Supplemental figures

Figure 1. Sub-cellular localization of MYC-tagged IBRDC2 in healthy and apoptotic cells

Typical examples of MYC-IBRDC2 localization in non-apoptotic (**A**) and apoptotic (**B**) cells are shown. HeLa cells were transfected with MYC-IBRDC2 (green on overlay images), and either were left untreated (**A**) or treated with 10μM ActD (**B**). Cells were immunostained with anti-MYC polyclonal antibody (Abcam) and, to reveal mitochondria, with anti-Tom20 monoclonal antibody (red on overlay images), and then analyzed by fluorescence microscopy. Bars: 20μm and 2.3μm (detail images).

Figure 2. C-terminal YFP fusion of IBRDC2

Typical examples of IBRDC2-YFP localization in non-apoptotic (**A**) and apoptotic (**B**) cells are shown. HeLa cells were transfected with IBRDC2-YFP (green on overlay images), and either were left untreated (**A**) or treated with 10μM ActD (**B**). To reveal mitochondria and apoptosis cells were immunostained with anti-cytochrome c monoclonal antibody (red on overlay images), and then analyzed by fluorescence microscopy. Bars: 20μm.

Figure 3. Sub-cellular localization of YFP-tagged predicted transmembrane domain of IBRDC2 (YFP-IBRDC2^{TM})

Typical examples of YFP-IBRDC2^{TM} localization in non-apoptotic (**A**) and apoptotic (**B**) cells are shown. HeLa cells were transfected with YFP-IBRDC2^{TM} (green on overlay images), and either were left untreated (**A**) or treated with 10μM ActD (**B**). To reveal mitochondria cells were immunostained with anti-Tom20 monoclonal antibody (red on overlay images), and then analyzed by fluorescence microscopy. Bars: 20μm and 2.3μm (detail images)

Figure 4. Effect of protein synthesis and proteasome inhibition on the levels of Bax

(**A**) Control RNAi and IBRDC2 RNAi HeLa cells were incubated with 2μg/ml CHX, and 30μM zVAD-fmk (to prevent cell death) for indicated periods. Expression levels of Bax and Tom20 were analyzed by Western blot. CHX-induced changes in Bax expression (Bax level) were quantified using ImageJ software and normalized to the levels of Tom20. HeLa (**B**), or WT and Bax-/-HCT116 (**C**) cells were incubated with 2μg/ml CHX or 10μM MG132 for 15hr followed by Western blot as indicated in the figure.

Figure 5. BN-PAGE analyses of Bax and IBRDC2

Mitochondrial fractions obtained from ActD- (**A**) or STS- (**B**) treated HeLa cells were analyzed by BN-PAGE (first dimension) combined with SDS-PAGE (second dimension) followed by Western blot of Bax and IBRDC2.

