

# Supplementary Material: Histone H2AX Is Involved in FoxO3a-Mediated Transcriptional Responses to Ionizing Radiation to Maintain Genome Stability

Stephane Tarrade, Tanya Bhardwaj, Matthew Flegal, Lindsey Bertrand, Ilya Velegzhaninov, Alexey Moskalev and Dmitry Klovov

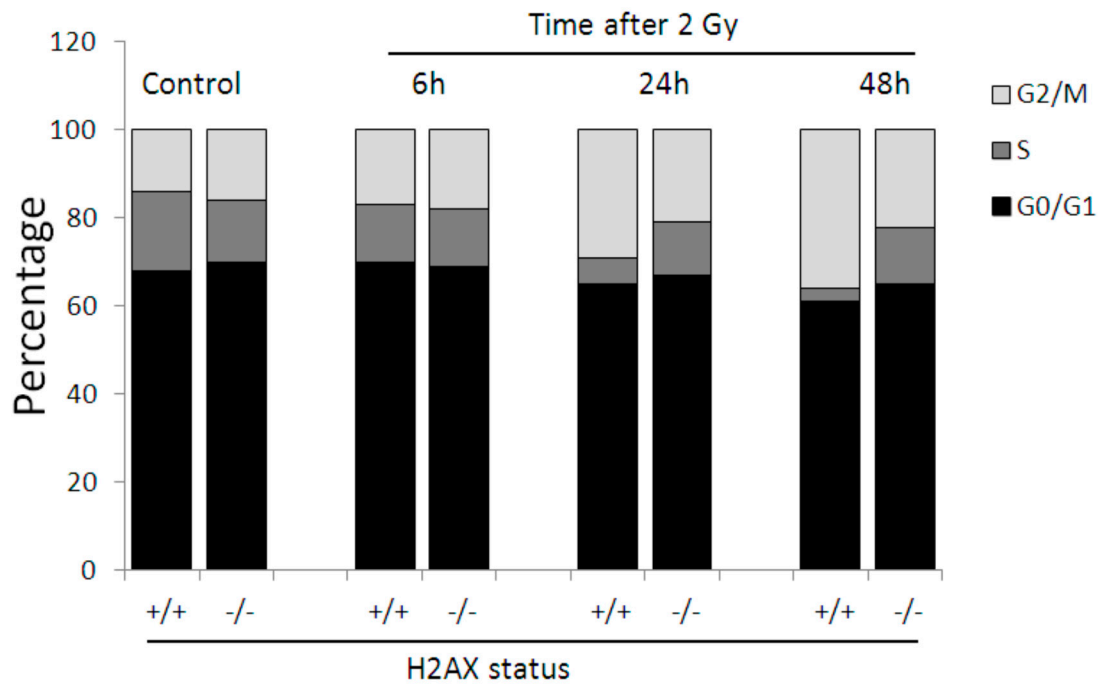
Table S1. Details of five pentaplex qPCR assays used in this study.

