

Supplementary Materials: Trpm8 expression in human and mouse castration resistant prostate adenocarcinoma paves the way for the preclinical development of TRPM8-based targeted therapies

Sacha Genovesi ¹, Riccardo Moro ¹, Beatrice Vignoli ², Dario De Felice ¹, Marco Canossa ¹, Rodolfo Montironi ³, Francesco Giuseppe Carbone ⁴, Mattia Barbareschi ⁴, Andrea Lunardi ^{1*,†}, and Alessandro Alaimo ^{1*,†}

¹ Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, 38123 Trento, Italy; sacha.genovesi@unitn.it (S.G.); riccardo.moro@studenti.unitn.it (R.MOR.); dario.defelice@unitn.it (D.D.F.); marco.canossa@unitn.it (M.C.)

² Department of Physics, University of Trento, 38123 Trento, Italy; beatrice.vignoli@unitn.it (B.V.)

³ Section of Pathological Anatomy, Polytechnic University of the Marche Region, School of Medicine, United Hospitals, 60126 Ancona, Italy; r.montironi@univpm.it (R.MON.)

⁴ Unit of Surgical Pathology, Santa Chiara Hospital, 38122 Trento, Italy; francescogiuseppe.carbone@apss.tn.it (F.G.C.); mattia.barbareschi@apss.tn.it (M.B.)

* Correspondence: andrea.lunardi@unitn.it (A.L.); alessandro.alaimo@unitn.it (A.A)

† A.L. and A.A. contributed equally as last authors.

