## Supplementary Material

## Supplementary table S1 : Biological parameters and clinical outcome of animals used in the experiments

**A) Early effects of chronic hyponatremia correction**

| | Control (Group 0) | Hypertonic NaCl 12 hrs (Group 1) | |
|---|---|---|---|
| | Before | Before treatment | 12 hrs after treatment |
| **Serum Na** | 115 ± 5 | 104 ±5 | 134 ± 7 |
| **Serum Urea** | 25 ± 5 | 32 ± 5 | 53 ± 6 |
| **Na increment** | NA | NA | 30 ±5 |

**B) Early effect of acute hyponatremia correction**

| | Control (Group 0) | Hypertonic NaCl 12 hrs (Group 2) | |
|---|---|---|---|
| | Before Treatment | Before treatment | 12 hrs after treatment |
| **Serum Na** | 115 ± 5 | 119 ± 2 | 145 ± 3 |
| **Serum Urea** | 25 ± 5 | 31 ± 9 | 44 ± 7 |
| **Na increment** | NA | NA | 26 ± 3 |

**C) Effect of chronic hyponatremia correction with NaCl versus Urea**

| | Hypertonic NaCl 24 hrs (Group 3) | | Urea 24 hrs (Group 4) | |
|---|---|---|---|---|
| | Before treatment | 24 hrs after treatment | Before treatment | 24 hrs after treatment |
| **Serum Na** | 112 ± 4 | 140 ± 8 | 113 ± 4 | 146 ± 8 |
| **Serum Urea** | 30 ± 6 | 45 ± 10 | 22 ± 3 | 192 ± 107 |
| **Na increment** | NA | 29 ± 4 | NA | 32 ± 6 |

**D) Effect of correction of hyponatremia on mortality**

| | Acute Hyponatremia | | Chronic Hyponatremia | | | |
|---|---|---|---|---|---|---|
| | Hypertonic NaCl 24 hrs Na | | Hypertonic NaCl 24 hrs | | Urea 24 hrs | |
| | Before treatment | 24 hrs after treatment | Before treatment | 24hrs after treatment | Before treatment | 24 hrs after treatment |
| **Serum Na** | 114 ± 6 | 144 ± 2 | 110 ± 5 | 138 ± 8 | 113 ± 6 | 141 ±6 |
| **Serum Urea** | 26 ± 3 | 37 ± 7 | 26 ± 4 | 40 ± 11 | 22 ± 4 | 104 ± 41 |
| **Na increment** | NA | 30 ± 7 | NA | 27 ± 7 | NA | 28 ± 5 |
| **Mortality day 5** | NA | 0/5 | NA | 3/5* | NA | 0/5 |

Na in mMol/L. Urea in mg/dl. Data are expressed in mean ± standard deviation. * 2 animals were moribund and were euthanized on day 3.

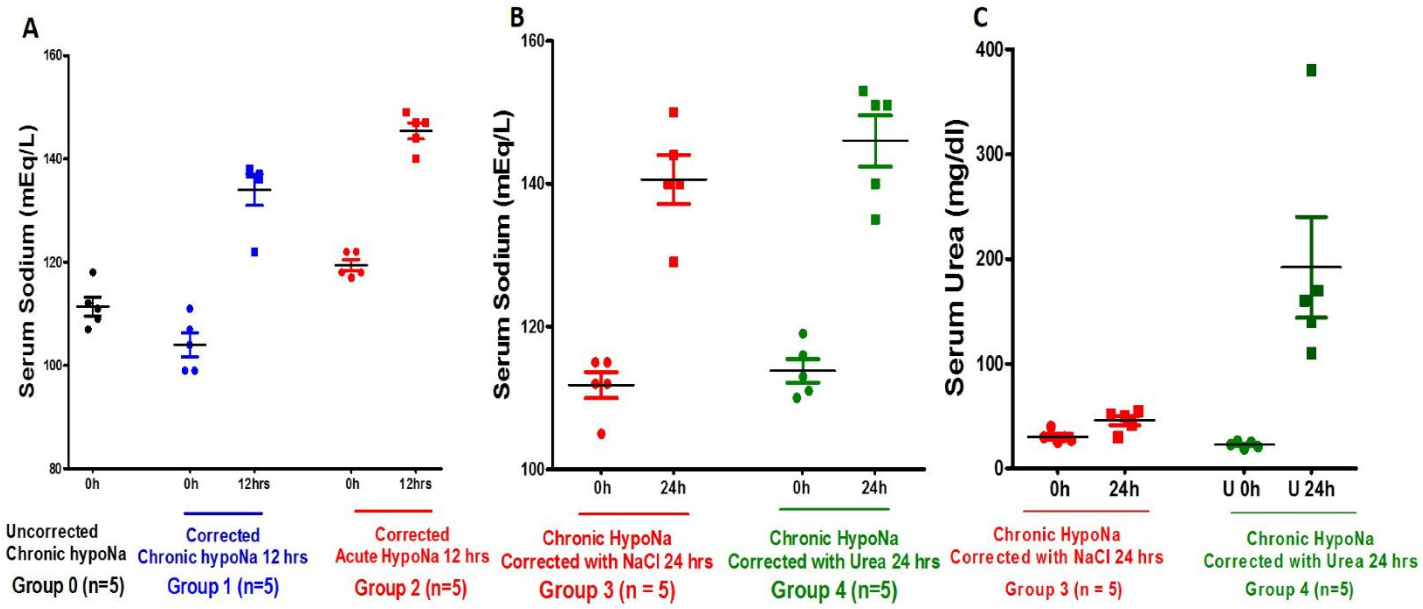
**Suplementary Table 2: List of antibodies used for immunohistochemistry (IHC), Immunofluorescence (IF) and Western blot (WB)**

