**Supplementary File for** 

Diabetic wound impaired injury-induced transient senescence in

adipose tissue that promotes the normal wound healing process

Authors: Arisa Kita MD1, Yuki Saito PhD2\*, Norihiro Miura3, Maki Miyajima PhD3,

Sena Yamamoto<sup>3</sup>, Tsukasa Sato<sup>3</sup>, Takatoshi Yotsuyanagi MD,PhD<sup>1</sup>, Mineko Fujimiya

MD, PhD<sup>2</sup>, and Takako S. Chikenji PhD <sup>2,3</sup>\*

**Affiliations:** 

1. Department of Plastic and Reconstructive Surgery, Sapporo Medical University, Sapporo,

Japan

2. Department of Anatomy, Sapporo Medical University School of Medicine, Sapporo, Japan

3. Graduate School of Health Sciences, Hokkaido University, Sapporo, Japan.

\*Corresponding authors:

Takako S. Chikenji

Address: North12 West5, Kitaku, Sapporo, 060-0812, Japan

Telephone: 011-706-3382 FAX: 011-706-3382

Email: chikenji@pop.med.hokudai.ac.jp

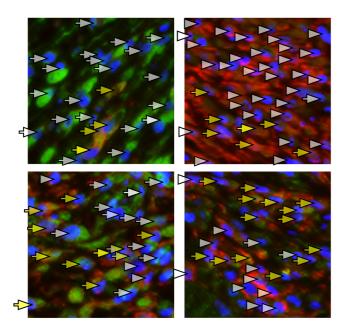
Yuki Saito

Address: South1 West17, Chuoku, Sapporo, 060-8556, Japan

Telephone: 011-611-2111 FAX: 011-618-4288

Email: <a href="mailto:vuki.saito@sapmed.ac.jp">vuki.saito@sapmed..ac.jp</a>

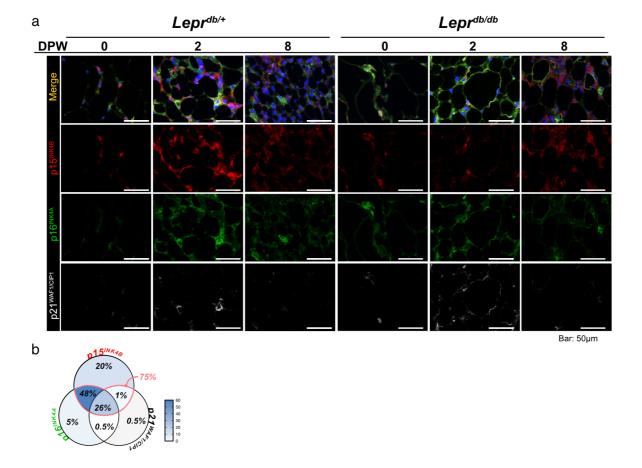
## **Supplementary Figures**



- PDGFRα positive⇒ α-SMA positivePDGFRα and α-SMA double-positive

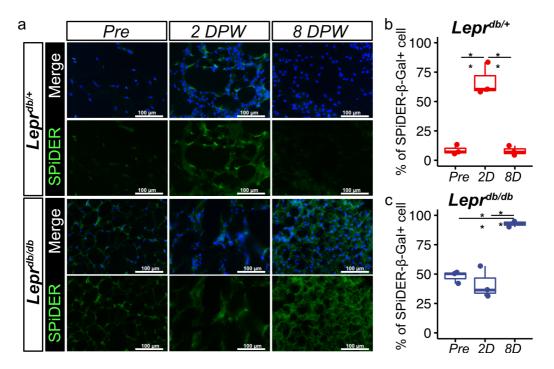
Supplementary Figure 1. Representative images of the definition of PDGFRa and a-SMA positive cells

White arrowheads indicate PDGFR $\alpha$ -positive cells, white arrows indicate  $\alpha$ -SMA-positive cells, and yellow arrows indicate cells positive for both PDGFR $\alpha$  and  $\alpha$ -SMA cells.



