doi:10.1038/nature11890

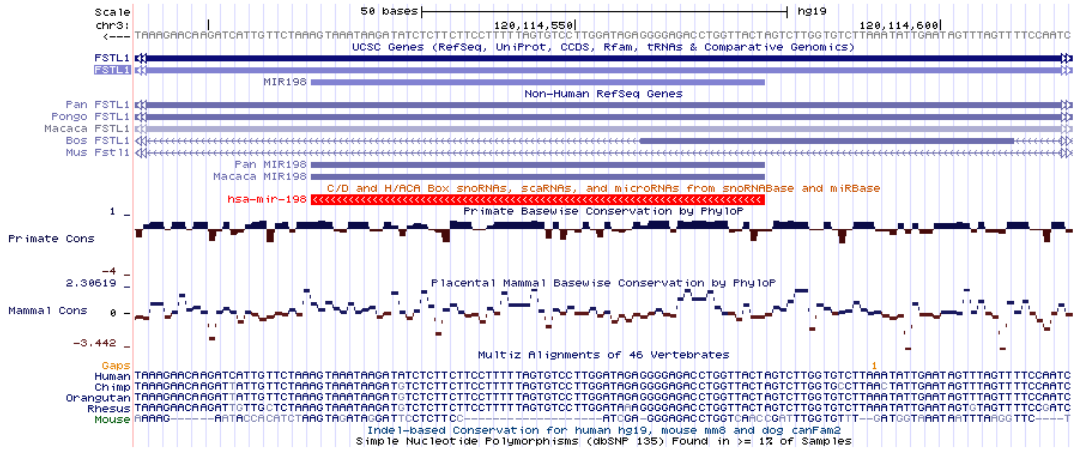


Figure S1: **Conservation map clearly indicates miR-198 is primate-specific**: Conservation across primates and mammals is shown. Pan-Chimpanzee, Pongo- Organgutan, Macaca-Rhesus macaque, Bos- Cattle and Mus- Mouse. Multiz alignment with primates and mouse orthologous sequence shows the lack of conservation in mouse.

Fig. S2

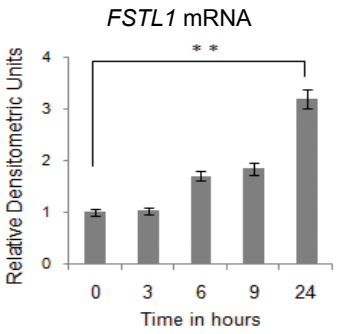


Figure S2: *FSTL1* mRNA levels upon injury: Densitometric analysis of band intensities from (Fig.1d), normalized against *RPLP0* intensity values. ***P<0.001.* Error bars represent s.d.

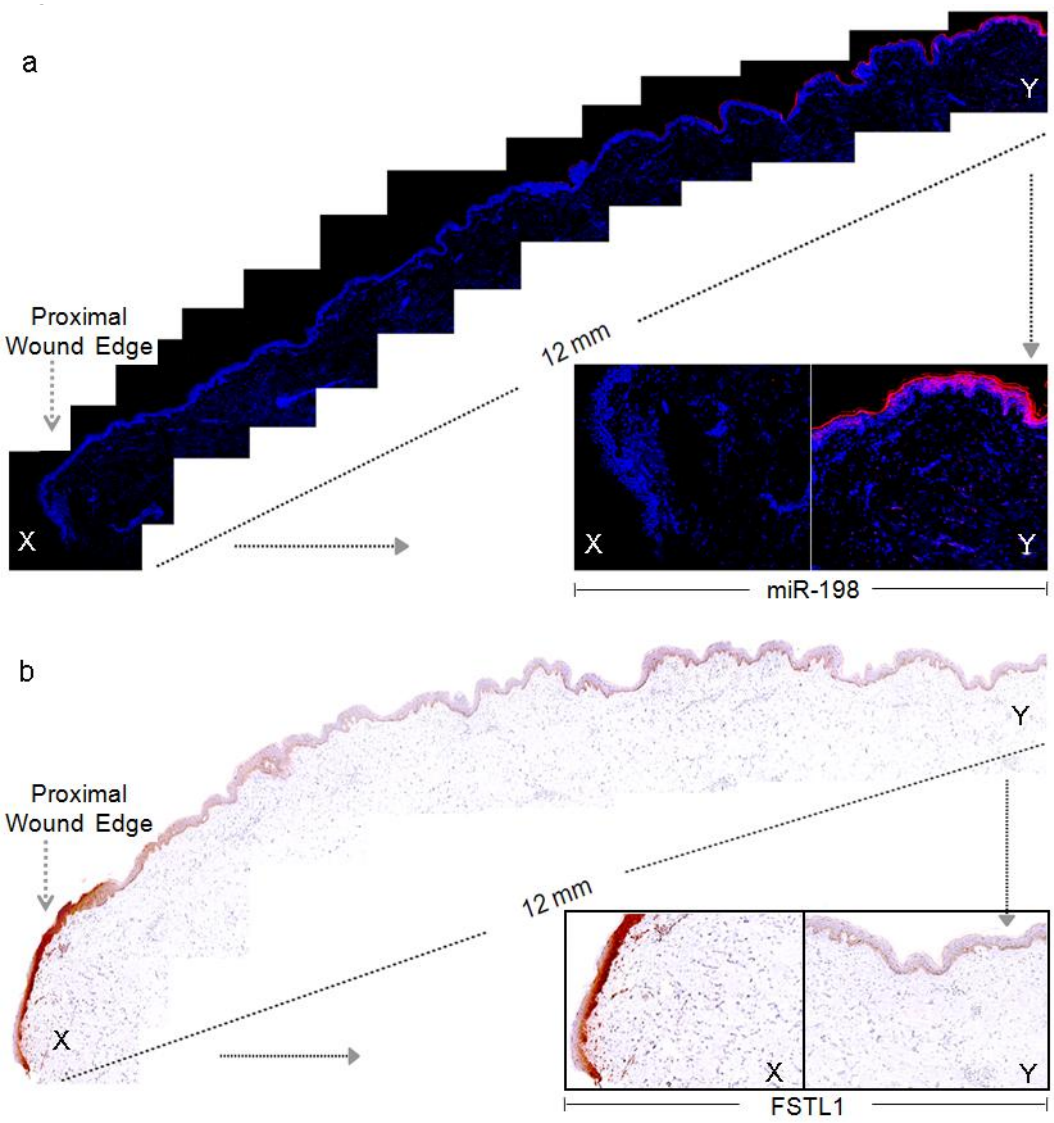


Figure S3: Reducing gradient of miR-198 expression and corresponding appearance of FSTL1 in the wound edge: a) *In situ* hybridization for miR-198 in skin section from *ex vivo* skin biopsy with the inner diameter of 25mm after 24h of injury response. *In situ* images were acquired contiguously from one wound edge (proximal) to the center of the biopsy (distal) and stitched manually. A gradient of injury response, resulting in down-regulation of miR-198 is observed from the proximal wound edge compared to the distal portion of the biopsy (< 12 mm). Inset x) magnified images of the proximal wound edge with no detectable miR198 and y) center of the biopsy with miR-198 expression. **b)** Immunohistochemistry for FSTL1 protein from the same *ex vivo* skin biopsy reveals expression at the proximal wound edge. Inset (x) and (y) magnified images of proximal and distal ends respectively (n=3).

