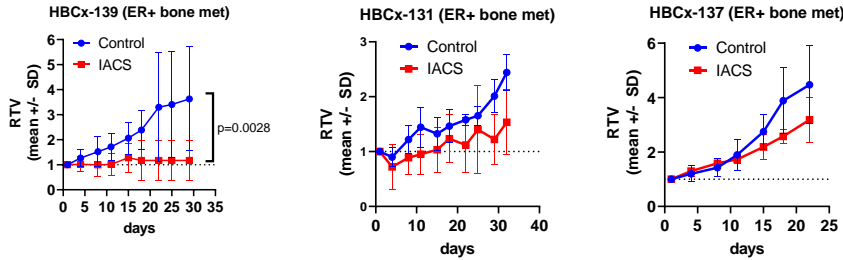


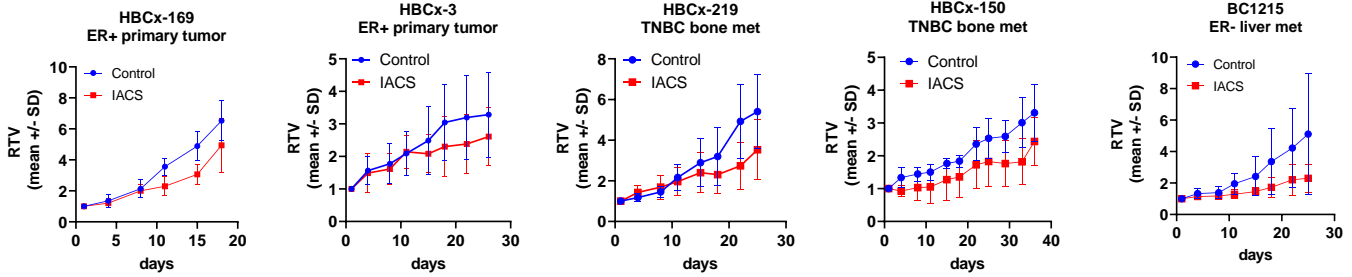
Supplemental information

Figure S1

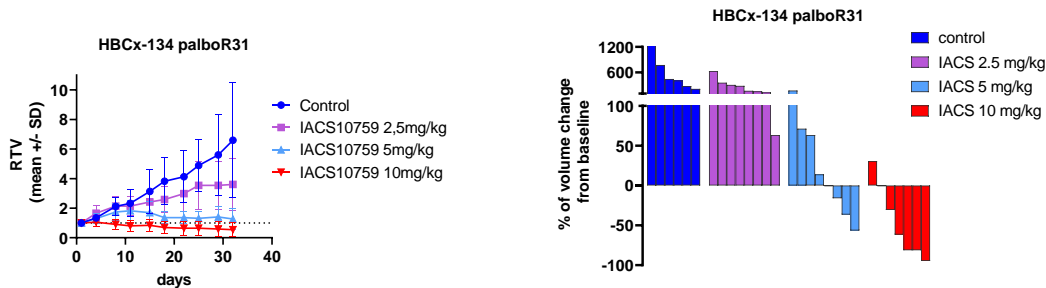
A



B



C



| Two-way RM ANOVA | | | | | |
|-------------------------------------------|---------------------------|--------------------|------------------|---------|------------------|
| Source of Variation | % of total variation | P value | P value summary | | |
| Row Factor TIME x Column Factor IACS dose | 19.13 | <0,0001 | **** | | |
| Row Factor TIME | 12.42 | <0,0001 | **** | | |
| Column Factor IACS dose | 35.25 | <0,0001 | **** | | |
| Subject | 21.57 | <0,0001 | **** | | |
| Tukey's multiple comparisons test | | | | | |
| day 32 | Predicted (LS) mean diff. | 95,00% CI of diff, | Below threshold? | Summary | Adjusted P Value |
| Control vs. IACS10759 2,5mg/kg | 2.985 | 1,480 to 4,490 | Yes | **** | <0,0001 |
| Control vs. IACS10759 5mg/kg | 5.328 | 3,823 to 6,833 | Yes | **** | <0,0001 |
| Control vs. IACS10759 10mg/kg | 6.05 | 4,500 to 7,600 | Yes | **** | <0,0001 |
| IACS10759 2,5mg/kg vs. IACS10759 5mg/kg | 2.344 | 0,9502 to 3,737 | Yes | *** | 0.0001 |
| IACS10759 2,5mg/kg vs. IACS10759 10mg/kg | 3.065 | 1,623 to 4,508 | Yes | **** | <0,0001 |
| IACS10759 5mg/kg vs. IACS10759 10mg/kg | 0.7217 | -0,7206 to 2,164 | No | ns | 0.5675 |

