Supplemental Materials

Molecular Biology of the Cell

Uetake and Sluder

Figure S1A. MCL1 knockdown. Asynchronous cultures were transfected with MCL1 siRNA and 48 hours later cells were collected for western blotting to quantify MCL1 expression levels. Also shown is MCL1 expression in parallel untransfected cells. Blots were probed with antibodies against MCL1 and alpha tubulin.

Figure S1B. Knockdown of FBW7 stabilizes MCL1 levels during prolonged prometaphase. Asynchronous cultures were transfected with FBW7 siRNA and 48 hours later treated with nocodazole for 3 hours. Mitotic shake off cells were then cultured another 3 hours in nocodazole so that all cells assayed spent 3-6 hours in prometaphase. The cells were collected during drug washout and prepared for western blotting to quantify MCL1 expression levels. Also shown is MCL1 expression in nocodazole treated untransfected cells. Blots were probed with antibodies against MCL1 and alpha tubulin.

Figure S1C. Knockdown of MCL1 reduces the temporal tolerance for prolonged prometaphase. Forty-eight hours after siRNA transfection, asynchronous cultures were treated with nocodazole for 6 hours and after drug washout, daughters of previously followed mothers were continuously followed. Significantly fewer daughters born of mothers spending < 1.5 hours in prometaphase proliferated relative to the basic experiment (Figure 2A): $p = 0.0020$. For the daughters born of mothers spending >1.5 hours in prometaphase, there was no significant increase in the proportion of cells that proliferated ($p = 1.0$).

Figure S1D. Transfection with control siRNA does not alter the relationship between prometaphase duration and daughter cell proliferation (compare to Figure 2A). Forty eight hours after siRNA transfection, asynchronous cultures were treated with nocodazole for 6 hours and after drug washout daughters of previously followed mothers were continuously followed. For daughters born of mothers spending < 1.5 hours in prometaphase, no significant decrease in the proportion of daughter proliferation relative to the basic experiment (Figure 2A): $p = 0.94$. For the daughters born of mothers spending >1.5 hours in prometaphase, there was no significant increase in the proportion of cells that proliferated ($p = 1.0$).

Figure S2. Inhibition of Bcl-xL, Bcl2, and Bcl-w activities during prolonged prometaphase does not reduce the temporal tolerance for prolonged prometaphase. Asynchronous cultures were treated with 1uM ABT-263 (Navitoclax) in combination with nocodazole for 6 hours and both drugs washed out. Upper panel: Daughters that proliferated are shown as light bars and those that arrested but remained alive as dark bars. Three arrested daughters (asterisk above bars) died 63-104 hours after nocodazole removal. Lower panel: Same preparations mother cells that died during prometaphase. Bar heights represent time in prometaphase before cell death. Included are 3 mothers whose progeny died within 2 hours of nocodazole removal (asterix above bars).

Figure S3A. Knockdown of Bak plus Bax allows some daughter cells to proliferate even though their mothers spent up to 4 hours in prometaphase. Forty eight hours after siRNA transfection, asynchronous cultures were treated with nocodazole for 6 hours, and

daughters were continuously followed thereafter. Significantly more daughters born of mothers spending > 1.5 hours in prometaphase proliferated relative to the basic experiment (Figure 2A): $p = 0.0012$. For the daughters born of mothers spending <1.5 hours in prometaphase, there was no significant decrease in the proportion of cells that proliferated ($p = 1.0$).

Figure S3B. Activated caspase 3 levels in mitotic cells from populations held in mitosis with 0.08 uM nocodazole for up to 30 minutes or up to 6 hours. Cultures were fixed and immunostained for cleaved caspase 3 with a monoclonal antibody specific for the large fragment of cleaved/activated caspase 3. The difference between these population means is significant at the $p < 0.00001$ level by unpaired t test (n=30 each).

Figure S3C. Antibody specificity: asynchronous cultures were treated with 1.6 uM nocodazole for 24 hours and then prepared for western blotting with the monoclonal antibody specific for the large fragment of cleaved/activated caspase 3. Only one band of proper molecular weight was detected.

