miR-500a-5p regulates oxidative stress response genes in breast cancer and predicts cancer survival

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Supplementary Figures

Figure S1. miR-500a-5p targets in two breast cancer cell lines.

Mir-500a-5p expression was modulated in MCF-7 and T47D cells by transfection of mimic and inhibitor. **a.** multidimensional scaling (MDS) of the normalized gene expression data was performed separately for MCF-7 (left panel) and T47D cells (right panel). Compare with the combined MDS plot shown in Figure 2b. **b.** 413 differentially expressed genes (DEGs) were obtained by comparing mimics vs. inhibitor in a linear regression model, and used for unsupervised clustering (left panel) and MDS plot (right panel). **c.** The same panel of 413 probes was used again for MDS plots separately for each cell line.

Figure S2. Target prediction with different algorithms.

a. Similar to Fig 2f, we searched for the overlap between our list of experimental targets ("Targets") and the targets predicted by miRDB ("miRDB"). In addition, we used the miRecords web tool which combines several target prediction algorithms [32]. For miR500a-5p we found a highly variable number of targets: RNAhybrid = 32724, PITA = 7251, miRanda = 3836, and miRtarget2 = 526. Of these, only 306 are common (corresponding to 236 annotated genes). The Venn diagram shows the overlap between these 236 genes ("miRecords") with "miRDB" and our identified "Targets". The overlap between our target list and miRecords is highly significant (7.6 times more than expected by chance, hypergeometric test p value < 1.095x10-19) using a conservative estimate of annotated human genes (20K). **b.** we tested for miRNA enrichment in our gene expression dataset, using the webtool MiRonTOP [13]. The resulting plots compare all annotated human miRNAs and their relationship with the full expression dataset of our in vitro experiment. The x-axis represents the enrichment (fold change) in putative targets for a given miRNA relative to the DEGs list, while the y-axis indicates the significance of that relationship. Negative relationships (gene downregulation after miRNA overexpression) are shown in green, while positive relationships are shown in red. For this particular analysis, we used the complementarity of the seed sequence to define a gene as a miRNA target, either in the UTRs (left panel) or the coding sequence (right panel). The position of miR500a-5p is indicated in both panels.

Figure S3. Functional pathway analyses.

We used the EnrichR [28,29] **(a)** and DAVID [30] **(b)** pathway/ontology tools to infer the functional role of the 369 identified targets of miR500a-5p (see Methods). Only the top categories are shown, ranked by their statistical significance.

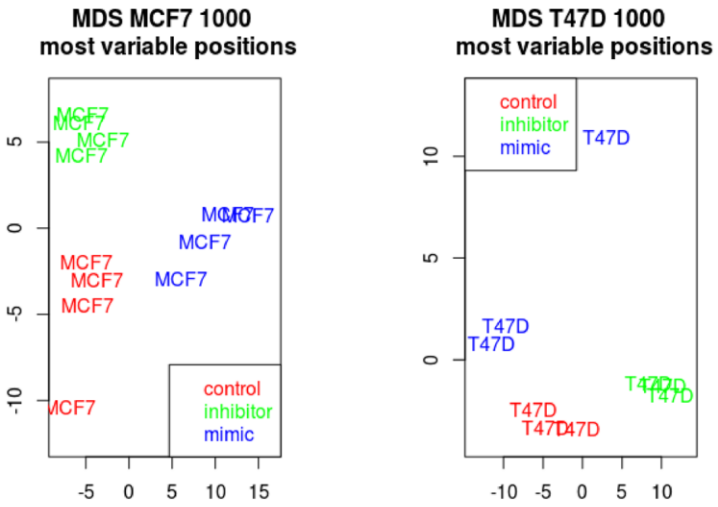
Figure S4. Survival analysis with identified miR-500a-5p targets (METABRIC dataset).

The breast cancer dataset METABRIC was used to test for survival prediction capacity of miR-500a-5p oxidative stress targets (Table 1) in ER+ **(a)** ER- **(b)**, and ER-/HER2- **(c)** breast cancer samples. Cox regression model was used for each gene to predict relapse-free survival. The three most significant associations (lowest p values) are shown. Samples are divided into Low (black) and High (red) expression groups for each gene. Hazard ratio (HR) and P value for each association are shown within each plot.

