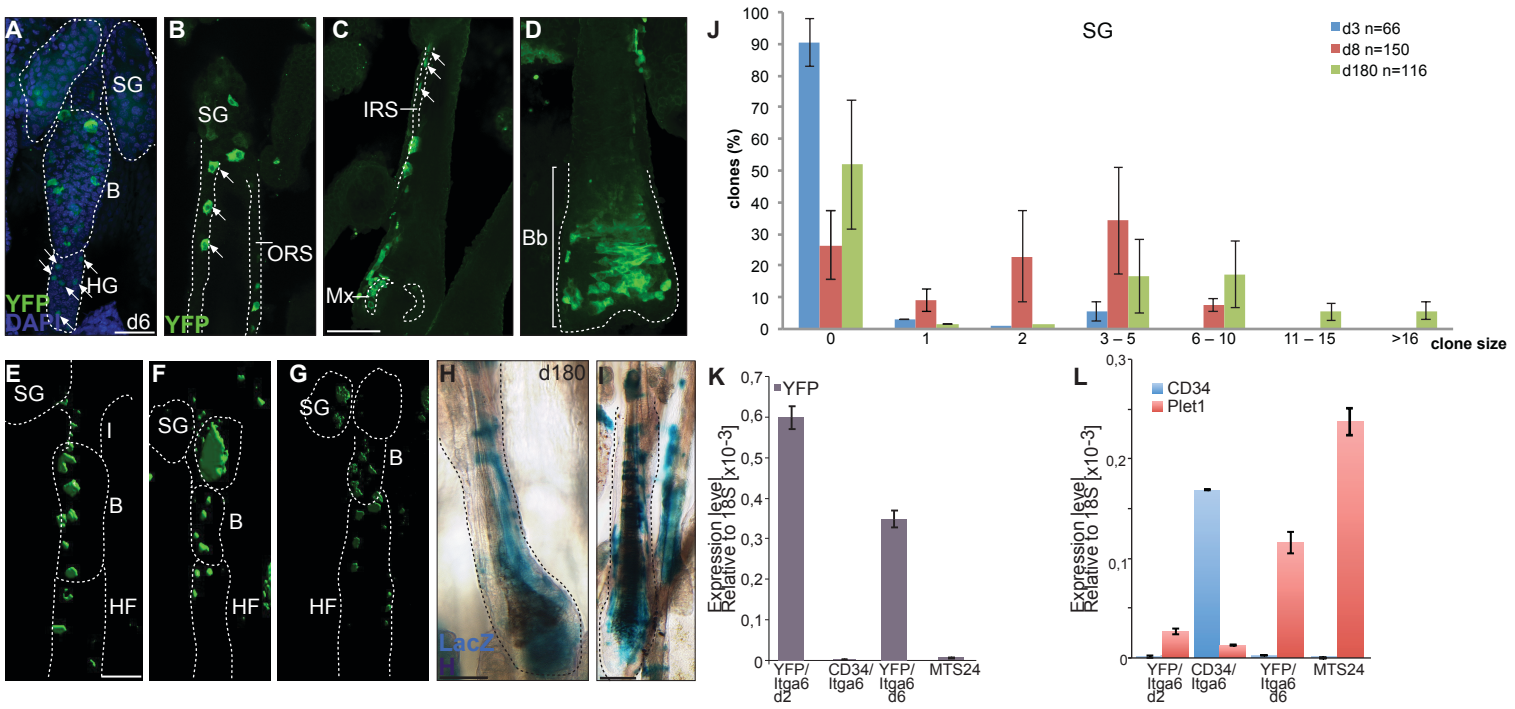

Suppl.Fig. S4
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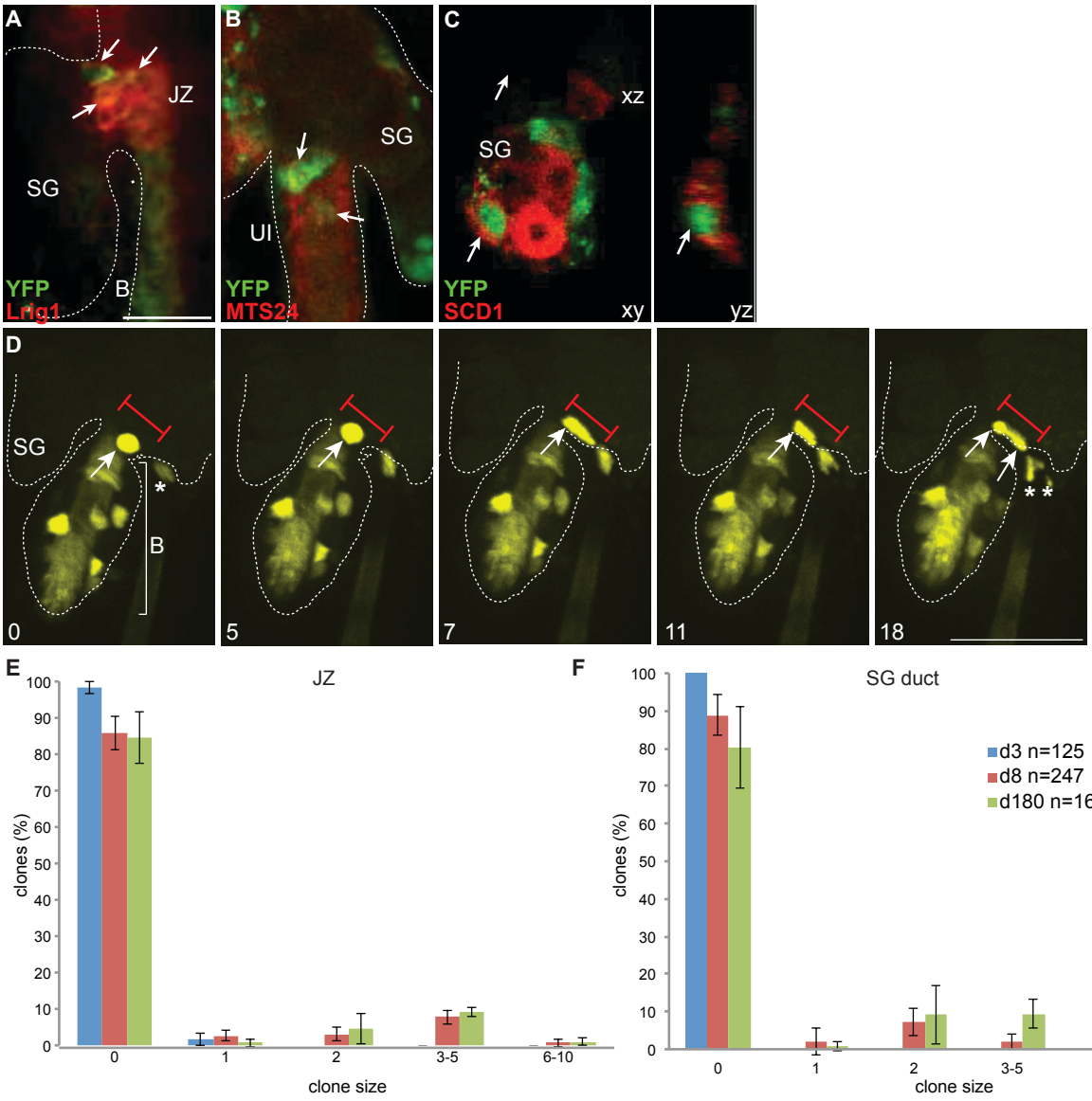

Suppl.Fig. S5
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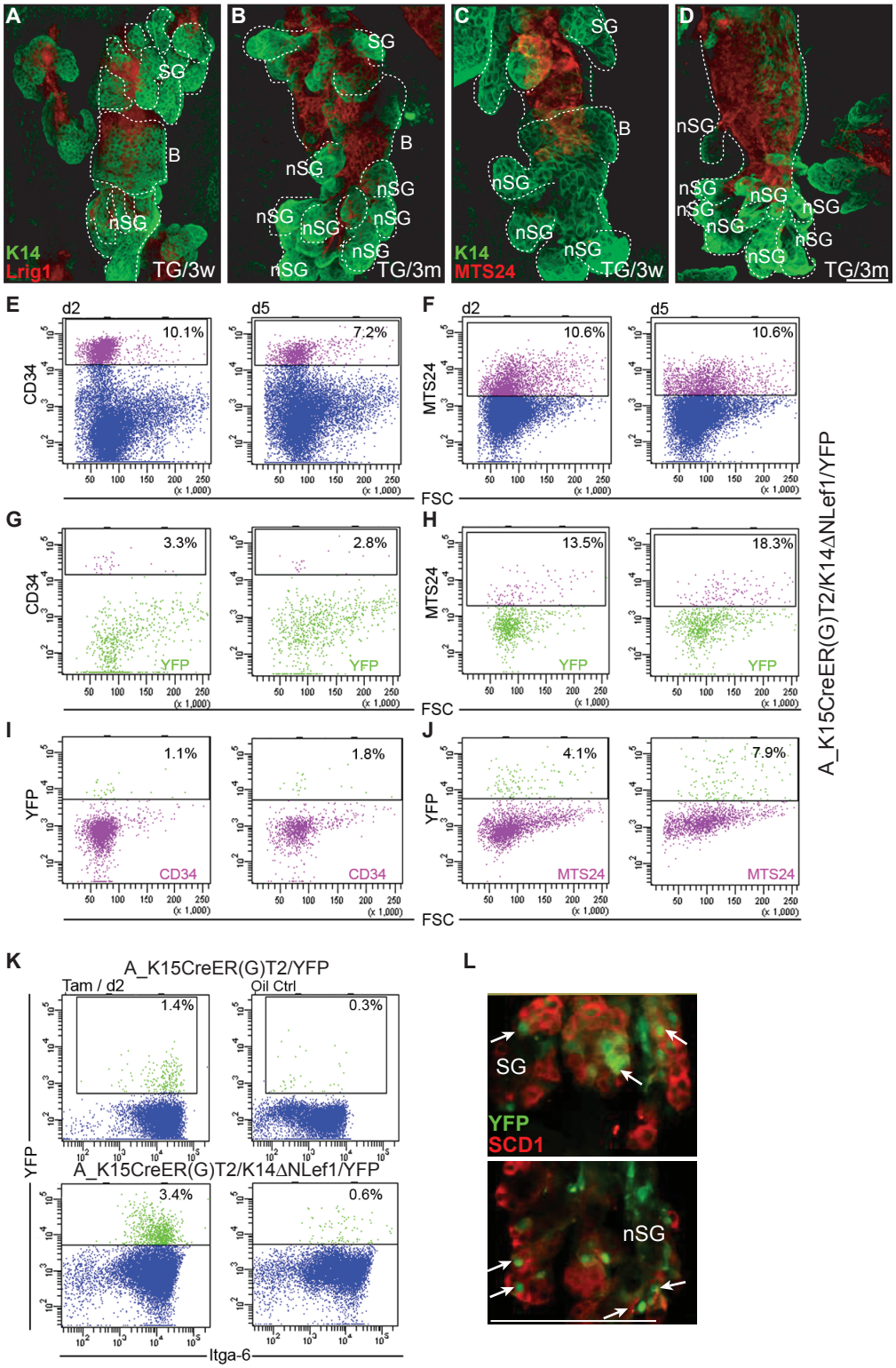

Suppl.Fig. S6
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gene	fragment size [bp]	Tm [°C]	for oligo 5´-3´	rev oligo 5´-3´
mc-myc	146	61	Mcmycfor-tgagcccctagtgctgcatgag	mcmycrev-atggagatgagcccgactccgac
mSCD1	183	61	mSCD1for-gcttccacaactaccaccacacct	mSCD1rev-tgtgactcccgtctccagttctctcaatcc
mBlimp1	115	59	mBlimp1Ex2for-ggcatccttaccaaggaacctgc	mBlimp1Ex3rev-gatgaggggtccaaagcgtg
