# TENOMODULIN INVOLVEMENT IN LATE TENDON REPAIR: ACHILLES TENDON INJURY IN TENOMODULIN-DEFICIENT AND WILDTYPE MICE

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Introduction:
The three stages of tendon repair are inflammation, proliferation and remodelling; each stage is marked with changes at molecular, cellular and structural levels. The aim of this study was to investigate Tenomodulin (Tnmd) involvement in tendon repair by implementing an Achilles tendon injury model in Tnmd-KO and WT animals. Histomorphometrical analysis at day 8 revealed inferior scar organization and areas filled with fat and blood vessels in KO tendons (Lin D. et al., Cell Death Dis. 2017). We hypothesize a worsening of the phenotype with time, because vasculature might unleash heterotopic ossification (HO).

Methods:
Surgical procedures were performed with skeletally mature Tnmd-KO and WT mice (6-months old). After skin incision, Achilles tendon was fully resected and directly reconstructed with a Kirchmayr-Kessler suture technique. A cerclage was inserted through the tibiofibular fork and calcaneus to avoid suture failure but allowing load transmission. Micro-CT (n=7-8), biomechanical testing (n=8-14) and voluntary running tests (n=7-10) were conducted 100 days after injury. Total HO surface and volume were measured. Biomechanical properties of uninjured and injured Achilles tendons were tested accordingly to the previously described protocol (Hochstrat E. et al., PLoS One, 2019). Tendon areas, maximum load, stiffness, static and dynamic E-modulus were analyzed. Finally, in order to test late tissue remodelling and functionality, operated animals ran voluntarily overnight and the total distance was recorded for a time lapse of 12 hours.

Results:
HO was detected in both genotypes and the distribution was bipolar in the mutant (at the myotendinous junction and enthesis) and continuous in the WT. Total HO surface and HO at the enthesis were significantly increased in the KO. Biomechanical testing revealed that injured tendons, regardless of genotype, were thicker, less stiff, and both static and dynamic E-modulus were significantly decreased compared to uninjured controls. Remarkably, higher stiffness and significantly increased static and dynamic E-modulus were measured in uninjured control KO tendons at 4%, 6% and 8% strain. Injured Tnmd-KO animals ran significantly less than their WT littermates.

Discussion:
Irrespective of genotype, tendon biomechanical properties were highly impaired upon injury. This can be explained with the detected HO. Significantly higher HO surface was detected in KO tendons, data that can be associated with augmented vasculature detected in the mutant at day 8. Uninjured control tendons from the KO were stiffer and possessed higher E-modulus, findings that go in line with our previous nanomechanical data (Dex S. et al., EBioMedicine,

2017). The ultimate test for tissue functionality is running, voluntary tests revealed that Tnmd-KO animals ran significantly less than WT littermates after injury. Based on these evidences we propose that Tnmd plays an important role in late tendon repair.