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MMP-10 Regulates Collagenolytic Activity of Alternatively Activated Resident Macrophages

Maryam G. Rohani[1,2,5,*], **Ryan S. McMahan**[1,3], **Maria V. Razumova**[4], **Angie L. Hertz**[1,2], **Maryelise Cieslewicz**[4], **Suzie H. Pun**[4], **Michael Regnier**[4], **Ying Wang**[1,2,5], **Timothy P. Birkland**[1,2], and **William C. Parks**[1,2,5]

[1]Center for Lung Biology, University of Washington, Seattle, WA USA

[2]Departments of Medicine, University of Washington, Seattle, WA USA

[3]Environmental and Occupational Health Sciences, University of Washington, Seattle, WA USA

[4]Bioengineering, University of Washington, Seattle, WA USA

[5]Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA USA

Abstract

MMP-10 is expressed by macrophages and epithelium in response to injury, but its functions in wound repair are unknown. We observed increased collagen deposition and skin stiffness in *Mmp10*$^{-/-}$ wounds with no difference in collagen expression or re-epithelialization. Increased collagen deposition in *Mmp10*$^{-/-}$ wounds was accompanied by less collagenolytic activity and reduced expression of specific metallocollagenases, particularly MMP-8 and MMP-13, where MMP-13 was the key collagenase. Ablation and adoptive transfer approaches and cell-based models demonstrated that the MMP-10-dependent collagenolytic activity was a product of alternatively activated (M2) resident macrophages. These data demonstrate a critical role for macrophage MMP-10 in controlling the tissue remodeling activity of macrophages and moderating scar formation during wound repair.

Introduction

Scar formation, the deposition of fibrous connective tissue within a wound bed, is a normal consequence of injury and provides temporary strength to damaged tissue. However, excessive, persistent scarring can lead to a variety of clinical problems, such as keloids, hypertrophic scars, and severe life-threatening fibrosis of lung, kidney, and liver. In these conditions, excess scarring is seemingly due to both an over-abundance of collagen production and inadequate resolution (McKleroy *et al*., 2013).

[*]**Corresponding Author** Maryam G. Rohani, Cedars-Sinai Medical Center, 8700 Beverly Blvd., AHSP A9403, Los Angeles, CA 90048 USA, Phone: 424-315-2379, Fax: 310-967-8370, maryam.rohani@cshs.org.

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Although far from being fully understood, resolution of scarring and fibrosis appears to be the responsibility of macrophages and, in particular, alternatively activated (M2) macrophages (Atabai *et al*., 2009; Madsen *et al*., 2013). Current models indicate that matrix turnover involves two sequential steps: limited extracellular proteolysis followed by uptake and lysosomal degradation (Madsen *et al*., 2011). For the first step, some matrix metalloproteinases (MMPs) cleave the large collagen fibrils into fragments that can be endocytosed and degraded intracellularly (Lee *et al*., 2006; Madsen *et al*., 2007; Wagenaar-Miller *et al*., 2007). MMPs can also contribute to resolution of scarring and fibrosis indirectly by shaping the proteolytic phenotype of cells (Giannandrea and Parks, 2014).

In response to injury, stromelysin-2 (MMP-10) is induced by epithelial cells and macrophages in many organs, including skin (Kassim *et al*., 2007; Koller *et al*., 2012; Saarialho-Kere *et al*., 1994). In collaboration with others, we reported that over-expression of active MMP-10 in basal keratinocytes did not affect re-epithelialization and led to only a minor phenotype in basement membrane deposition (Krampert *et al*., 2004). The function of MMP-10 during wound repair remains to be elucidated.

Like most tissues, skin contains a population of resident macrophages, and following injury, additional macrophages influx into the wound bed (Jameson *et al*., 2005). Macrophages have distinct roles during different phases of wound repair, but it is not clear how these roles are split between resident and recruited cells. Macrophages contribute to scar formation by producing profibrotic cytokines that activate resident fibroblasts and pericytes into ECM-producing myofibroblasts (Duffield, 2003; Wynn and Barron, 2010). Depletion of macrophages soon after injury impairs formation of vascularized granulation tissue and delays repair (Goren *et al*., 2009; Lucas *et al*., 2010). On the other hand, as demonstrated in liver injury, depletion of macrophages during later phases leads to an inability to resolve fibrotic matrix (Duffield *et al*., 2005; Ramachandran *et al*., 2012). Early phase macrophages are predominately classically-activated proinflammatory cells (M1-biased), whereas the resolution-phase cells tend to be alternatively activated, remodeling-competent macrophages (M2-biased) (Lichtnekert *et al*., 2013; Mosser and Edwards, 2008).

Here we report that scarring was increased in wounded skin of *Mmp10*$^{-/-}$ mice. We found that excess ECM was not associated with increased expression of collagen but rather with reduced metallo-collagenolytic activity of resident macrophages. As MMP-10 lacks collagenolytic activity, these findings indicate that this proteinase regulates the ECM degradative potential of macrophages.

Results

MMP-10 does not Affect Re-epithelialization

Using RNA from uninjured wildtype mouse skin, we detected a Ct range for *Mmp10* mRNA between 36–37 (Fig. S1), indicating essentially no or very low levels of expression. Similar to that reported earlier (Krampert *et al*., 2004), *Mmp10* was induced by day 3 post wounding, remained elevated at day 8, and began to decline by day 12 (Fig. S1). To examine the role of MMP-10 in re-epithelialization, we compared the rate of wound closure between wildtype and *Mmp10*$^{-/-}$ mice (Fig. S2). However, wound closure rates (i.e., slope

of the lines) did not differ between wildtype and *Mmp10*$^{-/-}$ wounds and both visual (Fig. S2) and histologic (not shown) analyses showed complete reepithelialization in both genotypes by day 8 post-injury.

MMP-10 Moderates Collagen Deposition

During wound healing, granulation tissue is replaced with newly deposited type I collagen forming a scar. Net collagen deposition is the sum of syntheses minus turnover. In skin wounds, collagen production begins 3–5 days after wounding and peaks around day 10 (Wu *et al*., 2003). Between days 3–8, we observed a trend for more deposition of collagen in *Mmp10*$^{-/-}$ wounds compared to wildtypes (not shown), which led to significantly elevated levels of accumulated collagen deposition by day 12 in null mice (Fig. 1a–c). Consistent with more collagen deposition, we found, using an established method that measures the dynamic mechanical strength of skin wounds (Jørgensen *et al*., 1993), that day-12 *Mmp10*$^{-/-}$ wounds were significantly stiffer than wildtype wounds (Fig. 1d). These data indicate that MMP-10 moderates scar formation. In intact skin, collagen levels did not differ significantly between wildtype (155 ± 26.8 μg/biopsy) and *Mmp10*$^{-/-}$ (165.7 ± 26.0) mice.

