Feb 10, 2026

# Optimizing Animal Randomization for In Vivo Experiments

Forked from a private protocol

DOI
https://dx.doi.org/10.17504/protocols.io.261ge19bov47/v1

Sylvain Carlioz[1]

[1]randmice

S Sylvain Carlioz
Stimunity



OPEN ACCESS

**DOI:** https://dx.doi.org/10.17504/protocols.io.261ge19bov47/v1

**External link:** https://randmice.com

**Protocol Citation:** Sylvain Carlioz 2026. Optimizing Animal Randomization for In Vivo Experiments. **protocols.io** https://dx.doi.org/10.17504/protocols.io.261ge19bov47/v1

**Manuscript citation:**
Bakhos Jneid *et al.*, Selective STING stimulation in dendritic cells primes antitumor T cell responses.*Sci. Immunol.***8**,eabn6612(2023).DOI:**10.1126/sciimmunol.abn6612**



**Protocol status:** Working
**We use this protocol and it's working**

**Created:** February 09, 2026

**Last Modified:** February 10, 2026

**Protocol Integer ID:** 242954

**Keywords:** randomization, mice, animal, animal randomization with randmice, animal randomization, randomization protocol, randmice, animals between the group, number of animal, animal, experiment, randomization, optimizing animal randomization for in vivo experiment, optimizing animal randomization, animals per group, combinations of animal assignment, experimental group, animal assignment, random assignment, laboratory animal, vivo experiment, mice, online tool randmice, testing combination, scale experiment, group, lowest covariate imbalance, distribution with the lowest covariate imbalance

## Abstract

This protocol describes a standardized procedure to distribute laboratory animals (mice, rats) into experimental groups using the online tool Randmice (https://randmice.com). The algorithm minimizes inter-group heterogeneity by iteratively testing combinations of animal assignments and selecting the distribution with the lowest covariate imbalance. This approach reduces operator bias, improves reproducibility, and supports compliance with the 3Rs principle (Replacement, Reduction, Refinement) as required by European Directive 2010/63/EU. The tool is optimized for small-scale experiments (N < 12 animals per group), where random assignment leads to high inter-group heterogeneity (Bertsimas et al., 2015).