Figure S3D. P38 activation after prolonged prometaphase in presence of caspase inhibitor. Asynchronous cultures were treated with 20uM z-VAD (OMe)-fmk for 12 hours to allow drug entry/cleavage. After inhibitor wash out, the cells were treated with nocodazole for 4 hours, and mitotic shake off cells were kept in nocodazole for another 2 hours. One and a half hours after nocodazole washout, the daughter cells were processed for western blotting to quantify phospho-p38 levels. As a control, asynchronous cultures

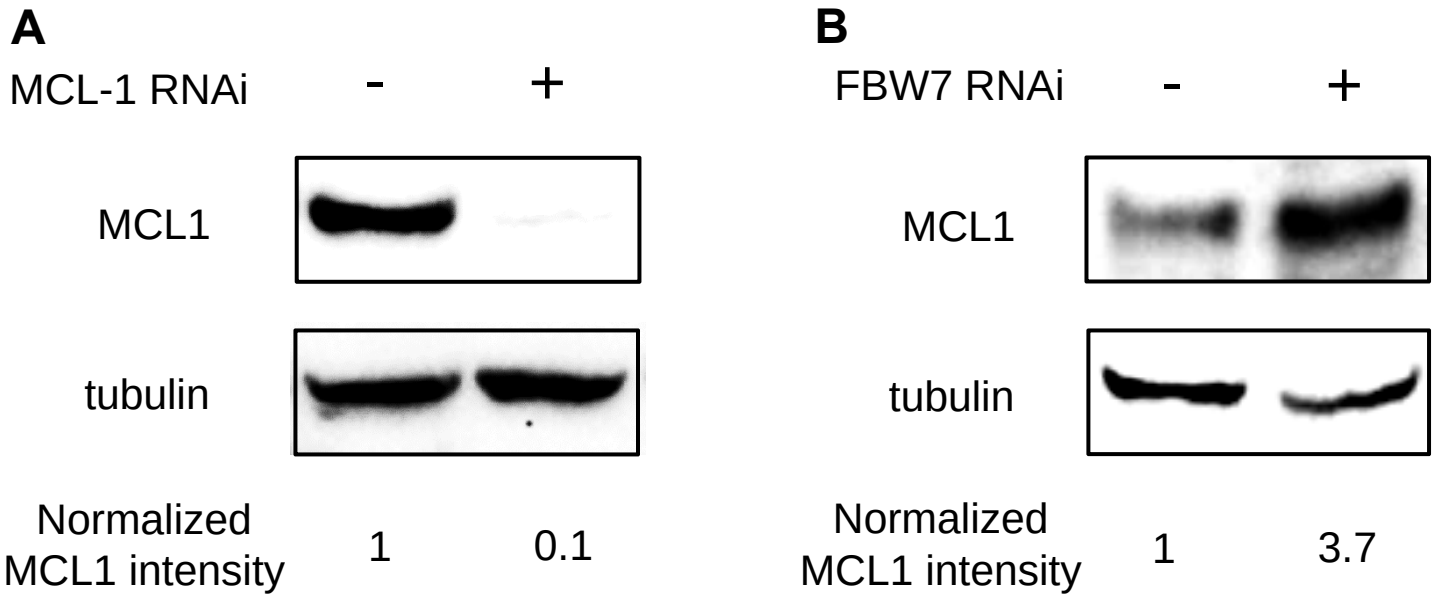
were treated same way with nocodazole but without 20uM z-VAD (OMe)-fmk pretreatment. For cultures treated with the caspase inhibitor, the levels of activated p38 were 42%, 64% and 48% those of the parallel control cultures (one example from three experiments shown here).

