

782 **Supplementary Material**

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784 **Tagging enhances histochemical and biochemical detection of Ran Binding Protein**

785 **9 *in vivo* and reveals its interaction with Nucleolin**

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807 **Supplementary Figures**

808

809 **Figure S1.** Generation of the *RanBP9-TT* mouse model by CRISPR/Cas9. **A)** Depiction
810 of guide RNA (sgRNA; light GREEN) selected to target the last codon of *RanBP9* and
811 relative on-target (MAROON) and off-target (LIGHT BROWN) scores (Benchling). **B)**
812 sgRNA sequence and scores (Benchling). **C)** Predicted off-target sites and scores using
813 the selected sgRNA (Benchling). **D)** Virtual PCR prediction for screening of positively
814 recombined *RanBP9-TT* KI animals. **E)** Sequence of primers used for mutant KI
815 screening and expected size (base pair) of amplicons for *WT* and *RanBP9-TT* animals.

816

817 **Figure S2.** IHC detection of *RanBP9* by α V5 or α HA in homozygous *RanBP9-TT* mice
818 compared with detection by α *RanBP9* specific antibody. Sections from same organs
819 shown in Figure 2. **A-D)** Cerebellum; **E-H)** Testis; **I-L)** Lung. Sections from indicated
820 organs from *RanBP9 WT* (**A, B, D, E, F, H, I, J, L**) and *RanBP9-TT* mice (**C, G, K**)
821 were stained with α V5 (**A, E, I**), α HA (**B, F, J**) or with Hematoxylin and Eosin (H&E)
822 (**C, D, G, H, K, L**). All pictures were taken with 40x objective and 10x eyepiece (400x).
823 Scale bar = 50um.

824

825 **Figure S3.** IHC detection of V5 in *RanBP9-TT* mice. **A-O)** Selection of indicated
826 organs/tissues from *RanBP9-TT* homozygous mice stained with α V5 specific antibody.
827 All pictures were taken with 40x objective and 10x eyepiece (400x). Scale bar = 50um.
828 EXCEPT: stomach (10x = 100x, scale bar = 200um); large intestine (10x = 100x, scale
829 bar = 200um); mammary gland (60x = 600x, scale bar = 20um).

830

831 **Figure S4.** IHC detection of HA in *RanBP9-TT* mice. **A-O)** Selection of indicated organs

832 and tissues from homozygous *RanBP9-TT* mice stained with α HA specific antibody.

833 All pictures were taken with 40x objective and 10x eyepiece (400x). Scale bar = 50um

834 EXCEPT: stomach (10x = 100x, scale bar = 200um); large intestine (10x = 100x, scale

835 bar = 200um); mammary gland (60x = 600x, scale bar = 20um).

836

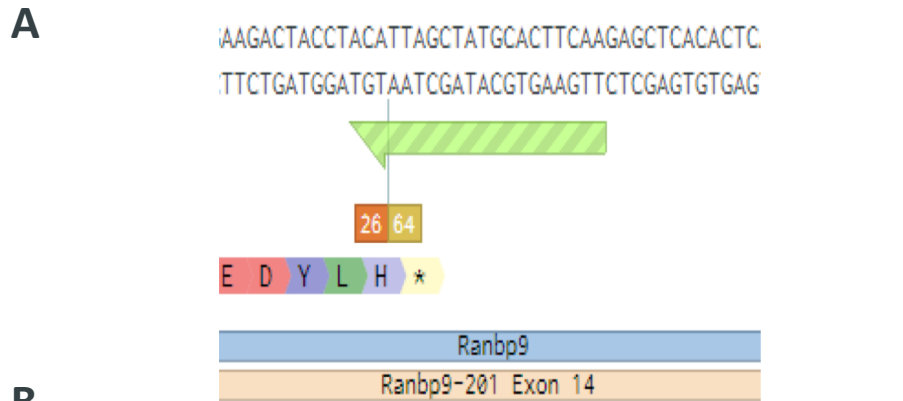
837

838 **Proteomic data.** The datasets generated and analyzed for this study are available in the

839 MassIVE repository [MSV000084462] at: (<ftp://massive.ucsd.edu/MSV000084462/>).

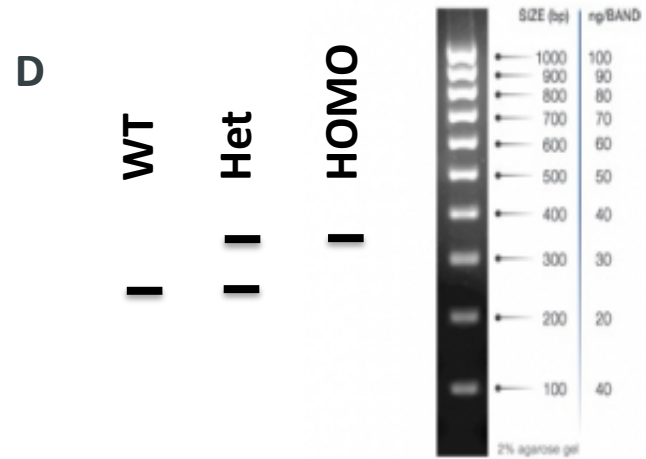
840 Alternatively, they are available from corresponding author on request.

Figure S1. Generation of the RanBP9-TT Mouse by CRISPR/Cas9



C

Sequence	PAM	Score	Gene	Locus
TTGAAGTGCATAGCTAATGT	AGG	100.0	Ranbp9 (ENSMUSG00000038546)	chr13:+43403563
AATAAGTGCATAGCTAATGT	TAG	2.3		chr13:-29291755
TTGATGTACACAGCTAATGT	GGG	1.5		chr11:+11476826
AAAAGTACATAGCTAATGT	TAG	1.5		chr18:+15335082
TTGATGTGCATGGCTAATGT	CAG	1.4		chr3:-63084635
TGGAAGAGCATAGCTAATGA	AGG	1.3	RP23-145F9.4 (ENSMUSG00000103083)	chr1:-171680603



E

Forward Primer: **AAACCCACAATCTGCCAAAG**
 Reverse Primer: **AAAGCGACAAAACCTGTCC**

WT: 256 bp
BP9-TT: 343 bp

Figure S2. IHC detection of RanBP9 by V5 in RanBP9-TT mice.