| | | | | Dilution | |
|---|---|---|---|---|---|
| **Primary Antibody** | **Species** | **Catalog Number** | **Manufacturer** | **IHC-IF** | **WB** |
| BiP | Mouse | sc376768 | SCBT | NA | 1/1000 |
| Calreticulin | Rabbit | HPA002242 | Sigma Aldrich | NA | 1/5000 |
| PDI | Rabbit | NB100-1921 | Novus Biological | NA | 1/5000 |
| pEIF2a | Rabbit | 07-760 | Millipore | NA | 1/1000 |
| pEIF2a | Goat | sc12412 | SCBT | 1/100 | NA |
| pEIF2a | Rabbit | ab32157 | Abcam | 1/50 | NA |
| CHOP | Mouse | PCRP-DDIT3-1A10-s | DSHB | 1/20 -1/50 | NA |
| CHOP | Rabbit | sc575 | SCBT | NA | 1/500 |
| pH2AX | Mouse | Cat 07164 | Merck-Millipore | 1/200 | NA |
| BAX | Rabbit | Poly6251 | BioLegend | NA | 1/1000 |
| BIM | Rabbit | 559685 | BD Biosciences | NA | 1/500 |
| BCL-2 | Mouse | 610538 | BD Biosciences | NA | 1/1000 |
| BCL-XL | Rabbit | 2H12 | BioLegend | NA | 1/500 |
| p62 SQTM1 | Rabbit | H00008878-M01 | Novus Biological | 1/200 -1/500 | |
| p62 SQTM1 | Mouse | NBP1-48320SS | Novus Biological | NA | 1/7500 |
| LC3 | Rabbit | NB600-1384SS | Novus Biological | NA | 1/7500 |
| ATG5-12 | Rabbit | NB110-53818S | Novus Biological | NA | 1/5000 |
| ALDH1L1 | Rabbit | ALDH1L1 | Encor | 1/200 | NA |
| GFAP | Rabbit | ab7260 | Abcam | 1/1000 | NA |
| GFAP clone GA5 | Mouse | G3893 | Sigma Aldrich | 1/500 | NA |
| Glutamine Synthase | goat | SC32557 | SCBT | 1/500 | NA |
| NeuN clone A60 | Mouse | MAB377 | Millipore | 1/1000 | NA |
| Olig 2 | Mouse | MABN50 | Millipore | 1/500 | NA |
| Olig 2 | Rabbit | NBP1-28667 | Novus Biological | 1/200 | NA |
| Ubiquitin Clone FK2 | Mouse | BML-PWL8810 | Enzo Lifesciences | 1/75 | 1/500 |
| ATF6 alpha | Rabbit | sc22799 | SCBT | 1/200 | NA |
| ATF4 | Rabbit | sc200 | SCBT | 1/200 | NA |
| XBP1 | Rabbit | sc7160 | SCBT | NA | 1/500 |

| **Secondary Antibody** | **Species** | **Catalog Number** | **Manufacturer** | **IHC -IF** | **WB** |
|---|---|---|---|---|---|
| Anti Mouse HRP | Donkey | 16017 | Pierce | 1/500 | 1/20000 |
| Anti Rabbit HRP | Donkey | 16035 | Pierce | 1/250 | 1/20000 |
| Anti Goat HRP | Donkey | sc2020 | SCBT | 1/100 | 1/7500 |
| Anti Mouse biotin | Horse | BA2000 | Vector Labs | 1/100 | |
| Anti Rabbit biotin | Goat | BA1000 | Vector Labs | 1/300 | |
| Anti goat biotin | Donkey | sc2042 | SCBT | 1/200 | |
| Apoptag Kit | NA | S7100 | Millipore | Per instruction | |
| Proteostat Kit | NA | Enz51035 | Enzo | Per instruction | |
| ABC kit | NA | PK6100 | Vector Labs | 1 | |
| FITC -streptavidin | NA | 21224 | Pierce | 1/200 | |
| Dylight 594 -Neutravidin | NA | 22842 | Pierce | 1/200 | |
| FITC - tyramide | NA | | Home Made Kit | 1/200 | |
| Alexa 594 - Tyramide | NA | | Home Made Kit | 1/200 | |
| DAPI | NA | D9542 | Sigma | 1/100000 | |

