October 20, 2014

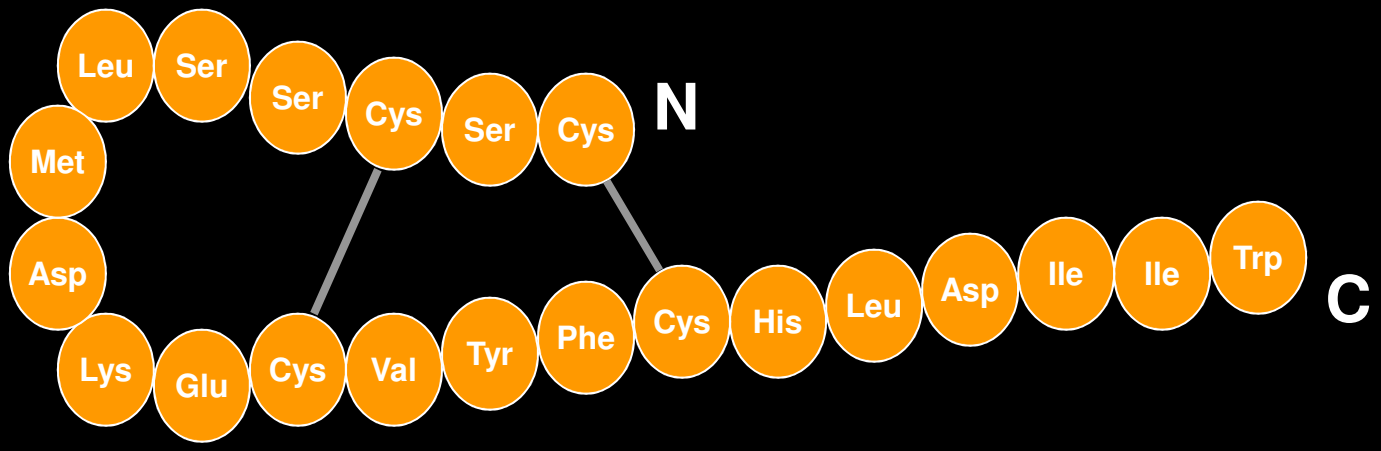
Endothelin signaling and actions in renal mesangium

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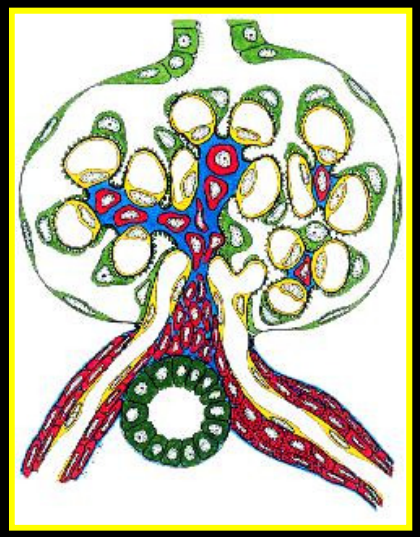
Medical College of Wisconsin



Pathophysiology of ET-1 in renal mesangium

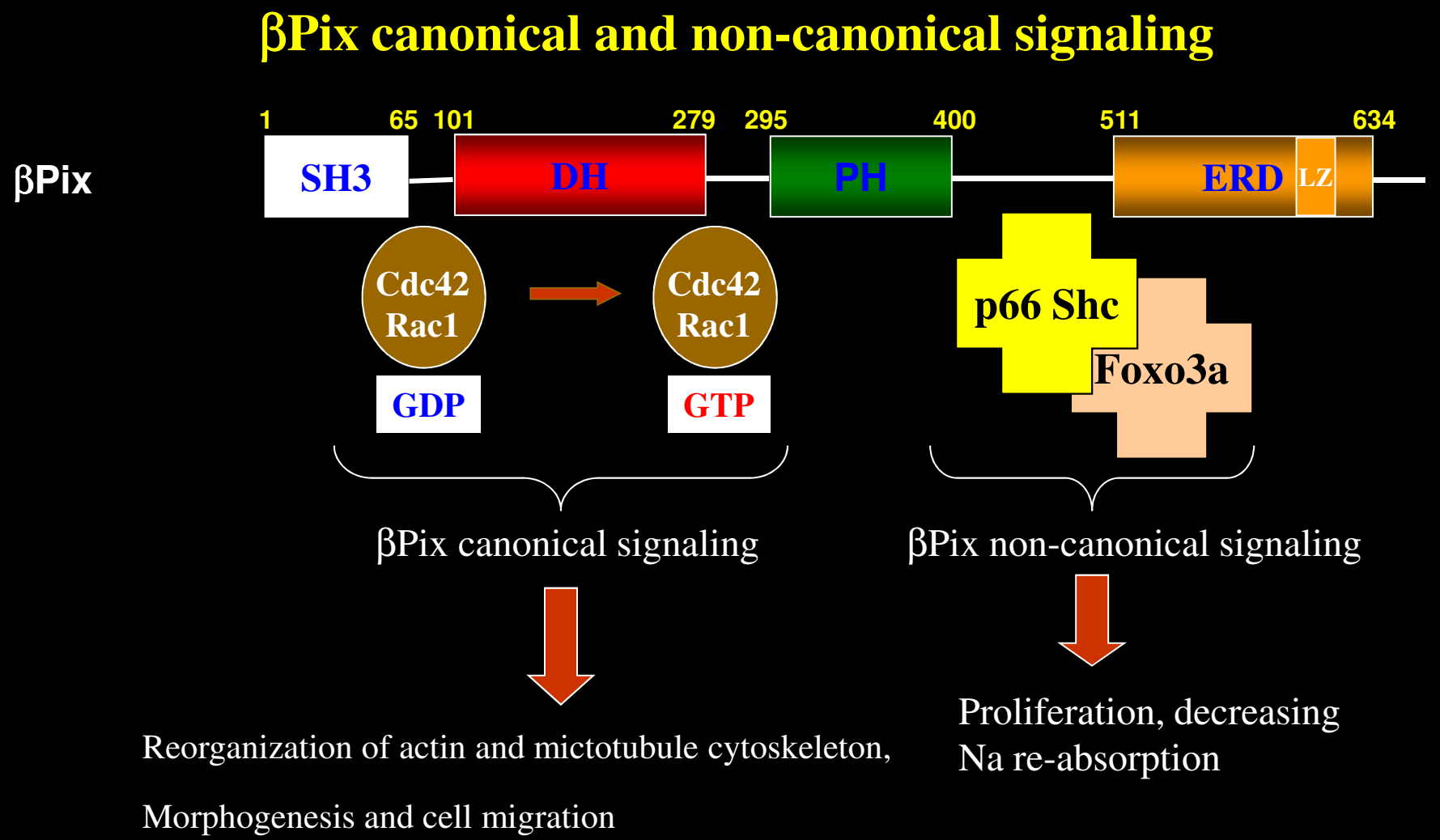
1. **Glomerulonephritis**
   - ET-1 production is increased
   - ET-1 increases mesangial cell proliferation and matrix accumulation
   - Blockade of ET-1 action reduces disease severity
2. **Diabetic Nephropathy**
   - ET-1 synthesis is increased by hyperglycemia (in vitro)
   - ET-1 blockade reduced albuminuria and increased glomerular filtration rate
3. **Hypertensive Glomerulosclerosis**
   - ET-1 production by mesangial cells is elevated in hypertension
   - Several agents enhance ET-1 release from SHR rats more than from WKY
   - ET-1 blockade reduced glomerular extracellular matrix accumulation

Epithelial cells
Endothelial cells
Mesangial cells



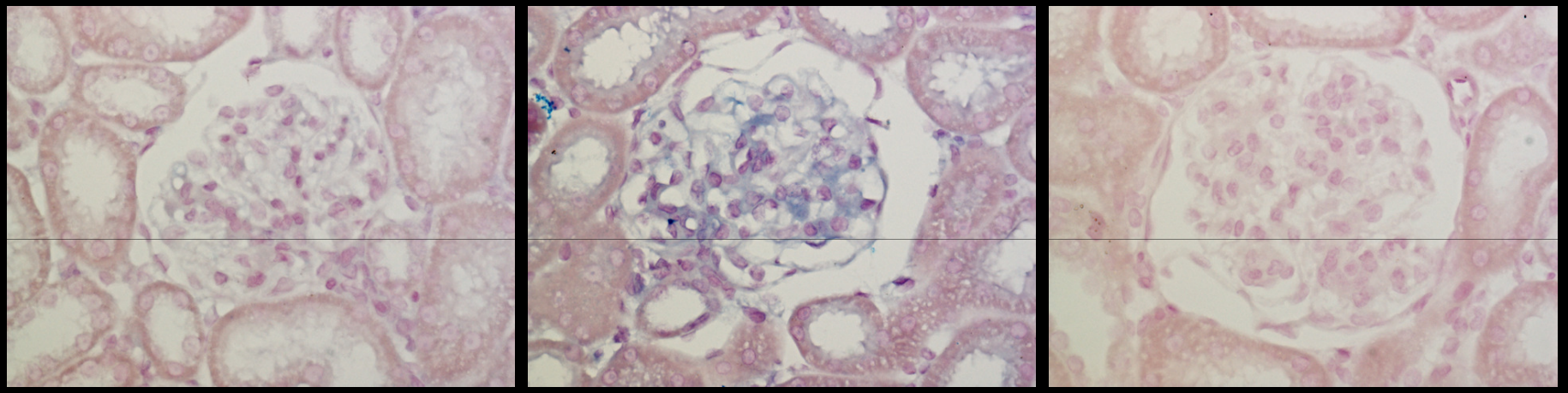
Mesangial cell ET-1 overactivity is observed in the pathogenesis of the three leading causes of ESRD, namely diabetes, hypertension and glomerulonephritis

Hypothesis" ET-1 signaling via a multiunit signaling complex including p66 Shc, β1Pix and FOXO3a is contributing to kidney pathologies associated with abnormal function of renal mesangial cells.



