Supplementary Information

# Prevention of respiratory syncytial virus infection with probiotic lactic acid bacterium *Lactobacillus gasseri* SBT2055

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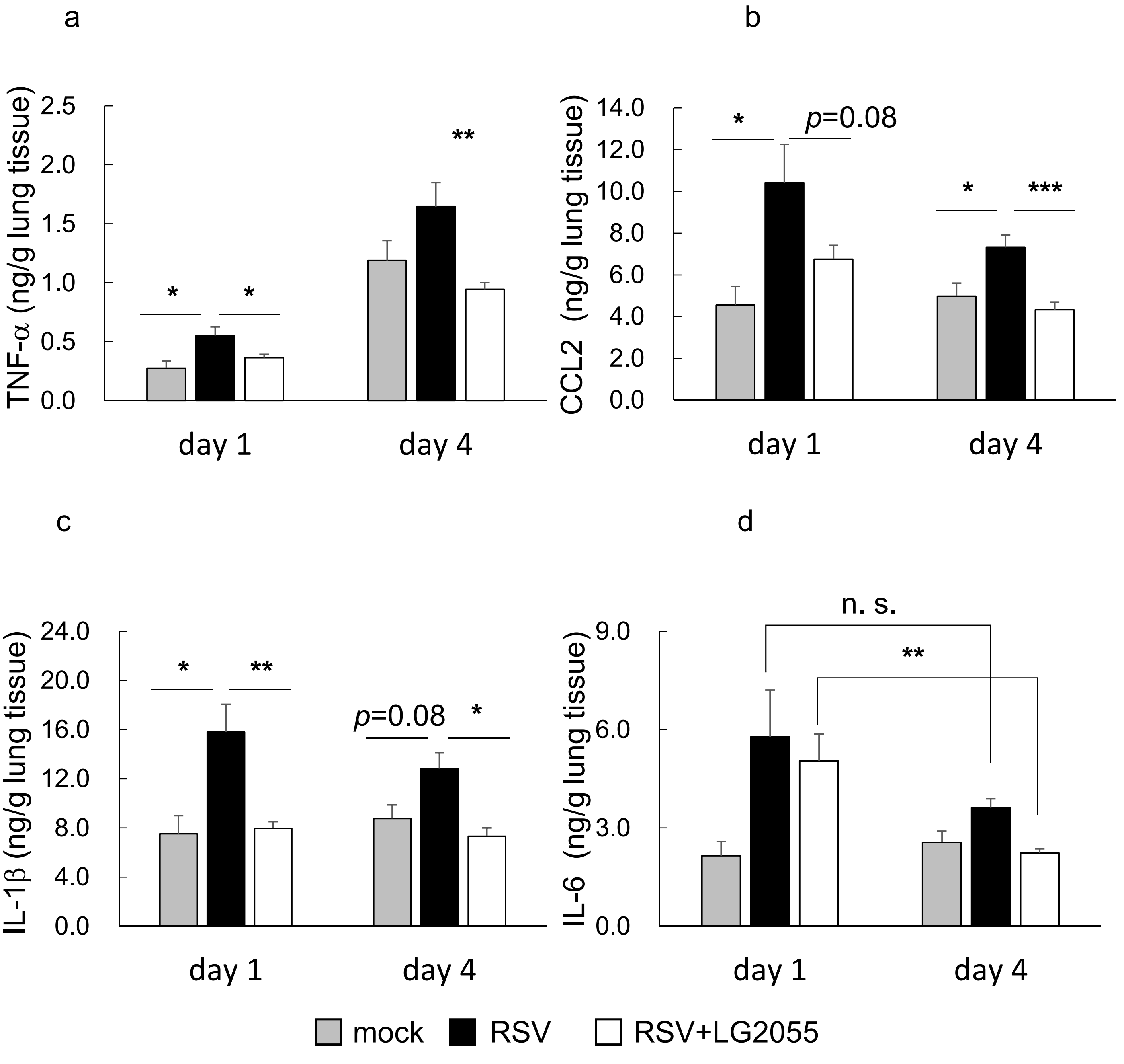
## Table S1 qPCR primer list used in this study

