Figure S1. Electrophoresis image of protein samples. Lane M contains protein markers, whereas lanes 1-6 are original protein samples. Samples in lanes 1, 3 and 5 (Patient 1 before, Patient 2 before, Patient 3 before) were extracted from granulation tissues before NPWT and samples in lanes 2, 4, 6 (Patient 1 after, Patient 2 after, Patient 3 after) were extracted from granulation tissues following NPWT for 1 week. NPWT, negative-pressure wound therapy.

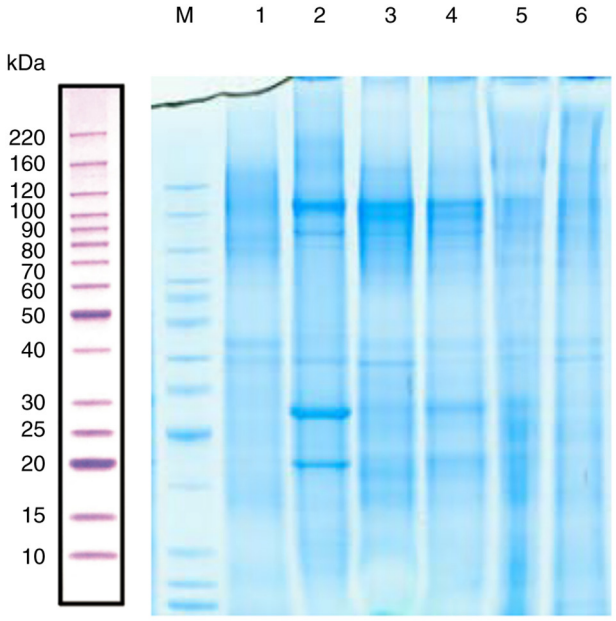

Figure S2. Validation of the protein expression levels of fibronectin. Foot granulation tissues from eight patients before and after NPWT were collected and subjected to western blotting. Primary antibodies against fibronectin were used to detect differences in protein levels. (A) Protein bands from four representative patients prior to NPWT (designated as minus symbol '-') and following NPWT (designated as plus symbol '+') are presented in the left panel. (B) Protein samples from the same patients were loaded next to each other. Then, eight pairs of protein samples from eight patients were analyzed by ImageJ and are depicted as paired dots in the middle panel. The gray level of the target protein was compared with the internal reference (β-actin) gray level to obtain the relative expression value. (C) The relative expression values of different proteins prior to and following NPWT are displayed by a bar graph (results are the mean ± SD). Paired t-tests were performed and significant changes are indicated by *P<0.001 vs. before NPWT group. NPWT, negative-pressure wound therapy.

