**Materials Design Analysis Reporting (MDAR)**

**Checklist for Authors**

The [MDAR framework](https://osf.io/xfpn4/) establishes a minimum set of requirements in transparent reporting mainly applicable to studies in the life sciences.

*eLife* asks authors to **provide detailed information within their article** to facilitate the interpretation and replication of their work. Authors can also upload supporting materials to comply with relevant reporting guidelines for health-related research (see [EQUATOR Network](http://www.equator-network.org/%20)), life science research (see the [BioSharing Information Resource](http://biosharing.org/)), or animal research (see the [ARRIVE Guidelines](http://www.plosbiology.org/article/info:doi/10.1371/journal.pbio.1000412) and the [STRANGE Framework](https://doi.org/10.1038/d41586-020-01751-5); for details, see *eLife*’s [Journal Policies](https://reviewer.elifesciences.org/author-guide/journal-policies)). Where applicable, authors should refer to any relevant reporting standards materials in this form.

For all that apply, please note **where in the article** the information is provided. Please note that we also collect information about data availability and ethics in the submission form.

**Materials:**

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| **Newly created materials** | **Indicate where provided: section/figure legend** | **N/A** |
| The manuscript includes a dedicated "materials availability statement" providing transparent disclosure about availability of newly created materials including details on how materials can be accessed and describing any restrictions on access. | All materials are available upon reasonable request to the corresponding author (statement in material and methods) |  |
|  |  |  |
| **Antibodies** | **Indicate where provided: section/figure legend** | **N/A** |
| For commercial reagents, provide supplier name, catalogue number and [RRID](https://scicrunch.org/resources), if available. | 1. anti-CD3 (clone HIT3a): (BD Biosciences Cat# 555336, RRID:AB\_395742)  2. anti-CD28 (clone 28.2): (BD Biosciences Cat# 555725, RRID:AB\_396068)  3. anti-ARP3 (clone FMS338): (Sigma-Aldrich Cat# A5979, RRID:AB\_476749)  4. anti-p16 ARC/ARPC5: (Synaptic Systems Cat# 305 011, RRID:AB\_887896)  5. anti-ARPC5L: (GeneTex Cat# GTX120725, RRID:AB\_11172404)  6. anti-ARPC1A: (Sigma-Aldrich Cat# HPA004334, RRID:AB\_1078215)  7. anti-ARPC1B: (Santa Cruz Biotechnology Cat# sc-137125, RRID:AB\_2289927)  8. anti-ARPC2: (abcam Cat# ab133315)  9. anti-ARPC3: (Sigma-Aldrich Cat# HPA006550, RRID:AB\_1854869)  10. anti-ARPC4: (Novus Cat# NBP1-69003, RRID:AB\_11015934)  11. anti-WAVE2: (Santa Cruz Biotechnology Cat# sc-373889, RRID:AB\_10917394)  12. anti-NWASP: (Sigma-Aldrich Cat# HPA005750, RRID:AB\_1854729)  13. anti-WASHC5: (Sigma-Aldrich Cat# HPA070916)  14. anti-Tubulin: (Cell Signaling Technology Cat# 3873, RRID:AB\_1904178)  15. anti-GAPDH: (Cell Signaling Technology Cat# 2118, RRID:AB\_561053)  16. anti-GAPDH: (Bio-Rad Cat# MCA4740, RRID:AB\_2107457)  17. anti-mCherry: (Novus Cat# NBP1-96752SS, RRID:AB\_11008969)  18. anti-mCherry: (Abcam Cat# ab167453, RRID:AB\_2571870)  19. anti-pTyr: (Santa Cruz Biotechnology Cat# sc-18182, RRID:AB\_670513)  20. anti-pSLP76: (Abcam Cat# ab75829, RRID:AB\_2136886)  21. Peroxidase AffiniPure Goat Anti-Rabbit IgG: (Jackson ImmunoResearch Labs Cat# 111-035-144, RRID:AB\_2307391)  22. Peroxidase AffiniPure Goat Anti-Mouse IgG: (Jackson ImmunoResearch Labs Cat# 115-035-003, RRID:AB\_10015289)  23. Goat anti-mouse Alexa Fluor 568: (Thermo Fisher Scientific Cat# A-21124, RRID:AB\_2535766)  24. Goat anti-rabbit Alexa Fluor 568: (Thermo Fisher Scientific Cat# A-11011, RRID:AB\_143157)  25. Goat anti-rabbit Alexa Fluor 647: (Thermo Fisher Scientific Cat# A-21246, RRID:AB\_2535814)  26. anti-Arp2: (Santa Cruz Biotechnology Cat# sc-137250, RRID:AB\_2221845)  27. anti-hnRNPL [4D11]: (Abcam Cat# ab6106, RRID:AB\_305294)  28. Brilliant Violet 421™ anti-human TNF-α Antibody (Biolegend, Cat#502932, RRID:AB\_10960738)  29. APC anti-human IL-2 antibody (Biolegend, Cat# 500311, RRID:AB\_315098)  30. FITC Mouse Anti-Human CD3 (BD Biosciences Cat# 561802, RRID:AB\_10893003)  31. ChromoTek RFP-Trap Magnetic Agarose (Proteintech, Cat# rtma-100, AB\_2631363) |  |
