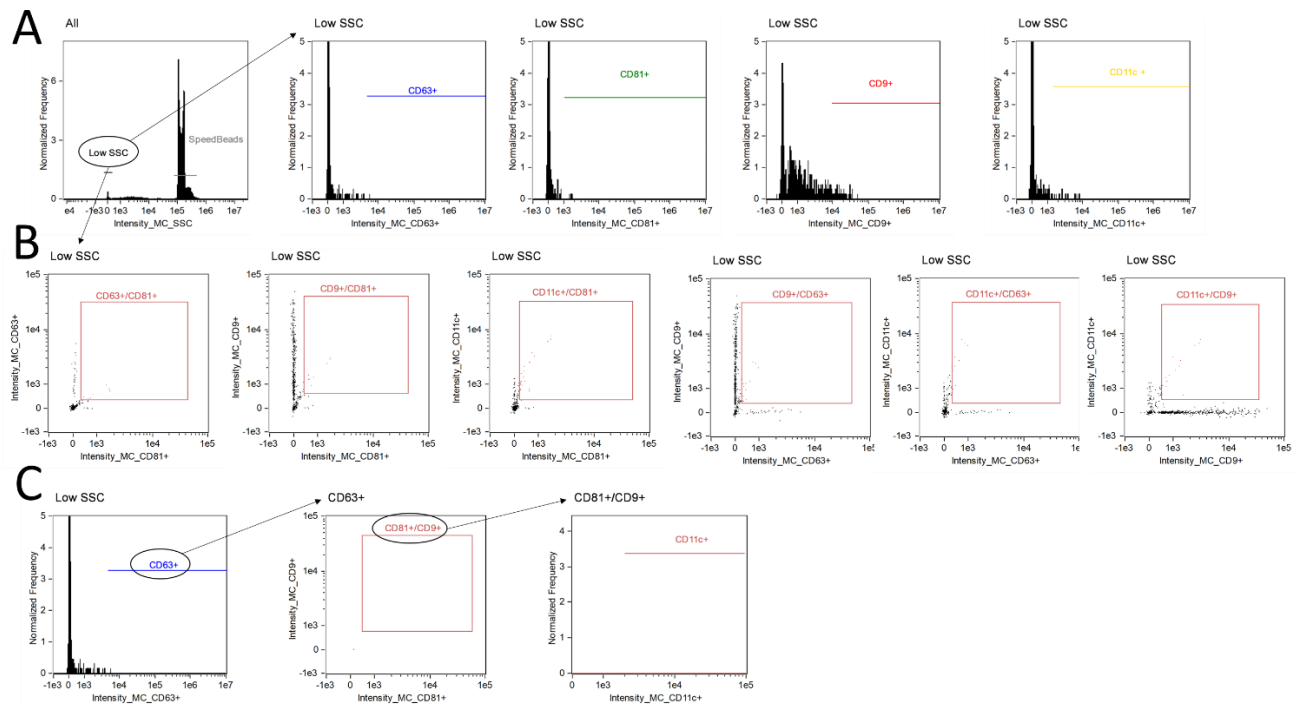**SUPPLEMENTARY**



**Figure S1. EVs gating strategy applied through imaging flow cytometry.** Representative figure of IFC EVs gating strategy by applying the "Intensity_MC" of SSC versus the "Normalized Frequency" in a histogram to identify the Low SSC area limited to the area under the Speed Beads. The graphs show single-positive EVs (A), double-positive EVs (B), and multiple-positive EVs (C) descending from the Low SSC area reporting the fluorescent-positive particles.

