Supplementary information

Vitamin Lipid Nanoparticles Enable Adoptive Macrophage Transfer for the Treatment of Multidrug-Resistant Bacterial Sepsis

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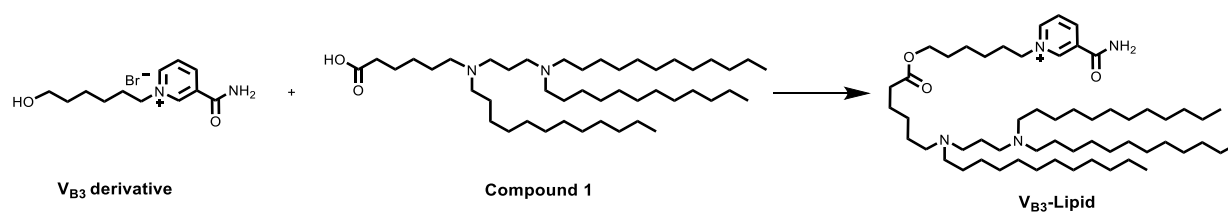
Supplementary Figure 1. Synthesis of V_{B3}-Lipid. Compound 1 (150 mg, 0.23 mmole) was dissolved in a mixture of 2 mL CH_2Cl_2 and 2 mL DMF. Vitamin B3 derivative (62 mg, 0.21 mmole), EDC (87 mg, 0.46 mmole) and DMPA (10 mg) were added to the solution. The resulting mixture was stirred at room temperature overnight. The reacting mixture was purified by column chromatography using a CombiFlash Rf system with a RediSep Gold Resolution silica column (Teledyne Isco) with gradient elution (CH_2Cl_2 and ultra) from 100% CH_2Cl_2 to 0% CH_2Cl_2 (ultra: CH_2Cl_2/MeOH/NH$_4$OH =75/22/3 by volume) to give 80 mg colorless oil V_{B3}-Lipid, yield 37%. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 10.75 (1H, s), 9.92 (1H, s), 9.17-9.16 (1H, d, J = 4), 8.94 (1H, s), 8.09-8.06 (1H, t, J = 4), 6.20 (1H, s), 4.88-4.85 (2H, t, J = 4), 4.06-4.03 (2H, t, J = 4), 2.47 (11H, m), 2.31-2.28 (2H, t, J = 4), 2.13 (2H, s), 1.64-1.63 (7H, m), 1.45 (12H, m), 1.26 (56H, s), 0.89-0.87 (9H, t, J = 4). MS (m/z): M$^+$ calcd. for $C_{57}H_{109}N_4O_3$, 897.8494; found: 897.8496.
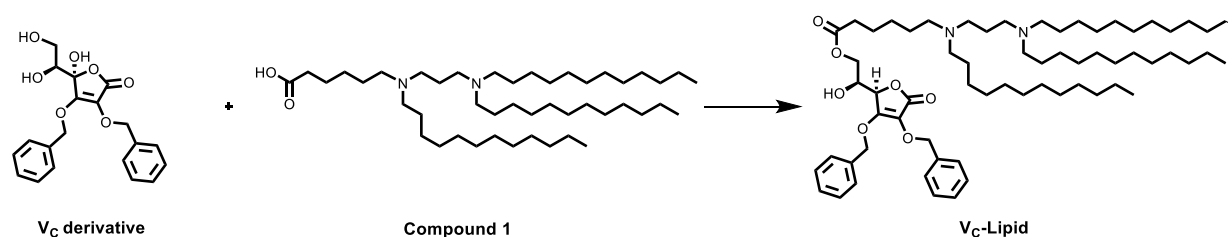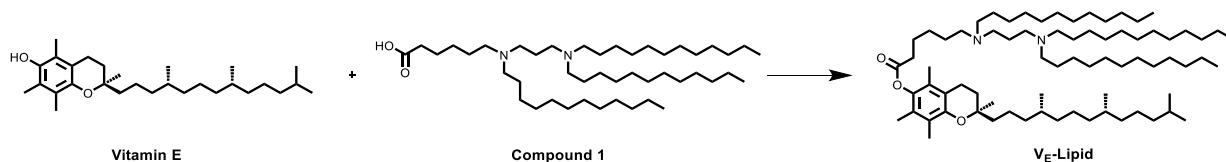