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| **DNA and RNA sequences** | **Indicate where provided: section/figure legend** | **N/A** |
| Short novel DNA or RNA including primers, probes: Sequences should be included or deposited in a public repository.    qRT-PCR primers as well as shRNA and CRISPR-sgRNA sequences are obtained from public/ respective company open databases, as mentioned in the materials & methods section. | qPCR primer sequences:  GAPDH  Fwd: GAAGGTGAAGGTCGGAGTC  Rev: GAAGATGGTGATGGGATTTC  ARP2  Fwd: CACCTGTGGGACTACACATTTG  Rev: TGGTTGGGTTCATAGGAGGTTC  ARP3  Fwd: TTCAACCATGTTCAGGGACTTTG  Rev: ACCACCACTCAATTCCTCACT  ARPC1A  Fwd: GCAGACCGCAATGCCTATGT  Rev: CCCACCAGTCATTTTCAGACTC  ARPC1B  Fwd: AAGGTGTCGGATCTTTTCAGCC  Rev: CCGCAGCTACTGCTGGATTC  ARPC2  Fwd: AAAGAGGGTGTACGGGAGTTT  Rev: TGGATGCCGGAAGATTTTCAAG  ARPC3  Fwd: GTGCAATTCCAAAAGCCAAGG  Rev: GGCTCATCACTTCATCTTCC  ARPC4  Fwd: AACGACACAACAAGCCGGAA  Rev: GGCACAAAATCTTCTCGATCTCA  ARPC5  Fwd: TGGTGTGGATCTCCTAATGAAGT  Rev: CACGAACAATGGACCCTACTC  ARPC5L  Fwd: TCTCCCGTCAACACCAAGAAT  Rev: GCCTGCTCAATCTCACTGCT  shRNA target sequences:  ARPC1A CCCTGGTGATCCTGAGAATTA  ARPC1B GCTGACCTTCATCACAGACAA  ARPC5 GTTCAATCTCTGGACAAGAAT  ARPC5L GAAAGTGCTCACAAACTTCAA  Single guideRNA (sgRNA) sequences:  ARPC5  sgRNA1: GCAGUGCUAUGUUACUGCAA  sgRNA2: CAAUGCUGCCUGCCCGGUCC  sgRNA3: UGACUCUUGGUGUUGAUAGG  ARPC5L  sgRNA1: UCGUCUGCAGGAGCGAGCCC  sgRNA2: ACUGCGCUGCUAUUUUCUGU  sgRNA3: AUUCGUCGAUGUCCACCCGG  NWASP  sgRNA1: GCAGACUAUGUCUUCAGCAG  sgRNA2: CUUAAAAAAUAAGAUCUCUG  sgRNA3: ACCUUUUGGGCCGUCGACAA  WAVE2  sgRNA1: CUUUCCAGGUAAAUAUGCAG  sgRNA2: UCGGUCGACCCUCUCAGCAA  sgRNA3: CUUCUCUCAGUGUCACUGCA  WASHC5  sgRNA1: GGAUGCUAAGCCAGAGCUAC  sgRNA2: UCUAGAUGAUCUCAAUGAAG  sgRNA3: CUUAUAUGGAGUUAUGCUAC |  |
|  |  |  |
| **Cell materials** | **Indicate where provided: section/figure legend** | **N/A** |
| Cell lines: Provide species information, strain. Provide accession number in repository OR supplier name, catalog number, clone number, OR RRID. | HEK293T cells (RRID CVCL\_0063) were used for lentivirus production.  Jurkat Tag (JTag) cells (RRID CVCL\_C831) , clone E6-1, stably expressing nuclear lifeact.GFP (Tsopoulidis et al 2019, Sci Immunol) used in all figures except Fig4 I-J, Fig5 C-D and Fig S5.  CEM derived A3.01 cells (RRIDCVCL\_6244) used for Fig4 I-J, Fig5 C-D and Fig S5.  Raji B cells (RRID CVCL\_0511) were used for Fig 1A-D. |  |
| Primary cultures: Provide species, strain, sex of origin, genetic modification status. | Peripheral blood mononuclear cells from anonymous blood donors received from the Heidelberg University Hospital Blood bank, were used in Fig 2 A-C. |  |
|  |  |  |
| **Experimental animals** | **Indicate where provided: section/figure legend** | **N/A** |
| Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository OR supplier name, catalog number, clone number, OR RRID. |  | x |
| Animal observed in or captured from the field: Provide species, sex, and age where possible. |  | x |
|  |  |  |
| **Plants and microbes** | **Indicate where provided: section/figure legend** | **N/A** |
| Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens). |  | x |
| Microbes: provide species and strain, unique accession number if available, and source. |  | x |
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| **Human research participants** | **Indicate where provided: section/figure legend) or state if these demographics were not collected** | **N/A** |
| If collected and within the bounds of privacy constraints report on age, sex, gender and ethnicity for all study participants. |  | **x** |