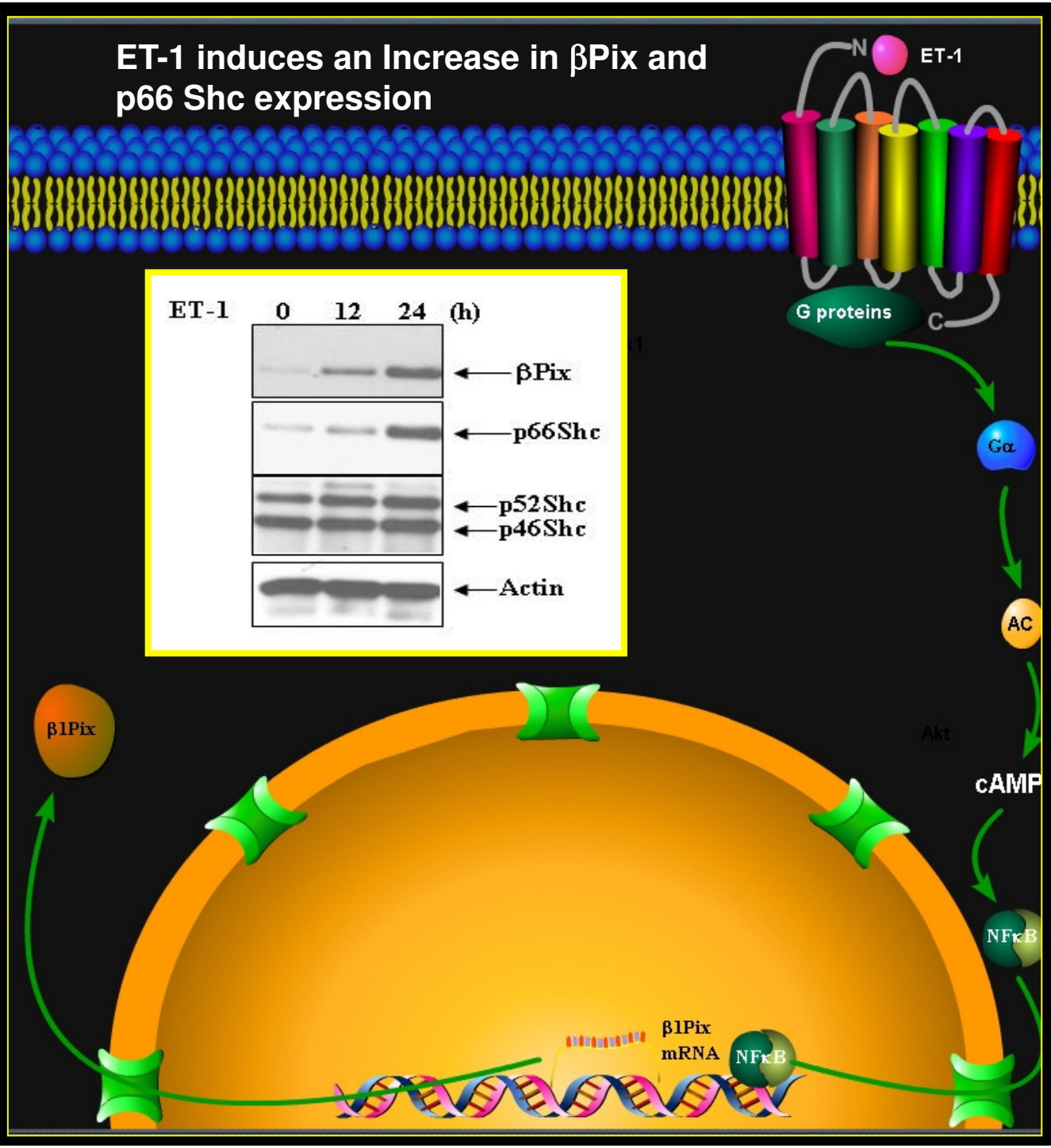
Expression of p66 Shc in glomeruli of rats with induced mesangial proliferative glomerulonephritis

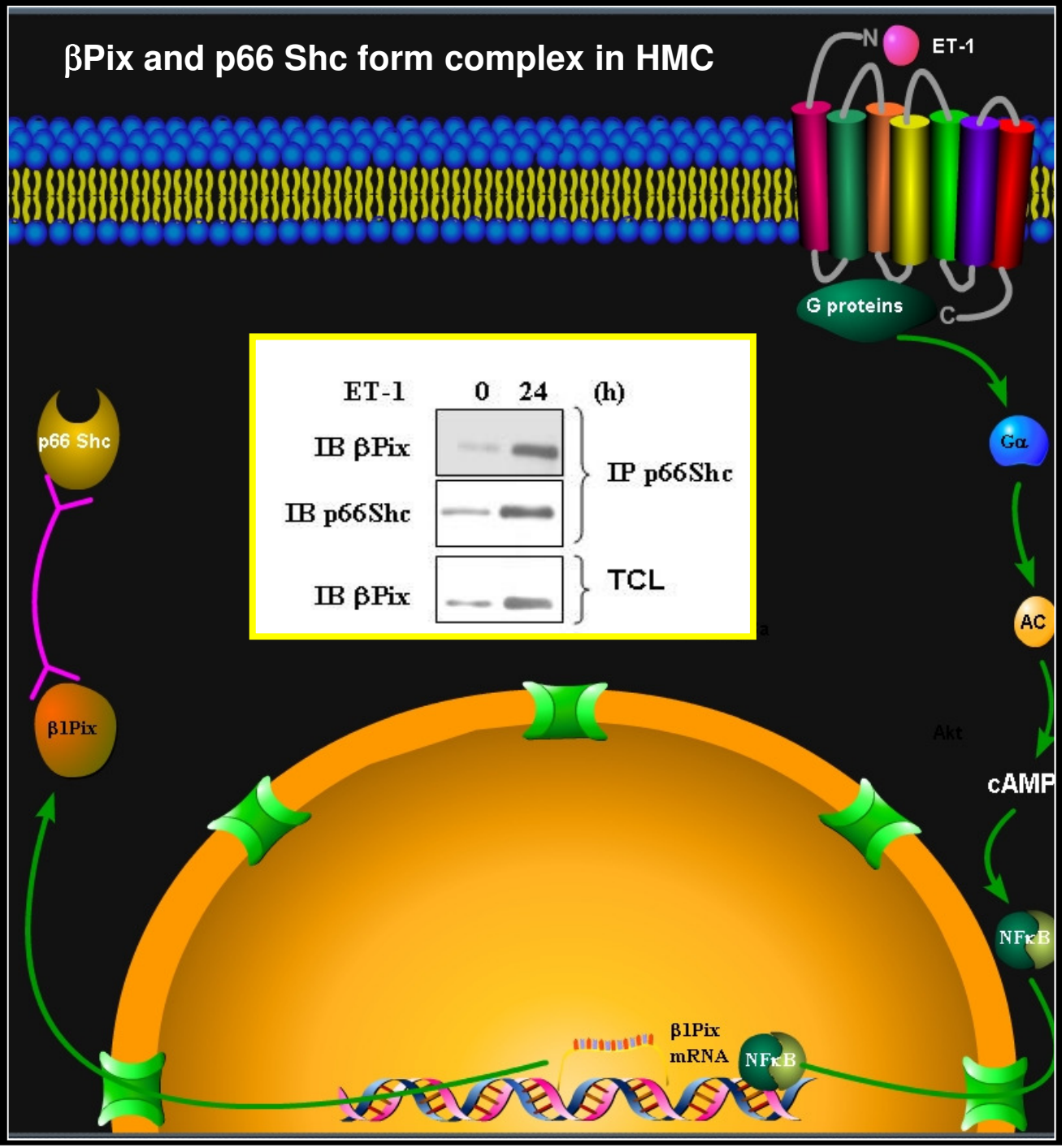
Control PBS | **Anti-Thy-1** | **Anti-Thy-1**



