

Supplementary Data

Transcriptomic changes associated with ERBB2 overexpression in colorectal cancer implicates a potential role of Wnt signaling pathway in tumorigenesis

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Supplementary Tables S5-S7

Supplementary Figure Legends S1-S7

Supplementary Figures S1-S7

Supplementary Table S5. Summary of the total activated cellular pathways identified by GSEA in *ERBB2*+ versus *ERBB2*- colorectal cancer (CRC) patients, CRC cell lines HCT116 and HT29, normal colon cell lines CCD33 and CCD841, and breast cancer (BC) patients (p < 0.05).

	C2	C5-BP	C5-MF	C6	C7
CRC Patients: 3 HER2- vs 11 HER2+	9	11	0	5	73
HCT116: 3 Ctrl EV vs 3 HER2+	6	3	0	0	6
HT29: 3 Ctrl EV vs 3 HER2+	21	24	2	0	42
CCD33 3 Ctrl EV vs 3 HER2+	0	0	0	0	0
CCD841 3 Ctrl EV vs 3 HER2+	2	0	4	0	0
BC Patients: 17 HER2+ vs 48 HER2-	908	1379	224	112	2017

Supplementary Table S6: Details of breast cancer datasets analyzed to determine correlation of *ERBB2*-mediated enrichment of pathways in breast and colon cancer patients. *N*, total number of breast cancer patients.

Data Set ID	Population	N	Comparison of Samples	
GSE29431	Caucasian/Spanish	21	13 HER2-	8 HER2+
GSE48391	Asian/Chinese	44	35 HER2-	9 HER2+
		65	48 HER2-	17 HER2+

Supplementary Table S7. The 26 common activated pathways among CRC and BC HER2+ versus HER2- patients obtained from Gene Set Enrichment Analysis. *ES*, enrichment score; *NES*, normalized ES; *NOM*, nominal; *Tag%*, the percentage of gene tags before (for positive ES) of after (for negative ES) the peak in the running enrichment score; *Gene %*, the percentage of genes in the gene list before (for positive ES) of after (for negative ES) the peak in the running enrichment score; *GO*, gene ontology.

Gene Sets	Class	SIZE	ES	NES	NOM p-val	Tag %	Gene %	Signal
GSE16385 UNTREATED VS 12H ROSIGLITAZONE TREATED MACROPHAGE DN	C7	17	0.58757	1.8687	0	0.471	0.127	0.413
GSE25088 WT VS STAT6 KO MACROPHAGE ROSIGLITAZONE AND IL4 STIM UP	C7	20	0.47997	1.6523	0.002299	0.7	0.426	0.405
GSE17721 PAM3CSK4 VS CPG 16H BMDC DN	C7	20	0.45969	1.5947	0.005319	0.5	0.274	0.366
GSE9006 TYPE 1 DIABETES AT DX VS 4MONTH POST DX PBMC DN	C7	25	0.48856	1.6076	0.006438	0.6	0.399	0.364
GSE39022 LN VS SPLEEN DC UP	C7	19	0.53179	1.6277	0.009546	0.632	0.317	0.435
GSE15659 NONSUPPRESSIVE TCELL VS ACTIVATED TREG UP	C7	30	0.45884	1.7067	0.01013	0.533	0.248	0.406
GSE26351 WNT VS BMP PATHWAY STIM HEMATOPOIETIC PROGENITORS UP	C7	27	0.46925	1.579	0.01295	0.63	0.396	0.384
GSE15624 CTRL VS 6H HALOFUGINONE TREATED CD4 TCELL DN	C7	26	0.49551	1.4309	0.01431	0.538	0.304	0.378
GSE7509 UNSTIM VS FCGRIB STIM DC UP	C7	22	0.48209	1.5959	0.02103	0.545	0.28	0.396
GSE37534 UNTREATED VS ROSIGLITAZONE TREATED CD4 TCELL PPARG1 AND FOXP3 TRASDUCED UP	C7	34	0.36357	1.4181	0.02529	0.765	0.548	0.35
GSE10147 IL3 VS IL3 AND CPG STIM PDC DN	C7	18	0.53648	1.5054	0.03178	0.556	0.241	0.424
GSE5542 UNTREATED VS IFNG TREATED EPITHELIAL CELLS 24H UP	C7	24	0.56425	1.5257	0.03272	0.667	0.33	0.451
GSE3982 MAST CELL VS NKCELL DN	C7	25	0.42148	1.4406	0.035	0.56	0.332	0.378
GSE5589 LPS VS LPS AND IL10 STIM MACROPHAGE 45MIN UP	C7	21	0.49249	1.4864	0.03846	0.619	0.337	0.414
GSE5542 UNTREATED VS IFNA AND IFNG TREATED EPITHELIAL CELLS 6H UP	C7	25	0.43579	1.444	0.04065	0.44	0.22	0.346
GSE34156 UNTREATED VS 24H NOD2 AND TLR1 TLR2 LIGAND TREATED MONOCYTE DN	C7	25	0.53741	1.5764	0.04816	0.52	0.18	0.431
GSE45365 CD8A DC VS CD11B DC IFNAR KO MCMV INFECTION UP	C7	17	0.50011	1.4465	0.04861	0.588	0.282	0.425
MYC UP.V1 UP	C6	24	0.48814	1.6908	0	0.667	0.392	0.409
ESC V6.5 UP EARLY.V1 UP	C6	24	0.45211	1.6871	0.04251	0.583	0.382	0.364
KIM RESPONSE TO TSA AND DECITABINE UP	C2	17	0.42292	1.462	0	0.471	0.252	0.354
PASINI SUZ12 TARGETS UP	C2	15	0.42068	1.4733	0.03937	0.467	0.276	0.34
GO RESPONSE TO BMP	C5	19	0.46386	1.671	0.01047	0.316	0.0963	0.287
GO CELLULAR COMPONENT ASSEMBLY INVOLVED IN MORPHOGENESIS	C5	16	0.52385	1.7083	0.01244	0.625	0.311	0.433
GO STEM CELL DIFFERENTIATION	C5	37	0.49366	1.711	0.01877	0.459	0.268	0.341
GO REGULATION OF STEM CELL DIFFERENTIATION	C5	21	0.57195	1.7104	0.03081	0.524	0.268	0.386
GO NEGATIVE REGULATION OF WNT SIGNALING PATHWAY	C5	29	0.38574	1.3922	0.04762	0.276	0.136	0.241

