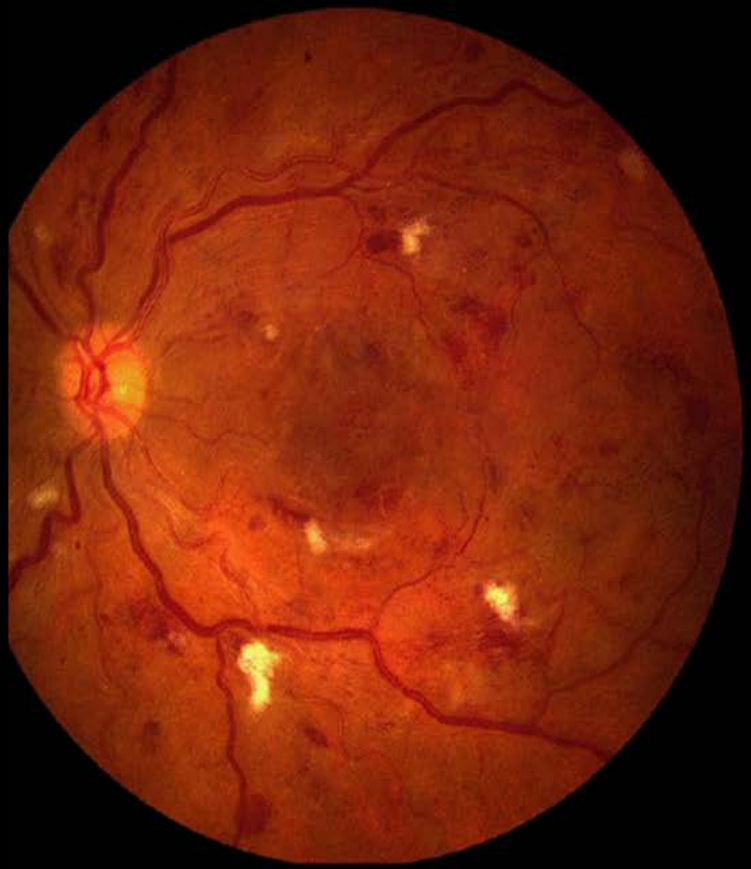
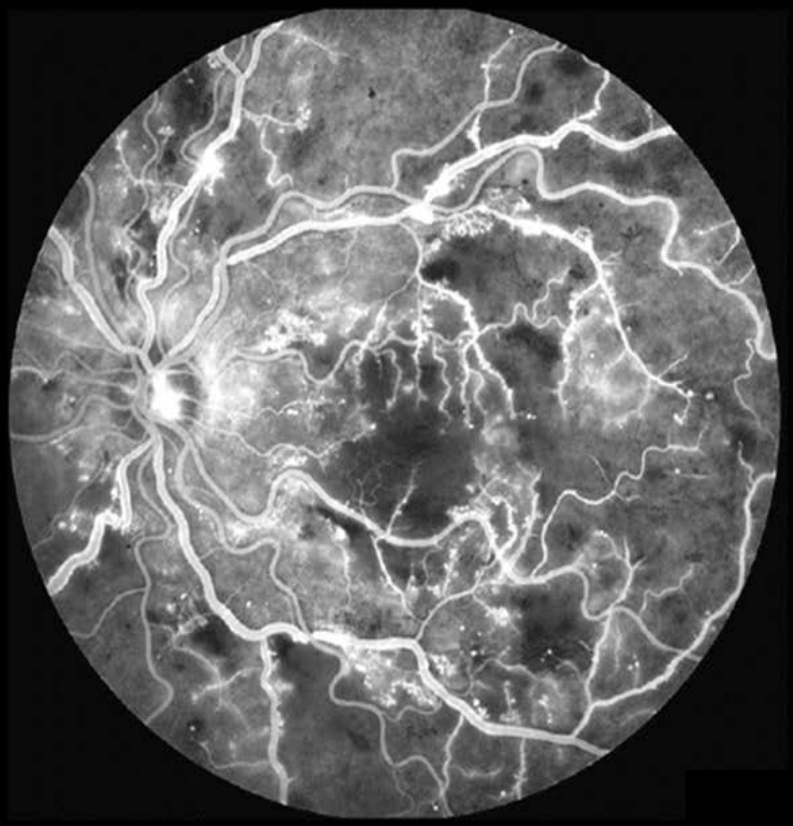
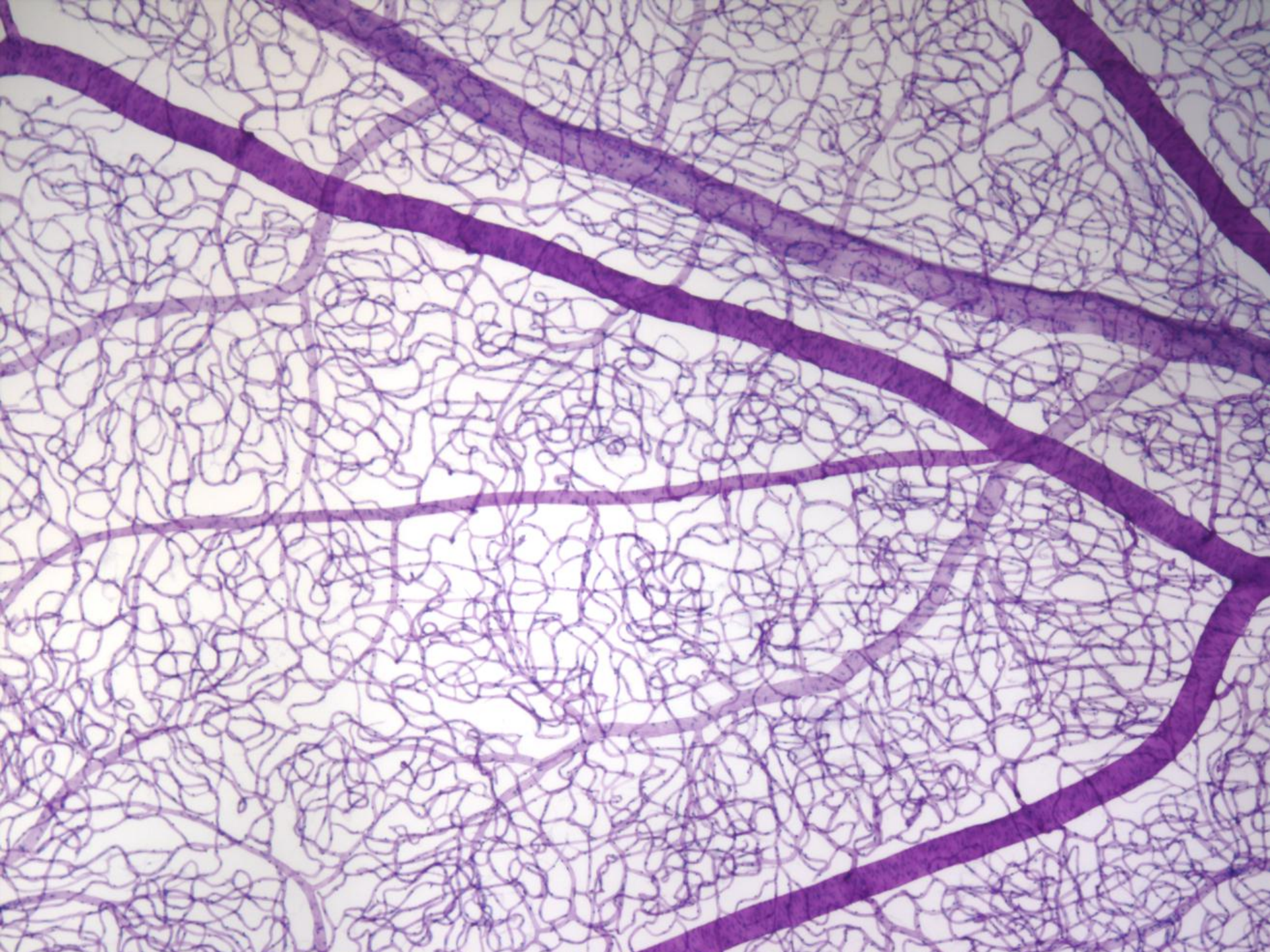
Inflammatory basis of diabetic retinopathy

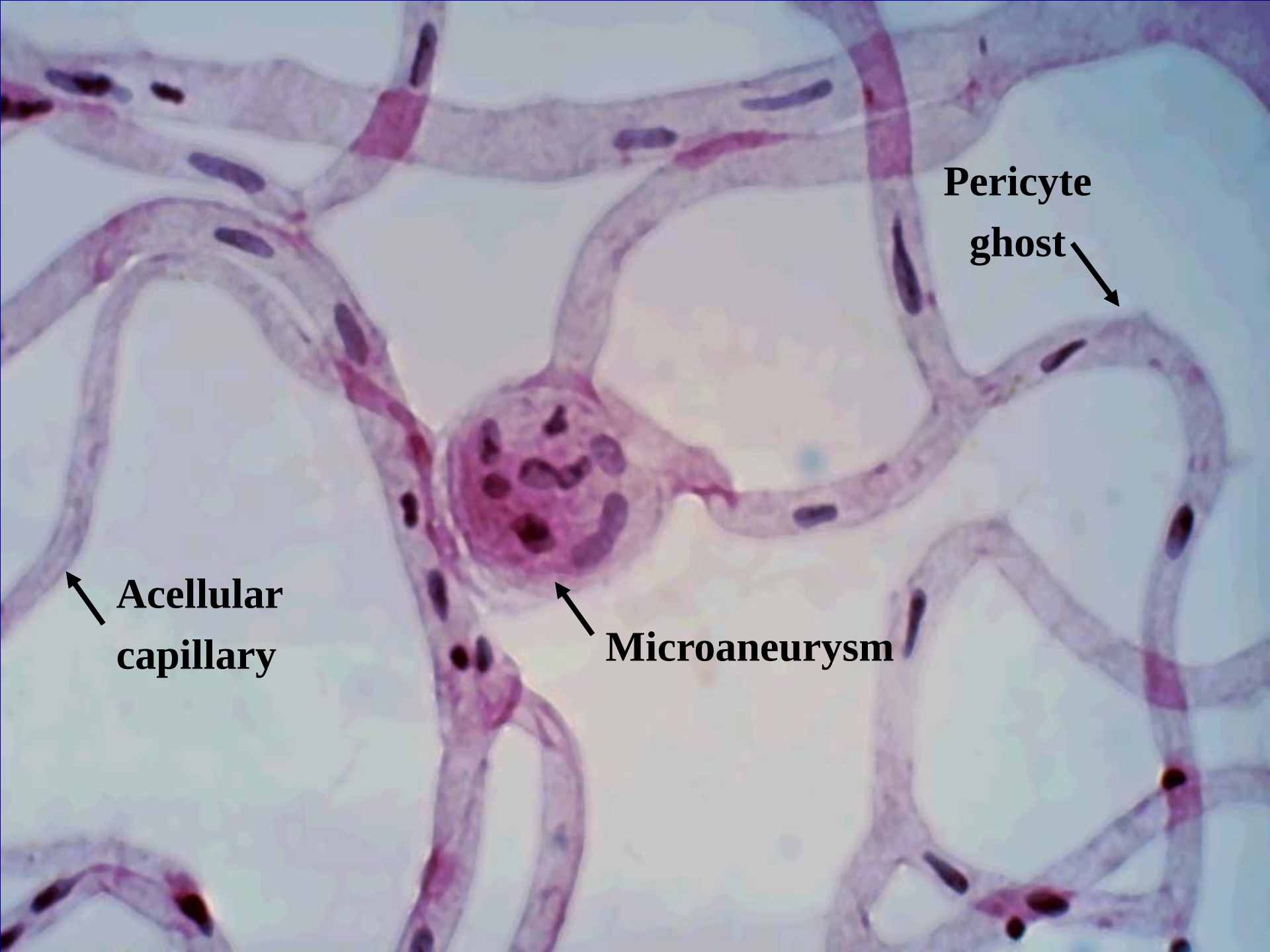
Tim Kern, PhD
Case Western Reserve University and
Stokes VA Hospital, Cleveland, OH

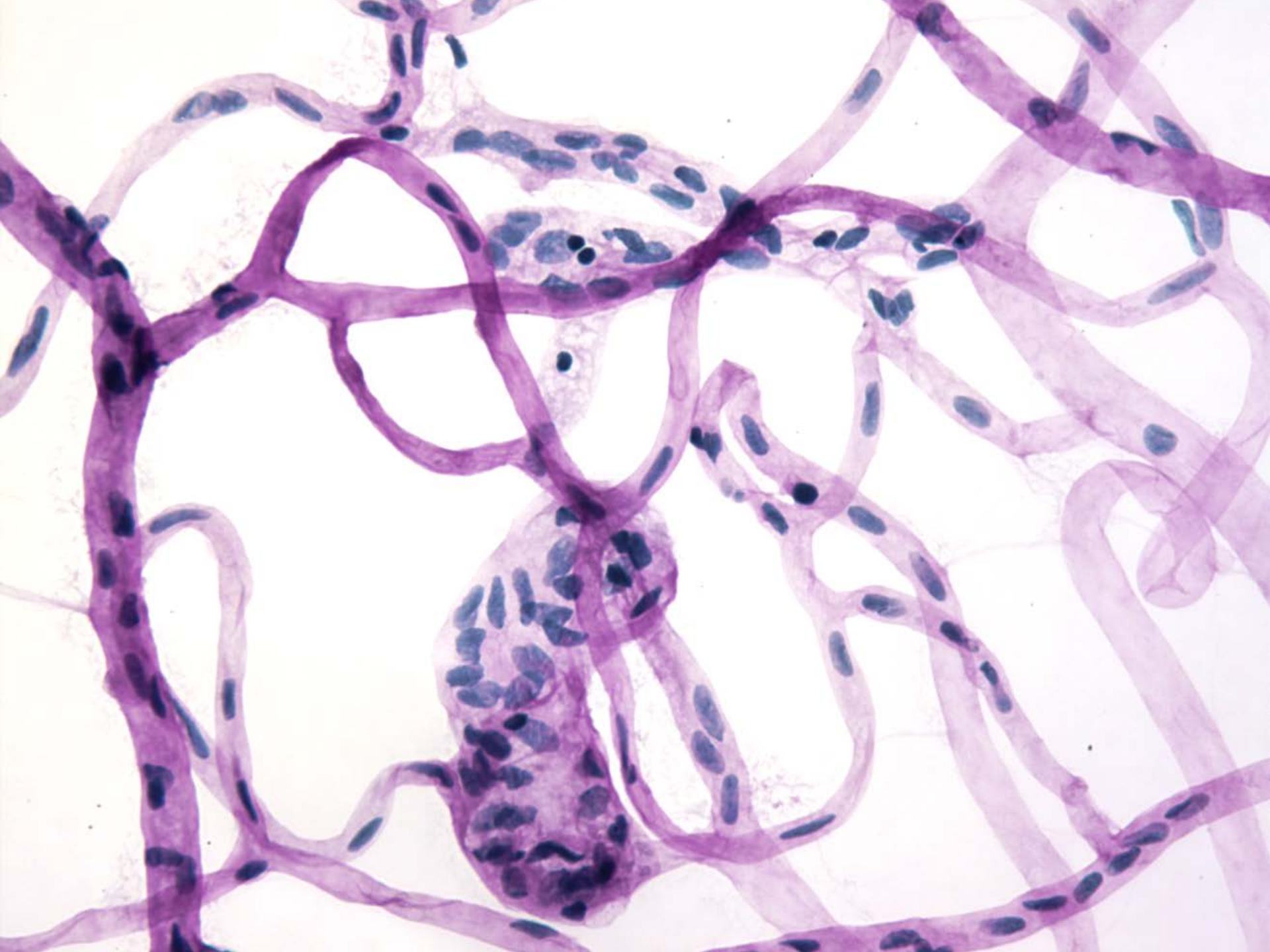
Diabetic retinopathy currently is diagnosed based on vascular abnormalities

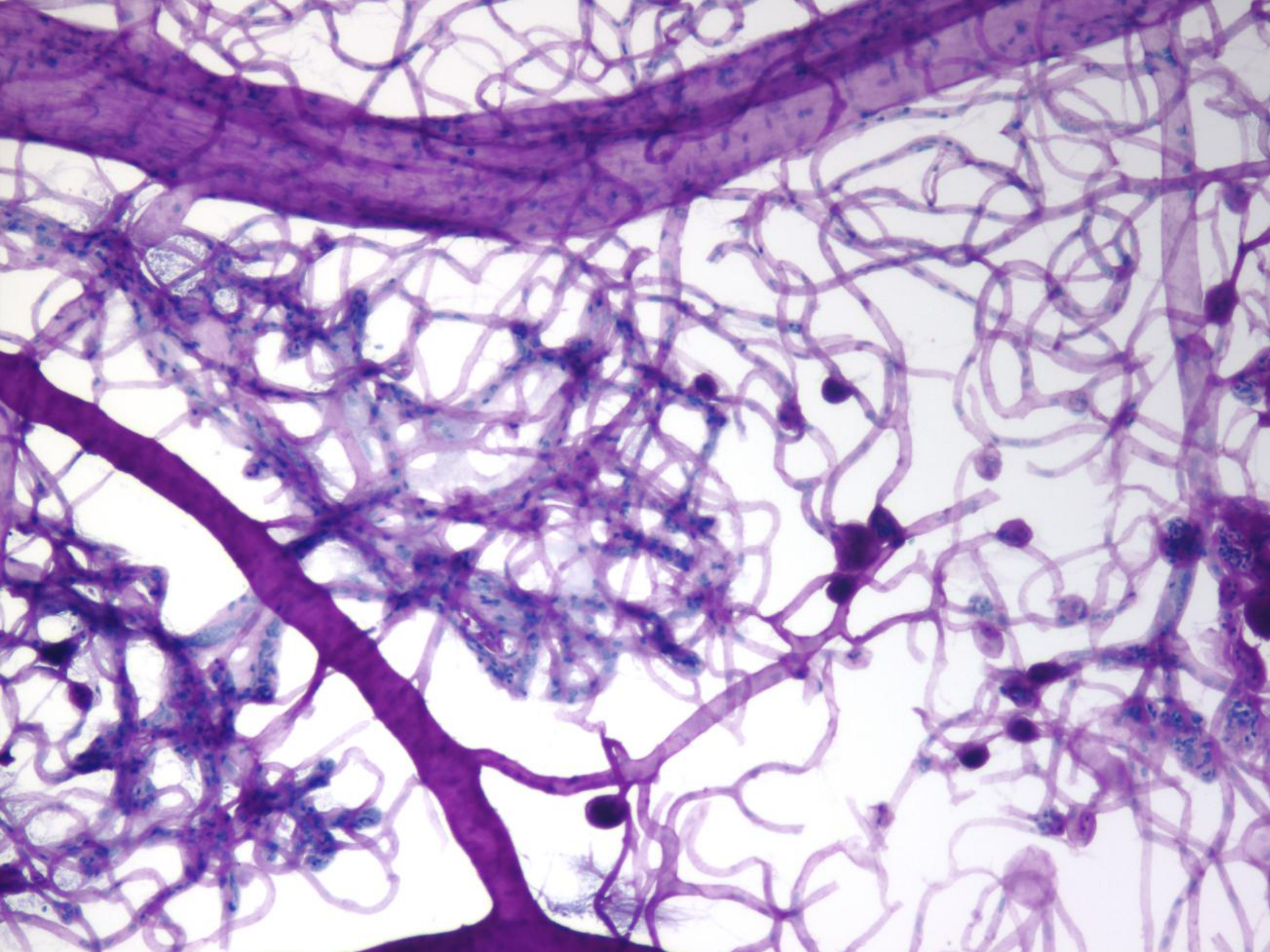


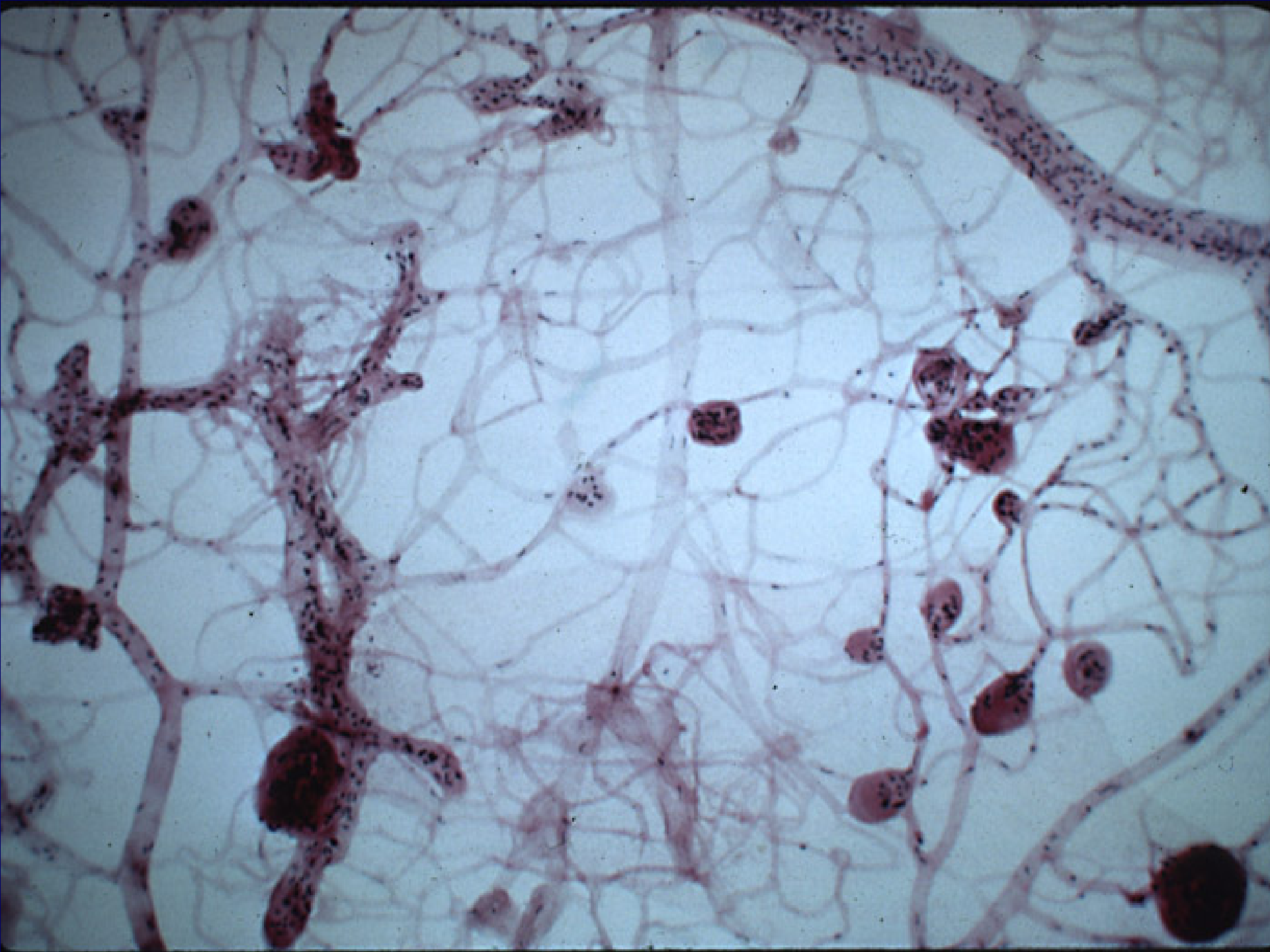





Pericyte
ghost
Acellular
capillary
Microaneurysm







CURRENT THERAPIES AGAINST DIABETIC RETINOPATHY

Laser photocoagulation
Anti-VEGF injections (intra-vitreal)
Corticosteroids

Improved glycemic control
Fibrates
Blood pressure medications

DIABETES

↓

ELEVATED BLOOD HEXOSE

↓

METABOLIC ABNORMALITIES

↓

NONPROLIFERATIVE RETINOPATHY

(especially capillary nonperfusion & degeneration)

↓

RETINAL ISCHEMIA

↓

PROLIFERATIVE RETINOPATHY

DIABETES

↓

ELEVATED BLOOD HEXOSE

↓

METABOLIC ABNORMALITIES

INFLAMMATION

↓

NONPROLIFERATIVE RETINOPATHY

(especially capillary nonperfusion & degeneration)

What is the molecular cause of capillary degeneration in diabetic retinopathy?

