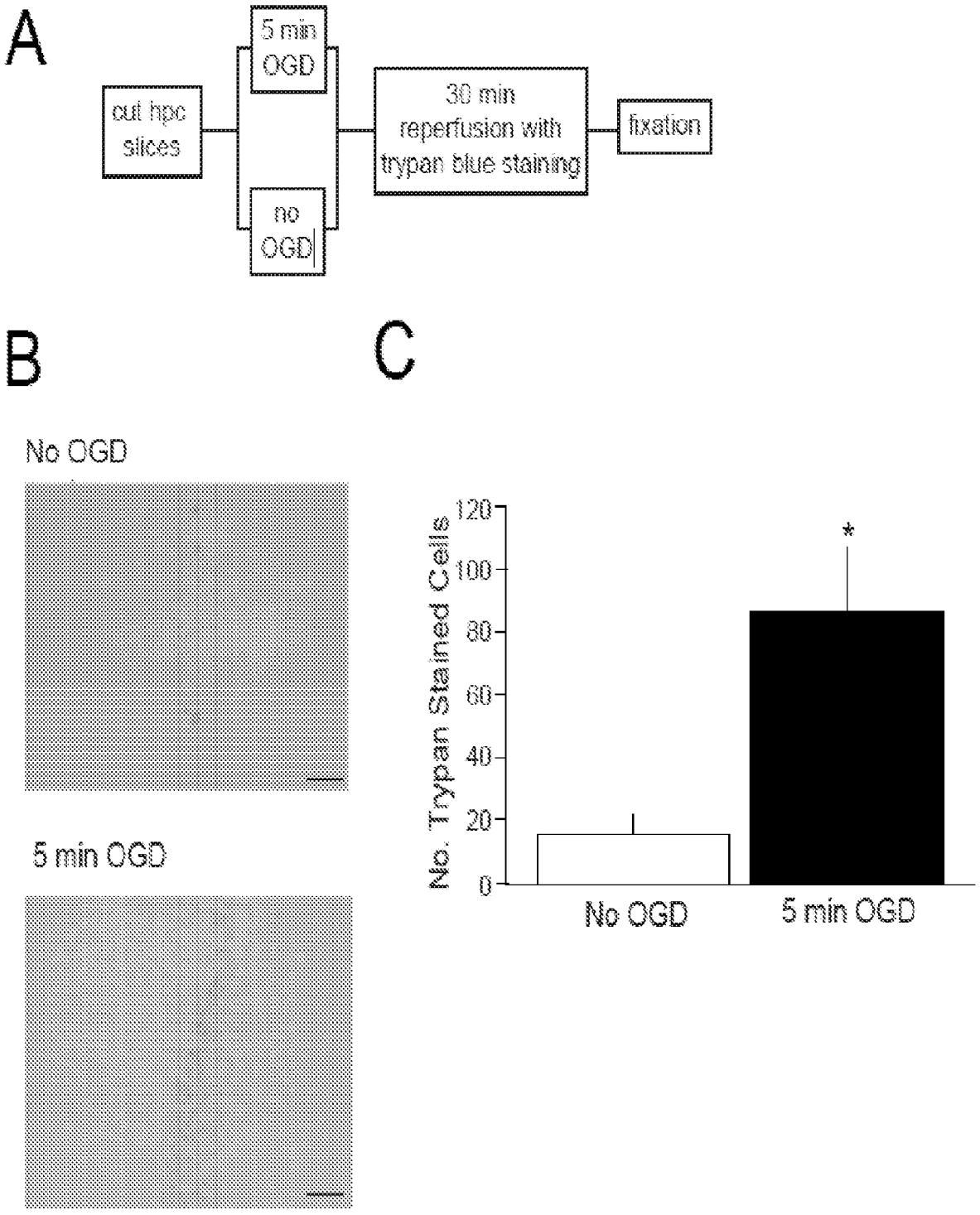

A
5 min OGD
cut hpc slices
30 min reperfusion with trypan blue staining
fixation
no OGD
B
C
No OGD
5 min OGD
No. Trypan Stained Cells
120
100
80
60
40
20
0
*
No OGD
5 min OGD

FIG. 1

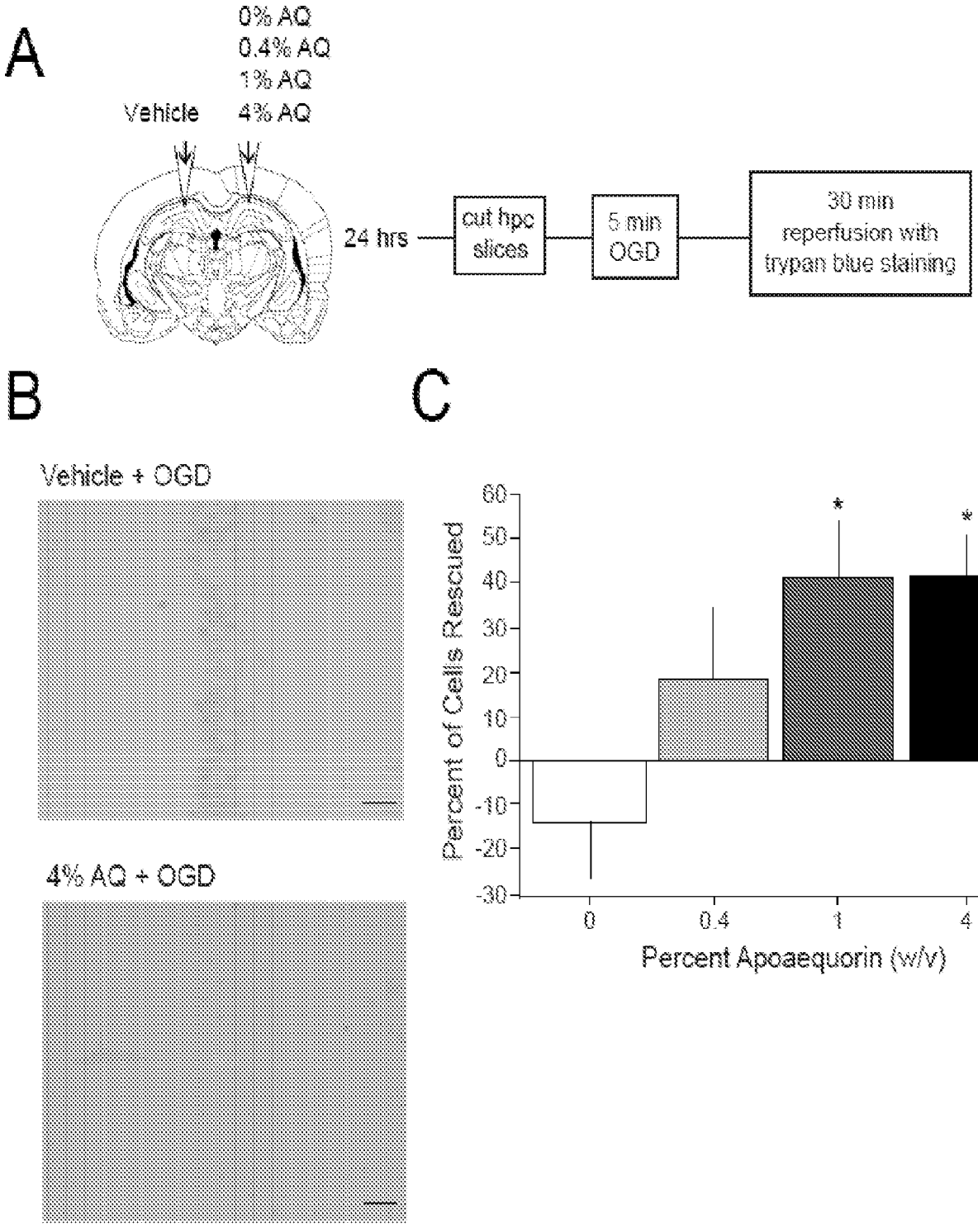

A
0% AQ
0.4% AQ
1% AQ
Vehicle
4% AQ
24 hrs
cut hpc slices
5 min OGD
30 min reperfusion with trypan blue staining
B
Vehicle + OGD
4% AQ + OGD
C
Percent of Cells Rescued
60
50
40
30
20
10
0
-10
-20
-30
0
0.4
1
4
Percent Apoaequorin (w/v)
*
*

