Figure 1a

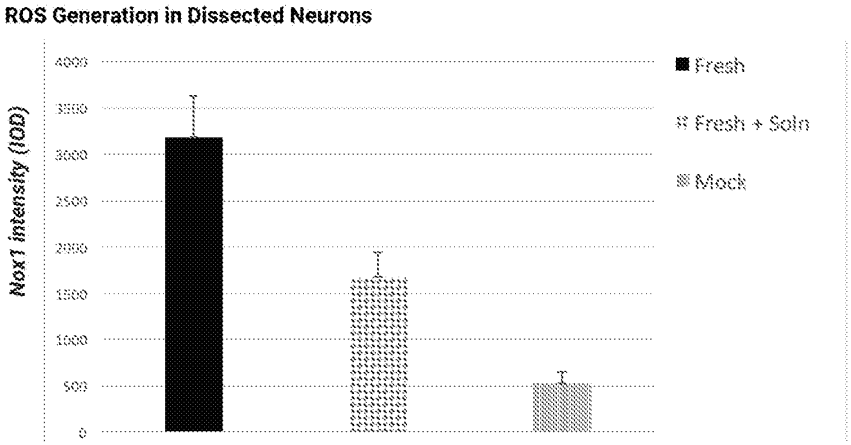

ROS Generation in Dissected Neurons
Nox1 intensity (IOD)
4000
3500
3000
2500
2000
1500
1000
500
0
Fresh
Fresh + Soln
Mock

Figure 1b

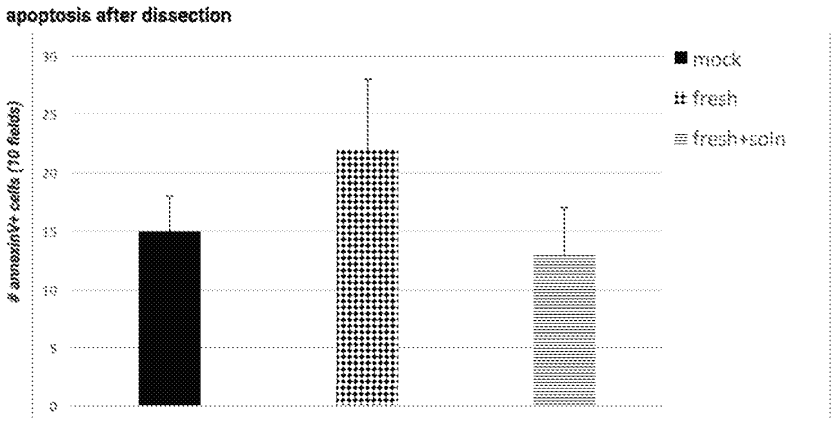

apoptosis after dissection
annexin V+ cells (10 fields)
0
5
10
15
20
25
30
mock
fresh
fresh+coln

