Supplementary Figure Legends

Supplementary Figure. 1. Quantitative reverse transcription PCR (qRT-PCR) analysis indicated that TNF-α mRNA was increased in the kidneys from postnatal day 7 *Pkd1*$^{flox/flox}$*:Ksp-Cre* mice (Flox) and postnatal day 28 *Pkd1*$^{nl/nl}$ mice compared to that in age matched *Pkd1* wild type kidneys. Each sample was run in triplicate in every experiment, and each experiment was repeated three times. The expression level of TNF-α was normalized to the expression level of actin. $p < 0.01$.

Supplementary Figure. 2. (**A**) The expression of cIAP1, RIPK1 and TRADD in complex I and the expression of caspase 8 and FADD in pro-death complex II were increased in postnatal *Pkd1* homozygous PN24 cells compared to that in postnatal *Pkd1* heterozygous PH2 cells. The expression of the upregulated proteins in the above cells was quantified from three independent immunoblots and was presented as the relative protein expression level standardized to actin in the bottom panel. $p < 0.05$. (**B** and **C**) The expression of cIAP1 and RIPK1 in complex I, the expression of FADD but not caspase 8 in pro-death complex II and the expression of FLIP were increased in kidneys from postnatal day 7 *Pkd1*$^{flox/flox}$*:Ksp-Cre* mice (Flox) and postnatal day 28 *Pkd1*$^{nl/nl}$ mice compared to that in age matched *Pkd1* wild type kidneys, respectively. The upregulation of proteins in *Pkd1* mutant kidneys was quantified from three independent immunoblots and was presented as the relative protein expression level standardized to actin in the bottom panel. $p < 0.05$ or 0.01.

Supplementary Figure. 3. The components of complex I and complex II did not response to TNF-α treatment in *Pkd1* wild type and null MEK cells.

Supplementary Figure. 4. (**A**) TNF-α treatment did not induce apoptosis in either *Pkd1* wild type or null MEK cells even at high concentration (200 ng/ml) by TUNEL assay. Scale bar, 50 μm. (**B**)

Treatment with TNF-α (50 ng/ml) plus NF-κB inhibitor, SN50 (18 or 36 μm), significantly decreased the viability indicated by methylene blue staining of *Pkd1* null MEK cells. However, TNF-α alone does not accelerate cell death

Supplementary Figure. 5. The TUNEL assay showed that Smac-mimetic significantly increased cystic epithelial cell death in *Pkd1* conditional knockout mouse kidneys at PN7 compared to age-matched DMSO treated controls. Scale bar on left panels, 20 μm. Scale bar on middle panels, 100 μm. Treatment with Smac-mimetic or DMSO did not induce apoptosis in *Pkd1* wild type mouse kidneys at PN7. Scale bar on right panels, 100 μm.

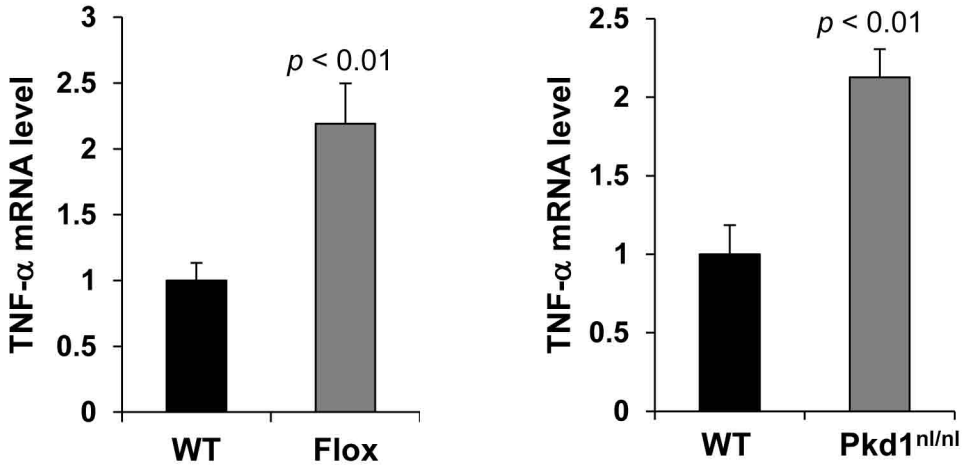
Supplementary Figure. 6. TUNEL assay showed that Smac-mimetic significantly increased death of cyst renal epithelial cells in P28 $Pkd1^{nl/nl}$ kidneys compared to age matched DMSO treated kidneys. Scale bar on left panels, 20 μm. Scale bar on middle panels, 100 μm. Treatment with Smac-mimetic or DMSO did not induce apoptosis in *Pkd1* wild type mouse kidneys at PN28. Scale bar right panels, 100 μm.

