# Supplementary Materials: A Small Molecule Stabilizer of the MYC G4-Quadruplex Induces Endoplasmic Reticulum Stress, Senescence and Pyroptosis in Multiple Myeloma

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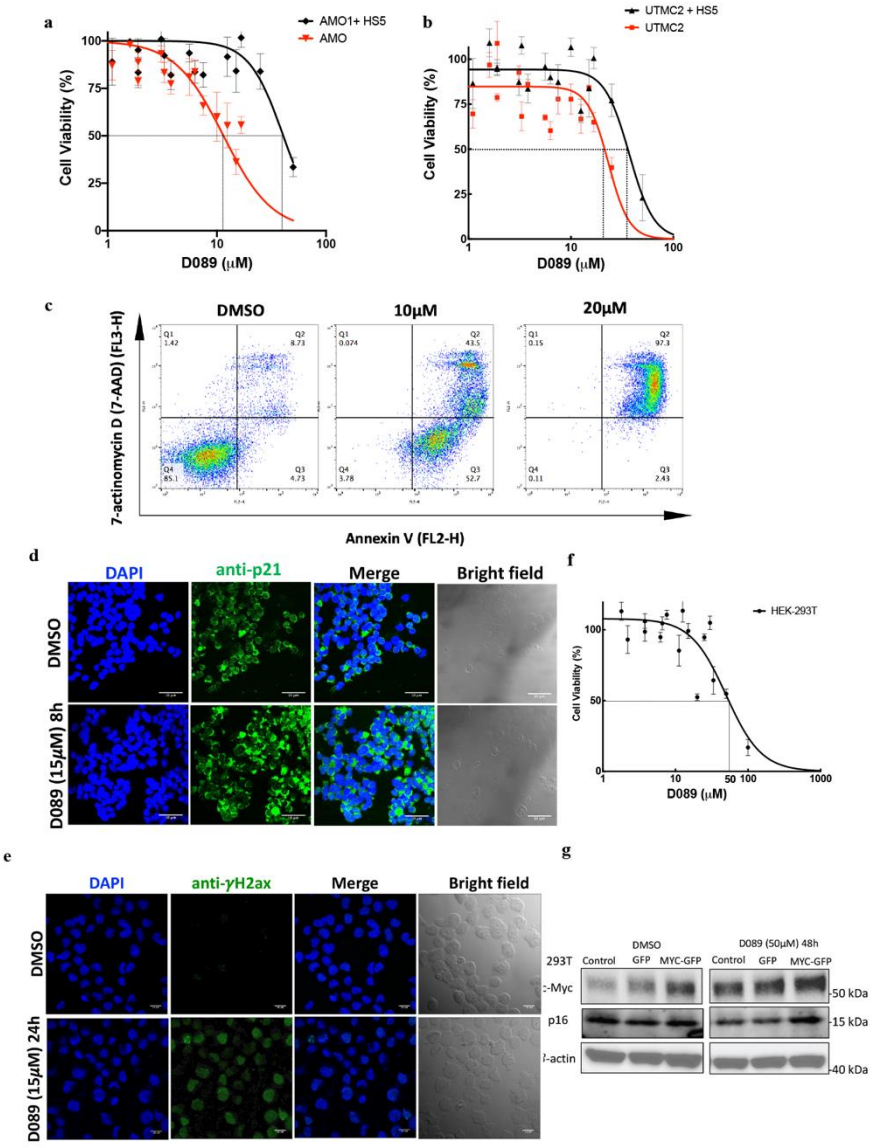


**Figure S1.** D089 mediated cytotoxicity in MM cells leads to senescence and cell death. (**a,b**) Cell viability of MM cell-lines AMO1 and UTMC2 in co-culture with HS-5- BMSCs after 48h of treatment with D089 (MTS assay). The data is presented as percentage of cell viability relative to untreated cells. Cytotoxicity of D089: for AMO1 cells either alone [($IC_{50}$) = 14 (11–16) μM] or in co-culture with BMSCs [$IC_{50}$ = 44 (37–52) μM]. Similarly, for UTMC2 cells either alone [($IC_{50}$) = 23 (20–27) μM] or in co-culture with BMSCs [$IC_{50}$ = 37 (30–47) μM]. (**c**) L363 cells were stimulated with different concentrations of D089 for 24 h and cell death was assessed with annexin-V and 7-AAD staining followed by flow cytometry. The double-positive staining in quadrant 2 (Q2) represents the percentage of cell death;

(**d,e**) L363 cells were treated with 15 μM D089 for 8 h and both p21 (d) and g-H2AX (e) protein expression were analyzed by confocal microscopy (p21 and g-H2AX in green); cells were counter stained with DAPI to detect nuclei (blue). Scale bars: 10 μm; Magnification: 63× objective lens. (**f**) Dose response of HEK293T cells assessed after 48 h of treatment with D089. The data are presented as percent of viable cells relative to untreated cells and the $IC_{50}$ concentration was 50 μM. (**g**) Immunoblotting of HEK293T cell lysates transiently transfected with CMV-c-MYC-IRES-GFP followed by D089 (50 μM) treatment for 24 or 48 h.

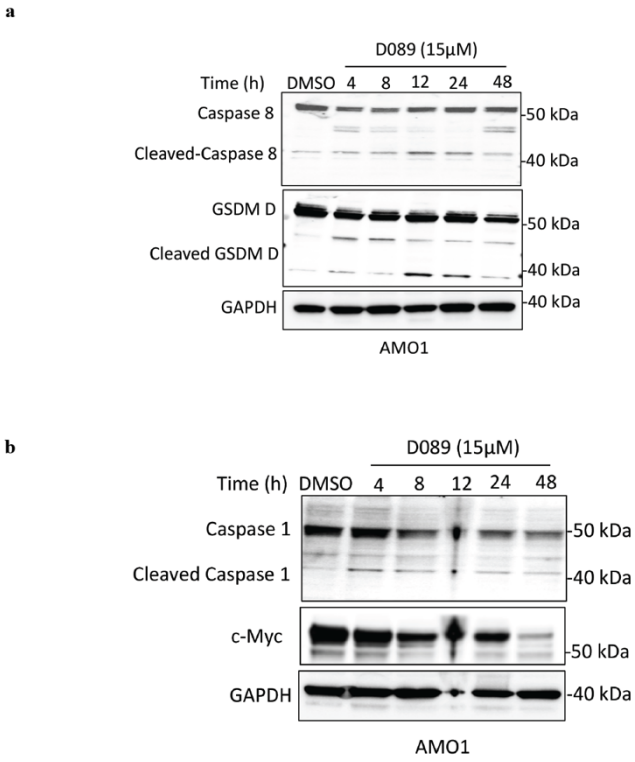


**Figure S2.** D089 triggers pyroptosis by inducing cleaved caspase 1, 8 and Gasdermin D. Immunoblotting of CASP8 and GSDM D (**a**) and MYC and CASP1 (**b**) after D089 (15 μM) treatment in AMO1 cells over 48 h. Cleaved products in both indicate active protein.