The carboxypeptidase angiotensin converting enzyme (ACE) shapes the MHC class I peptide repertoire

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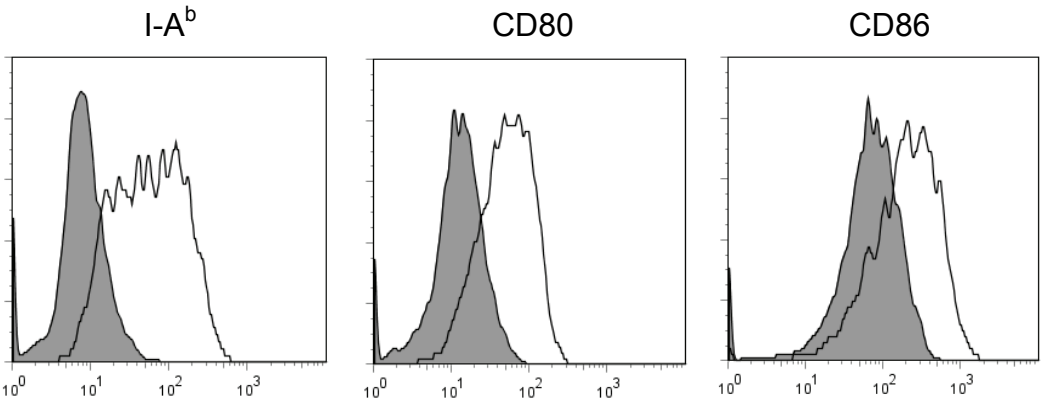
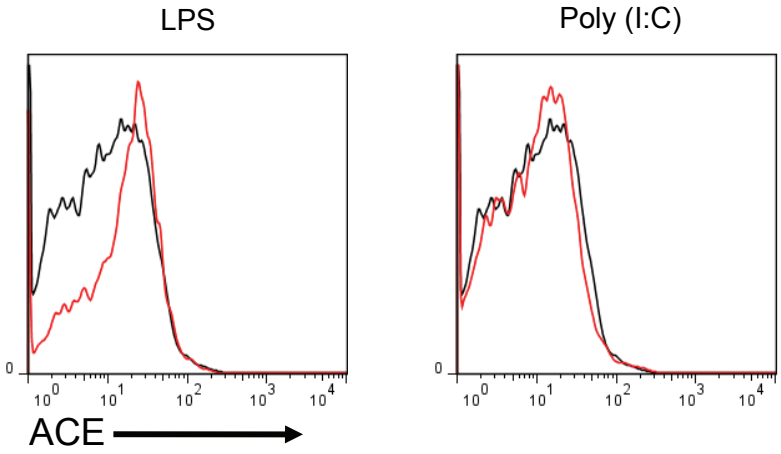
Supplementary Fig. 1



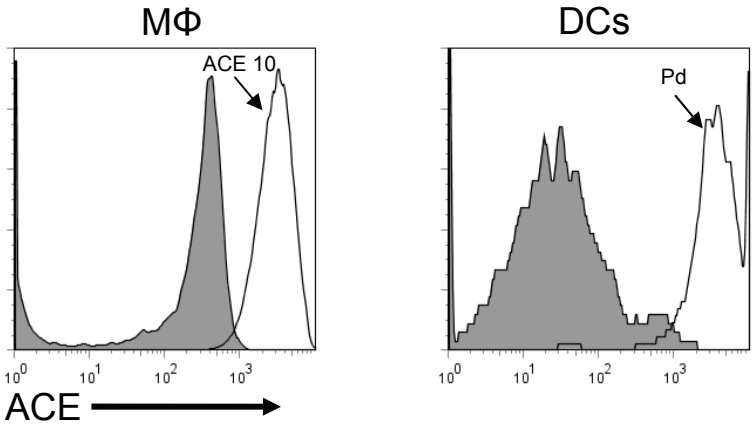
Supplementary Fig. 1 Expression of maturation associated surface markers in macrophages. Surface MHC class II (I-A^b), CD80 and CD86 on TPM (solid line) and M-CSF-induced MΦ (shaded) were evaluated by flow cytometry. These histograms are representative of four independent experiments.

