# DELAYED HAIR FOLLICLE MORPHOGENESIS AND HAIR FOLLICLE DYSTROPHY IN A LIPOATROPHY MOUSE MODEL OF Pparg TOTAL DELETION

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| Complete List of Authors: | Sardella, Chiara; Universite de Lausanne, Center for Integrative Genomics; Universita degli Studi di Pisa, Department of Clinical and Experimental Medicine  
Winkler, Carine; Universite de Lausanne, Center for Integrative Genomics  
Quignodon, Laure; Universite de Lausanne, Center for Integrative Genomics  
Hardman, Jonathan; University of Manchester, Centre for Dermatology Research, Institute of Inflammation and Repair  
Toffoli, Barbara; Universite de Lausanne, Center for Integrative Genomics  
Giordano Attianese, Greta Maria Paola; Universite de Lausanne, Center for Integrative Genomics  
Hundt, Jennifer Elisabeth; University of Luebeck, Dermatology, Allergology and Venerology  
Michalik, Liliane; University of Lausanne, CIG;  
Vinson, Charles ; National Cancer Institute, Center for Cancer Research  
Paus, Ralf; Centre for Dermatology Research, Institute of Inflammation and Repair, University of Manchester; University of Münster, Department of Dermatology  
Desvergne, Béatrice; Universite de Lausanne, Center for Integrative Genomics  
Gilardi, Federica; Universite de Lausanne, Center for Integrative Genomics |
| Keywords:         | adipose tissue, PPARγ, hair follicle, hair follicle morphogenesis |
Dear Dr Mark C. Udey, dear Editor,

We are very pleased to submit a revised version of the manuscript n° JID-2016-0816.R1 entitled “Delayed hair follicle morphogenesis and hair follicle dystrophy in a lipoatrophy mouse model of Pparg total deletion.” by Sardella et al. As you will see from the detailed response to the Editor, Deputy Editor and Reviewers, we have addressed all the minor comments, thus further improving our report. In particular, the main modifications concern the discussion, which has been modified according to the suggestions of the reviewer 3, and the nomenclature of the transgenic mice, which has been standardized according to Mouse Genome Database (MGD) Nomenclature Committee rules all along to manuscript.

A point-by-point summary of how we have addressed the Reviewer’s comments has been uploaded on the journal site in the apposite section. We also uploaded a letter signed by all authors who accepted all the introduced changes.

We thank you very much for your kind attention and are available for any questions you may have.

Sincerely,

Federica Gilardi
We thank the Editor, the Deputy Editor and the Reviewers for their comments that helped us to further improve our manuscript. To facilitate the revision, all the parts that have been modified in the text are highlighted in yellow in the revised version.

Below is a point-by-point summary of how we have addressed the comments.

**Editor**
The authors should provide a balanced presentation and discussion of their work, and incorporate the nomenclature changes alluded to by Reviewer 3 and the Section/Deputy Editor.

The discussion has been modified according to the suggestions of the reviewer 3 (see also answer below) and the nomenclature has been standardized all along the manuscript.

**Section/Deputy Editor: 1**
Comments to the Author:
The authors should make the nomenclature changes suggested by reviewer 2 to the manuscript for clarity.

The nomenclature has been standardized.

**Reviewer: 1**
Comments to the Author
The authors have improved the quality of their manuscript. The revision is nicely done.

Thanks.

**Reviewer: 3**
Comments to the Author
In this revision, authors significantly improved their work with several additional experiments. New studies, especially adipose-specific conditional deletion of Pparg, further support the assertion that dermal adipose tissue can modulate late stages of hair follicle morphogenesis. Importantly, additional studies help to understand that late skin defects of total Pparg deletion are likely caused by changes other than in dermal adipose. Authors should be commended on their effort.

Thanks.

In my opinion this work can be further improved, if the following issues are addressed:

1) The biggest remaining criticism relates to how authors' new data is positioned in the context of the current literature on hair follicle-adipose interplay. Some of the important literature on the role of dermal fat in wound healing, skin infection,
fibrosis and hair cycle modulation is only briefly mentioned in Discussion on page 12. Discussion concludes with the statement that further work is needed to fully unravel the molecular crosstalk between fat and hair follicles and how it relates to modulating hair morphogenesis. Overall, however, the narrative is worded in a manner that implies that dermal adipocytes as the lead signaling modulators of other skin processes and these in hair follicles, in particular.

I believe this discussion need to be rebalanced in view of the recent strong evidence for hair follicles as the primary drivers of dermal fat morphogenesis and cyclic remodeling in adult skin. Authors need to properly discuss several new works in this area, including on:
- the role of anagen hair follicles and hair-derived Shh ligands in driving skin adipogenesis during development and hypertrophy of skin fat during adult hair cycle (Genes Dev 2016; PMID: 27807033)
- the role of hair follicles and hair-derived BMP signals as critical drivers of new dermal fat regeneration in large skin wounds (Science 2017, PMID: 28059714)
- the role of epithelial WNT signaling and downstream BMP/IGF paracrine factors in driving skin adipogenesis during development (PNAS 2014; PMID: 24706781).

We thank the reviewer for this constructive comment, which has helped to improve the discussion. The second part of the discussion has been widened, by introducing the papers suggested by the reviewer and others. This adjustment has rebalanced the discussion, highlighting the reciprocal communication between HF and dermal adipose tissue in the different pathophysiological conditions, without focusing only on the effects of adipose tissue on HFs. Our data are now discussed also in light of the new evidence.

2) In light of new experiments, it becomes clear now that the defect in skin adipogenesis transiently delays normal hair follicle maturation during morphogenesis. Although authors’ own data does not provide insight into the mechanism of this defect, it will be appropriate to briefly speculate on this issue in Discussion. Authors should discuss if they think the defect is mediated by altered adipose-derived paracrine signaling regulators or, possibly, loss of trophic effect of the adipose tissue? The latter has been documented to take place in epicardial fat, where adipocytes directly supply adjacent cardiac muscle with free fatty acids.

Given space constrains, authors can free up space necessary for these discussions by consolidating their discussion of the role of Pparg, which is currently somewhat redundant between Introduction and Discussion.

The first part of the discussion, which was indeed a bit redundant with the introduction, has been shortened. Our data are now discussed in light of the new evidence introduced in the discussion, and our hypotheses about the potential players underlying the effect of adipose tissue on the timing of HF morphogenesis have been clarified.
3) Figures can be further improved:

3a) Mouse nomenclature labels have to be standardized throughout (see point #3 below)

Nomenclature has been standardized (see answer below)

3b) Orientation of skin histology panels should be standardized. It is standard for hair bulbs on skin histology to point to the left. Currently, panels are oriented in various directions. For example, compare Fig 1b vs. 1d. Problem with orientation are throughout the figures. Orientation on panels Fig. 4a, 5b is particularly distracting.