Figure S1: *in vivo* evaluation of IACS activity in PDX of bone ER positive metastasis (**A**), liver metastasis, TNBC bone metastases and ER+ primary tumours (**B**) and in the Palbociclib-resistant HBCx-134 palboR31 PDX (**C**). Mean +/- SD. HBCx-139: n= 7 (control and IACS); HBCx-131: n=3 (control) and n=5 (IACS); HBCx-137: n=5 (control and IACS); HBCx-169: n=5 (control and IACS); HBCx-3: n=8 (control) and n=9 (IACS); HBCx-219: n=6 (control and IACS); HBCx-150: n=4 (control) and n=5 (IACS); BC1215: n=4 (control and IACS). HBCx-131 palbo31: n=6 (control), n=8 (IACS 2.5 mg/kg and IACS 5 mg/kg), n=7 (IACS 10 mg/kg). The Turkey's multiple comparison test applied to the HBCx-134 palboR31 is shown under figure S1C (Two-way RM Anova). The difference between HBCx-139 control and treated group was analysed with the Mann Whitney test ($p = 0.0028$, two-tailed).

Figure S2

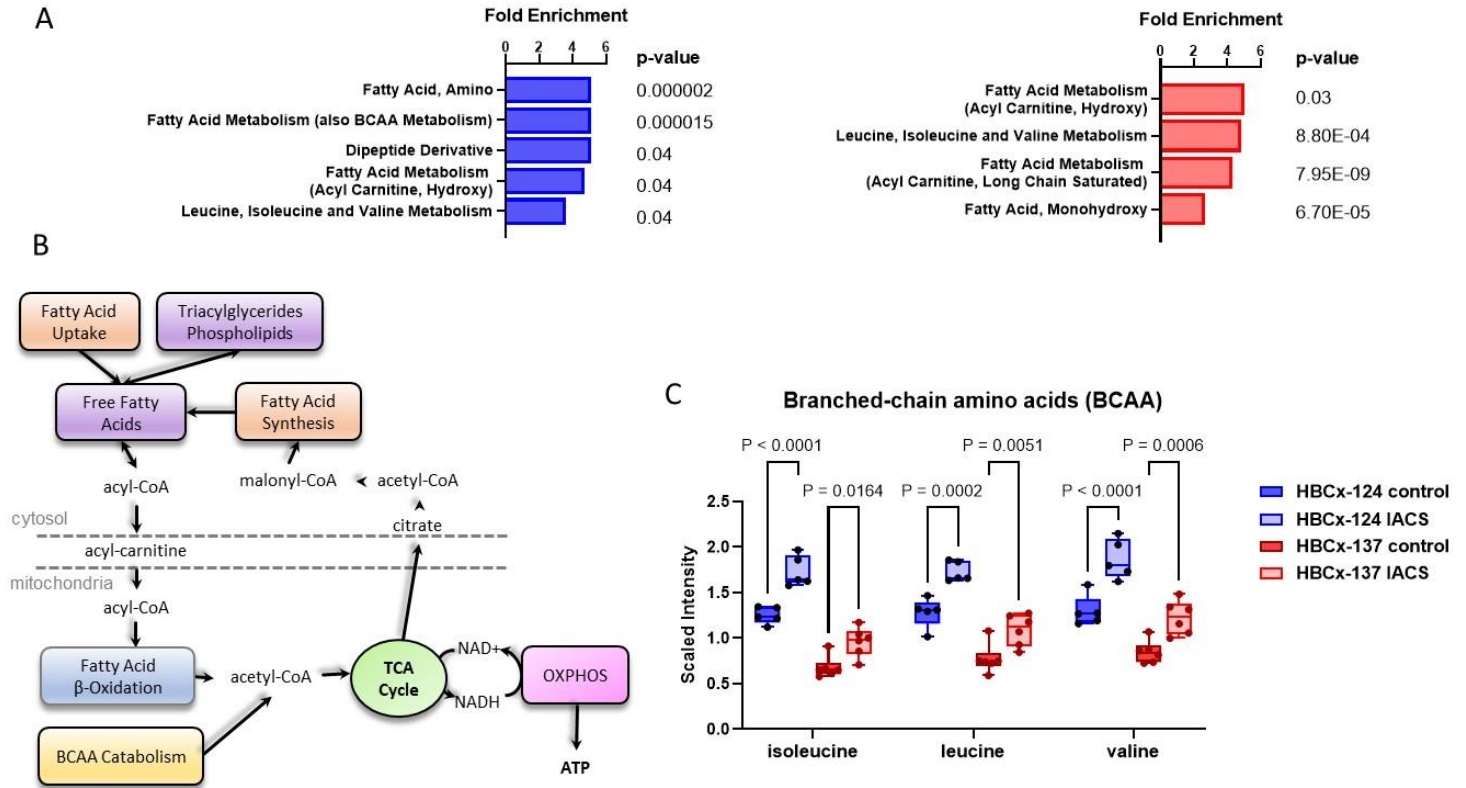


Figure S2. (A) Metabolic Pathway Enrichment Analysis of differentially expressed metabolites between IACS-treated and control xenografts of HBCx-124 and HBCx-137 PDX. p value were calculated with right-tailed Fisher's exact test (uncorrected). (B) Metabolic pathways showing the transport of fatty acids into mitochondria through the carnitine shuttle. In the mitochondria, these fatty acyl carnitines are progressively shortened by two carbons through rounds of β -oxidation, generating NADH, FADH₂, and acetyl-CoA. (C) Min/Max Whiskers plots showing the levels of branched-chain amino acids (BCAA) in HBCx-124 and HBCx-137 PDX control and IACS treated groups. n= 5 (HBCx-124 control and treated groups) and n=6 (HBCx-137 control and treated groups). Tukey's multiple comparisons test (2-way Anova).

