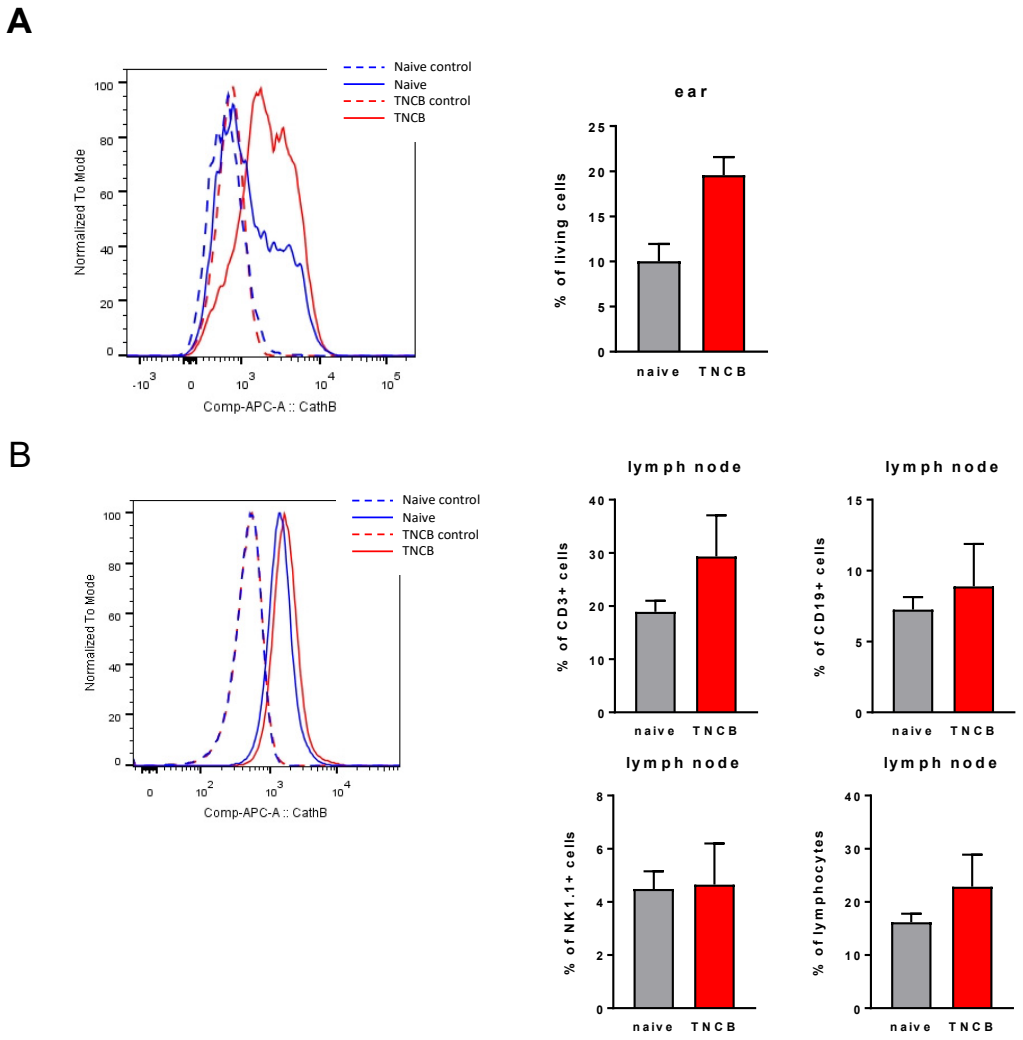
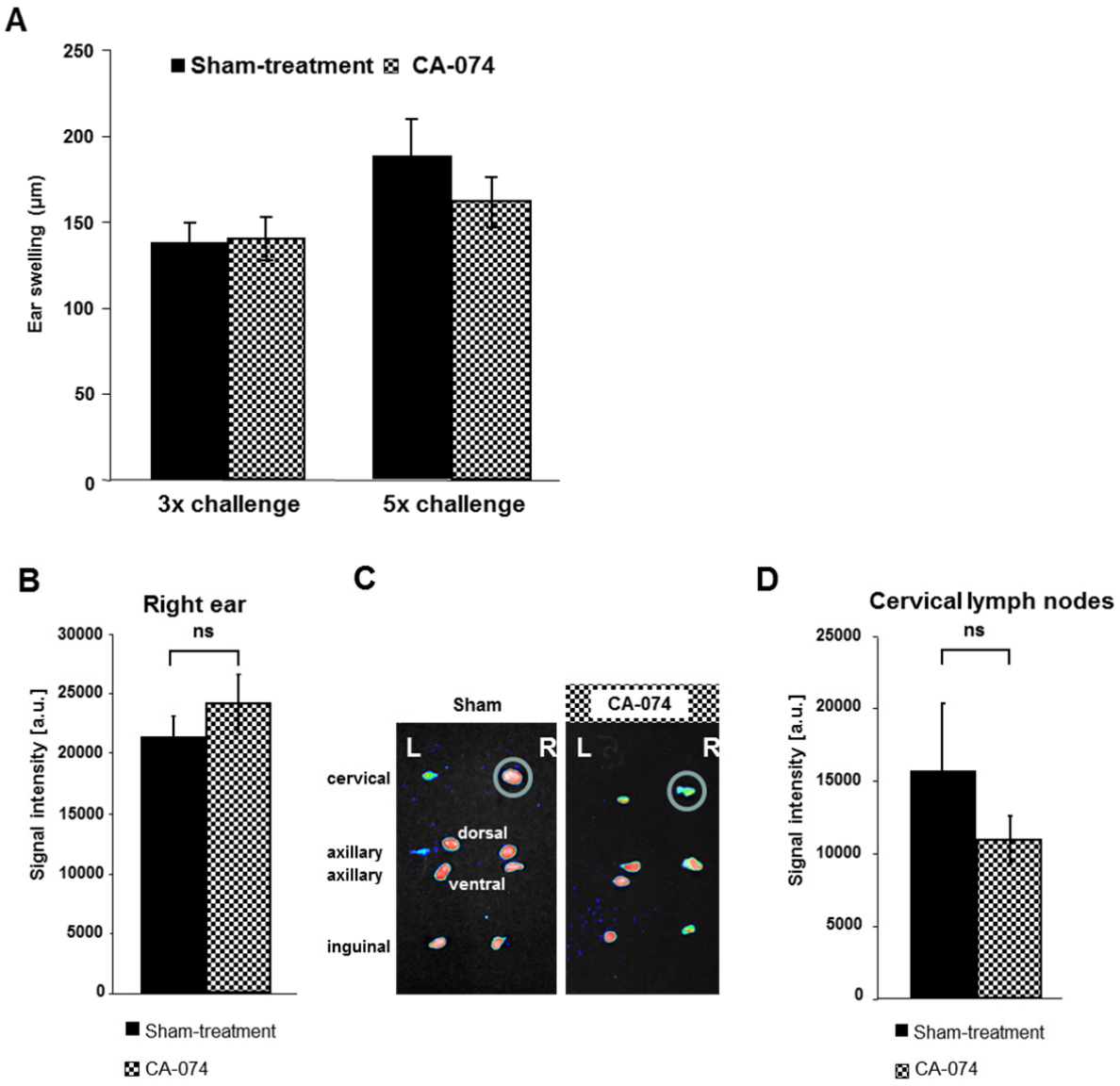
Supplementary Figures

