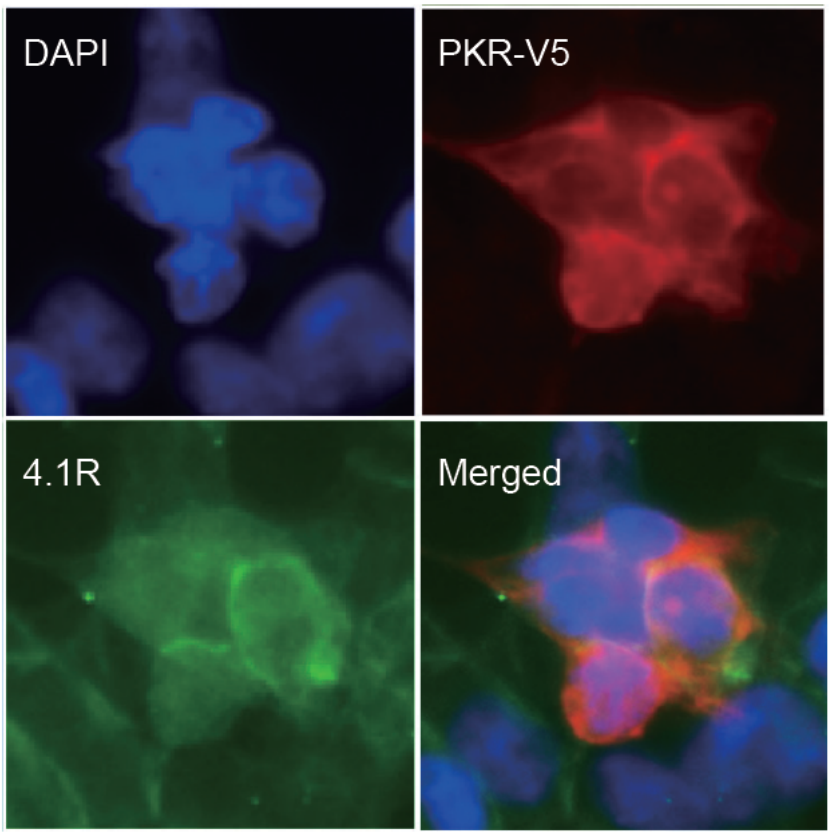
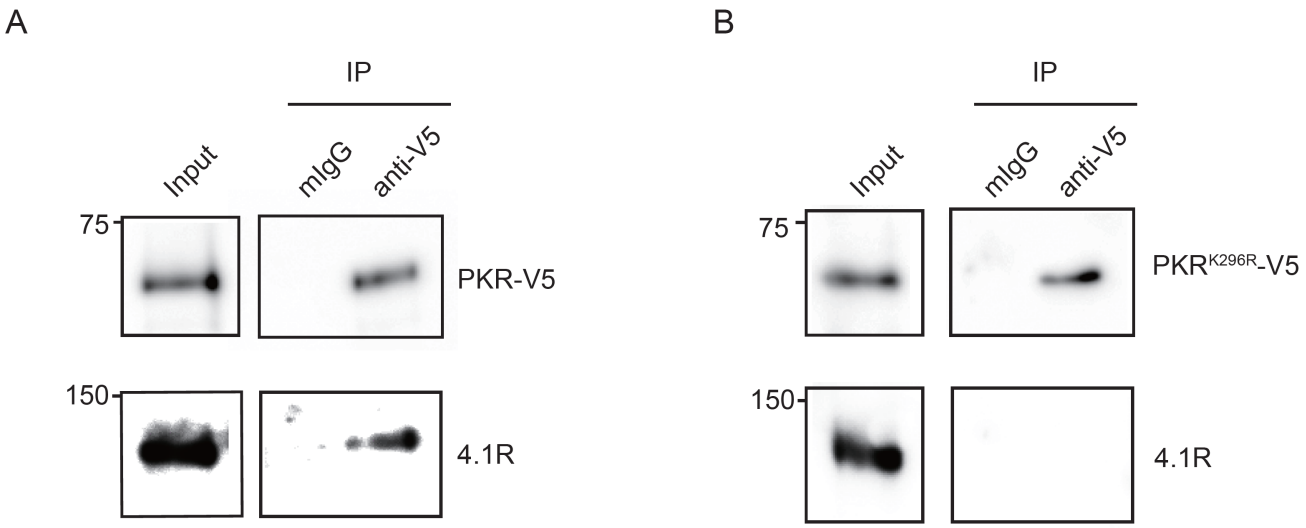
Supplementary figure 1:



Endogenous 4.1R and PKR proteins co-localize in the cell membrane.

The localization of 4.1R and PKR in HEK293FT cells was examined by immunofluorescence. HEK293FT cells were transfected with PKR-V5. The signals of PKR-V5 and the 4.1R proteins are shown in red and green, respectively. Details of the experimental methods were described previously.[1]

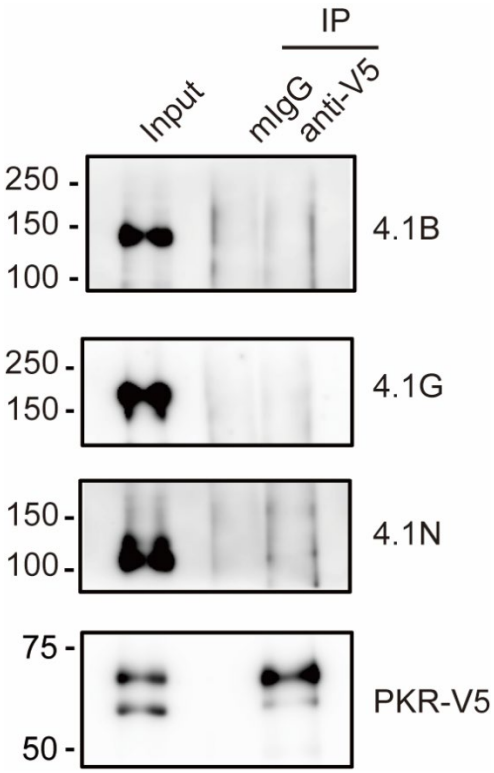
Supplementary figure 2:



Endogenous interaction between PKR and 4.1R.

HEK293FT cells were transfected with PKR-V5 (A) and PKR^{K296R}-V5 (B). Each bottom panel indicates the co-precipitations of endogenous 4.1R with PKR or PKR^{K296R} by IP-with anti-V5 or control antibody followed by WB with anti-4.1R antibody. Each top panel shows the result for PKR-V5. Details of the experimental methods were described previously.[1, 2]

