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## Supplemental Information

# Antibacterial Fusion Protein BPI21/LL-37 Modification Enhances the Therapeutic Efficacy of hUC-MSCs in Sepsis

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# Supplemental Information

**Supplemental Figures and Figure legends**

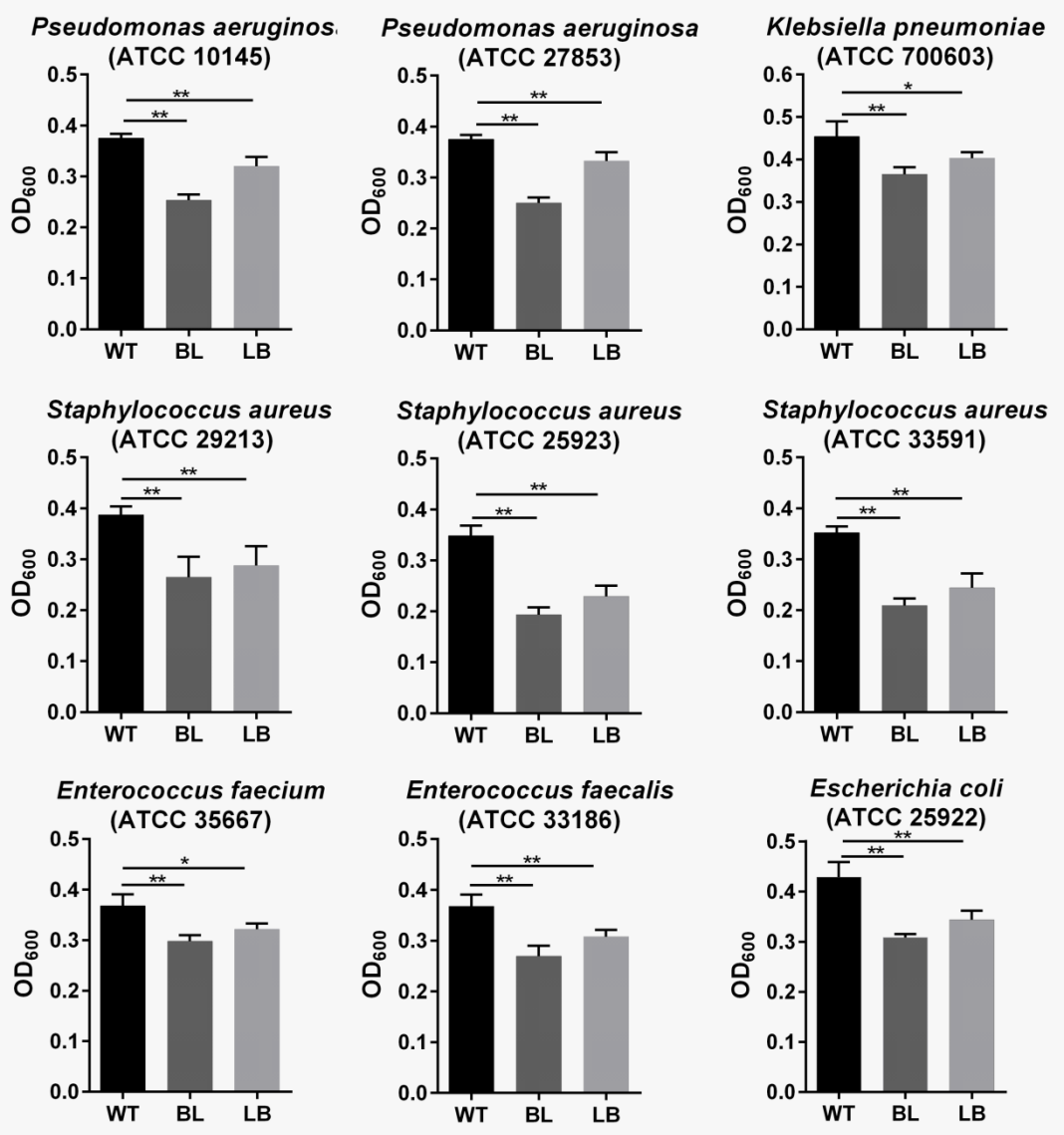


**Fig. S1. BPI21/LL-37 and LL-37/BPI21 fusion proteins enhance the antibacterial activity of hUC-MSCs *in vitro*.** The antibacterial activities of antibacterial fusion proteins secreted by engineered hUC-MSCs on gram-negative bacteria and gram-positive bacteria. The microplate reader detected the absorbance of each well at 600 nm and compared the OD600 difference of each group. The experiments were performed in quintuplicate, and the data are presented as the mean ± S.E.M. *p < 0.05, **p < 0.01. WT, wild-type hUC-MSCs; BL, BPI21/LL-37-engineered hUC-MSCs; LB, LL-37/BPI21-engineered hUC-MSCs.

