

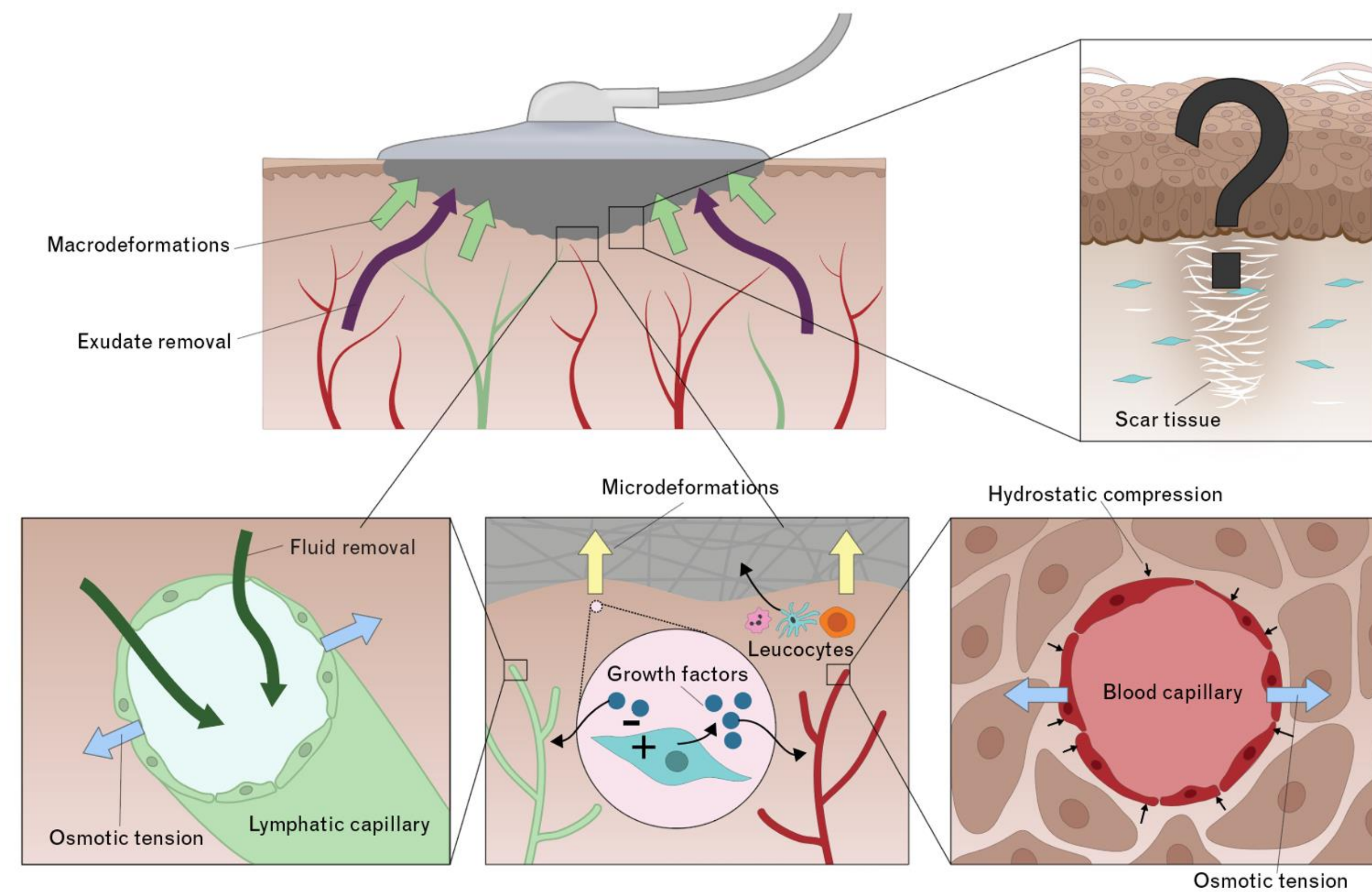
Continuous Negative Pressure Wound Therapy Regulates Healing And Fibrosis In Murine Excisional Wounds