MMP-10 does not Affect Collagen mRNA Levels

We assessed if increased collagen deposition in *Mmp10*$^{-/-}$ wounds was due to increased synthesis. Basal levels of *Col1a1* mRNA and its increase and decline in response to injury did not differ between genotypes (Fig. S3a). In addition, we found no significant difference in α-SMA protein levels, a marker of myofibroblasts, the major cell source of collagen synthesis in scars, between wildtype and *Mmp10*$^{-/-}$ wounds at days 3 (not shown), 8, or 12 (Fig. S3b). Furthermore, we found no difference in the proliferation rate, the basal or TGFβ1-stimulated expression of α-SMA, or collagen uptake between primary dermal fibroblasts from wildtype or *Mmp10*$^{-/-}$ mice (Fig. S4).

MMP-10 Promotes Collagen Degradation

The above data indicate that increased deposition in *Mmp10*$^{-/-}$ wounds was due to impaired turnover. In defined, *in vitro* degradation assays MMP-10 does not cleave native type I collagen (Parks *et al*., 2004; Sternlicht and Werb, 2001). Thus, we assessed if MMP-10 influences the overall collagenolytic activity in wounds. For this, wound samples were incubated with quenched-fluorescent type I collagen (DQ collagen) and release of fluorescence was measured as an indicator of total collagenase activity. Indeed, significantly less collagenase activity was released from *Mmp10*$^{-/-}$ wounds compared to wildtype samples (Fig. 2a).

We used biopsies of intact skin as an *ex vivo* injury model (Dumin *et al*., 2001). In wildtype explants, induction of *Mmp10* mRNA was detected at 72 h post-biopsy (Fig. S5a), similar to the *in vivo* pattern (Fig. S1). Using both DQ and native type I collagens, we found significantly less collagenase activity was released from *Mmp10*$^{-/-}$ explants compared to wildtype samples (Fig. 2b,c). The levels of collagenase activity released from wildtype and *Mmp10*$^{-/-}$ tissues and the differences between genotypes were comparable for both wound samples and explants (Fig. 2a,b). Because recruited inflammatory cells are not present in

explants, the close similarity in the data between these models indicates that the MMP-10-dependent collagenase activity was a product of cells residing in intact skin.

To identify the nature of the reduced collagenolytic activity, we treated wound samples with GM6001, a broad-spectrum metalloproteinase inhibitor. In the presence of GM6001, collagenase activity was reduced to the same level in both genotypes (Fig. 2d) indicating that MMP-10 promoted the expression and/or activity of a metallocollagenase(s). Addition of active recombinant MMP-10 (rh-MMP10) increased the level of collagenase activity released from *Mmp10*$^{-/-}$explants but had no effect on the activity from wildtype samples (Fig. 2e). Confirming its lack of collagenase activity, rh-MMP10 did not release fluorescence from DQ collagen (not shown).

MMP-10 Controls Expression of Collagenolytic MMPs

These data indicate that MMP-10 moderates scar formation and promotes resolution by controlling metallocollagenase activity. To identify the specific enzyme(s), we assessed the expression of mRNAs for *Mmp2, 8, 9, 13, 14,* and *16*, which encode known or suspected collagenases (Sabeh *et al*., 2009; Shi *et al*., 2008). In day-3 wound samples, we found reduced expression of *Mmp8* and *Mmp9* mRNAs in *Mmp10*$^{-/-}$ samples (Fig. 3). In day-8 wound samples, the levels of *Mmp2, Mmp13,* and *Mmp14* mRNAs were significantly lower in *Mmp10*$^{-/-}$ mice (Fig. 3). Similarly, in explants we found *Mmp8*, *Mmp9,* and *Mmp13* mRNAs were significantly reduced in *Mmp10*$^{-/-}$ samples (Fig. S5). These results suggest reduced expression of specific collagenolytic MMPs contributed to increased collagen accumulation in *Mmp10*$^{-/-}$ wounds.

MMP-10 Does Not Impact Macrophage Influx into Skin Wounds

To analyze the cellular mechanism by which MMP-10 governs collagen cleavage, we focused on macrophages. Although the similar levels of activity released from wound biopsies and explants (Fig. 2) suggested that MMP-10-dependent collagenolysis was a function of resident cells, impaired influx of macrophage could also have contributed to reduced collagenase activity in *Mmp10*$^{-/-}$ wounds. However, we found no difference in the number of resident macrophages between wildtype and *Mmp10*$^{-/-}$skin (Fig. S6a,b) or in the total number of macrophages (resident plus recruited) in wildtype and *Mmp10*$^{-/-}$ wounds (Fig. S6c,d). Furthermore, we detected no difference in the ability of wildtype and *Mmp10*$^{-/-}$ macrophages to migrate toward serum (Fig. S6e) or in macrophage chemotactic activity in wildtype and *Mmp10*$^{-/-}$ wounds (Fig. S6f). These data indicate that the reduction in collagenase activity in *Mmp10*$^{-/-}$ wounds was not due to fewer macrophages.

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To assess a functional role for macrophages, we depleted these cells in explants using clodronate liposomes (Fig. S7a). Whereas depletion of macrophages in wildtype explants significantly reduced total collagenase activity to the levels seen in *Mmp10*$^{-/-}$ samples, ablation did not further lower the reduced activity in *Mmp10*$^{-/-}$ tissue (Fig. 4a). Skin resident macrophages are categorized as alternatively activated (M2) cells (Davies *et al*., 2013), and M2 macrophages are considered to be the cells responsible for tissue remodeling (Madsen *et al*., 2013). Hence, we selectively ablated M2-like macrophages using the

M2pepKLA fusion peptide (Cieslewicz *et al*., 2013). The effectiveness of M2pepKLA was confirmed by reduced levels of MRC-1 (Fig. S7b), an M2 macrophage marker. Similar to the clodronate data, ablation of M2 macrophages with M2pepKLA significantly decreased collagenase activity in wildtype explants but not in *Mmp10*$^{-/-}$ samples (Fig. 4b).

