Appendix

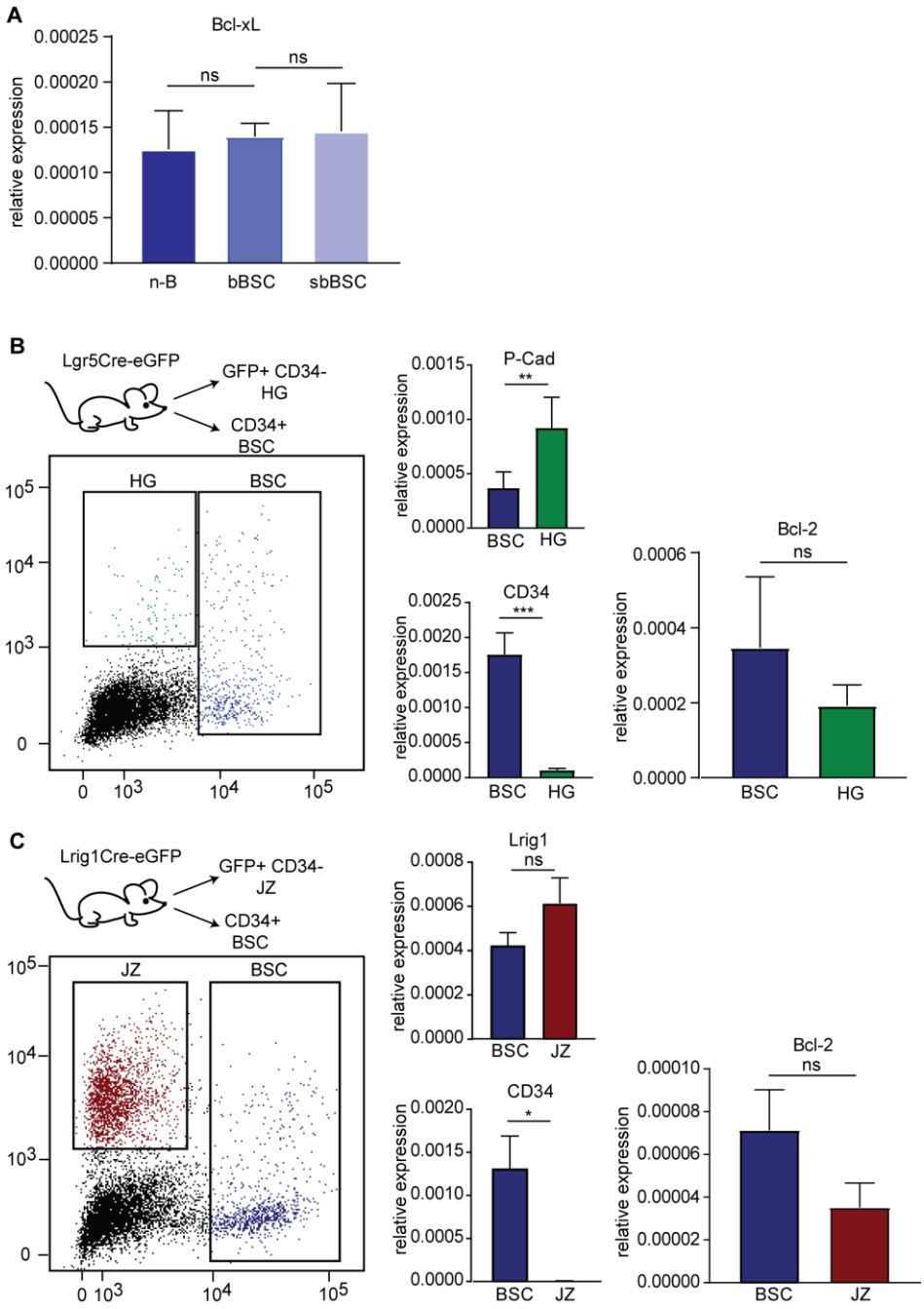
The anti-apoptotic Bcl-2 protein regulates hair follicle stem cell function

Anna Geueke, Giada Mantellato, Florian Kuester, Peter Schettina, Melanie Nelles, Jens Michael Seeger, Hamid Kashkar and Catherin Niemann

