

Figure S1. Nutlin-3a but not Nutlin-3b-pretreated MSCs exhibit reduced protection of FLT3/ITD AML cells from FI-700-induced apoptosis

Normal MSCs (N-MSC) were pretreated with 10 μ M Nutlin-3a or -3b for 24 hours. MOLM-13 cells were treated with 800 nM FI-700 for 24 hours in the presence or absence of MSCs. Asterisk (*) indicates significance at $P < 0.05$ (one-way ANOVA/Tukey).

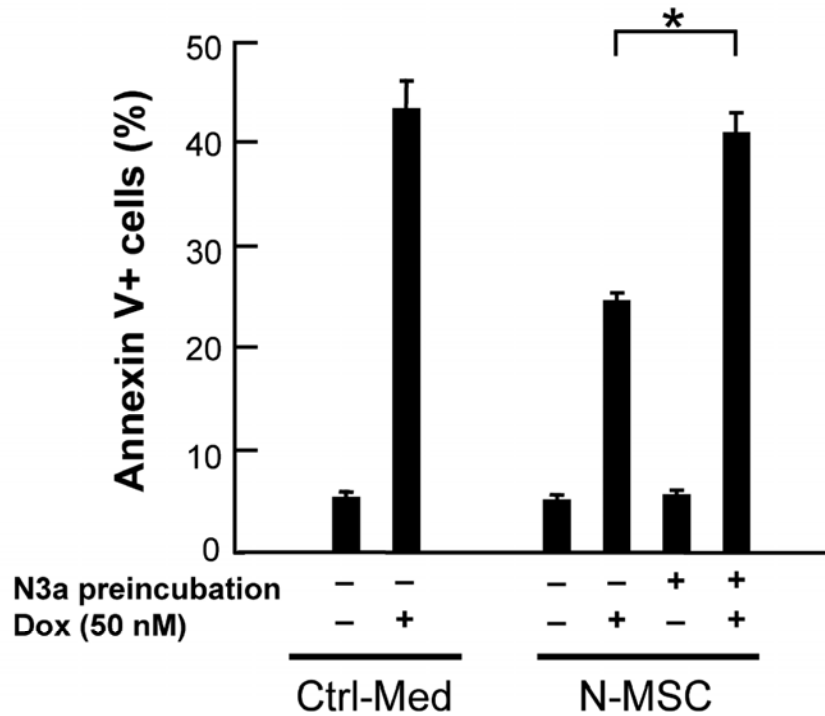


Figure S2. Nutlin-pretreated MSCs exhibit reduced protection of FLT3/ITD AML cells from doxorubicin-induced apoptosis

Normal MSCs (N-MSC) were pretreated with 10 μ M Nutlin-3a (N3a) for 24 hours. MOLM-13 cells were treated with 50 nM doxorubicin (Dox) for 48 hours in the presence or absence of normal MSCs. Asterisk (*) indicates significance at $P < 0.05$.

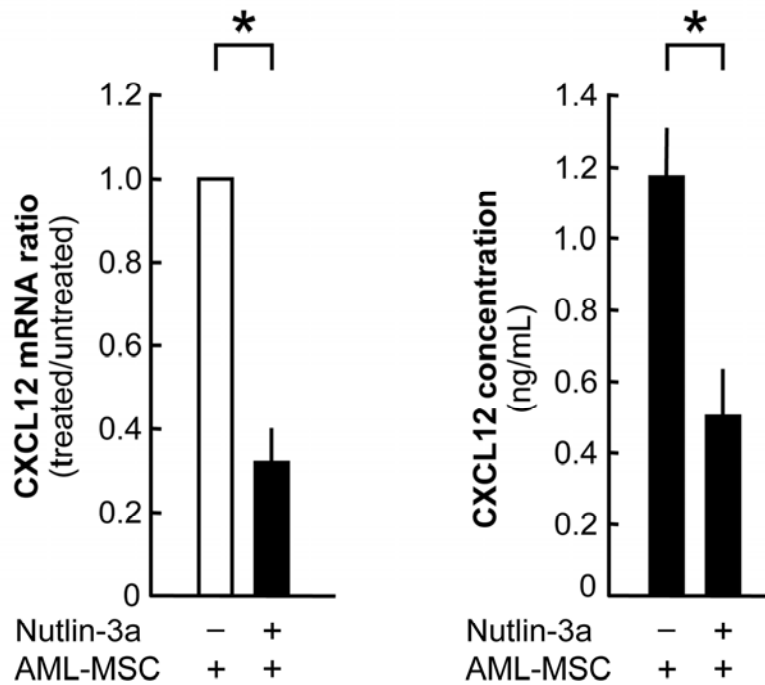


Figure S3. Nutlin-3a treatment reduces CXCL12 expression in AML-derived MSCs

MSCs from 5 AML patients (AML-MSC) were cultured for 48 hours in the presence or absence of 10 μ M Nutlin-3a, and CXCL12 mRNA levels relative to untreated cells (left) and CXCL12 concentrations in the culture medium (right) were determined. Results are expressed as mean \pm standard error. Asterisk (*) indicates significance at $P < 0.05$.