Figure S3

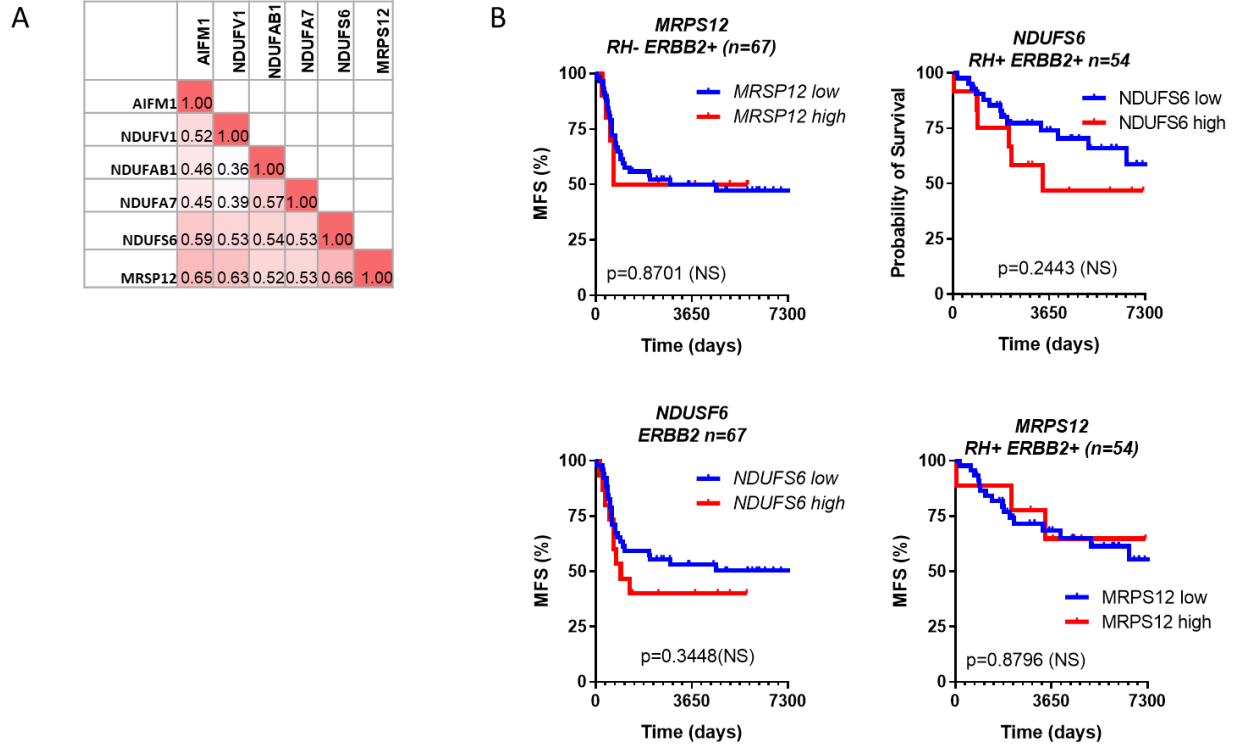


Figure S3. (A) Pearson correlation analysis of *AIFM1*, *NDUFV1*, *NDUFAB1*, *NDUFA7*, *NDUFS6* and *MRSP12* genes expression determined by RT-PCR analysis in 503 breast tumours. **(B)** Metastasis-free survival (MFS) of RH- ERBB2+ and RH+ ERBB2+ breast cancer patients according to *MRPS12* and *NDUFS6* low and high expression (optimal cut-off). P values were calculated with the Log-rank (Mantel-Cox) test.

Supplementary Tables

Table S1. Gene Set enrichment analysis of RNAseq data of IACS treated HBCx-124 xenografts as compared to control HBCx-124 xenografts. ES: enrichment score, NES: normalized enrichment score; NOM p val: nominal p value; FDR q value: p-value adjusted for the False Discovery Rate (FDR).

