

Supplementary material

Pannexin1-dependent and -independent protection by novel peptidomimetics against cardiac ischemia/reperfusion injury

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Short Title: Panx1-targeting peptidomimetics to prevent cardiac reperfusion injury

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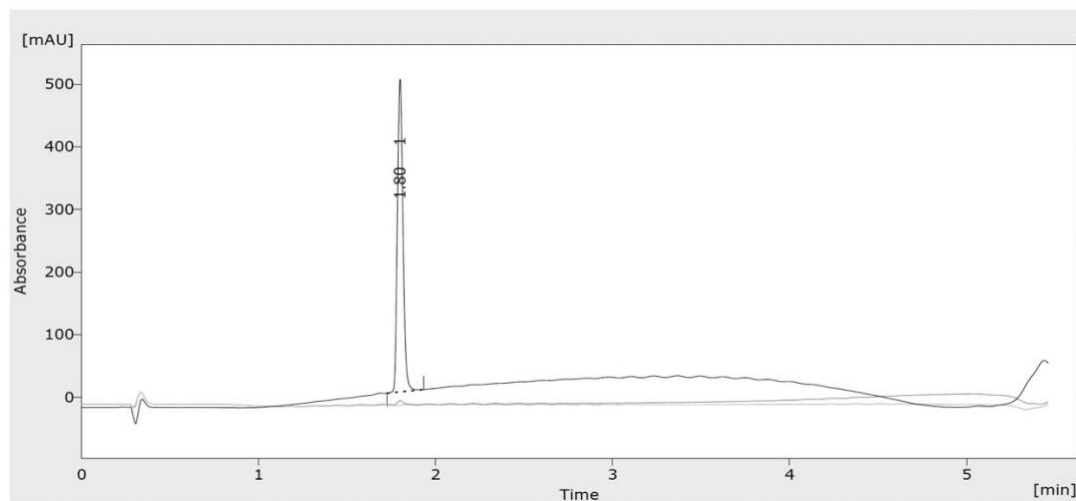
Table S1: Analytical data of synthesized peptides

	Sequence ^(a)		Formula	MW ^(b) (g.mol ⁻¹)	R _t ^(b) (min)	Yield (%)	Purity (%)	HRMS calculated	HRMS found
EL1	¹⁰ Panx	H-WRQAAFVDSY-OH	C ₅₈ H ₇₉ N ₁₅ O ₁₆	1917.9	1.87	6	96	1711.7304	1711.7303
EL1	SBL-PX1-42	Ac-SWRQA c [Pra FVD Azk]Y-NH ₂	C ₅₈ H ₇₉ N ₁₅ O ₁₆	1470.41	1.68	43	>99	1242.5907	1242.5928
EL2 Seq.1	SBL-PX1-62	H- <u>SL</u> SSLSEFV-NH ₂	C ₄₇ H ₇₅ N ₁₁ O ₁₈	1196.2	1.8	15	> 99	1082.5370	1082.5431
EL2 Seq.2	SBL-PX1-63	Ac-EFVCSIKSGI-NH ₂	C ₅₀ H ₈₂ N ₁₂ O ₁₅ S	1237.4	1.88	21	98	1123.5822	1123.5862
	SBL-PX1-64	H-EFVCSIKSGI-NH ₂	C ₄₈ H ₈₀ N ₁₂ O ₁₄ S	1309.3	1.69	23	> 99	1081.5715	1081.5752
EL2 Seq.3	SBL-PX1-65	Ac-SGILRNDSTV-NH ₂	C ₄₅ H ₇₉ N ₁₅ O ₁₇	1216.2	1.55	14	97	1102.5857	1102.5874
	SBL-PX1-66	H-SGILRNDSTV-NH ₂	C ₄₃ H ₇₇ N ₁₅ O ₁₆	1288.2	1.42	18	> 99	1060.5760	1060.5751
EL2 Seq.4	SBL-PX1-67	Ac-STVPDQFQCK-NH ₂	C ₅₁ H ₈₀ N ₁₄ O ₁₇ S	1306.4	1.53	29	> 99	1193.5625	1193.5626
	SBL-PX1-68	H-STVPDQFQCK-NH ₂	C ₄₉ H ₇₈ N ₁₄ O ₁₆ S	1379.4	1.45	27	> 99	1151.5519	1151.5527
EL2 Seq.5	SBL-PX1-70	H-QCKLIAVGIF-NH ₂	C ₅₁ H ₈₇ N ₁₃ O ₁₁ S	1318.4	2.03	11	95	1090.6447	1090.6433
	SBL-PX1-211	H-QSKLIAVGIF-NH ₂	C ₅₁ H ₈₇ N ₁₃ O ₁₂	1302.38	2.09	10	97	1074.6675	1074.6681
	SBL-PX1-213	H-K(Ahx)-QSKLIAVGIF-NH ₂	C ₆₃ H ₁₁₀ N ₁₆ O ₁₄	1657.74	2.05	18	96	1315.8466	1315.8477
EL2 Seq.6	SBL-PX1-136	H-QLLSV <u>I</u> N-NH ₂	C ₄₄ H ₇₃ N ₁₁ O ₁₁	1046.16	2.19	14	>99	932.5569	932.5572

