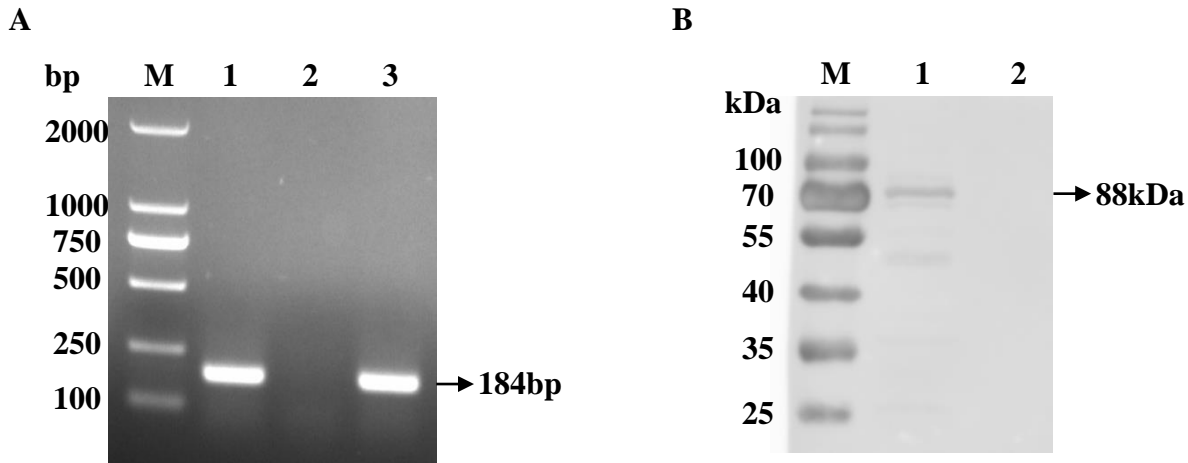
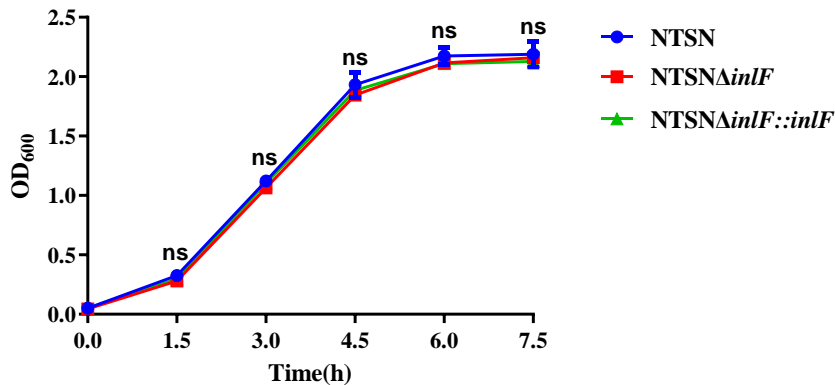


1.1 Supplementary Figures



Supplementary Figure 1. Verification of *inlF* gene deletion. (A) RT-PCR analysis to verify the deletion of *inlF*. M: DL2000 DNA marker; 1: PCR products of NTSN cDNA; 2: PCR products of NTSN Δ *inlF* cDNA; 3: PCR products of NTSN Δ *inlF*::*inlF* cDNA. (B) Western blot analysis of InlF protein expression by Lm NTSN and NTSN Δ *inlF*. M: Prestained protein Ladder; 1: proteins InlF from NTSN; 2: proteins from NTSN Δ *inlF*.



Supplementary Figure 2. The growth curve of Lm. Three initial OD₆₀₀-identical bacterium were cultured in BHI medium at 37°C. There was no difference among Lm NTSN, NTSN Δ *inlF* and NTSN Δ *inlF*::*inlF*.