Supplementary Fig. 2



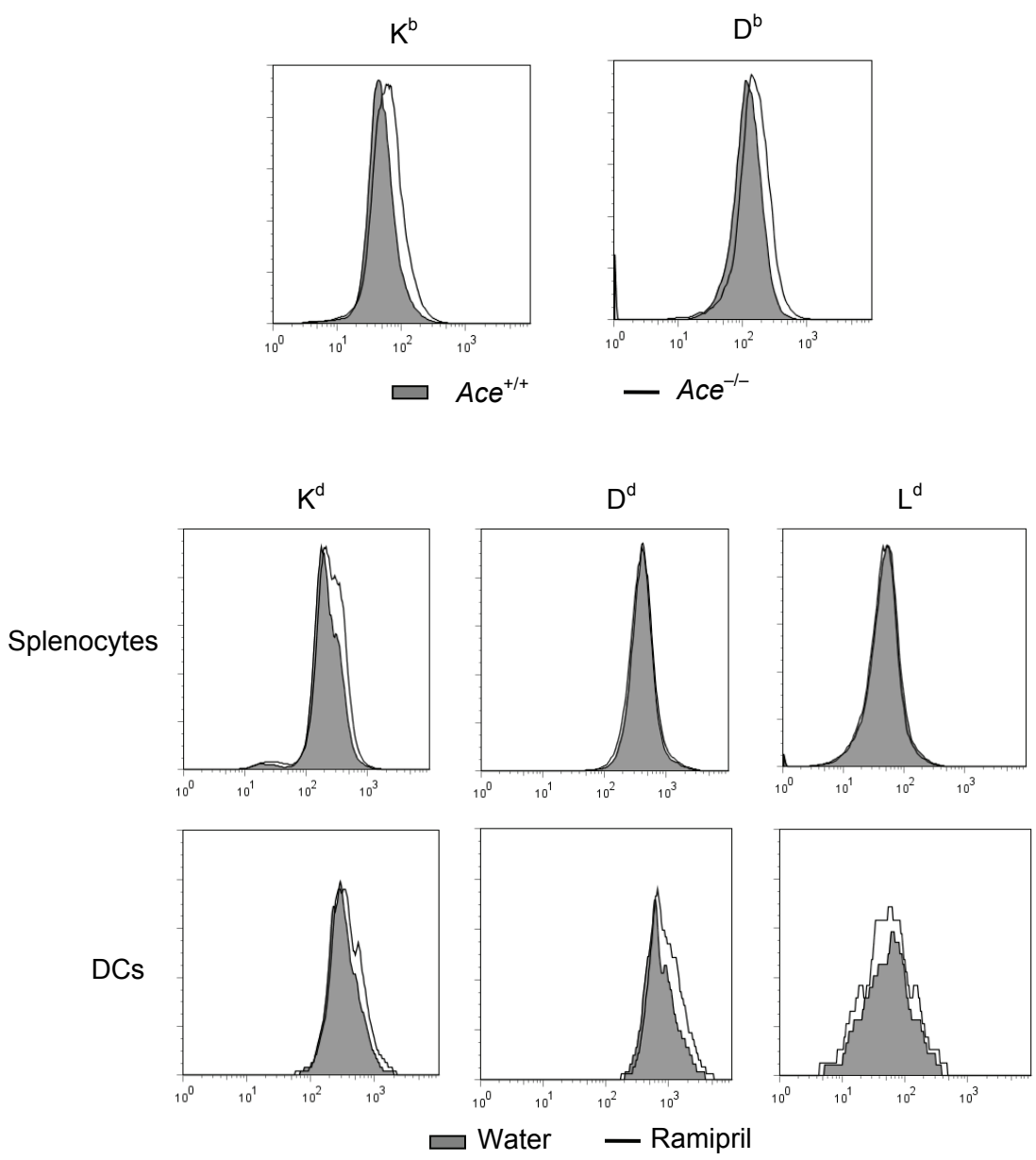
Supplementary Fig. 2 ACE expression in DCs after TLR ligand stimulation. ACE expression in bone marrow-derived DCs with (red) or without (black) LPS or Poly (I:C) stimulation for 24 h. Data are representative of 3 independent experiments.

Supplementary Fig. 3



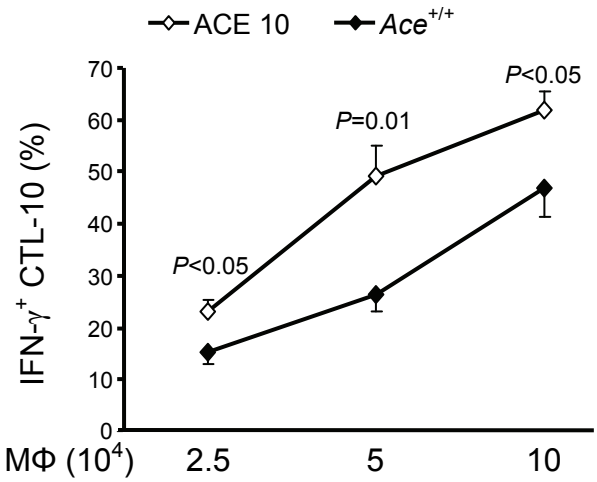
Supplementary Fig. 3 ACE expression by macrophages and DCs. Resident peritoneal F4/80$^+$ macrophages from ACE 10 mice (left) and splenic CD11c$^+$ DCs from Pd mice (right) were compared with equivalent cells from *Ace*$^{+/+}$ littermates (shaded). The histograms are typical of those obtained from at least 3 pairs of mice from each strain.