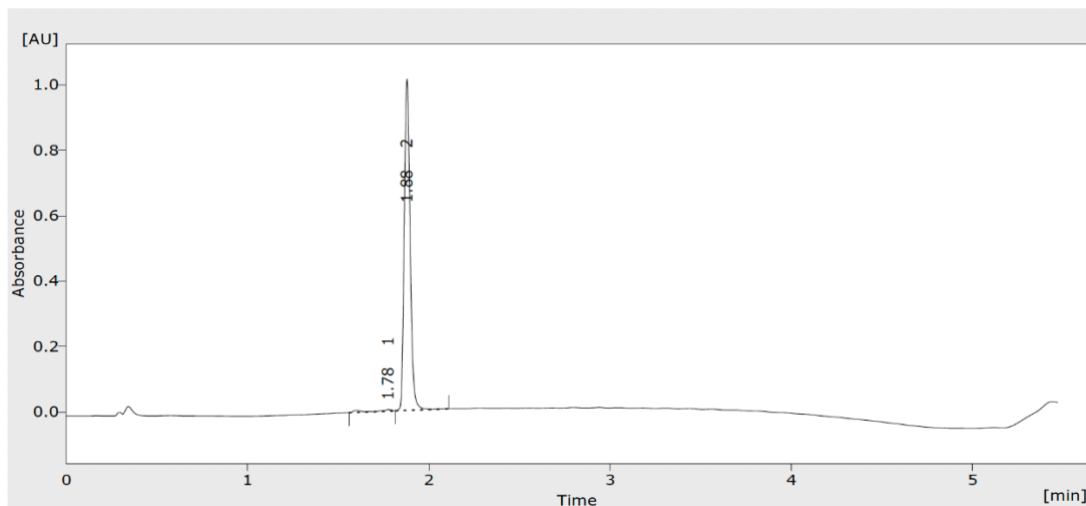
^(a) Underlined residues belong to the transmembrane domain. Compounds ¹⁰Panx and SBL-PX1-42 were previously reported (26). Residues between squared brackets are part of the peptide macrocycle. Ahx stands for 6-aminohexanoic acid; Azk and Pra stand for azido-lysine and propargylglycine precursors, respectively; ^(b) as TFA salts; ^(c) purity and retention time (R_t) are based on reversed-phase HPLC analysis.

