Mechanisms and experimental data of SGLT-2 inhibition

Theodoros Eleftheriadis
Prof. of Nephrology
Faculty of Medicine
University of Thessaly

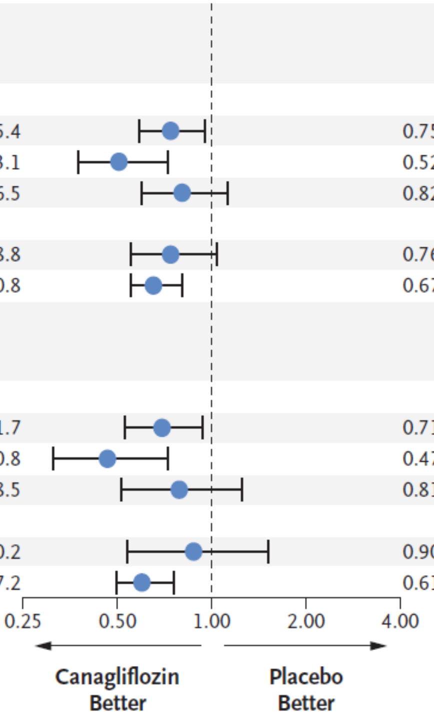
The NEW ENGLAND JOURNAL of MEDICINE
ESTABLISHED IN 1812 JUNE 13, 2019 VOL. 380 NO. 24

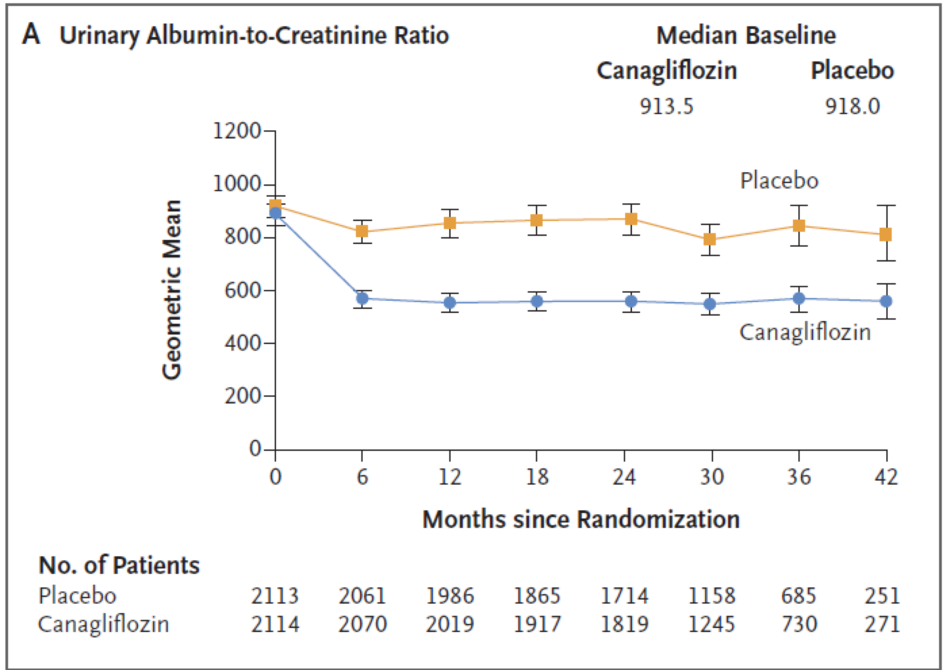
Canagliflozin and Renal Outcomes in Type 2 Diabetes and Nephropathy

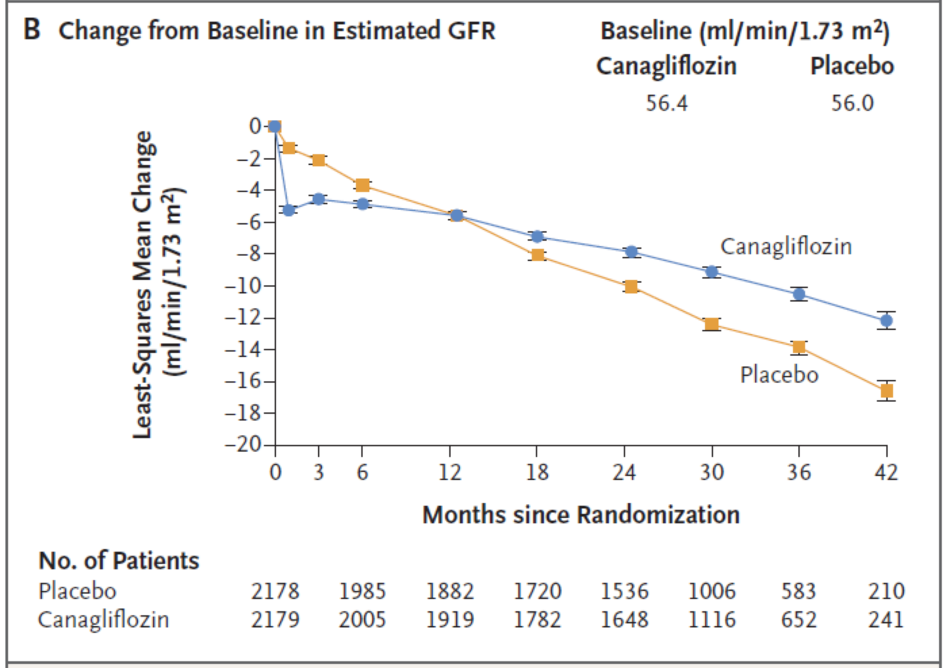
V. Perkovic, M.J. Jardine, B. Neal, S. Bompoint, H.J.L. Heerspink, D.M. Charytan, R. Ed
S. Bull, C.P. Cannon, G. Capuano, P.-L. Chu, D. de Zeeuw, T. Greene, A. Levin, C. Poll
H. Zhang, B. Zinman, G. Meininger, B.M. Brenner, and K.W. Mahaffey, for the CRED

CREDENCE population
Patients with **DKD** and eGFR ≥30 to <90 ml/min/1.73 m² **and** UACR >300 mg/g

Subgroup	Canagliflozin (no. of patients/total no.)	Placebo (no. of patients/total no.)	Canagliflozin (events/1000 patient-yr)	Placebo (events/1000 patient-yr)	Hazard Ratio (95% CI)	P Value for Interaction
Primary composite outcome of ESKD, doubling of serum creatinine, or renal or CV death						
Screening estimated GFR						0.11
30 to <45 ml/min/1.73 m²	119/657	153/656	72.2	95.4	0.75 (0.59–0.95)	
45 to <60 ml/min/1.73 m²	56/640	102/639	33.4	63.1	0.52 (0.38–0.72)	
60 to <90 ml/min/1.73 m²	70/905	85/904	29.9	36.5	0.82 (0.60–1.12)	
Baseline UACR						0.49
≤1000	69/1185	88/1163	22.0	28.8	0.76 (0.55–1.04)	
>1000	176/1017	252/1036	69.6	100.8	0.67 (0.55–0.81)	
Renal-specific composite outcome of ESKD, doubling of serum creatinine, or renal death						
Screening estimated GFR						0.18
30 to <45 ml/min/1.73 m²	85/657	115/656	51.6	71.7	0.71 (0.53–0.94)	
45 to <60 ml/min/1.73 m²	33/640	66/639	19.7	40.8	0.47 (0.31–0.72)	
60 to <90 ml/min/1.73 m²	35/905	43/904	14.9	18.5	0.81 (0.52–1.26)	
Baseline UACR						0.16
≤1000	29/1185	31/1163	9.2	10.2	0.90 (0.54–1.50)	
>1000	124/1017	193/1036	49.1	77.2	0.61 (0.49–0.76)	







The NEW ENGLAND JOURNAL of MEDICINE

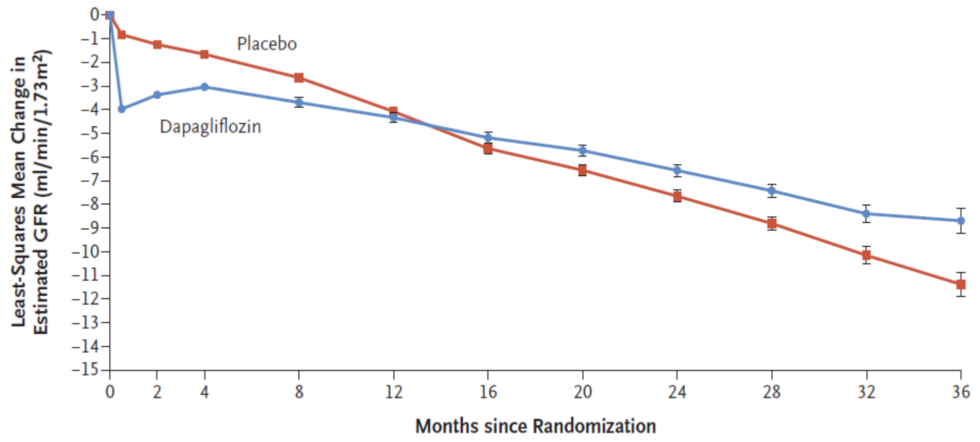
ORIGINAL ARTICLE

Dapagliflozin in Patients with Chronic Kidney Disease

Hiddo J.L. Heerspink, Ph.D., Bergur V. Stefánsson, M.D.,
Ricardo Correa-Rotter, M.D., Glenn M. Chertow, M
Fan-Fan Hou, M.D., Johannes F.E. Mann, M.D., Jo
Magnus Lindberg, M.Sc., Peter Rossing, M.D., C.
Roberto D. Toto, M.D., Anna-Maria Langkilde, M.D., a
for the DAPA-CKD Trial Committees and

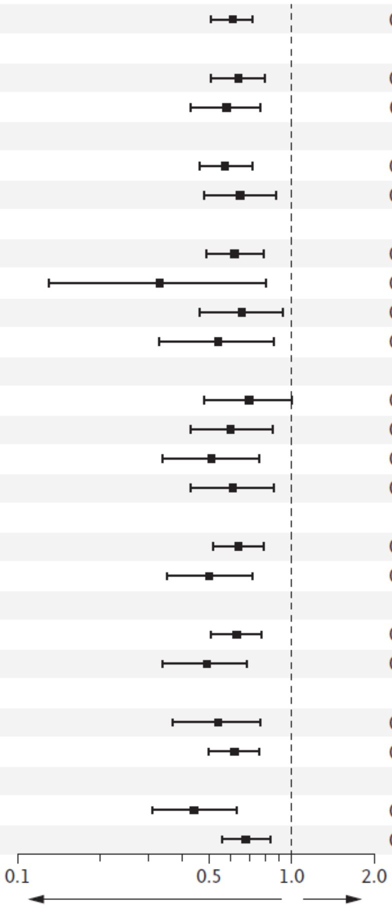
N Engl J Med 2020;383:1436-46.

DAPA-CKD population
Patients **with or without DKD** and eGFR >25 to <75 ml/min/1.73 m^2 **and** UACR ≥200 mg/g to ≤5000 mg/g

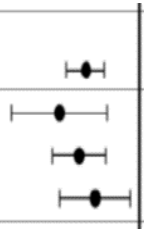


No. of Participants											
Placebo	2152	2029	1981	1866	1795	1753	1672	1443	935	447	157
Dapagliflozin	2152	2031	2001	1896	1832	1785	1705	1482	978	496	157

Subgroup	Dapagliflozin	Placebo	Hazard Ratio (95% CI)
	no. of participants/total no.		
All participants	197/2152	312/2152	0.61 (0.51–0.72)
Age			
≤65 yr	122/1247	191/1239	0.64 (0.51–0.80)
>65 yr	75/905	121/913	0.58 (0.43–0.77)
Sex			
Male	126/1443	209/1436	0.57 (0.46–0.72)
Female	71/709	103/716	0.65 (0.48–0.88)
Race			
White	110/1124	174/1166	0.62 (0.49–0.79)
Black	7/104	14/87	0.33 (0.13–0.81)
Asian	53/749	77/718	0.66 (0.46–0.93)
Other	27/175	47/181	0.54 (0.33–0.86)
Geographic region			
Asia	50/692	69/654	0.70 (0.48–1.00)
Europe	57/610	89/623	0.60 (0.43–0.85)
North America	35/401	69/412	0.51 (0.34–0.76)
Latin America	55/449	85/463	0.61 (0.43–0.86)
Type 2 diabetes			
Yes	152/1455	229/1451	0.64 (0.52–0.79)
No	45/697	83/701	0.50 (0.35–0.72)
Estimated GFR			
<45 ml/min/1.73 m^2	152/1272	217/1250	0.63 (0.51–0.78)
≥45 ml/min/1.73 m^2	45/880	95/902	0.49 (0.34–0.69)
Urinary albumin-to-creatinine ratio			
≤1000	44/1104	84/1121	0.54 (0.37–0.77)
>1000	153/1048	228/1031	0.62 (0.50–0.76)
Systolic blood pressure			
≤130 mm Hg	46/793	96/749	0.44 (0.31–0.63)
>130 mm Hg	151/1359	216/1403	0.68 (0.56–0.84)



Kidney outcome								
Overall	142/2152	243/2152	3.3	5.8	0.56 (0.45, 0.68)		4.7 (3.0, 6.4)	
UACR ≤113.0 mg/mmol	21/1104	51/1121	0.9	2.3	0.42 (0.25, 0.70)	0.59	2.6 (1.2, 4.1)	0.002
UACR >113.0 to ≤395.5 mg/mmol	71/881	130/883	4.1	7.7	0.52 (0.39, 0.69)		6.7 (3.7, 9.6)	
UACR >395.5 mg/g	50/167	62/148	16.7	27.7	0.62 (0.42, 0.90)		12.0 (1.4, 22.5)	



Waijer, Diabetologia, 2022

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Empagliflozin in Patients with Chronic Kidney Disease

The EMPA-KIDNEY Collaborative Group

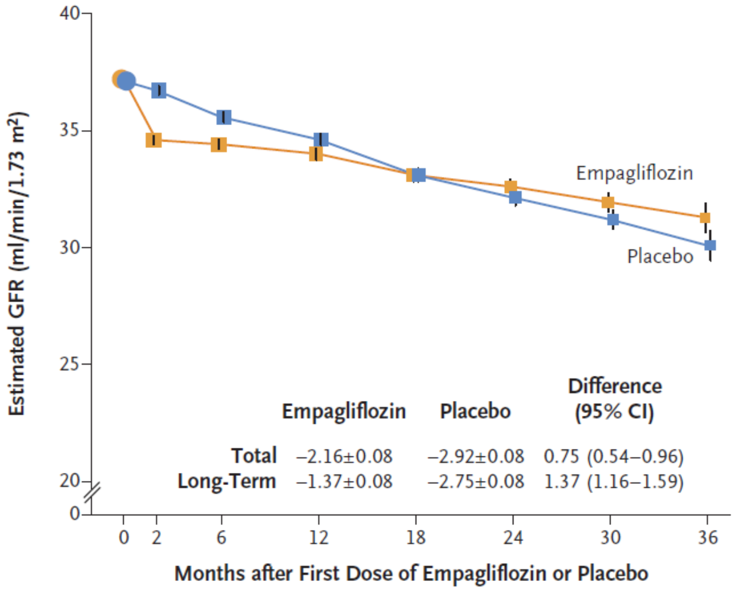
DOI: 10.1056/NEJMoa2204233

EMPA-KIDNEY population

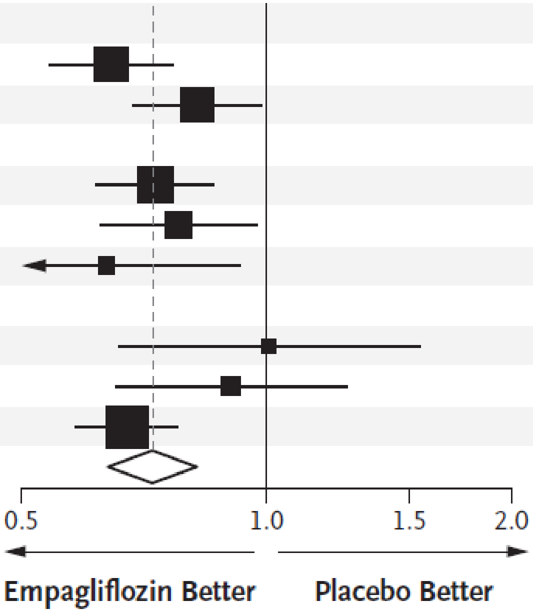
Patients **with or without DKD** and
eGFR ≥20 to <45 ml/min/1.73 m^2