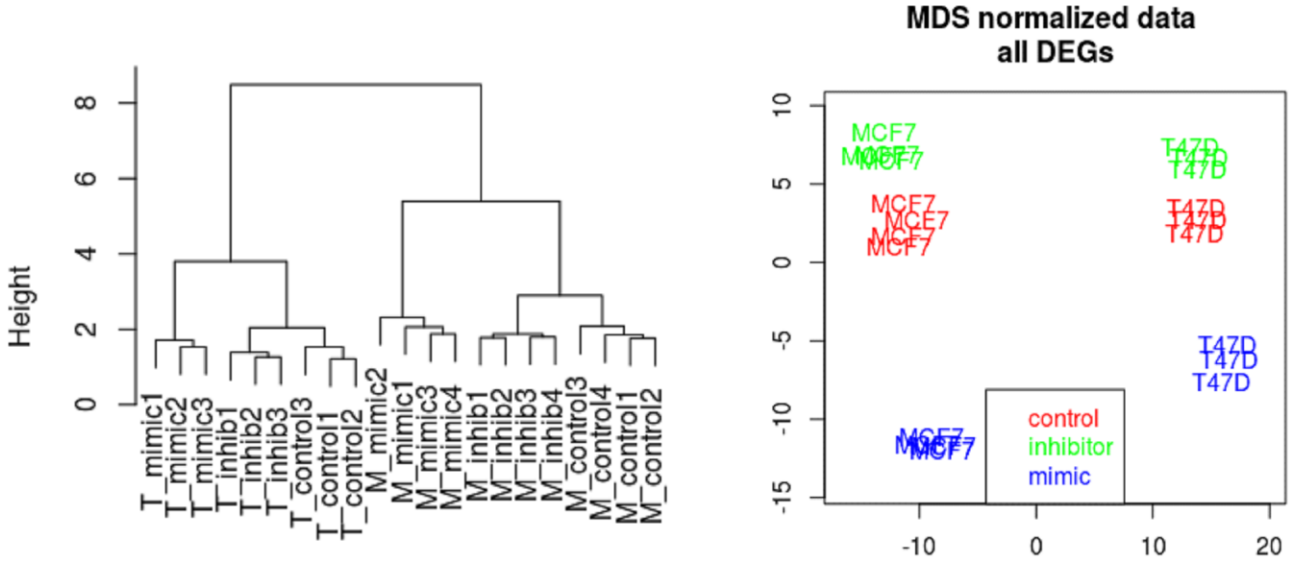
Figure S5. Survival analysis with miR-500a-5p targets (KM Plotter dataset).

The breast cancer dataset from Kaplan Meier Plotter (Gyorffy B. et al) was used to test for survival prediction capacity of miR-500a-5p oxidative stress targets (Table 1) in ER+ **(a)** ER- **(b)**, and ER-/HER2- **(c)** breast cancer samples. Cox regression model was used for each gene to predict relapse-free survival. Samples are divided into Low (black) and High (red) expression groups for each gene. Hazard ratio (HR) and P value for each association are shown within each plot.

a



b



c

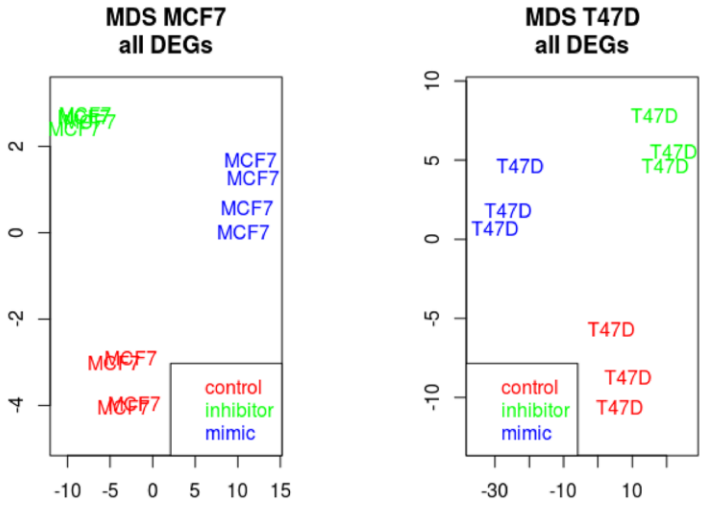
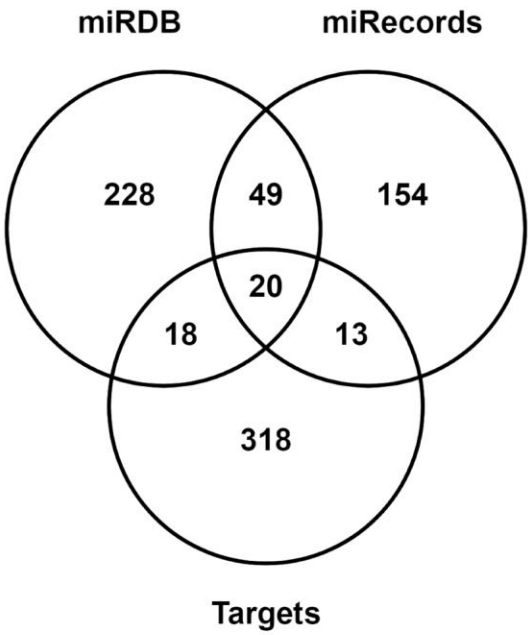


