mesenchymal progenitor cells (MPCs) towards osteochondral lineages, leading to heterotopic ossification (HO). Previous work has shown that reducing mechanical strain by immobilizing a limb prevents the formation of an aligned extracellular matrix (ECM), leading to altered MPC differentiation, however, the cellular mechanisms of matrix reorganization following injury remain unclear. Using novel bioinformatics approach, we identified discoidin domain receptor 2 (DDR2) as a key MPC specific tyrosine kinase receptor that interacts with the fibrillar collagen matrix. We hypothesized that DDR2 activation leads to regulation of ECM alignment and therefore serves as a novel upstream regulator of mechanotransductive signaling following musculoskeletal repair.

**Methods:** Heterotopic ossification was induced using a proven mouse model of 30% total body surface area burn with concurrent Achilles’ transection in Ddr2 deletion (Ddr2<sup>−/−</sup>) and littermate control mice on the C57/BL6J background. In separate experiments, ankle joint immobilizers were placed on injured mice for 1, 2 or 3 weeks after burn/tenotomy. Tissue from Ddr2 deleted, immobilized and mobile control mice were harvested for immunofluorescence histology, second harmonic generation of collagen fiber histology at 1, 3 and 9 weeks after injury (SHG) (n=4/group). HO site tissue was also harvested prior to injury and at 1 and 6 weeks after burn/tenotomy and processed for Single-Cell RNA sequencing (scRNA seq) and single nucleus assay for transposase-accessible chromatin (snATAC) using 10X Sequencing and downstream analysis using Seurat 4.0.1 and Signac 3.1.5.

**Results:** Single-cell RNA sequencing showed that genes for collagen type 1 and 3 and Ddr2 are highly upregulated in MPCs following injury. SnATAC showed increased open chromatin reads in the promoter of MPCs following injury (not shown). Utilizing SHG, we found that the collagen matrix is aligned at three-weeks following injury, but one week of limb immobilization was enough to prevent aligned collagen. The active phosphorylated form of DDR2 (pDDR2) was decreased in PDGFRα+ MPCs within immobilized samples, suggesting that DDR2 may affect matrix alignment. Ddr2<sup>−/−</sup> had more disorganized collagen matrix nine weeks following injury and significantly reduced HO.

**Conclusions:** This is the first work to alter collagen matrix organization by genetic modification and mobilization protocols and identifies DDR2 as a critical upstream receptor in matrix organization and aberrant progenitor differentiation following injury. DDR2 antagonism could have therapeutic potential in preventing heterotopic ossification following traumatic injury.

### 3 Multimodal Molecular Analysis Reveals Divergent Trajectories Of Wound Regeneration Versus Fibrosis

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**Purpose:** Scarring in the mouse dorsal dermis is mediated by pro-fibrotic, Engrailed-1 lineage-positive fibroblasts (EPFs). We recently showed that mechanotransduction blockade (YAP inhibition, using the drug verteporfin), results in complete wound regeneration, with full recovery of normal dermal appendages (hair follicles, glands), extracellular matrix (ECM) architecture, and tensile strength. This regenerative outcome following verteporfin treatment is mediated by Engrailed-1 lineage-negative fibroblasts (ENFs). The complex milieu of cell types and molecular signals involved in wound repair makes it difficult to study using any single data modality. Thus, we sought to use a holistic approach, incorporating multiple high-throughput, high-dimensional analyses, to define the divergent molecular events distinguishing typical scarring healing from verteporfin-induced wound regeneration.

**Methods:** C57BL/6J mice underwent dorsal splinted excisional wounding per standard protocol. Wounds were treated with local injection of either verteporfin or vehicle control (PBS) on POD 0. We harvested unwounded skin and wounds at POD 2, 7, 14, and 30 (n=5 mice/timepoint and treatment) and subjected wound cells to three analyses: single-cell RNA-sequencing (scRNA-seq, using 10X Genomics Chromium); timsTOF, a recently-developed, high-throughput proteomic sequencing platform; and a novel machine learning algorithm for quantitatively comparing ECM ultrastructure.

