Supplement to:
PERK Signaling Regulates Extracellular Proteostasis of an Amyloidogenic Protein During Endoplasmic Reticulum Stress

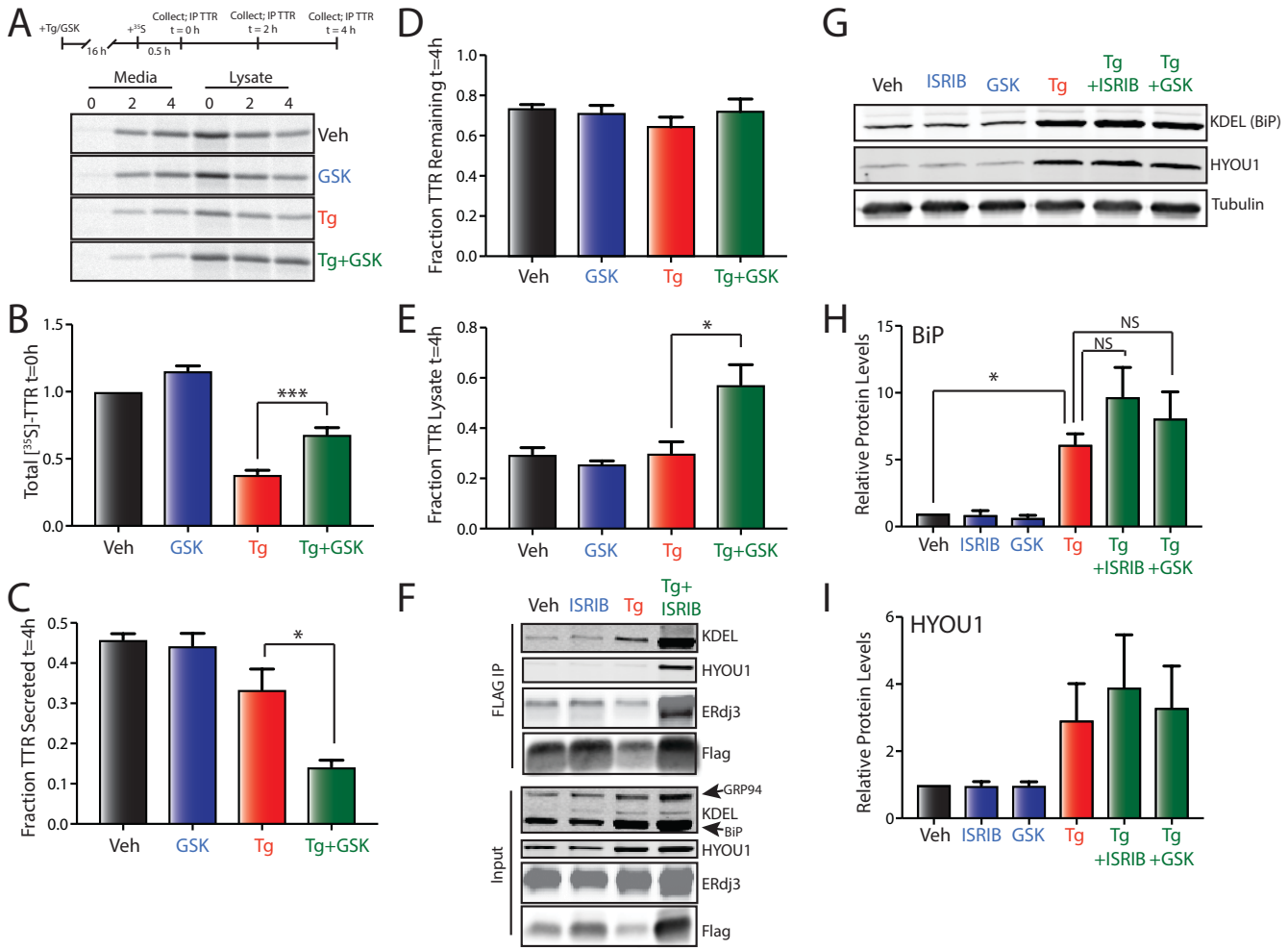Isabelle C. Romine and R. Luke Wiseman*

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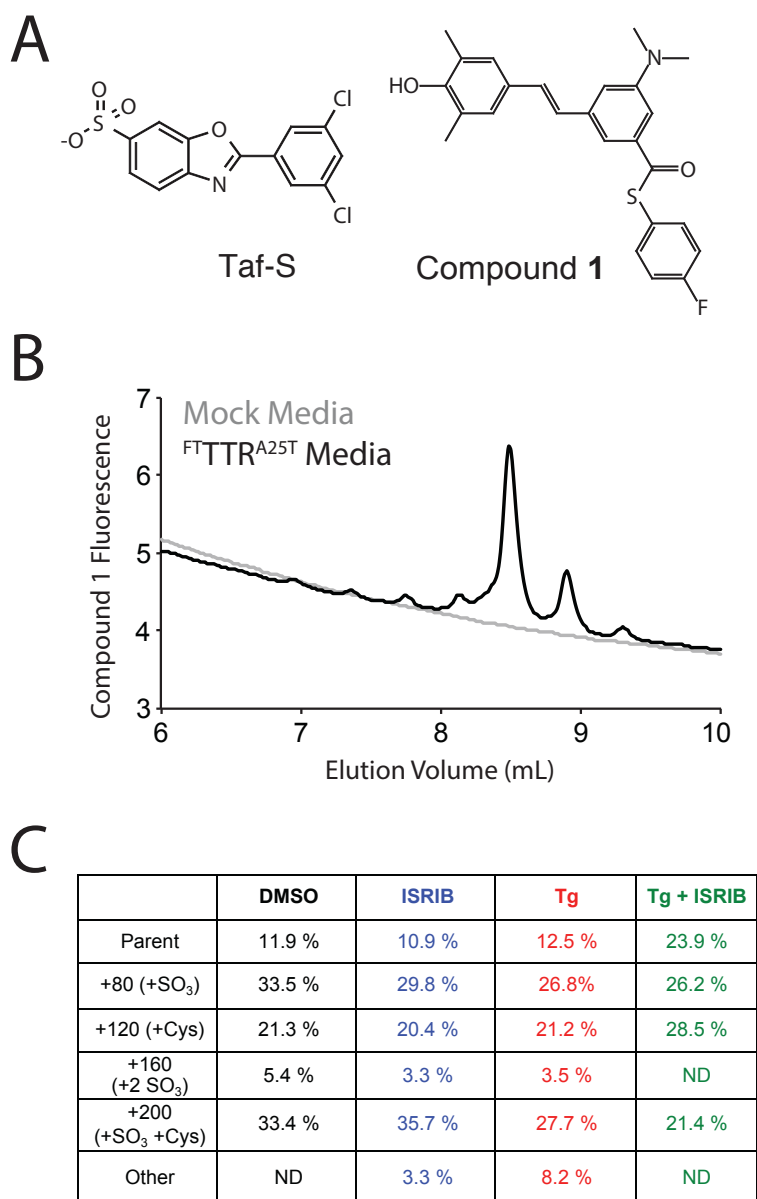Running Title: PERK regulates extracellular proteostasis

