

Supplemental Data

Supplemental Tables

Supplemental Table 1: Sarcomere Length (SL) shortening (% of baseline)

	[Drug]	0.5 Hz		1 Hz		2 Hz		3 Hz		4 Hz		Two-way ANOVA vs. NOS1 ^{-/-}
	(mM)	Mean ± SEM	N	Mean ± SEM	N	Mean ± SEM	N	Mean ± SEM	N	Mean ± SEM	N	
WT	—	4.8 ± 0.3	41	5.3 ± 0.4	39	6.9 ± 0.5	38	8.4 ± 0.5	38	9.3 ± 0.5	34	p < 0.0001
NOS1 ^{-/-} (1 st set)	—	4.3 ± 0.3	54	4.5 ± 0.3	58	5.2 ± 0.3	59	6.1 ± 0.4	56	5.9 ± 0.4	50	————
NOS1 ^{-/-} + HYD	0.1	3.7 ± 0.5	11	4.1 ± 0.4	12	6.1 ± 0.8	13	7.0 ± 1.1	10	6.8 ± 0.8	8	p = 0.395
NOS1 ^{-/-} + HYD	1	4.5 ± 0.6	11	5.5 ± 1.0	11	8.1 ± 1.2	11	8.7 ± 1.2	10	7.7 ± 0.8	8	p < 0.0001
NOS1 ^{-/-} + HYD	10	5.1 ± 1.0	7	5.9 ± 1.5	6	7.2 ± 1.0	8	8.7 ± 1.0	7	8.3 ± 0.7	4	p < 0.0001
NOS1 ^{-/-} + NTG	0.1	3.0 ± 1.1	4	3.4 ± 1.3	4	5.5 ± 1.4	2	6.2 ± 2.0	4	5.3 ± 1.8	4	p = 0.579
NOS1 ^{-/-} + NTG	1	3.8 ± 0.8	8	4.4 ± 1.2	8	5.7 ± 1.5	8	6.2 ± 1.7	5	6.8 ± 2.3	4	p = 0.745
NOS1 ^{-/-} + NTG	10	3.6 ± 0.7	12	3.6 ± 0.6	13	4.6 ± 0.8	13	5.9 ± 1.2	13	6.0 ± 1.3	9	p = 0.228
NOS1 ^{-/-} + HYD+NTG	0.1+0.1	3.5 ± 0.6	8	3.5 ± 0.7	8	5.1 ± 1.0	8	6.4 ± 0.8	8	6.9 ± 0.7	8	p = 0.777
NOS1 ^{-/-} + HYD+NTG	0.5+0.5	4.3 ± 0.6	8	5.2 ± 0.8	9	6.6 ± 1.0	9	8.1 ± 1.1	9	7.5 ± 0.7	6	p = 0.008
NOS1 ^{-/-} + HYD+NTG	10+10	4.3 ± 0.7	8	4.6 ± 0.7	9	6.4 ± 1.0	10	8.5 ± 1.0	10	8.4 ± 1.3	7	p = 0.004
NOS1 ^{-/-} (2 nd set)	—	4.4 ± 0.3	7	4.6 ± 0.3	7	5.3 ± 0.3	7	6.2 ± 0.4	7	6.0 ± 0.3	6	————
NOS1 ^{-/-} + ISDN	10	5.0 ± 0.9	7	4.2 ± 0.5	9	5.6 ± 0.9	9	5.7 ± 0.9	10	6.2 ± 0.9	9	p = 0.929
NOS1 ^{-/-} + HYD+ISDN	10+10	5.7 ± 0.8	8	5.1 ± 0.7	9	6.9 ± 1	9	7.3 ± 0.7	9	7.8 ± 0.6	11	p = 0.004

Supplemental Table 2: Cytosolic Ca²⁺ transient amplitude (Δ Ca²⁺, nM)

