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**Effect of Fetal Membrane-Derived Mesenchymal Stem Cell Transplantation
in Rats with Acute and Chronic Pancreatitis**

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Abstract

Objectives: Mesenchymal stem cells (MSCs) are a valuable cell source in regenerative medicine and can be isolated from fetal membranes (FMs), particularly amniotic membranes. We investigated the effect of rat FM-derived MSCs (rFM-MSCs) and human amnion-derived MSCs (hAMSCs) on the inflammatory reaction *in vitro* and therapeutic effects in rats with acute and chronic pancreatitis.

Methods: Effect of rFM-MSCs or hAMSC-conditioned medium was investigated *in vitro*. Acute pancreatitis was induced by intraductal injection of 4% taurocholate, and rFM-MSCs were transplanted intravenously. Chronic pancreatitis was induced by intravenous injection of 5 mg/kg dibutyltin dichloride, and hAMSCs were transplanted intravenously.

Results: The inflammatory reaction of macrophages induced by lipopolysaccharide and trypsin was significantly suppressed by rFM-MSC co-culture. Pancreatic acinar cell injury induced by cerulein was significantly ameliorated by hAMSC-conditioned medium. Pancreatic stellate cell activation induced by TNF- α was significantly decreased by hAMSC-conditioned medium. Transplantation of rFM-MSCs significantly

reduced the histological score and infiltration of CD68-positive macrophages in the rat pancreas. hAMSC transplantation significantly decreased the expression of MCP-1 and attenuated the downregulation of amylase expression in the pancreas.

Conclusions: Transplantation of FM-MSCs and AMSCs suppressed the inflammatory reaction of acute and chronic pancreatitis in rats.

Keywords; mesenchymal stem cells, acute pancreatitis, chronic pancreatitis, macrophages, fetal membrane, amnion

Introduction

Acute pancreatitis is a potentially fatal inflammatory disorder of the pancreas with an annual incidence of 5 to 50 per 100,000.^{1,2} Several inflammatory cytokines are known to be responsible for its pathogenesis; however, there is no pharmacological treatment to alleviate disease progression.^{3, 4} The mortality from acute pancreatitis accompanying organ failure and walled-off necrosis rises up to 30% despite the recent progress in surgical and endoscopic treatment.^{5, 6}

Chronic pancreatitis is an ongoing inflammatory disease of the pancreas that causes fibrosis and glandular atrophy, leading to exocrine and endocrine insufficiency.⁷ Patients with chronic pancreatitis have an increased risk of pancreatic adenocarcinoma and overall mortality.⁸ The incidence is increasing; however, there are no effective medical therapies to prevent this condition.⁹ Total pancreatectomy with autologous islet cell transfusion is the single definitive treatment but is associated with morbidity and impaired quality of life.^{10, 11}

Mesenchymal stem cells (MSCs) are multipotent cells that can differentiate into a variety of adult tissues.¹² Because of their ability to regulate several inflammatory

responses, MSCs have been investigated in many inflammatory disorders, such as graft-versus-host disease (GVHD) and Crohn's disease.¹³ MSCs can be isolated from adult bone marrow and adipose tissue; however, the limitation in resources remains to be solved.¹⁴ Recently, the fetal membrane (FM), consisting of amnion and chorion and generally discarded as medical waste after delivery, has been reported to be an abundant source of MSCs (FM-MSCs).^{15, 16} In small animals, transplantation of amnion-derived MSCs (AMSCs) alleviated inflammatory diseases, such as liver cirrhosis, hindlimb ischemia,¹⁷ myocarditis,^{18, 19} glomerulonephritis,²⁰ ischemia–reperfusion-induced kidney injury,²¹ and GVHD.²²

In this study, we investigated the effect of FM-MSC and AMSC on the inflammatory processes of macrophages, pancreatic acinar cells, and pancreatic stellate cells (PSCs) *in vitro* as well as the therapeutic effects in rats with acute and chronic pancreatitis.

Materials and Methods

Isolation and Expansion of Rat FM-MSCs and Human AMSCs

The Medical Ethical Committee of Hokkaido University Graduate School of Medicine, Sapporo, Japan approved this work, and all pregnant women gave written informed consent. Isolation and expansion of rat FM-MSCs were performed as described previously¹⁷. To obtain FMs, female Sprague–Dawley (SD) rats (15th day of pregnancy; Japan SLC, Hamamatsu, Japan) were sacrificed and the uterus was harvested and placed in phosphate-buffered saline (PBS; Life Technologies, Carlsbad, CA, USA). After separation from the placenta, FMs were minced with scissors and digested with type II collagenase solution (300 U/mL; Worthington Biochemicals, Lakewood, NJ, USA) for 1 h at 37°C in a water shaker. Enzyme activity was neutralized with α -minimal essential medium (α -MEM; Life Technologies) containing 10% fetal bovine serum (FBS; Life Technologies). After filtration through a mesh filter (100 μ m; BD Biosciences, Bedford, MA, USA) and centrifugation at 300 \times g for 5 min, dissociated FM cells were suspended in α -MEM culture medium supplemented with 10% FBS, 100 U/mL penicillin, and 100 μ g/mL streptomycin (Life Technologies) and then introduced into 100-mm dishes and

incubated at 37°C with 5% CO₂. A small number of cells developed into visible symmetric colonies by days 5 to 7. Non-adherent hematopoietic cells were removed and the medium was replaced. The adherent spindle-shaped MSC population was expanded until reaching 80% confluence. The passage was performed using 0.5% trypsin-ethylenediaminetetraacetic acid (Life Technologies). Isolation of human AMSCs were performed as described previously.²² In brief, human FMs were obtained during Caesarean deliveries, and the amnion was manually peeled from the chorion. Human AMSCs were isolated and expanded by digestion with collagenase type II (300 U/mL), followed by seeding in uncoated plastic dishes with α -MEM supplemented with 10% FBS, 100 U/mL of penicillin, and 100 μ g/mL of streptomycin. The cells were cultured and expanded as described above.

Inflammatory Reaction after Treatment with Lipopolysaccharide (LPS) and Trypsin in Macrophages

RAW264.7 cells [immortalized murine macrophage cells; American Type Tissue Collection (ATCC), Manassas, VA, USA] and rat FM-MSCs were cultured in a

humidified chamber at 37°C in a mixture of 95% air and 5% CO₂. RAW264.7 cells were stimulated with 100 ng/mL of LPS (Sigma-Aldrich, St. Louis, MO, USA) or 10 µM trypsin (Life Technologies) for 24 h under co-incubation with AMSCs (1/10 rat FM-MSCs to RAW264.7 cells). Subsequently, total RNA was collected and the expression of mouse TNF- α was measured by quantitative reverse-transcription polymerase chain reaction (qRT-PCR).

Inflammatory Reaction following Treatment with Cerulein in Pancreatic Acinar

Cells

Rat pancreatic acinar cells (AR42J; ATCC) were cultured in a humidified chamber at 37°C in a mixture of 95% air and 5% CO₂ in RPMI (Life Technologies) supplemented with 10% FBS, 100 U/mL of penicillin, and 100 µg/mL of streptomycin. AMSC-conditioned medium was collected by culturing sub-confluent human AMSCs with serum-free α -MEM for 48 h. AR42J cells (3×10^4 and 3×10^3 cells/well) were stimulated with 10 nM cerulein (Sigma-Aldrich) in AMSC-conditioned medium or serum-free α -MEM in 6-well and 96-well plates (Corning, New York, NY, USA),

respectively. In the control plate, AR42J cells were cultured without cerulein stimulation in serum-free α -MEM. After 12 h of incubation, total RNA was collected and expression levels of rat amylase, lipase, caspase-8, caspase-9, receptor-interacting protein kinase (RIPK), and X-linked inhibitor of apoptosis protein (XIAP) were measured by qRT-PCR. After a 24-h incubation period, the supernatant was collected for the amylase activity assay, the cells were harvested with trypsinization, and the cell number was counted with a hemocytometer. After 24 h of incubation of the 96-well plate, apoptosis was analyzed using a caspase-3/7 assay (Caspase-Glo 3/7 Assay; Promega, Fitchburg, WI, USA), according to the manufacturer's instructions.

Inflammatory Reaction after Treatment with TNF- α in Pancreatic Stellate Cells

(PSCs)

Pancreatic stellate cells (hPSC5) were isolated from the resected pancreatic tissue of a patient undergoing operation for pancreatic cancer as previously reported,²³ under the approval of the Ethics Committee of Tohoku University Graduate School of Medicine.

In brief, the resected human pancreatic tissue was minced with scissors and shaken in a

solution containing 0.03% collagenase P (Roche, Basel, Switzerland), 0.02% pronase (Roche), and 0.1% DNase I (Roche) at 37°C for 30 min. Digested tissue was pipetted through the narrow orifices and centrifuged. After washing, cells were seeded on tissue culture dishes coated with type I collagen (Corning) and maintained in Ham's F12/DMEM (Life Technologies) supplemented with 10% FBS, 100 U/mL of penicillin, and 100 µg/mL of streptomycin. hPSC5 cells were cultured in 6-well and 96-well plates (3.0×10^4 and 1.0×10^3 cells, respectively) and stimulated with 1 ng/mL rat recombinant TNF- α (R&D Systems, Minneapolis, MN, USA) in AMSC-conditioned medium or serum-free α -MEM. Following a 3-h incubation, total RNA was collected and expression levels of human MCP-1, IL-8, TGF- β , α -SMA were measured by qRT-PCR. After 24 h of incubation, cell proliferation was tested using CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega).

Induction of Acute Pancreatitis and Rat FM-MSC Transplantation

Acute pancreatitis was induced as previously described with a slight modification.²⁴ In brief, 8-week-old ACI rats (Japan SLC) were anesthetized by intraperitoneal injection of

50 mg/kg pentobarbital (Kyoritsu Seiyaku, Tokyo, Japan) and a 20-mm median laparotomy was performed. The proximal bile duct was temporarily occluded with a microvascular clip (Roboz Surgical Instrument, Gaithersburg, MD, USA), and the common bile duct was punctured with a 24-gauge needle. Then 2 mL/kg body weight 4% taurocholate (TCA) in PBS was infused at a constant rate for 1 min with an infusion pump (As One, Osaka, Japan). In the control group, rats were injected with PBS alone. After infusion, the microvascular clip and the injection needle were removed from the common bile duct and the abdomen was closed with Autoclip Wound Clips (BD Biosciences). Following this, 1×10^6 rat FM-MSCs suspended in 200 μ L of PBS were intravenously injected through the penile vein. All rats were sacrificed 4 days after cell transplantation. Blood samples were collected and serum levels of amylase and lipase were measured by an enzymatic method (SRL, Tokyo, Japan). The pancreas was removed and pancreatic sections were stained with hematoxylin and eosin, which were then graded in a blinded manner on a scale from 0 to 4 for the degree of edema, inflammation, hemorrhage, and necrosis, as described previously.²⁵

Induction of Chronic Pancreatitis and human AMSC Transplantation

Chronic pancreatitis was induced as described previously.²⁶ In brief, 6-week old SD rats were anesthetized by intraperitoneal injection of 50 mg/kg pentobarbital. Dibutyltin dichloride (DBTC; Schering AG, Berlin, Germany) was dissolved in ethanol (1 part) and then mixed with glycerol (2 parts) and dimethyl sulfoxide (2 parts).²⁶ The DBTC solution was administered into the left jugular vein with a syringe at 5 mg/kg body weight, according to a previous report.²⁷ In the control group, one volume of solvent was injected into the left jugular vein. One million human AMSCs suspended in 200 μ L of PBS were intravenously injected through the penile vein after 5 days of DBTC treatment. In the sham group, 200 μ L of PBS were injected similarly. All rats were sacrificed 14 days after cell transplantation. The tail of the pancreas was removed, fixed in 40 g/L of formaldehyde saline, embedded in paraffin, cut into 5- μ m sections, and stained with hematoxylin and eosin and Masson's trichrome. Another part of the pancreas was used for RNA isolation.

Immunohistochemical Analysis

Tissue sections were stained with anti-rat type I collagen antibody (dilution 1:50,000; LSL, Tokyo, Japan) for the assessment of collagen deposition. To assess pancreatic inflammation, tissue sections were stained with anti-rat CD3 (dilution 1:50; BD Biosciences) and CD68 (dilution 1:50; AbD Serotec, Kidlington, UK) antibodies. To assess the activation of PSCs, tissue sections were stained with anti-rat α -smooth muscle actin (α -SMA) antibody (dilution 1:800; Thermo Scientific, Waltham, MA, USA).

RNA Isolation and qRT-PCR

Total RNA of the rat pancreas or cultured cells was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany), and 1 μ g of the total RNA was reverse-transcribed into cDNA using the QuantiTect Reverse Transcription Kit (Qiagen). PCR amplification was performed using a 25- μ L reaction mixture containing 1 μ L of cDNA and 12.5 μ L of Platinum SYBR Green PCR Mix (Life Technologies). β -Actin mRNA amplified from the same samples served as the internal control. After initial denaturation at 95°C for 2 min, a two-step cycle procedure was used (denaturation at 95°C for 15 sec, annealing

and extension at 60°C for 1 min) for 40 cycles in a 7700 Sequence Detector (Applied Biosystems, Foster City, CA, USA). Gene expression levels were determined using the comparative threshold cycle (ddCt) method. Data were analyzed with Sequence Detection Systems software (Applied Biosystems). Primer sequences used in this study are shown in Table 1. Primers for human species were purchased from Qiagen (β -actin, Hs_Actb_1_SG; MCP-1, Hs_Ccl2_1_SG; IL-8, Hs_Il-8_1_SG; QuantiTect Primer Assay).

Statistical Analyses

Data are shown as means \pm SEM. Parameters among the groups were compared by one-way ANOVA, followed by a Tukey test. Differences were considered statistically significant at probability (P) levels of <0.05 .

Results

Effect of Rat FM-MSCs on Inflammatory Reaction in LPS- or Trypsin-Stimulated

Macrophages (Table 2)

We first examined the effect of co-culture of rat FM-MSCs on the inflammatory reactions in the RAW264.7 macrophages. Treatment with LPS markedly upregulated the expression of TNF- α ; rat FM-MSC co-culture significantly decreased expression of TNF- α . Treatment with trypsin also markedly upregulated expression of TNF- α ; rat FM-MSC co-culture significantly decreased expression of TNF- α .

Effect of Human AMSCs on Inflammatory Reaction in Cerulein-Stimulated Acinar

Cells (Table 3)

We next investigated the effect of AMSCs on acinar cell injury. Conditioned medium was obtained from AMSC culture and AR42J cells were treated with cerulein and cultured with AMSC-conditioned medium. Although cerulein stimulation did not affect the cell number, it significantly increased the expression of amylase from the acinar cells; however, AMSC-conditioned medium significantly decreased the secretion of

amylase. We then examined the expression of amylase and lipase from AR42J cells. Cerulein treatment significantly increased the expression of amylase and lipase, which were both significantly decreased by AMSC-conditioned medium. We also investigated the effect of AMSC-conditioned medium on cell injury signals. Cerulein treatment significantly increased caspase-3/7 activity and the expression of caspase-8 and caspase-9; however, AMSC-conditioned medium significantly decreased these. Treatment of cerulein also upregulated the expression of RIPK and XIAP, the key mediators of necrosis and AMSC-conditioned medium significantly decreased these.

Effect of Human AMSCs on Inflammatory Reaction in TNF- α -Stimulated

Pancreatic Stellate Cells (PSCs) (Table 4)

We next investigated whether AMSCs affect the activation of PSCs. TNF- α stimulation did not affect the proliferation of PSCs. Treatment with TNF- α significantly upregulated expression levels of MCP-1 and IL-8; however, AMSC-conditioned medium significantly decreased the expression. Expression levels of α -SMA and TGF- β were not affected by AMSC-conditioned medium (data not shown).

Effect of Rat FM-MSC Transplantation in Rats with Acute Pancreatitis

From the results of the *in vitro* experiments, we next investigated the effect of rat FM-MSCs in the acute pancreatitis model. Histological changes in the TCA group included edema, inflammation, and necrosis (Figure 4A). These findings were significantly ameliorated by rat FM-MSC transplantation. Immunohistological examination demonstrated that the number of infiltrated CD68-positive monocytes/macrophages was significantly higher in the TCA group and rat FM-MSC transplantation significantly decreased the number of CD68-positive cells (Figure 4B). However, the number of CD3-positive cells was not increased in the TCA group and was not affected by rat FM-MSC transplantation (Figure 4C). The upregulation of TNF- α and IL-6 by TCA in the pancreas tended to be suppressed by rat FM-MSC transplantation (Figure 4D and 4E). Serum levels of amylase and lipase were increased in the TCA group and they tended to be decreased by rat FM-MSC transplantation (Figure 4F and 4G).

Effect of Human AMSC Transplantation in Rats with Chronic Pancreatitis

We finally investigated the effect of human AMSCs in the chronic pancreatitis model.

Histological examinations showed that the fibrotic area, the expression of type I

collagen, the number of infiltrated CD68-positive monocytes/macrophages, and

α -SMA-positive stellate cells tended to be increased by DBTC treatment, and these

were not affected by human AMSC transplantation (Figure 5A–5D). However,

expression of amylase mRNA in the pancreas of DBTC-treated rats was significantly

decreased but was significantly increased by AMSC transplantation (Figure 5E).

