

1 **Supplementary materials**

2 **Tables**

3 Table S1. Characteristics of the patient population.

|   | <b>Total MF<br/>(29 cases)</b> | <b>JAK2<sup>V617F</sup><br/>(23 cases)</b> | <b>TN<br/>(6 cases)</b> |
|---|--------------------------------|--|-------------------------|
| Median age, years (range)                               | 72 (43-84)                     | 71 (43-82)                                 | 75 (60-84)              |
| Males, no. (%)  | 16 (55%)                       | 13 (52%)                                   | 3 (50%)                 |
| Median Hemoglobin, g/dl; median (range)                 | 11.3 (7.1-17.2)                | 11.5 (7.1-17.2)                            | 9.7 (7.5-14.3)          |
| Median Leukocytes, x 10 <sup>9</sup> /l; median (range) | 10.5 (2.5-40)                  | 10.5 (4.7-40)                              | 10.7 (2.5-27)           |
| Median Platelets, x 10 <sup>9</sup> /l; median (range)  | 168 (38-631)                   | 174 (48-631)                               | 121 (38-613)            |
| Median Lymphocyte x 10 <sup>9</sup> /l; median (range)  | 1.2 (0.2-11.7)                 | 1.1 (0.2-5.6)                              | 4.1 (0.4 -11.7)*        |
| Median Monocyte x 10 <sup>9</sup> /l; median (range)    | 0.5 (0.05-5)                   | 0.4 (0.05-2)                               | 0.7 (0.2-5.3)           |
| Blood blasts ≥1%  | 8 (27%)                        | 6 (26%)                                    | 2 (33%)                 |
| TSS >20   | 3 (10%)                        | 3 (13%)                                    | 0                       |
| Spleen ≥10 cm   | 13 (44%)                       | 11 (47%)                                   | 2 (33%)                 |
| <b>BM fibrosis, no. of patients (%)</b>                 |                                |  |                         |
| Grade 2   | 22 (75.8 %)                    | 16 (69.6%)                                 | 6 (100%)                |
| Grade 3   | 7 (24%)                        | 7 (30%)                                    | 0                       |
| <b>IPSS, no. of patients (%)</b>                        |                                |  |                         |
| Low   | 2 (6%)                         | 2 (8%)                                     | 0                       |
| Intermediate-1  | 11 (37%)                       | 8 (34%)                                    | 3 (50%)                 |
| Intermediate-2  | 7 (24%)                        | 6 (26%)                                    | 1 (17%)                 |
| High  | 9 (31%)                        | 7 (30%)                                    | 2 (33%)                 |
| <b>WHO Diagnosis</b>                                    |                                |  |                         |
| PMF   | 17 (58%)                       | 12 (52%)                                   | 5 (83%)                 |
| PPV-MF  | 10 (34%)                       | 10 (43%)                                   | 0                       |
| PET-MF  | 2 (6%)                         | 1 (4%)                                     | 1 (17%)                 |
| Unfavourable karyotype <sup>1</sup>                     | 11                             | 10   | 1                       |

4 <sup>1</sup> by DIPPS-plus

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7 Table S2. Pathway enrichment analysis for differentially expressed transcripts between EVs from TN  
8 and JAK2V617F-mutated patients.

| Source Pathway   | P value  | Genes  |
|--|----------|--|
| GO-BP Antigen processing and presentation  | 2.22E-07 | HLA-DQB1, HLA-DRB1, ULBP3, HLA-DRB4, HLA-B, HLA-DQA2, CD74, HLA-DQA1                                 |
| KEGG Antigen processing and presentation   | 6.55E-05 | HLA-DQB1, HLA-DRB1, HLA-DRB4, HLA-B, HLA-DQA2, CD74, HLA-DQA1  |
| GO-BP Interferon-gamma-mediated signaling pathway                                      | 2.31E-04 | HLA-DQB1, HLA-DRB1, HLA-DRB4, HLA-B, HLA-DQA2, HLA-DQA1  |
| KEGG Cell adhesion molecules (CAMs)  | 3.12E-04 | HLA-DQB1, ITGAL, HLA-DRB1, HLA-DRB4, CNTNAP2, HLA-B, HLA-DQA2, HLA-DQA1                              |
| GO-BP Immune response  | 4.63E-04 | HLA-DQB1, TNFRSF1B, TNFSF10, TNFSF4, HLA-DRB1, HRH2, HLA-DRB4, HLA-B, HLA-DQA2, CD74, HLA-DQA1, CTSG |
| GO-BP Regulation of immune response  | 0.0129   | ITGAL, ULBP3, TREML2, HLA-B, TYROBP, HCST  |
| GO-BP Calcium-dependent cell-cell adhesion via plasma membrane cell adhesion molecules | 0.0200   | PCDHB9, PCDHB14, PCDHB11   |
| GO-BP Homophilic cell adhesion via plasma membrane adhesion molecules                  | 0.0353   | PCDHB9, ROBO1, PCDHB14, PCDHB12, PCDHB11   |

