Supplemental Figures

The role of IAP antagonist proteins in the core apoptosis pathway of the mosquito disease vector *Aedes aegypti*

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Fig. S1. Expression of *mx* and *imp* was specifically silenced by addition of the corresponding dsRNA. (a) Levels of *mx* and *actin* mRNA were determined by RT-PCR at the indicated time points (hrs) following treatment with *mx* dsRNA, *cat* dsRNA or mock treatment. As a control, PCR was performed without the reverse transcription step to confirm the lack of DNA contamination in the cells (non-RT-PCR). (b) Levels of *imp* and *actin* mRNA were determined by RT-PCR at the indicated time points following treatment with *imp* dsRNA, *cat* dsRNA or mock treatment.

Fig. S2. Aag2 cells were treated with the indicated dsRNAs for 24 h, and the cells were then treated with 50 ng ml^{-1} of ActD (a) or 5 µg ml^{-1} of *Aeiap1* dsRNA (b). Twelve hours later, the levels of *mx, imp* and *actin* mRNA were determined by RT-PCR.

Fig. S3. Recombinant IAP antagonist proteins used in this study. (a) Conserved IAP-binding motifs (IBM) found at the N-termini of IAP antagonists. The tetrapeptide motif has the consensus sequence A-(V/I)-(A/P)-(F/Y). (b) Schematic of IAP antagonists and corresponding mutants constructed in this study. (c) Immunoblotting of IAP antagonist and mutant proteins with anti-His antibody.

Fig. S4. Recombinant AeIAP1 and truncation mutants used in this study. (a) Schematic of GST-tagged AeIAP1constructs. (b) Coommassie Blue staining of recombinant AeIAP1 full length and truncated proteins.

Fig. S5. Interaction between *Drosophila* Dronc and DIAP1. (a) *In vitro* translated Dronc was incubated with the indicated recombinant proteins or buffer alone, and protein complexes were purified using glutathione-agarose beads and examined by autoradiography. (b) Coommassie Blue staining of recombinant proteins used in the pull down assay. The two lanes shown for some of the proteins are merely different elution fractions obtained during purification.

Fig. S6. The P10 and S100 fractions isolated from mosquito Aag2, C6/36, or S2 cells were analyzed by immunoblotting with anti-cytochrome C and anti-actin antibodies.

Fig. S7. Fifty μg S100 lysate isolated from C6/36 cells was incubated with recombinant protein (Mx or IMP or control GB) (10 μM) prior to determining caspase activity using Ac-IETD-AFC as a substrate. The data shown represent the mean +/- SEM of three independent experiments (***P<0.0001; NS non-significant by Student's t test).