| GSEA REPORT FOR IACS TREATED HBCx-124 xenografts | | | | | |
|----------------------------------------------------------------------------------------------------------------|------|--------|--------|-----------|-----------|
| NAME | SIZE | ES | NES | NOM p-val | FDR q-val |
| REACTOME_ACTIVATION_OF_THE_MRNA_UPON_BINDING_OF_THE_CAP_BINDING_COMPLEX_AND_EIF5_AND_SUBSEQUENT_BINDING_TO_43S | 26 | 0.632 | 2.092 | 0.000 | 0.052 |
| REACTOME_RESPONSE_OF_EIF2AK4_GCN2_TO_AMINO_ACID_DEFICIENCY | 15 | 0.658 | 1.944 | 0.003 | 0.154 |
| REACTOME_EUKARYOTIC_TRANSLATION_INITIATION | 34 | 0.552 | 1.911 | 0.000 | 0.155 |
| REACTOME_FOXO_MEDIATED_TRANSCRIPTION_OF_CELL_DEATH_GENES | 15 | 0.668 | 1.848 | 0.000 | 0.234 |
| KEGG_ETHER_LIPID_METABOLISM | 18 | 0.626 | 1.843 | 0.000 | 0.197 |
| REACTOME_RESPONSE_OF_EIF2AK1_HRI_TO_HEME_DEFICIENCY | 15 | 0.650 | 1.837 | 0.005 | 0.177 |
| MED16_TARGET_GENES | 24 | 0.567 | 1.817 | 0.000 | 0.187 |
| REACTOME_PEROXISOMAL_PROTEIN_IMPORT | 57 | 0.454 | 1.814 | 0.000 | 0.170 |
| REACTOME_ATTENUATION_PHASE | 25 | 0.535 | 1.772 | 0.005 | 0.228 |
| KEGG_OTHER_GLYCAN_DEGRADATION | 15 | 0.607 | 1.765 | 0.010 | 0.218 |
| REACTOME_AMINO_ACIDS_REGULATE_MTORC1 | 50 | 0.461 | 1.761 | 0.000 | 0.205 |
| REACTOME_FOXO_MEDIATED_TRANSCRIPTION | 51 | 0.453 | 1.751 | 0.004 | 0.208 |
| GSEA REPORT FOR CONTROL HBCx-124 xenografts | | | | | |
| NAME | SIZE | ES | NES | NOM p-val | FDR q-val |
| HSD17B8_TARGET_GENES | 486 | -0.734 | -3.173 | 0.000 | 0.000 |
| HALLMARK_E2F_TARGETS | 195 | -0.763 | -3.096 | 0.000 | 0.000 |
| HALLMARK_G2M_CHECKPOINT | 186 | -0.753 | -2.995 | 0.000 | 0.000 |
| REACTOME_CELL_CYCLE_CHECKPOINTS | 241 | -0.692 | -2.839 | 0.000 | 0.000 |
| REACTOME_MITOTIC_SPINDLE_CHECKPOINT | 104 | -0.764 | -2.817 | 0.000 | 0.000 |
