Reagents and cell culture
Antibodies specific for caspase 3, PARP and GAPDH were purchased from Cell Signaling Technology Inc. (Beverly, MA). Caspase inhibitor z-VAD-fmk and ROS scavenger N-acetyl-L-cysteine (NAC) were purchased from Calbiochem (Gibbstown, NJ).

Mcl-1 gene expression: real-time quantitative RT-PCR
Real-time RT-PCR was performed using primer sets specific for human and rat Mcl-1, and an internal standard, 18S rRNA, in an ABI PRISM 7900 sequence detector (Foster City, CA) as described elsewhere. [38] RNK-16 cells or PBMC from NK-cell leukemia patients ($CD3^{-}CD56^{+}$ NK cells >80%) treated with 2.5, 5.0 and 10.0 uM for 24 hours were subjected to total RNA extraction by using TRIzoL Reagent (Invitrogen) followed the manufacturer's directions. First strand cDNA was synthesized from 2 ug purified total RNA using random hexamers and MMLV reverse transcription reagents (Invitrogen) in total volume of 20ul. A total of 1ug cDNA was applied in a 10ul PCR mix using a QuantiTect SYBR Green PCR kit (QIAGEN, Valencia CA). Amplification of triplicate cDNA template samples was then performed with denaturation for 15min at 95°C, followed by 40 PCR cycles of denaturation at 94°C for 15 s, annealing at 55°C for 30s, and extension at 72°C for 30s. A standard curve of cycle thresholds using serial dilutions of cDNA samples was established and used to calculate the relative abundance of the target gene between samples from patients and normal healthy controls. Values were normalized to the relative amounts of 18S mRNA, which were obtained from a similar standard curve. The changes in fluorescence of SYBR green dye in every cycle were monitored by the ABI 7900 system software and the threshold cycle (Ct) for each reaction was calculated. The relative amount of PCR products generated from each primer set was determined based on the threshold cycle or Ct value. [38] PCR analysis was performed on each cDNA sample at least twice. The following primers were used in the detection of Mcl-1: human Mcl-1 sense, 5′-CAAGGGAAGCTTTTCCTCTC-3′, antisense 5′-CATGGAGGCCAAGCCAAAGT-3′; rat Mcl-1 sense, 5′- ATGAATGAAAAGGGTGAATG -3′, antisense 5′-AAGTCACCACCATTGTTAGG-3′.

***In vitro* PP2A phosphatase assay**
Total PP2A activity was measured using a Malachite Green PP2A phosphatase assay kit (Millipore). The assay is based on dephosphorylation of a phosphopeptide substrate (K-R-pT-I-R-R); the released phosphate binds to malachite green, leading to increased absorbance at 650 nm. NKL cells were treated accordingly and scraped into phosphatase extraction buffer (20 mM imidazole-HCl, 2 mM EDTA, and 2 mM EGTA, pH 7.0, with 10 mg/ml aprotinin, leupeptin, 1 mM benzamidine, and 1 mM phenylmethylsulfonyl fluoride) and then sonicated for 10 s and centrifuged at 2000×*g* for 5 min. Resulting cell lysates were used for the phosphatase activity assay. Absorbance values at 650 nm were subtracted by that of negative control (no cell lysate added) using a Synergy HT Multi-Detection Microplate Reader (Bio-TEK).

Detection of Mcl-1 *in vivo*
Expression of Mcl-1 was detected in paraformaldehyde-fixed and paraffin-embedded spleen tissue sections using a Mcl-1 antibody, using immunohistochemical method as previously described. [39]

Figure S1. FTY720 induces apoptotic cell death in leukemic NK cells in a time-dependent manner

(A) Human NKL cell line was treated with 15 uM FTY720 or DMSO for 6, 16 and 24 h then cells were assayed for apoptosis by flow cytometry. (B) Rat RNK-16 cell line was treated with 15 uM FTY720 or DMSO for 6, 16 and 24 h then cells were assayed for apoptosis by flow cytometry. (C) PBMC from three individual chronic NK-cell leukemia patients (#5, 6, 7) were treated with 10 uM FTY720 or DMSO for 2, 6 and 24 hours then cells were assayed for apoptosis by flow cytometry. *$p<0.05$, **$p<0.005$, ***$p<0.0005$ indicate significant differences of FTY720 treated cells versus DMSO treated cells (Student's *t* test).