What cell types are involved in the development of diabetic retinopathy, and do those cells offer new ways to inhibit the retinopathy?

- **Leukocytes**
- **Photoreceptors**

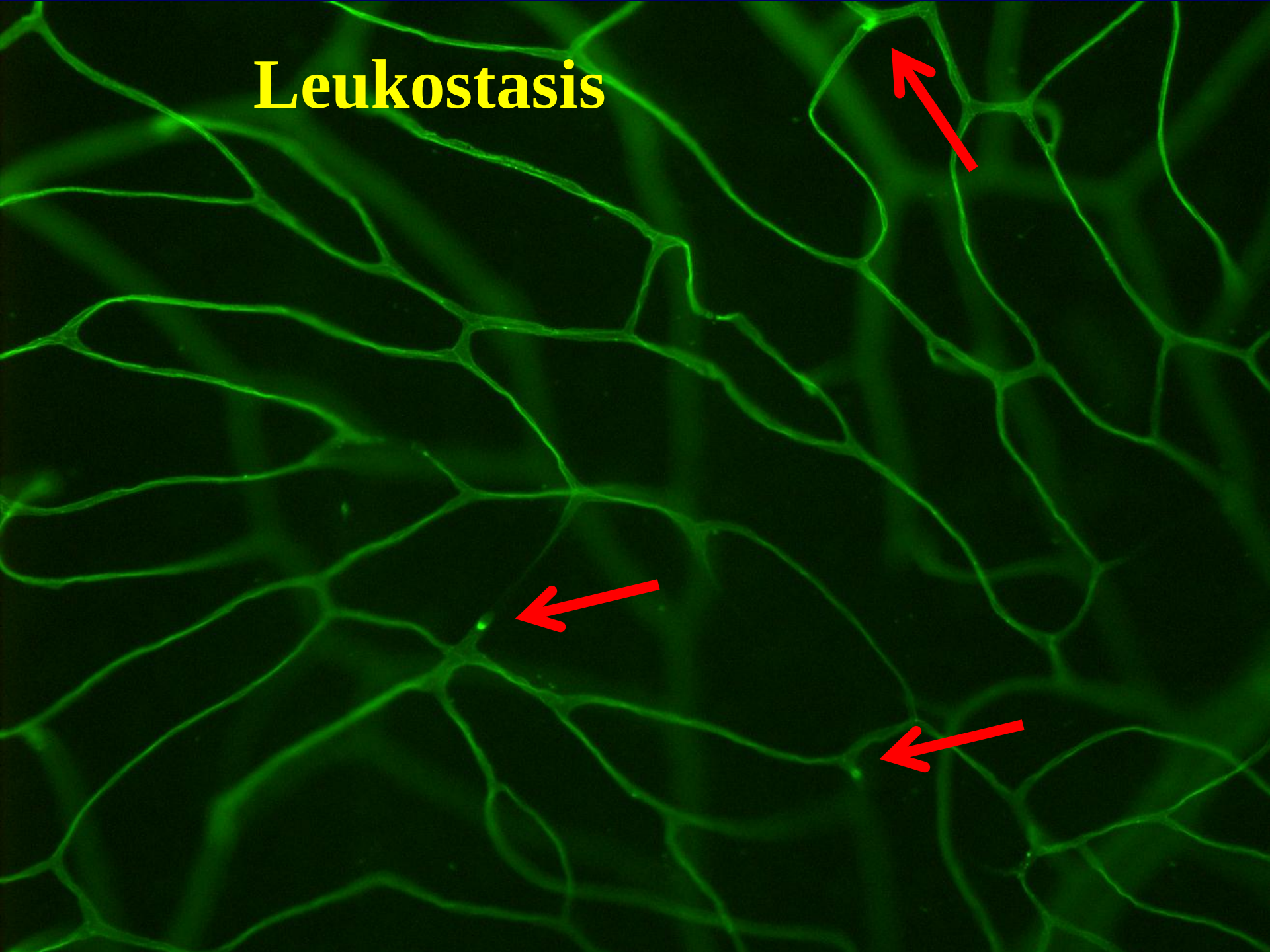
Study design

- Glycemic control
- Histology **8-10 months diabetes**

- iNOS, NO and nitrotyrosine **2-3 months diabetes**
- ICAM
- Leukostasis
- Superoxide generation by isolated retina
- NF-κB and C/EBP activation

- Cell culture studies

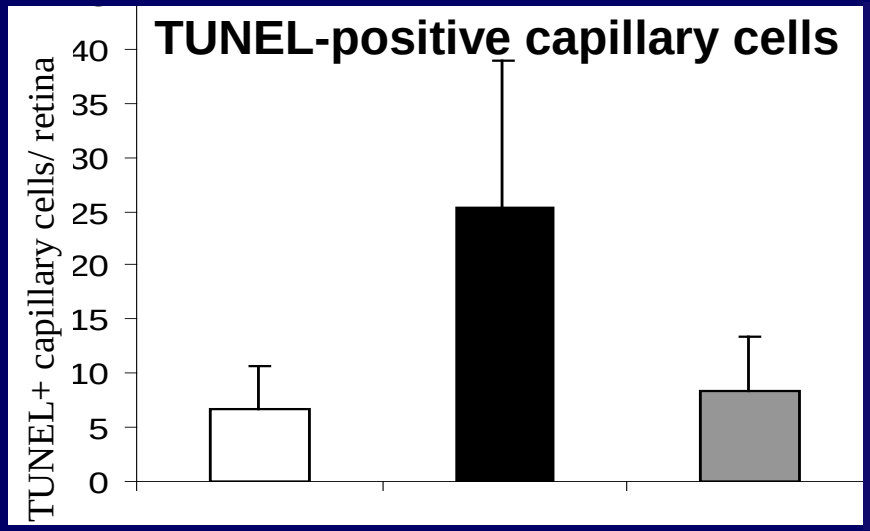
Leukostasis



Examples of glycemia in experimental animals: Effect of PJ-34 therapy

Group (n)	Duration (month)	Body weight (g)	Non-fast blood glucose (mg/L)	Glycated Hemoglobin(%)
Normal (10)	**3**	405 ± 27	62 ± 9	4.9 ± 1.0
Diabetes (10)	**3**	263 ± 19	**327 ± 32**	**10.7 ± 2.8**
D+PJ-34 (7)	**3**	268 ± 20	**288 ± 43**	**12.5 ± 1.1**
Normal (28)	**9**	575 ± 51	88 ± 26	4.0 ± 0.7
Diabetes (19)	**9**	314 ± 45	**340 ± 81**	**10.2 ± 1.4**
D+PJ-34 (19)	**9**	268 ± 31	**372 ± 78**	**9.9 ± 1.0**

PJ-34 inhibited diabetes-induced capillary cell apoptosis, and formation of acellular capillaries and pericyte ghosts

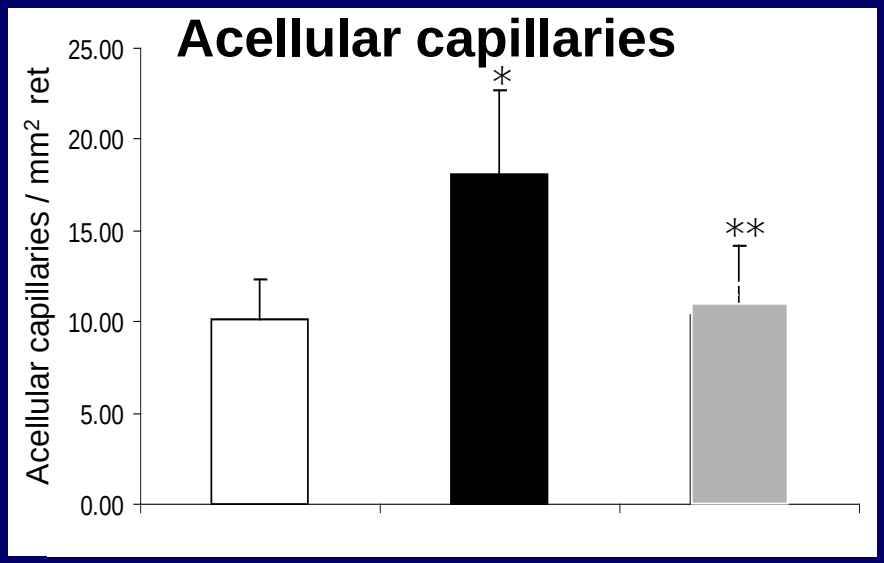


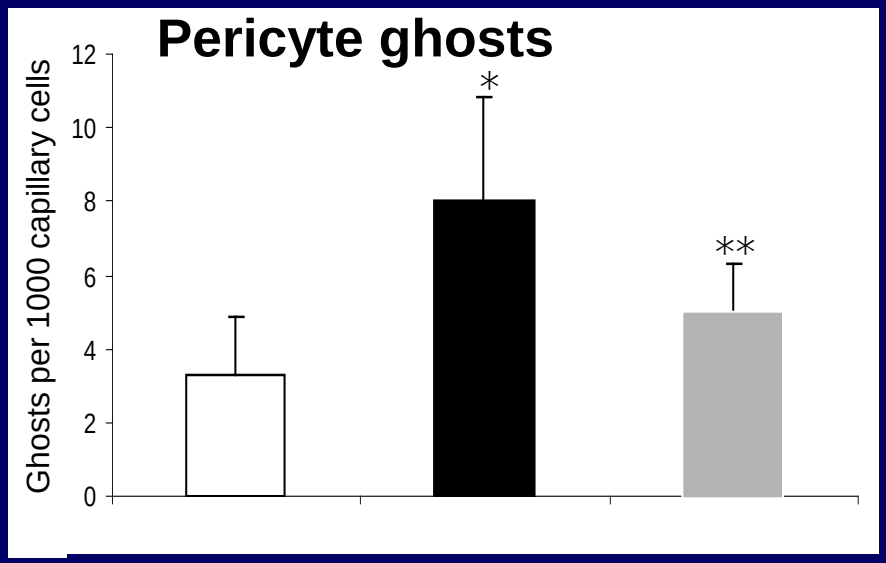
9 mos diabetes

Non-diabetes

Diabetes

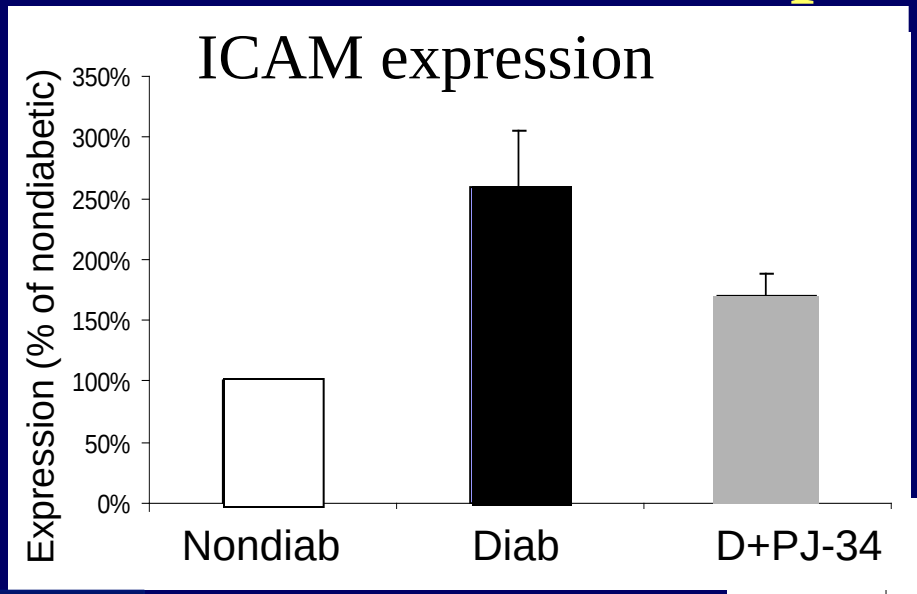
Diabetes+PJ-34

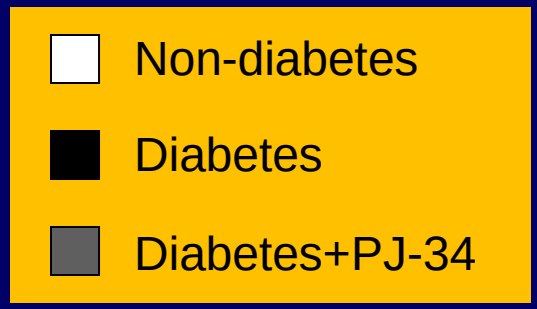


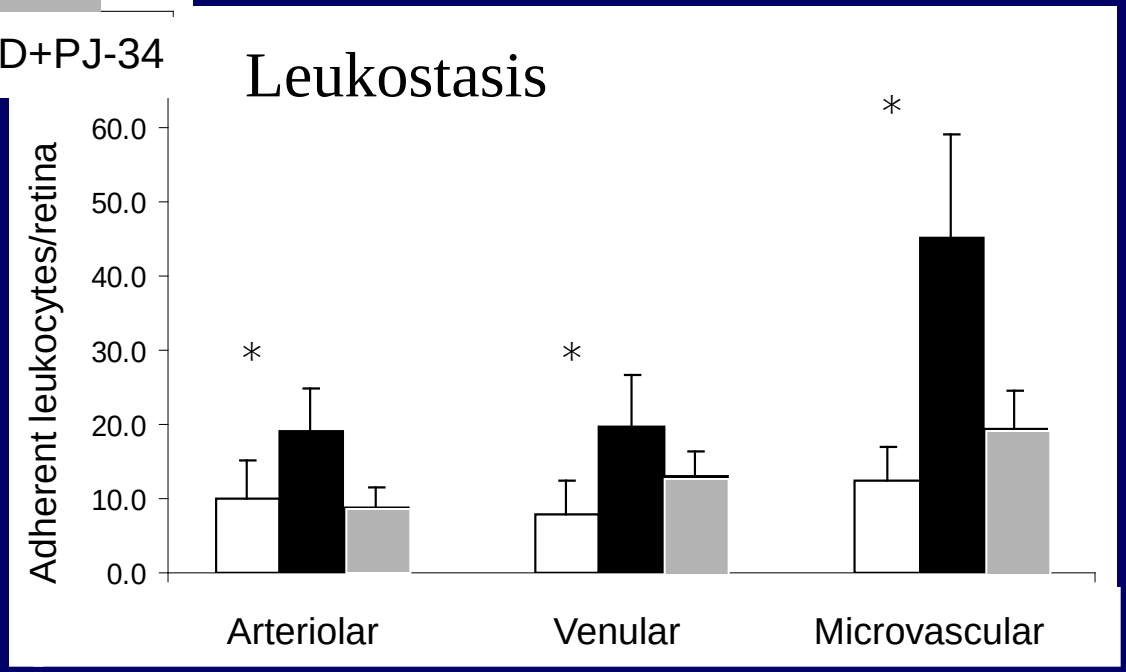


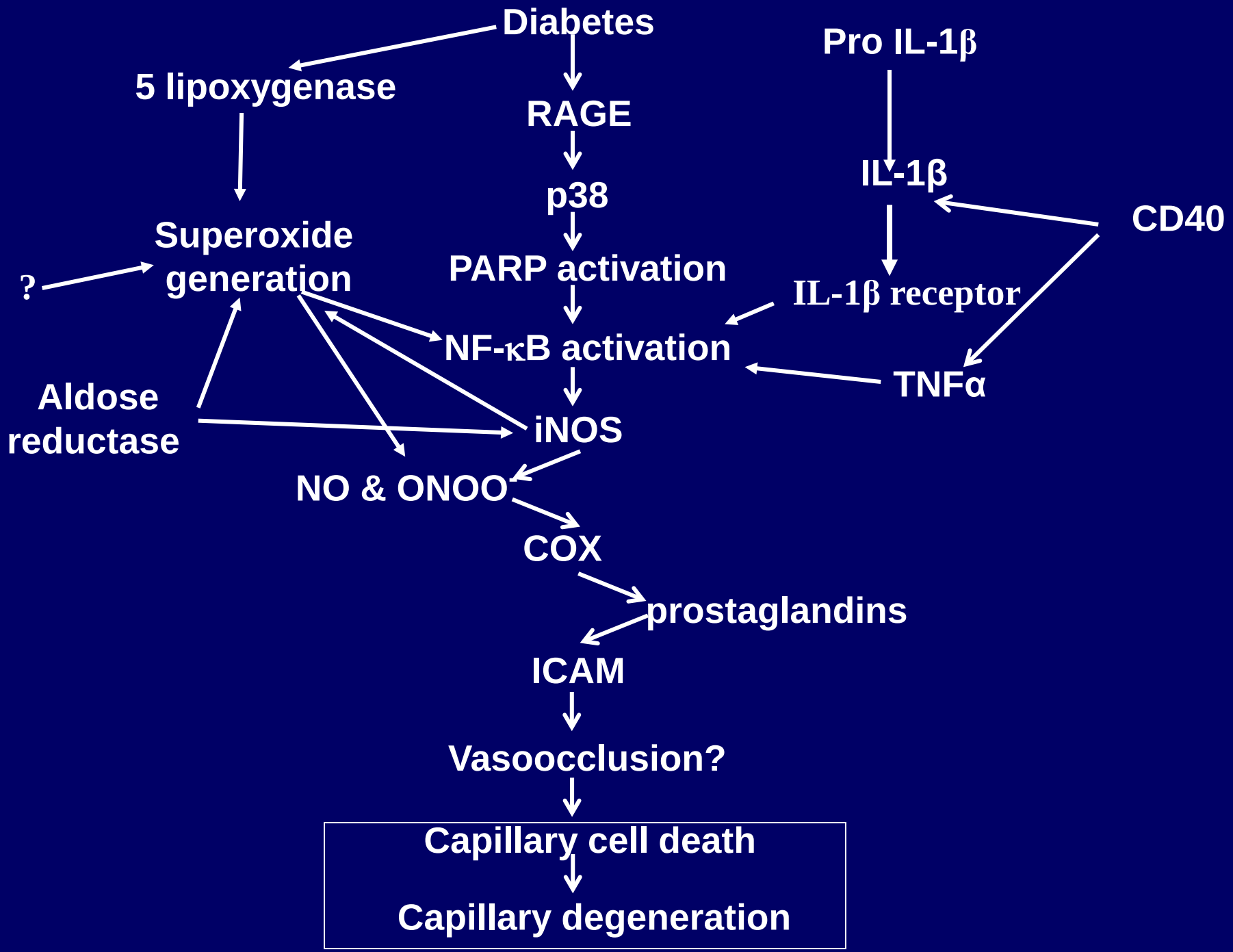
Zheng, Szabó, and Kern. Diabetes 53:2960, 2004

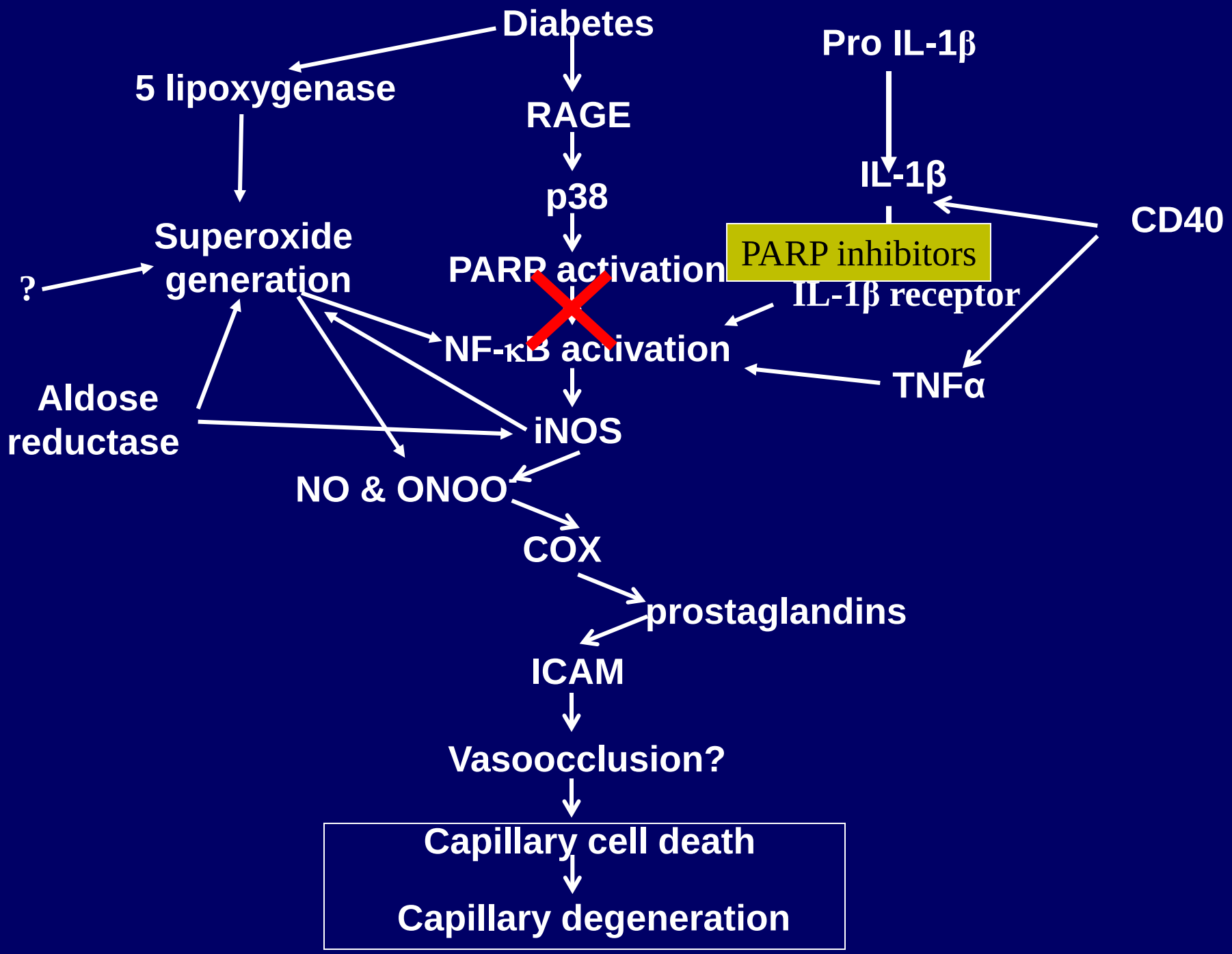
PJ34 inhibited the diabetes-induced increase in retinal ICAM expression and leukostasis








