SUPPLEMENTARY METHODS

Synthesis of CGK733-biotin and KU-55933. CGK733-biotin (**2**) was prepared by the synthetic route outlined in **Supplementary Figure 6b**. The compound **10** was converted into the corresponding amide **11** by reaction with Boc_2O and NH_4HCO_3 in the presence of pyridine in dioxane. Amide (**11**) was reacted with chloral in benzene to produce the coupling product, followed by chlorination using $SOCl_2$ to afford the compound **12**. Compound **12** was treated with KSCN in acetonitrile at room temperature, and coupled with 4-flroro-3-nitroaniline in acetonitrile to give the compound **13**. Alkylation of compound **13** with 4-bromobutyric acid ethyl ester produced the compound **14**, which upon treatment with biotin-PEO-LC-NH_2 afforded the desired CGK733-biotin derivative (**2**). KU-55933 (2-morpholin-4-yl-6-thianthren-1-yl-pyran-4-one; **3**) was prepared by the synthetic route outlined in **Supplementary Figure 6a**. Diketene (**4**) reacted with CCl_4 in the presence of (bis-4-t-butylcyclohexyl)peroxydicarbonate (BCHPO) by free-radical addition to give 4-chloro-4-(2,2,2-trichloroethyl)oxetan-2-one (**5**), followed by nucleophilic attack using morpholine and elimination of HCl to afford 5,5-dichloro-1-morpholin-4-ylpent-4-ene-1,3-dione (**7**). Ring closure of compound **7** under acidic condition furnished pyran-4-one ring (**8**), which was finally converted to 2-morpholin-4-yl-6-thianthren-1-ylpyran-4-one (**3**) by Suzuki coupling reaction. All the synthesized compounds were verified with mass spectrometry.

Plasmids and reagents. To generate retroviral bicistronic expression construct for $TRF2^{\Delta B\Delta M}$ and EGFP, CMV immediate-early promoter (excised from pcDNA3.1(+) (Invitrogen)), $TRF2^{\Delta B\Delta M}$ cDNA, IRES (excised from pIRES2-EGFP (Clontech)), and EGFP cDNA were cloned into the MCS of pBabe-puro. The retroviral construct for single expression of EGFP or $TRF2^{\Delta B\Delta M}$ was generated identically except without $TRF2^{\Delta B\Delta M}$ cDNA or IRES/EGFP cDNA, respectively. The construction of pMAGIC-N and pMAGIC-C was described previously[1]. The cDNAs of $TRF2^{\Delta B\Delta M}$

and mRFP are kind gifts from T. de Lange (The Rockefeller University, NY)[2] and R. Y. Tsien (University of California at San Diego, CA)[3]. pBabe-puro and the expression plasmid for PH-GFP were kindly provided by J. Campisi (Lawrence Berkeley National Laboratory, CA) and J. Chung (Korea Advanced Institute of Science and Technology, Korea)[4], respectively. Wortmannin, LY294002, U0126, SB202190, SB202474, and SB203580 were obtained from Calbiochem. Human recombinant PDGF-BB was purchased from Sigma.

Cell culture, transfection, and irradiation. BJ, HeLa, and U2OS cells were obtained from the American Type Culture Collection (ATCC). Phoenix-ampho (293T-derived retrovirus packaging cell line) and post-selection 184-strain HMEC (Ref. 5) cells were kindly provided by G. P. Nolan (Stanford University, CA) and M. R. Stampfer (Lawrence Berkeley National Laboratory, CA), respectively. HeLa, U2OS, and Phoenix-ampho cells were maintained in DMEM supplemented with 10% calf serum, 120 μg/ml penicillin, and 200 μg/ml streptomycin. BJ cells were maintained under identical conditions, except with 10% fetal bovine serum instead calf serum. BJ cultures were passaged using a 1:4 subculture regimen. Population doublings (PDs) were calculated from the cumulative cell number determined at each passage. HMECs were maintained in MEGM (Cambrex) supplemented with bovine pituitary extract, 5 μg/ml transferrin, and 10 mM isoproterenol. Transfection was performed with Lipofectamine 2000 reagent (Invitrogen) or PolyMAG-200 magnetofection reagent (Chemicell) according to the manufacturer's instructions. U2OS cells were exposed to ionizing radiation (15 Gy) and UV (50 J/m^2) by using a 137Cs radiation source (Mark I, model 68A Irradiator, JL Shepherd & Associates) and BLX-254 UV illuminator (Vilber Lourmat), respectively.

Retroviral transduction and FACS analysis. Phoenix-ampho cells were transfected with the retroviral expression plasmids or EGFP-fusion protein expression library. Retroviral supernatants from the transfected packaging cells were collected 48 h post-transfection, filtered through a 0.45

μm filter (Millipore), and used for infection of HeLa cells after supplementation with 4 μg/ml polybrene. Two days later, the infected cells were given puromycin (1 μg/ml) for three days for selection. The cells infected with the EGFP-fusion protein expression library were stripped off with trypsin-EDTA and subjected to FACS analysis on a Coulter Epics Altra flow cytometer (Beckman Coulter) to sort the EGFP-positive cells. These cells were incubated in a 16-well Lab-Tek chamber slide (Nunc) for expression cloning based on MAGIC technology.

***In vitro* kinase assays.** *In vitro* kinase assays were performed essentially as described previously for ATM (ref. 6), ATR (ref. 7), DNA-PK (ref. 8), ERK (ref. 9), CK1 (ref. 10), CDK2 (ref. 11), JNK (ref. 12), PKC (ref. 13), CAK (ref. 14), Chk1 (ref. 15), PKR (ref. 16), p38 (ref. 17), and PI3K (ref. 18). CGK733, wortmannin, LY294002, U0126, SB202190, SB202474, and SB203580 were added to the reaction mixture as indicated in the results.

Cytogenetics. G-banded chromosome preparations were made using standard methods[19]. Chromosomes were analyzed according to the criteria as described previously[20].

Immunoblotting. Proteins were separated by 10% SDS-PAGE and transferred to nitrocellulose membranes (Millipore). The membranes were blocked with 5% nonfat milk and probed with the respective antibodies. The following antibodies were used for immunoblotting: anti-phospho-ATM(S1981) (a kind gift from D. S. Lim, Korea Advanced Institute of Science and Technology, Korea), anti-ATM (Santa Cruz Biotechnology), anti-ATR (Santa Cruz Biotechnology), anti-phospho-p53(S15) (Cell Signaling Technology), anti-p53 (Cell Signaling Technology), anti-p21 (Santa Cruz Biotechnology), anti-p16 (BD Biosciences), anti-Akt (Santa Cruz Biotechnology), anti-phospho-Akt(T308) (Cell Signaling Technology), anti-phospho-Akt(S473) (Cell Signaling Technology), anti-Chk1 (Santa Cruz Biotechnology), anti-Chk2 (Santa Cruz Biotechnology), anti-phospho-Chk1(S345) (Cell Signaling Technology), anti-phospho-Chk2(T68) (Cell Signaling

Technology), and anti-actin (Santa Cruz Biotechnology) antibodies. Membranes were then incubated with HRP-conjugated anti-rabbit or anti-mouse IgG (Santa Cruz Biotechnology), and visualized using the ECL system (Pierce).

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