Figure S1. Linking TAT sequence to Mot-P2 enhanced its cytotoxicity. Ramos cells were treated with 50 $\mu$M Mot-P2 or Mot-P2-TAT or DMSO for 24 h in growth medium. Cells were washed, stained with propidium iodide and analyzed by flow cytometry. Percentage of cell death was calculated relative to DMSO-treated cells. n=3. $^{*}$P<0.05, Mot-P2 vs. Mot-P2-TAT. TAT, transactivator of transcription.

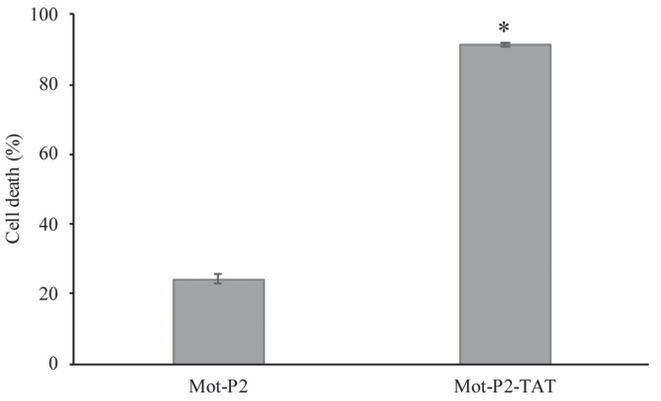


Figure S2. Mot-P2 and Mot-P7 uptake and intracellular distribution in carcinoma cells. PC3, SKOV3 and T47D cells were seeded in 4-well chamber slides and treated with biotinylated Mot-P2 or Mot-P7 (5 or 10 $\mu$M, respectively) for 5 min at 37˚C. Next, cells were washed, fixed and permeabilized. For nuclear staining, 300 nM DAPI staining solution was added for 5 min at room temperature. For peptide staining, cells were treated with 5 $\mu$g/ml FITC-conjugated streptavidin for 30 min at room temperature. Cell imaging was performed using a Leica-SP5 confocal microscope.

