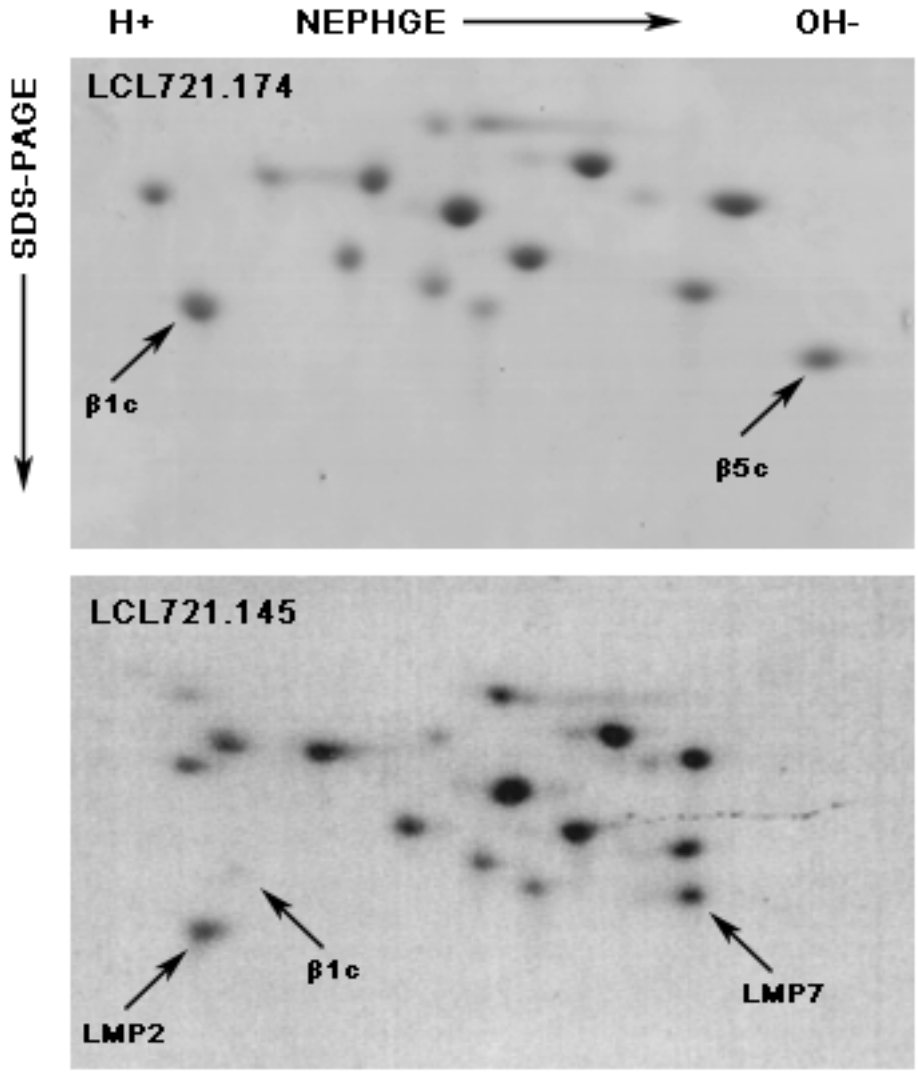
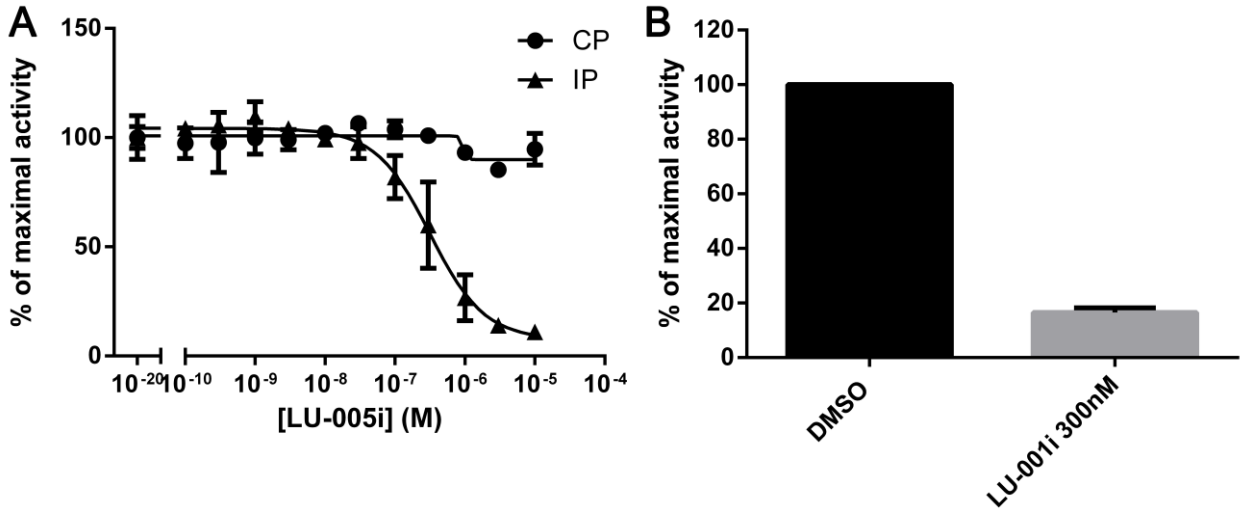
Supplementary Figures



