Vaccination-based immunotherapy to target profibrotic cells in liver and lung

Highlights
- During fibrosis, distinct genes become upregulated exclusively in fibrogenic cells
- The resulting “self-peptides” can be exploited for cytotoxic vaccination approaches
- Vaccination mounts T cell responses that reduces liver and lung fibrosis in mice
- Proof of principle for vaccination-based immunotherapies to treat fibrosis

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In brief
Fibrosis accounts for up to 45% of all deaths in the developed world. Stockmann and colleagues present a vaccination approach to ablate activated fibroblasts and ameliorate fibrosis in the liver and lungs without disturbing tissue homeostasis. These results provide a rationale for vaccination-based immunotherapies to treat fibrosis.
Fibrosis is the final path of nearly every form of chronic disease, regardless of the pathogenesis. Upon chronic injury, activated, fibrogenic fibroblasts deposit excess extracellular matrix, and severe tissue fibrosis can occur in virtually any organ. However, antifibrotic therapies that target fibrogenic cells, while sparing homeostatic fibroblasts in healthy tissues, are limited. We tested whether specific immunization against endogenous proteins, strongly expressed in fibrogenic cells but highly restricted in quiescent fibroblasts, can elicit an antigen-specific cytotoxic T cell response to ameliorate organ fibrosis. In silico epitope prediction revealed that activation of the genes Adam12 and Gli1 in profibrotic cells and the resulting "self-peptides" can be exploited for T cell vaccines to ablate fibrogenic cells. We demonstrate the efficacy of a vaccination approach to mount CD8+ T cell responses that reduce fibroblasts and fibrosis in the liver and lungs in mice. These results provide proof of principle for vaccination-based immunotherapies to treat fibrosis.

INTRODUCTION

Liver fibrosis with massive expansion of fibroblasts and accumulation of extracellular matrix (ECM) occurs upon chronic liver injury in the context of alcoholic and cholestatic liver disease as well as non-alcoholic fatty liver disease and chronic viral hepatitis (Pellicoro et al., 2014). Quiescent fibroblasts are important to maintain the structure and homeostasis of any solid tissue. However, expansion of activated, fibrogenic fibroblasts in response to chronic tissue damage impairs tissue function and ultimately results in organ failure (Rockey et al., 2015). Due to the lack of therapeutic interventions that directly target
excessive fibrosis, the global health burden of fibrotic disease including liver and lung fibrosis is tremendous (Rockey et al., 2015; Wynne, 2004). During fibrosis, distinct genes that are active during embryonic development but restricted afterward (Dulauroy et al., 2012; Gomez and Duffield, 2014; Kramann et al., 2015) become upregulated exclusively in fibrogenic cells that give rise to fibroblasts, e.g., the disintegrin Adam12 (Dulauroy et al., 2012) or the transcription factor Gli1 (Kramann et al., 2015). For instance, Adam12 is transiently upregulated in fibrogenic stromal cells before the subsequent acquisition expression of a myofibroblast phenotype and the expression of platelet-derived growth factor receptor alpha (PDGFR-α) and the myofibroblast marker alpha-smooth muscle actin (α-SMA) (Dulauroy et al., 2012). As a consequence, simultaneous protein expression of ADAM12 and such myofibroblast markers occurs at low frequency (Dulauroy et al., 2012). Moreover, the use of such specific genes for genetic ablation of fibrogenic cells after tissue injury has been shown to reduce fibrosis in mice (Dulauroy et al., 2012; Gomez and Duffield, 2014; Kramann et al., 2015). Although this is not a feasible approach in a clinical therapeutic setting, it suggests that such genes specifically “tag” fibrogenic cells and, hence, represent therapeutic targets.

Recently, chimeric antigen receptor (CAR) T cells, engineered to eradicate fibroblasts that express fibroblast activation protein (FAP) or senescent cells that express urokinase plasminogen activator surface receptor (uPAR), have been shown to be effective in reducing fibrosis in mouse models of myocardial injury (Aghajanian et al., 2019; Amor et al., 2020; Runik et al., 2022) and liver fibrosis (Amor et al., 2020), respectively. Moreover, breakthroughs in cancer immunotherapy have been achieved with US Food and Drug Administration (FDA)-approved vaccines that redirect cytotoxic T cells to recognize tumor-specific antigens and to eliminate cancer cells (Hollingsworth and Jansen, 2019; Sahin et al., 2017). MHC class I molecules on the surface of literally all body cells constantly present “self” peptides of intracellular proteins to T cells for immune surveillance (Yewdell et al., 2003). We reasoned that proteins encoded by genes specifically expressed in fibrogenic cells result in specific “self-peptides” on the surface on fibrogenic cells and can be turned into immunogenic “antigens” and targets for specific T cell responses upon specific immunization (Figure 1A, scheme). Therefore, we sought to determine whether fibrogenic cells could be effectively targeted by antifibrotic vaccination in murine models of liver fibrosis and lung fibrosis without affecting healthy tissues.

RESULTS

Prophylactic vaccination against an endogenous protein specific to fibrogenic cells elicits a specific T cell response and ameliorates liver fibrosis

The disintegrin metalloprotease ADAM12 has previously been shown to be expressed during embryonic development and muscle fibrosis but is expressed only at low levels—if at all—in most normal adult tissues in mice and humans (Dulauroy et al., 2012). We extended this finding by showing that ADAM12 is robustly expressed in fibrotic liver tissue from patients with cirrhosis and mice that underwent carbon tetrachloride (CCL4)-induced liver fibrosis (Kantari-Mimoun et al., 2015, 2017), as visualized by co-staining with collagen I (Col I), whereas normal human and mouse livers do not show ADAM12 expression (Figure 1B). As previously demonstrated in the context of muscle fibrosis, although ADAM12+ cells give rise to myofibroblasts, these cells sequentially acquire and only minimally co-express fibroblast markers (Dulauroy et al., 2012). In line with this, we observe only a fraction of ADAM12+ cells in fibrotic livers that co-express α-SMA (11%) and PDGFR-α (9.5%), whereas 77.65% of ADAM12+ cells express PDGFR-β and 86.76% express the hepatic stellate cell marker glial fibribrillary acidic protein (GFAP; Figure S1A). The minimal co-expression of express α-SMA and PDGFR-α is in line with the initial observation that the fibrogenic ADAM12+ cells only transiently upregulate ADAM12 and then gradually start to express fibroblast markers (Dulauroy et al., 2012). Furthermore, the high co-expression of ADAM12 and PDGFR-β as well as GFAP supports the concept that activated hepatic stellate cells are the major drivers of liver fibrosis upon engagement of PDGFR-β signaling (Kocabayoglu et al., 2015; Pizzvani et al., 1996; Wong et al., 1994). Thus, we wanted to test ADAM12 as a potential candidate for targeting fibrogenic fibroblasts in the liver.

Next, we sought to verify whether specific immunization against an endogenous protein expressed by fibrogenic cells, but not by quiescent fibroblasts or other critical cell types, can elicit an antigen-specific cytotoxic CD8+ T cell response. The immunogenicity of peptides from a given intracellular protein depends on the likelihood to be first processed by proteasomes into 8–11mer peptides (the size of peptides that can be presented by MHCI molecules to activate cytotoxic CD8+ T cells), 2nd delivered by the transporter associated with antigen processing (TAP) into the endoplasmic reticulum, and 3rd bound to MHC class I with high affinity (Kim et al., 2012; Peters and Sette, 2022) and liver fibrosis (Amor et al., 2020), respectively. We reasoned that proteins encoded by genes specifically expressed in fibrogenic cells result in specific “self-peptides” on the surface on fibrogenic cells and can be turned into immunogenic “antigens” and targets for specific T cell responses upon specific immunization (Figure 1A, scheme). Therefore, we sought to determine whether fibrogenic cells could be effectively targeted by antifibrotic vaccination in murine models of liver fibrosis and lung fibrosis without affecting healthy tissues.

Figure 1. Prophylactic vaccination against an endogenous protein specific to fibrogenic cells elicits a specific T cell response and ameliorates liver fibrosis

(A) Scheme of vaccination-based immunotherapy to target fibrosis.
(B) Images of ADAM12/collagen I immunostaining on healthy liver and fibrotic liver (for mouse, sample of CCL4-induced fibrosis and for human, sample of stage 4 cirrhosis).
(C) Scheme of prophylactic vaccination against liver fibrosis in mice.
(D) ADAM12-positive area on liver sections from sham- and CCL4-treated animals vaccinated with control vaccine (v-CTRL) or ADAM12 vaccine (v-A12) together with representative images of ADAM12 immunostaining (sham: v-CTRL, n = 3; v-A12, n = 3; CCL4: v-CTRL, n = 10; v-A12, n = 10). Data represented as fold change.
(E) α-SMA-positive area on liver sections from sham- and CCL4-treated animals vaccinated with v-CTRL or v-A12 and images of α-SMA immunostaining (sham: v-CTRL, n = 4; v-A12, n = 4; CCL4: v-CTRL, n = 16; v-A12, n = 15).
(F) Collagen content by hydroxyproline assay of liver tissue from sham- and CCL4-treated animals vaccinated with v-CTRL or v-A12 (sham: v-CTRL, n = 5; v-A12, n = 5; CCL4: v-CTRL, n = 20; v-A12, n = 20).
(G) Sirius red-positive area from sham- and CCL4-treated animals vaccinated with v-CTRL or v-A12 and images of Sirius red/fast green staining (sham: v-CTRL, n = 11; v-A12, n = 11; CCL4: v-CTRL, n = 20; v-A12, n = 20). Values are mean ± SEM, statistical test: one-way ANOVA. Scale bars in (B), (E), (G), and (H), 100 μm.
In *silico* epitope prediction revealed that ADAM12 is likely to pass all 3 steps and yield a set of 5 candidate peptides and potential CD8 T cell epitopes to be presented by MHC I molecules in the murine C57BL/6j background on the cell surface upon reactivation of ADAM12 (Figure S1C). The subsequent enzyme-linked immunospot (ELISpot) demonstrated interferon (IFN)-γ release from ADAM12-primed CD8+ T cells upon peptide re-exposure (Figure S1C), indicating an antigen-specific CD8+ T cell response and the accurate *in silico* prediction of immunogenic ADAM12 epitopes.