Supplementary Fig. 4



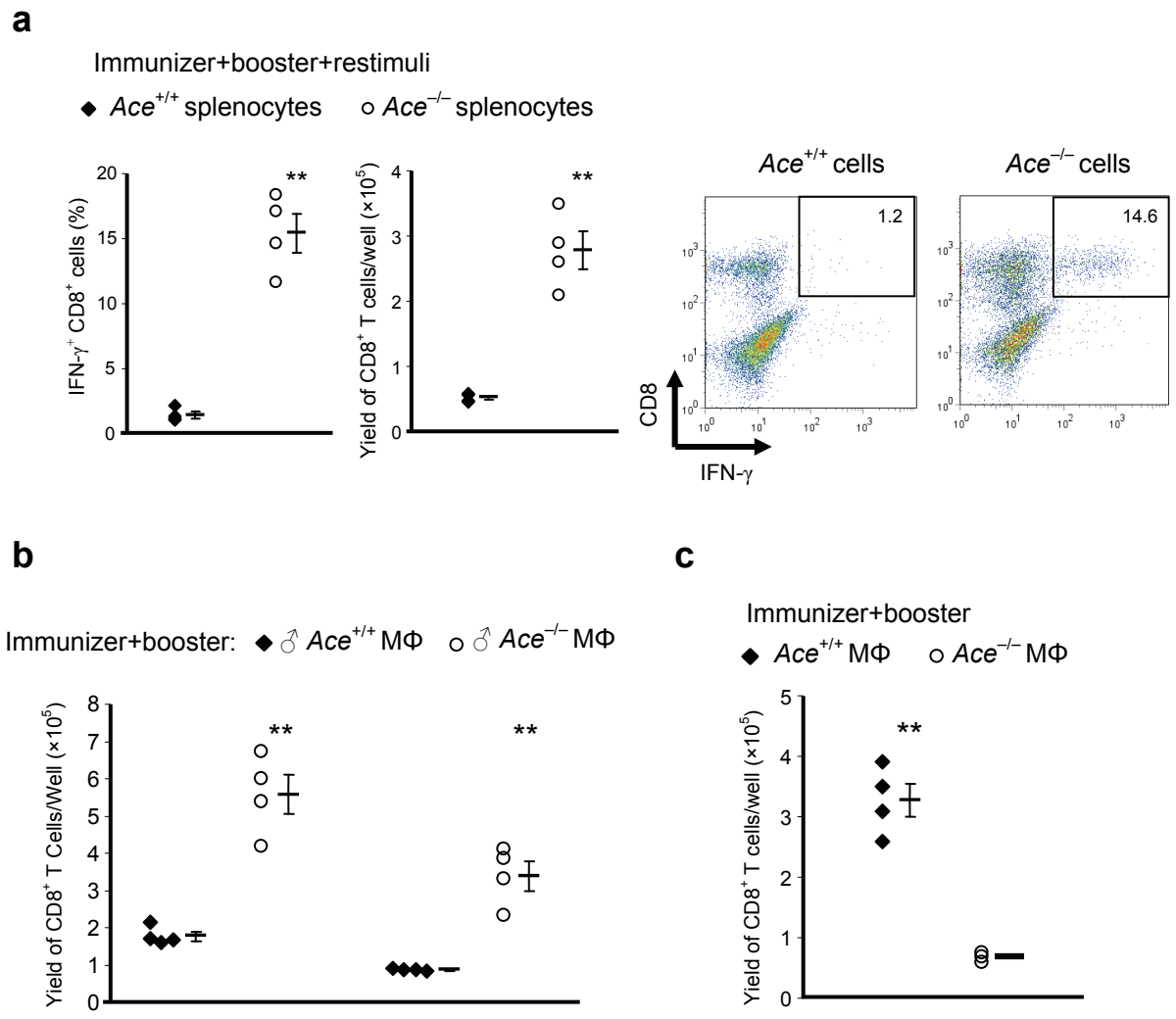
Supplementary Fig. 4 Surface MHC class I levels on the splenocytes. (**a**) K^b and D^b expressed by $Ace^{+/+}$ and $Ace^{-/-}$ total splenocytes. Data are representative of 5 pairs of mice. (**b**) K^d, D^d and L^d on the total splenocytes and splenic DCs of BALB/c mice treated with the ACE inhibitor ramipril or with water for 6 d. Data are representative of 6 pairs of mice.