Thanks for this comment. The orientation of the skin histology pictures has been now corrected.

3c) Given the importance of functional data from conditional Pparg deletion transgenic mice, it is surprising that it was placed into the supplement. To increase visibility of this data, at least portion of it should be moved from supplement figure S6 into one of the main figures.

We totally agree on the relevance of the data on Adipoq-Cre$^{tg/+}$;Pparg$^{fl/fl}$ mice. However, considering that the characterization of the skin defects of AZIP mice was much more complete, and due to space constraints for the main figures, we have let the data on Adipoq-Cre$^{tg/+}$;Pparg$^{fl/fl}$ in supplemental information.

4) Although relatively minor, nonetheless it is important that transgenic mouse nomenclature in the paper complies with the universally accepted standards. All transgenes should be italicized, and only first letter should be capitalized. For examples, Sox2-Cre (italicized) is the standard version. In the manuscript I can find non-italicized Sox2Cre, Sox2CRE with no dash. Similarly, italicized Adipoq-Cre is the standard version, as Adipoq is the official gene symbol. In the manuscript, authors use adiponectin-CRE. Adipoq-Cre;Pparg-flox/flox is the standard version of the new double transgenic that authors used. In the manuscript, authors use $\gamma F\Delta/\Delta$ and Pparg$\Delta/\Delta$ names among others. All these are non-standard and should be corrected both in the text and figures. Authors should consult Jackson Laboratory online resource for transgenic mouse nomenclature.

We agree on the importance of standard nomenclature and apologize for the lack of clarity. We have now corrected the standard name of fat-specific Pparg-null mice using Adipoq-Cre$^{tg/+}$; Pparg$^{fl/fl}$ when we mention these mice for the first time in the result section and all the figure legends, clearly explicating that we refer to these mice as to Pparg$^{fl/fl}$, as reported by Wang et al. (Wang et al., 2013), in the rest of the text and the figures.

For our total body Pparg-null mice, considering the complexity of the genotype of these mice, we clearly explain the exact genotype denomination is clearly explained in figure S1 and follows Mouse Genome Database (MGD)
Nomenclature Committee rules. The official name Sox2-Cre$^{tg/+}$;Pparg$^{\Delta/\Delta}$ is now shown when we mention the mice for the first time in the result section and the figure legends, indicating that thereafter we refer to these mice as to Pparg$^{\Delta/\Delta}$. All the confusing γ$^{\Delta/\Delta}$ and γ$^{F\Delta/\Delta}$ have been substituted in the figures with allele name (Pparg$^{\Delta/\Delta}$ and Pparg$^{F\Delta/\Delta}$, respectively).

Reference:
DELAYED HAIR FOLLICLE MORPHOGENESIS AND HAIR FOLLICLE DYSTROPHY IN A LIPOATROPHY MOUSE MODEL OF Pparg TOTAL DELETION

Chiara Sardella¹, Carine Winkler¹, Laure Quignodon¹, Jonathan A. Hardman², Barbara Toffoli¹, Greta Maria Paola Giordano Attianese¹, Jennifer E. Hundt³, Liliane Michalik¹, Charles R. Vinson⁴, Ralf Paus², Béatrice Desvergne¹ and Federica Gilardi¹

¹ Center for Integrative Genomics, Faculty of Biology and Medicine, University of Lausanne, CH-1005 Lausanne, Switzerland
² Centre for Dermatology Research, School of Biological Sciences, University of Manchester, Manchester, UK
³ Department of Dermatology, University of Lübeck, Lübeck, Germany
⁴ Center for Cancer Research, National Cancer Institute, Laboratory of Metabolism, Bethesda, MD 20892, USA
⁵ Current address: Department of Clinical and Experimental Medicine, Section of Endocrinology, University of Pisa, 56124 Pisa, Italy

Corresponding author:
Dr. Federica Gilardi
Center for Integrative Genomics, University of Lausanne
Genopode Building, CH-1015 Lausanne, Switzerland,
tel. 0041 21 6924146
fax. 0041 21 6925005
Mail: Federica.Gilardi@unil.ch

Short title: Role of adipose tissue in hair growth

Keywords: adipose tissue, PPARγ, hair follicle, hair follicle morphogenesis
Abstract

The peroxisome proliferator-activated receptor-gamma (PPARγ) regulates multiple aspects of skin physiology, including sebocyte differentiation, keratinocyte proliferation, epithelial stem cell survival, adipocyte biology, and inflammatory skin responses. However, the effects of its global deletion, namely of non-redundant key functions of PPARγ signaling in mammalian skin, are yet unknown, due to embryonic lethality. Herein, we describe the skin and hair phenotype of a novel whole body PPARγ-null mouse (PpargΔ/Δ), obtained by preserving PPARγ expression in the placenta. PpargΔ/Δ mice exhibited total lipoatrophy and complete absence of sebaceous glands. Right after birth, hair follicle (HF) morphogenesis was transiently delayed, along with reduced expression of HF differentiation markers and of transcriptional regulators necessary for HF development. Later, adult PpargΔ/Δ mice developed scarring alopecia and severe perifollicular inflammation. Skin analyses in other models of lipodystrophy, AZIP^{+/−} and Adipoq-Cre^{+/−};Pparg^{flo/flo} mice, coupled with skin graft experiments, demonstrated that the early defects observed in hair morphogenesis were caused by the absence of adipose tissue. In contrast, the late alteration of HF cycle and appearance of inflammation were only observed in Pparg^{Δ/Δ} mice and likely due to the lack sebaceous glands. Our findings underscore the increasing appreciation for the importance of adipose tissue-mediated signals in HF development and function.
**Introduction**

Peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear receptor activated by fatty acid metabolites and synthetic compounds such as thiazolidinediones (Desvergne et al., 2004, Straus and Glass, 2007, Varga et al., 2011). PPARγ was first described as the master regulator of adipogenesis and sebocyte differentiation (Barak et al., 1999, Rosen et al., 1999), but is also involved in metabolic homeostasis maintenance, in the control of immune cell differentiation, and in the inflammatory response regulation (reviewed in (Ahmadian et al., 2013, Desvergne et al., 2006)).

