**Fig.1-Workflow-EMT-Cell-Models**

Table

Description automatically generated with low confidence

***Figure 1.* Study workflow and a summary of the three EMT breast cell models.** **(A)** Workflow of the proteomic analysis of the three EMT breast cell models and metabolomic analysis after siRNA knockdown of the metabolic target, UGDH, in all the mesenchymal cell lines. Three EMT breast cell models (epithelial and mesenchymal pairs), namely, D492&D492M, HMLE&HMLEM, and PMC42LA&PMC42ET, were used in this study. The proteomic strategy was label-free quantification (LFQ) with each cell type in triplicates. The metabolomic strategy was targeted metabolomics in negative, positive, and basic modes. The upstream signaling regulation and downstream cellular functions of UGDH were also investigated in this study. **(B)** A comparison of the three EMT breast cell models.

**Locations:**

**Data used:**

**Fig.1.A (Workflow)**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Workflow”*

**Figure generated:**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Workflow”*

**Fig.1.B (EMT cell models)**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\9 ResearchLiteratureLibrary\_QIONG\Cell models for studying Breast cancer”*

D492:

Isolation, immortalization, and characterization of a human breast epithelial cell line with stem cell properties.

D492M:

Endothelial Induced EMT in Breast Epithelial Cells with Stem Cell Properties

ECM1 secreted by HER2-overexpressing breast cancer cells promotes formation of a vascular niche accelerating cancer cell migration and invasion. (non-tumorigenic)

HMLE:

ISOLATION AND GROWTH OF HUMAN MAMMARY EPITHELIAL CELLS (Stampfer 1985)

Human breast cancer cells generated by oncogenic transformation of primary mammary epithelial cells.

HMLEM:

Protein Kinase C α Is a Central Signaling Node and Therapeutic Target for Breast Cancer Stem Cells.

PMC42ET:

A New Human Breast Carcinoma Cell Line (PMC42) With Stem Cell Characteristics. I. Morphologic Characterization.

PMC42LA:

PMC42, A Novel Model for the Differentiated Human Breast

**Fig.2-EMT-Markers**

Chart

Description automatically generated

***Figure 2.* EMT markers in the three EMT breast cell models.** **(A-B)** Proteomic analysis of the three EMT models revealed cell lines with the same origin were more similar, and the D492 EMT model was closer to the HMLE model than the PMC42 model. **(C-H)** A list of known EMT markers consistently changed in all EMT models. RNA expression of CDH1 was downregulated **(C)**, while RNA expression of CDH2 was upregulated after EMT **(D)**. Protein levels of VIM **(E)**, LGALS1 **(F)**, SERPINE1 **(G)**,and PKP3 **(H)** were consistently altered in all EMT models. Student’s t-test, \*: p < 0.05; \*\*: p < 0.01; \*\*\*: p < 0.001.

**Locations:**

**Data used:**

**Fig.2.A&B**

*“C:/Users/lenovo/OneDrive - Háskóli Íslands/PC-HI/1 NTNU\_LFQ proteomic results/Second/OrgnizedResults”*

*"1 NTNU\_proteomics\_six cell lines\_no gene names\_28.12.2018.xlsx"*

**Fig.2.C&D (CDH1&2)**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH”*

*“RNA-Expression-CDH1-CDH2-UGDH.xlsx”*

*The location of the original data:*

*"C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\20 UGDH-Paper\1-WideType-D492-HMLE-PMC42"*

**Fig.2.E-G**

*"C:/Users/lenovo/OneDrive - Háskóli Íslands/PC-HI/1 NTNU\_LFQ proteomic results/Second/OrgnizedResults"*

"2 NTNU\_Proteomics\_D492 vs. D492M\_28.12.2018.xlsx"

"2 NTNU\_Proteomics\_HMLE vs. HMLE\_M\_28.12.2018.xlsx"

"2 NTNU\_Proteomics\_PMC42\_LA vs. PMC42\_ET\_28.12.2018.xlsx"

**Fig.2.H (PKP3)**

*C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH*

*“NTNU-LFQ-Expression-CTGF-FDFT1-PKP3.xlsx”*

The location of the original data:

*"C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Perseus”*

*“PerseusOutputData-NTNU-ThreeEMT-1.6.14.0-2021.01.11.xlsx"*

**Why did PKP3 and FDFT1 (did not use CTGF) be plotted separately?**

Because when using “BarPlotPlotting\_NTNU\_ThreeEMT\_2021.01.09.R” to plot, it uses these data:

*"C:/Users/lenovo/OneDrive - Háskóli Íslands/PC-HI/1 NTNU\_LFQ proteomic results/Second/OrgnizedResults"*

"2 NTNU\_Proteomics\_D492 vs. D492M\_28.12.2018.xlsx"

"2 NTNU\_Proteomics\_HMLE vs. HMLE\_M\_28.12.2018.xlsx"

"2 NTNU\_Proteomics\_PMC42\_LA vs. PMC42\_ET\_28.12.2018.xlsx"

There are missing values in these data, so I have to use the Perseus output data.