Figure S1

a


miRDB
miRecords
228
49
154
20
18
13
318
Targets

b

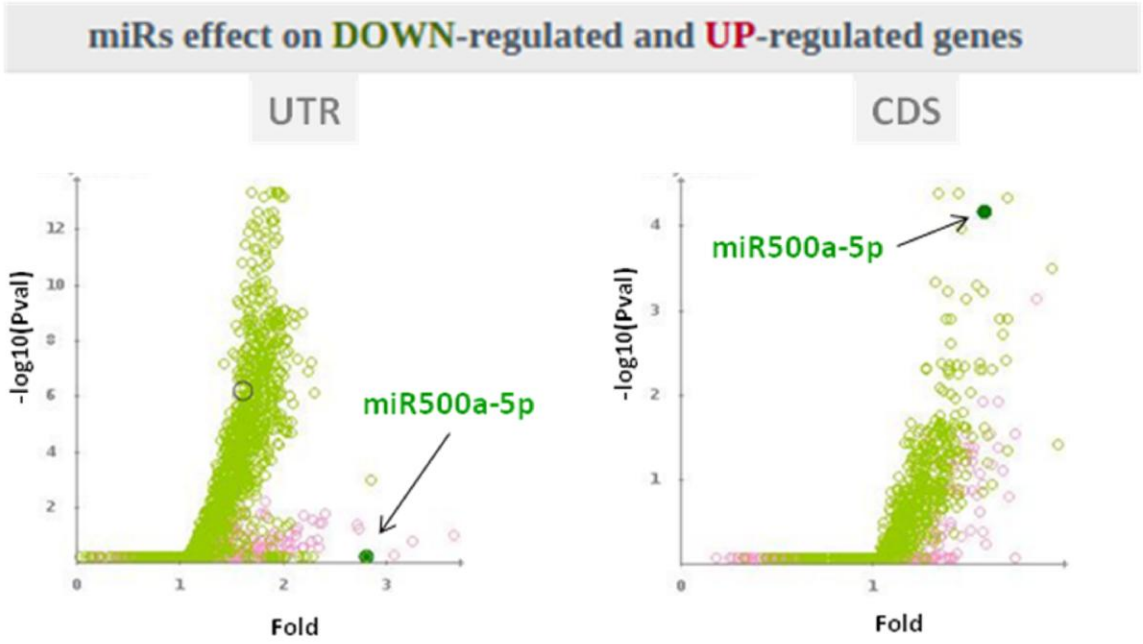
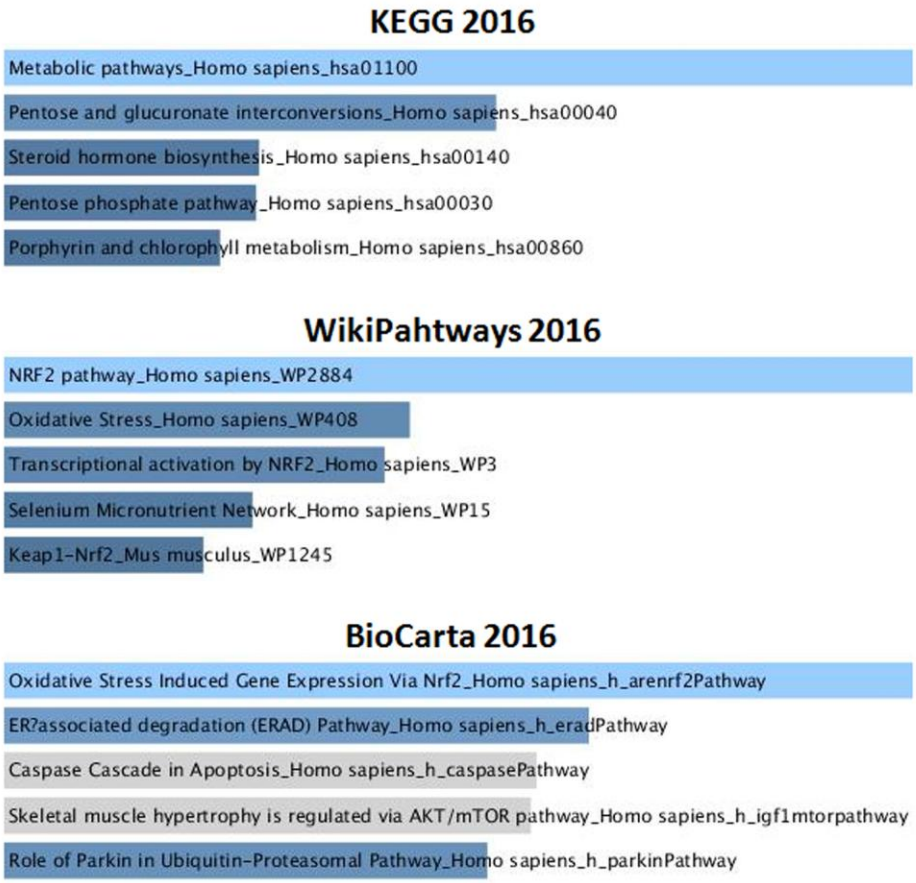