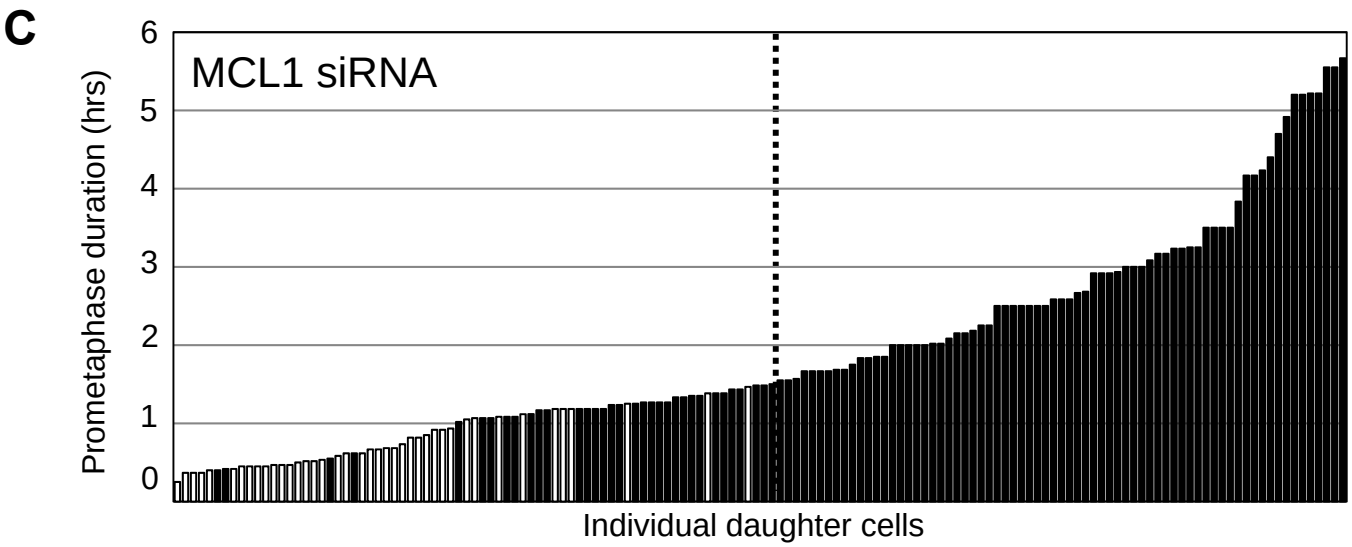
Figure S4A. External application of reduced glutathione just during prolonged prometaphase allows some daughter cells to proliferate even though their mothers spent between 1.5 and 4.5 hours in prometaphase. Asynchronous cultures were treated with 50 uM reduced glutathione in combination with nocodazole for 6 hours and both washed out at ambient oxygen levels. Significantly more daughters born of mothers spending > 1.5 hours in prometaphase proliferated relative to the basic experiment (Figure 2A): $p = 1.5 \times 10^{-6}$. For the daughters born of mothers spending <1.5 hours in prometaphase, there was no significant decrease in the proportion of cells that proliferated ($p = 1.0$).

Figure S4B. External application of oxidized glutathione just during prolonged prometaphase does not promote the proliferation of daughter cells born of mothers spending >1.5 hours in prometaphase. Asynchronous cultures were treated with 50 uM oxidized glutathione in combination with nocodazole for 6 hours and both washed out at ambient oxygen levels. For daughters born of mothers spending < 1.5 hours in prometaphase, no significant decrease in the proportion of daughter proliferation relative to the basic experiment (Figure 2A): $p = 1.0$. For the daughters born of mothers spending >1.5 hours in prometaphase, there was no significant increase in the proportion of cells that proliferated ($p = 1.0$).

Figure S4C. Incidence of DNA damage for cells held 6 – 10 hours in prometaphase (for the experiment shown in Figure 4B). Asynchronous populations were treated with nocodazole for 10 hours at 3% oxygen and after drug washout, daughter cells were continuously followed at ambient oxygen levels. Twelve hours after nocodazole washout, preparations were fixed for immunostaining of H2AX foci in the nuclei of the daughter cells**.** Upper panel: The open circles above the mother cell prometaphase duration line show daughters without H2AX foci and the filled circles below the time line show daughters with H2AX foci. Lower panels: Left pair shows a daughter with no H2AX foci; its mother spent 8 hours and 55 minutes in prometaphase. The right pair shows a daughter with H2AX foci; its mother spent 7 hours and 32 minutes in prometaphase. Phase contrast/fluorescence images, bar = 20 um.