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10 GO-BP, gene ontology biological process

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15 **Table S3. List of the selected miRNAs with their relative fold-changes (FC).**

| MF vs HD       |       | TN vs JAK2 <sup>V617F</sup> |       |
|----------------|-------|-----------------------------|-------|
| microRNA (miR) | FC    | microRNA (miR)              | FC    |
| miR-127-3p     | 9.25  | miR-122-5p                  | 2.55  |
| miR-15b-5p     | 6.69  | miR-365a-3p                 | 2.52  |
| miR-221-3p     | 5.62  | miR-744-5p                  | 2.47  |
| miR-19a-3p     | 4.07  | miR-27a-3p                  | 2.32  |
| miR-21-5p      | 2.53  | miR-548c-3p                 | 2.05  |
| miR-146a-5p    | 2.27  | miR-361-5p                  | -2.23 |
| miR-222-3p     | 2.18  | let-7b-5p                   | -2.45 |
| miR-24-3p      | 2.07  | miR-34a-5p                  | -4.50 |
| miR-155-5p     | 2.01  |                             |       |
| miR-202-3p     | -2.27 |                             |       |
| miR-212-3p     | -2.60 |                             |       |

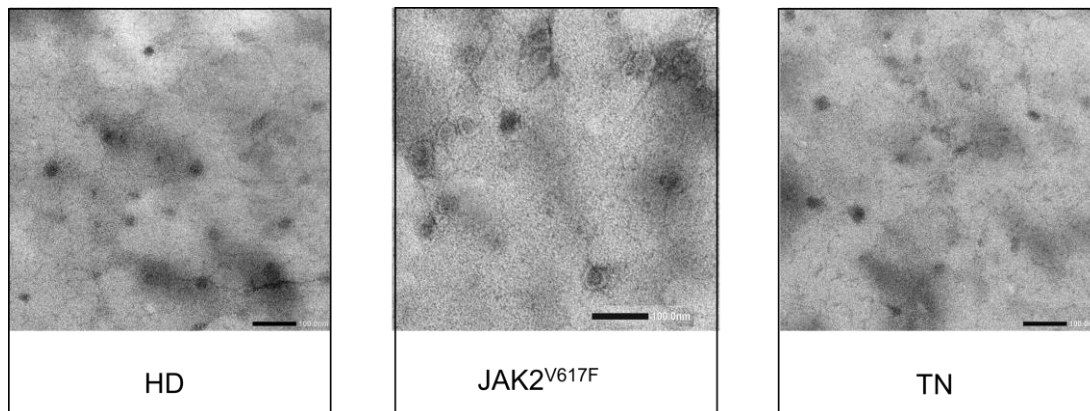
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17 MiRs validated by RT-qPCR are reported in red.

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44 **Figures**

45 Figure S1. EV characterization by TME and NTA

46 **a**



**b**

| Results | HD           | JAK2 <sup>V617F</sup> | TN           |
|---------|--------------|-----------------------|--------------|
| Mean    | 116 ± 13.4   | 119 ± 14.7            | 125.2 ± 14.1 |
| Mode    | 78.7 ± 13.4  | 85.4 ± 14.4           | 89.74 ± 19   |
| SD      | 52.8 ± 8.5   | 53.6 ± 9.1            | 52.34 ± 4.5  |
| D10     | 67.7 ± 8.6   | 67.8 ± 8.5            | 74.61 ± 14   |
| D50     | 101.2 ± 10.8 | 104.8 ± 13.6          | 111 ± 13.9   |
| D90     | 183.1 ± 24.6 | 185 ± 25.1            | 189.6 ± 16.3 |

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53 Figure S1. Characterization of EVs isolated from plasma by ultracentrifugation. (a) Whole-mount  
54 transmission electron micrograph of EVs displaying the characteristic EV morphology in each sample  
55 between HD, *JAK2V617F*-mutated and TN patients. b) Summary of data obtained by Nanosight  
56 equipment for each EV isolated by ultracentrifugation.