Figure 1c

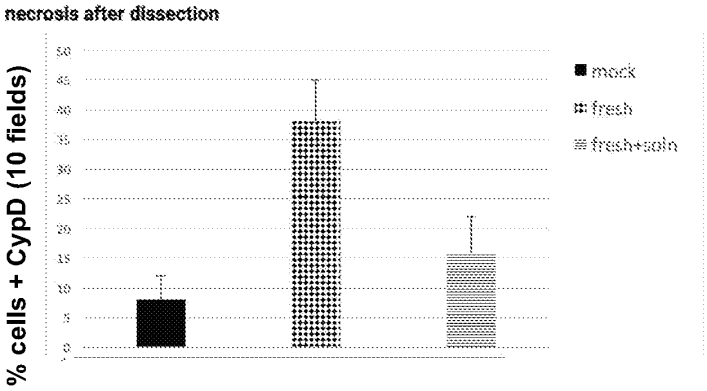

necrosis after dissection
% cells + CypD (10 fields)
mock
fresh
fresh+soln

Figure 2a

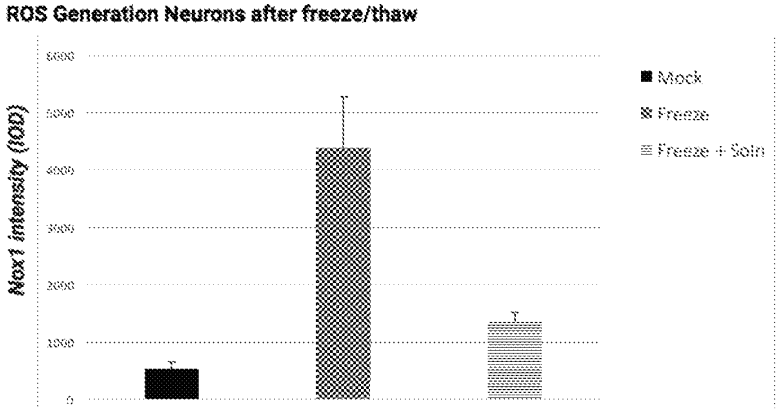

ROS Generation Neurons after freeze/thaw
Mock
Freeze
Freeze + Soln

Figure 2b

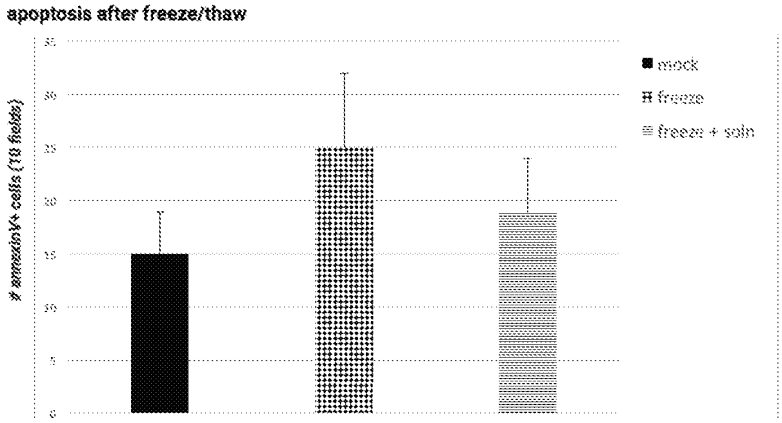

apoptosis after freeze/thaw
annexinV+ cells (10 fields)
mock
freeze
freeze + salin

Figure 2c

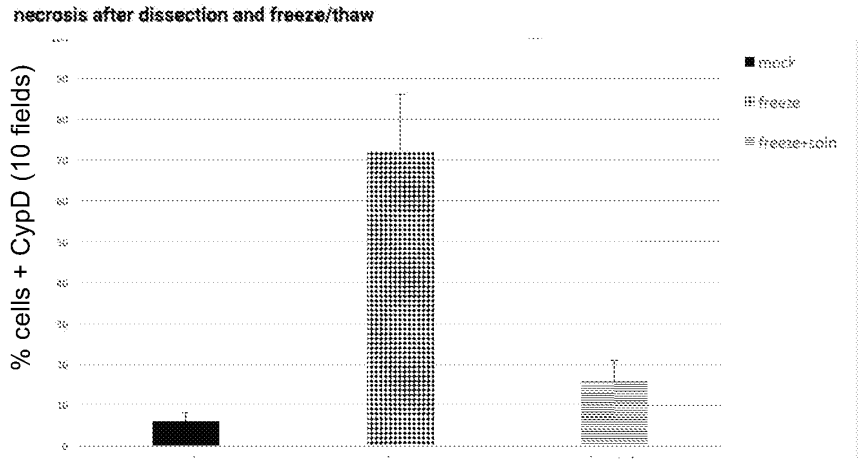

necrosis after dissection and freeze/thaw
% cells + CypD (10 fields)
mock
freeze
freeze+soln

Figure 3

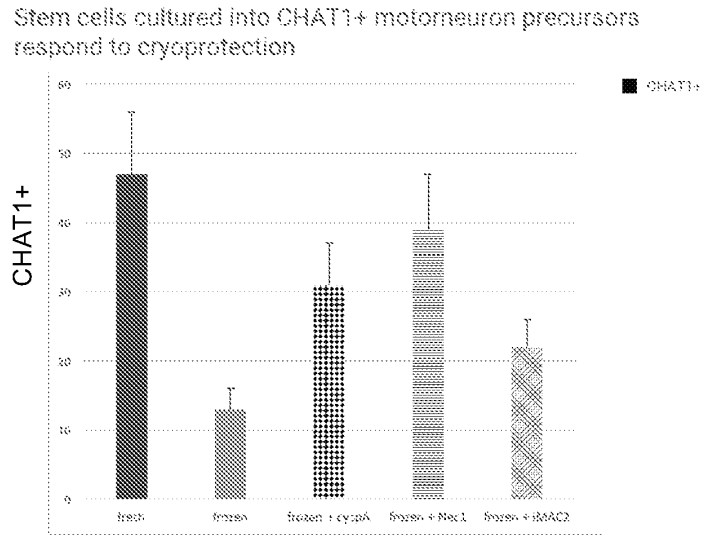

Stem cells cultured into CHAT1+ motorneuron precursors respond to cryoprotection
CHAT1+
CHAT1+
60
50
40
30
20
10
0
fresh
frozen
frozen + cypA
frozen + Nac1
frozen + RMAC2