In mammalian skin, besides its prominent expression in adipocytes (Chon and Pappas, 2015, Ramot et al., 2015), PPARγ is highly expressed in suprabasal keratinocytes (Icre et al., 2006), where it inhibits cell proliferation and promotes epidermal differentiation, thus regulating skin barrier permeability (Adachi et al., 2013). PPARγ is also expressed in pilosebaceous units, including, but not restricted to, hair matrix keratinocytes, dermal papilla cells, the inner root sheath (IRS) of the hair follicle (HF) (Billoni et al., 2000), and sebocytes (Dozsa et al., 2014). Murine hair morphogenesis starts prior to birth and is accomplished within the first 2 postnatal weeks, relying on stringently coordinated epithelial-mesenchymal interactions that ultimately result in differentiation of hair shaft, root sheaths and dermal papilla. Once formed, HFs undergo successive cycles of growth (anagen), regression (catagen) and rest (telogen) (Fuchs and Horsley, 2008, Muller-Rover et al., 2001, Paus et al., 1999, Schmidt-Ullrich and Paus, 2005, Sennett and Rendl, 2012, Sennett et al., 2015). The sebaceous gland (SG) duct is thought to impact on the disintegration of the upward moving IRS where both structures meet (Stenn and Paus, 2001), and SG function/dysfunction may directly influence HF epithelial stem cell compartment and the bulge (Stenn et al., 2008, Sundberg et al., 2000). Therefore, proper SG development and function is critical for HF physiology (Fuchs, 2007, Fuchs and Horsley, 2008).
The importance of PPARγ in HF function emerged with the finding that its targeted deletion in keratin 15+ murine HF epithelial stem cells leads to accumulation of pro-inflammatory lipids resulting in scarring alopecia and sebaceous gland atrophy in adult mice (Karnik et al., 2009). In human HFs, PPARγ activation enhances the expression of two keratins expressed by HF progenitor cells, Krt15 and Krt19 (Ramot et al., 2014). Moreover, continuous PPARγ signaling was proposed as a protective mechanism to maintain epithelial HF stem cells (Ramot et al., 2015). However, although PPARγ agonists exert beneficial effects in some patients with scarring alopecia (Mesinkovska et al., 2015, Mirmirani and Karnik, 2009), the role of PPARγ signaling in the development of focal lesions of scarring alopecia, in patients with lichen-planopilaris or frontal fibrosing alopecia, was questioned (Harries et al., 2013).

Therefore, appropriate mouse models are needed which permit one to comprehensively dissect the role of PPARγ in HF physiology and pathology. While mice with targeted, tissue-specific PPARγ deletion were developed (reviewed in (Ahmadian et al., 2013)) constitutive, whole-body PPARγ knockout mice are embryonically lethal (Barak et al., 1999). To overcome this limitation, we generated a whole body PPARγ-null mouse model (Ppargα/α), rescued from embryonic lethality by preserving PPARγ expression in the trophoblast (Nadra et al., 2010).

This mouse model permits one to study, for the first time, the consequences of the constitutive absence of functional PPARγ in the entire organism and to specifically dissect non-redundant key functions of PPARγ signaling in mammalian skin. Ppargα/α mice suffer from a generalized lipoatrophy, lack SGs, show delayed HF morphogenesis, and develop scarring alopecia as adults. Moreover, our results reveal a new role of the adipose tissue in HF morphogenesis.
Results

\( \text{Pparg}^{\Delta/\Delta} \) mice exhibit delayed postnatal hair growth, hair follicle dystrophy, and subsequent alopecia at adult stage

We generated \( \text{Sox2-Cre}^{\text{tg/+}}; \text{Pparg}^{\Delta/\text{em}\Delta} \) (\( \text{Pparg}^{\Delta/\Delta} \)) mice through specific epiblastic gene deletion (Figure S1). Consistent with the master role of PPAR\( \gamma \) in adipogenesis, \( \text{Pparg}^{\Delta/\Delta} \) mice were completely lipoatrophic. Two to three days after birth, mutant mice exhibited delayed hair appearance (Figure 1a). At postnatal day 8 (P8), the coats of mutant mice had a rough- appearance, with short hair over the entire body (Figure S2a). Thereafter, the skin appeared dry, with moderate white flaking, particularly highlighted after shaving (Figure S2b). At P28 and P49, mutant fur remained altered with shorter hair than control littermates (Figure 1a and S2a). With aging, all mutant mice displayed progressive hair loss, which occurred randomly (Figure 1a and S2c). In addition, one out of ten \( \text{Pparg}^{\Delta/\Delta} \) mice exhibited skin lesions (Figure S2d). No other macroscopic abnormalities were observed in tail hair, vibrissae, nails or teeth.

Histological analyses of young \( \text{Pparg}^{\Delta/\Delta} \) skin (P28) revealed hyperkeratosis and hyperplasia of the interfollicular epidermis (Figure 1b). The dermis showed increased cellularity, which suggested the presence of interstitial inflammation, while intradermal adipocytes and SGs were totally missing (Figure 1b and S3). The subcutaneous fat layer was also absent.

A thorough characterization of skin appendages in \( \text{Pparg}^{\Delta/\Delta} \) mice revealed general structural defects in HFs (details in Figure 1c), which resembled HFs damaged by chemotherapy (Hendrix et al., 2005). These defects were accompanied, at P28, by clusters of active phagocytic cells (MHC class II\(^+\)) around the HFs. Anti-CD45 staining of hematopoietic cells confirmed the presence, in \( \text{Pparg}^{\Delta/\Delta} \) skin, of inflammatory infiltrates, mainly composed of macrophages (F4/80\(^+\)) and neutrophils (Ly-6B.2\(^+\)) (Figure 1d,e). In contrast, no changes were observed in the expression of CD3 (\( \text{Cd3e} \)) (Figure S4), which suggested that inflammatory
infiltration was not sustained by T lymphocytes. These observations are reminiscent of human scarring alopecia, caused by a destructive inflammatory process that leads to cicatricial HFs as a consequence of irreversible HF stem cell damage (Harries et al., 2013, Harries and Paus, 2010), although the disease end stages, characterized by the complete absence of HF, was not fully recapitulated.

We thus explored hair morphogenesis and the subsequent hair cycling, with quantitative histomorphometry (Paus et al., 1999). At P1, hair morphogenesis score was significantly lower in Pparg$^{\Delta/\Delta}$ mice compared to control littermates (Figure 2a), which confirmed the significant delay in postnatal HF development. This difference became minor at P8. We next investigated hair cycling phases (Muller-Rover et al., 2001), and found that entry into catagen/first HF cycle was delayed in Pparg$^{\Delta/\Delta}$ skin at P17, with a lower percentage of HFs in catagen compared to control mice (Figure 2b). Conversely, at P28, HF number in late catagen was higher in Pparg$^{\Delta/\Delta}$ than in control skin. At P49, in control mice, nearly 80% of HFs was in telogen, while, in Pparg$^{\Delta/\Delta}$, most HFs remained in anagen or catagen.

Altogether, these observations indicated that the absence of PPARγ and/or adipose tissue-mediated signaling was responsible of two distinct effects. The early effects, occurring right after birth, affect HF development with transient delay in HF morphogenesis; the late effects are observed from P17 onwards, with the slow down of the hair cycle and HF dystrophy.