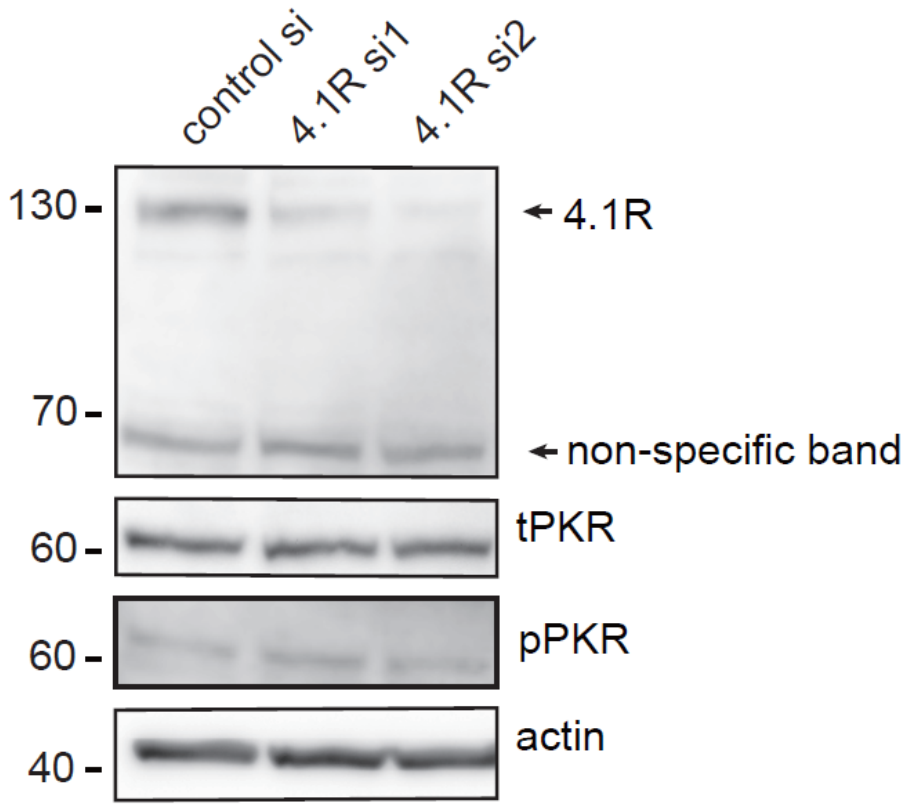
Supplementary figure 3:



Interaction between PKR and the 4.1 protein family.

HEK293FT cells were transiently transfected with PKR-V5. The top 3 panels indicate the co-precipitations of 4.1B, 4.1G, and 4.1N with PKR by IP with anti-V5 or control antibody followed by WB with anti-4.1B, 4.1G, and 4.1N antibodies, respectively. The bottom panel shows the result for PKR-V5. Details of the experimental methods were described previously.[1, 2]

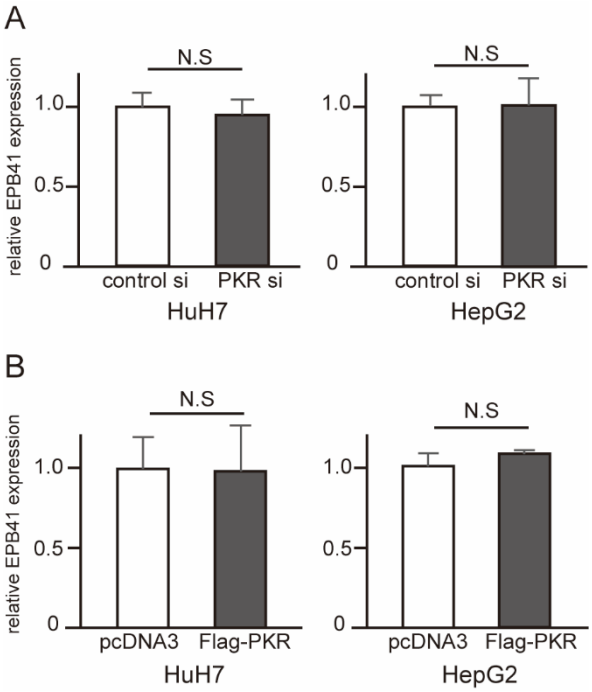
Supplementary figure 4:



Expression level of PKR in HuH7 cells after transfection of 4.1R siRNA.

HuH7 cells were transfected with control siRNA and two 4.1R siRNAs, followed by WB with anti-4.1R antibody (top panel), anti-total PKR antibody (2nd panel), anti-phospho-PKR (third panel), and anti-actin antibody (bottom panel).

Supplementary figure 5:

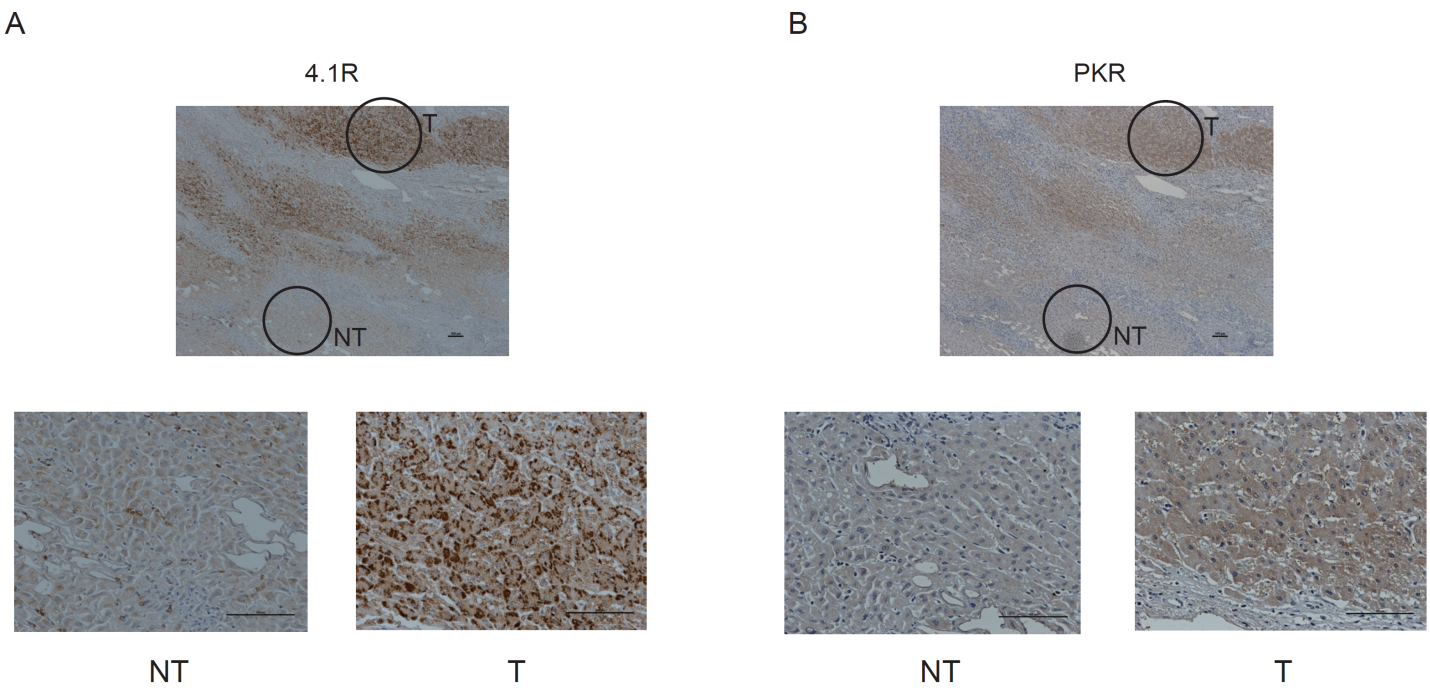


RNA expression of 4.1R after knockdown or overexpression of PKR in HCC cell lines.

(A) HuH7 and HepG2 cells were transfected with control siRNA or PKR siRNA, followed by real-time RT PCR. Relative changes of 4.1R RNA in PKR siRNA-transfected HuH7 cells (left panel) and HepG2 cells (right panel) are shown.

(B) HuH7 and HepG2 cells were transfected with or without Flag-PKR, followed by real-time RT PCR. Relative changes of 4.1R RNA by PKR overexpression in HuH7 cells (left panel) and HepG2 cells (right panel) are shown. Mean ± SD of four replicates. The fold changes of expressions of 4.1R were compared with control by the two-sided Student's *t*-test.

