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Supplementary Materials for

A molecular signature in the pannexin1 intracellular loop confers channel activation by the α1 adrenoreceptor in smooth muscle cells

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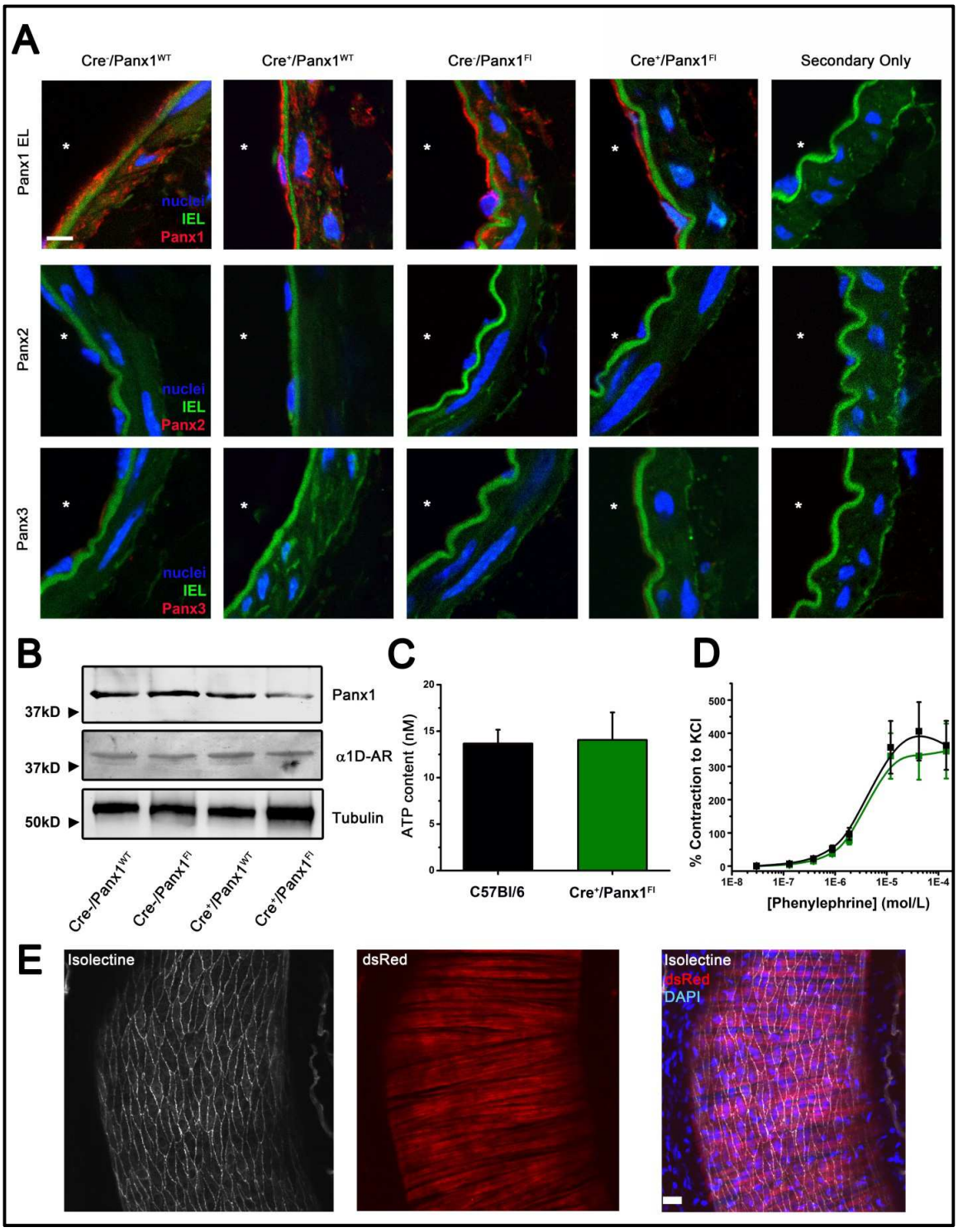