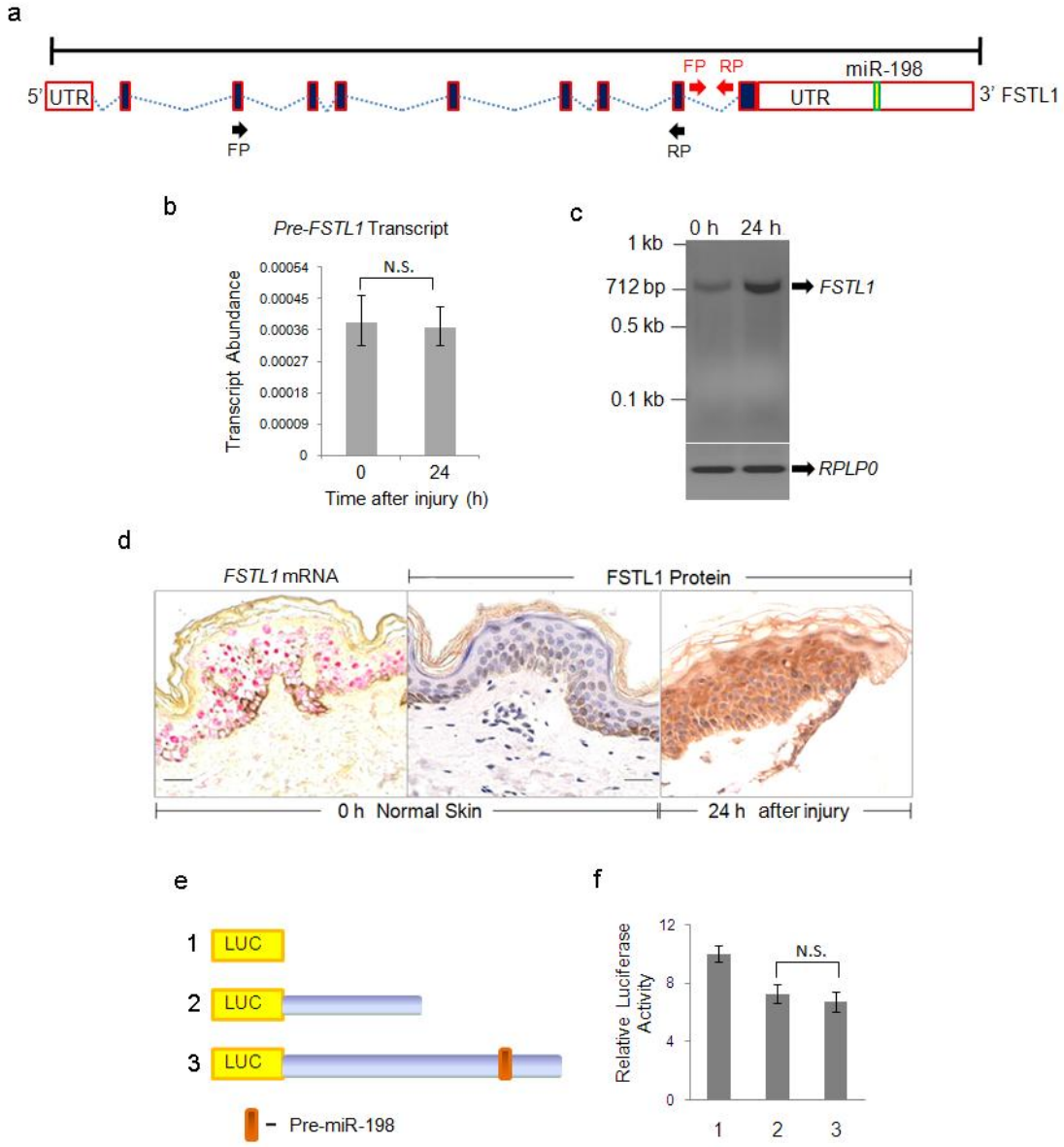


Figure S4: FSTL1 transcript can be processed to form mature miR-198 or can function as an *FSTL1* mRNA to make FSTL1 protein: **a)** Schematic of *FSTL1* showing the exon-intron boundaries and the 3'-UTR sequence encoding the miR-198. Primers used in semi-quantitative RT-PCR below are represented by the forward and reverse arrow marks. Primers in red are specific to introns whereas primers in black are specific to exons of pre-*FSTL1* transcript. **b)** Histogram representing quantification of pre-*FSTL1* transcript using intron specific primers in normal versus injured skin (24h after injury). N.S=not significant. **c)** Representative semi-quantitative-RT-PCR analysis of *FSTL1* mRNA using specific primers in explant samples after injury at indicated time points. (n=3) **d)** *In situ* hybridization with oligo probes (Panomics) on

normal skin at 0h (left panel) reveals nuclear localization of *FSTL1* mRNA that functions as pri-miRNA transcript. Immunohistochemical analysis of FSTL1 protein at 0h (middle) and 24h after injury (right) (n=5). Scale bar = 100 μM. **e)** Schematic diagram of the constructs used in luciferase assays. **f)** Luciferase assays with chimeric luciferase constructs, containing *FSTL1* 3'-UTR with (~2.7 Kb from stop codon) or without the pre-miR-198 (~0.9 Kb from stop codon) (NS-not significant) (n=3).

