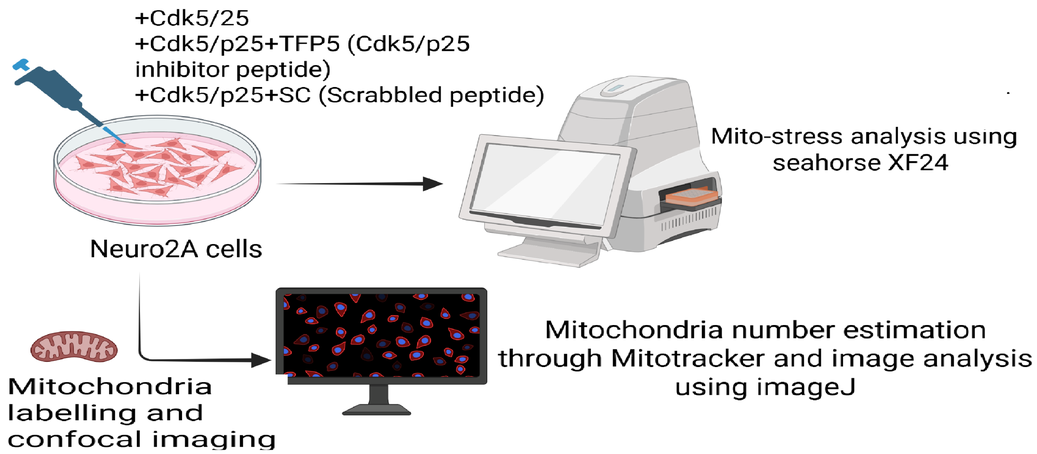
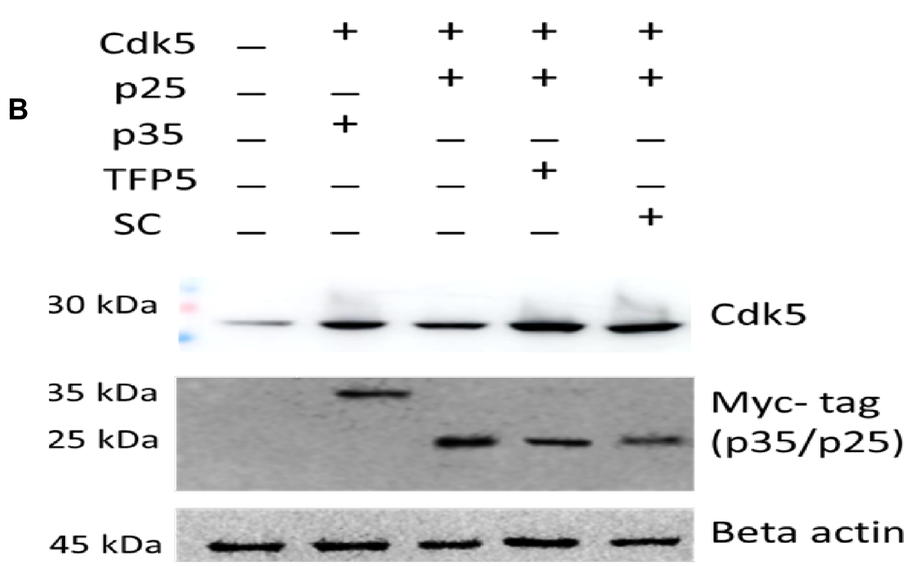
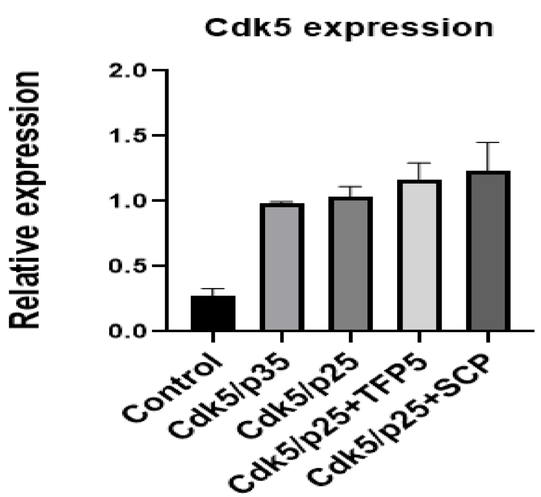
A



