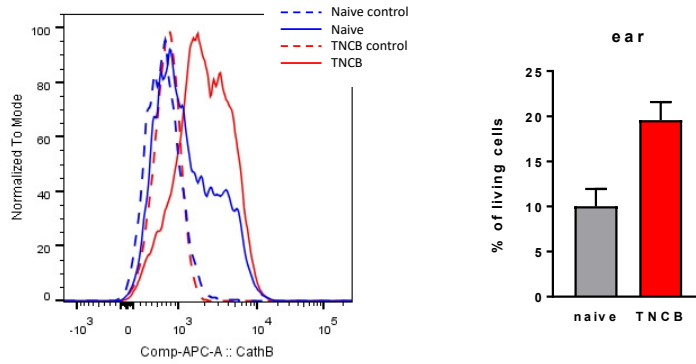


1 **Supplementary Figures**

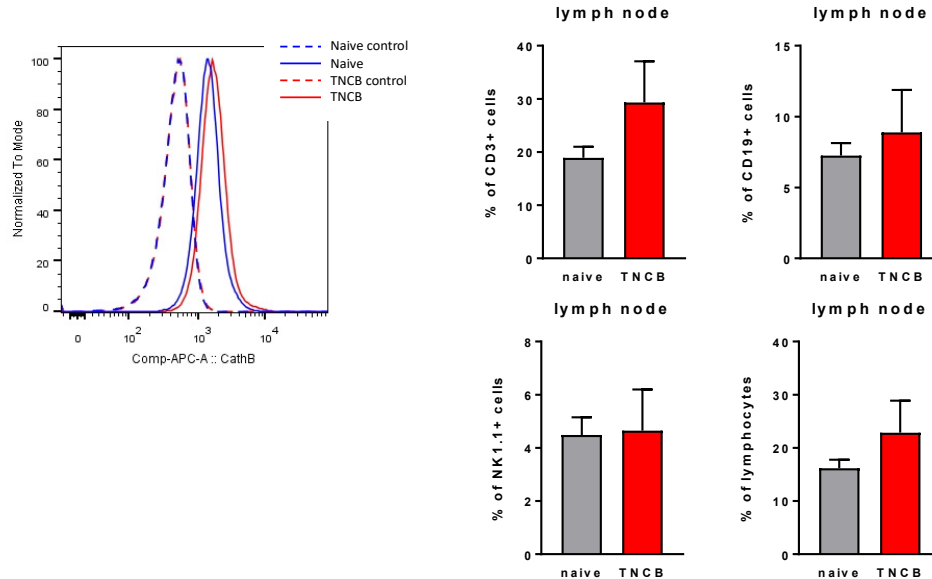
2

3 **Supplementary Fig. 1**

**A**



**B**



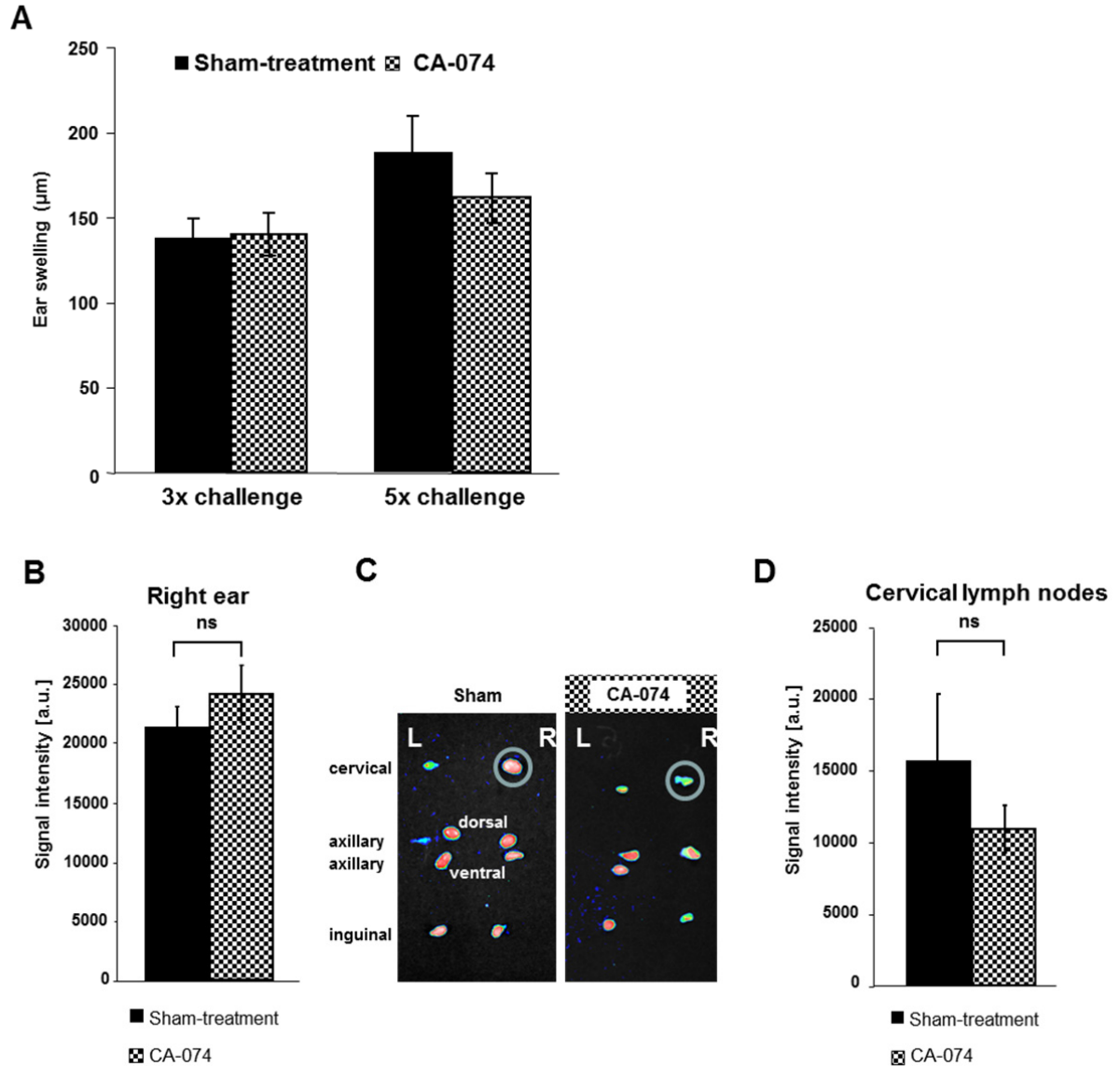
4

5 **A:** Intracellular cathepsin B flow cytometry analysis revealed a high cathepsin B expression in  
6 the leucocytes with high granularity (most probably neutrophils) isolated from the inflamed  
7 ears with acute cutaneous DTHR (n=4). **B:** An increase in the number of cathepsin B  
8 expressing T cells (CD3) and B cells (CD19) in the inflamed cervical lymph nodes (n=4) when  
9 compared to the lymph nodes derived from healthy mice (n=4) was determined in line with  
10 our fluorescence microscopy data. No inflammation induced change in cathepsin B  
11 expression was evident in NK cells (NK1.1).