The macrophage ablation data indicated that MMP-10 influenced the expression of collagenolytic MMPs in M2 macrophages. To test this idea, we isolated bone marrow-derived macrophages (BMDM) from wildtype and *Mmp10*$^{-/-}$ mice and assessed the expression of collagenolytic MMPs in naïve (M0) and M1 and M2-differentiated cells. Although the expression levels of metallocollagenases differed between M0 and M1 macrophages, these cell subtype-specific expression patterns did not differ between wildtype and *Mmp10*$^{-/-}$ cells (Fig. S8). However, we did find reduced expression of *Mmp8* and *Mmp13* mRNAs and elevated levels of *Mmp9* in *Mmp10*$^{-/-}$ M2 macrophages (Fig. 5a). These data agree with the reduced expression of *Mmp8* and *Mmp13* in *Mmp10*$^{-/-}$ wounds (Fig. 3) and explants (Fig. S5b). Antibody-clearance demonstrated that MMP-13 accounted for the bulk of collagenase activity produced by wildtype M2 macrophages (Fig. 5b).

Furthermore, polarization to M2-like macrophages increased the collagenase activity released from wildtype macrophages but had no effect on the activity released from *Mmp10*$^{-/-}$ macrophages (Fig. 5c). Expression of *Arg1* and *Fizz1* increased markedly in M2-differentiated macrophages (Gordon, 2003), but this well-characterized M2 response was not altered in *Mmp10*$^{-/-}$ cells (Fig. S9). These data indicate that the reduced levels of collagenase activity released from *Mmp10*$^{-/-}$ M2 macrophage was independent of the ability of *Mmp10*$^{-/-}$ M0 macrophages to respond to the M2 agonists.

To demonstrate that reduced collagenolytic activity of macrophages contributed to the enhanced scarring in *Mmp10*$^{-/-}$ wound, we transferred BMDM from wildtype MacGreen mice into *Mmp10*$^{-/-}$ recipients on day 10 post-injury. Tissues were harvested 2 days later (day 12 after wounding). EGFP^{+} macrophages were detected in both wildtype and *Mmp10*$^{-/-}$ wounds (Fig. S10), and similar numbers of F4/80^{+} macrophages were detected in wounds from both genotypes (Fig. 6a,b). We found that the influx of wildtype macrophages normalized the increased collagen deposition seen in *Mmp10*$^{-/-}$ wounds (Fig. 6c).

Discussion

We report that macrophage-derived MMP-10 serves a beneficial role in tissue repair by promoting resolution of scar tissue. Our findings indicate MMP-10 shaped scar deposition indirectly by controlling the collagenolytic activity of M2-biased resident macrophages without impacting collagen production by fibroblasts/myofibroblasts. More specifically, we found that MMP-10, which is not a collagenase, promoted the expression of MMP-8 and MMP-13, two metallocollagenases released by macrophages. However, we found that essentially all collagenolytic activity was due to MMP-13, suggesting that macrophage-derived MMP-8 functions in other processes. Indeed, data supporting that MMP-8 functions as a collagenase are not compelling. Both over-expression and deletion of *Mmp8* reduces fibrosis (Craig *et al*., 2013; Garcia-Prieto *et al*., 2010; Siller-Lopez *et al*., 2004).

Studies with *Mmp13*$^{-/-}$ mice complement our conclusions. The resolution of CCl_4-induced liver fibrosis is slowed in *Mmp13*$^{-/-}$ mice (Fallowfield *et al*., 2007) and accelerated in mice over-expressing this collagenase (Yang *et al*., 2014), and both of these studies linked MMP-13 production to scar-associated macrophages. Furthermore, excess type I collagen is deposited in bones of *Mmp13*$^{-/-}$ mice (Inada *et al*., 2004; Stickens *et al*., 2004). On the other hand, liver fibrosis due to bile duct ligation is reduced in *Mmp13*$^{-/-}$ mice (Uchinami *et al*., 2006). Regarding skin wounds, disparate findings have been reported for phenotypes in *Mmp13*$^{-/-}$ mice. Whereas Hattori *et al*. (2009) concluded that MMP-13 promotes closure of skin wounds, Hartenstein *et al*. (2006) found no difference in re-epithelialization between wildtype and *Mmp13*$^{-/-}$ mice. Although neither skin-wound study assessed scarring directly, Hattori *et al*. reported a reduction in both wound contraction and myofibroblast numbers in *Mmp13*$^{-/-}$ mice, findings that would suggest that MMP-13 has pro-fibrotic activities, too. As MMP-13 is expressed by many cells types, it is likely that this MMP can affect multiple, distinct processes within and among models.

Our findings indicate that MMP-10 controls collagen turnover by macrophages by promoting the expression of specific metallocollagenases, primarily MMP-13. As MMP-13 levels were reduced but not absent in *Mmp10*$^{-/-}$ macrophages, the residual enzyme likely contributed to the collagenase activity in *Mmp10*$^{-/-}$ samples and cells and may explain why we did not observe any differences in granulation tissue formation as seen in mice with a complete lack of MMP-13 (Toriseva *et al*., 2012). Complementary to our conclusion, the transcription factor ANKRD1 influences expression of *Mmp13* and *Mmp10* (Almodovar-Garcia *et al*., 2014), suggesting a link in the synthesis of these two MMPs.

In our studies, we used a model of normal skin repair, and we observed no differences in macrophage influx or in the expression of αSMA and type I collagen (or other markers of fibroblast activation) between wildtype and *Mmp10*$^{-/-}$ mice. In contrast, macrophage influx, αSMA expression, and fibrosis are elevated in *Mmp10*$^{-/-}$ mice in models of severe liver (Garcia-Irigoyen *et al*., 2013), colon, (Koller *et al*., 2012) and lung injury (unpublished findings). Although an explanation for these disparate findings is not yet apparent, we propose that the mechanisms by which MMP-10 affects fibrosis and macrophage influx are context dependent. Thus, the profound pro-inflammatory responses in liver/colon/lung models may lead to MMP-10-dependent roles in macrophage recruitment and activation that, in turn, stimulate myofibroblast differentiation and collagen production. In contrast, because the inflammatory response is less profound in skin wounds, MMP-10-dependent controls on macrophage influx could be modest and, hence, not easily detected in *Mmp10*$^{-/-}$ tissues.

Compared to the evidence supporting their roles in promoting fibrosis (Pellicoro *et al*., 2014), there are limited data on how macrophages function during resolution. We found that MMP-10 shapes the tissue-remodeling function of resident skin M2-like macrophages (Davies *et al*., 2013) by regulating expression of MMP-13. Reduced levels of this metallocollagenase were consistently seen among wound biopsies (Fig. 3), explants (Fig. S5b), and M2-polarized cultured macrophages (Fig. 5a). Reparative or M2-like macrophages are the dominant population of macrophages during the resolution phase (Duffield, 2014; Lichtnekert *et al*., 2013). M0 macrophages possessed baseline collagenolytic activity (Fig.