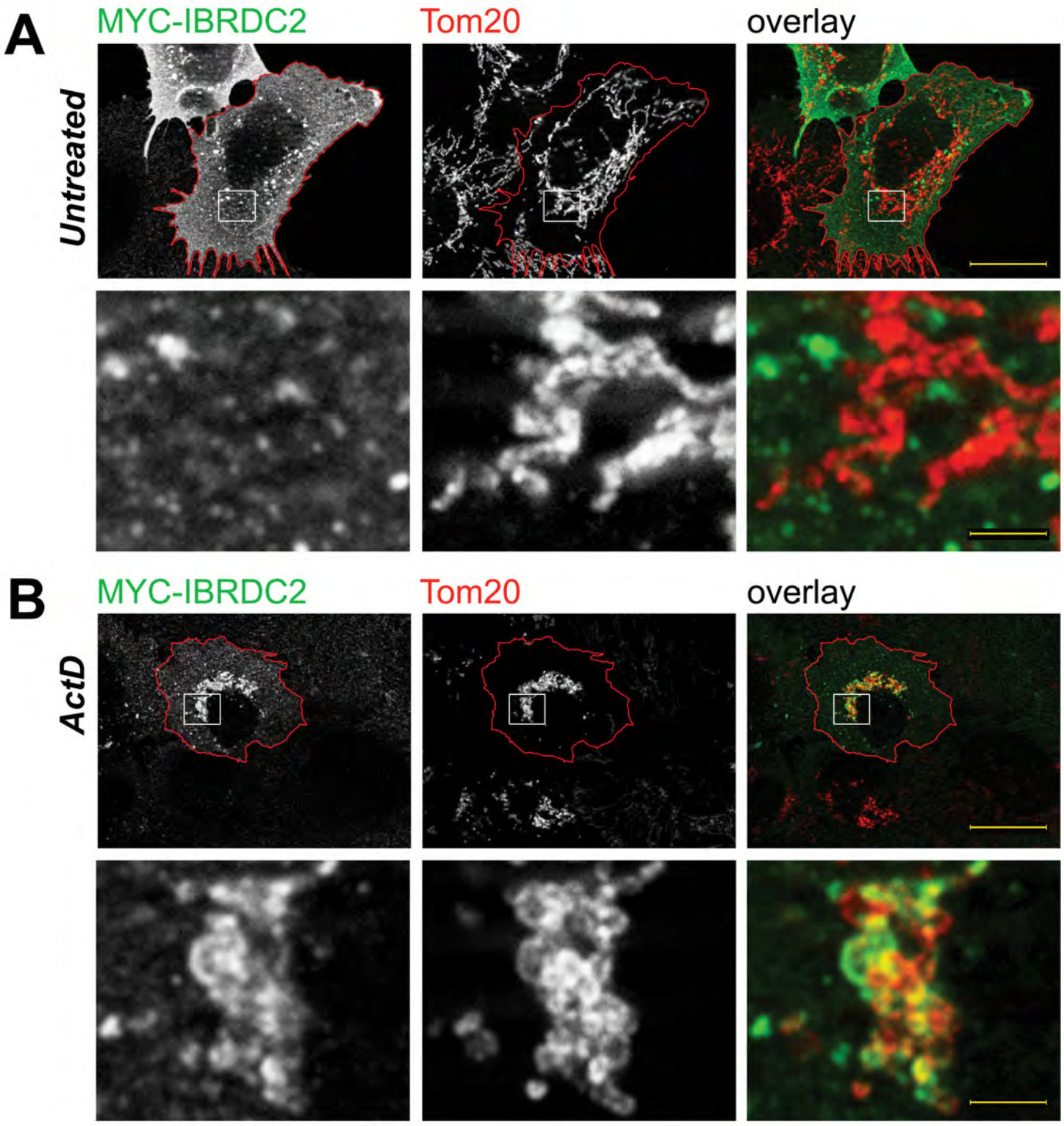
Figure 6. Effect of IBRDC2 overexpression on apoptosis

PARP cleavage (**A**), mitochondrial activation (**B**) and mitochondrial levels of Bax (**C**) in MYC-IBRDC2 or control pEYFP overexpressing HeLa cells. Cells were treated with ActD and STS, followed by Western blot of total cell lysates (**A**), or mitochondrial fractions (**B**, **C**) as indicated in the figure. In (**B**) mitochondrial fractions were treated with a chemical x-linker, 2mM BMH for 15 min on ice, prior to SDS PAGE and Western blot analyses. In (**A-C**) a single representative experiment is shown.