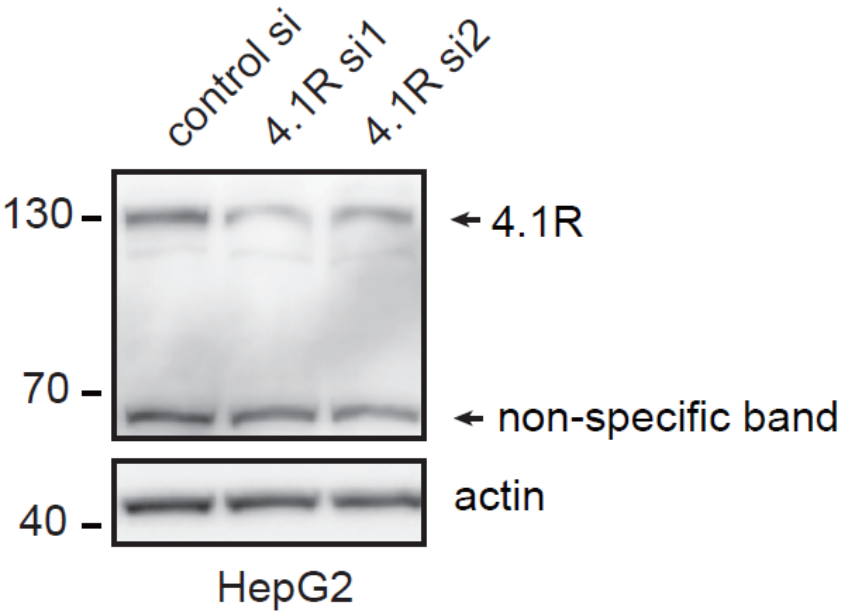
Supplementary figure 6:



Immunohistochemical staining for 4.1R and PKR in HCC patients.

Immunohistochemical staining for 4.1R (A) and PKR (B) was performed in tumor (T) and nontumor (NT) tissues. The hepatocytes of T and NT were stained with anti-4.1R or anti-PKR antibody. Lower panels show a magnified image of the field indicated by circles in the upper panel. Scale bar: 100 μm. Details of the experimental methods were described previously.[3]

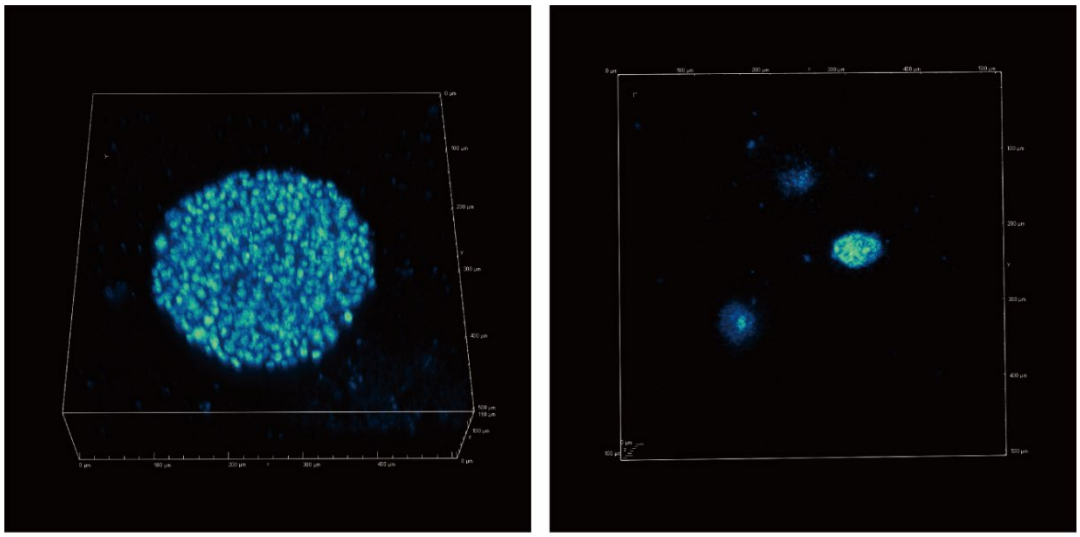
Supplementary figure 7:



Transfection efficiency of 4.1R siRNA in HepG2 cells was evaluated by WB.

HepG2 cells were transfected with control siRNA and two 4.1R siRNAs, followed by WB with anti-4.1R antibody (top panel) and anti-actin antibody (bottom panel).

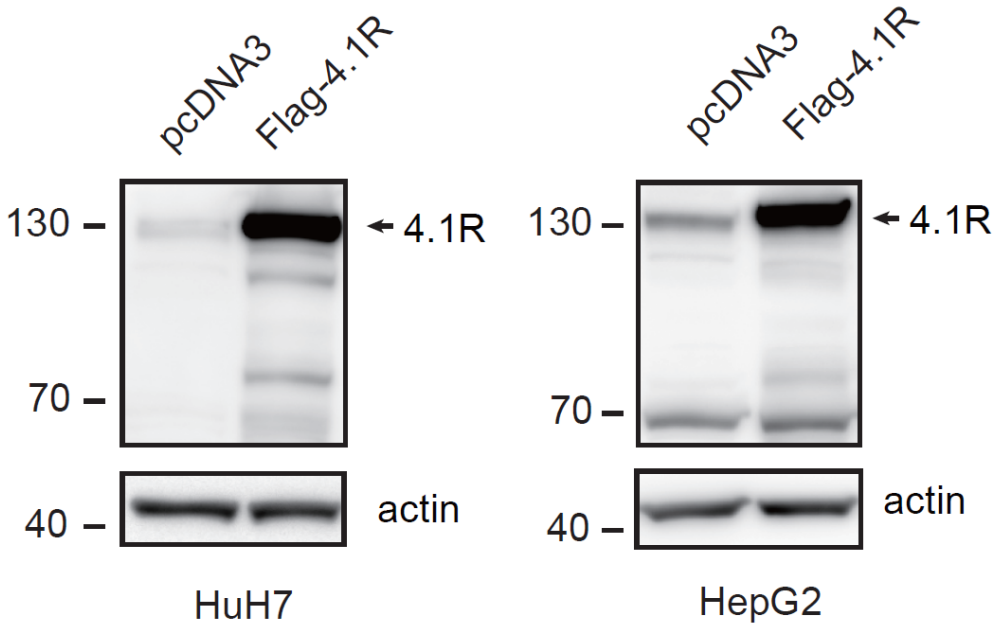
Supplementary figure 8:



3D reconstructed images of the nuclei in the HuH7 colony treated with 4.1R siRNA.

Using a confocal microscope, colonies were observed with the fluorescent signals from Hoechst 33342 staining nuclei of HuH7 cells.

Supplementary figure 9:



Transfection efficiency of 4.1R plasmid in HuH7 and HepG2 cells evaluated by WB.

HuH7 (left panel) and HepG2 cells (right panel) were transfected with or without Flag-4.1R, followed by WB with anti-4.1R antibody (top panel) and anti-actin antibody (bottom panel).

