Supplemental information

Microscopy-based single-cell proteomic profiling reveals heterogeneity in DNA damage response dynamics

Pin-Rui Su, Li You, Cecile Beerens, Karel Bezstarosti, Jeroen Demmers, Martin Pabst, Roland Kanaar, Cheng-Chih Hsu, and Miao-Ping Chien
Table S1. Statistics scores of ten different foci detection algorithms. Precision, recall and F1-score were shown

| Algorithm                  | Precision | Recall | F1-score |
|----------------------------|-----------|--------|----------|
| Simple Thresholding        | 0.016     | 0.495  | 0.031    |
| Regional Local Maximum     | 0.482     | 0.448  | 0.464    |
| Band-pass Filtering        | 0.208     | 0.192  | 0.200    |
| H-Dome Detection           | 0.526     | 0.347  | 0.418    |
| Kernel Density Estimation  | 0.284     | 0.347  | 0.312    |
| Local Comparison           | 0.812     | 0.741  | 0.775    |
| **Modified Local Comparison** | **0.935** | **0.727** | **0.818** |
| Locally Enhancing Filtering| 0.466     | 0.370  | 0.413    |
| Morphometry                | 0.048     | 0.721  | 0.091    |
| Multiscale Wavelets        | 0.026     | 0.865  | 0.051    |
Figure S1. Foci detection algorithm optimization and foci-dedicated image processing, related to Figure 1, 2 and STAR Methods. (A) Detailed steps of the modified local comparison algorithm. The modified kernels are Gaussian-like filters. Radius = 5 pixels. (B) The best sensitivity factor $\alpha$, 0.31, and radius of the filter size, 3 pixels, were optimized based on the highest F score and used in the modified local comparison algorithm for foci detection. The modified local comparison algorithm was used in the foci removal step in order to smoothen signals within nuclei but maintain sharpness of nuclei shape.
Figure S2. Evaluation of single-cell proteomics workflow, related to Figure 4 and STAR Methods. (A-i) Mass spectrometry setting optimization of automatic gain control (AGC) and maximum injection time (IT). (A-ii) Reporter ion intensity distribution of all sample channels. Standard deviations (std) of medians were listed. (B-i) Reporter ion intensity distribution of single-cell channels (with single cells) and blank channels (only PBS buffer). (B-ii) The reporter ion intensity of each protein across 9 channels were plotted. The reporter ion intensities were normalized by the median of each protein. The signals of individual single-cell channels were significantly higher than adjacent PBS channels (p ≤ 0.001, Student’s t-test). (B-iii) The signals from combined single cells were also significantly higher (p ≤ 0.001, Student’s t-test). (C-i) Evaluation of phototagging (PT) effect on proteomic data. Three types of samples were prepared: the cells without IR nor PT treatment (IR-PT⁻), the cells with only ionizing radiation (IR) treatment (IR+PT⁻) and the cells with both IR and PT (random tagging) treatment (IR+PT⁺). (C-ii) The histograms of mean log2 signal-to-noise (S/N) ratios of each protein of two types of sample: without or with PT. (C-iii) The histograms of mean coefficient of variation (CV) of summed S/N values of single-cell channels of each protein from the sample either without or with PT. The CV value was calculated from the proteins quantified in all single-cell channels. Each sample has four replicates. (D) Correlation of the label-free quantitation (LFQ) intensity of all proteins from the 200-cell samples treated with or without PT (random tagging). Pearson correlation analysis was performed and the coefficient (r) was indicated.
Figure S3. Evaluation of single-cell proteomics on irradiated (IR) samples, related to Figure 4 and STAR Methods. (A-i) The comparison of carrier cell composition between mixture of half non-IR cells (IR\(^-\)) and half IR (IR\(^+\)) cells, all non-IR cells, and all IR cells. (A-ii) The histograms of mean log2 signal-to-noise (S/N) ratios of each protein from three samples with different carrier cell compositions (non-IR & IR, only non-IR and only IR cells). (A-iii) The histograms of mean coefficient of variation (CV) of summed S/N values of single-cell channels of each protein from three samples with three different carrier cell compositions (non-IR & IR, only non-IR and only IR cells). The CV value was calculated from the proteins quantified in all single-cell channels. Each sample has four replicates. (B) Differential expression analysis of irradiated versus non-irradiated cells. (B-i) Single-cell samples (36 pairs from 12 repeats) and (B-ii) bulk-cell (1000-cell) samples (3 repeats). DNA damage response-related proteins (highlighted in green) that are differentially expressed comprise 33% and 29% of the total differentially expressed proteins for single-cell and bulk-cell samples, respectively. Dashed line shows the cutoff value of FDR at 0.05.
Figure S4. Single-cell analysis on Group 1 and Group 2 cells with FUNpro annotations, related to Figure 4 and STAR Methods. (A) UMAP (Uniform Manifold Approximation and Projection) plot of unsupervised clustering analysis on 40 pairs of Group 1 (non-tagged) and Group 2 (tagged) cells. (B) Cell cycle scoring analysis from Tirosh et al. was used to determine cell cycle phase of 80 cells in both Group 1 and Group 2 cells. (B-i) Relative cell cycle phase ratio of G1, S and G2/M cells from Group 1 (normal DDR) and Group 2 (abnormal DDR) cells; both N = 40 cells. (B-ii) G1 phase: both S score and G2M score are negative; S phase: S score is higher than G2M score; G2M phase: G2M score is higher than S score.
Figure S5. Characterization of Group 2 cells post irradiation, related to Figure 5. (A) A confocal microscopic image (left panel) of U2OS cells expressing 53BP1-mScarlet (red) or immunostained against anti-γH2AX antibodies (green). The cells were imaged after 24 h of ionizing radiation. Scale bar = 50 µm. Right panel: One representative zoomed-in image of Group 1 (one asterisk) or Group 2 (two asterisks) cell. Scale bar = 10 µm. (B) Bright field and fluorescent (53BP1) images of one representative Group 2 cell over the course of 2 days after irradiation. The foci were accumulated up to 48 h. Scale bar = 10 µm.