

**Online Supplement**

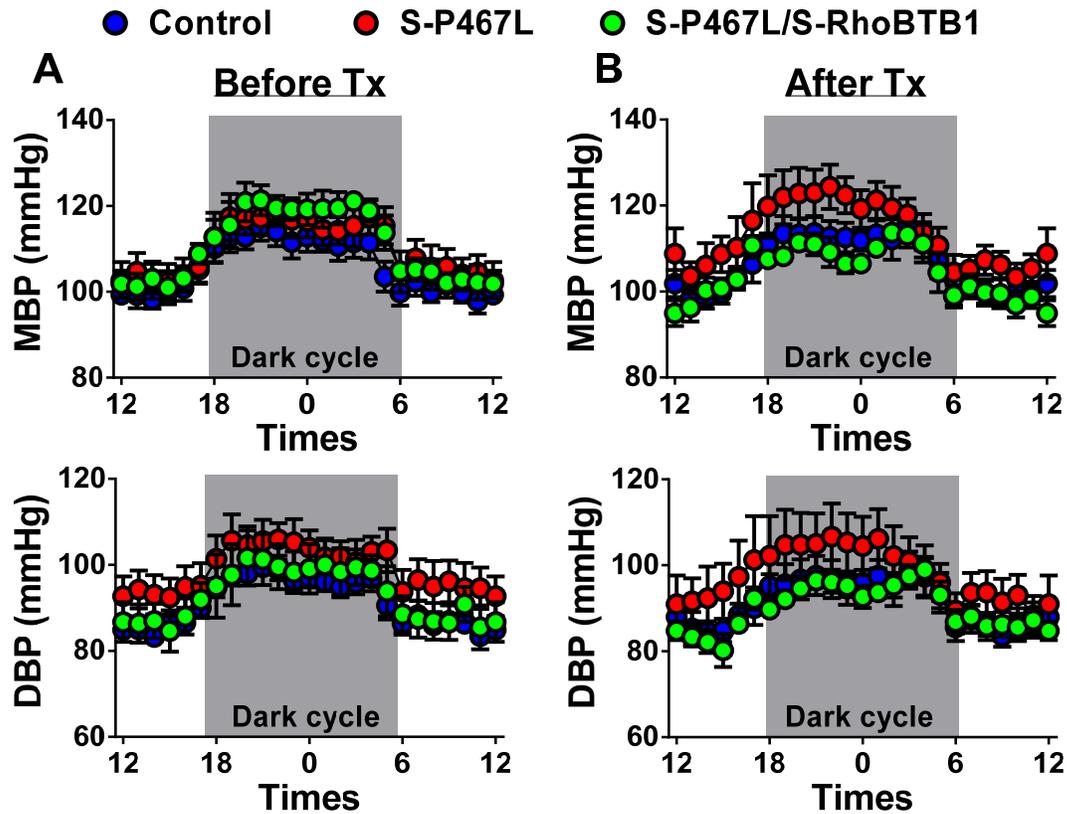
**RhoBTB1 Protects Against  
Hypertension and Arterial Stiffness by Restraining  
Phosphodiesterase 5 Activity**

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Debbie R. Davis, Henry L Keen, Frederick W. Quelle, and Curt D. Sigmund

**Supplemental Materials**

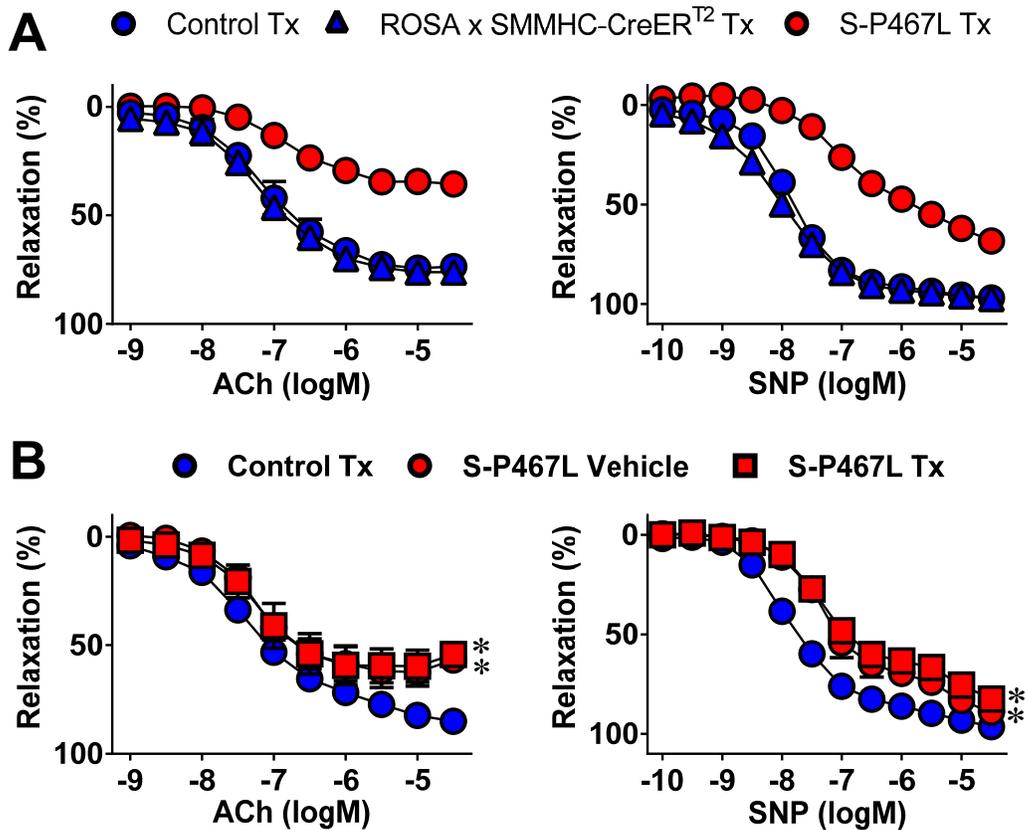
1. Supplemental Figures S1-S7

Supplemental Figures and Legends



**Figure S1.** Mean and Diastolic BP.

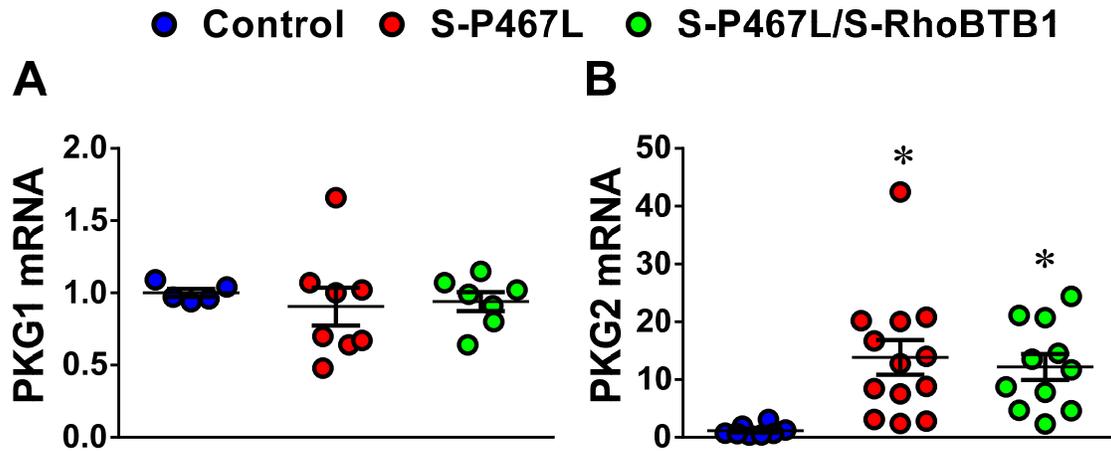
A-B) Mean and diastolic BP were measured by radiotelemetry for 1-week in mice (control) expressing a tamoxifen-inducible Cre recombinase (CreER<sup>T2</sup>) under the control of the smooth muscle myosin heavy chain promoter (n=8), S-P467L (n=8) and S-P467L/S-RhoBTB1 mice (n=8) before (A) or 3-4-weeks after Tx treatment (B). All data are mean±SEM. There were no significant difference detected.



**Figure S2.** Effect of *tdTomato* and Tamoxifen on Vascular Function.

A) Vascular relaxation in littermate non-transgenic mice (Control, n=3) or transgenic mice (ROSA x SMMHC- CreER<sup>T2</sup>) expressing the *tdTomato* reporter in smooth muscle by crossing ROSA26 reporter mice with mice expressing a tamoxifen-inducible Cre recombinase (CreER<sup>T2</sup>) under the control of the SMMHC promoter (n=4) 3-4 weeks after Tx treatment. A single curve from S-P467L mice was included as a reference. B) Vascular relaxation in Control (n=4) and S-P467L without or 3-4 weeks after Tx injection (B, n=4). Cumulative concentration-response curves for acetylcholine (ACh) or sodium nitroprusside (SNP) in aorta are shown. All data are mean±SEM.