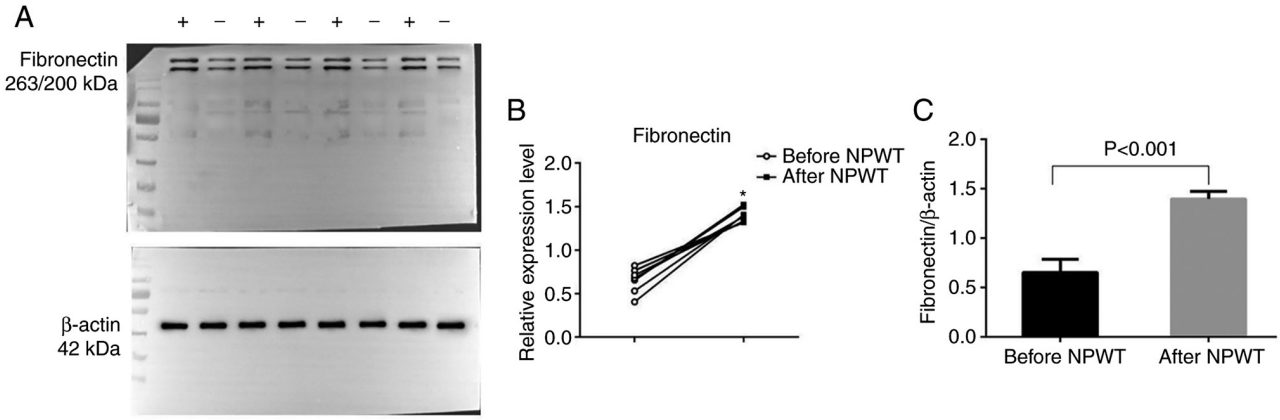

Figure S3. Validation of the protein expression levels of CTSS, PROS1, ITIH4 and PRDX2. Wound granulation tissues from four other patients with diabetic foot ulcers before and after NPWT (other than the four patients listed in Fig. 4) were collected and subjected to western blotting. Primary antibodies against (A) CTSS, (B) ITIH4, (C) PROS1 and (D) PRDX2 were used to detect differences in protein levels before NPWT (designated with a minus sign '-') and following NPWT (designated with a plus sign '+'). Protein samples from the same patient were loaded next to each other. CTSS, cathepsin; PROS1, protein S isoform 1; ITIH4, inter α-trypsin inhibitor heavy chain H4; PRDX2, peroxiredoxin-2; NPWT, negative-pressure wound therapy.

