Supplemental Figures

Supplemental Figure S1.

Western blot analysis on the 20 subcellular fractions obtained from discontinuous sucrose-gradient separation by using anti-CD44, anti-GADPH, anti-GM130, and anti-calnexin antibodies that are plasma membrane, cytosol, Golgi, and ER markers, respectively. Fractions number 14-15 and 11-12 were selected to represent PM and CM, respectively.

Supplemental Figure S2.

Relative HAS activity in PM or CM fractions after different treatments. Eighty percent confluent ECV cells were left untreated (control, white bars) or treated (black bars) with 10 μg/ml dithiothreitol (DTT), 4% ethanol (known to induce ER stress (1-2)) or cultured without serum (no FBS). DTT and ethanol are known to induce ER stress whereas FBS absence causes stress unrelated to ER. After 18 h of incubation, 20 μg of PM or CM fraction proteins were purified and analyzed for HAS activity. $^*P<0.01$ (ANOVA) control *vs*. treatments. Data represent mean ± SEM from two independent experiments of triplicate determinations.

Supplemental Figure S3.

Localization and analysis of CD44 after suramin treatment in ECV or OVCAR-3 cell lines. The cells were seeded on glass coverslips and left untreated or treated with 20 μg/ml. After 24 h of incubation, the cells were fixed with methanol and probed with primary monoclonal anti-CD44 antibody and secondary Cy2.2 conjugated antibodies. Images were taken by using Leica confocal microscope (upper panels). The grey levels across the plasma membranes were quantified by using ImageJ software and a typical result is shown (lower panels). The black lines represent the CD44 quantification in control cells whereas the red lines represent the CD44 quantification in suramin treated cells.

Supplemental Figure S4.

U937 adhesion on ECV or OVCAR-3 cells treated with DTT, ethanol or cultured without serum (no FBS). Eighty percent confluent cells were left untreated (control, white bars) or treated (black bars) with 10 μg/ml final concentration of DTT and 4% ethanol. After 18 h of incubation, $1x10^6$/well fluorescent U937 were added for 60 min. After washing with PBS, adherent U937 were counted under fluorescent microscope, and the number of cells per field is reported. In some experiments, before adding U937, ECV and OVCAR-3 cells were treated with 2U/μl hyaluronidase for 20 min (Hyal, grey bars). $^*P<0.05$ (ANOVA), data represent mean ± SEM from two independent experiments of triplicate determinations

Supplemental Figure S5.

Confocal immunolocalization of HA (*green*) and CD44 (*red*) on untreated or tunicamycin (10 μg/ml final concentration) or tunicamycin+suramin (20 μg/ml) treated OVCAR-3 pre-treated with hyaluronidase and fixed with methanol. The white bars represent 25 μm. The microphotographs show representative results of 3 different independent experiments.

