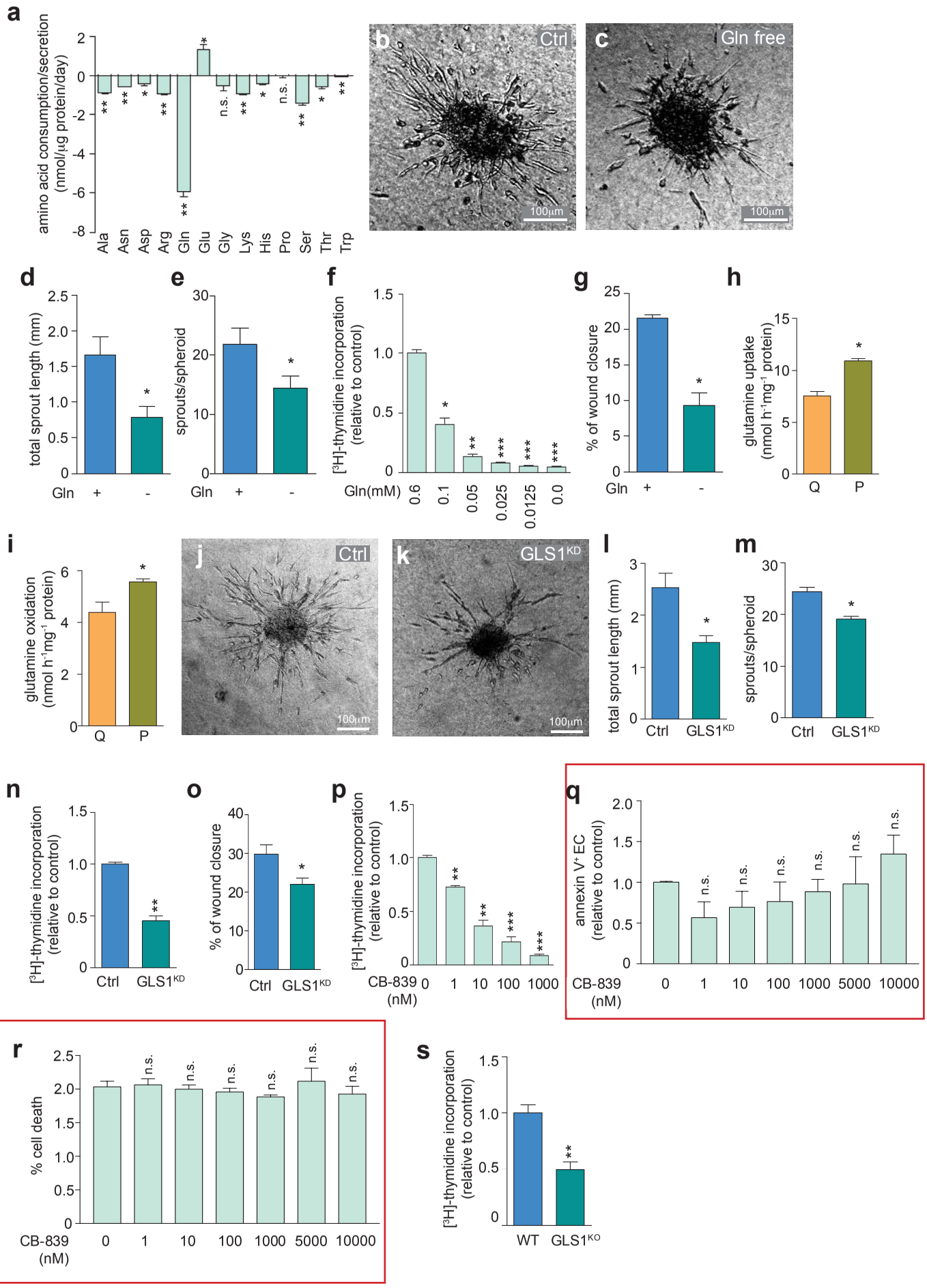
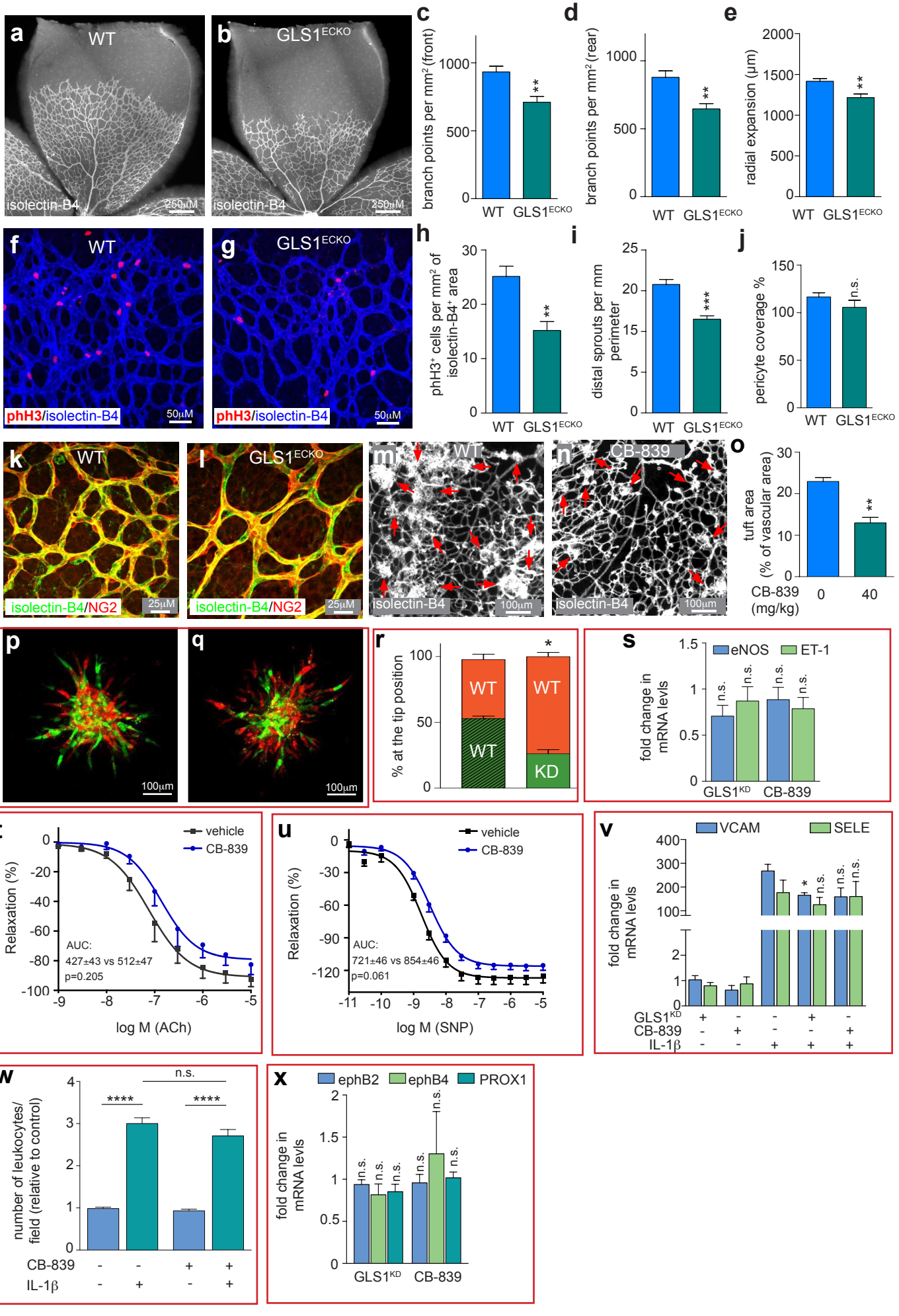
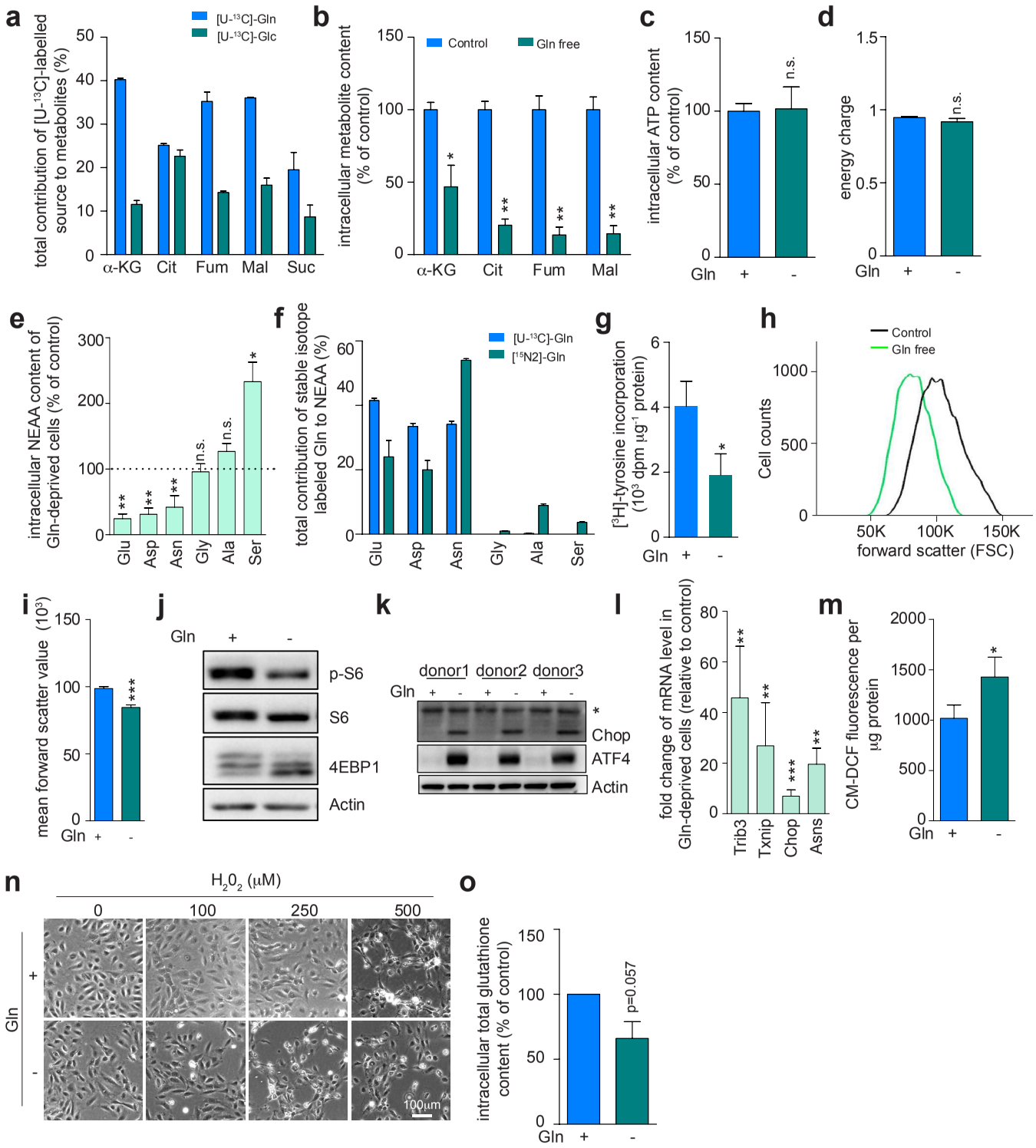
Article:
Huang, H, Vandekeere, S, Kalucka, J et al. (17 more authors) (2017) Role of glutamine and interlinked asparagine metabolism in vessel formation. The EMBO Journal, 36 (16). pp. 2334-2352. ISSN 0261-4189

