

Gene-edited murine cell lines for propagation of chronic wasting disease prions

Rupali Walia, Cheng Ching Ho, Chi Lee, Sabine Gilch and Hermann M. Schatzl

Supplementary Data

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CAD5_WT      CCTGGAGGGTGGAAACACCGGTGGAAGCCGGTATCCCAGGAGCCCTGGAGGCAAC
CAD5_KO2A    CCTGGAGGGTGGAAACACCGGTG-----
CAD5_KO2B    CCTGGAGGGTGGAAACACCGGTAT---CGGTATCCCAGGAGCCCTGGAGGCAAC
CAD5_KO2C    CCTGGAGGGTGGAAACACCGGTAT---CCCAGGAGCCCTGGAGGCAAC
*****.

CAD5_WT      CGTTACCCACCTCAGGGTGGCACCTGGGGGCAGCCCCACGGTGGTGGCTGGGGACAACCC
CAD5_KO2A    -----
CAD5_KO2B    CGTTACCCACCTCAGGGTGGCACCTGGGGGCAGCCCCACGGTGGTGGCTGGGGACAACCC
CAD5_KO2C    CGTTACCCACCTCAGGGTGGCACCTGGGGGCAGCCCCACGGTGGTGGCTGGGGACAACCC

CAD5_WT      CATGGGGGCAGCTGGGGACAACCTCATGGTGGTAGTTGGGGTCAGCCCCATGGCGGTGGA
CAD5_KO2A    -----
CAD5_KO2B    CATGGGGGCAGCTGGGGACAACCTCATGGTGGTAGTTG-----
CAD5_KO2C    CATGGGGGCAGCTGGGGACAACCTCATGGTGGTAGTTG-----

CAD5_WT      TGGGGCCAAGGAGGGGGTACCCATAATCAGTGAACAAGCCCAGCAAACCA
CAD5_KO2A    -----GAGGAGGGGGTACCCATAATCAGTGAACAAGCCCAGCAAACCA
CAD5_KO2B    -----GGGTCAGCCCCATGGCGGTGGAACAAGCCCAGCAAACCA
CAD5_KO2C    -----GGGTCAGCCCCATGGCGGTGGAACAAGCCCAGCAAACCA
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Supplementary Figure S1a: *Indel* signatures in CAD5-KO2 clone.

Genomic PCR was done from CAD5-KO2 cell clone using PrP-F and PrP-R primers and cloned into a bacterial mini library . Sequence comparison of ten bacterial inserts with the wild type control (CAD5_WT) shows : CAD5_KO2A having a large deletion between gRNA1 and gRNA2 (the same sequence appeared in 7/10 bacterial clones) while CAD5_KO2B and CAD5_KO2C had disruptions/smaller deletions at the gRNA1 and gRNA 2 site and appeared in 2/10 and 1/10 sequences respectively. Yellow and blue regions represent the gRNA1 and gRNA2 sites, respectively. Sequence in red represents *indels*.

CLUSTAL 2.1 multiple sequence alignment

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MEF_KO1      CCTGGAGGGTGGAAACACCGGTGGAAGCCGGTATCCCGGGCAGGGAAGCCCTGGAGGCAAC
MEF_KO2      CCTGGAGGGTGGAAACACCGGTGGAAGCCGGTATCCCGGGCAGGGAAGCCCTGGAGGCAAC
MEF_WT       CCTGGAGGGTGGAAACACCGGTGGAAGCCGGTATCCCGGGCAGGGAAGCCCTGGAGGCAAC
*****

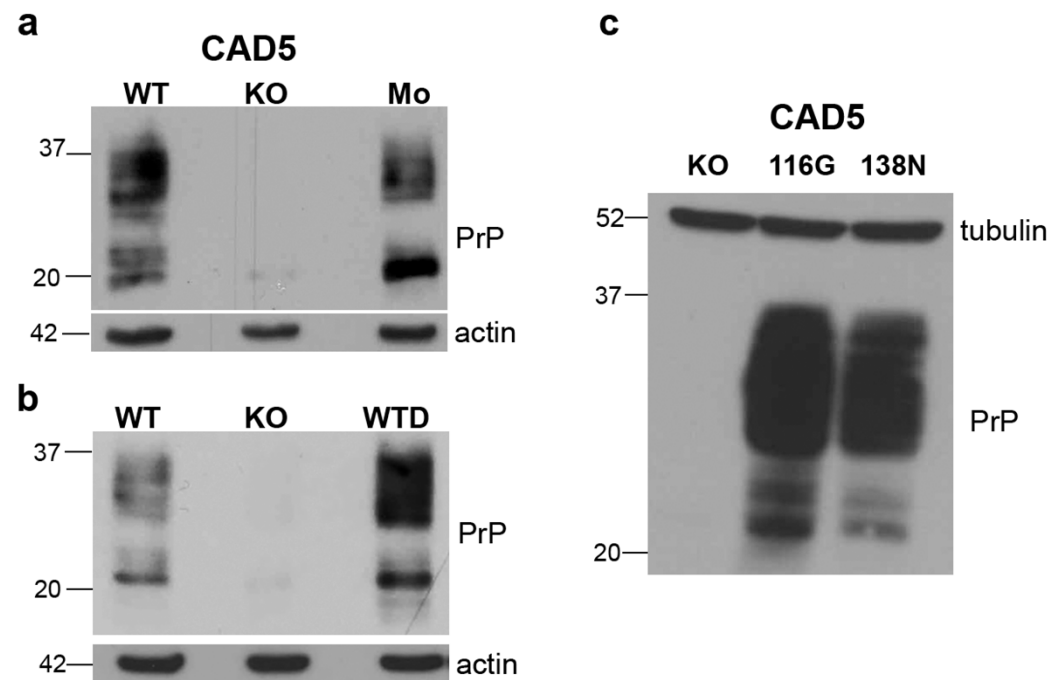
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MEF_KO2      CGTTACCCACCTCAGGGTGGCACCTGGGGGCAGCCCCACGGTGGTGGCTGGGGACAACCC
MEF_WT       CGTTACCCACCTCAGGGTGGCACCTGGGGGCAGCCCCACGGTGGTGGCTGGGGACAACCC
*****