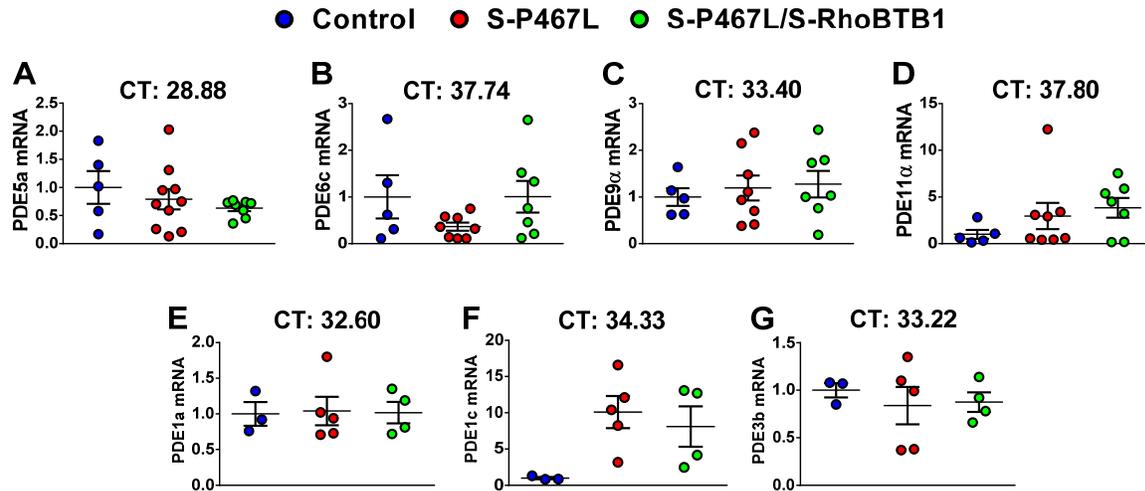
\**P*<0.05 vs. Control+Tx by two-way RM-ANOVA.



**Figure S3. PKG1 and PKG2 mRNA Expression**

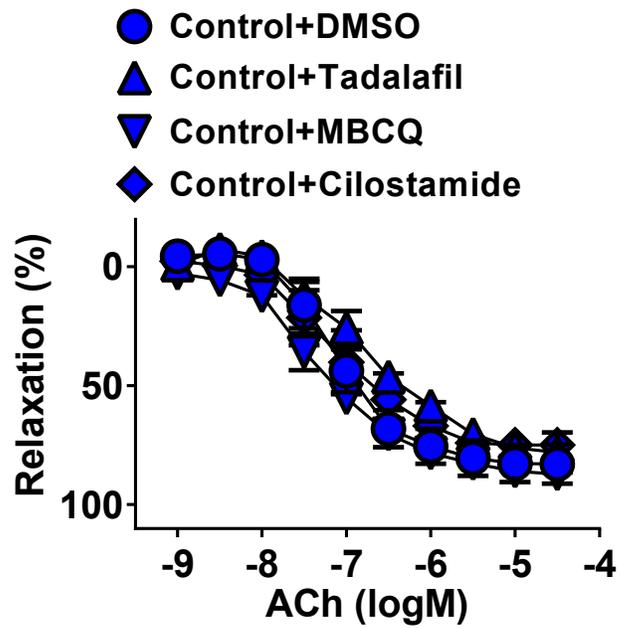
Relative mRNA expression of mouse PKG1 (A) and PKG2 (B) were determined by quantitative real-time RT-PCR in aorta from control, S-P467L and S-P467L/S-RhoBTB1 mice 3-4 weeks after Tx treatment. Data were normalized to the control value, set to 1.0. All data are mean±SEM.

\* $P < 0.05$  vs. Control by ANOVA.



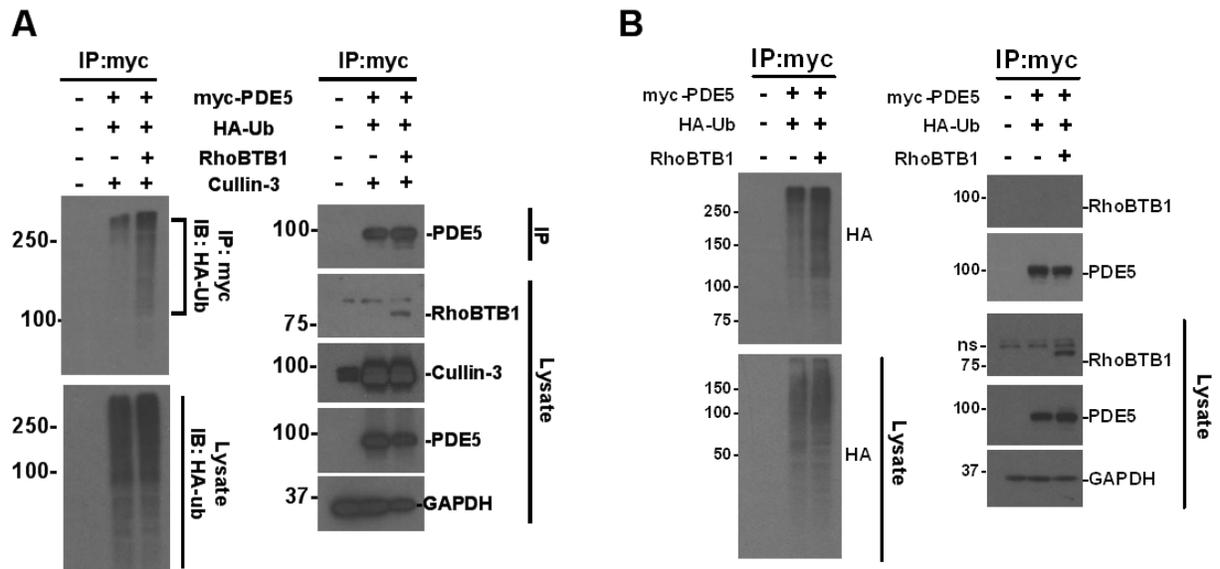
**Figure S4.** Expression of *PDE mRNAs*

Relative mRNA expression of mouse PDE5a (A), PDE6c (B), PDE9 $\alpha$  (C), PDE11 $\alpha$  (D), PDE1a (E), PDE1c (F) and PDE3b (G) were determined by quantitative real-time RT-PCR from aorta of control, S-P467L and S-P467L/S-RhoBTB1 mice after injection of Tx. Data were normalized to the control value, set to 1.0. All data are mean $\pm$ SEM. No significant differences were detected by one-way ANOVA.



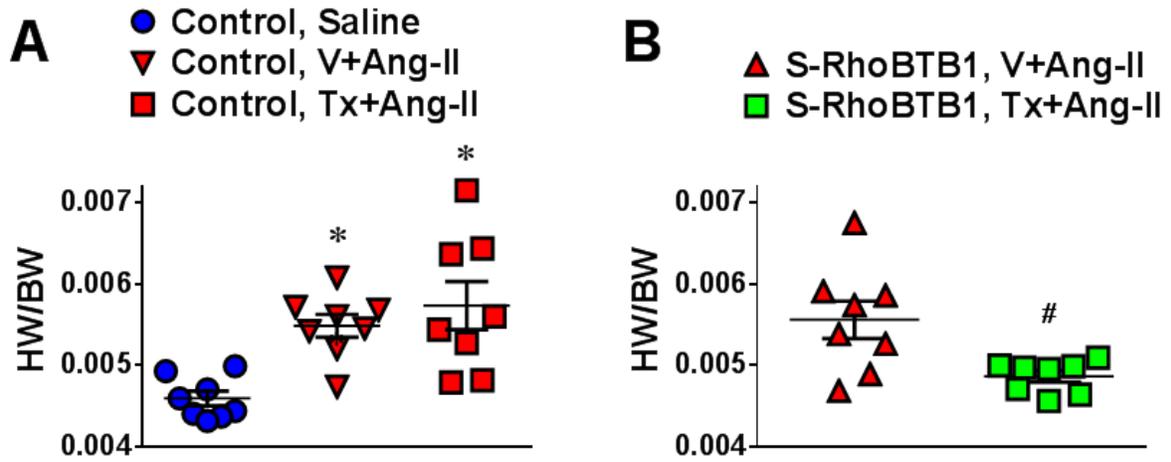
**Figure S5.** *Effect of PDE Inhibitors on Vascular Function in Normal Aorta*

Vascular function study in control aorta pretreated with DMSO, Tadalafil (100 nM), MBCQ (1  $\mu$ M) or Cilostamide (1  $\mu$ M). All samples are n=5. All data are mean $\pm$ SEM.