Pancreatic MCP-1 expression was significantly increased by DBTC treatment and

significantly decreased by human AMSC transplantation (Figure 5F). We also examined

the expression of IL-1 β and TGF- β ; however, this was not affected by human AMSC

transplantation (Figure 5G and 5H).

Discussion

In the present study, we demonstrated the following: (1) rat FM-MSC co-culture inhibited the inflammatory reaction of macrophages; (2) human AMSC-conditioned medium protected cell injury signals in pancreatic acinar cells; (3) human AMSC-conditioned medium downregulated inflammatory responses in PSCs; (4) rat FM-MSC transplantation improved acute pancreatitis in rats; and (5) human AMSC transplantation improved the upregulation of MCP-1 and downregulation of amylase levels in rats with chronic pancreatitis.

The present study demonstrated that AMSC-conditioned medium significantly reduced both the expression and secretion of amylase from acinar cells. In addition, AMSC-conditioned medium inhibited cell injury signals. Parenchymal cell death is a major pathological consequence of acute pancreatitis.²⁸ Consistent with our results, Meng et al. showed that umbilical cord-derived MSCs inhibited both apoptosis and necrosis of pancreatic acinar cells by reducing the levels of pro-inflammatory cytokines and increasing the level of anti-inflammatory cytokines.²⁹ However, the role of apoptosis in acute pancreatitis is still controversial. Mild acute pancreatitis is associated

with apoptosis, while severe acute pancreatitis is associated with necrosis.^{30, 31}

Considering that cerulein-induced acinar cell injury is relatively mild, AMSC would affect mild pancreatitis by inhibiting cell injury of pancreatic acinar cells.

Several reports demonstrated that transplantation of rat MSCs derived from bone marrow ameliorates rat acute pancreatitis.^{32, 33} Recently, therapeutic effects of human MSCs derived from bone marrow and umbilical cord on acute pancreatitis in rats have been reported.^{29, 34, 35} In our study, rat FM-MSCs led to lower histological scoring and suppressed inflammatory cell infiltration in the acute pancreatitis model. Immunohistochemical studies showed that the infiltration of CD68-positive cells was significantly reduced by FM-MSC transplantation. Our *in vitro* study demonstrated that rat FM-MSCs co-culture significantly decreased the expression level of TNF- α from RAW246.7 cells stimulated by both LPS and trypsin. Our findings suggest that the anti-inflammatory effect of FM-MSCs is mediated in part by suppressing the trypsin-activated macrophages. Yang et al. reported that the protective effect of umbilical cord MSCs on acute pancreatitis was time- and dose-dependent.³⁵ FM-MSC

transplantation with a larger number of cells could have potential to reduce inflammation more effectively.

Pancreatic stellate cells (PSCs) play a pivotal role in the pathogenesis of chronic pancreatitis.^{36, 37} In the normal pancreas, PSCs are round and quiescent. However, in response to pancreatic injury or inflammation triggered by various causes, PSCs undergo morphological and functional changes to become fibroblast-like cells. Activated PSCs promote pancreatic inflammation, angiogenesis, and fibrosis by expressing α -SMA, cytokines, chemokines, and cell adhesion molecules.³⁸ In our study, AMSC-conditioned medium significantly downregulated the expression level of MCP-1 and IL-8 in the PSCs. Inhibition of MCP-1 production in PSCs was reported to reduce pancreatic fibrosis;³⁹ therefore, AMSC could have the potential to ameliorate fibrosis in chronic pancreatitis. In the present study, however, TNF- α stimulation did not increase expression levels of α -SMA and TGF- β in PSCs. This is probably because hPSC5 cells were already activated, expressing high levels of α -SMA and TGF- β during the isolation and culture procedures. Further studies are required for investigating the effect of AMSC on the activation of PSCs.

In our study, human AMSC transplantation significantly downregulated the expression level of MCP-1 in the rat pancreas. Recruitment of macrophages plays an important role in the pathogenesis of chronic pancreatitis.⁴⁰ Recently, Zhou et al.⁴¹ reported that autologous transplantation of MSCs from Wharton's Jelly attenuated pancreatic fibrosis in a chronic pancreatitis rat model. Our study did not demonstrate any significant effect of AMSC transplantation in terms of reducing fibrosis. This may be due to the number of transplanted AMSCs being small, the timing of AMSC transplantation or sacrifice not being appropriate, or AMSCs themselves not having ample anti-fibrotic effect in the chronic pancreatitis model.

Recently, the therapeutic effect of AMSC transplantation for various diseases has been reported.^{42, 43} Furthermore, a first-in-human clinical trial of FM-MSC transplantation for nine patients with steroid-refractory acute GVHD demonstrated that the FM-MSCs appeared safe for intravenous infusion and the overall response rate in severe refractory acute GVHD was similar to that observed when using bone marrow-derived MSCs.⁴⁴ This is encouraging because the collection of AMSCs

involves procedures less invasive than those used for obtaining MSCs from bone marrow or adipose tissue from donors.

In conclusion, AMSC transplantation ameliorated inflammatory responses in a rat model of acute and chronic pancreatitis, possibly mediated by the suppression of the activity of macrophages and PSCs and by the suppression of acinar cell injury. Because FM has been treated as medical waste and can be obtained without use of any invasive procedure, human AMSC transplantation may represent a highly relevant therapeutic strategy for the treatment of acute and chronic pancreatitis.

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Figure Legends

Figure 1. Effect of rat FM-MSC transplantation in taurocholate (TCA)-induced acute pancreatitis in rats

Paraffin sections were stained with hematoxylin and eosin and a histological score was obtained (A). Immunohistochemical analyses of (B) CD68 and (C) CD3 were performed and the number of positive cells was counted in 10 sections per sample in each low-power field. Scale bars, 100 μ m. Expression of TNF- α (D) and IL-6 (E) were measured by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Serum levels of amylase (F) and lipase (G) were measured by an enzymatic method. The values were the mean \pm SEM. **P < 0.01 vs. the control group, ##P < 0.01 vs. the TCA group.

Figure 2. Effect of human amnion-derived mesenchymal stem cells (AMSC) transplantation in dibutyltin dichloride (DBTC)-induced chronic pancreatitis in rats

Paraffin sections were stained with Masson's trichrome staining. Fibrotic area was stained blue and calculated from the entire pancreas cross-sectional area (A).

Immunohistochemical analyses of (B) type I collagen, (C) α -smooth muscle actin (SMA) and (D) CD68 were performed and the stained area was calculated from the entire pancreas cross-sectional area. Scale bars, 100 μ m. RNA was obtained from the pancreatic tissue and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) for amylase (E), MCP-1 (F), IL-1 β (G), and TGF- β (H) was performed. The values were the mean \pm SEM. **P < 0.01, *P < 0.05 vs. Control, #P < 0.05 vs. DBTC.

Table 1. qRT-PCR primer sequences

Species	Gene	Primer sequence
Rat	Amylase	F: AATTGATCTGGGTGGTGAGC
		R: CTTATTTGGCGCCATCGATG
	Lipase	F: AGCCAGCACAAATCAACA
		R: TCAATGAAGCCGTGGATAA
	MCP-1	F: ATGCAGTTAATGCCCACTC
		R: TTCCTTATTGGGGTCAGCAC
	IL-1 β	F: GCTGACAGACCCCAAAGAT
		R: AGCTGGATGCTCTCATCTGG
	TGF- β	F: CCCCTGGAAAGGGCTCAACAC
		R: TCCAACCCAGGTCCTTCCTAAAGTC
	Caspase-8	F: ACTCGGCGACAGGTTACA
		R: GGCAGCCAGTTCTTCGTT

Caspase-9	F: ACGACCTGACTGCTAAGAAA
	R: AGCCATGAGAGAGGATGAC
RIPK	F: AC- CGCGCTGAGTACAATGA
	R: TCTCCACGATTATCCTTCCTT
XIAP	F: TGTGAGTGCTCAGAAAGATAAT
	R: TGCTTCTGCACACTGTTTACA
β -Actin	F: CCAACCGTGAAAAGATGACC
	R: ACCAGAGGCATACAGGGACA
Mouse	TNF- α
	F: CAGCCGATGGGTTGTACCTT
	R: ATAGCAAATCGGCTGACGGT
β -Actin	F: AGATCAAGATCATTGCTCCTCCTG
	R: GGGTGTA AACGCAGCTCAG

Table 2. Effect of rat FM-MSC co-culture on the expression of tumor necrosis factor (TNF)- α in RAW264.7 macrophages.

	Relative expression of mouse TNF- α
Control	1.012 (\pm 0.107)
LPS	11.62 (\pm 0.770)**
LPS+MSC	5.940 (\pm 0.504)**
Control	1.408 (\pm 0.408)
Trypsin	10.13 (\pm 1.872)**
Trypsin+MSC	5.624 (\pm 0.123) [#]

The values were the mean (\pm SEM). **P < 0.01 vs. Control, **P < 0.01 vs. LPS #P < 0.05 vs. Trypsin.

Table 3. Effect of human amnion-derived mesenchymal stem cells (hAMSC)-conditioned medium (CM) on infoammatory reaction in cerulean-stimulated rat pancreatic acinar cells.

	Control	Cerulein	Cerulein+CM
Cell count (10^6)	5.300 (± 0.954)	3.733 (± 0.809)	3.267 (± 0.555)
Amylase (IU/cell count/ 10^6)	1866 (± 6.8)	4023 (± 171.7) **	2673 (± 146.4) ##
Caspase-3/7 activity (Luminescence/ 10^5)	19.778 (± 1.199)	41.535 (± 1.094) **	25.178 (± 1.811) ##
Relative expression			
Amylase	1.001 (± 0.023)	3.708 (± 0.077) **	1.976 (± 0.089) ##
Lipase	1.012 (± 0.110)	33.59 (± 0.233) **	18.10 (± 0.476) ##
Caspase-8	1.001 (± 0.030)	1.694 (± 0.014) **	1.263 (± 0.062) ##
Caspase-9	1.001 (± 0.026)	2.057 (0.323) *	1.019 (0.010) #
RIPK	1.002 (± 0.042)	1.320 (± 0.117) *	1.222 (± 0.046) #
XIAP	1.000 (± 0.016)	1.290 (± 0.031) **	0.895 (± 0.006) ##

The values were the mean (\pm SEM). * $P < 0.05$, ** $P < 0.01$ vs. Control, # $P < 0.05$, ## $p < 0.01$ vs. Cerulein.

Table 4. Effect of human amnion-derived mesenchymal stem cells (hAMSC)-conditioned medium (CM) on inflammatory reaction in TNF- stimulated human pancreatic stellate cells (PSCs)

	Control	TNF	TNF + CM
MTS assay (absorbance)	1.000 (± 0.064)	0.915 (± 0.055)	0.768 (± 0.039)
Relative expression			
MCP-1	1.007 (± 0.084)	13.39 (± 1.124) **	2.328 (± 0.091) ##
IL-8	1.001 (± 0.034)	24.43 (± 0.542) **	17.04 (± 0.339) #

The values were the mean (\pm SEM). **P < 0.01 vs. Control, #P < 0.05, ##P < 0.01 vs. TNF- α .