Diabetes
5 lipoxygenase
RAGE
Pro IL-1β
IL-1β
p38
CD40
Superoxide generation
?
PARP activation
IL-1β receptor
NF-κB activation
TNFα
Aldose reductase
iNOS
NO & ONOO
COX
prostaglandins
ICAM
Vasoocclusion?
Capillary cell death
Capillary degeneration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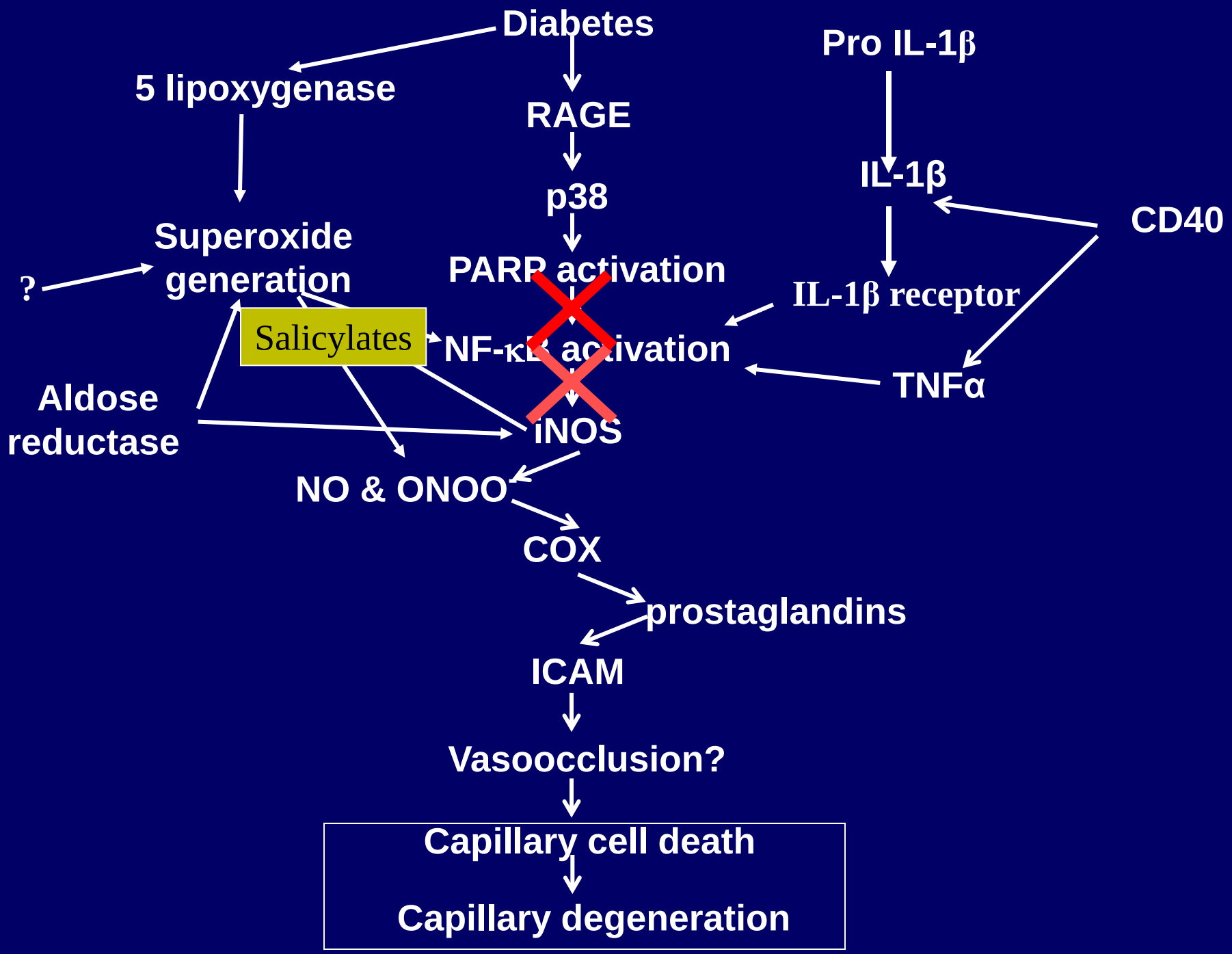
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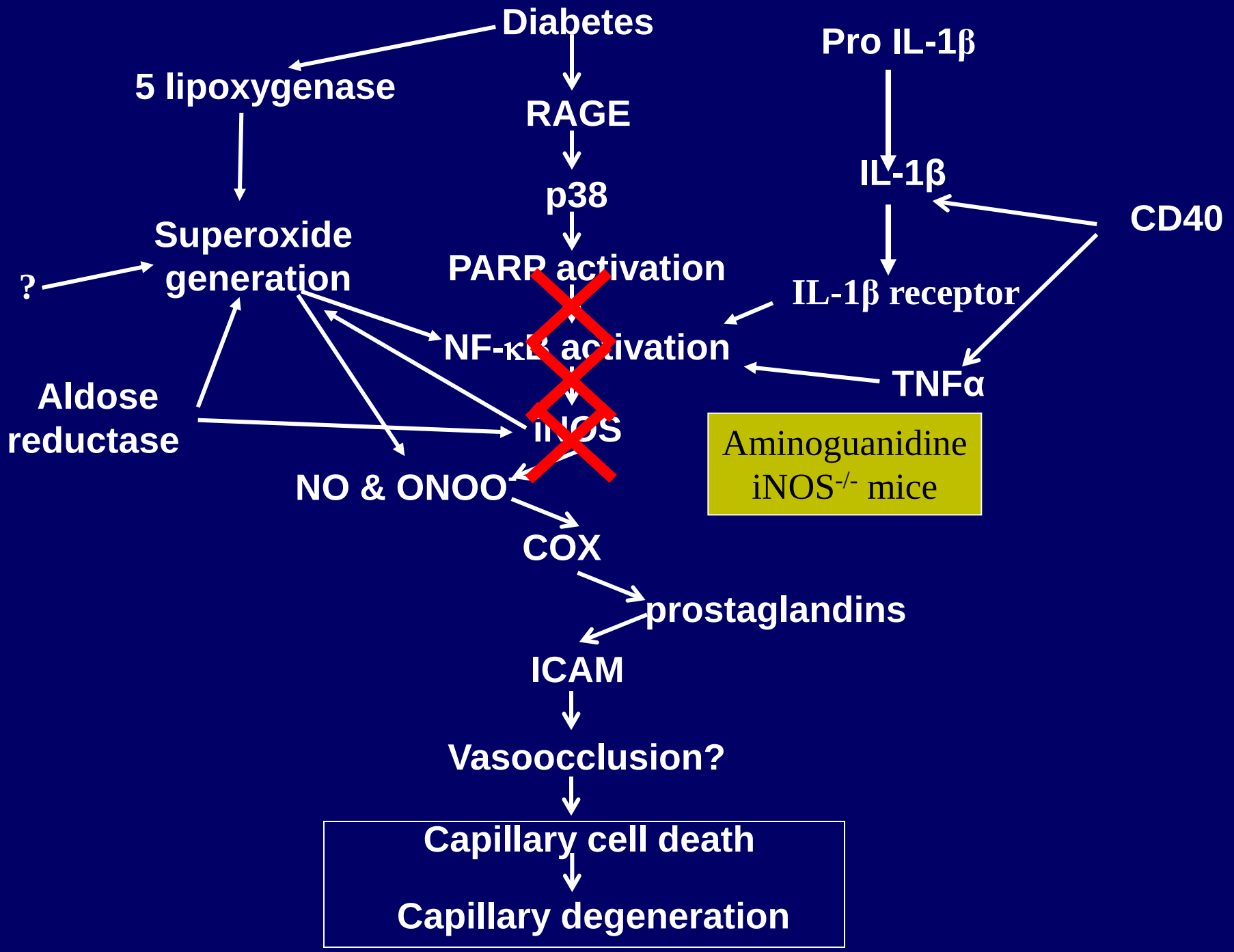
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NF-κB activation
TNFα
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iNOS
Aminoguanidine
iNOS-/- mice
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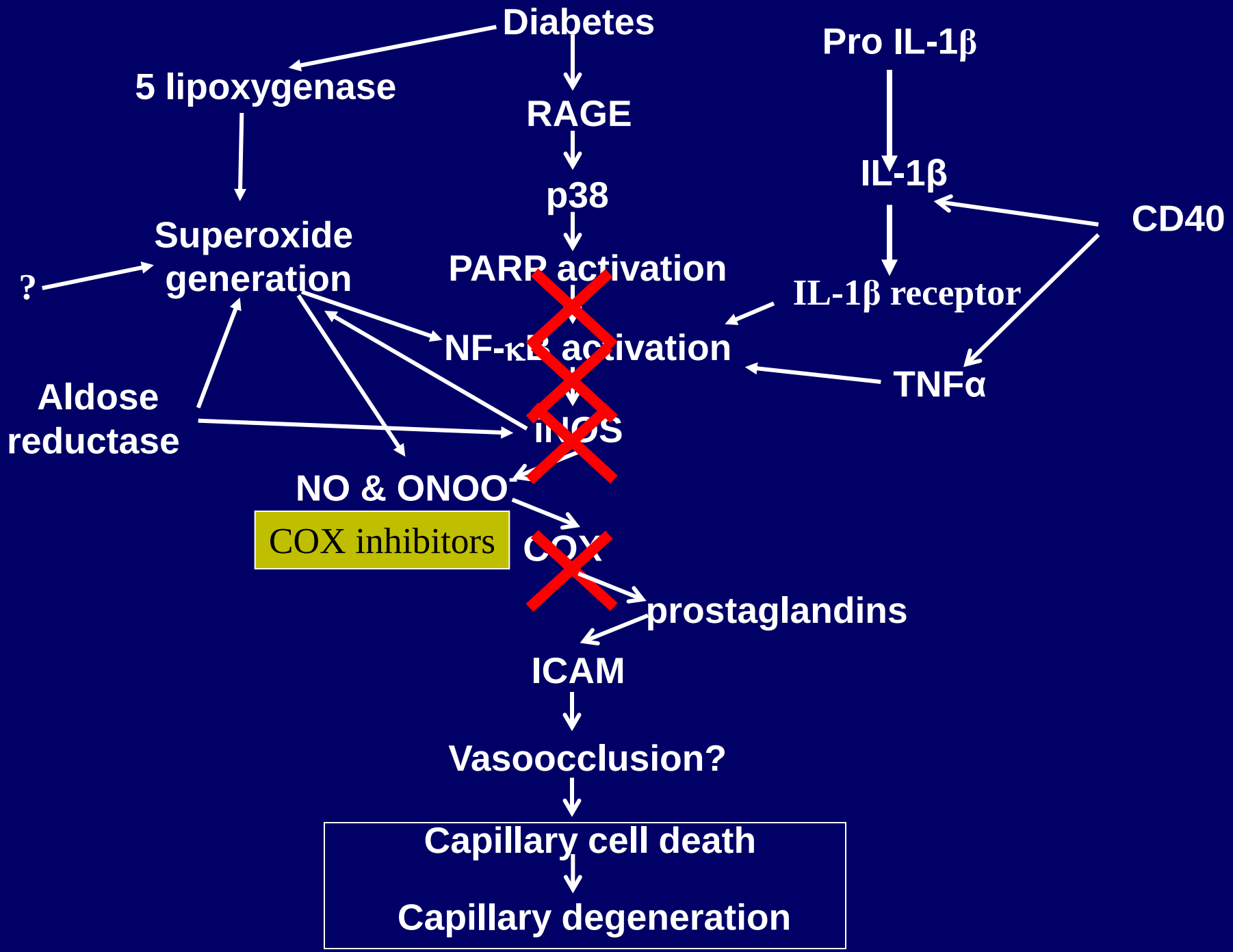
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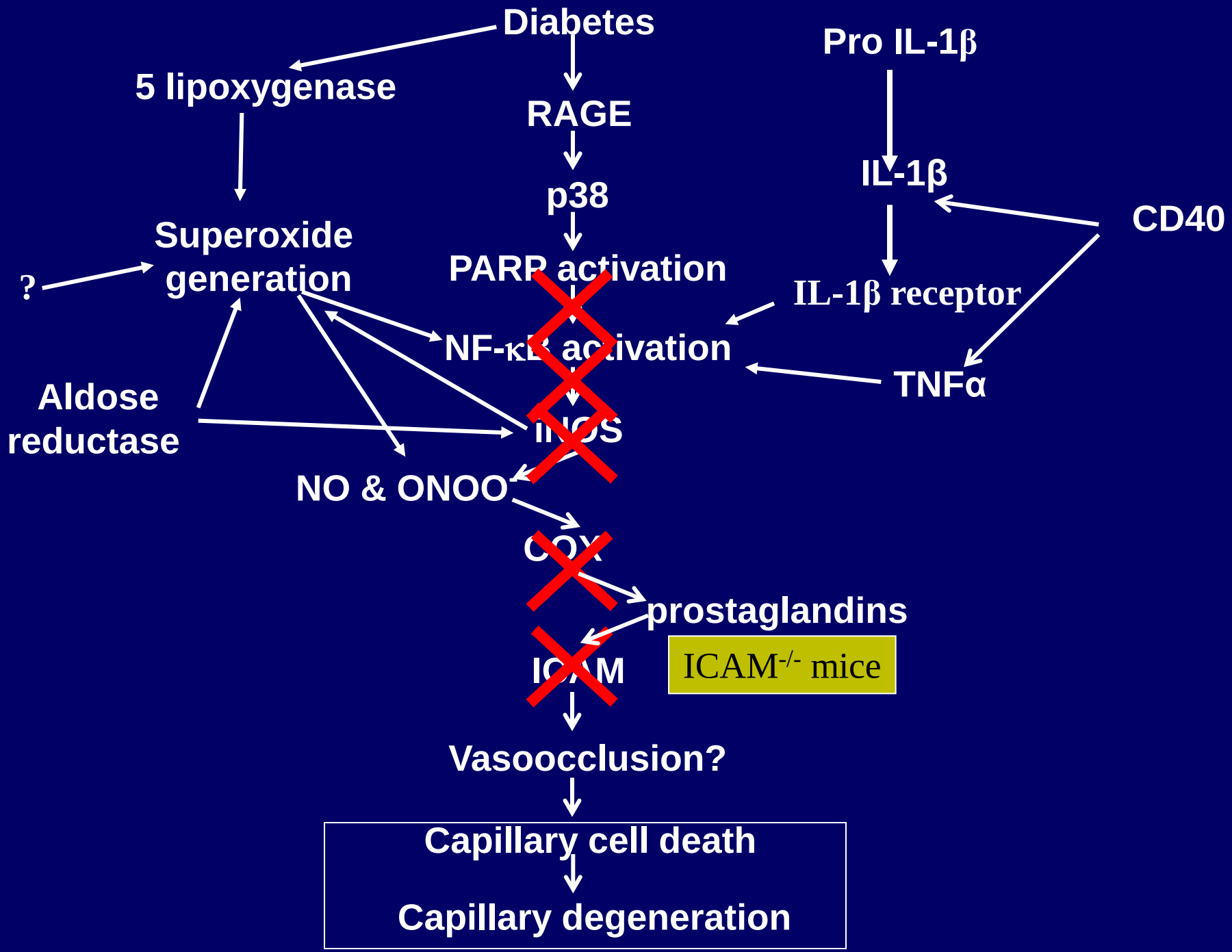
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ICAM
ICAM-/- mice
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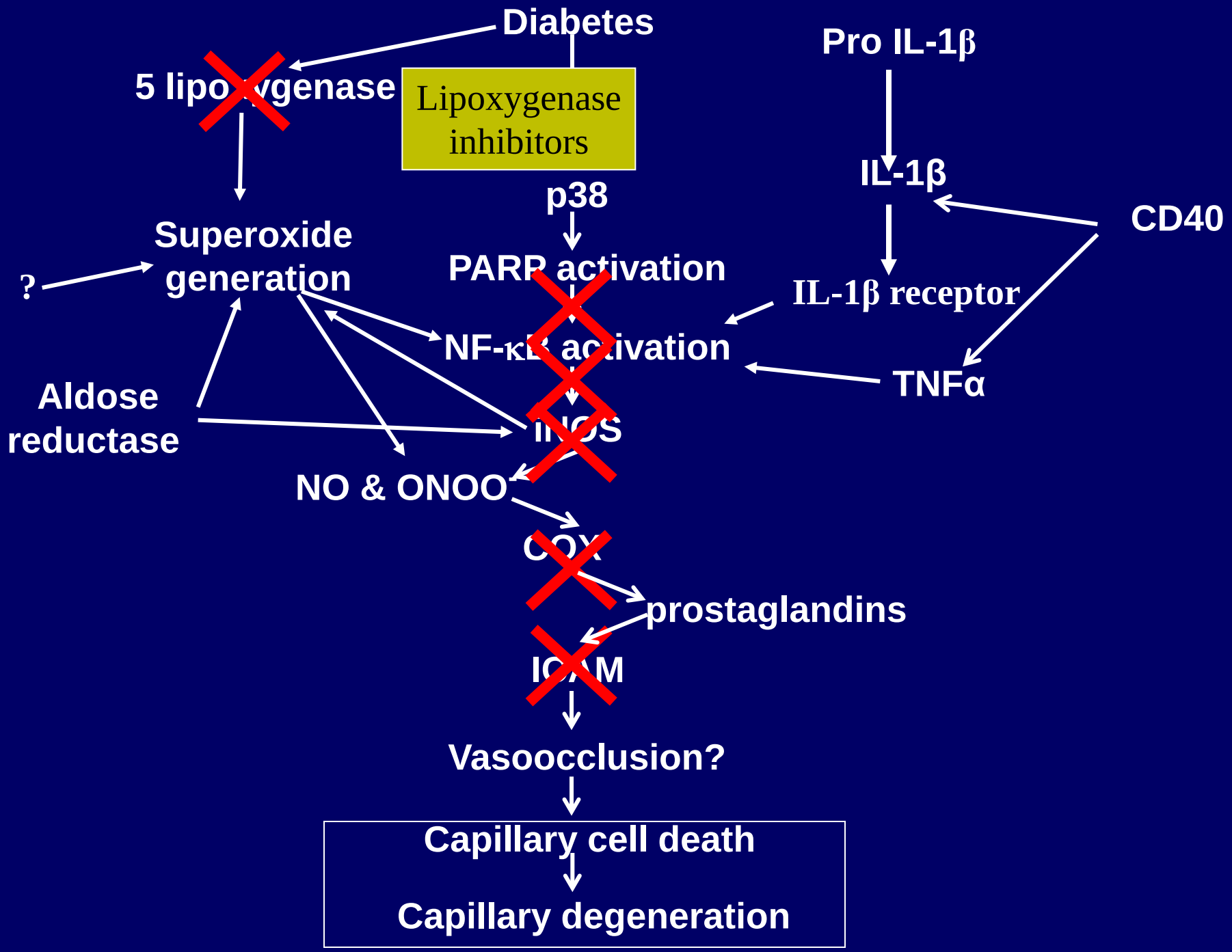
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Lipoxygenase inhibitors
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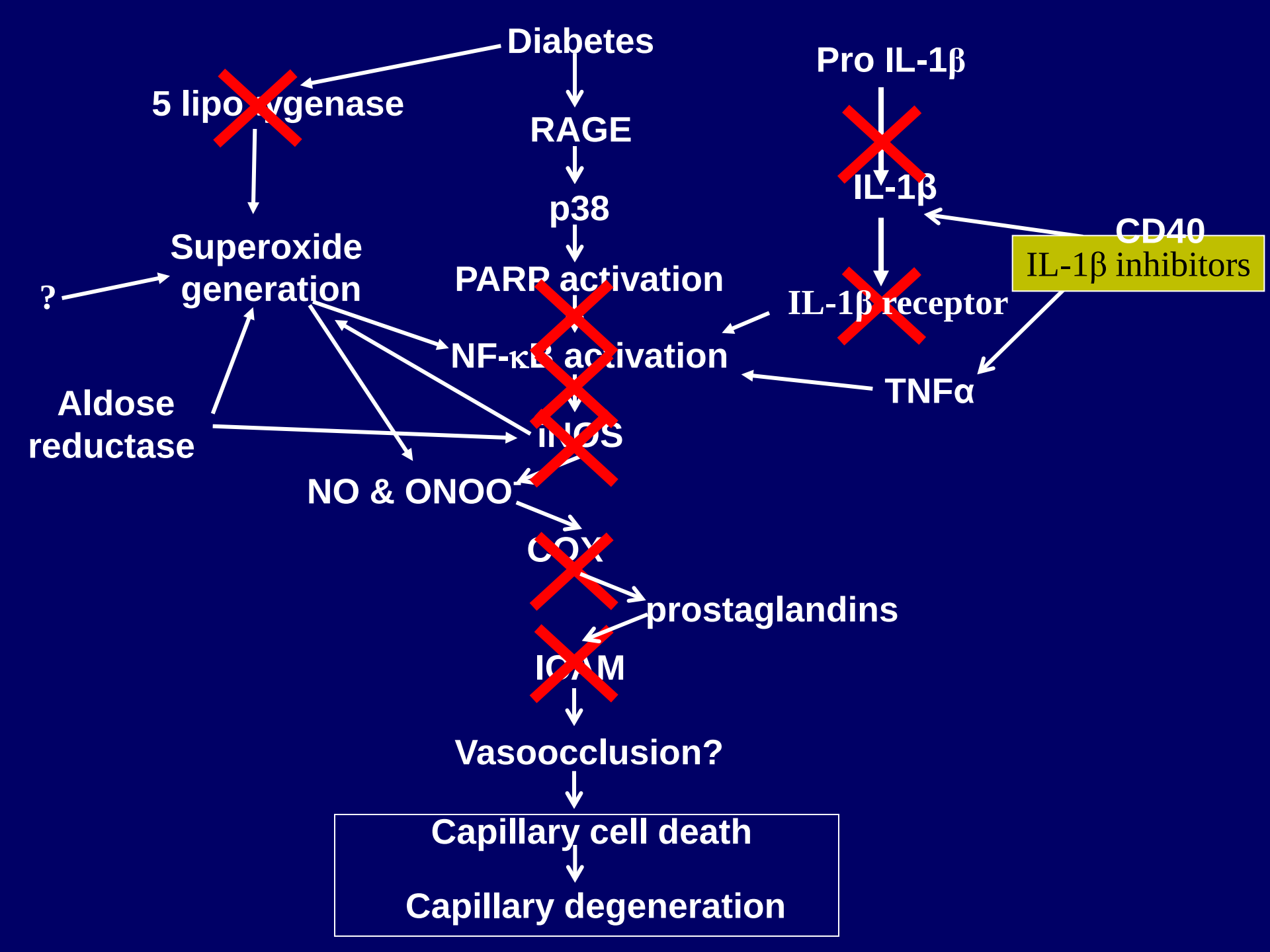
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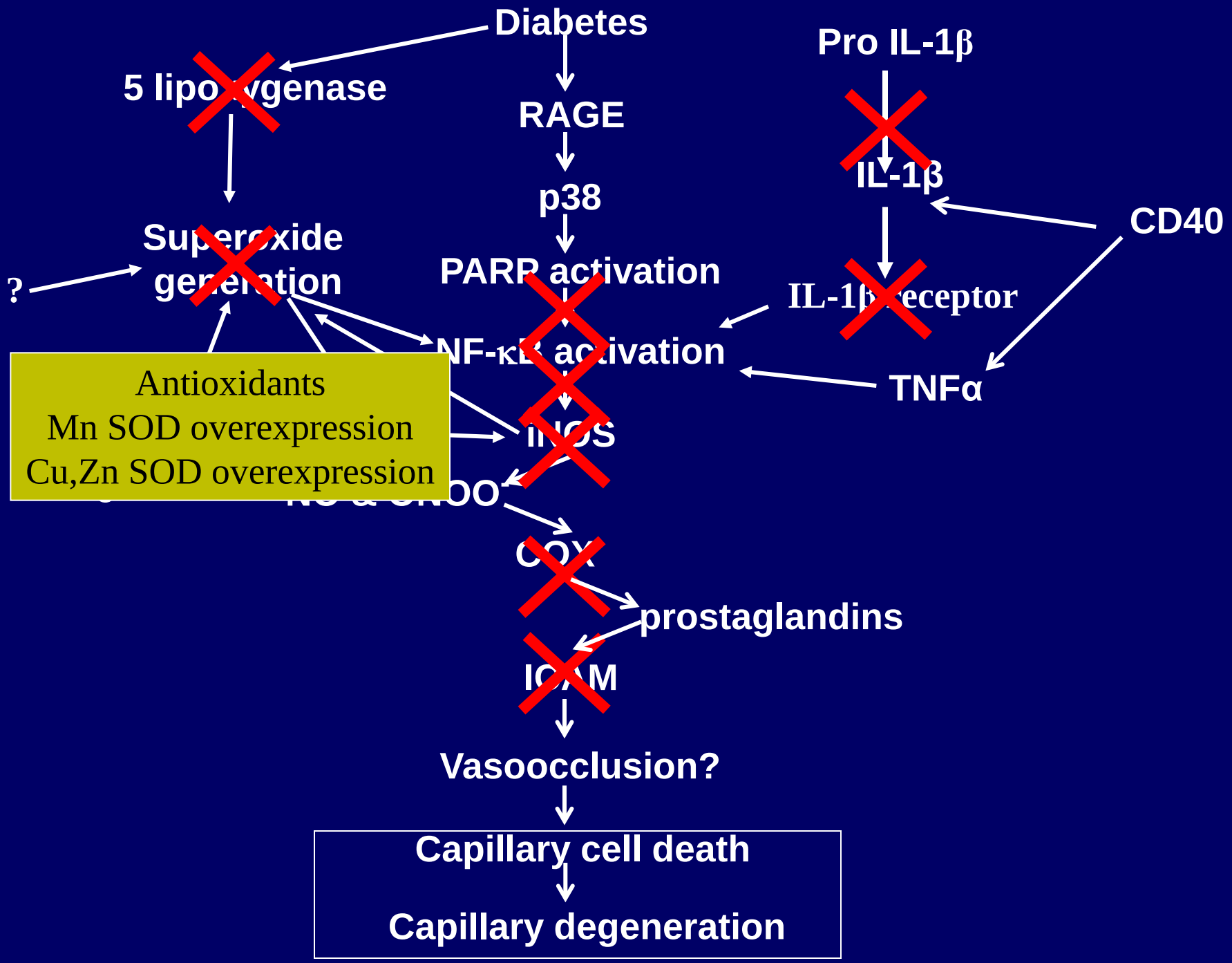
