**Supplementary Material**

**TITLE PAGE**

**Title:** ***Hippo pathway-related genes expression is deregulated in myeloproliferative neoplasms***

**Short title**: ***Hippo pathway gene differential expression in myeloproliferative neoplasms***

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Table S1 – Demographic and clinic-hematological data from patients with myeloproliferative neoplasms and healthy subjects.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Data** | **PV** | **ET** | **PMF** | **CTRL** |
| **(n=31)** | **(n=35)** | **(n=22)** | **(n=60)** |
| **Median age (range)** | 65 (45-83y) | 60 (20-85y) | 63.5 (41-80y) | 55 (20 -83y) |
| **Gender (male %)** | 14 (45.2) | 9 (25.7) | 15 (68.2) | 23 (38.3) |
| **Mutation *status* (%)** |  |  |  |  |
| *JAK2V617F* | 30 (96.8) | 15 (42.9) | 11 (50) | 0 (0) |
| *CALR* | 0 (0) | 12 (34.2) | 6 (27.3) | 0 (0) |
| *Negative* | 1 (3.2) | 8 (22.9) | 4 (18.2) | 0 (0) |
| *NA* | 0 (0) | 0 (0) | 1 (4.5) | 60 (100) |
| **Thrombotic event, n (%)** | 8 (25.8) | 7 (20) | 4 (18.2) | 60 (100) |
| *NA* | 3 (9.7) | 1 (2.9) | 0 (0) | 0 (0) |
| **Spleen size, n (%)** |  |  |  |  |
| *Normal* | 12 (38.7) | 20 (57.1) | 6 (27.3) | 60 (100) |
| *Splenomegaly* | 19 (61.3) | 14 (40) | 14 (63.6) | 0 (0) |
| *Splenectomy* | 0 (0) | 0 (0) | 2 (9.1) | 0 (0) |
| *NA* | 0 (0) | 1 (2.9) | 0 (0) | 0 (0) |
| **Blasts (%)** |  |  |  |  |
| *0* | 4 (12.9) | 13 (37.1) | 1 (4.5) |  |
| *1* | 8 (25.8) | 11 (31.3) | 13 (59.2) |  |
| *2* | 7 (22.6) | 7 (20) | 4 (18.2) |  |
| *3* | 5 (16.1) | 1 (2.9) | 2 (9.1) |  |
| *4* | 3 (9.7) | 1 (2.9) | 1 (4.5) |  |
| *5* | 0 (0) | 1 (2.9) | 1 (4.5) |  |
| *NA* | 4 (12.9) | 1 (2.9) | 0 (0) | 60 (100) |
| **LDH (range)** | 348 (211 - 1767) | 270 (136 - 1575) | 670.5 (323 - 1777) |  |
| *NA* | 2 | 2 | 0 | 60 (100) |
| **Hematological parameters, median (range)** |  |  |  |  |
| *WBC count, x103/mm³* | 9.7 (3.59 - 24.3) | 7.1 (2.9 - 49.4) | 7.38 (1.46 - 32.9) | NA |
| *RBC count, x106/mm³* | 6.01 (3.09 - 9.04) | 4.31 (2.98 - 7.24) | 3.68 (2.03 - 5.75) | NA |
| *Hemoglobin, g/dL* | 15.6 (11.1 - 22) | 13.1 (10 - 20.8) | 10.75 (5.88 - 15.9) | NA |
| *Hematocrit, %* | 48.2 (33.7 - 74) | 39.4 (30 - 51.7) | 32.95 (19.2 - 52.9) | NA |
| *PLT count, x103/mm³* | 449 (41.3 - 809) | 608 (259 - 1484) | 224 (27 - 1474) | NA |

PV = polycythemia vera; ET = essential thrombocythemia; PMF = primary myelofibrosis; CTRL = control; Y: years; CALR = calreticulin mutation; JAK2V617F: positive for JAK2V617F mutation; Negative = negative for JAK2V617F and CALR mutation; NA = data not available; LDH: lactate dehydrogenase median; WBC = white blood cells counts; RBC = red blood cells counts; PLT = platelets counts.