miRs effect on DOWN-regulated and UP-regulated genes
UTR
CDS
-log10(Pval)
miR500a-5p
Fold
-log10(Pval)
miR500a-5p
Fold

Figure S2

a



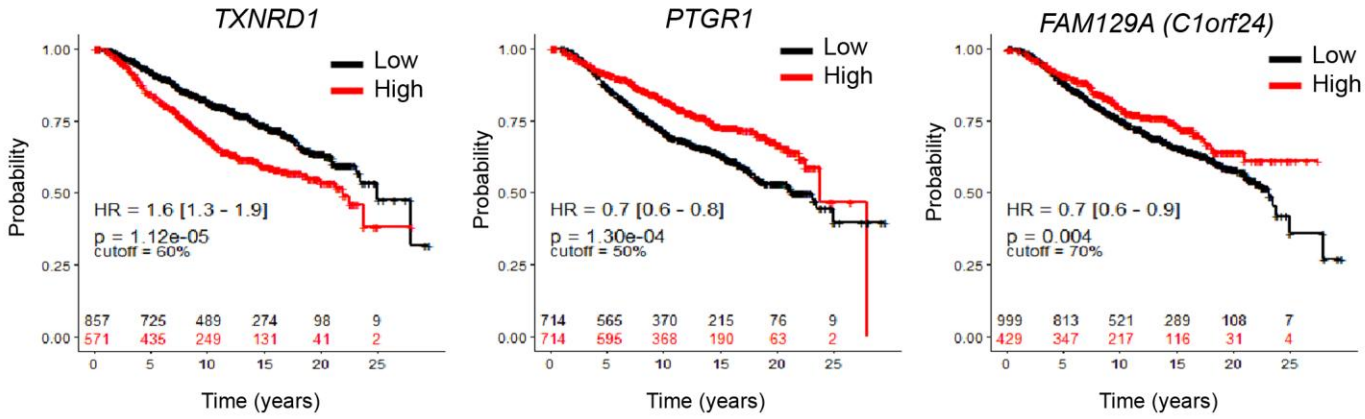
b

Functional Annotation Clustering

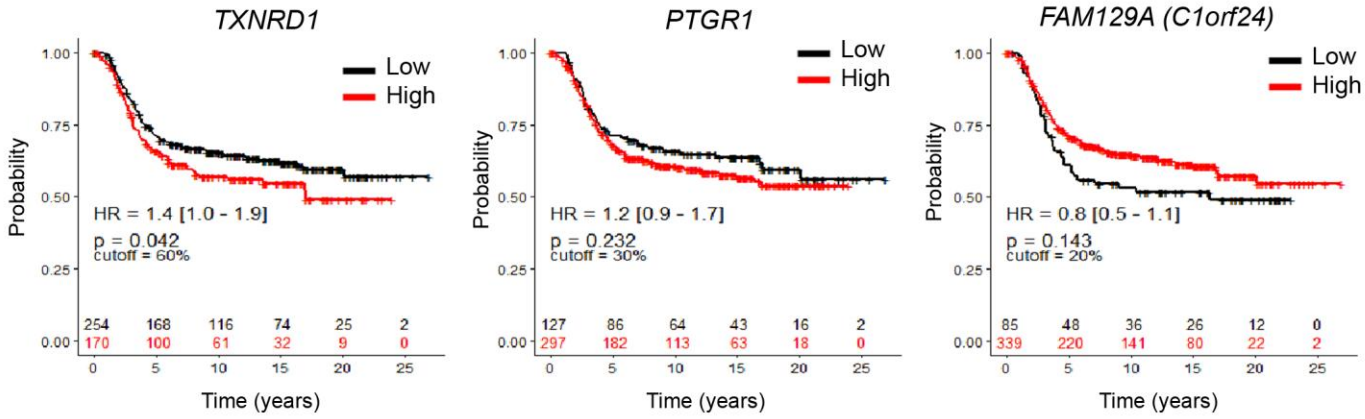
Enrichment Score: 7.02	G		Count	P_Value	Benjamini
oxidation-reduction process	RT		36	7.5E-12	1.1E-8
Oxidoreductase	RT		30	1.8E-9	2.6E-7