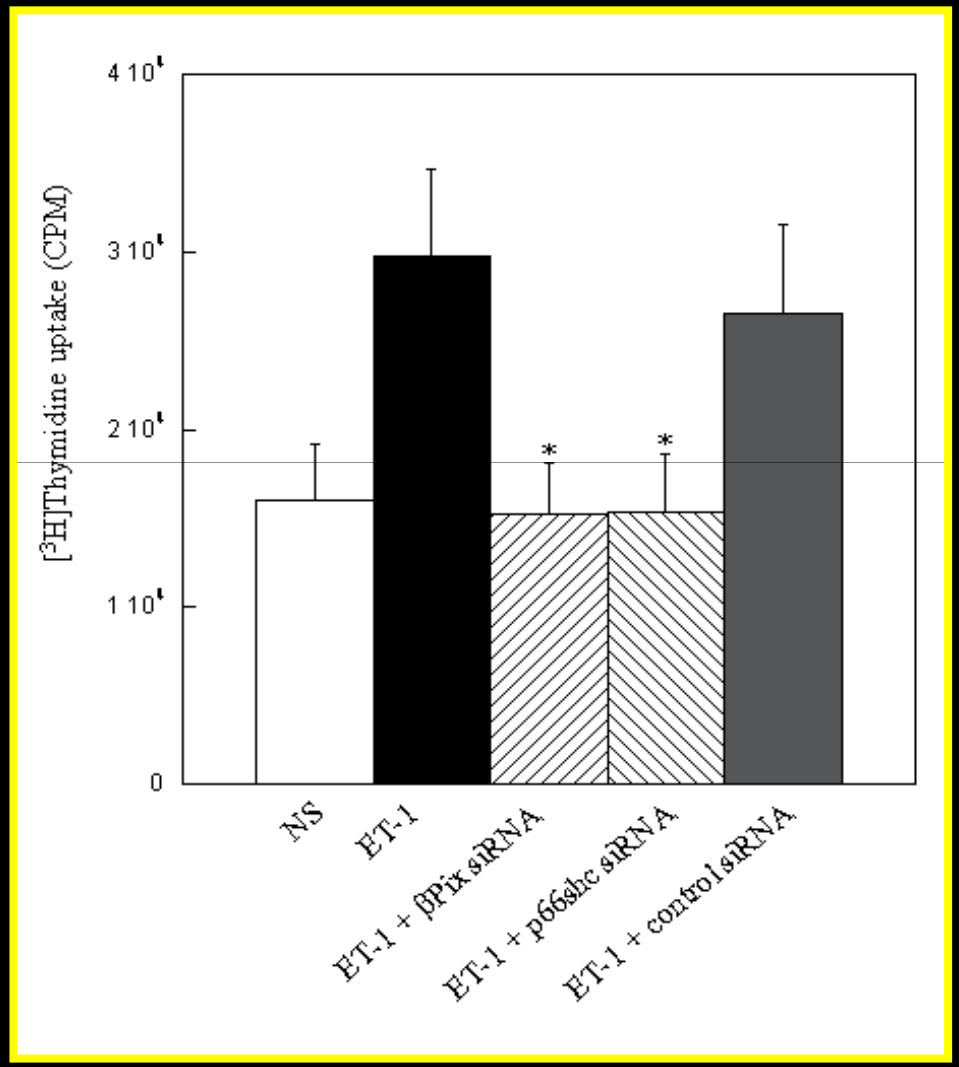
IHC p66Shc | **IHC p66Shc** | **IHC mouse serum**

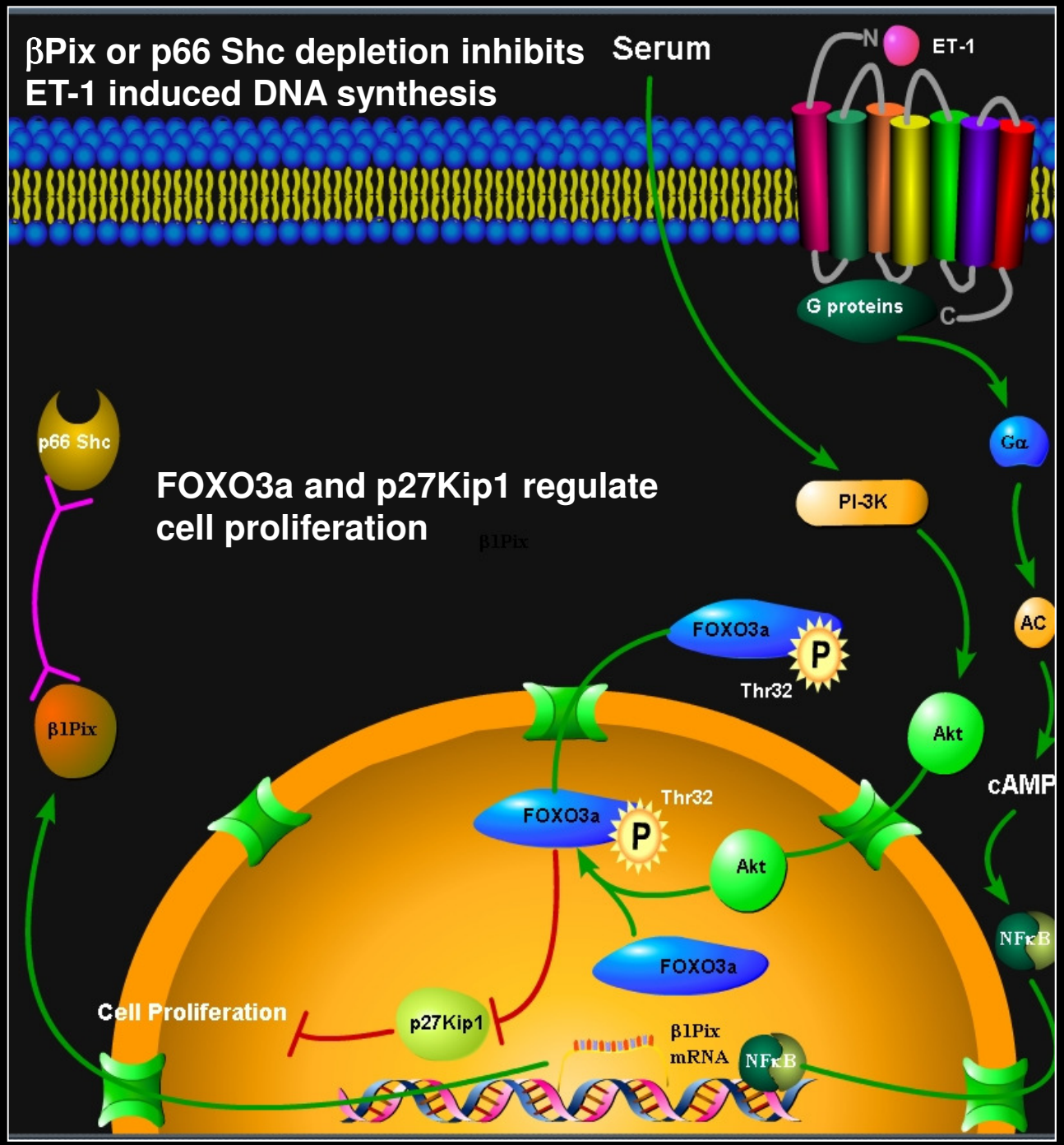
Probetex Anti-Thymocyte (Thy-1) Mesangial Proliferative Glomerulonephritis


ET-1 induces an Increase in βPix and p66 Shc expression
N
ET-1
G proteins
C
ET-1 0 12 24 (h)
βPix
p66Shc
p52Shc
p46Shc
Actin
Gα
AC
cAMP
Akt
β1Pix
NFκB
β1Pix mRNA
NFκB