Supplementary table 1: Antibodies used in this study

Antibody name	Product	Dilution ratio
Beta-actin	MBL (Tokyo, Japan) Cat#M177-3	1:2000
PKR	CST (Danvers, MA, USA) Cat#3072	1:1000
Phosphorylated PKR	Abcam (Cambridge, UK) Cat#ab32036	1:1000
4.1R	Proteintech (Rosemont, IL, USA) Cat#13014-1-AP	1:1000
Myc	CST Cat#2272	1:1000
Flag	Trans Genic (Fukuoka, Japan) Cat#KO602-S	1:1000

Supplementary table 2: Human siRNA sequences to inhibit PKR and 4.1R

siRNA name	siRNA sequence
siGENOME Human EIF2AK2 siRNA #1	CAAAUUAGCUGUUGAGAUA
siGENOME Human EIF2AK2 siRNA #2	GGAAAGACUUACGUUAUUA
siGENOME Human EPB41 siRNA #1	GAAAGUCUGUGUAGAACAU
siGENOME Human EPB41 siRNA #2	UGACACAGUUUAUGAAUGU

Reference

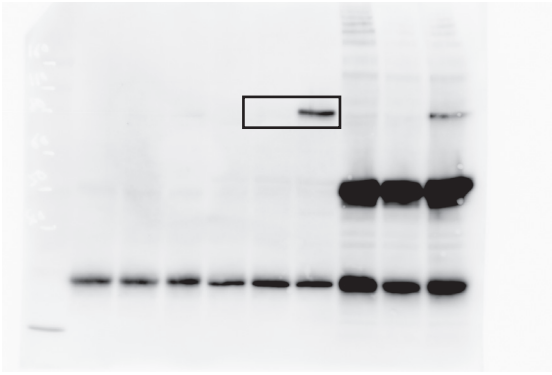
1. Funaki T, et al. CADM1 promotes malignant features of small-cell lung cancer by recruiting 4.1R to the plasma membrane. *Biochem Biophys Res Commun.* **534**, 172-178 (2021).

2. Ito T, et al. CADM1 associates with Hippo pathway core kinases; membranous co-expression of CADM1 and LATS2 in lung tumors predicts good prognosis. *Cancer Sci.* **110**, 2284-2295 (2019).

3. Koizumi M, et al. Apoptosis-associated speck-like protein containing a CARD regulates the growth of pancreatic ductal adenocarcinoma. Sci Rep. **16**, 22351 (2021).

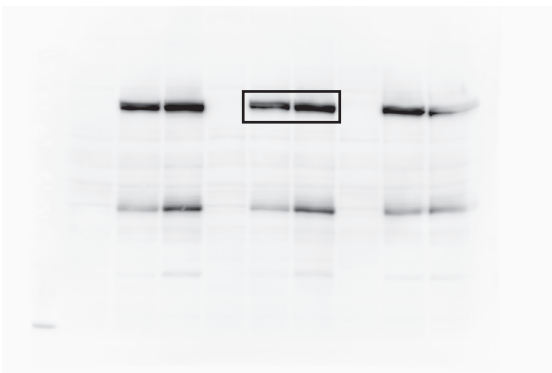
Presentation of full-length gels and blots in Figures
(Figures 1 and 2 and Supplementary Figures 2, 3, 4, 7 and 9)

Fig. 1A



IP: Flag
IB: Myc

IP: Flag
IB: Flag



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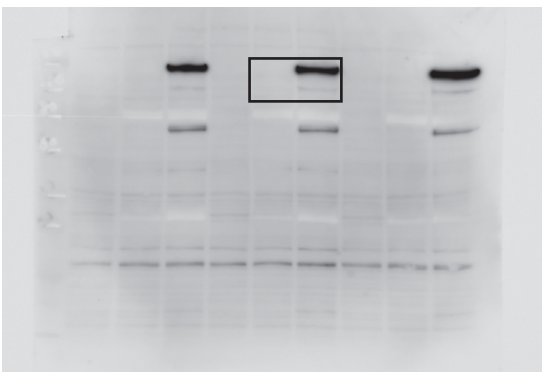

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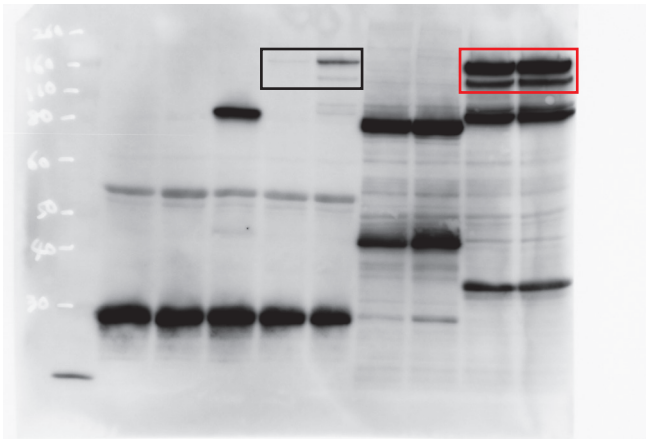
Fig. 1B



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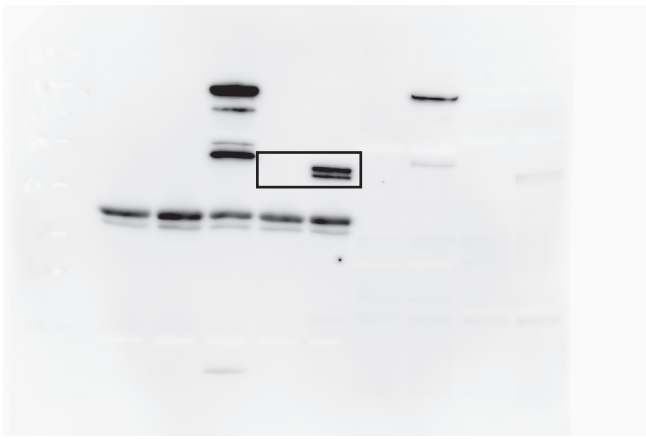

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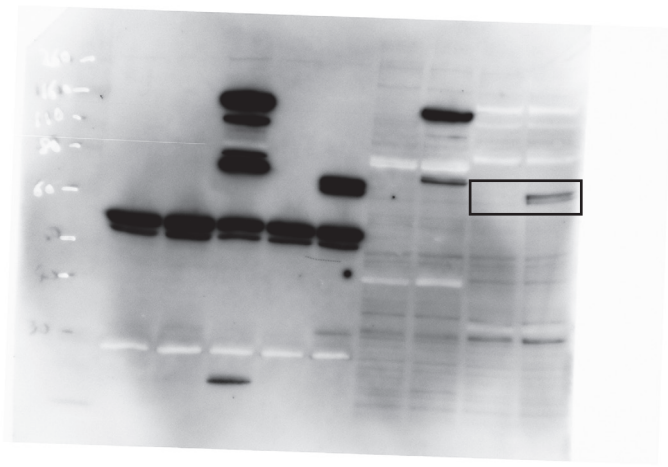

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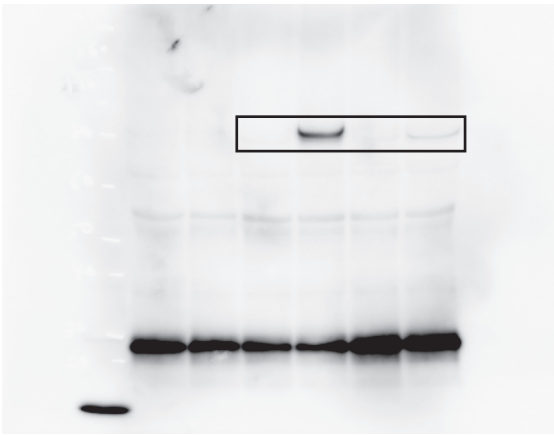
Fig. 1C



