Supplementary Experimental Procedures

MTT assay for cell viability

Cell viability was assayed by mitochondrial reduction of MTT (1-(4,5-Dimethylthiazol-2-yl)-3,5-diphenylformazan) (Sigma, St Louis, MO, USA) to blue formazan product [1]. Absorbance at 500 nm in cultures at time zero was used as a 100 % reference for treated cultures.

Immunoprecipitation

Immunoprecipitates were prepared from cerebellar granule neuron extracts using polyclonal antibody against SP4 (Santa Cruz Technology, Santa Cruz, CA, USA). Immunoprecipitated material was analyzed by immunoblotting with a monoclonal antibody against ubiquitinylated proteins (BioMol International, Plymouth Meeting, PA, USA) or polyclonal antibody against SP4.

[1] Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 1983 1983/12/16; **65**(1-2)**:** 55-63.

Supplementary Figures

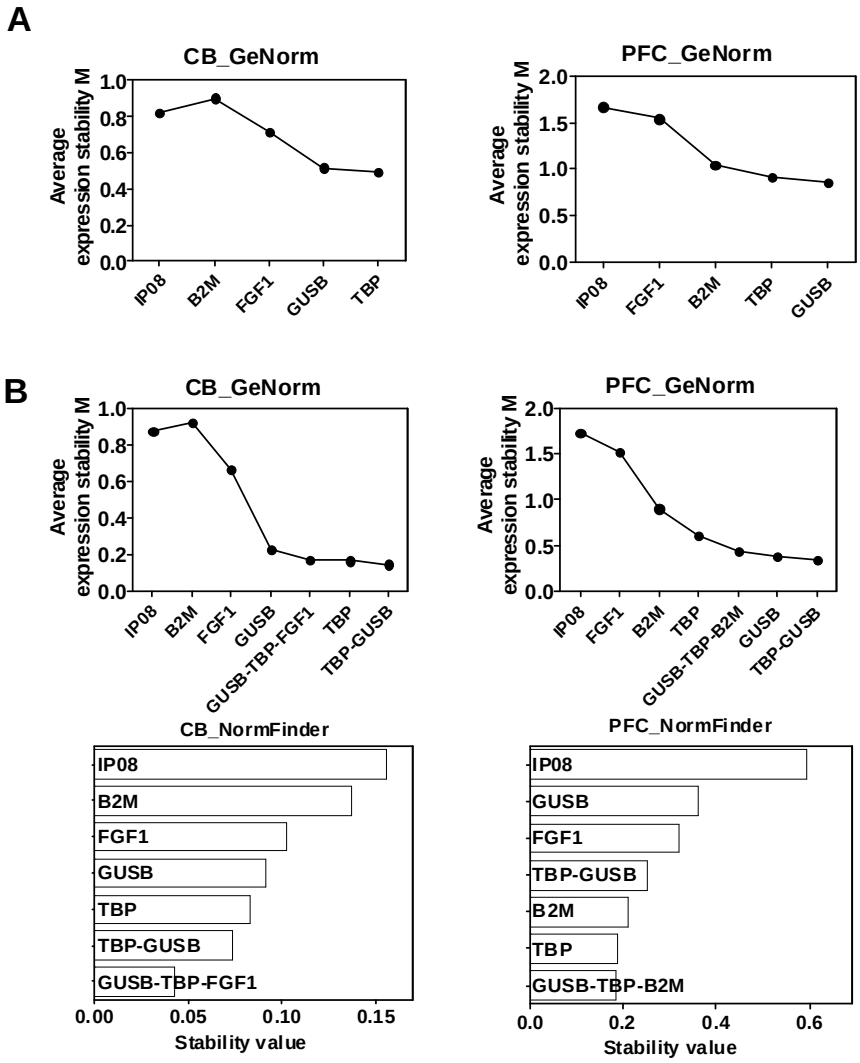

Figure S1.- Reference genes stability analysis. A total of five reference genes: importin 8 (*IP08*), fibroblast growth factor 1 (*FGF1*), TATA-binding protein (*TBP*), beta glucuronidase (*GUSB*) and Beta-2-microglobulin (*B2M*) were determined by RT-qPCR in postmortem brain samples from healthy individuals (Control, C, n= 10), and individuals with bipolar disease (Bipolar, BP, n=10). $2^{-\Delta CT}$ for all of them was calculated by subtracting to a reference healthy control sample. Then, stability analysis of the five selected genes was performed with GeNorm **(A)**. A geometrical mean of the three most stable genes *GUSB*, *TBP* and *FGF1,* in cerebellum (left panels); and *GUSB*, *TBP* and *B2M* in prefrontal cortex (right panels), was calculated, as well as the geometrical mean

