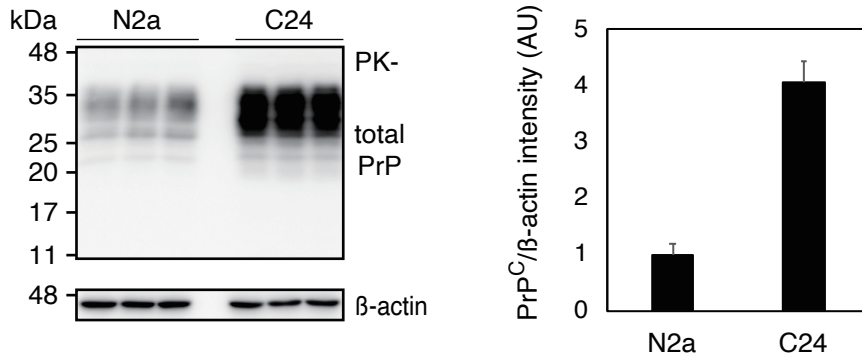


Supplementary Information

Neurotropic influenza A virus infection causes prion protein misfolding into infectious prions in neuroblastoma cells

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Supplementary Figure 1. PrP expression level of N2a and N2aC24 cells. (Left panels) Western blotting with 6D11 antibody of 40 μ g proteins from N2a (n = 3) and N2aC24 (n = 3) cells. β -actin was used as an internal control. (Right panel) Expression level of PrP^C in N2aC24 compared to those in N2a cells. AU, arbitrary units. Full length blots of Western blot images are shown in Supplementary Figure 5a, b.

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Prnp: ATGGCGAACC TTGGCTACTG GCTGCTGGCC CTCTTTGTGA CTATGTGGAC TGATGTCGGC 60
*****
C24: ATGGCGAACC TTGGCTACTG GCTGCTGGCC CTCTTTGTGA CTATGTGGAC TGATGTCGGC
*****
C24R1: ATGGCGAACC TTGGCTACTG GCTGCTGGCC CTCTTTGTGA CTATGTGGAC TGATGTCGGC

Prnp: CTCTGCAAAA AGCGGCCAAA GCCTGGAGGG TGGAAACACCG GTGGAAGCCG GTATCCCGGG 120
*****
C24: CTCTGCAAAA AGCGGCCAAA GCCTGGAGGG TGGAAACACCG GTGGAAGCCG GTATCCCGGG
*****
C24R1: CTCTGCAAAA AGCGGCCAAA GCCTGGAGGG TGGAAACACCG GTGGAAGCCG GTATCCCGGG

Prnp: CAGGGAAGCC CTGGAGGCAA CCGTTACCCA CCTCAGGGTG GCACCTGGGG GCAGCCCCAC 180
*****
C24: CAGGGAAGCC CTGGAGGCAA CCGTTACCCA CCTCAGGGTG GCACCTGGGG GCAGCCCCAC
*****
C24R1: CAGGGAAGCC CTGGAGGCAA CCGTTACCCA CCTCAGGGTG GCACCTGGGG GCAGCCCCAC

Prnp: GGTGGTGGCT GGGGACAACC CCATGGGGGC AGCTGGGGAC AACCTCATGG TGGTAGTTGG 240
*****
C24: GGTGGTGGCT GGGGACAACC CCATGGGGGC AGCTGGGGAC AACCTCATGG TGGTAGTTGG
*****
C24R1: GGTGGTGGCT GGGGACAACC CCATGGGGGC AGCTGGGGAC AACCTCATGG TGGTAGTTGG

Prnp: GGTGAGCCCC ATGGCGGTGG ATGGGGCCAA GGAGGGGGTA CCCATAATCA GTGGAACAAG 300
*****
C24: GGTGAGCCCC ATGGCGGTGG ATGGGGCCAA GGAGGGGGTA CCCATAATCA GTGGAACAAG
*****
C24R1: GGTGAGCCCC ATGGCGGTGG ATGGGGCCAA GGAGGGGGTA CCCATAATCA GTGGAACAAG

Prnp: CCCAGCAAAC CAAAAACCAA CCTCAAGCAT GTGGCAGGGG CTGCGGCAGC TGGGGCAGTA 360
*****
C24: CCCAGCAAAC CAAAAACCAA CCTCAAGCAT GTGGCAGGGG CTGCGGCAGC TGGGGCAGTA
*****
C24R1: CCCAGCAAAC CAAAAACCAA CCTCAAGCAT GTGGCAGGGG CTGCGGCAGC TGGGGCAGTA