**Figure generated:**

**Fig.2.A&B**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

*PCA:*

“PCAplot\_NTNU\_ThreeEMT\_Normalized\_2021.01.09.R”

*Dendrogram:*

“Dendrogram\_NTNU\_ThreeEMT\_Imputation\_2021.01.09.R”

**Fig.2.C&D (CDH1&2)**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

“BarPlot\_RNA-Expression-CDH1-CDH2-UGDH-2020.01.09.R”

**Fig.2.E-G**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

“BarPlotPlotting\_NTNU\_ThreeEMT\_2021.01.09.R”

**Fig.2.H (PKP3)**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

“BarPlot\_NTNU-LFQ-Expression-CTGF-FDFT1-PKP3-2020.01.13.R”

**All figures:**

*PCAplot: C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-PCA-Plot*

*Dendrogram - “C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH”*

*Barplots - “C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-EMTmarkers\Fig.1”*

**Note:**

1. Used “EMT-Markers-NTNU-ThreeEMT.R” to find the EMT targets. (first analysis)
2. Realized the data in “EMT-Markers-NTNU-ThreeEMT.R” is not good.
3. Used “EMT-Markers-NTNU-ThreeEMT\_V2.R” to find again the EMT targets
4. Did not added the missing EMT markers from the first analysis because they did not pass the FDR threshold in Perseus. (For the consistent markers)
5. Added the missing EMT markers for the inconsistent markers since they have passed the FDR cutoff in Perseus. - EGFR, S100A2, NDRG1

**Even though all bar plots for proteomic data had p values, the “\*” marks were calculated separately.**

**Fig.3-VolcanoPlots-Heatmap**

Diagram, schematic

Description automatically generated

***Figure 3.* Proteomic analysis of the three EMT breast cell models.** **(A)** The percentage of the significantly altered proteins in all EMT models (Permutation-based FDR < 0.05). **(B)** The up or down-regulation profile for all the significantly changed proteins in all EMT models. Based on the proteomic analysis, the log2(epithelial/mesenchymal ratio) along with its p value for each protein was plotted for the D492 model **(C)**, HMLE model **(D)**, and PMC42 model **(E)**. Proteins with FDR (Permutation-based) less than 0.05 and fold change more than 2 were colored. The horizontal dash lines indicated p value at 0.03, and the vertical dash lines showed a fold change at 2-fold. Labeled proteins had log2(fold change) more than 3. Proteins involved in metabolism with log2(fold change) more than 1 for D492 model, 1.5 for HMLE and PMC42 models were bold labeled. **(F)** It listed a list of proteins significantly changed (student’s t-test, p < 0.05) in all three EMT models and with the same direction for the up or down-regulation.

**Locations:**

**Data used:**

**Fig.3.A-B – Summary of the proteomic dataset**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-VolcanoPlot\Fig3AB”*

The data were based on:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Perseus”*

“PerseusOutputData-NTNU-ThreeEMT-1.6.14.0-2021.01.11.xlsx”

**Fig.3.C-E - Volcano**

*"C:/Users/lenovo/OneDrive - Háskóli Íslands/PC-HI/5-2 ProteomicPaper-UGDH/Perseus"*

*"PerseusOutputData-NTNU-ThreeEMT-1.6.14.0-2021.01.10.txt"*

**Fig.3.F - Heatmap**

*“C:/Users/lenovo/OneDrive - Háskóli Íslands/PC-HI/5-2 ProteomicPaper-UGDH/Perseus”*

*"PerseusOutputData-NTNU-Z-Score-1.6.14.0-2021.01.11\_Grouping.txt" – used 13 targets from this file (manually added GANAB (did not use) and FDFT1)*

**How to find the targets in figure 3F:**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

“Targets-MetabolicTargets-ThreeEMT\_2020.01.10.R”

**Output:**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH”*

*“NTNU\_ThreeEMT\_Targets\_2021.01.10.xlsx” – 12 targets*

**Figure generated:**

**Fig.3. A-B – Summary of the proteomic dataset**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-VolcanoPlot\Fig3AB”*

**Fig.3.C-E - Volcano**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

“VolcanoPlot\_LFQ\_NTNU\_ThreeEMT-2020.01.10.R”

**Fig.3.F - Heatmap**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

“Heatmap\_NTNU\_LFQ\_Valid\_Target-2020.01.11.R”

**All figures:**

*VolcanoPlot - “C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-VolcanoPlot”*

*Heatmap - “C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH”*

**Fig.4-GO-Annotation-KEGG-Enrichment**

Chart

Description automatically generated with medium confidence

***Figure 4.* Functional annotation of the GO terms and Reactome pathway analysis for the three EMT models.** **(A-C)** Functional annotation of the GO terms (BP) with DAVID platform (DAVID Bioinformatics Resources 6.8) for D492 model **(A)**, HMLE model **(B)**,and PMC42 model **(C)**. The GO terms were listed according to the -log10 p value in descending order. The numbers of genes in each GO term were also plotted as dots/line plots. **(D-F)** Reactome pathway analysis (Pathway browser version 3.7; Reactome database release: 75) for D492 model **(D)**, HMLE model **(E)**,and PMC42 model **(F)**. Data used for analysis were proteins significantly different in each EMT model (Permutation-based FDR < 0.05). Default settings in the DAVID and Reactome platforms were used. BP: Biological Process.

