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Epidermal melanocytes of segmental vitiligo show altered expression of E-cadherin, but not P-cadherin

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DEAR EDITOR : Vitiligo is the most common pigmentation disorder with a worldwide prevalence of 1%. The loss of melanocytes from the skin is the main clinical feature of patients with vitiligo, resulting in depigmentation macules¹. Vitiligo has been classified into two major forms: non-segmental vitiligo (NSV; accounting for approximately two thirds of overall vitiligo cases) and segmental vitiligo (SV, accounting for the rest)^{2, 3}. NSV lesions are generally bilateral or symmetrically scattered over the entire body. Onset may occur at any age, but most patients develop vitiligo before 40 years of age, and the depigmentation evolves over time². SV is characterized by its unilateral distribution, early onset, and rapid stabilisation⁴. The etiopathogenesis of NSV and SV has not been fully elucidated and it is possible that the two subtypes share common mechanisms for initiation of the disease, but diverge in the progression phase, as depigmentation rapidly stabilizes in SV, but not NSV. There is much data to support an autoimmune phase in the extension of vitiligo and mechanical and chemical trauma are known environmental stressors that accelerate disease expression. However, the events that initiate the loss of melanocytes are still a subject of debate.

We aim to explore cell adhesion defects in melanocytes before clinical lesions appear as an indicator of early events in pathogenesis of vitiligo. We have recently shown that E-cadherin, which mediates the adhesion between melanocytes and keratinocytes in the epidermis, is discontinuously/heterogeneously distributed within (or lost from) melanocyte membranes of NSV patients⁵. This alteration of E-cadherin distribution on melanocytes is observed in biopsies distant from the depigmentation macules, before clinical lesions appear, indicating that the alteration of E-cadherin distribution is an early event in the pathogenesis of vitiligo. Furthermore, we have shown that this primary defect that affects adhesion, combined with stress conditions, leads to the disappearance of melanocytes in human epidermal reconstructed skin and mouse models⁶. Here, we assessed whether alteration of E-cadherin is a common first step in the various subtypes of vitiligo by studying the distribution of E-cadherin on epidermal melanocytes of frozen skin biopsies from SV patients (taken from clinically normal looking skin) (see supplementary Table 1 for patient characteristics).

Briefly, we classified each melanocyte based on their E-cadherin labelling (BD Bioscience #610182) by immunochemistry. Type 1 melanocytes correspond to an homogenous distribution of E-cadherin, whereas Type 2 melanocytes correspond to heterogeneous distribution or lack of E-cadherin. We counted and analysed at least 50 melanocytes (TRP-2 shown in red, was used a marker for melanocytes; Santa Cruz SC-10451) per patient (and controls) for each staining (Fig. S1). In control patients, E-cadherin (shown in green) showed type 1 homogenous staining in 78% of melanocytes (Fig. 1a [panel 1]) with the 22% remaining cells showing type II staining (Fig. 1b). We observed a similar pattern of distribution at the melanocyte membrane for β -catenin (BD Bioscience #610154), a major cytoplasmic partner of E-cadherin (Fig. 1a [panel 2], Fig. 1b). The average percentages of the two types of stained melanocytes for E-cadherin and β -catenin are shown in Fig. 1b and Fig. S2. In contrast, melanocytes from SV patients showed only 43% and 46% type I staining for E-cadherin and β -catenin, with the remaining cells showing heterogenous or absent E-cadherin and β -catenin type II staining (Fig. 1a [panels 7,8] and Fig. 1b). There was no statistical difference for E-cadherin and β -catenin staining between the two vitiligo subtypes, NSV and SV (Fig. 1a [panels 4,5,7,8] and Fig. 1b). However, E-cadherin and β -catenin staining of melanocytes from control subjects was significantly different from that of NSV or SV patients (Fig. 1b, and Fig. S2). Thus, our results demonstrate that melanocytes of both vitiligo subtypes, SV and NSV, share the same altered distribution pattern for E-cadherin and β -catenin, which is different from that of melanocytes of healthy subjects.

