Supplemental Experimental Procedures

Cells and culture conditions

BJ-hTERT human fibroblasts were cultured according to the ATCC in low oxygen (2%) as previously described (Tu et al., 2011). The human melanoma cell lines UACC-62 and 451Lu (kind gift from Dr. Meenhard Herlyn) were cultured as previously described (Satyamoorthy et al., 1997).

Reagents, plasmids, and antibodies.

Cycloheximide was purchased from Sigma-Aldrich (St. Louis, MO). The ATM inhibitor KU55933 was purchased from Selleck Chemicals (Houston, TX). The ATR inhibitor VE822 was a kind gift from Dr. Eric Brown (University of Pennsylvania). pLKO.1-shRRM2 #2 and pLKO.1-shATR #1 and #2 were obtained from Open Biosystems (Waltham, MA). The mature sense sequences are: shATR #1: 5'-GCCGCTAATCTTCTAACATTA-3'; shATR #2: 5'-GCCAAAGTATTTCTAGCCTAT-3'. $HRAS^{G12V}$ plasmid was obtained from Addgene (Cambridge, MA). The following antibodies were obtained from the indicated suppliers: rabbit anti-phospho-ATR (Cell Signaling, Danvers, MA), goat anti-ATR (Santa Cruz Biotechnology), mouse anti-Chk1 (Santa Cruz, Dallas, TX), mouse anti-RAS (BD Biosciences, San Jose, CA), rabbit anti-RRM2B (Santz Cruz), mouse anti-HSP27 (Cell Signaling), and rabbit anti-HSP27 (Ser28) (Cell Signaling).

Retrovirus infections.

Retrovirus was packaged as described previously (Aird et al., 2013). Cells infected with viruses encoding the puromycin-resistance gene were selected in 1 μg/ml puromycin.

Reverse-Transcriptase quantitative PCR (RT-qPCR). RNA was extracted from cells with Trizol (Life Technologies, Grand Island, NY) and DNase treated using RNeasy columns (Qiagen, Venlo, Netherlands). Expression of mRNA levels for *c-myc* (primers from SABiosciences, Valencia, CA) was determined using SYBR green iScript (Bio-Rad, Hercules, CA) master mix on a Bio-Rad Chromo4 machine. β-2-microglobulin was used as an internal control.

Supplemental Table Legends

Supplemntal Table 1. ATM inactivation (mutation or deletion) is mutually exclusive from p53 inactivation (mutation or deletion)/c-MYC activation (mutation/amplification). Related to Figure 4.

Supplemental Figure Legends

Supplemental Figure 1. Knockdown of ATR does not overcome replication stress-induced senescence. Related to Figure 1.

(A) Primary IMR90 cells were infected with a control or shRRM2-expressing lentivirus. Drug-selected cells were stained for p-ATR (Ser428) expression, a marker of activated ATR, by immunofluorescence. DAPI staining was used to visualize nuclei. (B) Primary IMR90 cells were infected with a control or shRRM2-expressing lentivirus alone or in combination with 10μM KU55933, an ATM inhibitor. RRM2 protein levels were determined by immunoblotting. β-actin was used as a loading control. (C) Same as (B), but stained for SA-β-gal activity. 100 cells from each of the indicated groups were quantified for SA-β-Gal positive cells. (n=2) (D) Primary IMR90 cells were infected with a control or shRRM2-expressing lentivirus alone or in combination with two independent shATRs. RRM2, ATR, and Chk1 protein levels were determined by immunoblotting. β-actin was used as a loading control. (E) Same as (D), but SA-β-Gal activity was determined. (F) Quantitation of (E). 100 cells from each of the indicated groups were quantified for SA-β-Gal positive cells (n=3). (G) Same as (D) but cells were labeled with BrdU for 1 h, and BrdU incorporation was visualized by immunofluorescence. DAPI was used to visualize nuclei. Scale bars = 20μm. (H) Quantitation of (G). 200 cells from each