Figure S1: Effect of SMC deletion of Panx1 on Panx2 and Panx3 abundance, ATP content, α1DAR abundance, and phenylephrine-induced contraction of aortic rings. (**A**) Representative immunofluorescent labeling using an antibody directed against the extracellular loop of Panx1 (top row, red), against Panx2 (red, middle row), and against Panx3 (red, lower row) on cross sections of thoracodorsal arteries (TDAs) isolated from $Cre^{-}/Panx1^{WT}$ mice, $Cre^{-}/Panx1^{Fl}$ mice, $Cre^{+}/Panx1^{WT}$ mice, and $Cre^{+}/Panx1^{Fl}$ all injected with tamoxifen for ten days. The right panel shows a negative control with the secondary antibody only on a cross section of a thoracodorsal artery isolated from $Cre^{-}/Panx1^{WT}$ mice. The autofluorescence of the internal elastic lamina appears in green, and the nuclei were labelled with DAPI (blue). * indicates the lumen. Scale bar = 10μm. (**B**) Representative Western blot of isolated TDAs showing the abundance of Panx1 in $Cre^{+}/Panx1^{Fl}$ (top panel), or the abundance of α1DAR in $Cre^{-}/Panx1^{WT}$ mice, $Cre^{-}/Panx1^{Fl}$ mice, $Cre^{+}/Panx1^{WT}$ mice, and $Cre^{+}/Panx1^{Fl}$ all injected with tamoxifen for ten days (middle panel). Lower panel shows tubulin as a loading control. (**C**) Histogram of total ATP content measured in TDAs from C57Bl/6 (black) and $Cre^{+}/Panx1^{Fl}$ (green). (**D**) The contractile response of aortic rings isolated from control $Cre^{+}/Panx1^{Fl}$ (injected with peanut oil, black curve), or isolated from $Cre^{+}/Panx1^{Fl}$ injected with tamoxifen for ten days (green curve) in response to cumulative concentrations of phenylephrine. n=12-13 rings (6 mice in each group). (**E**) Representative images of TDAs isolated from a $Cre^{+}/tdTomato^{+}$ mouse showing the presence of dsRed only in the SMCs. Endothelial cells were labeled with isolectine and the nuclei are shown in blue. Scale bar = 20um.

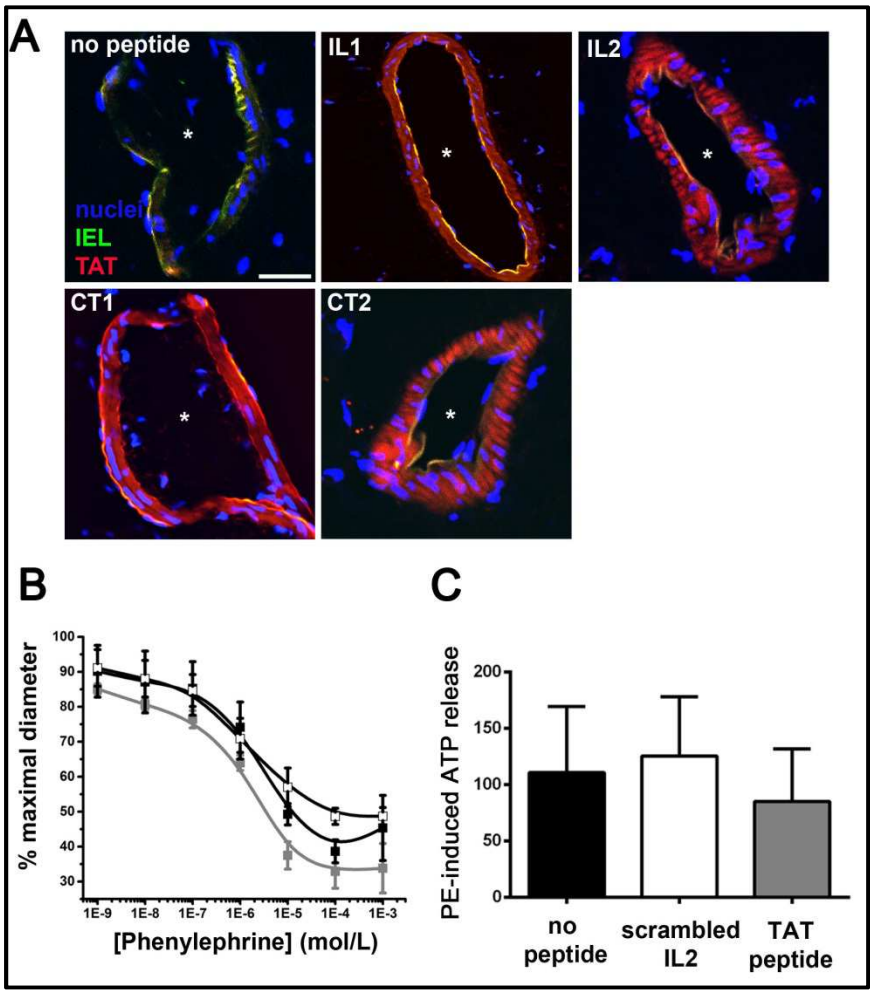

Figure S2: Effect of IL2 scrambled peptide and TAT peptide on phenylephrine-mediated constriction and ATP release. (**A**) Representative immunofluorescent labeling using an antibody directed against the TAT sequence (red) on cross sections of TDAs incubated with the IL1, IL2, CT1, or CT2 peptides. * indicates the lumen, green is the autofluorescence of the internal elastic lamina (IEL), and blue indicates the nuclei labeled with DAPI. Scale bar is 50μm. (**B**) Effect of scrambled IL2 peptide (grey curve) and TAT peptide (black curve with open boxes) on contraction of pressurized TDAs in response to cumulative concentrations of phenylephrine. n=5 for each condition. (**C**) The effect of no peptide (black) scrambled IL2 peptide (white) and TAT peptide (grey) was tested on phenylephrine-induced ATP release. Data are expressed as a percent increase in ATP concentration from unstimulated conditions. n=3-4.

