

## SUPPLEMENTAL INFORMATION

### SUPPLEMENTARY METHODS

#### *Cell culture*

Human PMSCs were isolated as previously reported.<sup>1</sup> Term placental tissue (38–40 weeks gestation) were obtained from healthy donor mothers after obtaining informed consent approval as reviewed by the institutional review board. PMSCs were cultured in low-glucose DMEM (Invitrogen-Thermo Fisher Scientific, MA, USA), with 10% FBS (Hyclone-Thermo Fisher Scientific), 100 U/ml penicillin, 100 g/ml streptomycin and 2 mM L-glutamine (all from Gibco-Thermo Fisher Scientific).<sup>1,2</sup>

#### *Mouse model of KP-induced bacterial pneumonia and PMSC injection*

A clinical *KP* strain of serotype 2 was isolated from sputum.<sup>3</sup> The strain was grown in 20 ml of LB broth (Alpha Biosciences, Baltimore, MD, USA) at 37°C on a shaker at 200 rpm. After overnight incubation, bacteria were then cultured in a 1:5 dilution for additional 4 hours at 37°C until an optical density of 0.9 at 600nm was reached to ascertain bacterial counts of  $4 \times 10^8$  colony-forming units (CFUs)/ml. Bacteria were then diluted in sterile PBS to a sublethal dose ( $5 \times 10^6$  CFUs/50  $\mu$ l) or lethal dose ( $5 \times 10^7$  CFUs/50  $\mu$ l) for intratracheal (i.t.) inoculation and maintained on ice until inoculation. Bacterial pneumonia was conducted in mice according to previous report.<sup>4</sup> Using protocols approved by the Institutional Animal Care and Use

Committee, 6-week old C57BL/6 mice were anesthetized with intraperitoneal a Zoletil/Xylazine mixture (Zoletil: 20-40 mg/kg, Xylazine: 5-10 mg/kg, both from Sigma-Aldrich), and then inoculated intratracheally with 50  $\mu$ l of the *KP* suspension. Appropriate dilutions of the inoculum were plated on LB agar plates to confirm the dose administered.<sup>5</sup> Control group of mice were inoculated with 50  $\mu$ l of PBS. PMSCs ( $3 \times 10^5$  cells in 100 $\mu$ l) or PBS were intravenously injected 2 hours after *KP* injection. Mice were euthanized with CO<sub>2</sub> at 4 hours, with peripheral blood collected for evaluation of bacterial load and lungs collected for evaluation of bacterial load, M $\Phi$  analyses, TNF- $\alpha$  detection, and histological evaluation.

#### *Enzyme-linked immunosorbent assay (ELISA)*

Lung-infiltrated immune cells were harvested after mice were infected with or without K2 *KP* for 2 hours, and then cultured in the presence or absence of PMSCs *ex vivo* for 2 hours additionally, with supernatant collected for TNF- $\alpha$  detection by ELISA according to manufacturer's instructions (eBioscience Systems).

#### *Histological analysis*

Lung lobes were excised and then fixed with 10% formalin overnight. Fixed lung lobes were sequentially dehydrated with 20% and 30% sucrose, then subsequently embedded in optical cutting temperature compound (OCT), followed by sectioning (10  $\mu$ m thick) and H&E staining. The scoring of lung tissue injury was performed by a different technician, and done

according to a previous report<sup>6</sup> in which interstitial damage, vasculitis, peri-bronchitis, edema, thrombus formation, and pleuritic were all evaluated—this evaluation then resulted in a score of 0 for normal/no injury, 1 = mild injury, 2 = moderate injury, 3 = severe injury, and 4 = very severe injury.

