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Renal dysfunction and metabolic syndrome: the chicken or the egg?

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**Academic Medical Center,
University of Amsterdam,
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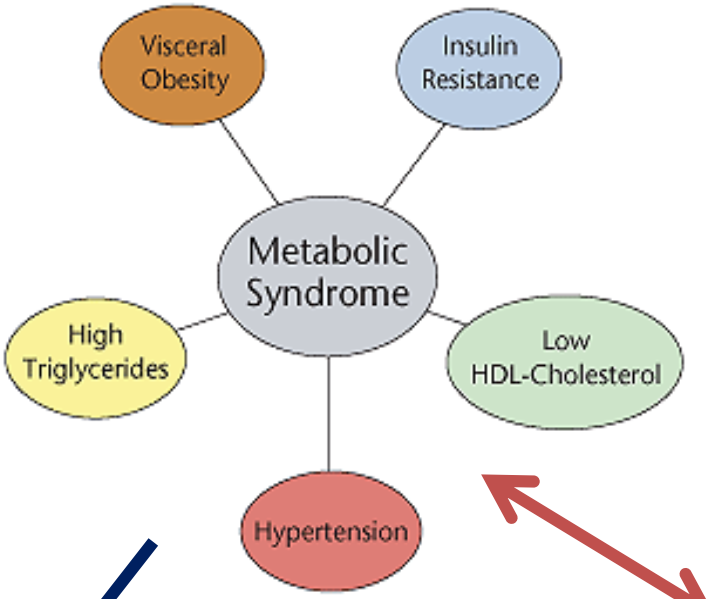
Metabolic syndrome



Type 2 Diabetes

Hearth disease

Stroke

Fatty Liver Disease

Renal dysfunction

Tubular atrophy,
Interstitial fibrosis,
Glomerulosclerosis

Metabolic syndrome & Renal dysfunction

- In 1974, first description of an association between Metabolic Syndrome & Nephrotic proteinuria.

basic research http://www.kidney-international.org

Nlrp3 is a key modulator of diet-induced nephropathy and renal cholesterol accumulation

Pieter J. Bakker[1], Loes M. Butter[1], Lotte Kors[1], Gwendoline J.D. Teske[1], Jan Aten[1], Fayyaz S. Sutterwala[2], Sandrine Florquin[1,3] and Jaklien C. Leemans[1]

[1]Department of Pathology, Academical Medical Center, Amsterdam, The Netherlands; [2]Department of Internal Medicine, Inflammation Program, University of Iowa, Iowa City, Iowa, USA and [3]Department of Pathology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands

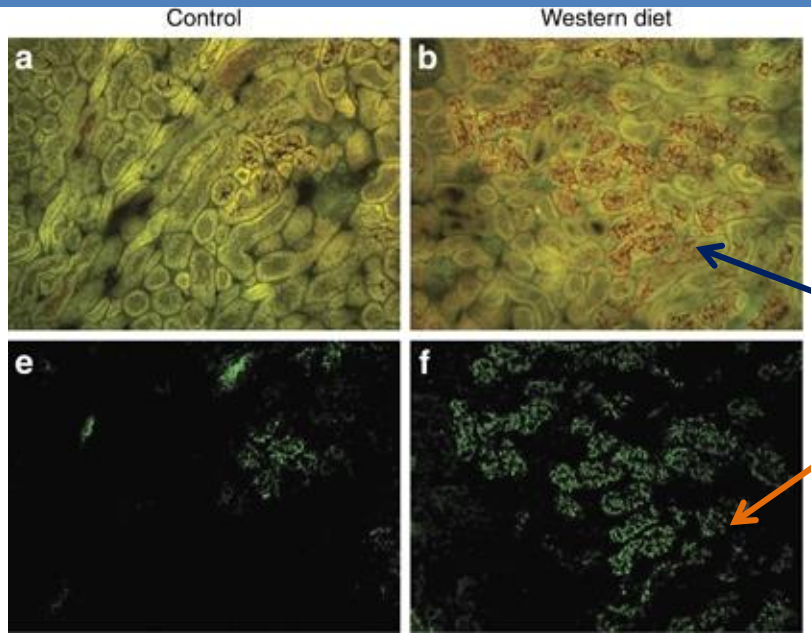
Western-diet →
- Weight gain
- Insulin resistance
- Dyslipidemia

↓

Renal pathology

- **Renal steatosis**
- **Cholesterol & phospholipid accumulation**
- **Renal inflammation & fibrosis**
- **Microalbuminuria**

Kidneys in Murine model of Metabolic syndrome



Nile Red positive vacuoles

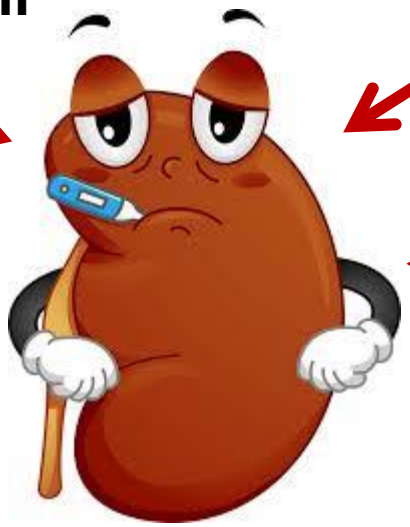
Phospholipid accumulation

Causes of renal dysfunction in Metabolic syndrome??

Glomerular hyperfiltration

Excess reabsorption

Physical compression by adipose tissue



Hyperlipidemia

Renal lipotoxicity

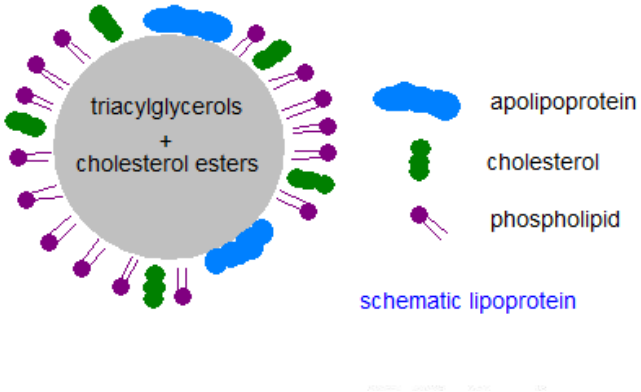
Effects of LDL on tubular epithelial cells?

RAAS activation

Glomerular and tubulointerstitial remodeling/injury

Experimental Approach

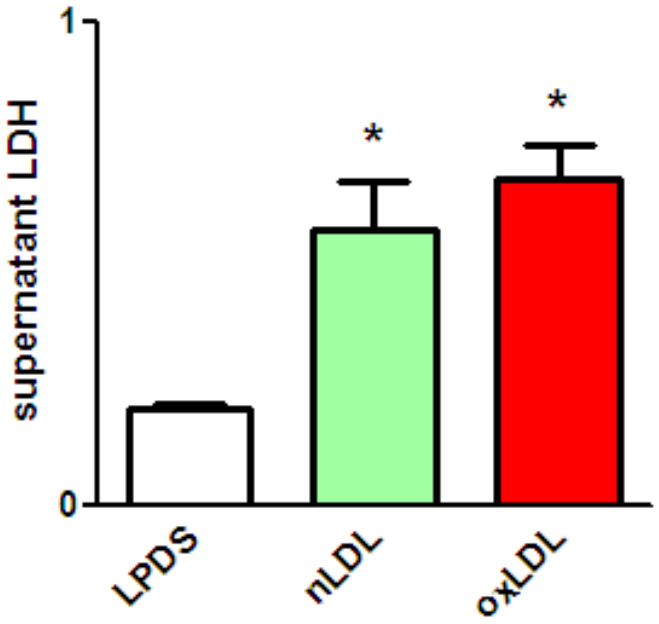
- Lipoproteins from plasma of human healthy donors.



Harmful effects of LDL on TECs

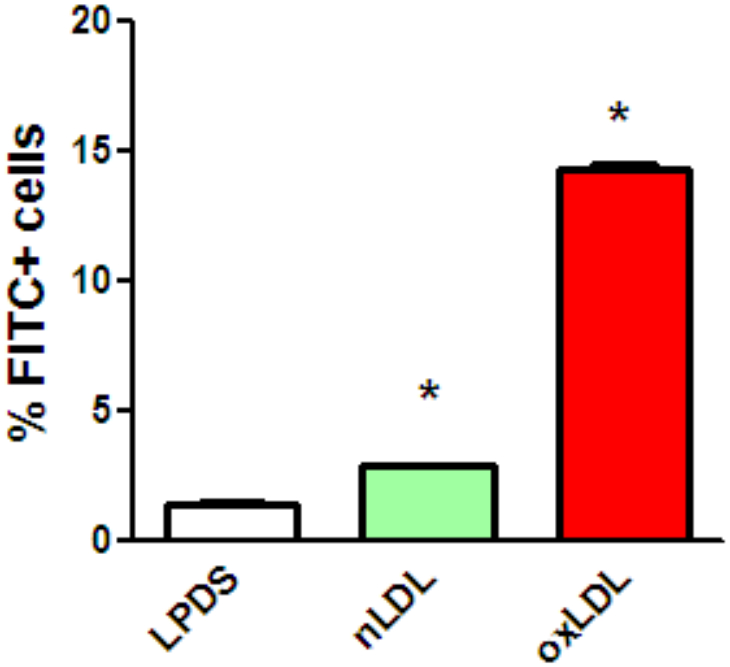
Lactate Dehydrogenase (LDH)