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TNFα
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Mn SOD overexpression
Cu,Zn SOD overexpression
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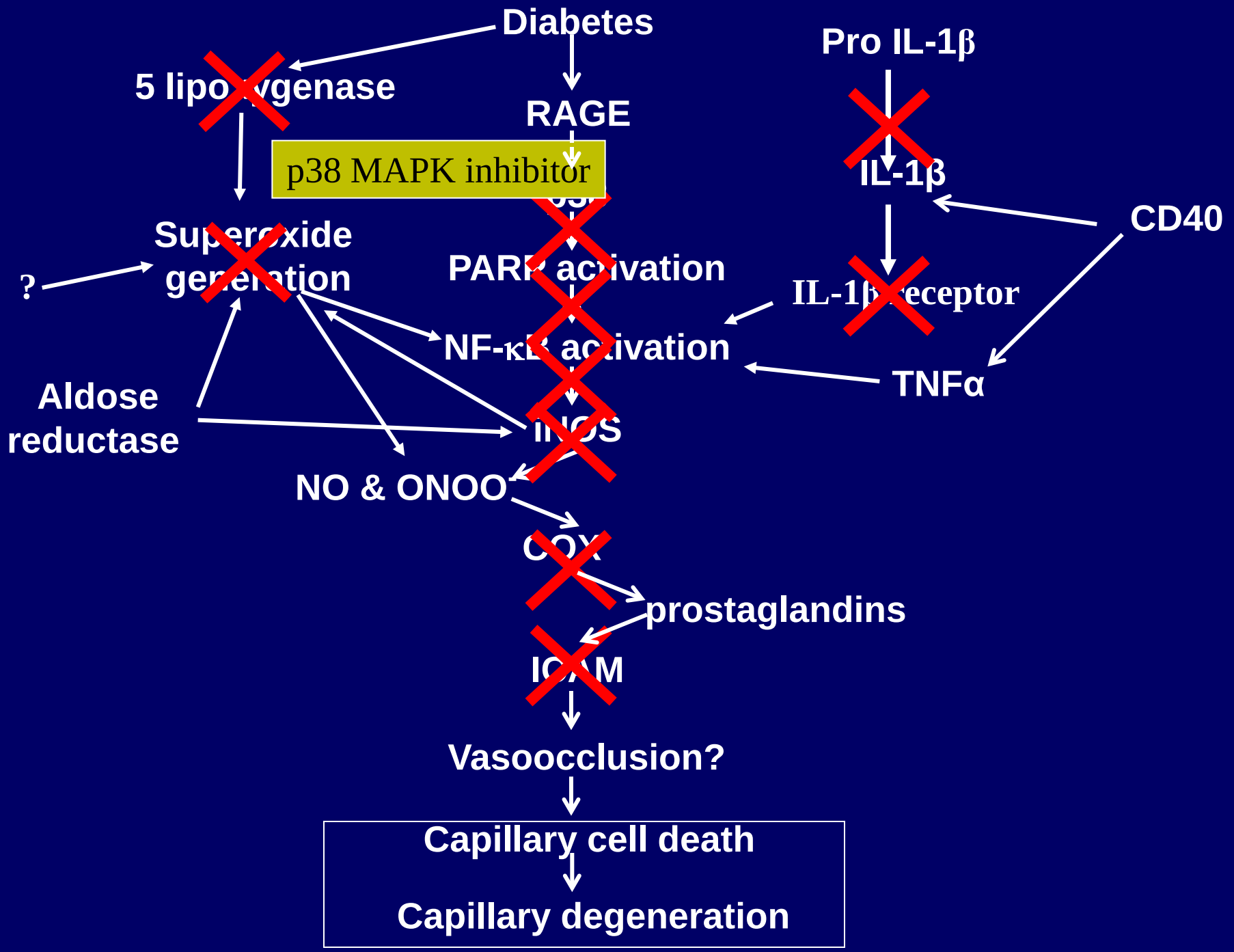
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IL-1β
CD40
Superoxide generation
PARP activation
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IL-1β receptor
NF-κB activation
TNFα
Aldose reductase
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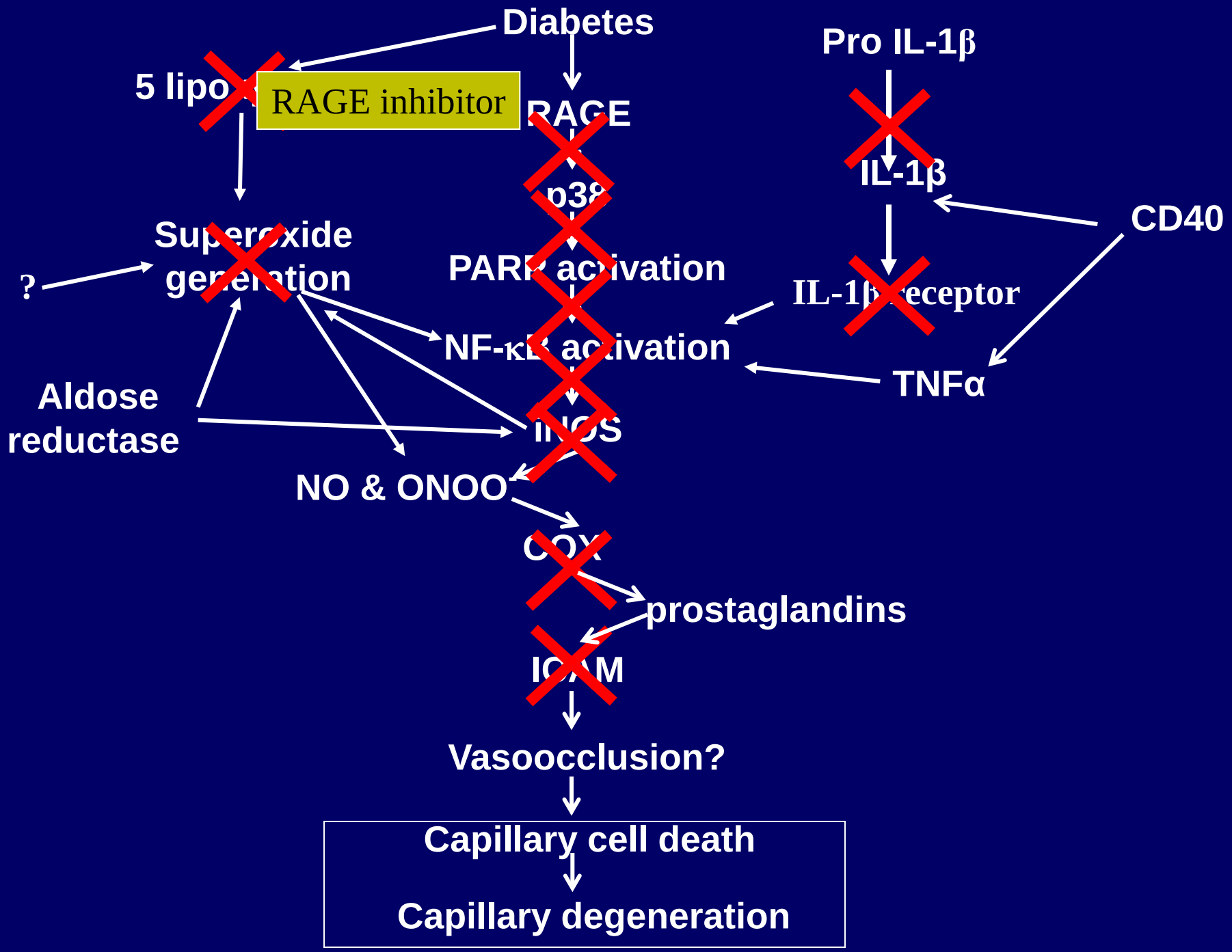
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5 lipo
RAGE inhibitor
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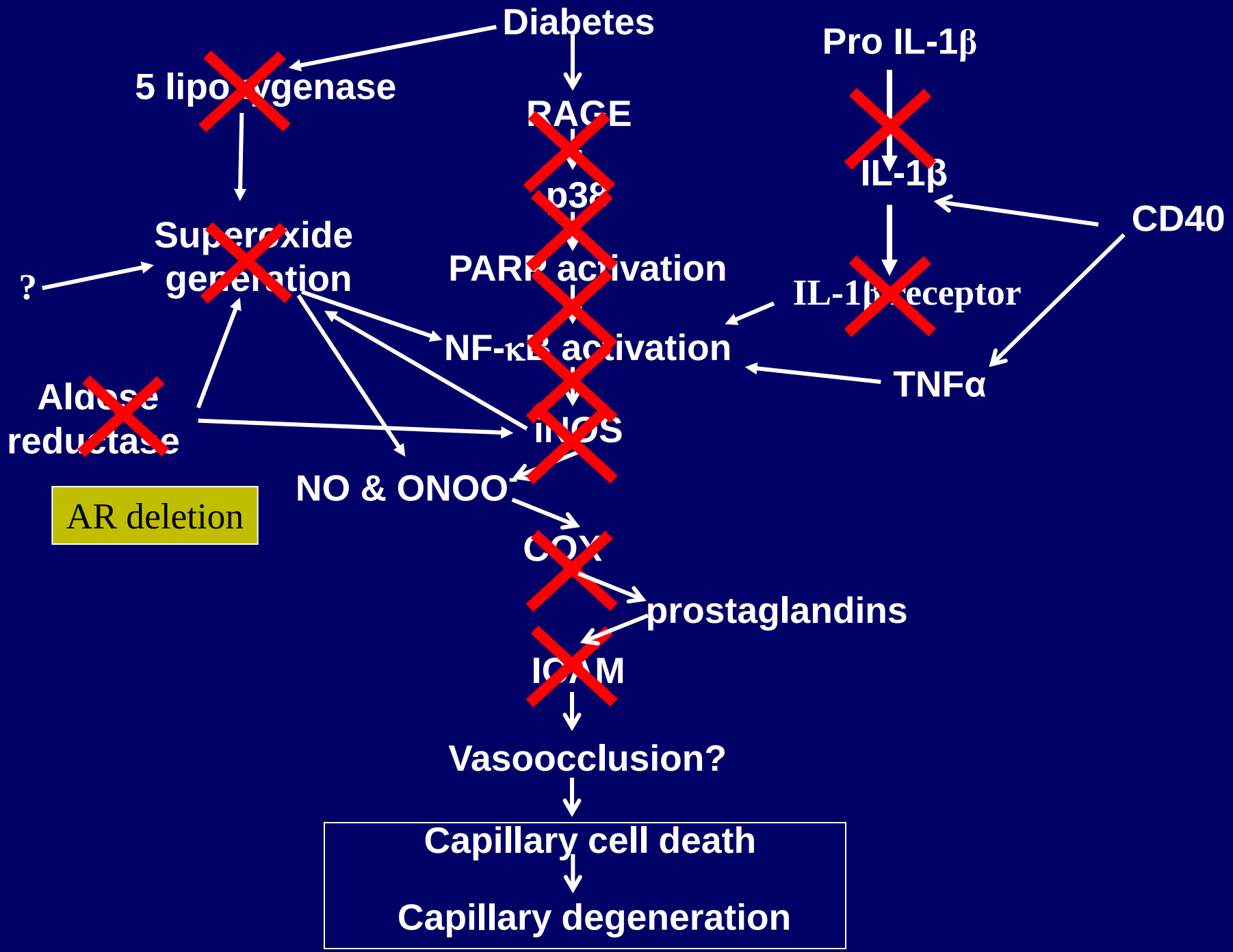
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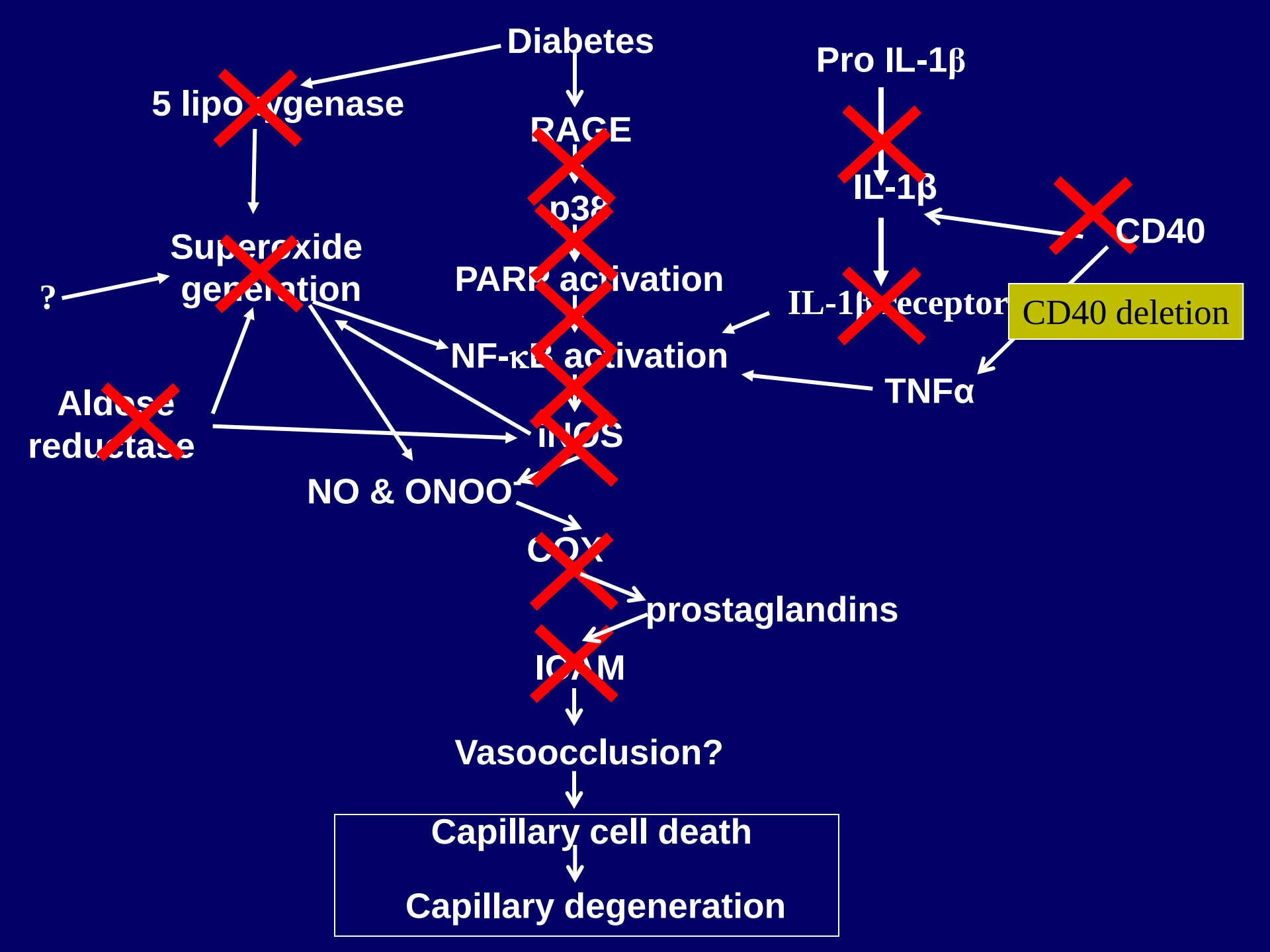
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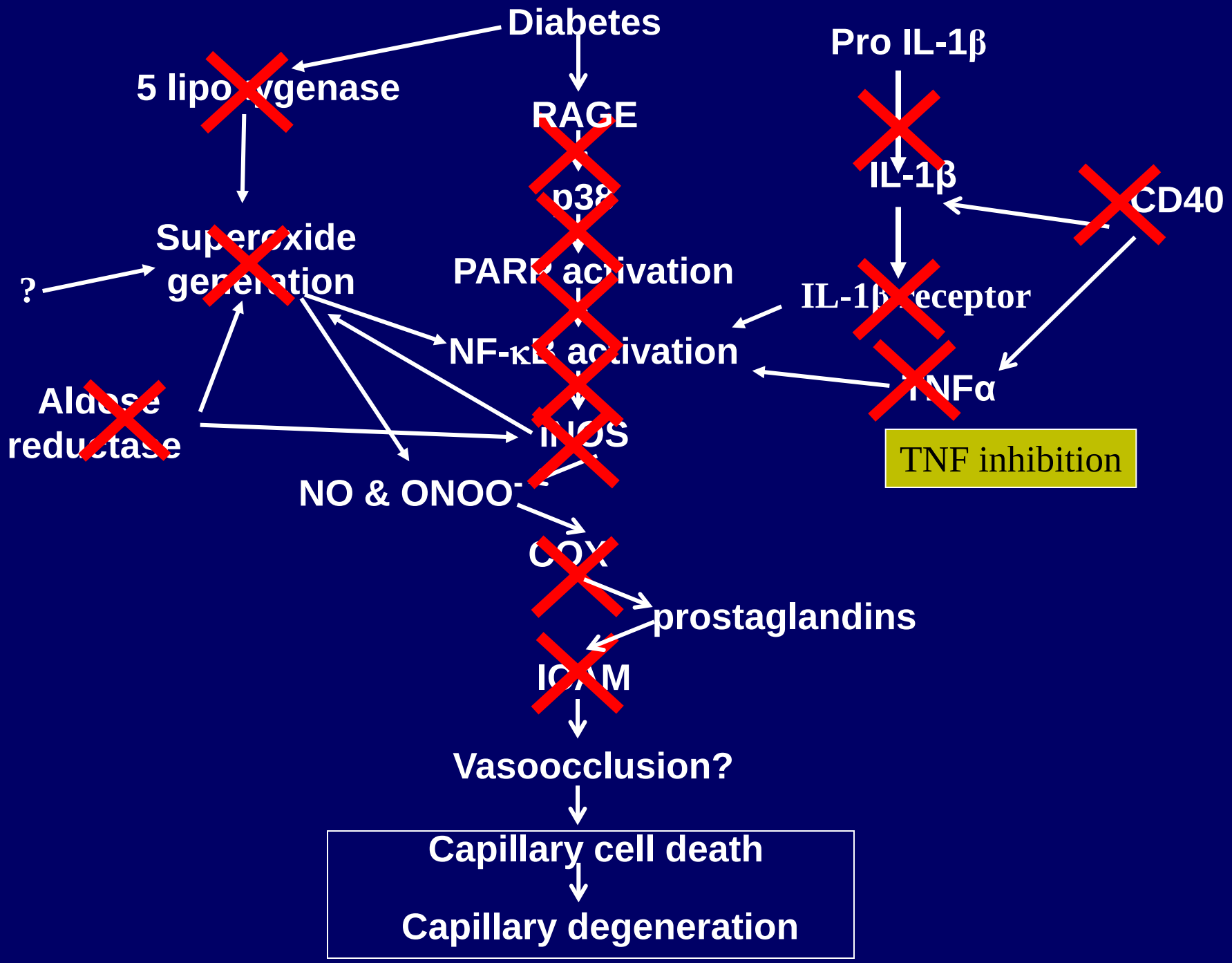
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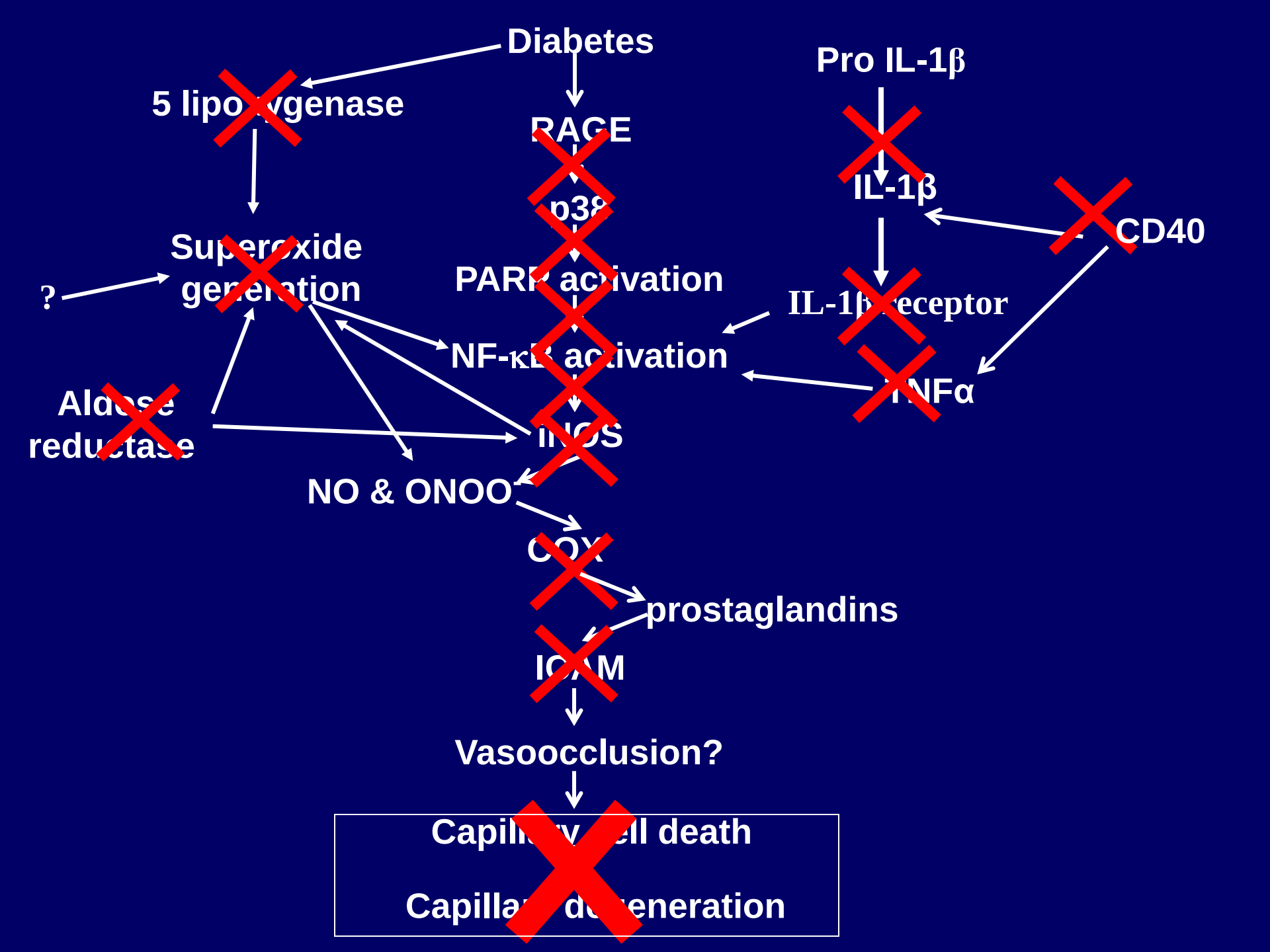
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What cell types have the biochemical abnormalities that cause capillary degeneration/dysfunction in diabetes?