Supplementary Fig. 1



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

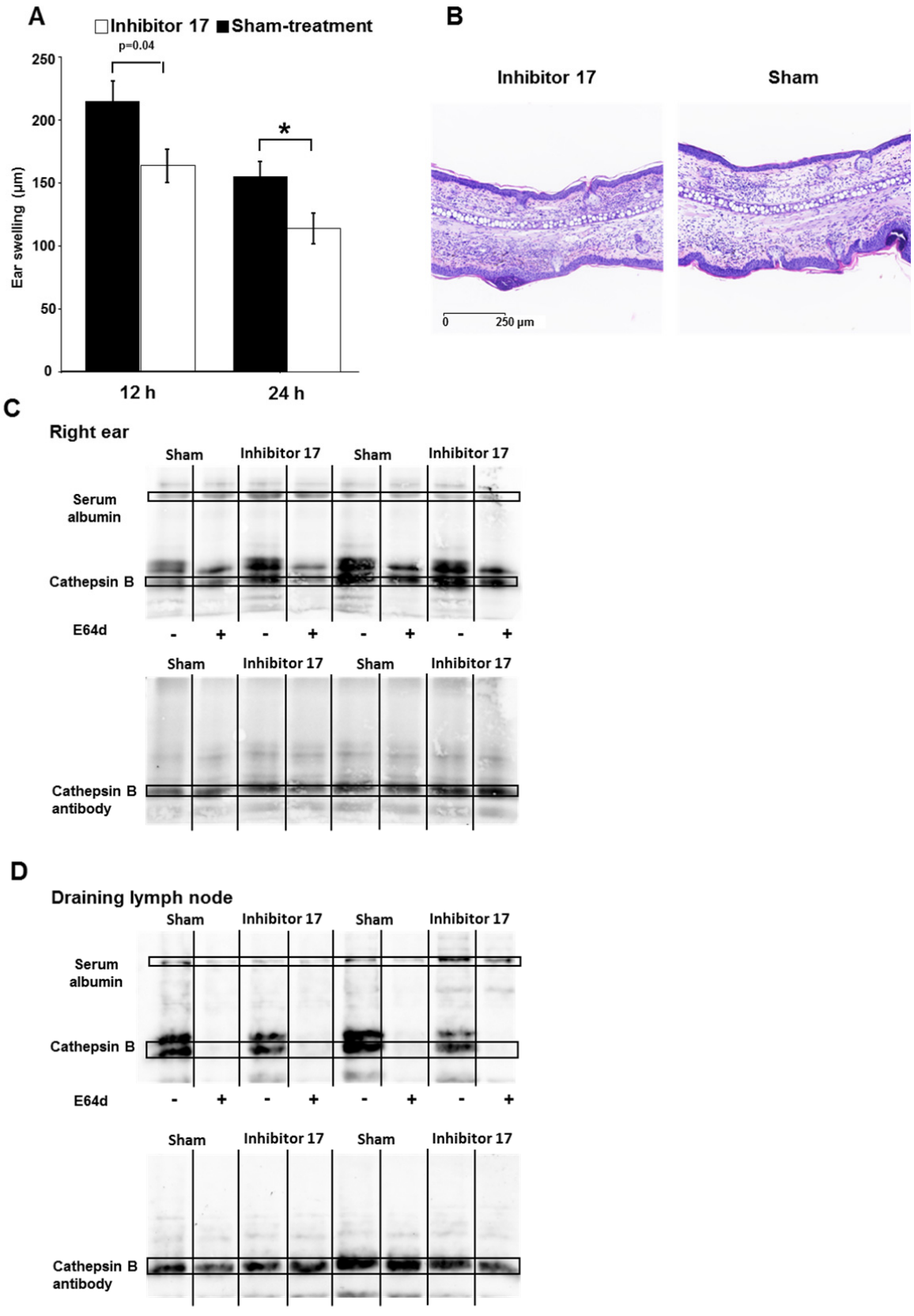
Supplementary Fig. 2



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

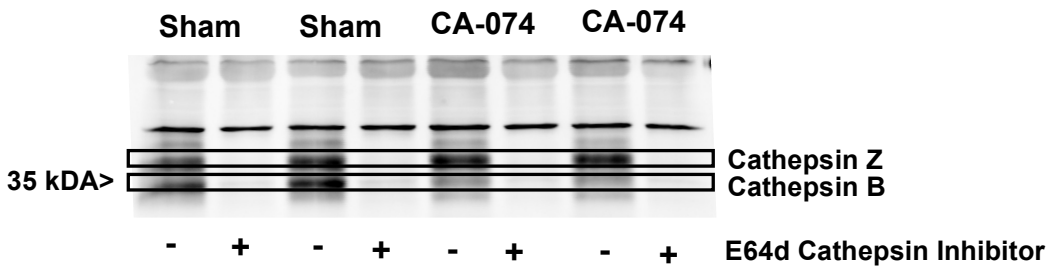
draining lymph nodes was slightly lower in CA-074-treated mice than in sham-treated mice (n=4).