Figure S2. FTY720 is not toxic to normal PBMC or normal NK cells

(A) PBMC samples from normal donors (n=8) were treated with 10 uM FTY720 or DMSO for 2, 4, and 6 hours then MTT assay was performed. (B). Normal NK cells were purified by negative selection using RosetteSep from 5 normal donors (90–100% cells were $CD3^{-}CD56^{+}$ NK cells by flow cytometry determined using a PE conjugated CD56 antibody) then treated with 10 uM FTY720 or DMSO for 24 hours and cells were assayed for apoptosis by flow cytometry. *$p<0.05$, **$p<0.005$ indicate significant differences of FTY720 treated cells versus DMSO treated cells (Student's *t* test).

Figure S3. FTY720 induced-apoptosis is caspase-dependent in leukemic NK cells

(A) Western blot analysis was performed for caspase 3 and PARP after 5 uM FTY720 or DMSO treatment of PBMC from an NK-cell leukemia patient for two and six hours. Data are representative of four independent experiments on PBMC from three NK-cell leukemia patients. (B) PBMC from an NK-cell leukemia patient were exposed to 10 uM FTY720, in the absence or presence of various concentrations of z-VAD-fmk, a pan caspase inhibitor. z-VAD-fmk was added 2 h before FTY720 treatment at the indicated concentration. Cell survival was determined 18 h later by the MTT assay. * $p<0.05$ and ** $p<0.005$ indicate significant differences of each dose of z-VAD-fmk treated cells compared to z-VAD-fmk untreated cells, respectively (Student's *t* test).

Figure S4. FTY720 regulates Mcl-1 independent of transcription

(A) RNK-16 cells were treated with DMSO or indicated doses of FTY720 for 24 h. Quantitative real time RT-PCR was performed to measure levels of Mcl-1 mRNA in these cells. (B) PBMC from an NK-cell patient ($CD3^{-}CD56^{+}$ >80%) were treated with DMSO or indicated doses of FTY720 for 24 h. Quantitative real time RT-PCR was performed to measure levels of Mcl-1 mRNA in these cells.

Figure S5. Mcl-1 degradation by FTY720 was through ROS production

RNK-16 cells were exposed to 10 uM FTY720 for 24 h, in the absence or presence of 1mM ROS scavenger N-acceyl-L-cysteine (NAC), and then cells were assayed for apoptosis by flow cytometry (A) or western blot was performed to examine Mcl-1 expression (B) NAC was added 2 h before FTY720 treatment.

Figure S6. FTY720 induced cell death is independent of PP2A in leukemic NK cells

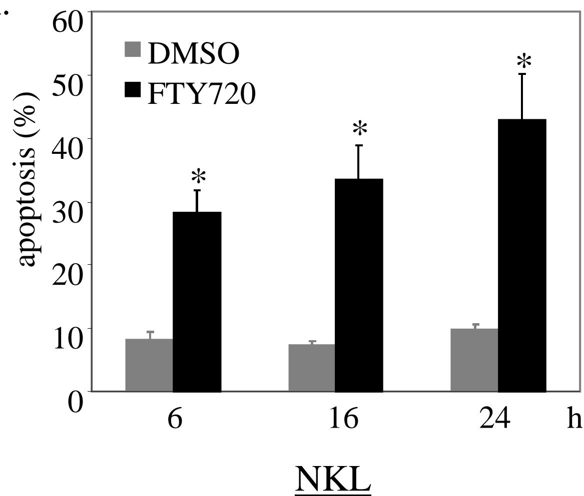
RNK-16 cells (A) or PBMC from an NK-cell leukemia patient ($CD3^{-}CD56^{+}$ >80%) (B) were exposed to 10 uM FTY720, in the absence or presence of Okadaic acid (OA), a PP2A inhibitor.