Fig. S5

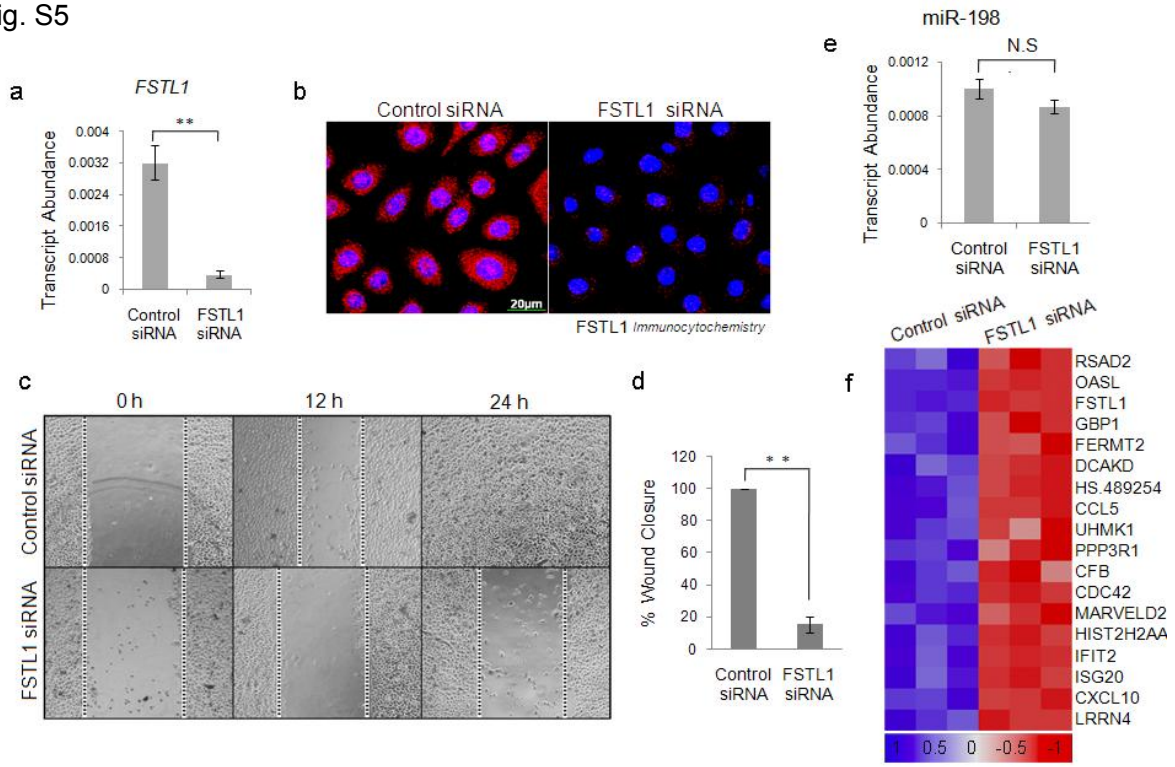


Figure S5: FSTL1 promotes keratinocyte migration: a) Histogram representing *FSTL1* transcript abundance in keratinocytes transfected with control non-targeting siRNA or gene-specific siRNA against *FSTL1* (n=3) ***P<0.001.* **b)** Immunocytochemistry on keratinocytes transfected with gene-specific siRNA against *FSTL1* shows down-regulation of FSTL1 protein expression (n=3). **c)** Representative images of migrating keratinocytes transfected with a control siRNA or *FSTL1*-specific siRNA at the indicated time points after scratch wound (n=5). **d)** Histograms representing relative wound closure in a scratch wound assay. By 24 hours, complete wound closure was observed in control transfected cells compared to knockdown of *FSTL1* leading to 15 ± 5 % wound closure (n=5) ***P<0.001.* Error bars represent s.d. **e)** Relative expression levels of mature miR-198 in keratinocytes transfected with *FSTL1* siRNA compared to control siRNA (N.S= not significant). **f)** Gene expression values of selected genes from microarray data of keratinocytes transfected with control non-targeting siRNA or *FSTL1* specific siRNA, represented as a heat-map. Expression values displayed in shades of red (low) or blue (high) relative to the individual mean value of the gene in a linear scale.

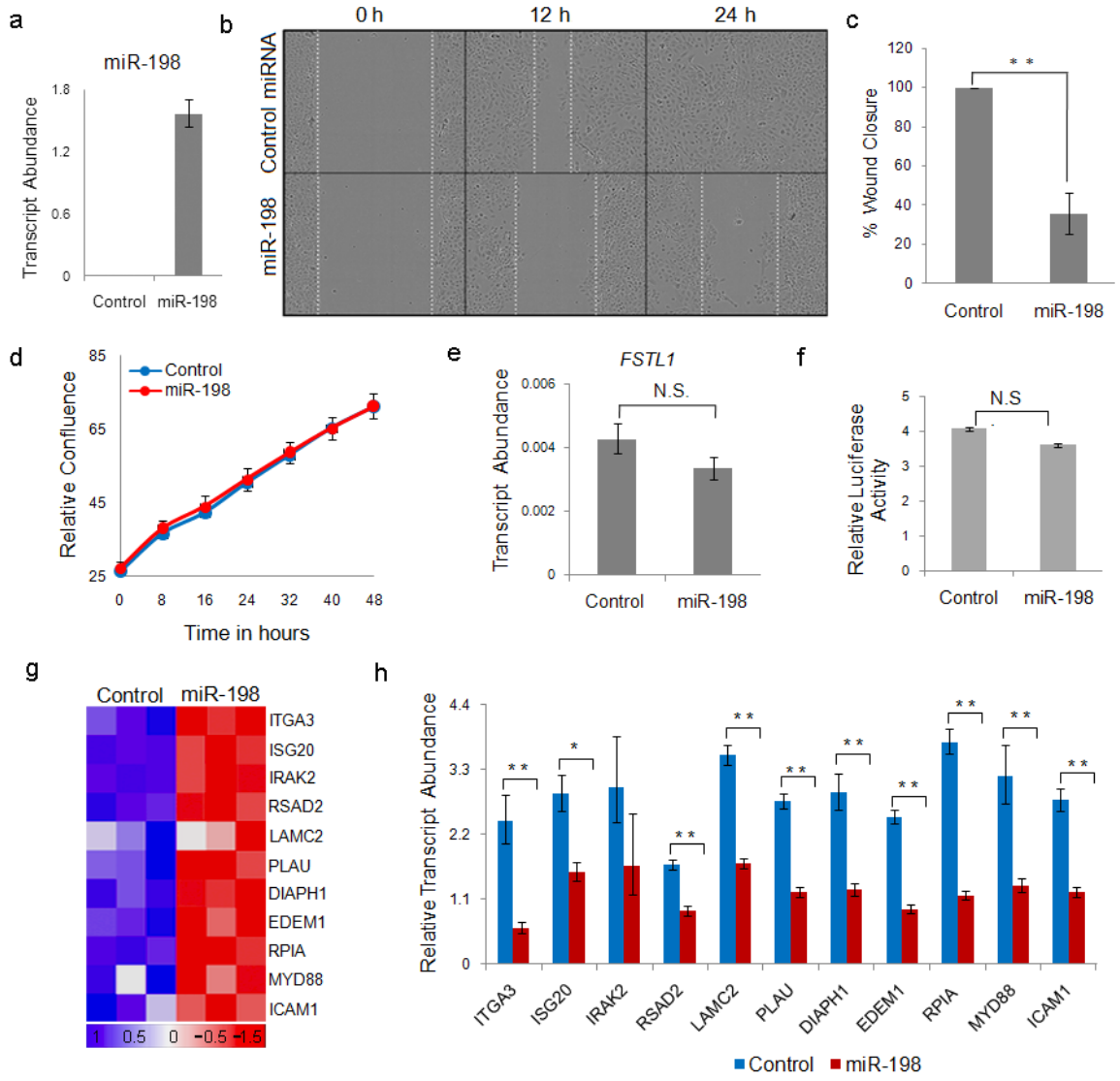


Figure S6: MicroRNA-198 inhibits keratinocyte migration independent of FSTL1: **a)** Histogram representing quantification of mature miR-198 in N/TERT-1 cells transfected with a control or miR-198 (n=3). **b)** Representative images of migrating keratinocytes transfected with non-targeting miRNA control (upper panel) or miR-198 (lower panel) at different time-points after scratch wounding. Dotted lines mark the migrating edge of the keratinocyte cell sheet (n=6). **c)** Histogram representing relative wound closure in the scratch wound assay. By 24 hours, complete wound closure was observed in control transfected cells, while over-expression of miR-198 resulted in 35 ± 10 % wound closure (n=6). ***P<0.001.* Error bars represent s.d. **d)** Proliferation of keratinocytes transfected with control or miR-198 assessed by relative cell confluence. Over-expression of miR-198 was not associated with any significant difference in proliferation (n=3). **e)** Relative levels of *FSTL1* mRNA in N/TERT-1 cells transfected with control