MEF_KO1      CATGGGGCAGCTGGGGACAACCTCATGGTGGTAGTTGGGGTCAGCCCCATGGCGGTGGA
MEF_KO2      CATGGGGCAGCTGGGGACAACCTCATGGTGGTAGTTGGGGTCAGCCCCATGGCGGTGGA
MEF_WT       CATGGGGCAGCTGGGGACAACCTCATGGTGGTAGTTGGGGTCAGCCCCATGGCGGTGGA
*****

MEF_KO1      TGGGGCCAAGGAGGGGGTACCATAATCAGTGGAAACAAGCCAGCAAACCAAAAACCAA
MEF_KO2      TGGGGCCAAG-----GGGTACCATAATCAGTGGAAACAAGCCAGCAAACCAAAAACCAA
MEF_WT       TGGGGCCAAGG-AGGGGGTACCATAATCAGTGGAAACAAGCCAGCAAACCAAAAACCAA
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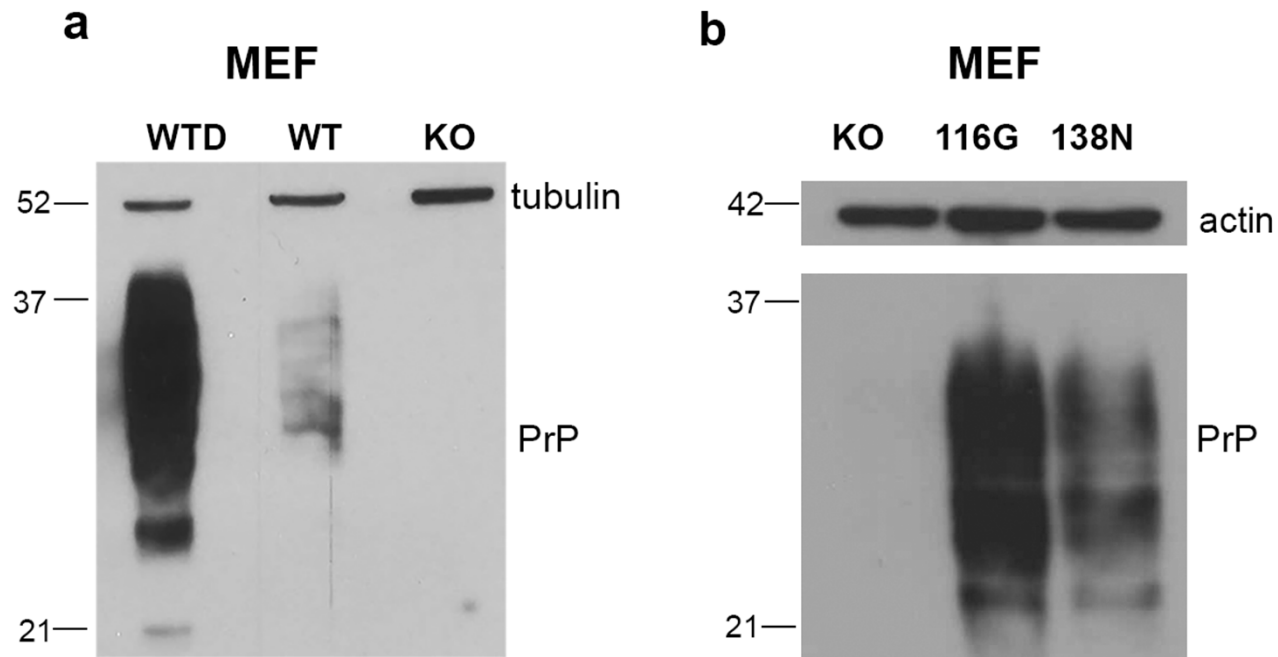
Supplementary Figure S1b: *Indel* signature in MEF-KO clones.

Genomic PCR was done from a representative CC9 clonal isolate of MEF cells using PrP-F and PrP-R primers. Sequence comparison of PCR amplicons (cloned into a bacterial mini library - KO1 and KO2) with the wild type (MEF_WT) shows *indels* at the gRNA2 target site. Yellow and blue regions represent the gRNA1 and gRNA2 sites, respectively. Sequence in red represents *indels*.