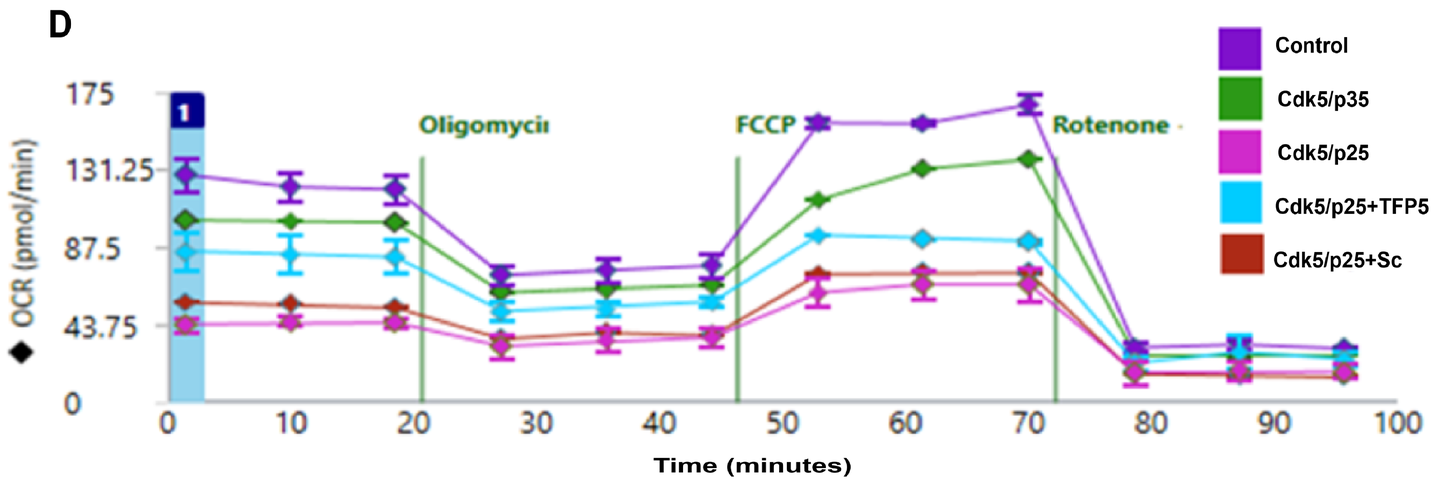
B



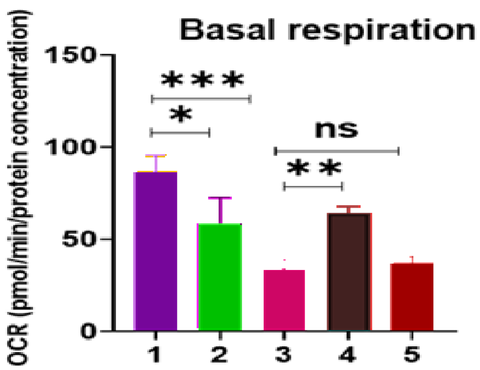
C



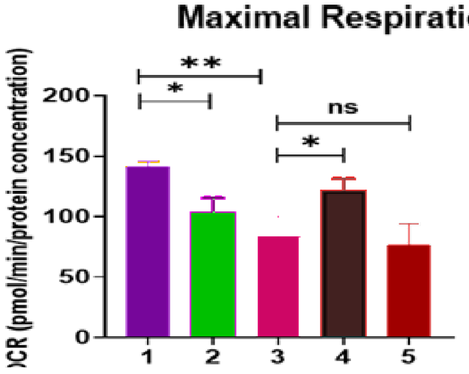
D



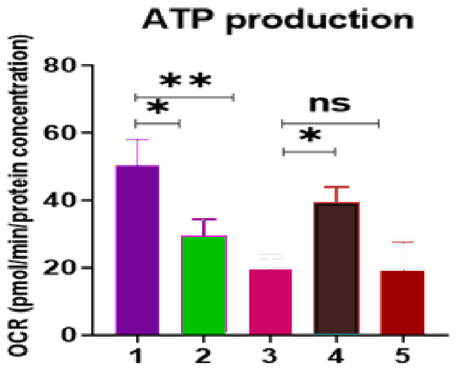
E



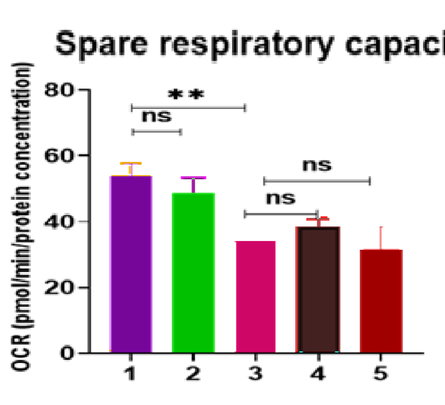
F



G

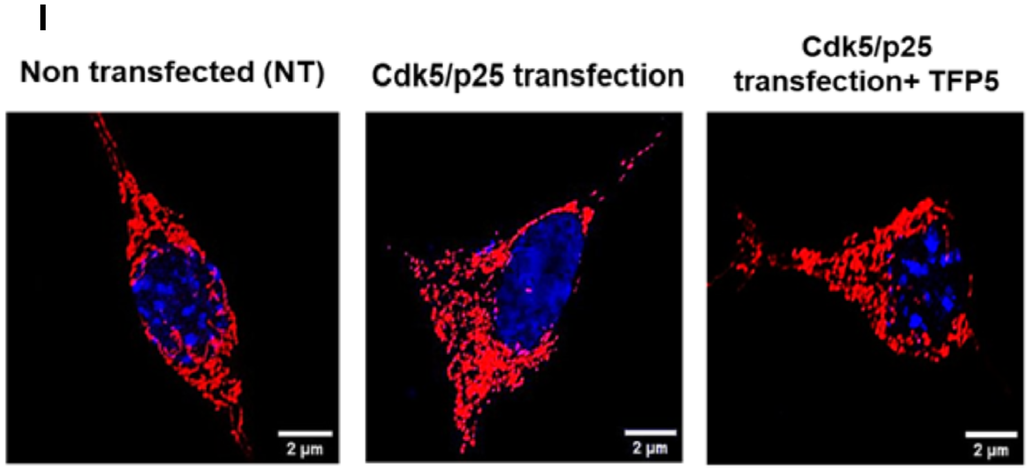


H

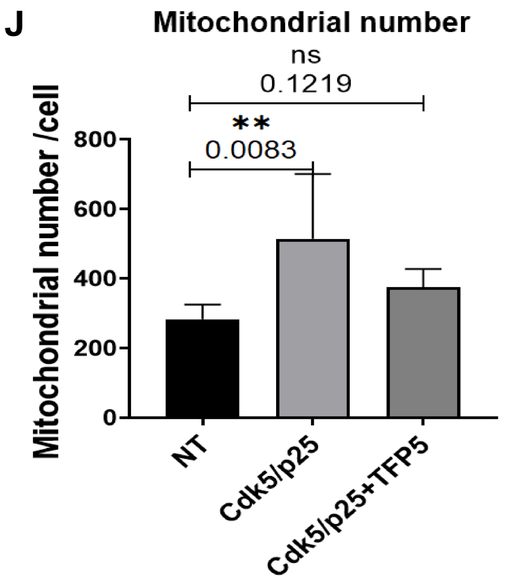


1. Control
2. Cdk5/p35
3. Cdk5/p25
4. Cdk5/p25+TFP5
5. Cdk5/p25+SC

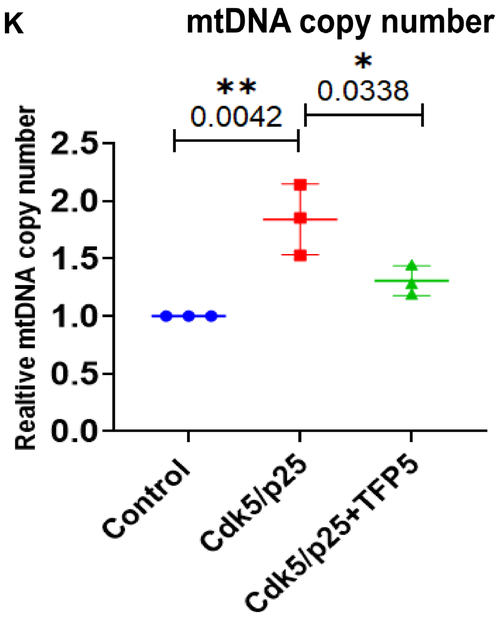
