CD13 is a Critical Regulator of Cell-cell Fusion in Osteoclastogenesis

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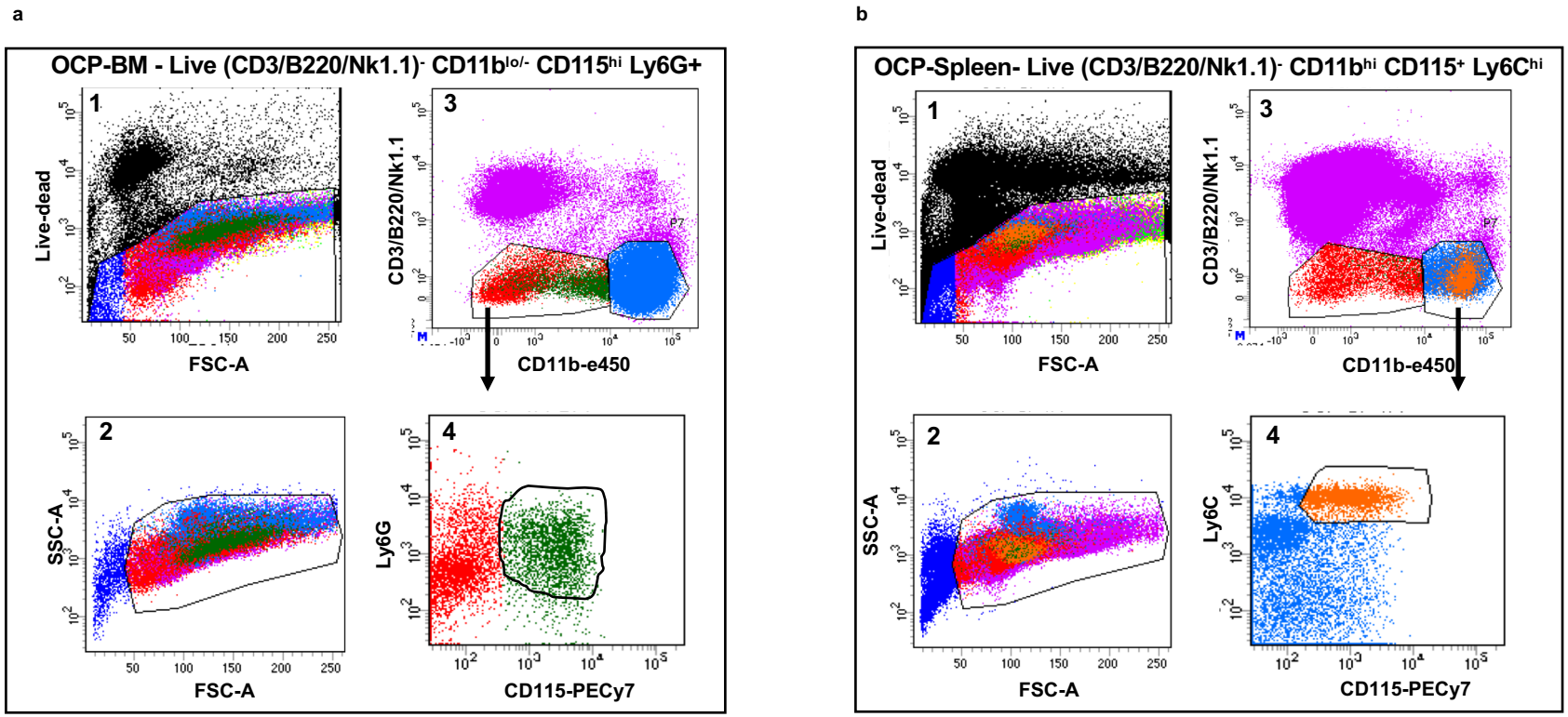

FIG S1. Representative image of flow sorting strategy of isolation of osteoclast progenitors from primary murine bone marrow or spleen from WT and CD13^{KO} mice. Live (CD3/B220/Nk1.1)- CD11b$^{lo/-}$ CD115^{hi} Ly6G^{+} OCP cells were sorted from BM (**a**) or Live (CD3/B220/Nk1.1)- CD11b^{hi} CD115^{+} Ly6C^{hi} OCP cells were sorted from spleen (**b**) to homogeneity using BD FACS ARIA and analyzed by FACS Diva version 9 (https://www.bdbiosciences.com/en-us/instruments/research-instruments/research-software/flow-cytometry-acquisition/facsdiva-software) and FlowJo (https://www.flowjo.com/) version 9.9.