FIG. 2

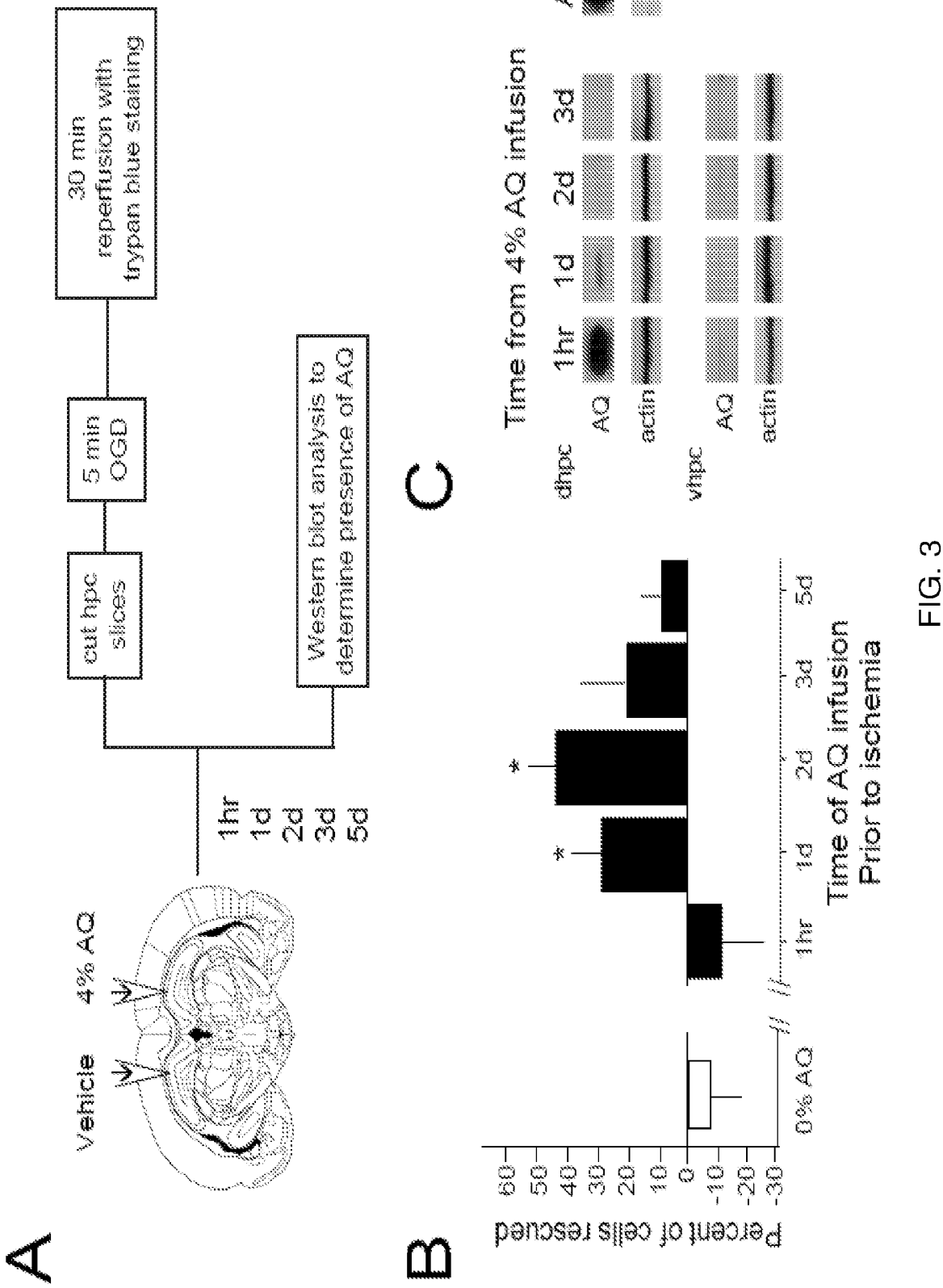

A
Vehicle
4% AQ
1hr
1d
2d
3d
5d
cut hpc slices
5 min OGD
30 min reperfusion with trypan blue staining
Western blot analysis to determine presence of AQ
B
Percent of cells rescued
60
50
40
30
20
10
0
-10
-20
-30
0% AQ
1hr
1d
2d
3d
5d
Time of AQ infusion Prior to ischemia
*
*
C
Time from 4% AQ infusion
dhpc
vhpc
1hr
1d
2d
3d
AQ
actin

FIG. 3

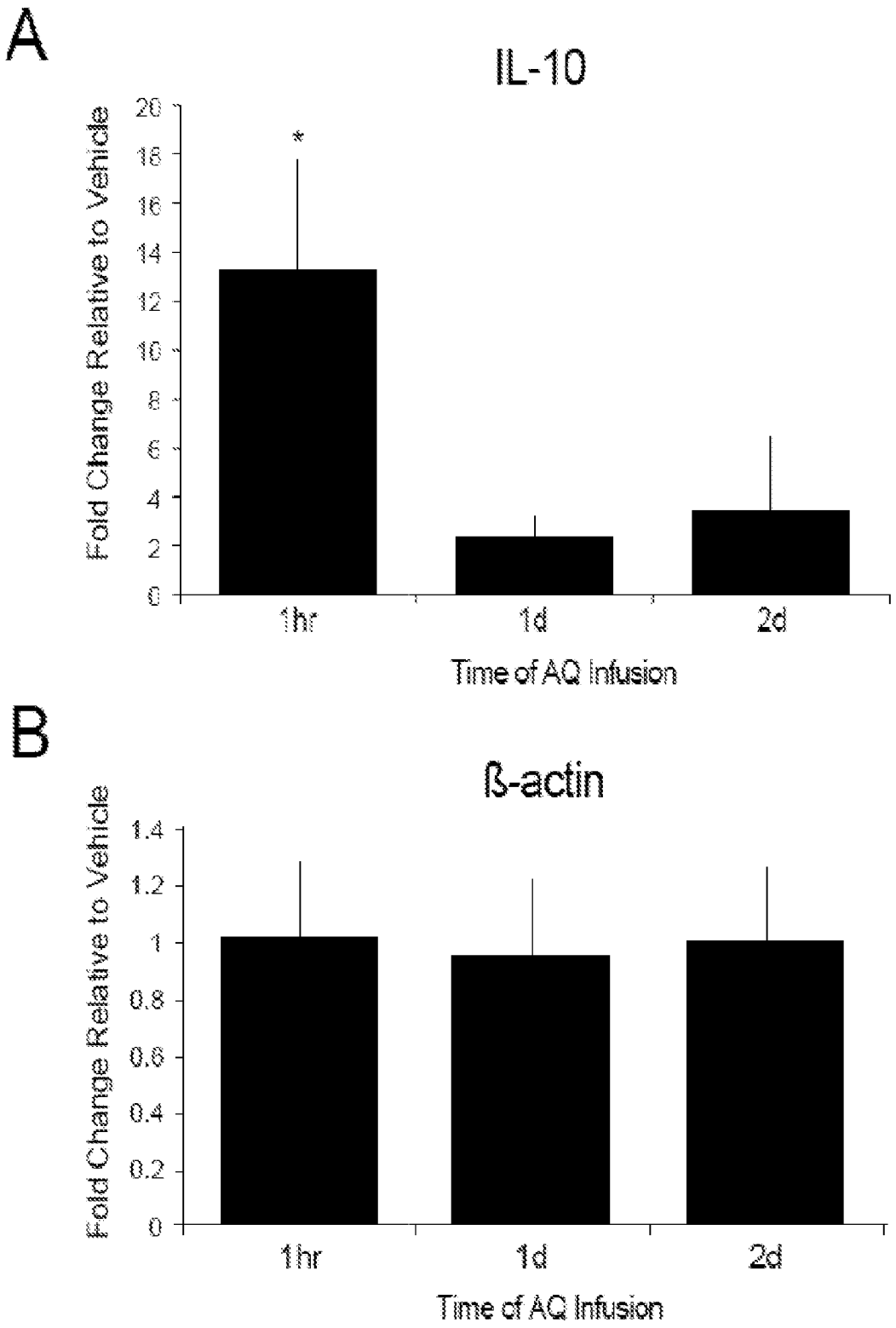

A
IL-10
Fold Change Relative to Vehicle
20
18
16
14
12
10
8
6
4
2
0
*
1hr
1d
2d
Time of AQ Infusion
B
ß-actin
Fold Change Relative to Vehicle
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1hr
1d
2d
Time of AQ Infusion