RP-HPLC chromatograms of the synthesized peptidomimetics

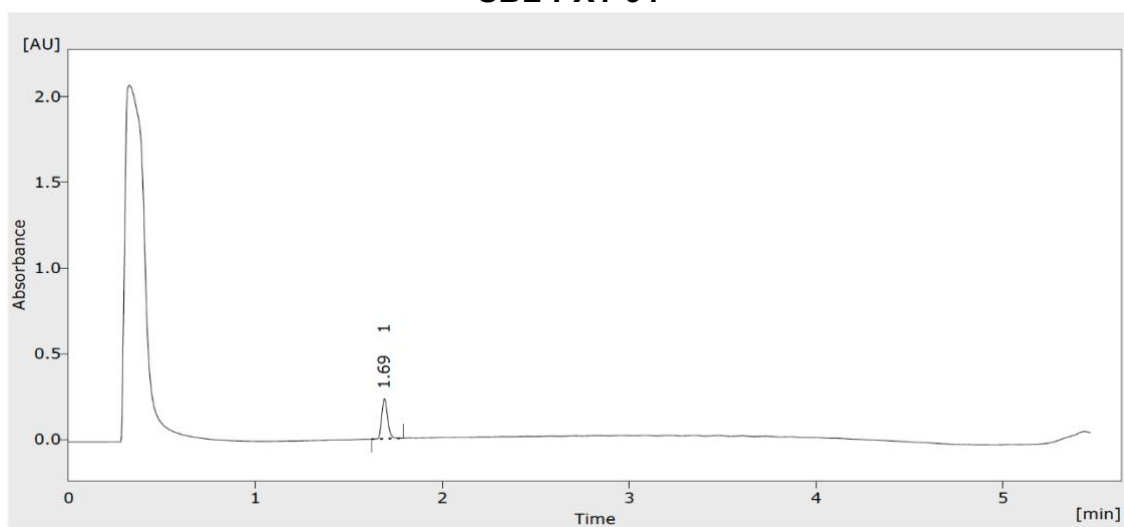
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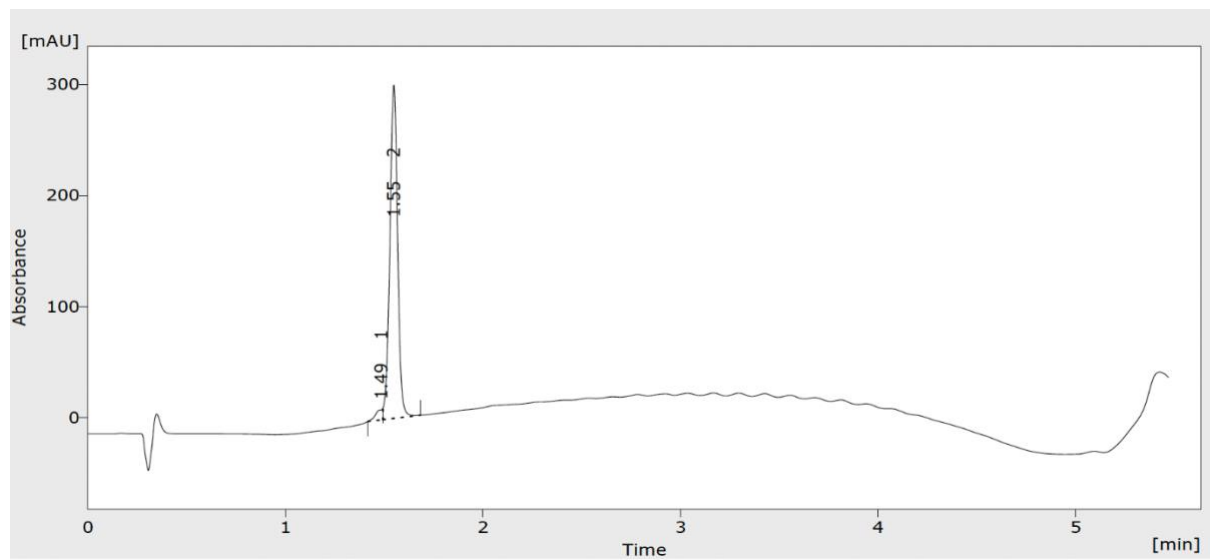
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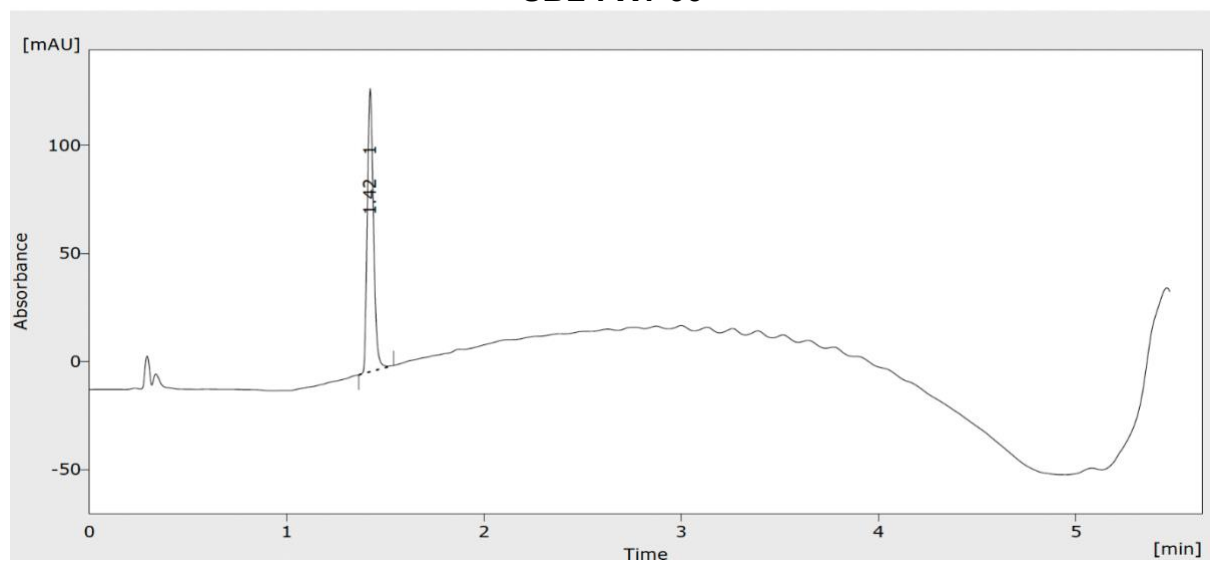
