Supplemental information

Sox9 regulates melanocytic fate decision

of adult hair follicle stem cells

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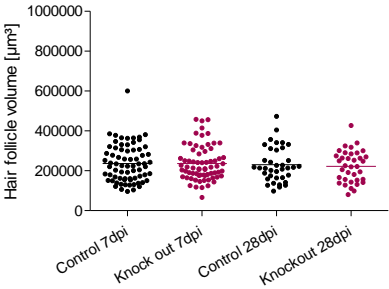
Supplemental figures

## *Figure S1: Volume of analyzed hair follicles, related to Figure 1 to 5.*

Quantification of the hair follicle volume, defined by the hair follicle area multiplied with the number of z-stacks of the microscope image, in Sox9KO and Ctrl mice showing a consistent size of analysed hair follicle volumes; n = 4 mice per group; one dot represents one hair follicle.

## Figure S2: Volume of analyzed interfollicular areas, related to Figure 5.
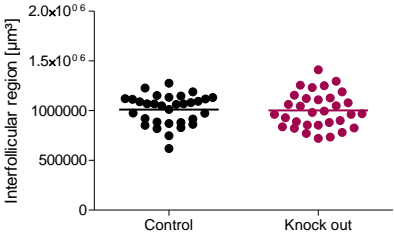
Quantification of the volume of the analysed interfollicular regions, defined by the interfollicular area multiplied with the number of z-stacks of the microscope image, in Sox9KO and Ctrl mice showing a consistent size of analysed interfollicular regions; n = 4 mice per group; one dot represents one interfollicular area.

Figure S3: Distribution of Nestin⁺/GFP⁺ cells in the HF, related to Figure 4.
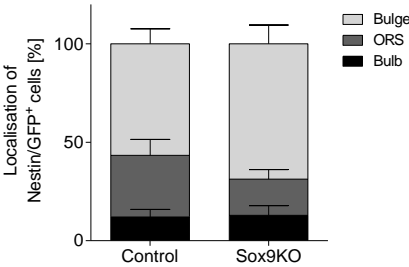

Quantitative analysis of the distribution of Nestin⁺/GFP⁺ cells through the HF in Sox9KO mice compared to Ctrl mice 7 dpi; n = 4 mice, 5-10 HF per mice; data are presented as mean ± SEM.

Figure S4: DCT expression level in cultivated melanocytes remains stable upon Sox9 inactivation, related to Figure 4.
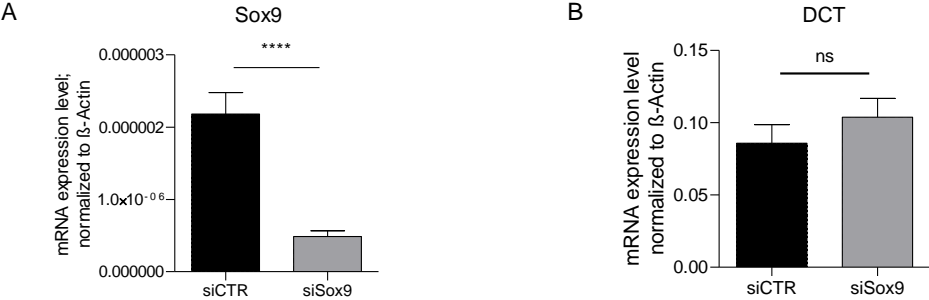
Sox9 (A) and DCT (B) mRNA expression levels normalized to β-Actin, measured with qRT-PCR after 96 h of transfection with siRNA targeting Sox9, compared to a control siRNA. Data are represented as mean ± SEM of n = 3.

