



# SPANDIDOS PUBLICATIONS

## Manuscript submission process



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## Online Submission

Spandidos Publications journals utilize an online submission and tracking system designed to provide a faster, more efficient service to authors.

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## Submission guidelines



- The principal aim of Spandidos Publications is to publish promptly original works of high quality in English.
- Manuscripts will be considered on the understanding that they report original work, or are review articles summarizing and interpreting progress in a thematic area and are not under consideration for publication by another journal.
- Manuscripts should be written in clear, concise English and should contain all essential data in order to make the presentation clear and the results of the study replicable.
- Authors who are not native speakers of English but would like to improve the English to ensure the meaning is clear can make use of the English Language Editing service offered by Spandidos Publications by accessing the following link: <https://www.spandidos-publications.com/languageediting>.
- All material submitted will be subject to review by appropriate referees selected by the Editorial Office and will be examined to detect inappropriate use of previously published material without attribution.
- Spandidos Publications utilizes iThenticate to screen submitted manuscripts against published studies and other relevant sources. iThenticate may also be utilized by authors to screen a manuscript prior to submission.
- Figures submitted will be subject to checks using the MOTUIN image authenticity detector.
- The Editors reserve the right to improve the grammar and style of manuscripts.
- The corresponding author is responsible for the submission on behalf of all authors.

Prior to submitting your manuscript, please ensure that it has been prepared according to the guidelines below.

### 1. Submission method

- Manuscripts may be submitted only by using the online submission system accessible via our website.
- Create a user account, log in and follow the onscreen directions.

## 2. Cover letter

Summarize briefly the important points of the submitted work including a brief description of the study to be submitted, that it is an original study presenting novel work, that it has not been previously submitted to or accepted by any other journal, that it has been approved by all authors, that ethics approval and written informed consent have been obtained, and explain whether any author has a conflict of interest.

## 3. Format of articles and reviews

### 3.1 General style

- Times New Roman. Font size 12. Spacing 1.5. Alignment Justified.
- Use a single tab on the first line of each new paragraph.
- Do not use page breaks or multiple returns between sections (one section should directly follow the previous one on the page).
- Do not insert page numbers or line numbers.
- Sub-headings and general headings should be presented in lower case letters (not capitals).
- Use British English or American English spellings throughout your manuscript, but not both.

### 3.2 Manuscripts

The first page should include:

- The title of the manuscript in sentence case. No abbreviations other than gene names or in common use.
- Full names and full postal addresses, but not including street names, of all authors and ORCID if desired.
- Affiliations of the authors indicated by numbers (not symbols), **and written in full, as in the example below:**

ZHIXUE ZHANG<sup>1</sup>, FANGXU TAO<sup>1-3</sup>, YINING LI<sup>2</sup>, YUDONG XIAO<sup>2</sup>, ZHISHU ZHANG<sup>4</sup> and JUN LIU<sup>1</sup>

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- Equal contribution indicated by asterisk.
- Name, full postal address, including street number and name, and e-mail address of the corresponding author(s).

- Abbreviations, if relevant.
- Key words (5-10).
- Running title preceded by the first author's name (maximum 100 characters with spaces, including the author's name). For example: PEARSON *et al*: REGULATION OF HER2 EXPRESSION BY NASCENT GROWTH FACTORS.

Manuscripts reporting experimental results must be divided into the following sections:

- Abstract. This section should have 150-300 words, be continuous (not structured) and without reference numbers. Abbreviations that appear once only, should be defined in full, unless they correspond to a gene name. If abbreviations appear more than once, the definition should be provided once, and then subsequently used throughout the Abstract.
- Introduction. The information in this section should always be referenced.
- Materials and methods
  - This section should include sufficient technical information to allow the experiments to be repeated. This implies that a full description of all the experiments described in Results and presented in the Figures/Tables is expected in this section. For each experiment, all steps (e.g., DNA and protein extraction, quantification, cloning, PCR and microscopy) need to be mentioned, along with instruments the analyses were performed on, reagents and methods (e.g., BCA method for protein quantification,  $\Delta\Delta Cq$  method for qPCR), and relevant citations. For specific details on our standards of reporting for individual techniques, please click [here](#).
  - For steps performed with commercialized kits, provide the full name of the kit, along with the full name and location (city, province or state if USA/Canada, and country) of the supplier, and state whether the protocol of the manufacturer was followed or explain any modifications made to the standard protocol. For PCRs, provide the name of the kit used, 5'-3' sequence of the primers, final concentration of all reagents in the reaction, and cycling conditions. Carefully review your text to ensure that the type of PCR, quantitative or semi-quantitative, is clearly explained. If the PCR is performed using cDNA synthesized from RNA samples by reverse transcription (RT), make sure that all steps are described, and refer to the method as RT-PCR or, if quantitative, as RT-qPCR. In relative quantification,  $\Delta\Delta C_t$  is referred to as  $\Delta\Delta Cq$ . When using the  $\Delta\Delta Cq$  method, this **must** be referenced. One suitable reference is: Livak and Schmittgen: Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta C_t$  method. *Methods* 25: 402-408, 2001. Manufacturers/suppliers/software details need to be provided for all reagents used (including chemicals), instruments (e.g. thermal cyclers, microscopes) and software, ideally accompanied by the corresponding kit number/model/version. For antibodies, include the type (monoclonal/polyclonal), species in which they were raised and targeted species (e.g., mouse anti-human). Please explain any antigen retrieval steps, mention the dilution used, and state the catalogue number and supplier. Please also state the temperature and duration of incubation. For centrifugation steps, provide centrifugal force units in x g rather than revolutions per minute (rpm).
  - For bioinformatic analyses: state the software used along with the relevant citation, unless the software is not published, in which case a website link can be provided. For microarray/RNA sequences, data downloaded from GEO or other databases, this needs to be clarified in the text, along with the corresponding accession number of the dataset. The use of software should be described with regards to the parameters (default, study-specific) and the applied thresholds; please explicitly name the parameters, e.g. 'association value'

or ‘false-positive rate’. For all software analysis of data from public databases, cite the database (along with date of access for databases as these are constantly updated), and species (e.g., human). If figures/tables contain data from a public database (e.g., Gene Ontology/KEGG), cite the source in the legend/title explicitly. For publically available sequences, provide the accession number.