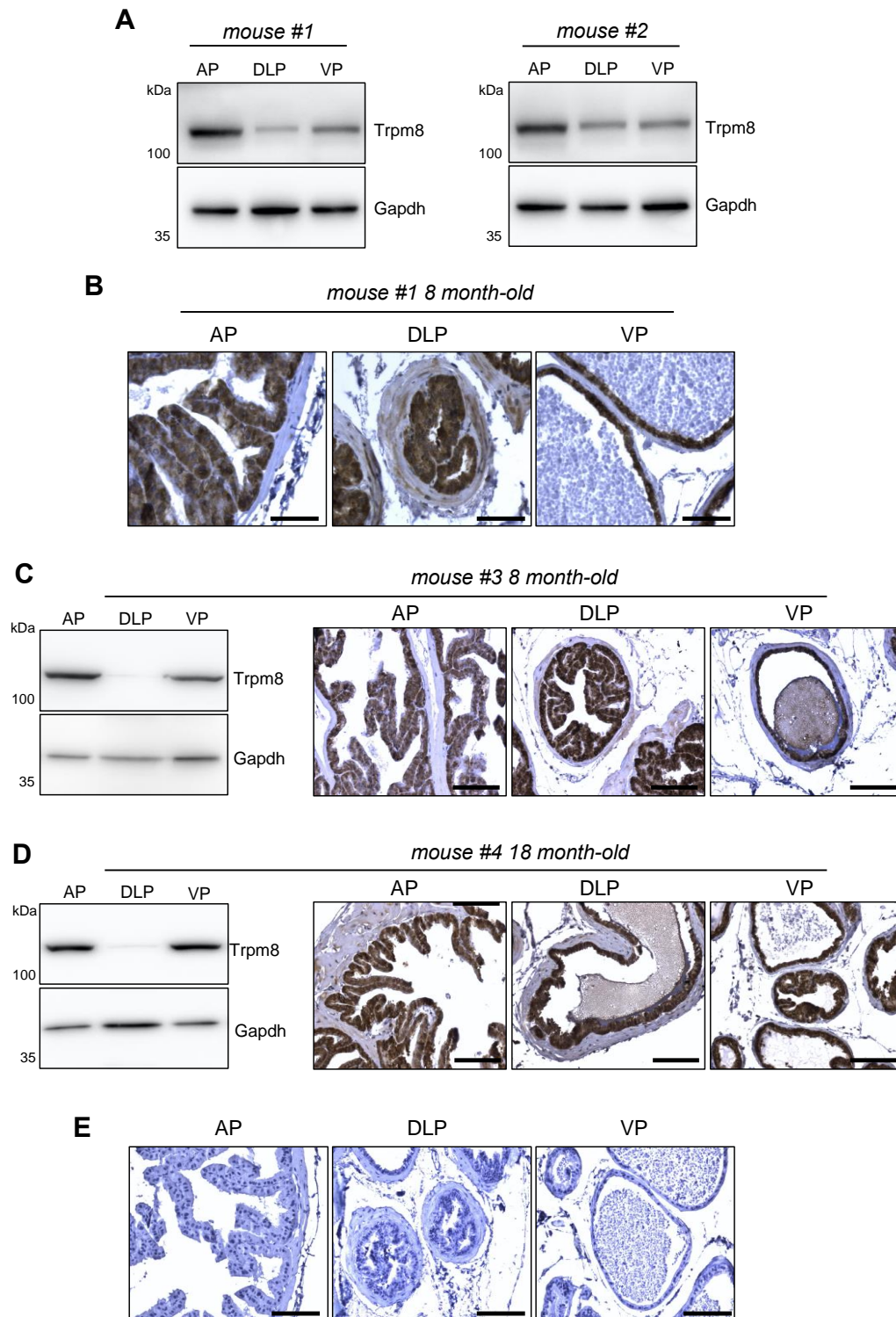


Figure S1. Characterization of Trpm8 expression in normal mouse prostate. **(A)** Western blot analysis of Trpm8 expression using a primary anti-TRPM8 antibody from Abcam (ab3243) which recognizes a different epitope compared to Alomone (ACC-049). Proteins were extracted from AP, DLP and VP lobes of 8 months-old (mouse #1) and 18 months-old (mouse #2) (related to Figure 1D and 1E, left). Gapdh was used as loading control. **(B)** 40x magnification of Figure 1D, right (mouse #1). Scale bars, 50 μ m. **(C-D)** Representative Western blots (left) and immunohistochemical localization (right) of Trpm8 in the AP, DLP and VP lobes obtained from a 8 months-old (**C**, mouse #3) and a 18 months-old (**D**, mouse #4) C57BL/6J mice. Gapdh was used as loading control for Western blot analysis. Scale bars, 100 μ m. **(E)** Sections of prostate lobes (mouse #1, related to Figure 1D) incubated with the secondary antibody alone confirming anti-TRPM8 antibody (ACC-049, Alomone labs) specificity.

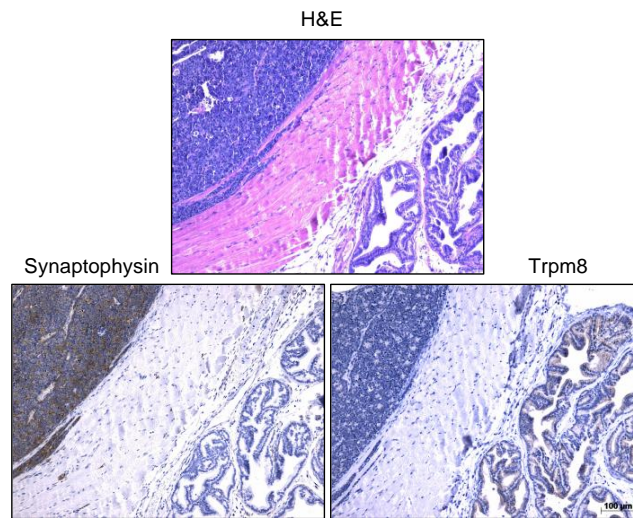


Figure S2. Trpm8 protein is expressed by adenocarcinoma but not by PCa cells in TRAMP models. Representative H&E (above) and immunostaining for Synaptophysin (below, left) and Trpm8 (below, right) of TRAMP mouse model specimens showing the absence of Trpm8 in neuroendocrine PCa cells. Scale bars, 100 μ m.