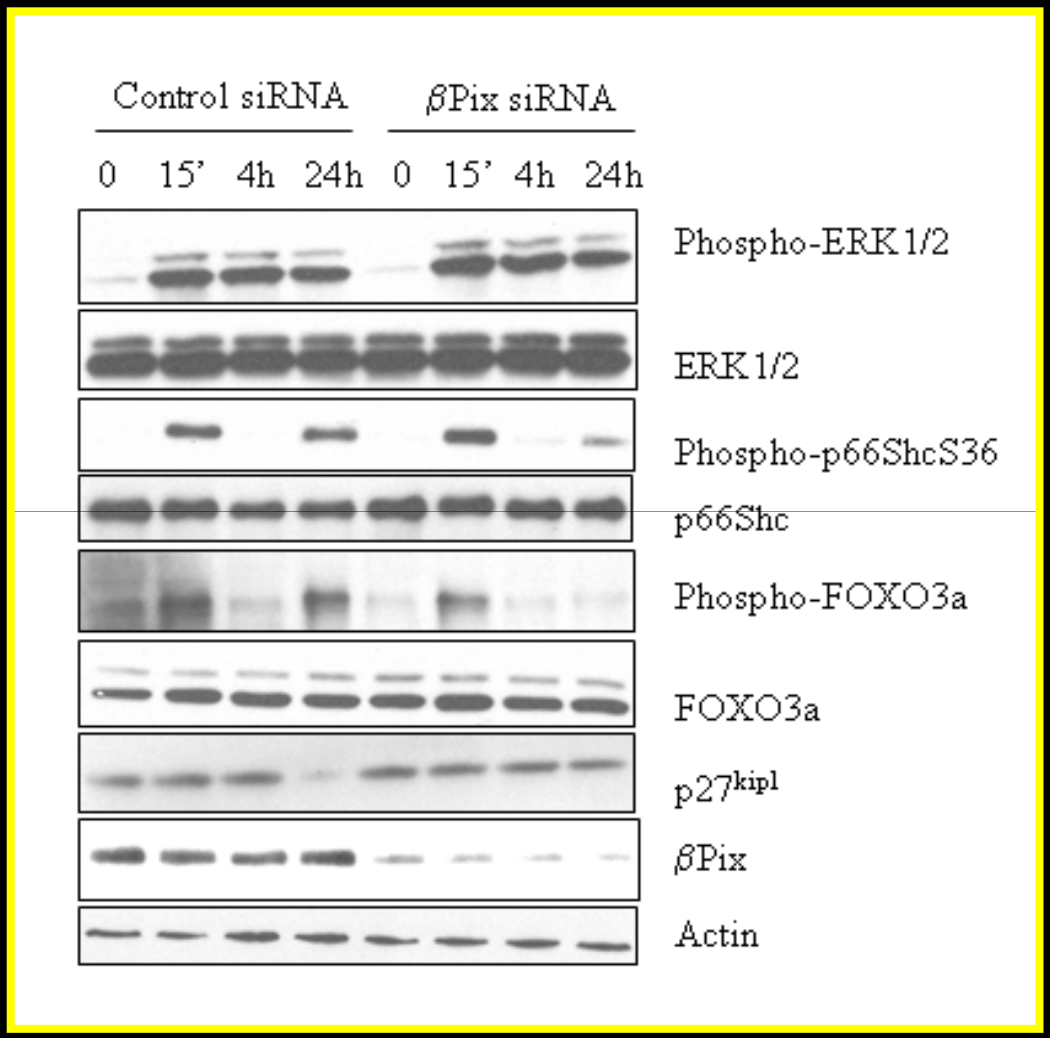

βPix and p66 Shc form complex in HMC
N
ET-1
G proteins
C
ET-1 0 24 (h)
IB βPix
IB p66Shc
IP p66Shc
IB βPix
TCL
p66 Shc
β1Pix
Gα
AC
Akt
cAMP
NFκB
β1Pix
mRNA
NFκB

βPix or p66 Shc depletion inhibits ET-1 induced DNA synthesis

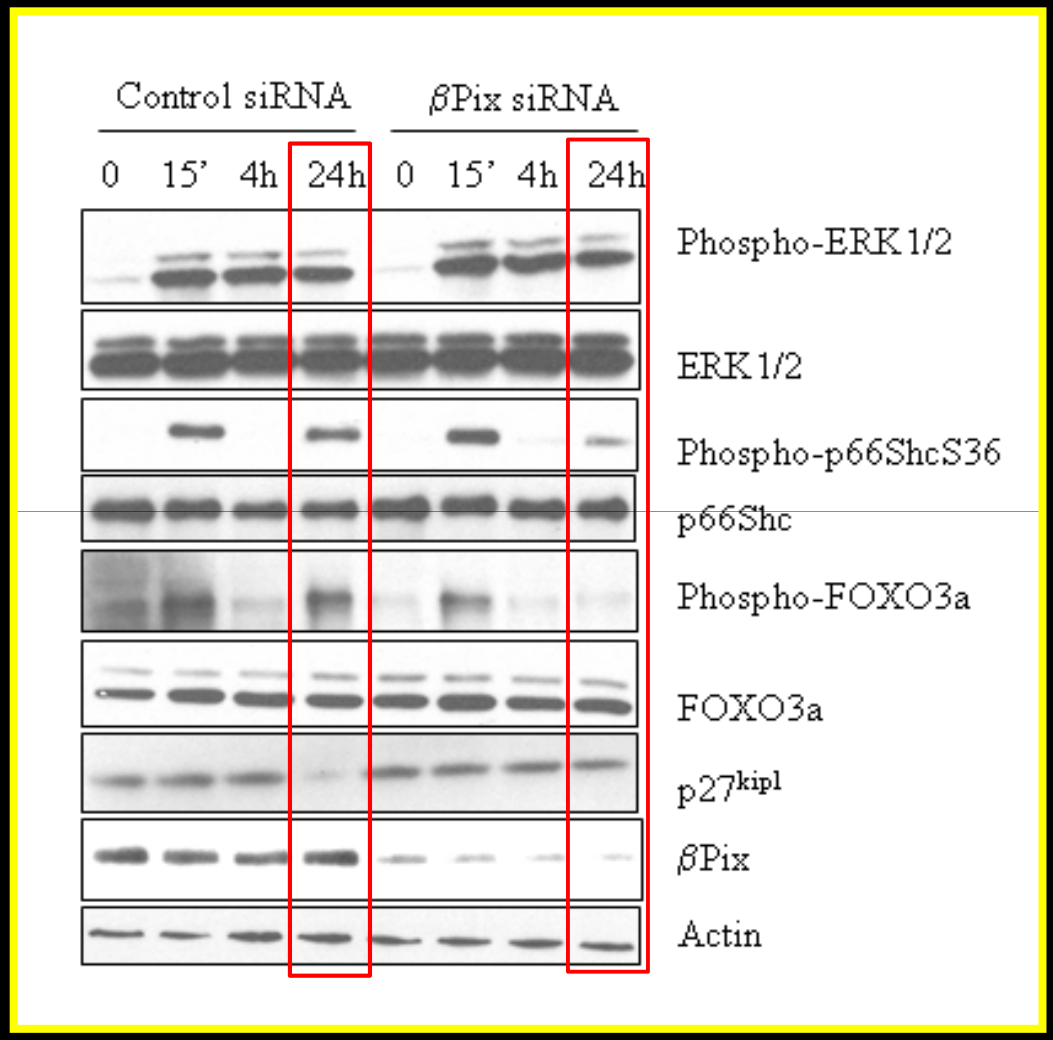


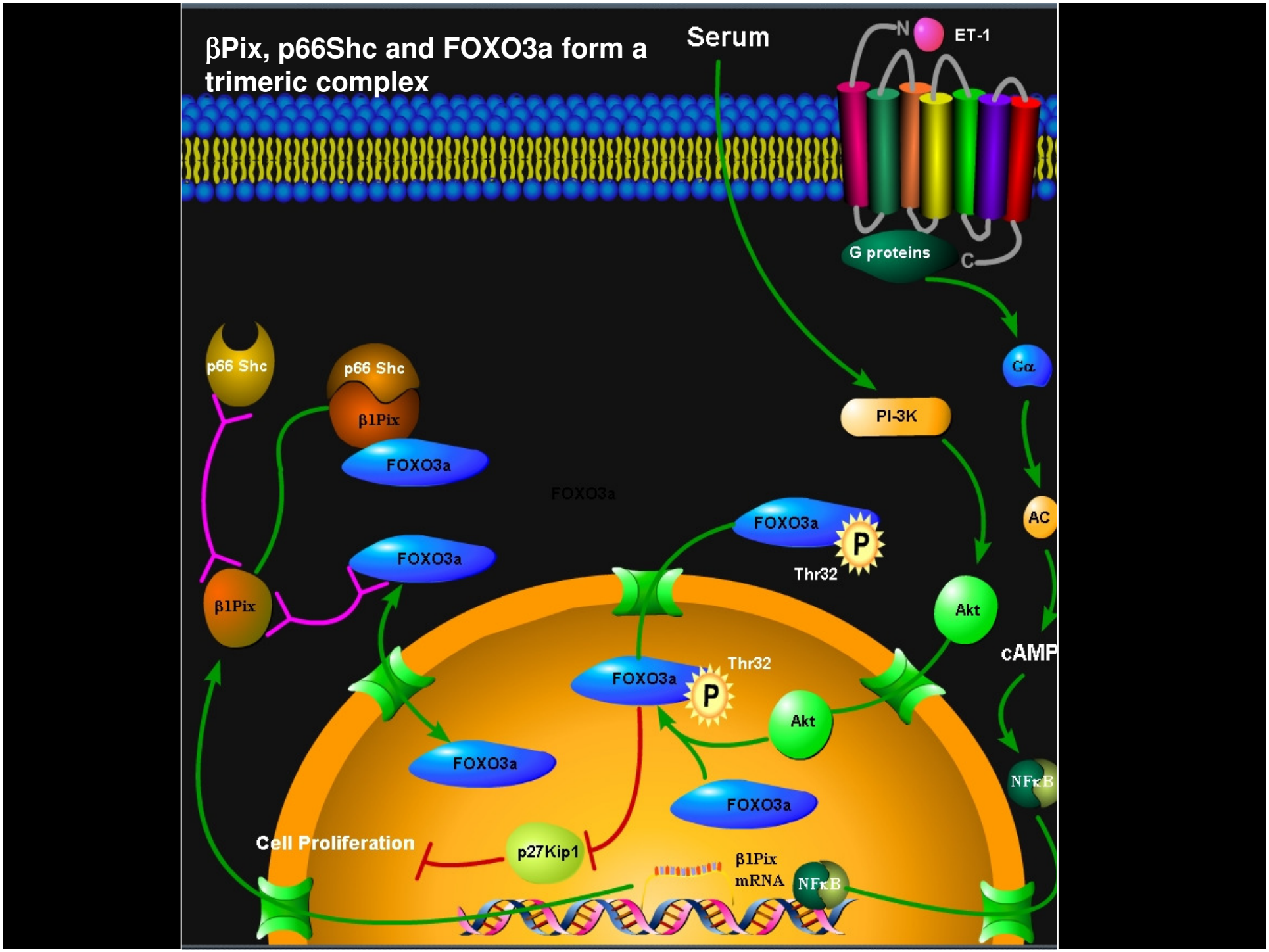

βPix or p66 Shc depletion inhibits ET-1 induced DNA synthesis
Serum
N
ET-1
G proteins
C
Gα
PI-3K
AC
cAMP
p66 Shc
β1Pix
FOXO3a and p27Kip1 regulate cell proliferation
β1Pix
FOXO3a
P
Thr32
Akt
FOXO3a
Thr32
P
Akt
NFκB
FOXO3a
Cell Proliferation
p27Kip1
β1Pix mRNA
NFκB