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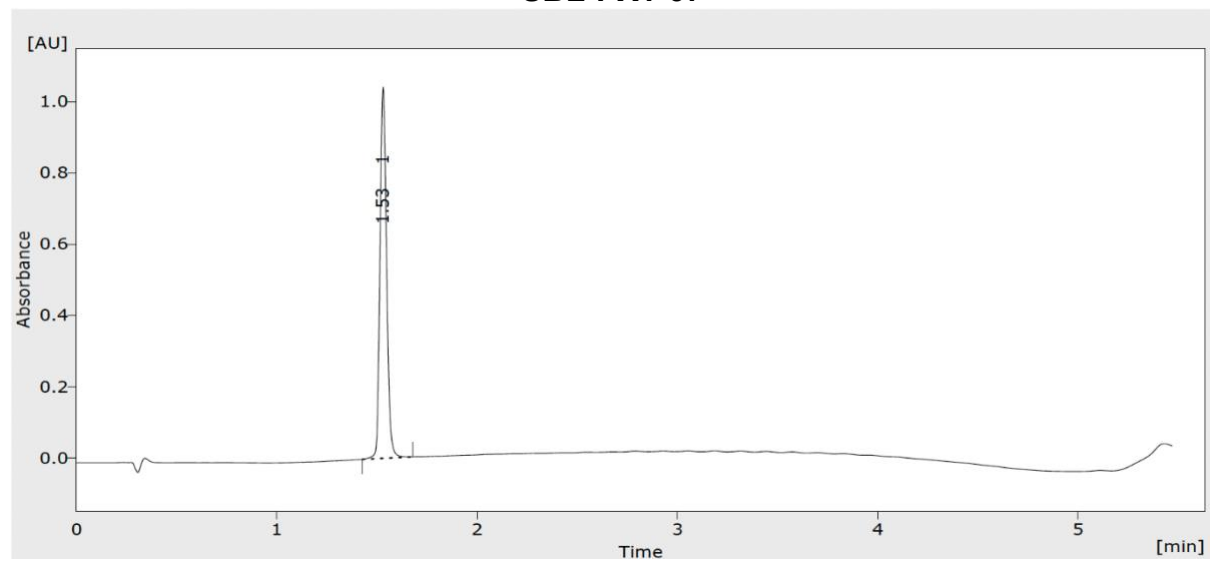
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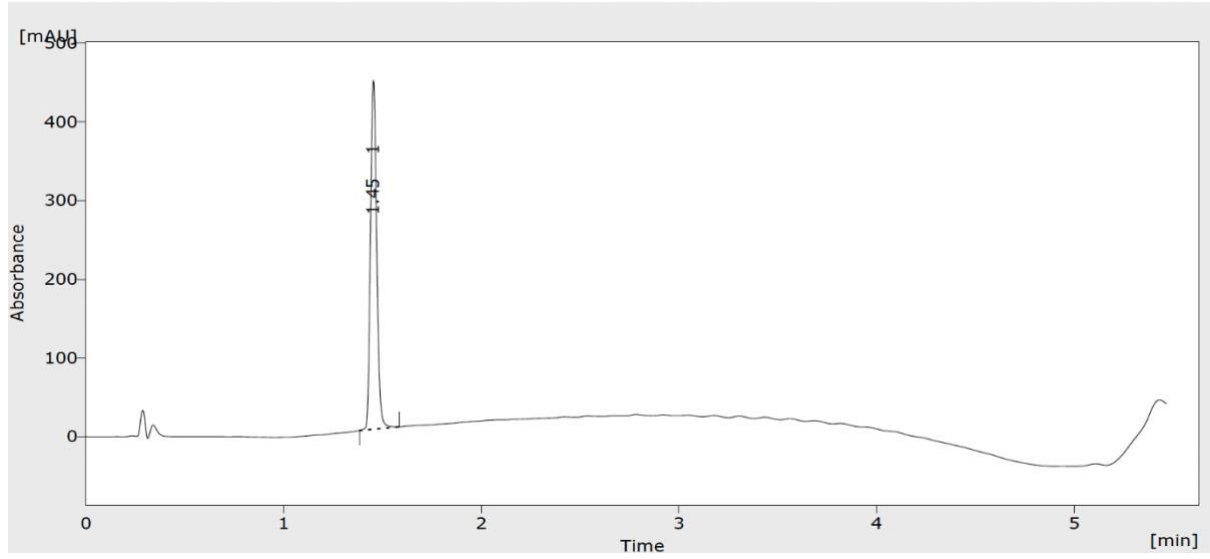
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