



Supplementary Material

Supplementary Figure 1.

A. Total protein isolated from AC L4 exosome, AC L4 exofree and sAg from AC L4 were measured by SDS-PAGE and stained using silver staining.



Supplementary Figure 2. Macrophage signature genes based on RNA-seq results.

A. Signature gene expression profile of BMDMs, including macrophage polarization markers, cytokines, chemokines and other secreted mediators. BMDMs were treated with PBS (ctr), free (AC



L4 exofree), IL-4, com (IL-4+AC L4 exofree) for 6h, 12h, and 24 h respectively. The horizontal axis represents genes, and vertical coordinates represents types of treatment.

Supplementary Figure 3. Metabolic reprogramming in AC L4 exofree-induced macrophage polarization.

A-B. BMDMs were cultured for 24 h in medium alone or treated with exofree, IL-13, IL-13+exofree, LPS, LPS+exofree, then their mitochondrial function was measured. Relative transcript levels for genes encoding key enzymes in β-oxidation (A) and nutrient uptake (B) are shown. **C.** Gene expression profile of BMDMs related to glycolysis metabolism were shown in a heatmap. BMDMs were treated with PBS (ctr), free (AC L4 exofree), IL-4, com (IL-4+AC L4 exofree) for 6h, 12h, and 24 h respectively. The horizontal axis represents genes, and vertical coordinates represents types of treatment. **D.** Proliferation of BMDMs was determined with CCK8 assay at 6, 12, 24, 48, 72 h after treatment of PBS, IL-4, exofree100, IL-4+exofree100. ^{&&&}P<0.001, compared with the control group; ^{###}P<0.001, compared with the IL-4 group; ***P<0.001, compared with the exofree100 group. 100 refer to 100µL AC L4 exofree. **E-F.** BMDMs were treated with PBS, exofree, IL-13, IL-13+exofree (**E**), and PBS, exofree, IL-4, IL-4+exofree (**F**) in combination of oligomycin, FCCP or 2-

DG for 24 h. The protein levels of Chi3l3 in the culture medium were measured using ELISA. *P < 0.05, **P < 0.01, ***P < 0.001. Data were presented as mean ± SD (Student's t-test).

Supplementary Figure 4. Chemical and physical property analysis of AC L4 exofree.

A. Protein expression level of Chi3l3 in the culture medium of BMDMs were measured in presence of PBS, exofree, exofree (50°C water bath, 15min), exofree (100°C water bath, 15min), IL-13, IL-13+exosome, IL-13+exosome (50°C water bath, 15min), IL-13+exosome (100°C water bath, 15min) for 24 h using ELISA. **B.** Protein expression level of Chi3l3 in the culture medium of BMDMs were measured in presence of PBS, AC exofree, AC exofree (100 µg/mL proteinase K treatment: 58°C water bath, 2 h; proteinase K inactivation: 100°C water bath, 15min), IL-13, IL-13+exosome (100 µg/mL proteinase K treatment: 58°C water bath, 2 h; proteinase K inactivation: 100°C water bath, 2 h; proteinase K inactivation: 100°C water bath, 2 h; proteinase K treatment: 58°C water bath, 2 h; proteinase K inactivation: 100°C water bath, 2 h; proteinase K treatment: 58°C water bath, 2 h; proteinase K inactivation: 100°C water bath, 2 h; proteinase K inactivation: 100°C water bath, 2 h; proteinase K treatment: 58°C water bath, 2 h; proteinase K inactivation: 100°C water bath, 15min) for 24 h using ELISA. **C.** Control (PBS) or AC L4 exofree medium was used unfractionated (whole) or as <3 kDa fraction to stimulate BMDMs as follows. And protein expression level of Chi313 in the culture medium of BMDMs were measured 24 h after the stimulation using ELISA. Data information: **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001. Data were presented as mean \pm SD (Student's t-test).

Supplementary Figure 5. A. Expression profile of significant TCGs in BMDMs at 6h, 12h, and 24h post different treatments. The horizontal axis represents genes, and vertical coordinates represents different treatments and time points. Free, IL-4, and Com represent treatments of AC L4 exofree, IL-4, and AC L4 exofree+IL-4 respectively. 6 h, 12 h, and 24 h represent time of the above stimulation. **B.** Expression patterns of TCGs. The x-axis represents time (with the unit as hour), and the y-axis represents the normalized gene expression levels.