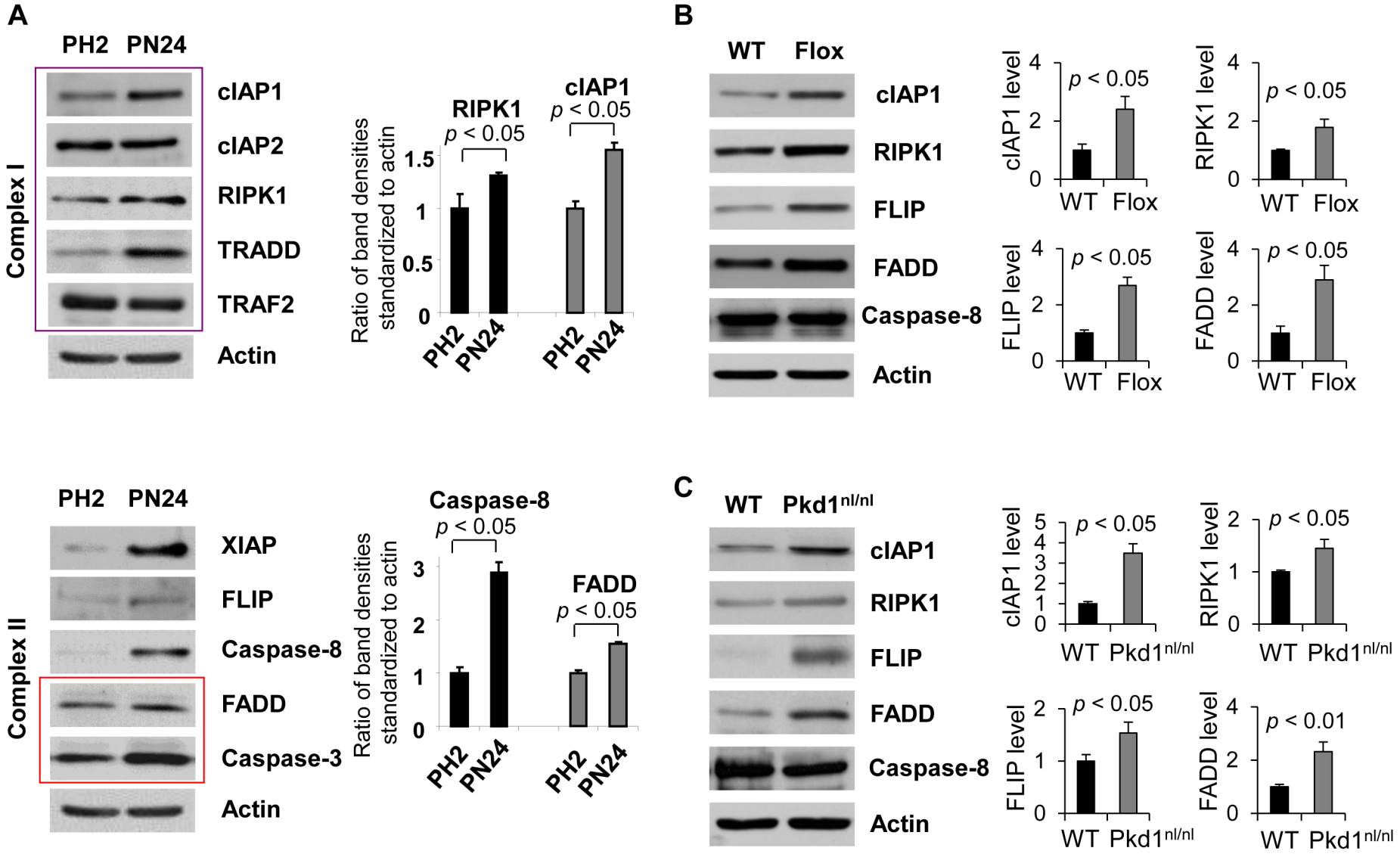
Supplementary Figure. 7. Smac-mimetic (GT13072) induced extremely rapid degradation of endogenous cIAP1, but had no effect on the expression of other components in complex I and II in *Pkd1* null renal epithelial cells.