Figure 1A_1

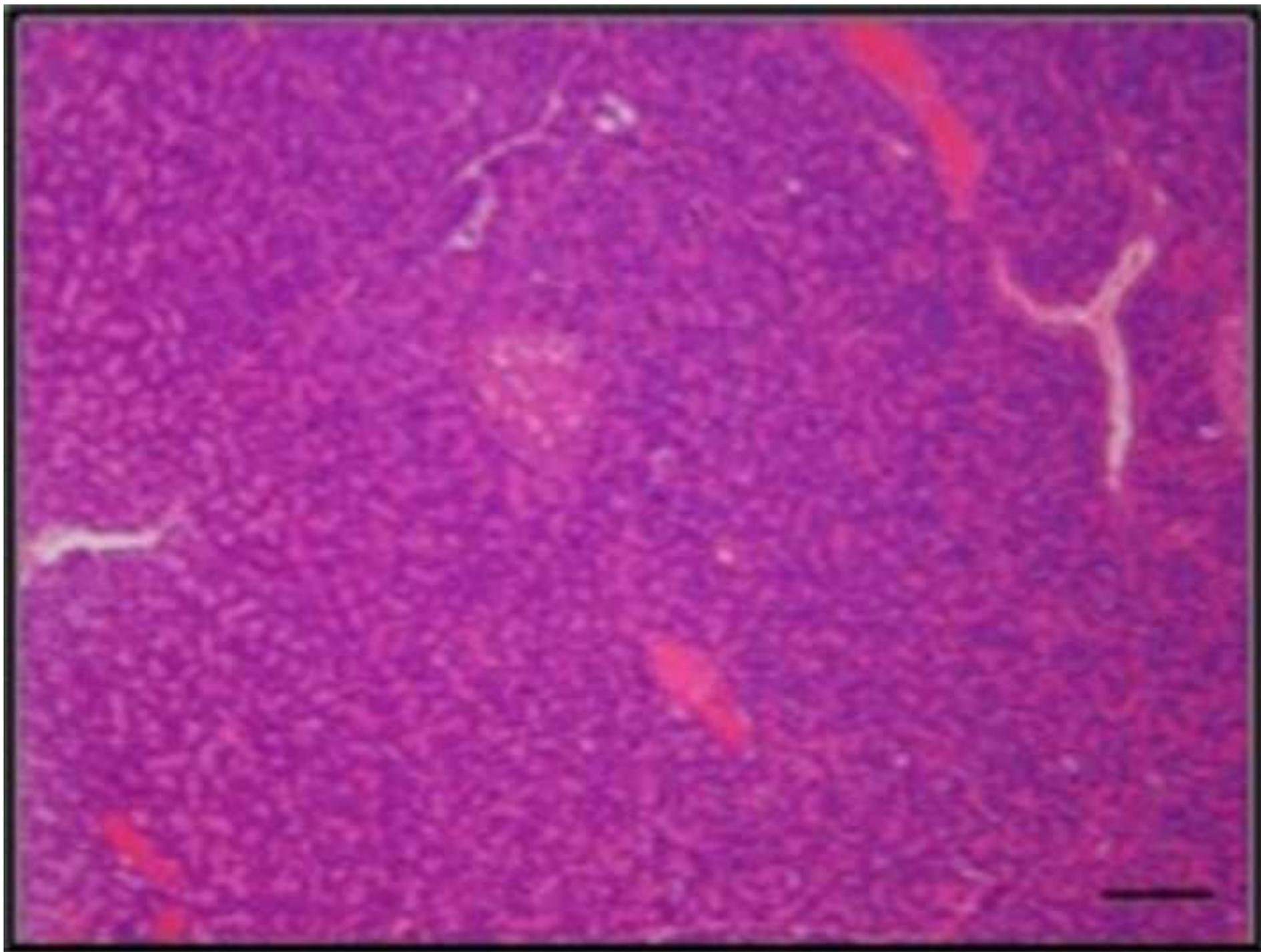


Figure 1A_2

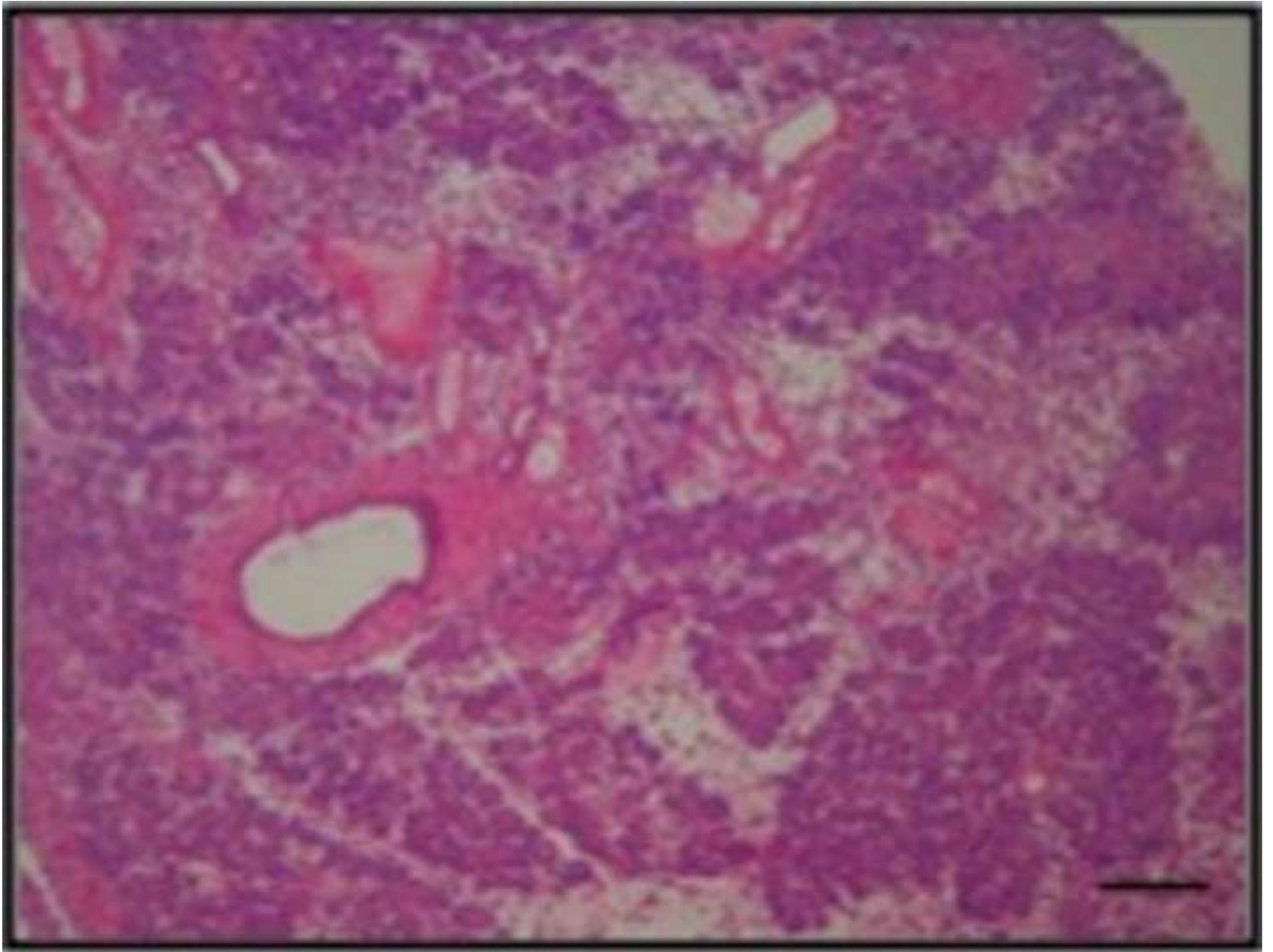


Figure 1A_3

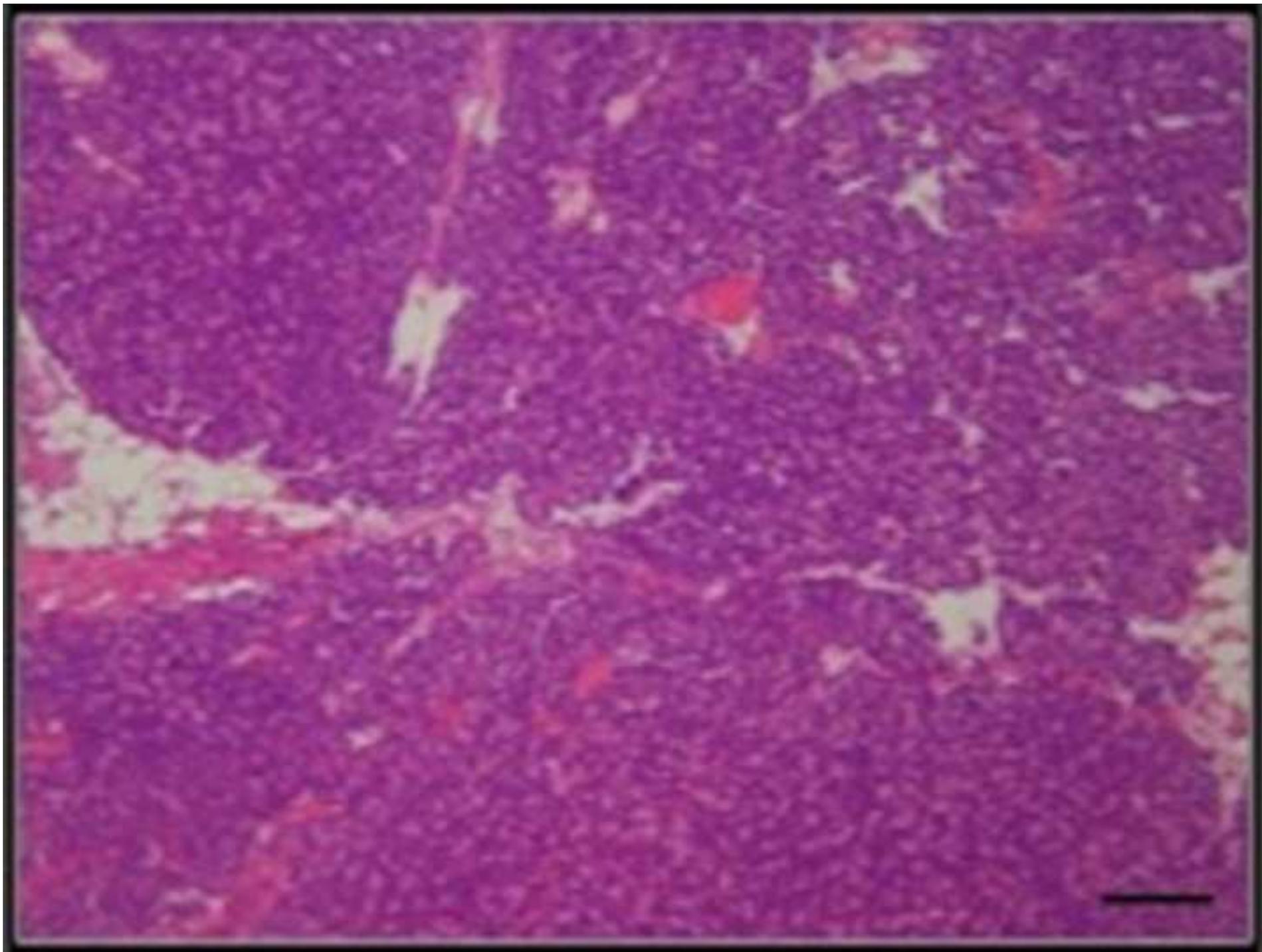


Figure 1A_4

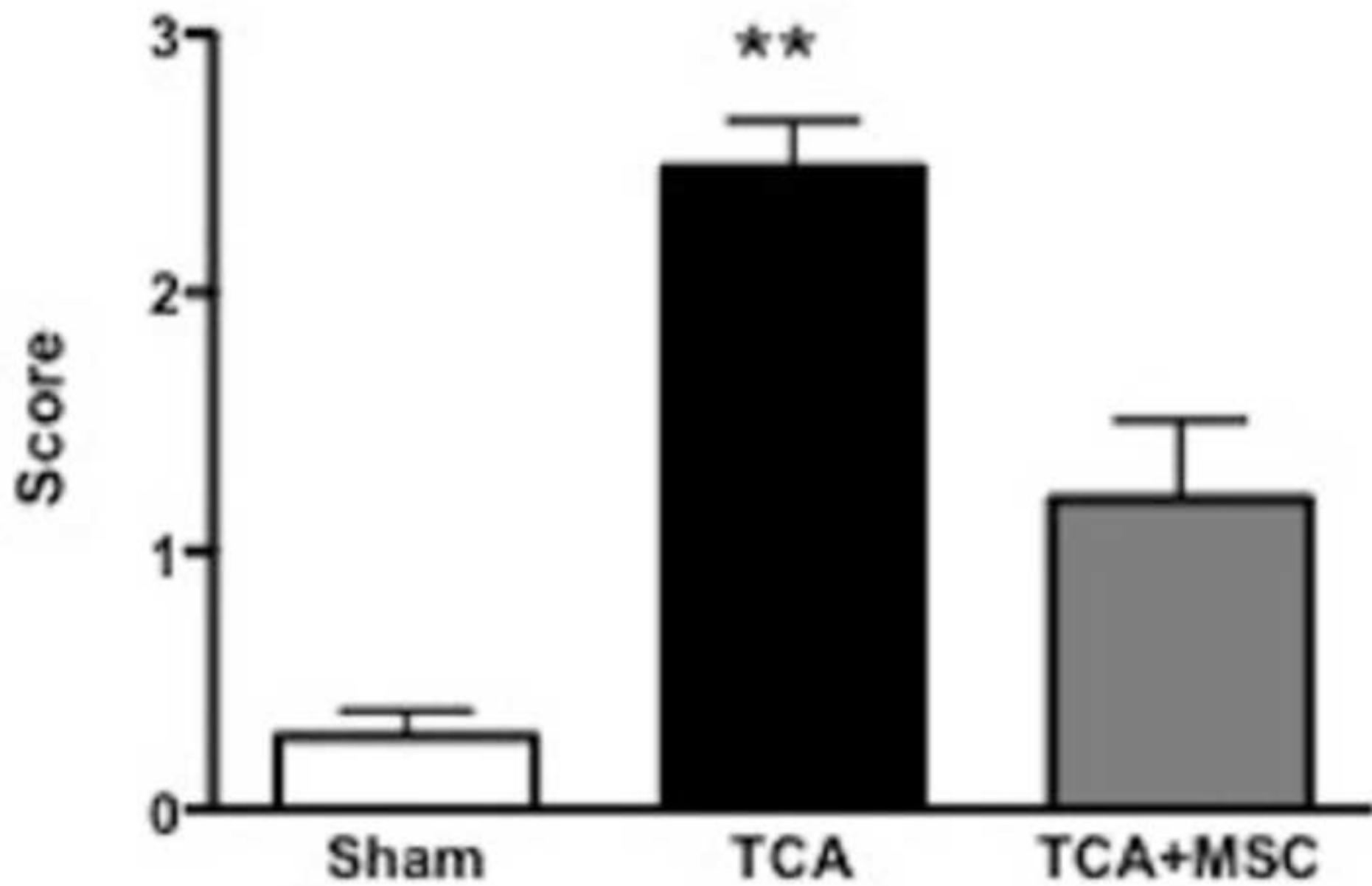


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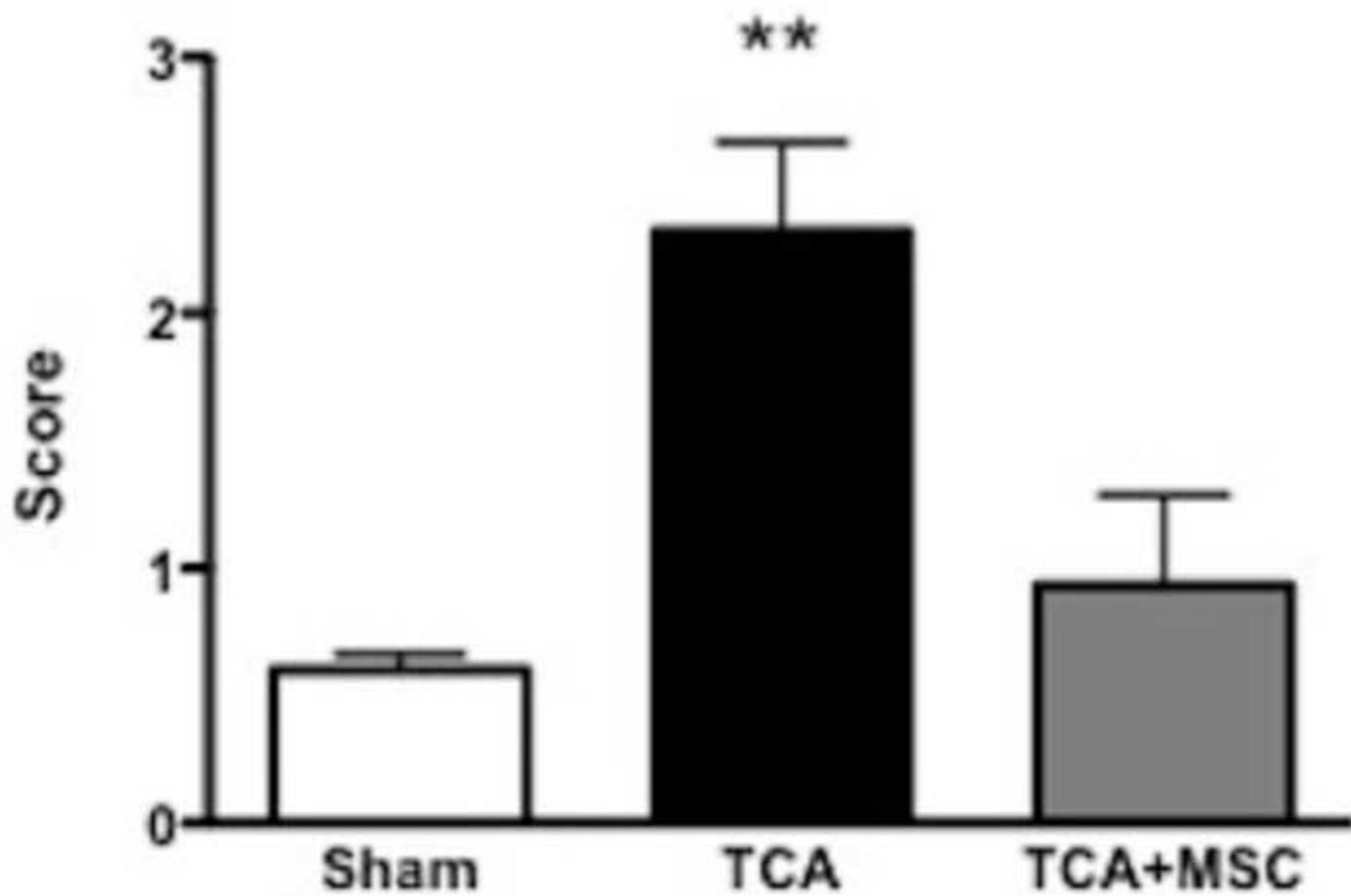


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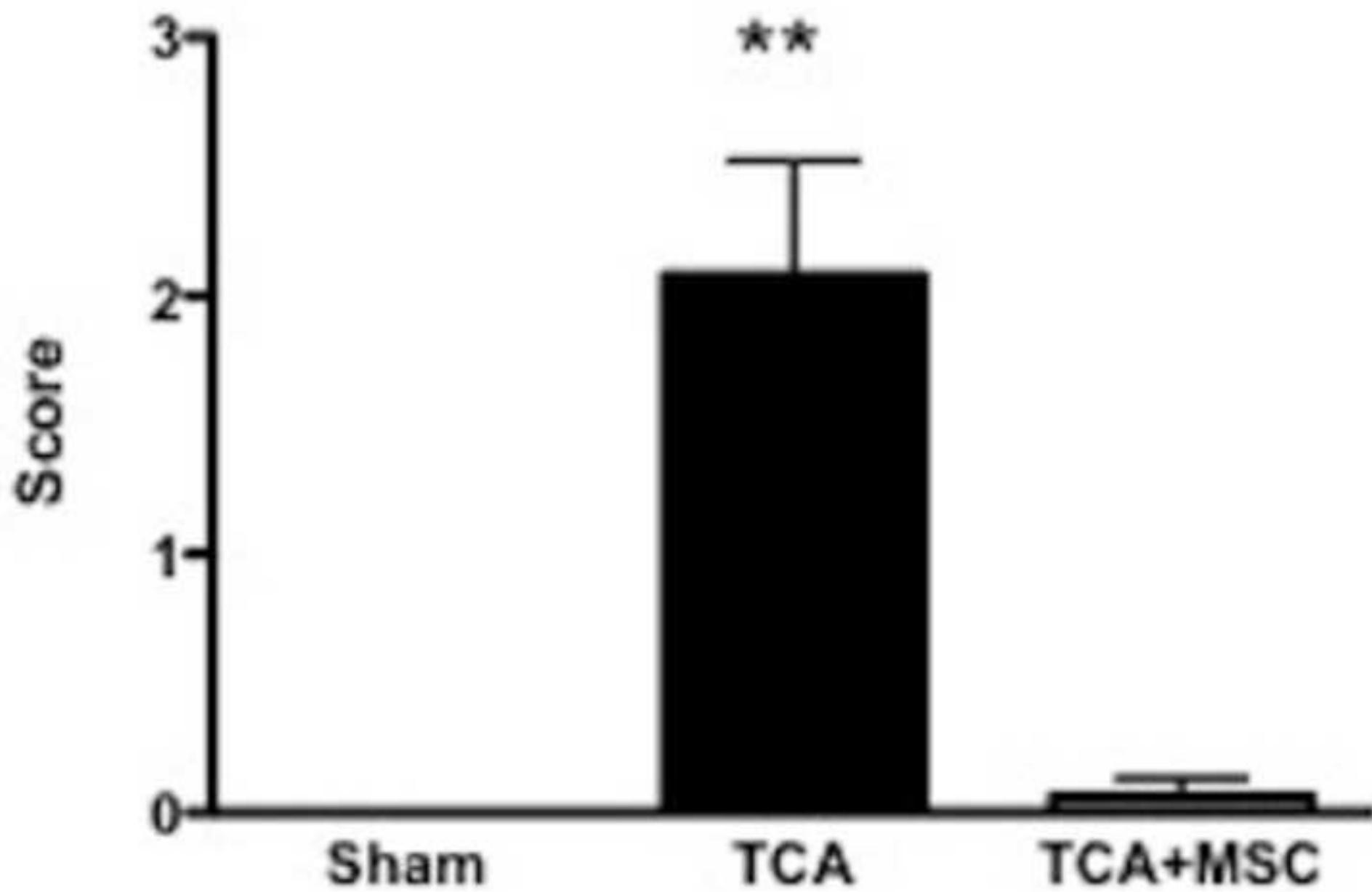