**HF terminal differentiation and homeostasis are defective in Pparg$^{\Delta/\Delta}$ mice**

We next evaluated changes in the expression of markers of different HF cell-populations during hair morphogenesis (P1-P5-P8). Overall, mRNA levels of K6irs (IRS marker), Tchh (expressed in IRS and hair shaft medulla), and hair keratins mHa2 and mHa3 (hair shaft cortex markers), were lower in Pparg$^{\Delta/\Delta}$ skin than in aged-matched controls, particularly at P5. These findings, further sustained by the trichohyalin protein expression pattern (Figure
S5a), confirmed a delay in the initial formation of Pparg\(\Delta/\Delta\) follicles (Figure 3a). Accordingly, at P5 we observed a significant down-regulation of the transcription factors Foxn1 and Msx2, both involved in regulating IRS and hair differentiation. Conversely, during hair morphogenesis, mutant and control mice showed similar mRNA levels of Alpl (dermal papilla marker), Krt5 (Outer Root Sheath – ORS marker), Krt15 and Cd200, (HF stem cells markers; Figure 3b,c,d) and no inflammation, as assessed at P1 and P8 (Figure S6).

We measured the expression of the same genes also during hair cycling (P17-P28-P49). The expression pattern of Alpl and Krt5 remained unaltered in Pparg\(\Delta/\Delta\) samples (Figure 3c). However, the asynchrony in hair cycles between control and Pparg\(\Delta/\Delta\) mice was particularly well illustrated by the mirror-image expression profiles of K6irs, Tchh, mHa2 and Msx2 at the three time points. Indeed, at P17, P28 and P49, control mRNA levels were low, high and then low, respectively; conversely, Pparg\(\Delta/\Delta\) mRNA levels were high, low, and then high respectively (Fig 3a,b). Trichohyalin protein showed a similar pattern of expression (Figure S5b). Cd200 and Krt15 mRNA levels were overall reduced during the different hair cycle stages of mutant mice, which suggested a progressive dysfunction occurring in HF stem cell compartment (Figure 3d), in concomitance with the appearance (from P17 onwards) of skin inflammation (Figure S6).

These results showed that the early delayed hair morphogenesis in Pparg\(\Delta/\Delta\) mice was mainly characterized by impaired IRS and HF terminal differentiation, while the late phenotype was a combination of inflammation and HF stem cell dysfunction, as assessed in the subsequent hair cycle.

**Lipodystrophy is associated to the early defective hair follicle postnatal differentiation**

Both the consequences of the total lack of PPAR\(\gamma\) and the lipoatrophy might contribute to determine the early HF phenotype observed in Pparg\(\Delta/\Delta\) mice. To clarify their respective
contribution, we generated fat-specific Pparg knockout mice (Adipoq-Cre\textsuperscript{tg/+};Pparg\textsuperscript{fl/fl}, referred to as Pparg\textsuperscript{F\Delta/\Delta}), as previously described (Wang et al., 2013). No mature adipocytes were detectable in Pparg\textsuperscript{F\Delta/\Delta} skin, while sebaceous glands were present and adipocyte precursor markers were expressed at normal levels (Fig S7a,b). As in Pparg\textsuperscript{\Delta/\Delta} mice, the expression of Tchh, mHa3, Foxn1, Msx2 at P5 was significantly reduced in Pparg\textsuperscript{F\Delta/\Delta} mice, and trichohyalin staining further reinforced the hypothesis that the lack of mature adipocytes delays HF morphogenesis (Fig S7c,d).

To exclude that this phenotype is specific to PPAR\textgamma–dependent lipodystrophy, we finally explored skin and HF phenotype in AZIP\textsuperscript{tg/+} mice, in which the Fabp4 promoter drives the expression of a C/EBP dominant negative protein (Moitra et al., 1998). These mice, which have no alteration in Pparg alleles, are characterized by a nearly complete ablation of mature adipocytes. In agreement with previous reports (Festa et al., 2011), we detected AZIP transgene expression in the dermis, but not in the epidermis of AZIP\textsuperscript{tg/+} mice, that excluded a contribution of keratinocyte defects to their skin phenotype (Fig S8a). As in Pparg\textsuperscript{\Delta/\Delta} mice, mature subcutaneous adipose tissue was undetectable in AZIP\textsuperscript{tg/+} mice (Figure 4d), while adipocyte precursor markers were unaltered (Figure S8b). In contrast to Pparg\textsuperscript{\Delta/\Delta}, but similar to Pparg\textsuperscript{F\Delta/\Delta}, AZIP\textsuperscript{tg/+} mice showed normal sebaceous glands (Figure 4d). Furthermore, AZIP\textsuperscript{tg/+} hair was not evident at P5 and looked sparse hair at P17 (Figure S9a). Quantitative histomorphometry showed that AZIP\textsuperscript{tg/+} HF morphogenesis was delayed (Figure 4a,b), as confirmed by the reduced expression of K6irs, Tchh, mHa2, mHa3 and Foxn1 at P5 (Figure 4c). In contrast, adult AZIP\textsuperscript{tg/+} mice were indistinguishable from their littermate controls (Figure S9a) and no CD45\textsuperscript{+} inflammatory cell or macrophage infiltration was observed in AZIP\textsuperscript{tg/+} skin (Figure 4d,e and S9b). Altogether, these results indicate that mature adipocytes contributed to coordinate HF differentiation during hair morphogenesis, but they were not required to maintain the late HF homeostasis.
To characterize at the molecular levels the differences lying at the very initial stage of the interaction between adipocytes and the forming HFs, knowing that mature adipocytes were detectable in control embryonic skin only from E17.5 onwards (Figure S10), we examined global gene expression in skin from \textit{Pparg}^{\Delta/\Delta} and control embryos at E17.5. We found only 31 differentially expressed genes, and, among these, 9 were still reduced at P1 and inhibited also in AZIP^{tg/+} (Table 1). As expected, several markers of mature adipocytes, including \textit{Fabp4}, \textit{Adipoq}, \textit{Plin1} and \textit{Rbp4}, were down-regulated in \textit{Pparg}^{\Delta/\Delta} skin. Moreover, thyroid stimulating hormone receptor (\textit{Tshr}) and bone morphogenic proteins (BMP)-binding endothelial regulator (\textit{Bmpr}), two genes potentially relevant for HF morphogenesis (Heinke et al., 2013, Moser et al., 2003), were significantly down-regulated.

