Expanded View Figures

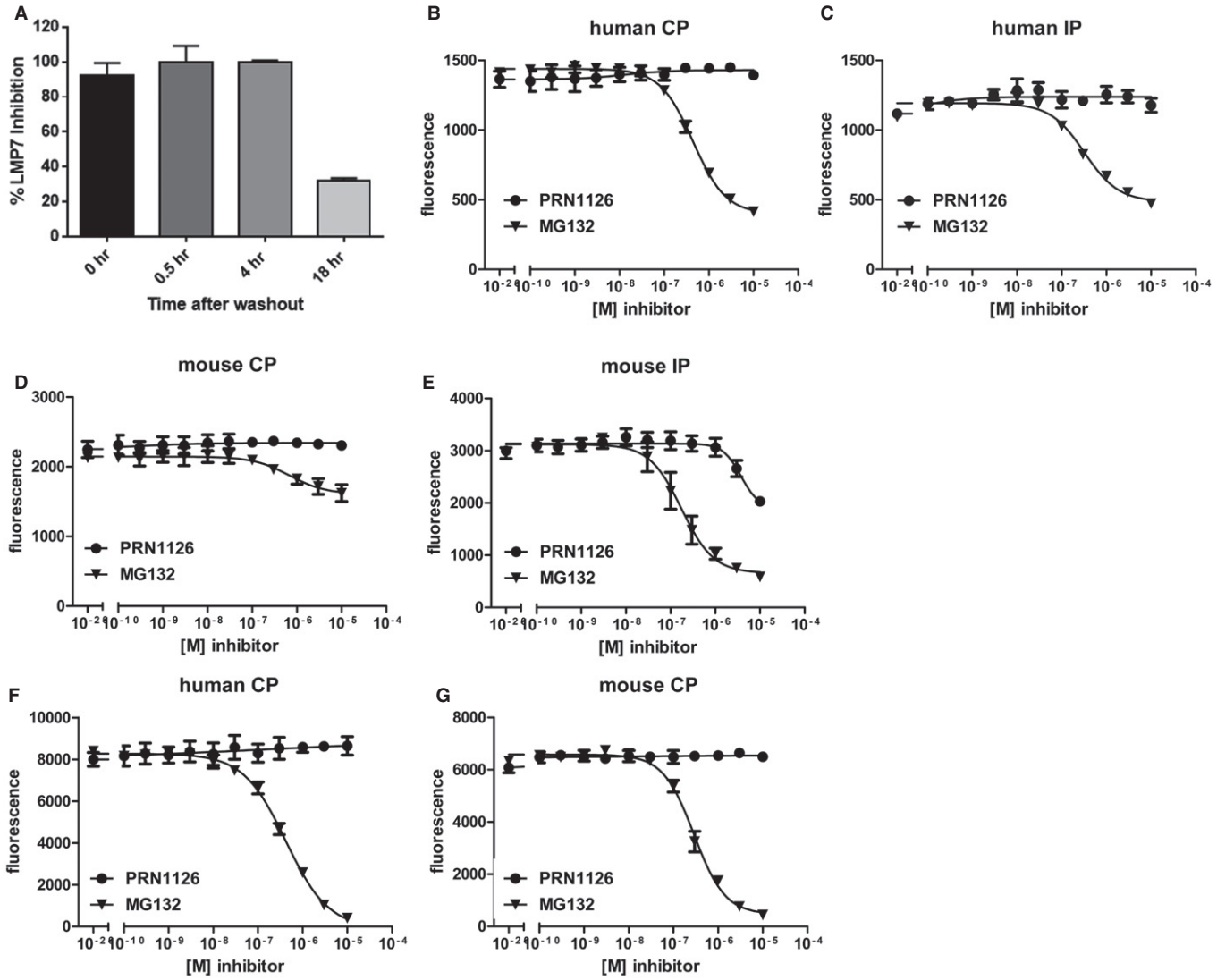

Figure EV1. Extended LMP7 inhibition in human PBMC.

A PBMCs were treated with PRN1126 at 1 μM for 1 h, washed to remove compound, and incubated in media for the time indicated. LMP7 inhibition was determined by hydrolysis of fluorogenic substrate (Suc-LLVY-AMC). Data points represent the mean ± s.d. (n = 3).

B–G Hydrolysis of fluorogenic substrates Bz-VGR-AMC for trypsin-like activity (B–E) or z-LLE-βNA for caspase-like activity (F, G) of human (B, C, F) or mouse (D, E, G) 20S constitutive proteasome (B, D, F, G) or immunoproteasome (C, E) at various concentrations of PRN1126 and MG132. Data are presented as the means of fluorescence ± s.d. from quadruplicate assays. The experiments were repeated three times with similar results.