Lineage I

Lineage II

```

07FF0776.4b  MAAHNNFKQLISIMVLSLEMLVIGQNNVEKATLAEFAEINQIFPDADLARGIRAV
NTSN.4b
Cl1p80459.4b
F2365.4b
J1-220.4b
J1816.4b
L312.4b
LL195.4b
SLCC2378.4e
SLCC2755.1/2
ATCC19117.4d
SLCC2482.7
SLCC2540.3b
SLCC5850.1/2
08-5578.1/2a
08-5923.1/2a
10403s.1/2a
EGD.1/2a
FSLR2561.1/2
EGDe.1/2a
SLCC7179.3a
SLCC2372.1/2
SLCC2479.3c
  
```

```

07FF0776.4b  LQKASVVDVVICBELESIKIVVAGERVASIQGIEYLINLEVININGCDDISPLSNVI
NTSN.4b
Cl1p80459.4b
F2365.4b
J1-220.4b
J1816.4b
L312.4b
LL195.4b
SLCC2378.4e
SLCC2755.1/2
ATCC19117.4d
SLCC2482.7
SLCC2540.3b
SLCC5850.1/2
08-5578.1/2a
08-5923.1/2a
10403s.1/2a
EGD.1/2a
FSLR2561.1/2
EGDe.1/2a
SLCC7179.3a
SLCC2372.1/2
SLCC2479.3c
  
```

Lineage I

Lineage II

```

07FF0776.4b  KLNLYYGFQKNTDISAGNLLNLRLEYNEDNLSISPLANLTKMYSLNGANNHLSDL
NTSN.4b
Cl1p80459.4b
F2365.4b
J1-220.4b
J1816.4b
L312.4b
LL195.4b
SLCC2378.4e
SLCC2755.1/2
ATCC19117.4d
SLCC2482.7
SLCC2540.3b
SLCC5850.1/2
08-5578.1/2a
08-5923.1/2a
10403s.1/2a
EGD.1/2a
FSLR2561.1/2
EGDe.1/2a
SLCC7179.3a
SLCC2372.1/2
SLCC2479.3c
  
```

```

07FF0776.4b  SFELSNMGTINYLIVVESKVKVDFPFIANLTLDSLSINNGYREDISPLASLTSHYHTAV
NTSN.4b
Cl1p80459.4b
F2365.4b
J1-220.4b
J1816.4b
L312.4b
LL195.4b
SLCC2378.4e
SLCC2755.1/2
ATCC19117.4d
SLCC2482.7
SLCC2540.3b
SLCC5850.1/2
08-5578.1/2a
08-5923.1/2a
10403s.1/2a
EGD.1/2a
FSLR2561.1/2
EGDe.1/2a
SLCC7179.3a
SLCC2372.1/2
SLCC2479.3c
  
```

Lineage I

Lineage II

```

07FF0776.4b  NQIETDIFVANTRINSIKIGNKIKTDLSPANLSQLMLEIGTGNQISDINAVKDLTKIK
NTSN.4b
Cl1p80459.4b
F2365.4b
J1-220.4b
J1816.4b
L312.4b
LL195.4b
SLCC2378.4e
SLCC2755.1/2
ATCC19117.4d
SLCC2482.7
SLCC2540.3b
SLCC5850.1/2
08-5578.1/2a
08-5923.1/2a
10403s.1/2a
EGD.1/2a
FSLR2561.1/2
EGDe.1/2a
SLCC7179.3a
SLCC2372.1/2
SLCC2479.3c
  
```

```

07FF0776.4b  MIVNVSQISDVSIVNQLSLELNQNNNGIENEDVIGGLTNTLFLSLGIDGIDRE
NTSN.4b
Cl1p80459.4b
F2365.4b
J1-220.4b
J1816.4b
L312.4b
LL195.4b
SLCC2378.4e
SLCC2755.1/2
ATCC19117.4d
SLCC2482.7
SLCC2540.3b
SLCC5850.1/2
08-5578.1/2a
08-5923.1/2a
10403s.1/2a
EGD.1/2a
FSLR2561.1/2
EGDe.1/2a
SLCC7179.3a
SLCC2372.1/2
SLCC2479.3c
  
