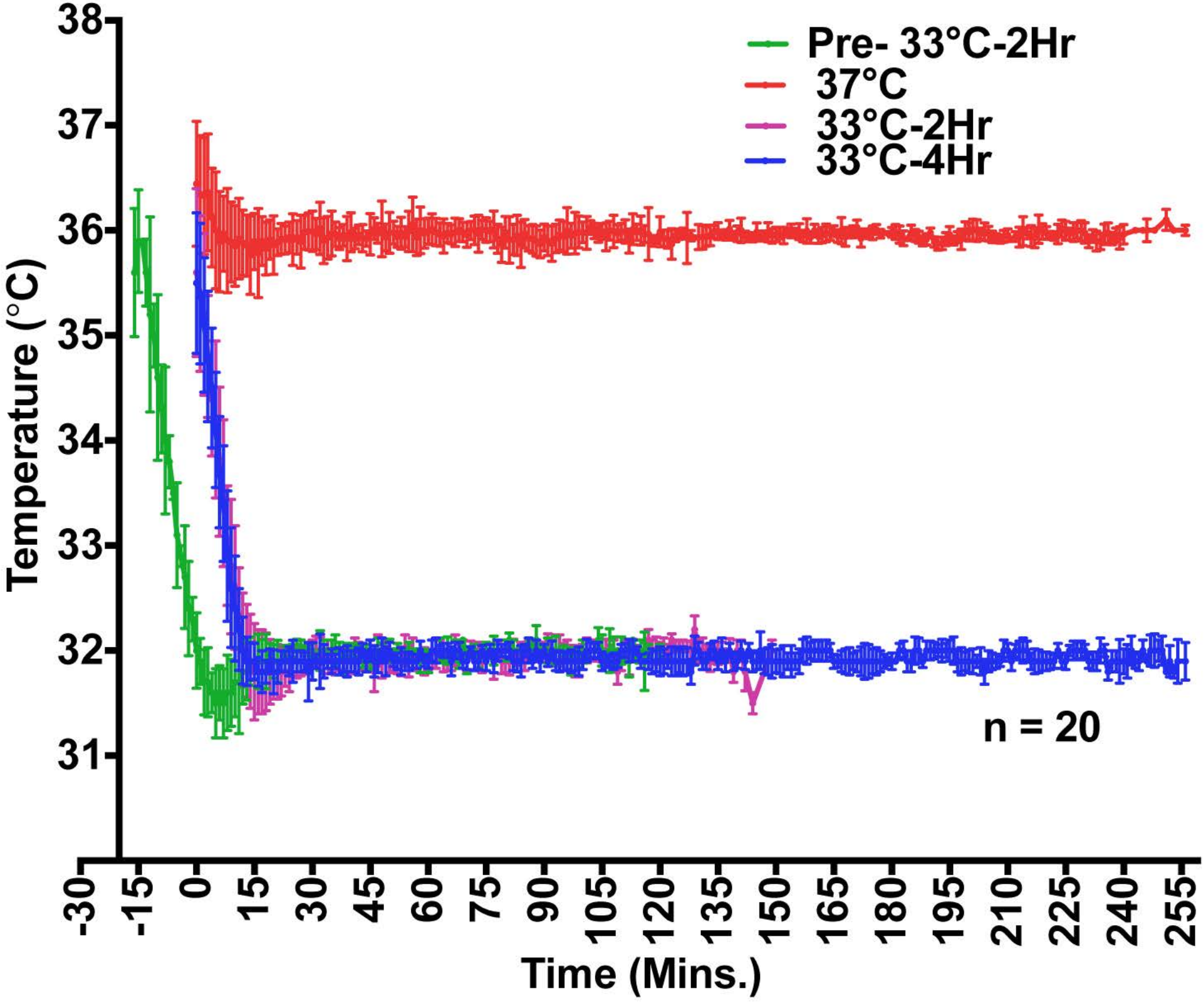
Supplemental Figure 1. Temperature Control in Perivascular Hypothermia Model. For each group in Fig. 1, animal temperature is monitored with a thermistor implanted in the temporalis muscle. Temperature rapidly dropped to 33°C in 12-14 min and was stably maintained within a tight range. The group maintained at 37°C includes subject (n=20) treated for 2 h and others (n=20) treated for 4 h. These control groups were combined after we found no differences in TTC stained lesion volumes. Data are presented as mean±SE at each 5 min interval.