of the indicated groups were quantified for BrdU positive cells (n=3). (I) Same as (D) but an equal number of cells were seeded in 6-well plates and focus formation was determined by crystal violet staining 14 days later. (J) Quantitation of (I). The intensity of foci formed by the indicated cells was quantified using NIH image J software (n=3) (K) Same as (D) but cells were examined for γH2AX and 53BP1 foci formation by immunofluorescence. (L) Quantitation of (K). 200 cells from each of the indicated groups were quantified for 53BP1 and γH2AX foci positive cells (n=3). (M) Same as (D) but single cell gel electrophoresis was performed and cells were stained using SYBR Green. (N) Quantitation of (M). The extent of DNA damage was quantified as Olive Moment using CometScore software (n=3). (O) Primary IMR90 cells were infected with a control or shRRM2-expressing lentivirus alone or in combination with 1μM VE822, an ATR inhibitor. RRM2 and Chk1 protein levels were determined by immunoblotting. β-actin was used as a loading control. (P) Same as (O) but 100 cells from each of the indicated groups were quantified for SA-β-Gal positive cells (n=2). (Q) Primary ATM patient fibroblasts were infected with an shRRM2-expressing lentivirus. RRM2 protein levels were determined by immunoblotting. β-actin was used as a loading control. (R) Same as (O), but stained for SA-β-Gal activity. (S) Quantitation of (R). 100 cells from each of the indicated groups were quantified for SA-β-Gal positive cells (n=3). (T) Primary ATR patient fibroblasts were infected with an shRRM2-expressing lentivirus. RRM2 protein levels were determined by immunoblotting. β-actin was used as a loading control. (U) Same as (T), but stained for SA-β-Gal activity. (V) Quantitation of (U). 100 cells from each of the indicated groups were quantified for SA-β-Gal positive cells (n=3). (W) BJ-hTERT cells were infected with an H-RAS^{G12V}-expressing retrovirus, and RAS and ATM protein levels were determined by immunoblotting. β-actin was used as a loading control. (X) Same as (W), but stained for SA-β-Gal activity. 100 cells from each of the

indicated groups were quantified for SA-β-Gal positive cells (n=3). *p<0.05 vs. control; **p<0.05 vs. shRRM2 or RAS alone. Error bars represent SEM. Scale bars = 10μm unless otherwise specified.

Supplemental Figure 2. Knockdown of ATR does not overcome replication stress induced by knockdown of RRM2. Related to Figure 2.

(A) Primary IMR90 cells were infected with a shRRM2-expressing lentivirus alone or in combination with a shATR-expressing lentivirus. DNA fiber analysis was conducted to observe replication fork dynamics in the indicated cells. The percentage of elongating, terminated, or newly fired replication forks was quantified in the indicated cells (n=3). (B) Primary IMR90 cells were infected with an shRRM2-expressing lentivirus alone or in combination with two independent shATR-expressing lentivirus. On day 1 post-drug selection, cells were labeled with BrdU for 15 min, and BrdU and γH2AX co-localization was determined by immunofluorescence using a confocal microscope. 200 cells from each of the indicated groups were quantified for BrdU and γH2AX co-localization positive cells (n=3). Note that more than 10/nuclei of co-localized BrdU and γH2AX foci was considered positive in the scoring. *p<0.05 vs. control. Error bars represent SEM.

Supplemental Figure 3. ATM knockdown-mediated senescence-bypass requires *de novo* dNTP synthesis; Knockdown of ATM shifts metabolism in multiple cell models. Related to Figure 3. (A) Schematic of de novo synthesis and salvage pathways to make dNTPs. G-6-P: glucose-6-phosphate; G6PD: glucose-6-phosphate dehydrogenase; 6-PG: 6-phosphogluconate. (B) Primary IMR90 cells were infected with a shATM-expressing lentivirus and simultaneously treated with 1μM 3-AP. Seven days later, SA-β-Gal activity was determined (left panel). Scale bars = 10μm. 100 cells from