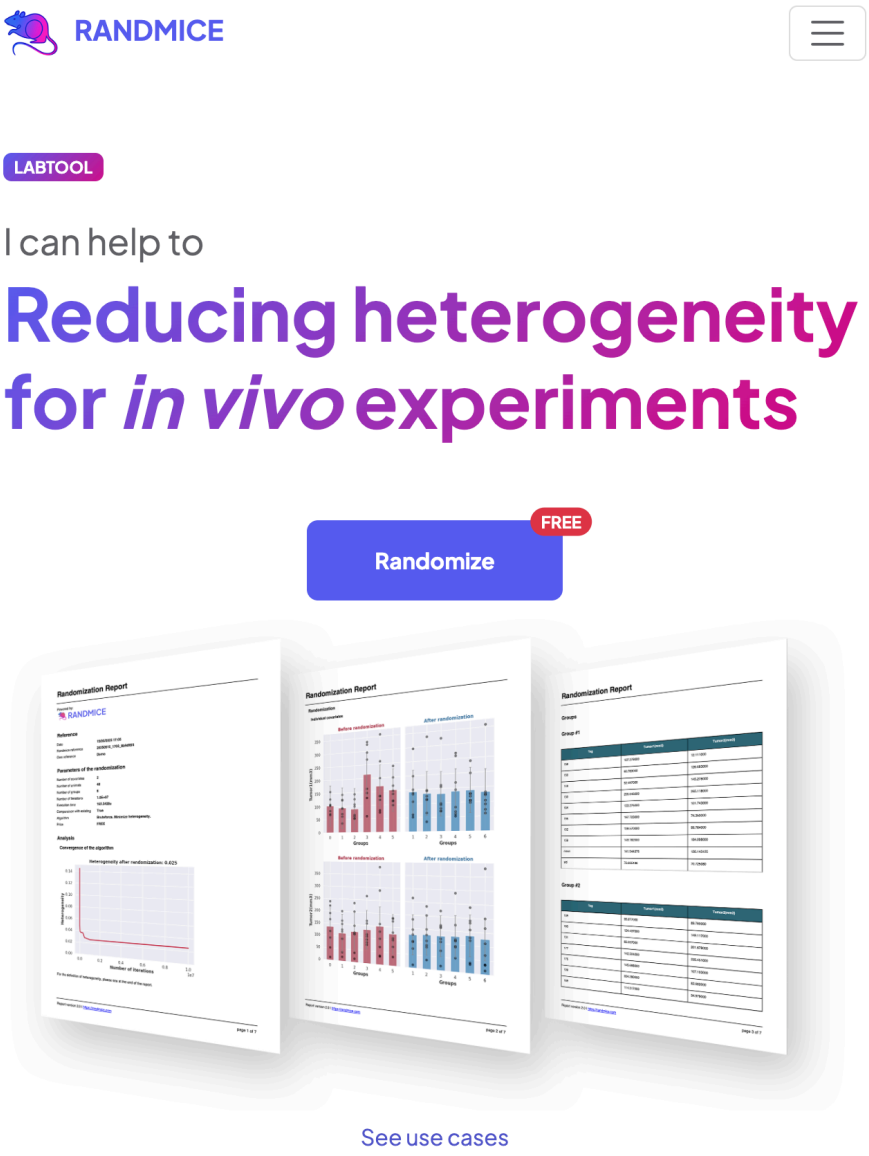
## Before randomization

1 Identify all animals with a unique tag

2 Measure the covariates to be balanced across groups. Example of common covariates are: body weight, tumor volume (left and/or right flank), blood pressure, any other quantitative variable relevant to the experiment

## Run randomization

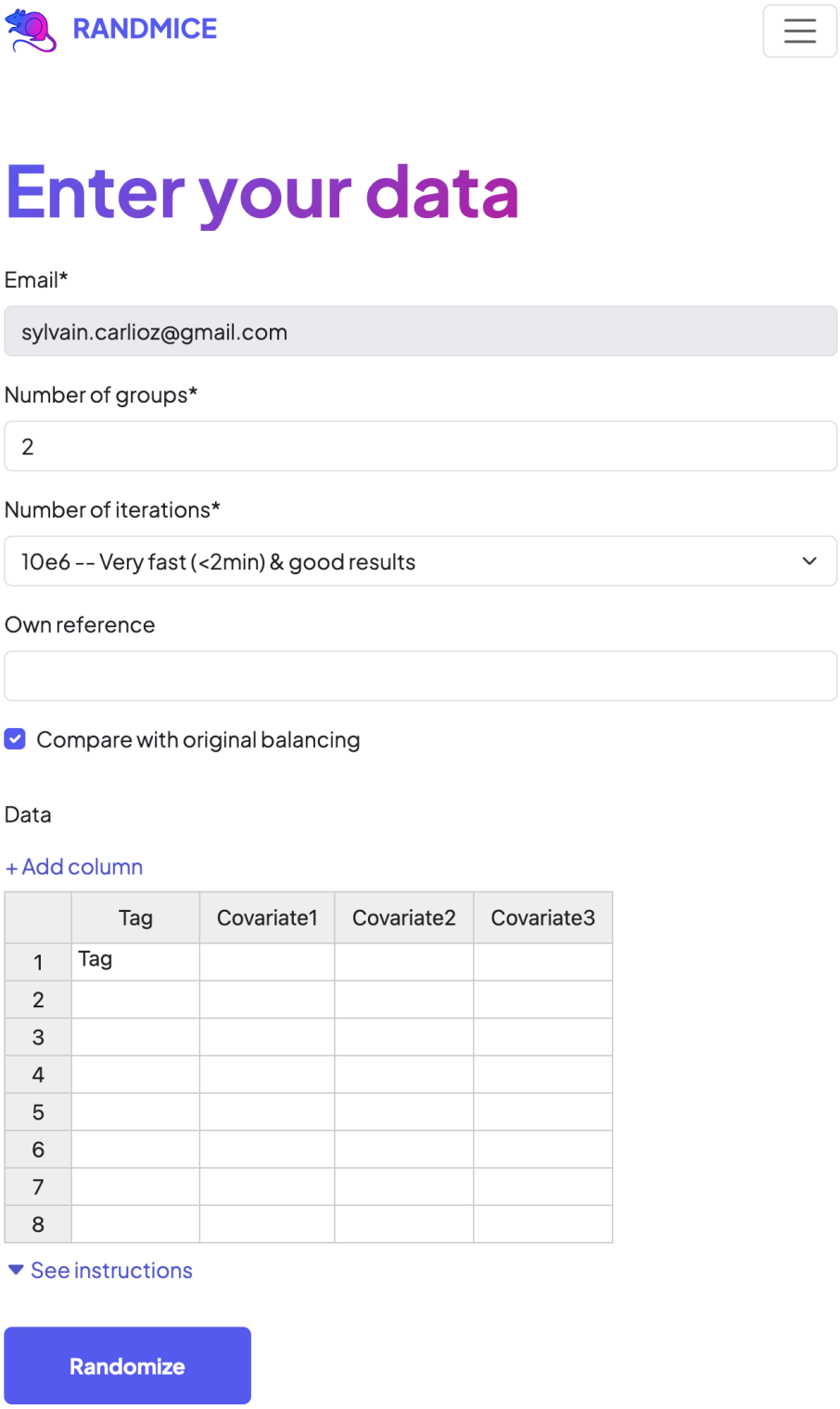
3 Open https://randmice.com via a web browser




