SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Comparison of MDM2 binding properties of RG7112 and nutlin-3a. Dose response curves have been generated using Biacore as described in Materials and Methods section.

Figure S2. Overlay of the crystal structures of MDM2 bound to RG7112 and Nutlin-2. The structure of the complex with Nutlin-2 (PDB accession code 1rv1), depicted with the magenta ribbon drawing for MDM2 and the stick figure of the antagonist with orange carbon atoms and other atom types as in Figure 1C (except that the bromine atoms are brown), has been superposed with the MDM2 structure (green ribbon drawing) with RG7112 (same color scheme as in Figure 1C except that the carbon atoms are colored cyan) based solely on the Cα positions of the two proteins. The root mean square deviation for the 79 out of 85 Cα positions that are within 1.5Å of each other is 0.58Å. The few small differences in the two protein structures are likely due primarily to the fact that they crystallized in two different crystal forms ($P2_13$ for Nutlin-2 and $C222_1$ for RG7112). The slight differences in the key elements of the two antagonists are likely due to the presence of the bromine atoms in Nutlin-2 compared to the chlorine atoms in RG7112 but may also be affected by the different space groups.

Figure S3. RG7112 is a non-genotoxic p53 activator. Exponentially proliferating RKO cancer cells were incubated with indicated concentrations of RG7112 and Doxorubicin (genotoxic control) or vehicle (DMSO) for 24 hours and the levels of total p53, p53 phosphorylated on Ser15 and MDM2 were determined by Western blotting in cell lysates.

Figure S4. Effect of RG7112 on the viability of HCT116 cancer cells and their p53-null clone HCT116R1 (1). Exponentially proliferating cells were incubated with indicated concentrations of RG7112 for 5 days and cell viability was measured by the MTT assay.

Figure S5. RG7112 blocks the proliferation of mouse NIH-3T3 cells. Early passage NIH-3T3 fibroblasts in log phase were incubated with the indicated concentrations of RG7112 or nutlin-3a for 24 hours and the cell cycle distribution of the cell populations

was determined by BrdU-labeling and cell cycle analysis. Percentage of cells in each phase of the cell cycle was determined and the changes in the S-phase cell population were plotted for each compound.

Table S1. Antitumor activity of RG7112 against a panel of solid tumor cells. Indicated cell lines representing diverse solid tumor types were incubated with a range of RG7112 concentrations for 5 days and cell number/viability determined by the MTT assay. RG7112 activity is expressed as IC50±SD is shown. * Indicates a single test done in triplicate.

1. Graves B, Thompson T, Xia M, Janson C, Lukacs C, Deo D, Di Lello P, Fry D, Garvie C, Huang KS, Gao L, Tovar C, Lovey A, Wanner J, Vassilev LT. Activation of the p53 pathway by small-molecule-induced MDM2 and MDMX dimerization. Proc Natl Acad Sci U S A. 2012;109:11788-93.