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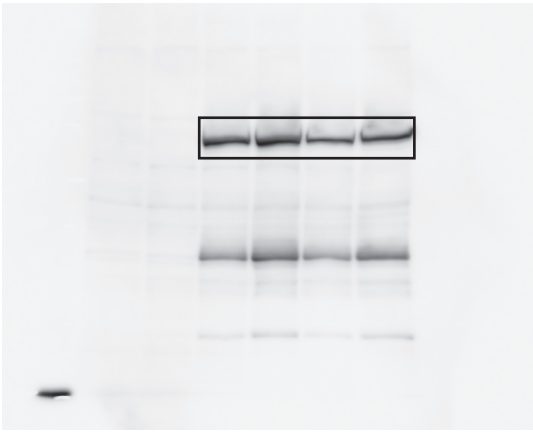

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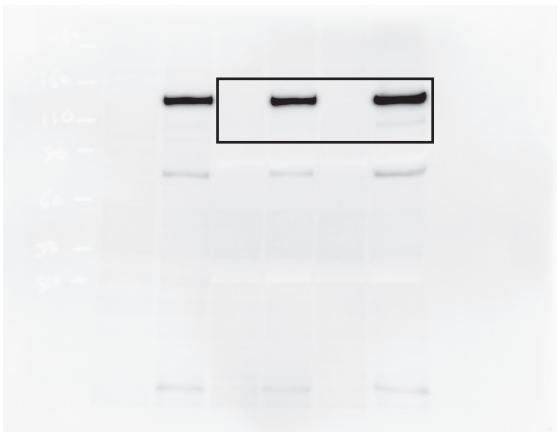

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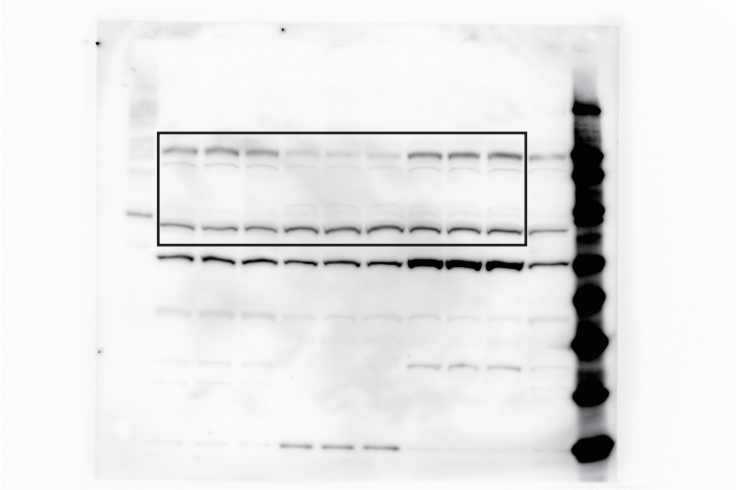
Figure 2A



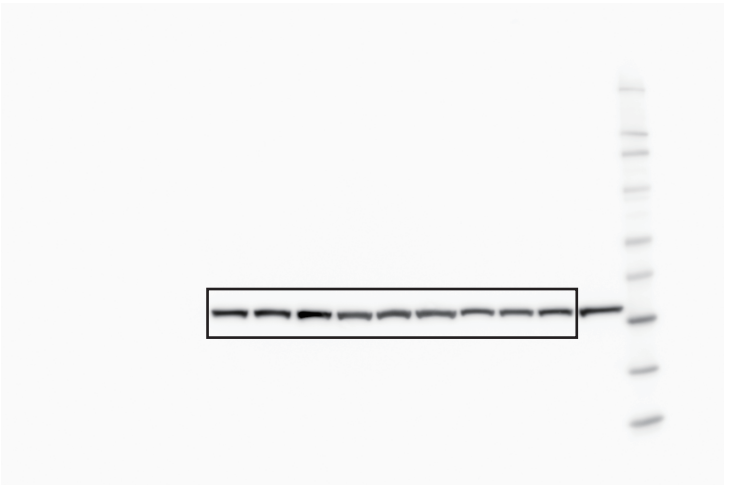
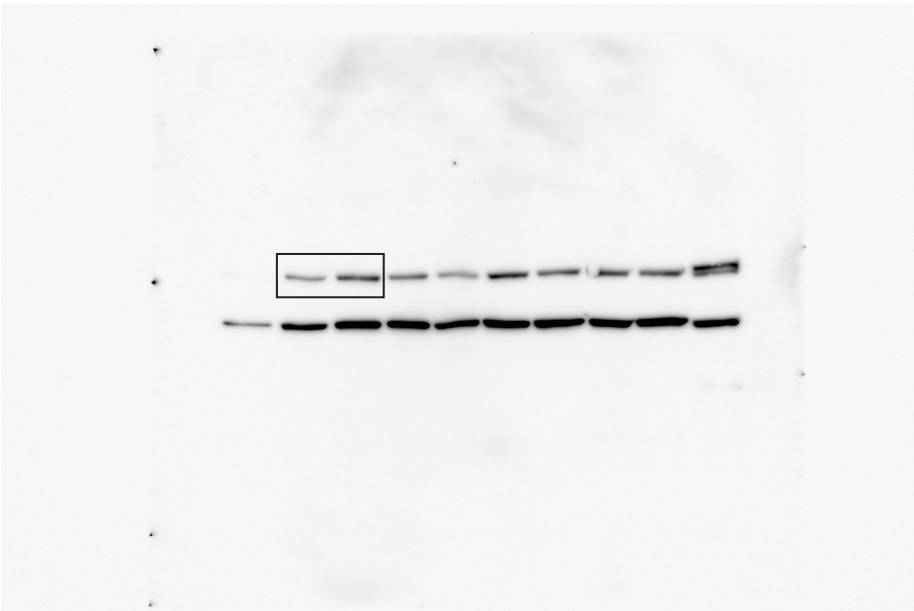
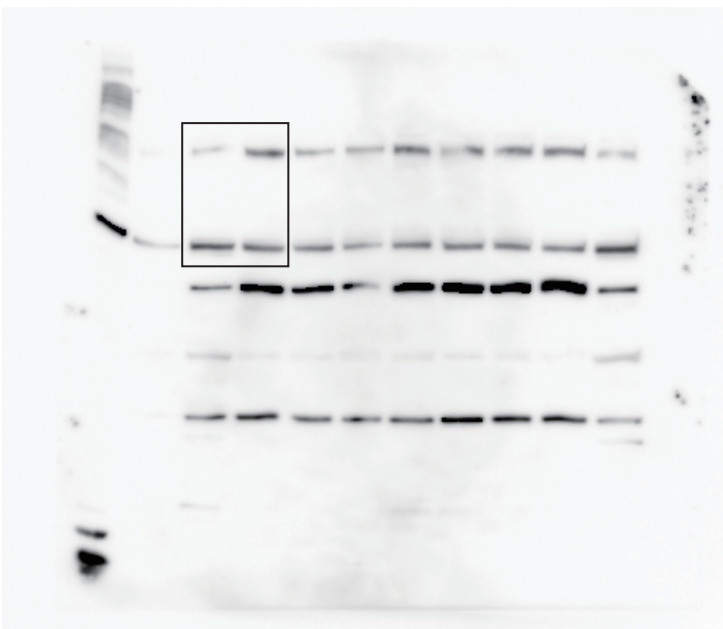