Supplementary Fig. 5



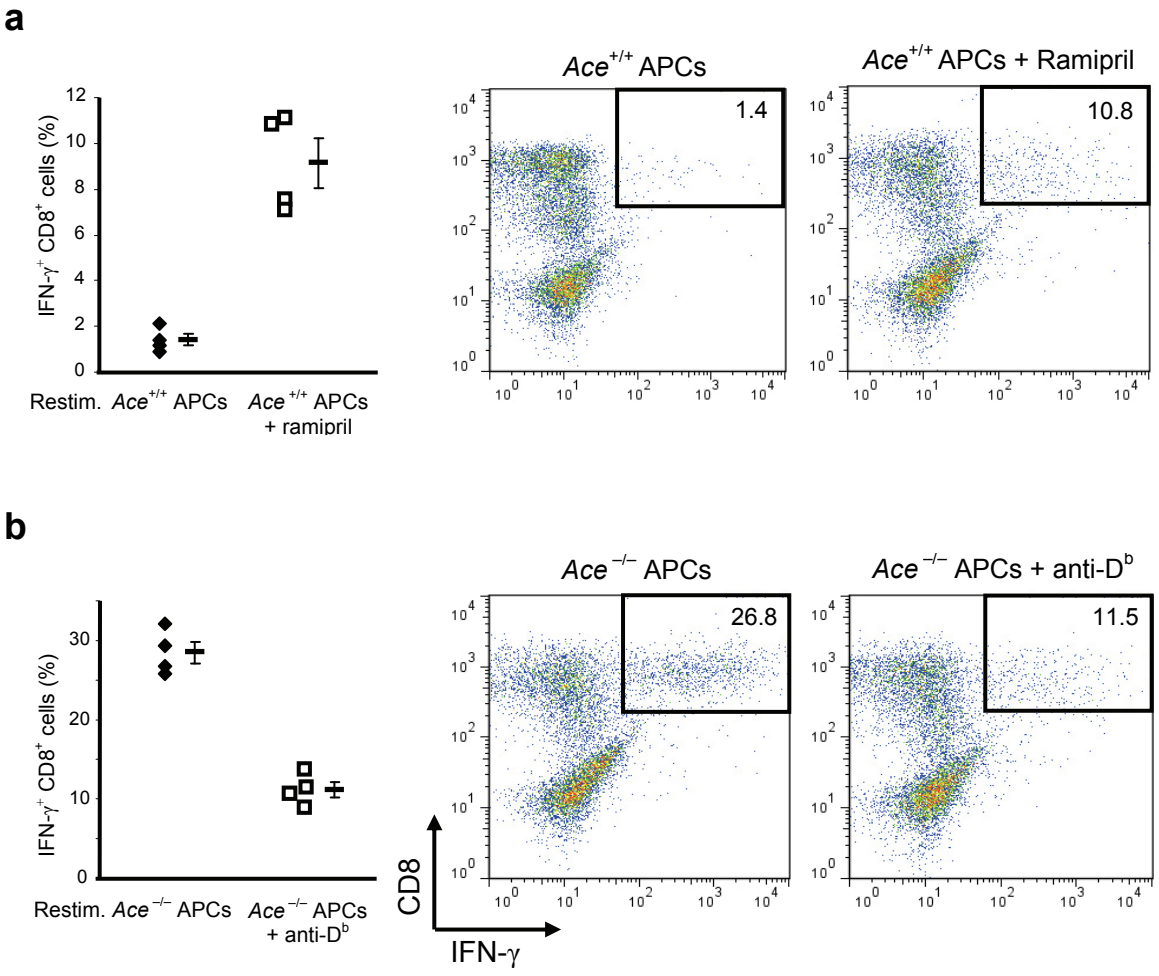
Supplementary Fig. 5 D^b-Uty presentation by ACE over-expressing cells. The indicated number of macrophages from male *Ace*$^{+/+}$ or ACE 10 mice were incubated with 1×10^5 Uty-specific CTLs, clone CTL-10. The percentage of IFN-γ^+ CTLs was determined by flow cytometry. n = 6.

Supplementary Fig. 6



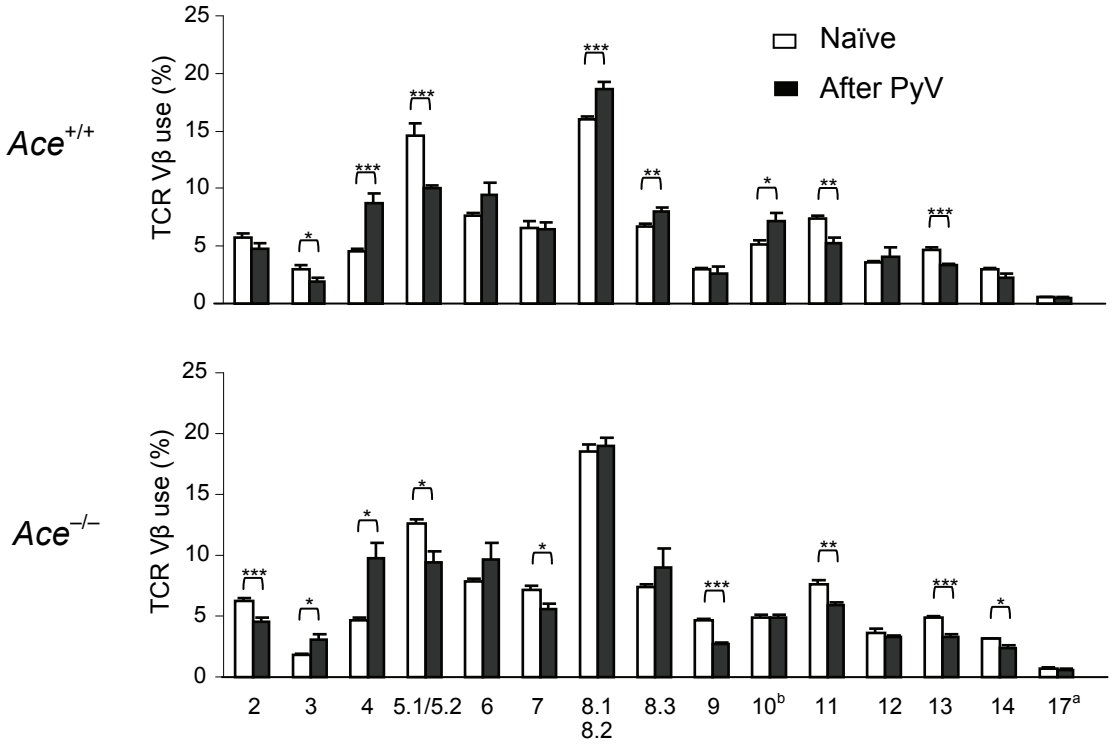
Supplementary Fig. 6 The effects of ACE on $CD8^+$ T cell repertoire. **(a)** $Ace^{+/+}$ mice were immunized, boosted and restimulated with splenocytes from $Ace^{+/+}$ or $Ace^{-/-}$ mice of the same gender. The percentage of IFN-γ secreting $CD8^+$ T cells and the yields of $CD8^+$ T cells responding to the indicated cells are shown. The right portion of the figure shows representative examples of IFN-γ expression by the $CD8^+$ T cells. (**b**) Supplemental to Fig. 4b. The yields of $CD8^+$ T cells responding to the indicated cells are shown. (**c**) Supplemental to Fig. 4c. The yields of $CD8^+$ T cells responding to the indicated cells are shown. $^{**}P<0.01$.