or miR-198 mimic. N.S: not significant (n=3). miR-198 over expression did not result in a significant change in *FSTL1* mRNA levels. **f)** Cells were co-transfected with *FSTL1* 3′-UTR luciferase reporter constructs either with miR-198 or a non-targeting, scrambled control. Normalized relative luciferase activities are shown as a bar diagram. Luciferase activity is expressed as the mean relative to controls N.S: not significant (n=3). **g)** Gene expression values of selected genes from microarray data of keratinocytes transfected with control or miR-198, represented as a heat-map. Expression values displayed in shades of red (low) or blue (high) relative to the individual mean value of the gene in a linear scale. **h)** Histogram representing relative transcript abundances (control versus miR-198 over-expression) of selected genes identified from microarrays and validated by qRT-PCR (n=3). Microarray data shows significant correlation with qRT-PCR results. **P < 0.05, **P < 0.001*. Student's t-test was used to calculate P value and error bars denote mean ± s.e.m.

Fig. S7

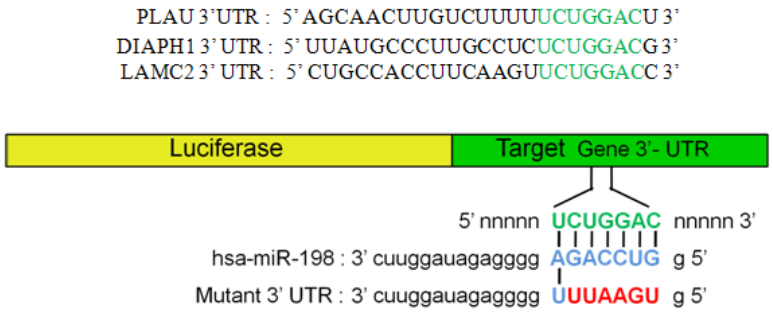
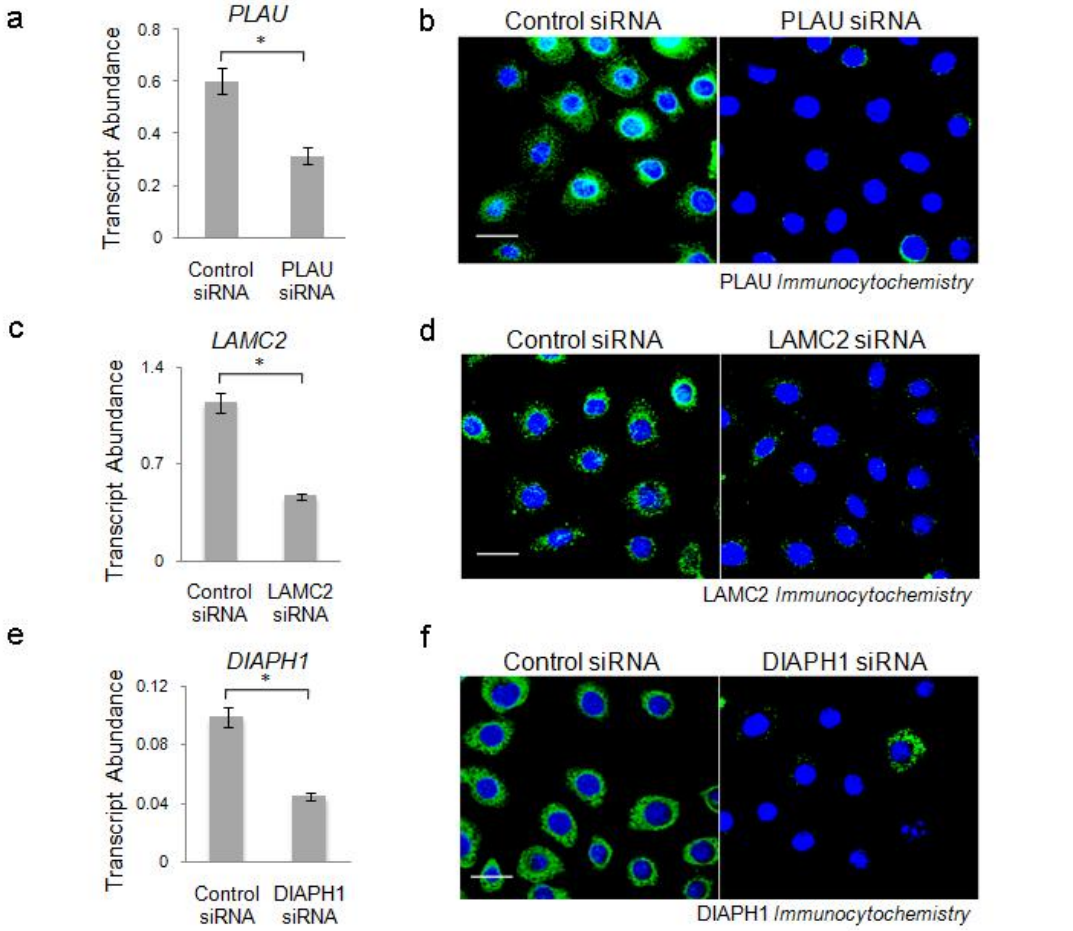
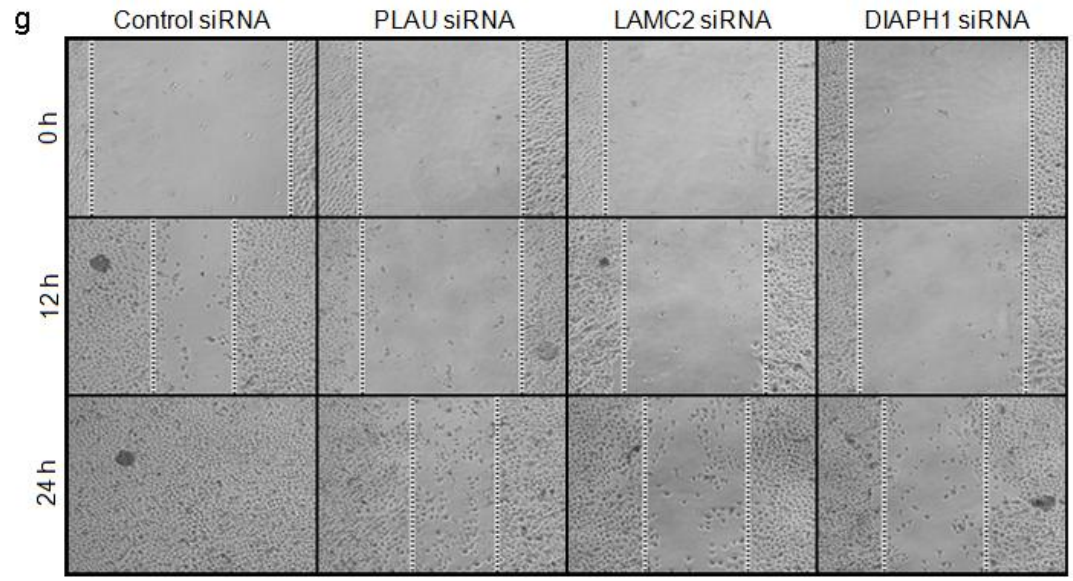