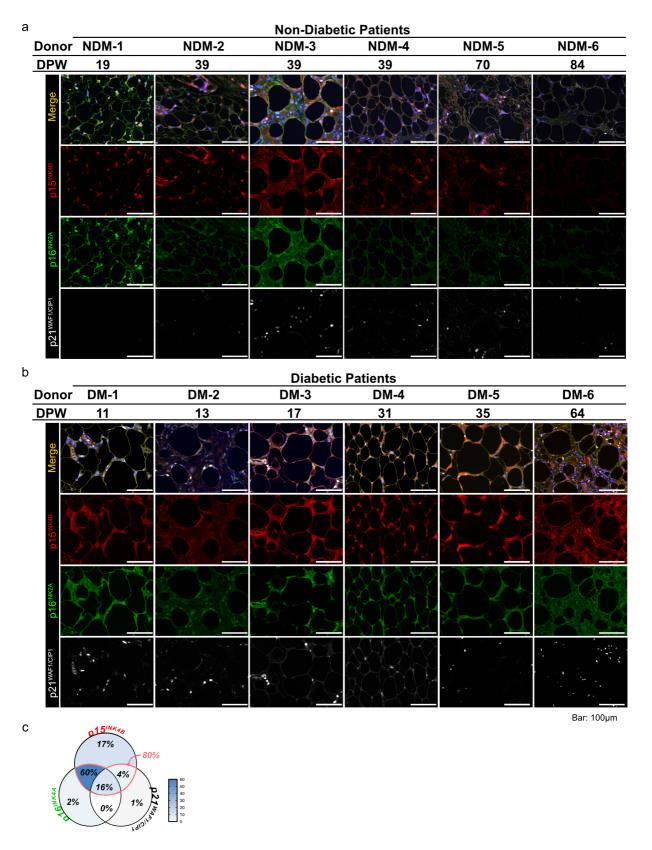
Supplementary Figure 2. p15<sup>INK4B</sup>, p16<sup>INK4A</sup>, and p21<sup>WAFI/CIPI</sup> expression in subcutaneous adipose tissue during wound healing in diabetic mice

(a) Representative images of p15INK4B, p16INK4A, and p21WAF1/CIP1 immunostaining of adipose tissue at pre-wounding, 2 DPW, and 8 DPW in Leprdb/+ and Leprdb/db mice and (b) Venn diagram showing proportions of cells positive for any or all of p15 $^{INK4B}$ , p16 $^{INK4A}$ , and p21 $^{WAF1/CIP1}$ .



Supplementary Figure 3. SPiDER-\(\beta\)-Gal staining in subcutaneous adipose tissue during wound healing in Lepr<sup>db/+</sup> and Lepr<sup>db/db</sup> mice

(a) Representative images of SPiDER- $\beta$ -Gal staining of adipose tissue, and (b–c) quantitative data for the percentage of SPiDER- $\beta$ -Gal–positive cells (n = 3 for each group) at prewounding, 2 DPW, and 8 DPW. Quantitative data are presented as box-and-whisker plots with IQRs and 1.5 times the IQR. p-values were determined using the Tukey method for one-way ANOVA (\*p < 0.05 and \*\*p < 0.001).



Supplementary Figure 4.  $p15^{INK4B}$ ,  $p16^{INK4A}$ , and  $p21^{WAFI/CIPI}$  expression in subcutaneous adipose tissue during wound healing in diabetic patients

(a–b) Representative images showing adipose tissue following immunostaining for p15 $^{INK4B}$ , p16 $^{INK4A}$ , and p21 $^{WAF1/CIP1}$ . Tissue samples were collected tissue during wound healing in

diabetic or non-diabetic patients, and (c) Venn diagram plot showing proportions of cells positive for any or all of  $p15^{INK4B}$ ,  $p16^{INK4A}$ , and  $p21^{WAF1/CIP1}$ .



## Supplementary Figure 5. Quantitative data from Proteome profiler antibody array

Quantitative data of Proteome profiler antibody array of SASP-containing culture media collected from organ culture of adipose tissue at pre-wounding, 2 DPW, and 8 DPW.

Supplementary Table.1 Specific primer sequence used for real-time PCR

Gene	Forward	Reverse	Size	Ascension Number
Cdkn2b	5' AATAACTTCCTACGCATTTTCTGC 3'	5' CCCTTGGCTTCAAGGTGAG 3'	64	NM_007670.4
Cdkn1a	5' GTACTTCCTCTGCCCTGCTG 3'	5' AGAAGACCAATCTGCGCTTG 3'	183	NM_007669.5
Trp53	5' AGCATCTTATCCGGGTGGAAG 3'	5' CCCATGCAGGAGCTATTACACA 3'	157	NM_011640.3
Serpine1	5' CCTCTTCCACAAGTCTGATGGC 3'	5' GCAGTTCCACAACGTCATACTCG 3'	119	NM_008871.2
Serpine2	5' ACTGTCTGCCATCATCCCTCAC 3'	5' GTAATGCCAAGGGCTTTCAGTGG 3'	150	NM_009255.4
IL6	5' TACCACTTCACAAGTCGGAGGC 3'	5' CTGCAAGTGCATCATCGTTGTTC 3'	116	NM_031168.2
Tgfb1	5' GCCTGAGTGGCTGTCTTTTGA 3'	5' CACAAGAGCAGTGAGCGCTGAA 3'	101	NM_011577.2
Pdgfra	5' GCAGTTGCCTTACGACTCCAGA 3'	5' GGTTTGAGCATCTTCACAGCCAC 3'	159	NM_001083316.2
Acta2	5' ACGCTGAAGTATCCGA 3'	5' CATTTTCTCCCGGTTGG 3'	162	NM_007392.3
Gapdh	5' AGGTCGGTGTGAACGGATTTG 3'	5' TGTAGACCATGTAGTTGAGGTCA 3'	123	NM_001289726.1
Atcb	5' CATTGCTGACAGGATGCAGAAGG 3'	5' TGCTGGAAGGTGGACAGTGAGG 3'	138	NM_007393.5