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Introduction

- Hypertrophic scarring is a fibroproliferative disorder that can result in significant functional and aesthetic morbidity
- Discoveries have linked mechanical signaling factor Yes-associated protein (YAP) and Engrailed-1 (En1) protein to fibrosis and scarring
- Negative Pressure Wound Therapy (NPWT) is a form of wound treatment using a device that creates a sub-atmospheric pressurized environment
- NPWT has been clinically shown to improve scarring, but biomolecular mechanisms remain unclear



Methods

- 20 obese and diabetic (db/db) mice received a 1x1 cm² full thickness excisional dorsal skin wound
- 10 mice each were treated with occlusive covering (Control, n=10) or NPWT (continuous, -125mmHg, 7 days; NPWT, n=10)
- Animals were sacrificed and harvested on day 10 for histological and molecular analysis

Results

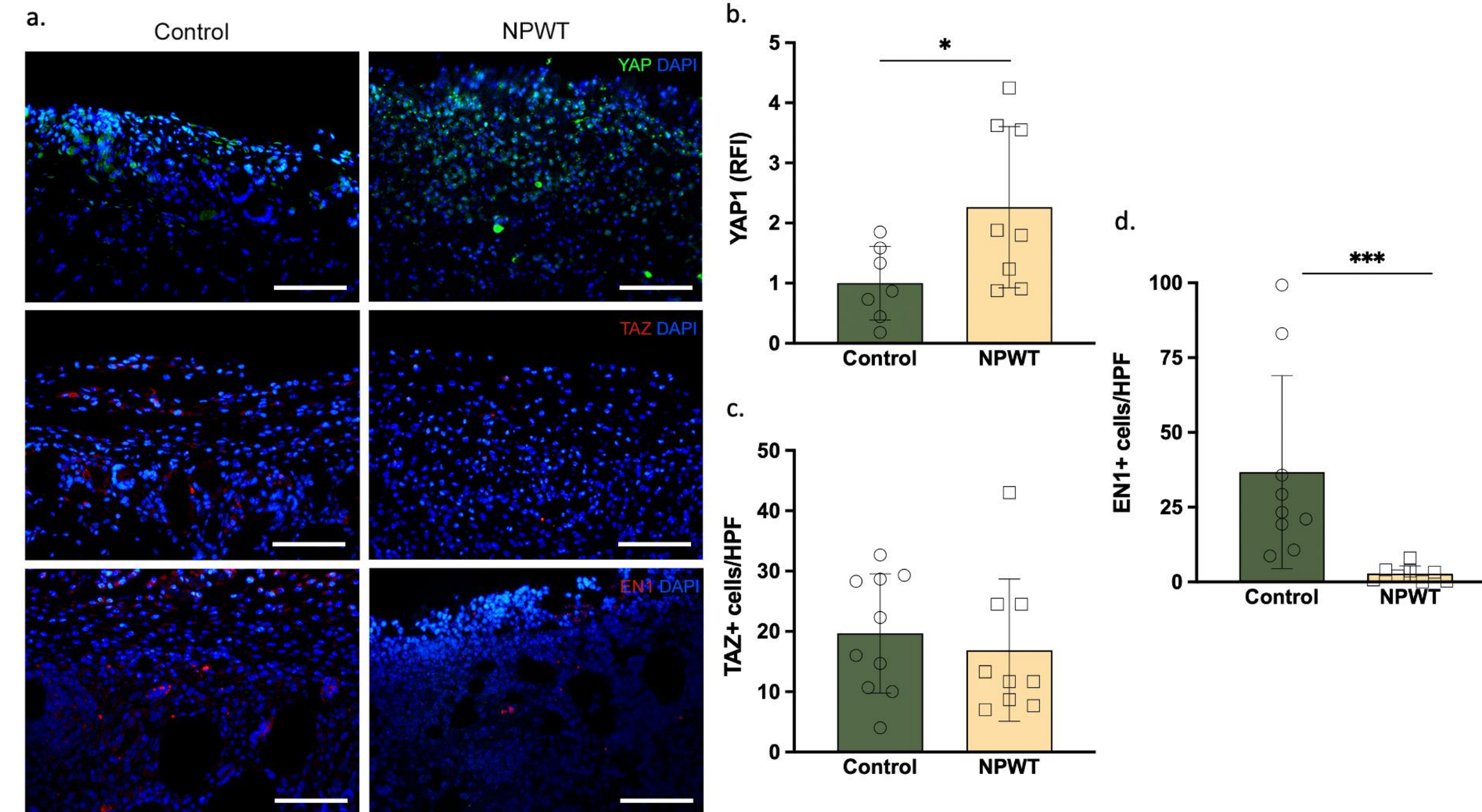


Figure 1. YAP, TAZ and EN1 levels. a. Immunofluorescent stained sections of the wound bed on Day 10. anti-YAP, anti-TAZ, or anti-EN1 and DAPI stained sections of the wound bed of the two groups on day 10. Scale bar=100 μ m. b. **Relative fluorescence intensity of YAP on Day 10.** There were significant differences in the presence of YAP between the two groups (NPWT: 2.3 $\pm$ 1.3 RFI vs. Control: 1.1 $\pm$ 0.6; p=0.04). c. **Percentage of TAZ +ve cells on Day 10.** No differences were noted in the presence of TAZ between the two groups. d. **Percentage of EN1 +ve cells on Day 10.** There were significant differences in the presence of EN1 (NPWT: 2.8 $\pm$ 2.6 %EN1+cells vs. Control: 37 $\pm$ 32 %EN1+cells; p=0.01). Number of circles/squares per bar indicates sample size (n=7-10). Each circle/square represents the average of three HPF measurements.

