

## Supplementary file 1

# Autophagy stimulation influenced the angiogenesis and metastasis behavior of human triple-negative breast cancer cells

Zahra Abbasi-Malati<sup>1</sup>, Çığır Biray Avcı<sup>2</sup>, Parisa Khanicheragh<sup>3</sup>, Zeinab Aliyari Serej<sup>1</sup>, Maryam Sabour Takanlou<sup>2</sup>, Leila Sabour Takanlou<sup>2</sup>, Seyed Ghader Azizi<sup>4</sup>, Reza Rahbarghazi<sup>5\*</sup>, Zohreh Sanaat<sup>6</sup>, Nafiseh Didar Khosrowshahi<sup>7</sup>, Hassan Amini<sup>8</sup>, Rasoul Hosseinpour<sup>9,10</sup>

<sup>1</sup>Department of Applied Cell Sciences, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

<sup>2</sup>Department of Medical Biology, Faculty of Medicine, Ege University, Izmir, Turkey

<sup>3</sup>Stem Cell and Regenerative Medicine Institute (SCARM), Tabriz University of Medical Sciences, Tabriz, Iran

<sup>4</sup>Clinical Immunology Research Center, Zahedan University of Medical Sciences, Zahedan, Iran

<sup>5</sup>Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

<sup>6</sup>Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

<sup>7</sup>Stem Cell and Tissue Engineering Research Laboratory, Sahand University of Technology, Tabriz, 51335-1996, Iran

<sup>8</sup>Department of General and Thoracic Surgery, Tabriz University of Medical Sciences, Tabriz, Iran

<sup>9</sup>Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran

<sup>10</sup>Department of Medical Nanotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

**Table S1.** Various signal transduction pathways related to autophagy

| Pathways                                                    | Genes                                                                                                                                                             |
|-------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Autophagic Vacuole Formation</b>                         | AMBRA1 (NYW1), ATG12, ATG16L1, ATG4A, ATG4B, ATG4C, ATG4D, ATG5, ATG9A, ATG9B, BECN1, GABARAP, GABARAPL1, GABARAPL2, IRGM, MAP1LC3A, MAP1LC3B, RGS19, ULK1, WIPI1 |
| <b>Vacuole Targeting</b>                                    | ATG4A, ATG4B, ATG4C, ATG4D, GABARAP                                                                                                                               |
| <b>Protein Transport</b>                                    | ATG10, ATG16L1, ATG16L2, ATG3, ATG4A, ATG4B, ATG4C, ATG4D, ATG7, ATG9A, GABARAP, GABARAPL2, RAB24                                                                 |
| <b>Autophagosome-Lysosome Linkage</b>                       | DRAM1, GABARAP, LAMP1, NPC1                                                                                                                                       |
| <b>Autophagy in Response to Other Intracellular Signals</b> | CTSD, CTSS, DRAM2 (TMEM77), EIF4G1, ESR1 (ER $\alpha$ ), GAA, HGS, MAPK14 (p38ALPHA), PIK3C3 (Vps34), PIK3R4, RPS6KB1, TMEM74, ULK2, UVRAG                        |

|                                                        |                                                                                                                                                                                                                                                                                                                                                            |
|--------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Ubiquitination</b>                                  | ATG3, ATG7, HDAC6                                                                                                                                                                                                                                                                                                                                          |
| <b>Proteases</b>                                       | ATG4A, ATG4B, ATG4C, ATG4D                                                                                                                                                                                                                                                                                                                                 |
| <b>Co-Regulators of Autophagy &amp; Apoptosis</b>      | AKT1, APP, ATG12, ATG5, BAD, BAK1, BAX, BCL2, BCL2L1 (BCLXL), BECN1, BID, BNIP3, CASP3, CASP8 (FLICE), CDKN1B (P27KIP1), CDKN2A (p16INK4a), CLN3, CTSB, CXCR4, DAPK1, DRAM1, EIF2AK3, FADD, FAS (TNFRSF6), HDAC1, HTT, IFNG, IGF1, INS, MAPK8 (JNK1), MTOR, NFKB1, PIK3CG, PRKAA1(AMPK), PTEN, SNCA, SQSTM1, TGFB1, TGM2, TNF, TNFSF10 (TRAIL), TP53 (p53) |
| <b>Chaperone-Mediated Autophagy</b>                    | HSP90AA1, HSPA8                                                                                                                                                                                                                                                                                                                                            |
| <b>Co-Regulators of Autophagy &amp; the Cell Cycle</b> | BAX, CDKN1B (P27KIP1), CDKN2A (p16INK4a), IFNG, PTEN, RB1, TGFB1, TP53 (p53)                                                                                                                                                                                                                                                                               |
| <b>Autophagy Induction by Intracellular Pathogens</b>  | EIF2AK3, IFNG, LAMP1                                                                                                                                                                                                                                                                                                                                       |

