SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLES

Table S1. Information on crow individuals used in the study
(Note: CC: *C.* (*corone*) *corone;* HC: *C.* (*corone*) *cornix*; T: torso, H: head; T-500: cross section at 500 µm above the dermal papilla; T-1000: cross section at 1000 µm above the dermal papilla; Ko: Konstanz, Germany; Up: Uppsala, Sweden; NA: not available)

individual	species	country	sex	sampling date	age [days] at sampling	nest	longitude	latitude	cross section analyses	longitudinal analyses	feather collecting	ringNr or altLabel
D_Ko_C27	CC	Germany	female	6.5.2014	19	D_Ko2014_N08	47.6702	9.1751	T-1000, T-500	H, T	8.2015, 8.2016	HF77356
D_Ko_C29	CC	Germany	female	6.5.2014	14	D_Ko2014_N04	47.7123	9.0835	H-1000		8.2015	HF77358
D_Ko_C31	CC	Germany	male	6.5.2014	21	D_Ko2014_N03	47.7217	9.0698	T-1000, T-500, H-1000	H, T	8.2015	HF77360
D_Ko_C36	CC	Germany	male	7.5.2014	16	D_Ko2014_N35	47.6673	9.2167	T-1000, T-500	H, T	8.2015	HF77365
D_Ko_C40	CC	Germany	male	7.5.2014	19	D_Ko2014_N36	47.6660	9.2138	H-1000, H-500		8.2015	HF77369
D_Ko_C58	CC	Germany	female	9.5.2014	19	D_Ko2014_N17	47.6810	9.1738	H-500		8.2015	HF77388
D_Ko_C59	CC	Germany	female	9.5.2014	21	D_Ko2014_N18	47.6808	9.1758	H-500		9.2015	HF77389
S_Up_H59	HC	Sweden	male	20.5.2014	<30	S_Up2014_N16	17.5091	59.9498	H-500		8.2015, 8.2016	80043805
S_Up_H60	HC	Sweden	female	20.5.2014	<30	S_Up2014_N02	17.5643	59.8916	T-1000, T-500, H-1000	H, T	8.2015	80043804
S_Up_H65	HC	Sweden	female	22.5.2014	<30	S_Up2014_N18	17.5324	59.9087	T-1000, T-500, H-500	H, T	8.2015	80043811
S_Up_H69	HC	Sweden	female	25.5.2014	25	S_Up2014_N25	17.6695	60.0341	T-1000, T-500, H-1000	H, T	8.2015	80043810
S_Up_H77	HC	Sweden	male	25.5.2014	23	S_Up2014_N06	17.5936	59.9137	H-1000		8.2015	80043813

