SUPPLEMENTAL METHODS

Additional protocols for generation of mouse model

Pups were weaned from the mother 3-4 weeks after birth. Both male and female mice of 8-10 weeks old were used for wound closure studies. Mouse genomic DNA was extracted from tail biopsies using the REDExtract-N-Amp kit (Sigma Aldrich, St Louis, MO), which is a combination of both DNA extraction and PCR analysis. Briefly, tail biopsies were incubated with the tissue preparation solution and then extraction solution from the kit at 55°C for 10 minutes, followed by 95°C for 3 minutes. After adding neutralization solution, the samples were stored at 4°C. For PCR analysis, 10μl of the PCR reaction mix from the kit was mixed with 4μl of the tissue extract, 2μl each of the forward and reverse primers and 4μl water. The nucleotide sequences for the primers were C forward (within the exon 8) ttctctgaaggactactgtaccagaatgaa; and C reverse (downstream of the exon 11) gtgtgggggtgcgcaaagaaaagacacatt. The reactions were carried out as suggested by the kit manufacturer. All control and treatment groups were age and gender matched to reduce variabilities. The two anti-Hsp90 antibodies used in this study were Hsp90α antibody from Novus Biologicals (NB120-2928, Littleton, CO) and Hsp90β antibody from StressMarq (H9010, Victoria, Canada). Please note that, while these antibodies do not cross react between human Hsp90α and human Hsp90β, we found that they cross react at lower degrees between mouse Hsp90α and mouse Hsp90β proteins. This was the reason why mass spec was utilized to confirm the identity of the mouse Hsp90 protein species in the Western blots (in Figure 3 and the text).

Western immunoblotting analysis of Hsp90 family proteins

The experimental procedures as described in details by Zou et al. (Zou et al., 2017) were utilized in this study.

Colloidal gold (individual) cell motility assay

The colloidal gold migration assay was conducted on isolated mouse fibroblasts as described previously (Cheng et al., 2008). The colloidal gold migration assay with a computer-assisted quantitation is currently the most sensitive and quantitative assay for testing pro-motility activity of a purified protein on individual cells, with the least influence of secreted molecules by surrounding neighbor cells. Data from multiple independent experiments ($n \geq 3$) were collected and calculated. Results of a representative experiment was shown (mean ± SD, $p < 0.05$).

Additional protocols for animals

All experiments using the pig model followed the procedures as described previously by us (O'Brien et al., 2014; Bhatia et al., 2016). Mice (both male and female mice of 8-10 weeks old) were anesthetized with a cocktail of ketamine and xylazine injected intraperitoneally, as well as isoflurane via inhalation (if necessary during preparation of the surgical procedures). Hair in the to-be-wounded area on the back of mice was shortened using an electrical shaver, followed by application of hair removal cream (Nair, Trenton, NJ). The exposed skin was cleaned with water and disinfected with betadine.

Care was taken to ensure the bandage was not too tight to prevent breathing or too loose to come off during the experiment. The mice were kept under a heating lamp until they woke up and housed individually until end of the experiment.

Additional protocols for Histology and Immunohistochemistry(IHC)

Fifteen randomly selected images under each condition were utilized to measure the relative distance of re-epithelialization under a specific condition or treatment, as previously reported by us (Cheng et al., 2011). In addition, the wound photographs will be presented to five "blinded" USC dermatology residents who have little knowledge of the experimental design and conditions. They will be asked to rate degree of the wound closure using a five-point scale, 0 = none; 1 = minimal or little difference (between treatment and placebo), 2 = moderate or visible difference, 3 = significant difference and 4 = dramatic change. IHC analyses included mouse monoclonal anti-Hsp90α antibody (1:200, Calbiochem, Darmstadt, Germany) and mouse monoclonal anti-keratin (pan) antibody (Abcam, Cambridge, MA) staining. Excessive amount of recombinant Hsp90α protein was used during IHC as the competitor for the antibody specificity control.