Table of contents

Appendix figures

Appendix Table

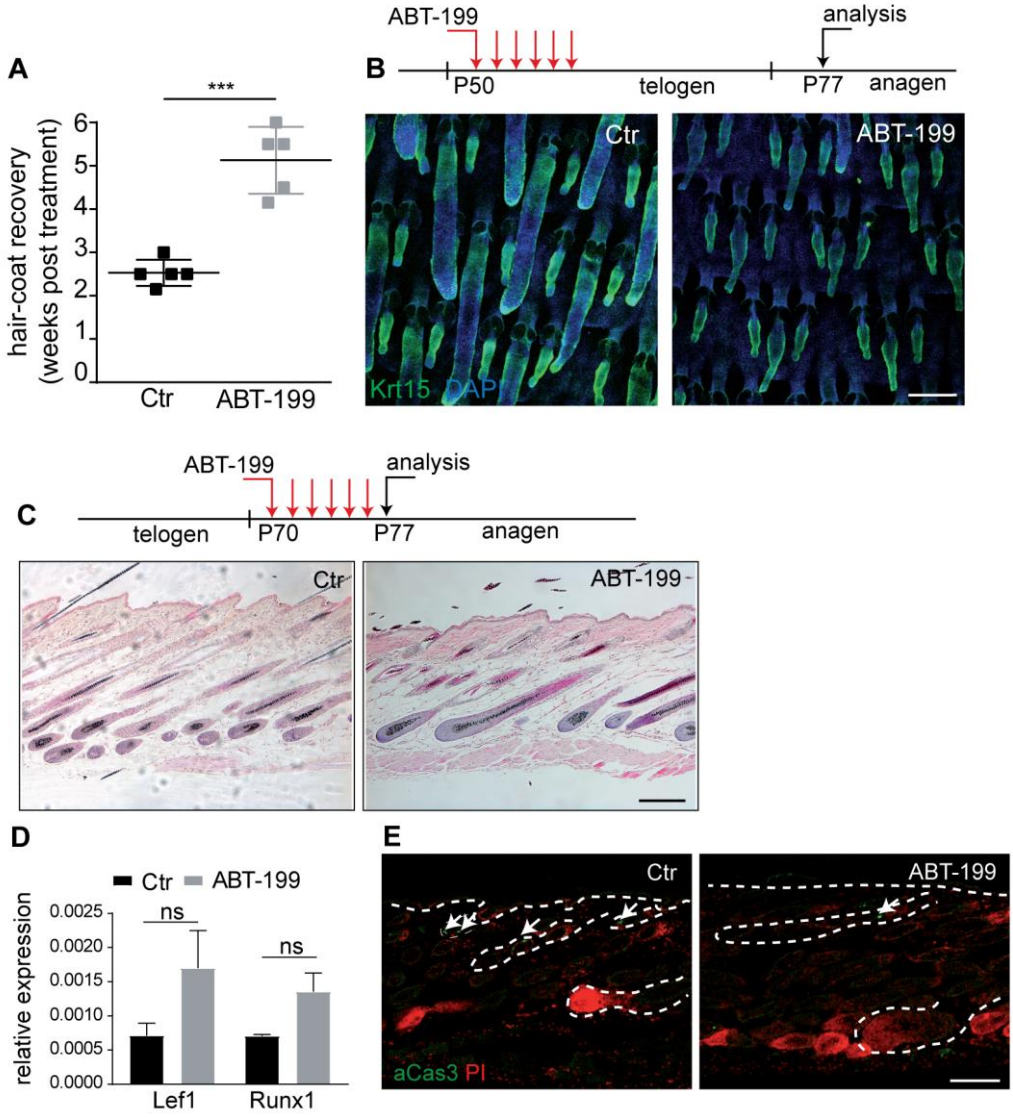


Appendix Figure S1: Bcl-xL expression in sorted bulge stem cells and Bcl-2 expression in hair germ and junctional zone.

A qRT-PCR for Bcl-xL mRNA expression in sorted non-bulge (n-B), basal BSC (bBSC) and suprabasal BSC (sbBSC) in second telogen mice (n=4 biological replicates, mean ± standard error of mean (SEM); ns= not significant, two-way ANOVA test).