each of the indicated groups were quantified for SA-β-Gal positive cells (right panel). (n=3) (C) Same as (B) but an equal number of cells were seeded in 6-well plates and focus formation was determined by crystal violet staining 14 days later (left panel). The intensity of foci formed by the indicated cells was quantified using NIH image J software (right panel). (n=3) (D) Primary IMR90 cells were infected with a shRRM2-expressing lentivirus alone or in combination with a shATM-expressing lentivirus. RRM2B protein levels were determined by immunoblotting. β-actin was used as a loading control. (E) Primary IMR90 cells were infected with a control or shRRM2-expressing lentivirus alone or in combination with 10μM ATM inhibitor KU55933, and media was harvested, and glucose consumption and lactate production were quantified (n=2). (F) Same as (E) but quantified for glutamine consumption and glutamate production (n=2). (G) Primary IMR90 cells were infected with a control or RAS-expressing retrovirus alone or in combination with an shATM (#1)-expressing lentivirus, and glucose uptake was determined by incubating cells with a fluorescent glucose analog (2NBDG) for 2 hours followed by flow cytometry. Cells were gated for high glucose uptake based on fluorescence. (n=3) (H) Same as (G) but media was harvested, and glucose consumption and lactate production were quantified (n=2). (I) Same as (H), but quantified for glutamine consumption and glutamate production (n=2). (J) WM793 or UACC-62 melanoma cells were infected with a shRRM2-expressing lentivirus alone or in combination with a shATM-expressing lentivirus, and glucose uptake was determined by incubating cells with a fluorescent glucose analog (2NBDG) for 2 hours followed by flow cytometry (n=3). (K) Same as (J) but media was harvested, and lactate production was quantified (n=3). (L) Same as (K) but media was harvested, and glutamine consumption was quantified (n=3). (M) Same as (L) but quantified for glutamate production (n=3). (N) Primary IMR90 cells were infected with a control or shRRM2-expressing lentivirus alone

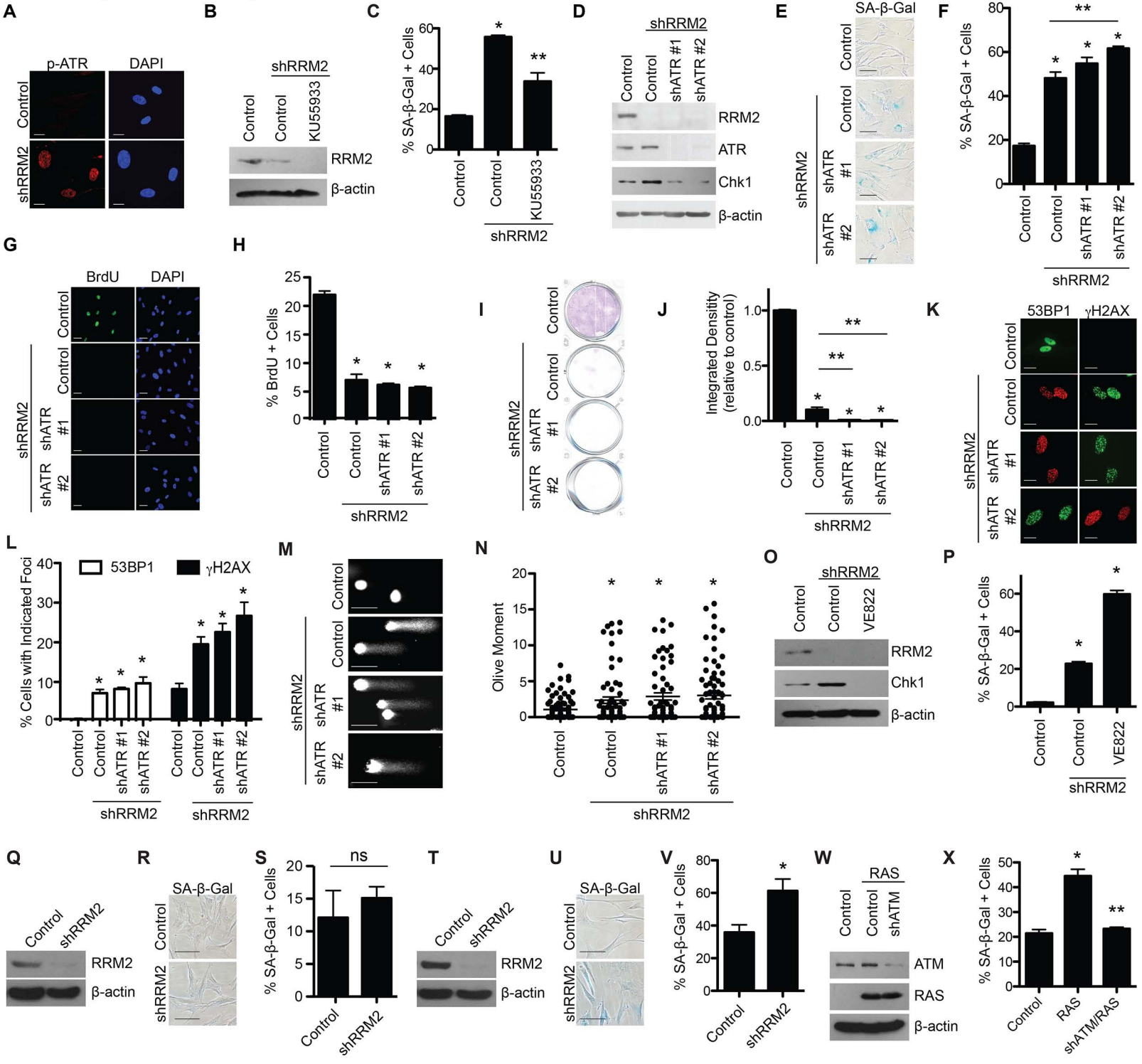
or in combination with 10μM ATM inhibitor KU55933, and G6PD activity was determined (n=2). (O) Primary IMR90 cells were infected with a control or RAS-expressing retrovirus alone or in combination with a shATM (#1)-expressing lentivirus, and G6PD activity was determined (n=2). (P) Primary IMR90 cells were infected with a shRRM2-expressing lentivirus alone or in combination with a shp53-expressing lentivirus, and G6PD activity was determined (n=3). (Q) Melanoma cells with known p53 status were infected with a shRRM2-expressing lentivirus alone or in combination with a shATM-expressing lentivirus, and RRM2, ATM, and G6PD protein expression was determined by immunoblotting. β-actin was used as a loading control. (R) Same as (Q) but G6PD activity was determined (n=3). (S) Same as (Q) but cells were examined for SA-β-Gal activity. (T) Quantification of (S). 100 cells from each of the indicated groups were quantified for SA-β-Gal positive cells (n=3). Scale bars = 5μm. (U) Primary IMR90 cells were infected with a control or shRRM2-expressing lentivirus alone or in combination with a shATM-expressing lentivirus, and total HSP27 and phosphorylated-HSP27 (Ser28) protein expression was determined by immunoblotting. β-actin was used as a loading control. *p<0.05 shRRM2 vs. control; #p<0.05 shATM/shRRM2, KU55933/shRRM2, or shATM/RAS vs. shRRM2 or RAS alone; **p<0.05 shp53/shRRM2 vs. shRRM2 alone. Error bars represent SEM.

