

Figure S1

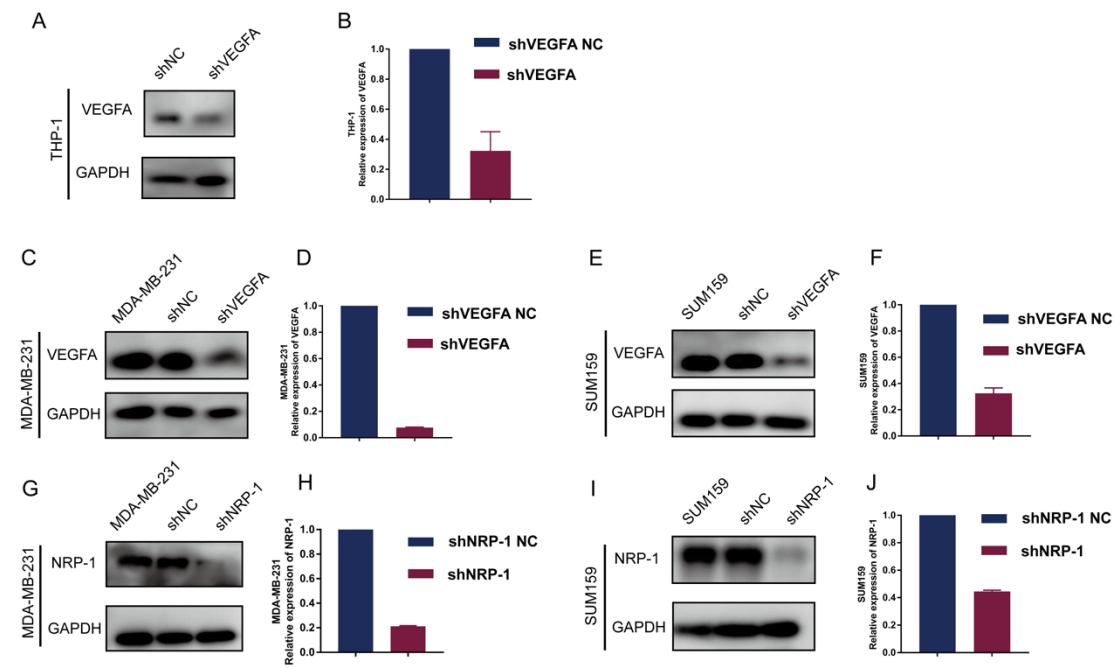


Figure S1 The efficiency of VEGFA and NRP-1 knockdown was verified by Western blotting and RT-qPCR

(A-B) RT-qPCR and Western blotting analysis of VEGFA knockdown efficiency in THP-1 cells. (C-F) RT-qPCR and Western blotting analysis of VEGFA knockdown efficiency in TNBC cells. (G-J) RT-qPCR and Western blotting analysis of NRP-1 knockdown efficiency in TNBC cells. Data are representative images and expressed as the mean \pm SD of each group of cells from three separate experiments.