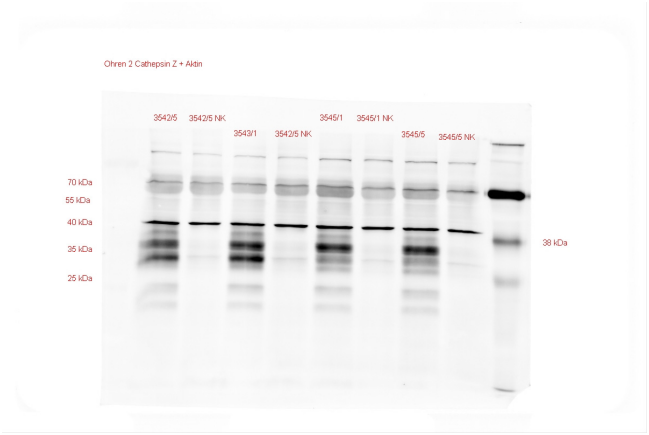
Supplementary Fig. 3



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

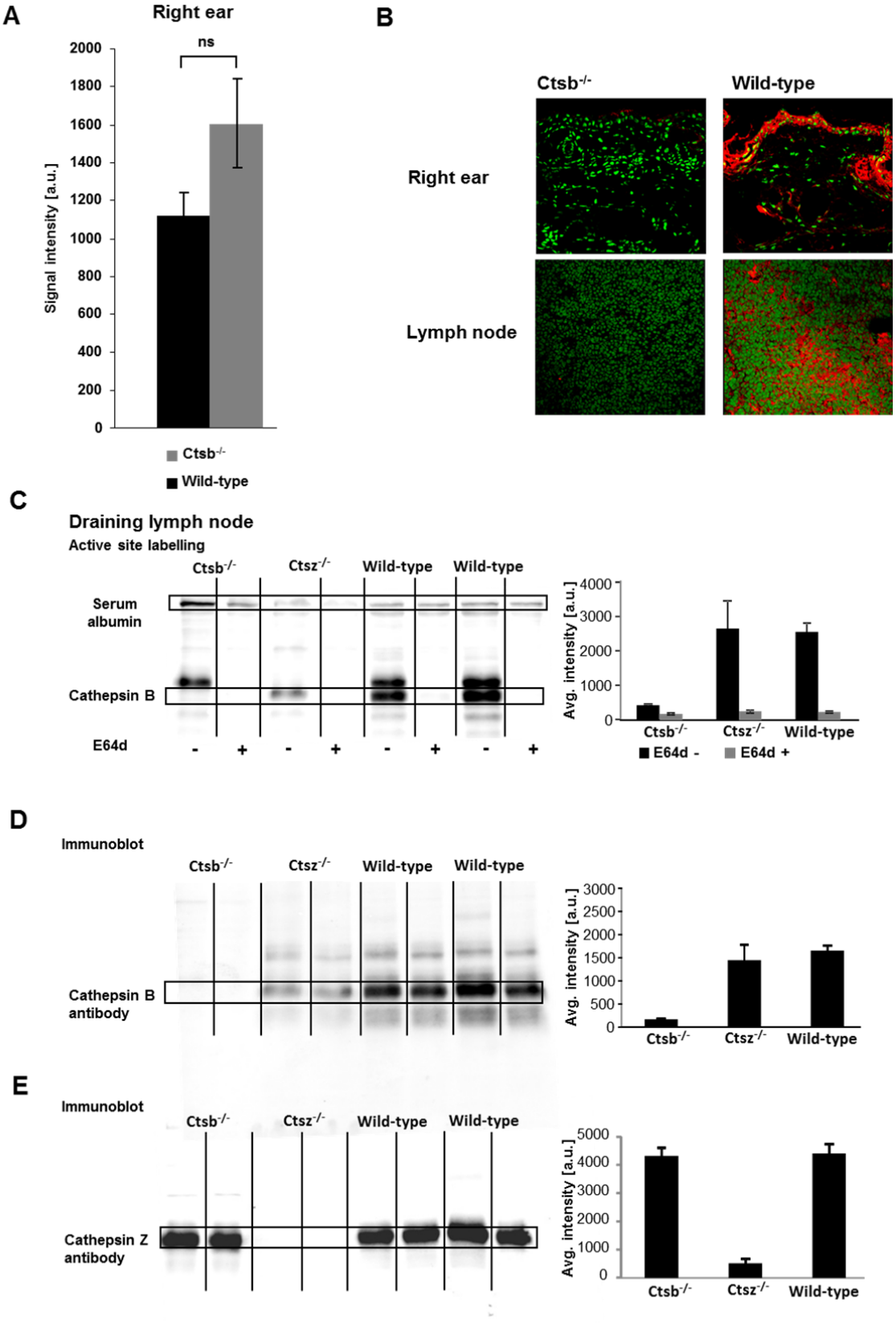
Supplementary Fig. 4





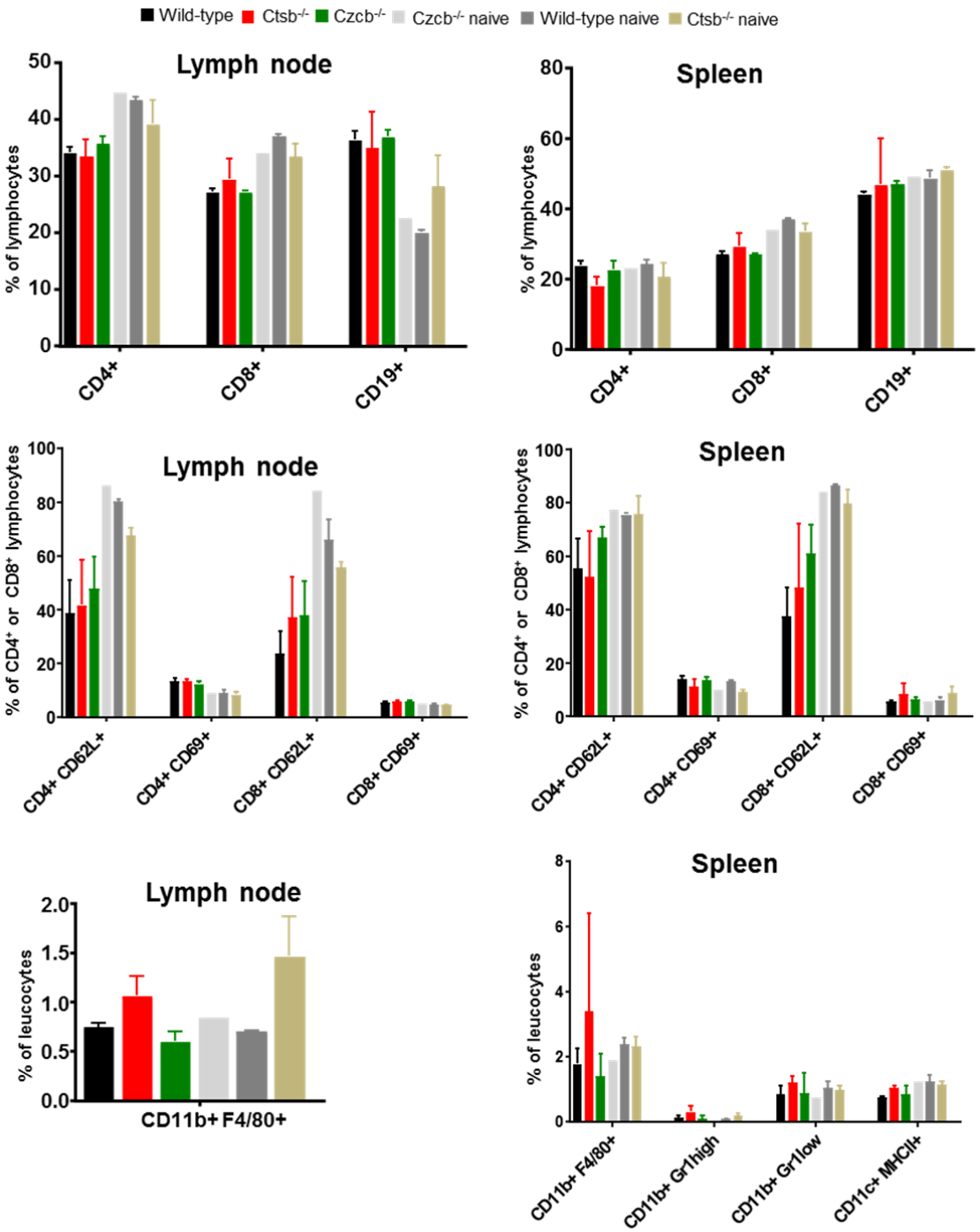
Active site labeling and western blot analysis of ear tissue derived from CA-074 (n=4) and sham-treated mice (n=4) 24 h after TNCB ear challenge revealed an impressively reduced expression of cathepsin B in inflamed ears of CA-074 treated mice when compared to inflamed ears derived from sham-treated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

