Figure 1

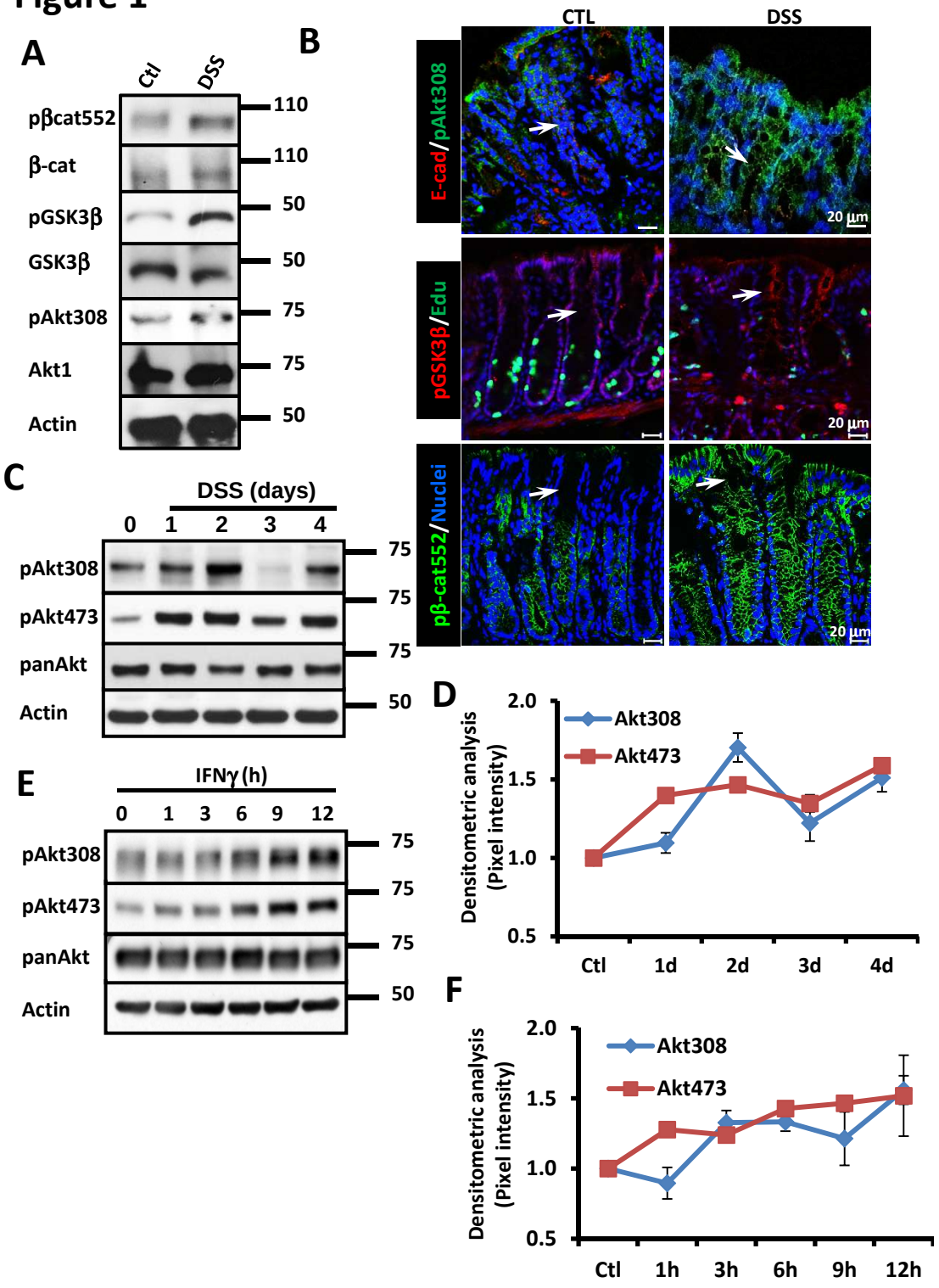

A
Ctl
DSS
pβcat552
β-cat
pGSK3β
GSK3β
pAkt308
Akt1
Actin
110
110
50
50
75
75
50
B
CTL
DSS
E-cad/pAkt308
pGSK3β/Edu
pβ-cat552/Nuclei
20 μm
C
DSS (days)
0 1 2 3 4
pAkt308
pAkt473
panAkt
Actin
75
75
75
50
D
Densitometric analysis (Pixel intensity)
Akt308
Akt473
2.0
1.5
1.0
0.5
Ctl 1d 2d 3d 4d
E
IFNγ (h)
0 1 3 6 9 12
pAkt308
pAkt473
panAkt
Actin
75
75
75
50
F
Densitometric analysis (Pixel intensity)
Akt308
Akt473
2.0
1.5
1.0
0.5
Ctl 1h 3h 6h 9h 12h

Figure 2

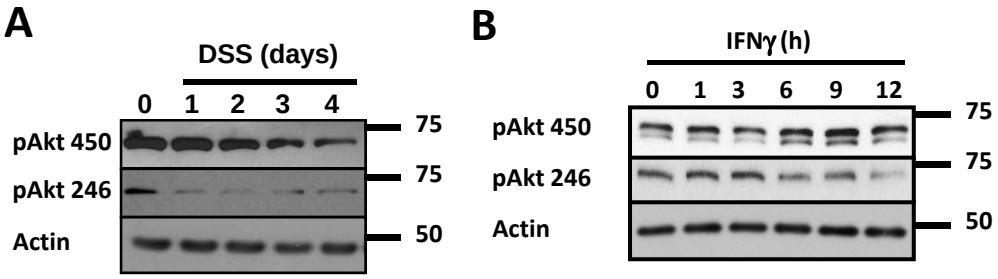

A
DSS (days)
0 1 2 3 4
pAkt 450
pAkt 246
Actin
75
75
50
B
IFNγ (h)
0 1 3 6 9 12
pAkt 450
pAkt 246
Actin
75
75
50