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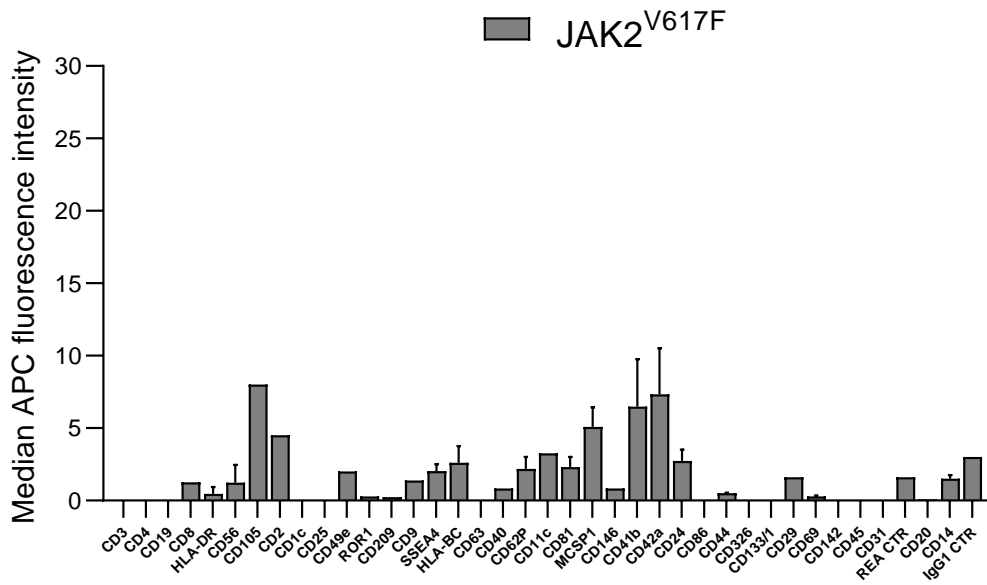
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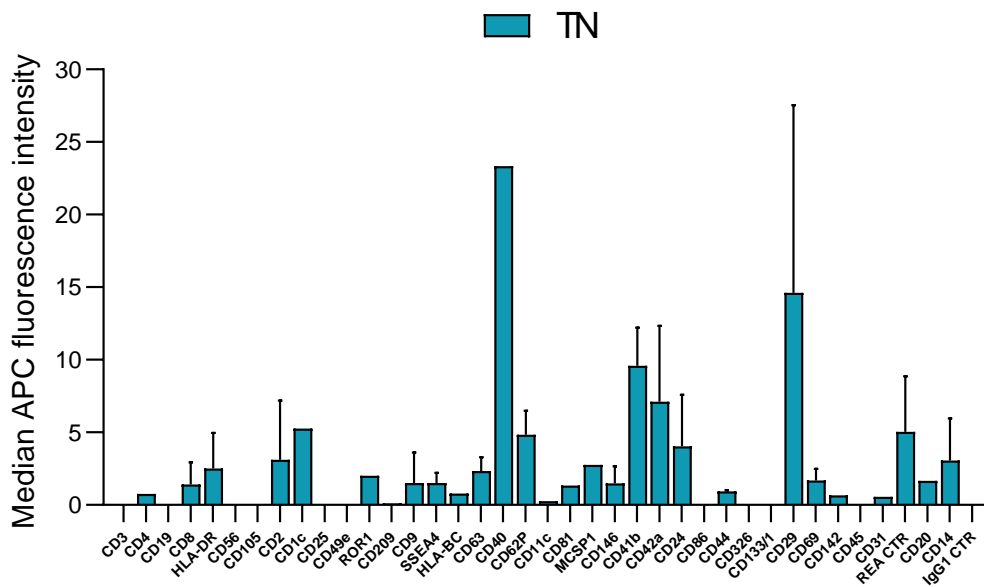
65 Figure S2. Flow cytometry characterization of EVs.