or

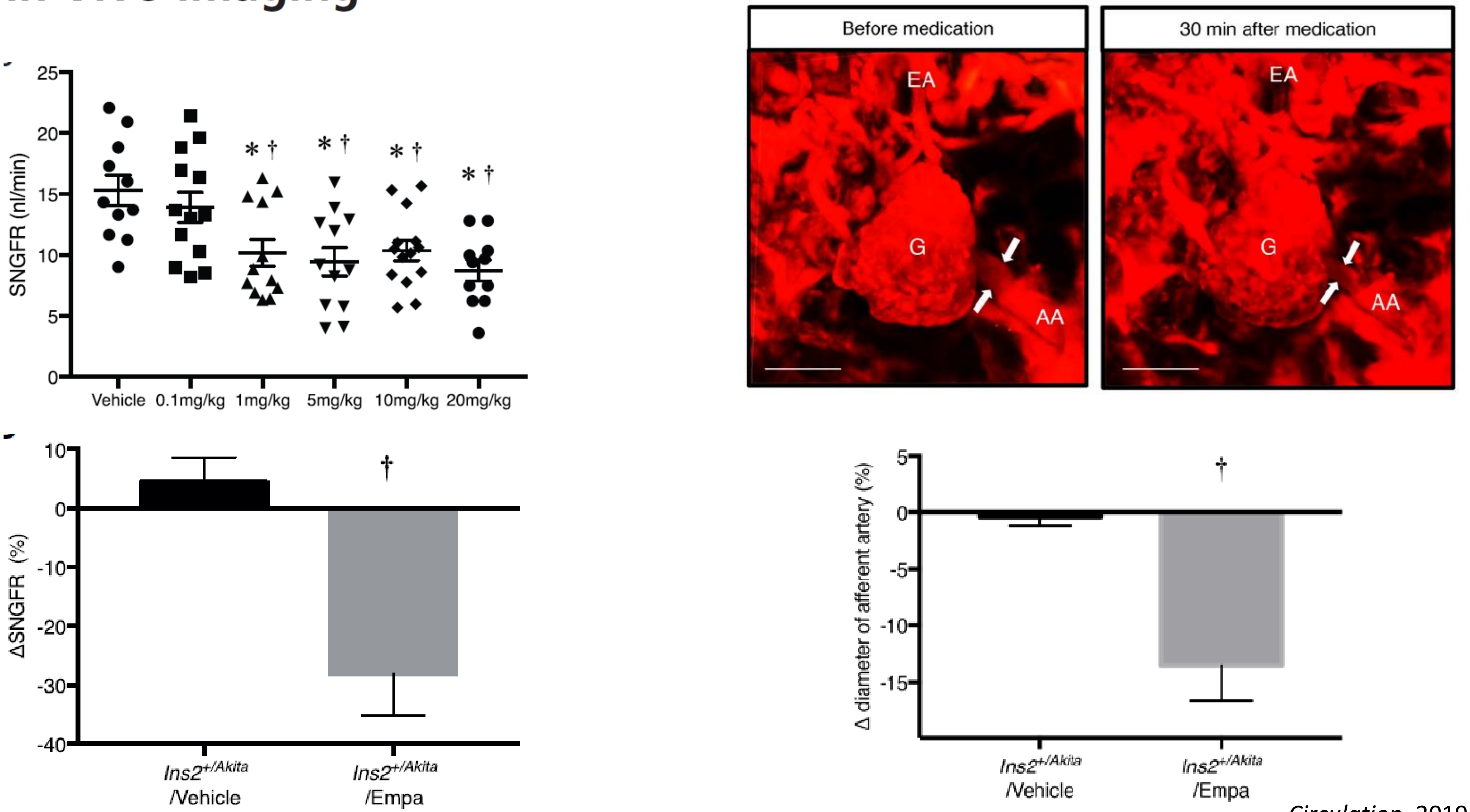
eGFR ≥45 to <90 ml/min/1.73 m^2
and UACR ≥200 mg/g



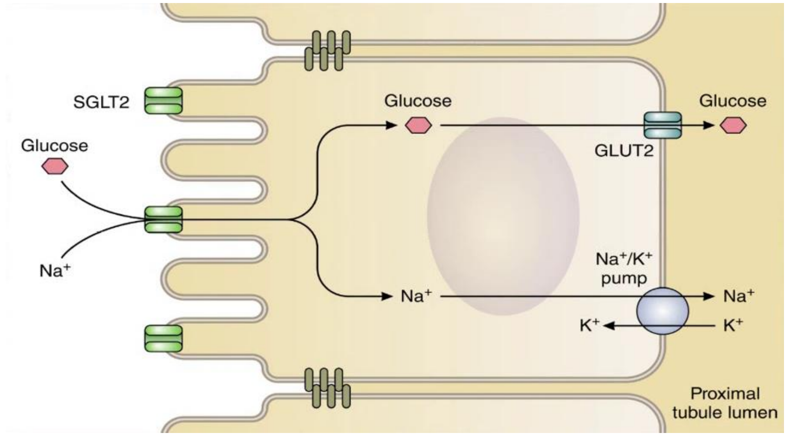
Subgroup	Empagliflozin	Placebo	Hazard Ratio for Progression of Kidney Disease or Death from Cardiovascular Causes (95% CI)
	no. of patients with event/total no.		
Diabetes mellitus			
Present	218/1525	306/1515	0.64 (0.54–0.77)
Absent	214/1779	252/1790	0.82 (0.68–0.99)
Estimated GFR			
<30 ml/min/1.73 m^2	247/1131	317/1151	0.73 (0.62–0.86)
≥30 to <45 ml/min/1.73 m^2	140/1467	175/1461	0.78 (0.62–0.97)
≥45 ml/min/1.73 m^2	45/706	66/693	0.64 (0.44–0.93)
Urinary albumin-to-creatinine ratio			
<30	42/665	42/663	1.01 (0.66–1.55)
≥30 to ≤300	67/927	78/937	0.91 (0.65–1.26)
>300	323/1712	438/1705	0.67 (0.58–0.78)
All patients	432/3304	558/3305	0.72 (0.64–0.82)

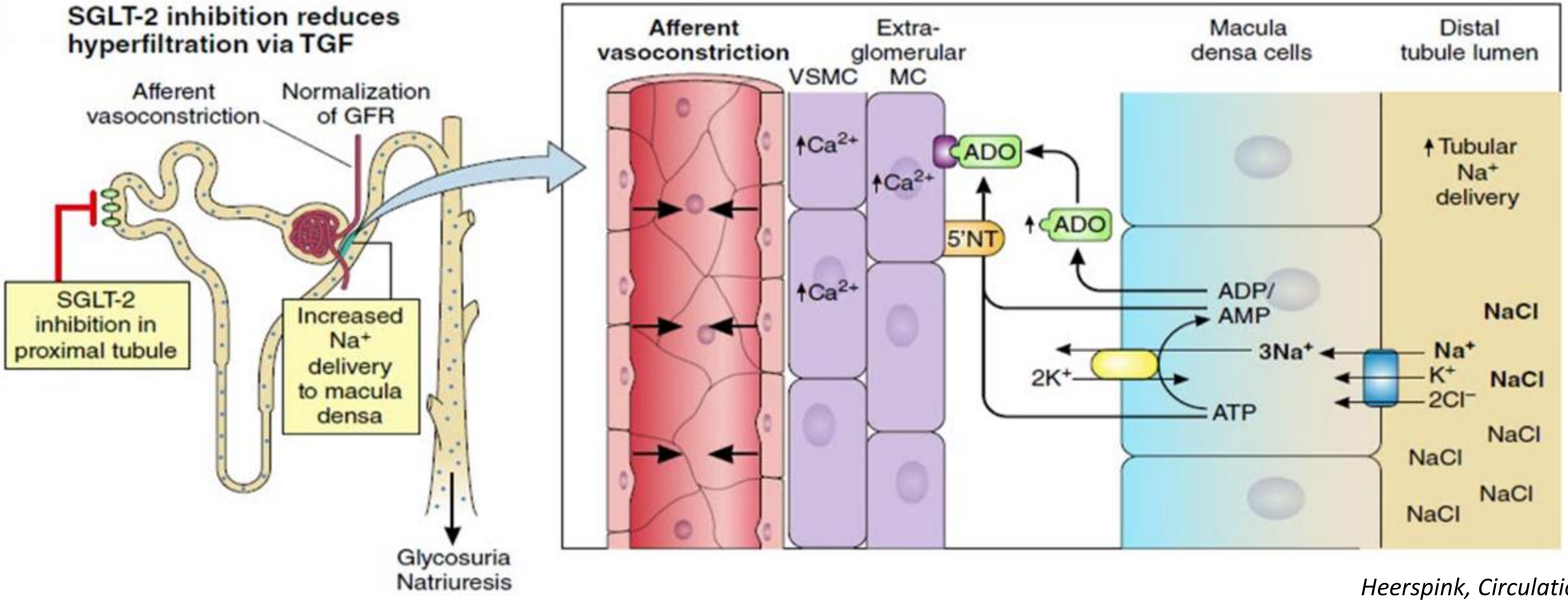


Evaluation of Glomerular Hemodynamic Function by Empagliflozin in Diabetic Mice Using In Vivo Imaging



Circulation. 2019;140:303–315





Heerspink, Circulation, 2016

SGLT2i

↑ tubuloglomerular feedback

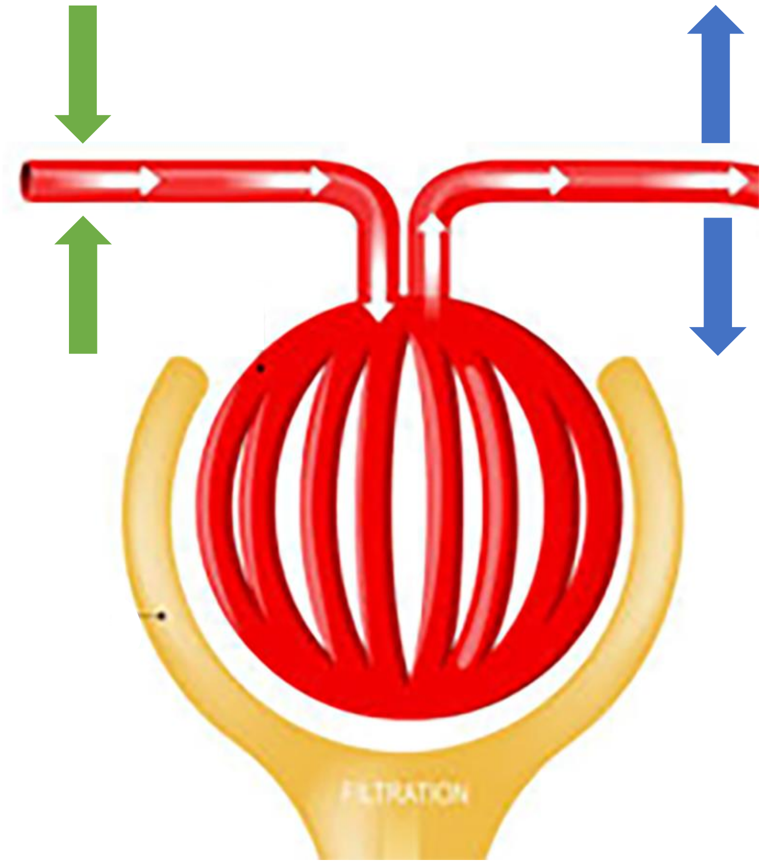
↑ afferent arteriole tone

↓ intraglomerular pressure

RASi

↓ efferent arteriole tone

↓ intraglomerular pressure



Am J Physiol Heart Circ Physiol 285: H2290–H2297, 2003.
First published September 4, 2003; 10.1152/ajpheart.00515.2003.

call for papers

Vascular Signaling by Free Radicals

Role of xanthine oxidoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress

Am J Physiol Regul Integr Comp Physiol 295: R1858–R1865, 2008.
First published October 15, 2008; doi:10.1152/ajpregu.90650.2008.

NADPH oxidase contributes to renal damage and dysfunction in Dahl salt-sensitive hypertension

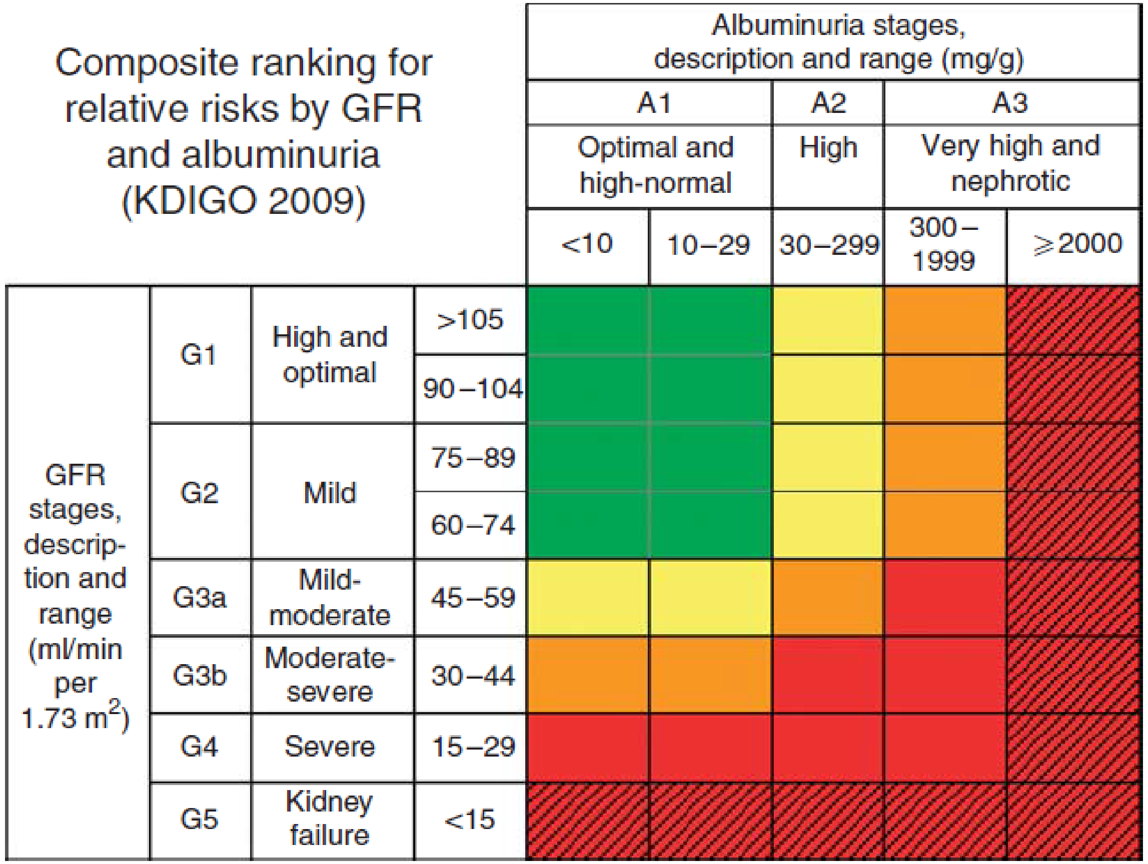
Am J Physiol Renal Physiol 296: F1239–F1244, 2009.
First published January 14, 2009; doi:10.1152/ajprenal.90521.2008.