# Supplementary figures and legends for Supplementary Figures

**Figure S1. Biological data from the animals studied in each group**

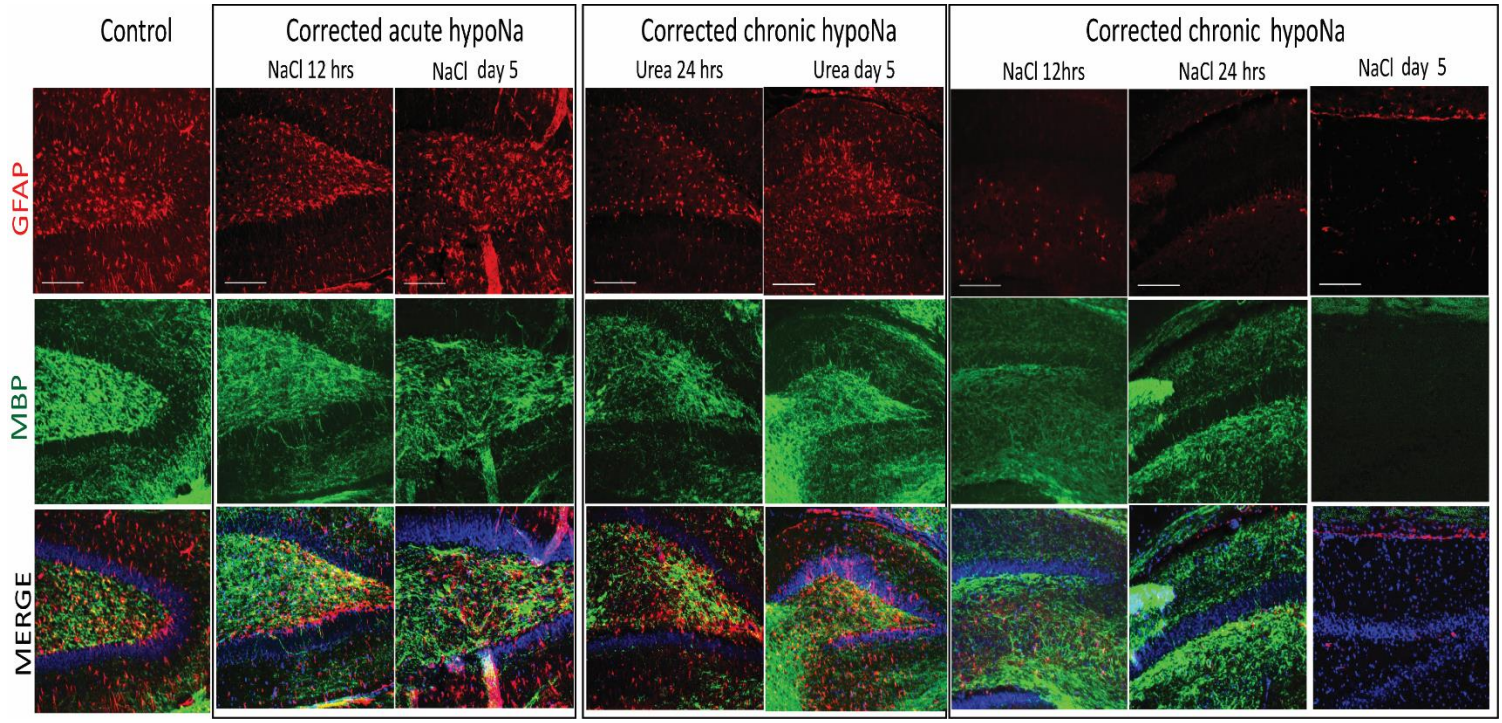



.

Graph A shows the serum sodium value in uncorrected chronic hyponatremic controls animals (Group 0) compared to animals that received hypertonic saline after 4 days - chronic - (Group 1) or 18 hours - acute - (Group 2) of hyponatremia and that were killed at 12 hours after injection of hypertonic saline. There is a brisk increase in the serum sodium value after administration of hypertonic saline ($p<0.001$ by paired t-test for serum sodium at 0hr vs 12 hrs in Group 1 and 2).

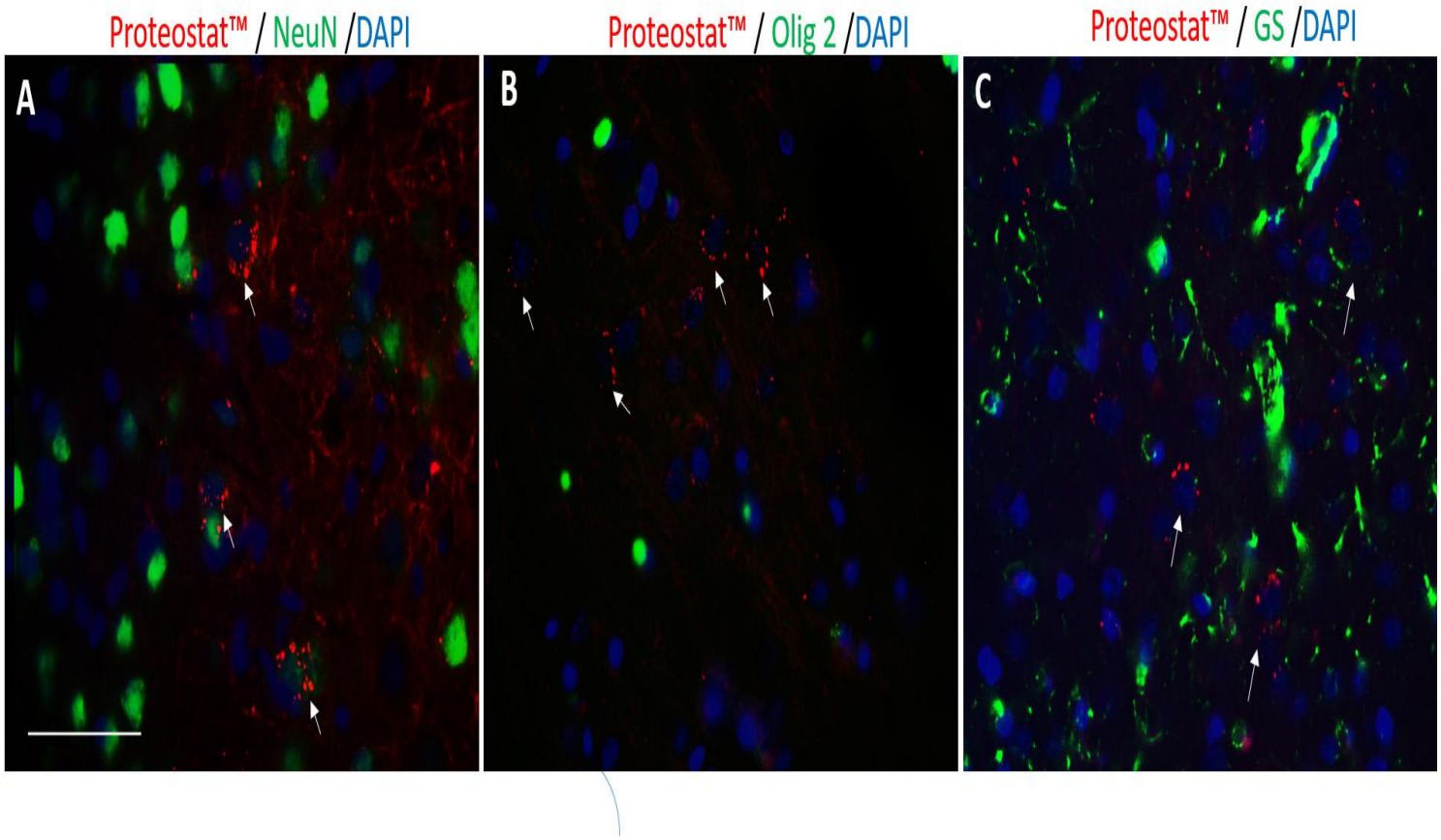
Graph B shows serum sodium before and 24 hrs after initiation of correction of chronic hyponatremia either with hypertonic saline (Group 3) or with urea (Group 4). Both groups had comparable degree of hyponatremia and sustained rapid correction of serum sodium correction ($p<0.001$ by paired t-test for serum sodium at 0hr vs 24 hrs in Group 3 and 4) with comparable final 24 hrs sodium increment. Graph C shows that animals corrected with urea had a higher level of blood urea compared to animals corrected with hypertonic saline (** $p< 0.001$ by unpaired t test for serum urea in group 3 versus group 4). HypoNa = hyponatremia