Figure 4a

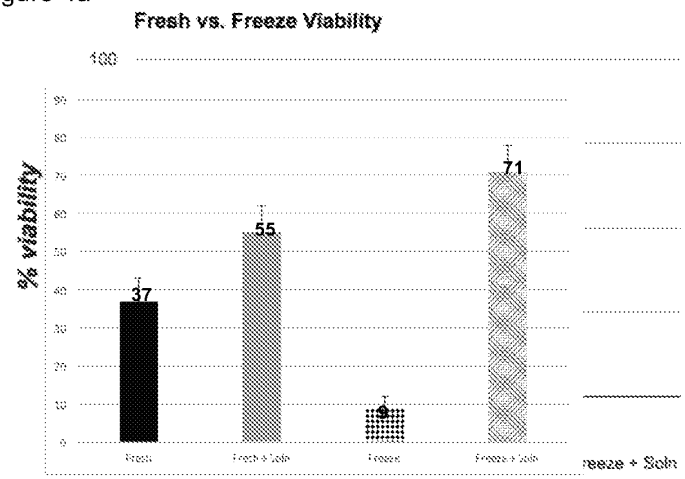

Fresh vs. Freeze Viability
% Viability
100
37
55
9
71
Fresh
Freeze
reeze + Soln

Figure 4b

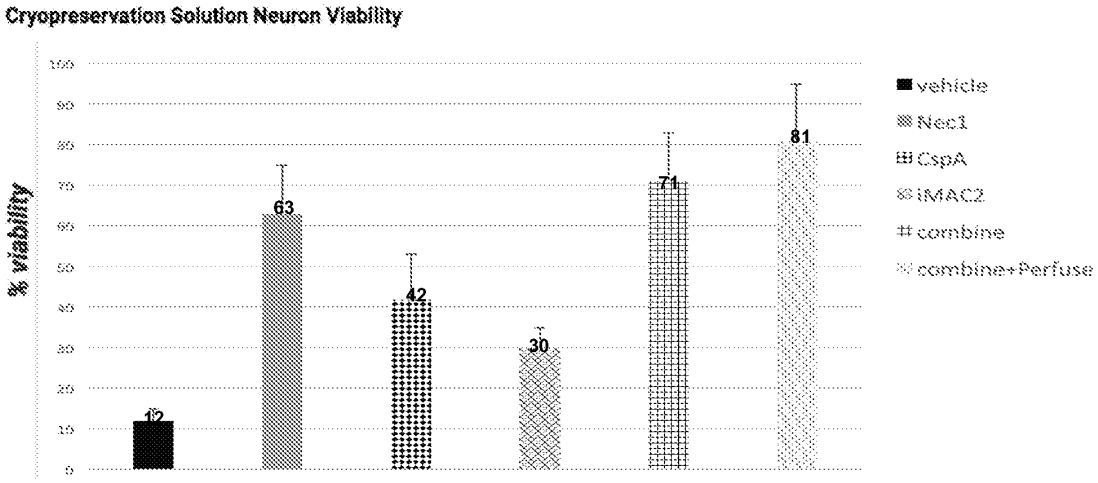

Cryopreservation Solution Neuron Viability
% viability
12
63
42
30
71
81
vehicle
Nec1
CspA
iMAC2
combine
combine+Perfuse

Figure 5

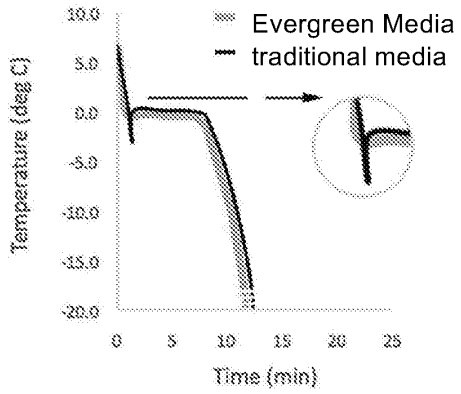

Evergreen Media
traditional media
Temperature (deg C)
10.0
5.0
0.0
-5.0
-10.0
-15.0
-20.0
0
5
10
15
20
25
Time (min)

