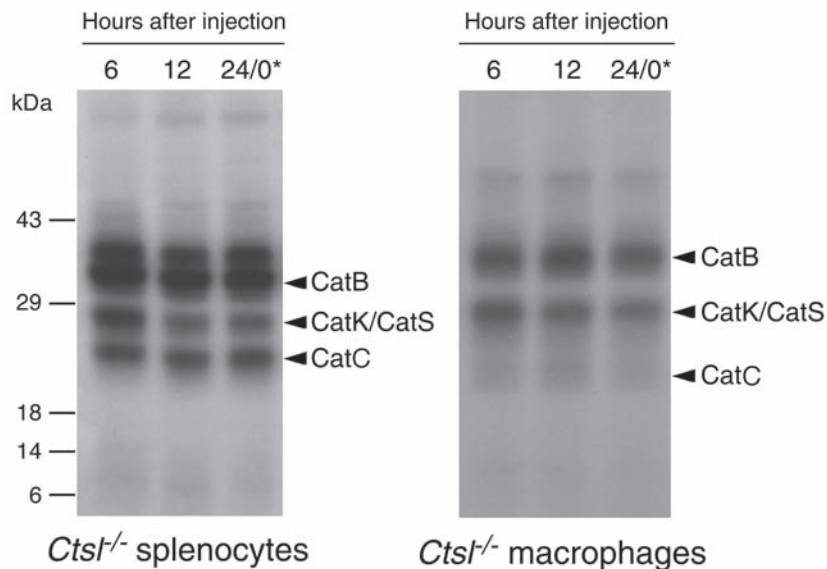


Supplementary Figure 1. Cysteine protease active site labeling in peritoneal macrophages treated with cell permeable CatB inhibitor CA074-methyl ester and CatL inhibitors CLIK195 and CLIK148. Macrophages were pre-incubated with different amounts of cathepsin inhibitors (indicated) for 3 hrs followed by addition of [125 I]-JPM-ethyl ester to the culture media. Cells were cultured overnight, lysed, protein concentrations determined, and equal amounts of protein (30 μ g/sample) were separated on a 12% SDS-PAGE. Gel was stained with Coomassie blue, destained, dried, and exposed to an X-ray film. Both CLIK148 and CLIK195 completely inhibited macrophage CatL activity at 10~20 μ M. The data from CA074me-treated macrophages were spliced from a separate gel.



Supplementary Figure 2. Cysteine protease cathepsin active site labeling in splenocytes and peritoneal macrophages from *Cts1*^{-/-} mice. Mouse splenocytes (left panel) and peritoneal macrophages (right panel) were isolated from *Cts1*^{-/-} mice 6, 12, and 24 hrs after CLIK195 injection (i.p. 100 mg/kg/day). Cells were lysed into pH5.5 buffer (J. Biol. Chem. 1992;267: 7258). Equal amounts of protein (30 μ g) from each sample was labeled with [¹²⁵I]-JPM and separated on a 12% SDS-PAGE. Known active cathepsins are indicated with arrowheads. These data indicate that CLIK195 has limited inhibitory activity to other cathepsins in mice treated with 100 mg/kg/day of this compound. *Since compound was injected every 24 hours, cells at 24 hours post-injection are equivalent to those before injection (0 hour).

Figure 1g. Top panel original figure.

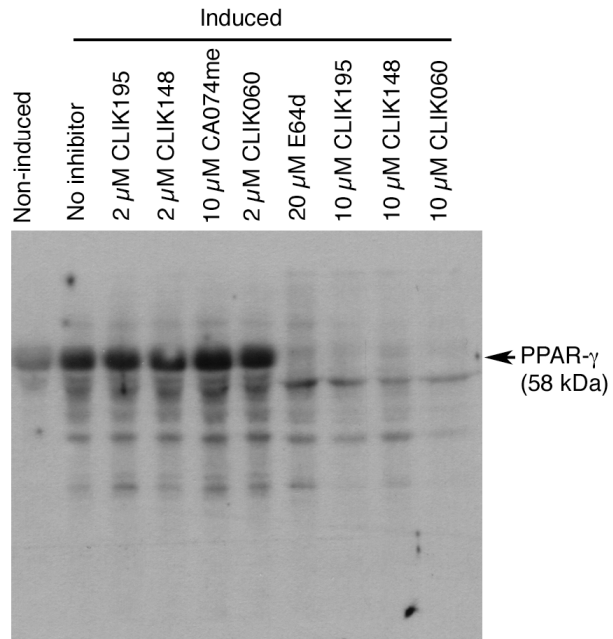


Figure 1g. Bottom panel original figure.