A +Tg/GSK ⊢16 h⊣ +^{35}S ⊢0.5 h⊣ Collect; IP TTR t = 0 h   Collect; IP TTR t = 2 h   Collect; IP TTR t = 4 h

Media | Lysate
0 2 4 | 0 2 4

Veh
GSK
Tg
Tg+GSK

B
Total [^{35}S]-TTR t=0h
1.5
1.0
0.5
0.0
Veh GSK Tg Tg+GSK
***

C
Fraction TTR Secreted t=4h
0.5
0.4
0.3
0.2
0.1
0.0
Veh GSK Tg Tg+GSK
*

D
Fraction TTR Remaining t=4h
1.0
0.8
0.6
0.4
0.2
0.0
Veh GSK Tg Tg+GSK

E
Fraction TTR Lysate t=4h
0.8
0.6
0.4
0.2
0.0
Veh GSK Tg Tg+GSK
*

F
Veh ISRIB Tg Tg+ISRIB
FLAG IP
KDEL
HYOU1
ERdj3
Flag
Input
GRP94
KDEL
BiP
HYOU1
ERdj3
Flag

G
Veh ISRIB GSK Tg Tg +ISRIB Tg +GSK
KDEL (BiP)
HYOU1
Tubulin

H
BiP
Relative Protein Levels
15
10
5
0
Veh ISRIB GSK Tg Tg +ISRIB Tg +GSK
* NS NS

I
HYOU1
Relative Protein Levels
6
4
2
0
Veh ISRIB GSK Tg Tg +ISRIB Tg +GSK

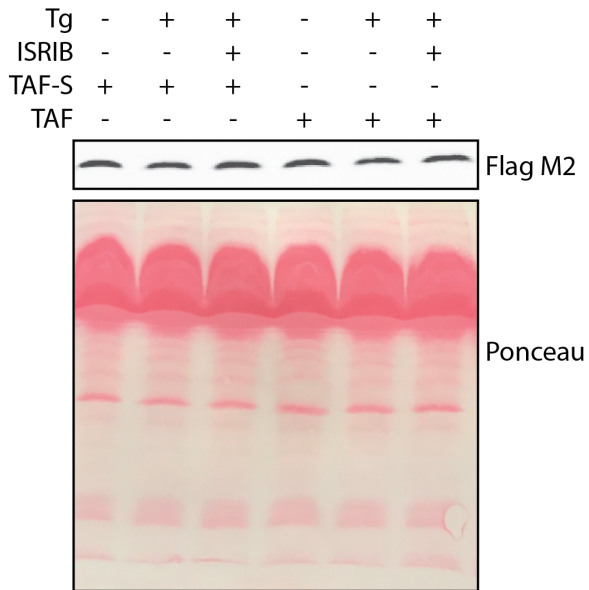**Supplemental Figure 1 (Relevant to Figure 1)**

A. Representative autoradiogram of [^{35}S]-labeled ^{FT}TTR^{A25T} in lysates and media collected from HEK293T cells pretreated for 16 h with thapsigargin (Tg; 500 nM) and/or GSK (300 nM). The protocol for the experiment is shown above.

B. Graph showing the total amount of [^{35}S]-labeled ^{FT}TTR^{A25T} at t = 0 h in HEK293T cells pretreated for 16 h with thapsigargin (Tg; 500 nM) and/or GSK (300 nM). Data are shown normalized to vehicle-treated cells. Error bars show SEM for n=3 independent experiments. A representative autoradiogram is shown in **Fig. S1A**.

C. Graph showing the fraction [^{35}S]-labeled ^{FT}TTR^{A25T} secreted at t = 4 h in HEK293T cells pretreated for 16 h with thapsigargin (Tg; 500 nM) and/or GSK (300 nM). Fraction secreted was calculated as described in **Materials and Methods**. Error bars show SEM for n=3 independent experiments. A representative autoradiogram is shown in **Fig. S1A**.

D. Graph showing the fraction [^{35}S]-labeled ^{FT}TTR^{A25T} remaining at t = 4 h in HEK293T cells pretreated for 16 h with thapsigargin (Tg; 500 nM) and/or GSK (300 nM). Fraction remaining was calculated as described in **Materials and Methods**. Error bars show SEM for n=3 independent experiments. A representative autoradiogram is shown in **Fig. S1A**.

E. Graph showing the fraction [^{35}S]-labeled ^{FT}TTR^{A25T} in lysates at t = 4 h in HEK293T cells pretreated for 16 h with thapsigargin (Tg; 500 nM) and/or GSK (300 nM). Fraction lysate was calculated as described in **Materials and Methods**. Error bars show SEM for n=3 independent experiments. A representative autoradiogram is shown in **Fig. S1A**.