Vascular

Neuroglial

Blood cells

Wildtype C57Bl/6J mouse

(Recipient)

Genetically modified mouse

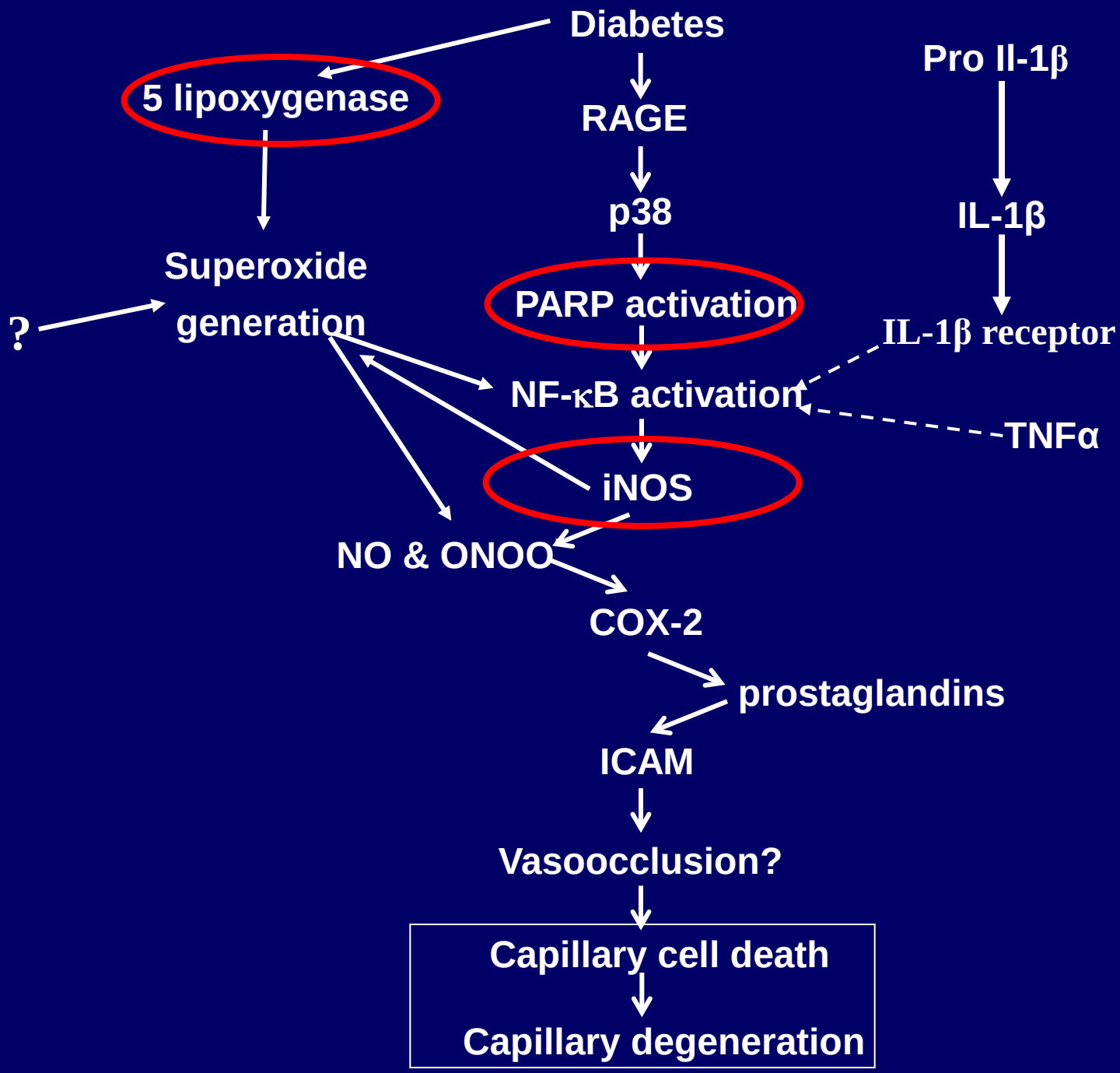
(iNOS$^{-/-}$, PARP$^{-/-}$, etc)

Isolate bone marrow

Inject into irradiated recipient

Irradiate (whole body) to kill bone marrow

Chimeric mouse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iNOS$^{-/-}$ → WT only marrow-derived cells **lack** iNOS

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Isolate bone marrow

Inject into irradiated recipient

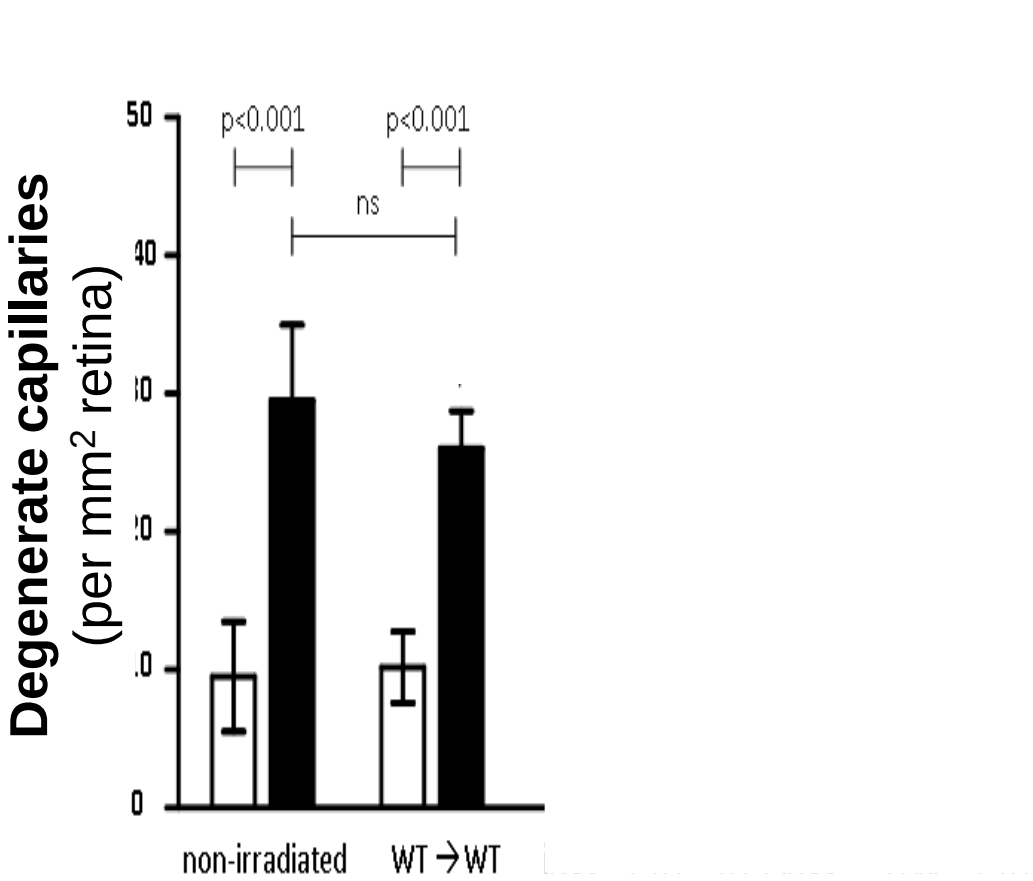
Irradiate (whole body) to kill bone marrow

Chimeric mouse

iNOS$^{-/-}$ → WT only marrow-derived cells **lack** iNOS

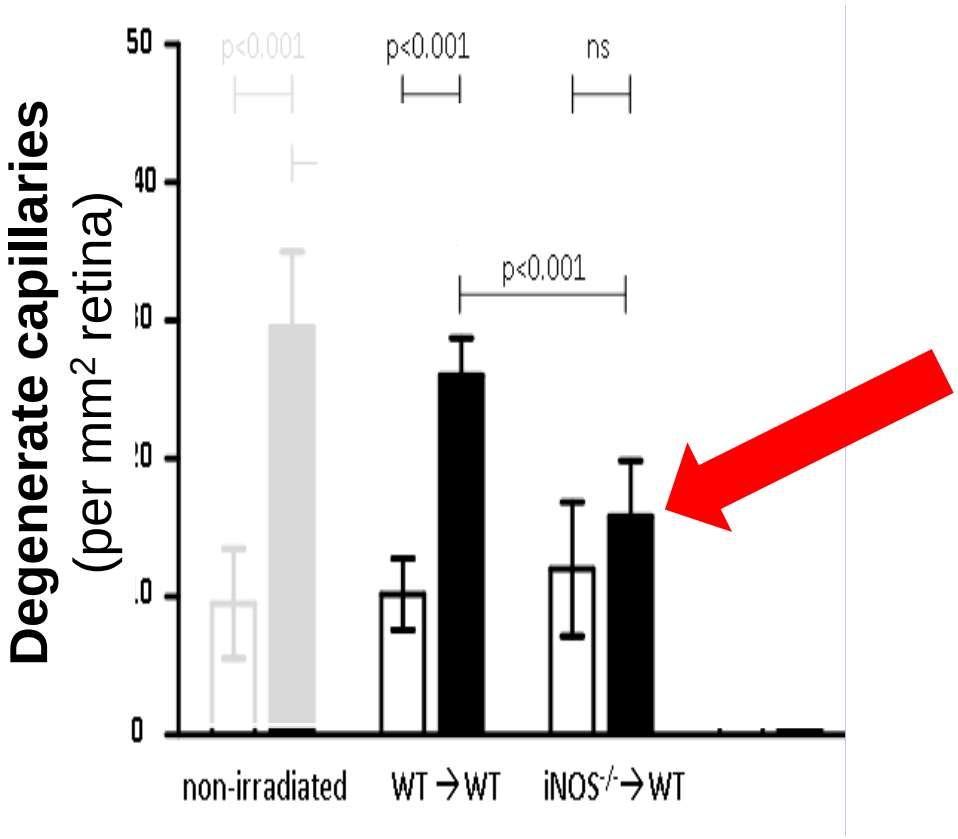
WT → iNOS$^{-/-}$ only marrow-derived cells **have** iNOS

Irradiation does not inhibit the process leading to diabetic retinopathy



Diabetes 2012;61:3294-3303

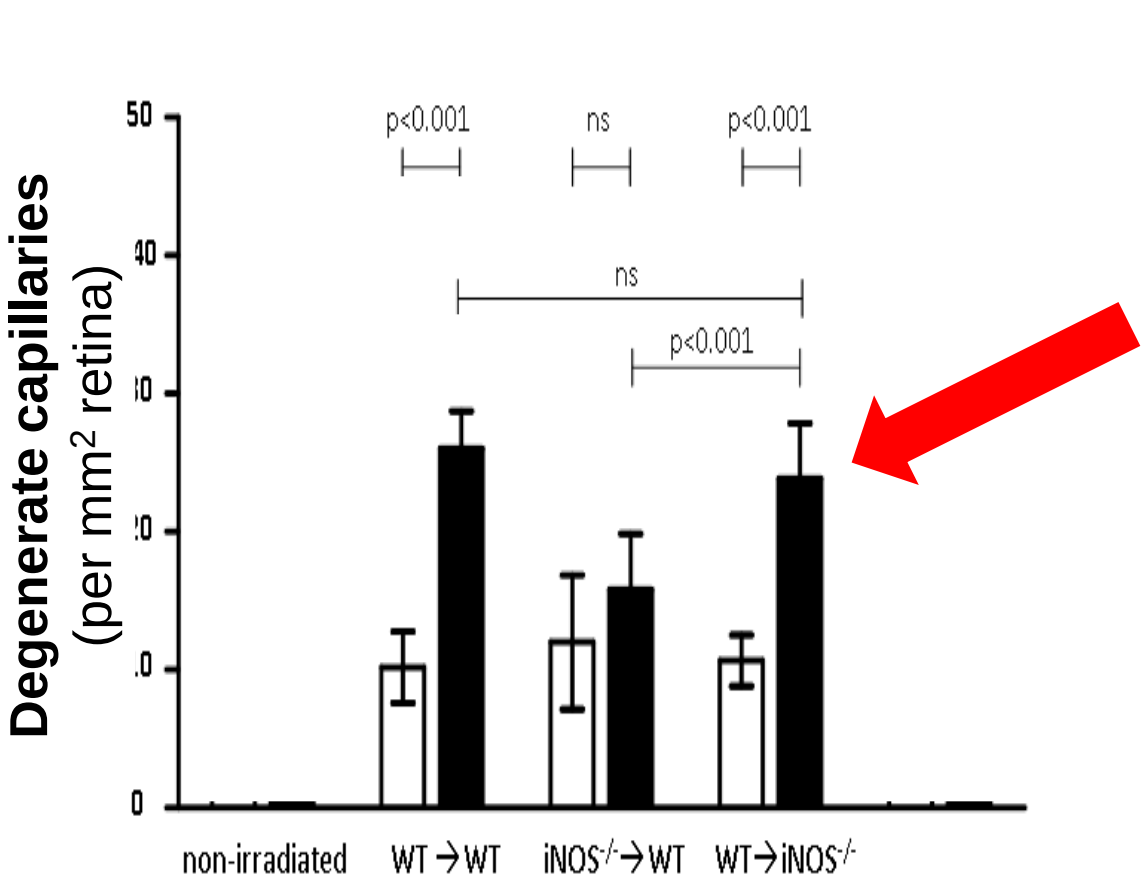
Deletion of iNOS from marrow-derived cells only inhibits DR…



Nondiabetic
Diabetic

Diabetes 2012;61:3294-3303

Deletion of iNOS from marrow-derived cells only inhibits DR…but deletion of iNOS from retinal cells only does not



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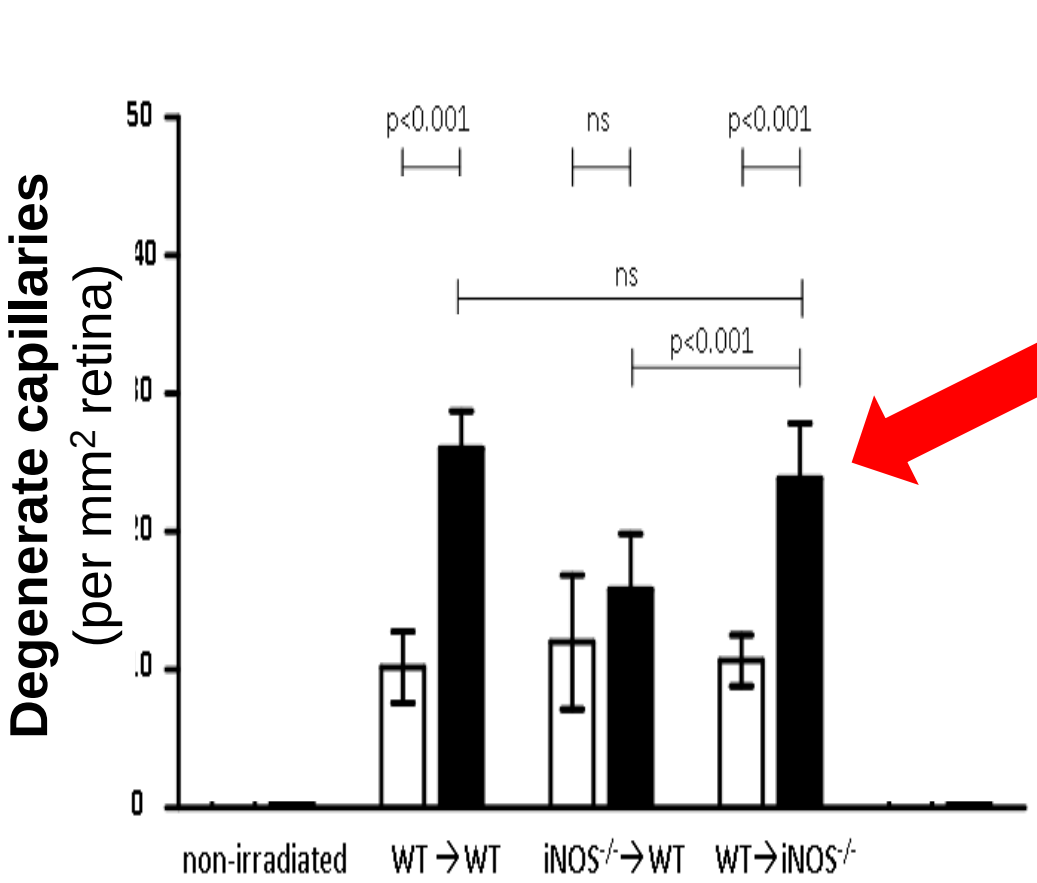


Nondiabetic
Diabetic

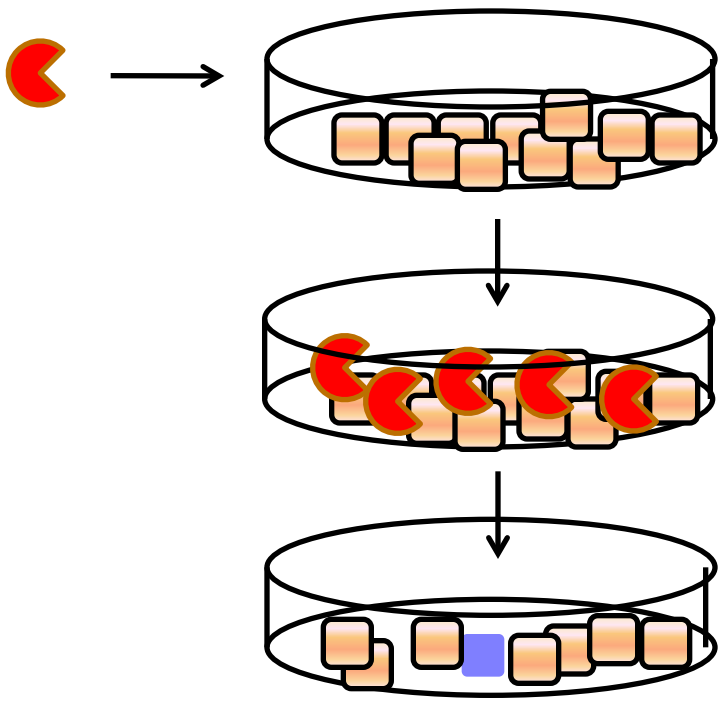
Deletion of iNOS or PARP only from leukocytes also inhibited:

- Oxidative stress in the retina
- Production of inflammatory proteins in the retina
- Leukostasis in the retina

Diabetes 2012;61:3294-3303

Co-culture of marrow-derived cells or leukocytes with retinal endothelial cells to measure leukocyte-mediated killing of endothelial cells

Leukocytes from diabetic or nondiabetic mice



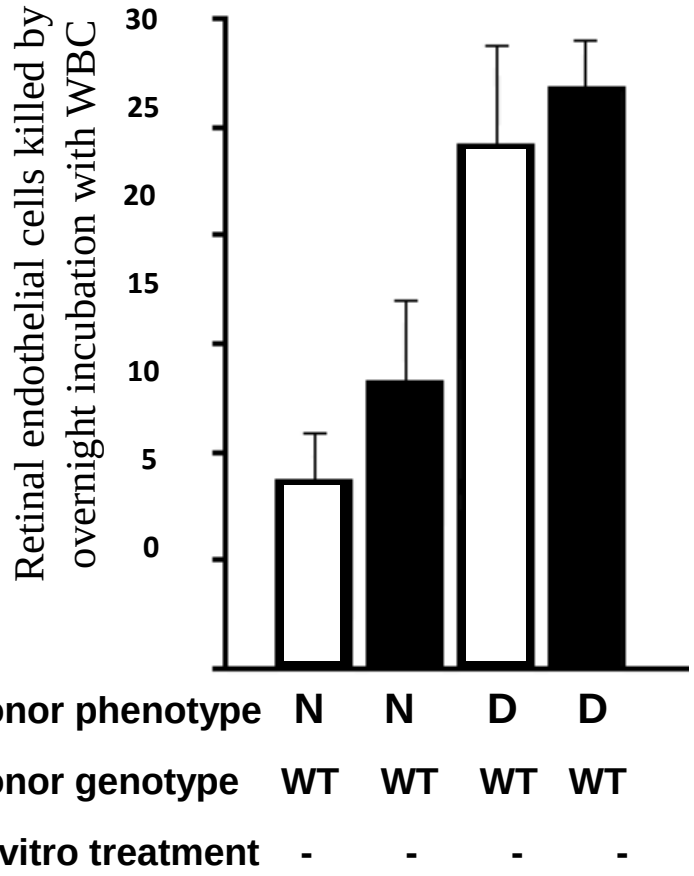
Transformed retinal endothelial cells
5 mM glucose
30 mM glucose