We tested *in vivo* immunization with GFP-expressing lentiviral vectors encoding the ADAM12 full-length protein (v-A12, vector construct in Figure S2A) with adjuvants (incomplete Freund’s adjuvant (IFA) plus the TLR9 agonist CpG oligodeoxynucleotides (ODN)) on day 0 (“prime”) and day 7 (“boost”) (Figure S2B, scheme), whereas control animals were injected with GFP-expressing lentiviral vectors (v-CTRL, vector construct in Figure S2A) and adjuvants. We chose a lentiviral vector approach to circumvent major limitations of peptide-based vaccines, namely restriction by only certain MHC class I haplotypes (Facciponte et al., 2014; Hollingsworth and Jansen, 2019), as well as to induce a robust multi-epitope based cytotoxic T lymphocyte response through sustained antigen presentation via MHC molecules and co-stimulation by dendritic cells to T cells (Dullaers and Thielemans, 2006; Pincha et al., 2010; Stripecke, 2009; Uhlig et al., 2015). 12 days after the initial immunization, splenocytes were isolated and exposed to dendritic cells that were engineered to express full-length ADAM12, followed by purification of CD8+ T cells and re-stimulation with a mix of the five synthetic ADAM12-derived peptides that we identified by *in silico* epitope prediction (Figure S1B). The subsequent in silico epitope prediction revealed that ADAM12 is likely to pass all 3 steps and yield a set of 5 candidate peptides and potential CD8 T cell epitopes to be presented by MHC I molecules in the murine C57BL/6j background on the cell surface upon reactivation of ADAM12 (Figure S1B). Next, we co-cultured murine activated splenocytes with dendritic cells engineered to express full-length ADAM12, followed by purification of CD8+ T cells and re-stimulation with a mix of the five synthetic ADAM12-derived peptides that we identified by *in silico* epitope prediction (Figure S1B). The subsequent enzyme-linked immunospot (ELISpot) demonstrated interferon (IFN)-γ release from ADAM12-primed CD8+ T cells upon peptide re-exposure (Figure S1C), indicating an antigen-specific CD8+ T cell response and the accurate *in silico* prediction of immunogenic ADAM12 epitopes.

Next, we performed bulk T cell receptor (TCR) α and β chain repertoire sequencing on sham-treated and fibrotic livers to further characterize the T cell response and to detect potential TCR clonotypes. In non-fibrotic, sham-treated animals, we observed low clonality (defined as a TCR sequence with a frequency > 1% and detectable in more than one sample of each treatment group) in both treatment groups (Figure 2A). 77% and 72.4% of the α chain sequences in v-CTRL and v-A12 mice, respectively, represented less than 1% of the clones and occurred in only one sample (Figure 2A). There was overlap between v-CTRL and v-A12 mice for a single α chain sequence (VVDGRGSAALGRLH), which is considered NKT cell receptor sequence according to the immune epitope database (IEDB) and expansion of two clones in the v-A12 group that occurred with a frequency of 4.6% (ALTANYYAQOGLT) and 1% (VVADGRGSAALGRLH; Figure 2A). In livers from v-CTRL mice, there was no clonality in the β chain sequences and no overlap with v-A12-vaccinated mice, which exhibited a single clone (ASSDRDR-GEQY; Figure 2A). Of note, in fibrotic livers, we observed increased TCR clonality (defined as a TCR sequence with a frequency > 1% and detectable in more than one sample of each treatment group) of liver-infiltrating T cells from v-A12- and control-vaccinated mice with minimal overlap to v-CTRL mice in two α chain sequences (VVDGRGSAALGRLH and VDGDRGSAALGRLH) (Figure 2B). This supports the concept that chronic liver disease results in distinctive antigen repertoires and expansion of disease-specific T cells (Liaskou et al., 2016; Dudek et al., 2021; Pfister et al., 2021). However, the vast majority of expanded clones from v-A12-vaccinated animals showed a unique sequence pattern (Figure 2B). Moreover, there was no overlap in the β chain sequences between v-A12-vaccinated and control mice (Figure 2B), suggesting a vaccination-induced expansion of antigen-specific TCR clonotypes. In addition to the clone VDGDRGSAALGRLH that was expanded in livers from v-CTRL and v-A12 mice, the most uniquely expanded TCR sequence clones in the liver from v-A12-vaccinated mice comprised 64% (AASSSGSWQLI) and 5% (ALSDFRTNAYKVI) of the sequenced population for the α chain and 84% (ASSLGQSNTEVF) and 15% (ASSYGGEQY) for the β chain (Figure 2B). We identified a total of 82 Vα and 39 Jα gene segments as well as 23 distinct Vβ gene segments and 11 distinct Jβ in the fibrotic livers from v-A12-vaccinated mice (Figure 2C). The sequenced TCRs from livers after v-A12 vaccination showed a predominant usage of the TRAV3-4, TRAV6D4, TRAV6D5, TRAV7D6, TRAV8D2, TRAV13D1, and TRAV14D3/DV8 and TRAJ2, TRAJ11, TRAJ22, TRAJ24, TRAJ26, TRAJ31, TRAJ41, and TRAJ45 α chain segments as well as TRBV13-2, TRBV13-3, TRBV19, and TRBV24 and TRBJ1-1, TRBJ1-5, TRBJ2-2, TRBJ2-3, and TRBJ2-7 β...
chain segments relative to v-CTRL vaccination (Figure 2C). This suggests that the enriched T cell clones are present in the liver as a result of the v-A12 vaccination. Moreover, splenic CD8+ and CD4+ T cells from v-A12-vaccinated mice with reduced liver fibrosis released IFN-γ upon re-exposure to ADAM12-expressing dendritic cells (Figures 2D and S1D), indicative of an

Figure 2. Antifibrotic vaccination results in the expansion of T cell receptor clonotypes in fibrotic livers
(A and B) Representation of specific CDR3a and specific CDR3b motifs detected in T cells from livers after prophylactic vaccination. Data represent CDR3s common for at least 2 samples from each treatment.
(C) Usage of V and J segments of TRA and TRB genes in T cells from liver of CCl4-treated animals. Data represented as a log2(fold change).
(D) Quantitative analysis of spot formation cells (SFC) in IFN-γ ELISPOT of CD8+ T cells populations purified from CCl4-treated, vaccinated animals and restimulated with dendritic cells presenting ADAM12-derived epitopes. Statistical test: two-tailed Student’s t test.
antigen-specific T cell response. In summary, we demonstrate successful prophylactic vaccination against murine liver fibrosis based on a fibroblast-specific transcript.

Therapeutic vaccination against endogenous fibroblast targets ameliorates established liver fibrosis

Fibrosis prevents parenchymal regeneration. However, there is evidence that human fibrotic disease is reversible if ablation of the fibroblast population is achieved (Cordero-Espinoza and Huch, 2018; Jun and Lau, 2018; Pellicoro et al., 2014). However, standard therapies that target fibrogenic cells in established fibrosis and, thus, allow for organ recovery are limited. Hence, we sought to evaluate whether a therapeutic vaccination scheme is successful in established liver fibrosis. For the therapeutic scheme, vaccination against ADAM12 was performed after 3 weeks of CCl4-treatment (Figure 3A, scheme), when liver fibrosis is already established as determined in pilot experiments (Figure S4A). Therapeutic vaccination against ADAM12 resulted in a decrease in the area covered by ADAM12-expressing cells (Figure 3B) and reduced tissue fibrosis as evidenced by a significant decrease in activated α-SMA-expressing fibroblasts (Figure 3C) and tissue collagen content (Figures 3D and 3E).

Therapeutic ADAM12 vaccination reduced the number of macrophages in fibrotic livers, whereas neutrophil counts remained unaffected (Figure S4B). In fibrotic livers, CD4* T cell counts were not higher upon v-A12 vaccination, whereas CD8* T cells were significantly increased, regardless of vaccination (Figure S4C). However, we detected an increase in splenic CD4* and CD8* effector and effector memory T cells, along with a decrease in naive T cells in ADAM12-vaccinated mice with ameliorated fibrosis (Figures S4D and S4E). Next, we performed killing assays with splenic and intrahepatic CD8* T cells from vaccinated mice with liver fibrosis and different target cells, including MHC I-deficient YAC-1 lymphoma cells with minimal ADAM12 expression (Figure S4F) as well as ADAM12-expressing primary hepatic stellate cells (HSC), NIH 3T3 fibroblasts (NIH 3T3), and NIH 3T3 fibroblasts engineered to express full-length ADAM12 (NIH 3T3 A12). We observed specific lysis of YAC-1 cells to a similar extent by CD8* T cells from v-CTRL and v-A12 mice (about 9% and 10% of specific lysis, respectively), which we therefore considered spontaneous baseline killing (Figure 3F). However, CD8* T cells from v-A12 mice showed increased specific lysis when exposed to primary HSC or NIH 3T3 A12 cells relative to CD8* T cells from v-CTRL mice (Figure 3F). Likewise, isolated intrahepatic CD8* T cells from v-A12 vaccinated mice with liver fibrosis showed increased specific killing primary HSC and NIH 3T3 A12 cells (Figure 3G). The subsequent ELISpot assay demonstrated increased IFN-γ release by splenic (Figure S4G) and more dominantly intrahepatic CD8* T cells (Figure S4H) from v-A12 vaccinated mice upon exposure to primary HSC and NIH 3T3 A12 cells. In summary, this suggested that successful vaccination against murine liver fibrosis involves an antigen-specific response from cytotoxic CD8* T cells.

Next, we sought to determine to which extend the vaccination-induced reduction in fibrosis in the therapeutic setting depended on cytotoxic T cells. To this end, we performed antibody-mediated depletion of CD8* T cells (ICD8) in mice with established liver fibrosis (Figure S4A) that started 48 h before the first therapeutic immunization of fibrotic mice and continued it for 3 weeks (Figure 4A, scheme). This approach resulted in a 60% reduction of CD8* T cells in the spleen and liver of v-CTRL- and v-A12-vaccinated mice (Figure 4B). In mice treated with an isotype antibody (iso) as control, v-A12-vaccination resulted in a decrease in ADAM12-expressing cells (Figure 4C) and α-SMA-expressing fibroblasts (Figure 4D) and tissue collagen content (Figures 4E and 4F). However, depletion of CD8* T cells impaired the antifibrotic effect of the v-A12 vaccine as evidenced by the level of ADAM12-expressing cells (Figure 4C), α-SMA-expressing fibroblasts (Figure 4D), and tissue collagen content (Figures 4E and 4F) similar to fibrotic mice immunized with v-CTRL. This suggests that the antifibrotic effect of v-A12 largely depends on cytotoxic CD8* T cells. In summary, we demonstrate that our vaccination approach based on a fibroblast-specific transcript is effective against liver fibrosis in a prophylactic and therapeutic setting.

