Supplemental Materials

Molecular Biology of the Cell

Sadaie et al.
SUPPLEMENTAL INFORMATION

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SUPPLEMENTAL FIGURE LEGENDS

SUPPLEMENTAL FIGURE S1. Increased large and spotty nuclei in RAS-induced senescent cells. (A) Oncogenic RAS$^{G12V}$-induced senescent (RIS) IMR90 cells were stained with DAPI and assessed for DNA content and nuclear size using a laser scanning cytometer (iCys). (B) Percentage of SAHF-positive senescent cells was manually counted. Prolif, proliferating. (C) 2-D plots for the indicated parameters measured by automated detection using ArrayScan. Cells were treated with library compounds either at 5 µM (top) or 3 µM (bottom) for 4 days. An arbitrary threshold for relative nuclear average area (compounds/DMSO) (representing ‘nuclear size’) were set at 1.2 for both concentrations, whereas thresholds for relative spot total area per nucleus (compounds/DMSO) (representing ‘spottiness’) were set at 3 or 2.5 for 5 µM or 3 µM libraries, respectively. (D) Screening of compounds at 3 µM. Score distributions of relative nuclear average area or relative spot total area per nucleus were shown as in Figure 1C. Number of hits identified by automated detection and subsequent visual inspection are summarized (right). *Compounds that gave counts of less than 100 nuclei. (E) Confocal images of IMR90 cells treated with indicated compounds (d3). Cells were stained using the indicated antibodies. LMNA, Lamin A. (F) Cell viability was determined by trypan blue exclusion assay after 24h incubation of IMR90 cells.
with indicated compounds at different concentrations.

**SUPPLEMENTAL FIGURE S2. Increased nuclear size in cells treated with the IRGs.**

Histograms show distribution of nuclear size in cells treated with the indicated kinase inhibitors as in Figure 2. Cells at d4 and d9 were stained with DAPI and analyzed by laser scanning cytometer.

**SUPPLEMENTAL FIGURE S3. Lamin B1 reduction in IRG hit-treated cells. (A)** IMR90 cells were treated with the indicated compounds as in Figure 2A, and the expression of the indicated proteins was detected by Western blotting. (B) Immunofluorescence with the indicated antibodies at day 9. (C) Immunofluorescence of Lamin B1 after one-day of treatment with ZM1. Representative percentage of nuclei with an irregular shape is shown on the right.

**SUPPLEMENTAL FIGURE S4. Treatment with IRG hits or ZM1 induces senescence in BJ cells. (A-F)** BJ cells were treated with indicated compounds as in Figure 2A, and assessed for nuclear shape (A), cell cycle profile (B), protein expression (C), BrdU incorporation (D),
SA-β-galactosidase activity (E), and colony forming capacity (F). Values are mean ± SEM from 3 independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001.

SUPPLEMENTAL FIGURE S5. Senescence induction in IMR90 cells with AZD1152 treatment. Cells were treated with 0.5 µM of AZD1152 as in Figure 2A and assessed for cell cycle and senescence assays as in Figure 4A and C.

SUPPLEMENTAL FIGURE S6. Full lanes in blots shown in Figure 3E. Two lanes indicated were removed in Figure 4E, because these compounds are not directly related to the current study. Note as expected both compounds, which inhibit ROCK, also failed to elicit exit from paclitaxel-induced M phase arrest.

SUPPLEMENTAL FIGURE S7. Treatment of quiescent cells with IRGs or ZM1 does not have an impact on their proliferation after releasing from quiescence. (A, B) IMR90 cells were synchronized at quiescence (G0 arrest) by a 3-day incubation in low serum (0.1%) (LS) medium, and treated subsequently with IRGs/ZM1 either in high serum (10%) (HS) or LS medium for 3 days. After the treatment, compounds were removed and cells were
incubated for 2 days in HS media and then assessed for nuclear shape. Percentages of cells that had irregular-shaped nuclei (A) and representative images of DAPI stained cells (B) are shown. Prolif, proliferating; Qui, quiescent. (C) Cells treated as shown in (A) with the indicated compounds were assessed for colony forming capacity. After removing compounds, all cells were maintained in normal medium (HS).