Figure 6a

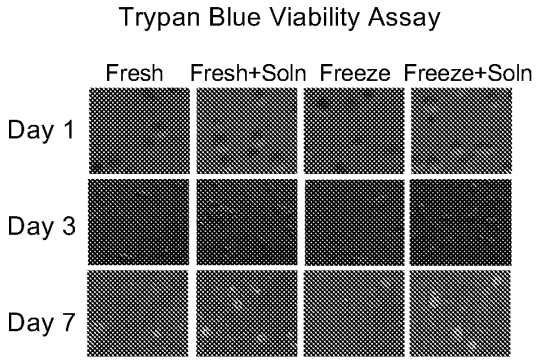


Figure 6b

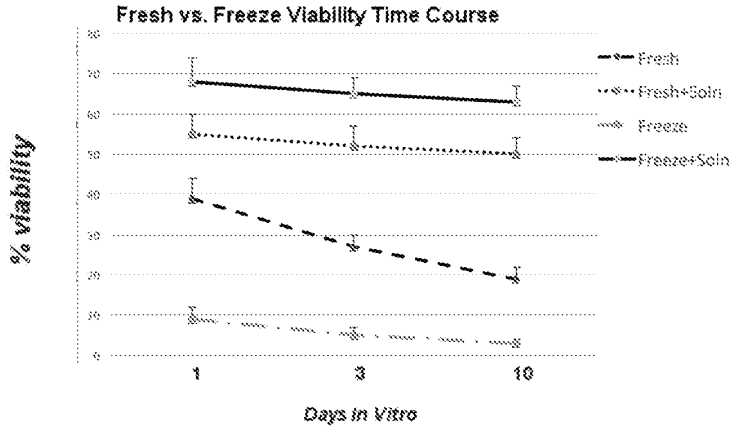


Figure 6c

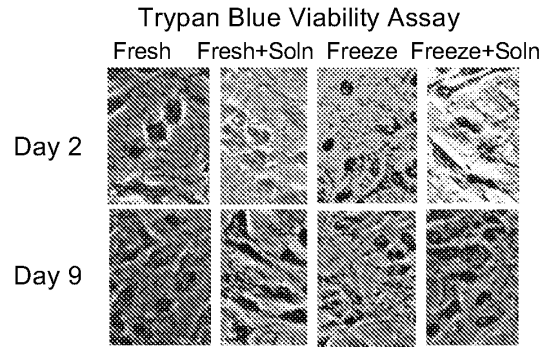


Figure 6d

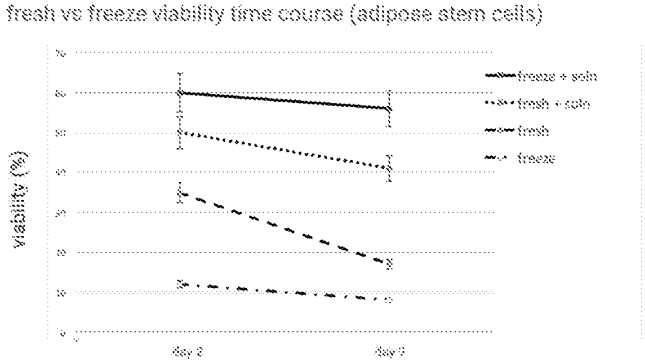


Figure 7a

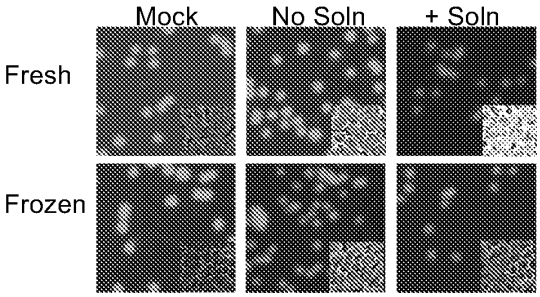


Figure 7b

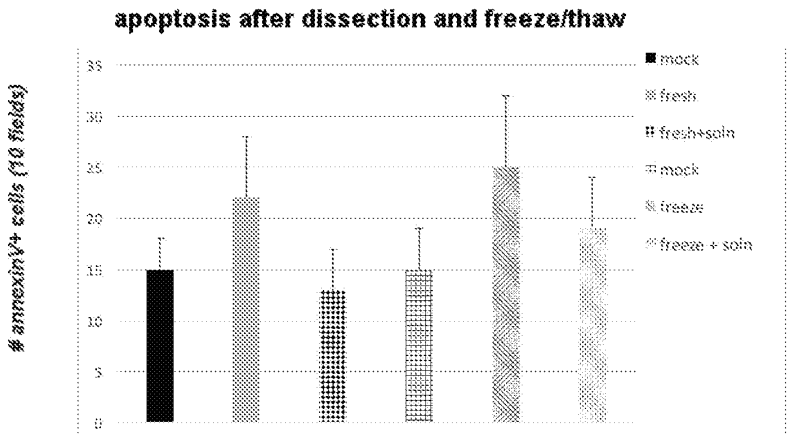