**Table S1. Protein concentrations in PFP samples and EVs fractions**

| Protein concentration [mg/mL] | | | |
|---|---|---|---|
| PFP samples | | EVs fractions | |
| IIM | HDs | IIM | HDs |
| 50.236 | 52.586 | 0.068 | 0.032 |
| 52.932 | 52.642 | 0.016 | 0 |
| 57.089 | 49.82 | 0.025 | 0 |
| 56.688 | 50.227 | 0.046 | 0.044 |
| 52.297 | 52.254 | 0.103 | 0.156 |
| 57.93 | 50.909 | 0.031 | 0.046 |
| 51.544 | 45.662 | 0.045 | 0.044 |
| 45.156 | 52.736 | 0.105 | 0.009 |
| 47.192 | 46.975 | 0.045 | 0.047 |
| 48 | 50.096 | 0.092 | 0.091 |
| 52.029 | 48.151 | 0.148 | 0.059 |
| 55.647 | 53.003 | 0.193 | 0.113 |
| 49.33 | 64.039 | 0.048 | 0.013 |
| 51.055 | 52.382 | 0.091 | 0.01 |
| 51.648 | 46.474 | 0.068 | 0.057 |
| 50.299 | 48.064 | 0.065 | 0.072 |
| 52.214 | 50.454 | 0.07 | 0.056 |
| 50.081 | 56.74 | 0.123 | 0.085 |

| | | | |
|---|---|---|---|
| 50.947 | 48.33 | 0.088 | 0.03 |
| 52.025 | 51.782 | 0.073 | 0.048 |
| 60.307 | 48.627 | 0.143 | 0.171 |
| 55.883 | 50.057 | 0.081 | 0.139 |
| 55.576 | 56.817 | 0.164 | 0.121 |
| 49.932 | 53.937 | 0.087 | 0.025 |
| 56.349 | 57.047 | 0.32 | 0.27 |
| 62.482 | 53.018 | 0.112 | 0.048 |
| 47.227 | 54.785 | 0.038 | 0.154 |
| 49.843 | 46.077 | 0.056 | 0.063 |
| 49.007 | 53.371 | 0.015 | 0.043 |
| 57.039 | 59.523 | 0.029 | 0.189 |
| 52.676 | 52.057 | 0.041 | 0.063 |
| 52.089 | 50.68 | 0.023 | 0.047 |
| 50.371 | 53.528 | 0.135 | 0.256 |
| 54.426 | 53.64 | 0.047 | 0.043 |
| 53.757 | 53.9 | 0.026 | 0.099 |
| 53.464 | 58.249 | 0.032 | 0.024 |
| 48.304 | 53.586 | 0.031 | 0.052 |
| 60.56 | 53.669 | 0.02 | 0.092 |
| 54.08 | 60.247 | 0.041 | 0.127 |
| 54.73 | 51.415 | 0.067 | 0.022 |
| 48.535 | 54.219 | 0.046 | 0.057 |
| 48.409 | 45.342 | 0.041 | 0.029 |
| 48.745 | 55.096 | 0.135 | 0.069 |
| 62.537 | 56.812 | 0.075 | 0.021 |
| 54.547 | 55.855 | 0.027 | 0.064 |
| 58.741 | 52.504 | 0.032 | 0.017 |
| 56.709 | 50.124 | 0.026 | 0.025 |
| 48.131 | 52.862 | 0.01 | 0.063 |
| 61.272 | 51.581 | 0.166 | 0 |
| 46.472 | 46.455 | 0.124 | 0.045 |
| 46.43 | 57.893 | 0.027 | 0.035 |
| 53.441 | 52.254 | 0.038 | 0.065 |
| 59.366 | 48.758 | 0.038 | 0.093 |
| 45.594 | 41.67 | 0.038 | 0.004 |
| 44.462 | 49.592 | 0.025 | 0.101 |
| 56.276 | 51.091 | 0.032 | 0.043 |
| 43.869 | 53.664 | 0.048 | 0.076 |
| 49 | 49.97 | 0.077 | 0.041 |
| 54.092 | 49.895 | 0.054 | 0.029 |
| 56.553 | 68.84 | 0.025 | 0.07 |
| 53.018 | 54.914 | 0.013 | 0.244 |
| 53.552 | 49.953 | 0 | 0.011 |
| 53.702 | 47.818 | 0.02 | 0.032 |
| 60.654 | 49.107 | 0.038 | 0.359 |
| 49.545 | 55.683 | 0.074 | 0.08 |

Protein concentration [mg/mL] before SEC isolation (PFP samples) and after SEC isolation (EVs fractions) in both IIM patients (n=65) and HDs (n=65).

