

## Supplementary materials

**Table S1. Parameters of AF4 for the separation of plasma-derived exosomes**

| Start time<br>(min) | End time<br>(min) | Duration<br>(min) | Mode         | Vx start<br>(mL/min) | Vx end<br>(mL/min) |
|---------------------|-------------------|-------------------|--------------|----------------------|--------------------|
| 0.00                | 1.00              | 1.00              | Elution      | 0                    | 0                  |
| 1.00                | 2.00              | 1.00              | Focus        | 0                    | 0                  |
| 2.00                | 7.00              | 5.00              | Focus+Inject | 0                    | 0                  |
| 7.00                | 9.00              | 2.00              | Focus        | 0                    | 0                  |
| 9.00                | 14.00             | 5.00              | Elution      | 3                    | 3                  |
| 14.00               | 34.00             | 20.00             | Elution      | 3                    | 0.1                |
| 34.00               | 44.00             | 10.00             | Elution      | 0.1                  | 0                  |

**Table S2. Intensity distribution of exosomes extracted by the three methods.**

### UC

| d (nm) | f(%) | f(cum.%) | d (nm) | f(%) | f(cum.%) | d(nm) | f(%) | f(cum.%) | d(nm) | f(%) | f(cum.%) |
|--------|------|----------|--------|------|----------|-------|------|----------|-------|------|----------|
| 1.0    | 0.0  | 0.0      | 7.1    | 0.0  | 0.0      | 51.0  | 1.9  | 22.3     | 363.7 | 2.0  | 83.9     |
| 1.1    | 0.0  | 0.0      | 7.7    | 0.0  | 0.0      | 55.1  | 2.0  | 24.3     | 393.5 | 1.9  | 85.8     |
| 1.2    | 0.0  | 0.0      | 8.4    | 0.0  | 0.0      | 59.6  | 2.1  | 26.4     | 425.7 | 1.8  | 87.6     |
| 1.3    | 0.0  | 0.0      | 9.0    | 0.3  | 0.3      | 64.5  | 2.2  | 28.6     | 460.5 | 1.6  | 89.2     |
| 1.4    | 0.0  | 0.0      | 9.8    | 0.4  | 0.7      | 69.8  | 2.3  | 30.9     | 498.1 | 1.5  | 90.7     |