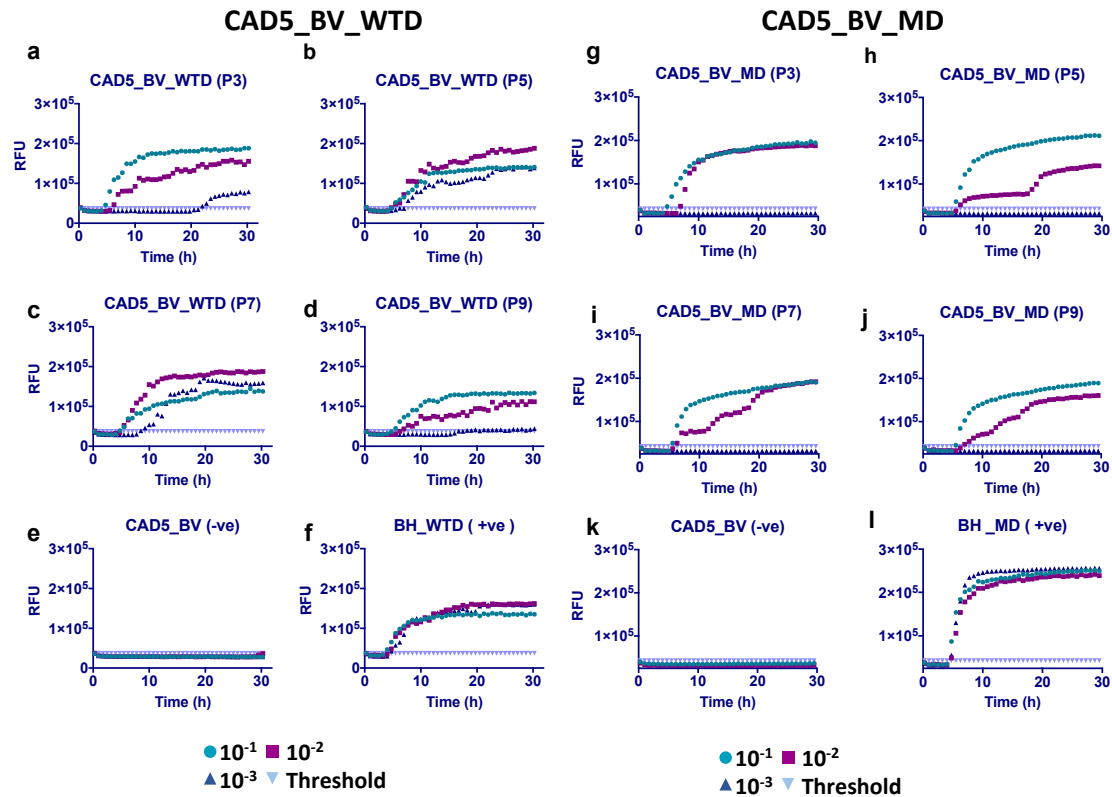
Supplementary Figure S2: Stable reconstitution of CAD5 PrP^{-/-} cells (KO2) with MoPrP and cervid WTD, 116G, and 138N PrP using lentiviral transduction.

(a) Western blot analysis showing expression of Mo-PrP in transduced cells. **(b)** Western blot showing the expression of wild-type WTD-PrP (white-tailed deer) in transduced cells. **(c)** Western blot showing expression of 116G and 138N-PrP in transduced cells. WT represents the parental wild-type CAD5 cells expressing murine PrP and KO represents the CAD5 knock-out background (KO2). The blots were probed with anti-PrP mAb 4H11 and actin or tubulin was used as a loading control



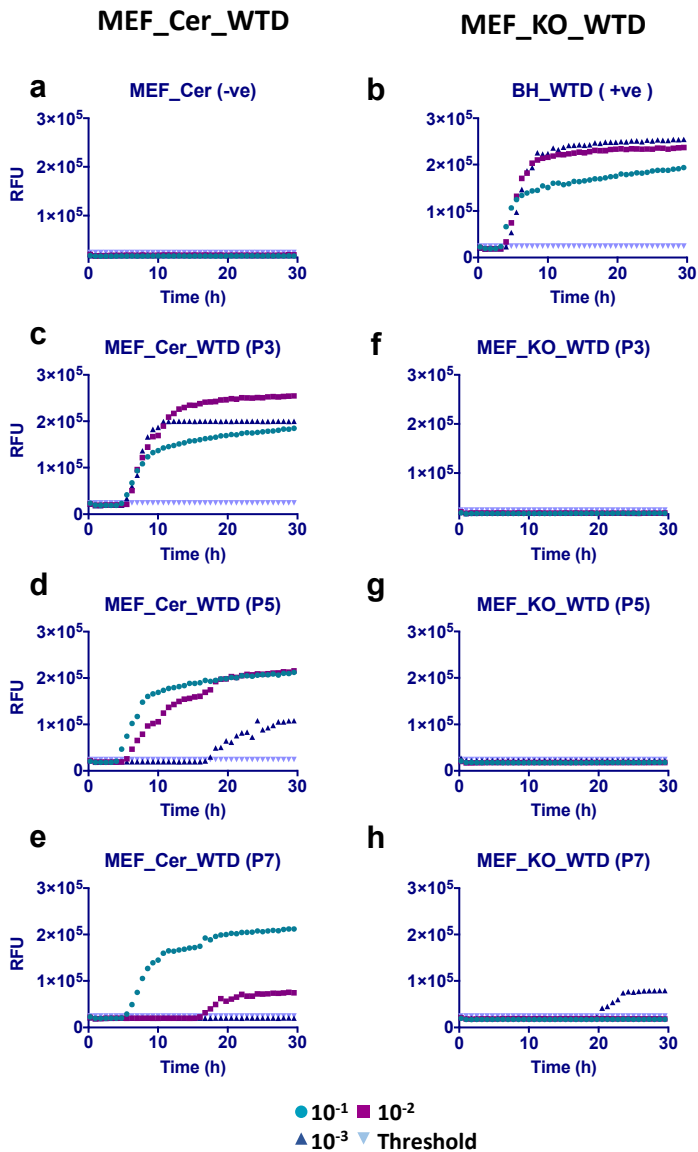
Supplementary Figure S3: Stable reconstitution of MEF PrP^{-/-} cells (KO1) with cervid WTD, 116G and 138N polymorphic PrP variants using lentiviral transduction.

