

SUPPLEMENTARY ONLINE DATA

ATR (ataxia telangiectasia mutated- and Rad3-related kinase) is activated by mild hypothermia in mammalian cells and subsequently activates p53

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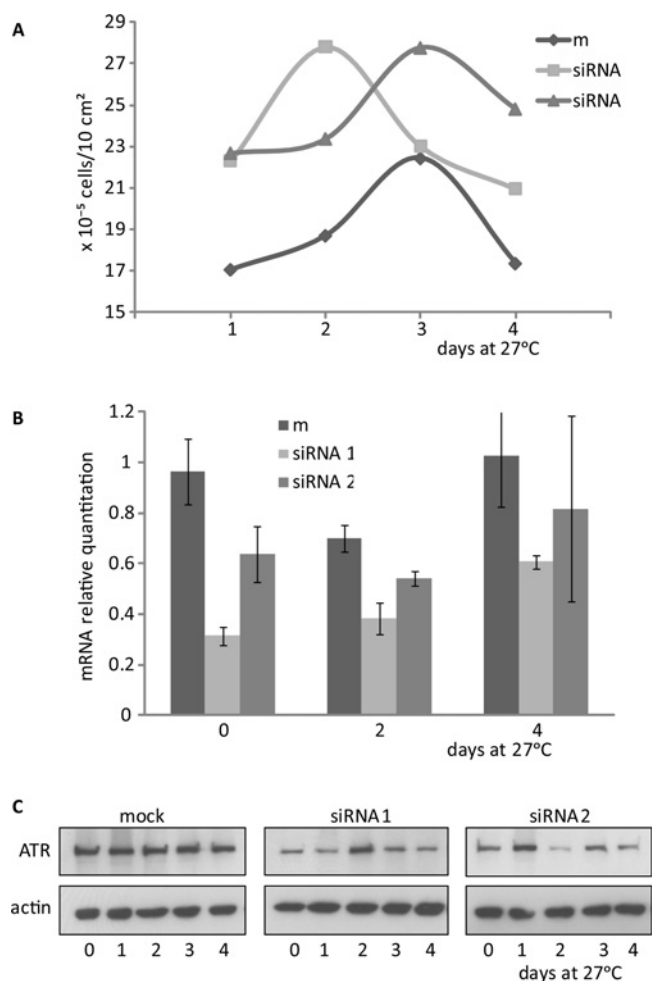


Figure S1 Effect of prolonged siRNA knockdown of ATR on cell proliferation at 27°C

CHO-K1 cells were transfected with 5 nM ATR siRNA as described in the main text and then maintained at 37°C for 48 h prior to transfer to 27°C (day 0 on the indicated time scales). Samples were prepared on the indicated days of maintenance at 27°C for cell counts (A), qRT-PCR quantification of *ATR* mRNA (B), and immunoblot detection of ATR protein (C). Mock transfections (m), siRNA1 and siRNA2 are as described in the Experimental section of the main text.

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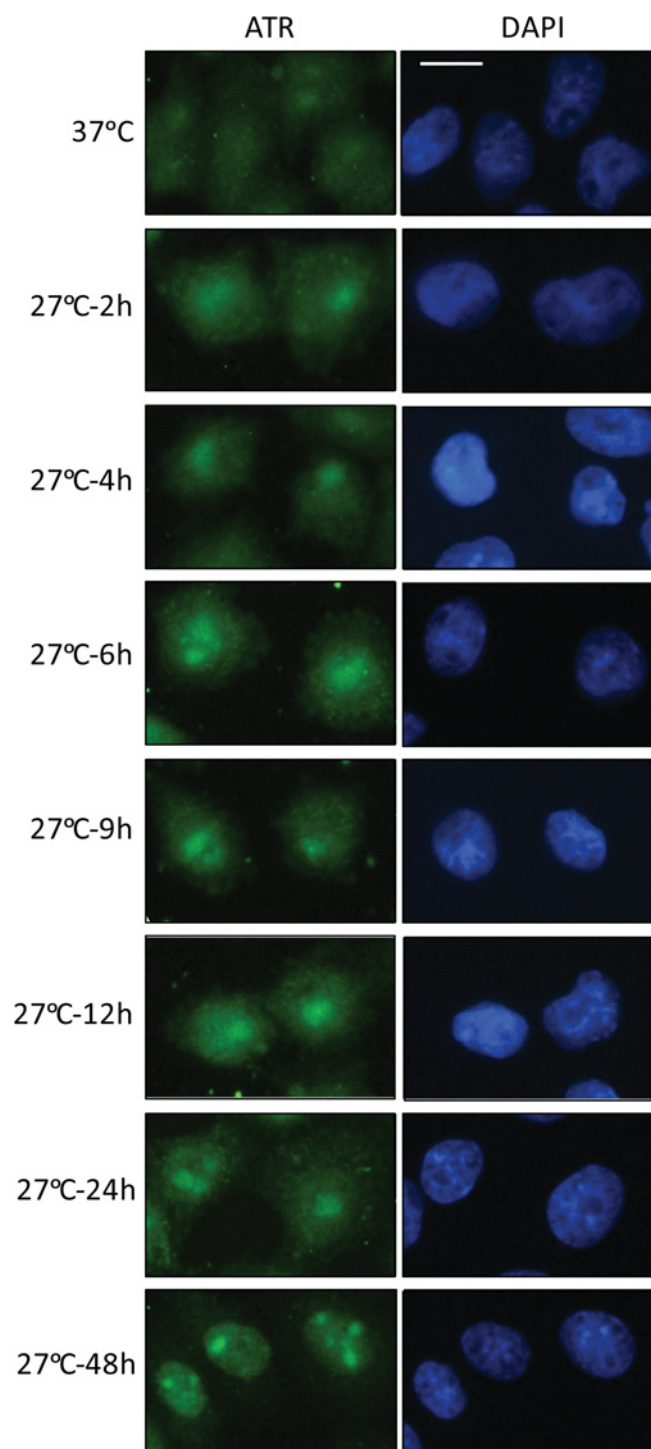