Figure S7: Schematic representation of chimeric luciferase constructs: The 3' UTR sequences containing miR-198 binding sites are shown (in green) for *PLAU, DIAPH1* and *LAMC2*. For the mutation of miR-198 binding sites in the target 3'UTR, the bases in the seed sequence (in blue) were mutated to the sequence shown in red. Mutations were restricted to the conversion of A to Gs and U to Cs and vice versa.


a
PLAU
Transcript Abundance
0.8
0.6
0.4
0.2
0
*
Control siRNA
PLAU siRNA
b
Control siRNA
PLAU siRNA
PLAU Immunocytochemistry
c
LAMC2
Transcript Abundance
1.4
0.7
0
*
Control siRNA
LAMC2 siRNA
d
Control siRNA
LAMC2 siRNA
LAMC2 Immunocytochemistry
e
DIAPH1
Transcript Abundance
0.12
0.08
0.04
0
*
Control siRNA
DIAPH1 siRNA
f
Control siRNA
DIAPH1 siRNA
DIAPH1 Immunocytochemistry


g
Control siRNA
PLAU siRNA
LAMC2 siRNA
DIAPH1 siRNA
0 h
12 h
24 h

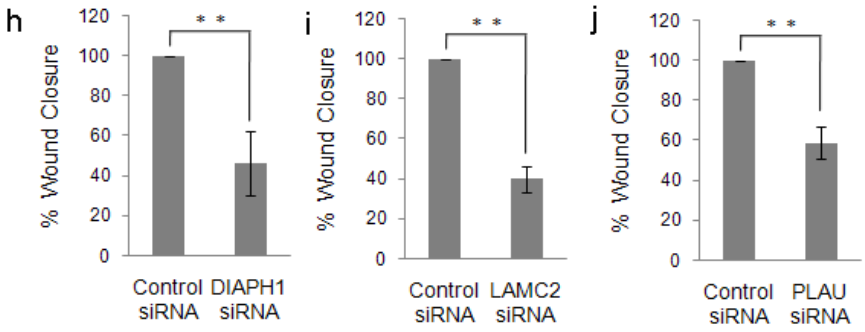


Figure S8: Knock-down of *DIAPH1*, *PLAU* and *LAMC2* significantly suppressed migration of keratinocytes: a-f) Quantification of mRNA and confirmation by immunocytochemistry using respective antibodies to assess relative knockdown efficiencies of *DIAPH1, PLAU* and *LAMC2* after transfection with specific siRNAs **P<0.05* (n=3) **g)** Representative images of migrating keratinocytes transfected with a control siRNA or *DIAPH1, PLAU* or *LAMC2* specific siRNA at the indicated time points after scratch wound (n=3). **h-j)** Histogram representing relative wound closure in a scratch wound assay. By 24 hours, knock-down of *DIAPH1, PLAU* and *LAMC2* resulted in 46 ± 16 %, 59 ± 8 % and 40 ± 6 % respectively, compared to complete wound closure in control siRNA transfected cells (n=3). ***P<0.001.* Error bars represent s.d.

Fig. S9

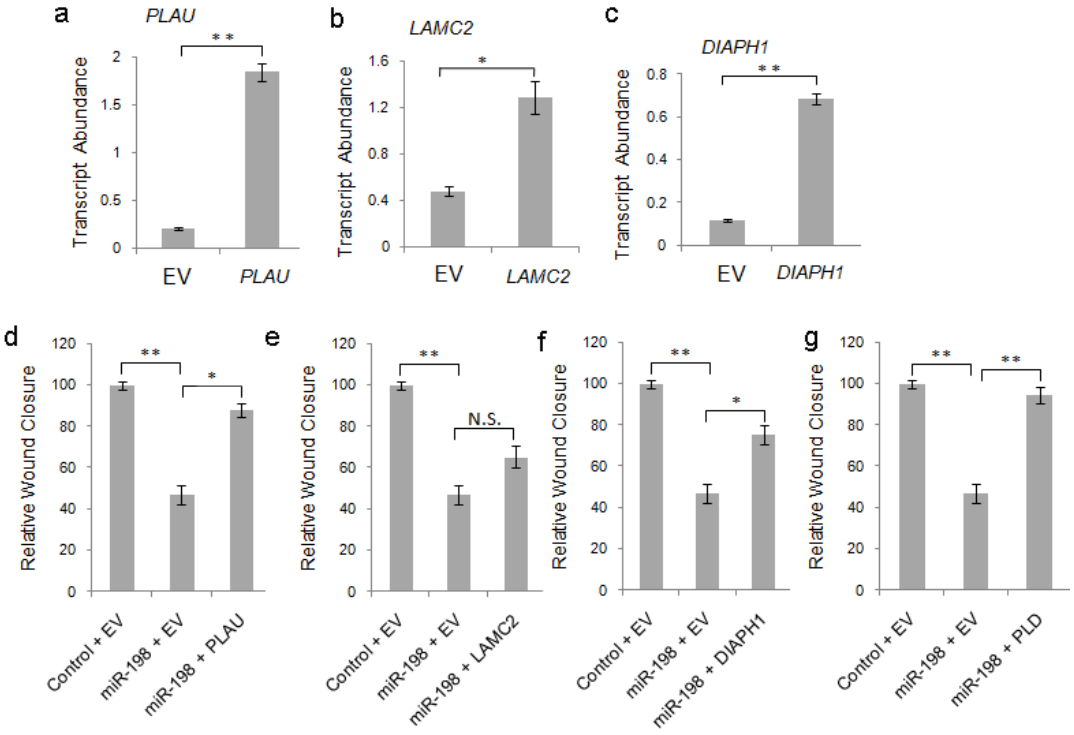


Figure S9: Over-expression of targets, in combination can significantly rescue the effect of miR-198: a-c) Histogram representing the relative transcript abundance of miR-198 targets in keratinocytes transfected with a control vector (EV) or constructs over-expressing *DIAPH1,*

LAMC2 and *PLAU*. **d-g)** Relative wound closure in keratinocytes co-transfected with miR-198 mimic and vectors over-expressing *PLAU, LAMC2* or *DIAPH1* individually or in combination (PLD) in equal ratios (n=3). **P<0.05, **P<0.001.* Error bars represent s.d.

Fig. S10

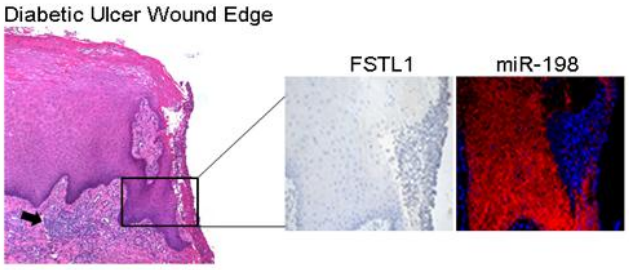


Figure S10: Chronic wound edge exhibits a defective switch: Tissue sections of chronic wounds stained with haematoxylin and eosin. Arrow and box indicates granulation tissue and wound edge respectively (n=8). Immunohistochemistry with FSTL1-antibody (left), or in situ hybridization with miR-198 probe (right), showing high levels of miR-198, but no FSTL1 at the wound edge of non-healing wounds.