Prnp: GTGGGGGGCC TTGGTGGCTA CATGCTGGGG AGCGCCATGA GCAGGCCAT GATCCATTTT 420
*****
C24: GTGGGGGGCC TTGGTGGCTA CATGCTGGGG AGCGCCATGA GCAGGCCAT GATCCATTTT
*****
C24R1: GTGGGGGGCC TTGGTGGCTA CATGCTGGGG AGCGCCATGA GCAGGCCAT GATCCATTTT

Prnp: GGCAACGACT GGGAGGACCG CTACTIONCGT GAAAACATGT ACCGCTACCC TAACCAAGTG 480
*****
C24: GGCAACGACT GGGAGGACCG CTACTIONCGT GAAAACATGT ACCGCTACCC TAACCAAGTG
*****
C24R1: GGCAACGACT GGGAGGACCG CTACTIONCGT GAAAACATGT ACCGCTACCC TAACCAAGTG

Prnp: TACTACAGGC CAGTGGATCA GTACAGCAAC CAGAACAAC TCGTGCACGA CTGCGTCAAT 540
*****
C24: TACTACAGGC CAGTGGATCA GTACAGCAAC CAGAACAAC TCGTGCACGA CTGCGTCAAT
*****
C24R1: TACTACAGGC CAGTGGATCA GTACAGCAAC CAGAACAAC TCGTGCACGA CTGCGTCAAT

Prnp: ATCACCATCA AGCAGCACAC GGTCACCACC ACCACCAAGG GGGAGAACTT CACCGAGACC 600
*****
C24: ATCACCATCA AGCAGCACAC GGTCACCACC ACCACCAAGG GGGAGAACTT CACCGAGACC
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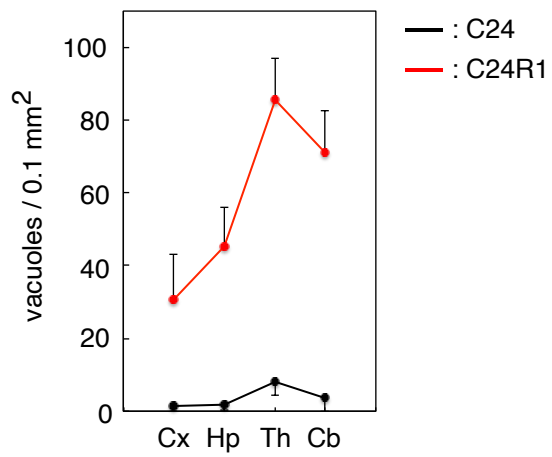
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*****
C24R1: GATGTGAAGA TGATGGAGCG CGTGGTGGAG CAGATGTGCG TCACCCAGTA CCAGAAGGAG

Prnp: TCCCAGGCCT ATTACGACGG GAGAAGATCC AGCAGCACCG TGCTTTTCTC CTCCCCTCCT 720
*****
C24: TCCCAGGCCT ATTACGACGG GAGAAGATCC AGCAGCACCG TGCTTTTCTC CTCCCCTCCT
*****
C24R1: TCCCAGGCCT ATTACGACGG GAGAAGATCC AGCAGCACCG TGCTTTTCTC CTCCCCTCCT

