

Supplemental Figure Legends

Supplemental Figure 1. TSCs are susceptible to *Lm* infection

(A,B) 5×10^5 TSCs were infected at MOI=100 with mid-log bioluminescent *Lm*. Bacterial growth was measured by bioluminescence (A) or colony forming units (B). (C) 5×10^5 TSCs were differentiated into either TGCs or SynTs with removal of growth factors (BM) or with treatment of 5 μ M RA, or 3 μ M CHIR. Each infection had six replicates.

Supplemental Figure 2. Nanoparticle tracking analysis of tEVs

(A-D) Example nanoparticle tracking analysis density plots giving the size distribution of tEVs. (A, B) Density plots for L-tEVs from uninfected (A) and *Lm*-infected (B) TSCs. Density plots for S-tEVs from uninfected (C) and *Lm*-infected (D) TSCs.

Supplemental Figure 3. tEVs infection does not change mouse resistance to *Lm*

(A) IVIS images of mice 72 hours after infection. (B) Quantification of bioluminescence radiance signifying bacterial growth in the mice. (C) Bacterial growth measured by plate counting serial dilutions of spleens. Non-parametric one-way ANOVA (Kruskal-Wallis test) was used for comparison of the groups.

Supplemental Table 1.

List of proteins that had twice the number of peptides identified in the S-tEVs from the infected TSCs vs. the S-tEVs from uninfected cells. Peptide counts were done in Scaffold Software. Included proteins had at least 10 peptide signatures in either EV condition.

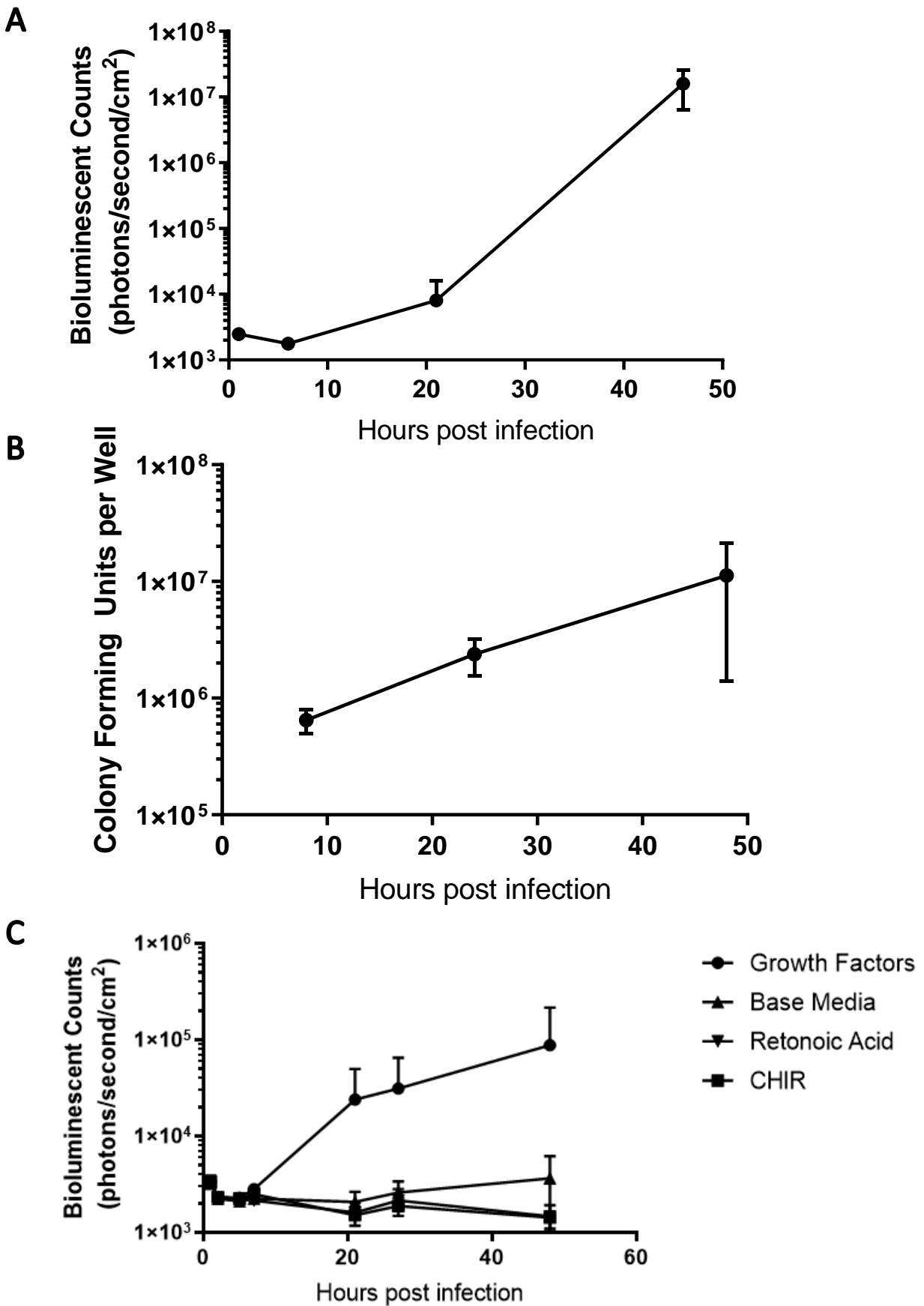
Supplemental Table 2.