Supplementary Figure Legends

Supplementary Figure S1. Schematic representation of the process used to select the HER2- and HER2+ breast cancer patient datasets.

Supplementary Figure S2. (A-D) Principal component analysis (PCA) was performed to determine batch effects among triplicate samples of CCD841 (A), CCD33 (B), HCT116 (C), and HT29 (D).

Supplementary Figure S3. (A-D) Volcano plot of differentially expressed genes between control (empty vector) and *ERBB2* overexpressing CCD841 (A), CCD33 (B), HCT116 (C), and HT29 (D) cells from input RNA-seq. Genes that are significantly upregulated and downregulated based on log2 fold change, respectively) in cells overexpressing *ERBB2* are demarcated by green and blue circles, respectively versus those that did not show a significant change in expression as grey circles ($p > 0.05$).

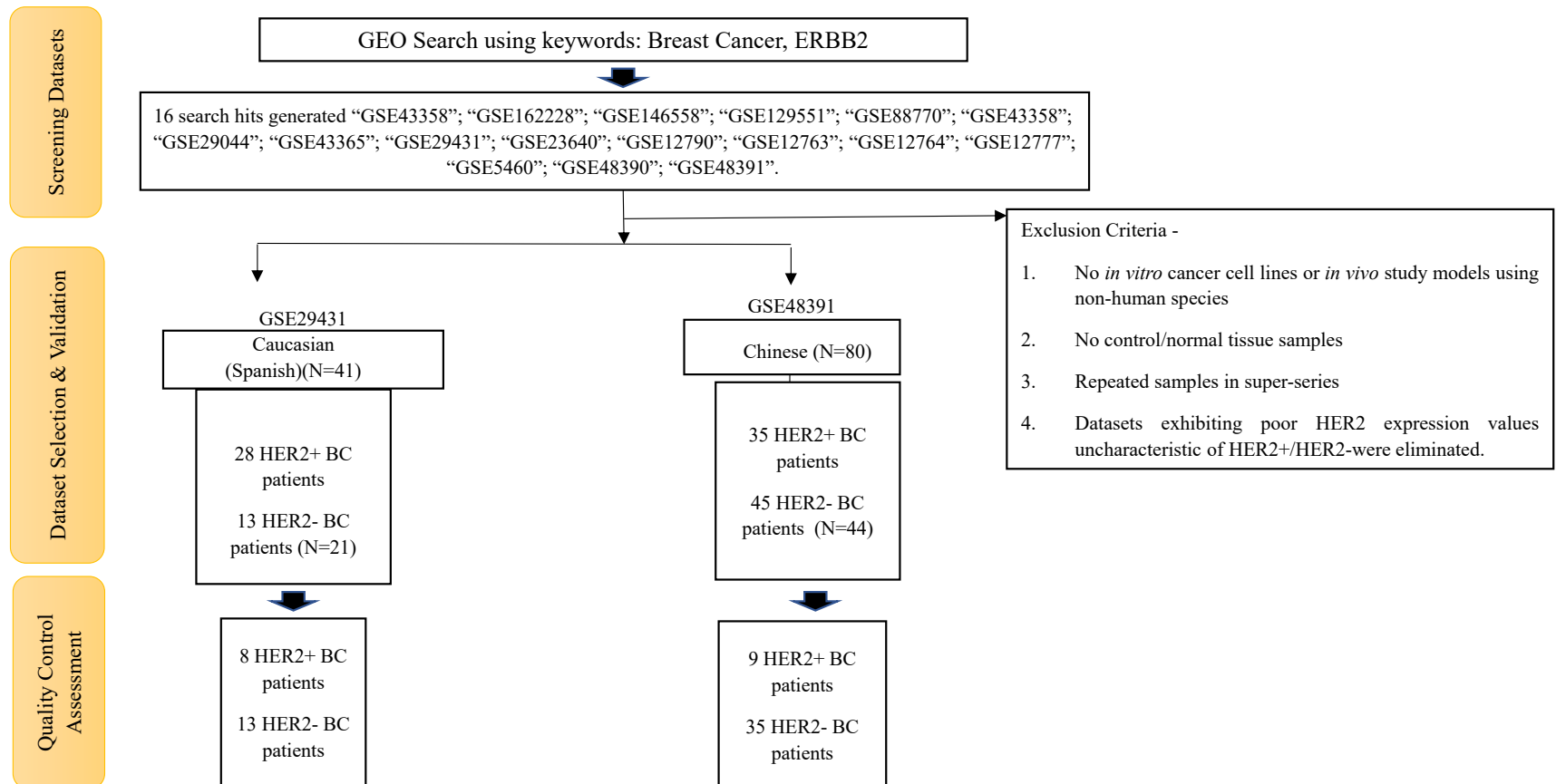
Supplementary Figure S4. Heat map of the differentially expressed genes between control (empty vector) and *ERBB2* overexpressing normal colon cell lines CCD841 (A) and CCD33 (B).