Supplementary Figure 2. Synthesis of V_C-Lipid. Compound 1 (150 mg, 0.23 mmole) was dissolved in 2 mL CH_2Cl_2. Vitamin C derivative (77 mg, 0.23 mmole), EDC (87 mg, 0.46 mmole) and DMPA (10 mg) were added to the solution. The resulting mixture was stirred at room temperature overnight. The reacting mixture was purified by column chromatography using a CombiFlash Rf system with a RediSep Gold Resolution silica column (Teledyne Isco) with gradient elution (CH_2Cl_2 and ultra) from 100% CH_2Cl_2 to 80% CH_2Cl_2 (ultra:

CH$_2$Cl$_2$/MeOH/NH$_4$OH =75/22/3 by volume) to give 105 mg colorless oil V$_C$-Lipid, yield 44%. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.40-7.25 (10H, m), 5.26-5.13 (4H, m), 4.67 (1H, s), 4.33-4.08 (3H, m), 2.65 (1H, s), 2.42 (12H, m), 1.67-1.60 (13H, m), 1.28 (57H, s), 0.92-0.89 (9H, t, J = 4). MS (*m/z*): [M+H]$^+$ calcd. C$_{65}$H$_{111}$N$_2$O$_7$, 1031.8391; found: 1031.8379.
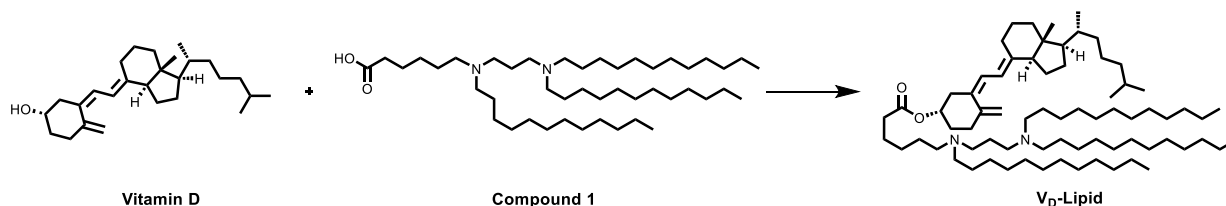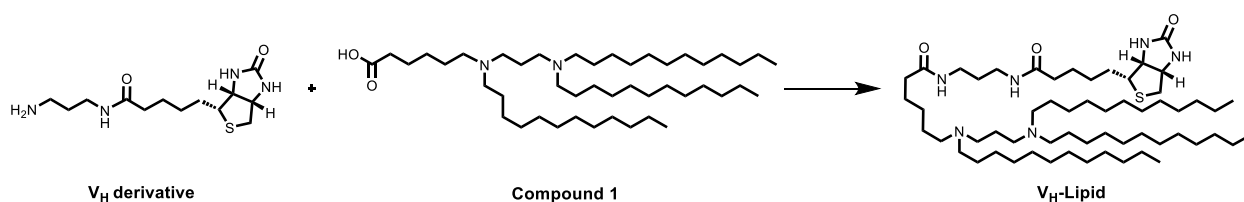

Supplementary Figure 3. Synthesis of V$_D$-Lipid. Compound 1 (150 mg, 0.23 mmole) was dissolved in 2 mL CH$_2$Cl$_2$. Vitamin D (83 mg, 0.23 mmole), EDC (87 mg, 0.46 mmole) and DMPA (10 mg) were added to the solution. The resulting mixture was stirred at room temperature overnight. The reacting mixture was purified by column chromatography using a CombiFlash Rf system with a RediSep Gold Resolution silica column (Teledyne Isco) with gradient elution (CH$_2$Cl$_2$ and ultra) from 100% CH$_2$Cl$_2$ to 85% CH$_2$Cl$_2$ (ultra: CH$_2$Cl$_2$/MeOH/NH$_4$OH =75/22/3 by volume) to give 40 mg colorless oil V$_D$-Lipid, yield 16%. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 6.22-6.19 (1H, d, J = 12), 6.04-6.02 (1H, d, J = 8), 5.06 (1H, s), 4.94 (1H, s), 4.84 (1H, s), 2.82-2.56 (12H, m), 2.38-2.28 (4H, m), 1.99-1.96 (5H, m), 1.67-1.49 (15H, m), 1.30-1.26 (71H, m), 0.93-0.87 (21H, m), 0.54 (2H, s). MS (*m/z*): [M+H]$^+$ calcd. for C$_{72}$H$_{135}$N$_2$O$_2$, 1060.0524; found: 1060.0529.