Supplementary Fig. 5



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

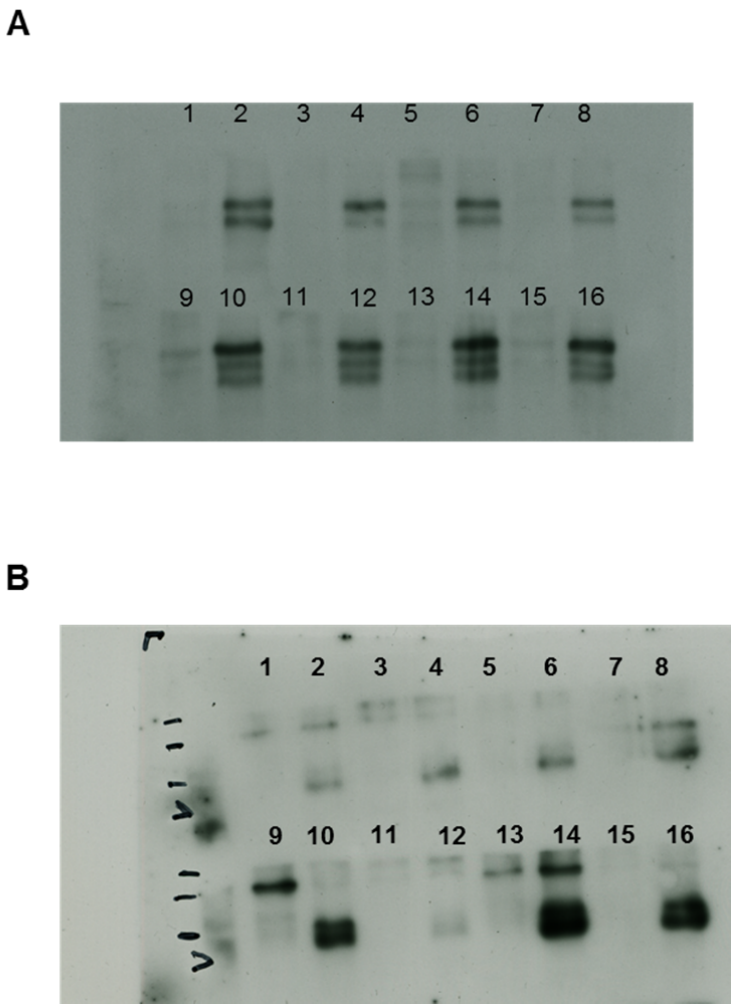
Supplementary Fig. 6



We performed flow cytometric analysis of inguinal and axillary lymph nodes (TNCB sensitization on the abdomen) and spleen tissue derived from naïve and TNCB-sensitized and challenged wild-type, Ctsb$^{-/-}$, and Ctsz$^{-/-}$ mice. Neither Ctsb$^{-/-}$ nor Ctsz$^{-/-}$ 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

