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Supplemental Material to:

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Identification of ribonucleotide reductase M2 as a potential target for pro-senescence therapy in epithelial ovarian cancer

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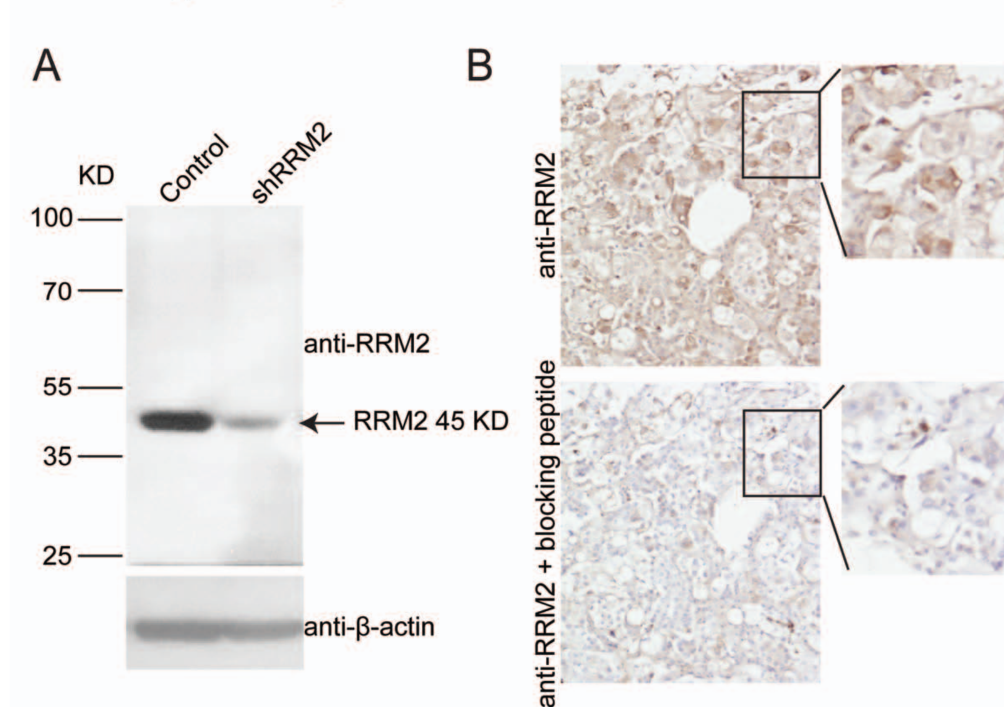