5c), and treatment with M2 agonists led to a significant increase in overall collagenase activity released by wildtype macrophages but had no effect on the levels from *Mmp10*$^{-/-}$ cells. These findings support our central conclusion that MMP-10 controls the collagenolytic activity of M2-biased macrophages. In agreement with our findings, Madsen *et al*. (2013) demonstrated that M2-like macrophages are the principal cells executing collagen turnover in skin.

Our findings indicated that M2-biased resident macrophages were responsible for scar resolution in excisional skin wounds. Of note, induction of *Arg1* and *Fizz1*, commonly used M2 markers, were not altered in IL-4/IL-13-treated *Mmp10*$^{-/-}$ macrophages, but a key function - tissue remodeling - attributed to this type of macrophage was markedly impaired. These findings add to the many caveats of the M1/M2 classification scheme and underscore that function is more important in defining macrophage subtype than expression of a few markers (Murray *et al*., 2014).

Excess scarring (fibrosis) can affect quality of life, with loss of tissue function, restriction of movement, and adverse psychological consequences (Brown *et al*., 2008). Although fibrotic conditions are oft considered to be irreversible, an understanding how macrophage activation is controlled and how excess ECM is cleared will shed light on means to manipulate these severe diseases. Results from our study demonstrate a role for MMP-10 in controlling the ability of M2-like macrophages to degrade fibrillar collagen during repair of skin wounds. Whether MMP-10 has a similar, beneficial role in mitigating fibrosis in disease settings has yet to be determined.

Materials and Methods

Animals

Mmp10$^{-/-}$ mice (C57BL/6J) (Kassim *et al*., 2007) and wildtype littermates (male and female) were used for these studies. All procedures were approved by the Office of Animal Welfare, University of Washington, and the IACUC, Cedars-Sinai.

Wounds and Explants

Mice were anesthetized, and their dorsal surface was shaved. Skin was pulled into a fold and laid on a plastic surface. Two full-thickness wounds were created by running a sterile 5-mm punch biopsy (Miltex Inc., New York, NY USA) through the fold. Four wounds were made per mouse and left uncovered. Mice were housed in individual cages. On indicated days post injury, wounds were excised with an 8-mm biopsy punch for further analysis.

RNA

Total RNA was isolated and specific transcripts were quantified by quantitative real-time PCR using TaqMan FAM-labeled probes (Applied Biosystems, Foster City, CA) as described (Rohani *et al*., 2014).

Hydroxyproline

Excised wounds were weighed and homogenized in deionized water (20 μl/ mg tissue). Equal volumes of homogenates were mixed with 12 N HCl (1:1) and hydrolyzed for 3 h at 120°C. Hydroxyproline was measured using a colorimetric assay (BD BioVision, Milpitas, CA). Data are presented as total micrograms hydroxyproline/biopsies.

Histology

Sections (4 μm) of paraffin-embedded tissues were stained with Masson's trichrome or processed for immunohistochemistry with anti F4/80 (BM8, eBioscience, San Diego, CA). VisioPharm software (Hørsholm, Denmark) was used to quantify the total area of F4/80 signal or trichrome staining, normalized to total surface area.

Biomechanics

Skin stiffness was measured as described (Rohani *et al*., 2014). Briefly, 12-d wound samples were dissected into strips and suspended on hooks attached to a force transducer (Aurora Scientific, model 400A) and a length controller (Aurora Scientific, model 308B). Tissue stiffness (dF/dL) was determined by imposing change in length of the skin strip and measuring the resultant change in force normalized per millimeter of sample width. On each strip of tissue, 4 steps of 5% length stretches were taken every 30 sec. Force and length signals were analyzed using custom LabView software.

Collagenase

Wound and explant samples were placed in the upper chambers of transwells in 50 μl, and 1% (v/v) quenched fluorescein-conjugated DQ™ type I collagen (1 μg/ml; Life Technologies, Grand Island, NY) was added to the bottom chambers in 500 μl of phenol red-free RPMI with 1% FBS and 1% penicillin/streptavidin. Collagenase activity was defined as the fluorescence detected in the bottom chamber (excitation 480 nm, emission 530 nm) minus fluorescence in blank controls (no sample in the upper chamber). For 3D matrix, we mixed native type I collagen (PurCol, Advanced BioMatrix, Carlsbad, CA) with DQ collagen and seeded explants onto the mixed matrix and assessed collagenase activity. For antibody depletion, we cultured samples with antibodies against MMP-8 (R&D Systems, Minneapolis, MN) or MMP-13 (Thermos Fisher Scientific, Waltham, MA). For macrophages, cells were incubated with phenol red-free RPMI containing 1% DQ collagen and 6 h.

Macrophage Depletion

Explants were cultured for 72 h with PBS- or clodronate liposomes (50 mg/ml; http://www.clodronateliposomes.org) added to the upper chamber. To deplete M2 macrophages, explants were cultured with 20 μg/ml M2pepKLA or a control scrambled peptide (scrM2pepKLA) (Cieslewicz *et al*., 2013).

Macrophage Culture

Isolation and activation of BMDM were done as described (Manicone *et al*., 2009). Briefly, marrow cells were cultured for 7 days in Mac medium. For M2 activation, BMDM were

stimulated with 10 ng/ml IL-4 and IL-13 for 48 h. For M1 activation, cells were cultured with 100 ng/ml *E. coli* LPS for 6 h, washed, and cultured for another 24 h. For adoptive transfer, macrophages were collected at day 5 after marrow harvest and suspended in PBS. Recipient mice received 7×10^6 BMDM in 50 μl PBS via retro orbital injection.