Cytotoxicity Assay



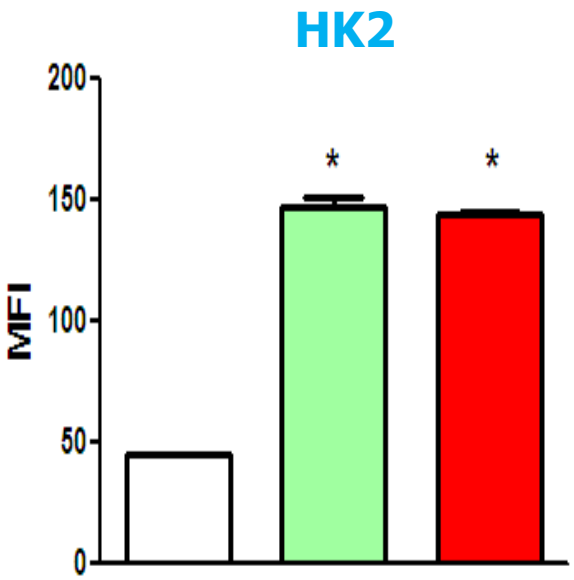
Apoptosis

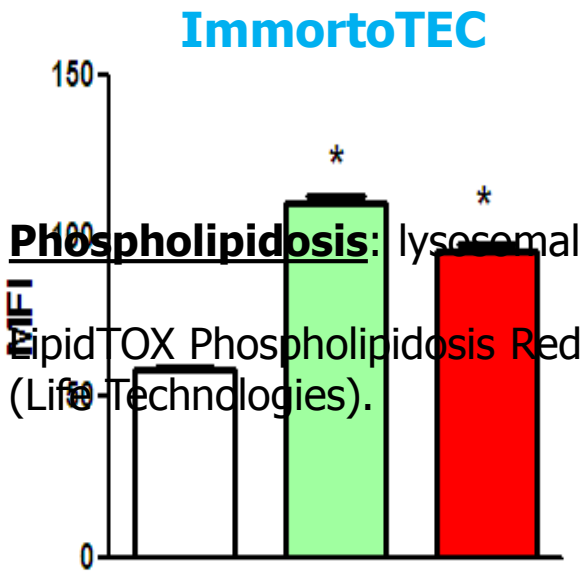
AnnexinV staining

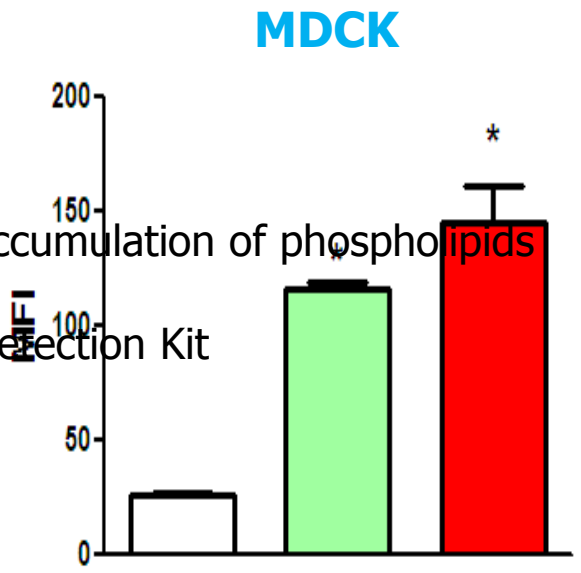


HK2 cells

LDL-induced phospholipidosis formation in TECs

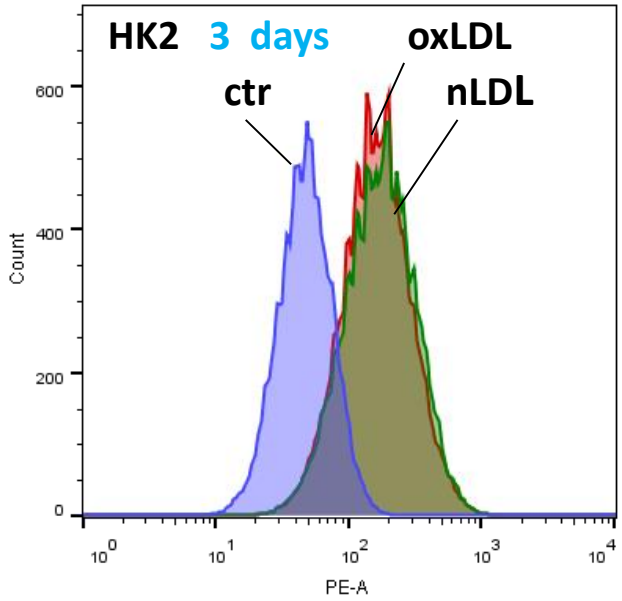


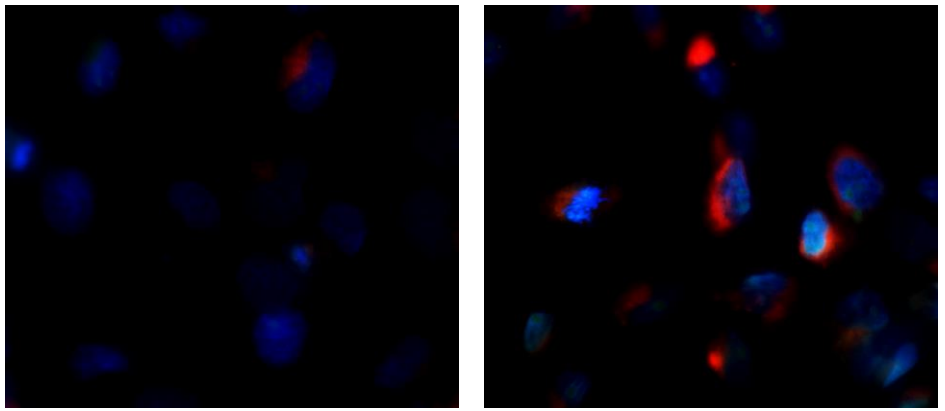




Phospholipidosis: lysosomal accumulation of phospholipids

LipidTOX Phospholipidosis Red Detection Kit (Life Technologies).





100x

HK2 ctr LDL

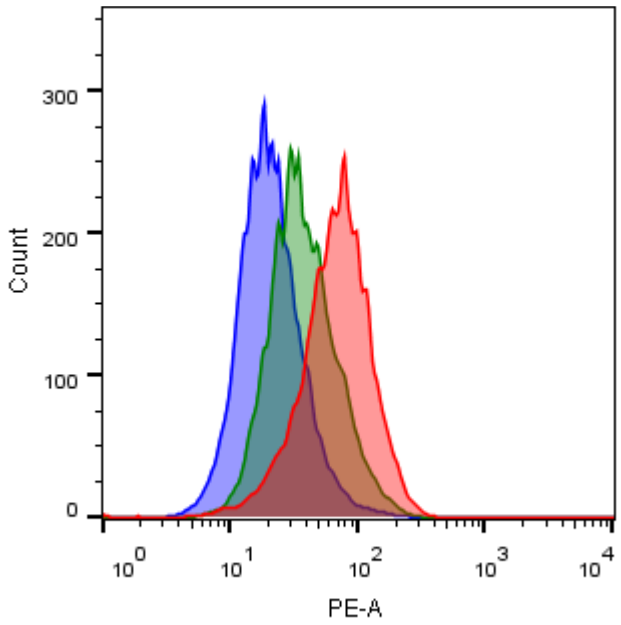
Enlargement of the lysosomal compartment upon LDL

