Supplemental Information

The TSC Protein Complex Regulates Melanogenesis through AKT-GSK3β-β-catenin-MITF signaling

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SUPPLEMENTAL SECTION INVENTORY

Figure S1 relates to manuscript Figure 1.

Figure S2 relates to manuscript Figure 2.

Figure S3 relates to manuscript Figure 3.

Figure S4 relates to manuscript Figure 4.

Figure S5 relates to manuscript Figures 5 and 6.

Figure S6 relates to manuscript Figures 7 and 8.

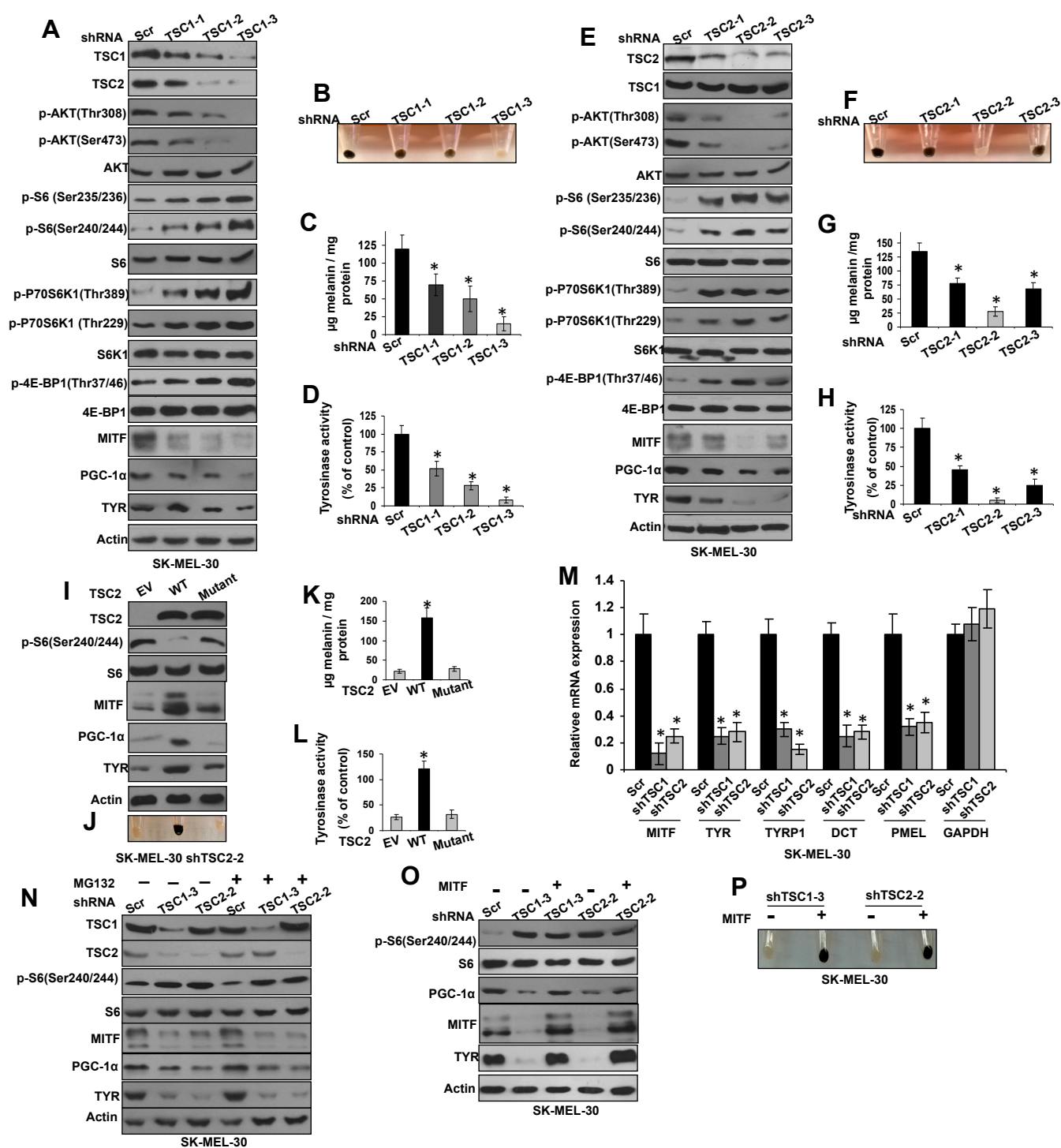# Fig. S1 related to Figure 1

SK-MEL-30

Figure S1. Disruption of the TSC Protein Complex Induces the Loss of Pigmentation in Melanocytes.

(A-D) Highly pigmented human melanoma cells (SK-MEL-30) were infected with lentiviruses expressing independent TSC1 shRNAs or independent TSC2 shRNAs or control (Scr) shRNA, and selected with puromycin. **(A)** Immunoblot analysis shows that TSC1 depletion leads to reduced MITF-M and its downstream targets PGC-1α and TYR, as well as mTORC1 activation. **(B)** Cell pellets from TSC1 depleted cells have reduced pigment. **(C)** TSC1 depleted cells have reduced melanin content. **(D)** TSC1 depleted cells have reduced tyrosinase activity. Data in **(C-D)** are presented as mean ± S.D. from at least three independent experiments. *, $p < 0.05$.

(E-H) Entirely similar to **A-D**, except that TSC2 shRNAs were expressed.

(I-L) TSC2 depleted SK-MEL-30 cells were reconstituted with lentiviruses containing wildtype TSC2 or patient derived TSC2 mutant (P419S) or empty vector control viruses (EV) and selected with puromycin. **(I)** Immunoblot analysis shows that wildtype TSC2 but not the TSC2 mutant rescues mTORC1 activation, MITF and PGC-1α expression, and TYR protein levels. **(J)** pigmentation; **(K)** melanin content; **(L)** tyrosinase activity; are also all rescued by WT TSC2. Data in **(K-L)** are presented as mean ± S.D. from at least three independent experiments. *, $p < 0.05$.

(M) Q-PCR analysis of gene expression. Expression of the indicated genes was measured in control or shRNA expressing cells by quantitative RT-PCR. GAPDH was a control. Data are presented as mean ± S.D. from at least three independent experiments. *, $p < 0.05$.

(N) Immunoblot analysis of lysates from TSC1 or TSC2 depleted cells treated with MG132 (25 μM) for 6 hours. MITF, PGC-1α, and TYR were not rescued by MG132 treatment.

(O-P) TSC1 or TSC2 depleted cells were infected with lentiviruses containing MITF-M or empty vector control (EV) and selected with puromycin, **(O)** Immunoblot analysis shows increased MITF and TYR expression in cells infected with MTIF-M lentivirus. **(P)** Ectopic expression of MITF rescues the pigmentation loss in TSC1 or TSC2 depleted cells.
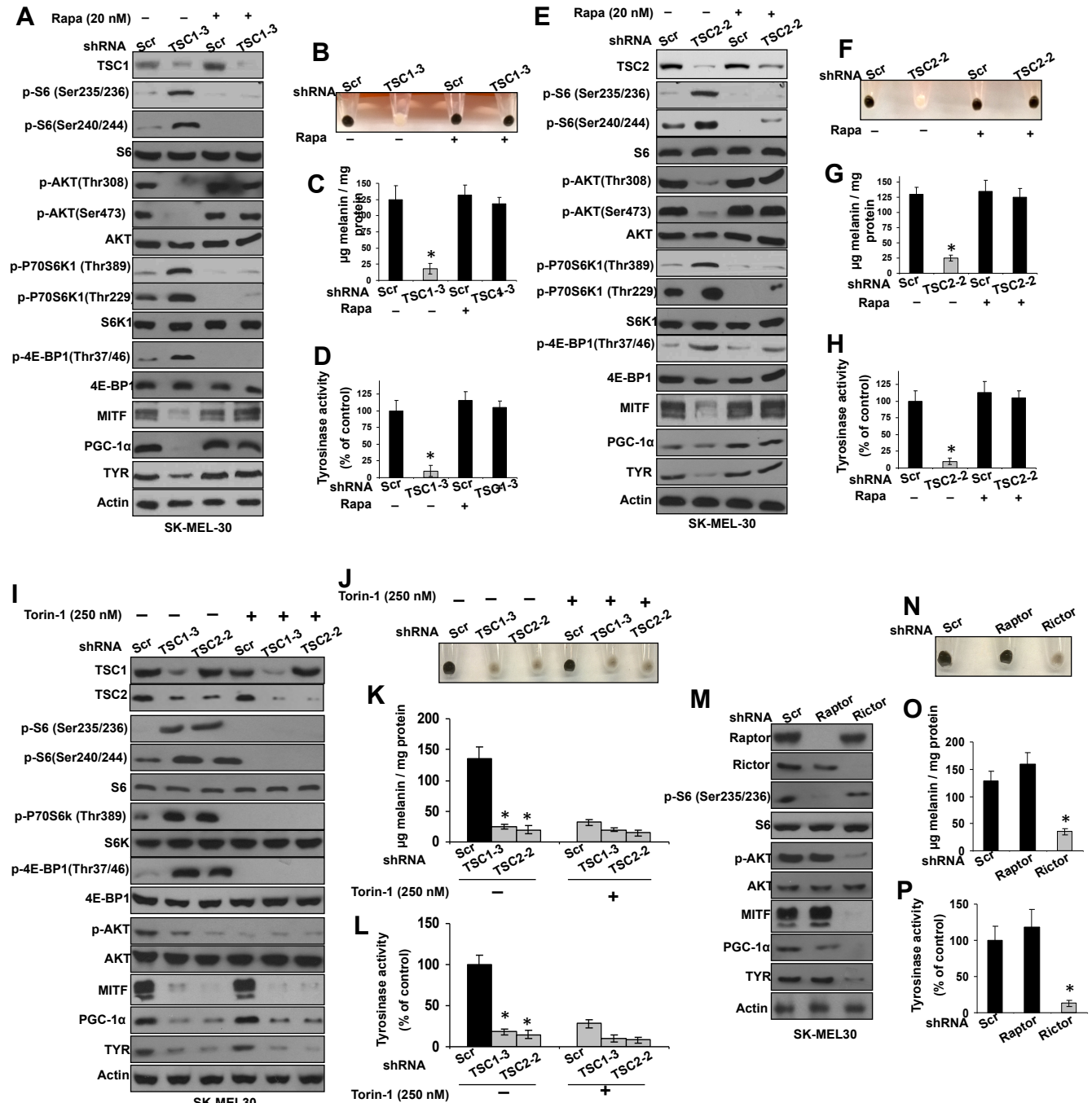
Fig. S2 related to Figure 2

SK-MEL-30

Figure S2. Rapamycin but not Torin-1 Restores the Loss of Pigmentation in TSC1 Protein Complex Deficient Human Melanoma Cells

(A-D) SK-MEL-30 cells stably expressing shTSC1 or control (Scr) were treated with rapamycin (20 nM) or vehicle control (DMSO) for 72 hours. **(A)** Immunoblot analysis shows recovery of MITF expression, suppression of mTORC1, and recovery of pAKT levels in cells treated with rapamycin. **(B)** pigmentation; **(C)** melanin content; **(D)** tyrosinase activity; are also all rescued by rapamycin. Data in **(C-D)** are presented as mean ± S.D. from at least three independent experiments. *, $p < 0.05$.

(E-H) Entirely similar to **A-D**, except with SK-MEL-30 cells with shTSC2 knockdown.

(I-L) SK-MEL-30 cells stably expressing shTSC1, shTSC2 or control (Scr) were treated with Torin1 (250 nM) or vehicle control (DMSO) for 72 hours. **(I)** Immunoblot analysis shows complete suppression of mTORC1 and mTORC2. **(J)** pigmentation; **(K)** melanin content; **(L)** tyrosinase activity; none are rescued by Torin1 treatment. Data in **(K-L)** are presented as mean ± S.D. from at least three independent experiments. *, $p < 0.05$.

(M-P) SK-MEL-30 cells were infected with control (Scr) or shRaptor or shRictor lentiviruses, and selected with puromycin for 6 days. **(M)** Immunoblot analysis shows that Rictor knockdown reduces MITF, PGC-1a, and TYR expression. **(N)** pigmentation; **(O)** melanin content; **(P)** tyrosinase activity; all show reduced pigmentation in Rictor knockdown cells. Data in **(O-P)** are presented as mean ± S.D. from at least three independent experiments. *, $p < 0.05$.
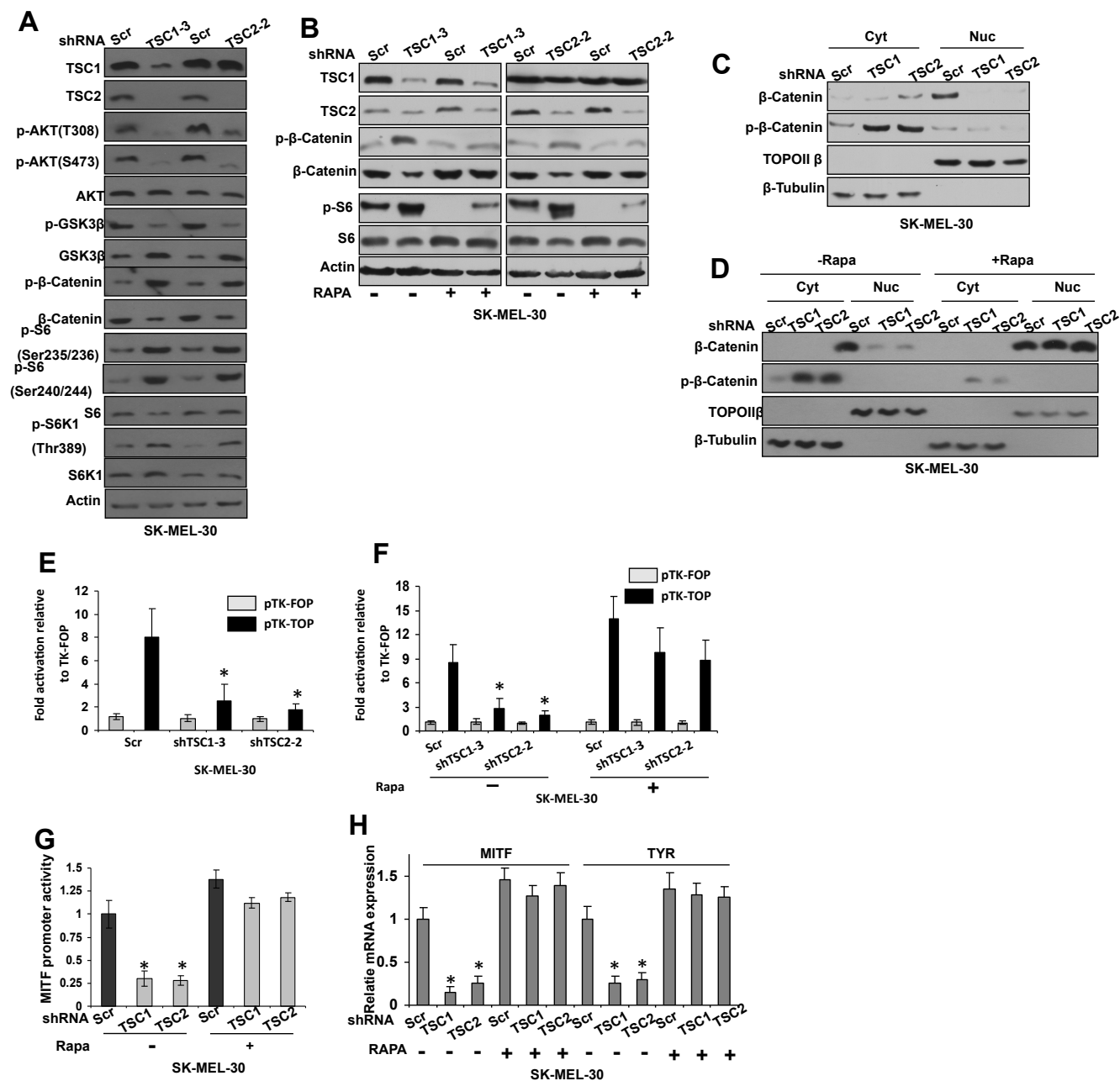
Fig. S3 related to Figure 3

Figure S3. Disruption of the TSC protein Complex Leads to GSK3β Activation and the Loss of β-catenin and MITF Transcription in Human Melanoma Cells.

(A) Immunoblot analysis of SK-MEL-30 cells expressing shTSC1 or shTSC2 or control (Scr) shows reduced p-AKT (Ser 473), reduced p-GSK3β (Ser9), increased GSK3β, reduced β-catenin and increased p-β-catenin (Ser 33,37/Thr 41).

(B) Immunoblot analysis of SK-MEL-30 cells expressing shTSC1 or shTSC2 or control (Scr), treated with rapamycin (20 nM) or vehicle (DMSO) for 72 hours shows recovery of β-catenin levels.

(C) Immunoblot analysis of cytosolic (Cyt) and Nuclear (Nuc) fractions shows loss of nuclear β-catenin in the TSC1 and TSC2 knockdown cells. TOPOII β and β-Tubulin serve as controls for nucleus and cytosol respectively.

(D) Entirely similar to **(C)**, except that cells on right were treated with rapamycin for 4 days, and β-catenin is seen again in the nuclear fraction.

(E) Graph of relative luciferase expression in SK-MEL-30 cells stably expressing shTSC1 or shTSC2 or control (Scr), transfected with TK-TOP or TK-FOP driven luciferase.

(F) Entirely similar to **(E)**, except that SK-MEL-30 cells were treated with rapamycin (20 nM) or vehicle (DMSO) for 72 hours prior to analysis.

(G) Graph of MITF promoter activity of SK-MEL-30 cells with TSC1 or TSC2 knockdowns treated with or without 20 nM rapamycin, measured using pGL3-Luciferase empty vector or MITF-PGL3-luciferase.

(H) Cells treated as in **(G)** were analyzed by quantitative RT-PCR for MITF-M and TYR mRNA levels.

Data in **(E-H)** are presented as mean ± S.D. from at least three independent experiments. *, $p < 0.05$.

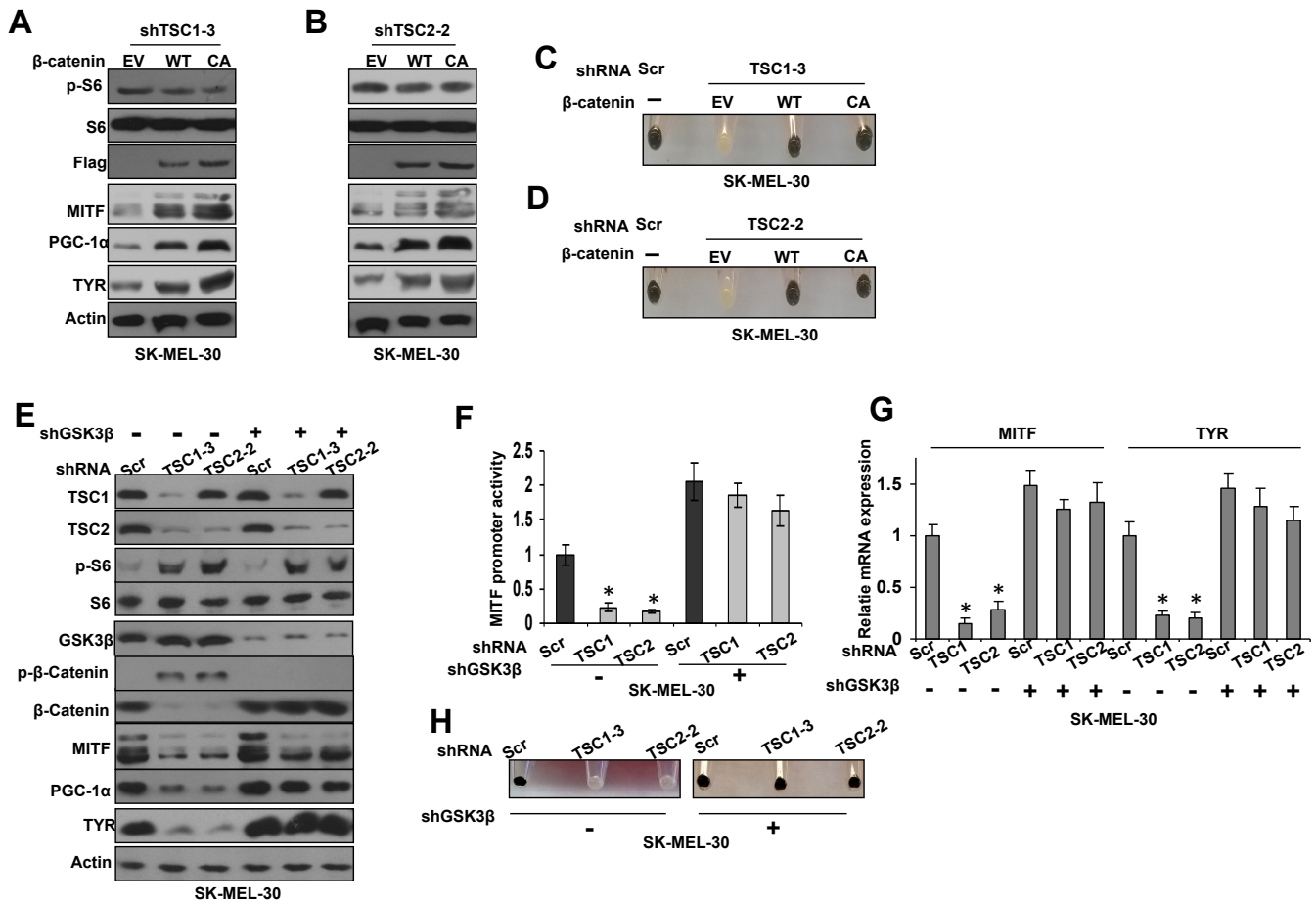# Fig. S4 related to Figure 4

Figure S4. Ecotopic Expression of β-catenin Restores Pigmentation in TSC Protein Complex Disrupted Human Melanoma Cells.

(A) Immunoblot analysis of SK-MEL-30 cells stably expressing shTSC1 or shTSC2 transduced to express wild type (WT) β-catenin or constitutively active (CA) β-catenin (S33Y) or empty vector control (EV). Note MITF expression in the WT and CA β-catenin expressing SK-MEL-30.

(B) Entirely similar to **(A)**, except that SK-MEL-30 are expressing shTSC2.

(C, D) Pigmentation of cells from **(A and B)**, respectively.

(E) Immunoblot analysis of of SK-MEL-30 cells stably expressing shTSC1 or shTSC2 transduced to express
GSK3β shRNA or control shRNA. Note marked enhancement of β-catenin expression in the GSK3β shRNA cells.

(F) Graph of lucerifase assay, assessed on the cells in **(E)** after transfection with pGL3-Luciferase empty vector or MITF-PGL3-luciferase.

(G) Q-RT-PCR analysis of MITF and TYR mRNA levels of the cells described in **(E)** shows marked enhancement of MITF and TYR expression by GSK3β shRNA

(H) Pigmentation of cells from **(E).**

Data in **(F-G)** are presented as mean ± S.D. from at least three independent experiments.

*, p < 0.05.

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Fig. S5 related to Figures 5 and 6
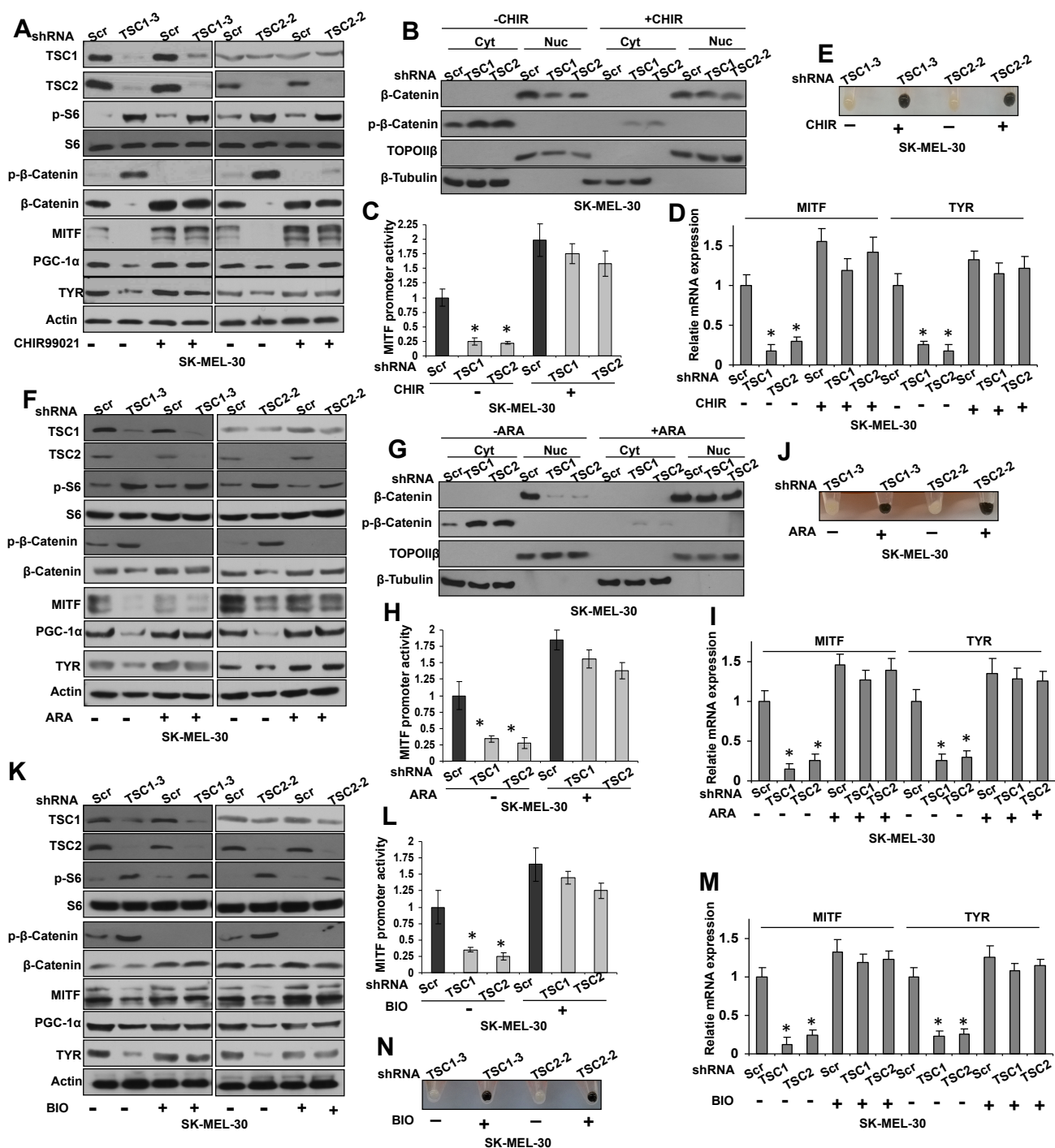
SK-MEL-30

Figure S5. TSC Protein Complex Regulates MITF Transcription and Pigmentation by Suppressing GSK3β in Human Melanoma Cells.

(A) Immunoblot analysis of SK-MEL-30 cells expressing shTSC1 or shTSC2 or control (Scr) after treatment with the GSK3β specific inhibitor CHIR-99021 (3 µM) for 5 days. Note recovery of β-catenin and MITF expression in cells treated with CHIR-99021.

(B) Immunoblot analysis shows recovery of nuclear β-catenin in SK-MEL-30 cells following treatment with CHIR-99021 (CHIR).

(C) Graph of MITF promoter activity of SK-MEL-30 cells with TSC1 or TSC2 knockdowns treated with or without 3 µM CHIR-99021 (CHIR), measured using pGL3-Luciferase empty vector or MITF-PGL3-luciferase.

(D) Cells treated as in **(C)** were analyzed by quantitative RT-PCR for MITF-M and TYR mRNA levels.

(E) Pigmentation is restored in cells treated with CHIR-99021 (CHIR).

Data in **(C-D)** are presented as mean ± S.D. from at least three independent experiments. *, $p < 0.05$.

(F-J) Entirely similar to **(A-E)**, except using the GSK3β specific inhibitor AR-A014418 (ARA) (5µM) for 5 days.

(K-N) Entirely similar to **(A, C-E)**, except using the GSK3β specific inhibitor BIO (2µM) for 5 days.

Fig. S6 related to Figures 7 and 8
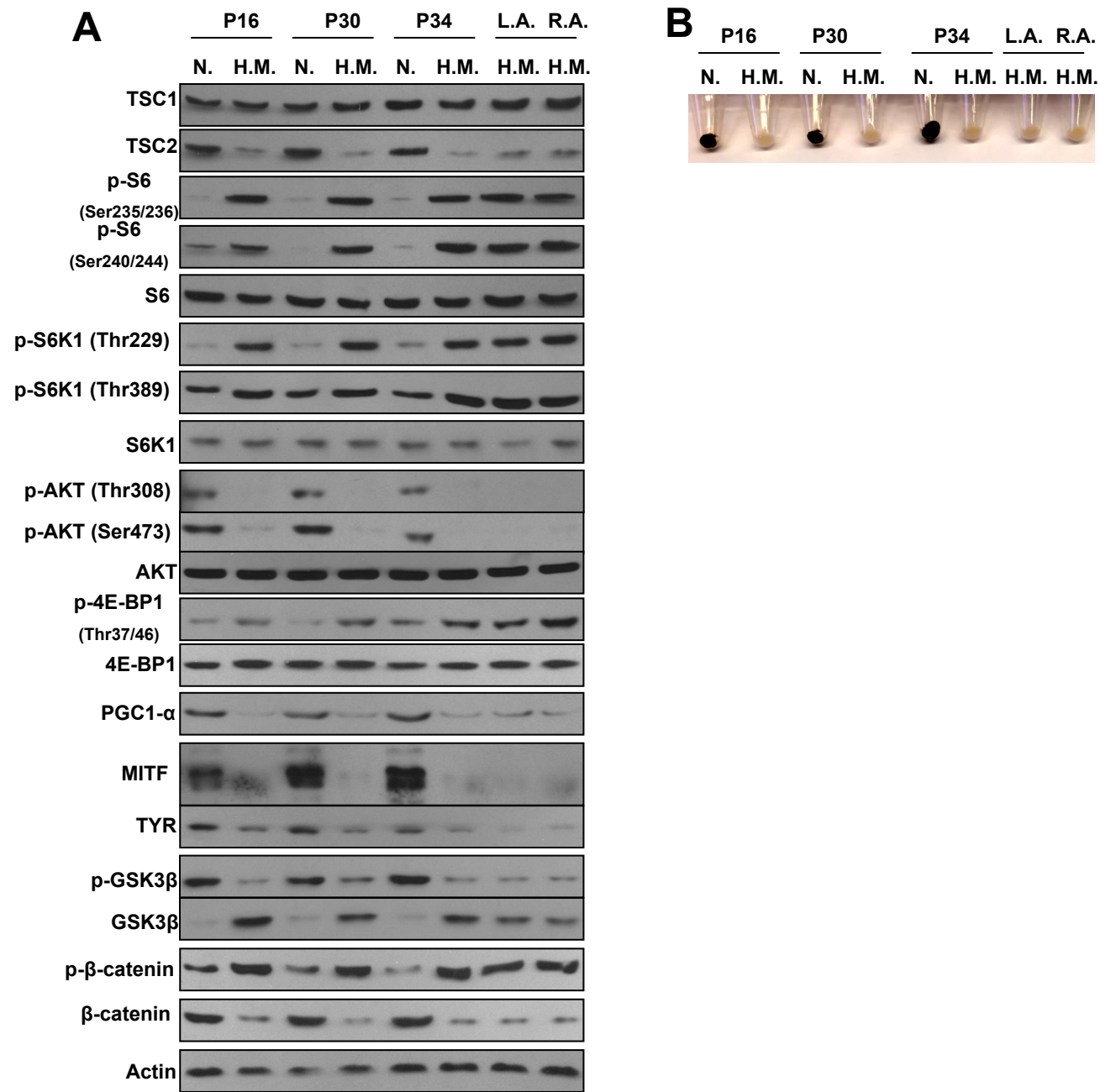
Figure S6. mTORC1 is Hyperactive in Melanocytes Isolated from TSC Patient Hypomelanotic Macules.
(A) Immunoblot analysis of primary melanocytes isolated from hypomelanotic macules (HMs) and nearby normal skin from 4 TSC patients, P16, P30, P34, and two lesions (L.A. and R.A.) from one patient. Note activation of mTORC1 in the melanocytes from the hypomelanotic macules (HMs) with reduction in MITF, PGC-1a, TYR, and β-catenin, with an increase in GSK3β. (B) Pigmentation of melanocytes analyzed in (A).

Full unedited blot images for Figures 1-8

Note: for all gels, blots were cut for different antibody staining and exposed on the same film

A

shRNA: Scr, TSC1-1, TSC1-2, TSC1-3

TSC1
TSC2
p-AKT(Thr308)
p-AKT(Ser473)
AKT
p-S6 (Ser235/236)
p-S6(Ser240/244)
S6
p-P70S6k (Thr389)
p-P70S6k (Thr229)
S6K1
p-4E-BP1(Thr37/46)
4E-BP1
PGC-1α
MITF
TYR
Actin

iHPM
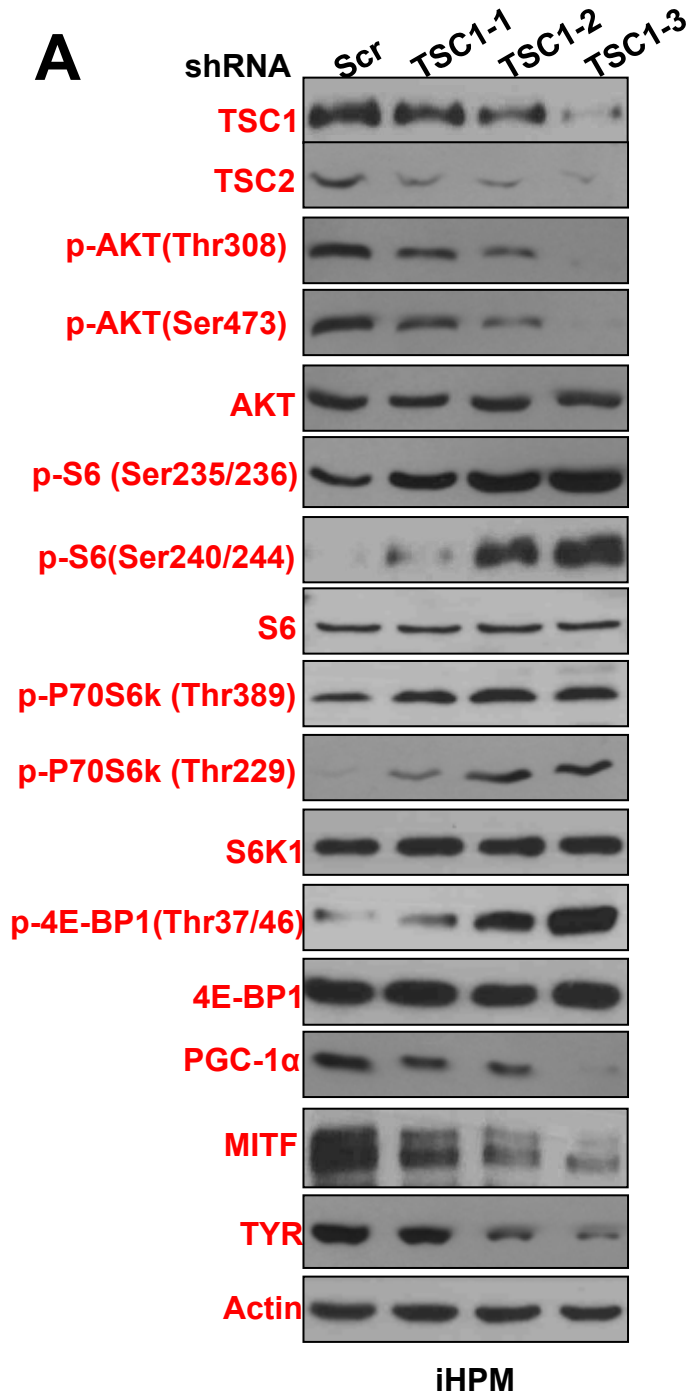
TSC1   p-S6(Ser240/244)   S6K1   4E-BP1   Actin

Run on same gel, **Actin** in the figure was used as the loading control

parallel gel 1

S6   MITF

Run on same gel, **S6** in the figure was used as the loading control, no extra loading control needed

parallel gel 2

p-AKT(Thr308)   p-AKT(Ser473)   AKT   p-S6 (Ser235/236)   PGC-1α   TYR

Run on same gel, **AKT** in the figure was used as the loading control, no extra loading control needed

parallel gel 3

TSC2   p-P70S6k (Thr229)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 4

p-P70S6k (Thr389)   p-4E-BP1(Thr37/46)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Figure 1

A
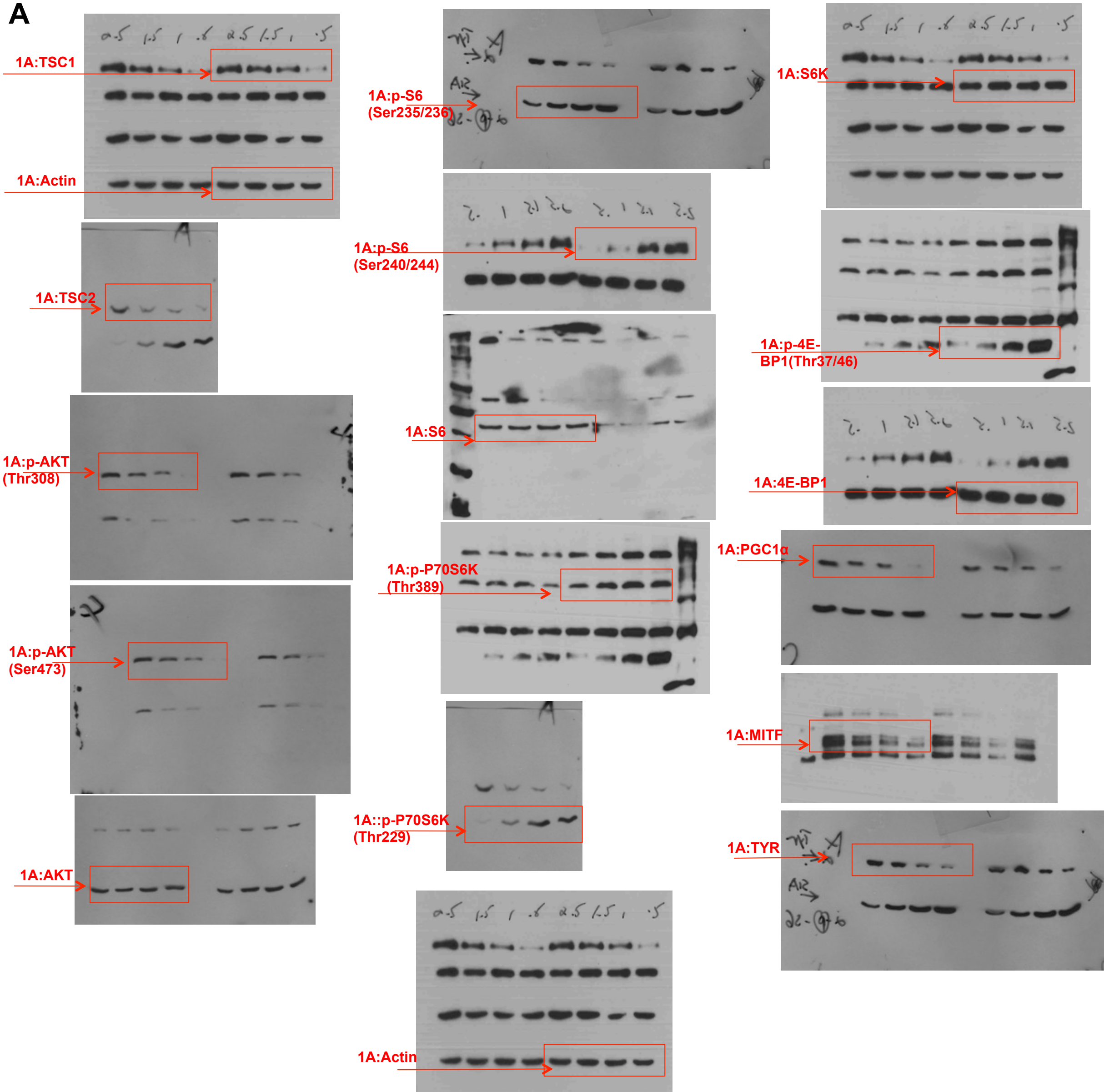
Full unedited gel for Figure 1A

Figure 1

Loading controls for parallel gels in figure 1A

parallel gel 1

1A: S6 was the control as shown in the figure

parallel gel 2

1A: AKT was the control as shown in the figure

parallel gel 3

1A:Actin

parallel gel 4

1A:Actin

E

shRNA   Scr   TSC2-1   TSC2-2   TSC2-3
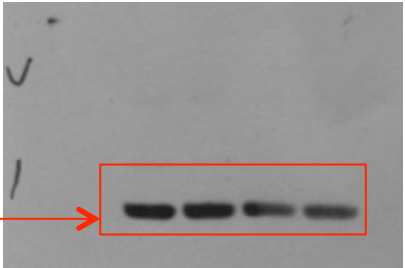
TSC2
TSC1
p-AKT(Thr308)
p-AKT(Ser473)
AKT
p-S6 (Ser235/236)
p-S6(Ser240/244)
S6
p-P70S6K1(Thr389)
p-P70S6K1(Thr229)
S6K
p-4E-BP1(Thr37/46)
4E-BP1
PGC-1α
MITF
TYR
Actin
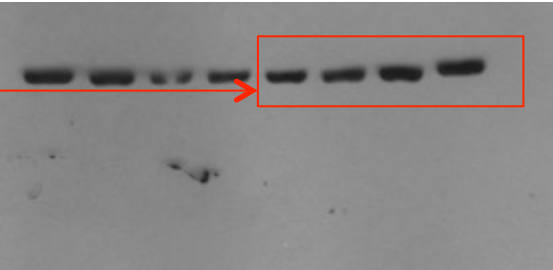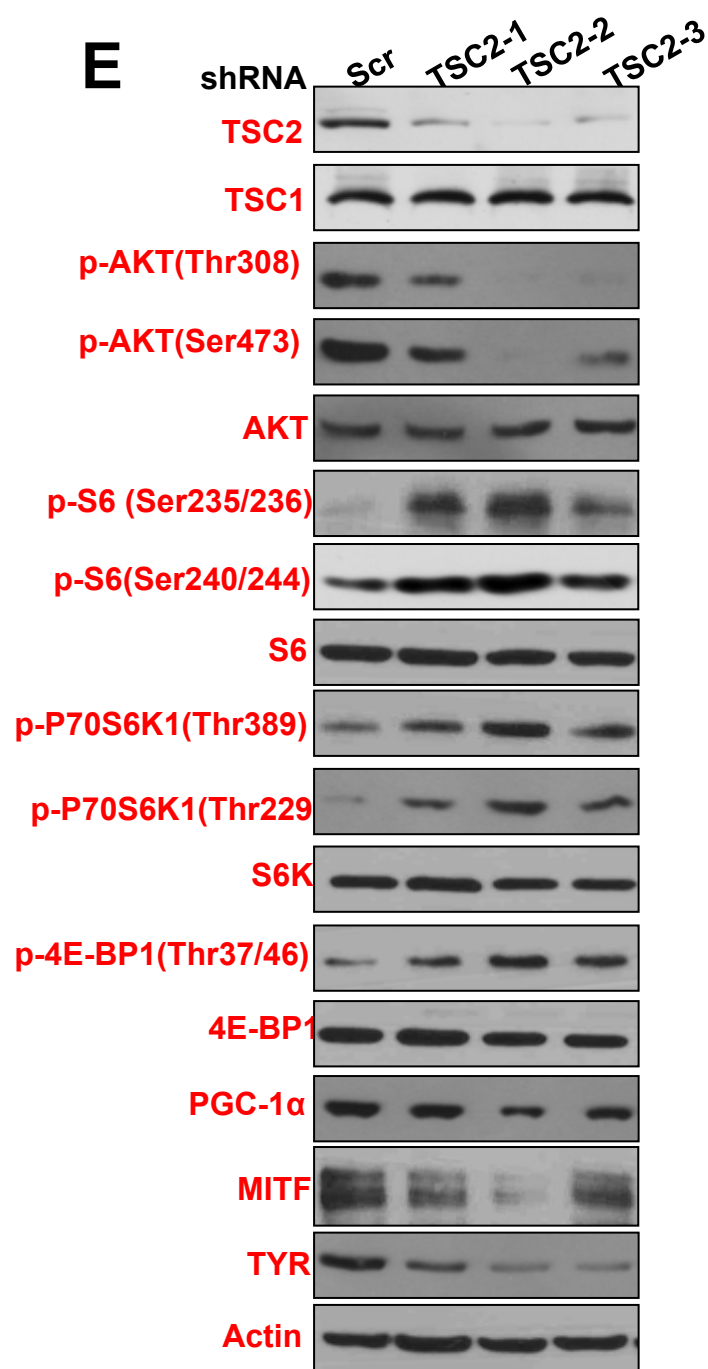
p-AKT(Thr308)   p-AKT(Ser473)   AKT   p-P70S6K1(Thr229)   PGC-1α   TYR

