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Supplemental Information for

Triggering of a Dll4-Notch1 loop impairs wound healing in diabetes

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This PDF file includes:

Section 1:

Gene Lists of Mouse Notch Signaling Target RT^2 Profiler PCR Array

Cell Fate Commitment
Hes1, Jag1, Runx2, Sox9.

Developmental Processes
Embryonic Development: Edn1, Efnb1, Foxc1, Hes1, Pbx1, Pcdh8, Pdgfra, Pdgfrb, Rbpj, Tcf15, Vegfa, Wnt4, Wnt5a.
Muscle Development: Edn1, Egr3, Foxc1, Hbegf (Dtr), Hey2, Igfbp3, Jag1, Pdgfb, Pdgfrb, Tnc.
Epidermis Development: Jag1, Krt14, Ptgs2 (COX2), Sox9, Tcf15.
Immune System Development: Id2, Jag1, Jun, Kitl (SCF), Pbx1, Runx1 (AML1), Sgpl1, Vegfa.
Nervous System Development: Bdnf, Ccnd2, Efna1, Efnb1, Egr3, Fabp7, Foxc1, Hes1, Hey1, Hey2, Heyl, Id3, Id4, Jag1, Kalrn, Nes, Pbx1, Rbpj, Rnd1, Vegfa.
Vasculature Development: Edn1, Efna1, Flt1 (VEGFR1), Foxc1, Hey2, Id1, Jag1, Jun, Rbpj, Runx1 (AML1), Sgpl1, Vegfa.
Kidney Development: Adamts1, Bdnf, Foxc1, Pbx1, Pdgfrb, Sgpl1.
Other Developmental Processes: Nrarp, S1pr3.

Signal Transduction
Notch Signaling: Crebbp (CBP), Foxc1, Hes1, Hey1, Hey2, Heyl, Jag1, Lfng, Rbpj, Snw1 (Skiip).
WNT Signaling: Ccnd1, Frzb (FRP-3), Fzd5, Wisp1, Wnt4, Wnt5a, Wnt6.

Apoptosis
Abl1, Bdnf, Foxc1, Gadd45b, Id3, Igfbp3, Jun, Kalrn, Ptgs2 (COX2), Sgpl1, Socs3, Sox9, Vegfa.

Cytokines
Cx3cl1, Cxcl12 (Sdf1), Cxcl1 (Gro1), Il33, Nampt.

Transcription Factors
Cbfa2t3, Clock, Crebbp (CBP), Egr3, Foxc1, Hey1, Hey2, Heyl, Jun, Msc, Pbx1, Rbpj, Runx1 (AML1), Runx2, Sox9, Tcf15.

Other Notch Signaling Targets
Fjx1, Gpsm2, Hspb8, Mark1, Rhov, Tec.

Section 2:

Primers used for SYBR green quantitative real-time PCR

Primer name: DNA sequence (5'->3'):

Mouse *Hey1*_F GCG CGG ACG AGAATG GAAA
Mouse *Hey1*_R TCA GGT GAT CCA CAG TCA TCT G
Mouse *Dll1*_F CCA AGT GCC AGT CAC AGA G
Mouse *Dll1*_R GCT TCC ATC TTA CAC CTC AGT C
Mouse *Dll4*_F TTC CAG GCAACC TTC TCC GA
Mouse *Dll4*_R ACT GCC GCT ATT CTT GTC CC
Mouse *Hes1*_F CCA GCC AGT GTC AAC ACG A
Mouse *Hes1*_R AAT GCC GGG AGC TAT CTT TCT
Mouse *PDGFRβ*_F AAG TGT GAG ACAATA GTG ACC CC
Mouse *PDGFRβ*_R CAT GGG TGT GCT TAAACT TTC G
Mouse *PBGD*_F GTG TTG CAC GAT CCT GAAACT
Mouse *PBGD*_R GTT GCC CAT CCT TTA TCA CTG TA
Mouse *SDF1*-F GAG AGC CAC ATC GCC AGA G
Mouse *SDF1*-R TTT CGG GTC AAT GCA CAC TTG
Mouse *CXCR4*-F AGC ATG ACG GAC AAG TAC C
Mouse *CXCR4*-R GAT GAT ATG GAC AGC CTT ACA C
Mouse *B2M*_F TTC TGG TGC TTG TCT CAC TGA
Mouse *B2M*_R CAG TAT GTT CGG CTT CCC ATT C
Mouse *Jagged1*_F CCT CGG GTC AGT TTG AGC TG
Mouse *Jagged1*_R CCT TGA GGC ACA CTT TGA AGT A
Mouse *Jagged2*_F CAC CAA TGA CTG CAA CCC T
Mouse *Jagged2*_R CGT GAA TAT GAC CAC TTC CTG
Mouse *VEGFR2*_F TTT GGC AAA TAC AAC CCT TCA GA
Mouse *VEGFR2*_R GCA GAA GAT ACT GTC ACC ACC
Mouse *VEGFR3*_F CTG GCA AAT GGT TAC TCC ATG A
Mouse *VEGFR3*_R ACA ACC CGT GTG TCT TCA CTG
Mouse *Notch1*_F CCA GCA GAT GAT CTT CCC GTA C
Mouse *Notch1*_R ACT GCC GCT ATT CTT GTC CC
Mouse *Notch2*_F ATG TGG ACG AGT GTC TGT TGC
Mouse *Notch2*_R GGA AGC ATA GGC ACA GTC ATC
Mouse *Notch3*_F TGC CAG AGT TCA GTG GTG G
Mouse *Notch3*_R CAC AGG CAA ATC GGC CAT C
Mouse *Notch4*_F ACG AGA GTA CCA CTC ATT CGG
Mouse *Notch4*_R TAA GAC CAA TGT TGT CCT CGC
Mouse *KRT14*_F CCA GGA GAT GAT CGG CAG
Mouse *KRT14*_R GAA GAT GAA AGG TGG GCG T
Mouse *β-actin*_F AAG ATC AAG ATC ATT GCT CCT C
Mouse *β-actin*_R GGA CTC ATC GTA CTC CTG
Human *Notch1*_F GAG GCG TGG CAG ACT ATG C
Human *Notch1*_R CTT GTA CTC CGT CAG CGT GA
Human *Notch2*_F GCC TTC GCC TCC TGT ACT C
Human *Notch2*_R GCC CAT TTA GGG GGT TGG T
Human *Notch3*_F ACA CAG GGC CAC TAT GTG AGA
Human *Notch3*_R CAC AGT CGT AAG TGA GGT CGC
Human *Notch4*_F GGG TGA GAC GTG CCA GTT TC