P-cadherin is the other type 1 cadherin expressed in melanocytes and located at the same E-cadherin locus in human and various mammals^{7,8}. The different expression pattern of E-cadherin (in the entire epidermis) and P-cadherin (only in the basal layer) (BD Bioscience #610228) is shown in Fig. S3. The modification of expression of E-cadherin could have some cis influence on the expression of P-cadherin. However we found that P-cadherin was homogeneously distributed throughout the plasma membrane of melanocytes (Fig. 1a [panels 3,6,9] and Fig. 1b) for controls as well as NSV and SV patients (91%, 86%, and 91%, respectively). P-cadherin staining of melanocytes for each patient is shown in Fig. S1c, and the average percentages for the two staining types are shown in Fig. S2. In conclusion, P-cadherin expression remained the same for both vitiligo subtypes and controls, although this adhesion molecule is highly similar to E-cadherin.

In conclusion, the two vitiligo subtypes, NSV and SV, showed the same reduction in E-cadherin expression in melanocyte relative to normal pigmented skin. This finding indicates that the changes of cell-adhesion mediated by E-cadherin in melanocytes are identical in both NSV and SV. The loss (or reduction) of E-cadherin from melanocyte membranes is therefore an early event of the disease and is directly involved in the initiation process of all vitiligo subtypes. This favours the cell-adhesion defect theory, called melanocytorrhagy⁹, not only for the initiation step of NSV, as originally proposed, but also for the SV subtype. In addition, P-cadherin expression remained the same for both vitiligo subtypes and controls, although this adhesion molecule is highly similar to E-cadherin. This observation demonstrates that alteration of E-cadherin distribution is an intrinsic defect of this protein and not a general defect affecting other cell-cell adhesion molecules.

Acknowledgements

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Conflicts of interest : none declared

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Figure legends

Figure 1. Loss of E-cadherin and β -catenin, but not P-cadherin, in NSV and SV.

a) Immunofluorescence of controls (1,2,3), NSV (4,5,6), and SV (7,8,9) SV for E-cadherin (1,4,7), β -catenin (2,5,8), and P-cadherin (3,6,9). TRP-2 was used as melanocyte marker and is shown in red; E-cadherin, β -catenin, and P-cadherin stainings are in green, and DAPI in blue. The white dotted line indicates the basal membrane. Scale bar = 10 μ m.

b) Average percentages of controls, NSV, and SV melanocytes (horizontally) showing type I and II staining for E-cadherin, β -catenin, and P-cadherin (vertically). Ten biopsies for each condition (control, NSV and SV) were analyzed and detailed results and statistical analyses are shown in Fig. S1 and S2. Patients provided signed informed consent before the study, which was approved by the relevant local ethics committees (Bordeaux CNIL #1545937 v.0 and Rabat Hospitals).