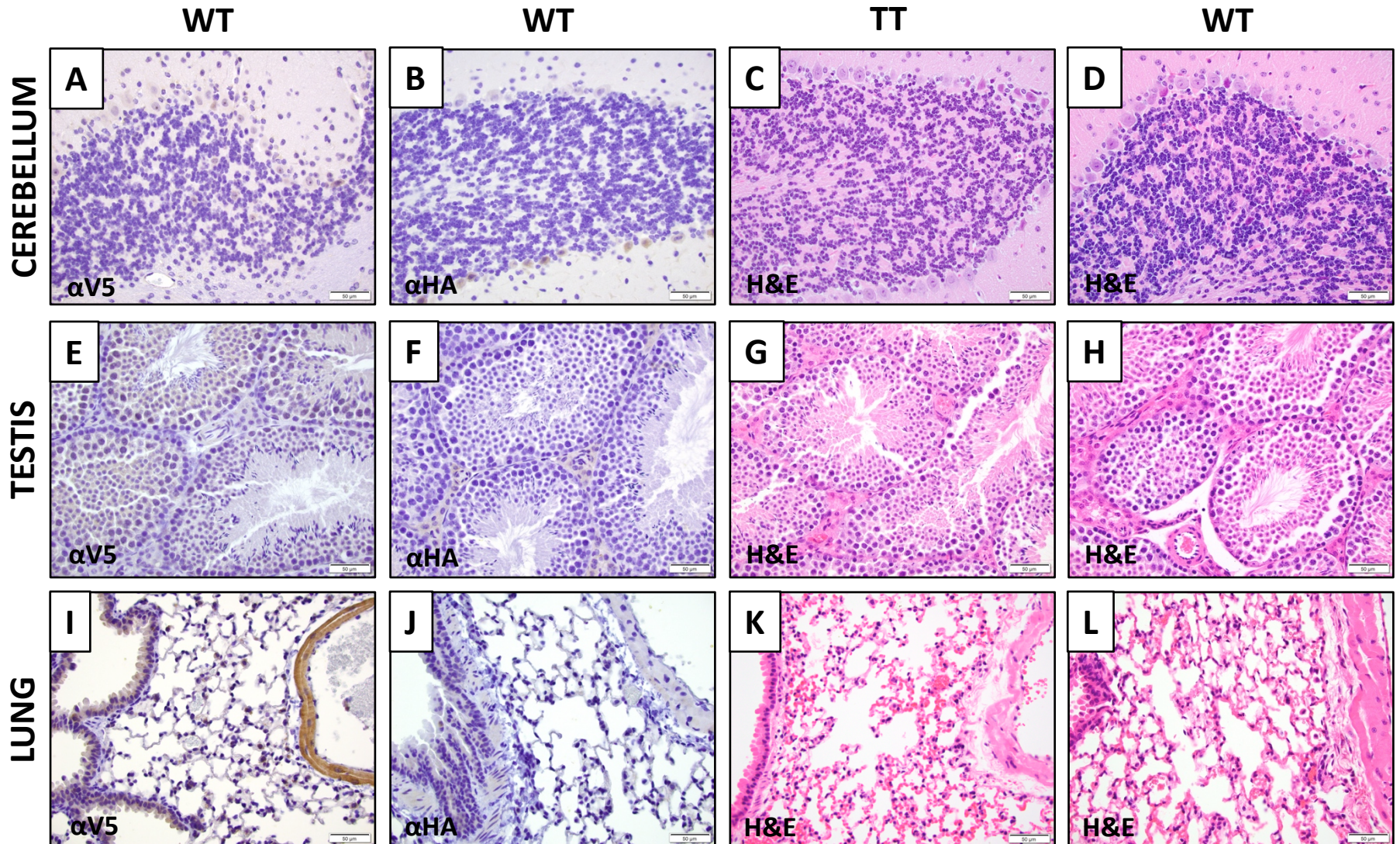


Figure S3. IHC detection of RanBP9 by V5 in RanBP9-TT mice.

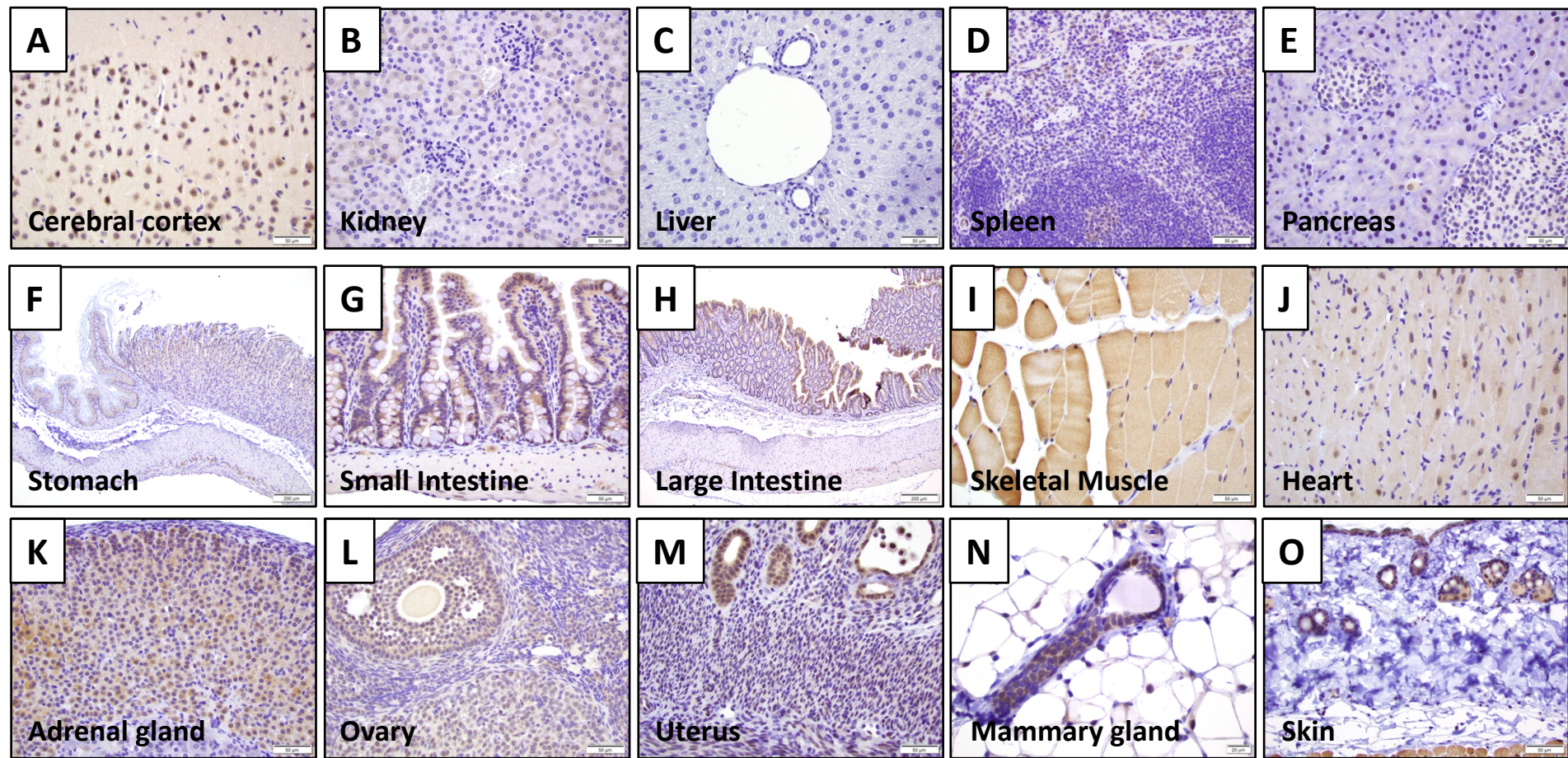
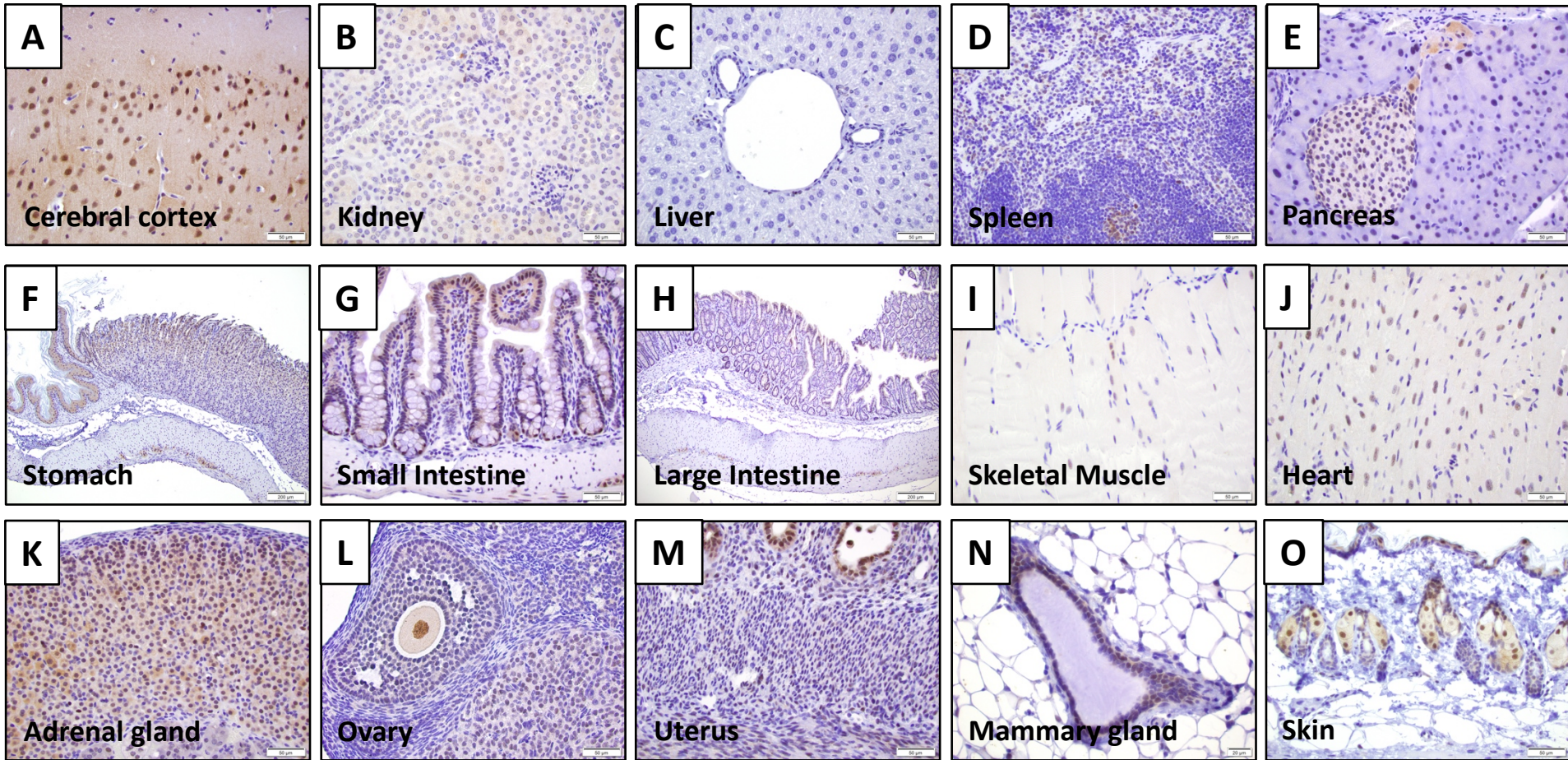