Renal tubulointerstitial fibrosis: common but never simple

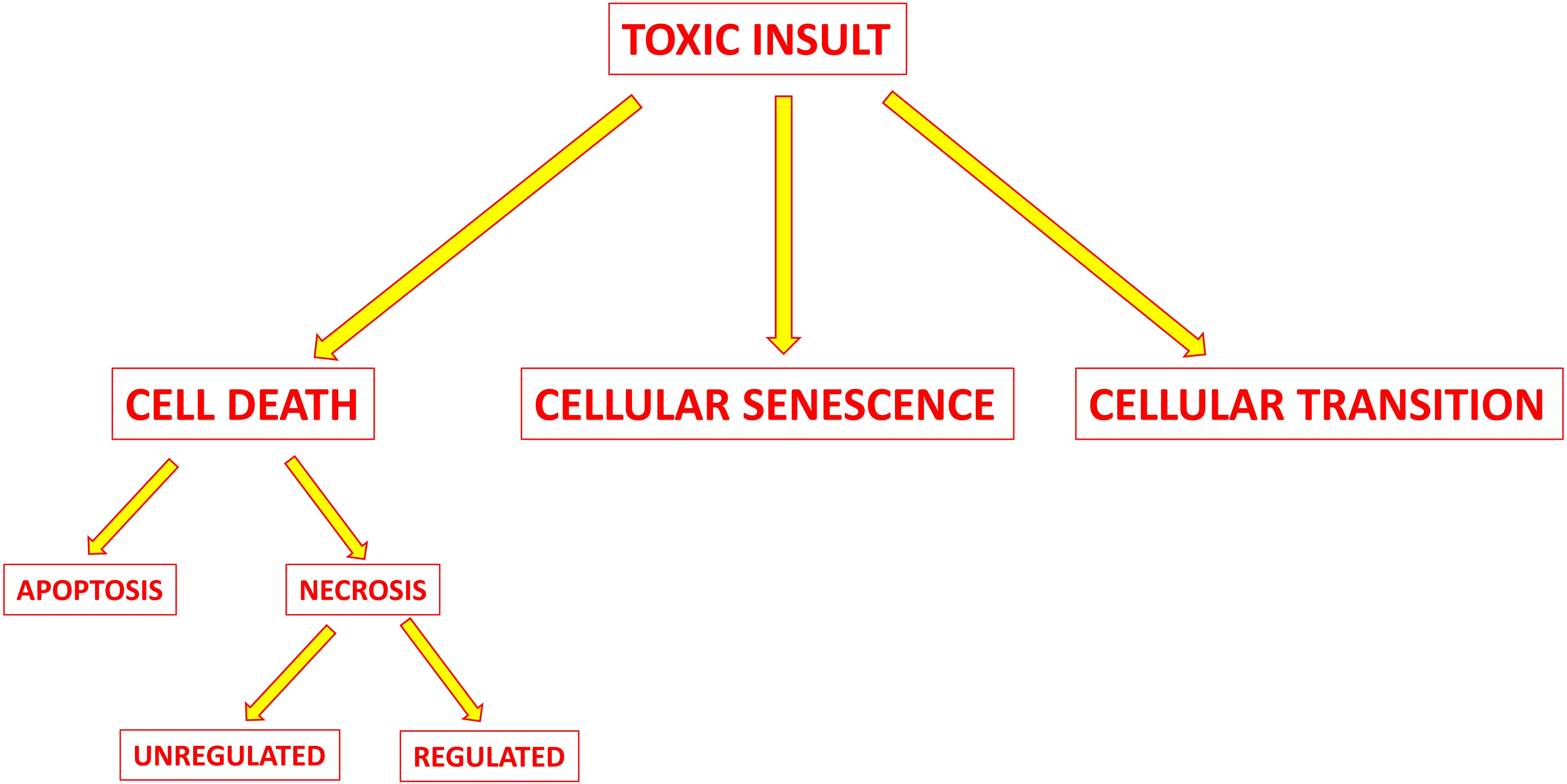
http://www.kidney-international.org pub

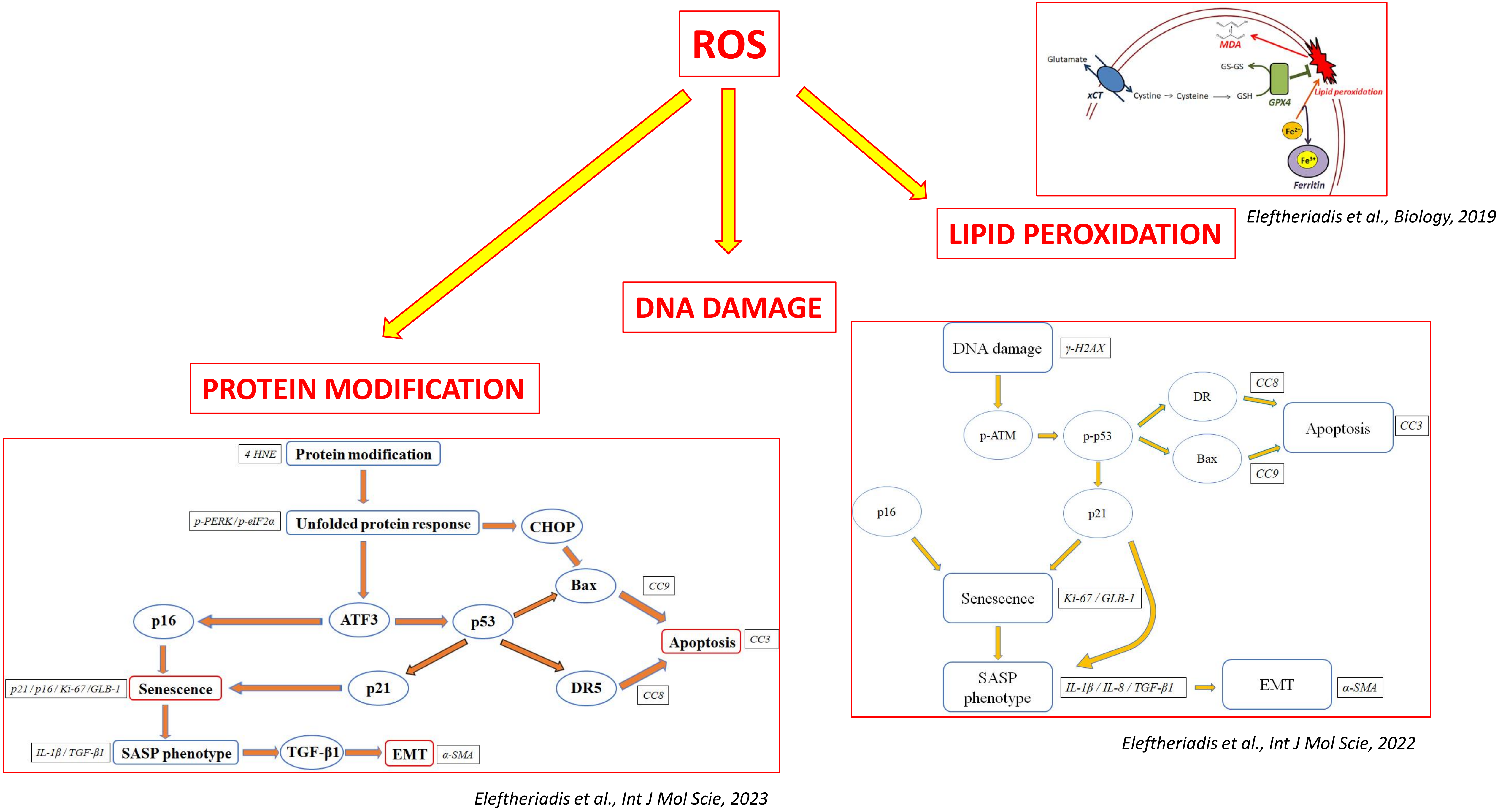
The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report

Andrew S. Levey[1], Paul E. de Jong[2], Josef Coresh[3], Meguid El Nahas[4], Brad C. Astor[3], Kunihiro Matsushita[3], Ron T. Gansevoort[2], Bertram L. Kasiske[5] and Kai-Uwe Eckardt[6]



Albuminuria toxicity in renal proximal tubular epithelial cells


TOXIC INSULT
CELL DEATH
CELLULAR SENESCENCE
CELLULAR TRANSITION
APOPTOSIS
NECROSIS
UNREGULATED
REGULATED


ROS
LIPID PEROXIDATION
Glutamate
xCT
Cystine → Cysteine → GSH
GS-GS
GPX4
MDA
Lipid peroxidation
Fe2+
Fe3+
Ferritin
Eleftheriadis et al., Biology, 2019
DNA DAMAGE
DNA damage
γ-H2AX
p-ATM
p-p53
DR
CC8
Bax
CC9
Apoptosis
CC3
p16
p21
Senescence
Ki-67 / GLB-1
SASP phenotype
IL-1β / IL-8 / TGF-β1
EMT
α-SMA
Eleftheriadis et al., Int J Mol Scie, 2022
PROTEIN MODIFICATION
4-HNE
Protein modification
p-PERK / p-eIF2α
Unfolded protein response
CHOP
Bax
CC9
ATF3
p16
p53
Apoptosis
CC3
p21/p16/Ki-67/GLB-1
Senescence
p21
DR5
CC8
IL-1β / TGF-β1
SASP phenotype
TGF-β1
EMT
α-SMA
Eleftheriadis et al., Int J Mol Scie, 2023

International Journal of *Molecular Sciences*

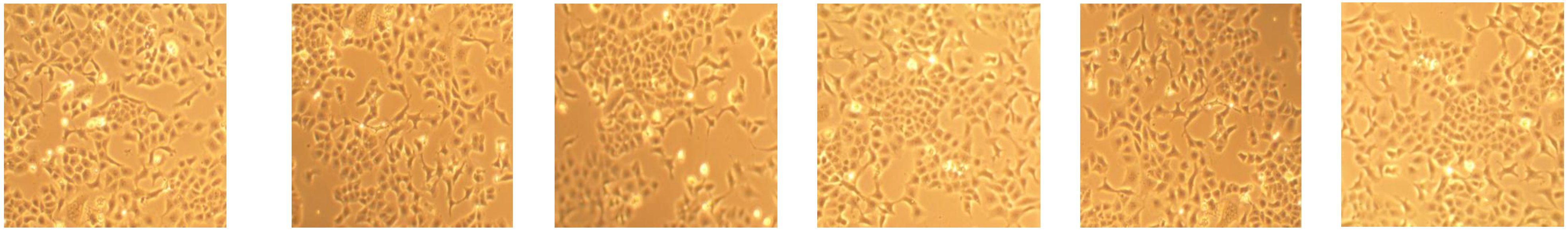
MDPI

Article

Routes of Albumin Overload Toxicity in Renal Tubular Epithelial Cells

Theodoros Eleftheriadis *,†, Georgios Pissas †, Spyridon Golfinopoulos, Maria Efthymiadi, Christina Poulianiti, Maria Anna Polyzou Konsta, Vassilios Liakopoulos and Ioannis Stefanidis

