Supplementary figures to Sulniute et al. “Plasminogen is a critical regulator of cutaneous wound healing” (Thromb Haemost 2016; 115.5)

**Suppl. Figure 1. Plg-deficiency delays re-epithelialisation of incision wounds.** Paraffin-sections from the incision wounds in wt and plg-deficient mice were taken from the middle part of the wounds and perpendicular, and stained with Masson’s Trichrome. The width of wounds was measured as a distance between keratinocytes on wound edges (at magnification 50x). The wounds in wt and plg-deficient mice were re-epithelialized at day 14 and 30, respectively.
Suppl. Figure 2. Plg-deficiency results in accumulation of neutrophils and fibrin in incision wounds long after reepithelialisation. Paraffin sections of incision wounds from wt and plg-deficient mice were stained for neutrophils (upper panel) and fibrin(ogen) (lower panel) and photos were taken (at magnification 50x). Stained areas were selected manually and quantified using Image-J software. There were only traces of neutrophils stained in wt samples at day 14, and no neutrophils were present in wt wounds at later time-points. The samples from wt mice were also negative for fibrin staining.
Suppl. Figure 3. Plg-deficiency results in accumulation of neutrophils and fibrin(ogen) in burn wounds long after reepithelialisation. Paraffin sections of burn wounds from wt mice and plg-deficient mice were stained for neutrophils (A) and fibrin(ogen) (B) and photos were taken at magnification 50x. Stained areas were selected manually and quantified using Image-J software. Wt mice had no neutrophils in the wounded area at day 60, and fibrin was not present from day 14.
**Suppl. Figure 4.** Plg supplementation to plg-deficient mice restores wound debridement. Burn wound was introduced to plg-deficient mice and the mice were supplemented daily with human plasminogen or PBS (as control) from day 0 (intravenous supplementation, A-F) or from day 5 (local administration, G-L). At post-wounding day 14, paraffin sections were prepared and stained for neutrophils (A, B, G, H) and fibrin (D, E, J, K). The accumulation of neutrophils (C and I) and fibrin (F and L) was quantified using Image-J software. Scale bar – 50 μm; magnification 200x.
Suppl. Figure 5. Five-days-long plasminogen supplementation to inflamed wounds in plg-deficient mice results in termination of the inflammation. Burn wound was introduced to plg-deficient mice and the mice were left untreated for 14 days. Then h-plg or PBS was injected locally around the wounded area daily, for 5 days (4 mice per group). Wound closure was quantified from digital photos (A). Paraffin sections from tissue samples taken at day 20 were stained for neutrophils (B and C) and fibrin (E, F). The accumulation of neutrophils and fibrin (D and G, respectively) was quantified using Image-J software. Scale bar – 50 μm; magnification 200x; arrow indicates the time point when plg supplementation started.
Suppl. Figure 6. Quantification of NGF-beta levels in burn wounds of plg-/- mice and plg-/- mice supplemented with human plasminogen (hplg). Burn wounds were induced in plg-/- mice and half of the mice were intravenously supplemented with hplg. At 24 h after burn, wound extracts were prepared and analyzed by reducing SDS-PAGE followed by western blot with antibodies against NGF-beta (Abcam, Cambridge, UK) and antibodies against beta-actin as internal control (Sigma-Aldrich, Stockholm, Sweden). Then NGF levels in wounds were quantified with ImageJ and shown as a ratio of band intensity of NGF-beta and beta-actin.