- For flow cytometry experiments authors are encouraged to adhere to MIFlowCyt guidelines (Lee J et al. (2008) MIFlowCyt: The minimum information about a flow cytometry experiment. *Cytometry* 73A: 926-930. doi: 10.1002/cyto.a.20623). Axis labels should include the marker and the dye used (rather than instrument-specific parameter descriptions such as FL-1H). The scaling (log/lin) should be clearly displayed. If most events are “piled up” on the plot axes, adjust scale or provide a different scale if necessary. If statistical analysis is provided, please clarify if it is the fluorescence intensity of the gated population (mean, median, Geo mean) or the proportion of cells within a specific gate that is examined.
- The source of material used and relevant ethical framework for all experiments should be clearly identified (ethics approval and/or written informed consent). For tissues, explain how these were collected, handled and stored, and where they were from. For bacterial strains or cells, provide the name and supplier. For studies on humans, a minimum of information is required: number of subjects, age range, gender ratio, health status, matching between controls and disease patients with regards to the above parameters. Please note that ‘normal’ should be avoided for controls; rather, the precise health status needs to be described, e.g., ‘healthy’, or ‘individuals with no recorded tumor complication’. For manuscripts presenting studies on humans and animals see 3.8 below.
- For statistical analyses: when statistical analyses have been performed, the following information should be provided: the name of the statistical test used, the n number for each analysis, the comparisons of interest, the alpha level and the actual P-value for each test. It should be clear which statistical test was used to generate every P-value. Error bars on graphs should be clearly labeled, and it should be stated whether the number following the  $\pm$  sign is a standard deviation or a standard error. The word ‘significant’ should only be used when referring to statistically significant results and should be accompanied by the relevant P-value. Significance indicators should be used on graphs and tables, and should be described in the figure or table legend, clearly indicating which groups are being compared.

If your study involves performing statistical analysis of datasets containing 3 or more groups, please note that the use of Student's t-test, Mann-Whitney U test or similar two-sample tests is not considered appropriate, as when these tests are used to perform multiple comparisons, the familywise error rate is raised to unacceptably high levels. These data should be analyzed using tests designed to control for type I error; the most popular tests for such comparisons are analysis of variance (ANOVA) followed by a post hoc test (e.g. Tukey, Bonferroni, Dunnett, etc.) for parametric data, or Kruskal-Wallis test followed by Dunn's test for non-parametric data.

- Please note that figure legends are not expected to contain information already described in Materials and methods, except for image-specific information, for example, for microscopy, mention the type of image, e.g., fluorescence and the original magnification if scale bars are not used. Legends should provide information concerning what is shown in the figure(s)/figure parts. The x- and y-axes of the graphs must be clearly explained in the legends, and when P-values are provided to indicate probability, the comparison to which these P-values refer must be clearly stated.

- If cell lines are used, authors are strongly encouraged to include the following information in the materials and methods section of their manuscript: i) Confirm that mycoplasma testing has been done for the cell lines used; ii) confirm that the cell lines used have been authenticated and state what method was used for the authentication; and iii) provide the source, supplier and, if available, catalogue number of all specific cell lines used in the study. The authors are strongly encouraged to submit a detailed methodology stating the maintenance and culture of cell lines according to international guidelines on good cell culture practice (fundamental techniques, mycoplasma contamination, passage number, etc.). Furthermore, information regarding misidentified or cross-contaminated cell lines must be provided and cross-checked from the International Cell Line Authentication Committee and ExPASy Cellosaurus databases in order to exclude their contamination with other cell lines or their incorrect identification. If a cell line has been previously reported to be contaminated or misidentified, an STR profile of the cell line used in the study must be available for evaluation by the journal's editor.
- Results
- Discussion
- Acknowledgements
- Funding
- Availability of data and materials
- Authors' contributions
- Ethics approval and consent to participate
- Patient consent for publication
- Competing interests
- Authors' information (optional)
- Use of artificial intelligence tools (to be added only when AI tools are used)
- References
- Figure legends
- Tables

For Review articles:

- Abstract. This section should have 150-300 words, be continuous (not structured) and without reference numbers.
- May have different sections and sub-headings according to the subject matter.
- The main headings of the review should be summarized as a numbered Contents section immediately following the Abstract.

### 3.3 Figures

Submission of figures to us implies that the images or parts thereof have not been published elsewhere (unless mentioned and/or cited in the text and permission has been obtained and provided to us).

Images showing any patient or patient's scans should not contain information that might identify them, unless you provide written permission from the patient allowing use of the specific image.

We accept that figures in our journals are rarely simple, and that certain adjustments are acceptable to help show experimental results clearly. The guiding principle when preparing digital artwork

should be to ensure that the version submitted to us is an honest and accurate representation of the original observation(s) and will not lead to possible misinterpretation of what was done experimentally.

The Editors may assess submitted images for unacceptable manipulation using forensic tools and other means. This might delay progress of your manuscript and/or lead to further investigations and action to preserve the integrity of the scientific record, such as not accepting or revoking a manuscript. We may request the original unmanipulated source files and may contact the author's institution for assistance with enquiries to establish probity. Our guidance builds on that described by Rossner and Yamada (1).