Figure S2

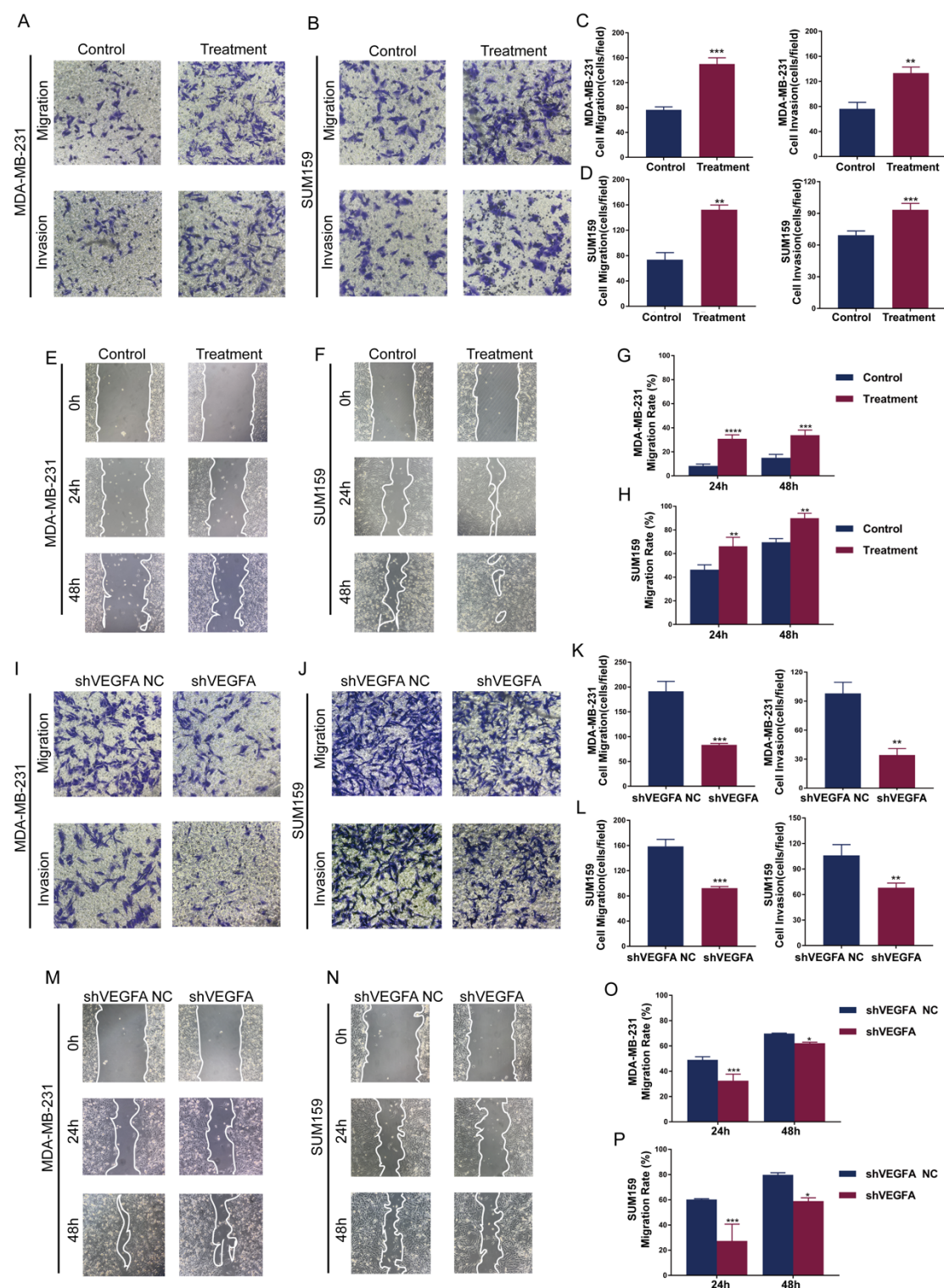


Figure S2 VEGFA regulates migration and invasion of TNBC cells

(A-H) The effect of exogenous hVEGFA₁₆₅ (10 ng/ml) on the migration and invasion of TNBC cells revealed by transwell and wound healing assay. (I-P) The effect of VEGFA knockdown on the migration and invasion of TNBC cells revealed by transwell and wound healing assay. Data are representative images and expressed as the mean \pm SD of each group of cells from three separate experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ vs. the controls.