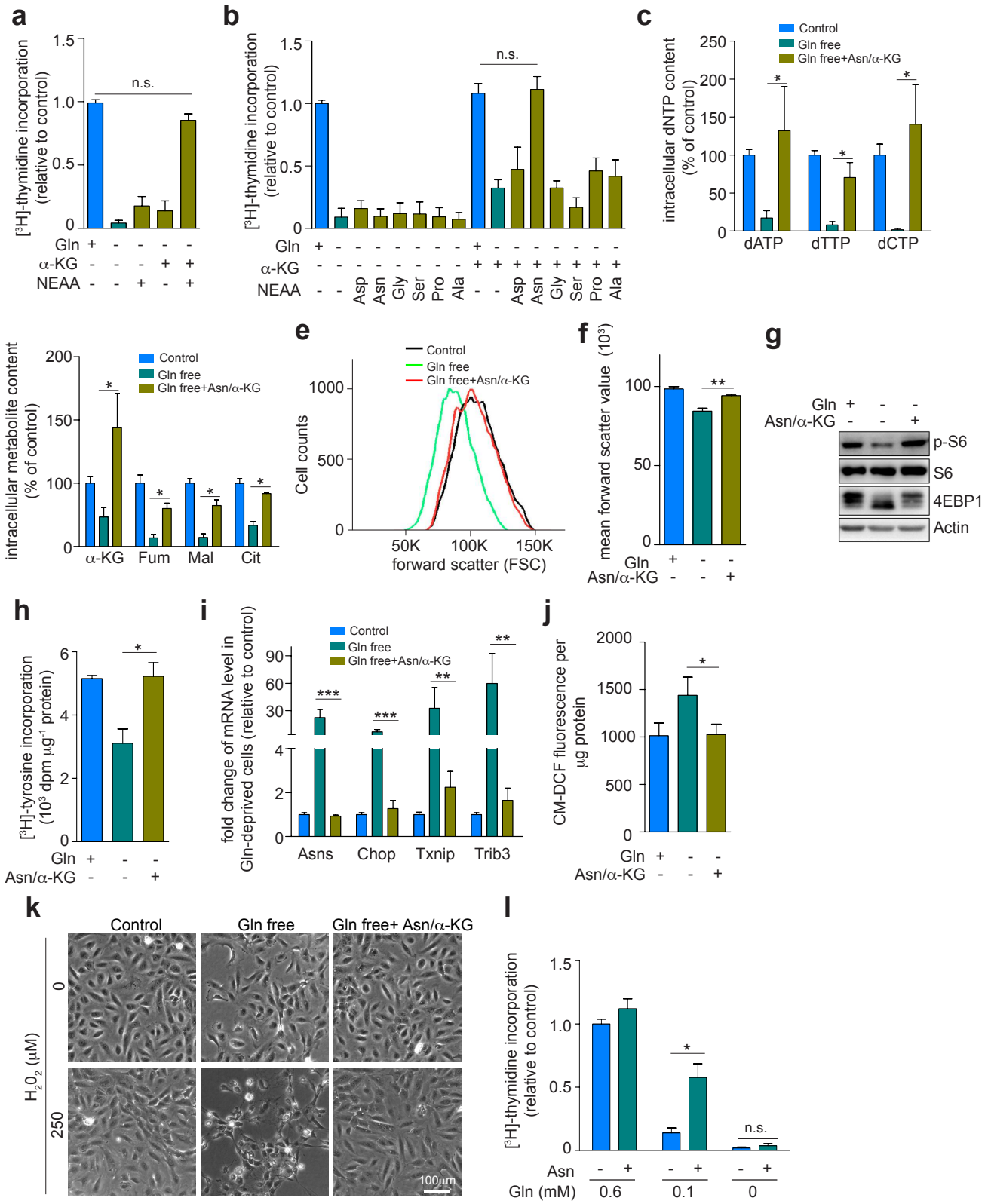
10.15252/embj.201695518


a
amino acid consumption/secretion (nmol/μg protein/day)
Ala Asn Asp Arg Gln Glu Gly Lys His Pro Ser Thr Trp
b Ctrl
c Gln free
100μm
d total sprout length (mm)
Gln + -
e sprouts/spheroid
f [3H]-thymidine incorporation (relative to control)
Gln(mM) 0.6 0.1 0.05 0.025 0.0125 0.0
g % of wound closure
h glutamine uptake (nmol h-1mg-1 protein)
Q P
i glutamine oxidation (nmol h-1mg-1 protein)
j Ctrl
k GLS1KD
l total sprout length (mm)
Ctrl GLS1KD
m sprouts/spheroid
n [3H]-thymidine incorporation (relative to control)
o % of wound closure
p [3H]-thymidine incorporation (relative to control)
CB-839 (nM) 0 1 10 100 1000
q annexin V+ EC (relative to control)
CB-839 (nM) 0 1 10 100 1000 5000 10000
r % cell death
CB-839 (nM) 0 1 10 100 1000 5000 10000
s [3H]-thymidine incorporation (relative to control)
WT GLS1KO