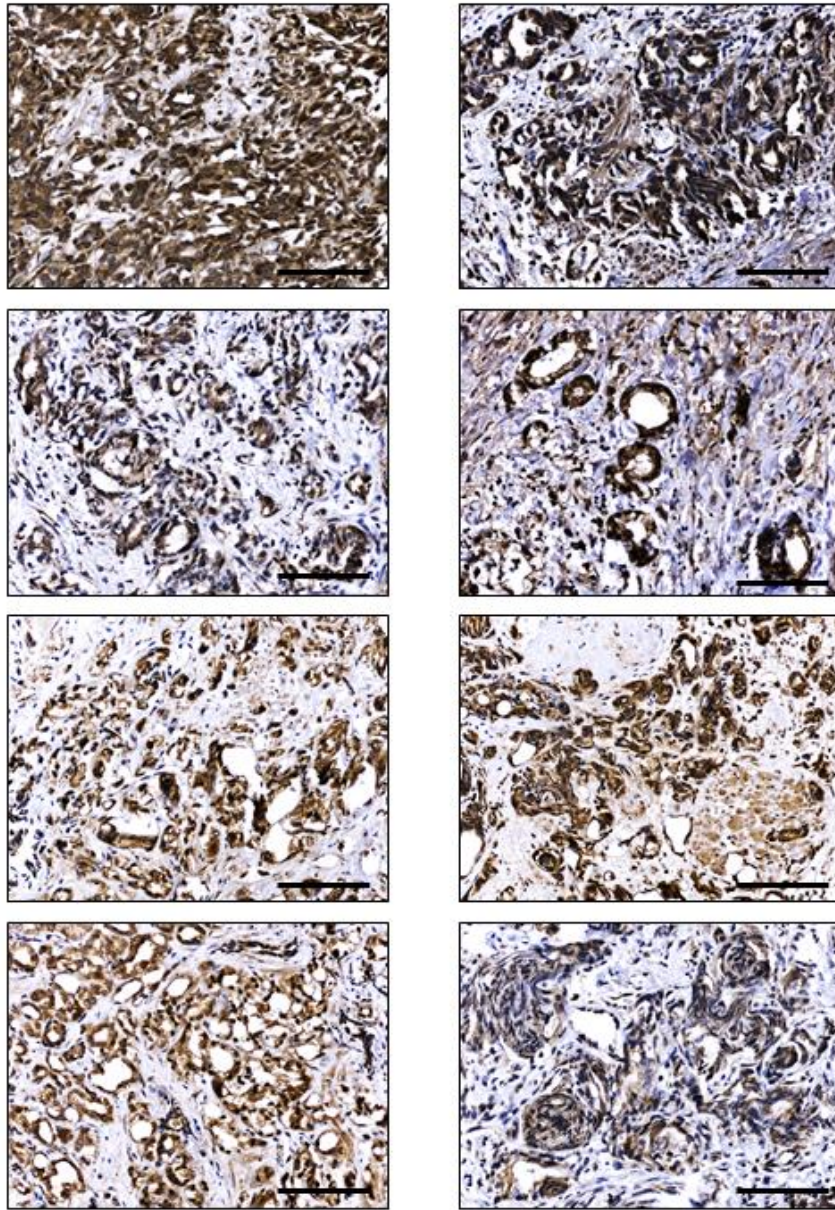


Figure S3. Characterization of Trpm8 expression in human castration resistant PCa samples. Immunostaining for TRPM8 in human CRPC specimens. Scale bars, 100 μ m

