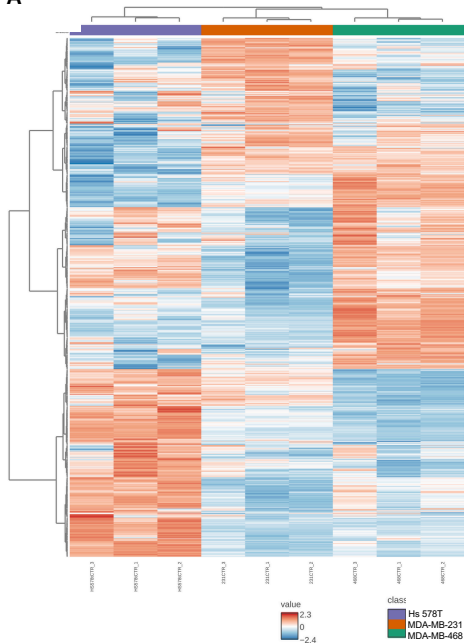
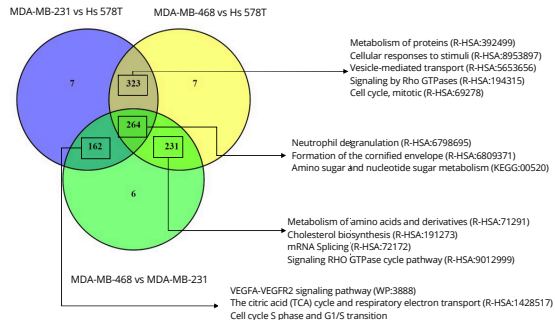


A



B



C

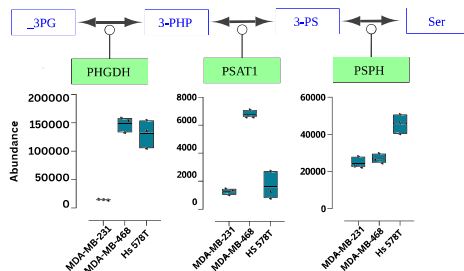
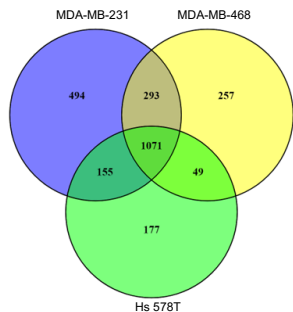


Figure S1. Heterogeneity of untreated TNBC cell line through proteome profiling A. Heatmap of hierarchical clustering analysis performed on protein profiles of TNBC cell panel; Hierarchical clustering was performed on normalized data using Euclidean distance measurement and WARD clustering method option; B. Venn diagram depicting proteins differentiating between cell lines and their involvement in metabolic pathways. Differentially expressed proteins were obtained using ANOVA test (corrected p-value threshold of 0.05) + Tukey's HSD post-hoc test; C. Proteins involved in de novo serine biosynthesis pathway and their expression in TNBC cell panel.

A Overview of intracellular proteome profiling

Intracellular proteome	MDA-MB-231	MDA-MB-468	Hs 578T
Total no. of proteins	2123	1739	1505
Reverse+	84	67	51
FDR dataset %	3.95	3.85	3.39
Final number of proteins	2013	1670	1452