Supplemental Figure 4. ATM knockdown cooperatively increases G6PD activity in a p53-dependent manner in multiple cell models; ATM knockdown upregulates c-MYC expression. Related to Figure 4. (A) WM793 melanoma cells were infected with an shRRM2-expressing lentivirus alone or in combination with a shp53-expressing lentivirus. Media was harvested and glucose consumption and lactate production were determined (n=3). (B) Same as (A) but for glutamine consumption and glutamate

production. (C) UACC-62 melanoma cells were infected with a shRRM2-expressing lentivirus alone or in combination with a shATM-expressing lentivirus, and c-MYC protein expression was determined by immunoblotting. β-actin was used as a loading control. (D) Primary IMR90 cells were infected with a shp53-expressing lentivirus, and c-MYC protein expression was determined by immunoblotting. β-actin was used as a loading control. (E) Primary IMR90 cells were infected with a shRRM2-expressing lentivirus alone or in combination with a shATM-expressing lentivirus, and *c-myc* mRNA expression was determined by qRT-PCR (n=3). (F) Same as (E) but p27 expression was determined by immunoblotting. β-actin was used as a loading control. (G) Same as (E) but c-MYC protein expression was determined after the indicated minutes treated with 10μg/ml cycloheximide (CHX). β-actin was used as a loading control. (H) Quantification of (G). Densitometric analysis was performed using NIH ImageJ software. (n=2) (I) WM793 melanoma cells were infected with a shc-MYC expressing lentivirus, and c-MYC protein expression was determined by immunoblotting. β-actin was used as a loading control. (J) WM793 cells were infected with a shRRM2 and shATM lentivirus together with a shc-MYC lentivirus. Media was harvested and glutamine consumption was determined. (n=2). *$p<0.05$ indicated cells vs. control; #$p<0.05$ shRRM2/shp53 vs. shRRM2 alone; **$p<0.05$ shRRM2 vs. shATM/shRRM2; ##$p<0.05$ shRRM2/shATM/shc-MYC vs. shRRM2/shATM. Error bars represent SEM.