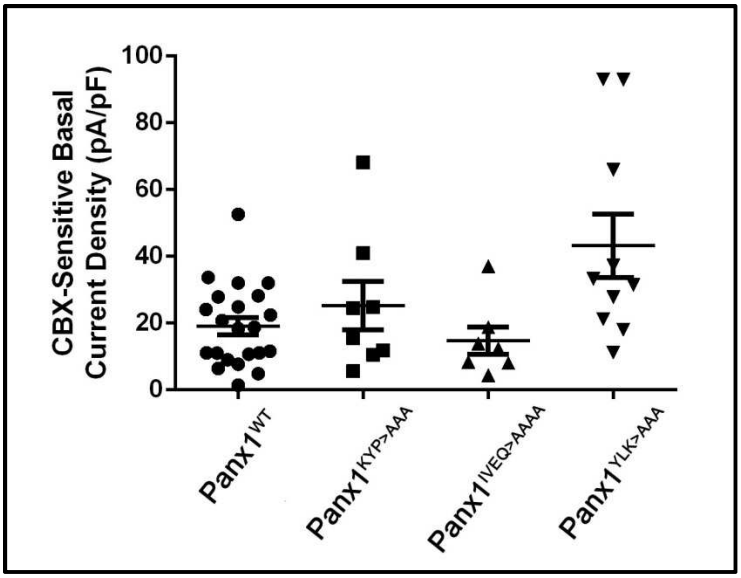

Figure S3: Effect of mutations in the Panx1 IL2 region on channel function. Carbenoxolone-sensitive currents measured in HEK293 cells cotransfected with α1DAR and $Panx1^{WT}$, $Panx1^{KYP>AAA}$, $Panx1^{IVEQ>AAAA}$, or $Panx1^{YLK>AAA}$ showing similar Panx1 currents produced by the different Panx1 mutants in unstimulated conditions. Results are expressed in current density (pA/pF).

Table S1: Contractile properties of aortic rings isolated from control $Cre^+/Panx1^{Fl}$ mice and $Cre^+/Panx1^{Fl}$ injected with tamoxifen for 10 days. The EC_{50} and E_{MAX} are calculated from the data presented in figure S1D. EC_{50} represents the concentration needed to produce 50% of the maximum effect (E_{MAX}). E_{MAX} is expressed as the percentage of constriction to KCl. Data are presented as mean ± sem.

	Phenylephrine	
	$Cre^+/Panx1^{FL}$ Peanut oil	$Cre^+/Panx1^{FL}$ With tamoxifen
EC_{50} (µmol/L)	3.39 ± 0.38	3.20 ± 0.32
E_{MAX}	368.8 ± 78.7	343.4 ± 69.4

Table S2: Effect of scrambled IL2 peptide and TAT peptide on constriction of TDAs in response to phenylephrine. The EC_{50} and E_{MAX} are calculated from the data presented in figure S2B. EC_{50} represents the concentration needed to produce 50% of the maximum effect (E_{MAX}). E_{MAX} is expressed as the percentage of maximal diameter. Data are presented as mean ± sem.

	Phenylephrine		
	No peptide	Scrambled IL2	TAT
EC_{50} (µmol/L)	2.98 ± 0.68	2.87 ± 1.97	1.63 ± 0.35
E_{MAX}	39.7 ± 6.75	47.5 ± 2.17	32.0 ± 6.21

Table S3: Primers used for the generation of $Panx1^{KYP>AAA}$, $Panx1^{IVEQ>AAAA}$, and $Panx1^{YLK>AAA}$ plasmids.

$Panx1^{KYP>AAA}$	forward	5'-GTGGGAGATATCTGAAAGCCACTTCGCGGCCGCAATCGTGGAGCAGTACTTGAAGAC-3'
	reverse	5'-GTCTTCAAGTACTGCTCCACGATTGCGGCCGCGAAGTGGCTTTCAGATATCTCCCAC-3'
$Panx1^{IVEQ>AAAA}$	forward	5'-CTGAAAGCCACTTCAAGTACCCAGCCGCGGCGGCGTACTTGAAGACAAAAAAGAACTC-3'
	reverse	5'-GAGTTCTTTTTTGTCTTCAAGTACGCCGCCGCGGCTGGGTACTTGAAGTGGCTTTCAG-3'
$Panx1^{YLK>AAA}$	forward	5'-CACTTCAAGTACCCAATCGTGGAGCAGGCCGCGGCGACAAAAAAGAACTCTAGTCATTTAATC-3'
	reverse	5'-GATTAAATGACTAGAGTTCTTTTTTGTCGCCGCGGCCTGCTCCACGATTGGGTACTTGAAGTG-3'