Cancer Cell *11*

Supplemental Data

Crosstalk between *Aurora-A* and *p53*:

Frequent Deletion or Downregulation of Aurora-A

in Tumors from *p53* Null Mice

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Supplemental Experimental Procedures

Detection of Apoptosis by Annexin V

Annexin V-FITC apoptosis detection kit was purchased from BD Biosciences Pharmingen. All procedures were carried out according to the manufacturer's instructions.

Synchronization Study

MEFs were plated in triplicate plates at $5x10^5$ cells. After 24h, cells were washed twice with DMEM and then incubated in DMEM without FCS for 24 h. Cells were allow to re-enter cell cycle by replacing the culture medium with that containing 10% FCS. Cells were harvested at several time points for flow cytometry.

Transfection and Establishment of Stable Cell Lines

p53-/- MEFs were infected with high-titre retroviral stocks produced by the transient transfection of 293T ecotropic Phoenix cells. After infection with the pBabe-Aurora-A allowing the expression of Aurora-A molecules. MEFs were selected with 1-2μg /ml of puromycin in the culture medium. The control for the experiment was the empty pBabe vector.

For growth (proliferation) curve determinations, cells were seeded into three-100 mm tissue culture plates at 3×10^5 cells per plate in DMEM (high glucose) supplemented with 10% FBS and penicillin-streptomycin. Cell numbers were determined every three days by Neubauer's chamber. Accumulative cell numbers were calculated at each passage.