| REACTOME_RESOLUTION_OF_SISTER_CHROMATID_COHESION | 114 | -0.745 | -2.814 | 0.000 | 0.000 |
| REACTOME_RHO_GTPASES_ACTIVATE_FORMINS | 128 | -0.707 | -2.724 | 0.000 | 0.000 |
| REACTOME_CELL_CYCLE_MITOTIC | 472 | -0.634 | -2.697 | 0.000 | 0.000 |
| PID_AURORA_B_PATHWAY | 36 | -0.838 | -2.649 | 0.000 | 0.000 |
| REACTOME_MITOTIC_PROMETAPHASE | 189 | -0.661 | -2.645 | 0.000 | 0.000 |
| REACTOME_MITOTIC_METAPHASE_AND_ANAPHASE | 219 | -0.647 | -2.637 | 0.000 | 0.000 |
| REACTOME_SEPARATION_OF_SISTER_CHROMATIDS | 175 | -0.664 | -2.634 | 0.000 | 0.000 |
| PID_PLK1_PATHWAY | 41 | -0.833 | -2.620 | 0.000 | 0.000 |
| PID_E2F_PATHWAY | 70 | -0.729 | -2.607 | 0.000 | 0.000 |
| PID_ATR_PATHWAY | 38 | -0.795 | -2.574 | 0.000 | 0.000 |
| RB_P107_DN.V1_UP | 115 | -0.678 | -2.573 | 0.000 | 0.000 |
| REACTOME_ACTIVATION_OF_ATR_IN_RESPONSE_TO_REPLICATION_STRESS | 37 | -0.804 | -2.558 | 0.000 | 0.000 |
| REACTOME_MITOTIC_G1_PHASE_AND_G1_S_TRANSITION | 141 | -0.648 | -2.525 | 0.000 | 0.000 |
| KEGG_CELL_CYCLE | 117 | -0.668 | -2.524 | 0.000 | 0.000 |
| REACTOME_DNA_REPLICATION | 123 | -0.660 | -2.511 | 0.000 | 0.000 |
| REACTOME_DEPOSITION_OF_NEW_CENPA_CONTAINING_NUCLEOSOMES_AT_THE_CENTROMERE | 24 | -0.858 | -2.503 | 0.000 | 0.000 |
| REACTOME_G2_M_CHECKPOINTS | 126 | -0.648 | -2.471 | 0.000 | 0.000 |
| REACTOME_G1_S_SPECIFIC_TRANSCRIPTION | 28 | -0.838 | -2.453 | 0.000 | 0.000 |
| REACTOME_M_PHASE | 334 | -0.583 | -2.452 | 0.000 | 0.000 |

Table S2. Gene Set enrichment analysis of RNAseq data of IACS resistant PDX as compared to IACS responder PDX. ES: enrichment score, NES: normalized enrichment score; NOM p val: nominal p value; FDR q value: p-value adjusted for the False Discovery Rate (FDR).