F. Representative immunoblot showing anti-FLAG IPs and inputs from lysates prepared from HEK293T cells expressing ^{FT}TTR^{A25T} and pretreated for 16 h with Tg (500 nM) and/or ISRIB (200 nM)
*indicates p<0.05; **indicates p<0.01 for a paired two-tailed t-test.

G. Representative immunoblot of lysates prepared from HEK293T cells pretreated for 16 hours with Tg (500 nM), ISRIB (200 nM), and/or GSK (300 nM), as indicated.

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H. Graph showing quantifications for BiP from immunoblots as shown in **Fig. 1G**. Data are shown normalized to vehicle-treated cells. Error bars show n=3. *indicates $p<0.05$.
I. Graph showing quantifications for HYOU1 from immunoblots as shown in **Fig. 1G**. Data are shown normalized to vehicle-treated cells. Error bars show n=3. *indicates $p<0.05$.
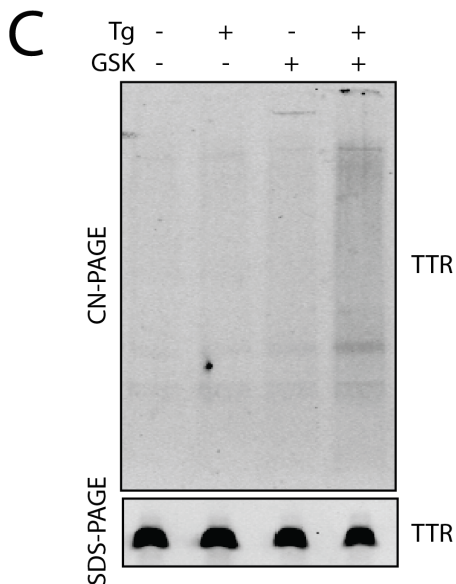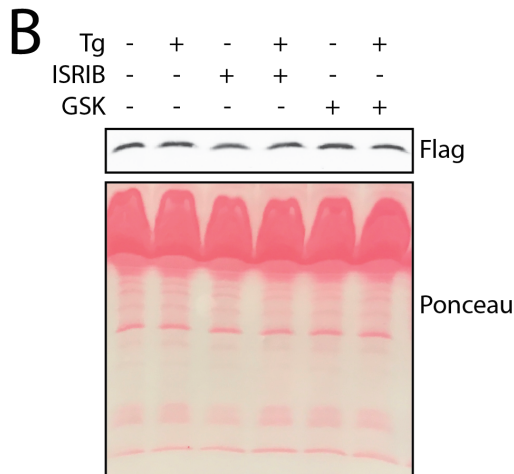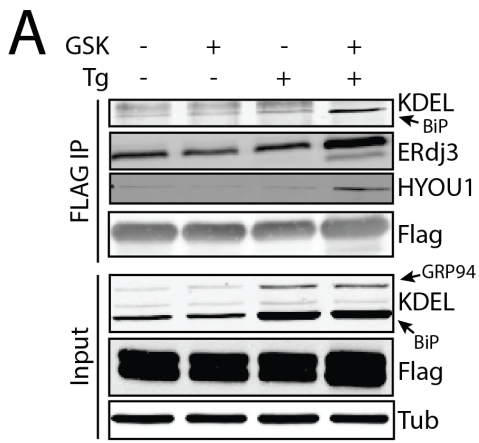
A

Taf-S

Compound **1**

B

C

	DMSO	ISRIB	Tg	Tg + ISRIB
Parent	11.9 %	10.9 %	12.5 %	23.9 %
+80 (+SO$_3$)	33.5 %	29.8 %	26.8%	26.2 %
+120 (+Cys)	21.3 %	20.4 %	21.2 %	28.5 %
+160 (+2 SO$_3$)	5.4 %	3.3 %	3.5 %	ND
+200 (+SO$_3$ +Cys)	33.4 %	35.7 %	27.7 %	21.4 %
Other	ND	3.3 %	8.2 %	ND

Supplemental Figure 2 (Relevant to Figure 2)
A. Structures of tafamidis-sulfonate (Taf-S) and the fluorogenic TTR ligand compound **1**.
B. Chromatogram of conditioned media prepared in the presence of Taf-S (10 µM) on mock-transfected HEK293T cells or HEK293T cells expressing $^{FT}TTR^{A25T}$. $^{FT}TTR^{A25T}$ tetramers were separated on anion exchange chromatography using the UPLC system and visualized by compound **1** fluorescence.
C. Table showing the relative populations of unmodified and posttranslationally modified $^{FT}TTR^{A25T}$ in conditioned media prepared on HEK293T cells treated for 16 h with Tg (500 nM) and/or ISRIB (200 nM). Please see *Materials and Methods* for further description of this experiment.

Tg	-	+	+	-	+	+
ISRIB	-	-	+	-	-	+
TAF-S	+	+	+	-	-	-
TAF	-	-	-	+	+	+

Flag M2

Ponceau

Supplemental Figure 3 (Relevant to Figure 3)
Representative immunoblot of $^{FT}TTR^{A25T}$ in conditioned media prepared in the presence of Taf-S (10 µM) or Taf (10 µM) on HEK293T cells treated for 16 h with Tg (500 nM) and/or ISRIB (200 nM). A ponceau stain of the immunoblot is shown as a control.

A

	GSK	−	+	−	+
	Tg	−	−	+	+

FLAG IP:
KDEL / BiP
ERdj3
HYOU1
Flag

Input:
GRP94 / KDEL / BiP
Flag
Tub

B

Tg	−	+	−	+	−	+
ISRIB	−	−	+	+	−	−
GSK	−	−	−	−	+	+

Flag
Ponceau

C

Tg	−	+	−	+
GSK	−	−	+	+

CN-PAGE — TTR
SDS-PAGE — TTR

Supplemental Figure 4 (Relevant to Figure 4).
  A. Representative immunoblot showing anti-FLAG IPs and inputs from lysates prepared from HEK293Tcells expressing $^{FT}TTR^{WT}$ and pretreated for 16 h with Tg (500 nM) and/or GSK (300 nM).
  B. Representative immunoblot of $^{FT}TTR^{WT}$ in conditioned media prepared in the presence of Taf-S (10 μM) on HEK293T cells treated for 16 h with Tg (500 nM), ISRIB (200 nM), or GSK (300 nM). A ponceau stain of the immunoblot is shown as a control.
  C. Representative CN-PAGE/immunoblot of $^{FT}TTR^{WT}$ aggregates in conditioned media prepared in the presence of Taf-S (10 μM) on HEK293T cells treated for 16 h with Tg (500 nM) and/or GSK (300 nM). A SDS-PAGE/immunoblot showing total $^{FT}TTR^{WT}$ is shown as a control.