(a) Western blot analysis showing the expression of wild type WTD-PrP (white-tailed deer) in transduced cells. **(b)** Western blot showing expression of 116G and 138N-PrP in transduced cells. WT represents the parental MEF wild-type cells expressing murine PrP and KO represents the MEF knock out cells (KO1). The blots were probed with anti-PrP mAb 4H11 and tubulin was used as a loading control.



Supplementary Figure S4: RT-QuIC analysis showing positive prion seeding activity in CAD5_BV cells with white-tailed deer (WTD) and mule deer (MD) CWD prions.

RT-QuIC assay was setup as done in Fig. 5. A positive seeding activity was seen up to passage P9 in cells infected with white-tailed deer (CAD5_BV_WTD) prions (**a-d**) as well as with mule deer (CAD5_BV_MD) CWD prions (**g-j**). (**e, k**) represent uninfected cell lysate at passage 3 (negative control). (**f, l**) 1% WTD- and MD-brain homogenate (BH) was used as positive control for the assay.



Supplementary Figure S5: RT-QuIC analysis showing positive prion seeding activity in MEF_Cer cells infected with WTD CWD prions and absence of seeding activity in infected knock out cells (MEF_KO_WTD).

RT-QuIC assay was setup as done in Fig. 5. A positive seeding activity was seen in passage P3-P7 in cells infected with white-tailed deer (MEF_Cer_WTD) prions **(c-e)**. No seeding activity was observed in infected non-reconstituted knock out (MEF_KO_WTD) cells **(f-h*)**. **(a)** represents uninfected cell lysate at passage 3 (negative control). **(b)** 1% WTD-brain homogenate (BH) was used as positive control for the assay.

* Sample h is negative as only one out of four replicates showed a weak signal. Samples are scored positive when at least 50% of the replicates (≥ 2 out of 4) reached a ThT fluorescence cut-off.

Supplementary Table S1: Oligonucleotides and primers used in this study

No.	Oligo/ Primer	Sequence
1	gRNA1	GGTGGAACACCGCTGGAAGC
2	gRNA2	GATTATGGGTACCCCCTCCT
3	PrPF	ATGGCGAACCTTGGCTACTGGCTG
4	PrPR	TCATCCCACGATCAGGAAGATG
5	LV- Mo-PrPF	TTTTTGAATTCGCCACCATGGCGAACCTTGGCTACTGGCTG
6	LV- Mo-PrPR	TTTTGGATCCTCATCCCACGATCAGGAAGATG
7	LV- BV-PrPF	GATGATGGATCCATGGCGAACCTCAGCTACTG
8	LV- BV-PrPR	ACGCGTTCATCCCACGATCAGGAAGA
9	LV- Cer-PrPF	GATGATGGATCCATGGTGAAAAGCCACATAGGC
10	LV- Cer-PrPR	ACGCGTCTATCCTACTATGAGAAAAAT

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Supplementary: Original Blots

