Figure 1

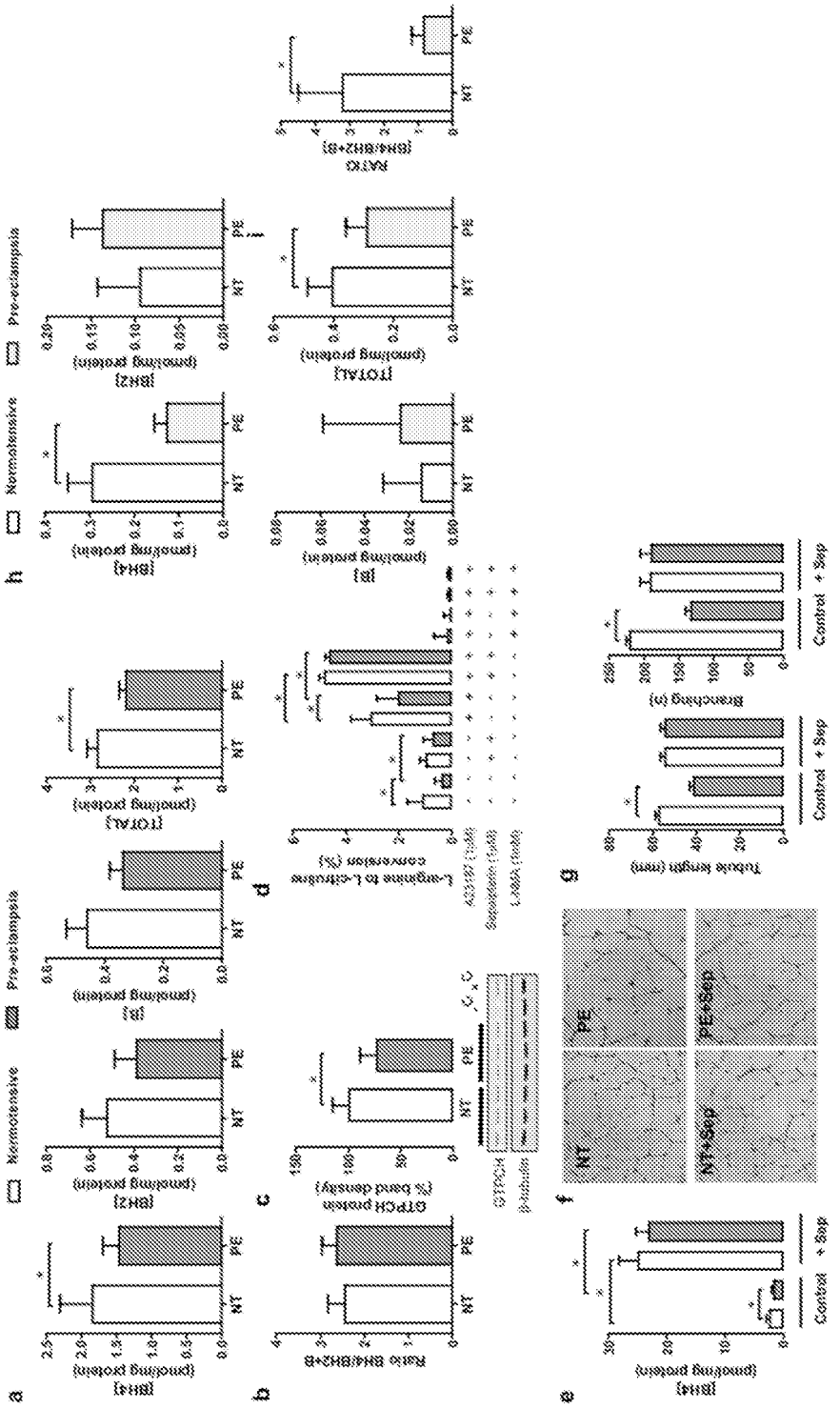


Figure 2

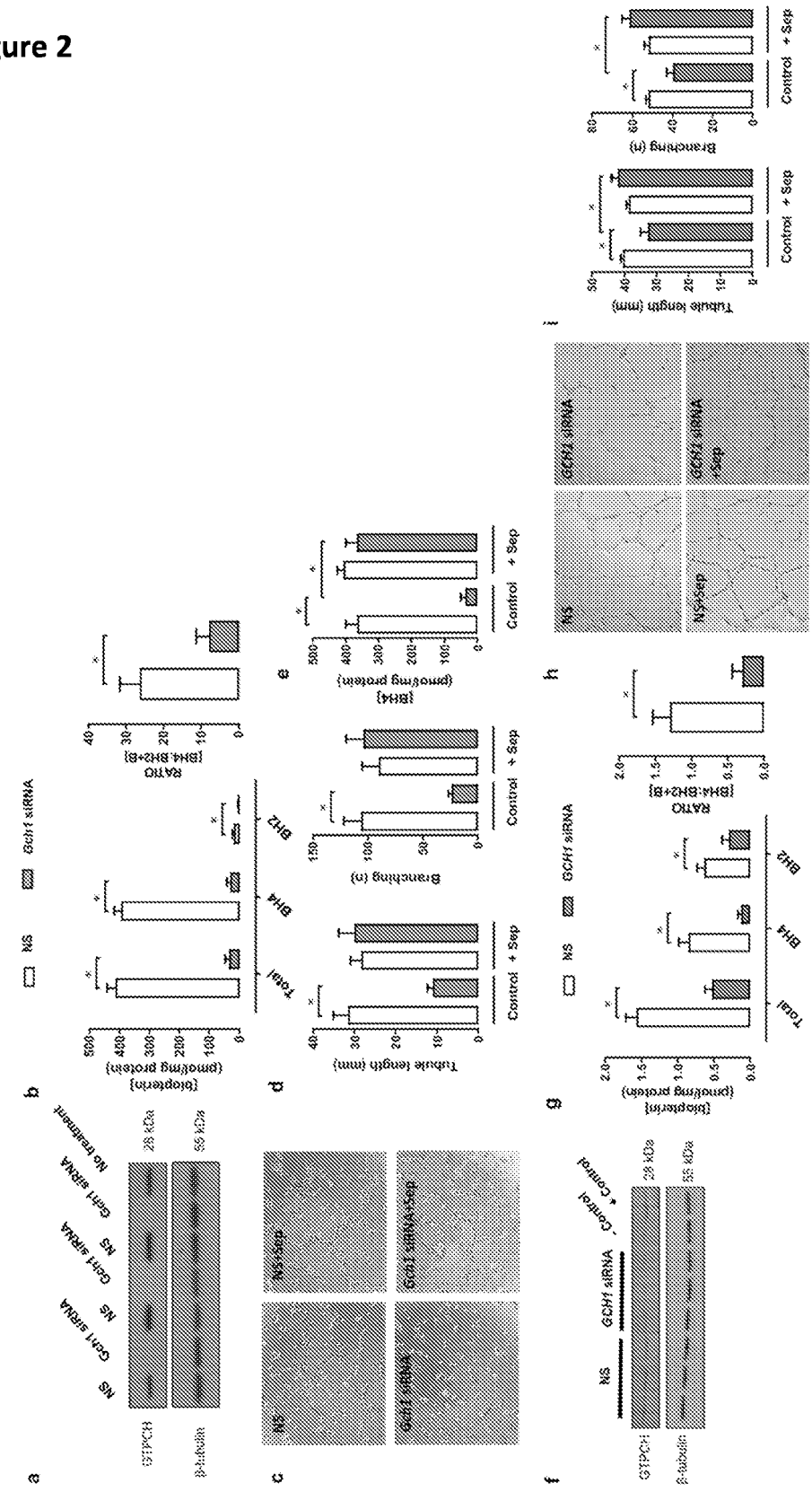

a
NS
Gch1 siRNA
NS
Gch1 siRNA
NS
Gch1 siRNA
No treatment
GTPCH
β-tubulin
28 kDa
55 kDa
b
NS
Gch1 siRNA
[biopterin] (pmol/mg protein)
[BH4] (pmol/mg protein)
RATIO [BH4:BH2+B]
Total
BH4
BH2
c
NS+Sep
Gch1 siRNA
Gch1 siRNA+Sep
d
Tubule length (mm)
Branching (n)
Control
+ Sep
e
[BH4] (pmol/mg protein)
f
NS
GCH1 siRNA
Control
+ Control
GTPCH
β-tubulin
28 kDa
55 kDa
g
NS
GCH1 siRNA
[biopterin] (pmol/mg protein)
RATIO [BH4:BH2+B]
Total
BH4
BH2
h
NS
NS+Sep
GCH1 siRNA
GCH1 siRNA +Sep
i
Tubule length (mm)
Branching (n)
Control
+ Sep