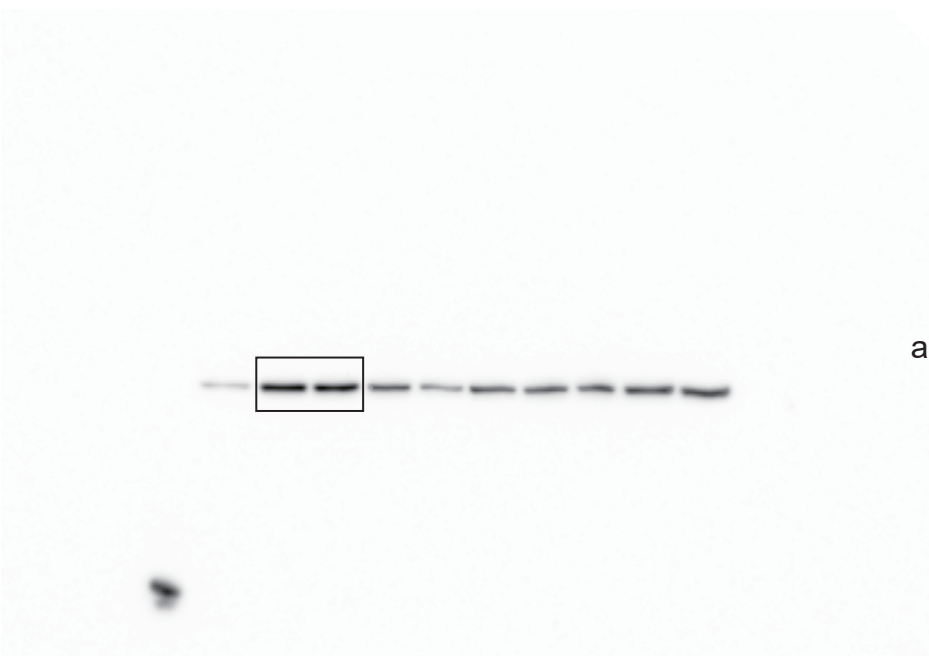
Fig. 2B



PKR

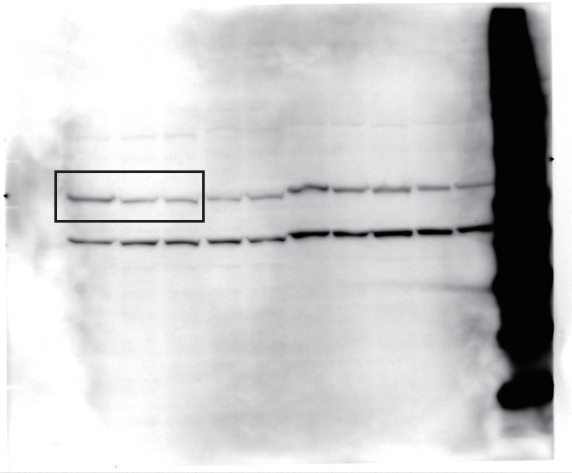


4.1R



actin

Fig. 2C



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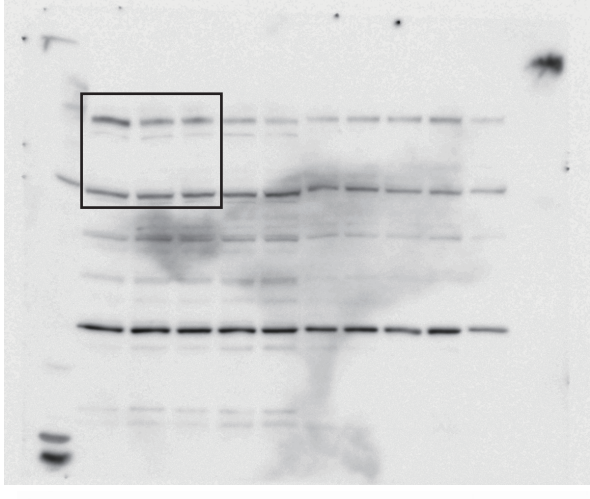

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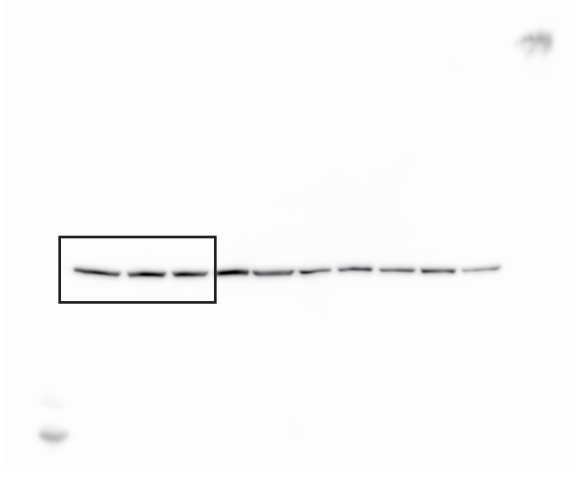