- If brightness, contrast or color balance is altered, the change should apply to the entire image shown and not a selected part. For images from gels or filters, ensure that details are not lost from bright areas or obscured in dark areas.
- No feature of a data image should be selectively enhanced, obscured, removed or added. If a composite image shows gels or blots with tracks from groups of samples analyzed separately (or from different exposures) then make the grouping obvious using black or white lines and explain this in the figure legend. The boundaries of individual panels of a tiled image should be marked.
- In the Methods or individual figure legends, outline the changes you made to images and how. For example, "Figure 99. Light microscopy of a frozen section of a lesion stained with toluidine blue. Original magnification x100. Uneven illumination was corrected using a control image as described (2)".

(1) Rossner M and Yamada KM: What's in a picture? The temptation of image manipulation. *J Cell Biol* 166: 11-15, 2004.

(2) Marty GD: Blank-field correction for achieving a uniform white background in brightfield digital photomicrographs. *BioTechniques* 42: 716-720, 2007.

### **3.3.1 File format**

- Acceptable
  - TIFF without layers and preferably using Lempel-Ziv-Welch (LZW) compression as it does not reduce image quality.
  - JPEG (only if originally saved at the highest quality).
- Unacceptable
  - Images imported or copy pasted into Word or PowerPoint.
  - BMP, GIF, PCT, PNG or low quality JPEG files originally saved at low quality.

### **3.3.2 Color mode**

- Acceptable:
  - Color figures: Use RGB as this will offer the best reproduction of your data in the final PDF version of your article on screen. CMYK mode is also acceptable. Fluorescence images must be submitted for publication in color.
  - Black and white figures and line art: grey scale mode or RGB mode.
  - Combination figures with color images and line art: RGB mode.
- PLEASE NOTE

- Color figures are welcome but must be submitted only if reproduction in color is intended (a charge will apply).
- There is a charge of Euro 390 per each published page containing color.
- Changing color figures to black and white following evaluation is NOT possible.

### 3.3.3 Image size

- Image size is measured in centimeters or inches
- Create your figures at the size (width) at which they will be printed:
  - 8.00 cm (3.15 in) wide for a single-column figure
  - 17.00 cm (6.70 in) maximum for a double-column (full page width) figure
  - Maximum height 20.00 cm (7.87 in)

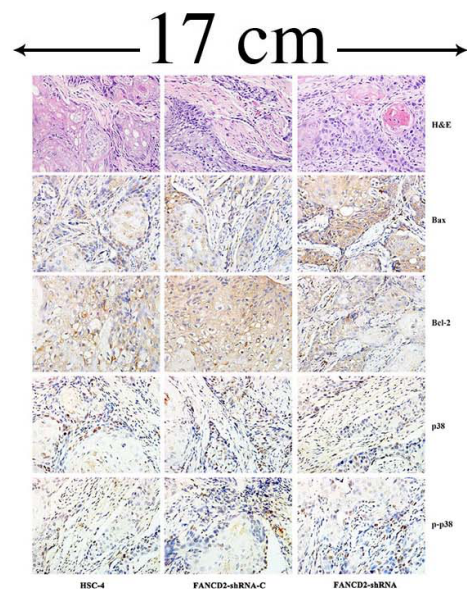


Figure 8. IHC staining of the tumors derived from HSC-4 cells and immunohistochemical analysis of the Bax, Bcl-2, p38 and p-p38 protein expression after radiotherapy. Conventional histopathological H&E staining showed that the three groups of tumors demonstrated the characteristics of squamous cell carcinoma (magnification,  $\times 400$ ). Positive expression of Bax and Bcl-2 was found in the cytoplasm, and positive expression of p38 was primarily found in the cytoplasm but also partly in the nucleus. Positive expression of p-p38 was observed only in the nucleus (magnification,  $\times 400$  EnVision method).



Figure 2. Grading of immunostaining intensities. Grading of immunostaining intensities was defined independently in the entire nucleus, nuclear bodies and cytoplasm. Arrows indicate the cells with representative immunostaining intensities corresponding to each grade.

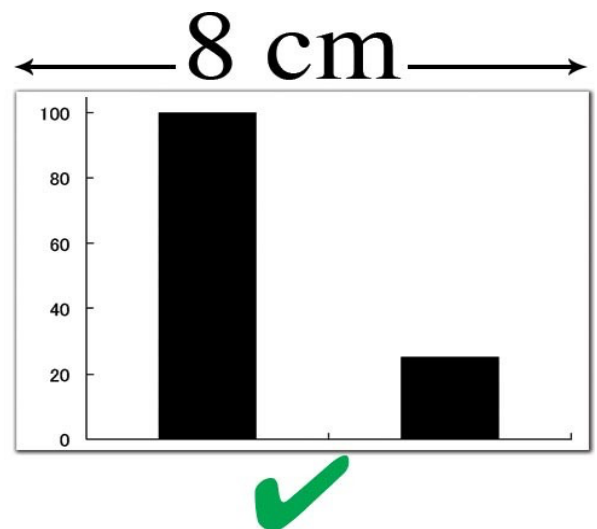
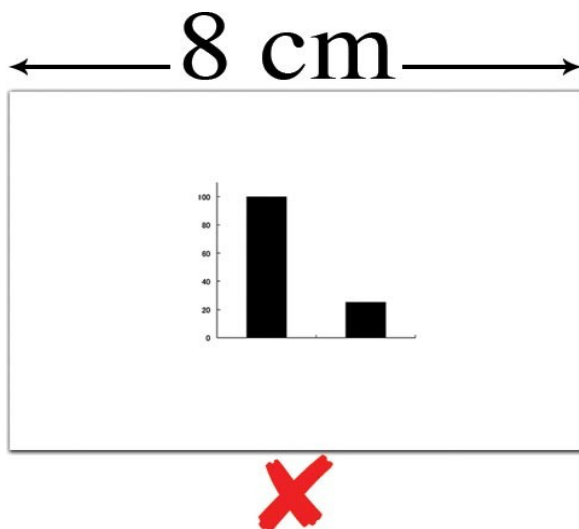
transfected into 293FT and HeLa cells using Lipofectamine LTX (Invitrogen). Cells transfected with vector GFP and vector GFP-WT1 were predicted to produce GFP and fusion protein

Table 1. Two patterns of experimental conditions used in western blotting.