B Functional analysis using GO ontologies

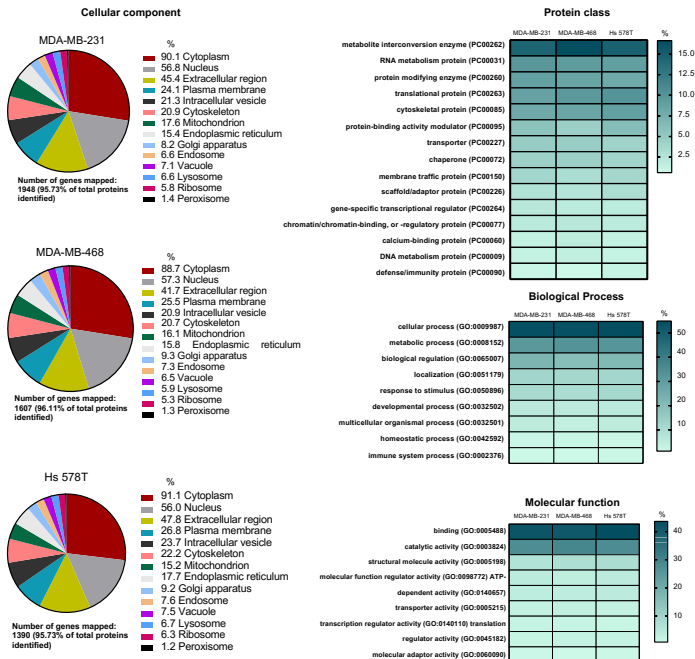
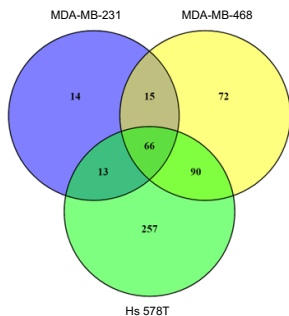


Figure S2. Overview of intracellular proteome profiles: A. Overview of the number of identified proteins and Venn diagram showing common and cell line particular proteins; B. Functional analysis using GO ontologies: cellular component- CC; biological process - BP and molecular function -MF;

A Overview of secreted proteome profiling

Secretome	MDA-MB-231	MDA-MB-468	Hs 578T
Total no proteins	112	250	441
Reverse+	3	6	10
FDR dataset %	2.61	2.40	2.32
Final number of proteins	108	243	426



B Functional analysis using GO ontologies

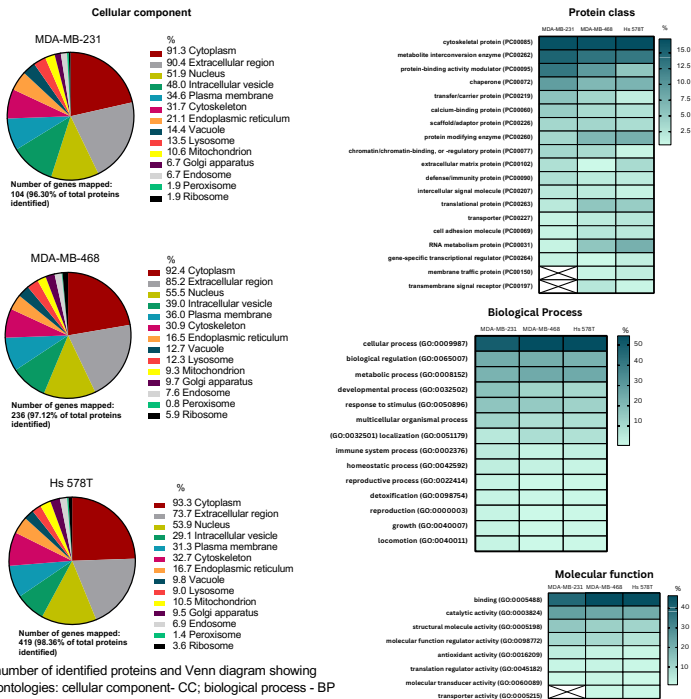


Figure S3. Overview of the secreted proteins profiles: A. Overview of the number of identified proteins and Venn diagram showing common and cell line particular proteins; B. Functional analysis using GO ontologies: cellular component- CC; biological process - BP and molecular function -MF;

