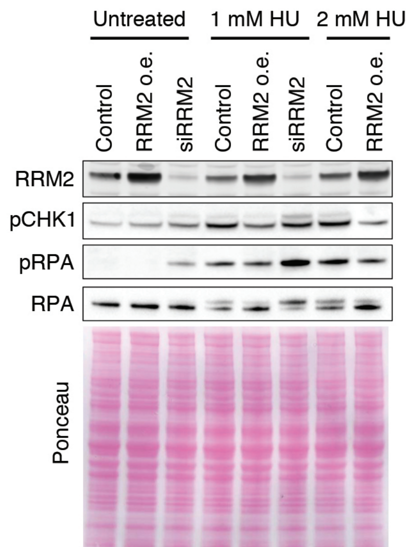

Fig. S1. Effect of RRM2 overexpression and depletion on CHK1 and RPA phosphorylation. Western blot illustrating the impact of RRM2 overexpression (RRM2 o.e.) or depletion (siRRM2) on RPA and CHK1 phosphorylation in U2OS cells. Ponceau is shown for a more global evaluation of the loading of this gel. HU was added for 3 hrs where indicated.

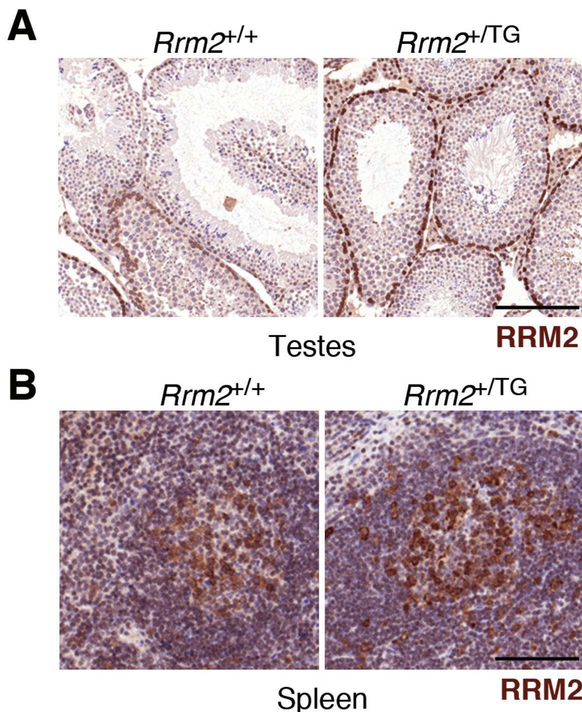

Fig. S2. Increased RRM2 levels in *Rrm2* transgenic tissues. Immunohistochemistry using RRM2 antibodies was performed on littermate wt and *Rrm2* transgenic tissues. The images illustrate RRM2 expression levels on the testes (**A**) and spleens (**B**) of these animals. Note that whereas RRM2 is cytoplasmic on spleen (and other organs), it is nuclear on testes, in agreement with the increase of nuclear RRM2 levels that has been observed in response to DNA damage. Scale bar indicates (black) indicates 100 μm.

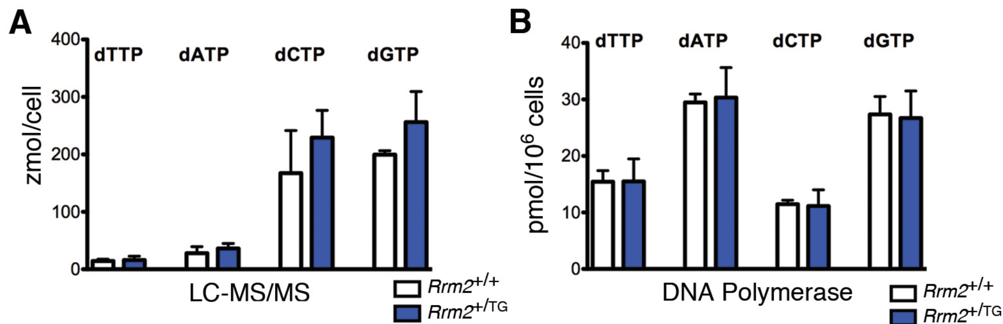

Fig. S3. Nucleotide concentrations in *Rrm2* transgenic cells. dNTP levels were measured by two independent methods: liquid chromatography coupled to tandem mass scpectrometry (LC-MS/MS) or a DNA Polymerase assay (see Methods). (**A**) LC-MS/MS analyses represent cytosolic dNTP levels and (**B**) DNA Polymerase assays whole-cel levels. Analysis were performed on wt and *Rrm2* transgenic littermate MEF lines from the same passage (n=3). Note: Whereas these methods have intrinsic variability due to the fact that different cellular fractions are used for analyses, as well as the effects of different solvents and calibrations, which limits comparison between experiments, each figure depicts one representative analysis where biological triplicates were analysed in parallel.