SUPPLEMENTARY FIGURES

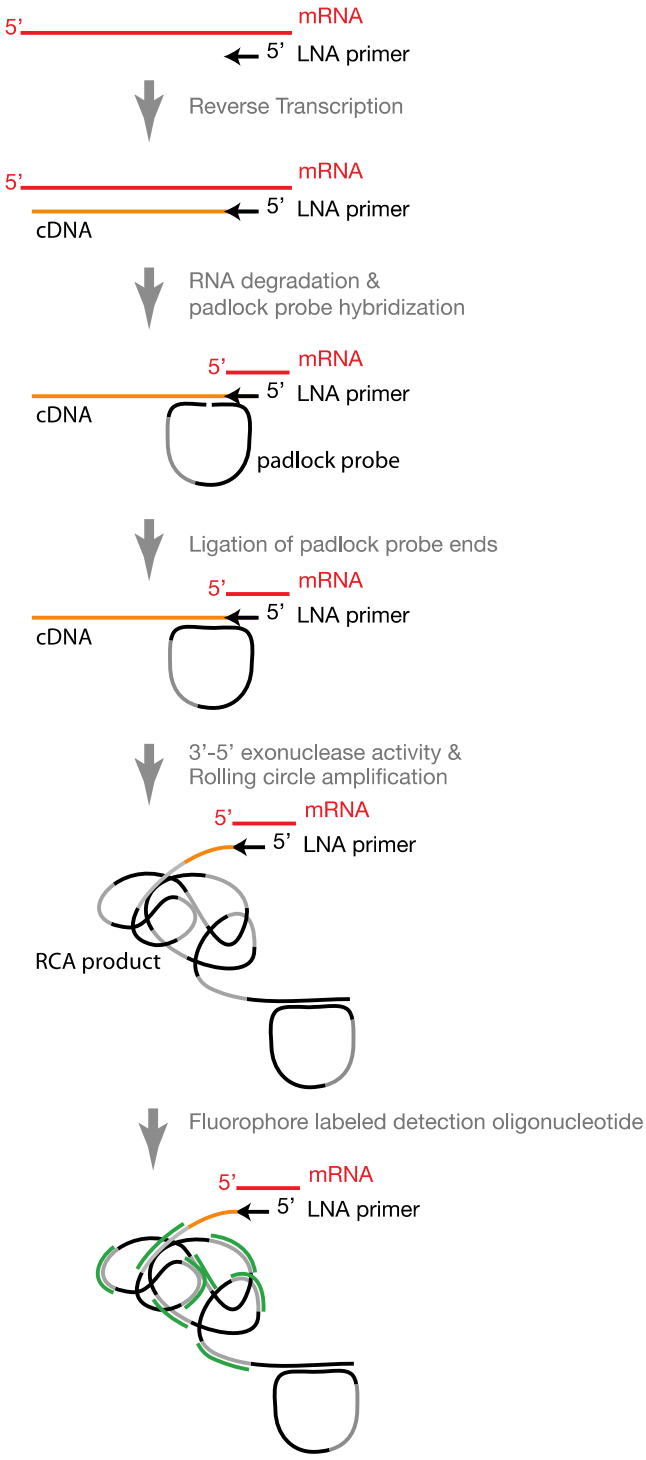

Figure S1: Schematic representation of the major processes of *in situ* mRNA quantification via padlock probe and rolling circle amplification. LNA primer (arrow in black): locked nucleic acid primer; strand in red: targeted native mRNA molecule; strand in orange: cDNA molecule; segment in grey: DNA region for the hybridization of fluorophore labeled detection oligonucleotides; strand in green: fluorophore labeled detection oligonucleotide.