Figure 1B_1

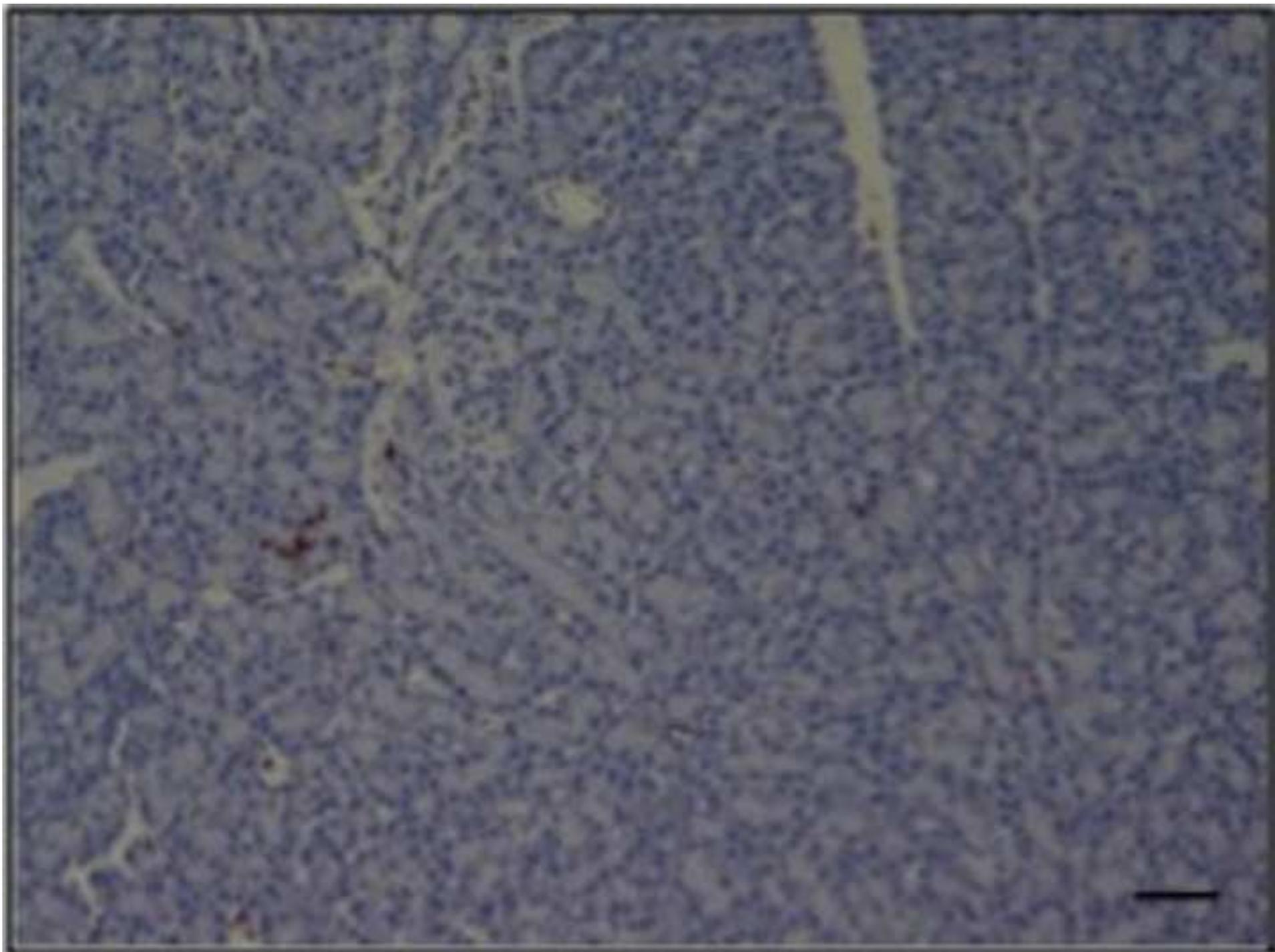


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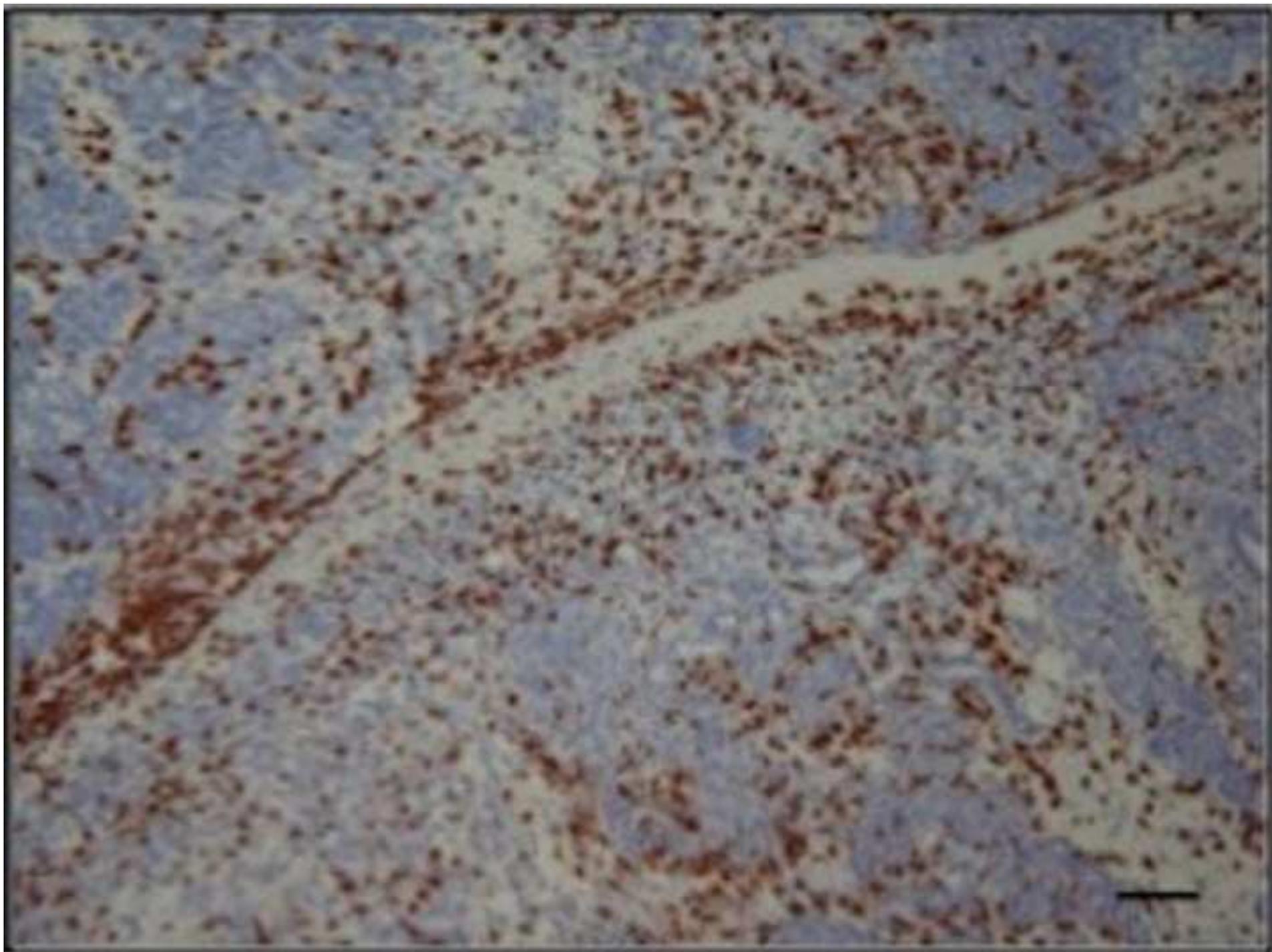


Figure 1B_3

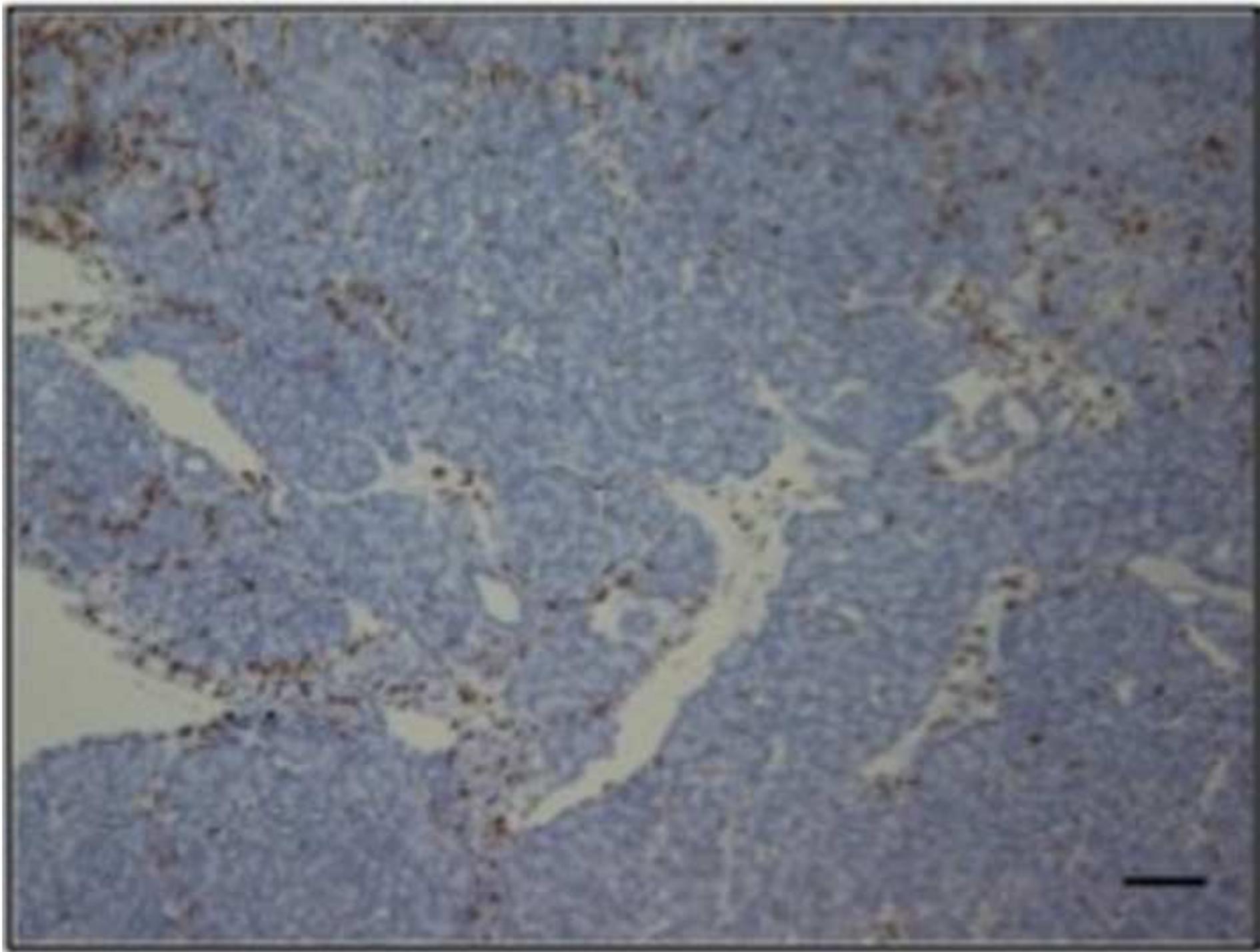


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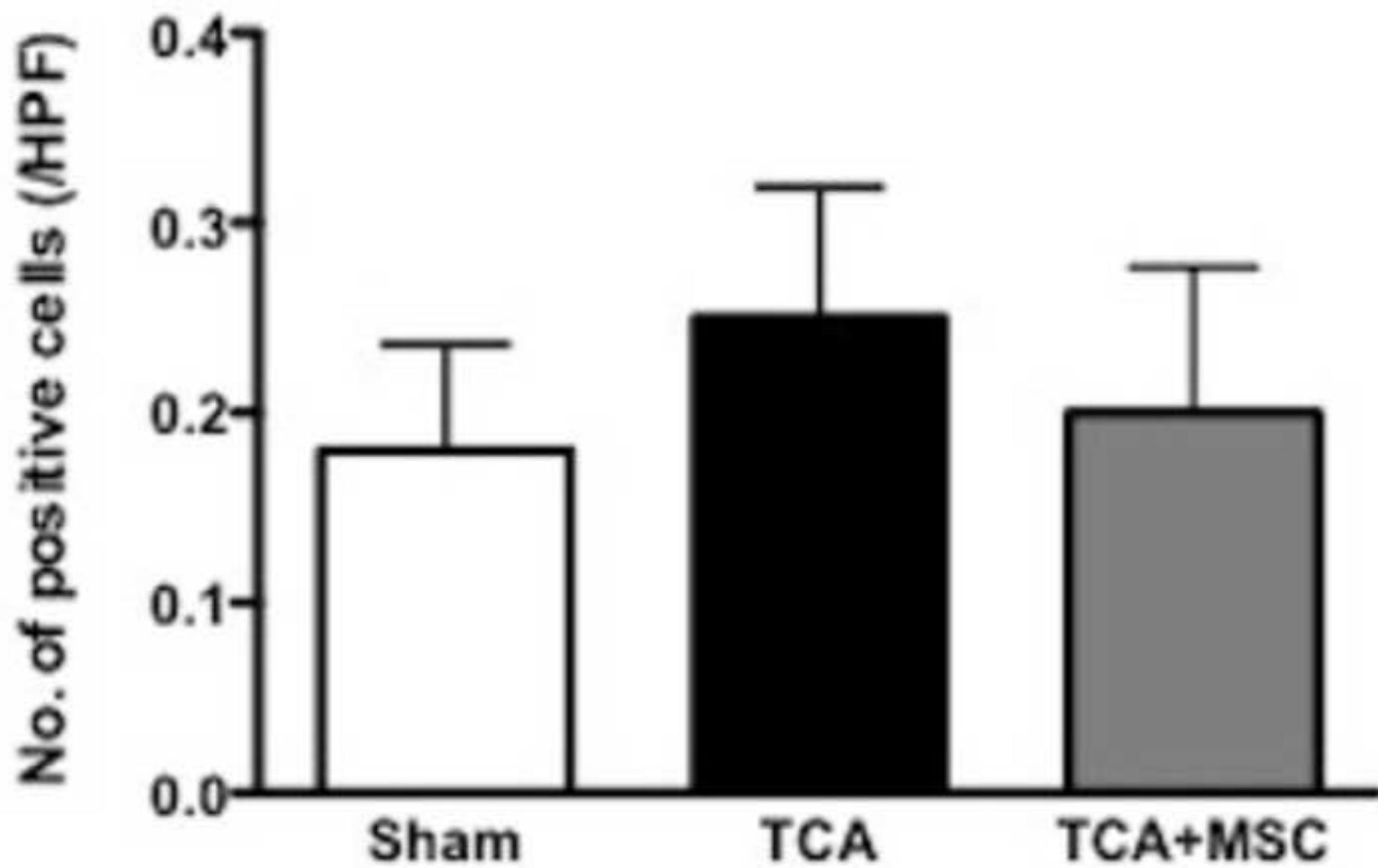