Supplementary Figure 4. Synthesis of V$_E$-Lipid. Compound 1 (150 mg, 0.23 mmole) was dissolved in 2 mL CH$_2$Cl$_2$. Vitamin E (99 mg, 0.23 mmole), EDC (87 mg, 0.46 mmole) and DMPA (10 mg) were added to the solution. The resulting mixture was stirred at room temperature

overnight. The reacting mixture was purified by column chromatography using a CombiFlash Rf system with a RediSep Gold Resolution silica column (Teledyne Isco) with gradient elution (CH_2Cl_2 and ultra) from 100% CH_2Cl_2 to 85% CH_2Cl_2 (ultra: CH_2Cl_2/MeOH/NH_4OH =75/22/3 by volume) to give 66 mg colorless oil V_E-Lipid, yield 26%. ^1H NMR (400 MHz, $CDCl_3$): δ = 2.83-2.57 (14H, m), 2.08 (3H, s), 2.00 (3H, s), 1.96 (3H, s), 1.81 (4H, m), 1.62 (5H, m), 1.54-1.52 (11H, m), 1.28-1.23 (67H, m), 1.14 (7H, m), 0.89-0.84 (24H, m). MS (*m/z*): [M+H]$^+$ calcd. for $C_{74}H_{141}N_2O_3$, 1106.0942; found: 1106.0944.

Supplementary Figure 5. Synthesis of V_H-Lipid. Compound 1 (100 mg, 0.15 mmole) was dissolved in a mixture of 3 mL THF. NHS (50 mg, 0.43 mmole) and DCC (80 mg, 0.39 mmole) were added to the solution that was stirred overnight. Vitamin H derivative (140 mg, 0.46 mmole) and 200 µL trimethylamine was added to the solution. The resulting mixture was stirred at room temperature overnight. The reacting mixture was purified by column chromatography using a CombiFlash Rf system with a RediSep Gold Resolution silica column (Teledyne Isco) with gradient elution (CH_2Cl_2 and ultra) from 100% CH_2Cl_2 to 75% CH_2Cl_2 (ultra: CH_2Cl_2/MeOH/NH_4OH =75/22/3 by volume) to give 60 mg colorless oil V_H-Lipid, yield 41%. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.11 (1H, s), 6.70 (1H, s), 5.98 (1H, s), 4.52-4.49 (1H, t, *J* = 4), 4.33-4.30 (1H, t, *J* = 4), 3.27-3.28 (4H, m), 2.75-2.60 (11H, m), 2.25-2.19 (4H, m), 1.74-1.64 (11H, m), 1.51-1.49 (11H, m), 1.28 (59H, s), 0.90-0.87 (9H, t, *J* = 4). MS (*m/z*): [M+H]$^+$ calcd. for $C_{58}H_{115}N_6O_3S$, 975.8751; found: 975.8629.

**RNA sequences**

RNA sequence that encodes cathepsin B:

AUGUGGUGGUCCUUGAUCCUUCUUUCUUGCCUGCUGGCACUGACCAGUGCCCAUG

ACAAGCCUUCCUUCCACCCGCUGUCGGAUGACCUGAUUAACUAUAUCAACAAACA

GAAUACAACAUGGCAGGCUGGACGCAACUUCUACAAUGUUGACAUAAGCUAUCU

GAAGAAGCUGUGUGGCACUGUCCUGGGUGGACCCAAACUGCCAGGAAGGGUUGC

GUUCGGUGAGGACAUAGAUCUACCUGAAACCUUUGAUGCACGGGAACAAUGGUC

CAACUGCCCGACCAUUGGACAGAUUAGAGACCAGGGCUCCUGCGGCUCUUGUUGG

GCAUUUGGGGCAGUGGAAGCCAUUUCUGACCGAACCUGCAUUCACACCAAUGGCC

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GUGUUUUCUGACUUCUUGACUUACAAAUCAGGAGUAUACAAGCAUGAAGCCGGU

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GUUCCCUACUGGCUGGCAGCCAACUCUUGGAACCUUGACUGGGGUGAUAAUGGCU

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RNA sequence that encodes a linker:

UUCGGAUUUCUGGGC

RNA sequence that encodes an antimicrobial peptide:

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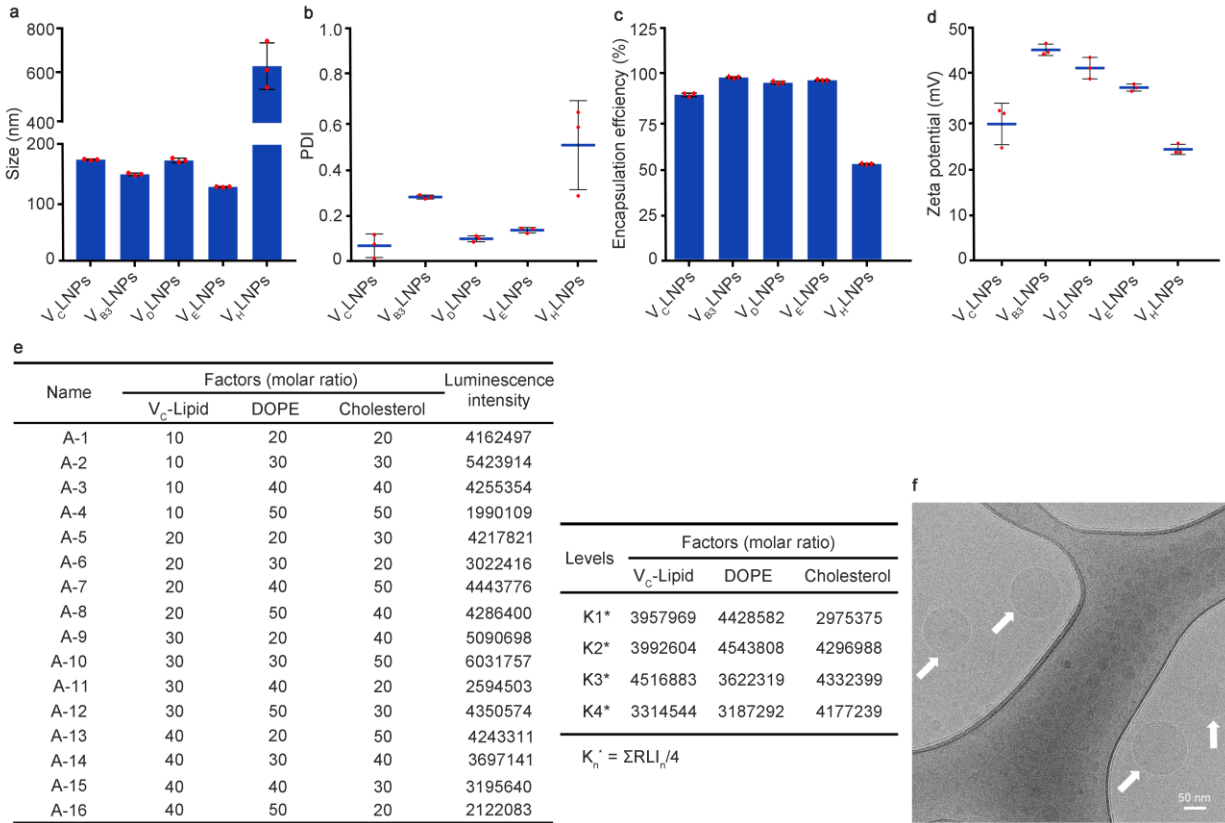
Supplementary Fig. 6. Screening, optimization, and characterization of VLNPs. a-d, Size, PDI, encapsulation efficiency, and zeta potential of VLNPs. **e**, Orthogonal array table $L_{16}(4)^4$ and K_n* values. **f**, Cryo-TEM image (Scale bar = 50 nm). Data in **f** are representative images from n = 3 independent experiments. Data in **a**, **b**, **c**, and **d** are from n = 3 biologically independent samples. All data are presented as mean ± s.d.
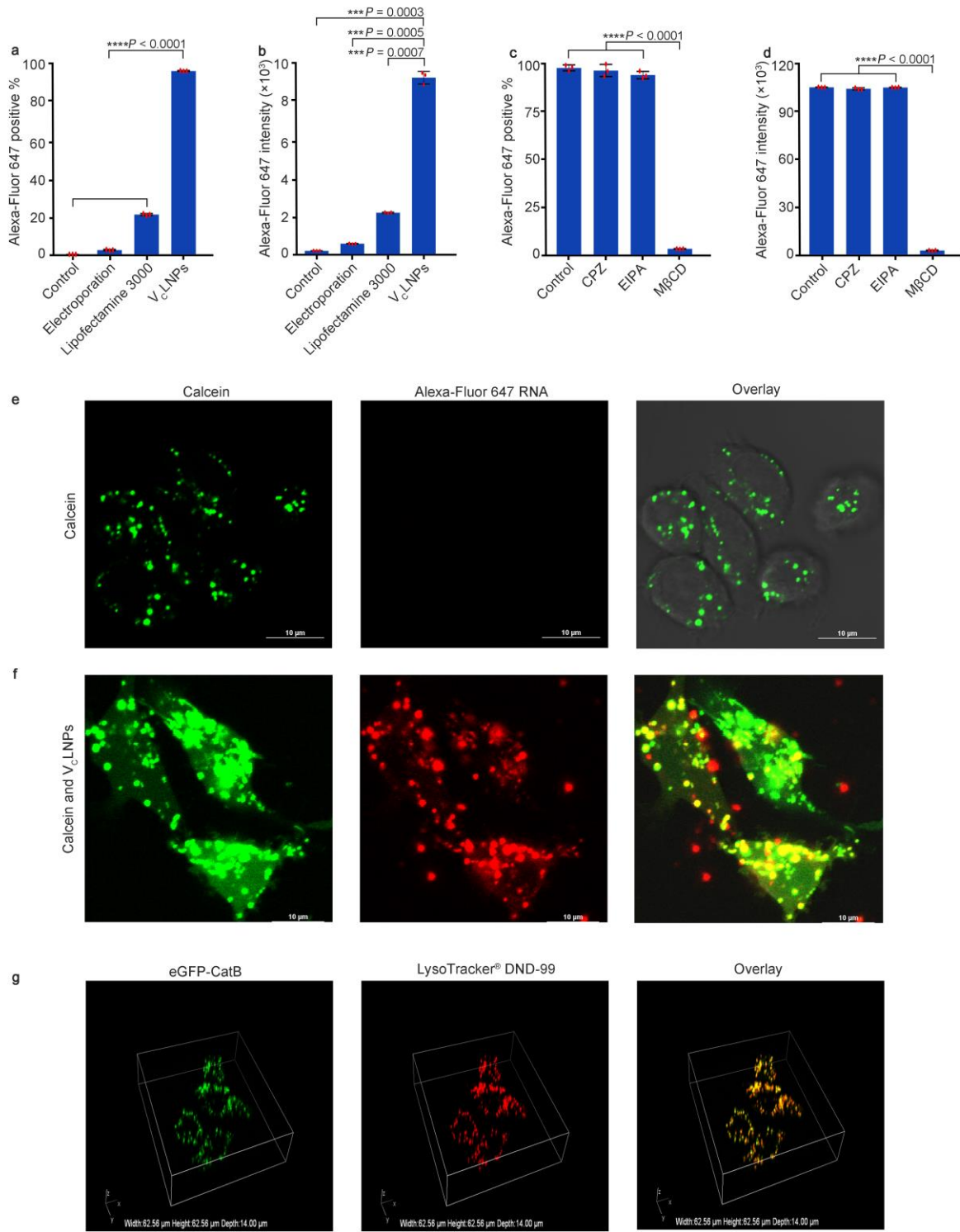
Supplementary Fig. 7. Cellular uptake, endocytic pathways, and endosomal escape. a, b,