Primary antibody	Amount of protein per lane (μg)		Dilution factor			
	A	B	Primary antibody	Secondary antibody	A	B
WT1 antibodies						
6F-H2	10	20	40	15	4000	1500
ab89901	10	20	4000	500	10000	7000
C-19	10	20	2000	150	10000	10000
GFP antibody	10	40	300	50	10000	3000
Actin antibody	5		800		10000	

Condition A and Condition B were predicted to provide weaker and stronger bands, respectively. Western blotting using the actin antibody was performed under a uniform condition throughout the study. A, Condition A; B, Condition B.

Empty white space surrounding a figure should NOT be included when calculating image size. Images should, therefore, be cropped (cut) as close to the outside edges of the figure as possible.



If a figure is too wide or contains too much information to be fit within 17 cm while keeping details clearly visible, figures must be divided into several clearly labeled separate parts.

### **3.3.4 Image resolution**

- Image resolution in this context is simply a measure of the number of pixels per inch (also called dots per inch, dpi) defining the image and does not relate to the quality of an image in terms of focus, contrast and legibility.
- Images must be clear, of good contrast and legible at the size they are to appear in the journal.
- Images should be AT LEAST 300 dpi, at the size at which they will be printed (8 or 17 cm wide).
- Insufficient image size and/or resolution (dpi) will result in poor quality (blurred) printed figures if they are upscaled.

### **3.3.5 Exporting/capturing/saving figures**

Figures may be produced by scanning, digital photography, or exporting from scientific software or a program such as PowerPoint.

- Scanning
  - Use a good quality scanner set to scan in RGB for color images or grey scale for line art or to scan gel images, at a resolution of at least 300 dpi and with the output file type set preferably to TIFF or JPG with the highest quality (lowest compression).
- Digital photographs
  - Set simple cameras to a ‘fine’ or ‘extra fine’ setting to help ensure that images have sufficient pixels.
  - Exporting
    - When exporting from scientific graphing software, choose settings to ensure the highest possible final size and resolution with lines of sufficient thickness to be seen at final printed size.
    - When exporting from PowerPoint, DO NOT choose ‘Save as TIFF’ from the Save as dialogue box as this will NOT result in an image of sufficiently high resolution. Instead, save the individual slide image as a PDF (from the Print dialogue box), THEN open the PDF with image editing software, such as Photoshop or GIMP, and when prompted specify 300 dpi resolution. Finally, save the resulting image as a TIFF (with LZW compression).
    - Note: figures initially scanned, photographed or exported at an insufficient size and resolution cannot be improved by upscaling, i.e., artificially increasing the resolution of a low-quality figure. Using image-editing software to keep the figure size the same while raising the dpi will NOT improve its quality.

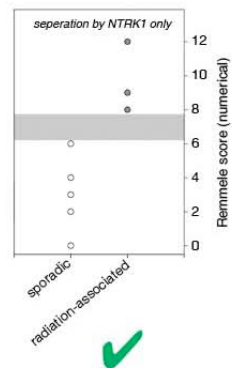
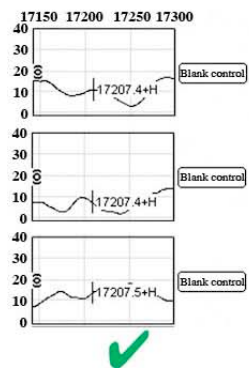
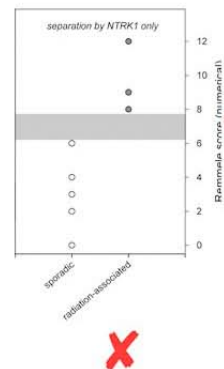
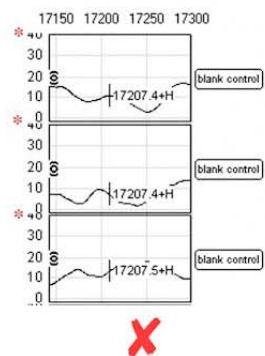
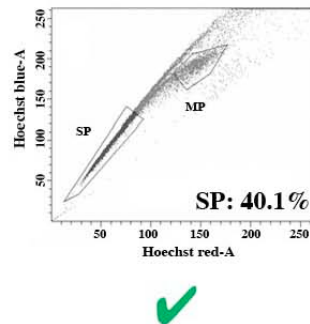
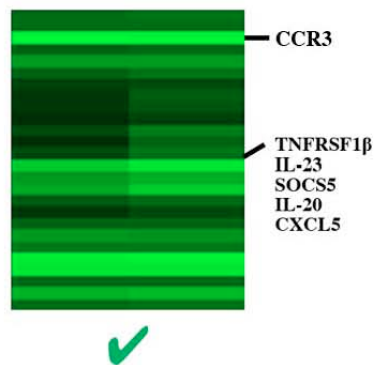
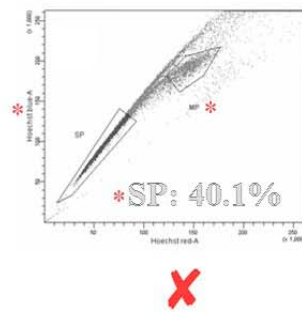
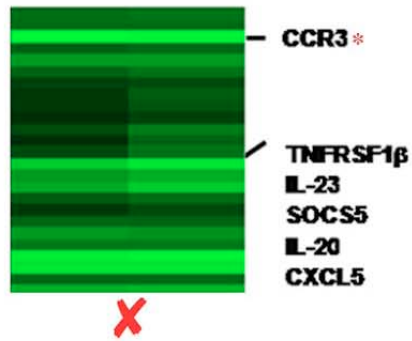
### **3.3.6 File size**

- If saved according to our guidelines, files will rarely exceed 10 MB.
- To reduce the file size of images:
  - Ensure figures are the exact width and height they should be for publication (not smaller), make sure the figures are saved at no more than 300 dpi.
  - Ensure that layers in the image have been flattened.
  - Save black and white figures as grey scale.