Fig. S11

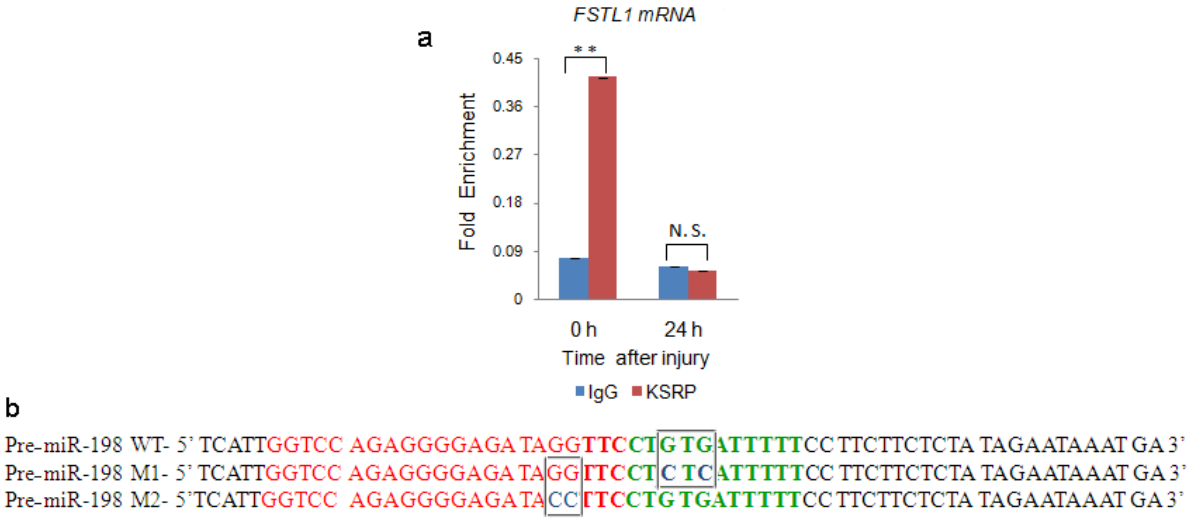


Figure S11: KSRP binds to the GUG motif of pre-miR-198 located in the 3'-UTR of FSTL1 mRNA in normal epidermal keratinocytes: a) RNA immunoprecipitations on epidermal lysates from explants, 0 hour or 24 hours after injury using anti-KSRP antibody or IgG control. Quantitative RT-PCR representing fold enrichment of transcripts reveals specific binding of KSRP to *FSTL1* transcript only at 0 hours but not 24 hours after injury (n=3). ***P < 0.001*. Student's t-test was used to calculate P value and error bars denote mean ± s.e.m. **b)** Wild type and mutant Pre-miR-198 sequences used in RNA gel retardation assay. Mature miR-198 sequence is marked in red, terminal loop sequence shown in green, whereas the mutated sequences are shown in blue within boxes.

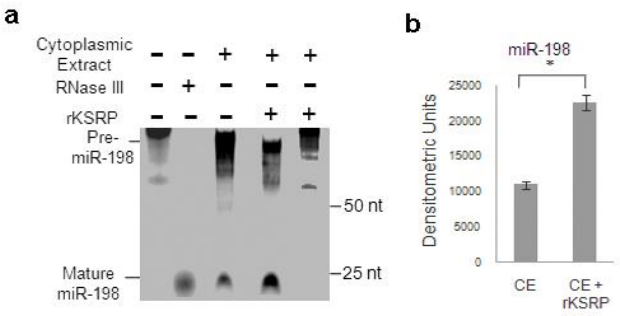


Figure S12: KSRP is essential for miR-198 processing: a) Efficient processing of pre-miR-198 in the presence of recombinant KSRP (rKSRP). *In vitro* synthesized pre-miR-198 transcripts were incubated with cytoplasmic extracts in the absence or presence of rKSRP. Cleaved mature miR-198 was detected by co-migration with the product released when pre-miR-198 was treated with RNase III. Pre-miR-198 mutant (with the CUC motif) in the last lane fails to form mature miR-198 (n=3). **b)** Histogram representing densitometric quantification of the mature miR-198 *$P<0.05$. Error bars represent s.d.

Fig. S13

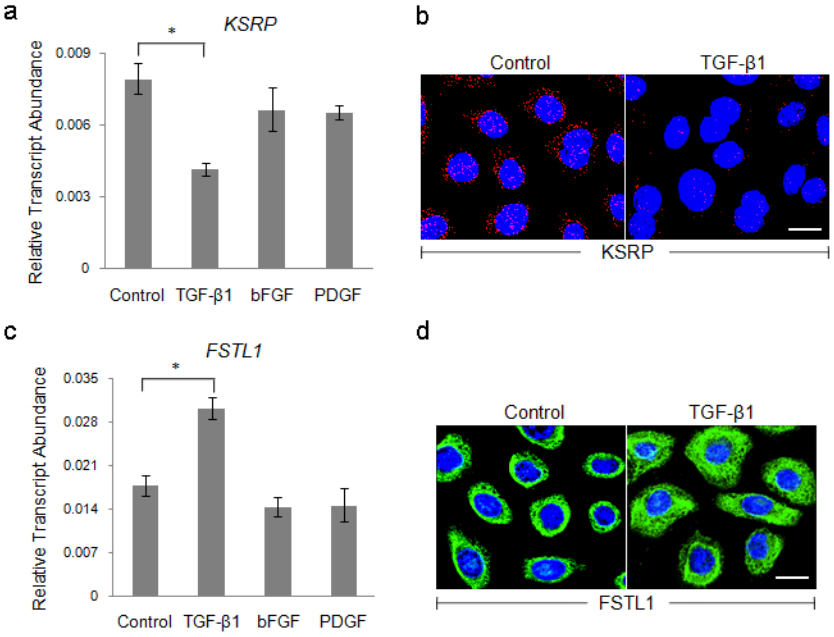


Figure S13: TGF-β1 down-regulates KSRP and facilitates FSTL1 expression: a) Relative transcript abundance of *KSRP* in keratinocytes treated with TGF-β1, FGF2 and PDGF-AB for 24hrs as measured by qRT-PCR *$P < 0.05$. **b)** Immunocytochemistry staining using KSRP-specific antibody shows a decrease in KSRP protein expression in TGF-β1 treated cells. **c)** Relative transcript abundance of *FSTL1* in keratinocytes treated with TGF-β1, FGF2 and PDGF-

AB for 24hrs as measured by qRT-PCR. **P < 0.05*. Student's t-test was used to calculate P value and error bars denote mean ± s.e.m. **d)** Immunocytochemistry staining using FSTL1-specific antibody shows increased FSTL1 protein expression in TGF-β1 treated cells (n=3).