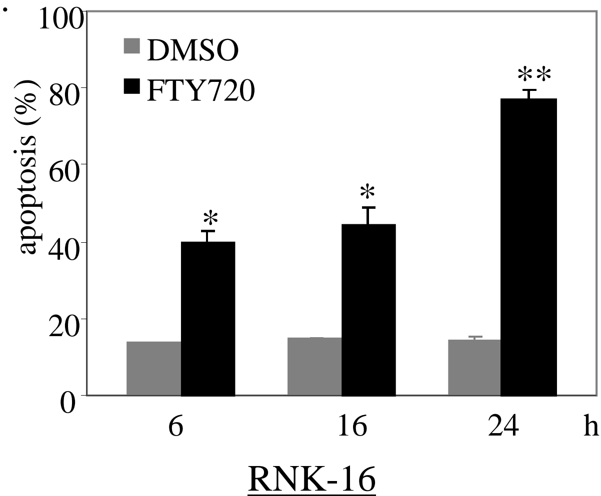
OA was added 2 h before FTY720 treatment at the indicated concentration. Cell survival was determined 18 h later by the MTT assay. (C) A PP2A immunoprecipitation phosphatase assay was performed in human NKL cells treated for 24 hours with DMSO or indicated doses of FTY720.

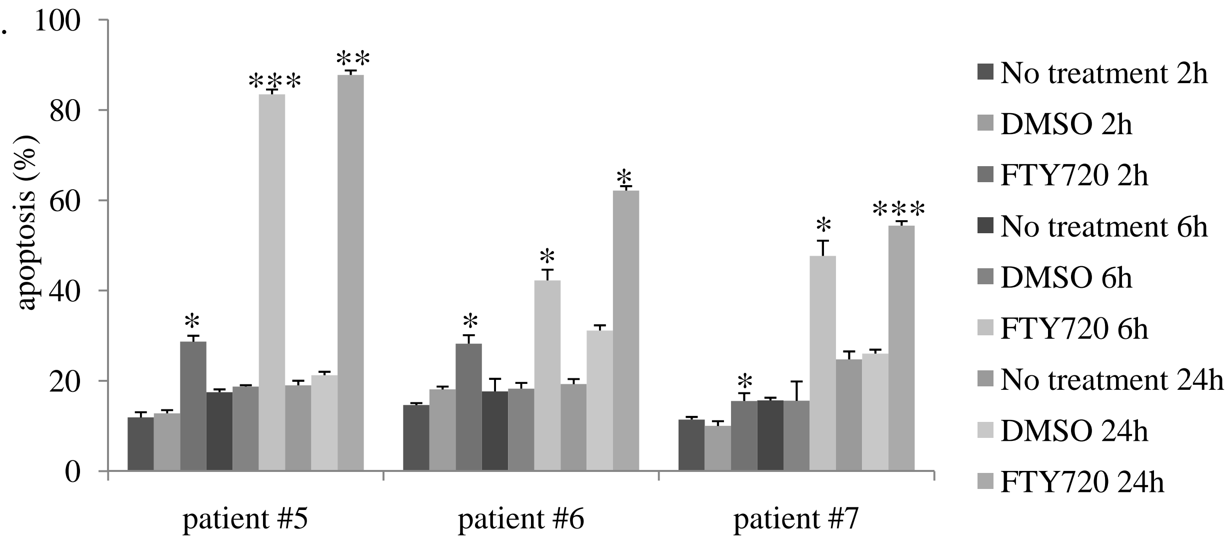
Figure S7. FTY720 induces complete remission in NK-cell leukemic rats
(A) The Kaplan–Meier survival curves for normal rats after treatment with 4.5mg/kg of FTY720 (n=16) or PBS (n=16) or leukemic rats after treatment with either 4.5mg/kg (n=16) or PBS (n=16), were plotted. (B) Maintenance of normal white blood counts, hemoglobin values and platelet counts in responding leukemic and normal rats treated with FTY720. 200ul of blood from leukemic rats treated with FTY720 (n=16) and PBS (n=16) and normal rats treated with FTY 720 (n=16) and PBS (n=16) was collected every week from tail veins of the animals and placed in EDTA K2 coated tubes, then CBC (complete blood count) analysis was performed. Arrow indicates the cessation of the treatment. (C) Flow cytometry was utilized to identify rat leukemic NK cells, which are $CD3^{-}CD8a^{+}$. Comparison of $CD3^{-}CD8a^{+}$ NK cells isolated from multiple tissues among normal rats treated with PBS (n=16) or FTY720 (n=16), leukemic rats treated with PBS (n=16) or leukemic rats responsive to FTY720 treatment (n=5) or unresponsive to FTY720 (n=11) .* $p<0.05$, ** $p<0.005$ ***, $p<0.0005$ indicates significance between leukemic rats responsive to FTY720 or unresponsive to FTY20 and leukemic rats treated with PBS (unpaired *t* test).

