a complex connective tissue disorder. In addition, the patients had severe intellectual disability that cosegregated with the connective tissue phenotype. A combination of computational methods demonstrated the impact of the missense mutation on structural stability, multimeric assembly, and dynamic behavior of the PYCR1 complex. We showed that Ala189 plays a pivotal role in maintaining the pentameric and decameric assembly of PYCR1, and that the p.Ala189Val mutation also leads to elimination of the critical inter- and intrasubunit interactions by affecting adjacent charged residues within the region in both pentameric and decameric conformations. These findings are predicted to result in loss of PYCR1 activity with severe phenotypic consequences.

CONFLICT OF INTEREST

The authors state no conflicts of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2016.10.007.

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Effects of Depilation Methods on Imiquimod-Induced Skin Inflammation in Mice

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TO THE EDITOR

Imiquimod (IMQ) acts as a toll-like-receptor-7/8 agonist and its topical application is used as a model for psoriasiform skin inflammation. In mice and humans, skin inflammation is characterized by infiltration of several immune cells (Drobits et al., 2012; Kalb et al., 2012; Palamara et al., 2004) and activation of the IL-23/IL-17/IL-22 axis (Riol-Blanco et al., 2014; van der Fits et al., 2009). Major histocompatibility complex-II upregulation on keratinocytes, hyperproliferation, parakeratosis, and increased vascularization are also observed recapitulating psoriatic hallmarks (Flutter and Nestle, 2013).

Hair removal is essential before IMQ is applied to the murine skin. Different laboratories use various depilation strategies such as razor shaving, depilation cream, or wax stripping. Shaving

Abbreviations: HF, hair follicle; IF, infundibulum; IFE, interfollicular epidermis; IMQ, imiquimod

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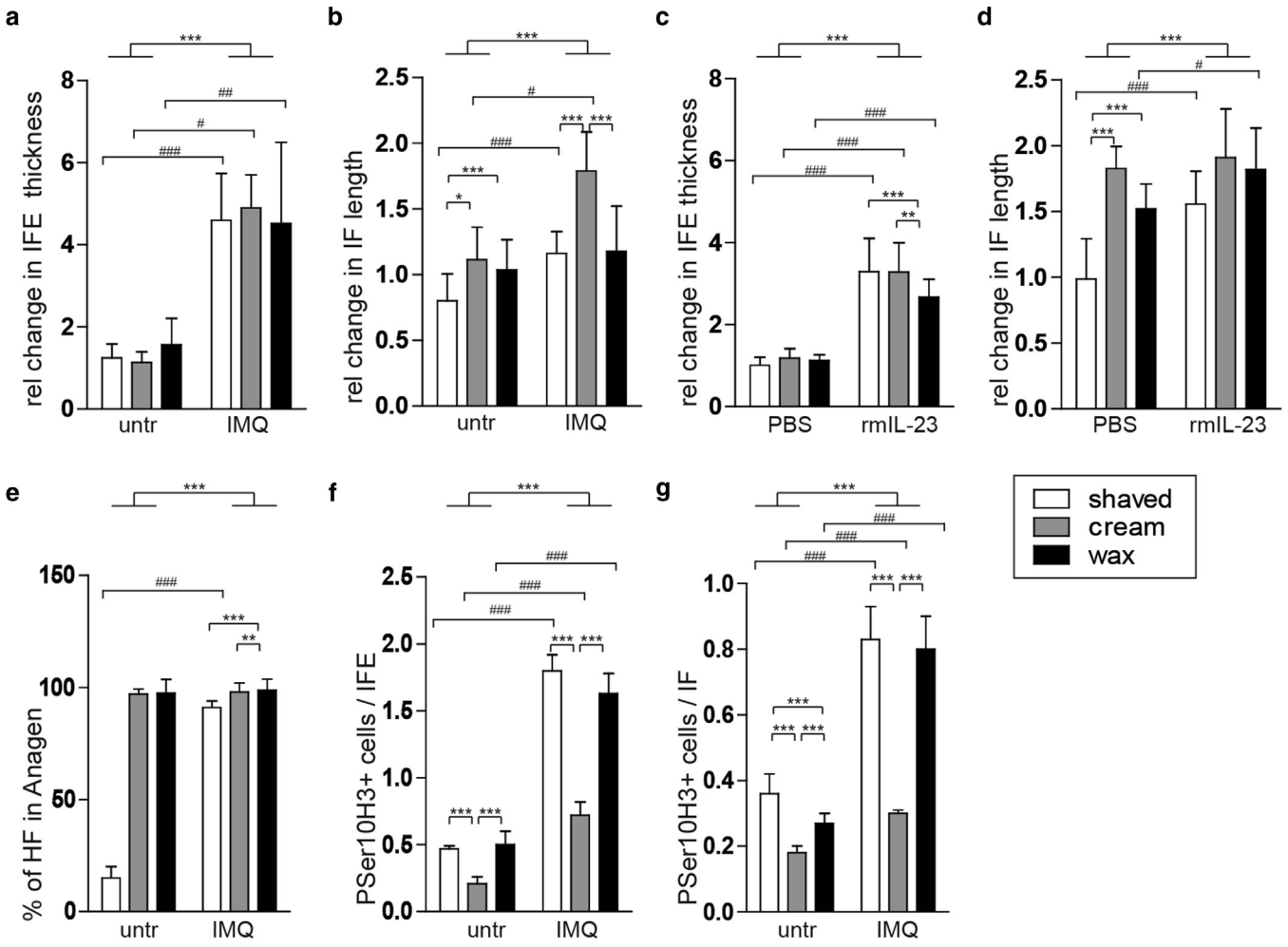