I



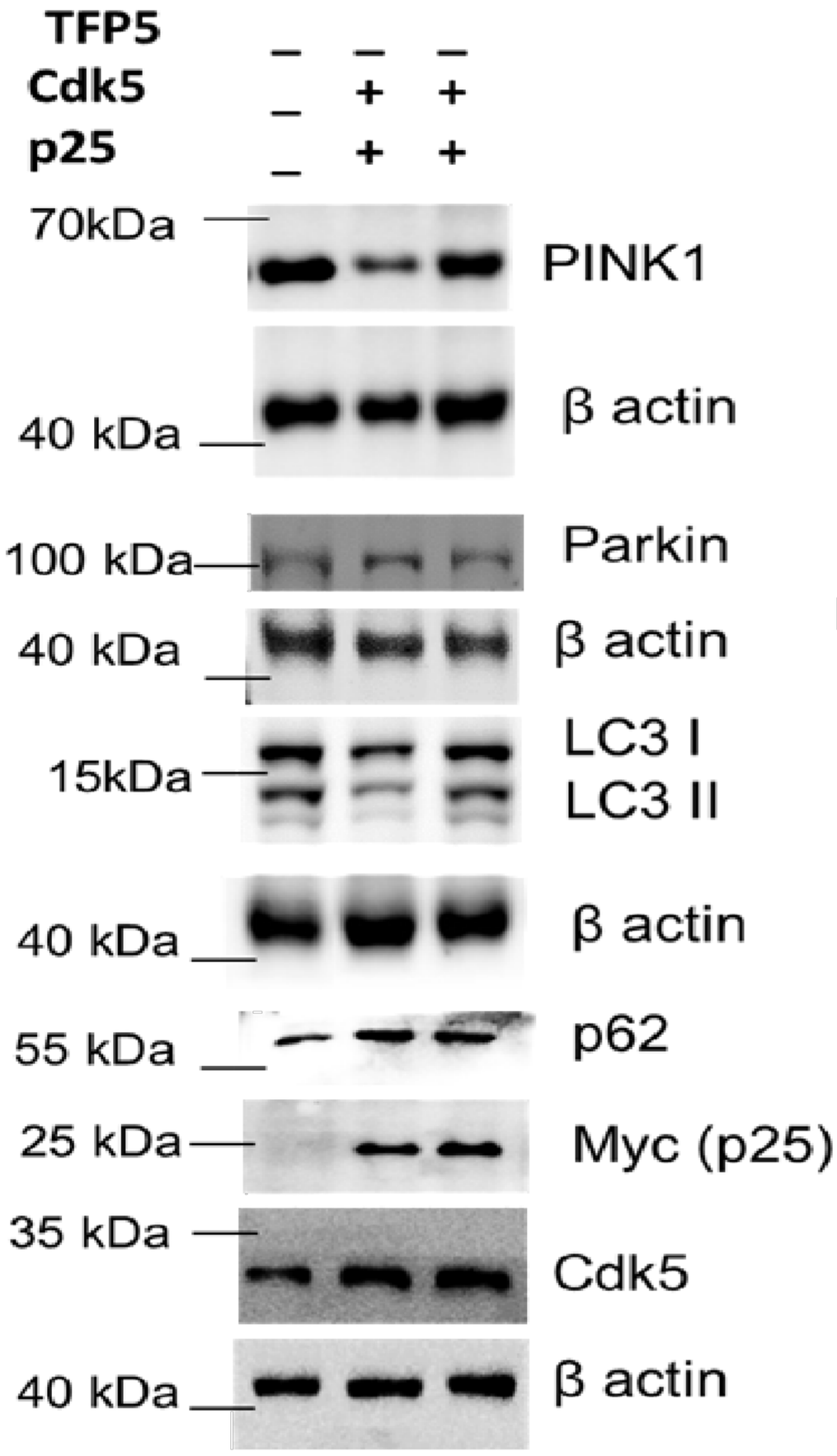
J



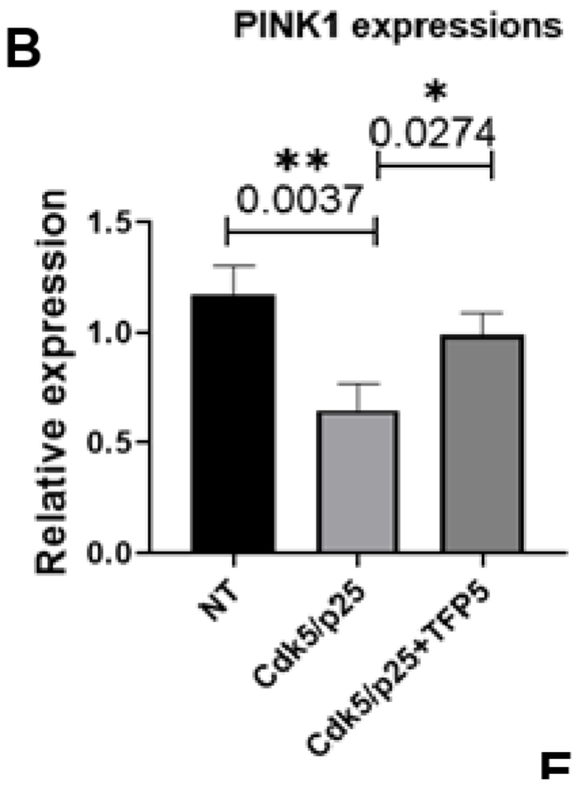
K



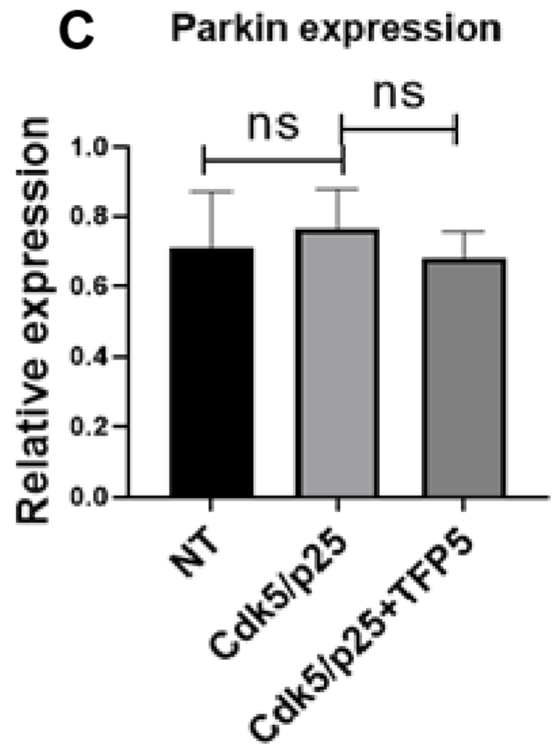
A


TFP5
Cdk5
p25
70kDa
PINK1
40 kDa
β actin
100 kDa
Parkin
40 kDa
β actin
15kDa
LC3 I
LC3 II
40 kDa
β actin
55 kDa
p62
25 kDa
Myc (p25)
35 kDa
Cdk5
40 kDa
β actin

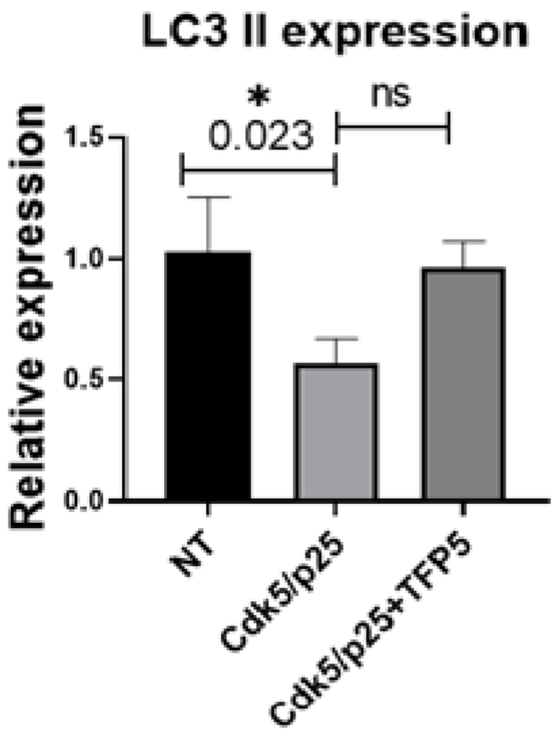
B


PINK1 expressions
Relative expression
0.0037
**
0.0274
*
NT
Cdk5/p25
Cdk5/p25+TFP5