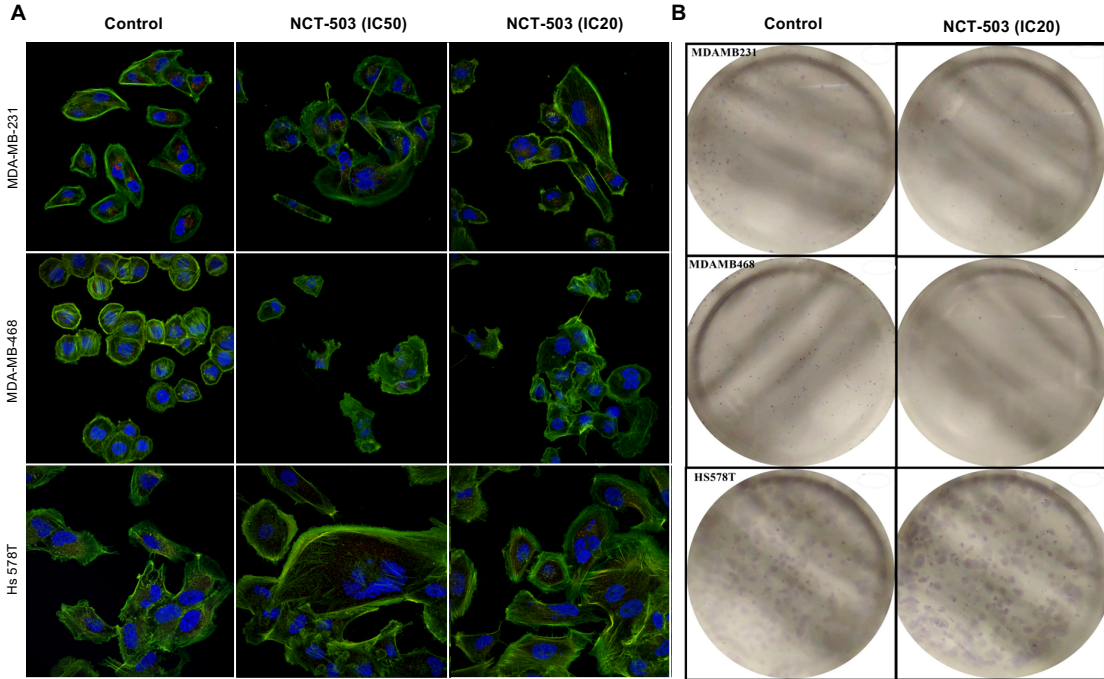
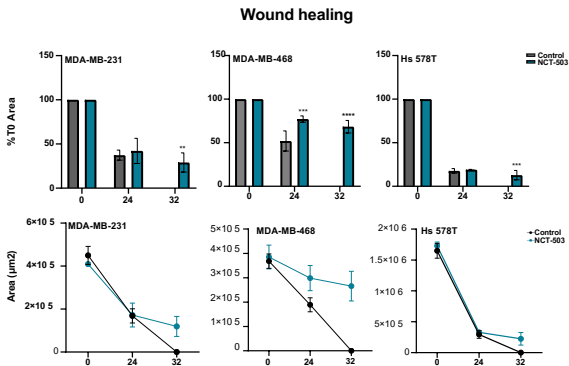


Figure S4. NCT-503 treatment impact on cellular properties of TNBC cell panel A. Impact of NCT-503 treatment on cell morphology at IC50 and IC20 dose, respectively for each TNBC cell line; blue- cell nucleus, green- cytoskeleton morphology; B. Effect of NCT 503 treatment on colony formation abilities of TNBC cell line panel