Figure 1C_1

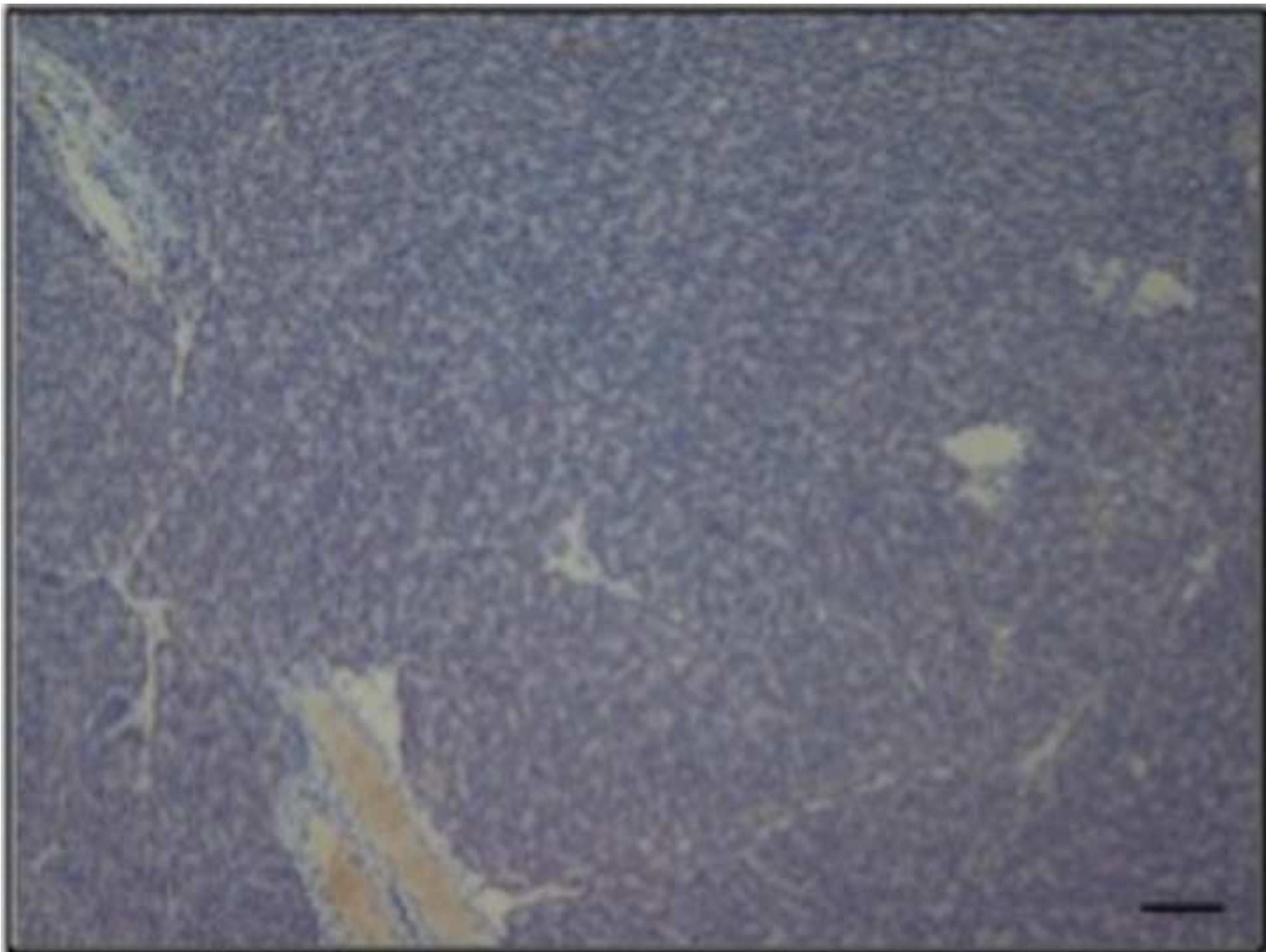


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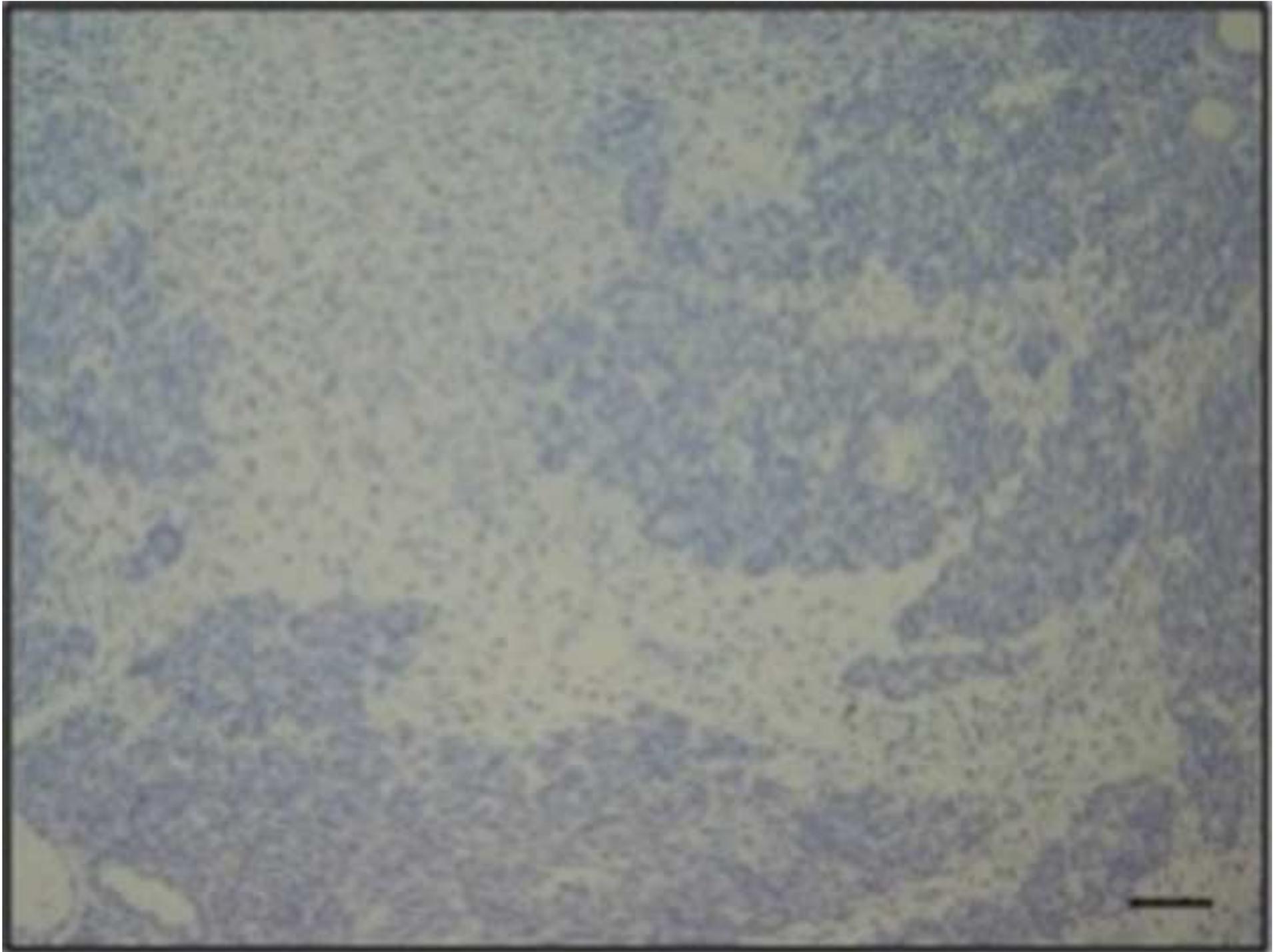


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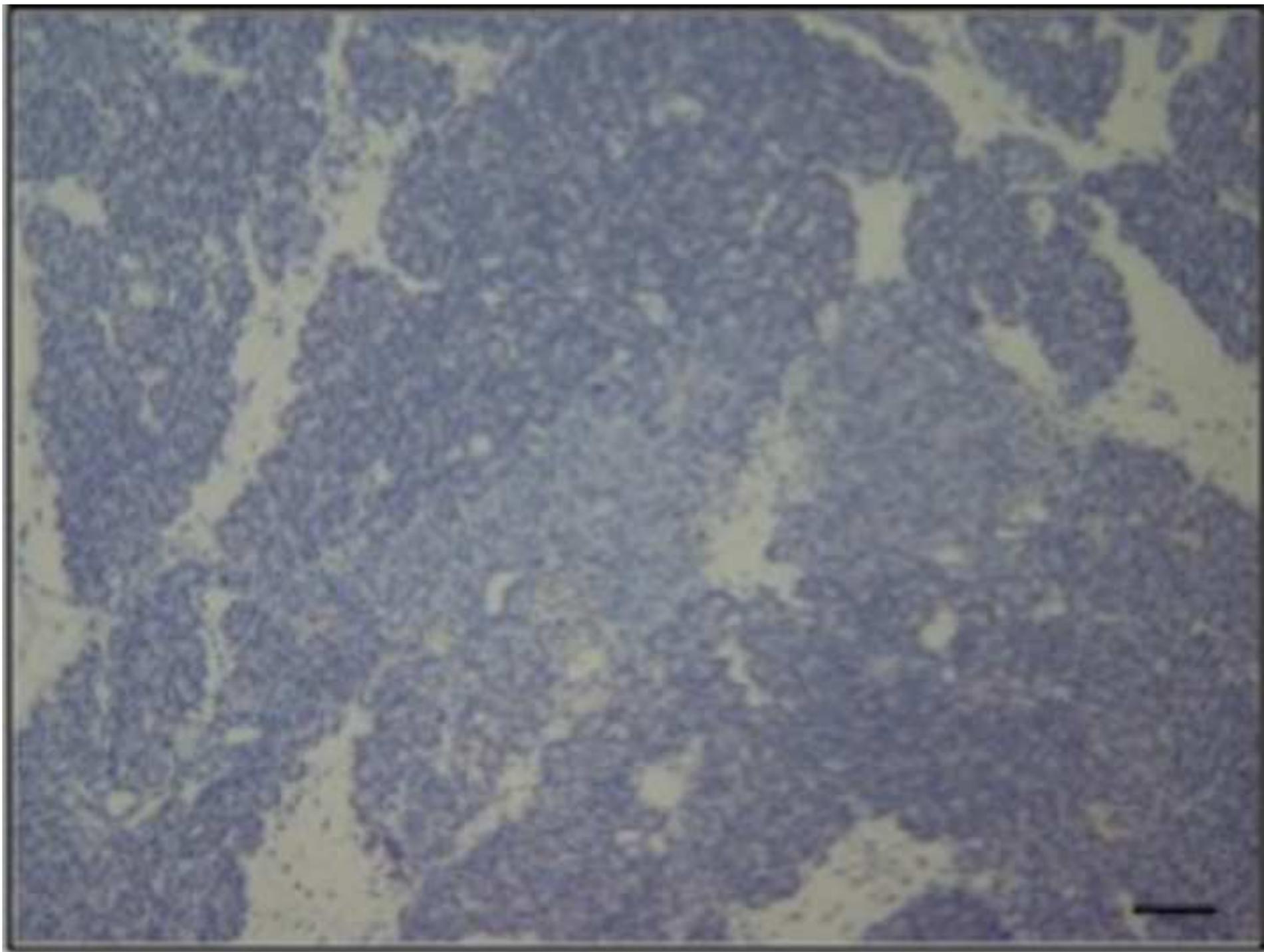


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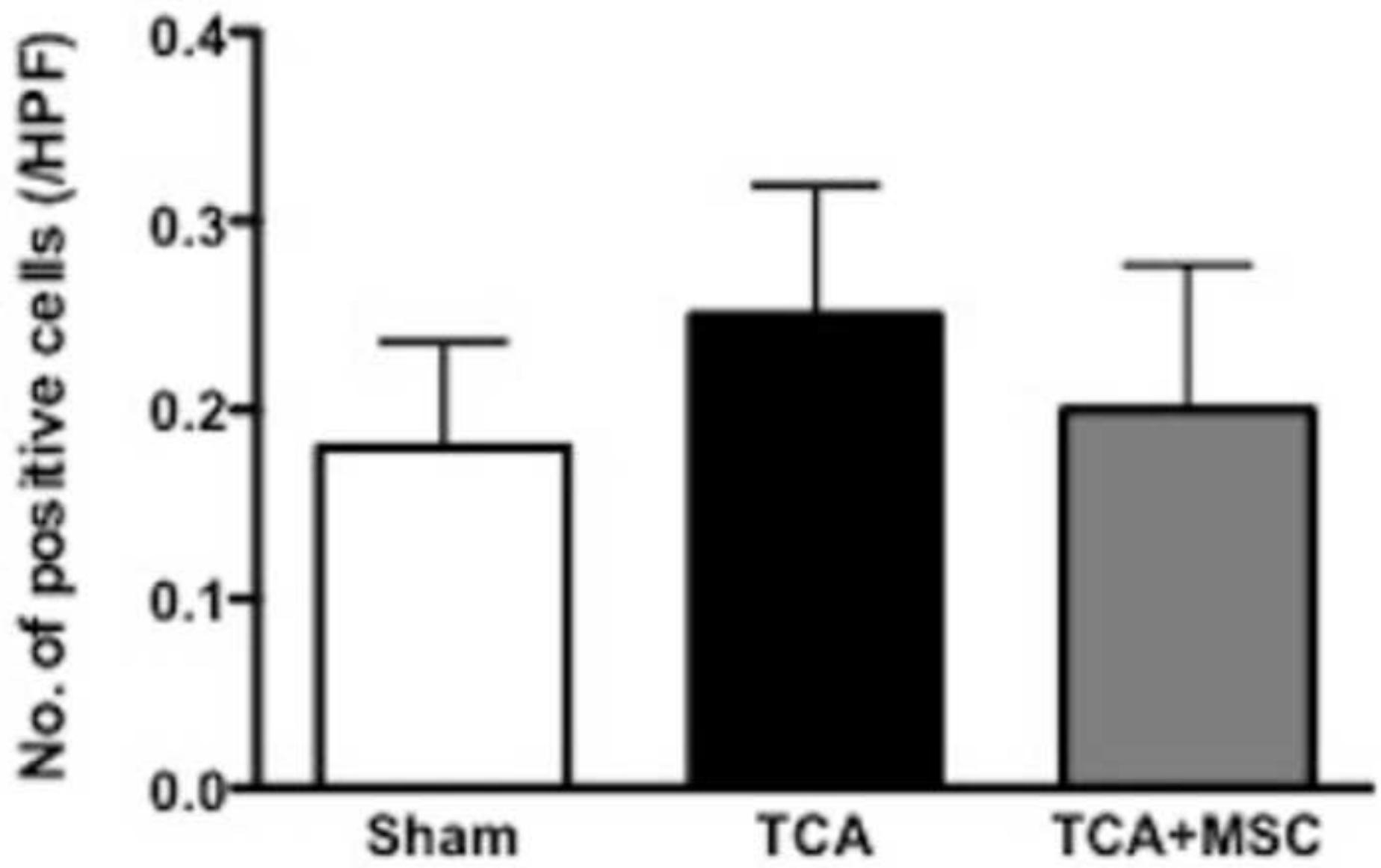


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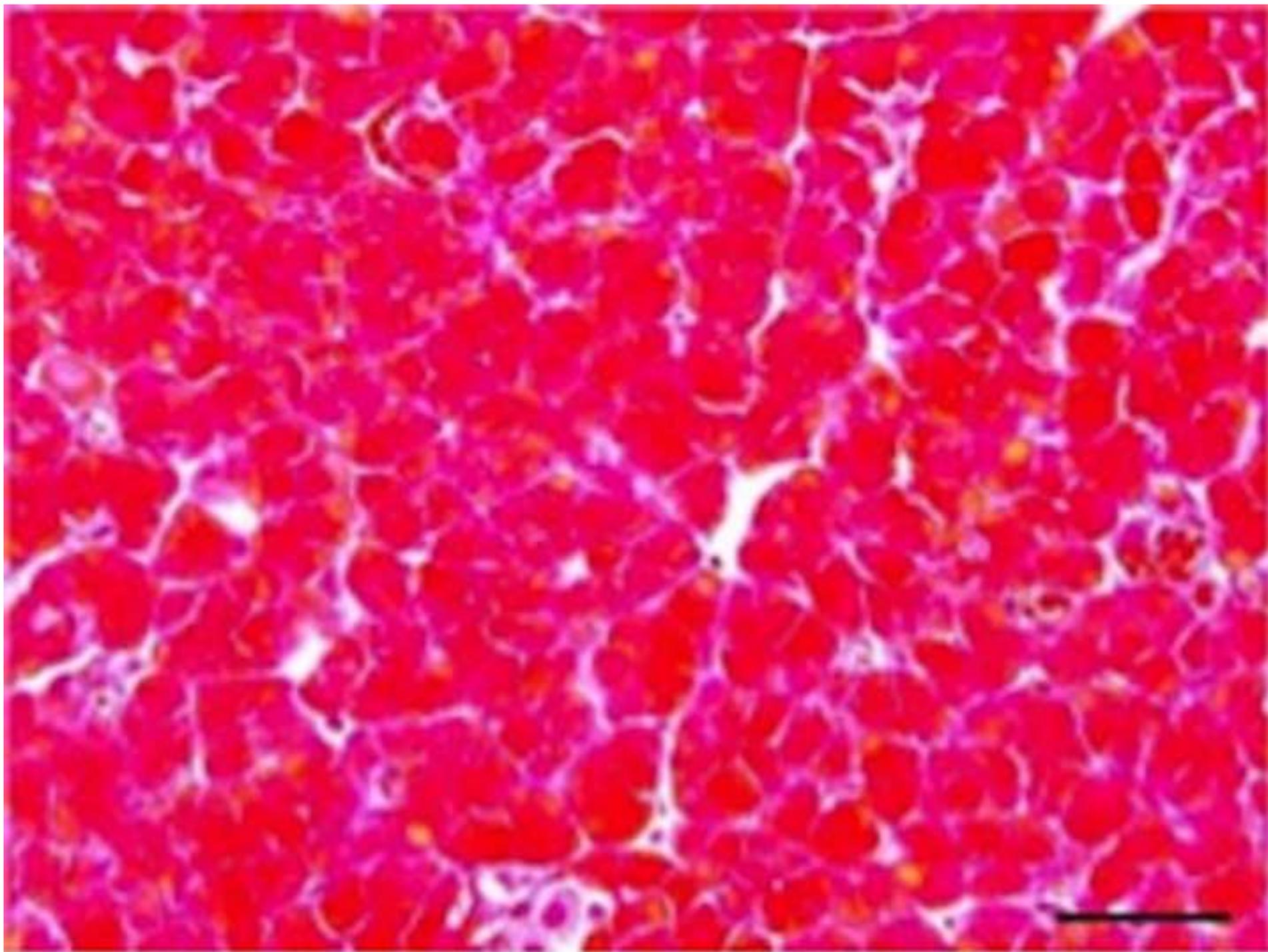