FIG. 4

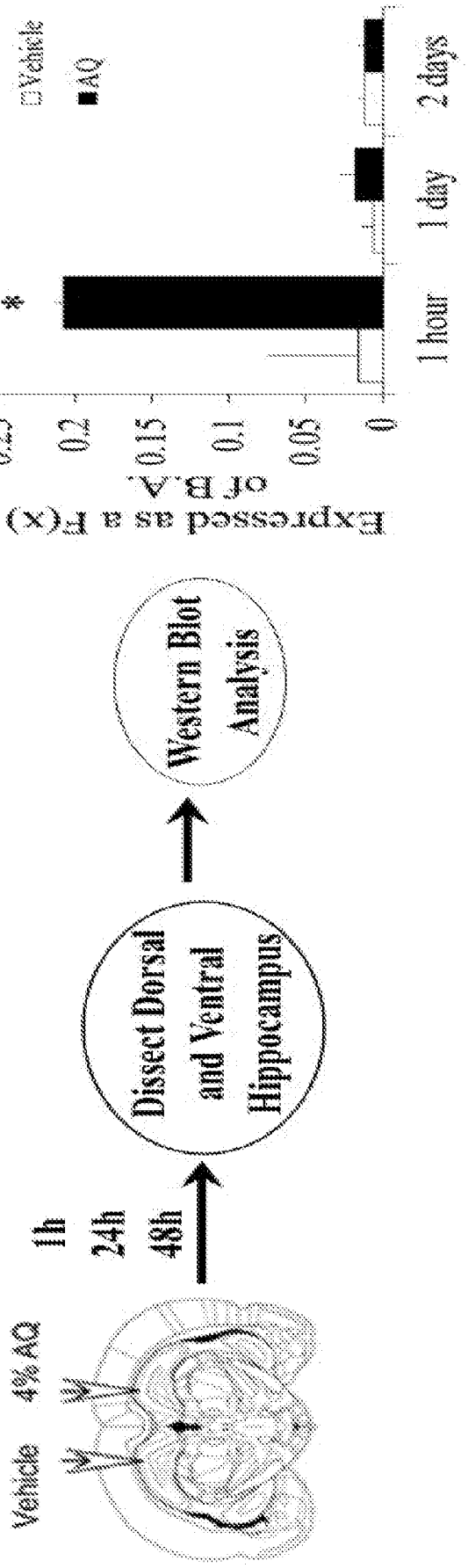

AQ
Vehicle
AQ
*
0.25
0.2
0.15
0.1
0.05
0
1 hour
1 day
2 days
Expressed as a F(x) of B.A.
Vehicle
4% AQ
1h
24h
48h
Dissect Dorsal and Ventral Hippocampus
Western Blot Analysis

FIG. 5

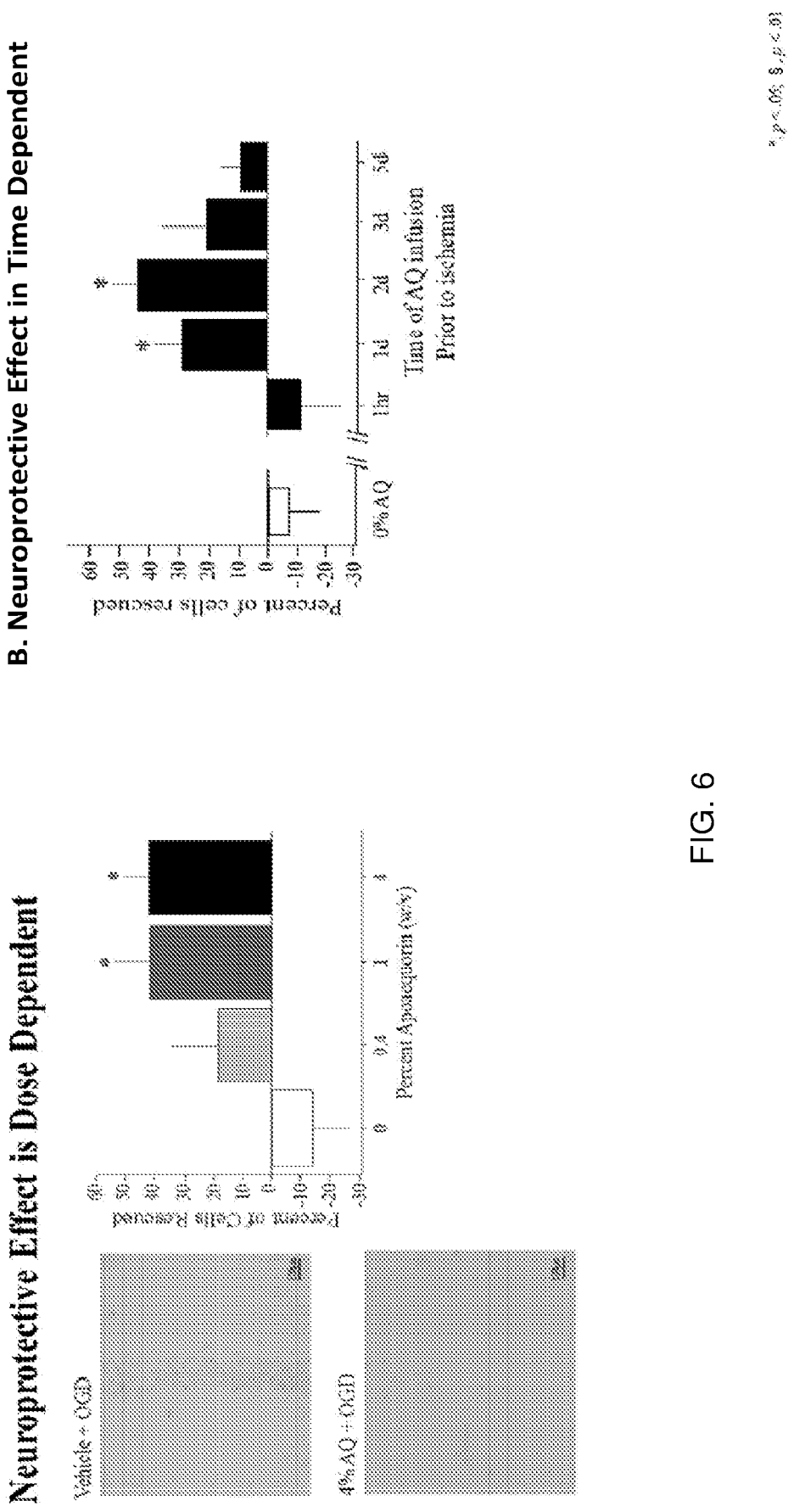

A. Neuroprotective Effect is Dose Dependent
Vehicle + OGD
4% AQ + OGD
Percent of Cells Rescued
Percent Apoaequorin (w/v)
B. Neuroprotective Effect in Time Dependent
Percent of cells rescued
0% AQ
1hr
3d
2d
3d
5d
Time of AQ infusion
Prior to ischemia
* p < .05; § p < .01