Statistical Analysis

Statistical analysis was performed using Prism 5 (GraphPad Software, Inc., La Jolla, CA). We used t-test to compare two groups, or two-way ANOVA followed by Bonferroni post-test to test the effect of two factors. Data are presented as mean ± SEM. A *p*-value less than 0.05 was considered statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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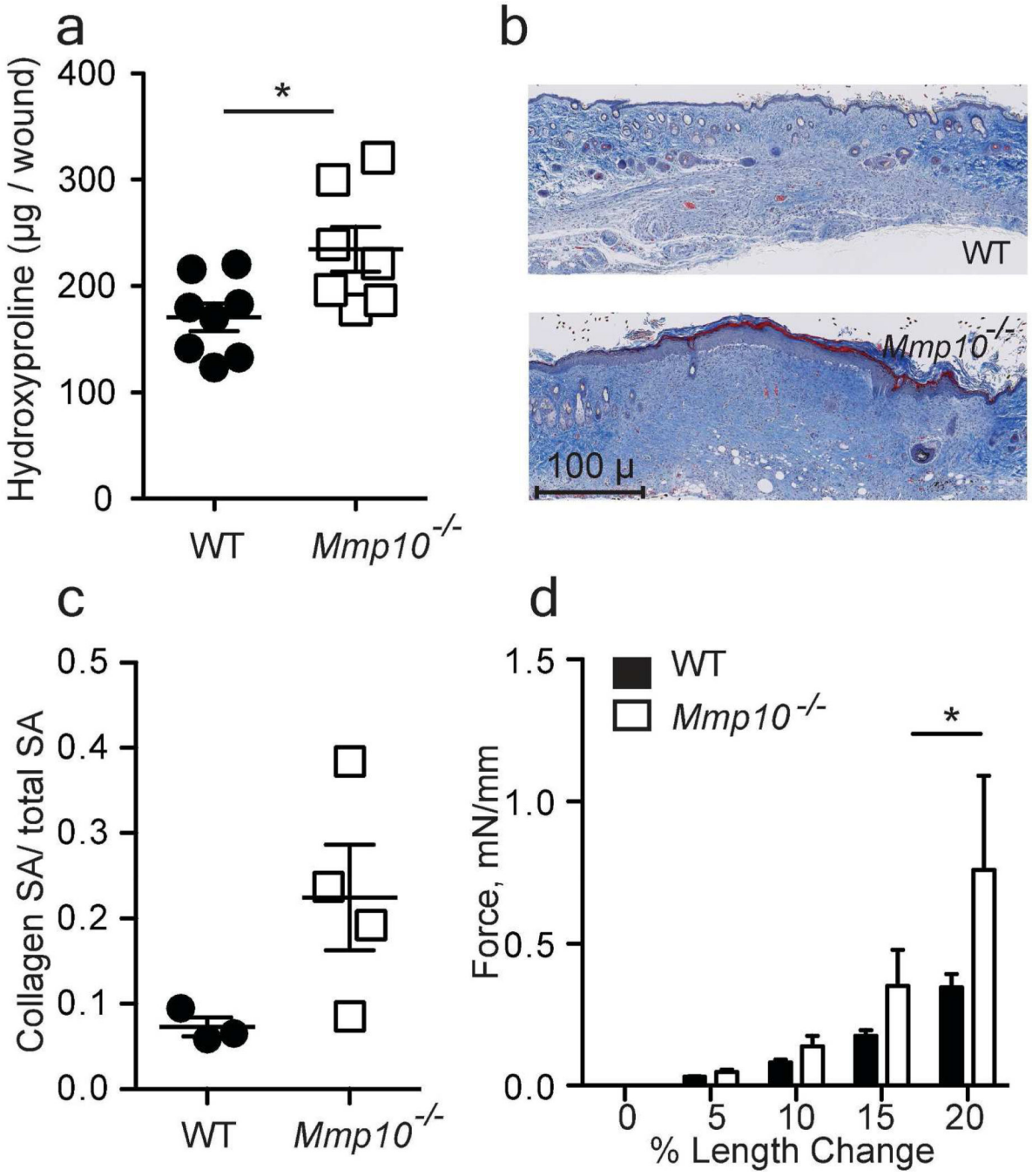

Figure 1. MMP-10 moderates collagen accumulation at the site of injury

(a) Uniform 8-mm wound samples were collected on day 12 post-injury, and hydroxyproline was quantified and normalized per wound sample (n = 8 mice/ genotype); WT = wildtype; * $p < 0.05$. (b) Representative images of day-12 wounds stained with Masson's trichrome. Scale bar = 100 µm. (c) Quantification of collagen density in Masson's trichrome stained samples. Data are the ratio of collagen-positive (blue) area to total surface area (SA). Each point represents samples from an individual mouse. (d) Tissue stiffness of wounds was

measured using 12-day wound samples. Data are presented as the amount of force change per percent of length change. (n = 4 WT, 3 *Mmp10*$^{-/-}$), * p <0.05.