Figure 1. Effects of different depilation methods on epidermal cell lineages following IMQ treatment. (**a**–**d**) Quantification of (**a**) IFE thickness and (**b**) IF length from IMQ treated mice and (**c**) IFE thickness and (**d**) IF length from rmIL-23 injected mice. Data show the relative change to shaved untreated (**a**–**b**) or shaved PBS injected skin (**c**–**d**). (**e**) Quantification of the percentage of anagen hair follicles at indicated treatments. (**f**–**g**) Quantification of numbers of PSer10H3 positive cells in (**f**) the IFE and (**g**) the IF of mice of indicated treatments. $*P < 0.05$, $**P < 0.005$, $***P < 0.001$ for 2-way ANOVA and Bonferroni post-test; $\#P < 0.05$, $\#\#P < 0.005$, $\#\#\#P < 0.001$ for Student's t test.

does not affect hair follicles (HF), whereas wax stripping results in depletion of HF stem cells leading to anagen entry (Amberg et al., 2016; Paus et al., 1994). The effects of depilation cream have not been carefully investigated yet.

We tested if these three different depilation strategies affect the inflammatory response by applying IMQ for 7 consecutive days on the back skin of late telogen (65-day-old) C57BL/6 mice 1 day after hair removal. All IMQ-treated mice exhibited splenomegaly as previously reported, which was not seen in untreated or vehicle-treated controls (Palamara et al., 2004) (Supplementary Figure S1a online). As it is important to distinguish infundibulum (IF) thickening from rete ridges found in human psoriatic skin, we analyzed IF and interfollicular epidermis (IFE), because both compartments harbor stem cells that can replenish injured epidermis. All treatment groups responded to IMQ with a similar increase in IFE thickening (Figure 1a, Supplementary Figure S1b). The IF was already longer in creamed and waxed mice compared with shaved mice and increased further after IMQ application in shaved and creamed, but not in waxed mice (Figure 1b). These results demonstrate that waxing masks the effects of IMQ on the IF.

We next investigated whether these distinct effects of hair removal methods were specific for IMQ or applied also to other triggers of skin inflammation such as rmIL-23 injection. Similar to IMQ treatment we found acanthosis of the IFE after intradermal rmIL-23 injection although waxing induced significantly less IFE thickening than shaving or creaming (Figure 1c, Supplementary Figure S1c). IF length was already increased by phosphate buffered saline injection in creamed and waxed compared with shaved mice (Figure 1d). Except for creamed mice, the IF increased further on rmIL-23 injection (Figure 1d). These results show that different hair removal methods affect skin morphology in two skin inflammation models and that the effects on IFE are less strong with rmIL-23 when compared with IMQ.

We recently showed that telogen HFs from shaved mice treated with IMQ started to enter anagen (Amberg et al., 2016). An analysis of HF stem cell activation by staining for the proliferation marker Ki67 or the M-phase