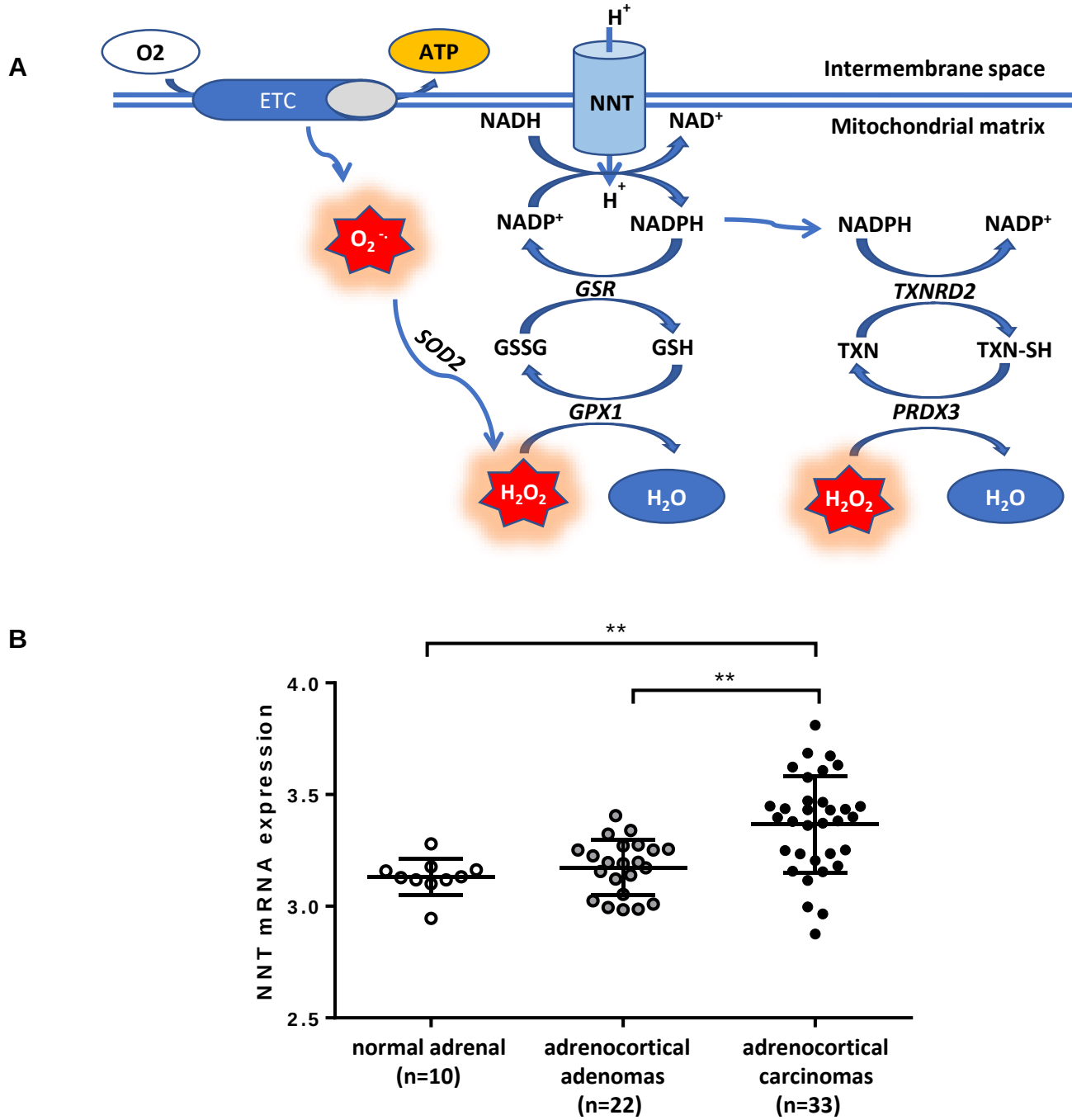
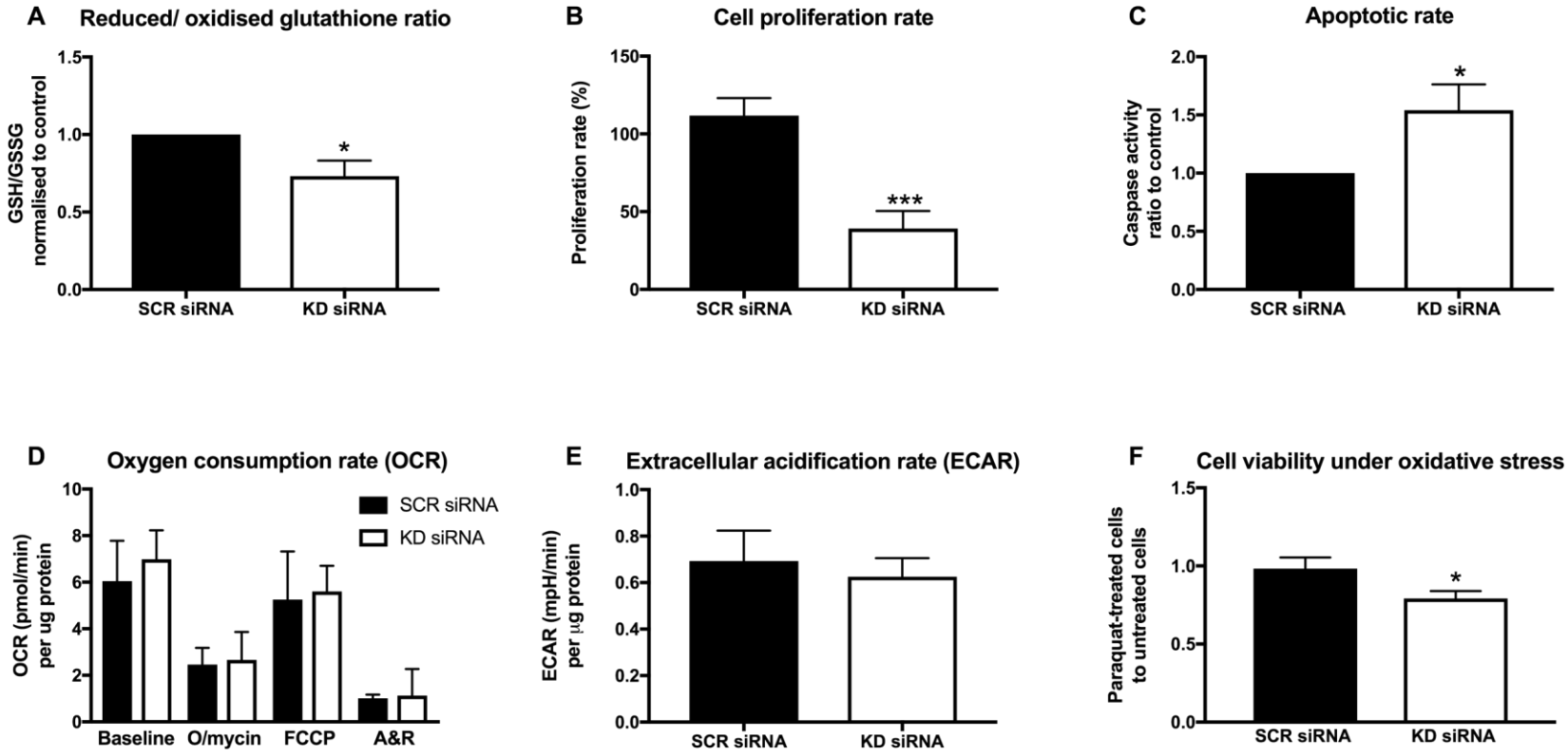
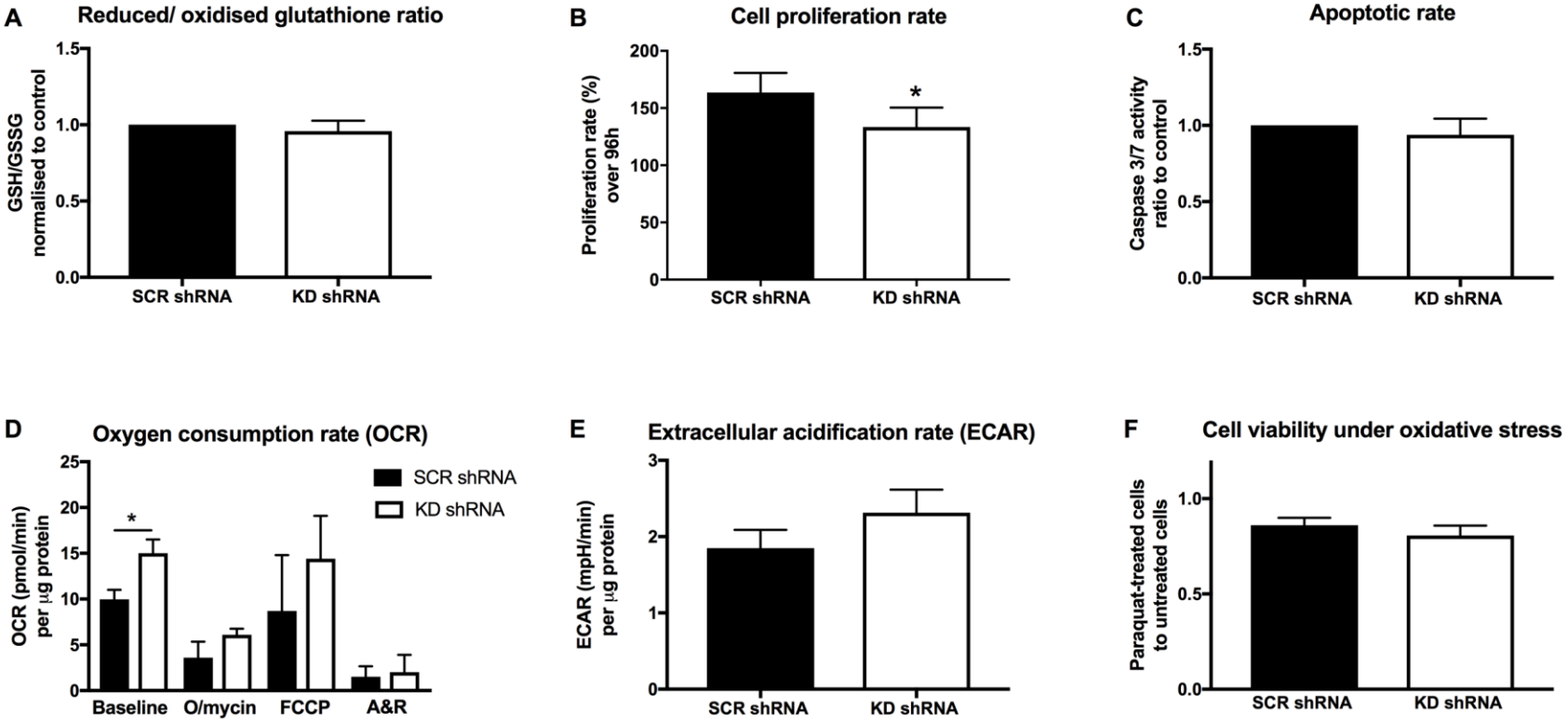
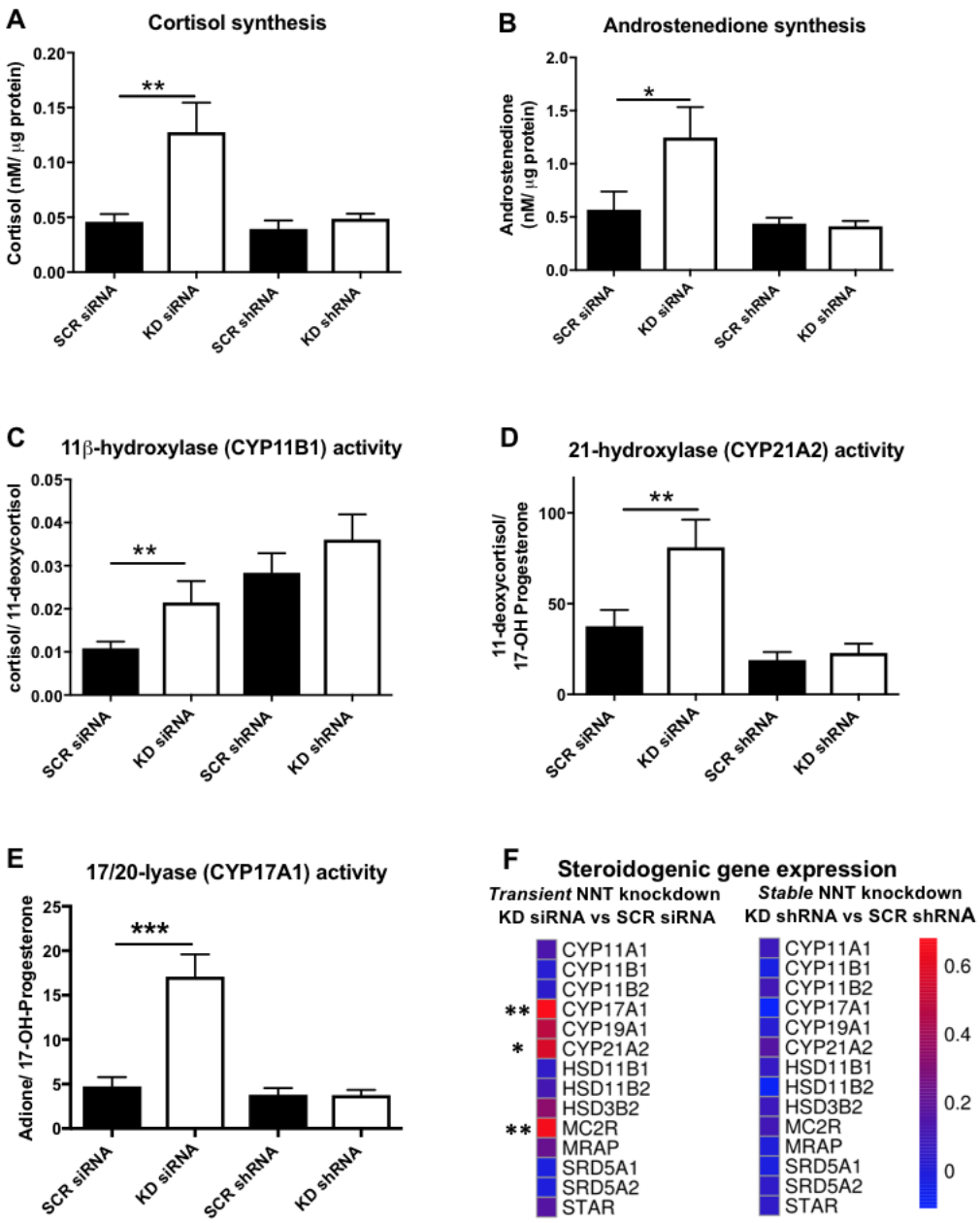
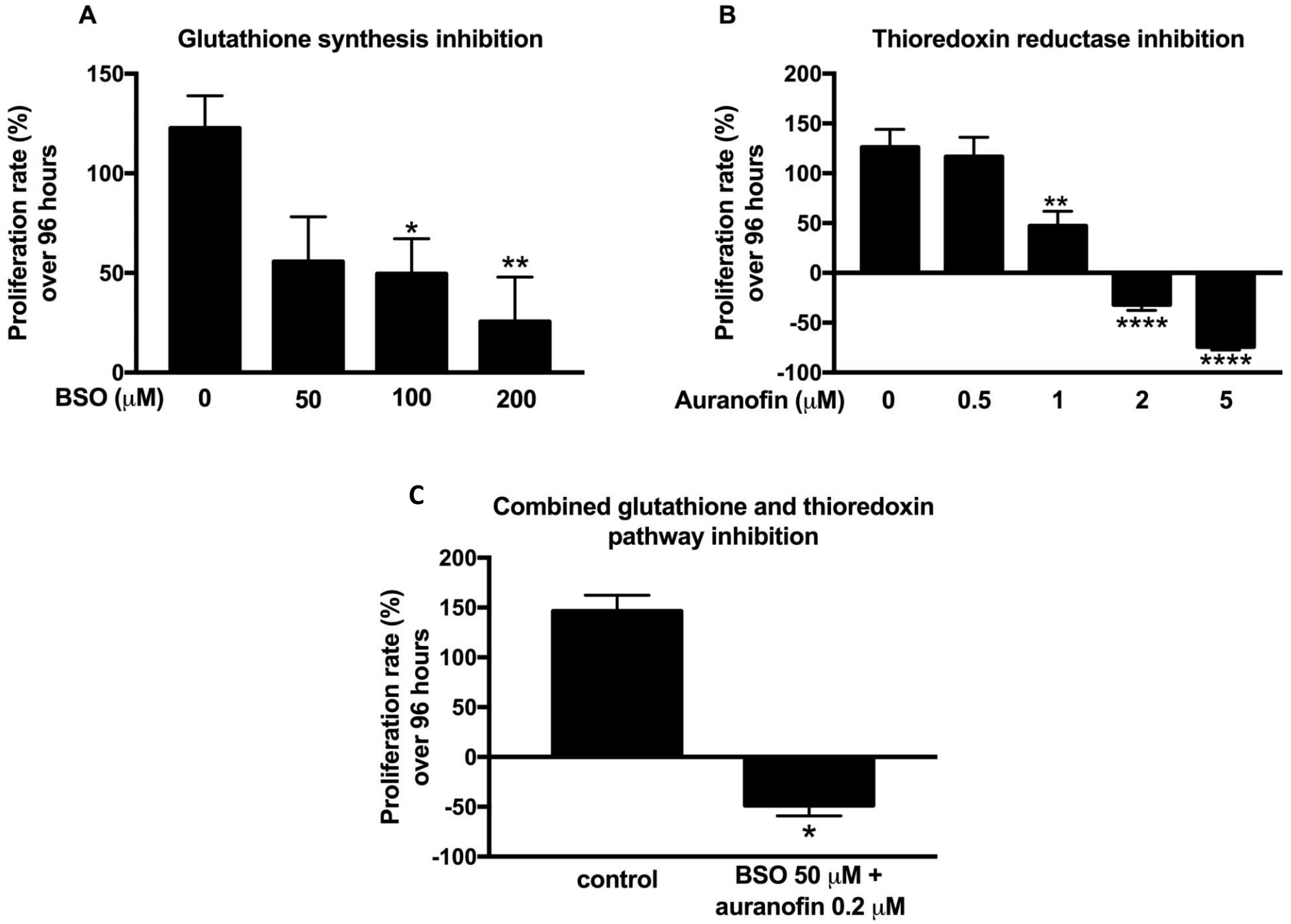
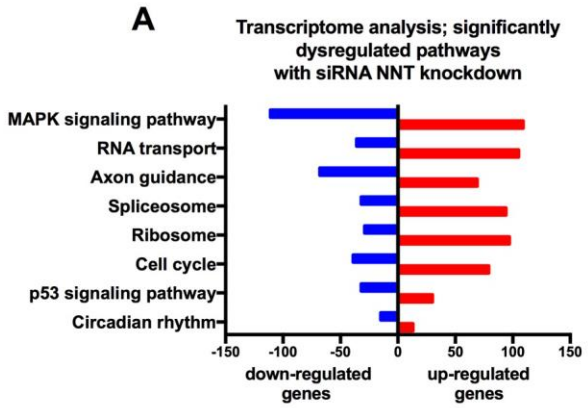

A
O2
ATP
ETC
H+
NNT
Intermembrane space
Mitochondrial matrix
NADH
NAD+
H+
NADP+
NADPH
O2·-
SOD2
GSR
GSSG
GSH
GPX1
H2O2
H2O
NADPH
NADP+
TXNRD2
TXN
TXN-SH
PRDX3
H2O2
H2O
B
**
**
NNT mRNA expression
4.0
3.5
3.0
2.5
normal adrenal (n=10)
adrenocortical adenomas (n=22)
adrenocortical carcinomas (n=33)

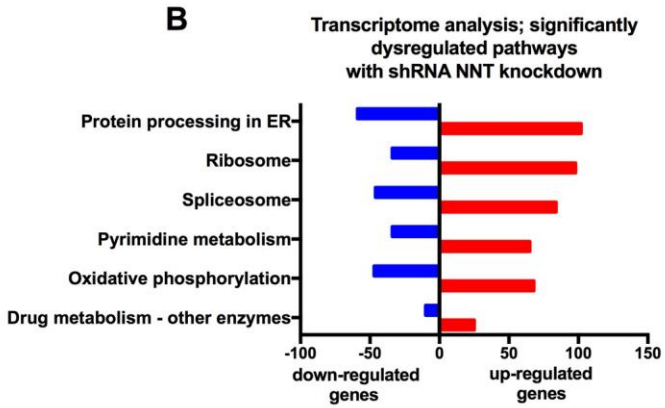

A
Reduced/ oxidised glutathione ratio