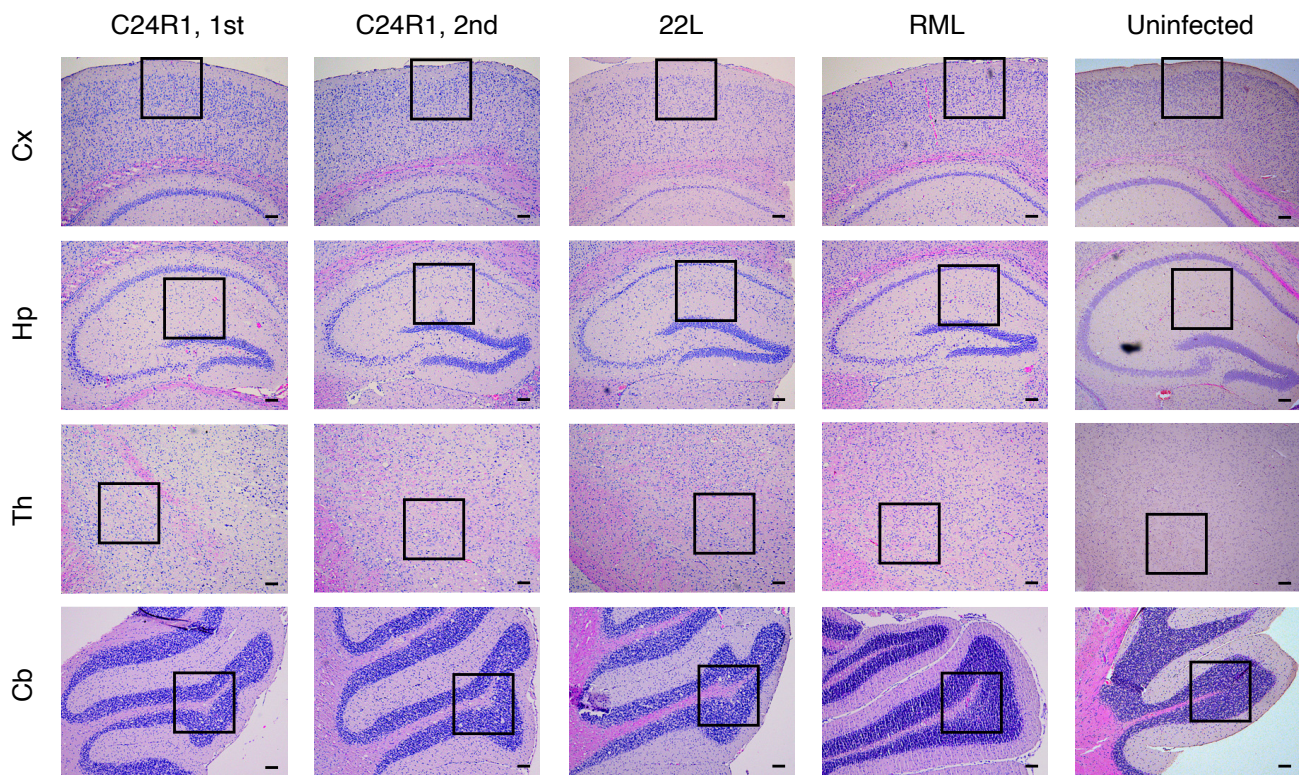
Prnp: GTCATCTCC TCATCTCCTT CCTCATCTTC CTGATCGTGG GATGA 765
*****
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C24R1: GTCATCTCC TCATCTCCTT CCTCATCTTC CTGATCGTGG GATGA

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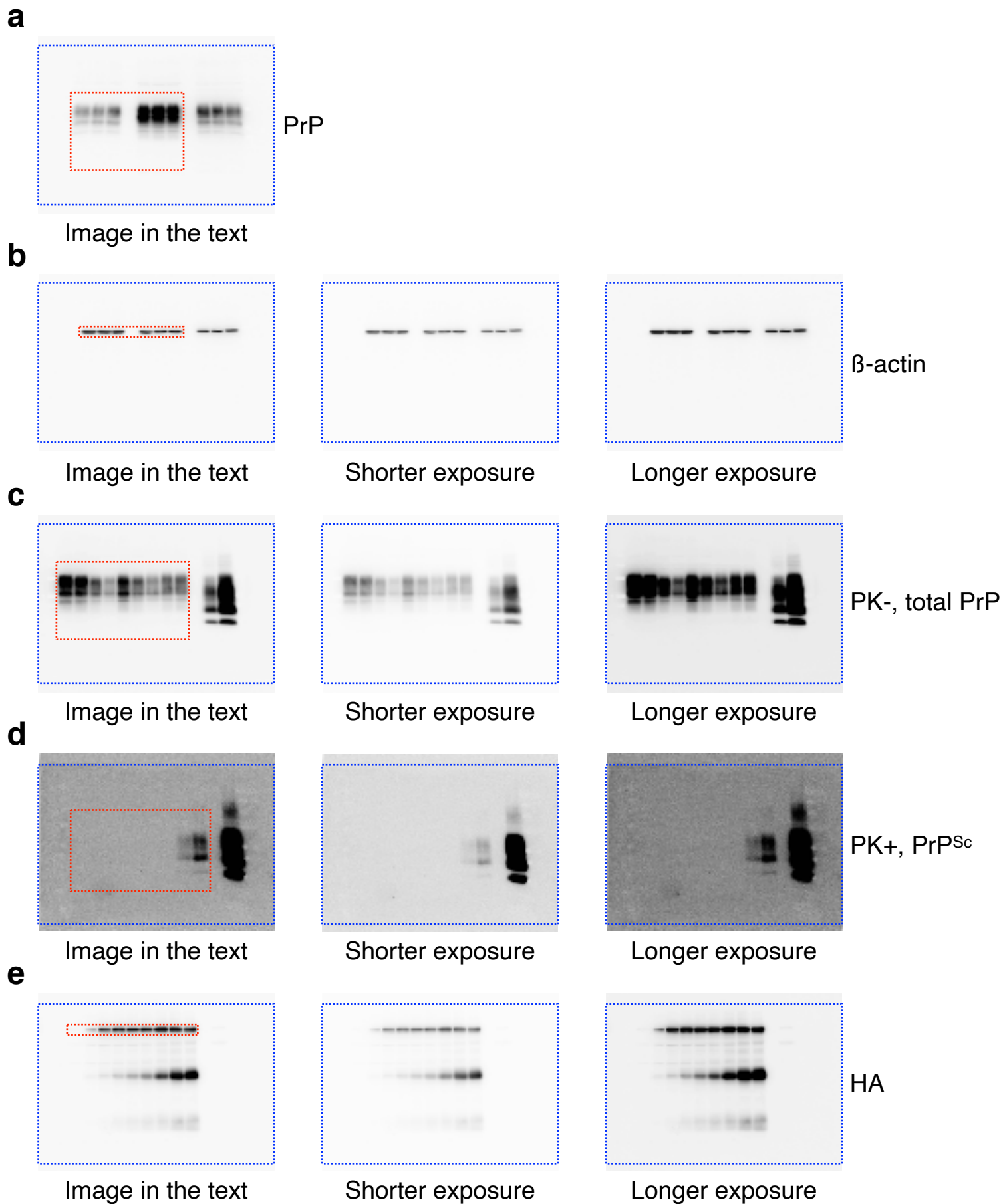
Supplementary Figure 2. No mutations detected in the PrP-coding sequences in N2aC24 (R1) cells. The PCR-amplified DNA fragments of the PrP-coding sequence were cloned from N2aC24 and N2aC24(R1) cells. Eight and 6 cloned fragments from N2aC24 and N2aC24(R1) cells were sequenced, respectively. All of them had identical sequences to each other, and to the PrP-coding sequence of the murine *Prnp* (GeneBank accession number BC006703.1). The sequences are shown from the initiation codon to the stop codon. *, identical nucleotides.



