Supplemental Information

Supplemental Materials and Methods

Cell culture: H2AX$^{-/-}$ MEFs were from Dr. Andre Nussenzweig (NIH, MD). MDC1$^{-/-}$ MEFs were from Dr. Junjie Chen (Yale Univ., CT). AT (ATM$^{-/-}$) fibroblasts and those complemented with ATM were provided by Dr. Y. Shiloh (Tel Aviv Univ., Israel). NBS1$^{-/-}$ cells were from Dr. J. Petrini (Sloan–Kettering Institute, NY). mTR$^{-/-}$ cells were kindly provided by Dr. R. DePinho (Harvard Univ., MA). MMR-deficient (hMLH1$^{-/-}$) HCT116 (parental) and isogenic MMR-corrected (corrected for hMLH1 expression by microcell transfer of an extra chromosome 3) HCT116:3-6 human colon cancer cells were provided by Dr. C.R. Boland (Baylor Univ., TX). HCT116 p53$^{-/-}$ cells were kindly provided by Dr. B. Vogelstein. hMLH1-deficient RKO cells were corrected for MMR with full length hMLH1 (RKO7) by us (Wagner et al 2008). HCT116:3-6 and RKO7 cells were knocked down for p53 using an shp53 SUPER lentiviral vector. MCF-7 cells were purchased from ATCC. Human bronchial epithelial cells (HBECs), immortalized by viral transduction of Cdk4 and hTERT (HBEC 3kt) and stably infected with small hairpin p53 (shp53) or R273H p53 mutant cDNA, were generously provided by Drs. J. Minna and M. Sato (Univ. of Texas Southwestern) (Sato et al 2006). Cell lines were maintained in 5% FBS containing DMEM (Hyclone) in a 10% CO_2-90% air humidified atmosphere. HBECs were maintained in KSFM defined media (Gibco). All cells were free from mycoplasma infection.

Western Blotting: Cells were harvested by RIPA extraction (0.1%SDS, 0.5% deoxycholate, 1% NP-40, 150 mM NaCl, 50 mM Tris pH 8.0), unless otherwise indicated. Nuclear extracts were performed with buffer A (10 mM HEPES, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1mM DTT and 0.5mM PMSF). NP-40 (0.5%) was added 15 minutes later. Nuclei were collected, and extracted in buffer C (20 mM HEPES, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, and 1 mM PMSF). Antibodies to human sCLU (B-5), mouse sCLU (M18), p53 (DO1), β-actin, and α-tubulin were from

Santa Cruz. Antibodies to phosphorylated Akt1 (S473), ERK-1/2 (Y204), and Chk1 (S317 and S345), as well as total Akt1, ERK-1/2, and Chk1 were from Cell Signaling Technology. Antibodies against phosphoryated IGF-1R and total IGF-1R were from Abcam and Millipore, respectively. GAPDH and Mdm2 antibodies were from EMD Biosciences.

Luciferase Assays: The p21-promoter luciferase reporter was from Dr. B. Vogelstein (el-Deiry et al 1993)

Site directed mutagenesis: Forward primers used in PCR reactions with full-length plasmid DNA were (backward primers were reverse complement):

S15A: 5' -GAGCCCCCTCTGGCTCAGGAAACATTTTCA-3';

S15D: 5'-GAGCCCCCTCTGGATCAGGAAACATTTTCA-3';

S15E: 5'-GAGCCCCCTCTGGAACAGGAAACATTTTCA-3';

S20A: 5'-CAGGAAACATTTGCAGACCTATGGAAACTACTTC-3';

S20E: 5'-CAGGAAACATTTGAAGACCTATGGAAACTACTTC-3';

IGF-1 NF-Y MUT: 5'- GCCCTAAAGGGATACATCCAATGCTGCCTG CCCCTCC - 3'

RT-PCR: Total RNA was isolated using RNeasy mini kits (Qiagen) and cDNA synthesized using high capacity cDNA archive kit (Applied Biosystems). IGF-1 forward: 5'-AACACCATCCATTTGGGAAA-3'; IGF-1 backward: 5'-TGACATATTGCCCCCATTTT-3'; β-actin forward: 5'-GGACTTCGAGCAAGAGATGG-3', β-actin backward: 5'-AGCACTGTGTTGGCGTACAG-3'.

ChIP primers: IGF-1 forward: 5'-TCTATTTCAGTTGGGTTTTACAGCT-3'; IGF-1 backward: 5'-CTCACTAGTGCTTCTGAAGTACAAAG-3'; p21 forward: 5'-CGACTCTTGTCCCCCAGGCT-3'; p21 backward: 5'-GGTCTCCTGTCTCCTACCAT-3'

Supplemental Figure Legends

Supplemental Figure 1, Activation of IGF-1-sCLU expression axis after IR. **A, B.** IGF-1-LUC (A) and CLU-LUC (B) are induced to a greater extent in RKO7 shp53 cells than in RKO7 SCR after IR exposure. Activity is normalized with 0 Gy for each cell line. *, ** $p \leq 0.02$. **C.** Lysates of cells from *A* were analyzed by immunoblotting. For *A and B*: Data are graphed as the mean +/- standard deviation (SD). RL, relative levels.