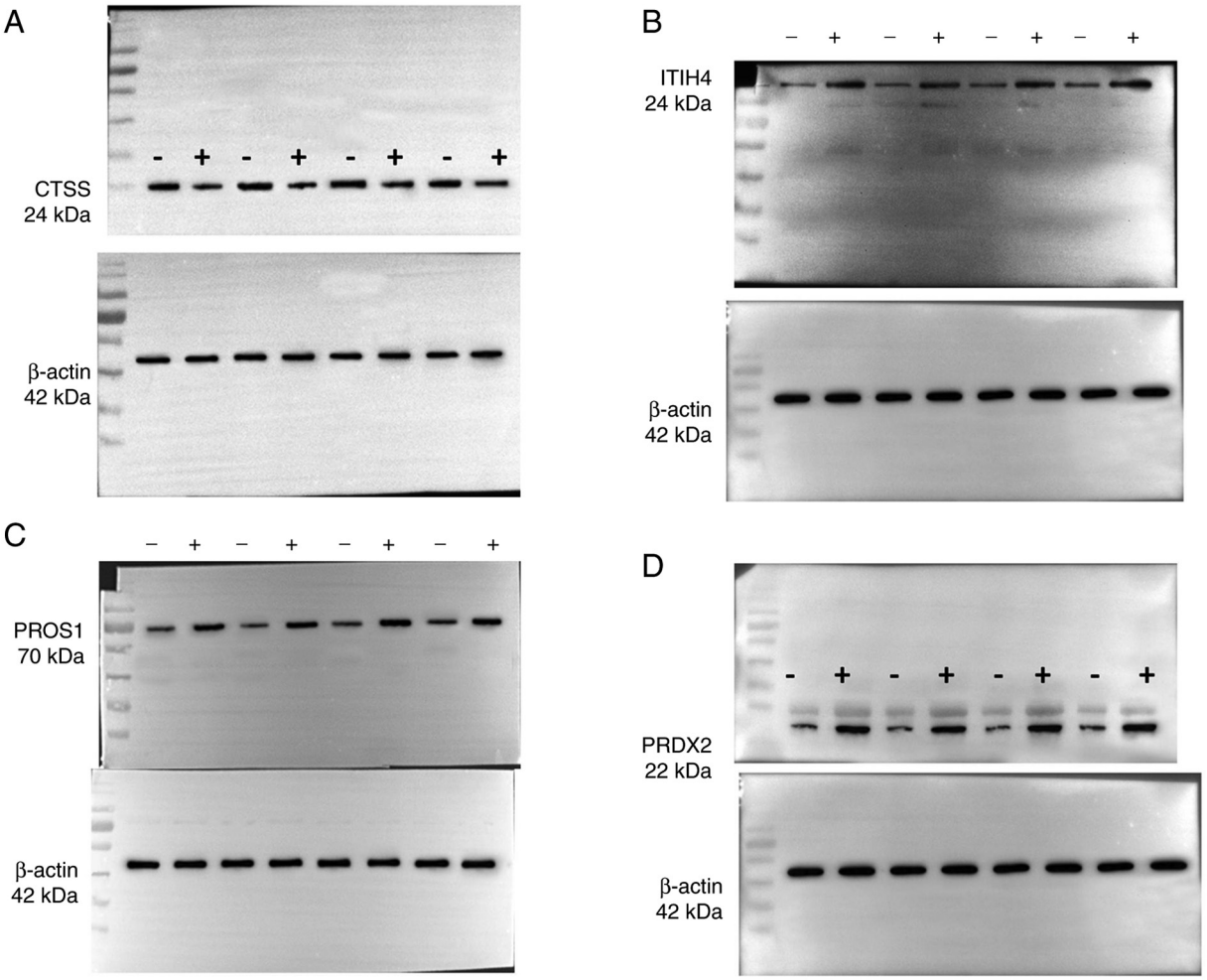

Figure S4. Detection of the activity of MMP2 and MMP9 by gelatin zymography. Granulation tissue samples from eight patients with diabetic foot ulcers prior to and following NPWT for 1 week were collected and the activity levels of (A) MMP-9 and (B) MMP-2 in granulation tissues were measured by gelatin zymography. Paired t-tests were performed. NPWT, negative-pressure wound therapy.

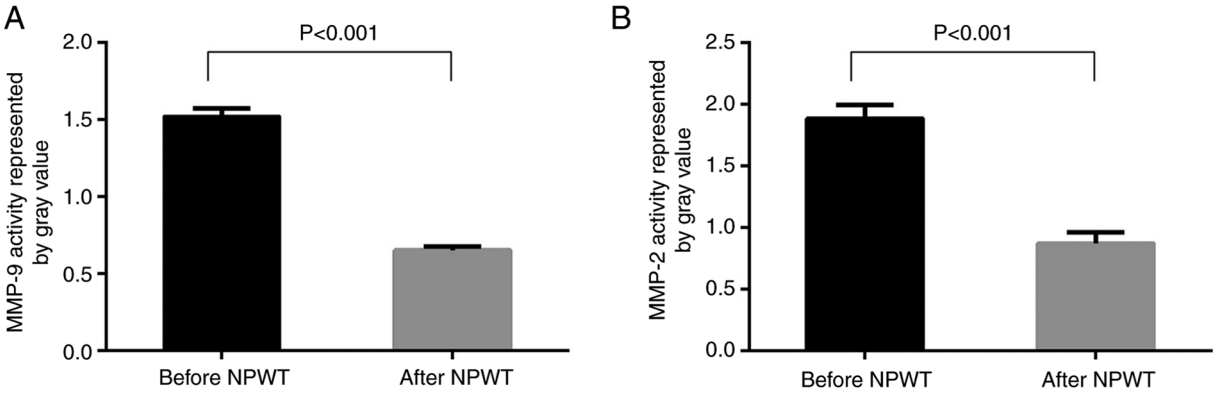

Table SI. Protein concentrations in samples from three patients who received label free quantitative mass spectrometry before and after negative-pressure wound therapy for 1 week.

Sample	Protein concentration, μg/μl	Total volume, μl	Total quantity, μg	Volume for electrophoresis, μl	Quality grade
Patient 1					
Before	2.93	400	1,173	11.93	A
After	2.26	400	906	15.46	A
Patient 2					
Before	3.62	400	1,449	9.66	A
After	3.13	400	1,254	11.17	A
Patient 3					
Before	1.67	350	586	20.92	A
After	2.61	400	1,046	13.39	A

A, passed the quality inspection, the protein band was clear, complete and uniform, the protein was not degraded and the total amount of protein could be satisfied ≥2 times.

Table SIII. List of differentially expressed proteins before and after negative-pressure wound therapy for 1 week (fold-change ≥1.2 or ≤0.7 difference).

