

# **Supplemental Material**

**Table S1. The use of conventional POTS therapy in the POTS group.**

	POTS (n=45)
Droxidopa	33.3
Beta-blockers	35.6
Procoralan	37.8
Midodrin	6.7
Orstanorm	4.4

Displayed as % of POTS subjects. No control subjects reported use of heart rate regulating or vasoactive medications. Data about current medications were missing for three POTS patients.

**Table S2. Serum activity against specific G-protein coupled receptors in POTS compared to healthy controls.**

	POTS (n = 48)	Controls (n = 25)	p-value
ADRA1	1.726 ± 2.157 *	0.923 ± 0.871 *	0.006
ADRB2	5.890 ± 5.641 ***	2.256 ± 1.615 *	<0.001
CHRM2	1.861 ± 3.940 ***	0.883 ± 1.135 *	0.007
OPRL1	0.955 ± 0.857 **	0.640 ± 0.992	0.009

Data was analysed with Independent samples t-test. Log-transformation was applied to calculate the p-values. Receptor activity is shown as the ratio between emitted light from cleaved substrate and non-cleaved substrate and calculated as mean value ± SD of the mean. Missing values: \*n=1; \*\*n=2; \*\*\*n=3. ADRA1: adrenergic alpha-1; ADRB2: adrenergic beta-2; CHRM2: muscarinic type-2; OPRL1: opioid-receptor-like 1.

**Table S3. Correlation between different G-protein coupled receptors in POTS patients (n=48).**

		ADRA1	ADRB2	CHRM2	OPRL1
ADRA1	Pearson Correlation	1	0.206	0.938**	0.418**
	p-value		0.181	< 0.001	0.004
	N	47	44	44	45
ADRB2	Pearson Correlation	0.206	1	0.445**	0.530**
	p-value	0.181		0.002	< 0.001
	N	44	45	44	45
CHRM2	Pearson Correlation	0.938**	0.445**	1	0.481**
	p-value	< 0.001	0.002		0.001
	N	44	44	45	45
OPRL1	Pearson Correlation	0.418**	0.530**	0.481**	1
	p-value	0.004	< 0.001	0.001	
	N	45	45	45	46

\*\* . Correlation is significant at the 0.01 level (2-tailed).

**Table S4. Correlation between different G-protein coupled receptors in controls (n=25).**

		ADRA1	ADRB2	CHRM2	OPRL1
ADRA1	Pearson Correlation	1	0.139	0.490*	0.610**
	p-value		0.549	0.024	0.002
	N	23	21	21	23
ADRB2	Pearson Correlation	0.139	1	-0.368	0.262
	p-value	0.549		0.101	0.228
	N	21	23	21	23
CHRM2	Pearson Correlation	0.490*	-0.368	1	0.082
	p-value	0.024	0.101		0.711
	N	21	21	23	23
OPRL1	Pearson Correlation	0.610**	0.262	0.082	1
	p-value	0.002	0.228	0.711	
	N	23	23	23	25

\*. Correlation is significant at the 0.05 level (2-tailed).

\*\*. Correlation is significant at the 0.01 level (2-tailed).

**Table S5. Orthostatic symptoms in relation to specific receptor activity in POTS (n=33) patients.**

Dependant Variable	Independent Variable	B (per SD)	p-value	Adjusted p-value
OHQ total	ADRA1	0.768	0.009	0.031
OHQ total	ADRB2	0.176	0.599	0.851
OHQ total	CHRM2	0.290	0.364	0.541
OHQ total	OPRL1	0.472	0.118	0.188
Vision	ADRA1	1.653	< 0.001	0.014
Vision	ADRB2	1.336	0.011	0.021
Vision	CHRM2	1.199	0.014	0.046
Vision	OPRL1	1.490	0.003	0.005
Dizziness	ADRA1	0.358	0.349	0.234
Dizziness	ADRB2	-0.219	0.586	0.338
Dizziness	CHRM2	-0.049	0.897	0.673
Dizziness	OPRL1	0.030	0.937	0.948
Weakness	ADRA1	0.607	0.093	0.343
Weakness	ADRB2	-0.100	0.795	0.609
Weakness	CHRM2	0.233	0.521	0.720
Weakness	OPRL1	0.292	0.433	0.481
Fatigue	ADRA1	0.196	0.583	0.546
Fatigue	ADRB2	-0.352	0.363	0.254
Fatigue	CHRM2	-0.245	0.493	0.449
Fatigue	OPRL1	-0.018	0.960	0.869
Concentration	ADRA1	0.529	0.264	0.702
Concentration	ADRB2	-0.042	0.934	0.792
Concentration	CHRM2	-0.076	0.871	0.682
Concentration	OPRL1	0.271	0.570	0.625
Head discomfort	ADRA1	0.699	0.186	0.323
Head discomfort	ADRB2	0.592	0.318	0.797
Head discomfort	CHRM2	0.481	0.384	0.600