| GSEA REPORT FOR IACS RESPONDERS | | | | | |
|------------------------------------|------|--------|--------|-----------|-----------|
| NAME | SIZE | ES | NES | NOM p-val | FDR q-val |
| HALLMARK_INTERFERON_ALPHA_RESPONSE | 94 | -0.488 | -2.271 | 0.000 | 0.000 |
| HALLMARK_ESTROGEN_RESPONSE_EARLY | 194 | -0.400 | -2.089 | 0.000 | 0.000 |
| HALLMARK_INTERFERON_GAMMA_RESPONSE | 184 | -0.356 | -1.864 | 0.000 | 0.004 |
| HALLMARK_ESTROGEN_RESPONSE_LATE | 195 | -0.342 | -1.802 | 0.000 | 0.005 |
| HALLMARK_PI3K_AKT_MTOR_SIGNALING | 97 | -0.366 | -1.726 | 0.000 | 0.008 |
| HALLMARK_XENOBIOTIC_METABOLISM | 179 | -0.320 | -1.691 | 0.000 | 0.009 |
| HALLMARK_PROTEIN_SECRETION | 93 | -0.351 | -1.628 | 0.000 | 0.014 |
| HALLMARK_OXIDATIVE_PHOSPHORYLATION | 183 | -0.313 | -1.626 | 0.004 | 0.013 |
| HALLMARK_ADIPOGENESIS | 183 | -0.301 | -1.573 | 0.000 | 0.018 |
| HALLMARK_IL6_JAK_STAT3_SIGNALING | 74 | -0.302 | -1.376 | 0.048 | 0.095 |
| HALLMARK_FATTY_ACID_METABOLISM | 148 | -0.257 | -1.309 | 0.041 | 0.149 |

| GSEA REPORT FOR IACS RESISTANT | | | | | |
|--------------------------------------------|------|-------|-------|-----------|-----------|
| NAME | SIZE | ES | NES | NOM p-val | FDR q-val |
| HALLMARK_E2F_TARGETS | 195 | 0.358 | 1.830 | 0.000 | 0.007 |
| HALLMARK_G2M_CHECKPOINT | 188 | 0.349 | 1.791 | 0.000 | 0.005 |
| HALLMARK_ANGIOGENESIS | 30 | 0.458 | 1.667 | 0.015 | 0.013 |
| HALLMARK_PANCREAS_BETA_CELLS | 30 | 0.468 | 1.664 | 0.006 | 0.010 |
| HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION | 188 | 0.307 | 1.593 | 0.000 | 0.018 |
| HALLMARK_MITOTIC_SPINDLE | 197 | 0.298 | 1.541 | 0.002 | 0.023 |
| HALLMARK_SPERMATOGENESIS | 107 | 0.314 | 1.465 | 0.007 | 0.045 |

Table S3. Characteristics of the 503 breast cancers

| | Number of patients | % patients | Number with metastases | % metastases | p value ^e |
|---------------------------------------------|--------------------|------------|------------------------|--------------|----------------------|
| <i>Total</i> | 503 | 100.0% | 202 | 40.16% | |
| <i>Age</i> | | | | | |
| ≤50 | 117 | 23.26% | 51 | 25.25% | 0.2895 (NS) |
| >50 | 386 | 76.74% | 151 | 74.75% | |
| <i>SBR histological grade^{a,b}</i> | | | | | |
| I | 58 | 11.84% | 12 | 5.94% | 0.0036 |
| II | 231 | 47.14% | 97 | 48.02% | |
| III | 201 | 41.02% | 93 | 46.04% | |
| <i>Lymph node status^c</i> | | | | | |
| 0 | 149 | 29.86% | 46 | 22.77% | <0.0001 |
| 1-3 | 242 | 48.50% | 85 | 42.08% | |
| >3 | 108 | 21.64% | 71 | 35.15% | |
| <i>Macroscopic tumor size^d</i> | | | | | |
| <25mm | 237 | 48.07% | 74 | 36.82% | <0.0001 |
| >25mm | 256 | 51.93% | 127 | 63.18% | |
| <i>ERα status</i> | | | | | |
| Negative | 169 | 33.6% | 72 | 35.64% | 0.0845 (NS) |
| Positive | 334 | 66.4% | 130 | 64.36% | |
| <i>PR status</i> | | | | | |
| Negative | 241 | 47.91% | 104 | 51.49% | 0.0349 |
| Positive | 262 | 52.09% | 98 | 48.51% | |
| <i>ERBB2 status</i> | | | | | |
| Negative | 382 | 75.94% | 149 | 73.76% | 0.2246 (NS) |
| Positive | 121 | 24.06% | 53 | 26.24% | |
| <i>Molecular subtypes</i> | | | | | |
| HR- ERBB2- | 95 | 18.89% | 36 | 17.82% | 0.0484 |
| HR- ERBB2+ | 67 | 13.32% | 34 | 16.83% | |
| HR+ ERBB2- | 287 | 57.06% | 113 | 55.94% | |
| HR+ ERBB2+ | 54 | 10.73% | 19 | 9.41% | |