12

13

1 **Supplementary Fig. 2**

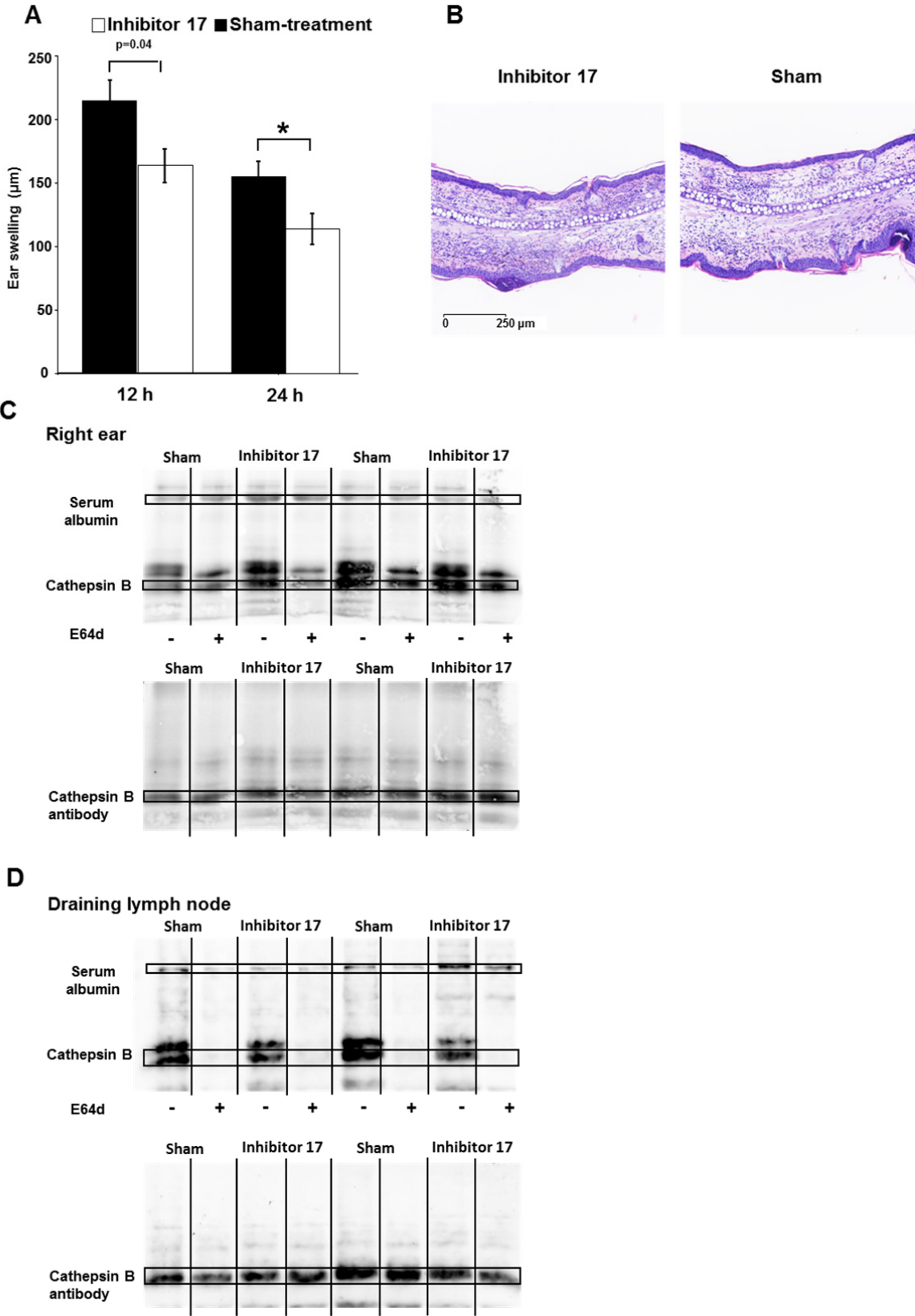


2

3 **A:** In chronic cutaneous DTHR after three or five TNCB challenges, no significant differences  
 4 in ear swelling response were observed between sham- and CA-074 treated mice (n=7). **B:** *In*  
 5 *vivo* optical imaging with the cathepsin-activatable probe CatB680 showed no significant  
 6 difference in signal intensity between mice treated on the right ear with CA-074 or sham  
 7 treatment (n=4). **C:** *Ex vivo* optical imaging showed reduced CatB680 signal intensity in  
 8 draining cervical lymph nodes (LNs) of CA-074-treated mice. Axillary and inguinal LNs showed  
 9 high signal intensities in both groups. **D:** The *ex vivo* CatB680 signal intensity in the cervical

- 1 draining lymph nodes was slightly lower in CA-074-treated mice than in sham-treated mice
- 2 (n=4).
- 3

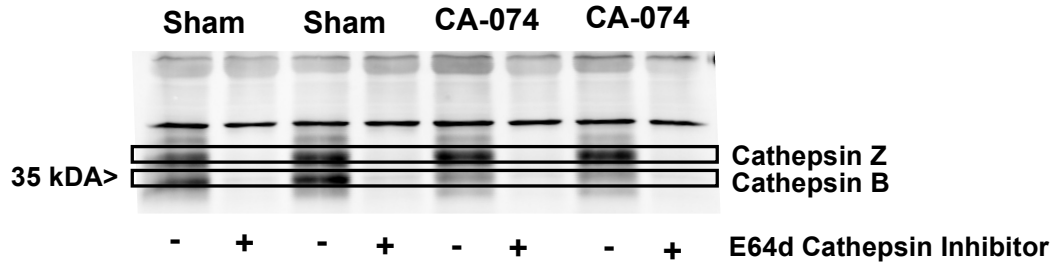
1 Supplementary Fig. 3



1 **A:** Topical treatment with inhibitor 17 reduced ear swelling 12 h and 24 h after TNCB  
2 challenge relative to that in sham-treated mice (n=8). **B:** Histological H&E staining of ear  
3 tissue derived from inhibitor 17- and sham-treated mice 24 h after challenge revealed  
4 reduced edema and leukocyte infiltration as a consequence of inhibitor 17 treatment. **C/D:**  
5 Differences between inhibitor 17- and sham-treated mice could not be detected by active  
6 site labeling or immunoblotting of tissue from ears as well as draining lymph nodes, most  
7 likely because of the reversible covalent binding of inhibitor 17 to cathepsin B (n=8).  
8