Figure S1


A
MCL-1 RNAi
-
+
MCL1
tubulin
Normalized MCL1 intensity
1
0.1
B
FBW7 RNAi
-
+
MCL1
tubulin
Normalized MCL1 intensity
1
3.7


C
MCL1 siRNA
Prometaphase duration (hrs)
0
1
2
3
4
5
6
Individual daughter cells

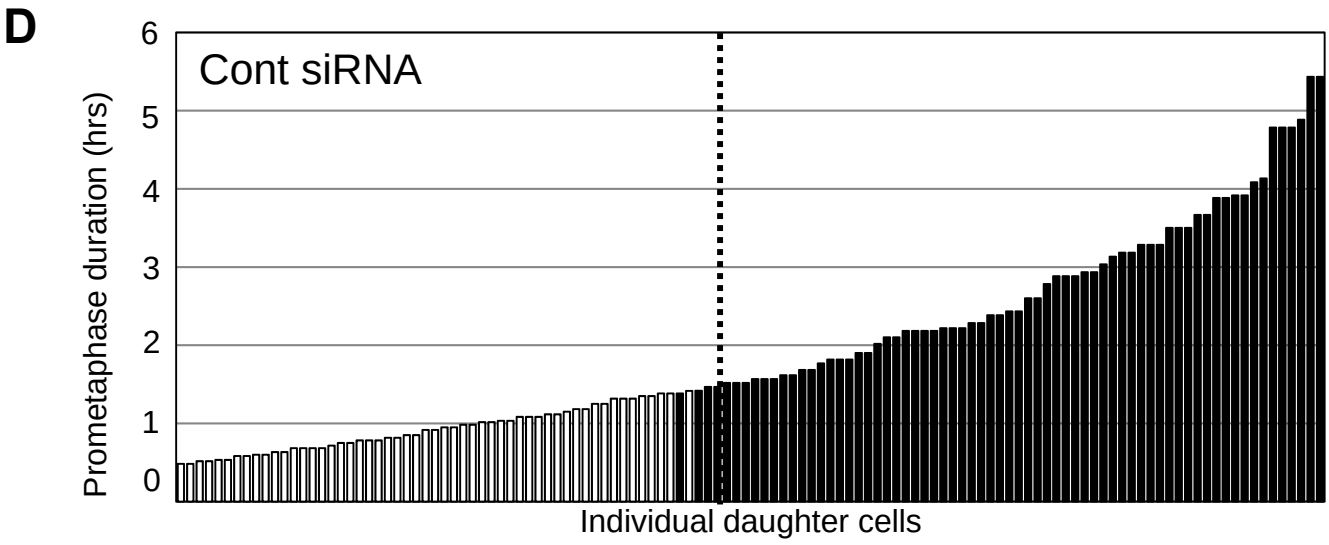

D
Cont siRNA
Prometaphase duration (hrs)
0
1
2
3
4
5
6
Individual daughter cells

Figure S2 Navitoclax (ABT-263)

