Supplementary Figure 1 - Gating Schematics for FACS Data

Growth Curve Analysis (Fig. 1b and Extended Data Fig. 1d) - Refer to Materials and Methods for Experimental Design



Gating for CountBright Absolute Counting Beads



Cell Cycle Analysis (Fig. 1c and Extended Data Fig. 1e) - Refer to Materials and Methods for Experimental Design



Apoptosis Analysis (Fig. 1d and Extended Data Fig. 1f) - Refer to Materials and Methods for Experimental Design



Cleaved Caspase 3-FITC

Cleaved Caspase 3-FITC

Supplementary Figure 2 - Uncropped Western Blots

Fig. 1a:

Exposure used for Enolase



Exposure used for HA-Cdk12



Hours

Anti-HA High Affinity Antibody (Roche 11867423001)

Approximate location membrane was cut.

Enolase I (CST 3810S)

Fig. 1f:



Figure 1e Uncropped Blots Control Lanes:

+Lane (Positive Control)= wild type mESCs treated with 1uM Doxorubicin for 6hrs -Lane (Negative Control)= wild type mESCs treated with DMSO (vehicle control) for 6hrs

Fig. 4d:

Cdk12∆ Cells		Cdk12∆ C	ells		Cdk12∆ Cells	
Pada +dox -dox 24hrs -dox 48hrs -dox 72hrs -dox 96hrs	kDa kDa +	-dox 24hrs -dox 48hrs	sult / xop- kDa	kDa +	-dox 24hrs -dox 48hrs -dox 72hrs -dox 96hrs	kDa
460 - 248 = - 171 + 177 - 71 - 55 - 41 -	$\begin{array}{c} 460 - \\ 268 - \\ 238 - \\ 150 - \\ 150 - \\ 171 + \\ 117 - \\ 76 - \\ 55 - \\ 52 - \\ 38 - \\ 11 - \\ 38 - \\ 11 - \\ 38 - \\ 11 - \\ 38 - \\ 11 - \\ 38 - \\ 11 - \\ 38 - \\ 11 - \\ 38 - \\ 11 - \\ 38 - \\ 11 - \\ 38 - \\ 11 - \\ 38 - \\ 11 - \\ 38 - \\ 11 - \\ 38 - \\ 11 - \\ 38 - \\ 11 - \\ 38 - \\ 11 - \\ 38 - \\ 11 - \\ 38 - \\ 11 - \\ 38 - \\ 11 - \\ 38 - \\ 11 - \\ 38 - \\ 11$			$\begin{array}{r} 460 \\ -268 \\ 238 \\ = \\ 171 \\ + \\ -1 \\ -1 \\ -1 \\ -1 \\ -1 \\ -1 \\ -1$		-225 - 150 - 102 - 76 - 52, -38
		/ A la a a sa	ah (00000)			4.24

ATR (CST 13934S)



Vinculin (Sigma V9131)

Fig. 4e:

	V5	V5 Tagged ATM clone 1					V5 Tagged ATM clone 7				
kDa	xop+	-dox 24hrs	-dox 48hrs	-dox 72hrs	-dox 96hrs	+dox	-dox 24hrs	-dox 48hrs	-dox 72hrs	-dox 96hrs	Contorl
160 - 218 = 238 = 171 + 171 + 171 - 71 - 71 - 55 -	· · ··································										

V5 (Life Technologies R96025)





Notes:

- (1) Control lane = lysate from untagged, Cdk12∆ cells (+Dox) shown as an antibody specificity control.
- (2) Second, independently-derived endogenous V5-tagged ATM clone also shown.

Fig. 4e (cont.):







Notes:

- (1) Control lane = lysate from untagged, Cdk12∆ cells (+Dox) shown as an antibody specificity control.
- (2) Second, independently-derived endogenous V5-tagged BRCA2 clone also shown.







Notes:

- (1) Control lane = lysate from untagged, Cdk12∆ cells (+Dox) shown as an antibody specificity control.
- (2) Second, independently-derived endogenous V5-tagged FANCD2 clone also shown.

Extended Data Fig. 1c:

Exposure used for Enolase



Hours

Anti-HA High Affinity Antibody (Roche 11867423001)

Approximate location membrane was cut.

Enolase I (CST 3810S)





Hours

Anti-HA High Affinity Antibody (Roche 11867423001)

Approximate location membrane was cut.

Enolase I (CST 3810S)

Extended Data Fig. 1h:

Total p53 Gel



pSer15-p53 Gel



Extended Data Fig. 1h Uncropped Blots Control Lanes:

+Lane (Positive Control)= wild type mESCs treated with 1uM Doxorubicin for 6hrs -Lane (Negative Control)= wild type mESCs treated with DMSO (vehicle control) for 6hrs

Extended Data Fig. 10c:

22RV1



PC-3



Note:

* indicates a band from a separate, prior antibody staining that did not strip off completely.

Extended Data Fig. 10c (cont.):

OVCAR4