Figure 3

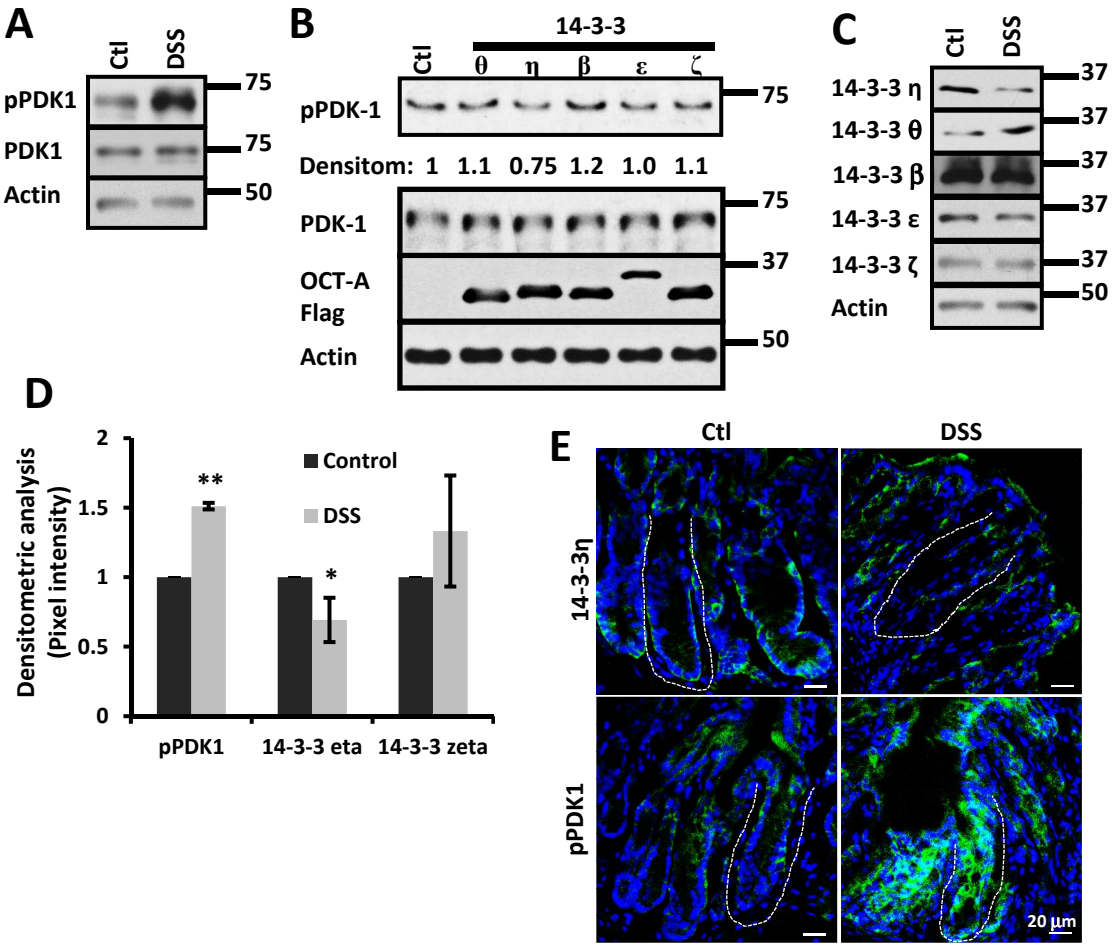

A
Ctl
DSS
pPDK1
PDK1
Actin
75
75
50
B
14-3-3
Ctl
θ
η
β
ε
ζ
pPDK-1
75
Densitom: 1 1.1 0.75 1.2 1.0 1.1
PDK-1
OCT-A Flag
Actin
75
37
50
C
Ctl
DSS
14-3-3 η
14-3-3 θ
14-3-3 β
14-3-3 ε
14-3-3 ζ
Actin
37
37
37
37
37
50
D
Densitometric analysis (Pixel intensity)
2
1.5
1
0.5
0
**
*
Control
DSS
pPDK1
14-3-3 eta
14-3-3 zeta
E
Ctl
DSS
14-3-3η
pPDK1
20 µm