Human *Notch4*_R CTG GGT GTC AAT GGA GAG GGA
Human *Dll1*_F TGC TGT GTC AGG TCT GGA G
Human *Dll1*_R CCT TCT TGT TGA CGA ACT CCT
Human *Dll4*_F AGC TGT AAG GAC CAG GAG
Human *Dll4*_R ACA TTC ACA AGC ATA GTT GG
Human *Jagged1*_F GTA ACA TAG CCC GAA ACA G
Human *Jagged1*_R ACC AGT TGT CTC CAT CCA
Human *Jagged2*_F CTC ACA ATA CCA ACG ACT G
Human *Jagged2*_R GAA CCC GAT CAC TTC CTG
Human *PBGD*_F AGG ATG GGC AAC TGT ACC
Human *PBGD*_R GTT TTG GCT CCT TTG CTC AG
Human *Hes1*_F CAC AGA AAG TCA TCA AAG CC
Human *Hes1*_R GTA TTA ACG CCC TCG CAC
Human *Hey1*_F TGA GTT CGG CTC TAG GTT CCA
Human *Hey1*_R GCG CTT CTC AAT TAT TCC TCT CC
Human *Hey2*_F GAG AAG ACT TGT GCC AAC TG
Human *Hey2*_R CGT CAA AGT AGC CTT TAC CC
Human *GNBL2*_F GAG TGT GGC CTT CTC CTC TG
Human *GNBL2*_R GCT TGC AGT TAG CCA GGT TC
Human *RPL32*_F CAT CTC CTT CTC GGC ATC A
Human *RPL32*_R AAC CCT GTT GTC AAT GCC TC
Rat *Hey1*_F TCG AGA AGC GCC GAC GAG ACC GA
Rat *Hey1*_R CAG CAG CGG GTG TGC GAT GTG TGG GT
Rat *Hes5*_F ACC GCA TCA ACA GCA GCA TT
Rat *Hes5*_R AGG CTT TGC TGT GCT TCA GG
Rat *PBGD*_F CAT GTA TGC TGT GGG TCA GG
Rat *PBGD*_R CAG GTA CAG TTG CCC ATC CT
Rat *B2 M*_F TCA CAC CCA CCG AGA CCG ATG T
Rat *B2 M*_R TCT CGG TCC CAG GTG ACG GTT T
Rat *Dll4*_F GAA ATT CAC TTA TCA GCC AA
Rat *Dll4*_R CAG GGG ATG GTG CAG GT
Rat *Jagged1*_F GCC CTG CGA GCC AAG GTG TG
Rat *Jagged1*_R CAC TCG TCG CGG GTG CAC TT

Section 3: Antibody information

Primary Antibodies for IHC and ICC			
Antibody	**Company**	**Catalog number**	**IHC/ICC dilution**
Dll4	Abcam	ab7280	1/200 (frozen)
Dll4	Novus Biologicals	NB600-892	1/100 (ICC)
Cleaved Notch1	Abcam	ab8925	1/200 (frozen), 1/100 (FFPE)
CD31	BD Biosciences	550274	1/200 (frozen)
CD31	Dianova	DIA310	1/100 (FFPE)
Ki67	Abcam	ab15580	1/1000 (frozen, FFPE)
PDGFRβ	Abcam	ab32570 (Y92)	1/50 (FFPE)
SDF-1	Abcam	ab25117	1/25 (FFPE)
VEGFR-2	R&D systems	AF644-SP	1/50 (FFPE)
VEGFR-3	R&D systems	AF743-SP	1/50 (FFPE)

Frozen: Frozen sections; ICC: immunocytochemistry

FFPE: Formalin-fixed paraffin embedded sections

Secondary Antibodies for IHC and ICC			
Antibody	**Company**	**Catalog number**	**IHC/ICC dilution**
Goat anti-Rabbit Alexa fluor 594	ThermoFisher Scientific	A11037	1/500
Goat anti-rat Alexa fluor 488	ThermoFisher Scientific	A11006	1/500
Donkey anti-goat Alexa fluor 488	ThermoFisher Scientific	A11055	1/500

Antibodies for western blotting			
Antibody	**Company**	**Catalog number**	**dilution**
Cleaved Notch1	Cell signaling	2421S	1/1000
Cleaved Notch1	Cell signaling	4147	1/500 – 1/200
Cleaved Notch2	Abcam	ab8926	1/500
Notch3 C-terminus	ThermoFisher	PA5-13203	1/500
Notch4 C-terminus	Millipore	07-189	1/500
Hes1	Abcam	ab71559	1/500
Myc	Cell Signaling	#2272S	1/500
PDGFRβ	Abcam	ab32570	1/5000
CXCR4	Abcam	ab124824	1/100
Beta-Actin	Sigma	A5441	1/5000
beta-actin	Abcam	ab8227	1/3000
IRDye 680RD Goat anti-rabbit	LI-COR	926-68071	1/20000
IRDye 800CW Goat anti-mouse	LI-COR	926-32210	1/20000

Section 4: Supplemental Methods

Laser microdissection followed by RNA purification and Quantitative RT-PCR

Frozen sections with 20µm thickness were obtained from KRT14-Cre;Notch1$^{fl/fl}$ mice or WT mice on RNase-free MMI Membrane Slides (Molecular Machines & Industries). Just before the laser microdissection, the slides were thawed and dehydrated in 70% ethanol for 30 seconds. The excess liquid was wiped away and the slide was immersed into Hemotoxylin containing 1U/µL RNase inhibitor (Applied Biosystems) for 30 seconds. The slides were then washed in 70% ethanol for 20 seconds, in RNase-free water for 10 seconds, counterstained with 0.2% Eosin containing 1U/µL RNase inhibitor for 10 seconds, washed in 70% ethanol and water and dried at room temperature.