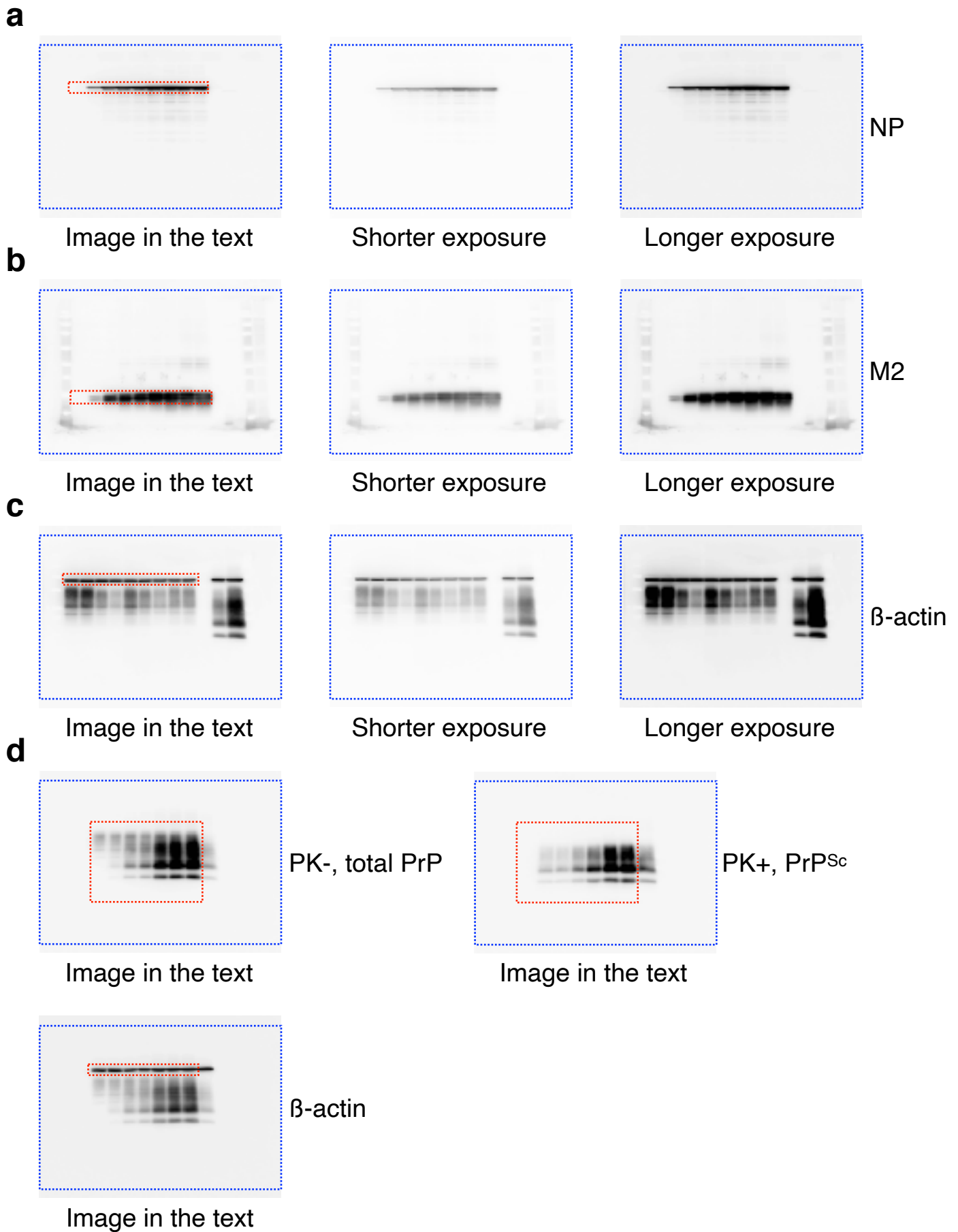
Supplementary Figure 3. Vacuole formation in the brains of terminally ill mice after inoculation with N2aC24R1 cell lysate. Vacuole counts at different brain areas of terminally ill mice in Fig. 3d. Cx, cerebral cortex; Hp, hippocampus; Th, thalamus; Cb, cerebellum.