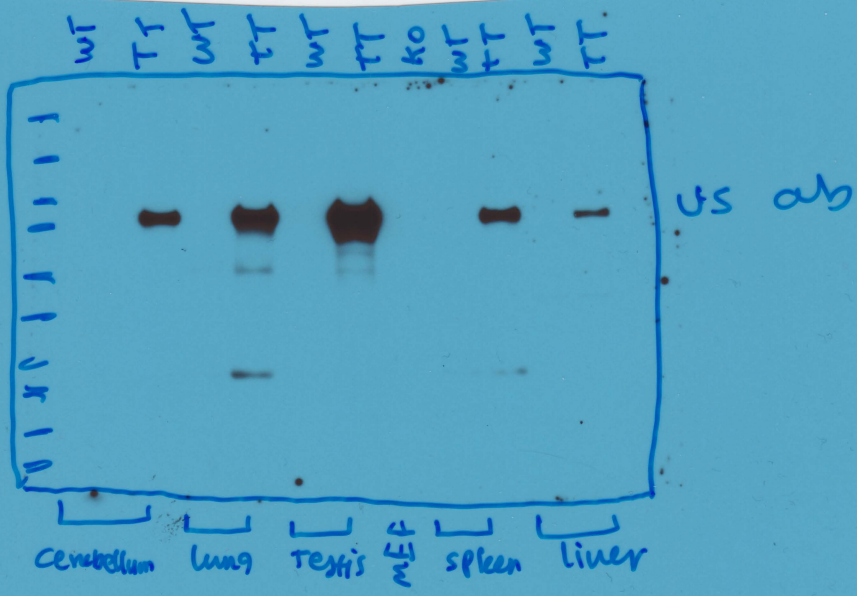
Figure S4. IHC detection of RanBP9 by HA in RanBP9-TT mice.



Supplementary Table 1. Primary antibodies used for this investigation

Name of the antibody	Company website/Validation data	Dilution		Amount
		WB	IHC	IP
1 rabbit aRanBP9 antibody, cat. nr. HPA050007	https://www.proteinatlas.org/ENSG00000010017-RANBP9/antibody#western_blot We have previously used and validate this antibody with RANBP9 KO cells (Palmieri et al., 2016; Tessari et al., 2018)	-----	-----	1:50
2 goat V5 antibody, Abcam cat. nr. ab95038	https://www.abcam.com/v5-tag-antibody-ab95038.html	-----	-----	1:350
3 rabbit HA, Cell Signaling cat. nr. C29F4	https://www.cellsignal.com/products/primary-antibodies/ha-tag-c29f4-rabbit-mab/3724	-----	-----	1:800
4 RANBP9, Abcam cat. nr. ab140627	https://www.abcam.com/ranbp9-antibody-epr9920b-ab140627.html	1:2,000	5% milk	-----
5 GAPDH, Cell Signaling cat. nr. 3683S	https://www.cellsignal.com/products/antibody-conjugates/gapdh-14c10-rabbit-mab-hrp-conjugate/3683	1:3,000	5% milk	-----
6 monoclonal HA-Agarose antibody, Sigma cat. nr. A2095-1ML	https://www.sigmaaldrich.com/catalog/product/sigma/a2095?lang=en&region=US	-----	-----	5ug
7 RMND5A, Novus Biologicals cat. nr. NBP1-92337	https://www.novusbio.com/products/rmnd5a-antibody_nbp1-92337	1:1,000	5% milk	-----
8 WDR26, Abcam cat. nr. ab85962	https://www.abcam.com/wdr26-antibody-ab85962.html	1:1,000	5% milk	-----
9 ARMC8, Proteintech cat. nr. 12653-1-AP	https://www.ptglab.com/products/ARMC8-Antibody-12653-1-AP.htm	1:1,000	5% milk	-----
10 Muskelin, Proteintech cat. nr. 14735-1-AP	https://www.ptglab.com/products/MKLN1-Antibody-14735-1-AP.htm	1:1,000	5% milk	-----
11 C20orf11 (a.k.a. GID8), Proteintech cat. nr. 24479-1-AP	https://www.ptglab.com/products/C20orf11-Antibody-24479-1-AP.htm	1:1,000	5% milk	-----
12 MAEA, R&D Systems cat. nr. AF7288	https://www.rndsystems.com/products/human-mouse-rat-emp-maea-antibody_af7288	1:2,000	5% milk	-----
13 RanBP10, Millipore SIGMA; cat. nr. SAB3500163	https://www.sigmaaldrich.com/catalog/product/sigma/sab3500163?lang=en&region=US	1:1,000	5% milk	-----
14 Nucleolin, D4C7O; Cell Signaling; rabbit mAB cat. nr. 14574	https://www.cellsignal.com/products/primary-antibodies/nucleolin-d4c7o-rabbit-mab/14574	1:2,000	5% milk	-----
15 V5 antibody, Invitrogen cat. nr. R96025	https://www.thermofisher.com/antibody/primary/target/v5%20tag	1:1,000	5% milk	-----
15 mouse Vinculin antibody, Abcam cat. nr. Ab130007	https://www.abcam.com/vinculin-antibody-vin-54-ab130007.html	1:2,000	5% milk	-----