B Mouse model, sorting strategy, FACS plot and qRT-PCR for P-Cad, Cd34 and Bcl-2 mRNA expression in hair germ (HG) and BSC of second telogen mice (n=5 biological replicates, mean ± standard error of mean (SEM); ns= not significant, ** p<0.01, *** p<0.001, two-tailed unpaired Student's t-test)

C Mouse model, sorting strategy, FACS plot and qRT-PCR for Lrig1, Cd34 and Bcl-2 mRNA expression in junctional zone (JZ) and BSC of second telogen mice (n=4 biological replicates, mean ± standard error of mean (SEM); ns= not significant, * p<0.01, two-tailed unpaired Student's t-test)



Appendix Figure S2: Bcl-2 inhibition during telogen, but not anagen, affects HF regeneration and apoptosis.

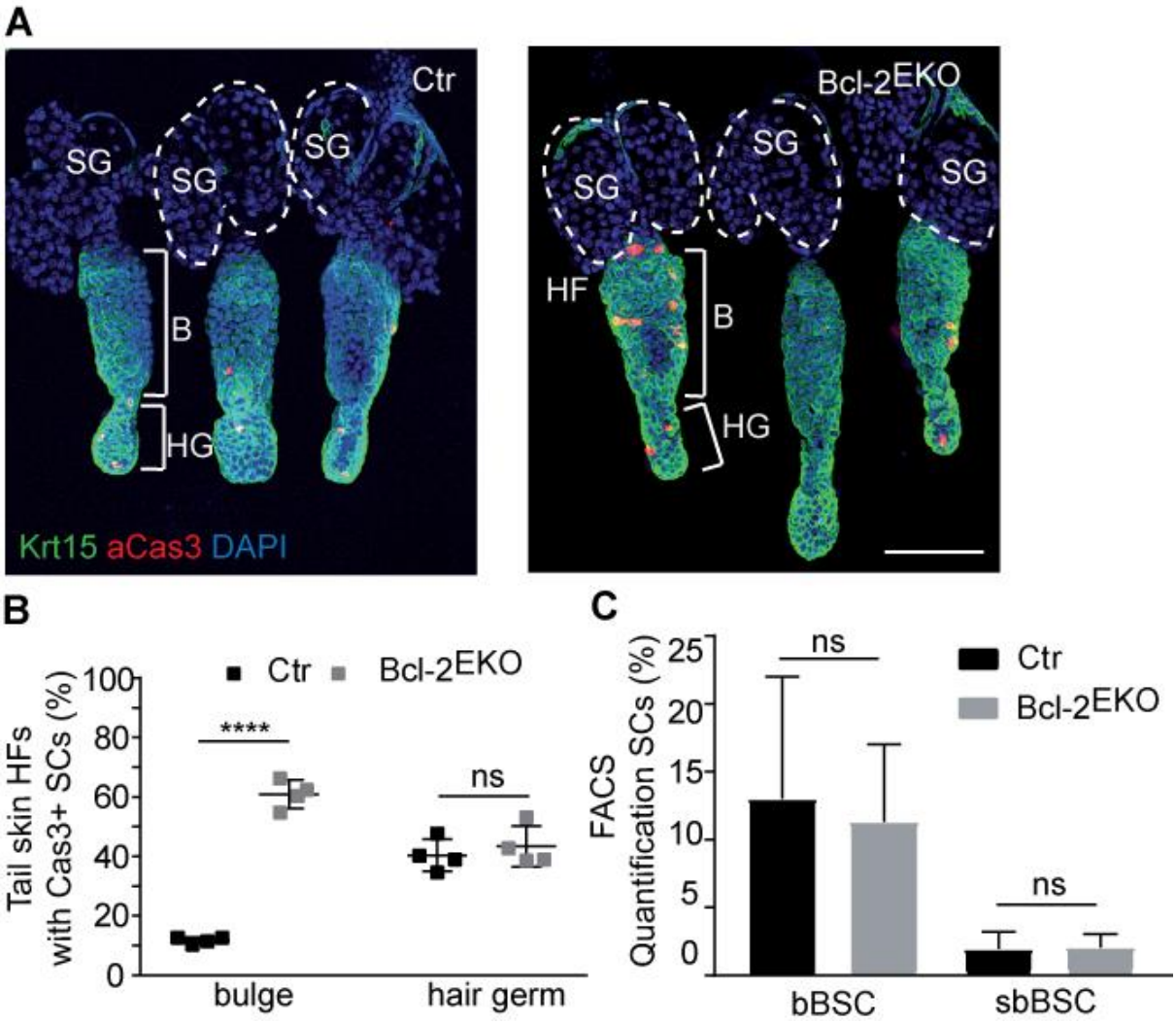
A Quantification of hair coat recovery in control (Ctr) and ABT-199 mice (n=5 biological replicates).