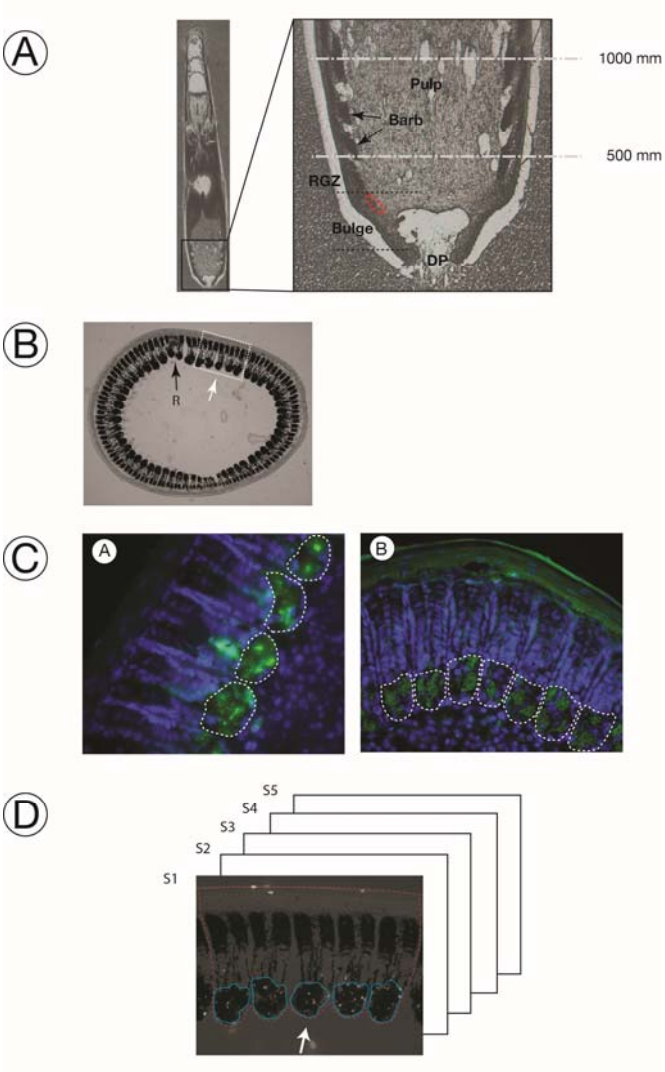

Figure S2: Description of the data set for mRNA *in situ* quantification
A) Longitudinal section of a carrion crow torso follicle indicating the location of cross sections at 500 µm and 1000 µm from the proximal end. DP: dermal papilla; RGZ: ramogenic zone; Dashed-line ellipse in red: collared bulge (CB). **B)** Example of a cross section indicating the location of five consecutive barb ridges (in white dashed-line square) next to the rachis used for *in situ* mRNA quantification (also see panel D). R: rachis. **C)** Histological definition of the barb ridge growth zone consisting predominantly of melanocytes (dotted lines). Images A and B represent cross-sections at 500 µm and 1000 µm from the distal end respectively. Melanocytes are visualized by immunostaining against the TYRP1 protein in green; nuclei stained with Hoechst 33342 are shown in blue. **D)** Example of five serial cross sections at 1000 µm above DP with *in situ* stained mRNA of the TYRP1 gene (white dots) forming the basis for melanocyte-specific mRNA quantification and statistical inference. The area enclosed in a red dashed line is for quantifying representing overall expression of mRNA transcripts on the given section (except cells in the pulp). The area on a given section for quantifying melanocyte-specific mRNAs is enclosed with light-blue line. The white arrow indicates the middle of selected five consecutive barb ridges as a reference across five sequential sections.

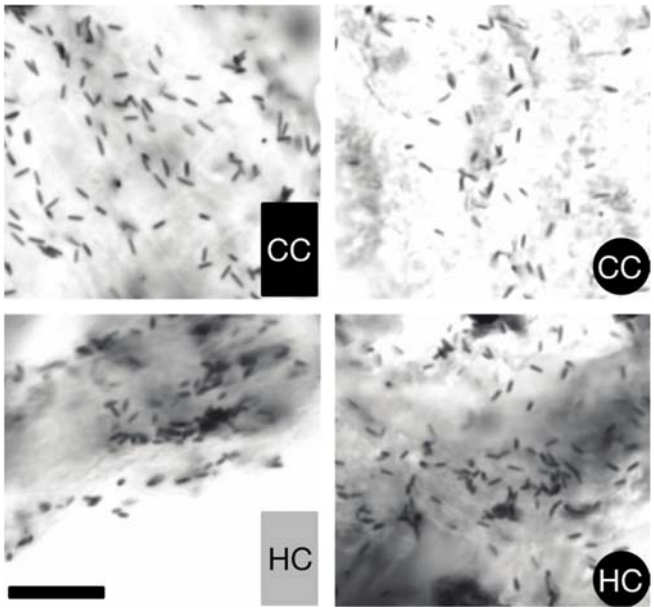

Figure S3: Images of separated melanosomes from barbule cells of carrion crow (CC) and hooded crow (HC) head (right) and torso (left). Each black, rod-shaped structure represents a mature melanosome packed with eumelanin. Symbol shape indicates sampling location on the body (circle: head, rectangle: torso); symbol colour imitates pigmentation intensity of the mature feather.; bar = 10 μm.

