INTRODUCTION
Psoriasis is a chronic inflammatory skin disease affecting approximately 2% of the population in the Western world (Di Meglio et al., 2014). The complex pathogenesis of psoriasis is based on a genetic predisposition that can give rise to chronic scaling cutaneous plaques with variable joint and cardiovascular involvement (Alwan and Nestle, 2015). Psoriatic skin lesions are characterized by a mixed inflammatory infiltrate consisting of neutrophils, dendritic cells (iDCs), macrophages, and T cells (Boehncke and Schön, 2015). Macrophages and DCs are of special immanent importance as antigen-presenting cells linking innate immune sensing and stimulation of adaptive immune responses (Nestle et al., 1994). Psoriatic skin comprises a network of DCs including a large population of 6-sulfo LacNAc (slan) DCs (Günther et al., 2012b; Hansel et al., 2011; Schäkel et al., 1998). They stand out by their capacity to secrete proinflammatory cytokines and stimulate T helper 1/T helper 17 T-cell responses, which drive hyperproliferation of keratinocytes and psoriatic inflammation (Eberle et al., 2016).

The immigration of inflammatory cells is orchestrated by a complex network of chemokines (Comerford et al., 2014). We have recently described that CXC chemokine ligand (CXCL) 16 is upregulated on keratinocytes and myeloid antigen-presenting cells in psoriatic skin and has the capacity to induce migration of CD8+ T cells in vivo (Günther et al., 2012a). CXCL16 is a membrane-bound chemokine released after proteolytic cleavage to exert its chemotactic function (Gough et al., 2004). The expression of the CXCL16 receptor (CXCR) 6 is not only restricted to CD8+ T cells, but is also found on type 1 polarized peripheral blood CD4+ T cells, γδ T cells, natural killer cells, natural killer T cells, monocytes, and neutrophils (Gaida et al., 2008; Huang et al., 2008; Kim et al., 2001; Unutmaz et al., 2000). Neutrophils might be especially important in the induction phase of psoriasis and contribute by secretion of proinflammatory cytokines, including IL-17, and the formation of neutrophil extracellular traps, which are rich in self-nucleic acids (NAS) (Brinkmann et al., 2004; Christophers et al., 2014; Keijser et al., 2014; Lin et al., 2011). The impact of CXCL16 on neutrophil recruitment in psoriasis has not yet been specifically addressed. Neutrophils are effector cells of the innate immune system and stimulation of innate immunity has increasingly been recognized as a potential initial trigger for psoriasis. It is known that psoriasis can be provoked or exacerbated by a variety of environmental factors, including the microbiome consisting of bacterial and viral pathogens colonizing the skin (Fry and Baker, 2007). Toll-like receptors (TLRs) recognize bacterial wall components or NAS. TLRs binding NAS are located in endosomal compartments to avoid unnecessary immune activation to self-NAs that are
continuously released by dying host cells or extruded by activated neutrophils (Akira et al., 2006). In psoriasis, the high prevalence of antimicrobial peptides can break protective mechanisms by complexing and stabilizing DNA and RNA not only from pathogens, but also from self-originating NAs. This activation has been recognized as a major innate immune pathway triggering psoriasis (Demaria et al., 2014).

We therefore aimed at investigating the effect of TLR stimulation on the regulation of CXCL16 expression. Hereby we could find a strong induction of CXCL16 after TLR ligation that led to an induction of neutrophil shape changes and deformation contributing to cell activation and recruitment.

RESULTS

TLR-mediated expression of CXCL16 on monocytes and slanDCs

We have previously shown that the chemokine CXCL16 is upregulated in psoriatic skin and mainly expressed on CD163+ macrophages and CD11c+ dendritic cells in the dermis (Gunther et al., 2012a). To further analyze the role of CXCL16 in the pathogenesis of psoriasis, we determined the CXCL16 expression on peripheral blood mononuclear cells (PBMCs) by flow cytometry. CXCL16 was mainly expressed on CD14+CD16low and CD14lowCD16+ monocytes and slanDCs (Figure 1a and Supplementary Figure S1 online). Interestingly, antigen-presenting cells from psoriasis patients showed a higher surface expression of CXCL16 compared with healthy controls (Figure 1a). This expression is sustained during migration in the skin, as we could detect CXCL16-expressing CD163+ monocytes/macrophages (Gunther et al., 2012a) and CXCL16-positive slanDCs in lesional psoriatic skin (Supplementary Figure S2 online).