SUPPLEMENTAL FIGURE S8. Validation of AURKB shRNAs. (A) Western blotting for the indicated proteins in IMR90 cells stably expressing four different shRNAs against AURKB in a miR30 backbone. RB-P, phosphorylated RB. V, miR30-based RNAi vector. (B) Colony formation assay in indicated cells. (C) Percentage of SA-β-gal-positive cells in the indicated cells. Sh-1, sh-AURKB-1; sh-2, sh-AURKB-2. Data are representative from at least two independent experiments. (D) Phase contrast and DAPI images of the cells expressing sh-AURKB-1 or sh-AURKB-2.

SUPPLEMENTAL FIGURE S9. Cell viability of tumour cell lines treated with IRG compounds. (A, B) HeLa cells (A) and H1299 cells (B) were treated with indicated compounds as in Figure 2A and cell viability was assessed by trypan blue exclusion. Cells
were replated 24h before the assay.

SUPPLEMENTAL FIGURE S10. The absence of SASP induction in senescent cells treated with IRG compounds. (A-B) IMR90 cells were treated with the indicated compounds as in Figure 2A, and the expression of the proteins shown was detected by Western blotting. As a control, HRAS<sup>G12V</sup>-induced senescent IMR90 cells were included.

SUPPLEMENTAL MOVIES S1-S3. IMR90 cells treated with compounds exit M phase without chromosome segregation. IMR90 cells expressing H2B-EYFP were synchronized at the G1/S border with double thymidine treatment, and released into medium containing DMSO or indicated compounds. Ten hours after the release, time-lapse images of H2B-EYFP were taken every 5 minutes. Movie S1. Representative time-lapse images of control cells treated with DMSO. Movie S2. Representative time-lapse images of cells treated with PDGFR-V (Large image field). Movie S3. Representative time-lapse images of cells treated with ZM1.
SUPPLEMENTAL FIGURE S1. Increased large and spotty nuclei in RAS-induced senescent cells. (A) Oncogenic RASG12V-induced senescent (RIS) IMR90 cells were stained with DAPI and assessed for DNA content and nuclear size using a laser scanning cytometer (iCys). (B) Percentage of SAHF-positive senescent cells was manually counted. Prolif, proliferating. (C) 2-D plots for the indicated parameters measured by automated detection using ArrayScan. Cells were treated with library compounds either at 5 μM (top) or 3 μM (bottom) for 4 days. An arbitrary threshold for relative nuclear average area (compounds/DMSO) (representing ‘nuclear size’) were set at 1.2 for both concentrations, whereas thresholds for relative spot total area per nucleus (compounds/DMSO) (representing ‘spottiness’) were set at 3 or 2.5 for 5 μM or 3 μM libraries, respectively. (D) Screening of compounds at 3 μM. Score distributions of relative nuclear average area or relative spot total area per nucleus were shown as in Figure 1C. Number of hits identified by automated detection and subsequent visual inspection are summarized (right). *Compounds that gave counts of less than 100 nuclei. (E) Confocal images of IMR90 cells treated with indicated compounds (d3). Cells were stained using the indicated antibodies. LMNA, Lamin A. (F) Cell viability was determined by trypan blue exclusion assay after 24h incubation of IMR90 cells with indicated compounds at different concentrations.
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## SUPPLEMENTAL TABLE S1. Optimized ArrayScan settings for detecting nuclear morphological changes.