FIG. S1

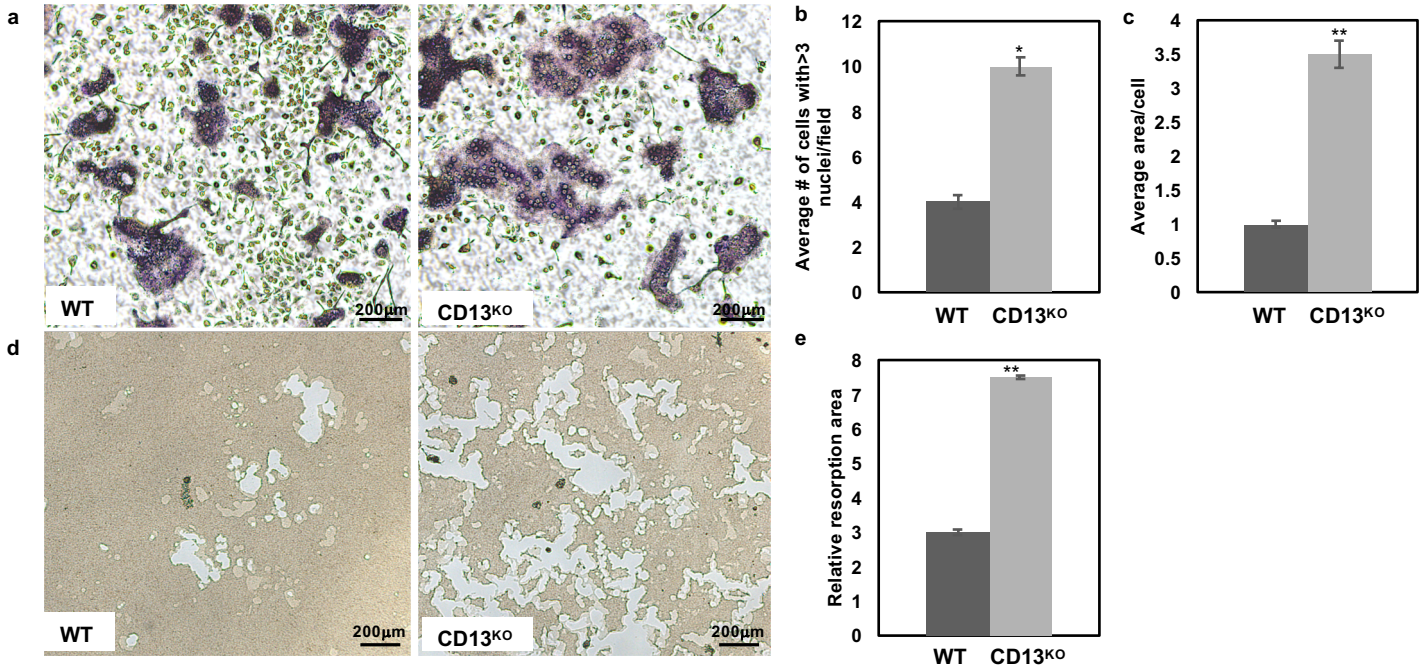

FIG S2. Formation of TRAP+ multinucleated BM-derived OCs isolated from aged mice are markedly enhanced in the absence of CD13. **a).** Primary murine BM derived OCs number and size /OC grown on tissue culture dish isolated from CD13^{KO} and WT at d5. **b**). Average # of cells with >3 nuclei/field and **c**). Average cell area per OCs in CD13^{KO} are significantly larger than the WT Ocs analyzed by and analyzed by using Zeiss Zen 2.0 Pro blue edition software (https://www.zeiss.com/content/dam/Microscopy/Downloads/Pdf/FAQs/zen2-blue-edition_installation-guide.pdf) . **d**). Area of resorption in CD13^{KO} and WT OCs grown on osteoplates for d10 by phase contrast imaging using Olympus cellSens Dimension V0118 software (Olympus Scientific) (https://www.olympus-lifescience.com/en/software/cellsens/) & quantified by Image J (https://imagej.nih.gov/ij/) (**e**) are shown. Scale bar; 200µm. Data represents +/- SEM of three independent experiments. N=6/genotype, **;p<0.01, *;p<0.05.