Run on same gel, **AKT** in the figure was used as the loading control, no extra loading control needed
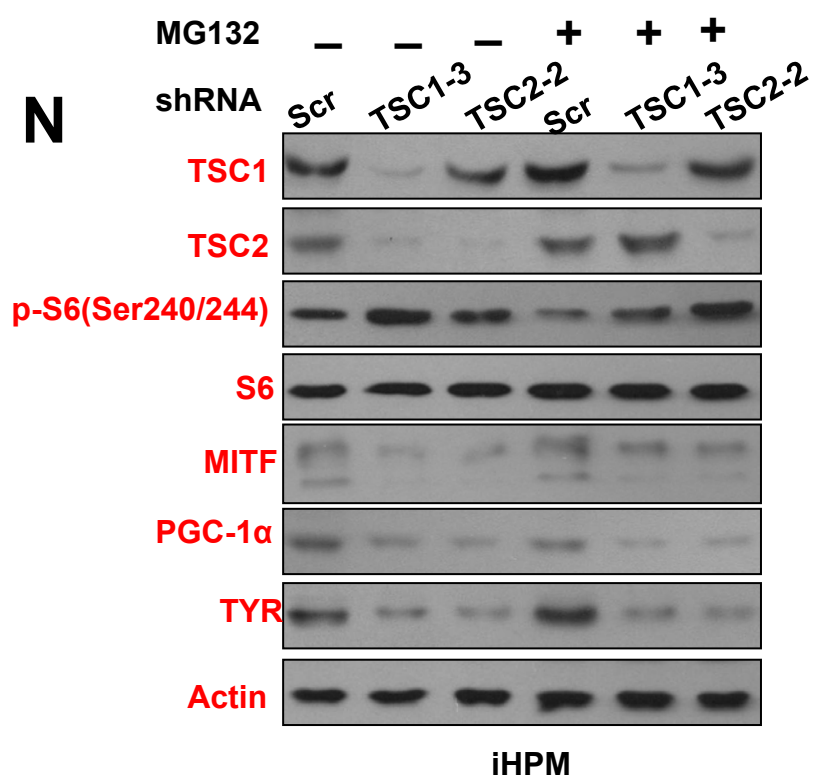
parallel gel 1

p-S6(Ser240/244)   S6   S6K   4E-BP1   Actin

Run on same gel, **Actin** in the figure was used as the loading control

parallel gel 2

TSC1

Run on same gel, **Actin** was used as the loading control (see uncropped gel image

parallel gel 3

TSC2

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 4

p-S6 (Ser235/236)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 5

p-P70S6K1(Thr389)   p-4E-BP1(Thr37/46)   MITF

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Figure 1

E

1E:TSC2

1E:TSC1

1E:p-AKT (Thr308)

1E:p-AKT (Ser473)

1E:AKT

1E:p-S6 (Ser235/236)

1E:p-S6 (Ser240/244)

1E:S6

1E:pP70S6K (Thr389)

1E:p-P70S6K (Thr229)

1E:S6K

1E:p-4EBP1 (Thr37/46)

1E:4E-BP1

1E:PGC1α

1E:MITF

1E:TYR

1E:Actin

Full unedited gel for Figure 1E
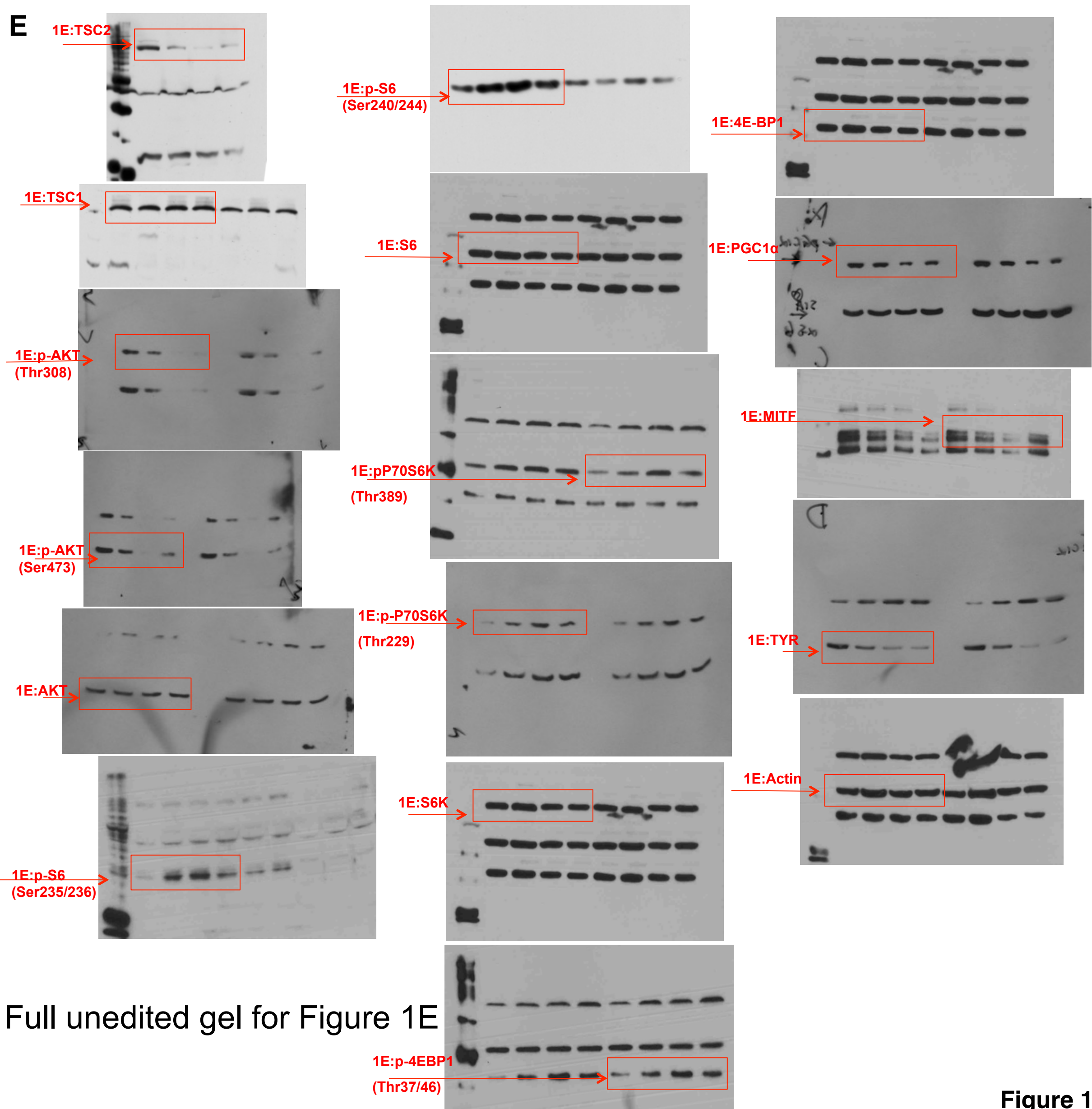
Figure 1

Loading controls for parallel gels in figure 1E

parallel gel 1

1E: Actin, as shown in the figure, was the control

Parallel **gel 2**

1E:Actin
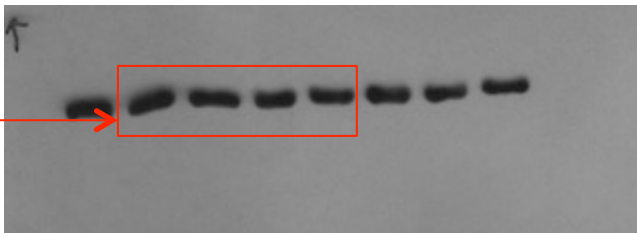
Parallel **gel 3**

1E:Actin
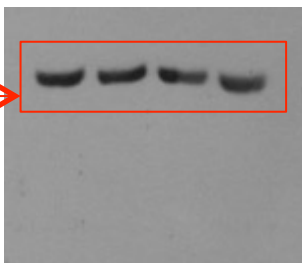
Parallel **gel 4**

1E:Actin
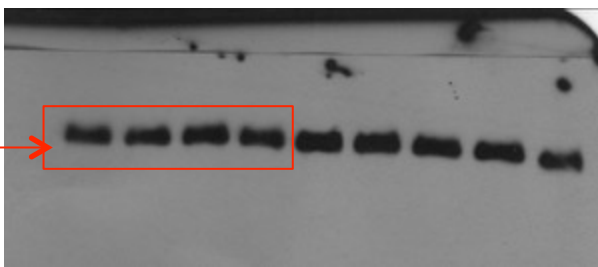
Parallel **gel 5**

1E:Actin
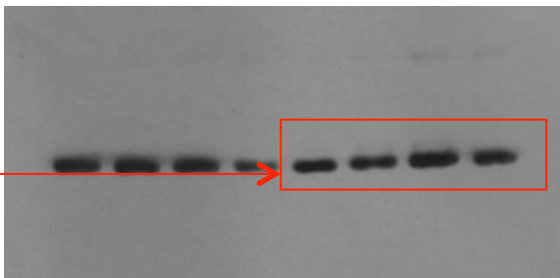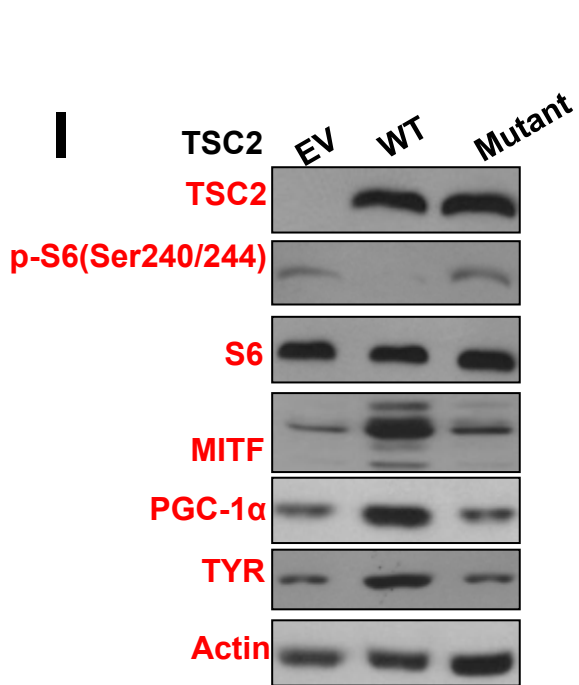
I

TSC2 | EV | WT | Mutant

TSC2
p-S6(Ser240/244)
S6
MITF
PGC-1α
TYR
Actin

PGC-1α   Actin

Run on same gel, **Actin** in the figure was used as the loading control

parallel gel 1

TSC2 p-S6(Ser240/244)   S6   MITF   TYR

Run on same gel, **S6** in the figure was used as the loading control, no extra loading control needed

Figure 1

Full unedited gel for Figure 1I

I

1I: TSC2

1I: p-S6
(Ser240/244)

1I: S6

1I: MITF

1I: PGC-1a

1I: TYR

1I: Actin
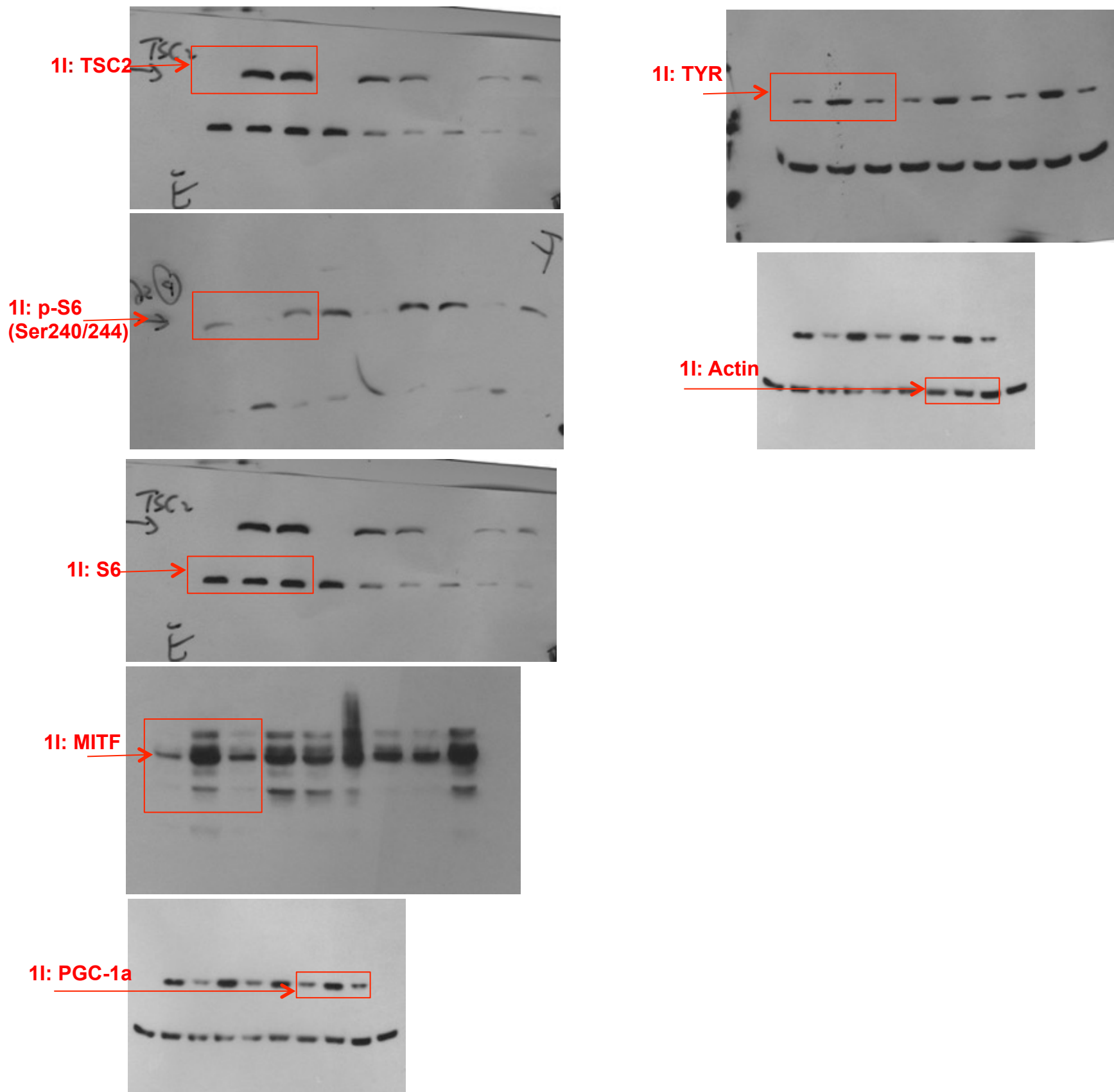
Figure 1

Loading controls for parallel gels in figure 1I

parallel gel 1
1I:

No extra loading control needed
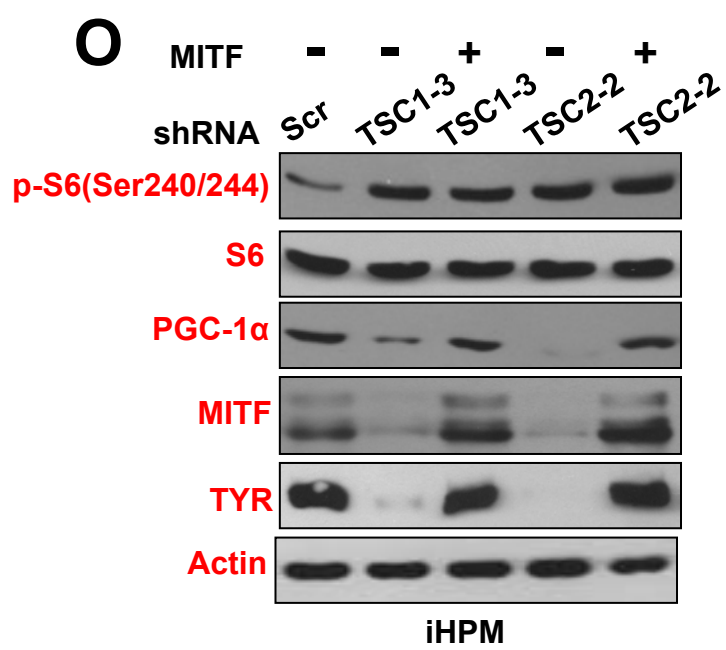
O

MITF: − − + − +

shRNA: Scr TSC1-3 TSC1-3 TSC2-2 TSC2-2

p-S6(Ser240/244)

S6

PGC-1α

MITF

TYR

Actin

iHPM

MITF   Actin
Run on same gel, **Actin** in the figure was used as the loading control

parallel gel 1

p-S6(Ser240/244)   S6   PGC-1α

Run on same gel, **S6** in the figure was used as the loading control, no extra loading control needed

parallel gel 2

TYR

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

N

MG132: − − − + + +

shRNA: Scr TSC1-3 TSC2-2 Scr TSC1-3 TSC2-2

TSC1

TSC2

p-S6(Ser240/244)

S6

MITF

PGC-1α

TYR

Actin

iHPM

TSC1   S6   Actin

Run on same gel, **Actin** in the figure was used as the loading control

parallel gel 1

TSC2   p-S6(Ser240/244)   MITF   PGC-1α   TYR

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Figure 1

Full unedited gel for Figure 1O&N

O

1O:pS6
(Ser240/244)

1O: MITF

1O: Actin

1O: S6

1O: TYR

1O: PGC-1a
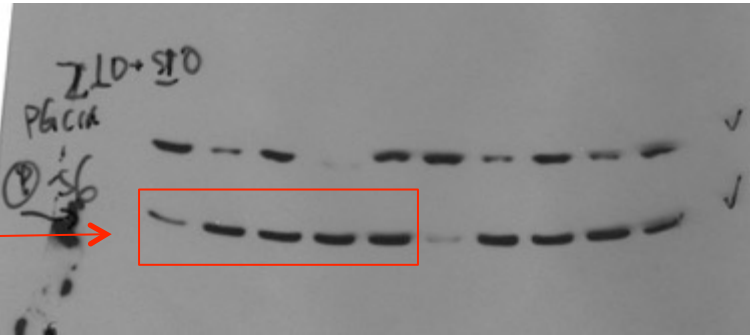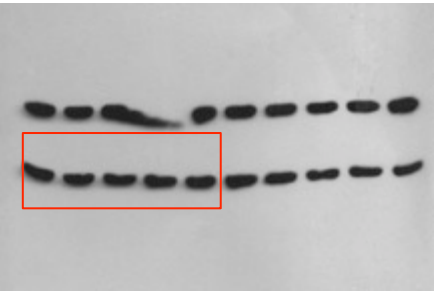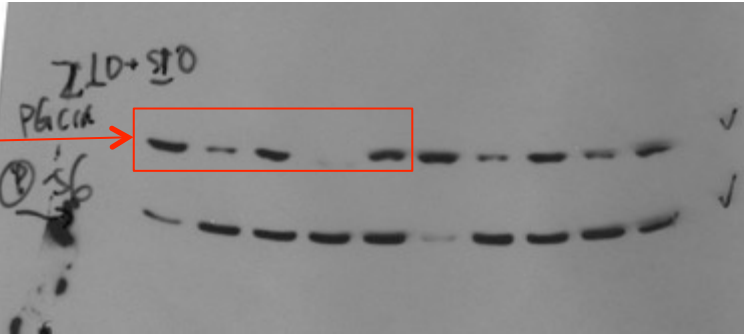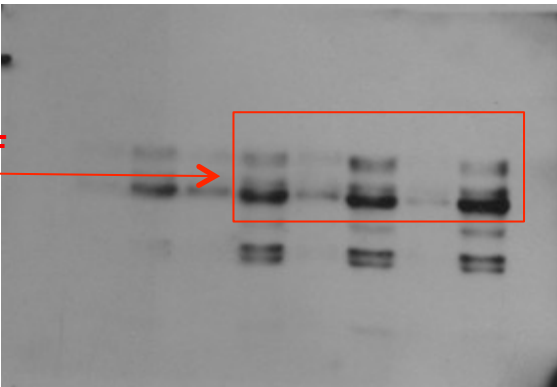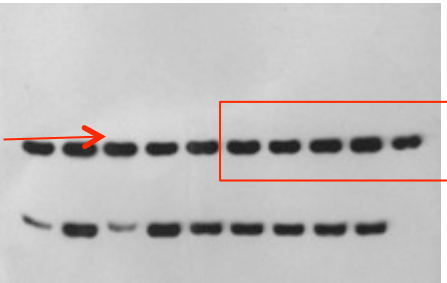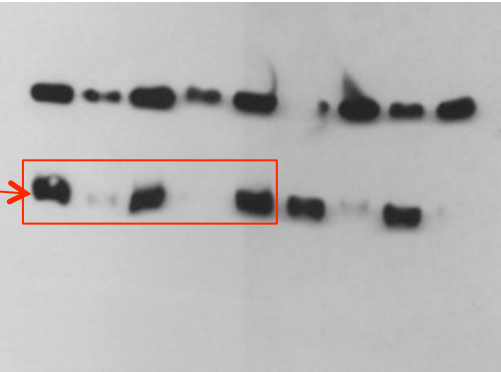
N
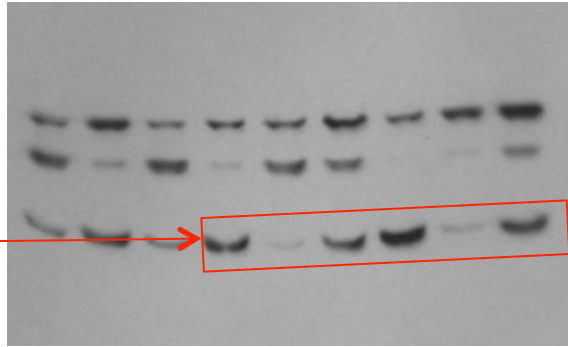
1N: TSC1

1N: S6
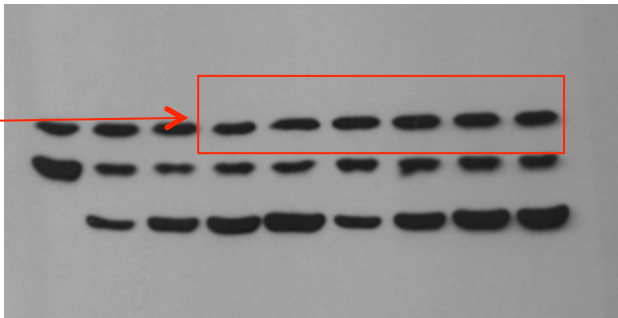
1N: TYR
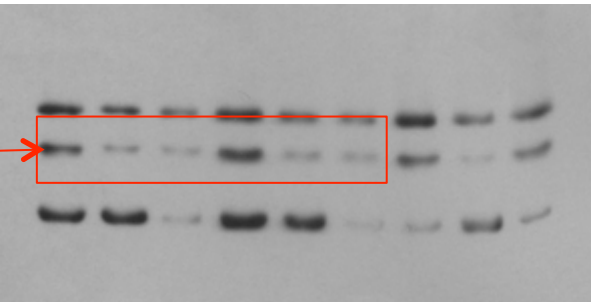
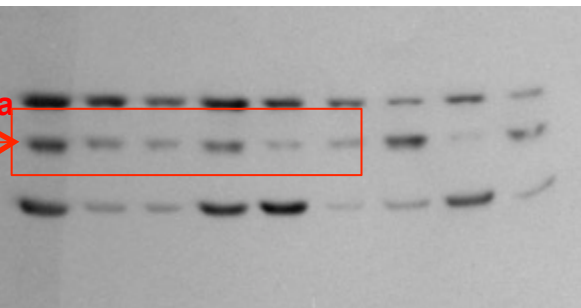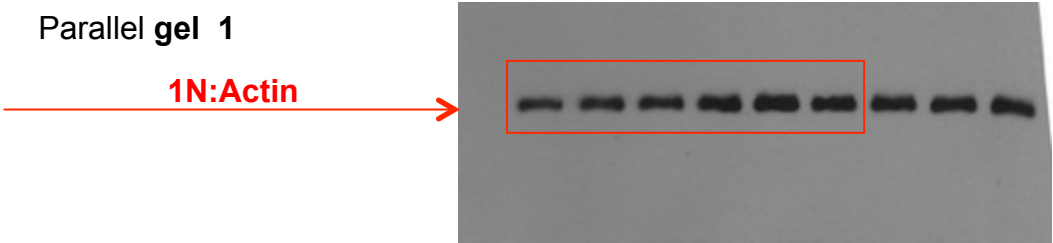1N: TSC2
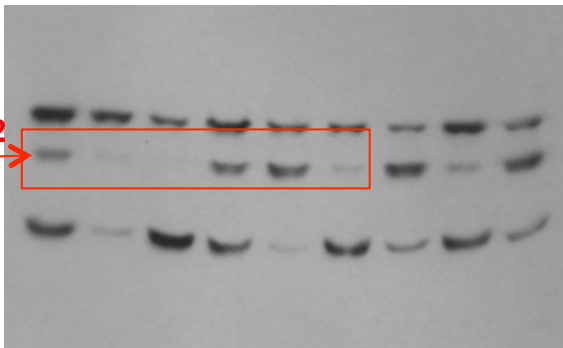
1N: MITF
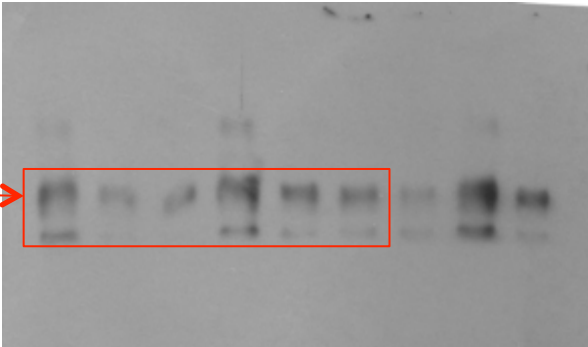
1N: Actin
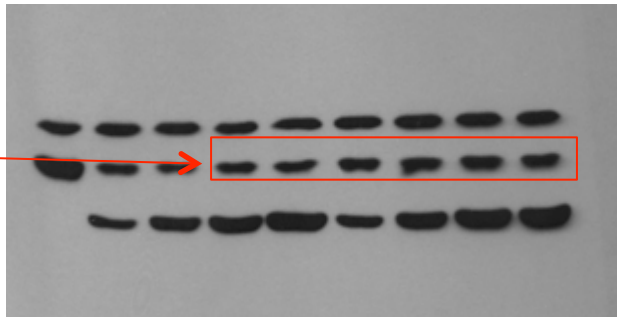
1N: p-S6
(Ser240/244)
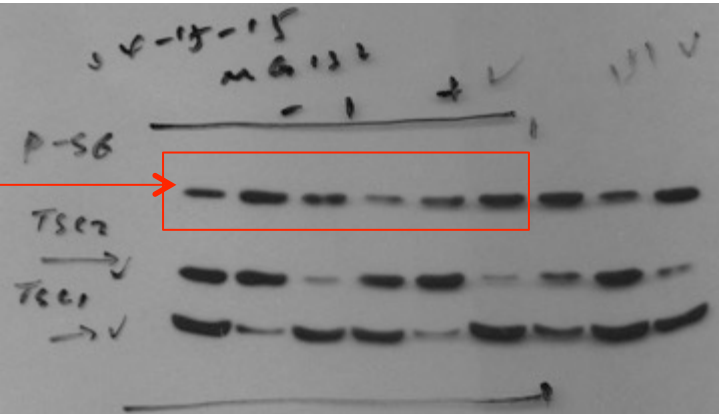
1N:PGC-1a

Figure 1
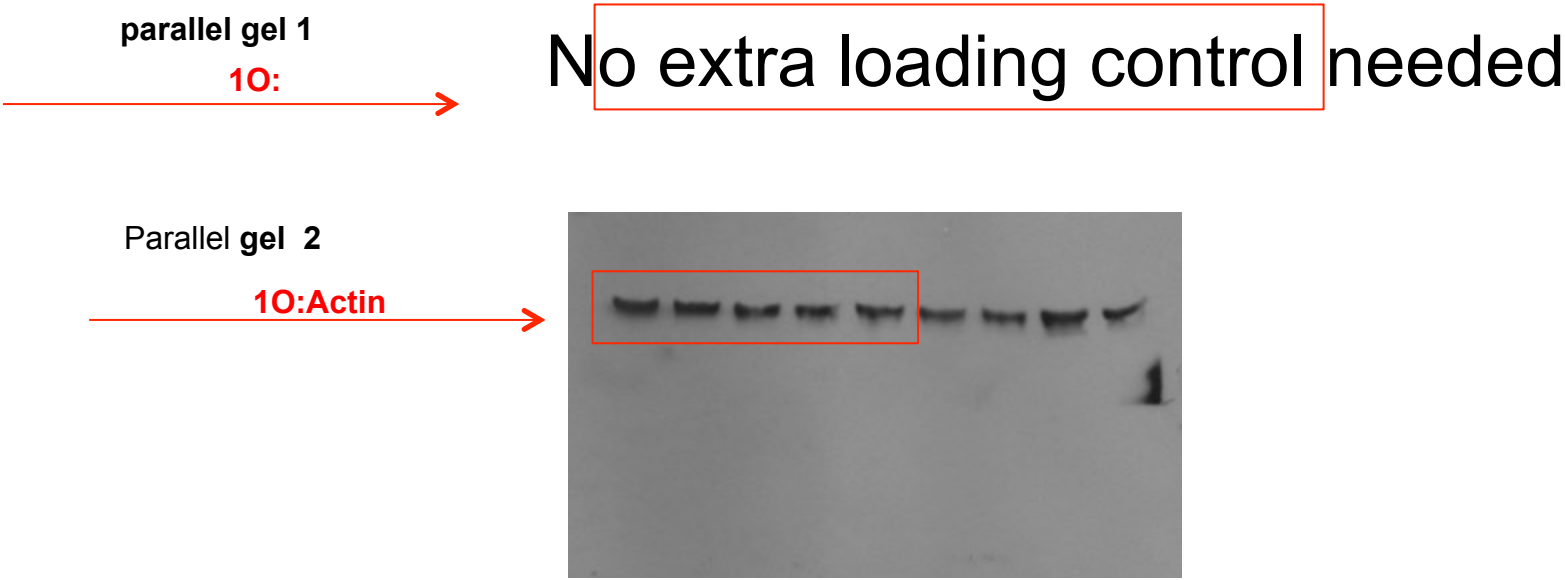
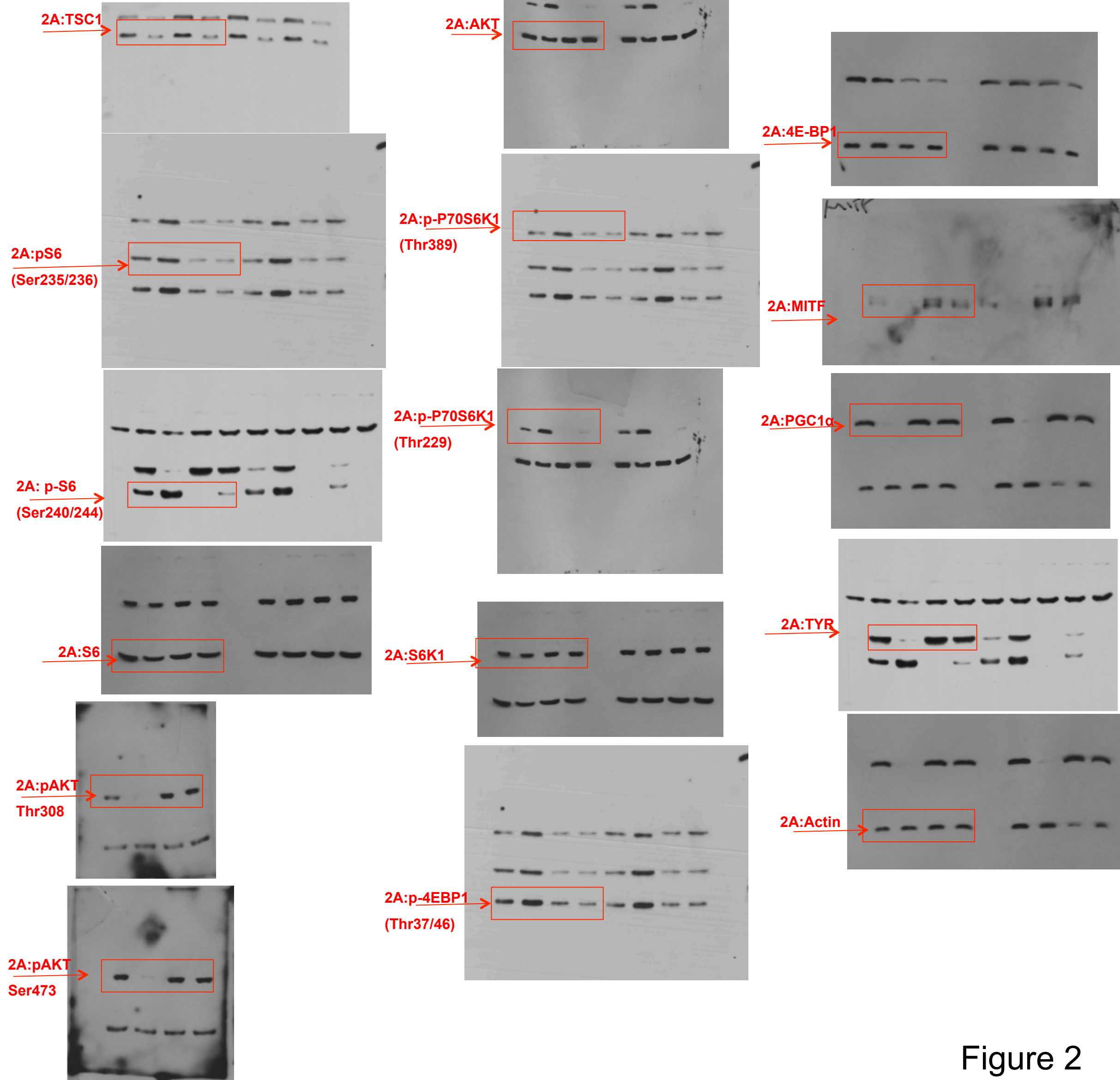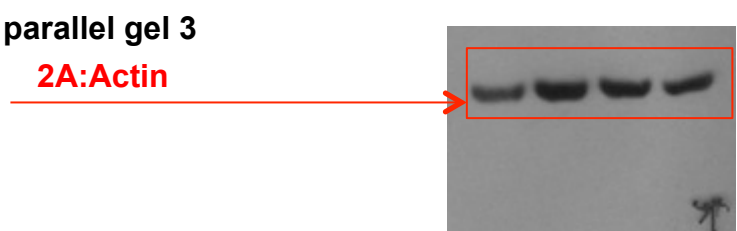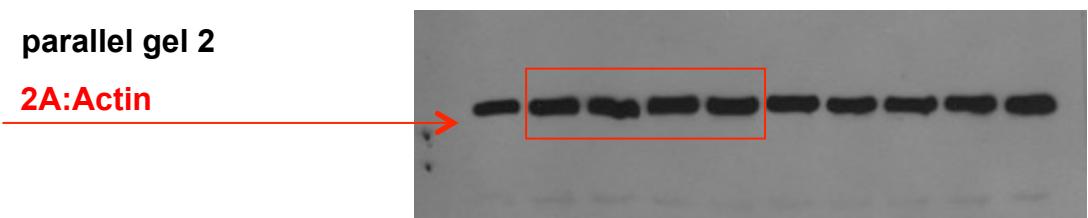# Loading controls for parallel gels in figure 1N

Parallel **gel 1**

     1N:Actin

Loading controls for parallel gels in figure 1O

parallel gel 1

     1O:

No extra loading control needed

Parallel **gel 2**

     1O:Actin

A

Rapa (20 nM) − − + +

shRNA Scr TSC1-3 Scr TSC1-3

TSC1

p-S6 (Ser235/236)

p-S6(Ser240/244)

S6

p-AKT(Thr308)

p-AKT(Ser473)

AKT

p-P70S6K (Thr389)

p-P70S6k (Thr229)

S6K

p-4E-BP1(Thr37/46)

4E-BP1

MITF

PGC-1α

TYR
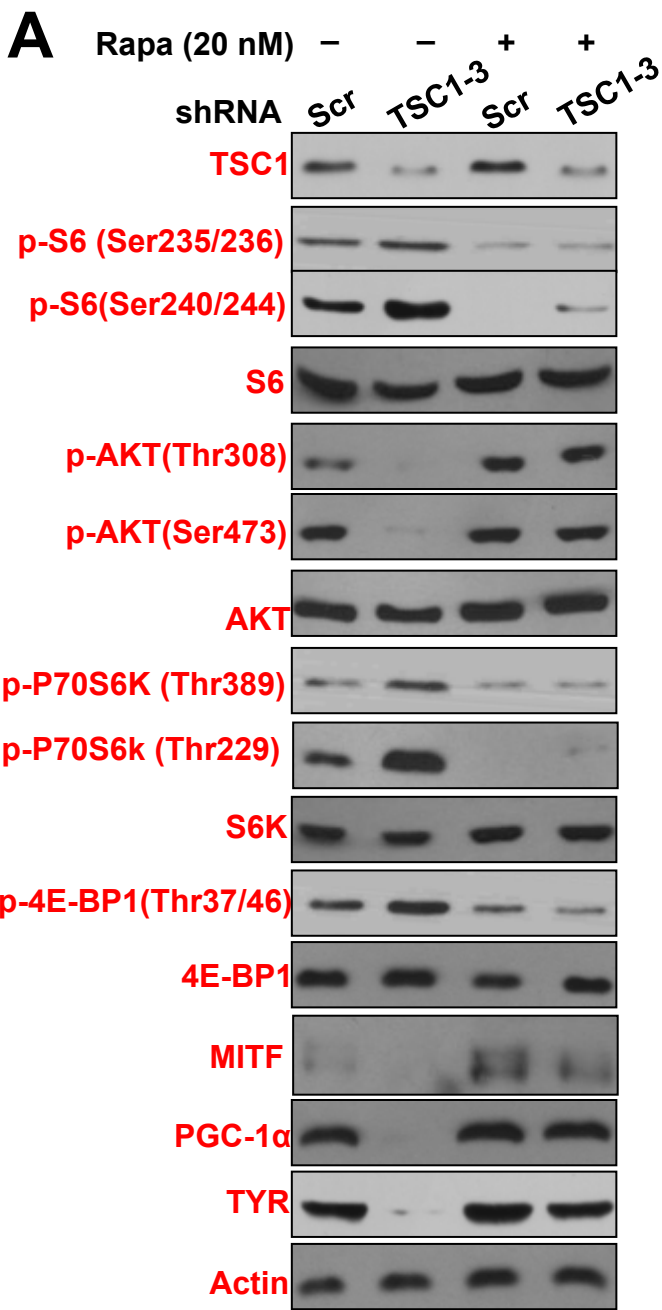
Actin

S6   AKT   p-P70S6k (Thr229)   S6K   4E-BP1   PGC-1α   Actin

Run on same gel, **Actin** in the figure was used as the loading control

parallel gel 1

TSC1   **p-S6 (Ser235/236)**   **p-P70S6K (Thr389)**   **p-4E-BP1(Thr37/46)**   **MITF**

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 2

p-S6(Ser240/244)   **TYR**

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 3

p-AKT(Thr308)   **p-AKT(Ser473)**

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)
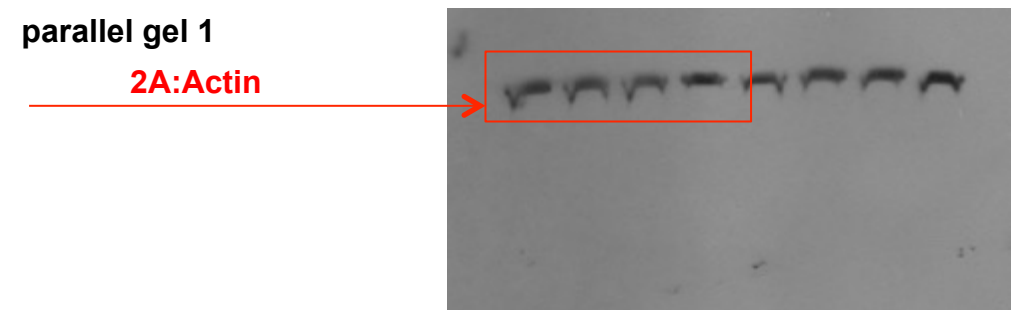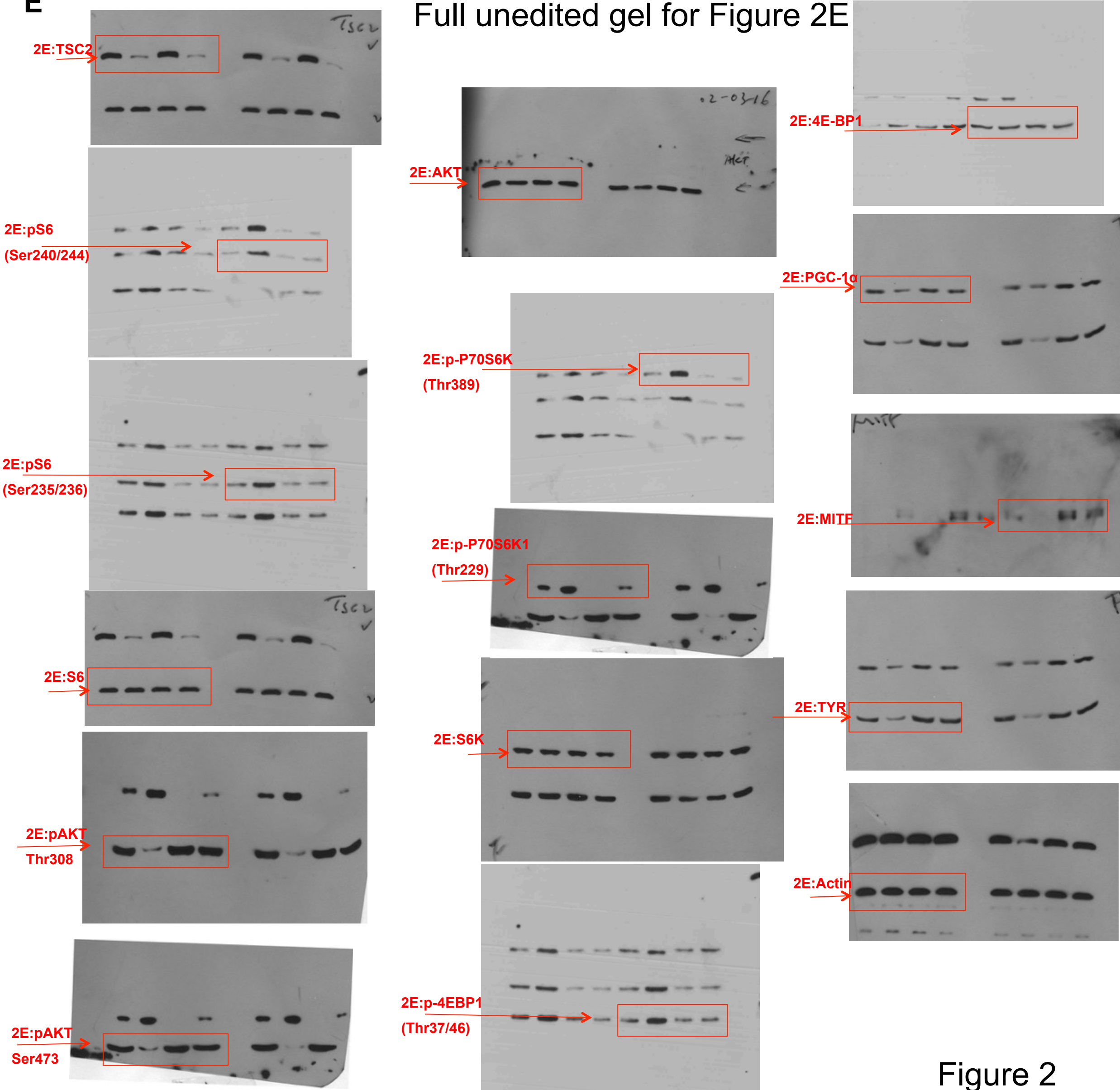
Figure 2