Figure 8a

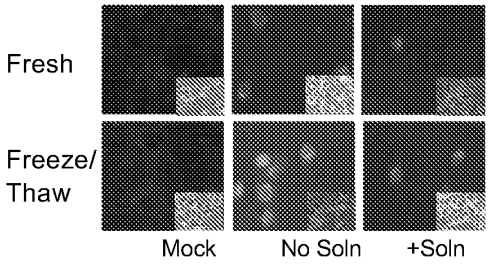


Figure 8b

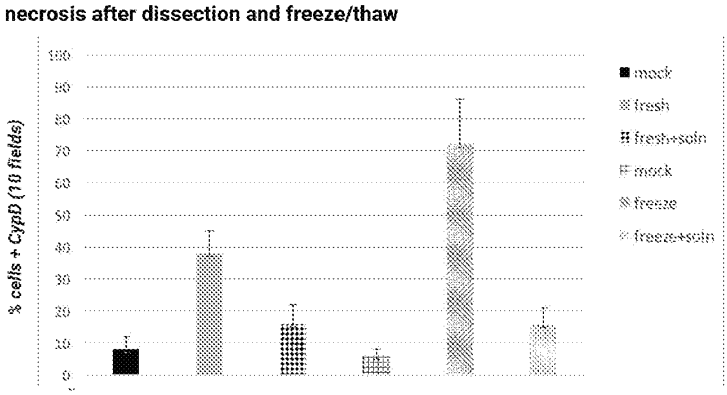


Figure 9a

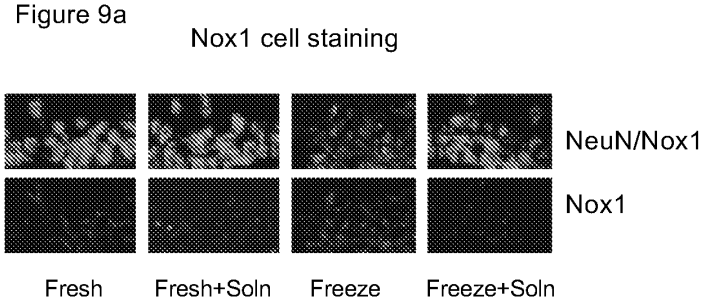


Figure 9b

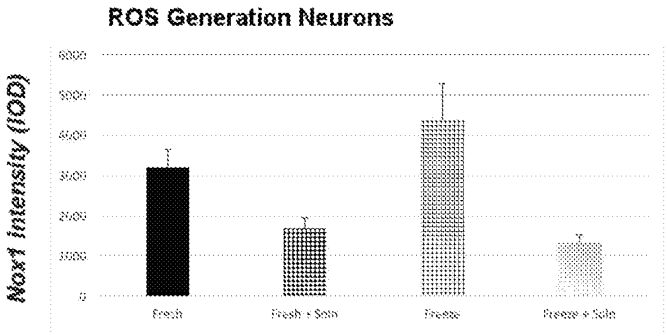


Figure 10a

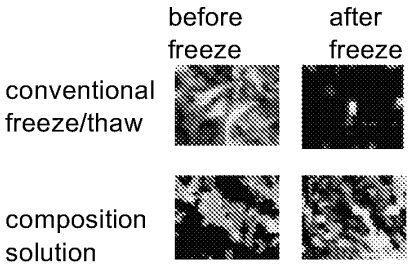


Figure 10b

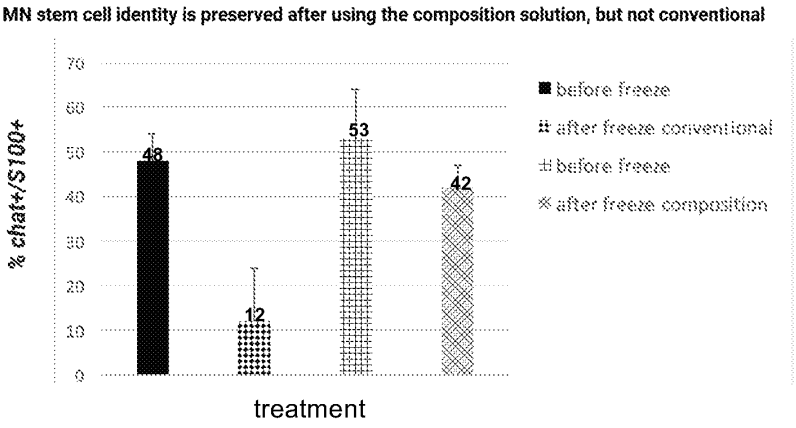


Figure 11a