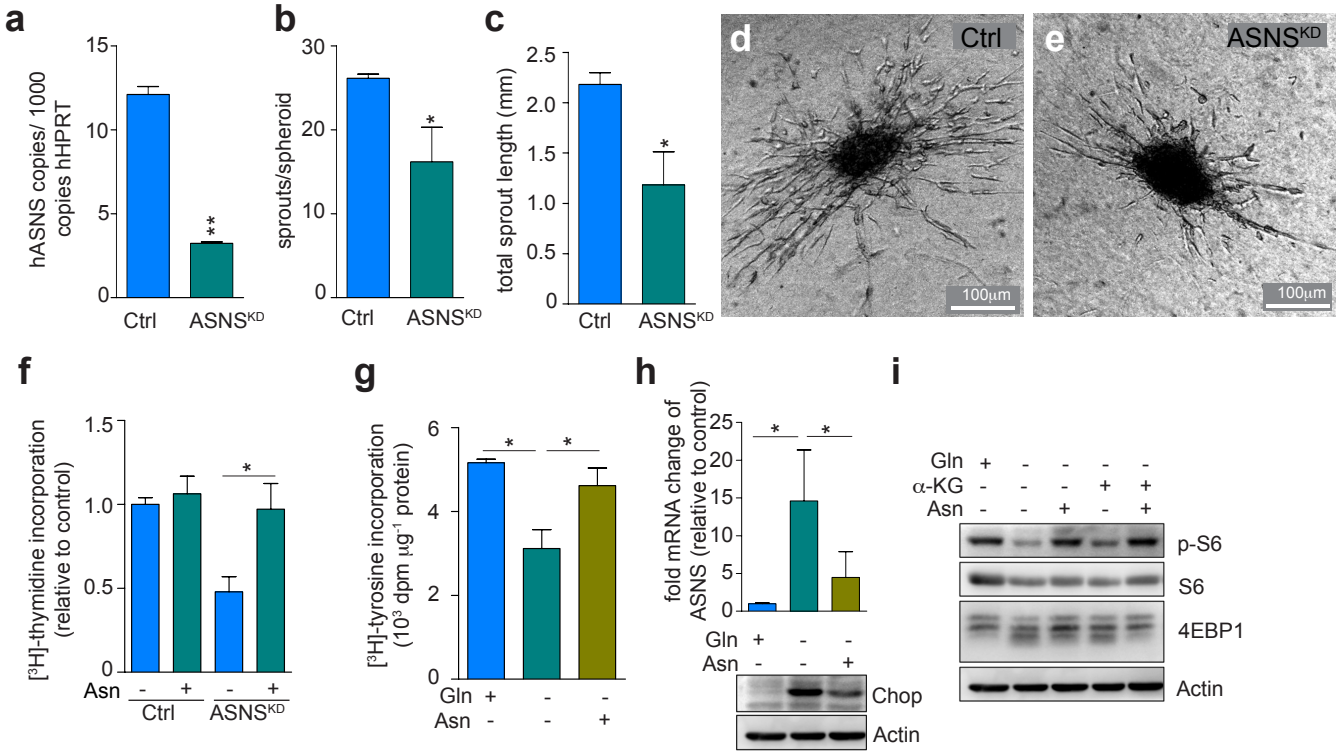

a
WT
isolectin-B4
250μM
b
GLS1ECKO
isolectin-B4
250μM
c
branch points per mm2 (front)
1000
500
0
**
WT
GLS1ECKO
d
branch points per mm2 (rear)
1000
500
0
**
WT
GLS1ECKO
e
radial expansion (μm)
2000
1500
1000
500
0
**
WT
GLS1ECKO
f
WT
phH3/isolectin-B4
50μM
g
GLS1ECKO
phH3/isolectin-B4
50μM
h
phH3+ cells per mm2 of isolectin-B4+ area
30
20
10
0
**
WT
GLS1ECKO
i
distal sprouts per mm perimeter
25
20
15
5
0
***
WT
GLS1ECKO
j
pericyte coverage %
150
100
50
0
n.s.
WT
GLS1ECKO
k
WT
isolectin-B4/NG2
25μM
l
GLS1ECKO
isolectin-B4/NG2
25μM
m
WT
isolectin-B4
100μm
n
CB-839
isolectin-B4
100μm
o
tuft area (% of vascular area)
30
20
10
0
**
CB-839 (mg/kg)
0
40
p
100μm
q
100μm
r
% at the tip position
100
50
0
WT
WT
WT
KD
*
s
fold change in mRNA levls
1.5
1
0.5
0
eNOS
ET-1
n.s.
n.s.
n.s.
n.s.
n.s.
GLS1KD
CB-839
t
vehicle
CB-839
Relaxation (%)
0
-20
-40
-60
-80
-100
AUC:
427±43 vs 512±47
p=0.205
-9
-8
-7
-6
-5
log M (ACh)
u
vehicle
CB-839
Relaxation (%)
0
-30
-60
-90
-120
AUC:
721±46 vs 854±46
p=0.061
-11
-10
-9
-8
-7
-6
-5
log M (SNP)
v
VCAM
SELE
fold change in mRNA levls
400
300
200
100
2
1
0
*
n.s.
n.s.
n.s.
n.s.
GLS1KD
CB-839
IL-1β
w
number of leukocytes/ field (relative to control)
4
3
2
1
0
n.s.
****
****
CB-839
IL-1β
x
ephB2
ephB4
PROX1
fold change in mRNA levls
2
1.5
1
0.5
0
n.s.
n.s.
n.s.
n.s.
n.s.
n.s.
GLS1KD
CB-839