Intro page at **randmice.com**

4 Login if you have an account. Otherwise, enter your email address to access the form.

5 You have now access to the form to enter your data




6 Enter the **Number of groups**

7 Choose the **Number of iterations** for the randomization. 10^6 is standard and run very fast (less than 2min) and generates good results. 10^7 is a bit longer and gives excellent results.

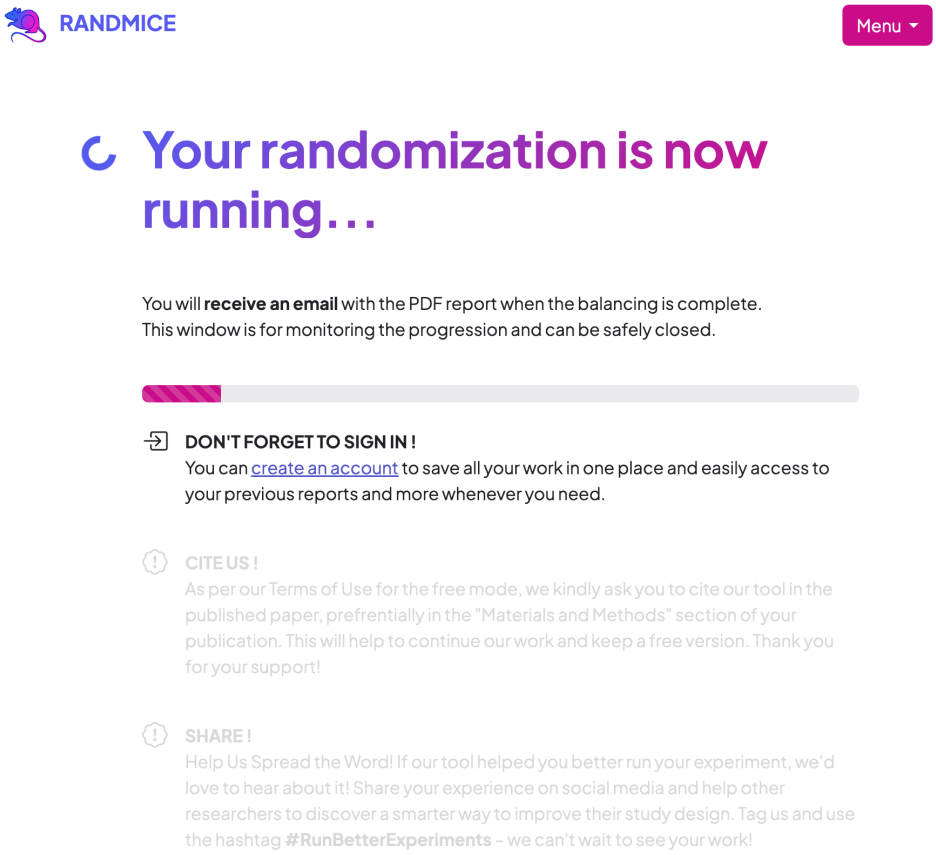
8 Fill your **Own reference** if needed

9 Check/uncheck **Compare with original balancing** if you want to compare your own randomization with the one from randmice.