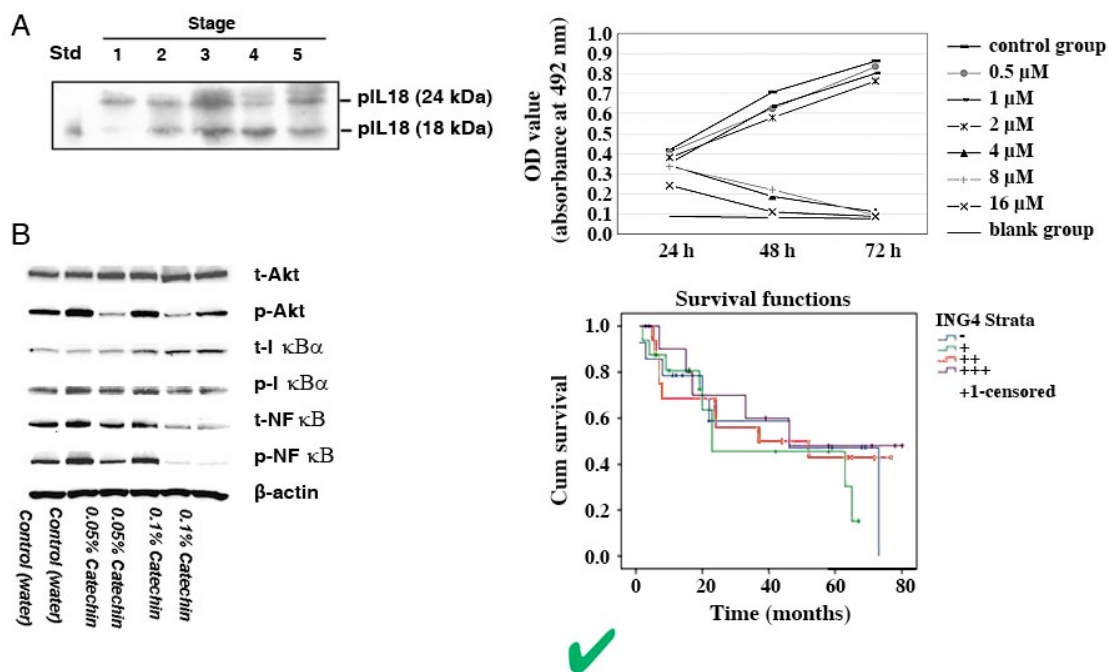
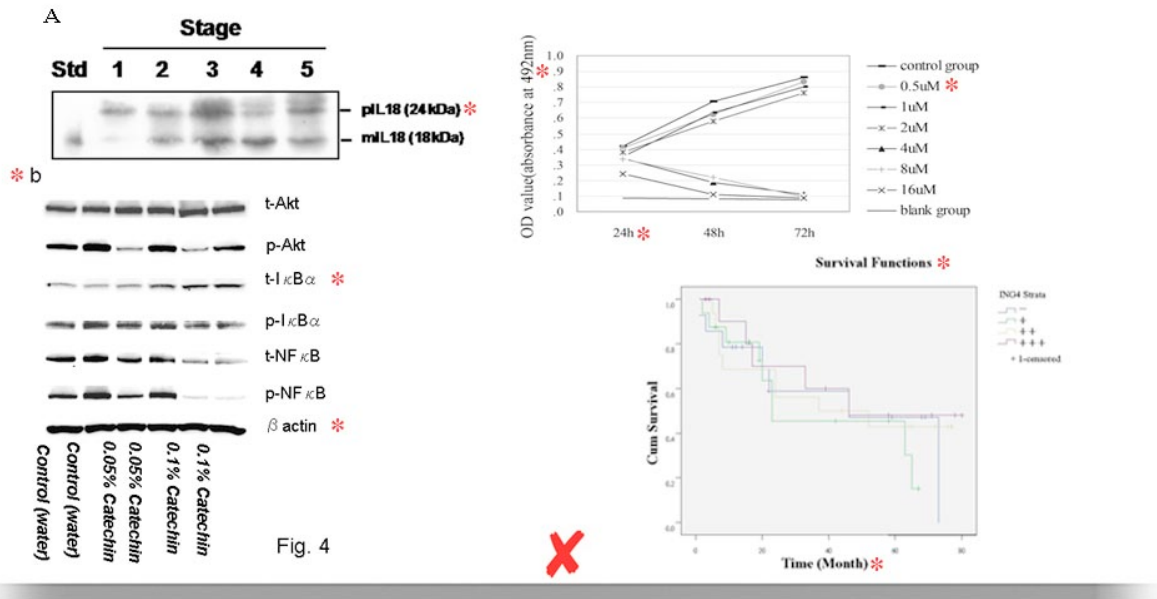
- Ensure that TIFF files are saved with LZW compression.
- Consider saving files as highest quality JPEGs. These may be smaller files than TIFF with LZW compression, but will lose some detail.
- Try using a compression or stuffing utility, such as WinZip or StuffIt.