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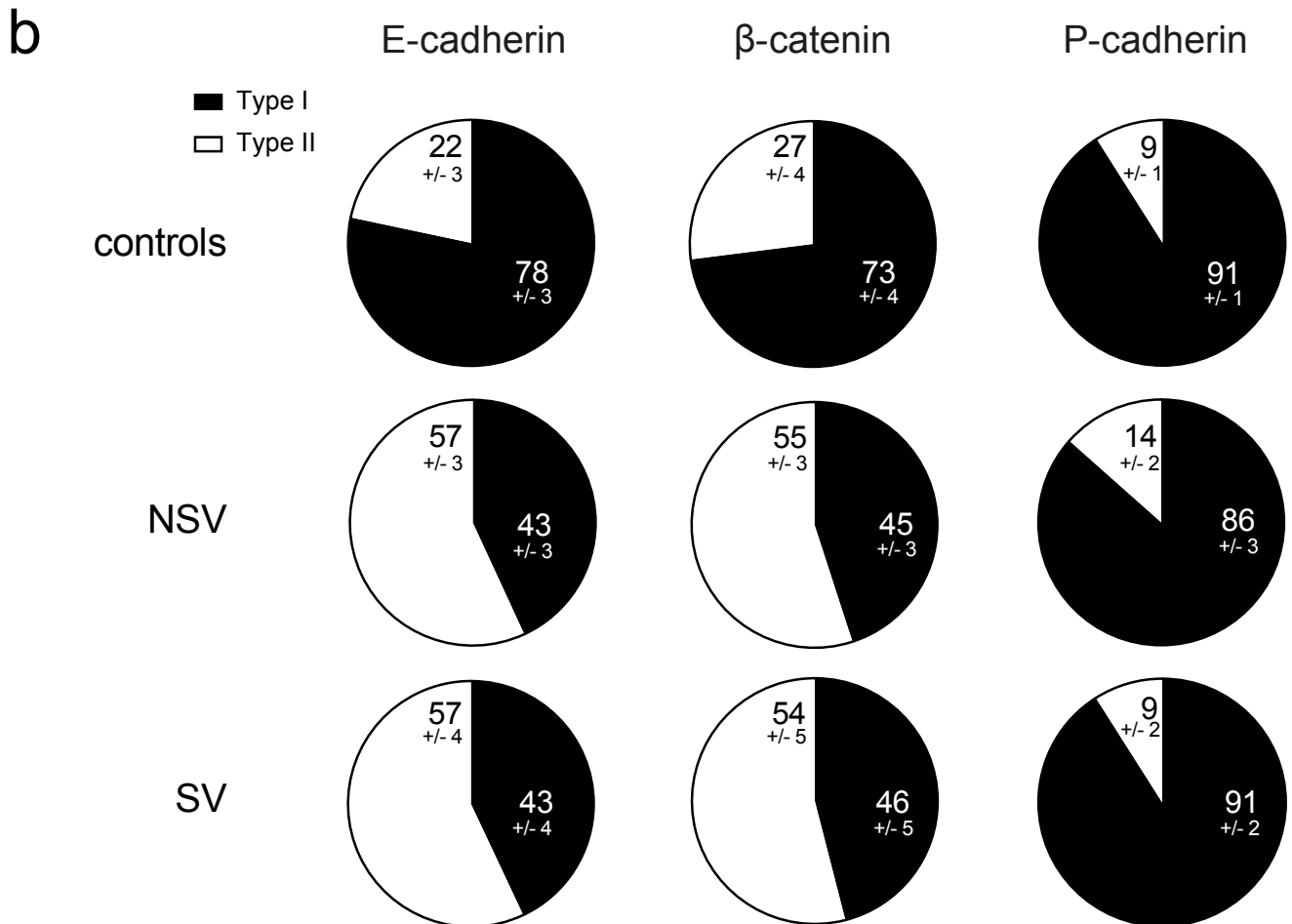
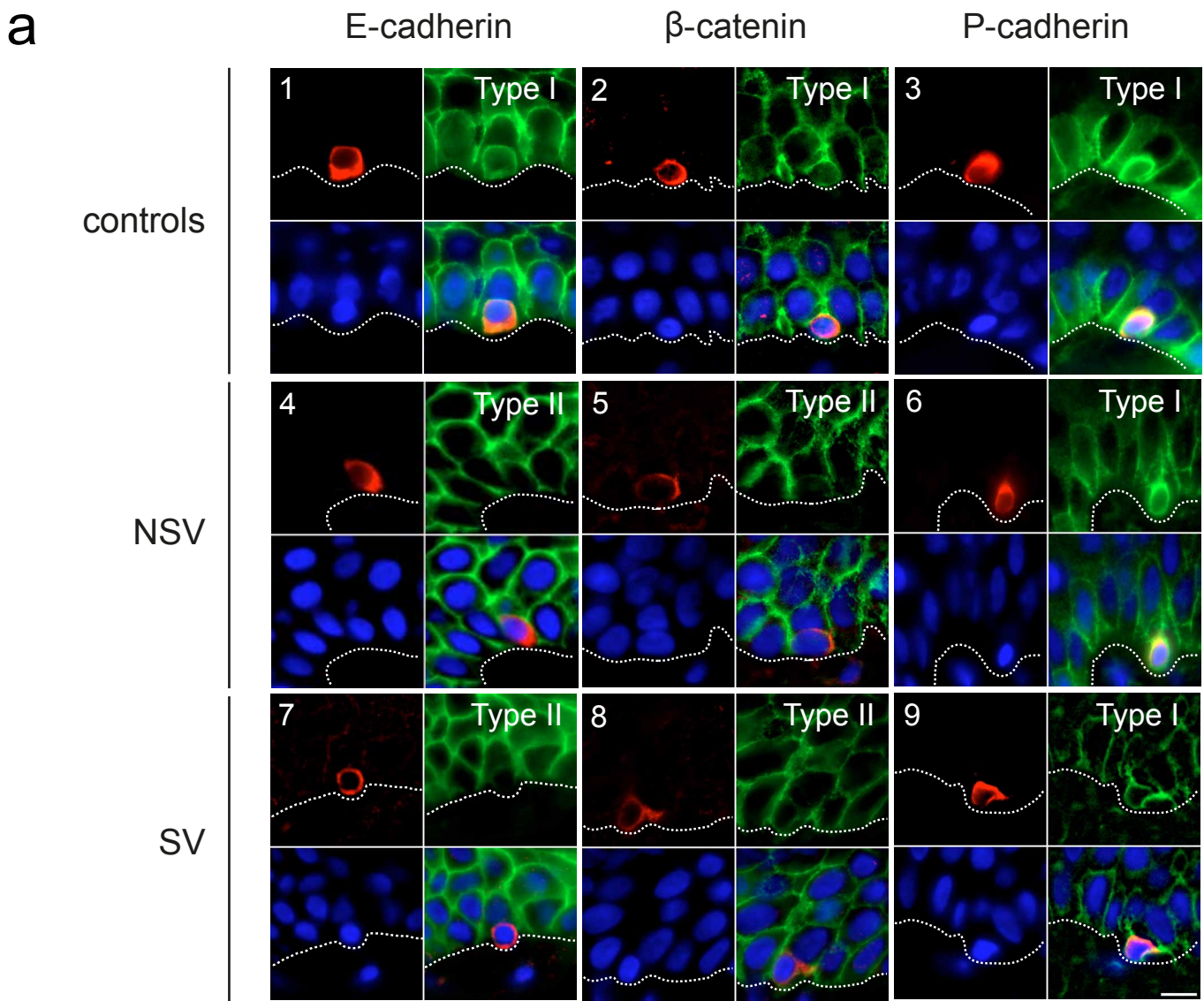
Supporting information