**Figure S6: PDE5 Ubiquitination**

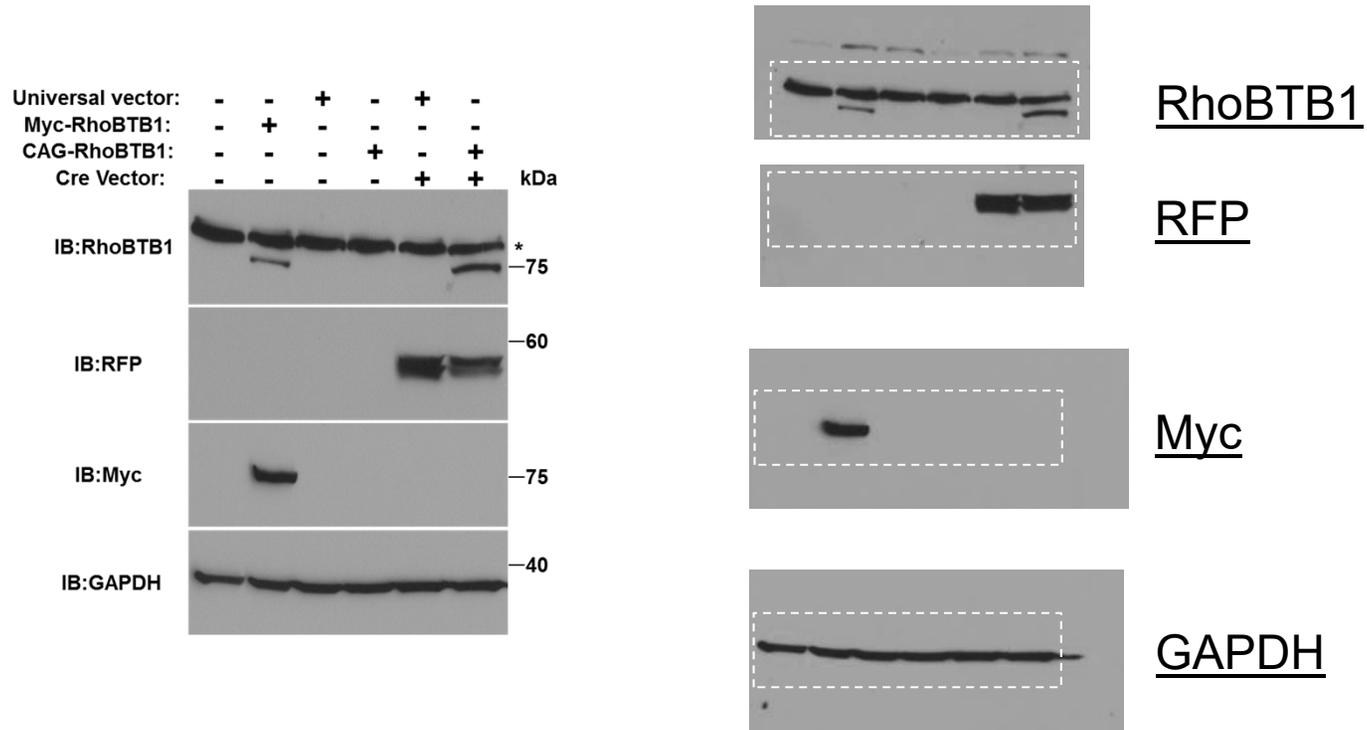
Ubiquitination of PDE5 in HEK293 transfected with vectors expressing myc-tagged PDE5, HA-tagged ubiquitin (Ha-Ub), untagged RhoBTB1 either with (A) or without (B) exogenous Cullin-3. Western blots are probed with the indicated antisera. IP and lysates are labeled. IP was performed under stringent denaturing conditions to ensure detection of only ubiquitinated PDE5. Under these conditions, RhoBTB1 is not pulled down by PDE5. Molecular weight markers were transferred from the original blots. Ns indicates non-specific band using the RhoBTB1 antisera.



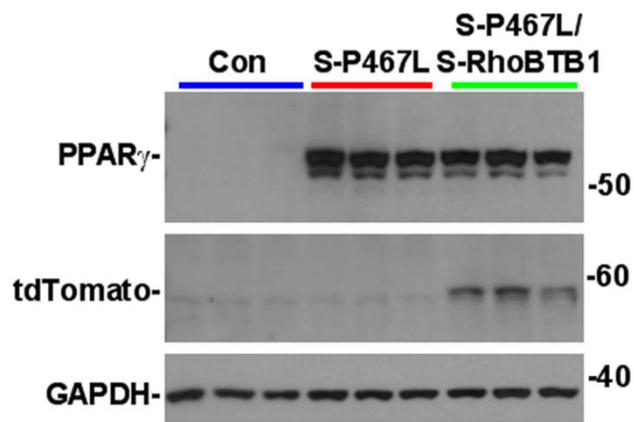
**Figure S7. Cardiac Hypertrophy**

Heart weight/body weight ratio was measured in Control (A) or S-RhoBTB1 (B) mice after injection of vehicle (V, corn oil) or Tamoxifen (Tx) and subsequent treatment with angiotensin-II (Ang-II) or saline for 14-days. All data are mean±SEM. \* $P < 0.05$  vs. Control-Saline; # $P < 0.05$  vs. S-RhoBTB1, V+Ang II by ANOVA.

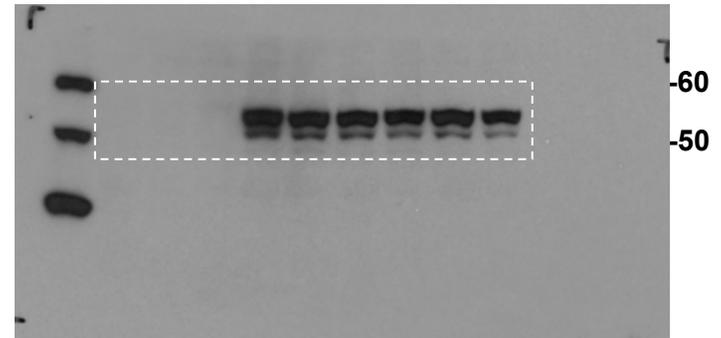
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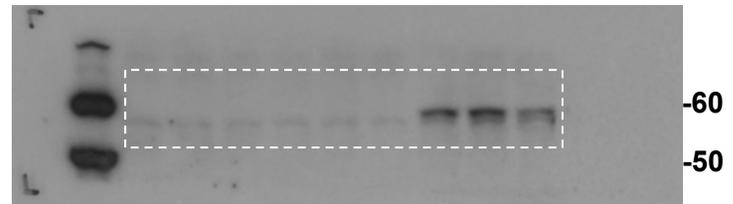
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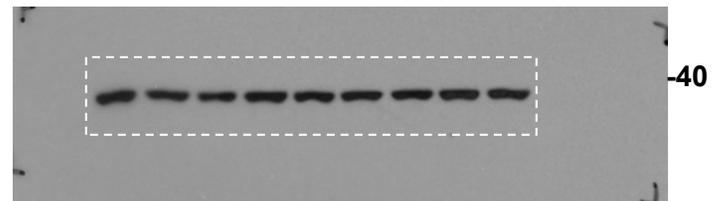
## PPAR $\gamma$



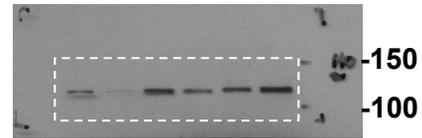
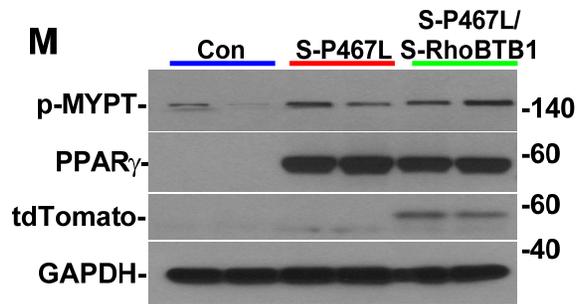
## Tdtomato



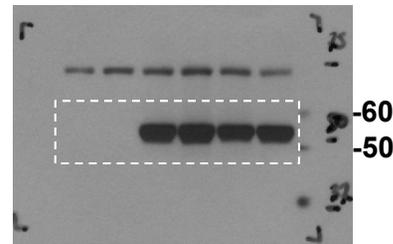
## GAPDH



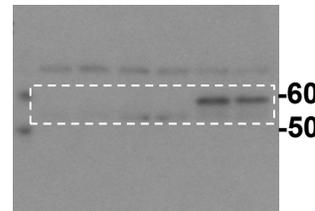
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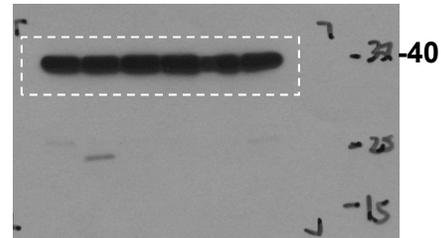
P-MYPT (Thr696)



PPAR $\gamma$

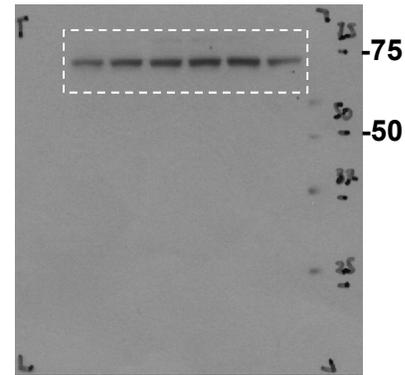
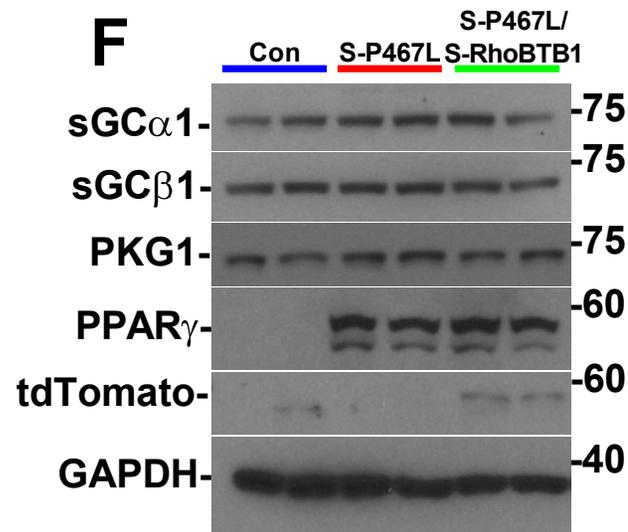