HK2 cells – day 3

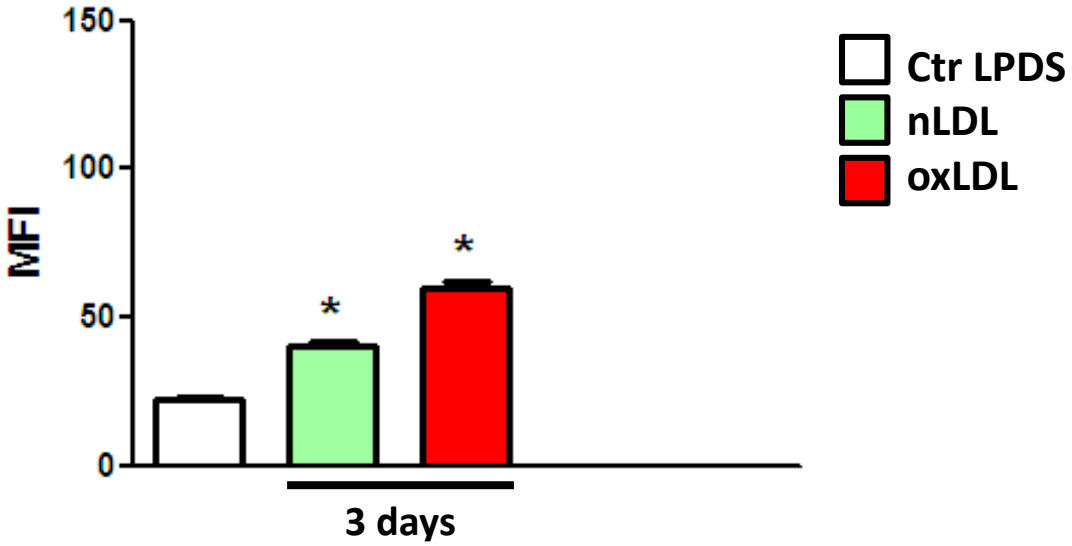
LysoTracker Red

labeling and tracking acidic organelles in live cells

3 days



LysoTracker Red MFI



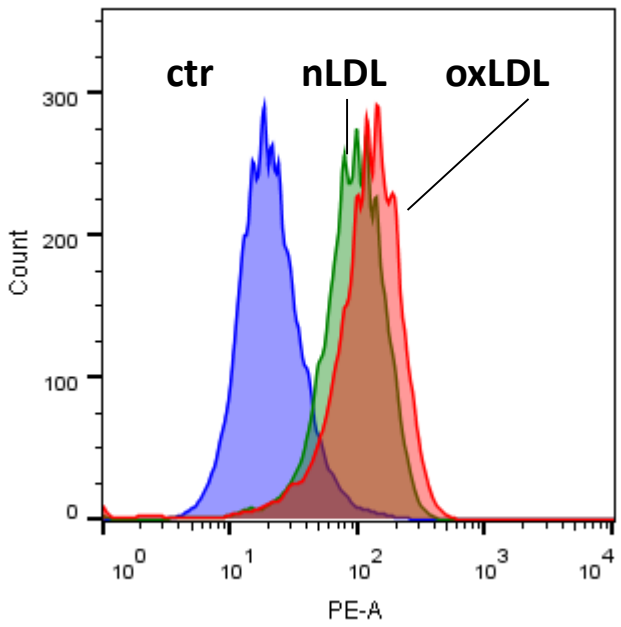
Enlargement of the lysosomal compartment upon LDL

HK2 cells – day 3/5

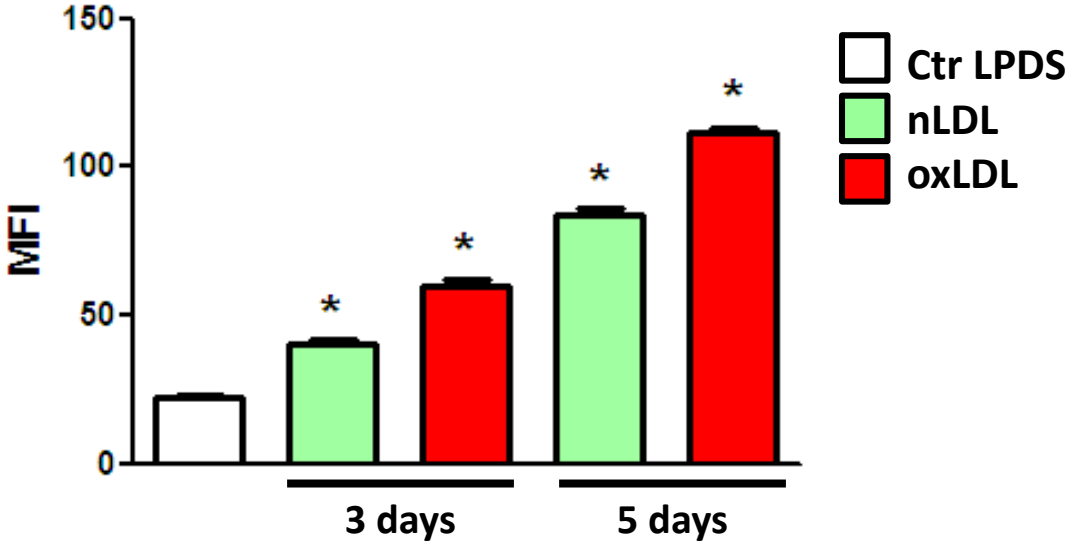
LysoTracker Red

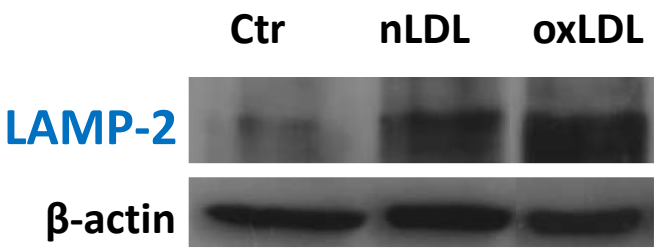
labeling and tracking acidic organelles in live cells

5 days



LysoTracker Red MFI

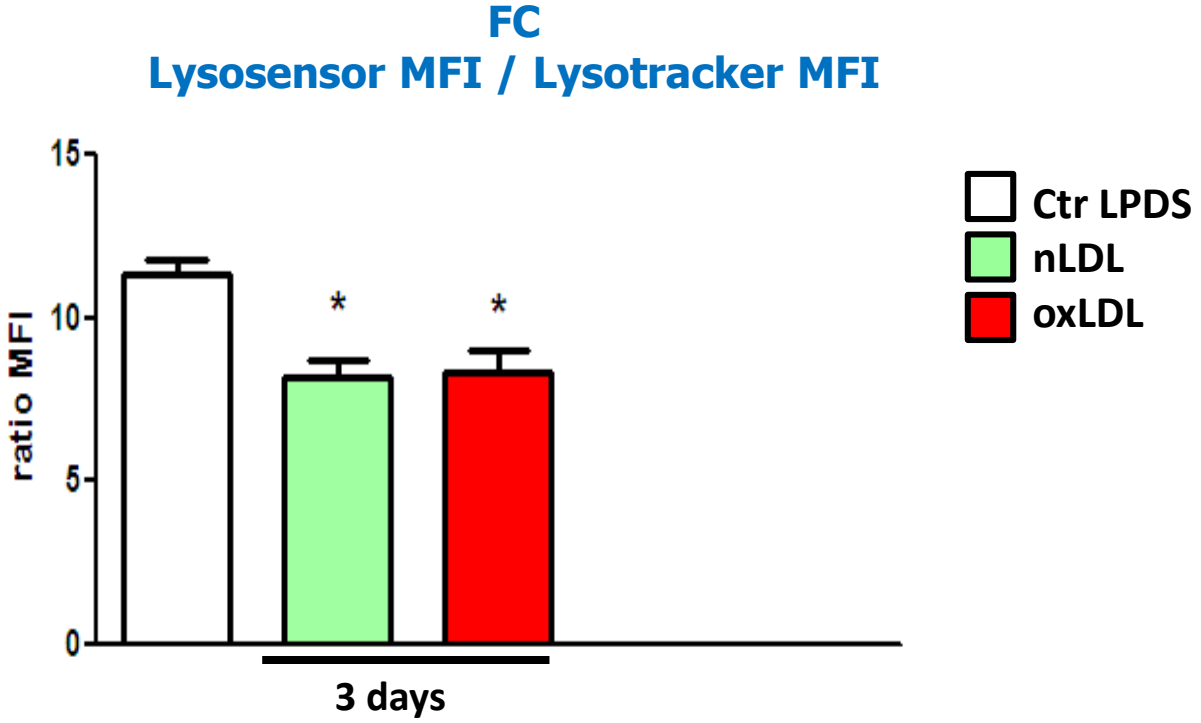




Impaired lysosomal acidification upon LDL

HK2 cells

LysoSensor Green: Low pH-dependent fluorescent dye
LysoTracker Red: Labeling acidic organelles