FIG 3 B
VS



HA ab

HA
FIG 3 C

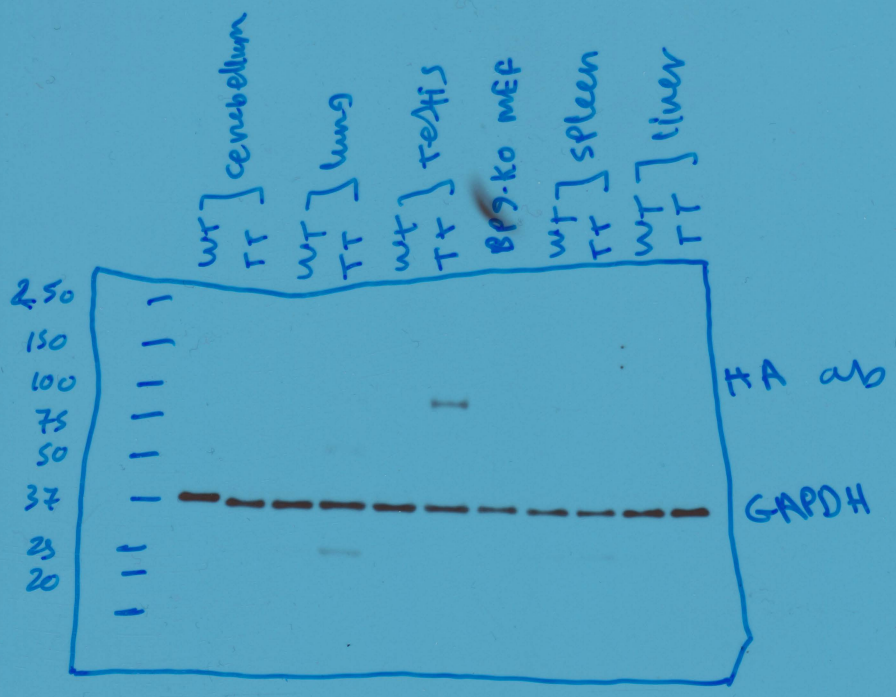
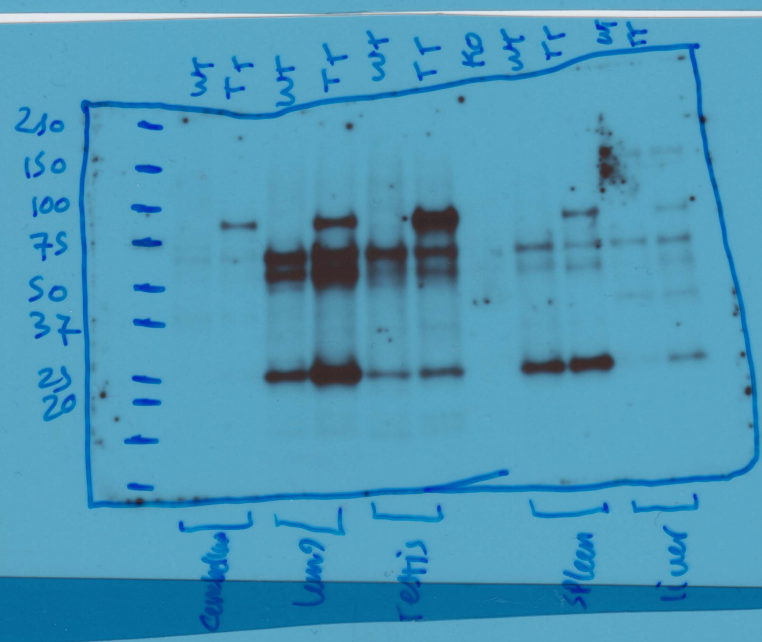