Figure 3

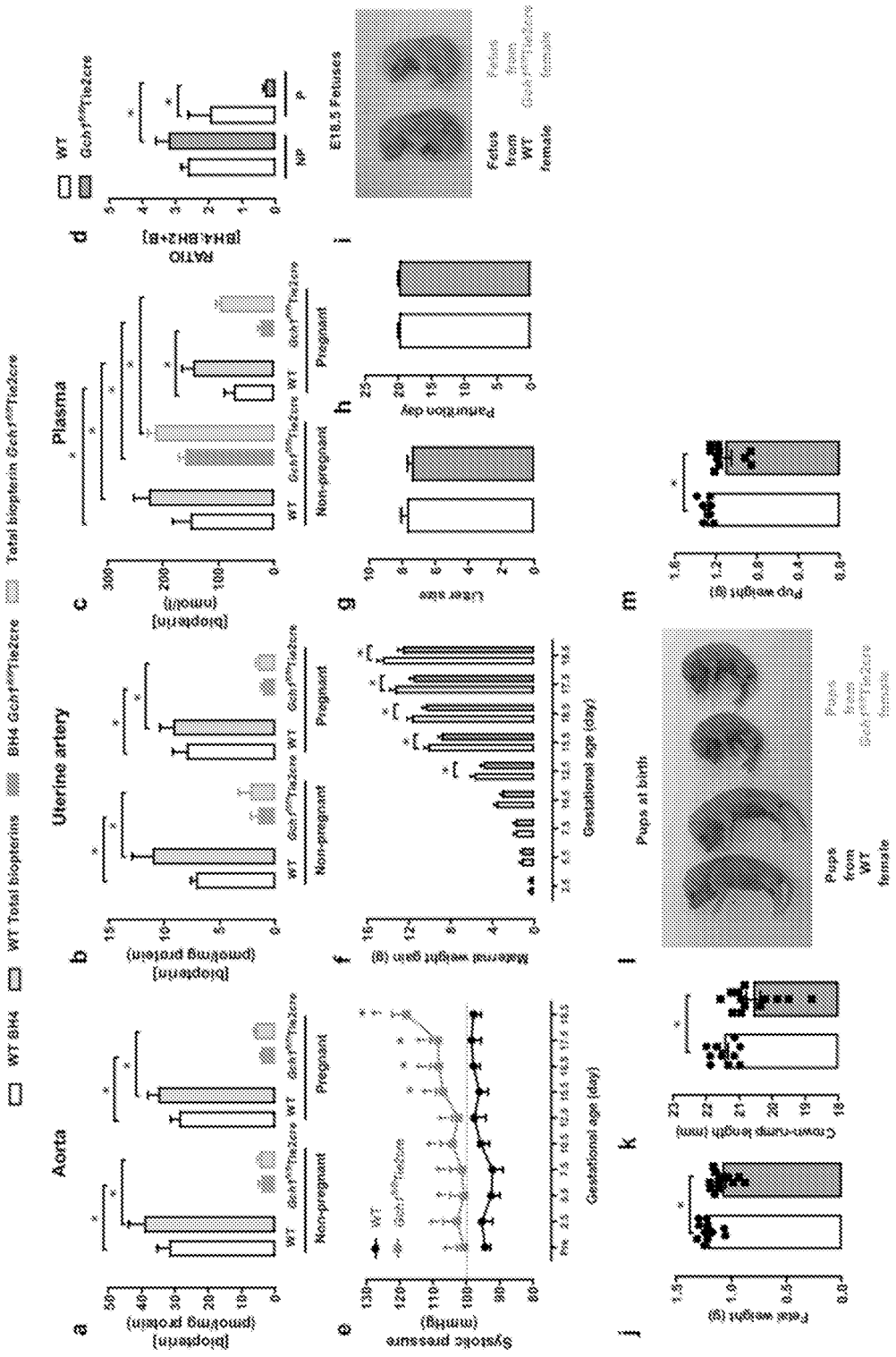


Figure 4

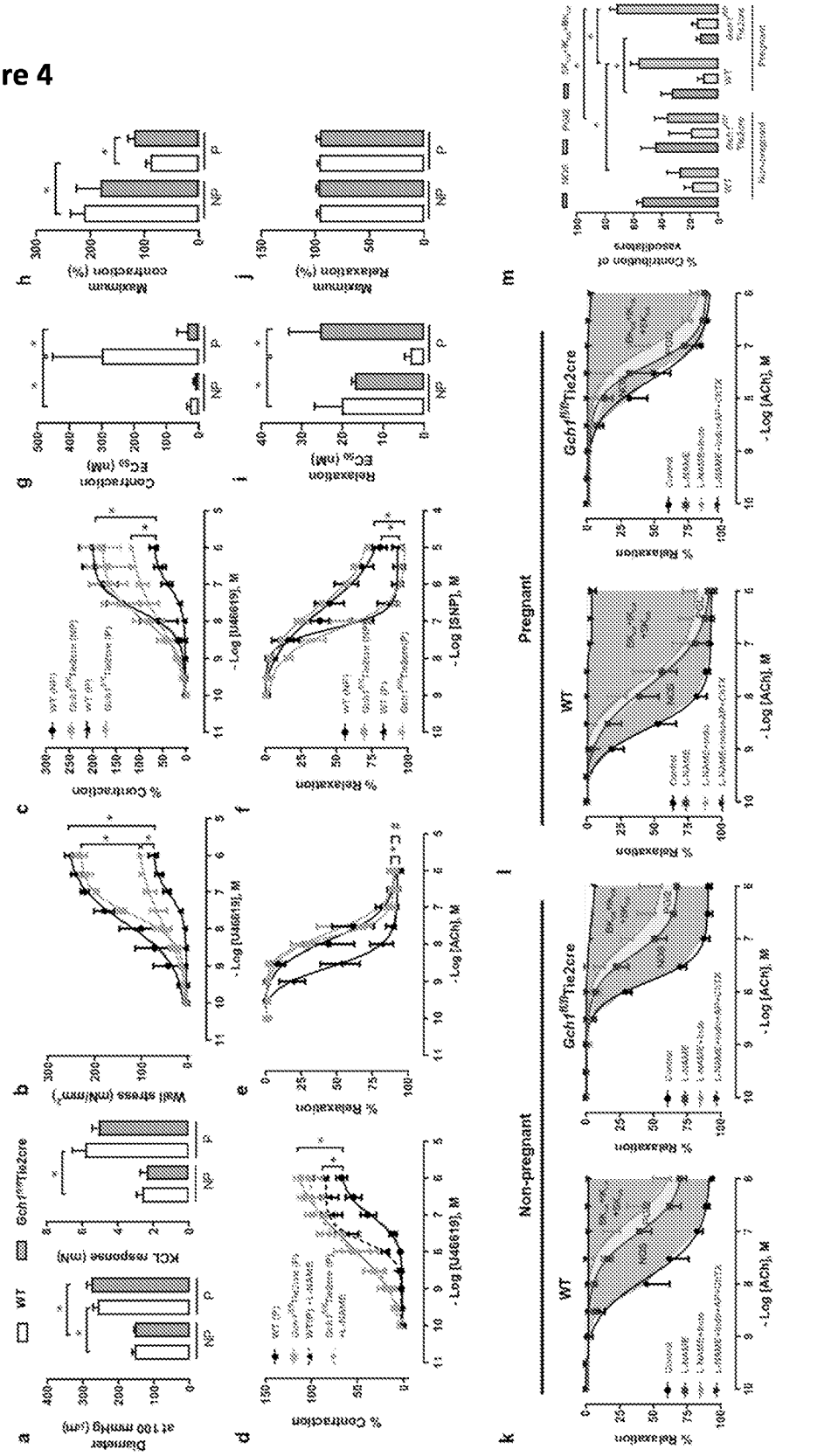


Figure 5

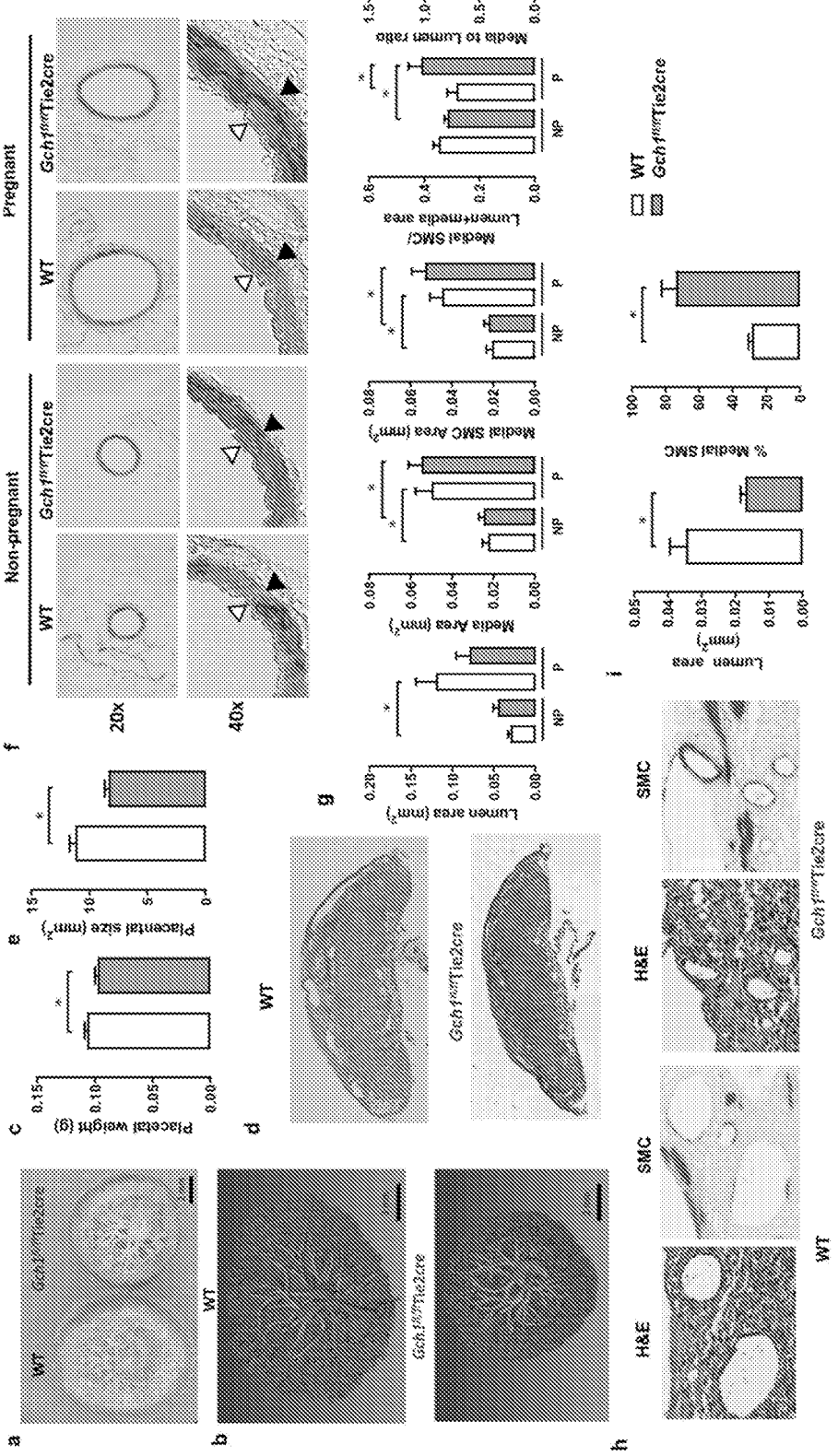

a
WT
Gch1fl/flTie2cre
b
WT
Gch1fl/flTie2cre
c
Placental weight (g)
0.15
0.10
0.05
0.00
*
d
WT
Gch1fl/flTie2cre
e
Placental size (mm2)
15
10
5
0
*
f
Non-pregnant
Pregnant
WT
Gch1fl/flTie2cre
20x
40x
g
Lumen area (mm2)
Media Area (mm2)
Media SMC Area (mm2)
Media/ Lumen+media area
Media to Lumen ratio
NP
P
*
h
H&E
SMC
WT
Gch1fl/flTie2cre
i
Lumen area (mm2)
% Medial SMC
WT
Gch1fl/flTie2cre
*

Figure 6

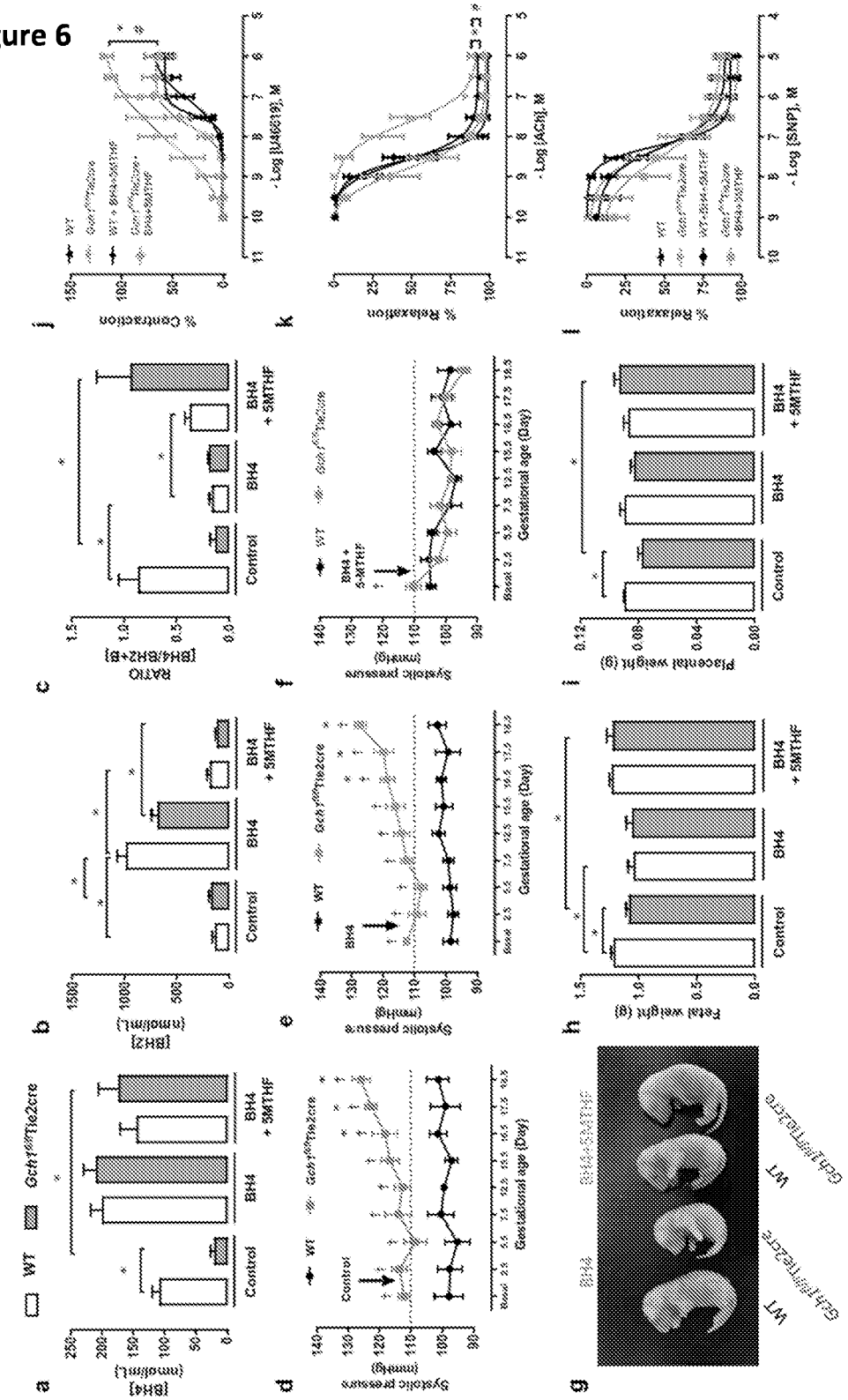

a
WT
Gch1fl/flTie2cre
[BH4] (nmol/mL)
Control
BH4
BH4 + 5MTHF
b
[BH2] (nmol/mL)
c
RATIO [BH4/BH2+B]
d
Systolic pressure (mmHg)
Gestational age (Day)
e
f
g
BH4
BH4+5MTHF
WT
Gch1fl/flTie2cre
h
Fetal weight (g)
i
Placental weight (g)
j
% Contraction
- Log [U46619], M
k
% Relaxation
- Log [ACh], M
l
- Log [SNP], M

Figure 7

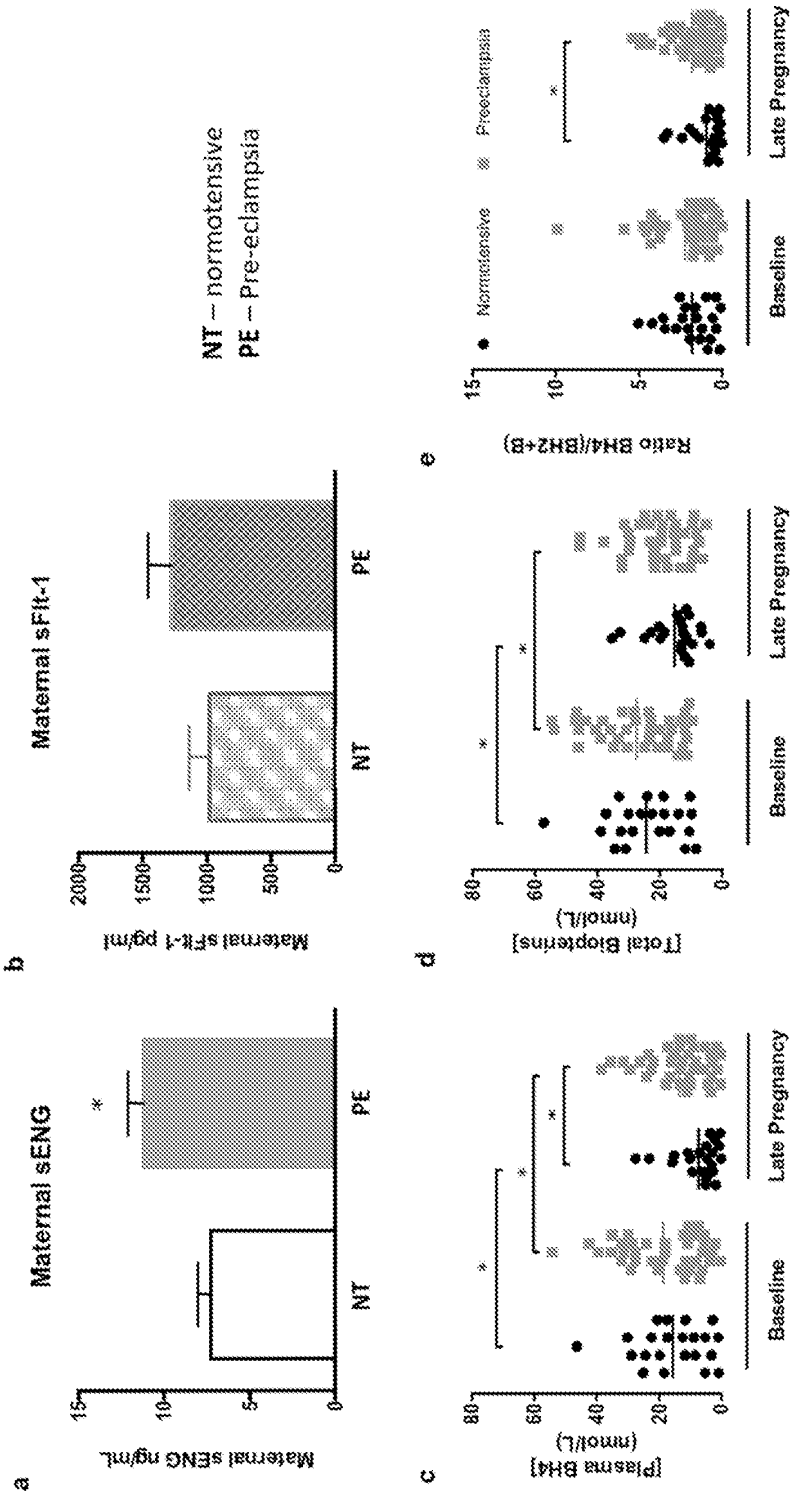

a
Maternal sENG
Maternal sENG ng/mL
15
10
5
0
NT
PE
*
b
Maternal sFlt-1
Maternal sFlt-1 pg/ml
2000
1500
1000
500
0
NT
PE
NT – normotensive
PE – Pre-eclampsia
c
[Plasma BH4]
(nmol/L)
80
60
40
20
0
Baseline
Late Pregnancy
d
[Total Biopterins]
(nmol/L)
80
60
40
20
0
Baseline
Late Pregnancy
e
Ratio BH4/(BH2+B)
15
10
5
0
Normotensive
Preeclampsia
Baseline
Late Pregnancy

Figure 8

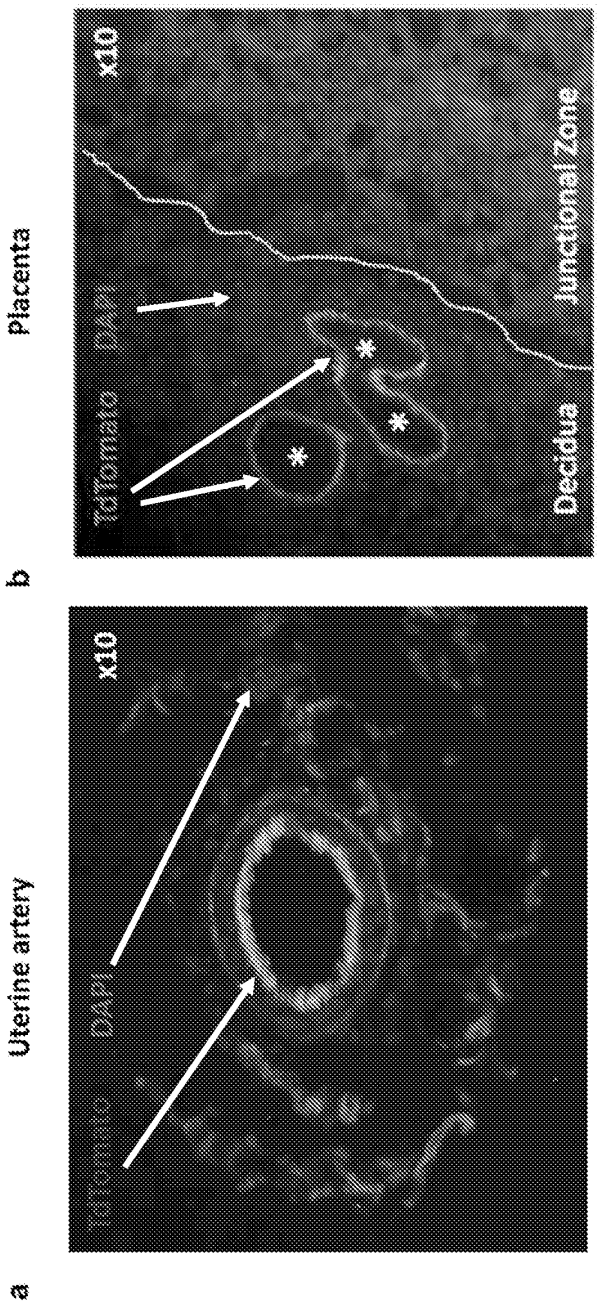

a
Uterine artery
DAPI
TdTomato
x10
b
Placenta
DAPI
TdTomato
Decidua
Junctional Zone
x10

Figure 9

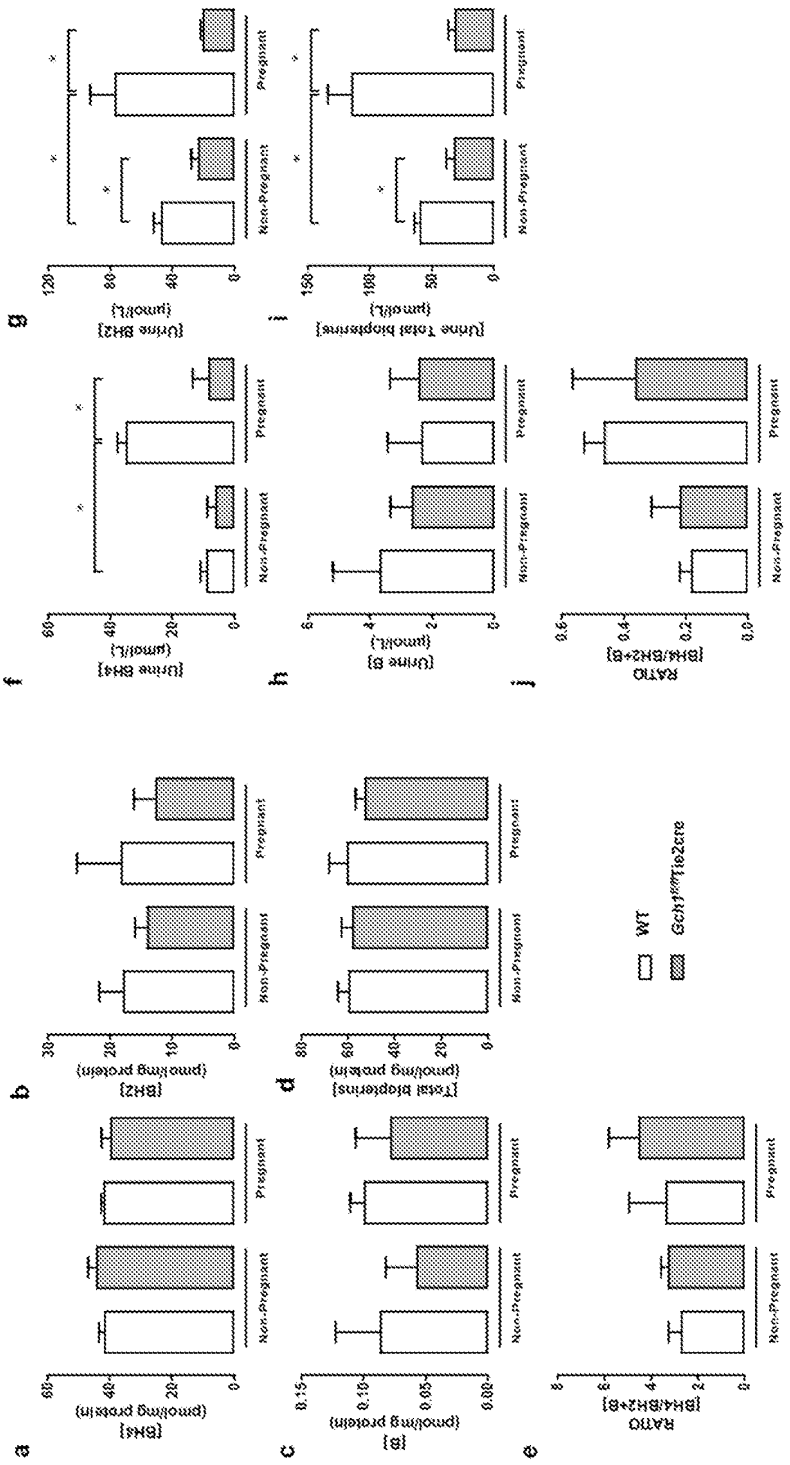

a
[BH4]
(pmol/mg protein)
b
[BH2]
(pmol/mg protein)
c
[B]
(pmol/mg protein)
d
Tissue biopterins
(pmol/mg protein)
e
RATIO
[BH4/BH2+B]
f
Urine BH4
(umol/L)
g
Urine BH2
(umol/L)
h
Urine B
(umol/L)
i
Urine Total biopterins
(umol/L)
j
RATIO
[BH4/BH2+B]
Non-Pregnant
Pregnant
WT
Gch1fl/flTie2cre