Effect of βPix depletion on ET-1 signaling

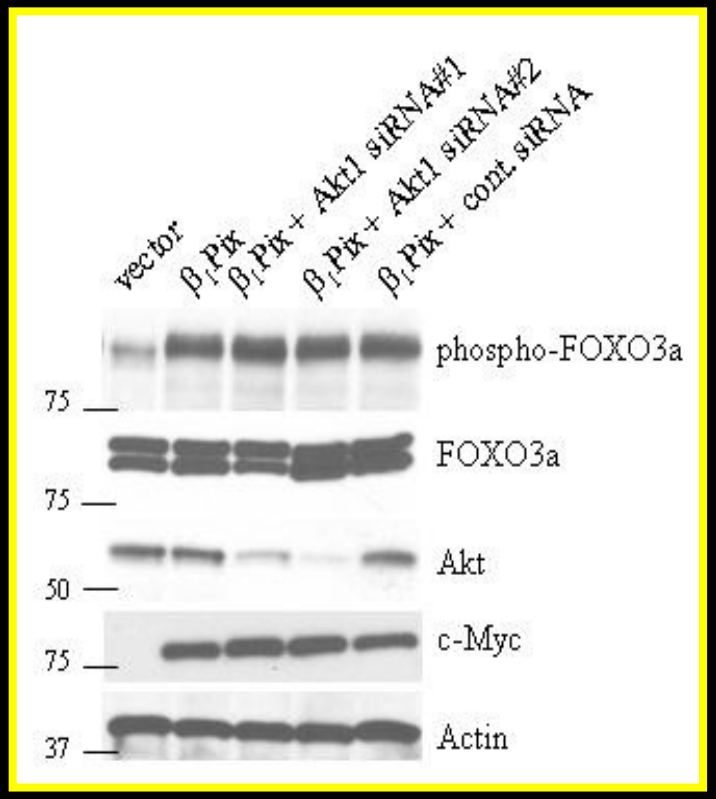


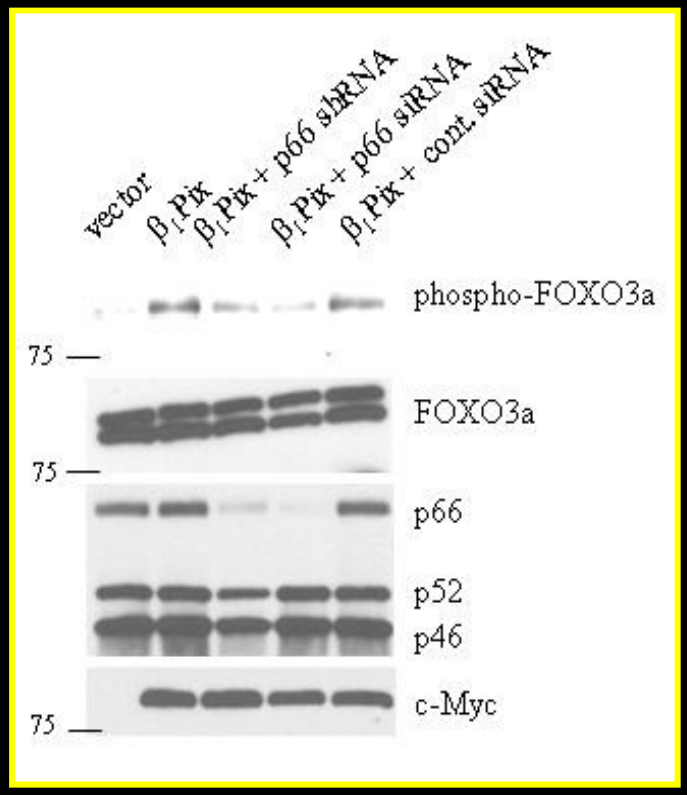
Effect of βPix depletion on ET-1 signaling

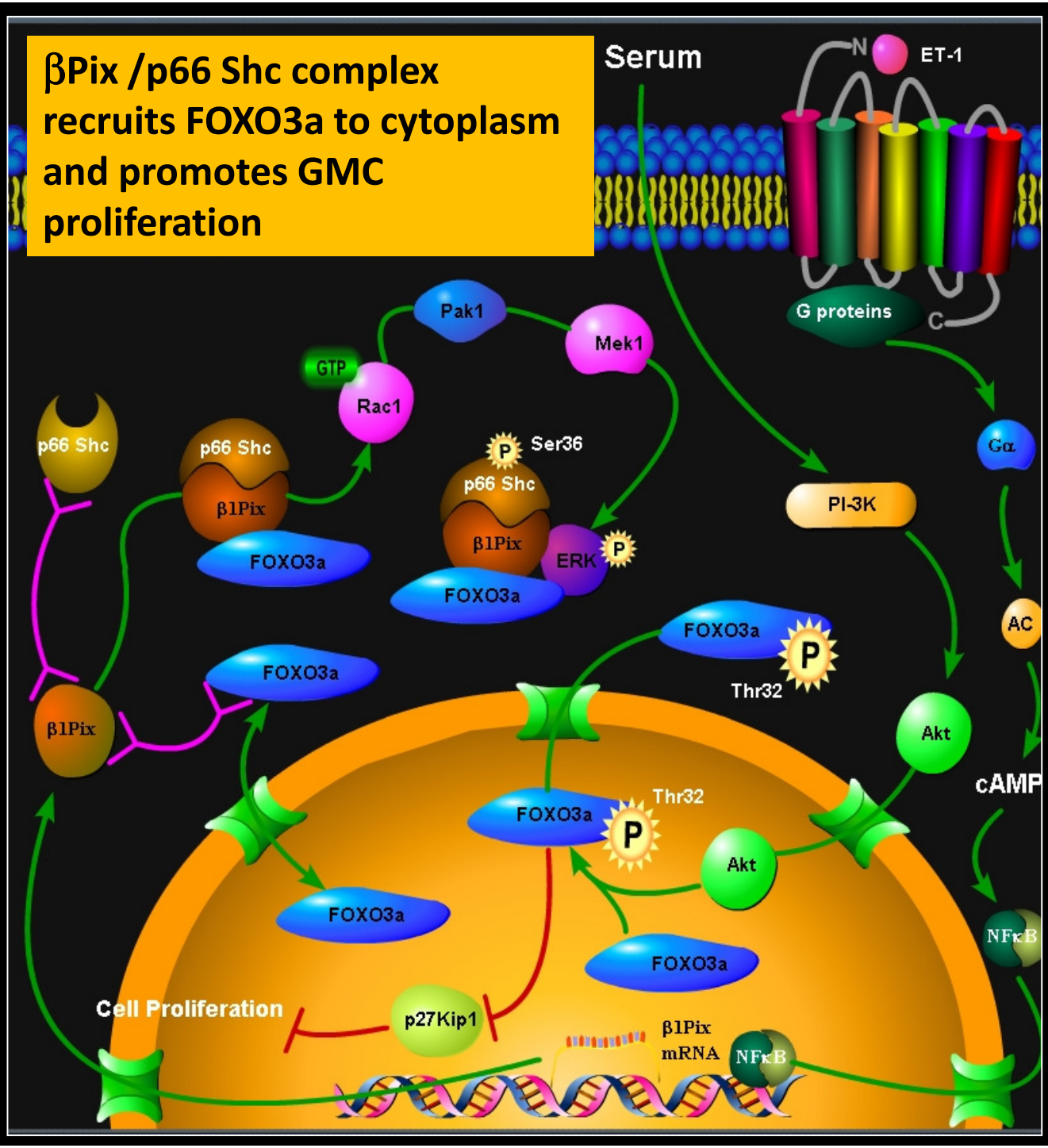



βPix, p66Shc and FOXO3a form a trimeric complex
Serum
N
ET-1
G proteins
C
Gα
PI-3K
AC
cAMP
p66 Shc
p66 Shc
β1Pix
FOXO3a
FOXO3a
FOXO3a
β1Pix
FOXO3a
P
Thr32
Akt
FOXO3a
Thr32
P
Akt
FOXO3a
FOXO3a
NFκB
Cell Proliferation
p27Kip1
β1Pix
mRNA
NFκB