FIG 3 C
GAPDH

FIG 3 A
GAPDH

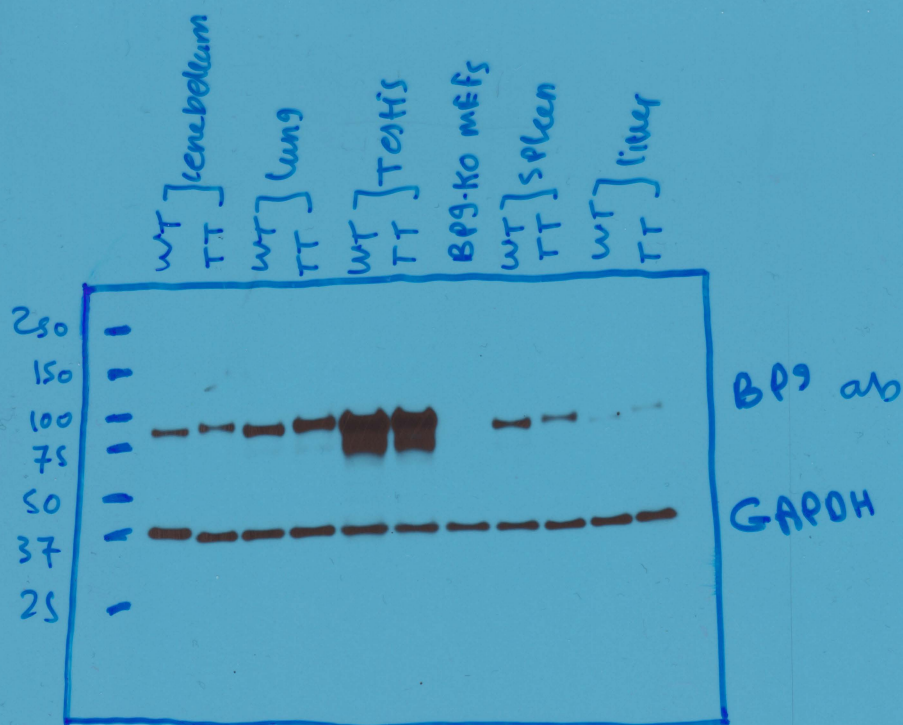


FIG 3 B
GAPDH

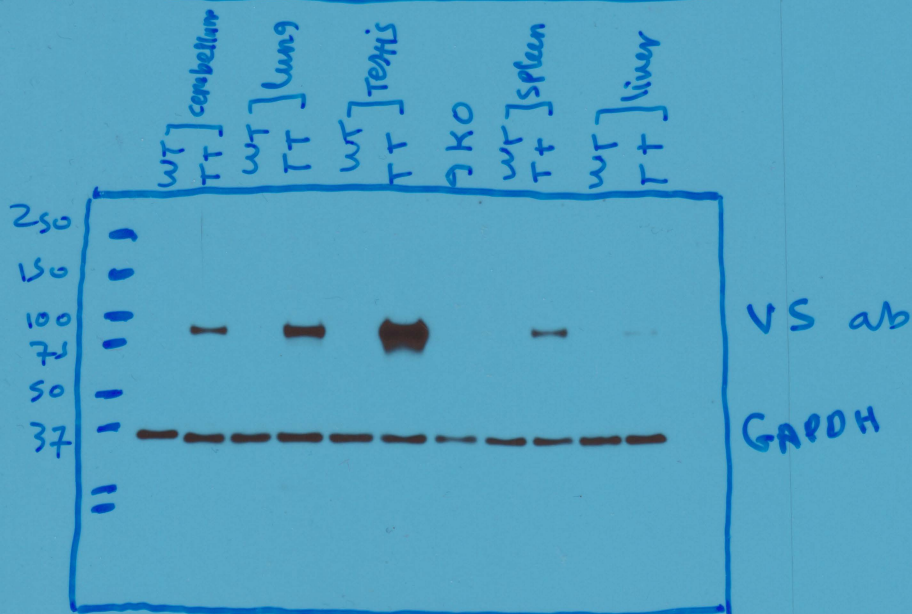


FIG 4A

ENB 1.16.20

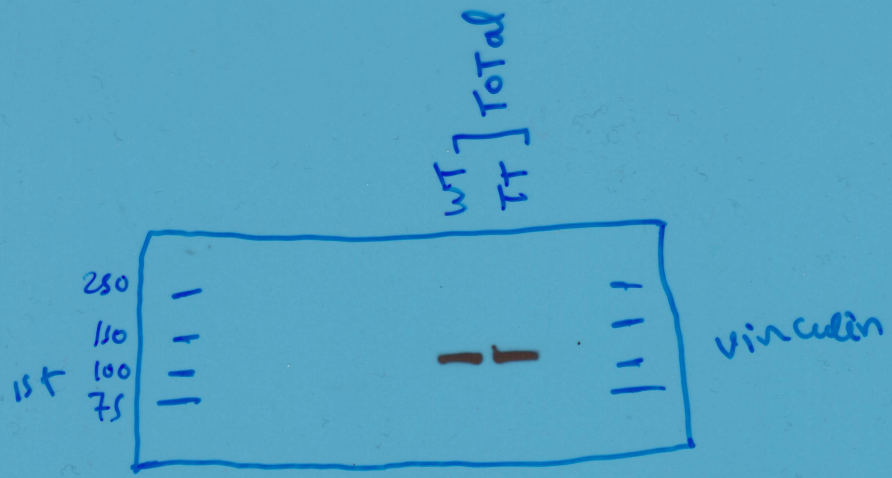


FIG 4A

ENB 1-16-20

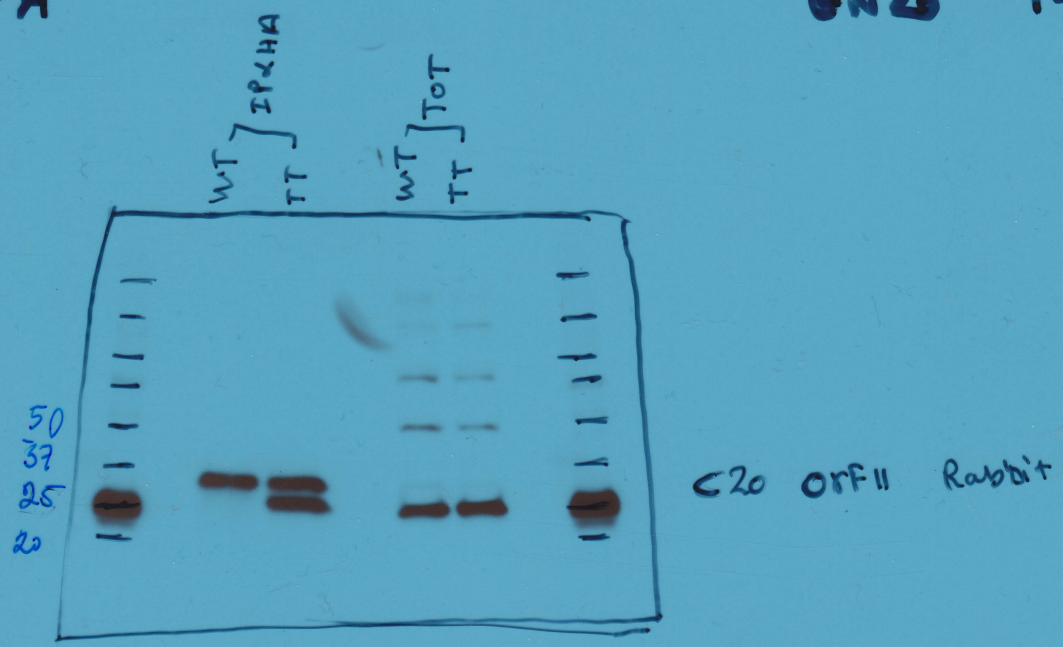
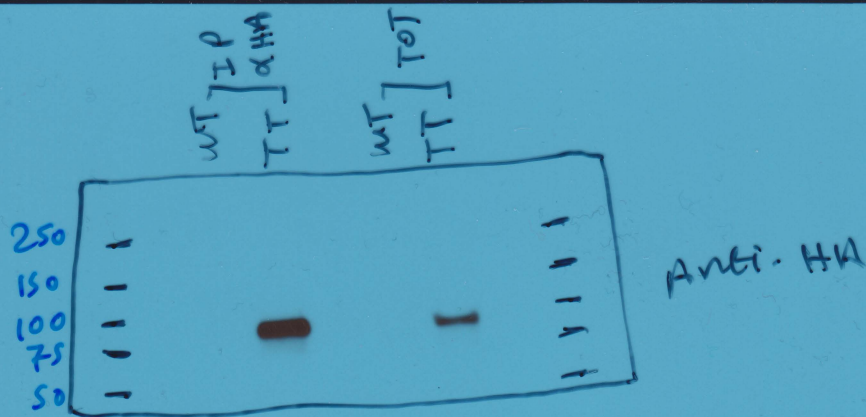


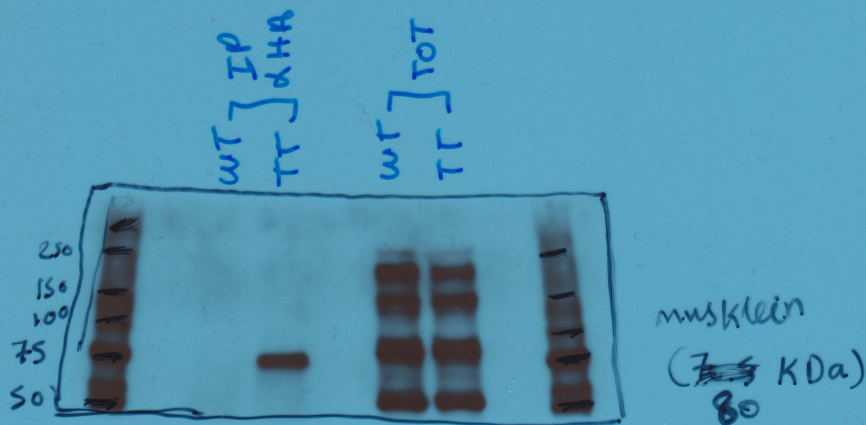
FIG 4A



ENZO 1-16-20

FIG 4A

ENZO 1-16-20



ENB 1-16-20

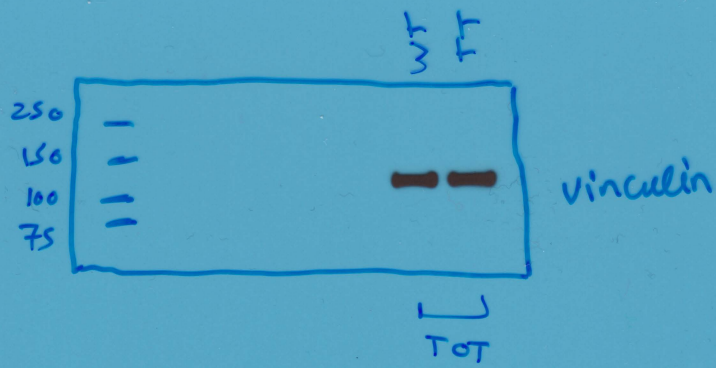
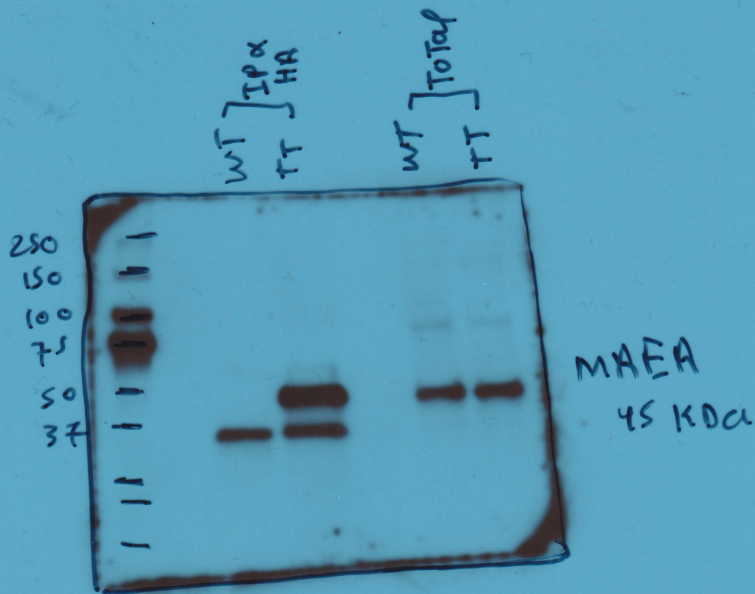