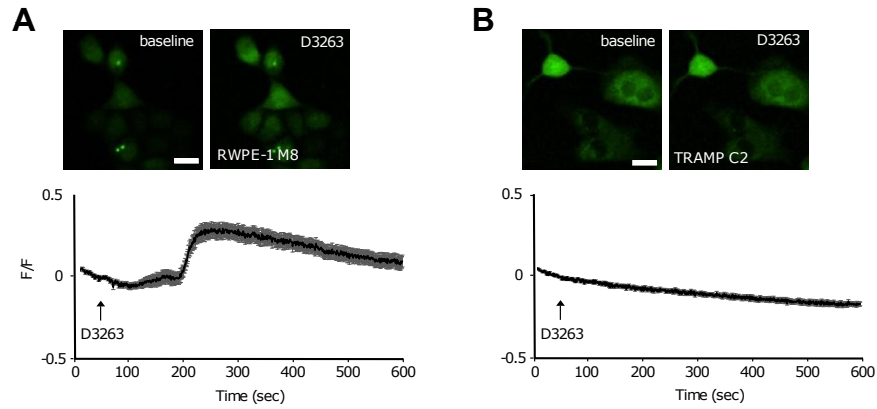


Figure S4. Fluorescence Calcium Imaging in prostate cells. **(A)** Panels show representative spinning disk confocal images of the Fluo-4 fluorescence for RWPE-1 M8 cells before (10 s) and after (180 s) treatment with D-3263. Histogram depicts the corresponding $\Delta F/F_-$ values. Data are mean \pm SEM (n=57 cells). **(B)** Panels show representative spinning disk confocal images of the Fluo-4 fluorescence for TRAMP C2 cells before (10 s) and after (180 s) treatment with D-3263. Histogram depicts the corresponding $\Delta F/F_-$ values. Data are mean \pm SEM (n=58 cells). Scale bars, 15 μ m.

Figure 1A

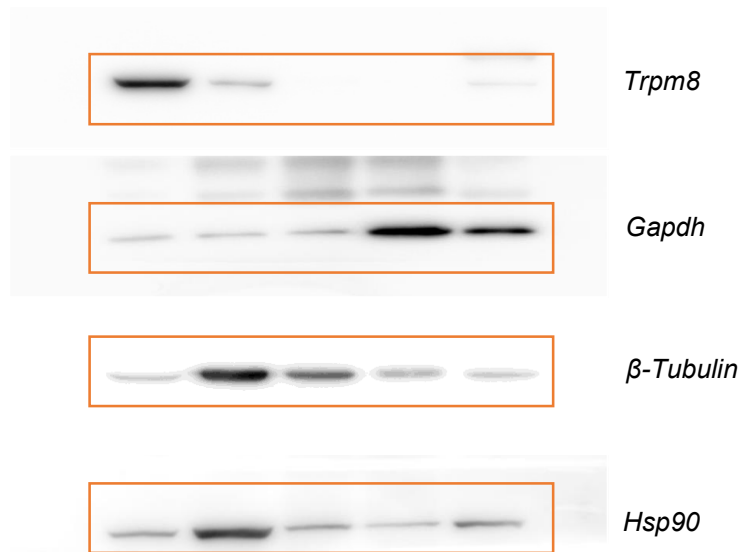


Figure 1B

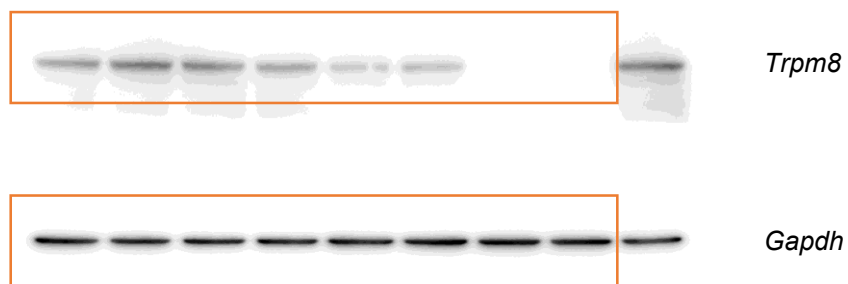


Figure 1C

Figure S1C

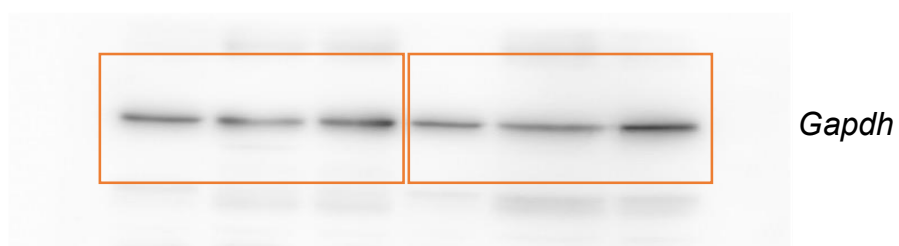
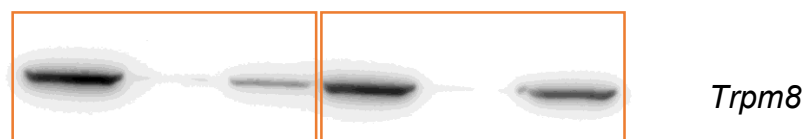