Vaccination against endogenous fibroblast targets prevents pulmonary fibrosis

Fibroblast populations across organs show remarkable heterogeneity (Lynch and Watt, 2018). Therefore, we aimed to test our vaccination approach in a different organ. Idiopathic pulmonary fibrosis (IPF) is usually fatal, with a median survival of less than 3 years (Murray et al., 2012). ADAM12 is expressed in fibrotic foci of the bleomycin-induced murine model of pulmonary fibrosis (Figure 5A) as demonstrated by co-stainings with collagen I (Col I) but is expressed, if at all, only at low levels in most normal tissues. Within fibrotic mouse lungs, 7% of ADAM12* cells co-express the myofibroblast marker α-SMA, whereas 12% and 37% of ADAM12* cells express PDGFR-α and PDGFR-β, respectively (Figure S5A). Immune inactivation against ADAM12 prior to the induction of fibrosis (Figure 5B, scheme) prevented bleomycin-induced pulmonary fibrosis almost completely as evidenced by a significant decrease in the area covered by ADAM12-expressing cells (Figure 5C), a reduction in α-SMA-expressing fibroblasts (Figure 5D), and normal tissue

Figure 3. Therapeutic vaccination against fibrogenic cells ameliorates established liver fibrosis

(A) Scheme of therapeutic vaccination against liver fibrosis.
(B) ADAM12-positive area on liver sections and images of ADAM12 immunostaining (sham: v-CTRL, n = 3; v-A12, n = 3; CCl4: v-CTRL, n = 5; v-A12, n = 5). Data represented as fold change.
(C) αSMA-positive area on liver sections and images of αSMA IHC (sham: v-CTRL, n = 9; v-A12, n = 9; CCl4: v-CTRL, n = 16; v-A12, n = 16).
(D) Sirius red-positive area on liver sections and images of Sirius red/fast green staining (sham: v-CTRL, v-A12, n = 16; CCl4: v-CTRL, n = 20; v-A12, n = 20).
(E) Collagen content by hydroxyproline assay of liver tissues (sham: v-CTRL, n = 5; v-A12, n = 5; CCl4: v-CTRL, n = 26; v-A12, n = 26).
(F and G) (F) Cytotoxic assay with purified splenic and (G) intrahepatic CD8* T cells from CCl4-treated animals vaccinated with v-CTRL or v-A12 with target cells (YAC-1, hepatic stellate cells [HSC], NIH 3T3, NIH 3T3 expressing ADAM12 [NIH 3T3 A12]); ratio effector: target 1:1 for 6 h. Data represented as % of specific lysis. Values are mean ± SEM, statistical test: one-way ANOVA (B–E), two-way ANOVA (F and G). Scale bars in (B), (C), (D), (F), and (G), 100 μm.
collagen content (Figures 5E and 5F). Although ADAM12 vaccination reduced the number of macrophages and CD4+ T cells in fibrotic lungs (Figure S2B), pulmonary CD8+ T cell counts were not significantly lower (Figure S5C).

Fibroblast populations even within a fibrotic organ show striking phenotypical diversity (Lynch and Watt, 2018). Given that vaccination targets among organs are likely to be heterogeneous, we aimed to test our approach in pulmonary fibrosis with an alternative vaccination target. The transcription factor GLI1 is expressed in fibrotic foci of the bleomycin-induced murine model of pulmonary fibrosis (Figure 6A) but is expressed only at low levels in most normal adult tissues in mice and humans (Kramann et al., 2015). We determined that within fibrotic mouse lungs, GLI1+ cells co-express the myofibroblast marker α-SMA (50% of GLI1+ cells), PDGFR-α (77% of GLI1+ cells), and PDGFR-β (37% of GLI1+ cells) (Figure S5D). In silico epitope prediction revealed that GLI1 is likely to yield a set of candidate peptides to be presented by MHC I molecules on the cell surface upon reactivation of GLI1 (Figure S5E). Thus, GLI1 is a potential vaccination candidate to target fibrogenic fibroblasts in the lung. Immunization with a GLI1-encoding lentiviral vectors with adjuvants prior to induction of fibrosis (Figure 6B, scheme) prevented bleomycin-induced pulmonary fibrosis almost completely as evidenced by a significant decrease in the area covered by GLI1-expressing cells (Figure 6C), a reduction in α-SMA-expressing fibroblasts (Figure 6D), and normal tissue collagen content (Figures 6E and 6F).

Although GLI1 vaccination reduced the number of macrophages in fibrotic lungs (Figure S5F), pulmonary CD4+ T cell and CD8+ T cell counts remained unaffected by GLI1 vaccination (Figure S5G). Finally, the reduction in tissue fibrosis was associated with elevated expression of surfactant protein A1 (Figure 6G) in fibrotic lungs from v-GLI1-vaccinated mice, indicating improved lung recovery. In summary, we demonstrate a vaccination approach to target murine lung fibrosis based on the fibroblast-specific transcripts Adam12 and Gli1.

Fibrosis vaccination does not adversely affect organ homeostasis and wound healing

In separate experiments, we performed preclinical safety tests and examined eventual autoimmunity damage and adverse effects of ADAM12 and GLI1 vaccination. We conducted histopathological evaluation of major organs from vaccinated mice at the Laboratory for Animal Model Pathology (LAMP) of the Vetuisse Faculty, University of Zurich, 1 week and 4 weeks after the prime/boost immunization. However, this did not reveal any obvious effect on the architecture of skin, heart, small intestine, skeletal muscle, spleen, pancreas, and kidney at the histological level (Figures 7A and 7B). No histological changes that could account for a side effect induced by the vaccines were identified in these organs. Furthermore, a blood analysis of vaccinated animals for markers of liver kidney and pancreas damage 4 weeks after the prime/boost immunization did not suggest organ damage (Figure 7C).

Previous reports have demonstrated that ADAM12+ and GLI1+ cells are located in the perivascular compartment. Therefore, we assessed whether immunization against ADAM12+ and GLI1+ results in pericyte loss or perivascular inflammation in blood vessels of the liver and lung under steady-state conditions and in the absence of fibrosis. The number of α-SMA+ perivascular cells around blood vessels of the liver periportal fields as well as pulmonary blood vessel with a diameter of 25–100 μm (Richard et al., 2014) were similar in sham livers of v-CTRL and v-A12 mice and sham lungs from v-CTRL, v-A12, and v-GLI1 mice (Figure S6A) and in the range of previous reports on pericyte coverage in these compartments (Richard et al., 2014; Strauss et al., 2017). Moreover, the pathologists did not observe perivascular inflammation, and this was further corroborated by a quantification of CD45+ leukocytes in the perivascular compartment of liver (Baek et al., 2014; Strowitzki et al., 2018) and lung (Summer et al., 2009) from immunized animals under steady-state conditions (Figure S6B).

Although mesenchymal GLI1 expression was observed in the colon as previously reported (Degirmenci et al., 2018), GLI1 and ADAM12 were minimally, if at all, expressed in the small intestine (Figure S6C). However, neither GLI1 nor ADAM12 vaccination resulted in changes in the mucosal relief including villous atrophy or loss of crypts at 1 week (Figures S6D–S6G) or 4 weeks after prime/boost immunization (Figures 7A and 7B).

Next, we evaluated potential vaccination-induced defects in skin wound healing 1 week and 4 weeks after the prime/boost immunization (Figures 7D, S6F, and S6G). No delay or defects in wound healing were observed in GLI1-vaccinated mice versus control-treated mice (Figures 7D and S6G). However, we noticed that 1 week after the prime/boost immunization, ADAM12 vaccination led to a transiently accelerated wound closure compared with control-treated mice (Figure S6F). This is consistent with the notion that ADAM12 expression in wound fibroblasts is associated with wound healing defects (Harsha et al., 2008).

Next, we decided to analyze more subtle immunization-related effects on skin regeneration that could eventually occur. To determine the degree of epidermal differentiation in vaccinated mice post injury, we analyzed the expression of keratin 5 (K5, a basally expressed keratin), keratin 1 (K1, a suprabasal keratin, expressed in spinous layer cells), and loricin (Lor, a
cornified envelope protein expressed in the granular layer) (Ja-
mora et al., 2005) in the wound bed and adjacent wound margins
(Figures S7A and S7B). Furthermore, we determined epidermal
thickness in the wound margins, based on K5 and Lor staining
(Figure S7C). However, the expression of K5, K1, and Lor as
well as epidermal thickness in the wound margins of ADAM12-
and GLI1-immunized mice were similar to that in control-vacci-
nated mice (Figures S7A–S7C).

Finally, we reasoned that tendon injury, where transient fibrosis
and ECM remodeling give way to repair and restitution, repre-
sents an important context to analyze the impact of ADAM12-
and GLI1-vaccination on repair. For this purpose, vaccinated
mice were subjected to a patellar tendon injury model 1 week af-
fter prime/boost immunization and allowed to heal for 4 weeks.
Both, ADAM12- and GLI1-vaccination allowed for normal recov-
ery from tendon injury similar to control-vaccinated mice
(Figures S7A–S7C).
Figure 6. Vaccination against GLI1 prevents pulmonary fibrosis

(A) Images of GLI1/collagen I immunostaining on healthy and fibrotic murine lungs (bleomycin-induced fibrosis).

(B) Prophylactic vaccination in a bleomycin model of lung fibrosis in mice.

(C) GLI1-positive area on lung sections from sham- and bleomycin-treated animals vaccinated with v-CTRL or GLI1 vaccine (v-GLI1) and images of GLI1 immunostaining (sham: v-CTRL, n = 3; v-GLI1, n = 3; bleomycin: v-CTRL, n = 6; v-GLI1, n = 7). Scale bars, 50 μm.