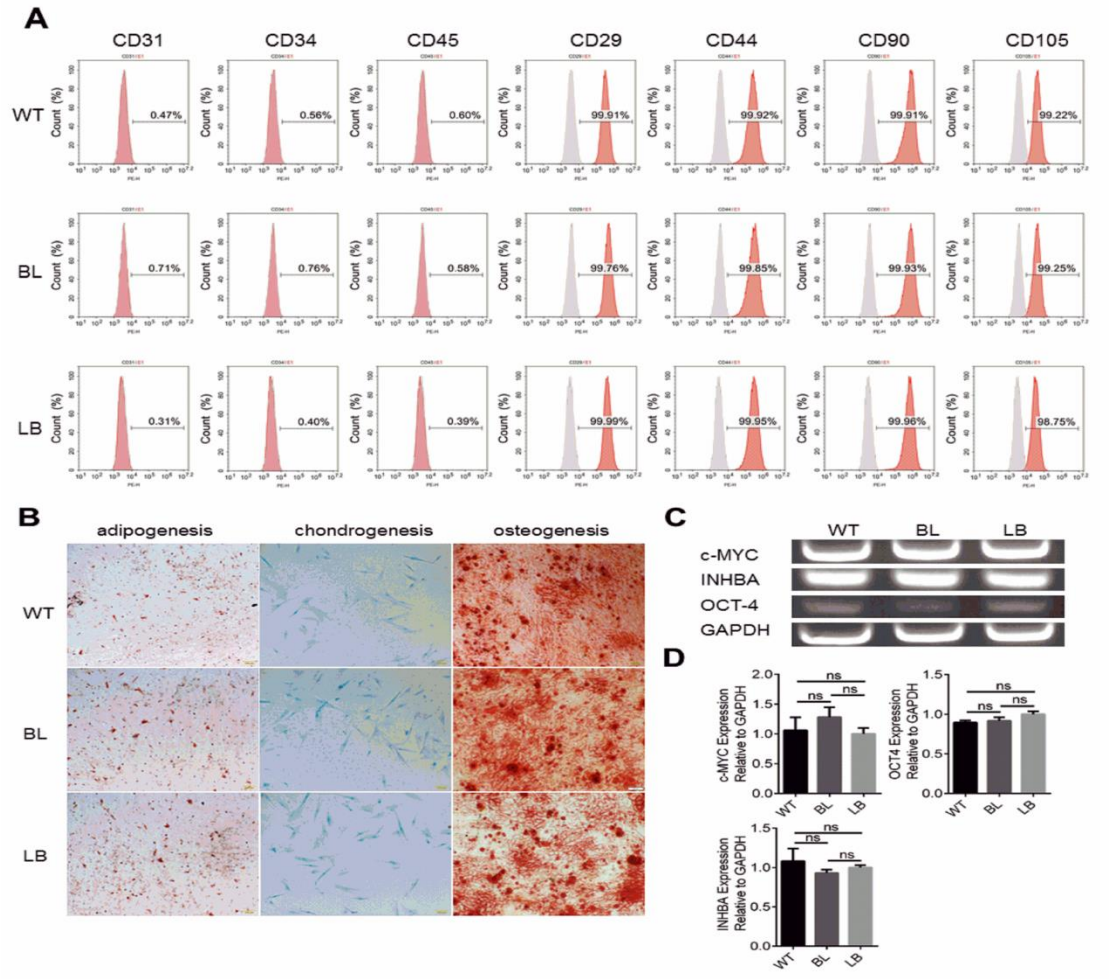


**Fig. S2. Characterization and stemness of hUC-MSCs engineered by antibacterial fusion proteins.** **(**A**)** The surface markers of engineered hUC-MSCs are seen using flow cytometry. (B) The three-lineage differentiation of engineered hUC-MSCs. Scale bar: 100 μm. (C) The gene expression of the stemness markers, including OCT-4, INHBA and c-MYC, in engineered hUC-MSCs using qPCR analysis. WT, wild-type hUC-MSCs; BL, BPI21/LL-37-engineered hUC-MSCs; LB, LL-37/BPI21-engineered hUC-MSCs. (D) Semi-quantification analysis of OCT-4, INHBA and c-MYC expression was performed. ns, not significant.

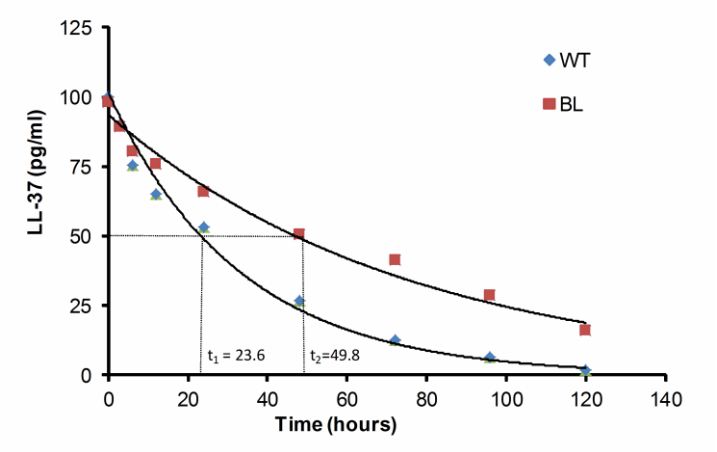


**Fig. S3. *In vitro* serum half-life of BPI21/LL-37 fusion peptides.** The concentrations of LL-37 standard and BPI21/LL-37 fusion peptides were measured at 9 time points (0, 3, 6, 12, 24, 48, 72, 96, 120 hours), and the half-lives were determined.

**Supplemental tables**

**Table S1 Primer sets used for real-time PCR**

| Gene | | Primer sequence (5′→3′) |
|---|---|---|