A Full unedited gel for Figure 2A
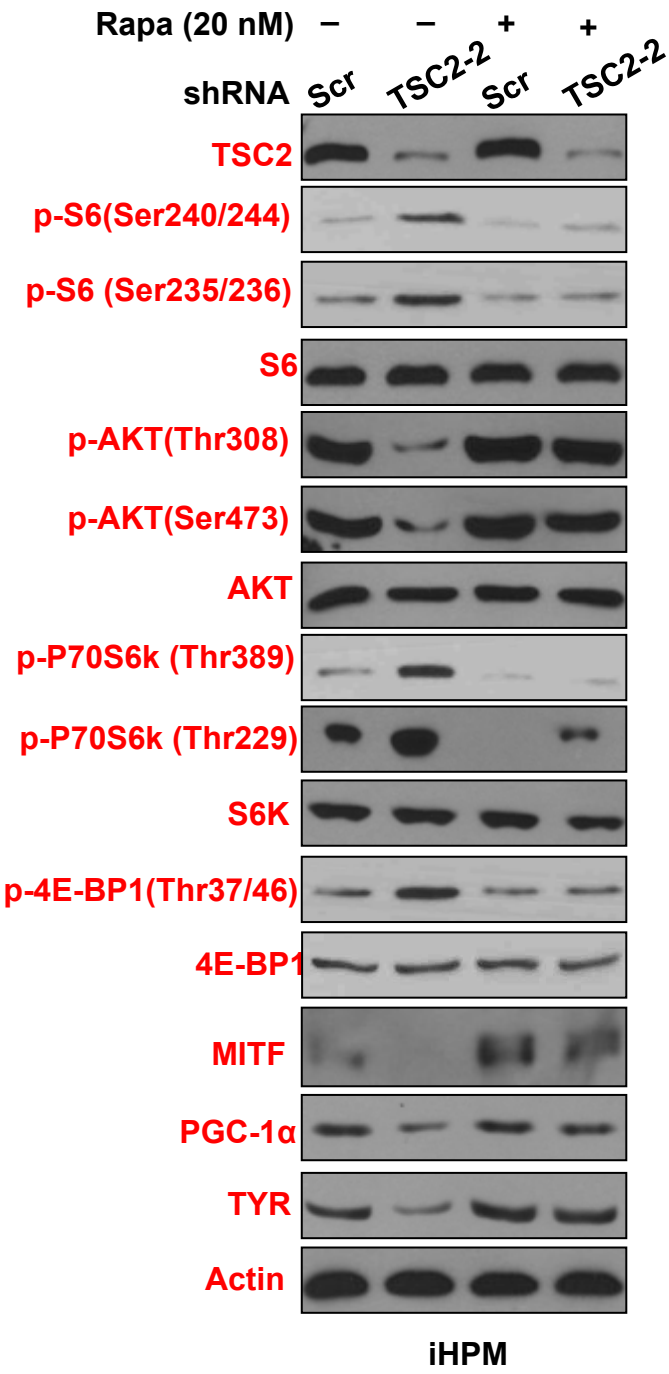
2A:TSC1

2A:AKT

2A:4E-BP1

2A:pS6
(Ser235/236)

2A:p-P70S6K1
(Thr389)

2A:MITF

2A: p-S6
(Ser240/244)

2A:p-P70S6K1
(Thr229)

2A:PGC1α

2A:S6

2A:S6K1

2A:TYR

2A:pAKT
Thr308

2A:p-4EBP1
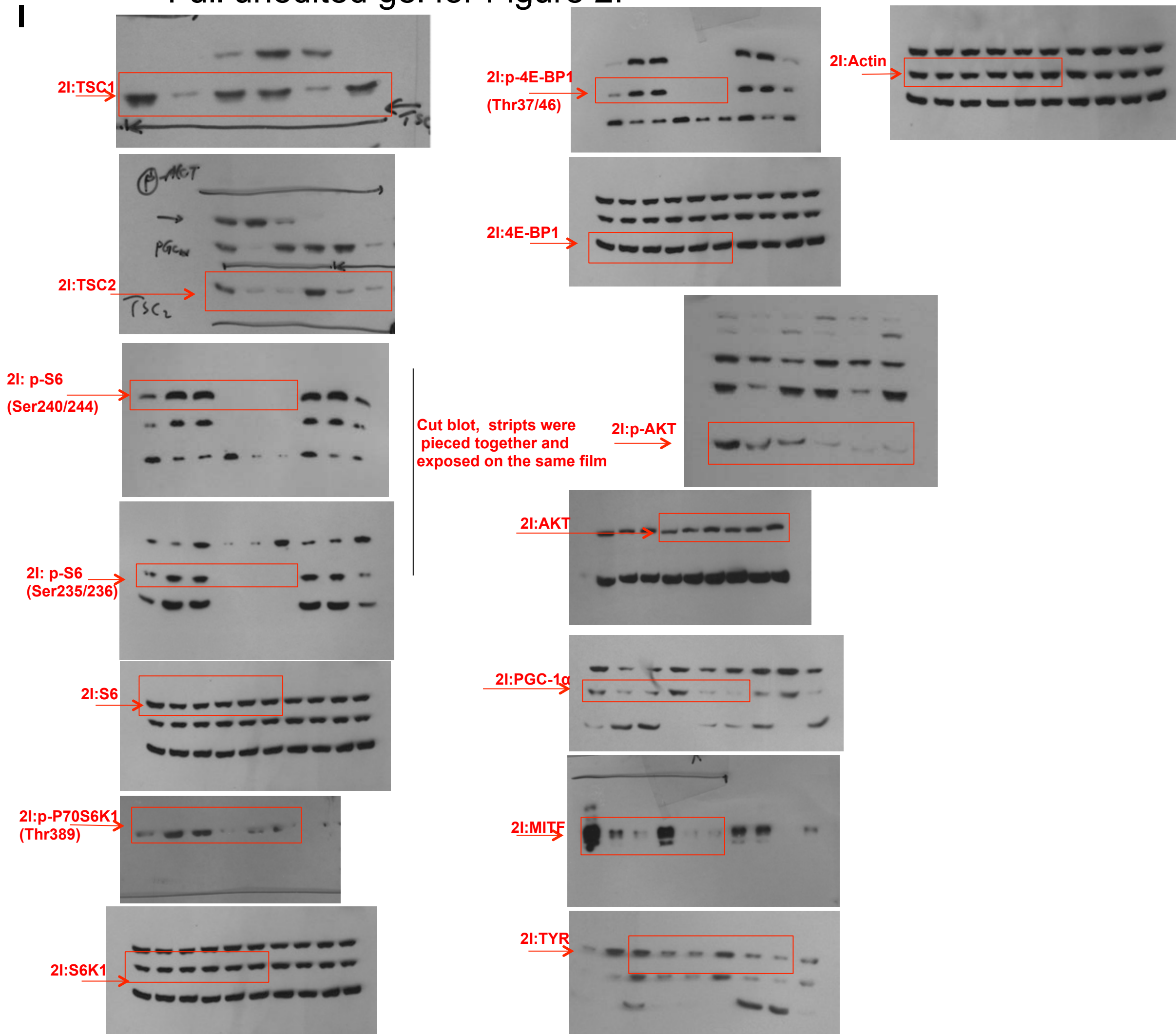(Thr37/46)

2A:Actin

2A:pAKT
Ser473

Figure 2

Loading controls for parallel gels in figure 2A
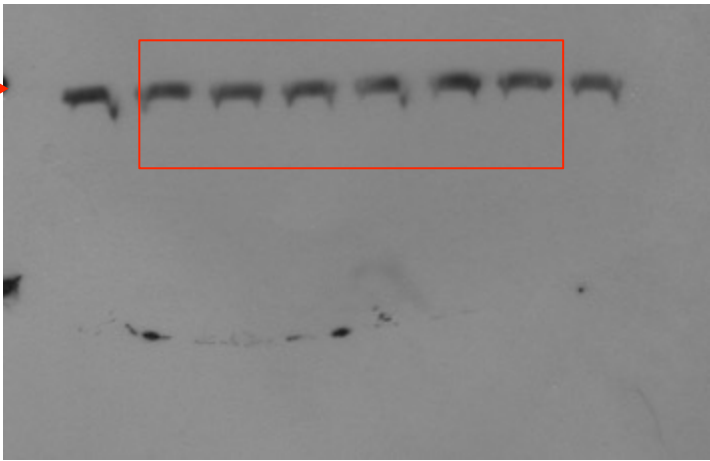
parallel gel 1

2A:Actin

parallel gel 2

2A:Actin

parallel gel 3

2A:Actin

E

Rapa (20 nM) − − + +

shRNA Scr TSC2-2 Scr TSC2-2

TSC2
p-S6(Ser240/244)
p-S6 (Ser235/236)
S6
p-AKT(Thr308)
p-AKT(Ser473)
AKT
p-P70S6k (Thr389)
p-P70S6k (Thr229)
S6K
p-4E-BP1(Thr37/46)
4E-BP1
MITF
PGC-1α
TYR
Actin

iHPM

TSC2	S6	p-AKT(Thr308)	p-AKT(Ser473)	AKT	p-P70S6k (Thr229)	S6K	PGC-1α	TYR	Actin

Run on same gel, **Actin** in the figure was used as the loading control

parallel gel 1

p-S6(Ser240/244)	p-S6 (Ser235/236)	p-P70S6k (Thr389)	p-4E-BP1(Thr37/46)	4E-BP1	MITF

Run on same gel, **4E-BP1** was used as the loading control (see uncropped gel image)

Figure 2

E

Full unedited gel for Figure 2E

2E:TSC2

2E:pS6 (Ser240/244)

2E:pS6 (Ser235/236)

2E:S6

2E:pAKT Thr308

2E:pAKT Ser473

2E:AKT

2E:p-P70S6K (Thr389)

2E:p-P70S6K1 (Thr229)

2E:S6K

2E:p-4EBP1 (Thr37/46)

2E:4E-BP1

2E:PGC-1α

2E:MITF

2E:TYR

2E:Actin

Figure 2

Loading controls for parallel gels in figure 2E

parallel gel 1

2E: ⟶ No extra loading control needed

I

Torin-1 (250 nM) − − − + + +

shRNA Scr TSC1-3 TSC2-2 Scr TSC1-3 TSC2-2

TSC1
TSC2
p-S6 (Ser235/236)
p-S6(Ser240/244)
S6
p-P70S6k (Thr389)
S6k
p-4E-BP1(Thr37/46)
4E-BP1
p-AKT
AKT
PGC-1α
MITF
TYR
Actin

M

shRNA Scr Raptor Rictor

Raptor
Rictor
p-S6 (Ser235/236)
S6
p-AKT
AKT
MITF
PGC-1α
TYR
Actin

iHPM

S6   S6k   4E-BP1   MITF   Actin

Run on same gel, **Actin** in the figure was used as the loading control

Parelle gel 1

TSC1   TSC2   p-P70S6k (Thr389)   p-AKT

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parelle gel 2

p-S6 (Ser235/236)   p-S6(Ser240/244)   p-4E-BP1(Thr37/46)   PGC-1α

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parelle gel 3

AKT

Run on same gel, **AKT in the figure** was used as the loading control

Parelle gel 4

TYR

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

p-AKT   Actin

Run on same gel, **Actin** in the figure was used as the loading control

Parelle gel 1

Raptor

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parelle gel 2

Rictor

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parelle gel 3

p-S6 (Ser235/236)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parelle gel 4

MITF

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parelle gel 5

PGC-1α   TYR

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

S6   AKT

No loading control needed

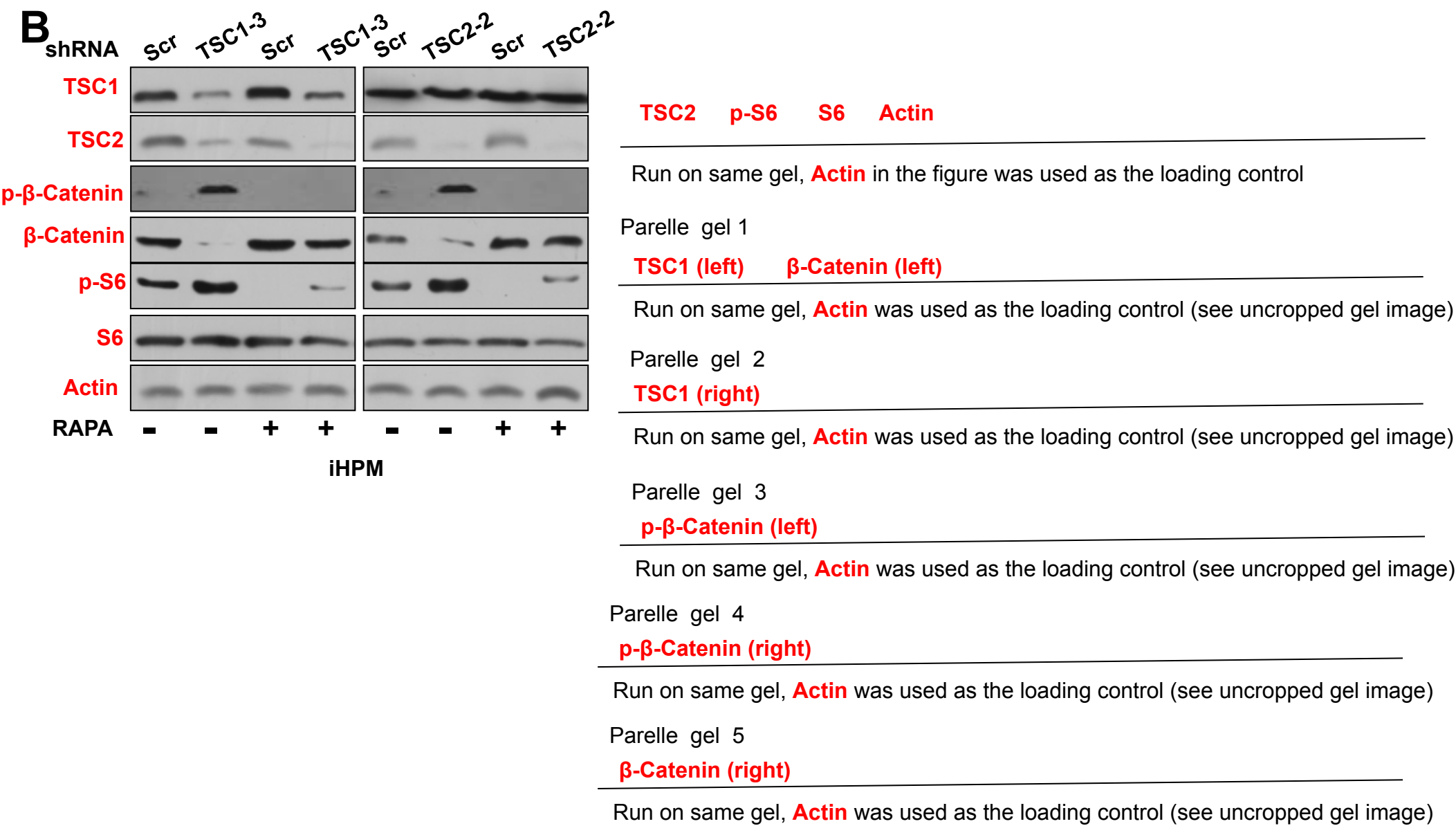Figure 2

Full unedited gel for Figure 2I

I

2I:TSC1

2I:TSC2

2I: p-S6
(Ser240/244)

2I: p-S6
(Ser235/236)

2I:S6

2I:p-P70S6K1
(Thr389)

2I:S6K1

Cut blot, stripts were
pieced together and
exposed on the same film

2I:p-4E-BP1
(Thr37/46)
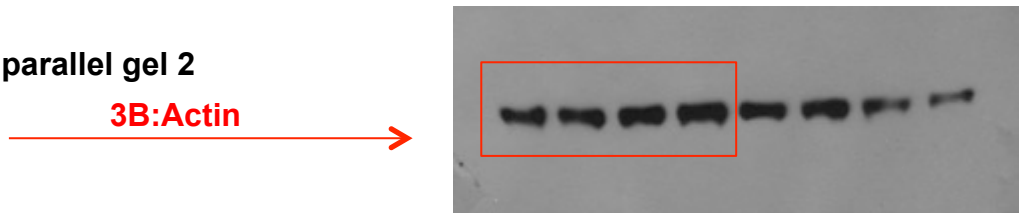
2I:4E-BP1

2I:p-AKT

2I:AKT

2I:PGC-1α

2I:MITF

2I:TYR

2I:Actin

Figure 2

Loading controls for parallel gels in figure 2I

Parallel **gel 1**

2I:Actin

Parallel **gel 2**

2I:Actin

Parallel **gel 3**

2I

No extra loading control needed

Parallel **gel 4**

2I:Actin

Full unedited gel for Figure 2M
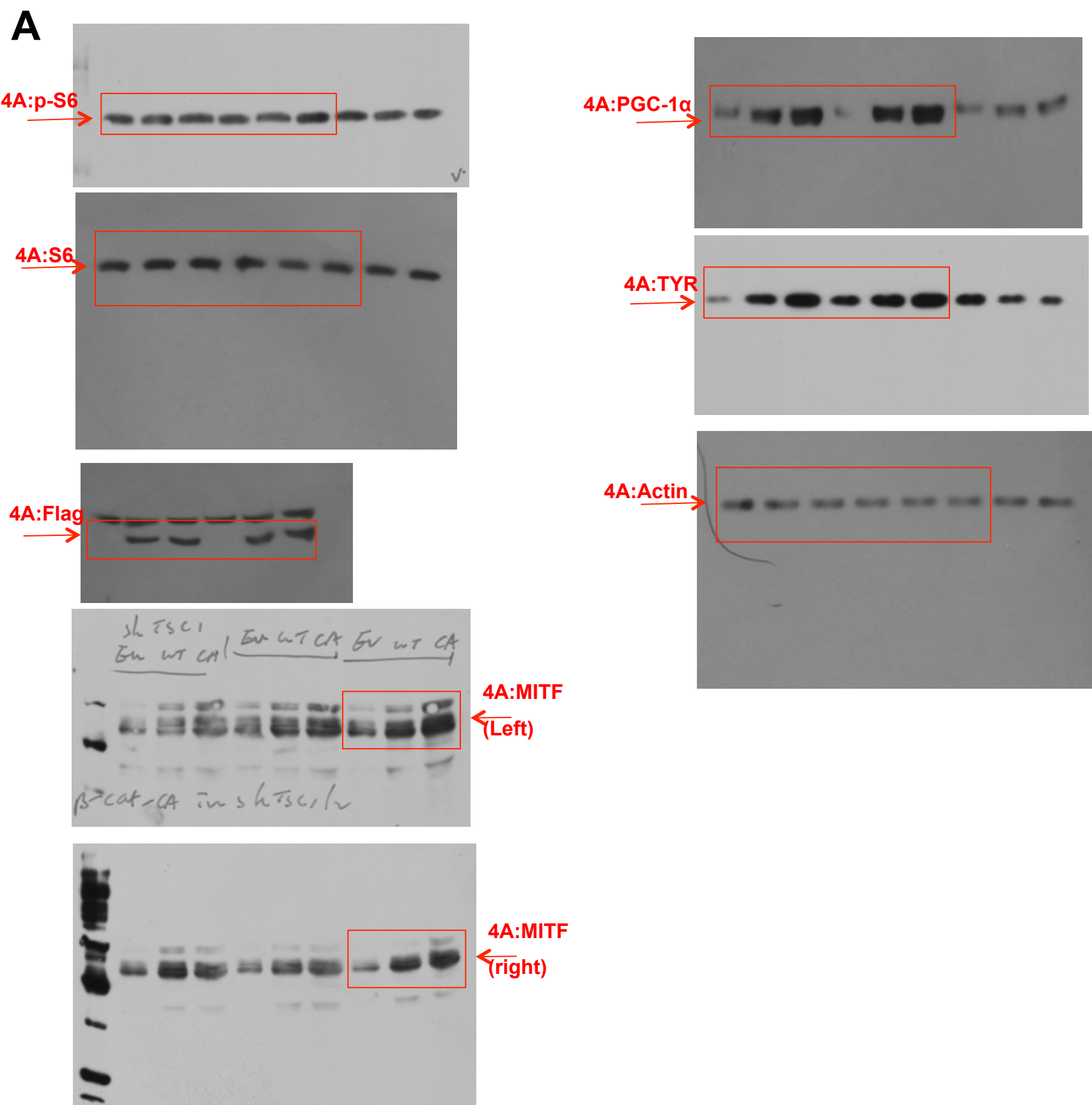

Figure 2

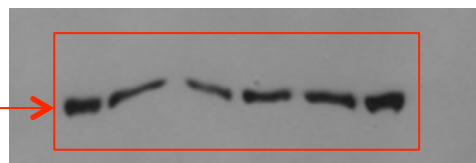# Loading controls for parallel gels in figure 2M

parallel gel 1

2M:Actin

parallel gel 2

2M:Actin

parallel gel 3

2M:Actin

Parallel gel 4

2M:Actin

Parallel gel 5

2M:Actin

A

shRNA   Scr   TSC1-3   Scr   TSC2-2

TSC1
TSC2
p-AKT(T308)
p-AKT(S473)
AKT
p-GSK3β
GSK3β
p-β-Catenin
β-Catenin
p-S6(Ser235/236)
p-S6(Ser240/244)
S6
p-S6K1 (Thr389)
S6K1
Actin
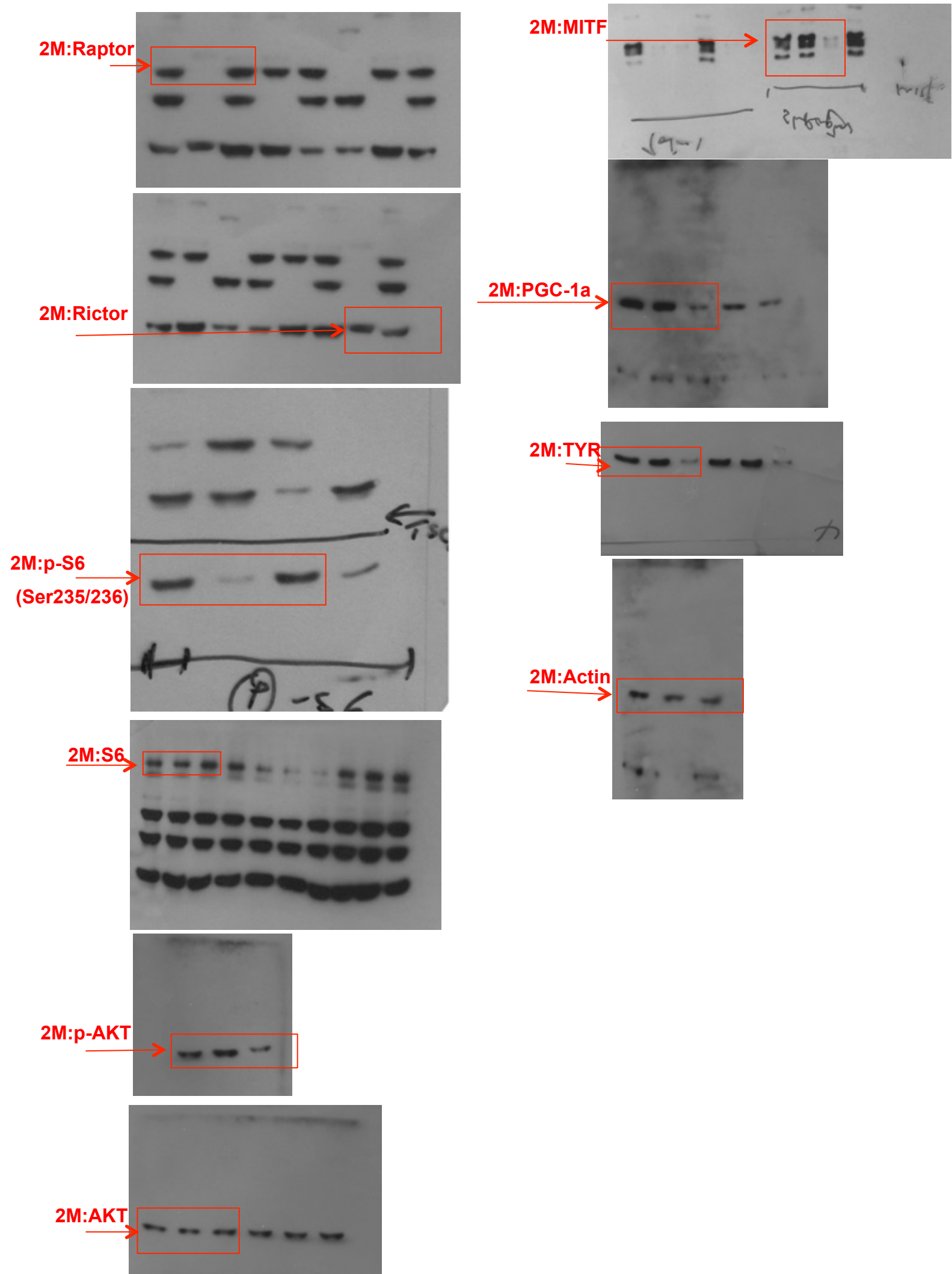
iHPM
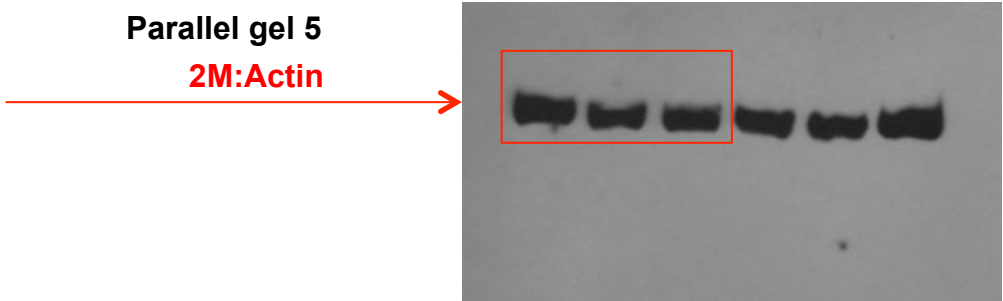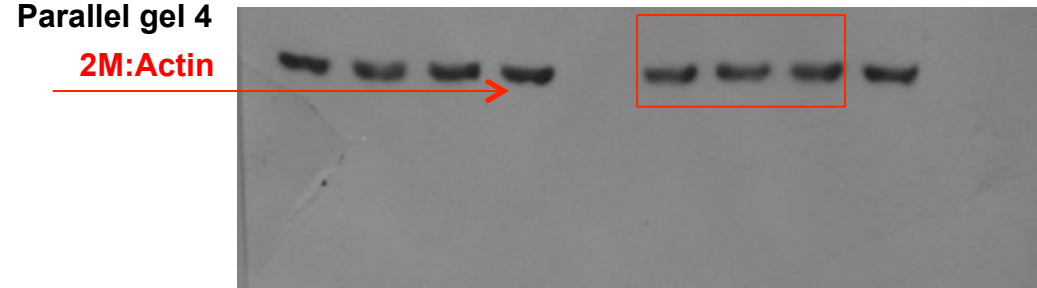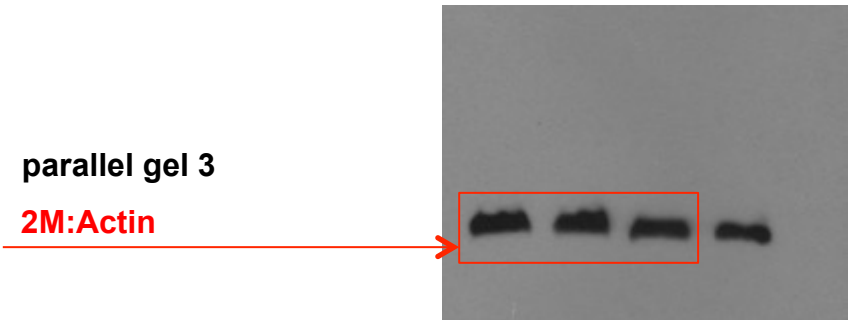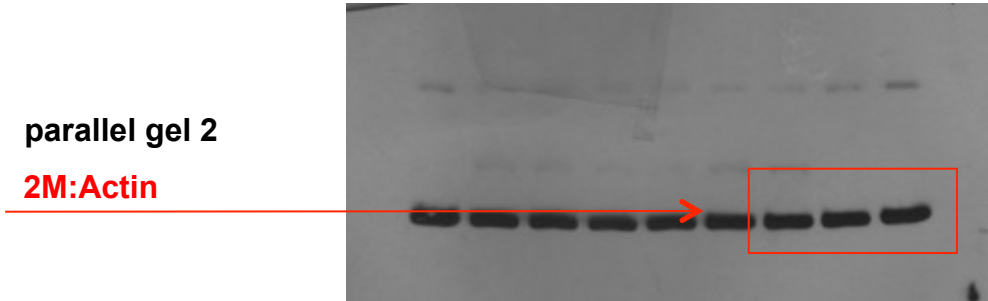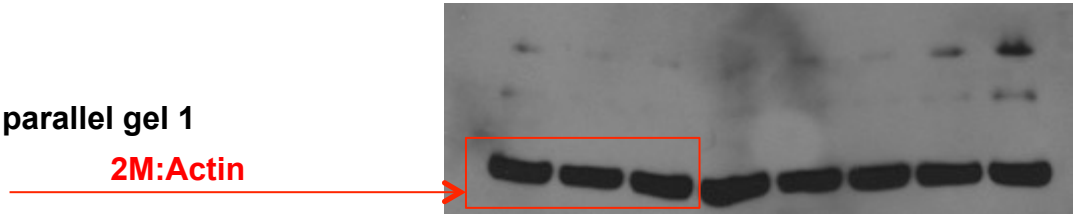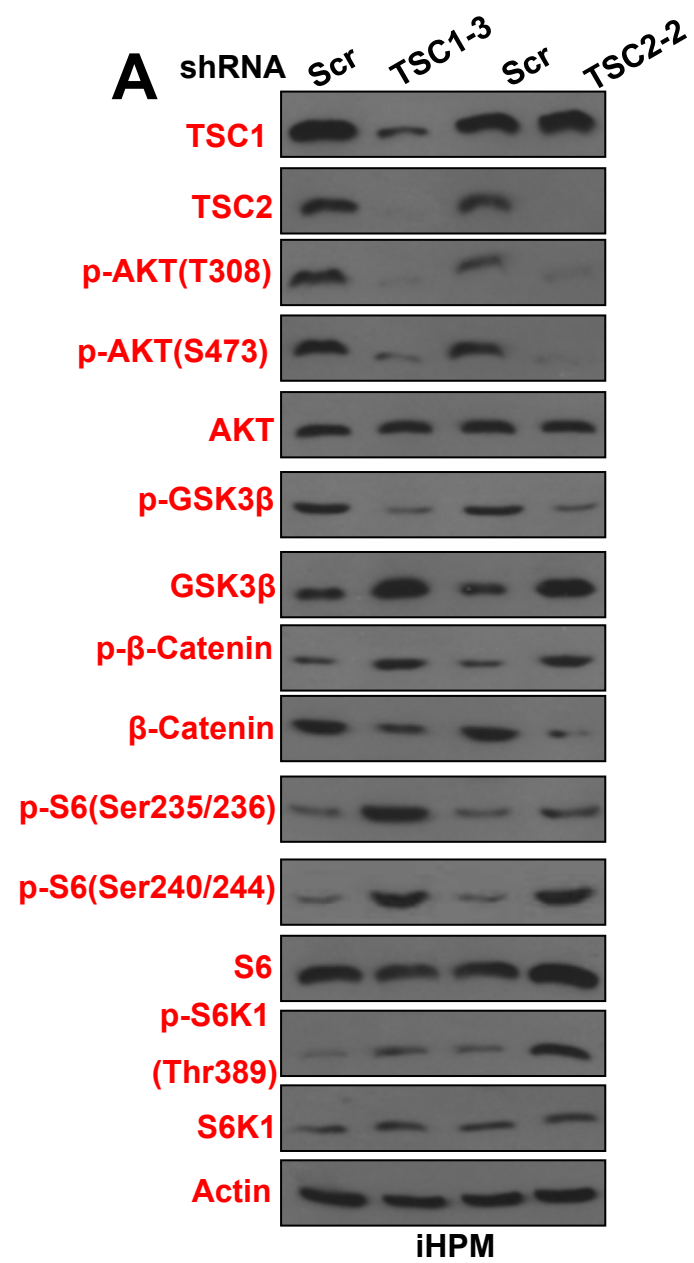
TSC1   TSC2   p-AKT(T308)   AKT   p-GSK3β   p-β-Catenin   p-S6(Ser235/236)   Actin

Run on same gel, **Actin** in the figure was used as the loading control

Parelle gel 1

p-AKT(S473)   GSK3β   β-Catenin   p-S6(Ser240/244)   S6   p-S6K1 (Thr389)   S6K1

Run on same gel, **S6** in the figure was used as the loading control

No extra loading control needed

Figure 3

A Full unedited gel for Figure 3A

3A:TSC1

3A:TSC2

3A:p-AKT T308

3A:p-AKT S473

3A:AKT

3A:p-GSK3β

3A:GSK3β

3A:p-β-catenin

3A:β-catenin

3A:p-S6 (s235/236)

3A:p-S6 (s240/244)

3A:S6

3A:p-S6K1(Thr389)

3A:S6K1

3A:Actin
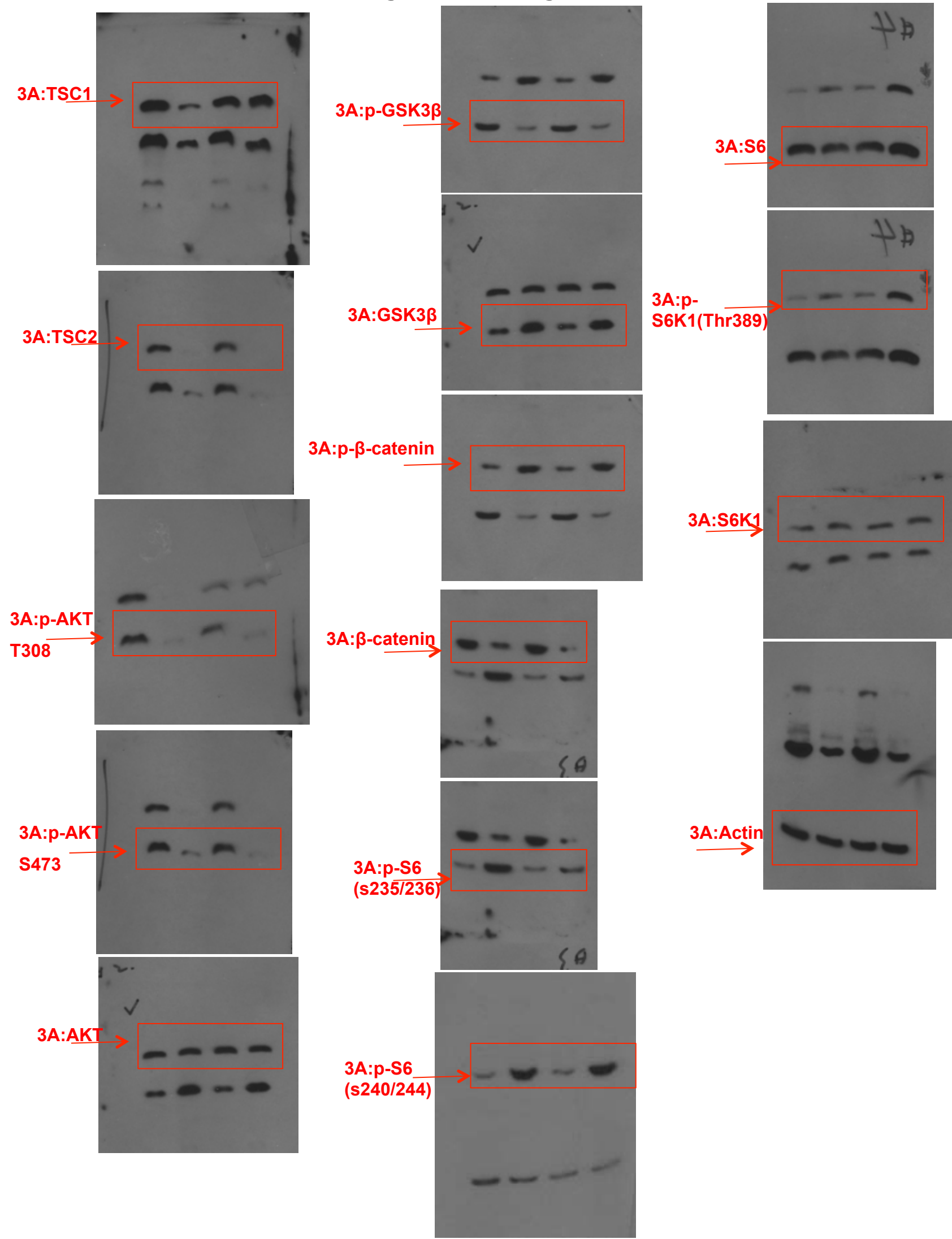
Figure 3

Loading controls for parallel gels in figure 3A

Parallel **gel 1**

3A → No extra loading control needed

B

shRNA	Scr	TSC1-3	Scr	TSC1-3	Scr	TSC2-2	Scr	TSC2-2
TSC1								
TSC2								
p-β-Catenin								
β-Catenin								
p-S6								
S6								
Actin								
RAPA	−	−	+	+	−	−	+	+

iHPM

TSC2   p-S6   S6   Actin

Run on same gel, **Actin** in the figure was used as the loading control

Parelle gel 1

   TSC1 (left)   β-Catenin (left)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parelle gel 2

   TSC1 (right)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parelle gel 3

   p-β-Catenin (left)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parelle gel 4

   p-β-Catenin (right)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parelle gel 5

   β-Catenin (right)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)
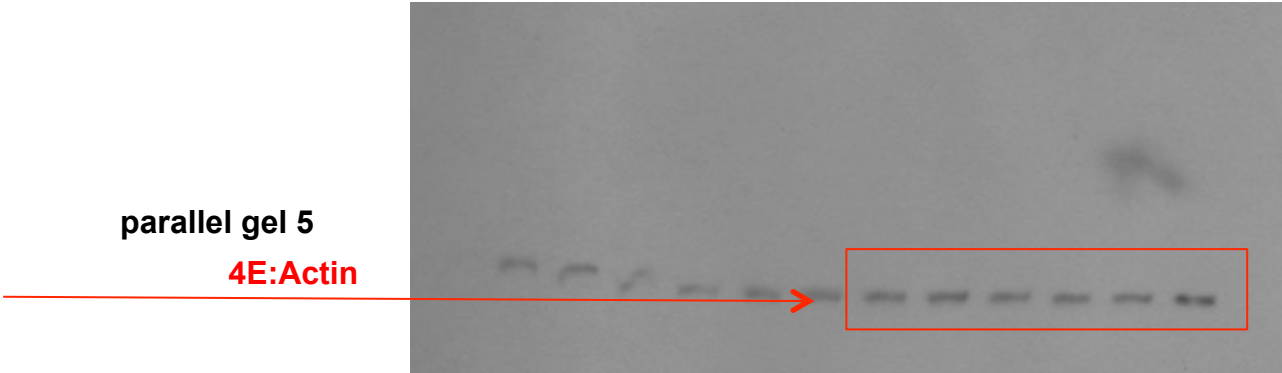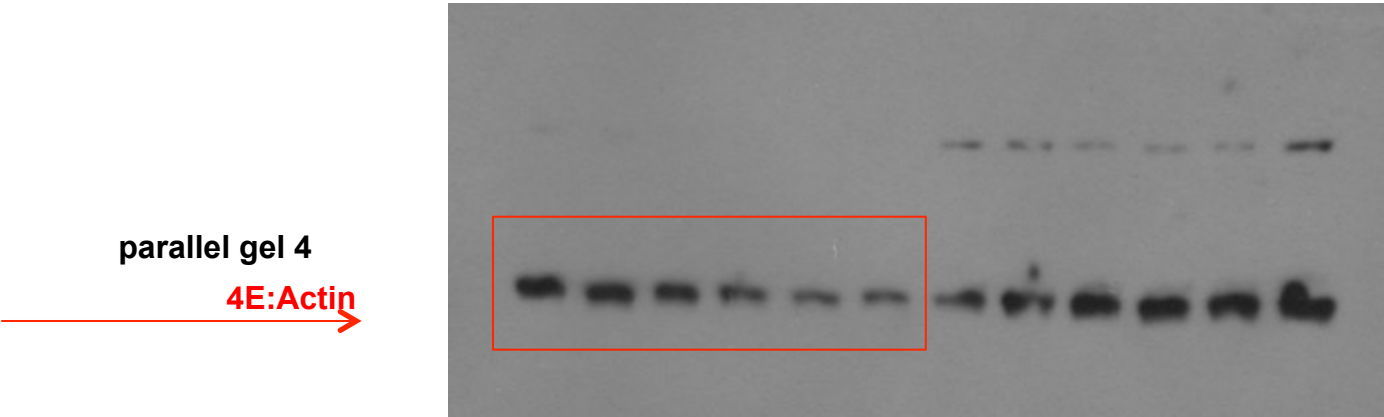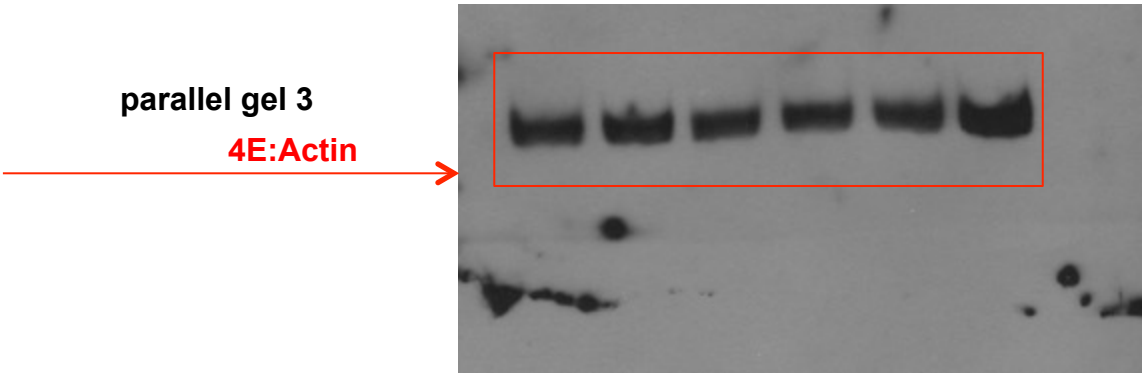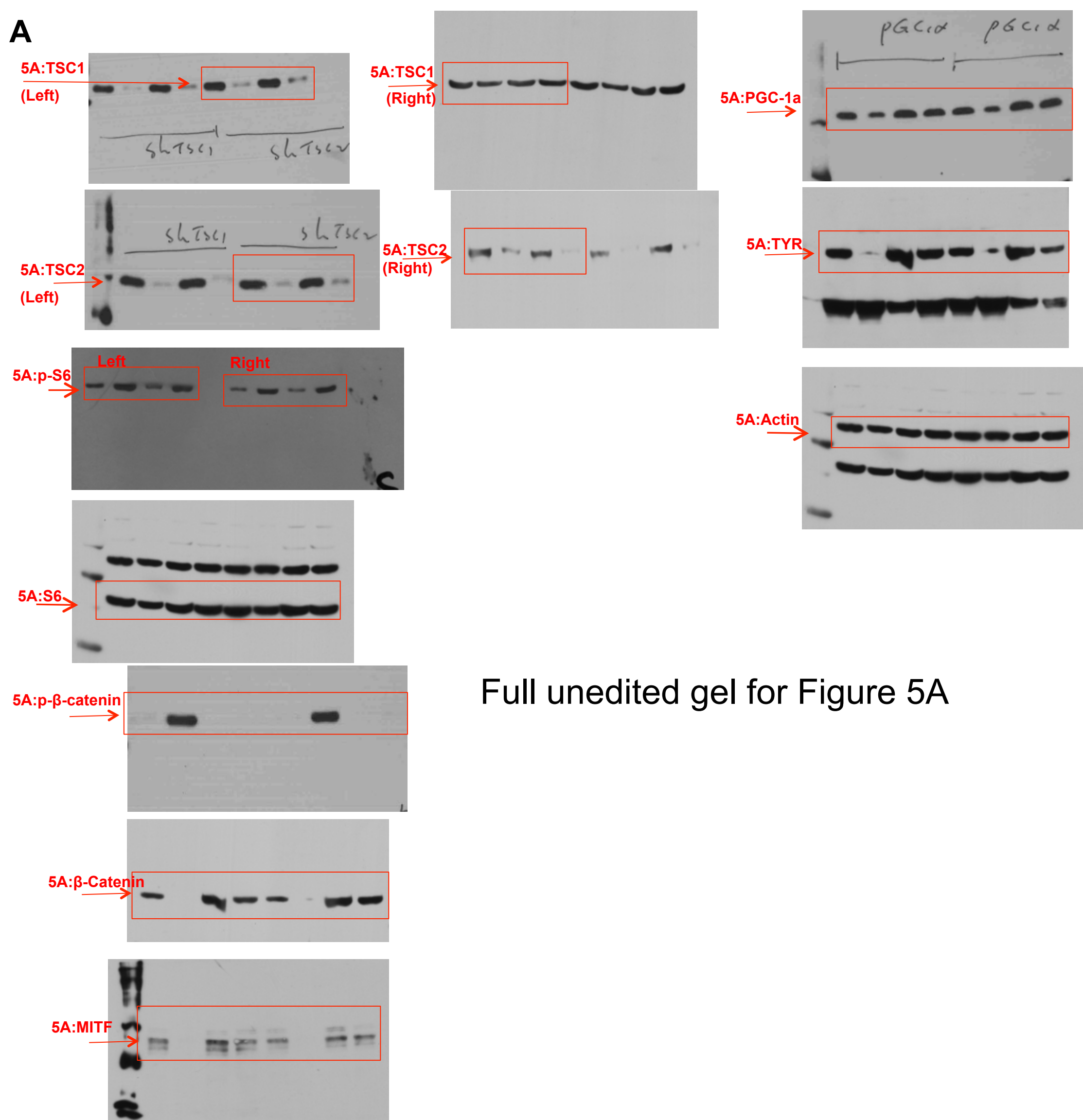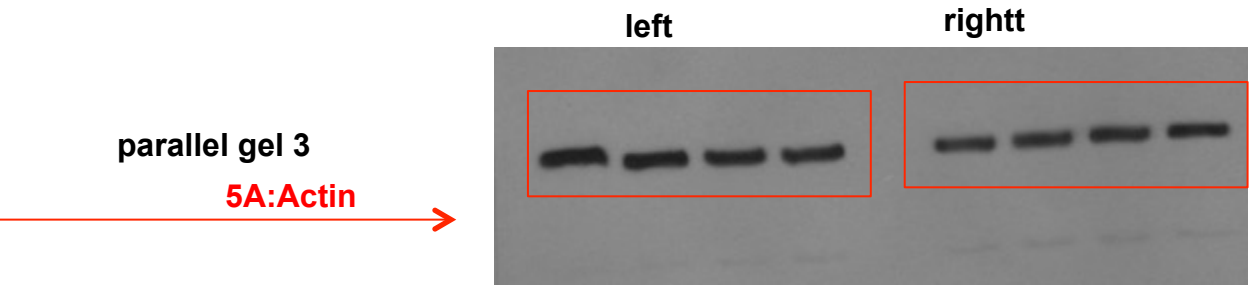
Figure 3