The laser microdissection was performed immediately after staining, using the MMI CellCut Laser Microdissection system (Molecular Machines & Industries). Briefly, the MMI Membrane Slide with the tissue section was inverted onto a clean glass slide and placed in the holder of the CellCut system microscope. The laser power was adjusted according to the thickness and toughness of the tissue section and the epidermal and dermal tissues were dissected and captured into separate MMI Isolation Caps (Molecular Machines & Industries) and immediately frozen on dry ice. RNA was isolated from the tissue pieces using the ARCTURUS PicoPure RNA Isolation kit (ThermoFisher Scientific). The cDNA was prepared using the SuperScript IV First-Strand Synthesis System (ThermoFisher Scientific). Quantitative RT-PCR was performed on a 7300 Real-Time PCR System using SYBR Green Master Mix (ThermoFisher Scientific). The primer sequences are presented in Supporting Information 2. β-actin or keratin 14 (KRT14) was used as internal control.

Table S1: Gene list with fold change and p value in RT2 Profiler PCR array

Gene Symbol	Refseq	Description	Fold Change	T-TEST
			db/db/Control	p value
Abl1	NM_009594	C-abl oncogene 1, non-receptor tyrosine kinase	4.36	0.0804
Adamts1	NM_009621	A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 1	2.36	0.1114
Bdnf	NM_007540	Brain derived neurotrophic factor	241.62	0.0160
Cbfa2t3	NM_009824	Core-binding factor, runt domain, alpha subunit 2, translocated to, 3 (human)	10.16	0.0452
Ccnd1	NM_007631	Cyclin D1	105.59	0.0995
Ccnd2	NM_009829	Cyclin D2	20.99	0.0114
Clock	NM_007715	Circadian locomotor output cycles kaput	4.24	0.0750
Crebbp	NM_001025432	CREB binding protein	11.97	0.0828
Cx3cl1	NM_009142	Chemokine (C-X3-C motif) ligand 1	8.37	0.0618
Cxcl1	NM_008176	Chemokine (C-X-C motif) ligand 1	305.77	0.0468
Cxcl12	NM_021704	Chemokine (C-X-C motif) ligand 12	18.69	0.0006
Edn1	NM_010104	Endothelin 1	2.84	0.0417
Efna1	NM_010107	Ephrin A1	4.35	0.0202
Efnb1	NM_010110	Ephrin B1	1.37	0.3015
Egr3	NM_018781	Early growth response 3	1.45	0.1763
Fabp7	NM_021272	Fatty acid binding protein 7, brain	285.56	0.0501
Fjx1	NM_010218	Four jointed box 1 (Drosophila)	6.41	0.0678
Flt1	NM_010228	FMS-like tyrosine kinase 1	23.16	0.0484
Foxc1	NM_008592	Forkhead box C1	1.6	0.2185
Frzb	NM_011356	Frizzled-related protein	16.63	0.0211
Fzd5	NM_022721	Frizzled homolog 5 (Drosophila)	157.19	0.0924
Gadd45b	NM_008655	Growth arrest and DNA-damage-inducible 45 beta	2.42	0.0650
Gpsm2	NM_029522	G-protein signalling modulator 2 (AGS3-like, C. elegans)	5.26	0.0664
Hbegf	NM_010415	Heparin-binding EGF-like growth factor	22.82	0.0064
Hes1	NM_008235	Hairy and enhancer of split 1 (Drosophila)	12.85	0.0054
Hey1	NM_010423	Hairy/enhancer-of-split related with YRPW motif 1	12.58	0.1451
Hey2	NM_013904	Hairy/enhancer-of-split related with YRPW motif 2	13.66	0.0209
Heyl	NM_013905	Hairy/enhancer-of-split related with YRPW motif-like	2.59	0.1186
Hspb8	NM_030704	Heat shock protein 8	6.67	0.0225
Id1	NM_010495	Inhibitor of DNA binding 1	-1.4	0.4581
Id2	NM_010496	Inhibitor of DNA binding 2	8.5	0.0016
Id3	NM_008321	Inhibitor of DNA binding 3	-1.27	0.9151
Id4	NM_031166	Inhibitor of DNA binding 4	54.57	0.0418
Igfbp3	NM_008343	Insulin-like growth factor binding protein 3	726.3	0.0044
Il33	NM_133775	Interleukin 33	8.98	0.0046
Jag1	NM_013822	Jagged 1	2.82	0.1440
Jun	NM_010591	Jun oncogene	5.52	0.0318
Kalrn	NM_001164268	Kalirin, RhoGEF kinase	2.67	0.1358
Kitl	NM_013598	Kit ligand	1.03	0.6487
Krt14	NM_016958	Keratin 14	2.57	0.0183
Lfng	NM_008494	LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase	24.14	0.0181
Mark1	NM_145515	MAP/microtubule affinity-regulating kinase 1	38.27	0.0578
Msc	NM_010827	Musculin	711.85	0.0322
Nampt	NM_021524	Nicotinamide phosphoribosyltransferase	16.44	0.0018
Nes	NM_016701	Nestin	14.76	0.0534
Nrarp	NM_025980	Notch-regulated ankyrin repeat protein	3.51	0.2010
Pbx1	NM_183355	Pre B-cell leukemia transcription factor 1	3.09	0.0014
Pcdh8	NM_021543	Protocadherin 8	49.32	0.2669
Pdgfb	NM_011057	Platelet derived growth factor, B polypeptide	7.06	0.0217
Pdgfra	NM_011058	Platelet derived growth factor receptor, alpha polypeptide	3.52	0.0107
Pdgfrb	NM_008809	Platelet derived growth factor receptor, beta polypeptide	4.59	0.0331
Ptgs2	NM_011198	Prostaglandin-endoperoxide synthase 2	14.04	0.1141
Rbpj	NM_009035	Recombination signal binding protein for immunoglobulin kappa J region	3.27	0.0856
Rhov	NM_145530	Ras homolog gene family, member V	1.62	0.2064
Rnd1	NM_172612	Rho family GTPase 1	38.06	0.0493
Runx1	NM_009821	Runt related transcription factor 1	3.52	0.0259
Runx2	NM_009820	Runt related transcription factor 2	5	0.0751