Figure 10

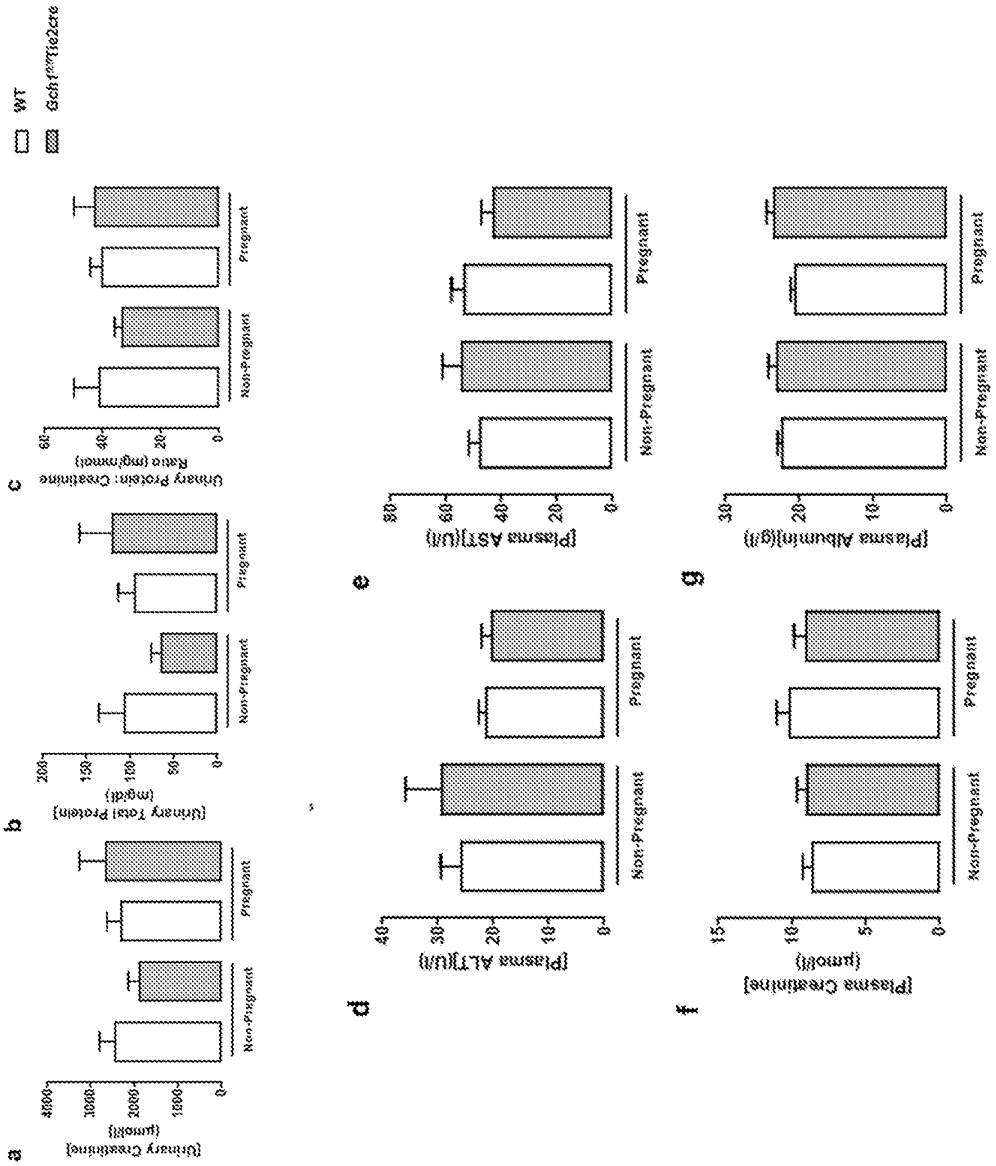

WT
Gch1fl/flTie2cre
a
Urinary Creatinine (μmol/l)
4000
3000
2000
1000
0
Non-Pregnant
Pregnant
b
Urinary Total Protein (mg/dl)
200
150
100
50
0
Non-Pregnant
Pregnant
c
Urinary Protein: Creatinine Ratio (mg/mmol)
60
40
20
0
Non-Pregnant
Pregnant
d
Plasma ALT](U/l)
40
30
20
10
0
Non-Pregnant
Pregnant
e
Plasma AST](U/l)
80
60
40
20
0
Non-Pregnant
Pregnant
f
Plasma Creatinine (μmol/l)
15
10
5
0
Non-Pregnant
Pregnant
g
Plasma Albumin](g/l)
30
20
10
0
Non-Pregnant
Pregnant

Figure 11

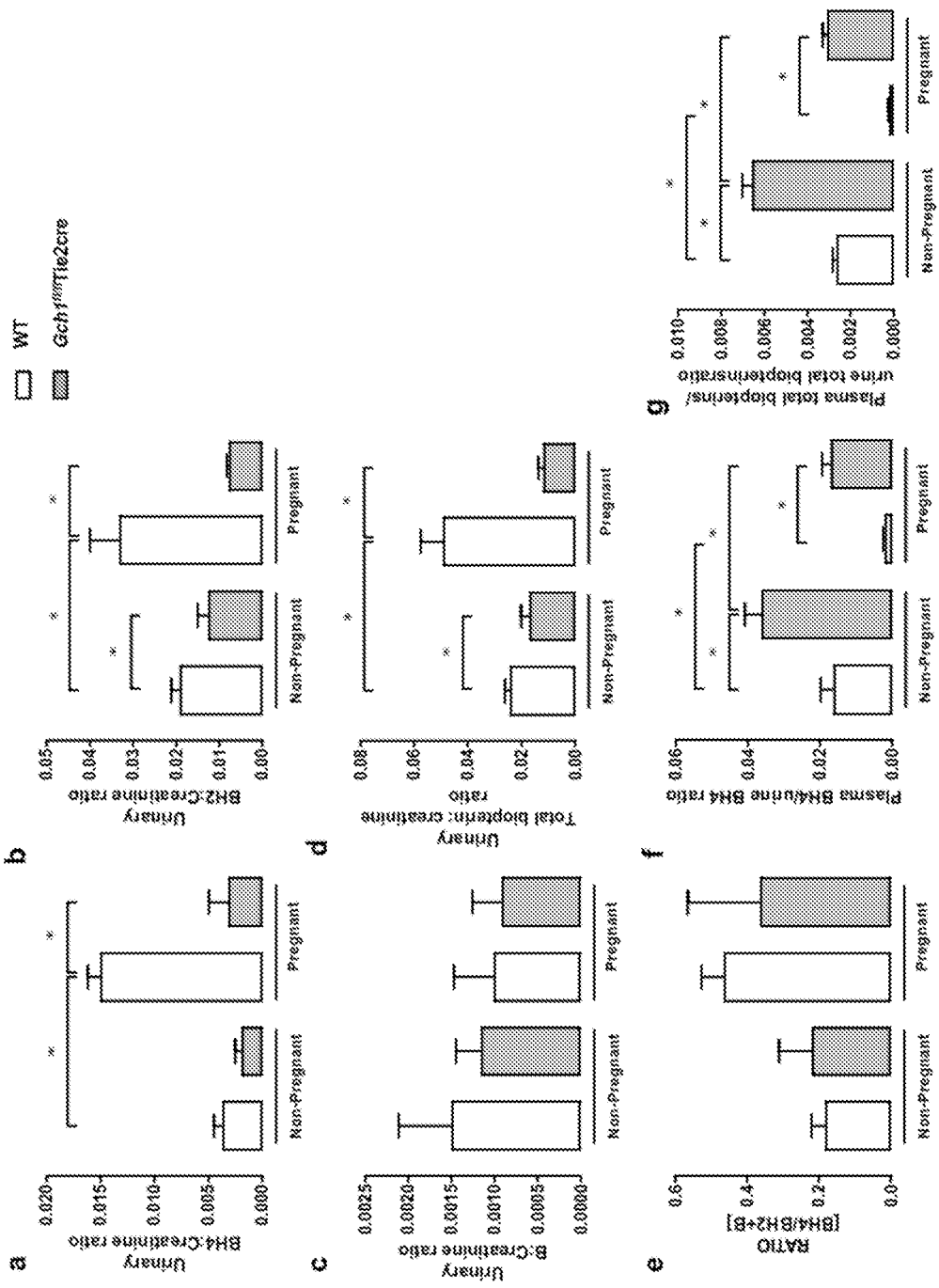

WT
Gch1fl/flTie2cre
a
Urinary BH4:Creatinine ratio
0.020
0.015
0.010
0.005
0.000
Non-Pregnant
Pregnant
b
Urinary BH2:Creatinine ratio
0.05
0.04
0.03
0.02
0.01
0.00
c
Urinary B:Creatinine ratio
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
d
Urinary Total biopterin: creatinine ratio
0.08
0.06
0.04
0.02
0.00
e
RATIO [BH4/BH2+B]
0.6
0.4
0.2
0.0
f
Plasma BH4/urine BH4 ratio
0.06
0.04
0.02
0.00
g
Plasma total biopterins/ urine total biopterin ratio
0.010
0.008
0.006
0.004
0.002
0.000

Figure 12

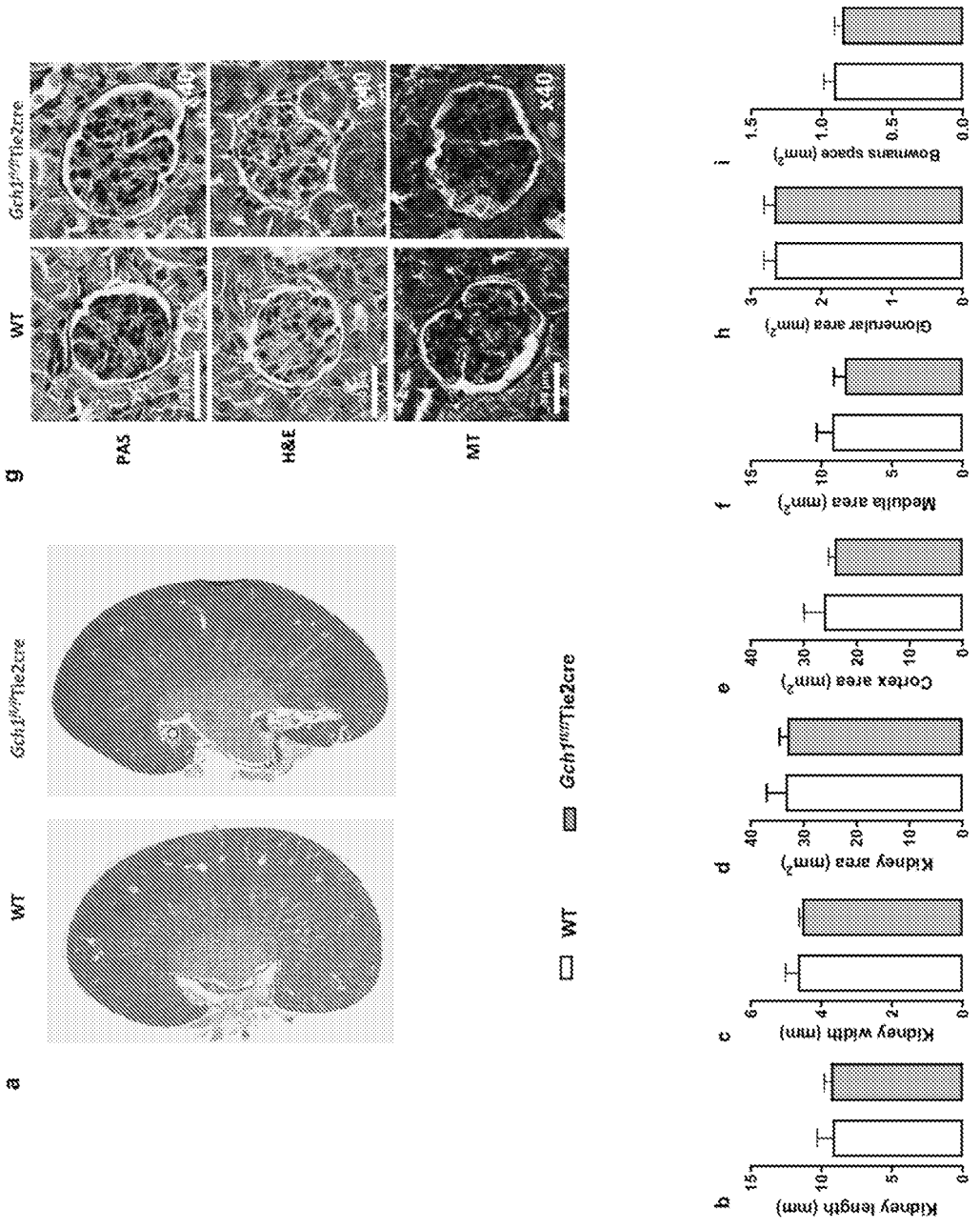

a
WT
Gch1fl/flTie2cre
WT
Gch1fl/flTie2cre
b
Kidney length (mm)
c
Kidney width (mm)
d
Kidney area (mm²)
e
Cortex area (mm²)
f
Medulla area (mm²)
g
WT
Gch1fl/flTie2cre
PAS
H&E
MT
×40
h
Glomerular area (mm²)
i
Bowmans space (mm²)

Figure 13

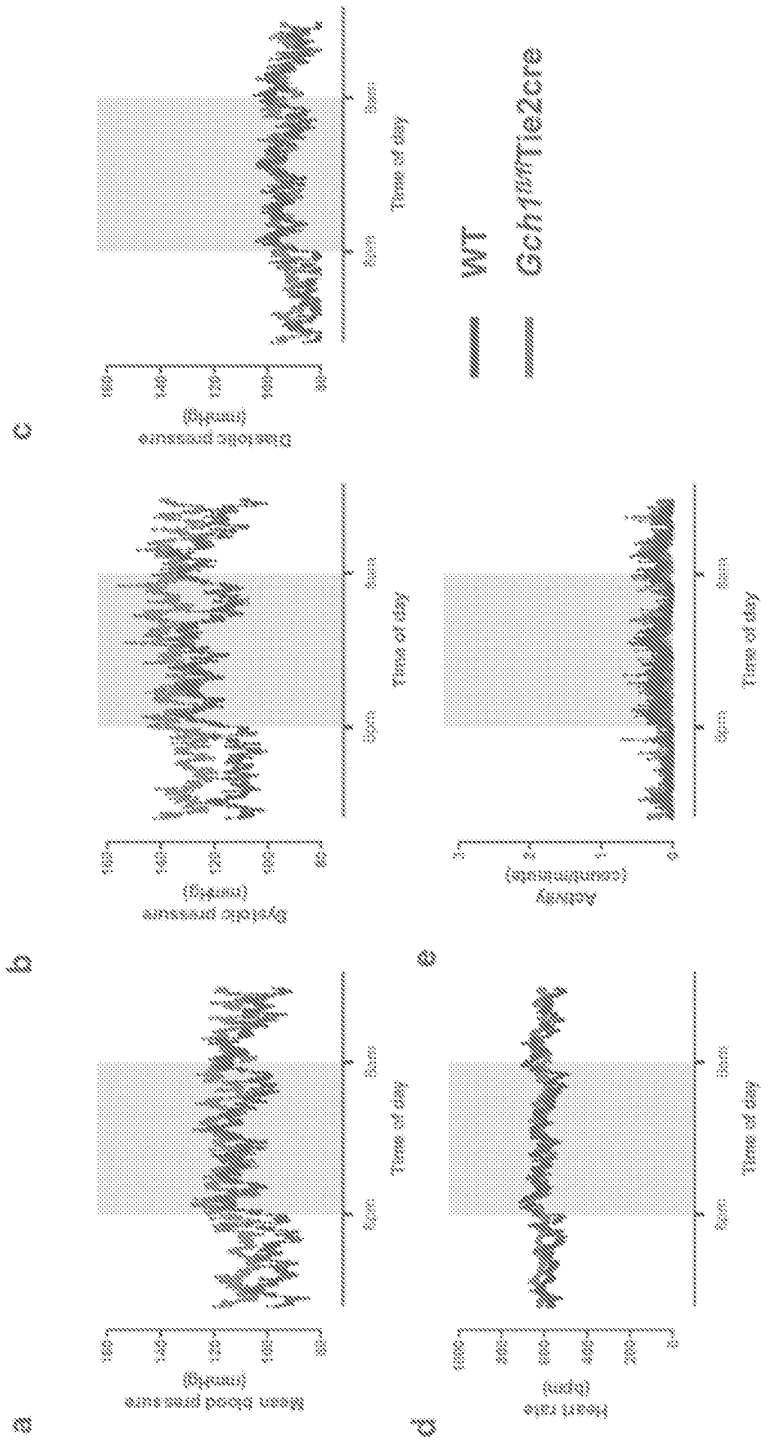

a
Mean blood pressure (mmHg)
b
Systolic pressure (mmHg)
c
Diastolic pressure (mmHg)
d
Heart rate (bpm)
e
Activity (counts/min/g)
Time of day
6pm
8am
WT
Gch1fl/flTie2cre