Tdtomato

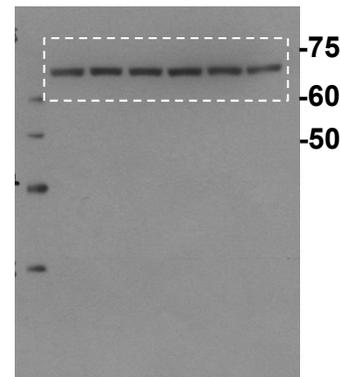


GAPDH

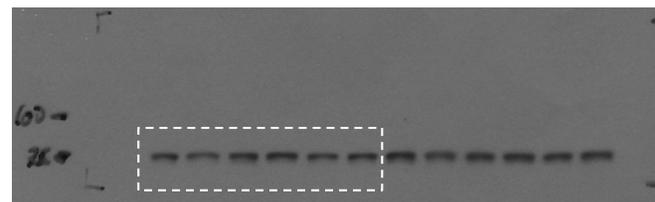
# Full unedited gel for Figure 4F TOP



sGC $\alpha$ 1

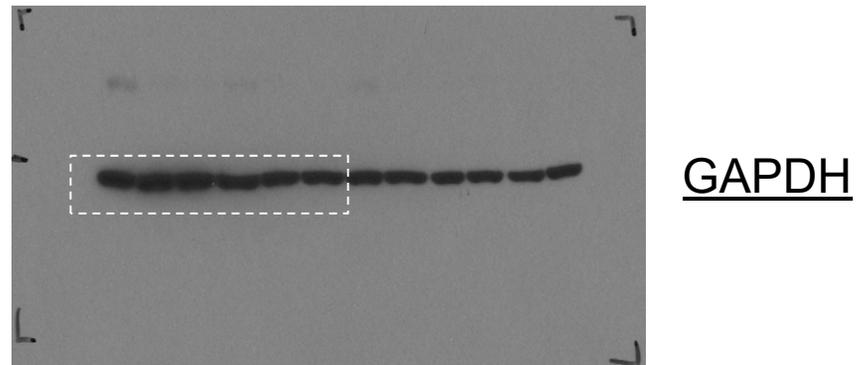
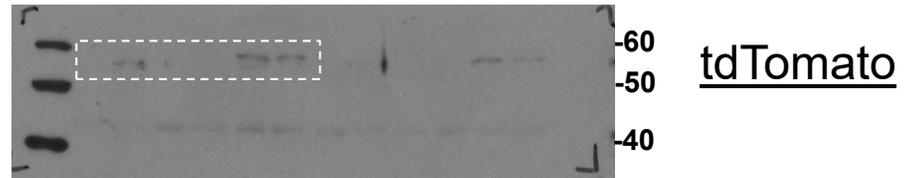
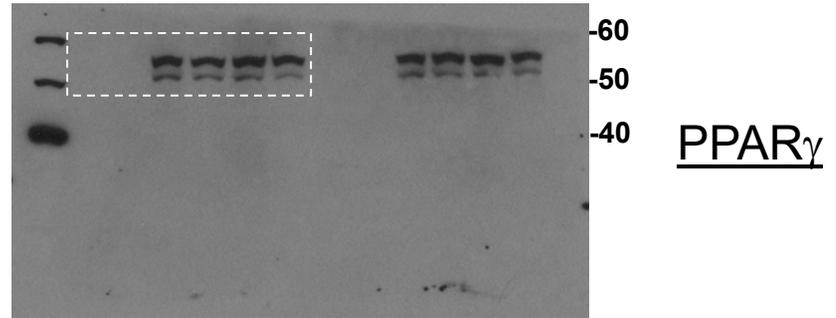
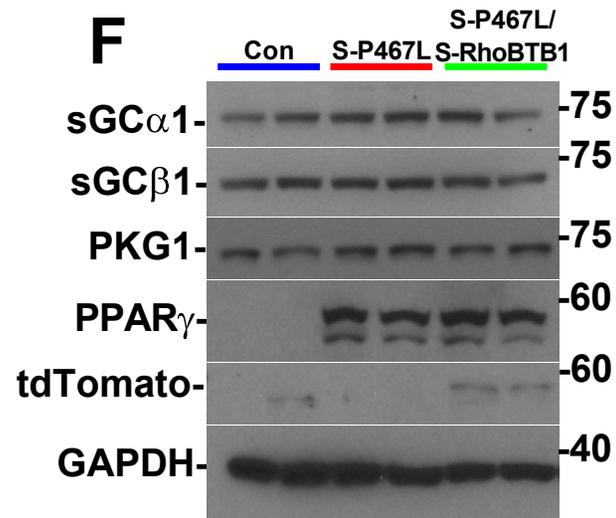


sGC $\beta$ 1

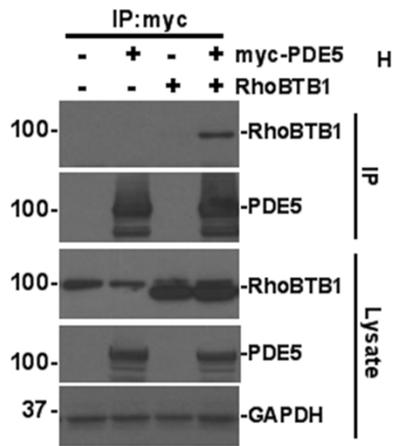


PKG1

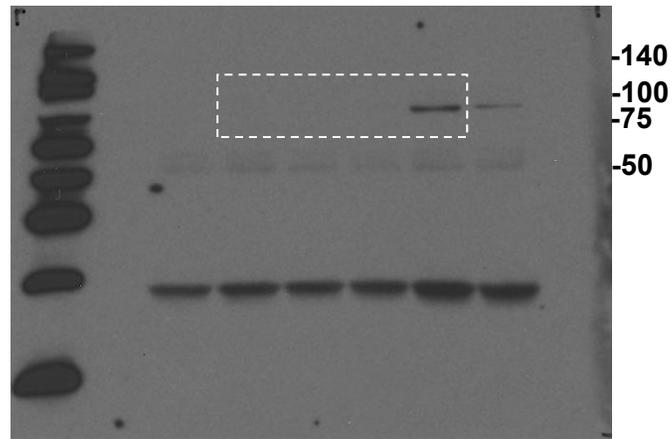
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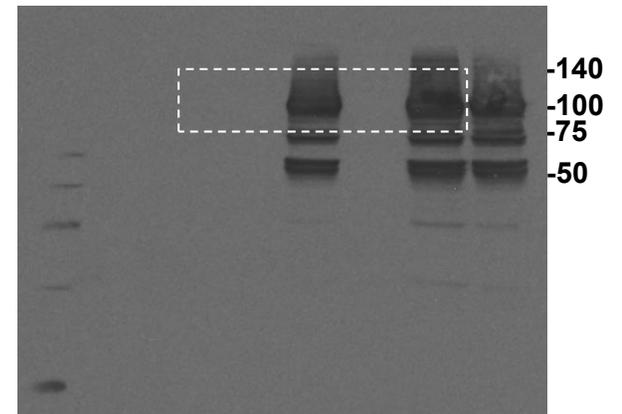
## Full unedited gel for Figure 6A