Gene ontology (GO) analysis with PANTHER was performed using the proteins identified in Table 1. The pathways that were associated with the proteins that were increased in tEVs from infected TSCs are listed, as well as the fold-enrichment scores, the P-values, and the false discovery rate (FDR).

Supplemental Table 3.

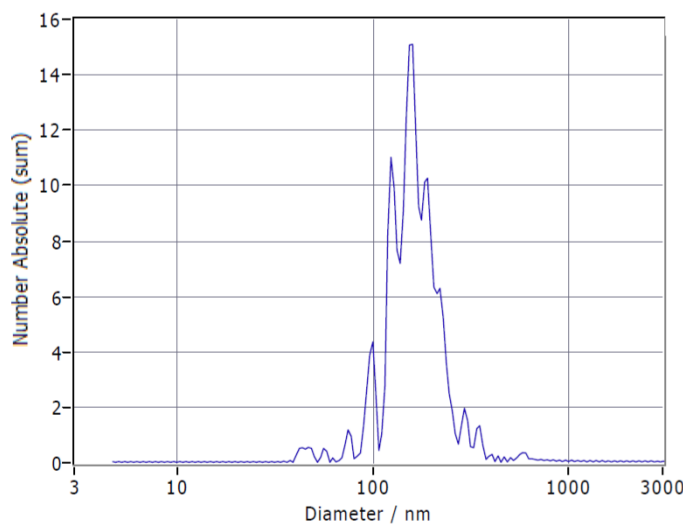
List of proteins that had twice the number of peptides identified in the S-tEVs from the uninfected TSCs vs. the S-tEVs from infected cells. Peptide counts were done in Scaffold Software. Included proteins had at least 10 peptide signatures in either EV condition.