Cellular uptake after treatment with electroporation, Lipofectamine 3000, or V$_C$LNPs . **a,**

Percentage of Alexa-Fluor 647 positive cells; **b**, Fluorescence intensity of cells. **c, d**, Cellular uptake in the presence of endocytic inhibitors, EIPA, MβCD, and CPZ, which inhibit macropinocytosis, caveolae-, and clathrin-mediated endocytosis, respectively. **c**, Percentage of Alexa-Fluor 647 positive cells; **d**, Fluorescence intensity of cells. **e, f**, Confocal microscopy of RAW264.7 cells incubated with calcein alone or calcein and V$_C$LNPs containing Alexa-Fluor 647 RNA. **e**, Calcein alone; **f**, Calcein and V$_C$LNPs containing Alexa-Fluor 647 RNA. **g**, 3D confocal microscopy images of RAW264.7 cells incubated with eGFP-CatB mRNA V$_C$LNPs. Data in **e, f, and g** are representative images from n = 3 independent experiments. Data in **a**, **b**, **c**, and **d** are from n = 3 biologically independent samples. All data are presented as mean ± s.d. Statistical significance was analyzed by the two-tailed Student's *t*-test. ***P < 0.001, ****P < 0.0001.
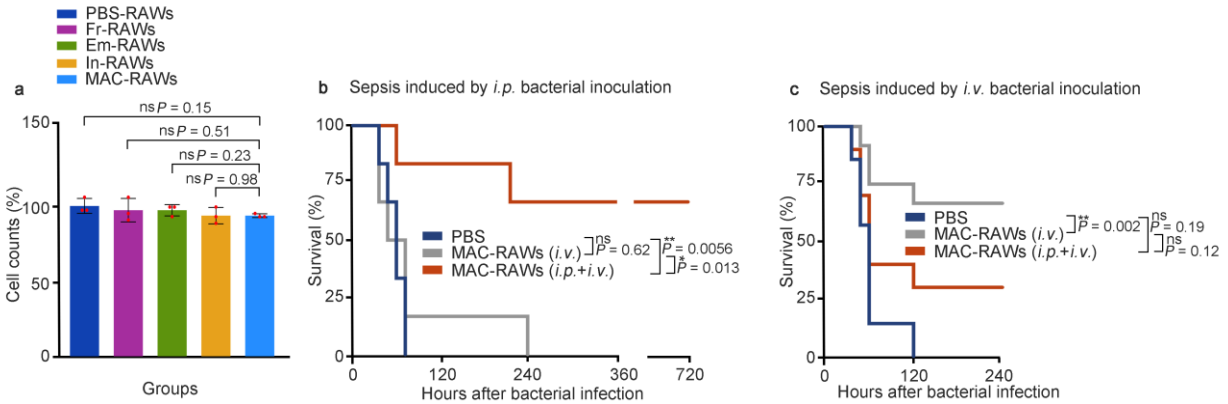
Supplementary Fig. 8. Cell counts from the *in vitro* bactericidal study and therapeutic effects of MAC-RAWs in MDRSA-induced sepsis mice with immunosuppression. a, The percentage of RAW264.7 cells normalized to the PBS-RAW group at 12 h. **b**, Percentage survival of sepsis mice induced by *i.p.