Band #1

Accession	Description	Score	Coverage
P07901	Heat shock protein HSP 90-alpha OS=Mus musculus GN=Hsp90aa1 PE=1 SV=4 - [HS90A_MOUSE]	559.44	52.25
Q71LX8	Heat shock protein 84b OS=Mus musculus GN=Hsp90ab1 PE=2 SV=1 - [Q71LX8_MOUSE]	407.85	47.24
O08601	Microsomal triglyceride transfer protein large subunit OS=Mus musculus GN=Mttp PE=2 SV=2 - [MTP_MOUSE]	244.65	41.95
P08113	Endoplasmin OS=Mus musculus GN=Hsp90b1 PE=1 SV=2 - [ENPL_MOUSE]	199.05	46.26
P35564	Calnexin OS=Mus musculus GN=Canx PE=1 SV=1 - [CALX_MOUSE]	88.60	16.41
A2A7A9	Hexose-6-phosphate dehydrogenase (Glucose 1-dehydrogenase) OS=Mus musculus GN=H6pd PE=4 SV=1 - [A2A7A9_MOUSE]	77.09	21.29
Q9JLF6	Protein-glutamine gamma-glutamyltransferase K OS=Mus musculus GN=Tgm1 PE=1 SV=2 - [TGM1_MOUSE]	54.38	12.15
Q01853	Transitional endoplasmic reticulum ATPase OS=Mus musculus GN=Vcp PE=1 SV=4 - [TERA_MOUSE]	52.39	15.38
Q8R0Y6	Aldehyde dehydrogenase family 1 member L1 OS=Mus musculus GN=Aldh1l1 PE=2 SV=1 - [AL1L1_MOUSE]	42.43	15.41
Q91ZX7	Prolow-density lipoprotein receptor-related protein 1 OS=Mus musculus GN=Lrp1 PE=1 SV=1 - [LRP1_MOUSE]	30.56	1.96
P57780	Alpha-actinin-4 OS=Mus musculus GN=Actn4 PE=1 SV=1 - [ACTN4_MOUSE]	25.04	7.46
Q8BHN3	Neutral alpha-glucosidase AB OS=Mus musculus GN=Ganab PE=1 SV=1 - [GANAB_MOUSE]	24.18	9.85
Q9JKX3	Transferrin receptor protein 2 OS=Mus musculus GN=Tfr2 PE=2 SV=2 - [TFR2_MOUSE]	23.62	11.15
E9Q1T0	Uncharacterized protein OS=Mus musculus GN=Vmn2r67 PE=4 SV=1 - [E9Q1T0_MOUSE]	20.94	2.23
Q3U6Y9	Putative uncharacterized protein OS=Mus musculus GN=Cd36 PE=2 SV=1 - [Q3U6Y9_MOUSE]	13.81	9.19
Q3U026	Putative uncharacterized protein OS=Mus musculus GN=Mogs PE=2 SV=1 - [Q3U026_MOUSE]	13.71	6.95
Q80VE7	Ctnnb1 protein (Fragment) OS=Mus musculus GN=Ctnnb1 PE=2 SV=1 - [Q80VE7_MOUSE]	13.17	5.60
D3YTQ5	Uncharacterized protein OS=Mus musculus GN=Acad11 PE=3 SV=1 - [D3YTQ5_MOUSE]	12.15	8.47
Q02257	Junction plakoglobin OS=Mus musculus GN=Jup PE=1 SV=3 - [PLAK_MOUSE]	12.09	4.03
Q99JX8	Psmd2 protein (Fragment) OS=Mus musculus GN=Psmd2 PE=2 SV=1 - [Q99JX8_MOUSE]	10.75	15.05
Q3TA19	Putative uncharacterized protein OS=Mus musculus GN=Slc4a1 PE=2 SV=1 - [Q3TA19_MOUSE]	10.12	8.10