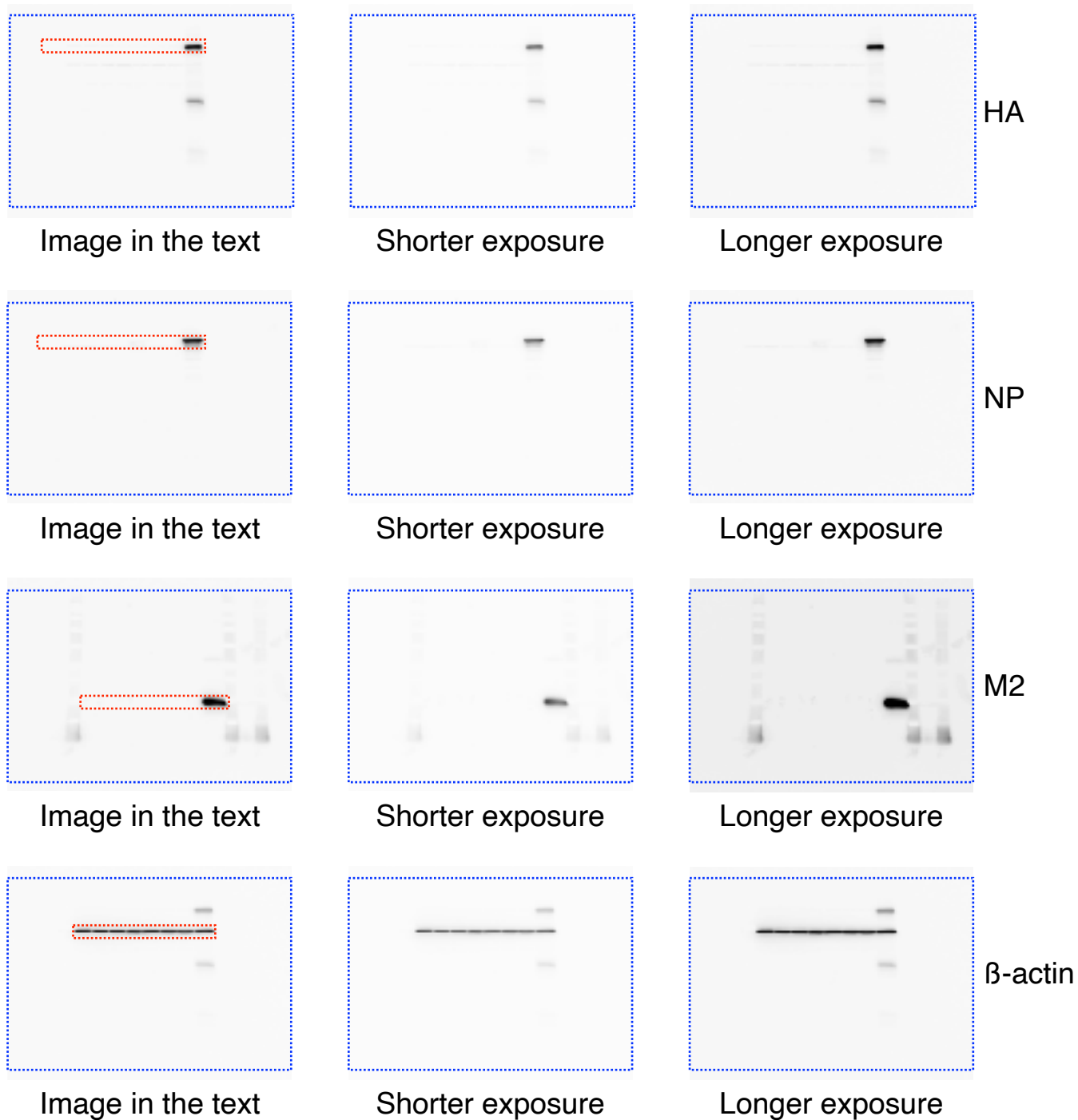
Supplementary Figure 4. Different pathological properties of prions in N2aC24R1 cells from RML and 22L prions. HE staining of brains in Fig. 5b at lower magnification. Uninfected brains were used as a control. Cx, cerebral cortex; Hp, hippocampus; Th, thalamus; Cb, cerebellum. Bars, 100 μ m.