| gene name | sense | antisense |
|---|---|---|
| *RSV-F* | GGAACAAGTTGTTGAGGTTTATGAATATGC | TTCTGCTGTCAAGTCTAGTACACTGTAGT |
| *Rpl19* | ATGAGTATGCTCAGGCTACAGA | GCATTGGCGATTTCATTGGTC |
| *HIST1H4* | TCCTGGAGAATGTGATACGG | TTGAGCGCGTAGACAACATC |
| *IFN-β* | TCCAGCACTGGGTGGAATGA | GGTACCTTTGCACCCTCCAG |
| *IFN-γ* | CTGCAGAGCCAGATTATCTC | CCTGTGGGTTGTTGACCTCA |
| *Oas1a* | GAAGAGGCTGATGTGTGGCT | TGTCCAGTTCTCTTCTACCTGC |
| *Mx1* | CCAACTGGAATCCTCCTGGAA | GCCGCACCTTCTCCTCATAG |
| *ISG15* | GAGCTAGAGCCTGCAGCAAT | TTCTGGGCAATCTGCTTCTT |
| *SRCAP* | CTCCACTGCTACCTCGTTTGGT | GGAAAATCCGTTCCAGGCGTTC |
| *GAPDH* | GTCTCCTCTGACTTCAACAGCG | ACCACCCTGTTGCTGTAGCCAA |

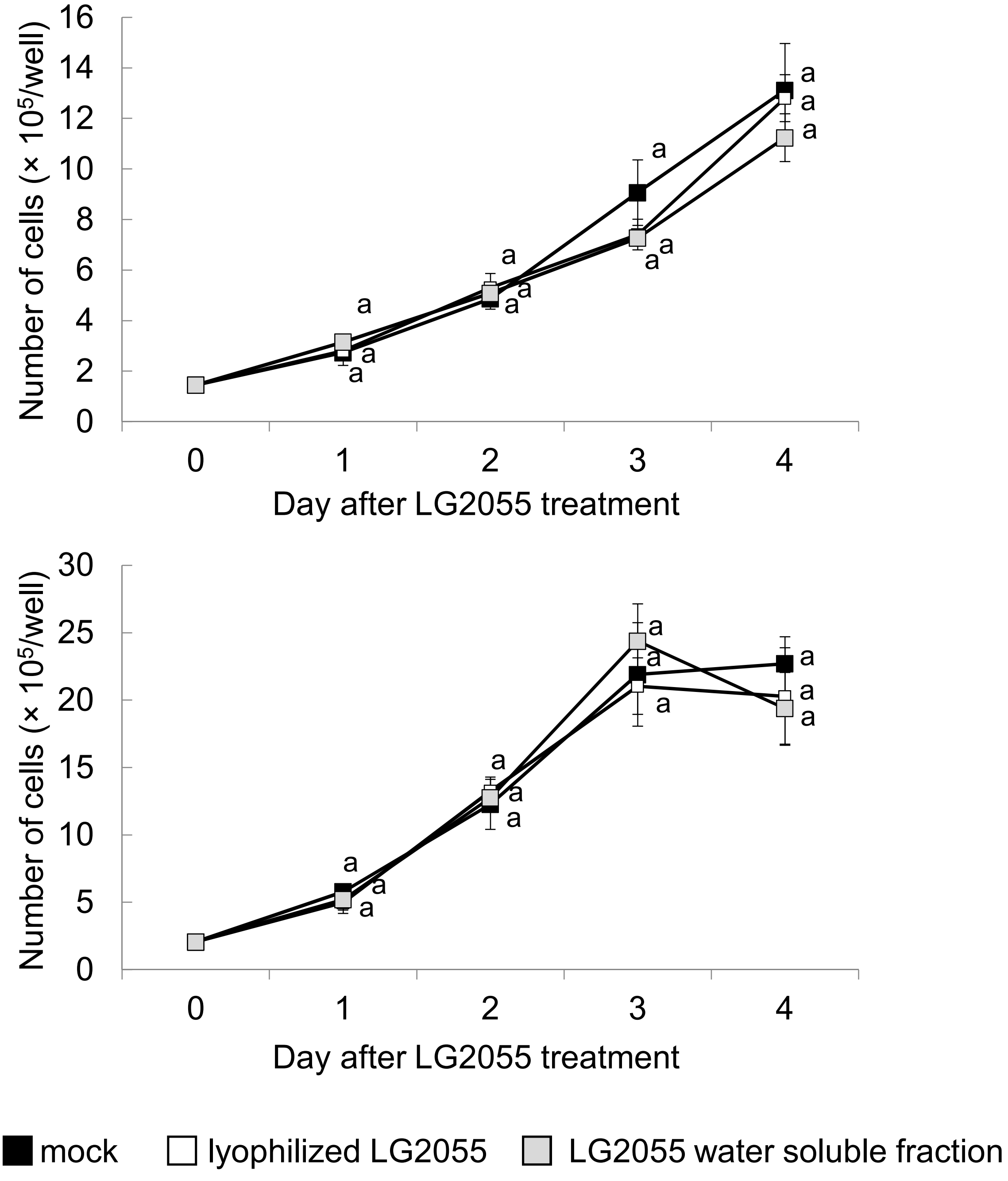
## Table S2 up- and down-regulated proteins by addition of LG2055 water-soluble fraction

