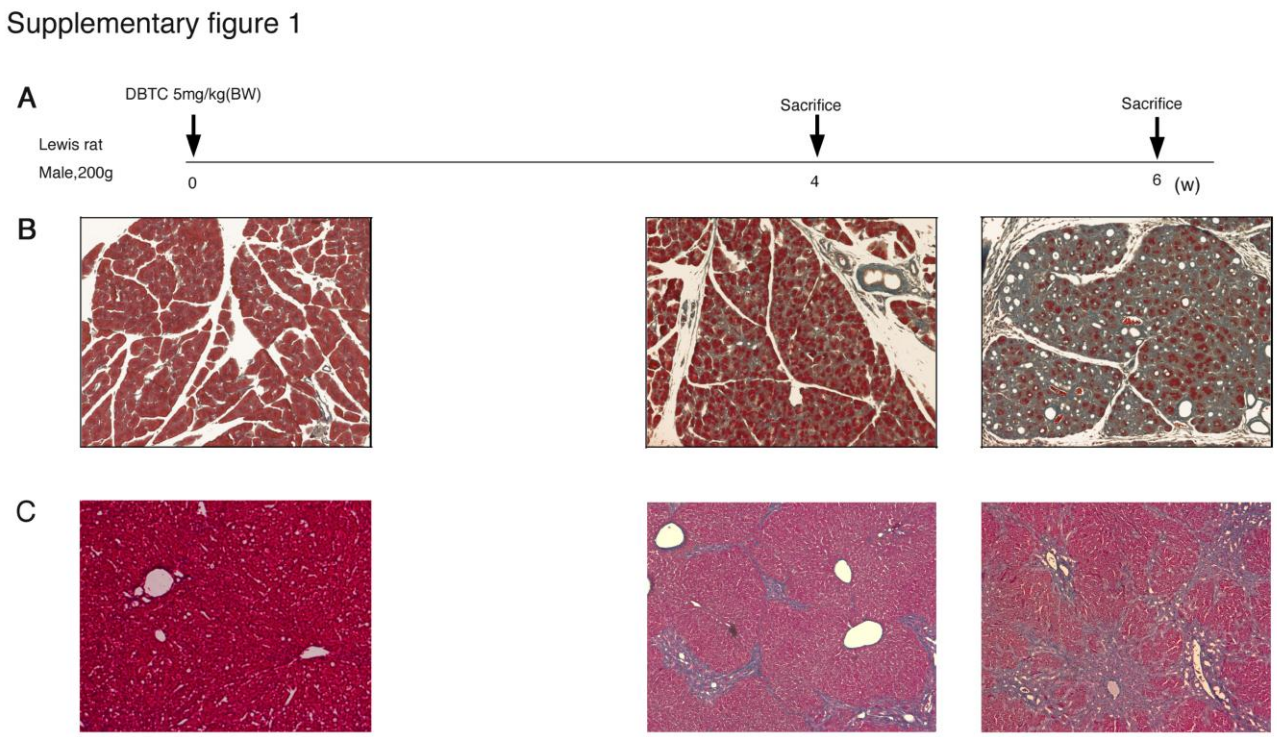
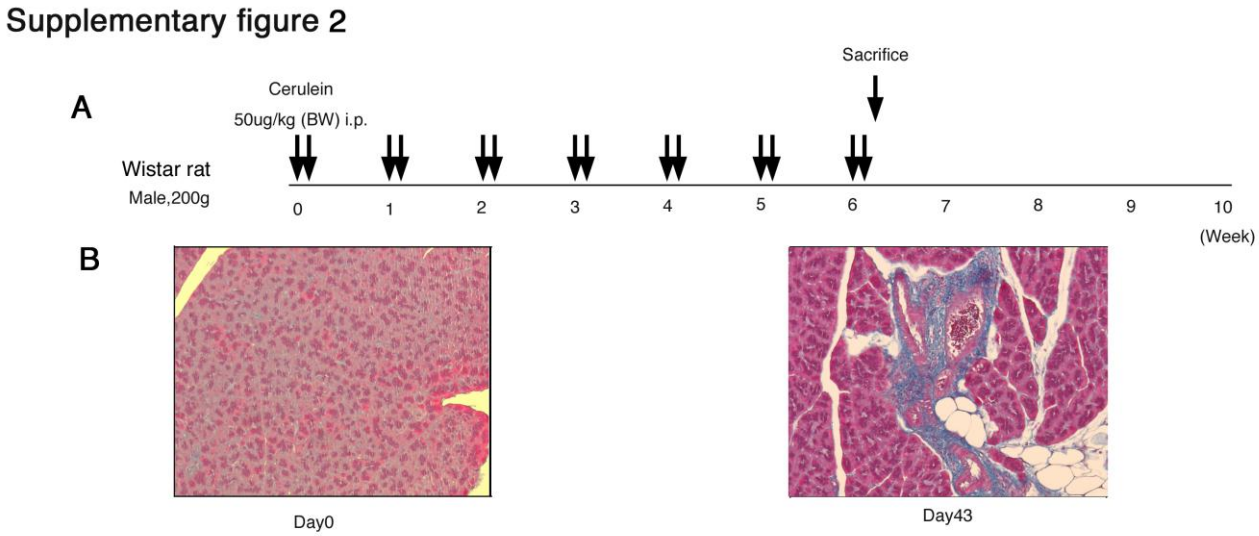
Supplementary figure 1. Induction of pancreatic fibrosis by DBTC treatment