Head discomfort	OPRL1	0.379	0.505	0.802
Standing long	ADRA1	0.867	0.037	0.041
Standing long	ADRB2	-0.003	0.994	0.985
Standing long	CHRM2	0.277	0.518	0.553
Standing long	OPRL1	0.637	0.136	0.144
Standing short	ADRA1	0.990	0.056	0.193
Standing short	ADRB2	-0.866	0.112	0.093
Standing short	CHRM2	-0.069	0.896	0.744
Standing short	OPRL1	-0.198	0.712	0.762
Walking long	ADRA1	1.690	0.001	0.023
Walking long	ADRB2	0.523	0.388	0.516
Walking long	CHRM2	0.773	0.173	0.377
Walking long	OPRL1	1.181	0.035	0.042
Walking short	ADRA1	1.117	0.042	0.107
Walking short	ADRB2	-0.001	0.999	0.290
Walking short	CHRM2	0.468	0.402	0.694
Walking short	OPRL1	0.480	0.393	0.635
OHSA	ADRA1	0.575	0.031	0.109
OHSA	ADRB2	0.257	0.371	0.765
OHSA	CHRM2	0.236	0.363	0.634
OHSA	OPRL1	0.401	0.143	0.276
OHDAS	ADRA1	0.960	0.009	0.022
OHDAS	ADRB2	0.078	0.849	0.930
OHDAS	CHRM2	0.299	0.436	0.533
OHDAS	OPRL1	0.543	0.159	0.191

Linear regression was performed to calculate the association between severity of symptoms and receptor activity. All analyses were adjusted for age. Adjusted p-value is defined as the p-value adjusted for the increase in heart rate from baseline to 3 minutes and for the decrease in systolic blood pressure from baseline to three minutes during orthostatic tests. B-values are shown as standard deviation from mean value. OHQ, orthostatic hypotension questionnaire; ADRA1, adrenergic receptor alpha 1; ADRB2, adrenergic receptor beta 2; CHRM2, Cholinergic receptor muscarinic 2; OPRL1, opioid receptor like 1; OHSA, Orthostatic Hypotension Symptom Assessment; OHDAS, Orthostatic Hypotension Daily Activity Scale.

**Figure S1. Heat map for the elevated cut-off values (above 75<sup>th</sup> percentile) derived from the control group shown and presented for each GPCR assays.**

ADRA1>75	ADRB2>75	CHRM2>75	OPRL1>75
0	1	0	1
0	1	0	1
0	1	0	1
0	1	0	1
1	1	1	0
0	1	1	0
0	1	1	0
1	1	1	1
0	1	1	1
0	1	0	1
0	1	1	1
0	1	1	1
0	1	1	1
1			
1			
1	0	0	1
0	0	0	0
1	0	0	0
1	1	1	1
0	0	1	0
1	1		1
0	1	0	1
0	1	1	1
	0	0	0
0	0	0	0
0	0	0	1
0	0	0	0
0	1	1	1
0	0	0	1
0	1	1	1
1	1	1	1
1	1	1	1
1	0	1	1
1	1	1	1
1	1	1	1
1	1	1	1
1	1	1	1
1	1	1	1
0	0	0	0
1	1	1	1
1	0	1	1
1		1	1
0	0	0	0
0	1	1	1
1	0	1	1
0	1	1	1
1	0	0	1
0	1	0	0
1	1	1	1
1	1	1	1

Heat map in which red and blue boxes indicate POTS patients with a greater or lower GPCR activity than the 75<sup>th</sup> percentile of controls respectively. ADRA1, adrenergic receptor alpha 1; ADRB2, adrenergic receptor beta 2; CHRM2, Cholinergic receptor muscarinic 2; OPRL1, opioid receptor like 1.