**Results:** Pseudotime analysis (Monocle3) of pooled scRNA-seq data revealed that fibroblasts followed two
distinct transcriptional trajectories, one characterized by mechanical activation (En-1 lineage-positive, “fibrotic” trajectory) and the other characterized by developmental and regenerative pathways (En-1 lineage-negative; Rspo1, Dkk2/3, Trps1). Cross-platform data integration confirmed that fibroblasts in the fibrotic trajectory correlated with myofibroblast proteomic signatures (Col1a1/2, Fn1, etc.) and fibrotic/scar ECM features. In contrast, fibroblasts in the regenerative trajectory negatively correlated with myofibroblast markers and were associated with a “basket-weave” ECM pattern quantitatively indistinguishable from that of unwounded skin. Our integrated dataset suggested an important role for Wnt pathway proteins in ENF-mediated skin regeneration, so we compared POD 14 scars and regenerated wounds by multiplexed in situ hybridization (RNA-Scope) for Rspo1 (Wnt agonist), Trps1 (master hair follicle regulator), Ank1 (YAP target gene), and Dpp4 (EPF marker). Quantification of RNA granules across thousands of cells using a custom image analysis pipeline revealed that ENF-mediated healing (low Dpp4) in YAP-inhibited (low Ank1) wounds yielded regeneration of functional hair follicles through Wnt-mediated pathway activation (high Rpos1, Trps1). These data suggest that YAP inhibition unlocks wound regeneration via Wnt-active, En-1 lineage-negative fibroblasts.

Conclusion: By studying regenerating (verteporfin-treated) versus scarring wounds across multiple healing timepoints and high-dimensional data modalities, we were able to profile fibrotic versus regenerative healing at unprecedented depth. Our integrated analysis revealed that dermal fibroblasts in these two wound settings exhibit distinct molecular trajectories defined by divergent transcriptomic, proteomic, and ultrastructural properties. Further, we found that wound regeneration in the context of verteporfin treatment is associated with suppression of mechanical signaling and activation of key Wnt pathway members including Trps1 (a gene with known hair follicle developmental roles). These results could have important implications for both the fundamental study of wound healing and potential anti-scarring therapeutic avenues.

Prolonged Tourniquet Use Following Blast Related Lower Extremity Injuries Increase Heterotopic Ossification in a Pre-clinical Model

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Purpose: Traumatic heterotopic ossification (tHO) has become a signature pathology affecting wounded military personnel who have sustained blast-associated traumatic amputations during the recent conflicts in Iraq and Afghanistan. Heterotopic ossification is characterized by the abnormal development of mature bone deposits in extra-skeletal sites such muscle, tendon, and soft tissues, leading to impaired wound healing, pain, reduced range of motion, and limited use of prostheses. While many factors influence the formation of tHO, the extended use of tourniquets to limit catastrophic hemorrhage during prolonged field care (PFC) has not been explored. Herein, we investigate the impact of tourniquet use following blast-related injury on ectopic bone formation.

Methods: Utilizing an established pre-clinical model of blast-associated complex lower limb injury and traumatic amputation, we evaluated the effects of extended tourniquet use on tHO formation. Male rats (11-12-week-old) were subjected to blast overpressure exposure, femur fracture, and soft tissue crush injury. A pneumatic tourniquet (250-300mmHg) was applied to the injured limbs for either 90- and 150-minutes followed by trans-femoral amputation. Limbs were assessed for HO formation using microCT. Analysis of muscle/soft-tissue osteogenesis-related gene transcripts and multiple serum inflammatory mediators were measured by using qRT-PCR and Luminex multiplex assays, respectively.

Results: At 12 weeks, volumetric analysis with microCT imaging revealed an 70% increase in total bone formation (P=0.007, n=11) near the site of injury in rats subjected to 150-minutes of tourniquet time compared to rats with no tourniquet time in the setting of blast-injuries. Rats subjected to 150-minute tourniquet usage and blast injury had increased expression of osteochondrogenic genes including Bmp2 (5.4-fold increase, P=0.01) as early as 6 hours