Figure S5: DCX expression in mice tail skin, related to Figure 5.
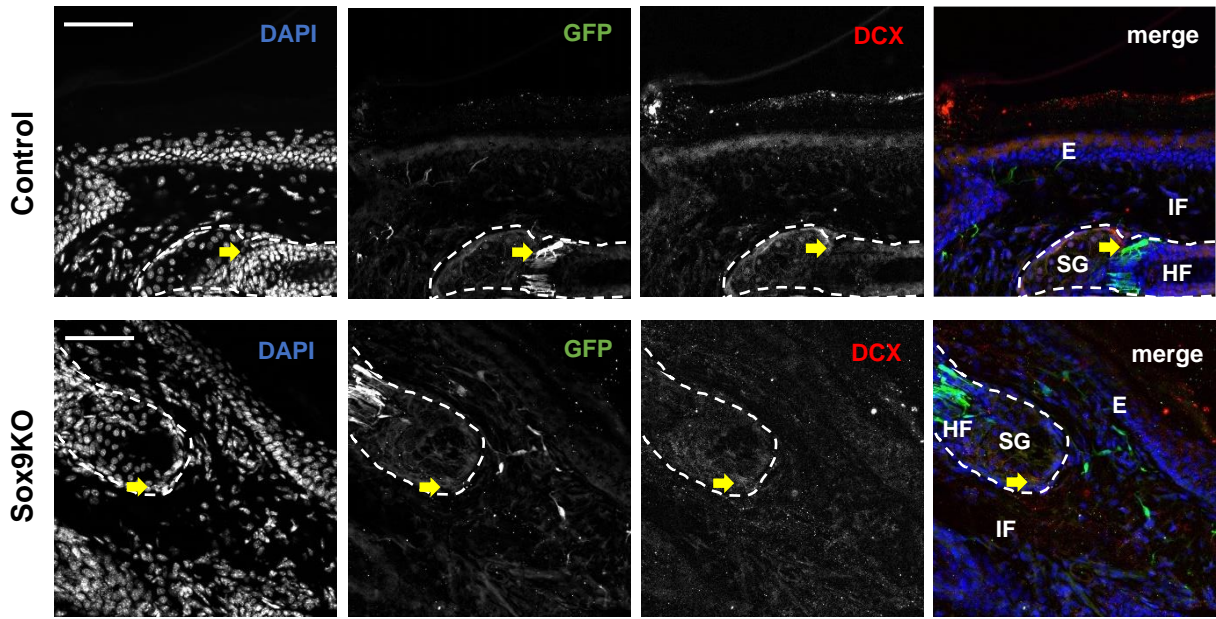
Immunohistochemical staining of GFP (green) and DCX (red) in the tail skin of Sox9KO mice and Ctrl mice 28 dpi; dashed line marks the hair follicle (HF); E = Epidermis, IF = Interfollicular area, SG = Sebaceous gland; n = 1 mouse, yellow arrows point towards DCX$^+$/GFP$^+$ cells; DAPI is depicted in blue (scale bar = 50 μm).