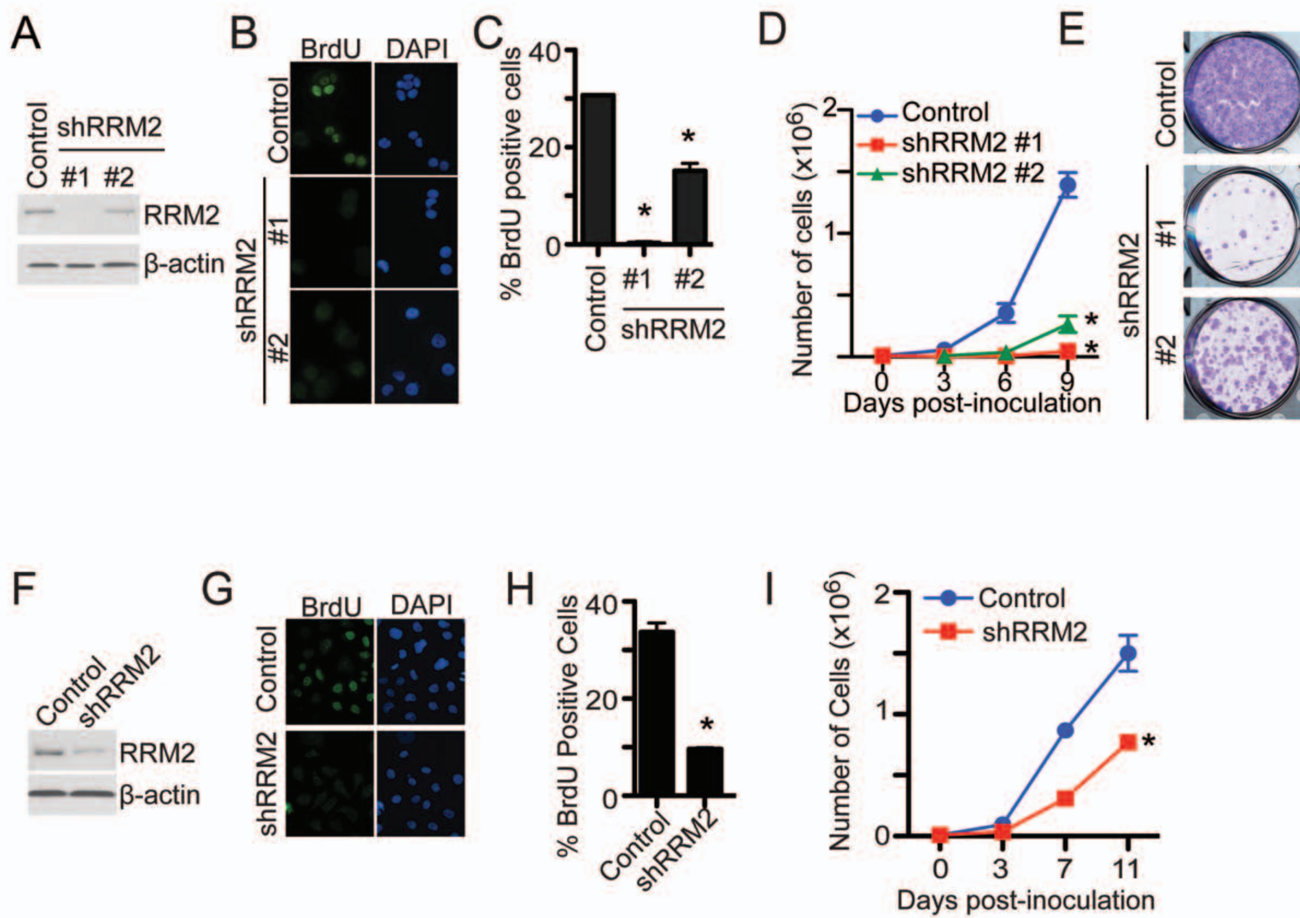
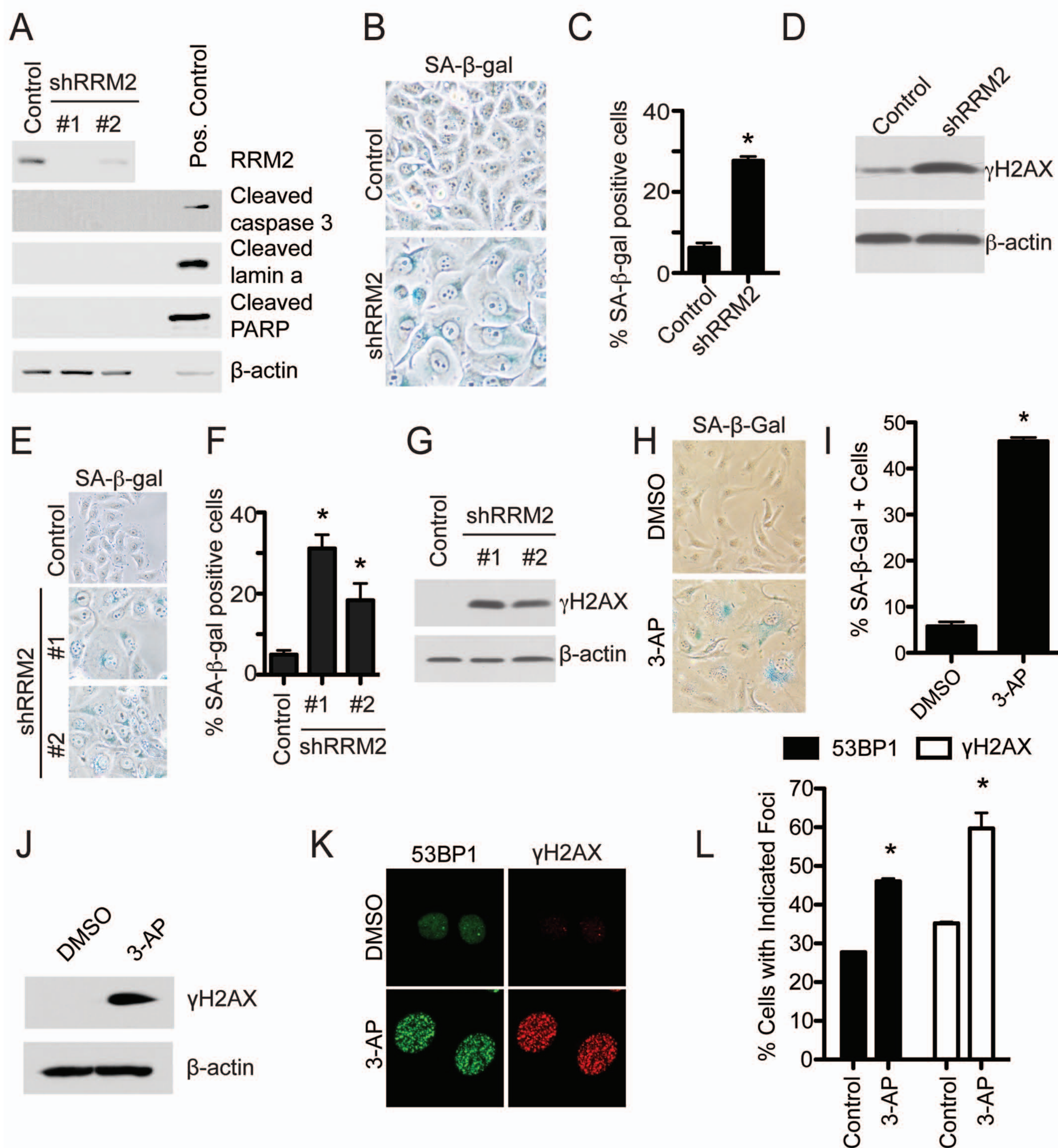
Aird et al.- Supplemental Figure 1



Aird et al.- Supplemental Figure 2



Aird et al.- Supplemental Figure 3



Supplementary Figure Legends

Supplemental Figure 1. The RRM2 antibody is highly specific for both immunoblotting and immunohistochemistry.