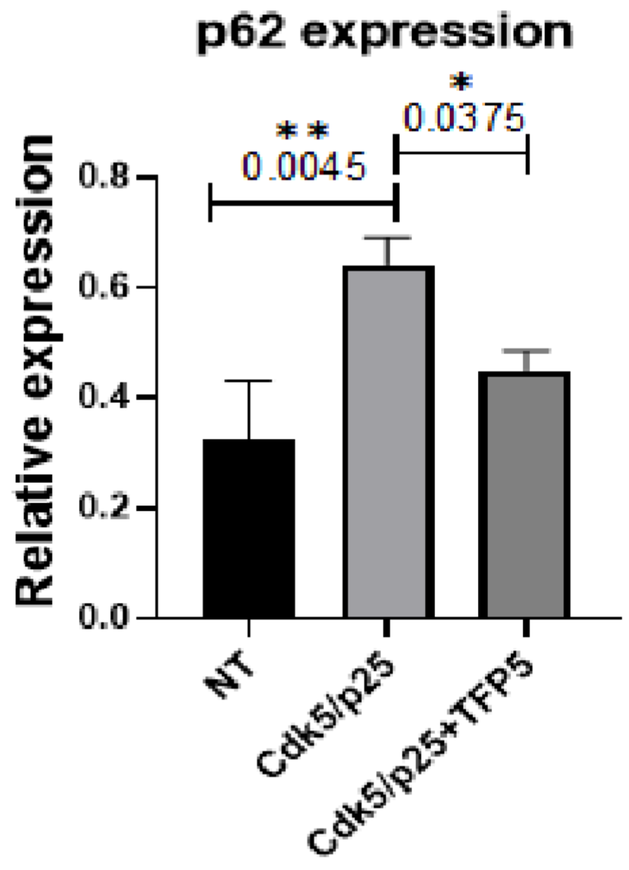
C


Parkin expression
Relative expression
ns
ns
NT
Cdk5/p25
Cdk5/p25+TFP5

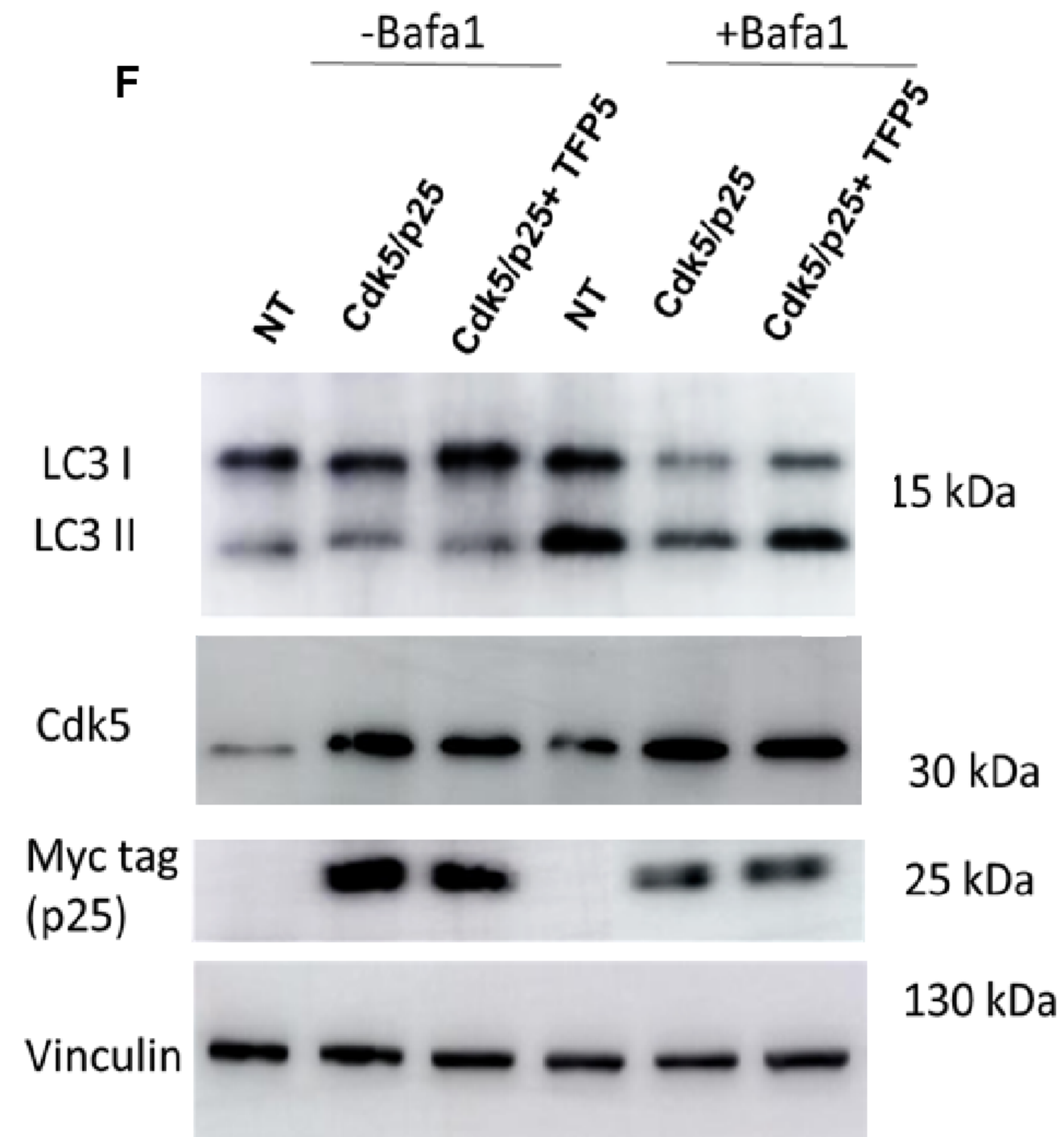
D


LC3 II expression
Relative expression
0.023
*
ns