Supplementary Fig. 7



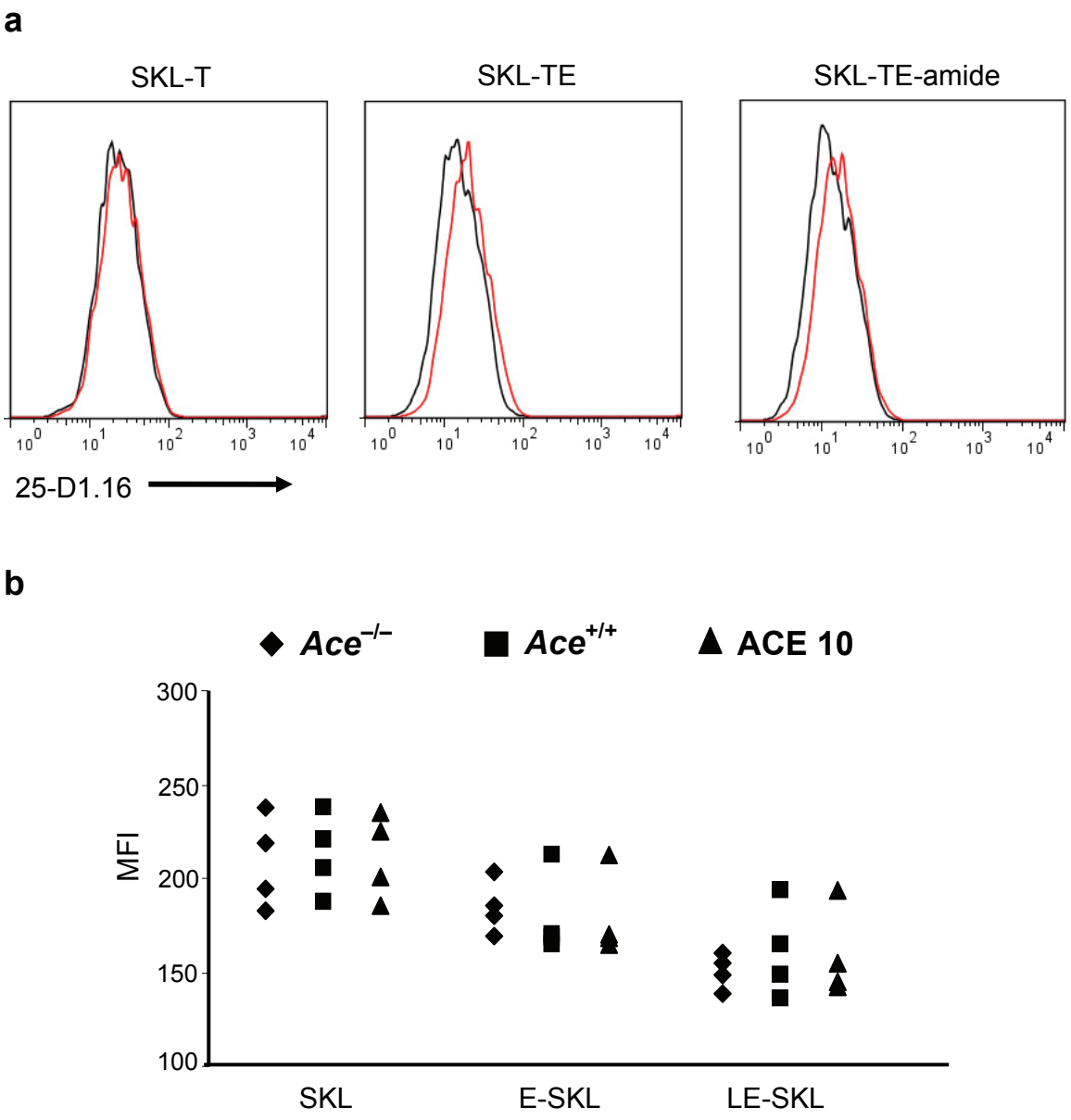
Supplementary Fig. 7 ACE inhibition changes MHC class I peptide pool. *Ace*$^{+/+}$ mice were immunized and boosted with MΦ from *Ace*$^{-/-}$ mice of the same gender. The splenocytes from the immunized mice were restimulated with either *Ace*$^{+/+}$ cells or the cells derived from ramipril-treated *Ace*$^{+/+}$ mice (**panel a**). In other experiments, cells were restimulated with *Ace*$^{-/-}$ cells or *Ace*$^{-/-}$ cells pretreated with anti-D^{b} antibody (**panel b**). The percentage of IFN-γ secreting CD8^{+} T cells was measured (left). The right portion of the figure shows representative histograms of the CD8^{+} T cell response. Data are pooled from 2 independent experiments.