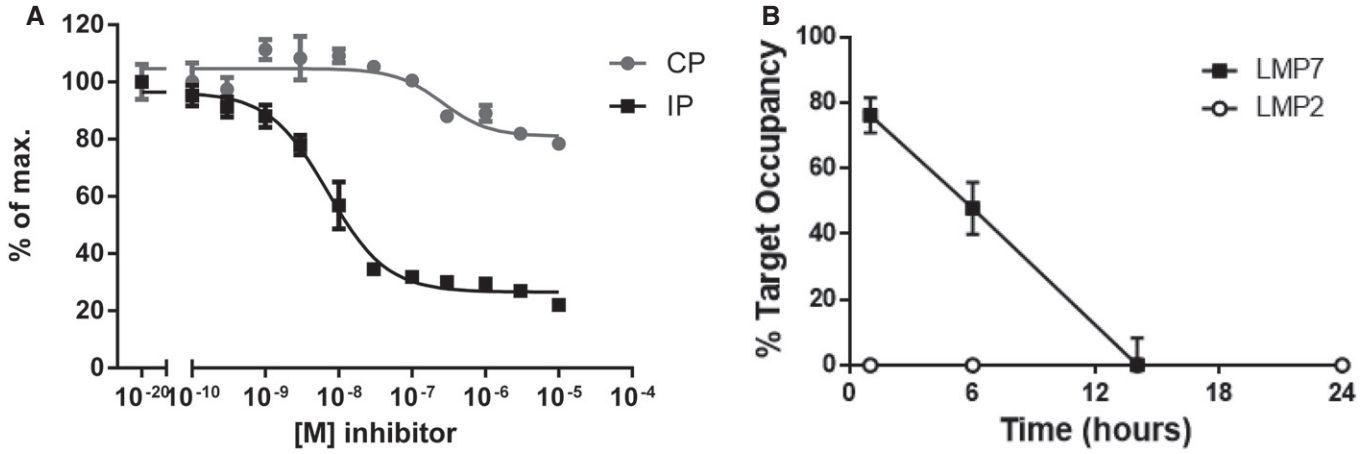

Figure EV2. PRN1126 cell permeability and target occupancy of LMP7 or LMP2 in mice.

A LCL721.174 cells expressing constitutive proteasomes (CP) or LCL721.145 cells expressing immunoproteasomes (IP) were treated with the indicated concentrations of the LMP7 inhibitor PRN1126. The chymotrypsin-like activity in the cells was determined by the hydrolysis of the cell-permeable fluorogenic proteasome substrate Meo-Suc-GLF-AMC. Depicted is the mean ± s.d. % of maximal hydrolytic activity of triplicate cultures. The highest fluorescence value was set to 100%.

B Mice were treated with a single dose of PRN1126 (40 mg/kg, s.c.) or vehicle. Spleens were harvested at the time points indicated, and the drug occupancy of LMP7 or LMP2 subunits was assessed in splenocytes with the ProCISE assay. Data points represent the means ± s.d. of three mice.

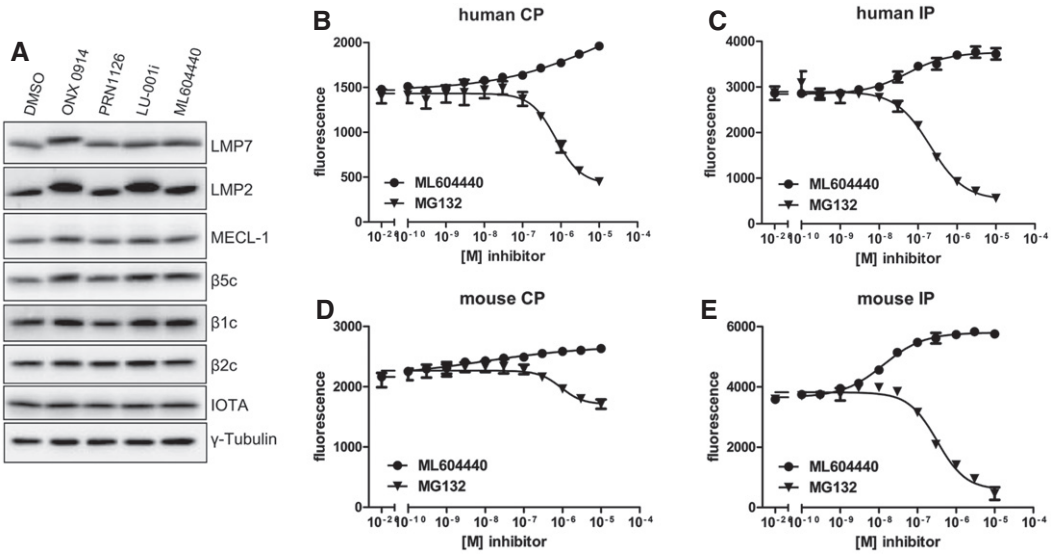

Figure EV3. Electrophoretic mobility shifts of IP subunits with different inhibitors.