Figure 14

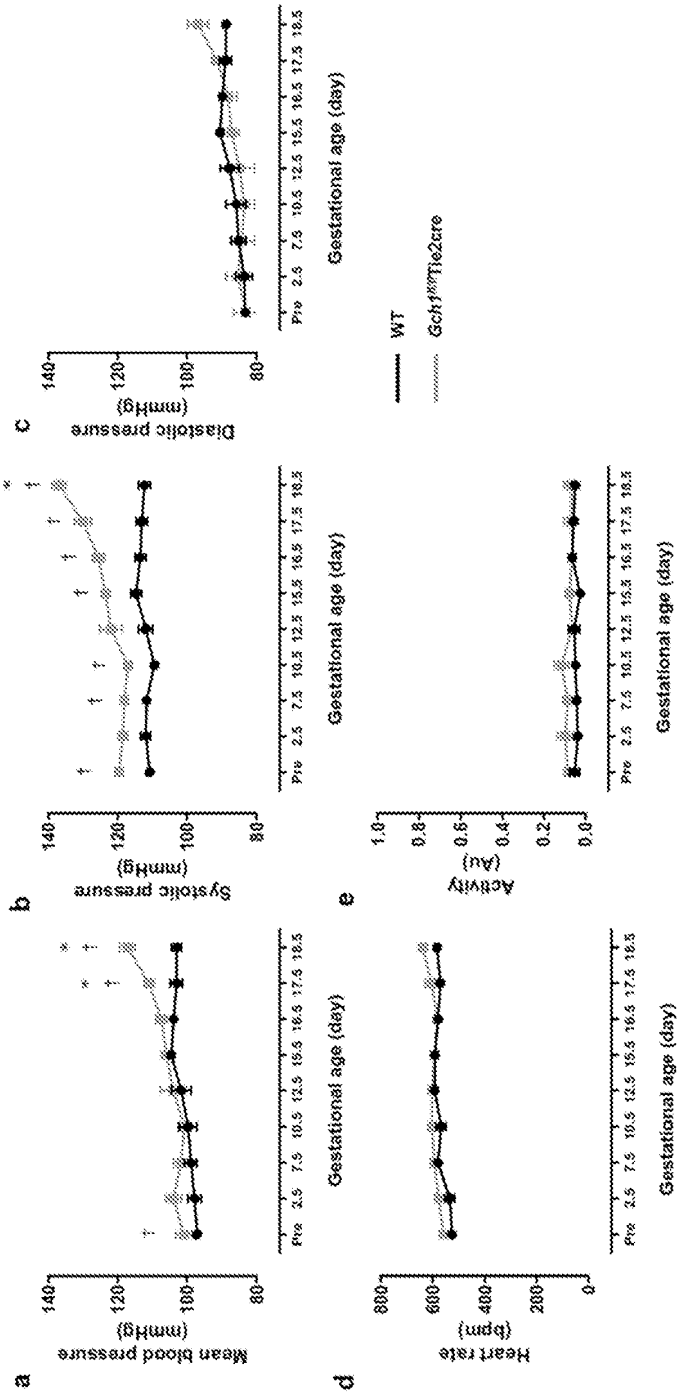

a
Mean blood pressure (mmHg)
b
Systolic pressure (mmHg)
c
Diastolic pressure (mmHg)
d
Heart rate (bpm)
e
Activity (Au)
Gestational age (day)
WT
Gch1fl/flTie2cre

Figure 15

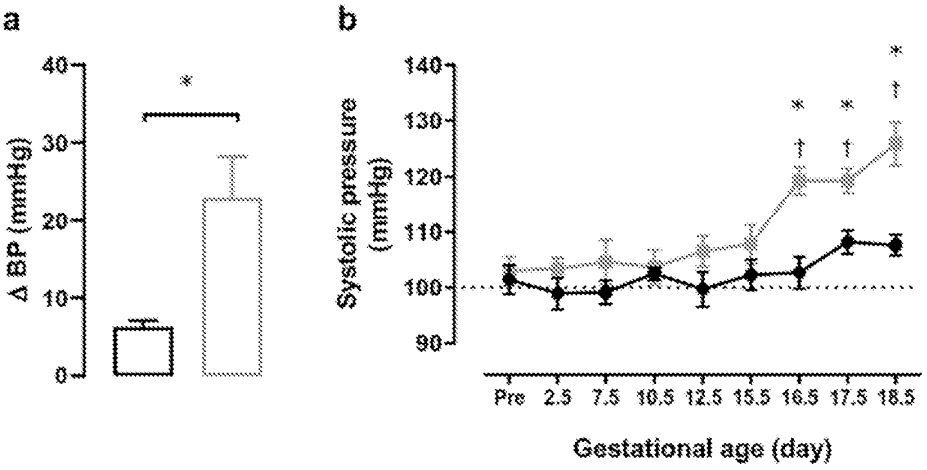

a
Δ BP (mmHg)
40
30
20
10
0
*
b
Systolic pressure (mmHg)
140
130
120
110
100
90
Pre
2.5
7.5
10.5
12.5
15.5
16.5
17.5
18.5
Gestational age (day)

Figure 16

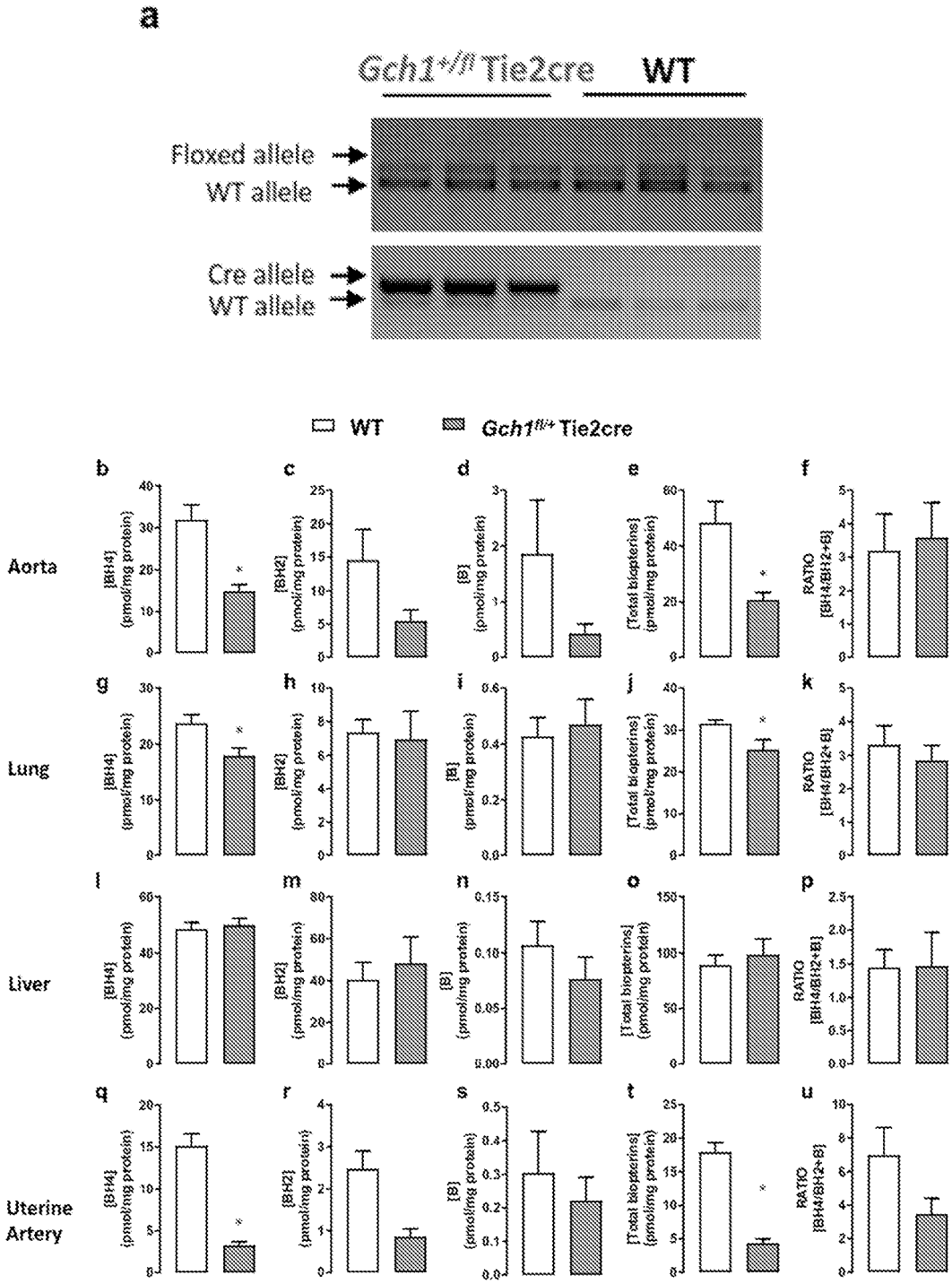

a
Gch1fl/fl Tie2cre
WT
Floxed allele
WT allele
Cre allele
WT allele
WT
Gch1fl/+ Tie2cre
Aorta
Lung
Liver
Uterine Artery
[BH4] (pmol/mg protein)
[BH2] (pmol/mg protein)
[B] (pmol/mg protein)
[Total biopterins] (pmol/mg protein)
RATIO [BH4/(BH2+B)]

Figure 17

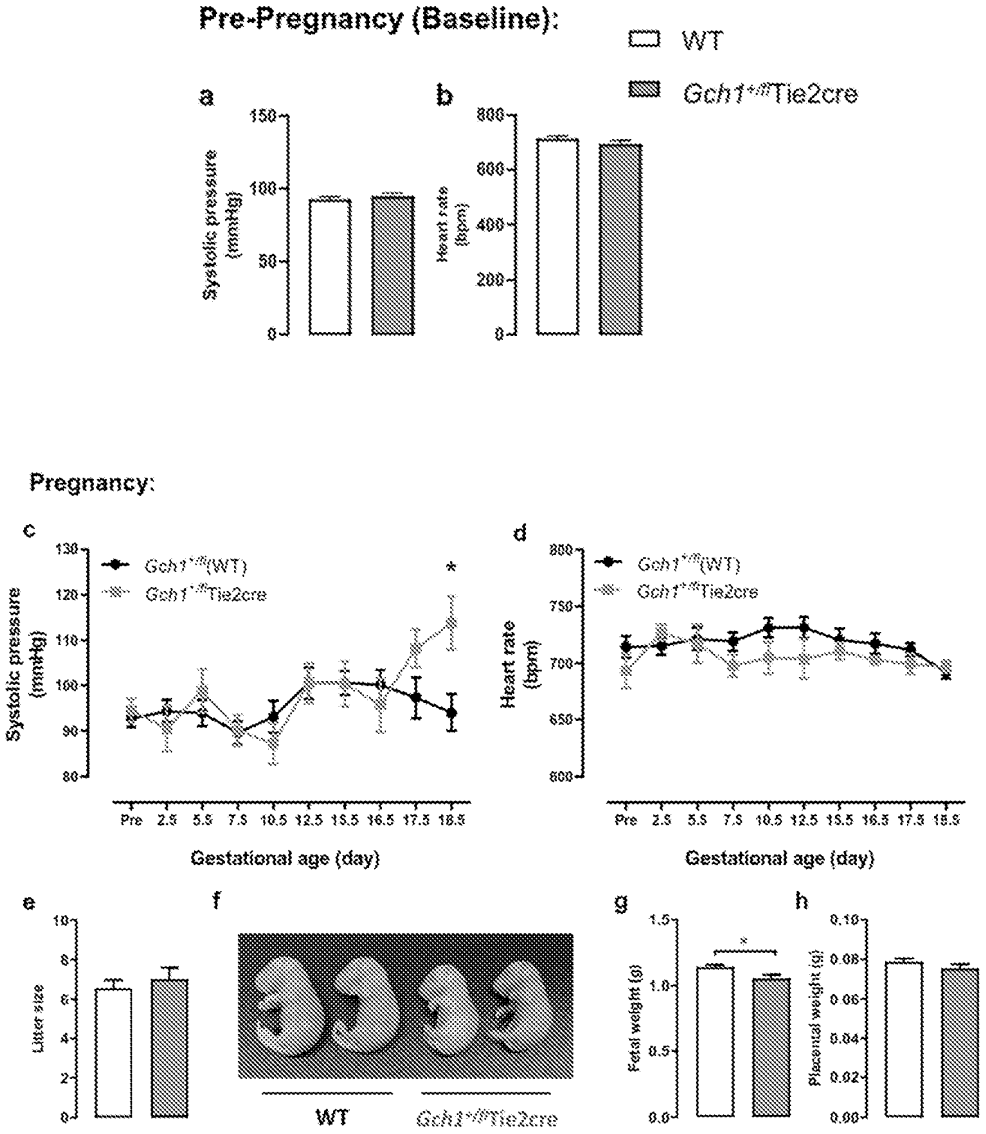

Pre-Pregnancy (Baseline):
WT
Gch1fl/flTie2cre
a
b
Systolic pressure (mmHg)
Heart rate (bpm)
Pregnancy:
c
d
Gch1fl/fl(WT)
Gch1fl/flTie2cre
Systolic pressure (mmHg)
Heart rate (bpm)
Pre 2.5 5.5 7.5 10.5 12.5 15.5 16.5 17.5 18.5
Gestational age (day)
e
f
g
h
Litter size
Fetal weight (g)
Placental weight (g)
WT
Gch1fl/flTie2cre