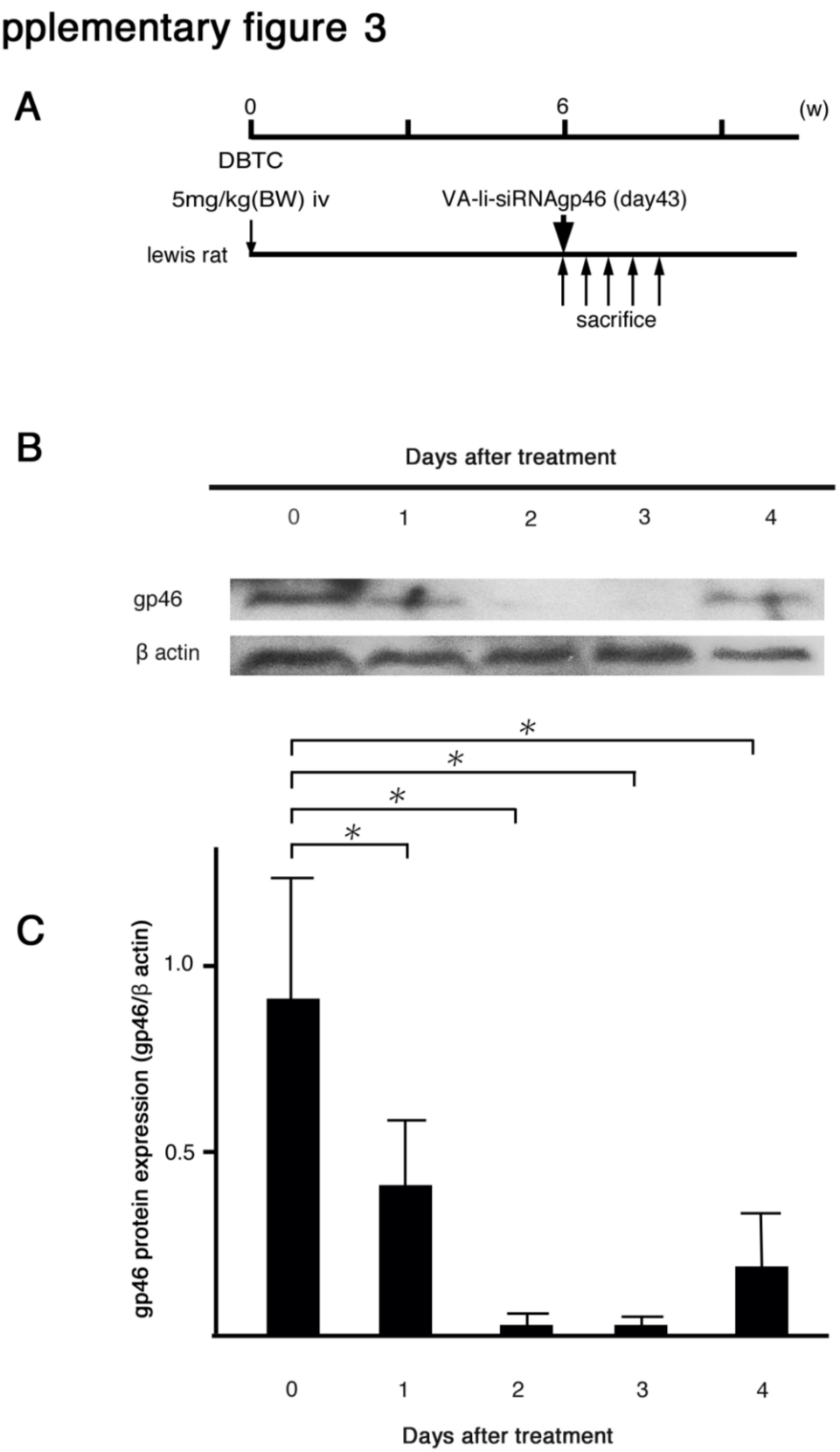
(***A***) Schedule of induction of pancreatic fibrosis by DBTC. DBTC (5 mg/kg body weight) was injected into the right jugular vein. At days 29, 43 after application of DBTC, rats were killed and the pancreases were obtained. ***(B)*** Representative photomicrographs of Azan-Mallory-stained pancreas section**s** at day 0, 29 and 43 after administration of DBTC. Pictures taken at original magnification (x 100). At day 29, early deposition of connective tissue was observed with a predominance in periductal areas. At day 43, the pancreatic tissue was characterized by extended interstitial and periductal fibrosis, cellular infiltrates, and tubular complexes. ***(C)*** Representative photomicrographs of Azan-Mallory-stained liver sections at day 0, 29, and 43 after administration of DBTC. Pictures were taken at original magnification (x 100).