B Full unedited gel for Figure 3B

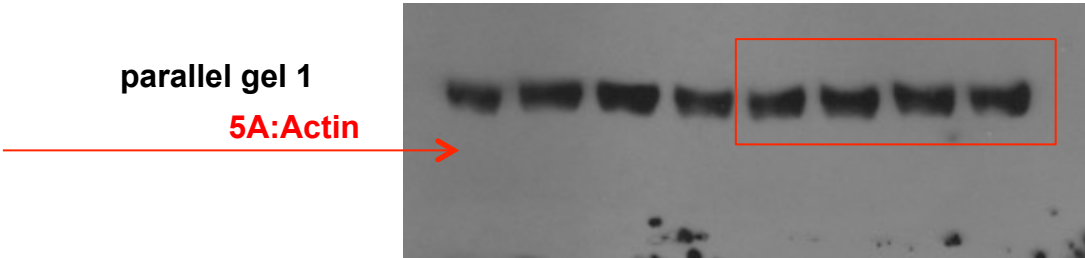

Figure 3

Loading controls for parallel gels in figure 3B

parallel gel 1

3B:Actin

parallel gel 2

3B:Actin

parallel gel 3

3B:Actin

Parallel gel 4

3B:Actin

Parallel gel 5

3B:Actin

C

	Cyt			Nuc		
shRNA	Scr	TSC1	TSC2	Scr	TSC1	TSC2

β-Catenin

p-β-Catenin

TOPOIIβ

β-Tubulin

iHPM

No extra loading needed

D

	-Rapa						+Rapa					
	Cyt			Nuc			Cyt			Nuc		
shRNA	Scr	TSC1	TSC2	Scr	TSC1	TSC2	Scr	TSC1	TSC2	Scr	TSC1	TSC2

β-Catenin

p-β-Catenin

TOPOIIβ

β-Tubulin

iHPM

No extra loading needed

Figure 3

C

3C:β-catenin

3C:p-β-catenin

3C:TOPOII β

3C:β-Tubulin

Full unedited gel for Figure 3C&D

D

3D:β-catenin

3D:p-β-catenin

3D:TOPOIIβ

3D:β-Tubulin

Figure 3

Loading controls for parallel gels in figure 3C&D

No extra loading control needed

A

β-catenin

shTSC1-3
EV WT CA

p-S6
S6
Flag
MITF
PGC1-α
TYR
Actin

iHPM

B

shTSC2-2
EV WT CA

iHPM

p-S6   S6   PGC1-α   TYR   Actin

Run on same gel, **Actin** in the figure was used as the loading control

Parallel gel 1

MITF (left)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parallel gel 2

MITF (right)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parallel gel 3

Flag

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Figure 4

Full unedited gel for Figure 4AB

A

4A:p-S6

4A:S6

4A:Flag

4A:PGC-1α

4A:TYR

4A:Actin

sh TSC1
Ev WT CA   Ev WT CA   Ev WT CA

4A:MITF
(Left)

β-cat-CA in sh TSC1/v
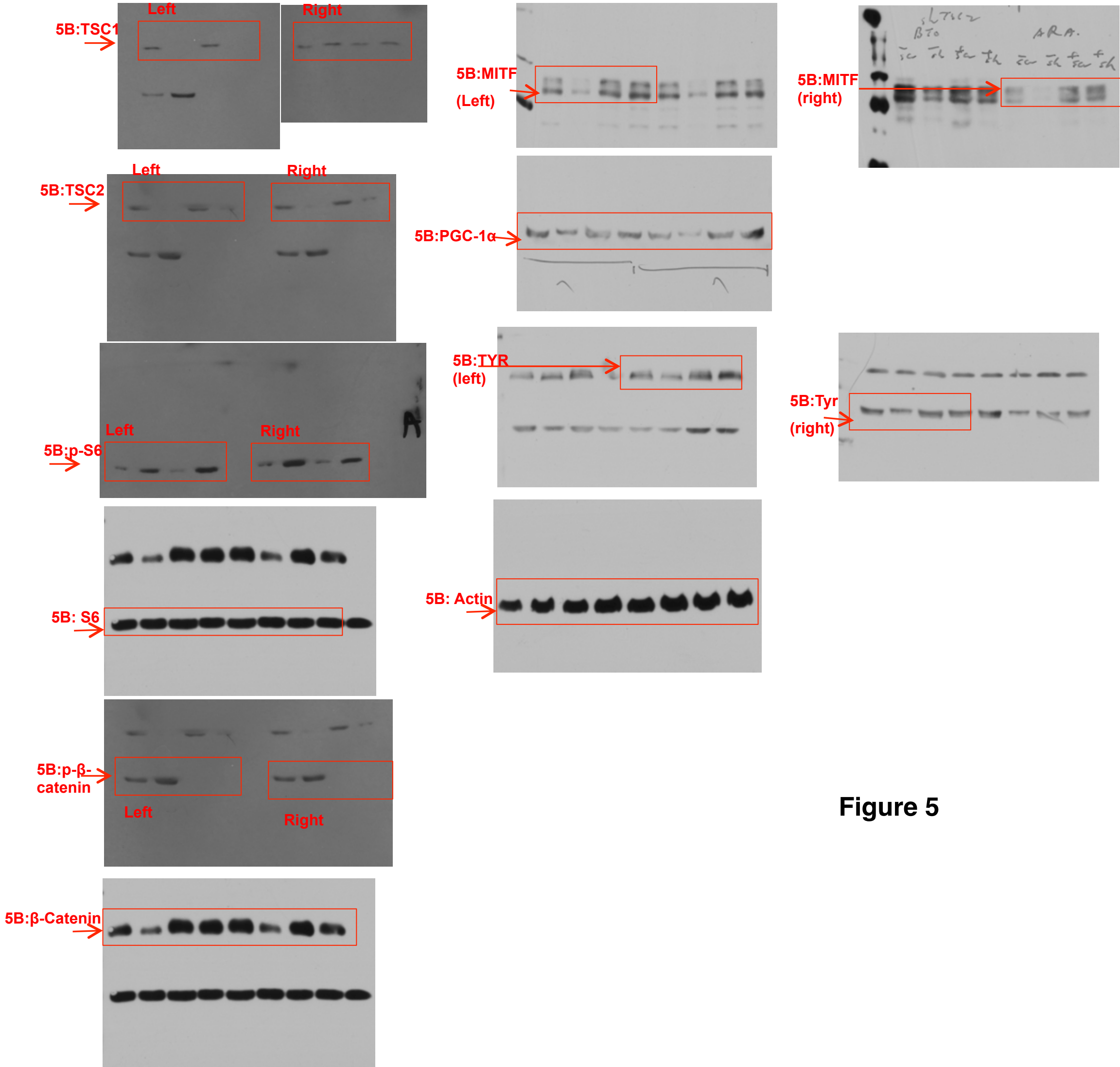
4A:MITF
(right)

Figure 4

Loading controls for parallel gels in figure 4AB

parallel gel 1

4A:Actin
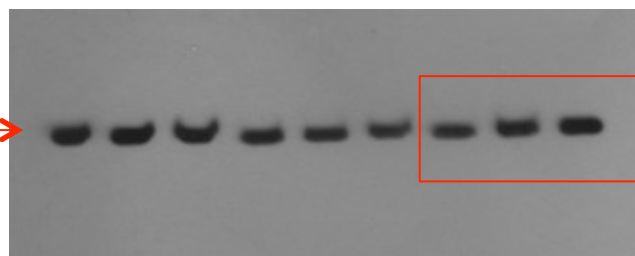
parallel gel 2

4A:Actin
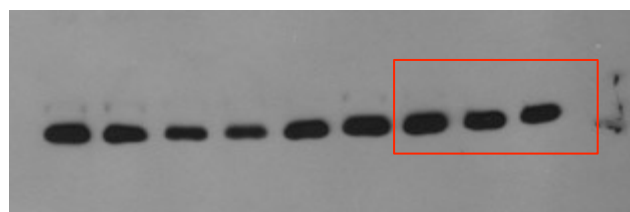
parallel gel 3

4A:Actin
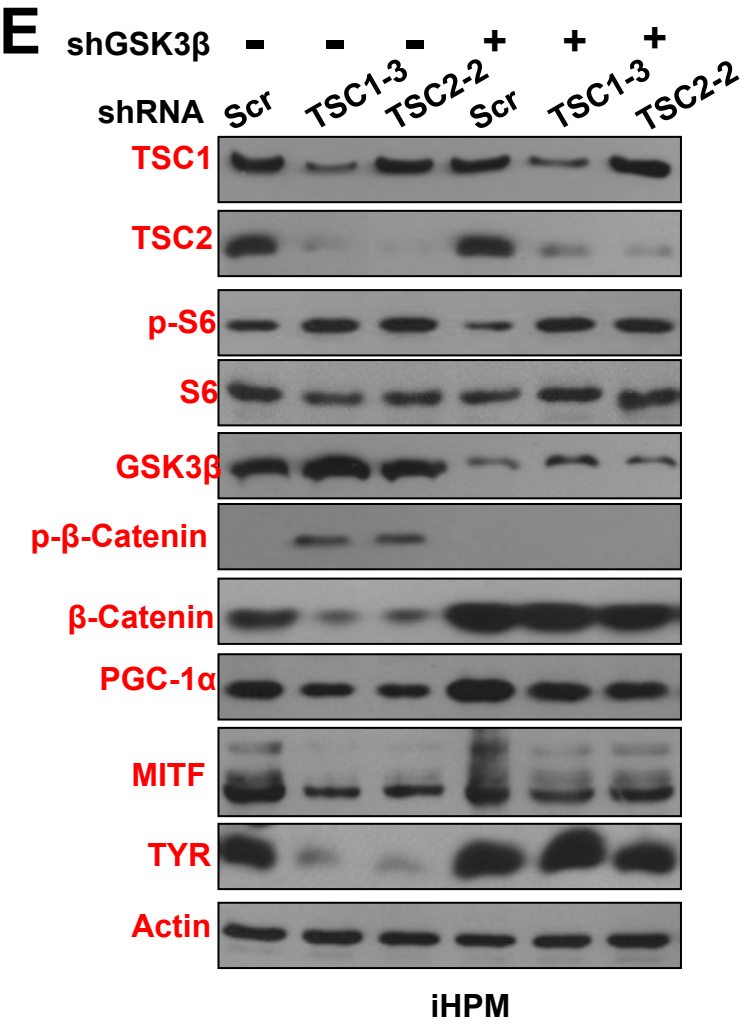
E

shGSK3β   −   −   −   +   +   +

shRNA   Scr   TSC1-3   TSC2-2   Scr   TSC1-3   TSC2-2

TSC1

TSC2

p-S6

S6

GSK3β

p-β-Catenin

β-Catenin

PGC-1α

MITF

TYR

Actin

iHPM

S6   Actin
_____

Run on same gel, **Actin** in the figure was used as the loading control

Parallel gel 1

TSC1   TSC2   GSK3β   PGC-1α   MITF
_____

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parallel gel 2

p-S6
_____

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parallel gel 3

p-β-Catenin
_____

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parallel gel 4

β-Catenin   TYR
_____

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parallel gel 5

TYR
_____

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Figure 4

Full unedited gel for Figure 4E

E
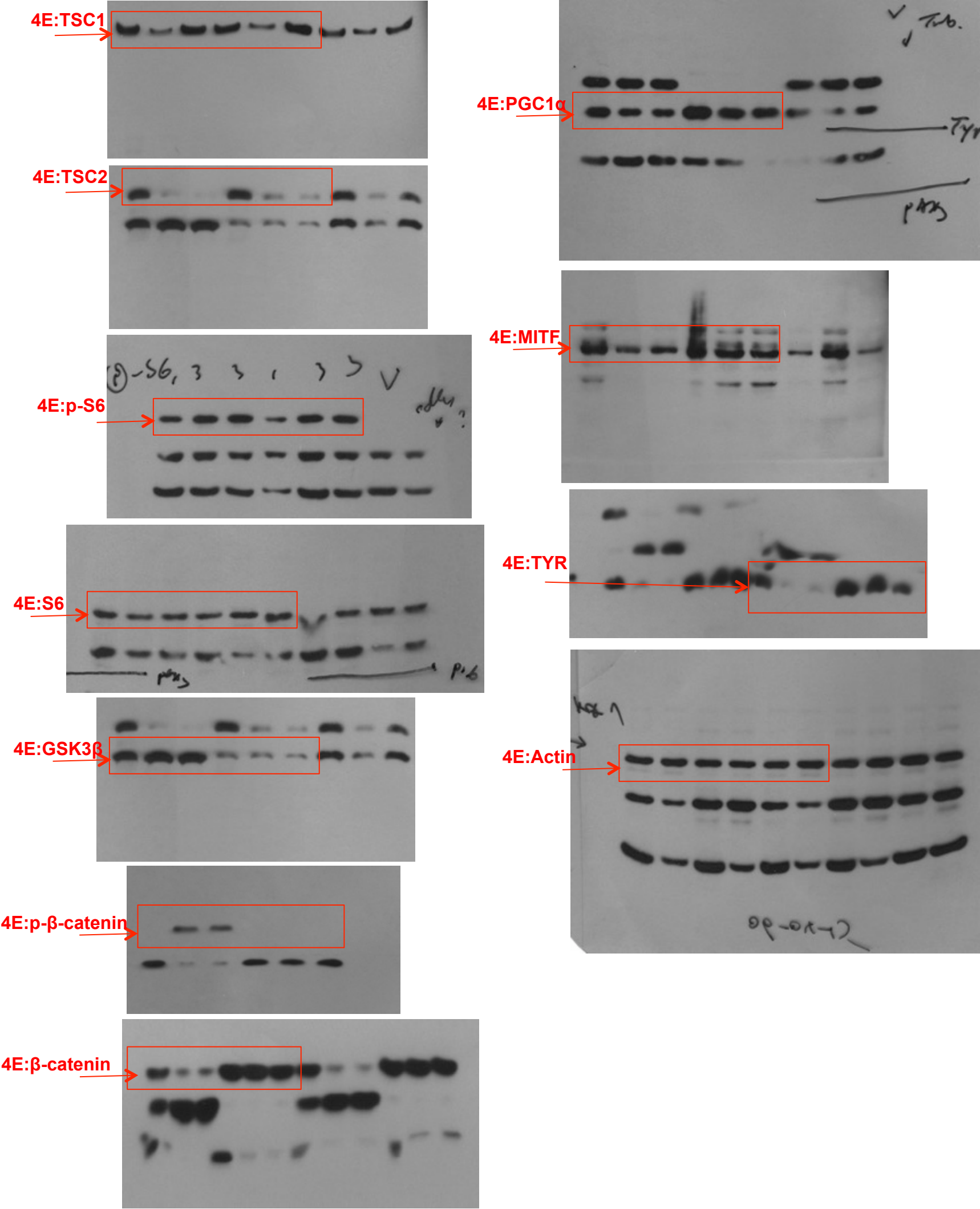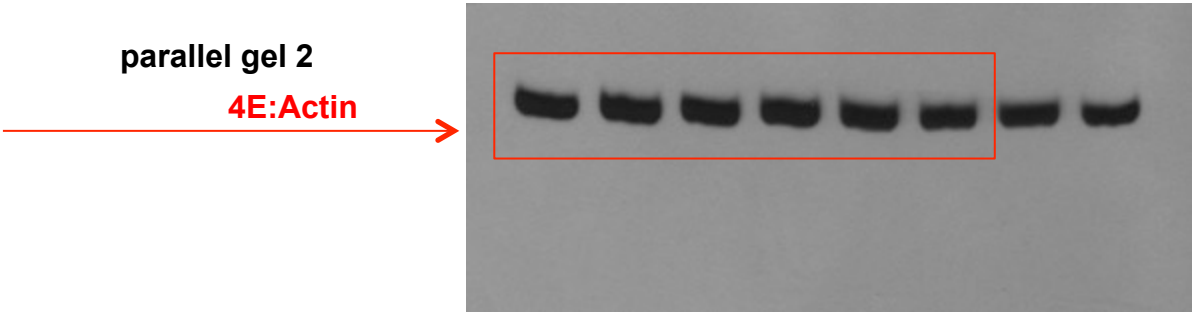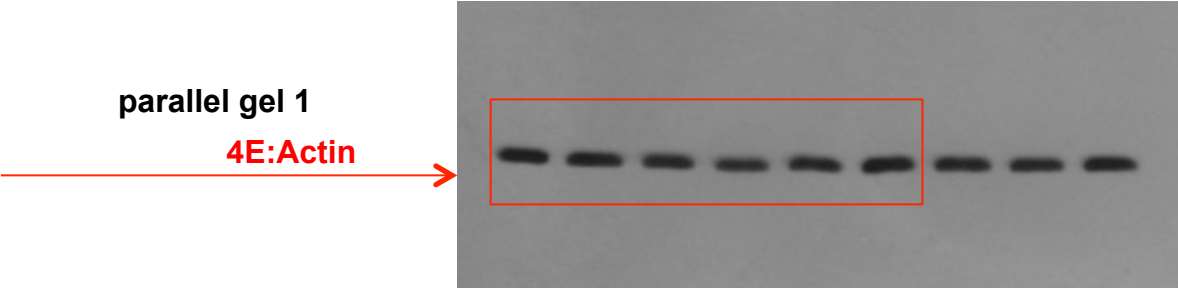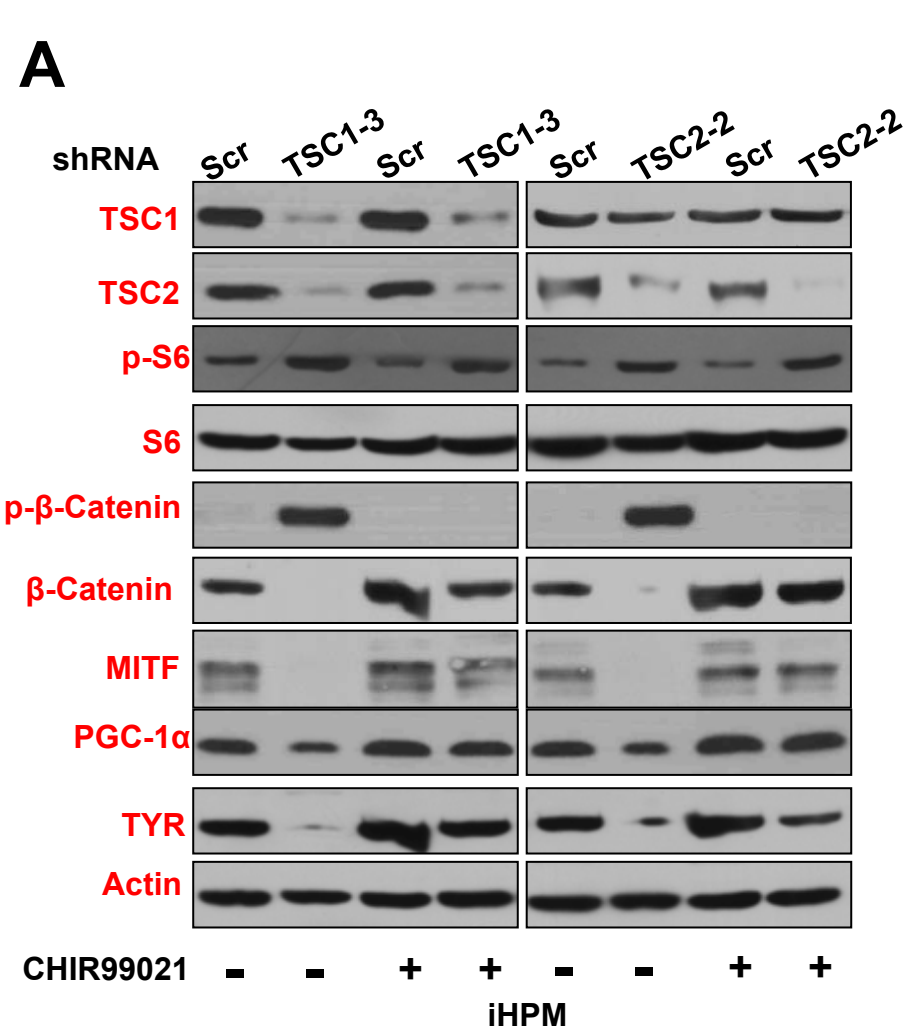
4E:TSC1

4E:TSC2

4E:p-S6

4E:S6

4E:GSK3β

4E:p-β-catenin

4E:β-catenin

4E:PGC1α

4E:MITF

4E:TYR

4E:Actin

Figure 4

Loading controls for parallel gels in figure 4E

parallel gel 1
4E:Actin

parallel gel 2
4E:Actin

parallel gel 3
4E:Actin

parallel gel 4
4E:Actin

parallel gel 5
4E:Actin

A

shRNA	Scr	TSC1-3	Scr	TSC1-3	Scr	TSC2-2	Scr	TSC2-2

TSC1

TSC2

p-S6

S6

p-β-Catenin

β-Catenin

MITF

PGC-1α

TYR

Actin

CHIR99021 − − + + − − + +

iHPM

S6   p-β-Catenin   β-Catenin   MITF   PGC-1α   TYR   Actin

Run on same gel, **Actin** in the figure was used as the loading control

Parelle gel 1

TSC1 (left)   TSC2 (left)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parelle gel 2

TSC1 (Right)   TSC2 (Right)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parelle gel 3

p-S6

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Figure 5
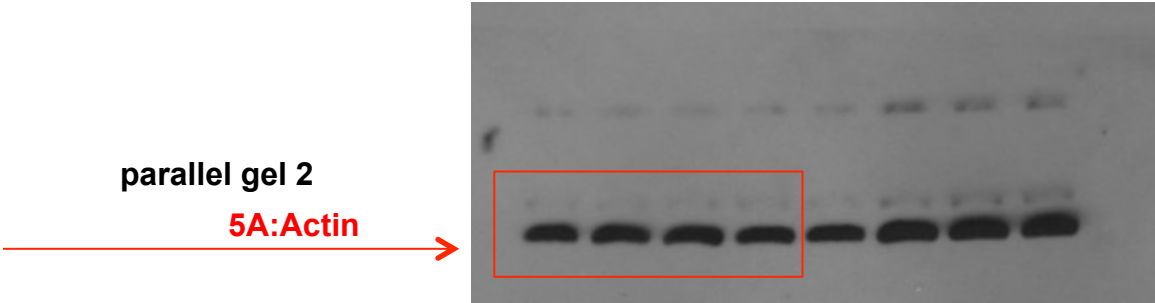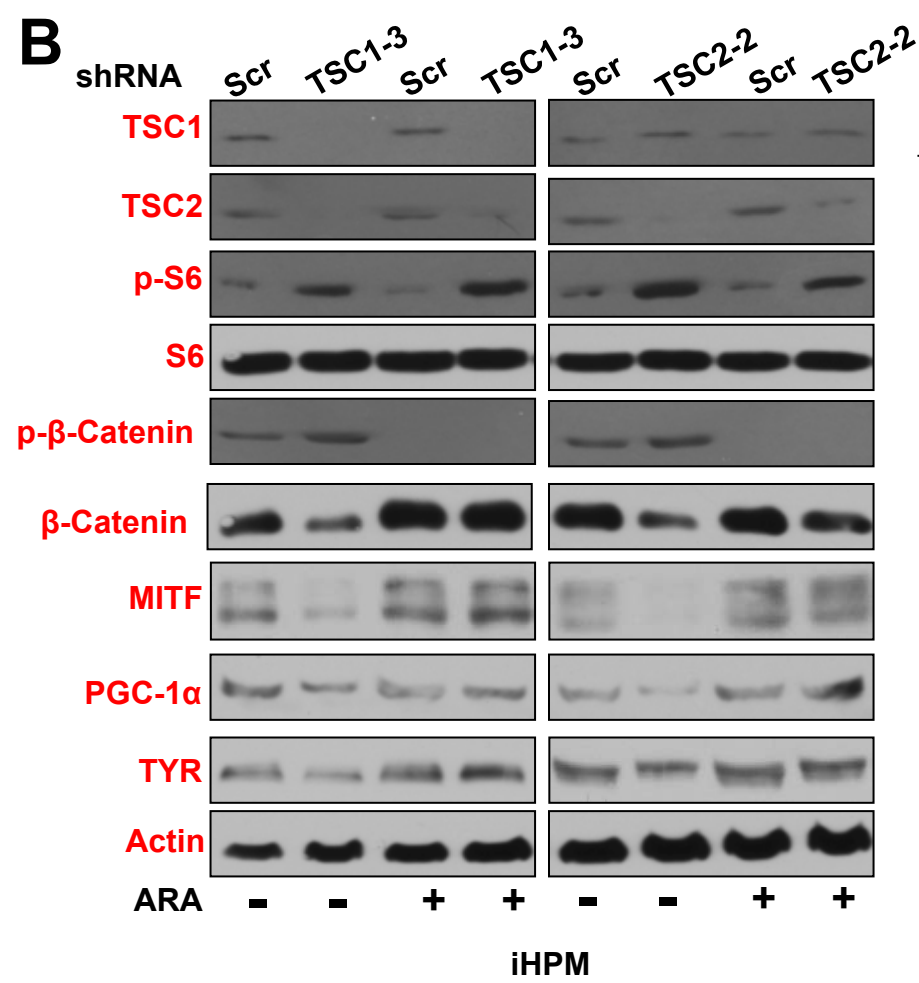
A

5A:TSC1 (Left)

5A:TSC1 (Right)

5A:PGC-1a

5A:TSC2 (Left)

5A:TSC2 (Right)

5A:TYR

5A:p-S6   Left   Right

5A:Actin

5A:S6

Full unedited gel for Figure 5A

5A:p-β-catenin

5A:β-Catenin

5A:MITF

Figure 5

Loading controls for parallel gels in figure 5A

parallel gel 1

5A:Actin

parallel gel 2

5A:Actin

parallel gel 3

5A:Actin

left

rightt

B

shRNA | Scr | TSC1-3 | Scr | TSC1-3 | Scr | TSC2-2 | Scr | TSC2-2

TSC1
TSC2
p-S6
S6
p-β-Catenin
β-Catenin
MITF
PGC-1α
TYR
Actin

ARA   −   −   +   +   −   −   +   +

iHPM

PGC-1α   Actin

Run on same gel, **Actin** in the figure was used as the loading control

Parallel gel 1

TSC1 (left)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parallel gel 2

TSC1 (Right)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parallel gel 3

TSC2   p-S6   p-β-Catenin

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parallel gel 4

TYR (left)   MITF(right)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parallel gel 5

TYR (right)   MITF (left)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parallel gel 6

S6   β-Catenin

Run on same gel, **S6** in the figure was used as the loading control, no extra loading control needed

Figure 5

Full unedited gel for Figure 5B

5B:TSC1

Left Right

5B:MITF (Left)

5B:MITF (right)

5B:TSC2

Left Right

5B:PGC-1α

5B:p-S6

Left Right

5B:TYR (left)

5B:Tyr (right)

5B: S6

5B: Actin

5B:p-β-catenin

Left Right

Figure 5

5B:β-Catenin

Loading controls for parallel gels in figure 5B

parallel gel 1

5B:Actin
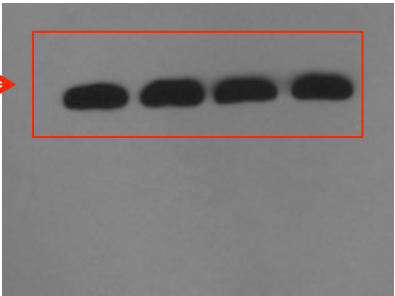
parallel gel 2

5B:Actin
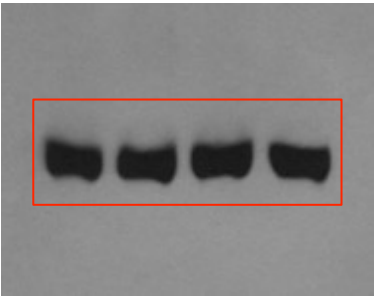
parallel gel 3

5B:Actin
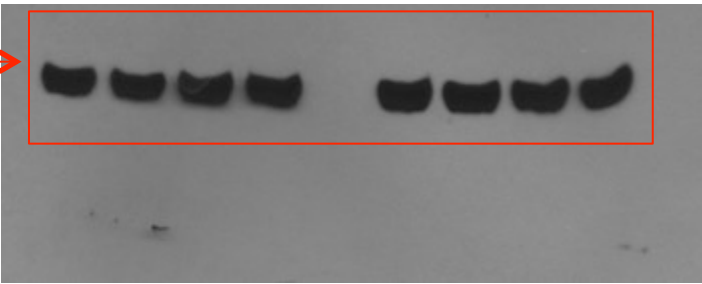
parallel gel 4

5B:Actin
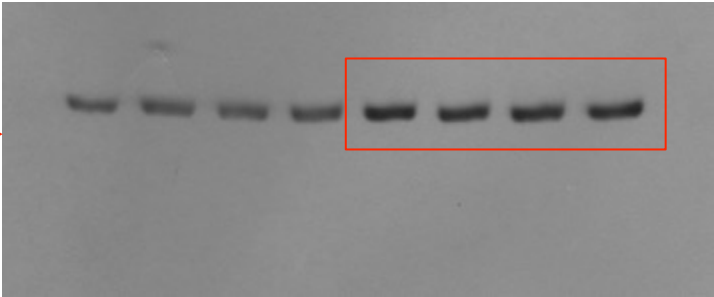
parallel gel 5

5B:Actin
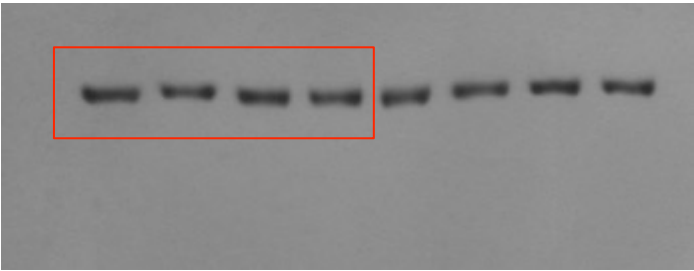
Parallel **gel 6**

5B

No extra loading control needed

C

shRNA	Scr	TSC1-3	Scr	TSC1-3	Scr	TSC2-2	Scr	TSC2-2

TSC1

TSC2

p-S6 (S235/236)

p-S6 (S240/244))

S6

p-β-Catenin

β-Catenin

MITF

PGC-1α

TYR

Actin

| BIO | − | − | + | + | − | − | + | |

iHPM
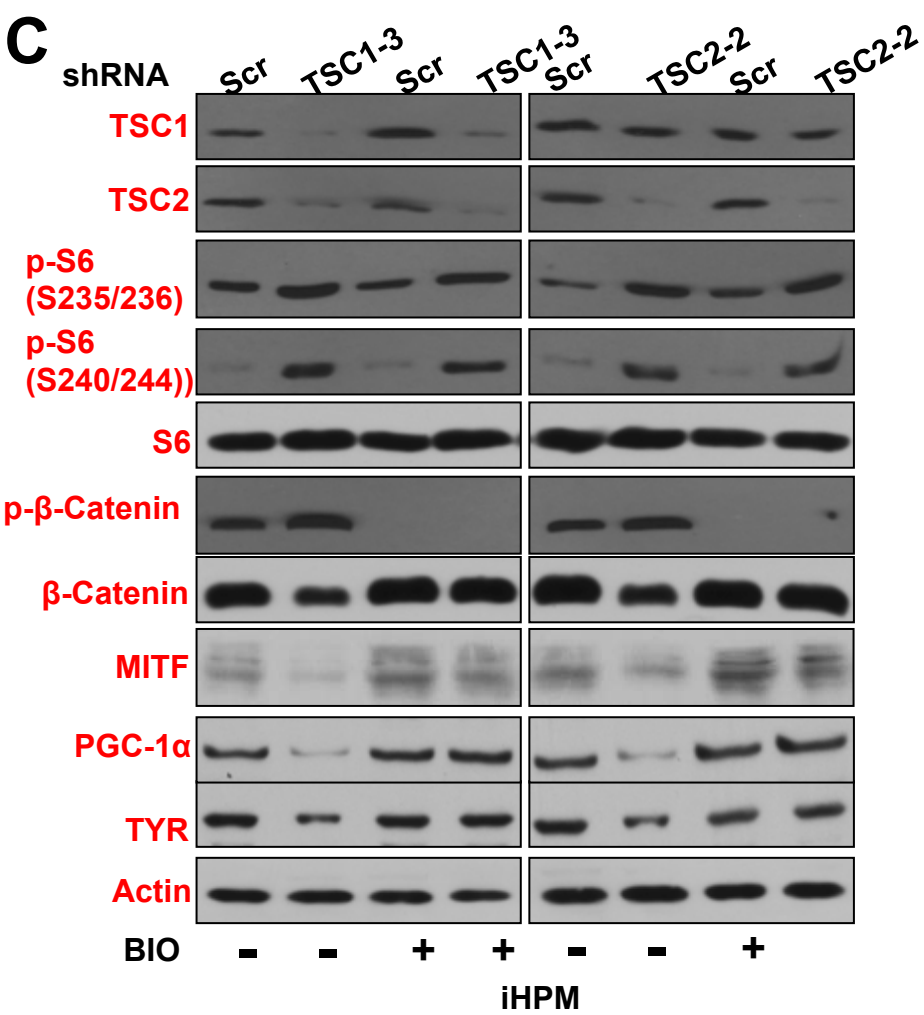
S6   β-Catenin   MITF   PGC-1α   TYR

Run on same gel, **S6** in the figure was used as the loading control, no extra loading control needed

Parallel gel 1

TSC1   TSC2   p-S6 (S235/236)   p-S6 (S240/244))   p-β-Catenin

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parallel gel 2

Actin

Run on same gel, **Actin** in the figure was used as the loading control , no extra loading is needed.

Figure 5

Full unedited gel for Figure 5C
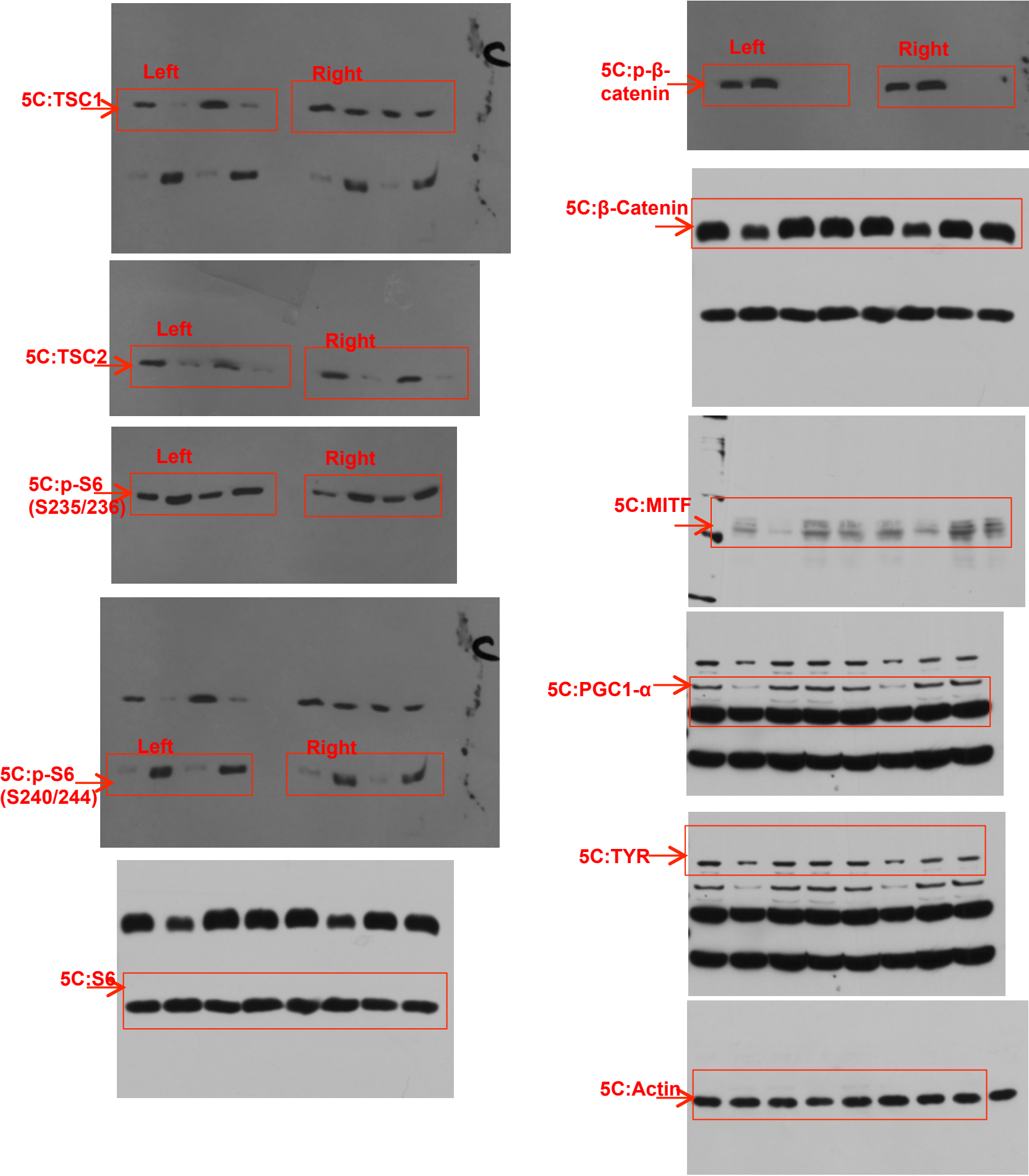
Figure 5

Loading controls for parallel gels in figure 5C

parallel gel 1

5C:Actin
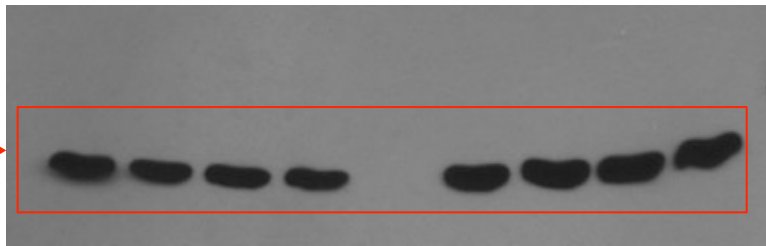
parallel gel 2

5C:Actin

No extra loading
needed

D
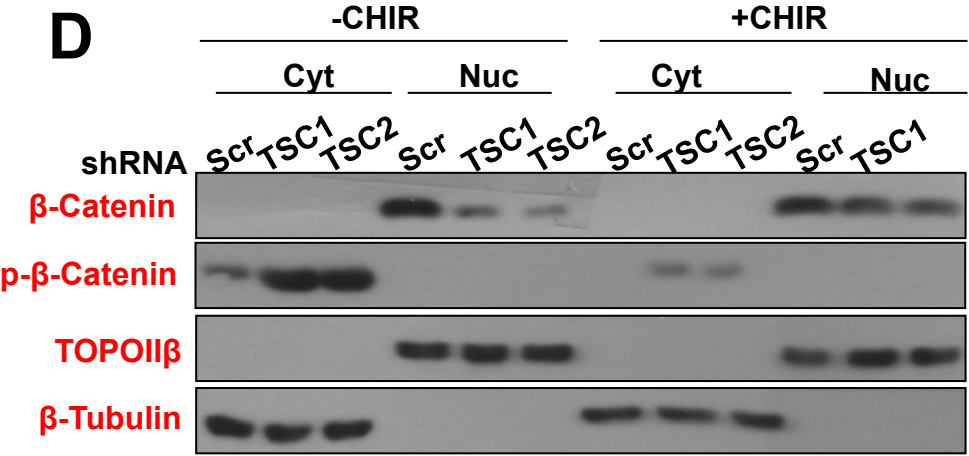
No extra loading needed

E

iHPM
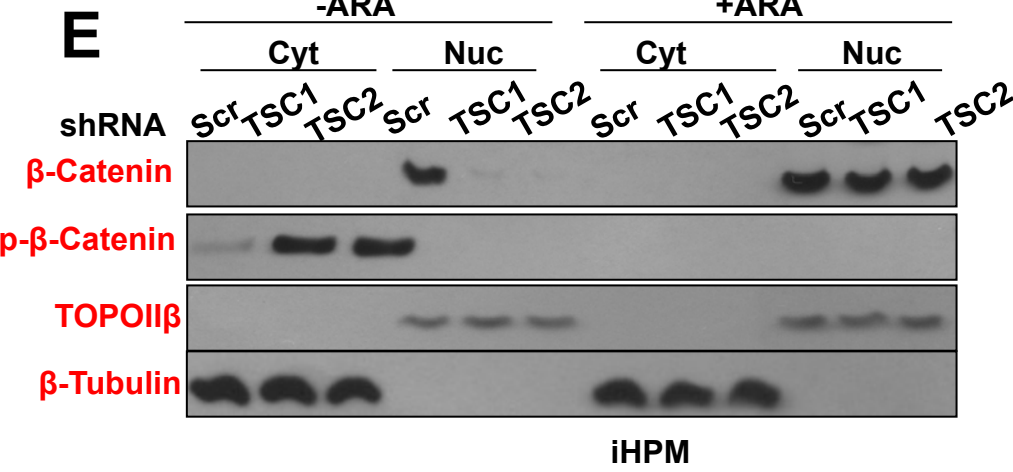
No extra loading needed

Figure 5

D

5B:β-catenin →
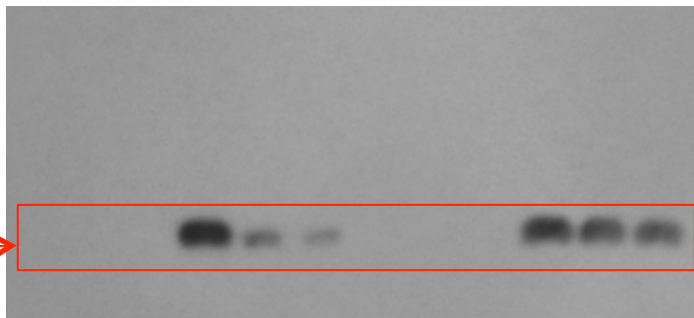
5B:p-β-catenin →
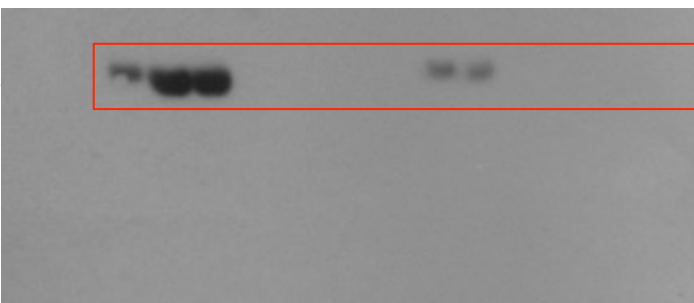
5B:TOPOIIβ →
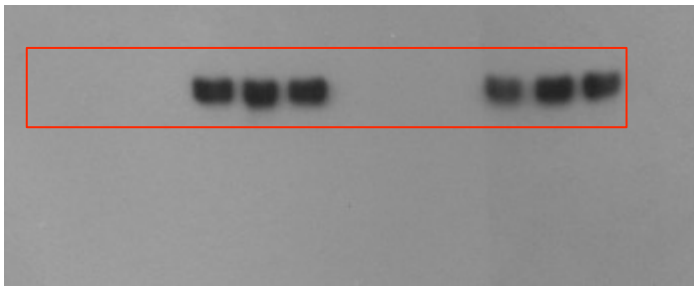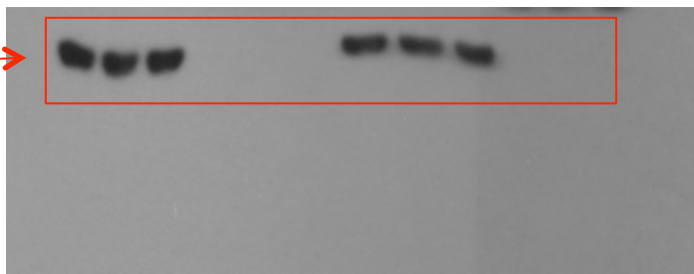
5B:β-Tubulin →