Fig. S14

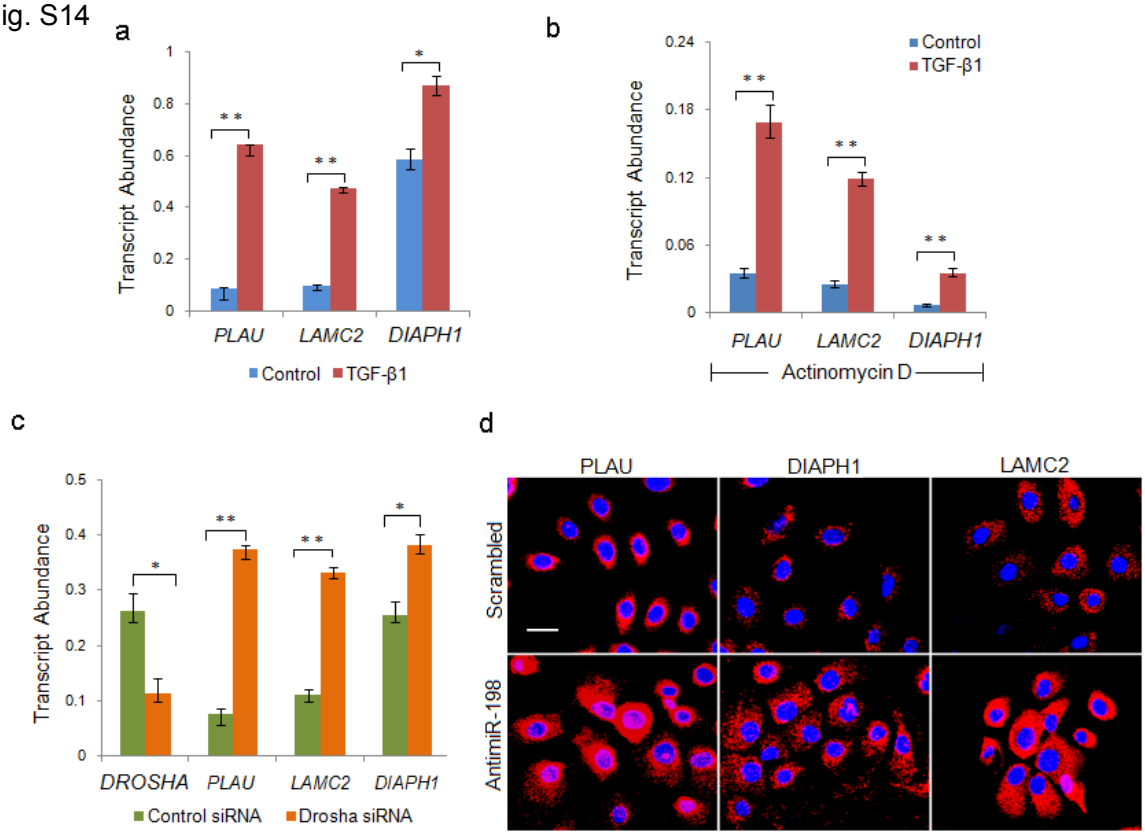


Figure S14: Increase in transcript stability of miR-198 targets upon treatment with TGF-β1: a) Histogram representing relative transcript abundance of *PLAU, LAMC2* and *DIAPH1* in keratinocytes treated with TGF-β1. **b)** Histogram representing relative transcript abundance of *PLAU, LAMC2* and *DIAPH1* in keratinocytes treated with TGF-β1 in the presence of actinomycin-D. **c)** Relative transcript abundance of *DROSHA* and *PLAU, LAMC2* and *DIAPH1* in keratinocytes transfected with a non targeting siRNA or siRNA against *DROSHA. *P < 0.05, **P < 0.001*. Student's t-test was used to calculate P value and error bars denote mean ± s.e.m. **d)** Immunocytochemistry for PLAU, LAMC2 and DIAPH1 on keratinocytes transfected with a control inhibitor or LNA inhibitor for mature miR198 shows a specific upregulation of miR-198 targets after inhibition of miR-198 (n=3).

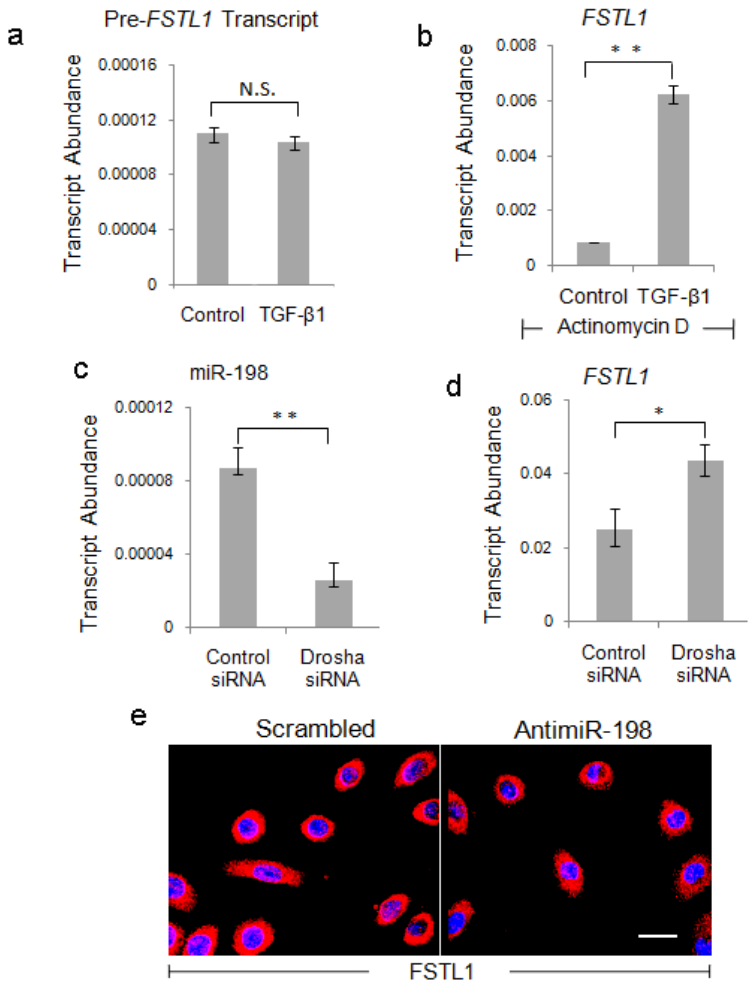


Figure S15: TGF-β1 facilitates FSTL1 expression: a) Histogram representing relative quantification of pre-*FSTL1* transcript detected by primers specifically amplifying the intronic region in keratinocytes treated with TGF-β1 (N.S= non significant) (n=3). **b)** Histogram representing relative transcript abundance of *FSTL1* in keratinocytes treated with TGF-β1 in the presence of actinomycin D. **c)** Histograms showing relative levels of mature miR-198 in keratinocytes transfected with a control siRNA or siRNA specific for human *DROSHA* (n=3), ***P < 0.001.* **d)** Histograms showing relative levels of *FSTL1* mRNA in keratinocytes transfected with a control siRNA or siRNA specific for human *DROSHA. *P < 0.05, **P < 0.001*. Student's t-test was used to calculate P value and error bars denote mean ± s.e.m. **e)** Immunocytochemistry for FSTL1 protein in keratinocytes transfected with control inhibitor or antimiR-198 shows no significant difference in protein levels (n=3).