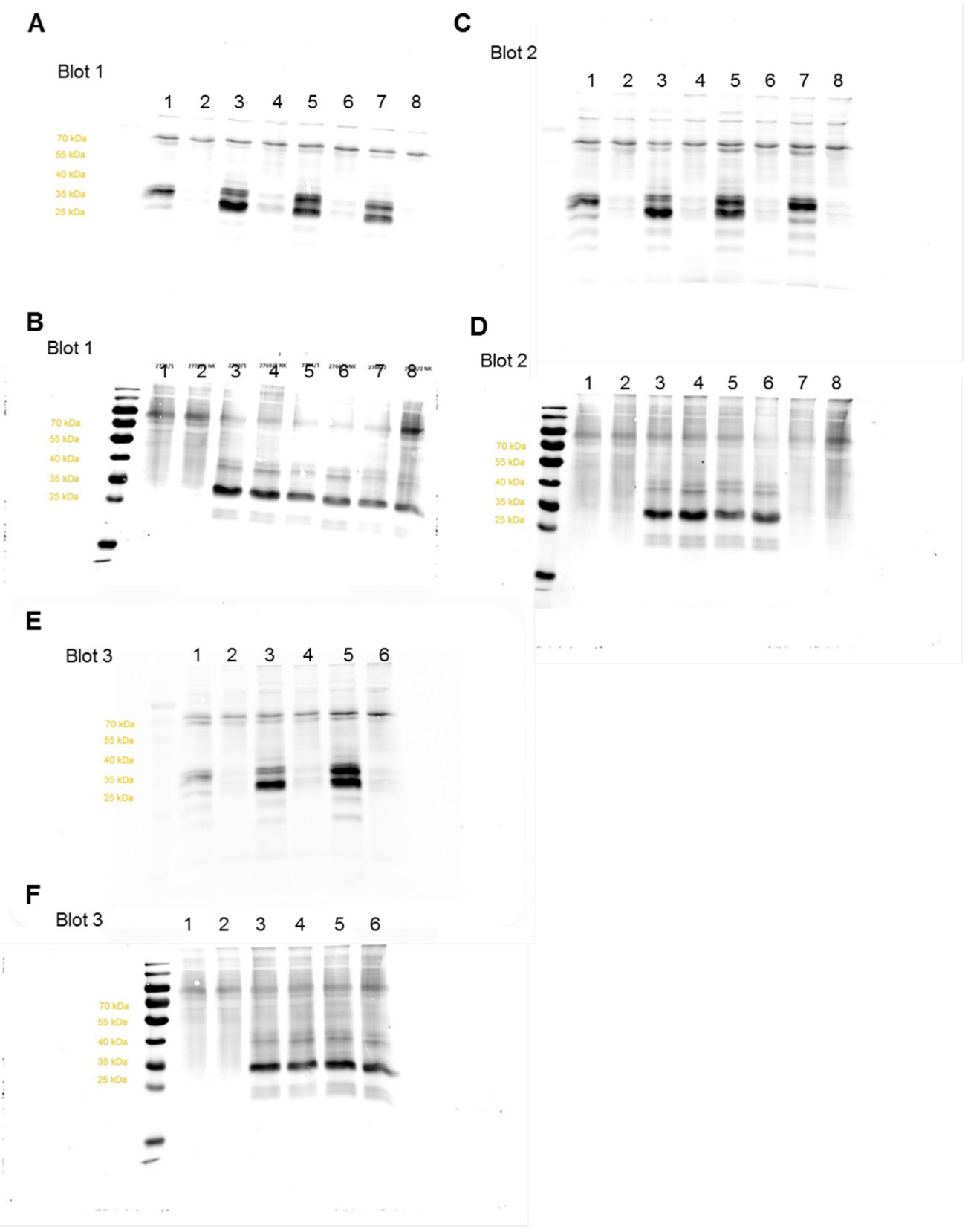
Supplementary Fig. 7



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

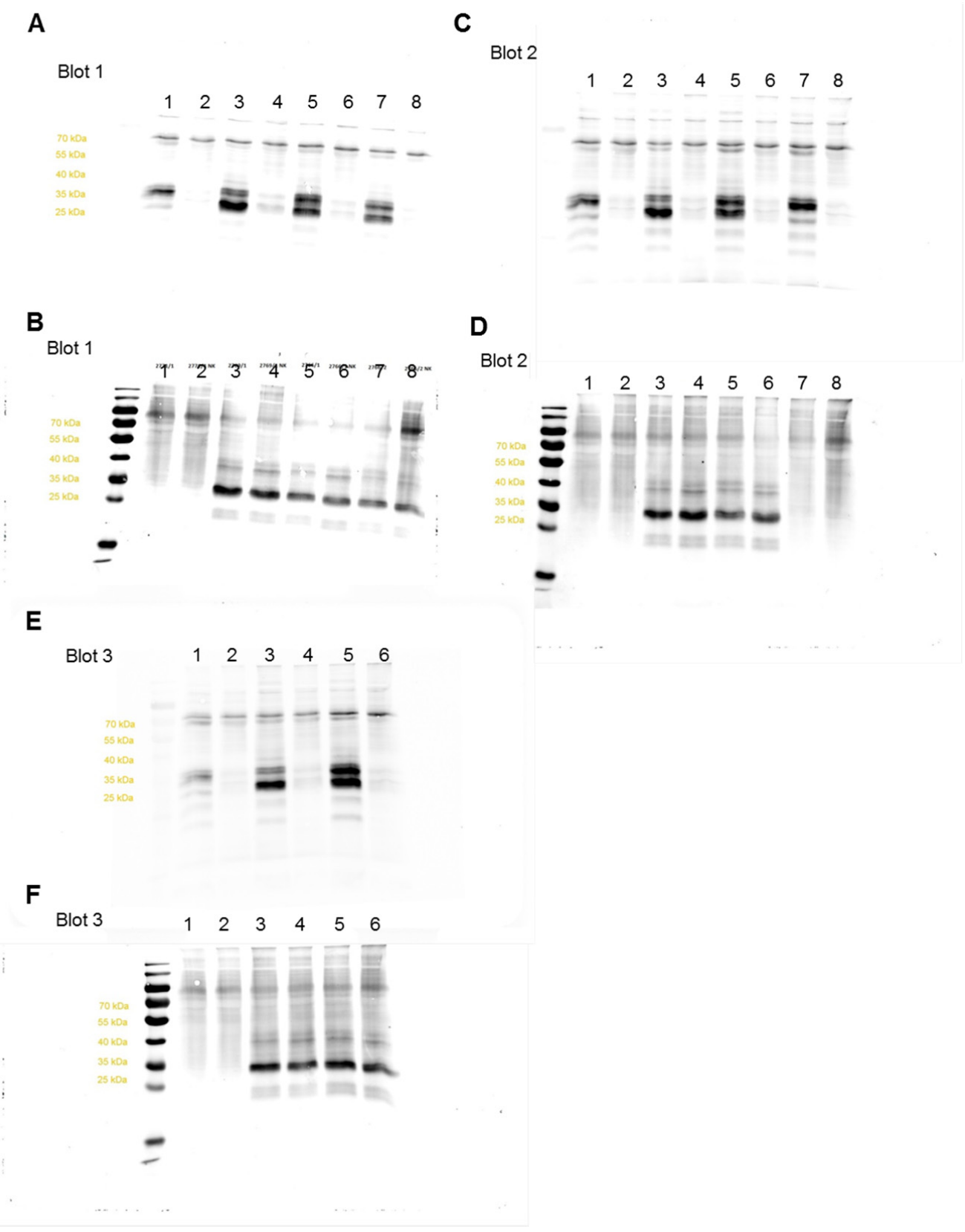
64d; lane 8, CA-074 treatment; lane 9, sham treatment + E-64d; lane 10, sham treatment; lane 11, sham treatment + E-64d (no proteases detectable, data not shown in Fig. 4D); lane 12, sham treatment (no proteases detectable, data not shown in Fig. 4D); lane 13, sham treatment + E-64d; lane 14, sham treatment;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