**Locations:**

**Data used:**

**Fig.4.A-C – DAVID - GO**

DAVID Bioinformatics Resources 6.8

Date: 2021.02.09

Procedures:

1. Go to Perseus Output data:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Perseus\”*

“PerseusOutputData-NTNU-ThreeEMT-1.6.14.0-2021.01.11.xlsx”

2. Find genes with significance "+" for each EMT model.

3. Copy these genes into DAVID for "Functional Annotation Chart" analysis, for GO-BP, GO-CC and GO-MF separately

4. Export the output and copy the output .txt file into an Excel file.

5. Use R file for plotting:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

“BarPlot-DoxPlot-GO-Annotation-2021.02.08.R”

**Fig.4.D-F – Reactome - pathway**

Reactome Version:

Reactome Database Release: 75

Pathway Browser Version: 3.7

Date: 2021.02.09

Procedures:

1. Go to Perseus Output data:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Perseus\”*

“PerseusOutputData-NTNU-ThreeEMT-1.6.14.0-2021.01.11.xlsx”

2. Find genes with significance "+" for each EMT model.

3. Copy these genes into Reactome for pathway enrichment.

4. Export the output .csv file

5. Use R file for plotting:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles\”*

“TreePlot\_Reactome\_Pathway\_Enrichment\_2021.02.09.R”

**Figure generated:**

**Fig.4.A-C – DAVID - GO**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

“BarPlot-DoxPlot-GO-Annotation-2021.02.08.R”

**Fig.4.D-F – Reactome - pathway**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles\”*

“TreePlot\_Reactome\_Pathway\_Enrichment\_2021.02.09.R”

**All figures:**

*GO annotation - C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\DAVID*

Reactome - C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Reactome

**Fig.5-MetabolicTargets**

Chart, bar chart

Description automatically generated

***Figure 5.* Metabolic enzymes consistently changed in all EMT models. (A-D)** Four targets involved in metabolism and identified in the proteomic analysis changed consistently in all EMT models. **(E)** RNA levels of UGDH in all EMT models were consistently higher in mesenchymal cell types. **(F)** UGDH protein levels in epithelial and mesenchymal cells were confirmed in another dataset also confirmed in another tumorigenic mesenchymal cell line. Student’s t-test, \*: p < 0.05; \*\*: p < 0.01; \*\*\*: p < 0.001.

**Locations:**

**Data used:**

**Fig.5.A (FDFT1)**

*C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH*

*“NTNU-LFQ-Expression-CTGF-FDFT1-PKP3.xlsx”*

The location of the original data:

*"C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Perseus”*

*“PerseusOutputData-NTNU-ThreeEMT-1.6.14.0-2021.01.11.xlsx"*

**Fig.5.B-D**

*"C:/Users/lenovo/OneDrive - Háskóli Íslands/PC-HI/1 NTNU\_LFQ proteomic results/Second/OrgnizedResults"*

*"2 NTNU\_Proteomics\_D492 vs. D492M\_28.12.2018.xlsx"*

*"2 NTNU\_Proteomics\_HMLE vs. HMLE\_M\_28.12.2018.xlsx"*

*"2 NTNU\_Proteomics\_PMC42\_LA vs. PMC42\_ET\_28.12.2018.xlsx"*

**Fig.5.E (UGDH RNA)**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH”*

*“RNA-Expression-CDH1-CDH2-UGDH.xlsx”*

*The location of the original data:*

*"C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\20 UGDH-Paper\1-WideType-D492-HMLE-PMC42"*

**Fig.5.F (UGDH D492HER2)**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-MetabolicTargets\pvalues”*

“UGDH\_Dundee\_LFQ\_SILAC.xlsx”

**Figure generated:**

**Fig.5.A (FDFT1)**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

“BarPlot\_NTNU-LFQ-Expression-CTGF-FDFT1-PKP3-2020.01.13.R”

**Fig.5.B-D**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

“BarPlotPlotting\_NTNU\_ThreeEMT\_2021.01.09.R”

**Note:**

“BarPlotPlotting\_NTNU\_ThreeEMT\_2021.02.22\_V3.R” for UGDH plotting since I normalized the expression to mesenchymal cell lines

**Fig.5.E (UGDH RNA)**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

“BarPlot\_RNA-Expression-CDH1-CDH2-UGDH-2020.01.09.R”

**Fig.5.F (UGDH D492HER2)**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5 ProteomicPaper\Figures&Tables in the paper\Figures\SupplementaryFig6”*

**Note:**

*Used “Targets-MetabolicTargets-ThreeEMT\_2020.01.10.R” to find the metabolic targets. Manually added “GANAB” (did not use this one) and “FDFT1”.*

*Output data from “Targets-MetabolicTargets-ThreeEMT\_2020.01.10.R”*

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH”*

“NTNU\_ThreeEMT\_Targets\_2021.01.10.xlsx”

