Supplemental Methods

Preparation of cell lysate and immunoprecipitation

Cells were washed with 1×PBS and resuspended in ice-cold 1% CHAPS lysis buffer (1% CHAPS, 50 mM Tris [pH 7.6], 120 mM NaCl, 1 mM EDTA, 1 mM Na_3VO4, 50 mM NaF, and 1 mM β-mercaptoethanol) with a cocktail of protease inhibitors (EMD Biosciences). Cells were lysed by sonication and centrifuged at 14,000×g for 10 min at 4°C. The resulting supernatant was collected as the total cell lysate. For immunoprecipitation, 1 mg of whole-cell extract was applied to 30 μl of protein A Sepharose slurry (Invitrogen) together with 5 μg of the specified antibodies or agarose-conjugated Bcl2 (Santa Cruz, CA). After overnight incubation at 4°C and stringency washes, elution was carried out by boiling in Laemmli buffer.

Quantum dot-based immunofluorescence (QD-IF)

Cells were washed with 1x PBS, fixed with cold methanol and acetone (1:1) for 10 min, and then blocked with 10% normal goat serum for 60 min at room temperature. The cells were incubated with mouse Bcl2 and rabbit hRRM1 or hRRM2 primary antibodies overnight at 4°C. After washing, samples were incubated with QD secondary antibody conjugates (QD 605 goat F(ab')2 anti-rabbit IgG; QD 705 goat F(ab')2 anti-mouse IgG, 1:50 dilution) in a cocktail solution at room temperature for 60 min. Cell nuclei were counterstained using 4,6-diamidino-2-phenylindole (DAPI). Mouse and rabbit IgG were used as negative controls. QD imaging and quantification procedures were performed as described (1). The Nuance™ fluorescence microscope system (CRi consolidated with Caliper, a PerkinElmer company, Hopkinton, MA) was used for quantification of the QD signals. All cubed image files were collected from culture cells at 10 nm wavelength intervals from 420-720 nm, with an auto exposure time per wavelength interval at 200~400x magnification. Taking the cube with a long wavelength band pass filter allowed transmission of all emission wavelengths above 420 nm. Both separated and combined QD images were obtained after establishing the QD spectral library and unmixing the image cube. For each cell sample, 10 cubes were taken. The background signal was

removed for accurate quantification of the QD signals. Cells were observed and signals were quantified by an Olympus microscope IX71 with a CRi Nuance spectral imaging and quantifying system (CRi Inc., Woburn, MA) (1). The percentage of Bcl2 and hRRM1 or hRRM2 co-localization was quantified by Nuance imaging software (Caliper/PerkinElmer), 10 randomly selected fields on the cell slides were calculated.

Cell viability assay

Apoptotic and viable cells were detected using an ApoAlert Annexin-V kit from Clontech according to the manufacturer's instructions. The percentage of viable cells or apoptotic cells was determined by fluorescence-activated cell sorting analysis.

References:

1. Huang DH, Su L, Peng XH, Zhang H, Khuri FR, Shin DM, et al. Quantum dot-based quantification revealed differences in subcellular localization of EGFR and E-cadherin between EGFR-TKI sensitive and insensitive cancer cells. Nanotechnology. 2009;20:225102.