Human RPTECs



Control

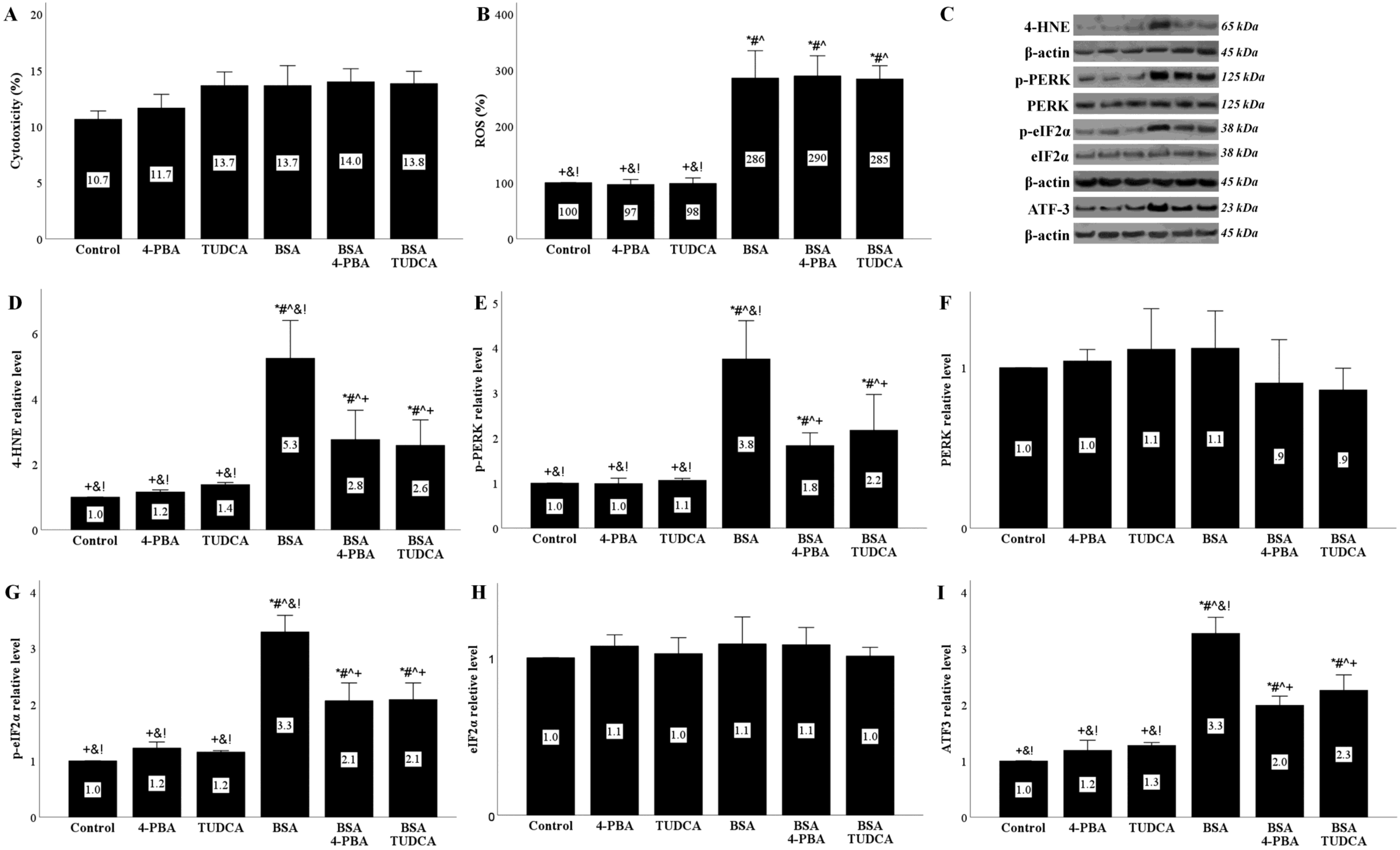
4-Phenylbutirate (4-PBA) *2mM*

Tauroursodeoxycholic (TUDCA) *2mM*

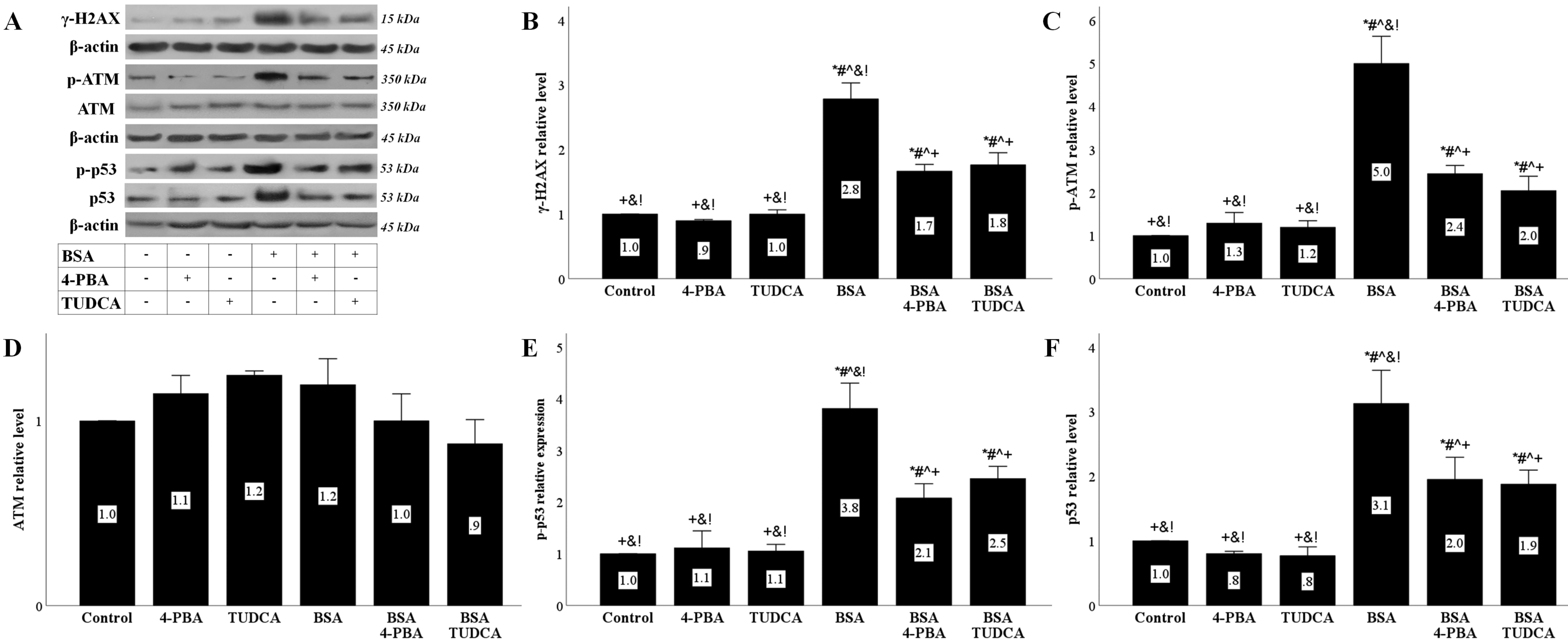
BSA *30 mg/mL*

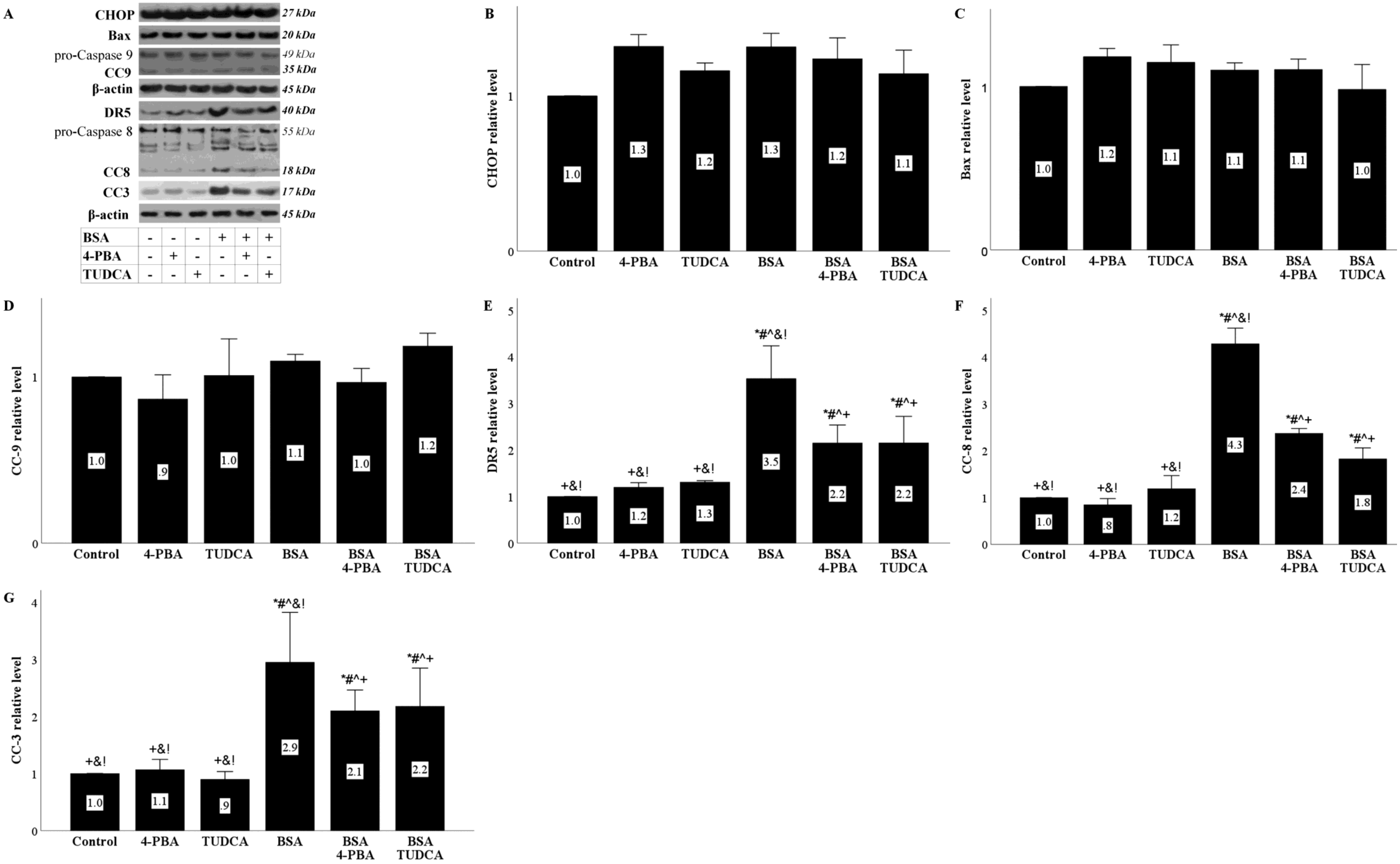
BSA 4-PBA

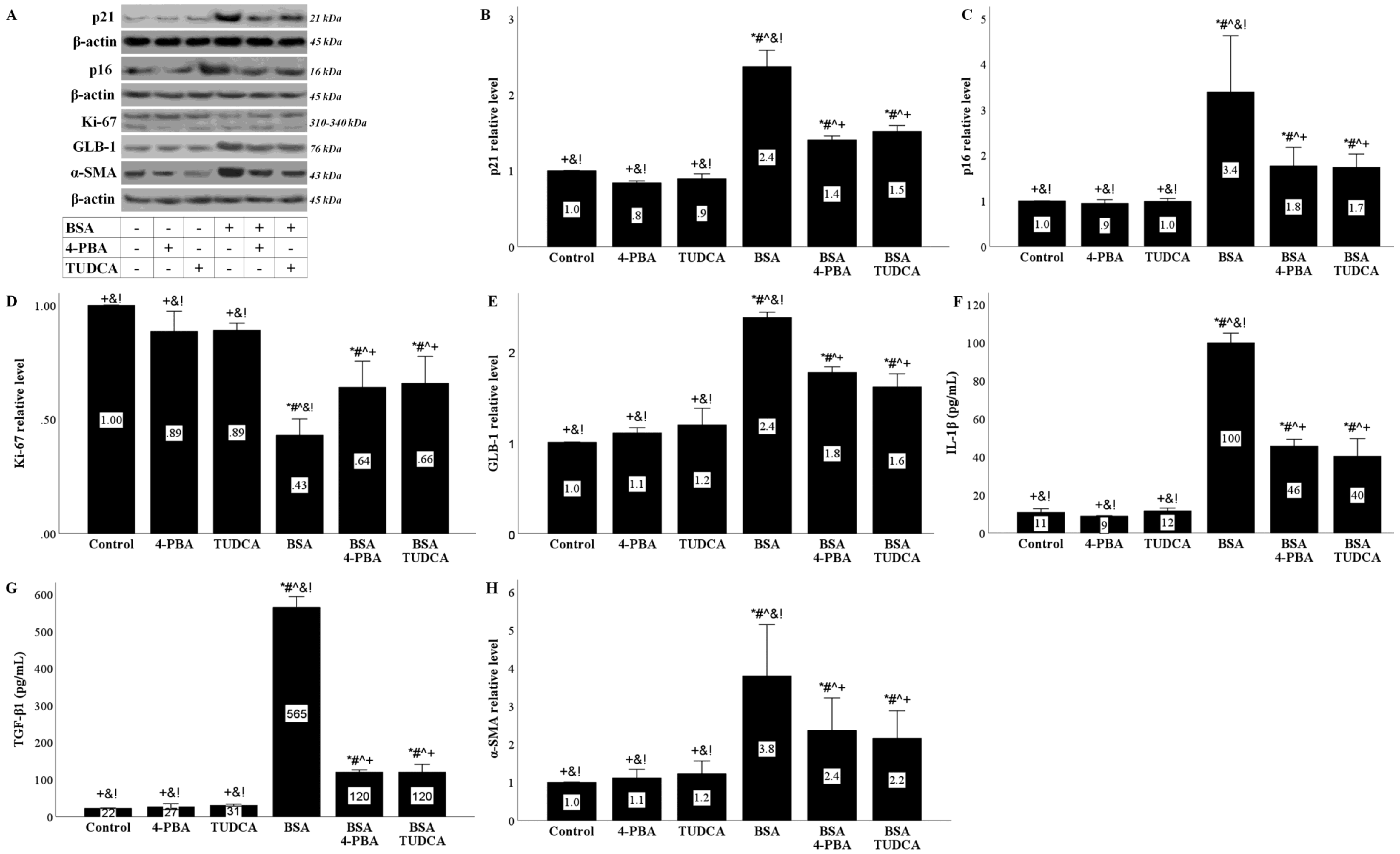
BSA TUDCA



A
Cytotoxicity (%)
10.7
11.7
13.7
13.7
14.0
13.8
Control
4-PBA
TUDCA
BSA
BSA 4-PBA
BSA TUDCA
B
ROS (%)
+&!
+&!
+&!
*#^
*#^
*#^
100
97
98
286
290
285
C
4-HNE 65 kDa
β-actin 45 kDa
p-PERK 125 kDa
PERK 125 kDa
p-eIF2α 38 kDa
eIF2α 38 kDa
β-actin 45 kDa
ATF-3 23 kDa
β-actin 45 kDa
D
4-HNE relative level
+&!
+&!
+&!
*#^&!
*#^+
*#^+
1.0
1.2
1.4
5.3
2.8
2.6
E
p-PERK relative level
+&!
+&!
+&!
*#^&!
*#^+
*#^+
1.0
1.0
1.1
3.8
1.8
2.2
F
PERK relative level
1.0
1.0
1.1
1.1
.9
.9
G
p-eIF2α relative level
+&!
+&!
+&!
*#^&!
*#^+
*#^+
1.0
1.2
1.2
3.3
2.1
2.1
H
eIF2α relative level
1.0
1.1
1.0
1.1
1.1
1.0
I
ATF3 relative level
+&!
+&!
+&!
*#^&!
*#^+
*#^+
1.0
1.2
1.3
3.3
2.0
2.3

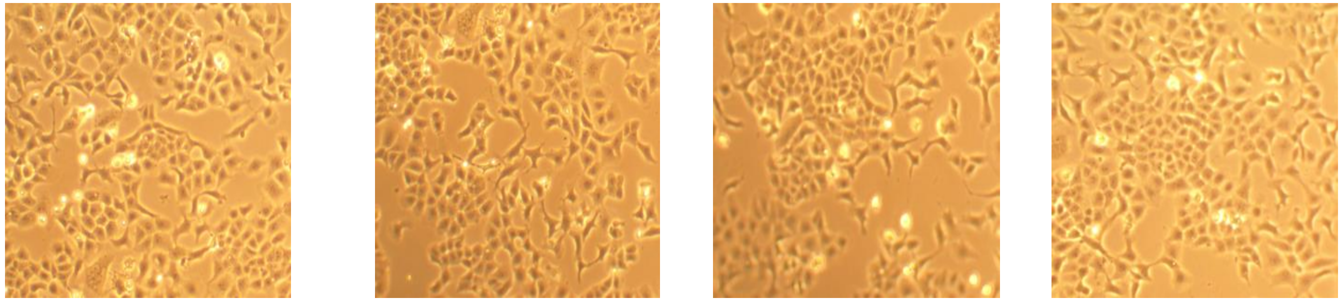

A
γ-H2AX
β-actin
p-ATM
ATM
β-actin
p-p53
p53
β-actin
15 kDa
45 kDa
350 kDa
350 kDa
45 kDa
53 kDa
53 kDa
45 kDa
BSA - - - + + +
4-PBA - + - - + -
TUDCA - - + - - +
B
γ-H2AX relative level
1.0 .9 1.0 2.8 1.7 1.8
+&! +&! +&! *#^&! *#^+ *#^+
C
p-ATM relative level
1.0 1.3 1.2 5.0 2.4 2.0
+&! +&! +&! *#^&! *#^+ *#^+
D
ATM relative level
1.0 1.1 1.2 1.2 1.0 .9
E
p-p53 relative expression
1.0 1.1 1.1 3.8 2.1 2.5
+&! +&! +&! *#^&! *#^+ *#^+
F
p53 relative level
1.0 .8 .8 3.1 2.0 1.9
+&! +&! +&! *#^&! *#^+ *#^+
Control
4-PBA
TUDCA
BSA
BSA 4-PBA
BSA TUDCA


A
CHOP 27 kDa
Bax 20 kDa
pro-Caspase 9 49 kDa
CC9 35 kDa
β-actin 45 kDa
DR5 40 kDa
pro-Caspase 8 55 kDa
CC8 18 kDa
CC3 17 kDa
β-actin 45 kDa
BSA - - - + + +
4-PBA - + - - + -
TUDCA - - + - - +
B
CHOP relative level
1.0 1.3 1.2 1.3 1.2 1.1
C
Bax relative level
1.0 1.2 1.1 1.1 1.1 1.0
D
CC-9 relative level
1.0 .9 1.0 1.1 1.0 1.2
E
DR5 relative level
+&! +&! +&! *#^&! *#^+ *#^+
1.0 1.2 1.3 3.5 2.2 2.2
F
CC-8 relative level
+&! +&! +&! *#^&! *#^+ *#^+
1.0 .8 1.2 4.3 2.4 1.8
G
CC-3 relative level
+&! +&! +&! *#^&! *#^+ *#^+
1.0 1.1 .9 2.9 2.1 2.2
Control
4-PBA
TUDCA
BSA
BSA 4-PBA
BSA TUDCA



A
p21
β-actin
p16
β-actin
Ki-67
GLB-1
α-SMA
β-actin
21 kDa
45 kDa
16 kDa
45 kDa
310-340 kDa
76 kDa
43 kDa
45 kDa
BSA - - - + + +
4-PBA - + - - + -
TUDCA - - + - - +
B
p21 relative level
C
p16 relative level
D
Ki-67 relative level
E
GLB-1 relative level
F
IL-1β (pg/mL)
G
TGF-β1 (pg/mL)
H
α-SMA relative level
Control
4-PBA
TUDCA
BSA
BSA 4-PBA
BSA TUDCA
+&!
*#^&!
*#^+

