nature portfolio

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Last updated by author(s):	Dec 11, 2023

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For	all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Confirmed
	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	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.
X	A description of all covariates tested
	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
X	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
×	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
x	Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
	Our web collection on statistics for high acids contains articles on many of the points above

Software and code

Policy information about availability of computer code

Data collection

No algorithms were used.

Data analysis

The LC-IMS-MS/MS data were analysed using FragPipe (version 20.0). Spectra were searched using MSFragger against the protein sequences of the human proteome (UP000005640, UniProtKB). Search results were validated using Percolator with MSBooster enabled rescoring and converged to false discovery rates of 1% on all levels.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a <u>data availability statement</u>. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The mass spectrometry data generated in this study have been deposited in the public database PRIDE (https://www.ebi.ac.uk/pride/) under the accession number PXD046777. Source data are provided with this paper.

Research involving human participants, their data, or biological	materia

Policy information at and sexual orientation		with

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6324184/

Validation

https://www.abcam.com/products/primary-antibodies/hiv1-p55--p24--p17-antibody-ab63917.html

https://www.licor.com/bio/reagents/irdye-800cw-goat-anti-mouse-igg-secondary-antibody

https://www.licor.com/bio/reagents/irdye-680rd-goat-anti-rabbit-igg-secondary-antibody

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

293T (F, CVCL_0063), Jurkat (M, CVCL_0065), and (CEM)A3.01 (F, CVCL_6244) were from ATCC. TZM-bl (F, CVCL_B478) and PM1 (M, CVCL_9472) were from the NIH HIV reagent program. THP-1 (M, CVCL_0006), U937 (M, CVCL_0007) and C8166 (M, CVCL_1099) were from internal laboratory stocks. PBMCs were isolated from buffy coats of three anonymous healthy donors from the German Red Cross.

Authentication

Some cell lines (293T, TZM-bl, THP-1, U937) were authenticated by STR testing from Eurofins Genomics. Others have not been tested.

Mycoplasma contamination

Some cell lines (Jurkat, PM1, A3.01) have tested negative for mycoplasma contamination by Eurofins Genomics. Others have not been tested.

Commonly misidentified lines (See ICLAC register)

None of the cell lines used in this study are listed in the ICLAC register $\nu 12$.