Given the elevated expression of CXCL16 on antigen-presenting cells in psoriasis patients, we asked which trigger factors could be responsible for induction of CXCL16. We observed that already spontaneous maturation in medium for 24 hours can upregulate expression of CXCL16 on CD14+CD16low monocytes (8.4-fold), CD14lowCD16+ monocytes (2.6-fold), and slanDCs (4-fold) compared with freshly isolated cells (Figure 1b). In psoriasis, activation of highly expressed TLRs by bacterial or viral infections can contribute to the initiation and maintenance of the disease (Carrasco et al., 2011; Fry and Baker, 2007; García-Rodríguez et al., 2013). Stimulation of differentiated mononuclear cells from psoriasis patients with the synthetic TLR ligands Pam2 (TLR2/6), Pam3 (TLR2/1), and R837 (TLR7) significantly enhanced the expression level of CXCL16 on the monocyte subpopulations CD14+CD16low (1.6-fold; Figure 1c) and CD14lowCD16+ (2-fold; Figure 1d) compared to unstimulated cells. This effect was not observed on slanDCs (Figure 1e). CXCL16 expression levels after TLR ligation were increased compared to healthy controls (Figure 1g). In contrast, stimulation with lipopolysaccharide (LPS; TLR4) and R848 (TLR8/7) inhibited CXCL16 expression by 60% on monocytes and DCs compared to unstimulated cells from psoriasis patients (Figure 1c–1e).

Chemotactic effects of CXCL16 are exerted by the soluble chemokine upon enzymatic cleavage of membrane bound CXCL16. Likewise to transmembrane chemokine expression, Pam2, Pam3, and R837 increased the CXCL16 secretion and LPS and R848 inhibited the release of CXCL16 (Figure 1f, 1g).

Tumor necrosis factor—α antagonists downregulate CXCL16 in vitro and in vivo

The pro-inflammatory cytokine tumor necrosis factor—α (TNF-α) is highly expressed in psoriasis (Tohyama et al., 2007) and is a strong inducer of CXCL16 expression and secretion in monocytes and slanDCs (Figure 2a, 2b). Blockade of TNF-α by treatment with etanercept completely inhibited TNF-α—induced CXCL16 expression and secretion in monocytes and slanDCs from psoriasis patients (Figure 2a, 2b). On mononuclear cells cultured in medium for 24 hours, the TNF-α blocker etanercept and adalimumab ameliorated the CXCL16 surface expression by 60% on slanDCs and by 40% on monocytes (Figure 2c). In addition, upon Pam2 or R837 stimulation, both inhibitors significantly decreased the CXCL16 surface expression on monocyte subpopulations (Figure 2c). In the PBMC supernatant, concentration of soluble CXCL16 was reduced by 20% after preincubation with TNF-α blockers (Figure 2c). In all experiments, the effects of etanercept and adalimumab were similar. To validate our findings in vivo, we analyzed skin biopsies from patients treated with adalimumab. We detected a decrease in CXCL16 expression in lesional skin after a 4-week treatment period (Figure 2d and Supplementary Table S1 online). This was comparable to the CXCL16 reduction by etanercept observed previously (Gunther et al., 2012a).

Keratinocytes from psoriasis patients secrete more CXCL16 upon TLR and TNF-α stimulation

Staining of CXCL16 in psoriatic lesions demonstrates chemokine expression not only in dermal inflammatory cells, but also a bright expression in the epidermis, which can result from CXCL16 expression and secretion by lesional keratinocytes (Figure 2d) (Gunther et al., 2012a). Analyzing primary keratinocytes from psoriatic lesions in comparison to healthy controls did not demonstrate significantly enhanced surface expression of CXCL16 (Figure 3a). However, the amount of CXCL16 secreted by keratinocytes from psoriatic patients after 24 hours of culture conditions and the CXCL16 protein levels analyzed by Western blot were significantly enhanced in psoriatic keratinocytes compared to healthy controls (Figure 3b, 3c). This suggests that production of CXCL16 in psoriatic epidermis is enhanced, resulting in a high turnover rate of transmembrane-expressed chemokine. Due to enhanced levels of ADAM10 in psoriatic epidermis (Oh et al., 2008), CXCL16 will be rapidly cleaved and secreted as indicated by the enhanced epidermal staining in lesional skin (Figure 2d).