Human
RPTECs

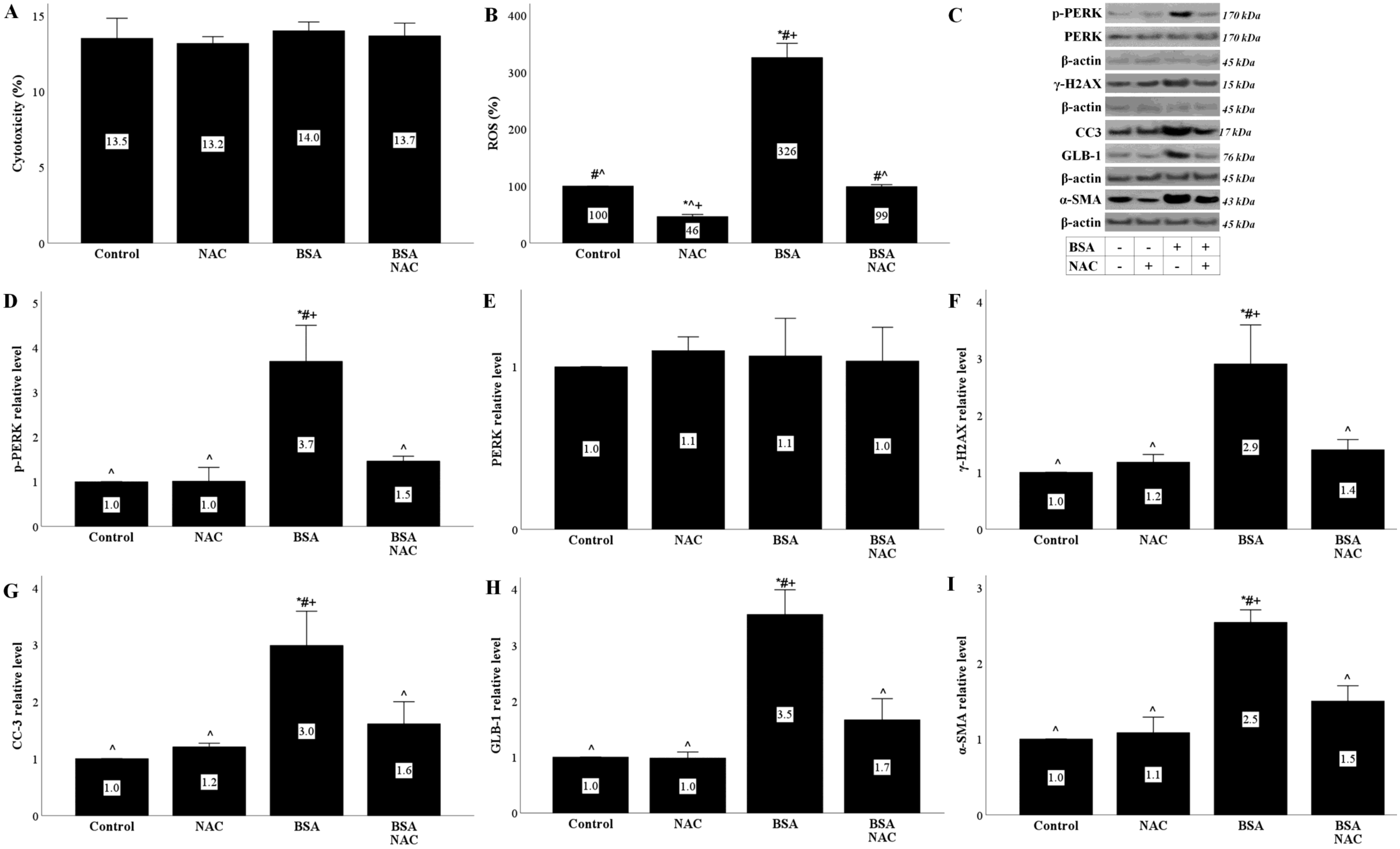


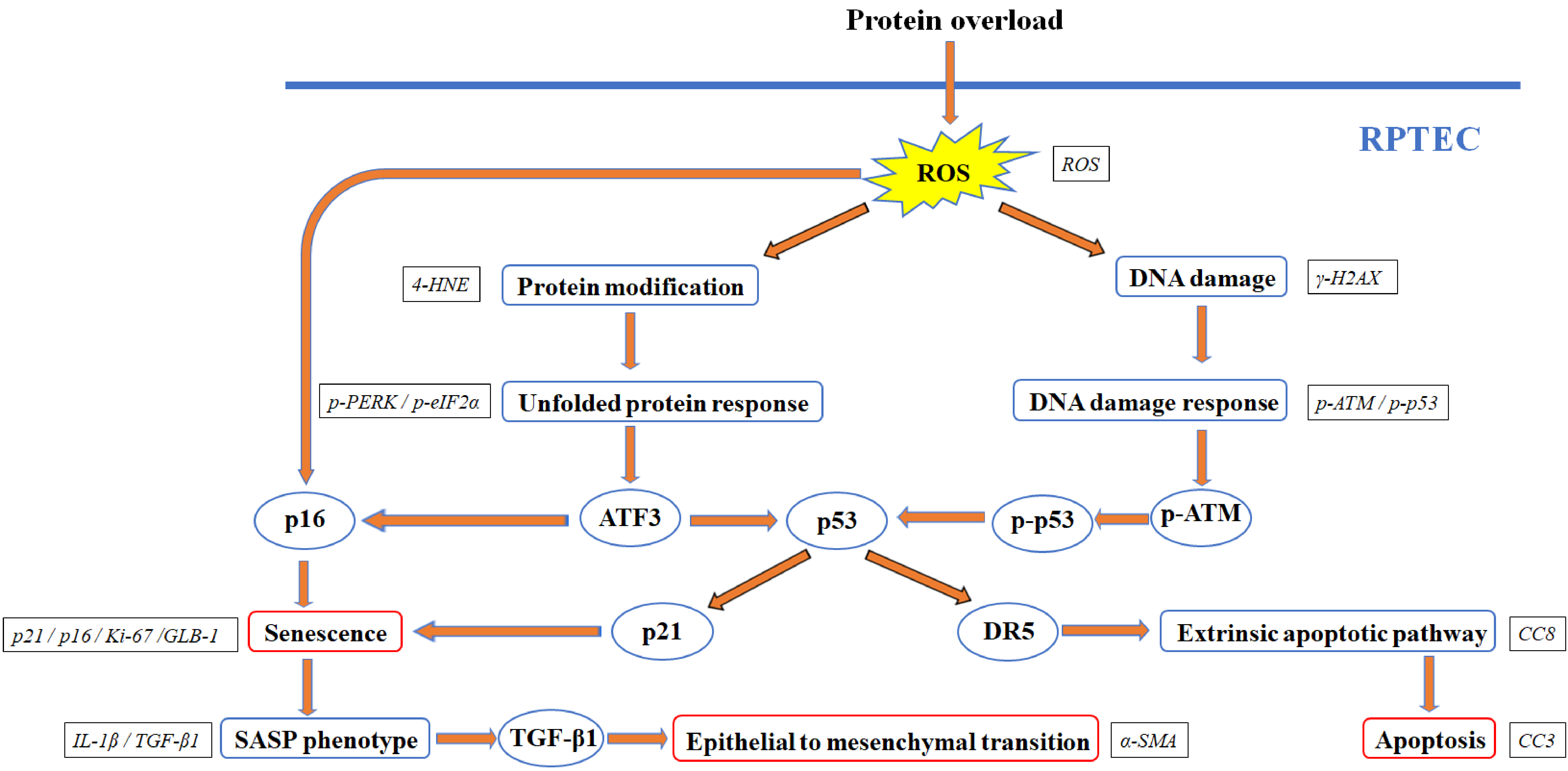
Control

N-acetylcysteine
(NAC)
3mg/mL

BSA

BSA
NAC


A
Cytotoxicity (%)
Control
NAC
BSA
BSA NAC
13.5
13.2
14.0
13.7
B
ROS (%)
100
46
326
99
C
p-PERK
170 kDa
PERK
170 kDa
β-actin
45 kDa
γ-H2AX
15 kDa
CC3
17 kDa
GLB-1
76 kDa
α-SMA
43 kDa
BSA - - + +
NAC - + - +
D
p-PERK relative level
1.0
1.0
3.7
1.5
E
PERK relative level
1.0
1.1
1.1
1.0
F
γ-H2AX relative level
1.0
1.2
2.9
1.4
G
CC-3 relative level
1.0
1.2
3.0
1.6
H
GLB-1 relative level
1.0
1.0
3.5
1.7
I
α-SMA relative level
1.0
1.1
2.5
1.5



Protein overload
RPTEC
ROS
ROS
4-HNE
Protein modification
DNA damage
γ-H2AX
p-PERK / p-eIF2α
Unfolded protein response
DNA damage response
p-ATM / p-p53
p16
ATF3
p53
p-p53
p-ATM
p21 / p16 / Ki-67 /GLB-1
Senescence
p21
DR5
Extrinsic apoptotic pathway
CC8
IL-1β / TGF-β1
SASP phenotype
TGF-β1
Epithelial to mesenchymal transition
α-SMA
Apoptosis
CC3

Glucose toxicity in renal proximal tubular epithelial cells

Banting Lecture 2004

The Pathobiology of Diabetic Complications

A Unifying Mechanism

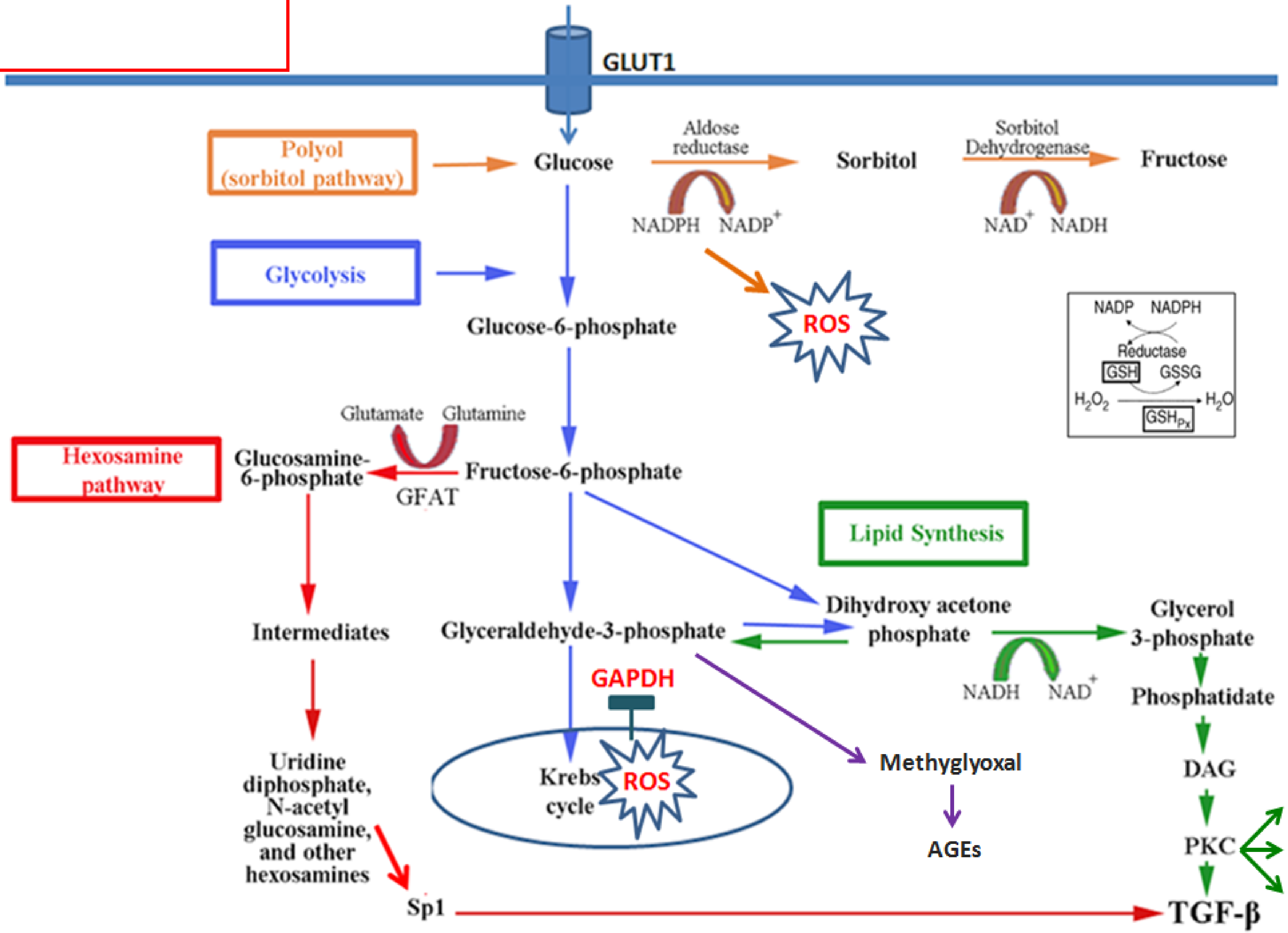
Michael Brownlee

Int Urol Nephrol (2016) 48:1731–1739
DOI 10.1007/s11255-016-1377-x

NEPHROLOGY - ORIGINAL PAPER

Activation of general control nonderepressible 2 kinase protects human glomerular endothelial cells from harmful high-glucose-induced molecular pathways

Theodoros Eleftheriadis[1] · Konstantina Tsogka[1] · Georgios Pissas[1] · Georgia Antoniadi[1] · Vassilios Liakopoulos[1] · Ioannis Stefanidis[1]

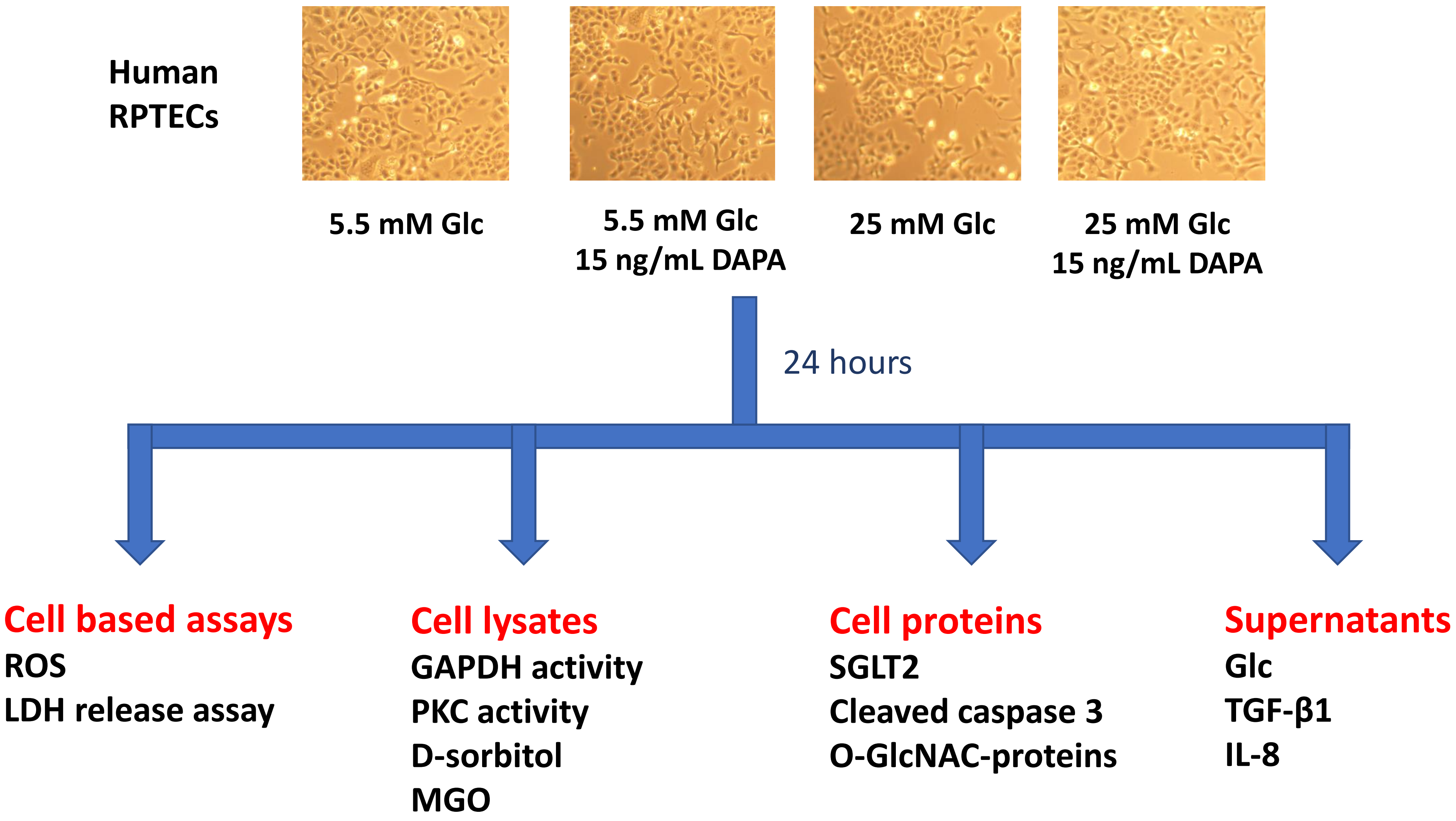


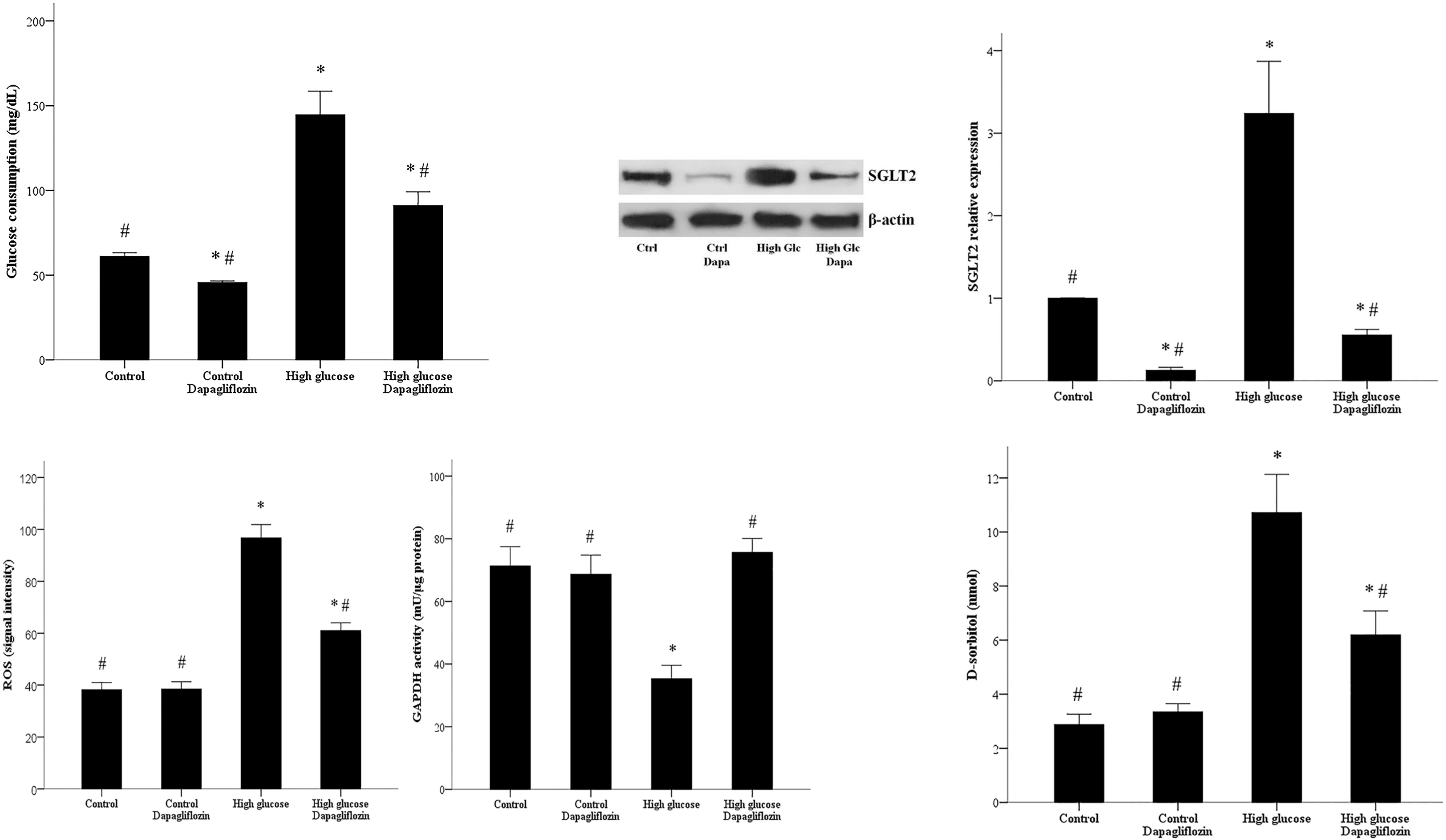
International Urology and Nephrology
https://doi.org/10.1007/s11255-020-02481-3