Figure 4

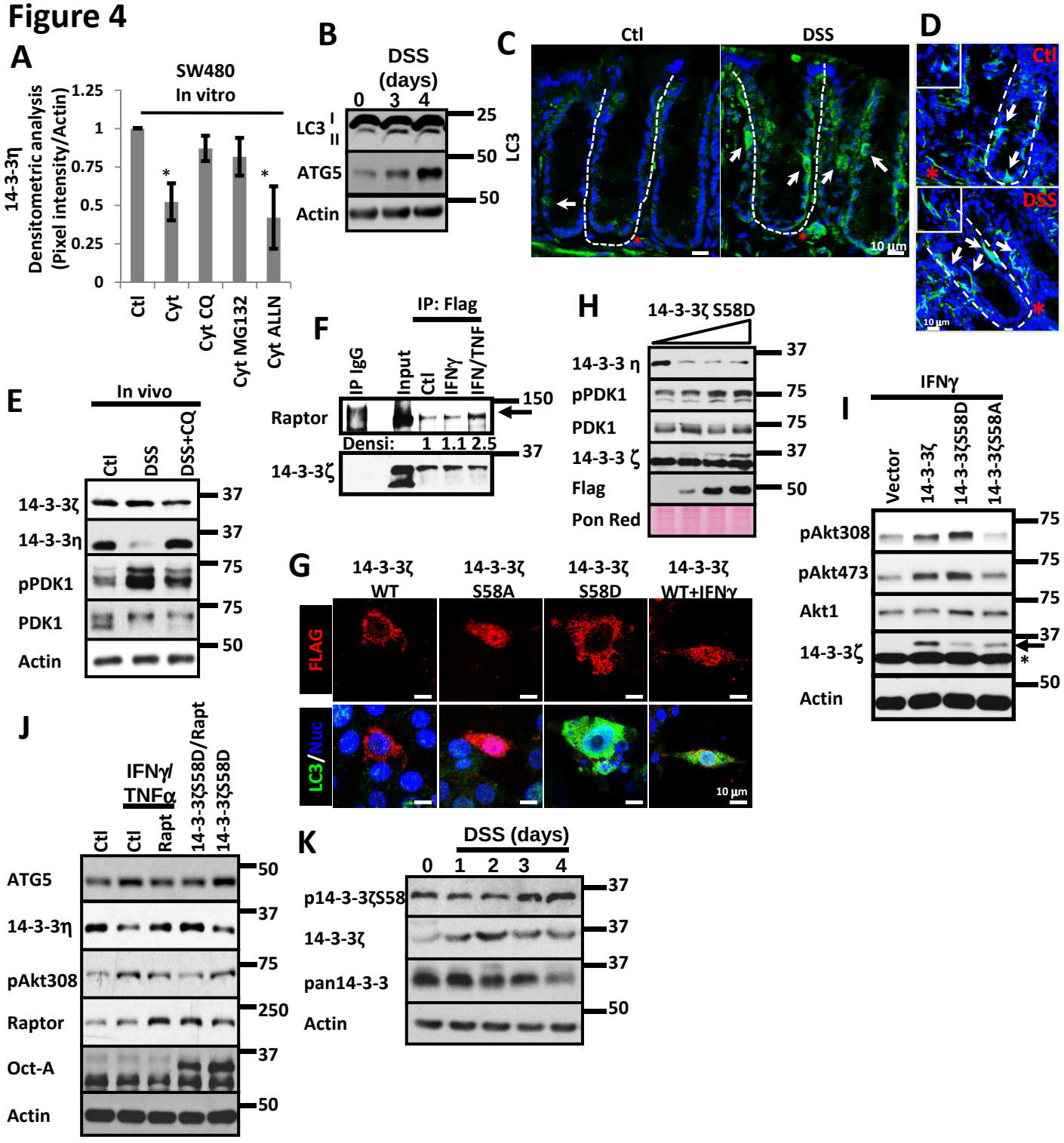

A
SW480
In vitro
14-3-3η
Densitometric analysis
(Pixel intensity/Actin)
1.25
1
0.75
0.5
0.25
0
Ctl
Cyt
Cyt CQ
Cyt MG132
Cyt ALLN
B
DSS
(days)
0 3 4
LC3 I
II
ATG5
Actin
25
50
50
C
Ctl
DSS
LC3
10 μm
D
Ctl
DSS
10 μm
E
In vivo
Ctl
DSS
DSS+CQ
14-3-3ζ
14-3-3η
pPDK1
PDK1
Actin
37
37
75
75
50
F
IP: Flag
IP IgG
Input
Ctl
IFNγ
IFN/TNF
Raptor
150
Densi: 1 1.1 2.5
14-3-3ζ
37
H
14-3-3ζ S58D
14-3-3 η
pPDK1
PDK1
14-3-3 ζ
Flag
Pon Red
37
75
75
37
50
I
IFNγ
Vector
14-3-3ζ
14-3-3ζS58D
14-3-3ζS58A
pAkt308
pAkt473
Akt1
14-3-3ζ
Actin
75
75
75
37
50
G
14-3-3ζ WT
14-3-3ζ S58A
14-3-3ζ S58D
14-3-3ζ WT+IFNγ
FLAG
LC3/Nuc
10 μm
J
IFNγ/ TNFα
Ctl
Ctl
Rapt
14-3-3ζS58D/Rapt
14-3-3ζS58D
ATG5
14-3-3η
pAkt308
Raptor
Oct-A
Actin
50
37
75
250
37
50
K
DSS (days)
0 1 2 3 4
p14-3-3ζS58
14-3-3ζ
pan14-3-3
Actin
37
37
37
50