Figure 1A Raw Data (Whole Gel Exposure)

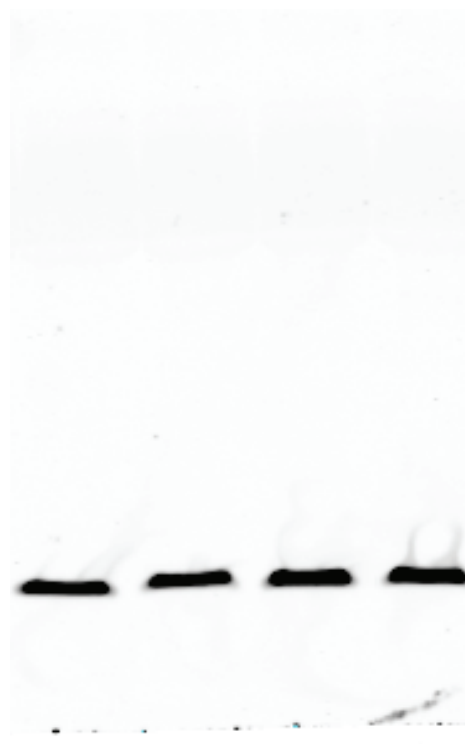Vehicle
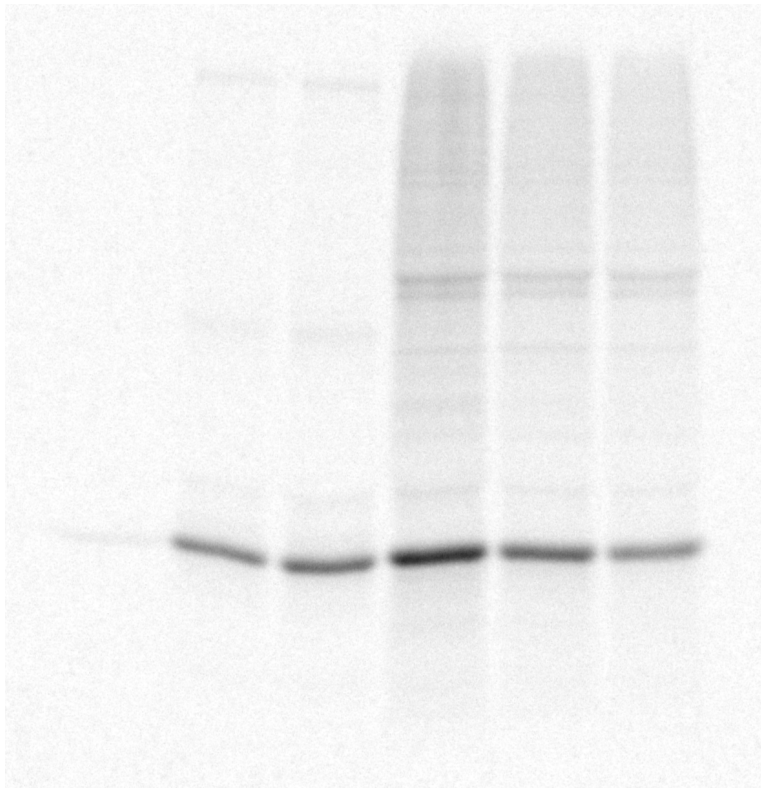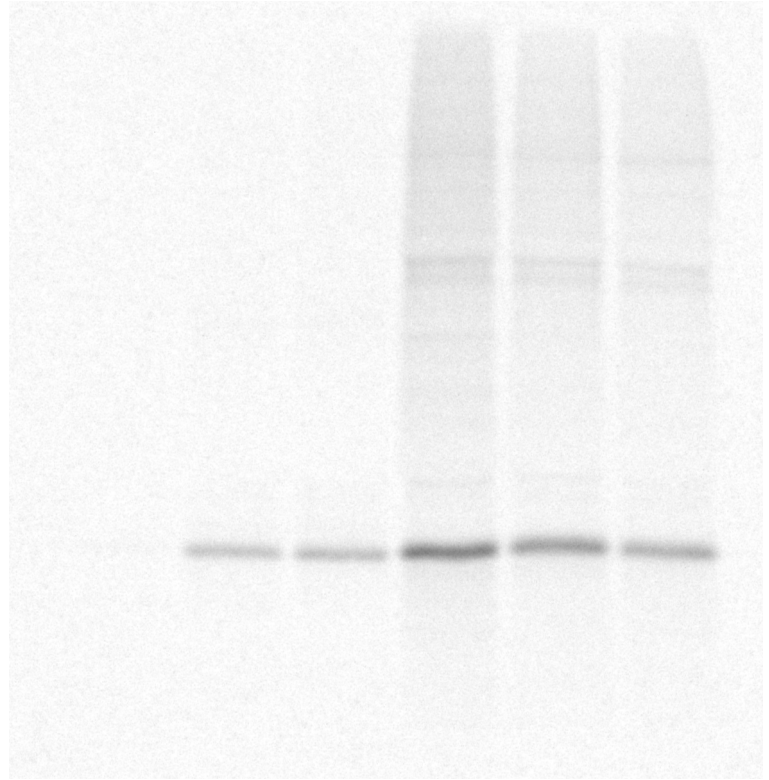
Thapsigargin