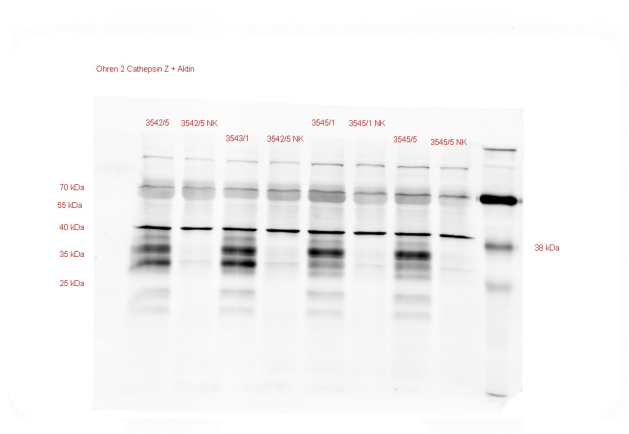
1 **Supplementary Fig. 4**

2



3

4

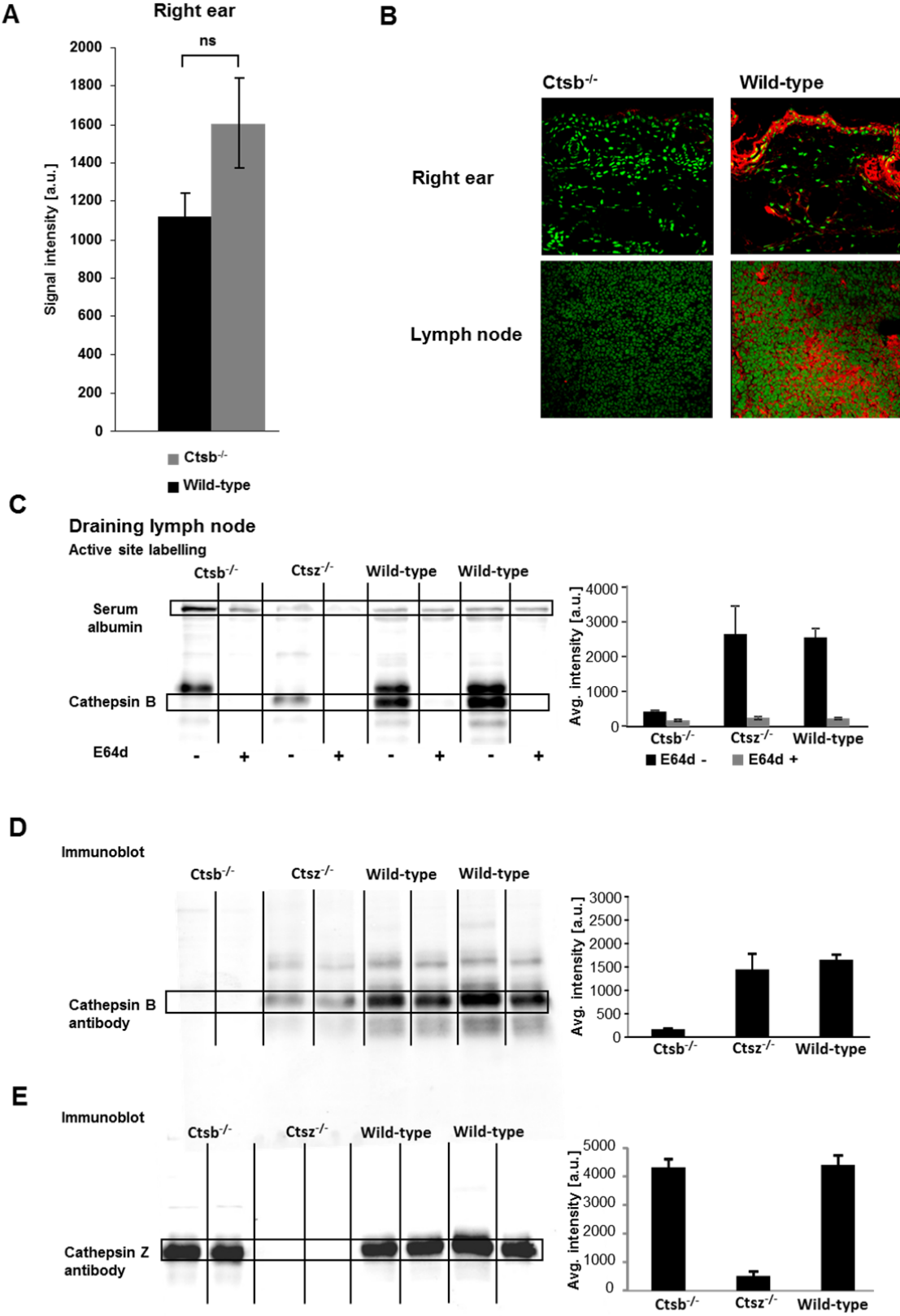


5

6 Active site labeling and western blot analysis of ear tissue derived from CA-074 (n=4) and  
7 sham-treated mice (n=4) 24 h after TNCB ear challenge revealed an impressively reduced  
8 expression of cathepsin B in inflamed ears of CA-074 treated mice when compared to  
9 inflamed ears derived from sham-treated littermates, but the expression of cathepsin Z in  
10 inflamed ears of CA-074 treated mice remained unaffected. A representative blot is shown  
11 here in this figure.

12

1 Supplementary Fig. 5



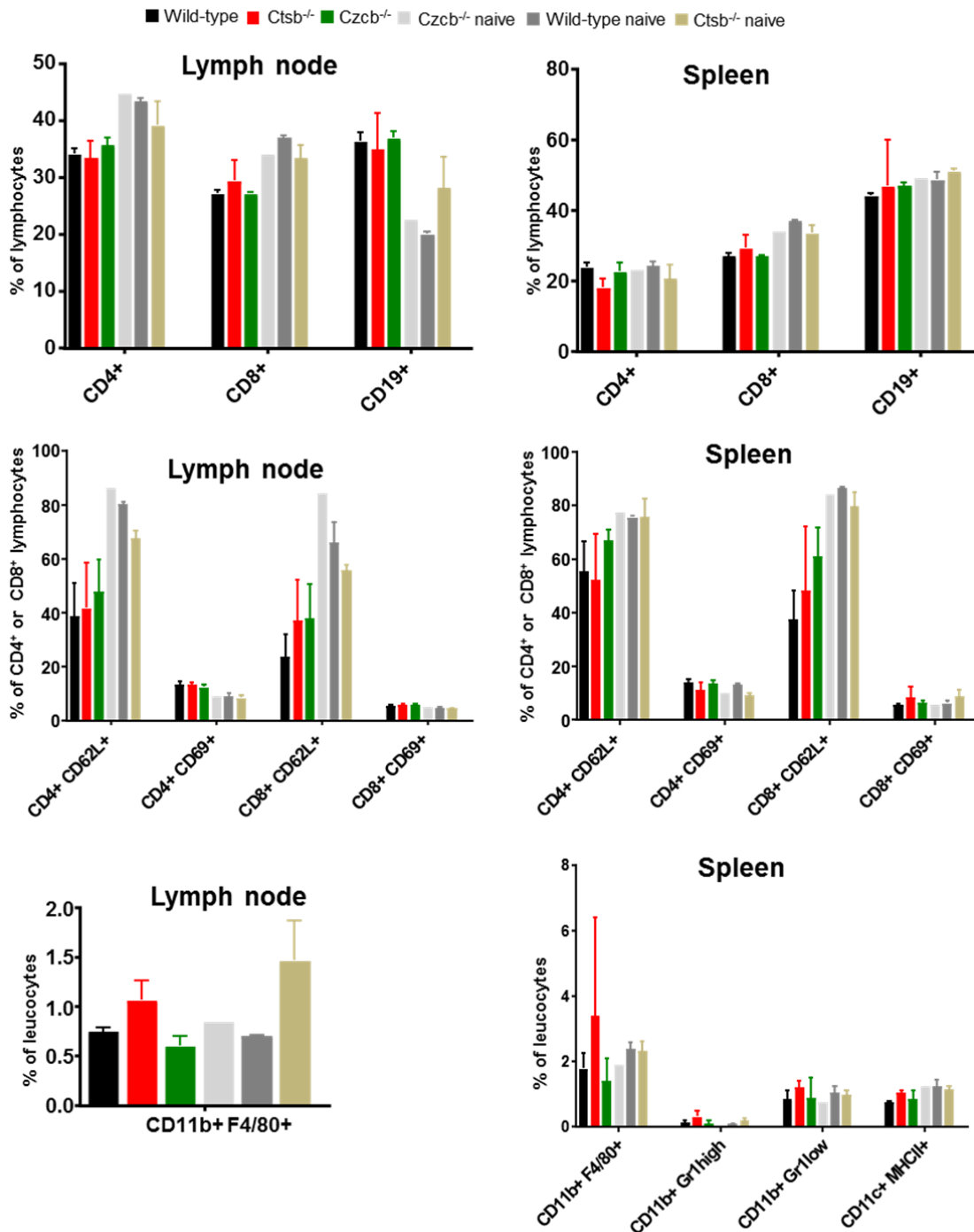
1

2 **A:** Optical imaging of *Ctsb*<sup>-/-</sup> mice and wild-type mice using the CatB680 probe revealed  
3 increased signal intensity in cathepsin B-deficient mice after the first challenge (1x challenge:  
4 n=10; 1x/3x challenge: n=7). **B:** Immunofluorescence staining of cathepsin B (red) in tissue  
5 from the right ear and draining lymph nodes of sensitized *Ctsb*<sup>-/-</sup> and wild-type mice 24 h  
6 after TNCB challenge revealed suppressed cathepsin B expression in *Ctsb*<sup>-/-</sup> mice (goat anti-  
7 cathepsin B antibody (Ab), 1:20; R&D Systems, Minneapolis, USA; visualized using Cy3-  
8 donkey anti-goat IgG Ab; Dianova, Hamburg, Germany). **C:** Active site labeling of draining  
9 lymph nodes harvested from *Ctsb*<sup>-/-</sup> mice revealed a diminished cathepsin B band density but  
10 equivalent cathepsin B expression in *Ctsz*<sup>-/-</sup> mice compared to that in wild-type mice (*Ctsb*<sup>-/-</sup>:  
11 n=4; *Ctsz*<sup>-/-</sup>: n=3; wild-type: n=4). **D:** Immunoblotting using a cathepsin B-specific antibody on  
12 the same membrane confirmed the bands detected by active site labeling and indicated  
13 normal cathepsin B expression in draining lymph nodes of *Ctsz*<sup>-/-</sup> mice, while cathepsin B  
14 expression in *Ctsb*<sup>-/-</sup> mice was diminished (*Ctsb*<sup>-/-</sup>: n=4; *Ctsz*<sup>-/-</sup>: n=3; wild-type: n=4). **E:**  
15 Cathepsin Z expression as detected by immunoblotting using a cathepsin Z-specific antibody  
16 was similar in draining lymph nodes of *Ctsb*<sup>-/-</sup> and wild-type mice, while cathepsin Z  
17 expression was virtually absent in *Ctsz*<sup>-/-</sup> mice (*Ctsb*<sup>-/-</sup>: n=4; *Ctsz*<sup>-/-</sup>: n=3; wild-type: n=4).

