SUPPLEMENTAL INFORMATION

Aggressive Triple Negative Breast Cancers have unique molecular signature on the basis of mitochondrial genetic and functional defects.

Manti Guha[1*] Satish Srinivasan[1], Pichai Raman[2], Yuefu Jiang[3], Brett A. Kaufman[3], Deanne Taylor[2], Dawei Dong[1], Rumela Chakrabarti[1], Martin Picard[6], Russ P. Carstens[5], Yuko Kijima[4], Mike Feldman[5], Narayan G Avadhani[1]

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1: TCGA analysis showing mtDNA content compared with the age of diagnosis.

Supplemental Figure S2: mtDNA content estimation in human tumors. (A-C) Relative mtDNA content between matched non tumor and tumor tissues in the Luminal A, Luminal B and HER2+ tissues as indicated in the figure. N= 22 paired. *p* values are indicated in the figure.

Supplemental Figure S3: Relative abundance of TFAM in in breast cancer cell lines. Box Plot showing the distribution of the relative *Tfam* mRNA levels in non-TNBC (T47D, MCF7 and SUM52PE) and TNBC basal cell lines (Hs578T, SUM1315, MDA-MB231 and HCC1806). 20ng of cDNA was used per reaction for real time PCR amplification using either *Tfam* or endogeneous gene *RPL13A*. The line inside each box indicates the mean.

Supplemental Figure S4: Relative abundance of mtDNA in primary human breast tumors.

Isolated total DNA from TNBC (n=6) and non TNBC (Luminal and HER2+, n= 28) were analyzed by multiplex qPCR for all pairings of ND1, ND4, and RNR1 primer-probe sets using optimized conditions. **(A)** Percent imbalance of ND1 and ND4 probes. Values above upper dashed lines indicate a loss of ND1 and gain of ND4 content. Values below the lower dashed lines indicate the inverse. **(B)** Percent imbalance of ND1 and RNR1 probes. Values above upper dashed lines indicate a loss of ND1 and gain of RNR1 content. Values below the lower dashed lines indicate the inverse.

Supplemental Figure S5: OXPHOS modulation among the breast tumor subtypes. **(A-B)** Box Plot representation of real time PCR analysis showing the relative mRNA levels of genes encoding mitochondrial proteins, SLC25A25 and UQCR11 in TNBC and non-TNBC cells. The line inside each box indicates the mean. **(C)** Real time PCR showing alterations in mitochondrial protein encoding gene UQCR11 in tumor tissues. **(D)** Box Plot representation of real time PCR analysis showing the relative distribution of Hexokinase 2 gene in TNBC and non-TNBC cells. The line inside each box indicates the mean. **(E)** Real time PCR showing alterations in hexokinase 2 gene expression in tumor tissues.

Supplemental Figure S6: TCGA analysis of ESRP1 and mtDNA levels among the tumor subtypes. **(A)** Box plot showing the distribution of the relative levels of ESRP1 in non-TNBC and TNBC cells analyzed by real time PCR using ESRP1 specific Taqman primer-probe pairs. 18S Taqman primer-probe pair was used as an internal control for

normalization. The line inside each box indicates the mean. (**B)** Distribution pattern of ESRP1 (FPKM) colored by PAM50 subtype (n=168). ANOVA *P*-value = 7.4e-06

(C) ESRP1 (FPKM) levels versus mtDNA levels in TNBC tumors. Red box indicates tumors with low mtDNA contents as well as low ESRP1 expression. ANOVA *P* value = 0.001113

Supplemental Figure S7: **TCGA analysis of tumors with BRCA1 defects correlated with mtDNA content. (A)** Waterfall plot showing distribution of mtDNA levels in 372 samples sorted by value and colored by presence (red) or absence (gray) of a BRCA1 mutation. BRCA1 mutation was reported in 10 of 372 samples used for analysis in this figure. **(B)** Distribution of BRCA1 mutations compared with mtDNA content represented as quartiles. Quartile 1 has the lowest mtDNA and Quartile 4 has the highest pathogenic BRCA1 mutations are typed in red. (n=10). **(C)** Table with actual BRCA1 mutations per sample.