```

Lineage I

Lineage II

```

07FF0776.4b  LASLRMDSDAFANCVIKKFARNFSEKLSVNNITSIDGIVLTFKTSNNGTVDAENVMN
NTSN.4b
Cl1p80459.4b
F2365.4b
J1-220.4b
J1816.4b
L312.4b
LL195.4b
SLCC2378.4e
SLCC2755.1/2
ATCC19117.4d
SLCC2482.7
SLCC2540.3b
SLCC5850.1/2
08-5578.1/2a
08-5923.1/2a
10403s.1/2a
EGD.1/2a
FSLR2561.1/2
EGDe.1/2a
SLCC7179.3a
SLCC2372.1/2
SLCC2479.3c
  
```

```

07FF0776.4b  SSFSLVFRVRYTFKQVUVGTSSTSYTGILIQINLEVDYVTFHIDNGSEVVKTVTEEDI
NTSN.4b
Cl1p80459.4b
F2365.4b
J1-220.4b
J1816.4b
L312.4b
LL195.4b
SLCC2378.4e
SLCC2755.1/2
ATCC19117.4d
SLCC2482.7
SLCC2540.3b
SLCC5850.1/2
08-5578.1/2a
08-5923.1/2a
10403s.1/2a
EGD.1/2a
FSLR2561.1/2
EGDe.1/2a
SLCC7179.3a
SLCC2372.1/2
SLCC2479.3c
  
```

Lineage I

Lineage II

```

07FF0776.4b  LLEFSEANPKQGYFDFGNYDABTGTRWDFTTQCMFANDMLIYAFHSVNSQVNDIDGG
NTSN.4b
Cl1p80459.4b
F2365.4b
J1-220.4b
J1816.4b
L312.4b
LL195.4b
SLCC2378.4e
SLCC2755.1/2
ATCC19117.4d
SLCC2482.7
SLCC2540.3b
SLCC5850.1/2
08-5578.1/2a
08-5923.1/2a
10403s.1/2a
EGD.1/2a
FSLR2561.1/2
EGDe.1/2a
SLCC7179.3a
SLCC2372.1/2
SLCC2479.3c
  
```

```

07FF0776.4b  VKNERVVYDILNEPIAFPKQYTFDGDWDYDGTGGNRKDFKIMMFANDVLYAFHTVSS
NTSN.4b
Cl1p80459.4b
F2365.4b
J1-220.4b
J1816.4b
L312.4b
LL195.4b
SLCC2378.4e
SLCC2755.1/2
ATCC19117.4d
SLCC2482.7
SLCC2540.3b
SLCC5850.1/2
08-5578.1/2a
08-5923.1/2a
10403s.1/2a
EGD.1/2a
FSLR2561.1/2
EGDe.1/2a
SLCC7179.3a
SLCC2372.1/2
SLCC2479.3c
  
```

Lineage I

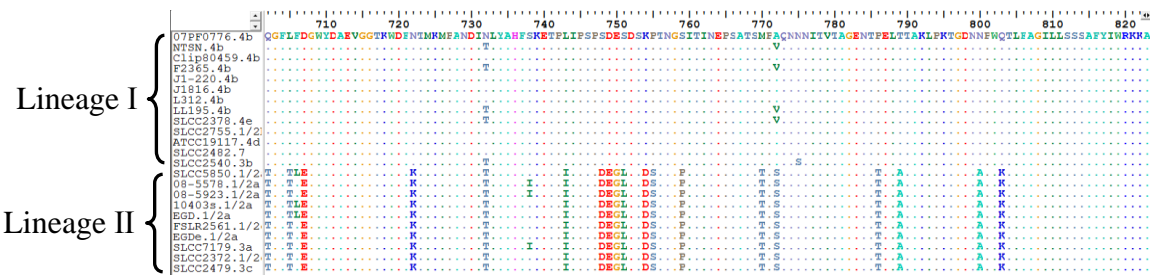
Lineage II

```

07FF0776.4b  QVNFDFIDGVAINEAIVDYLILNEFAFPFKQHTDFDHYDABTGGRWDFKIMMFANDV
NTSN.4b
Cl1p80459.4b
F2365.4b
J1-220.4b
J1816.4b
L312.4b
LL195.4b
SLCC2378.4e
SLCC2755.1/2
ATCC19117.4d
SLCC2482.7
SLCC2540.3b
SLCC5850.1/2
08-5578.1/2a
08-5923.1/2a
10403s.1/2a
EGD.1/2a
FSLR2561.1/2
EGDe.1/2a
SLCC7179.3a
SLCC2372.1/2
SLCC2479.3c
  