ISRIB
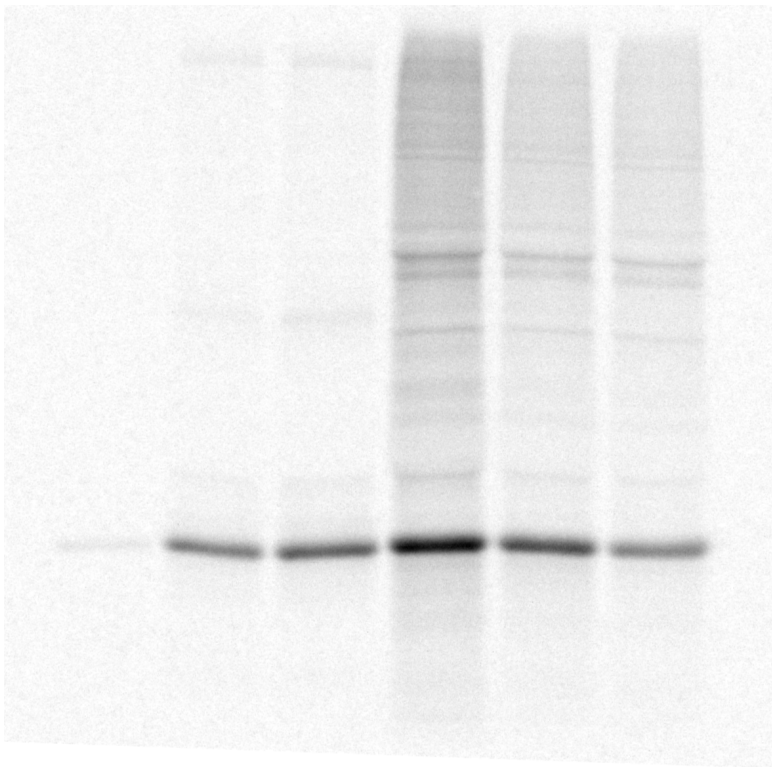
Thapsigargin and ISRIB
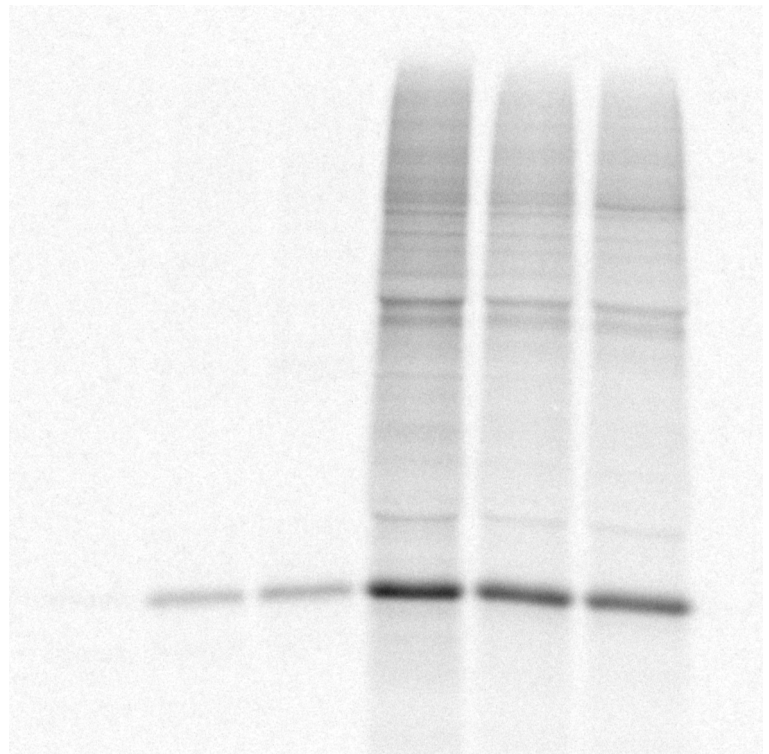
Figure 2E Raw Data (Whole Gels)