**Table S2.** Monitoring autophagy signaling pathway in human MDA-MB-231 cells using PCR array analysis

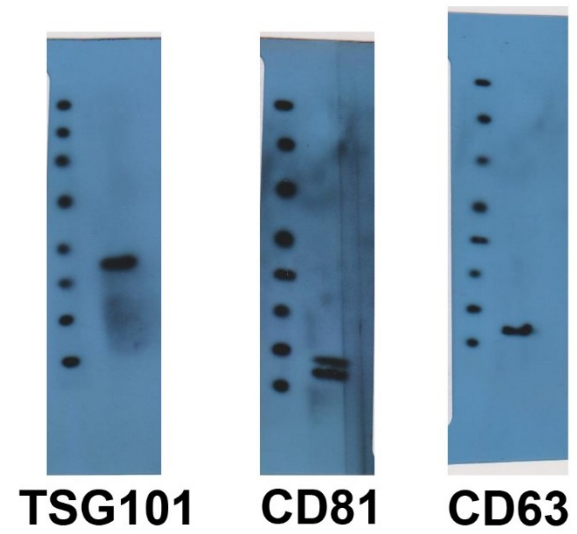
| Genes          | Experimental groups |          |           |        |          |
|----------------|---------------------|----------|-----------|--------|----------|
|                | Exos                | 3-MA     | 3-MA@Exos | Met    | Met@Exos |
| <i>AKT1</i>    | 1.06                | -7.47    | -5.03     | 3.17   | 13825.08 |
| <i>AMBRA1</i>  | -4.08               | 1.48     | 3.2       | 14.22  | 4.91     |
| <i>APP</i>     | -5.52               | 1.37     | -1.39     | 9.74   | 11.67    |
| <i>ATG10</i>   | -2.16               | -1.5E+08 | -1520.15  | -29.06 | 1.28     |
| <i>ATG12</i>   | -9.09               | -14.9    | -8.22     | 2.06   | 4284.76  |
| <i>ATG16L1</i> | -4.14               | 2.28     | -1.2      | 29.21  | 10.82    |
| <i>ATG16L2</i> | -3.36               | -6.91    | -4.38     | 2.49   | -146.52  |
| <i>ATG3</i>    | 1.08                | 3.38     | 3.27      | 19.16  | 3.13     |
| <i>ATG4A</i>   | -3.08               | -10.33   | -4.47     | 3.44   | 18.06    |
| <i>ATG4B</i>   | -3.99               | 1.19     | -2.68     | 8.9    | 4.21     |
| <i>ATG4C</i>   | -5.41               | -2.98    | 1.37      | -2.46  | 1.29     |
| <i>ATG4D</i>   | -149.44             | -1.58    | -9.71     | -20.34 | -1601.27 |
| <i>ATG5</i>    | -1.79               | -1.1     | -6.36     | 1.7    | 29634.7  |
| <i>ATG7</i>    | 2.32                | -114.05  | -781.44   | 1.5    | 91089.68 |
| <i>ATG9A</i>   | -6.95               | 1.46     | 1.42      | 23.14  | 8.43     |
| <i>ATG9B</i>   | -1.81               | -1.3     | -3.1      | 4.67   | 3.15     |
| <i>BAD</i>     | -2.18               | -3.84    | -4.63     | 3.15   | 4314.57  |