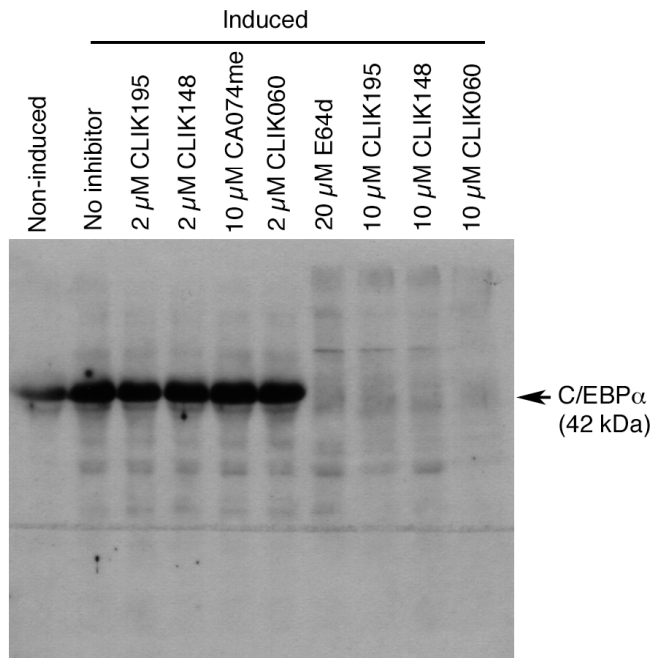


Figure 1j. Original figure.

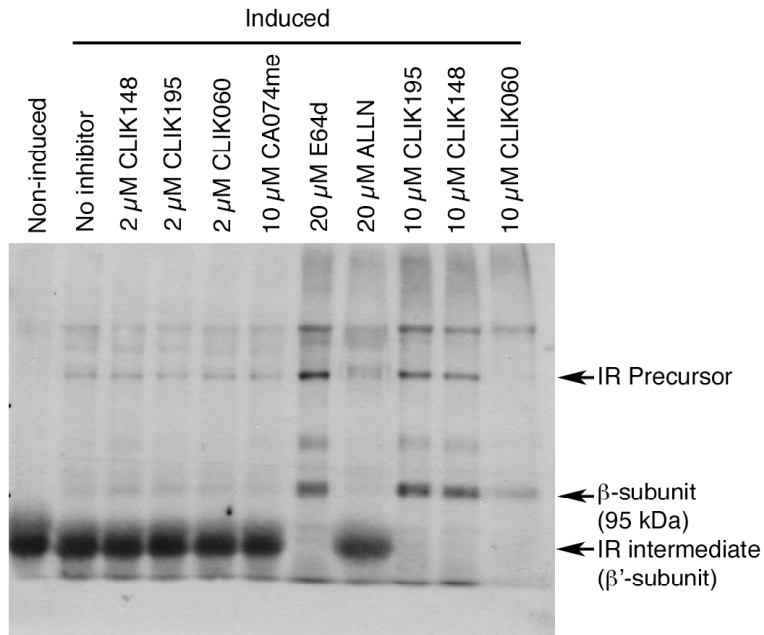
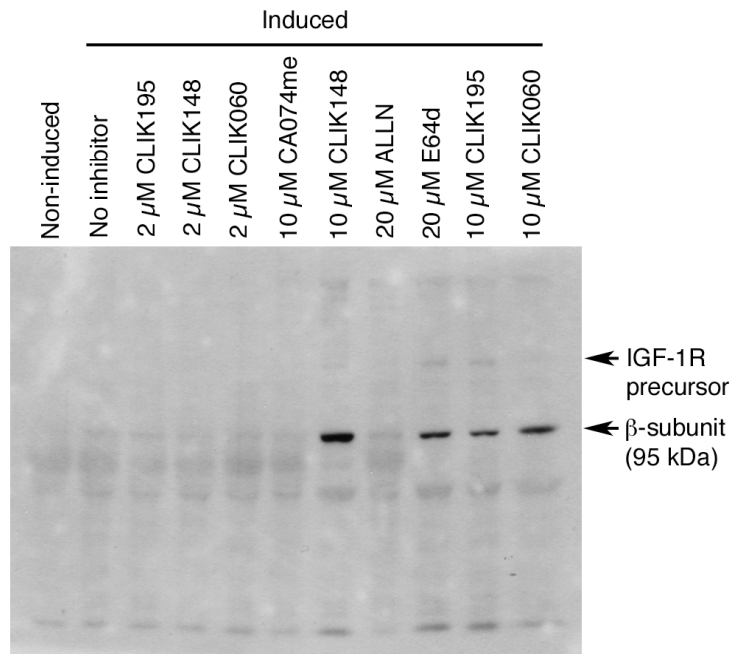
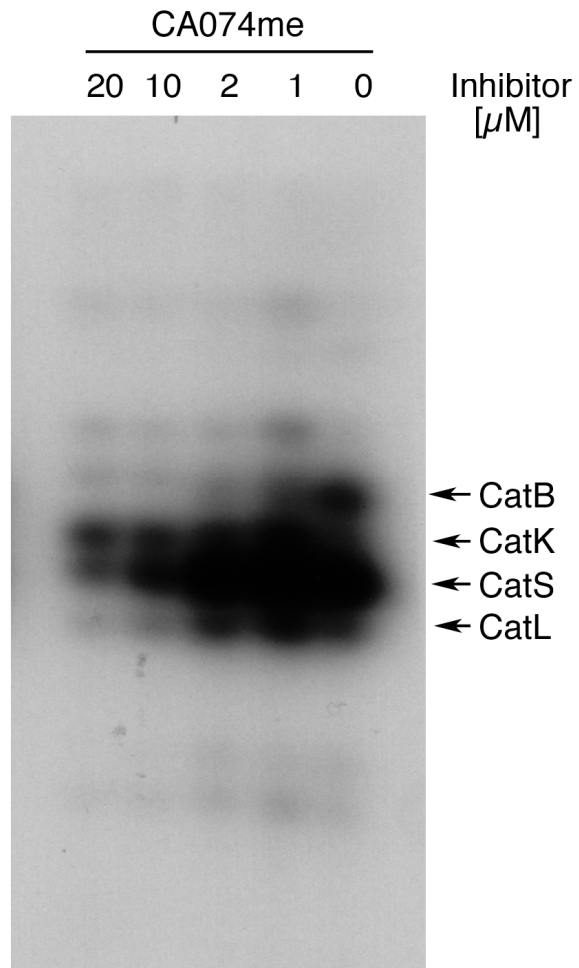