Figure 2d, 2e, 2f

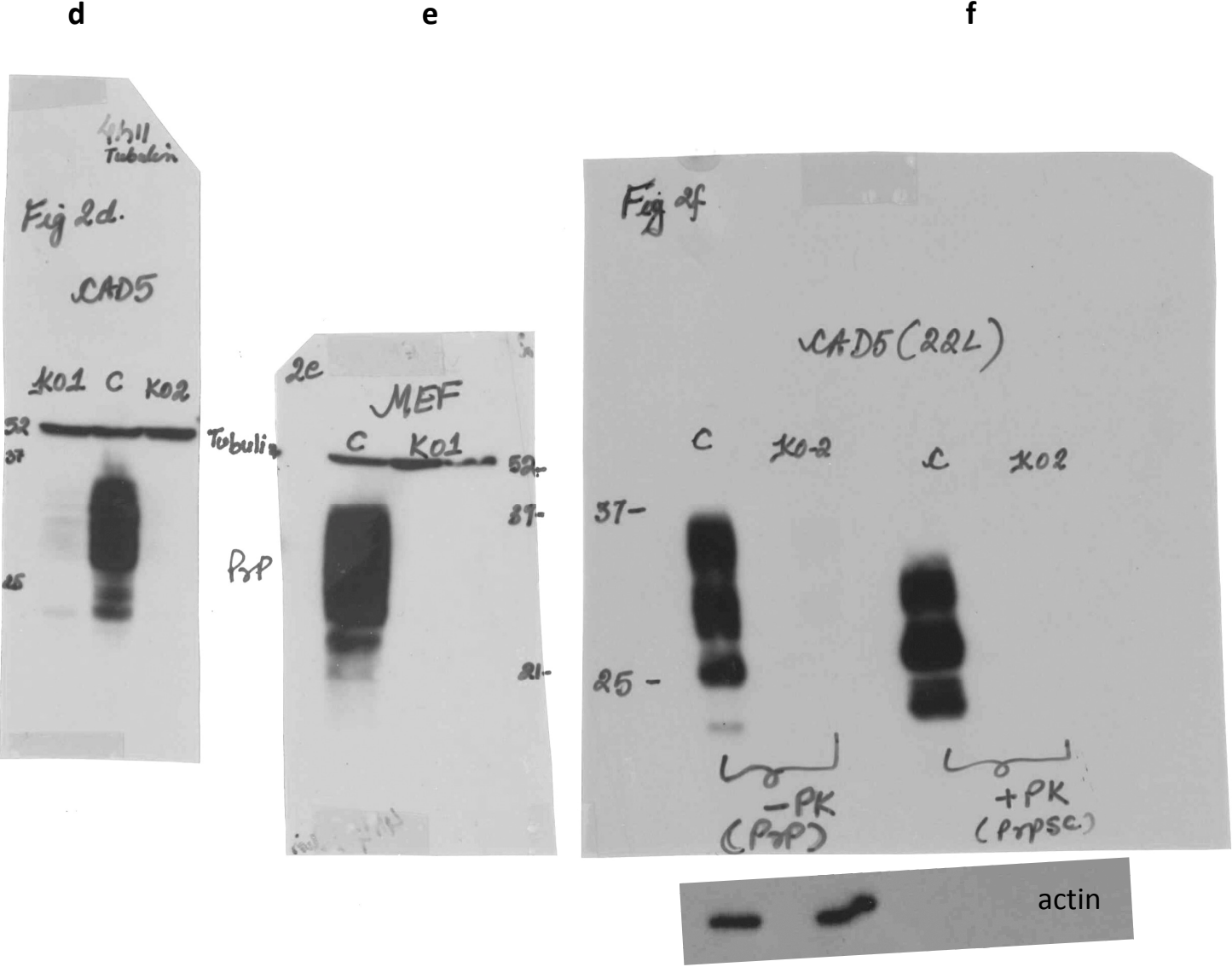
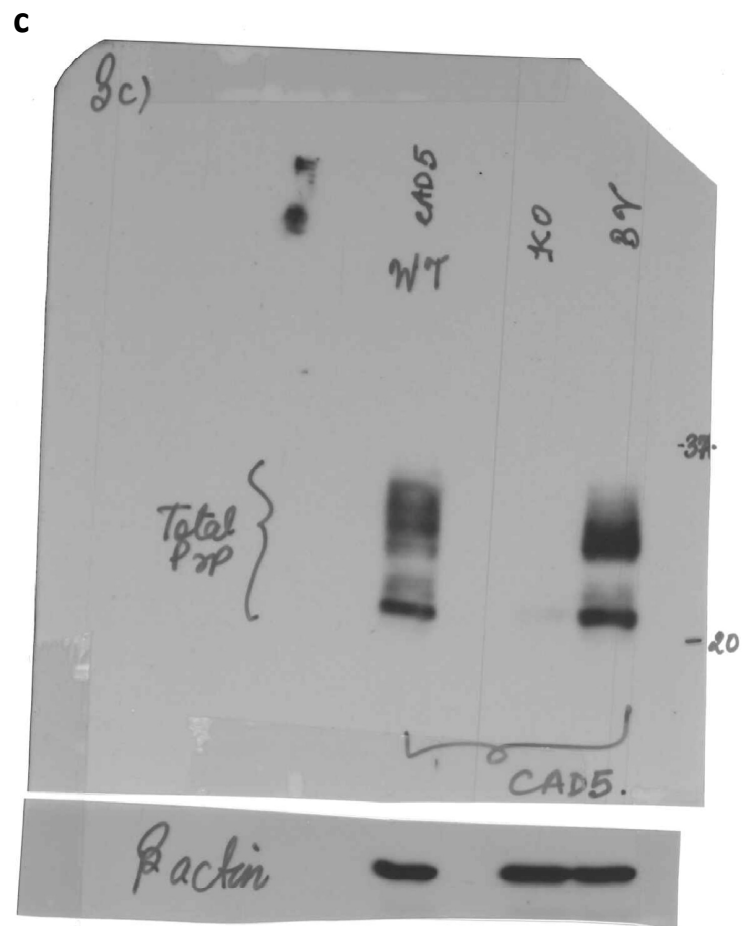


