

Life Sciences Reporting Summary

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For further information on the points included in this form, see [Reporting Life Sciences Research](#). For further information on Nature Research policies, including our [data availability policy](#), see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

▶ Experimental design

1. Sample size

Describe how sample size was determined.

Experiments were performed at least three different times. Sample size in mice experiments was estimated based in previous published experiments. At least three or more mice were used in each experimental point to allow data accuracy.

2. Data exclusions

Describe any data exclusions.

No data were excluded. So, no criteria were established to exclude any of the data generated.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All attempts at replication were successful.

4. Randomization

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

Samples or mice were not randomized. Cells were clonal cultures and mice belonged to inbred strains.

5. Blinding

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

The investigators were not blinded to group allocation during experiments and outcome assessment.

Note: all studies involving animals and/or human research participants must disclose 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 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 (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- The test results (e.g. p values) given as exact values whenever possible and with confidence intervals noted
- A summary of the descriptive statistics, including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars

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.

Statistic analysis: GraphPad Prism 5.03
Image Quantification: Image J
DNA microarray quantification: Agilent G2565BA Feature Extraction and BioRad iQ5 Optical System Software
Gene pathways analysis: GeneSpring, Ingenuity Pathway Analysis
Aortic images: VEVO 2100 version 1.5.0

For all studies, we encourage code deposition in a community repository (e.g. GitHub). Authors must make computer code available to editors and reviewers upon request. The *Nature Methods* [guidance for providing algorithms and software for publication](#) may be useful for any submission.

► 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 for-profit company.

The Plk1(lox) mouse strain will be shared under MTA.
Plk1 rat monoclonal antibody (clone POE125) will be shared under MTA.

9. Antibodies

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

mouse anti-phospho-Ser19 Myosin Light Chain 2 (MLC) (Cell Signaling, ref #3675).
mouse anti-phospho-Ser18/19 Myosin Light Chain 2 (MLC) (Cell Signaling, ref #3674).
rabbit anti-Ki67 (Master Diagnostica, clone SP6, ref #0003110QD).
rat monoclonal anti-Plk1 (clone POE125). Lab made antibody.
mouse monoclonal anti-Plk1 (clone PL6/PL2). ThermoFisher #33-1700).
rabbit anti-phospho-Ser10 Histone-H3 (Millipore, ref #06-570).
rabbit anti-phospho-Ser170 RacGAP1 (Active Motive, ref #39265-66).
rabbit anti-MYPT1 (Santa Cruz Biotechnologies, #sc-25618)
rabbit anti-phospho-MYPT1-Thr696 (CellSignaling, #5163)
rabbit anti-MLC (Cell Signaling, ref #3672)
mouse anti-alpha-tubulin (clone DM1A). (SIGMA #T9026).
mouse anti-GFP (Roche, clones 7.1/13.1 , ref #1 814 460).
mouse anti-RhoA/B/C (Millipore, clone 55 , ref #05-778).
mouse anti-myc tag (Santa Cruz Biotechnologies, clone 9E10, ref #sc-40).

10. Eukaryotic cell lines

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

Vascular Smooth Muscle Cells (VSMC) used in this study were extracted from aortas dissected from Plk1(lox/lox) and Plk1(+/lox) mice.

HEK293 cells were obtained from ATCC (CRL-3216)

b. Describe the method of cell line authentication used.

Murine VSMC were authenticated by analyzing expression of genes typically expressed in smooth muscle cells. HEK293 were not authenticated.

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

All cells are routinely tested for mycoplasma contamination

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

No commonly misidentified cells 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 details on animals and/or animal-derived materials used in the study.

Mice (*Mus musculus*) were maintained in a mixed 129/Sv x C57BL/6J background. Wild-Type female mice used in the Volasertib experiments are C57BL/6J background. Mice were housed at the pathogen-free animal facility of the Centro Nacional de Investigaciones Oncológicas (CNIO) and Centro Nacional de Investigaciones Cardiovasculares (CNIC), following the animal care standards of both institutions. All experiments and mice protocols were approved by the CNIO and CNIC Committee on Research Ethics and Animal Welfare.

Policy information about [studies involving human research participants](#)

12. Description of human research participants

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

This study did not involve any human research participants.