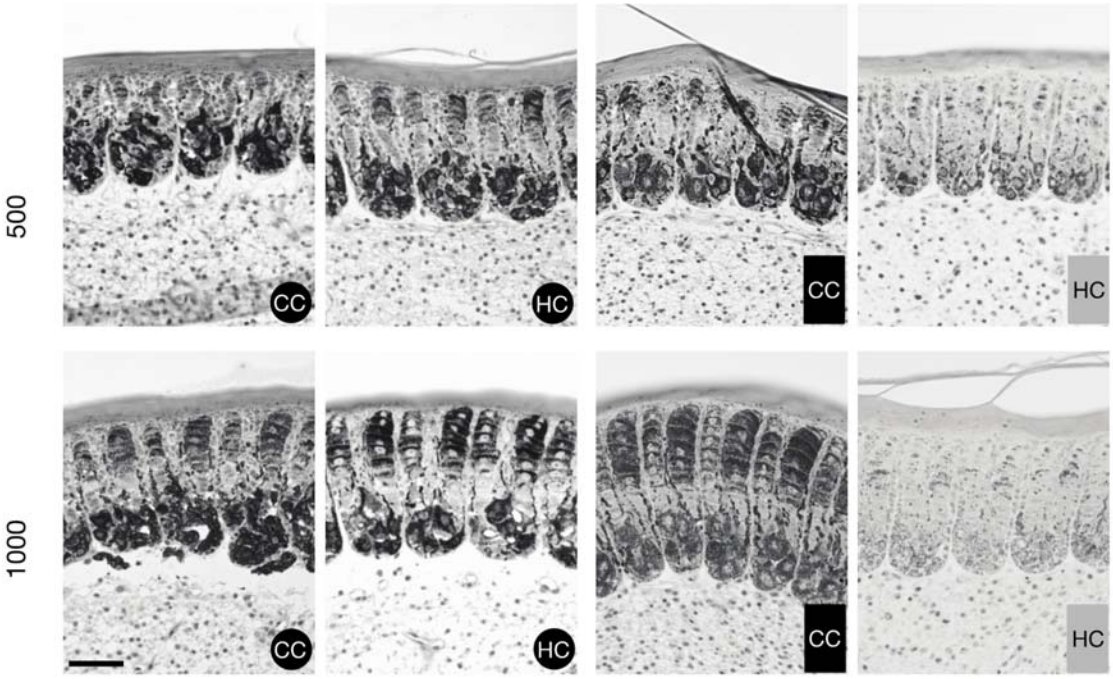

Figure S4: Cross-section images from ensheathed feather follicles at 500 µm (upper row) and 1000 µm (lower row) above the dermal papilla of carrion crows (CC) and hooded crows (HC). Shown are examples of the forth or fifth barb ridge (in the middle) posterior from the rachis. Follicles were sampled from the head region and on the ventral part of the torso. Symbol shape indicates sampling location on the body (circle: head, rectangle: torso); symbol colour imitates pigmentation intensity of the mature feather; bar = 50 µm.