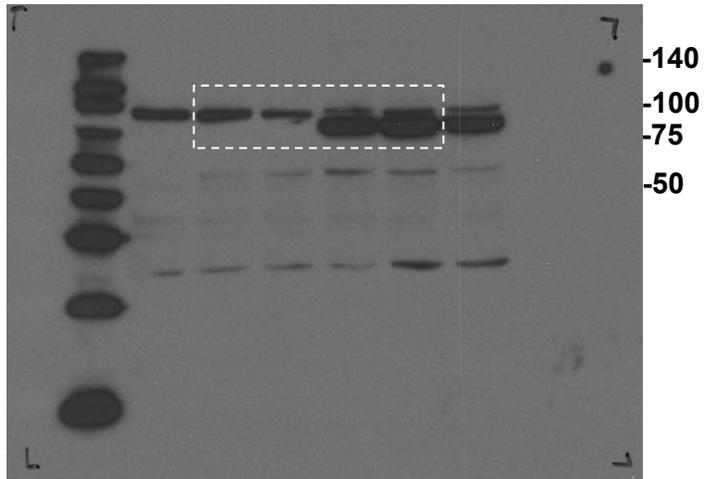
IP:myc, WB:RhoBTB1



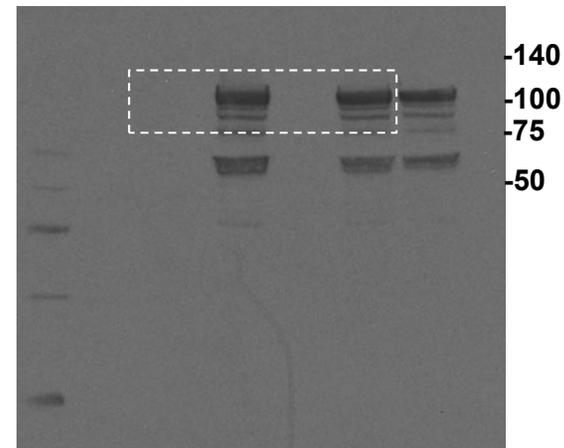
IP:myc, WB:PDE5



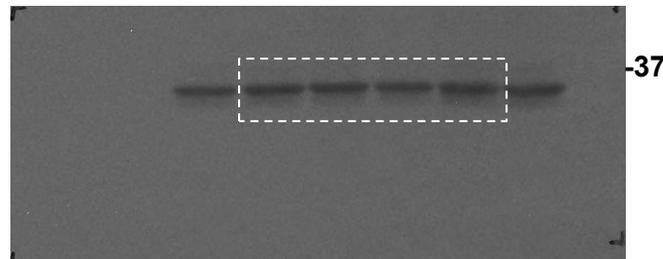
RhoBTB1



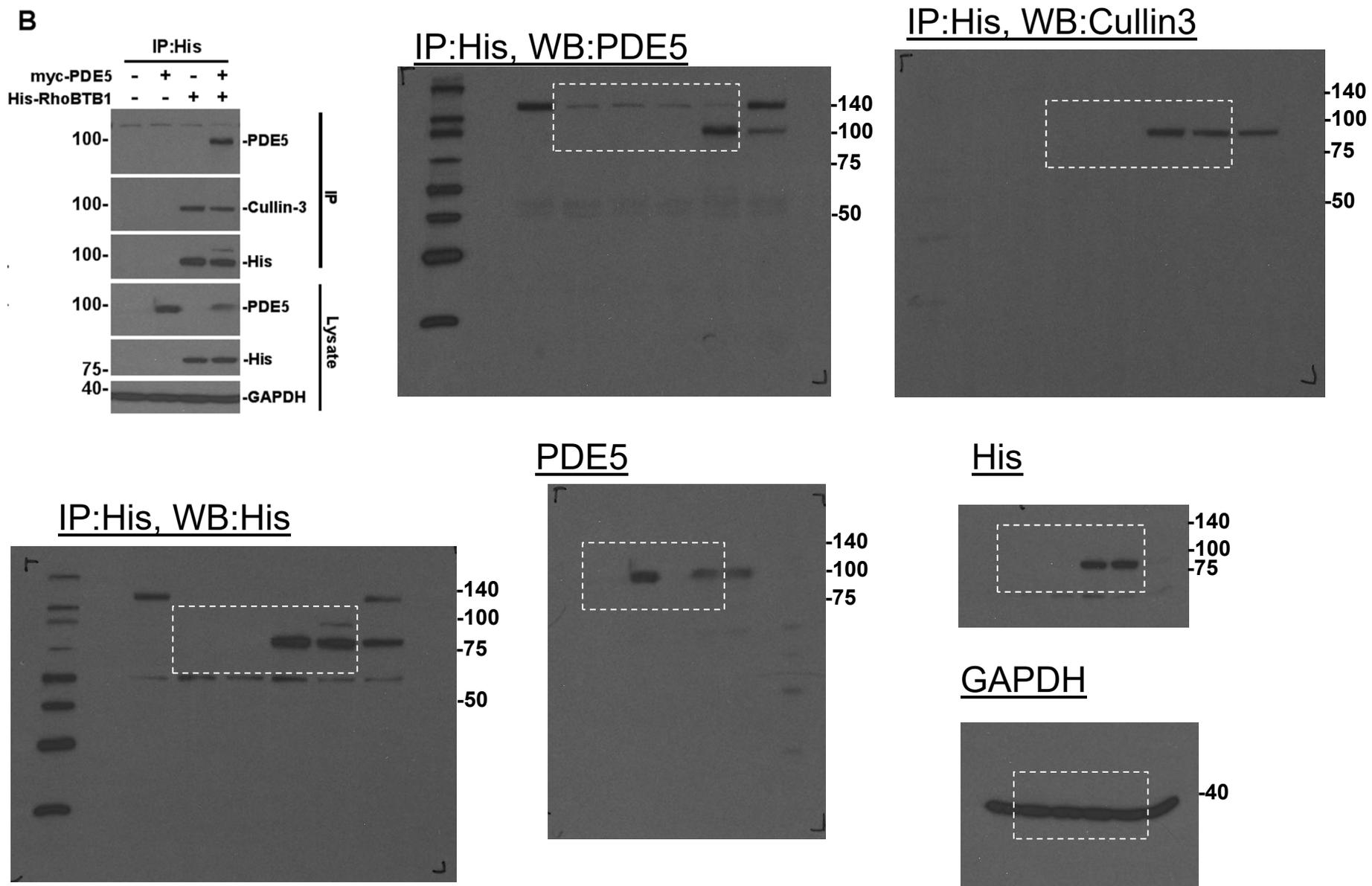
PDE5



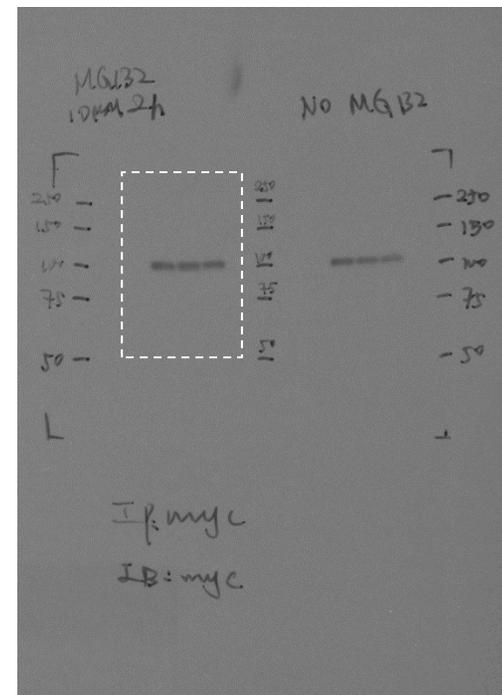
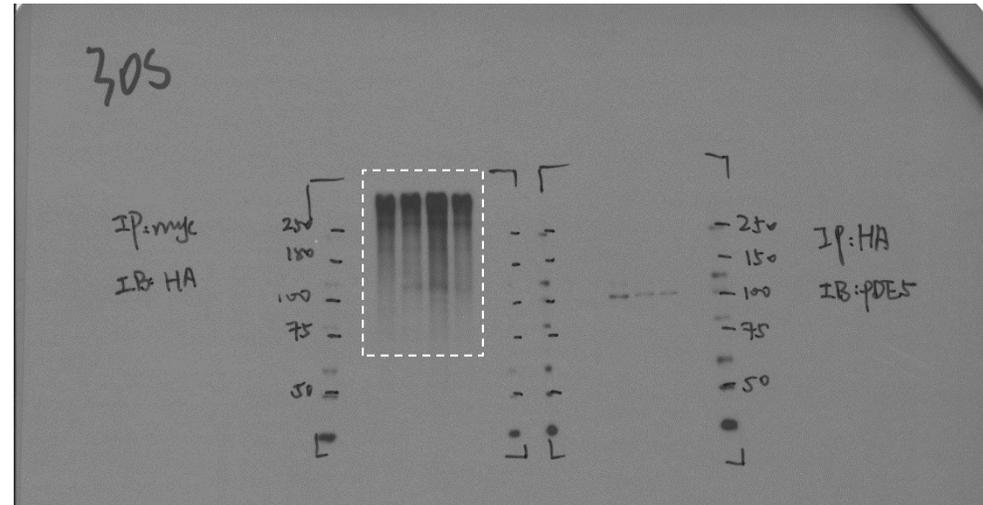
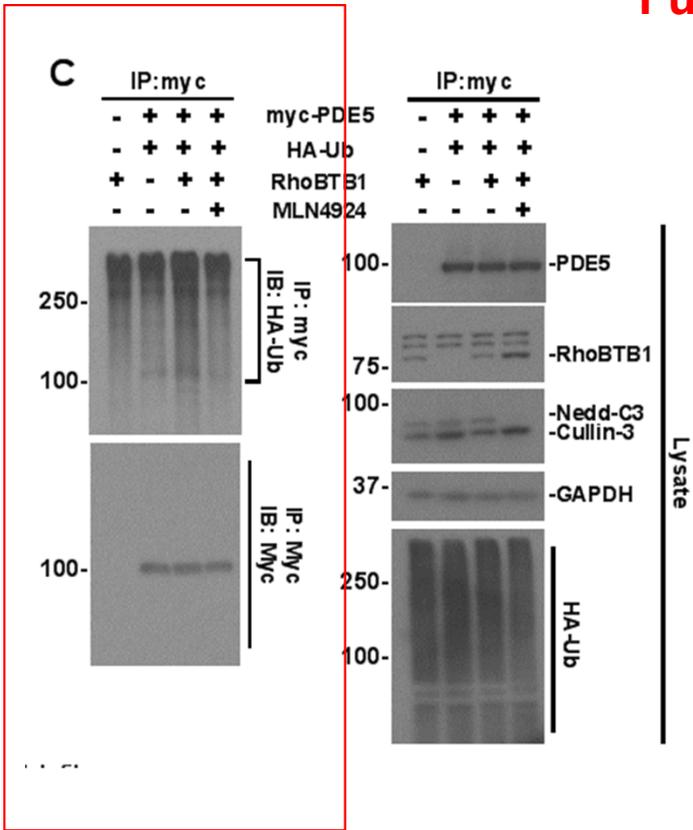
GAPDH