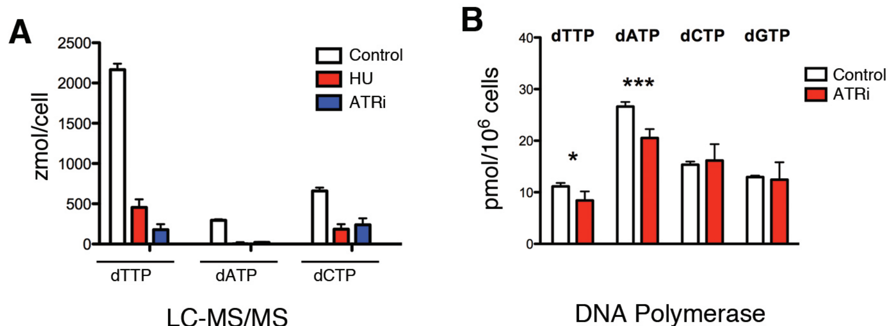

Fig. S4. Nucleotide concentrations in response to ATR inhibition. dNTP levels were measured by two independent methods: liquid chromatography coupled to tandem mass scpectrometry (LC-MS/MS) or a DNA Polymerase assay (see Methods). (**A**) LC-MS/MS analyses represent cytosolic dNTP levels and (**B**) DNA Polymerase assays whole-cel levels. This can contribute to the greater effects observed on cytosolic fractions which could be masked on whole cell extracts. Analysis were performed on NIH 3T3 cells exposed to HU (2 mM; 6 hrs) or ATRi (1 μM; 6 hrs) . In **A**, dGTP levels were undetectable. The greater effect of HU on dATP and its impact on dTTP levels is consistent with previous literature (Julias and Pathak, J Virol 1998). Note: Whereas these methods have intrinsic variability due to the fact that different cellular fractions are used for analyses, as well as the effects of different solvents and calibrations, which limits comparison between experiments, each figure depicts one representative analysis where biological triplicates were analysed in parallel.

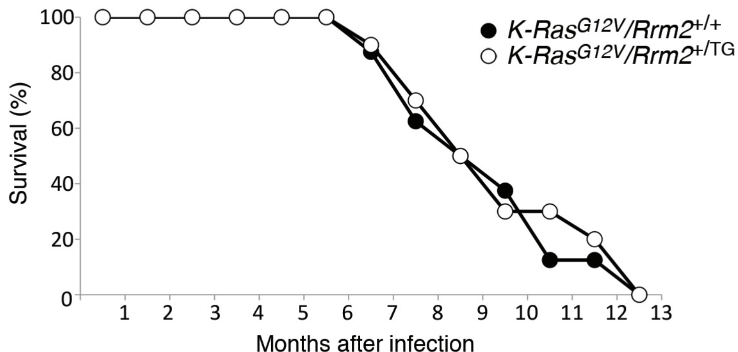

Fig. S5. Impact of the Rrm2 transgene on Ras-induced lung carcinogenesis. Survival of K-*Ras*^{LSLG12Vgeo}/RRM2$^{+/TG}$ (n=8, solid circles) and K-*Ras*^{LSLG12Vgeo}/RRM2$^{+/TG}$ (n=10, open circles) mice after intra-tracheal instillation with 1 × 106 pfu/mouse Ad-Cre virus. The model and procedure have been previously described (Blasco RB *et al* Cancer Cell 2011).

Supplemental Methods

Animal research

The linearized *Rrm2* vector (Genebridges) was used for the microinjection of fertilized oocytes. Transgenic animals were first identified by Southern Blot through standard procedures, and subsequently followed by PCR with primers amplifying a 600bp sequence from the vector (available upon request). Mice were kept under standard conditions at specific-pathogen free facility of the Spanish National Cancer Centre in a mixed C57BL/6-129/Sv background. All mouse work was performed in accordance with the Guidelines for Humane Endpoints for Animals Used in Biomedical Research, and under the supervision of the Ethics Committee for Animal Research of the “Instituto de Salud Carlos III”.

Whole-body imaging

Whole-body imaging was performed on anesthetized mice using the eXplore Vista PET-CT (GE Healthcare) and a 7-tesla Pharmascan (Bruker). MMWKS software (GE Healthcare) was used for the quantifications.