### 3.3.7 Figure labels

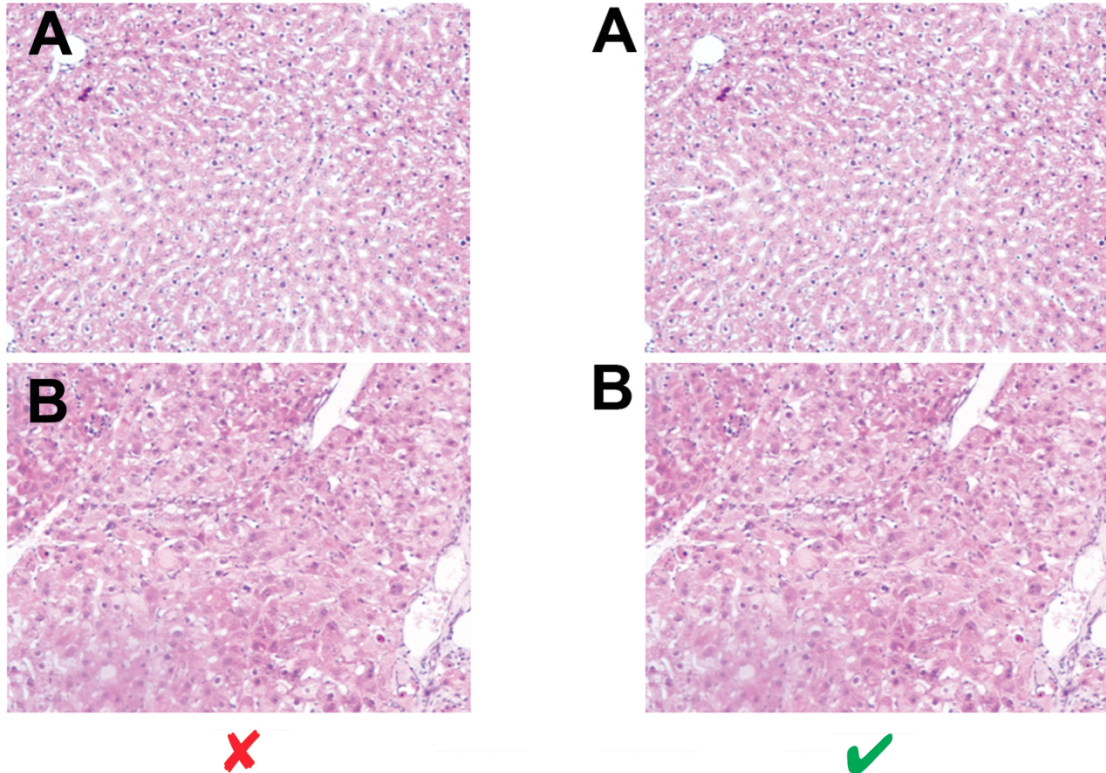
- Font size
  - Labels must be sized in proportion to the image, sharp, and clearly legible.
  - When figures are prepared at the correct size (8 or 17 cm at 300 dpi), the font size for labels should be 8-10 points.
  - If the figure is saved at a size larger than that needed for printing, the font size of labels must also be larger to maintain the correct proportions.
  - If labels cannot fit on an 8-cm-wide page unless the font size is smaller than 8 points, the figure must be prepared as a double column figure (14-17 cm wide). If labels cannot fit on the 17-cm-wide page unless the font size is smaller than 8 points, the figure must be split into several parts.
- Font style and appearance
  - Labels must be saved using standard fonts (Times New Roman, Times, Arial, Helvetica or Symbol font).
  - The labels should be of the same font and size in all figures. Also, the numbering should be of the same font and size in all figures.
  - Labels should be evenly spaced and aligned, easy to see (including exponential numbers around figure axes), and NOT faded, broken, or distorted by JPG compression artifact. Do NOT use light grey color lines or labels.
  - There must be strong contrast between labels and their background (e.g., labels placed over shaded bar graphs should be in a color that stands out against the shading, NOT blend in with it). Whenever possible, labels should be placed in black font on a white background. Consider using a black label with a white stroke applied to create contrast.
  - Letters of labels must NOT be overlapping, condensed, expanded, have unnecessary gaps between them or be otherwise irregularly spaced, and must NOT be stretched (distorted) horizontally or vertically.
  - Labels must NOT overlap or be concealed by other parts of the image, or be cropped (cut off) by the edge of the figure.



- Label styles and language
  - Labels must be prepared according to our in-house style, be phrased in accordance to the manuscript, and free of spelling and other language errors.
  - The first letter of each phrase, NOT each word, must be capitalized [e.g., ‘Overall survival (months)’ not ‘Overall Survival (Months)’ and not ‘overall survival (months)’].
  - Always use a leading zero (0) before decimal points: 0.5 NOT .5.
  - Decimal points must use a full stop/period (.) NOT a comma (,).
  - A space must be inserted before measurement units: 132 bp NOT 132bp, 5 mm NOT 5mm, 1 h NOT 1h.
  - Measurements must be written as:
    - second(s): sec
    - minute(s): min
    - hour(s): h
    - day(s): day(s)
    - week(s): week(s)
    - month(s): month(s)
    - micro:  $\mu$ ,  $\mu$  (available in Times and Helvetica) NOT u
    - liter(s): l NOT L
    - kilo Dalton: kDa NOT kD, Da, bp, kb
    - 5 units BUT 5 U/ml
      - Greek letters must be inserted using the correct Greek symbol (using Times, Helvetica or Symbol font), NOT written in full, i.e., alpha:  $\alpha$ ; beta:  $\beta$ ,  $\beta$ , (available in Times and Helvetica); and gamma:  $\gamma$ , etc.