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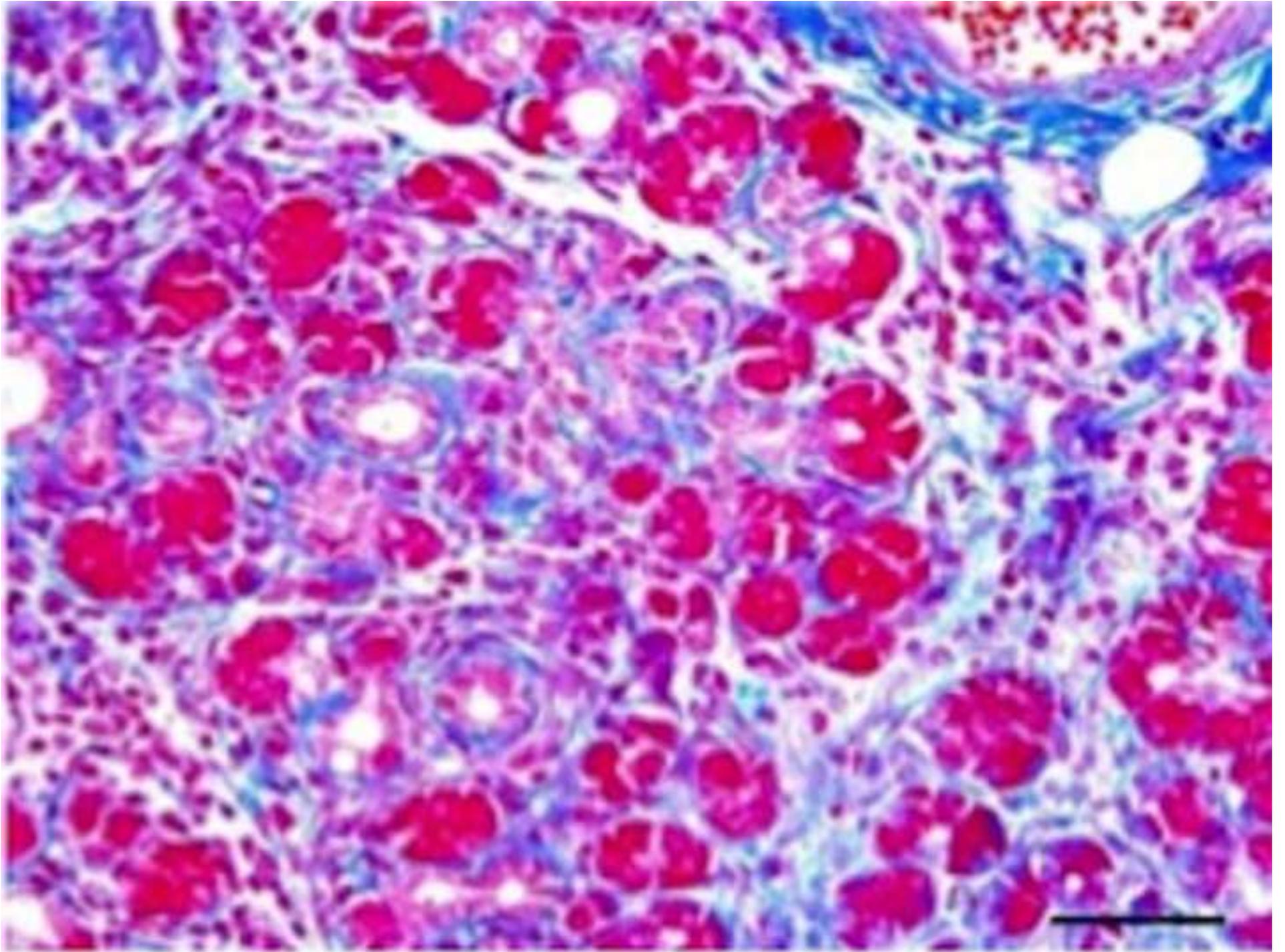


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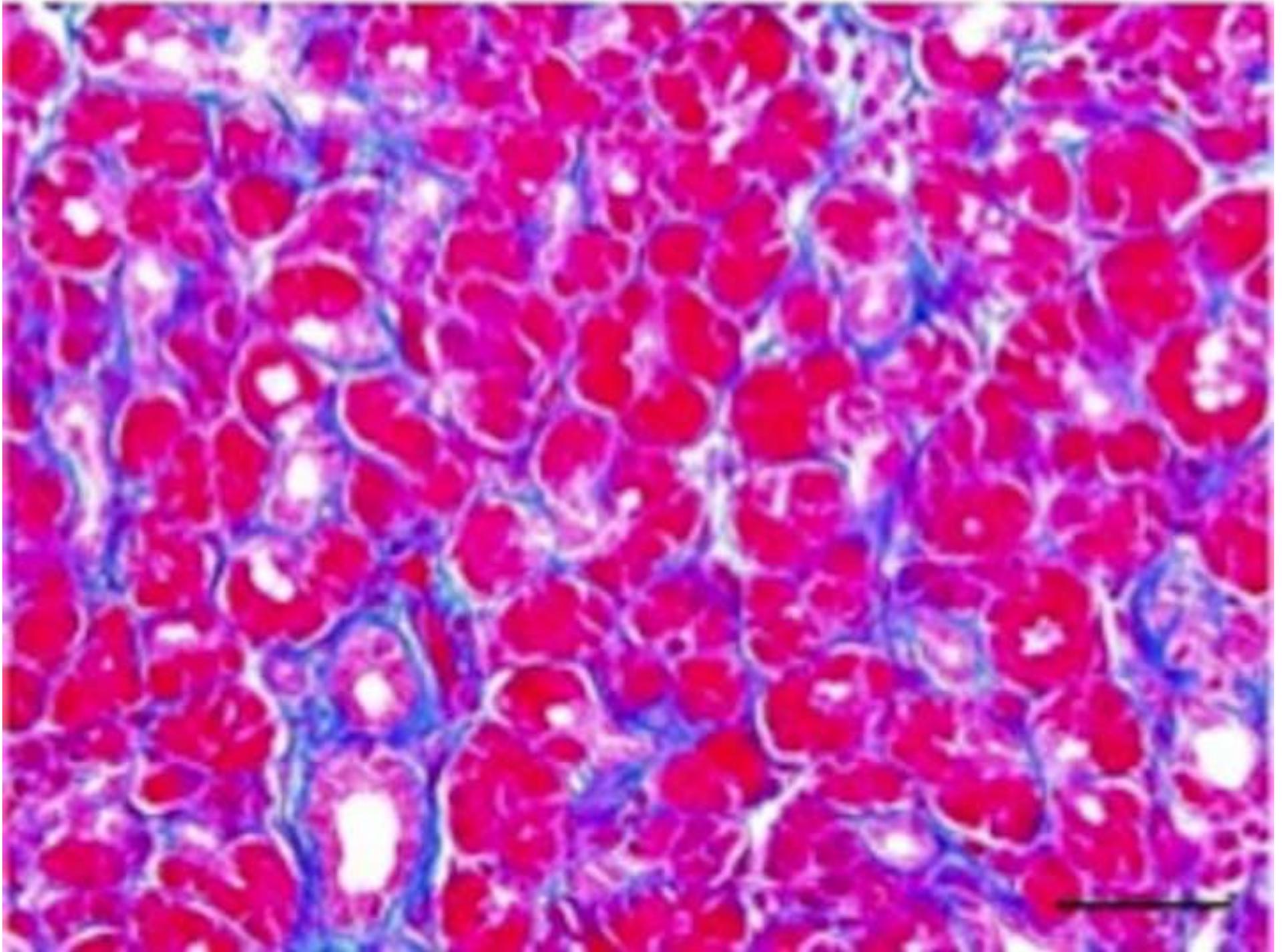


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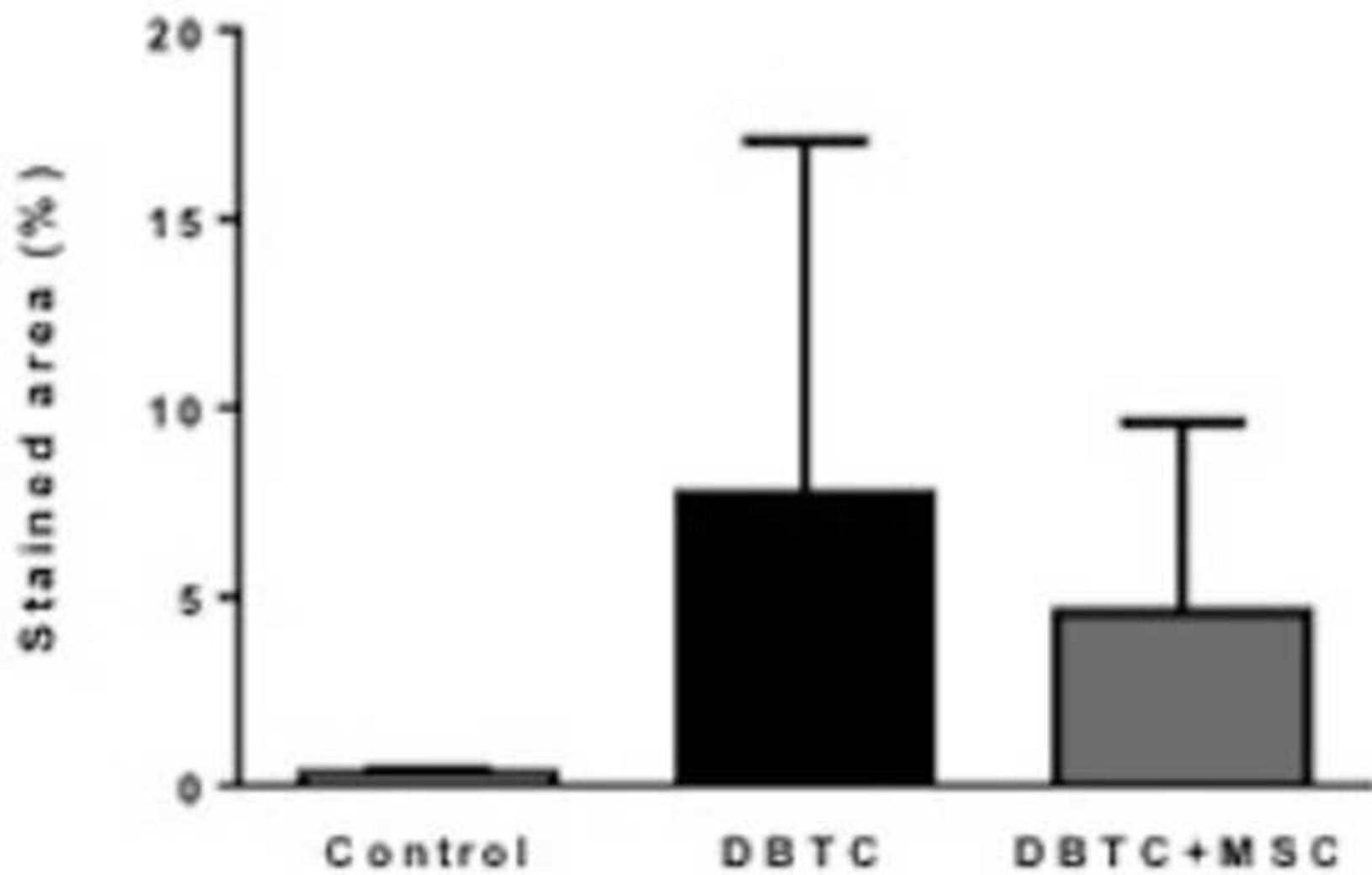


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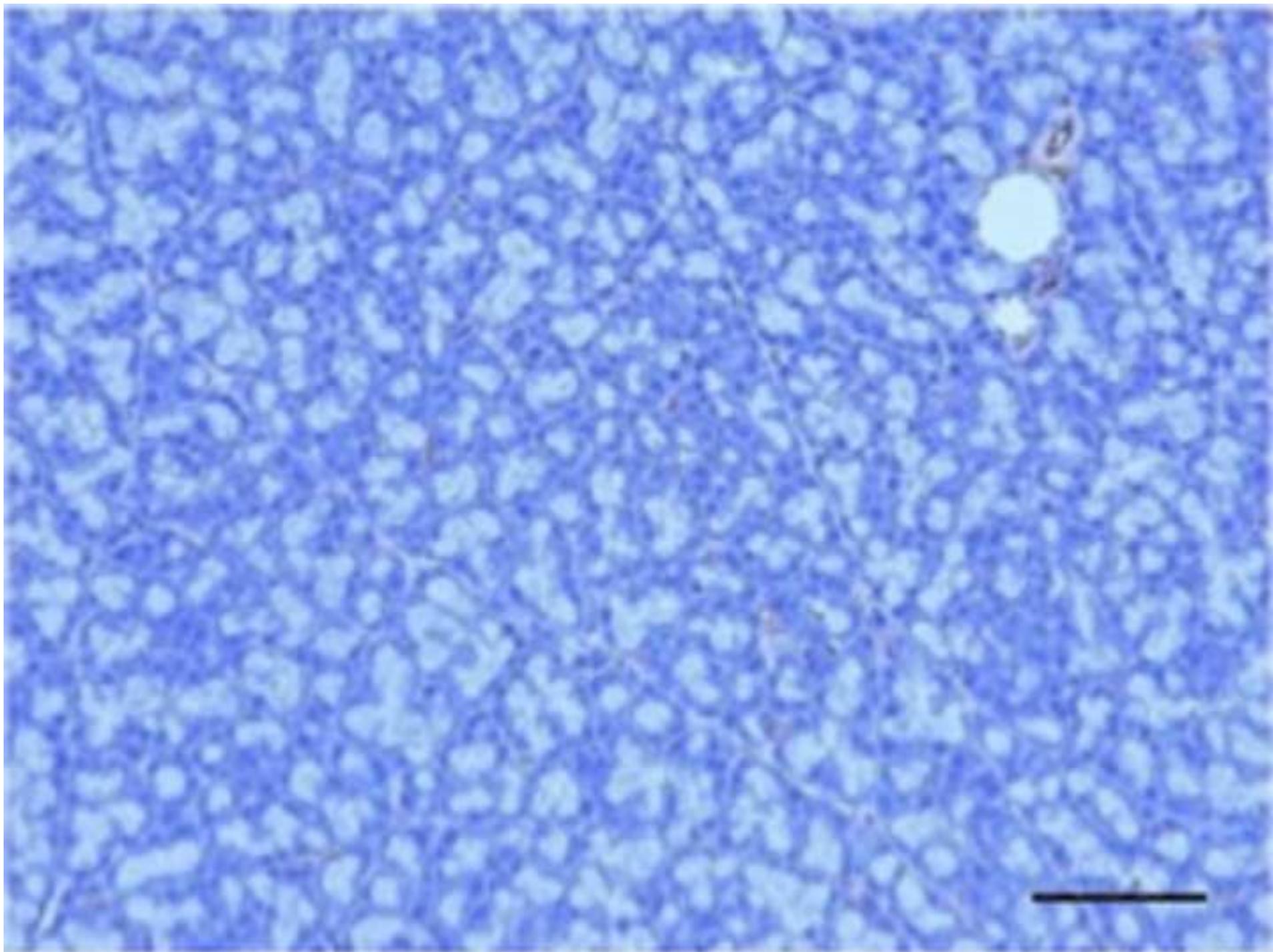


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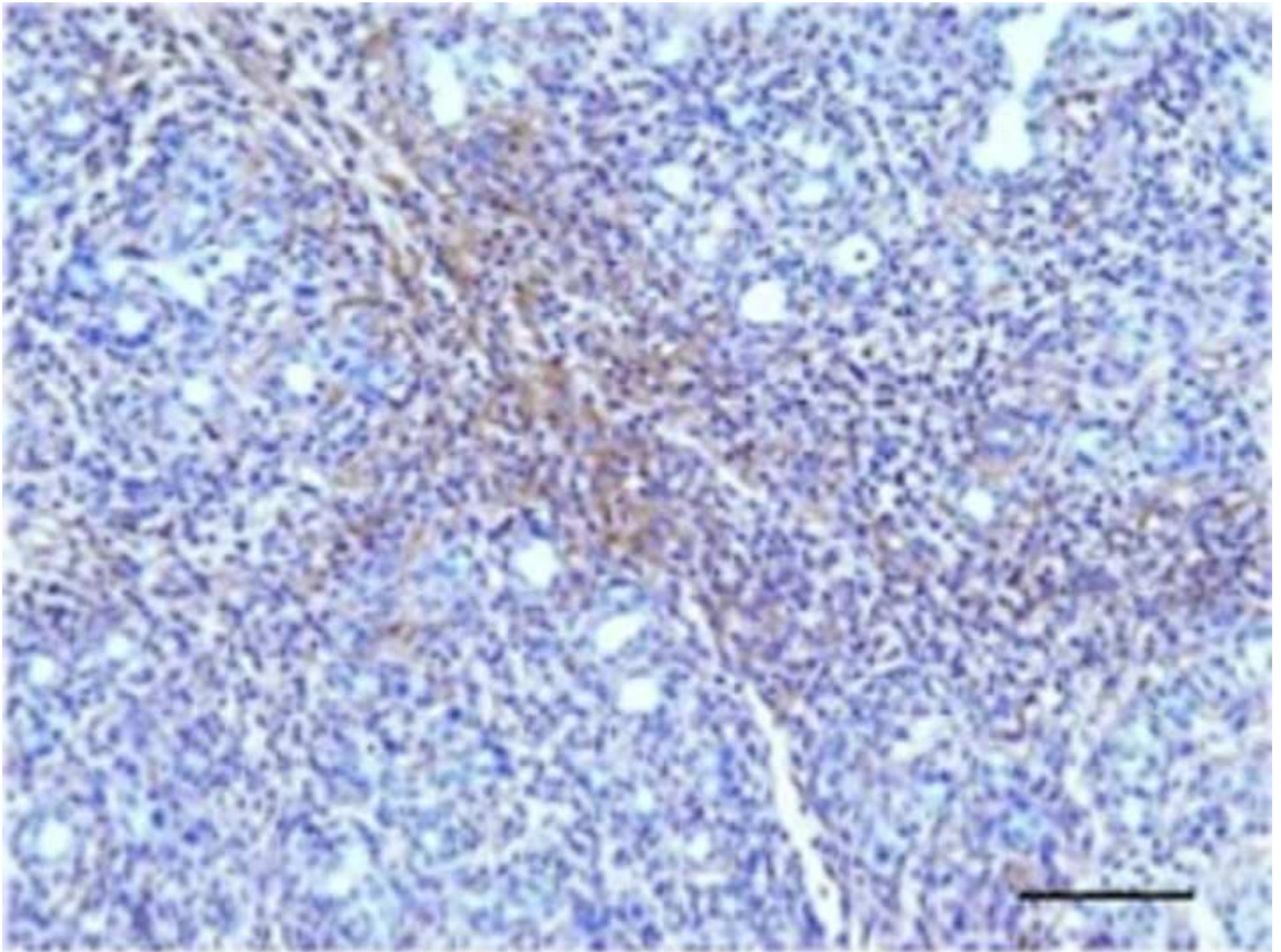