| Genes            | Experimental groups |          |           |       |          |
|------------------|---------------------|----------|-----------|-------|----------|
|                  | Exos                | 3-MA     | 3-MA@Exos | Met   | Met@Exos |
| <i>BAK1</i>      | -5.69               | -15.95   | -82.71    | -3.18 | 6248160  |
| <i>BAX</i>       | -1.57               | 2.48     | 1.48      | 12.37 | -6.04    |
| <i>BCL2</i>      | -7.28               | 2.92     | 1.01      | 25.09 | 7106.88  |
| <i>BCL2L1</i>    | 1.81                | 6.87     | 14.83     | 30.27 | 13079.32 |
| <i>BECN1</i>     | -1.89               | 3.65     | 2.14      | 11.76 | -3.08    |
| <i>BID</i>       | 6.01                | 3.61     | 6.92      | 4.83  | 470880   |
| <i>BNIP3</i>     | -5.34               | -1.55    | -1.93     | -2.32 | 39649.09 |
| <i>CASP3</i>     | -3.27               | -64.93   | -1.37     | 3.06  | 3348306  |
| <i>CASP8</i>     | -2.43               | -2.85    | -3.18     | 2.97  | 23090.31 |
| <i>CDKN1B</i>    | -2.36               | -1.54    | -23.26    | 9.54  | 155332.5 |
| <i>CDKN2A</i>    | -2.21               | -2.49    | -2.55     | 14.09 | 3315.48  |
| <i>CLN3</i>      | -9.98               | -1.03    | -4.72     | 6.69  | 3224.81  |
| <i>CTSB</i>      | 3.53                | -1.89    | 2.08      | 8.77  | 1.02     |
| <i>CTSD</i>      | -2.71               | -1.33    | -1.45     | 11.45 | 26340.59 |
| <i>CTSS</i>      | -4.12               | -4.82    | -4.56     | 3.26  | 2655.93  |
| <i>CXCR4</i>     | -4.59               | 2.35     | 1.49      | 15.77 | 5060.27  |
| <i>DAPK1</i>     | -42.72              | -753.85  | -63.56    | 1.71  | 571740.1 |
| <i>DRAM1</i>     | -2.65               | 1.48     | 2.69      | 5.32  | 1.66     |
| <i>DRAM2</i>     | -3.32               | -5.67    | -4.5      | 7.51  | 30467.85 |
| <i>EIF2AK3</i>   | -1.74               | -1.68    | -1.64     | 3.59  | 157500.8 |
| <i>EIF4G1</i>    | -2.82               | -2.42    | -7.67     | 1.22  | 127930.4 |
| <i>ESR1</i>      | -3.41               | -22.85   | -27.47    | 1.82  | 6186.9   |
| <i>FADD</i>      | -2.45               | -1.12    | -2.62     | 3.06  | 167.15   |
| <i>FAS</i>       | 874.73              | 15485.22 | -1.4      | 19.41 | 89476160 |
| <i>GAA</i>       | -4.41               | -7.01    | -7.31     | 2.06  | 9708.28  |
| <i>GABARAP</i>   | 2.25                | 2.95     | 2.66      | 14.48 | 486.06   |
| <i>GABARAPL1</i> | -4.68               | -3.89    | -7.46     | 1.93  | 2946.94  |

| Genes            | Experimental groups |          |           |         |          |
|------------------|---------------------|----------|-----------|---------|----------|
|                  | Exos                | 3-MA     | 3-MA@Exos | Met     | Met@Exos |
| <i>GABARAPL2</i> | -1.01               | 1.41     | -1.69     | 8.59    | 1.89     |
| <i>HDAC1</i>     | -1.7                | 1.99     | -49.87    | 4.02    | 1101.31  |
| <i>HDAC6</i>     | -1.49               | 1.45     | -3.14     | 8.49    | -4.39    |
| <i>HGS</i>       | -118.88             | 1.4      | -2.33     | 3.41    | 2.32     |
| <i>HSP90AA1</i>  | -2.93               | -1.98    | -4.06     | 5.45    | 4225.77  |
| <i>HSPA8</i>     | -1.14               | -18.48   | -46.85    | 1.33    | 762.71   |
| <i>HTT</i>       | -3.54               | -1.16    | -3.43     | 7.1     | 11074.85 |
| <i>IFNG</i>      | -5.78               | 2.47     | 1.28      | 33.11   | 70483/6  |
| <i>IGF1</i>      | 11.32               | 22.75    | -6.36     | 4.28    | 2835163  |
| <i>INS</i>       | -2.46               | -1.25    | -2.57     | 6.46    | -6.17    |
| <i>IRGM</i>      | -3.24               | 1.35     | -3.39     | -2.03   | 101070.3 |
| <i>LAMP1</i>     | -2.23               | -1.39    | -2.99     | 3.84    | 363.3    |
| <i>MAP1LC3A</i>  | -4.74               | -6.17    | -10.41    | 1.34    | 4225.77  |
| <i>MAP1LC3B</i>  | -2.2                | -1.29    | -4.08     | 4.35    | 2583.3   |
| <i>MAPK14</i>    | -3.37               | -1.21    | -10.63    | 2.03    | 29226.71 |
| <i>MAPK8</i>     | -2.75               | -2.07    | -1.88     | -139.44 | 7831.1   |
| <i>MTOR</i>      | 3.05                | 3.74     | 9.58      | 18.04   | 1.36     |
| <i>NFKB1</i>     | -3.17               | -10903.6 | -268.73   | 1.7     | 30679.77 |
| <i>NPC1</i>      | 2.05                | 2.31     | 4.35      | 16.85   | 1.64     |
| <i>PIK3C3</i>    | -2.32               | -1.77    | -4.72     | 4.27    | 701.84   |
| <i>PIK3CG</i>    | -5.11               | 1.43     | -1.27     | 15.71   | 6960.62  |
| <i>PIK3R4</i>    | -1.76               | -1.22    | -4.03     | 7.24    | -2.37    |
| <i>PRKAA1</i>    | -4.46               | -1.64    | -12.91    | 3.96    | 2393.66  |
| <i>PTEN</i>      | -2.91               | -1.35    | -26.54    | -2.33   | 968236.5 |
| <i>RAB24</i>     | -2.63               | 4.26     | 2.04      | 38.54   | 11.92    |
| <i>RBI</i>       | 3.27                | 8.79     | 29.04     | 37.38   | -2.34    |
| <i>RGS19</i>     | -8.21               | -2.2     | -3.92     | 3.91    | 3        |