```

```

07FF0776.4b  ELYAHFRIINVCANFDIDGQVINTEFLLENEFAFPFKQHTDFDHYDABTGGRWDFKIMMFANDV
NTSN.4b
Cl1p80459.4b
F2365.4b
J1-220.4b
J1816.4b
L312.4b
LL195.4b
SLCC2378.4e
SLCC2755.1/2
ATCC19117.4d
SLCC2482.7
SLCC2540.3b
SLCC5850.1/2
08-5578.1/2a
08-5923.1/2a
10403s.1/2a
EGD.1/2a
FSLR2561.1/2
EGDe.1/2a
SLCC7179.3a
SLCC2372.1/2
SLCC2479.3c
  
```



Supplementary Figure 3. Analysis of amino acid sequence alignments of InlF protein in lineage I and II. The amino acid sequences of 23 strains of InlF protein were downloaded from the NCBI database and compared by MEGA5 software. The total length of InlF protein in lineage I was 823 amino acids, and that in lineage II was 821. There were 114 heterotopias in the amino acid sequence of the two lineages, and the homology was 78%.

1.2 Supplementary Tables

Supplementary Table 1. Cell lines used in this study. The top five are nonphagocytes, and the last one is macrophage.

| Cells | Relevant characteristics | Reference/Source |
|---------|------------------------------------------------------|-----------------------------------|
| Caco-2 | Human epithelial colorectal adenocarcinoma cell line | ATCC CRL-2102 |
| NIH/3T3 | Mouse embryonic fibroblast cell | ATCC CRL-1658 |
| BRL-3A | Rat liver cell line | ATCC CRL-1442 |
| Hep G2 | Human liver cancer cell line | ATCC HB-8065 |
| HBMEC | Human brain microvascular endothelial cell | Cell Systems Corporation ACBRI376 |
| Hela | Human cervical cancer cell | ATCC CCL-2 |

Supplementary Table 2. Biochemical identification of Lm. 43 physiological and biochemical reactions of Lm were detected by automatic microorganism identification instrument. There was no difference among Lm NTSN and NTSN Δ *inlF*.

| Biochemical | NTSN | NTSN Δ <i>inlF</i> | NTSN Δ <i>inlF::inlF</i> |
|-------------|------|---------------------------|---------------------------------|
| AMY | + | + | + |
| PIPLC | + | + | + |
| dXYL | - | - | - |
| ADH1 | - | - | - |
| BGAL | - | - | - |
| AGLU | + | + | + |
| APPA | - | - | - |
| CDEX | + | + | + |
| PyrA | - | - | - |
| BGUR | - | - | - |
| AlaA | - | - | - |
| TyrA | + | + | + |
| dSOR | - | - | - |
| URE | - | - | - |
| POLYB | + | + | + |
| dGAL | - | - | - |
| dMAN | - | - | - |
| dMNE | + | + | + |
| MBdG | + | + | + |
| PUL | - | - | - |
| dTRE | + | + | + |
| ADH2s | - | - | - |
| AspA | - | - | - |
| BGAR | - | - | - |
| AMAN | + | + | + |

| | | | |
|-------|---|---|---|
| PHOS | - | - | - |
| LeuA | + | + | + |
| ProA | - | - | - |
| BGURr | - | - | - |
| AGAL | - | - | - |
| dRIB | - | - | - |
| ILATk | - | - | - |
| LAC | + | + | + |
| NAG | + | + | + |
| dMAL | + | + | + |
| BACI | + | + | + |
| NOVO | + | + | + |
| NC6.5 | + | + | + |
| Draf | - | - | - |
| O129R | + | + | + |
| SAL | + | + | + |
| SAC | + | + | + |
| OPTO | + | + | + |