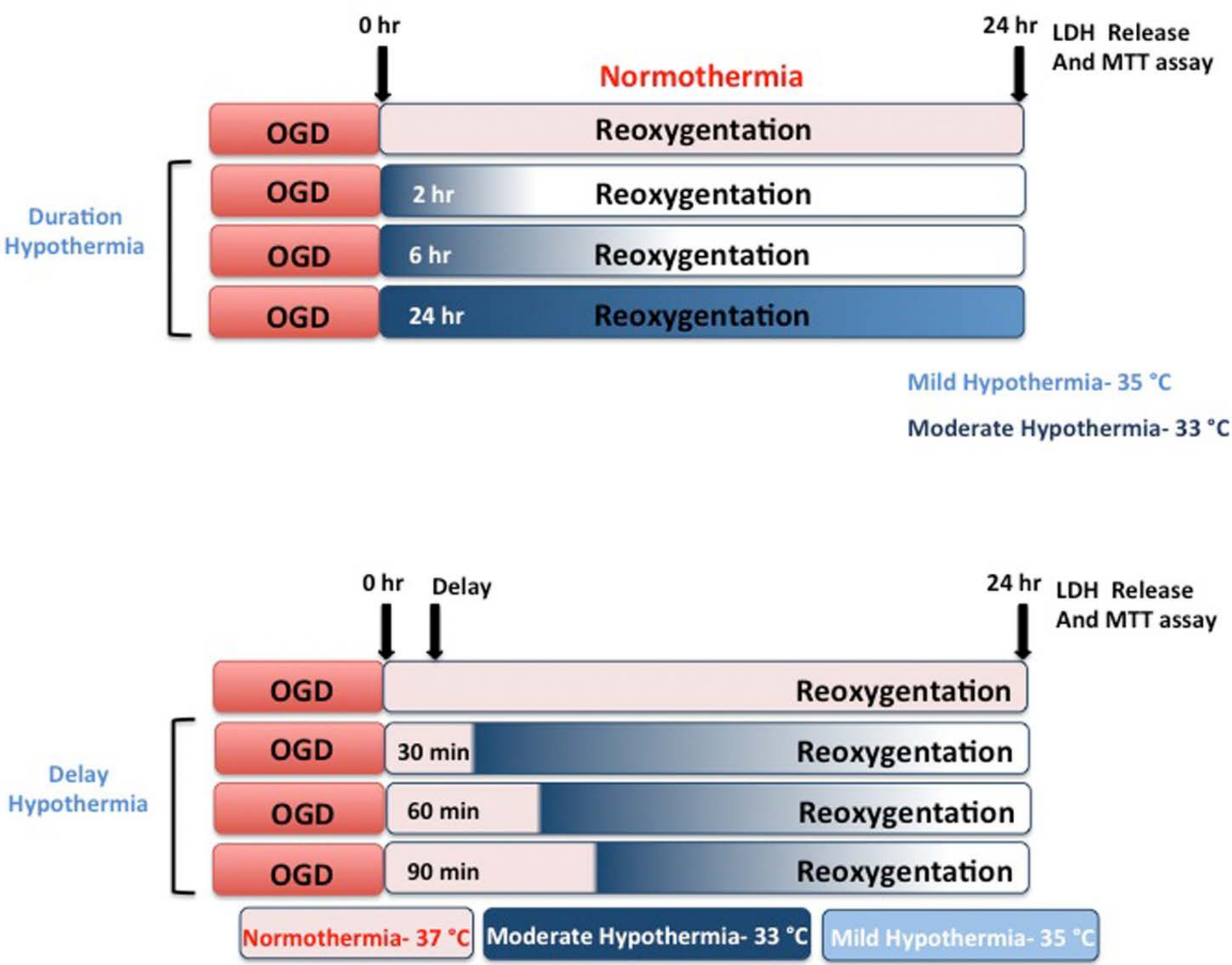
Supplemental Figure 2 . Experimental Design. To assess the effects of temperature depth, treatment delay, and treatment duration separately, we randomly assigned culture plates to one of the treatment paradigms shown. All plates (except controls) underwent OGD for a pre-defined time interval (neurons: 2 h, endothelial cells: 6 h, astrocytes: 10 h) and then the media was replaced and the plates were returned to normoxia. In the upper panel, the effect of TH duration was tested by applying (at random) 33°C or 35°C for (at random) 2, 6 or 24 h. In the lower panel, the effect of delay time was explored. After a randomly determined delay time (0, 30, 60, or 90 min) plates were randomized to an incubator maintained at 37, 35 or 33^{o}C for a randomly determined duration of TH (0, 2, 6, or 24 h). At the end of the hypothermia period, plates were then returned to 37^{o}C. At the end of the 24 h, each plate was assessed for cell viability using two assays, MTT viability and LDH release.