S1pr3	NM_010101	Sphingosine-1-phosphate receptor 3	2.13	0.1433
Sgpl1	NM_009163	Sphingosine phosphate lyase 1	1.1	0.4541
Snw1	NM_025507	SNW domain containing 1	9.26	0.0177
Socs3	NM_007707	Suppressor of cytokine signaling 3	4.36	0.0793
Sox9	NM_011448	SRY-box containing gene 9	20.25	0.4049
Tcf15	NM_009328	Transcription factor 15	214.77	0.0133
Tec	NM_001113460	Tec protein tyrosine kinase	3.99	0.0167
Tnc	NM_011607	Tenascin C	-1.02	0.5946
Vegfa	NM_009505	Vascular endothelial growth factor A	19.92	0.0152
Wisp1	NM_018865	WNT1 inducible signaling pathway protein 1	5.07	0.0448
Wnt4	NM_009523	Wingless-related MMTV integration site 4	1.53	0.2209
Wnt5a	NM_009524	Wingless-related MMTV integration site 5A	2.44	0.1311
Wnt6	NM_009526	Wingless-related MMTV integration site 6	8.01	0.0627

Table S2: Information of the patients with diabetes and Controls

Donor	Group	Gender	Age	HbA1c (mmol/mol)	Medication	Diagnosis	Duration of diabetes (years)
01	Diabetes	M	81	45	Insulin aspart, Insulin aspart protamin, acetylsalicylic acid, Imatinip, Bisoprolol, Furosemide, Fluoxacillin.	T2DM, DFU, Dyslipidemia, TIA, CAD, CML	35
03	Diabetes	M	77	63	Glipizide, Insulin aspart, Insulin aspart protamine, Prednisolone.	T2DM, DFU, Hypertension, COPD	13
04	Diabetes	F	57	63	Flucloxacillin, Sitagliptin, Repaglinide, Furosemide, Felodipine, Enalapril, Atorvastatin, Oxycondone	T2DM, DFU, Osteomyelitis, Chronic Charcot foot	10
05	Diabetes	M	60	74	Metformin, Insulin Apart, Insulin Glargine, Atenolol, Lisinopril, Fluoxacillin.	T2DM, DFU, Hypertension	15
06	Diabetes	M	66	45	Metformin, Insulin Apart, Insulin Glargine, Atenolol, Lisinopril, Fluoxacillin.	T2DM, DFU, Hypertension, Renal failure stage 3, TIA	15
07	Diabetes	M	70	51	Insulin Glargine	T2DM, DFU, Renal failure stage 3	13
22	Diabetes	M	69	-	Insulin Glargine, Metformin	T2DM, DFU, Hypertension, Dyslipidemia	19
23	Diabetes	M	86	61	Insulin aspart, Insulin aspart protamine	T2DM, DFU, Hypertension,	25
27	Diabetes	M	64	79	Insulin Glargine, Insulin Aspart, Acetylsalicylic acid, Furosemid, Metoprolol, Enalapril, Simvastatin, Ciprofloxacine	Dyslipidemia, Atrial fibrillation, TIA, T2DM, DFU, Hypertension, Dyslipidemia, Renal failure stage 3, COPD	31
30	Control	F	74	41	Enalapril, Felodipin, Bisoprolol, Atorvastatin, L-Thyroxine	Hypertension, Goiter, Breast cancer	-
31	Control	M	76	37	-		-
32	Control	F	58	35	Candesartan	Hypertension	-
33	Control	F	54	37	-	-	-
34	Control	F	71	44	L-Thyroxine	Hypothyreoidism	-
35	Control	M	71	41	Enalapril	Hypertension	-

36	Control	M	83	41		-	-
37	Control	F	74	43	Glucosamine	Osteoarthritis,	-
38	Control	M	48	36	D vitamin	-	-
39	Control	M	62	37	-	-	-
40	Control	F	73	39	-	Irritable bowel syndrome (IBS)	-
41	Control	F	67	44	Atorvastatin, Losartan.	Hypertension. Dyslipidemia,	-
42	Control	M	74	40	-	Benign prostatic hyperplasia (BPH)	-

T2DM: Type 2 Diabetes
DFU: Diabetes foot ulcer
TIA: Transcient ischemic attack
CAD: Chronic coronary artery disease
COPD: Chronic obstructive pulmonary disease

Supplemental figures and figure legends

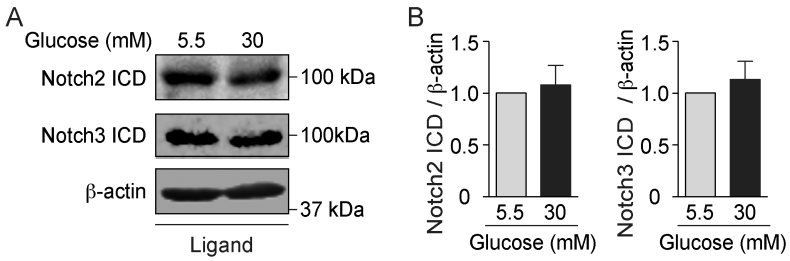

Fig. S1 (related to Fig. 3). High glucose levels do not affect the expression of Notch2 ICD or Notch3 ICD. (*A-B*) Primary human keratinocytes were exposed to 5.5 mM or 30 mM glucose for 24 h. Endogenous Notch2 ICD and Notch3 ICD protein levels were detected by Western blotting. Quantification is shown in panel *B* (n=4).