Supplementary Figure S5. Heat map of the differentially expressed genes between control (empty vector) and *ERBB2* overexpressing CRC cell line HCT116. Shown are all the differentially expressed genes (A) and the top 100 differentially expressed genes (B).

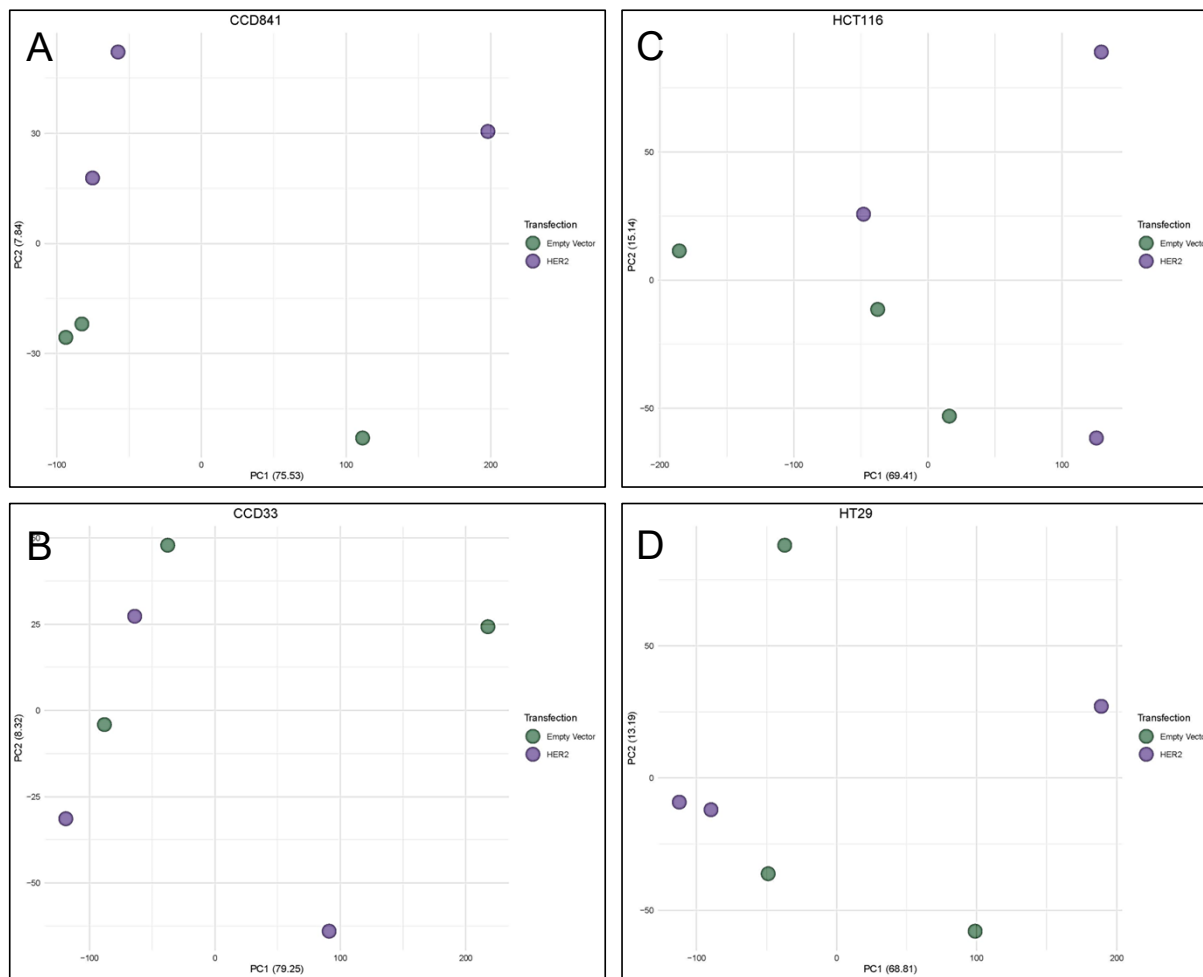
Supplementary Figure S6. Heat map of the differentially expressed genes between control (empty vector) and *ERBB2* overexpressing CRC cell line HCT116. Shown are all the differentially expressed genes (**A**) and the top 100 differentially expressed genes (**B**).

Supplementary Figure S7. (**A**) Heat map of the differentially expressed genes between *ERBB2* negative and positive CRC patients. (**B**) Relative *ERBB2* expressions in the 3 *ERBB2*- and 11 *ERBB2*+ patient samples as determined by whole transcriptome RNA-seq.