**Table S2. Mean concentration of positive EVs to the surface markers detected by imaging flow cytometry**

| | Total EVs samples | Males vs. females | IIM vs. HDs |
|---|---|---|---|
| **CD63+** | $2.65 \times 10^8 \pm 1.97 \times 10^8$ | $3.02 \times 10^8 \pm 2.11 \times 10^8$ vs. $2.47 \times 10^8 \pm 1.89 \times 10^8$ (p = 0.3193) | $2.91 \times 10^8 \pm 2.07 \times 10^8$ vs. $2.40 \times 10^8 \pm 1.85 \times 10^8$ (p = 0.3272) |
| **CD81+** | $1.53 \times 10^7 \pm 1.89 \times 10^7$ | $1.37 \times 10^7 \pm 1.68 \times 10^7$ vs. $1.61 \times 10^7 \pm 2.00 \times 10^7$ (p = 0.6457) | $1.62 \times 10^7 \pm 2.06 \times 10^7$ vs. $1.44 \times 10^7 \pm 1.73 \times 10^7$ (p = 0.7093) |
| **CD9+** | $6.17 \times 10^7 \pm 4.77 \times 10^7$ | $5.25 \times 10^7 \pm 2.38 \times 10^7$ vs. $6.64 \times 10^7 \pm 5.57 \times 10^7$ (p = 0.2910) | $5.89 \times 10^7 \pm 4.78 \times 10^7$ vs. $6.46 \times 10^7 \pm 4.83 \times 10^7$ (p = 0.6472) |
| **CD11c+** | $6.43 \times 10^6 \pm 7.21 \times 10^6$ | $6.08 \times 10^6 \pm 6.95 \times 10^6$ vs. $6.60 \times 10^6 \pm 7.42 \times 10^6$ (p = 0.7952) | $7 \times 10^6 \pm 7.78 \times 10^6$ vs. $5.85 \times 10^6 \pm 6.68 \times 10^6$ (p = 0.5413) |
| **CD63/CD81+** | $1.89 \times 10^6 \pm 2.70 \times 10^6$ | $1.94 \times 10^6 \pm 2.57 \times 10^6$ vs. $1.86 \times 10^6 \pm 2.80 \times 10^6$ (p = 0.9204) | $2.14 \times 10^6 \pm 3.06 \times 10^6$ vs. $1.63 \times 10^6 \pm 2.32 \times 10^6$ (p = 0.4675) |
| **CD63/CD9+** | $2.38 \times 10^6 \pm 1.74 \times 10^6$ | $2.28 \times 10^6 \pm 1.59 \times 10^6$ vs. $2.43 \times 10^6 \pm 1.82 \times 10^6$ (p = 0.7459) | $2.53 \times 10^6 \pm 1.75 \times 10^6$ vs. $2.23 \times 10^6 \pm 1.74 \times 10^6$ (p = 0.5071) |
| **CD81/CD9+** | $4.13 \times 10^5 \pm 6.24 \times 10^5$ | $4.98 \times 10^5 \pm 7.71 \times 10^5$ vs. $3.70 \times 10^5 \pm 5.42 \times 10^5$ (p = 0.4569) | $4.71 \times 10^5 \pm 6.06 \times 10^5$ vs. $3.54 \times 10^5 \pm 6.47 \times 10^5$ (p = 0.4711) |
| **CD63/CD11c+** | $3.76 \times 10^6 \pm 7.30 \times 10^6$ | $4.75 \times 10^6 \pm 9.68 \times 10^6$ vs. $3.27 \times 10^6 \pm 5.85 \times 10^6$ (p = 0.4632) | $4.40 \times 10^6 \pm 7.62 \times 10^6$ vs. $3.12 \times 10^6 \pm 7.04 \times 10^6$ (p = 0.5032) |
| **CD81/CD11c+** | $3.28 \times 10^5 \pm 6.22 \times 10^5$ | $4.40 \times 10^5 \pm 8.21 \times 10^5$ vs. $2.73 \times 10^5 \pm 4.97 \times 10^5$ (p = 0.3302) | $3.33 \times 10^5 \pm 5.50 \times 10^5$ vs. $3.24 \times 10^5 \pm 6.96 \times 10^5$ (p = 0.9555) |
| **CD9/CD11c+** | $2.34 \times 10^6 \pm 4.17 \times 10^6$ | $2.85 \times 10^6 \pm 5.30 \times 10^6$ vs. $2.08 \times 10^6 \pm 3.52 \times 10^6$ (p = 0.5028) | $2.71 \times 10^6 \pm 4.16 \times 10^6$ vs. $1.96 \times 10^6 \pm 4.21 \times 10^6$ (p = 0.4934) |
| **CD63/CD81/CD9+** | $2.77 \times 10^5 \pm 5.53 \times 10^5$ | $3.95 \times 10^5 \pm 7.81 \times 10^5$ vs. $2.21 \times 10^5 \pm 4.05 \times 10^5$ (p = 0.2630) | $2.77 \times 10^5 \pm 4.83 \times 10^5$ vs. $2.76 \times 10^5 \pm 6.22 \times 10^5$ (p = 0.9940) |