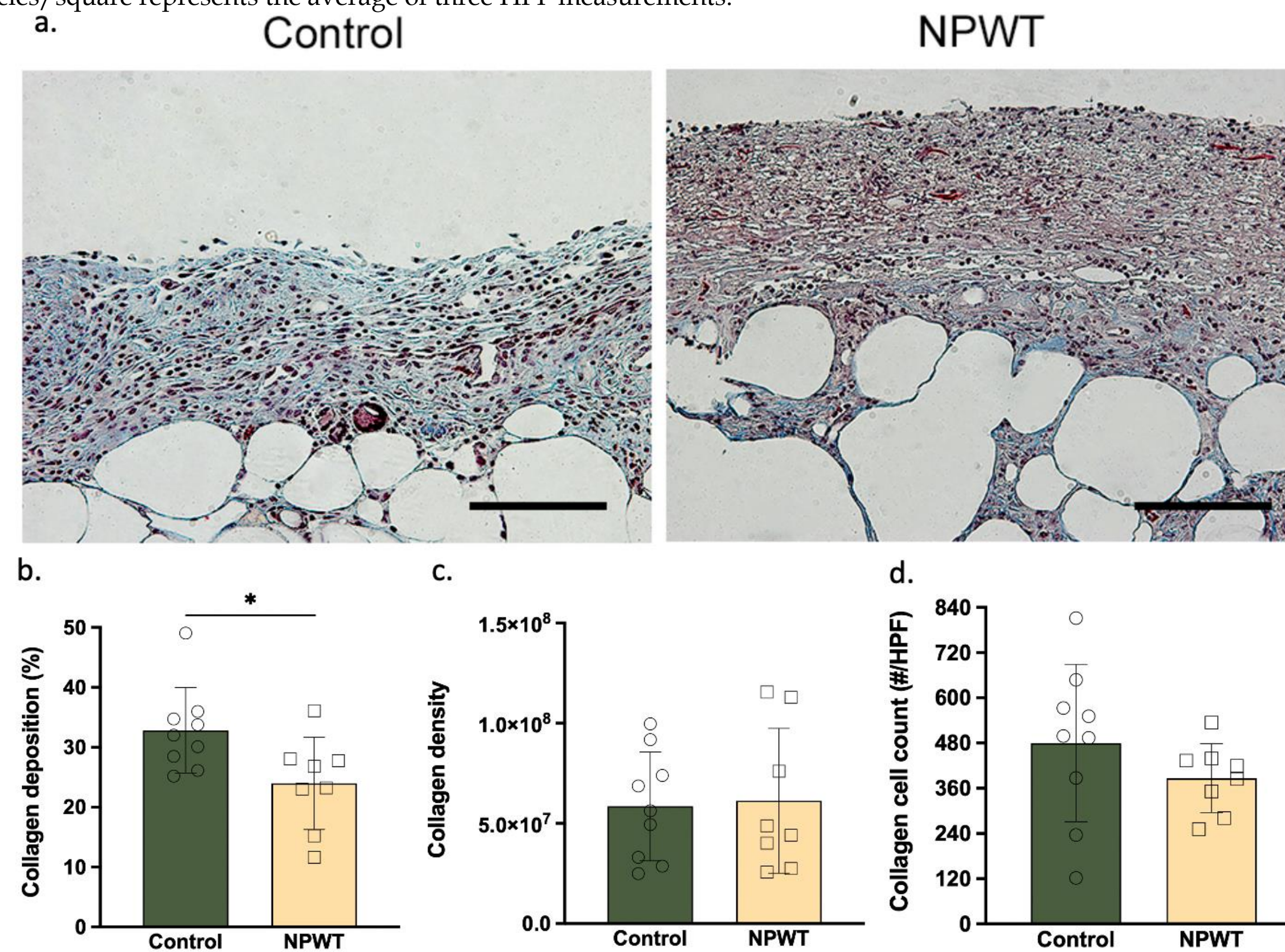


Figure 2. Collagen deposition on day 10. a. Representative sections. MT-stained wound beds of the two groups on day 10. Scale bar=100 μ m. b. **Collagen deposition.** Collagen deposition significantly differed between the two groups (NPWT: 24 $\pm$ 7.7% vs. Control: 33 $\pm$ 7.1; p=0.02). c. **Collagen density.** No significant differences were noted in collagen density between the two groups (NPWT: 6.1 $\pm$ 3.6 $\times 10^7$ vs. Control: 5.9 $\pm$ 2.7 $\times 10^7$; p = 0.86). c. **Collagen cell count.** No significant differences were noted in collagen cell count (NPWT: 387 $\pm$ 91 cells/HPF vs. Control: 480 $\pm$ 208 $\times 10^7$ cells/HPF; p = 0.26). Number of circles/squares per bar indicates sample size (n=8-9). Each circle represents the average of three HPF measurements.