By Christine Grill¹⁻³, Benzekri Laila⁴, Alain Rubod¹⁻³, Khaled Ezzedine^{5,6}, Alain Taieb^{5,6}, Yvon Gauthier^{5,6}, Lionel Larue^{1-3*\$}, and Véronique Delmas^{1-3S}

Fig S1. E-cadherin, β -catenin, and P-cadherin in SV vitiligo. Average percentages, per patient, of controls (blue) and SV (orange) melanocytes showing type I and II staining of (a) E-cadherin, (b) β -catenin, and (c) P-cadherin. Each bar represents one individual. The black horizontal lines indicate the average of the melanocytes.

Fig S2. E-cadherin and β -catenin, but not P-cadherin, are altered in vitiligo. Average percentages of controls (blue), NSV (red), and SV (orange) melanocytes showing type I and II staining of E-cadherin, β -catenin, and P-cadherin. *** P -value $< 10^{-3}$, **** P -value $< 10^{-4}$, ns: non significant.

Fig S3. E- and P-cadherin staining of healthy skin. Immunofluorescence staining of healthy skin with: (a) TRP-2 (red, melanocyte-specific marker), E-cadherin (green), and DAPI (blue), (b) TRP-2 (red), P-cadherin (green) and DAPI (blue). The white dotted line indicates the basal membrane. Scale bar = 10 μ m.