Figure 18

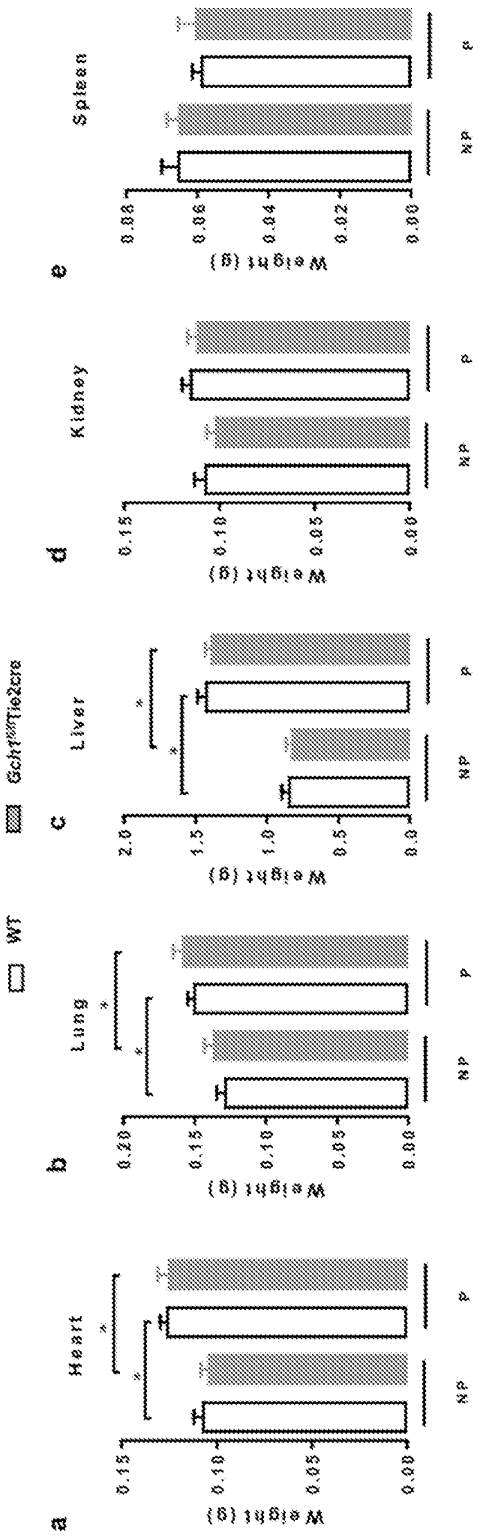

WT
Gchfl/fl Tie2cre
a
Heart
b
Lung
c
Liver
d
Kidney
e
Spleen
Weight (g)
NP
P
*

Figure 19

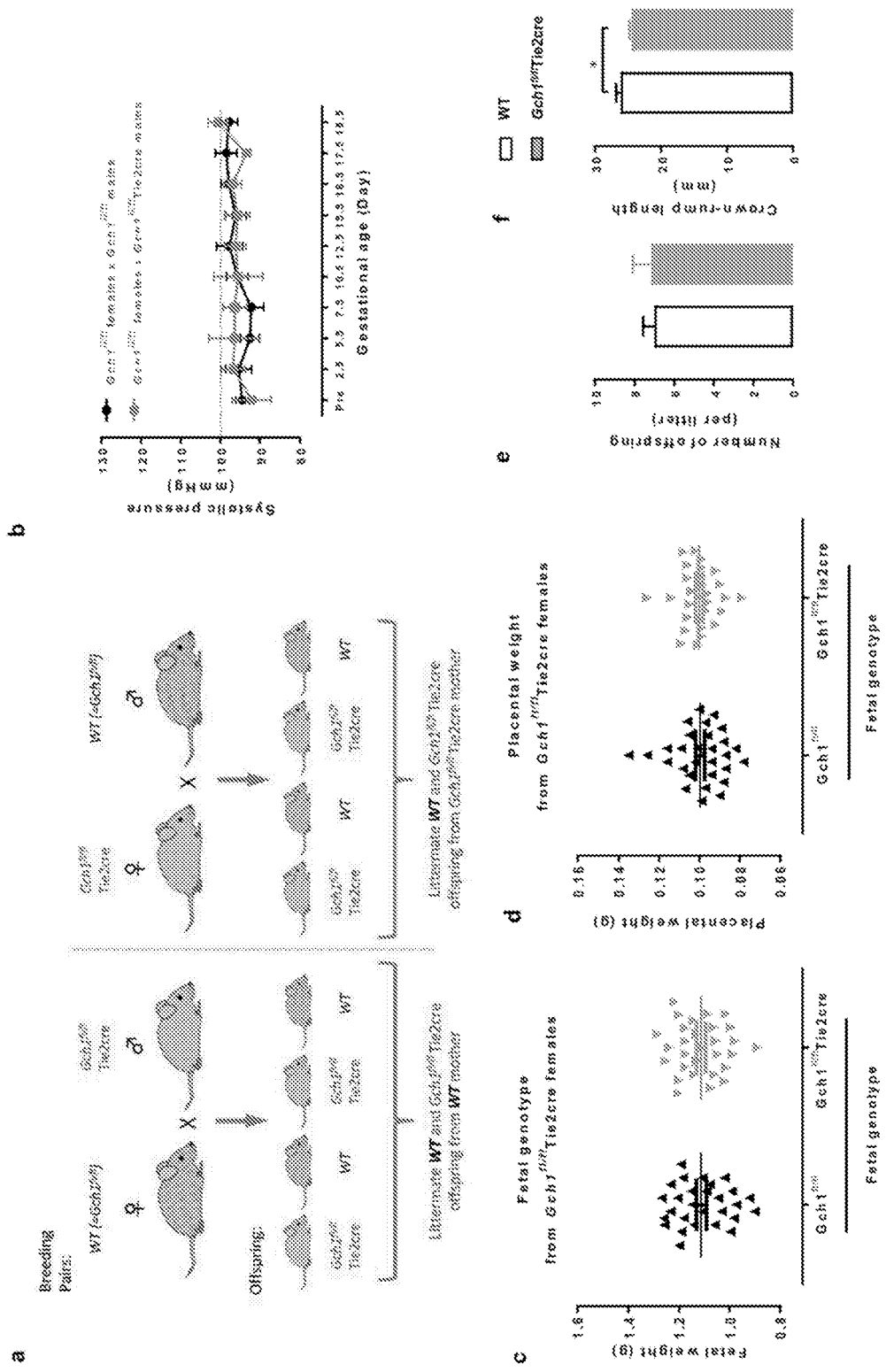

a
Breeding Pairs:
WT (+Gch1fl/fl)
Gch1fl/fl Tie2cre
Offspring
Gch1fl/+ Tie2cre
WT
Gch1fl/fl Tie2cre
Intermate WT and Gch1fl/fl Tie2cre offspring from WT mother
Intermate WT and Gch1fl/fl Tie2cre offspring from Gch1fl/fl Tie2cre mother
b
Systolic pressure (mmHg)
Gestational age (Day)
c
Fetal weight (g)
Fetal genotype
Gch1fl/+
Gch1fl/fl Tie2cre
d
Placental weight
from Gch1fl/fl Tie2cre females
Placental weight (g)
e
Number of offspring (per litter)
f
WT
Gch1fl/fl Tie2cre
Crown-rump length (mm)

Figure 20

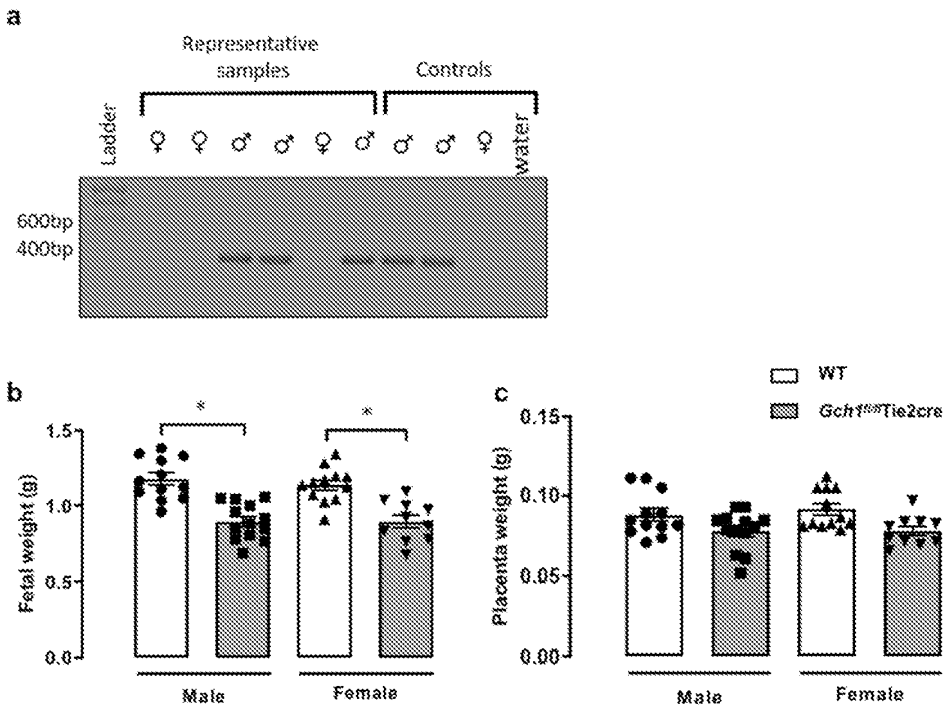

a
Representative samples
Controls
Ladder
water
600bp
400bp
b
Fetal weight (g)
1.5
1.0
0.5
0.0
*
*
Male
Female
c
Placenta weight (g)
0.15
0.10
0.05
0.00
WT
Gch1fl/flTie2cre
Male
Female

Figure 21

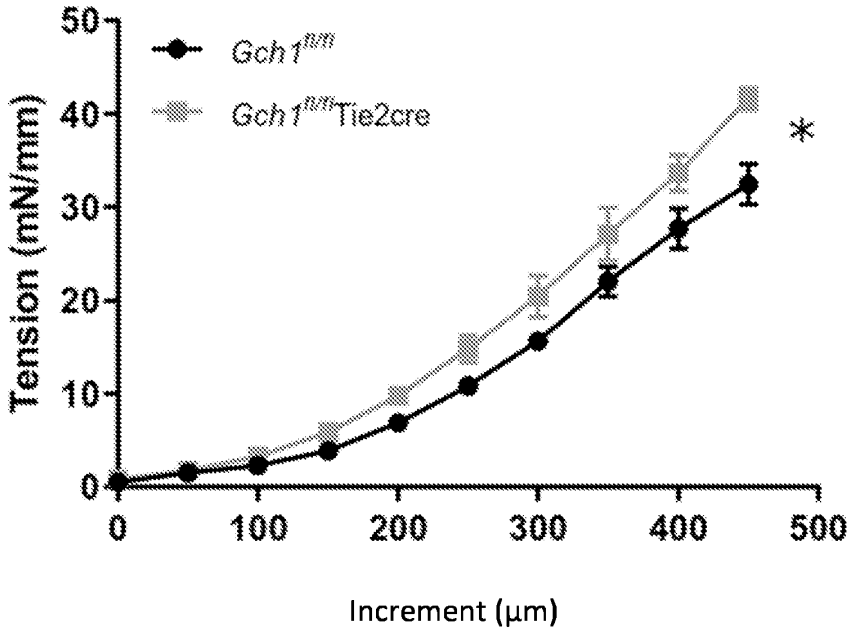

Gch1fl/fl
Gch1fl/flTie2cre
Tension (mN/mm)
50
40
30
20
10
0
0
100
200
300
400
500
Increment (µm)
*

Figure 22

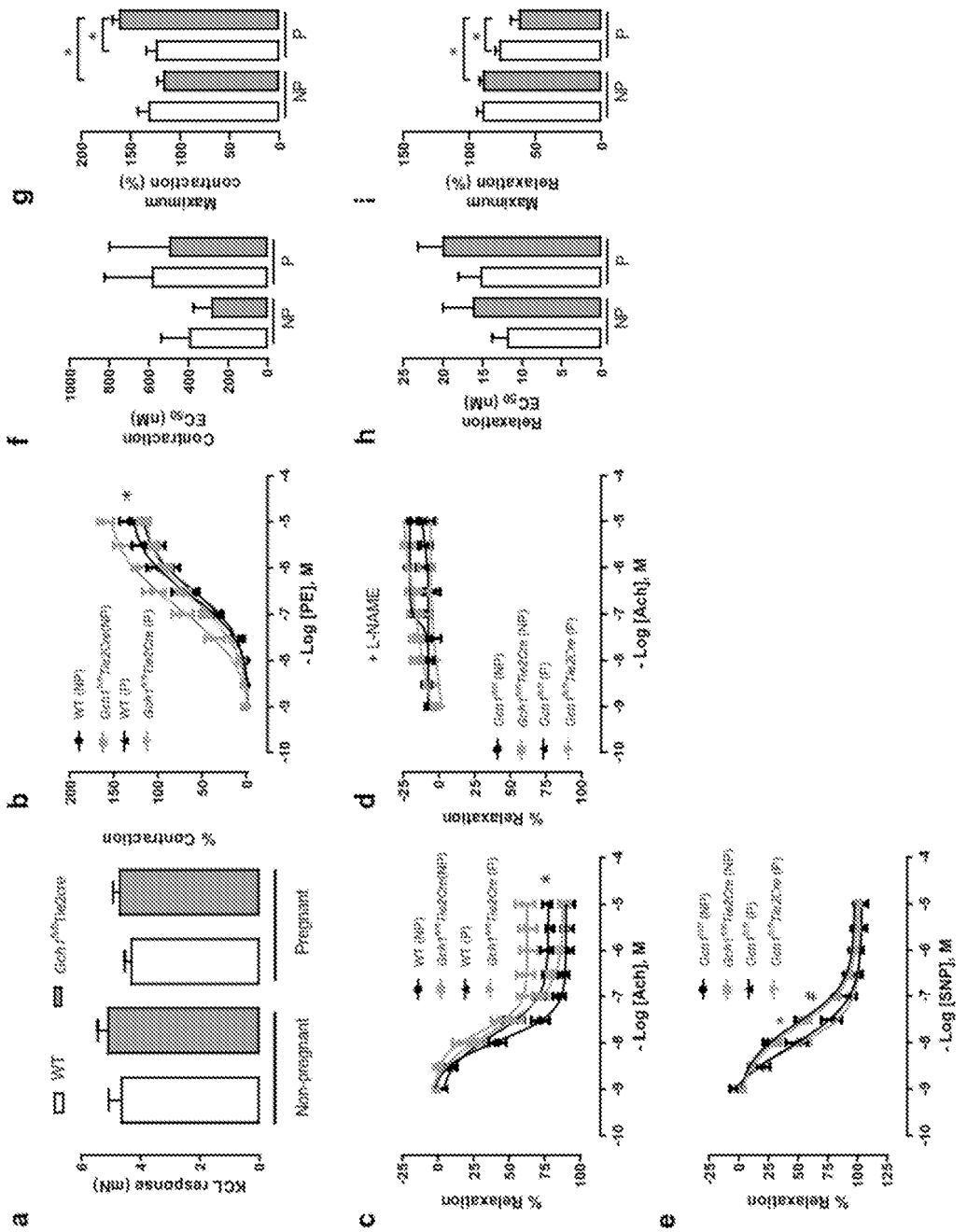

a
KCL response (mN)
WT
Non-pregnant
Pregnant
b
% Contraction
- Log [PE], M
c
% Relaxation
- Log [Ach], M
d
+ L-NAME
% Relaxation
- Log [Ach], M
e
% Relaxation
- Log [SNP], M
f
Contraction EC50 (nM)
NP
P
g
Maximum contraction (%)
NP
P
h
Relaxation EC50 (nM)
NP
P
i
Maximum Relaxation (%)
NP
P

Figure 23

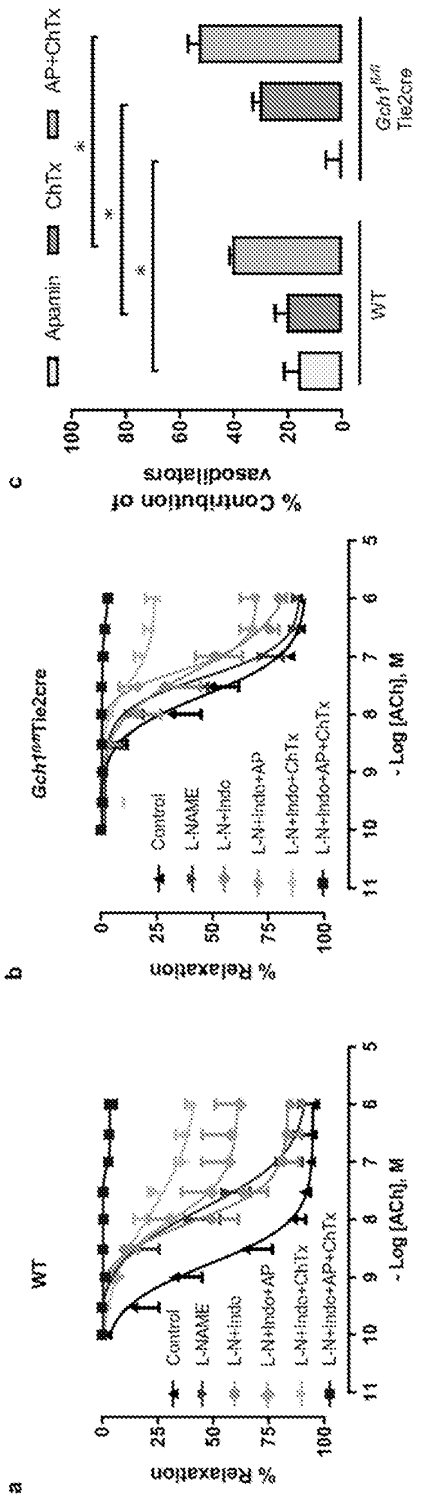

a
WT
% Relaxation
- Log [ACh], M
Control
L-NAME
L-N+indo
L-N+indo+AP
L-N+indo+ChTx
L-N+indo+AP+ChTx
b
Gch1fl/flTie2cre
% Relaxation
- Log [ACh], M
Control
L-NAME
L-N+indo
L-N+indo+AP
L-N+indo+ChTx
L-N+indo+AP+ChTx
c
% Contribution of vasodilators
Apamin
ChTx
AP+ChTx
WT
Gch1fl/fl Tie2cre

Figure 24

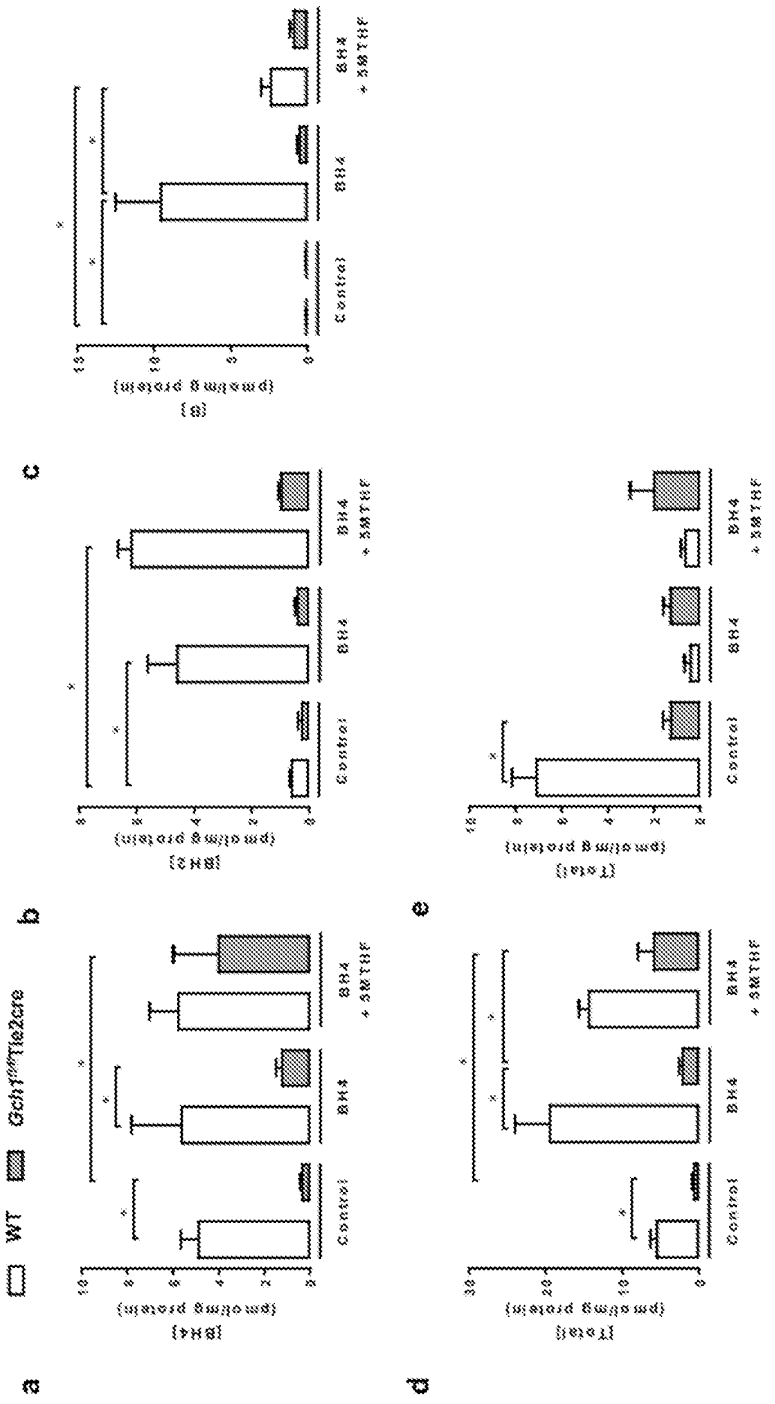

WT
Gch1fl/flTie2cre
a
b
c
d
e
Control
BH4
BH4 + 5MTHF
[BH4]
[BH2]
[Total]
(pmol/mg protein)