FIG. 6

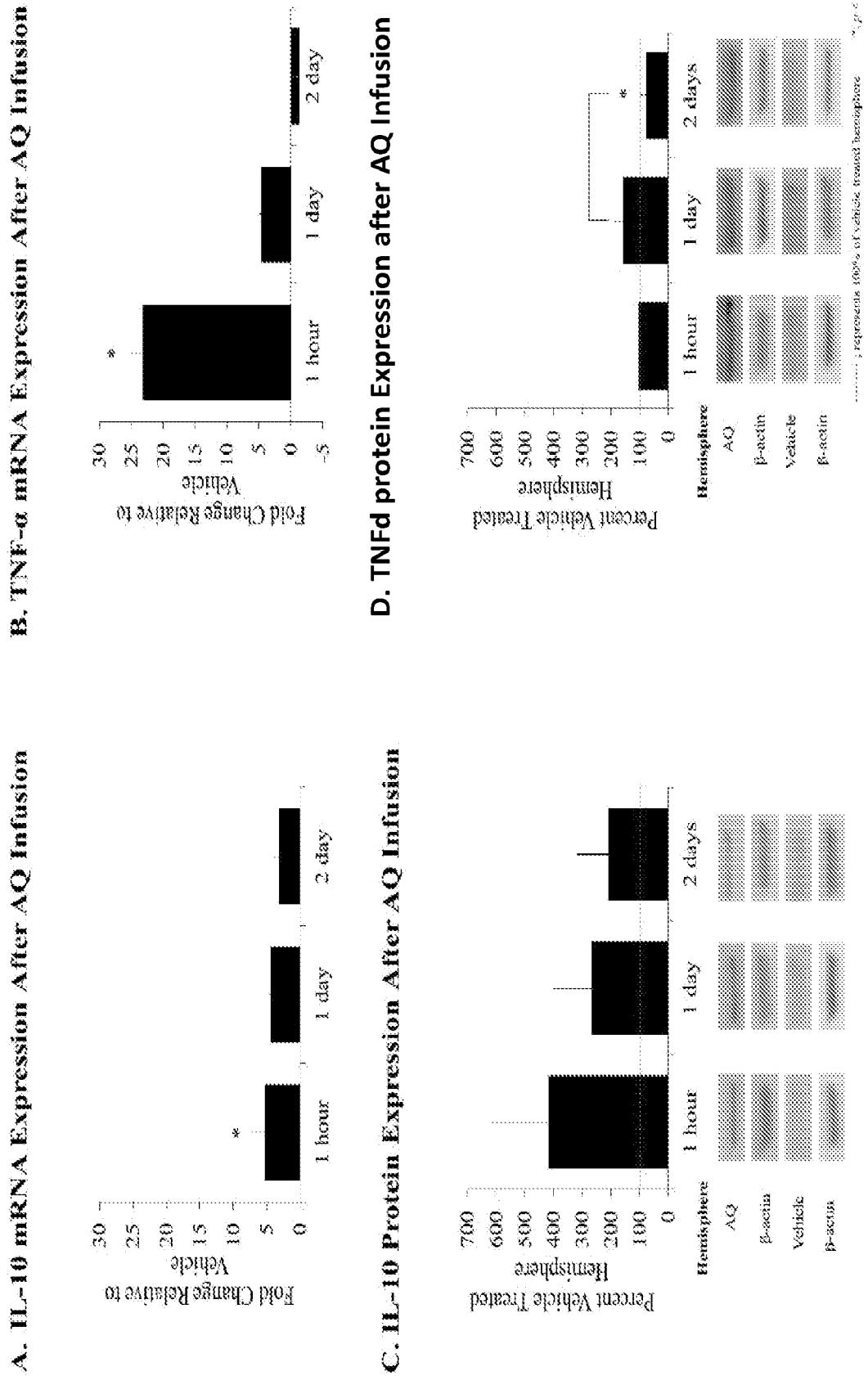

A. IL-10 mRNA Expression After AQ Infusion
Fold Change Relative to Vehicle
30
25
20
15
10
5
0
*
1 hour
1 day
2 day
B. TNF-α mRNA Expression After AQ Infusion
Fold Change Relative to Vehicle
30
25
20
15
10
5
0
-5
*
1 hour
1 day
2 day
C. IL-10 Protein Expression After AQ Infusion
Percent Vehicle Treated Hemisphere
700
600
500
400
300
200
100
0
1 hour
1 day
2 days
Hemisphere
AQ
β-actin
Vehicle
β-actin
D. TNFd protein Expression after AQ Infusion
Percent Vehicle Treated Hemisphere
700
600
500
400
300
200
100
0
*
1 hour
1 day
2 days
Hemisphere
AQ
β-actin
Vehicle
β-actin

FIG. 7

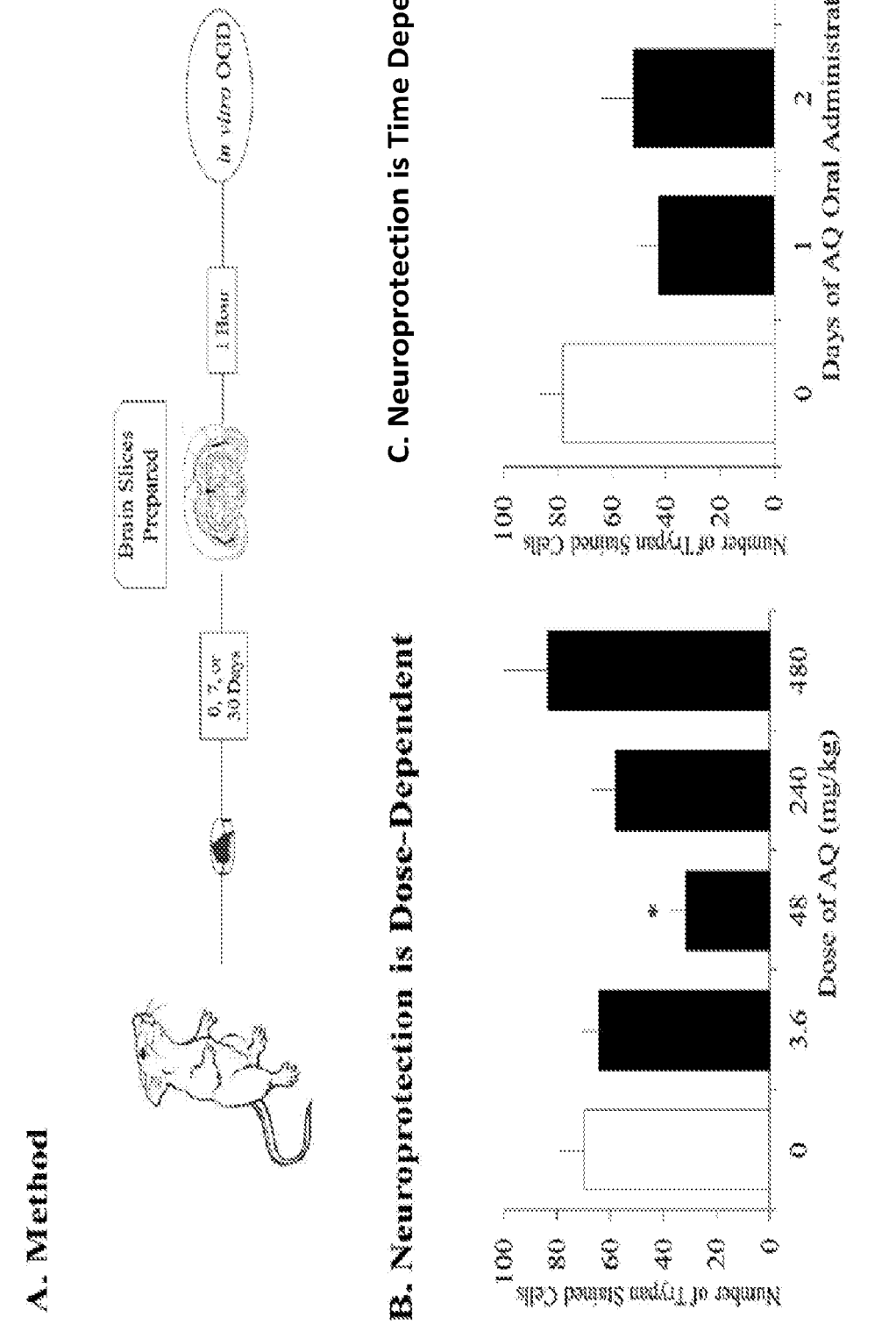

A. Method
Brain Slices Prepared
0, 7, or 30 Days
1 Hour
In vitro OGD
B. Neuroprotection is Dose-Dependent
Number of Trypan Stained Cells
100
80
60
40
20
0
0
3.6
48
240
480
Dose of AQ (mg/kg)
*
C. Neuroprotection is Time Dependent
Number of Trypan Stained Cells
100
80
60
40
20
0
0
1
2
7
Days of AQ Oral Administration
*