#### *Assessment of in vivo MΦ function*

Lung lobes were minced with scissors to 1mm-sized chunks and dissected with 50 U/ml DNase and 1 mg/ml type IV collagenase at 37°C for 30 minutes. An equal volume of heat inactivated FBS was added to stop the enzymatic reaction. Single-cell suspensions were harvested by filtering through 100 μm strainer, lysing red blood cells in hypotonic buffer (H<sub>2</sub>O with 0.15 M NH<sub>4</sub>Cl, 10 mM KHCO<sub>3</sub> and 0.1 mM Na<sub>2</sub>-EDTA) and washing with cold PBS. Then, the single-cell suspensions derived from lung lobes were stained with APC- or APC-Cy7-anti-mouse CD45, PerCP-anti-mouse CD11b, FITC- or PE-anti-mouse F4/80, APC- or FITC-anti-mouse CD206, PE-anti-mouse inducible nitric oxide synthase (iNOS), APC-anti-mouse arginase-1 (Arg1), PE-Cy7-anti-mouse TNF-α (all from eBioscience, San Diego, CA, USA), or FITC-anti-mouse CD11c, PE-Cy7-anti-mouse SIGLEC-F, 2'-7'-dichlorofluoresceindiacetate (DCFDA, Sigma-Aldrich, MO, USA) for 30 minutes according to manufacturer's instructions, then assessed by flow cytometry or analyzed with t-Distributed Stochastic Neighbor Embedding (t-SNE)-based algorithm. CD45 was first gated for analysis of the frequency of CD11b<sup>low</sup>F4/80<sup>+</sup> AMΦs and CD11b<sup>high</sup>F4/80<sup>+</sup> BMMΦs, and then either iNOS and Arg1, or CD206, TNF-α,

CD11c and SIGLEC-F were analyzed for the polarization phenotypes and further confirmation of both MΦs. Reactive oxygen species (ROS) production was assessed in gated AMΦs and BMMΦs by flow cytometry.

#### *In vivo depletion of AMΦs*

C57BL/6J mice were intratracheally injected with 100 µl of PBS or 15mM clodronate (Cayman Chemical, Ann Arbor, MI, USA).<sup>7</sup> After 48 hours, the frequency as well as absolute numbers of CD11b<sup>low</sup>F4/80<sup>+</sup> AMΦs and CD11b<sup>high</sup>F4/80<sup>+</sup> BMMΦs in lung tissues were assessed by flow cytometry. The efficacy for AMΦ depletion with clodronate was similar to use of liposomal clodronate at 3 days (data not shown), in line with previous report.<sup>7</sup>

#### *Bacterial phagocytosis assay*

Lung lobes from non-infected mice were harvested in RPMI (Invitrogen-Thermo Fisher Scientific)-based culture medium with 10% FBS, 100 U/ml penicillin, 100 g/ml streptomycin and 2 mM L-glutamine after animal euthanasia with CO<sub>2</sub>, washed with PBS and placed in petri dishes with 0.5 ml PBS. The lung tissues were minced with scissors to 1mm-sized chunks and dissected with 50 U/ml DNase and 1 mg/ml type IV collagenase at 37°C for 30 minutes. An equal volume of culture medium was added to stop the digestion reaction. Extracted cells were harvested by filtering with a 100 µm strainer, and red blood cells were lysed in hypotonic buffer (H<sub>2</sub>O with 0.15 M NH<sub>4</sub>Cl, 10 mM KHCO<sub>3</sub> and 0.1 mM Na<sub>2</sub>-EDTA, all components from Sigma-

Aldrich) and washed with cold PBS. Immune cells harvested from lung tissues were cultured with PMSCs for 2 hours, and then exposure to FITC-labeled *KP* at 37°C for 30 minutes. Suspended cells were collected and washed with cold PBS and then stained with APC-anti-mouse CD45, PerCP-anti-mouse CD11b, PE-anti-mouse F4/80. After further washing with PBS and fixing with 1% formaldehyde, CD45-gated cells were analyzed for the frequency of *KP* uptake in CD11b<sup>low</sup>F4/80<sup>+</sup> AMΦs and CD11b<sup>high</sup>F4/80<sup>+</sup> BMMΦs by flow cytometry.

#### *Bacterial growth assay*

*KP* growth was performed as we previously reported.<sup>8</sup> Briefly, *KP* suspensions were cultured for 2 hours and then measured by calculating colony-forming unit (CFU) in the presence or absence of PMSCs. 5 x 10<sup>5</sup> CFUs of live *KP* suspensions were added to culture medium or 2 x 10<sup>4</sup> PMSCs for 2-hour incubation, and then culture samples were serially diluted with PBS and plated on agar plates for calculation of bacterial counts.

#### *Determination of in vivo bacterial dissemination*

Lung lobes were harvested in 0.5ml cold PBS after animal euthanasia with CO<sub>2</sub>, and then fragmented with scissors and homogenized with syringes. Aliquots from lungs and blood samples were diluted with cold PBS and plated on LB agar plates (Alpha Biosciences) at 37°C. After overnight incubation, CFUs were calculated.