NADP	RT		17	5.4E-9	5.3E-7
nucleotide phosphate-binding region:NADP	RT		12	5.7E-9	4.5E-6
binding site:NADP	RT		7	2.4E-6	9.5E-4
oxidoreductase activity	RT		13	6.5E-5	3.2E-2
binding site:Substrate	RT		13	1.1E-3	1.9E-1

Figure S3

a



b



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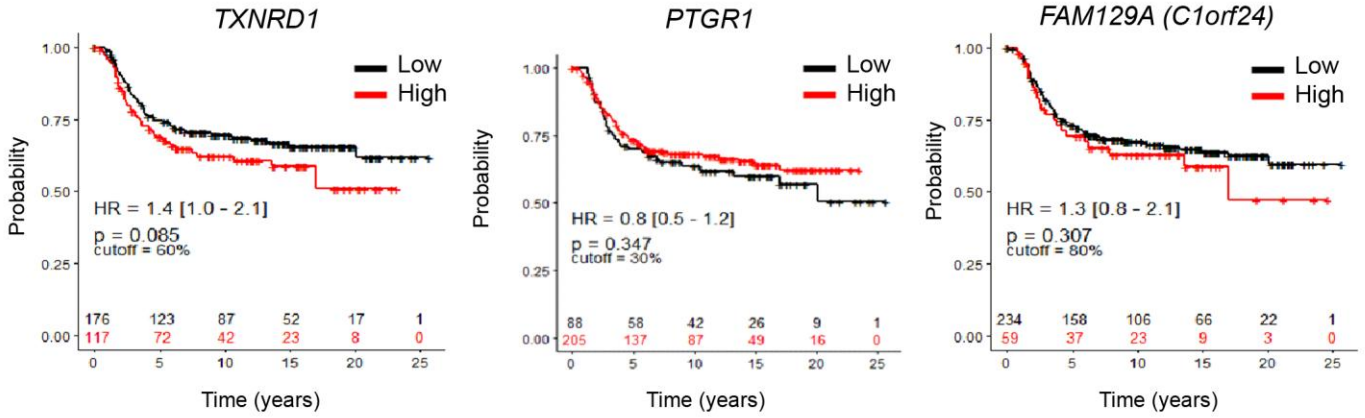
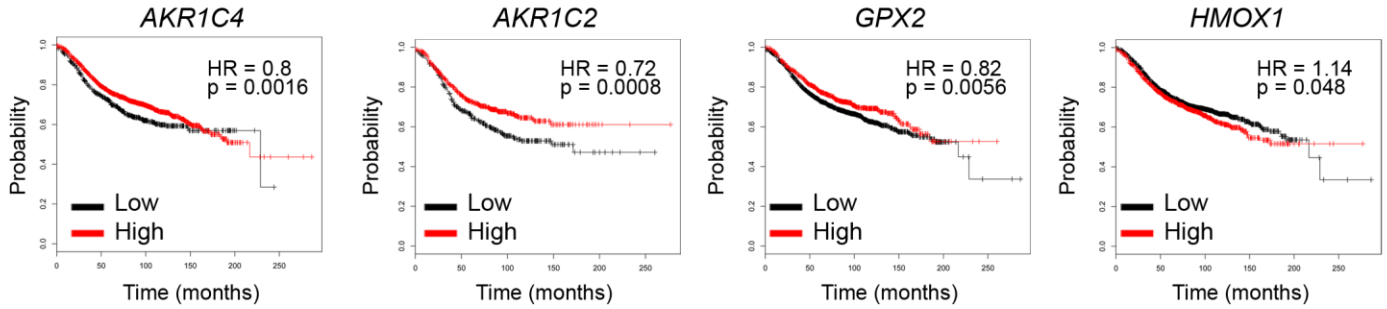