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LysoSensor Green: Low pH-dependent fluorescent dye
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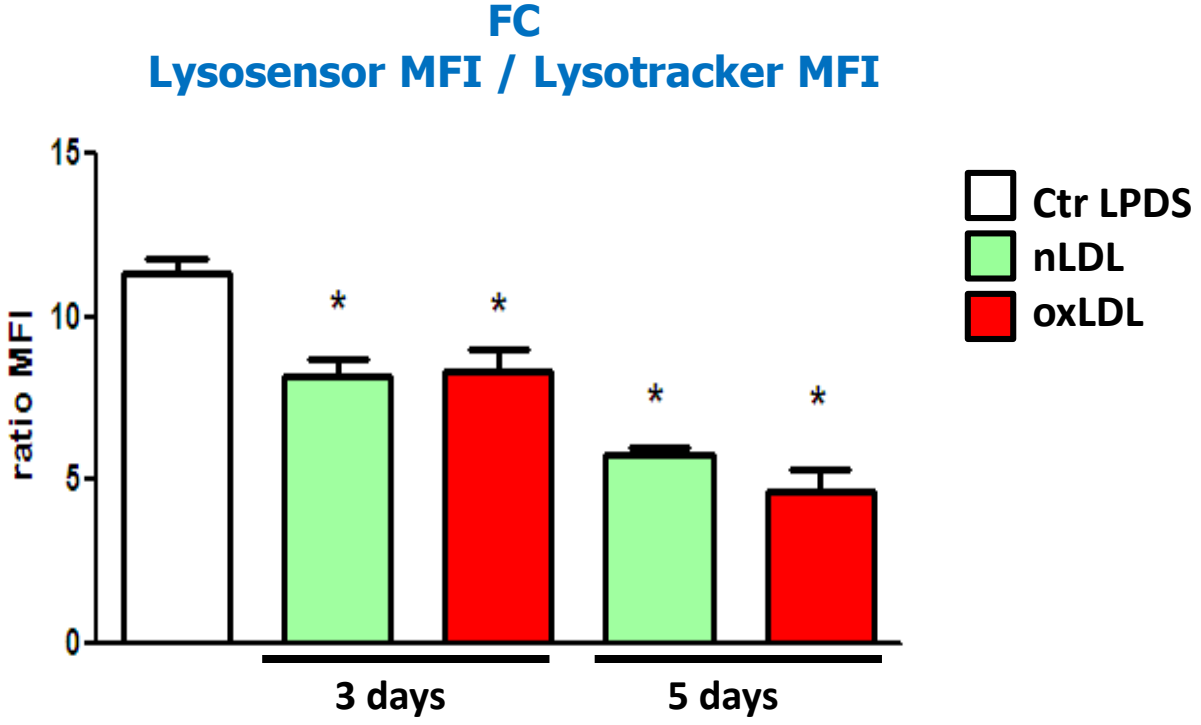
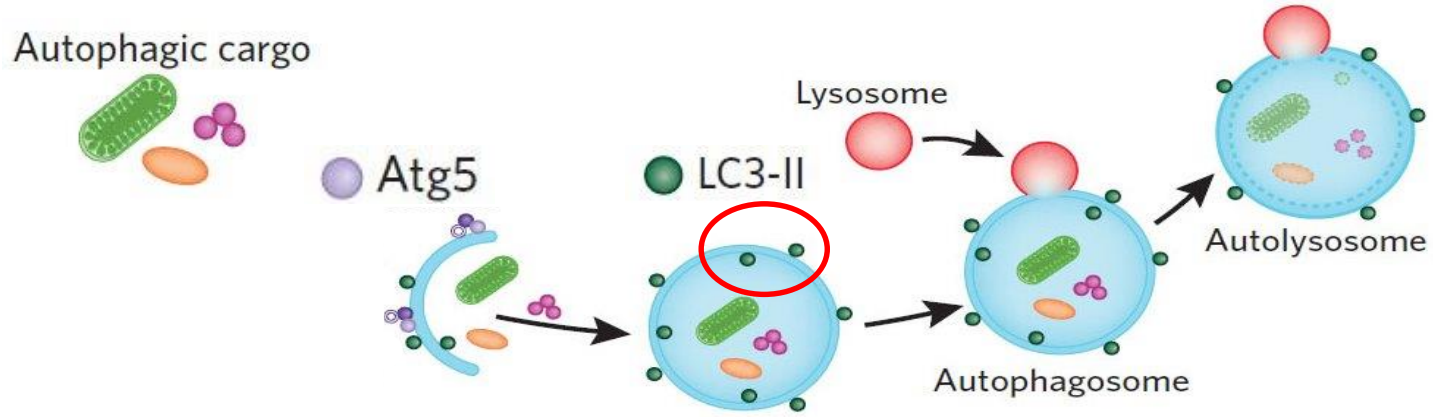
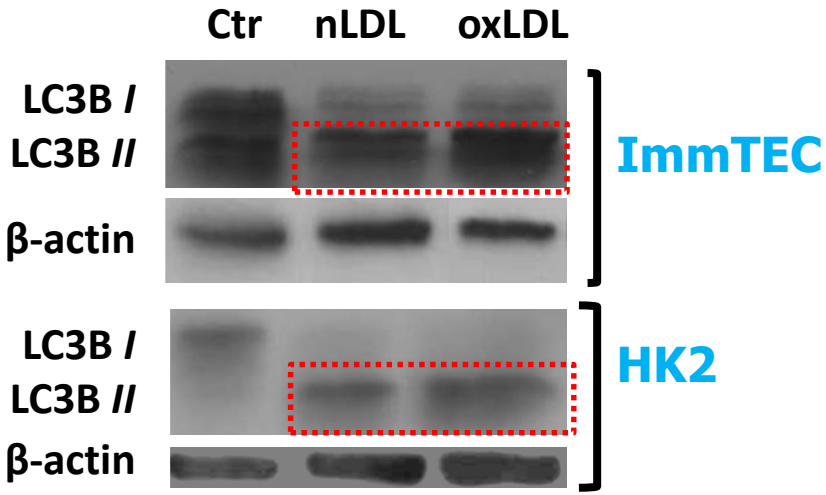


LDL-loading induces autophagy



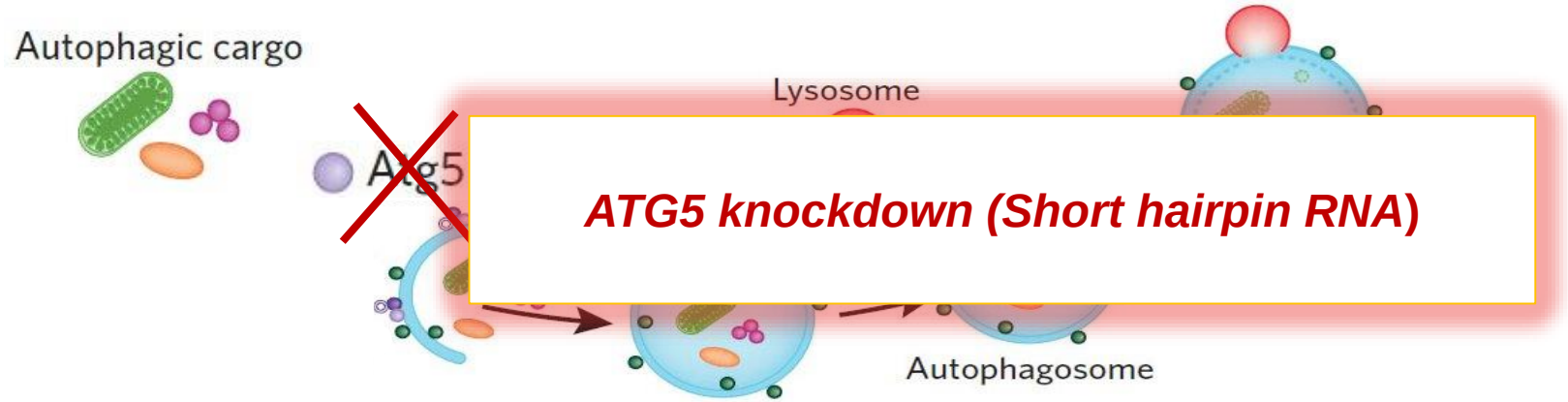
WB



n/oxLDL

increase in LC3-II
decrease in LC3-I expression

Autophagy as a protective mechanism

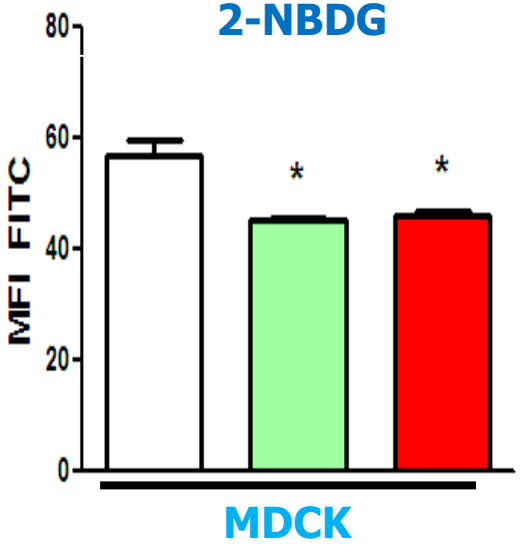


ATG5 knockdown (Short hairpin RNA)

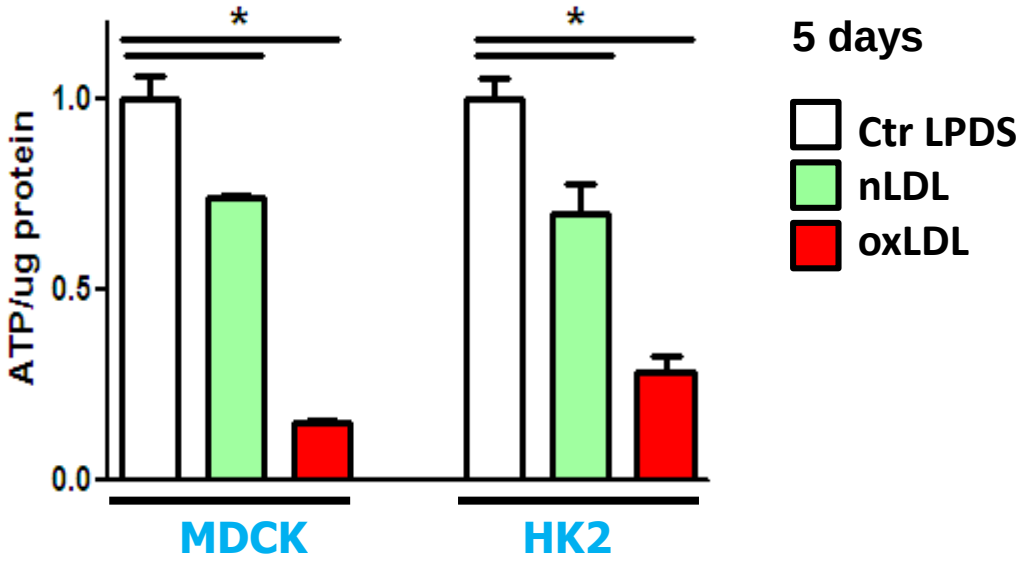
Without autophagy ⟹ **More Apoptosis**

Impaired function after n/oxLDL treatment: Less absorption , ATP , integral mitochondria