Band #2

Accession	Description	Score	Coverage
Q71LX8	Heat shock protein 84b OS=Mus musculus GN=Hsp90ab1 PE=2 SV=1 - [Q71LX8_MOUSE]	241.20	47.24
Q3TKB9	Putative uncharacterized protein (Fragment) OS=Mus musculus GN=Hsp90aa1 PE=2 SV=1 - [Q3TKB9_MOUSE]	76.97	19.96
Q8R0Y6	Aldehyde dehydrogenase family 1 member L1 OS=Mus musculus GN=Aldh1l1 PE=2 SV=1 - [AL1L1_MOUSE]	40.07	14.08
Q8BNF8	Putative uncharacterized protein OS=Mus musculus GN=Vcp PE=2 SV=1 - [Q8BNF8_MOUSE]	28.38	8.71
Q3TGJ9	Putative uncharacterized protein OS=Mus musculus GN=Gsn PE=2 SV=1 - [Q3TGJ9_MOUSE]	21.17	11.49
E9Q1T0	Uncharacterized protein OS=Mus musculus GN=Vmn2r67 PE=4 SV=1 - [E9Q1T0_MOUSE]	16.70	2.23
Q3TA19	Putative uncharacterized protein OS=Mus musculus GN=Slc4a1 PE=2 SV=1 - [Q3TA19_MOUSE]	14.71	8.10
D3Z4Y4	Uncharacterized protein OS=Mus musculus GN=Krt5 PE=3 SV=1 - [D3Z4Y4_MOUSE]	12.21	7.64
P35564	Calnexin OS=Mus musculus GN=Canx PE=1 SV=1 - [CALX_MOUSE]	11.65	6.77
Q80VE7	Ctnnb1 protein (Fragment) OS=Mus musculus GN=Ctnnb1 PE=2 SV=1 - [Q80VE7_MOUSE]	10.01	3.79