**Design:**

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| **Study protocol** | **Indicate where provided: section/figure legend** | **N/A** |
| If the study protocol has been pre-registered, provide DOI. For clinical trials, provide the trial registration number OR cite DOI. |  | x |
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| **Laboratory protocol** | **Indicate where provided: section/figure legend** | **N/A** |
| Provide DOI OR other citation details if detailed step-by-step protocols are available. |  | x |
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| **Experimental study design (statistics details) \*** | | |
| **For in vivo studies: State whether and how the following have been done** | **Indicate where provided: section/figure legend. If it could have been done, but was not, write “not done”** | **N/A** |
| Sample size determination |  | X |
| Randomisation |  | **X** |
| Blinding |  | X |
| Inclusion/exclusion criteria |  | X |
|  |  |  |
| **Sample definition and in-laboratory replication** | **Indicate where provided: section/figure legend** | **N/A** |
| State number of times the experiment was replicated in the laboratory. | Provided in figure legend |  |
| Define whether data describe technical or biological replicates. | Number of independent experiments and technical replicates provided in figure legend where applicable |  |
|  |  |  |
| **Ethics** | **Indicate where provided: section/submission form** | **N/A** |
| Studies involving human participants: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval. |  | x |
| Studies involving experimental animals: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval. |  | x |
| Studies involving specimen and field samples: State if relevant permits obtained, provide details of authority approving study; if none were required, explain why. | Ethics vote not required as blood samples were obtained from volunteers were fully anonymized |  |
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| **Dual Use Research of Concern (DURC)** | **Indicate where provided: section/submission form** | **N/A** |
| If study is subject to dual use research of concern regulations, state the authority granting approval and reference number for the regulatory approval. |  | x |