**Figure S2. Myelin (MBP in green) and astrocyte (GFAP in red) staining at different timepoint in the brain of animals in all the studied groups.**



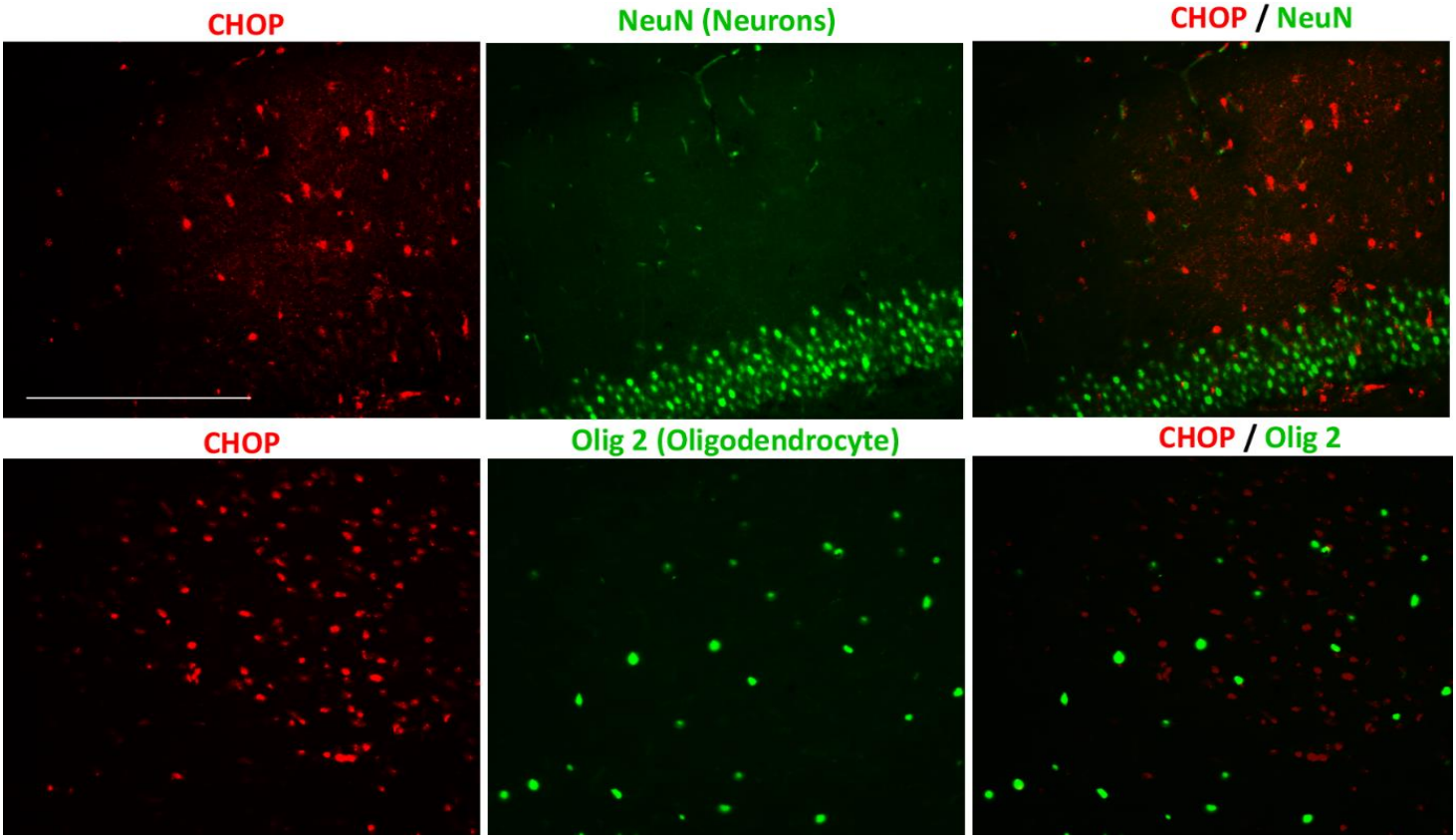

Astrocyte (GFAP in red) and myelin (MBP In green) were stained in the hippocampus of animals in the different groups at various time after the correction of hyponatremia. In control, (first column) there is no loss of astrocyte and myelin. Animals with acute hyponatremia (column 2 and 3) also displayed no loss of astrocyte or myelin when analyzed either 12 hrs or 5 days after acute hyponatremia was corrected. Correction of acute hyponatremia with urea did not resulted in astrocyte death or demyelination either 24 hrs or 5 days after the treatment was started. On the other hand, correction of hyponatremia with hypertonic saline (last 3 column) resulted in early astrocyte death (12 hrs and 24 hrs after the treatment) along with delayed myelin loss.