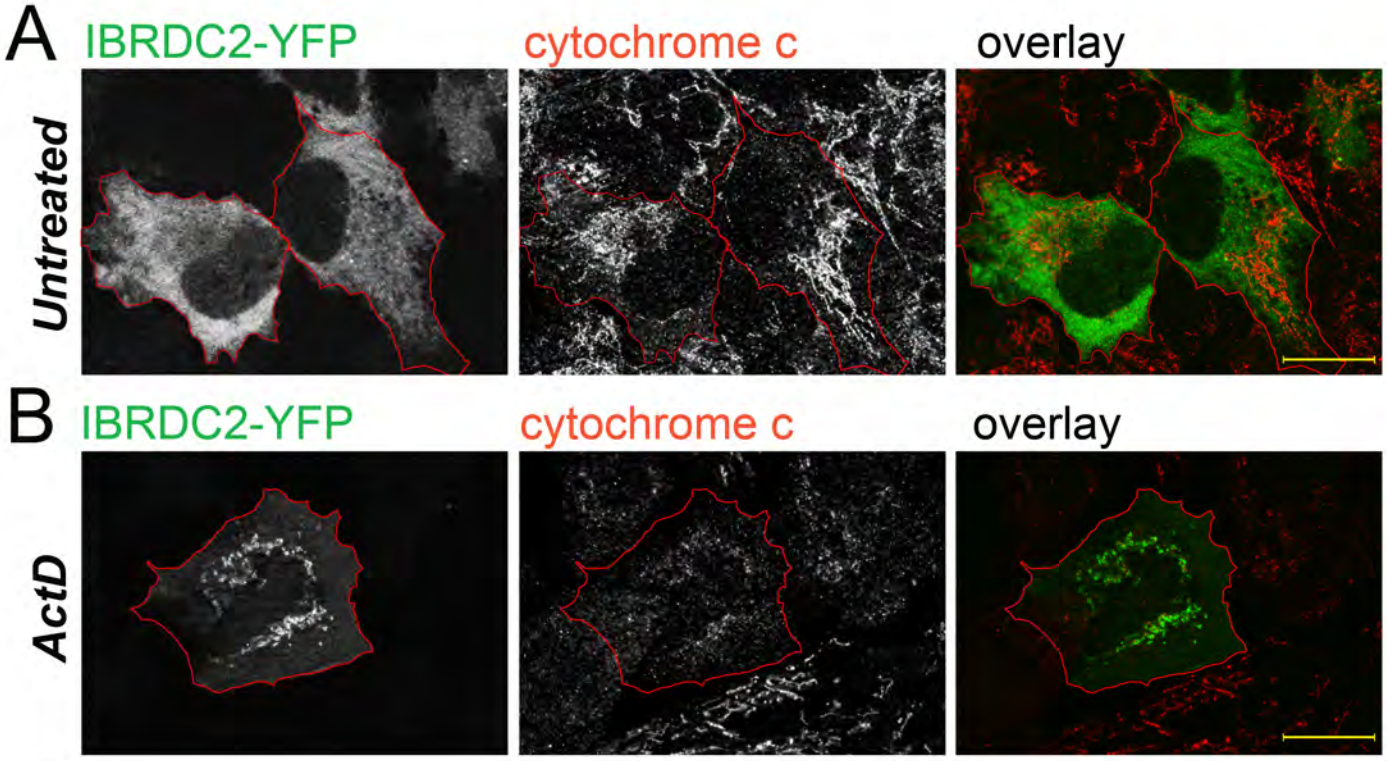
(**D**) PARP cleavage in IBRDC2 overexpressing WT, $Bax^{-/-}$, and $Bak^{-/-}$HCT116 cells

WT, $Bax^{-/-}$, and $Bak^{-/-}$HCT116 cells were transfected with either MYC-IBRDC2 (IBRDC2), or pEYFP control vector (Control), followed by the treatment with 10μM ActD, or 1μM STS for 10hr, and then Western blot analyses. The membranes were first blotted for IBRDC2 then for Bax and Tom20. Bak expression was tested using separate membranes.