A

| Decembinent Un Neve | β6 | β7 | | | ~~ | β8 | 1 | 39 | 0 | 2 | β10 |
|--------------------------------------|--------------------------|-------------------------------------|--|------------------------------|---|--------------------------------|--|---|--------------------------------|-----------------------|----------------------|
| Recombinant_hu_hexa | 1 | 10 | | 20 | 11 | 30 | | 4 <u>0</u> | 50 | | 60 |
| Recombinant_Hu_Hexa AC A0A0K0D6F2 | TLEKI | VVLVVS | VVTPGC | NQLPT | LESVEN | YTLTIN | 1DDQCL1 | LLSETV | WGALRO | GLETFS(| DTAMK |
| Human_Hexa_P06865 | TLEKI | VLVVS | VVTPGC | NQLPT | LESVEN | YTLTIN | IDDQCLI | LLSETV | WGALRO | GLETFS | QLVWK |
| Pecombinant Un Vera | ጥጥ | β11 | β12 | _ | β13 | | 0000 | α3 | 0.0.0 | β14 | |
| Recombinant_nu_nexa | | 7 0 | | 8 Q | | 90 | 10 | <u>, , , , , , , , , , , , , , , , , , , </u> | 110 | · | 120 |
| Recombinant_Hu_Hexa AC A0A0K0D6F2 | SAEG | FFINK | TEIEDF | PRFPH | RGLLLD | TSRHYI | LPLSSI | LDTL <mark>DV</mark> | MAYNKI MAMNKN | NVFHWE | ILVDD ILVDS |
| Human_Hexa_P06865 | SAEG | FFINK | TEIEDF | PRFPH | RGL <mark>LL</mark> D | TSRHYI | PLSSI | LDTL <mark>D</mark> V | MAYNK I | NVFHWE | ILVDD |
| Pocombinant Un Voya | | | α4 | 0 T | ጥጥ | 00000 | α5 | | β15 | | |
| Recombinant_nu_nexa | | 130 | | ~140 ⁻ | | 150 | 1 | eo. | 170 | | 180 |
| Recombinant_Hu_Hexa AC A0A0K0D6F2 | PSFP ESFP | ľesftf Vsttf | PELMRK POLSOV | GSYNP GAYSS | VTHIYT RHVYT | AQD <mark>VKE</mark> PKTIRI | EVIE <mark>YA</mark> I DILOYAI | R <mark>LRGIR</mark> RIRGIR | VLAEFI VIPEFI | DTPGHTI DLPGHT(| SWGP SWK |
| Human_Hexa_P06865 | P <mark>SFP</mark> | le <mark>s</mark> f <mark>tf</mark> | p ẽ l mrk | GS <mark>YN</mark> P | VT <mark>HIYT</mark> | AQD <mark>VKE</mark> | EVI <mark>ĒYAI</mark> | R <mark>LRGIR</mark> | VLAEFI | T <mark>PGHT</mark> I | ⊆ <mark>SW</mark> GP |
| Recombinant Hu Heya | | β16 | | β17 | | 000000 | α6 | 20000 | β18 | | |
| Kecombinant_nu_nexa | | 190 | | 200 | | 210 | 2: | 20 | 230 | | _ |
| Recombinant_Hu_Hexa AC_A0A0K0D6F2 | GIPG] GQPE] | LLTPCY LLT <mark>EC</mark> F | SG <mark>S</mark> EPS DA <mark>S</mark> KKP | GTFGP TYQNL | VNPSLN Vd ps ke | N T Y E F N E N F E F I | 4 S T F F L I L T K <mark>F F </mark> S I | EVSSVF EVVNAF | PDFYLF PDDFLF | ILGGDE\ ILGGDE\ | D Adyi |
| Human_Hexa_P06865 | GIPGI | LLTPCY 2 | SG <mark>S</mark> EPS | GTFGP | V N <mark>PS</mark> LN | NTYEFN | 4ST <mark>FF</mark> LI | EVSSVF | PDFYLF | LGGDE | 7D |
| Recombinant Hu Hexa | α7 0000 | | α8 000000 | 00 | 000 | α9 000000 | 000000 | 0 0 | β19 | α10 ►00000 | 2 |
| | 240 | | 250 | | 260 | 27 | 70 | 2 | 8 0 | 290 |) |
| Recombinant_Hu_Hexa AC_A0A0K0D6F2 | TE <mark>CWI</mark> | KS.NPE Kvl <mark>n</mark> vk | IQDFMR IQDFM <mark>k</mark> | KKGFG R <mark>KSFG</mark> | E D F K Q L N N T T L <mark>L</mark> | ESFYICENYFFI | ORVAE <mark>I</mark> : | IKNLPS | KRRMVE | WQEVFI | DNKVK DNN |
| Human_Hexa_P06865 | FT <mark>CWI</mark> 2 | S.NPE | IQDFMR | K <mark>K</mark> G FG | EDFKQ <mark>L</mark> | ESFYIC | 2 T L L D I V | VSSYG. | . <u>K</u> GY <mark>V</mark> V | WQEVFI | <mark>n</mark> k v k |
| Recombinant_Hu_Hexa | тт | β20 | | وووو | α11 <u>000000</u> | β2 | 1 → тт | тт | ٩ | α12 | тт |
| Decembinant Un Herra | 30 TODD | | 310 סבד היע | NVME | 320 TETVEV | | 330 | | | | |
| AC_AOAOKOD6F2 | KPNG | SIIQVW | KGNTHE | EILRE | VKAVTA | KGFNV] | | Y L N Y I K | | DETAGS | 5 |
| Human_Hexa_P06865 | IQPD | <u> </u> QVW | REDIPV | NY <u>MK</u> E | LELVTK | AGERAI | | LNRIS - | YGPDWE | DF. X V V F | SPLAF |
| Recombinant_Hu_Hexa | e e | 200 - | p22 | ▶ ТТ | ٩ | 2222 | فعفعف | 20000 | тт | معععه | 22222 |
| Recombinant Hu Hexa | E GTP | 50KALV | 370 | MWGEY | 380 VDNTNT | VPRIWE | 390 PRAGAVI | | NKLTSI | 410 TFAYE | RISH |
| AC_AOAOKOD6F2 | . SPS | | | MWCEV | . NSRSL | FKRLSS | 5 | IFS | QHLTLI | LKSTDO | GK |
| numan_Hexa_P00865 | с G <mark>T Б</mark> Т | JÄVATA | IGGEAC | ммсе́х | | V P KL W E | RAGAVI | ACKLWS | NALTSI | | гцгрн |
| Recombinant_Hu_Hexa | <u>وووو</u> | 200 | 420 | | | | | | | | |
| Recombinant Hu Hexa | FRC 🖪 | LRRGV | AOPLN | VGFCE | 440 OEFEOT | | | | | | |
| AC_AOAOKOD6F2 | FRC F | ETNEQ | ORKEMA | I | ~ 2 - | | | | | | |
| numan_nexa_r00005 | 3 | | NU Å I TIN | <u>-</u> GrCE 3 | verev. | | | | | | |