Figure S3

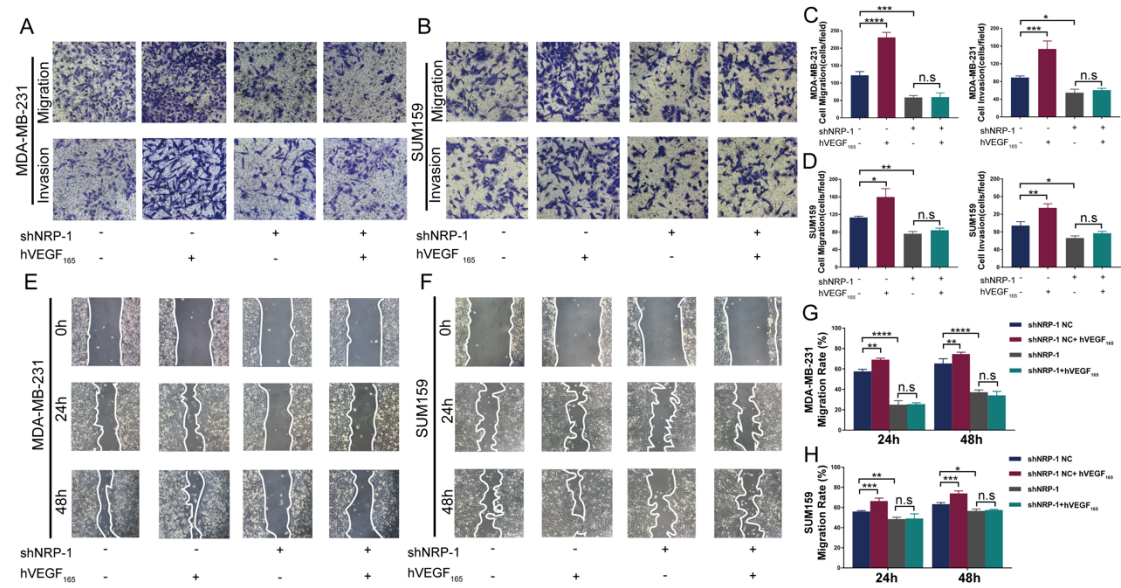


Figure S3 VEGFA modulates migration and invasion of TNBC cells in a NRP-1 dependent manner

(A-H) The effect of NRP-1 knockdown in the presence or absence of 10 ng/ml of hVEGF₁₆₅ on the migration and invasion of TNBC cells revealed by Transwell and wound healing assay. Data are representative images and expressed as the mean \pm SD of each group of cells from three separate experiments. n.s no significance, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ vs. the controls.

