# natureresearch

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## **Reporting Summary**

Nature Research 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 Research policies, see <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

#### Statistics

For	all st	atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Cor	firmed
	$\square$	The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement
	$\square$	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.
$\boxtimes$		A description of all covariates tested
$\boxtimes$		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)
$\boxtimes$		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>
	$\square$	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
$\boxtimes$		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
$\boxtimes$		Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
		Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

### Software and code

Policy information about availability of computer code					
Data collection	TBLASTN, CODEML, OLYMPUS BX53F, BECKMAN cytoflex, CytExpert 1.2, CFX Connect Real-Time System, Tanon-5200				
Data analysis	GraphPad Prism 6, ImageJ 1.45s, Microsoft Office Excel, PAML 4.7, MUSCLE, ModelTest, MrBayes v3.1.2, MEGA6				

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

#### Data

Life sciences

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 list of figures that have associated raw data

- A description of any restrictions on data availability

Provide your data availability statement here.

### Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Behavioural & social sciences Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

### Life sciences study design

An studies must disclose on these points even when the disclosure is negative.				
Sample size	No statistical methods were used to predetermine sample sizes. All sample numbers were stated in figure legends.			
Data exclusions	No data was excluded from the analyses.			
Replication	All attempts of replication were successful. We stated the number of replicates for each experiment in the figure legend.			
Randomization	All cells and mice were allocated randomly.			
Blinding	No blinding was performed in this study.			

### Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

MRI-based neuroimaging

#### Materials & experimental systems

#### Methods

ChIP-seq

Flow cytometry



### Antibodies

Antibodies used	All antibodies used in this study were stated in methods and figure legend. mouse monoclonal anti-ubiquitin antibody P4D1 (SC-8017, Santa Cruz, 1:500); mouse monoclonal anti-β-actin (A1978, Sigma, 1:10000); rabbit polyclonal anti-GAPDH (10494-1-AP, Proteintech, 1:10000); rabbit polyclonal anti-GAPDH (10494-1-AP, Proteintech, 1:10000); rabbit polyclonal anti-Insig-2, rabbit polyclonal anti-gp78 and mouse monoclonal anti-Myc (9E10) were prepared in our laboratory.
Validation	All the antibodies used in this paper were validated by immunoblotting and stated within the paper.

### Eukaryotic cell lines

Policy information about <u>cell lines</u>	
Cell line source(s)	All cells used in this study were stated in the method of this paper. CHO-7,1601 and C2C12 cells were obtained from ATCC. Muscle stem cells were obtained from mice muscle
Authentication	No further authentication of the cell lines was performed before use
Authentication	
Mycoplasma contamination	No test for mycoplasma contamination was performed.
Commonly misidentified lines (See <u>ICLAC</u> register)	None of these cell lines were utilized.

#### Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research				
Laboratory animals	All mice used in this study were stated in method and figure legend. Male, 8 week old C57BL/6J mice gp78-/- mice and Insig-2-/- mice were used.			
Wild animals	The study did not involve wild animals.			

Field-collected samples

The study did not involve field-collected samples.

Ethics oversight All animals were maintained and used in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Wuhan university.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

#### Flow Cytometry

#### Plots

Confirm that:

The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

All plots are contour plots with outliers or pseudocolor plots.

A numerical value for number of cells or percentage (with statistics) is provided.

#### Methodology

Sample preparation	Intracellular ROS level was determined using oxidation-sensitive DCFH-DA fluorescent dyes. Cells were washed twice with PBS and labeled on the culture plates with DCFH-DA for 30 min at 37 °C in serum-free medium. At the end of incubation, culture plates were trypsinized, resuspended in PBS and analyzed using a FACScan flow cytometer.
Instrument	BECKMAN cytoflex
Software	CytExpert 1.2
Cell population abundance	The mean fluorescence intensity of 10,000 cells was measured in each sample and corrected for autofluorescence from unlabeled cells.
Gating strategy	Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.