β_1Pix-induced FOXO3a phosphorylation requires p66Shc but not Akt



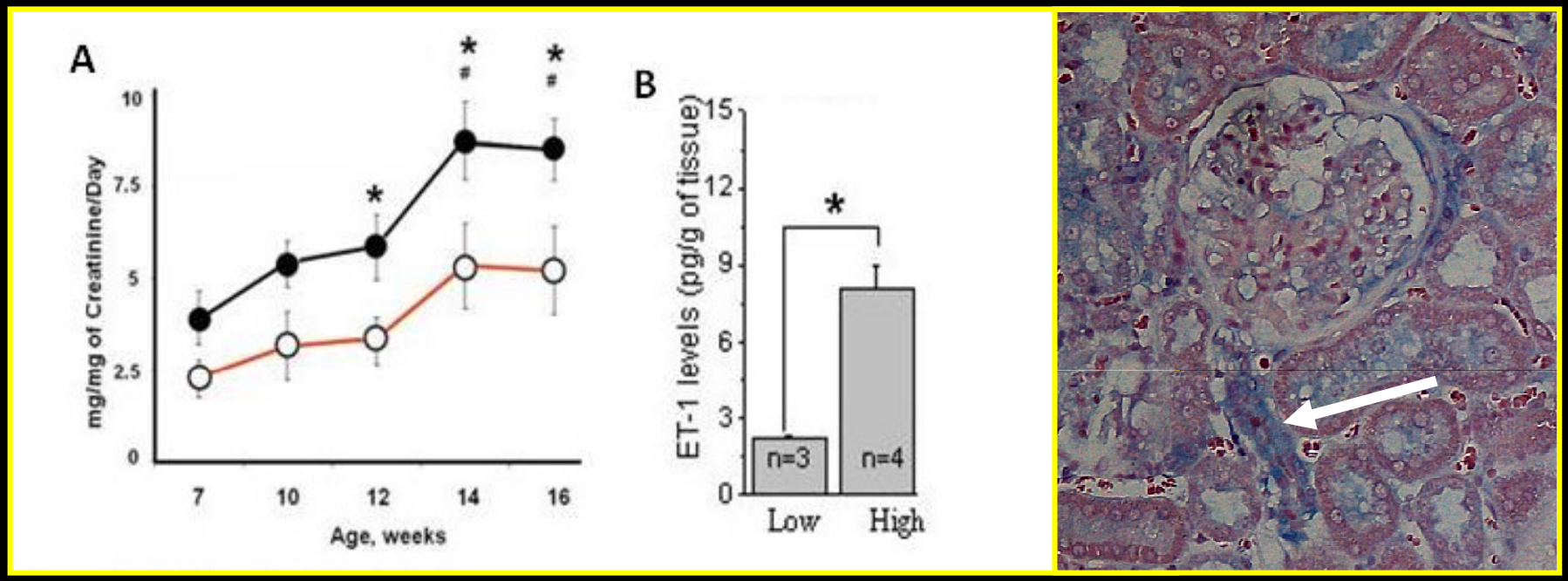


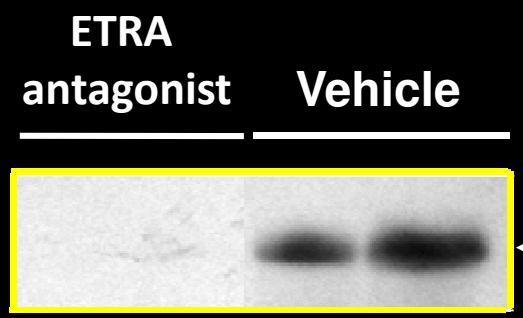

βPix /p66 Shc complex recruits FOXO3a to cytoplasm and promotes GMC proliferation
Serum
N
ET-1
G proteins
C
Pak1
Mek1
GTP
Rac1
p66 Shc
β1Pix
FOXO3a
P
Ser36
ERK
Gα
PI-3K
AC
cAMP
Akt
Thr32
NFκB
Cell Proliferation
p27Kip1
β1Pix mRNA

Conclusions I

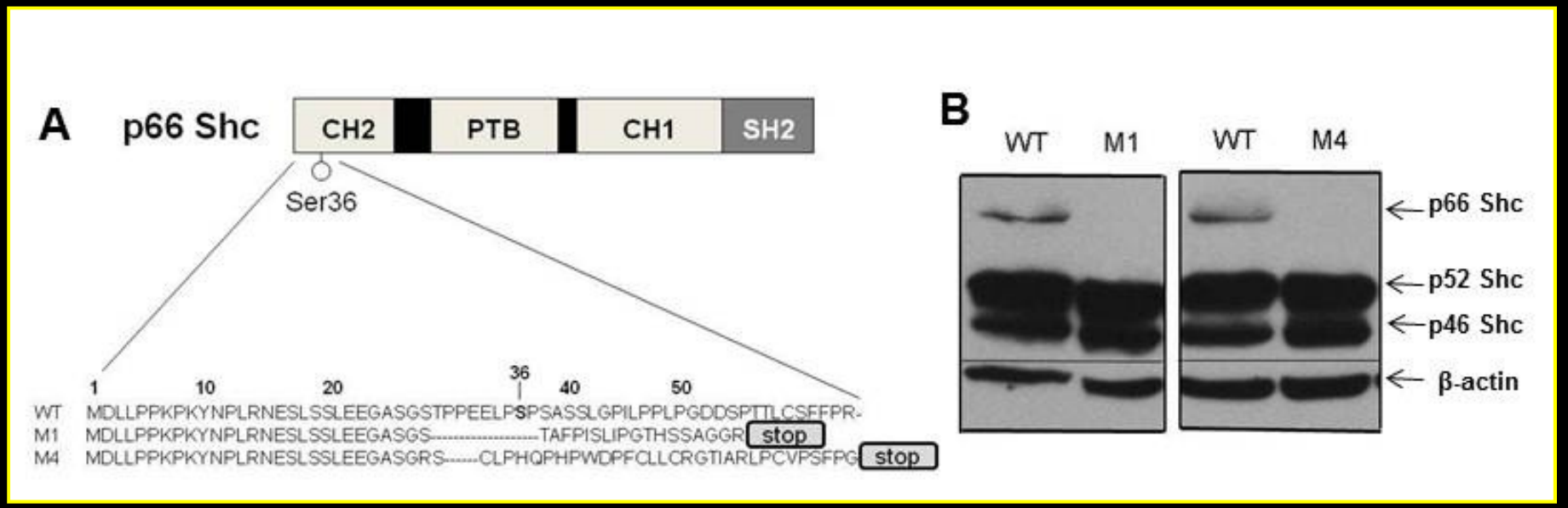
- β1Pix is a multi-purpose guanine nucleotide exchange factor which mediates ET-1 signaling as GEF for Cdc42 and also acts as a scaffolding protein
- ET-1 induced increased expression of adaptor protein p66 Shc which bound to β1Pix and promoted FOXO3a phosphorylation **and p27kip1 down-regulation** via previously unknown Akt-independent signaling pathway
- FOXO3a is recruited to p66 Shc-β1Pix complex with intricated effect upon cell proliferation and cell cycle
- siRNA downregulation of p66 Shc inhibited β1Pix - mediated effects in glomerular cells

ETRA receptor blockade attenuates progression of renal disease and p66 Shc expression in Dahl/SS rats on 1% salt diet