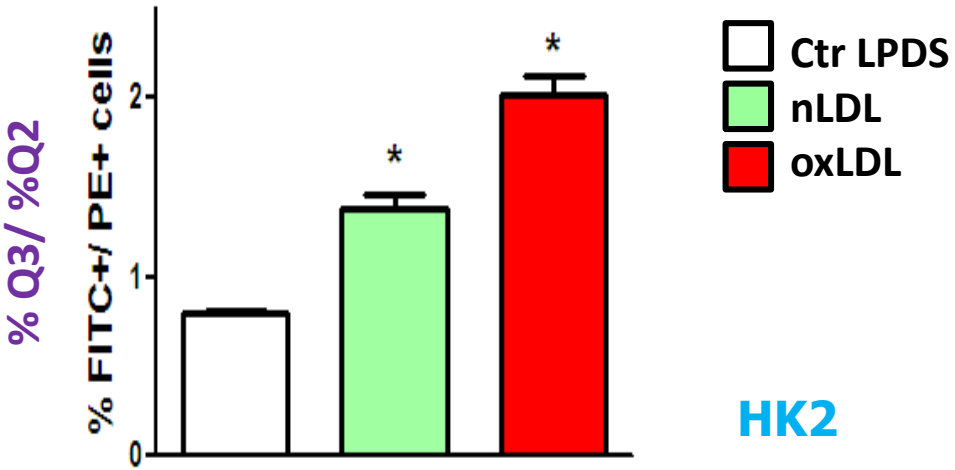
Glucose uptake
2-NBDG



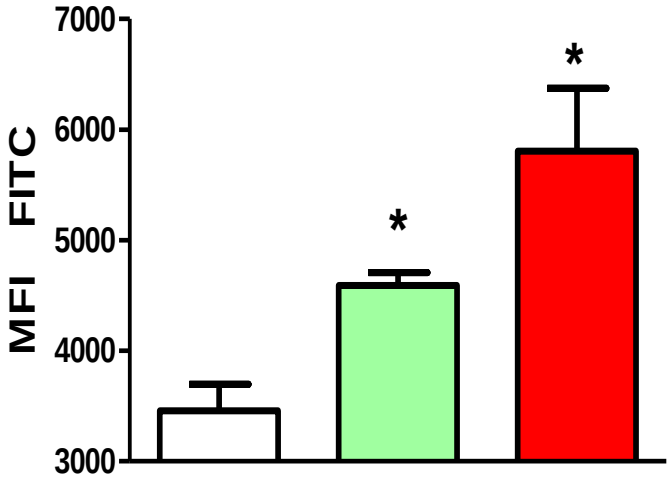
Intracellular ATP



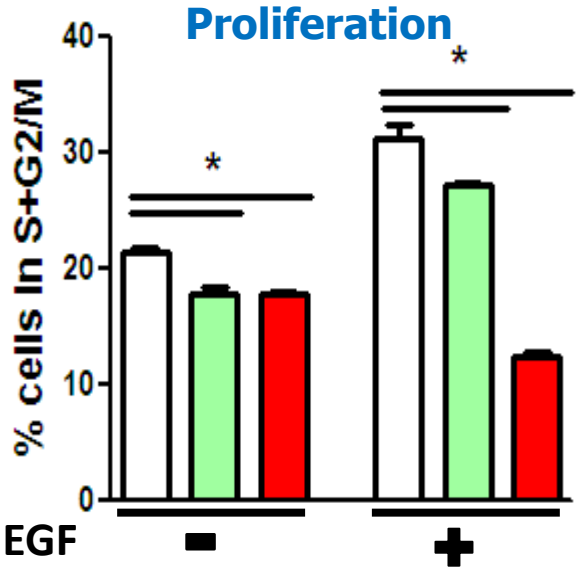
Mitochondrial damage

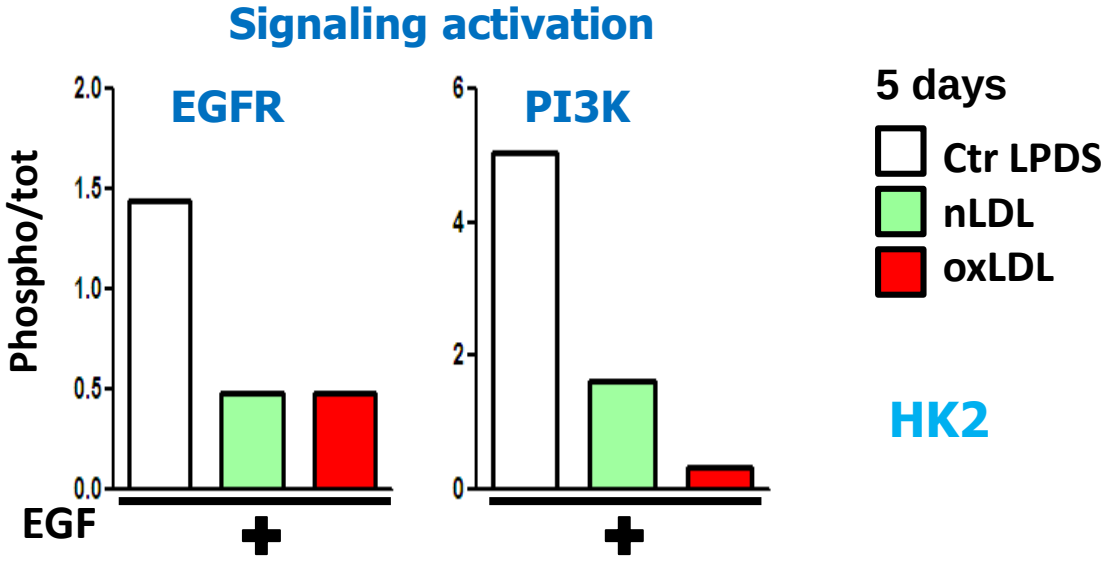


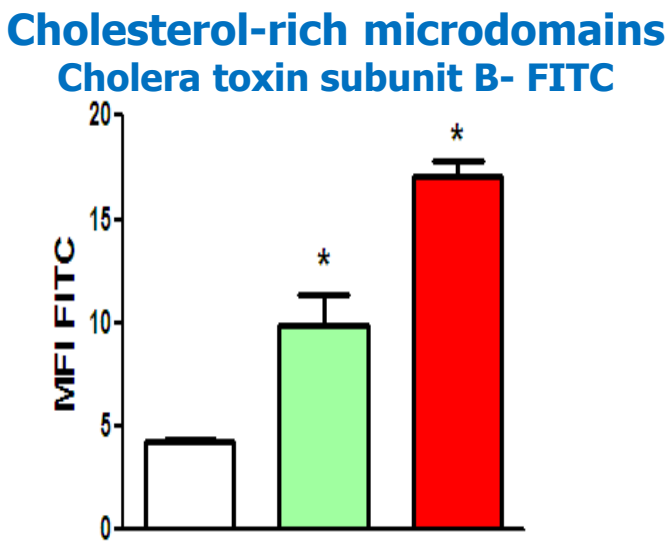
Oxidative stress - ROS

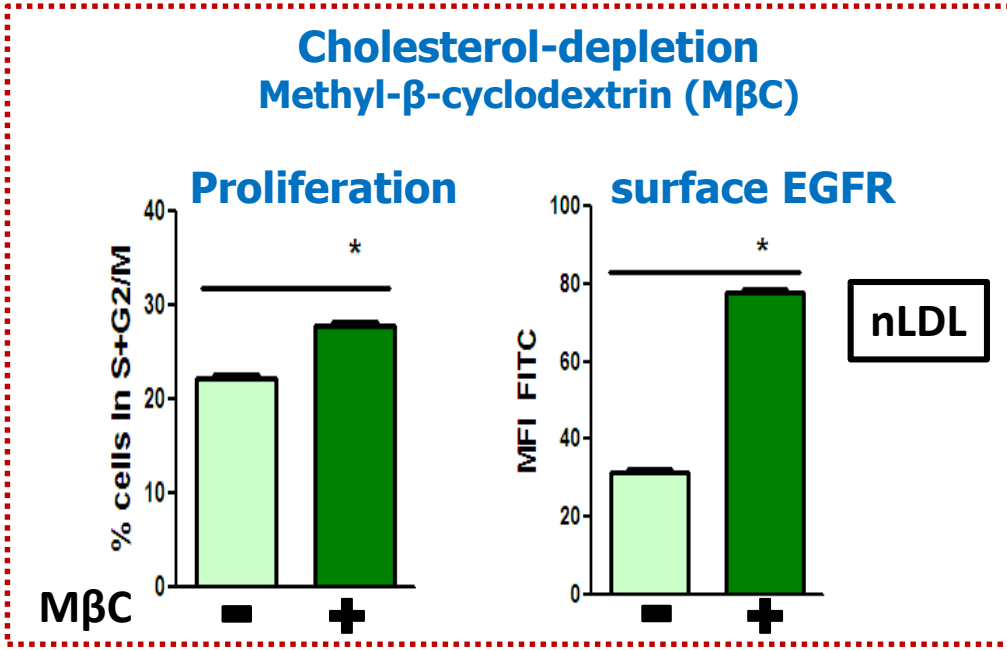


Impaired function after n/oxLDL treatment: Improper response to EGF









Low-grade inflammation in Metabolic syndrome

Nlrp3 is a key modulator of diet-induced nephropathy and renal cholesterol accumulation