Keratinocytes build the primary defense to the environment and constitutively express TLR 1, 2, 3, 4, and 6 (Baker et al., 2003; Begon et al., 2007; Lebre et al., 2007). Stimulating keratinocytes with the synthetic TLR agonists polyinosinic-polycytidylic acid (poly(IC); TLR3), Pam2 (TLR2/6), Pam3 (TLR2/1), or LPS (TLR4) for 24 hours did not influence cell surface expression of CXCL16 (Figure 3d), but stimulation with poly(IC) or Pam2 led to an increase in CXCL16 secretion (Figure 3e), indicating the influence of external trigger factors in CXCL16-mediated inflammation. The response after poly(IC) stimulation was significantly higher in keratinocytes from psoriasis patients (Figure 3b).
Besides monocytes and slanDCs, keratinocytes from psoriatic lesions also exhibited an increased production of soluble CXCL16 upon stimulation with the proinflammatory cytokine TNF-\(\alpha\) for 24 hours. This effect was completely inhibited by the TNF-\(\alpha\) blocker etanercept (Figure 3f).
TLR-dependent CXCL16 regulation is enhanced by type I IFN and inhibited by IL-10

TLR-mediated detection of NAs and production of type I IFN are linked to the induction of psoriasis (Kawai and Akira, 2007; Nestle et al., 2005). Because stimulation with the RNA ligands R837 (TLR7) on monocytes and poly(IC) (TLR3) on keratinocytes induces a signaling pathway that can lead to the production of type I IFN (Noppert et al., 2007), we first measured the concentration of type I IFN in the supernatants of PBMCs and keratinocytes by IFN reporter assay. Compared to unstimulated cells, high levels of type I IFN were detectable in supernatants of R837-stimulated PBMCs (Figure 4a) and poly(IC)-stimulated keratinocytes (Figure 4b) of psoriasis patients. Stimulation with IFN-beta resulted in an upregulation of CXCL16 on CD14<sup>+</sup>CD16<sup>-</sup> monocytes and slanDCs (Figure 4c), as well as an increased production of soluble CXCL16 by keratinocytes (Figure 4d) that was higher in patients with psoriasis compared with healthy controls suggesting cell intrinsic priming.

To further explore the mechanism of CXCL16 inhibition after stimulation of TLR4 and TLR7/8 on mononuclear cells, we analyzed supernatants of PBMCs from patients with psoriasis for secretion of IL-10, which has been suggested to downregulate CXCL16 (van Lieshout et al., 2009) and has been shown to be induced by TLR4 and TLR7/8 (Ghosh et al., 2006; Schildberger et al., 2013). Indeed, we detected elevated concentrations of IL-10 after LPS (TLR4) or R848 (TLR7/8) stimulation (Supplementary Figure S3a online). The inhibitory effect on CXCL16 was confirmed by dose-dependent stimulation of PBMCs with IL-10 for 24 hours. IL-10 (5 ng/ml) significantly decreased the secretion of soluble CXCL16 by 18% and the expression of transmembrane CXCL16 on CD14<sup>+</sup>CD16<sup>-</sup> monocytes by 44%.
CD14^{low}CD16^{+} monocytes by 31% and slanDCs by 38% (Supplementary Figure S3b, S3c).

CXCL16 enhances migratory and mechanical properties of CXCR6 and CXCL16 expressing neutrophils

Upregulation of CXCL16 in psoriasis can facilitate the recruitment of CXCR6^{+}CD8^{+} T cells into the skin (Gunther et al., 2012a). Besides T cells, neutrophils are important effector cells in psoriasis profoundly infiltrating into skin (Supplementary Figure S4 online), which secrete proinflammatory cytokines such as IL-1β, IL-6, IL-17, and IL-23 (Tecchio et al., 2014; Terui et al., 2000). Staining of neutrophils infiltrating psoriatic lesions revealed expression of the CXCL16 receptor CXCR6 on CD66b^{+} neutrophils (see Supplementary Figure S4a). In addition, neutrophils isolated from the peripheral blood of psoriasis patients expressed CXCR6 on their surface (see Supplementary Figure S4b), which was enhanced upon stimulation with phorbol 12-myristate 13-acetate.

After confirming the expression of CXCR6, we analyzed chemotaxis of isolated neutrophils in response to CXCL16 in vitro using a transwell migration assay. CXCL16 induced a dose-dependent migration of neutrophils in patients with psoriasis (Figure 5a), as well as healthy controls (data not shown). An important chemotactic factor for neutrophil attraction into psoriatic lesions is IL-8 (also known as CXCL8) (Schröder et al., 1992). Because chemotaxis of inflammatory cells is a redundant process, we investigated whether CXCL16 can potentiate IL-8-induced chemotaxis. Indeed, the simultaneous stimulation by CXCL16 and IL-8 resulted in an enhanced migratory response of neutrophils (Figure 5a).