Continued on next page

Band #3

Accession	Description	Score	Coverage
→ Q3TKB9	Putative uncharacterized protein (Fragment) OS=Mus musculus GN=Hsp90aa1 PE=2 SV=1 - [Q3TKB9_MOUSE]	223.15	46.40
Q9WVK4	EH domain-containing protein 1 OS=Mus musculus GN=Ehd1 PE=1 SV=1 - [EHD1_MOUSE]	149.67	57.87
Q71LX8	Heat shock protein 84b OS=Mus musculus GN=Hsp90ab1 PE=2 SV=1 - [Q71LX8_MOUSE]	105.68	28.04
Q8BH64	EH domain-containing protein 2 OS=Mus musculus GN=Ehd2 PE=1 SV=1 - [EHD2_MOUSE]	98.42	48.43
Q9D0I9	Arginyl-tRNA synthetase, cytoplasmic OS=Mus musculus GN=Rars PE=2 SV=2 - [SYRC_MOUSE]	94.31	42.42
Q8QZY1	Eukaryotic translation initiation factor 3 subunit L OS=Mus musculus GN=Eif3l PE=1 SV=1 - [EIF3L_MOUSE]	87.67	38.48
P11983	T-complex protein 1 subunit alpha OS=Mus musculus GN=Tcp1 PE=1 SV=3 - [TCPA_MOUSE]	82.93	47.12
Q76MZ3	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform OS=Mus musculus GN=Ppp2r1a PE=1 SV=3 - [2AAA_MOUSE]	78.90	31.75
P14685	26S proteasome non-ATPase regulatory subunit 3 OS=Mus musculus GN=Psmd3 PE=2 SV=2 - [PSMD3_MOUSE]	77.40	35.66
E9Q133	Uncharacterized protein OS=Mus musculus GN=Cct3 PE=4 SV=1 - [E9Q133_MOUSE]	76.76	48.13
P80317	T-complex protein 1 subunit zeta OS=Mus musculus GN=Cct6a PE=1 SV=3 - [TCPZ_MOUSE]	69.65	40.11
Q3UDS0	Putative uncharacterized protein (Fragment) OS=Mus musculus GN=Hspa8 PE=2 SV=1 - [Q3UDS0_MOUSE]	63.35	42.13
Q9QXY6	EH domain-containing protein 3 OS=Mus musculus GN=Ehd3 PE=1 SV=2 - [EHD3_MOUSE]	58.73	30.09
Q3V2Z4	Putative uncharacterized protein OS=Mus musculus GN=Anxa6 PE=2 SV=1 - [Q3V2Z4_MOUSE]	46.94	25.36
Q68FD5	Clathrin heavy chain 1 OS=Mus musculus GN=Cltc PE=1 SV=3 - [CLH_MOUSE]	46.49	10.81
Q8BMK4	Cytoskeleton-associated protein 4 OS=Mus musculus GN=Ckap4 PE=2 SV=2 - [CKAP4_MOUSE]	44.46	29.74
B7FAU9	Filamin, alpha OS=Mus musculus GN=Flna PE=4 SV=1 - [B7FAU9_MOUSE]	43.87	7.73
Q6NXX7	Abce1 protein (Fragment) OS=Mus musculus GN=Abce1 PE=2 SV=1 - [Q6NXX7_MOUSE]	41.13	24.00
Q91VL7	EH-domain containing 4 OS=Mus musculus GN=Ehd4 PE=2 SV=1 - [Q91VL7_MOUSE]	34.67	18.85
Q8VDD5	Myosin-9 OS=Mus musculus GN=Myh9 PE=1 SV=4 - [MYH9_MOUSE]	32.31	6.73
Q3TD51	Putative uncharacterized protein OS=Mus musculus GN=Picalm PE=2 SV=1 - [Q3TD51_MOUSE]	31.23	17.25
Q99K51	Plastin-3 OS=Mus musculus GN=Pls3 PE=1 SV=3 - [PLST_MOUSE]	29.26	18.25
E9QNJ1	Uncharacterized protein OS=Mus musculus GN=Ppp2r1b PE=4 SV=1 - [E9QNJ1_MOUSE]	28.39	19.30
P24527	Leukotriene A-4 hydrolase OS=Mus musculus GN=Lta4h PE=1 SV=3 - [LKHA4_MOUSE]	27.84	20.79
A1E2B8	Inducible heat shock protein 70 OS=Mus musculus GN=Hspa1a PE=3 SV=1 - [A1E2B8_MOUSE]	26.72	14.51
A3KGU5	Spectrin alpha 2 OS=Mus musculus GN=Spna2 PE=4 SV=1 - [A3KGU5_MOUSE]	26.53	4.76
P07724	Serum albumin OS=Mus musculus GN=Alb PE=1 SV=3 - [ALBU_MOUSE]	24.84	2.14
Q9CZK0	Putative uncharacterized protein (Fragment) OS=Mus musculus GN=Snx9 PE=2 SV=2 - [Q9CZK0_MOUSE]	20.23	18.29
Q5RKP4	Rpn1 protein (Fragment) OS=Mus musculus GN=Rpn1 PE=2 SV=1 - [Q5RKP4_MOUSE]	20.06	14.99
Q61833	Ribophorin OS=Mus musculus GN=Rpn2 PE=2 SV=1 - [Q61833_MOUSE]	19.35	14.66
Q3TEF0	Putative uncharacterized protein (Fragment) OS=Mus musculus GN=Cdc23 PE=2 SV=1 - [Q3TEF0_MOUSE]	17.78	12.06
Q3TIR3	Synembryn-A OS=Mus musculus GN=Ric8a PE=1 SV=2 - [RIC8A_MOUSE]	17.63	13.40
Q5XJY5	Coatomer subunit delta OS=Mus musculus GN=Arcn1 PE=2 SV=1 - [COPD_MOUSE]	17.00	10.76
Q3TE06	Putative uncharacterized protein (Fragment) OS=Mus musculus GN=Wdr1 PE=2 SV=1 - [Q3TE06_MOUSE]	16.33	13.10
P68373	Tubulin alpha-1C chain OS=Mus musculus GN=Tuba1c PE=1 SV=1 - [TBA1C_MOUSE]	16.09	16.04
D3Z4Y4	Uncharacterized protein OS=Mus musculus GN=Krt5 PE=3 SV=1 - [D3Z4Y4_MOUSE]	15.63	9.77
P04104	Keratin, type II cytoskeletal 1 OS=Mus musculus GN=Krt1 PE=1 SV=4 - [K2C1_MOUSE]	12.73	3.45
Q8VEH5	EPM2A-interacting protein 1 OS=Mus musculus GN=Epm2aip1 PE=2 SV=1 - [EPMIP_MOUSE]	12.57	11.39
Q6ZWS7	Putative uncharacterized protein OS=Mus musculus GN=Camk2g PE=2 SV=1 - [Q6ZWS7_MOUSE]	11.73	8.28
D3Z028	Uncharacterized protein OS=Mus musculus GN=Asns PE=4 SV=1 - [D3Z028_MOUSE]	10.40	11.02

Figure 2s. Excised bands from Figure 2E (with *) were subjected to protein extraction procedures (Cheng et al., 2008) to separate the proteins from polyacrylamide gel. TCA-extracted peptides were subjected mass spec analysis that revealed similarity profiles according to GenBank blast. Proteins with score greater than 10 are listed here. Arrows in red point the most likely genes related to each of the excised proteins.

