

Life Sciences Reporting Summary

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Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. [For final submission](#): please carefully check your responses for accuracy; you will not be able to make changes later.

▶ Experimental design

1. Sample size

Describe how sample size was determined.

Sample size was chosen based on the fact that we wanted to investigate pHis phosphorylation in 4 biological conditions. In order to compare those conditions we made technical triplicates (after digestion, before enrichment) of each condition, allowing us to perform a t-test in order to detect significant regulation. For the majority of the paper we did not perform quantitative analysis and primarily focused on identification of phosphorylation and more specifically for phosphohistidine.

2. Data exclusions

Describe any data exclusions.

No data was excluded from the analysis

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

Results were reproduced under 4 biological conditions, each consisting of technical triplicates. Identification of pHis peptides were reproduced in all conditions.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Randomization was not performed as in this study we mainly focused on the existence of the modification and methods to maintain this.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Blinding was not performed as in this study we mainly focused on the existence of the modification and methods to maintain this.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a Confirmed

- The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- The statistical test(s) used and whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- Test values indicating whether an effect is present
Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on [statistics for biologists](#) for further resources and guidance.

► Software

Policy information about [availability of computer code](#)

7. Software

Describe the software used to analyze the data in this study.

Raw data files were processed using the MaxQuant software (ver. 1.5.5.1)
Outputs were further processed with Perseus software (ver 1.5.6.0)

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). [Nature Methods guidance for providing algorithms and software for publication](#) provides further information on this topic.

► Materials and reagents

Policy information about [availability of materials](#)

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

No unique materials were used

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

No unique materials were used

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

No commonly misidentified cell lines were used

► Animals and human research participants

Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

No animals were used

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This study did not involve human research participants