Figure S8. FTY720 induces synergistic or additive apoptosis on leukemic NK cells
(A) Human NKL cell line was treated with 10 uM FTY720 in absence or presence of 10 uM C_6-ceramide nanaliposome for 16 h then cells were assayed for apoptosis by flow cytometry. (B) Rat RNK-16 cell line was treated with 5 uM FTY720 in absence or presence of 25 uM C_6-ceramide nanaliposome for 16 h then cells were assayed for apoptosis by flow cytometry. *$p<0.05$, **$p<0.005$ indicate significant differences of combinational treatment versus single agent treatment (Student's *t* test).

Figure S1







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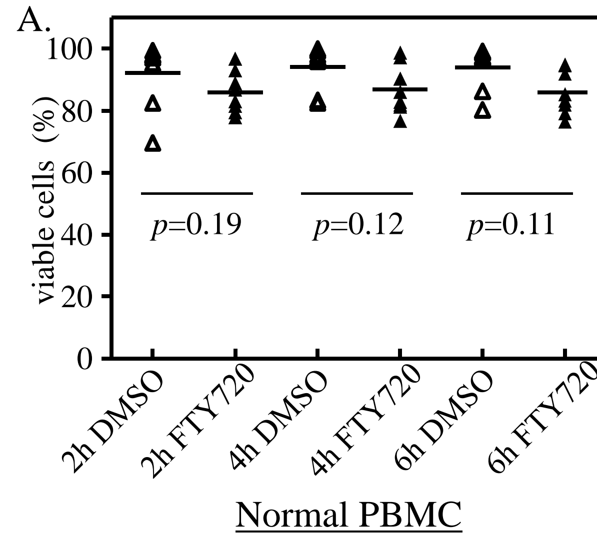

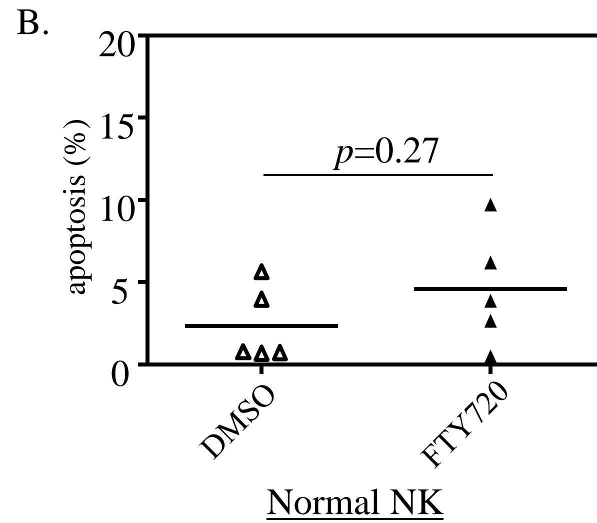

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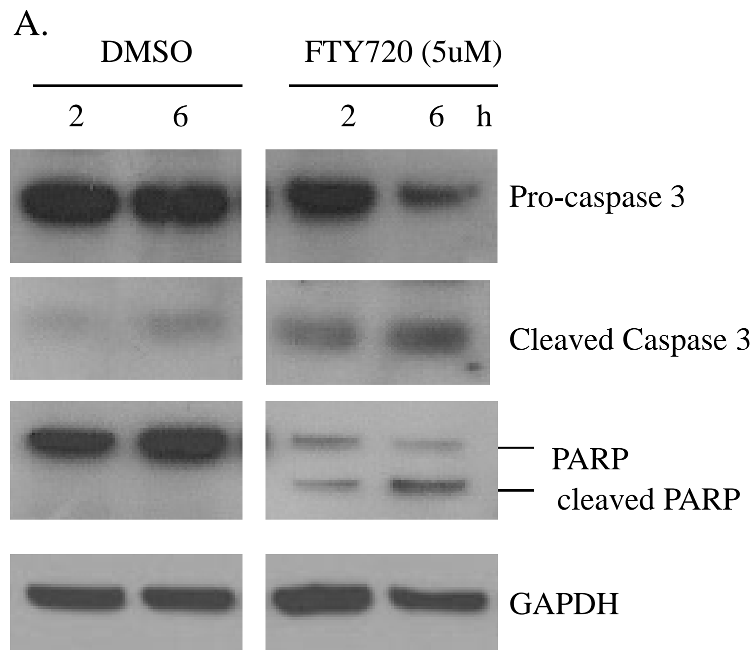