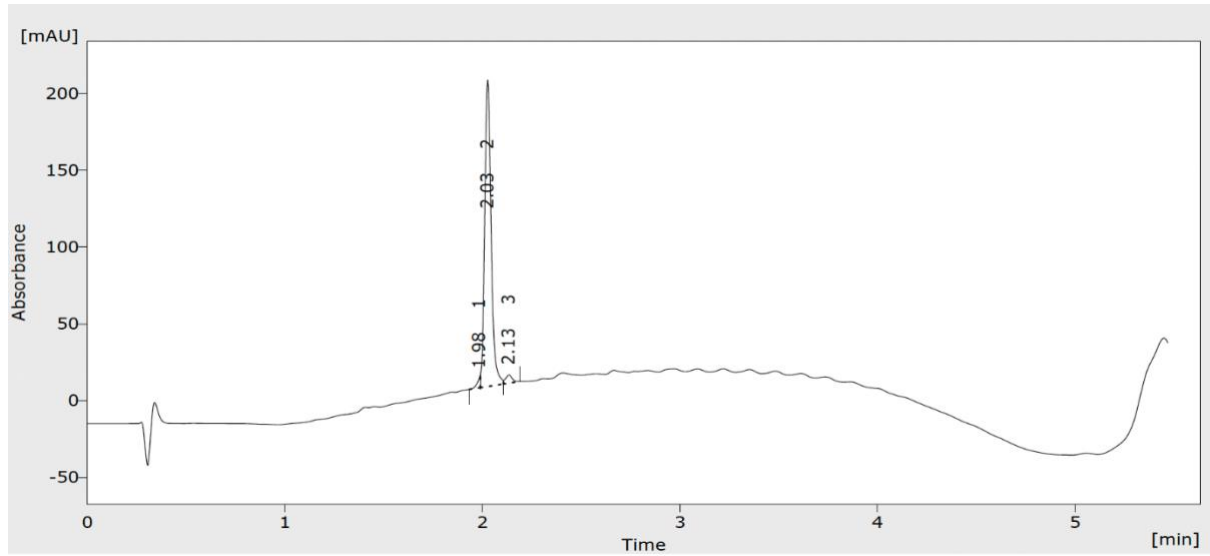
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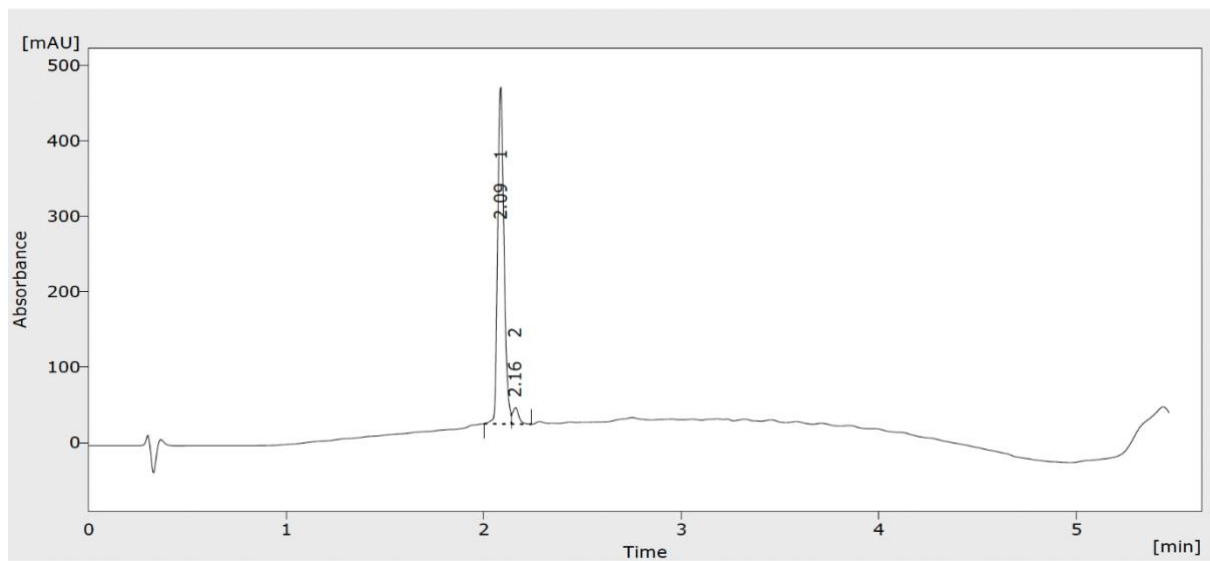
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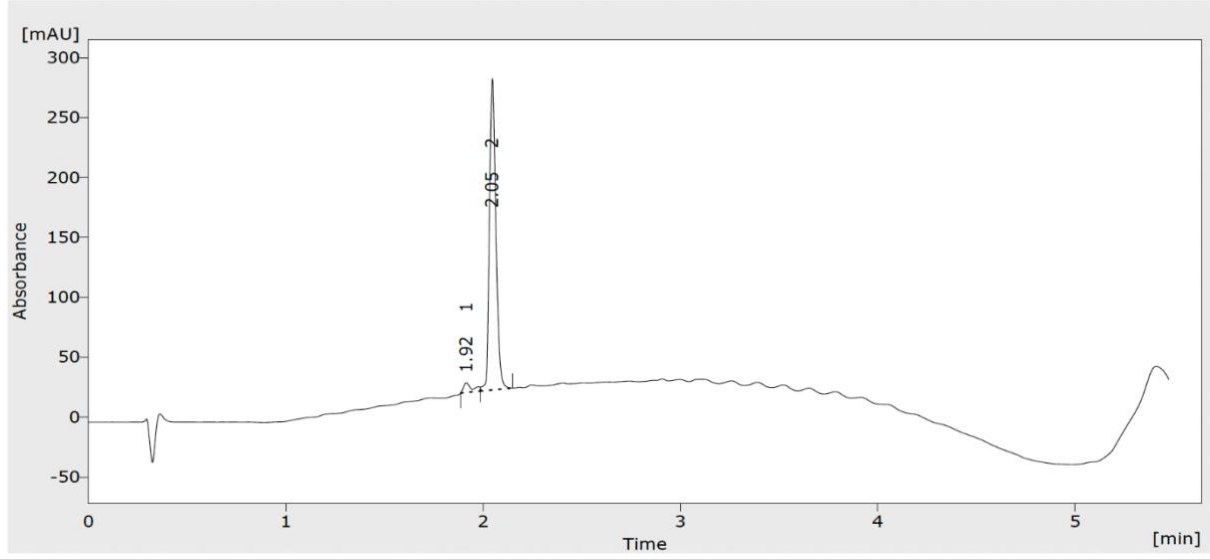
SBL-PX1-70