NT
Cdk5/p25
Cdk5/p25+TFP5

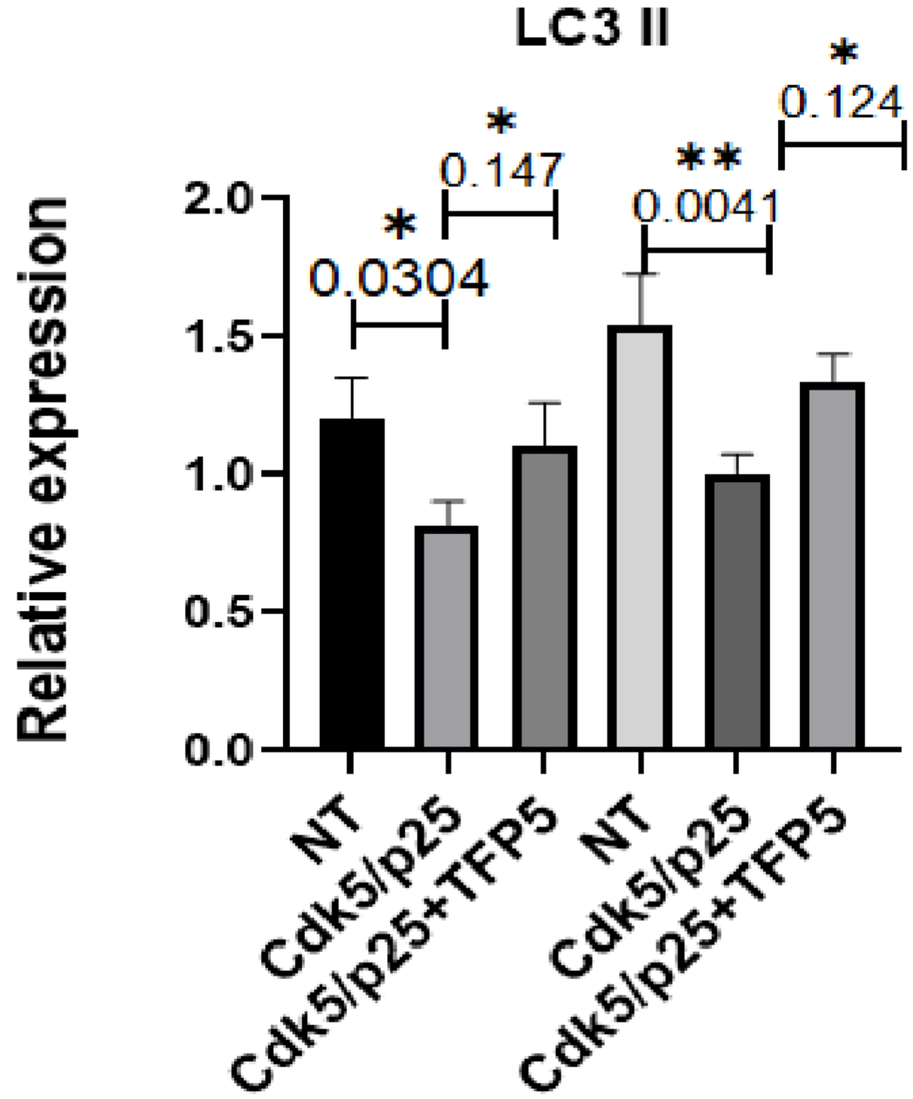
E

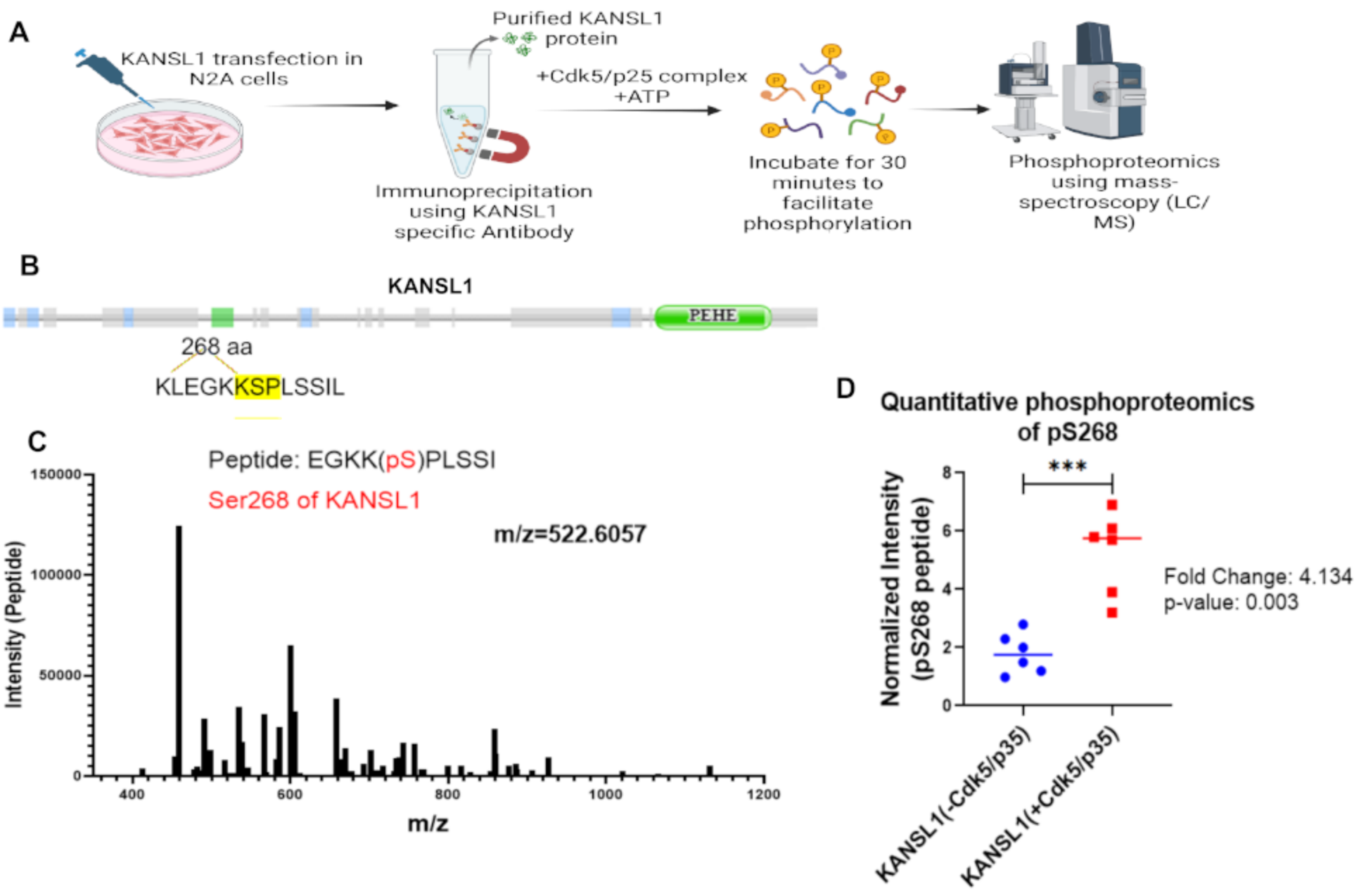

p62 expression
Relative expression
0.0045
**
0.0375
*
NT
Cdk5/p25
Cdk5/p25+TFP5