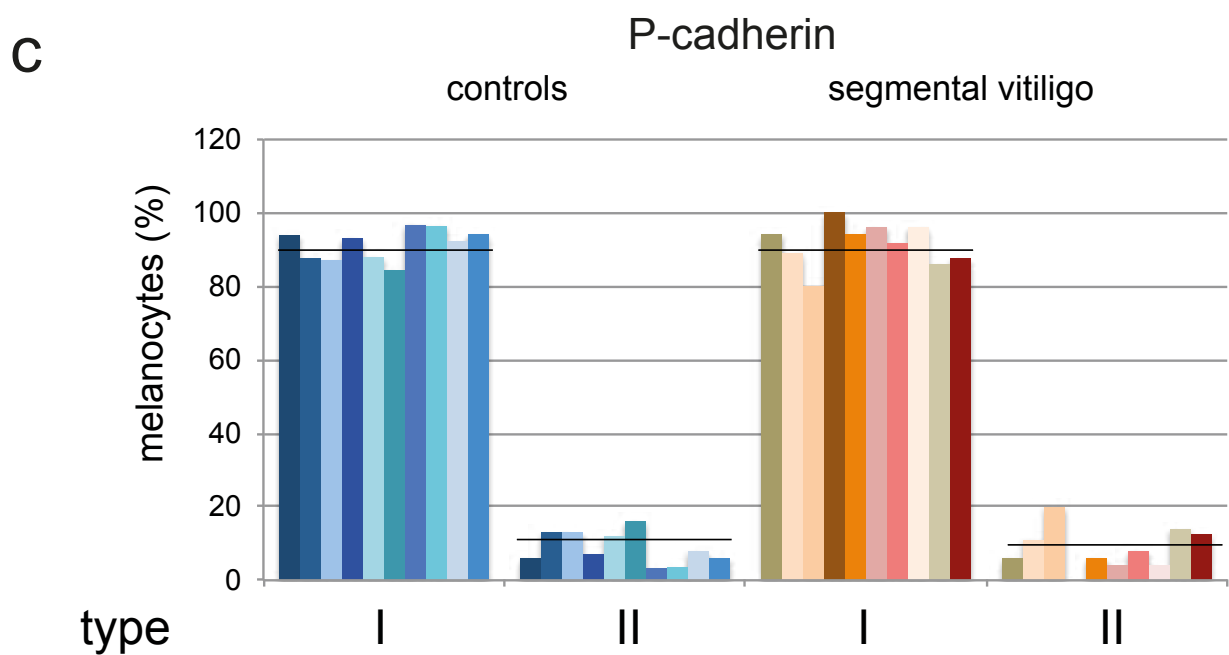
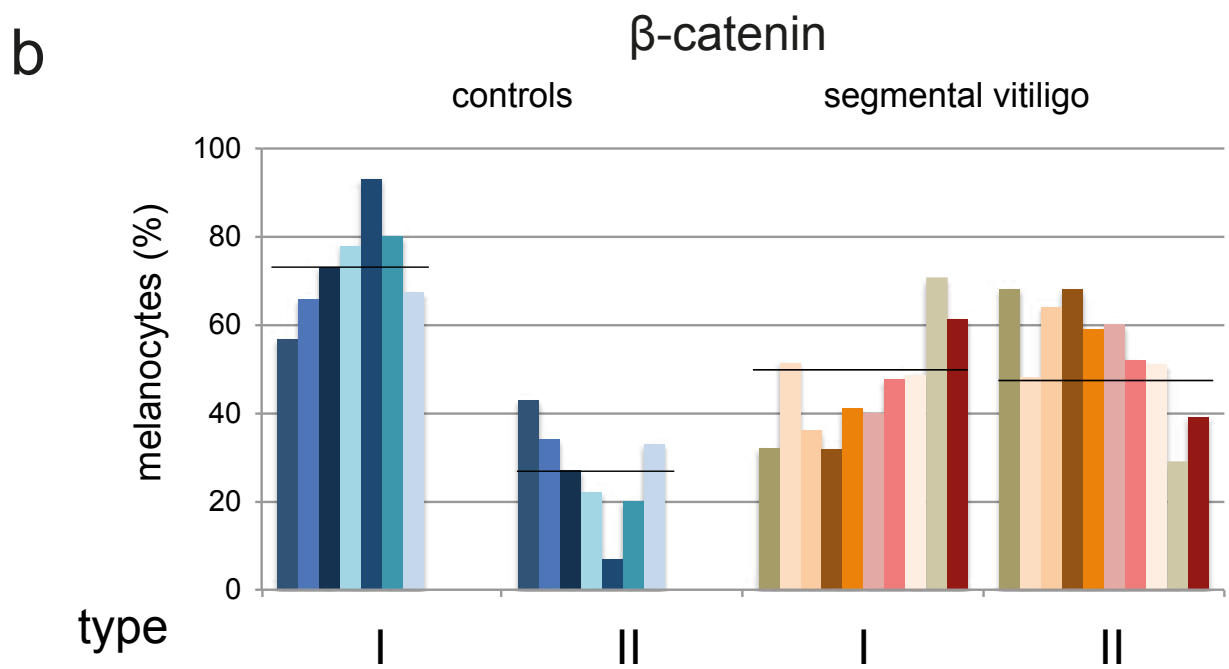
