Supplemental Materials

Molecular Biology of the Cell

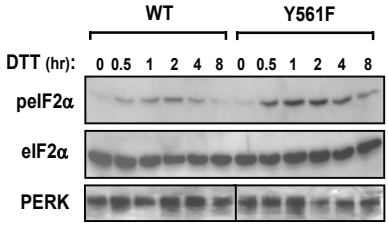
Yamani et al.

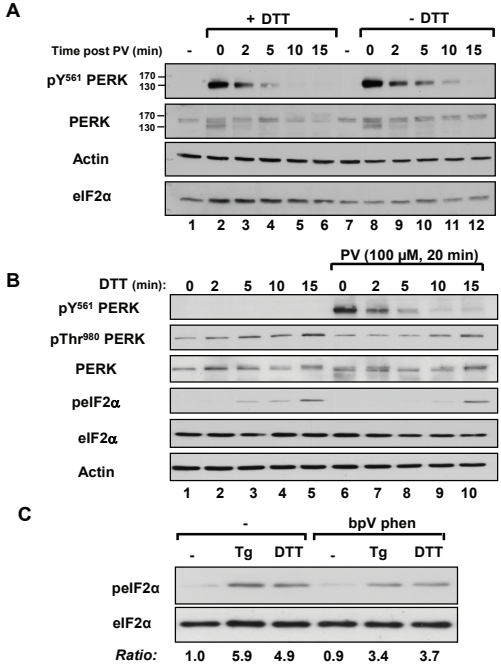
Figure S1. PERK Y561F mutation enhances ER stress-induced PERK-mediated peIF2αS^{51}. *PERK*$^{-/-}$ MEFs transiently expressing PERK WT or Y561F mutant were treated 48 hr later with 1 mM DTT for indicated times. Total cell lysates (50 µg protein) were subjected to immunoblotting using indicated antibodies. An inserted black line on PERK immunoblot specifies that PERK WT and Y561F samples were simultaneously processed, but from two different gels (n=3).

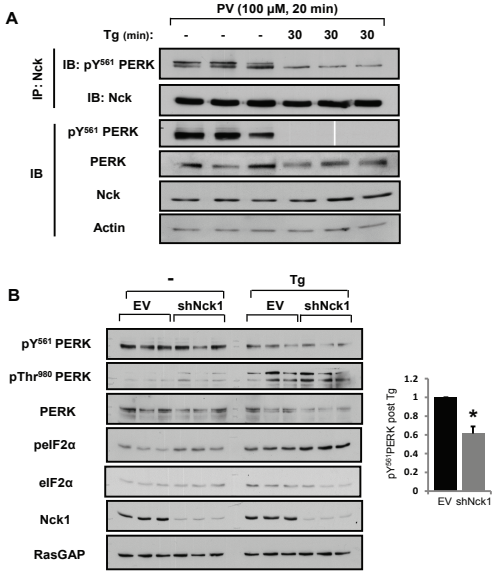
Figure S2. PERK phosphorylation at Y^{561} delays PERK activation and signaling in MIN6 cells. (A) MIN6 cells untreated (lanes 1 and 7) or pretreated with 100 µM PV for 20 min (lanes 2-6 and 8-12) were washed with PBS and then treated with 10 mM DTT (lanes 2-6) or not (lanes 8-12) for indicated times. Total cell lysates (50 µg protein) were subjected to immunoblotting with indicated antibodies. (B) MIN6 cells untreated (lanes 1-5) or pretreated with 100 µM PV for 20 min (lanes 6-10) were washed with PBS and then treated with 10 mM DTT for indicated times. Total cell lysates (50 µg protein) were subjected to immunoblotting with indicated antibodies. (C) Cos-1 cells untreated (-) or pretreated with pbVphen (100 µM, 10 min) were washed with PBS and then untreated (-) or treated with Tg (0.5 µM) or DTT (0.5 mM) for 20 min. Total cell lysates (50 µg protein) were subjected to immunoblotting using indicated antibodies. Ratios of peIF2αS^{51}/eIF2α established by densitometry quantification upon normalisation to untreated cells are reported. Data are representative of 3 independent experiments.