|         |       |      |         |       |      |         |        |      |        |     |       |
|---------|-------|------|---------|-------|------|---------|--------|------|--------|-----|-------|
| 1.5     | 0.0   | 0.0  | 10.6    | 0.4   | 1.1  | 75.5    | 2.4    | 33.3 | 538.9  | 1.4 | 92.1  |
| 1.6     | 0.0   | 0.0  | 11.4    | 0.4   | 1.5  | 81.7    | 2.5    | 35.8 | 582.9  | 1.2 | 93.4  |
| 1.7     | 0.0   | 0.0  | 12.4    | 0.5   | 2.0  | 88.3    | 2.5    | 38.3 | 630.6  | 1.2 | 94.5  |
| 1.9     | 0.0   | 0.0  | 13.4    | 0.5   | 2.5  | 95.6    | 2.6    | 40.9 | 682.2  | 1.0 | 95.6  |
| 2.0     | 0.0   | 0.0  | 14.5    | 0.6   | 3.0  | 103.4   | 2.7    | 43.6 | 738.0  | 0.9 | 96.5  |
| 2.2     | 0.0   | 0.0  | 15.7    | 0.6   | 3.7  | 111.8   | 2.7    | 46.3 | 798.4  | 0.8 | 97.3  |
| 2.4     | 0.0   | 0.0  | 16.9    | 0.7   | 4.3  | 121.0   | 2.7    | 49.0 | 863.7  | 0.7 | 98.0  |
| 2.6     | 0.0   | 0.0  | 18.3    | 0.7   | 5.1  | 130.9   | 2.7    | 51.8 | 934.3  | 0.6 | 98.7  |
| 2.8     | 0.0   | 0.0  | 19.8    | 0.8   | 5.8  | 141.6   | 2.8    | 54.5 | 1010.7 | 0.5 | 99.2  |
| 3.0     | 0.0   | 0.0  | 21.5    | 0.9   | 6.7  | 153.2   | 2.7    | 57.3 | 1093.4 | 0.4 | 99.6  |
| 3.3     | 0.0   | 0.0  | 23.2    | 0.9   | 7.7  | 165.7   | 2.7    | 60.0 | 1182.8 | 0.4 | 100.0 |
| 3.5     | 0.0   | 0.0  | 25.1    | 1.0   | 8.7  | 179.3   | 2.7    | 62.7 | 1279.6 | 0.0 | 100.0 |
| 3.8     | 0.0   | 0.0  | 27.2    | 1.1   | 9.8  | 193.9   | 2.7    | 65.3 | 1384.2 | 0.0 | 100.0 |
| 4.1     | 0.0   | 0.0  | 29.4    | 1.2   | 11.0 | 209.8   | 2.6    | 67.9 | 1497.4 | 0.0 | 100.0 |
| 4.5     | 0.0   | 0.0  | 31.8    | 1.3   | 12.3 | 226.9   | 2.5    | 70.5 | 1619.9 | 0.0 | 100.0 |
| 4.8     | 0.0   | 0.0  | 34.4    | 1.4   | 13.7 | 245.5   | 2.5    | 73.0 | 1752.4 | 0.0 | 100.0 |
| 5.2     | 0.0   | 0.0  | 37.2    | 1.5   | 15.2 | 265.6   | 2.4    | 75.3 | 1895.7 | 0.0 | 100.0 |
| 5.6     | 0.0   | 0.0  | 40.2    | 1.6   | 16.8 | 287.3   | 2.3    | 77.6 | 2050.8 | 0.0 | 100.0 |
| 6.1     | 0.0   | 0.0  | 43.5    | 1.7   | 18.5 | 310.8   | 2.2    | 79.9 | 2218.5 | 0.0 | 100.0 |
| 6.6     | 0.0   | 0.0  | 47.1    | 1.8   | 20.3 | 336.2   | 2.1    | 81.9 | 2400.0 | 0.0 | 100.0 |
| D(10%): | 33.20 | (nm) | D(50%): | 60.10 | (nm) | D(90%): | 111.00 | (nm) |        |     |       |