Supplementary Fig. 8



Supplementary Fig. 8 TCR repertoire change after polyomavirus infection. TCR Vβ usage was screened in *Ace*$^{+/+}$ and *Ace*$^{-/-}$ mice before and 8 d after polyomavirus (PyV) infection. Data, which are shown as mean + s.e.m., are pooled from 6 pairs of *Ace*$^{+/+}$ mice and 4 pairs of *Ace*$^{-/-}$ mice. **P*<0.05; ***P*<0.01; ****P*<0.005.

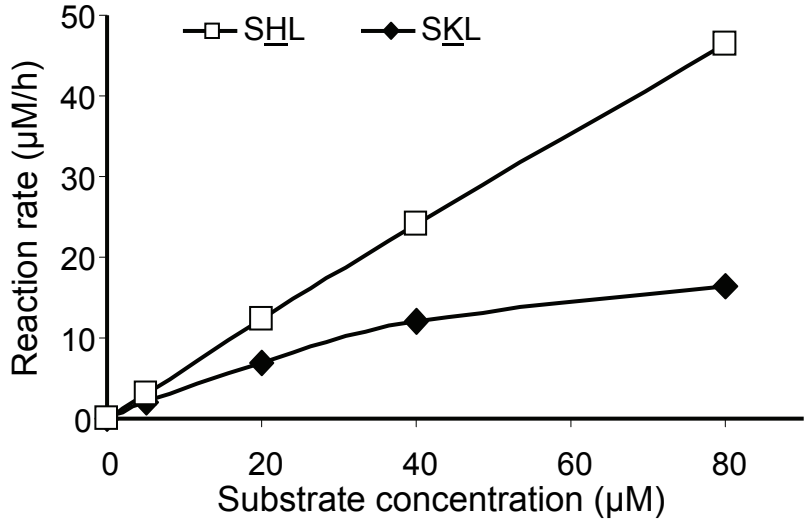
Supplementary Fig. 9



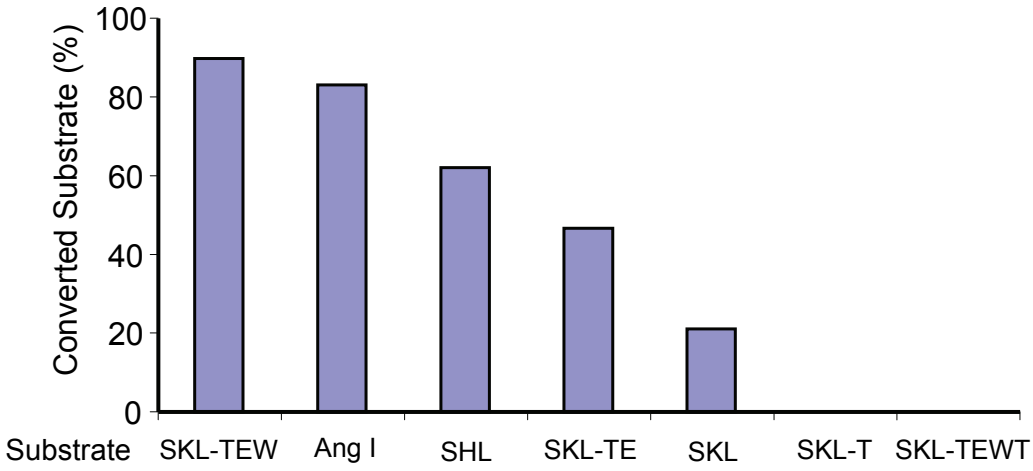
Supplementary Fig. 9 *In vivo* analysis of ACE catalytic activity. (**a**) Histograms of 25-D1.16 staining after *Ace*$^{+/+}$ MΦ (black) and ACE 10 MΦ (red) were pulsed with the indicated peptides. Data are representative of 3 independent experiments. (**b**) Macrophages from *Ace*$^{-/-}$, *Ace*$^{+/+}$ and ACE 10 mice were nucleofected with a GFP expressing construct and the indicated minigenes expressing SKL or SKL with 1- or 2-mer extension at the N-terminus. 25-D1.16 staining of GFP^{+} cells was evaluated 24 h later.