FIG. 8

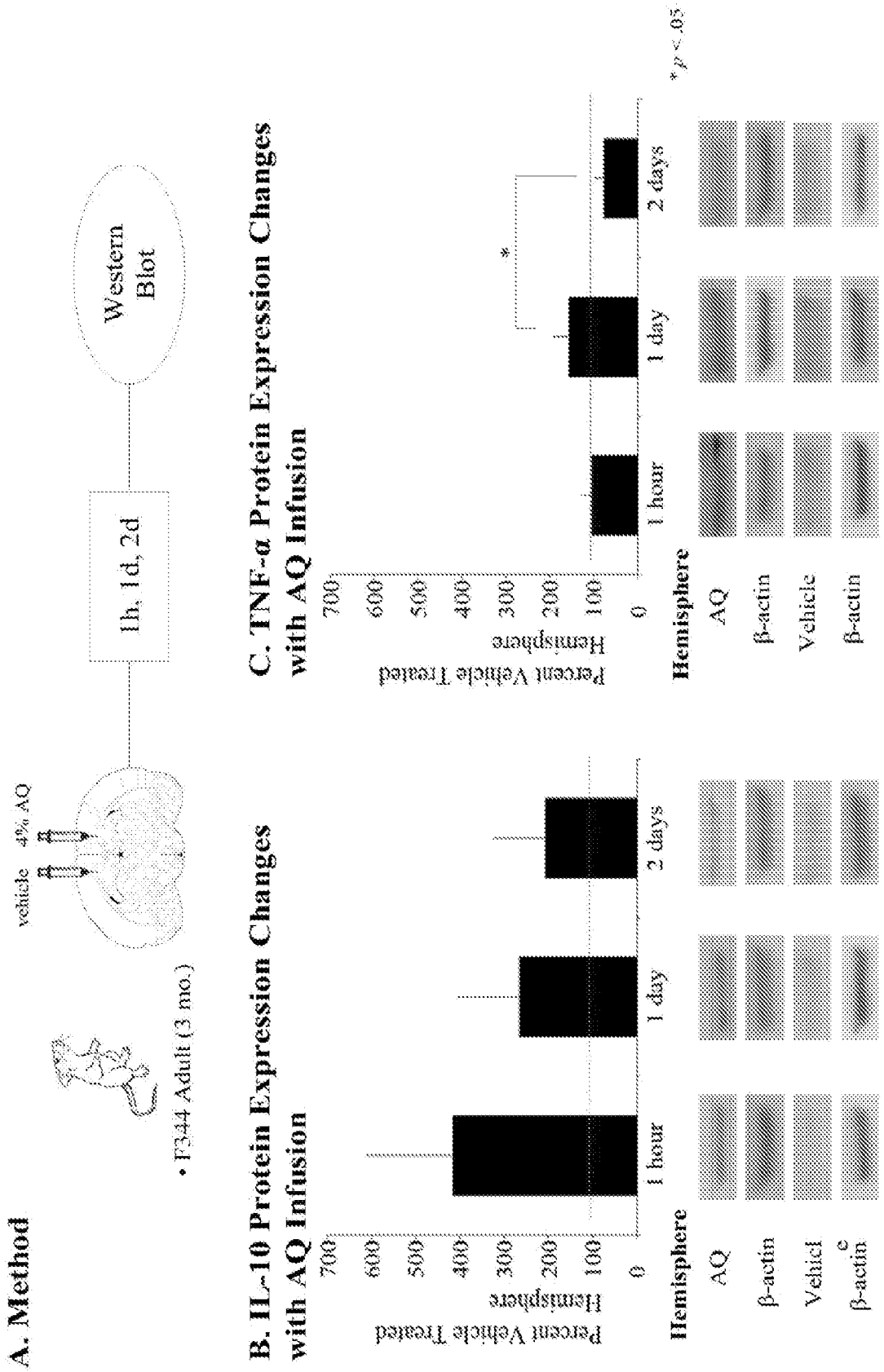

A. Method
vehicle
4% AQ
1h, 1d, 2d
Western Blot
• F344 Adult (3 mo.)
B. IL-10 Protein Expression Changes with AQ Infusion
Percent Vehicle Treated Hemisphere
700
600
500
400
300
200
100
0
1 hour
1 day
2 days
Hemisphere
AQ
β-actin
Vehicle
β-actin
C. TNF-α Protein Expression Changes with AQ Infusion
Percent Vehicle Treated Hemisphere
*
* p < .05

FIG. 9

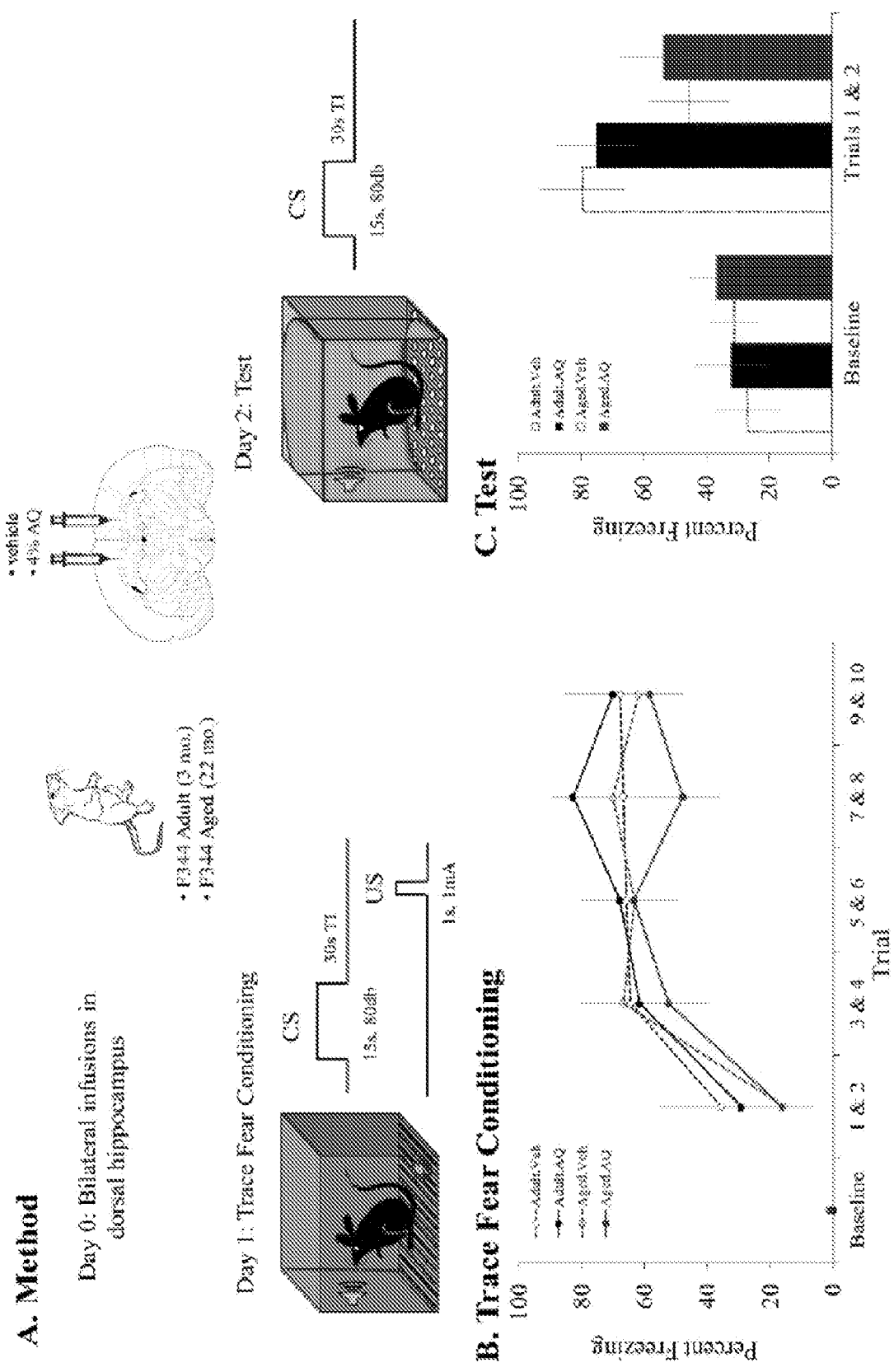

A. Method
Day 0: Bilateral infusions in dorsal hippocampus
• vehicle
• 4% AQ
• F344 Adult (3 mo.)
• F344 Aged (22 mo.)
Day 1: Trace Fear Conditioning
CS
30s TI
15s, 80db
US
1s, 1mA
Day 2: Test
CS
30s TI
15s, 80db
B. Trace Fear Conditioning
Percent Freezing
Trial
Baseline
1 & 2
3 & 4
5 & 6
7 & 8
9 & 10
C. Test
Percent Freezing
Baseline
Trials 1 & 2