### *Determination of in vivo expression of bacterial virulence genes*

Mice were i.t. injected with a  $5 \times 10^6$  CFUs of K2 *KP* followed by i.v. administration of PBS or  $3 \times 10^5$  PMSCs 2 hours later. At 4 hours after infection, lung lobes were excised, homogenized, serially diluted with PBS, and plated on agar plates for overnight culture. Finally, single colonies derived from each animal sample were picked up for PCR analyses. *KP* virulence genes including regulator of mucoid phenotype A (*rmpA*), activator for capsular polysaccharide synthesis (*rmpA2*), enterobactin synthase component B (*entB*) and last universal common ancestor (LUCA) as internal control were evaluated according to our previous report.<sup>9</sup>

### *RNA silencing*

The lentiviral system for IL1B knockdown was obtained from the Taiwan National RNAi Core Facility (Sinica, Taipei, Taiwan). To generate lentiviruses carrying short hairpin RNA (shRNA), we co-transfected pLKO.1-shLuc (negative control; target sequence, 5'-GCGGTTGCCAAGAGGTTCCAT-3') or pLKO.1-shIL1B (target sequence, 5'-CGGCCAGGATATAACTGACTT-3') with packaging vectors, pCMVdelR8.91 and pMD.G, into 293T cells according to protocols provided by the Taiwan National RNAi Core Facility. To knockdown IL1B expression, PMSCs were infected with 10 RIU/cell of prepared lentiviruses in the presence of 8 µg/ml protamine sulfate (Sigma-Aldrich).<sup>8</sup> After 24 hours of infection, the viral medium was replaced with 2 µg/ml puromycin (Sigma-Aldrich)-containing culture medium and cultured for 2 days, with the surviving cells used for experimentation.

### *Bioinformatic analyses*

Transcriptomic profiling databases were obtained from the National Center for Biotechnology Information-Gene Expression Omnibus database for bioinformatic analyses: GSE121970 generated from lung tissues of control mice or K2 *KP*-infected mice,<sup>10</sup> and GSE160163 generated from murine BMMΦs treated with or without TNF- $\alpha$ .<sup>11</sup> Principal component analysis (PCA) of transcriptomic profiles was performed to show the relatedness of whole transcriptomic profiles between control and *KP*-infected lung tissues using Partek® Flow® software (Partek, Inc., St. Louis, MO), while MΦ- or immune-related pathways in Gene Ontology (GO) Biological Processes were enriched by Metascape analysis; significance was set at  $P < 0.01$ .<sup>12</sup> The Molecular Activation Prediction (MAP) tool was used to interrogate networks and pathways with expression levels of involved genes by analyzing transcriptomic data of lung tissues in *KP*-infected mice versus control mice using Ingenuity Systems Pathway Analysis (IPA) software (QIAGEN, Hilden, Germany).

### REFERENCES

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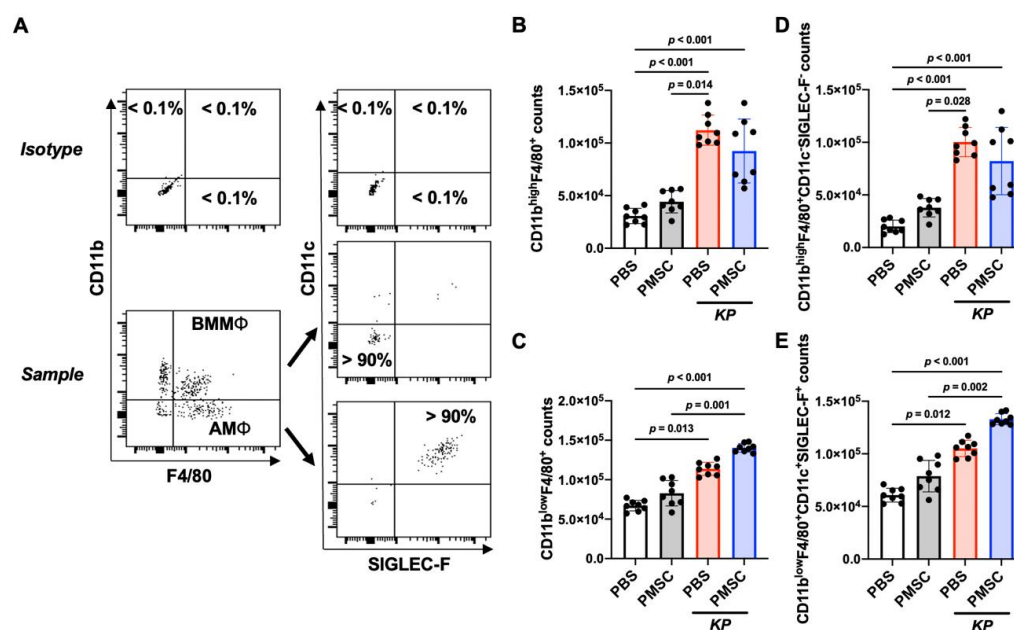
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## SUPPLEMENTARY FIGURES