* bacterial inoculation treated with PBS, MAC-RAWs (2 million cells *i.v.*), or MAC-RAWs (1 million cells *i.p.* + 1 million cells *i.v.*). n = 6 for each group. **c**, Percentage survival of sepsis mice induced by *i.v.* bacterial inoculation treated with PBS, MAC-RAWs (2 million cells *i.v.*), or MAC-RAWs (1 million cells *i.p.* +1 million cells *i.v.*). n = 7, 10, and 12 for PBS, MAC-RAWs (2 million cells *i.v.*), and MAC-RAWs (1 million cells *i.p.* + 1 million cells *i.v.*), respectively. Data in **a** are from n = 3 biologically independent samples. All data are presented as mean ± s.d. Statistical significance was analyzed by the two-tailed Student's *t*-test. Data in **b** and **c** were analyzed by the log-rank (Mantel–Cox) test. *$P < 0.05$, **$P < 0.01$; ns, not significant.
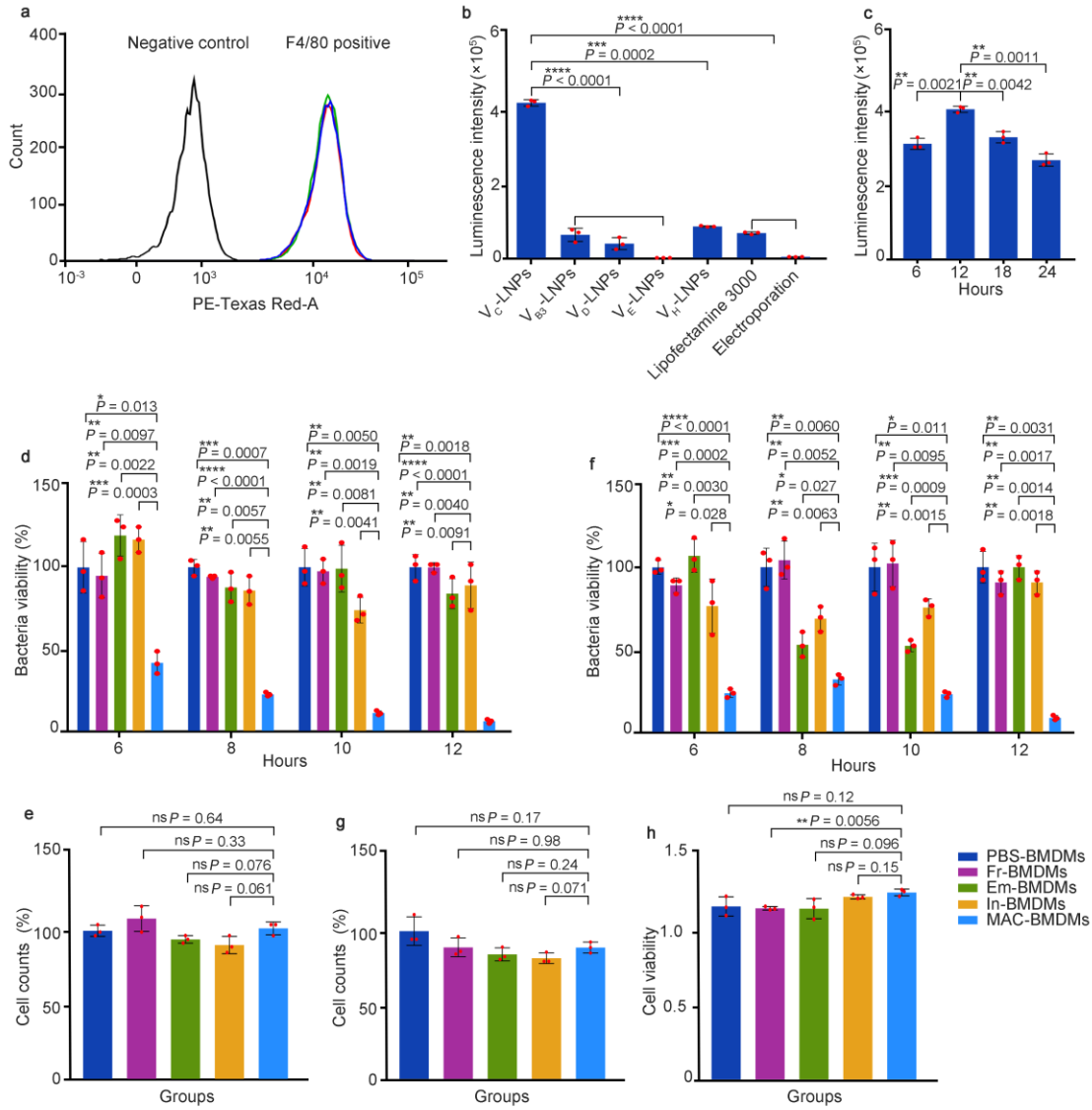
Supplementary Fig. 9. Screening of VLNPs in BMDMs and intracellular survival of MDR bacteria in BMDMs. a, F4/80, a macrophage maker, positive cells (83.5±0.7%). **b**, mRNA delivery efficiency of VLNPs in BMDMs. **c**, Expression kinetics of mRNA delivered by V_CLNPs in BMDMs. **d**, **f**, Intracellular survival of MDR bacteria in BMDMs treated by PBS (PBS-BMDMs), free AMP-CatB mRNA (Fr-BMDMs), empty V_CLNPs (Em-BMDMs), AMP-CatB mRNA V_CLNPs/CatB inhibitor II (In-BMDMs), and AMP-CatB mRNA V_CLNPs (MAC-BMDMs). **d**, MDRSA; **f**, MDR *E.coli*. **e**, **g**, The percentage of the BMDMs normalized to the