*Manually added “GANAB” (did not use this one) and “FDFT1” since they did not pass the significance in Perseus output data (GANAB: HMLE; FDFT1: PMC42). However, “FDFT1” passed the R calculated p value, so you can see that there is one “\*” for PMC42. Unfortunately, “GANAB” did not pass the R calculated p value.*

**All figures:**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-MetabolicTargets”*

**Note:**

Since no missing values for all the metabolic targets,

“BarPlotPlotting\_NTNU\_ThreeEMT\_2021.01.09.R”

Is the same as

“BarPlotPlotting\_NTNU\_ThreeEMT\_2021.02.17\_V2.R”

**Fig.6-Metabolomics-Targeted-siUGDH**

Diagram

Description automatically generated

***Figure 6.* Metabolomic changes after siRNA knockdown of UGDH in all three EMT models.** **(A-B)** Samples clustered together based on the differences of their metabolome from each EMT model **(A)** and D492 mesenchymal cells were closer to HMLE mesenchymal cells than PMC42 at metabolic level **(B)**. **(C)** The substrate of the reaction catalyzed by UGDH, UDP-Glucose, was increased with siRNA knockdown of UGDH in all cell types. **(D)** The product of the reaction catalyzed by UGDH, UDP-Glucuronate, was decreased with siRNA knockdown of UGDH in all cell types. **(E)** siRNA knockdown of UGDH significantly decreased glycerophosphocholine in all cell types. **(F)** Glycerophosphocholine level was higher in the non-tumorigenic D492M than the epithelial D492, and it was the highest in the tumorigenic mesenchymal D492HER2 **(G)** PPAR signaling was more active in mesenchymal cells with higher expression of UGDH. Student’s t-test, \*: p < 0.05; \*\*: p < 0.01; \*\*\*: p < 0.001.

**Locations:**

**Data used:**

**Fig.6.A - PCA**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\20 UGDH-Paper\3-siUGDH-D492M-HMLEM-PMC42ET\1-Metabolomics-Intracellular-BOX1\PCAplotting\_Data”*

MetabolomicData\_For\_PCA.xlsx

**Fig.6.B - Dendrogram**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\20 UGDH-Paper\3-siUGDH-D492M-HMLEM-PMC42ET\1-Metabolomics-Intracellular-BOX1\PCAplotting\_Data”*

MetabolomicData\_For\_PCA.xlsx

**Fig.6.C-E - metabolites**

*"C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\20 UGDH-Paper\3-siUGDH-D492M-HMLEM-PMC42ET\1-Metabolomics-Intracellular-BOX1”*

**Fig.6.F – GPC in D492, D492M and D492HER2 (wide type)**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\20 UGDH-Paper\3-siUGDH-D492M-HMLEM-PMC42ET\1-Metabolomics-Intracellular-BOX1\EMH-July2019-NEG-GPC”*

Protein amount normalization:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5 ProteomicPaper\Figures&Tables in the paper\Glycan precursor analysis\KDexperimentRepeat\_16.07.2019\QIONG”*

“AcidicNeg\_Knockdown\_Metabolomics\_3Scr\_template.xlsx”

**Fig.6.G - PPAR**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Signaling\Phosphoproteomics”*

pCanonicalPathways\_EM.xlsx

**Figure generated:**

**Fig.6.A - PCA**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\20 UGDH-Paper\3-siUGDH-D492M-HMLEM-PMC42ET\1-Metabolomics-Intracellular-BOX1\Rfiles”*

“PCAplot\_MetabolomicData\_2021.02.04.R”

**Fig.6.B - Dendrogram**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\20 UGDH-Paper\3-siUGDH-D492M-HMLEM-PMC42ET\1-Metabolomics-Intracellular-BOX1\Rfiles”*

“Dendrogram\_MetabolomicData\_2021.02.04.R”

**Fig.6.C-E - metabolites**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\20 UGDH-Paper\3-siUGDH-D492M-HMLEM-PMC42ET\1-Metabolomics-Intracellular-BOX1\Rfiles”*

BarPlot\_Metabolites\_2021.02.04.R

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\20 UGDH-Paper\3-siUGDH-D492M-HMLEM-PMC42ET\1-Metabolomics-Intracellular-BOX1\Figures”*

**Fig.6.F – GPC in D492, D492M and D492HER2 (wide type)**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\20 UGDH-Paper\3-siUGDH-D492M-HMLEM-PMC42ET\1-Metabolomics-Intracellular-BOX1\EMH-July2019-NEG-GPC”*

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Metabolomics\GPC”*

**Fig.6.G - PPAR**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

BarPlot-DoxPlot-IPA-2021.02.05.R

**Note:**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\20 UGDH-Paper\3-siUGDH-D492M-HMLEM-PMC42ET\1-Metabolomics-Intracellular-BOX1”*

**All figures:**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Metabolomics”*

**Fig.7-Functional-Assays**

Diagram

Description automatically generated

***Figure 7.* Functional analysis of UGDH in EMT. (A)** Kaplan-Meier plot of UGDH in basal breast cancer patients (The plot was downloaded from kmplot.com). **(B-C)** Cell proliferation slowed down with siRNA knockdown of UGDH in both non-tumorigenic D492M **(B)** and tumorigenic D492HER2 **(C)** cell types. **(D-E)** Cell invasion decreased with siRNA knockdown of UGDH in both non-tumorigenic D492M **(D)** and tumorigenic D492HER2 **(E)** cell types. **(F-G)** One of the main EMT regulators, SNAI1, was downregulated after siRNA knockdown of UGDH in both non-tumorigenic D492M **(F)** and tumorigenic D492HER2 **(G)** cell types. Student’s t-test, \*: p < 0.05; \*\*: p < 0.01; \*\*\*: p < 0.001.