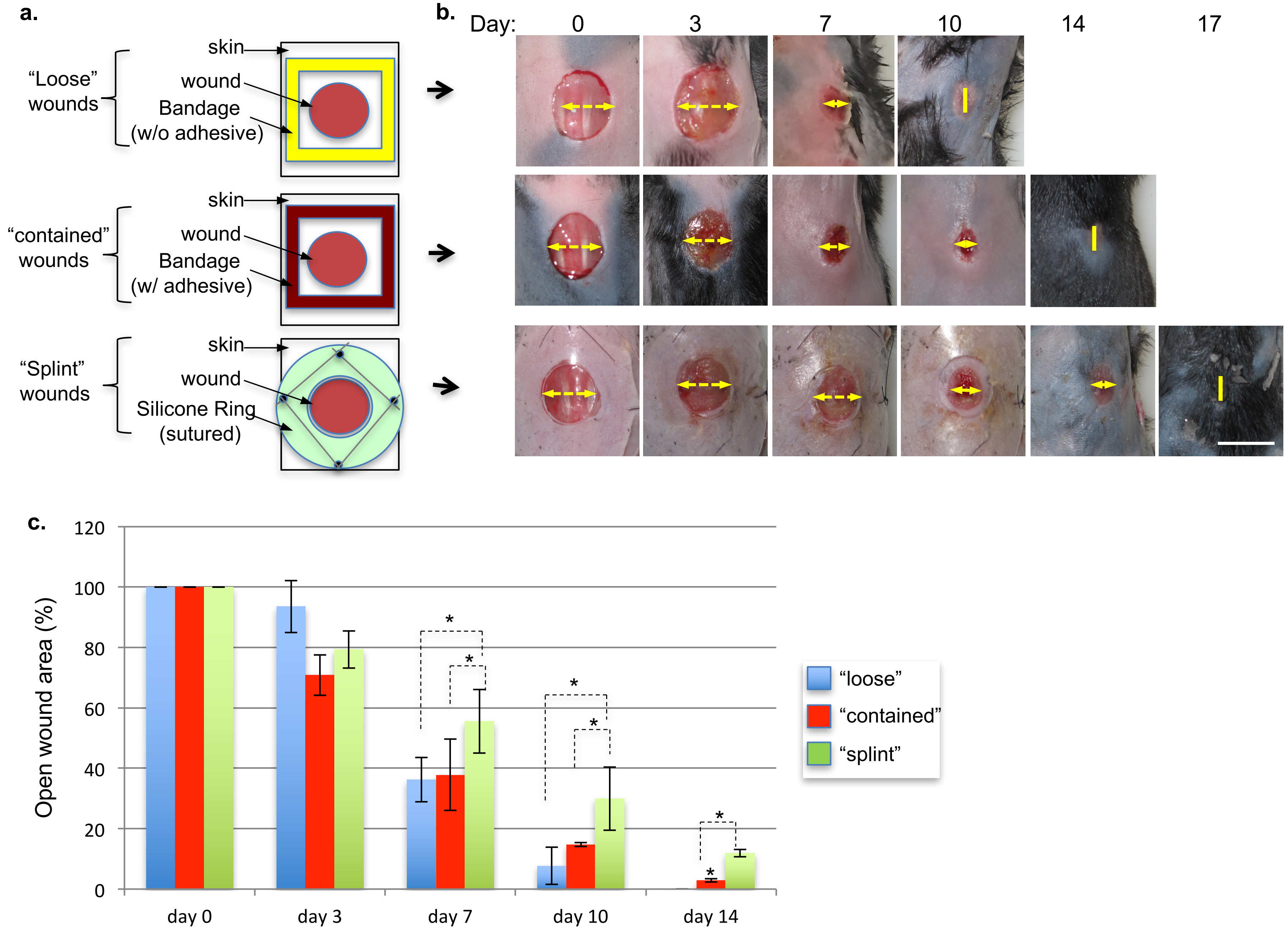
a.
"Loose" wounds
skin
wound
Bandage (w/o adhesive)
"contained" wounds
skin
wound
Bandage (w/ adhesive)
"Splint" wounds
skin
wound
Silicone Ring (sutured)
b.
Day:
0
3
7
10
14
17
c.
Open wound area (%)
120
100
80
60
40
20
0
day 0
day 3
day 7
day 10
day 14
*
"loose"
"contained"
"splint"