\textit{Skin graft experiments rescue the early delay in HF morphogenesis, but not the late skin phenotype observed in Pparg}^{\Delta/\Delta} \textit{mice}

To fully confirm the relevance of adipose tissue and its related systemic environment for a timely HF morphogenesis, we engrafted skin biopsies from \textit{Pparg}^{\Delta/\Delta} and control embryos onto immunocompromised \textit{Foxn1}^{nu/nu} mice. Until 21 days after engraftment, hair growth and density were similar in control and \textit{Pparg}^{\Delta/\Delta} grafts (Figure 5a). Notably, quantitative histomorphometry analysis performed 7 days after the engraftment showed a complete rescue of \textit{Pparg}^{\Delta/\Delta} delayed HF morphogenesis (Figure 5b,c), consistent with the recovery in the expression of \textit{K6irs}, \textit{Tchh}, \textit{mHa2}, \textit{mHa3}, \textit{Foxn1} and \textit{Msx2}, while \textit{Krt15} remained unaffected (Figure 5d). Interestingly, \textit{Pparg} and \textit{Adipoq} expression in the skin was still significantly reduced, thus arguing against the possible presence of local mature adipocytes within the graft. Similar results were obtained in skin grafts from AZIP^{tg/+} embryos (Figure S11). Collectively, these observations demonstrate that the altered environment induced by the lack
of adipose tissue is responsible for the early effects on HF morphogenesis observed in 
Pparg^{AA} and AZIP^{tg/+} mice.

In contrast, at later time points, hair loss and inflammation appeared specifically in 
Pparg^{AA} grafts (Figure 5a), as demonstrated by the increased inflammatory cell infiltration detected in 
Pparg^{AA} grafts after 38 days (Figure 5e,f), whereas no signs of inflammation were present in 
AZIP^{tg/+} grafts (Figure S12a,b). These results, consistent with the late appearance of 
inflammation only in Pparg^{AA} skin (Figure S6), suggested a direct responsibility of the lack 
of local PPARγ, and the associated absence of sebaceous glands, on the late HF phenotype of 
Pparg^{AA} mice, characterized by strong inflammatory response, HF cycle defects and alopecia.
Discussion

Our results highlight a dramatic skin phenotype in $Pparg^{AD}$ mice, characterized by two main defects at the level of HFs. Right after birth the complete absence of adipose tissue induces a transient delay of the postnatal HF morphogenesis. The late effects are seen after hair growth, when the lack of PPAR$\gamma$ in HF stem cells, together with the lack of sebaceous glands (SGs), slow hair cycle, severely alter HF morphology and induce skin inflammation.

The late $Pparg^{AD}$ skin phenotype reinforces previous reports that described PPAR$\gamma$ contribution to pilosebaceous unit homeostasis (Fu et al., 2010, Karnik et al., 2009, Nakahigashi et al., 2012, Zhang et al., 2006). In particular, PPAR$\gamma$ absence in bulge HF stem cells and the lack of SGs, explain, at least in part, the aberrant hair cycle and chronic inflammation observed in $Pparg^{AD}$ mice starting at P17, and reproduced in our graft experiments. However, additional contributions linked to PPAR$\gamma$ dysfunctions also in other cellular compartments cannot be excluded, in that they can induce environmental alterations with a potential impact on HF homeostasis. For instance, the milk produced by females lacking $Ppar$ in mammary glands is enriched in toxic lipids that provoke a strong alopecia in the breast-fed pups, starting from P16 (Wan et al., 2007), which shows how sensitive HFs might be to environmental cues. Furthermore, the role of adipose tissue, totally missing in $Pparg^{AD}$ mice, must be considered. Adipocytes are abundantly present in dermis (Driskell et al., 2014, Kruglikov and Scherer, 2016) and have been implicated in wound healing (Schmidt and Horsley, 2013), homeostatic temperature regulation (Kasza et al., 2014), cutaneous fibrosis (Marangoni et al., 2015) and protection against skin infections (Zhang et al., 2015).

The first indications of an adipocyte-HF axis came from mice with reduced intradermal adipose tissue, such as $Fatp4$ and $Dgat1$ and 2 deficient mice, exhibiting skin abnormalities and hair loss (Chen et al., 2002, Herrmann et al., 2003, Stone et al., 2004). However, those observations remained elusive due to additional sebocyte defects, which may have contributed
to the observed phenotype. More recently, independent reports have shown that intradermal preadipocytes and differentiated adipocytes secrete factors (Geyfman et al., 2015), such as PDGFA (Festa et al., 2011) and BMP2 (Plikus and Chuong, 2014, Plikus et al., 2008), respectively, that regulate the progression through HF cycling. Notably, recent evidence highlighted that the adipose tissue-HF axis works also in the opposite direction. Indeed, the appearance of dermal adipocytes in mid-anagen occurs right after the formation of HF transit-amplifying cells, generated by HF stem cells and secreting the Sonic Hedgehog (SHH) factor, which promotes adipocyte precursor proliferation (Zhang et al., 2016). Thus, the reciprocal communication between HF and dermal adipocytes ensures the proper progression through HF cycling. Moreover, in other pathophysiological conditions, such as wound healing, HF regeneration occurring in large skin wounds is required for the beneficial conversion of myofibroblasts to adipocytes through the activation of BMP signaling (Plikus et al., 2017).

During skin morphogenesis, while HF formation is not required for the induction and expansion of dermal adipocytes (Donati et al., 2014), our data clearly suggest that mature adipocytes are required for the early initial regulation of hair morphogenesis timing. First, hair growth is delayed in three mouse models with defects in adipocyte maturation, rather than in adipocyte precursors: $Ppar_{	ext{g}}^{\Delta/\Delta}$, AZIP$^{\text{tg/+}}$ and $Ppar_{	ext{g}}F^{\Delta/\Delta}$. Second, no defects are observed when $Ppar_{	ext{g}}^{\Delta/\Delta}$ or AZIP$^{\text{tg/+}}$ skin is engrafted in the recipient Foxn$^{\text{1nu/nu}}$ mice, which bear functional adipose tissue. Finally, HF stem cell markers are not altered during HF morphogenesis, which excludes a common mechanism for defective hair morphogenesis and the later occurrence of scarring alopecia.

The appearance of the first lipid-filled adipocytes below the dermis at E17.5 is consistent with previous observations (Birsoy et al., 2011, Driskell et al., 2013) and depends on the activation of the Wnt/βcatenin pathway in the epidermis occurring before, at E14.5 (Donati et al., 2014). This timing reinforces the hypothesis that adipose tissue-mediated signals might be implicated
during the last stages (i.e. late organogenesis and cytogenesis) of HF morphogenesis, rather than in the initiation phase, which occur sharply at E14.5 (Schmidt-Ullrich and Paus, 2005).