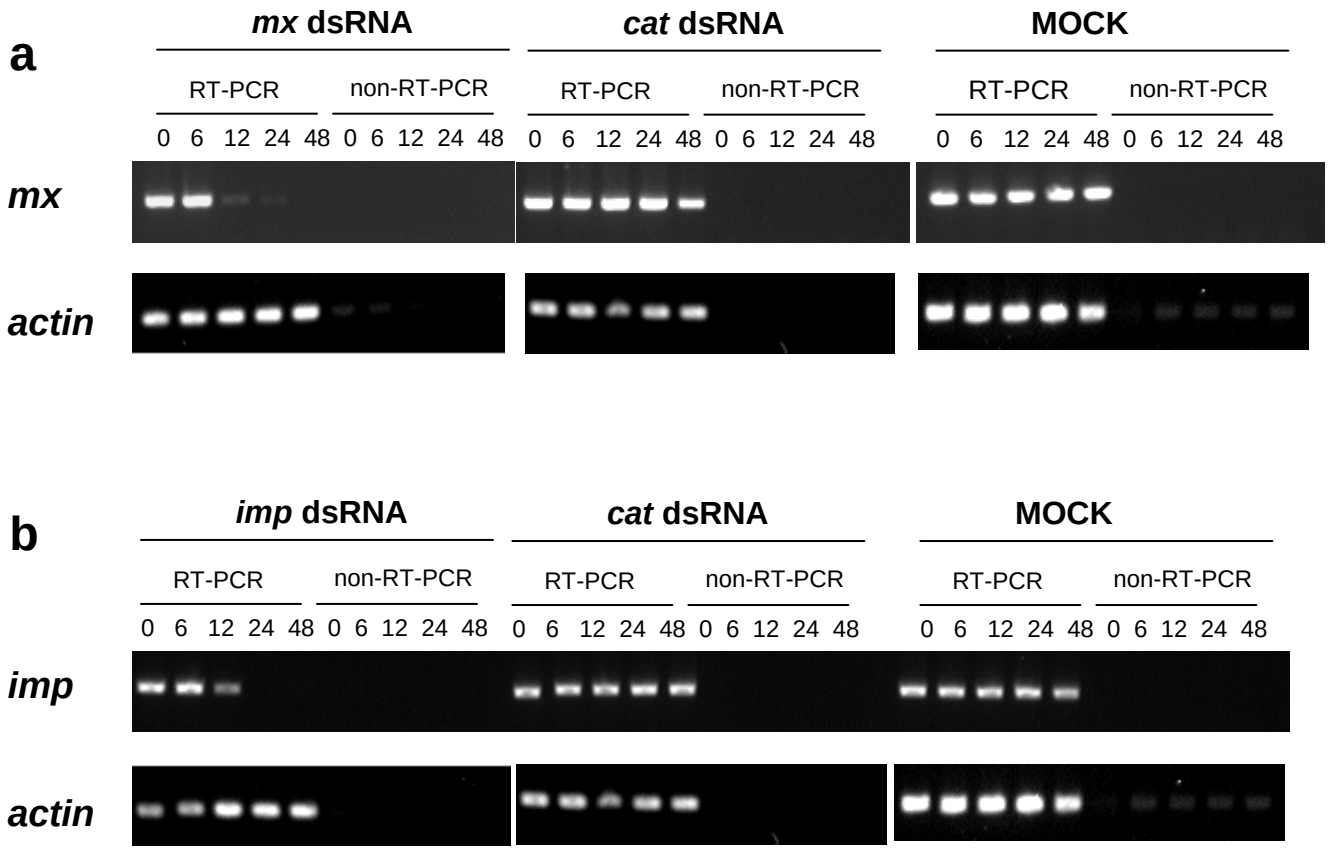

a
mx dsRNA
cat dsRNA
MOCK
RT-PCR
non-RT-PCR
0 6 12 24 48 0 6 12 24 48
mx
actin
b
imp dsRNA
cat dsRNA
MOCK
RT-PCR
non-RT-PCR
0 6 12 24 48 0 6 12 24 48
imp
actin