**Figure S3. Cellular distribution of protein aggregates**



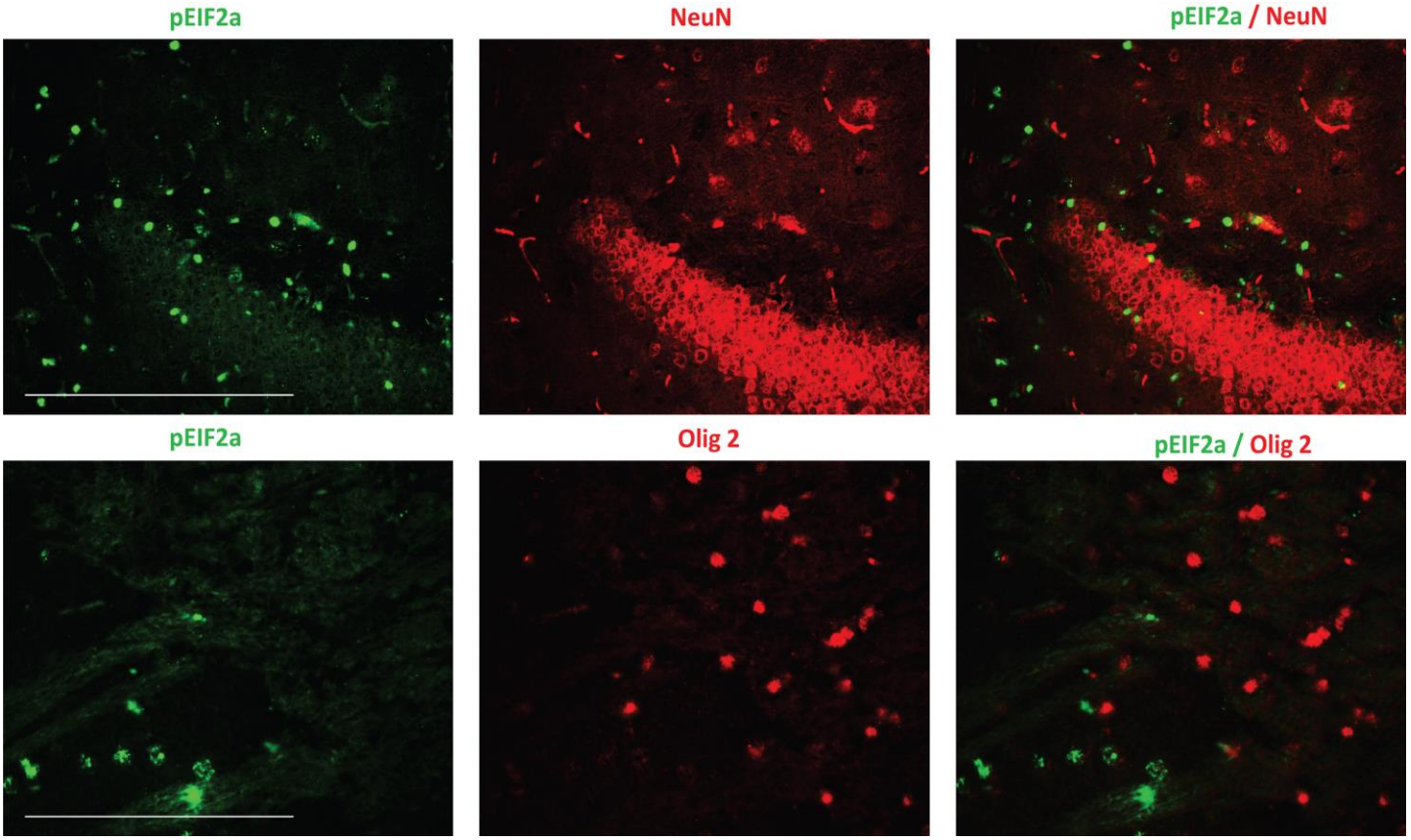

A shows that protein aggregates stained with proteostat in red are not present in neurons (NeuN positive stain). In B oligodendrocytes stained with Olig2 marker (in green) do no show positive co-localization with proteostat (in red). C: few cells expressing beaded glutamine synthase positive staining (white arrow) insoluble aggregates display perinuclear staining. Scale bar is set at 50 microns.