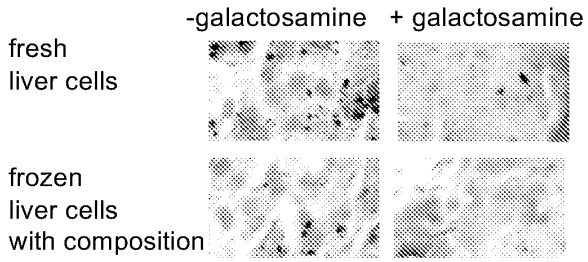


Figure 11b

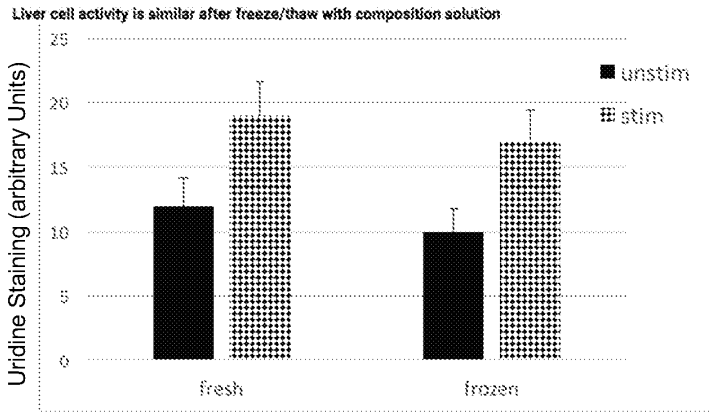


Figure 12

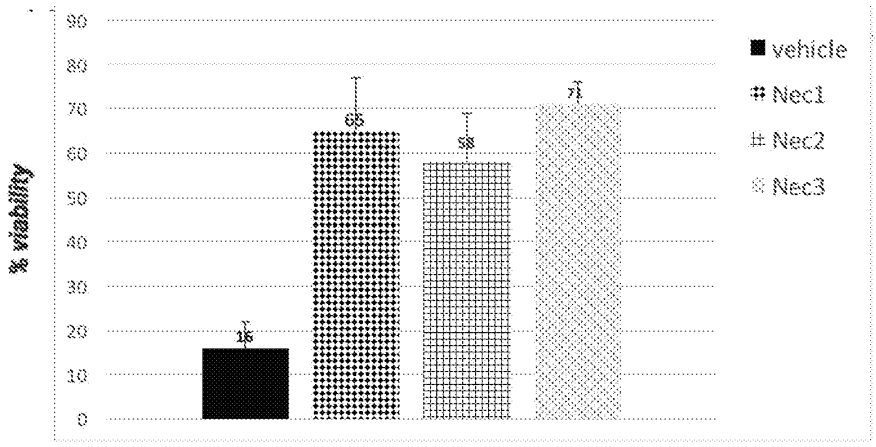

% viability
90
80
70
60
50
40
30
20
10
0
16
65
58
71
vehicle
Nec1
Nec2
Nec3

Figure 13a

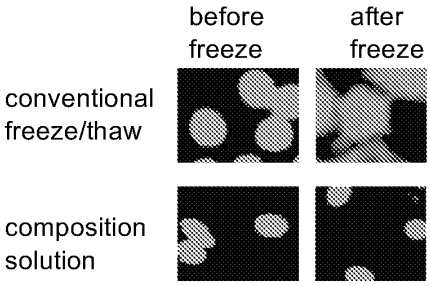
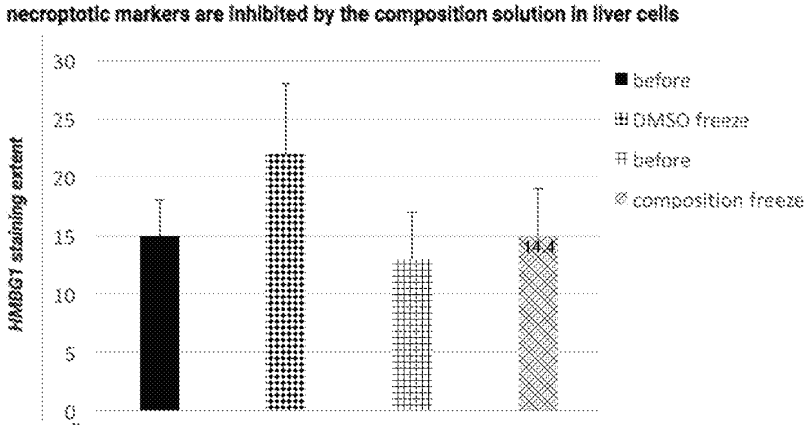