**Locations:**

**Data used:**

**Fig.7.A (KM-plot:)**

Go to website: https://kmplot.com/analysis/index.php?p=service

input gene UGDH

Auto select best cutoff

intrinsic subtype: basal

**Fig.7.B-C (Proliferation):**

Data used for Growth Curve plotting:

D492 & D492M & D492HER2

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\14 Knockdowns Experiments\Results\9 KD\_GrowthCurve”*

“KD\_GrowthCurve\_EMH\_Plotting\_2020.04.14.xlsx”

**Fig.7.D-E (Invasion\_siRNA1):**

Data of invasion location:

D492M:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\14 Knockdowns Experiments\Results\13 Invasion\Invasion\_UGDH\_2020.06\Invasion\_D492M\_Repeat-2020.07.28”*

D492HER2:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\14 Knockdowns Experiments\Results\13 Invasion\Invasion\_UGDH\_2020.06\Invasion\_D492HER2\_2020.06.05”*

**Fig.7 F-G (SNAI1\_siRNA1):**

Data Location:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\14 Knockdowns Experiments\Results\19 Knockdown-siRNA1&2-UGDH”*

**Figure generated:**

**Fig.7.A (KM-plot:)**

Website: <https://kmplot.com/analysis/index.php?p=service>

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-SurvivalCurve”*

**Fig.7.B-C (Proliferation):**

Rfile:

D492 & D492M

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\14 Knockdowns Experiments\Results\9 KD\_GrowthCurve\Rfiles”*

“KD\_GrowthCurve\_EMH\_24+48+12\_StartsFrom24h.R”

D492HER2

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\14 Knockdowns Experiments\Results\9 KD\_GrowthCurve\Rfiles”*

“KD\_GrowthCurve\_HER2highDensity\_StartsFrom48h.R”

**Fig.7.D-E (Invasion\_siRNA1):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-siUGDH-FunctionalAssay\Invasion”*

**Fig.7 F-G (SNAI1\_siRNA1):**

Figures location:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\14 Knockdowns Experiments\Results\19 Knockdown-siRNA1&2-UGDH\Figures”*

**All figures:**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-siUGDH-FunctionalAssay”*

**Fig.8-Signaling-PDGFRB-D492M**

Diagram, schematic

Description automatically generated

***Figure 8.* PDGFRB regulates UGDH via RELA in D492M.** **(A)** PDGFRB was highly expressed in mesenchymal cells than epithelial cells in the D492 model at the protein level. **(B)** PDGFD protein was highly secreted in mesenchymal cells than epithelial cells in the D492 model. **(C)** Motif enrichment of the phospho-proteome in the D492 EMT model suggested, amongst others, PKC kinase activity was highly enriched. **(D)** Knockdown efficiency of the PDGFRB with siRNA in D492M cell type. **(E)** RELA was downregulated after siRNA knockdown of PDGFRB in D492M. **(F)** UGDH was downregulated after siRNA knockdown of PDGFRB in D492M. **(G)** Knockdown efficiency of the RELA with siRNA in D492M. **(H)** UGDH was downregulated after siRNA knockdown of RELA in D492M. Student’s t-test, \*: p < 0.05; \*\*: p < 0.01; \*\*\*: p < 0.001.

**Locations:**

**Data used:**

**Fig.8.A (RPPA):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\3 RNA seq&RPPA\Second Set”*

“02\_Gunhild\_Maelandsmo\_\_Bylgja\_Himarsdottir\_D\_Qiong\_modified.xlsx”

**Fig.8.B (Secretome):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\1 DundeeProteomicDataSet\_14112017\ProteomicDataAnalysis\_Sophie&Erika\Sophie\Secretome\_D492vsD492her2”*

“Proteomics\_LFQ\_Secretome\_summary\_05.10.2018.xlsx”

**Fig.8.C (Motif enrichment-Phosphoproteomics):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\2 SILIC\_DundeePhosphoproteomicsDataset\Results\Phosphoproteomics\_1\Perseus\DataAnalysis\_Perseus”*

“3 Motifs enrichment\_31.10.2018.xlsx”

**Fig.8.D-F (siPDGFRB-D492M):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\14 Knockdowns Experiments\Results\24 KnockDown-siRNA-PDGFD-PDGFRB”*

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Signaling\PDGFRB-RELA-UGDH”*

“siPDGFRB-siRELA-UGDH.xlsx”

**Fig.8.G-H (siRELA-D492M\_siRNA1):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\14 Knockdowns Experiments\Results\26-KnockDown-siRNA-RELA”*