	[Drug]	0.5 Hz		1 Hz		2 Hz		3 Hz		4 Hz		Two-way ANOVA vs. NOS1 ^{-/-}
	(mM)	Mean ± SEM	N	Mean ± SEM	N	Mean ± SEM	N	Mean ± SEM	N	Mean ± SEM	N	
WT	—	160 ± 13	40	172 ± 16	38	211 ± 21	37	260 ± 28	36	316 ± 40	32	p = 0.045
NOS1 ^{-/-} (1 st set)	—	229 ± 16	50	229 ± 17	48	246 ± 16	49	268 ± 19	48	278 ± 21	40	————
NOS1 ^{-/-} + HYD	0.1	235 ± 23	12	230 ± 24	13	289 ± 32	14	280 ± 31	11	287 ± 33	9	p = 0.406
NOS1 ^{-/-} + HYD	1	266 ± 27	12	242 ± 23	12	266 ± 30	12	311 ± 36	10	334 ± 32	8	p = 0.057
NOS1 ^{-/-} + HYD	10	273 ± 21	6	270 ± 21	4	291 ± 39	7	362 ± 54	7	400 ± 82	5	p = 0.004
NOS1 ^{-/-} + NTG	0.1	248 ± 69	5	258 ± 97	5	241 ± 76	5	281 ± 132	5	304 ± 153	4	p = 0.566
NOS1 ^{-/-} + NTG	1	214 ± 18	12	231 ± 28	11	265 ± 30	11	251 ± 29	7	238 ± 26	6	p = 0.592
NOS1 ^{-/-} + NTG	10	196 ± 37	11	200 ± 31	12	217 ± 32	12	235 ± 35	12	190 ± 39	9	p = 0.018
NOS1 ^{-/-} + HYD+NTG	0.1+0.1	239 ± 32	9	226 ± 33	9	255 ± 47	9	272 ± 53	9	307 ± 40	7	p = 0.629
NOS1 ^{-/-} + HYD+NTG	0.5+0.5	264 ± 47	5	256 ± 49	5	298 ± 60	5	322 ± 61	6	329 ± 63	5	p = 0.081
NOS1 ^{-/-} + HYD+NTG	10+10	196 ± 28	9	198 ± 27	12	240 ± 33	12	291 ± 42	12	358 ± 69	9	p = 0.722
NOS1 ^{-/-} (2 nd set)	—	233 ± 14	7	235 ± 15	7	254 ± 15	7	275 ± 17	7	281 ± 19	6	————
NOS1 ^{-/-} + ISDN	10	209 ± 35	8	176 ± 27	10	198 ± 27	10	207 ± 31	11	216 ± 42	10	p = 0.005
NOS1 ^{-/-} + HYD+ISDN	10+10	214 ± 12	9	219 ± 17	11	239 ± 15	10	274 ± 17	11	293 ± 23	11	p = 0.496

Supplemental Table 3: Ca²⁺ transient decay time constant (τ ; msec)

	[Drug]	0.5 Hz		1 Hz		2 Hz		3 Hz		4 Hz		Two-way ANOVA vs. NOS1 ^{-/-}
	(mM)	Mean ± SEM	N	Mean ± SEM	N	Mean ± SEM	N	Mean ± SEM	N	Mean ± SEM	N	
(baseline to baseline ¹)												
WT	—	198 ± 9	14	161 ± 6	14	142 ± 5	14	125 ± 7	12	91 ± 4	9	p = 0.0003
NOS1 ^{-/-}	—	231 ± 13	17	184 ± 7	19	163 ± 12	18	143 ± 6	18	101 ± 6	13	————
NOS1 ^{-/-} + HYD	0.1	228 ± 16	8	212 ± 18	10	171 ± 8	11	146 ± 11	9	89 ± 5	7	p = 0.512
NOS1 ^{-/-} + HYD	1	225 ± 15	14	193 ± 16	19	157 ± 5	18	140 ± 5	16	96 ± 3	14	p = 0.735
NOS1 ^{-/-} + HYD	10	177 ± 15	11	145 ± 13	8	117 ± 7	10	118 ± 12	8	9 ± 9	5	p < 0.0001
NOS1 ^{-/-} + NTG	0.1	218 ± 12	5	181 ± 17	5	143 ± 16	5	124 ± 14	5	108 ± 15	3	p = 0.295
NOS1 ^{-/-} + NTG	1	208 ± 9	12	165 ± 7	11	131 ± 9	11	131 ± 13	7	86 ± 9	6	p = 0.003
NOS1 ^{-/-} + NTG	10	180 ± 7	10	156 ± 7	11	130 ± 4	11	127 ± 6	11	89 ± 6	7	p < 0.0001
NOS1 ^{-/-} + HYD+NTG	0.1+0.1	235 ± 15	3	171 ± 9	9	136 ± 4	9	121 ± 3	5	105 ± 13	4	p = 0.824
NOS1 ^{-/-} + HYD+NTG	0.5+0.5	227 ± 5	8	183 ± 7	9	162 ± 16	9	149 ± 13	7	93 ± 5	6	p = 0.188
NOS1 ^{-/-} + HYD+NTG	10+10	177 ± 7	9	145 ± 8	12	124 ± 8	11	117 ± 11	12	89 ± 4	8	p < 0.0001
(peak to baseline ²)												
WT	—	158 ± 6	29	141 ± 7	29	112 ± 6	28	93 ± 4	26	99 ± 6	25	p < 0.0001
NOS1 ^{-/-}	—	183 ± 6	36	161 ± 7	33	123 ± 5	33	105 ± 4	31	109 ± 5	29	————
NOS1 ^{-/-} + ISDN	10	130 ± 9	8	119 ± 7	10	106 ± 5	10	100 ± 5	10	95 ± 5	8	p < 0.0001
NOS1 ^{-/-} + HYD+ISDN	10+10	128 ± 8	11	117 ± 13	11	102 ± 6	10	118 ± 8	9	119 ± 11	4	p = 0.001

¹ set of τ measured from t_0 of the Ca²⁺ transient to baseline

² set of τ measured from peak of the Ca²⁺ transient to baseline