Fig. S1

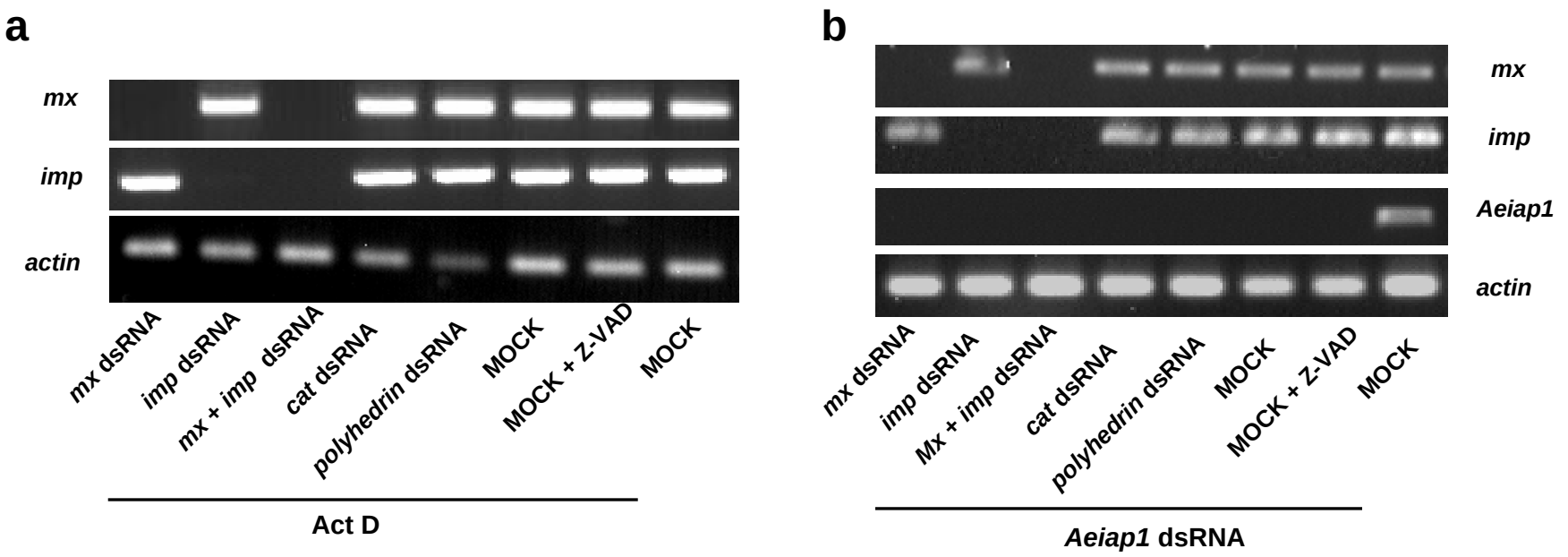

a
mx
imp
actin
mx dsRNA
imp dsRNA
mx + imp dsRNA
cat dsRNA
polyhedrin dsRNA
MOCK
MOCK + Z-VAD
MOCK
Act D
b
mx
imp
Aeiap1
actin
mx dsRNA