mElovl3	166	58	mElovl3for-ctgttgtctatcgttgttgg	mElovl3rev-gcttgaggcccactgtaaac
mKeratin6a	136	59	mK6aEx5for-atcgctgaggtcaaggcccagta	mK6aEx6rev-gcttggtgttgcgtaggtcg
mLgr6	153	59	mLgr6Ex15for-agggaacttggccctgtctc	mLgr6Ex16rev-ggatgaaagtcctcggcctg
mLrig1	118	62	mLrig1for-tgtttcccgaacggcctgcgta	MLrig1rev-tgctcagacggagagtcagcagtg
mLhx2	76	59	mLhx2for-gtaacgagaacgatgctgaac	mLhx2rev-ggtgcgcatgcgctttgtc
mCD34	133	61	mCD34Ex2for-ggacagcagtaagaccacaccagc	mCD34Ex3rev-aggcagtatgccagttgggga
mKeratin15	166	61	mK15Ex3for-tggagatgcagattgagcagctgaa	mK15Ex4rev-tgctccctcatctctgccagca
mItga6	218	60	mItga6Ex4for-aagtttggctcctgtcagcaagg	mItga6Ex5-ctgttagcaggaacgggcacga
mNFATc1	169	60.5	mNFATc1for-tgcgagcagtatctgtcggtgc	mNFATc1rev-tgtggtgagacttgggctgc
mPlet1	83	61	mPlet1for-ggctaacgtctctggtgggga	mPlet1rev-ctgctttcaggatcacgg
mSox9	178	60.5	mSox9for-ccttcatggtgtgggcgcag	mSox9rev-gggtggtctttcttgtgctgca
mTenascinC	172	62	mTenascinCEx6for-taccgccaaactggccttgctc	mTenascinCEx7rev-tgaaccaggtgatcagtgctgtgg
YFP	167	61	YFPfor-gtggtgcccatcctggtcga	YFPrev-taggccgaaggtggtcacgagg
mGapDH	223	55	mGapDHfor-acctttggcattgtggaagg	mGapDHrev-acacattgggggtaggaaca
m18S	65	55	m18Sfor-cctgccctttgtacac	m18Srev-cgatccgagggcctcac

Supplementary table 1
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