of the 2 most stable genes, TBP and GUSB for both areas. Then, the means were compared to all the genes in order to select the most stable normalization factor **(B)** by performing both GeNorm (top) and NormFinder (bottom). The geometrical mean of three genes was selected as the normalization factor for our samples. Smaller value under these analyses represents a more stable gene expression of the tested gene.

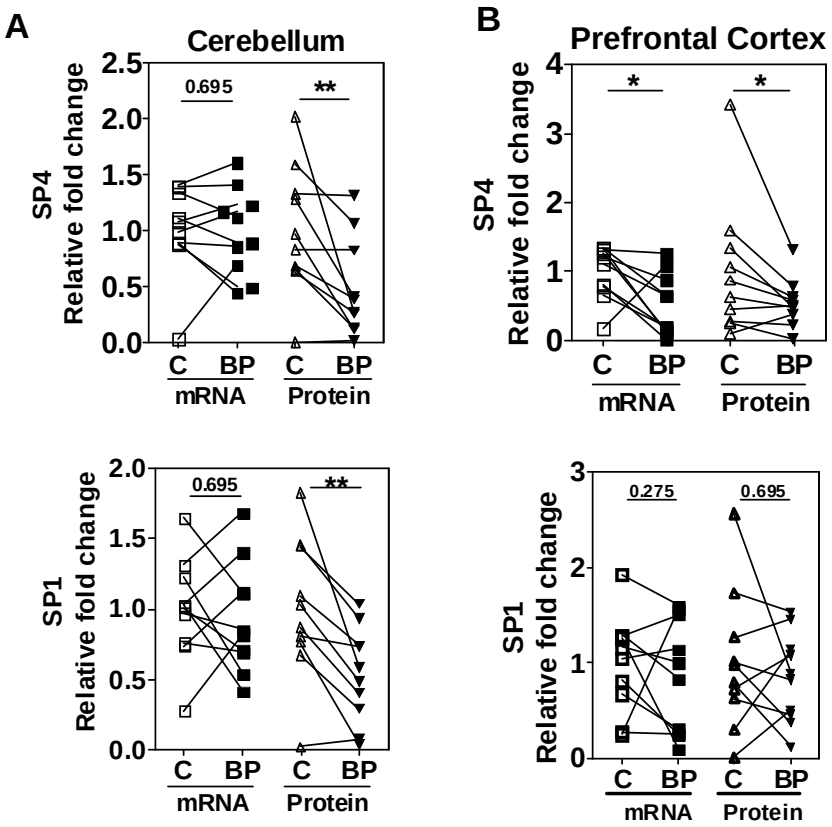

Figure S2.- SP4 and SP1 protein levels are reduced in postmortem cerebellum and also SP4 mRNA and protein levels in postmortem prefrontal cortex from bipolar disorder subjects. Protein and mRNA levels for SP4 (top) and SP1 (bottom) in cerebellum **(A)** and prefrontal cortex **(B)** are shown. Protein extracts from brain postmortem tissue of healthy individuals (Control, C, n= 10), and individuals with bipolar disease (Bipolar, BP, n=10) were immunoblotted for SP4, SP1 and GAPDH. The resultant bands were quantified by densitometry. SP4 and SP1 were normalized to GAPDH values and referred to a standard sample (healthy subject). Each value represents mean of two independent analyses. mRNA levels for *SP4* and *SP1* from the same subject samples were determined by RT-qPCR referred to a reference healthy control sample and normalized to the geometric mean of three reference genes: *GUSB*, *TBP* and *FGF1,* in cerebellum; and *GUSB*, *TBP* and *B2M* in prefrontal cortex. Each value represents mean of at least three independent analyses performed in duplicate. Statistical analysis was performed using Wilcoxon signed rank test for paired values ($p<0.01$-**, $p<0.05$-*).