| **Protein names** | **Gene names** | **Log2-fol** |
|---|---|---|
| E3 ubiquitin-protein ligase MYCBP2 | MYCBP2 | -4.504 |
| Helicase SRCAP | SRCAP | -4.487 |
| Death-associated protein kinase 2;Death-associated protein kinase 3 | DAPK2;DAPK3 | -4.101 |
| POU domain, class 3, transcription factor 2;POU domain, class 3, transcription factor 1;POU domain, class 3, transcr | POU3F2;POU3F1;POU3F3 | -3.878 |
| 60S ribosomal protein L15 | RPL15 | -3.808 |
| Histone H3.2 | HIST2H3A | -3.304 |
| Heterogeneous nuclear ribonucleoprotein U-like protein 2 | HNRNPUL2 | -3.187 |
| Tropomyosin alpha-3 chain | TPM3 | -3.130 |
| 3-beta-hydroxysteroid-Delta(8),Delta(7)-isomerase | EBP | -3.096 |
| Derlin-2 | DERL2 | -3.091 |
| Structural maintenance of chromosomes flexible hinge domain-containing protein 1 | SMCHD1 | -2.999 |
| Histone H1x | H1FX | -2.955 |
| ATP synthase subunit gamma, mitochondrial | ATP5C1 | -2.891 |
| BTB/POZ domain-containing protein KCTD16 | KCTD16 | -2.852 |
| N-terminal Xaa-Pro-Lys N-methyltransferase 1;N-terminal Xaa-Pro-Lys N-methyltransferase 1, N-terminally processed | NTMT1 | -2.787 |
| Fibroblast growth factor 8 | FGF8 | -2.711 |
| Transmembrane channel-like protein 1 | TMC1 | -2.687 |
| Protein NipSnap homolog 1 | NIPSNAP1 | -2.626 |
| Syntaxin-7 | STX7 | -2.583 |
| Helicase POLQ-like | HELQ | -2.487 |
| Catenin delta-1 | CTNND1 | -2.476 |
| ADP/ATP translocase 1 | SLC25A4 | -2.343 |
| UDP-glucose 6-dehydrogenase | UGDH | -2.310 |
| Splicing factor 3A subunit 3 | SF3A3 | -2.264 |
| Putative uncharacterized protein LRRC75A-AS1, mitochondrial | LRRC75A-AS1 | -2.161 |
| Protein mago nashi homolog;Protein mago nashi homolog 2 | MAGOH;MAGOHB | -2.144 |
| Splicing factor U2AF 26 kDa subunit;Splicing factor U2AF 35 kDa subunit | U2AF1L4;U2AF1 | -2.118 |
| Major vault protein | MVP | -2.077 |
| Coatomer subunit gamma-1 | COPG1 | -2.032 |