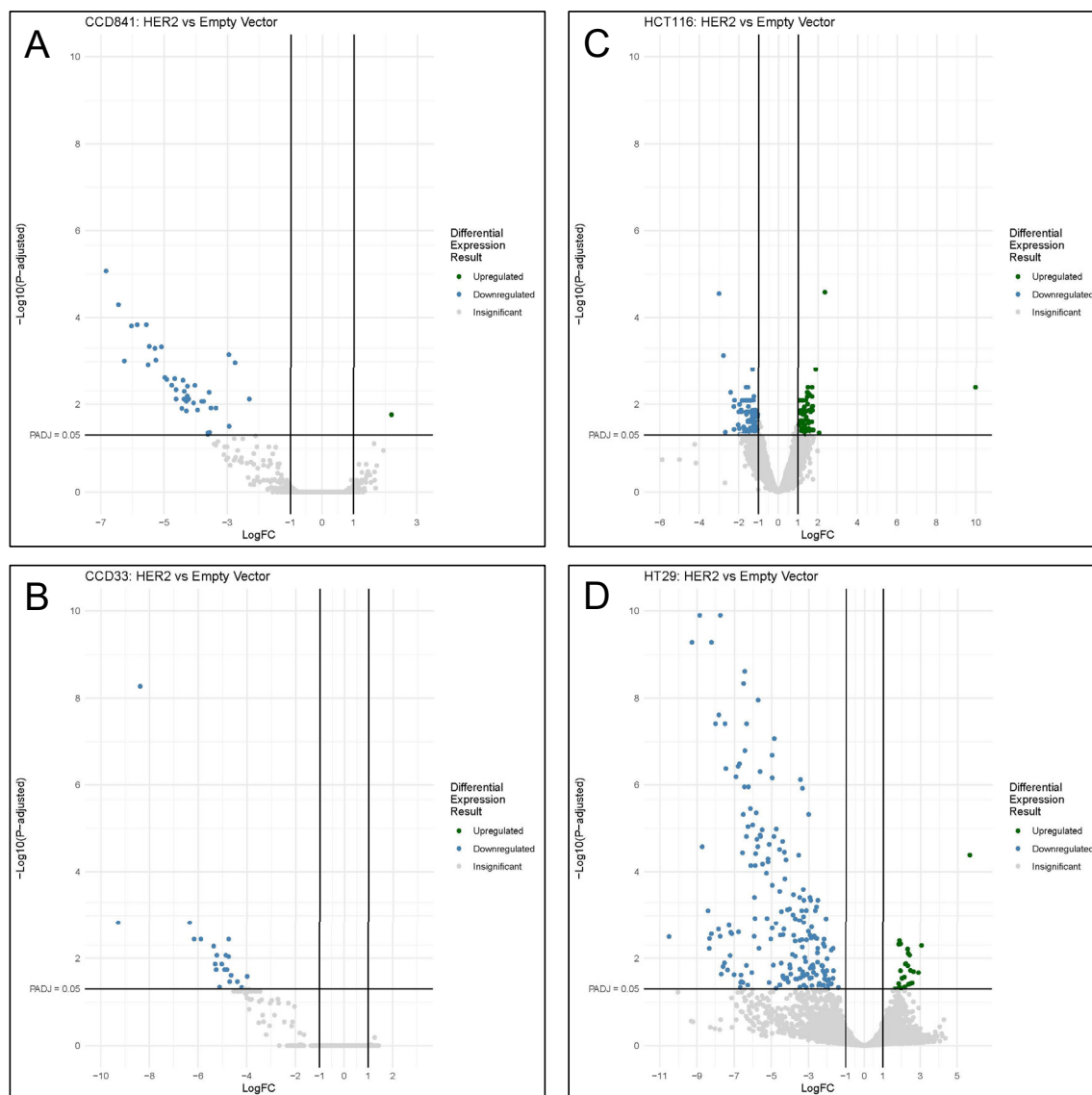
Supplementary Figure S1



Supplementary Figure S2