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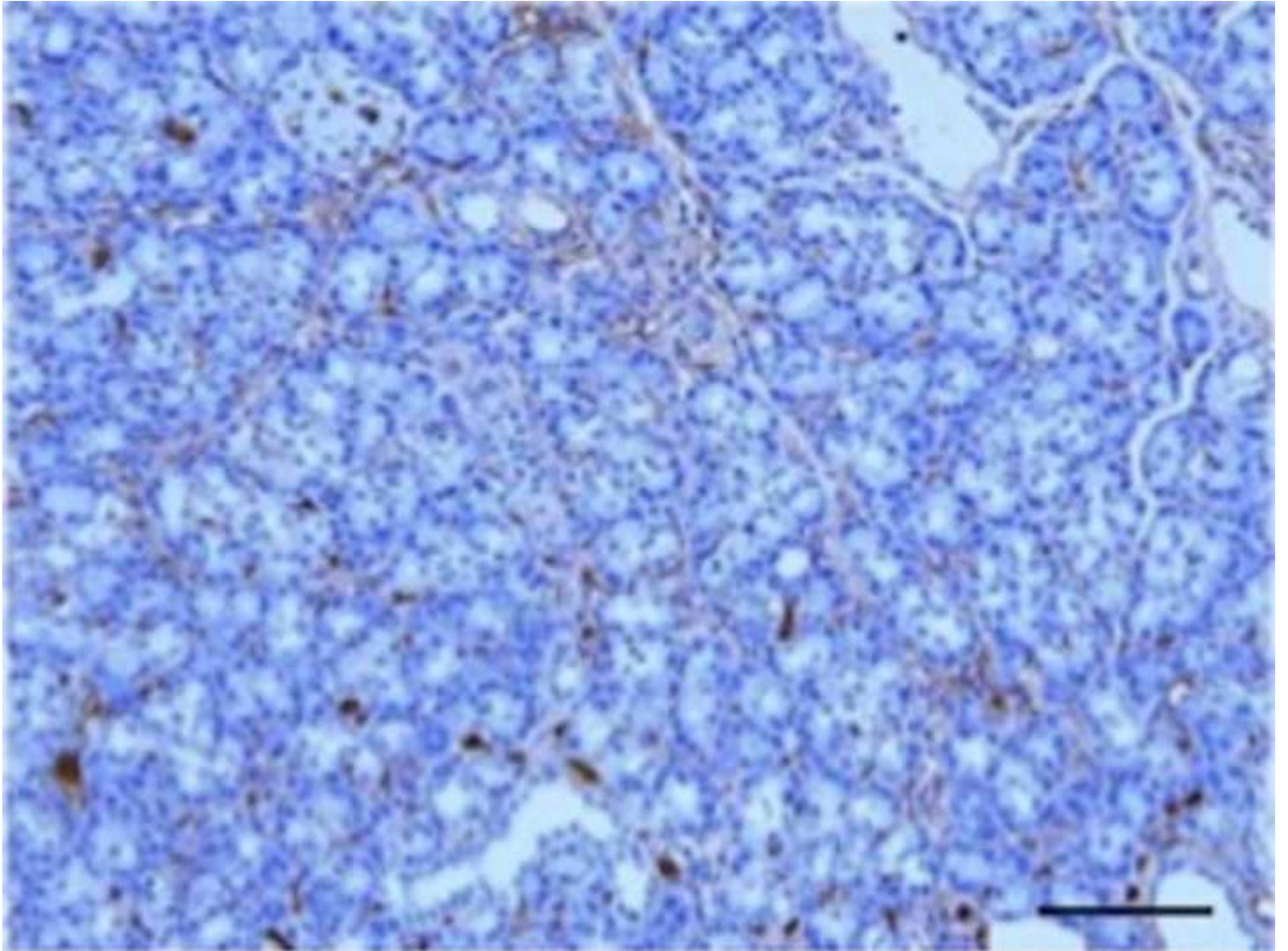


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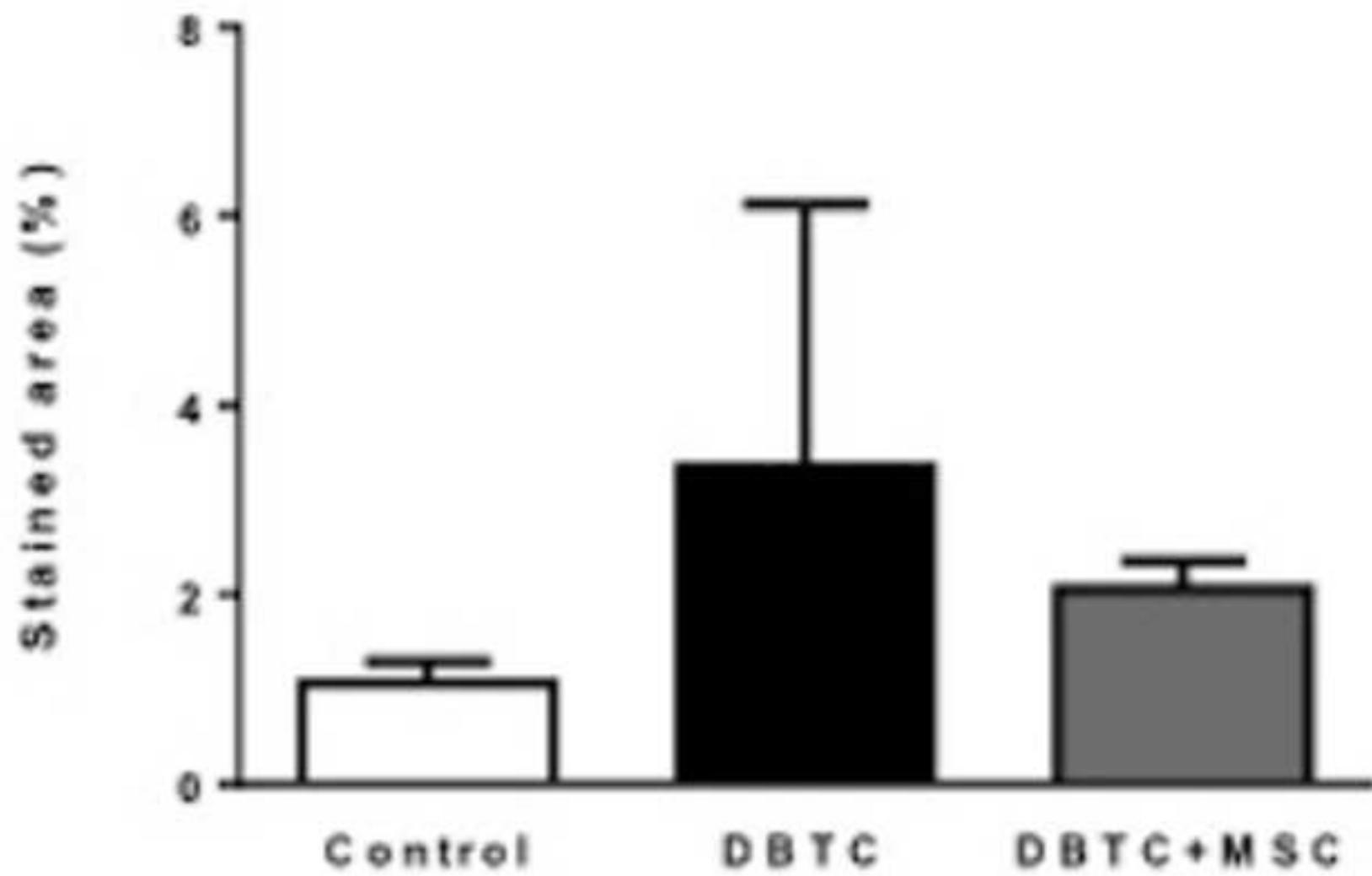


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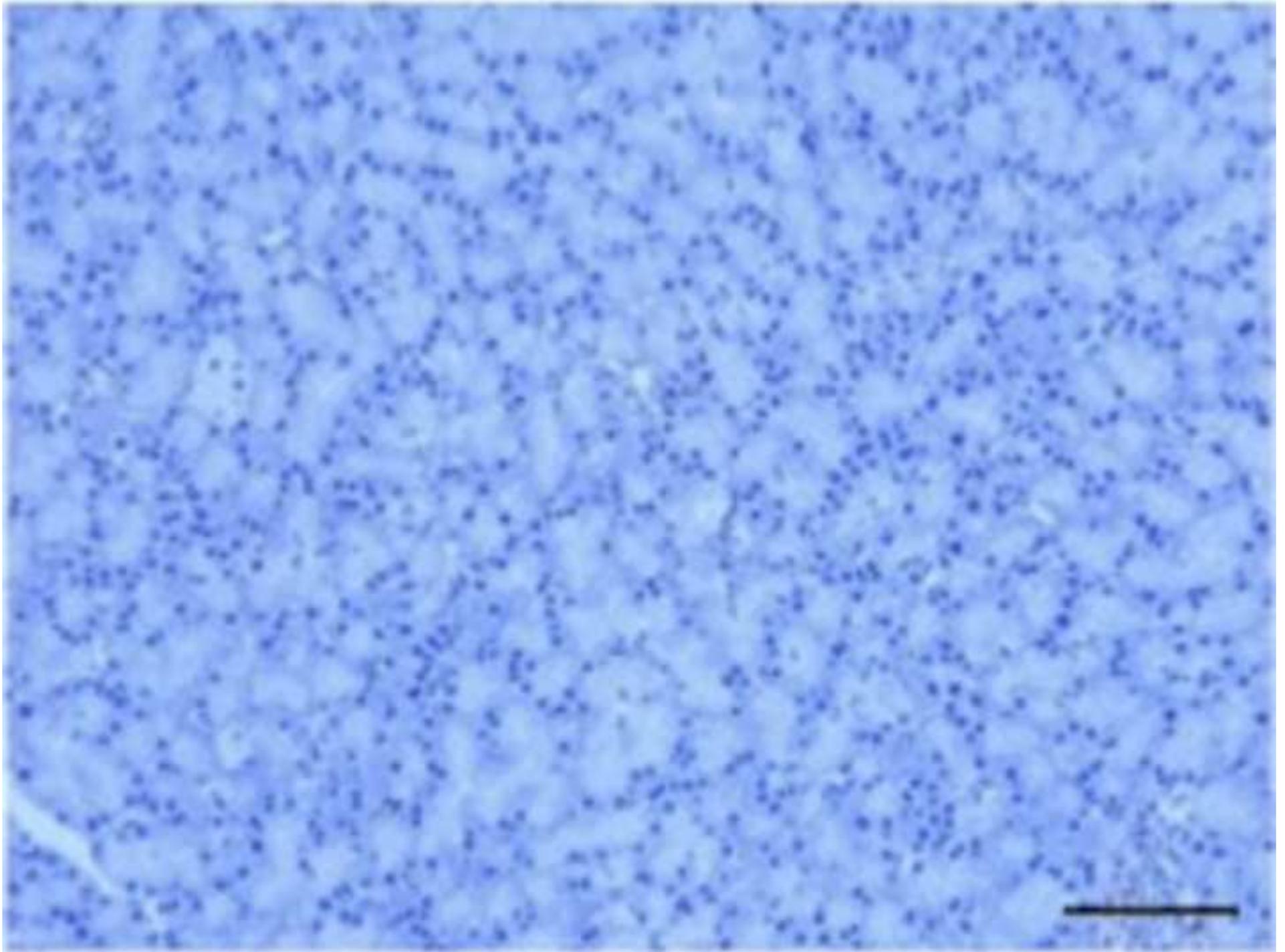


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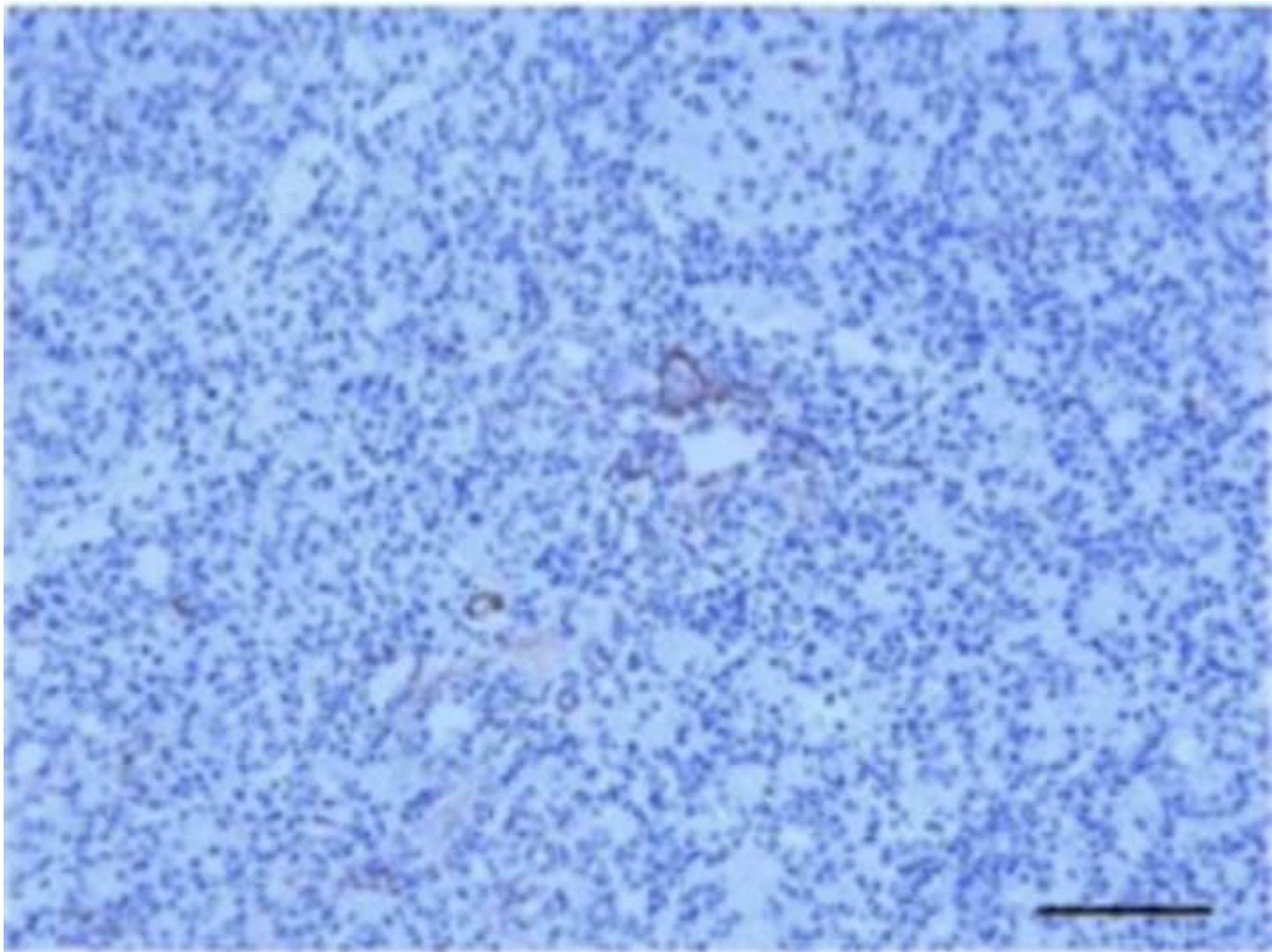