Figure S4

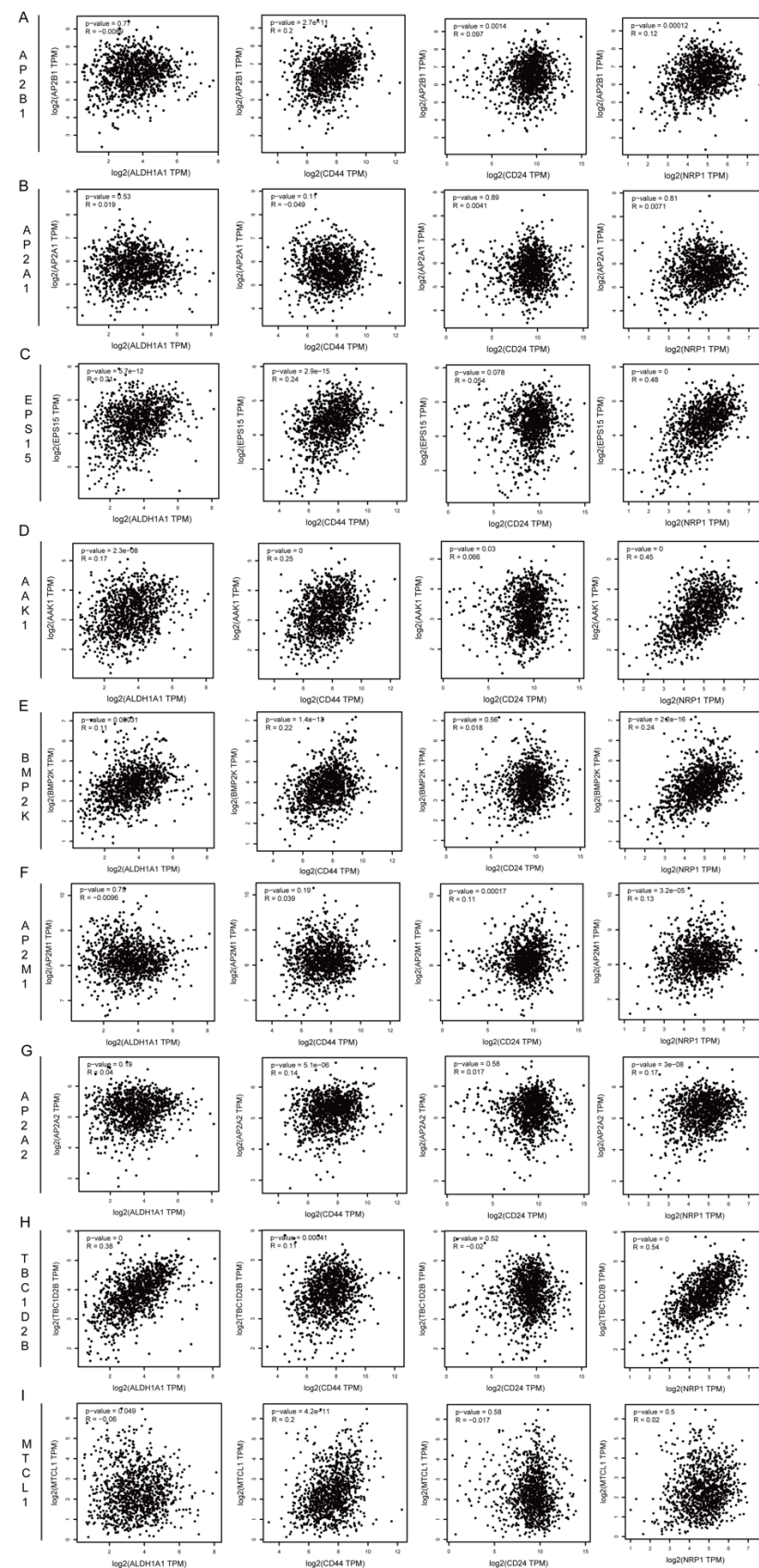


Figure S4 Correlations between MS target proteins and NRP-1 and cancer stem cell markers
(A-H) Correlations between MS target proteins and NRP-1 and cancer stem cell markers.