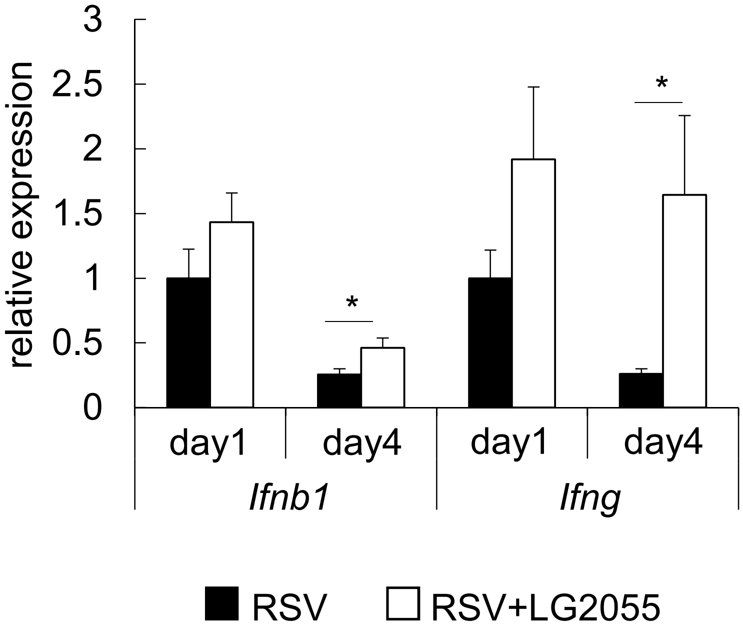
| **Protein names** | **Gene names** | **Log2-fol** |
|---|---|---|
| Protein monoglycylase TTLL8 | TTLL8 | 4.066 |
| Transcription elongation factor A N-terminal and central domain-containing protein 2 | TCEANC2 | 3.692 |
| Inorganic pyrophosphatase | PPA1 | 3.235 |
| Proline-rich basic protein 1 | PROB1 | 3.230 |
| Histone-lysine N-methyltransferase SUV420H1 | SUV420H1 | 3.132 |
| LIM domain and actin-binding protein 1 | LIMA1 | 3.097 |
| Retinoic acid receptor beta | RARB | 2.826 |
| Cystathionine beta-synthase | CBS | 2.776 |
| ATP-dependent RNA helicase DDX19A;ATP-dependent RNA helicase DDX19B | DDX19A;DDX19B | 2.672 |
| AP-2 complex subunit beta | AP2B1 | 2.548 |
| Isopentenyl-diphosphate Delta-isomerase 1 | IDI1 | 2.476 |
| Eukaryotic translation initiation factor 3 subunit M | EIF3M | 2.471 |
| Zinc finger protein 728 | ZNF728 | 2.444 |
| MICOS complex subunit MIC19 | CHCHD3 | 2.286 |
| Phosphatidylinositol 5-phosphate 4-kinase type-2 alpha | PIP4K2A | 2.192 |
| Prostaglandin G/H synthase 2 | PTGS2 | 2.182 |
| Transmembrane protein 2 | TMEM2 | 2.131 |
| Dynein heavy chain 2, axonemal | DNAH2 | 2.065 |
| Serine--tRNA ligase, cytoplasmic | SARS | 2.020 |



**Supplement Figure 1: Orally administered LG2055 reduced lung proinflammatory cytokines production at day1 and day4 postinfection with RSV.**
Mice were orally administered LG2055 ($2 \times 10^9$ cfu/head/day) or 25(%) trehalose solution for 21 d, and the mice were infected RSV or not infected. day1, mock: n=5, hRSV: n=9, RSV+LG2055: n=9, and day4, mock: n=5, RSV: n=10, RSV+LG2055: n=10. (a) TNF-α, (b) CCL2, (c) IL-1β, (d) IL-6 concentration in lung homogenates were measured using ELISA. Data are presented as means + SEM. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ (student's *t* test).



**Supplement figure 2: Cell growth assay with or without lyophilized LG2055**
Cells were prepared in 24-well dishes and treated with LG2055 or its soluble fraction. After 1,2,3 or 4 days incubation, each well was treated with Trypsin-EDTA (0.25%), phenol red (Thermo fisher Scientific) and the number of total cells were measured. (a) HEp-2 cell, (b) MLE12 cell. Data are presented as means±SD. Means with the same letter do not differ (*P* ≥ 0.05, Tukey-kramer test).



**Supplement Figure 3: IFN gene expression level in mouse lung at day 1 postinfection.** Mice were orally administered LG2055 (2 × 109 cfu/head/day) or a 25% trehalose solution for 21 d, and were then infected with RSV or not infected (mock: n=4, RSV: n=8 and RSV+LG2055: n=10). Gene expression in lung homogenates at day 1 postinfection was assessed by real-time PCR analysis. Data are presented as means + SEM. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ (student's *t* test).

Identities 9/12 (75%)
Positives 10/12 (83%)

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 HCV NS3   14 LGTIVTSLTGRD 25
              L TI+TSLT RD
hRSV NS2   23 LETIITSLT-RD 33
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**Supplement Figure 4: Result of alignment between HCV NS3 and hRSV NS2 by BLAST search**

Sequence homology between HCV and NS proteins of RSV was investigated by BLAST search. Sequence alignment between HCV NS3 and RSV NS2 was also assessed by BLAST search.