Veh   Tg   ISRIB   Tg+ ISRIB
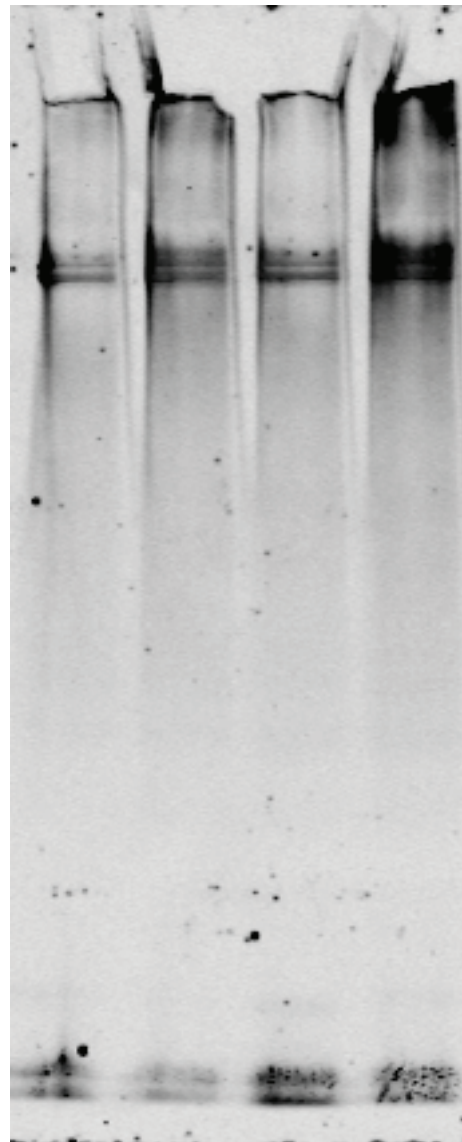
CN-PAGE
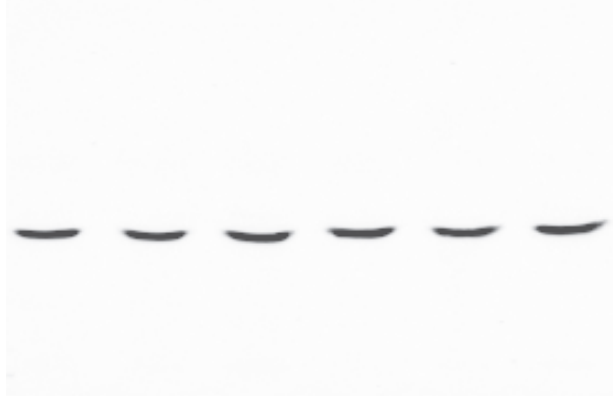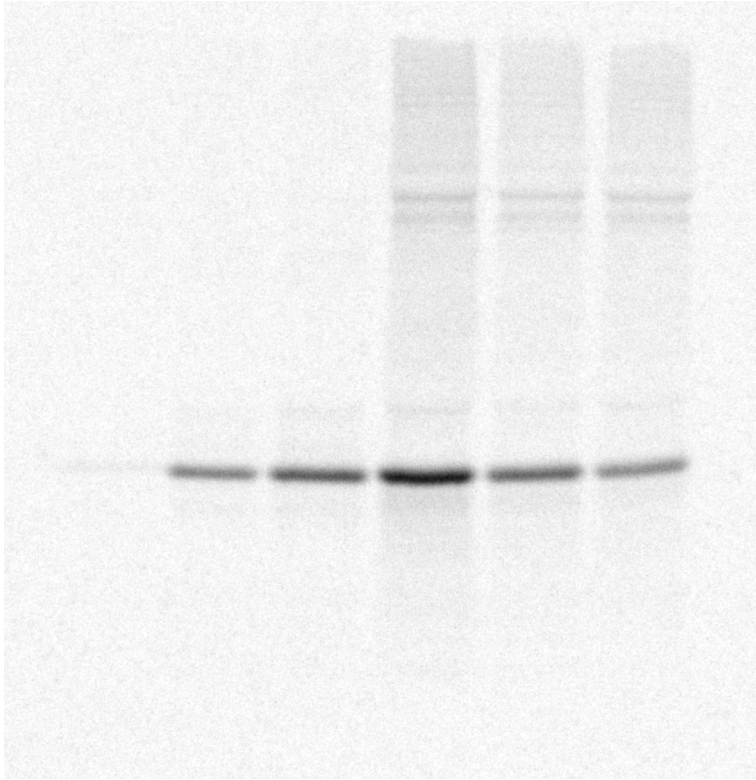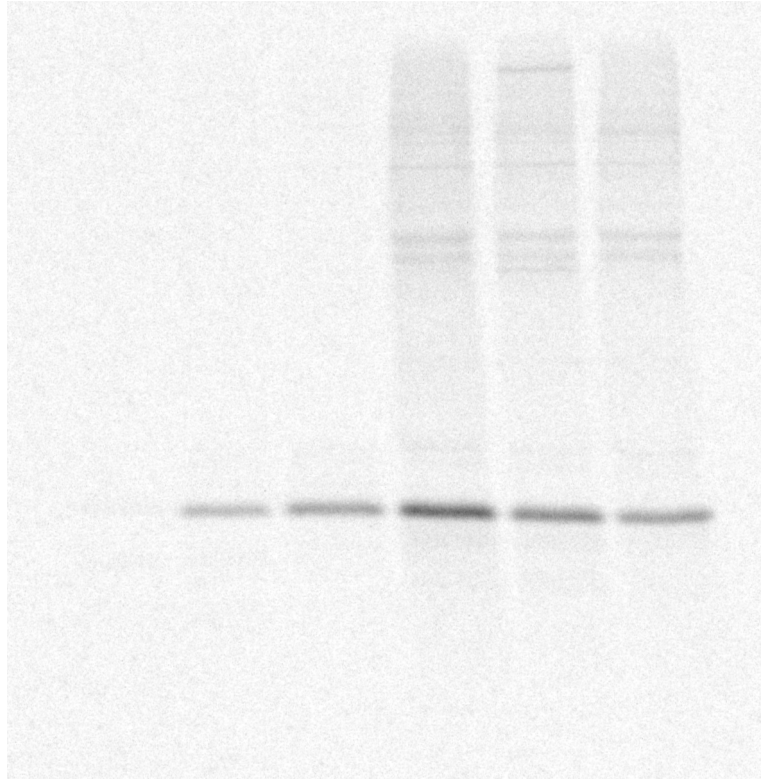IB:FLAG

SDS-PAGE
IB:FLAG

Figure 3C Raw Data (Whole Gels)
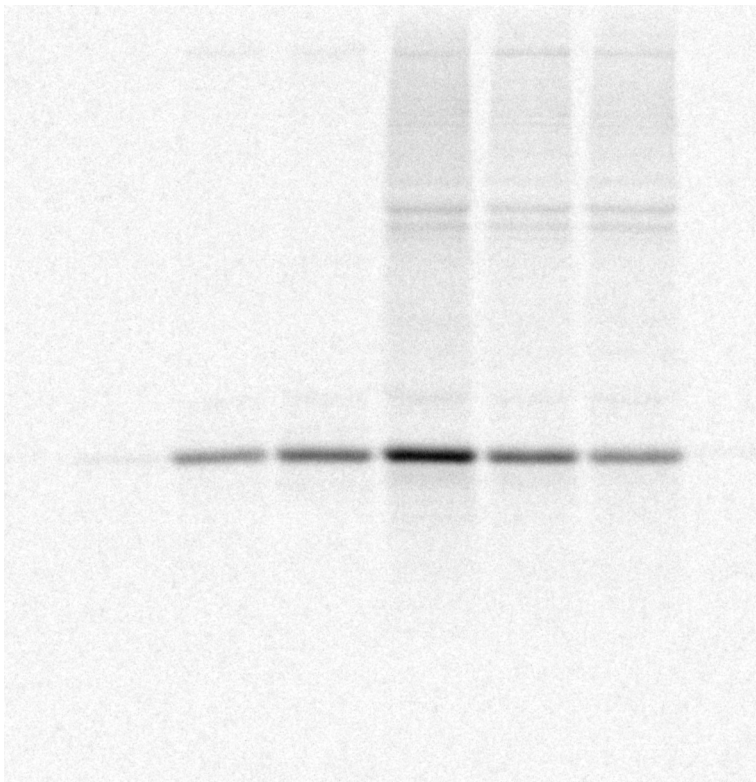
Figure S1A Raw Data (Whole Gel Exposure)