Figure 3c, 3f



f

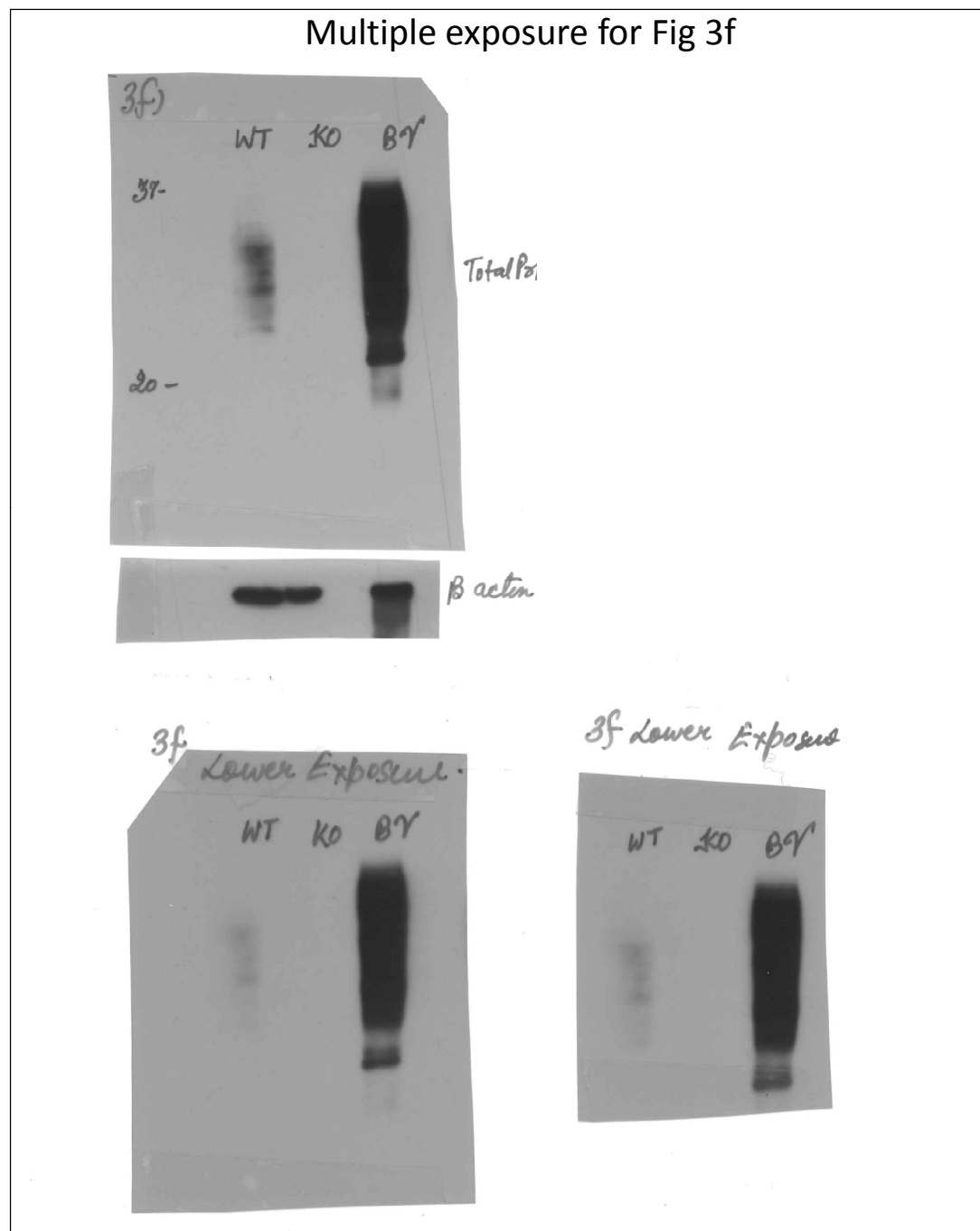
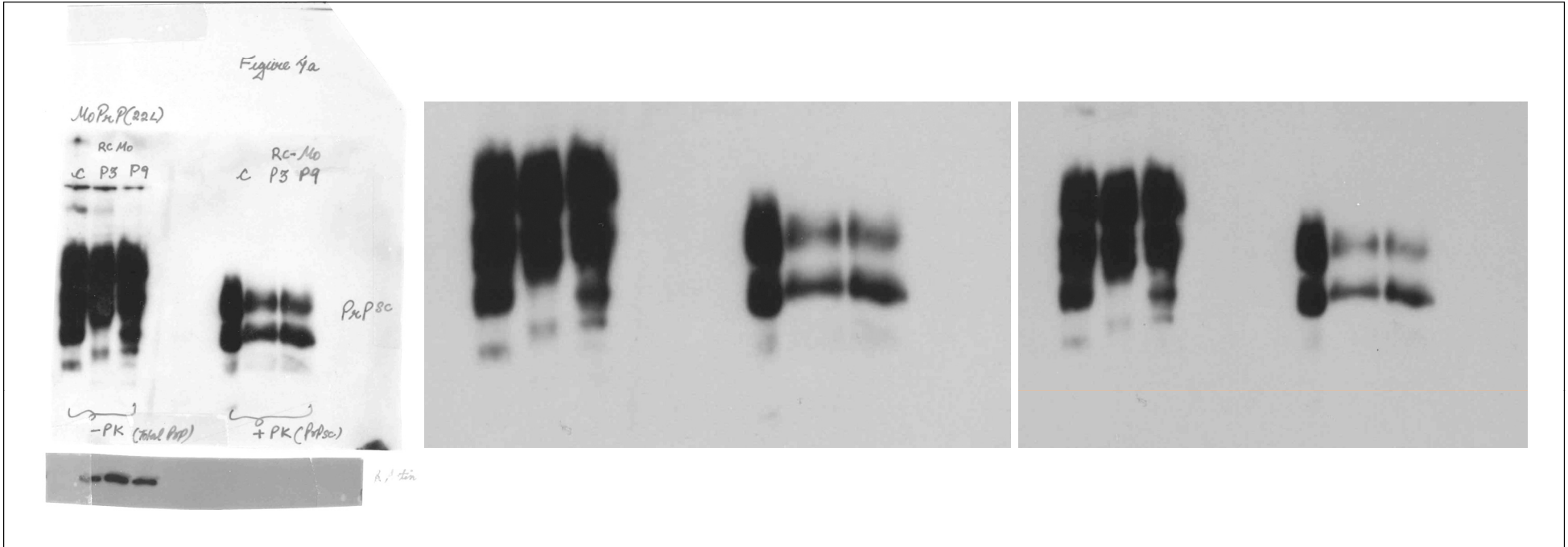