FIG 4B

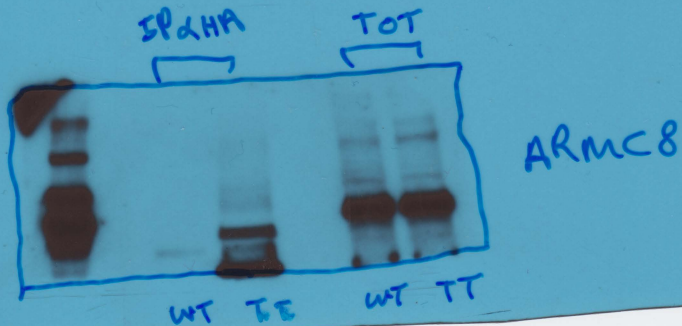
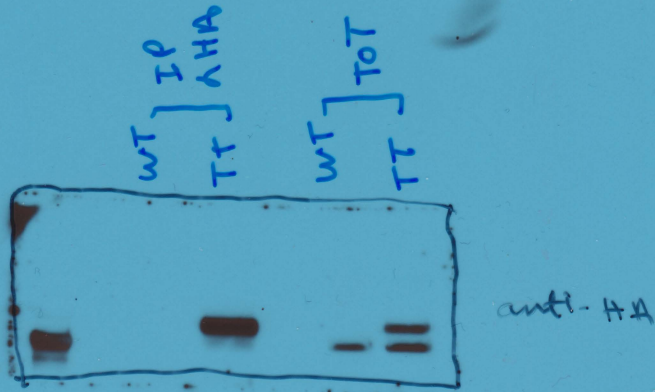


ENB 1-16-20

FIG 4B

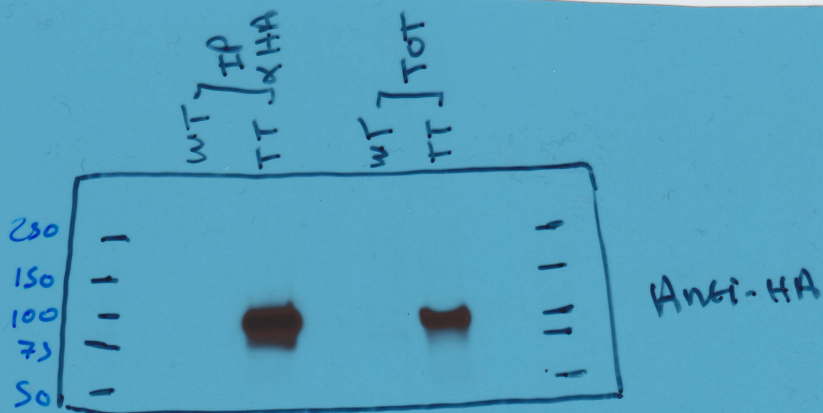
END 1-16-20

FIG 4B



END
1-16-20

FIG 4B



EMO 1-16-2

FIG 4C

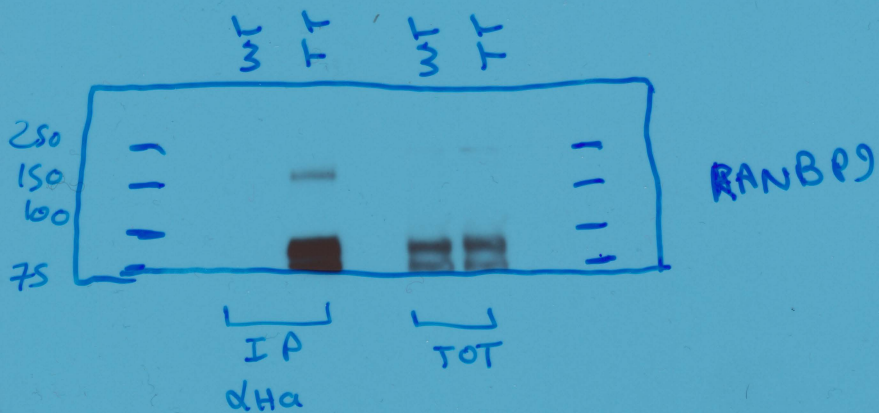
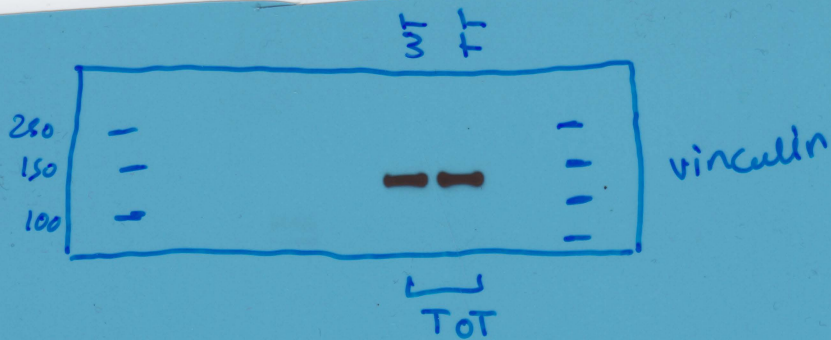


FIG 4c

EMO
1-16-2



END 1-16-20

FIG 4C

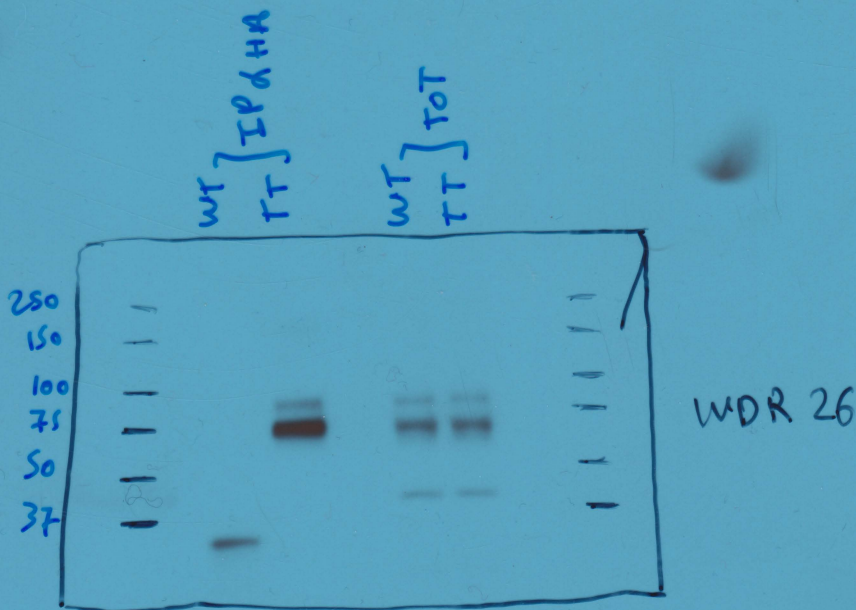
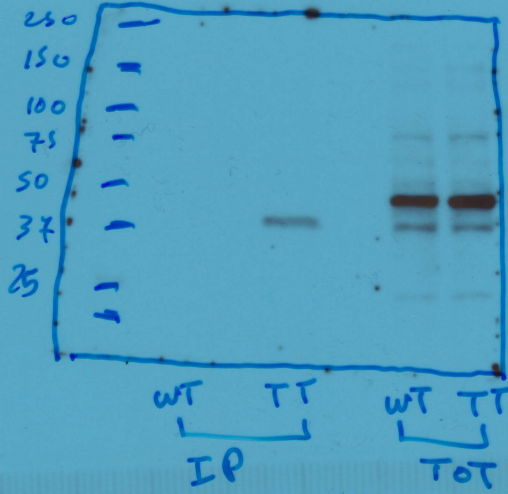