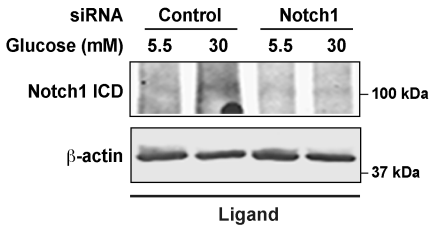

Fig. S2 (related to Fig. 3). The stimulatory effect of high glucose levels on Notch1 ICD expression is abolished by siRNA targeting Notch1. Primary human keratinocytes were exposed to 5.5 mM or 30 mM glucose for 24 h after transfection with control siRNA or siRNA targeting Notch1. Endogenous Notch1 ICD and β-actin protein levels were detected by Western blotting.

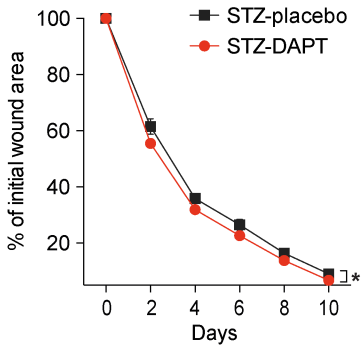

Fig. S3 (related to Fig. 4). Local inhibition of Notch signaling improves wound healing in STZ-induced diabetic mice. Full-thickness wounds were made on the dorsum of STZ-induced diabetic mice, and the wounds were treated locally with γ-secretase inhibitors DAPT (100μM) or DMSO (placebo) every second day. The wound healing rate was analyzed and shown as the percentage of the initial wound area (n=5-6). *, $P<0.05$ analyzed by Two-way ANOVA.

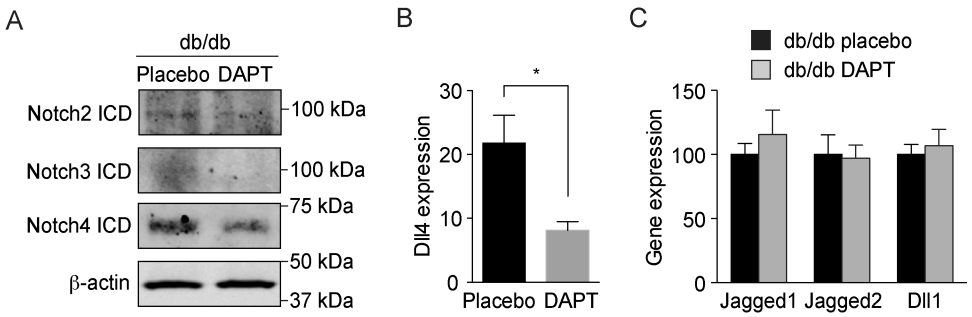

Fig. S4 (related with Fig. 4): Effects of DAPT on the expression of other Notch receptors and ligands in diabetic wounds. Full-thickness wounds were made on the dorsum of db/db mice, and the wounds were treated locally with γ-secretase inhibitor DAPT (100μM) or DMSO (placebo). **(*A*)** Western blotting results showing the expression of Notch2 ICD, Notch3 ICD, Notch4 ICD and β-actin in the wounds. **(*B*)** Dll4 expression was evaluated using fluorescent immunohistochemistry and the quantification results are shown (n=8-10). *, $P<0.05$. **(*C*)** Gene expression of Jagged1, Jagged2 and Dll1 were analyzed by QPCR (n=7-8).