18



1 **Supplementary Fig. 6**



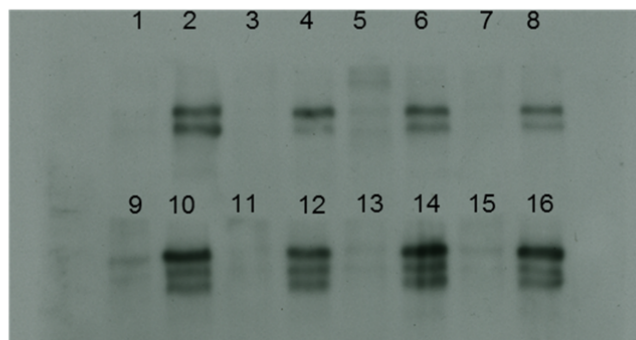
2

3 We performed flow cytometric analysis of inguinal and axillary lymph nodes (TNCB  
 4 sensitization on the abdomen) and spleen tissue derived from naive and TNCB-sensitized and  
 5 challenged wild-type, *Ctsb*<sup>-/-</sup>, and *Ctsz*<sup>-/-</sup> mice. Neither *Ctsb*<sup>-/-</sup> nor *Ctsz*<sup>-/-</sup> mice showed

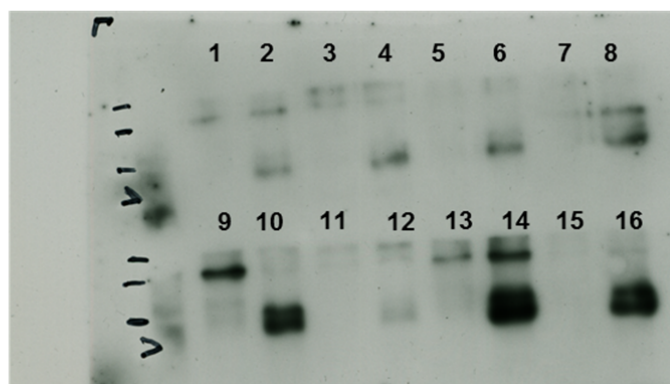
- 1 significant alterations in the composition of immune cells in the spleen or lymph nodes
- 2 relative to that in wild-type mice, either in the naïve state or 24 h after challenge (TNCB-
- 3 sensitized and challenged mice: n = 3-4; naïve mice: n = 1-3).
- 4

1 **Supplementary Fig. 7**

**A**



**B**



2

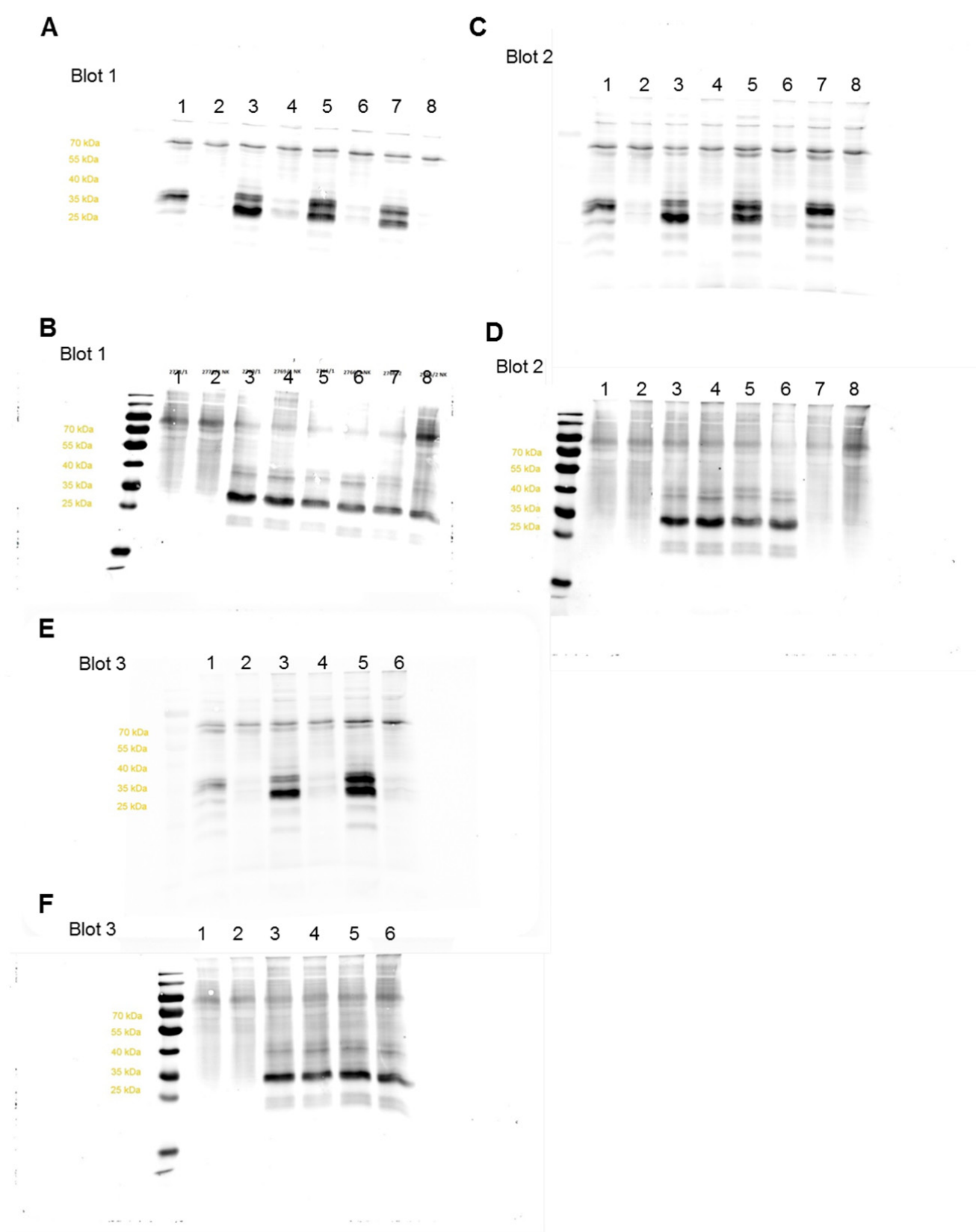
3 Original blots from Fig. 4C/D. **A:** Active site labeling blot (shown in Fig. 4C): lane 1, CA-074  
4 treatment + E-64d; lane 2, CA-074 treatment; lane 3, CA-074 treatment + E-64d; lane 4, CA-  
5 074 treatment; lane 5, CA-074 treatment + E-64d; lane 6, CA-074 treatment; lane 7, CA-074  
6 treatment + E-64d; lane 8, CA-074 treatment; lane 9, sham treatment + E-64d; lane 10, sham  
7 treatment; lane 11, sham treatment + E-64d; lane 12, sham treatment; lane 13, sham  
8 treatment + E-64d; lane 14, sham treatment; lane 15, sham treatment + E-64d; lane 16,  
9 sham treatment. **B:** Active site labeling blot (shown in Fig. 4D): lane 1, CA-074 treatment + E-  
10 64d; lane 2, CA-074 treatment; lane 3, CA-074 treatment + E-64d; lane 4, CA-074 treatment,  
11 lane 5, CA-074 treatment + E-64d; lane 6, CA-074 treatment; lane 7, CA-074 treatment + E-

1 64d; lane 8, CA-074 treatment; lane 9, sham treatment + E-64d; lane 10, sham treatment;  
2 lane 11, sham treatment + E-64d (no proteases detectable, data not shown in Fig. 4D); lane  
3 12, sham treatment (no proteases detectable, data not shown in Fig. 4D); lane 13, sham  
4 treatment + E-64d; lane 14, sham treatment; lane 15, sham treatment + E-64d; lane 16,  
5 sham treatment.