Figure 5

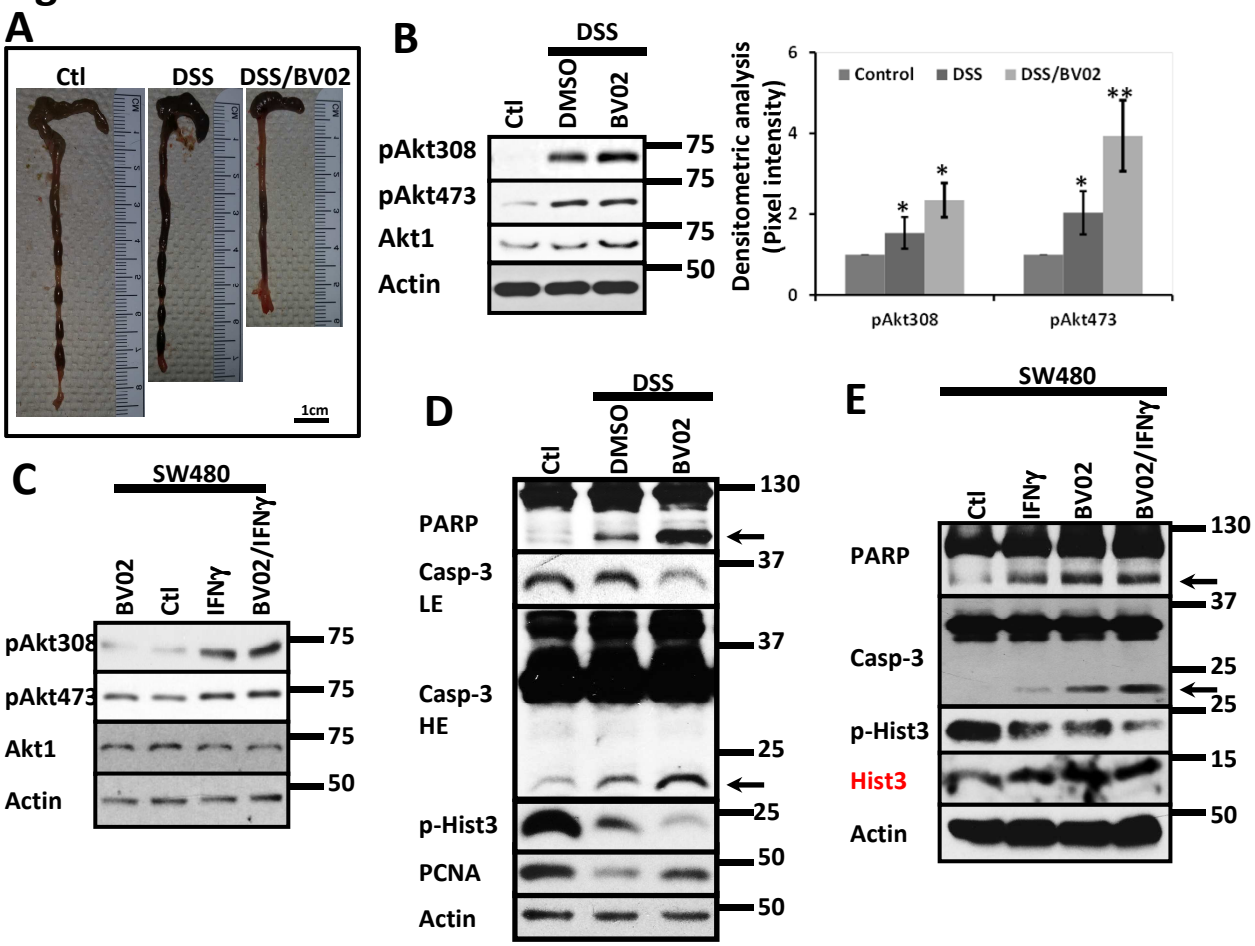

A
Ctl
DSS
DSS/BV02
1cm
B
DSS
Ctl
DMSO
BV02
pAkt308
pAkt473
Akt1
Actin
75
75
75
50
Densitometric analysis (Pixel intensity)
Control
DSS
DSS/BV02
pAkt308
pAkt473
C
SW480
BV02
Ctl
IFNγ
BV02/IFNγ
pAkt308
pAkt473
Akt1
Actin
75
75
75
50
D
DSS
Ctl
DMSO
BV02
PARP
Casp-3 LE
Casp-3 HE
p-Hist3
PCNA
Actin
130
37
37
25
25
50
50
E
SW480
Ctl
IFNγ
BV02
BV02/IFNγ
PARP
Casp-3
p-Hist3
Hist3
Actin
130
37
25
25
15
50