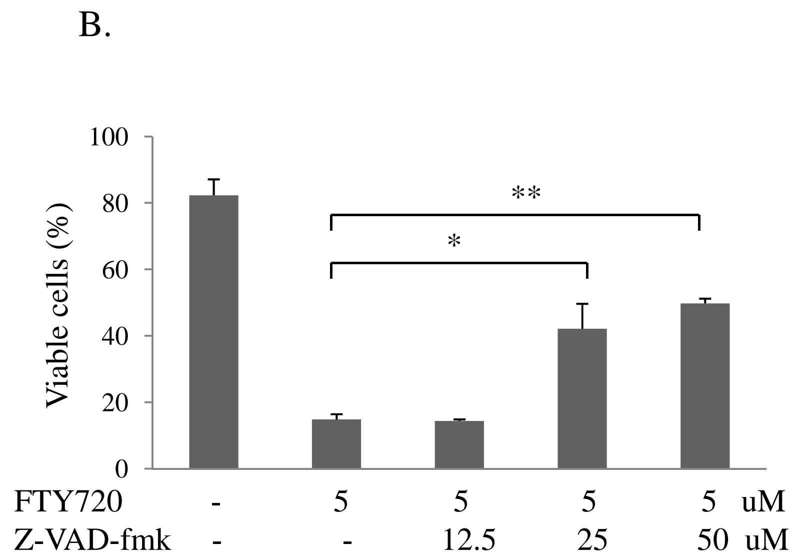

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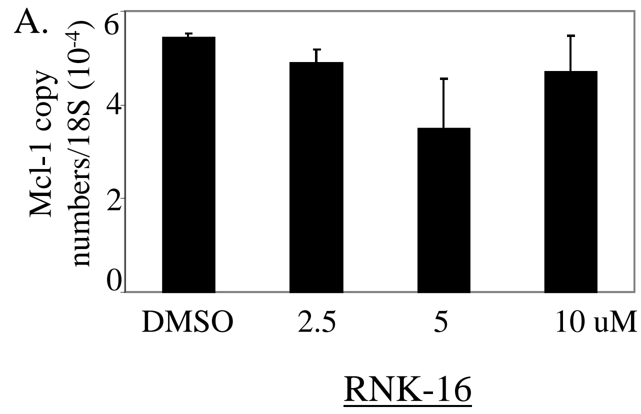


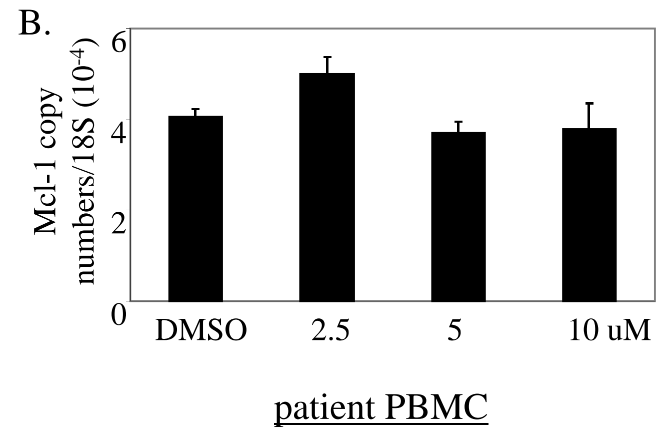
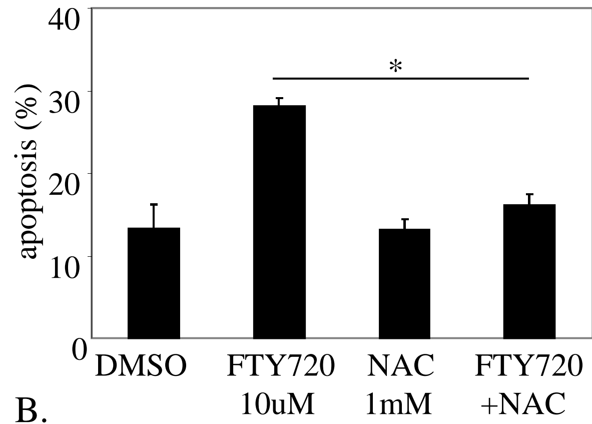


