#### <u>Materials Design Analysis Reporting (MDAR)</u> Checklist for Authors

The MDAR framework establishes a minimum set of requirements in transparent reporting applicable to studies in the life sciences (see Statement of Task: doi:10.31222/osf.io/9sm4x.). The MDAR checklist is a tool for authors, editors and others seeking to adopt the MDAR framework for transparent reporting in manuscripts and other outputs. Please refer to the MDAR Elaboration Document for additional context for the MDAR framework.

## **Materials**

Antibodies	Yes (indicate where provided: section/paragraph)	n/a
For commercial reagents, provide supplier	CD31:CST CD31 (PECAM-1) (89C2) Mouse mAb #3528	
name, catalogue number and RRID, if available.	CD34:CST CD34 (ICO115) Mouse mAb #3569	

Cell materials	Yes (indicate where provided: section/paragraph)	n/a
Cell lines: Provide species information, strain.	Lung cancer cells:H1299,A549,H358,H2170,H226,H1703	
Provide accession number in repository OR	Timing and details of latest authentication testing: April	
supplier name, catalog number, clone number,	2022.CT value of mycoplasma primers in each cell was	
OR RRID	greater than 30	
Primary cultures: Provide species, strain, sex of		n/a
origin, genetic modification status.		

Experimental animals	Yes (indicate where provided: section/paragraph)	n/a
Laboratory animals: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository <b>OR</b> supplier name, catalog number, clone number, <b>OR</b> RRID	Nude Mouse, BALB/C, male,6 weeks old, don't with genetic modification	
Animal observed in or captured from the field: Provide species, sex and age where possible		n/a
Model organisms: Provide Accession number in repository (where relevant) OR RRID		n/a

Plants and microbes	Yes (indicate where provided: section/paragraph)	n/a
<b>Plants:</b> provide species and strain, unique accession number if available, and source (including location for collected wild specimens)		n/a
Microbes: provide species and strain, unique accession number if available, and source		n/a

Human research participants	Yes (indicate where provide	ed: section/paragraph)	n/a
Identify authority granting ethics approval (IRB or	Affiliated Hospital of Jiangna	an University:LS2021019	
equivalent committee(s), provide reference number	Age(years)	62.9±7.9	-
for approval.	Sex, n(%)		
	Males	27(75%)	
	Females	9(25%)	
Provide statement confirming informed consent			n/a
obtained from study participants.			
Report on age and sex for all study participants.			n/a

### **Design**

Study protocol	Yes (indicate where provided: section/paragraph)	n/a
For clinical trials, provide the trial registration		n/
number <b>OR</b> cite DOI in manuscript.		а

Laboratory protocol	Yes (indicate where provided: section/paragraph)	n/a
Provide DOI or other citation details if detailed step-		n/
by-step protocols are available.		а

Experimental study design (statistics details)	Yes (indicate where provided: section/paragraph)	n/a
State whether and how the following have been	Sample size was determined according to experimental ,Random	
done, or if they were not carried out.	selection of required samples without subjectivity	
Sample size determination	Sample size was determined according to experimental	
Randomisation	Pathological sections of lung cancer patients were randomly	
Blinding		n/a
Inclusion/exclusion criteria		n/a

Sample definition and in-laboratory replication	Yes (indicate where provided: section/paragraph)	n/a
State number of times the experiment was replicated in laboratory	Three times	
Define whether data describe technical or biological replicates	technical replicates	

Ethics	Yes (indicate where provided: section/paragraph)	n/a
Studies involving human participants: State details of authority granting ethics approval (IRB or equivalent	Affiliated Hospital of Jiangnan University reference number:LS2021019	
committee(s), provide reference number for approval.	reference number.LS2021019	
Studies involving experimental animals: State details	Jiangnan university	
of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.	reference number:JN.No20211115c0240130[452]	
Studies involving specimen and field samples: State if		n/
relevant permits obtained, provide details of		а
authority approving study; if none were required, explain why.		