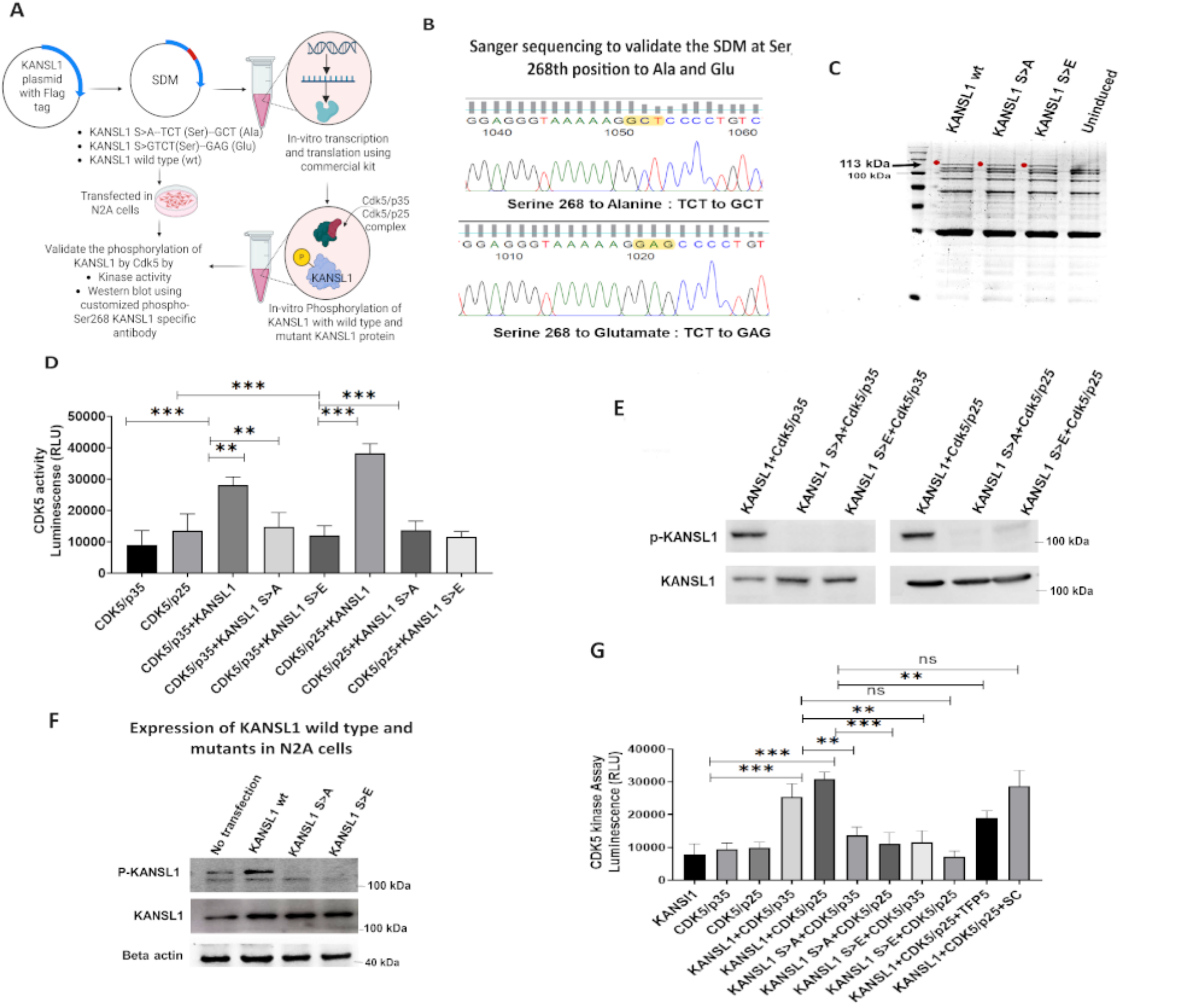
F

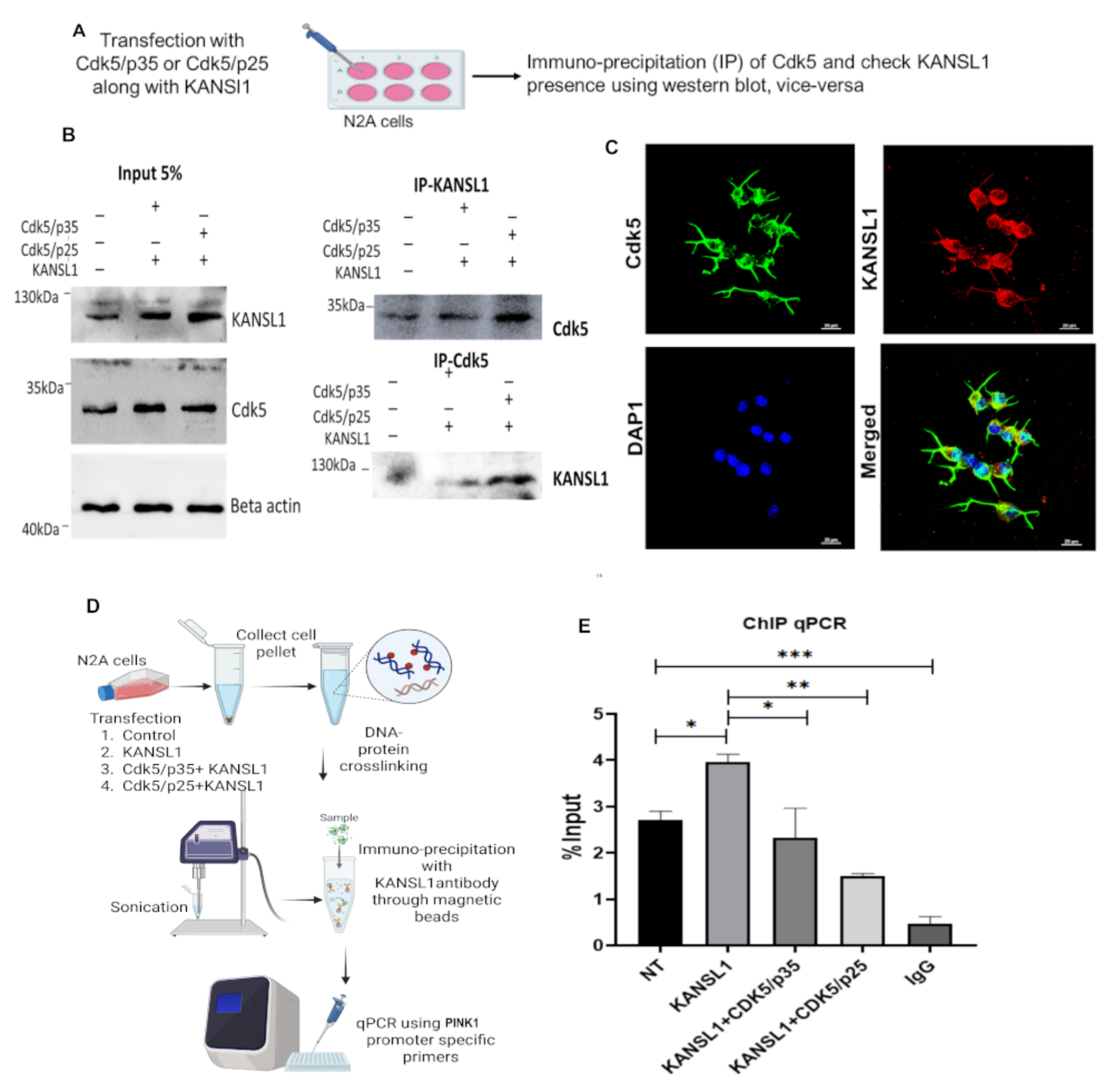

-Bafa1
+Bafa1
NT
Cdk5/p25
Cdk5/p25+ TFP5
NT
Cdk5/p25
Cdk5/p25+ TFP5
LC3 I
LC3 II
15 kDa
Cdk5
30 kDa
Myc tag
(p25)
25 kDa
Vinculin
130 kDa