Figure 25

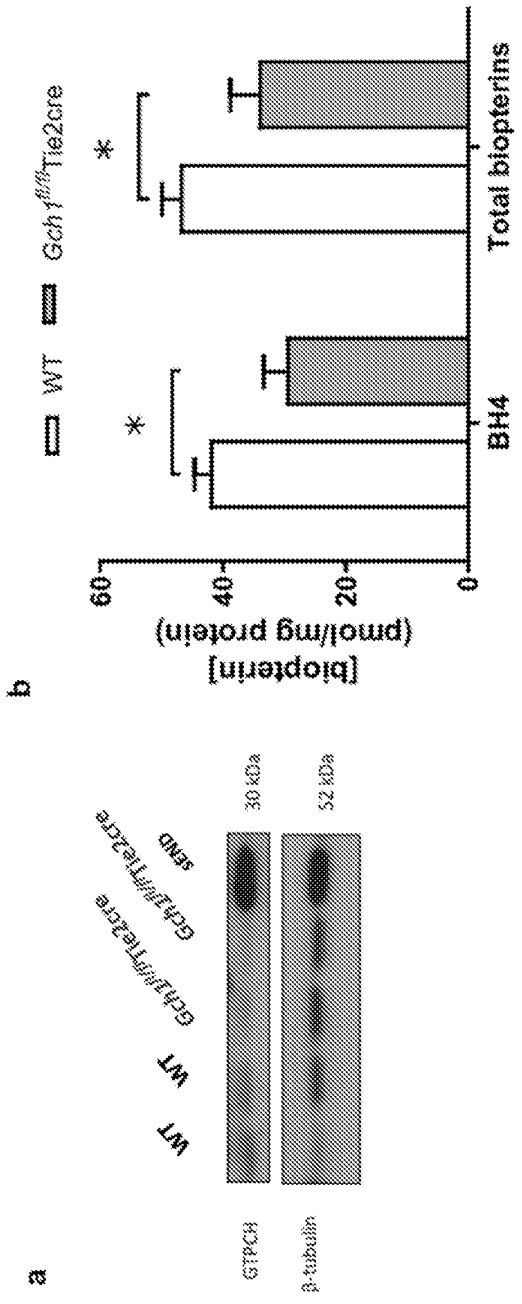

b
[biopterin]
(pmol/mg protein)
60
40
20
0
BH4
Total biopterins
WT
Gch1fl/flTie2cre
*
*
a
GTPCH
β-tubulin
30 kDa
52 kDa
WT
WT
Gch1fl/flTie2cre
Gch1fl/flTie2cre
sEND

Figure 26

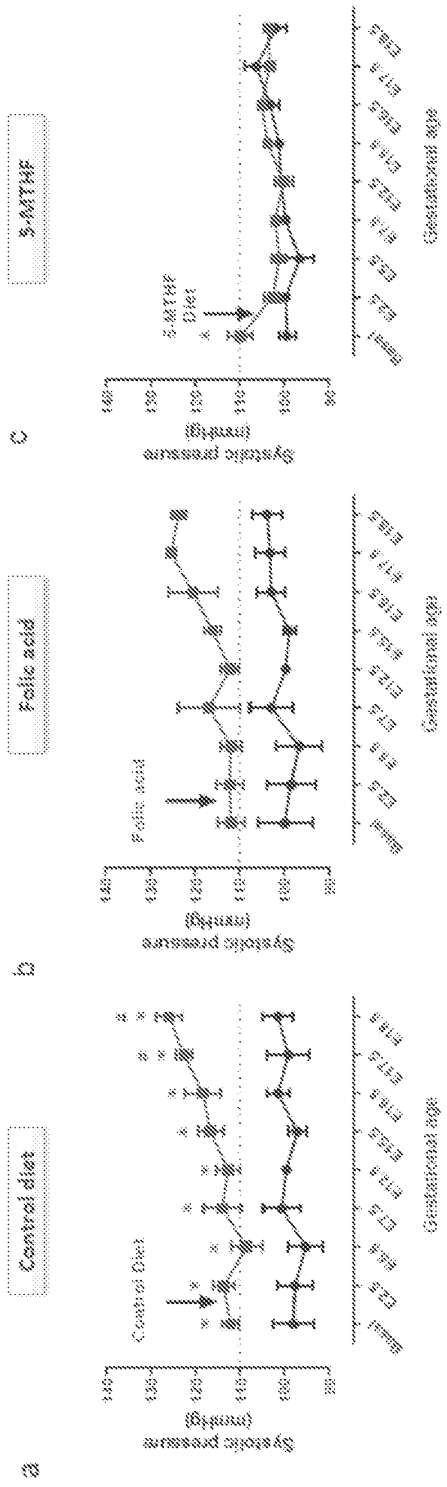

a
Control diet
Systolic pressure (mmHg)
Control Diet
Gestational age
b
Folic acid
Systolic pressure (mmHg)
Folic acid
Gestational age
c
5-MTHF
Systolic pressure (mmHg)
5-MTHF Diet
Gestational age

Figure 27

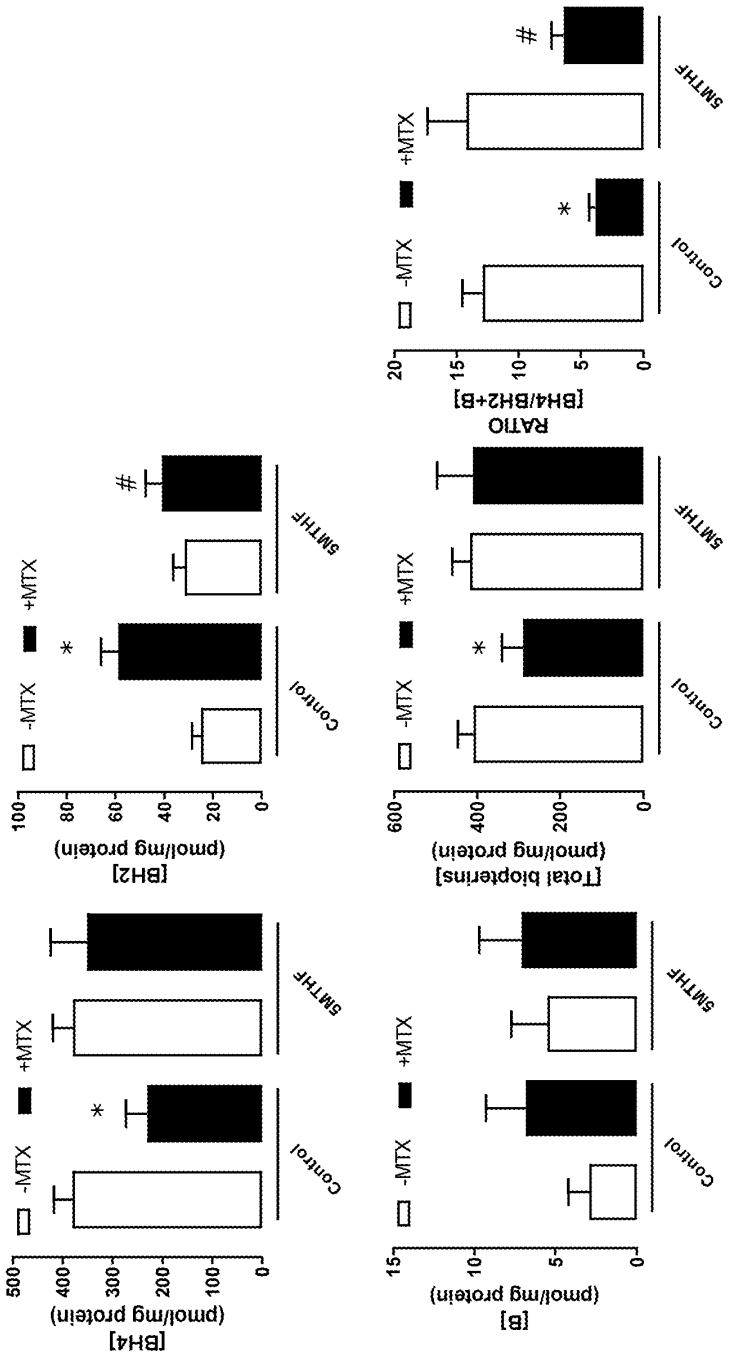

[BH4]
(pmol/mg protein)
500
400
300
200
100
0
-MTX
+MTX
*
Control
5MTHF
[BH2]
(pmol/mg protein)
100
80
60
40
20
0
-MTX
+MTX
*
#
Control
5MTHF
[B]
(pmol/mg protein)
15
10
5
0
-MTX
+MTX
Control
5MTHF
[Total biopterins]
(pmol/mg protein)
600
400
200
0
-MTX
+MTX
*
Control
5MTHF
RATIO
[BH4/BH2+B]
20
15
10
5
0
-MTX
+MTX
*
Control
5MTHF
#

Figure 28

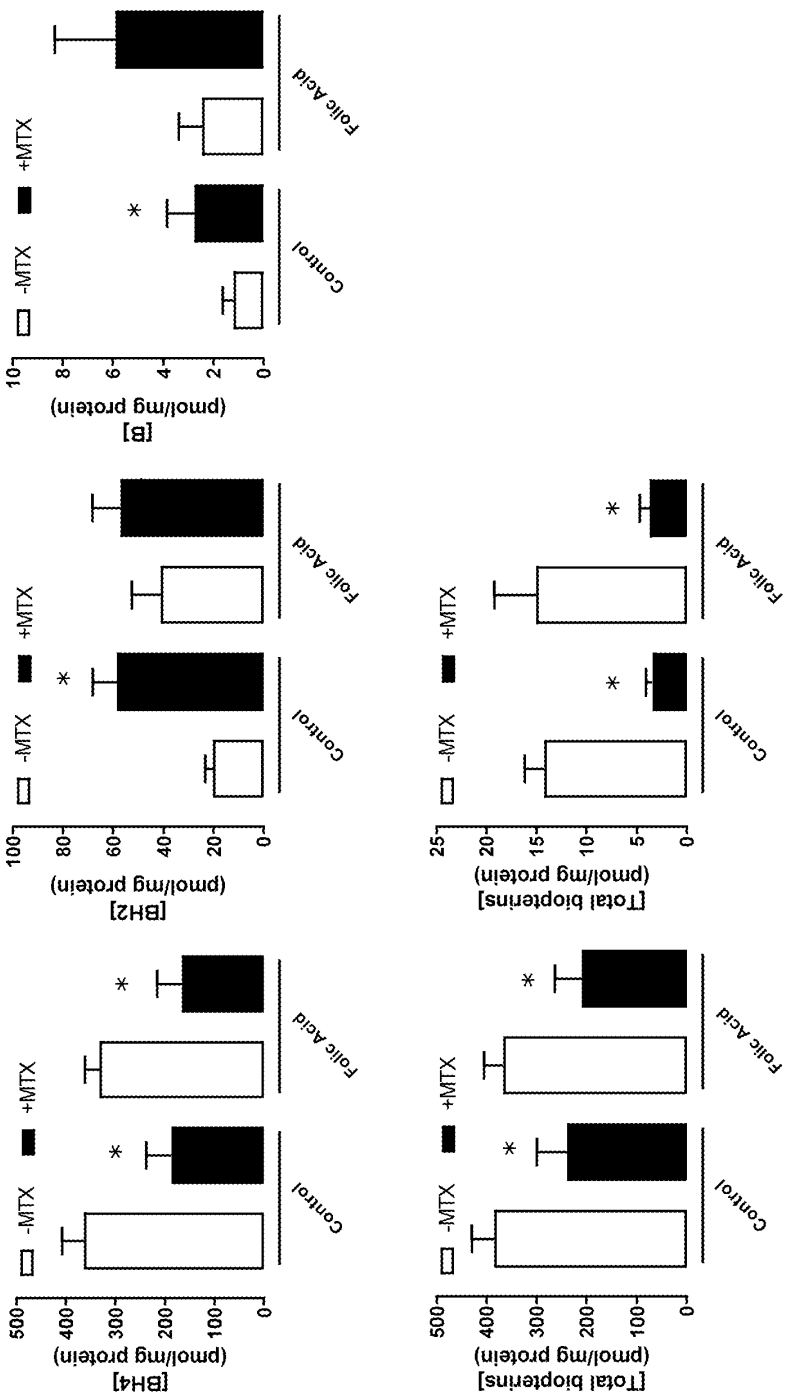

[B]
(pmol/mg protein)
0
2
4
6
8
10
-MTX
+MTX
Control
Folic Acid
*
[BH2]
(pmol/mg protein)
0
20
40
60
80
100
[Total biopterins]
(pmol/mg protein)
0
5
10
15
20
25
[BH4]
(pmol/mg protein)
0
100
200
300
400
500
[Total biopterins]
(pmol/mg protein)
0
100
200
300
400
500

Figure 29

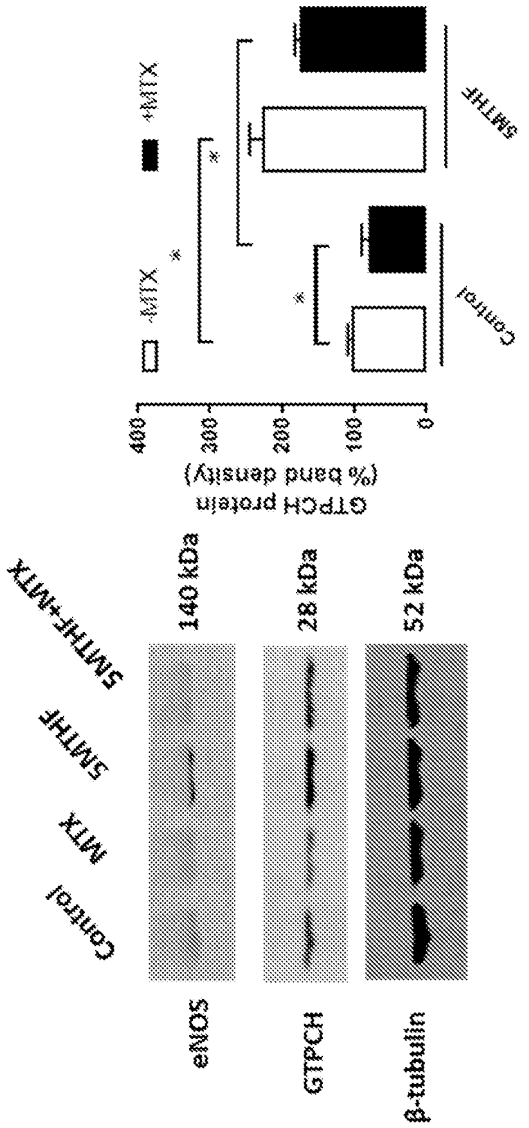

GTPCH protein
(% band density)
400
300
200
100
0
-MTX
+MTX
Control
5MTHF
*
*
*
140 kDa
28 kDa
52 kDa
Control
MTX
5MTHF
5MTHF+MTX
eNOS
GTPCH
β-tubulin

Figure 30

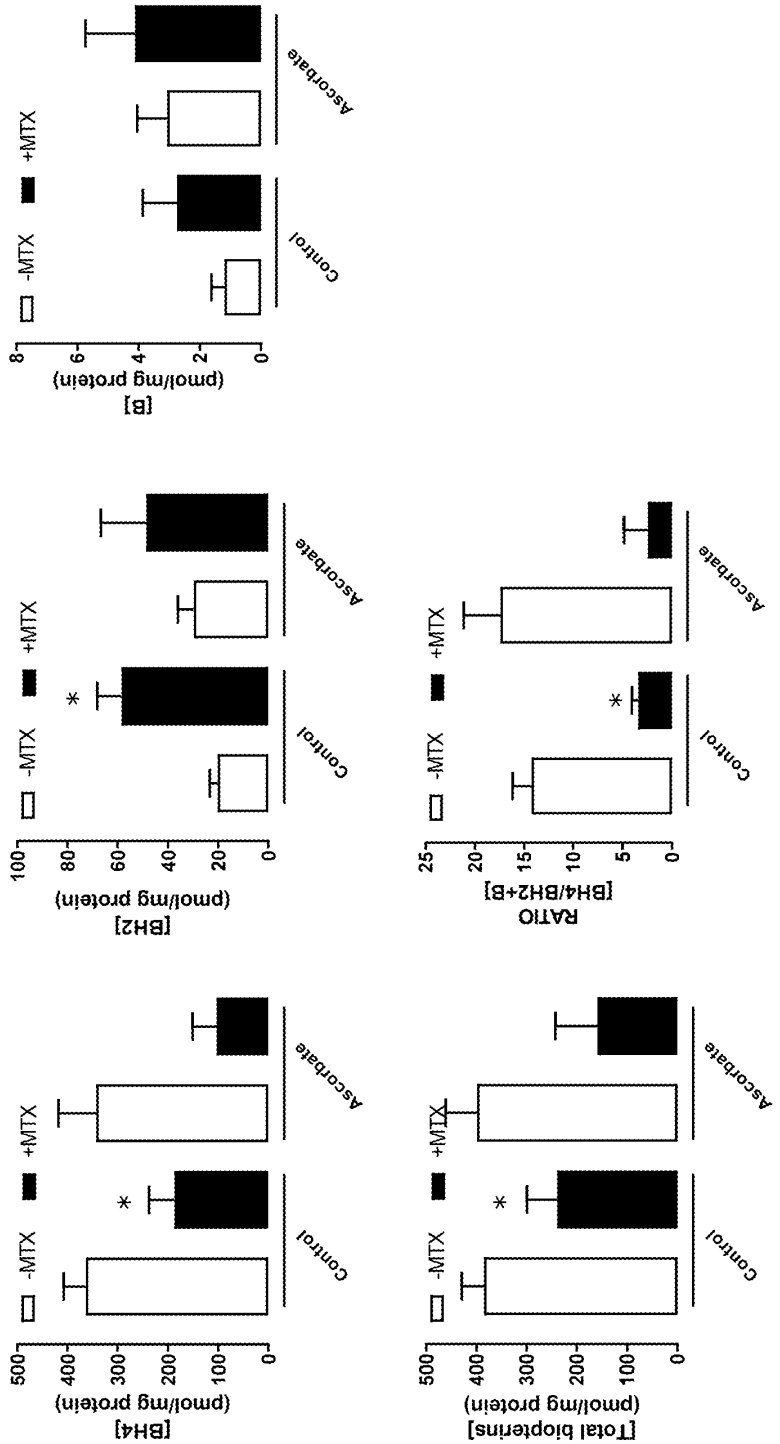

[B]
(pmol/mg protein)
[BH2]
(pmol/mg protein)
RATIO
[BH4/BH2+B]
[BH4]
(pmol/mg protein)
[Total biopterins]
(pmol/mg protein)
-MTX
+MTX
Control
Ascorbate
*