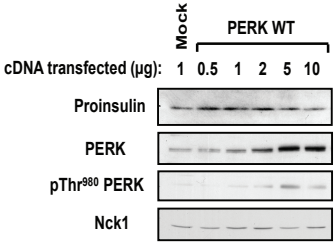
Figure S3. Nck interacts with PERK and modulates PERK activity. (A) MIN6 cells were pretreated with 100 µM PV for 20 min, then washed and either left untreated (-) or treated with 1 µM Tg for 30 min. Total cell lysates (500 µg protein) were subjected to Nck immunoprecipitation and Nck immunoprecipitates were subjected to immunoblotting with indicated antibodies. Total cell lysates (50 µg protein) were probed by immunoblotting with indicated antibodies. (B) LMP-EV and LMP-shNck1 MIN6 cells were pretreated with 100 µM PV for 20 min, then washed with PBS and either left untreated (-) or treated with 1 µM Tg for 10 min. Total cell lysates (50 µg protein) were subjected to immunoblotting using indicated antibodies. Bar chart shows the ratio of pY^{561} PERK determined by densitometry in Tg-treated compared to untreated cells. Data are mean ± SEM (*$P<0.007$) and are representative of 3 independent experiments.

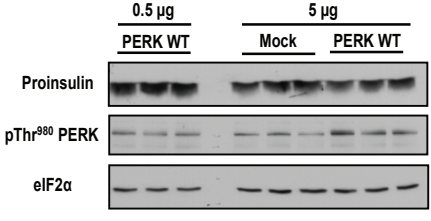
Figure S4. Overexpression of PERK modulates proinsulin levels in MIN6 cells. Total cell lysates (50 µg protein) from MIN6 cells transiently transfected with empty vector (Mock) or increasing amounts of plasmid encoding PERK WT were subjected to immunoblotting with indicated antibodies. Bar chart shows densitometry of proinsulin levels. Data are mean ± SEM (*$P<0.001$). Data are representative of 3 independent experiments.

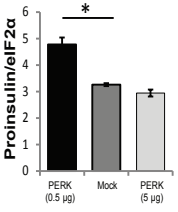

WT
Y561F
DTT (hr):
0 0.5 1 2 4 8 0 0.5 1 2 4 8
peIF2α
eIF2α
PERK


A
+ DTT
- DTT
Time post PV (min)
- 0 2 5 10 15 - 0 2 5 10 15
pY561 PERK
170
130
PERK
170
130
Actin
eIF2α
1 2 3 4 5 6 7 8 9 10 11 12
B
PV (100 μM, 20 min)
DTT (min):
0 2 5 10 15 0 2 5 10 15
pY561 PERK
pThr980 PERK
PERK
peIF2α
eIF2α
Actin
1 2 3 4 5 6 7 8 9 10
C
-
bpV phen
- Tg DTT - Tg DTT
peIF2α
eIF2α
Ratio:
1.0 5.9 4.9 0.9 3.4 3.7


A
PV (100 μM, 20 min)
Tg (min):
- - - 30 30 30
IP: Nck
IB: pY561 PERK
IB: Nck
IB
pY561 PERK
PERK
Nck
Actin
B
-
Tg
EV
shNck1
EV
shNck1
pY561 PERK
pThr980 PERK
PERK
peIF2α
eIF2α
Nck1
RasGAP
pY561PERK post Tg
1.2
1
0.8
0.6
0.4
0.2
0
*
EV shNck1


Mock
PERK WT
cDNA transfected (μg): 1 0.5 1 2 5 10
Proinsulin
PERK
pThr980 PERK
Nck1


0.5 μg
5 μg
PERK WT
Mock
PERK WT
Proinsulin
pThr980 PERK
eIF2α


*
Proinsulin/eIF2α
0
1
2
3
4
5
6
PERK (0.5 μg)
Mock
PERK (5 μg)