10 Enter your data. The 1st fill is a header that describes each column. It can be any text. Tag must be unique identifier. See instructions link for more information.

11 Click **Randomize** when everything is ready.

The randomization will proceed. You can close the window if you want, the report will be sent in your mailbox when it is ready.




12 In your mailbox, you will find the report.

Below is an example:

# Randomization Report

Powered by:
RANDMICE

## Reference

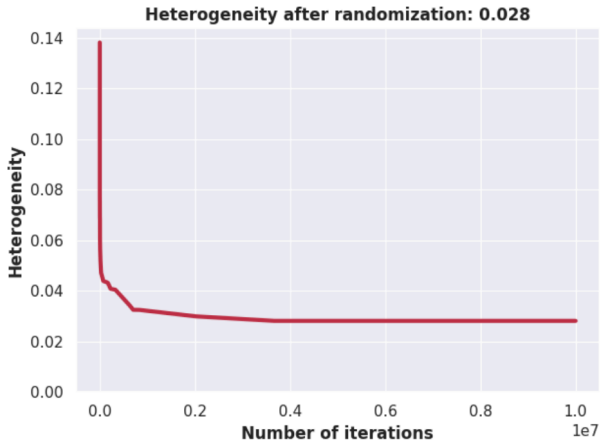
| | |
|---|---|
| Date | **23/05/2025 12:54** |
| Randmice reference | **20250523_1244_dece2d2d** |
| Own reference | |

## Parameters of the randomization

| | |
|---|---|
| Number of covariates | **2** |
| Number of animals | **48** |
| Number of groups | **6** |
| Number of iterations | **1.0E+07** |
| Execution time | **617.0227s** |
| Comparaison with existing | **True** |
| Algorithm | **Bruteforce. Minimize heterogeneity.** |
| Price | **FREE** |

## Analysis

### Convergence of the algorithm

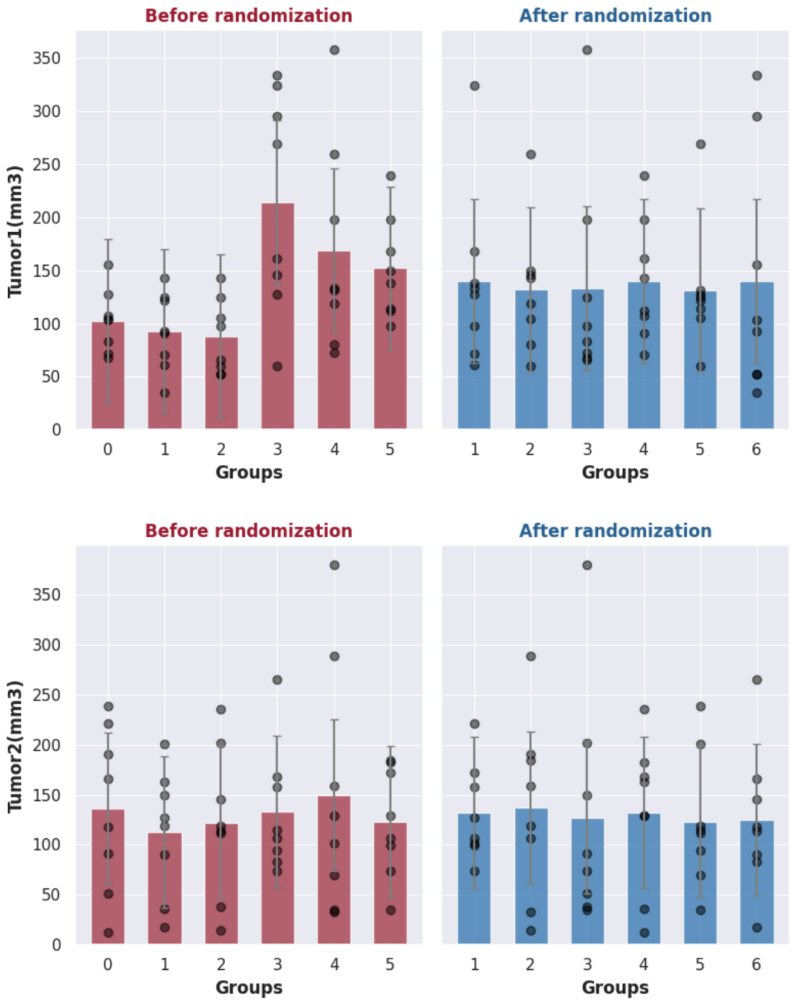



For the definition of heterogeneity, please see at the end of the report.