(D) αSMA-positive area on lung sections from sham- and bleomycin-treated animals vaccinated with v-CTRL or v-GLI1 and images of αSMA IHC (sham: v-CTRL, n = 3; v-GLI1, n = 3; bleomycin: v-CTRL, n = 6; v-GLI1, n = 7).

(E) Sirius red-positive area on lung sections from sham- and bleomycin-treated animals vaccinated with v-CTRL or v-GLI1 and images of Sirius red/fast green staining (sham: v-CTRL, n = 3; v-GLI1, n = 3; bleomycin: v-CTRL, n = 6; v-GLI1, n = 7).

(F) Collagen content by hydroxyproline assay on lung samples from sham- and bleomycin-treated animals vaccinated with v-CTRL or v-GLI1 (sham: v-CTRL, n = 3; v-GLI1, n = 3; bleomycin: v-CTRL, n = 6; v-GLI1, n = 7).

(G) Gene expression analysis of Sftpa1 in lung samples from sham- and bleomycin-treated animals vaccinated with v-CTRL or v-GLI1 (sham: v-CTRL, n = 3; v-GLI1, n = 3; bleomycin: v-CTRL, n = 6; v-GLI1, n = 7). Values are mean ± SEM, statistical test: one-way ANOVA. Scale bars in (A), (C), (D), (E), 100 μm.

(Figures S7D and S7E). As assessed by second harmonic generation microscopy of tendon fibers (Figures S7D and S7E), the density and organization of healed collagen fibers in tendons from injured, vaccinated mice after recovery were comparable with the contralateral sham-injured tendon, which served as control. In summary, this suggests at least no severe vaccination-induced defects in tendon repair.
Figure 7. Fibrolytic vaccination has no adverse effects

(A) 4 weeks post-vaccination histological analysis of spleen, pancreas, heart, kidney, small intestine, skeletal muscle, and skin from animals vaccinated with v-CTRL or v-A12.

(B) 4 weeks post-vaccination histological analysis of spleen, pancreas, heart, kidney, small intestine, skeletal muscle, and skin from animals vaccinated with v-CTRL or v-GLI1.

(C) 4 weeks post-vaccination blood diagnostics of animals vaccinated with v-A12 and v-GLI1 with clinical chemistry parameters of liver, kidney, and pancreas.

(D) Top: scheme of the experimental strategy for wound healing model in animals vaccinated with v-CTRL or v-A12. Bottom left: quantification of individual wound areas at day 2 and 5 post injury in animals vaccinated with v-CTRL or v-A12 relative to their respective initial size at day 0 (v-CTRL, n = 17; v-A12, n = 19). Bottom right: scheme of the experimental strategy for wound healing model in animals vaccinated with v-CTRL or v-GLI1. Left: quantification of individual wound areas at day 2 and 5 post injury in animals vaccinated with v-CTRL or v-GLI1 relative to their respective initial size at day 0 (v-CTRL, n = 17; v-GLI1, n = 15). Values are mean ± SEM, statistical test: Student’s t test. Scale bars in (A) and (B), 100 μm.
wild-type mice, indicating that ADAM12- and GLI1-expressing cells could be involved in tendon fibrosis (Figures S7F and S7G).

In summary, this suggests that our immunization approach to target fibrogenic cells does not have severe adverse effects with regard to the homeostasis and microarchitecture of major organs or physiological healing responses.

DISCUSSION

Importantly, it is not the function of the fibrogenic cell-specific transcripts but the “tagging function” that we exploit for a vaccination approach. Therefore, conventional approaches such as small molecule inhibitors to inhibit the function of such endogenous molecules are likely to be less suitable. However, it is important to consider that not every fibrogenic cell-specific transcript will give rise to sufficiently immunogenic epitopes. Indeed, in silico epitope prediction for the fibrogenic transcription factor FoxD1 (Gomez and Duffield, 2014) revealed that FoxD1 is unlikely to yield immunogenic peptides to be presented on MHC class I molecules (data not shown).

Despite successful fibrolitic vaccination, fibrosis was only partially reduced upon vaccination, and fibroblast populations across organs and even within a fibrotic tissue show remarkable heterogeneity (Lynch and Watt, 2018). Therefore, vaccination targets within a fibrotic organ are likely to be diverse, and profiling of activated fibroblasts will identify alternative antigens, which may be effective T cell vaccination targets in patients with organ fibrosis. Moreover, the outcome of T cell-based immunotherapies can fail due to an immunosuppressive environment and the expression of checkpoint inhibitor molecules in the target tissue (Devaud et al., 2013), which need to be further characterized.

We show that the depletion of CD8+ T cells completely abolishes the antifibrotic effect of v-A12 vaccination in the CCl4-induced toxic liver fibrosis, whereas the absence of CD8+ T cells has no impact on the degree of fibrosis in v-CTRL-immunized mice. This indicates that in CCl4-induced liver fibrosis, CD8+ T cell responses are per se not pathogenic but can be leveraged by targeted immunization to generate protective T cell immunity. However, in the context of non-alcoholic steatohepatitis (NASH), it has been recently demonstrated that CD8+ T cell depletion ameliorates liver damage by removing asegressive T cells with distinct pathogenic profile that target metabolically altered hepatocytes (Dudek et al., 2021). Therefore, it is tempting to speculate whether in the NASH context, fibroblast-targeting vaccines can redirect the cytolytic machinery (Aghajanian et al., 2019; Amor et al., 2020; Rurik et al., 2022). A potential advantage of the vaccination approach is the possibility to attack endogenous factors that are not expressed on the cell surface, including cytoplasmic or nuclear proteins, including transcription factors that are specific for fibrogenic cells. Modern vaccination approaches, including the recently emerged mRNA vaccines, can combine several target epitopes (Hollingsworth and Jansen, 2019; Sahin et al., 2017; Tureci et al., 2018) and therefore hold the potential to manufacture personalized vaccines with a cocktail of targets for patients with distinct types of organ fibrosis. However, before this tailored vaccine approach can be translated to humans, efficacy and safety profiles need to be determined.

Tremendous progress in the prevention and therapy of infectious diseases as well as certain cancers have been achieved through vaccination. Here, we suggest that vaccination may be used as preventive and curative immunotherapeutic approach for organ fibrosis, one of the most frequent forms of mortality.

Limitations of the study

We have not undertaken single-cell analysis to gain more insight into additional mechanisms of fibrosis regression and liver regeneration. Although we demonstrate that ablation of fibrogenic cells by cytotoxic CD8+ T cells is the dominant response to the vaccination, we have not studied in depth the involvement of other immune cell subsets in the regression of fibrosis, e.g., macrophage populations. Likewise, in addition to fibrolysis that allows for liver regeneration, we have not investigated to which extent the vaccine affects the expansion of endogenous liver
stem and progenitor cell populations that contribute to parenchymal regeneration. With regard to liver fibrosis, it is important to point out that we have not tested the vaccination approach in other models of liver fibrosis, namely NASH, with a different pathogenesis.

Although we demonstrate T cell reactivity against a in silico-predicted set of 5 peptides after vaccination with a lentiviral vector encoding for full-length ADAM12 vaccination, we have not performed vaccinations with the individual peptides to show in vivo efficacy of the peptides. Finally, a rigorous validation and testing of vaccination targets for the human setting will be needed to facilitate translation and clinical applications of vaccination-based approaches.

**STAR Methods**

Detailed methods are provided in the online version of this paper and include the following:

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**Supplemental Information**

Supplemental information can be found online at https://doi.org/10.1016/j.stem.2022.08.012.

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**Author Contributions**

M.S. designed and conducted experiments, analyzed data, prepared the figures, and wrote the manuscript. J.C. and E.K. conducted experiments, analyzed data, and prepared the figures. S.N., Z.F., E.N., J.M.M.R., F.S., A.H., G.M., E.Y.H., and R.K. conducted experiments and analyzed data. D.G. and J.D. analyzed data. C.B., T.S., T.I., N.T., C.T., and E.T. provided reagents and edited the manuscript. A.W., S.W., J.L., and L.S. provided conceptual input and edited the manuscript. V.S. and C.M. provided reagents, technical support, conceptual input, and edited the manuscript. C.F.-B., E.P., O.D., J.S., and C.J. provided technical support, conceptual input, supervised experiments, analyzed data, and edited the manuscript. C.S. designed and conducted experiments, analyzed and interpreted data, wrote the manuscript, and supervised and directed the project.

**Declaration of Interests**

The authors declare no competing interests.

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STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Rabbit anti-mouse alpha smooth muscle actin (αSMA) antibody | Abcam | Cat# 5694; RRID: AB_2223021 |
| Mouse anti-mouse alpha smooth muscle actin -Cy3 (αSMA) antibody (C1A4) | Sigma-Aldrich | Cat# C6198; RRID: AB_476856 |
| Rabbit anti-mouse ADAM12 antibody | Thermo Fisher Scientific | Cat# PA5-50594; RRID: AB_2636047 |
| Goat anti-mouse ADAM12 antibody | Novus Biology | Cat# NB300-889; RRID: AB_10001047 |
| Rabbit anti-mouse ADAM12 antibody | Proteintech | Cat# 14139-1-AP; RRID: AB_2289230 |
| Rabbit anti-Collagen I antibody | Abcam | Cat# ab34710; RRID: AB_731684 |
| Goat anti-mouse PDGFRα antibody | R&D Systems | Cat# AF1062; RRID: AB_2236897 |
| Rabbit anti-mouse PDGFRβ antibody (2BE1) | Cell Signaling Technology | Cat# 3169; RRID: AB_2162497 |
| Mouse anti-mouse PDGFRβ antibody (D-6) | Santa Cruz Biotechnology | Cat# sc-374573; RRID: AB_1099092 |
| Rabbit anti-Glial Fibrillary Acidic Protein | Agilent | Cat# 20334; RRID: AB_10013382 |
| Goat anti-human/mouse GLI1 antibody | R and D Systems | Cat# AF3455; RRID: AB_2247710 |
| Rabbit anti-GLI1 antibody | Sigma-Aldrich | Cat# 665996 |
| Goat anti-mouse Albumin | Abcam | Cat# ab19194; RRID: AB_777886 |
| Mouse anti-mouse β-actin (8H10D10) | Cell Signaling Technology | Cat# 3700; RRID: AB_2242334 |
| Rabbit anti-Loricrin | Jamora lab-generated | N/A |
| Chicken anti-Keratin 5 | Jamora lab-generated | N/A |
| Rabbit anti-Keratin 1 | Jamora lab-generated | N/A |
| Anti-mouse CD8α monoclonal antibody | Veronika Sexl lab provided | N/A |
| Donkey anti-Rabbit IgG (H+L) _Cy3 | Jackson ImmunoResearch Labs | Cat# 711-165-152; RRID: AB_2307443 |
| Goat anti-Mouse IgG1 (H+L), Alexa Fluor 546 | Thermo Fisher Scientific | Cat# A-21123; RRID: AB_2553765 |
| Donkey anti-Goat IgG (H+L), Alexa Fluor 488 | Thermo Fisher Scientific | Cat# A-11055; RRID: AB_2534102 |
| Goat anti-Rabbit IgG (H+L), Alexa Fluor 488 | Thermo Fisher Scientific | Cat# A-11034; RRID: AB_2576217 |
| Goat anti-Chicken IgY (H+L), Alexa Fluor 568 | Thermo Fisher Scientific | Cat# A-11041; RRID: AB_2534098 |
| **Cell Lines**       |        |            |
| **Cell Lines**       |        |            |