FIG 4C

END 1-16-20

FIG 4D

RMNDSA



ENR 1-16-20

ENR 1-16-20

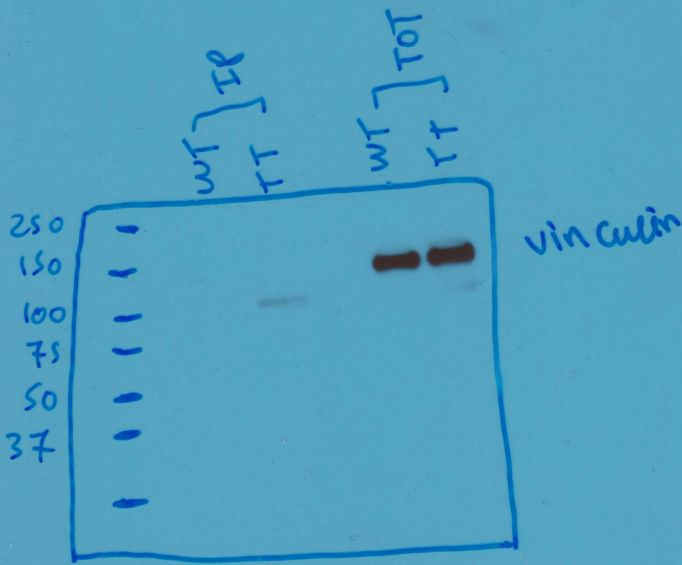
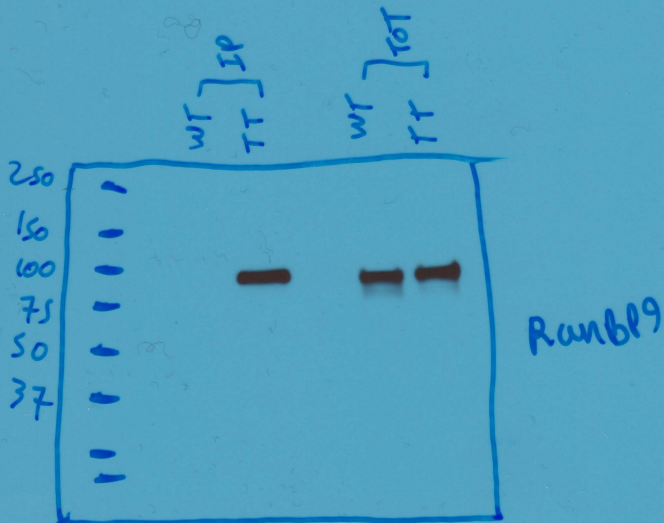