imp dsRNA
Mx + imp dsRNA
cat dsRNA
polyhedrin dsRNA
MOCK
MOCK + Z-VAD
MOCK
Aeiap1 dsRNA

Fig. S2

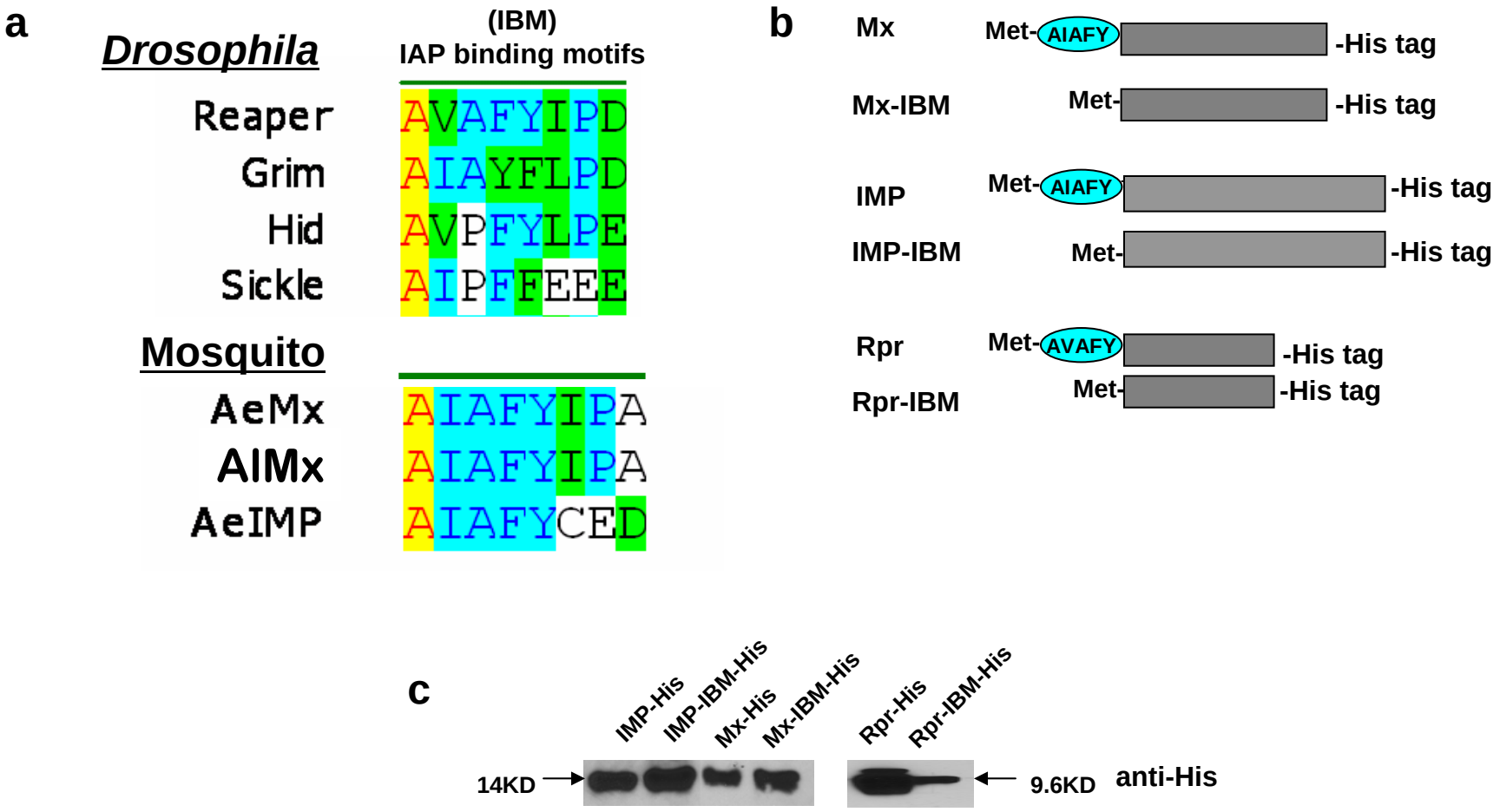
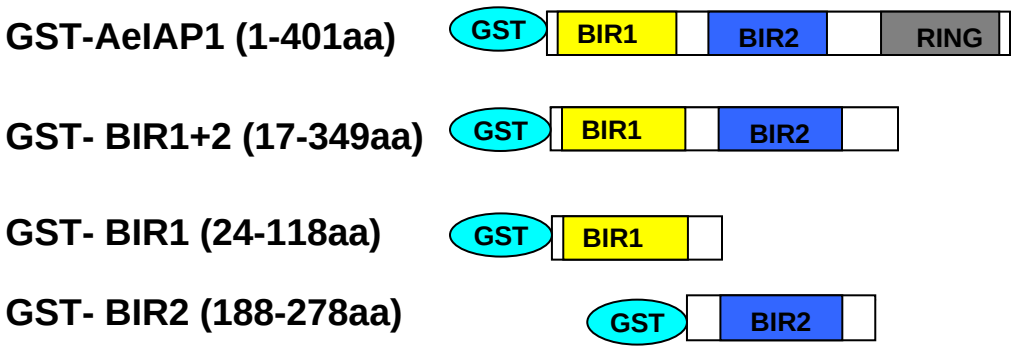