Figure 6

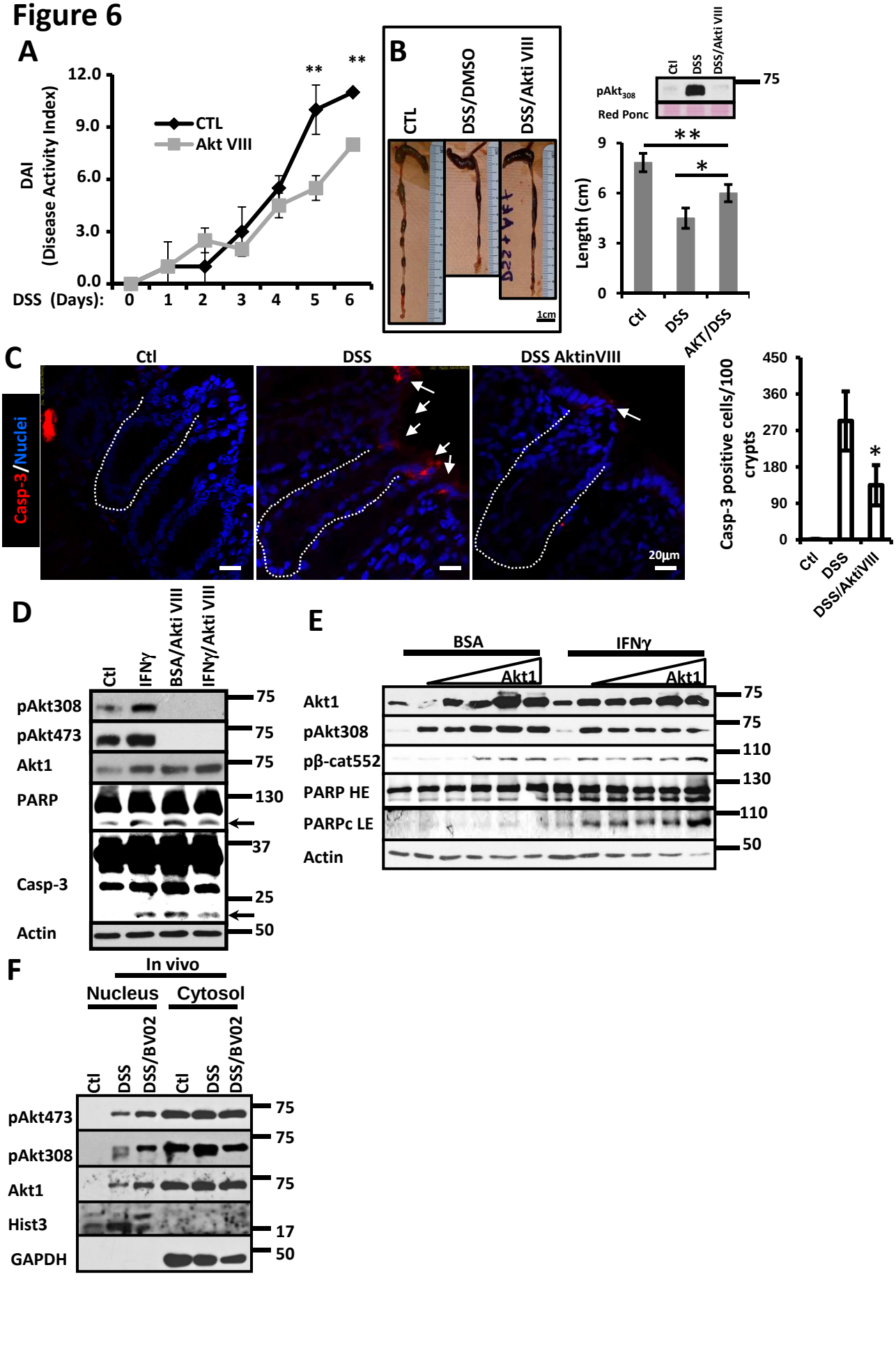
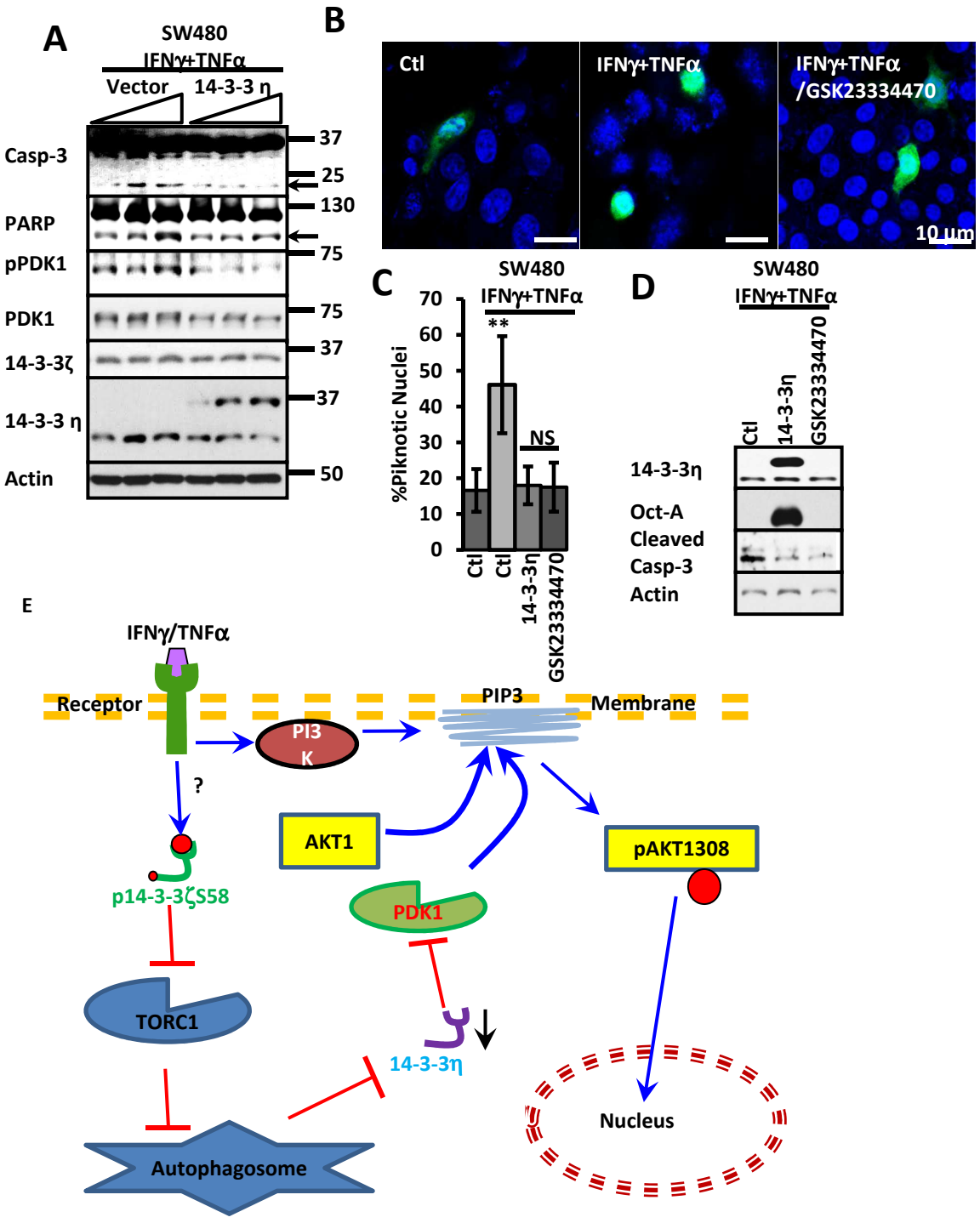
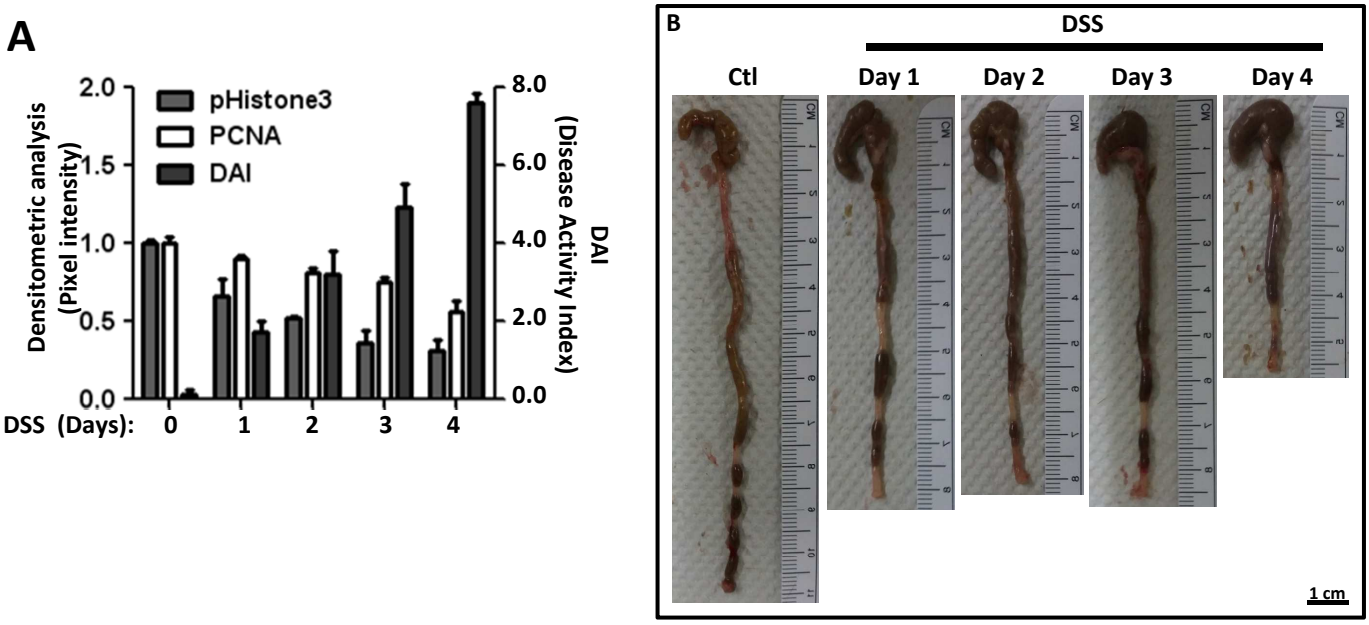