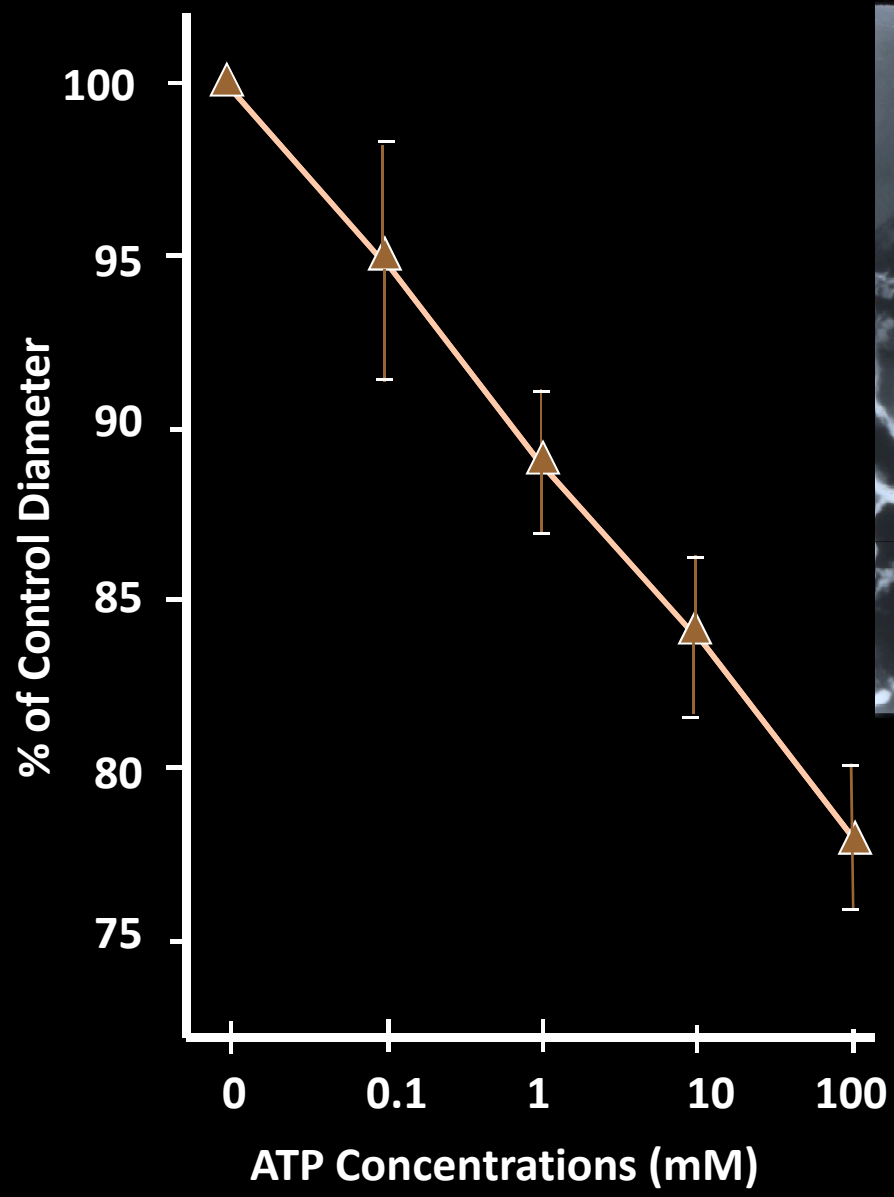


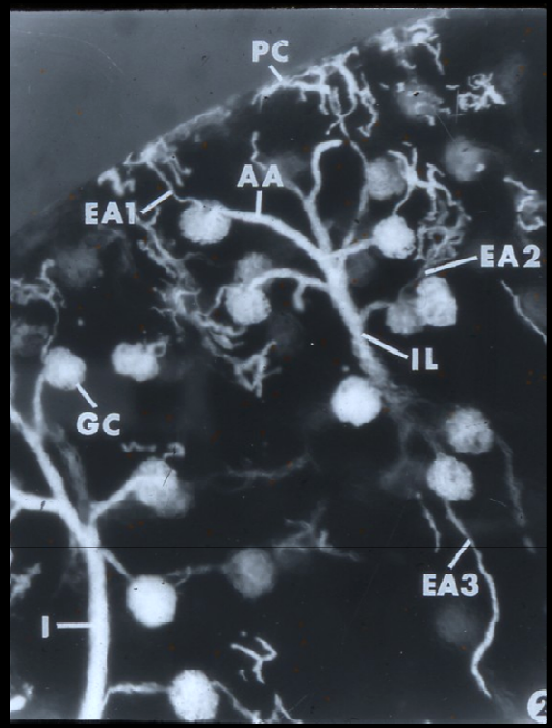
Generation of rat strains deficient in p66 Shc using engineered Zinc Finger Nucleases



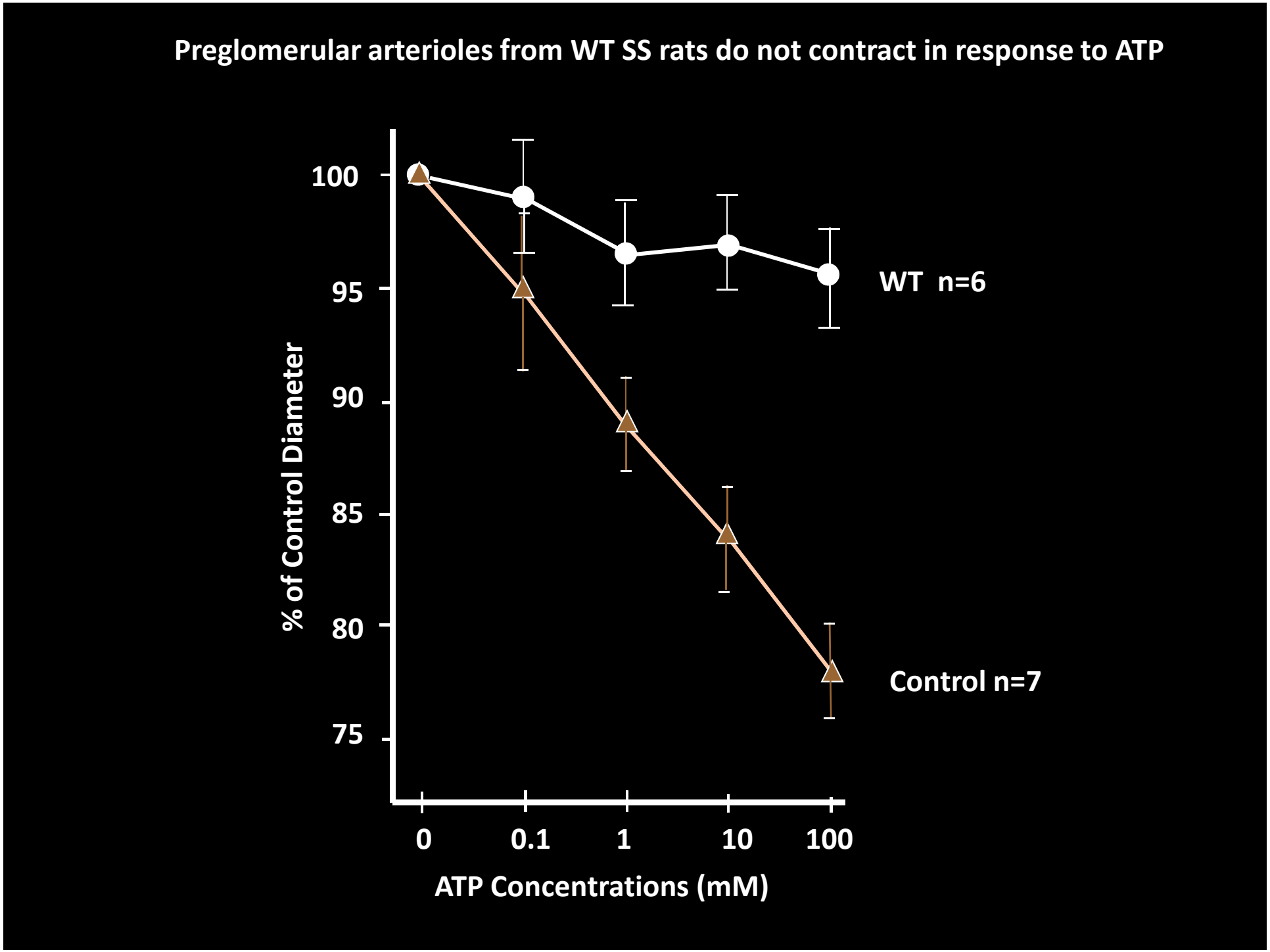
N-terminal sequence of p66 Shc protein in genetically modified rats and expression of Shc isoforms in renal tissue of M1 and M4 rats

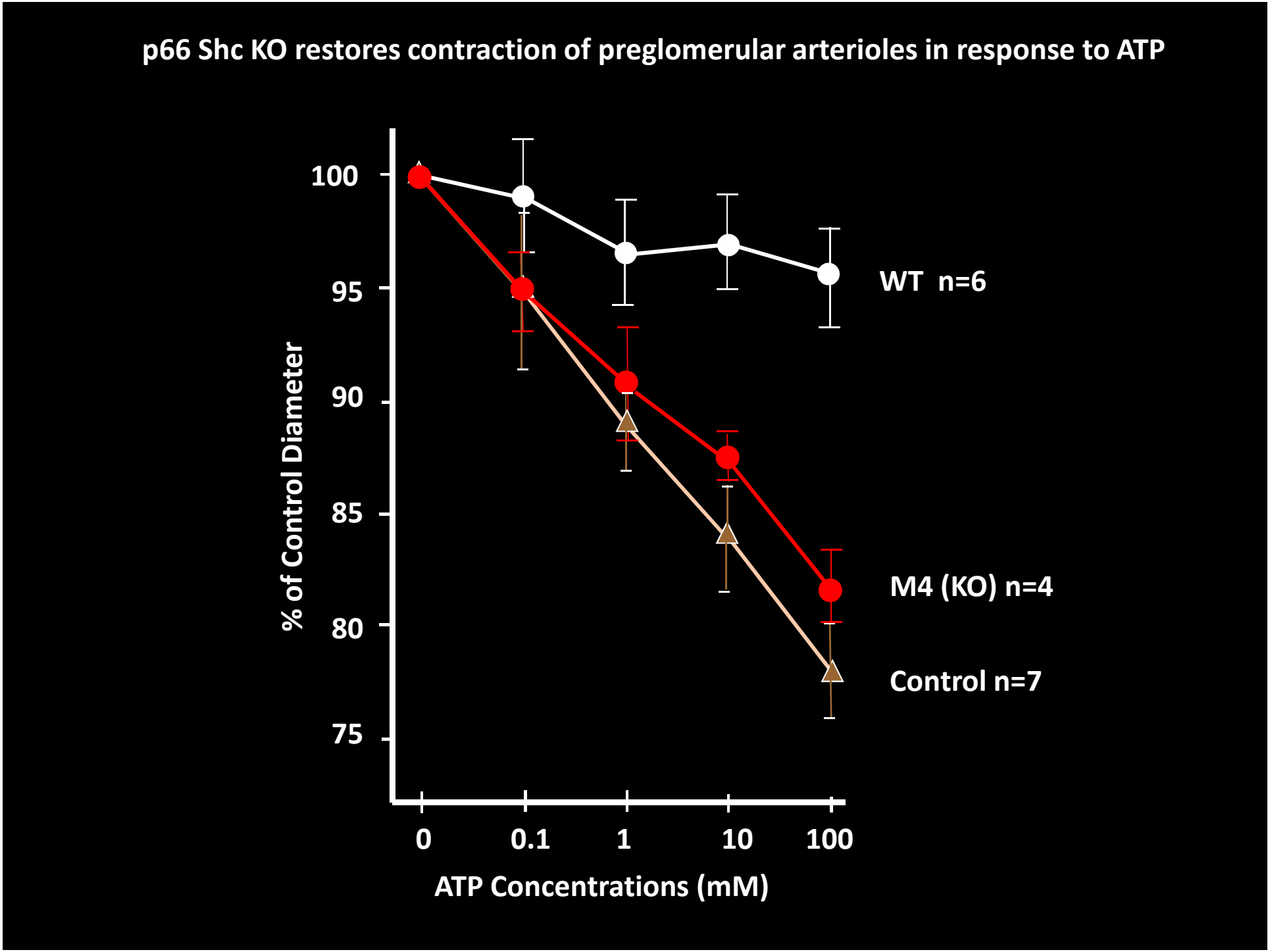
Preglomerular arterioles from Wistar Kyoto rats contract in response to ATP



