Materials Design Analysis Reporting (MDAR) 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: page no/section/legend)	n/a
For commercial reagents, provide supplier name, catalogue number and RRID, if available.	Yes • Supplement pp 9-10 / "Analysis of B6 thymocyte development in vitro" ○ CD24 antibody, BD Biosciences, Cat# 553146, RRID:AB_394661 ○ Pacific Blue™ anti-mouse CD4 antibody, BioLegend, Cat# 100531, RRID:AB_493374 ○ PE anti-mouse CD8b.2 antibody, BioLegend, Cat# 140408, RRID:AB_10644002 ○ Ly-6A/E (Sca-1) Monoclonal Antibody (D7), FITC, eBioscience, Cat# 11-5981-81, RRID:AB_465332 ○ Rat Anti-CD117 Monoclonal Antibody, Allophycocyanin Conjugated, Clone 2B8, BD Biosciences Cat# 553356, RRID:AB_398536 ○ APC anti-mouse CD45 antibody, BioLegend Cat# 103112, RRID:AB_312977 ○ CD25 Monoclonal Antibody (PC61.5), PE-Cyanine5, eBioscience, Cat# 15-0251-82, RRID:AB_468733 ○ Rat Anti-Mouse CD44 Monoclonal Antibody, APC-Cy7 Conjugated, (BD Biosciences Cat# 560568, RRID:AB_1727481 ○ Brilliant Violet 711™ anti-mouse CD4 antibody, BioLegend, Cat# 100549, RRID:AB_11219396 ○ PerCP/Cyanine5.5 anti-mouse CD8a antibody, BioLegend, Cat# 100734, RRID:AB_2075238 ○ PE anti-mouse CD28 antibody, BioLegend Cat# 122010, RRID:AB_604078 ○ PE anti-mouse TCR beta chain antibody, BioLegend, Cat# 109207, RRID:AB_313430	

Cell materials	Yes (indicate where provided: page no/section/legend)	n/a

Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID	Yes • pp 7 / para.3 / lines 1-4: ○ OP9-DL4 stromal cell line: DOI: 10.4049/jimmunol.1000782 ○ OP9-DL4-MHC-deficient cell line: DOI: 10.1074/jbc.M116.752865 • pp 8 / para 2 / lines 6-7: ○ SCID.adh cells are thymic lymphoma cells isolated from SCID mice adapted for *in vitro* growth as in DOI: 10.1073/pnas.1504971112 • pp 8 / para 2 / line 2: ○ OP9-DL4-scVSV/H-2Kb: DOI: 10.1074/jbc.M116.752865 • Supplement pp 2 / "Molecular cloning and inclusion bodies expression of proteins" ○ Escherichia coli (for protein expression), One Shot BL21 Star (DE3), Thermo Fisher Scientific, Cat. No. C601003, Lot 1908669 ○ Escherichia coli (for cloning), One Shot™ MAX Efficiency™ DH5α-T1 R Thermo Fisher Scientific, Cat. No. 12297016	
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Yes • Supplement pp 9 / "Mice" ○ Mouse, C57BL/6NTac, No gender preference, Wild Type (+/+) ○ Mouse, B6.129S6-Rag2< tm1Fwa >N12, No gender preference, Homozygous (-/-)	

Experimental animals	**Yes (indicate where provided: page no/section/legend)**	**n/a**
Laboratory animals: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID	Yes • Supplement pp 9 / "Mice" ○ Mouse, C57BL/6NTac, E14.5, No gender preference, Wild Type (+/+), Taconic, stock number B6 ○ Mouse, B6.129S6-Rag2< tm1Fwa >N12, E14.5, No gender preference, Homozygous (-/-), Taconic stock number RAGN12	
Animal observed in or captured from the field: Provide species, sex and age where possible		X
Model organisms: Provide Accession number in repository (where relevant) **OR** RRID		X

Plants and microbes	**Yes (indicate where provided: page no/section/legend)**	**n/a**
Plants: provide species and strain, 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

Human research participants	**Yes (indicate where provided: page no/section/legend)**	**n/a**
Identify authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.		X

Provide statement confirming informed consent obtained from study participants.		X
Report on age and sex for all study participants.		X

Design

Study protocol	Yes (indicate where provided: page no/section/legend)	n/a
For clinical trials, provide the trial registration number **OR** cite DOI in manuscript.		X

Laboratory protocol	Yes (indicate where provided: page no/section/legend)	n/a

Provide DOI or other citation details if detailed step-by-step protocols are available.	Yes
	• pp 9 / para. 1 / line 4-5:
	  ○ Transduction of hematopoietic stem cells with TCR beta chain:DOI: 10.1073/pnas.1504971112
	• Supplement pp 2 / "Molecular cloning and inclusion bodies expression of proteins"
	  ○ DOI: 10.1074/jbc.M117.813493
	  ○ DOI: 10.1073/pnas.1504971112
	• Supplement pp 6 / "Structural determination, refinement and structural analysis"
	  ○ DOI: 10.1006/jmbi.1993.1648 (shape complementarity)
	• Supplement pp 9 / "Analysis of B6 thymocyte development in vitro"
	  ○ DOI: 10.1073/pnas.1504971112
	  ○ DOI: 10.1074/jbc.M116.752865
	• Supplement pp 10 / "N15β mutant functional analysis"
	  ○ DOI: 10.1074/jbc.M116.752865
	  ○ DOI: 10.1073/pnas.1504971112
	• Supplement pp 11 / "TCR β chain repertoire analysis of DN3 and DN4 thymocytes."
	  ○ DOI: 10.3389/fimmu.2013.00456	