A
DAI (Disease Activity Index)
12.0
9.0
6.0
3.0
0.0
DSS (Days): 0 1 2 3 4 5 6
CTL
Akt VIII
**
**
B
CTL
DSS/DMSO
DSS/Akti VIII
1cm
pAkt308
Red Ponc
Ctl
DSS
DSS/Akti VIII
75
Length (cm)
9
6
3
0
**
*
Ctl
DSS
AKT/DSS
C
Ctl
DSS
DSS AktinVIII
Casp-3/Nuclei
20µm
Casp-3 positive cells/100 crypts
450
360
270
180
90
0
*
Ctl
DSS
DSS/AktiVIII
D
Ctl
IFNγ
BSA/Akti VIII
IFNγ/Akti VIII
pAkt308
pAkt473
Akt1
PARP
Casp-3
Actin
75
75
75
130
37
25
50
E
BSA
IFNγ
Akt1
Akt1
Akt1
pAkt308
pβ-cat552
PARP HE
PARPc LE
Actin
75
75
110
130
110
50
F
In vivo
Nucleus
Cytosol
Ctl
DSS
DSS/BV02
Ctl
DSS
DSS/BV02
pAkt473
pAkt308
Akt1
Hist3
GAPDH
75
75
75
17
50

Figure 7

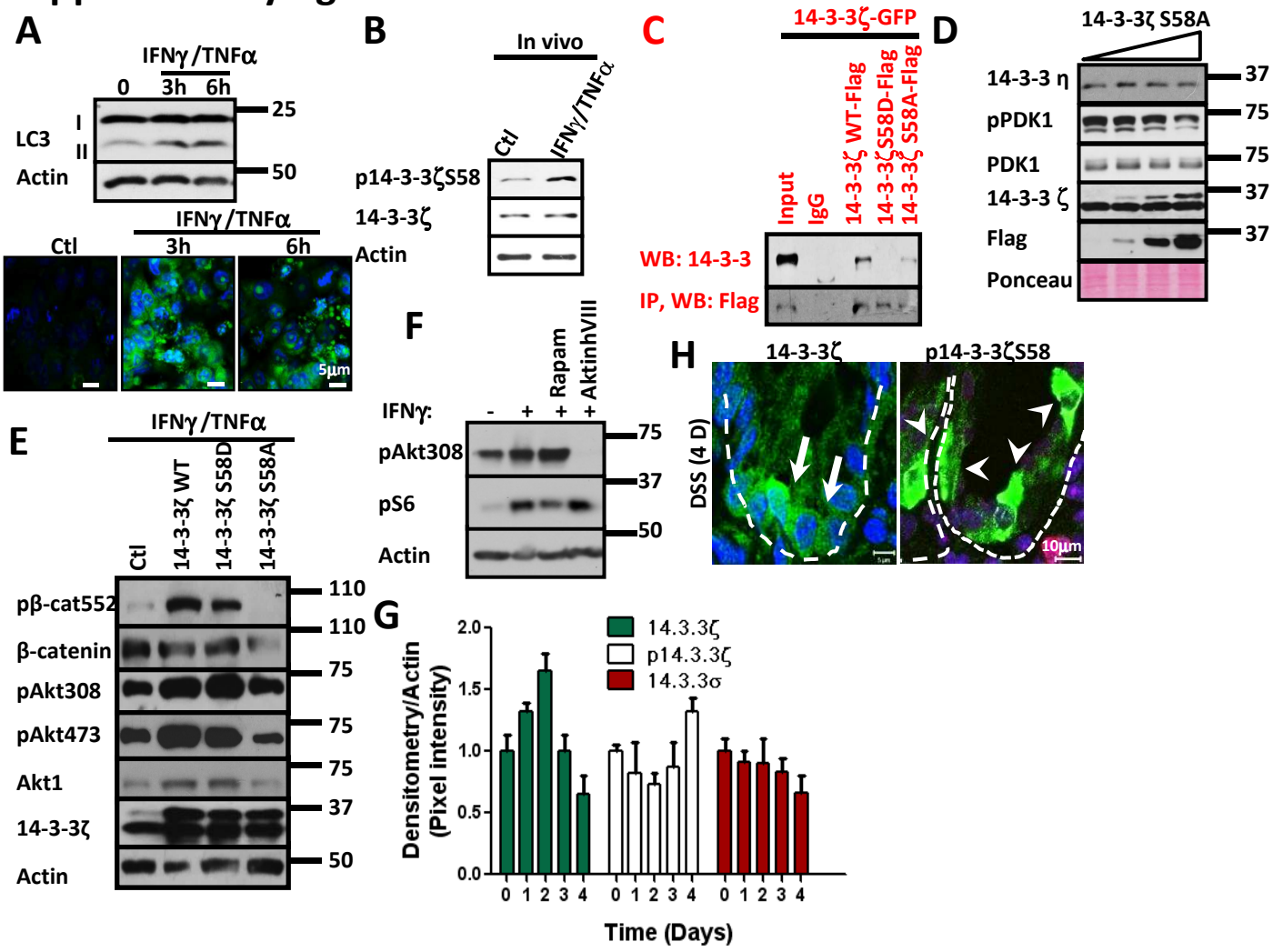

A
SW480
IFNγ+TNFα
Vector
14-3-3 η
Casp-3
PARP
pPDK1
PDK1
14-3-3ζ
14-3-3 η
Actin
37
25
130
75
75
37
37
50
B
Ctl
IFNγ+TNFα
IFNγ+TNFα
/GSK23334470
10 μm
C
SW480
IFNγ+TNFα
%Piknotic Nuclei
70
60
50
40
30
20
10
0
**
NS
Ctl
Ctl
14-3-3η
GSK23334470
D
SW480
IFNγ+TNFα
Ctl
14-3-3η
GSK23334470
14-3-3η
Oct-A
Cleaved
Casp-3
Actin
E
IFNγ/TNFα
Receptor
PIP3
Membrane
PI3
K
?
AKT1
pAKT1308
p14-3-3ζS58
PDK1
TORC1
14-3-3η
Nucleus
Autophagosome