A



B

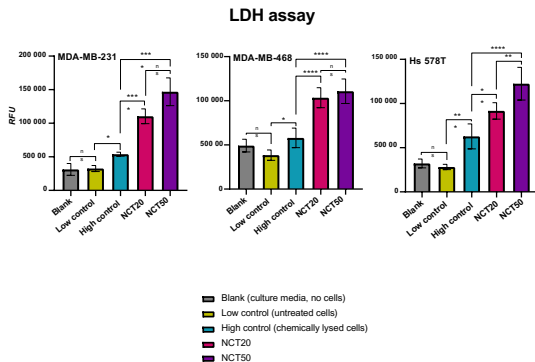
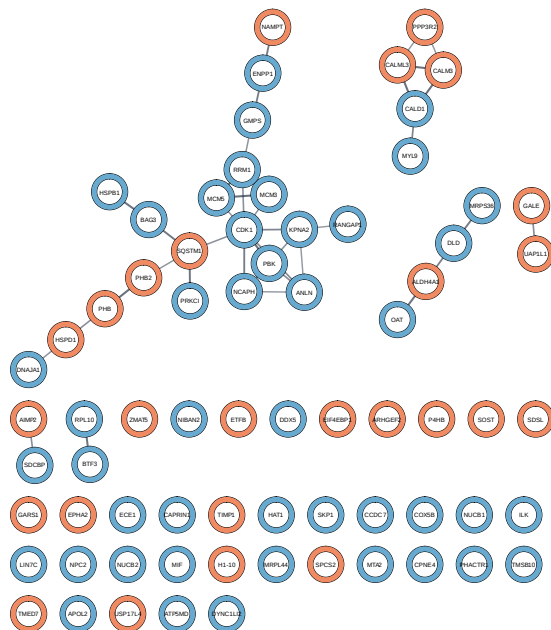
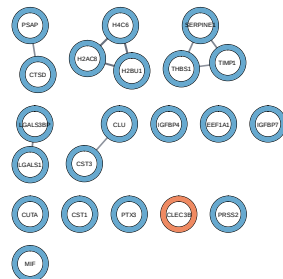


Figure S5 In vitro assays of NCT-503 treatment impact on TNBC cell lines. A. wound healing assay; B. Culture media release LDH assay

MDA-MB-231 intracellular DEPs



MDA-MB-231 secreted DEPs



STRING App

Full string network retrieval; confidence cutoff: 0.7, no additional interactors

MDA-MB-231 intracellular DEPs

Network Stats
 number of nodes: 71
 number of edges: 39
 average node degree: 1.1
 avg. local clustering coefficient: 0.319
 expected number of edges: 20
PPI enrichment p-value: 1.20e-04

MDA-MB-231 secreted DEPs

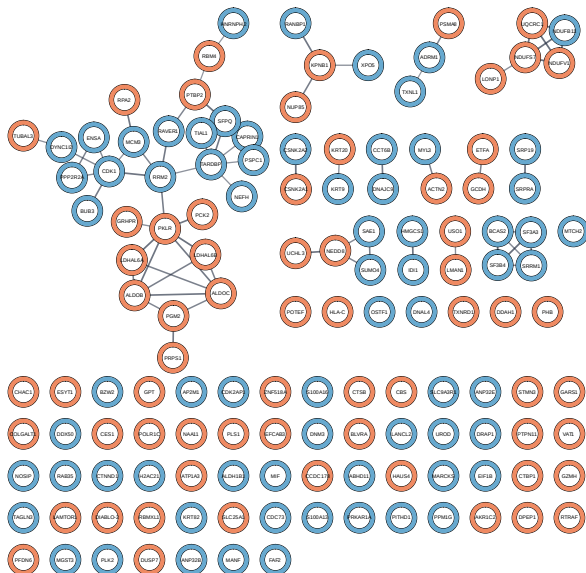
Network Stats
 number of nodes: 21
 number of edges: 9
 average node degree: 0.857
 avg. local clustering coefficient: 0.571
 expected number of edges: 2
PPI enrichment p-value: 5.18e-05

Legend

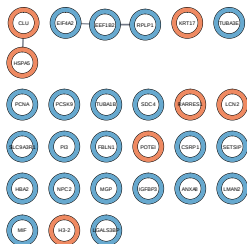
Highest condition: Control (CTR) NCT-503

Figure S6. Protein-protein interaction (PPI) network retrieved using StringApp for DEPs induced by NCT-503 treatment in MDA-MB-231 cell line

MDA-MB-468 intracellular DEPs



MDA-MB-468 secreted DEPs



STRING App

Full string network retrieval; confidence cutoff: 0.7, no additional interactors

MDA-MB-468 intracellular DEPs

Network Stats
 number of nodes: 135
 number of edges: 68
 average node degree: 1.01
 avg. local clustering coefficient: 0.375
 expected number of edges: 38
PPI enrichment p-value: 1.03e-05