Supplementary Figure 5. The original western full blots. **a**, Full blot image for Supplementary Figure 1 (Upper panel). **b**, Full blot images for Supplementary Figure 1 (Lower panel). **c**, Full blot images for Figure 1b (Upper left panel). **d**, Full blot images for Figure 1b (Lower left panel). **e**, Full blot images for Figure 1b (Upper right panel). Blue-dotted rectangles show the membrane edges. Red-dotted rectangles show the images in the text.

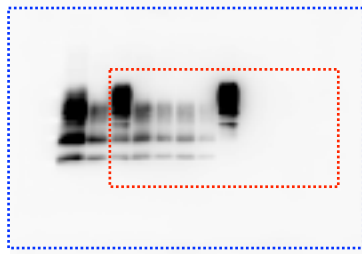


Supplementary Figure 6. The original western full blots. **a**, Full blot images for Figure 1b (Upper middle right panel). **b**, Full blot images for Figure 1b (Lower middle right panel). **c**, Full blot images for Figure 1b (Lower right panel). **d**, Full blot images for Figure 1e. Blue-dotted rectangles show the membrane edges. Red-dotted rectangles show the images in the text.



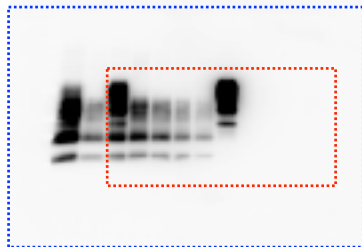
Supplementary Figure 7. The original western full blots. Full blot images for Figure 1f. Blue-dotted rectangles show the membrane edges. Red-dotted rectangles show the images in the text.

a



2nd experiment

Image in the text



3rd experiment

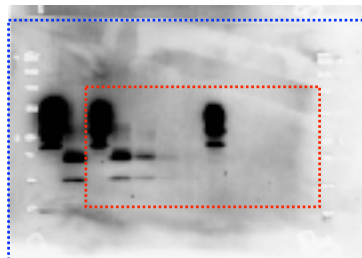
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4th experiment