A Ficoll-enriched lymphocytes from C57BL/6 mice were treated with DMSO, 300 nM ONX 0914, 300 nM PRN1126, 300 nM LU-001i, or 300 nM ML604440 for 2 h *in vitro*. SDS–PAGE and immunoblotting against indicated proteins were performed. Shown are representative Western blots out of two independent experiments with similar outcome.

B–E Hydrolysis of the fluorogenic substrate Bz-VGR-AMC for trypsin-like activity of human (B, C) or mouse (D, E) 20S constitutive proteasome (B, D) or immunoproteasome (C, E) at various concentrations of ML604440 and MG132. Data are presented as the means of fluorescence ± s.d. from quadruplicate assays. The experiments were repeated three times with similar results.

Source data are available online for this figure.

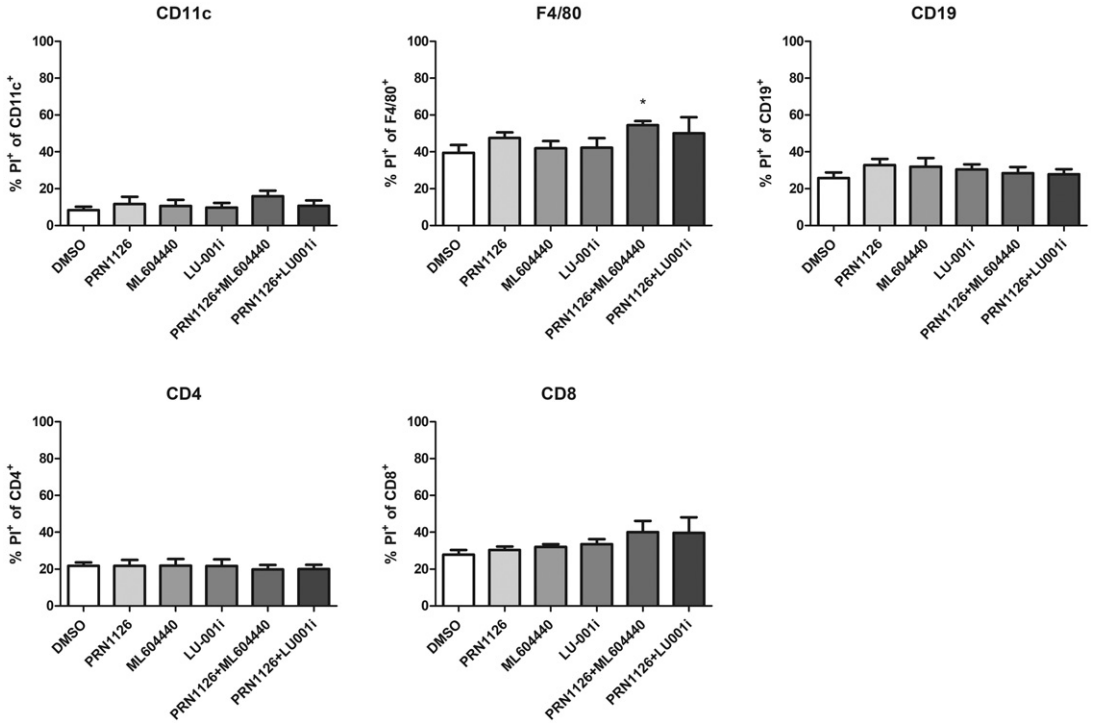

Figure EV4. Effect of different inhibitors on cell viability.
Splenocytes derived from C57BL/6 mice were treated with DMSO, 300 nM PRN1126, 300 nM ML604440, 300 nM LU-001i, 300 nM PRN1126 + 300 nM ML604440, and 300 nM PRN1126 + 300 nM LU-001i for 24 h. Cell death (PI^+) of $CD11c^+$, $F4/80^+$, $CD19^+$, $CD4^+$, and $CD8^+$ was analyzed by flow cytometry. Shown are the means of propidium iodide-positive (PI^+) populations ± s.e.m. from splenocytes derived from three different mice ($n = 3$) measured in triplicates. All data were statistically compared to the DMSO-treated group. *$P < 0.05$. One-way ANOVA.