| 5plex ID                             | Gene Name/ID <sup>a</sup>           | Sequence/Probe Type                  | T <sub>m</sub> (°C) | Validation Result |
|--------------------------------------|-------------------------------------|--------------------------------------|---------------------|-------------------|
| #1                                   | <i>CDKN1A</i> /<br>NM_007669        | For: 5'-ACCGTGGGTGTCAAAGCA-3'        | 67                  | Passed            |
|                                      |                                     | Rev: 5'-GTGCGGGCTAAGGGTAGA-3'        | 65                  |                   |
|                                      |                                     | Probe: FAM—BHQ1                      | -                   |                   |
|                                      | <i>SOD2</i> /<br>NM_013671          | 5'-TTAGTGGGTCTGACTCCAGCCC-3'         | 69                  | Passed            |
|                                      |                                     | For: 5'-CGCCTGCCATGAGTACCAT-3'       | 66                  |                   |
|                                      |                                     | Rev: 5'-CGAGGTCATCTTGCCAAACTTG-3'    | 67                  |                   |
|                                      |                                     | Probe: CAL Fluor Gold 540—BHQ1       | -                   |                   |
|                                      | <i>CAT</i> /<br>NM_009804           | 5'-ACTGAGTCTAATGTTTAGGCCTTCCAGTGT-3' | 70                  | Passed            |
|                                      |                                     | For: 5'-ACAACGCTGAGAAGCCTAAGA-3'     | 67                  |                   |
|                                      |                                     | Rev: 5'-GCTGAGCACCGGAGTTACAG-3'      | 67                  |                   |
|                                      | <i>GAPDH</i> /<br>NM_008084         | Probe: CAL Fluor Red 610—BHQ2        | -                   | Passed            |
|                                      |                                     | 5'-ACCTACACGCAGGCCGGCTCTC-3'         | 70                  |                   |
|                                      |                                     | For: 5'-GTGTCCGTCGTGGATCTGA-3'       | 67                  |                   |
|                                      | <i>ACTB</i> /<br>NM_007393          | Rev: 5'-CACCACCTTCTTGATGTCATCATAC-3' | 67                  | Passed            |
|                                      |                                     | Probe: Quasar 670—BHQ2               | -                   |                   |
| 5'-CGTGCCGCCTGGAGAAACCT-3'           |                                     | 70                                   |                     |                   |
| For: 5'-GACAGGATGCAGAAGGAGATTACTG-3' |                                     | 66                                   |                     |                   |
| <i>DDB2</i> /<br>NM_028119           | Rev: 5'-GCTCAGGAGGAGCAATGATC-3'     | 66                                   | Passed              |                   |
|                                      | Probe: Quasar 705—BHQ2              | -                                    |                     |                   |
|                                      | 5'-TGGCTCCTAGCACCATGAAGATCA-3'      | 70                                   |                     |                   |
|                                      | For: 5'-GGCCACAGCCTCCATAGATC-3'     | 66                                   |                     |                   |
| <i>BBC3</i> /<br>NM_010786           | Rev: 5'-GTGAGGCAGTGAGTAGAGGAA-3'    | 67                                   | Passed              |                   |
|                                      | Probe: FAM—BHQ1                     | -                                    |                     |                   |
|                                      | 5'-AGTGAAGATTTGGGACCTGCGCCAAA-3'    | 70                                   |                     |                   |
|                                      | For: 5'-TGCCTTAAAGCTTCAGAGACAAG-3'  | 66                                   |                     |                   |
| #2                                   | <i>PPARG</i> /<br>NM_011146         | Rev: 5'-CCCACCGCCTCCTATTCAA-3'       | 67                  | Failed, excluded  |
|                                      |                                     | Probe: CAL Fluor Gold 540—BHQ1       | -                   |                   |
|                                      |                                     | 5'-CAAGAACACTCCTCCCACGTGACA-3'       | 70                  |                   |
|                                      | <i>GAPDH</i> /<br>NM_008084         | For: 5'-CTCAGGCAGATCGTCACAGA-3'      | 66                  | Passed            |
| Rev: 5'-TCAGCGGGTGGGACTTTC-3'        |                                     | 66                                   |                     |                   |
| Probe: CAL Fluor Red 610—BHQ2        |                                     | -                                    |                     |                   |
| 5'-TGCAGCTACTGCATGTGATCAAGAA-3'      |                                     | 69                                   |                     |                   |
| <i>ACTB</i> /<br>NM_007393           | For: 5'-CCTGGAGAAACCTGCCAAGTATG-3'  | 67                                   | Passed              |                   |
|                                      | Rev: 5'-AGCCCAAGATGCCCTTCAG-3'      | 67                                   |                     |                   |
|                                      | Probe: Quasar 670—BHQ2              | -                                    |                     |                   |
|                                      | 5'-CAAGAAGGTGGTGAAGCAGGCATCT-3'     | 70                                   |                     |                   |
| #3                                   | <i>DDIT3</i> /<br>NM_007837         | For: 5'-GTGACGTTGACATCCGTAAAGAC-3'   | 66                  | Passed            |
|                                      |                                     | Rev: 5'-GCCTGGGTACATGGTGGTA-3'       | 66                  |                   |
|                                      |                                     | Probe: Quasar 705—BHQ2               | -                   |                   |
|                                      | <i>MDM2</i> /<br>NM_010786          | 5'-ATGCCAACACAGTGCTGTCTGGT-3'        | 70                  | Passed            |
| For: 5'-GAGCTGGAAGCCTGGTATGAG-3'     |                                     | 66                                   |                     |                   |
| Rev: 5'-GGACGCAGGGTCAAGAGTAG-3'      |                                     | 66                                   |                     |                   |
| <i>MDM2</i> /<br>NM_010786           | Probe: FAM—BHQ1                     | -                                    | Passed              |                   |
|                                      | 5'-TGCAGGAGGTCCTGTCTCAGAT-3'        | 70                                   |                     |                   |
|                                      | For: 5'-GTGGGCTGCAGAGAAGAC-3'       | 66                                   |                     |                   |
| <i>MDM2</i> /<br>NM_010786           | Rev: 5'-CATGCGTGCATCCATTTGAAGATC-3' | 66                                   | Passed              |                   |
|                                      | Probe: CAL Fluor Gold 540—BHQ1      | -                                    |                     |                   |
|                                      | 5'-CAGCCAGTAAAGGCACCTGCTGT-3'       | 70                                   |                     |                   |