Supplemental Figure 10

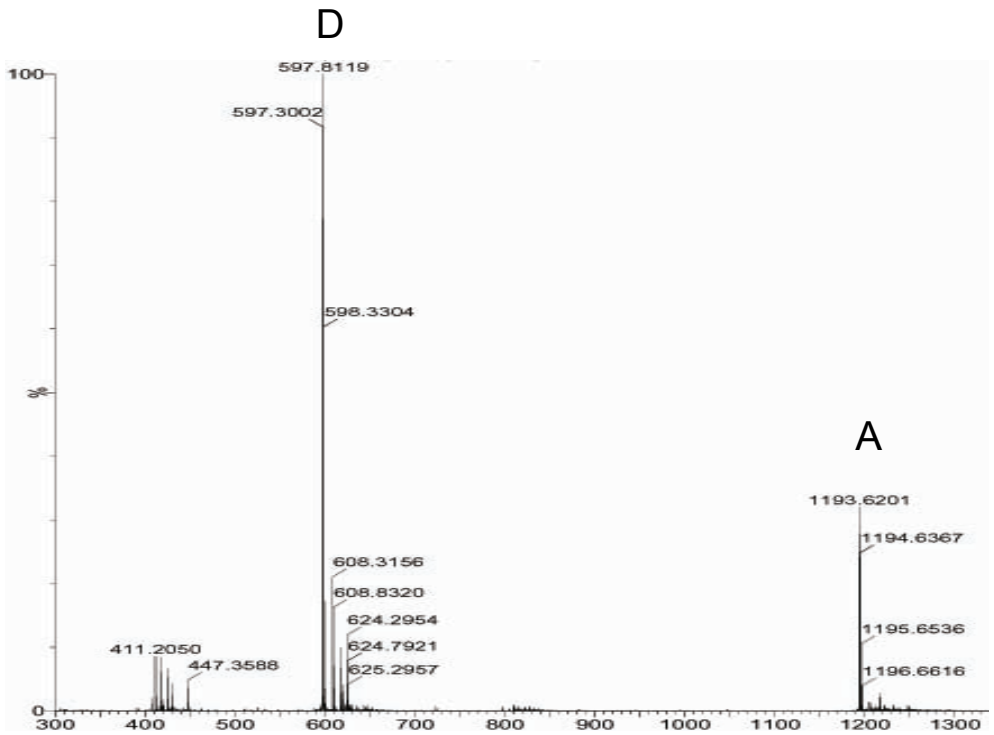
a



b



c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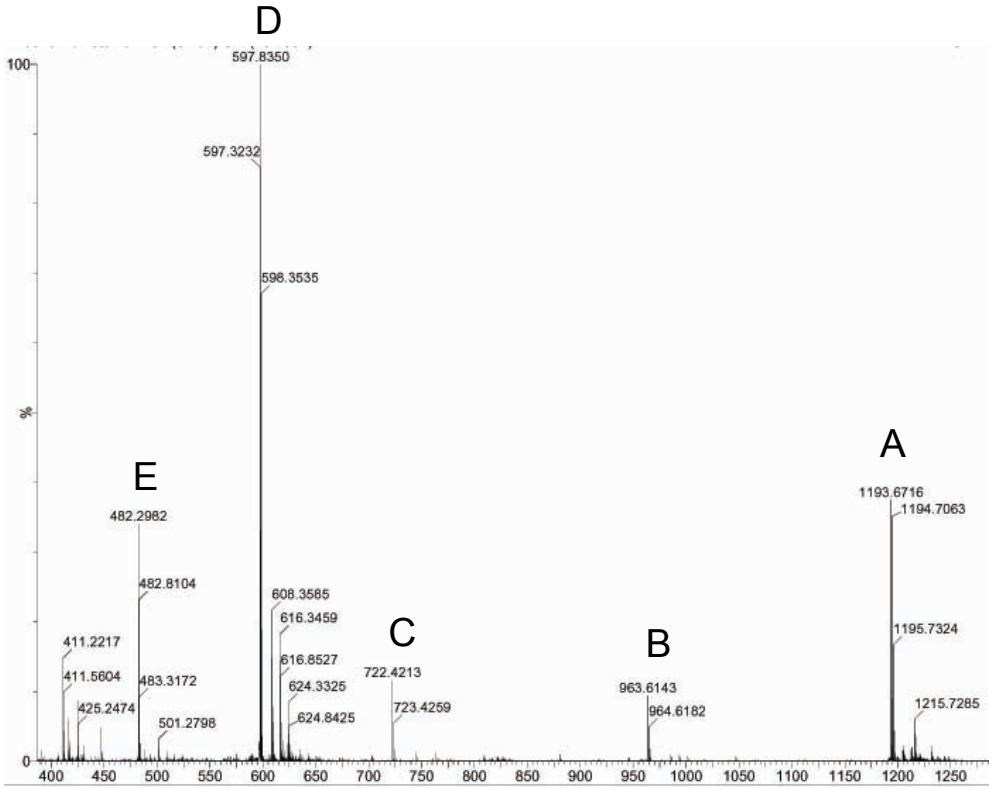
597.3002
598.3304
608.3156
608.8320
624.2954
624.7921
625.2957
411.2050
447.3588
A
1193.6201
1194.6367
1195.6536
1196.6616
%
100
0
300
400
500
600
700
800
900
1000
1100
1200
1300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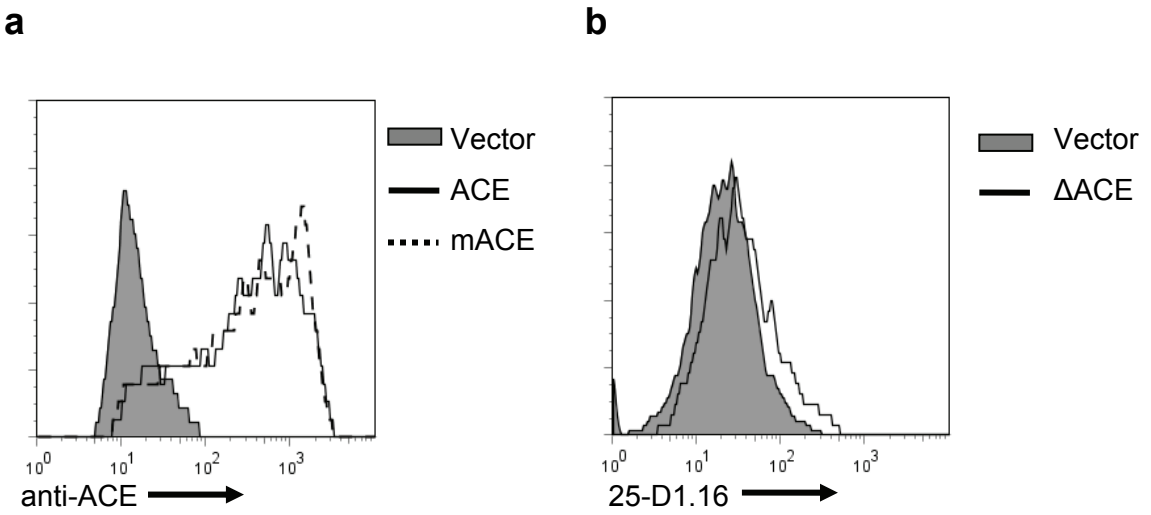
597.3232
598.3535
E
482.2982
482.8104
411.2217
411.5604
425.2474
483.3172
501.2798
608.3585
616.3459
616.8527
624.3325
624.8425
C
722.4213
723.4259
B
963.6143
964.6182
A
1193.6716
1194.7063
1195.7324
1215.7285
%
100
0
400
450
500
550
600
650
700
750
800
850
900
950
1000
1050
1100
1150
1200
1250

Supplementary Fig 10. *In vitro* biochemical analysis of ACE catalytic activity. (**a**) The indicated concentration of SKL or SHL was incubated with ACE for 1 h at 37°C. The reaction was stopped by EDTA and 0°C. The N-terminal 6-mer, SIINFE, was quantitated by LC-MS, and the representative Michaelis–Menten kinetics were drawn. (**b**) 20 μM of the indicated peptides, including angiotensin I (Ang I), were incubated with ACE for 1 