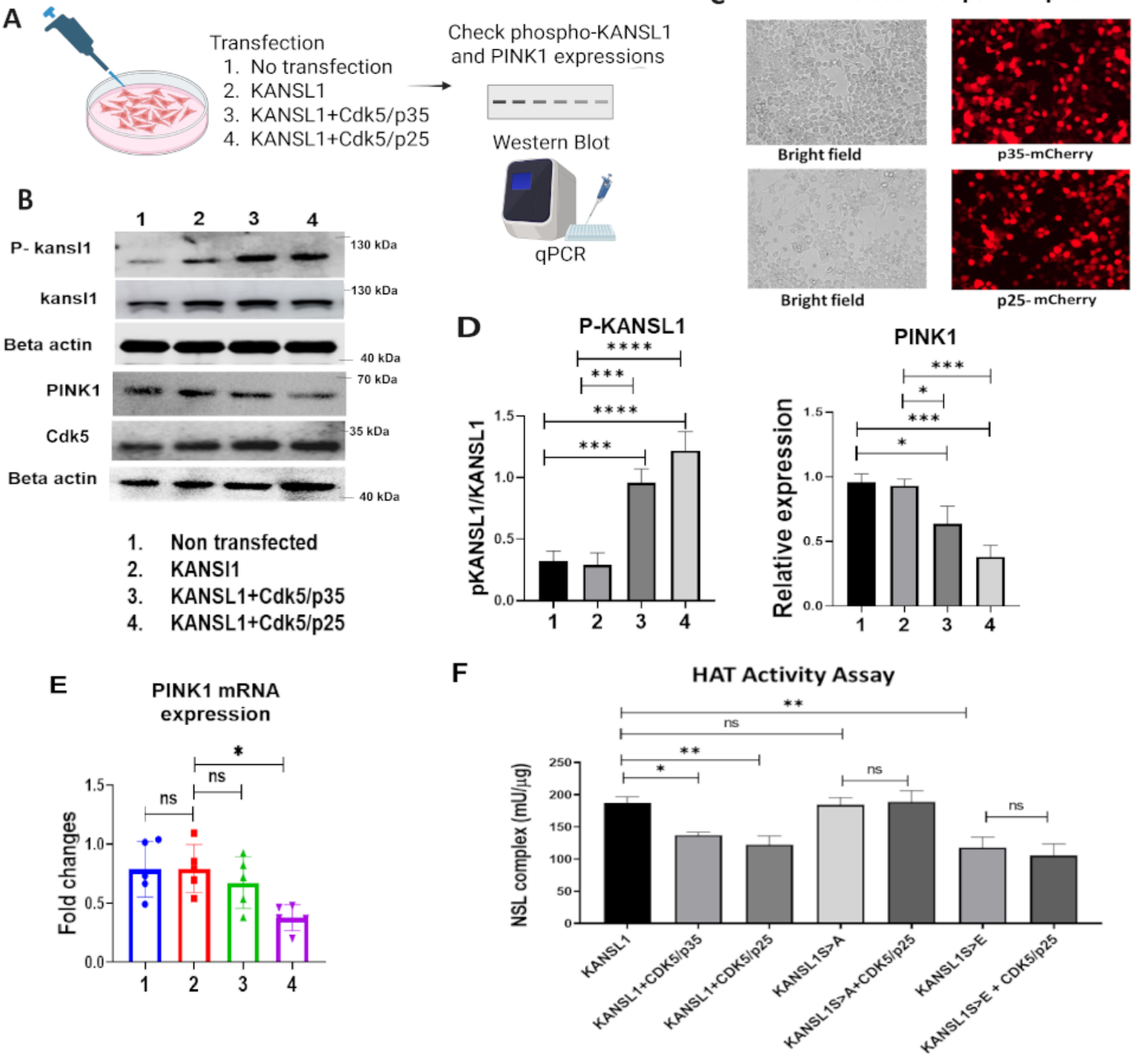
G

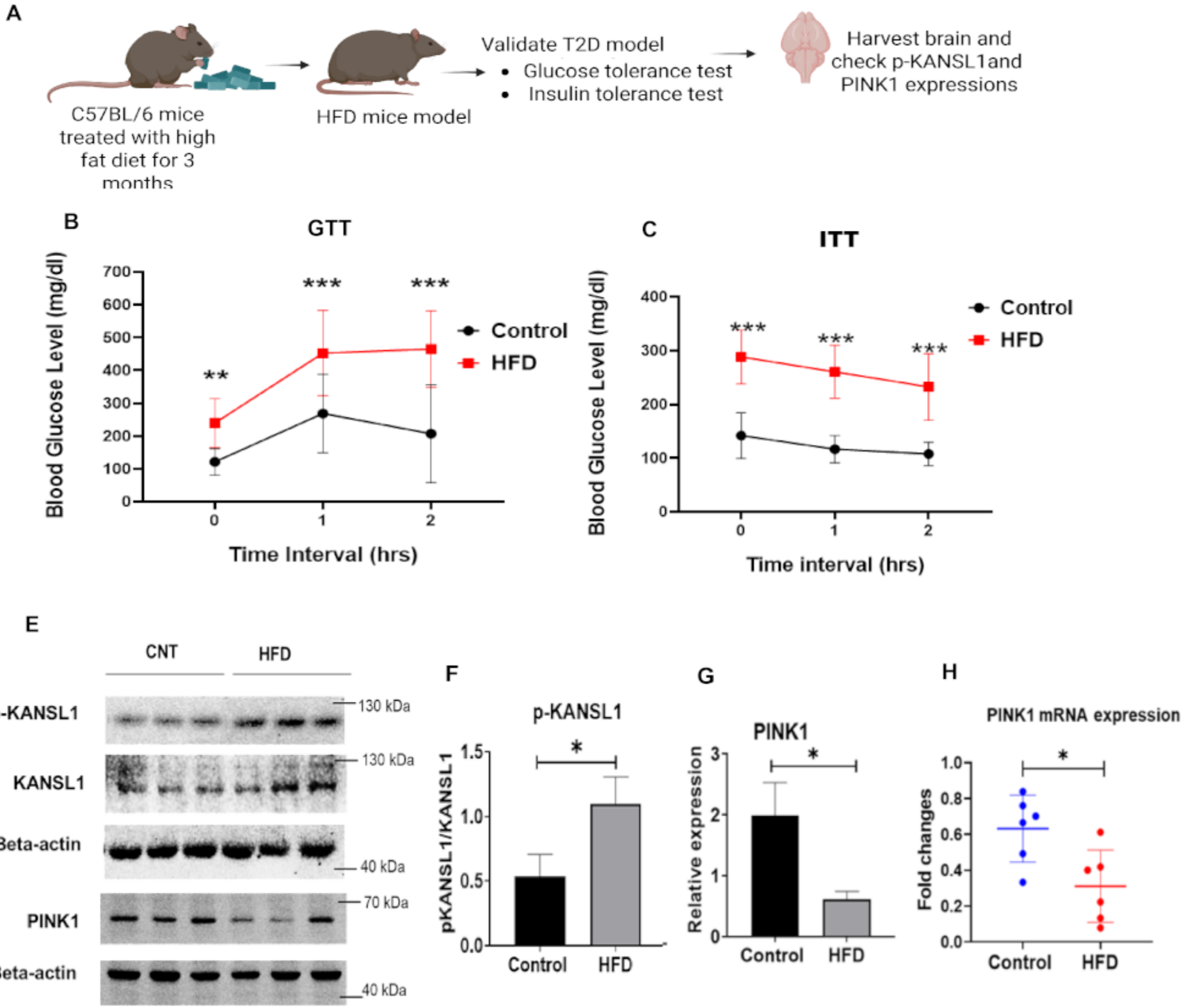

LC3 II
Relative expression
0.0304
*
0.147
*
0.0041
**
0.124
*
NT
Cdk5/p25
Cdk5/p25+TFP5
NT
Cdk5/p25
Cdk5/p25+TFP5
-Bafa1
+Bafa1