(A) OVCAR5 EOC cells were infected with a shRRM2 encoding puromycin-resistant lentivirus or control. Cells were selected with 3μg/ml puromycin and harvested after 7 days. RRM2 protein expression was determined by immunoblotting. Shown is a full membrane immunoblotting. Note that there is only one band whose intensity is weakened by shRRM2 expression showing that the antibody is specific for immunoblotting.

(B) The specificity of the RRM2 antibody for IHC was determined by incubating consecutive sections of a EOC specimen with the anti-RRM2 antibody without (top panel) or with (bottom panel) a specific RRM2 blocking peptide. Note that the staining intensity is significantly decreased in the sample incubated with the blocking peptide, indicated the specificity of the antibody for IHC.

Supplemental Figure 2. RRM2 knockdown suppresses the growth of human EOC cells.

(A) PEO1 EOC cells were infected with control or two different shRRM2-encoding puromycin lentivirus and selected with 1 μg/ml puromycin. The expression of RRM2 was determined in drug-selected cells by immunoblotting. β-actin was used as a loading control.

(B) Same as (A) but labeled with 10μM BrdU for 30 minutes, and the incorporated BrdU was visualized by immunofluorescence staining. DAPI counterstaining was used to visualize cell nuclei.

(C) Quantification of (B) 200 cells from each of the indicated groups were examined for BrdU incorporation. Mean of three independent experiments with SEM. $*p < 0.01$ compared with controls.

(D) Same as (C) but an equal number of cells (1000 cells/well) were seeded in 6-well plates and the number of cells was counted at the indicated time points. Mean of three independent experiments with SD. $*p<0.05$ compared with controls.

(E) Same as (D) but after 2 weeks of culture, the plates were stained with 0.05% crystal violet in PBS to visualize focus formation. Shown are representative images of three independent experiments.

(F) OVCAR5 EOC cells were infected with control or an shRRM2 (#1) encoding puromycin lentivirus and selected with 3 μg/ml puromycin. The expression of RRM2 was determined in drug-selected cells by immunoblotting. β-actin was used as a loading control.

(G) Same as (F) but labeled with 10μM BrdU for 30 minutes, and the incorporated BrdU was visualized by immunofluorescence staining. DAPI counterstaining was used to visualize cell nuclei.

(H) Quantification of (G) 200 cells from each of the indicated groups were examined for BrdU incorporation. Mean of three independent experiments with SEM. $* p < 0.01$ compared with controls.

(I) Same as (F) but an equal number of cells (1000 cells/well) were seeded in 6-well plates and the number of cells was counted at the indicated time points. Mean of three independent experiments with SD. $*p<0.05$ compared with controls.

Supplemental Figure 3. RRM2 inhibition induces cellular senescence in human EOC cells, which correlates with an increase in DNA damage.

(A) OVCAR5 cells were infected with control or two individual shRRM2 encoding lentivirus and selected with 3 μg/ml puromycin. After 7 days in culture, the expression of RRM2 and the apoptotic markers such as cleaved caspase 3, cleaved lamin a, and cleaved PARP p85 were determined by immunoblotting. β-actin was used as a loading control. Note that OVCAR5 cells expressing an shRNA to the human EZH2 gene, which has previously been demonstrated to induce apoptosis, was used as a positive control.

(B) Same as (A) but the control and shRRM2 (#1) expressing cells were stained for SA-β-gal activity.

(C) Quantification of (B). 200 cells from each of the indicated groups were examined for SA-β-gal activity. Mean of three independent experiments with SEM. *$p<0.05$ compared with controls.

(D) Same as (A) but γH2AX expression was determined by immunoblotting. β-actin was used as a loading control.

(E-G) Same as (B-D) but for PEO1 EOC cells. *$p<0.05$ compared with controls.

(H) SKOV3 cells were treated with or without 1μM 3-AP. After 7 days in culture, cells were stained for SA-β-gal activity.

(I) Quantification of (H). 200 cells from each of the indicated groups were examined for SA-β-gal activity. Mean of three independent experiments with SEM. *$p<0.05$ compared with DMSO control.

(J) Same as (H) but γH2AX expression was determined by immunoblotting. β-actin was used as a loading control.

(K) Same as (H) but cells were examined for 53BP1 and γH2AX foci formation by immunofluorescence.

(L) Quantification of (K). 200 cells from each of the indicated groups were examined for foci formation. Mean of three independent experiments with SEM. *$p<0.05$ compared with DMSO control.