Figure S5

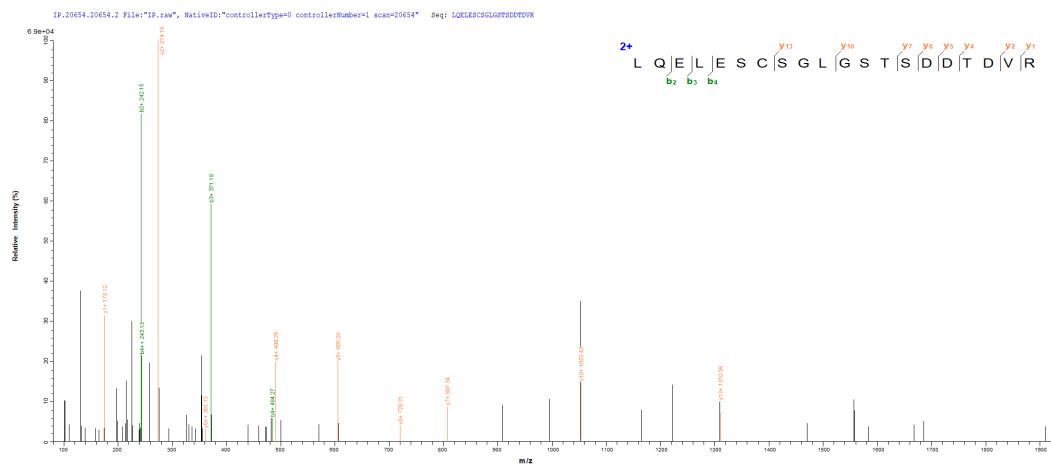


Figure S5 Identification of GAPVD1 peptide using LC-MS/MS

Figure S6

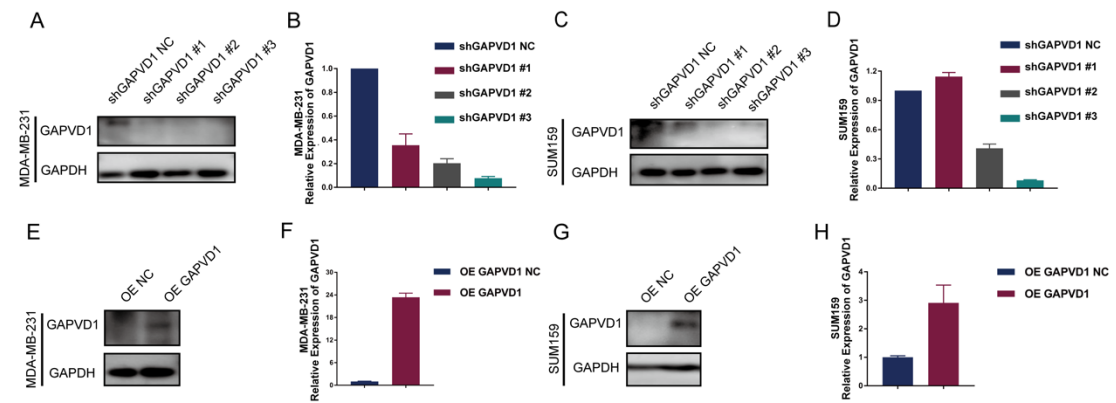


Figure S6 The efficiency of GAPVD1 silencing and overexpression was verified by Western blotting and RT-qPCR

(A-D) RT-qPCR and Western blotting analysis of GAPVD1 knockdown efficiency in TNBC cells, shRNA sequence #2 and #3 creating higher knockdown efficiency and were used for subsequent experiments. (E-H) RT-qPCR and Western blotting analysis of GAPVD1 overexpression efficiency in TNBC cells. Data are representative images and expressed as the mean \pm SD of each group of cells from three separate experiments.