FIG. S2

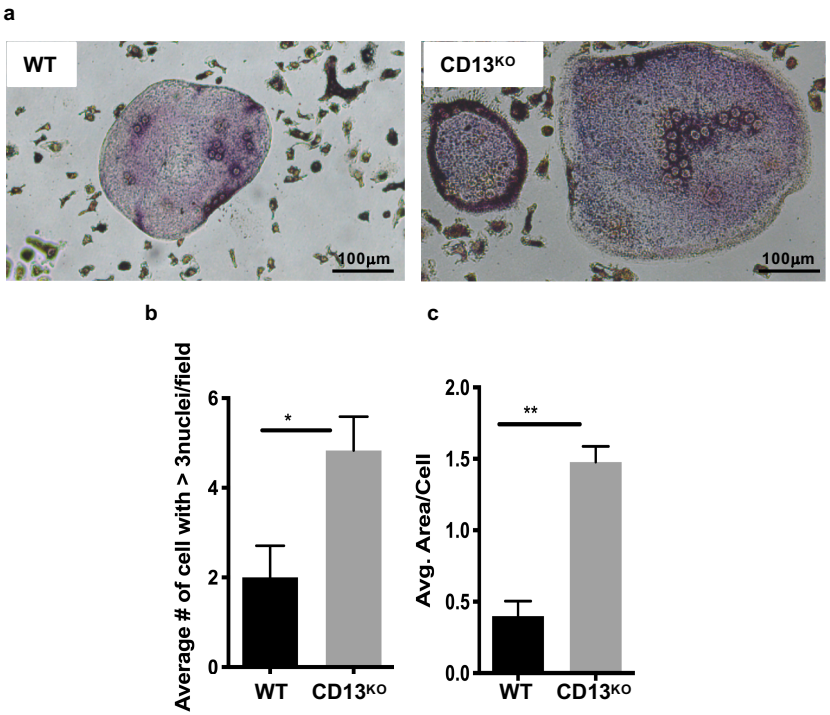

FIG S3. Increased TRAP+ splenic multinucleated OCs in the absence of CD13 in vitro. a). Flow-sorted primary murine spleen-derived OCP grown on plastic in the presence of M-CSF and RANKL led to enhanced OCs size and number of nuclei/OC in $CD13^{KO}$ cells compared to WT at d7. **b**). Average # of cells with >3 nuclei/field and **c**). Average cell area per OC in $CD13^{KO}$ are significantly larger than the WT cells analyzed by using Zeiss Zen 2.0 Pro blue edition software (https://www.zeiss.com/content/dam/Microscopy/Downloads/Pdf/FAQs/zen2-blue-edition_installation-guide.pdf) . Scale bar; 100µm. Data represents +/- SEM of three independent experiments. N=6/genotype, **;p<0.01.

FIG. S3

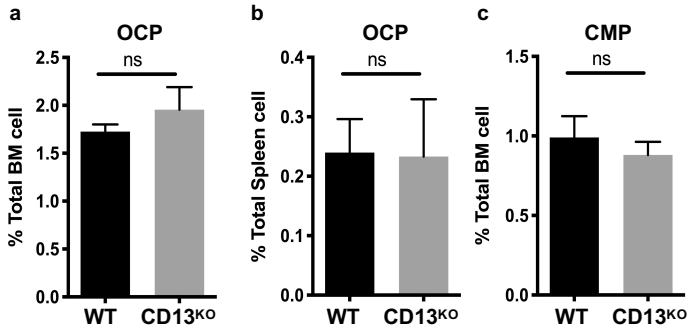

FIG S4. Osteoclast progenitors with osteoclastogenic potential are similar in WT and CD13^{KO} BM and periphery. Flow cytometric analysis of OCP profile of WT and CD13^{KO} mice indicated by CD3^{-}, B220^{-}, NK1.1^{-}, CD11b$^{-/lo}$, CD115^{+}, CD117^{+} in the BM (**a**), and CD3^{-}, B220^{-}, NK1.1^{-}, CD11b^{+}, Ly6G^{-}, Ly6C^{+}, CD115^{+} in spleen (**b)**, and common myeloid progenitor population indicated by lin^{-} c-kit^{+} Sca-1^{-} CD34^{+} in the BM analyzed by FlowJo software version 9.9 (https://www.flowjo.com/) (**c**). Data represents +/- SEM of three independent experiments. N=6/genotype.