Supplemental Table. Treatment Effect Sizes. The treatment effect size was computed as the difference in cell viability at each combination of temperature depth, treatment delay, and duration. For all cells, the mean±SD treatment effect size was largest for 33ºC vs. normothermia, followed by the effect for 35°C vs. normothermia, but both effects were much greater that the treatment effect size comparing 33 vs. 35ºC. To illustrate the differences among the treatment effect sizes, the ratio of each treatment vs. normothermia effect size was compared to the effect size in the 33 vs. 35 comparison. Thus, considering neurons, the treatment effect size of 33 vs. normo was 4.8x larger than the treatment effect size of 33 vs. 35; similarly, 35 vs. normo was 3.9x that of 33v35. Across all cell types, deeper hypothermia to 33°C was associated with larger effect sizes, compared to 35°C, and much larger (4.8 to 18x) effect sizes than 33 v 35.

Supplementary Table. Treatment effect sizes

Cell Type	Delay	Duration	33 v normo	35 v normo	33 v 35
Neurons	0	2	44.38	36.27	8.11
	0	6	50.97	43.13	7.84
	0	24	57.90	56.08	1.83
	30	2	29.15	27.65	1.50
	30	6	34.53	31.52	3.01
	30	24	39.87	34.16	5.71
	60	2	34.48	22.98	11.50
	60	6	43.63	25.27	18.36
	60	24	46.24	34.02	12.23
	90	2	-1.48	9.05	-10.53
	90	6	29.15	18.00	11.15
	90	24	40.45	18.98	21.47
Mean±SD			37.4±14.9	29.8±12.4	7.7±8.4
Ratio			4.8	3.9	1.0
Astrocytes	0	2	41.55	0.19	41.36
	0	6	45.97	20.72	25.25
	0	24	56.33	14.12	42.21
	30	2	22.23	14.39	7.84
	30	6	22.64	22.67	-0.03
	30	24	39.60	26.30	13.30
	60	2	20.94	11.74	9.20
	60	6	24.79	12.02	12.77
	60	24	39.87	16.36	23.51
	90	2	28.36	8.23	20.13
	90	6	38.73	16.01	22.72
	90	24	50.36	18.62	31.73
Mean±SD			35.9±11.9	15.11±6.9	2.1±1.3
Ratio			18.0	7.2	1.0
Endothelial cells	0	2	-93.41	-87.99	6.17
	0	6	-92.54	-62.59	47.85
	0	24	-92.52	-91.75	0.84
	30	2	-90.42	-70.02	29.14
	30	6	-79.60	-74.18	7.31
	30	24	-90.50	-86.13	5.08
	60	2	-86.16	-77.27	11.51
	60	6	-92.22	-71.99	28.10
	60	24	-91.52	-85.28	7.31
	90	2	-89.12	-69.65	27.97
	90	6	-86.72	-68.82	26.00
	90	24	-93.81	-87.77	6.89
Mean±SD			-71.4±3.2	-61.9±7.6	9.6±7.2
Ratio			7.4	6.4	1.0


Pre- 33°C-2Hr
37°C
33°C-2Hr
33°C-4Hr
n = 20
Temperature (°C)
38
37
36
35
34
33
32
31
-30
-15
0
15
30
45
60
75
90
105
120
135
150
165
180
195
210
225
240
255
Time (Mins.)


0 hr
24 hr
LDH Release
And MTT assay
Normothermia
OGD
Reoxygentation
Duration
Hypothermia
OGD
2 hr
Reoxygentation
OGD
6 hr
Reoxygentation
OGD
24 hr
Reoxygentation
Mild Hypothermia- 35 °C
Moderate Hypothermia- 33 °C
0 hr
Delay
24 hr
LDH Release
And MTT assay
OGD
Reoxygentation
Delay
Hypothermia
OGD
30 min
Reoxygentation
OGD
60 min
Reoxygentation
OGD
90 min
Reoxygentation
Normothermia- 37 °C
Moderate Hypothermia- 33 °C
Mild Hypothermia- 35 °C