h, respectively, and compared to the reaction without ACE. The percentage of hydrolyzed substrate was then calculated. (**c**) Upper: the spectrum of SKL-TE without ACE degradation. Lower: the spectrum of SKL-TE after 0.5 h ACE degradation; (A) SKL-TE +1 *m/z*; (B) SKL +1 *m/z*; (C) SIINFE +1 *m/z*; (D) SKL-TE +2 *m/z*; (E) SKL +2 *m/z*.

Supplementary Fig. 11



Supplementary Fig. 11 The mutated ACE constructs. (**a)** L.K^b cells were transfected with an empty vector or the construct expressing either wild-type ACE or catalytic domain mutated ACE (mACE). A polyclonal ACE antibody was used to detect the expression of the constructs. (**b**) An SKL-TE minigene was transfected to L.K^b cells which were also co-transfected with either an empty vector or a construct expressing N-terminal signal truncated ACE (ΔACE). SKL presentation was evaluated with the 25-D1.16 antibody. Representative histograms from at least 3 independent experiments are shown for a and b.

Supplementary Table 1 Peptides used in ACE catalytic assays

Symbol	Sequence
SKL	SIINFEKL
SHL	SIINFEHL
SKL-T	SIINFEKLT
SKL-TE	SIINFEKLTE
SKL-TE-amide	SIINFEKLTE-amide
E-SKL	ESIINFEKL
LE-SKL	LESIINFEKL