**Figure S4. CHOP is not expressed in neurons or oligodendrocytes in ODS**



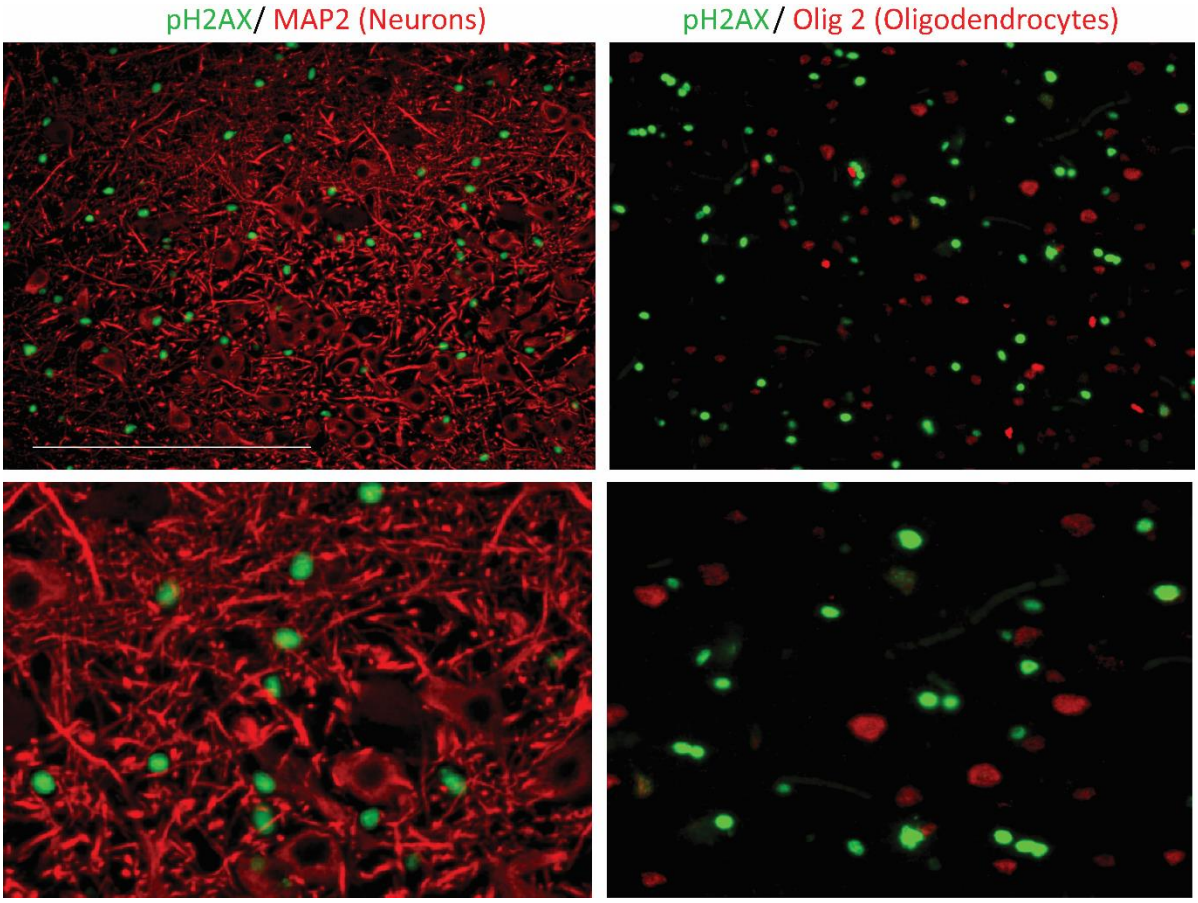

Representatives images of co-immunofluorescences for CHOP (DDIT3/GADD153) in red and neurons (NeuN) or oligodendrocytes (Olig2) both in green showing that upon rapid correction of chronic hyponatremia there no neurons or oligodendrocytes expressing the ER stress marker CHOP. Scale bar us set at 200 microns.