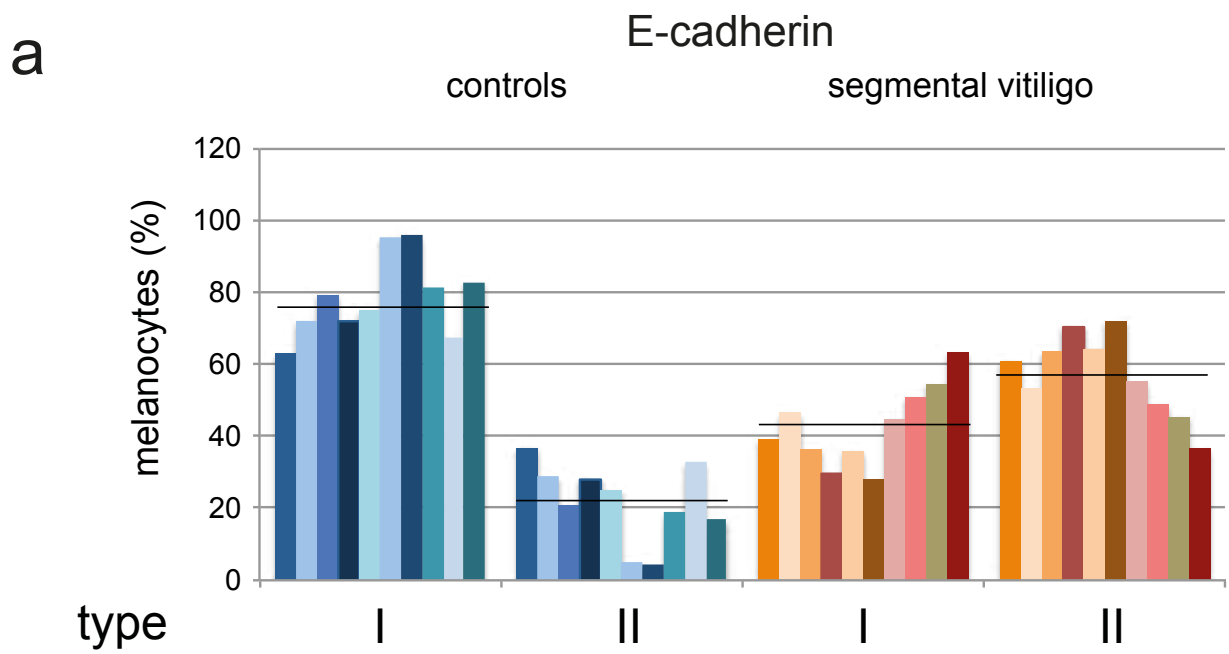