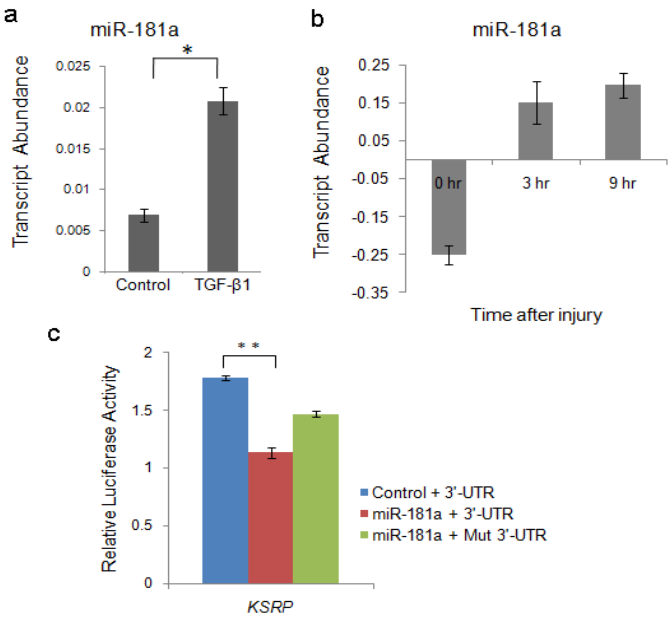


Figure S16: KSRP is a target of miR-181a: a) Histogram representing relative transcript abundance of miR-181a in keratinocytes treated with TGF-β1 or control. Treatment with TGF-β1 induces miR-181a expression (n=3). **b)** Expression of miR-181a in epidermal keratinocytes after injury at indicated time points. **c)** Validation of *KSRP* as a direct target of miR-181a by luciferase reporter assays. Cells were co-transfected with wild type or mutant *KSRP* 3′-UTR luciferase reporter constructs as indicated, either with miR-181a or non-targeting scrambled control (n=3). Normalized relative luciferase activities are shown as a bar diagram. Luciferase activity is expressed as mean relative to controls. **P < 0.05, **P < 0.001*. Student's t-test was used to calculate P value and error bars denote mean ± s.e.m.

Fig. S17

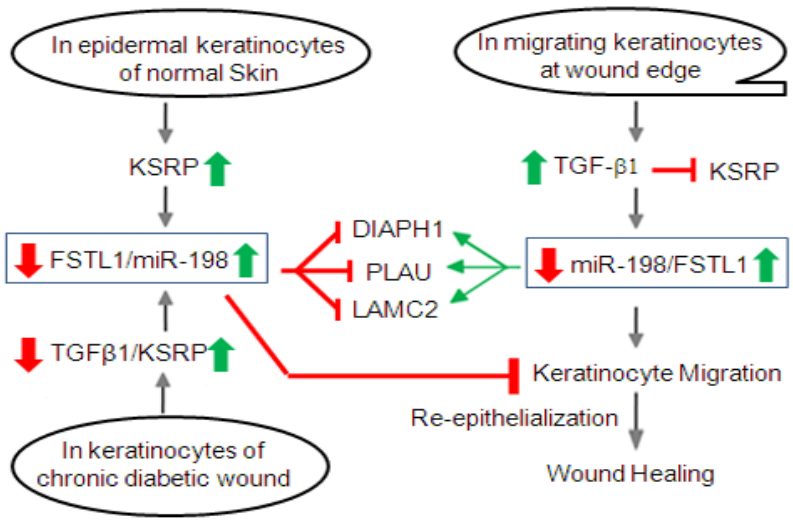


Figure. S17: Model depicting the mechanism of regulatory-switch in normal versus chronic wounds.

2. Supplementary tables

a: List of primers used for RNA quantification

	Forward primer	Reverse primer	Remarks
FSTL1 3'-UTR	gagttggccctgtctcttctt	ctttcccactctcttcctgct	Spans the precursor miR198
FSTL1 ORF	aatccaagatctgtgccaatg	gctgtacagacccaatttcca	Binds to Exon 3 and Exon 9
FSTL1 primary transcript	gggatctctgggaatggaata	acactgataggccacaaatgc	Binds within Intron 10
ITGA3	taaatggctgggctaccctat	gggtccgcttaaagaagtcac	
ISG20	tgagggagagatcaccgatta	gctcatgtcctctttcagtgc	
IRAK2	caagtgattctcctgcctcag	tcaagcctgtaatcccaacac	
RSAD2	gtgcctggatttcatgtcagt	atgcttgctttctctgagctg	
LAMC2	ctgggttgtgcacatttcttt	aaatacagaagcaaggcagca	
LAMC2 ORF	agtggaaggagagctggaaag	gaccagcccctcttcatctac	Binds within ORF sequence
PLAU	gcttgtccaagagtgcatggt	agggctggttctcgatggt	
PLAU ORF	tcactggctttggaaaagaga	gtggtgacttcagagccgtag	Binds within ORF sequence
DIAPH1	aggaagcatgagggcaactat	cccaggaatagtccaaaggag	
DIAPH1 ORF	agctgccacagatgaaaaaga	tcttggggtcaaagaggaagt	Binds within ORF sequence
EDEM1	tgaaaaggtagggctgagtga	gcagggaagaggcactagaat	
RPIA	gagcggtatggtatggaatga	gcatttctggtcaactgcttc	
MYD88	gcatgatcttgttgaggcatt	atggcaaatatcggcttttct	
ICAM1	gcactatgcagctccagtttc	caagactgcagtgaaccatga	
KRT14	catgagtgtggaagccgacat	gcctctcagggcattcatctc	
KHSRP	ctgttttgtttggcgagagag	gagacacagaacaggcgagag	
HuR	ttgtaagtcaccgccagtacc	tcacatggtcatggtcaaaga	
RPLP0	cagattggctacccaactgtt	gggaaggtgtaatccgtctcc	

b: List of primers used for amplifying miR198 target 3'UTRs:

Red represents restriction enzymes used for cloning purpose

	Forward primer	Reverse primer
DIAPH1	tcca gagctc ctaaggaagcagggagcaaat	tcca tctaga gcccacccacttctcttttag
LAMC2	cca gagctc gggtgtgagaatgatcaagga	cca tctaga cccagctgaagtgtgagtagg
PLAU	tcca gagctc taggctctgcacagatggatt	tcca tctaga gccccaggagtgacctataac
KHSRP	tcca gagctc aggctcaatgaatcgaatgaa	tcca tctaga tacaacacctggtccaaggaa
FSTL1	cca gagctc atcccagcatcttctccactt	cca tctaga taattgggggaaaggaaacc

c. Probe sequences used for in situ hybridization:

Probe	Probe Sequence	Vendor
miR-198	GAACCTATCTCCCCTCTGGACC	Exiqon
FSTL1	Probeset covers region 2106 to 3714 in mRNA	Panomics
Scramble	GTGTAACACGTCTATACGCCCA	Exiqon