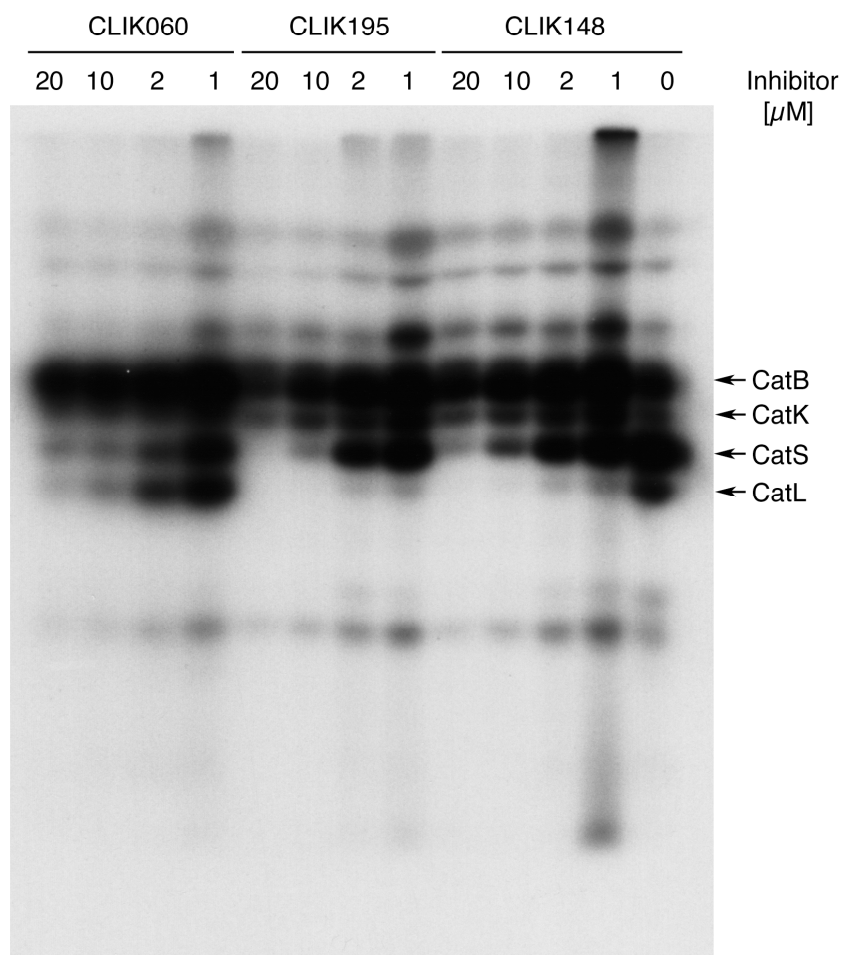
Figure 1k. Original figure.