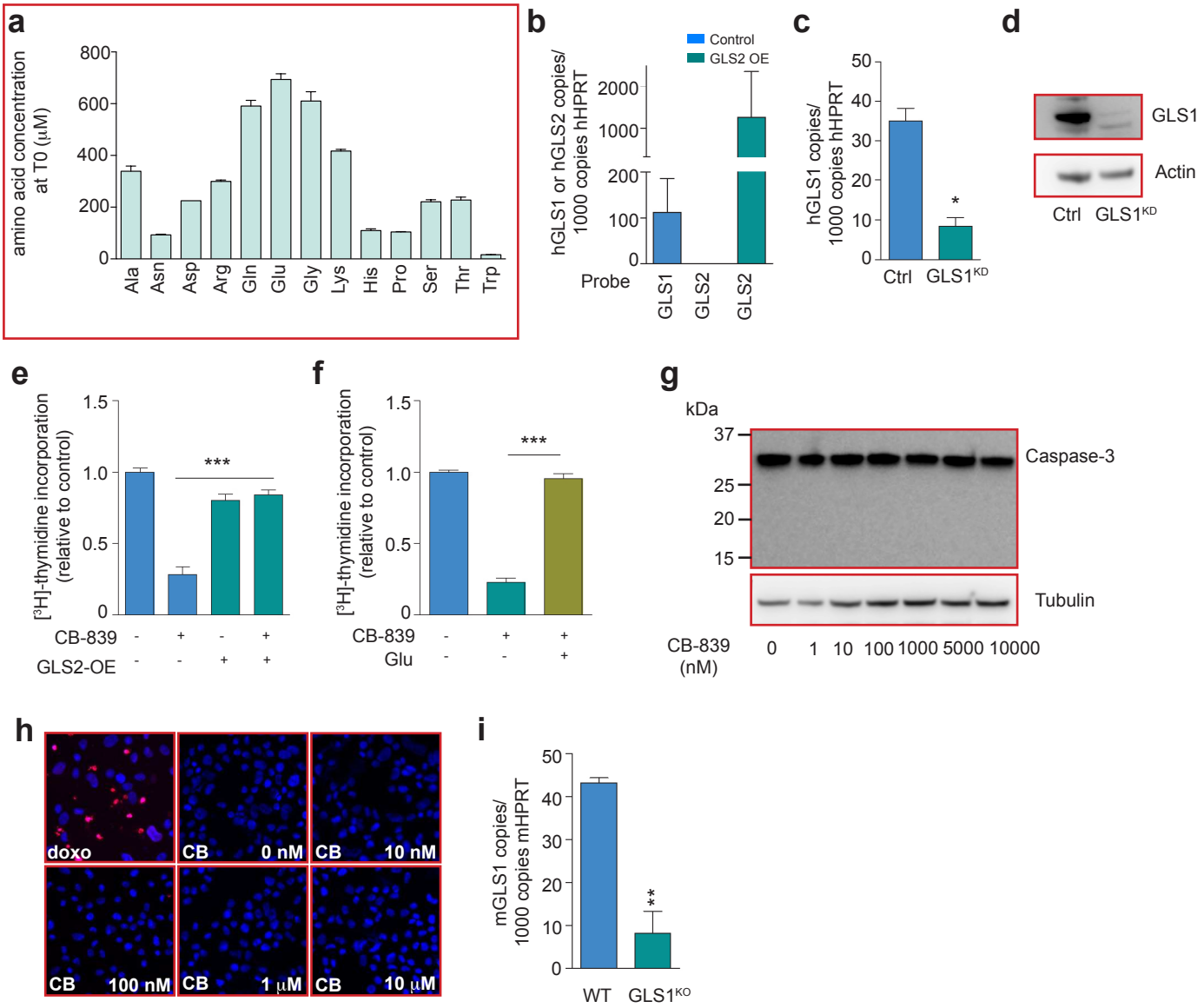
Huang *et.al* Figure-3

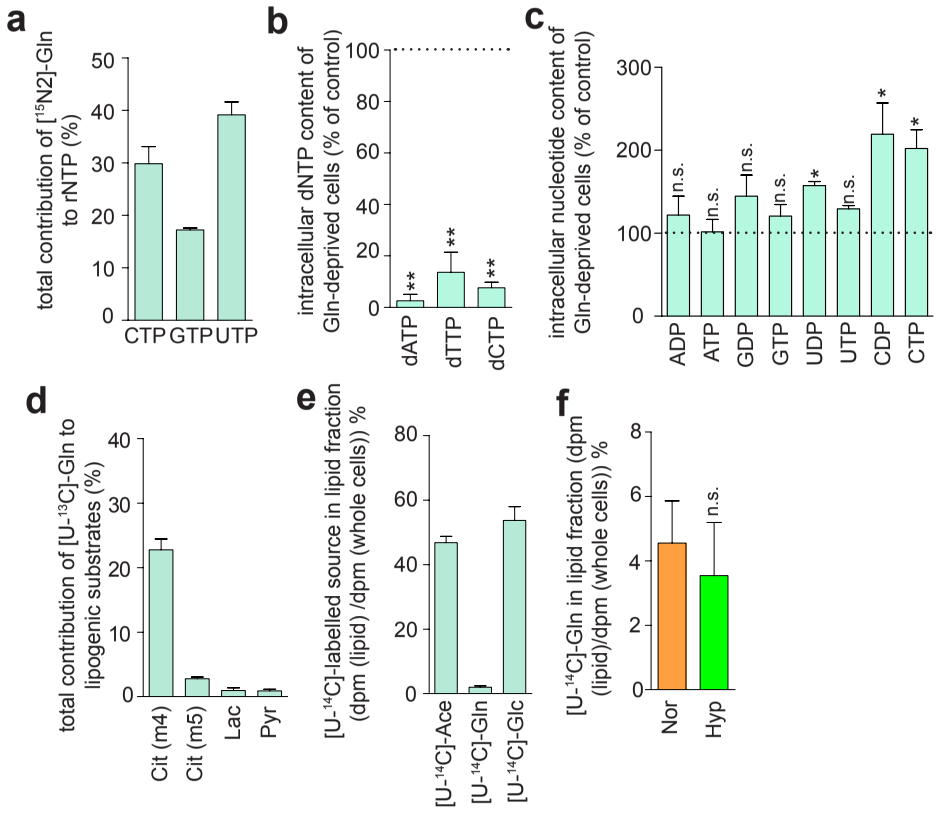


Huang *et.al* Figure-4

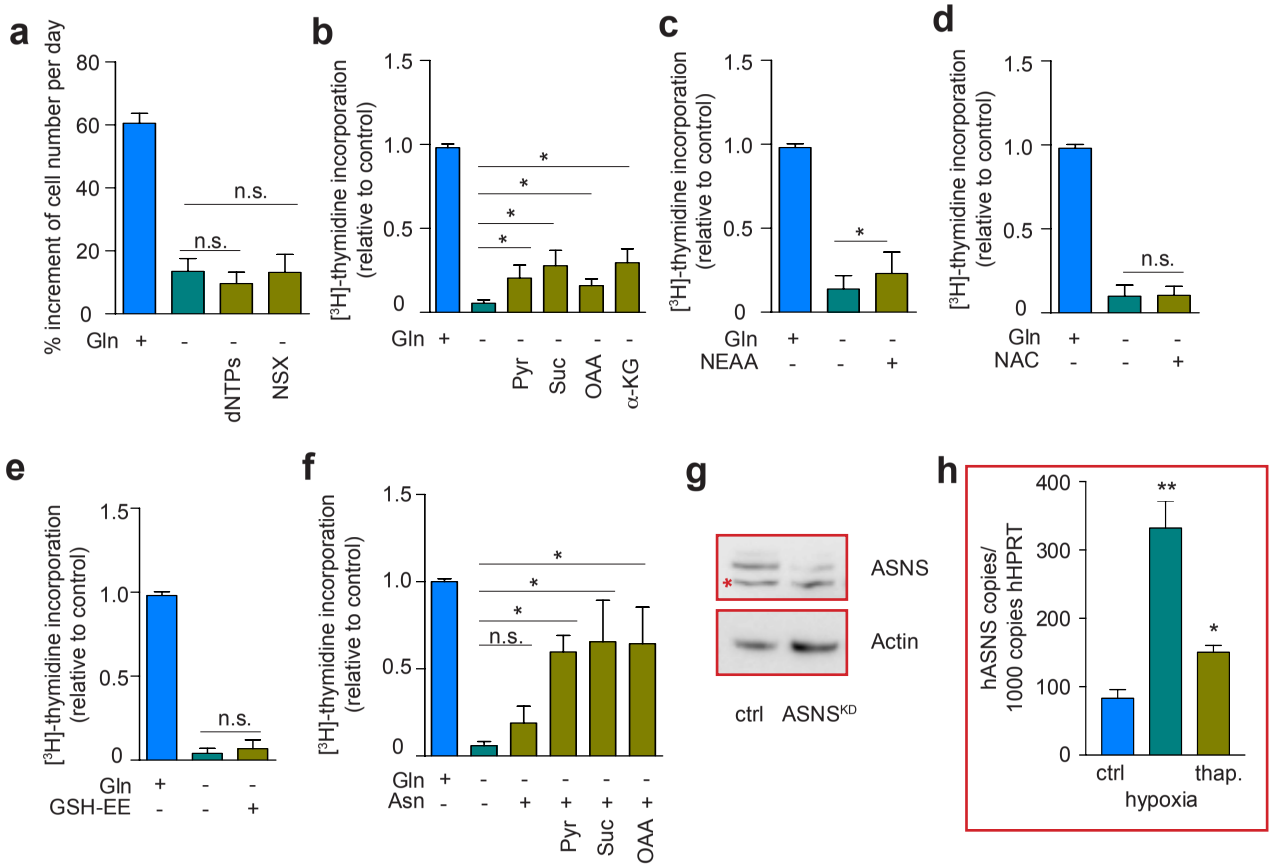


Huang *et.al* Figure-5


a
amino acid concentration at T0 (μM)
800
600
400
200
0
Ala
Asn
Asp
Arg
Gln
Glu
Gly
Lys
His
Pro
Ser
Thr
Trp
b
Control
GLS2 OE