SBL-PX1-211



SBL-PX1-213



SBL-PX1-62

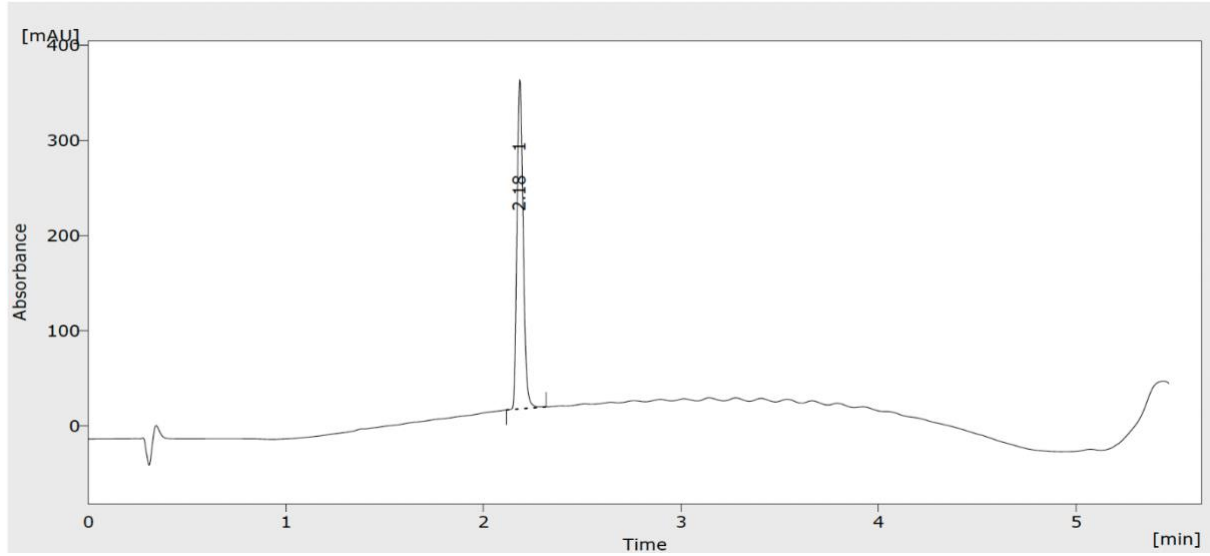


Table S2: Cardiac function is affected by SBL-PX1-211

	LVDP (mmHg)	+dP/dt (mmHg/sec)	-dP/dt (mmHg/sec)	HR (bpm)
Stabilization	103.1 ± 8.396	2545 ± 357.8	-1696 ± 192.3	283.5 ± 21.33
After 45 minutes washout	43.15 ± 24.44	1137 ± 627.8	-685.9 ± 390.4	121.4 ± 46.31

Cardiac function at the end of stabilization and after 45 min wash-out of 90 μ M SBL-PX1-211. N=5. Data are shown as mean $\pm$ SEM.

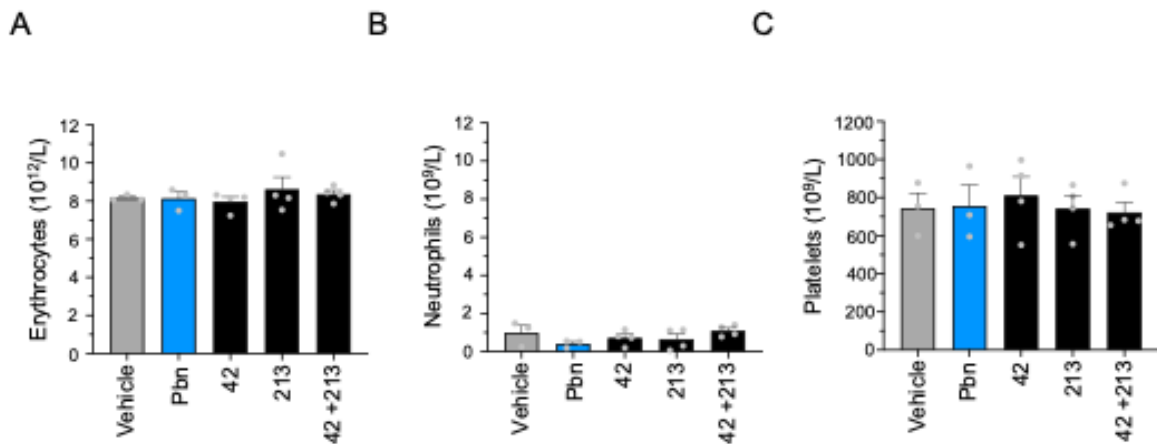


Figure S2: EL1 and EL2 peptidomimetics do not affect blood cell count

Erythrocyte (**A**), neutrophil (**B**) and platelet (**C**) numbers in blood samples of WT mice taken at 24 hours after cardiac I/R. Mice were treated at 5 min before reperfusion with a single intravenous injection of vehicle, 2.5 mM Pbn, 90 μ M SBL-PX1-42, 1 μ M SBL-PX1-213 or a combination thereof. Data are shown as mean $\pm$ SEM.