FIG 4D



ENR 1-16-20

FIG 4D

Enzo 1-16-20

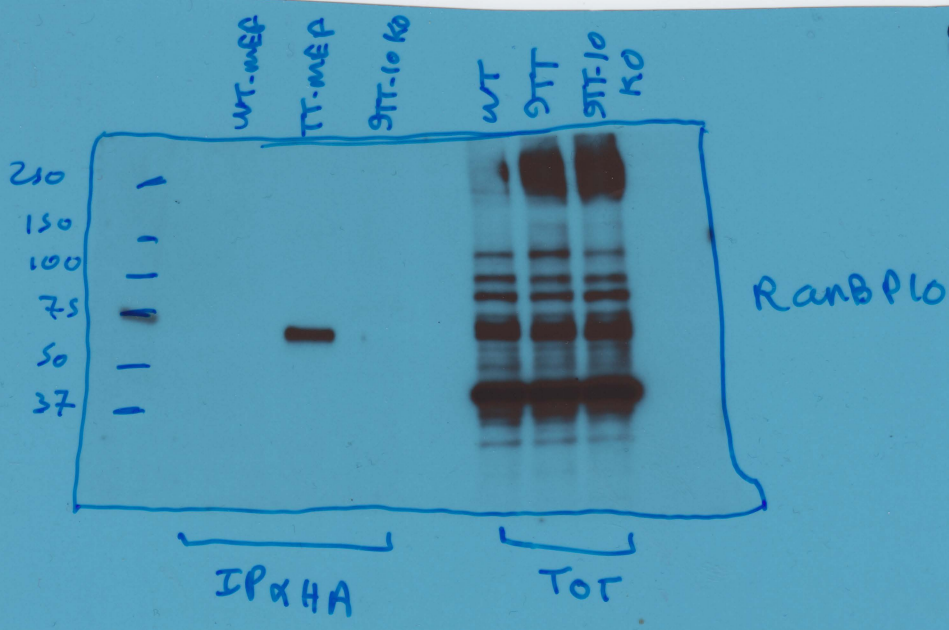


FIG 4E

Enzo 1-16-20

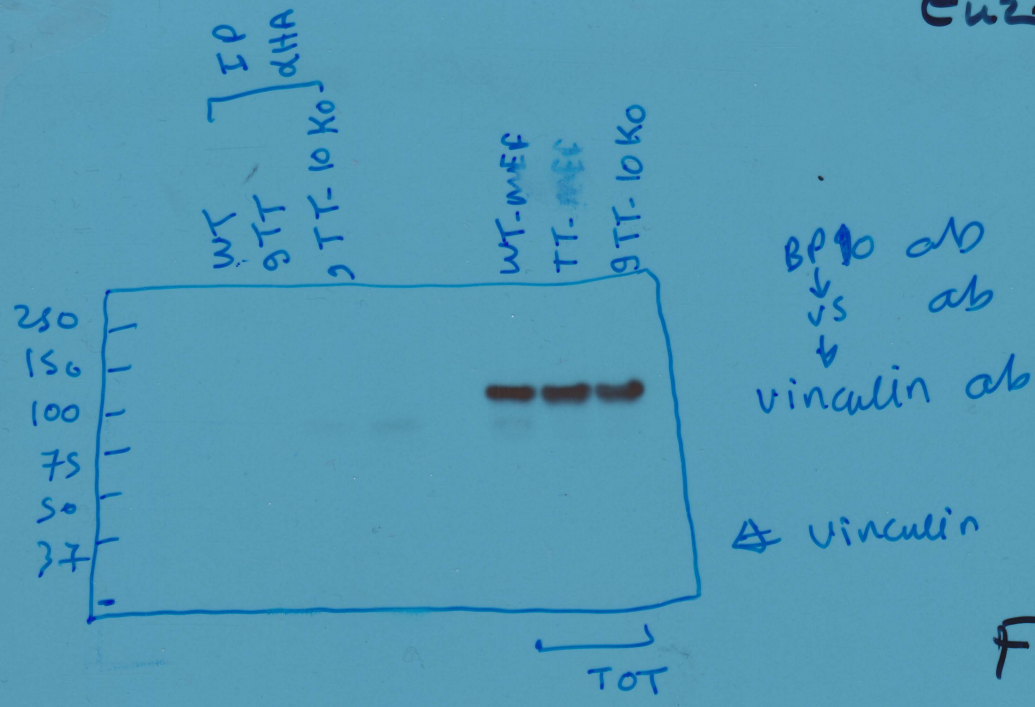


FIG 4E

Enzo 1-16-20

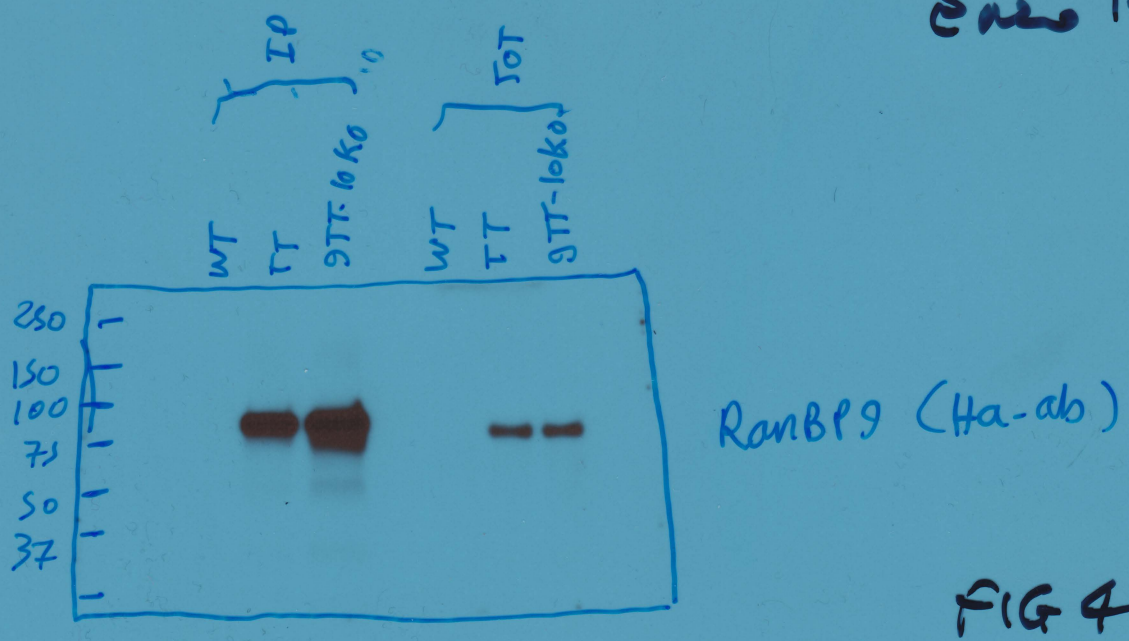
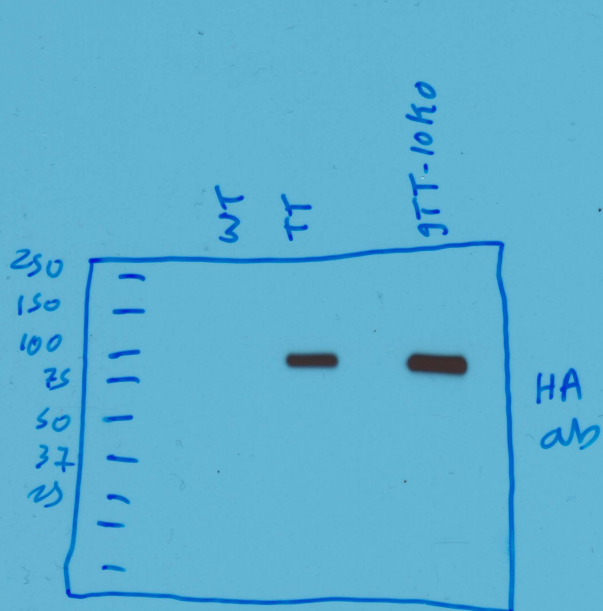


FIG 4E



HA IP

IP α Ha

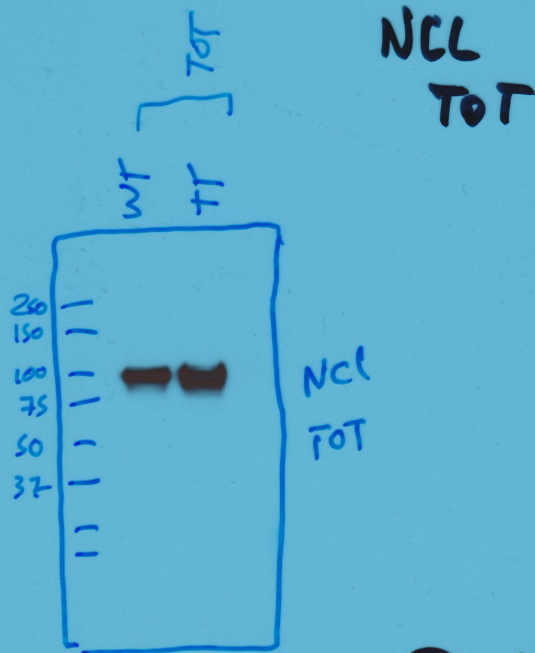
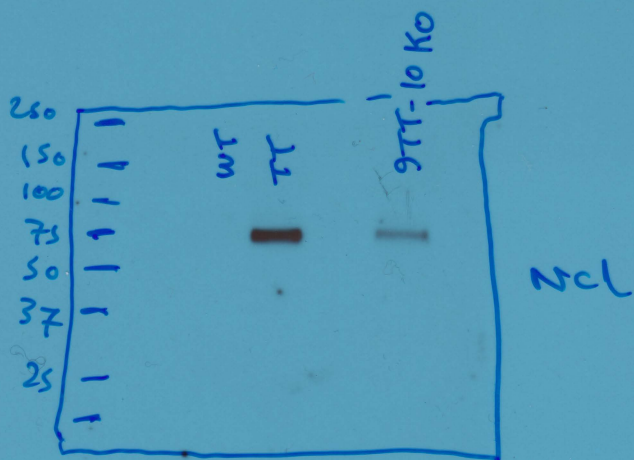


FIG 4F

FIG 4F

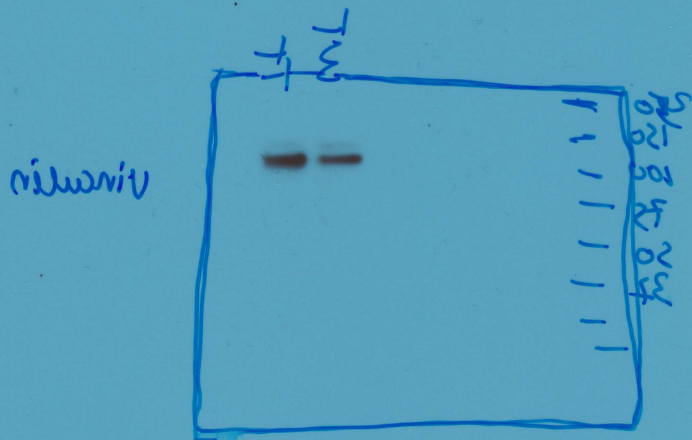


NCL IP

IP α HA

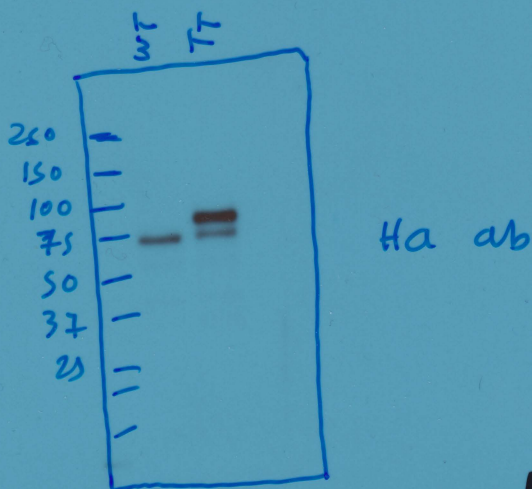
ENL 1-16-20

FIG 4E



5-01-1 Tot Nucleonin

FIG 4F



HA tot

1-16-20
EN2