Supplementary figure 2. Induction of pancreatic fibrosis by cerulein treatment



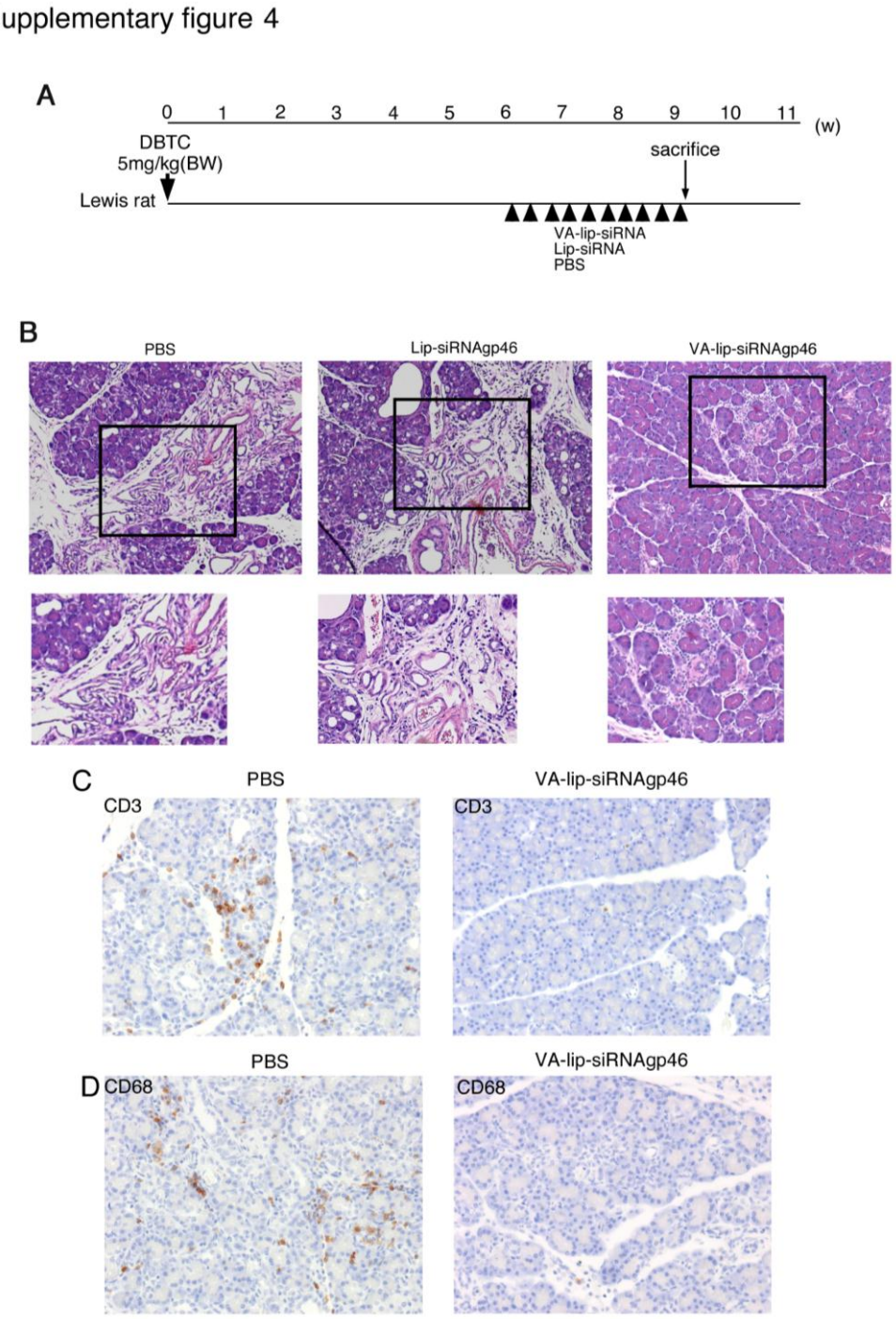
(A) Schedule of induction of pancreatic fibrosis by cerulein. Cerulein (50 μg/kg body weight) were induced in rats by 2 intraperitoneal injections of cerulein (50 μg/kg) given 1 hour apart. At day 43 after the first application of cerulein, rats were killed and the pancreases were removed. ***(B)*** Representative photomicrographs of Azan-Mallory–stained pancreas section at day 0 and 43 after application of DBTC. Pictures taken at original magnification (x 100). At day 43, deposition of connective tissue was observed with a predominance of periductal areas.

Supplementary figure 3. Duration of suppressive effect of siRNAgp46 on gp46 expression in aPSCs