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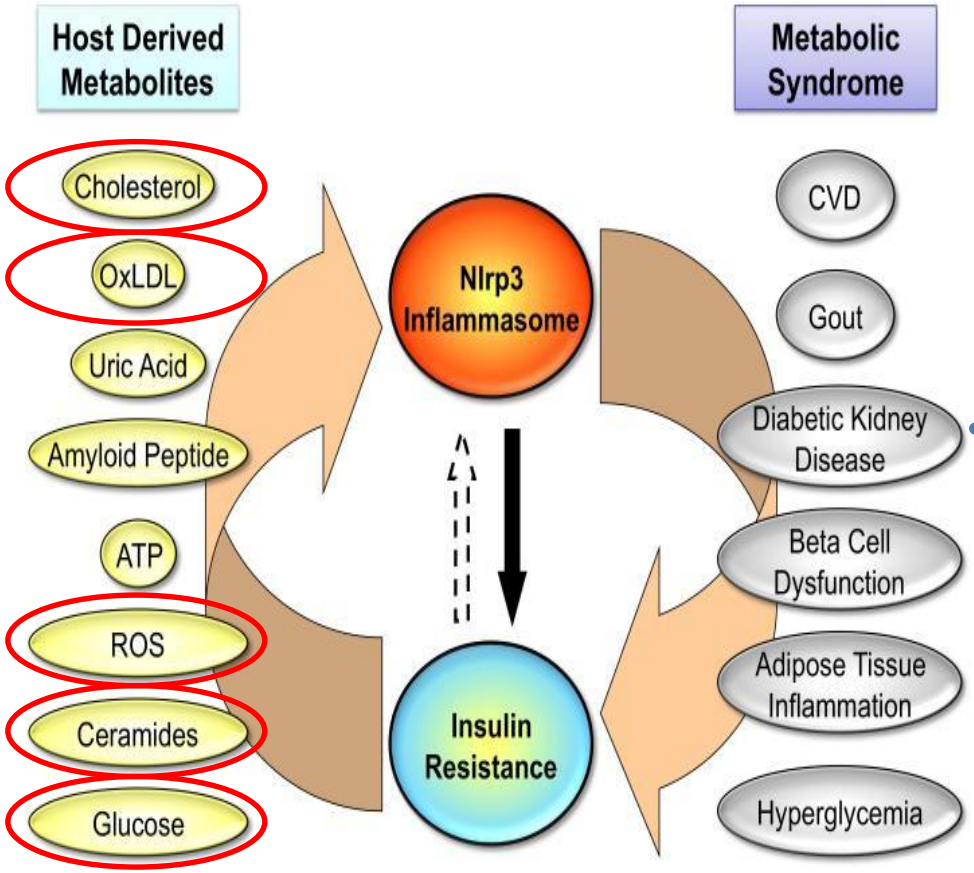
NLRP3 deficiency protects against

- **renal steatosis**
- **cholesterol & phospholipid accumulation**
- **fibrosis**
- **macrophage influx**
- **microalbuminuria**

NLRP3 mediates high fat diet-induced metabolic renal injury

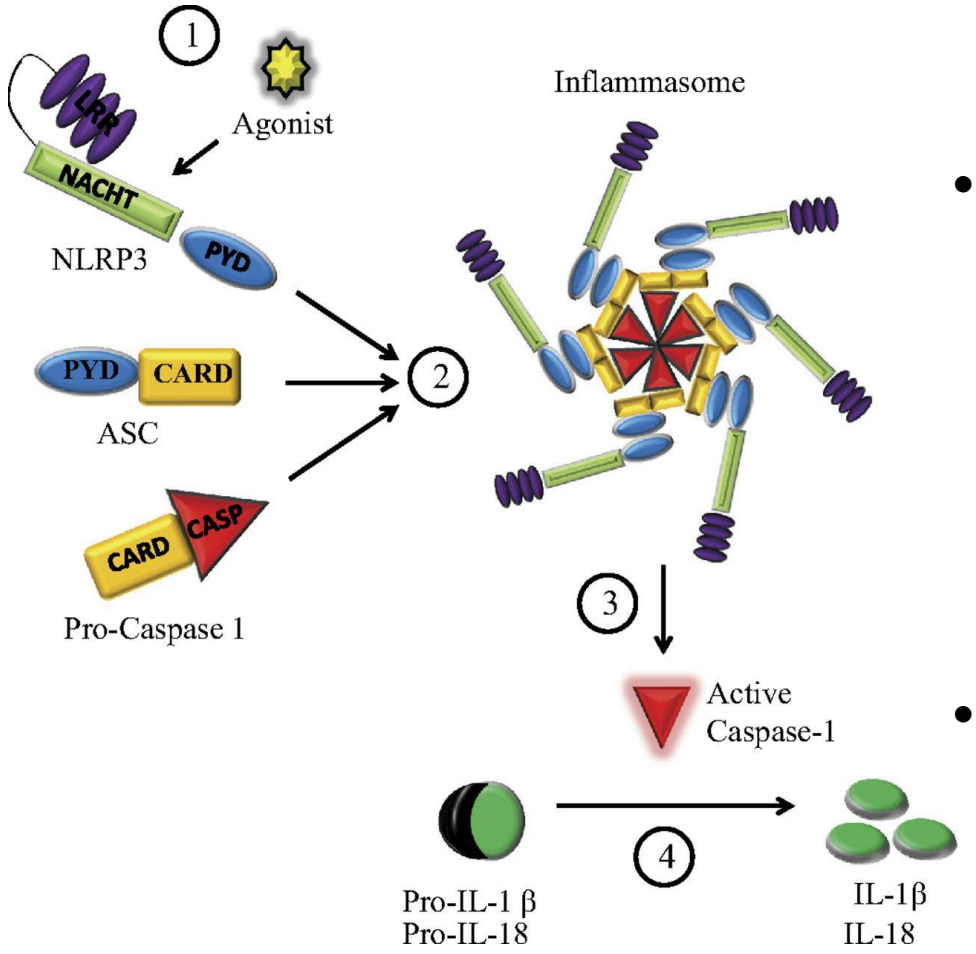
Low-grade grade metabolic inflammation: NLRP3 inflammasome



- Activated in CKD & diabetic nephropathy (DN)
- Triggers onset of DN
- Mediates diet-induced nephropathy & renal cholesterol accumulation

Mori et al., Nat. Med., 2011

Low-grade grade metabolic inflammation: NLRP3 inflammasome



NLRP3 Inflammasome

- Cytoplasmic **innate immune** multiprotein complex
  - Nod-like receptor protein 3 NLRP3,
  - adaptor ASC,
  - pro-caspase-1

- **IL-1β** and **IL-18** maturation

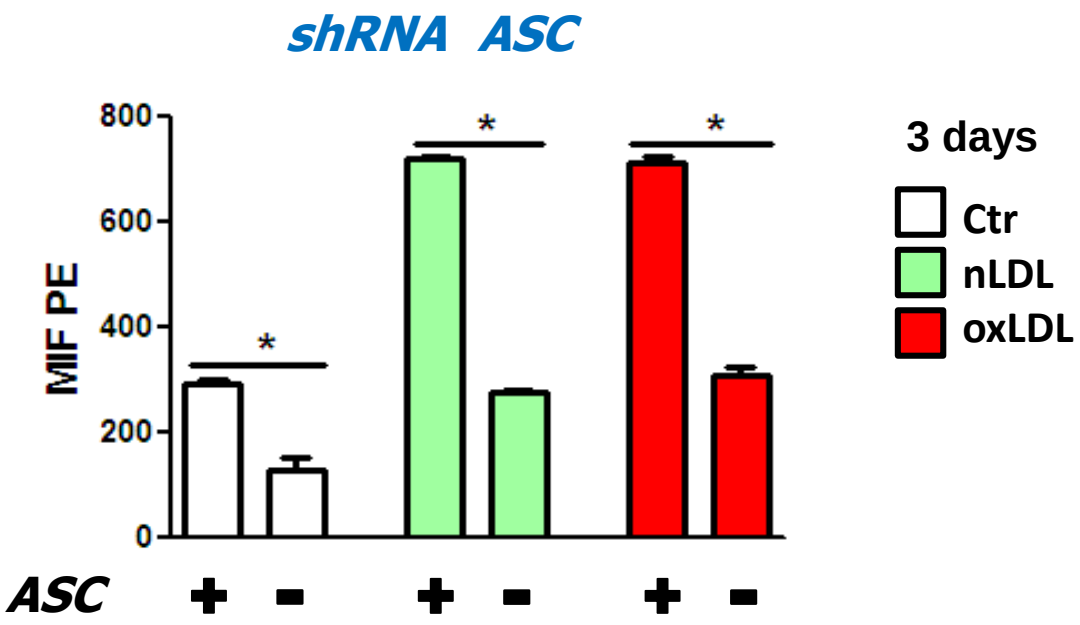
dos Santos et al., Am. Journ. Physiol., 2012