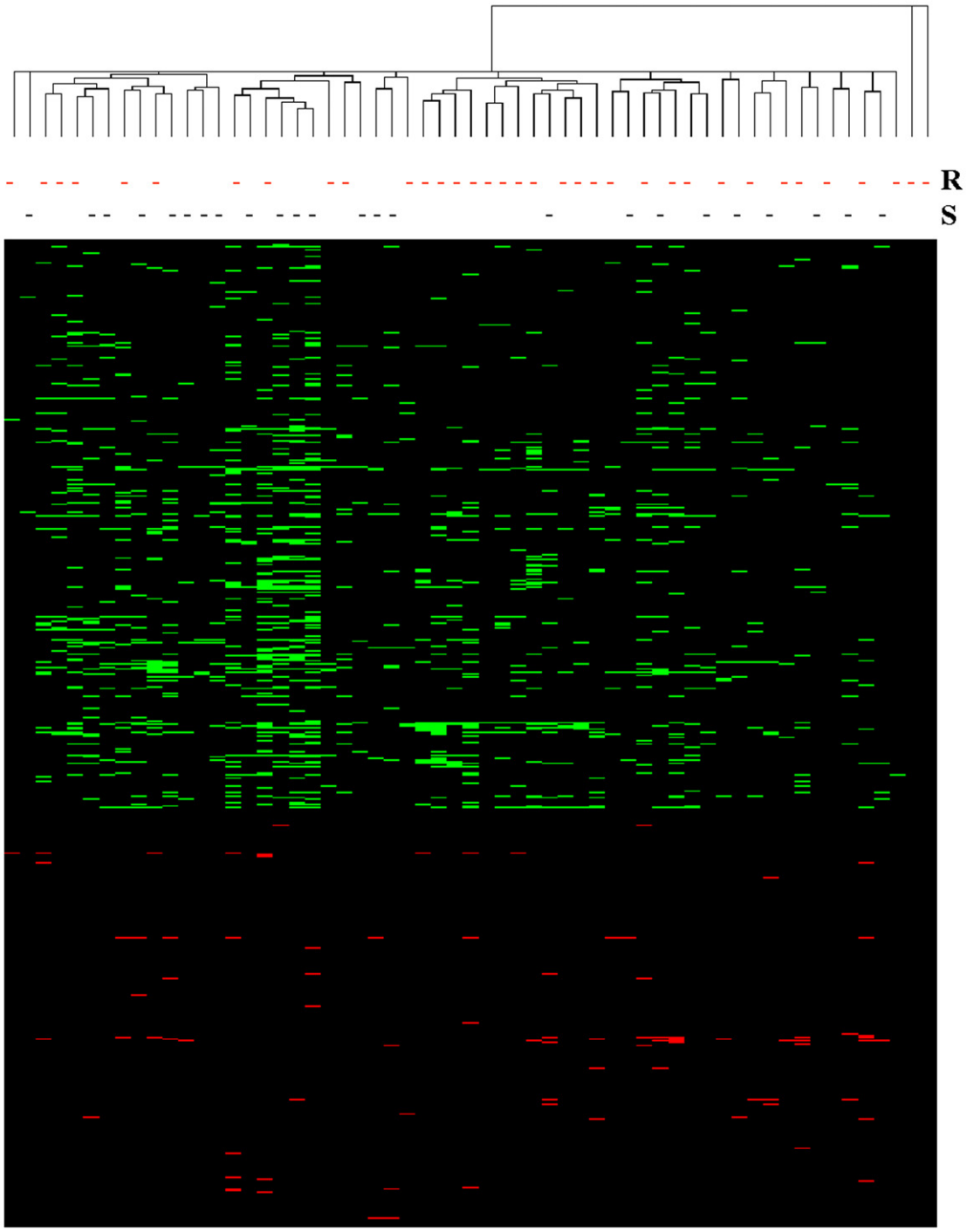

Figure S1. Comparison of Genetic Changes between Radiation-Induced and Spontaneous Thymic Lymphomas from p53-/- Mice

Unsupervised clustering of genomic alterations from CGH array analysis of tumors from p53 null mice. Tumors labeled NS are from unirradiated mice, and this labeled NR are from irradiated animals.

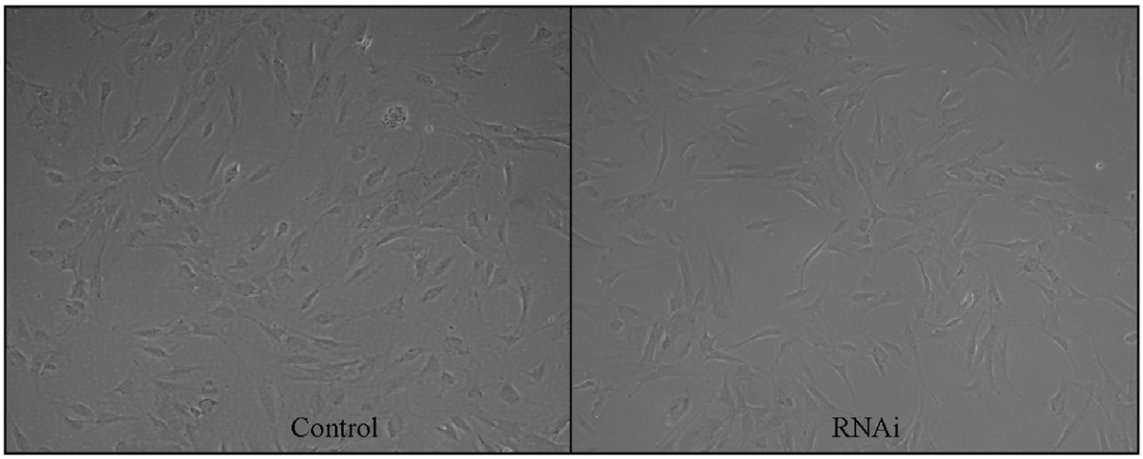

Figure S2. Cell Morphology Was Not Changed in p53 Null MEFs Treated with Aurora-A RNAi