FIG. 10

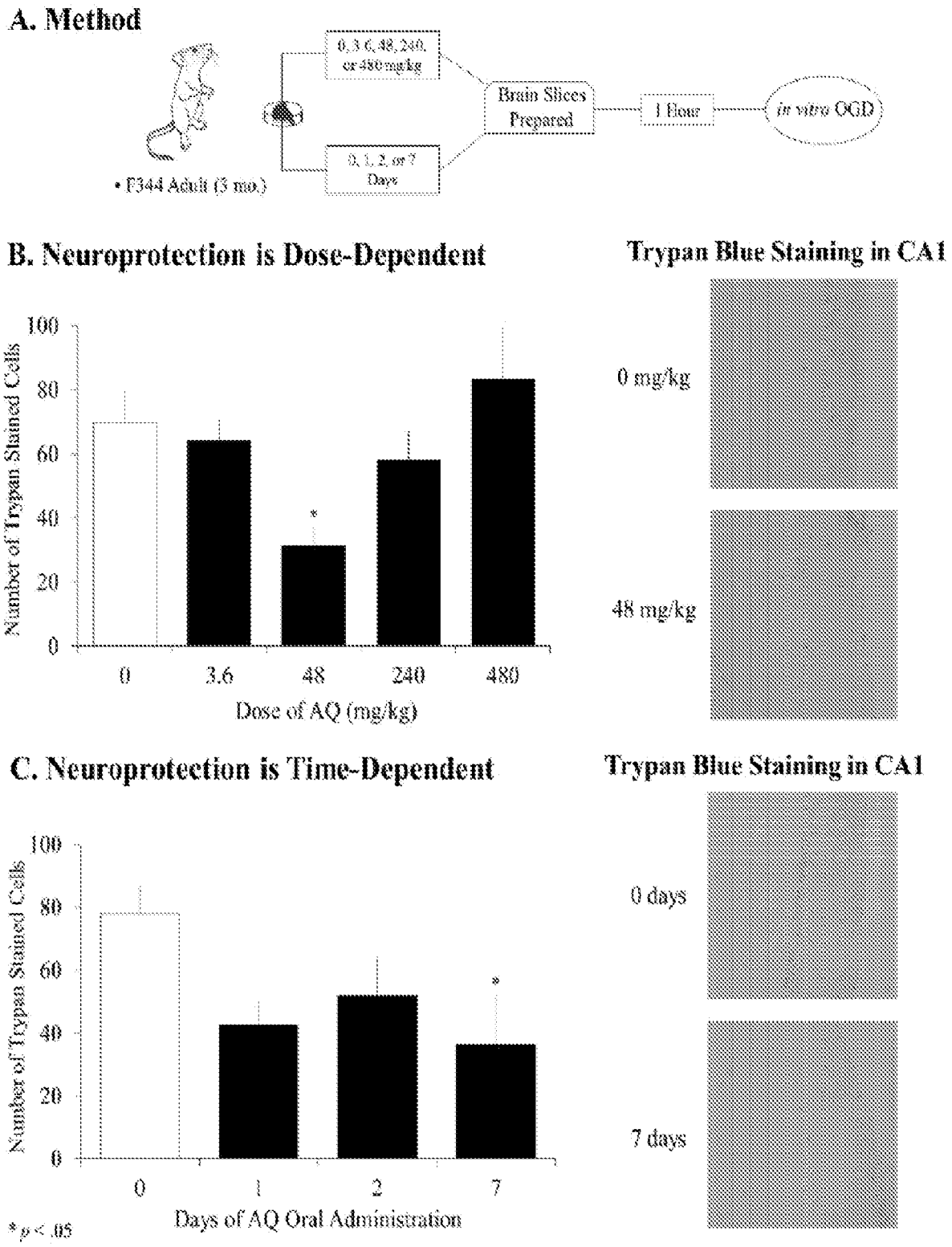

A. Method
0, 3.6, 48, 240, or 480 mg/kg
Brain Slices Prepared
1 Hour
in vitro OGD
0, 1, 2, or 7 Days
• F344 Adult (3 mo.)
B. Neuroprotection is Dose-Dependent
Trypan Blue Staining in CA1
Number of Trypan Stained Cells
100
80
60
40
20
0
0
3.6
48
240
480
Dose of AQ (mg/kg)
0 mg/kg
48 mg/kg
C. Neuroprotection is Time-Dependent
Trypan Blue Staining in CA1
Number of Trypan Stained Cells
100
80
60
40
20
0
0
1
2
7
Days of AQ Oral Administration
0 days
7 days
* p < .05

FIG. 11

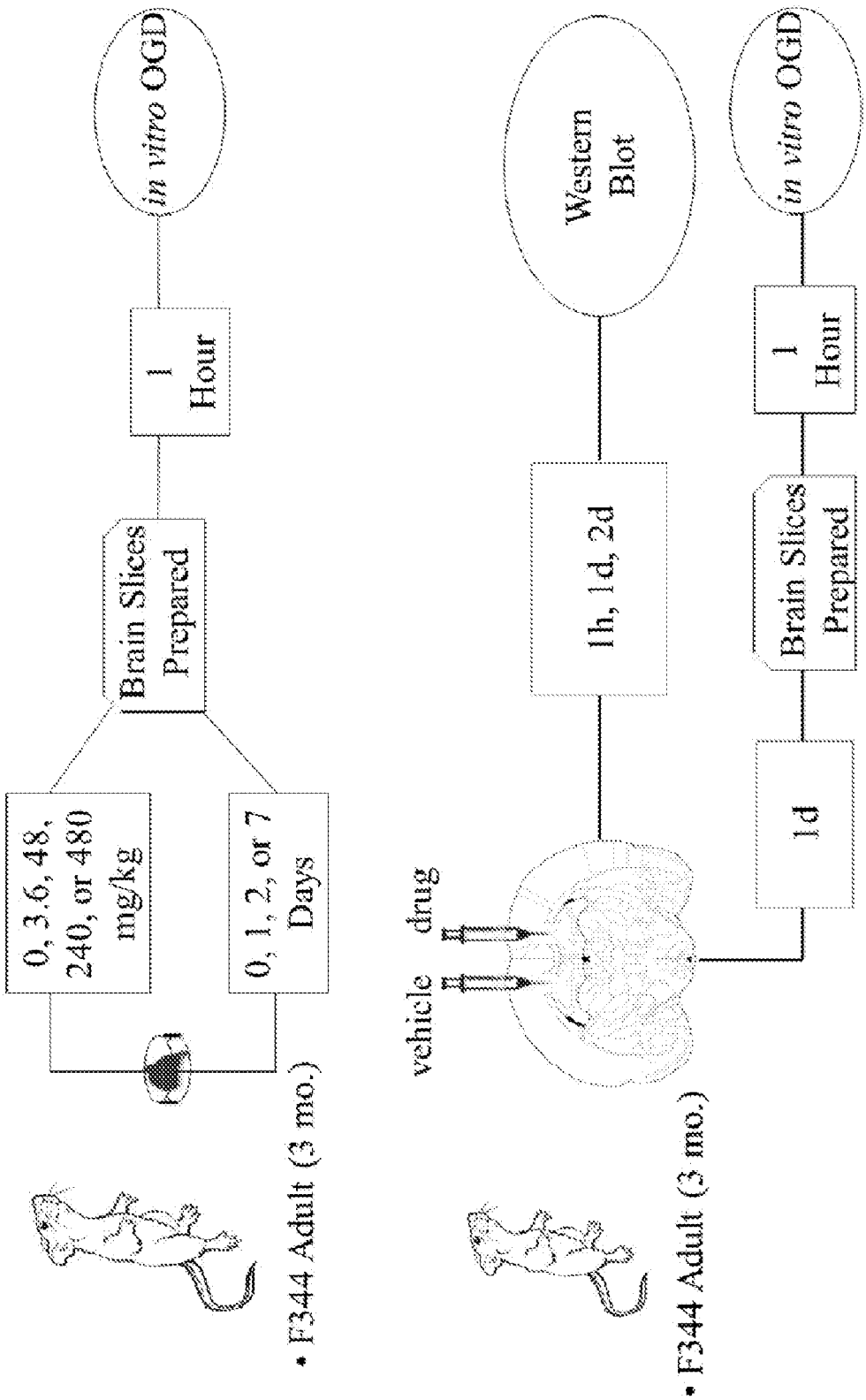

• F344 Adult (3 mo.)
0, 3.6, 48, 240, or 480 mg/kg
0, 1, 2, or 7 Days
Brain Slices Prepared
1 Hour
in vitro OGD
• F344 Adult (3 mo.)
vehicle
drug
1h, 1d, 2d
Western Blot
1d
Brain Slices Prepared
1 Hour
in vitro OGD