Supplemental Figure 2, IGF-1 signaling is upregulated after DNA damage. A. mRNA was isolated from RKO7 cells exposed to DNA damaging agents (IR, 1, 2.5 5 Gy; H_2O_2, 50 and 100 μM, 5 h; VP16, 5 and 10 μM, 5 h; TPT, 2 and 4 μM, 5 h; UT, DMSO) and assessed using semi-quantitative RT-PCR. **B.** Mock or IR-treated RKO7 cells were assessed for IGF-1 ligand by ELISA 48 h after treatment. **C.** MCF-7 cells induce Erk phosphorylation (pErk) after exposure to DNA damaging agents: IR, 5 Gy; H_2O_2, 50 μM for 5 h; VP16, 10 μM for 5 h; TPT, 2.2 μM for 5 h. Controls received DMSO (UT). Whole cell extracts were harvested at the indicated times for western analyses of phosphorylated ERK (pERK) and total ERK. Note 24 h and 48h are reversed for TPT treatment. **D.** $H2AX^{-/-}$ and $H2AX^{+/+}$ MEFs were analyzed for IGF-1 expression by FACS. **E.** $H2AX^{-/-}$ and $H2AX^{+/+}$ MEFs induce IGF-1 signaling when treated with IGF-1 (10 ng/mL, 1 h) and analyzed for phospho-IGF-1R and phospho-Akt by immunoblotting. **F.** $MDC1^{-/-}$ and $MDC1^{+/+}$ MEFs were treated with 1 μM AG1024 and collected 48 h later for sCLU expression by immunoblotting. **G.** $mTR^{-/-}$ MEFs were treated with 2-6 μM AG1024 and collected 48 h later for sCLU expression by immunoblotting. For *B and D*: Data are graphed as the mean +/- standard deviation (SD). *$p \leq 0.05$.

Supplemental Figure 3, Genomically unstable cells have heightened DNA damage signaling. A. Exposure to TPT leads to elevated phospho-ATM and γ-H2AX in S phase that is blocked by aphidicolin. *Left*, FACS assessments of $pATM^{S1981}$ and γ-H2AX in MCF-7 cells treated with 4.4 μM TPT with or without aphidicolin (1 μg/ml, 2 h). Specific phases of the cell cycle are indicated on the dot plots. *Right*, graph of data. Data are graphed as the mean +/- standard deviation (SD), n=3. **B.** H2AX, MDC1, and mTR deficient (-/-) cells have elevated ATR signaling, shown by higher phospho-Chk1 in the deficient cells, compared to WT (+/+) MEFs.

Supplemental Figure 4, AAI blocks ATM signaling in human and mouse cells A. AAI pre-treatment (2 h) blocks phospho-ATM and γ-H2AX in MCF-7 cells 1 h after IR exposure (10 Gy). Boxes indicate cells that stained positive for the indicated antibody and the percentages of cells in these boxes were enlarged on the right. **B.** AAI pre-treatment (2 h) blocks phospho-ATM in MEFs 1 h after IR exposure (10 Gy). Outlined area indicates cells that stained positive for the indicated antibody and the percentages of cells in these areas were enlarged on the right. RL, relative levels.

Supplemental Figure 5, ATM and p53 expression determine sCLU induction after IR A. $ATM^{-/-}$ and $ATM^{+/-}$ fibroblasts were treated with IGF-1 (10 ng/ml, 3-72 h) and whole cell extracts analyzed by Western. **B.** Immortalized human ATM deficient (ATM-/-) or ATM reconstituted (ATM+) cells induced IGF-1 signaling after exposure to IGF-1 (10 ng/ml, 1 h). Cells were examined for phospho-IGF-1R and phospho-Akt by western blot. **C.** phospho-Chk1 was induced by 25 or 50 J/m^2 UV in ATM-/- and ATM+ cells. **D, E.** HCT116 $p53^{-/-}$ cells were co-transfected with vector only (VO), WT p53 CMV expression plasmid (WT), serine 15 mutants (Serine 15 to Alanine (S15A), Aspartate (S15D), or Glutamate (S15E)), or serine 20

mutants (Serine 20 to Alanine (S20A) or Glutamate (S20E)), IGF-1-LUC or CLU-LUC, and RSV-β-Gal. Luciferase activities were measured 48 h after transfection. *Lower*, western analysis of lysates confirm p53 expression. Data were graphed as the means +/- standard deviation (SD). **F.** HCT116 p53-/- cells were co-transfected with the p21-promoter luciferase reporter (p21-LUC) and mutant p53 constructs from *D* and *E*. Cells were harvested 48 h later for luciferase activity measurements. *Right*, western blot of p53 expression. For *D, E, and F*: Data were graphed as the means +/- SD. */**/** p53 WT vs p53 mutant.

*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.005$.

References

el-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM *et al* (1993). WAF1, a potential mediator of p53 tumor suppression. *Cell* **75:** 817-825.

Sato M, Vaughan MB, Girard L, Peyton M, Lee W, Shames DS *et al* (2006). Multiple oncogenic changes (K-RAS(V12), p53 knockdown, mutant EGFRs, p16 bypass, telomerase) are not sufficient to confer a full malignant phenotype on human bronchial epithelial cells. *Cancer Res* **66:** 2116-2128.

Wagner MW, Li LS, Morales JC, Galindo CL, Garner HR, Bornmann WG *et al* (2008). Role of c-Abl kinase in DNA mismatch repair-dependent G2 cell cycle checkpoint arrest responses. *J Biol Chem* **283:** 21382-21393.