Supplementary Fig. 8



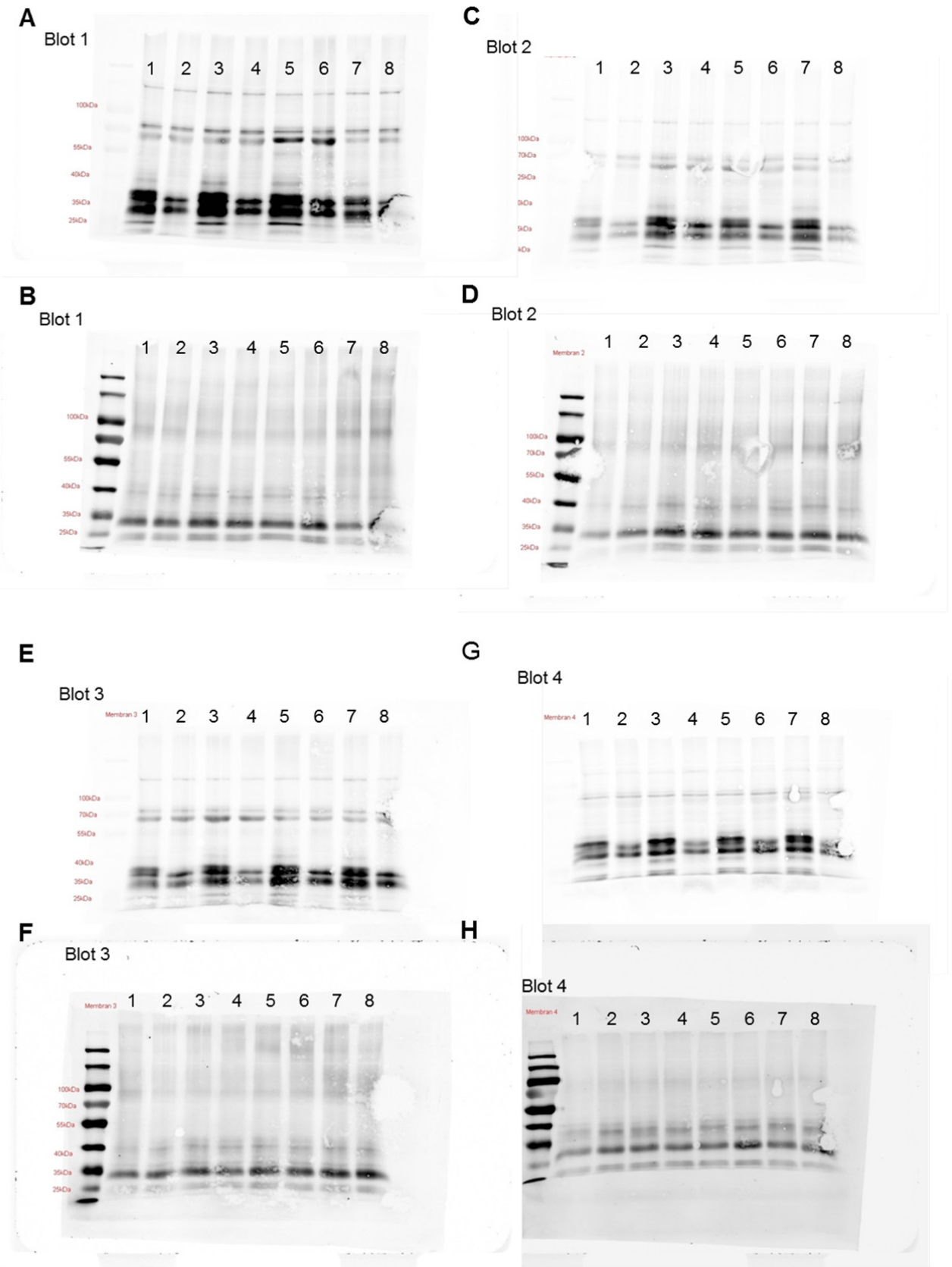
Original blots from Fig. 5 C/D. **A:** Blot 1, active site labeling (shown in Fig. 5C): lane 1, $Ctsb^{-/-}$; lane 2, $Ctsb^{-/-}$ + E-64d; lane 3, $Ctsz^{-/-}$; lane 4, $Ctsz^{-/-}$ + E-64d; lane 5, wild-type; lane 6, wild-type + E-64d; lane 7, wild-type; lane 8, wild-type + E-64d. **B:** Blot 1, cathepsin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