**PEG**

| d (nm) | f(%) | f(cum.%) | d (nm) | f(%) | f(cum.%) | d(nm) | f(%) | f(cum.%) | d(nm) | f(%) | f(cum.%) |
|--------|------|----------|--------|------|----------|-------|------|----------|-------|------|----------|
| 1.0    | 0.0  | 0.0      | 5.4    | 0.0  | 0.0      | 29.3  | 2.9  | 22.7     | 158.2 | 1.3  | 95.1     |
| 1.1    | 0.0  | 0.0      | 5.8    | 0.0  | 0.0      | 31.3  | 3.0  | 25.7     | 169.3 | 1.1  | 96.3     |
| 1.1    | 0.0  | 0.0      | 6.2    | 0.0  | 0.0      | 33.5  | 3.2  | 28.9     | 181.1 | 1.0  | 97.2     |
| 1.2    | 0.0  | 0.0      | 6.6    | 0.0  | 0.0      | 35.8  | 3.3  | 32.1     | 193.8 | 0.8  | 98.1     |
| 1.3    | 0.0  | 0.0      | 7.1    | 0.0  | 0.0      | 38.3  | 3.4  | 35.5     | 207.3 | 0.7  | 98.7     |
| 1.4    | 0.0  | 0.0      | 7.6    | 0.0  | 0.0      | 41.0  | 3.5  | 39.0     | 221.8 | 0.5  | 99.3     |
| 1.5    | 0.0  | 0.0      | 8.1    | 0.0  | 0.0      | 43.9  | 3.5  | 42.6     | 237.3 | 0.4  | 99.7     |
| 1.6    | 0.0  | 0.0      | 8.7    | 0.0  | 0.0      | 46.9  | 3.6  | 46.2     | 253.9 | 0.3  | 100.0    |
| 1.7    | 0.0  | 0.0      | 9.3    | 0.0  | 0.0      | 50.2  | 3.6  | 49.8     | 271.6 | 0.0  | 100.0    |
| 1.8    | 0.0  | 0.0      | 9.9    | 0.0  | 0.0      | 53.7  | 3.6  | 53.4     | 290.6 | 0.0  | 100.0    |
| 2.0    | 0.0  | 0.0      | 10.6   | 0.0  | 0.0      | 57.5  | 3.6  | 56.9     | 310.9 | 0.0  | 100.0    |
| 2.1    | 0.0  | 0.0      | 11.4   | 0.3  | 0.3      | 61.5  | 3.5  | 60.4     | 332.6 | 0.0  | 100.0    |
| 2.2    | 0.0  | 0.0      | 12.2   | 0.4  | 0.7      | 65.8  | 3.4  | 63.9     | 355.8 | 0.0  | 100.0    |
| 2.4    | 0.0  | 0.0      | 13.0   | 0.6  | 1.3      | 70.4  | 3.3  | 67.2     | 380.6 | 0.0  | 100.0    |
| 2.6    | 0.0  | 0.0      | 13.9   | 0.7  | 2.0      | 75.3  | 3.2  | 70.5     | 407.2 | 0.0  | 100.0    |
| 2.8    | 0.0  | 0.0      | 14.9   | 0.9  | 2.9      | 80.6  | 3.1  | 73.6     | 435.7 | 0.0  | 100.0    |
| 2.9    | 0.0  | 0.0      | 15.9   | 1.1  | 4.0      | 86.2  | 3.0  | 76.5     | 466.1 | 0.0  | 100.0    |
| 3.2    | 0.0  | 0.0      | 17.0   | 1.3  | 5.2      | 92.2  | 2.8  | 79.3     | 498.7 | 0.0  | 100.0    |
| 3.4    | 0.0  | 0.0      | 18.2   | 1.5  | 6.7      | 98.6  | 2.6  | 82.0     | 533.5 | 0.0  | 100.0    |
| 3.6    | 0.0  | 0.0      | 19.5   | 1.7  | 8.4      | 105.5 | 2.4  | 84.4     | 570.8 | 0.0  | 100.0    |
| 3.9    | 0.0  | 0.0      | 20.9   | 1.9  | 10.3     | 112.9 | 2.3  | 86.7     | 610.7 | 0.0  | 100.0    |

|         |       |      |         |       |      |         |        |      |       |     |       |
|---------|-------|------|---------|-------|------|---------|--------|------|-------|-----|-------|
| 4.1     | 0.0   | 0.0  | 22.3    | 2.1   | 12.4 | 120.8   | 2.1    | 88.7 | 653.3 | 0.0 | 100.0 |
| 4.4     | 0.0   | 0.0  | 23.9    | 2.3   | 14.6 | 129.2   | 1.9    | 90.6 | 698.9 | 0.0 | 100.0 |
| 4.7     | 0.0   | 0.0  | 25.6    | 2.5   | 17.1 | 138.2   | 1.7    | 92.3 | 747.8 | 0.0 | 100.0 |
| 5.1     | 0.0   | 0.0  | 27.3    | 2.7   | 19.8 | 147.9   | 1.5    | 93.8 | 800.0 | 0.0 | 100.0 |
| D(10%): | 20.70 | (nm) | D(50%): | 50.40 | (nm) | D(90%): | 126.30 | (nm) |       |     |       |