Supplemental Figure 1

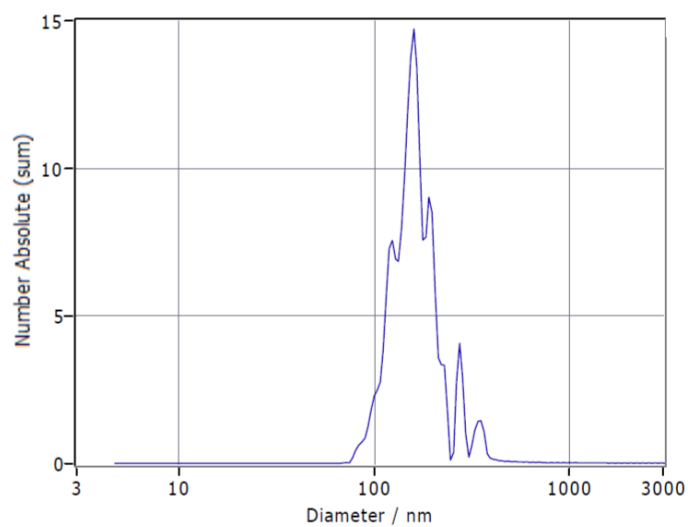


Supplemental Figure 2

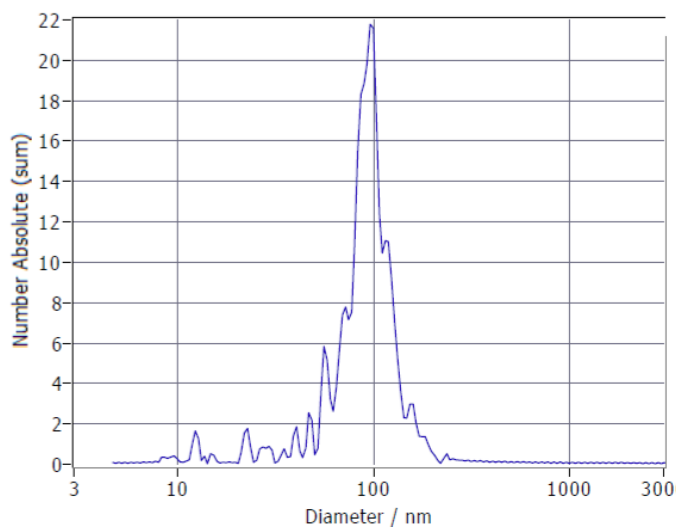
A



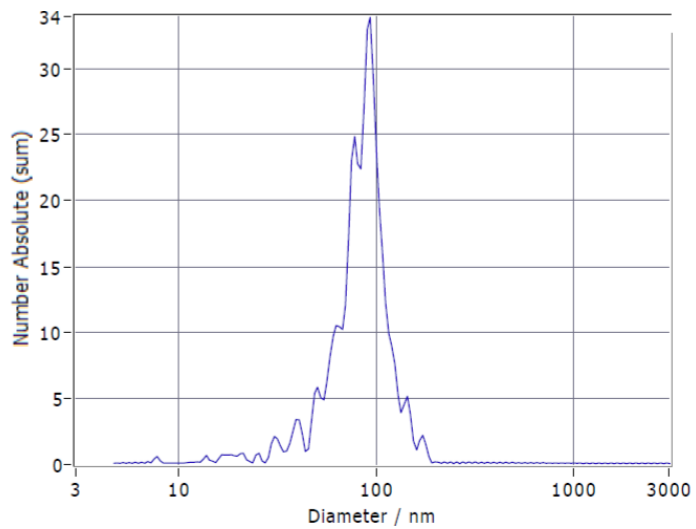