GSH/GSSG normalised to control
1.5
1.0
0.5
0.0
*
SCR siRNA
KD siRNA
B
Cell proliferation rate
Proliferation rate (%)
150
100
50
0
***
SCR siRNA
KD siRNA
C
Apoptotic rate
Caspase activity ratio to control
2.0
1.5
1.0
0.5
0.0
*
SCR siRNA
KD siRNA
D
Oxygen consumption rate (OCR)
OCR (pmol/min) per ug protein
10
8
6
4
2
0
SCR siRNA
KD siRNA
Baseline
O/mycin
FCCP
A&R
E
Extracellular acidification rate (ECAR)
ECAR (mpH/min) per µg protein
1.0
0.8
0.6
0.4
0.2
0.0
SCR siRNA
KD siRNA
F
Cell viability under oxidative stress
Paraquat-treated cells to untreated cells
1.5
1.0
0.5
0.0
*
SCR siRNA
KD siRNA

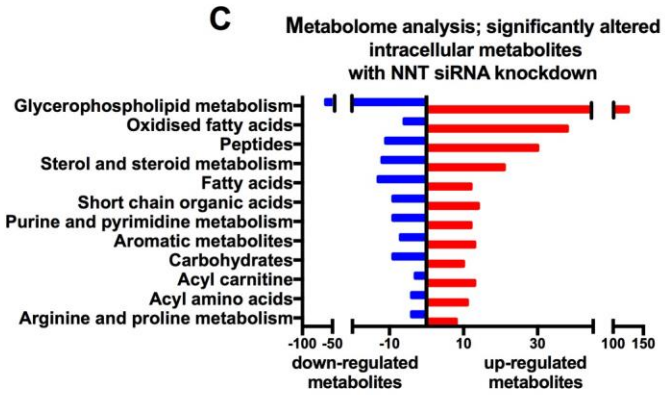

A
Reduced/ oxidised glutathione ratio
GSH/GSSG normalised to control
1.5
1.0
0.5
0.0
SCR shRNA
KD shRNA
B
Cell proliferation rate
Proliferation rate (%) over 96h
200
150
100
50
0
*
SCR shRNA
KD shRNA
C
Apoptotic rate
Caspase 3/7 activity ratio to control
1.5
1.0
0.5
0.0
SCR shRNA
KD shRNA
D
Oxygen consumption rate (OCR)
OCR (pmol/min) per µg protein
25
20
15
10
5
0
*
SCR shRNA
KD shRNA
Baseline
O/mycin
FCCP
A&R
E
Extracellular acidification rate (ECAR)
ECAR (mpH/min) per µg protein
3
2
1
0
SCR shRNA
KD shRNA
F
Cell viability under oxidative stress
Paraquat-treated cells to untreated cells