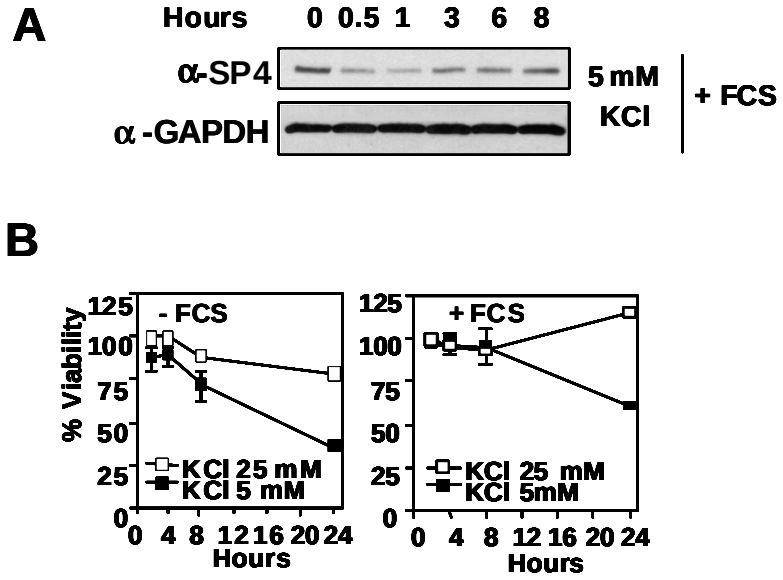

Figure S3.- SP4 protein stability is regulated by depolarization. Cerebellar granule neurons obtained from P6 rat pups and maintained in culture for 7 days were incubated for the indicated times in medium containing 25 or 5 mM of KCl in the presence of serum (A) or in the presence (B, left panel) and absence of serum (B, right panel) as described in experimental procedures. Cells were lysed and protein extracts were immunoblotted with antibody against SP4 or GAPDH (A) or cell viability (B) was determined by MTT reduction and normalized to the absorbance at time zero and performed in triplicate. Values represent mean ± standard deviation.

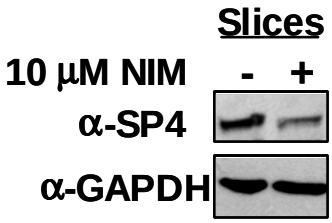

Figure S4.- Inhibition of calcium influx in cerebellar organotypic cultures decreased SP4 protein levels. Cerebellar slices from P9 rat pups were maintained in vitro for 3 days and then treated with 10μM nimodipine (NIM) for 24h. Protein extracts were resolved by SDS/electrophoresis and probed with a polyclonal antibody against SP4 or GAPDH as indicated.