B Timeline experimental ABT-199 daily treatment in telogen (P50-55) and analysis in subsequent anagen (postnatal day P77) and immunofluorescence staining of tail whole mounts (P77) for keratin 15 (Krt15, green) and DAPI (blue) in control (Ctr) and ABT-199 treated mice. Scale bars, 200 µm.

C Timeline experimental ABT-199-treatment (P70-75) and analysis (P77) in anagen and histology of backskin sections of control (Ctr) and ABT-199 treated mice at P77. Scale bar, 200 µm.

D qRT-PCR for mRNA expression of the transcription factors Lef1 and Runx1 in epidermis of ABT-199 treated (P70-75) and control mice at P77 (n=3 biological replicates, mean ± standard error of mean (SEM); ns=not significant, two-tailed unpaired Student's t-test)**.**

E Immunofluorescence staining of anagen backskin HF for activated Caspase 3 (aCas3, green) and PI (red) in control (Ctr) and Bcl-2 inhibitor (ABT-199) treated mice at P77. Scale bar, 200 µm.

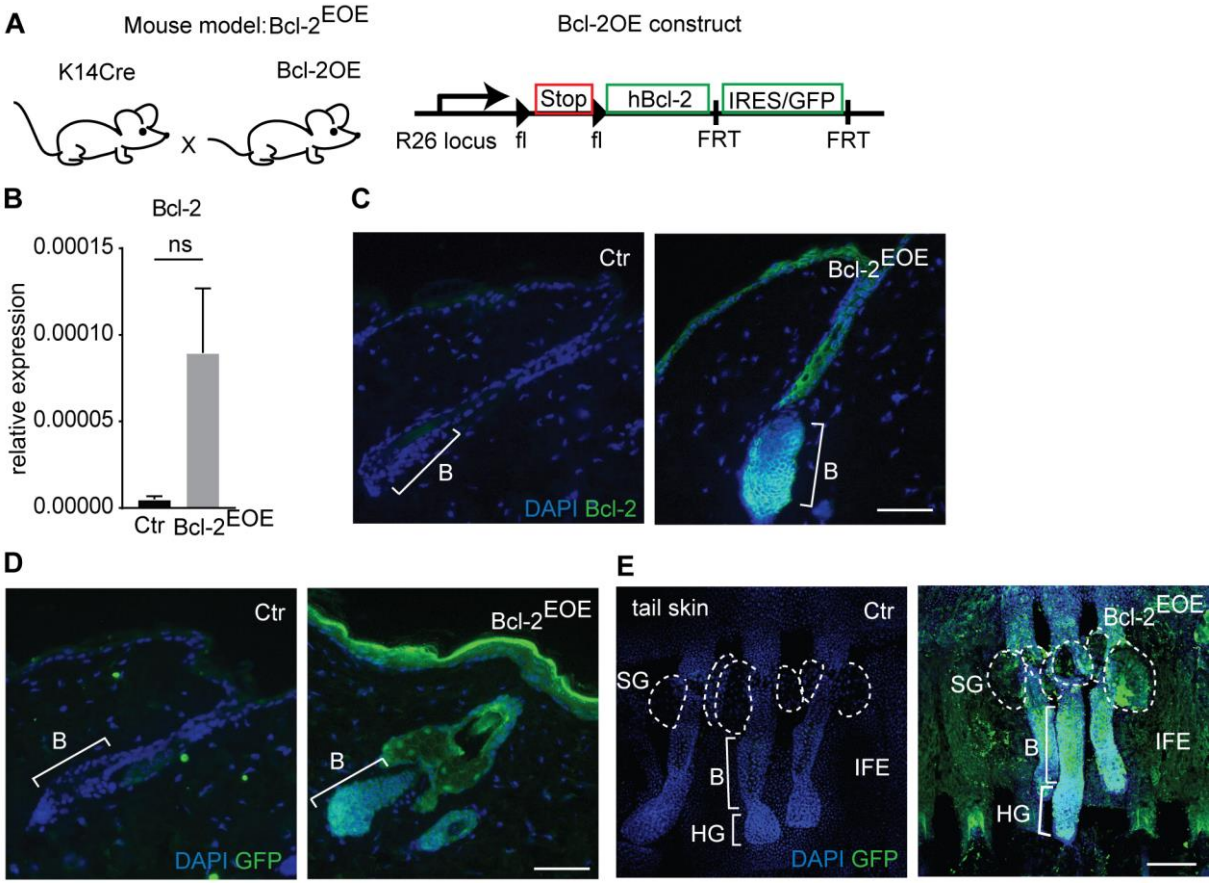


Appendix Figure S3: BSC apoptosis in epidermal Bcl-2 KO mice.

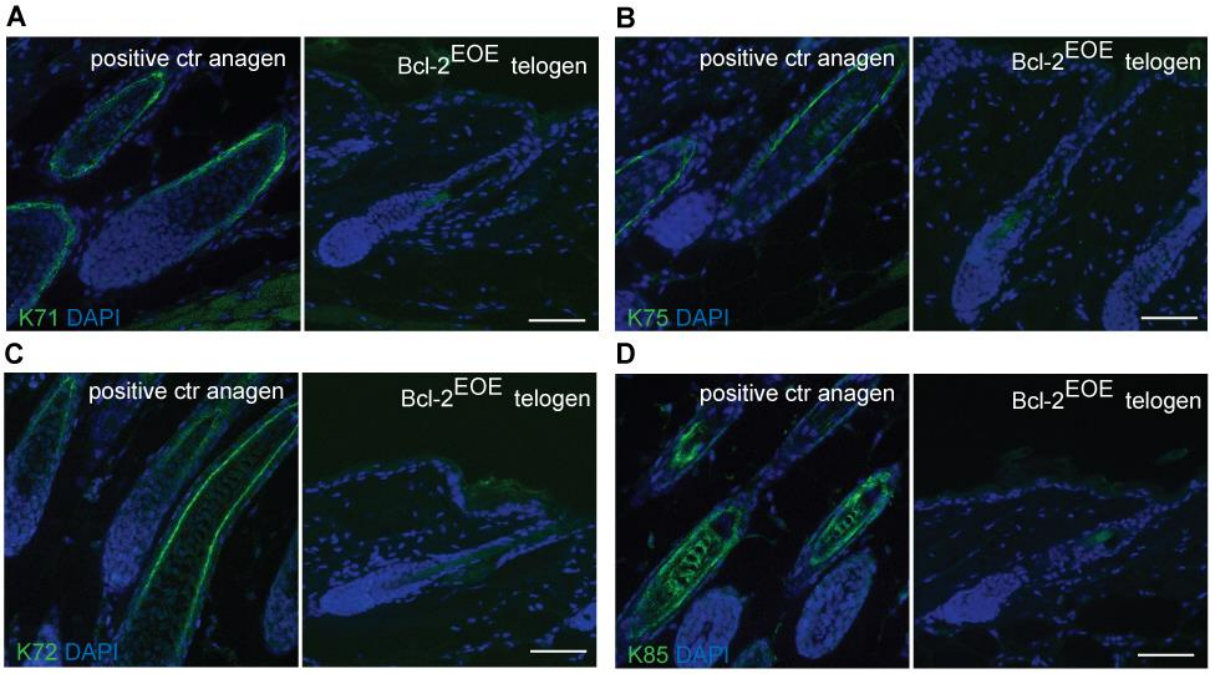
A Immunofluorescence staining of telogen tail whole mounts for keratin 15 (Krt15, green), activated caspase 3 (aCas3, red) and DAPI (blue) in control (Ctr) and Bcl-2^{EKO} mice. B: bulge, HF: hair follicle, HG: secondary hair germ, SG: sebaceous gland. Scale bar, 100 µm.

