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Supplemental Information

Biochemical and Electrophysiological Modification of Amyloid Transthyretin on Cardiomyocytes

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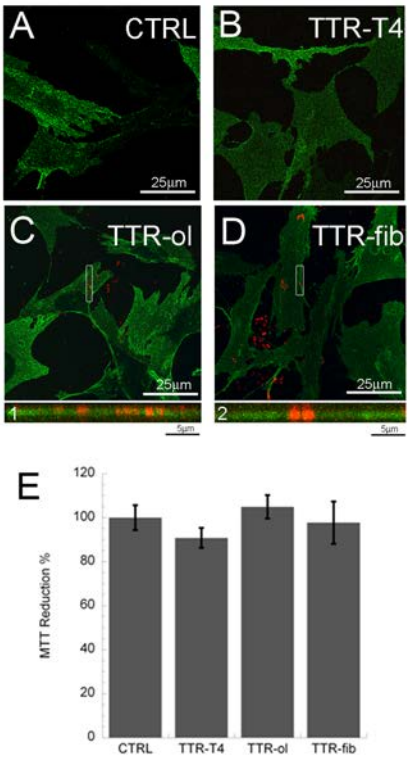

FIGURE S1. Effect of TTR variants on HDFα human cells. Confocal images of Human dermal fibroblast (HDFα) exposed for 24 h to protein buffer (A), 10 μM TTR-T4 (B), TTR-ol (C) and TTR-fib (D). The cells were stained with cholera toxin (green) and then were fixed, permeabilized and stained with anti-TTR antibody (red). 1,2: z-projection of the ROIs selected. E: Cytotoxic effect of TTR-T4, TTR-ol and TT-fib to HDFα cells performed by the MTT reduction assay. The values shown are normalized against the buffer control (n=3, mean ±s.d.).

Table S I: Cumulative calcium fractional variation relative to vehicle in HL-1 cardiomyocytes