Figure S2 The intracellular localization of ATR changes during the early stages of hypothermia at 27 °C

Immunofluorescence detection of ATR and nuclei [visualized using DAPI (4',6-diamidino-2-phenylindole)] in CHO-K1 cells maintained at 37 °C and during the first 48 h after transfer to 27 °C. Scale bar, 10 μ m.

Table S1 Description of statistically significantly different metabolites

PA, phosphatidic acid; PE, phosphatidylethanolamine; PG, prostaglandin; PI, phosphatidylinositol; PS, phosphatidylserine.

(a) Class 1 (control maintained at 37 °C for 6 h with no treatment) compared with Class 2 (control maintained at 37 °C for 6 h with BEL treatment).

| P value | Fold difference | Lipid identification | Lipid class |
|---------|-------------------------|----------------------|-------------------------------|
| 0.00395 | 0.33 (lower in control) | PG(18:0/20:4) | Diacylglycerophosphoglycerols |
| 0.00395 | 0.35 (lower in control) | PG(18:0/22:6) | Diacylglycerophosphoglycerols |
| 0.00395 | 0.66 (lower in control) | Unidentified | Unidentified |
| 0.00395 | 0.58 (lower in control) | PI(18:0/18:0) | Diacylglycerophosphoinositols |
| 0.01041 | 0.66 (lower in control) | Unidentified | Unidentified |
| 0.01041 | 0.43 (lower in control) | PG(17:0/17:0) | Diacylglycerophosphoglycerols |
| 0.01631 | 0.73 (lower in control) | Unidentified | Unidentified |
| 0.01631 | 0.70 (lower in control) | Unidentified | Unidentified |
| 0.01631 | 0.76 (lower in control) | Unidentified | Unidentified |
| 0.02498 | 0.69 (lower in control) | Unidentified | Unidentified |

(b) Class 1 (control maintained at 37 °C for 6 h with no treatment) compared with Class 3 (maintained at 27 °C for 6 h).

| P value | Fold difference | Lipid identification | Lipid class |
|---------|-------------------------|----------------------|-----------------------------|
| 0.00395 | 0.32 (lower in control) | Unidentified | Unidentified |
| 0.00395 | 0.34 (lower in control) | PS(18:0/18:1) | Diacylglycerophosphoserines |
| 0.01041 | 0.42 (lower in control) | PA(16:0/18:1) | Diacylglycerophosphates |
| 0.02498 | 0.40 (lower in control) | Unidentified | Unidentified |

(c) Class 1 (control maintained at 37 °C for 6 h with no treatment) compared with Class 4 (maintained at 32 °C for 6 h).