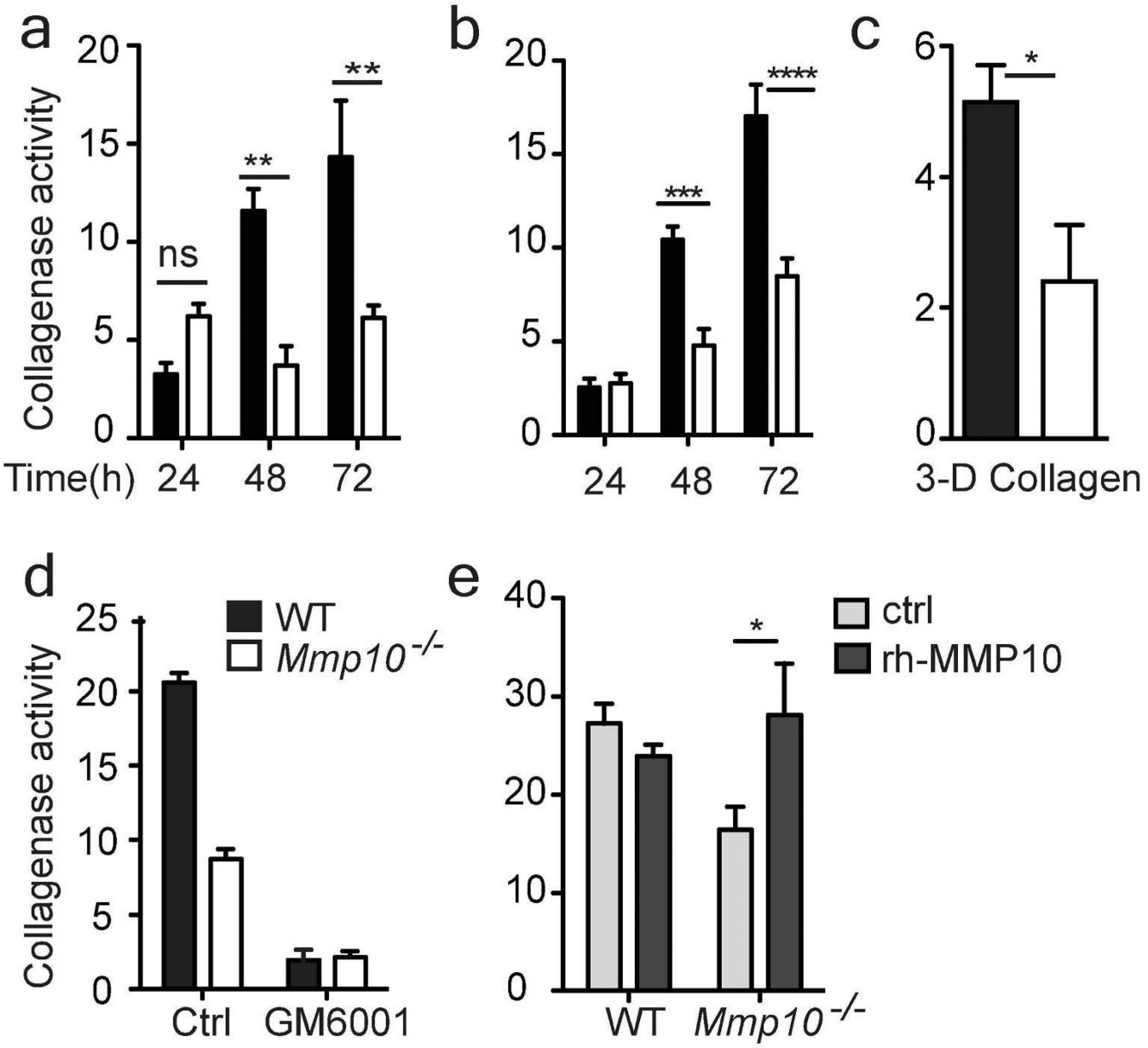

Figure 2. Reduced collagenase activity in *Mmp10*$^{-/-}$ wounds

(a) Day-8 wounds were incubated with DQ-collagen for 72 h. Collagen degradation was measured every 24 h (n = 3/genotype). (b) Collagenase activity from explants of normal skin (n = 7 WT, 6 *Mmp10*$^{-/-}$). (c) Skin explants were seeded on FITC-conjugated type I collagen gels. Collagenase activity was measured in conditioned medium at 72 h (n = 5/genotype). (d) Explants were incubated with 25 μM GM6001, and collagenase activity was assessed 72 h later (n = 4 WT, 3 *Mmp10*$^{-/-}$). (e) Explants were treated with rh-MMP10 (100 ng/ml) or vehicle (ctrl), and collagenase activity was assessed 72 h later (n=5/genotype). **p* <0.05, ** *p* <0.01, *** *p* <0.001, **** *p* <0.0001.

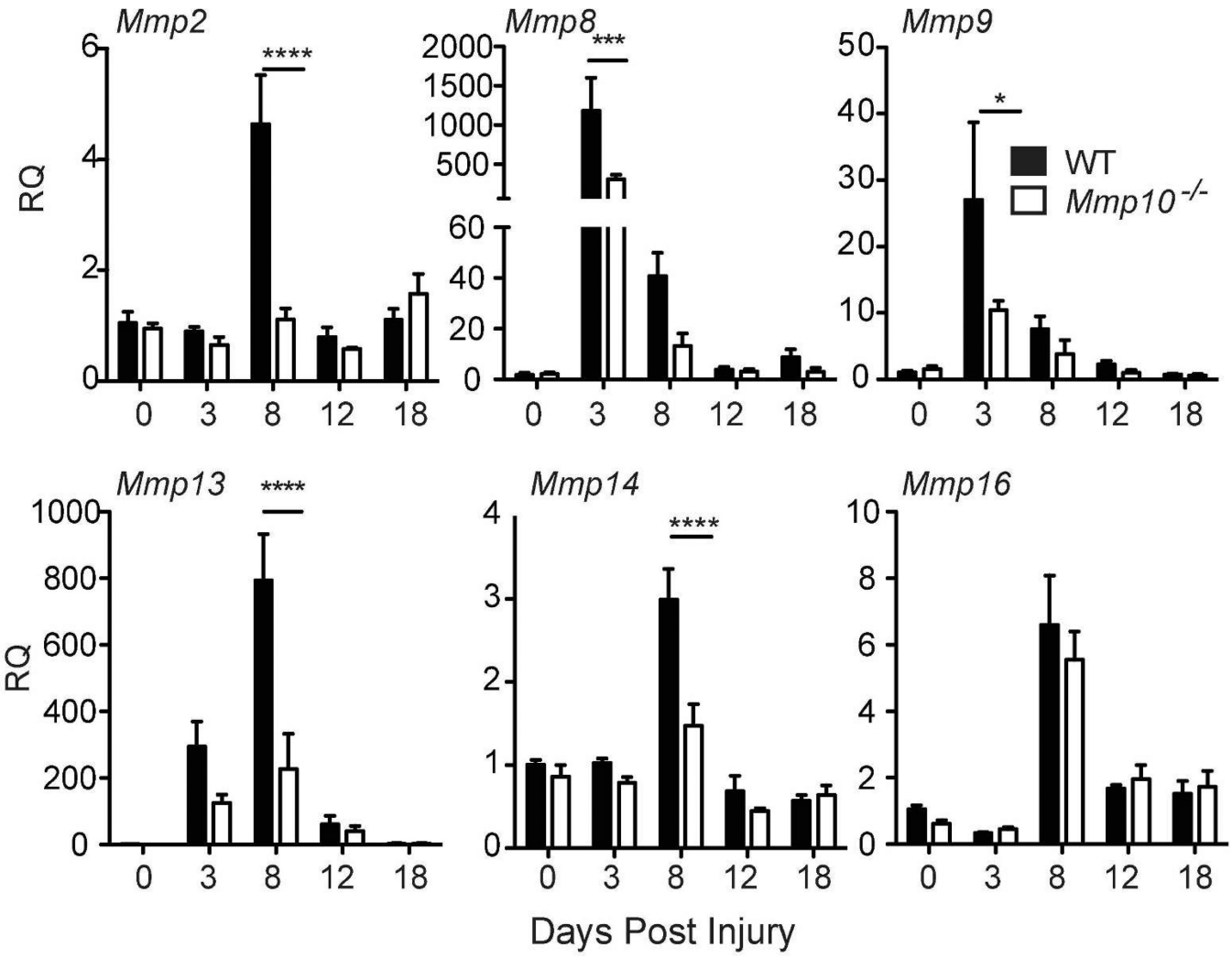

Figure 3. MMP-10 influences expression of collagenolytic MMPs

Expression of mRNAs for *Mmp* 2, 8, 9, 13, 14, and 16 were quantified in wound samples harvested at the indicated days. Data shown are the ratio compared to non-wounded wildtype (WT) samples and normalized to *Hprt* (n≥ 4/ genotype). **p* <0.05, ** *p* <0.01, *** *p* <0.001, **** *p* <0.0001.