Figure 13b



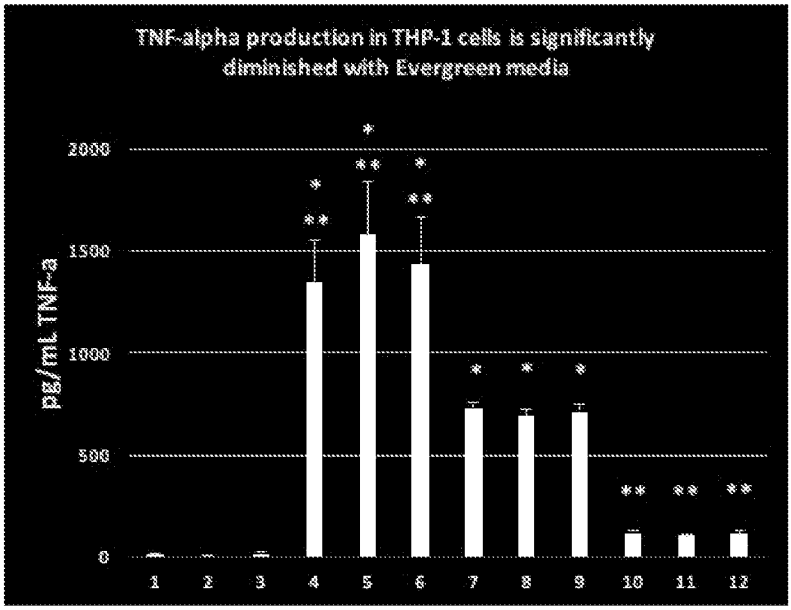


FIGURE 14

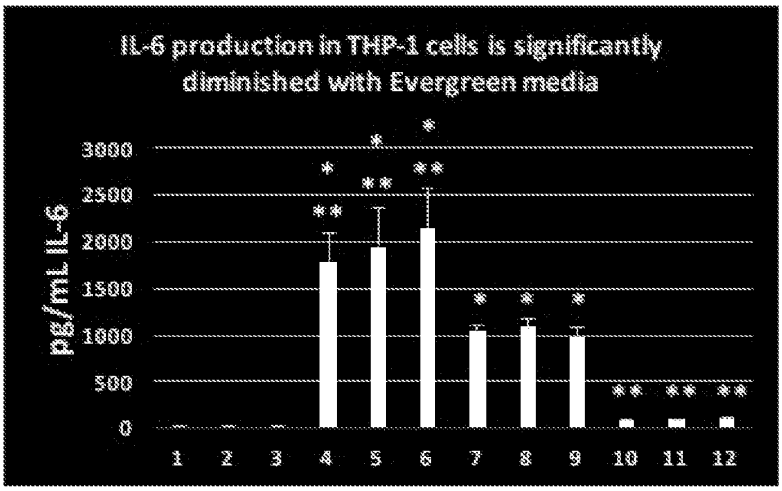


FIGURE 15

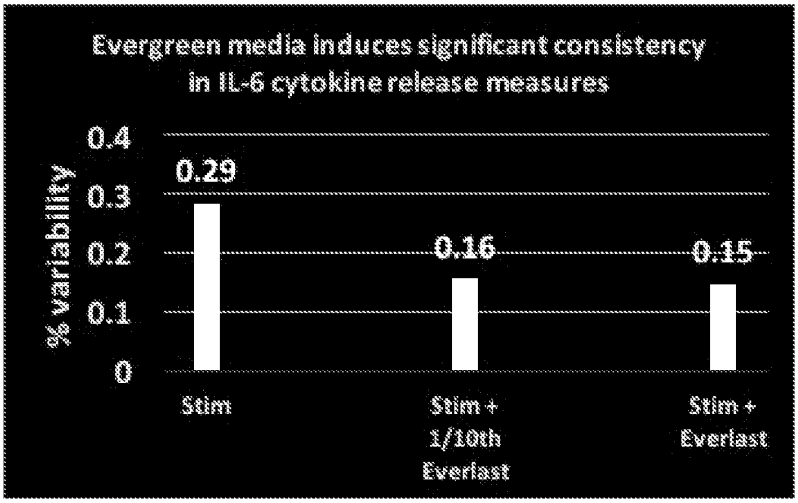


FIGURE 16

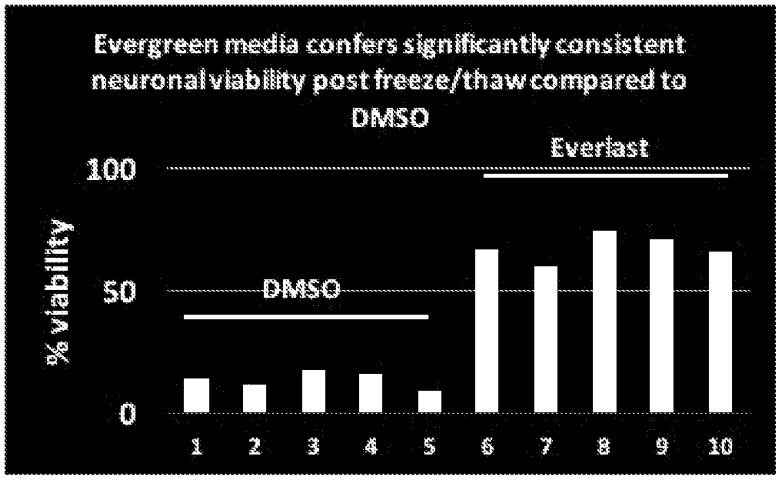


FIGURE 17

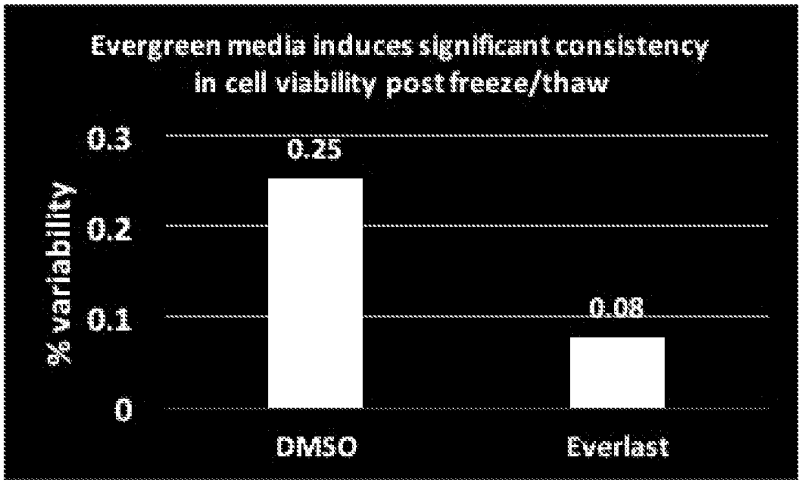

Evergreen media induces significant consistency in cell viability post freeze/thaw
% variability
0.3
0.2
0.1
0
0.25
0.08
DMSO
Everlast