Supplementary Figure 6. A. Multiple sequence alignment of human and AC Hexa protein.

| Primer | Sequence (5'-3') |
|---------------------------|---------------------------|
| m- <i>Chi3l3</i> -qPCR -F | CTGAATGAAGGAGCCACTGA |
| m- <i>Chi3l3</i> -qPCR -R | AGCCACTGAGCCTTCAACTT |
| m-β-actin-qPCR -F | GGCATCCTGACCCTGAAGTA |
| m-β-actin-qPCR -R | CTCTCAGCTGTGGTGGTGAA |
| m-Arg1-qPCR-F | CCAGAAGAATGGAAGAGTCAGTGT |
| m-Arg1-qPCR-R | GCAGATATGCAGGGAGTCACC |
| m- <i>Nos2</i> -qPCR-F | CACCAAGCTGAACTTGAGCG |
| m-Nos2-qPCR-R | CGTGGCTTTGGGCTCCTC |
| m-Arg1-qPCR-F | CCAGAAGAATGGAAGAGTCAGTGT |
| m-Arg1-qPCR-R | GCAGATATGCAGGGAGTCACC |
| m- <i>IL-12b</i> -qPCR-F | GGACATCATCAAACCAGACC |
| m- <i>IL-12b</i> -qPCR-R | GAATTGTAATAGCGATCCTGAG |
| m- <i>IL-1b</i> -qPCR-F | TTCAGGCAGGCAGTATCACTC |
| m- <i>IL-1b</i> -qPCR-R | GAAGGTCCACGGGAAAGACAC |
| m- <i>IL-6</i> -qPCR-F | GTTGCCTTCTTGGGACTGATG |
| m- <i>IL-6</i> -qPCR-R | GGGAGTGGTATCCTCTGTGAAGTCT |
| m-IL-10-qPCR-F | AGCCTTATCGGAAATGATCCAGT |
| m- <i>IL-10</i> -qPCR-R | GGCCTTGTAGACACCTTGGT |

