Supplemental Methods and Materials

Antibodies

Rabbit polyclonal antibodies to phosphorylated Stat 3, Stat3, and secondary anti-rabbit HRP were obtained from Cell Signaling Technology (Danvers, MA). Western blotting was performed according to standard procedures with overnight incubation at 4°C with primary antibodies and development by ECL.

Animals

All animal protocols are approved by the Johns Hopkins University Animal Care and Use Committee. C57BL/6J (stock # 00664), and IL-6 null mice (B6.129S2-Il6^{tm1Kopf}/J ; stock # 002650) were obtained from The Jackson Laboratory. All animal colonies were maintained within animal facilities with standard humidity, 12 hour light/dark cycle and laboratory diet *ad libitum*. Both male and female mice were used in the experiments and gender did not impact the results. All comparisons are between mice of identical strain and treatments except for experimental intervention.

Wound Induced Hair Neogenesis (WIHN)

1 cm^2 full-thickness wounds on the backs of 21-day old male and female mice were created as previously described (Ito *et al.*, 2007; Nelson *et al.*, 2013). Numbers of regenerated hair follicles were quantified in the re-epithelialized skin by non-invasive confocal scanning laser microscopy (CSLM) as published (Fan *et al.*, 2011; Nelson *et al.*, 2015). For our statistical analysis, we used a paired t-test between our experimental groups and considered significant if $p < 0.05$. Our data followed an approximately normal distribution.

Inhibition of P-Stat3 was achieved with the specific Jak/Stat pharmacological inhibitor cucurbitacin I. Cucurbitacin I (Tocris Biosciences/R&D Systems, Minneapolis MN) was dissolved in 10% EtOH/PBS to a final concentration of 1mg/mL prior to injection. Healing wounds were injected 7 days after wounding with 2mg/kg cucurbitacin I. Control injections were vehicle only. WIHN was measured by CSLM on WD20-WD24. Cucurbitacin I was confirmed to decrease phospho-STAT3 (P-Stat3) levels *in vivo* by western blot (Nelson et al., 2015).

Quantitative real-time PCR (qRT-PCR)

Mouse skin was harvested prior to wounding and throughout wounding as described (Nelson *et al.*, 2013). RNA was isolated with RNeasy Fibrous Mini Kit (Qiagen, Valencia, CA) with DNase I digestion followed by conversion to cDNA using the High Capacity RNA-to-cDNA kit (Life Technologies, MD). qRT-PCR was performed for genes of interest (OsM and IL-11) and 18S rRNA ribosomal unit (housekeeping genes) using inventoried TaqMan reagents. Differences in gene expression were assessed by comparative $\Delta\Delta$CT values with fold change calculations. Statistical analysis was done with a paired *t*-test and considered significant if $p < 0.05$.

ELISA

IL-6 protein levels were assayed by ELISA (R&D Systems, Minneapolis, MN) for non-wounded and wounded murine skin at times indicated. A minimum of three independent mice were used for each time point. Statistical analysis was done with a paired *t*-test and considered significant if $p < 0.05$.

References

Fan C, Luedtke MA, Prouty SM*, et al.* (2011) Characterization and quantification of wound-induced hair follicle neogenesis using in vivo confocal scanning laser microscopy. *Skin Res Technol* 17:387-97.

Ito M, Yang Z, Andl T*, et al.* (2007) Wnt-dependent de novo hair follicle regeneration in adult mouse skin after wounding. *Nature* 447:316-20

Nelson AM, Loy DE, Lawson JA*, et al.* (2013) Prostaglandin D(2) Inhibits Wound-Induced Hair Follicle Neogenesis through the Receptor, Gpr44. *J Invest Dermatol* 133:881-9.

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