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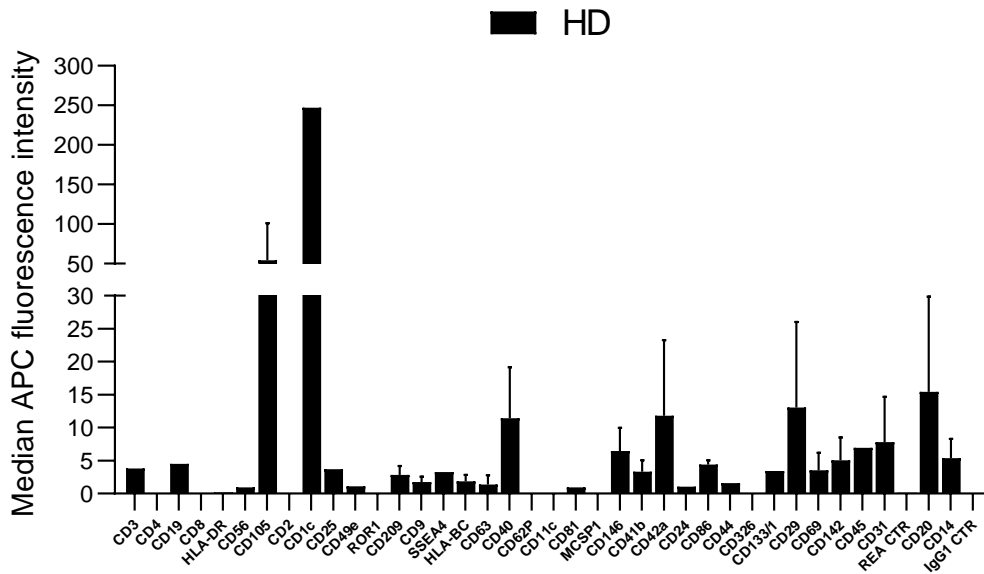
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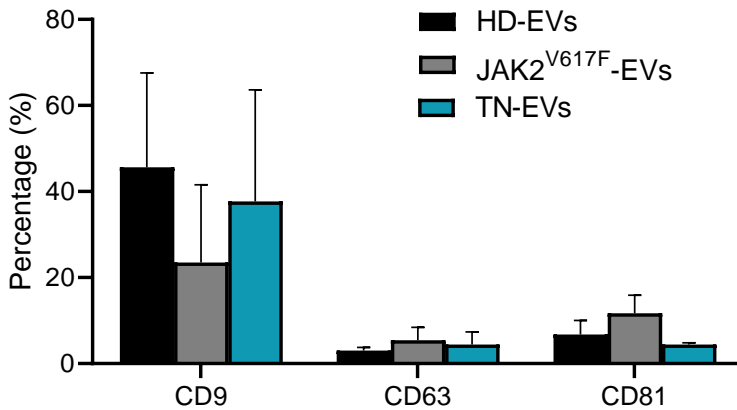
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81 Figure S2. Surface marker profiles of plasma-derived EV from MF patients and HD. Background  
 82 corrected APC median signal intensities after incubation of plasma-derived EVs from 3  
 83 JAK2<sup>V617F</sup>-mutated patients (a) or 3 TN patients (b) and 3 HD (c). EVs from plasma with 39 capture  
 84 antibody bead populations, followed by staining with a cocktail of anti-CD9-, anti-CD63- and  
 85 anti-CD81-APC antibodies. REA, mlgG1 indicate isotype control-beads. d) CD9, CD63, CD81  
 86 frequency in EVs by flow cytometry.

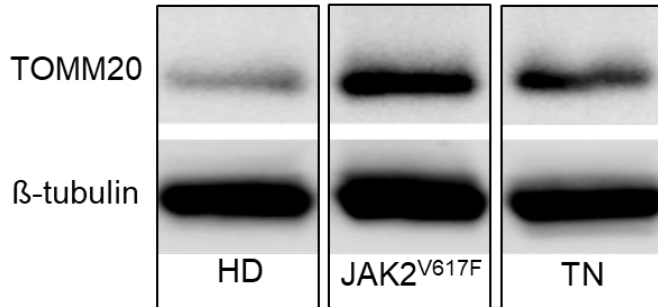
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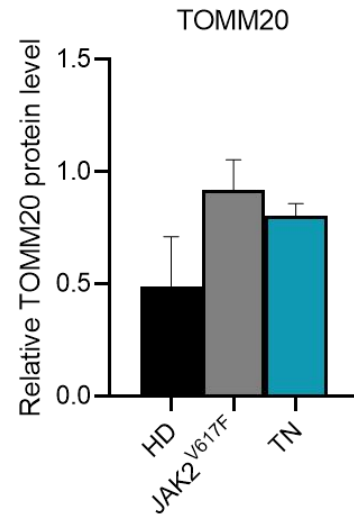
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90 Figure S3. Representative western blot and quantification graph for TOMM20

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94 a) Representative western blot of EVs preparation from HD, JAK2<sup>V617F</sup>-mutated, and TN  
95 patient-derived plasma.  $\beta$ -tubulin was used as a loading control. b) Graphic representation of  
96 quantification for TOMM20 expression normalized to the relative  $\beta$ -tubulin from HD (n=3),  
97 JAK2<sup>V617F</sup>-mutated (n=3), and TN (n=2) patients. The translocase of outer mitochondrial membrane  
98 20 (TOMM20) protein level was determined using the ImageJ analysis software and normalized to  
99  $\beta$ -tubulin, taken as an index of loading control. Histograms show the mean  $\pm$  SEM.