Mean concentration [EVs/mL] ± SD of single, double and triple positive EVs to the surface markers in EVs samples (n=60), males (n=20) vs. females (n=40), and IIM patients (n=30) vs. HDs (n=30).

**Table S3. EVs concentration and size by nanoparticles tracking analysis measurements**

| | Total EVs samples | Males vs. females | IIM vs. HDs |
|---|---|---|---|
| **EVs concentration [EVs/mL]** | $1.51 \times 10^{10} \pm 1.06 \times 10^{10}$ | $1.46 \times 10^{10} \pm 9.28 \times 10^9$ vs. $1.54 \times 10^{10} \pm 1.12 \times 10^{10}$ (p = 0.6737) | $1.71 \times 10^{10} \pm 1.29 \times 10^{10}$ vs. $1.31 \times 10^{10} \pm 7.17 \times 10^9$ (p = 0.0306 *) |
| **EVs mean size (nm)** | $201.6 \pm 19.04$ | $198.6 \pm 19.20$ vs. $203.1 \pm 18.88$ (p = 0.1989) | $199 \pm 20.02$ vs. $204.1 \pm 17.78$ (p = 0.1227) |
| **EVs mode size (nm)** | $153.4 \pm 18.40$ | $151.8 \pm 19.04$ vs. $154.2 \pm 18.11$ (p = 0.4718) | $151.9 \pm 19.81$ vs. $154.8 \pm 16.89$ (p = 0.3706) |

Mean EVs concentration [EVs/mL] ± SD, mean size (nm ± SD), and mode size (nm ± SD) measured by NTA in total EVs samples analysed (n=130), males (n=44) vs. females (n=86), and IIM patients (n=65) vs. HDs (n=65).

**Table S4. EVs concentrations measured by nanoparticles tracking analysis.**

| EVs concentration [EVs/mL] | |
|---|---|
| **IIM** | **HDs** |
| $1.76 \times 10^{10}$ | $1.43 \times 10^{10}$ |
| $1.56 \times 10^{10}$ | $1.96 \times 10^{10}$ |
| $2.47 \times 10^{10}$ | $1.19 \times 10^{10}$ |
| $1.76 \times 10^{10}$ | $1.6 \times 10^{10}$ |
| $1.98 \times 10^{10}$ | $1.57 \times 10^{10}$ |
| $1.08 \times 10^{10}$ | $1.36 \times 10^{10}$ |
| $1.57 \times 10^{10}$ | $1.71 \times 10^{10}$ |
| $1.02 \times 10^{10}$ | $1.51 \times 10^{10}$ |
| $8.5 \times 10^9$ | $6.35 \times 10^9$ |
| $1.09 \times 10^{10}$ | $9.46 \times 10^9$ |
| $2.09 \times 10^{10}$ | $1.46 \times 10^{10}$ |
| $3.45 \times 10^{10}$ | $3.45 \times 10^{10}$ |
| $1.9 \times 10^{10}$ | $1.99 \times 10^{10}$ |
| $1.81 \times 10^{10}$ | $1.15 \times 10^{10}$ |
| $1.26 \times 10^{10}$ | $8.62 \times 10^9$ |
| $2.36 \times 10^{10}$ | $6.95 \times 10^9$ |
| $4.54 \times 10^{10}$ | $3.23 \times 10^{10}$ |
| $1.88 \times 10^{10}$ | $1.79 \times 10^{10}$ |
| $3.97 \times 10^{10}$ | $2.17 \times 10^{10}$ |
| $1.29 \times 10^{10}$ | $2.66 \times 10^{10}$ |
| $2.08 \times 10^{10}$ | $6.94 \times 10^9$ |
| $6.52 \times 10^{10}$ | $9.99 \times 10^9$ |
| $8.56 \times 10^{10}$ | $3.03 \times 10^{10}$ |
| $7.59 \times 10^9$ | $9.14 \times 10^9$ |
| $1.16 \times 10^{10}$ | $1.13 \times 10^{10}$ |