FIG. S4

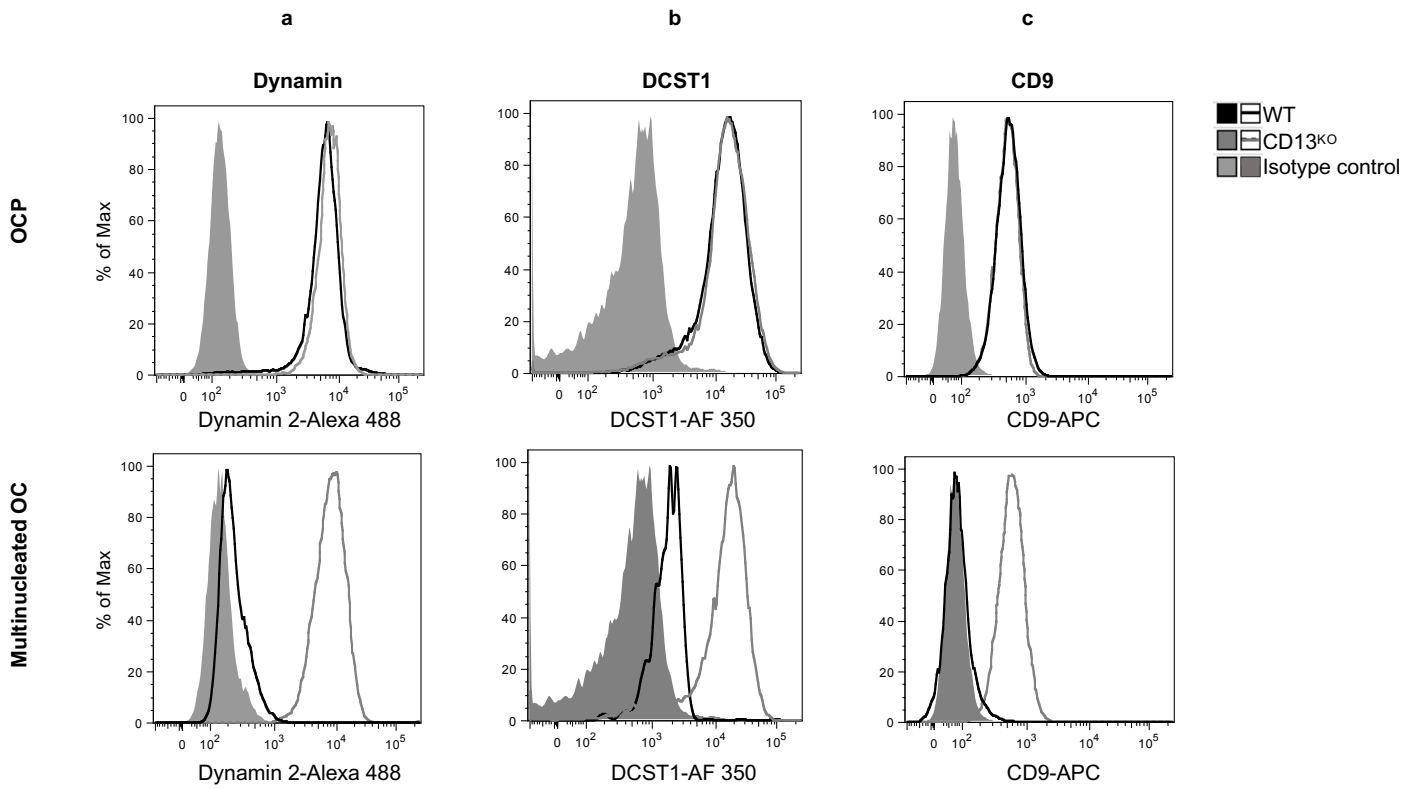

FIG S5. Representative histogram of dynamin, DCST1 and CD9 surface expression in osteoclast progenitors (OCP) and multinucleated OC isolated from WT and CD13^{KO} mice. Abundance at the surface was analyzed by staining with goat anti-rabbit dynamin2-Alexa 488 (**a**), rabbit anti-mouse DCST1-Alexa fluor 350 (**b**) and rat anti-mouse CD9-APC (**c**) followed by flow cytometry and analyzed by FlowJo software version 9.9 (https://www.flowjo.com/). Goat IgG-Alexa 488 (**a**) or rabbit IgG–AF350 (**b**) or rat IgG APC (**c**) was used as isotype control. N=3/genotype.