Figure S1D

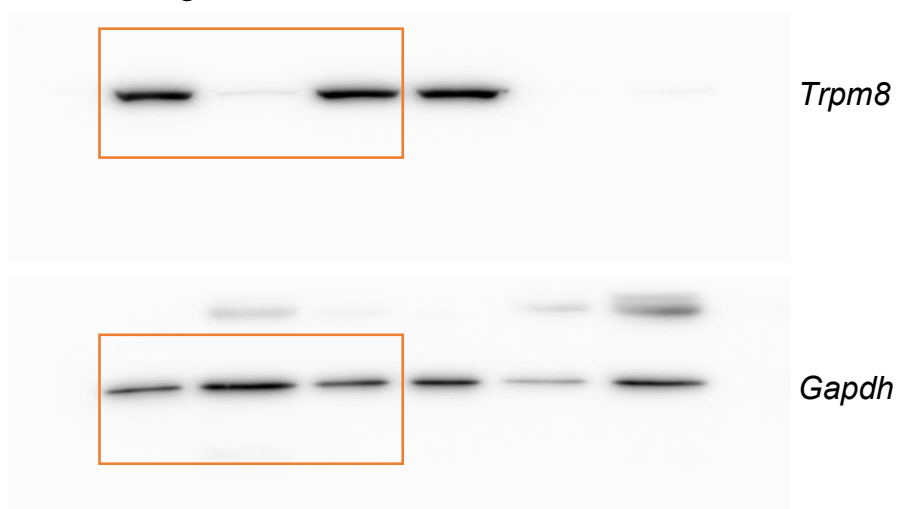


Figure 1D



Figure S1A

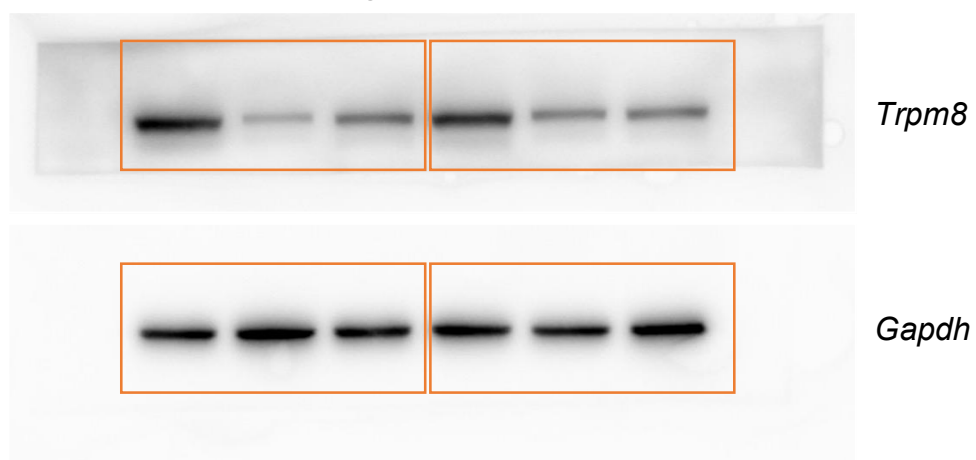


Figure 4B