Dual Use Research of Concern (DURC)	Yes (indicate where provided: section/paragraph)	n/a
If study is subject to dual use research of concern,		n/
state the authority granting approval and reference		а
number for the regulatory approval		

### **Analysis**

Attrition	Yes (indicate where provided: section/paragraph)	n/a
State if sample or data point from the analysis is		n/a
excluded, and whether the criteria for exclusion were		
determined and specified in advance.		

Statistics	Yes (indicate where provided: section/paragraph)	n/a
Describe statistical tests used and justify choice of	GraphPad Prism 8.0 tests for normal distribution and	
tests.	homogeneity of variance were conducted. The data are	
	expressed as the mean $\pm$ the standard error of the	
	mean (SEM) and were analyzed by Student's t test (two	
	groups) or one-way analysis of variance (ANOVA) with	
	Tukey's post hoc test (more than two groups). The data	
	with skewed distributions were tested by nonparametric	
	tests. The results with a P value < 0.05 were considered	
	statistically significant.	

Data Availability	Yes (indicate where provided: section/paragraph)	n/a
State whether newly created datasets are available,	No new datasets were created	
including protocols for access or restriction on		
access.		
If data are publicly available, provide accession		n/a
number in repository or DOI or URL.		
If publicly available data are reused, provide	Lung cancer single-cell sequencing data were obtained	
accession number in repository or DOI or URL, where	from a recent study.DOI: 10.1038/s41467-021-22801-0	
possible.	,	

Code Availability	Yes (indicate where provided: section/paragraph)	n/a
For all newly generated code and software essential	the DEGs were identified using the FindMarkers	
for replicating the main findings of the study:	function in the "Seurat" package of R software	
State whether the code or software is available.		n/a
If code is publicly available, provide accession		n/a
number in repository, or DOI or URL.		

#### Reporting

Adherence to community standards	Yes (indicate where provided: section/paragraph)	n/a
MDAR framework recommends adoption of		
discipline-specific guidelines, established and		
endorsed through community initiatives. Journals		
have their own policy about requiring specific		
guidelines and recommendations to complement		
MDAR.		
State if relevant guidelines (eg., ICMJE, MIBBI,	ICMJE guidelines were followed, as the journal follows	
ARRIVE) have been followed, and whether a checklist	ICMJE recommendations for publication.	
(eg., CONSORT, PRISMA, ARRIVE) is provided with	·	
the manuscript.		

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# The ARRIVE guidelines 2.0: author checklist

## The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including:	
		a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated.	
		b. The experimental unit (e.g. a single animal, litter, or cage of animals).	
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used.	
		b. Explain how the sample size was decided. Provide details of any <i>a priori</i> sample size calculation, if done.	
Inclusion and exclusion criteria	3	<ul> <li>Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established a priori. If no criteria were set, state this explicitly.</li> </ul>	
		b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so.	
		c. For each analysis, report the exact value of <i>n</i> in each experimental group.	
Randomisation	4	<ul> <li>State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence.</li> </ul>	
		<ul> <li>Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.</li> </ul>	
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes).	
		b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	
Statistical methods	7	Provide details of the statistical methods used for each analysis, including software used.	
		b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight.	
		b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including:	
		a. What was done, how it was done and what was used.	
		b. When and how often.	
		c. Where (including detail of any acclimatisation periods).	
		d. Why (provide rationale for procedures).	
Results	10	For each experiment conducted, including independent replications, report:	
		<ul> <li>a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range).</li> </ul>	
		b. If applicable, the effect size with a confidence interval.	

# The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

ltem		Recommendation	Section/line number, or reason for not reporting
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	
Background	12	<ul><li>a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach.</li><li>b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.</li></ul>	
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	
Animal care and monitoring	16	<ul> <li>a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress.</li> <li>b. Report any expected or unexpected adverse events.</li> <li>c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.</li> </ul>	
Interpretation/ scientific implications	17	<ul><li>a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature.</li><li>b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.</li></ul>	
Generalisability/ translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	
Data access	20	Provide a statement describing if and where study data are available.	
Declaration of interests	21	<ul><li>a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated.</li><li>b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.</li></ul>	