Experimental study design (statistics details)	Yes (indicate where provided: page no/section/legend)	n/a
State whether and how the following have been done**, or** if they were not carried out.		
Sample size determination	Not carried out	
Randomisation	Not carried out	
Blinding	Not carried out	
Inclusion/exclusion criteria	No inclusion/exclusion criteria were implemented	

Sample definition and in-laboratory replication	Yes (indicate where provided: page no/section/legend)	n/a

State number of times the experiment was replicated in laboratory	Yes
	• Fig. 4, panel B, legend
	  ○ 4 times
	• Fig. 4, panel C, legend
	  ○ 5 times
	• Fig. 4, panel E, legend
	  ○ 3 times	

Define whether data describe technical or biological replicates	biological	

Ethics	Yes (indicate where provided: page no/section/legend)	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.	Yes • Supplement pp 9 / "Mice" ○ Dana-Farber Cancer Institute IRB/Institutional Animal Care and Use Committee (IACUC): Protocols # 03-138, 04-113	
Studies involving specimen and field samples: State if relevant permits obtained, provide details of authority approving study; if none were required, explain why.		X

Dual Use Research of Concern (DURC)	**Yes (indicate where provided: page no/section/legend)**	**n/a**
If study is subject to dual use research of concern, state the authority granting approval and reference number for the regulatory approval		X

Analysis

Attrition	Yes (indicate where provided: page no/section/legend)	n/a
State if sample or data point from the analysis is excluded, and whether the criteria for exclusion were determined and specified in advance.		X

Statistics	Yes (indicate where provided: page no/section/legend)	n/a

Describe statistical tests used and justify choice of tests.	Yes
	• Fig. 4, panels B, E:
	  ○ Parametric statistics (mean, standard, deviation, standard error of the mean) with comparison using Student's t test and 2-tailed probability determination.
	• Fig. 4, Panel C; Fig. S10:
	  ○ Box-and-Whisker plots with quartiles, median, mean, outliers and range.	

Data Availability	Yes (indicate where provided: page no/section/legend)	n/a

State whether newly created datasets are available, including protocols for access or restriction on access.	Yes
	• Fig. S8.
	  ○ NMR data: available upon request
	• Fig. 4 panel C
	  ○ preTCR β chain unique clonotypes: provided as a MS Excel file (Table S7)	

If data are publicly available, provide accession number in repository or DOI or URL.	Yes • pp 12 / "Data and materials availability" ○ X-ray data: Protein Data Bank accession codes: 6WL2, 6WL3, 6WL4, 7JI2	

If publicly available data are reused, provide accession number in repository or DOI or URL, where possible.	Yes
	• Fig. 2. / panel A, B legend
	  ○ PDB structures: 3OF6, 1KPU, 2CKB
	• pp 6 / para 2 / lines 3-4
	  ○ PDB structures on (Fig. 3C): 1VAC, 7JI2, 1KPV, 1VAD
	• Fig. S4 / panel A legend
	  ○ NMR data: DOIs: 10.1074/jbc.M117.813493, 10.1073/pnas.1504971112
	• Fig. S4 / panel E legend
	  ○ NMR data: DOI: 10.1007/s10858-019-00234-8
	• Fig. S6 / legend
	  ○ PDB structures: 3OF6, 6JXR
	• Fig. S7 / legend
	  ○ PDB structures: 1FYT, 3TZV, 1YPZ
	• Table S6
	  ○ PDB structures: 1DUZ,5VUE, 4NT6, 1LEG, 1VGK, 5T7G, 6DIG, 6HBY, 3LQZ, 1IAO, 6MNN, 1K2D, 1D9K, 3BZE, 1YDP, 3VJ6, 4GUP, 1NEZ, 1YPZ, 1ZS8, 1T7V, 1A6Z, 6C97, 1ZT4, 1KCG, 1HYR, 1JE6, 3D2U	

Code Availability	**Yes (indicate where provided: page no/section/legend)**	**n/a**
For all newly generated code and software essential for replicating the main findings of the study:		X
State whether the code or software is available.		X
If code is publicly available, provide accession number in repository, or DOI or URL.		X

Reporting

Adherence to community standards	**Yes (indicate where provided: page no/section/legend)**	**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.		X
State if relevant guidelines (eg., ICMJE, MIBBI, ARRIVE) have been followed, and whether a checklist (eg., CONSORT, PRISMA, ARRIVE) is provided with the manuscript.		X