1.0
0.5
0.0
SCR shRNA
KD shRNA

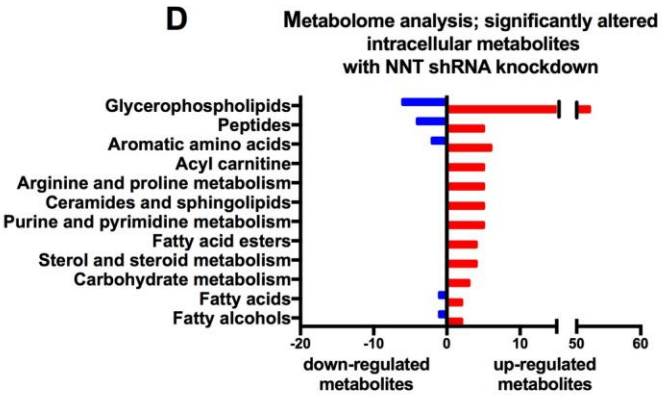

A
Cortisol synthesis
Cortisol (nM/ μg protein)
0.20
0.15
0.10
0.05
0.00
**
SCR siRNA
KD siRNA
SCR shRNA
KD shRNA
B
Androstenedione synthesis
Androstenedione (nM/ μg protein)
2.0
1.5
1.0
0.5
0.0
*
SCR siRNA
KD siRNA
SCR shRNA
KD shRNA
C
11β-hydroxylase (CYP11B1) activity
cortisol/ 11-deoxycortisol
0.05
0.04
0.03
0.02
0.01
0.00
**
SCR siRNA
KD siRNA
SCR shRNA
KD shRNA
D
21-hydroxylase (CYP21A2) activity
11-deoxycortisol/ 17-OH Progesterone
100
50
0
**
SCR siRNA
KD siRNA
SCR shRNA
KD shRNA
E
17/20-lyase (CYP17A1) activity