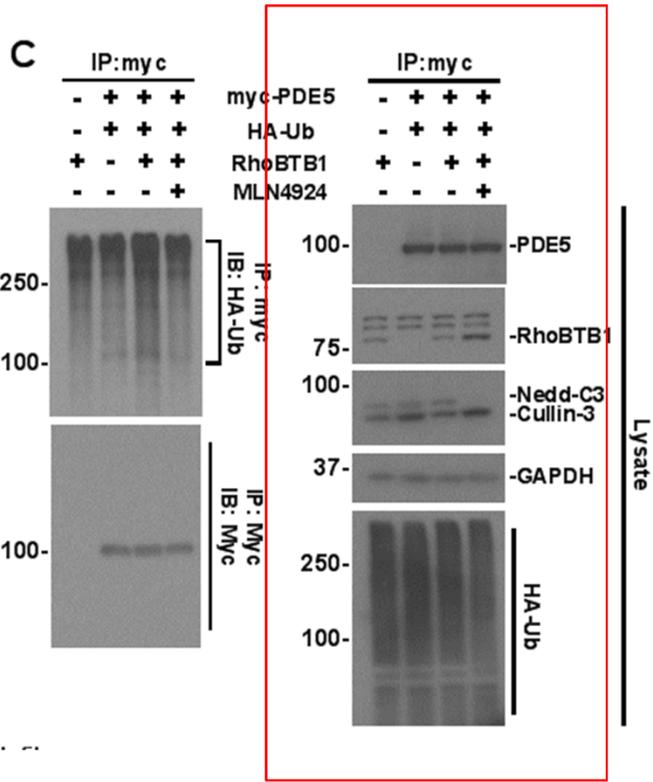


## Full unedited gel for Figure 6B

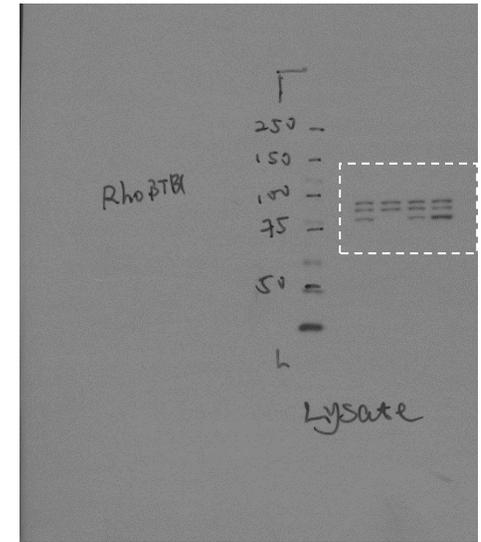
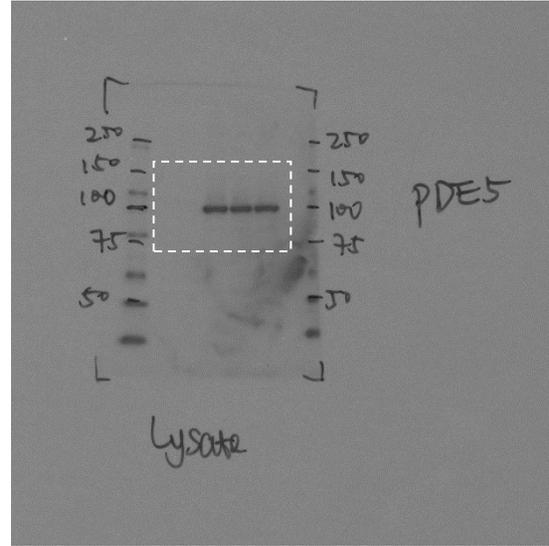


## Full unedited gel for Figure 6C Left



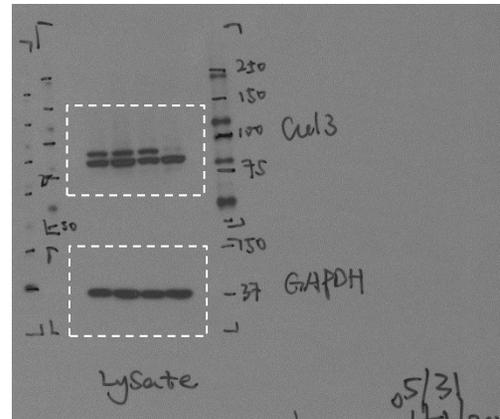
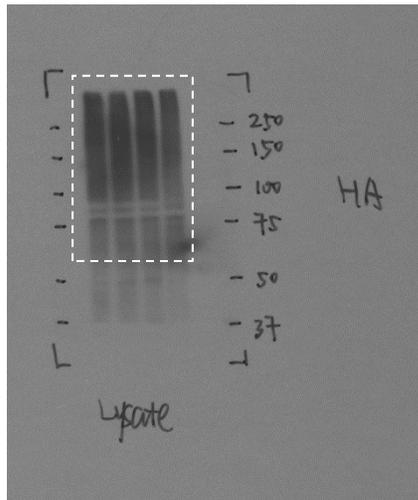


**Full unedited gel for Figure 6C Right**



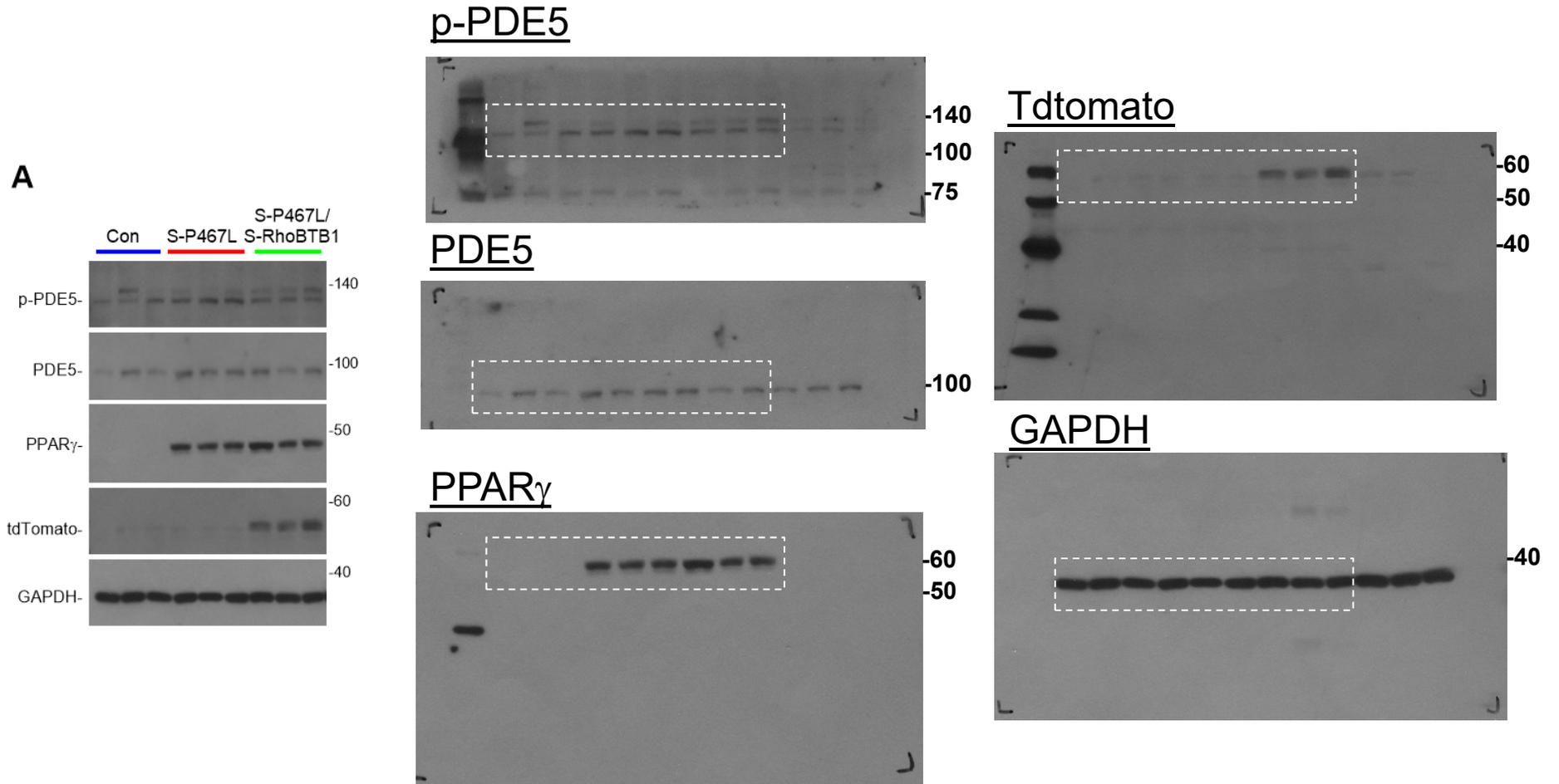
Probe with RhoBTB1  
Then reprobe with PDE5