Image in the text

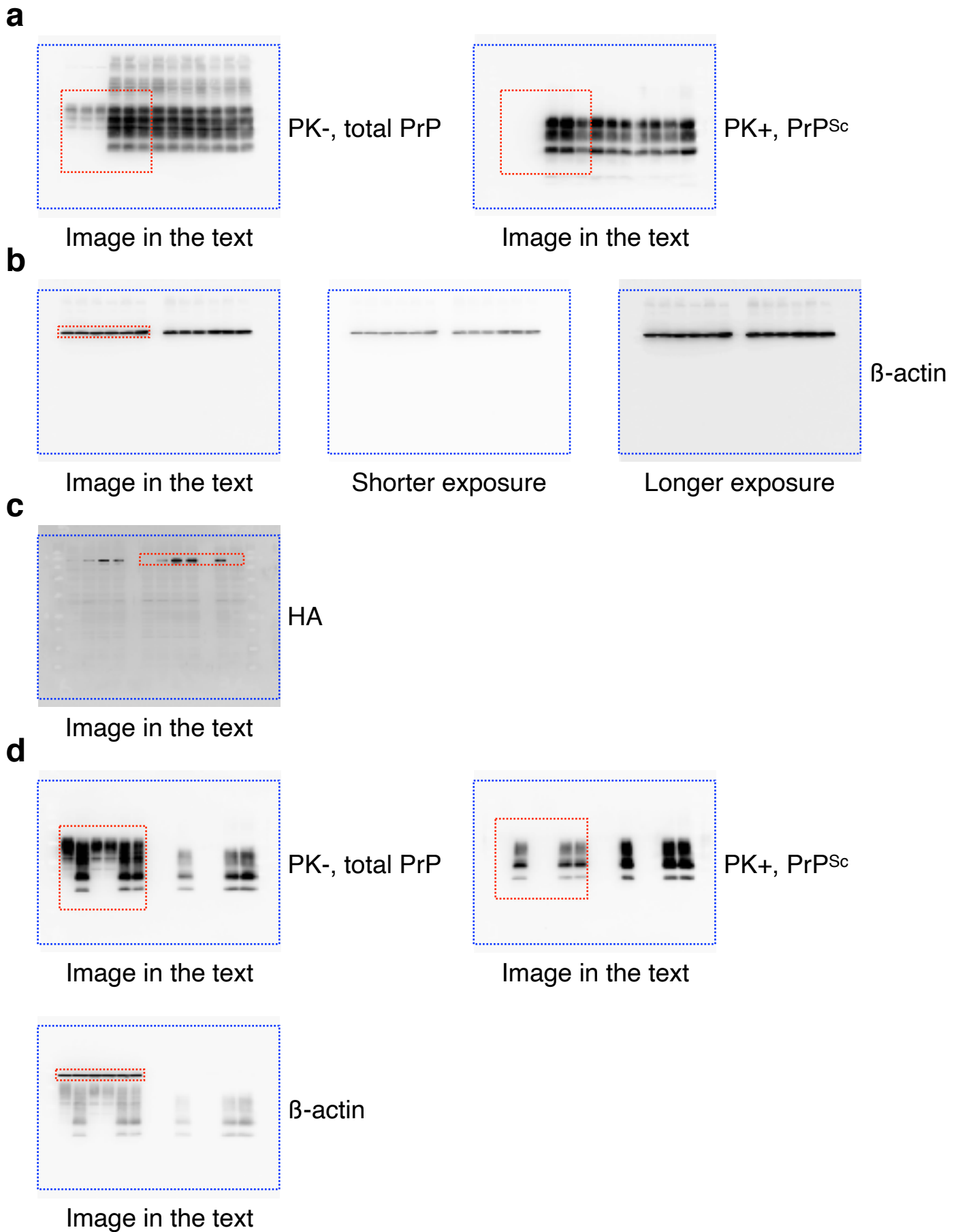
b



PrP

Image in the text

Supplementary Figure 8. The original western full blots. a, Full blot images for Figure 2a. **b**, Full blot image for Figure 2b. Blue-dotted rectangles show the membrane edges. Red-dotted rectangles show the images in the text.



Supplementary Figure 9. The original western full blots. **a**, Full blot images for Figure 3b (Upper left and Right panel). **b**, Full blot images for Figure 3b (Lower left panel). **c**, Full blot image for Figure 4a. **d**, Full blot image for Figure 4b. Blue-dotted rectangles show the membrane edges. Red-dotted rectangles show the images in the text.

a

Image in the text

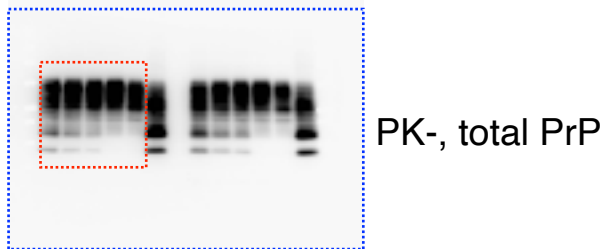
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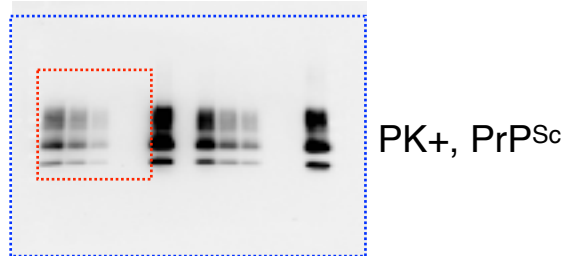


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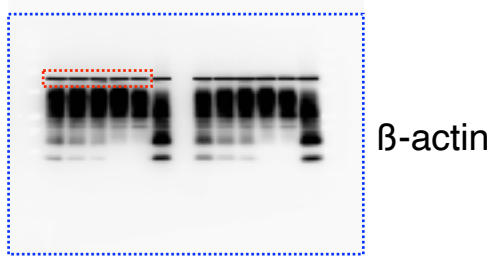