**Figure S5. pEIF2a is not expressed in neurons or oligodendrocytes after rapid correction of chronic hyponatremia.**



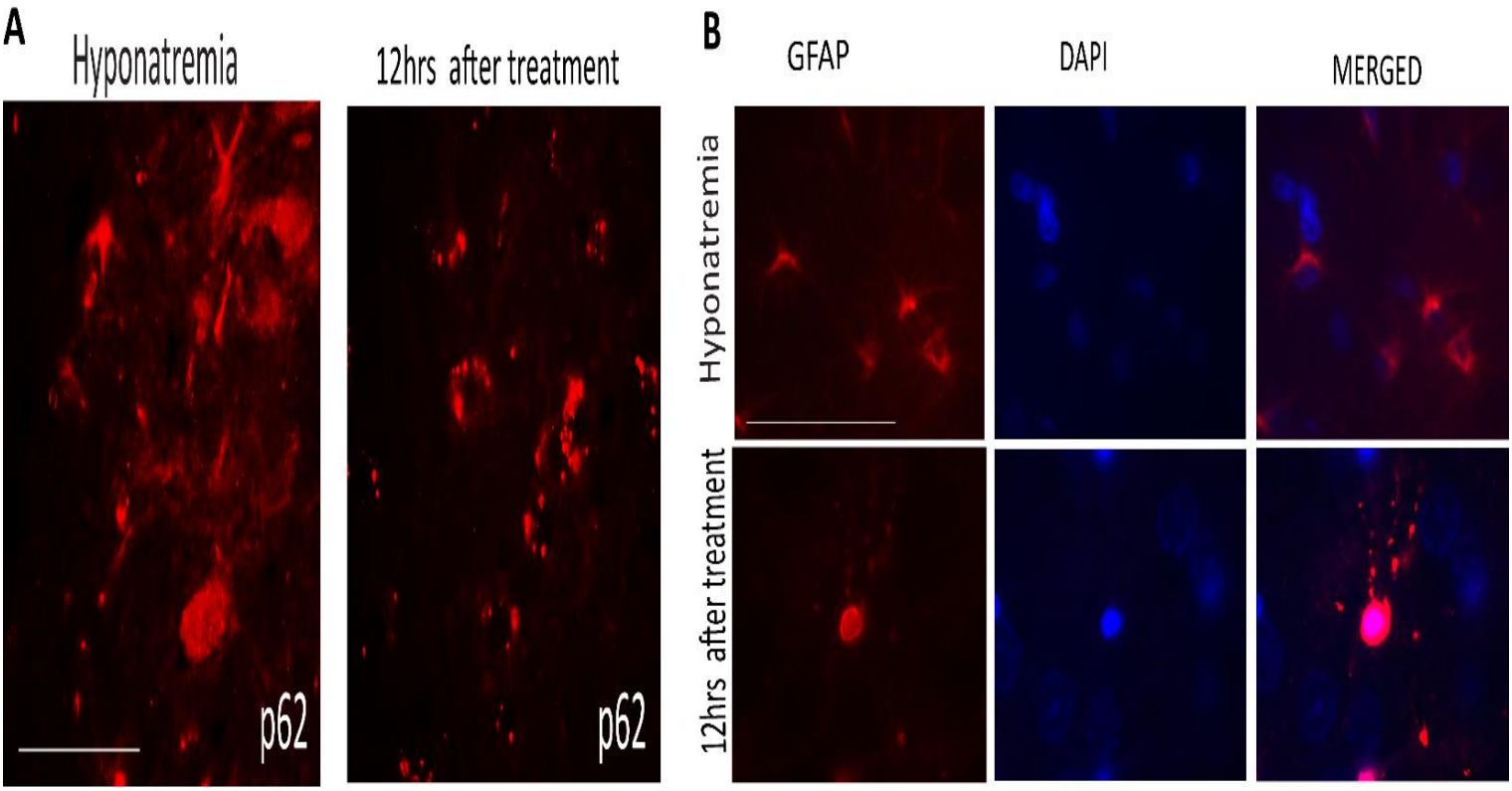

Double immunofluorescence for phosphorylated EIF2a (pEIF2a) in green and neurons (NeuN) or oligodendrocytes (Olig2) markers (both in red) showing that pEIF2a does not co-localizes with neurons or oligodendrocytes after rapid correction of chronic hyponatremia. Scale bar is set at 200 microns.

**Figure S6. DNA damage marker phosphorylated H2AX ( pH2AX or ɣH2AX) is not expressed in neurons or oligodendrocytes after rapid correction of chronic hyponatremia.**




Double staining for pH2AX (in green ) and neuron marker MAP 2 or oligodendrocyte marker OLIG 2 both in red showing no expression of pH2AX in neurons or oligodendrocytes 12 hours after the correction of chronic hyponatremia. Scale bar is set at 200 microns. Higher magnification is shown in the lower panel (not at scale).

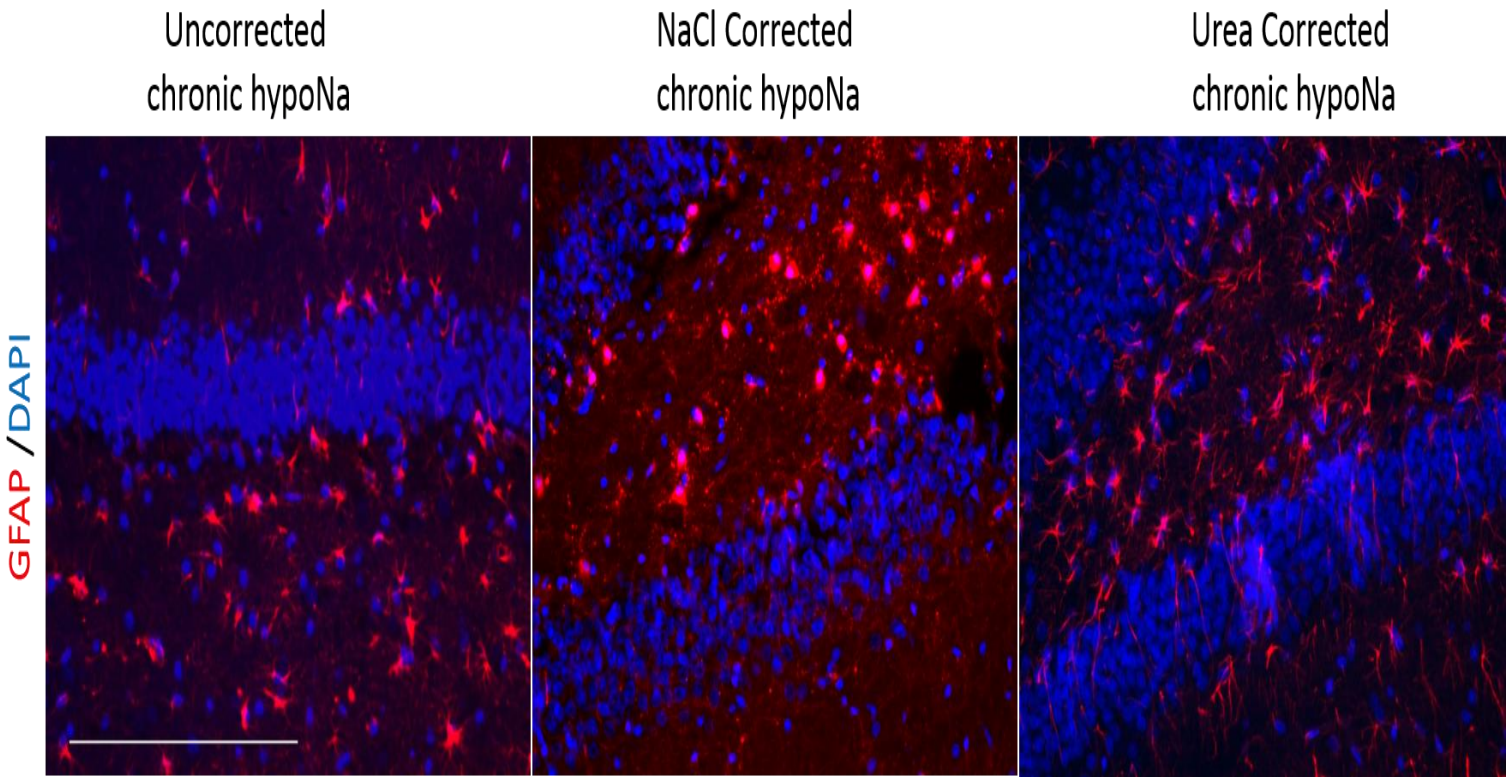
**Figure S7: Rapid correction of chronic hyponatremia induces p62 perinuclear accumulation and clasmatodendrosis**




In A: p62 staining is seen as punctate perinuclear aggregates in the brain of animals with chronic hyponatremia, corrected with hypertonic saline while only homogenous staining is present in control animals. Scale bar at 50 microns