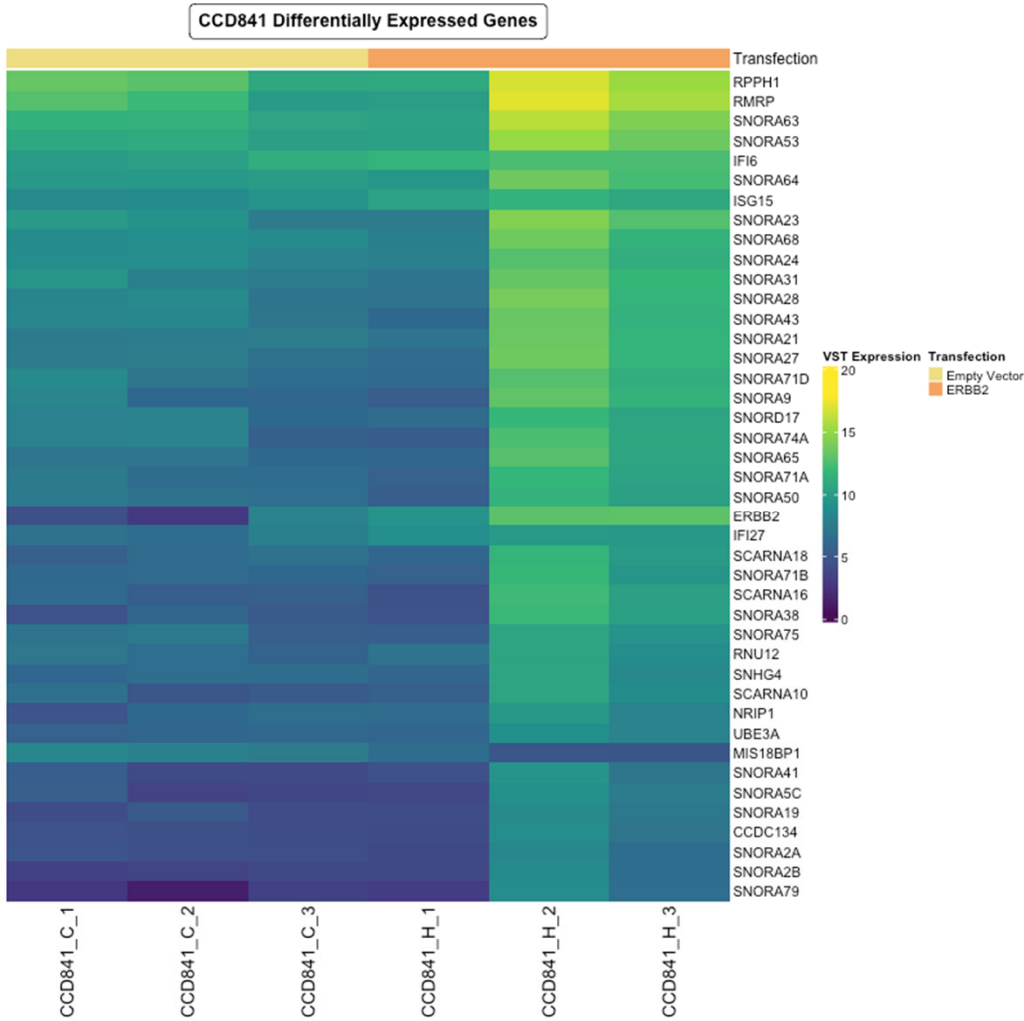


Supplementary Figure S3

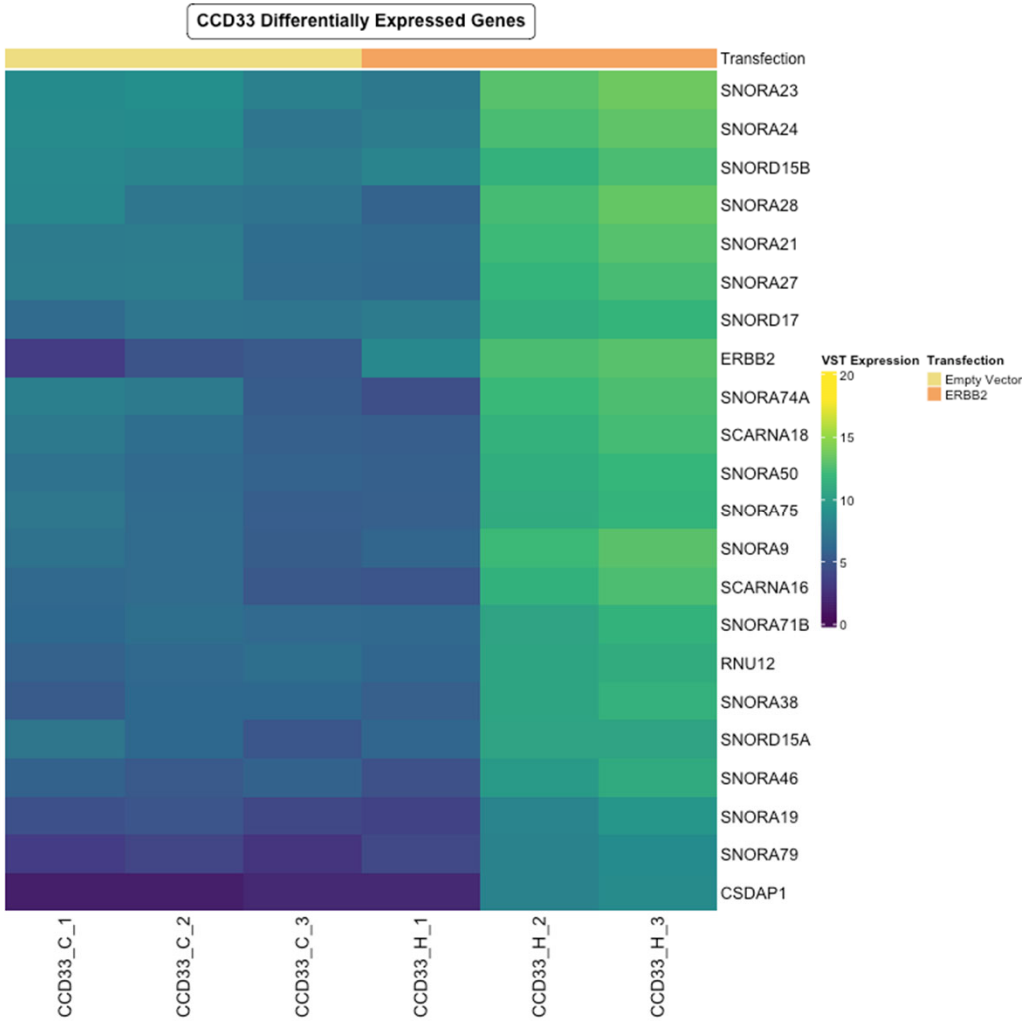


Supplementary Figure S4