hGLS1 or hGLS2 copies/ 1000 copies hHPRT
2000
1000
200
100
0
Probe
GLS1
GLS2
GLS2
c
hGLS1 copies/ 1000 copies hHPRT
50
40
30
20
10
0
*
Ctrl
GLS1KD
d
GLS1
Actin
Ctrl
GLS1KD
e
[3H]-thymidine incorporation (relative to control)
1.5
1.0
0.5
0
***
CB-839
GLS2-OE
f
[3H]-thymidine incorporation (relative to control)
1.5
1.0
0.5
0
***
CB-839
Glu
g
kDa
37
25
20
15
Caspase-3
Tubulin
CB-839 (nM)
0
1
10
100
1000
5000
10000
h
doxo
CB 0 nM
CB 10 nM
CB 100 nM
CB 1 μM
CB 10 μM
i
mGLS1 copies/ 1000 copies mHPRT
50
40
30
20
10
0
**
WT
GLS1KO

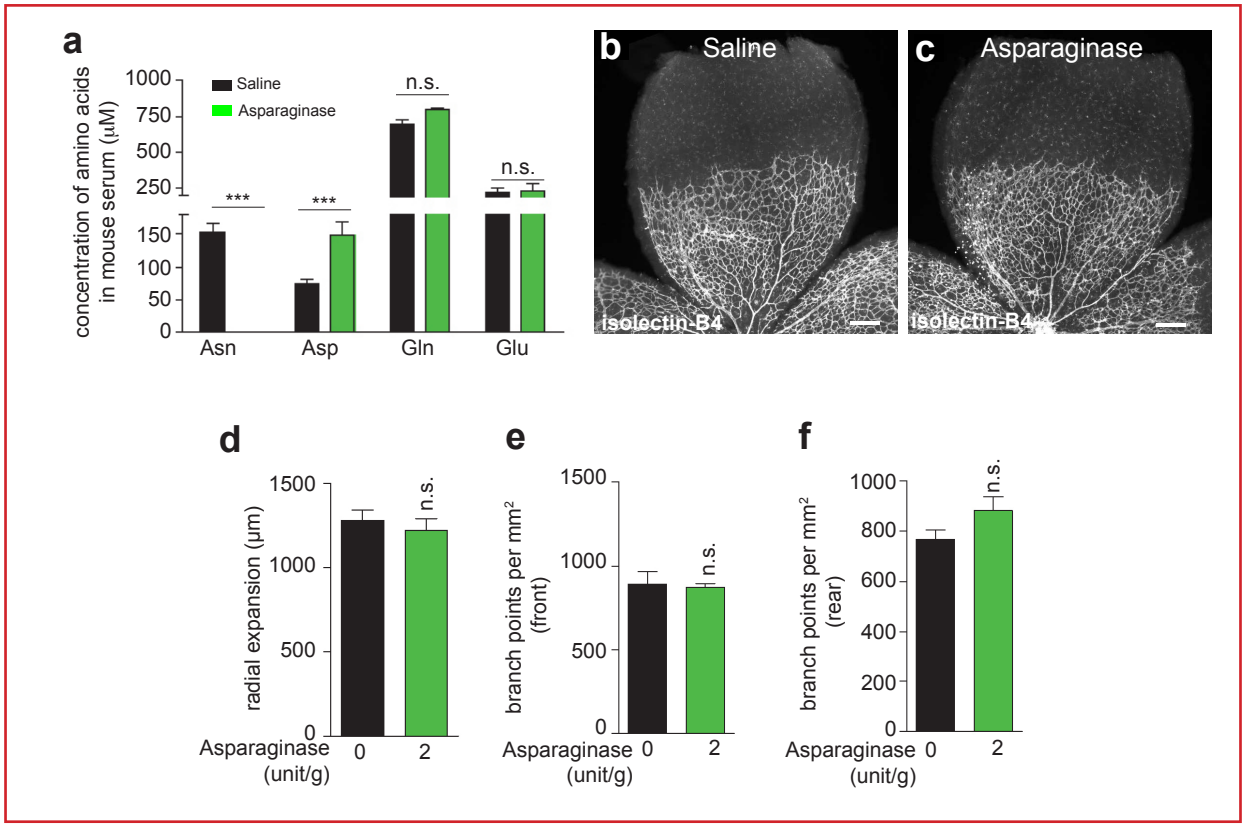


Huang *et.al* Figure-S2



Huang *et.al* Figure-S3

scale bar =250 micron



Huang *et.al* Figure-S4