Figure S7

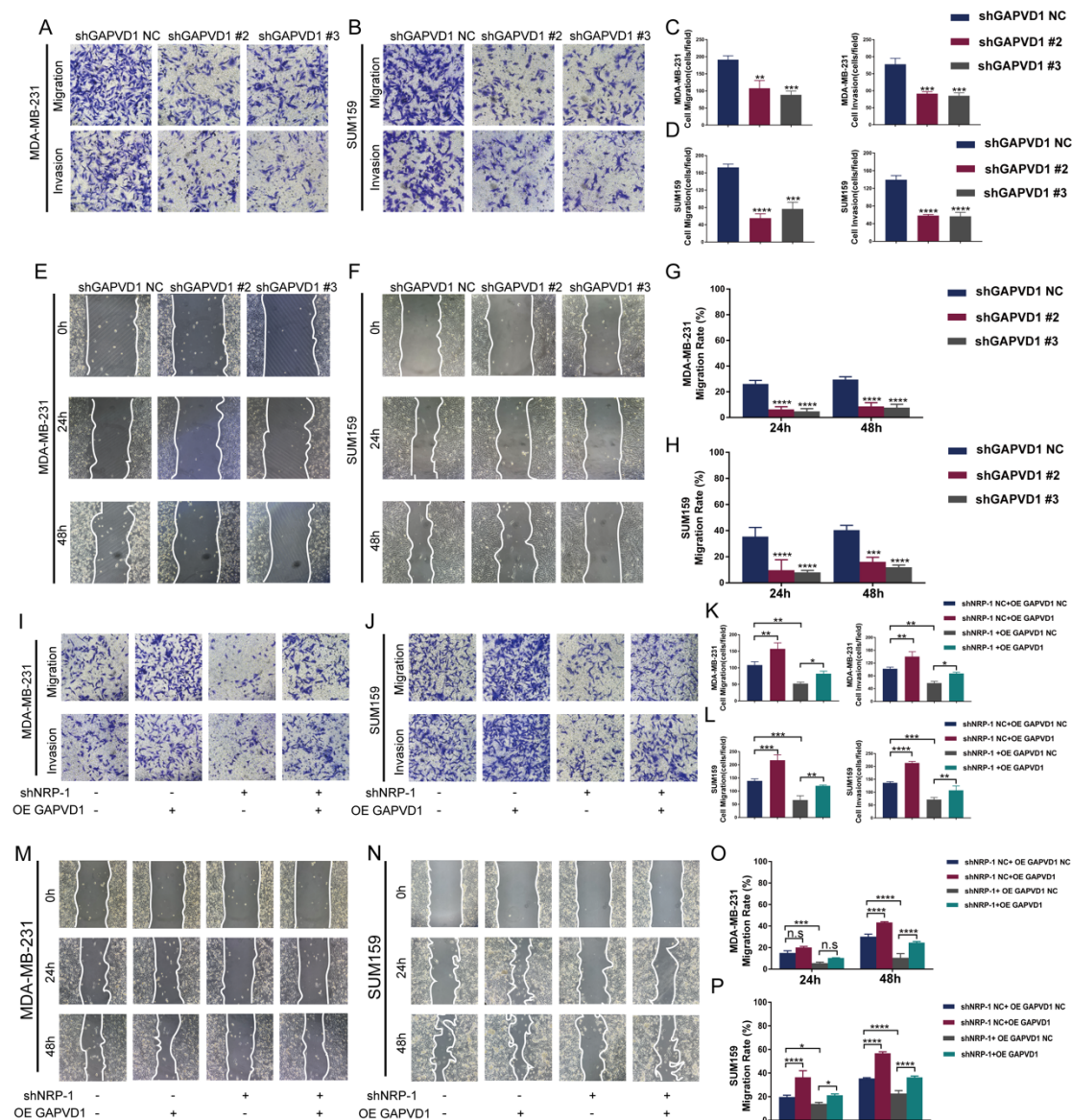


Figure S7 GAPVD1 regulates migration and invasion of TNBC cells

(A-H) The effect of GAPVD1 knockdown on the migration and invasion of TNBC cells revealed by Transwell and wound healing assay. (I-P) Migration and invasion revealed by Transwell and wound healing assay after GAPVD1 overexpression in control and NRP-1-silencing TNBC cells. Data are representative images and expressed as the mean \pm SD of each group of cells from three separate experiments. n.s no significance, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ vs. the controls.

Table S3 The Overlap Genes Between GeneCard and GEO Dataset

Gene Symbol	Gene Title	adj.P.Value	logFC
IL1B	interleukin 1 beta	3.80E-07	1.10E+01
GDF15	growth differentiation factor 15	4.30E-07	9.87
IL1RN	interleukin 1 receptor antagonist	1.72E-06	5.38
S100A9	S100 calcium binding protein A9	3.62E-06	-4.55
HBEGF	heparin binding EGF like growth factor	3.98E-06	3.17
S100A8	S100 calcium binding protein A8	6.71E-06	-6.67
PPBP	pro-platelet basic protein	7.68E-06	6.36
FGF2	fibroblast growth factor 2	9.45E-06	4.57
ISM1	isthmin 1, angiogenesis inhibitor	1.27E-05	3.71
ANGPTL4	angiopoietin like 4	1.29E-05	4.91
CCL13	C-C motif chemokine ligand 13	1.44E-05	4.97
TNFSF10	tumor necrosis factor superfamily member 10	2.32E-05	-3.27
CCL2	C-C motif chemokine ligand 2	2.68E-05	5.35
EBI3	Epstein-Barr virus induced 3	2.77E-05	3.31
S100A11	S100 calcium binding protein A11	3.13E-05	2.08
AREG	amphiregulin	3.52E-05	7.48
CCL4	C-C motif chemokine ligand 4	4.11E-05	4.58
CCL7	C-C motif chemokine ligand 7	4.54E-05	7.27
PDGFA	platelet derived growth factor subunit A	4.97E-05	5.83
VEGFC	vascular endothelial growth factor C	5.31E-05	3.02
GDF11	growth differentiation factor 11	6.04E-05	-2.35
CCL8	C-C motif chemokine ligand 8	6.31E-05	5.7
VEGFA	vascular endothelial growth factor A	9.43E-05	3.06
CCL26	C-C motif chemokine ligand 26	1.06E-04	2.14
CCL3	C-C motif chemokine ligand 3	2.07E-04	2
BDNF	brain derived neurotrophic factor	2.38E-04	2.34
S100A10	S100 calcium binding protein A10	2.52E-04	2.31
WNT7B	Wnt family member 7B	2.70E-04	-2.23
INHBE	inhibin beta E subunit	2.86E-04	2.95
NRG1	neuregulin 1	2.87E-04	5.77
S100A12	S100 calcium binding protein A12	3.08E-04	-3.39
ANGPTL6	angiopoietin like 6	3.20E-04	2.04
CCL20	C-C motif chemokine ligand 20	3.34E-04	8.3
TNFSF15	tumor necrosis factor superfamily member 15	3.54E-04	4.4
CXCL8	C-X-C motif chemokine ligand 8	3.65E-04	8.91
TGFB3	transforming growth factor beta 3	4.02E-04	3.61
FST	folliculin	5.03E-04	2.39
IL36G	interleukin 36, gamma	5.23E-04	3.77
TCHH	trichohyalin	5.96E-04	-2.93
TNFSF13B	tumor necrosis factor superfamily member 13b	7.47E-04	-2.28
BMP8A	bone morphogenetic protein 8a	7.71E-04	2.4