NEPHROLOGY - ORIGINAL PAPER

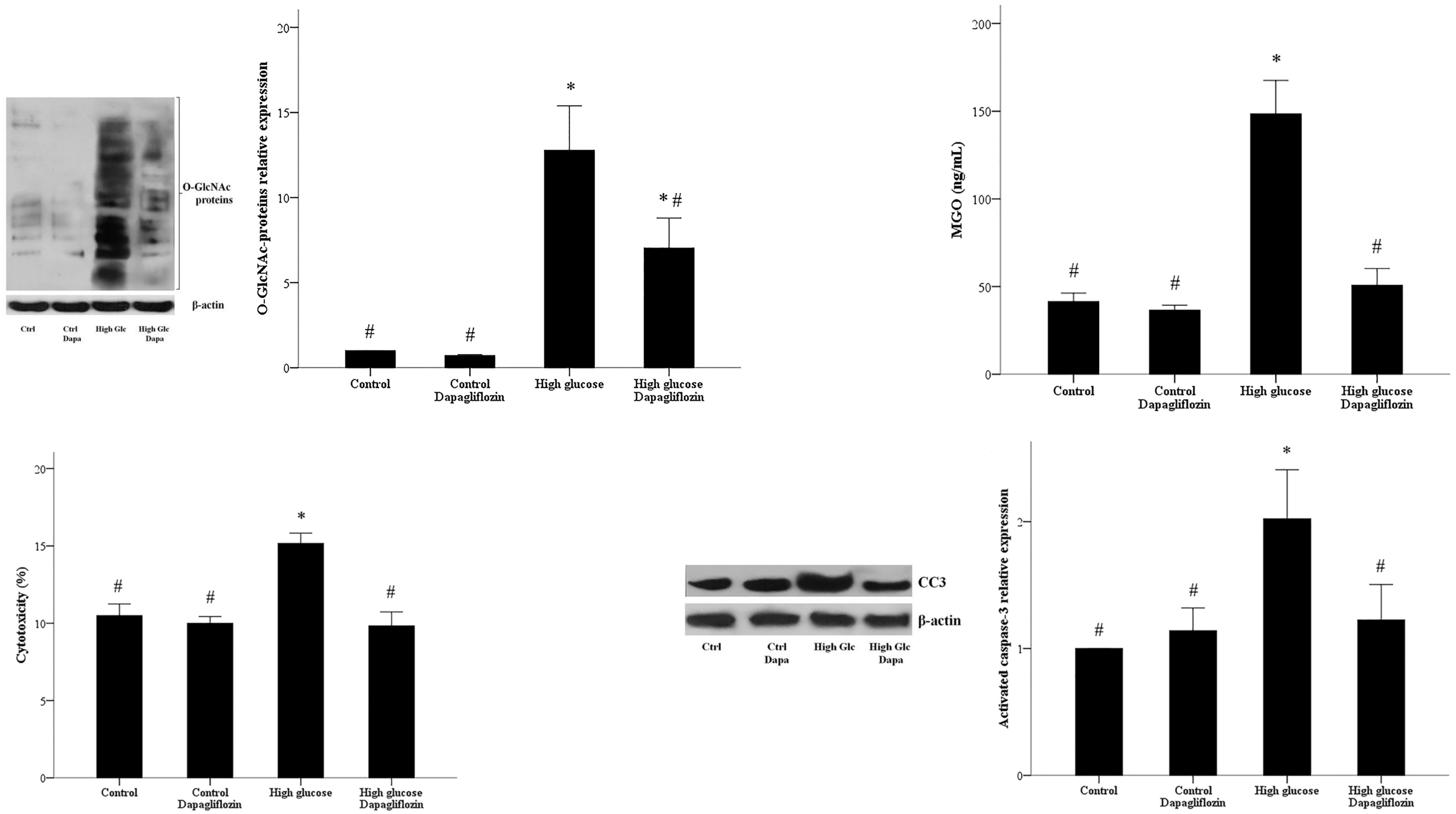
A unifying model of glucotoxicity in human renal proximal tubular epithelial cells and the effect of the SGLT2 inhibitor dapagliflozin

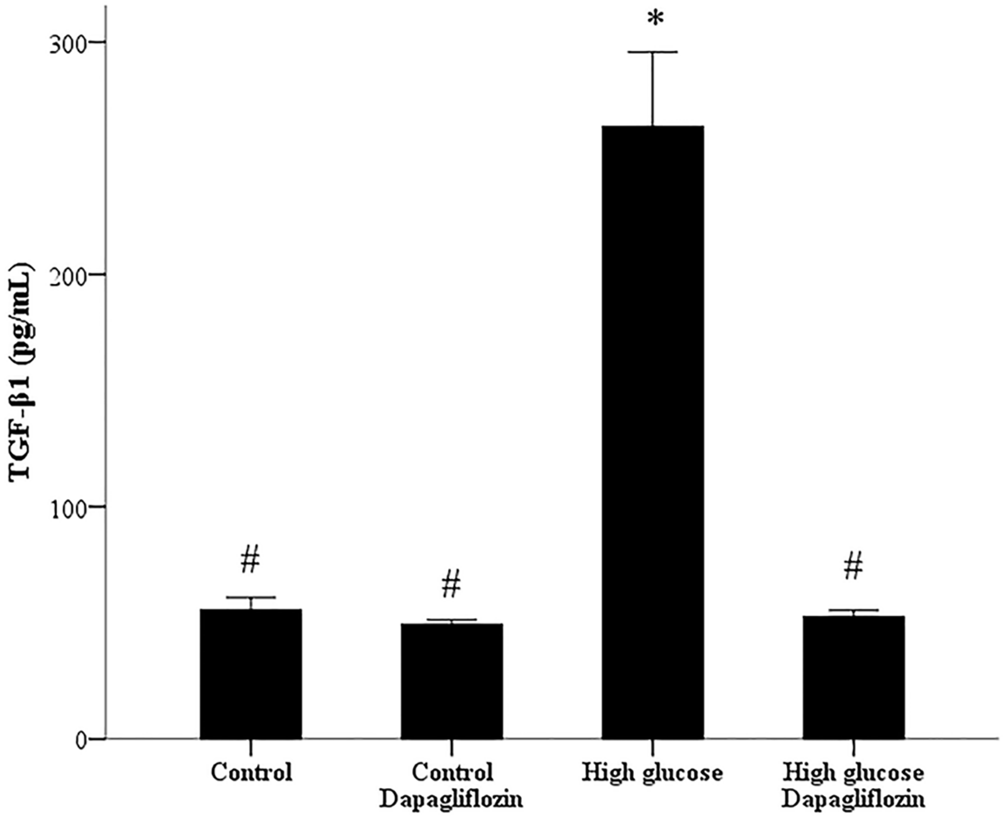
Theodoros Eleftheriadis[1] · Georgios Pissas[1] · Konstantina Tsogka[1] · Evdokia Nikolaou[1] · Vassilios Liakopoulos[1] · Ioannis Stefanidis[1]



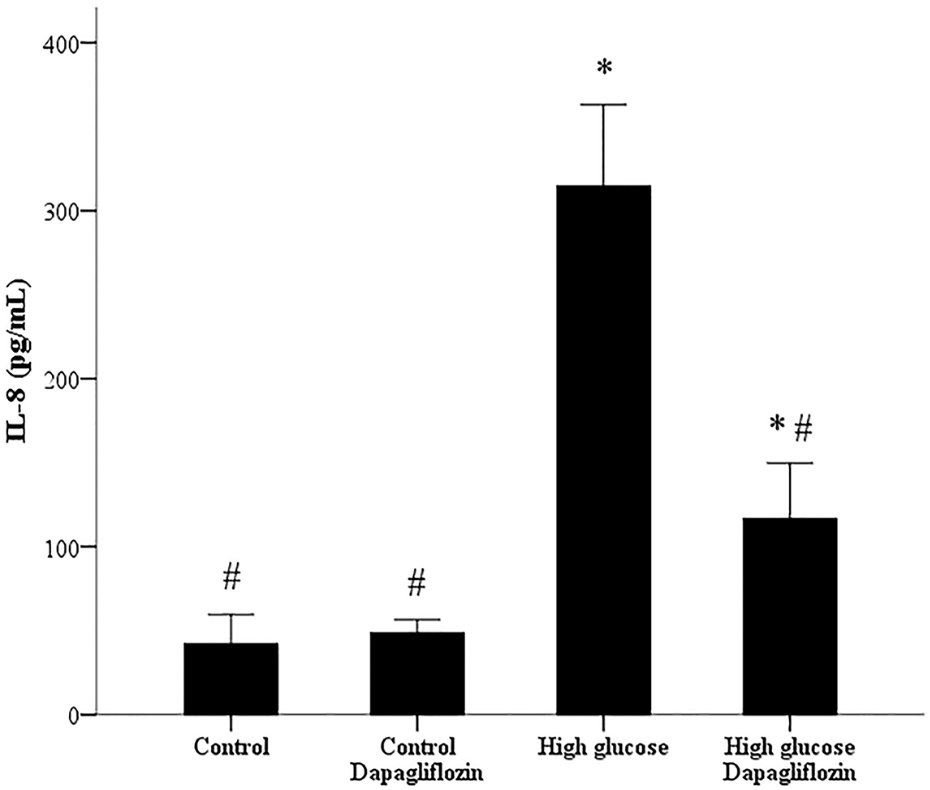


Glucose consumption (mg/dL)
200
150
100
50
0
#
* #
*
* #
Control
Control Dapagliflozin
High glucose
High glucose Dapagliflozin
SGLT2
β-actin
Ctrl
Ctrl Dapa
High Glc
High Glc Dapa
SGLT2 relative expression
4
3
2
1
0
ROS (signal intensity)
120
100
80
60
40
20
GAPDH activity (mU/µg protein)
D-sorbitol (nmol)
12
10
8
6

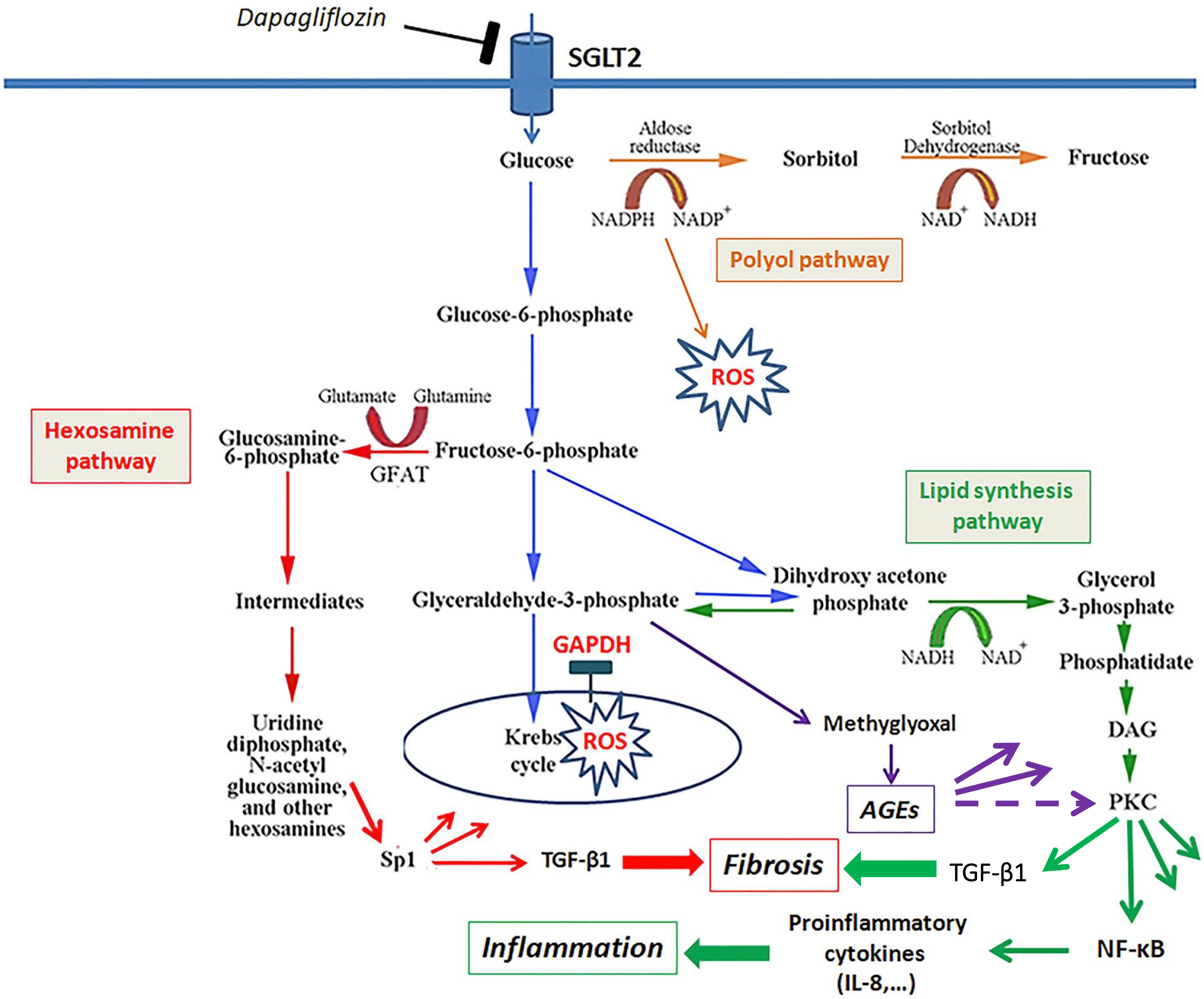

O-GlcNAc proteins
β-actin
Ctrl
Ctrl Dapa
High Glc
High Glc Dapa
O-GlcNAc-proteins relative expression
Control
Control Dapagliflozin
High glucose
High glucose Dapagliflozin
MGO (ng/mL)
Cytotoxicity (%)
CC3
Activated caspase-3 relative expression



TGF-β1 (pg/mL)
300
200
100
0
*
#
#
#
Control
Control Dapagliflozin
High glucose
High glucose Dapagliflozin



IL-8 (pg/mL)
400
300
200
100
0
*
*#
#
#
Control
Control Dapagliflozin
High glucose
High glucose Dapagliflozin