**Analysis:**

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| **Attrition** | **Indicate where provided: section/figure legend** | **N/A** |
| Describe whether exclusion criteria were pre-established. Report if sample or data points were omitted from analysis. If yes, report if this was due to attrition or intentional exclusion and provide justification. |  | x |
|  |  |  |
| **Statistics** | **Indicate where provided: section/figure legend** | **N/A** |
| Describe statistical tests used and justify choice of tests. | Provided in each figure legend and in the materials and methods section where applicable |  |
|  |  |  |
| **Data availability** | **Indicate where provided: section/submission form** | **N/A** |
| For newly created and reused datasets, the manuscript includes a data availability statement that provides details for access (or notes restrictions on access). | Source data uploaded on university repository and link provided in the data availability statement in the manuscript |  |
| When newly created datasets are publicly available, provide accession number in repository OR DOI and licensing details where available. |  | x |
| If reused data is publicly available provide accession number in repository OR DOI, OR URL, OR citation. | Described in M&M section: PMID 28091601 & GEO accession no. GSE126030 (Fig. 2B-C & S2C-E) |  |
|  |  |  |
| **Code availability** | **Indicate where provided: section/figure legend** | **N/A** |
| For any computer code/software/mathematical algorithms essential for replicating the main findings of the study, whether newly generated or re-used, the manuscript includes a data availability statement that provides details for access or notes restrictions. |  | x |
| Where newly generated code is publicly available, provide accession number in repository, OR DOI OR URL and licensing details where available. State any restrictions on code availability or accessibility. |  | x |
| If reused code is publicly available provide accession number in repository OR DOI OR URL, OR citation. |  | x |

**Reporting:**

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives.

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| **Adherence to community standards** | **Indicate where provided: section/figure legend** | **N/A** |
| State if relevant guidelines (e.g., ICMJE, MIBBI, ARRIVE, STRANGE) have been followed, and whether a checklist (e.g., CONSORT, PRISMA, ARRIVE) is provided with the manuscript. |  | x |

\* We provide the following guidance regarding transparent reporting and statistics; we also refer authors to [Ten common statistical mistakes to watch out for when writing or reviewing a manuscript](https://doi.org/10.7554/eLife.48175).

**Sample-size estimation**

* You should state whether an appropriate sample size was computed when the study was being designed
* You should state the statistical method of sample size computation and any required assumptions
* If no explicit power analysis was used, you should describe how you decided what sample (replicate) size (number) to use

**Replicates**

* You should report how often each experiment was performed
* You should include a definition of biological versus technical replication
* The data obtained should be provided and sufficient information should be provided to indicate the number of independent biological and/or technical replicates
* If you encountered any outliers, you should describe how these were handled
* Criteria for exclusion/inclusion of data should be clearly stated
* High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

**Statistical reporting**

* Statistical analysis methods should be described and justified
* Raw data should be presented in figures whenever informative to do so (typically when N per group is less than 10)
* For each experiment, you should identify the statistical tests used, exact values of N, definitions of center, methods of multiple test correction, and dispersion and precision measures (e.g., mean, median, SD, SEM, confidence intervals; and, for the major substantive results, a measure of effect size (e.g., Pearson's r, Cohen's d)
* Report exact p-values wherever possible alongside the summary statistics and 95% confidence intervals. These should be reported for all key questions and not only when the p-value is less than 0.05.

**Group allocation**

* Indicate how samples were allocated into experimental groups (in the case of clinical studies, please specify allocation to treatment method); if randomization was used, please also state if restricted randomization was applied
* Indicate if masking was used during group allocation, data collection and/or data analysis