6

7

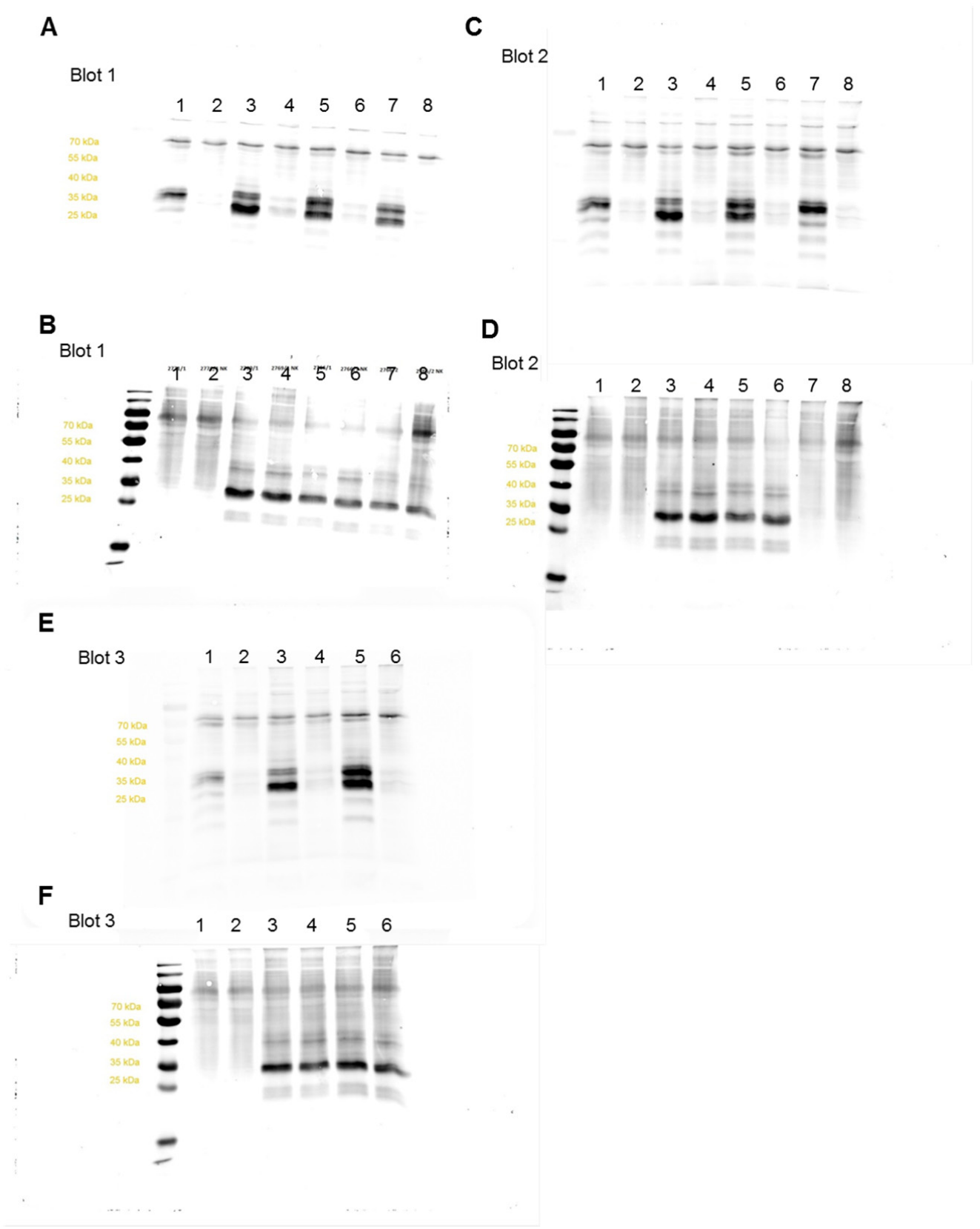
1 **Supplementary Fig. 8**



2

1 Original blots from Fig. 5 C/D. **A:** Blot 1, active site labeling (shown in Fig. 5C): lane 1, *Ctsb*<sup>-/-</sup>;  
2 lane 2, *Ctsb*<sup>-/-</sup> + E-64d; lane 3, *Ctsz*<sup>-/-</sup>; lane 4, *Ctsz*<sup>-/-</sup> + E-64d; lane 5, wild-type; lane 6, wild-  
3 type + E-64d; lane 7, wild-type; lane 8, wild-type + E-64d. **B:** Blot 1, cathepsin B Western blot  
4 (shown in Fig. 5D): lane 1, *Ctsb*<sup>-/-</sup>; lane 2, *Ctsb*<sup>-/-</sup> + E-64d; lane 3, *Ctsz*<sup>-/-</sup>; lane 4, *Ctsz*<sup>-/-</sup> + E-64d;  
5 lane 5, wild-type; lane 6, wild-type + E-64d; lane 7, wild-type; lane 8, wild-type + E-64d. **C:**  
6 Membrane 2, active site labeling: lane 1, *Ctsb*<sup>-/-</sup>; lane 2, *Ctsb*<sup>-/-</sup> + E-64d; lane 3, *Ctsz*<sup>-/-</sup>; lane 4,  
7 *Ctsz*<sup>-/-</sup> + E-64d; lane 5, wild-type; lane 6, wild-type + E-64d; lane 7, *Ctsb*<sup>-/-</sup>; lane 8, *Ctsb*<sup>-/-</sup> + E-  
8 64d. **D:** Blot 2, cathepsin B Western blot: lane 1, *Ctsb*<sup>-/-</sup>; lane 2, *Ctsb*<sup>-/-</sup> + E-64d; lane 3, *Ctsz*<sup>-/-</sup>;  
9 lane 4, *Ctsz*<sup>-/-</sup> + E-64d; lane 5, wild-type; lane 6, wild-type + E-64d; lane 7, *Ctsb*<sup>-/-</sup>; lane 8, *Ctsb*<sup>-/-</sup>  
10 <sup>-/-</sup> + E-64d. **E:** Blot 3, active site labeling: lane 1, *Ctsb*<sup>-/-</sup>; lane 2, *Ctsb*<sup>-/-</sup> + E-64d; lane 3, *Ctsz*<sup>-/-</sup>;  
11 lane 4, *Ctsz*<sup>-/-</sup> + E-64d; lane 5, wild-type; lane 6, wild-type + E-64d. **F:** Blot 3, cathepsin B  
12 Western blot: lane 1, *Ctsb*<sup>-/-</sup>; lane 2, *Ctsb*<sup>-/-</sup> + E-64d; lane 3, *Ctsz*<sup>-/-</sup>; lane 4, *Ctsz*<sup>-/-</sup> + E-64d; lane  
13 5, wild-type; lane 6, wild-type + E-64d.  
14

