

## - SUPPORTING INFORMATION -

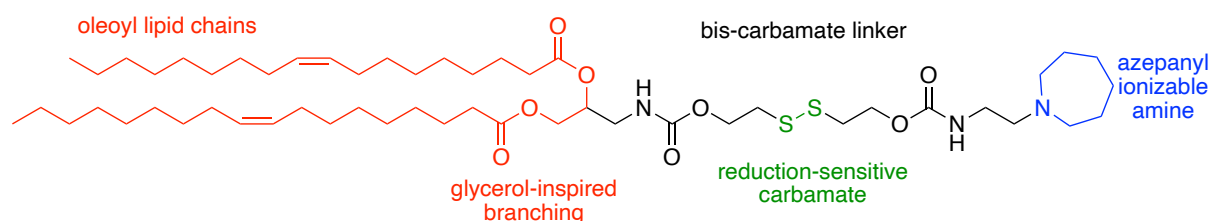
### LIPID NANOPARTICLE ENCAPSULATION EMPOWERS POLY(I:C) TO ACTIVATE CYTOPLASMIC RLRs AND THEREBY INCREASES ITS ADJUVANTICITY

*Alexander Lamoot,<sup>\*1</sup> Sonia Jangra,<sup>\*2,3</sup> Gabriel Laghlali,<sup>2,3</sup> Prajakta Warang,<sup>2,3</sup> Gagandeep Singh,<sup>2</sup> Lauren A. Chang,<sup>2,3,4</sup> Seok-Chan Park,<sup>2,3,5,6</sup> Gagandeep Singh,<sup>2,3</sup> Kim De Swarte,<sup>7,8</sup> Zifu Zhong,<sup>1</sup> Benoit Louage,<sup>1</sup> Emily De Lombaerde,<sup>1</sup> Tingting Ye,<sup>1</sup> Yong Chen,<sup>1</sup> Sara Cuadrado-Castano,<sup>2,3</sup> Stefan Lienenklaus,<sup>9</sup> Niek Sanders,<sup>10</sup> Bart N. Lambrecht,<sup>7,8</sup> Adolfo García-Sastre,<sup>2,3,11,12,13</sup> Michael Schotsaert,<sup>#,2,3,14,15</sup> Bruno G. De Geest<sup>#1</sup>*

- <sup>1</sup> Department of Pharmaceutics, Ghent University, 9000 Ghent, Belgium.
- <sup>2</sup> Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA
- <sup>3</sup> Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA
- <sup>4</sup> Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA
- <sup>5</sup> Laboratory of Pathology, College of Veterinary Medicine, Jeonbuk National University, Iksan, 54596, Korea
- <sup>6</sup> Biosafety Research Institute, College of Veterinary Medicine, Jeonbuk National University, Iksan, 54596, Korea
- <sup>7</sup> Laboratory of Mucosal Immunology, VIB-UGent Center for Inflammation Research, Ghent University, 9000 Ghent, Belgium
- <sup>8</sup> Department of Internal Medicine and Pediatrics, Faculty of Medicine and Health Sciences, Ghent University, 9000 Ghent, Belgium; Department of Respiratory Medicine, Ghent University Hospital, 9000 Ghent, Belgium.
- <sup>9</sup> Institute for Laboratory Animal Science and Institute of Immunology, Hannover Medical School, 30625 Hannover, Germany.
- <sup>10</sup> Laboratory of Gene Therapy, Ghent University, 9820 Merelbeke, Belgium.
- <sup>11</sup> Department of Medicine, Division of Infectious Diseases, Icahn School of Medicine at Mount Sinai, New York, NY, United States.
- <sup>12</sup> The Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, United States.
- <sup>13</sup> Department of Pathology, Molecular and Cell-Based Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, United States.
- <sup>14</sup> Icahn Genomics Institute, Icahn School of Medicine at Mount Sinai, New York, NY, United States.
- <sup>15</sup> Department of Immunology and Immunotherapy, Icahn School of Medicine at Mount Sinai, New York, NY, United States.

\* authors with equal contribution

# corresponding authors: [Michael.Schotsaert@mssm.edu](mailto:Michael.Schotsaert@mssm.edu) , [Br.DeGeest@Ugent.be](mailto:Br.DeGeest@Ugent.be)



**Figure S1.** Molecular structure of the ionizable lipid S-Ac7-DOG.

**Table S1.** LNP composition

|                                      | LNP(poly(I:C)-Rho) |        |                    |
|--------------------------------------|--------------------|--------|--------------------|
|                                      | LNP(poly(I:C))     | LNP(-) | LNP(poly(I:C)-Rho) |
| <b>N/P ratio*</b>                    | 5:1                | 5:1    | 5:1                |
| <b>Target flow rate ratio**</b>      | 2:1                | 2:1    | 2:1                |
| <b>Total formulation volume (mL)</b> | 5                  | 5      | 5                  |
| <b>Amount poly(I:C) (µg)</b>         | 250                | -      | -                  |
| <b>Amount poly(I:C)-Rho (µg)</b>     | -                  | -      | 250                |
| <b>S-Ac7-DOg (mol%)</b>              | 50                 | 50     | 50                 |
| <b>DOPE (mol%)</b>                   | 10                 | 10     | 9.9                |
| <b>Cholesterol (mol%)</b>            | 38.5               | 38.5   | 38.5               |
| <b>DSG-PEG<sub>2000</sub> (mol%)</b> | 1.5                | 1.5    | 1.5                |

\*Target molar charge ratio (mol S-Ac7-DOg: mol poly(I:C))

\*\*aqueous phase volume : organic phase volume