Figure 31

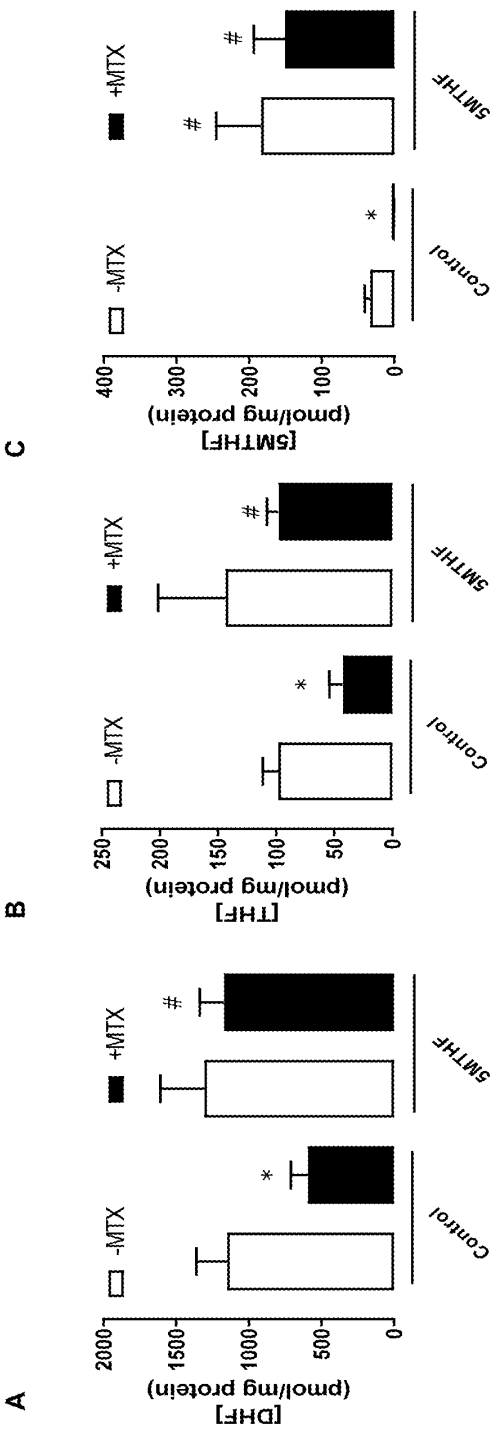

A
[DHF]
(pmol/mg protein)
2000
1500
1000
500
0
-MTX
+MTX
Control
5MTHF
*
#
B
[THF]
(pmol/mg protein)
250
200
150
100
50
0
-MTX
+MTX
Control
5MTHF
*
#
C
[5MTHF]
(pmol/mg protein)
400
300
200
100
0
-MTX
+MTX
Control
5MTHF
*
#

Figure 32

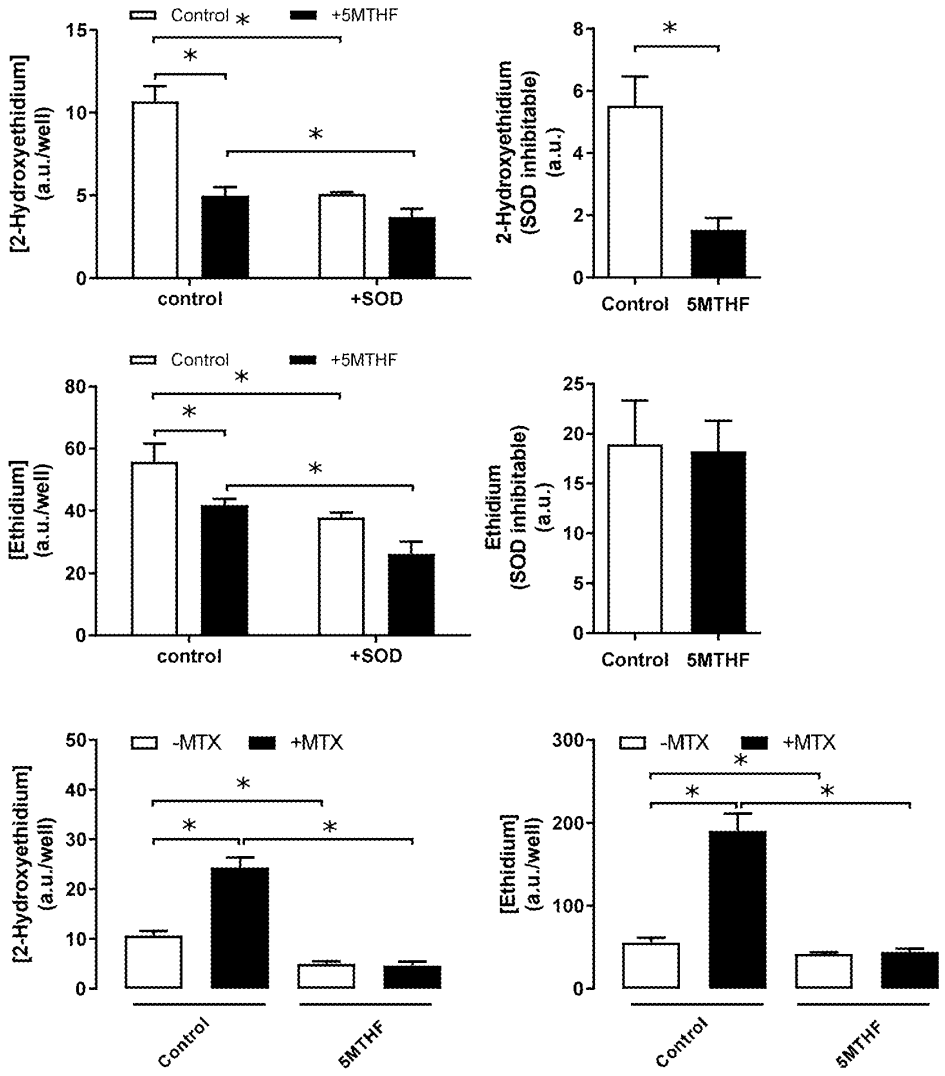

Control
+5MTHF
[2-Hydroxyethidium] (a.u./well)
control
+SOD
2-Hydroxyethidium (SOD inhibitable) (a.u.)
Control
5MTHF
[Ethidium] (a.u./well)
Ethidium (SOD inhibitable) (a.u.)
-MTX
+MTX
5MTHF

Figure 33

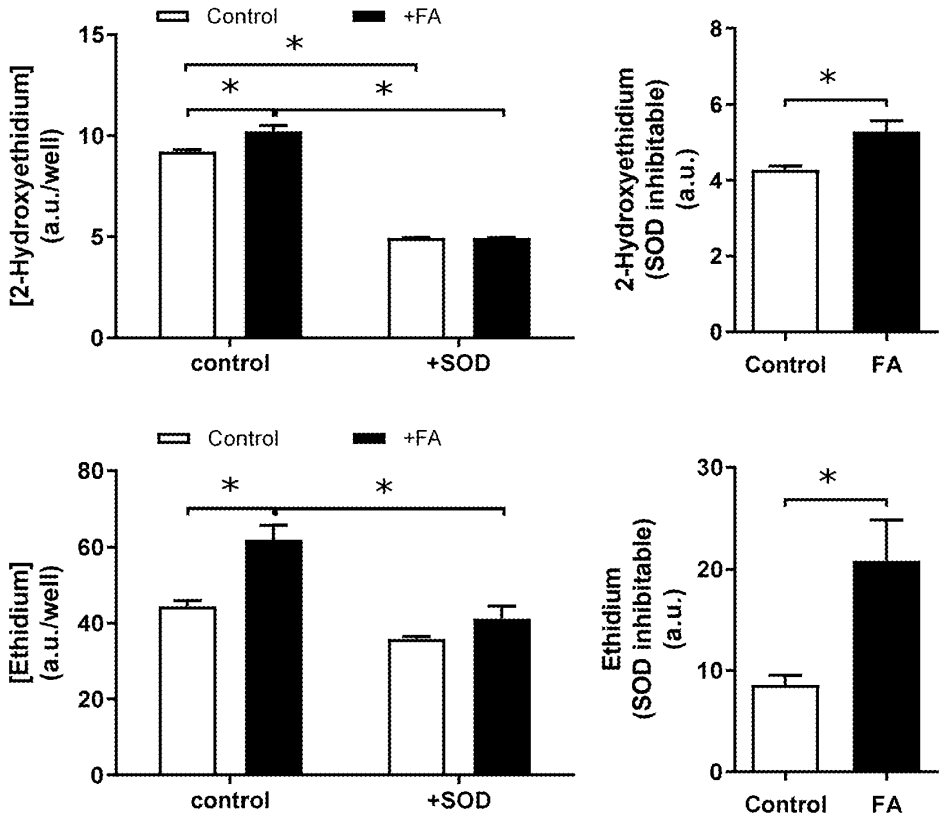

Control
+FA
[2-Hydroxyethidium] (a.u./well)
15
10
5
0
control
+SOD
2-Hydroxyethidium (SOD inhibitable) (a.u.)
8
6
4
2
0
Control
FA
[Ethidium] (a.u./well)
80
60
40
20
0
Ethidium (SOD inhibitable) (a.u.)
30
20
10
0

Figure 34

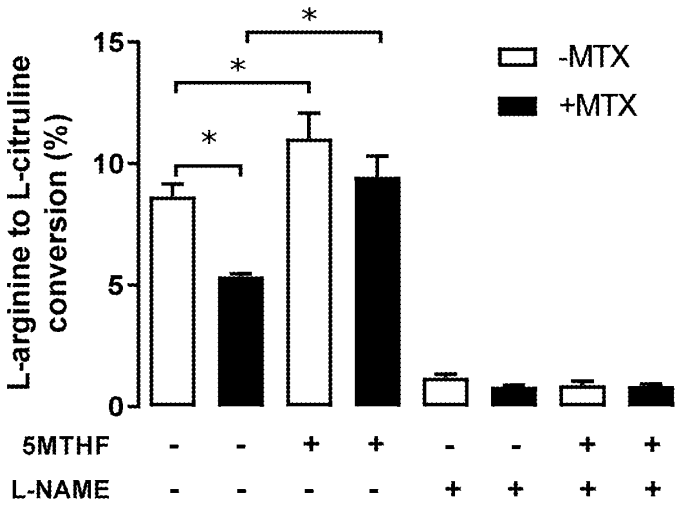

L-arginine to L-citruline conversion (%)
15
10
5
0
*
*
*
-MTX
+MTX
5MTHF - - + + - - + +
L-NAME - - - - + + + +

Figure 35

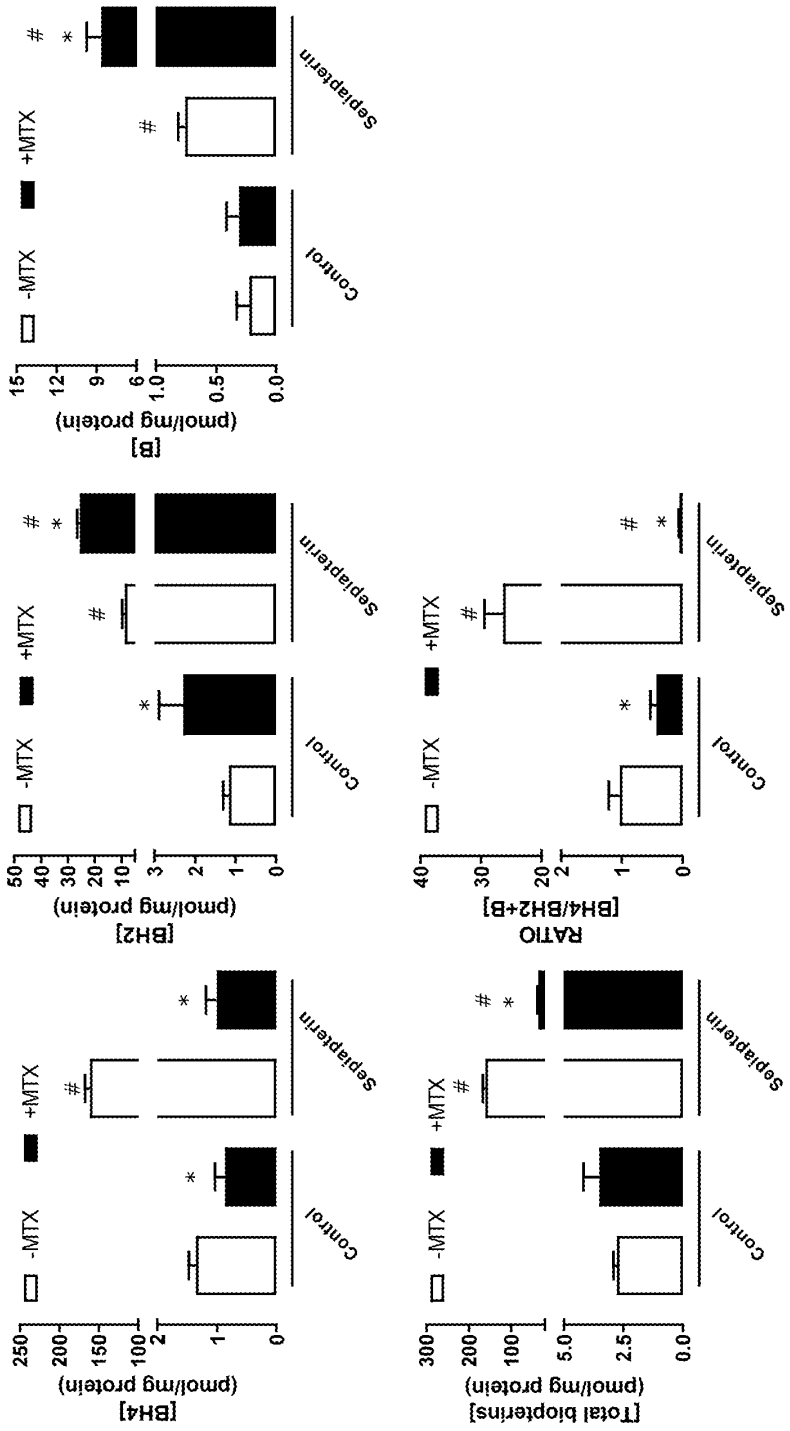

[Total biopterins]
(pmol/mg protein)
[BH4]
(pmol/mg protein)
RATIO
[BH4/BH2+B]
[BH2]
(pmol/mg protein)
[B]
(pmol/mg protein)
-MTX
+MTX
Control
Sepiapterin