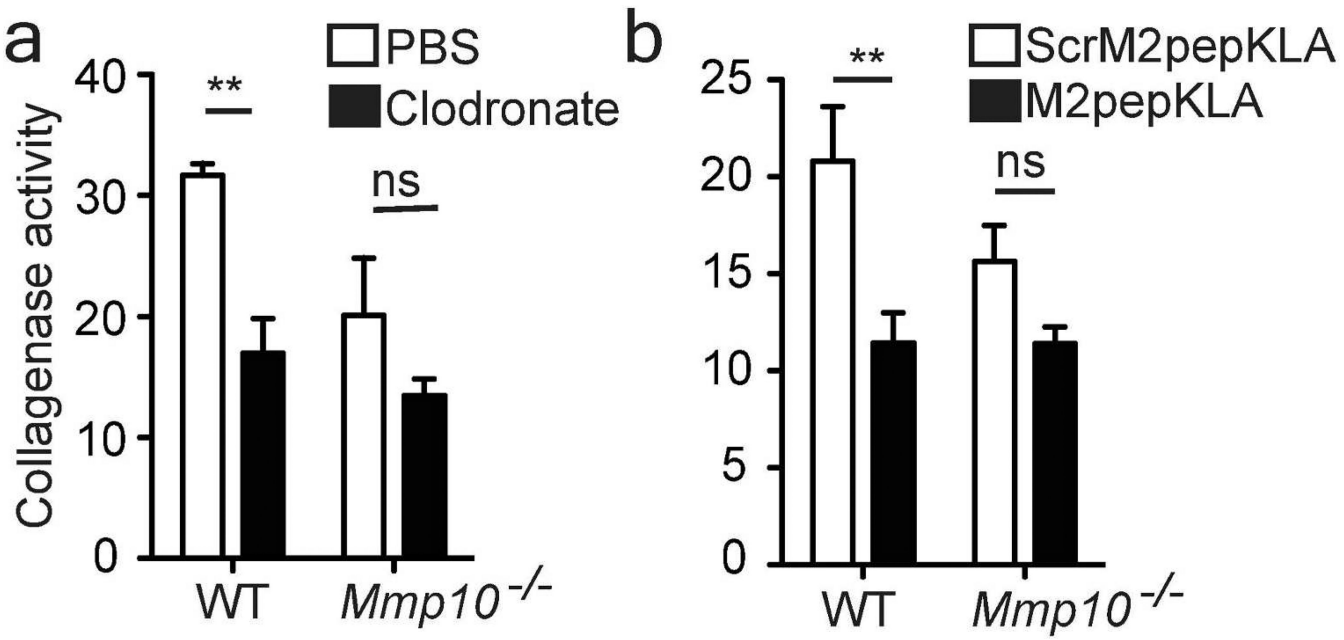

Figure 4. MMP-10 regulates collagenase activity of tissue macrophages

Wildtype (WT) and *Mmp10*$^{-/-}$ skin explants were cultured in the upper chambers of a 24-transwell plate containing (a) PBS or clodronate liposomes (n = 3/genotype) or (b) the apoptosis-inducing peptide M2pepKLA or ScrM2peptideKLA (20 μg/ml; n=5/genotype). FITC-conjugated type I collagen was added to the medium in the lower chamber, and collagenase activity was measured 72 h later. ** p <0.01.