| OCT-4 | F | GACAACAATGAGAACCTTCAGGAGA |
| | R | TTCTGGCGCCGGTTACAGAACCA |
| c-MYC | F | GCTCCTGGCAAAAGGTCAGA |
| | R | CCAAGACGTTGTGTGTTCGC |
| INHBA | F | TCATCACGTTTGCCGAGTCA |
| | R | TGTTGGCCTTGGGGACTTTT |
| GAPDH | F | GTGGTCTCCTCTGACTTCAACA |
| | R | CTCTTCCTCTTGTGCTCTTGCT |

**Table S2 Effect of MSCs by tail intravenous injection on body weight (gram ) in mice**

| Time (days) | NC(n=5) | MSCs(n=5) | P value |
|---|---|---|---|
| 1 | 21.82±0.75 | 22.04±0.96 | NS |
| 5 | 22.84±0.90 | 23.12±1.14 | NS |
| 10 | 24.02±0.78 | 24.38±0.88 | NS |
| 15 | 25.32±0.68 | 25.28±0.79 | NS |
| 20 | 27.34±0.37 | 27.36±0.67 | NS |

NS, not significant.

**Table S3 Effect of MSCs by tail intravenous injection on organ weight (gram) in mice**

| Organ | NC(n=5) | MSCs(n=5) | P value |
|---|---|---|---|
| Heart | 0.170 ±0.004 | 0.171 ±0.003 | NS |
| Liver | 1.964 ±0.056 | 1.971 ±0.062 | NS |
| Spleen | 0.125 ±0.004 | 0.126 ±0.004 | NS |
| Lung | 0.203 ±0.005 | 0.201 ±0.006 | NS |
| Kidney | 0.481 ±0.018 | 0.481 ±0.014 | NS |

NS, not significant.