Table S1. Cont.

| 5plex ID            | Gene Name/ID <sup>a</sup>          | Sequence/Probe Type                | T <sub>m</sub> (°C) | Validation Result |
|---------------------|------------------------------------|------------------------------------|---------------------|-------------------|
| #3                  | TNFSF10/<br>NM_009425              | For: 5'-AGGCAACTGGAGCTAAAGACAAG-3' | 66                  | Failed, excluded  |
|                     |                                    | Rev: 5'-CCACCCTGTTCTCACAGATTC-3'   | 67                  |                   |
|                     |                                    | Probe: CAL Fluor Red 610—BHQ2      | -                   |                   |
|                     | GAPDH/<br>NM_008084                | 5'-TAAGCTGGTGTACCCCGATGGAGC-3'     | 70                  | Passed            |
|                     |                                    | For: 5'-CCTGGAGAAACCTGCCAAGTATG-3' | 67                  |                   |
|                     |                                    | Rev: 5'-AGCCCAAGATGCCCTTCAG-3'     | 67                  |                   |
|                     | ACTB/<br>NM_007393                 | Probe: Quasar 670-BHQ2             | -                   | Passed            |
|                     |                                    | 5'-CAAGAAGGTGGTGAAGCAGGCATCT-3'    | 70                  |                   |
|                     |                                    | For: 5'-GTGACGTTGACATCCGTAAGAC-3'  | 66                  |                   |
| #4                  | FOXO3A/<br>NM_019740               | Rev: 5'-GCTCAGGAGGAGCAATGATC-3'    | 66                  | Passed            |
|                     |                                    | Probe: Quasar 705—BHQ2             | -                   |                   |
|                     |                                    | 5'-AAGACCTACAGAGAAAACCCCTTGCCAA-3' | 69                  |                   |
|                     | GADD45α/<br>NM_007836              | For: 5'-GCAAAGCAGACCCTCAAACCTG-3'  | 66                  | Passed            |
|                     |                                    | Rev: 5'-CGGCGTGGGAGTCTCAAAG-3'     | 66                  |                   |
|                     |                                    | Probe: CAL Fluor Gold 540—BHQ1     | -                   |                   |
|                     | DDB1/<br>NM_015735                 | 5'-TGGAAGGATCCTGCCTAAGTCAACTTA-3'  | 68                  | Passed            |
|                     |                                    | For: 5'-CACACTGAGCGAAAGACAGAAC-3'  | 66                  |                   |
|                     |                                    | Rev: 5'-GGCGCTGATATCTAGGAAAC-3'    | 65                  |                   |
| GAPDH/<br>NM_008084 | Probe: CAL Fluor Red 610—BHQ2      | -                                  | Passed              |                   |
|                     | 5'-CAGCCACAGGCTTCATCGATGGT-3'      | 70                                 |                     |                   |
|                     | For: 5'-GCCAAGGTCATCCATGACAAC-3'   | 67                                 |                     |                   |
| ACTB/<br>NM_007393  | Rev: 5'-TCACGCCACAGCTTCCAG-3'      | 67                                 | Passed              |                   |
|                     | Probe: Quasar 670—BHQ2             | -                                  |                     |                   |
|                     | 5'-TGCTCTGGCTCCTAGCACCA-3'         | 69                                 |                     |                   |
| #5                  | HSPA1B/<br>NM_010478               | For: 5'-GACAGGATGCAGAAGGAGATTAC-3' | 66                  | Passed            |