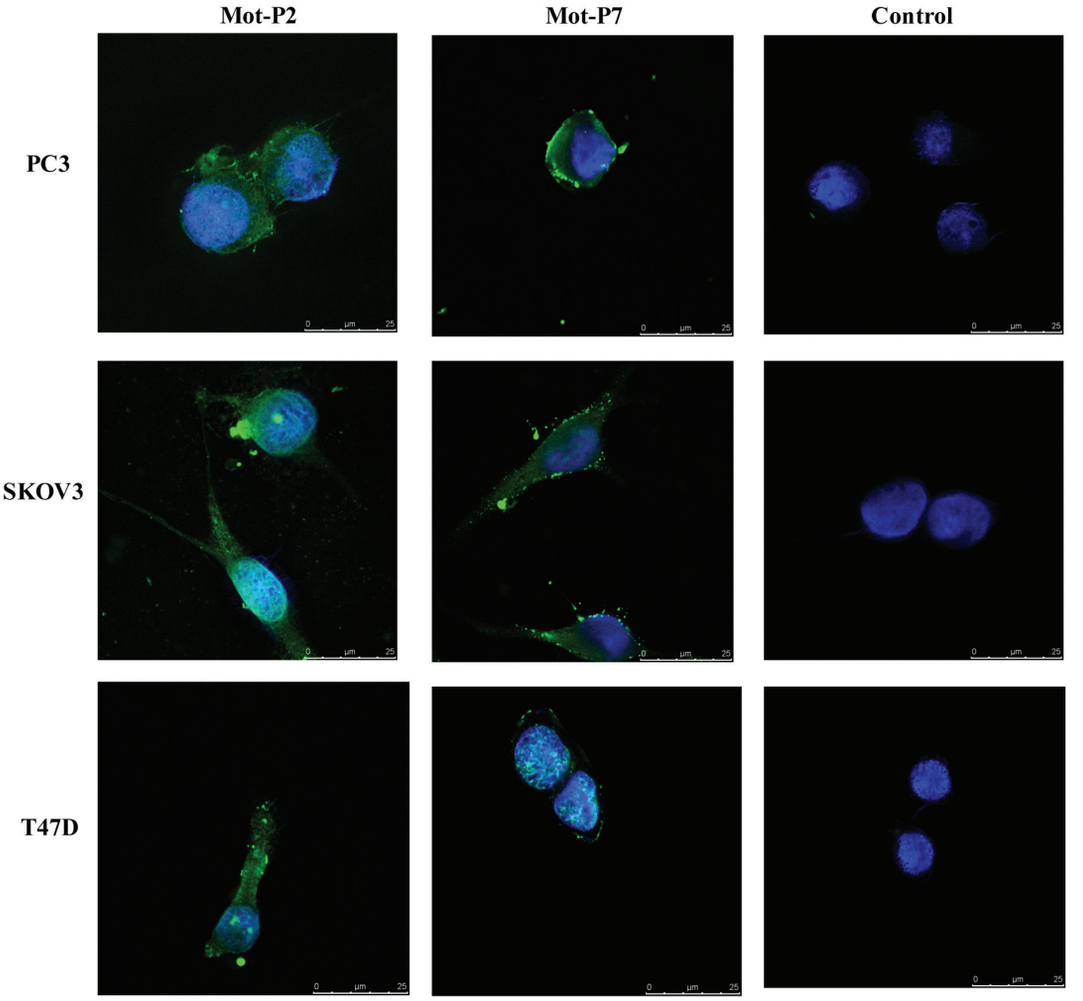


Figure S3. Kinetics of LDH release relative to ATP loss in cells treated with Mot-P2 and Mot-P7. (A and B) Ramos, (C and D) PC3, (E and F) T47D and (G and H) SKOV3 cells were treated with 25 $\mu$M Mot-P2 (A,C,E,G) or 100 $\mu$M Mot-P7 (B,D,F,H) for the indicated times at 37˚C. Next, cells were analyzed for ATP levels (black lines) and LDH release (grey lines). Data were expressed as RLU ± SD (ATP) or RFU ± SD (LDH). Maximal LDH release is indicated in each figure by the symbol ■. LDH, lactate dehydrogenase; RFU, relative fluorescence unit; SD, standard deviation; RLU, relative luminescence unit.