| Category         | Parameter                | Setting                        | Comments                                                                 |
|------------------|--------------------------|--------------------------------|--------------------------------------------------------------------------|
| **Assay**        | Assay algorithm          | SpotDetector.V3               | Name of BioApplication – optimized for spot detection                    |
|                  | Protocol name            | SAHF_compounds_20x_Spots       |                                                                           |
|                  | # channels               | 2 (both DAPI)                 | Allows collection of two images with differing saturation               |
|                  | Form Factor              | Falcon 96 well                | Microplate template                                                      |
| **Image Acquisition** | Objective              | 20x                            | Highest magnification available to permit detection of distinct puncta   |
|                  | Acquisition camera mode  | Standard                       | Pixel resolution 1024x1024; 2x2 binning                                   |
|                  | AutoFocus camera mode    | AutoFocus                      | Pixel resolution 1024x1024; 4x4 binning (faster focusing)               |
| **Scan Limits**  | Max Fields for Well      | 60                             | Maximum number of images taken per well                                  |
|                  | Min Objects for Well     | 600                            | Min # of objects (nuclei) to detect each well                           |
|                  | Max Sparse Fields for Well | 4                    | # of images taken of ‘Sparse wells’ before moving to next well          |
|                  | Min Objects for Field    | 5                              | ‘Sparse well’ defined as one with <5 nuclei                             |
| **Channel 1: Nuclei** | Dye                    | XF100 - Hoechst               | Name of fluorescence filter                                             |
|                  | Exposure                 | Fixed: 40% saturation          | These settings set the threshold for detecting nuclei as single objects (i.e. whole nuclei). Typical exposure time = 0.054secs. Threshold depends on signal intensity. |
|                  | Object identification    | Fixed Threshold: 20 – 100      |                                                                           |
|                  | ObjectAreaCh1            | Min: 200                      | Defines min and max size of a nucleus in pixels (eliminates debris and large nuclear clumps) |
|                  |                          | Max: 2500                     |                                                                           |
|                  | ObjectAvgIntenCh1        | Min: 0                        | Rejects very bright objects – most likely debris                        |
|                  |                          | Max: 2000                     |                                                                           |
| **Channel 2: Spots** | Dye                    | XF100 - Hoechst               | Name of fluorescence filter                                             |
|                  | Exposure                 | Fixed: 25% saturation          | Using a lower saturation helps improve sensitivity of spot detection. Typical exposure = 0.034secs |
|                  | Object identification    | Fixed Threshold: 20 – 100      |                                                                           |
|                  | SpotAreaCh2              | Min: 0                        | Defines min and max size of spots in pixels (improves assay sensitivity by eliminating larger areas of nucleus with different DAPI intensities) |
|                  |                          | Max: 30                       |                                                                           |
| **Assay Parameters** | Use reference wells     | 1                              | Turns on function which allows data to be expressed as % responders vs control wells. (Responder value set as 2SDs away from control) |
|                  | SpotDetectRadiusCh2      | 3                              | Determines the size of spots (in pixels) to be detected – helps to increase signal:noise by eliminating large variances in nuclear staining being detected as spots |
|                  | SpotSmoothFactorCh2      | 0                              | Turns off smoothing so that only bright spots with large contrast of background are detected – improves signal:noise |
|                  | RejectBorderObjectsCh1   | 1                              | Rejects all nuclei at edge of image                                     |
|                  | ObjectSegmentationCh1    | 0-7                            | Splits clumped nuclei into individual objects – important for assay sensitivity as SAHF phenotype is not 100% penetrant, and nuclear area is an important indicator of senescence |
|                  | Background CorrectionCh1 | 35                             | Improves signal:noise                                                   |
|                  | Background CorrectionCh2 | 10                             |                                                                           |
### SUPPLEMENTAL TABLE S2. Size hits (nucleus average area)

| Compound name* | Primary target kinases* | 3 µM | 5 µM |
|----------------|-------------------------|------|------|
| Aminopurvalanol A | Cdk1/cyclin B, Cdk2/cyclin A, Cdk2/cyclin E, Cdk5/p35 | II   | L    |
| Aurora Kinase Inhibitor II | Aurora Kinase | | |
| Gö 6976 | PKC | L | |
| Herbimycin A, Streptomyces sp. | P60^Src | L | |
| JAK3 Inhibitor VI | JAK3 | I | |
| Met Kinase Inhibitor | met kinase activity | I | |
| Rho Kinase Inhibitor IV | ROCK II | I | I |
| SU9516 | Cdk2/A | II | II |
| BAY 11-7082 | TNF-α-inducible phosphorylation of IkBα | L | Toxic |
| Cdk2 Inhibitor IV, NU6140 | Cdk1/cyclin B | I | I |
| GSK-3 Inhibitor XIII | GSK-3 | II | Toxic |
| IC261 | CK1δ | L | |
| JAK Kinase Inhibitor I | murine JAK1 | II | I |
| Kenpaullone | Gsk-3β | II | II |
| PDGF RTK Inhibitor | PDGFR | I | I |
| SU6656 | Src | I | I |
| (Cut-off threshold) | | (≥1.2) | (≥1.2) |