UniProt ID	Symbol	Protein name	Unique peptides	Peptide spectral matches	Sequence coverage	Score SEQUEST HT	Group after, average ± SD	Group before, average ± SD	Fold-change, group after/ group before	P-value
A6XND5	N/A	Ribonuclease T2	2	2	8	24.31	557,012.94± 62,001.65	1,431,626.92± 324,985.50	0.39	0.0464
Q32MZ4	LRRFIP1	Leucine-rich repeat flightless-interacting protein 1	5	5	12	24.56	621,341.23± 236,015.69	998,421.33± 88,009.54	0.62	0.0424
P25774	CTSS	Cathepsin S	7	7	29	64.34	8,066,671.50± 351,671.53	12,681,873.58± 1,354,147.96	0.64	0.0236
Q96TA1	FAM129B	Niban-like protein 1	4	4	10	21.43	2,268,520.16± 71,020.03	1,833,576.62± 1,054,772.86	1.24	0.0042
B2RMS9	ITIH4	Inter-α-trypsin inhibitor heavy chain H4	24	24	32	318.95	87,069,011.06± 16,128,237.94	68,534,836.79± 26,363,291.92	1.27	0.0305
P27824	CANX	Calnexin	16	16	30	325.05	75,348,397.66± 2,939,805.79	59,183,631.89± 8,300,527.87	1.27	0.0305
Q96PD5	PGLYRP2	N-acetylmuramoyl-L-alanine amidase	8	8	24	66.24	3,815,843.05± 300,505.19	2,841,144.72± 772,719.35	1.34	0.0361
Q5HYM3	DKFZp686C0249	Oxysterol-binding protein	3	3	3	8.14	976,174.16± 181,395.25	707,457.33± 128,669.12	1.38	0.0370
O94905	ERLIN2	Erlin-2	5	5	17	34.17	9,068,423.17± 379,728.69	6,231,080.83± 955,013.62	1.46	0.0232
Q96QK1	VPS35	Vacuolar protein sorting-associated protein 35	12	12	18	94.35	15,226,381.13± 129,474.46	9,608,542.04± 1,554,550.60	1.58	0.0254
P02649	APOE	Apolipoprotein E	19	19	58	282.04	132,119,591.70± 8,510,223.28	80,400,064.01± 29,121,302.81	1.64	0.0265
J3KPF3	SLC3A2	4F2 cell-surface antigen heavy chain	5	5	12	51.64	4,717,363.07± 751,357.59	2,789,432.41± 884,032.39	1.69	0.0362
P27169	PON1	Serum paraoxonase/arylesterase 1	4	4	11	28.29	5,978,831.69± 1,163,530.89	3,488,327.46± 1,878,285.49	1.71	0.0442
R4GMU1	H6PD	GDH/6PGL endoplasmic bifunctional protein	2	2	2	4.99	2,659,571.85± 726,641.33	1,482,982.36± 629,914.33	1.79	0.0124
D3DP46	SPCS3	Signal peptidase complex subunit 3	3	3	18	22.81	6,349,524.27± 446,570.17	3,538,589.04± 160,053.26	1.79	0.0129
Q6ZNJ1	NBEAL2	Neurobeachin-like protein 2	4	4	2	18.99	1,100,566.49± 396,842.86	610,782.77± 59,744.60	1.80	0.0081
C8C504	HBB	β-globin	2	18	90	2,370.14	2,827,043,3030.00± 181,608,580.00	15,555,683,691.00± 5,239,372,641.08	1.82	0.0203
P54709	ATP1B3	Sodium/potassium-transporting ATPase subunit β-3	4	4	18	33.89	4,043,601.23± 612,672.31	2,015,560.42± 1,117,886.11	2.01	0.0321

Table SIII. Continued.