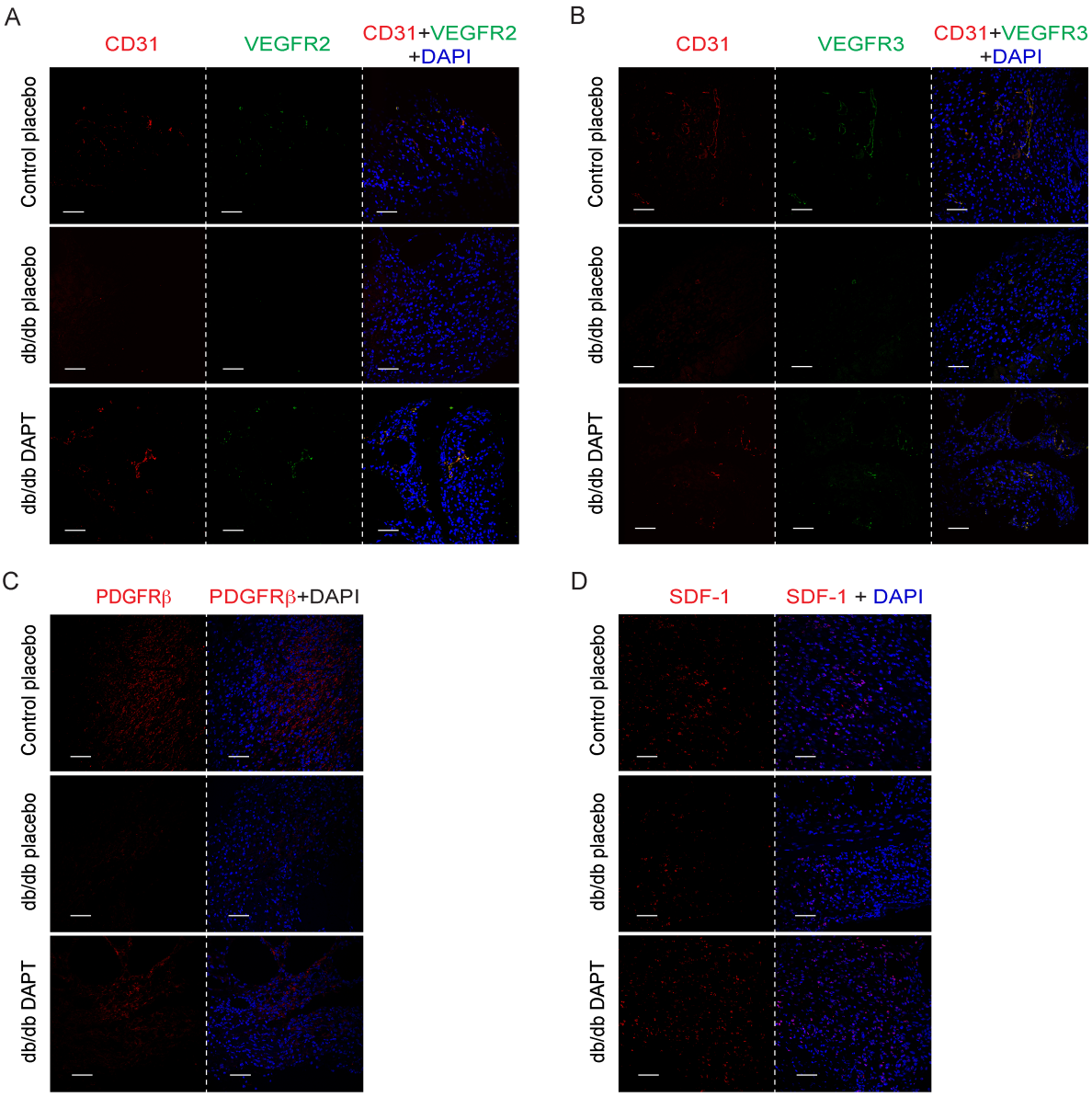

Fig. S5 (related to Fig. 4). Inhibition of Notch signaling improves angiogenesis in diabetic mice. (*A-B*) Representative images of immunohistochemcal staining for CD31 (red), VEGFR2 (green), VEGFR3 (green), DAPI (blue) and merged images. **(*C-D*)** Representative images of immunohistochemistry for PDGFRβ (red), SDF-1 (red), DAPI (blue) and merged images.

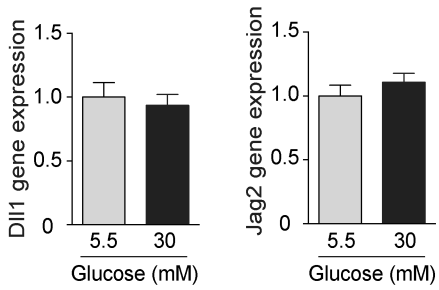

Fig. S6 (related to Fig. 5). High glucose levels do not affect the gene expression of *Dll1* or *Jagged2*. Relative Dll1 and Jagged2 (Jag2) gene expression in human primary keratinocytes exposed to 5.5 mM or 30 mM glucose (n=7).

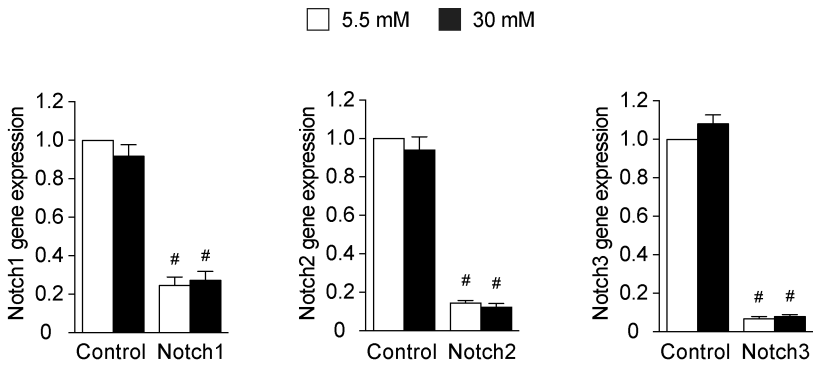

Fig. S7 (related with Fig. 5): RNA interference efficacy in primary human keratinocytes. Relative Notch1, Notch2, and Notch3 mRNA levels in keratinocytes transfected with siRNA for *Notch1*, *Notch2*, *Notch3* or control siRNA (n=4-5). **#,** $P<0.05$ compared with the corresponding Control siRNA.

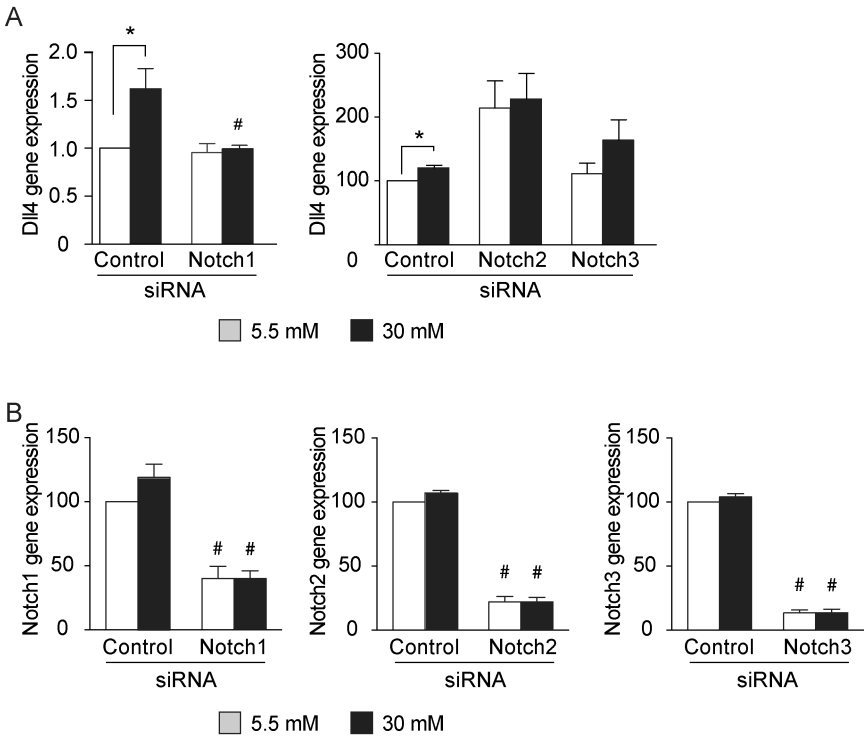

Fig. S8 (related with Fig. 5): Induction of Dll4 by high glucose levels is dependent on Notch1 in keratinocytes – results from another set of siRNA. (*A* and *B*) Relative Dll4, Notch1, Notch2, and Notch3 mRNA levels in keratinocytes transfected with siRNA for *Notch1*, *Notch2*, *Notch3* or control siRNA (n=3). *, $P<0.05$ compared with cells exposed to 5.5 mM glucose; #, $P<0.05$ compared with the corresponding Control siRNA.