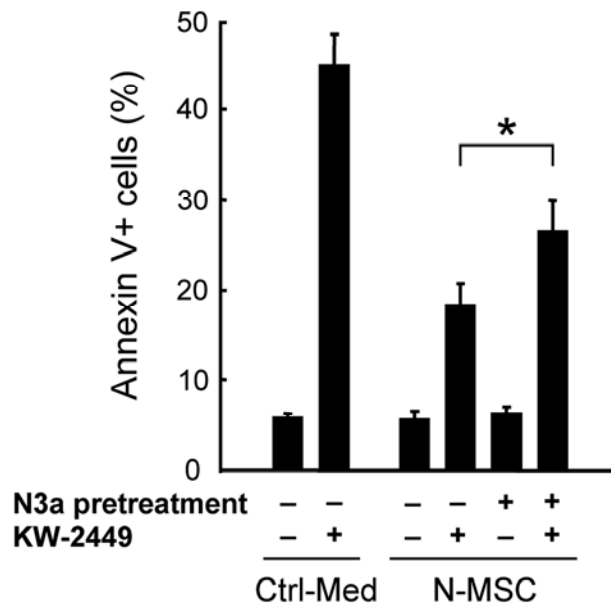


Figure S4. Nutlin-3a-pretreated MSCs exhibit reduced protection of FLT3/ITD AML cells from KW-2449-induced apoptosis

Normal MSCs (N-MSC) were pretreated with 10 μ M Nutlin-3a for 24 hours. MOLM-13 cells were treated with 400 nM KW-2449 for 24 hours in the presence or absence of MSCs.

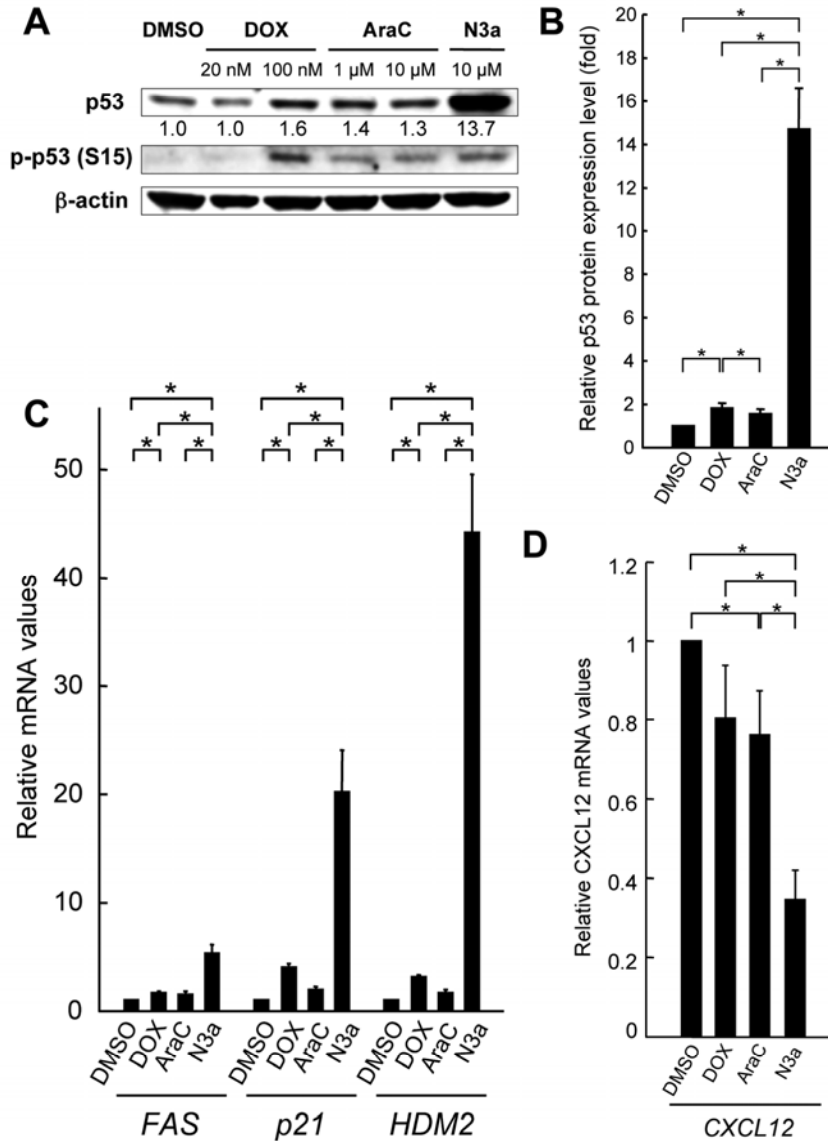


Figure S5. Nutlin-3a more potently induces p53-mediated responses compared to doxorubicin or AraC in MSCs

Four normal MSC samples were treated for 48 hours with 100 nM doxorubicin (DOX), 10 μM AraC, 10 μM Nutlin-3a (N3a) or vehicle (DMSO). (A) Expression of p53 protein was determined by Western blotting. A representative case is shown. (B) p53 protein expression levels relative to a loading control, β-actin, were determined in each sample and then compared to the reference DMSO-treated sample. Samples treated with Nutlin-3a expressed significantly higher levels of p53 than DMSO-, doxorubicin- or AraC-treated cells. (C–D) p53-target gene activation (C) and CXCL12 repression (D) in response to doxorubicin, AraC or Nutlin-3a in 4 MSC samples. Ratios represent doxorubicin, AraC or Nutlin-3a values divided by DMSO values. Results are expressed as mean ± SEM. Asterisk (*) indicates significance at $P < 0.05$.