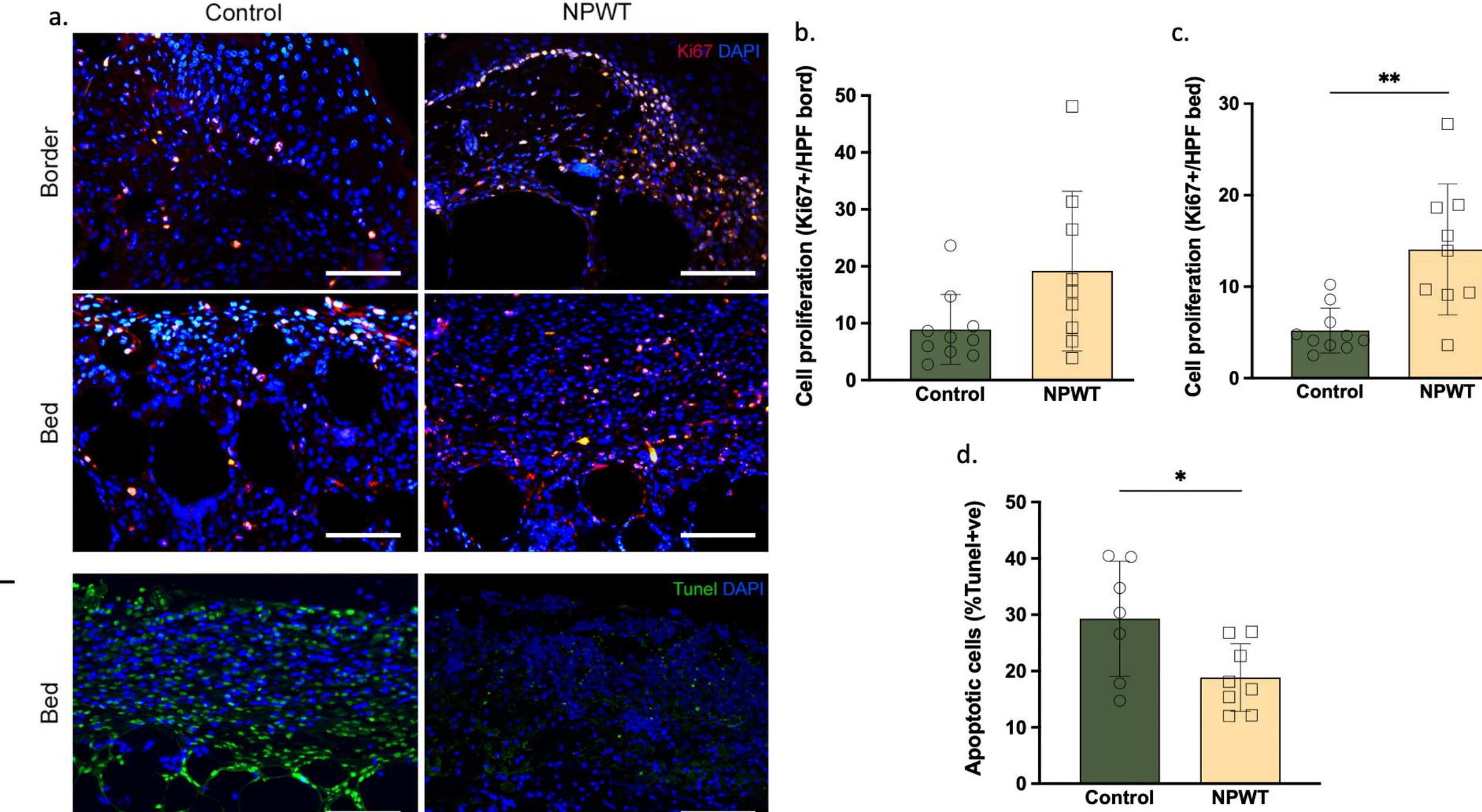


Figure 3. Cell turnover on day 10. a. Representative sections. anti-Ki67, TUNEL and DAPI stained sections of the wound bed and border of the two groups on day 10. Scale bar=100 μ m. b. **Cellular proliferation of the bed on Day 10.** There were significant differences in cellular proliferation of the wound bed between the two groups (NPWT: 14 $\pm$ 7.2 %Ki67+cells/HPF vs. Control: 5.2 $\pm$ 2.4 %Ki67+cells; p=0.002). c. **Cellular proliferation of the border on Day 10.** There were significant differences in cellular proliferation of the wound border between the two groups (NPWT: 19 $\pm$ 14 %Ki67+cells/HPF vs. Control: 7.3 $\pm$ 3.5 %Ki67+cells; p=0.03). d. **Cellular apoptosis on Day 10.** There were significant differences in cellular apoptosis between the two groups (NPWT: 19 $\pm$ 6 %TUNEL+/HPF vs. Control: 29 $\pm$ 10 %TUNEL+/HPF; p=0.03). Number of circles/squares per bar indicates sample size (n=8-10). Each circle represents the average of three HPF measurements.