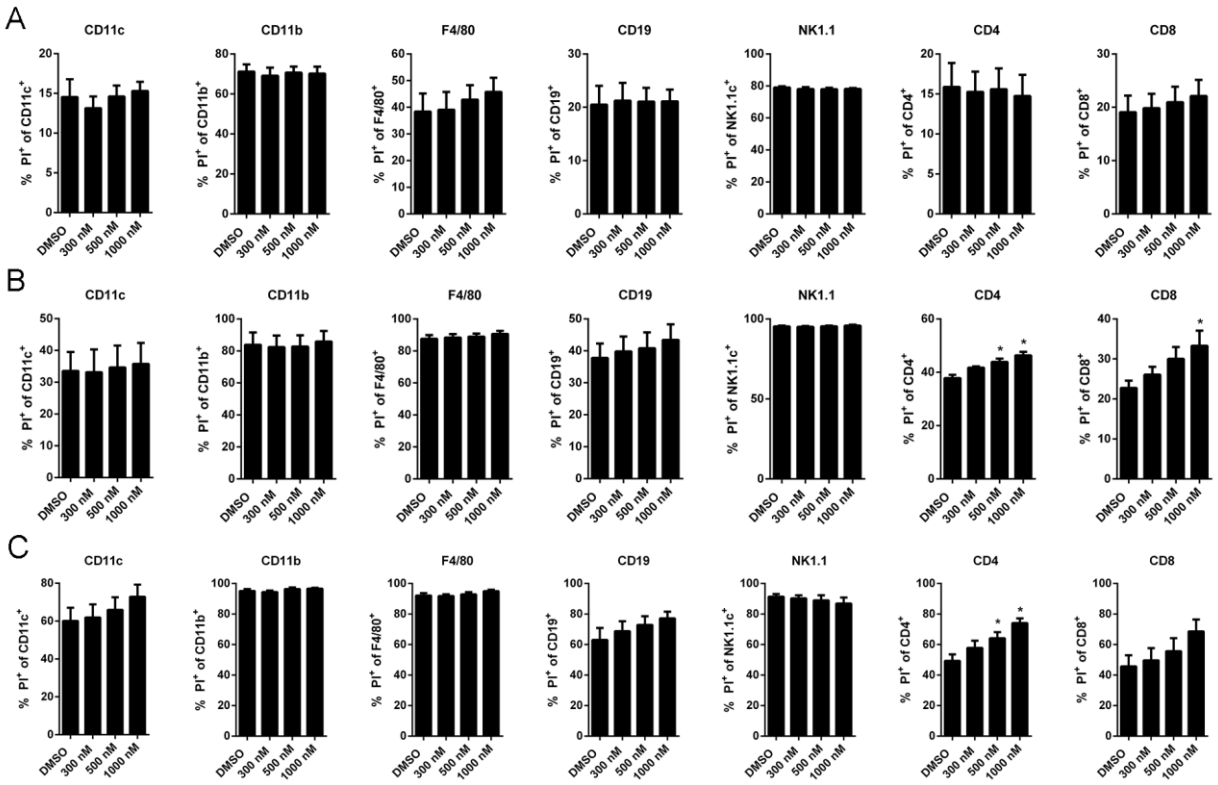
Supplementary Figure 1. Proteasome composition of LCL721.174 cells and LCL721.145 cells

NEPHGE/SDS-PAGE analysis of 70 µg 20S proteasome purified from LCL721.174 cells (CP) (top) LCL721.145 cells (IP) (bottom). The proteins were visualized by coomassie stain. The positions of proteasome subunits LMP2, LMP7, β1c, and β5c are indicated.