In addition, immunohistochemical staining of psoriatic lesions revealed expression of CXCL16 by neutrophils in the upper dermis (Figure 5b) and in dermal located blood vessels (Figure 5c) (see also Supplementary Table S1). Western blot analysis of lysed neutrophils isolated from blood of patients with psoriasis confirmed expression of CXCL16 similar to the known expression of CXCL16 on antigen-presenting mononuclear cells (Figure 5d). Stimulation of neutrophils with IL-8 for 24 hours induced secretion of soluble CXCL16 (Figure 5e), suggesting a positive feedback loop.

As transmigration into tissue requires activation and mechanical deformation of neutrophils, we further investigated the effect of the chemoattractant IL-8 and CXCL16 on mechanical properties of neutrophils using a recently described technique called real-time deformability cytometry.
Hereby, cells are deformed in a microfluidic channel constriction and analysis of morphological and mechanical parameters is performed in real-time at rates up to 1,000 cells/s (Otto et al., 2015). First, we analyzed size (projected area of the cell) and shape of neutrophils. The latter is estimated by the area ratio of convex hull and contour of the cell. Compared to healthy neutrophils, untreated neutrophils from psoriasis patients were larger and had an increased area ratio indicating an irregular shape (Figure 6a, 6b). This can be interpreted as sign of pre-activation. Next we analyzed the deformation D, as described in Otto et al. (2015). Untreated neutrophils from psoriasis patients showed a higher deformation (Figure 6a, 6b). IL-8 (50 ng/ml) induced a significant increase in area, area ratio, and deformation of neutrophils that was observed to a similar extent in neutrophils isolated from healthy controls or patients with psoriasis (Figure 6a, 6b). Instead, an increased deformation upon CXCL16 ligation (100 ng/ml) was recorded on healthy neutrophils only, but was less strong compared with IL-8 (Figure 6a, 6b). The increased deformation of round, inactivated neutrophils (area ratio of 1.0:1.05) from healthy donors upon CXCL16 and IL-8 stimulation indicates cell softening (Figure 6c). Simultaneous stimulation with both chemokines had no additional effect on activation or deformation (Figure 6b, 6c).