FIG. 12

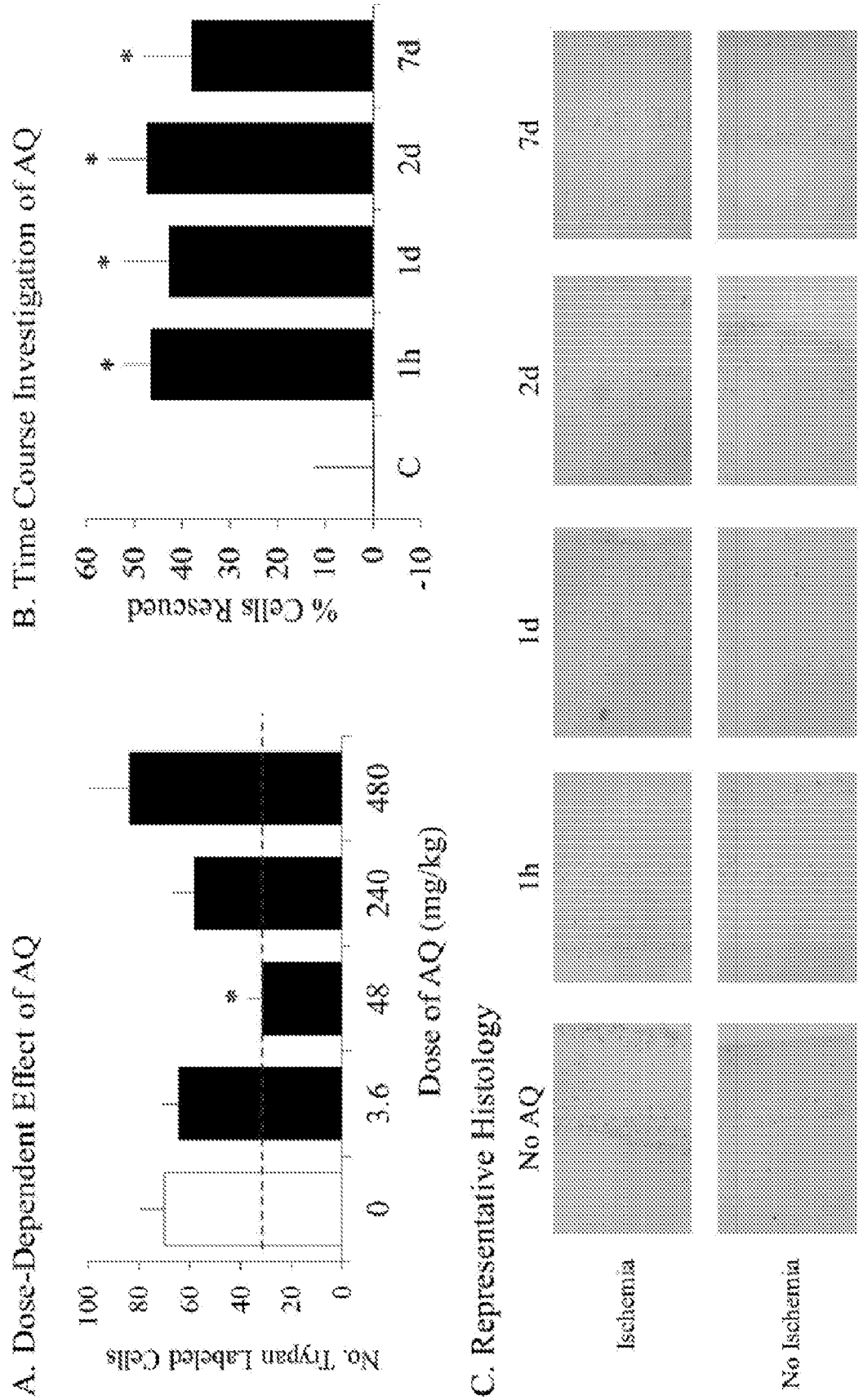

A. Dose-Dependent Effect of AQ
No. Trypan Labeled Cells
100
80
60
40
20
0
0
3.6
48
240
480
Dose of AQ (mg/kg)
*
B. Time Course Investigation of AQ
% Cells Rescued
60
50
40
30
20
10
0
-10
C
1h
1d
2d
7d
*
C. Representative Histology
No AQ
1h
1d
2d
7d
Ischemia
No Ischemia

FIG. 13

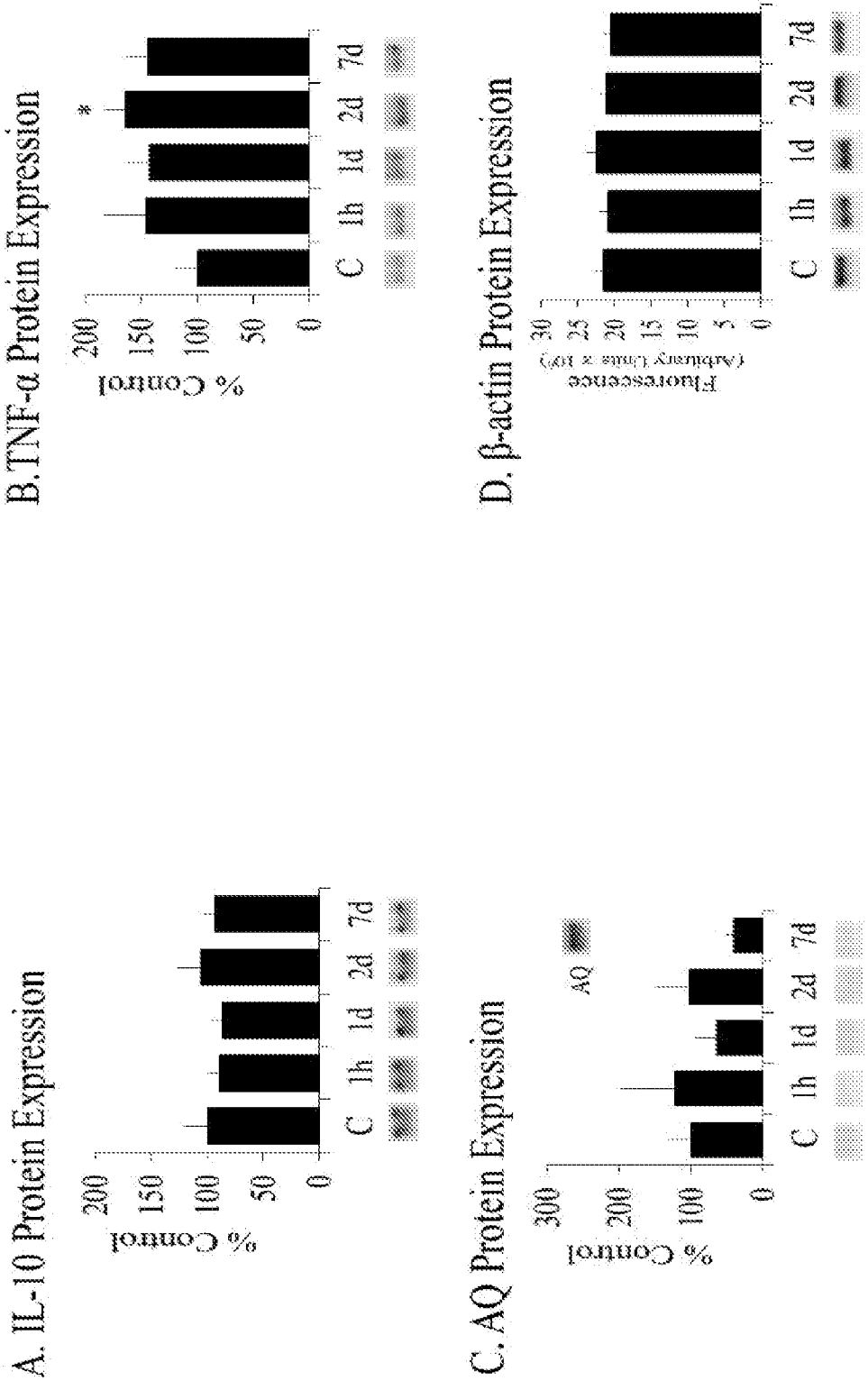

A. IL-10 Protein Expression
% Control
200
150
100
50
0
C 1h 1d 2d 7d
B. TNF-α Protein Expression
% Control
200
150
100
50
0
*
C 1h 1d 2d 7d
C. AQ Protein Expression
% Control
300
200
100
0
AQ
C 1h 1d 2d 7d
D. β-actin Protein Expression
Fluorescence
(Arbitrary Units x 10³)
30
25
20
15
10
5
0
C 1h 1d 2d 7d