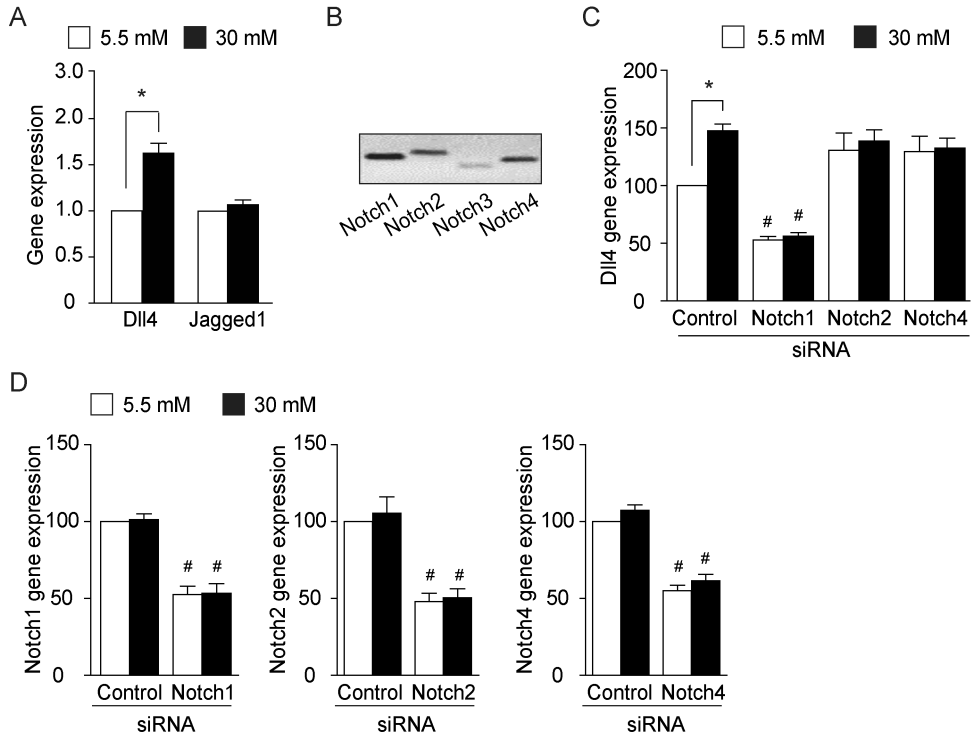

Fig. S9 (related with Fig. 5): Induction of Dll4 by high glucose levels is dependent on Notch1 in HDMEC. (*A*) Relative Dll4 and Jagged1 gene expression in HDMECs exposed to 5.5 mM or 30 mM glucose (n=5). **(*B*)** Agarose gel image showing the gene expression of Notch1, 2, 3, and 4 in HDMEC. **(*C* and *D*)** Relative Dll4, Notch1, Notch2, and Notch4 mRNA levels in HDMECs transfected with siRNA for *Notch1*, *Notch2*, *Notch4* or control siRNA (n=3). *, $P<0.05$ compared with cells exposed to 5.5 mM glucose; **#,** $P<0.05$ compared with the corresponding Control siRNA.

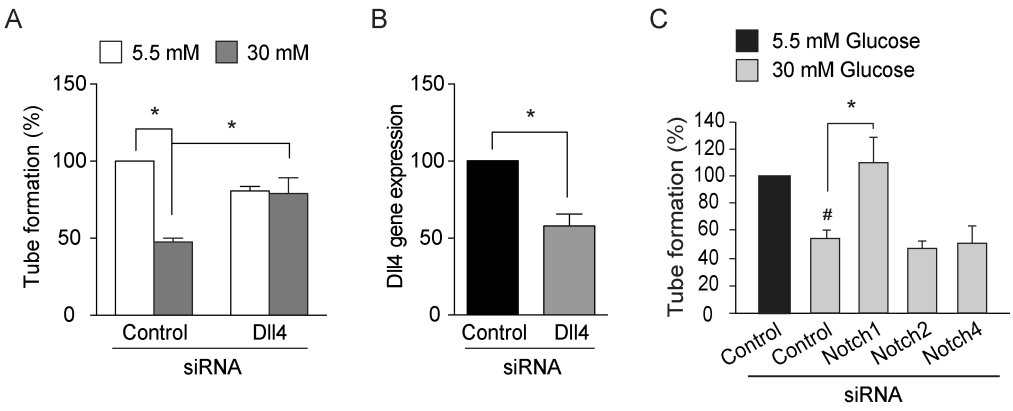

Fig. S10 (related with Fig. 5): High glucose levels inhibit angiogenesis via activation of a positive Dll4-Notch1 feedback loop. HDMECs were transfected with Control siRNA or siRNA silencing Dll4, Notch1, Notch 2, or Notch 4. The cells were cultured in medium containing 5.5 mM or 30 mM glucose for 24 h prior to the *in vitro* angiogenesis assay wherein tube formation was evaluated. **(*A* and *C*)** Evaluation of tube formation of HDMECs (n=3-5). **(*B*)** The relative gene expression of Dll4 in HDMECs that were transfected with control siRNA (Control) or siRNA silencing *Dll4* (n=5). *, $P<0.05$. #, $P<0.05$ comparing with cells exposed to 5.5 mM glucose.