Table S3 Characteristics of the 503 breast cancers. **a** Scarff Bloom Richardson classification, **b** Information available for 490 patients, **c** Information available for 499 patients, **d** Information available for 493 patients, **e** Metastasis-free survival (Log-rank Mantel-Cox test)

Table S4

| | | Total population (%) | Low MRPS12 | High MRPS12 | p-value | Total population (%) | Low NDUFS6 | High NDUFS6 | p-value ^a |
|---------------------------------------------|-------------------|------------------------|-------------------|--------------------|-------------------|------------------------|-------------------|--------------------|----------------------|
| | | Number of patients (%) | | | | Number of patients (%) | | | |
| Age | <i>Total</i> | 503 (100) | 443 (88.07) | 60 (11.93) | | 503 (100) | 419 (83.3) | 84 (16.7) | |
| | ≤50 | 117 (23.26) | 99 (84.62) | 18 (15.38) | 0.1880 | 117 (23.26) | 94 (80.34) | 23 (19.66) | 0.3274 |
| | >50 | 386 (76.74) | 344 (89.12) | 42 (10.88) | | 386 (76.74) | 325 (84.2) | 61 (15.80) | |
| SBR histological grade <i>b,c</i> | <i>I</i> | 58 (11.84) | 57 (98.28) | 1 (1.72) | 0.0002 | 58 (11.84) | 56 (96.55) | 2 (3.45) | 0.0004 |
| | <i>II</i> | 231 (47.14) | 211 (91.34) | 20 (8.66) | | 231 (47.14) | 198 (85.71) | 33 (14.29) | |
| | <i>III</i> | 201 (41.02) | 164 (81.59) | 37 (18.41) | | 201 (41.02) | 153 (76.12) | 48 (23.88) | |
| Lymph node status <i>d</i> | <i>0</i> | 149 (29.86) | 129 (86.58) | 20 (13.42) | 0.7995 | 149 (29.86) | 126 (84.56) | 23 (15.44) | 0.8627 |
| | <i>1-3</i> | 242 (48.5) | 215 (88.84) | 27 (11.16) | | 242 (48.50) | 200 (82.64) | 42 (17.36) | (NS) |
| | <i>>3</i> | 108 (21.64) | 95 (87.96) | 13 (12.04) | | 108 (21.64) | 91 (84.26) | 17 (15.74) | |
| Macroscopic tumor size <i>e</i> | ≤ 25mm | 237 (48.07) | 217 (91.56) | 20 (8.44) | 0.0202 | 237 (48.07) | 203 (85.65) | 34 (14.35) | 0.1895 |
| | > 25mm | 256 (51.93) | 217 (84.77) | 39 (15.23) | | 256 (51.93) | 208 (81.25) | 48 (18.75) | |
| | | | | | | | | | |
| ERα status | <i>Negative</i> | 169 (33.6) | 136 (80.47) | 33 (19.53) | 0.0002 | 169 (33.6) | 128 (75.74) | 41 (24.26) | 0.0012 |
| | <i>Positive</i> | 334 (66.4) | 307 (91.92) | 27 (8.08) | | 334 (66.4) | 291 (87.13) | 43 (12.87) | |
| PR status | <i>Negative</i> | 241 (47.91) | 203 (84.23) | 38 (15.77) | 0.0108 | 241 (47.91) | 189 (78.42) | 52 (21.58) | 0.0049 |
| | <i>Positive</i> | 262 (52.09) | 240 (91.60) | 22 (8.40) | | 262 (52.09) | 230 (87.79) | 32 (12.21) | |
| ERBB2 status | <i>Negative</i> | 382 (75.94) | 341 (89.27) | 41 (10.73) | 0.1416 | 382 (75.94) | 325 (85.08) | 57 (14.92) | 0.0574 |
| | <i>Positive</i> | 121 (24.06) | 102 (84.30) | 19 (15.70) | | 121 (24.06) | 94 (77.69) | 27 (22.31) | |
| Molecular subtypes | <i>HR- ERBB2-</i> | 95 (18.89) | 73 (76.84) | 22 (23.16) | 0.0001 | 95 (18.89) | 71 (74.74) | 24 (25.26) | 0.0040 |
| | <i>HR- ERBB2+</i> | 67 (13.32) | 57 (85.07) | 10 (14.93) | | 67 (13.32) | 52 (77.61) | 15 (22.39) | |
| | <i>HR+ ERBB2-</i> | 287 (57.06) | 268 (93.38) | 19 (6.62) | | 287 (57.06) | 254 (88.50) | 33 (11.50) | |
| | <i>HR+ ERBB2+</i> | 54 (10.73) | 45 (83.33) | 9 (16.67) | | 54 (10.73) | 42 (77.78) | 12 (22.22) | |
| MKI67 mRNA expression <i>h,j</i> | <i>median</i> | 8.35 (0.28-79.79) | 7.54 (0.28-79.79) | 21.64 (2.25-57.67) | <0.0001 | 8.35 (0.28-79.79) | 7.48 (0.28-79.79) | 16.13 (2.21-71.97) | <0.0001 |
| | | | | | | | | | |
| Metastasis | <i>No</i> | 301 (59.84) | 274 (91.03) | 27 (8.97) | 0.0125 | 301 (59.84) | 260 (86.38) | 41 (13.62) | 0.0238 |
| | <i>Yes</i> | 202 (40.16) | 169 (83.66) | 33 (16.34) | | 202 (40.16) | 159 (78.71) | 43 (21.29) | |