PBS-BMDM group at 12 h. **e**, MDRSA; **g**, MDR *E.coli*. **h**, Cytotoxicity of V$_C$LNPs encapsulating AMP-CatB mRNA in BMDMs was determined by the MTT assay. Data in **a-h** are from n = 3 biologically independent samples. All data are presented as mean ± s.d. Statistical significance was analyzed by the two-tailed Student's *t*-test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$; ns, not significant.
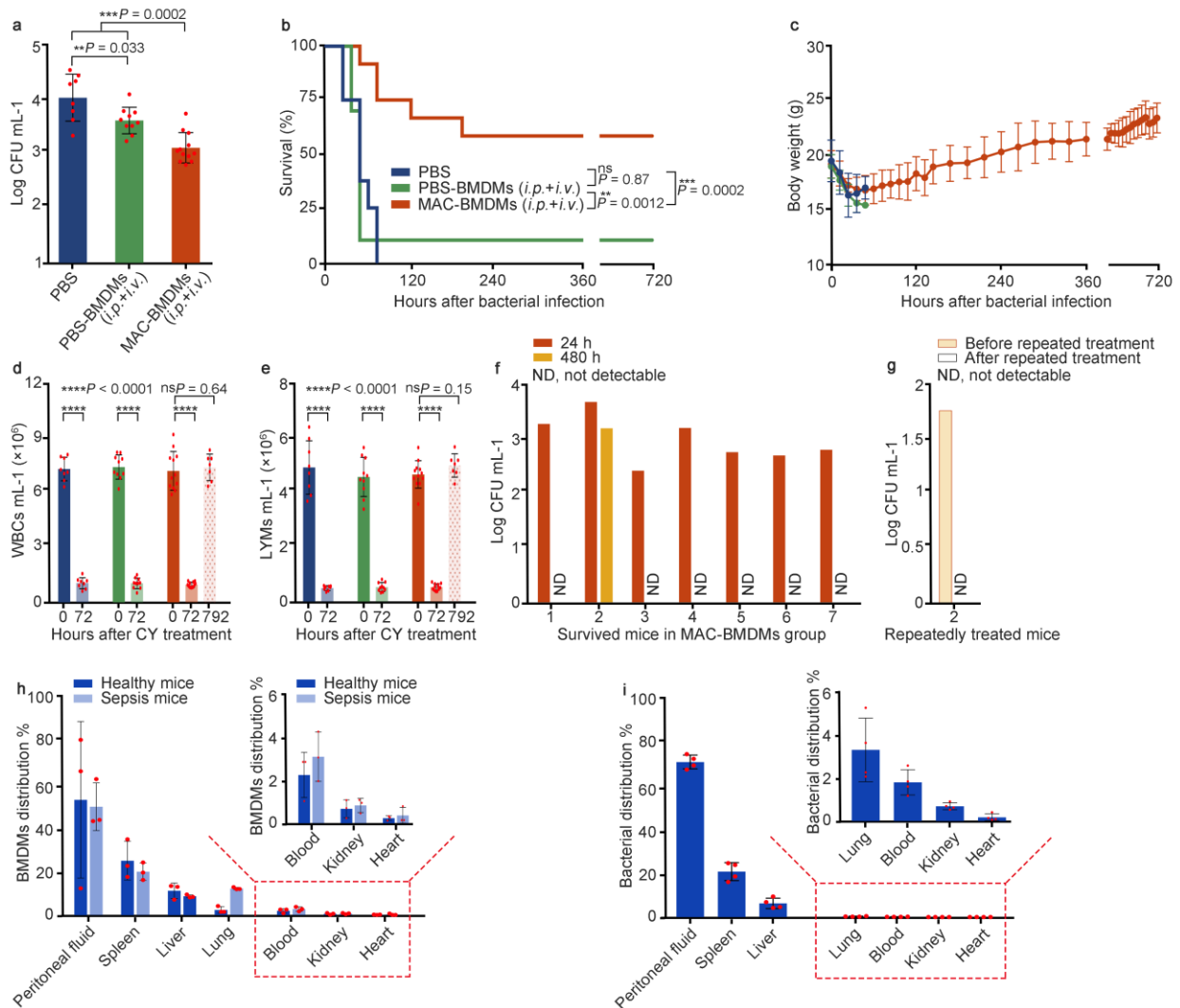
Supplementary Fig. 10. Therapeutic effects of MAC-BMDMs in MDRSA-induced sepsis mice with immunosuppression. a, Bacterial burden in blood at 24 h after cell transfer. n = 8, 10,