1:5 ratio of leukocytes to endothelial cells
24 hr co-culture

Count endothelial cells that are dead (stained with trypan blue) relative to number of healthy (unstained) endothelial cells

Diabetes 2012;61:3294-3303

Leukocytes from diabetic mice kill more retinal endothelial cells in high glucose *in vitro*



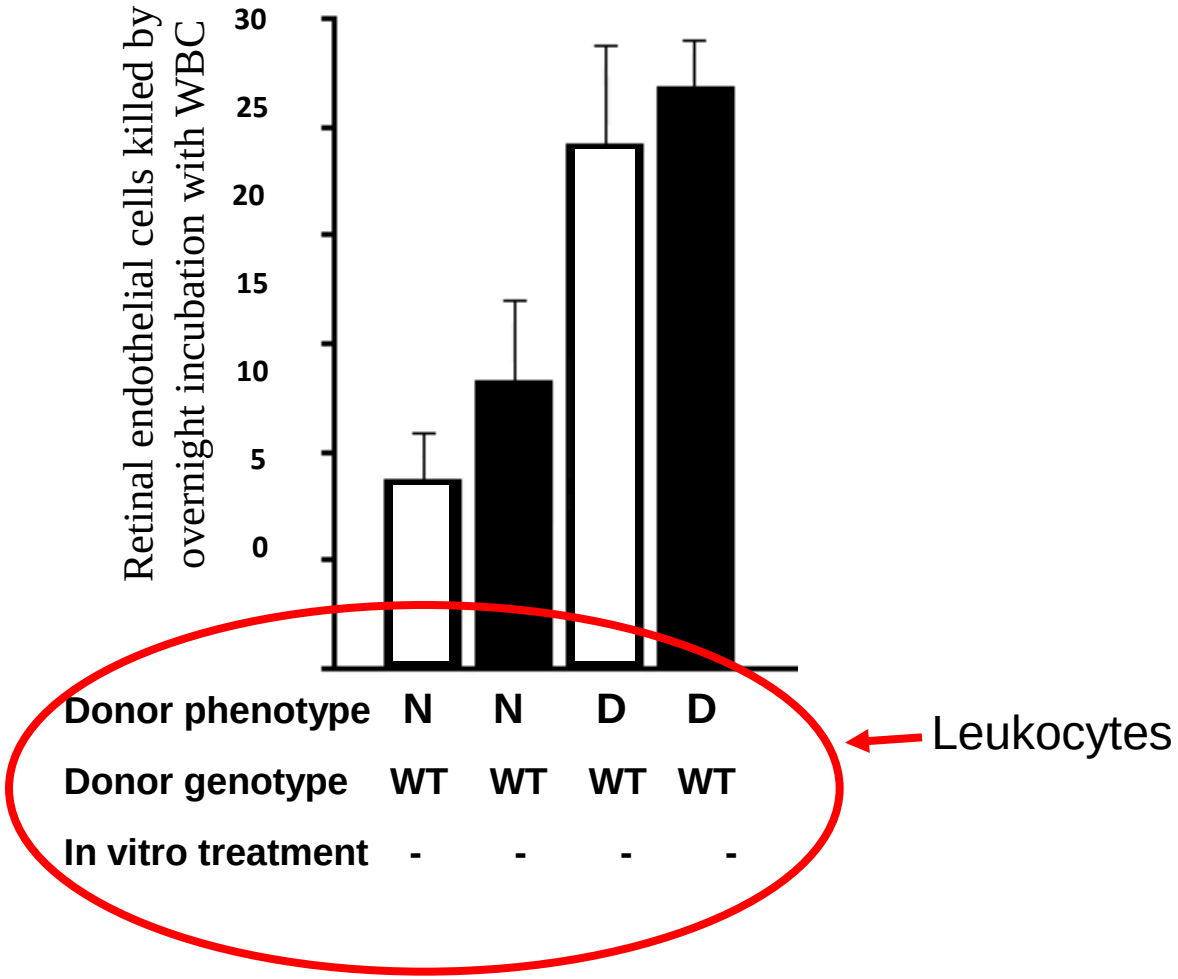
Endothelial cells incubated in:

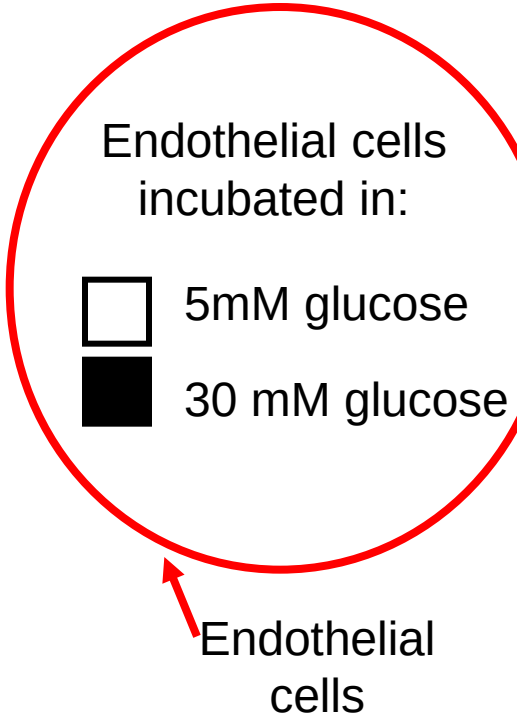
5mM glucose

30 mM glucose

Diabetes 2012;61:3294-3303

Leukocytes from diabetic mice kill more retinal endothelial cells in high glucose *in vitro*





Diabetes 2012;61:3294-3303

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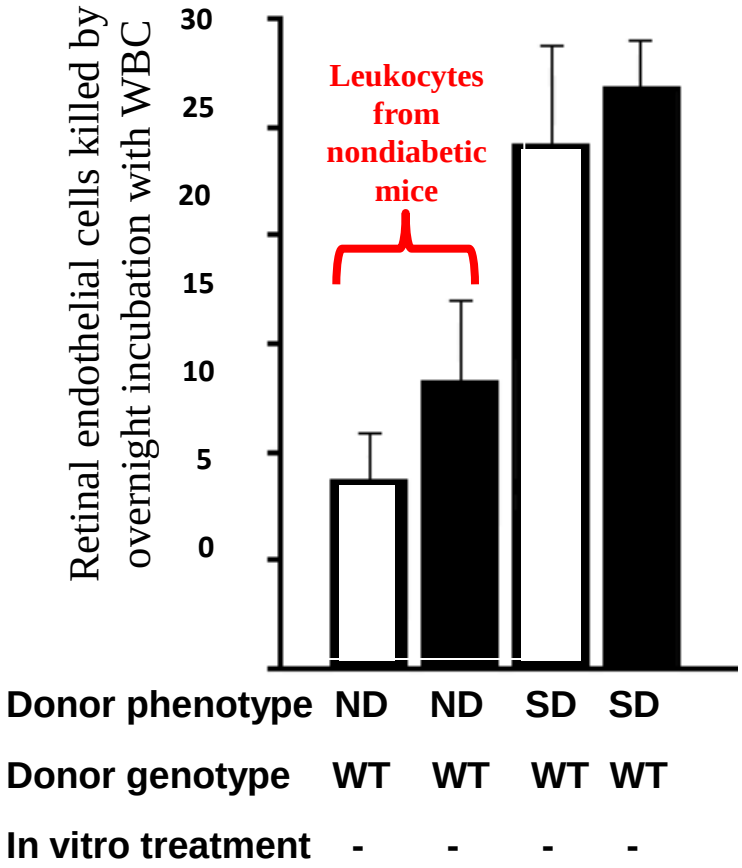


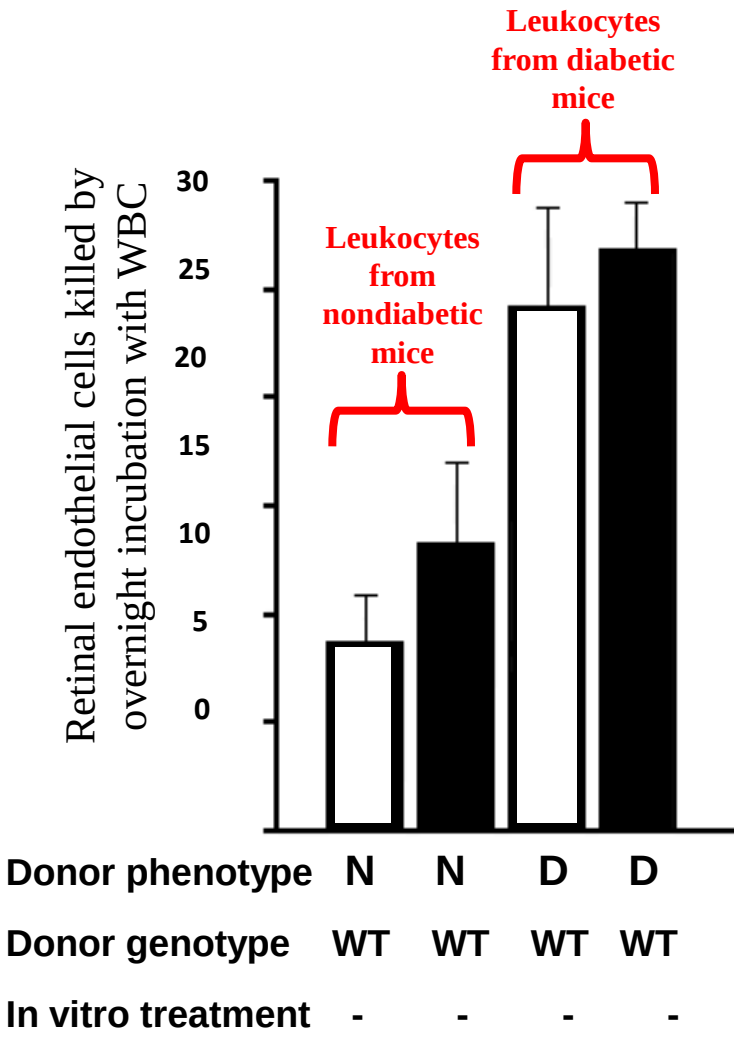
Endothelial cells incubated in:

5mM glucose

30 mM glucose

Diabetes 2012;61:3294-3303

Leukocytes from diabetic mice kill more retinal endothelial cells in high glucose *in vitro*



Endothelial cells incubated in:

5mM glucose

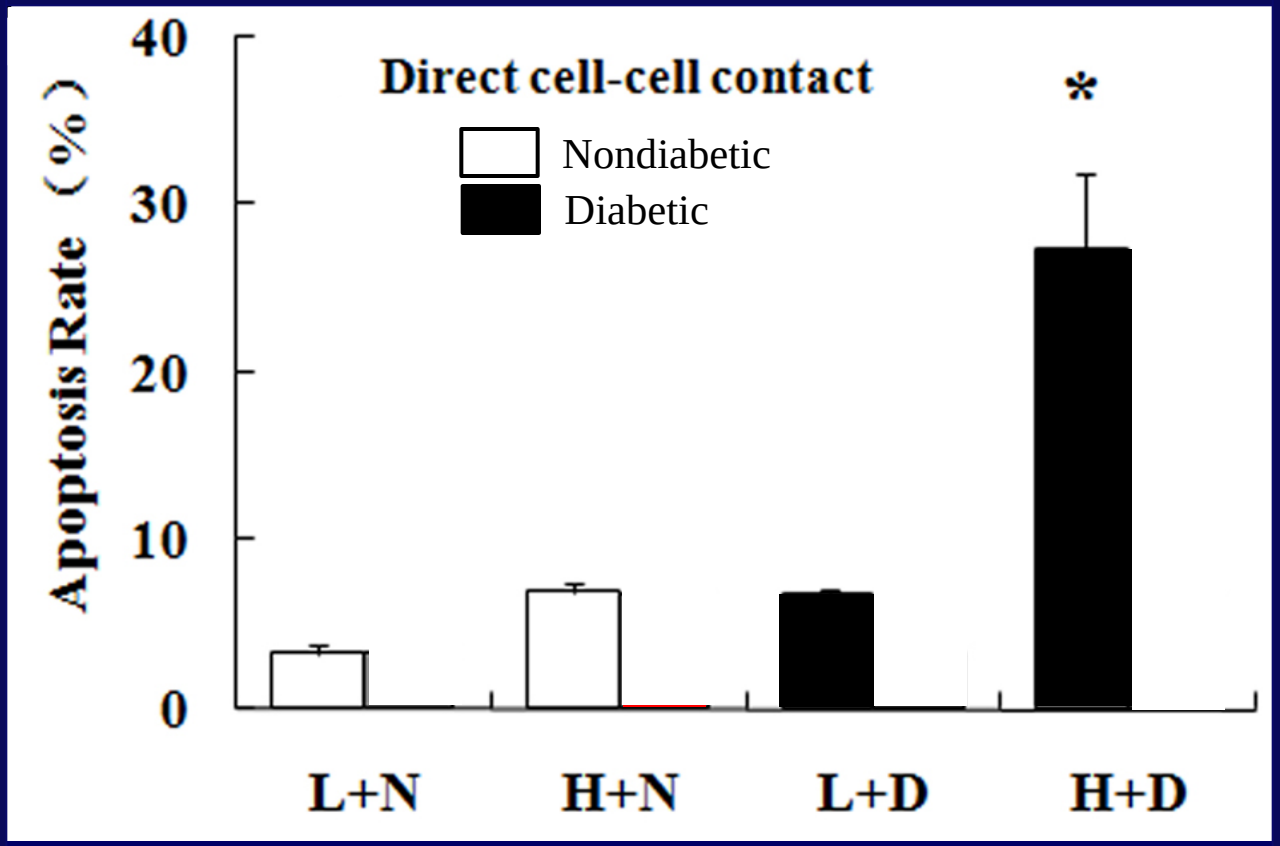
30 mM glucose

Diabetes 2012;61:3294-3303

The animal studies indicate that capillary degeneration that occurs in diabetic retinopathy and possibly neuropathy are caused by leukocytes.

The same leukocyte-mediated killing of endothelial cells occurs in diabetic patients.

Leukocytes from diabetic humans likewise kill retinal endothelial cells via direct contact

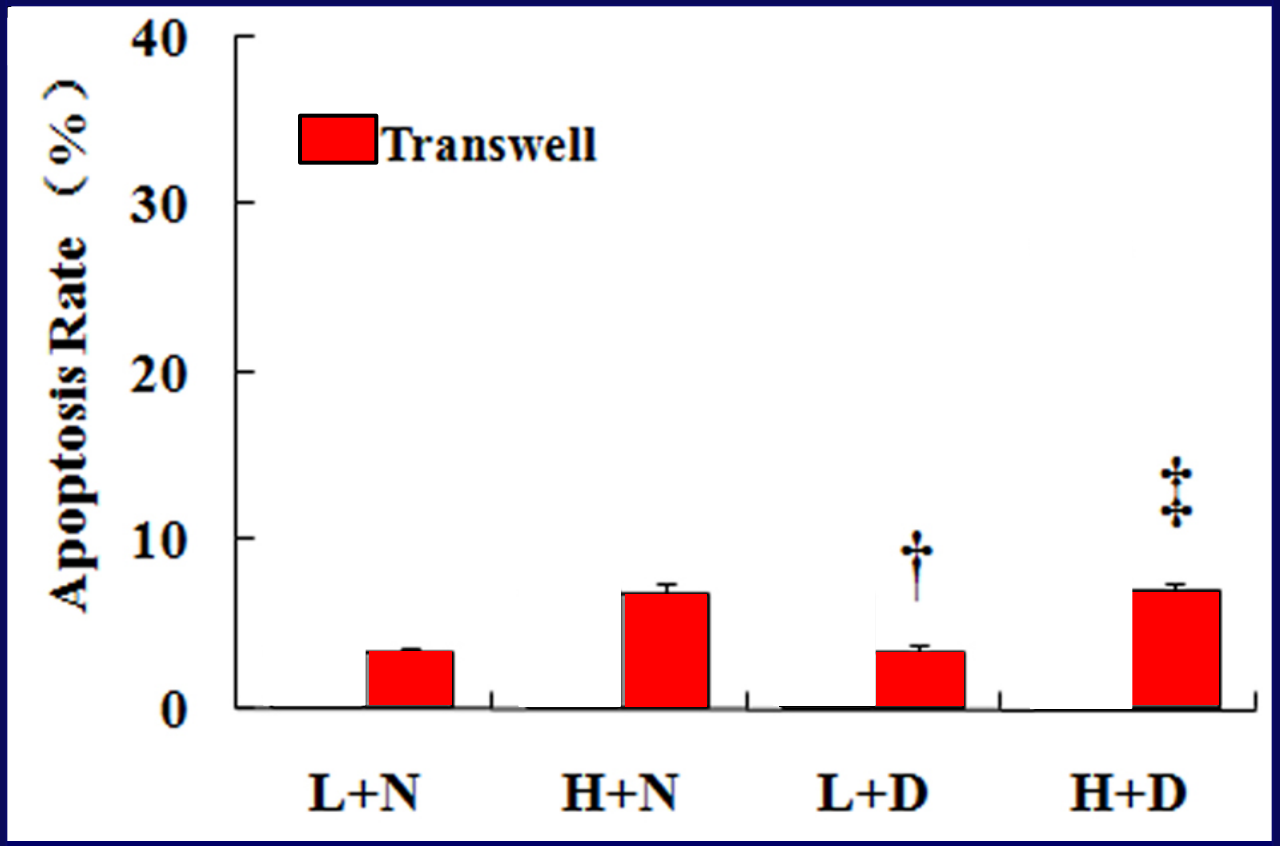


L; human retinal endothelial cells incubated in 5mM glucose
H; human retinal endothelial cells incubated in 25mM glucose

N; leukocytes from nondiabetic patients
D; leukocytes from diabetic patients

Mol Vis. 2013 19:2092-105

Leukocytes from diabetic humans likewise kill retinal endothelial cells via direct contact



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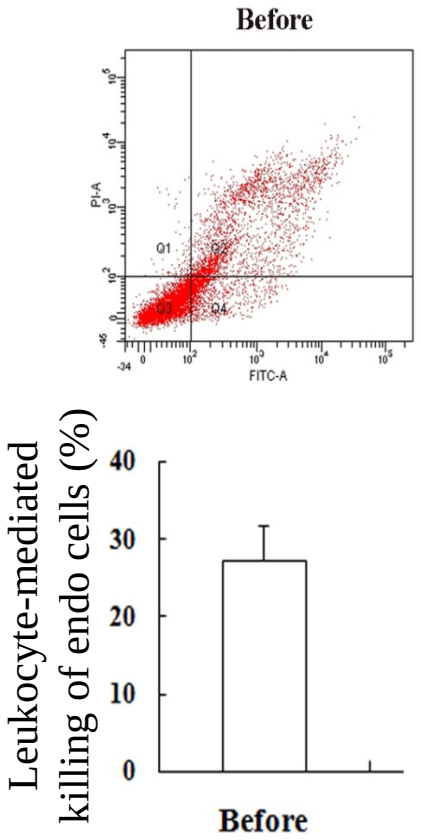
Mol Vis. 2013 19:2092-105

In vivo inhibition of leukocyte-mediated killing of retinal endothelial cells in diabetic patients

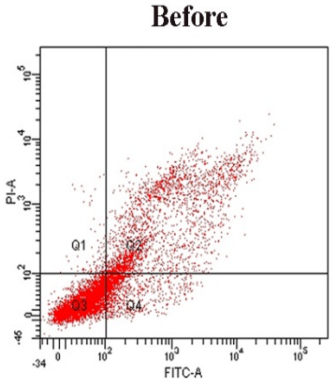
1. Collect blood from diabetic patients, and measure leukocyte-mediated killing of human retinal

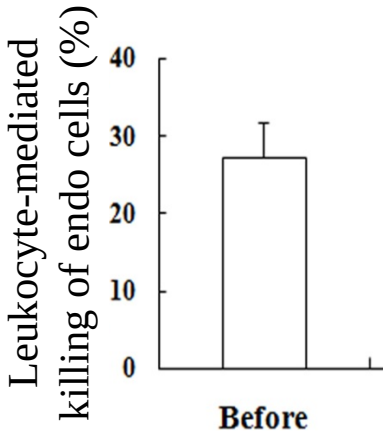


Molecular Vision 2013; **19**:2092-2105

In vivo inhibition of leukocyte-mediated killing of retinal endothelial cells in diabetic patients

1. Collect blood from diabetic patients, and measure leukocyte-mediated killing of human retinal endothelial cells (Before).
2. Ask patients to consume the anti-oxidant and anti-inflammatory agent, berberine, for one month.

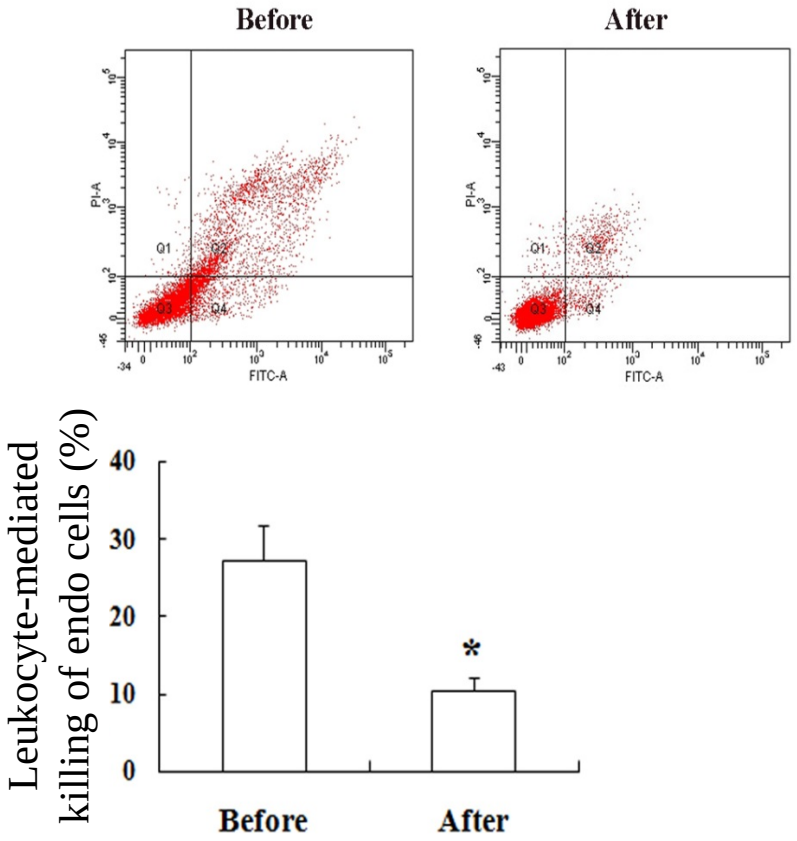




Molecular Vision 2013; **19**:2092-2105

In vivo inhibition of leukocyte-mediated killing of retinal endothelial cells in diabetic patients

1. Collect blood from diabetic patients, and measure leukocyte-mediated killing of human retinal endothelial cells (Before).
2. Ask patients to consume the anti-oxidant and anti-inflammatory agent, berberine, for one month.
3. Recollect blood from the same patients, and re-measure leukocyte-mediated killing of endothelial cells (After).