Figure 36

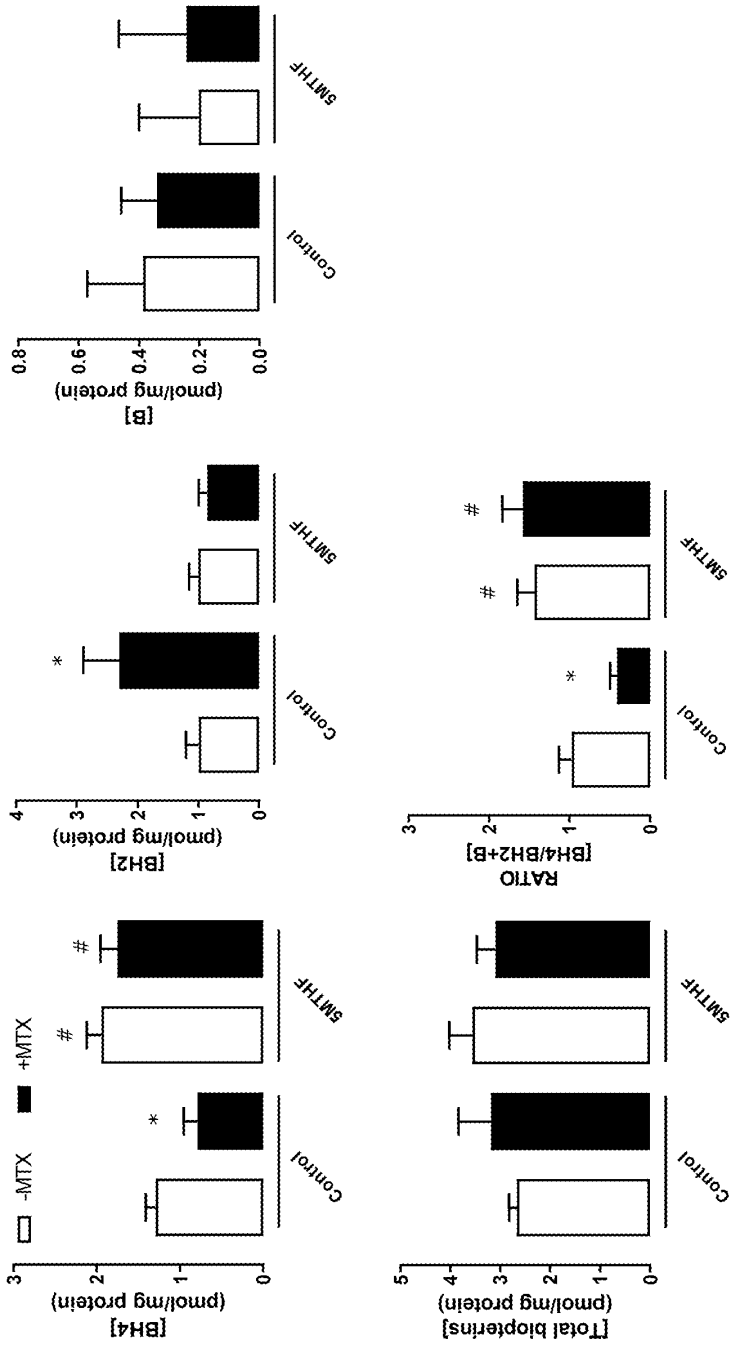

[Total biopterins]
(pmol/mg protein)
RATIO
[BH4/BH2+B]
[BH4]
(pmol/mg protein)
[BH2]
(pmol/mg protein)
[B]
(pmol/mg protein)
-MTX
+MTX
Control
SMTHF
*
#

Figure 37

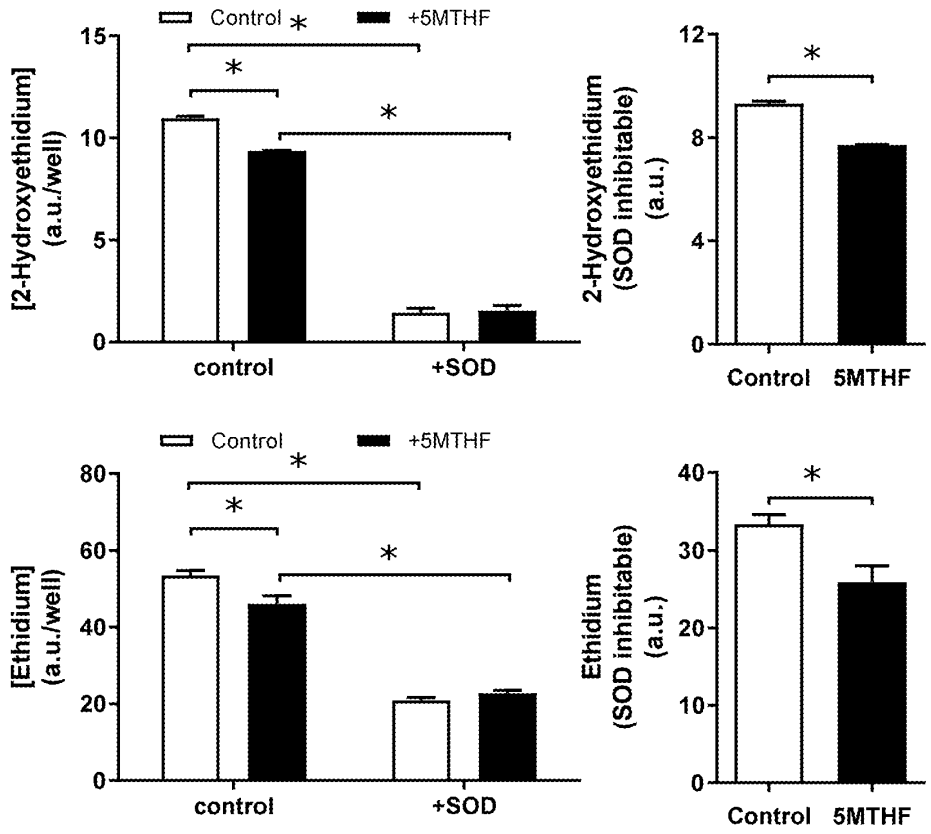

Control
+5MTHF
[2-Hydroxyethidium] (a.u./well)
15
10
5
0
control
+SOD
2-Hydroxyethidium (SOD inhibitable) (a.u.)
12
8
4
0
Control
5MTHF
[Ethidium] (a.u./well)
80
60
40
20
0
control
+SOD
Ethidium (SOD inhibitable) (a.u.)
40
30
20
10
0
Control
5MTHF

Figure 38

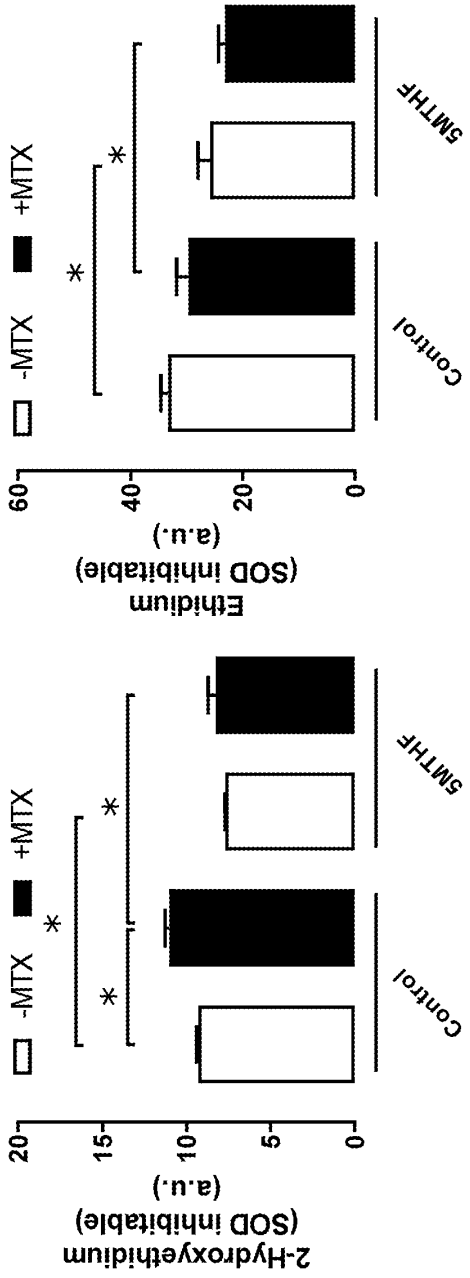

2-Hydroxyethidium
(SOD inhibitable)
(a.u.)
0
5
10
15
20
-MTX
+MTX
*
*
*
Control
5MTHF
Ethidium
(SOD inhibitable)
(a.u.)
0
20
40
60
-MTX
+MTX
*
*
*
Control
5MTHF

Figure 39

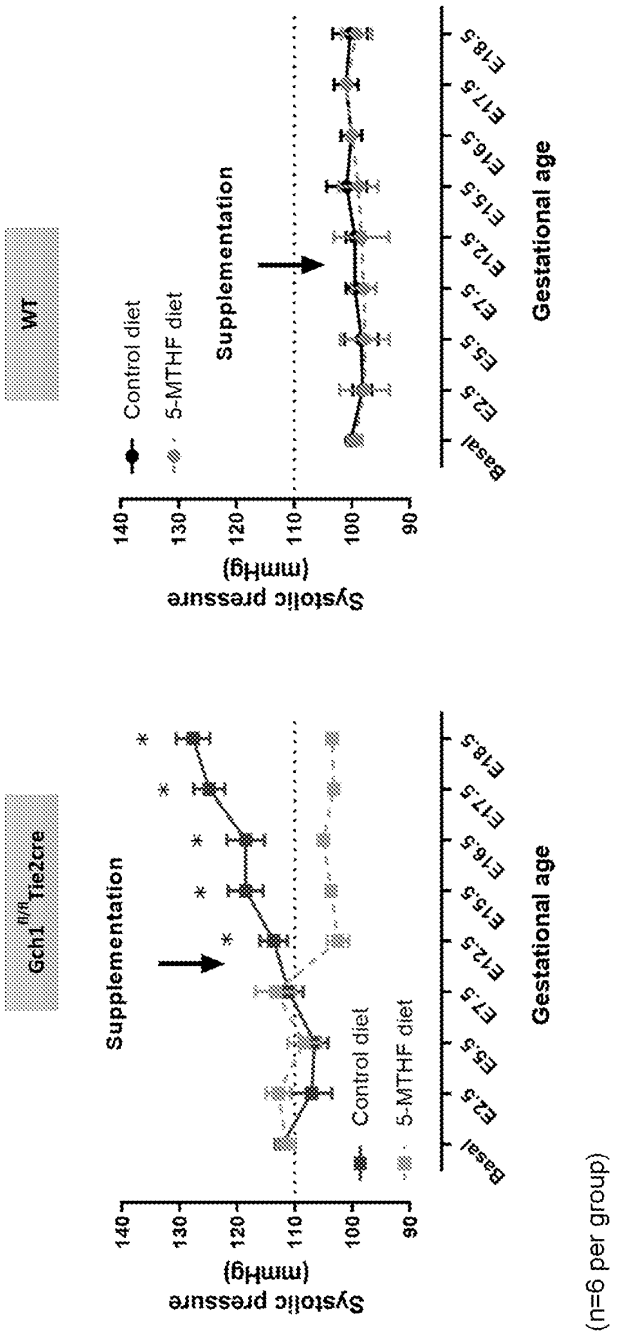

WT
Gch1fl/flTie2cre
Control diet
5-MTHF diet
Supplementation
Systolic pressure (mmHg)
140
130
120
110
100
90
Basal
E2.5
E3.5
E7.5
E12.5
E15.5
E16.5
E17.5
E18.5
Gestational age
(n=6 per group)

Figure 40

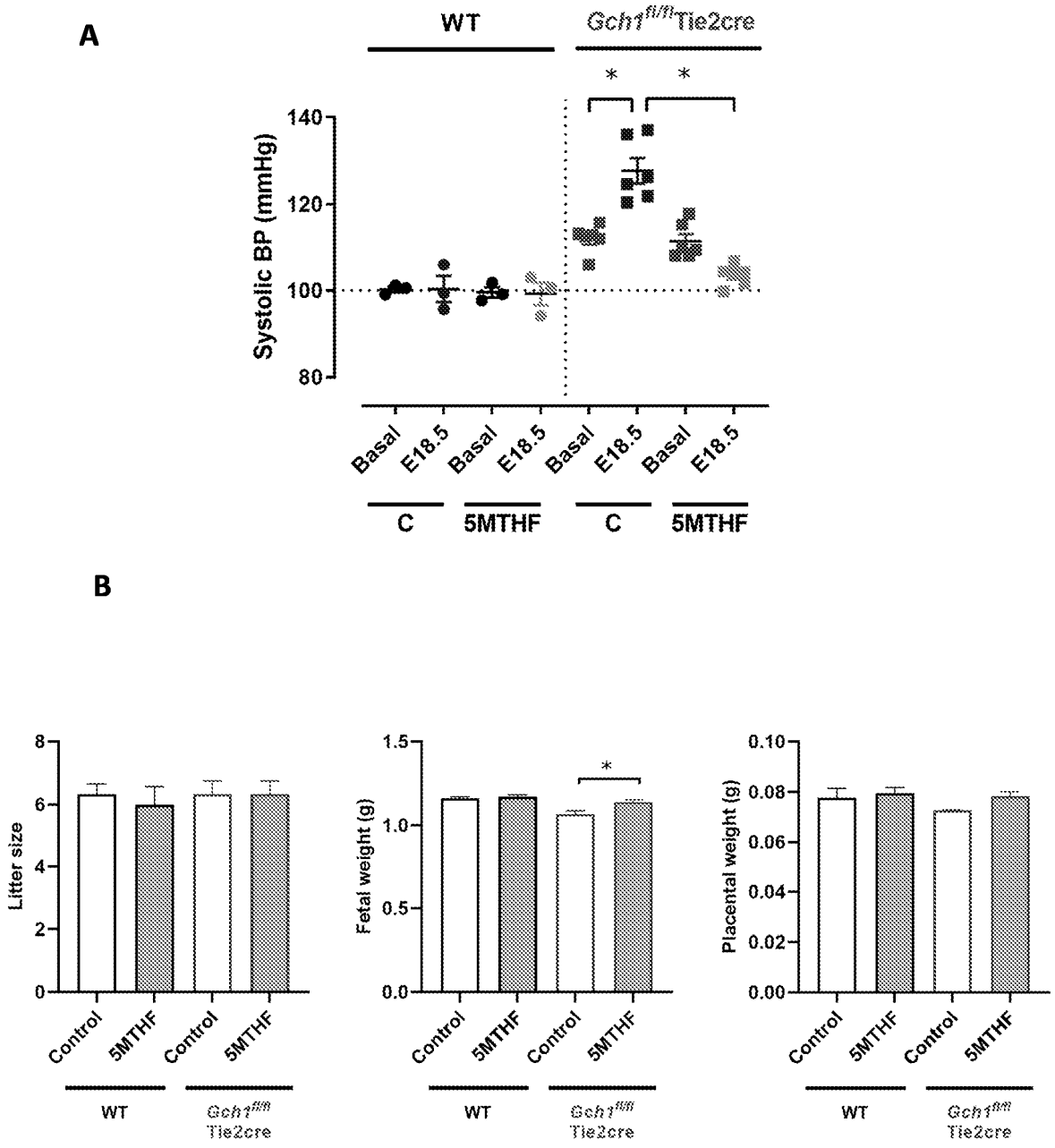
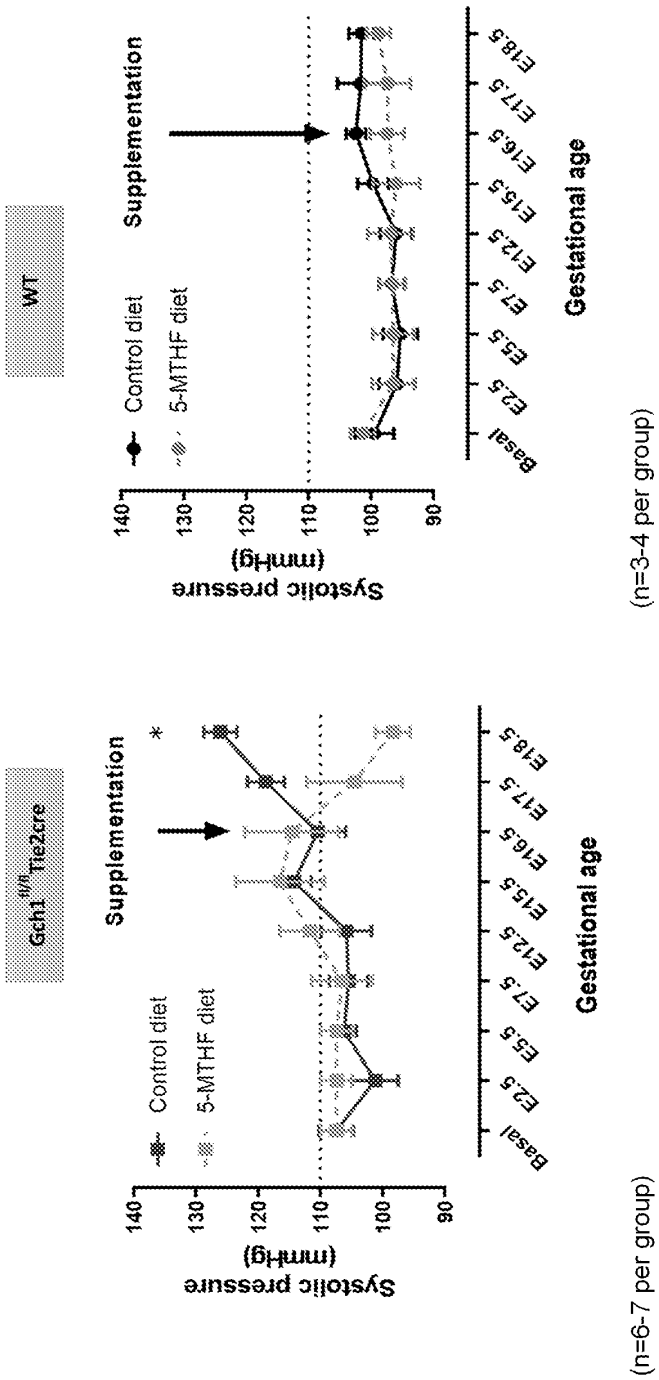

A
WT
Gch1fl/flTie2cre
*
*
Systolic BP (mmHg)
140
120
100
80
Basal
E18.5
Basal
E18.5
Basal
E18.5
Basal
E18.5
C
5MTHF
C
5MTHF
B
Litter size
8
6
4
2
0
Control
5MTHF
Control
5MTHF
WT
Gch1fl/fl Tie2cre
Fetal weight (g)
1.5
1.0
0.5
0.0
*
Control
5MTHF
Control
5MTHF
WT
Gch1fl/fl Tie2cre
Placental weight (g)
0.10
0.08
0.06
0.04
0.02
0.00
Control
5MTHF
Control
5MTHF
WT
Gch1fl/fl Tie2cre

Figure 41


WT
Gch1fl/flTie2cre
Supplementation
Control diet
5-MTHF diet
Systolic pressure (mmHg)
140
130
120
110
100
90
Basal
E2.5
E5.5
E7.5
E12.5
E15.5
E16.5
E17.5
E18.5
Gestational age
*
(n=3-4 per group)
(n=6-7 per group)