E

5G:β-catenin →
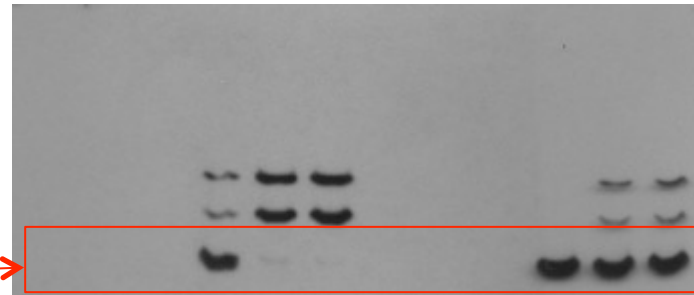
5B:p-β-catenin →
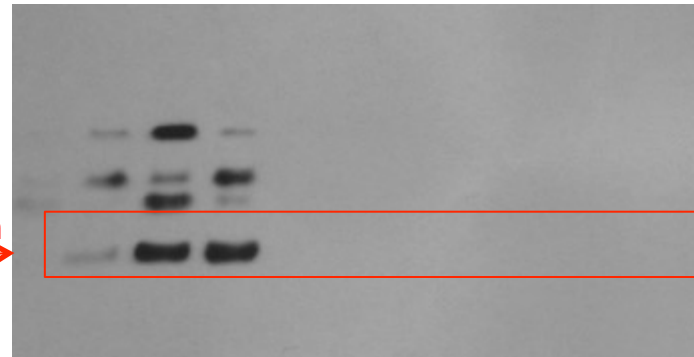
5G:TOPOIIβ →
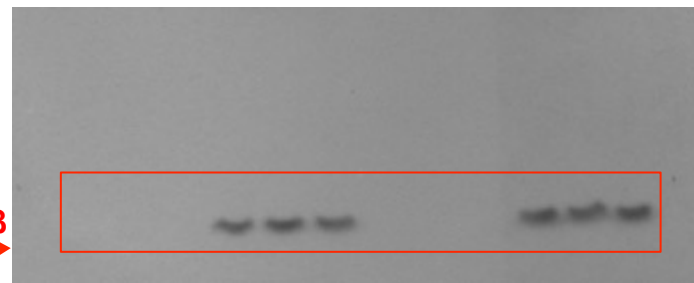
5G:β-Tubulin →
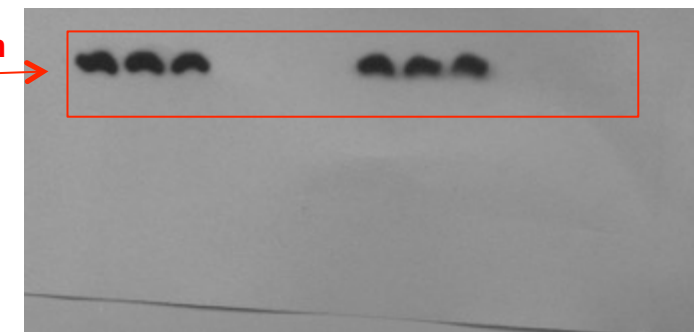
Full unedited gel for Figure5D&E

Figure 5

Loading controls for parallel gels in figure 5D&E

No extra loading control needed

A

P35   P38

Normal   HM   Normal   HM

TSC1
TSC2
p-S6 (Ser235/236)
p-S6 (Ser240/244)
S6
p-4E-BP1(Thr37/46)
4E-BP1
p-S6K1(T229)
p-S6K1(T389)
S6K
p-AKT(Thr308)
p-AKT(Ser473)
AKT
MITF
PGC-1α
TYR
p-GSK3β
GSK3β
p-β-catenin
β-catenin
Actin
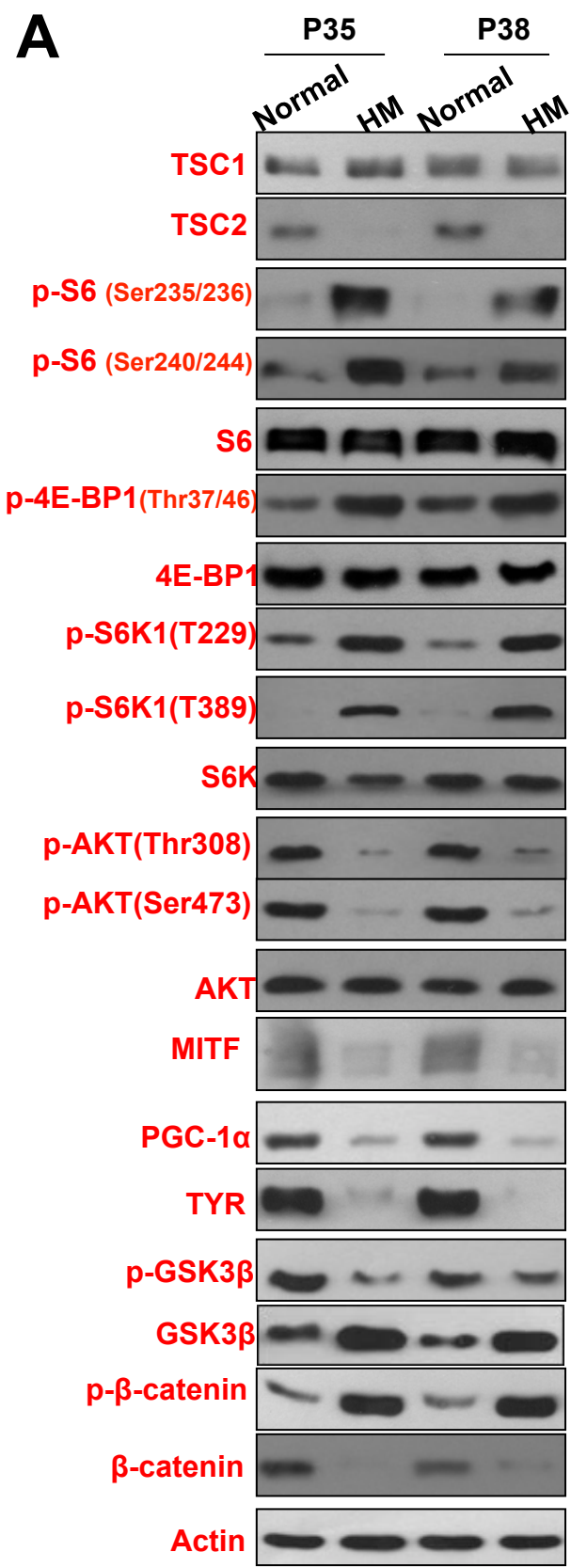
p-S6 (Ser235/236)   p-S6 (Ser240/244)   S6   4E-BP1   AKT   MITF   TYR

Run on same gel, **S6** in the figure was used as the loading control, no extra loading control needed

Parallel gel 1

TSC1   TSC2   p-4E-BP1(Thr37/46)   PGC-1α   GSK3β   β-catenin

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parallel gel 2

p-S6K1(T229)   p-AKT(Ser473)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parallel gel 3

p-S6K1(T389)   p-AKT(Thr308)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parallel gel 4

p-GSK3β

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parallel gel 5

β-catenin

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Figure 7

A

Full unedited gel for Figure 7A

7A: TSC1
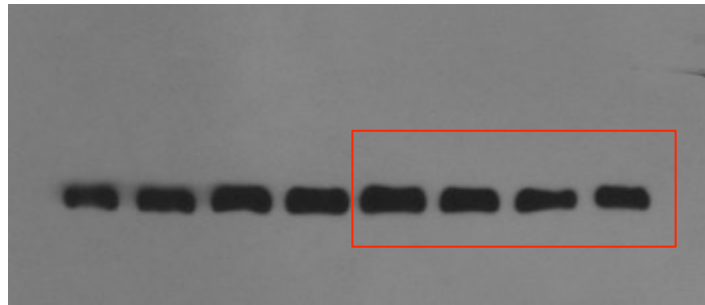
7A: TSC2

7A: p-S6 (Ser235/236

7A: p-S6 (Ser240/244)

7A:S6

7A: p-4E-BP1 (Thr37/46)

7A: 4EBP1

7A:p-S6K1 (T229)

7A:p-S6K1 (T389)

7A:S6K

7A: p-AKT (Thr308)

7A: p-AKT (Ser473)

7A:AKT

7A:MITF

7A:PGC-1α

7A:TYR

7A:p-GSK3β

7A:GSK3β

7A:p-β-catenin

7A:β-catenin

7A:Actin
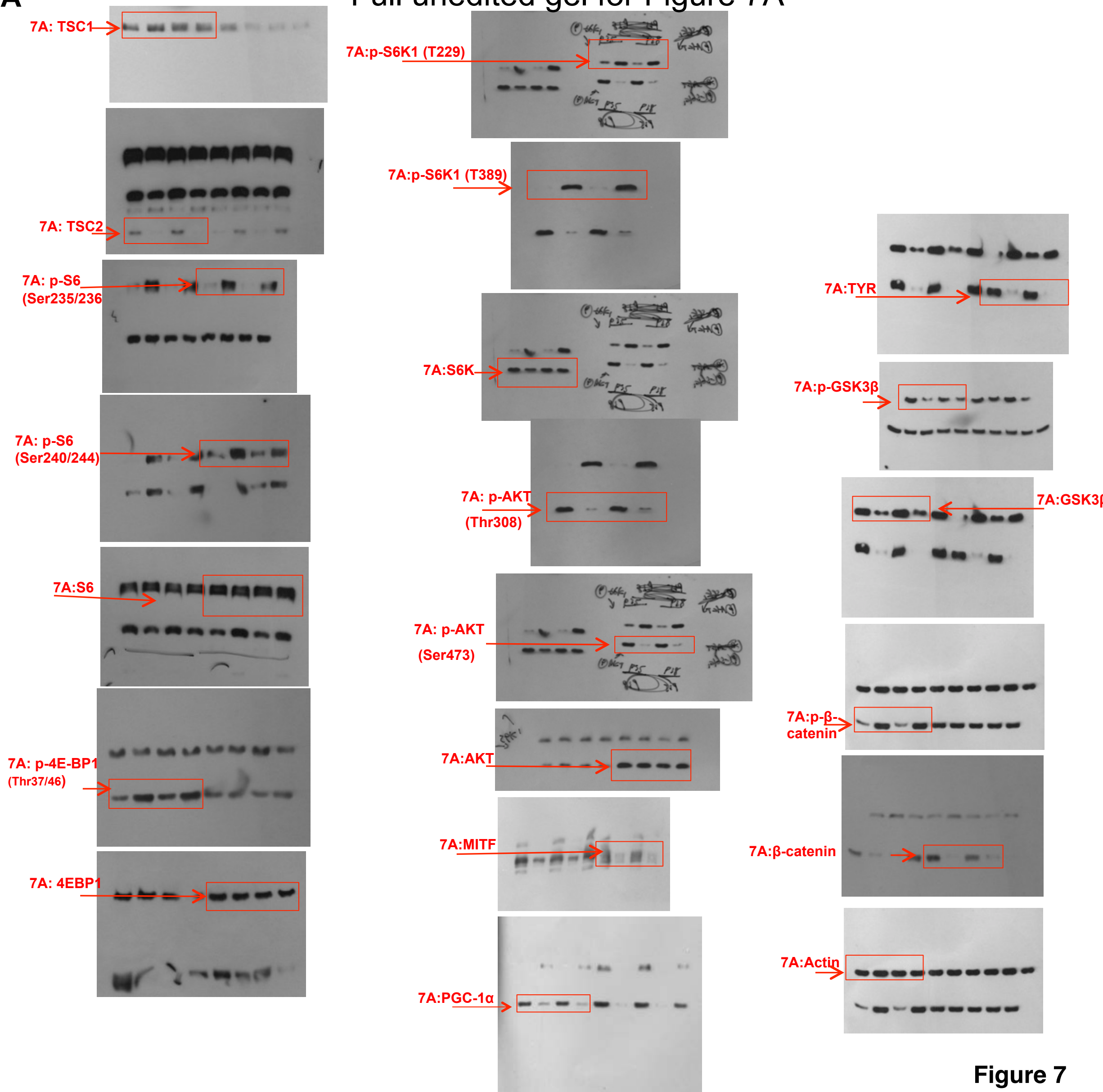
Figure 7

Loading controls for parallel gels in figure 7A

parallel gel 1

7A: Actin

parallel gel 2

7A: Actin

parallel gel 3

7A: Actin

parallel gel 4

7A: Actin

parallel gel 5

7A:Actin

D

	P35		P38	
	Normal	HM	Normal	HM

TSC1
TSC2
p-S6 (Ser235/236)
p-S6 (Ser240/244)
S6
p-4E-BP1(Thr37/46)
4E-BP1
Actin
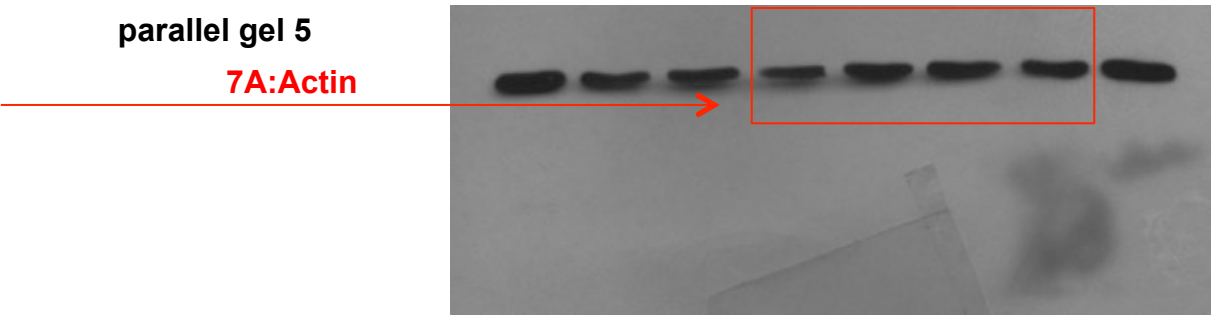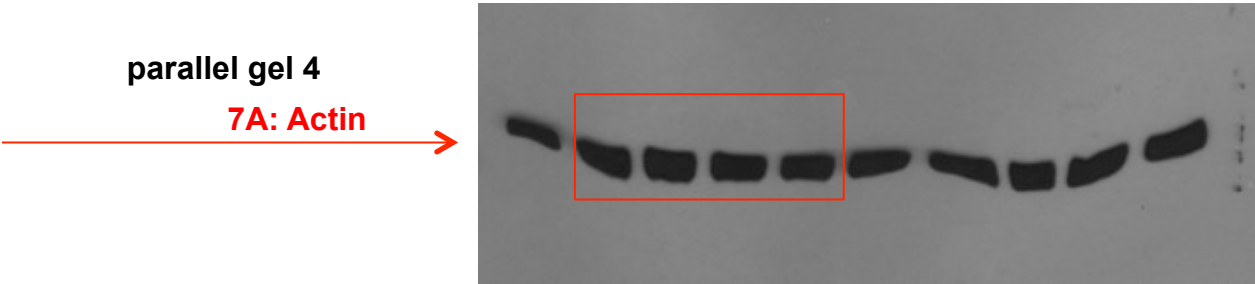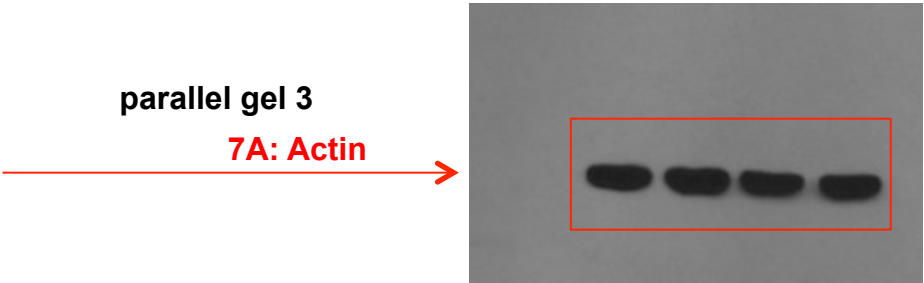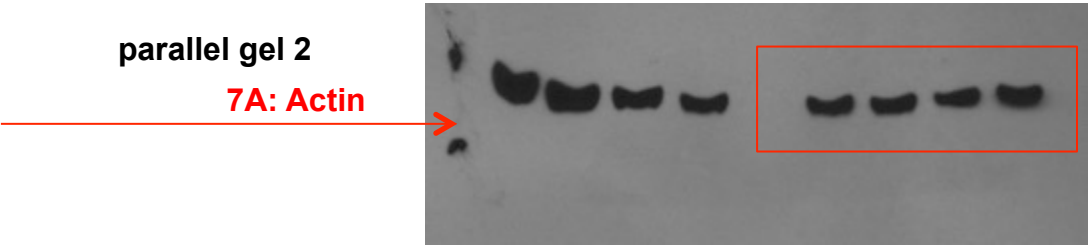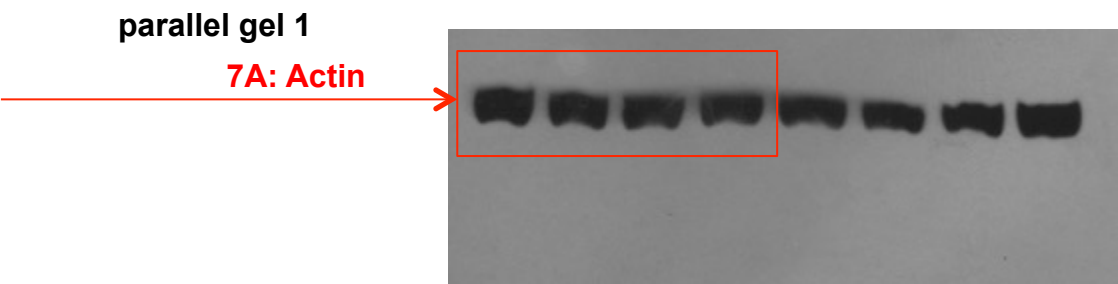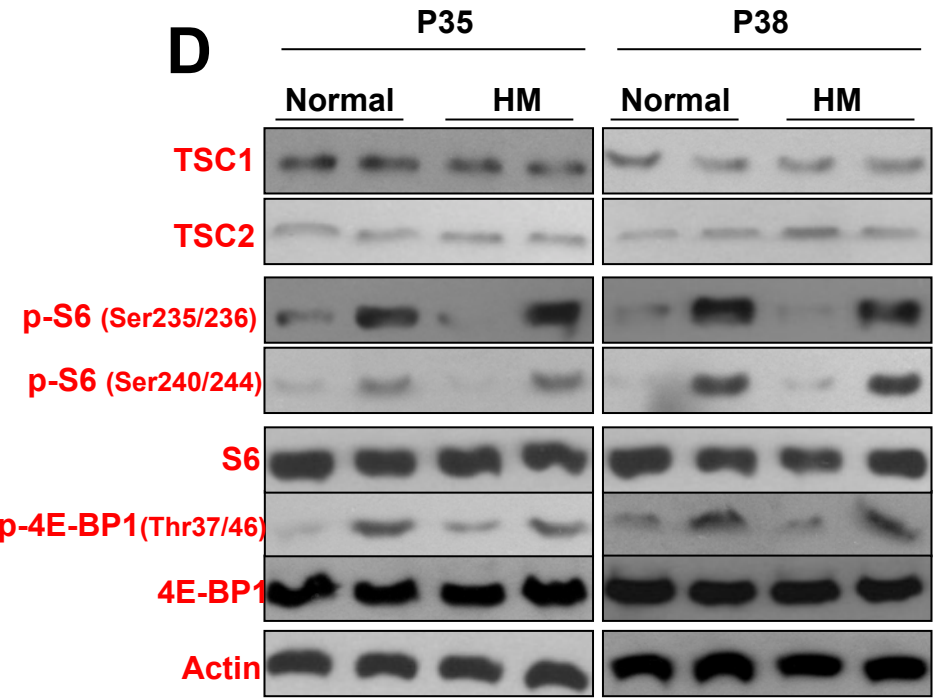
TSC2   S6   p-4E-BP1(Thr37/46)   Actin

Run on same gel, **Actin** in the figure was used as the loading control
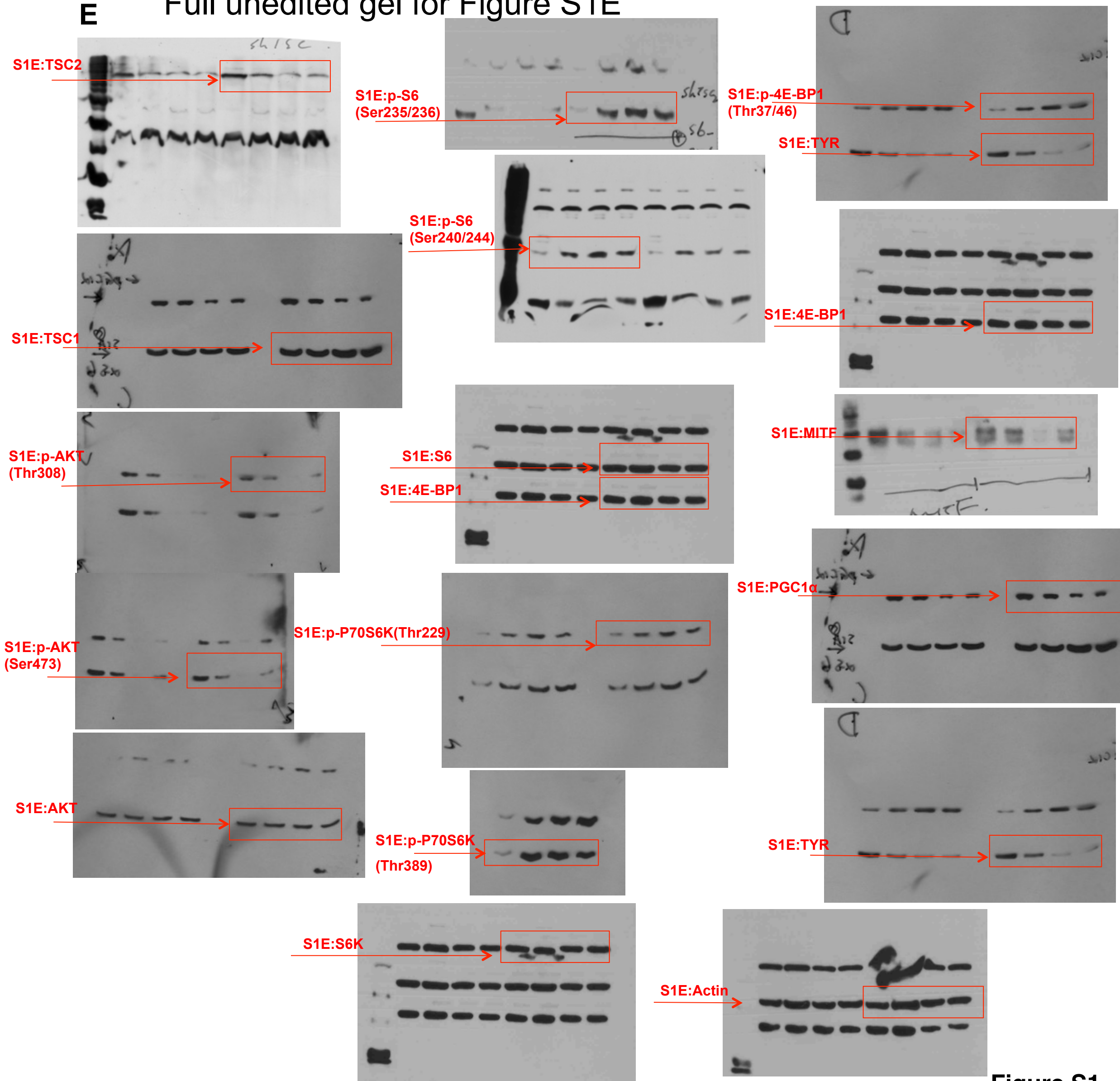
Parallel gel 1

TSC1 (P35)   p-S6 (Ser235/236) (P35)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parallel gel 2

p-S6 (Ser240/244)

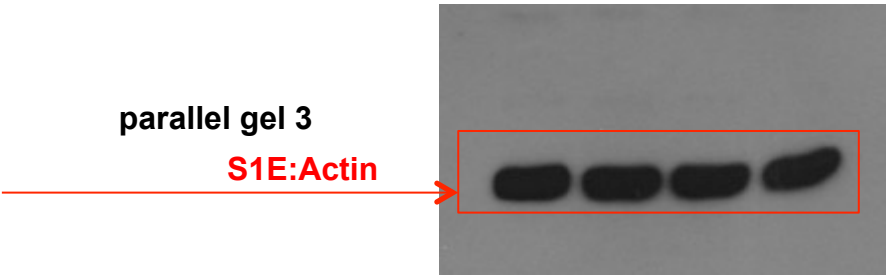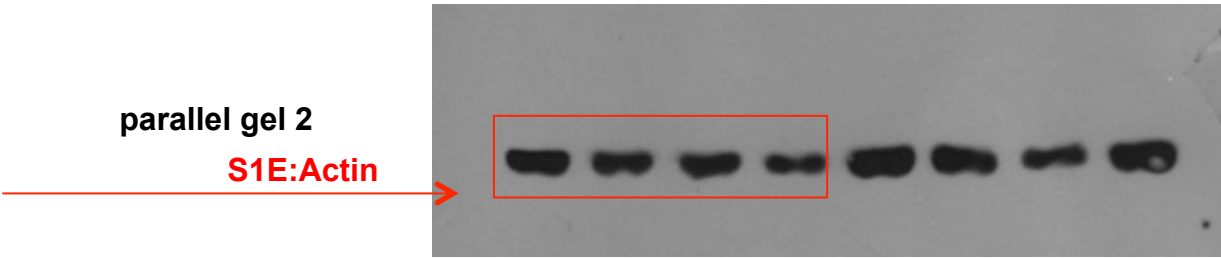Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parallel gel 3

TSC1 (P38)   p-S6 (Ser235/236) (P38)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Parallel gel 4

4E-BP1(P35, P38)

Run on same gel, **4E-BP1** was used as the loading control , no extra loading control needed)

Parallel gel 5

p-S6 (Ser235/236) (P35)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Figure 7

Full unedited gel for Figure 7D
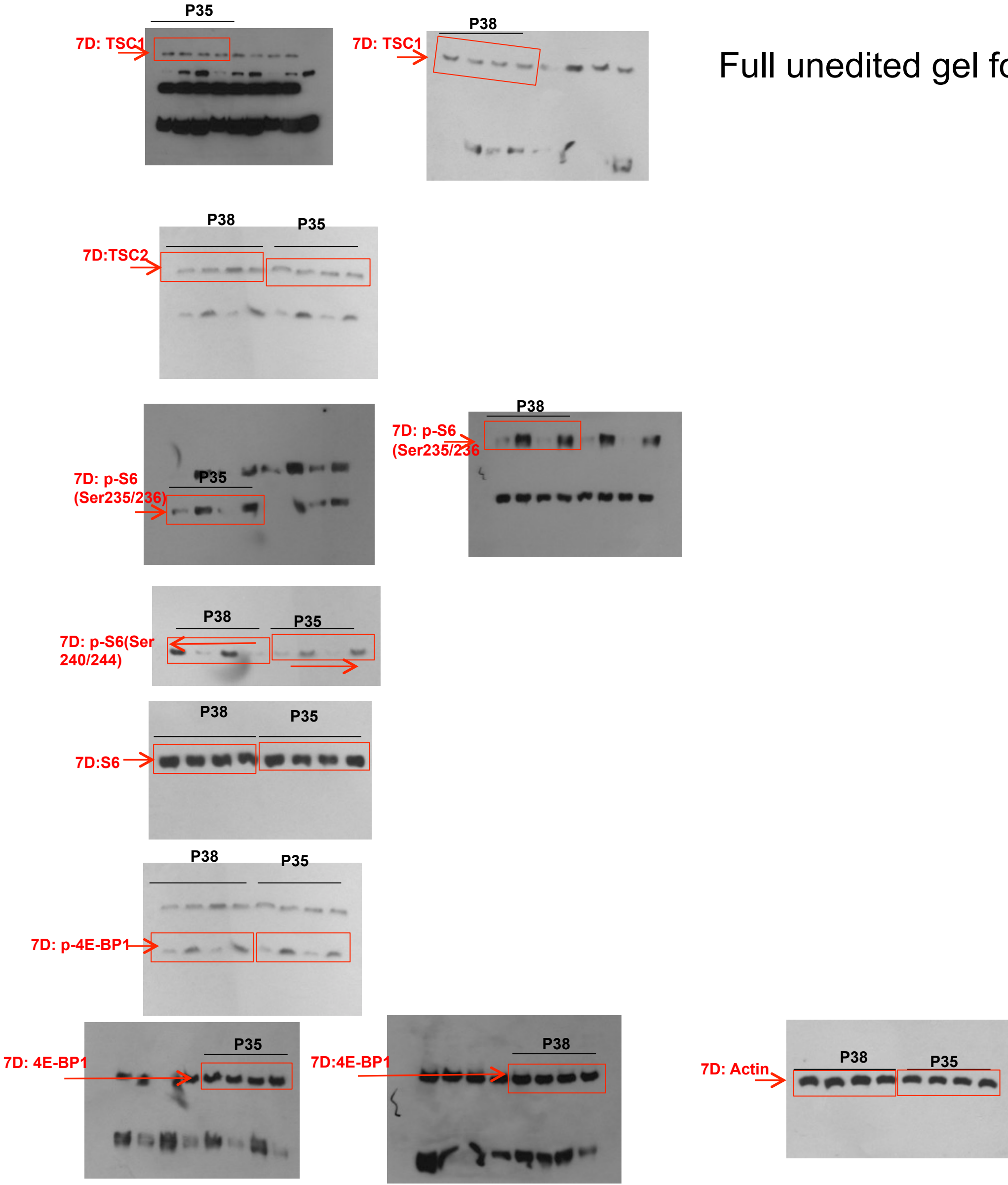
Figure 6

Loading controls for parallel gels in figure 7D

parallel gel 1

7D: Actin

parallel gel 2

7D: Actin

parallel gel 3
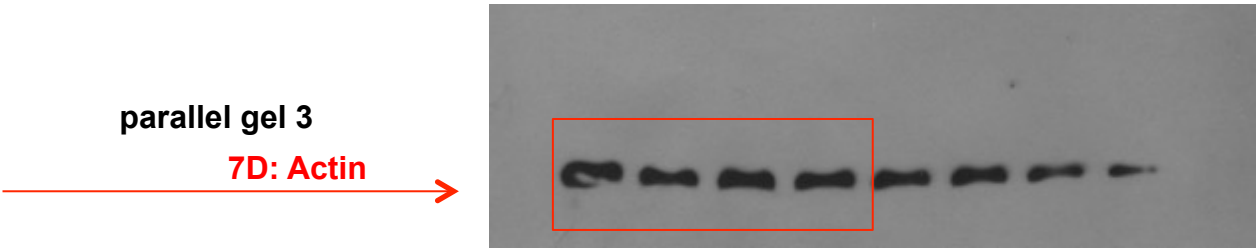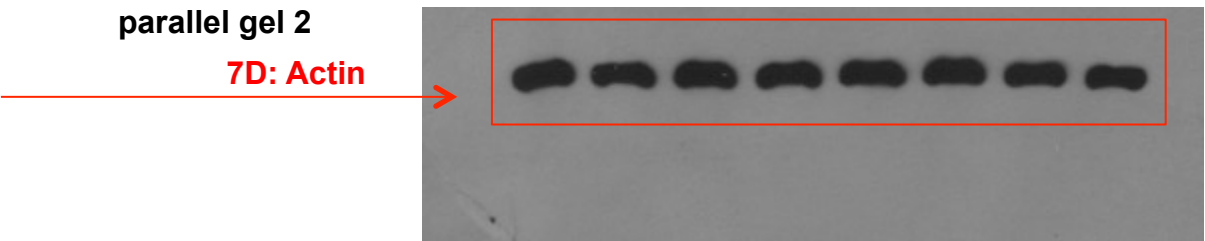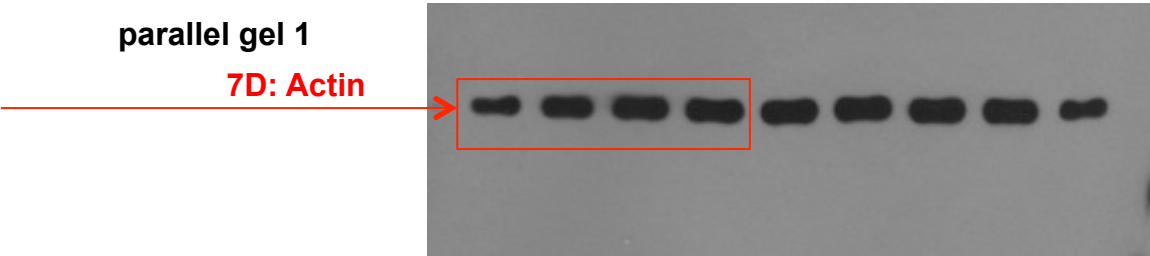
7D: Actin

parallel gel 4

7D: Actin

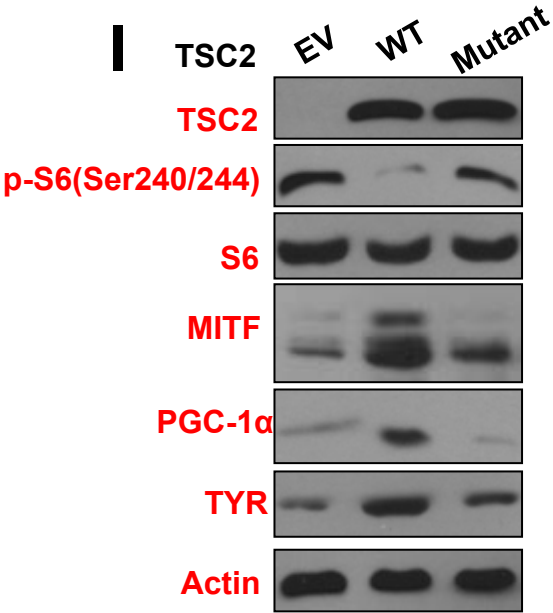No extra loading control needed

parallel gel 5

7D:Actin
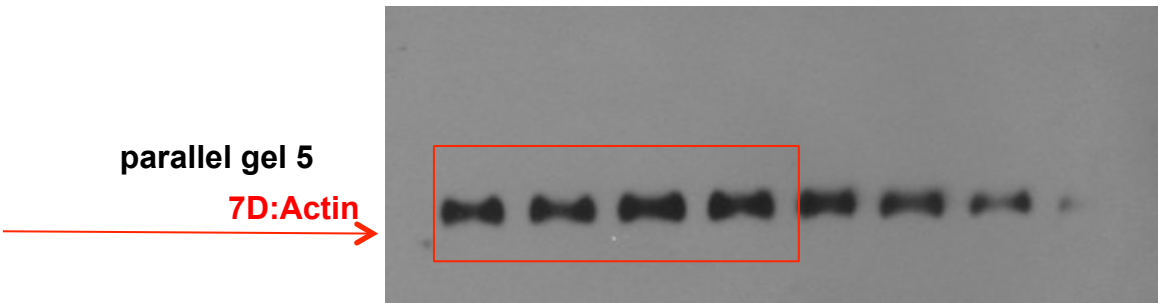
Full unedited gels for supplemental figures

Note: for all gels, blots were cut for different antibody staining and exposed on the same film

A

shRNA: Scr, TSC1-1, TSC1-2, TSC1-3

TSC1
TSC2
p-AKT(Thr308)
p-AKT(Ser473)
AKT
p-S6 (Ser235/236)
p-S6(Ser240/244)
S6
p-P70S6k (Thr389)
p-P70S6k (Thr229)
S6K
p-4E-BP1(Thr37/46)
4E-BP1
MITF
PGC-1α
TYR
Actin

SK-MEL-30

TSC1   TSC2   p-S6(Ser240/244)   S6K   p-4E-BP1(Thr37/46) 4E-BP1 MITF Actin

Run on same gel, **Actin** in the figure was used as the loading control

p-AKT(Thr308)   p-AKT(Ser473)   AKT S6 PGC-1α   TYR

Run on same gel, **AKT** in the figure was used as the loading control

parallel gel 1

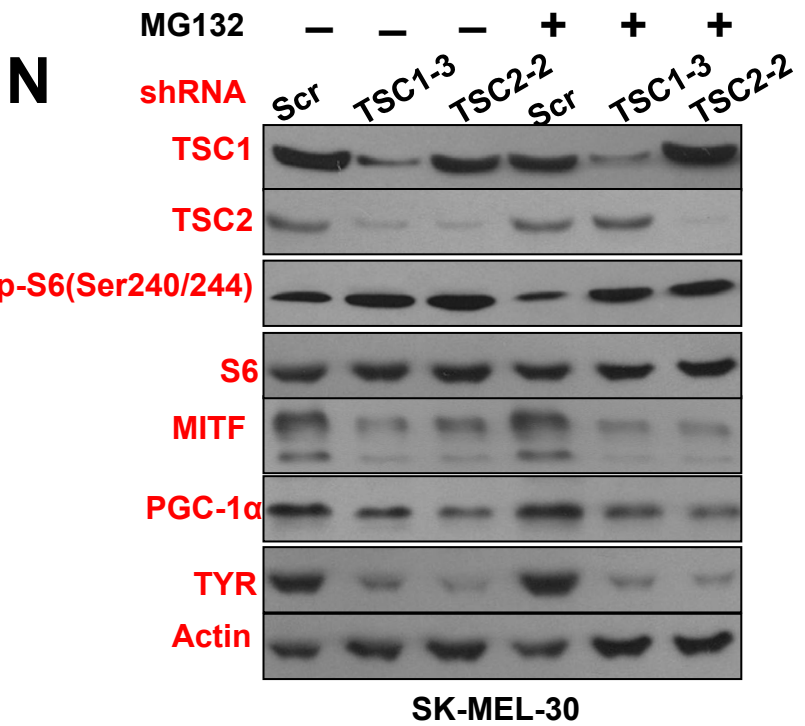p-S6 (Ser235/236)   p-P70S6k (Thr389)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 2

p-P70S6k (Thr229)
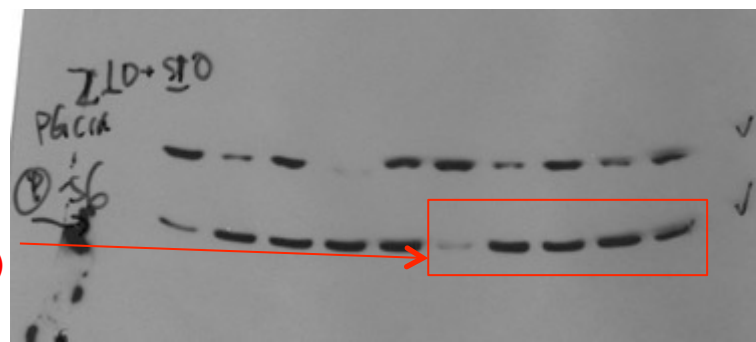
Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Figure S1

Full unedited gel for Figure S1A

A

S1A:TSC1

S1A:TSC2

S1A:p-AKT (Thr308)

S1A:p-AKT (Ser473)

S1A:AKT

S1A:p-S6 (Ser235/236)

1A:p-S6 (Ser240/244)

S1A:S6

S1A:p-P70S6K(Thr389)

S1A:p-P70S6K(Thr229)

S1A:S6K

S1A:P4E-BP1 (Thr37/46)

1A:4E-BP1

S1A:MITF

S1A:PGC1α

S1A:TYR

S1A:Actin

Blots were cut for different antibody staining and exposed on the same film
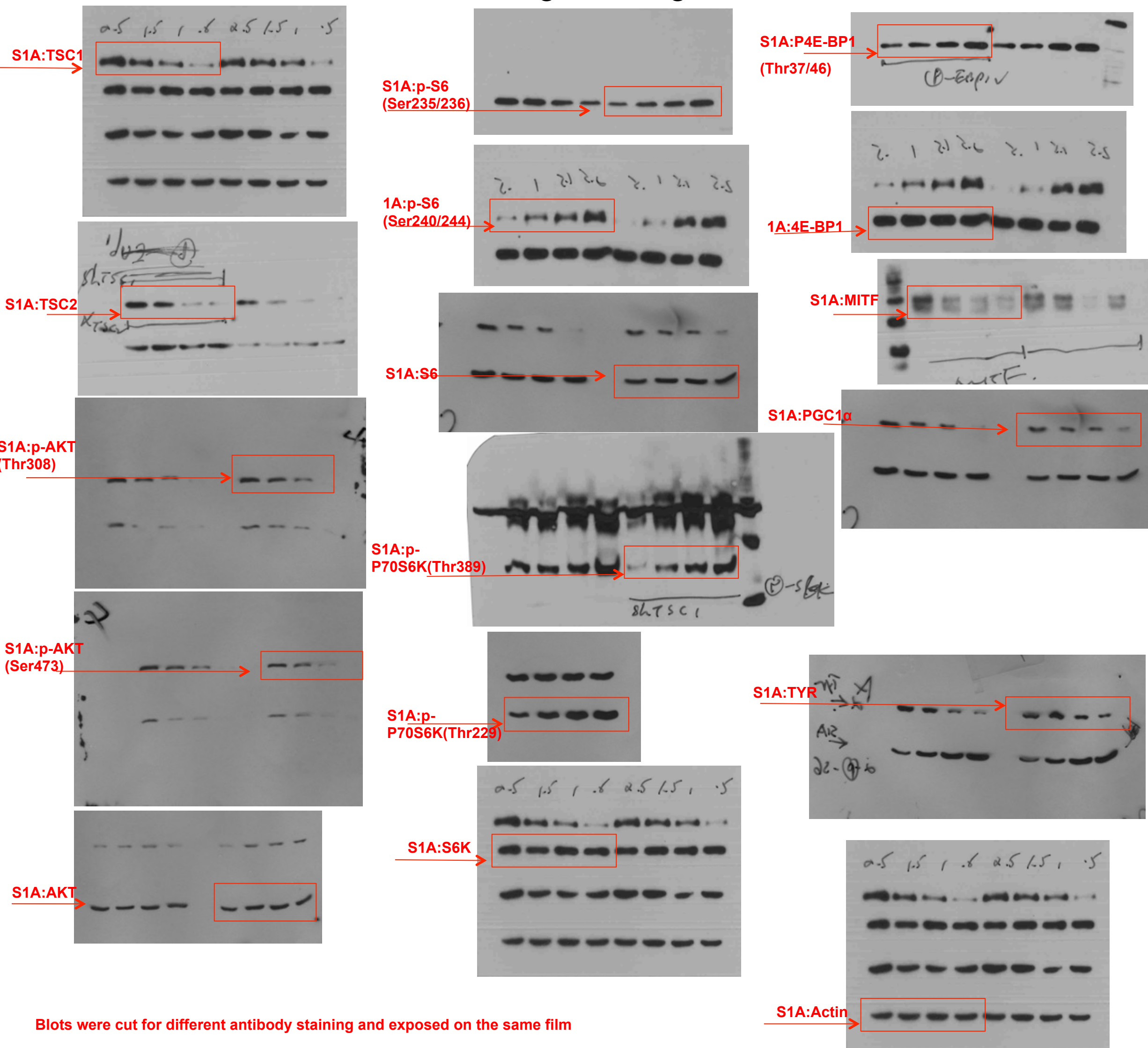
Figure S1

Loading controls for parallel gels in figure S1A

parallel gel 1
S1A:Actin →

parallel gel 2
S1A:Actin →

E

shRNA Scr TSC2-1 TSC2-2 TSC2-3

TSC2
TSC1
p-AKT(Thr308)
p-AKT(Ser473)
AKT
p-S6 (Ser235/236)
p-S6(Ser240/244)
S6
p-P70S6K(Thr389)
p-P70S6K(Thr229)
S6K
p-4E-BP1(Thr37/46)
4E-BP1
MITF
PGC-1α
TYR
Actin
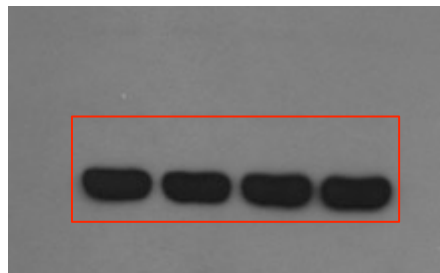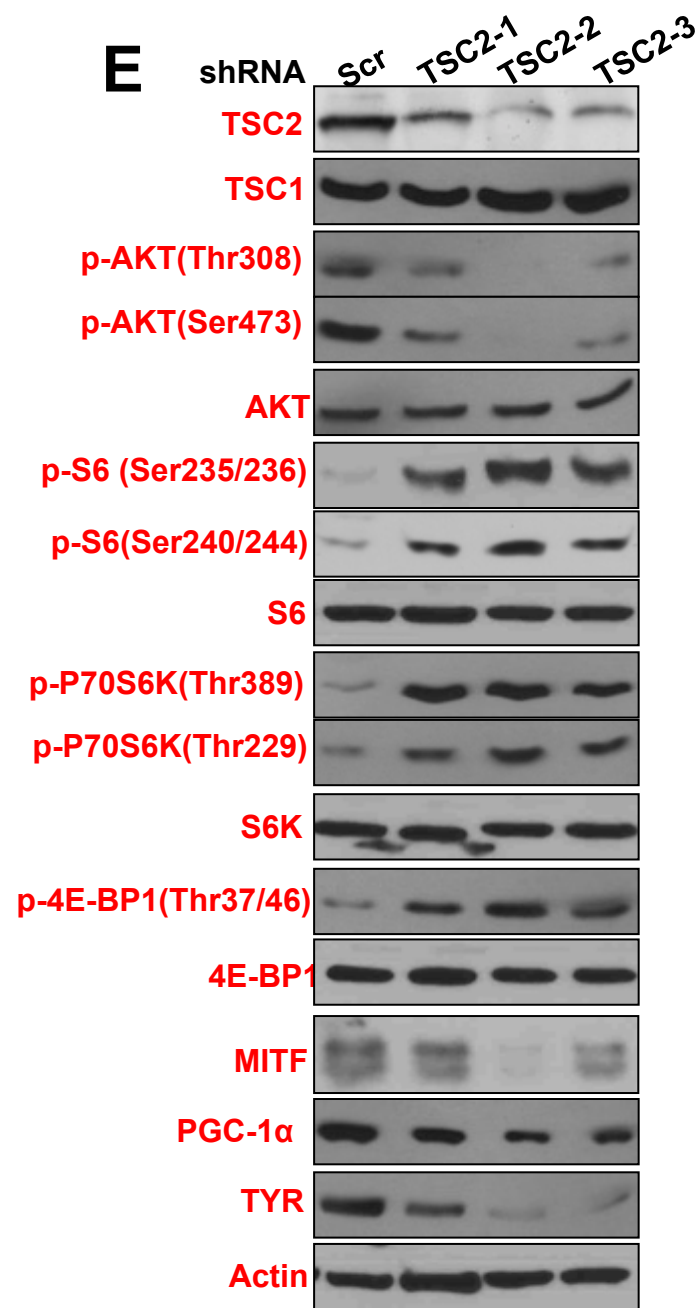
p-S6 (Ser235/236)   S6   4E-BP1   Actin   S6K