Table S2 – Demographic and clinic-hematological data from patients with myeloproliferative neoplasms and healthy subjects who donated bone marrow for isolation of CD34+ cells.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Data** | **PV** | **ET** | **PMF** | **CTRL** |
| **(n=15)** | **(n=20)** | **(n=7)** | **(n=15)** |
| **Median age (range)** | 61y (45-80) | 59.5y (35-81) | 68y (41-80) | 30y (16 -54) |
| **Gender (male %)** | 8 (50) | 9 (35) | 6 (85.7) | 8 (53.3) |
| **Mutation *status* (%)** |  |  |  |  |
| *JAK2V617F* | 15 (93.8) | 9 (45) | 2 (28.6) | 0 (0) |
| *CALR* | 0 (0) | 3 (15) | 4 (57.1) | 0 (0) |
| Negative | 1 (6.2) | 8 (40) | 1 (14.3) | 0 (0) |
| *NA* | 0 (0) | 0 (0) | 0 (0) | 60 (100) |

PV = polycythemia vera; ET = essential thrombocythemia; PMF = primary myelofibrosis; CTRL = control; Y: years; CALR = calreticulin mutaion; JAK2V617F: positive for JAK2V617F mutation; Negative = negative for JAK2V617F and CALR mutation; NA = data not available.

Table S3 - TaqMan probes used to quantify the expression of Hippo pathway target genes and the reference genes.

|  |  |
| --- | --- |
| **Gene** | **Reference TaqMan Life**  **Technologies** |
| *MST1* | Hs00360684\_m1 |
| *MST2* | Hs00169491\_m1 |
| *SAV1* | Hs00560416\_m1 |
| *LATS1* | Hs00177987\_m1 |
| *LATS2* | Hs00324396\_m1 |
| *MOB1A* | Hs00217172\_m1 |
| *MOB1B* | Hs01397675\_m1 |
| *TAZ* | Hs00794094\_m1 |
| *YAP* | Hs00371735\_m1 |
| *- ACTIN* | 4326315E |
| *GAPDH* | Hs99999905\_m1 |

Table S4 - Sequence of the oligonucleotides used to quantify the expression of apoptosis-related genes and the reference genes.

|  |  |  |
| --- | --- | --- |
| **Gene** | **Sense primer** | **Antisense primer** |
| *BAD* | GGTAGGAGCTGTGGCGACT | CCGAGTGAGCAGGAAGACTC |
| *BAK* | ACAAACTGGCCCAACAGAAC | TCTGGCCCTACACGTCTACC |
| *BCL-2* | ACGAGTGGGATGCGGGAGATGTG | GCGGTAGCGGCGGGAGAAGTC |
| *BCL-XL* | TGGATGTCAGGTCACTGAA | CTGAATCGGAGATGGAGACC |
| *YAP* | TTGAGAACAATGACGACCAATAGCT | GCTGCTCATGCTTAGTCCACCTGT |
| *-ACTIN* | GACAGCAGTCGGTTGGACC | CAGGTAAGCCCTGGCTGC |
| *GAPDH* | GGAGAAGGCTGGGGTCAT | GTCCTTCCACGATACCAAAGTT |

