

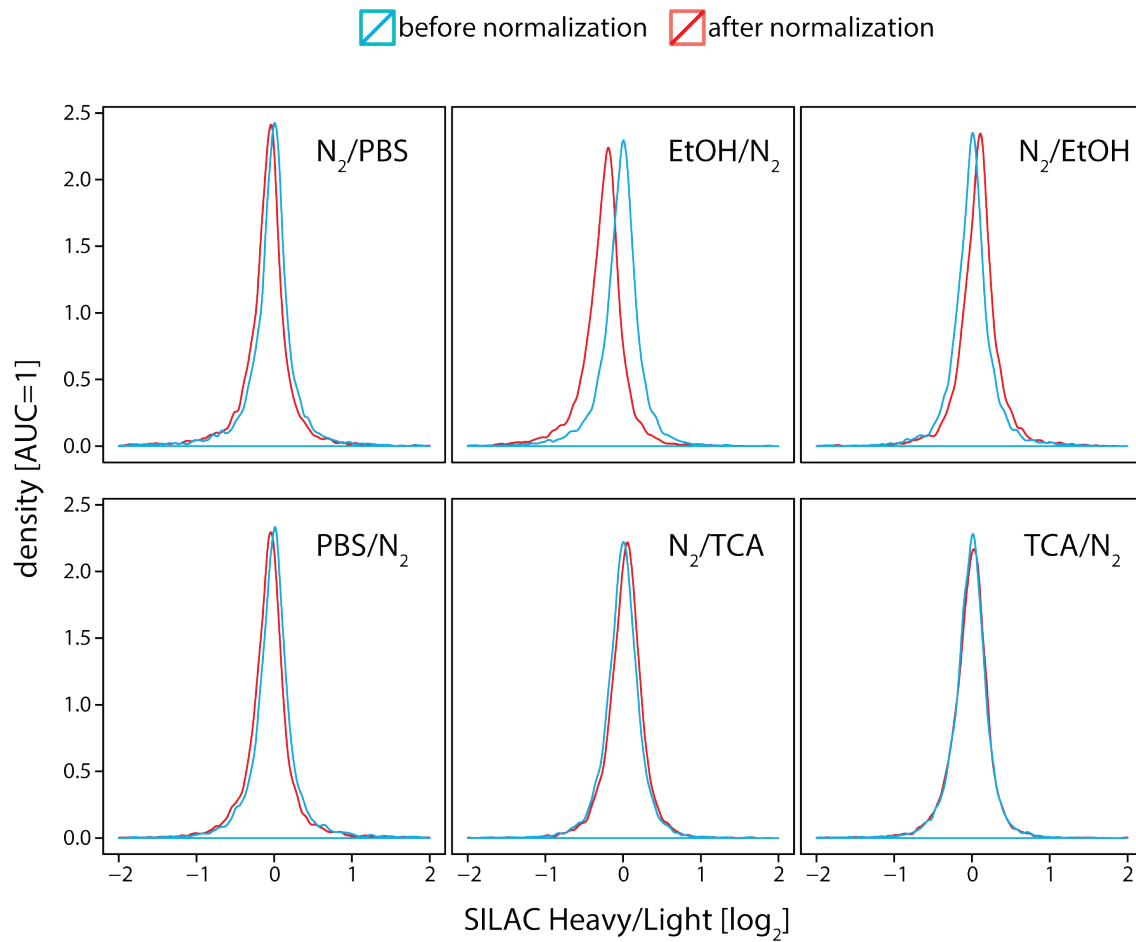


Figure S1. Comparison of sample losses between PBS, EtOH, TCA-based and liquid nitrogen sample collection protocols. Comparison of normalized and non-normalized log₂(FC) distributions for identified peptides in PBS, EtOH, TCA-based and liquid nitrogen sample collection protocols showing minimal changes across different protocols. Small difference observed for EtOH is likely due to the mixing errors.