Run on same gel, **Actin** in the figure was used as the loading control

TSC1   p-AKT(Thr308)   p-AKT(Ser473)   AKT   p-P70S6K(Thr229)   p-4E-BP1(Thr37/46)   PGC-1α TYR

Run on same gel, **AKT** in the figure was used as the loading control

parallel gel 1

TSC2

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 2

p-S6(Ser240/244)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 3

p-P70S6K(Thr389)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 4

MITF

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Figure S1

E

Full unedited gel for Figure S1E

S1E:TSC2

S1E:p-S6 (Ser235/236)

S1E:p-4E-BP1 (Thr37/46)

S1E:TYR

S1E:p-S6 (Ser240/244)

S1E:4E-BP1

S1E:TSC1

S1E:p-AKT (Thr308)

S1E:S6

S1E:4E-BP1

S1E:MITF

S1E:p-AKT (Ser473)

S1E:p-P70S6K(Thr229)

S1E:PGC1α

S1E:AKT

S1E:p-P70S6K (Thr389)

S1E:TYR

S1E:S6K

S1E:Actin

Blots were cut for different antibody staining and exposed on the same film

Figure S1

Loading controls for parallel gels in figure S1E

parallel gel 1

S1E:Actin

parallel gel 2

S1E:Actin

parallel gel 3

S1E:Actin

parallel gel 4

S1E:Actin
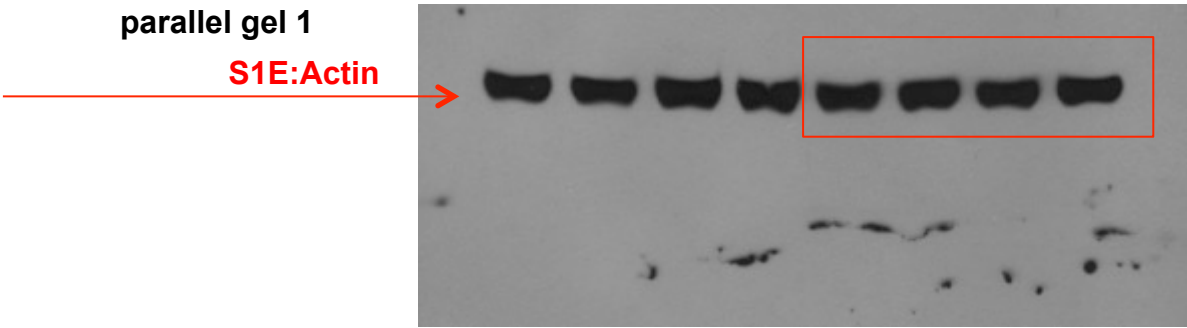
TSC2   MITF   Actin

Run on same gel, **Actin** in the figure was used as the loading control

parallel gel 1

p-S6(Ser240/244)   **PGC-1α**

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 2

S6   **TYR**

Run on same gel, **S6** in the figure was used as the loading control, no extra loading control needed

Figure S1

Full unedited gel for Figure S1I

For all gels, Blots were cut for different antibody staining and exposed on the same film

I

S1I: TSC2

S1I:p-S6 (Ser240/244)

S1I:S6

S1I:MITF

S1I:PGC1α

S1I:TYR
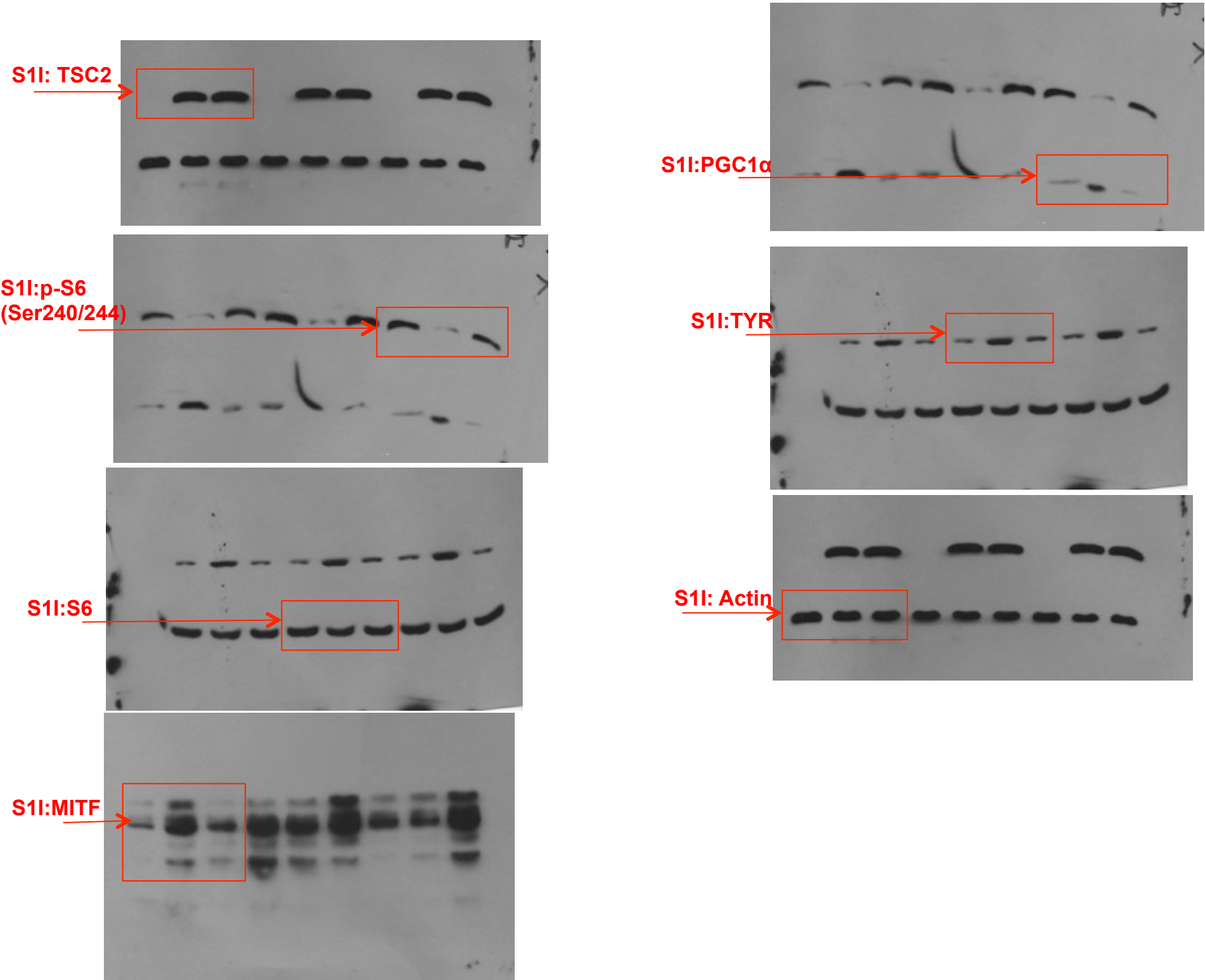
S1I: Actin

Figure S1

Loading controls for parallel gels in figure S1I

parallel gel 1

S1I:Actin
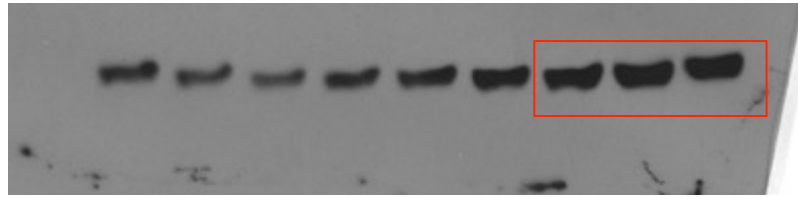
parallel gel

S1I:

No extra loading control needed

O

MITF	−	−	+	−	+
shRNA	Scr	TSC1-3	TSC1-3	TSC2-2	TSC2-2

p-S6(Ser240/244)
S6
PGC-1α
MITF
TYR
Actin
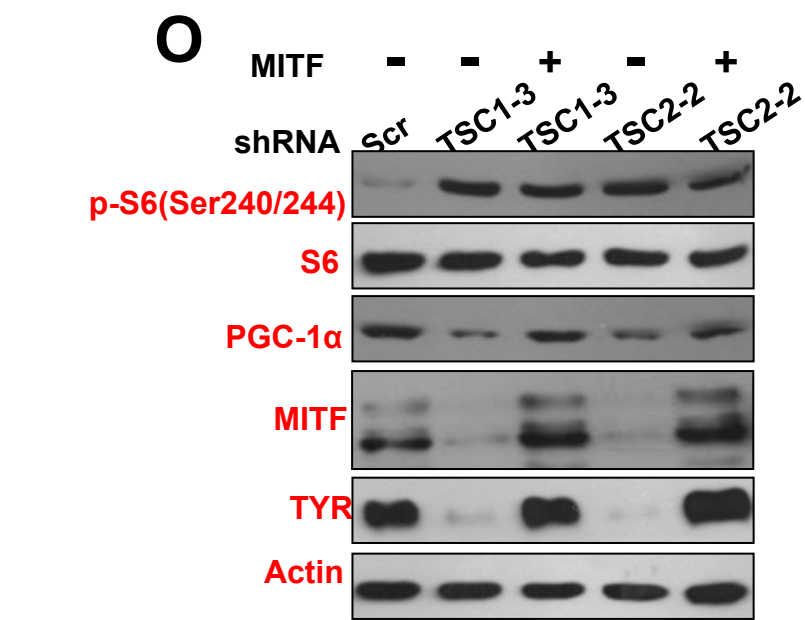
p-S6(Ser240/244)   S6   PGC-1α   Actin

Run on same gel, **Actin** in the figure was used as the loading control

parallel gel 1

MITF

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 2

TYR

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

N

MG132	−	−	−	+	+	+
shRNA	Scr	TSC1-3	TSC2-2	Scr	TSC1-3	TSC2-2

TSC1
TSC2
p-S6(Ser240/244)
S6
MITF
PGC-1α
TYR
Actin

SK-MEL-30

p-S6(Ser240/244)   S6   TYR   Actin

Run on same gel, **Actin** in the figure was used as the loading control

parallel gel 1

TSC1   TSC2   MITF   PGC-1α

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Figure S1

O Full unedited gel for Figure S1O

Blots were cut for different antibody staining and exposed on the same film
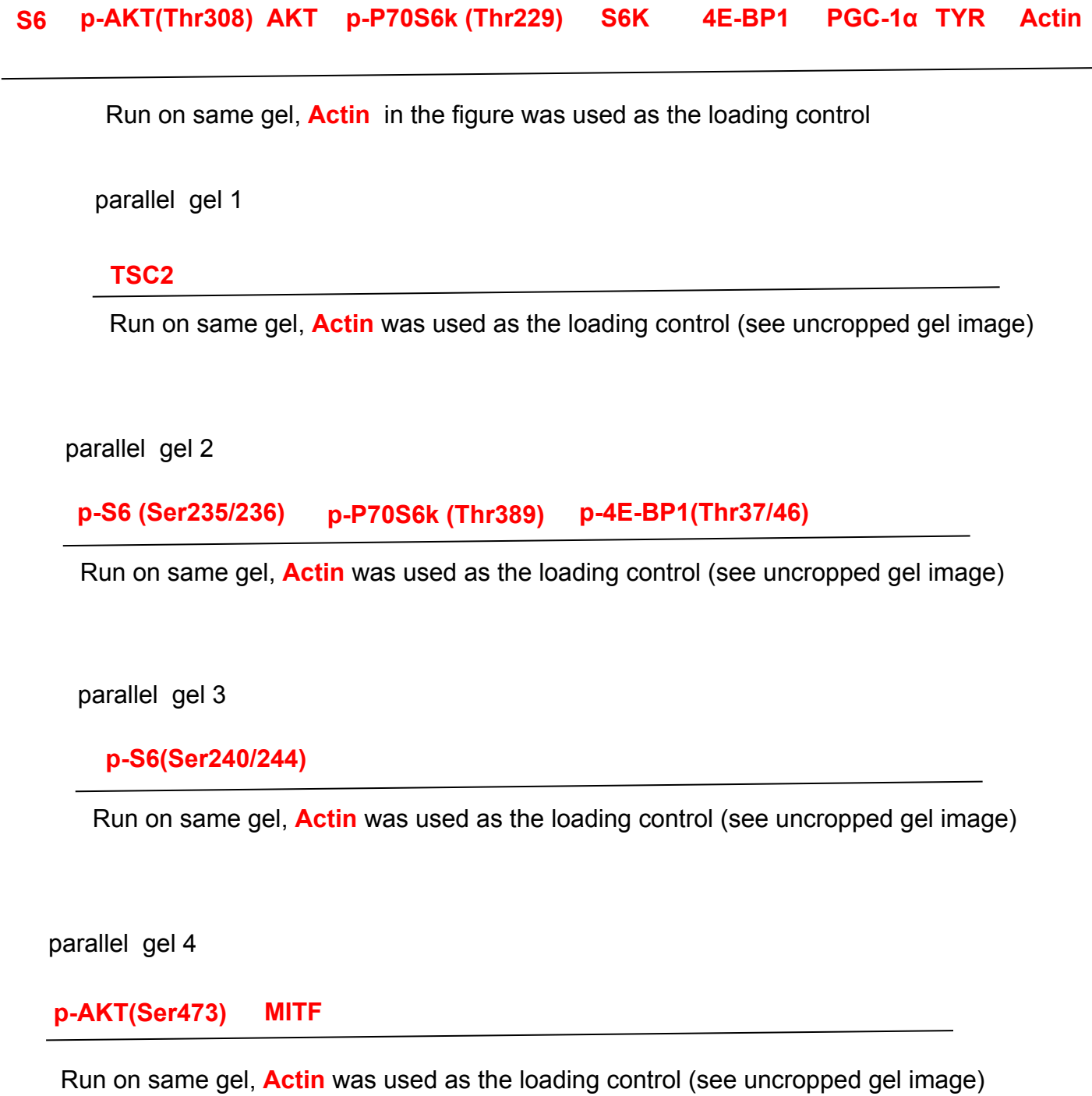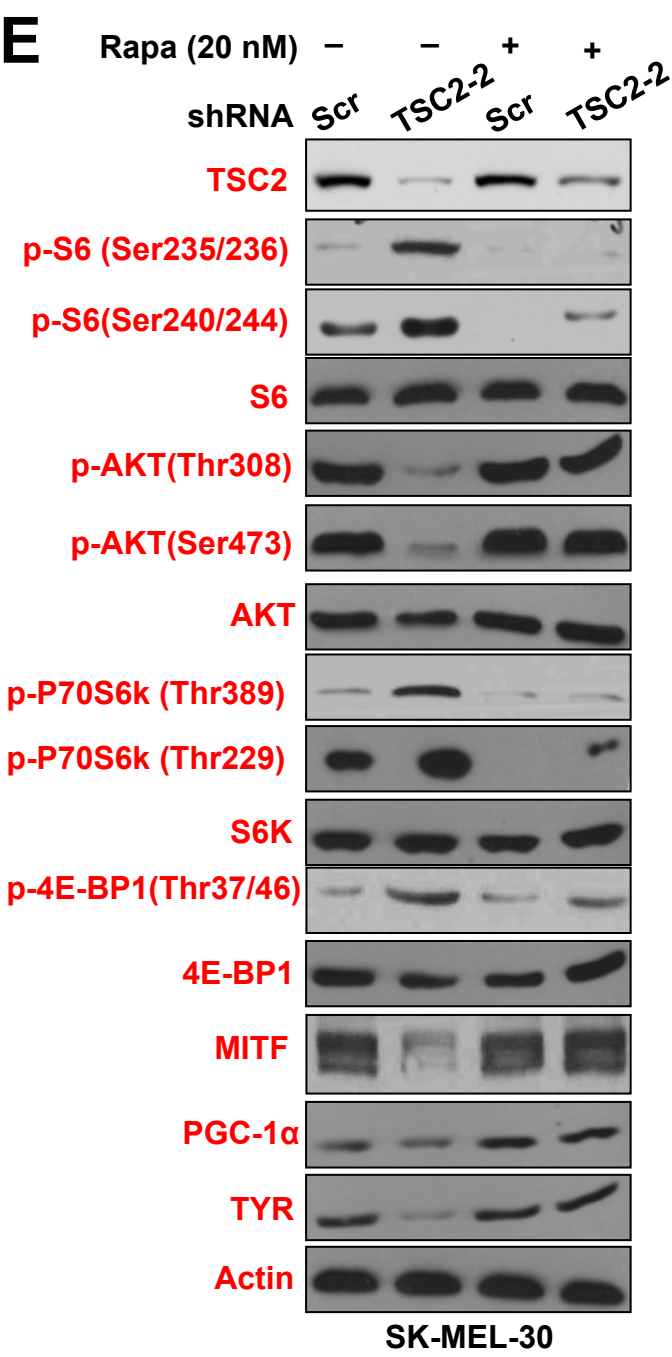
S1O:PGC1a

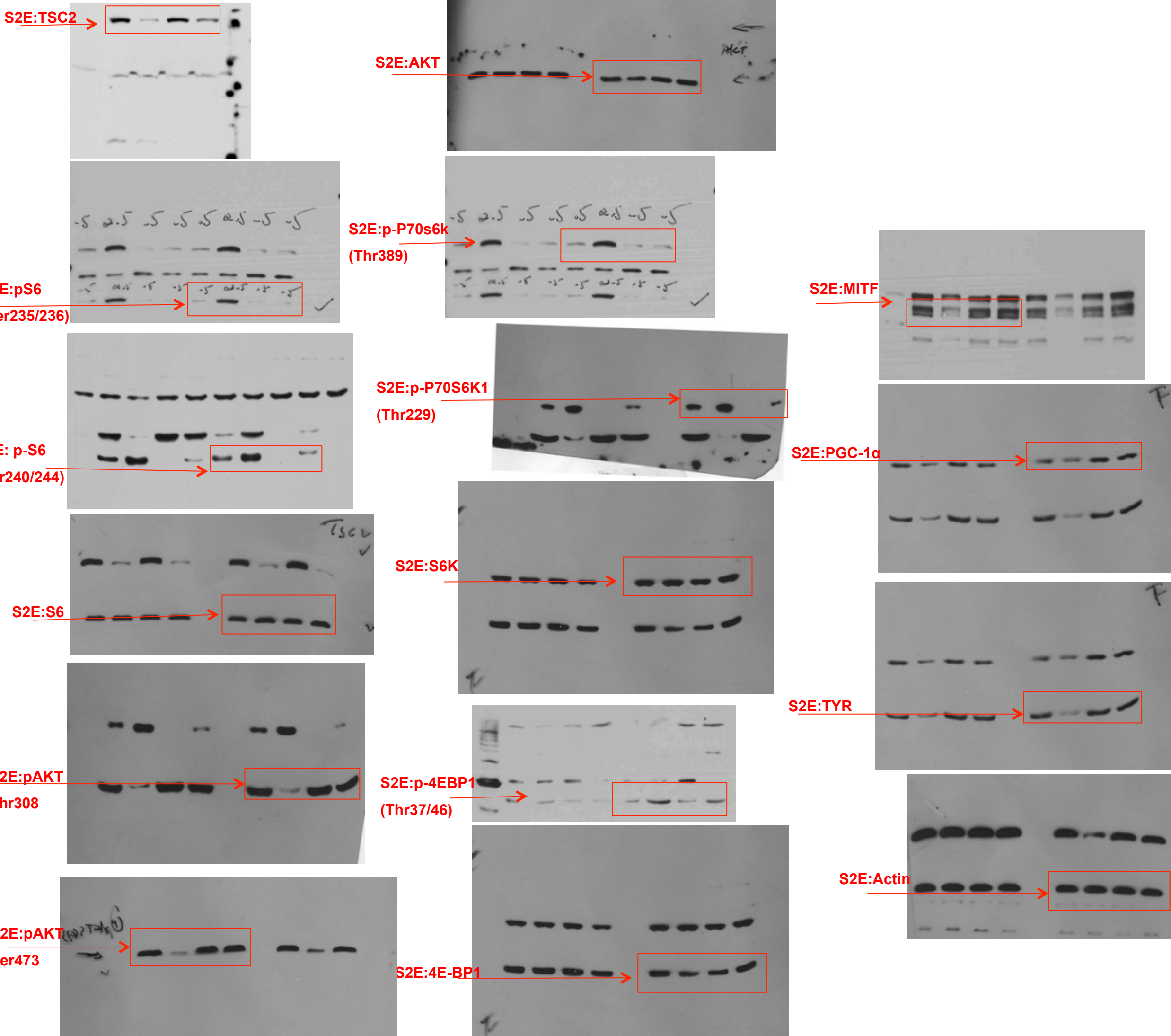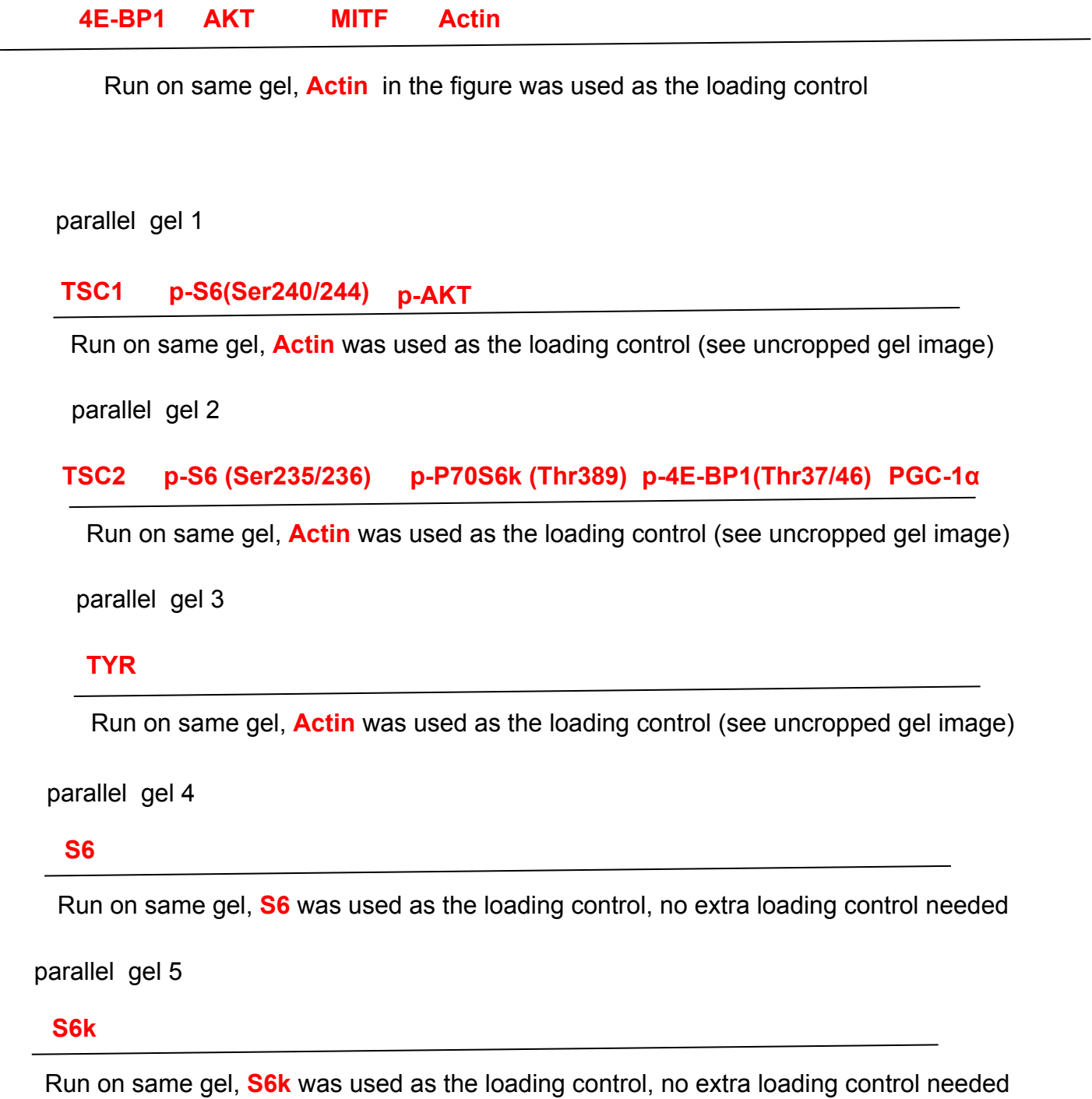S1O:Actin
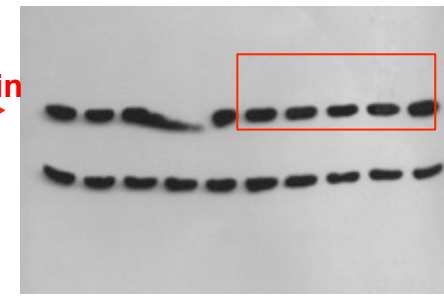
S1O: p-S6 (Ser240/244)

S1O: S6
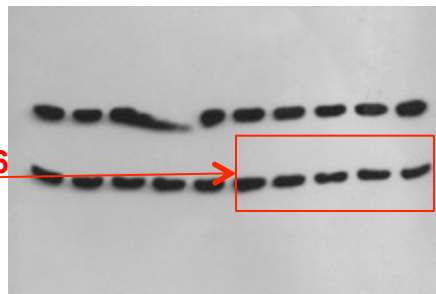
S1O:MITF
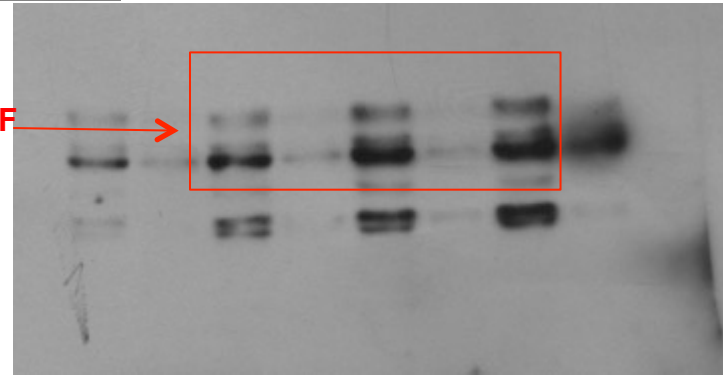
S1O:TYR
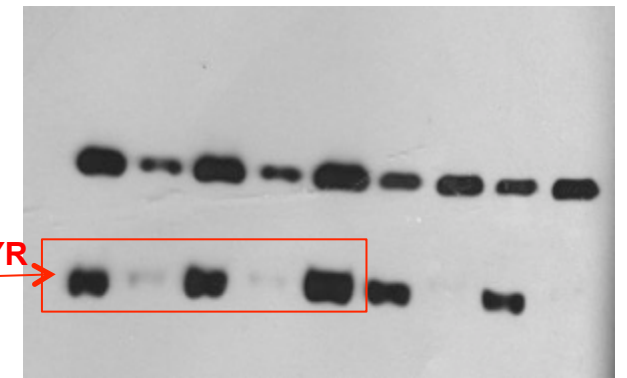
N Full unedited gel for Figure S1N

Blots were cut for different antibody staining and exposed on the same film

S1N: TSC1
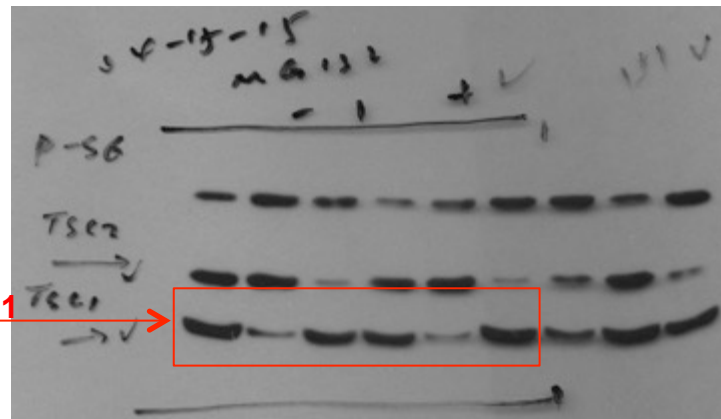
S1N: TSC2
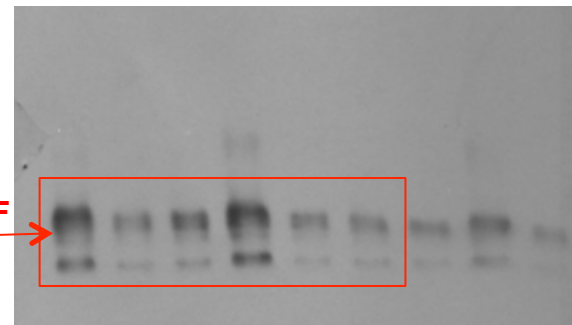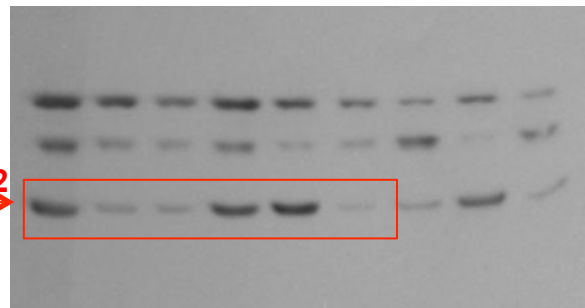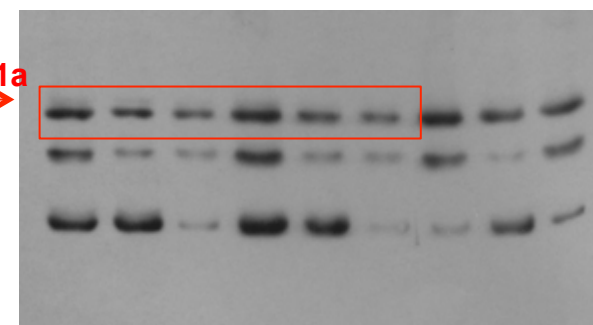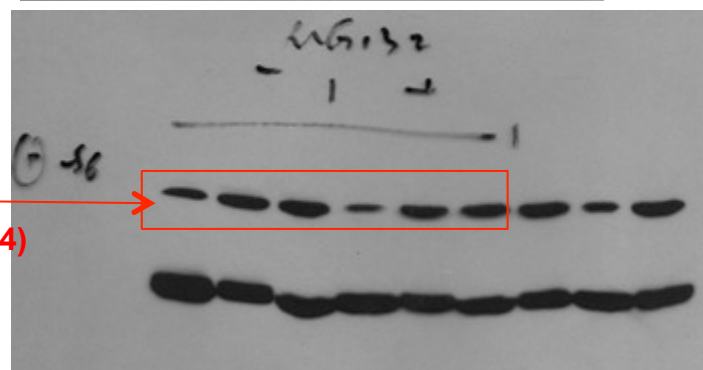
S1N: p-S6 (Ser240/244)

S1N:S6
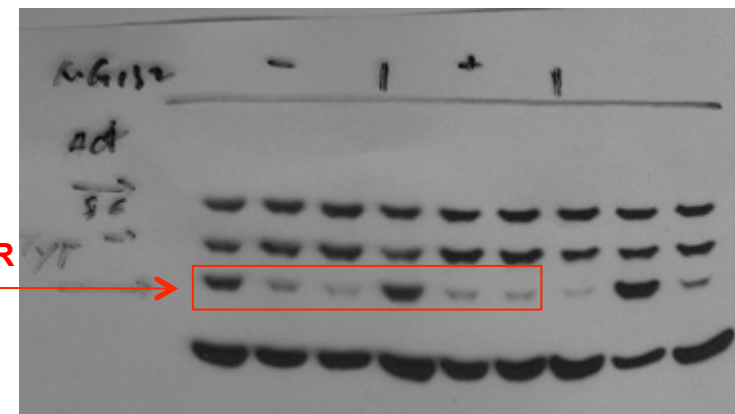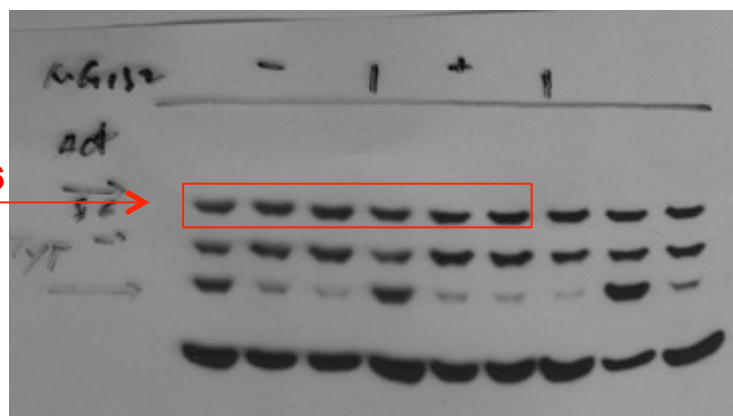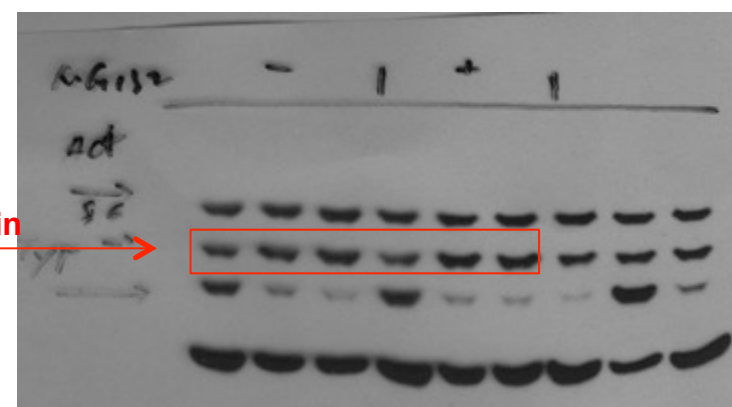
S1N: MITF

S1N: PGC-1a

S1N:TYR

S1N:Actin

Figure S1

Loading controls for parallel gels in figure S1O

parallel gel 1
S1O:Actin

parallel gel 2
S1O:Actin

Loading controls for parallel gels in figure S1N

parallel gel 1
S1N:Actin

A

Rapa (20 nM)   −   −   +   +

shRNA   Scr   TSC1-3   Scr   TSC1-3

TSC1

p-S6 (Ser235/236)

p-S6(Ser240/244)

S6

p-AKT(Thr308)

p-AKT(Ser473)

AKT

p-P70S6k (Thr389)

p-P70S6k (Thr229)

S6K

p-4E-BP1(Thr37/46)

4E-BP1

MITF

PGC-1α

TYR
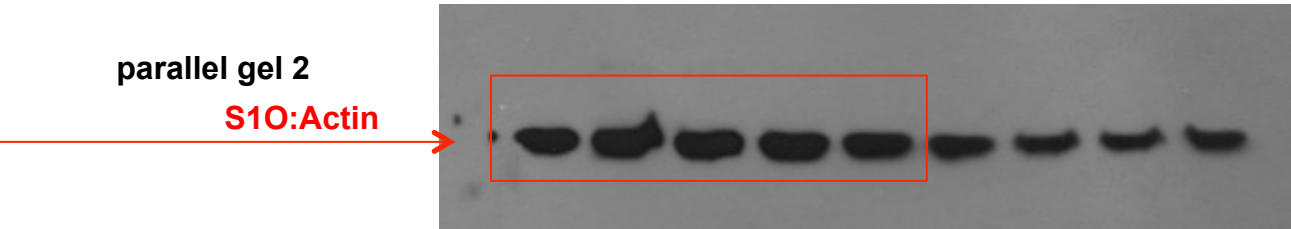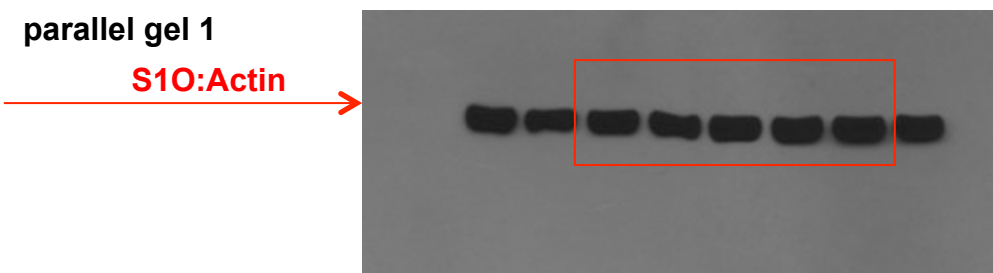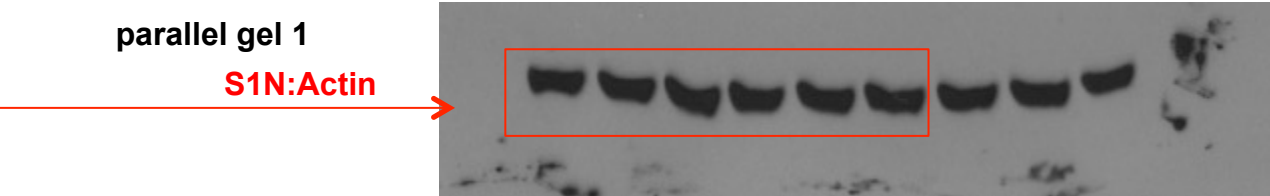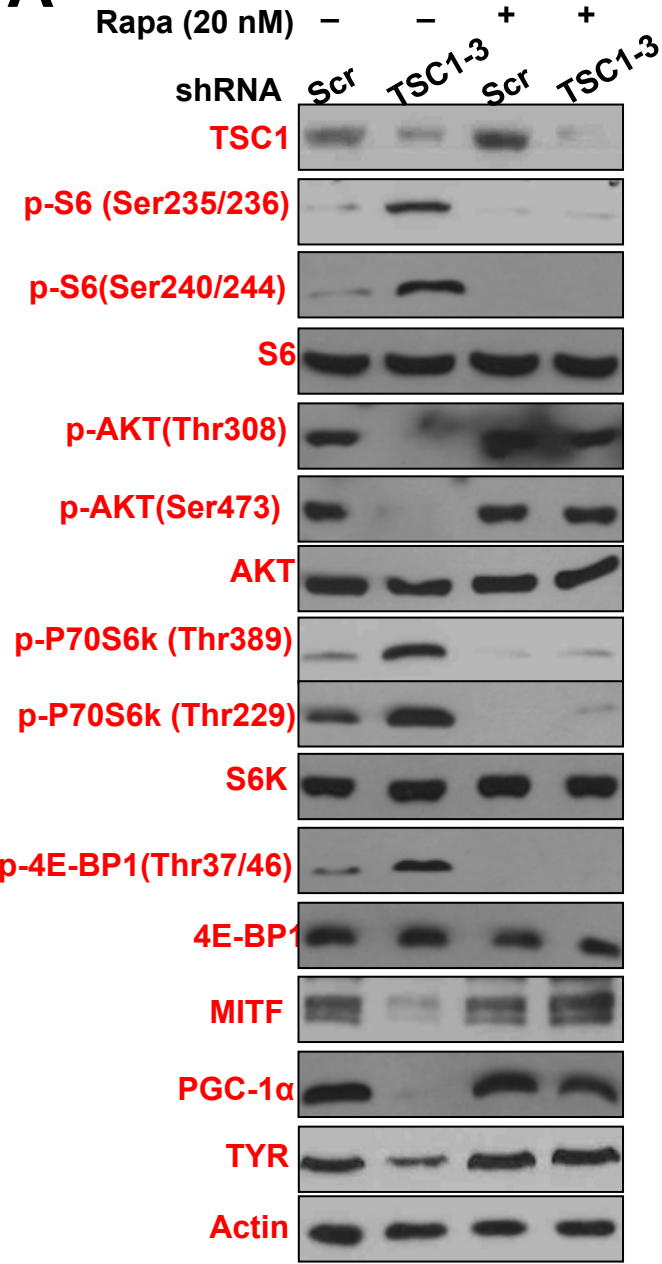
Actin

p-S6 (Ser235/236) p-P70S6k (Thr389)   Actin
_____

Run on same gel, **Actin** in the figure was used as the loading control

parallel gel 1

TSC1
_____

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 2

p-S6(Ser240/244)   p-AKT(Thr308)   p-AKT(Ser473)   p-4E-BP1(Thr37/46)
_____

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 3

MITF   TYR
_____

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 4

S6   AKT p-P70S6k (Thr229)   S6K   4E-BP1 PGC-1α
_____

Run on same gel, **AKT** was used as the loading control. No extra loading control needed

Figure S2

Full unedited gel for Figure S2A

A

S2A:TSC1

S2A:p-S6
(Ser235/236)

S2A:p-S6
(Ser240/244)

S2A:S6

S2A:pAKT
Thr308

S2A:pAKT
Ser473

S2A:AKT

S2A:p-P70S6K
(Thr389)

S2A:p-P70S6K1
(Thr229)