Although our study does not completely clarify the molecular players involved in the cross-talk adipocyte-HF at this stage of HF development, we found two interesting candidates of possible adipose-derived paracrine regulators: Tshr and Bmper, whose expression was defective in Pparg<sup>Δ/Δ</sup> skin. Both Tshr and Bmper are expressed by adipocytes (Bell et al., 2000, Mastrogiannaki et al., 2016) and might potentially influence HF development through the regulation of keratins (Bodo et al., 2008), or interfering with BMP signaling (Heinke et al., 2013, Moser et al., 2003), respectively. However, we cannot exclude that the lack of dermal adipocytes alters the availability of metabolites required for energy metabolism of surrounding organs (e.g. HFs), similarly to what was observed for fatty acids provided to heart by epicardial fat (Talman et al., 2014). Interestingly, in Pparg<sup>Δ/Δ</sup> and AZIP<sup>tg/+</sup> skin grafts experiments, HF morphogenesis is rescued even though adipocytes are not always evident in graft sections and Adipoq expression remained down-regulated. Thus, at the current state, the involvement of systemic cues triggered by the lack of adipose tissue in fine-tuning HF morphogenesis seems plausible.
Material and methods

In vivo experiments: Animal experiments were approved by the animal experimentation commission of Canton of Vaud, accordingly to the European Community Council Directives (86/609/EEC).

Mouse models: Pparg floxed allele (formerly PPARγL2, hereafter called Pparg^f) and Pparg-null allele (PPARγL−, hereafter called Pparg^n) were previously described (Imai et al., 2004). Matings to generate Pparg^Δ/Δ using Sox2-Cre^w/+ mice (Jackson Laboratory) are shown in Figure S1. AZIP/F1 mice (AZIP^w/+), and fat-specific Pparg-null mice (Adipoq-Cre^w+;Pparg^f/f) were generated as previously described (Moitra et al., 1998)(Wang et al., 2013).

Skin grafts: Full thickness skin (1cm^2) was removed from the torso of E18.5 Pparg^Δ/Δ, AZIP^w/+ and respective control littermates and grafted onto wounds on the back of Foxn1^nu/nu mice, after removal of epidermis and dermis. Grafts were performed in at least eight mice per genotype.

Histology: Full thickness back skin was either fixed in 4% PFA and paraffin embedded or frozen in OCT (Sakura, Torrance, CA). 4μm paraffin sections were stained with hematoxylin and eosin or with anti-F4/80 (ab6640, Abcam, Cambridge, MA), anti-Ly-6B.2 alloantigen (MCA771GA, AbD Serotec, Oxford, UK), anti-Trichohyalin (ab58755, Abcam) and anti-Iba1 (019-19741, Wako Chemicals, Richmond, VA), followed by detection with DAB or fluorescent secondary antibody. For quantitative histomorphometry, HF morphogenesis was evaluated according to the stages defined in Paus et al. (Paus et al., 1999) and hair cycle was evaluated at P17 (catagen), P28 (anagen) and P49 (telogen), according to the Muller-Rover classification (Magerl et al., 2001). Cryo-sections were stained with Oil Red O (Sigma-Aldrich) or with anti-CD45PE (103105, BioLegend, San Diego, CA), anti-MHC class II (T-2106, BMA Biomedicals, Switzerland), anti-Caveolin1 (3267, Cell Signaling,
Danvers, MA), and HCS LipidTOX (H34476, Molecular Probes, Eugene, OR). Staining quantification was performed with Image J.

**Gene expression analyses:** full thickness skin samples were snap frozen in TRIzol (Invitrogen) and RNA was extracted following manufacturer’s instructions. For microarrays, 100ng of total RNA were analyzed on Mouse Gene 1.0ST arrays according to manufacturer’s instructions (Affymetrix). Statistical analyses were performed with R and various Bioconductor packages ([http://www.Bioconductor.org](http://www.Bioconductor.org)). Normalized expression signals were calculated from Affymetrix CEL files using RMA normalization method. Data are accessible at [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=shizumsgrdsnjeh&acc=GSE85497](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=shizumsgrdsnjeh&acc=GSE85497).

For quantitative RT-PCR analysis, retrotranscription was performed using iScript cDNA synthesis kit (Bio-Rad, Laboratories, Hercules, CA). Real-time PCR was performed using the FastStart Universal SYBR Green Master (Roche Applied Science, Indianapolis, IN) in an ABI Prism 7900 Sequence Detection System (Life Technologies, Carlsbad, CA). Primer sequences are available upon request.

**Statistical analyses** were performed with Student t test or two-way ANOVA (Bonferroni post-test analysis) for comparison of two or multiple groups, respectively, using Prism 5.0 (GraphPad, San Diego, CA). Differences with P-values < 0.05 were considered statistically significant.
Conflict of Interest

The authors state no conflict of interest.

Acknowledgments

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Table 1. qPCR gene expression analysis of differentially regulated genes in the skin of *Pparg*^ΔΔ^ and AZIP^{tg/+} mice.

<table>
<thead>
<tr>
<th>PATHWAY</th>
<th>GENE</th>
<th>Fold Change <em>Pparg</em>^ΔΔ^ vs CTL 1</th>
<th>Fold Change AZIP^{tg/+} vs WT 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E17.5</td>
<td>P1</td>
</tr>
<tr>
<td>PPAR TARGETS / ADIPOSE TISSUE FUNCTION</td>
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<td>-204</td>
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<td></td>
<td>Plin1</td>
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<td>-10.5</td>
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<td>-1.6</td>
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<td>-4</td>
</tr>
<tr>
<td></td>
<td>Enpep</td>
<td>-9</td>
<td>-1.6</td>
</tr>
</tbody>
</table>

1Gene expression evaluated by qPCR in the skin of *Pparg*^ΔΔ^ and control mice at E17.5 (n=4) and P1 (n=7)  
2Gene expression evaluated by qPCR in the skin from AZIP^{tg/+} and the respective control mice at E17.5 (n=6).
Figure Legends

Figure 1: Spontaneous skin and hair phenotype in $Pparg^{AlA}$ mice

(a) Pictures of Sox2-Cre$^{tg/+}$;$Pparg^{Al/emA}$ ($Pparg^{AlA}$) and control (CTL) mice at P3, P49 and 6 months. (b) Hematoxylin & Eosin and Oil Red O staining of back skin sections from $Pparg^{AlA}$ and CTL mice at P28. (c) Giemsa-staining revealing dystrophic features of the HF (arrows) at P28: follicular plugging (black), perifollicular inflammation (fuchsia), HF disruption (blue) or deformation (red) with widened hair canal (yellow), irregular melanin banding pattern (green) and extrafollicular deposits of melanin (brown). (d) Immunostaining with anti-MHC II (activated phagocytic cells), anti-F4/80 (macrophages), anti-Ly-6B.2 (neutrophils), anti-CD45 (leukocytes). (e) Quantification of the stainings in d. Data expressed as mean ± SEM (n=3).

Scale bar =100 µm except in panels Cii, Ciii and Cv (=50µm).