**DISCUSSION**

Chemokines orchestrate the overboarding immune activation in psoriasis, which leads to hyperproliferation of keratinocytes accompanied by infiltrating immune cells. Psoriasis can be provoked by a variety of environmental factors, which include the microbiome colonizing the skin. TLRs are the most important class of innate immune receptors recognizing pathogen-associated molecular patterns (Akira et al., 2006). Here, we demonstrate that ligation of TLR2/6 and TLR2/1 on monocytes and keratinocytes upregulate CXCL16 secretion. TLR2/6 can be engaged by diacylated lipopeptides from Gram-positive bacteria, such as *Staphylococcus aureus* (Pietrocola et al., 2011). Colonization with *S. aureus* has been reported in 60% of psoriatic skin lesions and correlated with the psoriasis area severity index score (Balci et al., 2009; Tomi et al., 2005). TLR2 can also be engaged by other Gram-positive bacteria, such as streptococcal organism. This could be pathogenically relevant in cases of psoriasis induced by *Streptococcus pyogenes* throat infection. Invading pathogens could access antigen-presenting cells in the tonsils, which migrate through lymphatics and blood vessels into the skin and activate the immune response (Baker et al., 2006). Besides exogenous factors, there are many endogenous TLR ligands, such as antimicrobial peptides and proteins, overexpressed in psoriatic skin. This overexpression may trigger inflammation in psoriasis by activation of proinflammatory cytokines and chemotaxis toward immune cells (Lai and Gallo, 2009). S100 proteins, such as S100A7, S100A8, and S100A9, as well as human β-defensin 2 and 3, antimicrobial ribonuclease RNase 7, lysozyme, and cathelicidin LL37, were identified to be highly produced by keratinocytes from patients with psoriasis vulgaris (Buchau and Gallo, 2007). We have shown that stimulation of TLR3 and TLR7 can lead to CXCL16 upregulation. TLR3 is thought to recognize double-stranded RNA, while TLR7 has been implicated in single-stranded RNA recognition. These ligands could originate from bacteria or self-NAs. Cationic antimicrobial peptides, such as LL37 and β-defensin, which are
overexpressed in psoriatic skin (Lande et al., 2015) can form complexes with free self-DNA and self-RNA. These self-RNA—antimicrobial peptide complexes protect NAs from degradation and can activate human cells through TLR7 (Ganguly et al., 2009; Lande et al., 2007). TLR7 is expressed on macrophages and slanDCs (Hansel et al., 2013). The importance of TLR7 ligation for triggering psoriasis has been demonstrated best by the observation that topical application of the TLR7 agonist imiquimod to the skin exacerbates human psoriasis (van der Fits et al., 2009). TLR7 and TLR9 are
also expressed by plasmacytoid DCs, which sense RNA and DNA released by dying bacteria and host cells coupled with antimicrobial peptides resulting in type I IFN secretion (Lande et al., 2007; Meller et al., 2015). Plasmacytoid DCs have been described as important innate immune cells in the initiation phase of psoriasis and are well known as the most potent type I IFN producing immune cells (Nestle et al., 2005; Takagi et al., 2016). Interestingly, our data revealed that CXCL16 can be induced by type I IFN, which implicates its importance in an early phase of psoriasis induction. Type I IFN is induced IRF-3/7-dependent after ligation of TLR3 and TLR7, whereas stimulation of TLR2/1 and TLR2/6 activates the MyD88- and NF-κB-dependent pathway (Kawai and Akira, 2007) and could explain the TLR2-mediated upregulation of CXCL16 in keratinocytes (see Supplementary Figure S5 online). CXCL16 could be induced by both TLR signaling pathways highlighting its importance in the innate immune response against pathogen-associated molecular patterns, including microbes and self-NAs. Importantly, both types of TLR signaling leading to CXCL16 upregulation were...
induced in keratinocytes. Their function as epidermal barrier with first contact to the microbiome further implicates CXCL16 upregulation as an initial inflammatory mechanism in psoriasis. Furthermore, TNF-α is a potent stimulator of CXCL16 expression (Gunther et al., 2012a), and we could show that TNF-antagonizing drugs ameliorate CXCL16 expression in vivo and in vitro even upon TLR2 and TLR7 ligation supporting the role of CXCL16 in the pathogenesis of the disease. In rare cases, triggering psoriasis under TNF blockade can be observed (Joyau et al., 2012), which could be explained by type I IFN triggering the disease, including an upregulation of CXCL16 upon TLR2/1, 2/6, or TLR7 stimulation.

Neutrophils are important effector cells in psoriasis that characteristically invade the stratum corneum and lead to inflammation by secretion of neutrophil extracellular traps, whereby they extrude web-like chromatin strands complexed with antimicrobial peptides, including β-defensin and LL37 (Hoffmann and Enk, 2016). In addition, activated neutrophils secret prestored enzymes and proinflammatory mediators that are indispensable for the development of psoriasis (Schön et al., 2017). The attraction of neutrophils to the skin is mediated by a network of chemotactic factors secreted by keratinocytes and immune cells, of which IL-8 is one of the most potent regarding neutrophil activation. However, blocking of IL-8 alone in a clinical trial did not sufficiently control the disease, indicating that a concerted action of chemoattractants might be responsible for inducing neutrophil migration (Abgenix, 2002). Here, we demonstrated that CXCL16 can stimulate chemotaxis of neutrophils and additionally enhance the effect of IL-8 on neutrophil attraction. These findings reveal CXCL16 as an important chemokine mediating neutrophil migration through the tissue toward the epidermis of early psoriatic lesions (Christophers et al., 2014).

During the process of tethering and rolling at the vessel wall, neutrophils become activated and eventually transmigrate through the endothelium accompanied by squeezing through microvascular constriction (Ley et al., 2007; Seely et al., 2003). Using real-time deformability cytometry that permits short time-scales deformation (~1 ms) mimicking the rapid transmigration process across narrow constrictions we could conclude that neutrophils from blood of patients with psoriasis are pre-activated and more prone to deformation upon shear stress. This could be an advantage for efficient transmigration into the skin additionally supported by IL-8 and therefore enhance the inflammatory process of psoriasis. Shape changes and increased deformation induced by CXCL16 and IL-8 stimulation of neutrophils from healthy controls directly implicate an effect on the cellular cytoskeleton (Sham et al., 1993; Westlin et al., 1992). In combination with the known increased compliance and a low advection time of differentiated neutrophils (Ekpenyong et al., 2012), this could promote entering narrow constrictions, such as the vascular endothelium. Interestingly, neutrophilic granules also contain chemotactic factors, which are released upon stimulation and contribute to the vicious cycle of inflammation (Cassatella et al., 1997). Our experiments demonstrated that neutrophils in psoriatic lesions contain prestored CXCL16 and stimulation of neutrophils by IL-8—induced secretion of this chemotactant, which could further contribute to maintain psoriatic inflammation.