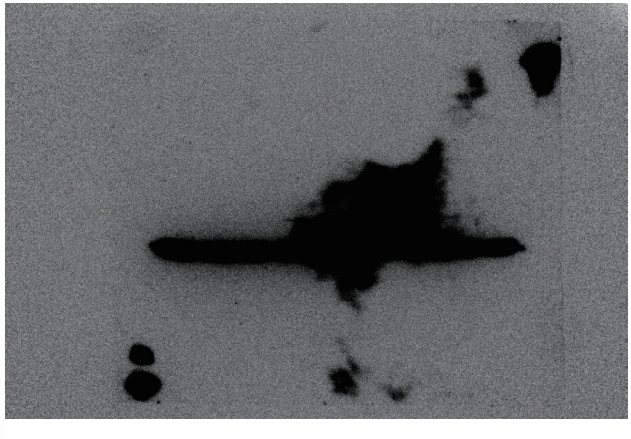

actin

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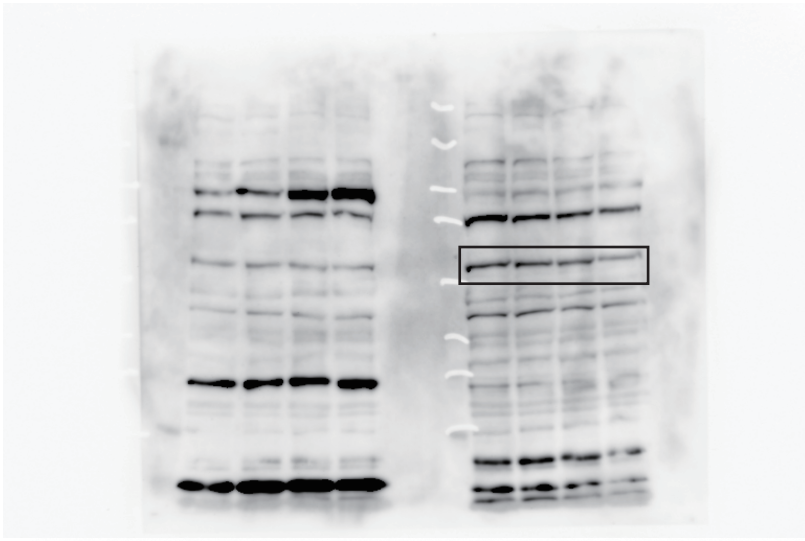
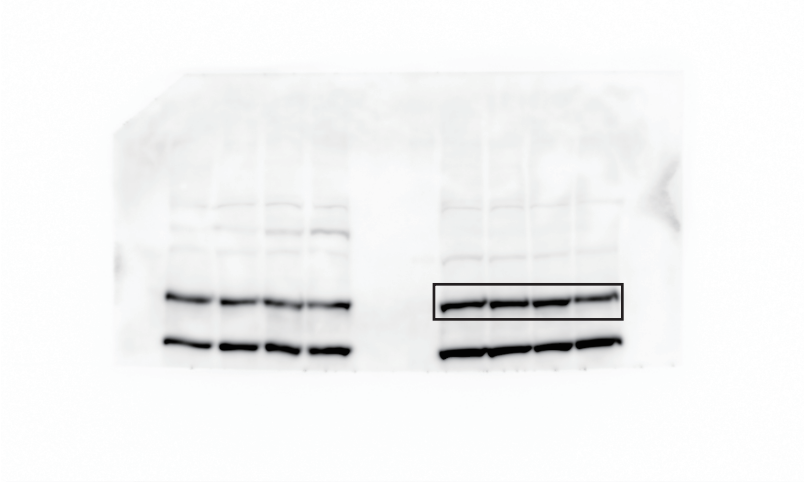
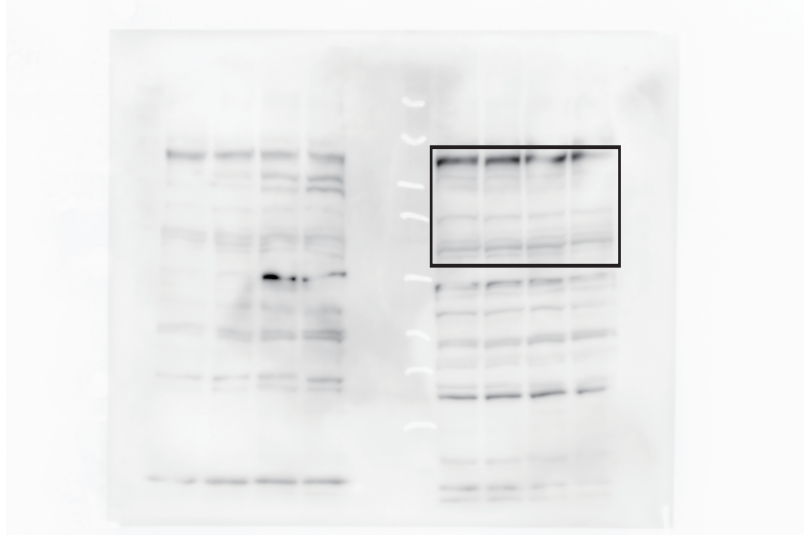
Fig. 2D



pPKR



tPKR



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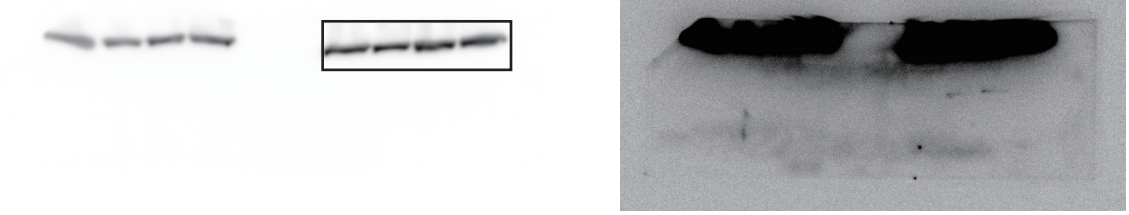

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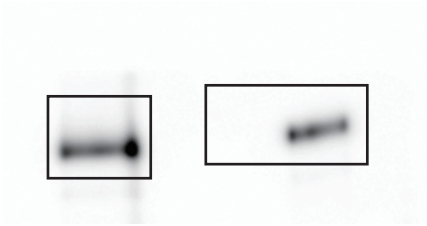
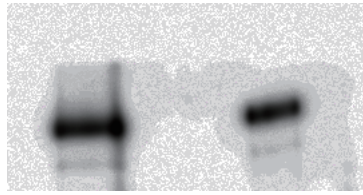
Supplementary Fig. 2





PKR-V5

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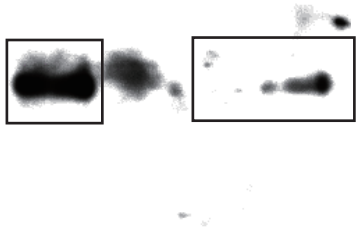

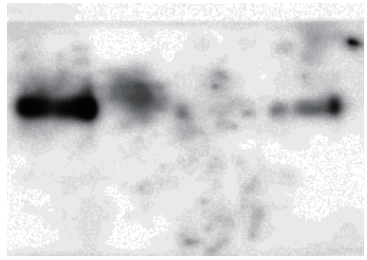

4.1R

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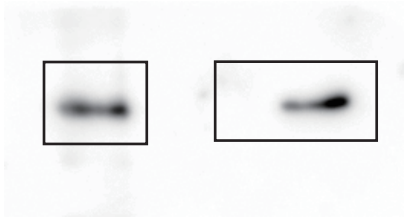
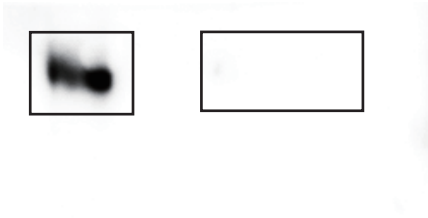
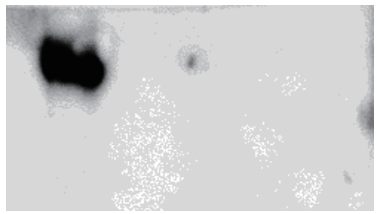


PKR^{K296R}-V5





4.1R

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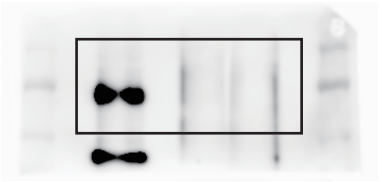
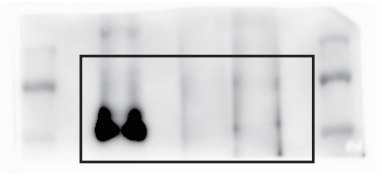
Supplementary Fig. 3

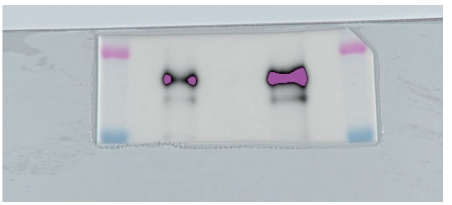


4.1B

4.1G



4.1N



PKR-V5

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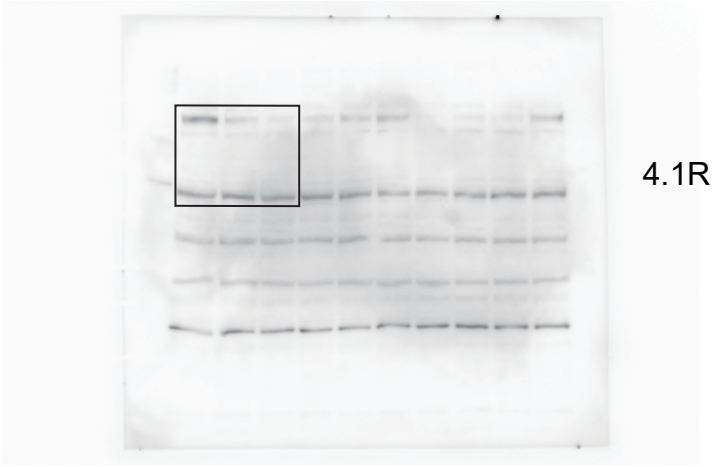
Supplementary Fig. 4