a
Drosophila
(IBM)
IAP binding motifs
Reaper
Grim
Hid
Sickle
AVAFYIPD
AIAYFLPD
AVPFYLPE
AIPFFEEE
Mosquito
AeMx
AlMx
AeIMP
AIAFYIPA
AIAFYIPA
AIAFYCED
b
Mx
Met-
AIAFY
-His tag
Mx-IBM
Met-
-His tag
IMP
Met-
AIAFY
-His tag
IMP-IBM
Met-
-His tag
Rpr
Met-
AVAFY
-His tag
Rpr-IBM
Met-
-His tag
c
IMP-His
IMP-IBM-His
Mx-His
Mx-IBM-His
Rpr-His
Rpr-IBM-His
14KD
9.6KD
anti-His

Fig. S3

a


GST-AeIAP1 (1-401aa)
GST
BIR1
BIR2
RING
GST- BIR1+2 (17-349aa)
GST
BIR1
BIR2
GST- BIR1 (24-118aa)
GST
BIR1
GST- BIR2 (188-278aa)
GST
BIR2

b

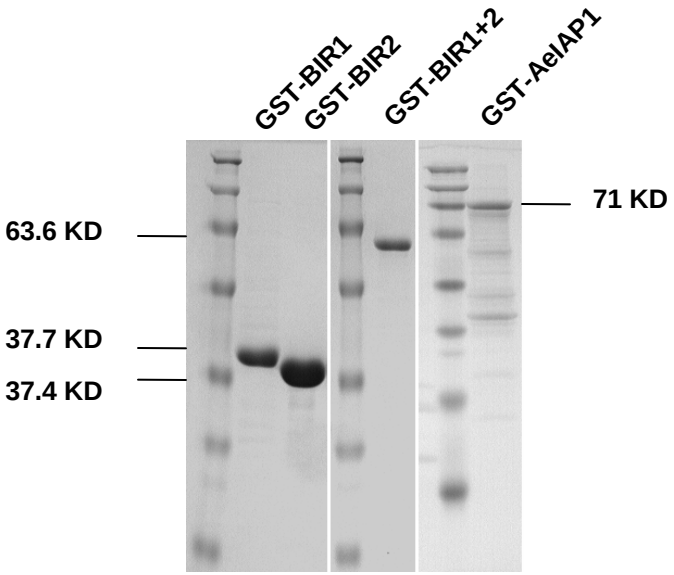

GST-BIR1
GST-BIR2
GST-BIR1+2
GST-AeIAP1
63.6 KD
37.7 KD
37.4 KD
71 KD

Fig. S4

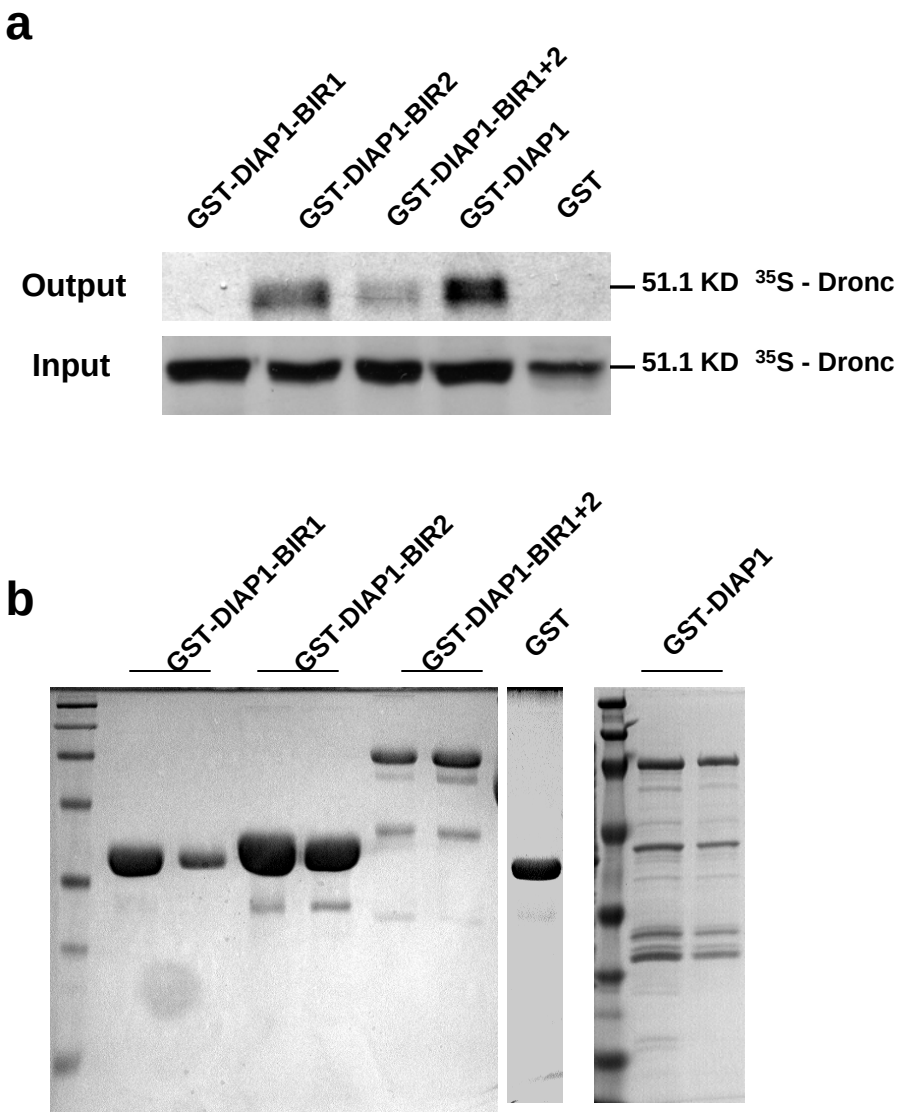

a
GST-DIAP1-BIR1
GST-DIAP1-BIR2
GST-DIAP1-BIR1+2
GST-DIAP1
GST
Output
51.1 KD 35S - Dronc
Input
51.1 KD 35S - Dronc
b
GST-DIAP1-BIR1
GST-DIAP1-BIR2
GST-DIAP1-BIR1+2
GST
GST-DIAP1