Figure S5

A.



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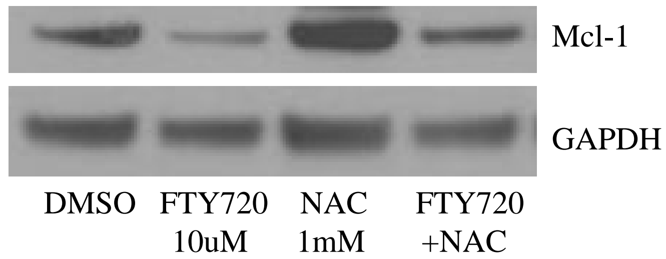

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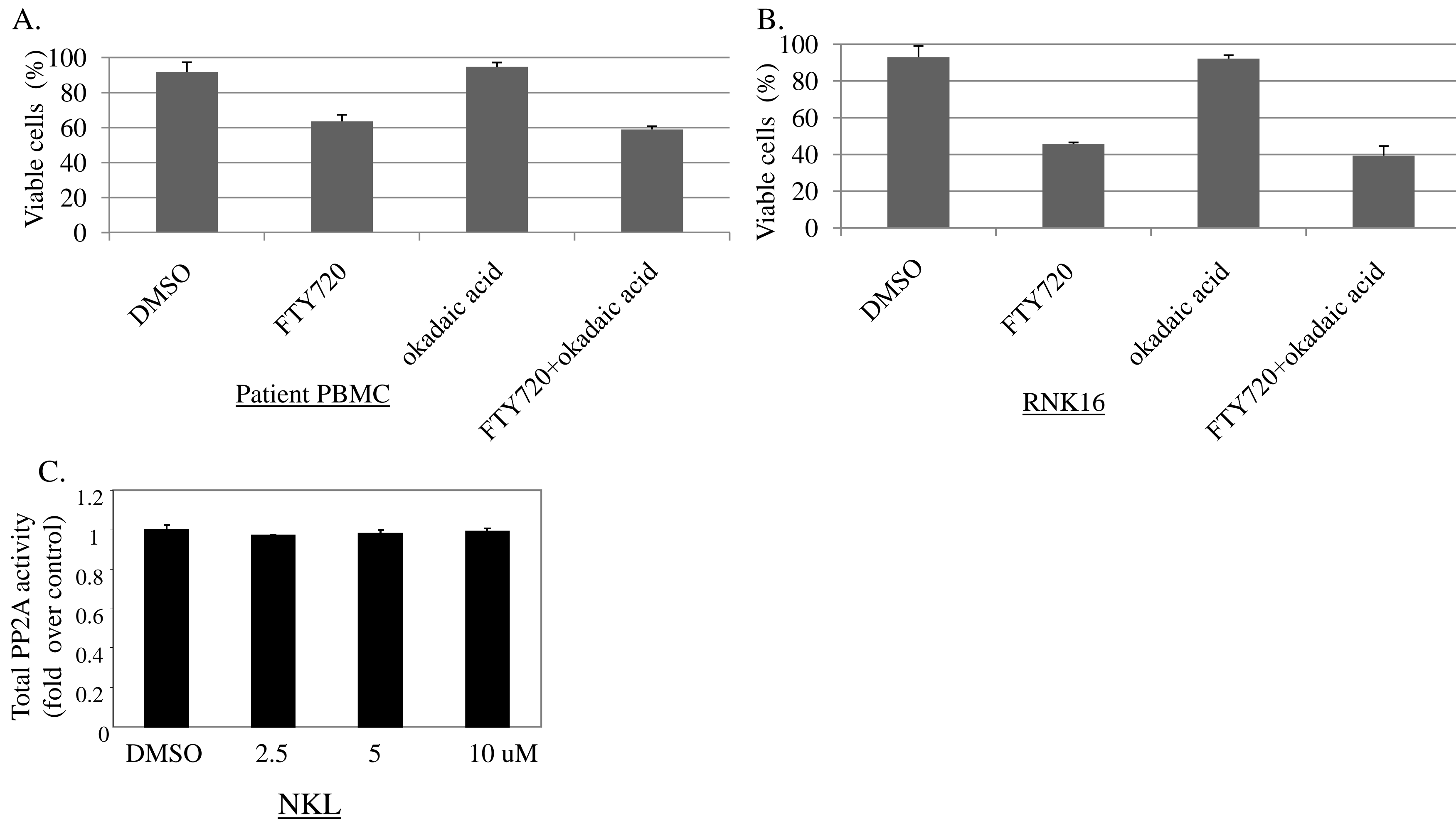