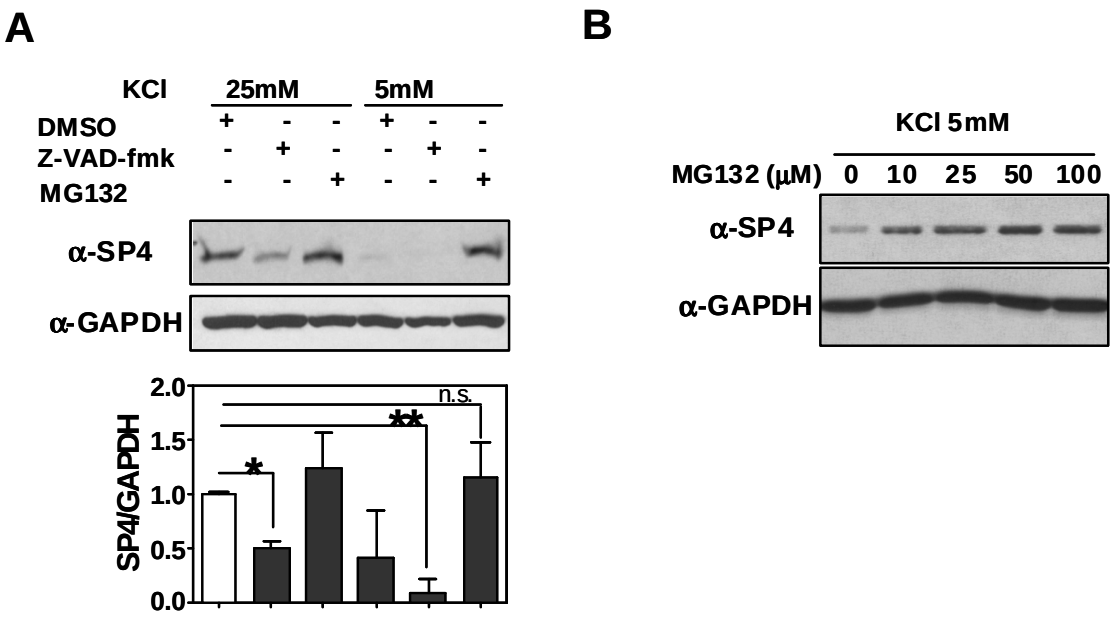

Figure S5.- SP4 levels are restored upon proteasome inhibition. (A) Cerebellar granule neurons were exposed to control vehicle (DMSO), 100 μM MG-132 or z-VAD-fmk 80 μM 1 hour before and after changing into a culture medium with 25 mM or 5 mM KCl in the presence of serum as indicated. **(B)** Cerebellar granule neurons were switched to medium containing 5 mM of KCl and exposed to the indicated concentration of MG132. After 1 hour of incubation, cells were lysed and protein extracts were immunoblotted with antibody against SP4 or GAPDH. Values represent mean ± standard deviation of two independent experiments. Statistical analysis was performed using two tailed t-test ($p<0.05$- *, $p<0.01$- **, n.s., not significant).

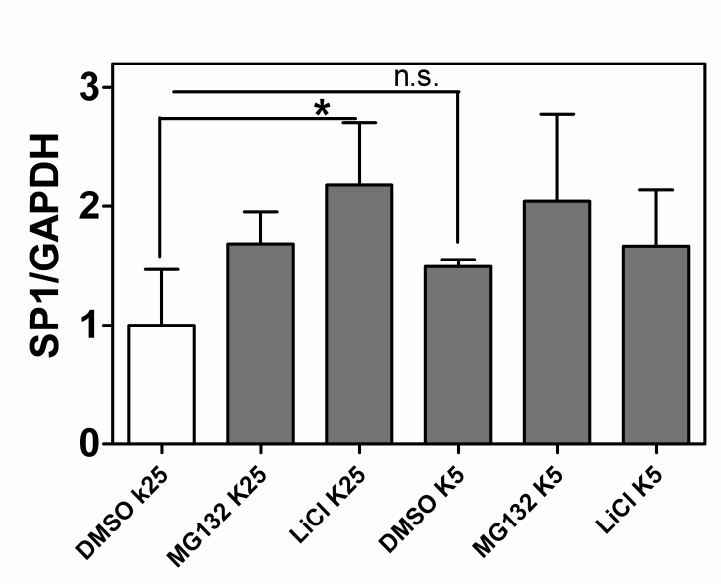

Figure S6.- Lithium increases SP1 protein levels in depolarizing conditions. Cerebellar granule neurons maintained 7 days in vitro were incubated with 100μM MG132, 5mM LiCl, or DMSO before and after switching cell medium to 25mM or 5mM KCl with serum for 1 hour. Immunoblot analysis of cellular extracts was performed with antibodies against SP1 and GAPDH (Figure 4). SP1 levels were analyzed by densitometry and normalized to GAPDH levels. Values represent mean ± standard deviation of three independent experiments. Statistical analysis was performed using two tailed t-test ($p<0.05$- *, n.s., not significant).