Dapagliflozin
SGLT2
Glucose
Aldose reductase
Sorbitol
Sorbitol Dehydrogenase
Fructose
NADPH
NADP+
NAD+
NADH
Polyol pathway
ROS
Glucose-6-phosphate
Glutamate
Glutamine
Hexosamine pathway
Glucosamine-6-phosphate
GFAT
Fructose-6-phosphate
Lipid synthesis pathway
Intermediates
Glyceraldehyde-3-phosphate
Dihydroxy acetone phosphate
Glycerol 3-phosphate
GAPDH
NADH
NAD+
Phosphatidate
Uridine diphosphate, N-acetyl glucosamine, and other hexosamines
Krebs cycle
ROS
Methylglyoxal
DAG
AGEs
PKC
Sp1
TGF-β1
Fibrosis
TGF-β1
Inflammation
Proinflammatory cytokines (IL-8,...)
NF-κB

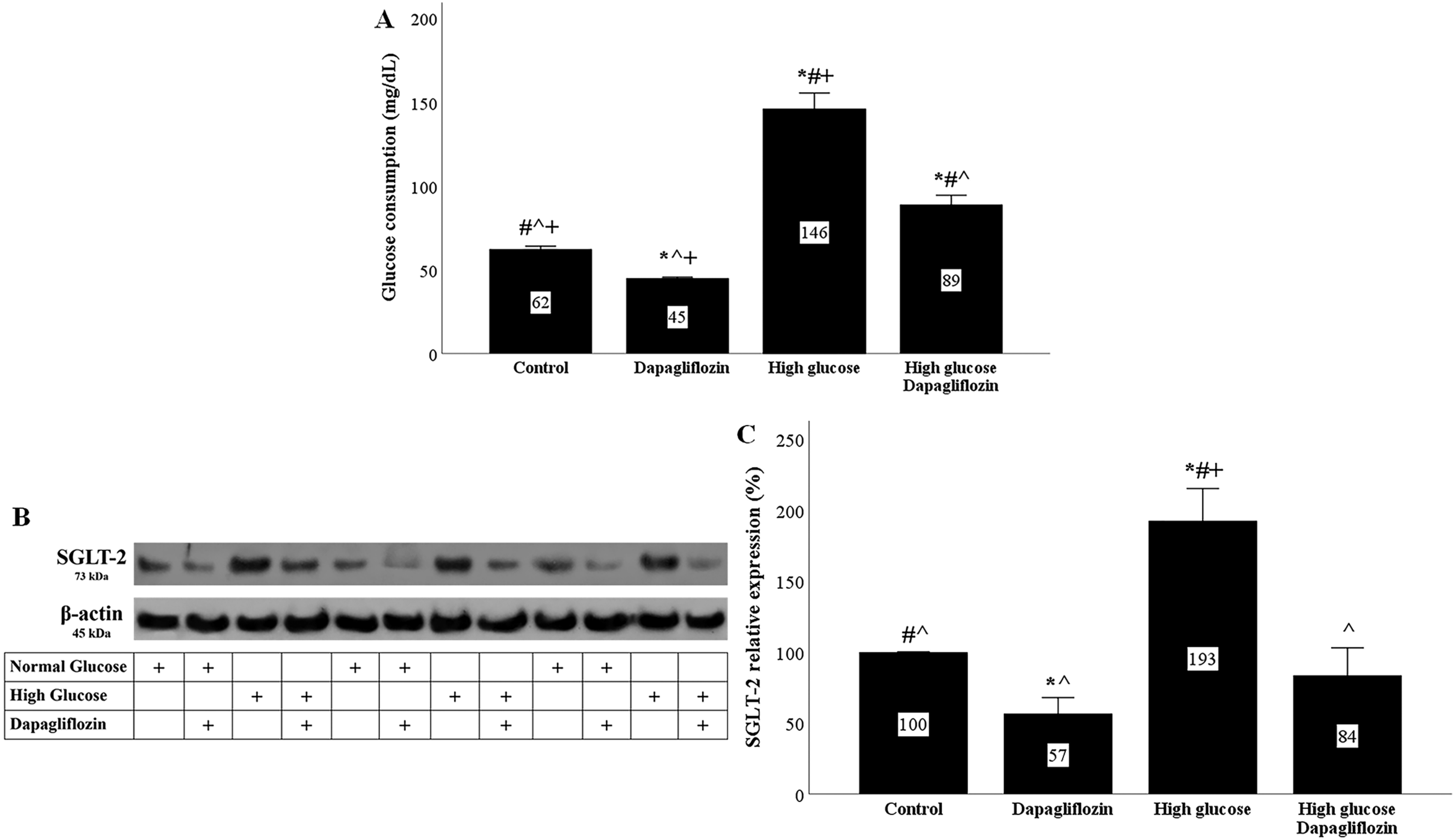
International Journal of *Molecular Sciences*

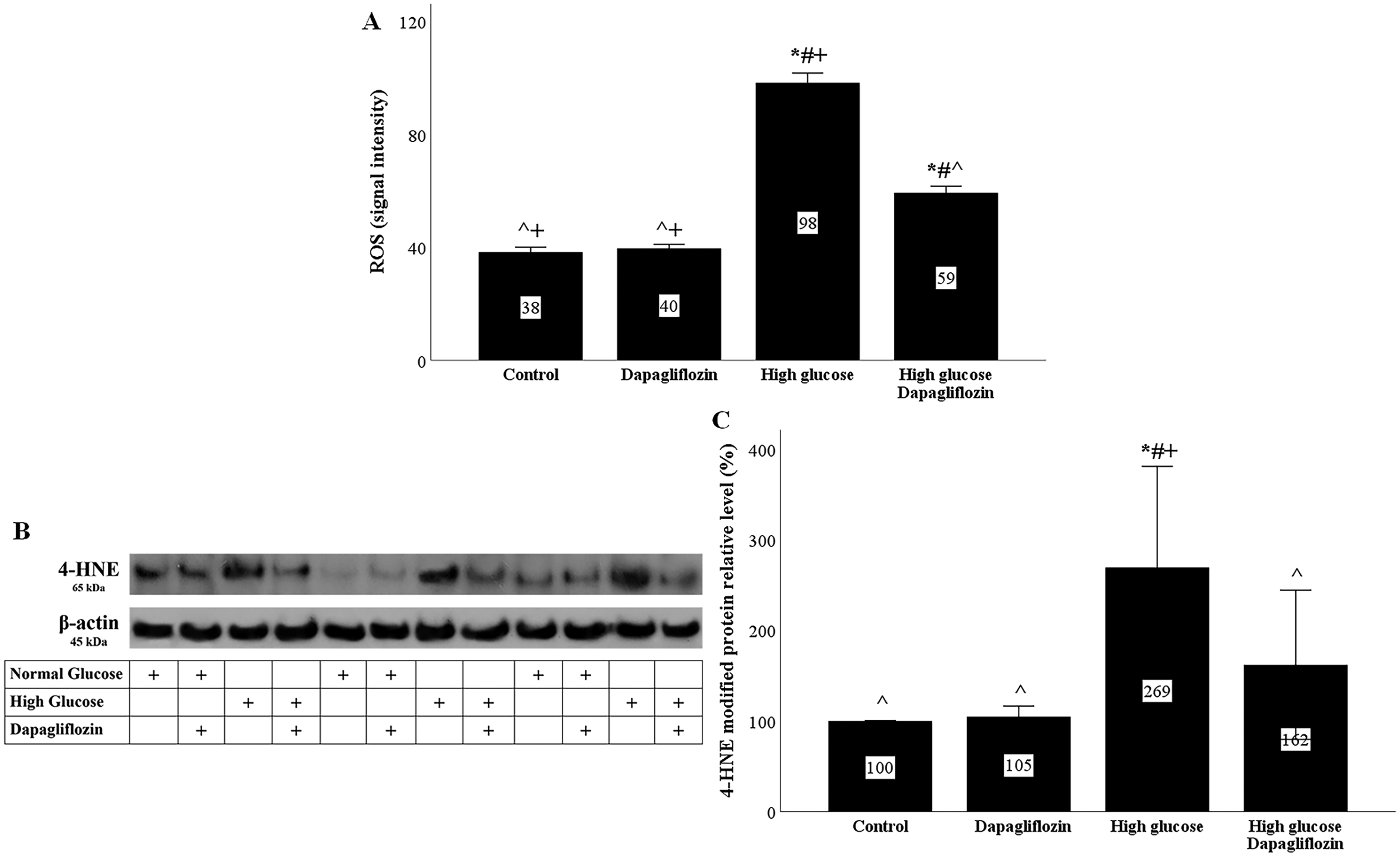
MDPI

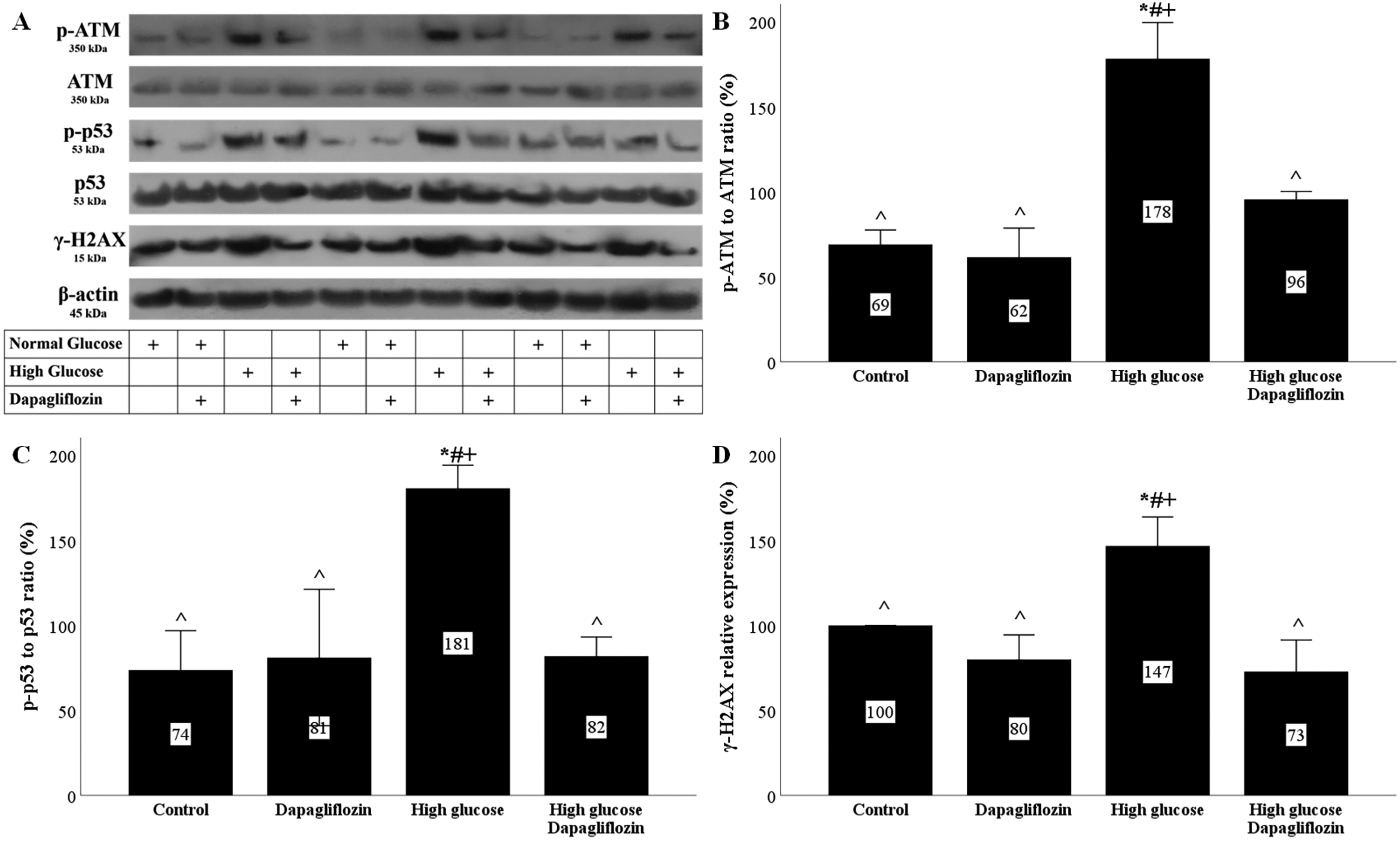
Article

Dapagliflozin Prevents High-Glucose-Induced Cellular Senescence in Renal Tubular Epithelial Cells

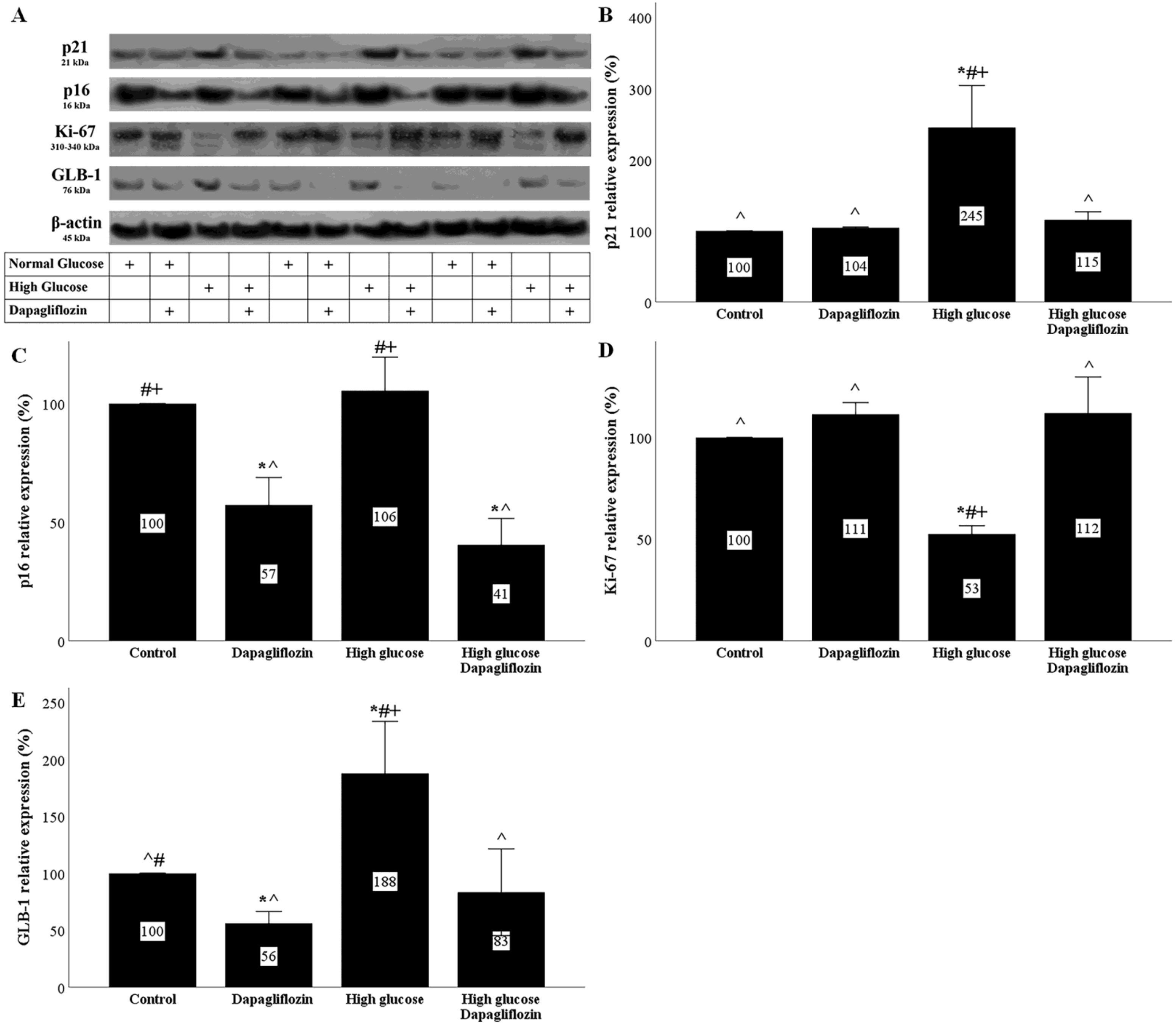
Theodoros Eleftheriadis *,†, Georgios Pissas †, Georgios Filippidis, Maria Efthymiadi, Vassilios Liakopoulos and Ioannis Stefanidis