MDA-MB-468 secreted DEPs

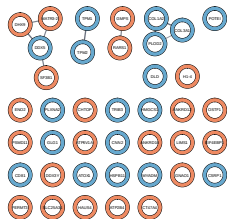
Network Stats
 number of nodes: 28
 number of edges: 3
 average node degree: 0.214
 avg. local clustering coefficient: 0.143
 expected number of edges: 2
PPI enrichment p-value: 0.371

Legend

Highest condition:  Control (CTR)  NCT-503

Figure S7. Protein-protein interaction (PPI) network retrieved using StringApp for DEPs induced by NCT-503 treatment in MDA-MB-468 cell line

Hs 578T intracellular DEPs



STRING App

Full string network retrieval; confidence cutoff: 0.7, no additional interactors

Hs 578T intracellular DEPs

Network Stats

number of nodes: 40
number of edges: 9
average node degree: 0.45
avg. local clustering coefficient: 0.258
expected number of edges: 3
PPI enrichment p-value: 0.00466

Hs 578T secreted DEPs

Network Stats

number of nodes: 207
number of edges: 481
average node degree: 4.65
avg. local clustering coefficient: 0.465
expected number of edges: 162
PPI enrichment p-value: < 1.0e-16

Legend

Highest condition:  Control (CTR)  NCT-503

Hs 578T secreted DEPs

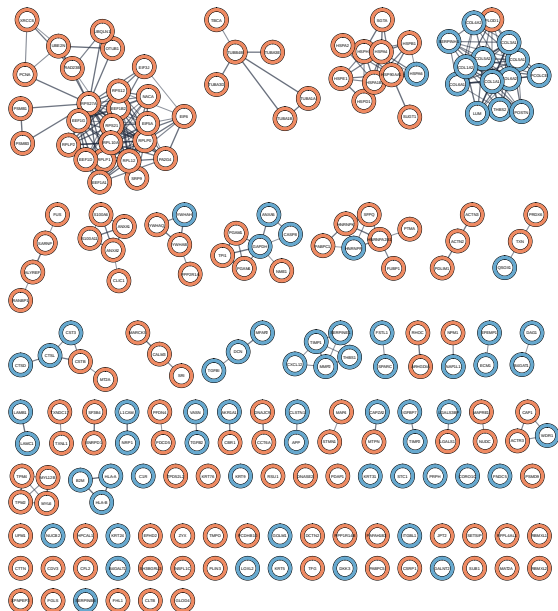


Figure S8. Protein-protein interaction (PPI) network retrieved using StringApp for DEPs induced by NCT-503 treatment in Hs 578T cell line