Adione/ 17-OH-Progesterone
25
20
15
10
5
0
***
SCR siRNA
KD siRNA
SCR shRNA
KD shRNA
F
Steroidogenic gene expression
Transient NNT knockdown
KD siRNA vs SCR siRNA
Stable NNT knockdown
KD shRNA vs SCR shRNA
CYP11A1
CYP11B1
CYP11B2
** CYP17A1
CYP19A1
* CYP21A2
HSD11B1
HSD11B2
HSD3B2
** MC2R
MRAP
SRD5A1
SRD5A2
STAR
0.6
0.4
0.2
0

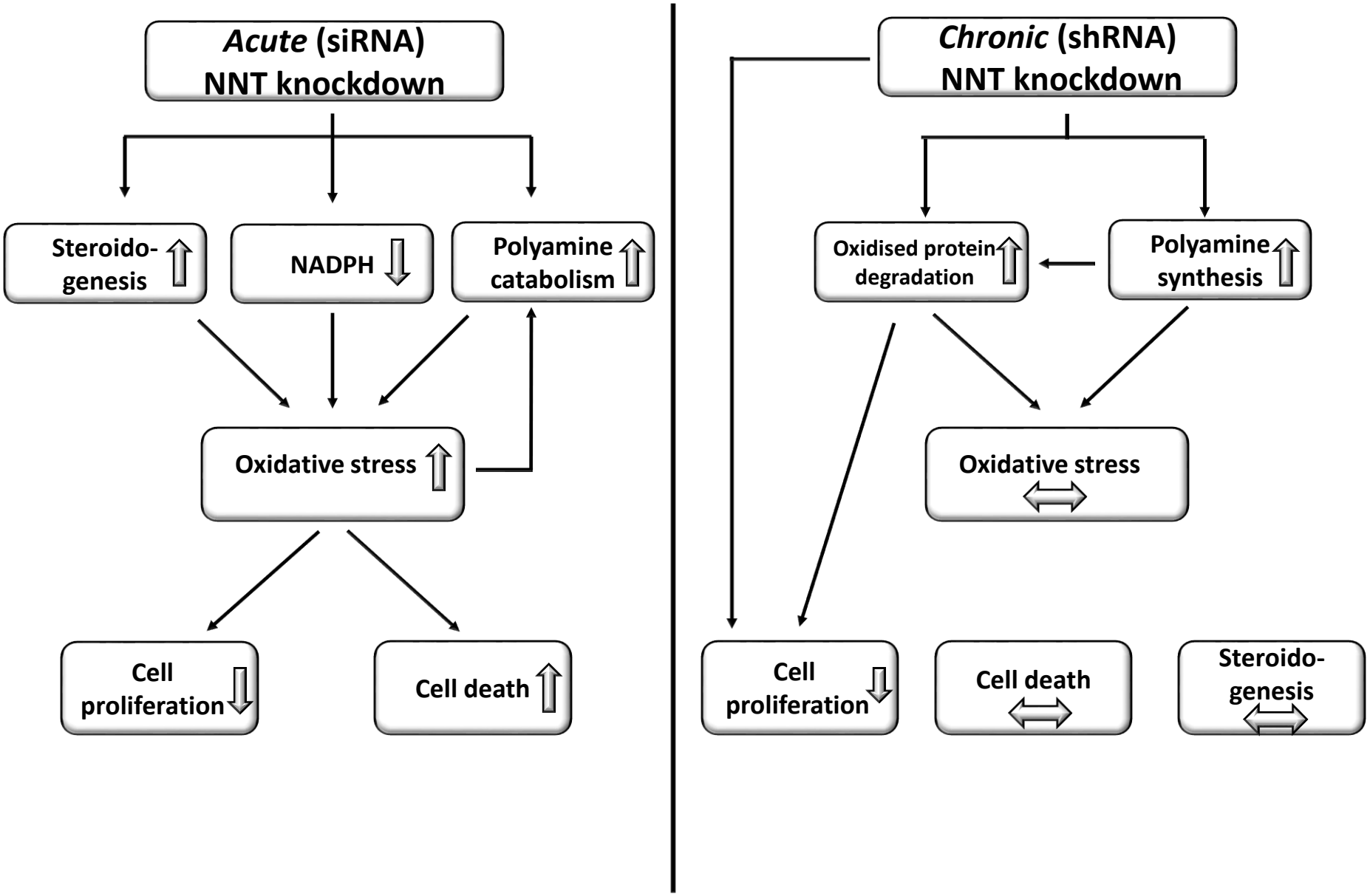

A
Glutathione synthesis inhibition
Proliferation rate (%) over 96 hours
150
100
50
0
*
**
BSO (μM) 0 50 100 200
B
Thioredoxin reductase inhibition
Proliferation rate (%) over 96 hours
200
150
100
50
0
-50
-100
**
****
****
Auranofin (μM) 0 0.5 1 2 5