Vehicle

Thapsigargin

GSK
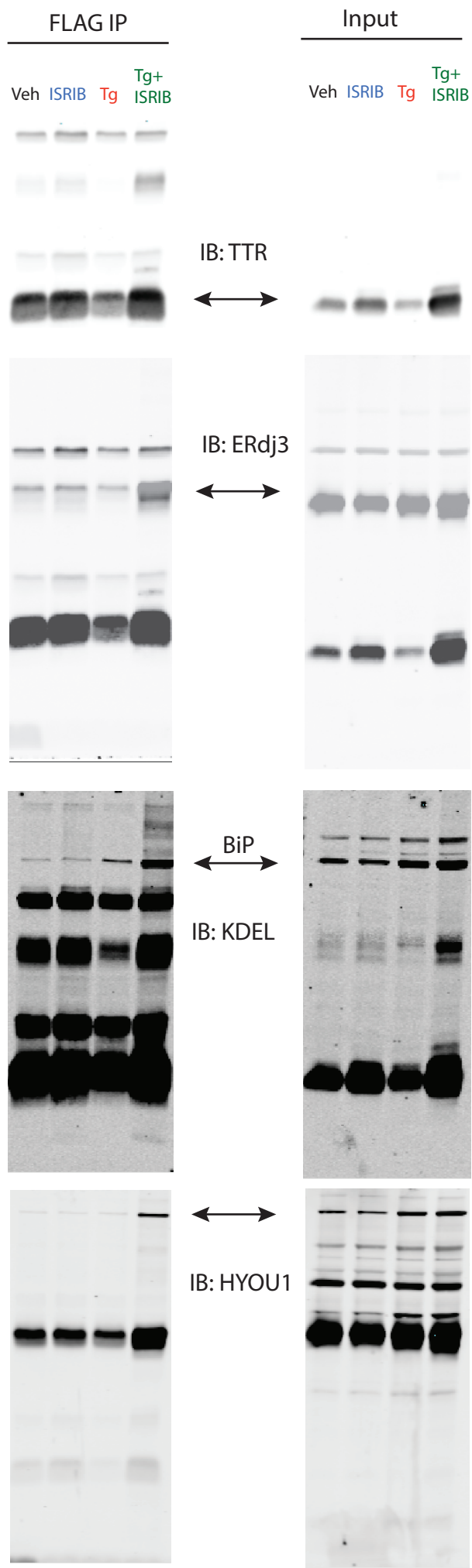

Thapsigargin and GSK
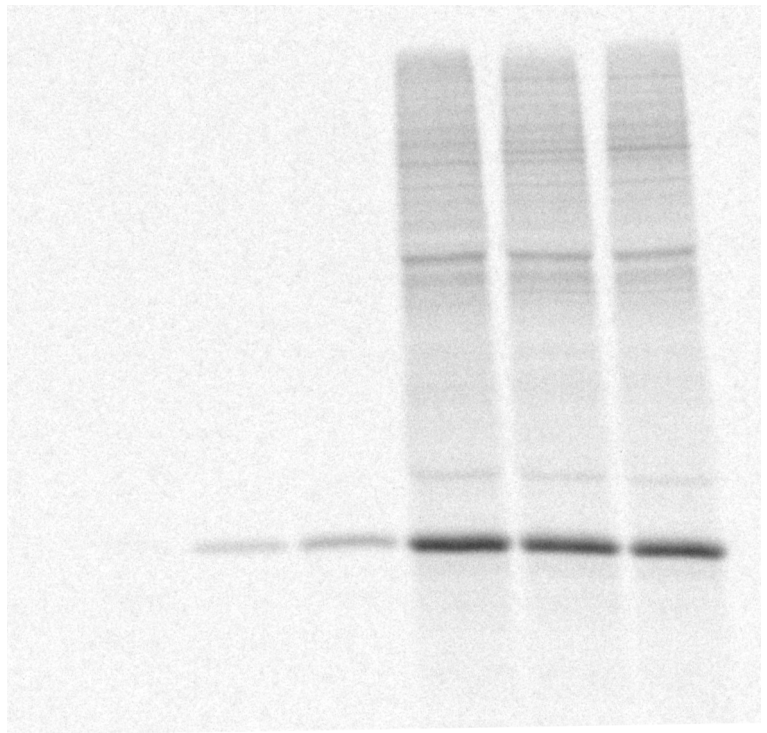
Figure S1F Raw Data (Whole Gels

Figure S3 Raw Data (Whole Gels)