Figure S7

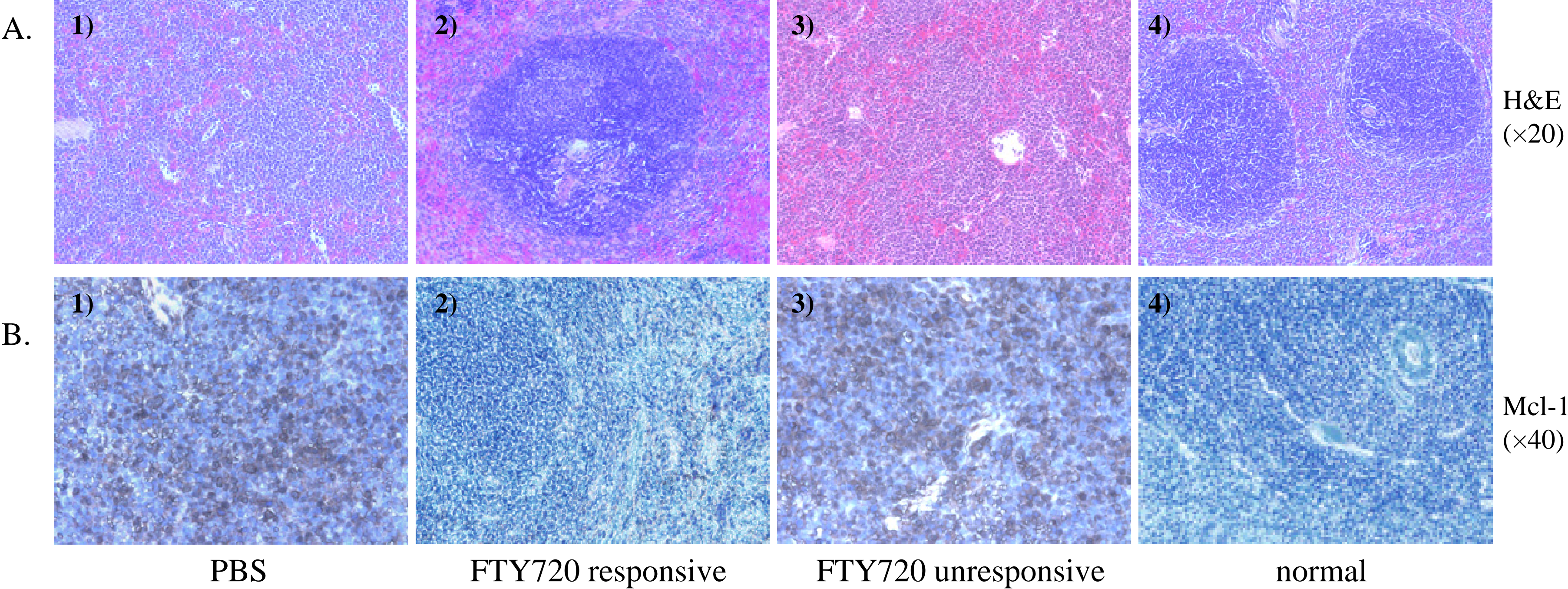

Figure S8