Online Supplement

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

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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- CreERT²) expressing the tdTomato reporter in smooth muscle by crossing ROSA26 reporter mice with mice expressing a tamoxifen-inducible Cre recombinase (CreERT²) 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), PDE9α (C), PDE11α (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±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 μM) or Cilostamide (1 μM). All samples are n=5. All data are mean±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.
Full unedited gel for Figure 1B

Universal vector: - - + - + -
Myc-RhoBTB1: - + - - - -
CAG-RhoBTB1: - - - + - +
Cre Vector: - - - + + +

IB:RhoBTB1
IB:RFP
IB:Myc
IB:GAPDH

RhoBTB1
RFP
Myc
GAPDH
Full unedited gel for Figure 1E
Full unedited gel for Figure 2M

- P-MYPT (Thr696)
- PPARγ
- Tdtomato
- GAPDH
Full unedited gel for Figure 4F TOP

- sGCα1
- sGCβ1
- PKG1
Full unedited gel for Figure 4F Bottom
Full unedited gel for Figure 6A
Full unedited gel for Figure 6B
Full unedited gel for Figure 6C Left
Probe with RhoBTB1
Then reprobe with PDE5

Dual probe Cul3 and GAPDH
Full unedited gel for Figure 7A

A

Con  S-P467L  S-RhoBTB1

-140
-100
-75

p-PDE5

PDE5

PPARγ

Tdtomato

-40

GAPDH

-40
Full unedited gel for Figure 7B Right Bottom

His-RhoBTB1

PDE5
Full unedited gel for Figure S6A

A

IP: myc, WB: HA

- IP: myc
- WB: HA

- myc-PDE5
- HA-Ub
- RhoBTB1
- Cullin-3

- Lysate

IP: myc, WB: PDE5

- IP: myc
- WB: PDE5

- Lysate

RhoBTB1

- IP: myc
- WB: HA Lysate

- IP: myc
- WB: HA

- Cullin3

- Lysate

PDE5

- IP: myc
- WB: HA

- Cullin3

- Lysate

GAPDH

- IP: myc
- WB: HA

- Cullin3

- Lysate

- IP: myc
- WB: HA

- Cullin3

- Lysate

- GAPDH

- IP: myc
- WB: HA

- Cullin3

- Lysate

- IP: myc
- WB: HA

- Cullin3

- Lysate

- GAPDH

- IP: myc
- WB: HA

- Cullin3

- Lysate

- IP: myc
- WB: HA

- Cullin3

- Lysate

- GAPDH

- IP: myc
- WB: HA

- Cullin3

- Lysate

- IP: myc
- WB: HA

- Cullin3

- Lysate

- GAPDH

- IP: myc
- WB: HA

- Cullin3

- Lysate

- IP: myc
- WB: HA

- Cullin3

- Lysate

- GAPDH
Full unedited gel for Figure S6B

### IP: myc, WB: HA
- IP: myc, WB: HA
- IP: myc, WB: RhoBTB1
- IP: myc, WB: PDE5

### WB: HA
- WB: HA
- WB: RhoBTB1
- WB: PDE5
- WB: GAPDH

### WB: RhoBTB1
- WB: RhoBTB1
- WB: PDE5
- WB: GAPDH

### WB: PDE5
- WB: PDE5
- WB: GAPDH

### WB: GAPDH
- WB: GAPDH
- WB: PDE5

### Full Gel
- Gel for Figure S6B with indicated bands for IP: myc and WB: RhoBTB1, HA, PDE5, and GAPDH.

### Table

<table>
<thead>
<tr>
<th>Description</th>
<th>Condition 1</th>
<th>Condition 2</th>
</tr>
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<tbody>
<tr>
<td>myc-PDE5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HA-ub</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>RhoBTB1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HA</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Legend:**
- IP: myc (immunoprecipitation with myc antibody)
- WB: HA (Western blotting with HA antibody)
- WB: PDE5 (Western blotting with PDE5 antibody)
- WB: RhoBTB1 (Western blotting with RhoBTB1 antibody)
- WB: GAPDH (Western blotting with GAPDH antibody)

**Notes:**
- Bands are visualized across lanes for different conditions.
- Full gel for detailed analysis.