A

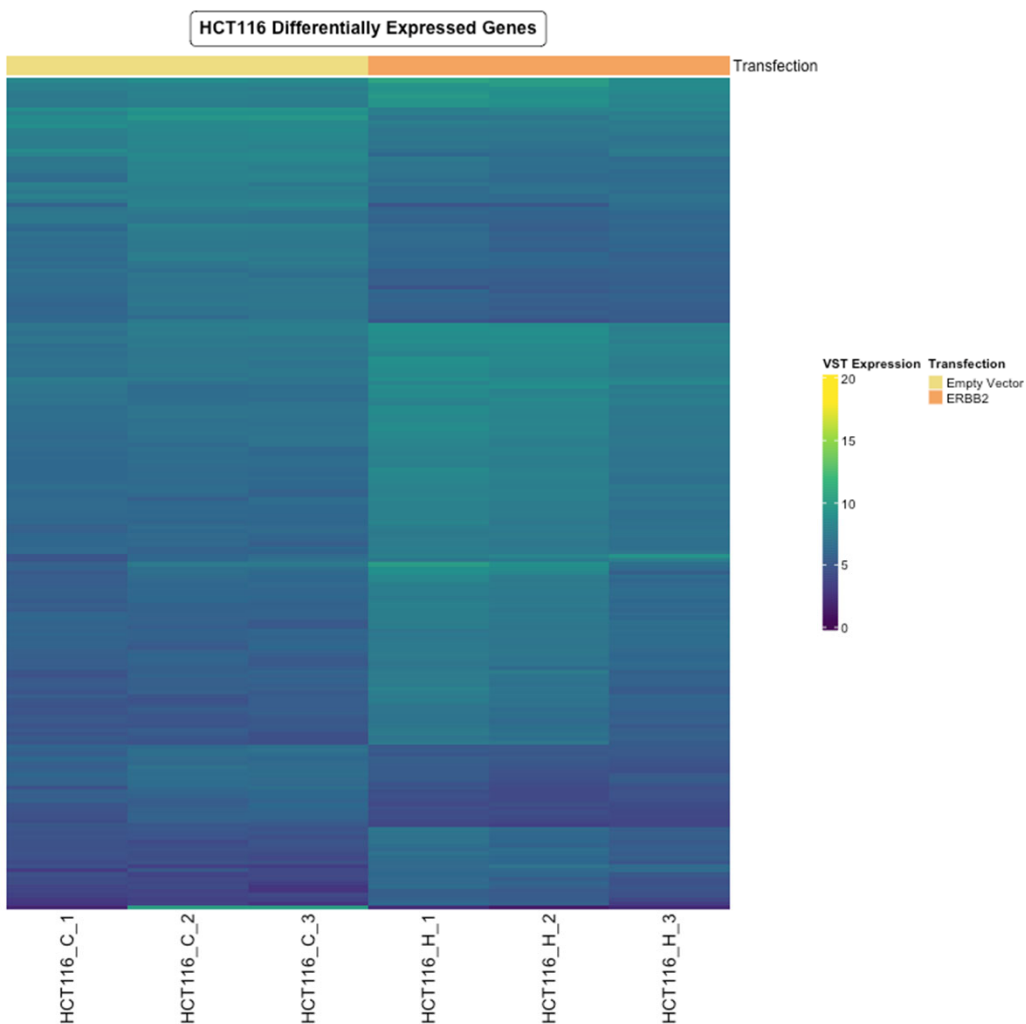


B

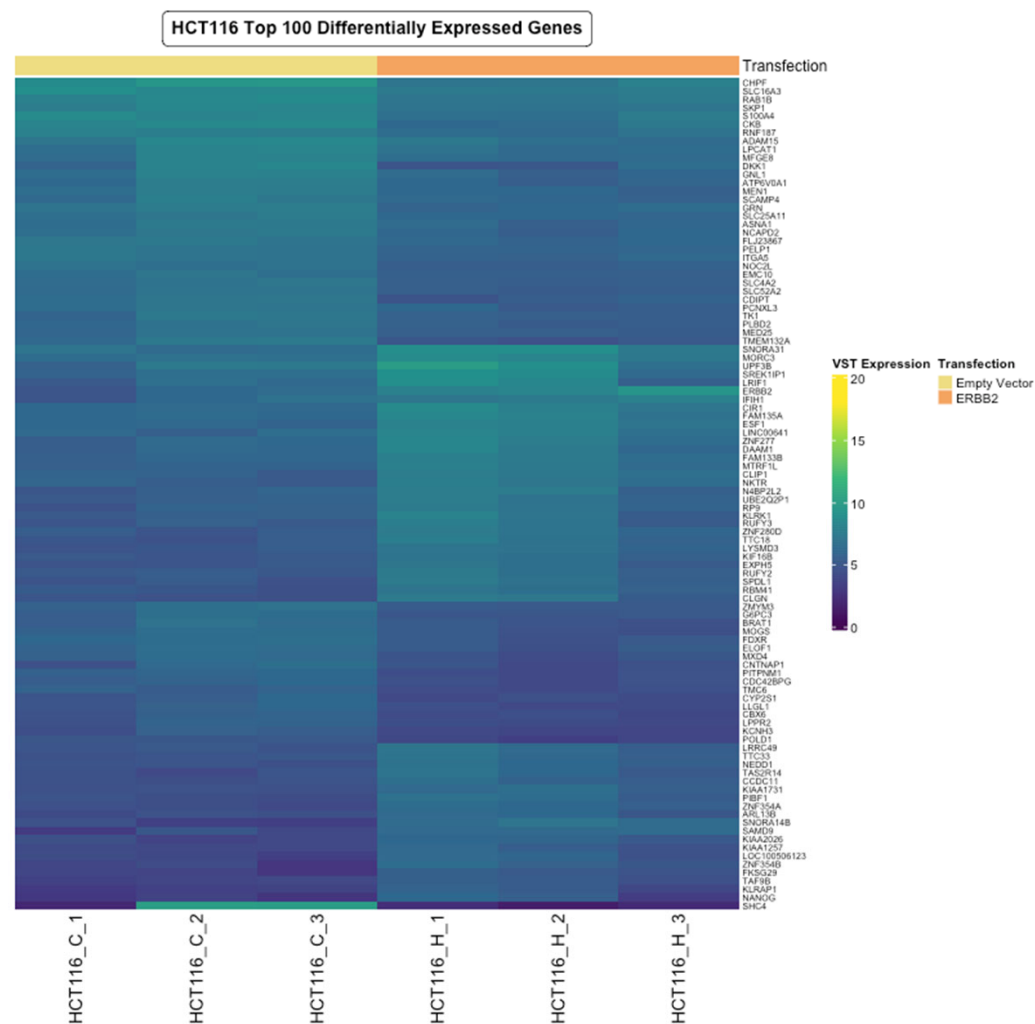