| P value | Fold difference | Lipid identification | Lipid class |
|---------|----------------------------|---------------------------------------------------------------------------|----------------------------------------------|
| 0.00395 | 5.88 higher in hypothermia | Unidentified | Unidentified |
| 0.00395 | 2.08 higher in hypothermia | Unidentified | Unidentified |
| 0.00395 | 2.86 higher in hypothermia | PS(18:0/18:1) | Diacylglycerophosphoserines |
| 0.00395 | 3.22 higher in hypothermia | Unidentified | Unidentified |
| 0.00395 | 1.82 higher in hypothermia | Unidentified | Unidentified |
| 0.00395 | 2.13 higher in hypothermia | Unidentified | Unidentified |
| 0.00395 | 2.33 higher in hypothermia | Unidentified | Unidentified |
| 0.00395 | 2.22 higher in hypothermia | Unidentified | Unidentified |
| 0.00395 | 2.50 higher in hypothermia | PI(18:0/18:0) | Diacylglycerophosphoinositols |
| 0.00395 | 2.27 higher in hypothermia | Unidentified | Unidentified |
| 0.00649 | 3.13 higher in hypothermia | PA(16:0/18:1) | Diacylglycerophosphates |
| 0.00649 | 1.61 higher in hypothermia | Unidentified | Unidentified |
| 0.00649 | 1.88 higher in hypothermia | Unidentified | Unidentified |
| 0.01041 | 1.69 higher in hypothermia | PE(16:0/18:3) | Diacylglycerophosphoethanolamines |
| 0.01041 | 1.96 higher in hypothermia | 1-Tetrahexanoyl-2-(8-[3]-ladderane-octanyl)-sn-glycerophosphoethanolamine | 1-Acyl-2-alkylglycerophosphoethanolamines |
| 0.01041 | 1.89 higher in hypothermia | Unidentified | Unidentified |
| 0.01041 | 1.59 higher in hypothermia | PE(16:0/22:6) | Diacylglycerophosphoethanolamines |
| 0.01041 | 1.47 higher in hypothermia | Unidentified | Dialkylglycerophosphoglycerols |
| 0.01041 | 1.75 higher in hypothermia | Unidentified | Unidentified |
| 0.01041 | 1.92 higher in hypothermia | PI(16:0/18:0) | Diacylglycerophosphoinositols |
| 0.01631 | 1.82 higher in hypothermia | PA(16:0/16:0) | Diacylglycerophosphates |
| 0.01631 | 1.85 higher in hypothermia | Unidentified | Unidentified |
| 0.01631 | 1.54 higher in hypothermia | PS(17:0/20:4) | Diacylglycerophosphoserines |
| 0.01631 | 1.49 higher in hypothermia | Unidentified | Unidentified |
| 0.01631 | 1.72 higher in hypothermia | Unidentified | Unidentified |
| 0.01631 | 1.62 higher in hypothermia | Unidentified | Unidentified |
| 0.02498 | 1.72 higher in hypothermia | PE(P-16:0/22:6) | 1Z-alkenyl-2-acylglycerophosphoethanolamines |
| 0.02498 | 1.54 higher in hypothermia | PS(18:0/18:1) | Diacylglycerophosphoserines |
| 0.02498 | 1.47 higher in hypothermia | PS (20:0/18:2) | Diacylglycerophosphoserines |
| 0.02498 | 1.82 higher in hypothermia | PG(18:0/20:4) | Diacylglycerophosphoglycerols |
| 0.02498 | 1.82 higher in hypothermia | PG(18:0/22:6) | Diacylglycerophosphoglycerols |
| 0.02498 | 1.52 higher in hypothermia | PI(16:0/18:1) | Diacylglycerophosphoinositols |
| 0.02498 | 1.56 higher in hypothermia | PS (22:1/18:3) | Diacylglycerophosphoserines |
| 0.03737 | 1.92 higher in hypothermia | Unidentified | Unidentified |
| 0.03737 | 1.61 higher in hypothermia | PS(16:0/18:1) | Diacylglycerophosphoserines |
| 0.03737 | 1.92 higher in hypothermia | Unidentified | Unidentified |
| 0.04461 | 1.89 higher in hypothermia | Unidentified | Unidentified |

Table S1 Continued

(d) Class 3 (maintained at 27 °C for 6 h) compared with Class 5 (recovery at 37 °C for 2 h after a temperature of 27 °C for 6 h).

| <i>P</i> value | Fold difference | Lipid identification | Lipid class |
|----------------|----------------------------|----------------------|-------------------------------|
| 0.00649 | 4.23 higher in hypothermia | PS(18:0/18:1) | Diacylglycerophosphoserines |
| 0.02498 | 2.48 higher in hypothermia | PA(16:0/18:1) | Diacylglycerophosphates |
| 0.02498 | 3.23 higher in hypothermia | Unidentified | Diacylglycerophosphoinositols |
| 0.02498 | 2.55 higher in hypothermia | Unidentified | Unidentified |
| 0.02498 | 2.63 higher in hypothermia | Unidentified | Unidentified |
| 0.02498 | 2.98 higher in hypothermia | Unidentified | Unidentified |
| 0.02498 | 2.37 higher in hypothermia | Unidentified | Unidentified |
| 0.03737 | 2.97 higher in hypothermia | PI(16:0/18:0) | Diacylglycerophosphoinositols |
| 0.03737 | 2.25 higher in hypothermia | PS (22:1–18:3) | Diacylglycerophosphoserines |
| 0.03737 | 2.24 higher in hypothermia | Unidentified | Unidentified |
| 0.03737 | 2.33 higher in hypothermia | Unidentified | Unidentified |
| 0.03737 | 2.75 higher in hypothermia | Unidentified | Unidentified |