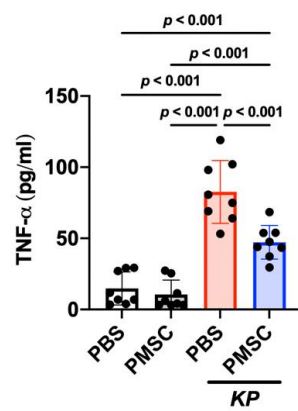
Figure S1



**Supplementary Figure 1. Human placenta mesenchymal stem cells (PMSCs) prevent CD11b<sup>high</sup>F4/80<sup>+</sup> or CD11b<sup>high</sup>F4/80<sup>+</sup>CD11c<sup>-</sup>SIGLEC-F<sup>-</sup> bone marrow-derived macrophages (BMM $\Phi$ s) but preserve CD11b<sup>low</sup>F4/80<sup>+</sup> or CD11b<sup>low</sup>F4/80<sup>+</sup>CD11c<sup>+</sup>SIGLEC-F<sup>+</sup> alveolar macrophages (AM $\Phi$ s) in KP-infected lungs.** (A) Representative data for frequency analysis of recruited BMM $\Phi$ s and resident AM $\Phi$ s in lung lobes as assessed with flow cytometry. C57BL/6J mice were intratracheally (i.t.) injected with  $5 \times 10^6$  CFUs of K2 KP followed by intravenous (i.v.) administration of PBS or  $3 \times 10^5$  PMSCs 2 hours later. Single-cell suspensions derived from lung lobes were harvested at 4 hours after infection, and then stained with anti-CD45, anti-CD11b, anti-F4/80, anti-CD11c, and anti-SIGLEC-F for flow cytometric analysis. Gating for CD45<sup>+</sup> cells was first performed, with subsequent frequency analysis for

CD11b<sup>high</sup>F4/80<sup>+</sup> BMMΦs and then the CD11c<sup>-</sup>SIGLEC-F<sup>-</sup> population, as well as CD11b<sup>low</sup>F4/80<sup>+</sup> AMΦs and then the CD11c<sup>+</sup>SIGLEC-F<sup>+</sup> population. Isotype control antibodies related to all used antibodies were mixed to serve as a control for each gating protocol. (B and C) Pooled data for analyses of absolute counts of CD11b<sup>high</sup>F4/80<sup>+</sup> BMMΦs and CD11b<sup>low</sup>F4/80<sup>+</sup> AMΦs, respectively, in uninfected and infected lungs with or without PMSC treatment (n = 8 for each group). (D and E) Pooled data for analyses of absolute counts of CD11b<sup>high</sup>F4/80<sup>+</sup> CD11c<sup>-</sup>SIGLEC-F<sup>-</sup> BMMΦs and CD11b<sup>low</sup>F4/80<sup>+</sup> CD11c<sup>+</sup>SIGLEC-F<sup>+</sup> AMΦs, respectively, in uninfected and infected lungs with or without PMSC treatment (n = 8 for each group). Data are shown as mean ± SD.