(Continued on next page)
Continued

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Anti-mouse Ly-6G (1A8) | BD Biosciences | Cat# 746448; RRID: AB_2743751 |
| Anti-mouse Ly-6C (HK1.4) | BioLegend | Cat# 128035; RRID: AB_2562352 |

**Chemicals, peptides, and recombinant proteins**

| TLR9 agonist CpG oligodeoxynucleotides | InvivoGen | Cat# ODN1826 |
| Incomplete Freund’s adjuvant | Sigma-Aldrich | Cat# F5506 |
| Carbon tetraChloride | Sigma-Aldrich | Cat# 289116-100ML |
| Bleomycin | Merck-Milipore | Cat# 203408 |
| Buprenorphine (Temgesic) | Indivior Schweiz AG, | Cat# 6664853 |
| Hoechst 33342 | Life Technologies | Cat# H3570 |
| Liberase TL | Roche | Cat# 5401020001 |
| DNAel | Roche | Cat# 04716728001 |
| Optiprep | STEMCELL technology | Cat# 07820 |
| RIPA Lysis and Extraction Buffer | Thermo Fischer Scientific | Cat# 89900 |
| Protease inhibitor tablet | Roche | Cat# C775C25 |

**Biological samples**

| Human liver samples | George Pompidou Hospital | N/A |
| Human biceps tendon | Amro Ahmed Hussien Ahmed lab provided | N/A |
| Fra-2tg mice achilles tendon | Amro Ahmed Hussien Ahmed lab provided | N/A |

**Critical commercial assays**

| Liver Dissociation Kit | Miltenyi Biotech | Cat# 130-105-807 |
| Lung Dissociation Kit | Miltenyi Biotech | Cat# 130-095-927 |
| TransIT-X2® Dynamic Delivery System | MirusBio | Cat# MIR 6000 |
| DC protein assay kit | Bio-rad | Cat# 5000116 |
| Murine IFN-γ ELISpot assay | Diaclone | Cat# 862.031.010S |
| LIVE/DEAD® Fixable Aqua Dead Cell Stain Kit | Thermo Fisher Scientific | Cat# L34957 |
| CD8a+ (Ly-2) T Cell Isolation Kit (mouse) | Miltenyi | Cat# 130-104-075 |
| CD4+ (L3T4) T Cell Isolation Kit (mouse) | Miltenyi | Cat#130-117-043 |
| VECTASTAIN Elite ABC-HRP Kit | Vectorlabs | Cat# VEC-PK-6101 |
| Avidin/Biotin Blocking Kit | DAKO | Cat# X0590 |
| DAB Peroxidase Substrate Kit | Vectorlabs | Cat# SK-4100 |
| RNasey Mini Kit (250) | QIAGEN | Cat# 74106 |
| High-Capacity cDNA Reverse Transcription Kit | Applied Biosystems™ | Cat# 4368814 |
| LightCycler® 480 SYBR Green I Master MIX | Roche | Cat# 04887352001 |

**Experimental models: Organisms/strains**

| Mouse: C57BL/6JR | JANVIER LABS | N/A |
| Mouse: Fra-2tg mice | Amro Ahmed Hussien Ahmed lab provided | N/A |

**Experimental models: Lentiviral vectors**

| pLV.EF1.TurboGFP.WPRE – CTRL vaccine (v-CTRL) | FLASH THERAPEUTICS | N/A |
| pLV.EF1.Adam12.T2A.TurboGFP – ADAM12 vaccine (v-A12) | FLASH THERAPEUTICS | N/A |
| pLV.EF1.mGlI1.T2A.TurboGFP - GLI-1 vaccine (V-GLI1) | FLASH THERAPEUTICS | N/A |

**Experimental models: Cell lines**

| NIH 3T3 cell line | ATCC | ATCC number: CRL-1658 |
| YAC-1 cell line | Veronika Sexl lab provided | N/A |
| MutuDC1940 cell line | Hans Acha-Orbea lab provided | Cat# ACC 635 |

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RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Christian Stockmann (christian.stockmann@anatomy.uzh.ch).

Materials availability
This study did not generate new unique reagents nor mouse lines.

Data and code availability
T cell receptor sequencing data have been deposited at GEO and are publicly available as of the date of publication. Accession numbers are listed in the key resources table.

This paper does not report original code.

Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animal models
Female wild type C57BL/6JRj mice aged 8-10 weeks were purchased from JANVIER LABS (Le Genest-Saint-Ise, France) for liver fibrosis, lung fibrosis, skin wound healing and patellar tendon injury studies. Mice were randomly allocated to different treatment groups, and the investigator was blinded to the group allocation during the experiment as well as during the analysis. Fra2Tg transgenic mice were generated according to previous study (Renoux et al., 2020) and mice with established fibrotic phenotype in tendon at the age between 16–24 weeks old and their wild-type controls were for tendon fibrosis (Hussien et al., 2021). All mice were housed at standard housing conditions (22 °C, 12 h light/dark cycle), with ad libitum access to chow diet (18 % proteins, 4.5 % fibers, 4.5 % fat, 6.3 % ashes, Proviミ Kliba SA) and water. Health status of all mouse lines was regularly monitored according to FELASA guidelines. All animal experiments were approved by the local animal ethics committee (Kantonales Veterинаrsamt Zürich, licenses ZH170/2018, and ZH219/2017), and performed according to local guidelines (TschV, Zurich) and the Swiss animal protection law (TschG).
Human samples
Immunohistochemical analysis was performed on human liver samples from patients with a non-viral aetiology of fibrosis/cirrhosis. Liver tissue specimens were obtained from surgical liver biopsies or liver resection in the context of gastrointestinal malignancies. Histological samples were analyzed and classified as fibrotic or cirrhotic by the pathology department at George Pompidou Hospital according to the Metavir classification. F4 liver specimens showed cirrhosis, periportal and septal fibrosis. Uninvolved liver segments from surplus resected liver served as nonfibrotic control. Human biceps tendon were gifts from Amro Ahmed Hussien lab and were measured as healthy and tendinopathy from Tierspital grading cohort. Ethics approval was obtained from the local ethics committee and patients gave informed consent.

Cell lines
The MutuDC1940 and YAC-1 cell line was grown in Iscove’s Modified Dulbecco’s Medium + GlutaMAX supplemented with 10% FBS, 10 mM HEPES, 55 µM 2-Mercaptoethanol at 37°C in a humidified atmosphere of 5% CO2 in air. To generate NIH 3T3 Adam12 cell line, NIH 3T3 cells were transduced with lentiviral particles pLV.EF1.Adam12.T2A.TurboGFP – ADAM12 at MOI 1. Passage 4 post-transduction was sorted to isolate high GFP+ cells using FACSaria II 5L (BD). The NIH 3T3 Adam12 cell line was grown in DMEM/F12 + GlutaMAX Medium supplemented with 10% FBS at 37°C in a humidified atmosphere of 5% CO2 in air.

METHOD DETAILS

Mouse immunization
Mice were injected twice (prime vaccination and a second boost vaccination 7 days later) subcutaneously in the neck region with 2×10^7 TU of each vector formulated in 200 µL of 50% Incomplete Freund’s adjuvant (Sigma-Aldrich, F5506), 50 µg TLR9 agonist Cpg oligodeoxynucleotides (InvivoGen, ODN 1826) in LAL water. Recombinant lentiviral vectors were purchased from FLASH THERAPEUTICS (France) at minimal titer of 2.5×10^9 TU/mL. Vectors used in following study:

1) pLV.EF1.TurboGFP.WPRE – CTRL vaccine (v-CTRL);
2) pLV.EF1.Adam12.T2A.TurboGFP – ADAM12 vaccine (v-A12);
3) pLV.EF1.mGli1.T2A.TurboGFP – GLI1 vaccine (v-GLI1).

For detailed description of the vectors see Figure S2A and key resources table.

Liver fibrosis mouse model, prophylactic setup
For carbon tetrachloride (CCl4; Sigma-Aldrich, 289116-100ML) treatment, mice were injected intraperitoneally (300 µL of CCl4/kg body weight suspended in olive oil three times a week, with a total volume of 100 µL per injection) for 6 weeks. 8 weeks old C57BL/6JRj female mice from JANVIER LABS were allotted into 4 treatment groups: 1) Sham-treated and vaccinated with v-CTRL; 2) Sham-treated and vaccinated with v-A12; 3) CCl4-treated and vaccinated with v-CTRL; 4) CCl4-treated and vaccinated with v-A12. Mice were immunized (see mouse immunization section) on day 9 (prime) and day 2 (boost) before the start of the CCl4 or sham treatment. For sham treatment, mice were injected intraperitoneally with 100 µL olive oil three times a week for 6 weeks. For a scheme of the experimental setup, see Figure 1C. Body weight was measured every 2 days and tissue samples were collected on day 42.