S2A:P70S6K1

S2A:p-4E-BP1

S2A:4E-BP1

S2A:MITF

S2A:PGC1α

S2A:TYR
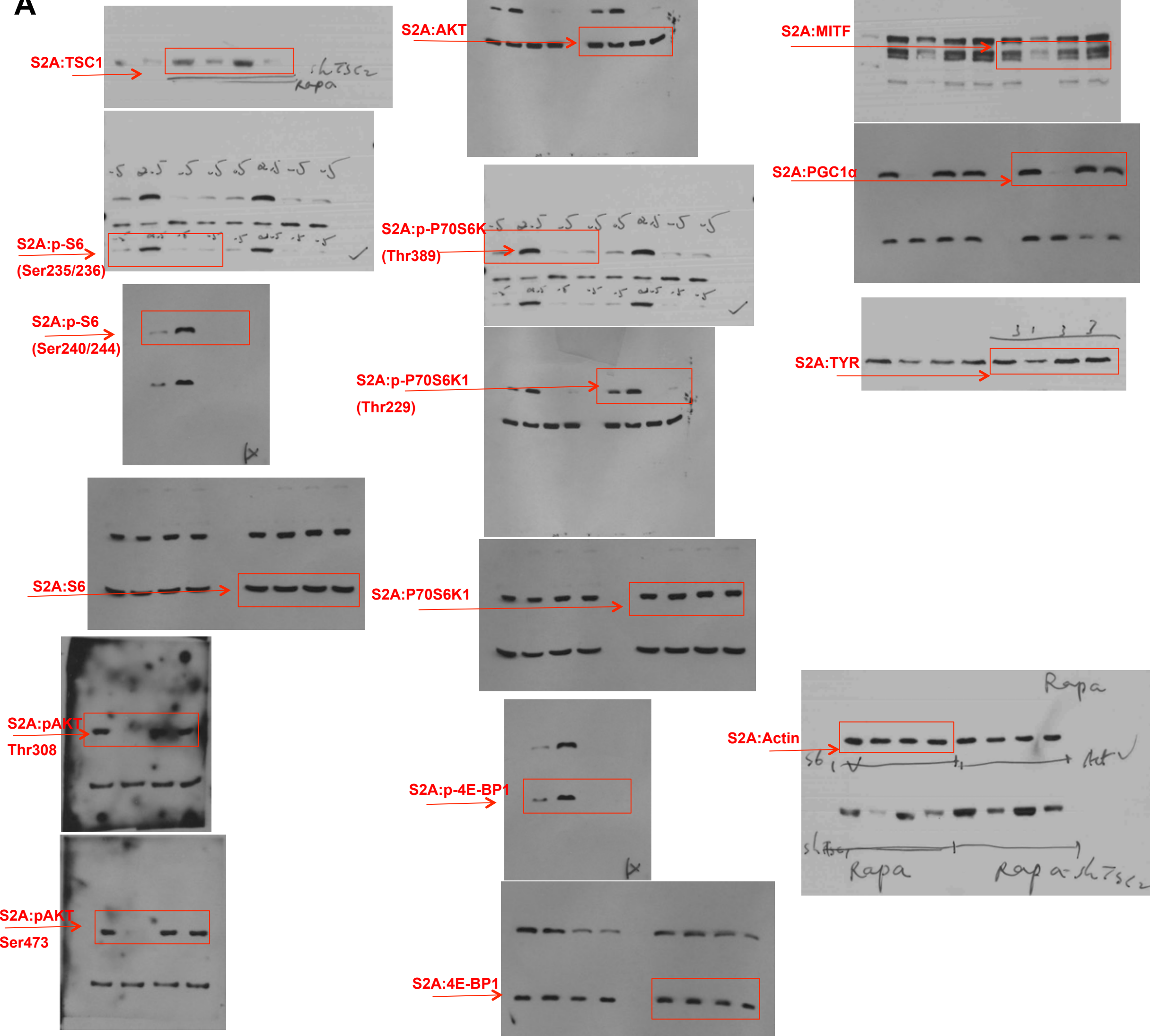
S2A:Actin

Blots were cut for different antibody staining and exposed on the same film

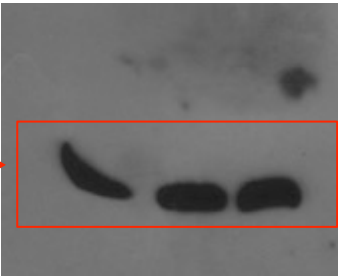**Figure S2**

Loading controls for parallel gels in figure S2A

parallel gel 1

S2A:Actin →
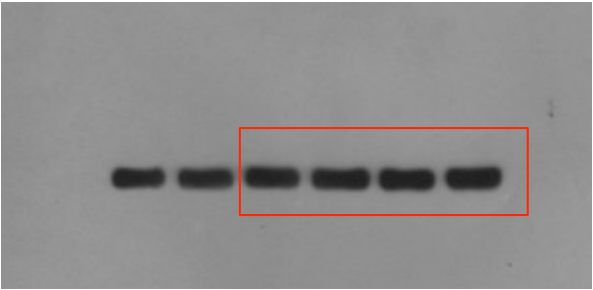
parallel gel 2

S2A:Actin →
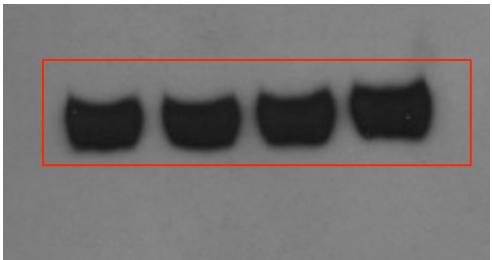
parallel gel 3

S2A:Actin →

parallel gel 4

S2A →

No extra loading control needed

E

Rapa (20 nM) − − + +

shRNA Scr TSC2-2 Scr TSC2-2

TSC2
p-S6 (Ser235/236)
p-S6(Ser240/244)
S6
p-AKT(Thr308)
p-AKT(Ser473)
AKT
p-P70S6k (Thr389)
p-P70S6k (Thr229)
S6K
p-4E-BP1(Thr37/46)
4E-BP1
MITF
PGC-1α
TYR
Actin

SK-MEL-30

S6   p-AKT(Thr308) AKT   p-P70S6k (Thr229)   S6K   4E-BP1   PGC-1α TYR   Actin

Run on same gel, **Actin** in the figure was used as the loading control

parallel gel 1

TSC2

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 2

p-S6 (Ser235/236)   **p-P70S6k (Thr389)**   **p-4E-BP1(Thr37/46)**

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 3

p-S6(Ser240/244)

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 4

p-AKT(Ser473)   **MITF**

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

Figure S2

E Full unedited gel for Figure S2E

S2E:TSC2

S2E:AKT

S2E:pS6
(Ser235/236)

S2E:p-P70s6k
(Thr389)

S2E:MITF

S2E: p-S6
(Ser240/244)

S2E:p-P70S6K1
(Thr229)

S2E:PGC-1α

S2E:S6

S2E:S6K

S2E:pAKT
Thr308

S2E:p-4EBP1
(Thr37/46)

S2E:TYR

S2E:pAKT
Ser473

S2E:4E-BP1

S2E:Actin

Figure S2

Loading controls for parallel gels in figure S2E

parallel gel 1

S2E:Actin →

parallel gel 2

S2E:Actin →

parallel gel 3

S2E:Actin →

parallel gel 4

S2E:Actin →

I

Torin-1 (250 nM)   −   −   −   +   +   +

shRNA   Scr   TSC1-3   TSC2-2   Scr   TSC1-3   TSC2-2

TSC1

TSC2

p-S6 (Ser235/236)

p-S6(Ser240/244)

S6

p-P70S6k (Thr389)

S6K

p-4E-BP1(Thr37/46)

4E-BP1

p-AKT

AKT

MITF

PGC-1α

TYR

Actin

SK-MEL30

4E-BP1   AKT   MITF   Actin

Run on same gel, **Actin** in the figure was used as the loading control

parallel gel 1

TSC1   p-S6(Ser240/244)   p-AKT

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 2

TSC2   p-S6 (Ser235/236)   p-P70S6k (Thr389)   p-4E-BP1(Thr37/46)   PGC-1α

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 3

TYR

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 4

S6

Run on same gel, **S6** was used as the loading control, no extra loading control needed

parallel gel 5

S6k

Run on same gel, **S6k** was used as the loading control, no extra loading control needed

Figure S2

Full unedited gel for Figure S2I

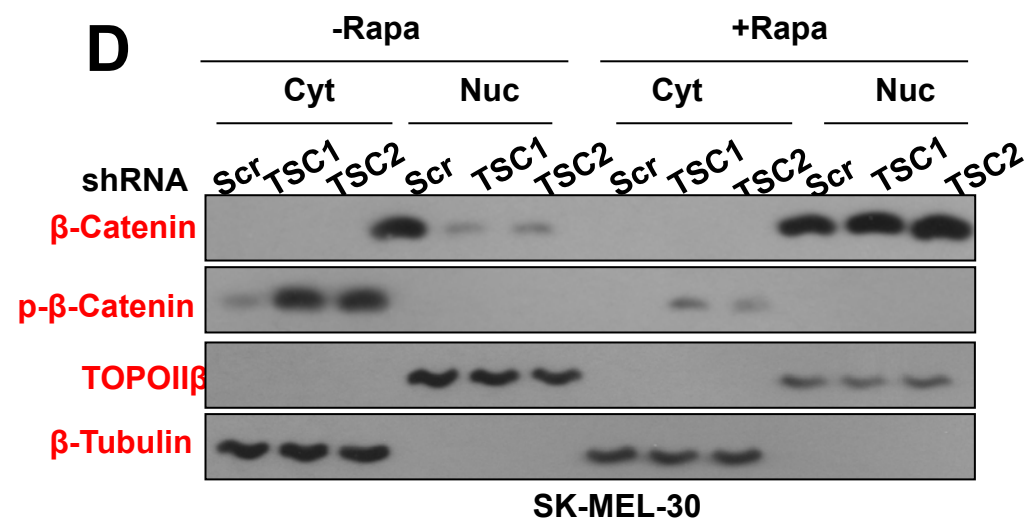Blots were cut for different antibody staining and exposed on the same film

S2I:TSC1

S2I:p-4E-BP1
(Thr37/46)

S2I:TSC2
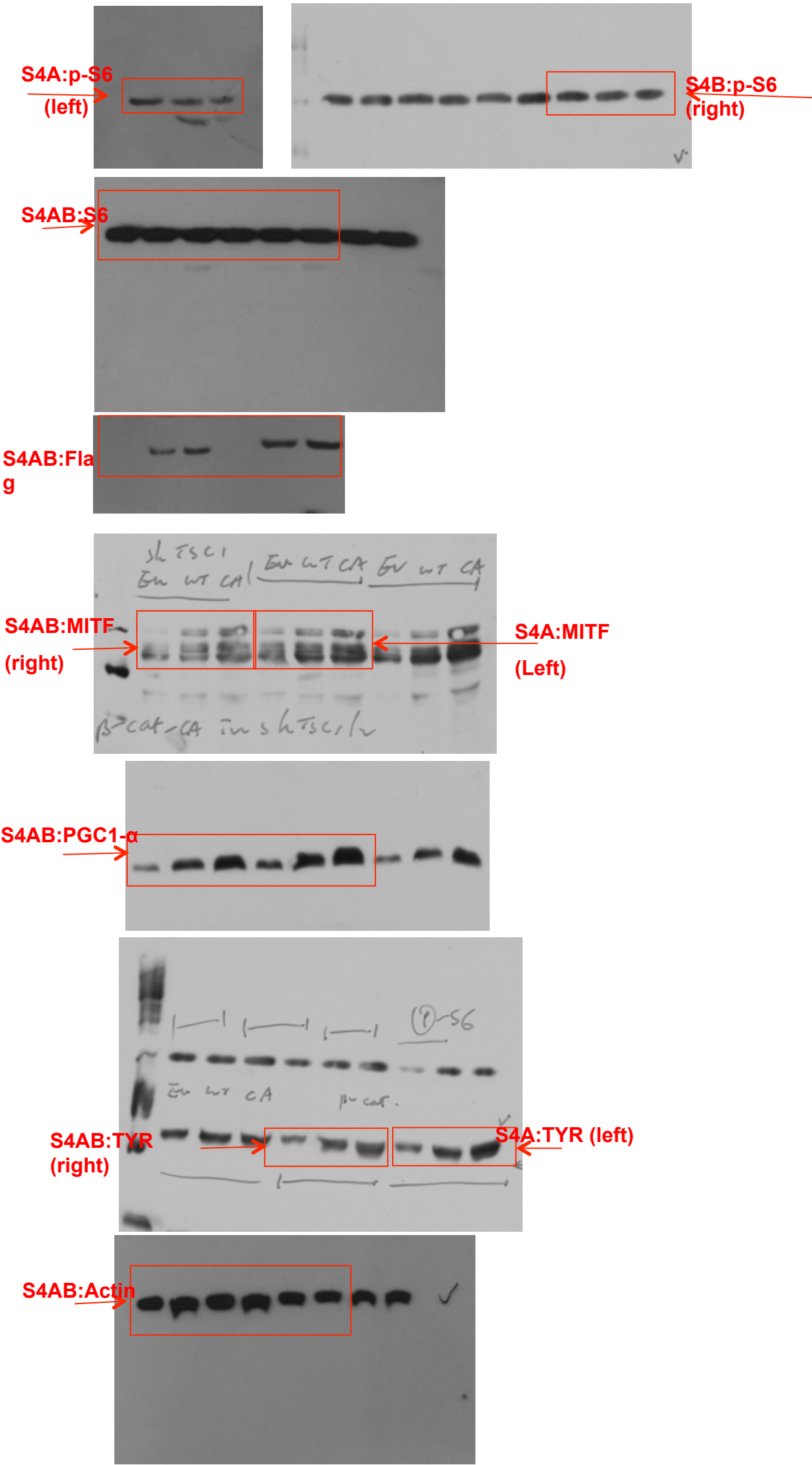
S2I:4E-BP1

S2I:p-S6
(Ser235/236)

S2I:p-AKT

S2I:p-S6
(Ser240/244)

S2I: AKT

S2I:S6

S2I:MITF

S2I:p-P70S6k
(Thr389)

S2I:PGC-1a

S2I:S6K

S2I: TYR

S2I:Actin

Figure S2

Loading controls for parallel gels in figure S2I

parallel gel 1

S2I:Actin

parallel gel 2

S2I:Actin

parallel gel 3

S2I:Actin

parallel gel 4

S2I:

No extra loading control needed

parallel gel 5

S2I:

No extra loading control needed

M

shRNA   Scr   Raptor   Rictor

Raptor

Rictor

p-S6 (Ser235/236)

S6

p-AKT

AKT

MITF

PGC-1α

TYR

Actin

SK-MEL30

parallel gel 1

Raptor

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 2

p-S6 (Ser235/236)   **p-AKT**

Run on same gel, **Actin** was used as the loading control (see uncropped gel image)

parallel gel 3

S6   **MITF**

Run on same gel, **S6** in the figure was used as a loading control, no extra loading control needed

parallel gel 4

AKT   **PGC-1α**   **TYR**

Run on same gel, **AKT** in the figure was used as a loading control, no extra loading control needed

parallel gel 5

Actin

Run on same gel, **Actin** in the figure was used as the loading control, no extra loading control needed

Full unedited gel for Figure S2M

M

S2M:Raptor

S2M:Rictor

S2M:PGC-1a

p-S6
(Ser235/236)

S2M:Tyr

S2M:S6

S2M:Actin

S2M:p-AKT

S2M:AKT

S2M:MITF
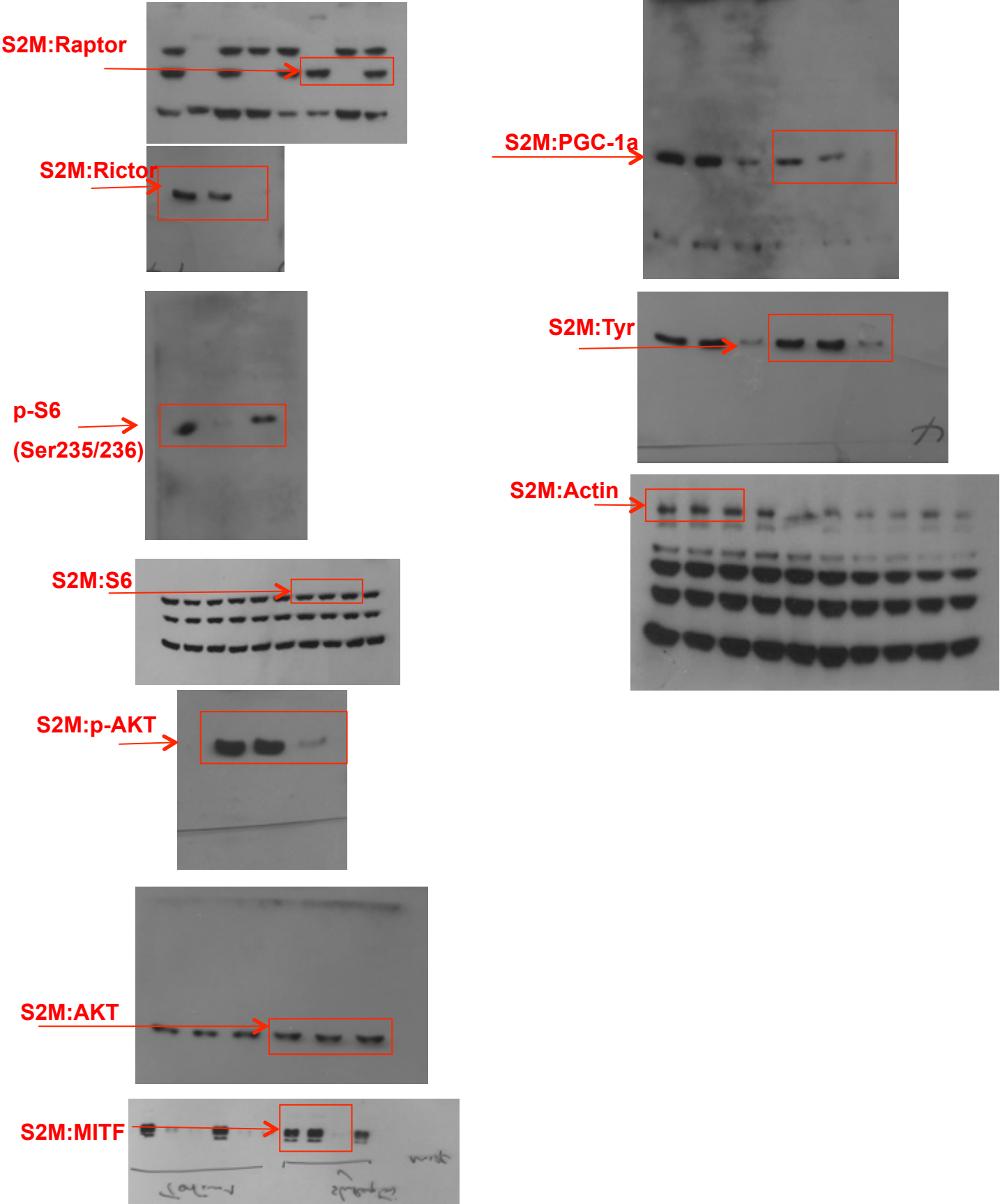
Figure S2

Loading controls for parallel gels in figure S2M

parallel gel 1
S2M:Actin
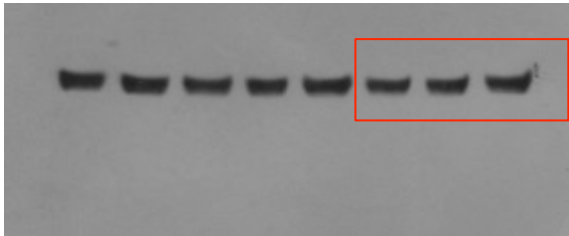
parallel gel 2
S2M:Actin

parallel gel 3
S2M:

No extra loading control needed

parallel gel 4
S2MI:

No extra loading control needed

parallel gel 5
S2M:

No extra loading control needed

A

shRNA Scr TSC1-3 Scr TSC2-2

TSC1
TSC2
p-AKT(T308)
p-AKT(S473)
AKT
p-GSK3β
GSK3β
p-β-Catenin
β-Catenin
p-S6 (Ser235/236)
p-S6 (Ser240/244)
S6
p-S6K1 (Thr389)
S6K1
Actin

SK-MEL-30
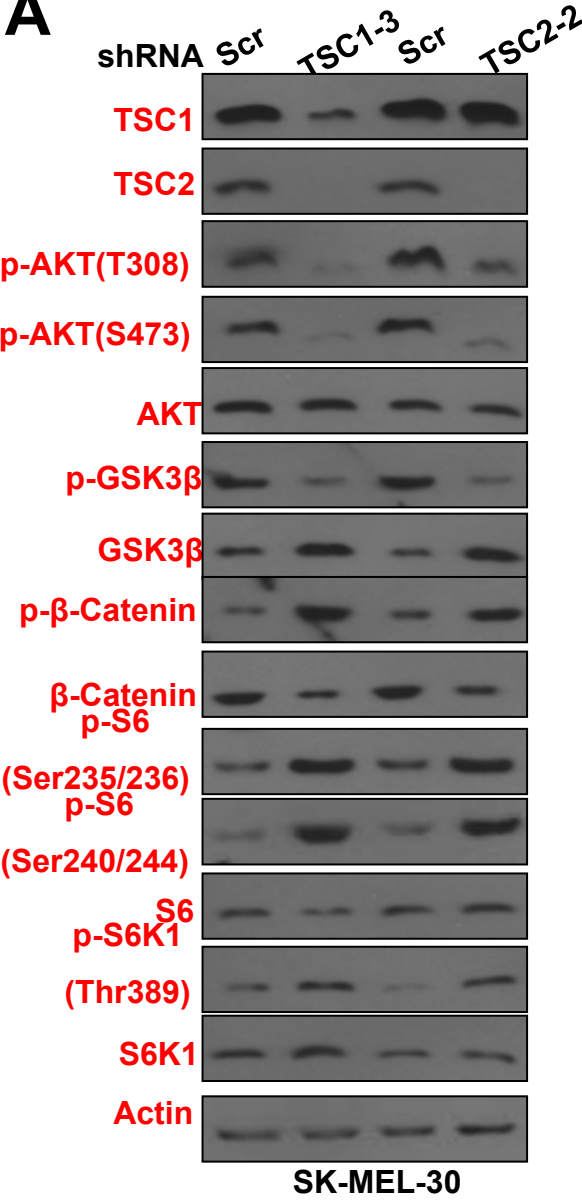
parallel gel 1

TSC1   TSC2   p-AKT(T308)   p-AKT(S473)   AKT

Run on same gel, **AKT** in the figure was used as a loading control, no extra loading control needed

parallel gel 2

p-GSK3β   GSK3β   p-β-Catenin   β-Catenin   p-S6 (Ser235/236)   p-S6 (Ser240/244)   S6

Run on same gel, **S6** in the figure was used as a loading control, no extra loading control needed

parallel gel 3

p-S6K1 (Thr389)   S6K1   Actin

Run on same gel, **Actin** in the figure was used as the loading control, no extra loading control needed

Figure S3

A Full unedited gel for Figure S3A   Blots were cut for different antibody staining and exposed on the same film

S3A:TSC1

S3A:TSC2

S3A:p-AKT
T308

S3A:p-AKT
S473

S3A:AKT

S3A:p-GSK3β

S3A:GSK3β

S3A:p-β-catenin

S3A:β-catenin

S3A:p-S6
(s235/236)

3A:p-S6
(s240/244)

S3A:S6

S3A:p-S6K1(Thr389)

S3A:S6K1

S3A:Actin

Figure S3

Loading controls for parallel gels in figure S3A

parallel gel 1
S3A: No extra loading control needed

parallel gel 2
S3A: No extra loading control needed

parallel gel 3
S3A: No extra loading control needed

B

SK-MEL-30
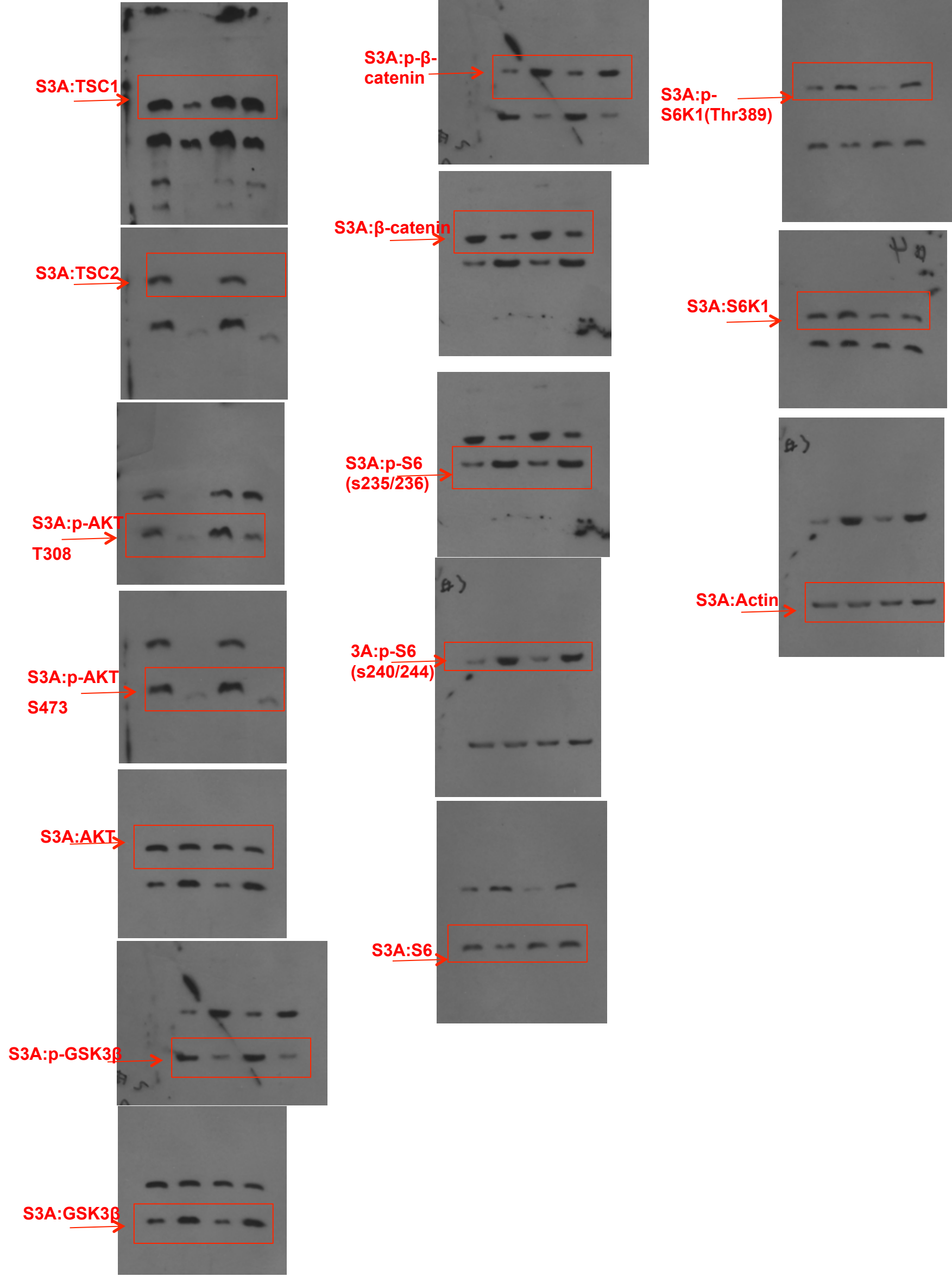
TSC2   p-β-Catenin   β-Catenin   Actin
_____

Run on same gel, **Actin** in the figure was used as a loading control

parallel gel 1

TSC1 (left)
_____

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 2

TSC1 (right)
_____

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 3

p-S6   **S6**
_____

Run on same gel, **S6** in the figure was used as the loading control, no extra loading control needed

Figure S3

B Full unedited gel for Figure S3B Blots were cut for different antibody staining and exposed on the same film
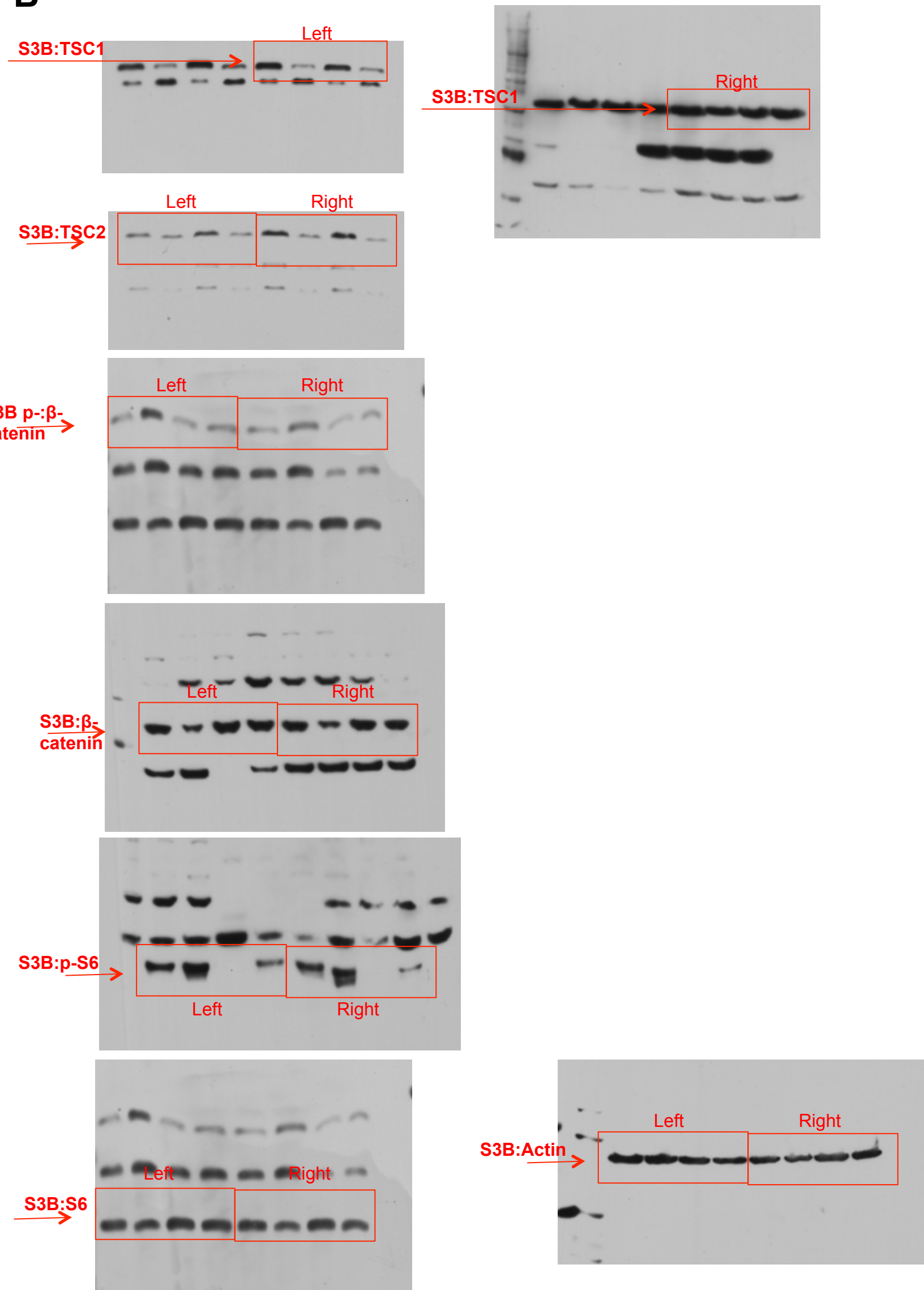
Figure S3

Loading controls for parallel gels in figure S3B
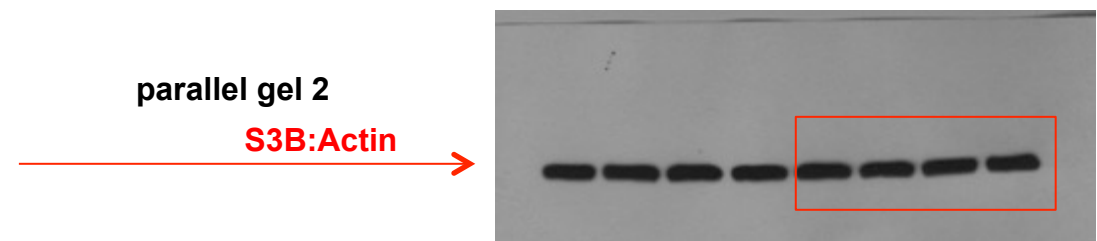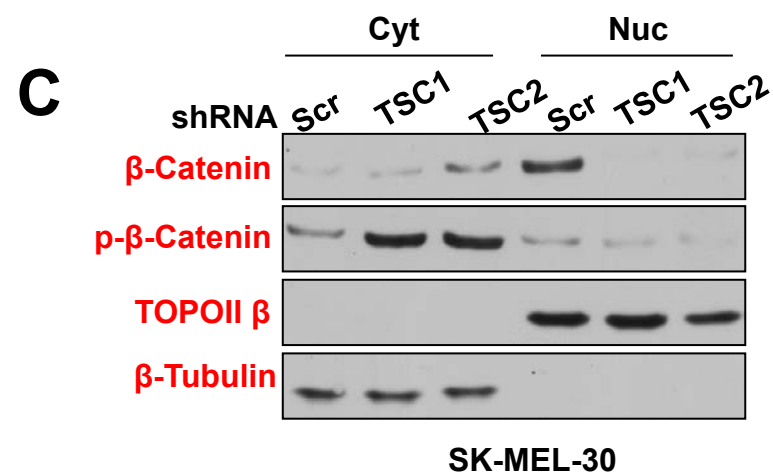
parallel gel 1

S3B:Actin →

parallel gel 2

S3B:Actin →

parallel gel 3

S3B:Actin → No extra loading control needed

C

Cyt | Nuc

shRNA | Scr | TSC1 | TSC2 | Scr | TSC1 | TSC2

β-Catenin
p-β-Catenin
TOPOII β
β-Tubulin

SK-MEL-30

All run on the same gel, no extra loading needed

D

-Rapa | +Rapa

Cyt | Nuc | Cyt | Nuc

shRNA | Scr | TSC1 | TSC2 | Scr | TSC1 | TSC2 | Scr | TSC1 | TSC2 | Scr | TSC1 | TSC2

β-Catenin
p-β-Catenin
TOPOIIβ
β-Tubulin

SK-MEL-30

All run on the same gel, no extra loading needed

Figure S3

Full unedited gel for Figure S3C&D

C

S3C:β-catenin

S3C:p-β-catenin

S3C:TOPOIIβ

S3C:β-Tubulin

D

S3D:β-catenin

S3D:p-β-catenin

S3D:TOPOIIβ

S3D:β-Tubulin
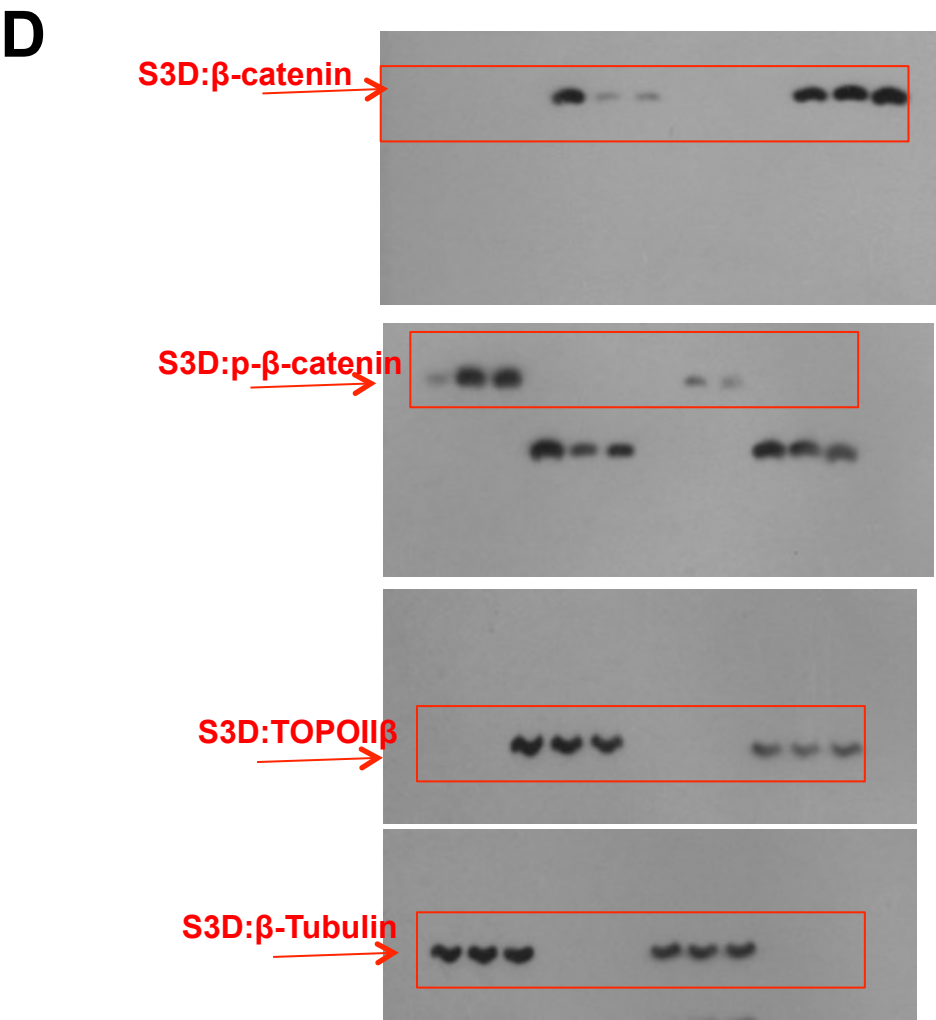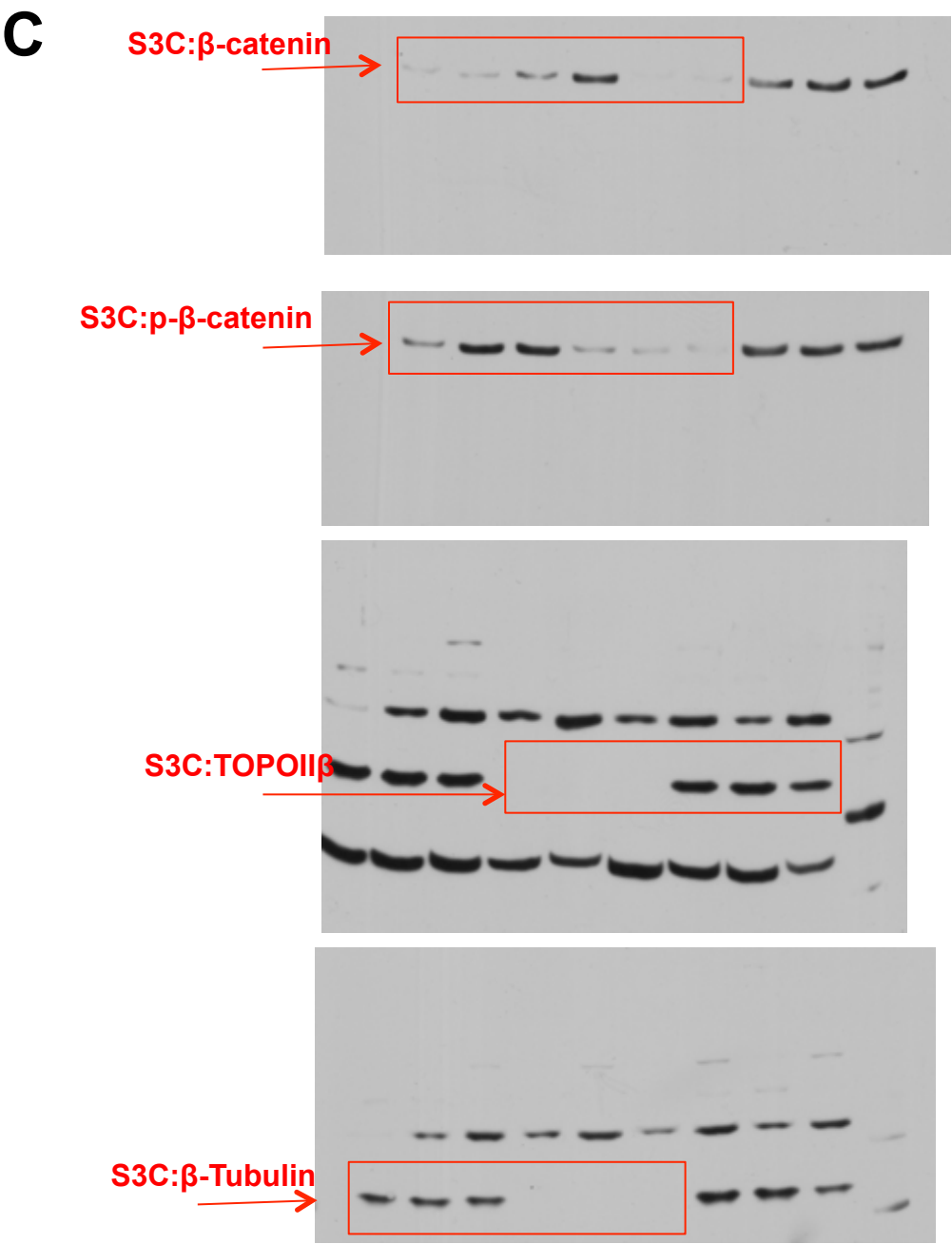
Figure S3

Loading controls for parallel gels in figure S3C&D

All run on the same gel, no
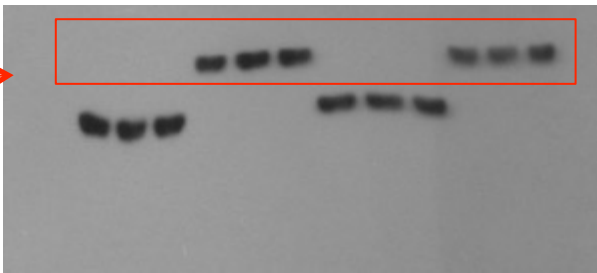extra loading needed

A

shTSC1-3

β-catenin EV WT CA

p-S6

S6

Flag

MITF

PGC-1α

TYR

Actin

SK-MEL-30

B

shTSC2-2

EV WT CA

SK-MEL-30
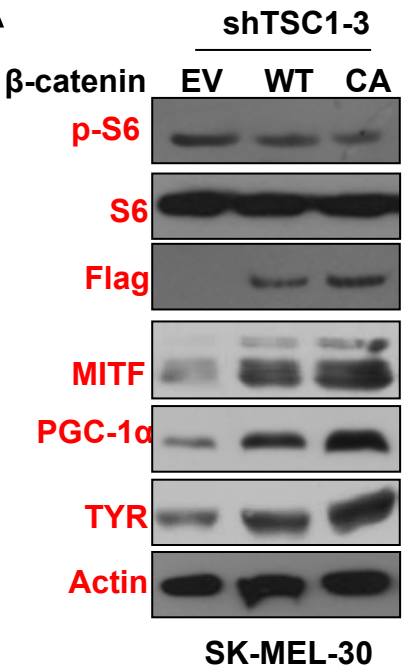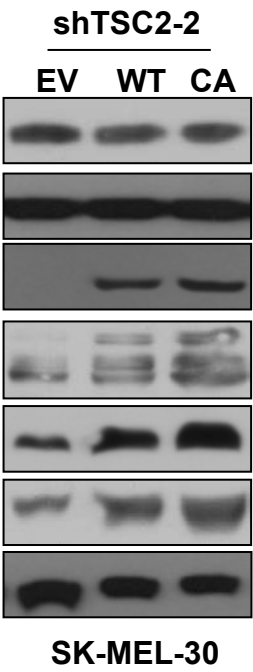
S6   Actin

Run on same gel, **Actin** in the figure was used as a loading control

parallel gel 1

p-S6 (left)

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 2

p-S6 (right)

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 3

Flag

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 4

MITF

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 5

PGC-1α

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 6

TYR

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

Figure S4

Full unedited gel for Figure S4AB

Blots were cut for different antibody staining and exposed on the same film

S4A:p-S6 (left)

S4B:p-S6 (right)

S4AB:S6

S4AB:Flag

S4AB:MITF (right)

S4A:MITF (Left)

S4AB:PGC1-α

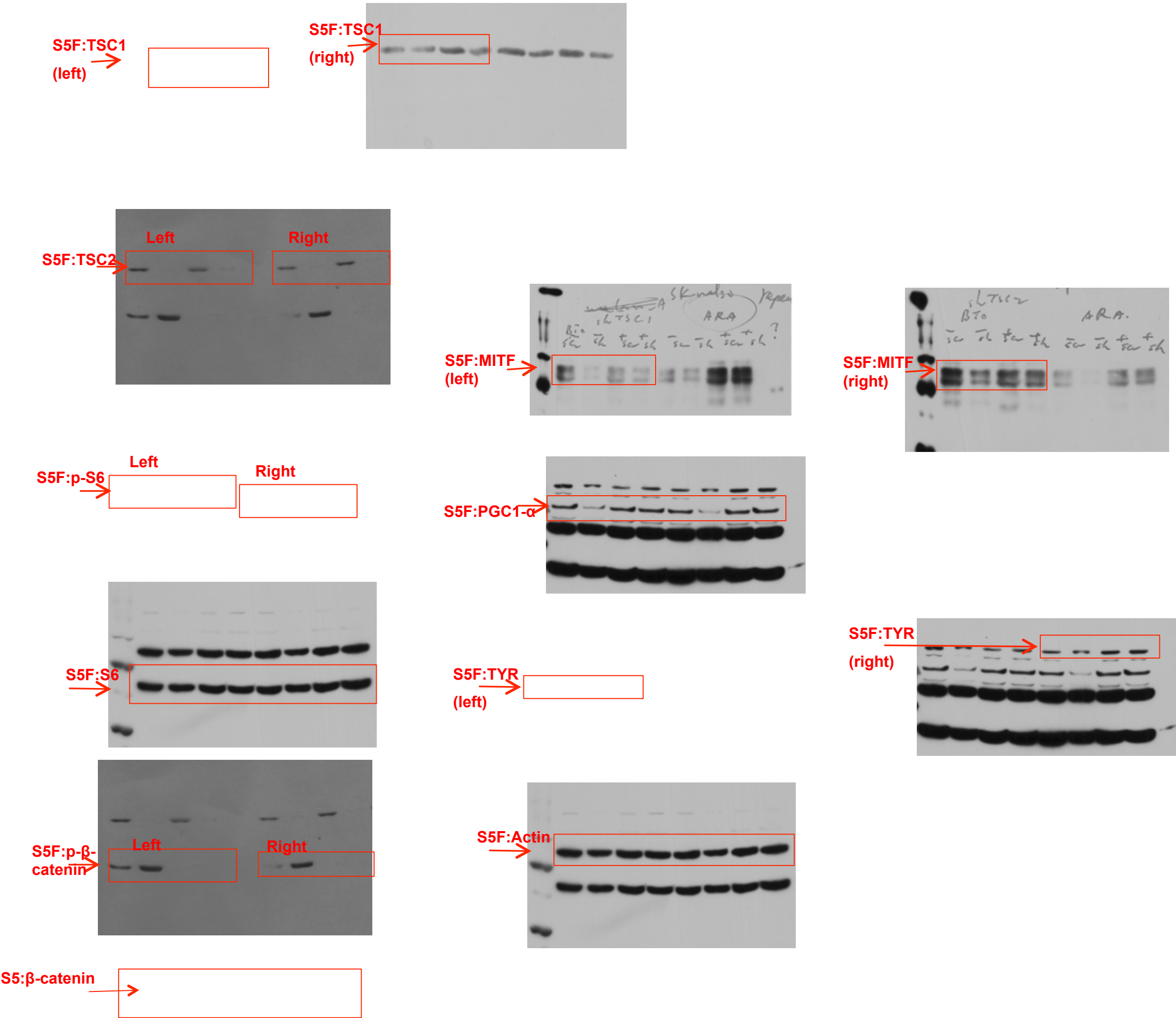S4AB:TYR (right)