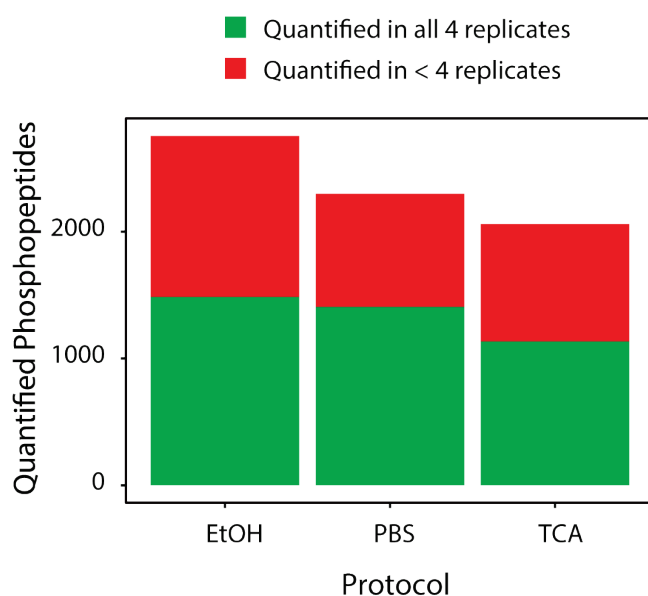


Figure S2. Distribution of quantified phosphopeptides in osmotic shock experiments. A total of 3683 phosphopeptides were quantified using all sample collection protocols. The number of phosphopeptides quantified in < 4 replicates and in all 4 replicates represent 2754/1485 (EtOH), 2298/1406 (PBS) and 2059/1135 (TCA), respectively.

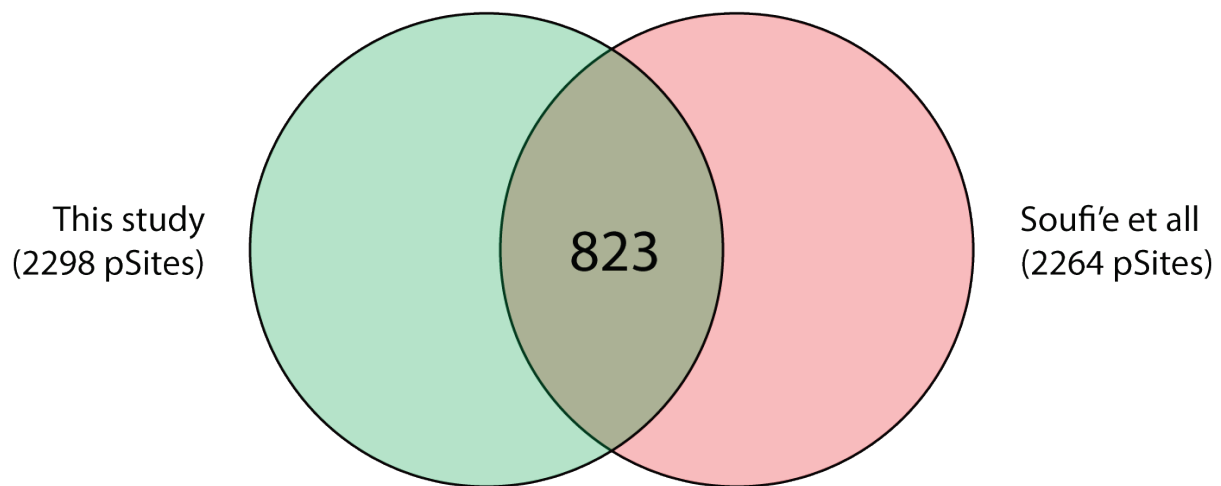


Figure S3. Overlap of phosphosite identification between this study and that of Soufi *et al*²¹.

In Soufi *et al.*, samples were collected in cold PBS at 5 and 20 min following cell incubation in 0.4 M NaCl. The overlap of identified phosphopeptides between both studies corresponds to 35%. The relatively small overlap observed here could be explained by differences in experimental and data analysis workflows including MS instrumentation and fragmentation modes.