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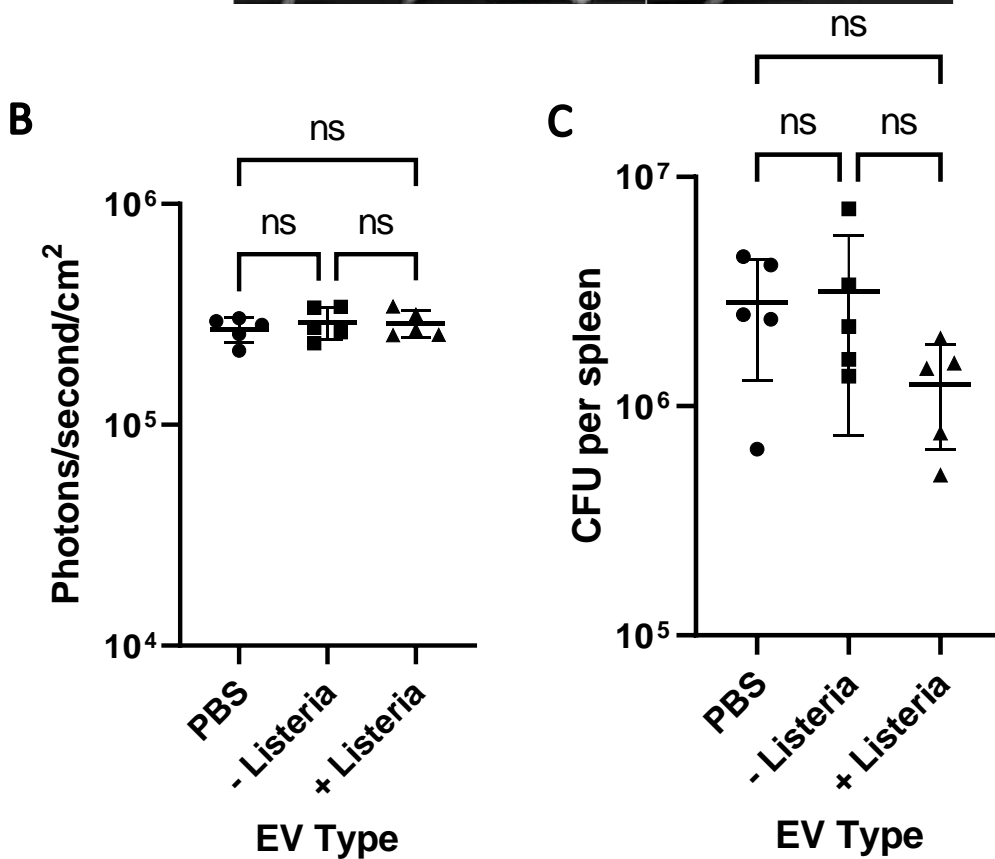
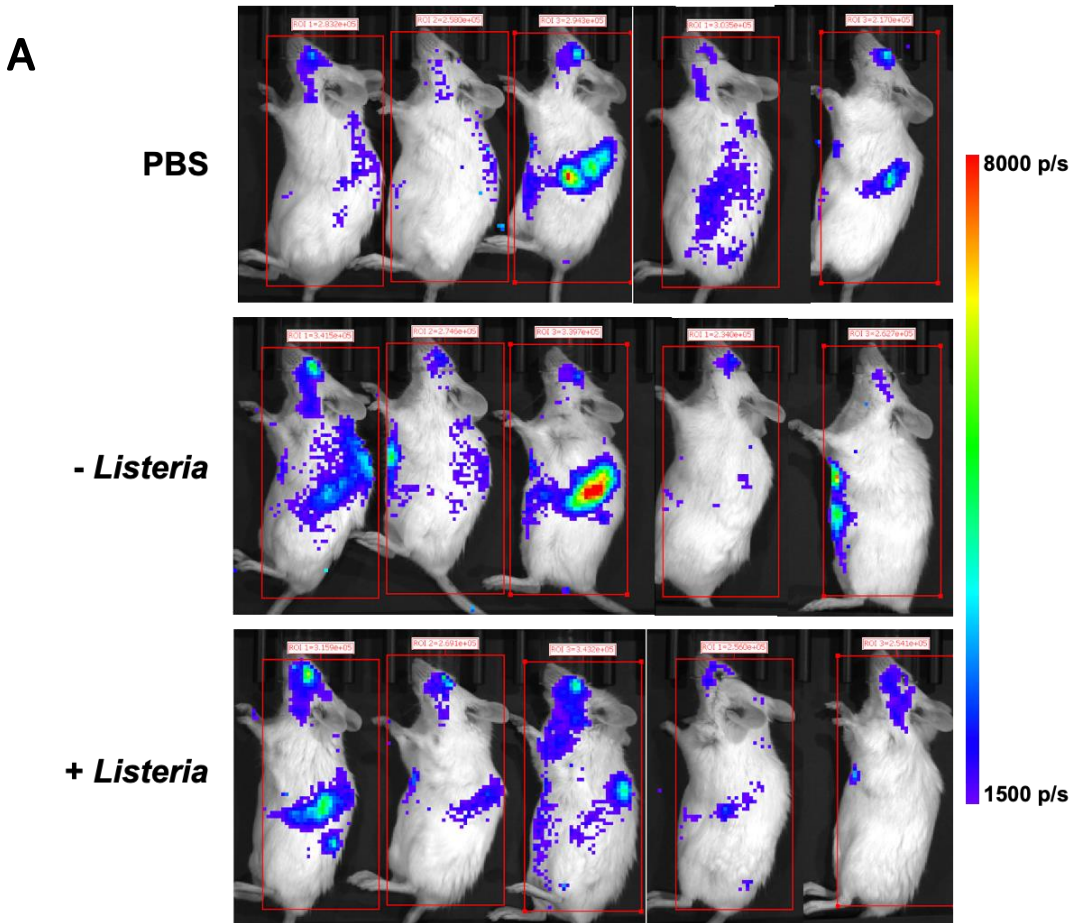
C