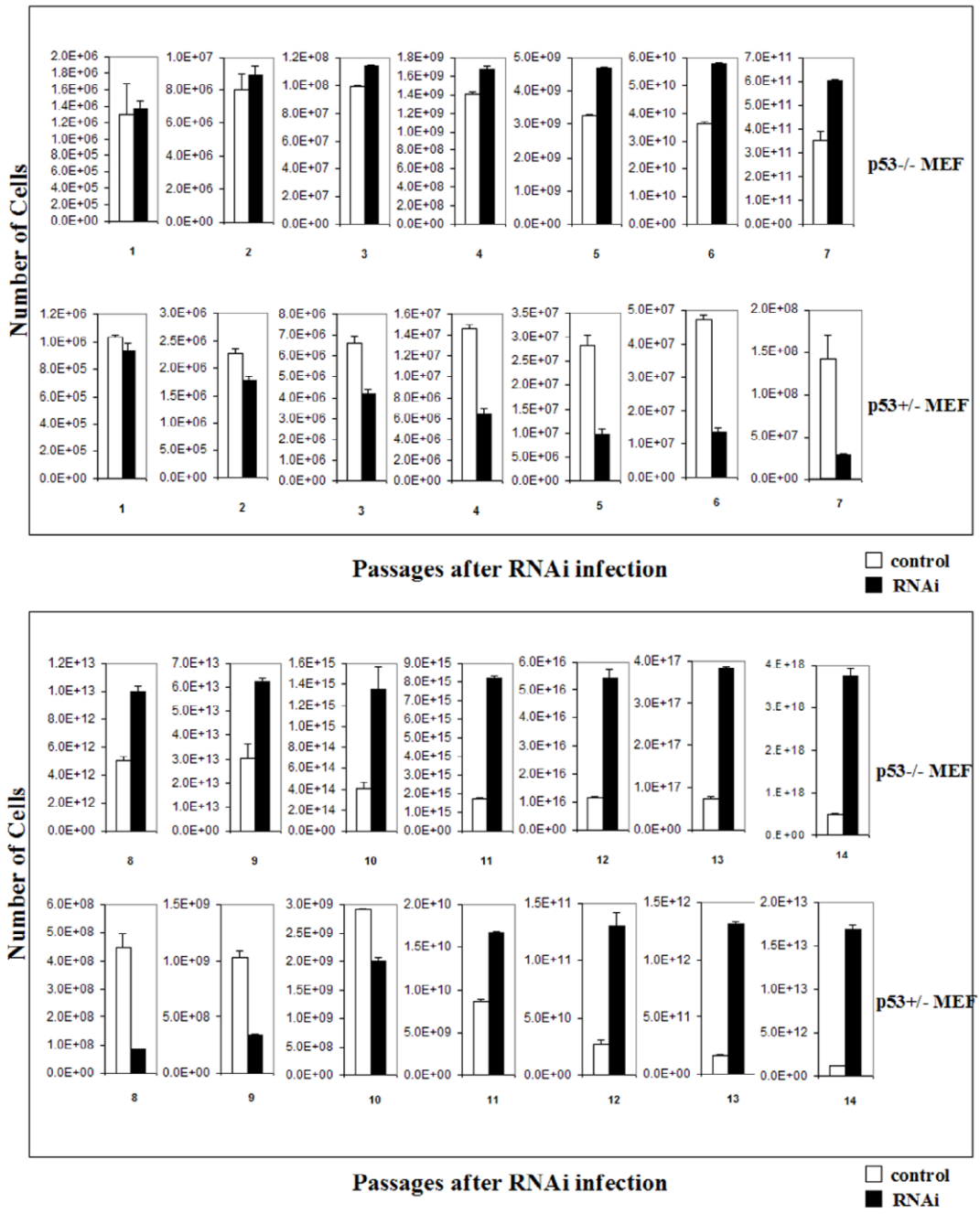

Figure S3. Actual Number of Cells in Each Passage

Standard 3T3 cell culture protocol was used to characterize the proliferative potentials of *p53+/-* and *p53-/-*control MEFs or MEFs with Aurora-A RNAi. Down-regulation of *Aurora-A* in p53+/- MEFS produces a clear growth disadvantage at early passages, but growth advantage at late passages, compared with their untransfected counterparts. p53 null MEFs transfected with the same RNAi construct showed similar behaviour independently of the presence of *Aurora-A* RNAi at early passages, and produced a clear growth advantage at late passages.

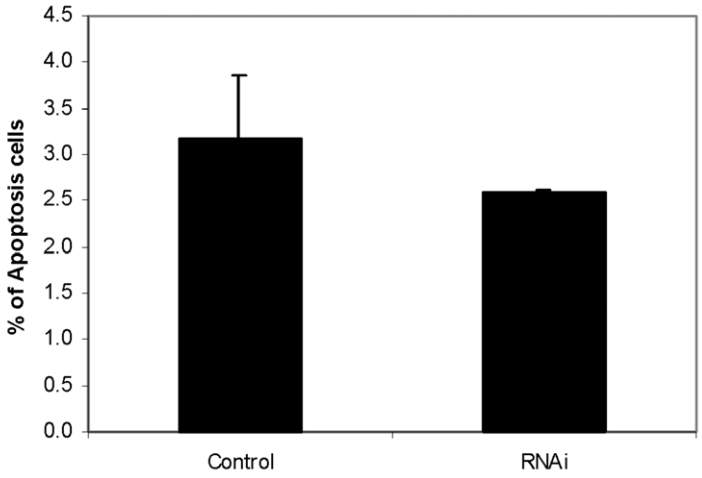

Figure S4. Percentage of Apoptosis Cells in p53-/- MEFs with or without Aurora-A RNAi

AnnexinV staining and FACS analysis were performed as described in Methods. Experiments were repeated at least three times and the average number of Annexin V positive cells was calculated.