| Genes          | Experimental groups |       |           |         |          |
|----------------|---------------------|-------|-----------|---------|----------|
|                | Exos                | 3-MA  | 3-MA@Exos | Met     | Met@Exos |
| <i>RPS6KB1</i> | -3.96               | 1.09  | -1.35     | 4.96    | 24070.86 |
| <i>SNCA</i>    | -5.77               | -5.41 | -5.5      | 2.54    | -1.11    |
| <i>SQSTM1</i>  | -2.87               | -5.1  | -5.62     | 3.82    | 1.07     |
| <i>TGFB1</i>   | -2.08               | -2.5  | -1.14     | 4.45    | 2.2      |
| <i>TGM2</i>    | -8.21               | 1.16  | -1.67     | 17.48   | 13.98    |
| <i>TMEM74</i>  | -2.88               | -4.33 | -3.14     | 3.62    | 16670.39 |
| <i>TNF</i>     | -3.08               | -1.24 | -2.64     | 4.67    | -5.3     |
| <i>TNFSF10</i> | -1.13               | -1.45 | -4.63     | 5.46    | -4.3     |
| <i>TP53</i>    | -2.23               | 1.54  | -11.31    | 1.96    | 1.34     |
| <i>ULK1</i>    | -2.39               | 1.12  | -1.33     | 27.96   | 2.2      |
| <i>ULK2</i>    | -2.81               | -2.76 | -17.27    | 3.2     | 21844.77 |
| <i>UVRAG</i>   | 1.18                | 1.12  | -2.1      | 3.62    | 5.22     |
| <i>WIP1</i>    | -2.95               | -1.28 | 1.2       | -1.93   | 27650.16 |
| <i>ACTB</i>    | 1.38                | 1.64  | 5.74      | -1.33   | 168.31   |
| <i>B2M</i>     | -1.79               | -7.91 | -55.33    | -324.27 | 5592260  |
| <i>GAPDH</i>   | -1.89               | -1.83 | -5.24     | -1.97   | -2.69    |
| <i>HPRT1</i>   | 3.51                | 3.71  | 6.73      | 5.84    | -19.77   |
| <i>RPLP0</i>   | -2.56               | -3.32 | -7.36     | -2.22   | -3.17    |

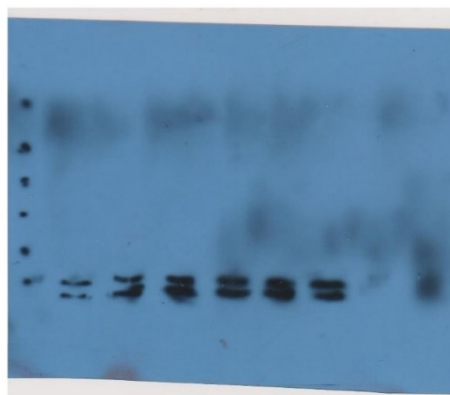
Fold-change values in human MDA-MB-231 cells treated with free Met and 3-MA, or Met-, and 3-MA-loaded Exos were calculated using the  $2^{-\Delta\Delta C_t}$  methods related to the non-treated control group. Differences in expression more than twofold were accepted as the cut-off value (n = 3).



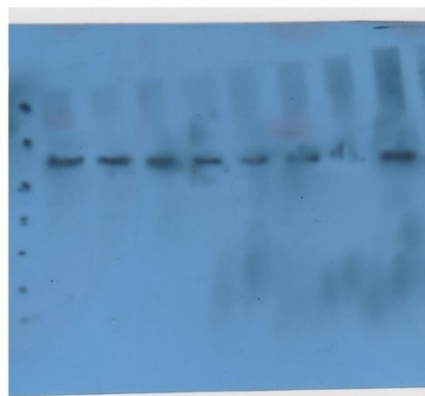
**Figure S1. Full-length blots related to exosome markers**



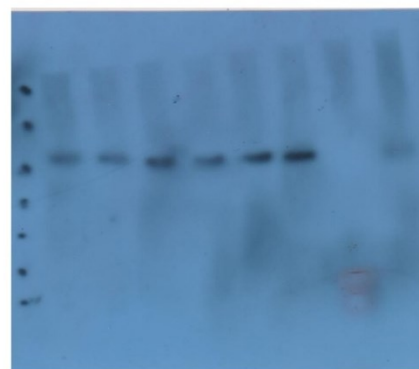
**Beta actin**



**LC3**



**p62**



**Beclin**

**Figure S2. Full-length blots associated with autophagy response.**