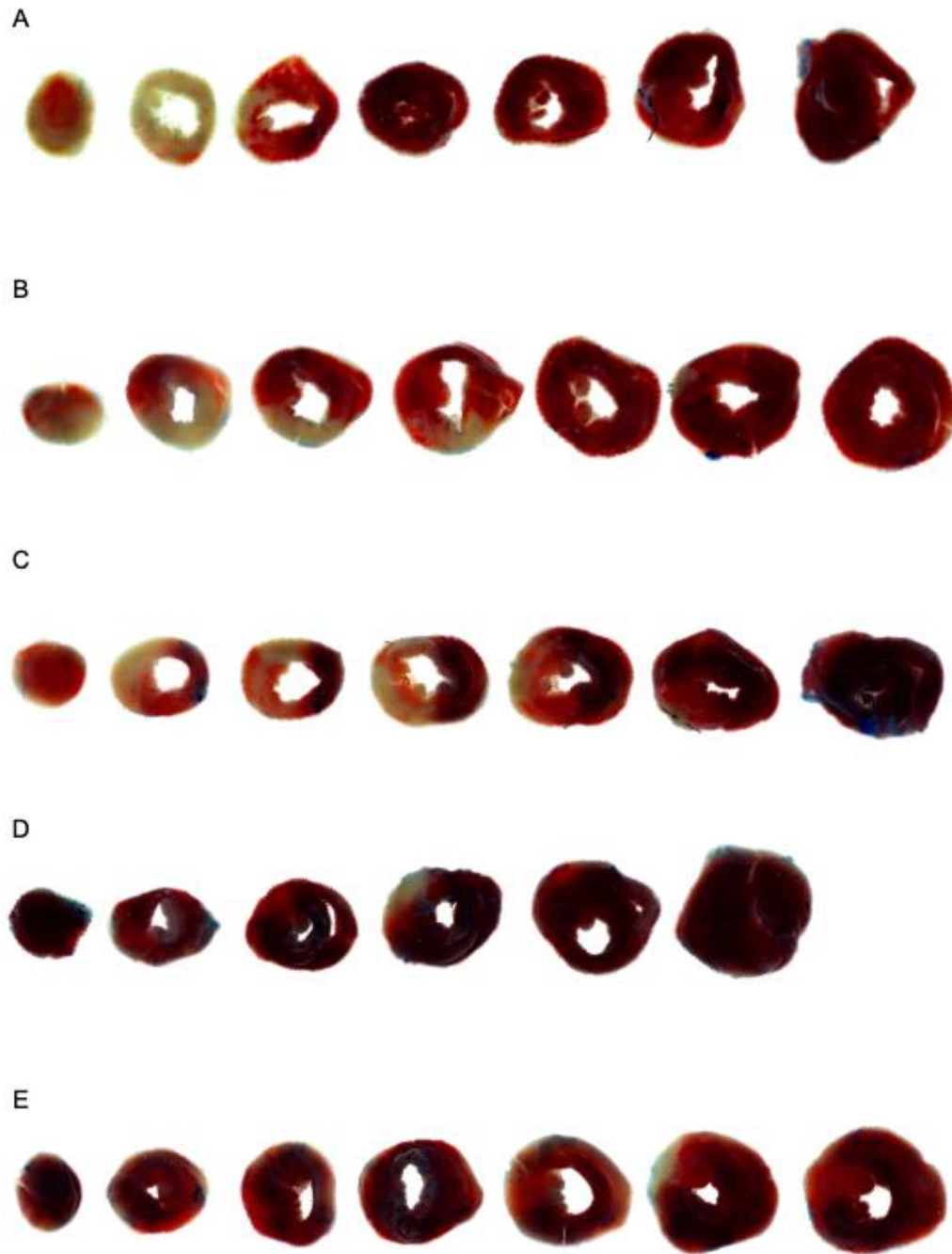


Figure S1: Evans blue and TTC staining on WT hearts

Representative examples of WT heart slices stained with Evans blue and TTC 24 hours after cardiac I/R. Mice were treated at 5 min before reperfusion with a single intravenous injection of vehicle (**A**), 2.5 mM Pbn (**B**), 90 μ M SBL-PX1-42 (**C**), 1 μ M SBL-PX1-213 (**D**) or a combination thereof (**E**).

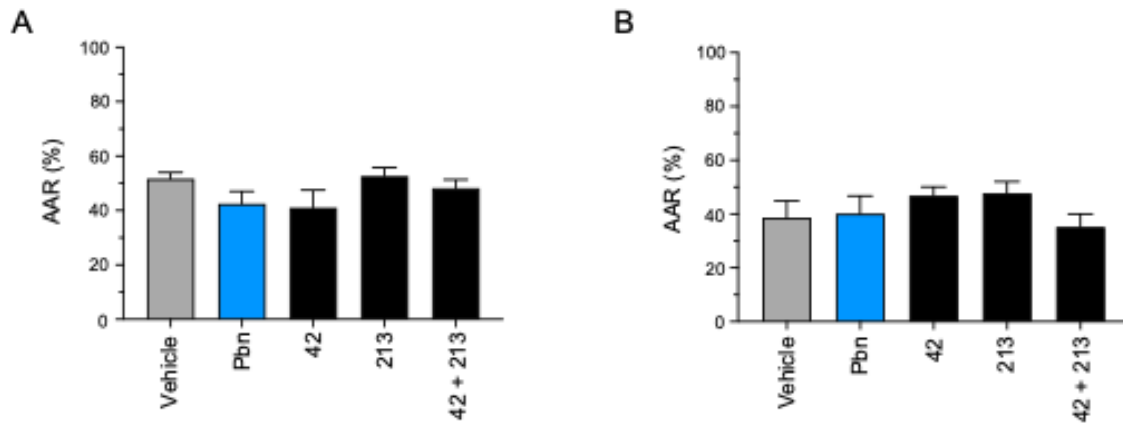


Figure S2: Area at risk (AAR) in WT and *Panx1*^{-/-} mice after cardiac I/R

AAR in WT (**A**) and *Panx1*^{-/-} mice (**B**) measured at 24 hours after cardiac I/R. Mice were treated at 5 min before reperfusion with a single intravenous injection of vehicle, 2.5 mM Pbn, 90 μ M SBL-PX1-42, 1 μ M SBL-PX1-213 or a combination thereof. Data are shown as mean $\pm$ SEM.