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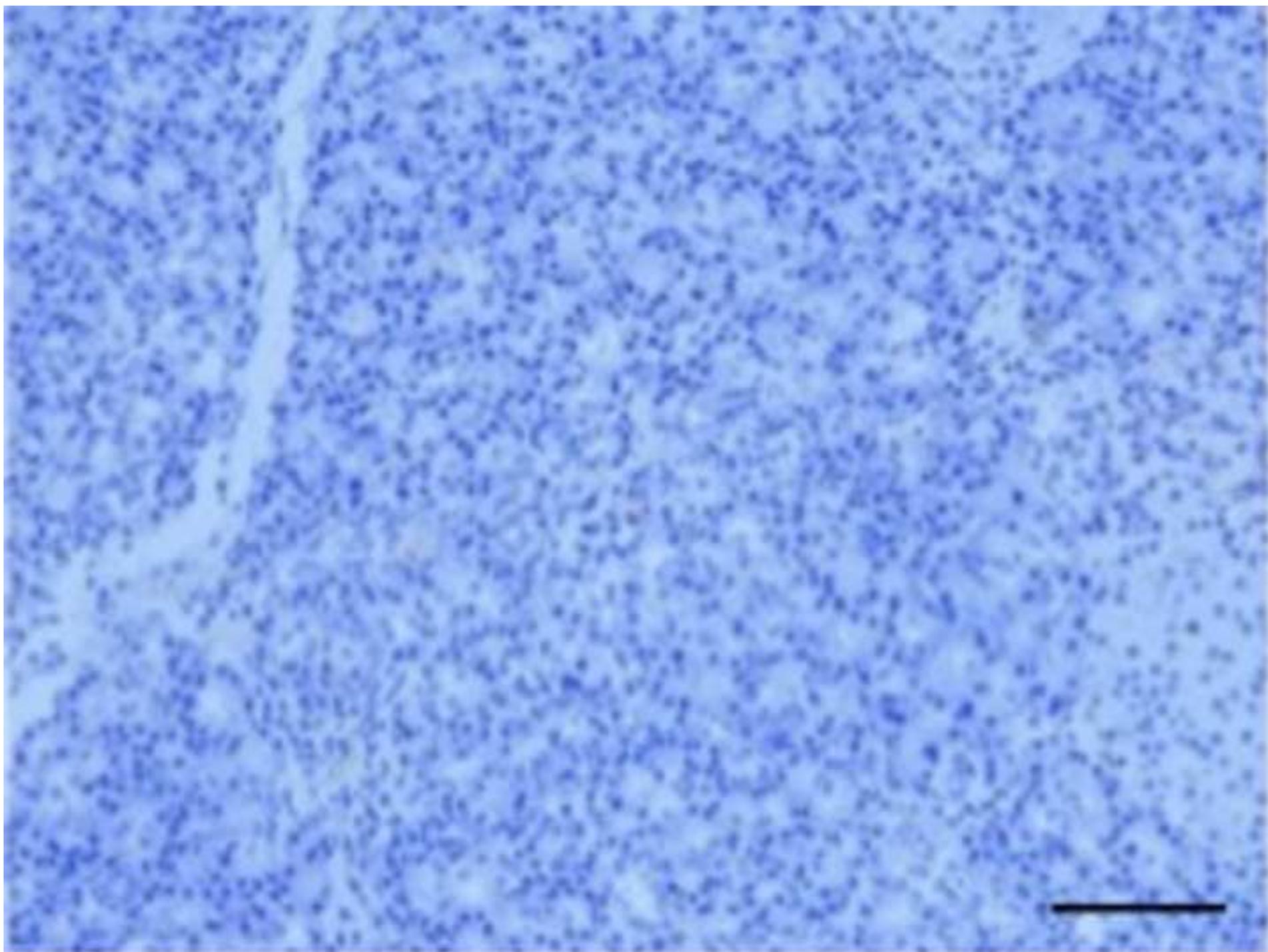


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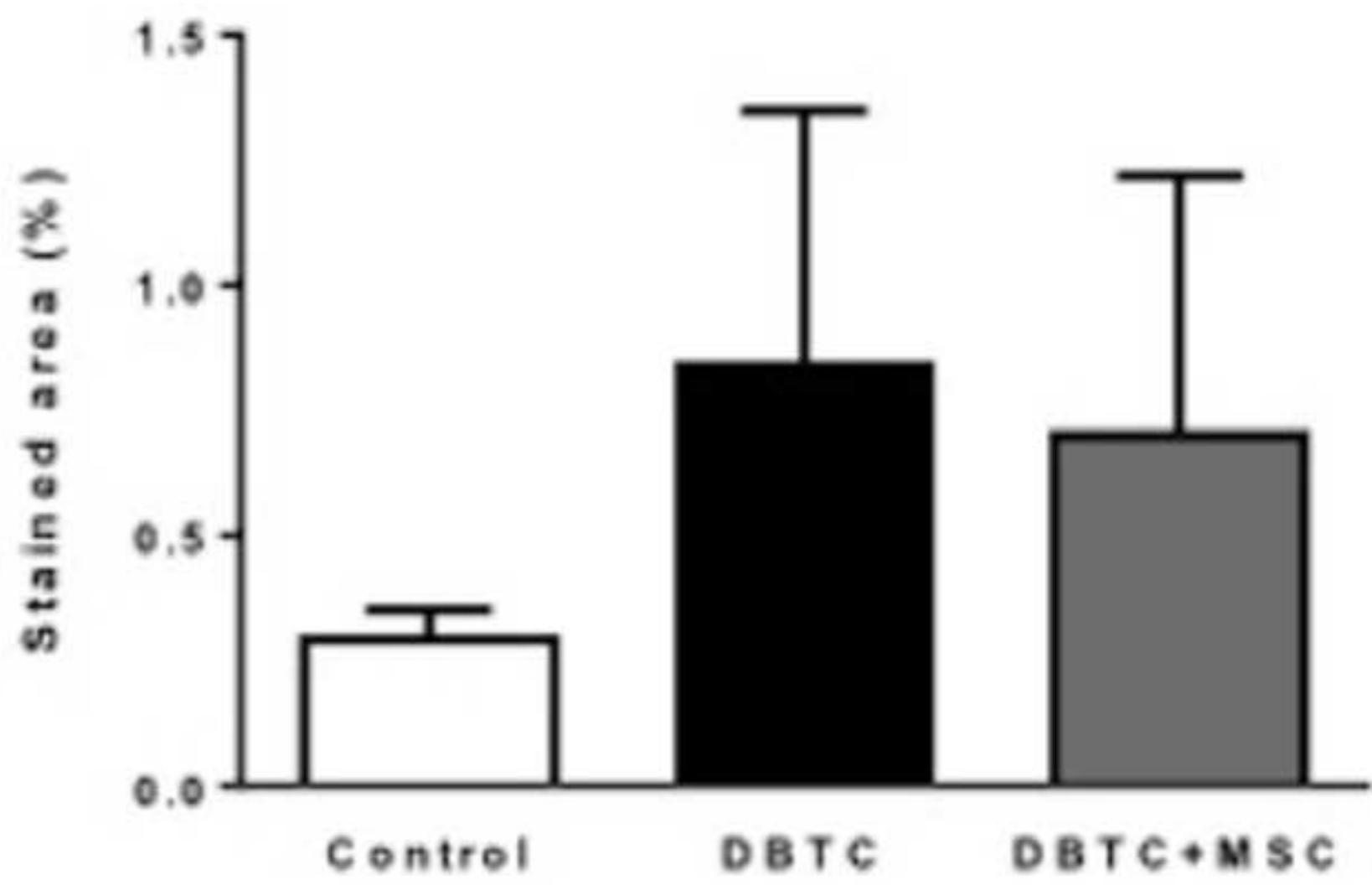


Figure 2D_1

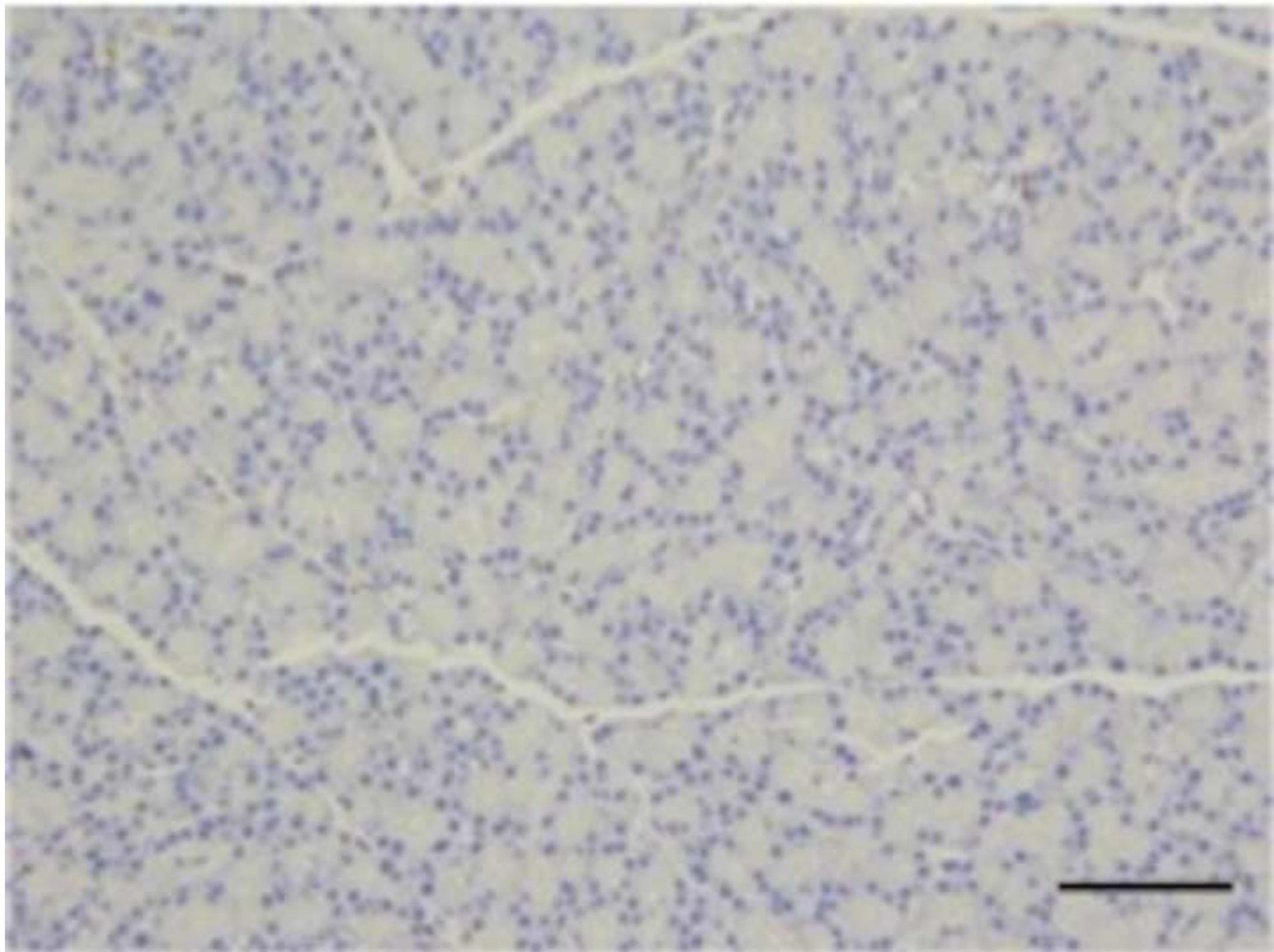


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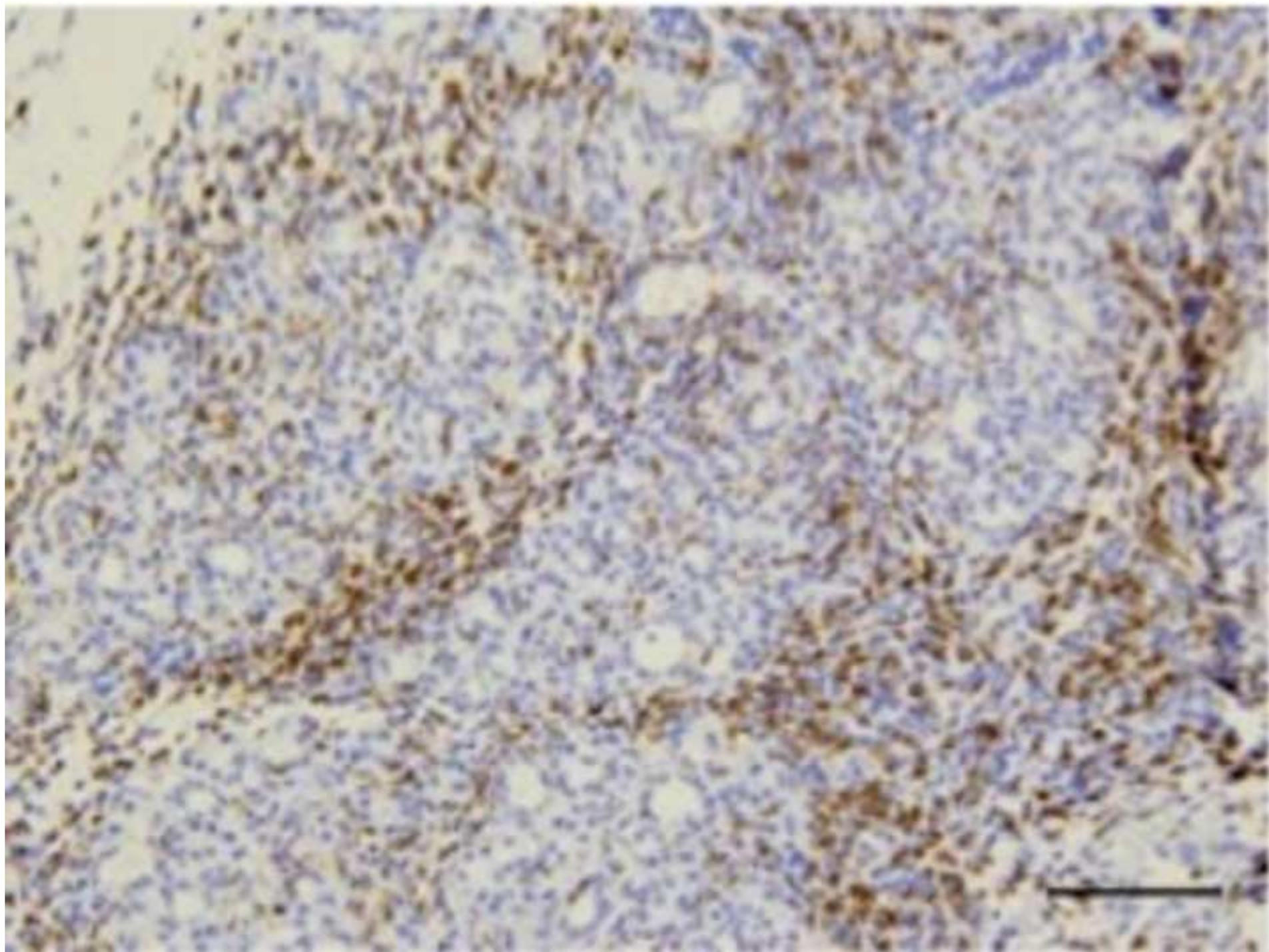


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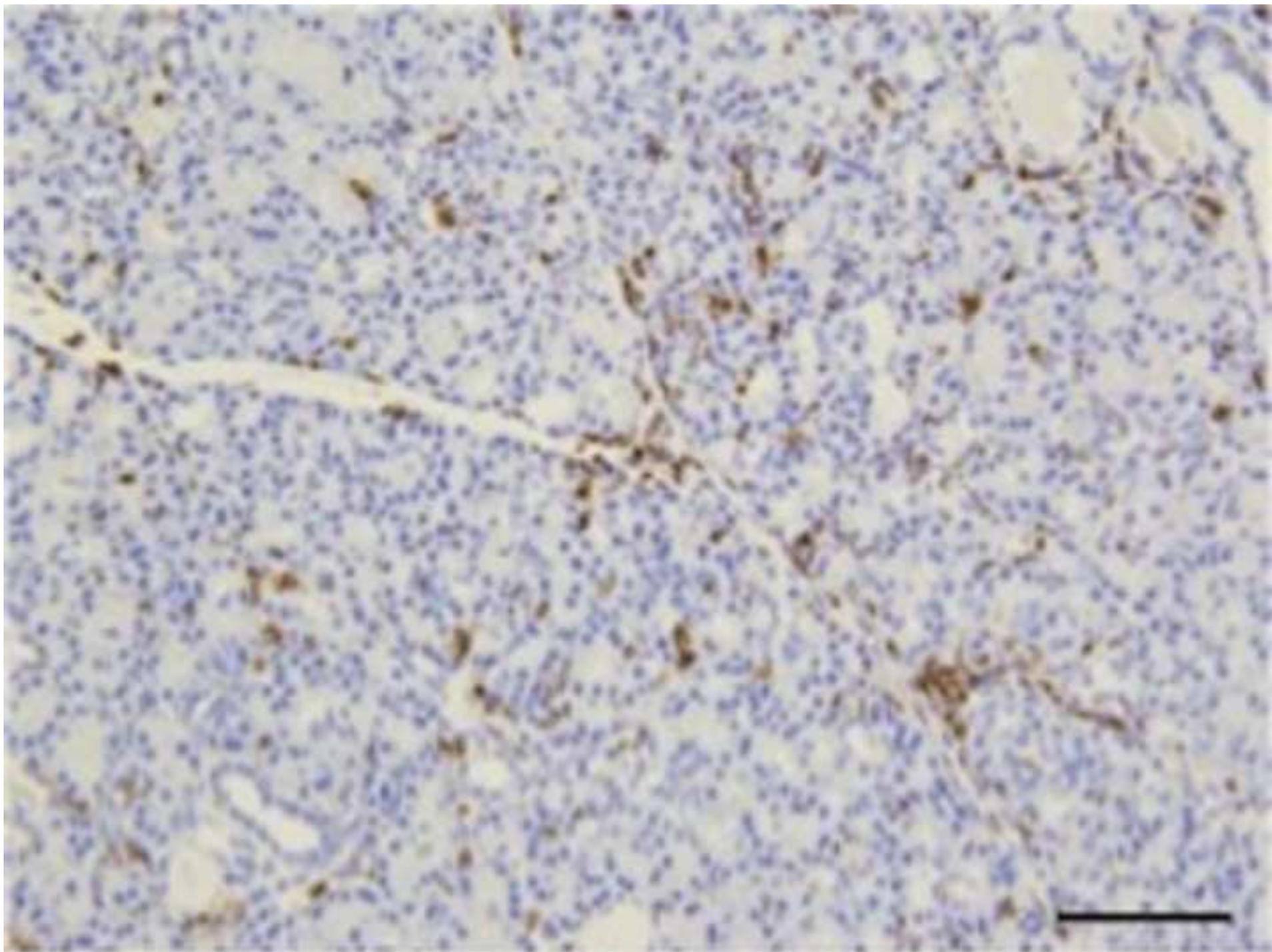


Figure 2D_4