Supplementary Figure S5

A

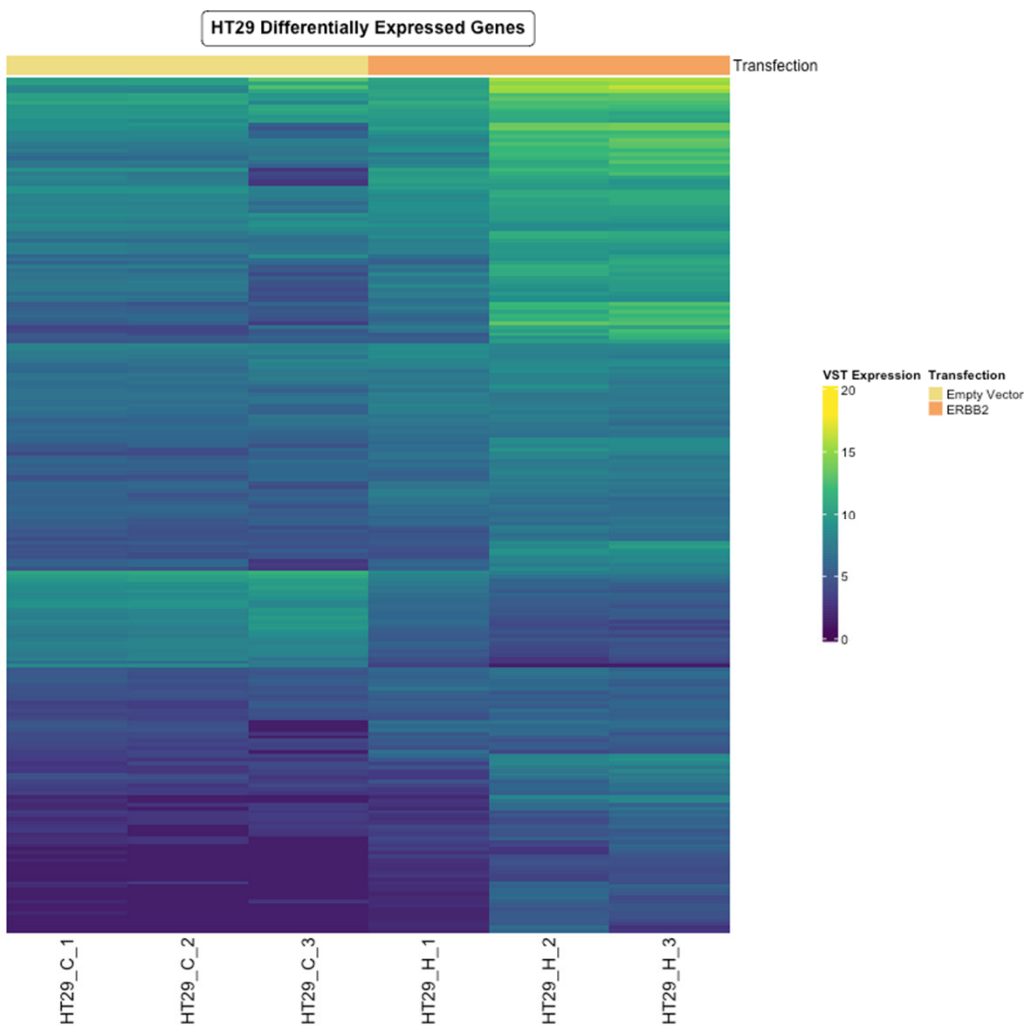


B

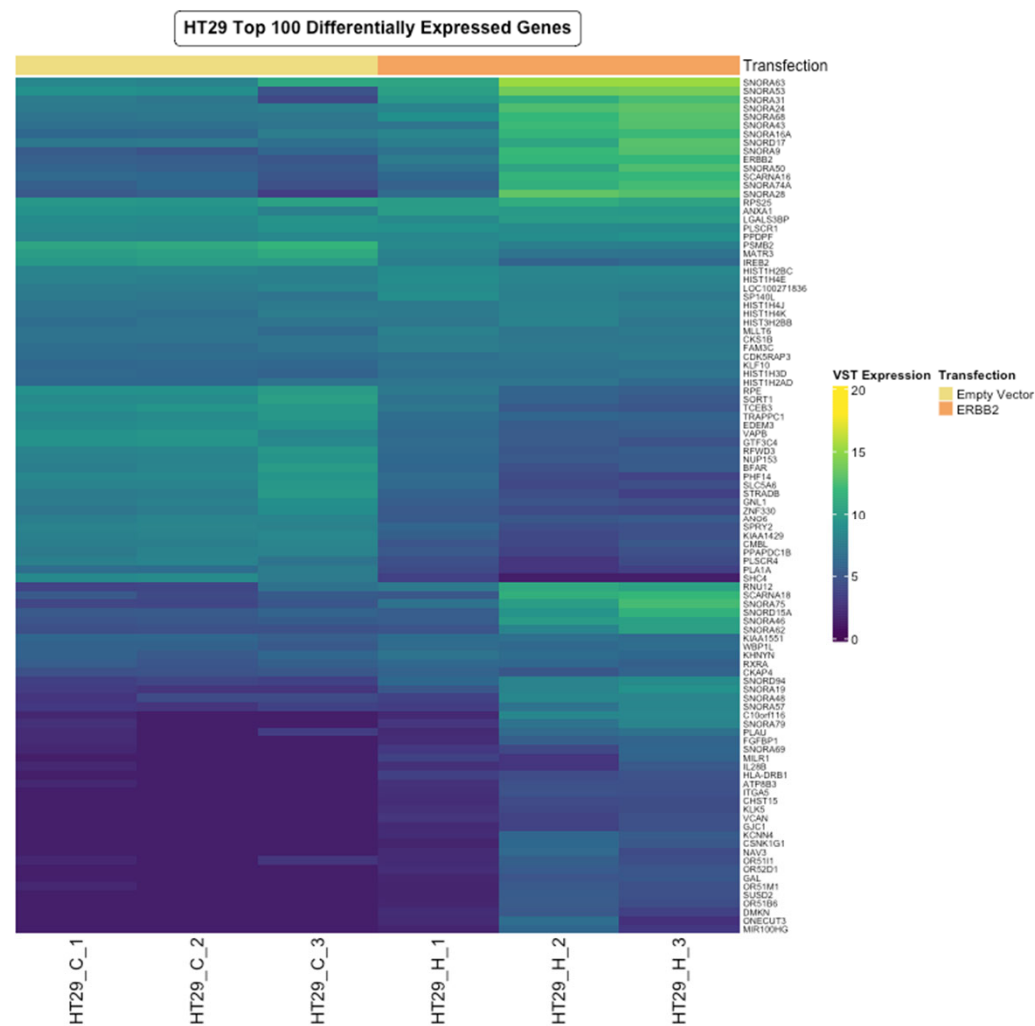