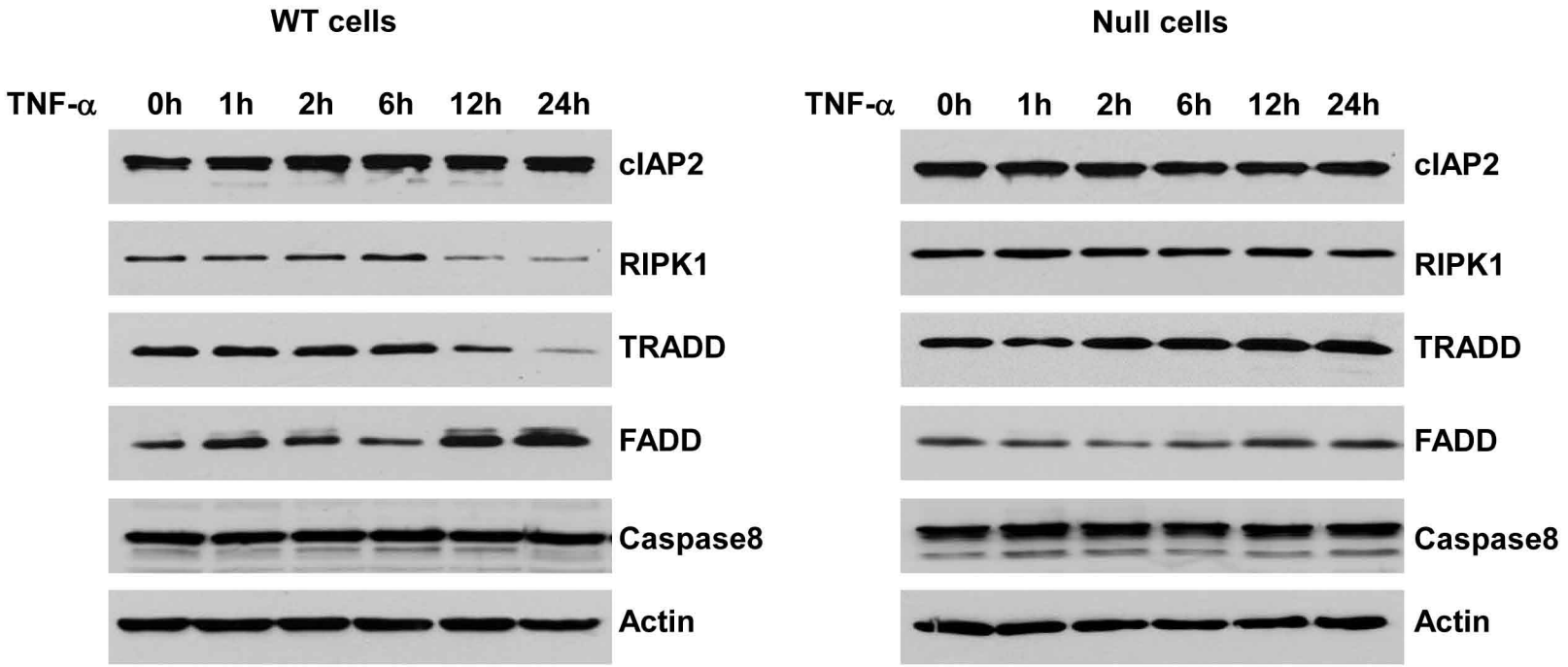
Supplementary Figure. 8. Smac-mimetic induced TNF-α dependent degradation and cleavage of FLIP in *Pkd1* null MEK cells but not that in *Pkd1* wild type MEK cells. (**A** and **B**) Western blot analysis of cIAP1 and/or FLIP expression in whole cell lysates of *Pkd1* wild-type and null MEK cells treated with Smac-mimetic plus TNF-α (**A**), Smac-mimetic alone (**B**).