FIG. S5

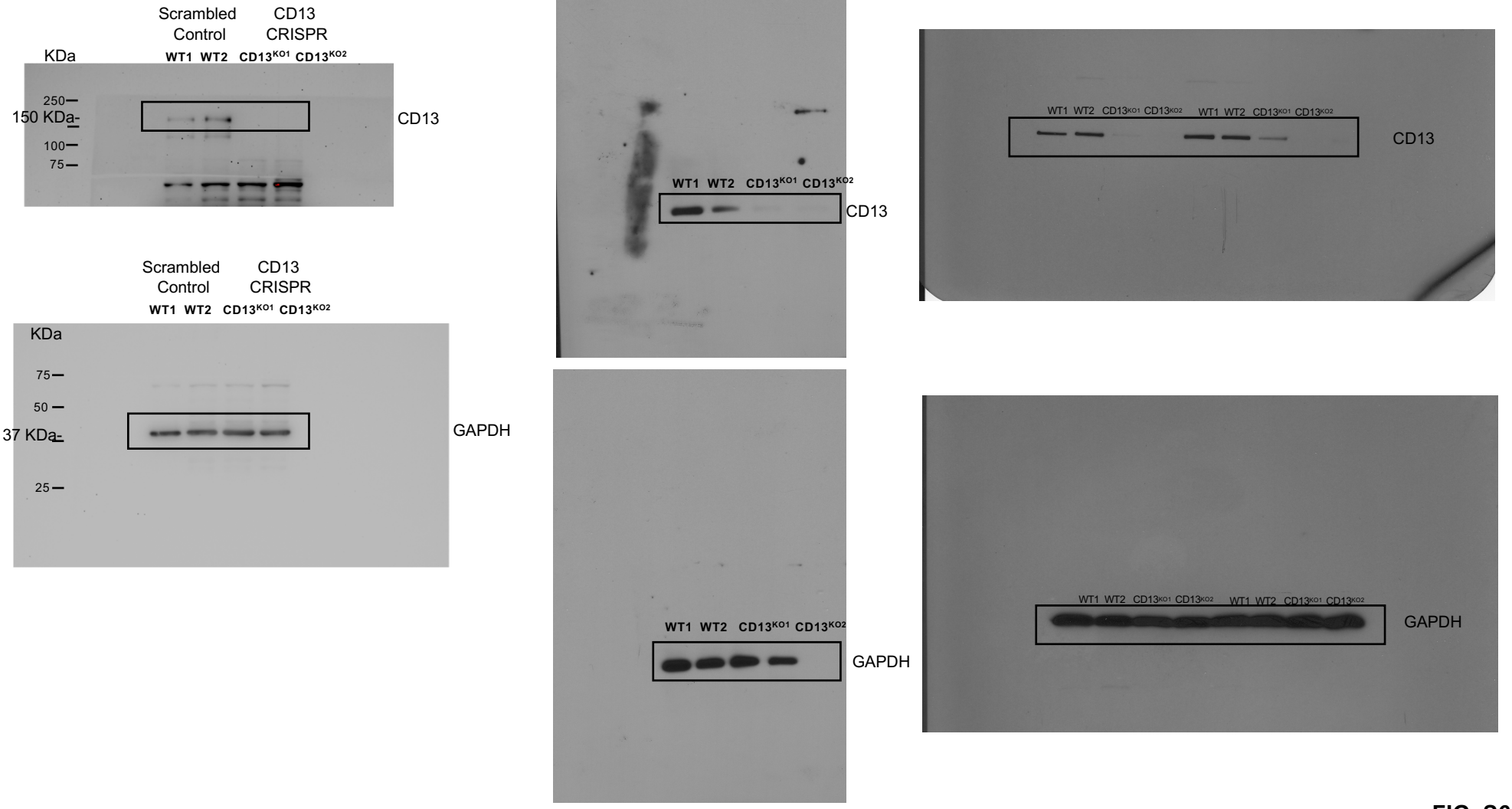

FIG. S6

FIG S6. Full-length individual blots and cropped replicates of Fig. 5f.