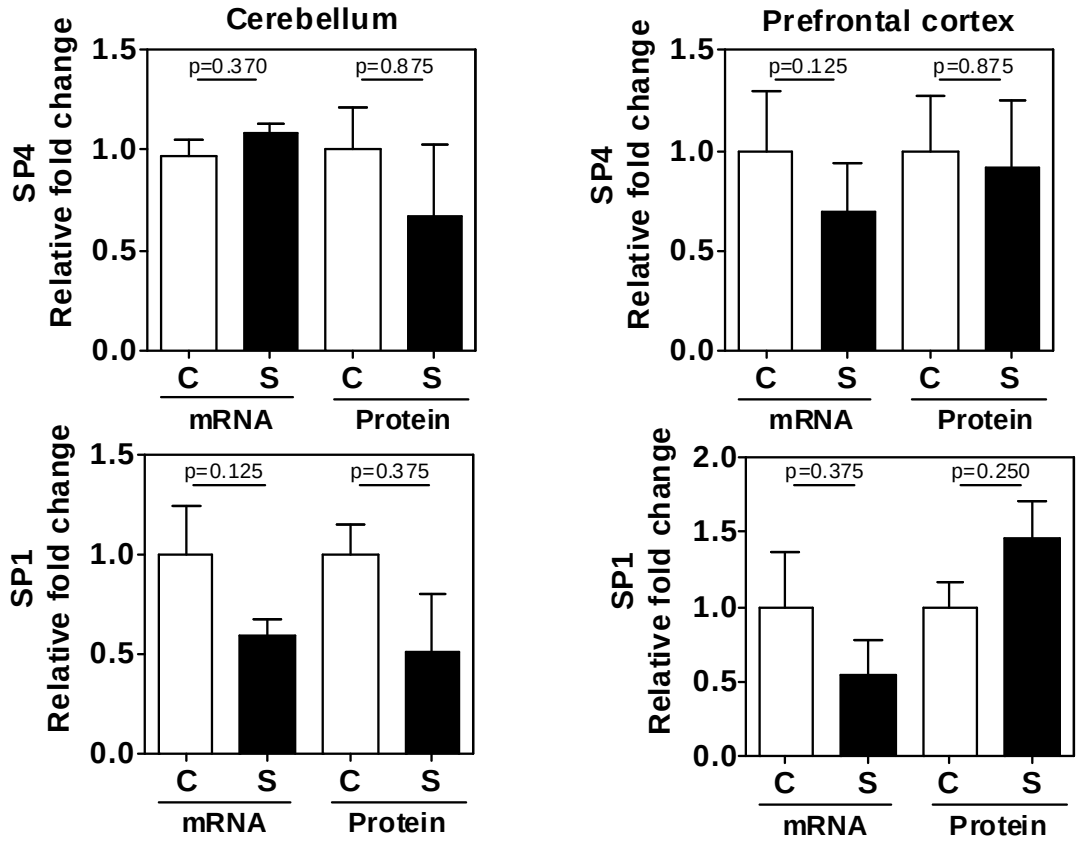

Figure S7.- SP1 and SP4 levels are not altered in cerebellum or prefrontal cortex from suicide subjects compared to controls from the Brain Tissue Collection of the University of Basque Country. Protein and mRNA levels for SP4 and SP1 in cerebellum **(A)** and prefrontal cortex **(B)** are shown. Protein extracts from postmortem cerebellum (CB) and prefrontal cortex (PFC) of control subjects (C, n=4, age [years] 41 ± 12, postmortem delay [hours] 13 ± 8; pH 6.67 ±0.47, RIN 8 ± 0.56 (CB) and 7.67 ±0.32 (PFC); mean ±SD) and matching subjects that committed suicide (S, n=4 age [years] 42 ± 13, postmortem delay [hours] 24 ± 7, pH 6.72 ±0.29, RIN 7.82 ± 0.64 (CB) and 5.67 ± 1.61 (PFC); mean ±SD) were immunoblotted for SP4, SP1 and GAPDH. The resultant bands were quantified by densitometry; SP4 and SP1 were normalized to GAPDH values. Values represent mean ± standard error of the mean of two independent analyses. mRNA levels for SP4 and SP1 from the same subject samples were determined by RT-qPCR and normalized to B2-microglobulin (B2M). Graphs represent mean ± standard error of the mean of values from at least three independent analyses performed in

duplicate. Statistical analysis was performed using Wilcoxon signed rank test for paired values, p values are shown for each group.