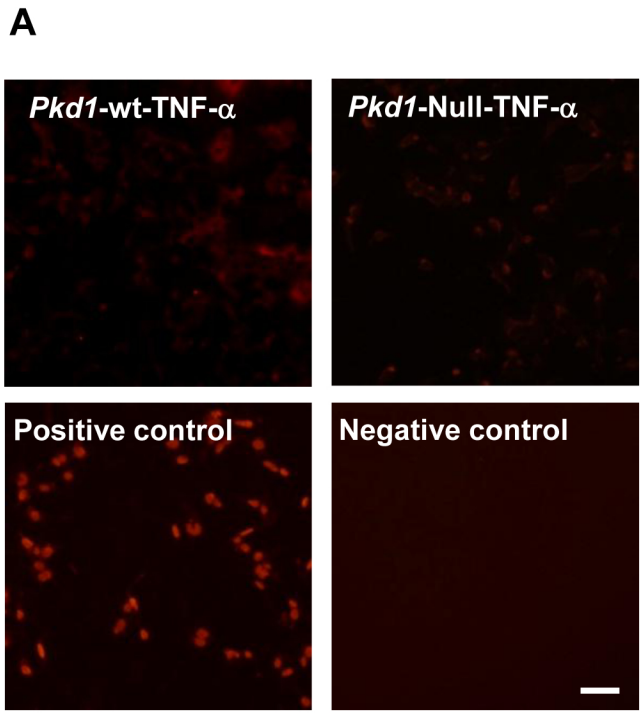
Supplementary Figure. 9. Knockdown c-Myc with siRNA decreased the expression of the components in complex I but did not affect the expression of the components in complex II in *Pkd1* mutant MEK cells. The expression of c-Myc and the components of complex I and II were analyzed by Western blot from whole cell lysates of *Pkd1* mutant (Null) MEK cells transfected with c-Myc siRNA for 48 hours.