Fig. S5

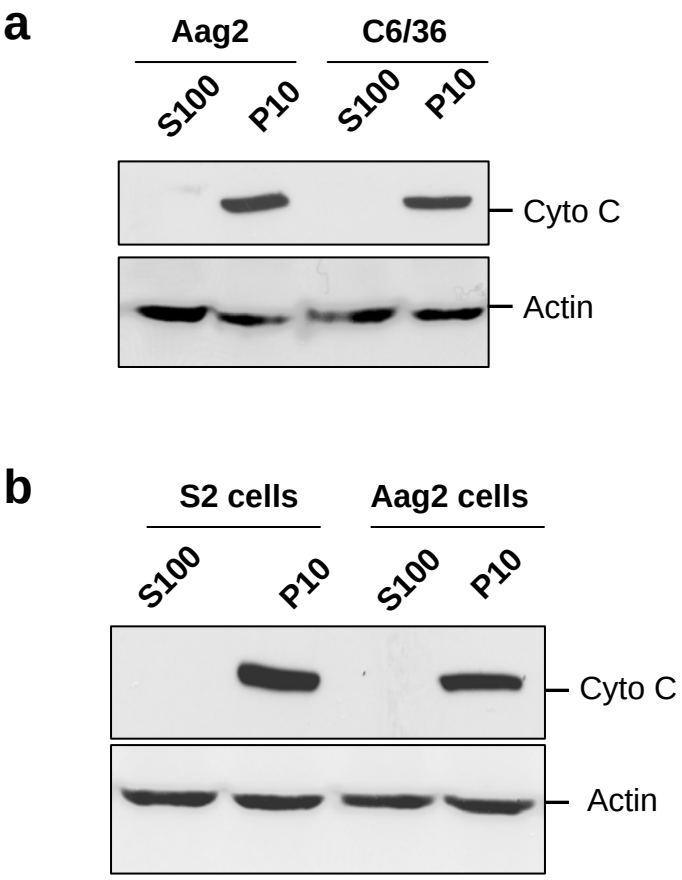

a
Aag2
C6/36
S100
P10
S100
P10
Cyto C
Actin
b
S2 cells
Aag2 cells
S100
P10
S100
P10
Cyto C
Actin

Fig. S6

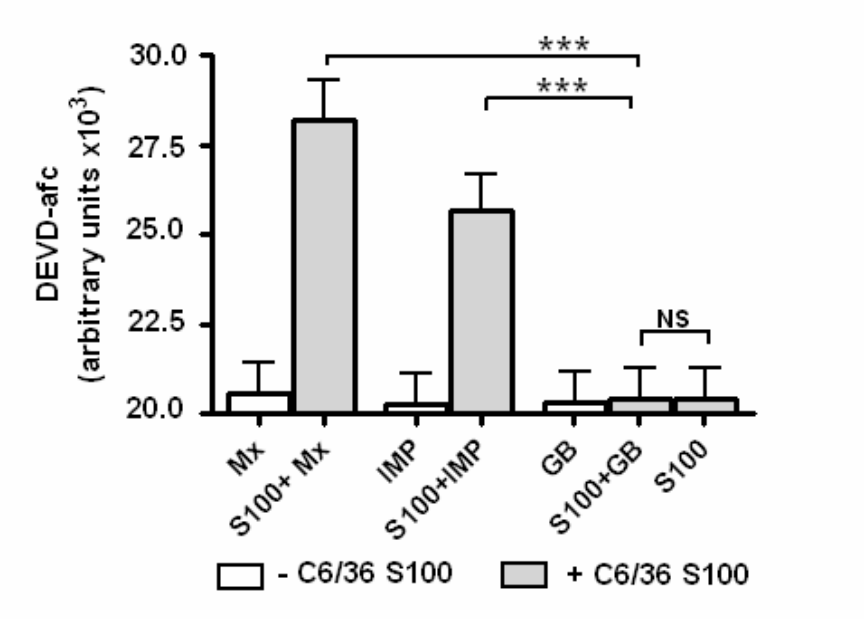

DEVD-afc
(arbitrary units x10^3)
30.0
27.5
25.0
22.5
20.0
***
***
NS
Mx
S100+ Mx
IMP
S100+IMP
GB
S100+GB
S100
- C6/36 S100
+ C6/36 S100

Fig. S7