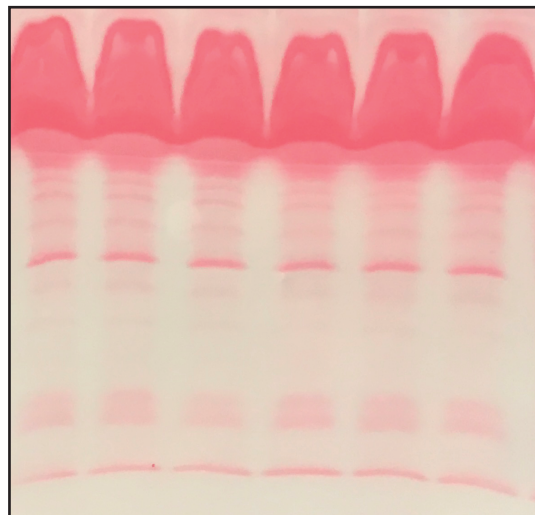| Tg	-	+	+	-	+	+
ISRIB	-	-	+	-	-	+
TAF-S	+	+	+	-	-	-
TAF	-	-	-	+	+	+
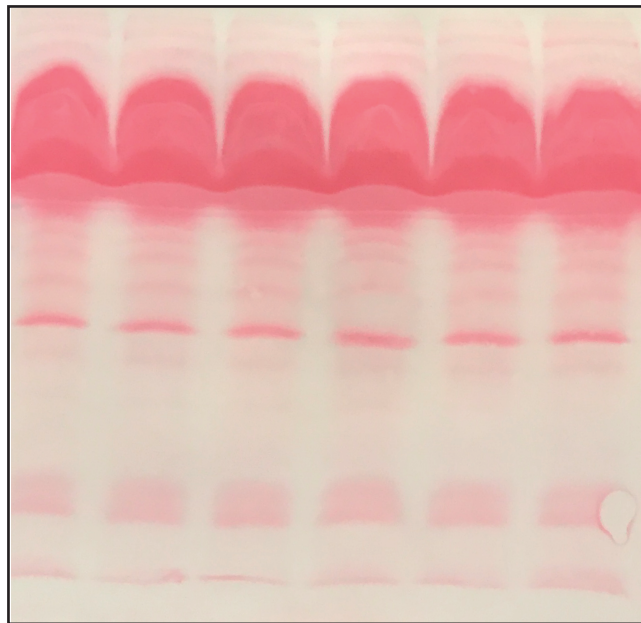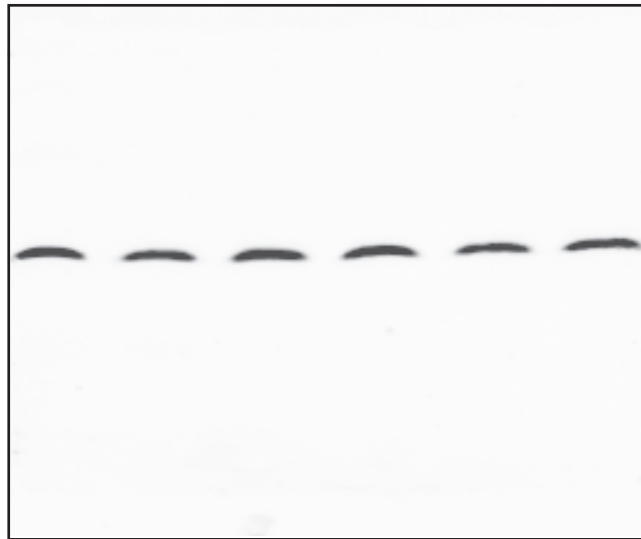
Figure S4A Raw Data (Whole Gels)

FLAG IP

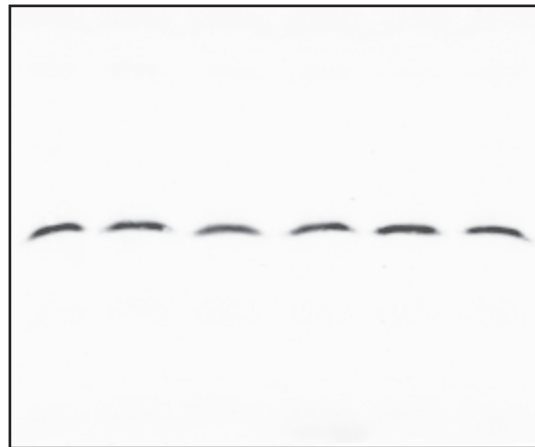|				
GSK	-	+	-	+
Tg	-	-	+	+

IB: KDEL

IB: ERDJ3

IB: HYOU1

IB: FLAG
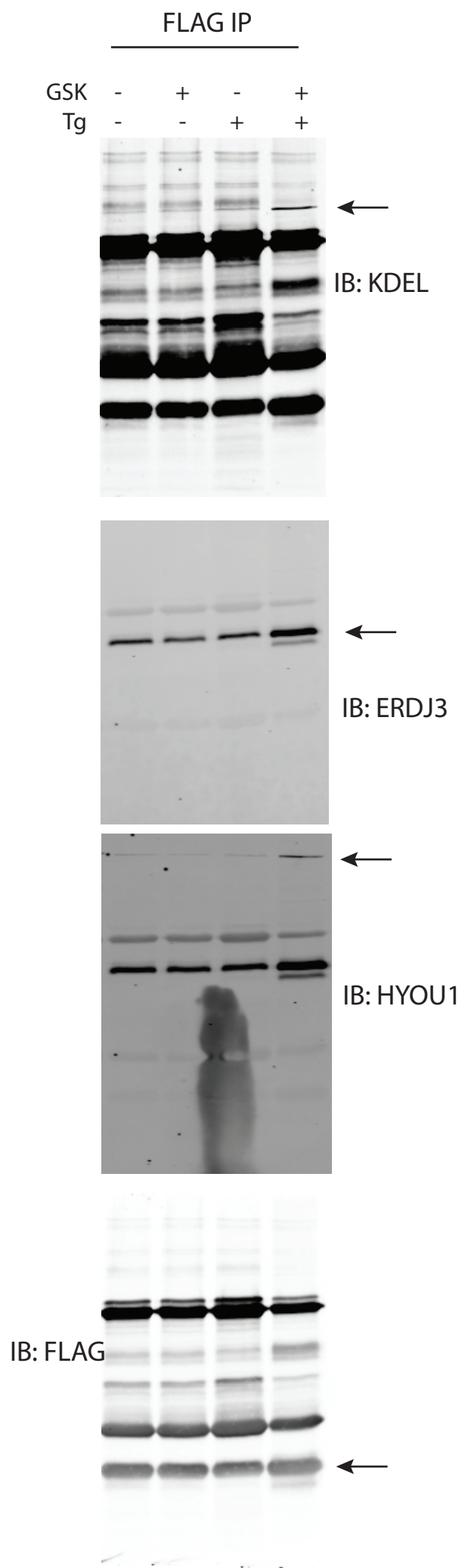
Figure S4B Raw Data (Whole Gels)

Figure S4C Raw Data (Whole Gels)