Aird et al. Supplemental Table 1.

Study	% ATM inactivation (#)	% p53 inactivation/ c-MYC activation (#)	% ATM inactivation/p53 inactivation/c-MYC activation (#)
Lung Adenocarcinoma (Broad)	11.5% (21)	5.5% (10)	0% (0)
Lung Adenocarcinoma (TCGA)	10.4% (24)	5.2% (12)	0% (0)
Colorectal Adenocarcinoma (TCGA)	12.3% (26)	2.8% (6)	0% (0)
Uterine Corpus Endometrial Carcinoma (TCGA)	12.9% (31)	7.1% (17)	0.4% (1)
Breast Invasive Carcinoma (TCGA)	3.7% (18)	10.1% (49)	0.6% (3)
Head and Neck Squamous Cell Carcinoma (TCGA)	4.3% (13)	10.9% (33)	0.6% (2)
Lung Squamous Cell Carcinoma (TCGA)	5.1% (9)	5.1% (9)	0.6% (1)
Pancreatic Adenocarcinoma (TCGA)	8.2% (4)	10.2% (5)	0% (0)
Prostate Adenocarcinoma (TCGA)	5.6% (14)	1.6% (4)	0.4% (1)
Prostate Adenocarcinoma (Michigan)	11.5% (7)	9.8% (6)	1.6% (1)
Stomach Adenocarcinoma (TCGA)	11.0% (24)	6.8% (15)	0.5% (1)
Uterine Carcinosarcoma (TCGA)	5.3% (3)	17.9% (10)	0% (0)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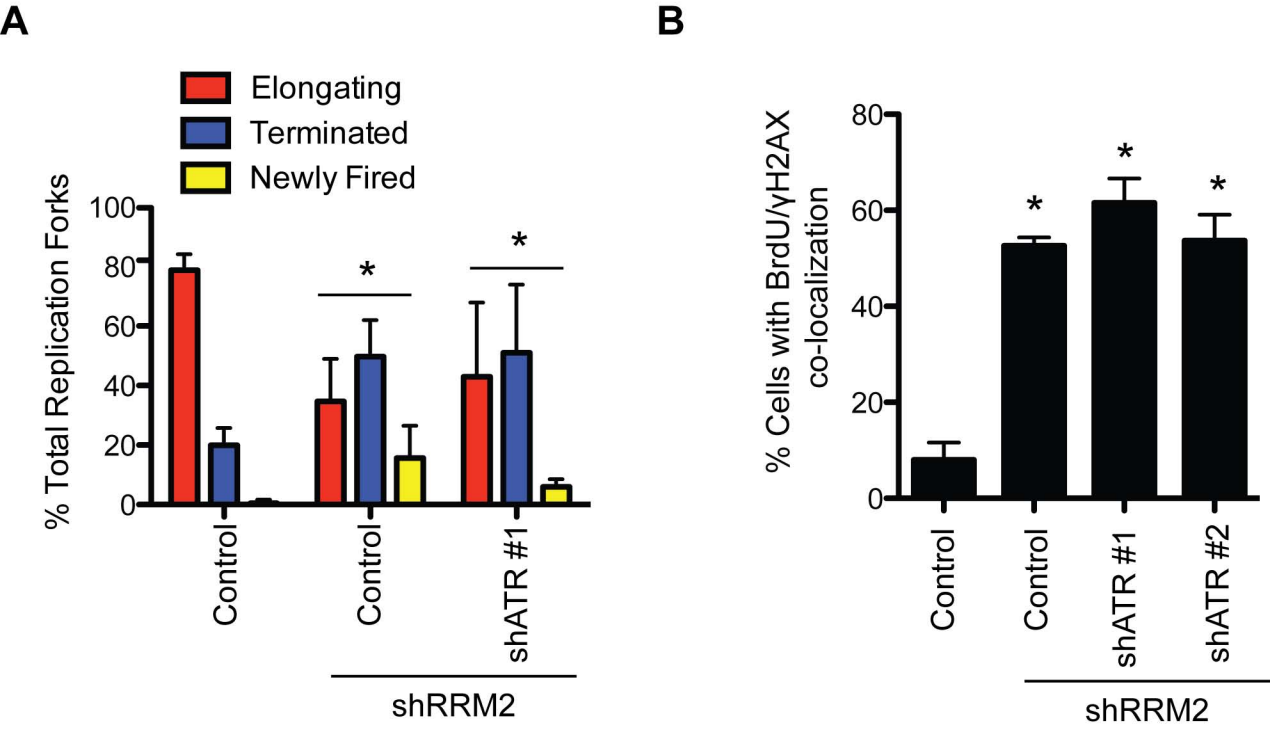
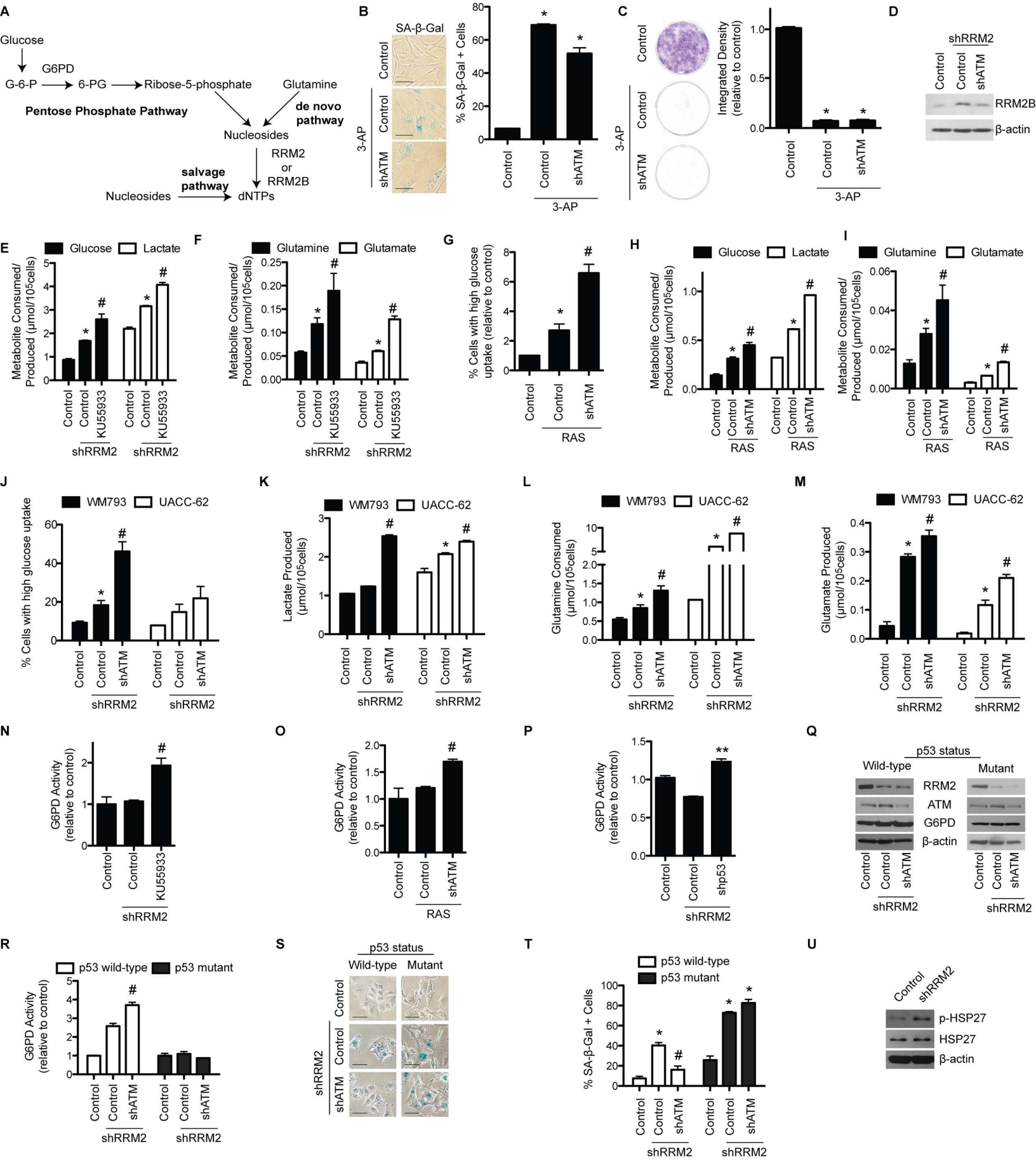
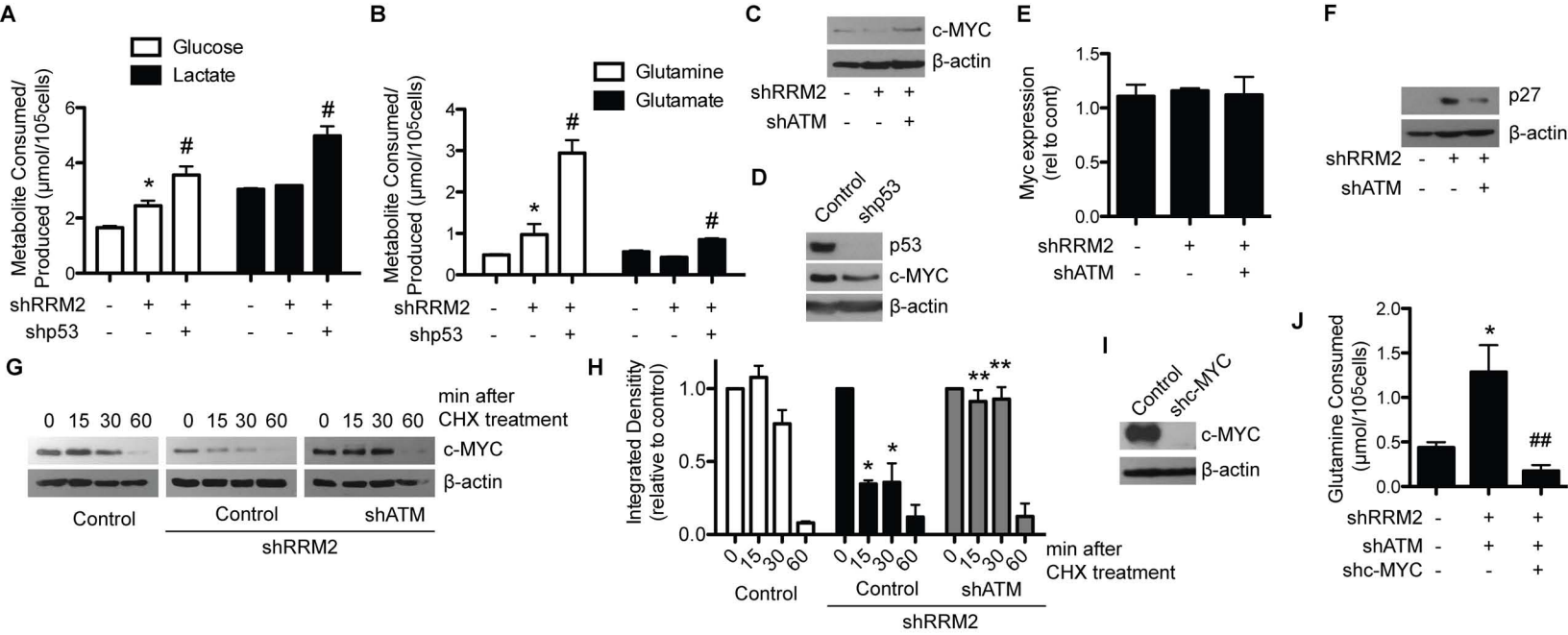
shATM/RAS

Aird et al. - Figure S2; Related to Figure 2



Aird et al. - Figure S3; Related to Figure 3

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shp53 - - + - - +
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Glutamine
Glutamate
Metabolite Consumed/ Produced (μmol/10^5cells)
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shp53 - - + - - +
C
c-MYC
β-actin
shRRM2 - + +
shATM - - +
D
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shp53
p53
c-MYC
β-actin
E
Myc expression (rel to cont)
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shATM - - +
F
p27
β-actin
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c-MYC
β-actin
Control
Control
shATM
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Integrated Density (relative to control)
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min after CHX treatment
Control
Control
shATM
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shc-MYC
c-MYC
β-actin
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Glutamine Consumed (μmol/10^5cells)
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shATM - + +
shc-MYC - - +