Supplementary Table 1 Sequences of the primers used for qPCR

| m-Acox1-qPCR-F | GCCAGGACTATCGCATGATT |
|-------------------------|-------------------------|
| m-Acox1-qPCR-R | GCCCAACTGTGACTTCCATC |
| m- <i>Cpt1b</i> -qPCR-F | CCAGACCCATACACCGACAG |
| m- <i>Cpt1b</i> -qPCR-R | GTCTCAGAGCCTCCCGACTA |
| m- <i>Cpt1c</i> -qPCR-F | TCTTCACTGAGTTCCGATGGG |
| m- <i>Cpt1c</i> -qPCR-R | ACGCCAGAGATGCCTTTTCC |
| m-Acadm-qPCR-F | AGGGTTTAGTTTTGAGTTGACGG |
| m-Acadm-qPCR-R | CCCCGCTTTTGTCATATTCCG |
| m-Acadl-qPCR-F | CTTTTCCTCGGAGCATGACA |
| m-Acadl-qPCR-R | GACCTCTCTACTCACTTCTCCAG |
| m-Adipoq-qPCR-F | TGTTCCTCTTAATCCTGCCCA |
| m-Adipoq-qPCR-R | CCAACCTGCACAAGTTCCCTT |
| m- <i>CD36</i> -qPCR-F | ATGGGCTGTGATCGGAACTG |
| m- <i>CD36</i> -qPCR-R | GTCTTCTCAATAAGCATGTCTCC |
| m- <i>Lpl</i> -qPCR-F | GGGAGTTTGGCTCCAGAGTTT |
| m- <i>Lpl</i> -qPCR-R | TGTGTCTTCAGGGGGTCCTTAG |
| m-slc27a1-qPCR-F | CGCTTTCTGCGTATCGTCTG |
| m-slc27a1-qPCR-R | GATGCACGGGATCGTGTCT |

Supplementary Table 2 Summary table of the treatments and the time of experiment in Figure 3 L and Figure 3M.

BMDMs were pretreated in 0~24h as shown, the medium was discarded at 24h. Then, BMDMs were washed with PBS for three times, and restimulated as shown below. Finally, the 24~48h-BMDMs culture medium was collected for Chi313 ELISA analysis.

| Group serial number | 0-24 h stimulation | Wash step | 24-48 h stimulation | |
|---------------------|--------------------|---------------------------|---------------------|--|
| 1 | PBS | wash with PBS for 3 times | PBS | |
| 2 | PBS | wash with PBS for 3 times | exofree | |
| 3 | PBS | wash with PBS for 3 times | IL-4 | |
| 4 | PBS | wash with PBS for 3 times | exofree+IL-4 | |
| 5 | exofree | wash with PBS for 3 times | PBS | |
| 6 | exofree | wash with PBS for 3 times | exofree | |
| 7 | exofree | wash with PBS for 3 times | IL-4 | |
| 8 | exofree | wash with PBS for 3 times | exofree+IL-4 | |
| 9 | IL-4 | wash with PBS for 3 times | PBS | |
| 10 | IL-4 | wash with PBS for 3 times | exofree | |
| 11 | IL-4 | wash with PBS for 3 times | IL-4 | |
| 12 | IL-4 | wash with PBS for 3 times | exofree+IL-4 | |
| 13 | exofree+IL-4 | wash with PBS for 3 times | PBS | |
| 14 | exofree+IL-4 | wash with PBS for 3 times | exofree | |
| 15 | exofree+IL-4 | wash with PBS for 3 times | IL-4 | |
| 16 | exofree+IL-4 | wash with PBS for 3 times | exofree+IL-4 | |