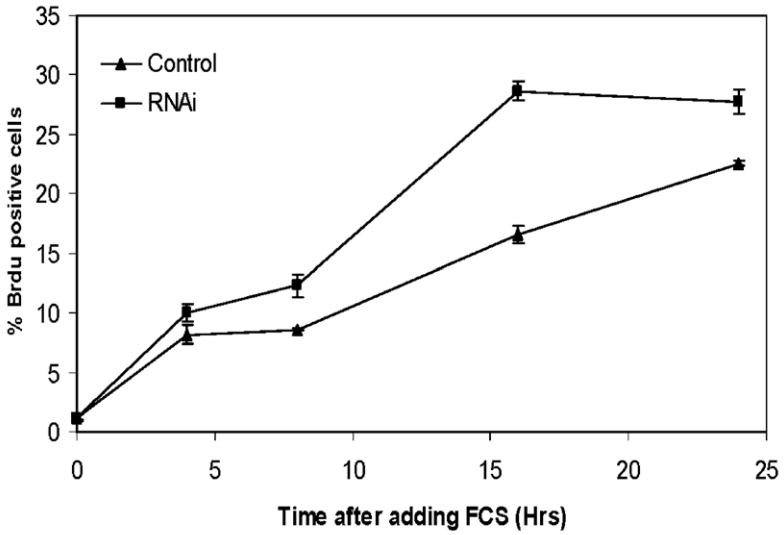

Figure S5. Percentage of Cells that Re-enter Cell Cycle in p53-/- MEFs with or without Aurora-A RNAi after Adding FCS

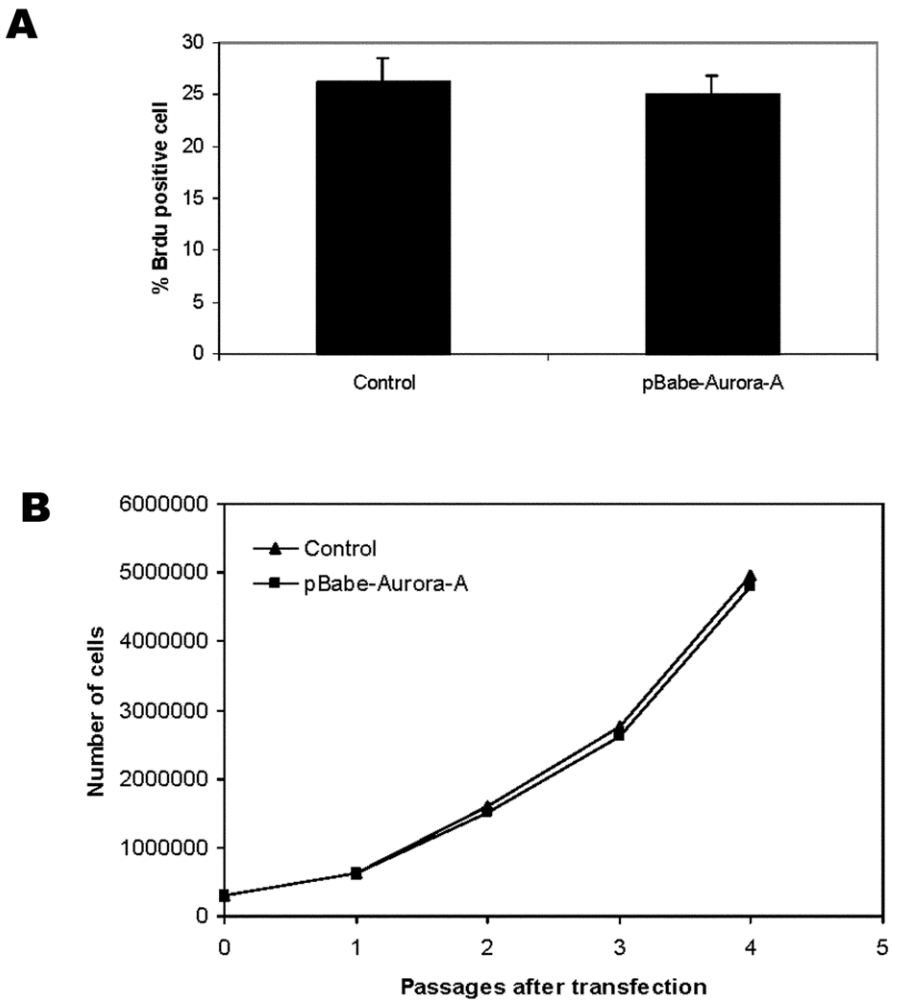

Figure S6. Overexpression of Aurora-A in p53-/- MEF Does Not Give Growth Advantage

A: Percentage of Brdu positive cells. B: Proliferation assay using standard 3T3 culture protocol.