1 **Supplementary Fig. 9**

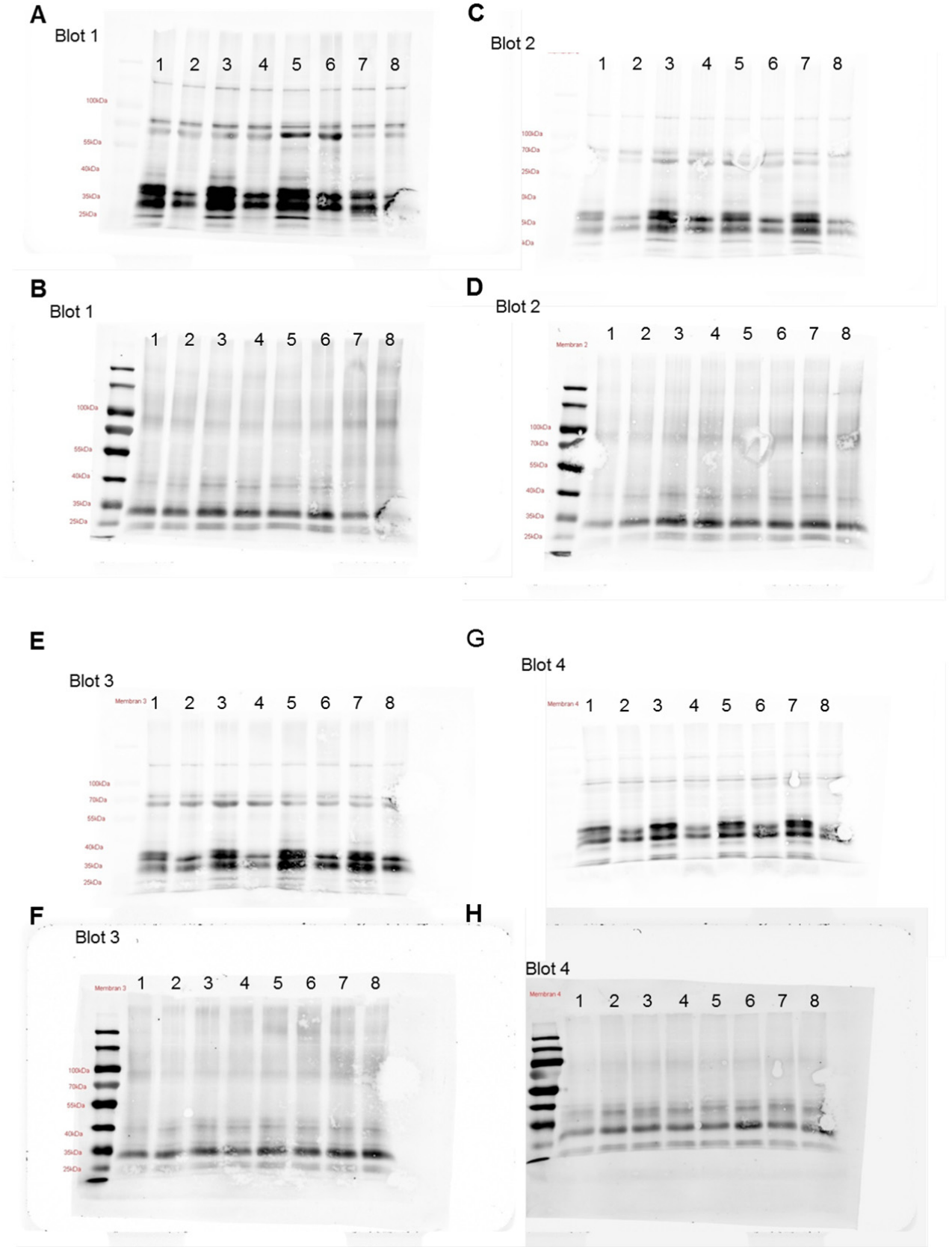


2

1 Original blots from Fig. 5E. **A:** Blot 1, active site labeling: lane 1,  $Ctsb^{-/-}$ ; lane 2,  $Ctsb^{-/-}$  + E-64d;  
2 lane 3,  $Ctsz^{-/-}$ ; lane 4,  $Ctsz^{-/-}$  + E-64d; lane 5, wild-type; lane 6, wild-type + E-64d; lane 7, wild-  
3 type; lane 8, wild-type + E-64d. **B:** Blot 1, cathepsin Z Western blot (shown in Fig. 5E): lane 1,  
4  $Ctsb^{-/-}$ ; lane 2,  $Ctsb^{-/-}$  + E-64d; lane 3,  $Ctsz^{-/-}$ ; lane 4,  $Ctsz^{-/-}$  + E-64d; lane 5, wild-type; lane 6,  
5 wild-type + E-64d; lane 7, wild-type; lane 8, wild-type + E-64d. **C:** Blot 2, active site labeling:  
6 lane 1,  $Ctsb^{-/-}$ ; lane 2,  $Ctsb^{-/-}$  + E-64d; lane 3,  $Ctsz^{-/-}$ ; lane 4,  $Ctsz^{-/-}$  + E-64d; lane 5, wild-type;  
7 lane 6, wild-type + E-64d; lane 7,  $Ctsb^{-/-}$ ; lane 8,  $Ctsb^{-/-}$  + E-64d. **D:** Blot 2, cathepsin Z  
8 Western blot: lane 1,  $Ctsb^{-/-}$ ; lane 2,  $Ctsb^{-/-}$  + E-64d; lane 3,  $Ctsz^{-/-}$ ; lane 4,  $Ctsz^{-/-}$  + E-64d; lane  
9 5, wild-type; lane 6, wild-type + E-64d; lane 7,  $Ctsb^{-/-}$ ; lane 8,  $Ctsb^{-/-}$  + E-64d. **E:** Blot 3, active  
10 site labeling: lane 1,  $Ctsb^{-/-}$ ; lane 2,  $Ctsb^{-/-}$  + E-64d; lane 3,  $Ctsz^{-/-}$ ; lane 4,  $Ctsz^{-/-}$  + E-64d; lane  
11 5, wild-type; lane 6, wild-type + E-64d. **F:** Blot 3, cathepsin Z Western blot: lane 1,  $Ctsb^{-/-}$ ;  
12 lane 2,  $Ctsb^{-/-}$  + E-64d; lane 3,  $Ctsz^{-/-}$ ; lane 4,  $Ctsz^{-/-}$  + E-64d; lane 5, wild-type; lane 6, wild-  
13 type + E-64d.  
14



1 **Supplementary Fig. 10**



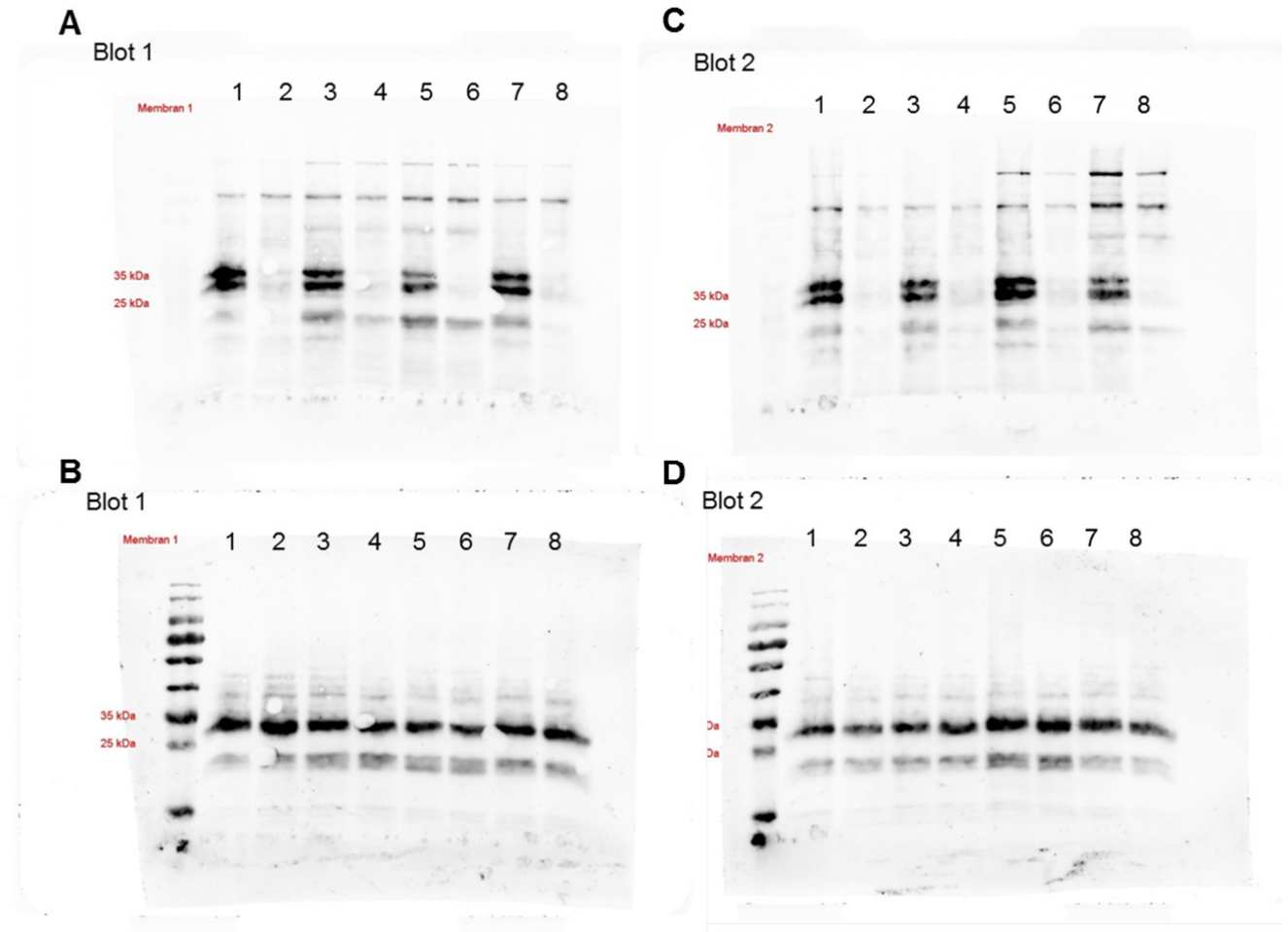
2

1

2 Original blots from Supplementary Fig. 2C. **A:** Blot 1, active site labeling: lane 1, sham  
3 treatment; lane 2, sham treatment + E-64d; lane 3, inhibitor 17; lane 4, inhibitor 17 + E-64d;  
4 lane 5, sham treatment; lane 6, sham treatment + E-64d; lane 7, inhibitor 17; lane 8,  
5 inhibitor 17 + E-64d. **B:** Blot 1, cathepsin B Western blot: lane 1, sham treatment; lane 2,  
6 sham treatment + E-64d; lane 3, inhibitor 17; lane 4, inhibitor 17 + E-64d; lane 5, sham  
7 treatment; lane 6, sham treatment + E-64d; lane 7, inhibitor 17; lane 8, inhibitor 17+ E-64d.  
8 **C:** Blot 2 active site labeling: lane 1, sham treatment; lane 2, sham treatment + E-64d; lane 3,  
9 inhibitor 17; lane 4, inhibitor 17 + E-64d; lane 5, sham treatment; lane 6, sham treatment +  
10 E-64d; lane 7, inhibitor 17; lane 8, inhibitor 17 + E-64d. **D:** Blot 2, cathepsin B Western blot:  
11 lane 1, sham treatment; lane 2, sham treatment + E-64d; lane 3, inhibitor 17; lane 4,  
12 inhibitor 17 + E-64d; lane 5, sham treatment; lane 6, sham treatment + E-64d; lane 7,  
13 inhibitor 17; lane 8, inhibitor 17 + E-64d. **E:** Blot 3, active site labeling: lane 1, sham  
14 treatment; lane 2, sham treatment + E-64d; lane 3, inhibitor 17; lane 4, inhibitor 17 + E-64d;  
15 lane 5, sham treatment; lane 6, sham treatment + E-64d; lane 7, inhibitor 17; lane 8,  
16 inhibitor 17 + E-64d. **F:** Blot 3, cathepsin Z Western blot, cathepsin B Western blot: lane 1,  
17 sham treatment; lane 2, sham treatment + E-64d; lane 3, inhibitor 17; lane 4, inhibitor 17 +  
18 E-64d; lane 5, sham treatment; lane 6, sham treatment + E-64d; lane 7, inhibitor 17; lane 8,  
19 inhibitor 17 + E-64d. **G:** Blot 3, active site labeling: lane 1, sham treatment; lane 2, sham  
20 treatment + E-64d; lane 3, inhibitor 17; lane 4, inhibitor 17 + E-64d; lane 5, sham treatment;  
21 lane 6, sham treatment + E-64d; lane 7, inhibitor 17; lane 8, inhibitor 17 + E-64d. **H:** Blot 3,  
22 cathepsin Z Western blot, cathepsin B Western blot: lane 1, sham treatment; lane 2, sham  
23 treatment + E-64d; lane 3, inhibitor 17; lane 4, inhibitor 17 + E-64d; lane 5, sham treatment;  
24 lane 6, sham treatment + E-64d; lane 7, inhibitor 17; lane 8, inhibitor 17 + E-64d.