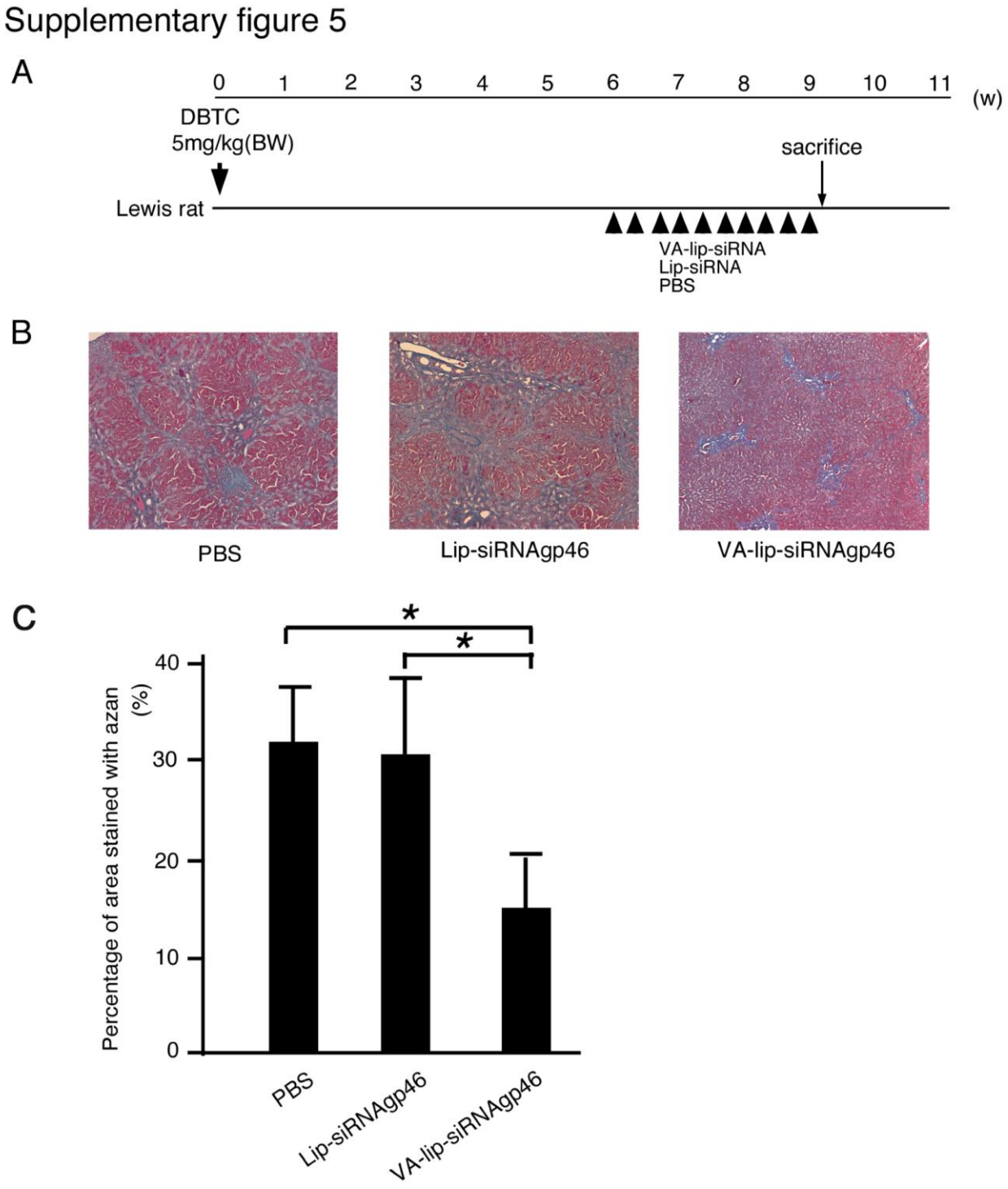
(A) The time course of gp46 expression in a rat with DBTC-induced pancreatic fibrosis treated with VA-lip-siRNAgp46 (siRNA 0.75 mg/kg, one injection at day 43, n=15). Western blotting ***(B)*** and quantitative densitometry analysis ***(C)*** were carried out to measure the expression of gp46 at the time points indicated. The expression of gp46 was normalized to that of β-actin. *$p < 0.05$ vs day 0.

Supplementary figure 4. Effect of VA-lip-siRNAgp46 treatment on pancreatic inflammation of DBTC-treated rats



(A) Schedule of VA-lip-siRNAgp46 treatment in rats with DBTC-induced pancreatic fibrosis. Samples were obtained from DBTC-treated rats that received 10 injections of VA-lip-siRNAgp46 (siRNA doses of 0.75 mg/kg, 3 times every other day), Lip-siRNAgp46 or PBS alone (n= 10 per group).
(*B*) Representative photomicrographs of pancreas section. Representative photomicrographs of Hematoxylin-eosin stained pancreas section (original magnification x 100). Magnified images (x200) corresponding to the areas enclosed in boxes are presented as indicated. ***(C)*** Representative immunohistochemical staining images of T cells stained with anti CD3 antibody (original magnification x 50). ***(D)*** Representative immunohistochemical staining images of macrophages stained with anti CD68 antibody (original magnification x 200). Results from each group of 10 rats were essentially similar (B-D).

