**Publication:**

[D-Serine disrupts Cbln1 and GluD1 interaction and affects Cbln1-dependent synaptic effects and nocifensive responses in the central amygdala](https://link.springer.com/article/10.1007/s00018-024-05554-z)

**Western Blot Imaging and Densitometric Quantification:**

As described in the Methods section, Western blots were treated with SuperSignal™ West Pico PLUS substrate (Thermo Fisher) and developed using the ChemiDoc imaging system (Bio-Rad). Optical density was analyzed using ImageJ and normalized to GAPDH or β-actin as loading controls.

For densitometric analysis, the blot images were opened in ImageJ, and a rectangular selection was drawn around the largest band in the blot to determine a consistent area for measurement. This rectangle was then applied to each band within the blot, and the intensity was measured using the “Measure” tool in ImageJ. This process yielded the “Area” (which remains constant across all bands) and the “Band Intensity” (signal intensity for individual bands/samples).

To account for background noise, the same rectangular selection was used to measure background intensity by placing it in an empty region above or below the bands. The measured intensity values were exported as a CSV file. The same process was applied to GAPDH or β-actin blots for internal control normalization. The net intensity for each band was calculated by subtracting the background intensity from the band intensity for both the protein of interest and the internal control.

For normalization, the net intensity of each protein band was divided by the corresponding net intensity of the internal control band. To standardize the data across blots, all values were normalized to the average of the control group, which was set to 1. The normalization formula used was:

Sample value × 1

Normalized value =

Average control group value

For example, if a sample had a raw intensity of 2.55 and the control group average was 2.4114, the calculation would be:

2.55 × 1

= 1.06

2.4114

All blots were normalized in this manner, and the data are presented as Mean ± S.E.M.