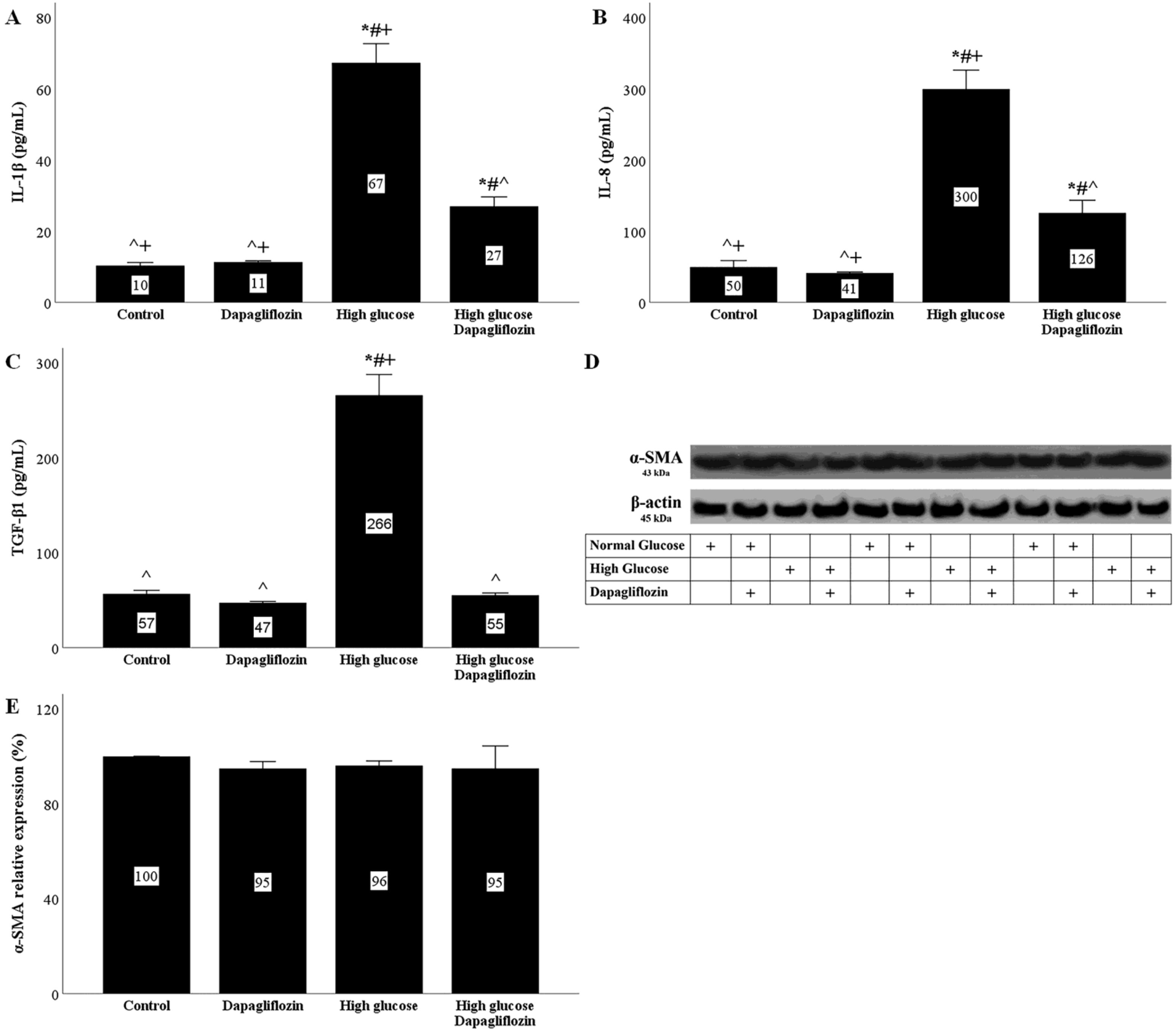

A
ROS (signal intensity)
120
80
40
0
^+
^+
*#+
*#^
38
40
98
59
Control
Dapagliflozin
High glucose
High glucose Dapagliflozin
B
4-HNE
65 kDa
β-actin
45 kDa
Normal Glucose
High Glucose
Dapagliflozin
C
4-HNE modified protein relative level (%)
400
300
200
100
0
^
^
*#+
^
100
105
269
162
Control
Dapagliflozin
High glucose
High glucose Dapagliflozin

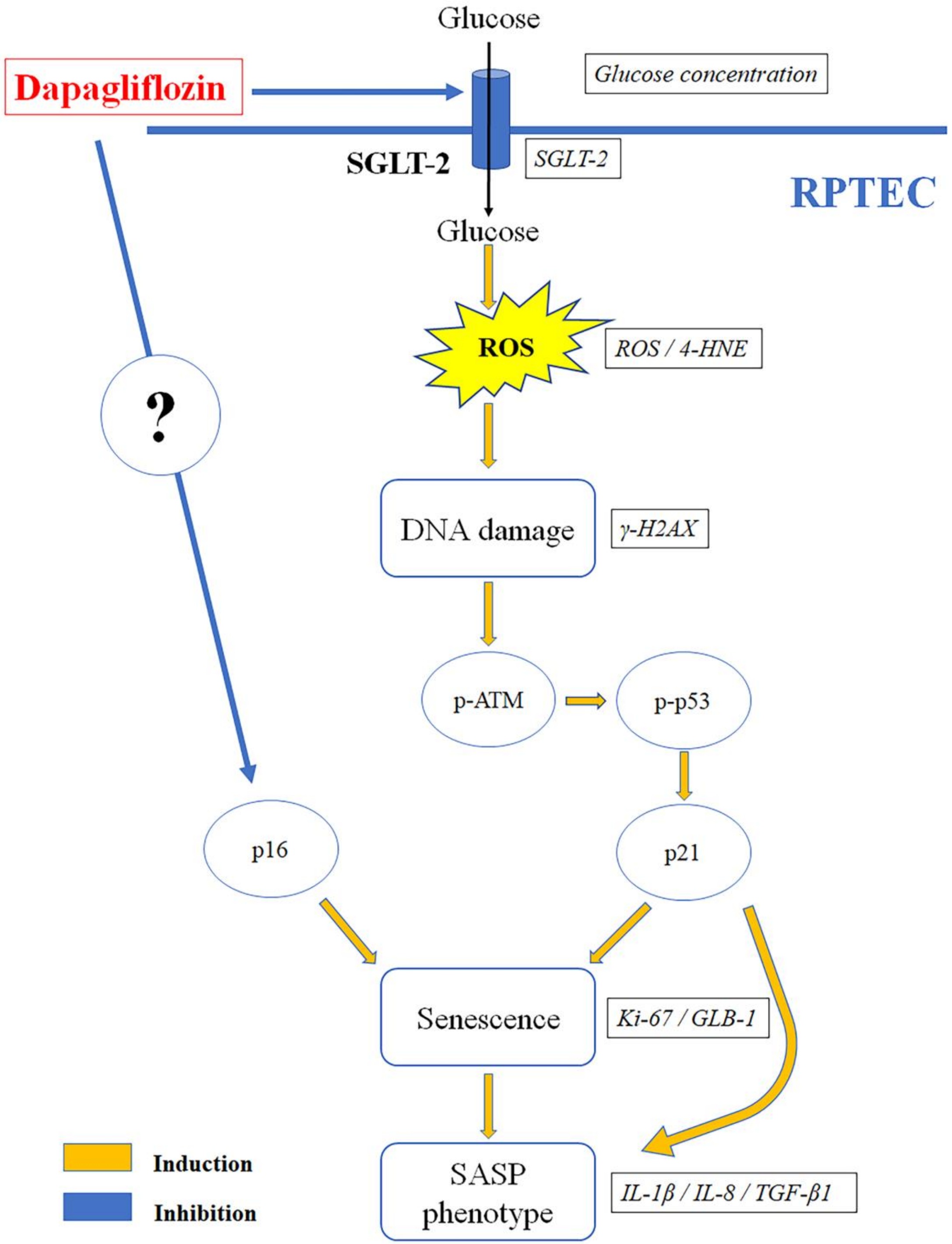


A
p-ATM
350 kDa
ATM
350 kDa
p-p53
53 kDa
p53
53 kDa
γ-H2AX
15 kDa
β-actin
45 kDa
Normal Glucose
High Glucose
Dapagliflozin
B
p-ATM to ATM ratio (%)
*#+
^
69
62
178
96
Control
Dapagliflozin
High glucose
High glucose Dapagliflozin
C
p-p53 to p53 ratio (%)
74
81
181
82
D
γ-H2AX relative expression (%)
100
80
147
73



A
p21
21 kDa
p16
16 kDa
Ki-67
310-340 kDa
GLB-1
76 kDa
β-actin
45 kDa
Normal Glucose
High Glucose
Dapagliflozin
B
p21 relative expression (%)
*#+
^
100
104
245
115
C
p16 relative expression (%)
#+
*^
100
57
106
41
D
Ki-67 relative expression (%)
*#+
100
111
53
112
E
GLB-1 relative expression (%)
^#
*^
*#+
100
56
188
83
Control
Dapagliflozin
High glucose
High glucose Dapagliflozin


A
IL-1β (pg/mL)
0
20
40
60
80
10
11
67
27
^+
^+
*#+
*#^
Control
Dapagliflozin
High glucose
High glucose Dapagliflozin
B
IL-8 (pg/mL)
0
100
200
300
400
50
41
300
126
^+
^+
*#+
*#^
Control
Dapagliflozin
High glucose
High glucose Dapagliflozin
C
TGF-β1 (pg/mL)
0
100
200
300
57
47
266
55
^
^
*#+
^
Control
Dapagliflozin
High glucose
High glucose Dapagliflozin
D
α-SMA
43 kDa
β-actin
45 kDa
Normal Glucose
High Glucose
Dapagliflozin
E
α-SMA relative expression (%)
0
40
80
120
100
95
96
95
Control
Dapagliflozin
High glucose
High glucose Dapagliflozin

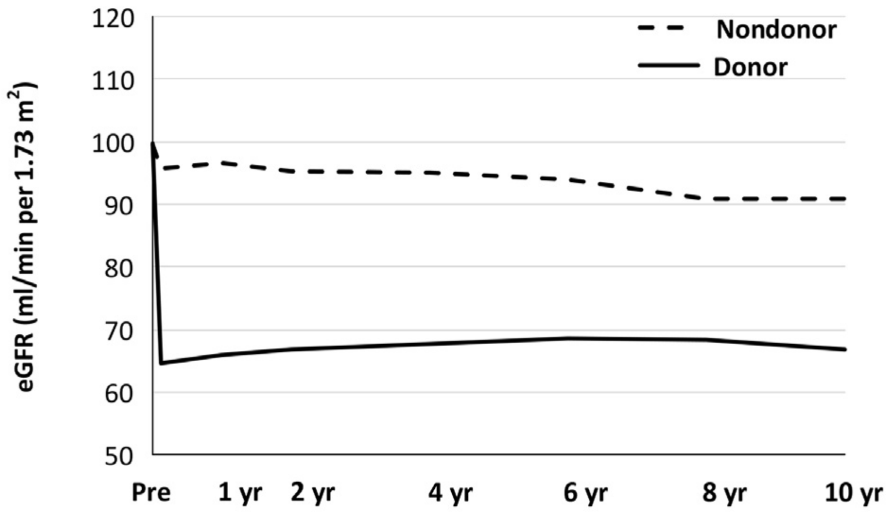

Glucose
Dapagliflozin
Glucose concentration
SGLT-2
SGLT-2
RPTEC
Glucose
ROS
ROS / 4-HNE
?
DNA damage
γ-H2AX
p-ATM
p-p53
p16
p21
Senescence
Ki-67 / GLB-1
SASP phenotype
IL-1β / IL-8 / TGF-β1
Induction
Inhibition

Changes in kidney function follow living donor nephrectomy

Kidney International (2020) **98,** 176–186

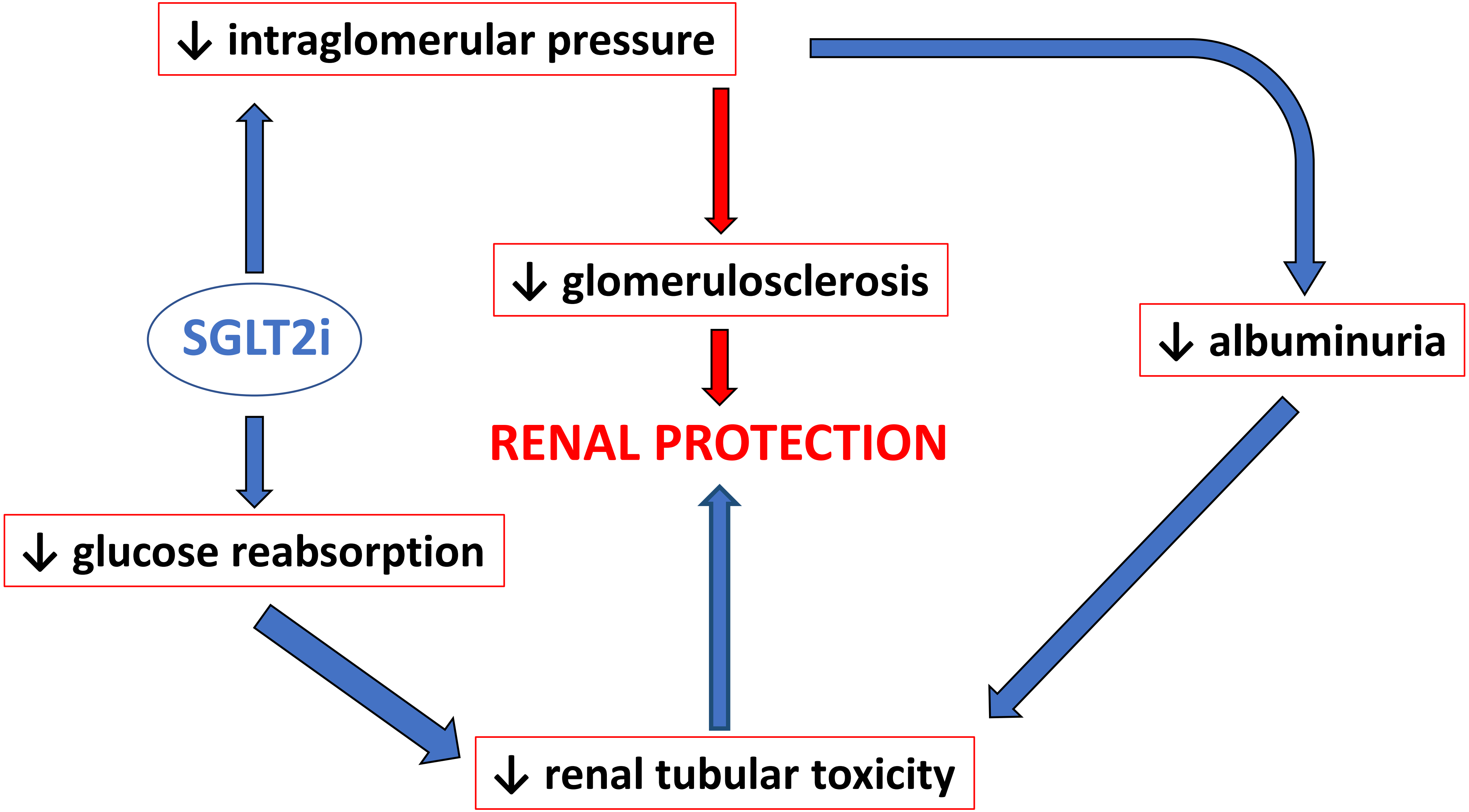
A prospective controlled study of metabolic and physiologic effects of kidney donation suggests that donors retain stable kidney function over the first nine years

Kidney International (2020) **98,** 168–175



- 6 months after nephrectomy the GFR is stabilized at the 70% of its initial value

In case of decreased renal mass, the single nephron GFR increases upregulating glucose load per nephron. Thus, it is likely that the dapagliflozin protection against glucotoxicity is extended in CKD patients without diabetes mellitus.


↓ intraglomerular pressure
↓ glomerulosclerosis
SGLT2i
↓ albuminuria
RENAL PROTECTION
↓ glucose reabsorption
↓ renal tubular toxicity


ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ - ΤΜΗΜΑ ΙΑΤΡΙΚΗΣ
ΥΓΕΙΑ
ΚΑΙ
ΑΣΚΛΗΠΙΟ
ΝΙΚΑΙΕΙC
ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ


ΠΕΡΙΦΕΡΕΙΑΚΟ
ΠΑΝΕΠΙΣΤΗΜΙΑΚΟ
ΓΕΝΙΚΟ
ΝΟΣΟΚΟΜΕΙΟ
ΛΑΡΙΣΑΣ