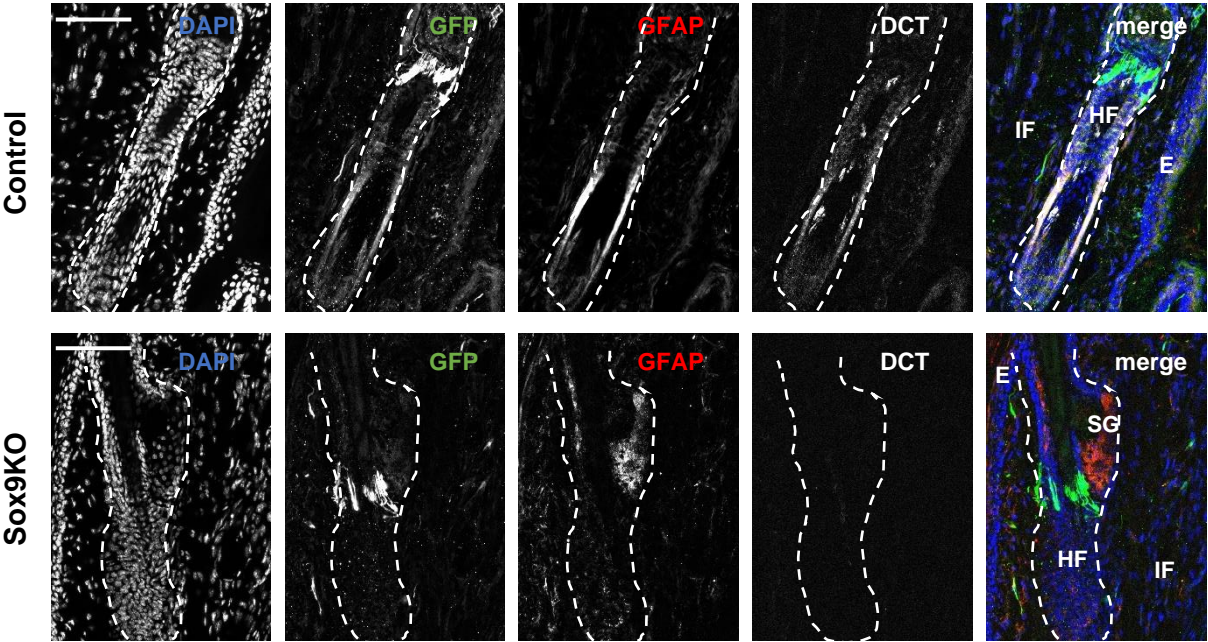**Figure S6: ß-III-Tublin expression in mice tail skin, related to Figure 5.**
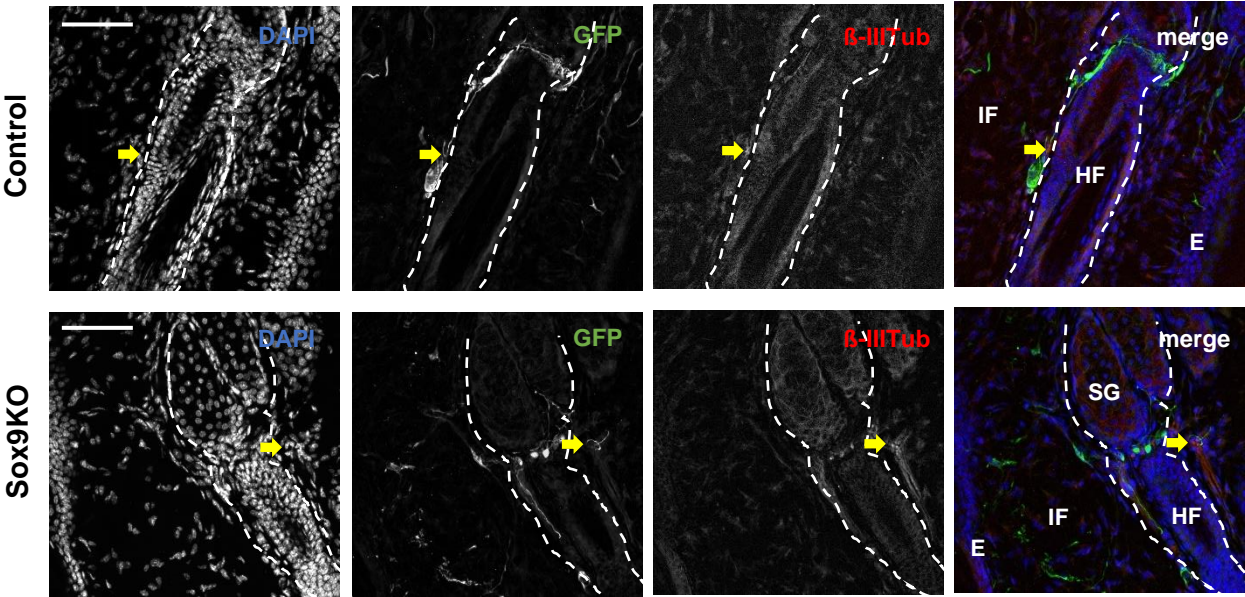
Immunohistochemical staining of GFP (green) and Beta-III-Tubulin (red) in the tail skin of Sox9KO mice and Ctrl mice 28 dpi; dashed line marks the hair follicle (HF); E = Epidermis, IF = Interfollicular area, SG = Sebaceous gland; n = 1 mouse, yellow arrows point towards Beta-III-Tubulin[+]/GFP^{+} cells; DAPI is depicted in blue (scale bar = 50 μm).

Figure S7: GFAP expression in mice tail skin, related to Figure 5.