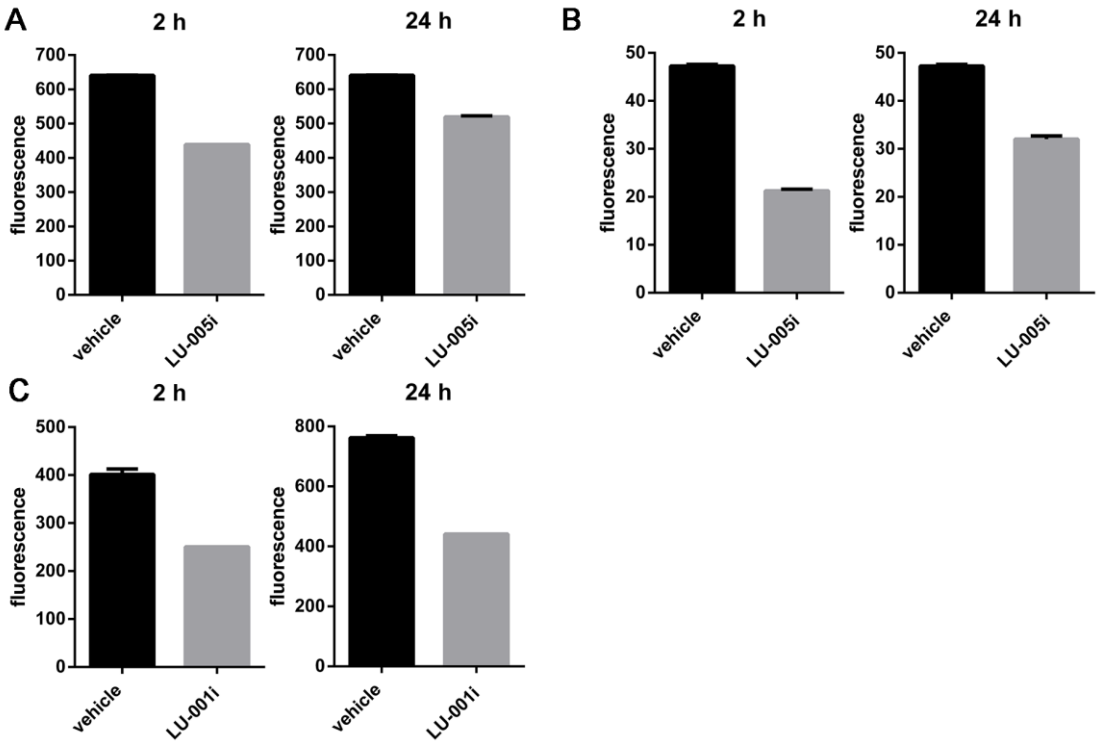
Supplementary Figure 2. Cell permeability of LU-005i and LU-001i

(A) LCL721.174 cells (CP) or LCL721.145 cells (IP) were treated with the indicated concentrations of LU-005i. The chymotrypsin-like activity in the cells was determined by the hydrolysis of the cell permeable substrate Meo-Suc-GLF-AMC. Depicted is the mean ± SD % of maximal activity of triplicate cultures. The highest fluorescence value was set to 100%. (B) LCL721.145 (contain IP) cells were incubated with 300 nM LU-001i or DMSO overnight. Proteasomes of crude lysates of these cells were immunoprecipitated and assayed for the hydrolysis of the fluorogenic substrate Ac-PAL-AMC (LMP2-activity). Depicted is the mean ± SD % of maximal activity of triplicate cultures. The highest fluorescence value was set to 100%. Experiments were performed twice with a similar outcome. These experiments were only performed to demonstrate the cell permeability of LU-005i and LU-001i. Therefore, the data were not statistically analyzed.



Supplementary Figure 3. Effect of LU-005i on cell death

Splenocytes derived from C57BL/6 mice were treated with DMSO, 300 nM LU-005i, 500 nM LU-005i, or 1000 nM LU-005i for 24 h (A), 48 h (B), or 72 h (C). Cell death (PI^+) of $CD11c^+$, $CD11b^+$, $F4/80^+$, $CD19^+$, $NK1.1^+$, $CD4^+$, and $CD8^+$ was analysed by flow cytometry. Shown are the means of PI^+ populations ± SEM from splenocytes derived from 5 different mice (n=5). All data were statistically compared to the DMSO treated group. *$p < 0.05$.



Supplementary Figure 4. Activity of LU-005i and LU-001i in mice

C57BL/6 mice were treated with LU-005i (A, B) (15 mg/kg), LU-001i (C) (15 mg/kg), or vehicle for 2 h or 24 h. Proteasomes of crude lysates of splenocytes derived from these mice were immunoprecipitated and assayed for the hydrolysis of the fluorogenic substrate Ac-PAL-AMC (LMP2-acitivity) (A, C) or Suc-LLVY-AMC (chymotrypsin-like activity) (B). Depicted is the mean ± SD of the fluorescence of two mice per group measured in duplicates. Experiments were performed twice with a similar outcome. These experiments were only performed to demonstrate the activity of LU-005i and LU-001i in mice. Therefore, the data were not statistically analyzed.