- Figures may be divided into separate sections. Each section may be saved as a separate file (clearly indicated in file name) or included together in one file (with parts clearly labeled).
- Separate parts of a figure should be labeled using just A, B, C, NOT 1A, 1B, 1C.
- Figure sections may be divided and subdivided as follows:
  - A, B, C
  - A a,b,c; B a,b,c; C a,b,c
  - A a-1, a-2, b-1, b-2; B, a-1, a-2, b-1, b-2
- The number of the figure must NOT be included in the image, especially if placed on the overlapping part of the image. Instead, the file itself should be named using the figure number.
- A, B, Cs must be placed to the top left of each section of the figure, NOT overlapping the image.



### 3.3.8 Figure appearance

- Figure backgrounds must be white. Grey backgrounds (or backgrounds of any other color) are NOT acceptable.
- White space surrounding figures should be cropped so that the image is as close to the edges of the page as possible.
- Figures and specific sections of figures should NOT be surrounded by borders (frames).
- Figures should NOT be stretched out of proportion (distorted) horizontally or vertically.
- Yellow must NOT be used for lines in diagrams. Any darker color may be used instead.
- Line art should be dark, and lines and labeling thick enough to be clearly visible, even at small sizes.
- Charts, graphs and diagrams should NOT use more than 5 shades of grey. Patterns are acceptable.
- In charts, graphs and diagrams, unnecessary colors should be avoided (e.g., color that does not impart any additional information and is used for slight emphasis only, or color that can be replaced by shades of grey, patterns or shapes).

### 3.3.9 Copyright

- If a figure or table has been published previously (even if you were the author of the manuscript), copyright permission for re-use of the figure or table will often be required.
- You must acknowledge the original source and submit written permission from the copyright holder to reproduce the material where necessary.
- As an author of your manuscript, you are responsible for obtaining permissions to use material owned by others.

### 3.4 Figure legends

- Figure legends should be listed one after the other, as part of the text document and separate from the figure files.
- Figure legends must begin with a brief title for the whole figure and continue with a short description of each panel or part.
- All symbols (e.g. asterisks, hashtags) used to indicate significant differences in the figures must be defined accordingly in the figure legend.
- All error bars must be defined in the figure legend.
- Legends should not contain any details of the methods.

### 3.5 Tables

- Each table should be submitted on a separate Word file.
- Times New Roman. Font size 12. Spacing 1.5.
- Label using Roman numerals, i.e. Table I, Table II, etc.
- Include a short title.
- All symbols and abbreviations should be defined immediately below the table.

### 3.6 Supplementary material

Supplementary data and other materials can now be submitted to all of our journals to support and enhance research manuscripts. The material should be directly relevant to your paper and can include information in the form of audio, video, tables and figures. Supplementary data should be submitted together with the original manuscript, as these data will undergo the peer review process as well.

Acceptable file types are: JPG, JPEG, EPS, TIFF, TIF, DOC, DOCX, ODT, ODF and for video/audio: MKV, MOV, AVI, MPG, MPEG, MP4.

Please note that supplementary materials should be referenced in the text as: 'Fig. S1', 'Table SI, Table SII e.t.c', 'Data S1' or 'Appendix S1'. Supplementary video/audio clips should be called 'Supplementary\_Data1.mp4'.

### 3.7 Nomenclature and abbreviations

- Naming of chemicals should follow that given in Chemical Abstracts Service.
- Use standard abbreviations where possible. Use the generic name of any drug unless making claims about a specific brand or formulation.
- New abbreviations must be defined at first usage.
- When reporting sequence variants and phenotypes, please follow the recommendations of the Human Variome Project Consortium for describing sequence variants (Human Genome Variation Society) and phenotypes (Human Phenotype Ontology).

### 3.8. Characterization of chemical and biomolecular materials

Manuscripts submitted to *Spandidos publications* must contain adequate data to support their assignment of identity and purity for each new compound described in the manuscript. Authors should provide a statement confirming the source, identity and purity of known

compounds that are key in their study, even if they are purchased or resynthesized using published methods.

#### 3.8.1 Chemical identity

Chemical identity for organic and organometallic compounds should be established through spectroscopic analysis. Standard peak listings for  $^1\text{H}$  NMR and proton-decoupled  $^{13}\text{C}$  NMR spectra should be provided for all new compounds. Other NMR data such as  $^{31}\text{P}$  NMR or  $^{19}\text{F}$  NMR should be reported when appropriate. For new materials, authors should also provide mass spectral data to support molecular weight identity. UV or IR spectral data may be reported for the identification of characteristic functional groups, when appropriate. Melting-point ranges should be provided for crystalline materials. Specific rotations may be reported for chiral compounds. For known compounds, references rather than detailed procedures should be provided, unless the authors followed a modification of the published methods.

#### 3.8.2 Combinatorial compound libraries

Standard characterization data for a diverse panel of library components should be included in manuscripts describing the preparation of combinatorial libraries.

#### 3.8.3 Biomolecular identity

If direct structural analysis of new biopolymeric materials (e.g. oligosaccharides, peptides, nucleic acids) by NMR spectroscopy is not possible, authors must provide evidence of identity based on sequence (when appropriate) and mass spectral characterization.

#### 3.8.4 Biological constructs

Authors should be able to provide sequencing or functional data that validates the identity of their biological constructs (plasmids, fusion proteins, site-directed mutants) upon request.

#### 3.8.5 Sample purity

Evidence of sample purity must be shown for each new compound. For organic and organometallic compounds, purity may be demonstrated by high-field  $^1\text{H}$  NMR or  $^{13}\text{C}$  NMR, while elemental analysis is encouraged for small molecules. Quantitative analytical methods, including chromatographic (e.g. GC, HPLC) or electrophoretic analyses may be used for small molecules and polymeric materials.