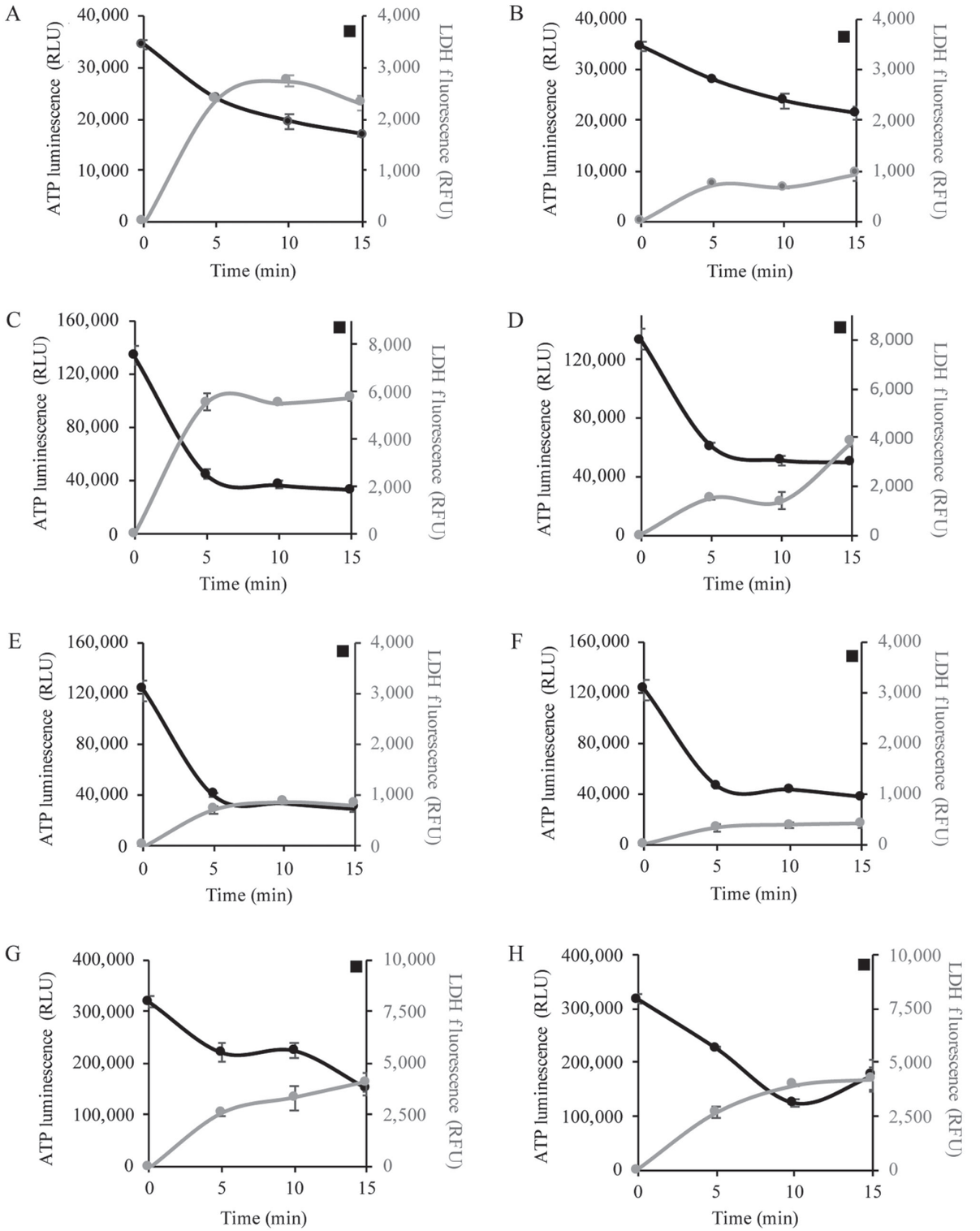


Figure S4. Growth curves of cells treated with Mot-P2 or Mot-P7. Ramos cells were grown with sub-toxic doses of Mot-P2 or Mot-P7 (5 or 25 $\mu$M, respectively) or DMSO as a control in growth medium. After 48 or 72 h, cells were collected, stained with PI and the number of viable cells (PI-negative) was determined by flow cytometry. Data are expressed as the number of viable cells. n=2. P<0.05, each peptide vs. DMSO-treated cells.

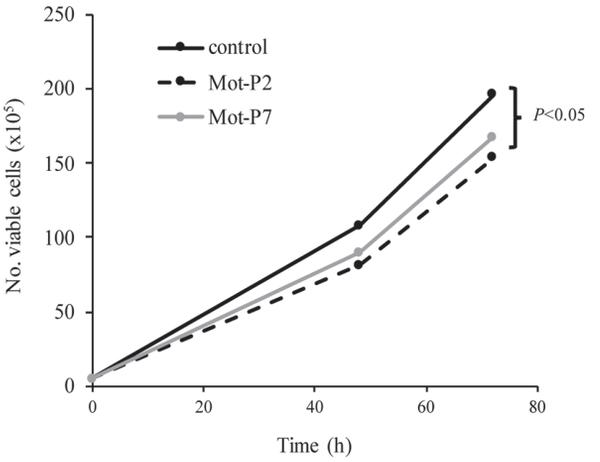
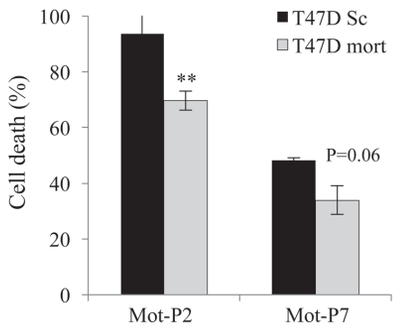
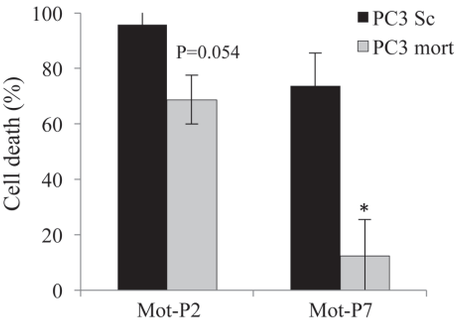


Figure S5. Silencing of mortalin lowered peptide cytotoxicity in T47D and PC3 cells. T47D and PC3 cells were transfected by electroporation with mort siRNA or Sc siRNA as a control for 48 h. Transfected cells were collected by trypsinization, washed and counted. Cells were placed in test tubes and treated with 25 $\mu$M Mot-P2 or 100 $\mu$M Mot-P7 for 30 min at 37˚C. Then, cells were centrifuged at 250 x g for 6 min at 4˚C and supernatant was collected. Extent of LDH release, as a measure of cell death, was quantified and percentage of cell death was calculated. $^{*}$P<0.05, $^{**}$P<0.01 vs. Sc siRNA-transfected cells. Cell transfection was confirmed by western blotting. Mort, mortalin; Sc, scramble; si, small interfering; LDH, lactate dehydrogenase.