FIGURE 18



FIGURE 19A

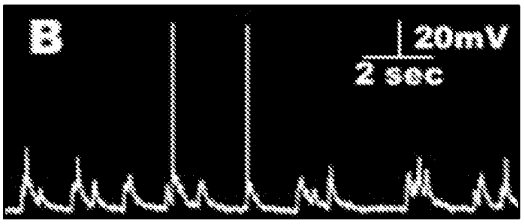


FIGURE 19B

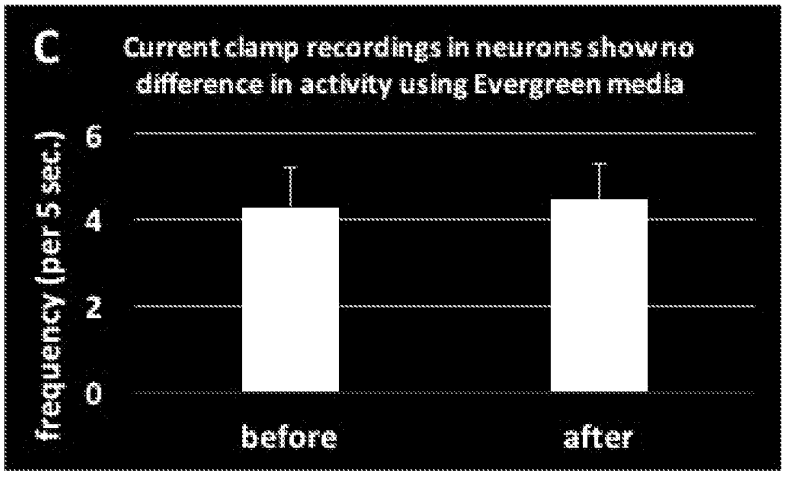
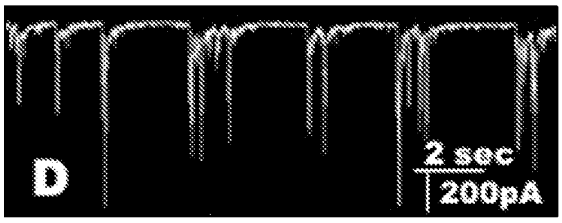
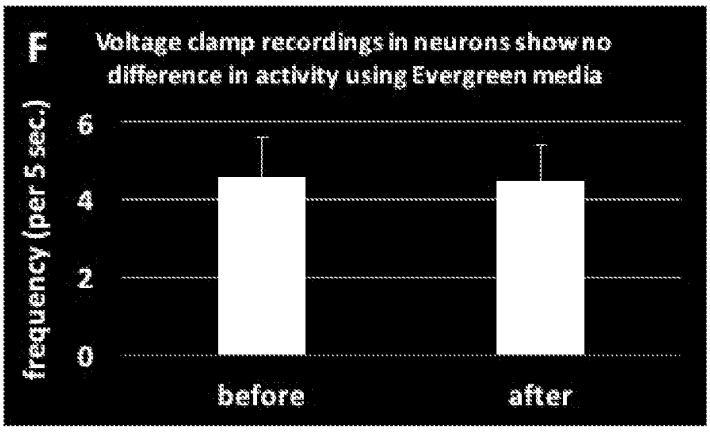


FIGURE 19C


D
2 sec
200pA


E
2 sec
200pA


F
Voltage clamp recordings in neurons show no difference in activity using Evergreen media
frequency (per 5 sec.)
6
4
2
0
before
after

19F

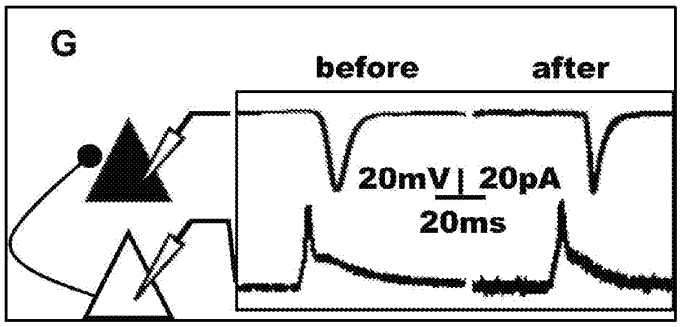


FIGURE 19G

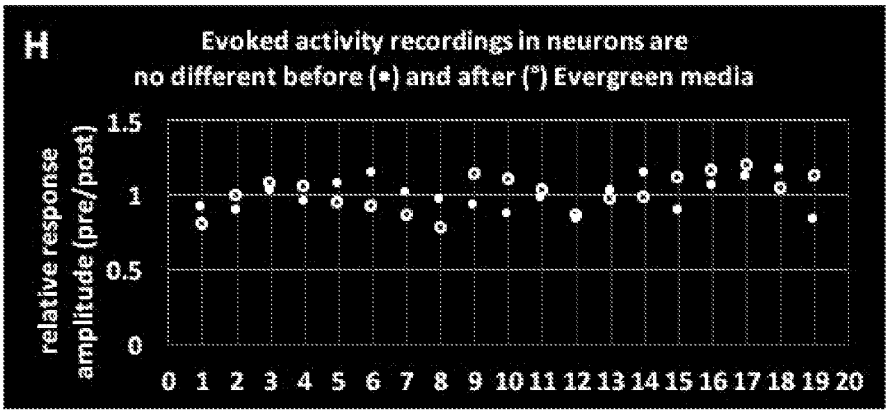


FIGURE 19H