# Randomization Report

**Randomization**

**Individual covariates**



# Randomization Report

## Groups

### Group #1

| Tag | Tumor1(mm3) | Tumor2(mm3) |
|---|---|---|
| 145 | 71.241000 | 221.675000 |
| 150 | 60.783000 | 126.683000 |
| 153 | 127.070000 | 157.969000 |
| 197 | 323.870000 | 74.359000 |
| 164 | 133.275000 | 101.743000 |
| 142 | 97.687000 | 106.331000 |
| 185 | 167.752000 | 171.788000 |
| 152 | 138.570000 | 99.764000 |
| mean | 140.031000 | 132.539000 |
| std | 77.125159 | 45.084718 |

### Group #2

| Tag | Tumor1(mm3) | Tumor2(mm3) |
|---|---|---|
| 176 | 104.02100 | 190.393000 |
| 172 | 143.27800 | 118.588000 |
| 178 | 59.56500 | 14.132000 |
| 175 | 145.68800 | 107.103000 |
| 193 | 259.33200 | 289.193000 |
| 168 | 80.05200 | 158.372000 |
| 184 | 119.25800 | 32.801000 |

## Randomization Report

| | | |
|---|---|---|
| 159 | 149.78200 | 184.098000 |
| mean | 132.62200 | 136.835000 |
| std | 56.71387 | 83.572646 |

### Group #3

| Tag | Tumor1(mm3) | Tumor2(mm3) |
|---|---|---|
| 191 | 67.403000 | 90.791000 |
| 146 | 83.222000 | 51.581000 |
| 183 | 124.437000 | 149.117000 |
| 129 | 97.728000 | 38.022000 |
| 131 | 66.057000 | 201.678000 |
| 163 | 358.042000 | 379.814000 |
| 166 | 72.153000 | 34.606000 |
| 195 | 197.723000 | 74.350000 |
| mean | 133.345625 | 127.494875 |
| std | 94.282813 | 109.660727 |

### Group #4

| Tag | Tumor1(mm3) | Tumor2(mm3) |
|---|---|---|
| 158 | 107.279000 | 12.111000 |
| 194 | 90.961000 | 35.823000 |
| 133 | 70.334000 | 163.191000 |
| 177 | 142.504000 | 235.451000 |
| 167 | 161.079000 | 167.860000 |

## Randomization Report

| | | |
|---|---|---|
| 140 | 197.967000 | 128.944000 |
| 151 | 239.128000 | 182.265000 |
| 200 | 112.041000 | 129.517000 |
| mean | 140.161625 | 131.895250 |
| std | 53.323849 | 69.934167 |

### Group #5

| Tag | Tumor1(mm3) | Tumor2(mm3) |
|---|---|---|
| 181 | 127.291000 | 238.556000 |
| 180 | 121.916000 | 200.236000 |
| 173 | 124.263000 | 112.227000 |
| 154 | 105.722000 | 118.632000 |
| 137 | 60.306000 | 114.396000 |
| 187 | 269.400000 | 94.729000 |
| 155 | 131.393000 | 70.000000 |
| 169 | 114.317000 | 34.979000 |
| mean | 131.826000 | 122.969375 |
| std | 56.131387 | 62.132613 |

### Group #6

| Tag | Tumor1(mm3) | Tumor2(mm3) |
|---|---|---|
| 196 | 155.176000 | 117.370000 |
| 170 | 103.717000 | 165.791000 |
| 132 | 93.085000 | 17.473000 |

## Randomization Report

| | | |
|---|---|---|
| 139 | 35.077000 | 89.780000 |
| 136 | 52.562000 | 113.906000 |
| 149 | 52.497000 | 145.278000 |
| 130 | 295.646000 | 265.118000 |
| 199 | 334.280000 | 82.682000 |
| mean | 140.255000 | 124.674750 |
| std | 107.254764 | 67.529551 |

## Randomization Report

### Material and methods

Algorithm: **Bruteforce. Minimize heterogeneity.**

Animals were randomized using Randmice (https://randmice.com) on the basis of minimizing the heterogeneity between the groups. The algorithm randomly distribute all animals between the different groups and calculates the mean of each covariates and minimize the difference between the maximum and the minimum of all groups. Between 10^6 to 10^8 iterations can be performed to find the optimum.