Supplementary Fig. 9



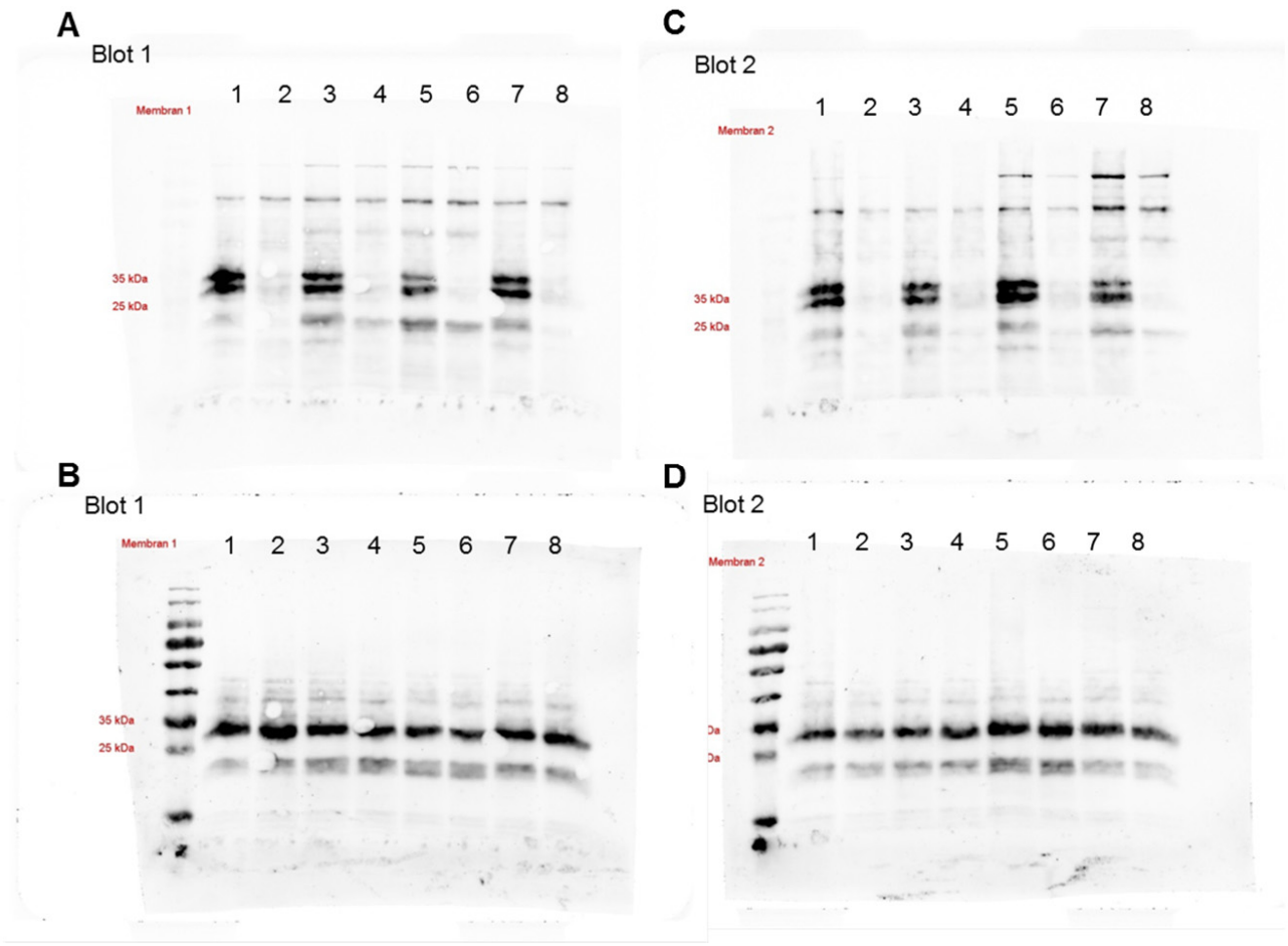
Original blots from Fig. 5E. **A:** Blot 1, active site labeling: lane 1, $Ctsb^{-/-}$; lane 2, $Ctsb^{-/-}$ + E-64d; lane 3, $Ctsz^{-/-}$; lane 4, $Ctsz^{-/-}$ + E-64d; lane 5, wild-type; lane 6, wild-type + E-64d; lane 7, wild-type; lane 8, wild-type + E-64d. **B:** Blot 1, cathepsin Z Western blot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