Figure S2

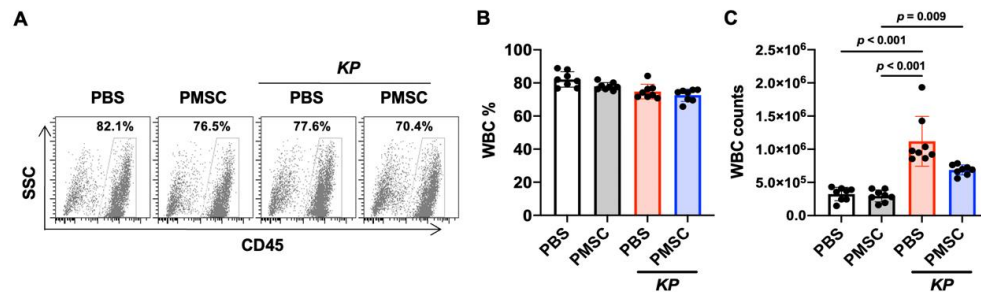
**Supplementary Figure 2. Human PMSCs decrease levels of secreted TNF- $\alpha$  in *KP*-infected**

**lungs.** Mice were i.t. infected with  $5 \times 10^6$  CFUs of K2 *KP* followed by i.v. administration of PBS

or  $3 \times 10^5$  PMSCs 2 hours later. At 4 hours after infection, mice were sacrificed and lung tissues

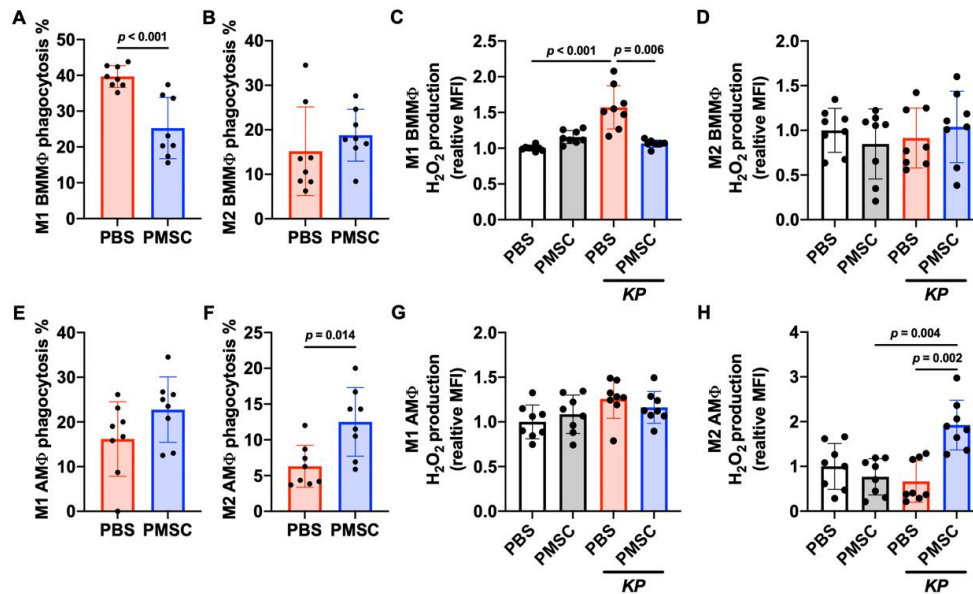
were collected for homogenization for assessment of TNF- $\alpha$  by ELISA (n = 8 for each group).

Figure S3



**Supplementary Figure 3. Human PMSCs decrease the pulmonary infiltration of white blood cells (WBCs) in mice with pneumonia induced by clinically isolated *Klebsiella pneumoniae* (KP)-serotype K2.** (A & B, n=8 for each group) Representative and pooled data, respectively, of frequency of WBCs in lung lobes of each experimental group as assessed by flow cytometry. Gating for CD45<sup>+</sup> cells was performed and analyzed for frequency. (C, n=3, 3, 5, 5) Absolute number of WBCs in lung lobes of each experimental group as assessed by flow cytometry with gating for CD45<sup>+</sup> cells. Data are shown as mean ± SD.

Figure S4



**Supplementary Figure 4. Human PMSCs downregulate capabilities of phagocytosis and respiratory burst in M1-type BMMΦs while upregulating these antibacterial functions in M2-based AMΦs in mice with KP pneumonia.**

(A and B) Capabilities of *KP* phagocytosis in

M1 and M2 BMMΦs harvested from lung tissues co-cultured with or without PMSCs for 2

hours as assessed with subsequent addition of FITC-labeled *KP* for 30 minutes at 37°C.