Supplementary Figure 1. Left panel original figure.



Supplementary Figure 1. Right panel original figure.



Supplementary Table 1: Energy expenditure analysis in cathepsin L-deficient mice.

Parameters	<i>Ctsl</i>^{+/+} (n=6)	<i>Ctsl</i>^{-/-} (n=6)	P Values*
Food Consumption (gram/24 hrs)	3.25 ± 0.37	3.7 ± 1.0	0.74
Water Consumption (ml/24 hrs)	5.83 ± 0.60	7.5 ± 2.5	0.56
Fecal Output (gram/24 hrs)	2.20 ± 0.24	2.6 ± 0.6	0.57
Urine Volume (ml/24 hrs)	1.96 ± 0.34	1.2 ± 0.2	0.12
O ₂ Consumption (ml/min)	3.49 ± 0.49	3.78 ± 0.18	0.61
CO ₂ Production (ml/min)	2.31 ± 0.37	2.43 ± 0.15	0.78
Metabolic Rate** (cal/min)	16.34 ± 2.32	17.58 ± 0.85	0.64

*Mann Whitney test.

**Metabolic rate = $V_{CO_2} \times 1.106 + V_{O_2} \times 3.941$.

Supplementary Table 2: Energy expenditure analysis in CLIK195-treated male C57BL/6 mice.

Parameters	C57BL/6 (+DMSO, n=8)	C57BL/6 (+CLIK195, n=7)	P Values*
Food Consumption (gram/24 hrs)	3.20 ± 0.30	3.90 ± 0.20	0.07
Water Consumption (ml/24 hrs)	5.00 ± 0.76	6.86 ± 0.88	0.16
Fecal Output (gram/24 hrs)	2.10 ± 0.20	2.20 ± 0.10	0.60
Urine Volume (ml/24 hrs)	1.80 ± 0.30	1.40 ± 0.20	0.56
O ₂ Consumption (ml/min)	3.14 ± 0.15	3.11 ± 0.12	>0.99
CO ₂ Production (ml/min)	1.99 ± 0.11	1.83 ± 0.11	>0.99
Metabolic Rate** (cal/min)	14.57 ± 0.68	14.23 ± 0.59	0.67

*Mann Whitney test.

**Metabolic rate = $V_{CO_2} \times 1.106 + V_{O_2} \times 3.941$.

Supplementary Table 3: Energy expenditure analysis in CLIK195-treated female *ob/ob* mice.

Parameters	<i>ob/ob</i> (+DMSO, n=6)	<i>ob/ob</i> (+CLIK195, n=6)	P Values*
Food Consumption (gram/24 hrs)	3.53 ± 0.27	4.00 ± 0.57	0.58
Water Consumption (ml/24 hrs)	4.50 ± 0.84	4.00 ± 1.24	0.79
Fecal Output (gram/24 hrs)	1.72 ± 0.15	2.23 ± 0.42	0.43
Urine Volume (ml/24 hrs)	1.46 ± 0.18	1.12 ± 0.36	0.54
O ₂ Consumption (ml/min)	3.15 ± 0.11	2.85 ± 0.03	0.07
CO ₂ Production (ml/min)	1.97 ± 0.08	1.95 ± 0.04	0.91
Metabolic Rate** (cal/min)	14.59 ± 0.52	13.41 ± 0.15	0.10

*Mann Whitney test.

**Metabolic rate = $V_{CO_2} \times 1.106 + V_{O_2} \times 3.941$.

Supplementary Table 4: Energy expenditure analysis in CLIK195-treated male *ob/ob* mice.

Parameters	<i>ob/ob</i> (+DMSO, n=6)	<i>ob/ob</i> (+CLIK195, n=6)	P Values*
Food Consumption (gram/24 hrs)	4.00 ± 0.28	4.50 ± 0.18	0.21
Water Consumption (ml/24 hrs)	8.33 ± 0.56	8.00 ± 0.61	0.73
Fecal Output (gram/24 hrs)	1.97 ± 0.09	2.20 ± 0.18	0.37
Urine Volume (ml/24 hrs)	2.06 ± 0.13	2.23 ± 0.27	0.67
O ₂ Consumption (ml/min)	3.55 ± 0.43	3.05 ± 0.05	0.34
CO ₂ Production (ml/min)	2.48 ± 0.36	2.07 ± 0.06	0.48
Metabolic Rate** (cal/min)	16.71 ± 2.09	14.30 ± 0.27	0.34

*Mann Whitney test.

**Metabolic rate = $V_{CO_2} \times 1.106 + V_{O_2} \times 3.941$.