Table S4: Relationship between NDUFS6 and MRPS12 transcripts levels and classical clinical biological parameters in a series of 503 breast cancer. NS: not significant; ^a Chi-squared Test (one-sided); ^b Scarff Bloom Richardson classification; ^c Information available for 490 patients; ^d Information available for 499 patients; ^e Information available for 493 patients; ^f Information available for 500 patients; ^h Information available for 502 patients; ⁱ Information available for 428 patients; ^j Mann Whitney Test (two-tailed).

Table S5: sequences of primers used in the RT-PCR analysis of OXPHOS associated genes

| Gene | Upper primer (5' to 3') | Lower primer (5' to 3') |
|------------------|---------------------------|----------------------------|
| PDCD8 (AIFM1) | CTCTAACATCTGGGTGGCAGGA | ACTCACAACAGCGTGATCATGG |
| NDUFV1 | CCCGCAGACGTGGGAGTGTT | TACCTCCACGGCGGCAGATT |
| NDUFAB1 | GTTTGGACCAAGTGGAGATTATCAT | TGTGGACACATTAACCTTTTCAGCAT |
| NDUFA7 | GCTGCAGCTACGCTACCAGGAGAT | ATTGTTGGAGAGCTTGTGGCTAGGA |
| NDUFS6 | CACACTGGCCAGGTTTATGATGAT | TGGCAAAGTTTTTCATTCACCTCTT |
| MRPS12 | CCCTAACTTGTGGCCCAGCTCT | CAGGCGGTGCATCTGGTTCA |
| TBP | TGCACAGGAGCCAAGAGTGAA | CACATCACAGCTCCCCACCA |