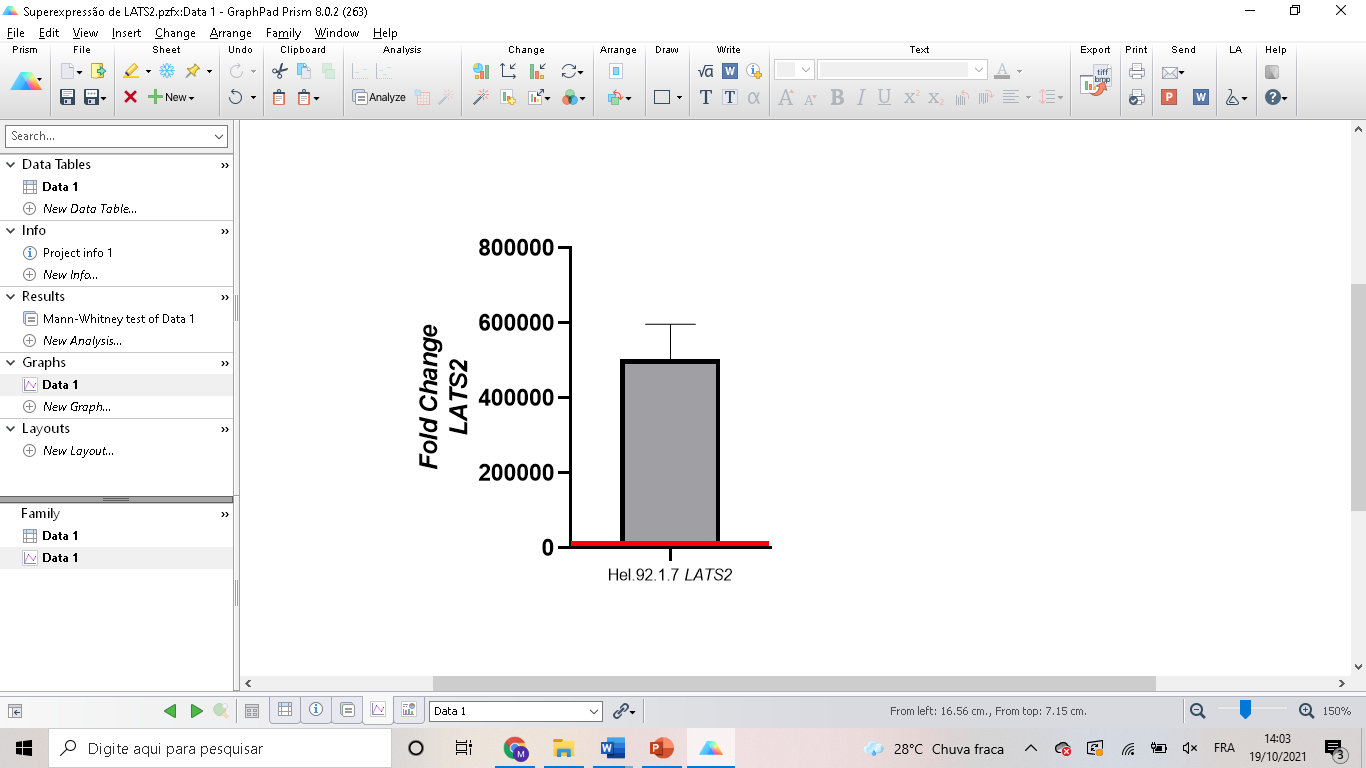


Figure S1 - *LATS2* overexpressionin HEL.92.1.7 cells confirmed by real time RT-PCR. The red line represents the *LATS2* gene expression in HEL.92.1.7 MOCK cells transfected with empty pcDNA 3.1.



Figure S2 - Hippo pathway-related genes expression in CD34+ cells from patients with polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), and healthy subjects (CT). Tukey’s statistical test. p<0.05 indicates significant differences.



Figure S3- Expression of Hippo pathway-related genes according to the mutation *status* in patients with primary myelofibrosis (PMF) and essential thrombocythemia (ET). A) Comparative analysis of *MST1*, *MOB1A* and *MOB1B* expression in JAK2V617F+, JAK2V617F-, CALR+, or CALR- PMF patients. JAK2V617F+ PMF patients expressed less *MST1*, *MOB1A* and *MOB1B* than JAK2V617F- and CALR+ PMF patients. CALR+ PMF patients expressed more *MOB1B* than CALR- PMF patients. B) Comparative analysis of *MOB1B* and *SAV1* expression in CALR+, CALR- and double negative (DN; JAK2V617F‑/CALR‑) ET patients. CALR+ ET patients expressed more *MOB1B* and *SAV1* than CALR- ET patients and DN ET patients. Only the groups of patients with significant differences were presented. Data are expressed as relative expression.



Figure S4 – Pearson’s correlation test among Hippo pathway-related genes, apoptosis-related genes, and clinic-hematological data from patients with essential thrombocythemia. The circle size indicates the strength of the test in each comparison. Red color means negative correlation and blue color means positive correlation. Only statistically significant correlations (p<0.05) were represented in the correlation heatmap.



Figure S5 – Pearson’s correlation test among Hippo pathway-related genes, apoptosis-related genes, and clinic-hematological parameters data from patients with polycythemia vera. The circle size indicates the strength of the test in each comparison. Red color means negative correlation and blue color means positive correlation. Only statistically significant correlations (p<0.05) were represented in the correlation heatmap.



Figure S6 – Pearson’s correlation test among Hippo pathway-related genes, apoptosis-related genes, and clinic-hematological parameters data from patients with primary myelofibrosis. The circle size indicates the strength of the test in each comparison. Red color means negative correlation and blue color means positive correlation. Only statistically significant correlations (p<0.05) were represented in the correlation heatmap.