Liver fibrosis mouse model, therapeutic setup
8 weeks old C57BL/6JRj female mice from JANVIER LABS were allotted as above. Mice were immunized (see mouse immunization section) 3 (prime) and 4 weeks (boost) after the start of CCl4 or sham treatment. For CCl4 treatment, mice were injected intraperitoneally with carbon tetrachloride (CCl4) (300 µL of CCl4/kg body weight suspended in olive oil three times a week, with a total volume of 100 µL per injection) for 6 weeks. For sham treatment, mice were injected intraperitoneally with 100 µL olive oil three times a week for 6 weeks. For a scheme of the experimental setup, see Figure 2A. Body weight was measured every 2 days and tissue samples were collected on day 42.

Liver fibrosis mouse model with CD8+ T cell depletion, therapeutic setup
8 weeks old C57BL/6JRj female mice from JANVIER LABS were allotted as above. Mice were immunized (see mouse immunization section) 3 (prime, day 19) and 4 weeks (boost, day 26) after the start of CCl4 or sham treatment. For CCl4 treatment, mice were injected intraperitoneally with CCl4 (300 µL of CCl4/kg body weight suspended in olive oil three times a week, with a total volume of 100 µL per injection) for 6 weeks. For sham treatment, mice were injected intraperitoneally with 100 µL olive oil three times a week for 6 weeks. For CD8a+ T cell depletion, mice were injected intraperitoneally with anti-CD8a monoclonal antibody (10 mg/kg body weight; kindly provided by Prof. Veronika Sexl from Vienna) at day 18, 23, 25, 30, 32, 37.

For a scheme of the experimental setup, see Figure 3A. Body weight was measured every 2 days and tissue samples were collected on day 42.
Lung fibrosis mouse model

8 weeks old C57BL/6JRj female mice from JANVIER LABS were allotted into 4 groups. Mice were treated as follows: 1) Sham-treated and vaccinated with v-CTRL; 2) Sham-treated and vaccinated with v-A12 or v-GL1; 3) Bleomycin-treated and vaccinated with v-CTRL; 4) Bleomycin-treated and vaccinated with v-A12 or v-GL1. Mice were immunized (see mouse immunization section) on day 9 (prime) and day 2 (boost) before the start of the Bleomycin (Merck-Millipore, 203408) or sham treatment. For Bleomycin treatment, mice were treated once a day by oropharyngeal aspiration of 25 μL of Bleomycin at 0.5 mg/kg body weight for 5 following days over first week. For sham treatment, mice were treated once a day by oropharyngeal aspiration of 25 μL of phosphate-buffered saline for 5 following days over first week. For an experimental scheme see Figure 3B. Body weight was measured every 2 days and tissue samples were collected on day 28.

ADAM12 and GLI1 expression by antigen presenting cells

The MutuDC1940 cell line was a kind gift from Hans Acha-Orbea. Plasmids containing CDS of TurboGFP (pcDNA.EF1.TurboGFP), murine Adam12 (NM_007400.2) (pcDNA.EF1.Adam12.T2A.TurboGFP) and GLI1 (NM_010296.2) (pcDNA.EF1.mGli1.T2A.TurboGFP) were purchased from FLASH THERAPEUTIC (France). MutuDC1940 were transfected with the expression vectors with TransIT-X2® Dynamic Delivery System (MirusBio, MIR 6000) according to the manufactures protocol. Cells were collected 48h post transfection for further ELISpot experiments.

Tissue dissociation

For isolation of splenic immune cells: the spleen was removed and mechanically disrupted, filtered through a 70-μm strainer and red blood cells were lysed by a 3-min incubation with 1x RBC Lysis Buffer (BioLegend, 420301) on ice. Splenic cells were then diluted 1:10 with cold PBS and filtered through a 40-μm strainer. For isolation of hepatic immune cells: the mouse liver was processed with the Liver Dissociation Kit (Miltenyi Biotec, 130-105-807) according to the manufacturer’s instructions. For isolation of pulmonary immune cells: the mouse lung was processed with the Lung Dissociation Kit (Miltenyi Biotec, 130-095-927) according to the manufacturer’s instructions. The dissociated cells were further processed and stained as described in the Flow cytometry section.

Flow cytometry

Single-cell suspension of liver tissue and spleen were obtained and stained. Cell viability was measured using LIVE/DEAD® Fixable Aqua Dead Cell Stain Kit (Thermo Fisher, L34957). The following mAbs from eBioscience or BD-Biosciences or BioLegend were used: anti-CD8a (53-6-7; 100730), anti-CD62L (MEL-14; 104436), anti-CD44 (IM7; 103049), anti-CD45 (30-F11; 564225), anti-CD4 (GK1.5; 564667, 564667), anti-CD3 (145-2C11; 562286), anti-F4/80 (BM8; 123128), anti-CD11c (N418; 117310), anti-Ly-6G (1A8; 746448), anti-Ly-6C (HK1.4; 128035), anti-CD4 (GK1.5; 564667, 564667), anti-CD11b (M1/70; 741722), and relevant isotype controls. Flow cytometry was carried out on a BD FACSymphony®X Flow Cytometer. Data were analysed using FlowJo v10 (Treestar), (see Figures S3A and S3B for full gating strategy).

In silico epitope prediction for murine Adam12 and Gli1

Prediction of antigen processing and presentation through the MHC class I pathway was performed with the prediction tool from Immune Epitope Database Analysis Resource. Briefly, to identify potential MHC-I ligands, i.e. peptides that are naturally processed from their source proteins and presented by MHC class I molecules, the T Cell Epitopes - Processing Prediction (Proteasomal cleavage/TAP transport/MHC class I combined predictor) tool was used (http://tools.iedb.org/processing/) and fed with the respective protein sequences. Adam12 protein reference sequence: Q61824, Gli1 protein reference sequence: P47806. For predicted Adam12 derived epitopes see Figure S1A and for predicted GLI1 derived epitopes see Figure S6A.

T cell receptor sequencing

Sequencing with analysis of CDR3α (complementarity determining region 3 α motif and specific CDR3 β (complementarity determining region 3 β) and usage of V and J segments of TRA and TRB genes in T cells was performed on livers from CCl4-treated animals vaccinated with ADAM12 vaccine (v-A12) or CTRL vaccine (v-CTRL) in the prophylactic setup. Sequencing and bioinformatic analysis were performed by iRepertoire (Huntsville, AL 35806, USA).

ELISpot assays

The frequency of IFN-γ-secreting T cells in response to a specific stimulus was determined with the mouse IFN-gamma ELISPot assay (Diaclone, 862.031.010S). Splenocytes were isolated, then, CD8+ T cells (CD8α (Ly-2) MicroBeads, mouse, Miltenyi, 130-117-044) and CD4+ T cells (CD4 (L3T4) MicroBeads, mouse, Miltenyi, 130-117-043) were purified. Next, 1 × 10⁵ CD8+ T cells or 1 × 10⁵ CD4+ T cells were cocultured with previously transfected 1 × 10⁵ MutuDC1940 cells (see ADAM12 and GLI1 expression by antigen presenting cells section). The detection part of the experiment was conducted according to manufacturer’s instructions. The number of spots was evaluated in blinded fashion by AID EliSpot/FluoroSpot Reader System operated by AID EliSpot Software Version 7.0. Parameters and were adjusted following the International Harmonization Guidelines for ELISPOT plate evaluation. The frequency of positive (IFN-γ-producing) cells per the total number of CD4+ or CD8+ T cells was calculated after the number of spots in control wells had been subtracted from that in experimental wells. Additional control wells for the assay included reagents alone.
CD8a+ T cells were purified using CD8a+ T Cell Isolation Kit (Miltenyi, 130-104-075) and LS Column (Miltenyi, 130-042-401), and a 4 mL of 40% OptiPrep diluted in HBSS. Finally, 0.5 mL HBSS was added over the intermediate phase. Tube was centrifuged at 37 °C, 5% CO2 incubator. After 20 h, MutuDC 1940 dendritic cells engineered to express ADAM12 protein or control MutuDC 1940 were added to splenocytes at ratio 1:1 in 1 mL of fresh growing media (RPMI 1640 + Glutamax, 10% FBS, 55 μM 2-Mercaptoethanol) and placed in a humidified 37 °C, 5% CO2 incubator. After 5 days of culture, the CD8a+ T cells (CD8a LU-2) MicroBeads, mouse, Miltenyi, 130-117-044) were purified and used for ELISpot assays. Briefly, 1 x 10^5 of purified CD8a+ T cells were restimulated with a mix of the 5 in silico predicted peptides (see Figure S1A) at a concentration of 2.5 μg/mL for each peptide. Additional control wells for the assay included reagents alone (blank), nonstimulated, purified CD8a+ T cells (spontaneous IFN-γ production) and CD8a+ T cells stimulated with phorbol myristate acetate (PMA) at 1 ng/mL and ionomycin at 500 ng/mL as positive control.

**T cell restimulation with peptides**

6-well plate were coated with 5 μg/mL solution of anti-CD3e (eBioscience, 16-003182) for 2h at 37 °C. Then, 1 x 10^6/mL of a single splenocytes from C57BL/6JR mice were seeded into precoated wells in 2 mL of fresh growing media (RPMI 1640 + Glutamax, 10% FBS, 55 μM 2-Mercaptoethanol) and placed in a humidified 37 °C, 5% CO2 incubator. After 20 h, MutuDC 1940 dendritic cells engineered to express ADAM12 protein or control MutuDC 1940 were added to splenocytes at ratio 1:1 in 1 mL of fresh growing media (RPMI 1640 + Glutamax, 10% FBS, 55 μM 2-Mercaptoethanol) and placed in a humidified 37 °C, 5% CO2 incubator. After 5 days of culture, the CD8a+ T cells (CD8a LU-2) MicroBeads, mouse, Miltenyi, 130-117-044) were purified and used for ELISpot assays. Briefly, 1 x 10^5 of purified CD8a+ T cells were restimulated with a mix of the 5 in silico predicted peptides (see Figure S1A) at a concentration of 2.5 μg/mL for each peptide. Additional control wells for the assay included reagents alone (blank), nonstimulated, purified CD8a+ T cells (spontaneous IFN-γ production) and CD8a+ T cells stimulated with phorbol myristate acetate (PMA) at 1 ng/mL and ionomycin at 500 ng/mL as positive control.