Figure 4a , 4b

Multiple exposure for Fig 4a and 4b

a



b

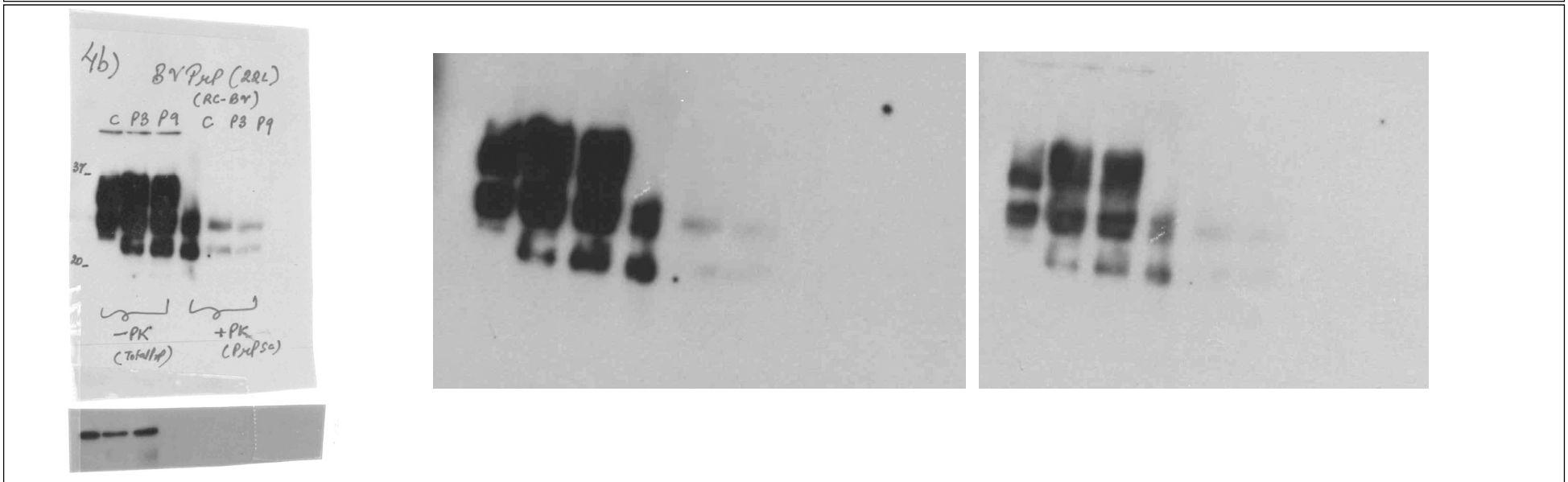


Figure S2 a, b, c

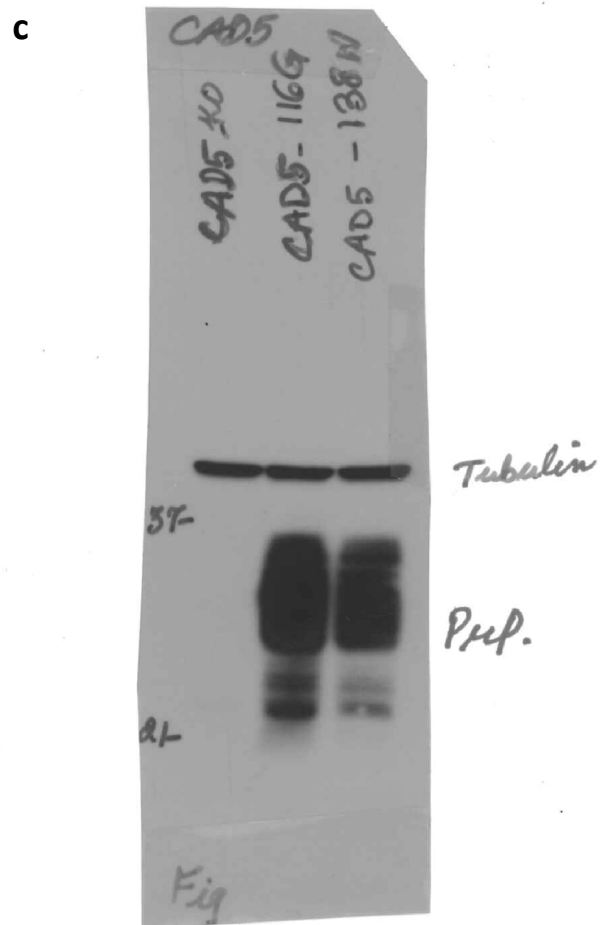