D



Supplemental Figure 3



Supplemental Table 1

| Protein Name | - Listeria | + Listeria | + Listeria/- Listeria |
|--|-------------------|-------------------|------------------------------|
| PEG10 | 0 | 6.33 | Infinite |
| 60S ribosomal protein L10 | 0 | 6.67 | Infinite |
| 60S ribosomal protein L3 | 0.33 | 11.33 | 34 |
| 60S ribosomal protein L5 | 0.33 | 9 | 27 |
| Myb-binding protein 1A | 0.33 | 7.67 | 23 |
| 40S ribosomal protein S3a | 0.33 | 5 | 15 |
| Nucleophosmin | 1 | 11 | 11 |
| Clathrin heavy chain 1 | 2.33 | 25.33 | 10.86 |
| 60S ribosomal protein L4 | 2 | 19.33 | 9.67 |
| Tubulin beta-5 chain | 2.33 | 22.33 | 9.57 |
| 60S ribosomal protein L6 | 1 | 8.67 | 8.67 |
| 60S ribosomal protein L7a | 1 | 8 | 8 |
| 60S ribosomal protein L17 | 1.33 | 10.33 | 7.75 |
| Major vault protein | 1 | 6.67 | 6.67 |
| Heterogenous nuclear Ribonucleoprotein | 1.33 | 7.67 | 5.75 |
| Basement membrane-specific heparan sulfate proteoglycan | 3.33 | 18.67 | 5.6 |
| 40S ribosomal protein 1A | 2 | 8.67 | 4.33 |
| Histone H2AX | 5.67 | 22.33 | 3.94 |

| | | | |
|--------------------------|-------|-------|------|
| 60S ribosomal protein L7 | 1.67 | 5.67 | 3.4 |
| Tubulin alpha-1B | 6 | 14.67 | 2.44 |
| GAPDH | 6 | 13.33 | 2.22 |
| Histone H2B | 11.67 | 23.33 | 2 |
| Histone H4 | 6.67 | 13.33 | 2 |

Supplemental Table 2

| GO biological process | fold Enrichment | raw P-value | FDR |
|--|------------------------|--------------------|------------|
| ribosomal large subunit assembly | > 100 | 2.44E-08 | 3.84E-05 |
| maturation of LSU-Rrna | > 100 | 4.11E-08 | 5.40E-05 |
| maturation of LSU-rRNA from tricistronic rRNA transcript | > 100 | 1.58E-04 | 4.89E-02 |
| ribosomal large subunit biogenesis | > 100 | 8.98E-15 | 1.41E-10 |
| ribosome assembly | 62.69 | 5.88E-07 | 4.63E-04 |
| cytoplasmic translation | 41.57 | 5.67E-05 | 2.23E-02 |
| nucleosome assembly | 36.77 | 8.06E-05 | 2.76E-02 |
| ribosome biogenesis | 30.19 | 8.31E-12 | 6.54E-08 |
| translation | 26.64 | 2.46E-11 | 1.29E-07 |
| peptide biosynthetic process | 25.01 | 4.25E-11 | 1.67E-07 |
| rRNA processing | 23.2 | 2.26E-06 | 1.55E-03 |
| ribonucleoprotein complex assembly | 23.04 | 2.71E-05 | 1.22E-02 |
| rRNA metabolic process | 22.13 | 2.83E-06 | 1.78E-03 |
| ribonucleoprotein complex subunit organization | 22.1 | 3.18E-05 | 1.35E-02 |
| ribonucleoprotein complex biogenesis | 21.56 | 1.54E-10 | 4.84E-07 |
| amide biosynthetic process | 19.42 | 3.80E-10 | 9.97E-07 |
| non-membrane-bounded organelle assembly | 19.06 | 5.80E-07 | 4.81E-04 |
| peptide metabolic process | 17.7 | 8.45E-10 | 1.90E-06 |

| | | | |
|---|-------|----------|----------|
| negative regulation of transferase activity | 14.94 | 1.41E-04 | 4.54E-02 |
| ncRNA processing | 13.62 | 2.85E-05 | 1.25E-02 |
| regulation of translation | 12.85 | 3.75E-05 | 1.55E-02 |
| posttranscriptional regulation of gene expression | 12.08 | 7.77E-06 | 4.22E-03 |
| cellular amide metabolic process | 11.93 | 2.49E-08 | 3.56E-05 |
| ncRNA metabolic process | 11.14 | 7.31E-05 | 2.62E-02 |
| regulation of cellular amide metabolic process | 11.12 | 7.39E-05 | 2.59E-02 |
| cellular protein-containing complex assembly | 11.1 | 3.12E-07 | 2.73E-04 |

Supplemental Table 3

| Protein Name | - Listeria | + Listeria | + Listeria/ - Listeria |
|---|-------------------|-------------------|-----------------------------------|
| Keratin type 1 cytoskeleton (A6BLY7) | 11 | 3 | 0.27 |
| Trypsin | 66.67 | 23.33 | 0.35 |
| Lactotransferrin | 9.33 | 4 | 0.43 |
| Keratin type 1 cytoskeleton (E9Q0F0) | 14.33 | 7 | 0.49 |