### SEC

| d (nm) | f(%) | f(cum.%) | d (nm) | f(%) | f(cum.%) | d(nm) | f(%) | f(cum.%) | d(nm) | f(%) | f(cum.%) |
|--------|------|----------|--------|------|----------|-------|------|----------|-------|------|----------|
| 1.0    | 0.0  | 0.0      | 5.6    | 0.0  | 0.0      | 31.0  | 2.2  | 7.3      | 173.0 | 0.6  | 99.6     |
| 1.1    | 0.0  | 0.0      | 6.0    | 0.0  | 0.0      | 33.3  | 2.7  | 10.0     | 185.3 | 0.4  | 100.0    |
| 1.1    | 0.0  | 0.0      | 6.4    | 0.0  | 0.0      | 35.6  | 3.2  | 13.3     | 198.5 | 0.0  | 100.0    |
| 1.2    | 0.0  | 0.0      | 6.8    | 0.0  | 0.0      | 38.2  | 3.7  | 17.0     | 212.6 | 0.0  | 100.0    |
| 1.3    | 0.0  | 0.0      | 7.3    | 0.0  | 0.0      | 40.9  | 4.2  | 21.1     | 227.7 | 0.0  | 100.0    |
| 1.4    | 0.0  | 0.0      | 7.9    | 0.0  | 0.0      | 43.8  | 4.6  | 25.7     | 243.9 | 0.0  | 100.0    |
| 1.5    | 0.0  | 0.0      | 8.4    | 0.0  | 0.0      | 46.9  | 4.9  | 30.6     | 261.3 | 0.0  | 100.0    |
| 1.6    | 0.0  | 0.0      | 9.0    | 0.0  | 0.0      | 50.2  | 5.2  | 35.7     | 279.9 | 0.0  | 100.0    |
| 1.7    | 0.0  | 0.0      | 9.7    | 0.0  | 0.0      | 53.8  | 5.4  | 41.1     | 299.8 | 0.0  | 100.0    |
| 1.9    | 0.0  | 0.0      | 10.3   | 0.0  | 0.0      | 57.6  | 5.5  | 46.6     | 321.1 | 0.0  | 100.0    |
| 2.0    | 0.0  | 0.0      | 11.1   | 0.0  | 0.0      | 61.7  | 5.5  | 52.1     | 343.9 | 0.0  | 100.0    |
| 2.1    | 0.0  | 0.0      | 11.9   | 0.0  | 0.0      | 66.1  | 5.5  | 57.6     | 368.4 | 0.0  | 100.0    |
| 2.3    | 0.0  | 0.0      | 12.7   | 0.0  | 0.0      | 70.8  | 5.3  | 62.9     | 394.6 | 0.0  | 100.0    |
| 2.4    | 0.0  | 0.0      | 13.6   | 0.0  | 0.0      | 75.9  | 5.1  | 68.0     | 422.7 | 0.0  | 100.0    |

|         |       |      |         |       |      |         |        |      |       |     |       |
|---------|-------|------|---------|-------|------|---------|--------|------|-------|-----|-------|
| 2.6     | 0.0   | 0.0  | 14.6    | 0.0   | 0.0  | 81.2    | 4.8    | 72.9 | 452.7 | 0.0 | 100.0 |
| 2.8     | 0.0   | 0.0  | 15.6    | 0.0   | 0.0  | 87.0    | 4.5    | 77.4 | 484.9 | 0.0 | 100.0 |
| 3.0     | 0.0   | 0.0  | 16.7    | 0.0   | 0.0  | 93.2    | 4.1    | 81.5 | 519.4 | 0.0 | 100.0 |
| 3.2     | 0.0   | 0.0  | 17.9    | 0.0   | 0.0  | 99.8    | 3.7    | 85.2 | 556.4 | 0.0 | 100.0 |
| 3.4     | 0.0   | 0.0  | 19.2    | 0.0   | 0.0  | 106.9   | 3.3    | 88.5 | 595.9 | 0.0 | 100.0 |
| 3.7     | 0.0   | 0.0  | 20.6    | 0.0   | 0.0  | 114.6   | 2.8    | 91.3 | 638.3 | 0.0 | 100.0 |
| 4.0     | 0.0   | 0.0  | 22.0    | 0.4   | 0.4  | 122.7   | 2.4    | 93.6 | 683.7 | 0.0 | 100.0 |
| 4.2     | 0.0   | 0.0  | 23.6    | 0.6   | 1.0  | 131.4   | 1.9    | 95.6 | 732.4 | 0.0 | 100.0 |
| 4.5     | 0.0   | 0.0  | 25.3    | 1.0   | 2.0  | 140.8   | 1.5    | 97.1 | 784.4 | 0.0 | 100.0 |
| 4.9     | 0.0   | 0.0  | 27.1    | 1.3   | 3.3  | 150.8   | 1.2    | 98.2 | 840.2 | 0.0 | 100.0 |
| 5.2     | 0.0   | 0.0  | 29.0    | 1.8   | 5.1  | 161.5   | 0.8    | 99.1 | 900.0 | 0.0 | 100.0 |
| D(10%): | 33.20 | (nm) | D(50%): | 60.10 | (nm) | D(90%): | 111.00 | (nm) |       |     |       |