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Signaling\PDGFRB-RELA-UGDH”*

“siPDGFRB-siRELA-UGDH.xlsx”

**Figure generated:**

**Fig.8.A (RPPA):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Signaling\RPPA”*

**Fig.8.B (Secretome):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Signaling\Secretome”*

**Fig.8.C (Motif enrichment-Phosphoproteomics):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Signaling\Phosphoproteomics\Perseus”*

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

“BarPlot-DoxPlot-Phosphoproteomics-Perseus-Motif-Enrichment-2021.02.17.R”

**Fig.8.D-F (siPDGFRB-D492M):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Signaling\PDGFRB-RELA-UGDH\Figures”*

“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”

“BoxPlot\_Function\_siPDGFRB\_2021.02.07.R”

**Fig.8.G-H (siRELA-D492M\_siRNA1):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Signaling\PDGFRB-RELA-UGDH\Figures”*

“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”

“BoxPlot\_Function\_siPDGFRB\_2021.02.07.R”

**S.Fig.1-Cell-Phenotypes**

A picture containing text, different

Description automatically generated

***Supplementary figure 1.*** Phenotypes of all cell lines in the three EMT breast cell models used in this study.

**Locations:**

**Data used:**

*“Y:\Students\Qiong\IC capture”*

**Figure generated:**

*“Y:\Students\Qiong\IC capture”*

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Cell-Pictures”*

**S.Fig.2-Data-Validation**

![Chart, scatter chart

Description automatically generated]()

***Supplementary figure 2.*** The accuracy and validity of the proteomic analysis in this study were confirmed by comparing the current data for the D492 EMT model to our previous published proteomic data. The correlation between these two datasets was 0.936.

**Locations:**

**Data used:**

Only D492 proteomic data (LFQ NTNU and LFQ Dundee)

LFQ NTNU:

*“C:/Users/lenovo/OneDrive - Háskóli Íslands/PC-HI/5-2 ProteomicPaper-UGDH/Perseus”*

"PerseusOutputData-NTNU-ThreeEMT-1.6.14.0-2021.01.11.xlsx"

LFQ Dundee:

*"F:/DundeeAnalysisResults/All datasets\_organized/Proteo&Phosphoproteo\_QIONG\_2017-2018"*

"Dundee\_LFQ\_valid\_Log2\_imputated\_p.value\_t.stat\_q.value\_mean\_22.11.2018.xlsx"

**Figure generated:**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-ScatterPlot”*

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

“ScatterPlot\_NTNUvsDundee\_D492s\_2021.02.07.R”

**S.Fig.3-EMT-Markers-not-consistent**

Chart

Description automatically generated

***Supplementary figure 3.*** A list of known EMT markers which were inconsistently altered comparing among the three EMT models. Student’s t-test, \*: p < 0.05; \*\*: p < 0.01; \*\*\*: p < 0.001.

**Locations:**

**Data used:**

*"C:/Users/lenovo/OneDrive - Háskóli Íslands/PC-HI/1 NTNU\_LFQ proteomic results/Second/OrgnizedResults"*

"2 NTNU\_Proteomics\_D492 vs. D492M\_28.12.2018.xlsx"

"2 NTNU\_Proteomics\_HMLE vs. HMLE\_M\_28.12.2018.xlsx"

"2 NTNU\_Proteomics\_PMC42\_LA vs. PMC42\_ET\_28.12.2018.xlsx"

**Note:**

**It is better to use:**

"2-1 2018-12-28 17-51 Two.groups.statistics.LFQ.xlsx"

"2-2 2018-12-28 17-51 Two.groups.statistics.LFQ.xlsx"

"2-3 2018-12-28 17-52 Two.groups.statistics.LFQ.xlsx"

**Figure generated:**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-EMTmarkers”*

**Note:**

The EMT markers were found via:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

“EMT-Markers-NTNU-ThreeEMT.R” and

“EMT-Markers-NTNU-ThreeEMT\_V2.R” – (EGFR, S100A2, NDRG1, because they have “NA” in their dataset, so they did not be found in “EMT-Markers-NTNU-ThreeEMT.R”)

**S.Fig.4-GO-Annotation-CC-MF**

Chart, line chart

Description automatically generated

***Supplementary figure 4.*** Functional annotation of the GO terms (CC and MF) on the DAVID (DAVID Bioinformatics Resources 6.8) platform for each EMT model. Data used for analysis were proteins significantly different in each EMT model (Permutation-based FDR < 0.05). Default settings were used for the analysis. The GO terms were listed according to the -log10 p value in descending order. The numbers of genes in each GO term were also plotted as dots/line plots. CC: Cellular Component; MF: Molecular Function.