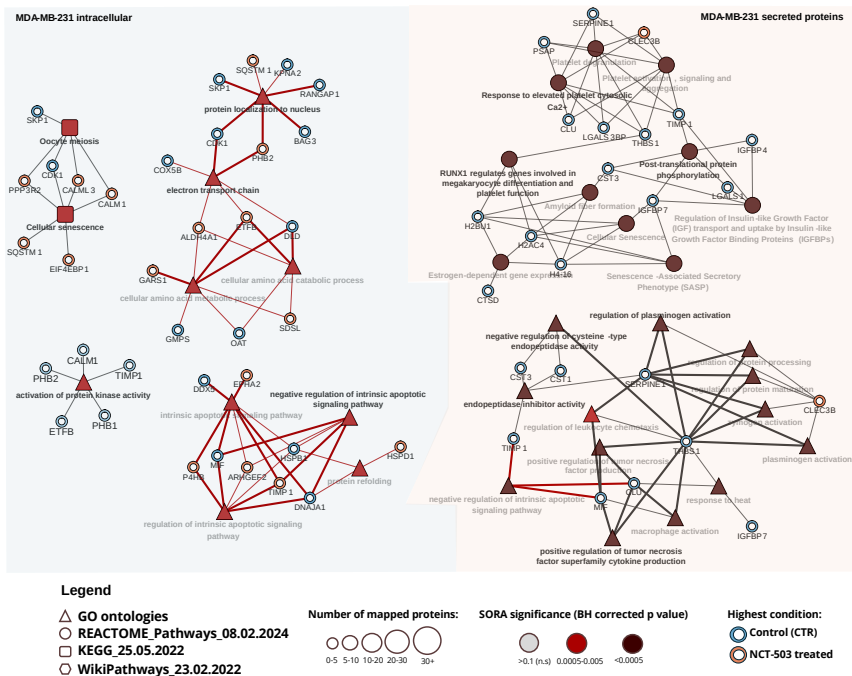
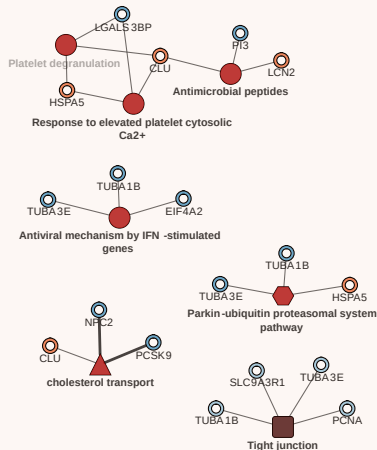
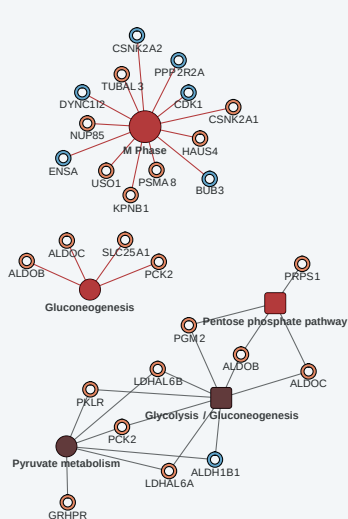


Figure S9. Enrichment analysis of MDA-MB-231 DEPs using ClueGO Enrichment tool



Legend

- △ GO ontologies
- REACTOME_Pathways_08.02.2024
- KEGG_25.05.2022
- WikiPathways_23.02.2022

Number of mapped proteins:



SORA significance (BH corrected p value)



Highest condition:

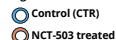
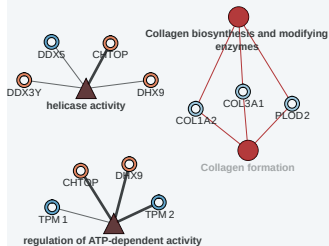
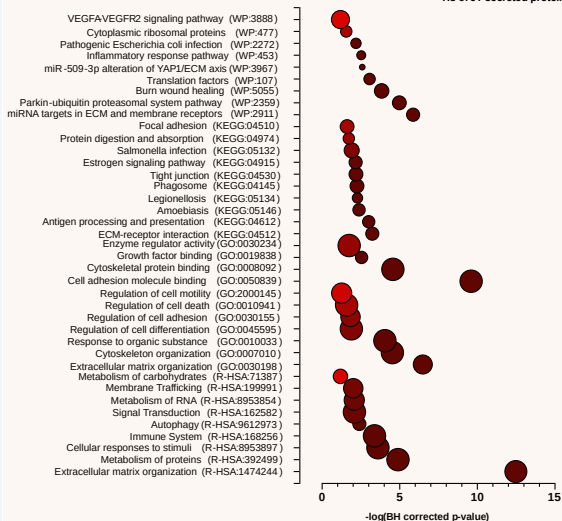