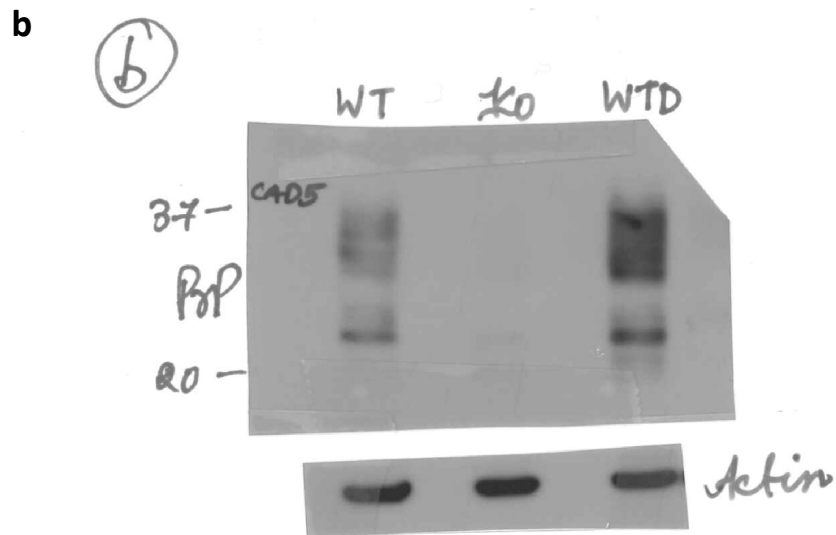
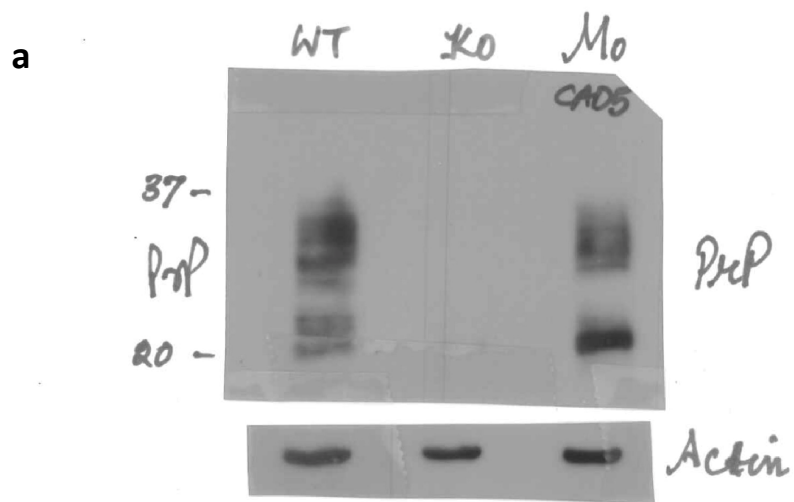
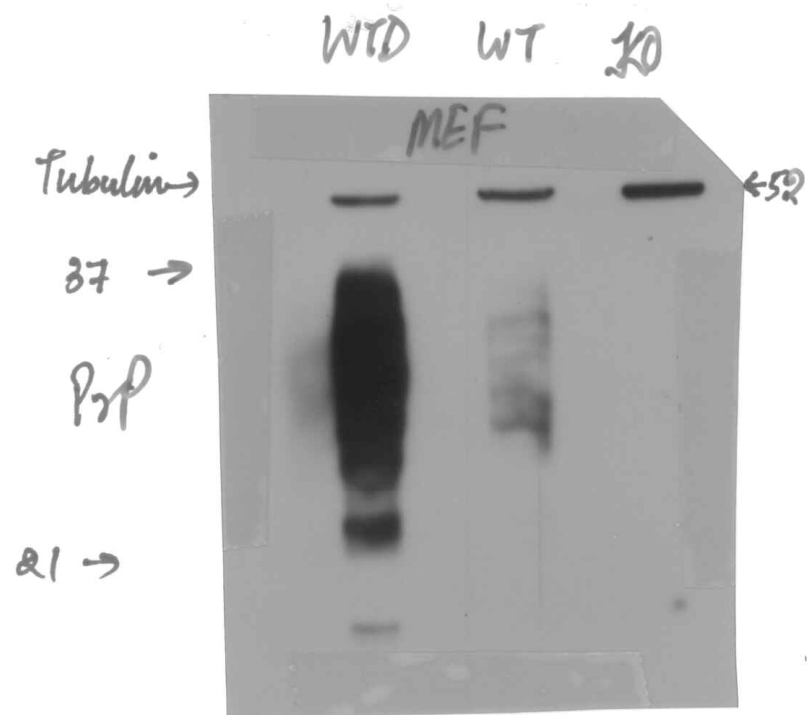


Figure S3 a, b

a



b