(e) Class 4 (maintained at 32 °C for 6 h) compared with Class 6 (recovery at 37 °C for 2 h after a temperature of 32 °C for 6 h).

| <i>P</i> value | Fold difference | Lipid identification | Lipid class |
|----------------|----------------------------|------------------------------------------------------------------------------------|-------------------------------------------|
| 0.003948 | 2.96 higher in hypothermia | PS(18:0/18:1) | Diacylglycerophosphoserines |
| 0.006485 | 2.80 higher in hypothermia | Unidentified | Unidentified |
| 0.006485 | 2.31 higher in hypothermia | PI(16:0/18:1) | Diacylglycerophosphoinositols |
| 0.006485 | 2.52 higher in hypothermia | Unidentified | Unidentified |
| 0.006485 | 2.16 higher in hypothermia | Unidentified | Unidentified |
| 0.006485 | 2.4 higher in hypothermia | Unidentified | Unidentified |
| 0.006485 | 2.5 higher in hypothermia | Unidentified | Unidentified |
| 0.006485 | 2.12 higher in hypothermia | Unidentified | Unidentified |
| 0.006485 | 2.39 higher in hypothermia | Unidentified | Unidentified |
| 0.006485 | 2.46 higher in hypothermia | Unidentified | Unidentified |
| 0.006485 | 2.43 higher in hypothermia | Unidentified | Unidentified |
| 0.006485 | 2.33 higher in hypothermia | Unidentified | Unidentified |
| 0.016309 | 1.91 higher in hypothermia | Unidentified | Unidentified |
| 0.016309 | 1.9 higher in hypothermia | 1,2-Dihexadecanoyl- <i>sn</i> -glycero-3-phosphosulfocholine | Glycerophospholipids |
| 0.016309 | 1.80 higher in hypothermia | Unidentified | Dialkylglycerophosphoglycerols |
| 0.016309 | 2.07 higher in hypothermia | PS(17:0/20:4) | Diacylglycerophosphoserines |
| 0.016309 | 2.14 higher in hypothermia | PI(16:0/18:1) | Diacylglycerophosphoinositols |
| 0.016309 | 2.02 higher in hypothermia | Unidentified | Unidentified |
| 0.016309 | 2.81 higher in hypothermia | PI(16:0/18:0) | Diacylglycerophosphoinositols |
| 0.016309 | 2.27 higher in hypothermia | PS (22:1/18:3) | Diacylglycerophosphoserines |
| 0.016309 | 2.20 higher in hypothermia | Unidentified | Unidentified |
| 0.016309 | 2.07 higher in hypothermia | Unidentified | Unidentified |
| 0.016309 | 2.24 higher in hypothermia | PI(18:0/18:0) | Diacylglycerophosphoinositols |
| 0.024975 | 1.96 higher in hypothermia | Unidentified | Unidentified |
| 0.024975 | 2.00 higher in hypothermia | Unidentified | Unidentified |
| 0.024975 | 1.91 higher in hypothermia | PS(18:0/18:1) | Diacylglycerophosphoserines |
| 0.024975 | 1.70 higher in hypothermia | PE(18:0/22:6) | Diacylglycerophospho-ethanolamines |
| 0.02846 | 2.11 higher in hypothermia | Unidentified | Unidentified |
| 0.037373 | 1.68 higher in hypothermia | 1-Tetrahexanoyl-2-(8-[3]-ladderane-octanyl)- <i>sn</i> -glycerophosphoethanolamine | 1-Acyl,2-alkylglycerophosphoethanolamines |
| 0.037373 | 1.76 higher in hypothermia | Unidentified | Unidentified |
| 0.037373 | 1.77 higher in hypothermia | PE(16:0/22:6) | Diacylglycerophosphoethanolamines |
| 0.037373 | 1.72 higher in hypothermia | Unidentified | Unidentified |
| 0.037373 | 1.68 higher in hypothermia | Unidentified | Unidentified |
| 0.0455 | 5.73 higher in hypothermia | Unidentified | Unidentified |
| 0.0455 | 2.38 higher in hypothermia | PS(18:1/18:2) | Diacylglycerophosphoserines |
| 0.0455 | 2.32 higher in hypothermia | Unidentified | Unidentified |