B Quantification of tailskin hair follicles (HF) with activated caspase 3 (aCas3$^{+)}$ cells in the bulge and hair germ of control (Ctr) and Bcl-2^{EKO} mice (n=4 biological replicates; ; mean ± standard deviation (SD); **** p<0.0001, ns=not significant, two-tailed unpaired Student's t-test).

C FACS quantification of bBSC (basal BSCs) and sbBSC (suprabasal BSCs) of control (Ctr) and Bcl-2^{EKO} mice (n=4 biological replicates; mean ± standard deviation (SD); ns=not significant, two-tailed unpaired Student's t-test).

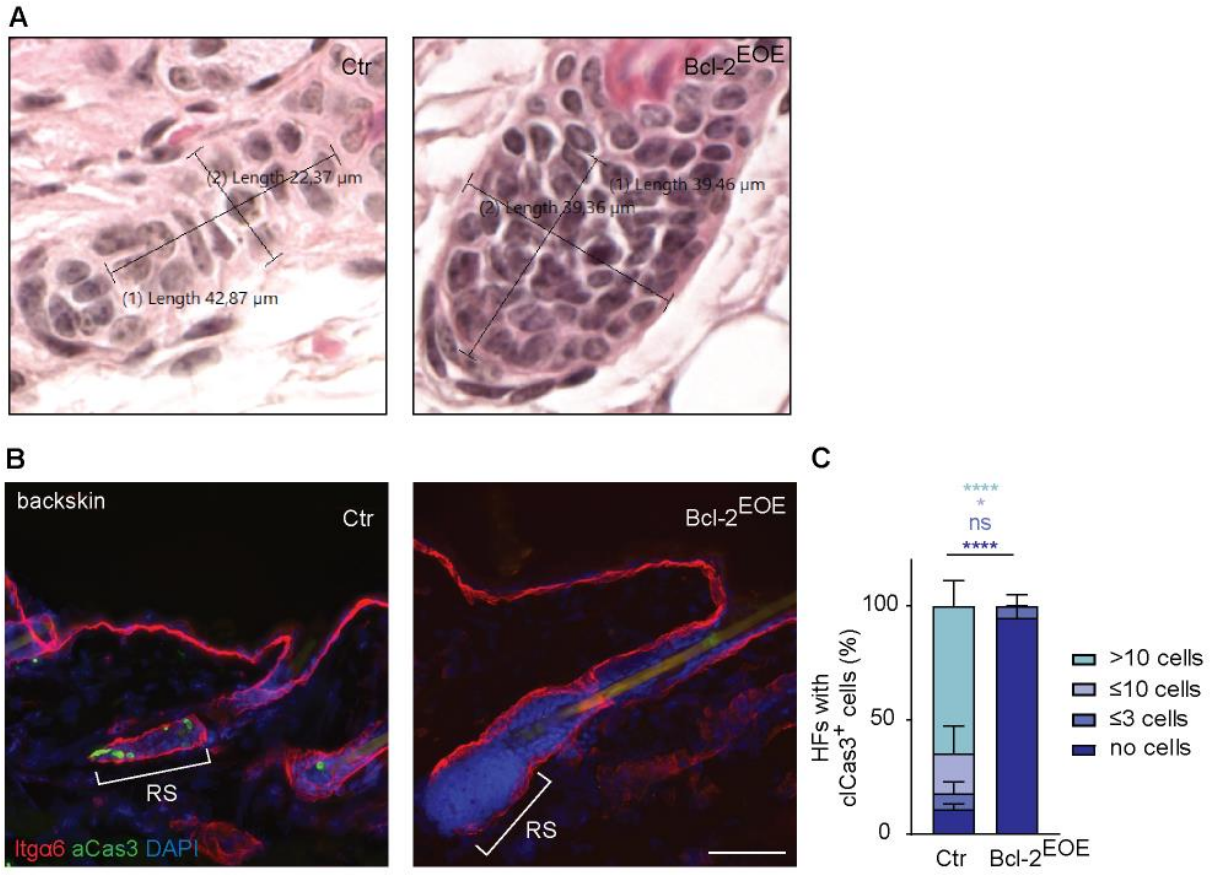


Appendix Figure S4: Genetic Bcl-2 overexpression in epidermal compartments.
A Experimental strategy generating Bcl-2^{EOE} mice.
B qRT-PCR for mRNA expression of hBcl-2 in epidermis of telogen control (Ctr) and Bcl-2^{EOE} mice (n=4 biological replicates; mean ± standard error of mean (SEM); ns=not significant, two-tailed unpaired Student's t-test).
C Immunofluorescence staining of telogen backskin sections backskin sections for Bcl-2 (green) and DAPI (blue) in control (Ctr) and Bcl-2^{EOE} mice. B: bulge Scale bar 100 µm.
D,E Immunofluorescence staining of telogen backsckin sections **(D)** and tail whole mounts **(E)** for GFP (green) and DAPI (blue) in control (Ctr) and Bcl-2^{EOE} mice. Dashed lines and SG: sebaceous glands; B: bulge; HG: hair germ; IFE: interfollicular epidermis. Scale bar 100 µm.



Appendix Figure S5: Abnormal bulge Bcl-2^{EOE} hair follicles do not express hair keratins.

A-D Immunofluorescence staining for hair keratins K71 **(A)**, K75 **(B)**, K72 **(C)**, K85 **(D)** (all in green) and DAPI (blue) in anagen control (ctr) hair follicles of wild-type mice and Bcl-2^{EOE} telogen hair follicles. Scale bars 100 µm.