External solution (n)	**mean±s.e.m.**	**P value (Tukey's multiple comparisons test)**
Veh (51) vs. TTT-ol (26)	$-9.1\cdot10^{-5}$±2.0 vs. 42.9±3.8	<0.001
Veh (51) vs. TTR-fib (17)	$-9.1\cdot10^{-5}$±2.0 vs. 22.3±1.5	<0.001
Veh (51) vs. TTR-ol 0Ca (16)	$-9.1\cdot10^{-5}$±2.0 vs. 16.3±1.2	<0.01
Veh (51) vs. TTR-fib 0Ca (41)	$-9.1\cdot10^{-5}$±2.0 vs. 11.6±2.6	<0.001
Veh (51) vs. TTR-T4 0Ca (42)	$-9.1\cdot10^{-5}$±2.0 vs. -11.7±1.0	<0.001
Veh (51) vs. TTR-ol Rya (14)	$-9.1\cdot10^{-5}$±2.0 vs. 20.7±1.4	<0.001
TTR (12) vs. TTR-ol (26)	2.5±1.8 vs. 42.9±3.8	<0.001
TTR (12) vs. TTR-fib (17)	2.5±1.8 vs. 22.3±1.5	<0.05
TTR-ol (26) vs. TTR-fib (17)	42.9±3.8 vs. 22.3±1.5	<0.001
TTR-ol (26) vs. TTR-T4 (33)	42.9±3.8 vs. 4.0±3.0	<0.001
TTR-ol (26) vs. TTR-ol 0Ca (16)	42.9±3.8 vs. 16.3±1.2	<0.001
TTR-ol (26) vs. TTR-fib 0Ca (41)	42.9±3.8 vs. 11.6±2.6	<0.001
TTR-ol (26) vs. TTR-T4 0Ca (42)	42.9±3.8 vs. -11.7±1.0	<0.001
TTR-ol (26) vs. TTR-ol Rya (14)	42.9±3.8 vs. 20.7±1.4	<0.001
TTR-ol (26) vs. TTR-ol 2APB (23)	42.9±3.8 vs. 6.2±0.4	<0.001
TTR-ol (26) vs. TTR-fib Rya (20)	42.9±3.8 vs. -0.8±2.7	<0.001
TTR-ol (26) vs. TTR-fib 2APB (18)	42.9±3.8 vs. 9.1±1.2	<0.001
TTR-ol (26) vs. TTR-T4 Rya (29)	42.9±3.8 vs. -7.4±1.5	<0.001
TTR-ol (26) vs. TTR-T4+2APB (33)	42.9±3.8 vs. -6.9±1.6	<0.001
TTR-fib (17) vs. TTR-T4 (33)	22.3±1.5 vs. 4.0±3.0	<0.01
TTR-fib (17) vs. TTR-T4 0Ca (42)	22.3±1.5 vs. -11.7±1.0	<0.001
TTR-fib (17) vs. TTR-ol 2APB (23)	22.3±1.5 vs. 6.2±0.4	<0.05
TTR-fib (17) vs. TTR-fib Rya (20)	22.3±1.5 vs. -0.8±2.7	<0.001
TTR-fib (17) vs. TTR-T4 Rya (29)	22.3±1.5 vs. -7.4±1.5	<0.001
TTR-fib (17) vs. TTR-T4 2APB (33)	22.3±1.5 vs. -6.9±1.6	<0.001
TTR-T4 (33) vs. TTR-T4 0Ca (42)	4.0±3.0 vs. -11.7±1.0	<0.001
TTR-T4 (33) vs. TTR-ol Rya (14)	4.0±3.0 vs. 20.7±1.4	<0.05
TTR-ol 0Ca (16) vs. TTR-T4 0Ca (42)	16.3±1.2 vs. -11.7±1.0	<0.001
TTR-ol 0Ca (16) vs. TTR-fib Rya (20)	16.3±1.2 vs. -0.8±2.7	<0.05
TTR-ol 0Ca (16) vs. TTR-T4 Rya (29)	16.3±1.2 vs. -7.4±1.5	<0.001
TTR-ol 0Ca (16) vs. TTR-T4 2APB (33)	16.3±1.2 vs. -6.9±1.6	<0.001
TTR-fib 0Ca (41) vs. TTR-T4 0Ca (42)	11.6±2.6 vs. -11.7±1.0	<0.001
TTR-fib 0Ca (41) vs. TTR-T4 Rya (29)	11.6±2.6 vs. -7.4±1.5	<0.001
TTR-fib 0Ca (41) vs. TTR-T4 2APB (33)	11.6±2.6 vs. -6.9±1.6	<0.001
TTR-T4 0Ca (42) vs. TTR-ol Rya (14)	-11.7±1.0 vs. 20.7±1.4	<0.001
TTR-T4 0Ca (42) vs. TTR-ol 2APB (23)	-11.7±1.0 vs. 6.2±0.4	<0.001
TTR-T4 0Ca (42) vs. TTR-fib 2APB (18)	-11.7±1.0 vs. 9.1±1.2	<0.001
TTR-ol Rya (14) vs. TTR-fib Rya (20)	20.7±1.4 vs. -0.8±2.7	<0.01
TTR-ol Rya (14) vs. TTR-T4 Rya (29)	20.7±1.4 vs. -7.4±1.5	<0.001
TTR-ol Rya (14) vs. TTR-T4 2APB (33)	20.7±1.4 vs. -6.9±1.6	<0.001
TTR-ol 2APB (23) vs. TTR-T4 Rya (29)	6.2±0.4 vs. -7.4±1.5	<0.05
TTR-ol 2APB (23) vs. TTR-T4 2APB (33)	6.2±0.4 vs. -6.9±1.6	<0.05
TTR-fib 2APB (18) vs. TTR-T4 Rya (29)	9.1±1.2 vs. -7.4±1.5	<0.01
TTR-ol 2APB (23) vs. TTR-T4 2APB (33)	6.2±0.4 vs. -6.9±1.6	<0.01

Table S II: Amplitude fractional variation of calcium transient relative to initial value in mice ventricular cardiomyocytes