NLRP3 activation in metabolic overloading

Silencing inflammasome components

Phospholipidosis

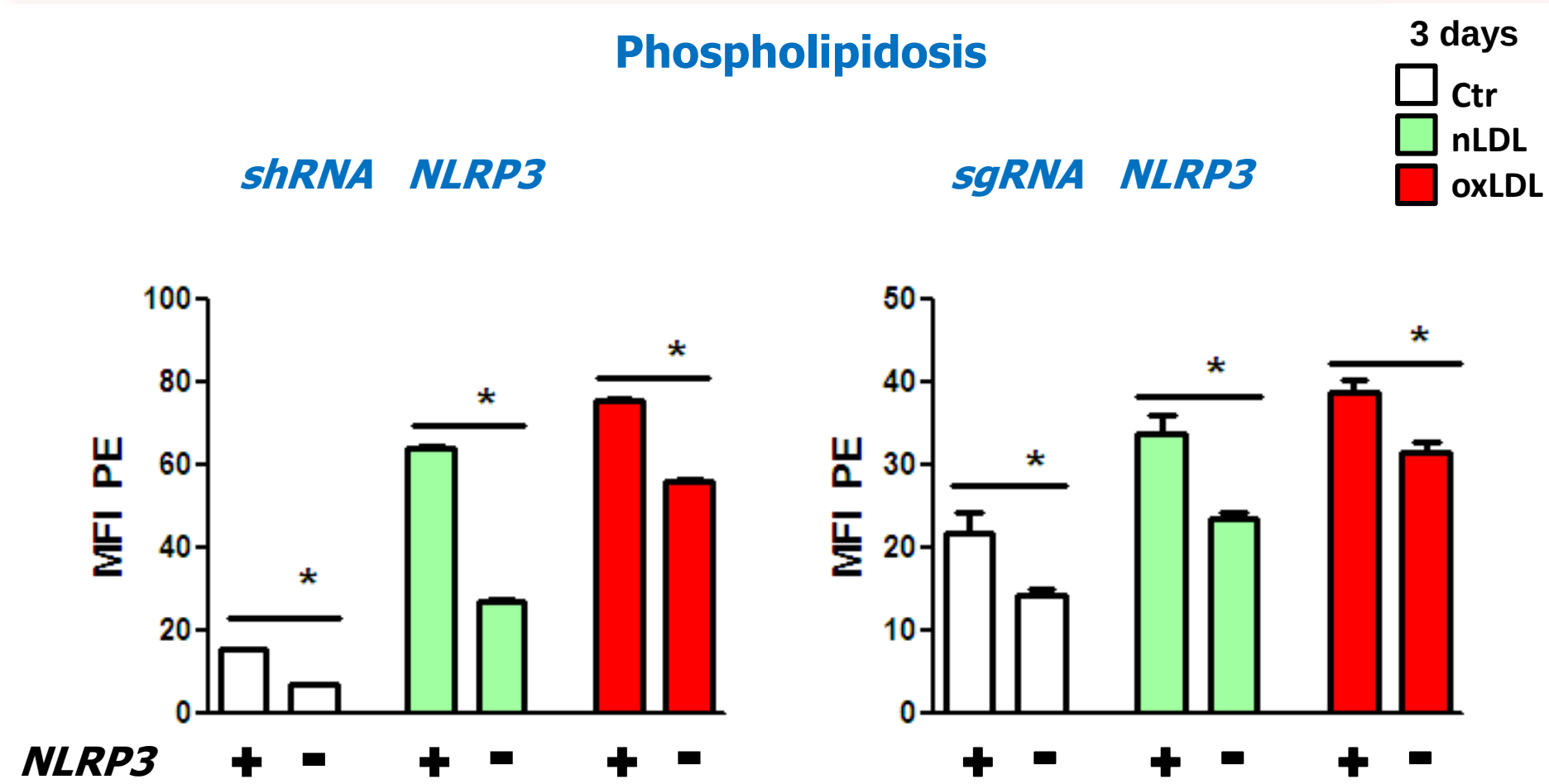
HK2



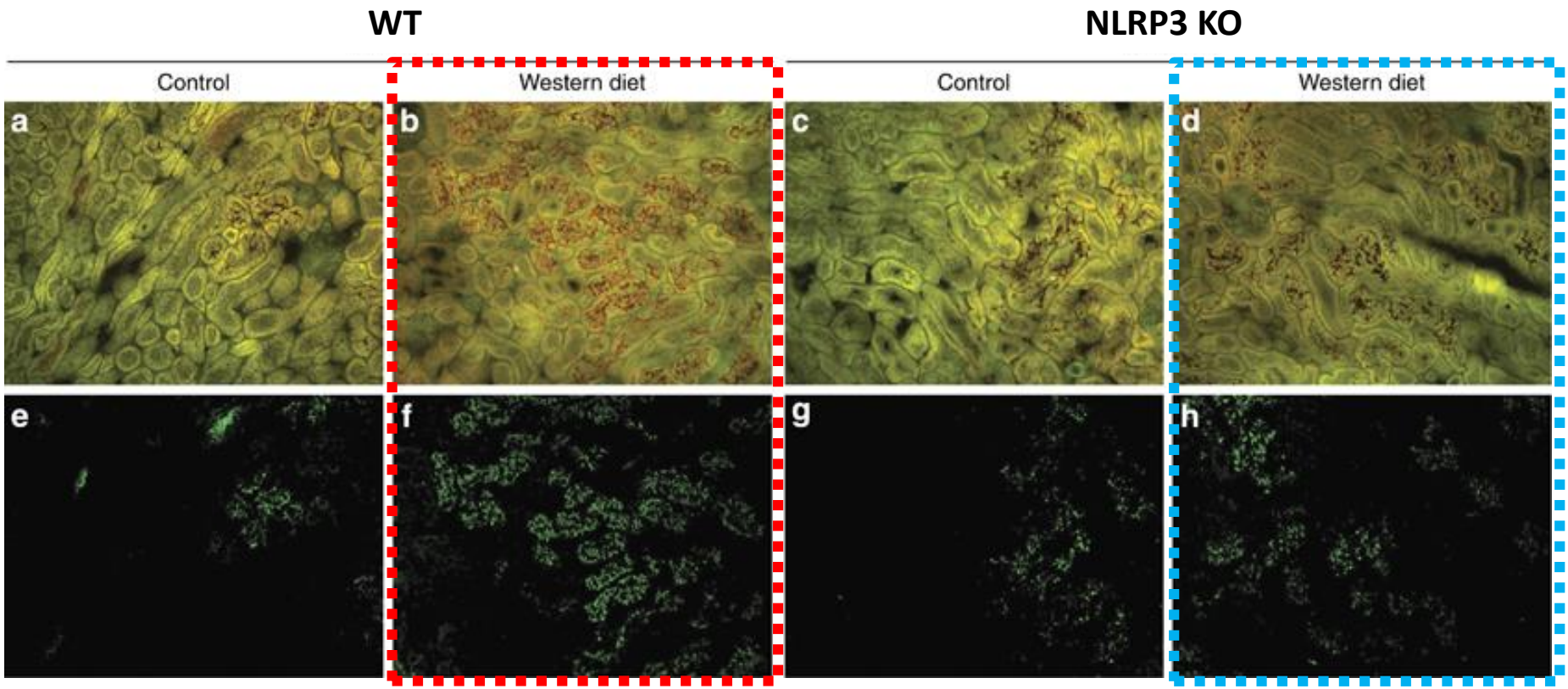
NLRP3 activation in metabolic overloading

Without NLRP3/ASC complex → **Less Phospholipidosis**

Phospholipidosis

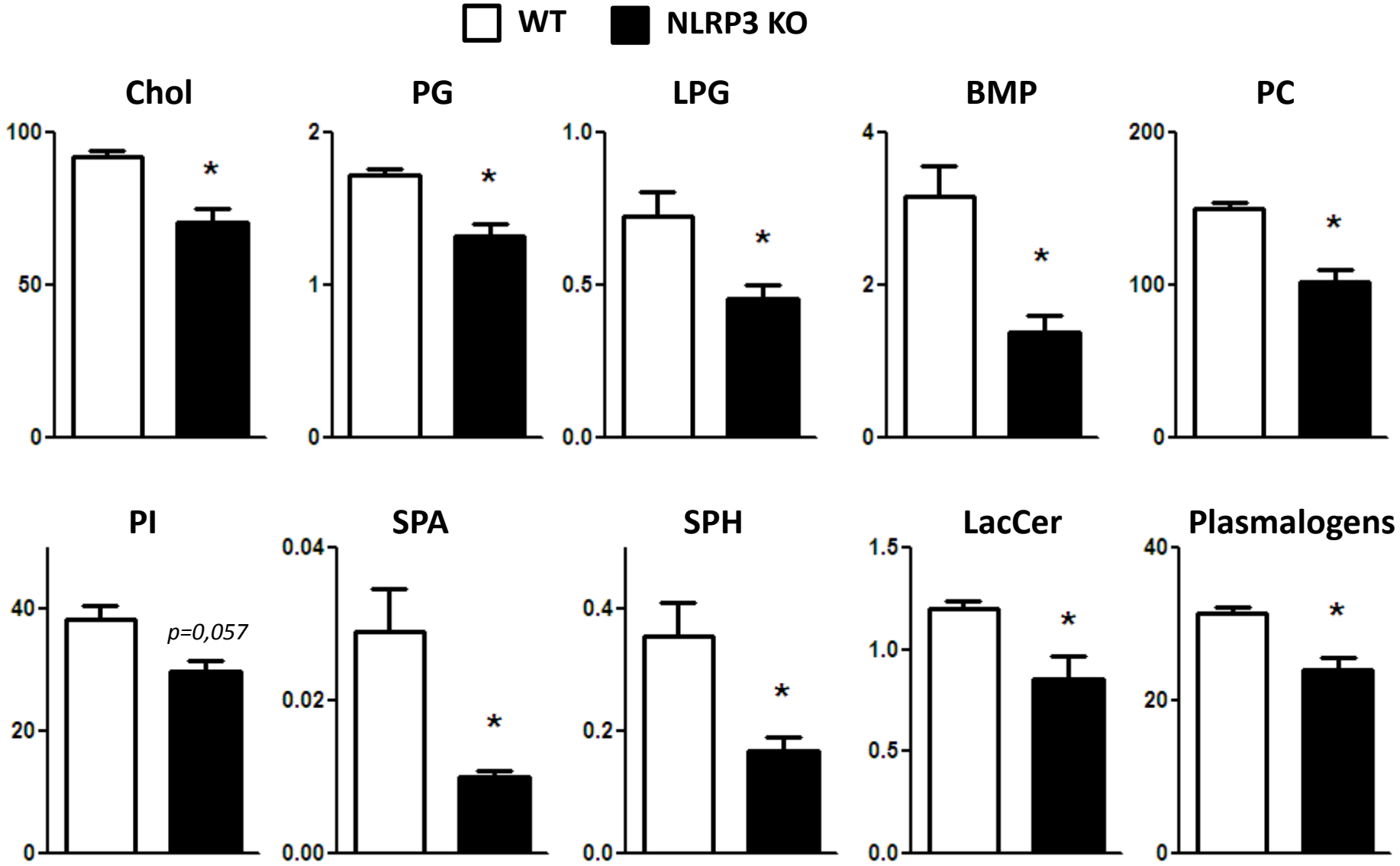


Absence of NLRP3 prevent excessive intracellular lipid deposition in proximal tubules