C
Combined glutathione and thioredoxin pathway inhibition
Proliferation rate (%) over 96 hours
200
150
100
50
0
-50
-100
*
control
BSO 50 μM + auranofin 0.2 μM


A
Transcriptome analysis; significantly dysregulated pathways with siRNA NNT knockdown
MAPK signaling pathway
RNA transport
Axon guidance
Spliceosome
Ribosome
Cell cycle
p53 signaling pathway
Circadian rhythm
-150 -100 -50 0 50 100 150
down-regulated genes
up-regulated genes


B
Transcriptome analysis; significantly dysregulated pathways with shRNA NNT knockdown
Protein processing in ER
Ribosome
Spliceosome
Pyrimidine metabolism
Oxidative phosphorylation
Drug metabolism - other enzymes
-100 -50 0 50 100 150
down-regulated genes
up-regulated genes


C
Metabolome analysis; significantly altered intracellular metabolites with NNT siRNA knockdown
Glycerophospholipid metabolism
Oxidised fatty acids
Peptides
Sterol and steroid metabolism
Fatty acids
Short chain organic acids
Purine and pyrimidine metabolism
Aromatic metabolites
Carbohydrates
Acyl carnitine
Acyl amino acids
Arginine and proline metabolism
-100 -50 -10 10 30 100 150
down-regulated metabolites
up-regulated metabolites


D
Metabolome analysis; significantly altered intracellular metabolites with NNT shRNA knockdown
Glycerophospholipids
Peptides
Aromatic amino acids
Acyl carnitine
Arginine and proline metabolism
Ceramides and sphingolipids
Purine and pyrimidine metabolism
Fatty acid esters
Sterol and steroid metabolism
Carbohydrate metabolism
Fatty acids
Fatty alcohols
-20 -10 0 10 50 60
down-regulated metabolites
up-regulated metabolites


Acute (siRNA)
NNT knockdown
Steroido-genesis
NADPH
Polyamine catabolism
Oxidative stress
Cell proliferation
Cell death
Chronic (shRNA)
NNT knockdown
Oxidised protein degradation
Polyamine synthesis
Oxidative stress
Cell proliferation
Cell death
Steroido-genesis