External solution (n)/time (minute)	**mean±s.e.m.**	**P value (Tukey's multiple comparisons test)**
Veh (18)/0 vs. Veh (18)/5	-0.012±0.012 vs. -8.59±2.90	ns
Veh (18)/0 vs. Veh (18)/10	-0.012±0.012 vs. -36.09±4.27	<0.001
Veh (18)/0 vs. Veh (18)/15	-0.012±0.012 vs. -49.85±4.29	<0.001
Veh(18)/0 vs. Veh (18)/20	-0.012±0.012 vs. -60.62±4.19	<0.001
Veh (18)/0 vs. TTR-ol (14)/0	-0.012±0.012 vs. $-1.33\cdot10^{-5}\pm1.66\cdot10^{-5}$	ns
Veh (18)/0 vs. TTR (13)/0	-0.012±0.012 vs. $2.059\cdot10^{-5}\pm4.038\cdot10^{-5}$	ns
Veh (18)/0 vs. TTR-fib (19)/0	-0.012±0.012 vs. $6.25\cdot10^{-4}\pm4.93\cdot10^{-4}$	ns
Veh (18)/5 vs. Veh (18)/10	-8.59±2.90 vs. -36.09±4.27	<0.05
Veh (18)/5 vs. Veh (18)/15	-8.59±2.90 vs. -49.85±4.29	<0.001
Veh (18)/5 vs. Veh (18)/20	-8.59±2.90 vs. -60.62±4.19	<0.001
Veh (18)/5 vs. TTR-fib (19)/5	-8.59±2.90 vs. 18.39±5.64	<0.05
Veh (18)/10 vs. Veh (18)/15	-36.09±4.27 vs. -49.85±4.29	ns
Veh (18)/10 vs. Veh (18)/20	-36.09±4.27 vs. -60.62±4.19	ns
Veh (18)/10 vs. TTR-ol (14)/10	-36.09±4.27 vs. -2.01±7.90	<0.001
Veh (18)/10 vs. TTR-fib (19)/10	-36.09±4.27 vs. -9.82±7.87	<0.05
Veh (18)/15 vs. Veh (18)/20	-49.85±4.29 vs. -60.62±4.19	ns
Veh (18)/15 vs. TTR-ol (14)/15	-49.85±4.29 vs. -15.68±7.57	<0.001
Veh (18)/15 vs. TTR (13)/15	-49.85±4.29 vs. -44.51±6.13	ns
Veh (18)/15 vs. TTR-fib (19)/15	-49.85±4.29 vs. -30.47±26.14	ns
Veh (18)/20 vs. TTR-ol (14)/20	-60.62±4.19 vs. -26.72±7.88	<0.001
Veh (18)/20 vs. TTR (13)/20	-60.62±4.19 vs. -56.19±5.74	ns
Veh (18)/20 vs. TTR-fib (19)/20	-60.62±4.19 vs. -46.49±7.83	ns
TTR-ol (14)/0 vs. TTR (13)/0	$-1.33\cdot10^{-5}\pm1.66\cdot10^{-5}$ vs. $2.06\cdot10^{-5}\pm4.04\cdot10^{-5}$	ns
TTR-ol (14)/0 vs. TTR-fib (19)/0	$-1.33\cdot10^{-5}\pm1.66\cdot10^{-5}$ vs. $6.25\cdot10^{-4}\pm4.93\cdot10^{-4}$	ns
TTR-ol (14)/5 vs. TTR (13)/5	54.47±10.30 vs. -18.50±4.85	<0.001
TTR-ol (14)/5 vs. TTR-fib (19)/5	54.47±10.30 vs. 18.39±5.64	<0.001
TTR-ol (14)/10 vs. TTR (13)/10	-2.01±7.90 vs. 29.26±4.48	ns
TTR-ol (14)/10 vs. TTR-fib (19)/10	-2.01±7.90 vs. -9.82±7.87	ns
TTR-ol (14)/15 vs. TTR (13)/15	-15.68±7.57 vs. -44.51±6.13	ns
TTR-ol (14)/15 vs. TTR-fib (19)/15	-15.68±7.57 vs. -30.47±26.14	ns
TTR-ol (14)/20 vs. TTR (13)/20	-26.72±7.88 vs. -56.19±5.74	ns
TTR-ol (14)/20 vs. TTR-fib (19)/20	-26.72±7.88 vs. -46.49±7.83	ns
TTR (13)/0 vs. TTR-fib (19)/0	$2.06\cdot10^{-5}\pm4.04\cdot10^{-5}$ vs. $6.25\cdot10^{-4}\pm4.93\cdot10^{-4}$	ns
TTR (13)/5 vs. TTR-fib (19)/5	-18.50±4.85 vs. 18.39±5.64	<0.001
TTR (13)/10 vs. TTR-fib (19)/10	29.26±4.48 vs. -9.82±7.87	ns
TTR (13)/15 vs. TTR-fib (19)/15	-44.51±6.13 vs. -30.47±26.14	ns
TTR (13)/20 vs. TTR-fib (19)/20	-56.19±5.74 vs. -46.49±7.83	ns

