

Supplemental information

Adiponectin Signaling Regulates Urinary Bladder Function by Blunting Smooth Muscle Purinergic Contractility

Zhaobo Luo,¹ Ali Wu,¹ Simon Robson,^{3,5} Seth L. Alper,^{1,2,4} and Weiqun Yu^{1,2,4*}

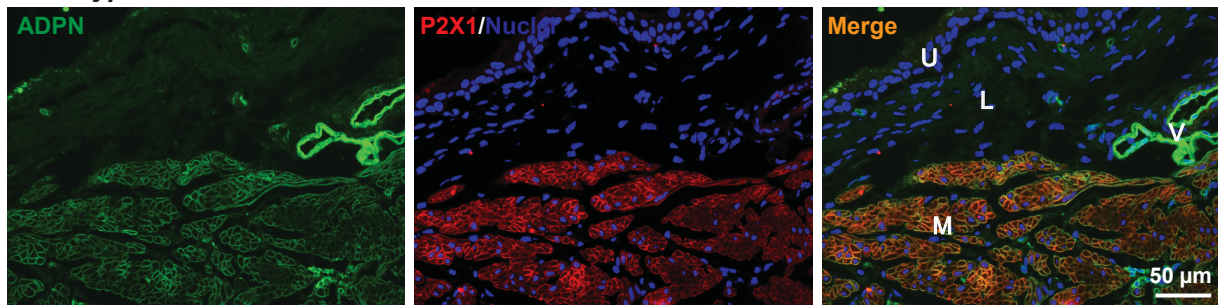
¹Division of Nephrology and Departments of ²Medicine and ³Anesthesia, Beth Israel Deaconess

Medical Center, Boston, MA 02215; ²Departments of ⁴Medicine and ⁵Anesthesia, Harvard

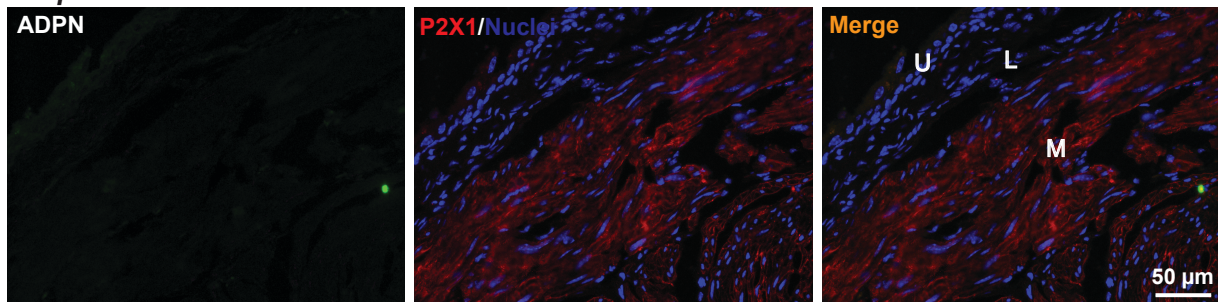
Medical School, Boston, Massachusetts 02115

Supplemental Figure 1. Adiponectin protein is absent from *Adpn*^{-/-} mice BSM cells. **A:** *Wild-type*) and **B:** *Adpn*^{-/-} bladder tissues immunostained with anti-adiponectin antibody (green) and anti-P2X1 antibody (red). ADPN is localized in BSM layer (**A**) and completely absent from *Adpn*^{-/-} bladder tissue (**B**). The P2X1 signal is significantly decreased in *Adpn*^{-/-} mice BSM cells. Nuclei were labeled with DAPI (blue). U: urothelial cells; L: lamina propria; M: smooth muscle; V: blood vessel. **C:** Western blot of adiponectin protein detected a specific band above 25KD in *wild-type* bladder absent from *Adpn*^{-/-} bladder (n=6), with GAPDH loading control (n=6).