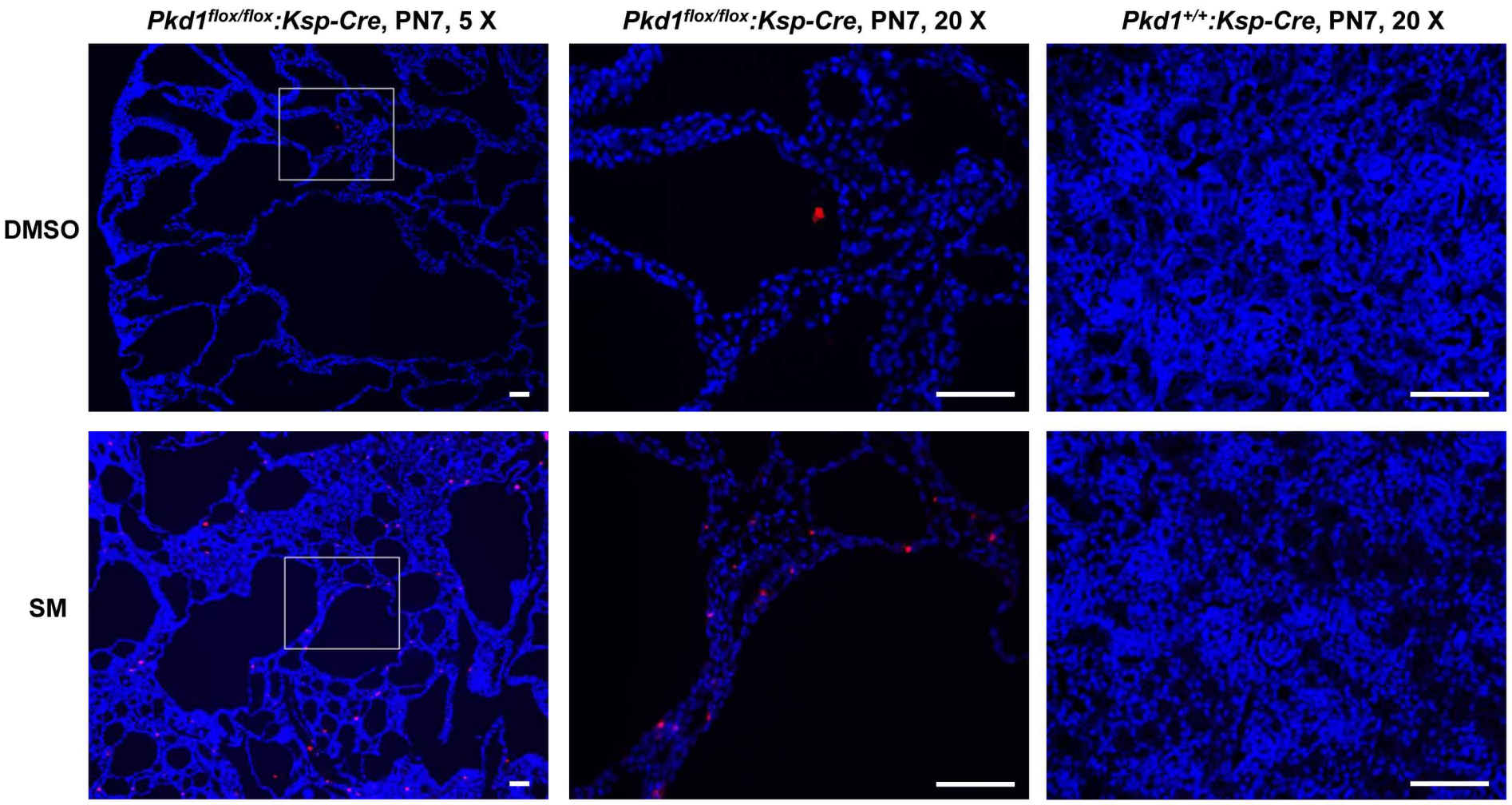
Supplemental Figure 1

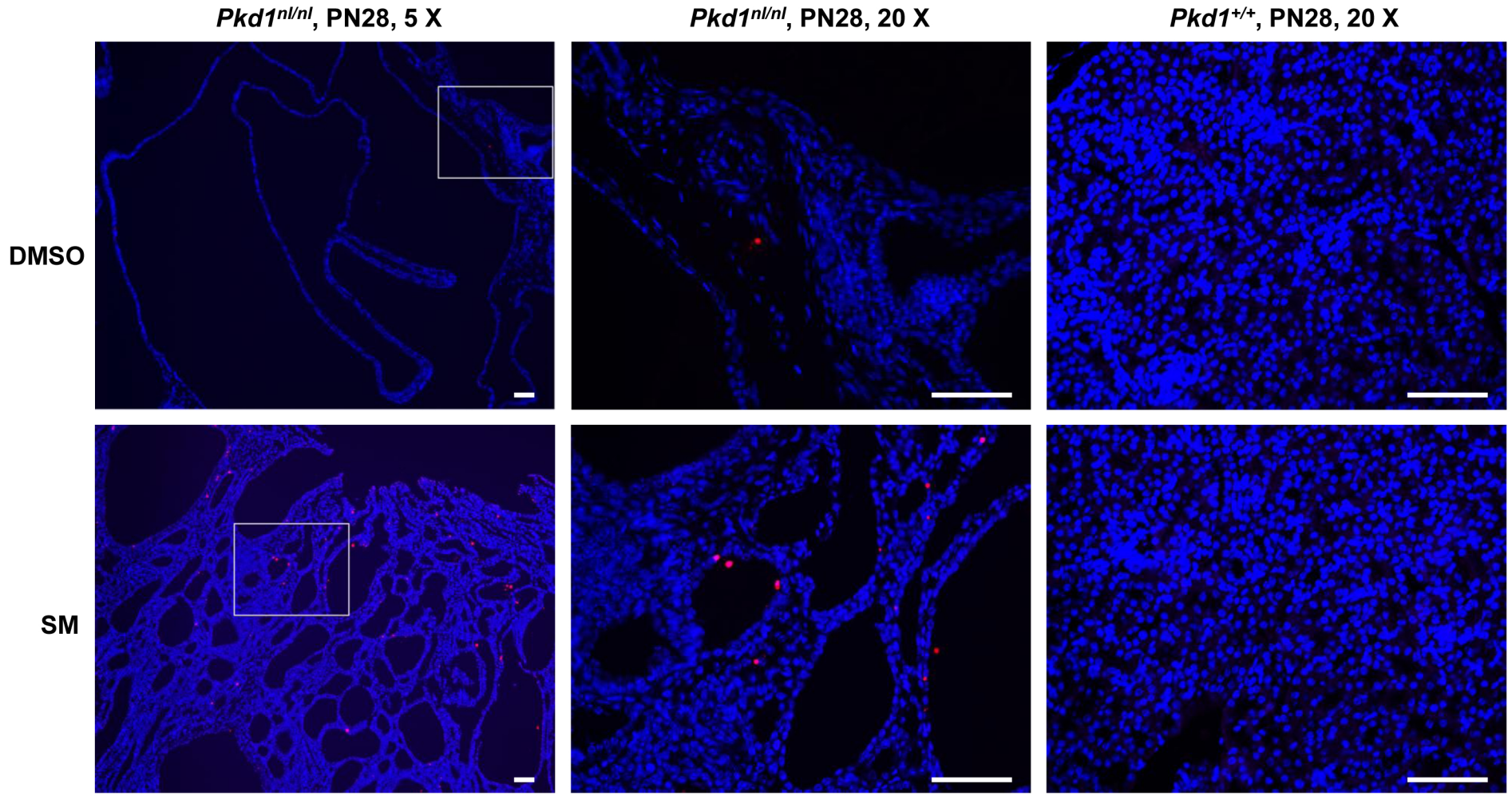


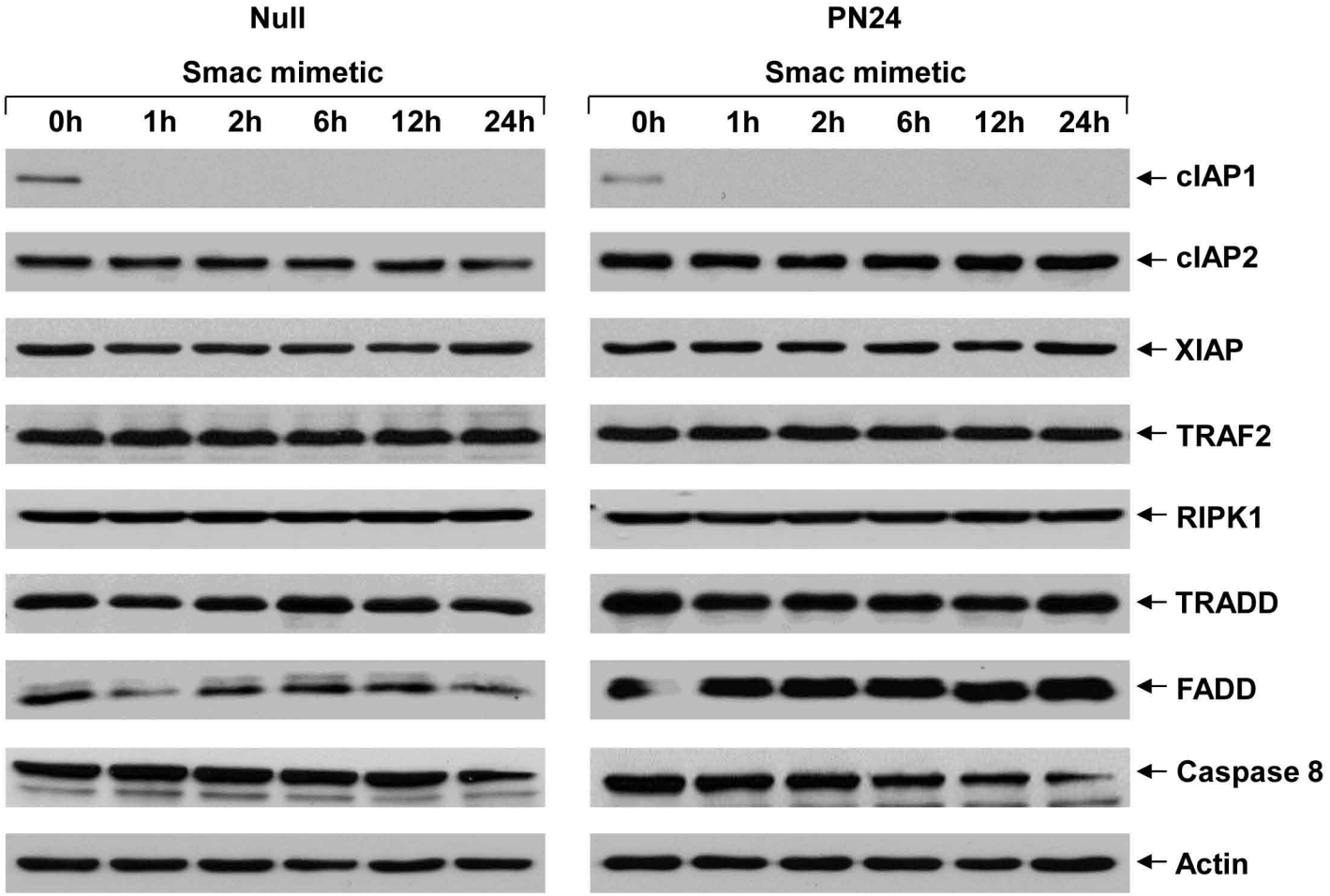

A
PH2
PN24
Complex I
cIAP1
cIAP2
RIPK1
TRADD
TRAF2
Actin
Ratio of band densities standardized to actin
RIPK1
p < 0.05
cIAP1
p < 0.05
1.5
1
0.5
0
PH2
PN24
PH2
PN24
Complex II
XIAP
FLIP
Caspase-8
FADD
Caspase-3
Actin
Ratio of band densities standardized to actin
Caspase-8
p < 0.05
FADD
p < 0.05
3
2
1
0
PH2
PN24
PH2
PN24
B
WT
Flox
cIAP1
RIPK1
FLIP
FADD
Caspase-8
Actin
cIAP1 level
p < 0.05
RIPK1 level
p < 0.05
FLIP level
p < 0.05
FADD level
p < 0.05
4
2
0
WT
Flox
C
WT
Pkd1nl/nl
cIAP1
RIPK1
FLIP
FADD
Caspase-8
Actin
cIAP1 level
p < 0.05
RIPK1 level
p < 0.05
FLIP level
p < 0.05
FADD level
p < 0.01
5
4
3
2
1
0
WT
Pkd1nl/nl