Hits identified by Arrayscan are highlighted in grey

I: Irregular (Type I)

II: Irregular (Type II)

L: Large

Toxic: gives count of less than 100 nuclei per well

* as shown in the Merck Millipore website
**SUPPLEMENTAL TABLE S3. Spotty hits (spot total area per nucleus)**

| Compound name* | Primary target kinases* | 3 µM | 5 µM |
|----------------|-------------------------|------|------|
| Aminopurvalanol A | Cdk1/cyclin B, Cdk2/cyclin A, Cdk2/cyclin E, Cdk5/p35 | - | - |
| Aurora Kinase Inhibitor II | Aurora Kinase | S | - |
| Chelerythrine Chloride | PKC | S | Toxic |
| Cdk2 Inhibitor III | Cdk2/A, Cdk2/E | S | S |
| EGFR Inhibitor | EGFR | S | S |
| GTP-14564 | Class III receptor tyrosine kinases | S | - |
| Herbimycin A, Streptomyces sp. | P60°src | - | - |
| JAK3 Inhibitor VI | JAK3 | - | - |
| MK2a Inhibitor | Mk2α | S | S |
| Rho Kinase Inhibitor IV | ROCK II | S | S |
| SU9516 | Cdk2/A | - | - |
| BAY 11-7082 | TNF-α-inducible phosphorylation of IκBα | - | Toxic |
| Cdk2 Inhibitor IV, NU6140 | Cdk1/cyclin B | - | - |
| EGFR/ErbB-2/ErbB-4 Inhibitor | EGFR/ErbB-2/ErbB-4 | S | - |
| GSK-3 Inhibitor XIII | GSK-3 | - | Toxic |
| IC261 | CK1ε | S | - |
| JAK Inhibitor I | murine JAK1 | - | - |
| JNK Inhibitor IX | JNK2, JNK3 | - | - |
| Kenpaullone | Gsk-3β | - | - |
| PDGF RTK Inhibitor | PDGFR | - | - |
| SB220025 | P38 MAPK | - | - |
| SU6656 | Src | - | - |

(Cut-off threshold) (*≥2.5) (*≥3.0)

Hits identified by Arrayscan are highlighted in grey

S: Spotty
- Not obvious

Toxic: gives count of less than 100 nuclei per well

* as shown in the Merck Millipore website
SUPPLEMENTAL TABLE S4. Failed mitosis under the treatment with IRGs

| Treatment | Normal mitosis (%) | Failed mitosis (%) | Number of mitosis events |
|-----------|--------------------|--------------------|--------------------------|
|           |                    | Single nucleus     | Multiple                 |
|           |                    | Irregular | Smooth |               |
| DMSO      | 92.0               | 0.0       | 2.0    | 6.0       | 50       |
| RhoK-IV   | 0.0                | 75.7      | 21.6   | 2.7       | 37       |
| PDGFR-V   | 0.0                | 90.7      | 5.6    | 3.7       | 54       |
| Cdk2-IV   | 0.0                | 72.1      | 23.0   | 4.9       | 61       |
| ZM1       | 0.0                | 74.3      | 22.9   | 2.9       | 35       |

Fate of the nuclei which underwent chromosome condensation and subsequent decondensation was categorized
Normal mitosis: two equal-sized nuclei were formed
Irregular: single irregular-shaped nucleus
Smooth: single nucleus without irregularity
Multiple: binuclei with equal- or unequal-sized nuclei, or more than two nuclei including both irregular and normal shape nuclei
Movies of H2B:YFP expressing cells under the treatment with indicated compounds were used for the analysis (three fields per well were captured)