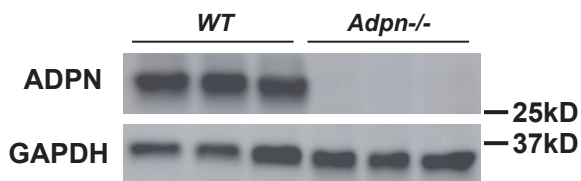
A Wild type



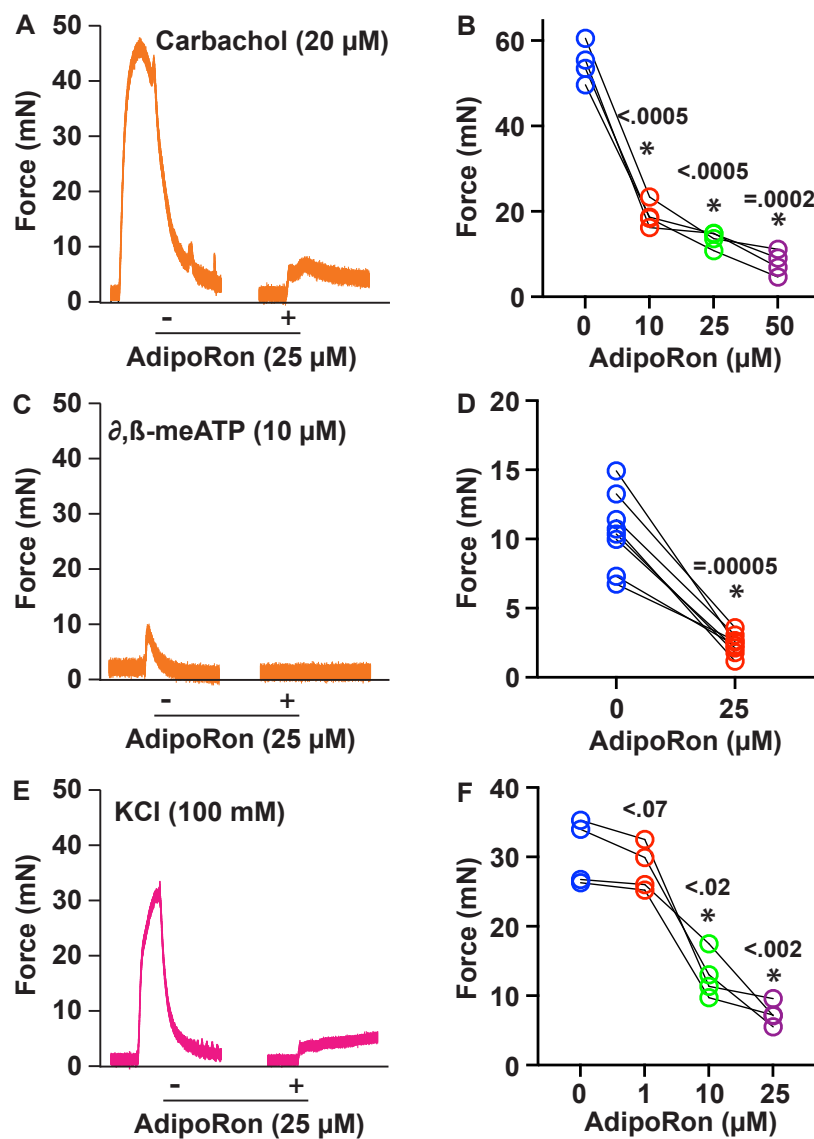
B *Adpn*^{-/-}



C



Supplemental Figure 2. Adiponectin receptor agonist AdipoRon inhibited mouse BSM contractile force in a dose-dependent manner. Representative *wild-type* male BSM contraction traces in response to carbachol (A), α , β -meATP (C), and KCl (E), before and after exposure to AdipoRon. B, D, and F: Summarized data corresponding to experiments in formats of panels A, C and E (n=4-8). Data show individual symbols and line plots for each sample. Paired student *t*-test, *P* values above data points. *, *P*<0.05.



Supplemental Table 1. Antibody information

Antibody	Company	Catalog	Host	Application
Adiponectin	R&D system	AF1119	Goat	WB, IF
Ki67	Invitrogen	14-5698-82	Rat	IF
Alkaline Phosphatase	R&D system	AF2910	Goat	WB
ENTPD1	Invitrogen	MA5-32707	Rabbit	WB
ENTPD2	R&D system	AF5797	Sheep	WB
CHRM3	Invitrogen	PA585322	Rabbit	WB
P2X1	Alomone laboratory	APR-001	Rabbit	WB, IF
α SMA	Cell Signaling Technology	19245S	Rabbit	WB
TAGLN	Cell Signaling Technology	40471S	Rabbit	WB
SMMHC	Proteintech	21404-1-AP	Rabbit	WB
5'-Nucleotidase	R&D system	MAB44881	Rat	WB
Insulin Receptor β	Cell Signaling Technology	23413S	Rabbit	WB
p-AKT	Cell Signaling Technology	9271T	Rabbit	WB
AKT	Cell Signaling Technology	9272S	Rabbit	WB
p-Erk1/2	Cell Signaling Technology	9101S	Rabbit	WB
Erk1/2	ABclonal	A4782	Rabbit	WB
AMPK- α	Cell Signaling Technology	2532S	Rabbit	WB
GAPDH	ABclonal	A19056	Rabbit	WB