|                     |                                    | Rev: 5'-GCTCAGGAGGAGCAATGATC-3'    | 66                  |                   |
|                     |                                    | Probe: Quasar 705—BHQ2             | -                   |                   |
|                     | G6PC/<br>NM_008061                 | 5'-CTGCTGGCTCTCCCGGTGTG-3'         | 70                  | Failed, excluded  |
|                     |                                    | For: 5'-GCAAGCCCAGGCTAGAGATC-3'    | 67                  |                   |
|                     |                                    | Rev: 5'-TTTCCCAGCCGGAAGATTC-3'     | 66                  |                   |
|                     | BCL2L11/<br>NM_207681              | Probe: CAL Fluor Gold 540—BHQ1     | -                   | Passed            |
|                     |                                    | 5'-TGAGAATGCTCTTGCGGTGCA-3'        | 70                  |                   |
|                     |                                    | For: 5'-TTGCCAAGGCCACTTGTG-3'      | 66                  |                   |
| GAPDH/<br>NM_008084 | Rev: 5'-GGGAGCCGAATGGTATAGGTAAG-3' | 66                                 | Passed              |                   |
|                     | Probe: CAL Fluor Red 610—BHQ2      | -                                  |                     |                   |
|                     | 5'-CAGGACACTCGCTGGCTACCG-3'        | 70                                 |                     |                   |
| ACTB/<br>NM_007393  | For: 5'-CCTGGAGAAACCTGCCAAGTATG-3' | 66                                 | Passed              |                   |
|                     | Rev: 5'-GCCCAAGATGCCCTTCAGT-3'     | 67                                 |                     |                   |
|                     | Probe: Quasar 670—BHQ2             | -                                  |                     |                   |
| ACTB/<br>NM_007393  | 5'-CAAGAAGGTGGTGAAGCAGGCATCT-3'    | 70                                 | Passed              |                   |
|                     | For: 5'-GTGACGTTGACATCCGTAAGAC-3'  | 66                                 |                     |                   |
|                     | Rev: 5'-GCTCAGGAGGAGCAATGATC-3'    | 66                                 |                     |                   |
| ACTB/<br>NM_007393  | Probe: Quasar 705—BHQ2             | -                                  | Passed              |                   |
|                     | 5'-ATGCCAACACAGTGCTGTCTGGTG-3'     | 70                                 |                     |                   |
|                     | For: 5'-GCAAAGCAGACCCTCAAACCTG-3'  | 66                                 |                     |                   |

<sup>a</sup> NCBI RefSeq accession number. # designates the number of an assay. Abbreviations: BHQ, black hole quencher; FAM, 5-carboxyfluorescein.



**Figure S1.** Distribution of cell cycle phases in MEF *H2AX*<sup>+/+</sup> vs. MEF *H2AX*<sup>-/-</sup> cells under control and irradiated condition. Subconfluent cells were sham- or  $\gamma$ -irradiated with 2 Gy followed by fixation in 70% methanol at indicated time points and stored at -20 °C. Fixed cells were subsequently rehydrated, stained with DAPI (4',6-diamidino-2-phenylindole) and analyzed using the Cell Lab Quanta SC flow cytometer (Beckman Coulter). DAPI (DNA content) fluorescence were triggered by an mercury arc lamp and detected and measured in the FL1 (525BP) channel. Readings from at least 5000 cells were collected and analyzed by Quanta Analysis software (Beckman Coulter) to calculate fraction of cells in various cell cycle phases. Data are the means of two independent experiments.