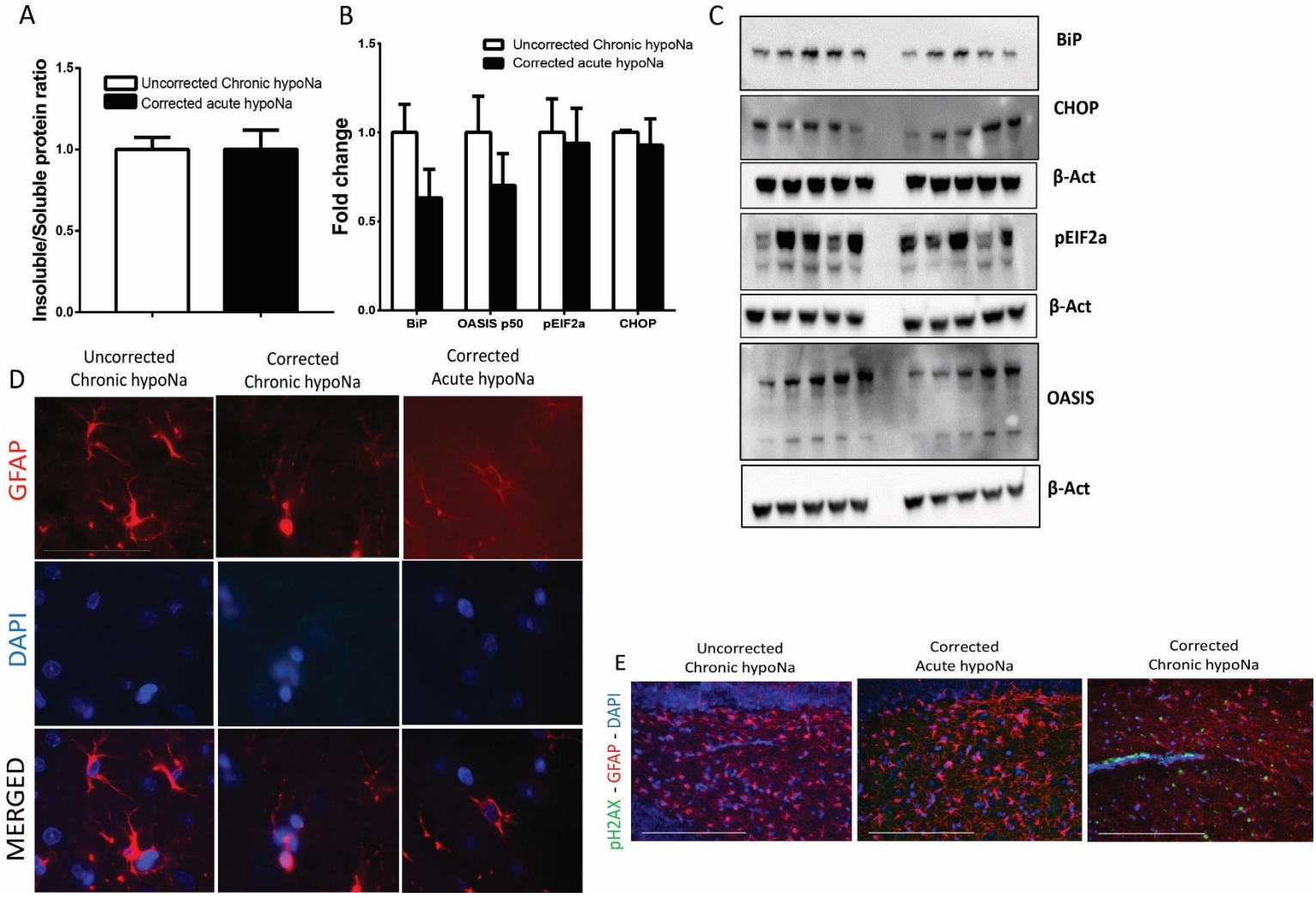
In B: top panel shows GFAP in red and nuclear DAPI in blue in hyponatremic controls animals. GFAP straining shows a very fine and regular staining of the astrocytic foot processes and nuclear staining reveal no nuclear condensation. In contrast 12 hrs after the correction of serum sodium (bottom panel) GFAP staining appear as perinuclear dense deposit with granular and beaded foot processes (typical feature of clasmatodendrosis or astrocytic autophagic cell death). Note that the nucleus of the clamatodendrocytic astrocytes appear very dense. Scale bar is at 50 microns

**Figure S8: Rapid correction of chronic hyponatremia with urea does not induces astrocyte clasmatodendrosis**




Brain of the uncorrected animals (left panel) or animals treated with urea (far right panel) did not display signs of astrocyte beading (clasmatodendrosis) in contrast to the astrocyte seen in brain of animals treated with hypertonic saline (midle image where beading of astrocyte is evident). Scale bar is set at 200 microns.

**Figure S9: Rapid correction of acute hyponatremia does not induce insoluble protein accumulation, UPR /ER stress or cell death**




In A, quantification of the ratio of insoluble to soluble proteins in animals after correction of acute hyponatremia compared to chronic hyponatremic controls shows that rapid correction of acute hyponatremia does not induces insoluble protein accumulation.

In B and C, western blot for key marker of UPR/ER stress reveals that correcting acute hyponatremia is not accompanied by significant changes in expression of pEIFa and CHOP (p= 0.71 for CHOP and 0.8 for pEIF2a). A non significant reduction in the protein amount of BiP and OASIS is seen after correction of acute hyponatremia compared to chronic hyponatremic controls (p=0.14 for BiP and 0.39 for OASIS by unpaired t-test n=5 for each group).

In D, representative high magnification images of GFAP staining after uncorrected chronic hyponatremia, rapidly corrected acute hyponatremia and rapidly corrected chronic hyponatremia. Beading of astrocytes processes with nuclear densification (clasmatodendrosis) only upon correction of chronic hyponatremia and is not seen with correction of acute hyponatremia or in uncorrected chronic hyponatremic controls animals.

In E, no staining for the DNA damage marker pH2AX is seen in animals when acute hyponatremia is corrected compared to when chronic hyponatremia is rapidly corrected