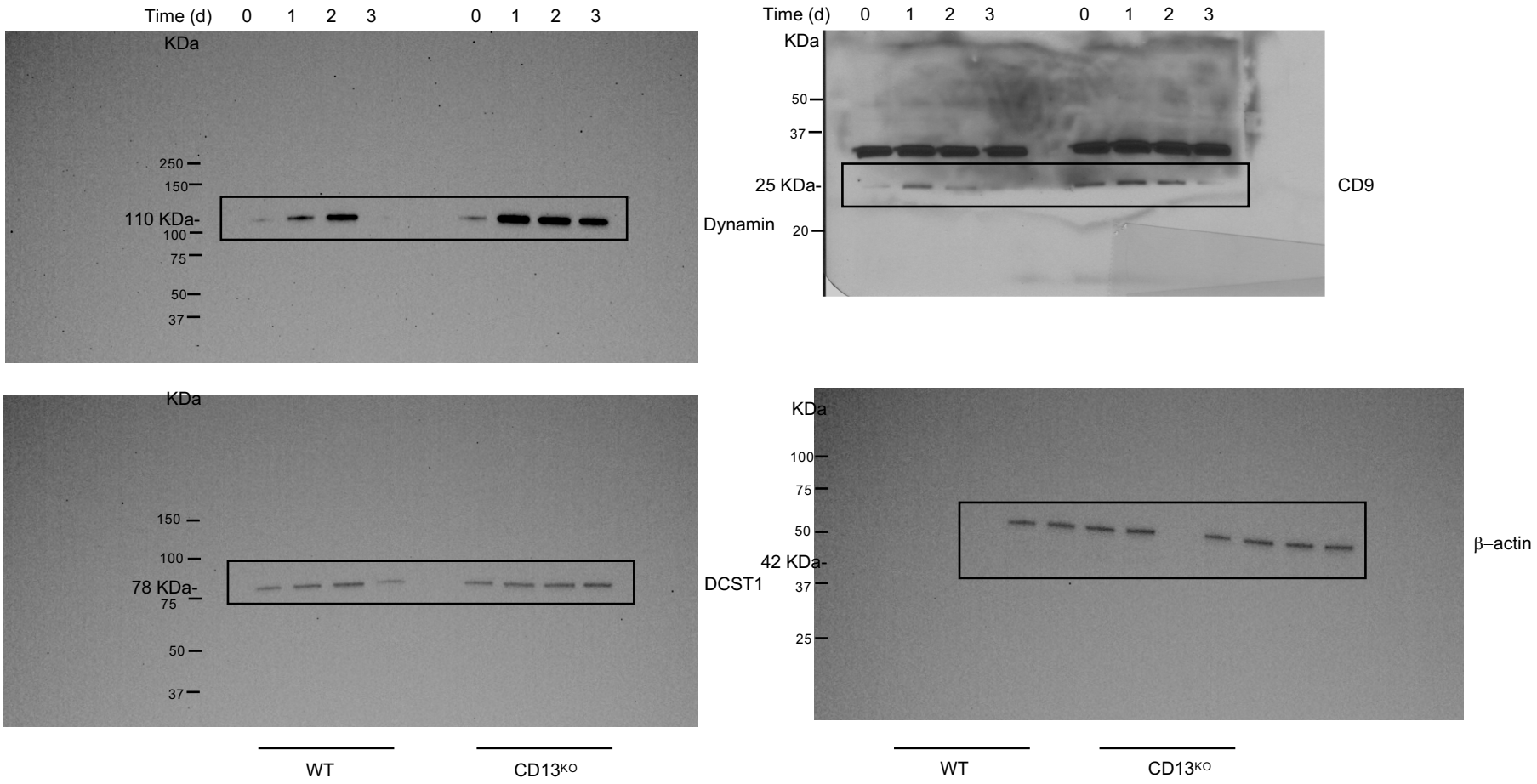

FIG S7. Full-length individual blots of Fig. 7a.

FIG. S7

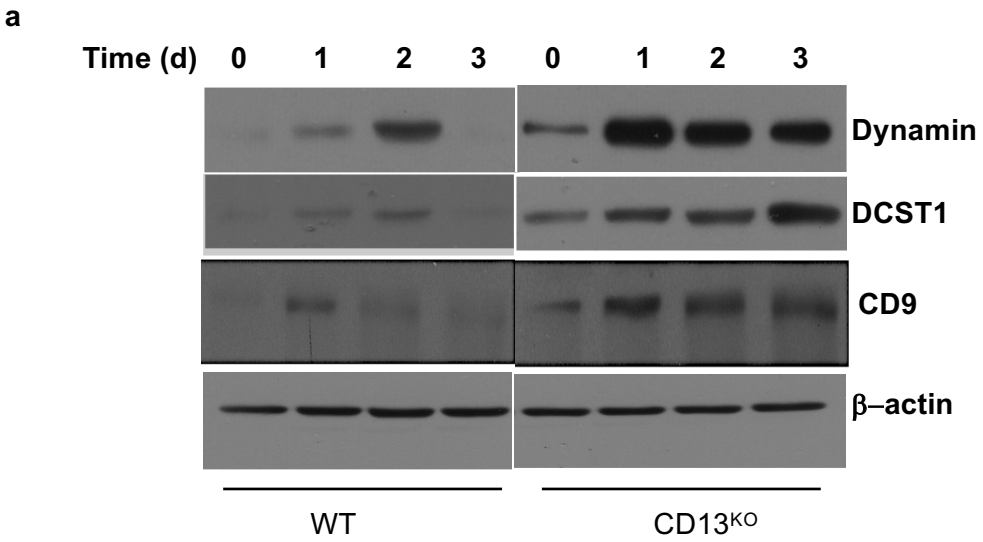

FIG S8. Cropped replicates of Fig. 7a.