Supplementary figure 1



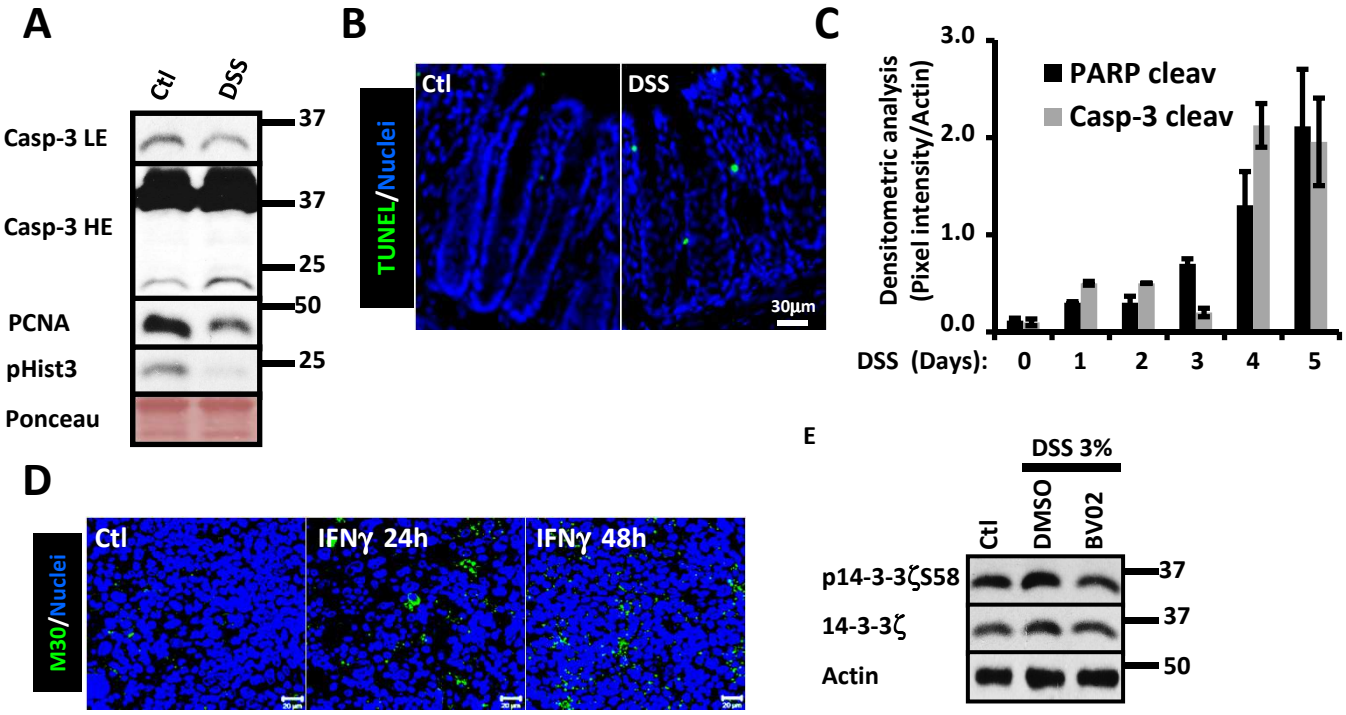
DSS induced inflammation decreases cell proliferation and colon length. (A) Effects of DSS-induced inflammation on cell proliferation markers (PCNA, pHistone3) and disease activity (Disease Activity Index (DAI)). C57BL/6J were treated with DSS 3% dissolved in drinking water for 1-4 days. Relative values for PCNA and pHistone3 obtained from densitometric analysis were normalized to actin. DAI was analyzed as previously reported (Laukoetter et al, 2007). n=6. (B) Representative image of colon tissue obtained from control and colitic mice. C57BL/6J mice were treated with DSS 3% dissolved in drinking water for 1-4 days. n=6. Bar= 1cm.

