APPENDIX

1. Reagents for cell culture experiments

1.1 Minimum Essential Medium (MEM)

MEM powder for 1 L

NaHCO3	2.2	g

Add 3BH_2O to 1,000 ml and adjust pH to 7.2

1.2 Ham's F-12 Nutrient Mixture medium

F-12 powder for 1 L

NaHCO3	1.17	g

Add 3BH_2O to 1,000 ml and adjust pH to 7.2

1.3 Culture medium

Mix Minimum Essential Medium (MEM)/Ham's F-12 Nutrient Mixture (1:1) supplemented with 10% fetal bovine serum (FBS), 1mM sodium pyruvate, 1 mM non-essential amino acid, and 25 mg/ml penicillin/25 U/ml streptomycin**.**

1.4 Serum free medium

Mix Minimum Essential Medium (MEM)/Ham's F-12 Nutrient Mixture (1:1) supplemented with 1mM sodium pyruvate, 1 mM non-essential amino acid, and 25 mg/ml penicillin/25 U/ml streptomycin**.**

1.5 0.01 M Phosphate-buffered saline (PBS; 10X).

NaCl	80	g
KCl	2	g
Na_2HPO_4	14.4	g
KH_2PO_4	2.4	g

Add 3BH_2O to 1,000 ml and sterilize the solution by autoclaving

To prepare 1 x PBS, diluted to 100 ml 10 x PBS with 900 ml ddH_2O and adjust pH to 7.4.

1.6 1 xTrypLE

10X TrypLE	1	ml
1XPBS buffer	9	ml

1.7 0.1% Trypan blue

0.4% trypan blue	2.5	ml
1XPBS buffer	7.5	ml

1.8 MTT solution (1 mg/ml)

MTT	0.01	mg

Add 1XPBS buffer to 10 ml and filter sterile by 0.45 µm sterile filter membrane. Protected from light and stored at 4°C.

2. Reagents for Western blot analysis

2.1 NP40 lysis buffer

Tris pH 7.4	0.24	g
NaCl	0.35	g
NaDC	0.10	g
NP-40	0.50	ml
EDTA	0.01	g

Add 3B H_2O to 40 ml, adjust pH to 7.4

For protein extraction, mix Np40 lysis buffer with 1% protease phosphatase inhibitor cocktail.

2.2 4 x **Running gel buffer (1.5M Tris-HCl, pH 8.8)**

Tris	36.6	g

Add 3BH_2O, adjust to pH 8.8 with HCl and adjust volume to 200 ml.

2.3 4x **Stacking gel buffer (0.5M Tris-Cl, pH 6.8)**

Tris	3	g

Add 3B H_2O, adjust to pH 6.8 with HCl and adjust volume to 50 ml.

2.4 30% Bis-acrylamide solution

Acrylamide	29	g
Bis	1	g

Add 3B H_2O to 200 ml, filtered, protected from light and store at 4°C.

2.5 10% Sodium dichosylsulphate (10% SDS)

SDS	10	g

Add 3B H_2O to 100 ml dissolved with 3B H_2O to the final volume 100 ml.

2.6 10% Ammonium persulfate (10% APS)

APS	0.1	g

Add 3B H_2O to 1 ml. Protected from light and stored at 4°C. (Use within 7 days)

2.7 6x Loading dye buffer

1 M Tris-HCl pH 6.8	3.75	ml
SDS	1	g
Glycerol	5	ml
10% Bromophenol Blue	30	µl

Add ddH_2O to 9 ml and store at 4°C

Before use, add β-mercapto ethanol 1: 6X Loading dye buffer 9

2.8 10xRunning Electrophoresis buffer

Tris-HCl	30.28	g
Glycine	144.13	g
SDS	10	g

Add dd H_2O to 1,000 ml. The working concentration (1×) was diluted with dd H_2O.

2.9 Transfer buffer

1 xRunning Electrophoresis buffer	600	ml
Methanol	150	ml

2.10 1x Phosphate buffer saline with 0.1% tween-20 (1xPBST)

NaCl	8	g
KCl	0.2	g
Na_2HPO_4	1.44	g
KH_2PO_4	0.24	g
Tween-20	1	ml
Add dd H_2O to 1,000 ml		

2.11 Gel (12.5 %) Preparation for 1.5 mm glass

Seperating gel (lower gel)

4 x running gel buffer	2.5	ml
Bis/acrylamide solution	4.2	ml
3B H_2O	3.2	ml
10% SDS	100	µl
10% APS	65	µl
TEMED	5	µl

Stacking gel (upper gel)

4x stacking gel buffer	830	µl
Bis/acrylamide solution	440	µl
3B H_2O	2.03	ml
10% SDS	33	µl
10% APS	25	µl
TEMED	3	µl

2.12 Ponceau S solution (0.1%w/v)

Ponceau S	0.1	g

Add 30 % acetic acid to 100 ml.

2.13 Blocking buffer

5% (w/v) non-fat milk

non-fat milk	2.5	g

Add 1XPBT to 50 ml.