FIG. 14

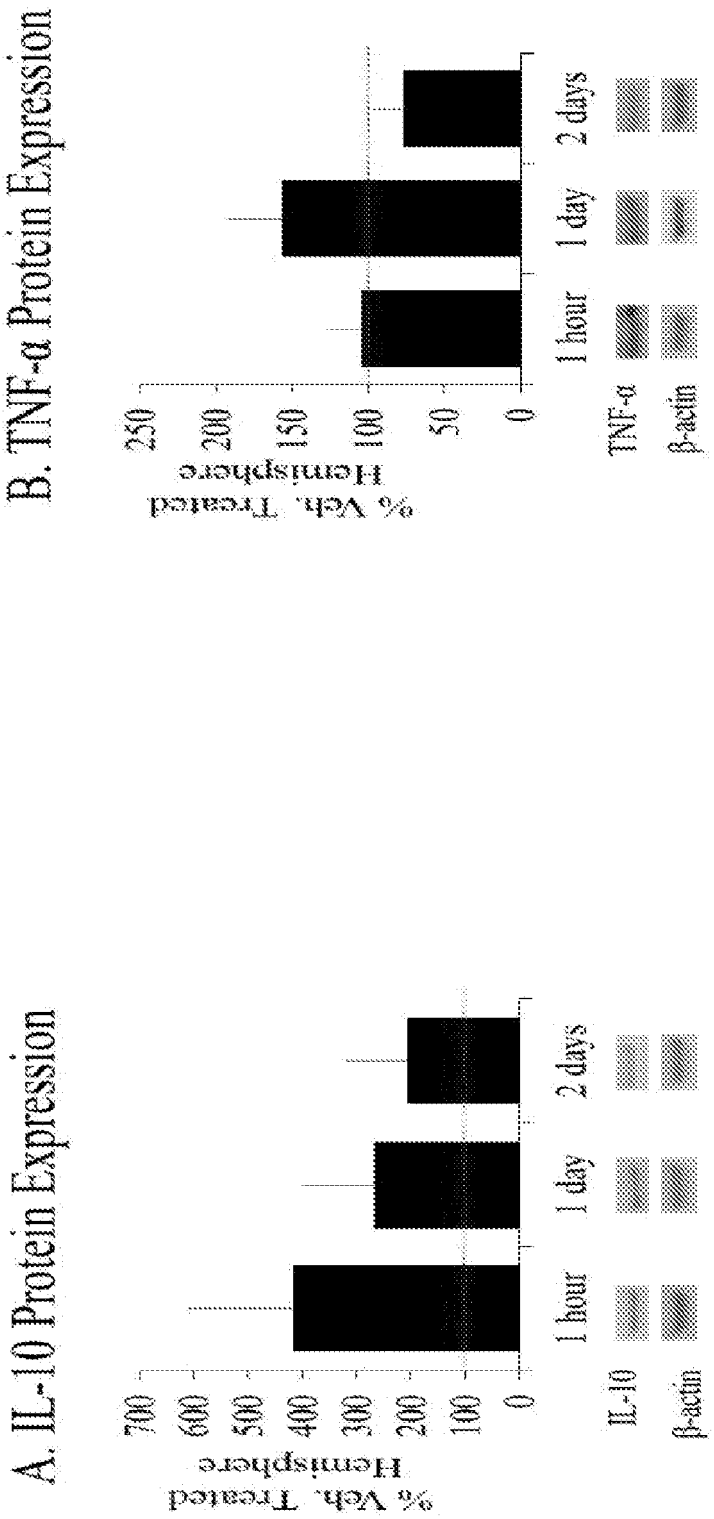

B. TNF-α Protein Expression
% Veh. Treated Hemisphere
250
200
150
100
50
0
1 hour
1 day
2 days
TNF-α
β-actin
A. IL-10 Protein Expression
% Veh. Treated Hemisphere
700
600
500
400
300
200
100
0
1 hour
1 day
2 days
IL-10
β-actin

FIG. 15

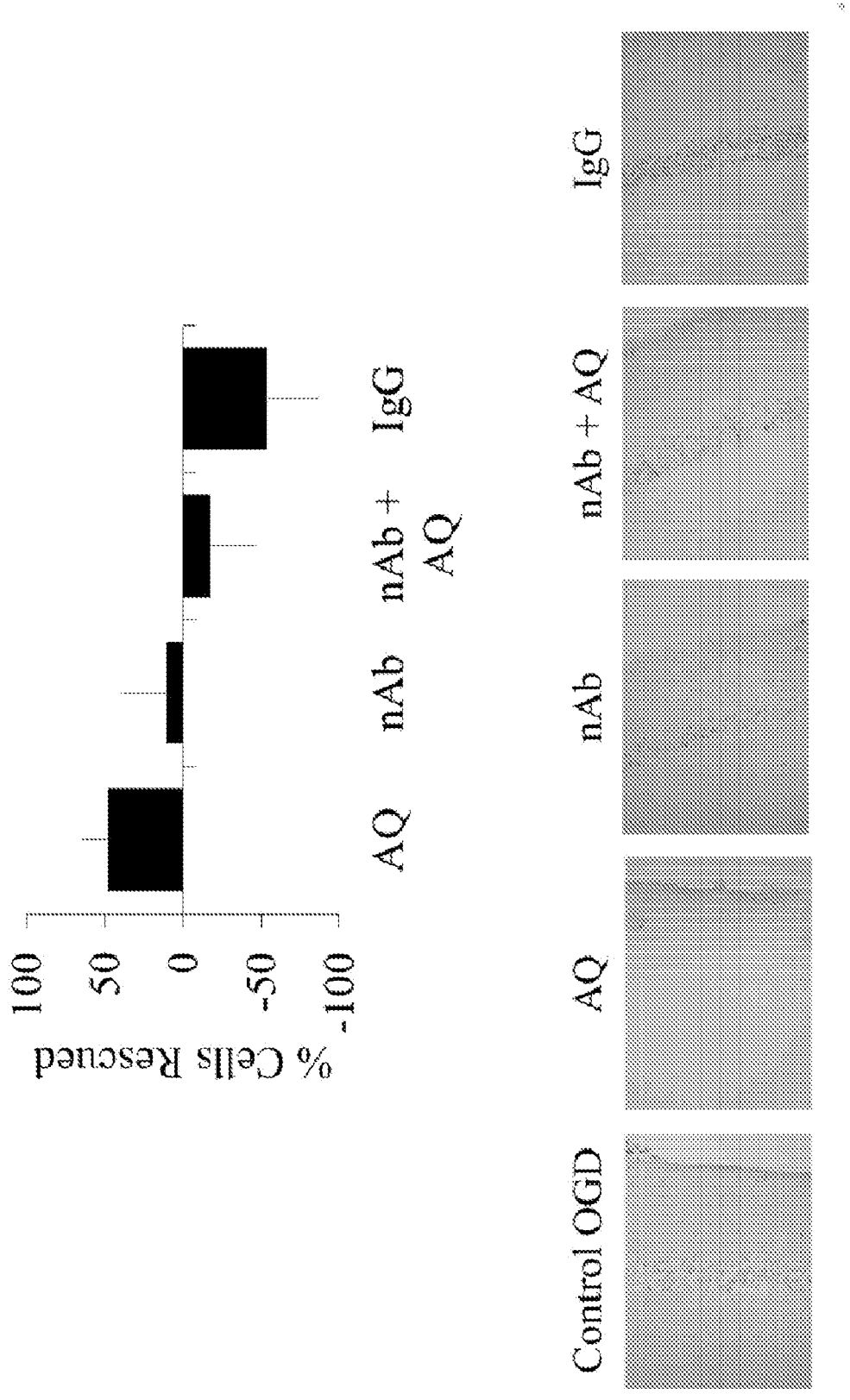

% Cells Rescued
100
50
0
-50
-100
AQ
nAb
nAb + AQ
IgG
Control OGD
AQ
nAb
nAb + AQ
IgG

FIG. 16