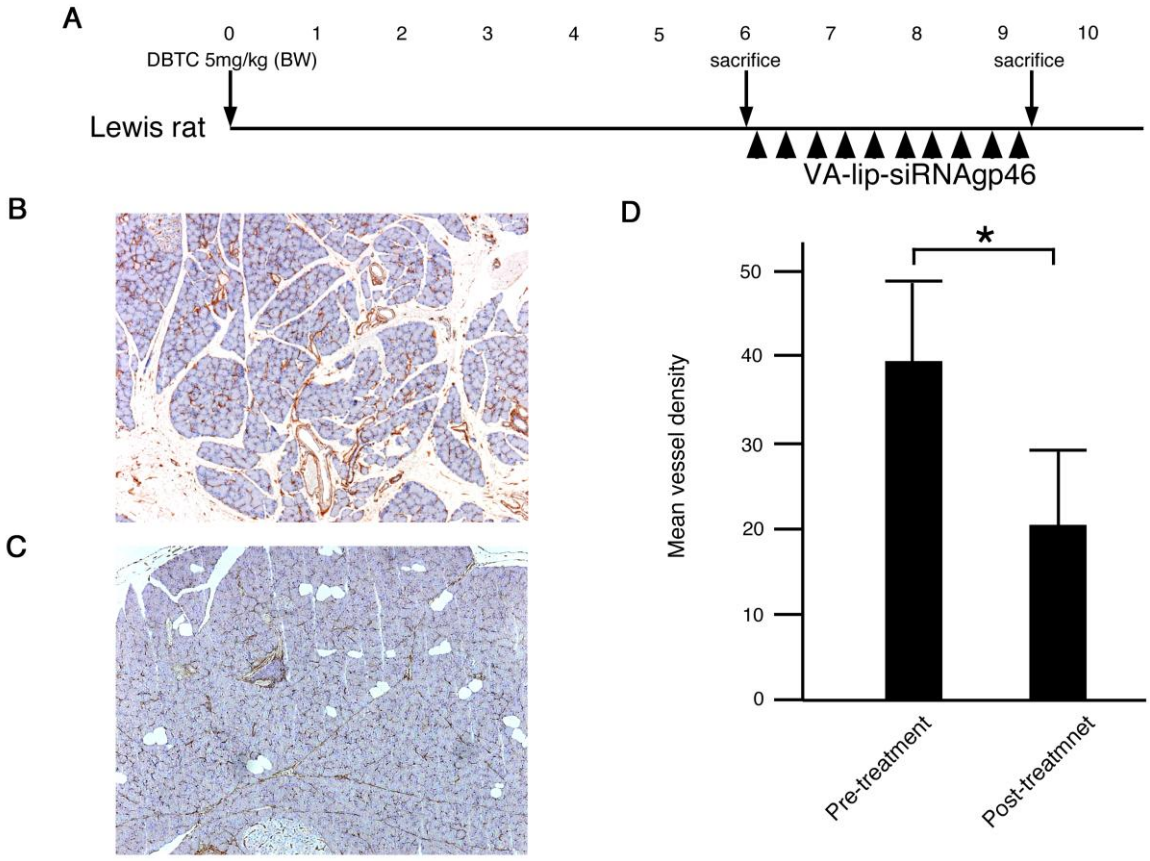
Supplementary figure 5. Effect of i.v. injected VA-lip-siRNAgp46 on DBTC-induced hepatic fibrosis



(A) Schedule of VA-lip-siRNAgp46 treatment in rats with DBTC-induced hepatic fibrosis. Samples were obtained from DBTC-treated rats that received 10 injections of VA-lip-siRNAgp46, Lip-siRNA gp46 (siRNA doses of 0.75 mg/kg, 3 times every other day), or PBS alone (n = 10 per group). ***(B)*** Representative photomicrographs of Azan-Mallory-stained pancreas sections (original magnification x 100). ***(C)*** Azan-Mallory–positive staining area assessed by computerized image analysis. Data were obtained from 6 randomly selected fields in each of 10 rats from 3 groups and represent the mean ± SD. *$p < 0.05$ vs VA-lip-siRNAgp46 treated- DBTC rat.