**CD8a+ T cell purification**

CD8a+ T cells were purified using CD8a+ T Cell Isolation Kit (Miltenyi, 130-104-075) and LS Column (Miltenyi, 130-042-401), and a MidiMACSTM Separator (Miltenyi, 130-042-302). Purified CD8a+ T cells were expanded for 7 days in precoated plates with anti-mouse CD3e, clone 145-2C11 (Ultra-LEAF™ format, Biolegend, Cat. No. 100359) and anti-mouse CD28, clone 37.51 (Ultra-LEAF™ format, Biolegend, Cat. No. 102121) at 10 μg/mL in PBS and cultured in Iscove’s Modified Dulbecco’s Medium+GlutaMAX supplemented with 10% FBS, 10 mM HEPES, 55 μM 2-Mercaptoethanol at 37 °C in a humidified atmosphere of 5% CO2 in air.

**Hepatic stellate cell (HSCs) isolation**

Liver tissues were placed in gentleMACS C Tubes (Miltenyi, 130-093237) with 5 mL of HBSS/HEPES (w/ Ca²⁺, w/ Mg²⁺, 10 mM HEPES). Next, tissues were cut into small pieces in size of 1–2 mm². Then, HBSS/HEPES was supplemented with 0.55 Wünsch U/mL of Liberase TL (Roche, 05401020001) and 200 KU/mL of DNAse I (Roche, 04716728001) and digestion reaction was performed at 37 °C with continuous shaking for 1 h. At the end of incubation, C Tubes were attached upside down onto the sieve of the gentleMACS Dissociator and run twice for program D. Cell suspensions were filtered through 70 μm Cell Strainer and cells were washed twice with HBSS (w/o Ca²⁺, w/o Mg²⁺) buffer containing 2% FBS. Then, HSCs were purified by gradient density centrifugation. Briefly, cell pellet was resuspended in 8 mL of 40% OptiPrep (STEMCELL Technologies, 07820) diluted in HBSS and added dropwise on top of the 4 mL of 40% OptiPrep diluted in HBSS. Finally, 0.5 mL HBSS was added over the intermediate phase. Tube was centrifuged at 1400g for 20 min at 4 °C without break and at a slow acceleration. Then the thick white band, the layer beneath the HBSS layer, was gently collected and washed with HBSS (w/o Ca²⁺, w/o Mg²⁺) buffer containing 2% FBS. Red blood cells were lysed with RBC lysis buffer for 5 min on ice. Finally, cell pellet was resuspended in warm DMEM/F12 + GlutaMAX Medium supplemented with 10% FBS and cultured at 37 °C in a humidified atmosphere of 5% CO2 in air.

**In vitro cytotoxicity assays**

In vitro cytotoxicity assays were performed with purified and expanded CD8a+ T cells and the NIH 3T3 Adam12 expressing cells, HSC and YAC-1 target cell lines. Target cells were washed and labelled with CFSE (ThermoFisher, C34554). Following the washing steps, CD8a+ T cells were co-cultured with target cells at E:T ratios of 1:1 for 6 h at 37 °C in air. The target cells were identified as CFSE+, and effector cells were identified as CD3ε+ or CD8ε+. Data were analyzed using FlowJo v10 (Treestar). The target cells were identified as CFSE+, and effector cells were identified as CFSE−. The dead target cells were identified as CFSE‘Live/Dead’. Spontaneous death was defined as the proportion of dead target cells cultured alone (negative control), and this value was subtracted from the proportion of dead target cells cocultured with effector cells. Post-assay supernatants were used in ELISpot assay in order to determine the IFNγ secretion.

**Hydroxyproline assay**

Hydroxyproline content was quantified colorimetrically from tissue samples by using the chloramine T method as described by Bergheim et al. (Bergheim et al., 2006) with minor modifications. In brief, the sample was weighed and then homogenized in 600 μL pre-chilled distilled H2O. The lysate was mixed with 100 μL of 50% trichloroacetic acid (TCA, Sigma-Aldrich, 91228) and let sit on ice for 20 min and centrifuged at 6000 rpm for 10 min. The supernatant was removed and the clay-like pellet was washed three times with 1 mL ice-cold 100% ETOH. Next pellet was air dried and hydrolyzed in 500 μL of 6 M HCl at 105 °C for 20h. The supernatant was filtered to remove debris. 40 mL of the supernatant was added to an Eppendorf tube containing 10 μL of 10M NaOH and incubated with 450 μL of chloramine T solution (one part of 7% chloramine T in 50% N-propanol and four parts citrate/acetate buffer [pH 6.0, 695 mM sodium acetate, 128 mM trisodium citrate, and 29 mM citric acid, with 38.5% isopropanol]) at room temperature (20–22 °C) for 30 min. Then, 500 μL of Ehrlich’s solution (1.4 M dimethylaminobenzaldehyde in 33% perchloric acid and 67% isopropanol)
was added and incubated at 65°C for 20 min. After cooling, absorbance was measured at 560 nm. The hydroxyproline content was quantified using a standard curve of high-purity hydroxyproline (Sigma-Aldrich, 56250).

**Sirius Red/Fast Green**

Collagen fibers were detected in murine liver samples. Briefly, samples were deparaffinated, washed with distilled water and then stained with Hematoxylin for 45 seconds, followed by washing with distilled water. Then, samples were incubated in 0.04% Fast Green for 15 min, washed with distilled water and then incubated in 0.1% Fast Green and 0.04% Sirius Red in saturated picric acid for 30 min, followed by washing with 0.5% glacial acid twice. Then, samples were dehydrated and mounted with DPX mounting media (Sigma-Aldrich, 06522). Collagen fibers appeared red, while the non-collagen proteins were green. Slide scanner was performed with the microscope Zeiss Axio Scan.Z1, and Image J was used to quantify the collagen content of the whole section.

**Immunohistochemistry**

At the day of sample collection, animals were euthanized using CO₂ and samples were excised post-mortem, fixed in 4% neutral-buffered formaldehyde solution overnight at 4°C. And then samples were proceeded to ethanol dehydration and paraffin infiltration, followed by paraffin embedding in blocks. Paraffin blocks were processed into sections of 5 μm. Sections were deparaffinized and subjected to a heat-induced antigen retrieval step with citrate buffer (Sigma-Aldrich, C9999) or 1x Tris-EDTA, according to antibody instructions. Cryoblocks were also prepared with fresh tissue samples embedded in OCT (Sakura, Tissue-Tek® O.C.T™ Compound, 4583) in a suitable tissue mold. Samples were cut into 10μm-thick sections in the cryostat at -18°C (Cryostat NX70). Cryosections were fixed with 2% neutral-buffered formaldehyde solution for 1 min. For immunofluorescence, FFPE sections were permeabilized with 0.5% Triton™ X-100 (Sigma-Aldrich, 9002-93-1) in PBS for 10 min and were blocked at RT for 1 hour in blocking buffer (5% Normal Donkey Serum or 5% Normal Goat Serum in PBS containing 0.05% Tween-20, 0.2% Triton™ X-100, 0.2% Bovine serum albumin (Sigma-Aldrich, A4503-50G), 0.2% Gelatin type A from porcine skin (Sigma-Aldrich, 9000-70-8), 0.2% Casein (Fisher Scientific, 9000-71-9) and 0.001% Sodium azide in 1X TBS); cryosections were permeabilized with 0.5% Triton™ X-100 in PBS for 10 min and blocked with 5% Normal Donkey serum in PBST for 3 hours. Next, primary antibodies (listed in the key resources table) were applied in blocking buffer overnight at 4°C, followed by incubation with corresponding secondary antibodies (listed in the key resources table) in blocking buffer for 1h at room temperature. For double staining, second primary antibodies were diluted in blocking buffer and incubated for 1.5 hours at room temperature and followed by corresponding secondary antibody incubation. DAPI (Thermo Fisher, 19158656) was used as nuclear counterstain at a 1 μg/mL working concentration. 0.03% Sudan Black B (Sigma-Aldrich, 4197-25-5) in 70% ethanol was used to quench endogenous fluorescence and the slides were mounted with Epredia™ Immu-Mount™ media (Fisher Scientific, 9990402). Immunohistochemistry staining was processed as described in the immunofluorescence staining section with the following modifications: activity of endogenous peroxidase was blocked using 0.3% H₂O₂ (Sigma-Aldrich, 7722-84-1) in methanol for 10 min and endogenous biotin was blocked using Avidin/Biotin blocking kit (DAKO, X0590). The VECTASTAIN® Elite ABC-HRP Kit (Vectorlabs, VEC-PK-6101) was used according to the manufacturer’s protocol (VectorLabs), and then DAB Peroxidase Substrate Kit (Vector Laboratories, SK-4100) was applied for visualization. Samples were counterstained with hematoxylin for 30 sec, followed by washing with running cold water for 15 min. Samples were dehydrated and then mounted with DPX mounting media. Immunofluorescence-stained and IHC/DAB-stained sections were imaged using either a DMI 6000B microscope (Leica) or an Axio Scan.Z1 slide scanner (Zeiss). Image analysis and quantifications were performed using ImageJ 1.49 (National Institutes of Health, USA) and ZEN (Zeiss) imaging software.

**Immunoblot analysis**

Liver tissues were collected and lysed with RIPA Lysis and Extraction Buffer (Thermo Fisher, 89900) supplemented with protease inhibitor tablet (Roche Applied Science, C755C25). Lysates were centrifuged at 10,000 g for 10 min at 4°C. Supernatant was collected, and protein concentration was measured using the DC protein assay kit (Bio-rad, 5000116). 5-10 μg of total protein was loaded in a 15-well pre-casted gradient gel (Bio-Rad, 456-1096). Proteins were transferred onto a PVDF membrane (Bio-rad, 170-4156) with a semi-dry system and subsequently blocked for 1 hour at room temperature with 5% milk in 0.1% TBS-Tween. Membranes were incubated overnight at 4°C with primary antibodies: Adam12 (Proteintech, 14139-1-AP, 1:500), Albumin (Abcam #ab19194, 1:100) and β-actin (Cell Signaling, 3700, 1:1000). The appropriate secondary antibodies (1:5000) for anti-rabbit (IRDye® 800CW Donkey anti-Rabbit IgG Secondary Antibody, LI-COR, 926-32213), anti-goat (IRDye® 800CW Donkey anti-Goat IgG Secondary Antibody, LI-COR, 926-32214) and anti-mouse (IRDye® 800CW Donkey anti-Mouse IgG Secondary Antibody, 926-32212, LI-COR) IgG fluorescence-linked antibodies were used for fluorescent detection of proteins. Membranes were scanned with an Odyssey® DLx Imaging System (LI-COR) and quantified using Fiji (ImageJ 1.53c).