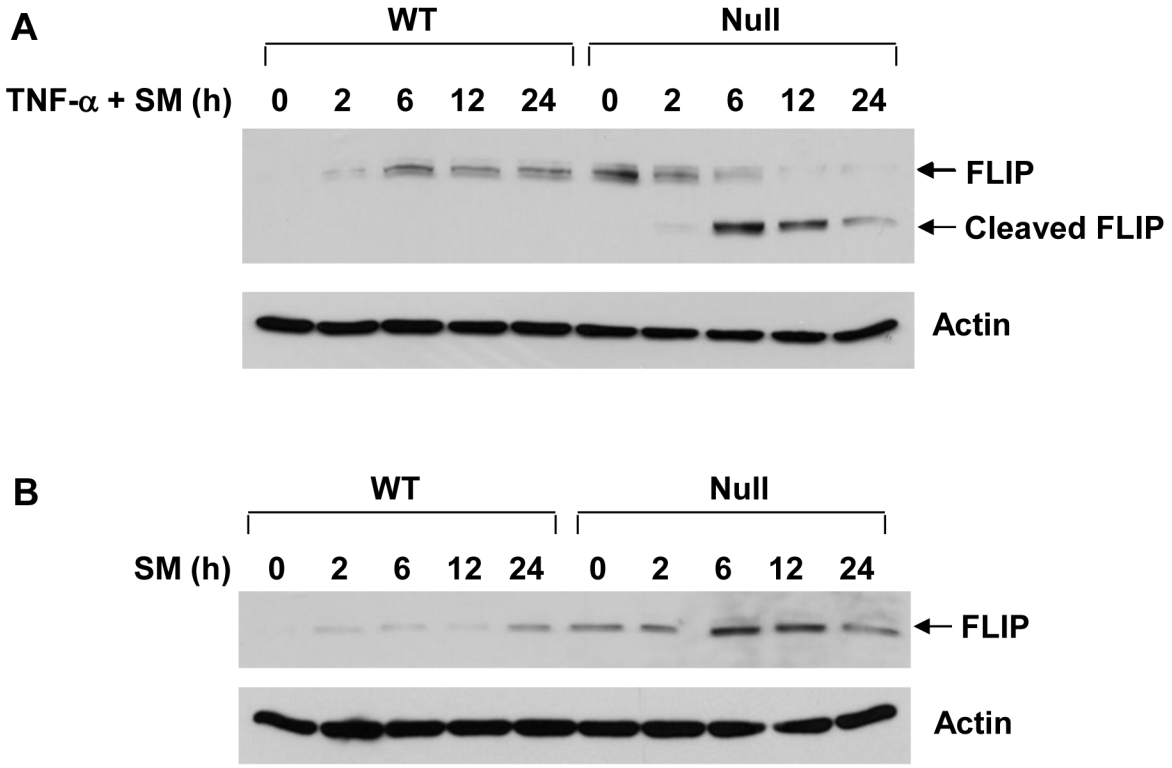

WT cells
TNF-α
0h
1h
2h
6h
12h
24h
cIAP2
RIPK1
TRADD
FADD
Caspase8
Actin
Null cells
TNF-α
0h
1h
2h
6h
12h
24h
cIAP2
RIPK1
TRADD
FADD
Caspase8
Actin

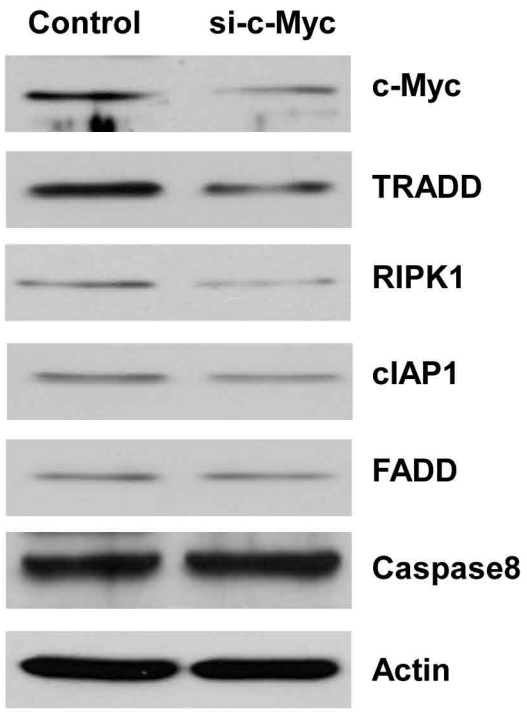

A
Pkd1-wt-TNF-α
Pkd1-Null-TNF-α
Positive control
Negative control
B
Null
Untreated
TNF-α (50 ng/ml)
SN50 (36 μM)
TNF-α + SN50 (18 μM)
TNF-α + SN50 (36 μM)
TNF-α + SM (1 μM)


Pkd1$^{flox/flox}$*:Ksp-Cre*, PN7, 5 X
Pkd1$^{flox/flox}$*:Ksp-Cre*, PN7, 20 X
Pkd1$^{+/+}$*:Ksp-Cre*, PN7, 20 X
DMSO
SM


Pkd1$^{nl/nl}$, PN28, 5 X
Pkd1$^{nl/nl}$, PN28, 20 X
Pkd1$^{+/+}$, PN28, 20 X
DMSO
SM


Null
Smac mimetic
0h
1h
2h
6h
12h
24h
PN24
Smac mimetic
0h
1h
2h
6h
12h
24h
cIAP1
cIAP2
XIAP
TRAF2
RIPK1
TRADD
FADD
Caspase 8
Actin


A
WT
Null
TNF-α + SM (h)
0 2 6 12 24 0 2 6 12 24
FLIP
Cleaved FLIP
Actin
B
WT
Null
SM (h)
0 2 6 12 24 0 2 6 12 24
FLIP
Actin


Control
si-c-Myc
c-Myc
TRADD
RIPK1
cIAP1
FADD
Caspase8
Actin