**Supplemental Materials and Methods**

**Flow cytometry analysis**

The surface markers of MSCs were stained as previously described [1-3], and the MSCs showed the positive expression of CD29, CD44, CD90 and CD105 and were negative for CD31, CD34 and CD45. The MSCs were divided into different groups in 1.5 ml microcentrifuge tubes, and all pellets were resuspended in PBS to a concentration of 1 x $10^5$ cells per 100 μl of PBS. Then, 5 μl of an antibody against the MSCs marker was added to 195 μl of the resuspended cells. The surface of MSCs markers were stained with PE-conjugated monoclonal antibodies (mAbs; CD29-PE, CD31-PE,CD34-PE, CD44-PE, CD45-PE, CD90-PE and CD105-PE; BD Biosciences) at 4℃ in the dark for 30 min. Non-specific isotype-matched antibodies were used to control for non-specific staining. After staining, cells were washed with PBS. Then, the MSCs were analyzed by a flow cytometer (ACEA Biosciences, Hangzhou, China).

**Multilineage differentiation potential and the stemness of engineered hUC-MSCs**

The multilineage differentiation potential of the MSCs was determined as previously described [4]. The 4th passage BL- or LB-hUC-MSCs were seeded into 12-well plates at a density of 1 × $10^5$ cells/well and were cultured to a confluency of 70%. The chondrogenesis, osteogenesis, and adipogenesis of MSCs was induced using commercial OriCell$^{TM}$ chondrogenic, osteogenic and adipogenic differentiation media (Cyagen Biosciences Inc., Guangzhou, China), respectively, according to the manufacturer's instructions. After 2-4 weeks, oil red O staining, Alcian blue staining and alizarin red S staining were performed to assess adipogenesis, chondrogenesis and osteogenesis, respectively. In addition, the mRNA expression levels of stemness genes, such as OCT-4, INHBA and c-MYC, were detected by RT-PCR. The primer sequences are listed in Table S1.

**The serum half-life of BPI21/LL-37 fusion peptides in *vitro***

To detect the serum half-life of BPI21/LL-37 fusion peptides, the mice were randomly divided into two groups (WT-hUC-MSCs vs BL-hUC-MSCs). MSCs were administered to mice by tail intravenous injections (2 ×$10^5$ cells per mouse in 200 μl of PBS). Mice blood and serum were collected at 12 hours. The concentration of BPI21/LL-37 in the mice serum was determined using ELISA kit (TSZ, USA). Simultaneously, LL-37 standard was added to the serum of WT-hUC-MSCs treatment group, and its concentration was set equal to BPI21/LL-37 in the mice serum after MSCs

administration. Then, the serum of two groups were added to 24-well culture plate and incubated at 37 ℃. Serum samples were collected at 9 time points (0, 3, 6, 12, 24, 48, 72, 96, 120 hours). The concentration of LL-37 in the samples was detected by ELISA (TSZ, USA).

**Effect of MSCs on body and organ weight (gram) in mice**

To detect the effect of MSCs in C57BL/6J mice, the animals were randomly divided into three groups (PBS vs BL-hUC-MSCs). BL-hUC-MSCs were administered to mice by tail intravenous injections ($2\times10^5$ cells per mouse in 200 μl of PBS). The body mass was weighed once every 5 days. Then, the heart, kidney, liver, spleen and lung were collected and weighed at 20 days after BL-hUC-MSCs transplantation for analysis.

**Supplemental References:**


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3. Song, Y.,H. Dou,X. Li,X. Zhao,Y. Li,D. Liu,J. Ji,F. Liu,L. Ding,Y. Ni, et al.(2017). Exosomal miR-146a Contributes to the Enhanced Therapeutic Efficacy of Interleukin-1beta-Primed Mesenchymal Stem Cells Against Sepsis. Stem Cells.35: 1208-1221.
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