References

1. Chen, G., Ma, C., Bower, K.A., Shi, X., Ke, Z., and Luo J. (2008) *J Neurosci Res.* **86**, 937-946

2. Yoshida, H. (2007) *FEBS Journal* **274**, 630-658

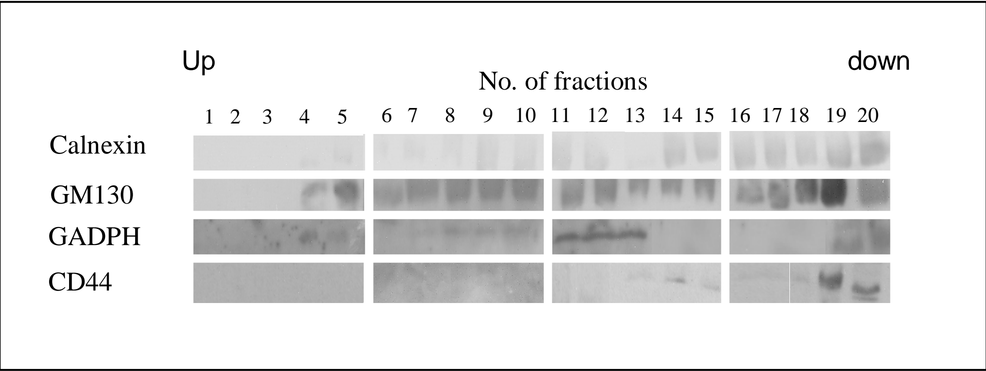


Figure S1

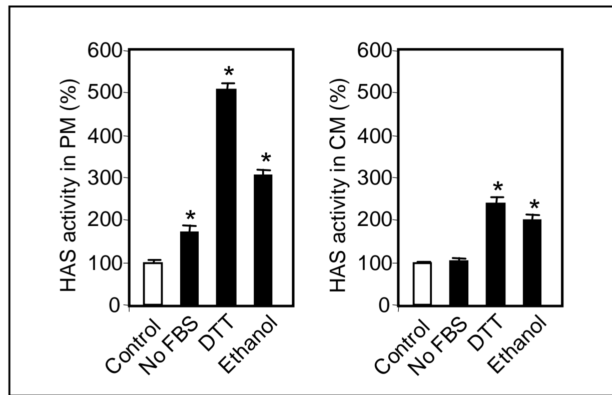


Figure S2

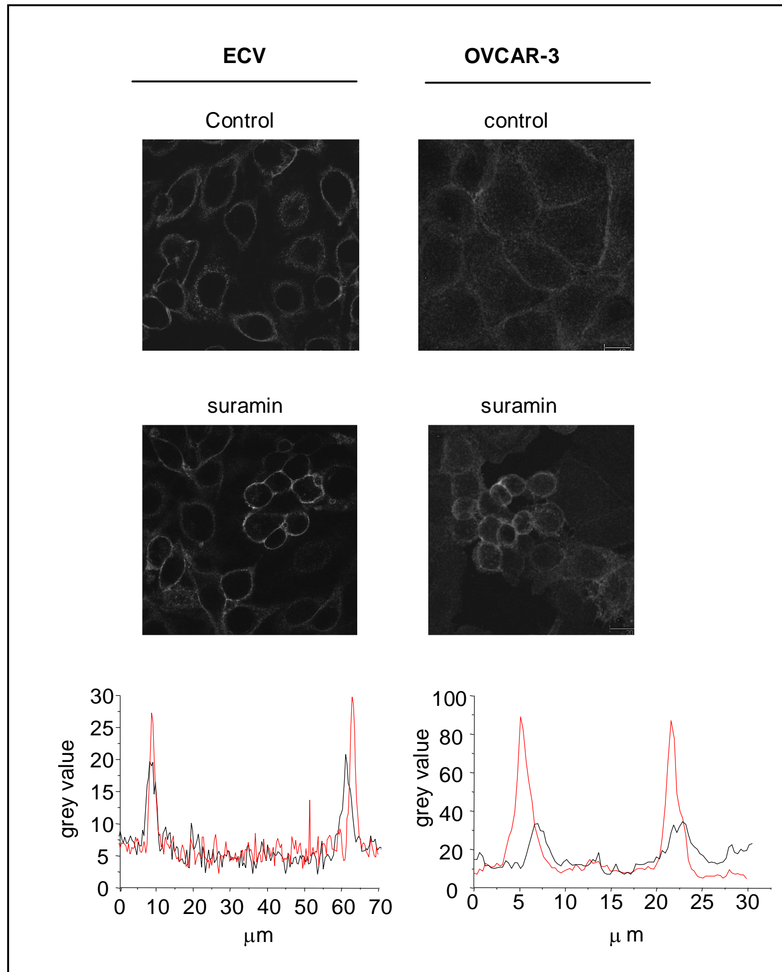
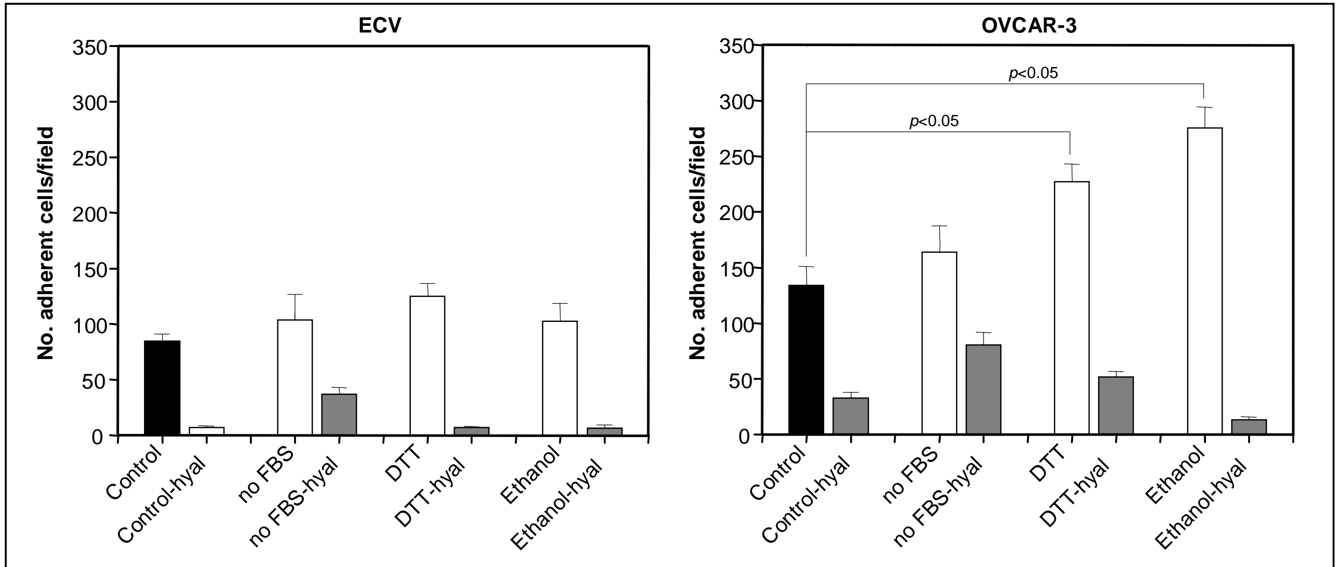