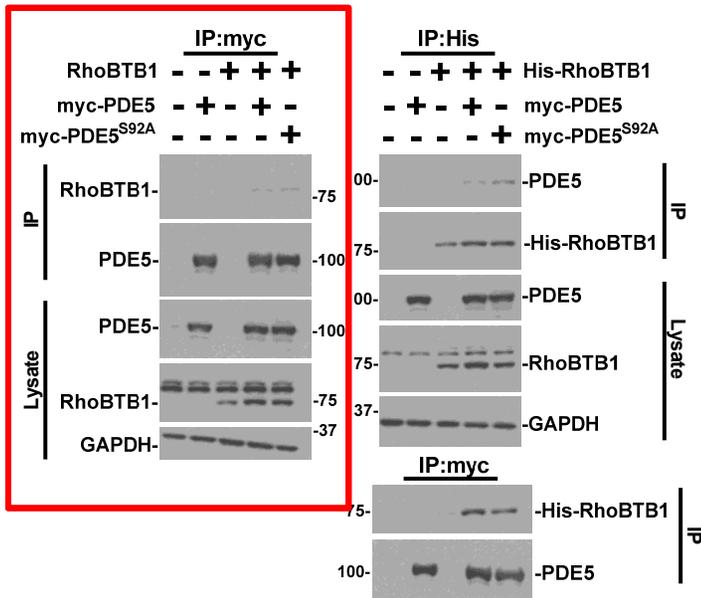
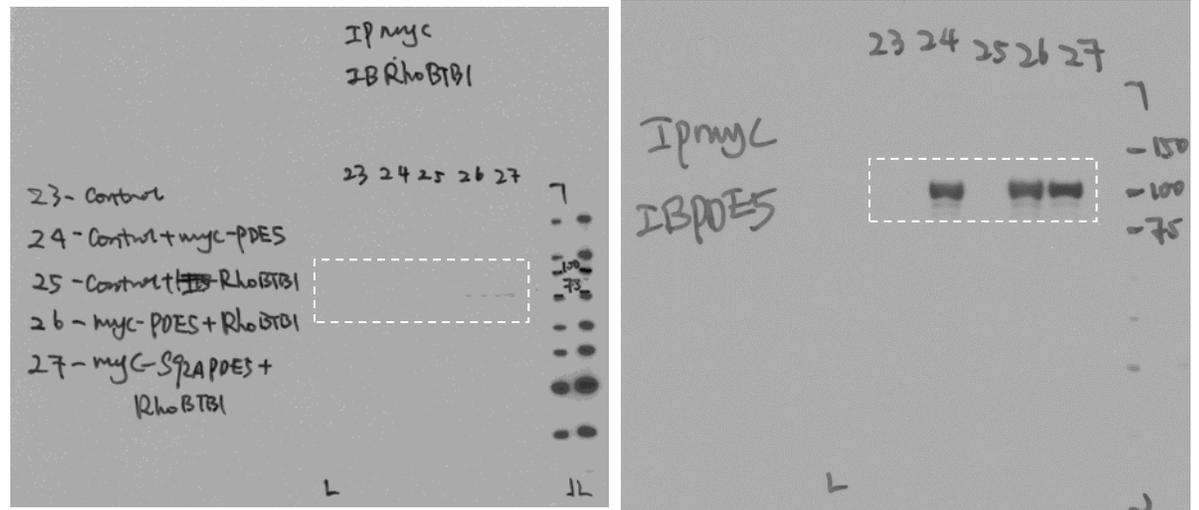
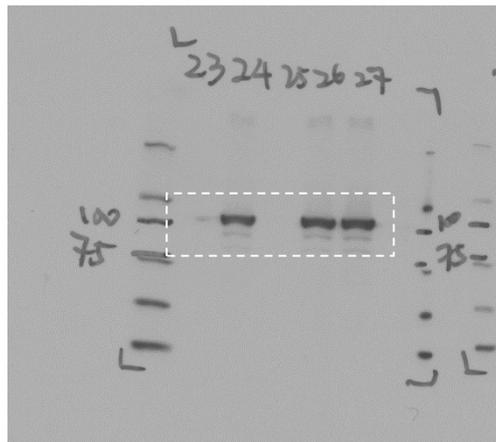
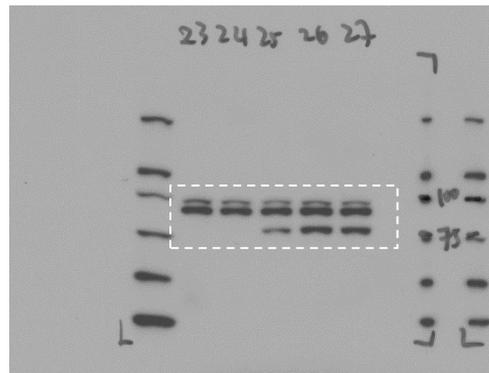
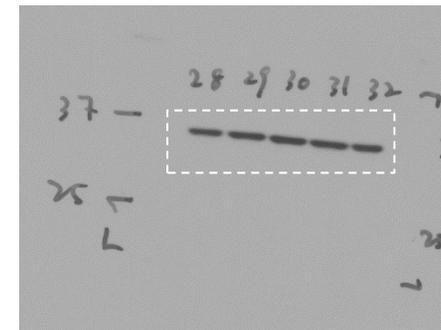
HA-Ub



Dual probe Cul3 and  
GAPDH

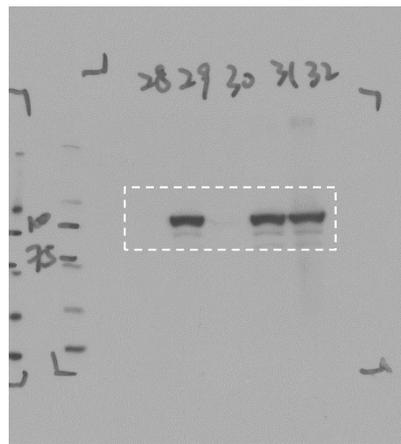
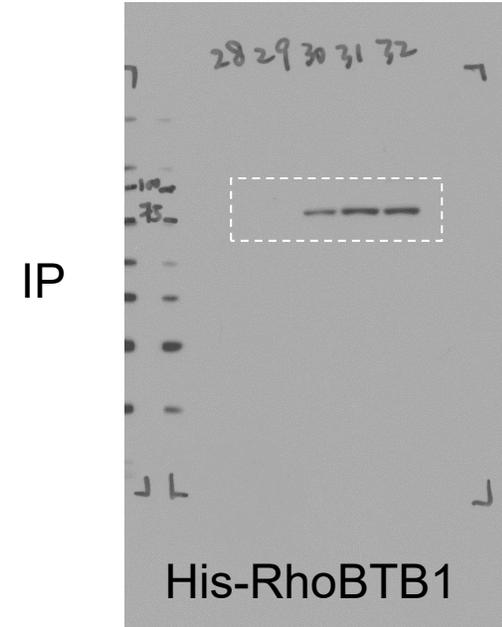
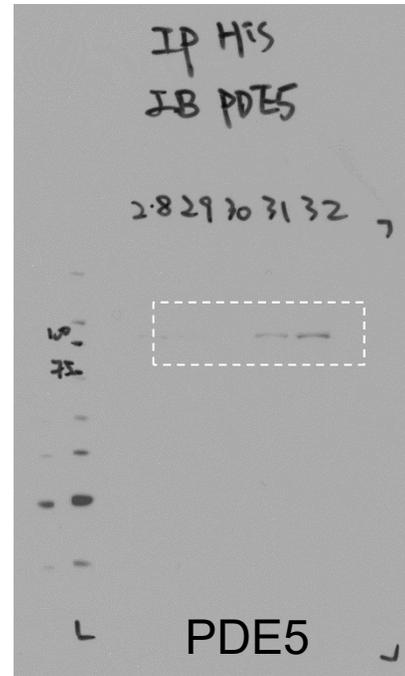
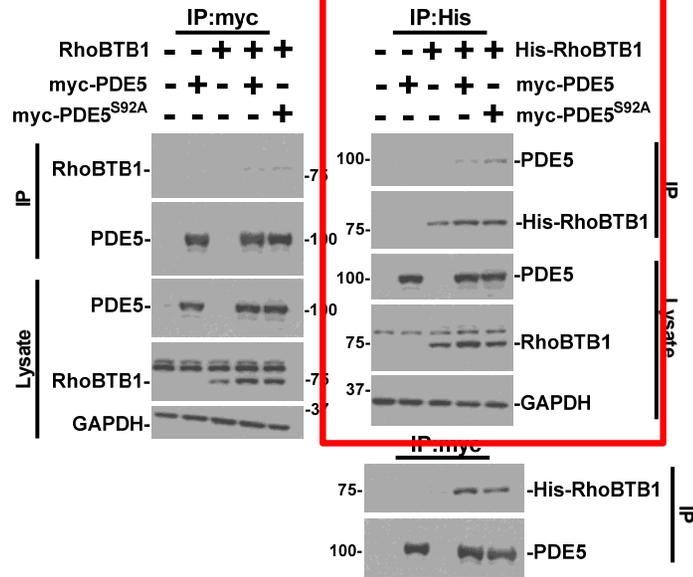
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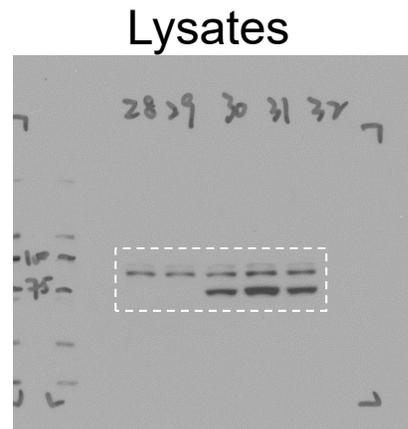
**B****Full unedited gel for Figure 7B Left****RhoBTB1****PDE5****Lysates****IP****PDE5****RhoBTB1****GAPDH**