25

1 **Supplementary Fig. 11**

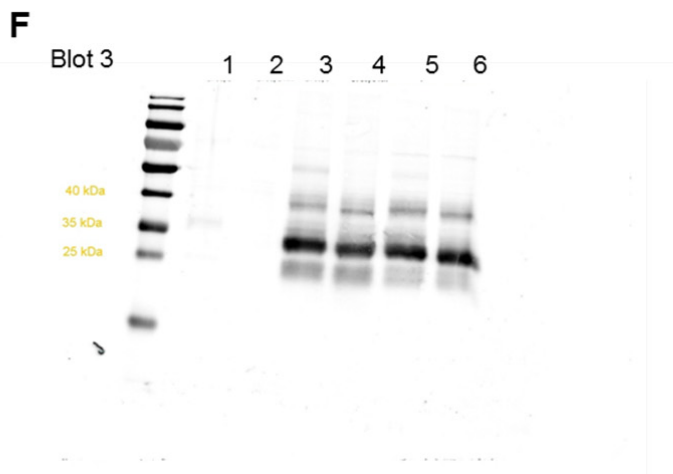
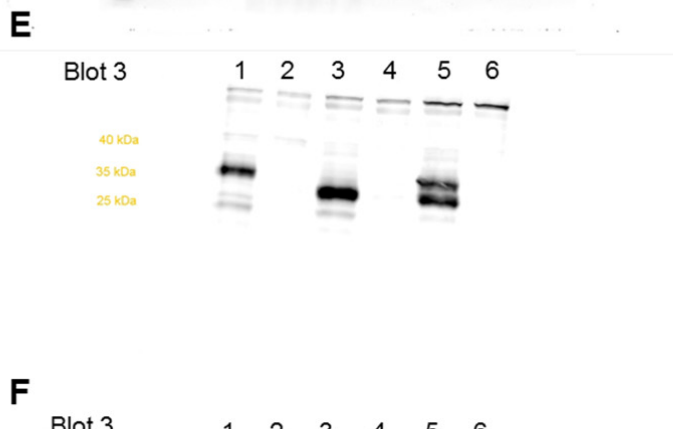
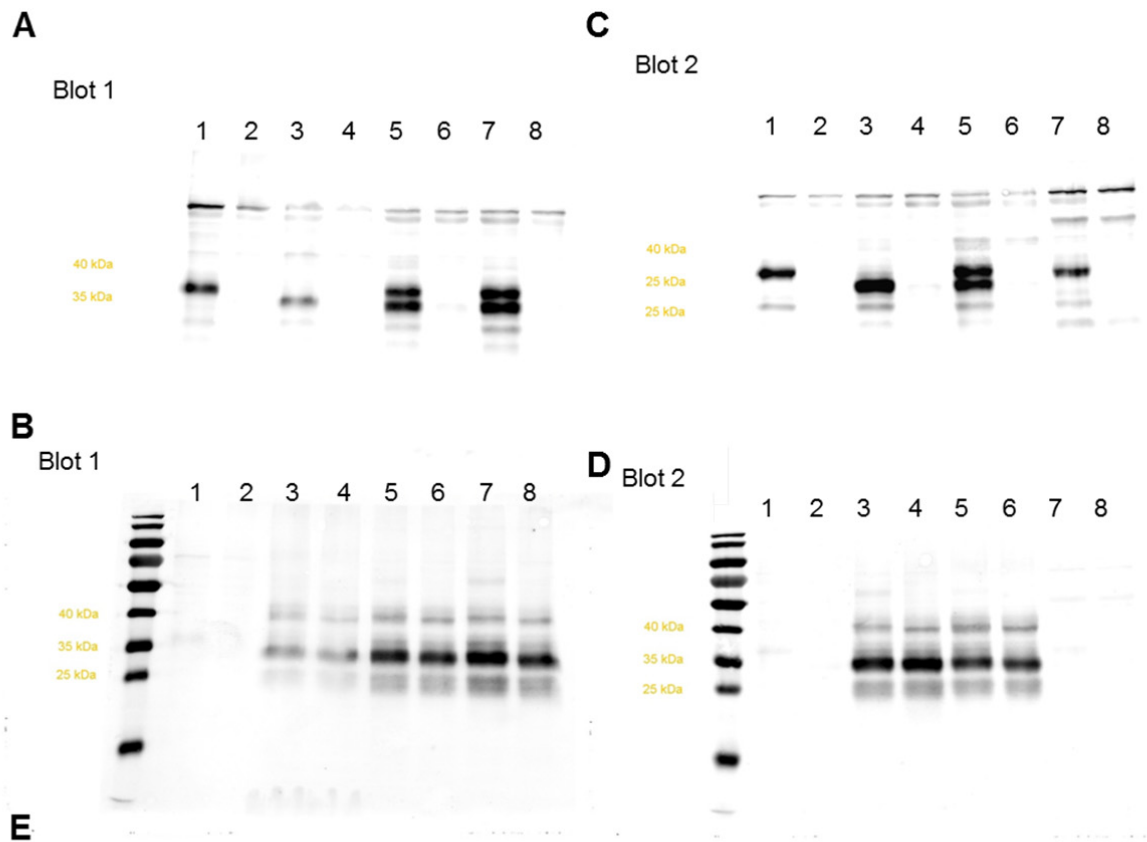


2

3 Original blots from Supplementary Fig. 2D. **A:** Blot 1, active site labeling: lane 1, sham  
4 treatment; lane 2, sham treatment + E-64d; lane 3, inhibitor 17; lane 4, inhibitor 17 + E-64d;  
5 lane 5, sham treatment; lane 6, sham treatment + E-64d; lane 7, inhibitor 17, lane 8, inhibitor  
6 17 + E-64d. **B:** Blot 1, cathepsin B Western blot: lane 1, sham treatment; lane 2, sham  
7 treatment + E-64d; lane 3, inhibitor 17; lane 4, inhibitor 17 + E-64d; lane 5, sham treatment;  
8 lane 6, sham treatment + E-64d; lane 7, inhibitor 17; lane 8, inhibitor 17+ E-64d. **C:** Blot 2,  
9 active site labeling: lane 1, sham treatment; lane 2, sham treatment + E-64d; lane 3, inhibitor  
10 17; lane 4, inhibitor 17 + E-64d; lane 5, sham treatment; lane 6, sham treatment + E-64d;  
11 lane 7, inhibitor 17; lane 8, inhibitor 17 + E-64d. **D:** Blot 2, cathepsin B Western blot: lane 1,

- 1 sham treatment; lane 2, sham treatment + E-64d; lane 3, inhibitor 17; lane 4, inhibitor 17 +
- 2 E-64d; lane 5, sham treatment; lane 6, sham treatment + E-64d; lane 7, inhibitor 17; lane 8,
- 3 inhibitor 17 + E-64d.
- 4