Supplementary figure 6. Microvessel density before and after administration of VA-lip-siRNAgp46 treatment on pancreas of DBTC-treated rats

Supplementary figure 6



(A) Schedule of VA-lip-siRNAgp46 treatment in rats with DBTC-induced pancreatic fibrosis. Samples were obtained from DBTC-treated rats on day 42 before treatment of VA-lip-siRNAgp46 and from DBTC-treated rats that received 10 injections of VA-lip-siRNAgp46 on day 65 (siRNA doses of 0.75 mg/kg, 3 times every other day (n= 10 per group). ***(B)*** Representative immunohistochemical staining images of vessels stained with anti CD34 antibody on day 42. Pictures were taken at original magnification (x 50). ***(C)*** Representative immunohistochemical staining images of vessels stained with anti CD34 antibody on day 65. Pictures were taken at original magnification (x 50). ***(D)*** MVD was analyzed as described in the Materials and Methods. Mean ± SD of 10 rats per group. *$p < 0.05$

Supplementary table 1

Inflammatory parameters in the DBTC pancreas treated with VA-lip-siRNAgp46 or Lip-siRNAgp46 or PBS

	DBTC rat (Day 43)	PBS (Day 65)	Lip-siRNAgp46 (Day 65)	VA-lip-siRNAgp46 (Day 65)	P value of comparison between VA-lip-siRNA and other groups
Inflammatory cell infiltration	2.9 ± 0.6	2.6 ± 0.5	2.3 ± 0.5	0.8 ± 0.3	< 0.01
Fatty change	1.8 ± 0.5	1.5 ± 0.3	1.5 ± 0.4	0.5 ± 0.2	< 0.01
Tubular complex	2.1 ± 0.8	2.0 ± 0.8	1.8 ± 0.5	0.9 ± 0.2	< 0.01

Values are means ± SD (n=10)

Note. The grade of inflammation was defined according to a system reported previously (Takano S, et al. Effects of stress on the development of chronic pancreatitis. Pancreas 1992;7:548-555). Histological findings of chronic pancreatitis such as inflammatory cell infiltration,tubular complexes, and fatty changes were calculated as the sum of the lesion size scores per rat. Lesion size scores were as follows:
0 = absent; 1 = lesion appearing slightly in the lobule or intralobular region ; 3 = lesion evident across the lobule and intralobule region or showing destruction of lobular architecture. Value are means ± SD (n=10).

Supplementary methods

Immunohistochemical staining for macrophages and T cells.
Immunohistochemical staining for CD68 (macrophages) and CD3 (T cells) was performed by the dextran polymer method using monoclonal anti CD68 antibody (1: 100, DAKO), monoclonal anti CD3 antibody (1: 100, Nichirei Biosciences, Tokyo, Japan) and an Envision Kit (Dako), followed by color development with DAB and nuclear staining with Gill's hematoxylin solution. The grade of inflammation was defined according to a criteria reported previously.[1] Histological findings of chronic pancreatitis such as inflammatory cell infiltration, tubular complexes, and fatty changes were calculated as the sum of the lesion size scores per rat. Lesion size scores were as follows: 0 = absent; 1 = lesion appearing slightly in the lobule or intralobular region; 3 = lesion evident across the lobule and intralobule region or showing destruction of lobular architecture.

Measurement of microvascular density (MVD)
For evaluation of MVD, sections of pancreas tissue from the DBTC treated rat were stained with polyclonal anti CD34 antibody (1: 50, R&D Systems, Minneapolis, MN) as described in previous section, 8 fields of the stained section randomly selected were numerized at a magnification of ×200 through microscopy. The number of CD34 positive structures in each numerized image was then counted. The mean MVD for each section was calculated as the mean of the counts, after exclusion of the lowest and highest values measured.[2]

Supplementary reference

1. Takano S, Kimura T, Yamaguchi H, et al. Effects of stress on the development of chronic pancreatitis. Pancreas 1992;7:548-555.

2. Weidner N, Semple JP, Welch WR, et al. Tumor angiogenesis and metastasis--correlation in invasive breast carcinoma. N Eng J Med 1991;324:1-8.