Full unedited gel for Figure 7B Right TOP

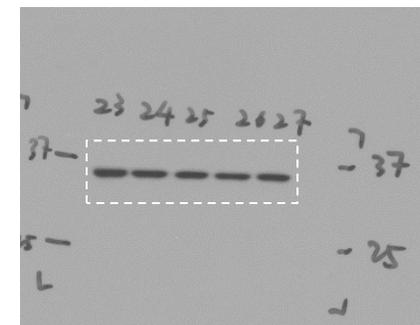
B



PDE5



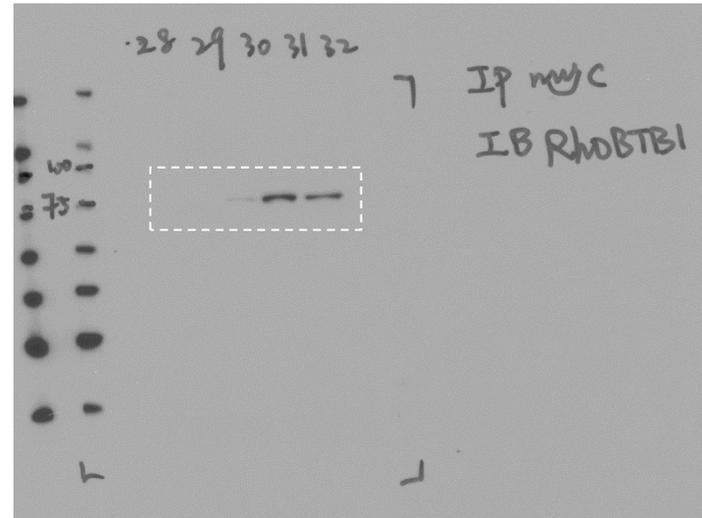
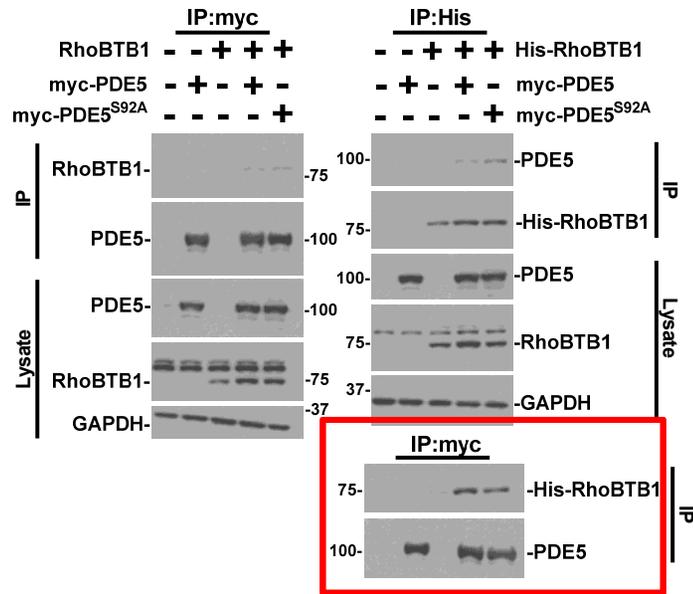
RhoBTB1



GAPDH

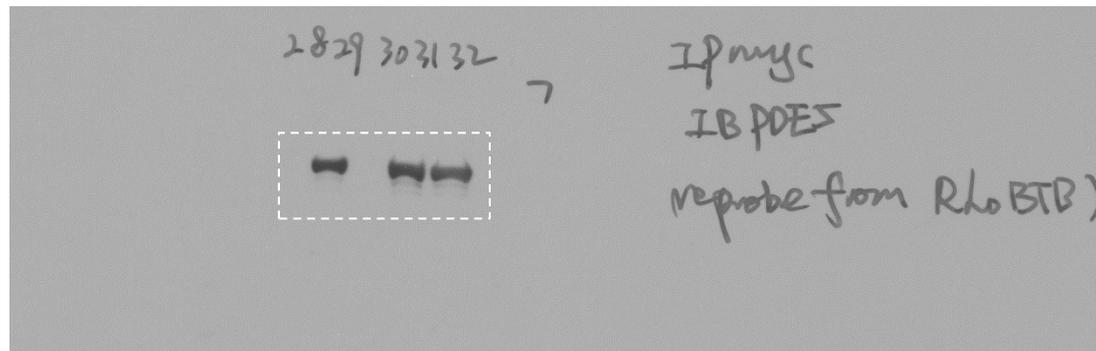
B

Full unedited gel for Figure 7B Right Bottom



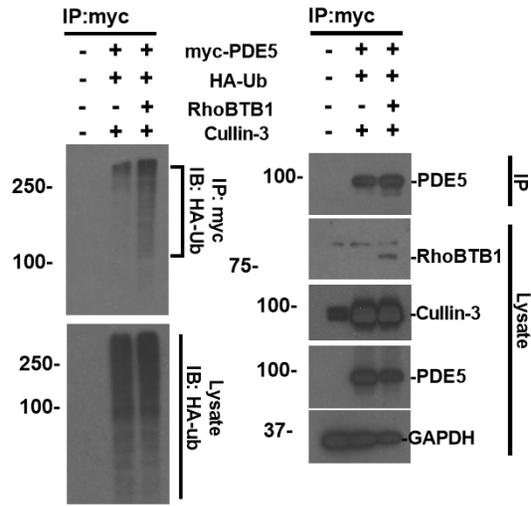
His-RhoBTB1

PDE5

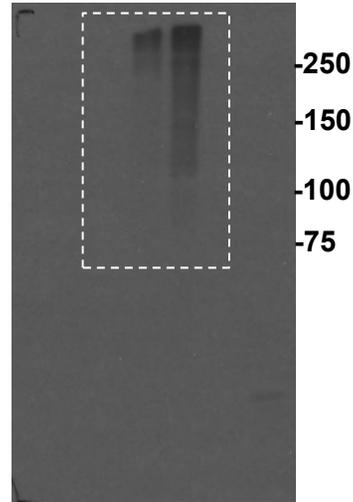


## Full unedited gel for Figure S6A

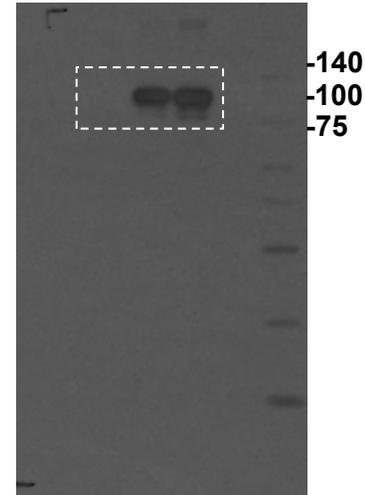
**A**



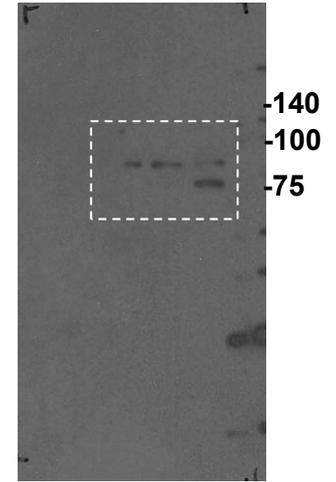
IP:myc, WB:HA



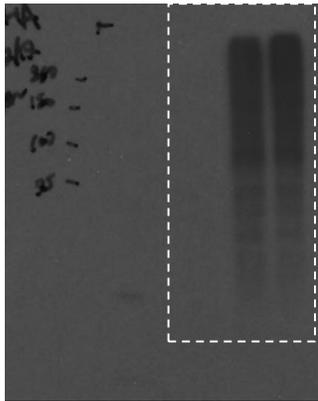
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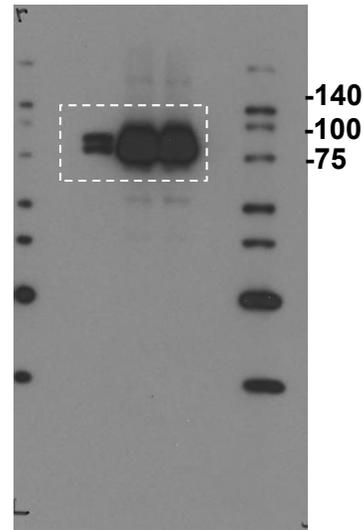
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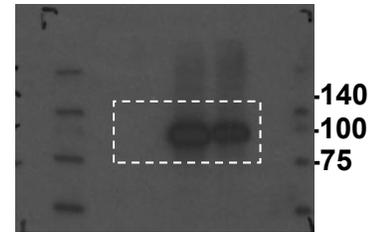
IP:myc, WB:HA Lysate



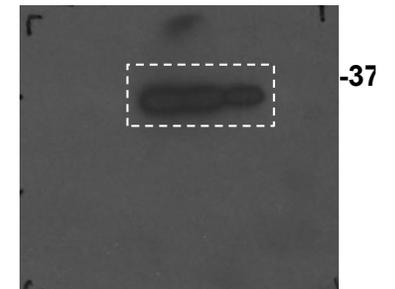
Cullin3



PDE5



GAPDH



**Full unedited gel for Figure S6B**

**B**