Appendix Figure S6: Bcl-2 blocks apoptosis during hair follicle regression.

A High magnification of hair follicles demonstrating measurements of length and width of the retracting strand in catagen (P19) from control (Ctr, left) and Bcl-2^{EOE} mice (right) using Olympus software.

B Immunofluorescence staining for integrin a6 (Itgα6, red), activated caspase 3 (aCas3, green) and DAPI (blue) of catagen backskin hair follicles of control (Ctr) and Bcl-2^{EOE} mice. RS: retracting strand. Scale bar, 50 µm

C Quantification of backskin hair follicles with aCas3^{+} (n=3 biological replicates; mean ± standard deviation (SD); **** p<0.0001, ns=not significant, two-tailed unpaired Student's t-test).

Target	Sequence (5'→3')
Bcl-xL	For: GGAGAACGGCGGCTGGGATA Rev: GGCCACAGTCATGCCCGTCA
Bim	For: CGGATCGGAGACGAGTTCA Rev: TTCCAGCCTCGCGGTAATCA
Bmf	For: CTGGCATCACAACTCGGA Rev: CCTCTGACTGGAACACATC
Bmp6	For: GAGCATCAGCACAGAGAC Rev: TTCCAGCCAACCTTCTTC
CD34	For: GGACAGCAGTAAGACCACACCAGC Rev: AGGCAGTATGCCAGTTGGGGA
Fgf18	For: CCCAGGACTTGAATGTGCTT Rev: ACTGCTGTGCTTCCAGGTTC
Foxc1	For: CACTCGGTGCGGGAAATGT Rev: GTGCGGTACAGAGACTGACTG
Foxp1	For: AACAGCAGCAGCAACAAC Rev: TCATCATAGCCACTGACACG
K14	For: GCAGCAGAACCAGGAGTA Rev: GAGAGGATGAGGAGAATTGG
K24	For: TCCTTTCAGCCACTTGTG Rev: TCTCGAAGGCAGAGTTCA
Lef1	For: CGCCACCGATGGAGATGA Rev: CTAAGTCGCCCTCCTCTTC
Lgr6	For: GTATGAACAACCTCACGGAGC Rev: TTGGAGGCCAGAGAATGCC
Lrig1	For: TGTTTCCCGAACGGCCTGCGTA Rev: TGCTCAGACGGAGAGTCAGCAGTG
Nfatc1	For: AACGCCCTGACCACCGATAGCACT Rev: CCCGGGTGCCTTCCGTCTCATA
P-Cad	For: ATCGTGGGAGGTGATGATGG Rev: GTGTTGGTCCTGAGCCTCAA
Runx1	For: CTTCCTCTGCTCCGTGCTA Rev: TCTCGCTACCTGGTTCTTCAT
Runx2	For: CAAGTAGCCAGGTTCAAC Rev: TAGCTCTGTGGTAAGTGG
18S	For: ATCAGATACCGTCGTAGT Rev: CTTTAAGTTTCAGCTTTGC

Appendix Table S1: Primers for qRT-PCR analysis