Table S3: BLAST analysis for motif similarities with SBL-PX1-213

Name	Homology	Region of the protein	Sequence ID
Transmembrane protein 185A	Query 4 LIAVGI 9 LIAVGI Sbjct 85 LIAVGI 90	Transmembrane helical region	A2AF53.1
Transmembrane protein 185B	Query 4 LIAVGI 9 LIAVGI Sbjct 85 LIAVGI 90	Transmembrane helical region	Q8R3R5.1
Hydrocephalus-inducing protein	Query 6 AVGIF 10 AVGIF Sbjct 2940 AVGIF 2944	Intracellular domain	Q80W93.2
Voltage-gated potassium channel regulatory subunit KCNF1	Query 6 AVGIF 10 AVGIF Sbjct 332 AVGIF 336	Transmembrane helical region	Q7TSH7.1
LHFPL tetraspan subfamily member 2 protein	Query 6 AVGIF 10 AVGIF Sbjct 100 AVGIF 104	Transmembrane helical region	Q8BGA2.1
V-type proton ATPase subunit F	Query 3 KLI AV 7 KLI AV Sbjct 6 KLI AV 10	Intracellular domain	Q9D1K2.2
Tetratricopeptide repeat protein 8	Query 5 IAVGI 9 IAVGI Sbjct 406 IAVGI 410	Intracellular domain	Q8VD72.1
Intraflagellar transport protein 88 homolog	Query 3 KLI AV 7 KLI AV Sbjct 329 KLI AV 333	Intracellular domain	Q61371.2
Partitioning defective 3 homolog B	Query 3 KLI AV 7 KLI AV Sbjct 74 KLI AV 78	Intracellular domain	Q9CSB4.2
Alpha-2A adrenergic receptor	Query 4 LIAVG 8 LIAVG Sbjct 411 LIAVG 415	Transmembrane helix 6 (411-413) Extracellular domain (414-415)	Q01338.2

Ectonucleoside triphosphate diphosphohydrolase 1 or CD39	Query 4 LIAVG 8 LIAVG Sbjct 32 LIAVG 36	Transmembrane helical region	<u>P55772.1</u>
WD repeat and coiled-coil-containing protein	Query 4 LIAVG 8 LIAVG Sbjct 392 LIAVG 396	Intracellular domain	<u>Q6NV72.2</u>
Cilia- and flagella-associated protein 251	Query 4 LIAVG 8 LIAVG Sbjct 770 LIAVG 774	Intracellular domain	<u>E9Q743.1</u>
Rapamycin-insensitive companion of mTOR	Query 4 LIAVG 8 LIAVG Sbjct 169 LIAVG 173	Intracellular domain	<u>Q6QI06.2</u>
Cell surface glycoprotein MUC18 or CD146	Query 4 LIAVGIF 10 L+ VGIF Sbjct 424 LVSVGIF 430	Extracellular region	<u>Q8R2Y2.1</u>
PDZK1-interacting protein 1	Query 4 LIAVGIF 10 LIAV +F Sbjct 34 LIAVAVF 40	Transmembrane helical region	<u>Q9CQH0.1</u>
Glutamate receptor ionotropic, NMDA 1	Query 3 KLIAVGIF 10 KL+ VGI+ Sbjct 360 KLVQVGIIY 367	Extracellular region	<u>P35438.1</u>
Collagen alpha-1(VII) chain	Query 3 KLIAVGI 9 KL AVGI Sbjct 170 KLFVAVGI 176	Extracellular protein	<u>Q63870.3</u>
Sulfate transporter or Solute carrier family 26 member 2	Query 5 IAVGIF 10 I VGIF Sbjct 160 ISVGIF 165	Intracellular domain	<u>Q62273.1</u>
Protein ABHD1	Query 1 QSKLIAVGI 9 Q L AVGI Sbjct 202 QAPLLAVGI 210	Intracellular domain	<u>Q9QZC8.2</u>

Solute carrier family 15 member 4 or Peptide/histidine transporter 1	Query 5 IAVGIF 10 IAVG F Sbjct 406 IAVGMF 411	Transmembrane helical region	<u>Q91W98.1</u>
Phosphatidylinositol 4-kinase alpha	Query 5 IAVGIF 10 IA GIF Sbjct 82 IALGIF 87	Intracellular domain	<u>E9Q3L2.2</u>
Solute carrier family 5 member 4B or Low affinity sodium-glucose cotransporter	Query 4 LIAVGI 9 LIAV I Sbjct 433 LIAVSI 438	Transmembrane helical region	<u>Q91ZP4.2</u>

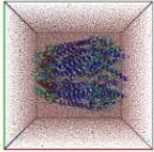
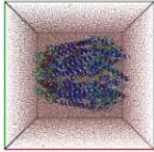
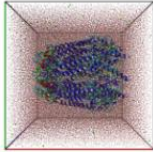
SBL-PX1-42		SBL-PX1-213		COMBINED	
					
Protein	7 monomers	Protein	7 monomers	Protein	7 monomers
Peptide	1	Peptide	1	Peptide	2
Element Cl	193	Element Cl	211	Element Cl	208
Element Na	171	Element Na	187	Element Na	183
Water	65998 molecules	Water	72109 molecules	Water	70675 molecules
Total n° of atoms	237982	Total n° of atoms	256076	Total n° of atoms	252163

Figure S5: Parameters of the three generated systems for MD simulations