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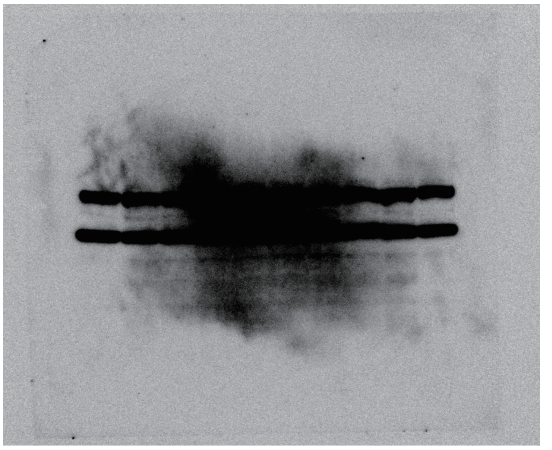

tPKR

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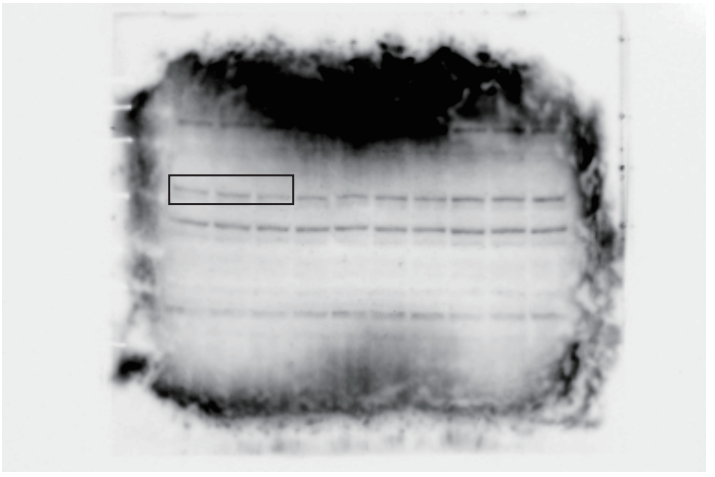
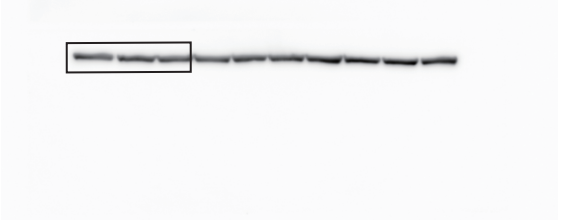


pPKR



actin

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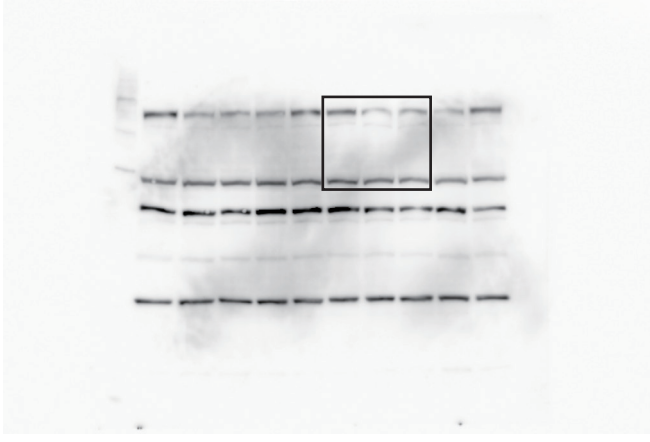
Supplementary Fig. 7



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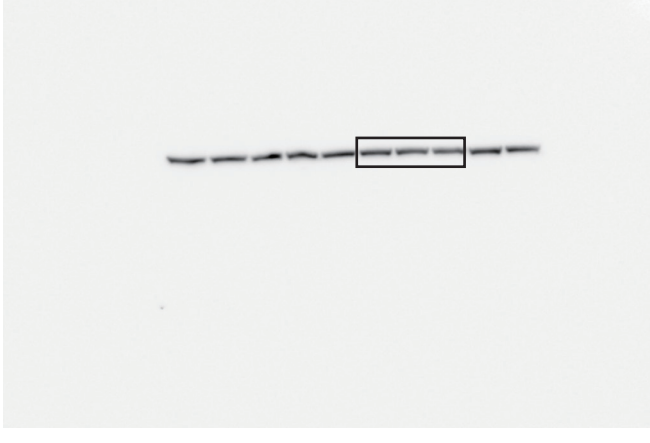
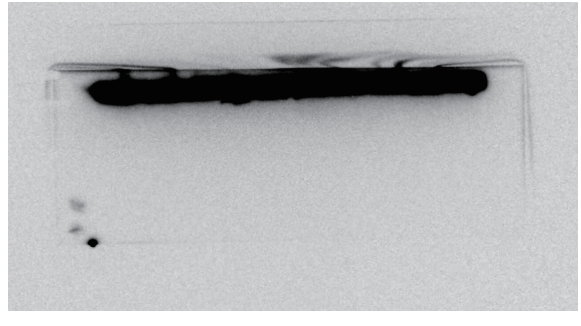
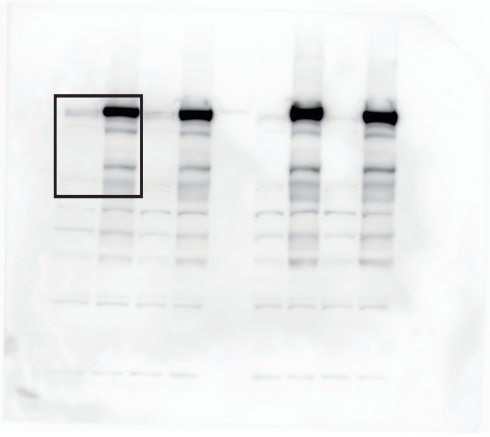




actin

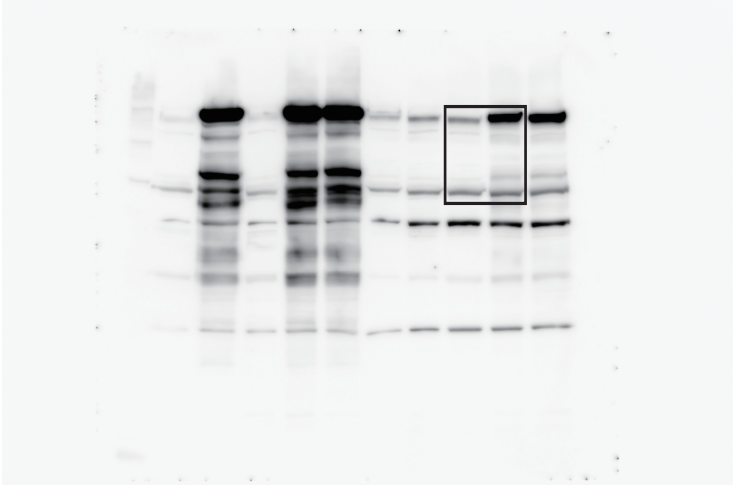
Image modified to reveal edges

Supplementary Fig. 9



HuH7
4.1R

HuH7
actin



HepG2
4.1R

HepG2
actin