Figure 2: Delayed hair follicle morphogenesis and abnormal hair follicle cycling in $Pparg^{AlA}$ mice

Quantitative histomorphometry of Giemsa-stained back skin cryosections from Sox2-Cre$^{tg/+}$;$Pparg^{Al/emA}$ ($Pparg^{AlA}$) and control (CTL) mice collected at the indicated postnatal days

(a) Hair morphogenesis analysis at P1 and P8: representative pictures (left panel, scale bar =100µm); hair morphogenesis score (central panel); % of HF's found in the different hair morphogenesis stages (right panel; n=6). (b) Hair cycle progression analysis at P17 (catagen), P28 (anagen) and P49 (anagen): representative pictures (left panel, scale bar =100µm); hair cycle score (central panel); % of HF's found in the different hair cycle stage (right panel; n=4-5). Score values are expressed as mean ± SEM. * and *** represent p<0.05 and p<0.001 respectively.
Figure 3: Molecular markers of the hair follicle cell populations are dysregulated in Pparg<sup>∆/∆</sup> mice during hair morphogenesis and cycling

qPCR analysis of gene expression in skin from Sox2-Cre<sup>tg/+;</sup>Pparg<sup>∆/em∆</sup> (Pparg<sup>∆/∆</sup>; red bars) and control (CTL; blue bars) mice; Hair Morphogenesis (empty bars): P1 (n=5), P5 (n=6) and P8 (n=5); Hair Cycle (filled bars): P17, P28 and P49 (n=4). (a) keratin 72 (K6irs), trichohyalin (Tchh); murine type I hair keratins mHa2 and mHa3; (b) forkhead box N1 (Foxn1), homeobox msh-like 2 (Msx2); (c) alkaline phosphatase (Alpl), keratin 5 (Krt5). (d) Keratin 15 (Krt15) and Cd200. Data are normalized to Eef1α1 and expressed as mean ± SEM. *, ** and *** represent p<0.05, p<0.01 and p<0.001 respectively, with respect to control expression at the same time point.

Figure 4: Delayed hair follicle morphogenesis in AZIP<sup>tg/+</sup> lipodystrophic mice

Analysis of back skin sections from AZIP<sup>tg/+</sup> and WT mice (a) Hematoxylin & Eosin staining Scale bar=100µm (b) Quantitative histomorphometry at P1. Hair morphogenesis score (left); % of HFs found in the different hair morphogenesis stages (right panel; n=9-12). (c) Gene expression of keratin 72 (K6irs), trichohyalin (Tchh), murine type I hair keratins mHa2 and mHa3 and forkhead box N1 (Foxn1) in skin from AZIP<sup>tg/+</sup> and WT mice (P5). Data are normalized to Eef1α1 and expressed as mean ± SEM (n=7). (d) Oil Red O staining and CD45 immunostaining (leucocytes, in red) and (e) CD45 quantification. Scale bar=200µm. *, ** and *** represent p<0.05, p<0.01 and p<0.001 respectively, with respect to control at the same time point.

Figure 5: Skin graft experiments rescue Pparg<sup>∆/∆</sup> delayed hair morphogenesis, but not late skin inflammation
(a) Representative images of skin grafts from Sox2-Cre<sup>tg/+</sup>;Pparg<sup>Δ/Δ</sup> (Pparg<sup>Δ/Δ</sup>) and control (CTL) embryos on Foxn1<sup>nu/nu</sup> mice. (b) Quantitative histomorphometry 7 days after engraftment. (c) Hair morphogenesis score (left); % of HFs in the different hair morphogenesis stages (right; n=10-11). (d) Gene expression at day 7 of keratin 72 (K6irs), trichohyalin (Tchh); murine type I hair keratins mHa2 and mHa3; forkhead box N1 (Foxn1), homeobox msh-like 2 (Msx2); Keratin 15 (Krt15), Pparg and adiponectin (Adipoq). Data normalized to Eef1α1 and expressed as mean ± SEM (n=8). (e) CD45 (leucocytes) and F4/80 (macrophages) immunostaining (red) at day 38 and (f) their quantification. Scale bar=200μm. * and ** represent p<0.05 and p<0.01, respectively. γγ
Figure 1

189x237mm (300 x 300 DPI)
Figure 2

100x127mm (300 x 300 DPI)
Figure 3

90x192mm (300 x 300 DPI)
Figure 4
Figure 5

187x171mm (300 x 300 DPI)
DELAYED HAIR FOLLICLE MORPHOGENESIS AND HAIR FOLLICLE DYSTROPHY IN A LIPOATROPHY MOUSE MODEL OF *Pparg* TOTAL DELETION

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SUPPLEMENTARY FIGURES
Figure S1. Mating strategy and genotypes of $Pparg^{\Delta/\Delta}$ mice and their littermates

The Sox2-Cre transgene must be transmitted by a male mouse. Sox2-Cre$^{tg/+}$: carrier of one Sox2-Cre expressing allele. Sox2-Cre$^{+/+}$: no transmission of the Sox2-Cre transgene; $Pparg^{+}$: wild-type allele; $Pparg^{fl}$: functional $Pparg$ allele carrying 2 flox sites flanking exon 1 and 2 of $Pparg$; $Pparg^{\Delta}$: $Pparg$ allele deleted of the genomic region encompassing exon 1 and exon 2, resulting in a non functional allele; $Pparg^{em\Delta}$: $Pparg$ allele deleted during development in all the cells forming the embryo (epiblast deletion) but still present in the trophoblastic cells allowing a normal placental development. The last line gives the corresponding simplified names.
Figure S2: Skin and hair phenotype in \( Pparg^{\Delta/\Delta} \) mice
(a) Representative pictures of Sox2-Cre\(^{tg/+}\);\( Pparg^{\Delta/m\Delta} \) (\( Pparg^{\Delta/\Delta} \)) and littermates taken at P3, P8 and P28. (b) Representative pictures of 4-5 weeks old \( Pparg^{\Delta/\Delta} \) and littermate control mice just shaved on the back. The shaved area is less recognizable in \( Pparg^{\Delta/\Delta} \), due to their shorter hair, but shows flaking skin. (c) Representative pictures of patchy hair loss in 6 months old \( Pparg^{\Delta/\Delta} \) and control mice (CTL). (d) Representative pictures of skin lesions in \( Pparg^{\Delta/\Delta} \) mice.
Figure S3: Lack of sebaceous glands in \( Pparg^{\Delta/\Delta} \) skin
Oil Red O staining of back skin cryosections from \( Sox2-Cre^{tg/+};Pparg^{\Delta/em\Delta} \) \( (Pparg^{\Delta/\Delta}) \) and control (CTL) mice at P28. Yellow arrows indicate the sebaceous glands in control mice and the place where sebaceous glands should be in \( Pparg^{\Delta/\Delta} \) mice. Scale bar= 50µm.