Supplementary Table 3. Primers for quantitative real-time PCR analysis.

| Primer code | Primers Sequences (5'-3') |
|--------------------|----------------------------------|
| InlA1 | ACACTACACCACCTTCCG |
| InlA2 | GCATTATCGCTATCACCA |
| InlB1 | GAGCGTTAGCAGGACTTA |
| InlB2 | TCGCCATCATCACTTATT |
| InlF1 | CCGCTAAATGAGCCAGTA |
| InlF2 | GCCCAGTTGTAAAGTCCC |
| Gyrb1 | AGACGCTATTGATGCCGATGA |
| Gyrb2 | GTATTGCGCGTTGTCTTCGA |
| TNF- α -F | TCTCATTCCCTGCTTGTGG |

| | |
|------------------|----------------------|
| TNF- α -R | ACTTGGTGGTTTGCTACGA |
| IL-1 β -F | GCCACCTTTTGACAGTGATG |
| IL-1 β -R | TGATGTGCTGCTGCGAGA |
| IL-10-F | ACCTGGTAGAAGTGATGCC |
| IL-10-R | GACACCTTGGTCTTGGAG |
| IL-6-F | TACCACTCCCAACAGACC |
| IL-6-R | CATTTCCACGATTTCCCAGA |
| GAPDH-F | CAAATTCAACGGCACAGTCA |
| GAPDH-R | TTAGTGGGGTCTCGCTCC |

Supplementary Table 4. Primers for mutant and complementary strain's construction.

| Primer code | Primers sequences (5'-3') ^a | Digestion sites | Size of PCR product (bp) |
|-----------------|-------------------------------------------------|-----------------|--------------------------|
| <i>inlFa</i> -F | CTGTCGACAAAGTGGTTCGGGAAATCCCG | <i>Sal</i> I | 501 |
| <i>inlFa</i> -R | GGATATACTGGGTTTTTTAATTTAGTACCT TCCTTCAAC | | |
| <i>inlFb</i> -F | GTTGAAGGAAGGTACTAAATTA AAAAACC CAGTATATCC | <i>Kpn</i> I | 702 |
| <i>inlFb</i> -R | GCGGTACCAAACA AAAAATCG | | |
| <i>inlFW</i> 1 | AGTTAGCTTGGATTTTAACGCT | | 4071/1599 |
| <i>inlFW</i> 2 | GGGTGAAATTGACTTAACCAAAACT | | |
| <i>inlFc</i> -F | TTGTAAAACGACGGCCAGTGAATTCAGGC TTATCCAATCCACT | <i>Eco</i> RI | 2195 |
| <i>inlFc</i> -R | TTCTTCTGTTACAGTTTTTACTGCGTTTGTA TTGCCGTCTAT | | |
| <i>inlFd</i> -F | ATAGACGGCAATACAAACGCAGTAAAAA CTGTAACAGAAGAA | <i>Xba</i> I | 1956 |
| <i>inlFd</i> -R | TGCATGCCTGCAGGTCGACTCTAGAGCGG GTGAAATTGACTTA | | |
| <i>inlFW</i> 3 | CGGATTGACGGTAGAAT | | 4460/1990 |
| <i>inlFW</i> 4 | AAATGTCGCCGATAAAC | | |

^aThe inlFa-F and inlFb-R primers contain homologous fragments of the pGEM-T vector. The restriction endonuclease used are marked with the following lines.