Table S III: Baseline fractional variation of calcium transient relative to initial value in mice ventricular cardiomyocytes

Time (min)	Vehicle (mean±s.e.m.) n=18	TTR (mean±s.e.m.) n=13	TTR-ol (mean±s.e.m.) n=14	**TTR-fib (mean±s.e.m.) n=19**

0	-0.0075±0.0074	$2.9\cdot10^{-10}\pm7.5\cdot10^{-9}$	$-1.4\cdot10^{-6}\pm5.2\cdot10^{-6}$	$9.6\cdot10^{-8}\pm4.9\cdot10^{-4}$
5	3.1±0.6	-1.5±0.7	1.2±1.5	3.0±5.6
10	0.2±1.7	1.4±0.7	0.078±0.33	6.7±7.9
15	13.0±5.4	3.2±1.6	13.0±7.4	5.0±26.1
20	23.2±6.4	16.1±3.0	19.4±9.9	10.6±7.8

Table S IV: Time to peak fractional variation of calcium transient relative to initial value measured in mice ventricular cardiomyocytes

Time (min)	Vehicle (mean±s.e.m.) n=18	TTR (mean±s.e.m.) n=13	TTR-ol (mean±s.e.m.) n=14	**TTR-fib (mean±s.e.m.) n=19**
0	$9.7\cdot10^{-7}\pm1.2\cdot10^{-6}$	$1.5\cdot10^{-6}\pm1.5\cdot10^{-6}$	$1.9\cdot10^{-7}\pm1.1\cdot10^{-6}$	$4.7\cdot10^{-7}\pm9.6\cdot10^{-7}$
5	23.3±13.5	10.9±7.7	11.4±3.5	6.7±3.8
10	32.7±18.5	15.8±6.5	19.7±10.9	8.6±7.1
15	85.6±52.8	26.8±12.8	60.3±10.2	60.0±6.7
20	225.8±83.1	189.2± 26.3	166.3±16.7	150.73±12.4

Table S V: 50% decay time fractional variation of calcium transient relative to initial value in mice ventricular cardiomyocytes

Time (min)	Vehicle (mean±s.e.m.) n=18	TTR (mean±s.e.m.) n=13	TTR-ol (mean±s.e.m.) n=14	**TTR-fib (mean±s.e.m.) n=19**
0	$8.3\cdot10^{-7}\pm1.03\cdot10^{-6}$	$2.3\cdot10^{-7}\pm4.0\cdot10^{-7}$	$3.1\cdot10^{-8}\pm3.3\cdot10^{-7}$	$5.9\cdot10^{-7}\pm3.3\cdot10^{-7}$
5	14.0±12.6	-2.3±1.6	-3.9±3.0	5.0±2.4
10	17.0±15.3	-7.4±2.8	5.3±5.7	7.4±4.8
15	40.2±23.3	10.6±8.6	16.8±6.1	6.8±4.7
20	202.8±81.4	125.8± 35.1	110.6±25.6	100.4±24.6