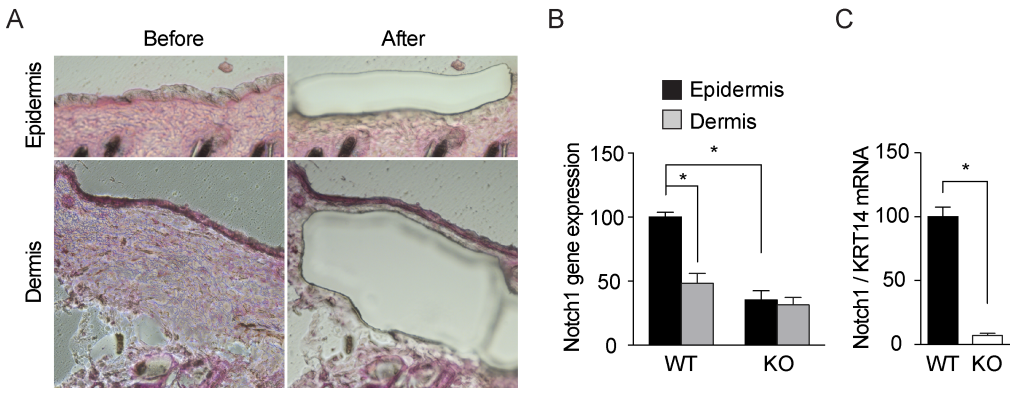

Fig. S11 (related with Fig. 6): Notch1 gene expression in the epidermis and dermis of the skin-specific Notch1-knockout *KRT14-Cre;Notch$^{fl/fl}$* (KO) mice and Wild-type (WT) mice. (*A*) Representative images of the epidermis and dermis before and after Laser microdissection. **(*B*)** Relative Notch1 gene expression in epidermis and dermis prepared using Laser microdissection (n=3). *, $P<0.05$. **(*C*)** Notch1 gene expression normalized to KRT14 gene expression in epidermis from WT and KO mice (n=3). *, $P<0.05$.

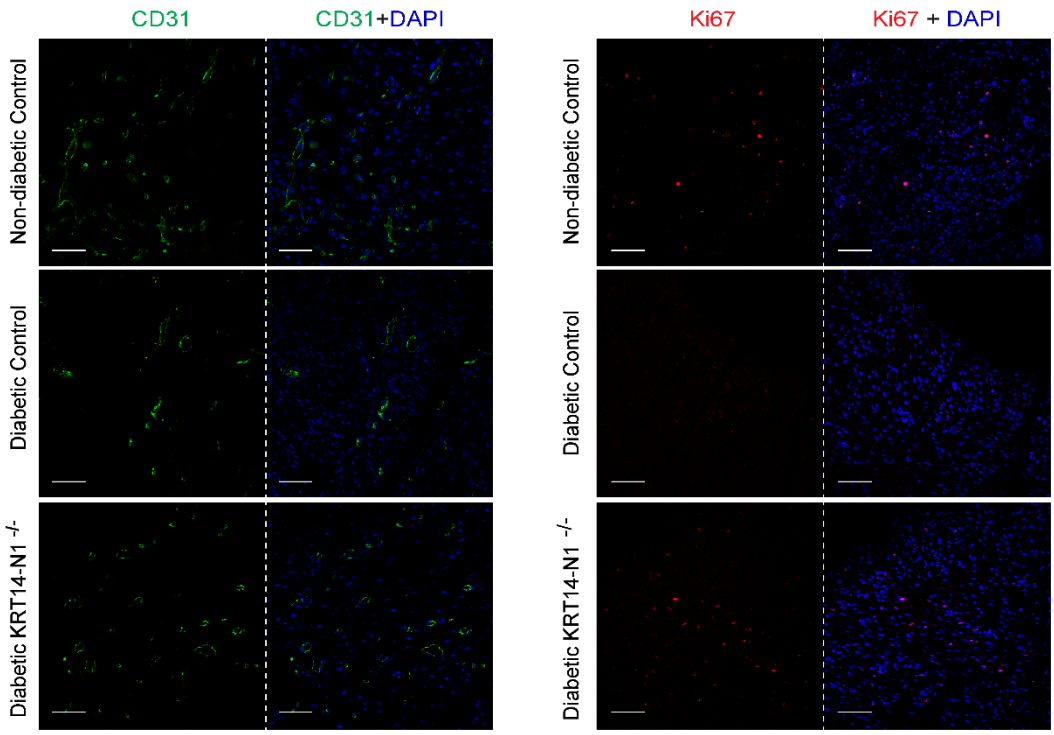

Fig. S12 (related to Fig. 6). Notch1 gene ablation in the skin promotes angiogenesis and proliferation in diabetic wound. Representative images of immunohistochemical staining for CD31 (green), Ki67 (red) and DAPI (blue) in wounds from non-diabetic Control mice, diabetic Control mice and diabetic *KRT14-Notch1*$^{-/-}$ mice.

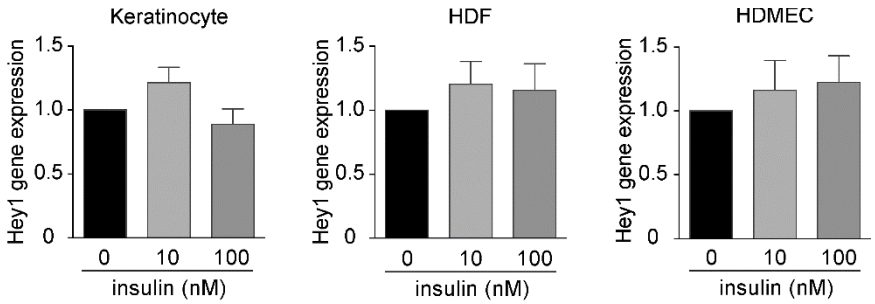

Fig. S13. Effect of insulin on Notch target gene expression. Primary human keratinocytes (n=3), human dermal fibroblasts (HDF, n=3) and human dermal microvascular endothelial cells (HDMEC, n=5), starved before, were exposed to different concentrations of insulin or vehicle. Relative Hey1 mRNA expression levels are shown.