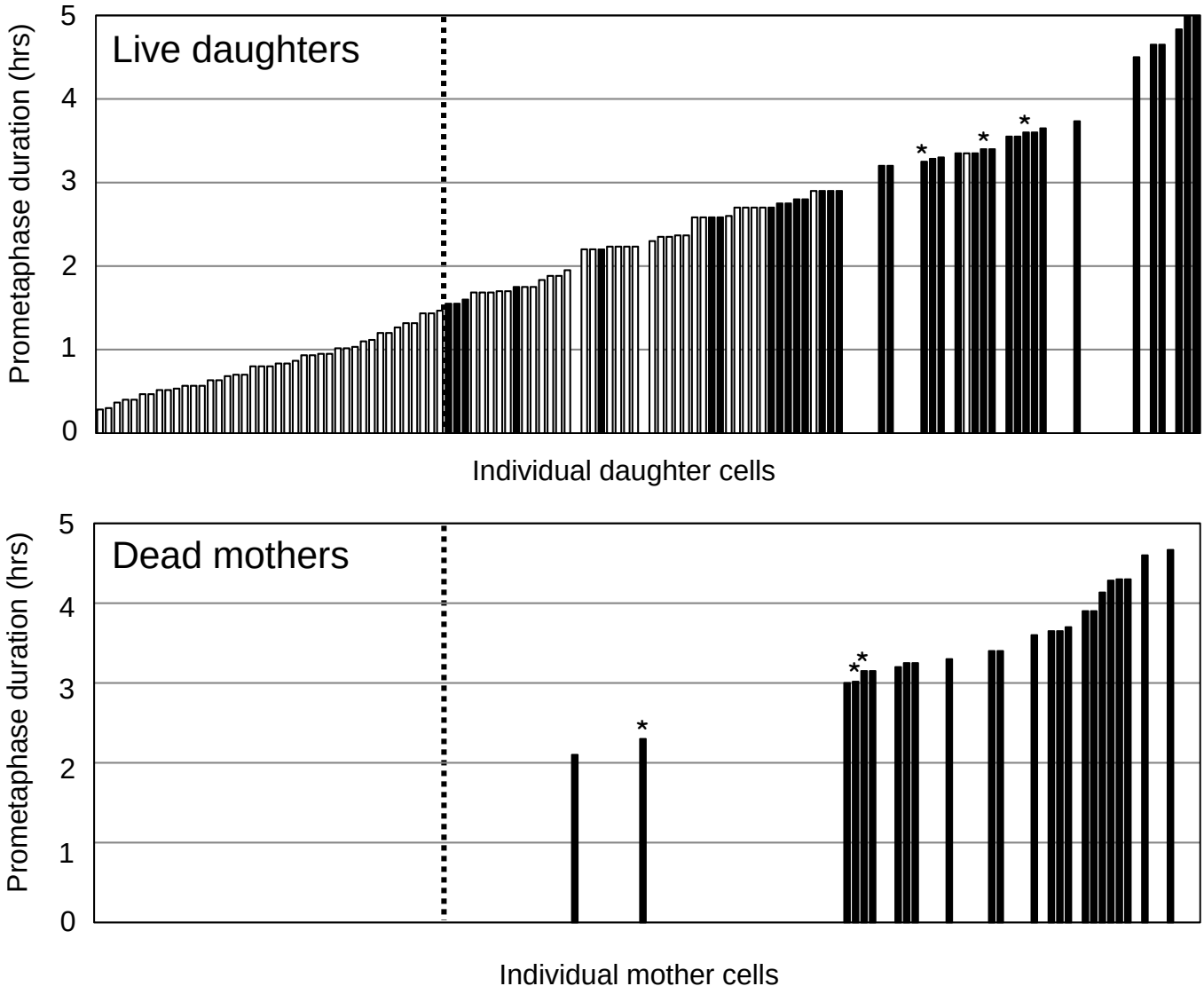


Figure S3

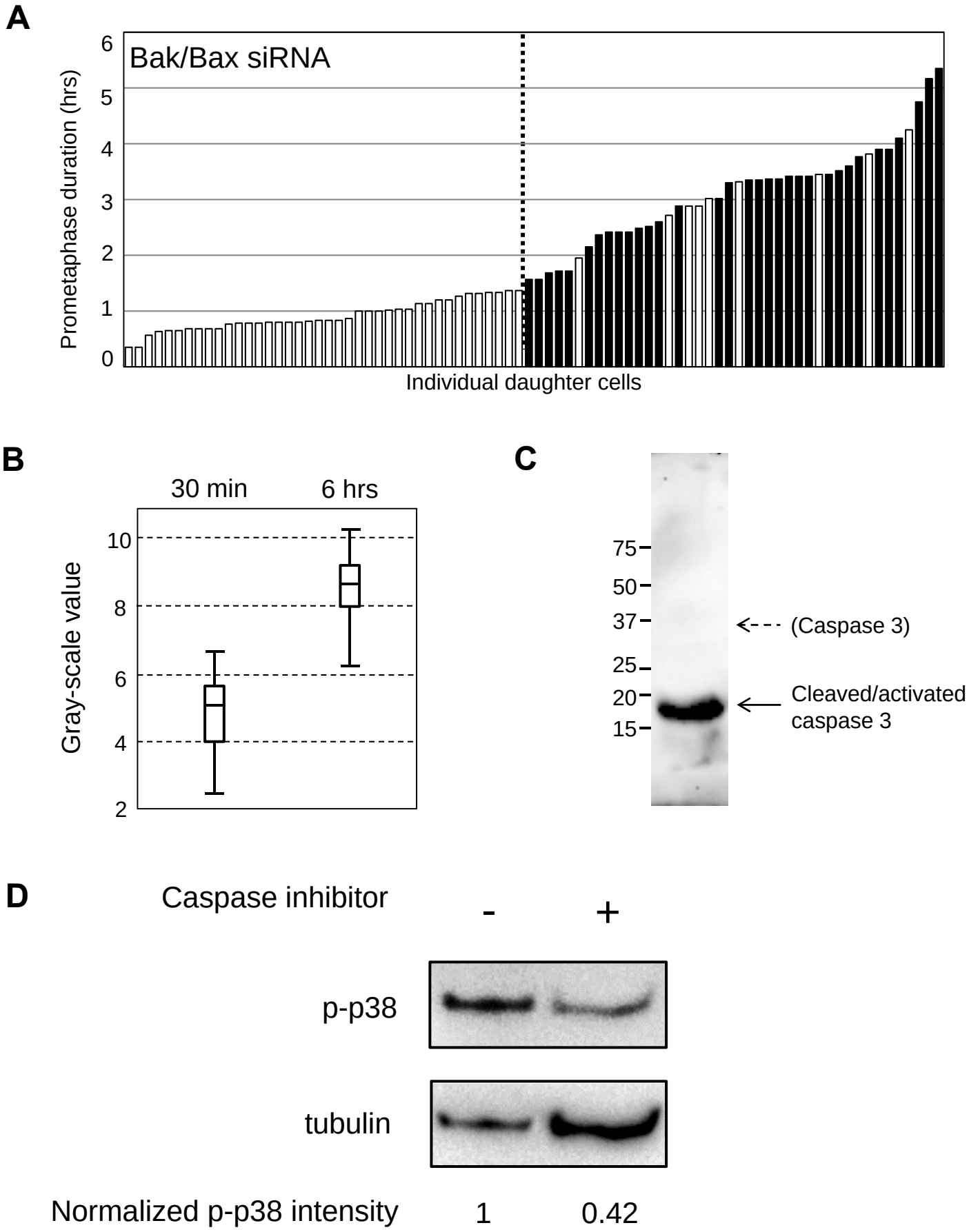
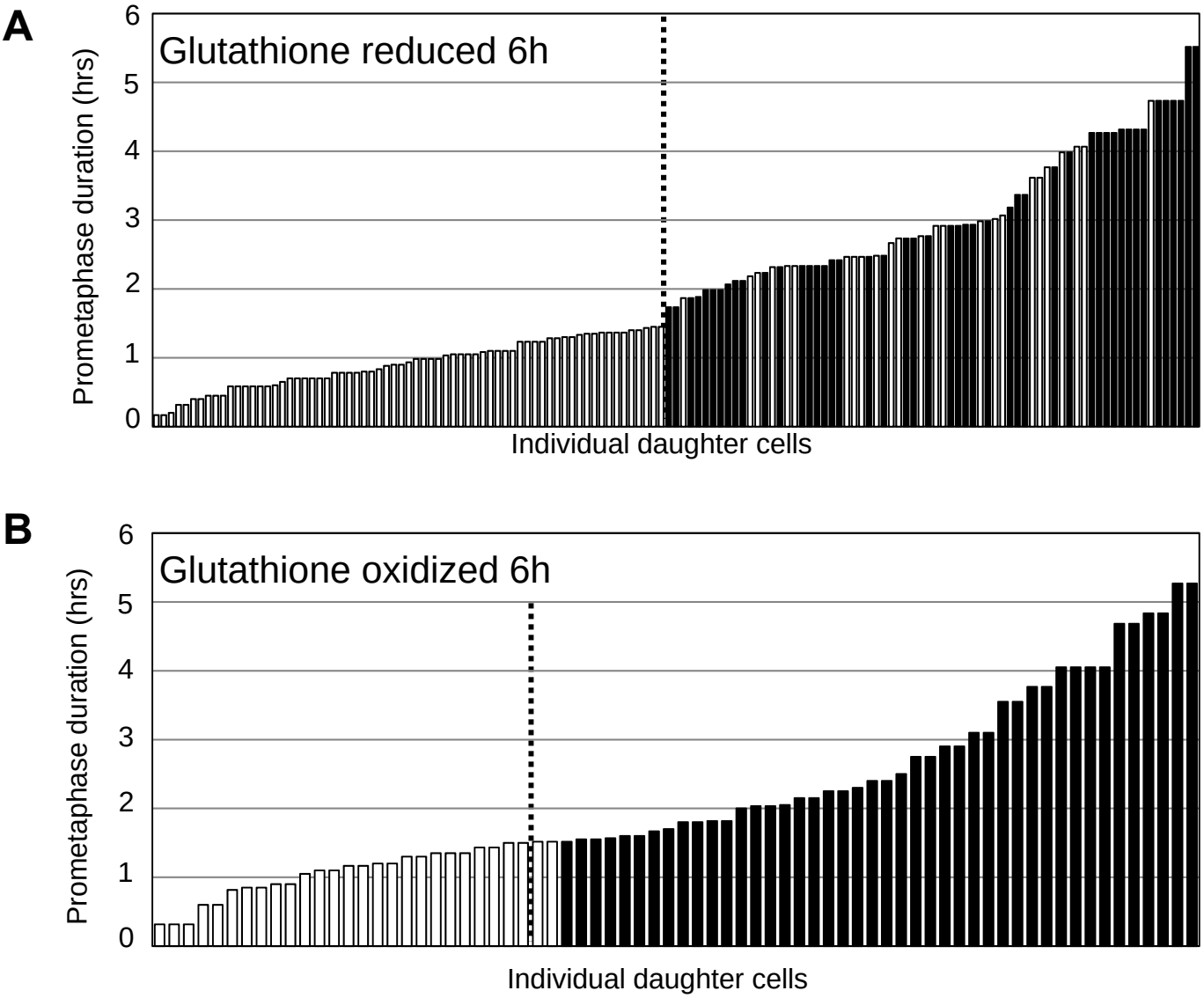

A
Bak/Bax siRNA
Prometaphase duration (hrs)
0
1
2
3
4
5
6
Individual daughter cells
B
30 min
6 hrs
Gray-scale value
2
4
6
8
10
C
75
50
37
25
20
15
(Caspase 3)
Cleaved/activated caspase 3
D
Caspase inhibitor
-
+
p-p38
tubulin
Normalized p-p38 intensity
1
0.42

Figure S4


A
Glutathione reduced 6h
Prometaphase duration (hrs)
0
1
2
3
4
5
6
Individual daughter cells
B
Glutathione oxidized 6h
Prometaphase duration (hrs)
0
1
2
3
4
5
6
Individual daughter cells
C
Mother cell
prometaphase
(hr)
6
7
8
9
10
No DNA damage
(26/50)
DNA damage
(24/50)
H2A.X
Phase
8:55
7:32