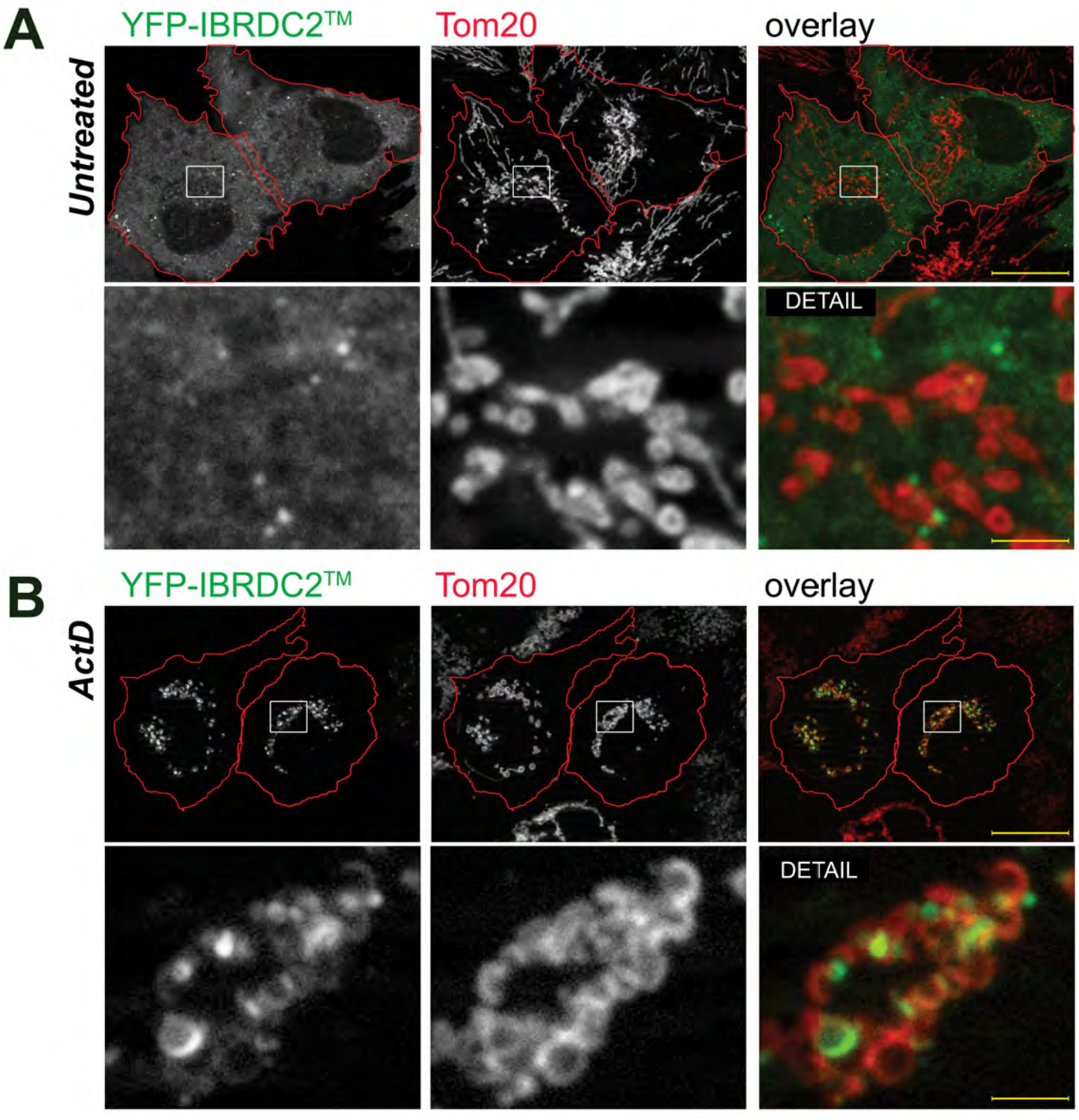
The data show that in contrast to IBRDC2 downregulation, overexpression of this protein has only a slight effect on PARP cleavage. A similar minor effect of IBRDC2 overexpression on Bax translocation to the mitochondria and mitochondrial oligomerization of Bax was also detected. Furthermore, overexpression of IBRDC2 did not affected apoptosis activation in HCT116 WT, HCT116 $Bax^{-/-}$, and HCT116 $Bak^{-/-}$ cells.


A
MYC-IBRDC2
Tom20
overlay
Untreated
B
MYC-IBRDC2
Tom20
overlay
ActD

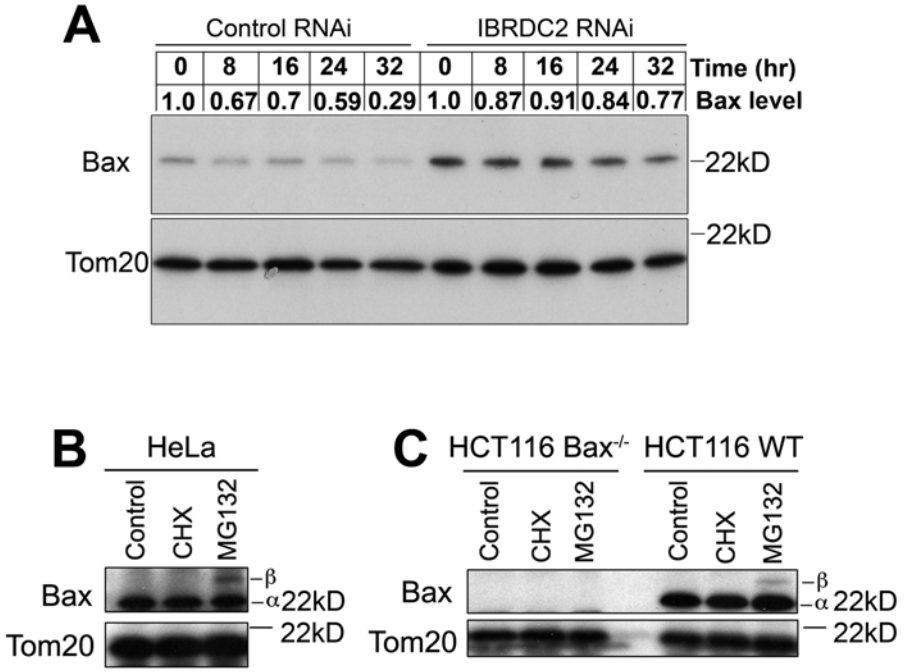
Figure_S1


A
IBRDC2-YFP
cytochrome c
overlay
Untreated
B
IBRDC2-YFP
cytochrome c
overlay
ActD