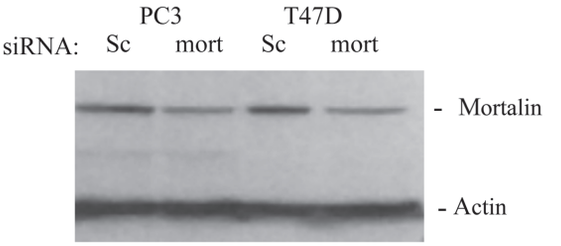



Figure S6. Rituximab antibody titration with complement. Ramos cells were treated with increasing concentrations of RTX (0-20 $\mu$g/ml) for 30 min at 4˚C. Next, cells were incubated with 50% normal human serum for 60 min at 37˚C. Percentage of cell death was measured by propidium iodide inclusion and flow cytometry. RTX, rituximab.

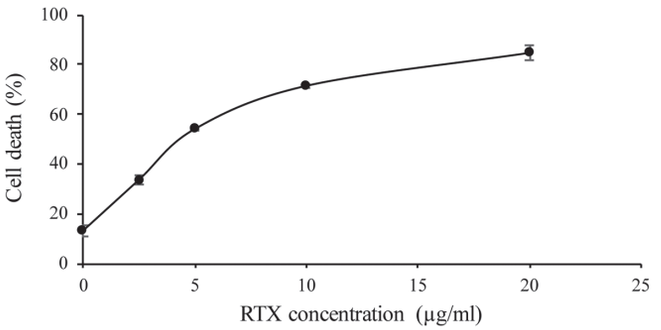


Figure S7. Rituximab antibody binding to peptide-treated cells. (A and B) Ramos cells were incubated with the indicated concentrations of (A) Mot-P2 or (B) Mot-P7 for 30 min at 37˚C. Next, cells were labeled with RTX (2 $\mu$g/ml; 30 min at 4˚C), followed by Alexa-flour 488-conjugated anti-human IgG antibodies. MFI of bound RTX was determined by flow cytometry and presented as MFI ± standard deviation. n=3. RTX, rituximab; MFI, mean fluorescence intensity.

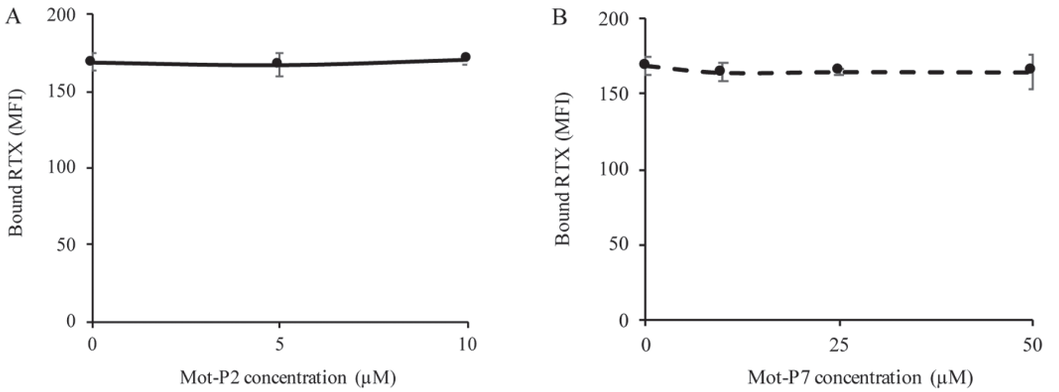


Table SI. Lethal dose 50 of mortalin mimetic peptides on lymphoma cells.

| Peptide[a] | Ramos | Raji | Z-138 |
|---|---|---|---|
| Mot-P2 | 26 | 28 | 30 |
| Mot-P7 | 63 | 40 | 61 |
| Mot-P8 | 547 | 1728 | 574 |
| Mot-P10 | 22 | 130 | 97 |
| Mot-P14 | 48 | 87 | 232 |
| Mot-P16 | 92 | 123 | 262 |

[a]TAT-linked mortalin peptides. Table presents the concentration of peptide (in $\mu$M) causing 50% cell death. Cells were treated with peptides for 24 h at 37˚C. Dose-dependence curves of cell death are presented in Fig. 1.