SUPPLEMENTARY INFORMATION

Exosomes released by breast cancer cells under mild hyperthermic stress possess immunogenic potential and modulate polarization in vitro in macrophages
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Supplementary Figure S1: Melt curve analysis of Arg-1 (red), iNOS (teal), and GAPDH (green) from RT-PCR samples from BMDMs and RAW264.7 cells. Representative of 3 independent experiments.
Supplementary Figure S2: (a, c, e) Cell viability of EMT-6, 4T1, and RAW 264.7 cells after treatment at different temperatures (41-45° C). (b, d, f) Cell viability of EMT-6, 4T1, and RAW 264.7 cells after treatment at different temperatures (41-45° C) converted to CEM43 equivalent minutes. Cell viability of untreated cells was considered as 100%.
Supplementary Figure S3: (a, b, c) Flow cytofluorimetric analysis of individual replicates of untreated and treated EMT-6, 4T1, and RAW 264.7 cells treated with TD50 at 43º C assessed by Annexin V-FITC-PI assay [Data represented in Figure 1. (c-d)]. (d) Cell viability profile of RAW 264.7 cells after treatment at TD50 43º C.
Supplementary Figure S4: NTA analysis of three different isolates of 4T1 derived exosomes of (a-c) untreated control and (d-f) hyperthermia treated set [Data represented in Figure 2, Table 3].
Supplementary Figure S5: NTA analysis of three different isolates of EMT-6 derived exosomes of (a-c) untreated control and (d-f) hyperthermia treated set [Data represented in Figure 2, Table 3].
Supplementary Table S1: Wound healing profile of EMT-6 in homeostatic conditions and after hyperthermic stress. [Data represented in Figure 4 (a)].

| Experimental set          | Replicate 1 | Replicate 2 | Replicate 3 |
|---------------------------|-------------|-------------|-------------|
|                           | EMT-6_C     | EMT-6_HT    | EMT-6_C     | EMT-6_HT    | EMT-6_C     | EMT-6_HT    |
| Length of wound (in µm)   | 1435.3      | 1643.5      | 1211.8      | 1700.0      | 1276.5      | 1864.7      |
| Length of wound after 24h (in µm) | 469.8      | 691.3      | 370.6      | 747.3      | 476.5      | 764.7      |
| Length of wound recovered after 24h (in µm) | 965.6      | 952.2      | 841.2      | 952.8      | 800.0      | 1100.0      |
| % Recovery                | 67.3        | 57.9        | 69.4        | 56.0        | 62.7        | 59.0        |

Supplementary Table S2: Wound healing profile of 4T1 in homeostatic conditions and after hyperthermic stress. [Data represented in Figure 4 (b)].

| Experimental set          | Replicate 1 | Replicate 2 | Replicate 3 |
|---------------------------|-------------|-------------|-------------|
|                           | 4T1_C       | 4T1_HT      | 4T1_C       | 4T1_HT      | 4T1_C       | 4T1_HT      |
| Length of wound (in µm)   | 1343.5      | 1291.4      | 1141.2      | 1488.34     | 923.6       | 1359.0      |
| Length of wound after 24h (in µm) | 195.6      | 378.4      | 323.6      | 517.68      | 200.0       | 464.7       |
| Length of wound recovered after 24h (in µm) | 1147.8     | 913.0     | 817.7      | 970.66      | 723.6       | 894.3       |
| % Recovery                | 85.4        | 70.7        | 71.6        | 65.2        | 78.4        | 65.8        |
Supplementary Figure S6: Bright-field images of wound healing profile of EMT-6 and 4T1 in homeostatic conditions and after hyperthermic stress. (a-b). Wound healing capacity of EMT-6 and 4T1 cells in response to hyperthermia in EMT-6 and 4T1 cells. Student's t-test was used for comparison of the data between the two groups (n=3).
Supplementary Figure S7: Wound healing profile of RAW 264.7 in homeostatic conditions and after hyperthermic stress with or without the presence of positive control (HT=hyperthermia treatment, +ve control=LPS treatment, +ve control+Hyperthermia= LPS, and hyperthermia treatment).
Supplementary Figure S8: Epi-fluorescent microscopy of untreated negative control, positive control (100 ng LPS-treated), control exosome treated RAW 264.7 cells, and post-hyperthermia derived exosome treated RAW 264.7 cells after 24 hours treatment. The cytoskeleton was stained with Alexa Fluor™ 488 Phalloidin (scale bar represents 50 μm). The exosomes used for treatments were derived from 4T1 or EMT-6 cells, as indicated on the figure.
Supplementary Figure S9: Epi-fluorescence microscopy images of RAW 264.7 cells at 20 × magnification showing the distribution of Nile Red-stained exosomes around and within the cells. The cells were incubated with Nile Red-stained exosomes for 0 hours, 3 hours, 6 hours. The cytoskeleton was stained with Alexa Fluor™ 488 Phalloidin (scale bar represents 50 μm).
**Supplementary Figure S10:** Epi-fluorescence microscopy images of RAW 264.7 cells at 20× magnification showing the distribution of Nile Red-stained exosomes around and within the cells. Cells were incubated with Nile Red-stained exosomes for 0 h, 3 h, 6 h, 12 h, and 24 h. The cytoskeleton was stained with Alexa Fluor™ 488 Phalloidin (scale bar represents 50 μm).
**Supplementary Figure S11:** Western blot analysis of Hsp70, CD9, CD63 proteins in exosomes from control untreated or hyperthermia treated EMT-6 and 4T1 cells of three different isolates.