Molecular Vision 2013; **19**:2092-2105

What is the molecular cause of capillary degeneration in diabetic retinopathy?

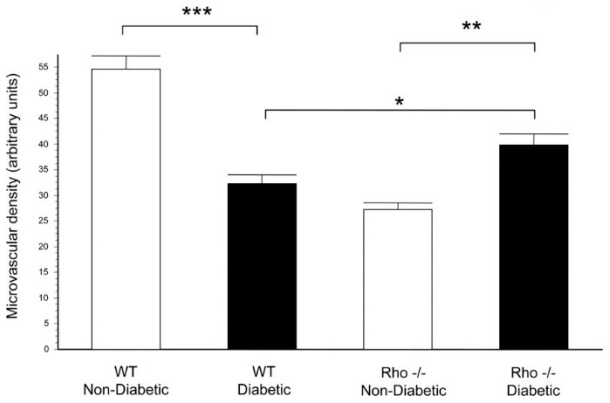
What cell types are involved in the development of diabetic retinopathy, and do those cells offer new ways to inhibit the retinopathy?

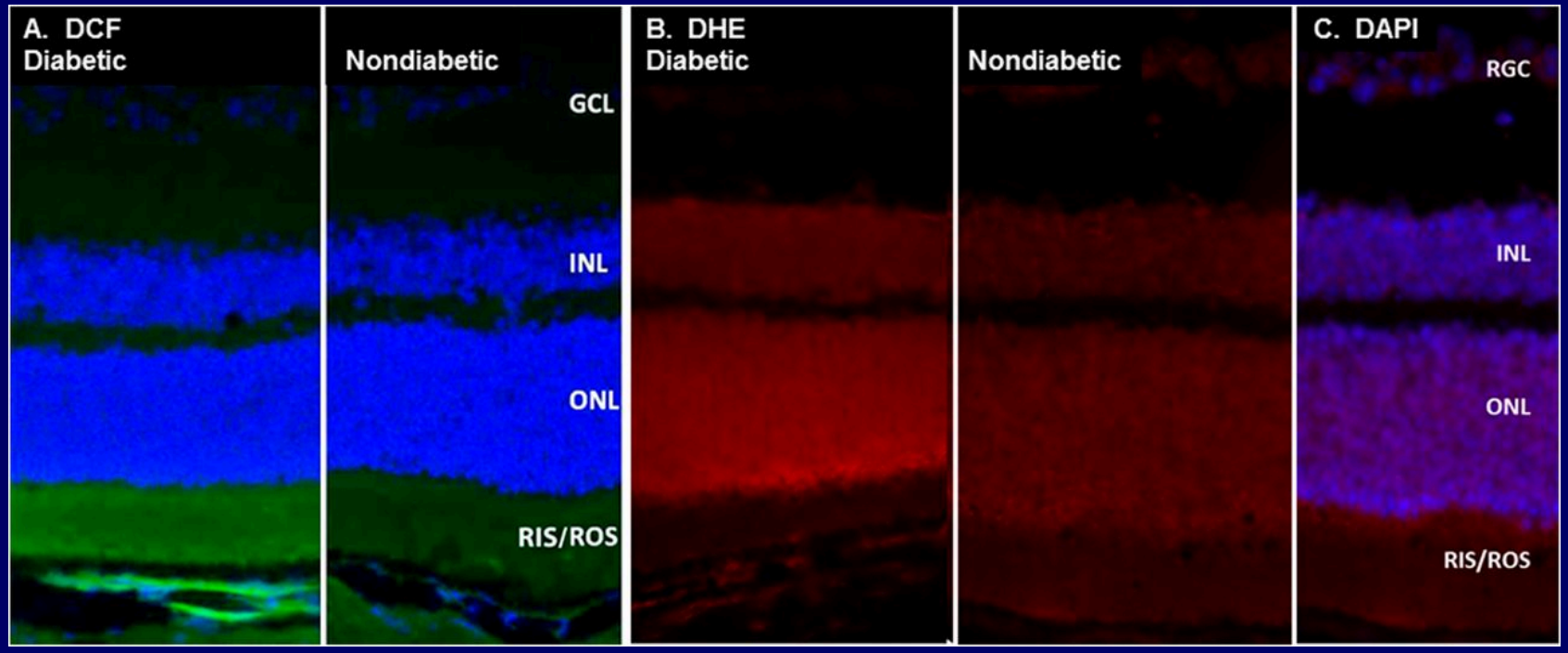
Leukocytes

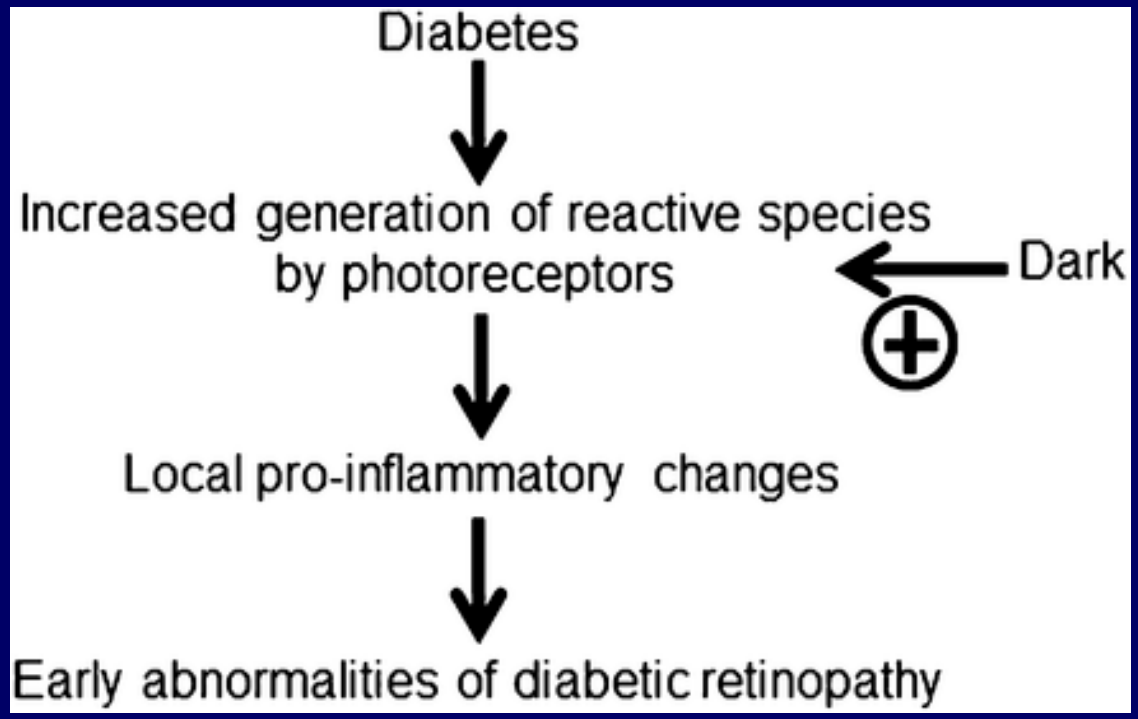
Photoreceptors

Photoreceptors and diabetic retinopathy

- Laser photocoagulation has beneficial effects on advanced diabetic retinopathy in patients
- Patients having retinitis pigmentosa have been reported to have less diabetic retinopathy. ***Br J Ophthalmol*. 2001;85(3):366-70**
- Continuous low intensity light all night inhibits retinal edema. ***Eye (Lond).* 2010;24:1149-55 and 2011;25:1546-54.**
- Density of retinal vasculature was preserved in diabetic rhodopsin$^{-/-}$ animals. ***Invest. Ophthalmol. Vis. Sci*. 47:5561-68, 2006**

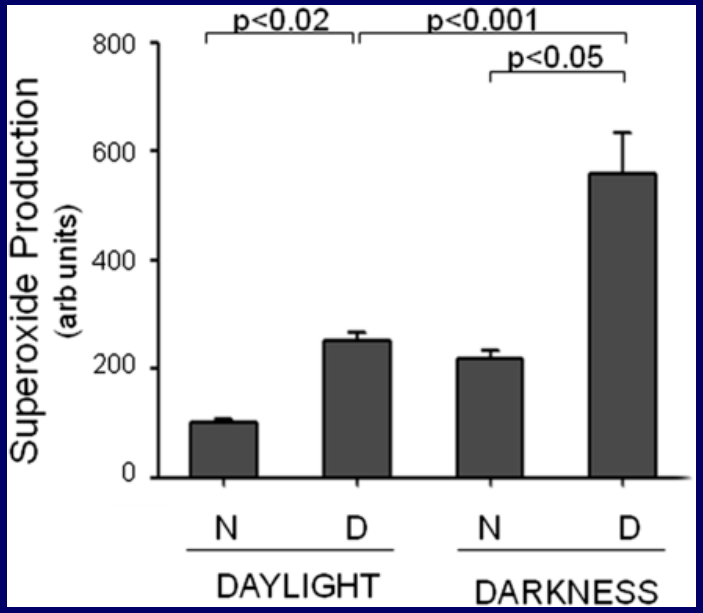




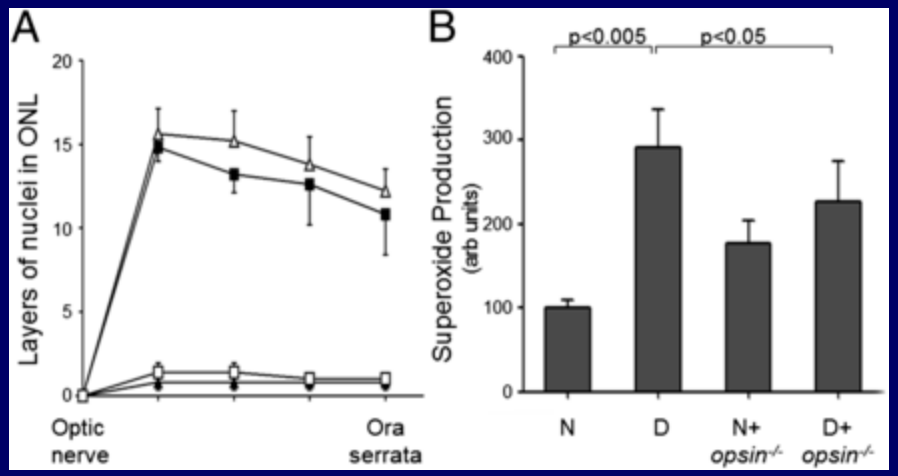


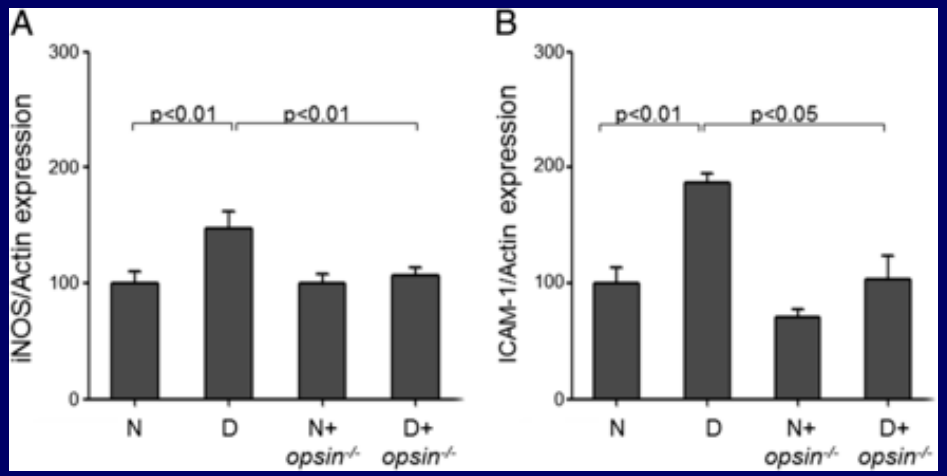
PNAS U S A. 2013 110:16586-91

Photoreceptor cells cause the oxidative stress and secondary inflammatory response in diabetes



Photoreceptor degeneration occurs in animals lacking opsin

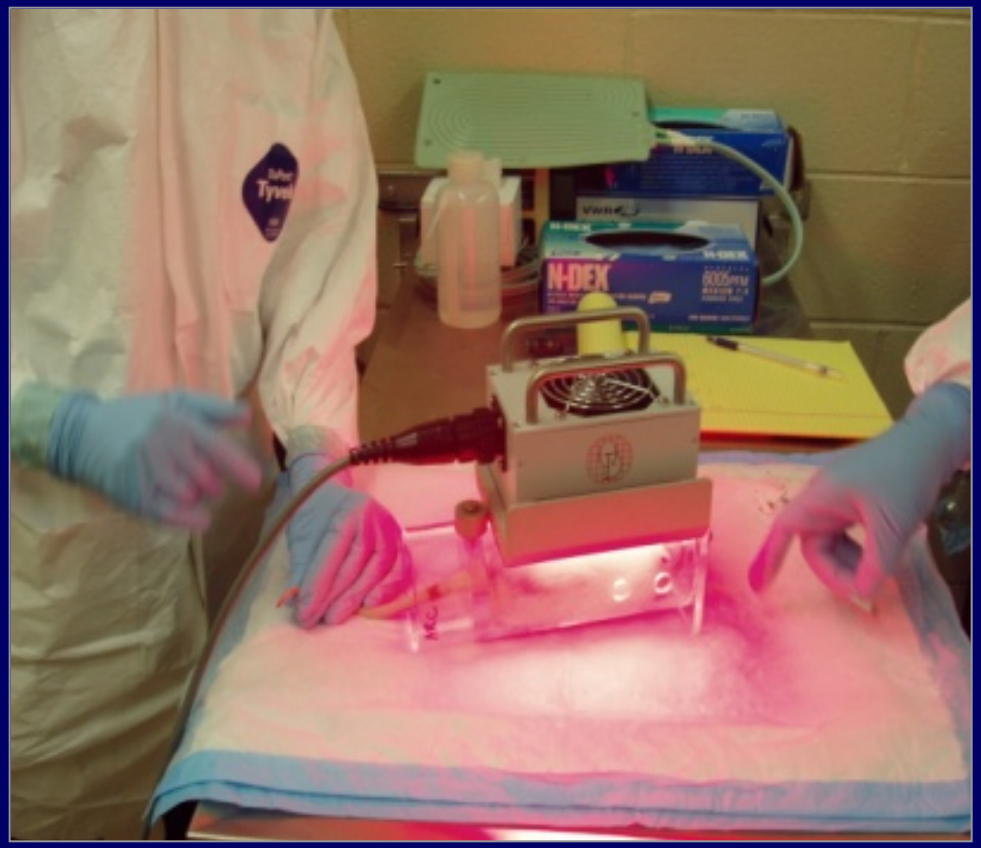




Low-intensity far-red light inhibits early lesions that contribute to diabetic retinopathy: in vivo and in vitro

Low intensity far-red light (photobiomodulation): effects on the retina in diabetic rats

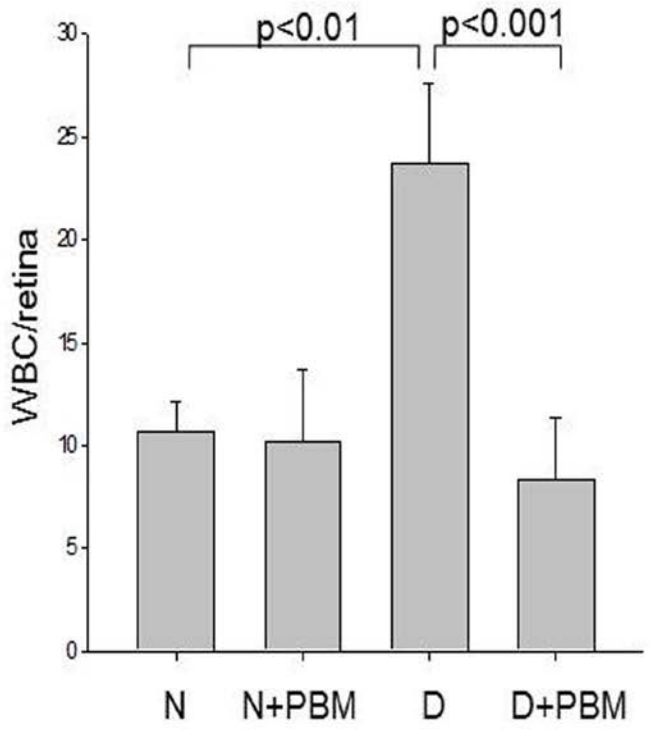
Light treatment is only 4 minutes per day

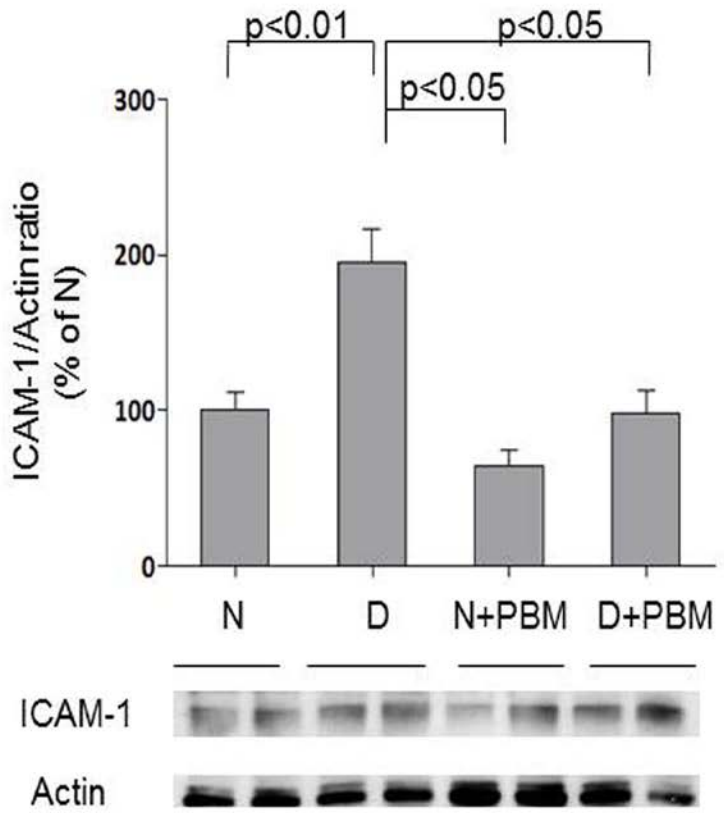


670 nm
6 joules cm^2 per session
7days/wk
10wks therapy

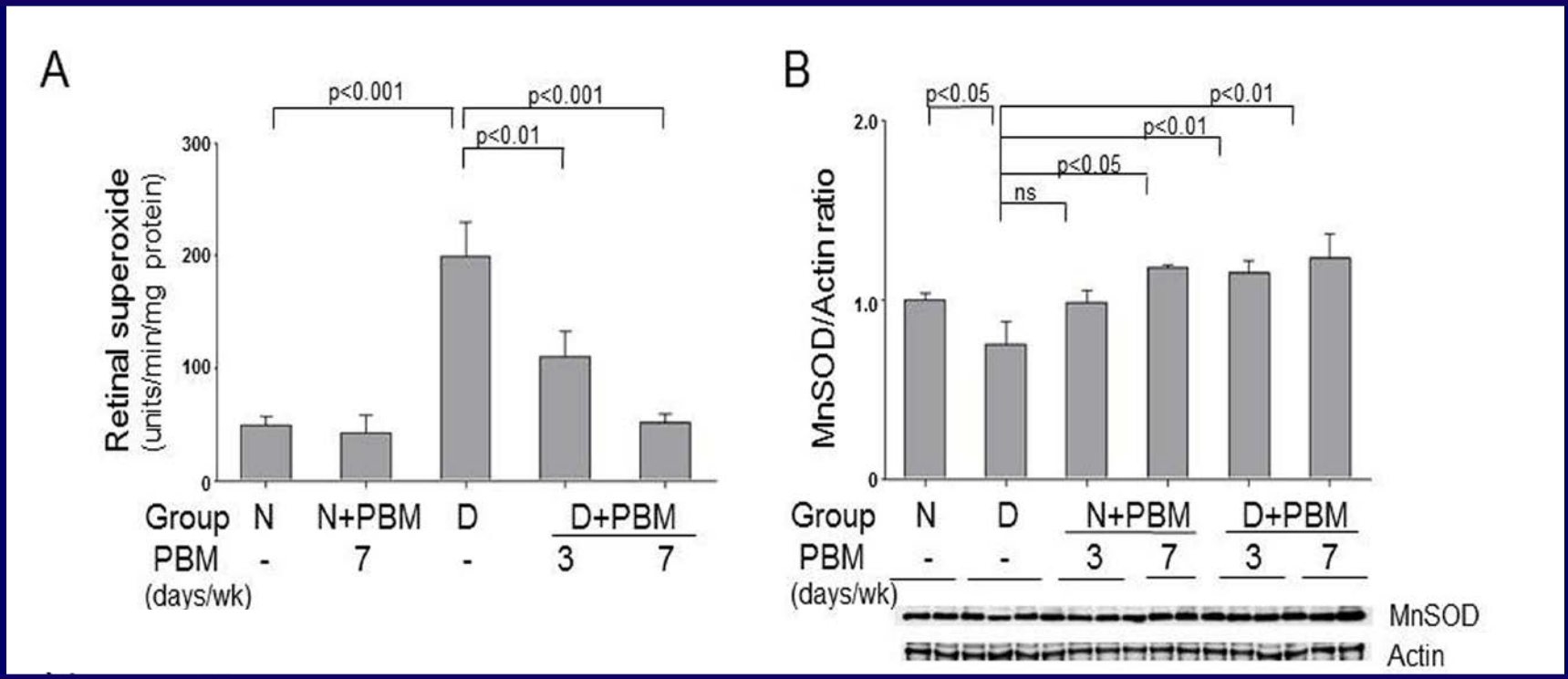
Diabetes was induced approx 8 days before LED therapy was initiated

Light therapy inhibited the diabetes-induced increase in leukostasis in retinal microvessels and induction of ICAM