13 You will also receive a CSV file to import if needed

| | Group | Tag | Tumor1(mm3) | Tumor2(mm3) |
|---|---|---|---|---|
| 1 | 1 | 145 | 71.241 | 221.675 |
| 2 | 1 | 150 | 60.783 | 126.683 |
| 3 | 1 | 153 | 127.07 | 157.969 |
| 4 | 1 | 197 | 323.87 | 74.359 |
| 5 | 1 | 164 | 133.275 | 101.743 |
| 6 | 1 | 142 | 97.687 | 106.331 |
| 7 | 1 | 185 | 167.752 | 171.788 |
| 8 | 1 | 152 | 138.57 | 99.764 |
| 9 | 2 | 176 | 104.021 | 190.393 |
| 10 | 2 | 172 | 143.278 | 118.588 |
| 11 | 2 | 178 | 59.565 | 14.132 |
| 12 | 2 | 175 | 145.688 | 107.103 |
| 13 | 2 | 193 | 259.332 | 289.193 |
| 14 | 2 | 168 | 80.052 | 158.372 |
| 15 | 2 | 184 | 119.258 | 32.801 |
| 16 | 2 | 159 | 149.782 | 184.098 |
| 17 | 3 | 191 | 67.403 | 90.791 |
| 18 | 3 | 146 | 83.222 | 51.581 |
| 19 | 3 | 183 | 124.437 | 149.117 |
| 20 | 3 | 129 | 97.728 | 38.022 |
| 21 | 3 | 131 | 66.057 | 201.678 |
| 22 | 3 | 163 | 358.042 | 379.814 |
| 23 | 3 | 166 | 72.153 | 34.606 |
| 24 | 3 | 195 | 197.723 | 74.35 |
| 25 | 4 | 158 | 107.279 | 12.111 |
| 26 | 4 | 194 | 90.961 | 35.823 |

| | | | | |
|---|---|---|---|---|
| 27 | 4 | 133 | 70.334 | 163.191 |
| 28 | 4 | 177 | 142.504 | 235.451 |
| 29 | 4 | 167 | 161.079 | 167.86 |
| 30 | 4 | 140 | 197.967 | 128.944 |
| 31 | 4 | 151 | 239.128 | 182.265 |
| 32 | 4 | 200 | 112.041 | 129.517 |
| 33 | 5 | 181 | 127.291 | 238.556 |
| 34 | 5 | 180 | 121.916 | 200.236 |
| 35 | 5 | 173 | 124.263 | 112.227 |
| 36 | 5 | 154 | 105.722 | 118.632 |
| 37 | 5 | 137 | 60.306 | 114.396 |
| 38 | 5 | 187 | 269.4 | 94.729 |
| 39 | 5 | 155 | 131.393 | 70.0 |
| 40 | 5 | 169 | 114.317 | 34.979 |
| 41 | 6 | 196 | 155.176 | 117.37 |
| 42 | 6 | 170 | 103.717 | 165.791 |
| 43 | 6 | 132 | 93.085 | 17.473 |
| 44 | 6 | 139 | 35.077 | 89.78 |
| 45 | 6 | 136 | 52.562 | 113.906 |
| 46 | 6 | 149 | 52.497 | 145.278 |
| 47 | 6 | 130 | 295.646 | 265.118 |
| 48 | 6 | 199 | 334.28 | 82.682 |
| 49 | | | | |

## Protocol references

Bakhos Jneid *et al.*, Selective STING stimulation in dendritic cells primes antitumor T cell responses.*Sci. Immunol.***8**,eabn6612(2023).DOI:**10.1126/sciimmunol.abn6612**