#### 3.8.6 Spectral data

Detailed spectral data for new compounds should be provided in the Materials and methods section. Figures containing spectra must be made available to the Editor upon request. The authors should explain how specific, unambiguous NMR assignments were made in the Materials and methods section.

#### 3.8.7 Crystallographic data for small molecules

Authors reporting new structures of small molecules from crystallographic analysis must be able to provide a standard crystallographic information file (.cif); structure factors for each structure; and a structural figure with probability ellipsoids upon request. The structure factors and structural output should be checked using International Union of Crystallography [checkCIF](#). Crystallographic data for small molecules should be submitted

to the Cambridge Structural Database, and the deposition number must be referenced in the manuscript.

#### 3.8.8 Macromolecular structural data

Manuscripts reporting new structures should contain a table summarizing structural and refinement statistics, and the different programs used in the analysis should be mentioned and referenced. To assess the quality of the structural data, a stereo image of a portion of the electron density map (for crystallography papers); of the superimposed lowest energy structures (>10; for NMR papers); or of the entire structure (as a backbone trace) if the reported structure represents a novel overall fold should be provided upon request. For cryo-EM structures, a representative micrograph showing individual particles should be provided at submission. Protein structures should be deposited in the Protein Data Bank PDB (<https://www.rcsb.org/>) and the deposition number must be referenced in the manuscript.

Table I. Data collection and refinement statistics.

Crystal name	
Data collection	
Space group	
Cell dimensions	
$a, b, c$ (Å)	
$\alpha, \beta, \gamma$ (°)	
Resolution (Å)	##() <sup>a</sup>
$R_{\text{sym}}$ or $R_{\text{merge}}$	##()
$I/\sigma I$	##()
Completeness (%)	##()
Redundancy	##()
Refinement	
Resolution (Å)	
No. reflections	
$R_{\text{work}}/R_{\text{free}}$	
No. atoms	
Protein	
Ligand/ion	
Water	
$B$ -factors	
Protein	
Ligand/ion	
Water	
R.m.s. deviations	
Bond lengths (Å)	
Bond angles (°)	

<sup>a</sup>Values in parentheses are for the highest-resolution shell. The number of crystals for each structure should be indicated in the table legend.

Ramachandran statistics should be included in the Materials and methods section, at the end of Refinement subsection. The wavelength of data collection, temperature and beamline should be specified in the Materials and methods sections.

### 3.8.9 Chemical structures

Structures of compounds should be prepared using a drawing program, such as ChemDraw. Please ensure to use the following settings (ACS Style sheet in ChemDraw):

- Chain angle: 120°
- Bond spacing: 18% of width
- Fixed length: 14.4 pt (0.508 cm, 0.2 in.)
- Bold width: 2.0 pt (0.071 cm, 0.0278 in.)
- Line width: 0.6 pt (0.021 cm, 0.0084 in.)
- Margin width: 1.6 pt (0.056 cm, 0.0222 in.)
- Hash spacing: 2.5 pt (0.088 cm, 0.0347 in.)
- Font: Arial/Helvetica
- Size: 10 pt

### 3.9 References

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  - Spandidos DA and Wilkie NM: Malignant transformation of early passage rodent cells by a single mutated human oncogene. *Nature* 310: 469-475, 1984.
  - Spandidos A, Wang X, Wang H and Seed B: PrimerBank: A resource of human and mouse PCR primer pairs for gene expression detection and quantification. *Nucleic Acids Res* 38: D792-D799, 2010.
  - Barbu CG, Arsene AL, Florea S, Albu A, Sirbu A, Martin S, Nicolae AC, Burcea-Dragomiroiu GTA, Popa DE, Velescu BS, *et al*: Cardiovascular risk assessment in osteoporotic patients using osteoprotegerin as a reliable predictive biochemical marker. *Mol Med Rep* 16: 6059-6067, 2017.
  - Hall A, Morris JDH, Price B, Lloyd A, Hancock JF, Gardener S, Houslay MD, Wakelam MJO and Marshall CJ: The function of the mammalian Ras proteins. In: *Ras oncogenes*. Spandidos DA (ed.) Plenum Publ. Corp., New York, pp99-104, 1989.
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- Funding
- Availability of data and materials
- Authors' contributions
- Ethics approval and consent to participate
- Patient consent for publication
- Competing interests
- Authors' information (optional)
- Use of artificial intelligence tools (to be included only when AI tools are used)

If any of the sections are not relevant to your manuscript, please include the heading and write 'Not applicable' for that section.

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A list of recommended repositories is featured below; consulting the Registry of Research Data Repositories (<http://www.re3data.org/>) may also be useful in this regard.

### **List of suggested repositories**

<b>Type of data</b>	<b>Repositories</b>
Research output in general (including supplementary figures and data, and multimedia adjuncts)	Figshare SimTK
Protein sequences	UniProt
Macromolecular structure	Worldwide Protein Data Bank (wwPDB) Biological Magnetic Resonance Data Bank (BMRB)
Crystallographic data for small molecules	The Cambridge Structural Database
DNA and RNA sequences	DNA DataBank of Japan (DDBJ) European Nucleotide Archive (ENA) Gene Expression Omnibus (GEO) China National GeneBank DataBase (CNGBdb)
Sanger sequencing	GenBank
Deep sequencing	Sequence Read Archive

Genetic polymorphisms	dbSNP  dbVAR  European Variation Archive (EVA)
Linked genotype and phenotype data	dbGAP  European Genome-phenome Archive (EGA)
Microarray data (must be compliant with Minimum Information About a Microarray Experiment, or MIAME)	Gene Expression Omnibus (GEO)  ArrayExpress
Flow cytometry	<u>FlowRepository</u>

### Authors' contributions

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