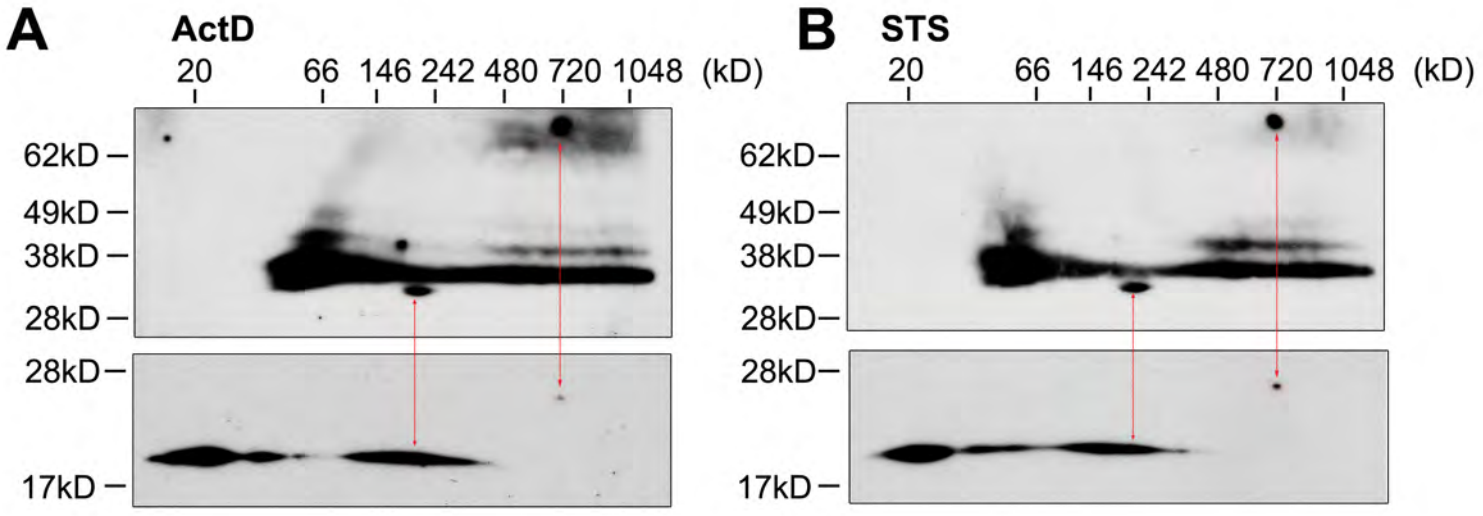
Figure_S2


A
YFP-IBRDC2™
Tom20
overlay
Untreated
DETAIL
B
YFP-IBRDC2™
Tom20
overlay
ActD
DETAIL