Image in the text

c

Image in the text

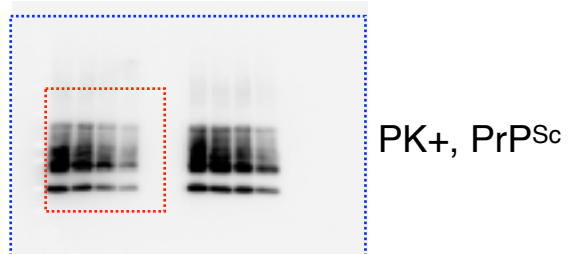


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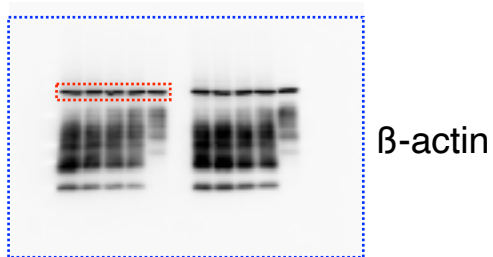
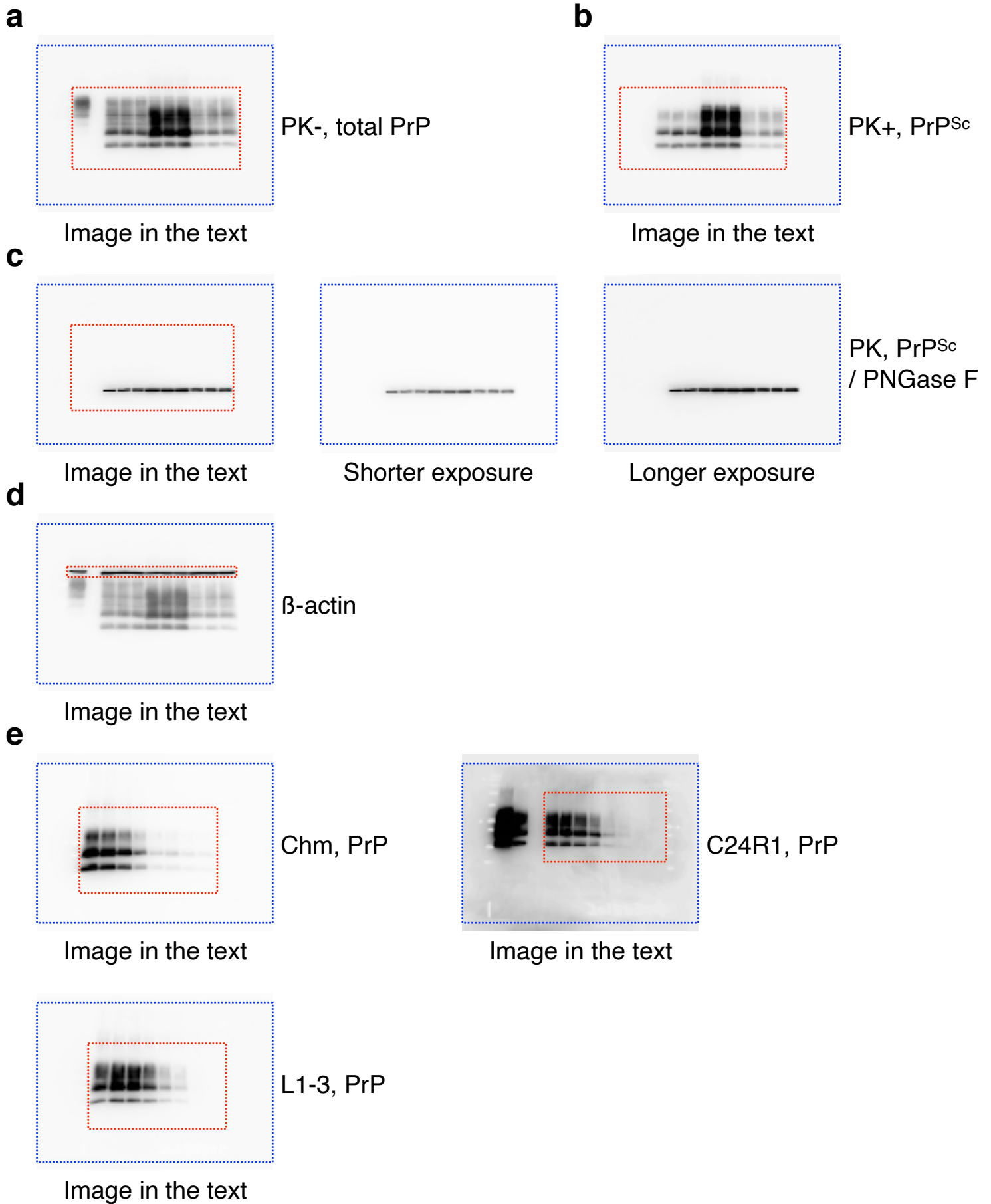


Image in the text

Supplementary Figure 10. The original western full blots. a, Full blot image for Figure 4c. **b**, Full blot images for Figure 4c (Left panels). **c**, Full blot images for Figure 4c (Right panels). Blue-dotted rectangles show the membrane edges. Red-dotted rectangles show the images in the text.



Supplementary Figure 11. The original western full blots. **a**, Full blot image for Figure 6a (Upper left panel). **b**, Full blot image for Figure 6a (Middle panel). **c**, Full blot images for Figure 6a (Right panel). **d**, Full blot image for Figure 6a (Lower left panel). **e**, Full blot images for Figure 6c. Blue-dotted rectangles show the membrane edges. Red-dotted rectangles show the images in the text.