FIG. S8

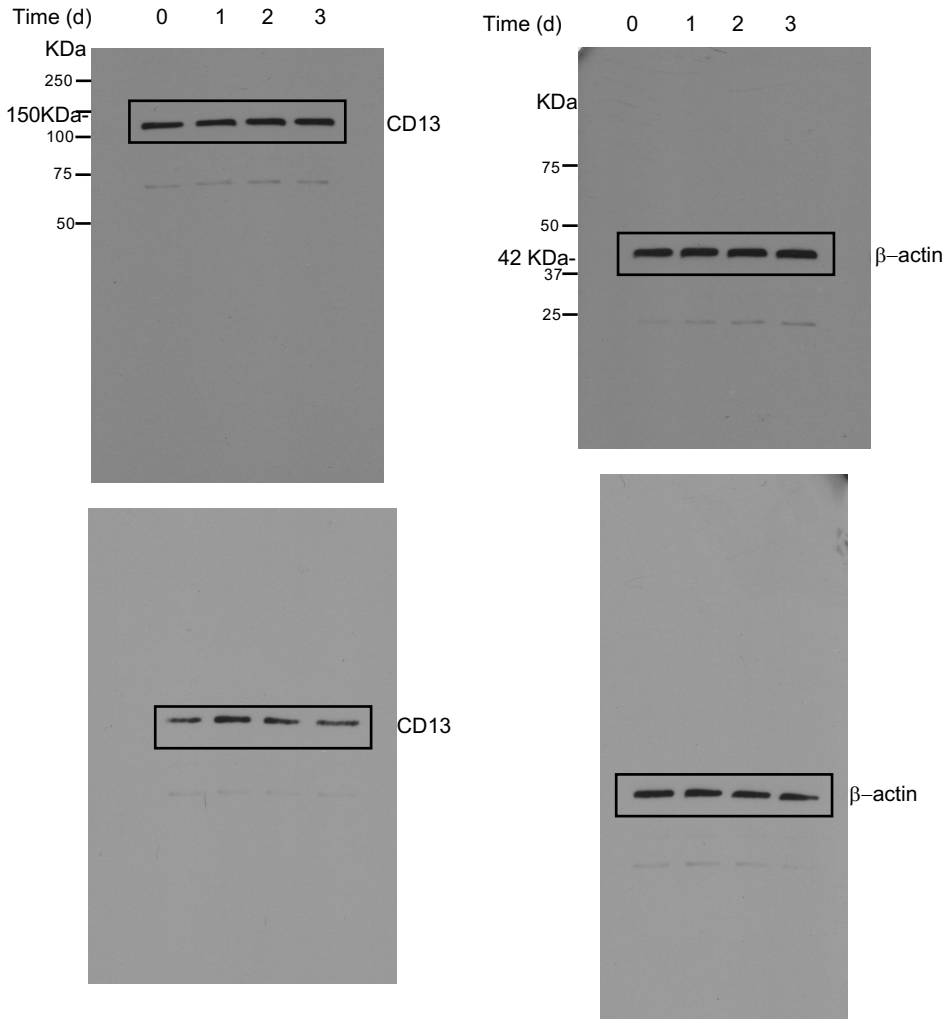

FIG S9. Full-length individual blots of Fig. 7c.