Supplementary Fig. 10



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

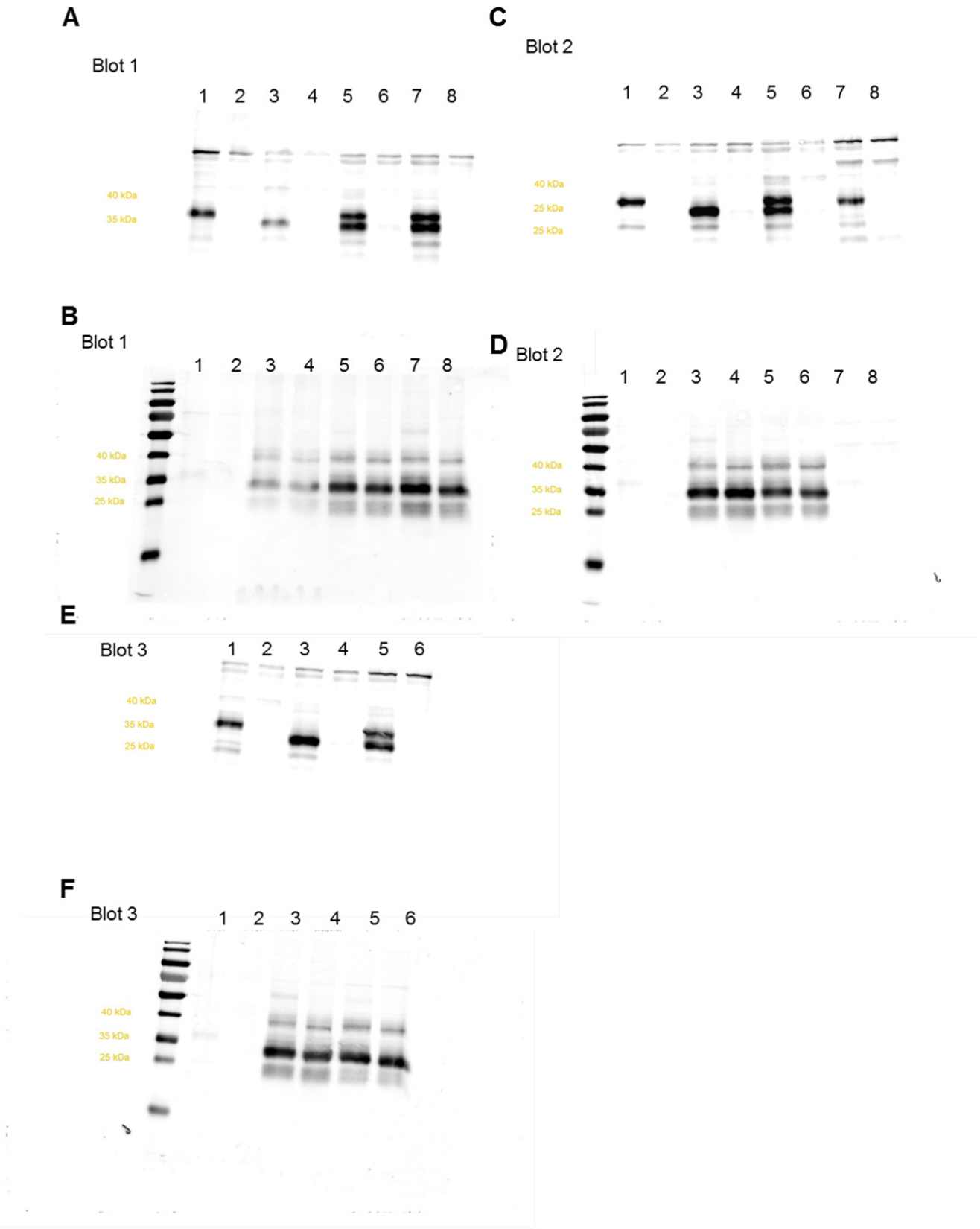
Supplementary Fig. 11



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

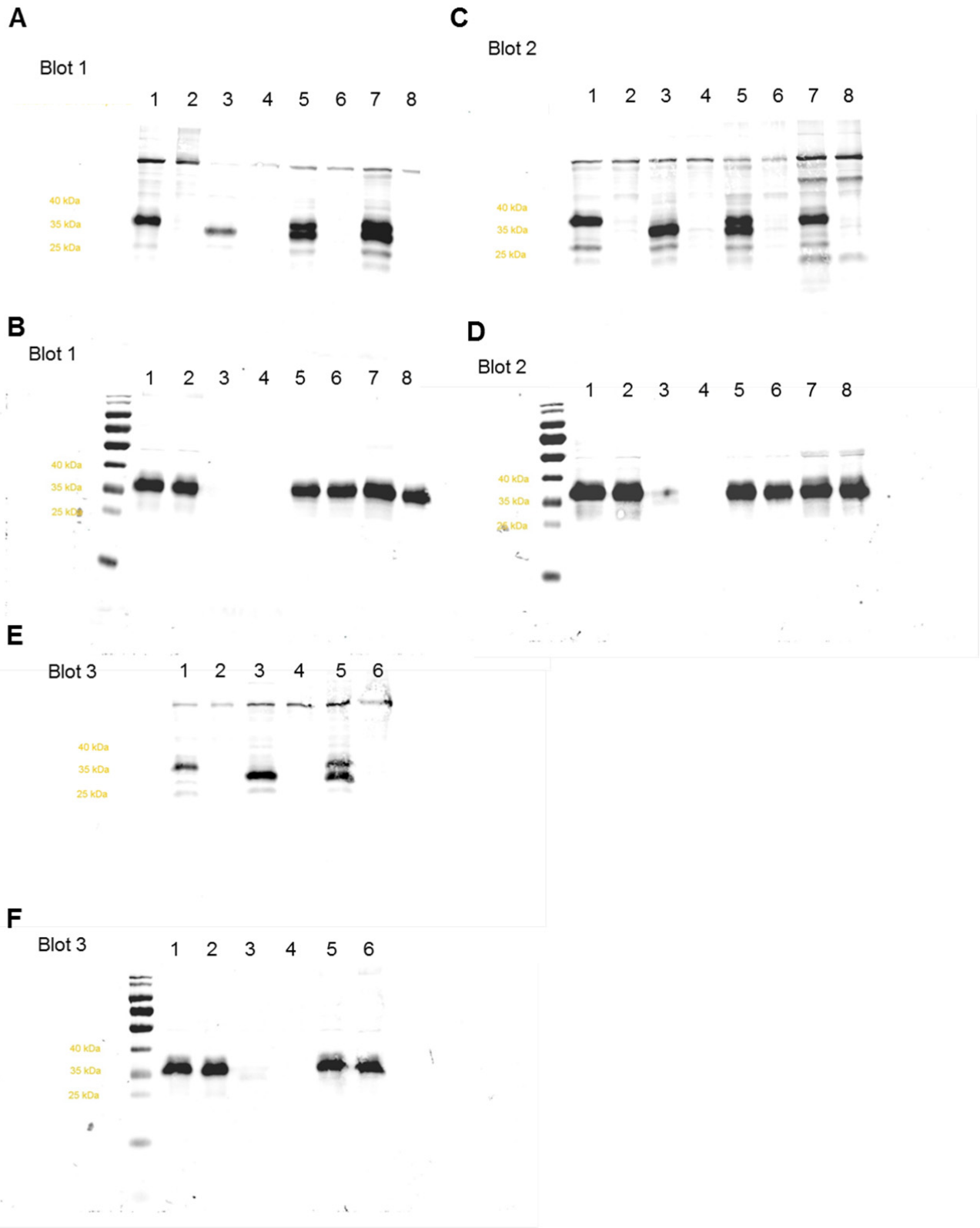
sham treatment; lane 2, sham treatment + E-64d; lane 3, inhibitor 17; lane 4, inhibitor 17 + E-64d; lane 5, sham treatment; lane 6, sham treatment + E-64d; lane 7, inhibitor 17; lane 8, inhibitor 17 + E-64d.

Supplementary Fig. 12



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

Supplementary Fig. 13



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