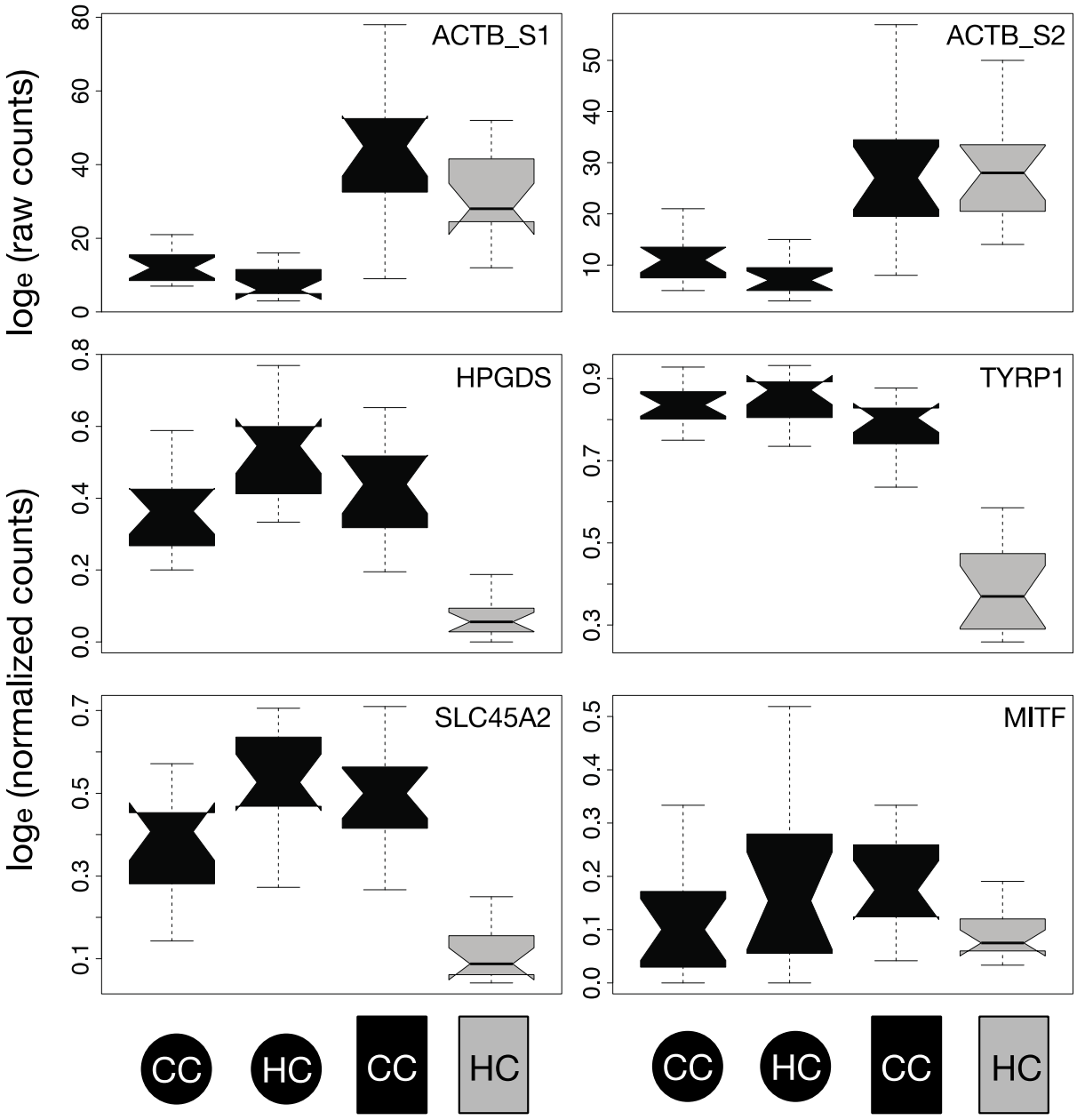

Figure S5: Notched boxplot of raw counts (ACTB gene) or normalized *in situ* RCA signals of targeted mRNA transcripts (HPGDS, SLC45A2, TYRP1, MITF) in melanocytes at 1000 µm above the dermal papilla of head and torso in carrion crow (CC) and hooded crow (HC). Symbol shape indicates sampling location on the body (circle: head, rectangle: torso); symbol colour imitates pigmentation intensity of the mature feather. (n = 30)

SUPPLEMENTARY MOVIES

Movie S1: Three-dimensional reconstruction of melanocytes, presenting in the ventral (top) to dorsal (bottom) direction. Each object of the same color represents a melanocyte. Spherical objects in yellow within melanocytes represent their nuclei. Major portion of a melanocyte where its nucleus locates is within the growth zoon of barb ridges, and dendritic cytoplasm extends toward down to barbule plate (not shown).

Movie S2: A whole-mounted barb of carrion crows (CC) in PBS showing dendritic cytoplasm filled with black melanosome of eumelanin connecting from ramus (on the left) to barbule plate (on the right).