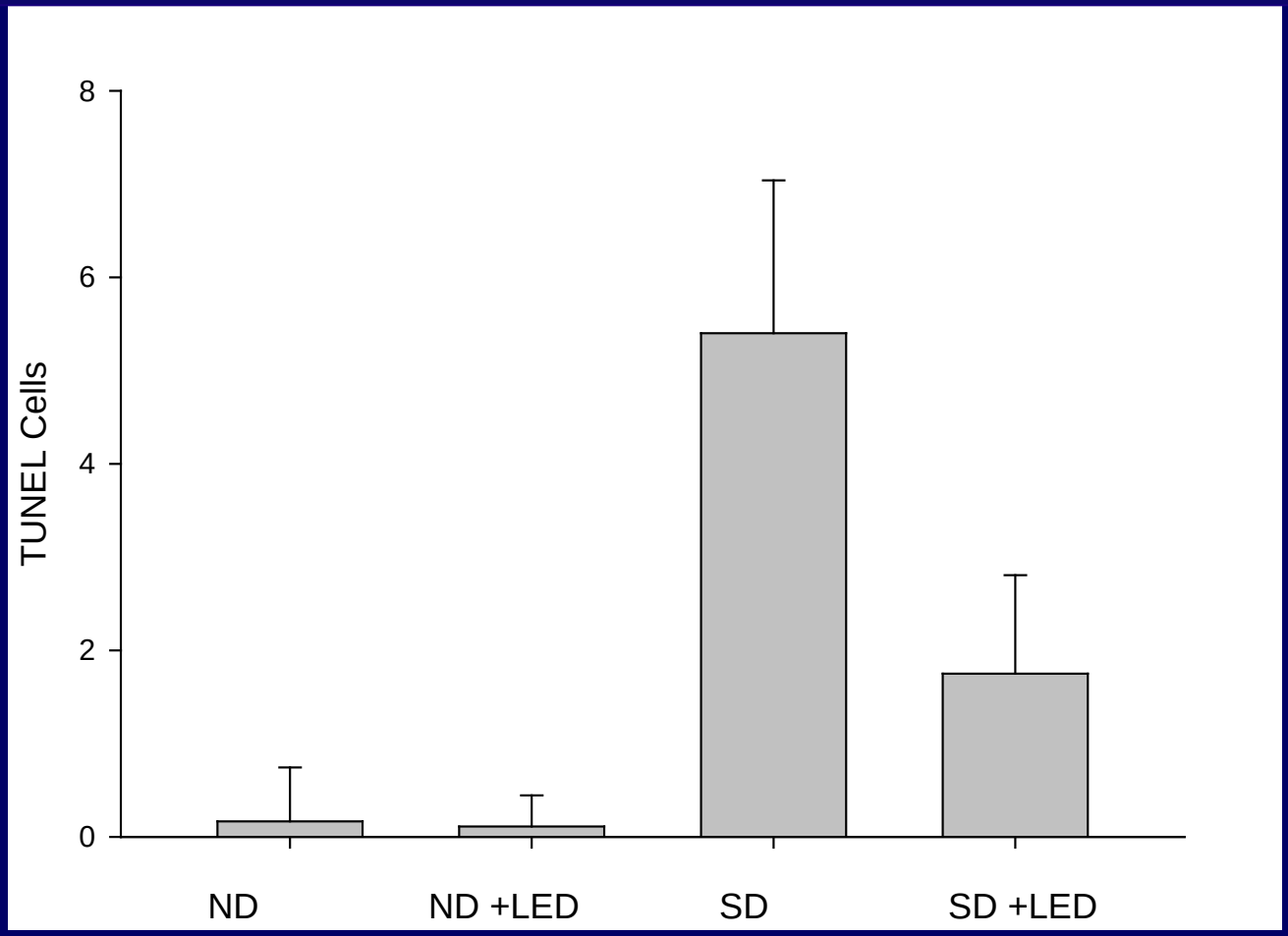




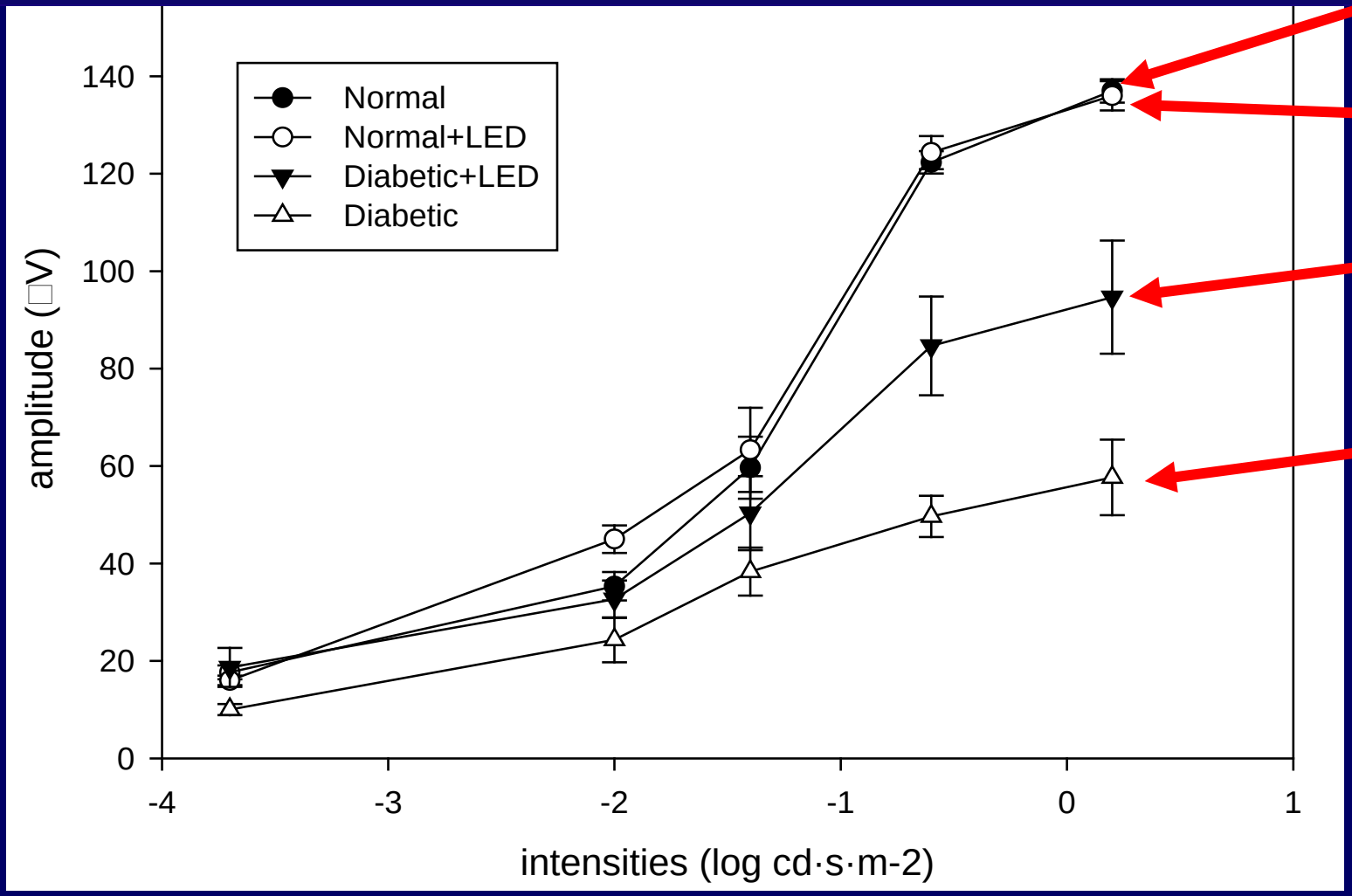
Light therapy inhibits the diabetes-induced increase in retinal production of superoxide and inhibits loss of SOD



Light therapy inhibited diabetes-induced apoptosis of retinal ganglion cells in vivo



Light therapy partially inhibited diabetes-induced loss in retinal function (photopic response)



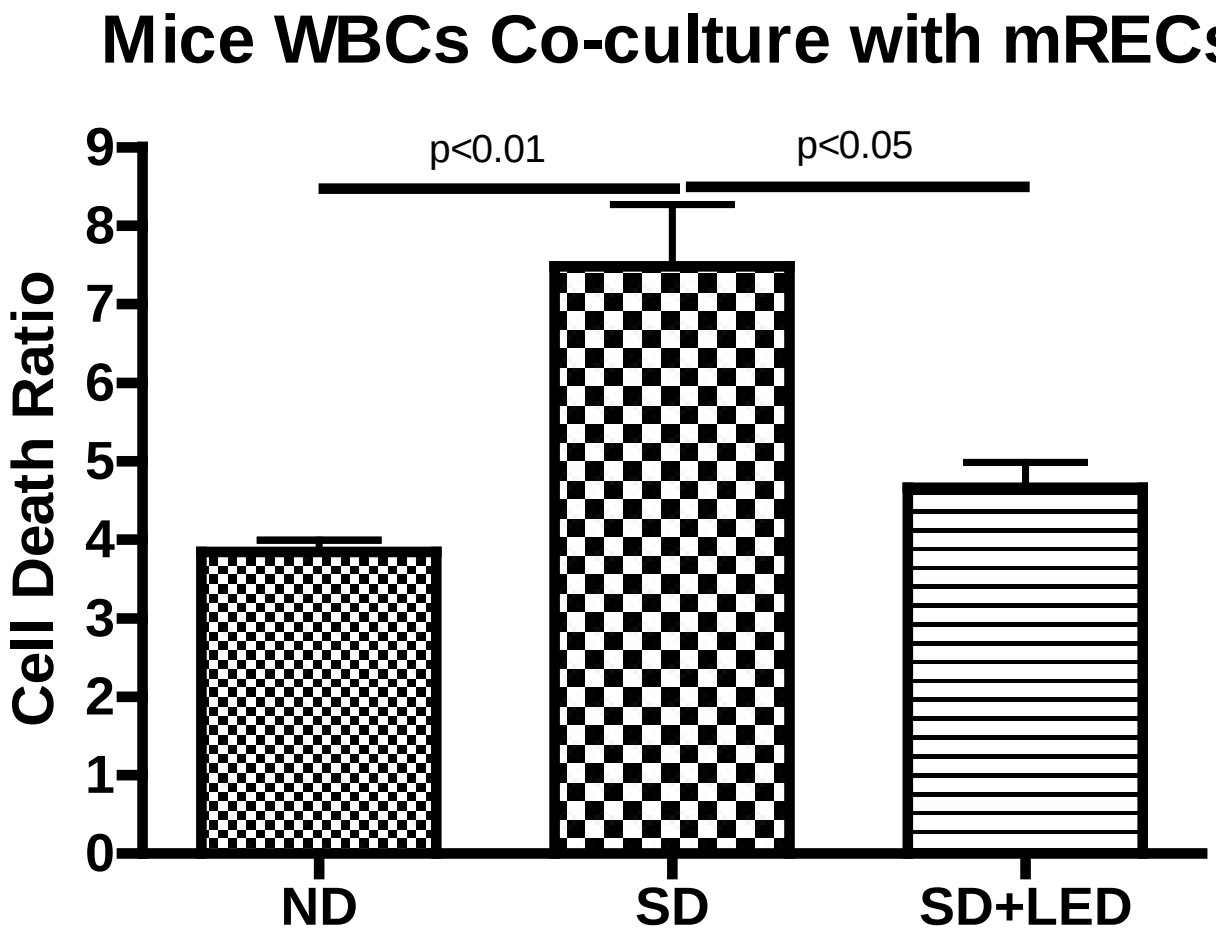
Nondiabetic

Nondiabetic +LED

Diabetic +LED

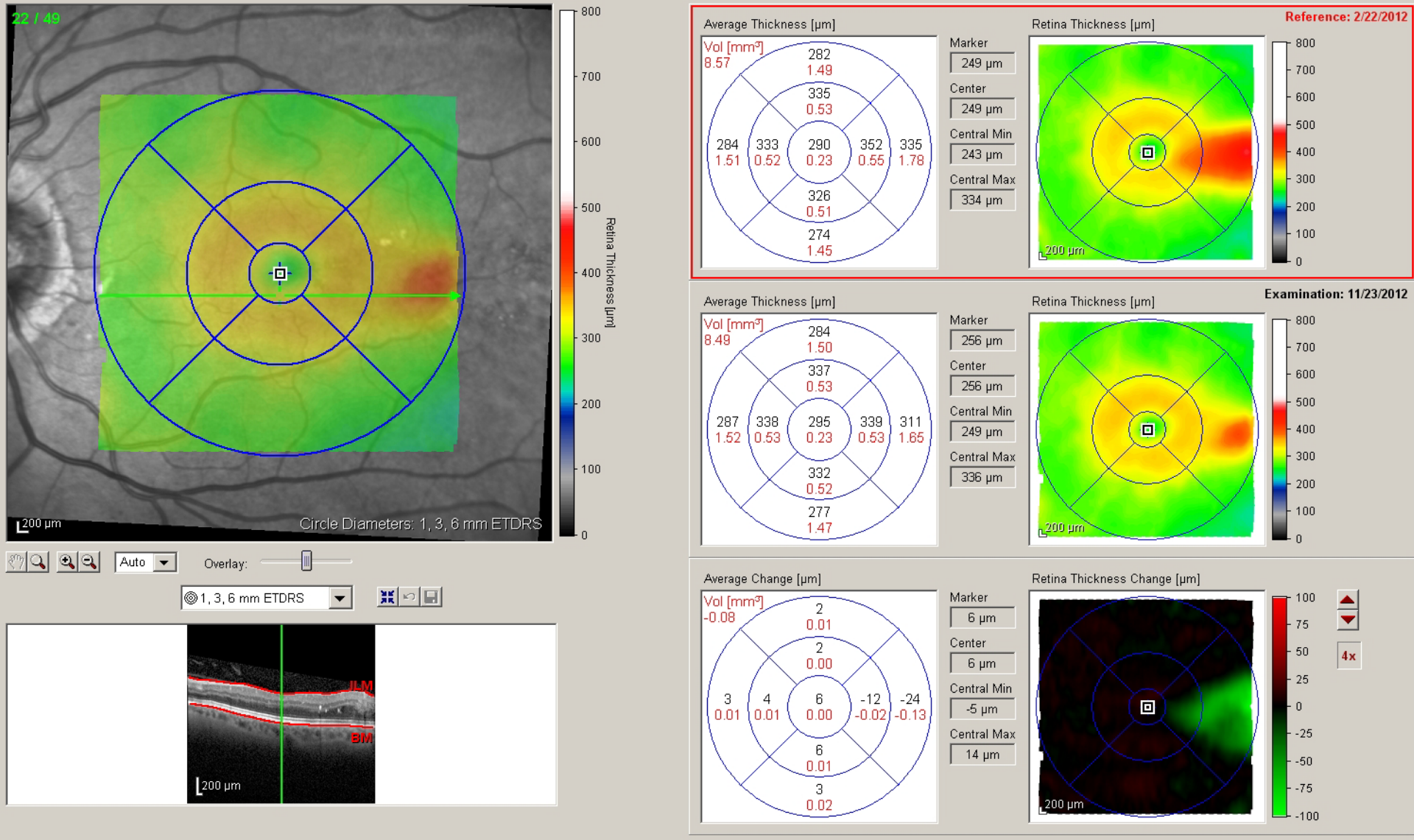
Diabetic

Far-red light also inhibits leukocyte-mediated degeneration of retinal endothelial cells

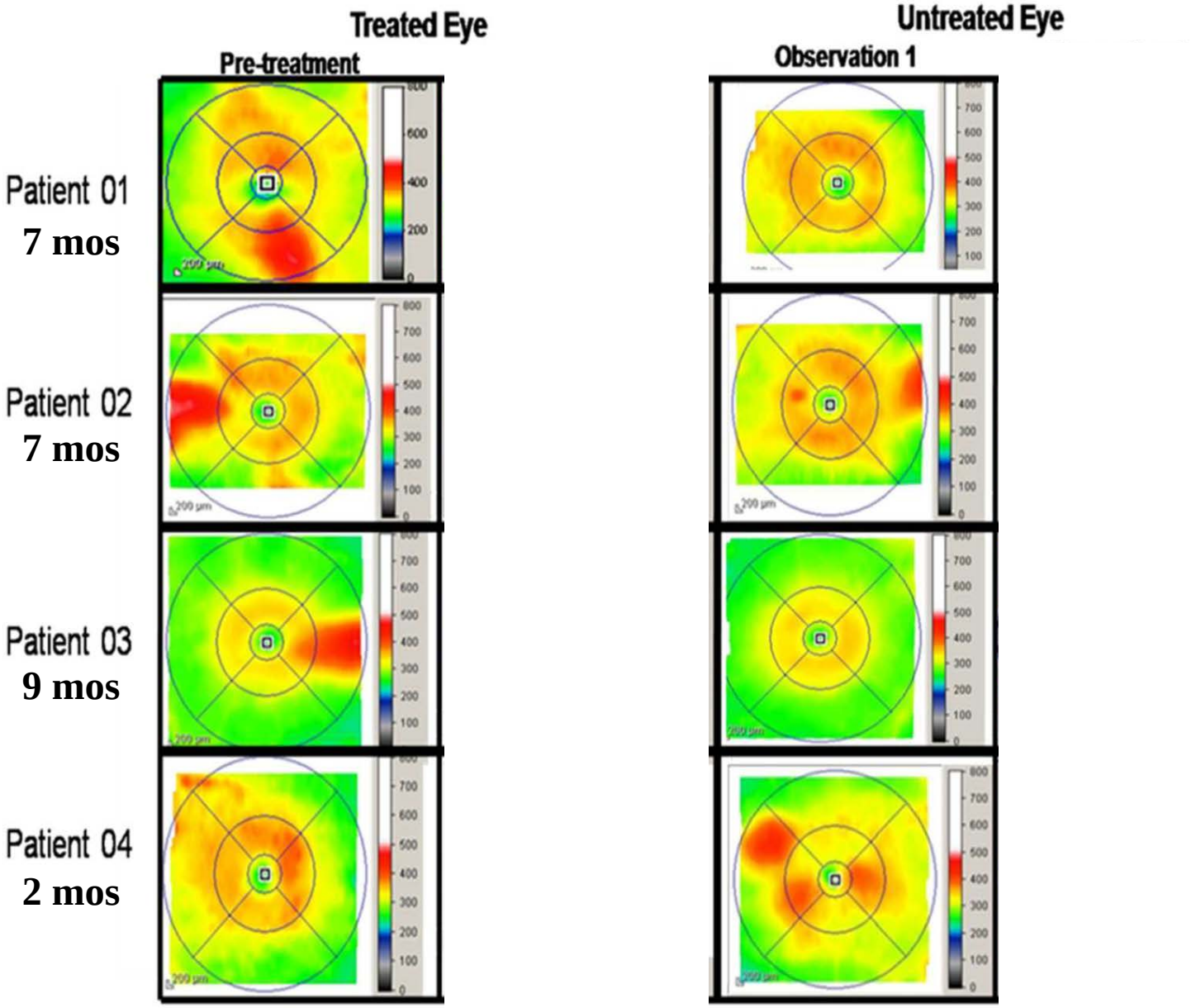


Light treatment is only 4 minutes per day

Optical coherence tomography assessment of macular edema in patients



Reversal of non-center involved macular edema using 3 min/day of far-red light



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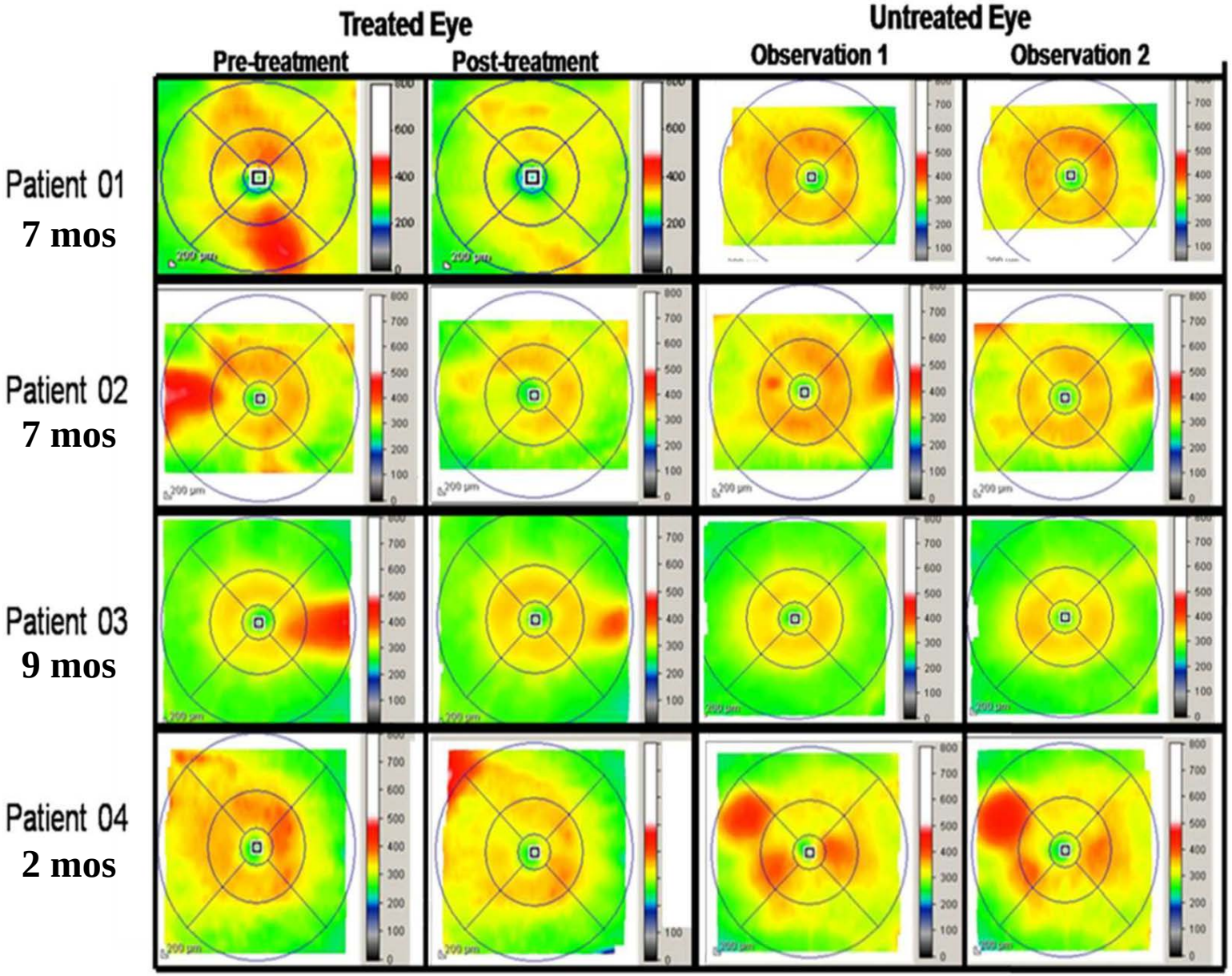

Conclusions

- Immune/inflammatory processes play a critical role in the development of at least the vascular lesions of early diabetic retinopathy.
- The inflammation seems to be derived, or at least initiated by, marrow-derived cells (notably neutrophils). Retinal cells contribute also, but their contribution needs to be studied more.
- Future investigations on the pathogenesis of diabetic complications need to expand beyond the traditional vascular-specific view of complications, to include also immune cells.
- We are investigating the possibility that leukocyte studies might be used in the future to predict the likelihood that a particular patient will progress to advanced complications, and to predict their response to particular therapies.