In conclusion, these findings suggest that upregulation of CXCL16 by innate immune sensing upon TLR ligation and type I IFN stimulation in psoriasis does not only have a stimulating effect on CXCL16-mediated migration of neutrophils, but also alters their mechanical properties facilitating transmigration into tissue. Additionally, IL-8—induced secretion of CXCL16 by neutrophils supports the positive feedback loop (see Supplementary Figure S6 online). Targeting CXCL16 could therefore be a potential therapeutic target in psoriasis.

**MATERIALS AND METHODS**

**Patients**

Blood and skin samples were taken from patients with psoriasis vulgaris (mean ± standard deviation age 47.9 ± 15.1 years), who did not receive any systemic treatment with immunosuppressive drugs for at least 2 weeks. Diagnosis of psoriasis vulgaris was confirmed by clinical and histologic criteria. Blood from age-matched healthy volunteers was used as control. The investigational protocols (EK396112011 and 539122015) were approved by the Ethics Committee of the University Hospital of the Technical University Dresden according to the Declaration of Helsinki, and patients provided written informed consent for this study.

**In vitro stimulation of PBMCs**

PBMCs were isolated from heparinized whole blood from psoriasis patients and healthy controls by density gradient centrifugation using Biocoll separating solution (Biochrom, Berlin, Deutschland). PBMCs were cultured in RPMI 1640 medium (Gibco LifeTechnologies, Carlsbad, CA) containing 10% fetal calf serum (Sigma-Aldrich, St. Louis, MO), 2 mM l-glutamine, 1% nonessential amino acids, 100 U/ml penicillin, and 100 mg/ml streptomycin (Biochrom, Cambridge, UK) with or without therapeutic concentrations of 10 μg/ml etanercept or 5 μg/ml adalimumab at 1 × 10⁶ cells/ml in 6-well plates for 6 hours at 37°C in 5% CO₂. Synthetic TLR agonists (100 ng/ml Pam2CSK4, 100 ng/ml Pam3CSK4, 1 μg/ml imiquimod (R837), 1 μg/ml R848 (all from InvivoGen), 100 ng/ml LPS (Sigma-Aldrich), or recombinant human proteins TNF-α (InvivoGen), IFN-beta and IL-10 (both from PeproTech, Rocky Hill, NJ) were added for additional 18 hours. Determination of transmembrane and soluble CXCL16 was performed by flow cytometry and ELISA.

**In vitro stimulation of keratinocytes**

Primary human keratinocytes were derived from skin biopsies of psoriasis patients or healthy controls. The epidermis was separated from dermis by dispase treatment over night at 4°C. Keratinocytes were extracted by trypsin incubation at 37°C (Sham et al., 1993). Until confluence (C6/C14) were added for additional 18 hours. Determination of transmembrane and soluble CXCL16 was performed by flow cytometry and ELISA.
Flow cytometry
For flow cytometric analysis, cells were incubated with fluorescent primary and secondary antibodies (Supplementary Table S2 online) and analyzed on a FACSCalibur (BD Biosciences, San Jose, CA).

ELISA
Soluble CXCL16 in cell-free supernatants was quantified using Duoset ELISA assay (R&D Systems, Minneapolis, MN).

Statistical analysis
Data are presented as bars (indicating mean ± standard deviation) or box plots (indicating medians, whiskers: 5th to 95th percentiles and standard deviations). Statistical analysis was performed using repeated-measures one-way analysis of variance (in normally distributed values tested by Shapiro-Wilk test) with Bonferroni’s multiple-comparison post-hoc test, unless otherwise indicated, with the help of GraphPad Prism 6 (GraphPad Software, San Diego, CA) or using linear mixed-effects models with ShapeOut (Mueller et al., 2017). In all cases, *P < 0.05 was considered to be statistically significant (**P < 0.01, ***P < 0.001).

CONFLICT OF INTEREST
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