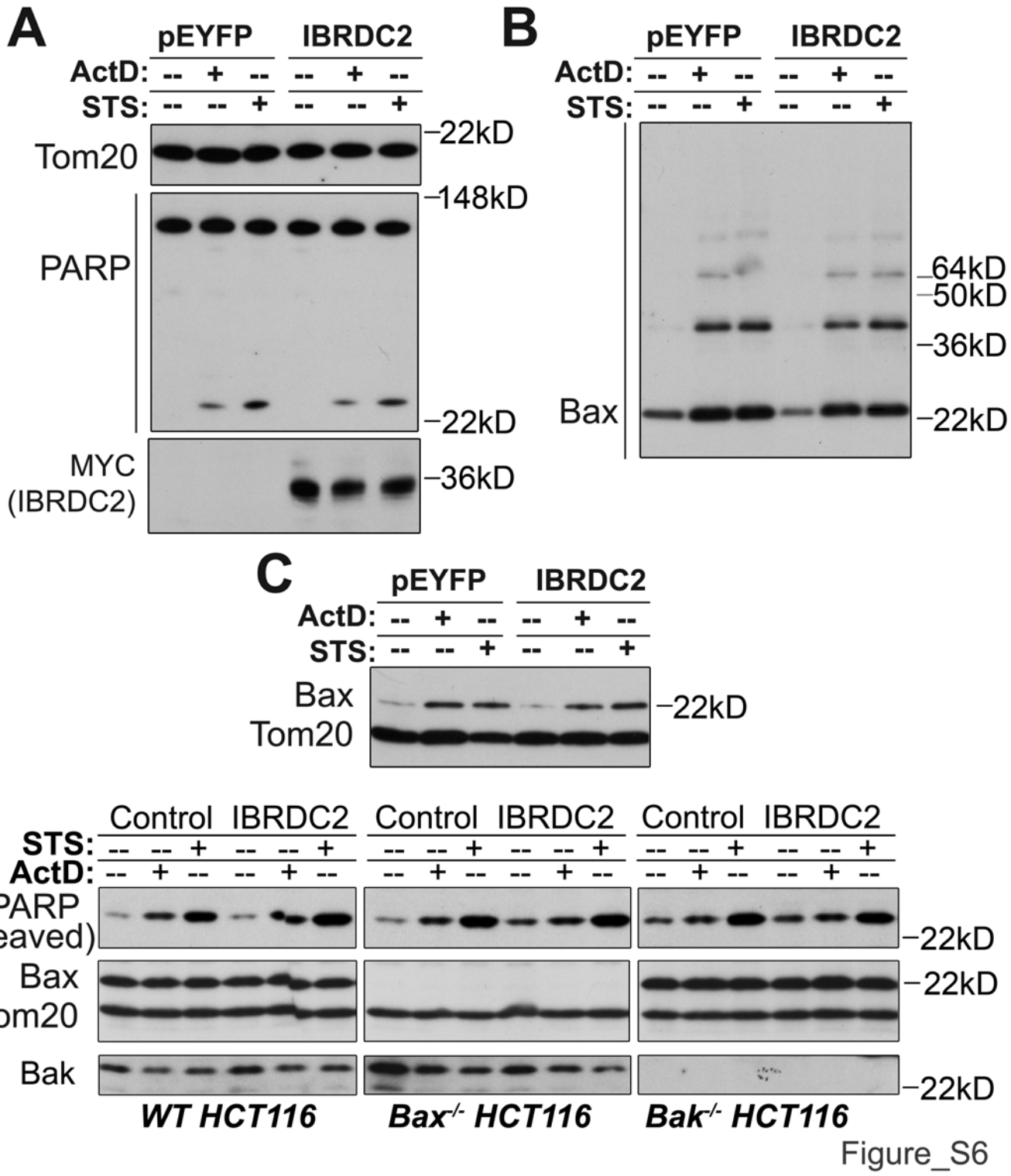
Figure_S3


A
Control RNAi
IBRDC2 RNAi
0 8 16 24 32 0 8 16 24 32 Time (hr)
1.0 0.67 0.7 0.59 0.29 1.0 0.87 0.91 0.84 0.77 Bax level
Bax
22kD
Tom20
22kD
B
HeLa
Control
CHX
MG132
Bax
β
α22kD
Tom20
22kD
C
HCT116 Bax-/-
HCT116 WT
Control
CHX
MG132
Control
CHX
MG132
Bax
β
α22kD
Tom20
22kD

Figure_S4


A
ActD
20
66
146
242
480
720
1048
(kD)
62kD
49kD
38kD
28kD
28kD
17kD
B
STS
20
66
146
242
480
720
1048
(kD)
62kD
49kD
38kD
28kD
28kD
17kD

Figure_S5


A
pEYFP
IBRDC2
ActD: -- + -- -- + --
STS: -- -- + -- -- +
Tom20
22kD
148kD
PARP
22kD
MYC
(IBRDC2)
36kD
B
pEYFP
IBRDC2
ActD: -- + -- -- + --
STS: -- -- + -- -- +
64kD
50kD
36kD
Bax
22kD
C
pEYFP
IBRDC2
ActD: -- + -- -- + --
STS: -- -- + -- -- +
Bax
Tom20
22kD
D
Control
IBRDC2
Control
IBRDC2
Control
IBRDC2
STS: -- -- + -- -- +
ActD: -- + -- -- + --
PARP
(cleaved)
22kD
Bax
Tom20
22kD
Bak
22kD
WT HCT116
Bax-/- HCT116
Bak-/- HCT116

Figure_S6