Immunohistochemical staining of GFP (green), GFAP (red) and DCT (white) in the tail skin of Sox9KO mice and Ctrl mice 28 dpi; dashed line marks the hair follicle (HF); E = Epidermis, IF = Interfollicular area, SG = Sebaceous gland; DAPI is depicted in blue (scale bar = 50 μm).

Figure S8: Sox10 expression in GFP+ cells in the IF, related to Figure 5.
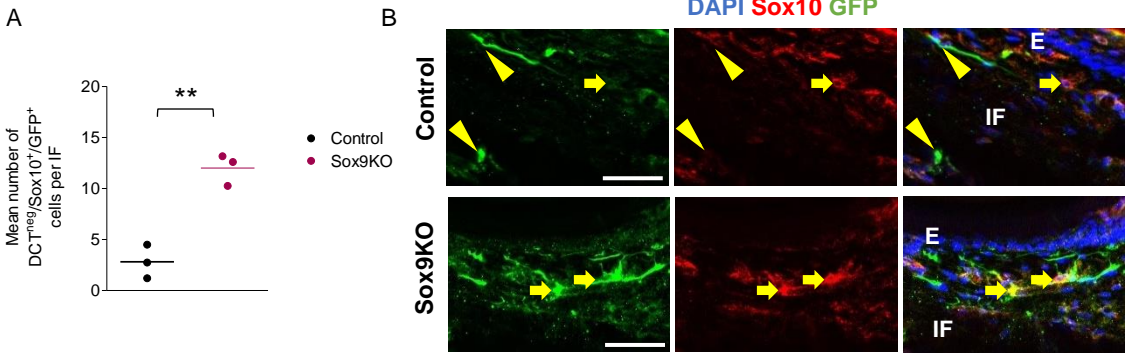
(A) Quantification of the mean cell number of DCT^{neg}/Sox10$^+$/GFP$^+$ cells per IF in Sox9KO mice and Ctrl mice 28 dpi; n = 3 mice per group; 4 IF per mice; data are presented as mean ± SEM. (B) Immunohistochemical staining of GFP (green) and Sox10 (red) in the tail skin of Sox9KO mice and Ctrl mice 28 dpi; DAPI is depicted in blue (scale bar = 30 µm).

Supplemental tables

Table S1: Mean ratio of cells expressing specific marker over a specific cell population, related to Figure 1 to 6.

Region	Timepoint dpi; Genotype	Marker protein	Mean of double positive cells over all GFP+cells (±SEM) [%]	Mean of triple positive cells over all Nestin+/GFP+ cells (±SEM) [%]	Mean of triple positive cells over all DCT+/GFP+ cells (±SEM) [%]	Number of analysed HF per mice (n=4 mice)
HF	7; Ctrl	Sox9+	47.0 (± 5.0)	46.7 (± 6.9)	56.2 (± 6.5)	5-10
HF	7; Sox9KO	Sox9+	18.7 (± 1.4)	-	-	8
HF	7; Ctrl	DCT+	-	45.2 (± 6.5)	-	5-8
HF	7; Sox9KO	DCT+	-	10.0 (± 2.0)	-	6-11
HF	7; Ctrl	DCT-	-	54.8 (± 6.5)	-	5-8
HF	7; Sox9KO	DCT-	-	90.0 (± 2.0)	-	6-11

Quantitative immunohistochemical analysis of adult Nestin-CreER[T2]; GFP mice (Ctrl) and Nestin-CreER[T2]; Sox9^{fl/fl}; GFP mice (Sox9KO). Values in the table represent the mean number of double/triple positive cells over the analysed cell population (GFP^{+} cells, Nestin[+]/GFP^{+} cells, DCT[+]/GFP^{+} cells) at 7 dpi in the hair follicle (HF). Cells were counted on 12-18 µm thick z-stack images and on 4-6 sections for n = 4 mice (in a specific number of hair follicles per mice).

Table S2: Raw cDNA-Array data (log2signal) of Sox9 and β-Actin (ACTB) in melanoblast-related cells (MB) and normal human epidermal melanocytes (NHEM), n=3, related to Figure 3.