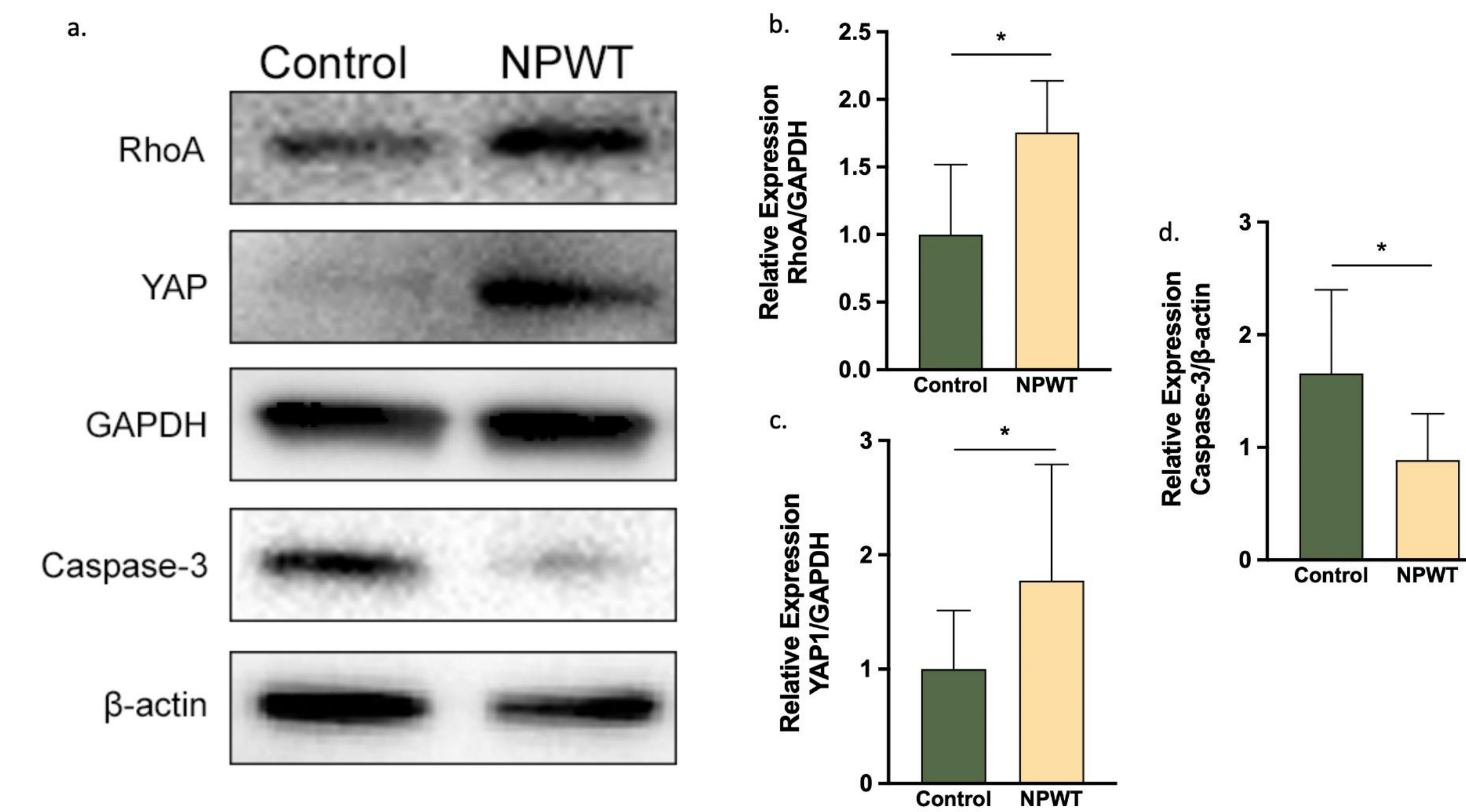
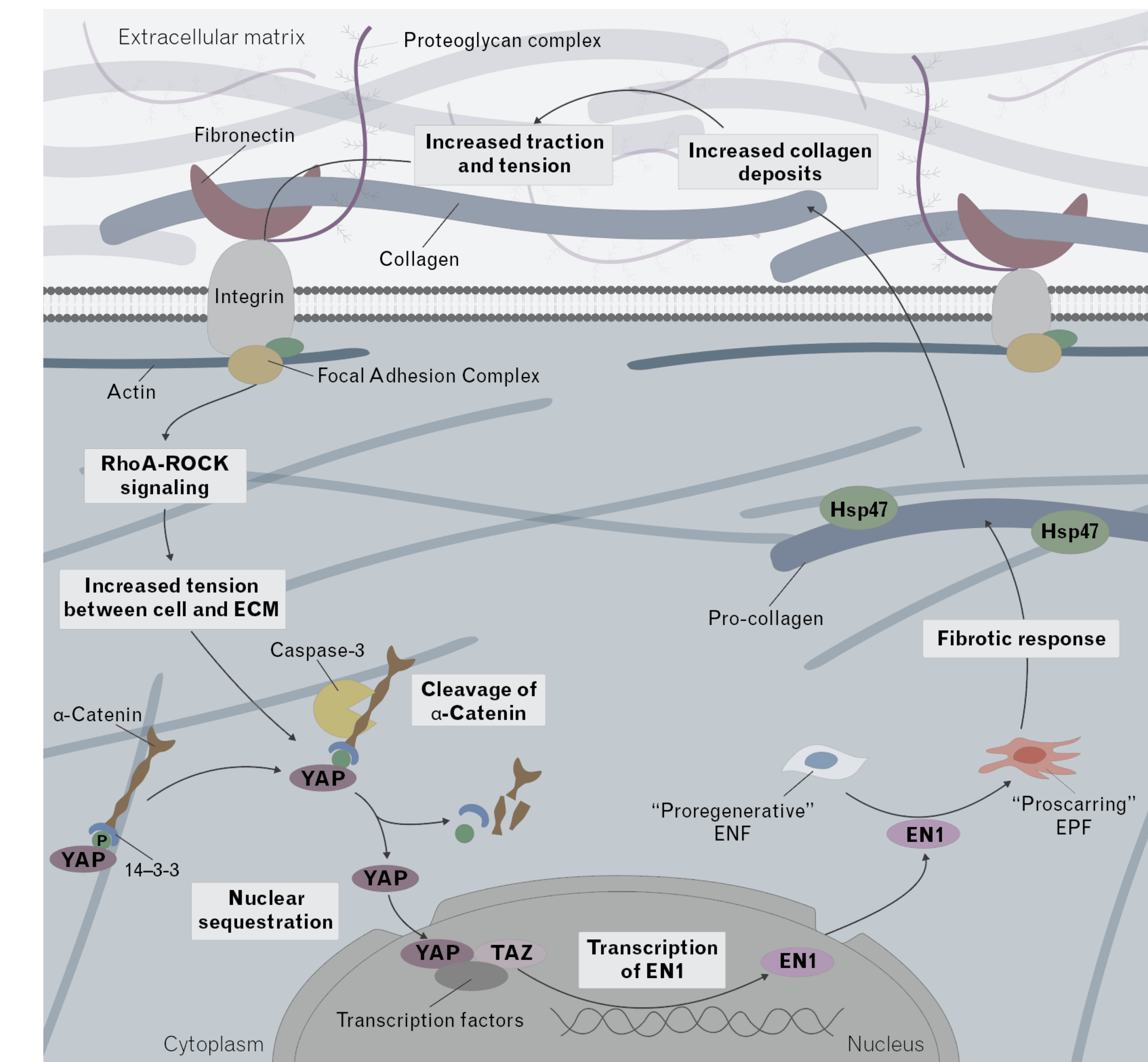


Figure 4. YAP, RhoA and Caspase-3 expression. a. Representative Western blots. Expression of YAP, RhoA and Caspase-3 in the two groups on day 10. b. **Relative expression of RhoA.** Densitometric analysis of ratios of RhoA to GAPDH (n = 6; p=0.03). Levels are indicated as bar graphs. The NPWT group had significantly higher RhoA levels than the Control group. c. **Relative expression of YAP.** Densitometric analysis of ratios of YAP to GAPDH (n = 6; p=0.02). Levels are indicated as bar graphs. The NPWT group had significantly higher YAP levels than the Control group. d. **Relative expression of Caspase-3.** Densitometric analysis of ratios of Caspase-3 to β -Actin (n = 6; p=0.03). Levels indicated as bar graphs. The Control group had significantly higher Caspase-3 levels than the NPWT group.

Conclusion

- NPWT decreases Caspase-3, a pro-apoptotic factor linked to keloid formation
- Decreased Caspase-3 prevents nuclear translocation of cytoplasmic YAP
- Lack of YAP nuclear sequestration leads to decreased En1 transcription and decreased pro-fibrotic response



Acknowledgment

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Disclosure

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