**Locations:**

**Data used:**

DAVID Bioinformatics Resources 6.8

Date: 2021.02.09

Procedures:

1. Go to Perseus Output data:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Perseus\”*

“PerseusOutputData-NTNU-ThreeEMT-1.6.14.0-2021.01.11.xlsx”

2. Find genes with significance "+" for each EMT model.

3. Copy these genes into DAVID for "Functional Annotation Chart" analysis, for GO-BP, GO-CC and GO-MF separately

4. Export the output and copy the output .txt file into an Excel file.

5. Use R file for plotting:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

“BarPlot-DoxPlot-GO-Annotation-2021.02.08.R”

**Figure generated:**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

“BarPlot-DoxPlot-GO-Annotation-2021.02.08.R”

**S.Fig.5-UGDH-KD-Efficiency**

Diagram, schematic

Description automatically generated

***Supplementary figure 5.*** **Knockdown efficiency of UGDH with two siRNAs.** **(A)** Knockdown efficiency of UGDH with two siRNAs compared to the scramble control in the metabolomics experiments with D492M, HMELM, and PMC42ET. KD: Knock Down. **(B-E)** Knockdown efficiency of UGDH with two siRNAs in D492M **(B-C)** and D492HER2 **(D-E)**. Student’s t-test, \*: p < 0.05; \*\*: p < 0.01; \*\*\*: p < 0.001.

**Locations:**

**Data used:**

**S.Fig.5A – KD efficiency for Metabolomics**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\20 UGDH-Paper\1-WideType-D492-HMLE-PMC42”*

“D492-D492M-HMLE-HMLEM-PMC42LA-PMC42ET\_16.11.2020-CDH1-CDH2-UGDH.xlsx”

**S.Fig.5B-E – KD efficiency for D492HER2**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\14 Knockdowns Experiments\Results\19 Knockdown-siRNA1&2-UGDH”*

**Figure generated:**

**S.Fig.5A – KD efficiency for Metabolomics**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-siUGDH-FunctionalAssay\GeneExpressions\CYP1B1&SNAI1”*

The location of the original figures:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\14 Knockdowns Experiments\Results\19 Knockdown-siRNA1&2-UGDH\Figures”*

**S.Fig.5B-E – KD efficiency for D492HER2**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

“BarPlot\_KDefficiency-UGDH-Mesenchymal-Metabolomics\_2020.01.11.R”

**All figures:**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-UGDH-KD-Efficiency”*

**S.Fig.6-** **Functional-Assays**

Diagram

Description automatically generated

***Supplementary figure 6.* Functional analysis of UGDH in EMT. (A-C)** Kaplan-Meier plot of FDFT1, SORD, and TSTA3 in basal breast cancer patients (The plot was downloaded from kmplot.com). **(D)** Glycerophosphocholine level was decreased after siUGDH treatment in tumorigenic D492HER2. **(E-F)** Cell invasion decreased with the second siRNA knockdown of UGDH in both non-tumorigenic D492M **(E)** and tumorigenic D492HER2 **(F)** cell types. **(G-H)** One of the main EMT regulators, SNAI1, was downregulated after the second siRNA knockdown of UGDH in both non-tumorigenic D492M **(G)** and tumorigenic D492HER2 **(H)** cell types. Student’s t-test, \*: p < 0.05; \*\*: p < 0.01; \*\*\*: p < 0.001.

**Locations:**

**Data used:**

**S.Fig.6.A-C – Kaplan-Meier plots for FDFT1, SORD and TSTA3**

From <https://kmplot.com/analysis/index.php?p=service>

**S.Fig.6.D – GPC in D492HER2 (siUGDH)**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\20 UGDH-Paper\3-siUGDH-D492M-HMLEM-PMC42ET\1-Metabolomics-Intracellular-BOX1\EMH-July2019-NEG-GPC”*

Protein amount normalization:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5 ProteomicPaper\Figures&Tables in the paper\Glycan precursor analysis\KDexperimentRepeat\_16.07.2019\QIONG”*

“AcidicNeg\_Knockdown\_Metabolomics\_3Scr\_template.xlsx”

**S.Fig.6E-F (Invasion\_siRNA2):**

Data of invasion location:

D492M:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\14 Knockdowns Experiments\Results\13 Invasion\Invasion\_UGDH\_2020.06\Invasion\_D492M\_Repeat-2020.07.28”*

D492HER2:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\14 Knockdowns Experiments\Results\13 Invasion\Invasion\_UGDH\_2020.06\Invasion\_D492HER2\_2020.06.05”*

**S.Fig.6G-H (SNAI1\_siRNA2):**

Data Location:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\14 Knockdowns Experiments\Results\19 Knockdown-siRNA1&2-UGDH”*

**S.Fig.6F (Migration) – did not use.**

Migration:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\14 Knockdowns Experiments\Results\12 Migration\”*

**Figure generated:**

**S.Fig.6.A-C – Kaplan-Meier plots for FDFT1, SORD and TSTA3**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-SurvivalCurve”*

**S.Fig.6.D – GPC in D492HER2 (siUGDH)**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\20 UGDH-Paper\3-siUGDH-D492M-HMLEM-PMC42ET\1-Metabolomics-Intracellular-BOX1\EMH-July2019-NEG-GPC”*

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Metabolomics\GPC”*

**S.Fig.6E-F (Invasion\_siRNA2):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-siUGDH-FunctionalAssay\Invasion”*

**S.Fig.6G-H (SNAI1\_siRNA2):**

Figures location:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\14 Knockdowns Experiments\Results\19 Knockdown-siRNA1&2-UGDH\Figures”*