Gene Symbol	01_A_NHEM_RMA_log2Signal	02_A_MB_RMA_log2Signal	03_B_NHEM_RMA_log2Signal	04_B_MB_RMA_log2Signal	05_C_NHEM_RMA_log2Signal	06_C_MB_RMA_log2Signal
SOX9	6,38	6,62	6,34	6,60	6,25	6,58
ACTB	12,81	12,67	12,81	12,55	12,84	12,72

Raw cDNA-Array data (Affymetrix cDNA-Array, log2signal) of Sox9, FoxD3 and β-Actin in melanoblast-related-cells (MB) and normal human epidermal melanocytes (NHEM). Parts of the cDNA-Array has been published previously.[1]

Table S3: Mean number of cells per HF/HF-region expressing a specific marker, related to Figure 1 to 6.

Region	Timepoint dpi; Genotype	Marker protein	Mean number of cells per HF (±SEM)	Number of analysed HF/IF per mice (n=4 mice)
HF	7; Ctrl	GFP+	63.1 (± 2.0)	20-23
HF	7; Sox9KO	GFP+	58.1 (± 2.7)	20-21
HF	7; Ctrl	Nestin+/GFP+	28.2 (± 2.6)	13
HF	7; Sox9KO	Nestin+/GFP+	32.3 (± 1.7)	12
HF	7; Ctrl	DCT+/GFP+	18.9 (± 3.8) (Bulge/ORS) 10.2 (± 0.8) (Bulb)	5-8
HF	7; Sox9KO	DCT+/GFP+	8.6 (± 1.3) (Bulge/ORS) 6.1 (± 0.9) (Bulb)	6-12
HF	7; Ctrl	DCT+/Nestin+/GFP+	6.5 (± 0.9) (Bulge)	5-8
HF	7; Sox9KO	DCT+/Nestin+/GFP+	1.9 (± 0.5) (Bulge)	6-12
HF	7; Ctrl	DCT+	68.3 (± 5.3)	12-15
HF	7; Sox9KO	DCT+	42.9 (± 6.0)	12
HF	7; Ctrl	Ki67+/GFP+	18.5 (± 2.7)	8
HF	7; Sox9KO	Ki67+/GFP+	18.1 (± 1.5)	8
HF	7; Ctrl	Ki67+/Nestin+/GFP+	1.6 (± 0.3) (Bulge)	4-8
HF	7; Sox9KO	Ki67+/Nestin+/GFP+	4.8 (± 0.4) (Bulge)	4-12
HF	7; Ctrl	Ki67+/DCT+/GFP+	13.3 (± 2.4)	6-12
HF	7; Sox9KO	Ki67+/DCT+/GFP+	5.9 (± 1.5)	6-12
HF	7; Ctrl	DCT-/Nestin+/GFP+	8.0 (± 1.3) (Bulge)	5-8

HF	7; Sox9KO	DCT-/Nestin+/GFP+	16.3 (± 1.5) (Bulge)	6-11
IF	28; Ctrl	GFP+	19.4 (± 1.8)	4
IF	28; Sox9KO	GFP+	31.0 (± 2.9)	4
IF	28; Ctrl	DCT-/GFAP+/GFP+	8.4 (± 0.9)	4
IF	28; Sox9KO	DCT-/GFAP+/GFP+	16.3 (± 1.6)	4

Quantitative immunohistochemical analysis of adult Nestin-CreER^{T2}; GFP mice (Ctrl) and Nestin-CreER^{T2}; Sox9$^{fl/fl}$; GFP mice (Sox9KO). Values in the table represent the mean number of single/double/triple positive cells per hair follicle (HF) or interfollicular region (IF) at 7 or 28 dpi. Cells were counted on 12-18 μm thick z-stack images and on 4-6 sections for n = 4 mice (in a specific number of hair follicles/IF areas per mice).

References

1.	Linck-Paulus, L., L. Lammerhirt, D. Voller, K. Meyer, J.C. Engelmann, R. Spang, N. Eichner, G. Meister, S. Kuphal, and A.K. Bosserhoff. (2021). Learning from Embryogenesis-A Comparative Expression Analysis in Melanoblast Differentiation and Tumorigenesis Reveals miRNAs Driving Melanoma Development. *J Clin Med*. 10(11), 10.3390/jcm10112259.