Figure S10. Enrichment analysis of MDA-MB-468 DEPs using ClueGO Enrichment tool

Hs 578T intracellular

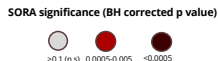


Hs 578T secreted proteins



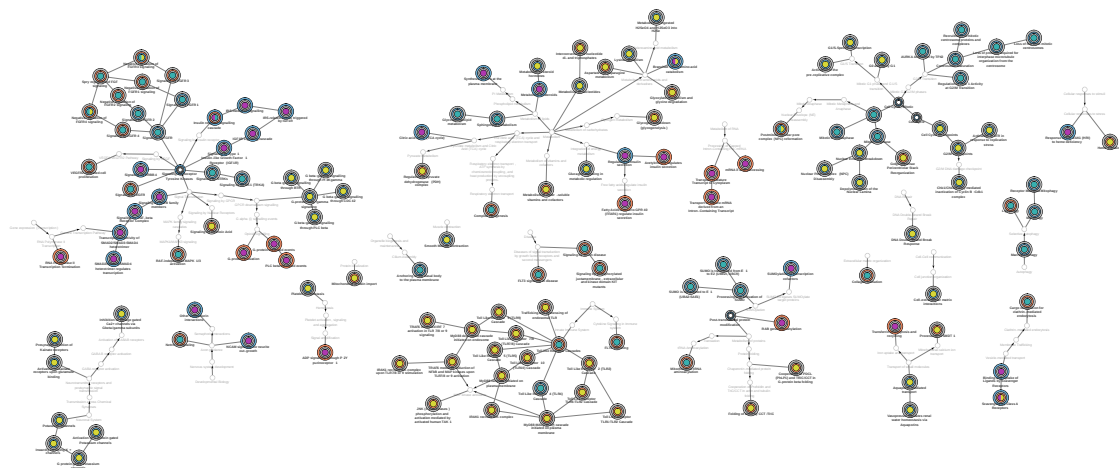
Legend

- △ GO ontologies
- REACTOME_Pathways_08.02.2024
- KEGG_25.05.2022
- WikiPathways_23.02.2022



- Highest condition:
- Control (CTR)
 - NCT-503 treated

Figure S11. Enrichment analysis of Hs 578T DEPs using ClueGO Enrichment tool



Legend:

Rings (inner to outer):

direction GSEA MDA-MB-231

direction GSEA MDA-MB-468

direction GSEA Hs 578T

Significant Down Significant Up

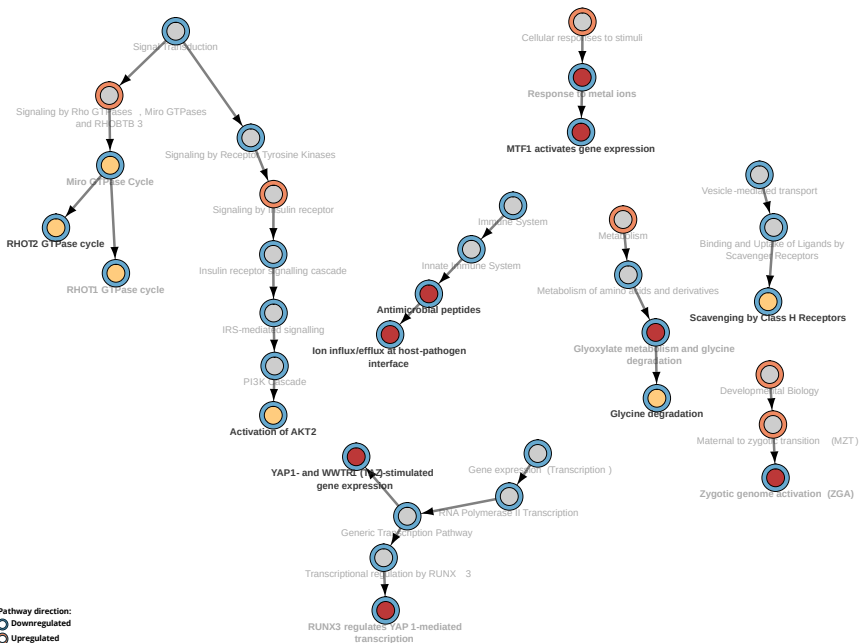
Not significant

MDA-MB-231

MDA-MB-468

Hs 578T

Figure S12. Network representation of Reactome GSEA pathways for the intracellular proteome of TNBC cell lines treated with NCT-503



ReactomeGSEA analysis: Hs 578T intracellular proteins

Legend:

Pathway significance (BH corrected p-value)