**S.Fig.6F (Migration) – did not use.**

Migration:

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-siUGDH-FunctionalAssay\Migration”*

**S.Fig.7-Signaling-PDGFRB**

Diagram, schematic

Description automatically generated

***Supplementary Figure 7.* PDGFRB regulates UGDH via RELA.** **(A)** PDGFRB was highly expressed in the tumorigenic mesenchymal cell line, D492HER2, based on the RPPA analysis. **(B)** Knockdown efficiency of the PDGFRB with siRNA in D492HER2 cell type. **(C)** RELA was downregulated after siRNA knockdown of PDGFRB in D492HER2. **(D)** UGDH was downregulated after siRNA knockdown of PDGFRB in D492HER2. **(E)** Knockdown efficiency of the RELA with the second siRNA in D492M cell type. **(F)** UGDH was downregulated after siRNA knockdown of RELA in D492M with the second siRNA. **(G)** Knockdown efficiency of the RELA with the first siRNA in D492HER2 cell type. **(H)** No significant change in UGDH was observed after the siRNA knockdown of RELA with the first siRNA in D492HER2. **(I)** Knockdown efficiency of the RELA with the second siRNA in D492HER2 cell type. **(J)** UGDH was downregulated after siRNA knockdown of RELA in D492HER2 with the second siRNA. Student’s t-test, \*: p < 0.05; \*\*: p < 0.01; \*\*\*: p < 0.001.

**Locations:**

**Data used:**

**Deleted S.Fig.7A-B**

**S.Fig.7.A-B (PDGFRB-RNAseq):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\16 HMLE”*

“pnas.1618298114.sd01.xlsx”

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\3 RNA seq&RPPA”*

“zDiff\_PMC42\_ET-EGF\_vs\_LA-EGF\_counts.txt”

**S.Fig.7.A (PDGFRB-RPPA-D492HER2):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\3 RNA seq&RPPA\Second Set”*

“02\_Gunhild\_Maelandsmo\_\_Bylgja\_Himarsdottir\_D\_Qiong\_modified.xlsx”

**S.Fig.7.B-D (siPDGFRB-D492HER2):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\14 Knockdowns Experiments\Results\24 KnockDown-siRNA-PDGFD-PDGFRB”*

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Signaling\PDGFRB-RELA-UGDH”*

“siPDGFRB-siRELA-UGDH.xlsx”

**S.Fig.7.E-F (siRELA-D492M\_siRNA2):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\14 Knockdowns Experiments\Results\26-KnockDown-siRNA-RELA”*

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Signaling\PDGFRB-RELA-UGDH”*

“siPDGFRB-siRELA-UGDH.xlsx”

**S.Fig.7.G-J (siRELA-D492HER2\_siRNA1&2):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\4 Experiments documents\_QIONG\14 Knockdowns Experiments\Results\26-KnockDown-siRNA-RELA”*

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Signaling\PDGFRB-RELA-UGDH”*

“siPDGFRB-siRELA-UGDH.xlsx”

**Figure generated:**

**Deleted S.Fig.7A-B**

**S.Fig.7.A-B (PDGFRB-RNAseq):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Signaling\RNAseq\_HMLEM\_PMC42ET”*

**S.Fig.7.A (PDGFRB-RPPA-D492HER2):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Signaling\RPPA”*

**S.Fig.7.B-D (siPDGFRB-D492HER2):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Signaling\PDGFRB-RELA-UGDH\Figures”*

“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”

“BoxPlot\_Function\_siPDGFRB\_2021.02.07.R”

**S.Fig.7.E-F (siRELA-D492M\_siRNA2):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Signaling\PDGFRB-RELA-UGDH\Figures”*

“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”

“BoxPlot\_Function\_siPDGFRB\_2021.02.07.R”

**S.Fig.7.G-J (siRELA-D492HER2\_siRNA1&2):**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Figures-Signaling\PDGFRB-RELA-UGDH\Figures”*

“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”

“BoxPlot\_Function\_siPDGFRB\_2021.02.07.R”

**Table 1**

***Table 1.*** A List of EMT targets was significantly different (student’s t-test, p < 0.05) in all EMT models. Literature related to each target in terms of EMT was also listed. Changes of these targets in another mesenchymal cell type with tumorigenicity were consistent with the findings in this study.

Table

Description automatically generated

**Locations:**

**Data used:**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Rfiles”*

“Targets-MetabolicTargets-ThreeEMT\_2020.01.10.R”

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH”*

“NTNU\_ThreeEMT\_Targets\_2021.01.10.xlsx” – 12 targets

Manually added two targets: GANAB and FDFT1

**Figure generated:**

*“C:\Users\lenovo\OneDrive - Háskóli Íslands\PC-HI\5-2 ProteomicPaper-UGDH\Table.1”*

**Supplementary Table 1**

***Supplementary Table 1.*** A List of primers used in this study.

*Table

Description automatically generated*

**Supplementary Data 1**

Dataset directly from NTNU

**Supplementary Data 2**

Perseus output dataset

**Supplementary Data 3**

Phosphoproteomic dataset of D492 model