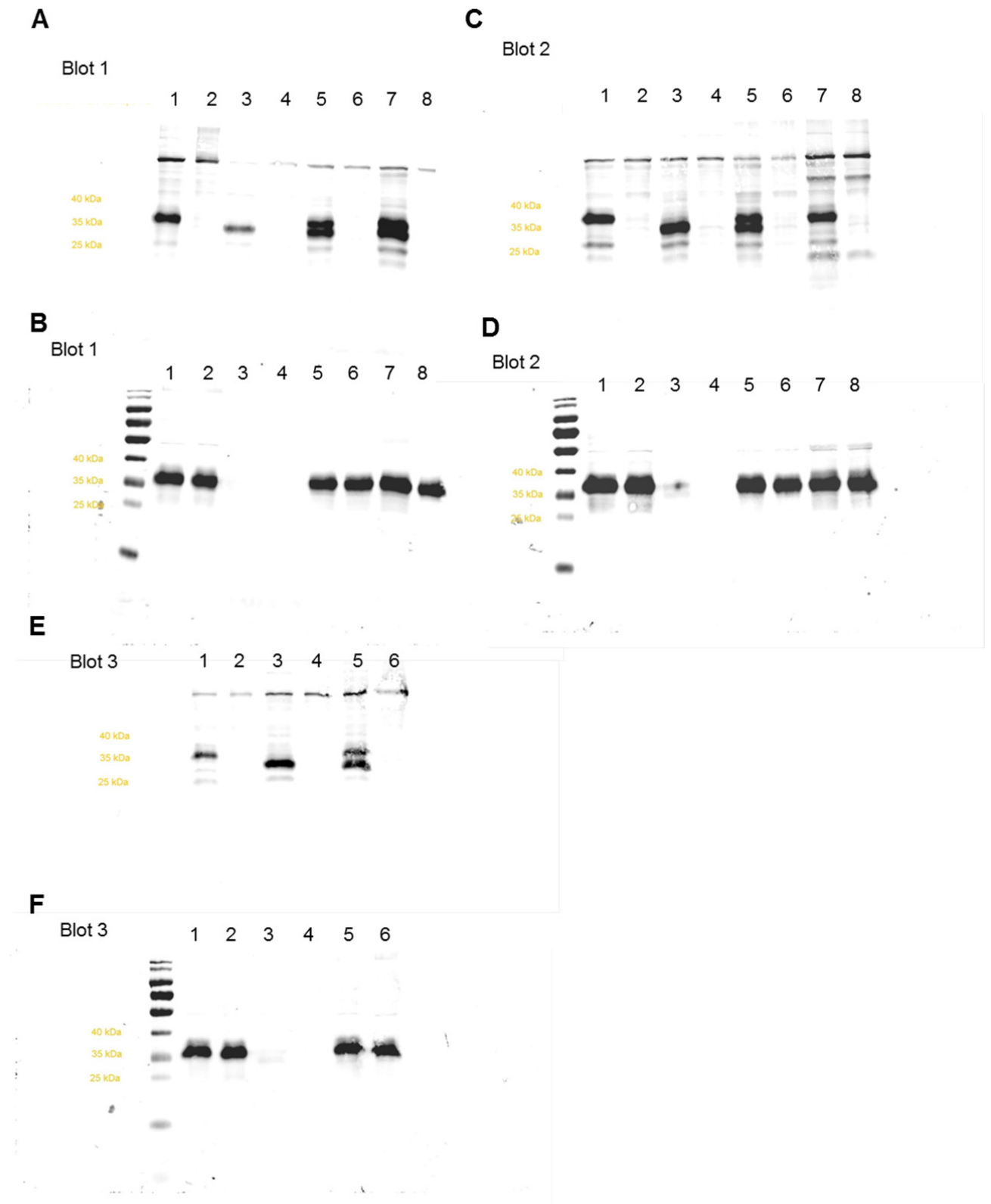
1 **Supplementary Fig. 12**



2

1 Original blots from Supplementary Fig. 3C/D. **A:** Blot 1, active site labeling (shown in  
2 Supplementary Fig. 3C): lane 1, *Ctsb*<sup>-/-</sup>; lane 2, *Ctsb*<sup>-/-</sup> + E-64d; lane 3, *Ctsz*<sup>-/-</sup>; lane 4, *Ctsz*<sup>-/-</sup> + E-  
3 64d; lane 5, wild-type; lane 6, wild-type + E-64d; lane 7, wild-type; lane 8, wild-type + E-64d.  
4 **B:** Blot 1, cathepsin B Western blot (shown in Supplementary Fig. 3D): lane 1, *Ctsb*<sup>-/-</sup>; lane 2,  
5 *Ctsb*<sup>-/-</sup> + E-64d; lane 3, *Ctsz*<sup>-/-</sup>; lane 4, *Ctsz*<sup>-/-</sup> + E-64d; lane 5, wild-type; lane 6, wild-type + E-  
6 64d; lane 7, wild-type; lane 8, wild-type + E-64d. **C:** Membrane 2, active site labeling: lane 1,  
7 *Ctsb*<sup>-/-</sup>; lane 2, *Ctsb*<sup>-/-</sup> + E-64d; lane 3, *Ctsz*<sup>-/-</sup>; lane 4, *Ctsz*<sup>-/-</sup> + E-64d; lane 5, wild-type; lane 6,  
8 wild-type + E-64d; lane 7, *Ctsb*<sup>-/-</sup>; lane 8, *Ctsb*<sup>-/-</sup> + E-64d. **D:** Blot 2, cathepsin B Western blot:  
9 lane 1, *Ctsb*<sup>-/-</sup>; lane 2, *Ctsb*<sup>-/-</sup> + E-64d; lane 3, *Ctsz*<sup>-/-</sup>; lane 4, *Ctsz*<sup>-/-</sup> + E-64d; lane 5, wild-type;  
10 lane 6, wild-type + E-64d; lane 7, *Ctsb*<sup>-/-</sup>; lane 8, *Ctsb*<sup>-/-</sup> + E-64d. **E:** Blot 3, active site labeling:  
11 lane 1, *Ctsb*<sup>-/-</sup>; lane 2, *Ctsb*<sup>-/-</sup> + E-64d; lane 3, *Ctsz*<sup>-/-</sup>; lane 4, *Ctsz*<sup>-/-</sup> + E-64d; lane 5, wild-type;  
12 lane 6, wild-type + E-64d. **F:** Blot 3, cathepsin B Western blot: lane 1, *Ctsb*<sup>-/-</sup>; lane 2, *Ctsb*<sup>-/-</sup> +  
13 E-64d; lane 3, *Ctsz*<sup>-/-</sup>; lane 4, *Ctsz*<sup>-/-</sup> + E-64d; lane 5, wild-type; lane 6, wild-type + E-64d.  
14

1 **Supplementary Fig. 13**



2

1 Original blots from Supplementary Fig. 3E. **A:** Blot 1, active site labeling: lane 1, *Ctsb*<sup>-/-</sup>; lane  
2 2, *Ctsb*<sup>-/-</sup> + E-64d; lane 3, *Ctsz*<sup>-/-</sup>; lane 4, *Ctsz*<sup>-/-</sup> + E-64d; lane 5, wild-type; lane 6, wild-type + E-  
3 64d; lane 7, wild-type; lane 8, wild-type + E-64d. **B:** Blot 1, cathepsin Z Western blot (shown  
4 in Fig. 5E): lane 1, *Ctsb*<sup>-/-</sup>; lane 2, *Ctsb*<sup>-/-</sup> + E-64d; lane 3, *Ctsz*<sup>-/-</sup>; lane 4, *Ctsz*<sup>-/-</sup> + E-64d; lane 5,  
5 wild-type; lane 6, wild-type + E-64d; lane 7, wild-type; lane 8, wild-type + E-64d. **C:** Blot 2,  
6 active site labeling: lane 1, *Ctsb*<sup>-/-</sup>; lane 2, *Ctsb*<sup>-/-</sup> + E-64d; lane 3, *Ctsz*<sup>-/-</sup>; lane 4, *Ctsz*<sup>-/-</sup> + E-64d;  
7 lane 5, wild-type; lane 6, wild-type + E-64d; lane 7, *Ctsb*<sup>-/-</sup>; lane 8, *Ctsb*<sup>-/-</sup> + E-64d. **D:** Blot 2,  
8 cathepsin Z Western blot: lane 1, *Ctsb*<sup>-/-</sup>; lane 2, *Ctsb*<sup>-/-</sup> + E-64d; lane 3, *Ctsz*<sup>-/-</sup>; lane 4, *Ctsz*<sup>-/-</sup> +  
9 E-64d; lane 5, wild-type; lane 6, wild-type + E-64d; lane 7, *Ctsb*<sup>-/-</sup>; lane 8, *Ctsb*<sup>-/-</sup> + E-64d. **E:**  
10 Blot 3, active site labeling: lane 1, *Ctsb*<sup>-/-</sup>; lane 2, *Ctsb*<sup>-/-</sup> + E-64d; lane 3, *Ctsz*<sup>-/-</sup>; lane 4, *Ctsz*<sup>-/-</sup> +  
11 E-64d; lane 5, wild-type; lane 6, wild-type + E-64d. **F:** Blot 3, cathepsin Z Western blot: lane 1,  
12 *Ctsb*<sup>-/-</sup>; lane 2, *Ctsb*<sup>-/-</sup> + E-64d; lane 3, *Ctsz*<sup>-/-</sup>; lane 4, *Ctsz*<sup>-/-</sup> + E-64d; lane 5, wild-type; lane 6,  
13 wild-type + E-64d.

14

15