Inmunohistochemistry

Tissues were fixed in formalin and embedded in paraffin for subsequent processing. Consecutive 2.5-µm sections were treated with citrate for antigenic recovery and processed for immunohistochemistry with RRM2 (Santa Cruz) and Ki67 (Master Diagnostica) antibodies. Whole-embryo IHCs were scanned and digitalized with a MIRAX system (Zeiss) for further analysis.

Cell culture

MEF from E13.5 d.p.c. embryos were generated by standard methods and grown in Dulbecco’s Minimum Essential Media (DMEM, Invitrogen) supplemented with 15% FBS (Lonza). For all experiments, MEF were used at a low passage (<3) and grown in 5% oxygen to minimize exposure to reactive oxygen species. For ERFS breakage analyses, resting splenic B cells were isolated with anti-CD43 microbeads (anti-Ly48; Miltenyi Biotech), cultured in the presence of LPS (25 µg/ml; Sigma), IL-4 (5 ng/ml; Sigma) and RP105 (2.5 µg/ml; BD) and metaphases from these cultures processed for ERFS expression by FISH described (Barlow et al. 2013). Hydroxyurea (Sigma) was added at the indicated concentrations. esiRNA sequences for firefly luciferase and *Rrm2* were obtained from SIGMA and transfected following manufacturers instructions. The plasmid coding for RRM2 and its empty vector were obtained from the Precision LentiORF™ collection (Dharmacon).

High Throughput Microscopy

MEF were grown on µCLEAR bottom 96-well plates (Greiner Bio-One). Immunofluorescence was performed using standard procedures with γH2AX (Millipore) or phospho-RPA32 (S4/S8) (Bethyl) antibodies. Analysis of DNA Replication by EdU incorporation was done using click-it (Invitrogen) following manufacturers instructions. The assay for measuring cell viability by HTM using TO-PRO®-3 and Hoechst 33342 has been described before (Eguren et al.

2014). Briefly, MEF were exposed to 1μM TO-PRO®-3 and 5ug/ml Hoechst 33342 (Invitrogen) for 30 min. In all cases, images were automatically acquired from each well using an Opera High-Content Screening System (Perkin Elmer). A 20x magnification lens was used and images were taken at non-saturating conditions. Images were segmented using DAPI or Hoechst 33342 signals to generate masks matching cell nuclei from which the mean signals for the rest of the stainings (γH2AX, pRPA, EdU or TO-PRO®-3) were calculated. Data were represented with the use of the Prism software (GraphPad Software).

Immunoblotting

For protein extracts, cells were washed once with PBS, and lysed in RIPA buffer (Tris-HCl 50 mM, pH 7.4, NP-40 1%, Na-deoxycholate 0.25%, NaCl: 150 mM, EDTA 1 mM) containing protease and phosphatase inhibitors (Sigma). Samples were resolved by SDS-PAGE and analyzed by standard Western blotting techniques. Antibodies against RRM2 (Santa Cruz), RRM1 (Cell Signaling), ATR (Serotec) and β-actin (Sigma) were used. Protein blot analyses were performed on the LICOR platform (Biosciences).

dNTP measurements

For **LC-MS/MS** analysis of cytosolic fractions the instrument consisted of an Acquity Ultra Performance LC and a Xevo TQ using an ESI source operated in negative mode (Waters, Sollentuna, Sweden). 200 *μ*l of 60% acetonitrile in water containing 100 mM ammonium acetate and 10 mM ammonium phosphate was added to five million cells followed by 10 min of sonication using an Ultrasonic Cleaner 5510 (Branson Ultrasonics, Danbury, CT) to lyse cell membranes and precipitate the proteins. After centrifugation, 20 *μ*l of the supernatant was injected on a ZIC-cHILIC column (100x2.1 mm, 3 *μ*m, Merck-Millipore, Sollentuna, Sweden) termostated at 30 °C. dNTPs were separated using a six minute gradient from 65 to 50% acetonitrile at a flow rate of 400 *μ*l/min.

For **DNA Polymerase** assays two million MEF were resuspended in 60% methanol, frozen in liquid nitrogen and boiled for 3 minutes. Samples were evaporated until dryness in a speedvac and whole cell levels of dNTPs were determined using the DNA polymerase assay previously described (Desler et al. 2007) .

References:

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- Eguren M, Alvarez-Fernandez M, Garcia F, Lopez-Contreras AJ, Fujimitsu K, Yaguchi H, Luque-Garcia JL, Fernandez-Capetillo O, Munoz J, Yamano H et al. 2014. A synthetic lethal interaction between APC/C and topoisomerase poisons uncovered by proteomic screens. *Cell Rep* **6**: 670-683.