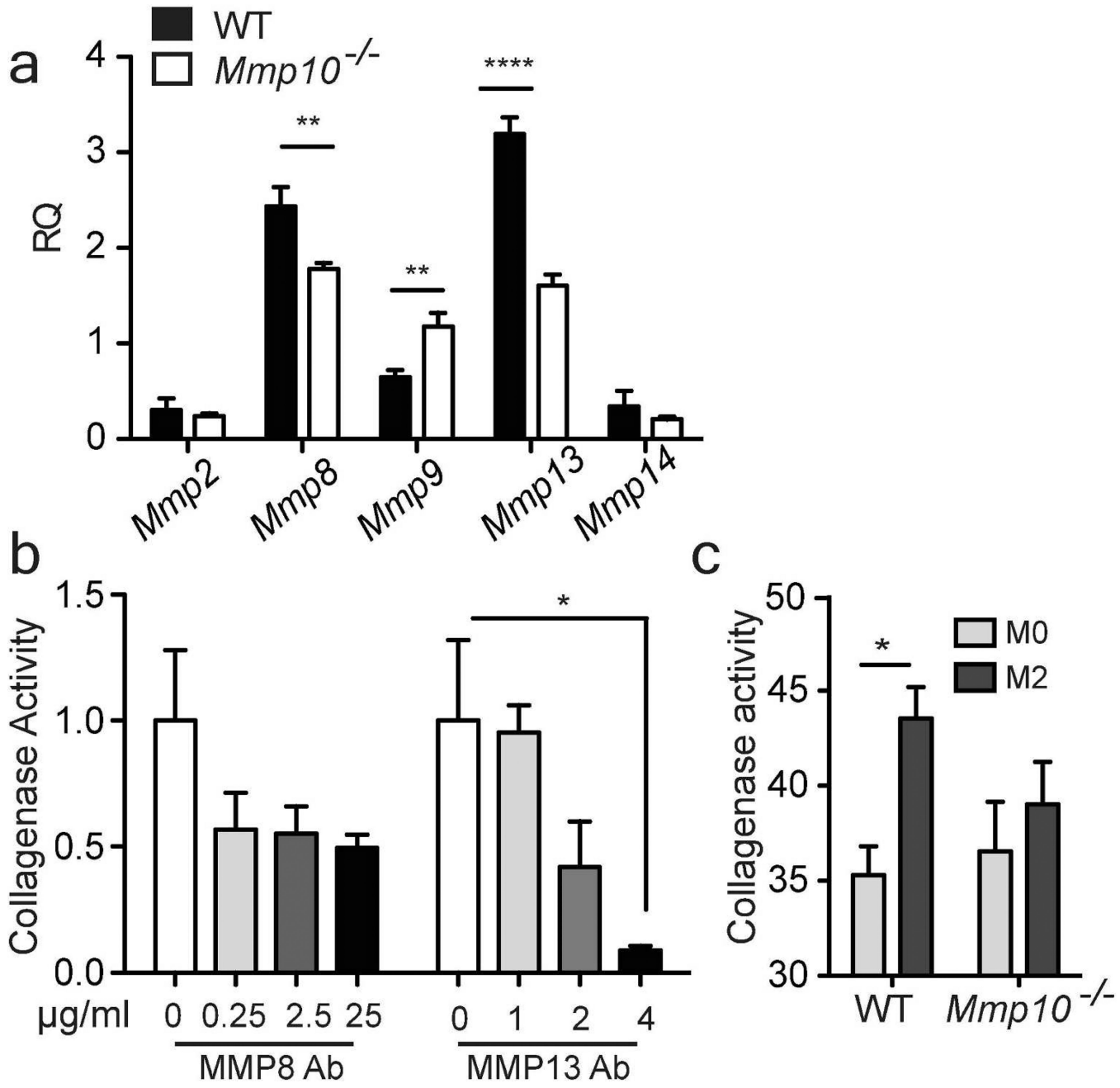

Figure 5. MMP10 influences expression of collagenolytic MMPs and activity in M2 macrophages

(a) Total RNA was isolated from M2-polarized wildtype and *Mmp10*$^{-/-}$ BMDMs and analyzed by qRT-PCR. Data are presented as fold change relative to naïve BMDM from wildtype mice. (b) Wildtype skin explants were cultured in the upper chambers of 24-transwell plates. DQ-collagen was added to the lower chamber containing antibody against MMP-8 or, antibody against MMP-13. Collagenase activity was measured 72 h later. Values are presented as the ratio of untreated samples. n = 3, *p <0.05, **p <0.01. (c) Naïve and M2-polarized BMDMs were incubated with DQ-collagen for 6 h. Supernatants were assayed for collagenase activity. (n = 4 wildtype, 5 *Mmp10*$^{-/-}$).

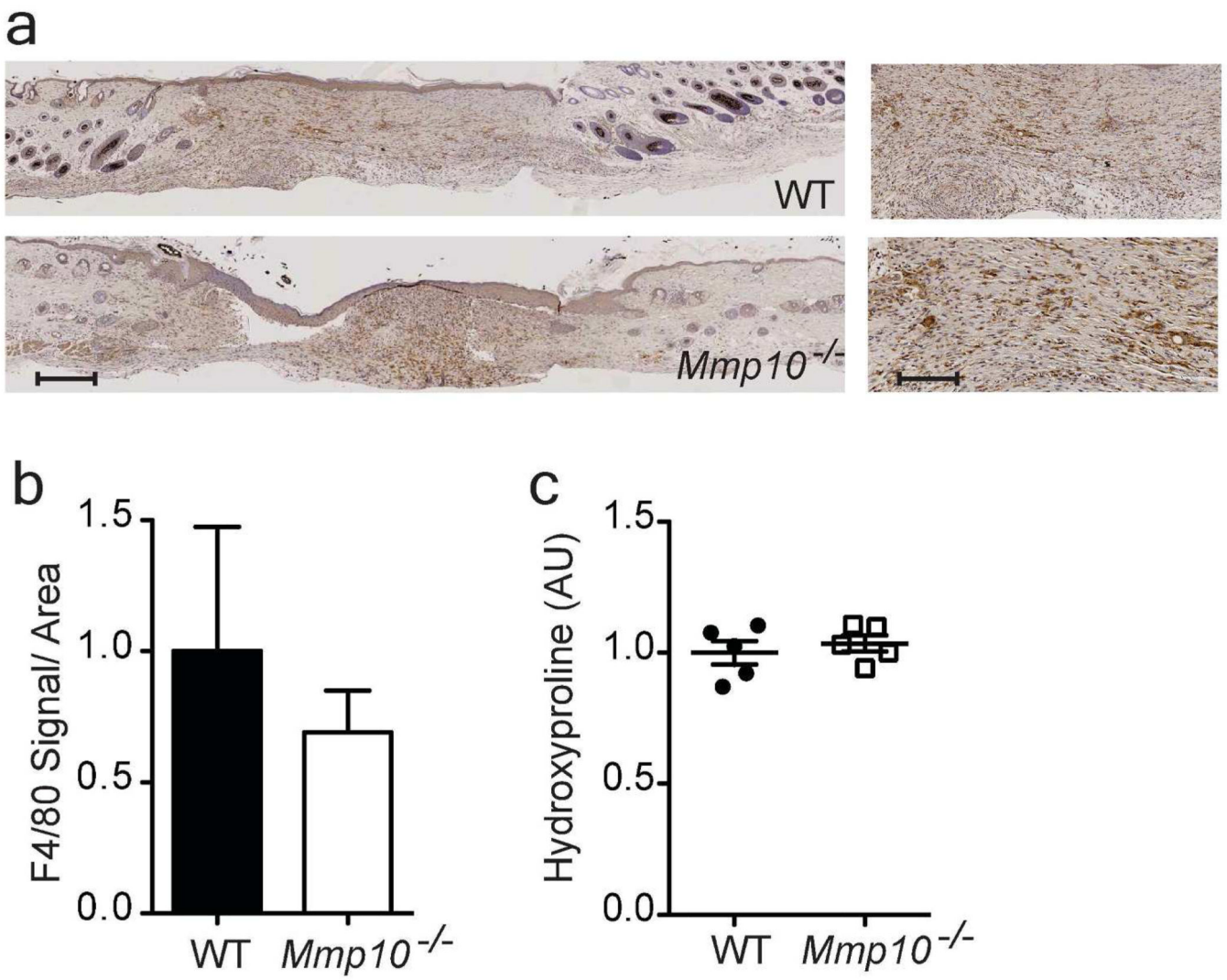

Figure 6. Adoptive transfer of wildtype macrophages normalizes collagen deposition in *Mmp10*$^{-/-}$ wounds

Wildtype (WT) and *Mmp10*$^{-/-}$ mice received 7 × 10^6 GFP$^+$ BMDM via retro orbital injection at day 10 post-wounding. Wounds were harvested on day 12. (a) Representative images of 12-d wounds sections stained for F4/80. Scale bar = 500 μm (left), 60 μm (right) (b) The total area of F4/80-positive signal was quantified and normalized to total tissue surface area (n = 5/genotype). (c) Hydroxyproline was quantified, normalized per wound sample, and expressed relative to wildtype samples (n=5 mice/genotype).