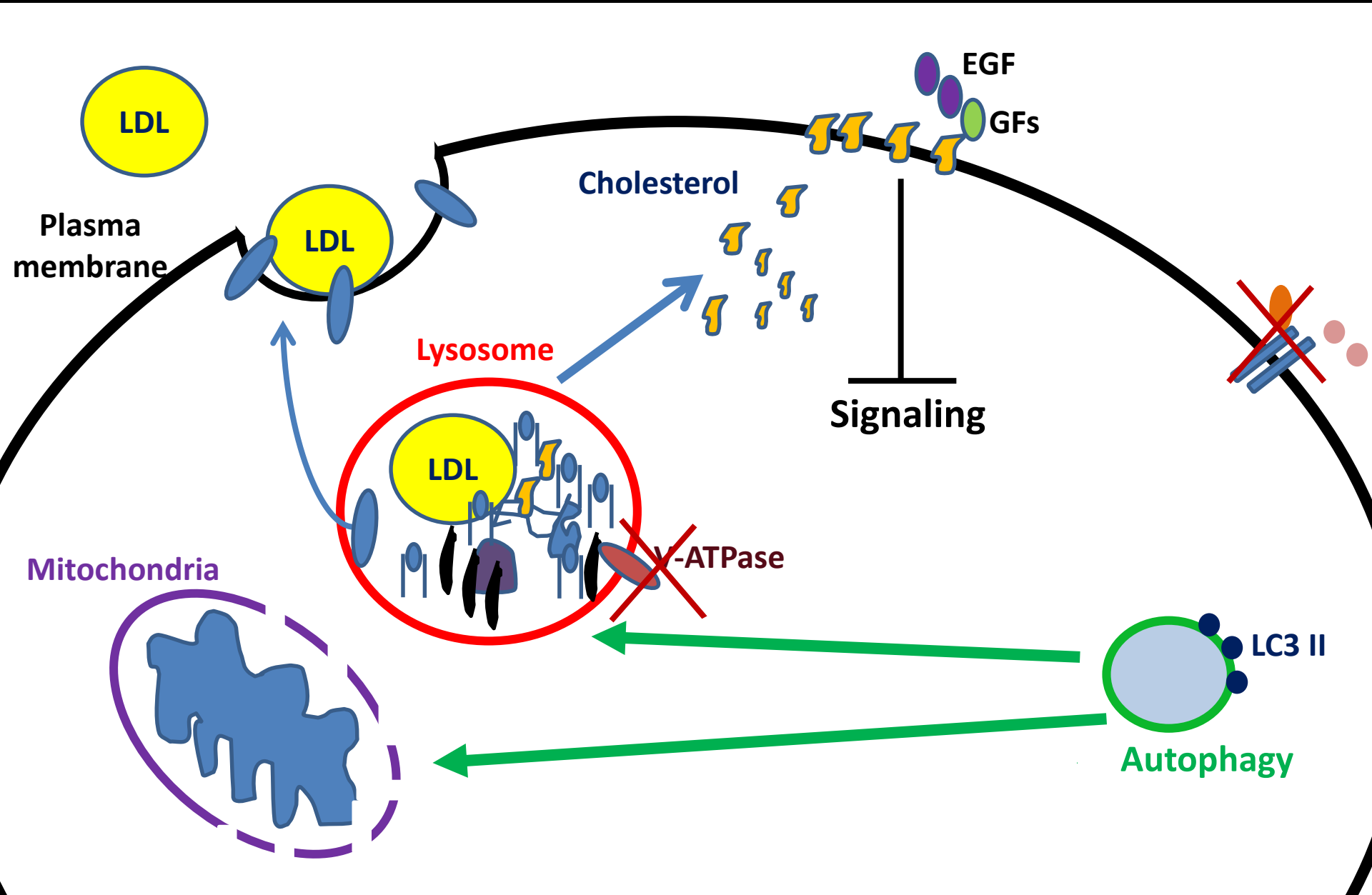


Nile Red staining

Absence of NLRP3 attenuates Western-diet-induced lipid accumulation in kidneys



PROPOSED MECHANISM



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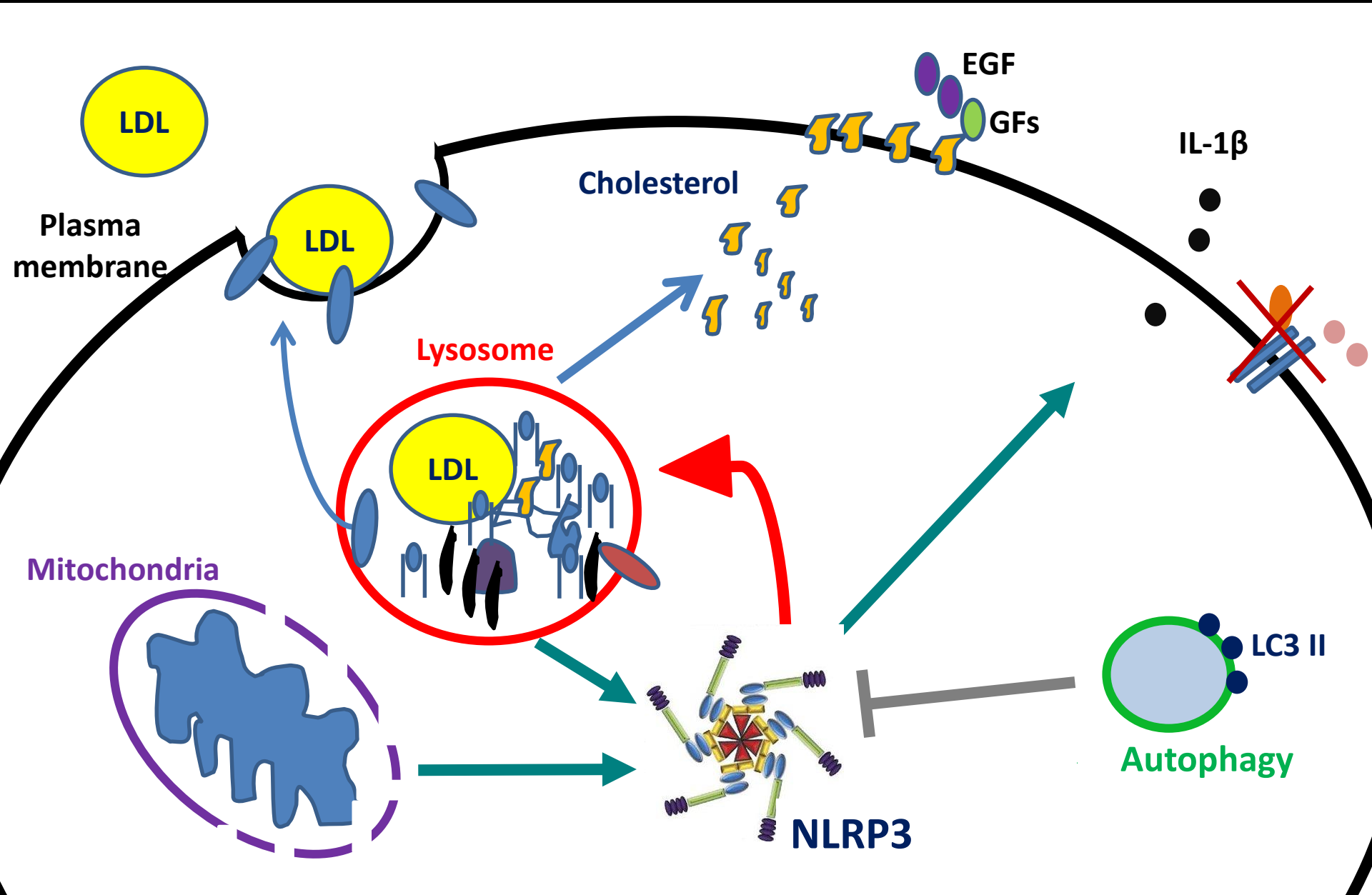

Acknowledgement

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Germany

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NIERSTICHTING
Leven gaat voor.

NWO Vici
Nederlandse Organisatie voor Wetenschappelijk Onderzoek