>0.1 (n.s) 0.05-0.005 0.005-0.0005 <0.0005

Pathway direction:

Downregulated Upregulated

Figure S15. Network representation of ReactomeGSEA analysis performed on the intracellular protein profiles of NCT-503 treatment and control group of Hs 578T

ReactomeGSEA analysis: MDA-MB-231 secreted proteins

Legend:

Pathway significance (BH corrected p-value)

● >0.1(n.s)
 ● 0.05-0.005
 ● 0.005-0.0005
 ● <0.0005

Pathway direction:

○ Downregulated
○ Upregulated

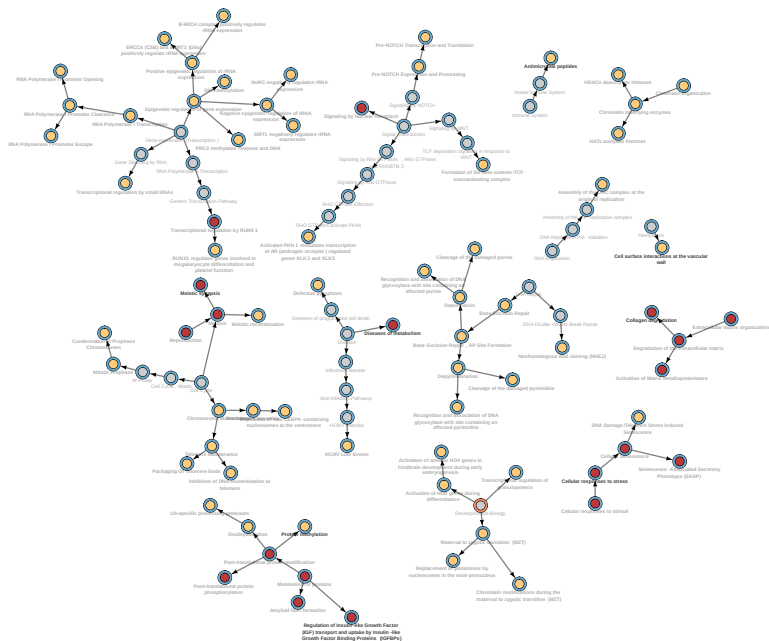
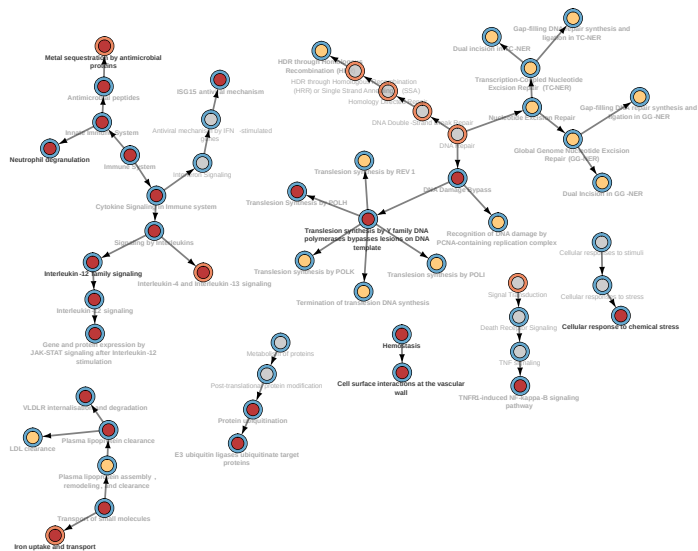


Figure S17. Network representation of ReactomeGSEA analysis performed on the secreted protein profiles of NCT-503 treatment and control group of MDA-MB-231



ReactomeGSEA analysis: MDA-MB-468 secreted proteins

Legend:

Pathway significance (BH corrected p-value)

● >0.1 (n.s.) ● 0.05-0.005 ● 0.005-0.0005 ● <0.0005

Pathway direction:

● Downregulated ● Upregulated

Figure S18. Network representation of ReactomeGSEA analysis performed on the secreted protein profiles of NCT-503 treatment and control group of MDA-MB-468