S4A:TYR (left)

S4AB:Actin

Figure S4

Loading controls for parallel gels in figure S4AB

parallel gel 1
S4AB:Actin

parallel gel 2
S4AB:Actin

parallel gel 3
S4AB:Actin

parallel gel 4
S4AB:Actin

parallel gel 5
S4AB:Actin

parallel gel 6
S4AB:Actin

4E

shGSK3β   −   −   −   +   +   +

shRNA   Scr   TSC1-3   TSC2-2   Scr   TSC1-3   TSC2-2

TSC1

TSC2

p-S6

S6

GSK3β

p-β-Catenin

β-Catenin

MITF

PGC-1α

TYR

Actin

SK-MEL-30
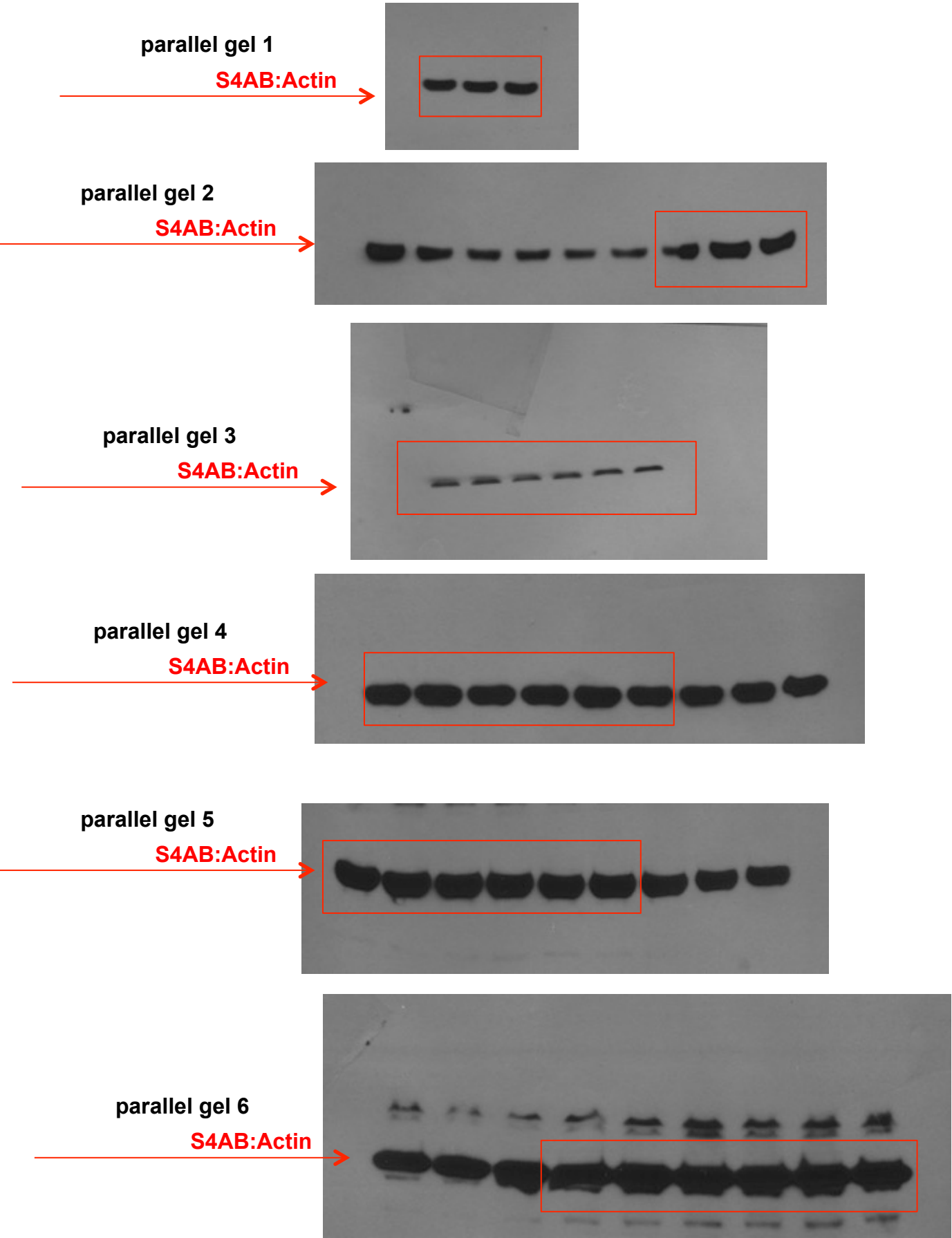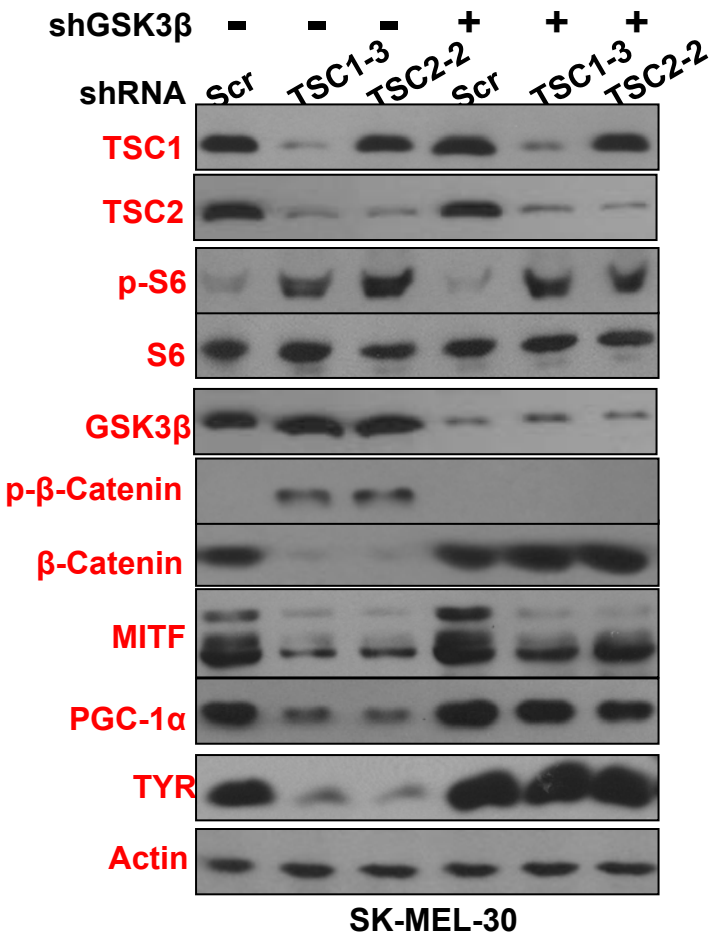
TSC1	TSC2	S6 GSK3β MITF PGC-1α

Run on same gel, **S6** in the figure was used as a loading control

parallel gel 1

p-S6

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 2

p-β-Catenin

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 3

β-Catenin

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 4

TYR

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 5

Actin

Run on same gel, **Actin** in the figure was used as a loading control, no extra loading needed

Full unedited gel for Figure S4E

E **Blots were cut for different antibody staining and exposed on the same film**

S4E:TSC1

S4E:GSK3β

S4E:Actin

S4E:TSC2

S4E: p-β-catenin

S4E:β-catenin

S4E:p-S6

S4E:MITF

S4E:S6

S4E:PGC1α

S4E:TYR

Figure S4

Loading controls for parallel gels in figure S4E

parallel gel 1

S4E:Actin

parallel gel 2

S4E:Actin

parallel gel 3

S4E:Actin

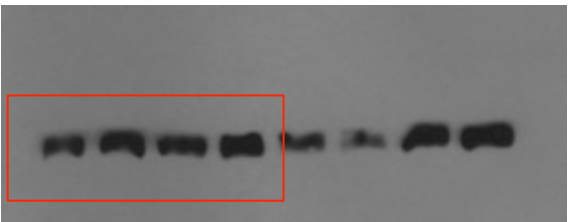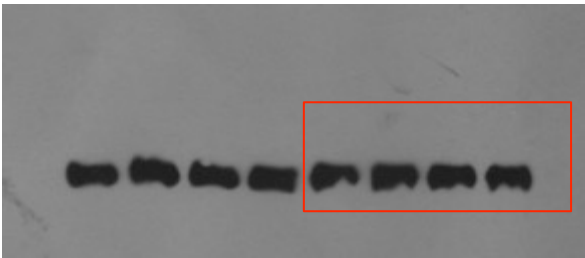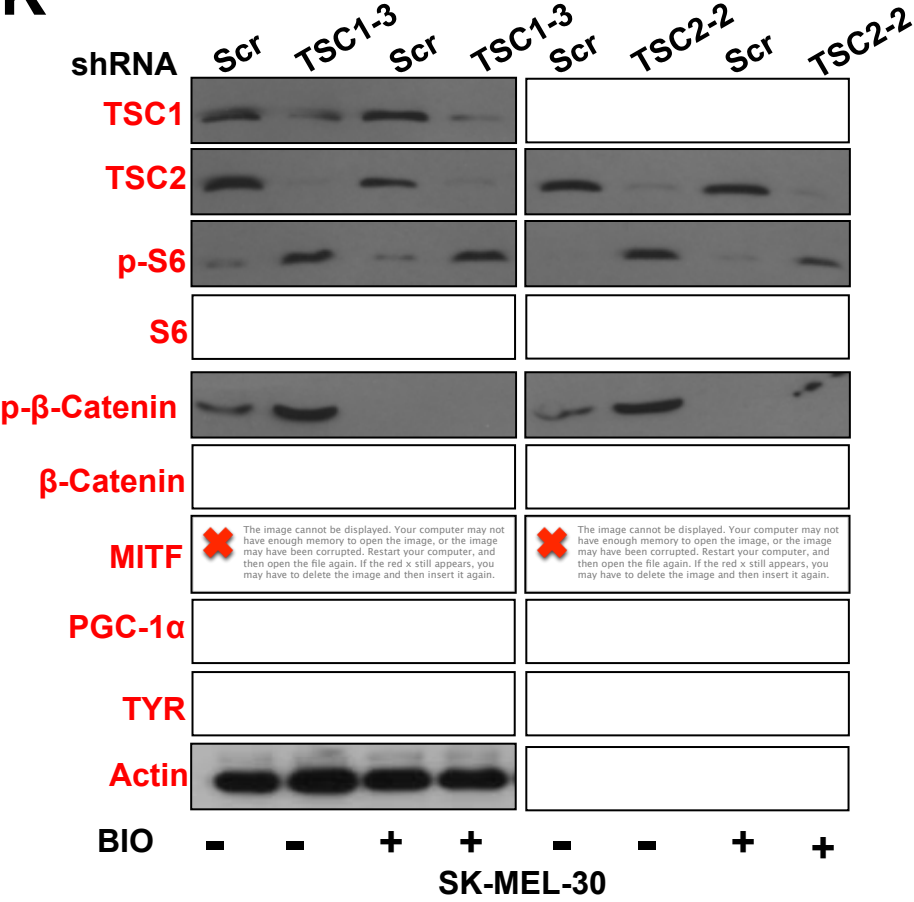parallel gel 4

S4E:Actin

parallel gel 5

S4E:Actin

No extra loading control needed

A

shRNA	Scr	TSC1-3	Scr	TSC1-3	Scr	TSC2-2	Scr	TSC2-2

TSC1				
TSC2				
p-S6				
S6				
p-β-Catenin				
β-Catenin				
MITF				
PGC-1α				
TYR				
Actin				

CHIR99021 − − + + − − + +

SK-MEL-30

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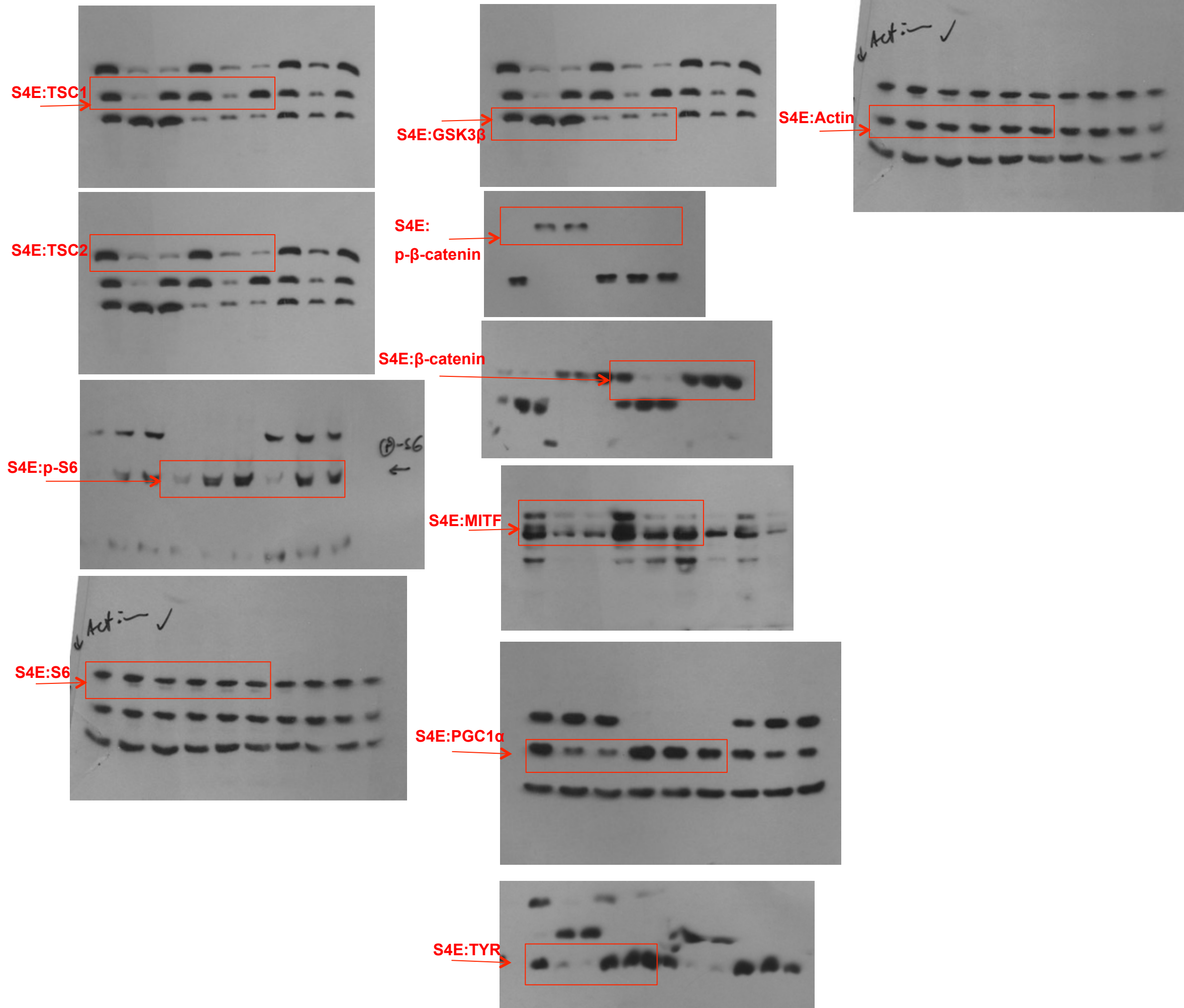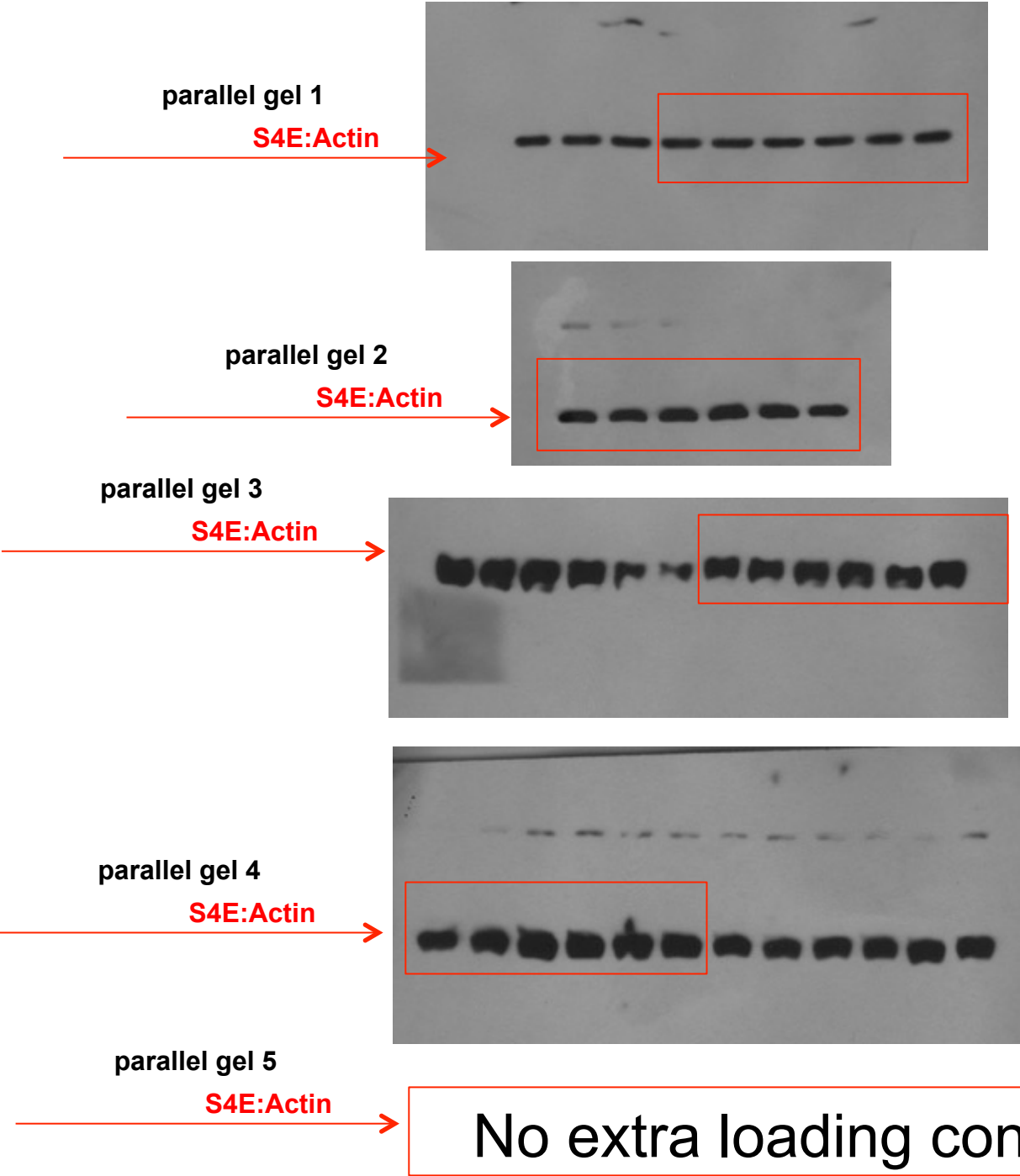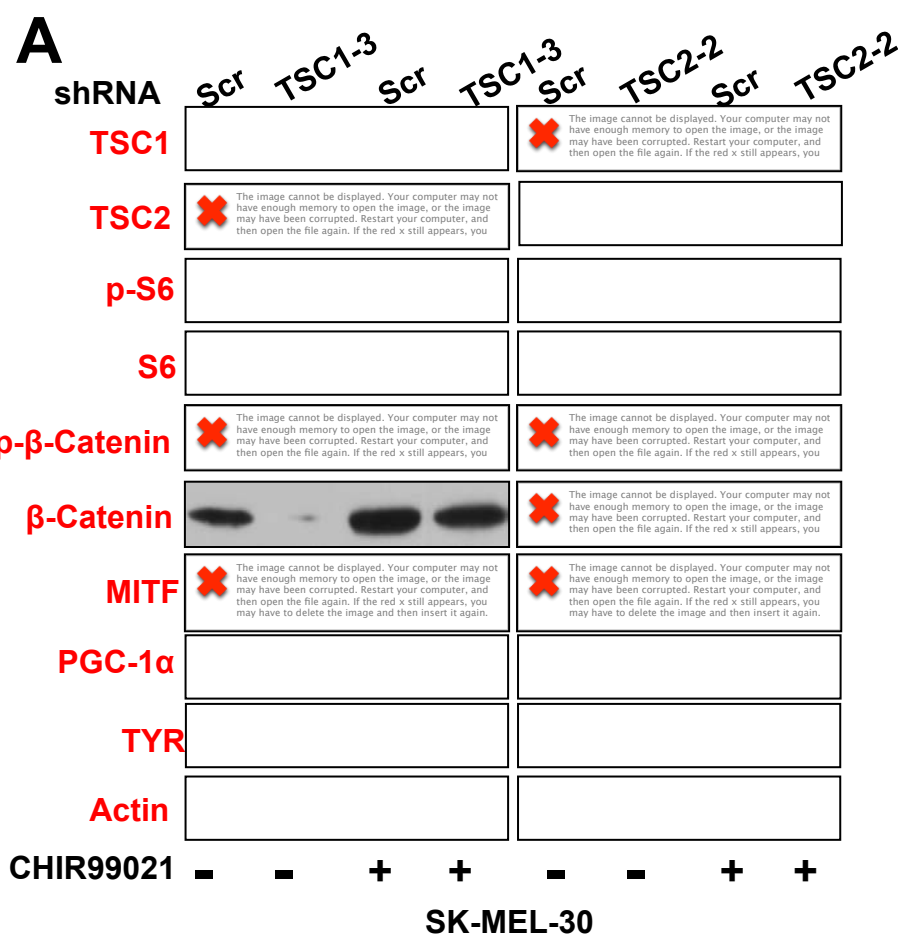
p-β-Catenin   β-Catenin   MITF PGC-1α   Actin

Run on same gel, **Actin** in the figure was used as a loading control

parallel gel 1

TSC1 (left)   TSC2 (left)   TYR (left)

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 2

TSC1 (right)

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 3

TSC2 (right)

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 4

p-S6 (left)

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 5

p-S6 (right)

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 6

TYR (right)

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 7

S6

Run on same gel, **S6** in the figure was used as a loading control, no extra loading needed

Figure S5

A Full unedited gel for Figure S5A

Blots were cut for different antibody staining and exposed on the same film

S5A:TSC1 (Left)

S5A:TSC1 (Right)

S5A:TSC2 (Left)

S5A:TSC2 (Right)

S5A:p-S6 (Left)

S5A:p-S6 (Right)

S5A:S6

Left

Right

S5A:TYR (Left)

S5A:p-β-catenin

S5A:TYR (Right)

A:β-Catenin

S5A:Actin

S5A:MITF

S5A:PGC-1α

Figure S5

Loading controls for parallel gels in figure S5A

parallel gel 1

S5A:Actin →

parallel gel 2

S5A:Actin →

parallel gel 3

S5A:Actin →

parallel gel 4

S5A:Actin →

parallel gel 5

S5A:Actin →

parallel gel 6
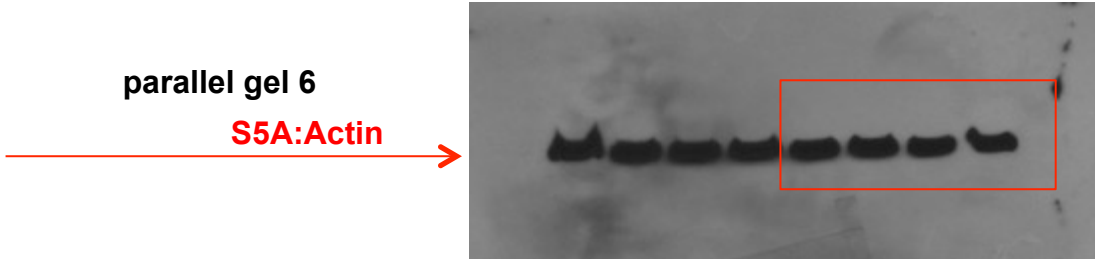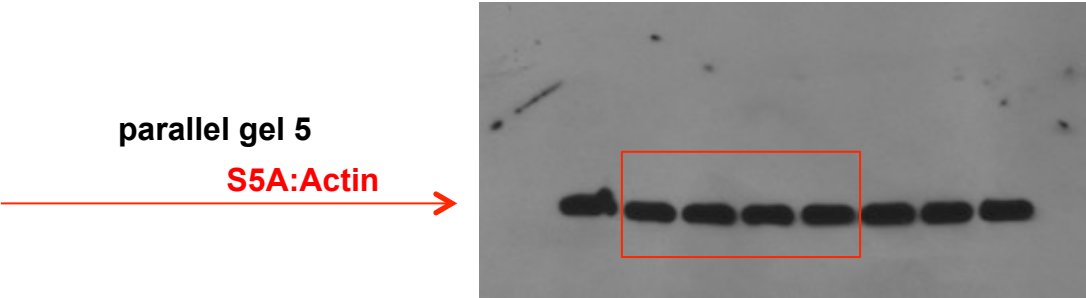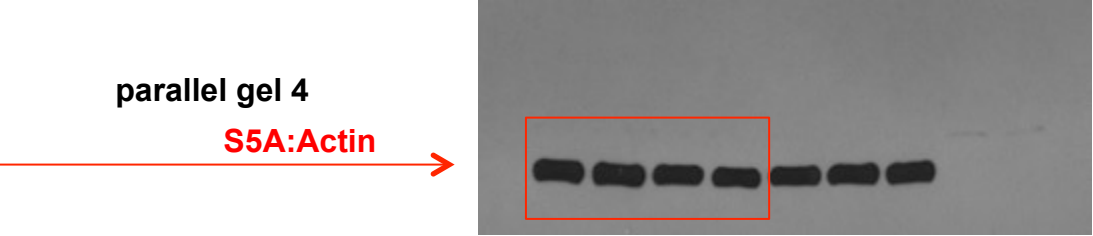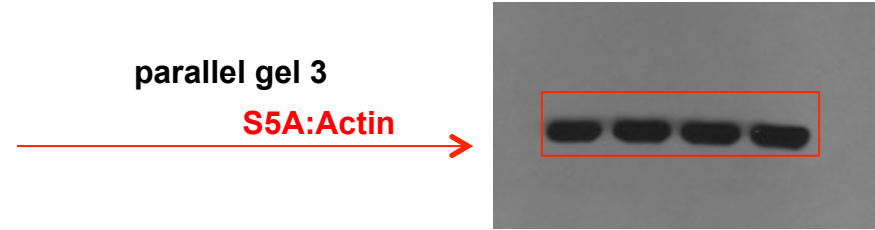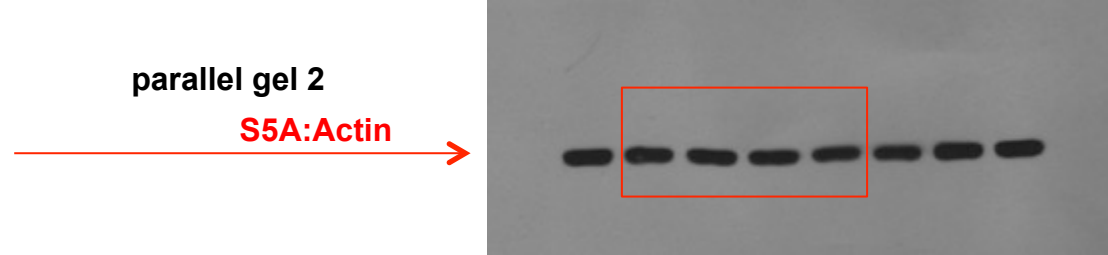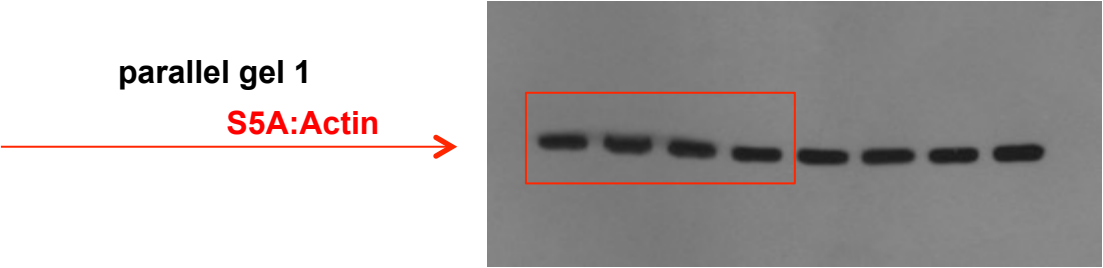
S5A:Actin →

parallel gel 7

S5A:Actin → No extra loading control needed
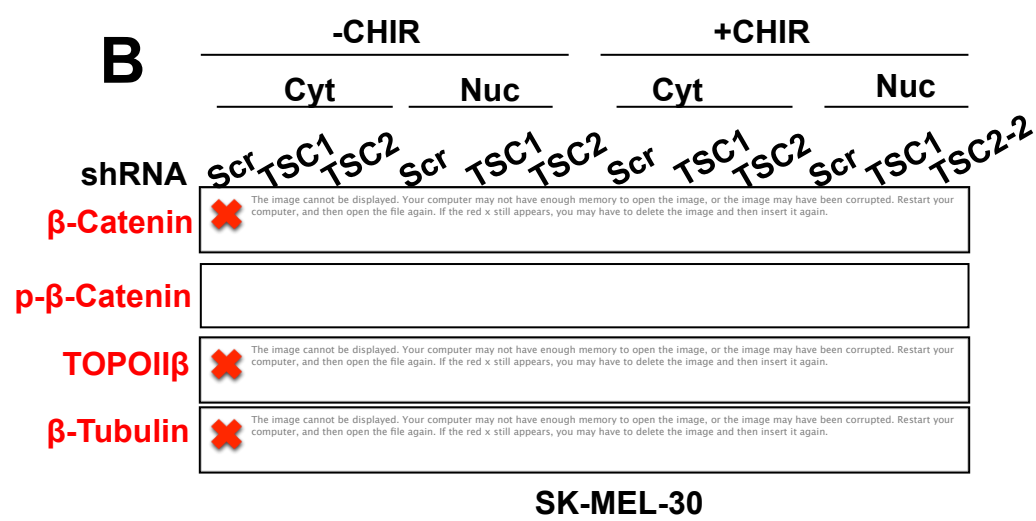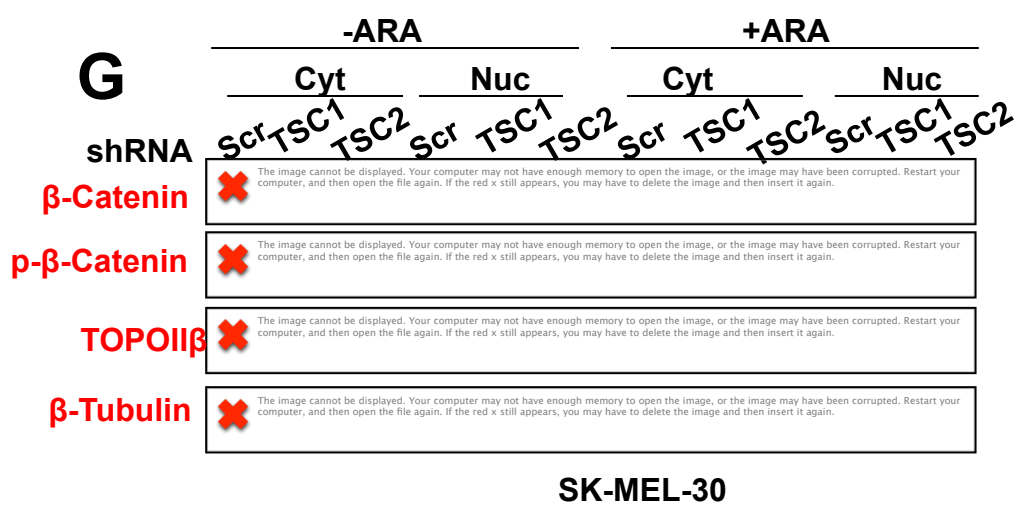
B

-CHIR | +CHIR

Cyt | Nuc | Cyt | Nuc

shRNA: Scr TSC1 TSC2 Scr TSC1 TSC2 Scr TSC1 TSC2 Scr TSC1 TSC2-2

β-Catenin

p-β-Catenin

TOPOIIβ

β-Tubulin

SK-MEL-30

All run on the same gel, no extra loading needed

G

-ARA | +ARA

Cyt | Nuc | Cyt | Nuc

shRNA: Scr TSC1 TSC2 Scr TSC1 TSC2 Scr TSC1 TSC2 Scr TSC1 TSC2

β-Catenin

p-β-Catenin

TOPOIIβ

β-Tubulin

SK-MEL-30

All run on the same gel, no extra loading needed

Figure S5

Full unedited gel for Figure S5B&G

B

S5B:β-catenin
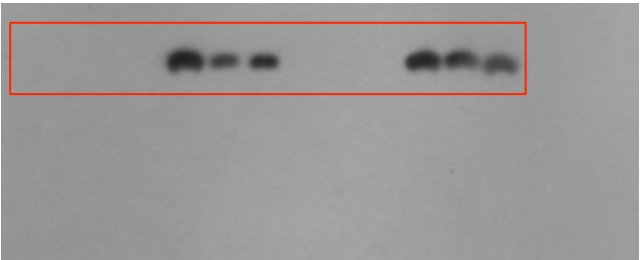
S5B:p-β-catenin
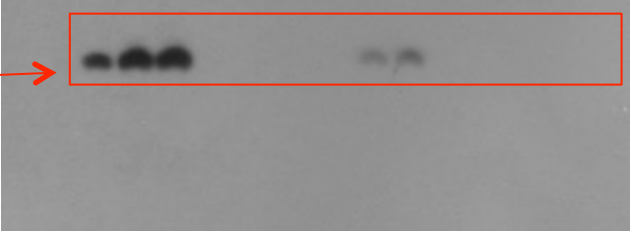
S5B:TOPOIIβ
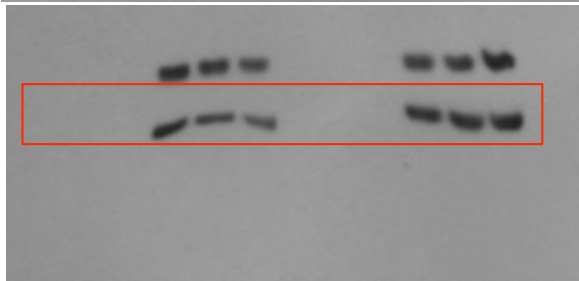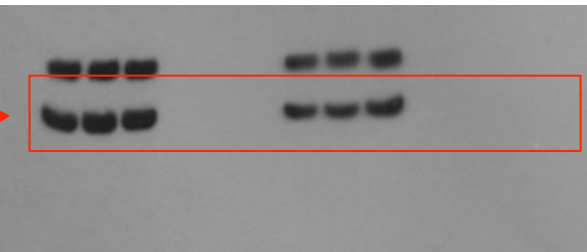
S5B:β-Tubulin

G

S5G:β-catenin
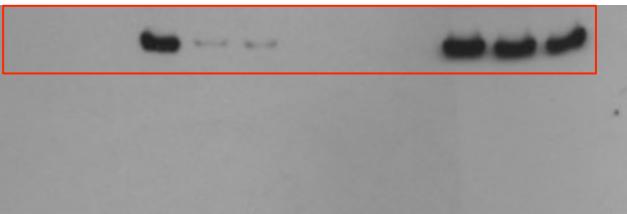
S5G:p-β-catenin

S5G:TOPOIIβ

S5G:β-Tubulin
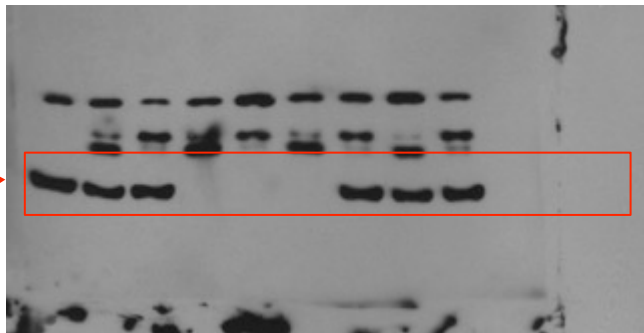
Figure S5

Loading controls for parallel gels in figure S5B&G

All run on the same gel, no
extra loading needed

F

shRNA	Scr	TSC1-3	Scr	TSC1-3	Scr	TSC2-2	Scr	TSC2-2
TSC1								
TSC2								
p-S6								
S6								
p-β-Catenin								
β-Catenin								
MITF								
PGC-1α								
TYR								
Actin								
ARA	-	-	+	+	-	-	+	+

SK-MEL-30
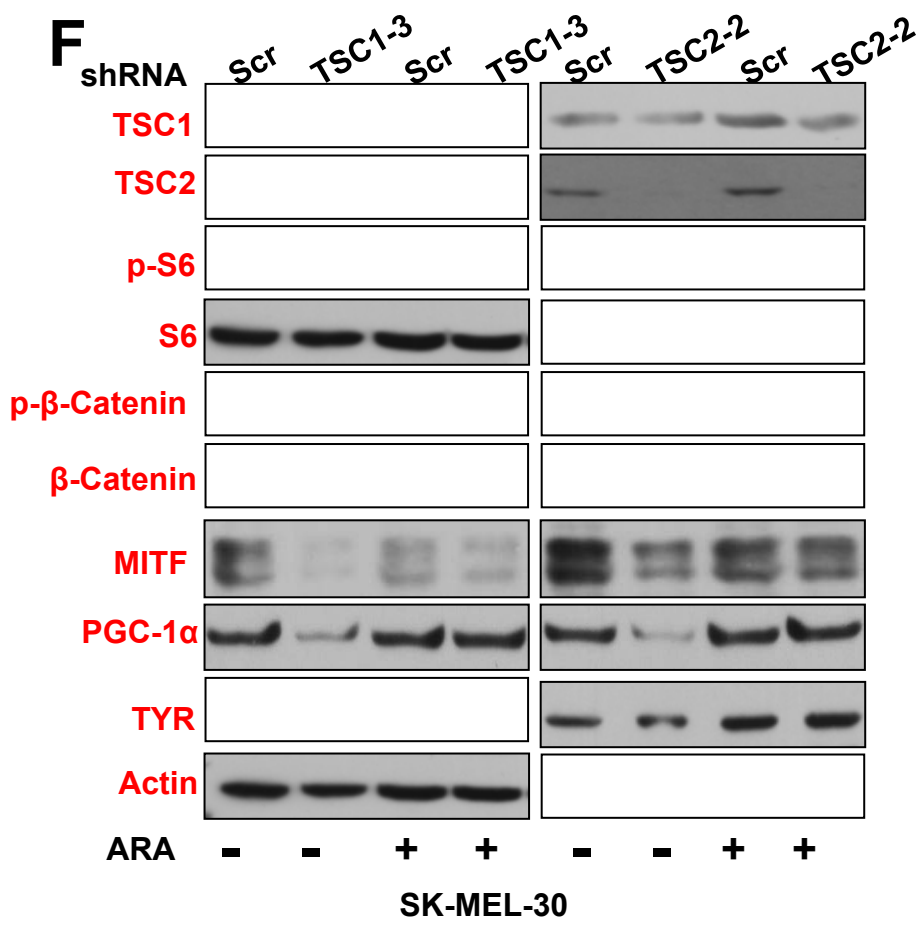
S6   β-Catenin   Actin

Run on same gel, **Actin** in the figure was used as a loading control

parallel gel 1

TSC2   p-S6   p-β-Catenin

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 2

TSC1 (left)

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 3

TSC1 (right)

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 4

MITF (left)   TYR (left)

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 5

MITF (right)

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 6

TYR (right)

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

Figure S5

Full unedited gel for Figure S5F

F

Blots were cut for different antibody staining and exposed on the same film

S5F:TSC1 (left)

S5F:TSC1 (right)

S5F:TSC2

Left   Right

S5F:MITF (left)

S5F:MITF (right)

S5F:p-S6

Left   Right

S5F:PGC1-α

S5F:S6

S5F:TYR (left)

S5F:TYR (right)

S5F:p-β-catenin

Left   Right

S5F:Actin

S5:β-catenin

Figure S5

Loading controls for parallel gels in figure S5F

parallel gel 1

S5F:Actin

parallel gel 2

S5F:Actin

parallel gel 3

S5F:Actin

parallel gel 4

S5F:Actin

parallel gel 5

S5F:Actin

parallel gel 6

S5F:Actin

K

shRNA	Scr	TSC1-3	Scr	TSC1-3	Scr	TSC2-2	Scr	TSC2-2
TSC1								
TSC2								
p-S6								
S6								
p-β-Catenin								
β-Catenin								
MITF								
PGC-1α								
TYR								
Actin								
BIO	−	−	+	+	−	−	+	+

SK-MEL-30

S6   β-Catenin   MITF   PGC-1α   TYR   Actin

Run on same gel, **Actin** in the figure was used as a loading control

parallel gel 1

TSC1 (left)

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 2

TSC1 (right)

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

parallel gel 3
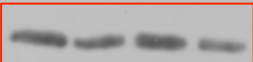
TSC2   p-S6   p-β-Catenin

Run on same gel, **Actin** was used as a loading control, (see uncropped gel image

Figure S5

K

S5K:TSC1
(Left)

S5K:TSC1
(right)

Full unedited gel for Figure S5K

S5K:TSC2

Left   Right

S5K:MITF

S5K:p-S6

Left   Right

S5K:PGC-1α

S5K:Actin

S5K:TYR

S5K:S6

S5K:Actin

S5K:p-β-catenin

Left   Right

S5K:β-catenin

Figure S5

Loading controls for parallel gels in figure S5K

parallel gel 1

S5K:Actin

parallel gel 2

S5K:Actin

parallel gel 3

S5K:Actin

Figure S6

Blots were cut for different antibody staining and exposed on the same film

A

S6A: TSC1

S6A: TSC2

S6A:p-S6
(Ser240/244)

S6A:p-S6
(Ser235/236)

S6A:S6

S6A:p-S6K1
(T229)

S6A:S6K1

S6A:p-AKT
(Thr308)

S6A:p-AKT
(Ser473)

S6A:AKT

S6A:p-S6K1
(T389)

Figure S6

Blots were cut for different antibodies and exposed on the same film

S6A:TYR

Full unedited gel for Figure S6A

S6A:p-GSK3β

S6A:GSK3β

S6A:p-4E-BP1
(Thr37/46)

S6A:p-β-catenin

S6A:4E-BP1

S6A:β-catenin

S6A:PGC1-a

S6A:Actin

S6A:MITF
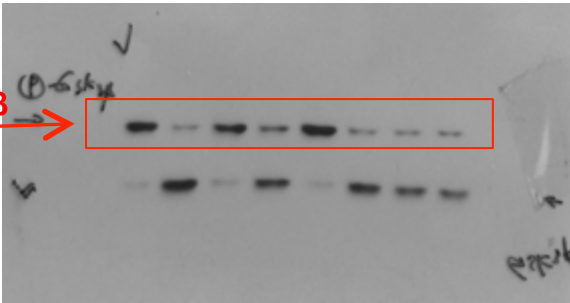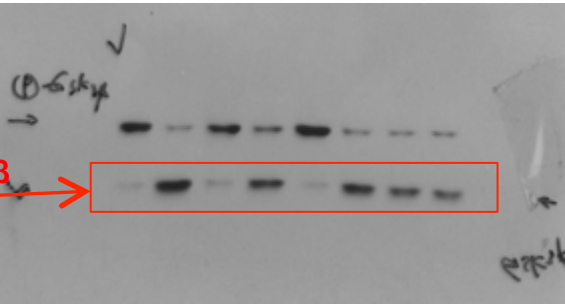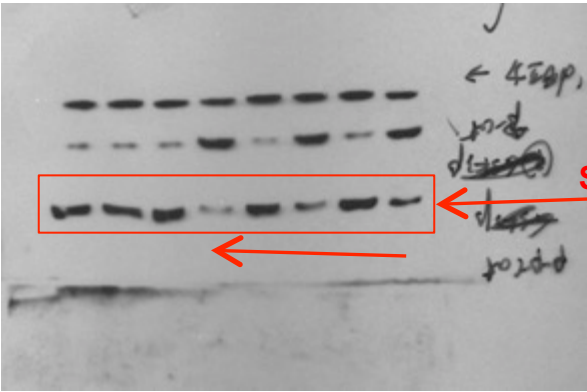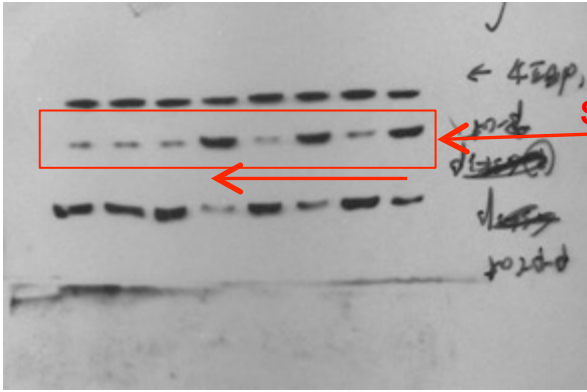
Figure S6

Loading controls for parallel gels in figure S6A

parallel gel 1
S6A: No extra loading control needed

parallel gel 2
S6A: No extra loading control needed

parallel gel 3
S6A: No extra loading control needed