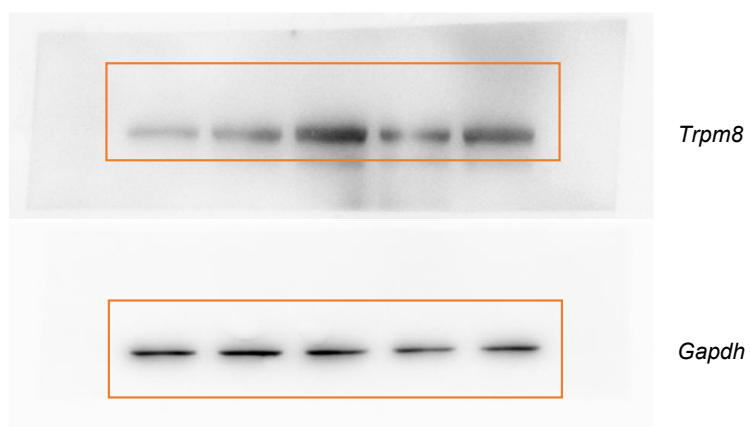


Figure 4C

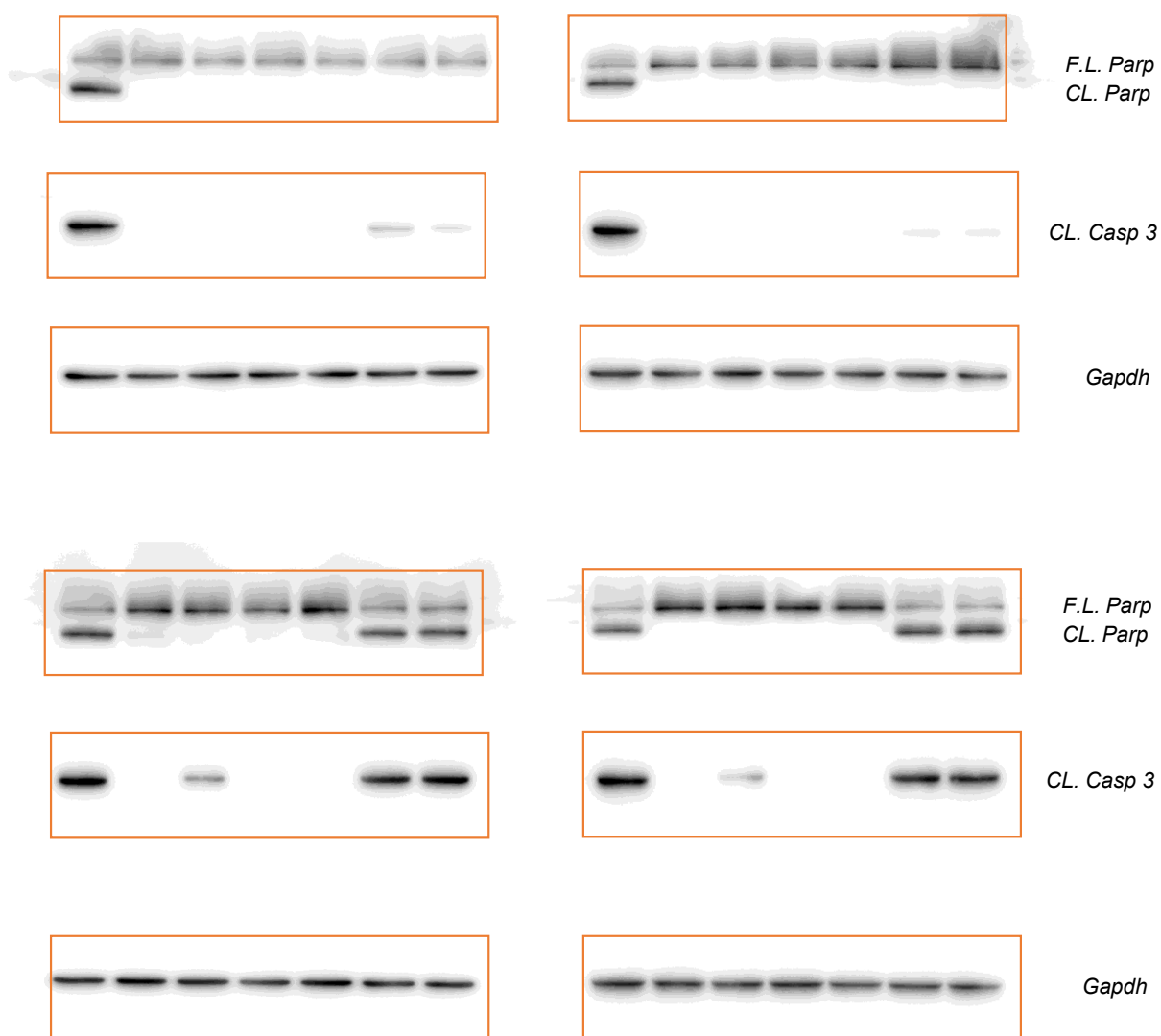


Figure S5. Uncropped western blot figures.