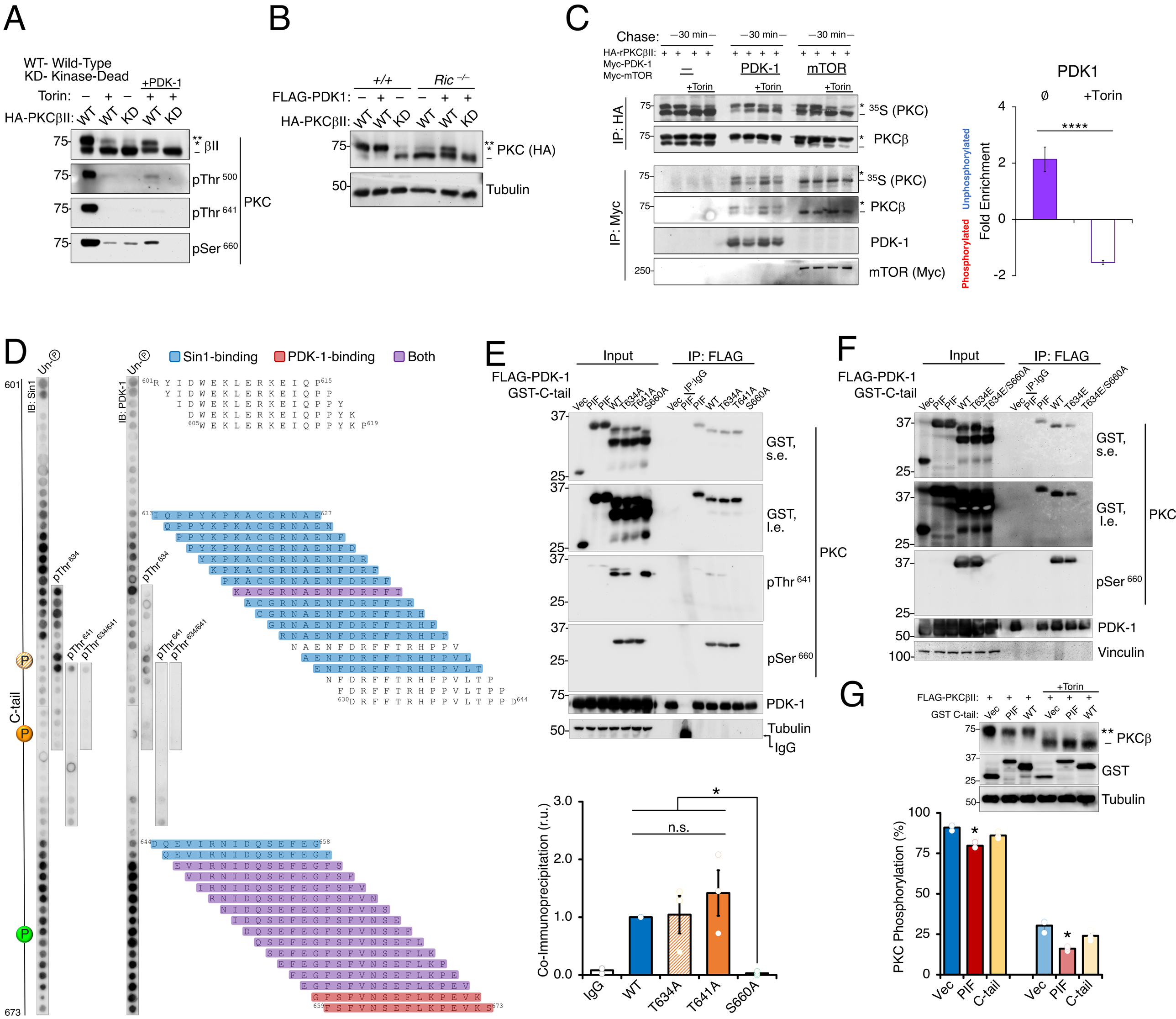
A
WT- Wild-Type
KD- Kinase-Dead
+PDK-1
Torin: – + – + +
HA-PKCβII: WT WT KD WT KD
75
βII
pThr 500
pThr 641
pSer 660
PKC
B
+/+
Ric −/−
FLAG-PDK1: – + – – + +
HA-PKCβII: WT WT KD WT WT KD
75
PKC (HA)
50
Tubulin
C
Chase: −30 min− −30 min− −30 min−
HA-rPKCβII: + + + + + + + + + + + +
Myc-PDK-1 —
Myc-mTOR PDK-1 mTOR
+Torin +Torin +Torin
IP: HA
IP: Myc
75
35S (PKC)
PKCβ
PDK-1
250
mTOR (Myc)
PDK1
Ø +Torin
****
Fold Enrichment
Unphosphorylated
Phosphorylated
D
Un-P
IB: Sin1
IB: PDK-1
Sin1-binding
PDK-1-binding
Both
601
673
C-tail
pThr 634
pThr 641
pThr 634/641
E
Input
IP: FLAG
FLAG-PDK-1
GST-C-tail
Vec PIF PIF WT T634A T641A S660A
IP:IgG
GST, s.e.
GST, l.e.
pThr 641
pSer 660
PKC
PDK-1
Tubulin
IgG
Co-Immunoprecipitation (r.u.)
n.s.
IgG WT T634A T641A S660A
F
T634E T634E/S660A
PDK-1
Vinculin
G
FLAG-PKCβII:
GST C-tail:
Vec PIF WT Vec PIF WT
PKCβ
GST
Tubulin
PKC Phosphorylation (%)
Vec PIF C-tail Vec PIF C-tail