| | |
|---|---|
| $1.68 \times 10^{10}$ | $8.68 \times 10^{9}$ |
| $1.3 \times 10^{10}$ | $3.8 \times 10^{10}$ |
| $1.16 \times 10^{10}$ | $1.34 \times 10^{10}$ |
| $7.25 \times 10^{9}$ | $1.09 \times 10^{10}$ |
| $2.03 \times 10^{10}$ | $1.28 \times 10^{10}$ |
| $1.27 \times 10^{10}$ | $8.9 \times 10^{9}$ |
| $7.75 \times 10^{9}$ | $6.6 \times 10^{9}$ |
| $8.31 \times 10^{9}$ | $9.57 \times 10^{9}$ |
| $1.65 \times 10^{10}$ | $1.1 \times 10^{10}$ |
| $9.7 \times 10^{9}$ | $1.48 \times 10^{10}$ |
| $1.95 \times 10^{10}$ | $7.84 \times 10^{9}$ |
| $2.74 \times 10^{10}$ | $2.61 \times 10^{10}$ |
| $2.16 \times 10^{10}$ | $1.26 \times 10^{10}$ |
| $1.39 \times 10^{10}$ | $1.33 \times 10^{10}$ |
| $1.41 \times 10^{10}$ | $7.15 \times 10^{9}$ |
| $1.5 \times 10^{10}$ | $5.24 \times 10^{9}$ |
| $2.03 \times 10^{10}$ | $1.47 \times 10^{10}$ |
| $1.49 \times 10^{10}$ | $1.34 \times 10^{10}$ |
| $1.04 \times 10^{10}$ | $6.47 \times 10^{9}$ |
| $1.06 \times 10^{10}$ | $1.13 \times 10^{10}$ |
| $1.25 \times 10^{10}$ | $9.04 \times 10^{9}$ |
| $1.35 \times 10^{10}$ | $1.79 \times 10^{10}$ |
| $1.08 \times 10^{10}$ | $6 \times 10^{9}$ |
| $7.6 \times 10^{9}$ | $8.38 \times 10^{9}$ |
| $4.72 \times 10^{9}$ | $1.4 \times 10^{10}$ |
| $7.65 \times 10^{9}$ | $9.79 \times 10^{9}$ |
| $7.09 \times 10^{9}$ | $8.26 \times 10^{9}$ |
| $1.2 \times 10^{10}$ | $2.2 \times 10^{10}$ |
| $1.48 \times 10^{10}$ | $4.28 \times 10^{9}$ |
| $1.13 \times 10^{10}$ | $6.83 \times 10^{9}$ |
| $7.22 \times 10^{9}$ | $1.2 \times 10^{10}$ |
| $1.72 \times 10^{10}$ | $6.57 \times 10^{9}$ |
| $9.99 \times 10^{9}$ | $1.05 \times 10^{10}$ |
| $1.18 \times 10^{10}$ | $9.82 \times 10^{9}$ |
| $1.37 \times 10^{10}$ | $9.3 \times 10^{9}$ |
| $2.5 \times 10^{10}$ | $1.07 \times 10^{10}$ |
| $8.96 \times 10^{9}$ | $8.63 \times 10^{9}$ |
| $1.52 \times 10^{10}$ | $5.95 \times 10^{9}$ |
| $1.83 \times 10^{10}$ | $1.01 \times 10^{10}$ |
| $7.86 \times 10^{9}$ | $1.11 \times 10^{10}$ |

EVs concentration [EVs/mL] measured by NTA in IIM patients (n=65) and HDs (n=65).