Phagocytic capabilities of M1 and M2 BMMΦs were measured by detecting for the frequency of

FITC<sup>+</sup> cells in CD45<sup>+</sup>CD11b<sup>high</sup>F4/80<sup>+</sup>CD206<sup>-</sup> and CD45<sup>+</sup>CD11b<sup>high</sup>F4/80<sup>+</sup>CD206<sup>+</sup>, respectively

(n = 8 for each group). (C and D) Assessment of M1 and M2 BMMΦ respiratory burst as

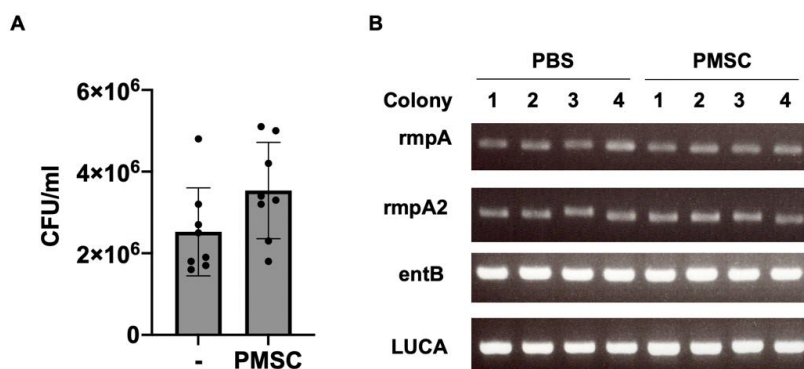
measured by reactive oxygen species (ROS) production of H<sub>2</sub>O<sub>2</sub>. Single-cell suspensions

derived from lung tissues were incubated with 10 μM of 2'-7'-dichlorofluoresceindiacetate

(DCFDA) for 30 minutes, and then analyzed by flow cytometry to assess ROS levels in

CD45<sup>+</sup>CD11b<sup>high</sup>F4/80<sup>+</sup>CD206<sup>-</sup> and CD45<sup>+</sup>CD11b<sup>high</sup>F4/80<sup>+</sup>CD206<sup>+</sup> BMMΦs (n = 8 for each group). (E and F) Capabilities of *KP* phagocytosis in M1 and M2 AMΦs derived from lung tissues co-cultured with or without PMSCs for 2 hours as assessed with subsequent addition of FITC-labeled *KP* for 30 minutes at 37°C. Phagocytic capabilities of M1 and M2 AMΦs were measured by detecting for the frequency of FITC<sup>+</sup> cells in CD45<sup>+</sup>CD11b<sup>low</sup>F4/80<sup>+</sup>CD206<sup>-</sup> and CD45<sup>+</sup>CD11b<sup>low</sup>F4/80<sup>+</sup>CD206<sup>+</sup>, respectively (n = 8 for each group). (G and H) Assessment of M1 and M2 AMΦ respiratory burst as measured by ROS production of H<sub>2</sub>O<sub>2</sub>. Single-cell suspensions derived from lung tissues were incubated with 10 μM of DCFDA for 30 minutes, and then analyzed by flow cytometry to assess ROS levels in CD45<sup>+</sup>CD11b<sup>low</sup>F4/80<sup>+</sup>CD206<sup>-</sup> and CD45<sup>+</sup>CD11b<sup>low</sup>F4/80<sup>+</sup>CD206<sup>+</sup> AMΦs (n = 8 for each group).

Figure S5



**Supplementary Figure 5. Human PMSCs negligibly affect the virulence of *KP*.** (A) *KP*

growth as assessed by measurement of colony-forming unit (CFU) in the presence or absence of

PMSCs.  $5 \times 10^5$  CFUs of live *KP* suspension were added to culture medium or  $2 \times 10^4$  PMSCs

for 2-hour incubation, and then culture samples were serially diluted with PBS and plated on

agar plates for calculation of bacterial counts. (B) PCR analyses of *KP* virulence genes including

regulator of mucoid phenotype A (*rmpA*), activator for capsular polysaccharide synthesis

(*rmpA2*), enterobactin synthase component B (*entB*), with last universal common ancestor

(LUCA) as internal control. Mice were i.t. injected with a  $5 \times 10^6$  CFUs of K2 *KP* followed by i.v.

administration of PBS or  $3 \times 10^5$  PMSCs 2 hours later. At 4 hours after infection, lung lobes were

excised, homogenized, serially diluted with PBS, and plated on agar plates for overnight culture.

Finally, single colony derived from each animal sample was picked up for PCR analyses.