Figure S3



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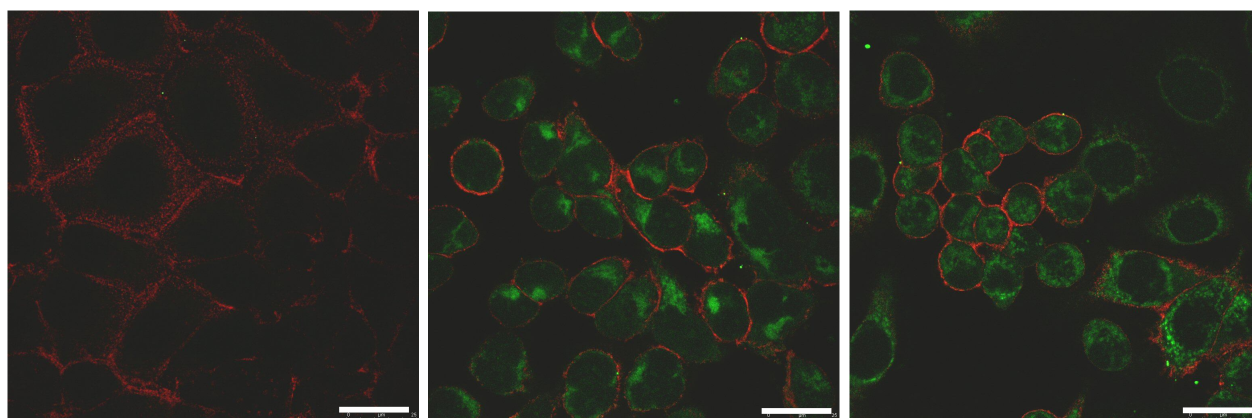

Figure S5