Figure S4: Gene expression analysis of inflammatory markers in \( Pparg^{\Delta/\Delta} \) skin at P28
Gene expression analysis of markers of immune cells, chemokine (C-C motif) ligand 2 \( (Ccl2) \) for macrophages, neutrophil elastase \( (Elane) \) for neutrophils, CD3 \( (Cd3e) \) for T lymphocytes. RNA was isolated from total skin of \( Sox2-Cre^{tg/+};Pparg^{\Delta/em\Delta} \) \( (Pparg^{\Delta/\Delta}) \) and control mice at P28 \( (n=4) \). Data are normalized to \( Eef1\alpha1 \) and expressed as mean ± SEM. *** represents p value <0.001.
Figure S5: Trichohyalin protein expression is desynchronized in \textit{Pparg}^{Δ/Δ} skin
Representative images of IF staining of trichohyalin (red) in skin from \textit{Sox2-Cre}^{tg/+};\textit{Pparg}^{Δ/emΔ} (\textit{Pparg}^{Δ/Δ}) and littermate controls (CTL) at different stages of HF morphogenesis (a) and of HF cycle (b) Nuclei are stained in blue. Scale bar=100µm.
Figure S6: Inflammatory infiltrate appears in PparγΔ/Δ skin starting from P17 onwards

(a) Staining with antibody against major histocompatibility complex class II (MHC II) of skin sections from Sox2-Cre<sup>tg/l</sup>; Pparγ<sup>Δ/Δ</sup> (Pparγ<sup>Δ/Δ</sup>) and control (CTL) mice. Scale bar=100µm (b) DAB staining quantification, normalized to the skin area of each section. Data are expressed as mean ± SEM. * represents p<0.05 (c) Gene expression analysis of skin from Pparγ<sup>Δ/Δ</sup> (γ<sup>Δ/Δ</sup>; red bars) and control (CTL; blue bars) mice at different stages of hair morphogenesis: P1 (n=5), P5 (n=6) and P8 (n=5); and hair cycle: P17, P28 and P49 (n=4). mRNA levels of Chemokine (C-C motif) ligand 2 (Ccl2), as macrophage marker, are shown. Data were normalized to Eef1α1 and expressed as mean ± SEM. *** represents p<0.001.
Figure S7: HF morphogenesis phenotype of fat-specific *Pparg* knock-out mice

(a) Oil Red O staining of back skin cryosections from *Adipoq-Cre*<sup>tg/+;Pparg<sup>fl/fl</sup> (<i>Pparg<sup>ΔΔ</sup></i>) and control (CTL) mice at P28. Yellow arrows indicate the sebaceous glands in *Pparg<sup>ΔΔ</sup>* mice.

(b) Gene expression analysis in full thickness skin of preadipocyte-markers, kruppel like factor 4 (*Klf4*), early growth response 2 (*Egr2*) and preadipocyte factor 1 (*Pref1*) in *Pparg<sup>ΔΔ</sup>* and control (CTL) mice at P1 (n=4 for CTL, n=3 for *Pparg<sup>ΔΔ</sup>*)). Data are normalized to *Eef1α1* and expressed as mean ± SEM. * represents p<0.05.

(c) Gene expression analysis in full thickness skin of keratin 72 (*K6irs*), trichohyalin (*Tchh*); murine type I hair keratins *mHa2* and *mHa3*; forkhead box N1 (*Foxn1*), homeobox msh-like 2 (*Msx2*), Keratin 15 (*Krt15*), *Ppary* (*Pparg*) and adiponectin (*Adipoq*) in *Pparg<sup>ΔΔ</sup>* and control (CTL) mice at P5 (n=4 for CTL, n=3 for *Pparg<sup>ΔΔ</sup>*)). Data are normalized to *Eef1α1* and expressed as mean ± SEM. * represents p<0.05.

(d) Representative images of IF staining of trichohyalin (red) in skin from *Pparg<sup>ΔΔ</sup>* and control (CTL) mice at P5. Nuclei are stained in blue. Scale bar=200µm.
Figure S8: Skin expression of the AZIP transgene and of preadipocyte markers in AZIP\textsuperscript{tg/+} mice

(a) Epidermis and dermis fractions were separated from the skin of AZIP\textsuperscript{tg/+} and littermate wild type control mice. Gene expression analysis of Keratin 10, as a marker of epidermis, and of the AZIP transgene (AZIP tg) were evaluated. (b) Gene expression analysis of preadipocyte-markers, kruppel like factor 4 (Klf4), early growth response 2 (Egr2) and preadipocyte factor 1 (Pref1). RNA was isolated from total skin of Pparg\textsuperscript{Δ/Δ} AZIP\textsuperscript{tg/+} and the respective control mice at P1 (n=7 for CTL, n=5 for Pparg\textsuperscript{Δ/Δ}, n=4 for AZIP\textsuperscript{tg/+} and WT). Data are normalized to Eef1α1 and expressed as mean ± SEM. ** represents p value <0.01.
Figure S9: AZIP\textsuperscript{tg/+} mice show delayed hair growth, but no skin inflammation at adult stage

(a) Representative pictures of AZIP\textsuperscript{tg/+} and littermate controls taken at P5, P17 and 6 months. (b) IF staining of Iba1\textsuperscript{*} macrophages (red) in the skin of 6 months old AZIP\textsuperscript{tg/+} and littermate controls (nuclei are stained in blue). Skin from \textit{Ppar\textsuperscript{gΔ/Δ}} (right panel) was used as positive control. Scale bar=200µm.
Figure S10: Mature adipocytes appear at E17.5 during embryogenesis

Frozen sections of embryos at E14.5 and E17.5 from Sox2-Cre\textsuperscript{tg/+};Pparg\textsuperscript{Δ/Δ} and control (CTL) mice were stained in double immunofluorescence with rabbit anti-caveolin 1 (green) and HCS LipidTOX (red) to mark mature adipocytes, as described in Le Lay et al. 2010. Scale bar=200µm.
Figure S11: Role of adipose tissue for hair follicle postnatal differentiation
(a) Representative images of skin grafts from AZIP\textsuperscript{tg/+} and wild-type (WT) embryos on Foxn1\textsuperscript{nu/nu} mice, 7, 14, 21 and 38 days after engraftment. Scale bar=200\textmu m (b) Representative picture 7 days after engraftment and (c) Quantitative histomorphometry analysis of hair morphogenesis. Hair morphogenesis score (left panel); % of HFs found in the different hair morphogenesis stages (right panel; n=6). (d) Gene expression of keratin 72 (K6irs), trichohyalin (Tchh); murine type I hair keratins mHa2 and mHa3; forkhead box N1 (Foxn1), homeobox msh-like 2 (Msx2); Keratin 15 (Krt15), Pparg and adiponectin (Adipoq). Data are normalized to Eef1a1 and expressed as mean ± SEM (n=8). * represents p<0.05.
Figure S12: No inflammation in AZIP\textsuperscript{tg/+} grafts
38 days after engraftment: (a) CD45 (leucocytes) and F4/80 (macrophages) immunostaining (red) and (b) their quantification. Scale bar=100µm.