and 12 live mice for PBS, PBS-BMDM (*i.p.* + *i.v.*), and MAC-BMDM (*i.p.* + *i.v.*) groups, respectively. **b**, Percentage survival of mice with sepsis induced by *i.p.* bacterial inoculation. **c-e**, The BWs, WBCs, and LYMs of mice. **c**, BWs; **d**, WBCs; **e**, LYMs. Data in **b**, **c**, **d**, and **e** (except time point 792 h), n = 8, 10, and 12 for PBS, PBS-BMDM (*i.p.* + *i.v.*), and MAC-BMDM (*i.p.* + *i.v.*) groups, respectively. **f**, **g**, Bacterial burden in the blood of each survived mouse treated by MAC-BMDMs. The number of mice in the groups of PBS, PBS-BMDM, and MAC-BMDM were 8, 10, and 12, respectively. **h**, BMDMs distribution in the peritoneal fluid, blood, and major organs 6 h after *i.p.* + *i.v.* administration in healthy mice or sepsis mice. **i**, Bacterial distribution in the peritoneal fluid, blood, and major organs 6 h after *i.p.* bacterial inoculation. Data in **a**, **c**, **d**, **e**, **h**, and **i** are presented as mean ± s.d. Statistical significance was analyzed by the two-tailed Student's *t*-test. Data in **b** were analyzed by the log-rank (Mantel–Cox) test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$; ns, not significant; ND, not detectable.