UniProt ID	Symbol	Protein name	Unique peptides	Peptide spectral matches	Sequence coverage	Score SEQUEST HT	Group after, average ± SD	Group before, average ± SD	Fold-change, group after/ group before	P-value
Q9Y277	VDAC3	Voltage-dependent anion-selective channel protein 3	7	8	26	111.11	19,767,066.19± 1,862,700.59	9,796,914.48± 1,503,182.10	2.02	0.0098
V9HW21	HEL-76	Epididymis luminal protein 76	10	10	51	179.00	74,243,761.91± 1,134,145.51	34,800,784.62± 15,872,894.65	2.13	0.0199
A5PL32	APOL1	APOL1 protein	3	3	9	17.92	3,002,596.36± 48,821.68	1,335,697.00± 695,600.58	2.25	0.0248
A0A0S2Z4L3	PROS1	Protein S isoform 1	13	13	23	176.93	32,768,921.47± 6,634,685.59	14,474,664.70± 4,911,785.95	2.26	0.0477
P32119	PRDX2	Peroxiredoxin-2	14	15	71	482.97	270,556,638.70± 10,511,743.15	109,068,801.70± 59,316,218.38	2.48	0.0179
M0QXB5	ETHE1	Persulfide dioxygenase ETHE1, mitochondrial	3	3	14	9.00	5,307,099.09± 1,160,690.86	2,134,016.72± 184,626.69	2.49	0.0136
A0A024R5H8	RAB6A	RAB6A, member RAS oncogene family, isoform CRA_b	3	4	27	43.72	2,333,473.00± 33,254.72	926,057.74± 399,487.11	2.52	0.0114
B4DJ38	N/A	cDNA FLJ56092, highly similar to Pentatricopeptide repeat protein 1	2	2	3	26.38	2,793,258.53± 522,296.07	1,107,095.92± 201,523.33	2.52	0.0327
Q59GX2	N/A	Solute carrier family 2 (Facilitated glucose transporter), member 1 variant	3	4	8	57.41	11,660,008.39± 2,326,958.04	4,364,300.99± 2,889,228.12	2.67	0.0031
P20851	C4BPB	C4b-binding protein β chain	4	4	18	23.13	4,415,318.10± 708,342.46	1,600,820.80± 323,582.09	2.76	0.0184
Q59FP5	N/A	Spectrin, β, erythrocytic (includes spherocytosis, clinical type I) variant	54	55	31	492.77	112,325,140.60± 585,967.99	38,919,300.82± 23,045,744.25	2.89	0.0452
P02549	SPTA1	Spectrin α chain, erythrocytic 1	64	64	37	608.40	122,563,447.50± 1,253,4709.08	40,346,596.05± 30,671,204.40	3.04	0.0318
E2RVJ0	SLC4A1	Anion exchange protein	19	19	30	352.06	193,868,754.50± 56,105,542.44	59,502,751.08± 31,645,326.52	3.26	0.0334
A8K2X4	N/A	cDNA FLJ75401, highly similar to Homo sapiens endoglin (Osler-Rendu-Weber syndrome 1), mRNA	2	2	3	14.04	3,168,604.09± 778,562.04	885,229.99± 153,183.20	3.58	0.0383
Q8NE71	ABCF1	ATP-binding cassette sub-family F member 1	2	2	3	23.77	796,805.90± 16,024.40	220,298.29± 21,623.43	3.62	0.0228
P16452	EPB42	Erythrocyte membrane protein band 42	13	14	25	112.48	20,174,064.87± 4,930,507.74	3,422,142.07± 3,852,422.45	5.90	0.0026

Table SIII. Continued.

UniProt ID	Symbol	Protein name	Unique peptides	Peptide spectral matches	Sequence coverage	Score SEQUEST HT	Group after, average ± SD	Group before, average ± SD	Fold-change, group after/ group before	P-value
Q9H4B7	TUBB1	Tubulin β-1 chain	4	8	16	292.15	2,597,526.31± 760,821.00	432,204.24± 311,802.49	6.01	0.0212
O43236	SEPTIN4	Septin-4	2	2	5	9.67	911,032.15± 53,682.90	127,084.98± 10,822.76	7.17	0.0348

N/A, not applicable.

Table SVIII. Comparison of serum CTSS, ITIH4, PROS1 and PRDX2 levels before NPWT and after NPWT for 1 week.

Variables	Before NPWT	After NPWT	P-value
CTSS, pg/ml	4,650.51±1,139.60	4,133.26±1,145.33	0.027
ITIH4, pg/ml	48.90±29.11	62.87±32.27	<0.001
PROS1, μg/ml	4,804.52±1,181.70	6,267.02±1,351.90	<0.001
PRDX2, ng/ml	35.56±13.90	52.85±13.81	0.003

Data are presented as the mean ± SD. NPWT, negative-pressure wound therapy; CTSS, cathepsin S; ITIH4, inter α-trypsin inhibitor heavy chain H4; PROS1, protein S isoform 1; PRDX2, peroxiredoxin-2.