Supplementary figure 2



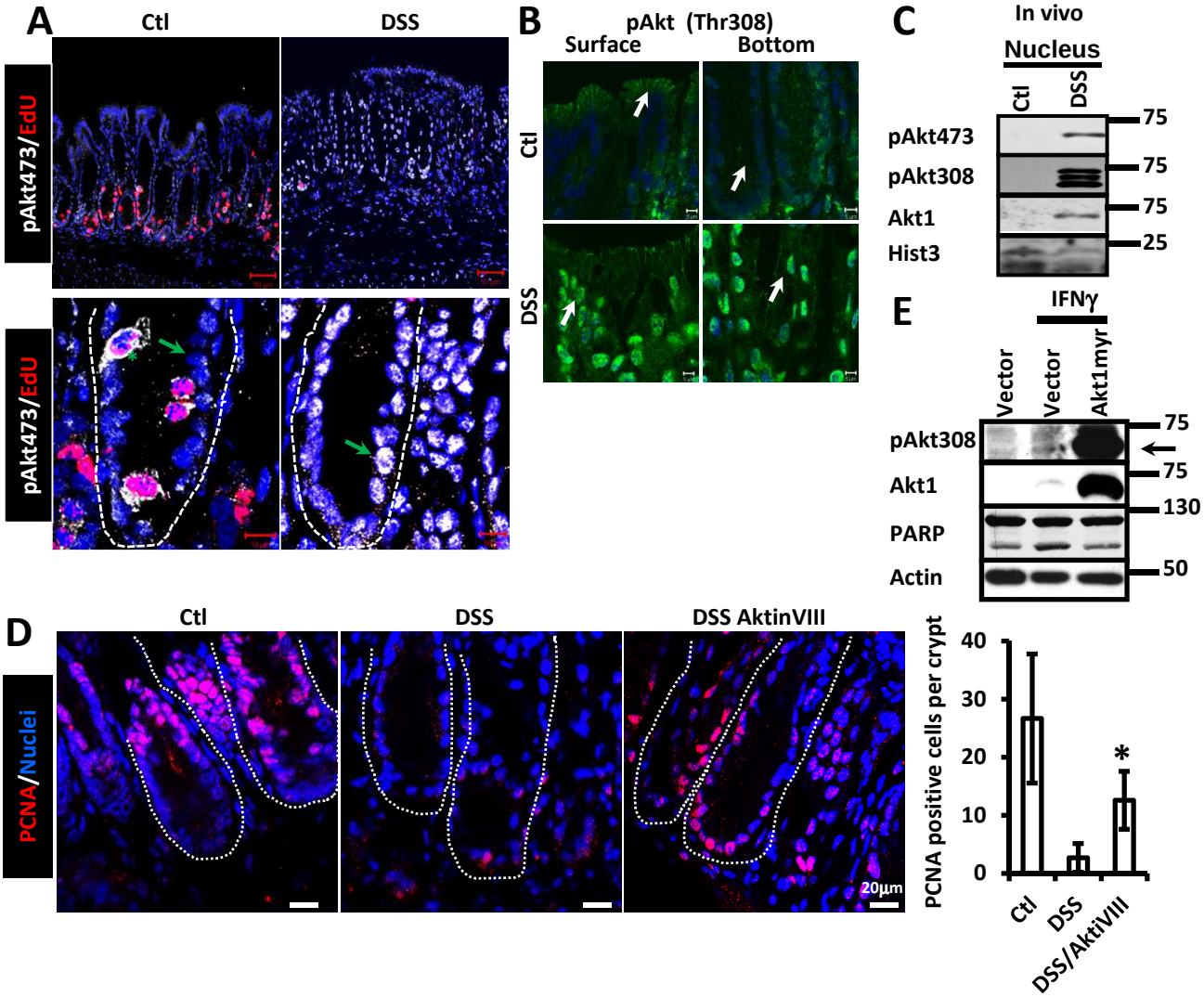
14-3-3ζ and p14-3-3ζS58 increased in the mucosa of colitic mice and regulate Akt activation. (A, *upper panel*) LC3I and LC3II were analyzed in SW480 cells treated with cytokines. Actin was used as loading control. (A, *lower panel*) Acridine orange (green) was used to analyze the presence of autophagosomes. Nuclei blue. Bar= 5µm. (B) p14-3-3ζS58 and p14-3-3ζ were analyzed in colonic cell lysates of C57BL/6J mice injected intraperitoneally with IFNγ/TNFα. Actin was used as loading control. (C) 14-3-3ζWT-Flag, 14-3-3ζWT-Flag and 14-3-3ζWT-Flag were precipitated from cells cotransfected with a plasmid expressing 14-3-3ζ-GFP plus 14-3-3ζWT-Flag, 14-3-3ζWT-Flag or 14-3-3ζWT-Flag. Westernblot against pan-14-3-3 and Flag were performed. (D) 14-3-3η, pPDK1, PDK1, 14-3-3ζ and Flag were analyzed in SW480 cells expressing increasing concentrations of 14-3-3 ζ S58A. Ponceau read was used as loading control. (E) pβ-cat552, β-catenin, pAkt308, pAkt473, Akt1 and14-3-3ζ were analyzed in SW480 cells transfected with 14-3-3 ζWT, 14-3-3 ζ S58D and 14-3-3 ζ S58A and treated with IFNγ (12h). Actin was used as loading control. (F) pAkt308 and pS6 were analyzed in SW480 cells treated with IFNγ, IFNγ plus rapamycin (200 nM) and IFNγ plus Akt Inhib VIII (2.12µM). IFNγ was carried out for 12h. Actin was used as loading control. (G) 14-3-3 ζ, p14-3-3 ζ S58 and 14-3-3σ were analyzed in the mucosa of mice treated with DSS 1-4 days. Densitometric analysis is shown. n=6. (H) The distribution of 14-3-3ζ and p14-3-3ζS58 was analyzed in the mucosa of colitic mice by immunofluorescence. 14-3-3ζ and p14-3-3ζS58, green. Highly enriched cells are marked by arrow (14-3-3ζ) and arrowhead (p14-3-3ζS58). Discontinuous line denotes crypt shape. Nuclei, blue. EdU= red Bar= 10µm.

Supplementary figure 3



Inflammation enhances apoptosis in IECs. (A) Caspase-3, PCNA and pHist3 were analyzed in control and DSS treated mice. C57BL/6J were treated with DSS 3% dissolved in drinking water for 4 days. Low Exposure (LE) and High Exposure (HE) for caspase-3 are shown. Ponceau red was used as loading control. (B) Apoptosis was analyzed in the mucosa of control and DSS treated mice by Tunel staining (green). C57BL/6J were treated with DSS 3% dissolved in drinking water for 1-4 days. Nuclei, blue. Bar=30µm. (C) Graph shows relative densitometric values for PARP and Casp3 cleavage in the mucosa of DSS treated animals. C57BL6/J mice received 3% DSS in drinking water for 1-5 days. (D) Apoptosis was analyzed in SW480 cells treated with 100U/ml of IFNγ by M30 staining (green). Nuclei, blue. Bar=20µm. (E) 14-3-3ζ and p14-3-3ζS58 levels were analyzed in the mucosa of mice treated with DSS/DMSO or DSS/BV02. C57BL6/J mice received 3% DSS in drinking water for 4 days. Mice were injected daily with 10 mg/Kg of weight of BV02 via peritoneum. Actin was used as loading control.

Supplementary figure 4



Akt accumulates in the nucleus of IECs and regulates cell proliferation during inflammation. (A) pAkt 473(White) was analyzed by immunofluorescence in samples of mice exposed to DSS 4 days. pAkt473 is observed in cytosol in proliferating cells green star and nuclear staining is observed in non-proliferating cells green arrow. EdU, red. Nuclei, Blue. Bar= 10µm. (B) pAkt 308 (Green) was analyzed by immunofluorescence in samples of mice exposed to DSS 2 and 4 days. pAkt308 is observed at membrane (White arrow) and nucleus. Nuclei, Blue. Bar=10µm. (C) pAkt 473, pAkt308 and Akt1 presence were analyzed in nuclear fractions of IECs obtained from mice exposed to DSS 4 days. pAkt473, pAkt308 and Akt1 were enriched in nuclear fractions of colitic mice. Histone 3 was used as marker for nuclear fraction. (D)Cell proliferation was analyzed in the mucosa of control and mice treated with DSS/DMSO or DSS/Akt inhibitor VIII by PCNA staining (red). Crypts are marked by dotted line. C57BL6/J mice received 3% DSS in drinking water for 4 days. Mice were injected daily with 10 mg/Kg of weight of Akt inhibitor VII via peritoneum. Nuclei, blue. Bar=20µm. Quantification is showed in the graph. **p<0.05; **p<0.001 (E) pAkt308, Akt1 and PARP cleavage were analyzed in SW480 cells expressing Akt1 myristoylated (Akt1.myr) that were exposed to IFNγ for 36h. Actin was used as loading control.