Figure 2s

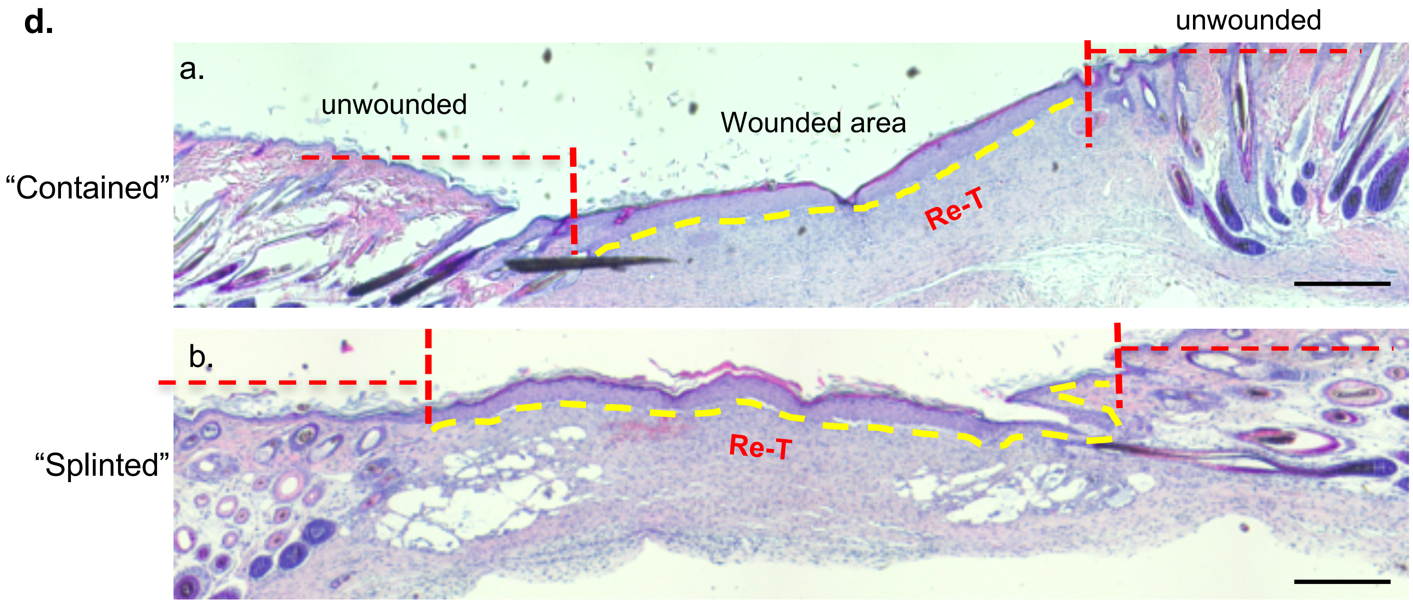

Figure 2s. Comparison of wound healing rate among three types of commonly used mouse wound models. (a) Schematic illustration of the three types of full-thickness excision wounds. (b) Images of 8 mm wound healing over time. (c) Quantitation of wound closure based on triplicates of a representative experiment. (d) Biopsies from 14-day "contained" wounds and 17-day "splinted" wounds were subjected H&E staining. Re-T, re-epithelialized tongue. These experiments were repeated three times. n = 3, * $p \leq 0.05$. Scale bar for b, 1 cm, for d, 0.4 mm