A
KANSL1 transfection in N2A cells
Purified KANSL1 protein
+Cdk5/p25 complex +ATP
Immunoprecipitation using KANSL1 specific Antibody
Incubate for 30 minutes to facilitate phosphorylation
Phosphoproteomics using mass-spectroscopy (LC/MS)
B
KANSL1
PEHE
268 aa
KLEGKKSPLSSIL
C
Peptide: EGKK(pS)PLSSI
Ser268 of KANSL1
m/z=522.6057
Intensity (Peptide)
150000
100000
50000
0
400
600
800
1000
1200
m/z
D
Quantitative phosphoproteomics of pS268
***
Normalized Intensity (pS268 peptide)
8
6
4
2
0
Fold Change: 4.134
p-value: 0.003
KANSL1(-Cdk5/p35)
KANSL1(+Cdk5/p35)


A
KANSL1 plasmid with Flag tag
SDM
KANSL1 S>A–TCT (Ser)–GCT (Ala)
KANSL1 S>GTCT(Ser)–GAG (Glu)
KANSL1 wild type (wt)
In-vitro transcription and translation using commercial kit
Transfected in N2A cells
Cdk5/p35
Cdk5/p25
complex
KANSL1
Validate the phosphorylation of KANSL1 by Cdk5 by
Kinase activity
Western blot using customized phospho-Ser268 KANSL1 specific antibody
In-vitro Phosphorylation of KANSL1 with wild type and mutant KANSL1 protein
B
Sanger sequencing to validate the SDM at Ser 268th position to Ala and Glu
GGAGGGTAAAAAAGGCTCCCCTGTC
1040
1050
1060
Serine 268 to Alanine : TCT to GCT
GGAGGGTAAAAAAGGAGCCCCTGT
1010
1020
Serine 268 to Glutamate : TCT to GAG
C
KANSL1 wt
KANSL1 S>A
KANSL1 S>E
Uninduced
113 kDa
100 kDa
D
CDK5 activity Luminescense (RLU)
50000
40000
30000
20000
10000
0
***
**
CDK5/p35
CDK5/p25
CDK5/p35+KANSL1
CDK5/p35+KANSL1 S>A
CDK5/p35+KANSL1 S>E
CDK5/p25+KANSL1
CDK5/p25+KANSL1 S>A
CDK5/p25+KANSL1 S>E
E
KANSL1+Cdk5/p35
KANSL1 S>A+Cdk5/p35
KANSL1 S>E+Cdk5/p35
KANSL1+Cdk5/p25
KANSL1 S>A+Cdk5/p25
KANSL1 S>E+Cdk5/p25
p-KANSL1
KANSL1
100 kDa
F
Expression of KANSL1 wild type and mutants in N2A cells
No transfection
KANSL1 wt
KANSL1 S>A
KANSL1 S>E
P-KANSL1
KANSL1
Beta actin
100 kDa
40 kDa
G
CDK5 kinase Assay Luminescence (RLU)
40000
30000
20000
10000
0
ns
**
***
KANSl1
CDK5/p35
CDK5/p25
KANSL1+CDK5/p35
KANSL1+CDK5/p25
KANSL1 S>A+CDK5/p35
KANSL1 S>A+CDK5/p25
KANSL1 S>E+CDK5/p35
KANSL1 S>E+CDK5/p25
KANSL1+CDK5/p25+TFP5
KANSL1+CDK5/p25+SC


A
Transfection with Cdk5/p35 or Cdk5/p25 along with KANSl1
N2A cells
Immuno-precipitation (IP) of Cdk5 and check KANSL1 presence using western blot, vice-versa
B
Input 5%
Cdk5/p35
Cdk5/p25
KANSL1
130kDa
KANSL1
35kDa
Cdk5
40kDa
Beta actin
IP-KANSL1
35kDa
Cdk5
IP-Cdk5
130kDa
KANSL1
C
Cdk5
KANSL1
DAP1
Merged
D
N2A cells
Collect cell pellet
Transfection
1. Control
2. KANSL1
3. Cdk5/p35+ KANSL1
4. Cdk5/p25+KANSL1
DNA-protein crosslinking
Sample
Sonication
Immuno-precipitation with KANSL1antibody through magnetic beads
qPCR using PINK1 promoter specific primers
E
ChIP qPCR
% Input
NT
KANSL1
KANSL1+CDK5/p35
KANSL1+CDK5/p25
IgG


A
Transfection
1. No transfection
2. KANSL1
3. KANSL1+Cdk5/p35
4. KANSL1+Cdk5/p25
Check phospho-KANSL1 and PINK1 expressions
Western Blot
qPCR
B
1 2 3 4
P- kansl1
kansl1
Beta actin
PINK1
Cdk5
Beta actin
130 kDa
130 kDa
40 kDa
70 kDa
35 kDa
40 kDa
1. Non transfected
2. KANSl1
3. KANSL1+Cdk5/p35
4. KANSL1+Cdk5/p25
C
Transfection of p35 and p25
Bright field
p35-mCherry
Bright field
p25- mCherry
D
P-KANSL1
pKANSL1/KANSL1
PINK1
Relative expression
E
PINK1 mRNA expression
Fold changes
ns
ns
*
F
HAT Activity Assay
NSL complex (mU/µg)
KANSL1
KANSL1+CDK5/p35
KANSL1+CDK5/p25
KANSL1S>A
KANSL1S>A+CDK5/p25
KANSL1S>E
KANSL1S>E + CDK5/p25


A
C57BL/6 mice treated with high fat diet for 3 months
HFD mice model
Validate T2D model
Glucose tolerance test
Insulin tolerance test
Harvest brain and check p-KANSL1and PINK1 expressions
B
GTT
Blood Glucose Level (mg/dl)
Time Interval (hrs)
Control
HFD
C
ITT
Blood Glucose Level (mg/dl)
Time interval (hrs)
Control
HFD
E
CNT
HFD
p-KANSL1
130 kDa
KANSL1
130 kDa
Beta-actin
40 kDa
PINK1
70 kDa
Beta-actin
40 kDa
F
p-KANSL1
pKANSL1/KANSL1
Control
HFD
G
PINK1
Relative expression
Control
HFD
H
PINK1 mRNA expression
Fold changes
Control
HFD