Figure S4

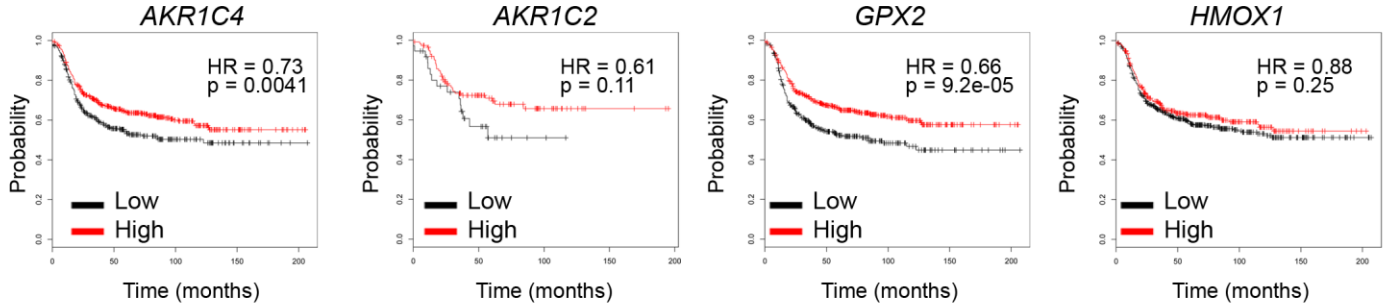
a

ER Positive - Relapse-free survival



b

ER Negative - Relapse-free survival



c

ER Negative / HER2 negative - Relapse-free survival

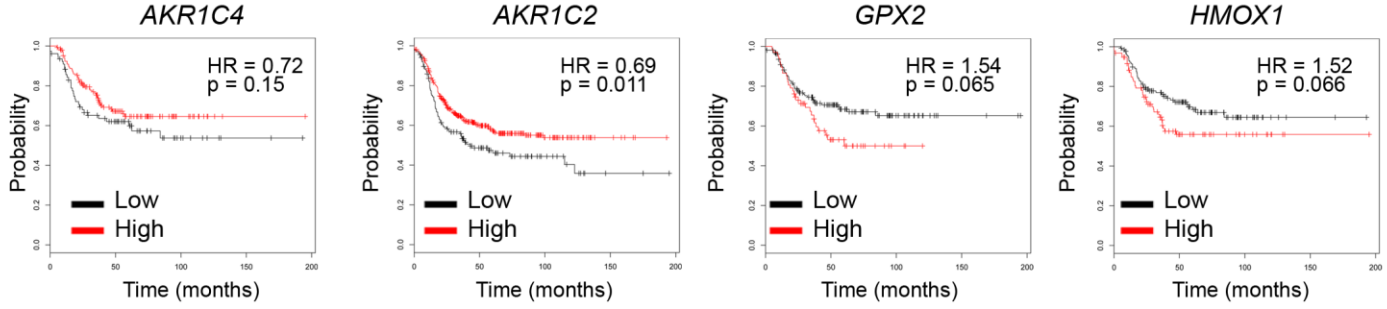


Figure S5