Figure S1

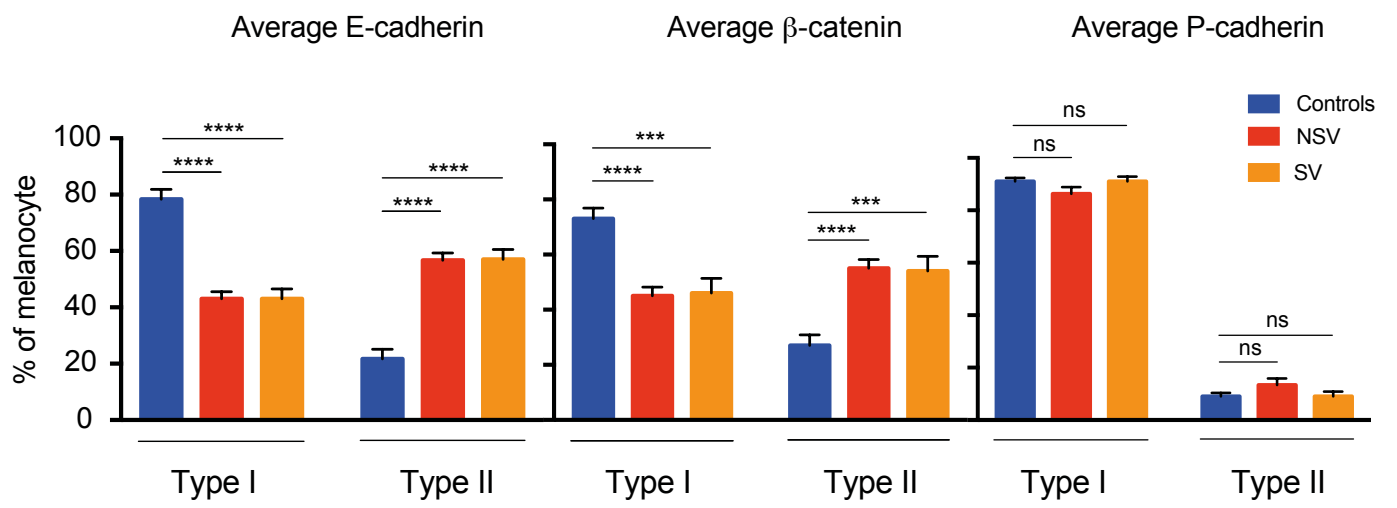


Figure S2

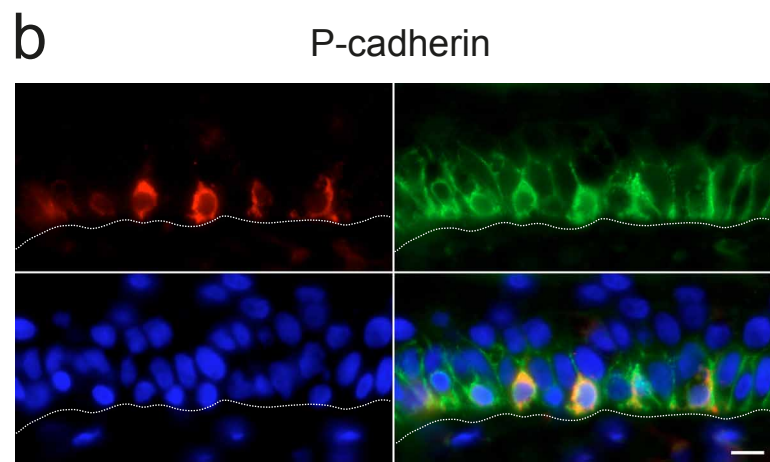
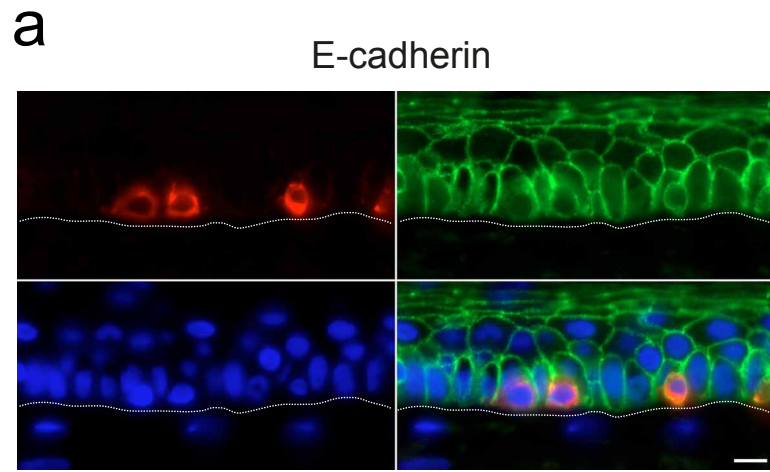


Figure S3