Supplementary Figure S6

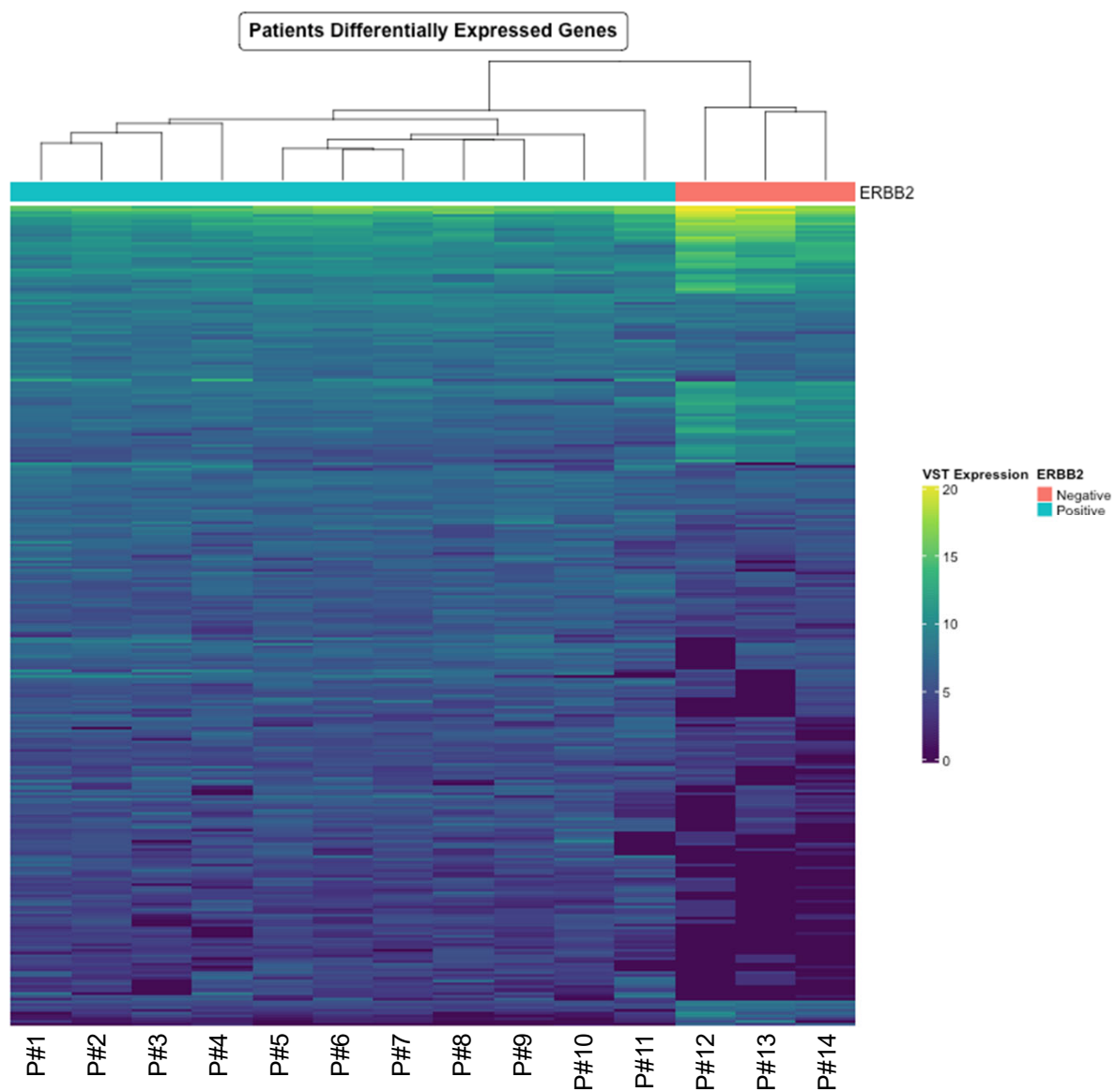
A



B



A



B

Supplementary Figure S7