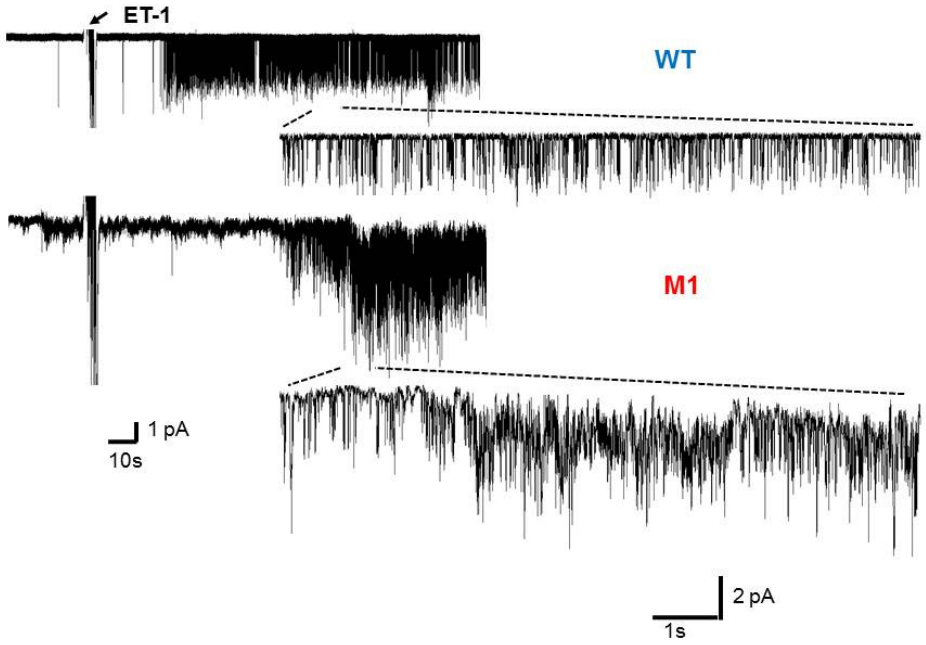


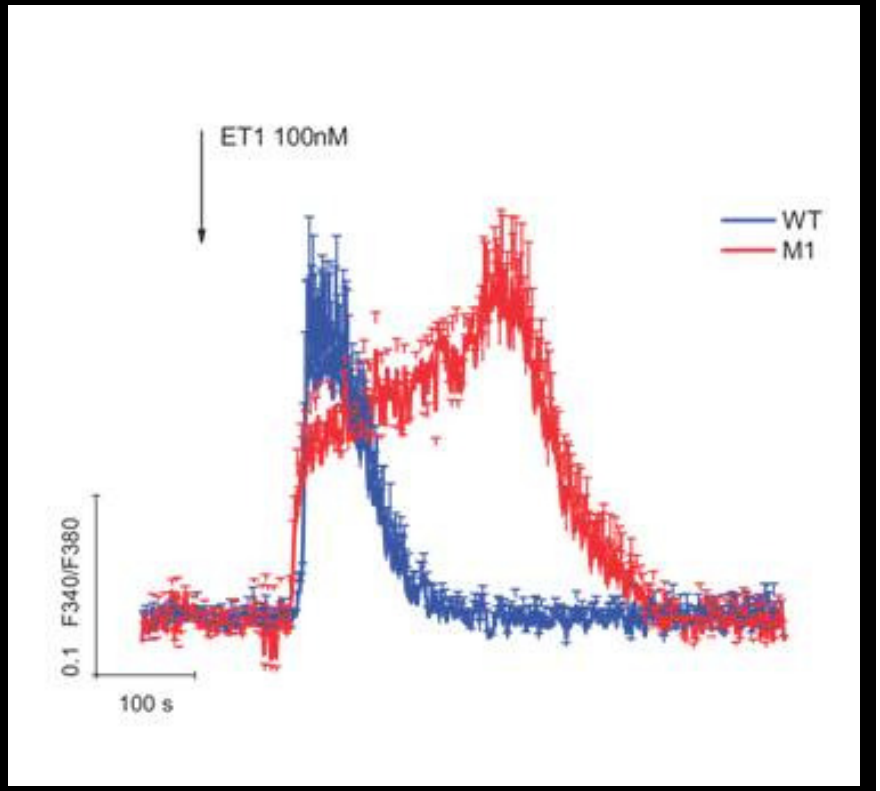
Control n=7


Preglomerular arterioles from WT SS rats do not contract in response to ATP
100
95
90
85
80
75
% of Control Diameter
WT n=6
Control n=7
0
0.1
1
10
100
ATP Concentrations (mM)


p66 Shc KO restores contraction of preglomerular arterioles in response to ATP
100
95
90
85
80
75
% of Control Diameter
0
0.1
1
10
100
ATP Concentrations (mM)
WT n=6
M4 (KO) n=4
Control n=7

p66 Shc actions on TRPC channels activity and Calcium intracellular concentration mediated by ET-1 application

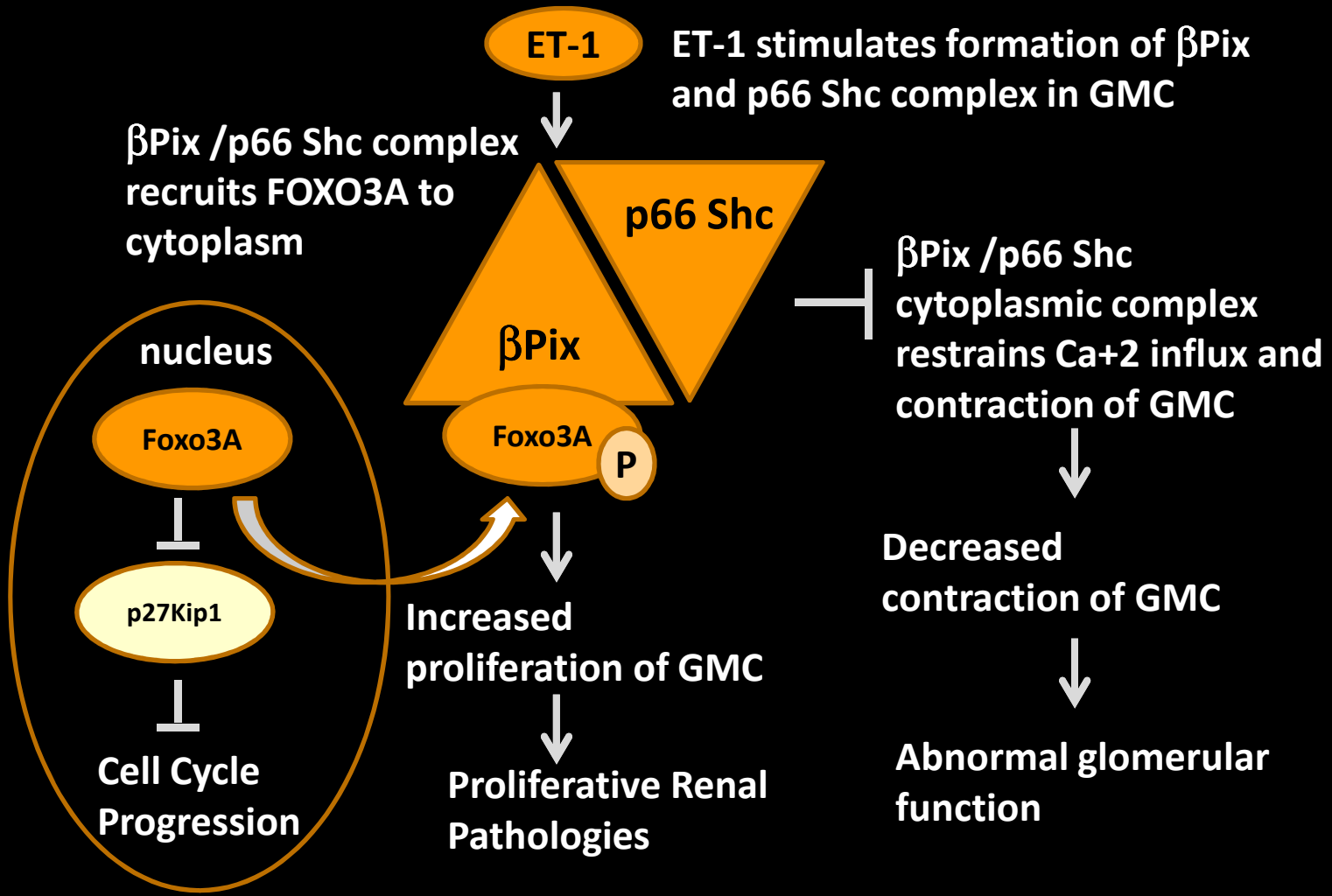




Conclusions II

- p66 Shc knockout enhances ET-1-mediated activation of TRPC-like channels
- p66 Shc knockout enhances ET-1-mediated increase of intracellular Calcium concentration
- p66 Shc knockout restores the autoregulation of afferent glomerular arterioles by increasing cellular contractility

Schematic representation of proposed mechanisms of p66 Shc-mediated ET-1 signaling in renal mesangium



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