**Gene expression by quantitative PCR**

The cells were homogenized in RLT buffer (Qiagen). Total RNA was isolated with Qiagen RNA extraction kits following the manufacturer’s instructions. For real-time PCR analysis, the isolated RNA was reversely transcribed (High-Capacity cDNA Reverse Transcription Kit; Applied Biosystems™, 4368814). For PCR reactions, SYBR Green I Master MIX (LightCycler® 480 SYBR Green I Master; Roche; 04887352001) was used. 10 ng cDNA was used as template to determine the relative amount of mRNA by real-time PCR (Roche Detection System). PCR conditions were as follows: 95°C for 10 min followed by 45 cycles of 95°C for 15 seconds and
Cantonal veterinary office, general anesthesia was induced by 4% Isoflurane in 70% O2 and subsequently maintained.

Loricrin expression starts (Lee et al., 2017). For the pathological evaluation of major organs, unwounded skin, heart, small intestine, skeletal muscle, spleen, pancreas and kidney from vaccinated mice were removed and analyzed. A full macroscopic examination was performed in each animal and the respective organs were sampled for histological assessment. The tissues were fixed in 4% formaldehyde, dehydrated through graded ethanol series, cleared in xylene and embedded in paraffin before the production of serial sections. Sections (6 μm) were cut on a microtome and mounted between a microscopy glass slide and a cover slip with a drop of PBS. Images were acquired with an upright Leica TCS MP DIVE FALCON two-photon microscope, equipped with a tunable laser and four spectrally tunable non-descanned photon-counting hybrid detectors. A x25/1.0 NA water-immersion objective was used. Second harmonic generation signal associated with collagen and keratin was imaged by SHG microscopy 28 days post-injury. Patellar tendon was isolated from mice and stored at -80°C. Samples were fixed in 10% Formalin for 24 hours, dehydrated through graded ethanol series, infiltrated, and embedded in paraffin. Sections (2 μm) were stained with HE and routinely stained with loricrin and as a nuclear counterstain at a dilution of 1:1000, for 1 hour at RT. The sections were mounted on a slide in Citrate buffer (10mM Sodium Citrate, 0.05% Tween 20, pH 6.0) at 60°C for 1 hour. Sections were stained following standard immunofluorescence protocols. Briefly, sections were blocked at RT for 1 hour in blocking solution (2.5% FBS, 2.5% BSA, 0.2% Triton X-100, 0.3 M glycine, 1% gelatin, in PBS). Sections were then incubated with the primary antibodies diluted in blocking buffer (listed in the key resources table), at 4°C overnight. This was followed by incubation with secondary antibodies, along with Hoechst 33342 for nuclear counterstain. Image analysis was performed in ImageJ. SHG intensity was quantified in ImageJ. GLCM texture features were calculated using ImageJ with the Texture Analyzer plugin to determine the entropy.

**Wound healing studies and histopathological assessment of major organs**

Mice were vaccinated twice (prime vaccination and a second boost vaccination 7 days later also see section on mouse immunization). 2 days after the last vaccination (see also experimental schemes in Figure 7D), following a protocol approved by the Zurich cantonal veterinary office, general anesthesia of the animals was induced by 4% Isoflurane in 70% O2 and subsequently maintained using 3% Isoflurane. The back skin was shaved, thoroughly cleaned, and disinfected prior the surgery using 70% ETHOH solution. Pre-operative analgesia was applied using a subcutaneous injection of Buprenorphine (0.1 mg/kg) (Temgesic, Indivior Schweiz AG, 6664853). Four circular full-thickness excisional wounds of 6 mm of diameter were generated on the lower back skin of each animal, two on each side, 1 cm of the midline of the animal and roughly 2 cm apart from each other. Post-operative analgesia was performed by a 5-day treatment of Buprenorphine through the drinking water. Wound area was determined every two days under isoflurane anesthesia for each wound by caliper measures taken from day 0 to day 6 post-surgery. The animals were monitored daily and criteria for early termination were: persistent non-closure of wounds or presence of pus in the wounds, persistent inactivity, persistent low responsiveness to handling, persistent skin tenting and persistent hunching, trembling, rapid and labored breathing and weight loss >15%. The area at different time points after wounding were then reported to the initial area at day 0 for each wound. At day 6 post-wounding, the animals were euthanized and the wound tissue was harvested. Paraffin embedded skin from the dorsal side of the mouse were sectioned on a microtome to obtain 10 μM sections. Sections were de-paraffinized, rehydrated, and incubated in 1× PBS for 5 minutes. Note that sections stained with loricrin were subjected to heat-induced antigen retrieval by immersion in Sodium Citrate buffer (10mM Sodium Citrate, 0.05% Tween 20, pH 6.0) at 60°C for 1 hour. Sections were stained following standard immunofluorescence protocols. Briefly, sections were blocked at RT for 1 hour in blocking solution (2.5% FBS, 2.5% BSA, 0.2% Triton X-100, 0.3 M glycine, 1% gelatin, in PBS). Sections were then incubated with the primary antibodies diluted in blocking buffer (listed in the key resources table), at 4°C overnight. This was followed by incubation with secondary antibodies, along with Hoechst 33342 for nuclear counterstain. Image analysis was performed in ImageJ. SHG intensity was quantified in ImageJ. GLCM texture features were calculated using ImageJ with the Texture Analyzer plugin to determine the entropy.

**Biochemical analysis**

Serum albumin, total bilirubin, alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), enzymatic creatinine (EC), uric acid (US), urea and α-amylase were measured using the UniCel DxC 800 Chemistry Analyzer by Zurich Integrative Rodent Physiology (ZIRP) according to the manufacturer’s instructions.

**Patellar tendon injury model**

Mice were anesthetized and surgery was performed aseptically under analgesia with the aid of a dissecting microscope. Both hind limbs were shaved and aseptically prepped. Next, longitudinal skin incisions were made to expose the patellar tendon in each limb. The tendon was punctured using a beveled 25 Gauge needle that was passed through the tendon at a 45° angle while centered medially and laterally. The contralateral left limb was sham-operated with the uninjured but exposed tendon subsequently serving as an experimental control (David et al., 2013; Gilday et al., 2014). Progression of the tendon healing process was assessed by second harmonic generation microscopy 28 days post-injury. Patellar tendon was isolated from mice and stored at -80°C. Samples were mounted between a microscopy glass slide and a cover slip with a drop of PBS. Images were acquired with an upright Leica TCS MP DIVE FALCON two-photon microscope, equipped with a tunable laser and four spectrally tunable non-descanned photon-counting hybrid detectors. A x25/1.0 NA water-immersion objective was used. Second harmonic generation signal was imaged by SHG microscopy. A x25/1.0 NA water-immersion objective was used. Second harmonic generation signal was imaged by SHG microscopy. A x25/1.0 NA water-immersion objective was used. Second harmonic generation signal was imaged by SHG microscopy. A x25/1.0 NA water-immersion objective was used. Second harmonic generation signal was imaged by SHG microscopy. A x25/1.0 NA water-immersion objective was used. Second harmonic generation signal was imaged by SHG microscopy.

**Tendon fibrosis in Fra2Tg mouse model**

Fra2Tg transgenic mice showed an established fibrotic phenotype in tendon at the age between 16 – 24 weeks old and their wild-type controls were used in the study (Hussien et al., 2021). After euthanizing the animals, Achilles tendons were freshly dissected, including calcaneus and gastrocnemius muscle complexes. Dissected tissues were embedded in OCT without fixation and were cryosectioned at 8 μm thickness.
QUANTIFICATION AND STATISTICAL ANALYSIS

Image analysis
Collagen content quantification was performed by quantifying the Sirius red signal via Fiji (ImageJ 1.53c) on RGB images as follow: Image/Color/Colour Deconvolution/User values (defined accordingly to the set of staining). Equal threshold setting (Image/Adjust/Threshold) was applied on a set of analyzed samples in order to determine percentage of SiriusRed positive area. For immunohistochemistry images, quantification was performed using Fiji (ImageJ 1.53c) on RGB images as follow: Image/Color/Colour Deconvolution/H DAB. Equal threshold setting (Image/Adjust/Threshold) was applied on a set of analyzed samples in order to determine percentage of DAB positive area. Perivascular inflammation was assessed on tissue sections with the pan-hematopoietic marker CD45 in periportal blood vessels of the liver and pulmonary blood vessels ranging from 50 to 150 μm. Perivascular inflammatory cells were defined as extraluminal CD45+ cells in juxtaposition to the vascular wall. For immunofluorescence images, quantification was performed using Fiji (ImageJ 1.53c). Threshold was applied in order to determine areas covered by staining of interest within analyzed image. Equal threshold setting was applied to the analyzed images. Pericytes were identified by α-SMA positive cells in periportal blood vessels of the liver and pulmonary blood vessels ranging from 50 to 150 μm. Pericyte coverage was quantified by calculating α-SMA positive cells per periportal blood vessel.

Statistical analysis
All statistical analysis was performed using GraphPad software v9. All data represent mean ± SEM of the corresponding number of samples. All experiments in this study were repeated at least three times. All sample number (n) of corresponding biological samples can be found in the figure legends. The value of n represents the number of mice per group except as follows: n represents the number of images from 3-10 mice per group in Figures 1G, 7D, S1A, S5A, and S5D; n represents the number of wounds from 5 mice per group in Figures 7D, S6F, and S6G. Statistical tests such as two-tailed Student’s t-test (Figures 2D, S1D, S4A, S4G, S4H, S6A, and S6B), one-way analysis of variance (ANOVA) (Figures 1D–1G, 3B–3E, 4B–4F, 5C–5F, 6C–6G, S1C, S2D–S2G, S3C–S3F, S4B–S4E, S5B, S5C, S5F, S5G, and S7H) and two-way ANOVA analysis (Figures 3F, 3G, 7D, S6F, and S6G) were used. P values less than 0.05 were considered statistically significant.