FIG. S9

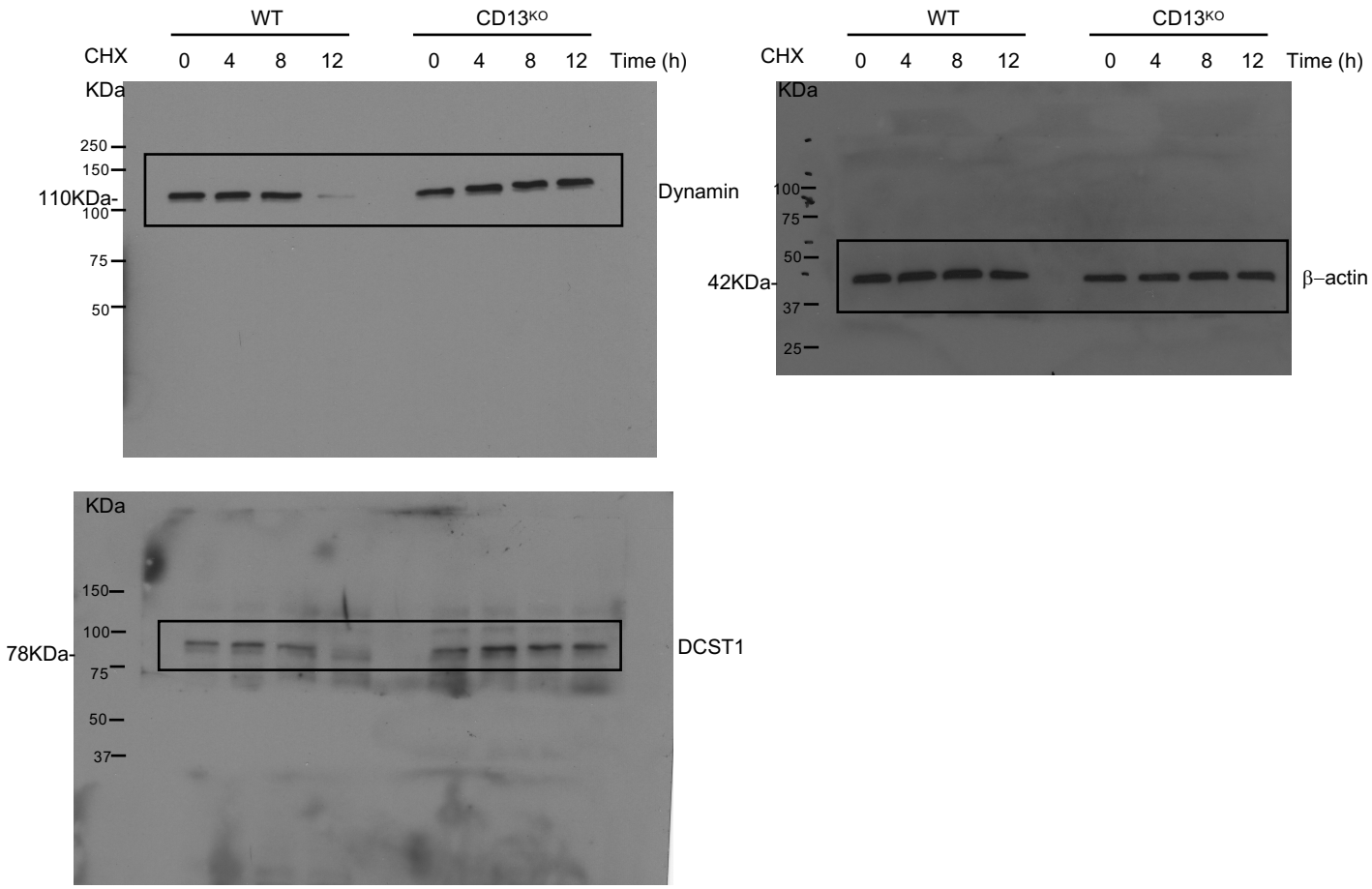

FIG S10. Full-length individual blots of Fig. 8b.

FIG. S10

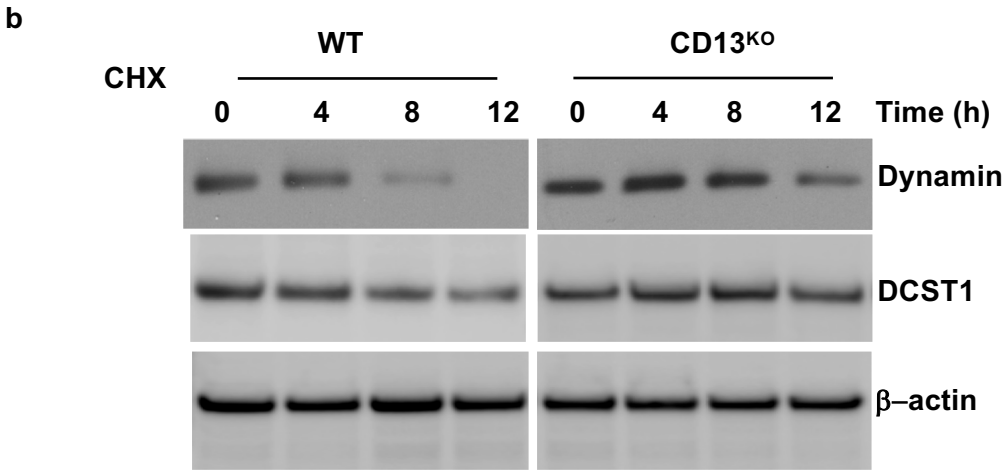

FIG S11. Cropped replicates of Fig. 8b.

FIG. S11