IL1A	interleukin 1 alpha	1.78E-03	5.53
S100A16	S100 calcium binding protein A16	2.76E-03	2.68
KITLG	KIT ligand	2.90E-03	2.95
HGF	hepatocyte growth factor	2.96E-03	-3.39
CCL17	C-C motif chemokine ligand 17	3.93E-03	2.22
INHBA	inhibin beta A subunit	4.17E-03	4.89
PDGFD	platelet derived growth factor D	4.92E-03	-2.66
CXCL1	C-X-C motif chemokine ligand 1	5.21E-03	7.06
WNT5A	Wnt family member 5A	5.47E-03	4.95
WNT5B	Wnt family member 5B	5.81E-03	3.52
CSF1	colony stimulating factor 1	5.81E-03	2.29
CXCL2	C-X-C motif chemokine ligand 2	6.56E-03	5.98
CXCL3	C-X-C motif chemokine ligand 3	8.04E-03	4.32
IL16	interleukin 16	8.77E-03	-2.03

Table S5 Top 10 Proteins Binding to NRP-1

Gene names	Unique peptides IP	Sequence coverage [%]	Score	Sequence coverage IgG [%]	Sequence coverage IP [%]	Intensity IgG	Intensity IP
AP2B1	21	48.3	323.31	0	48.3	0	27116000000
AP2A1	30	42.9	323.31	0	42.9	0	20496000000
EPS15	25	34.4	323.31	0	34.4	0	3683000000
AAK1	12	34.8	223.64	0	34.8	0	2969000000
BMP2K	22	25	221.4	0	25	0	2896300000
AP2M1	15	34.6	203.38	0	34.6	0	12804000000
AP2A2	19	31.6	179.93	0	31.6	0	3488900000
GAPVD1	22	15.1	166.86	0	15.1	0	1938000000
TBC1D2B	15	18.7	128.84	0	18.7	0	1379700000
MTCL1	15	10	123.48	0	10	0	568670000

Table S6 Correlations Between NRP-1, GAPVD1 and Pathological Parameters in TNBC Patients

Variables	NRP-1 Staining		<i>P</i>	GAPVD1 Staining		<i>P</i>
	Low expression	High expression		Low expression	High expression	
Age						
< 51	11	17	> 0.99999	12	16	> 0.99999
≥51	8	14		9	13	
Location						
Left	4	8	> 0.99999	3	9	0.2919
Right	7	15		10	12	
Unknown	4	12		4	12	
Tumor Size						
T1-2	17	17	0.0134*	18	16	0.0321*
T3-4	2	14		3	13	
Lymph Node						
N0	18	21	0.0352*	17	22	0.7412
N1	1	10		4	7	
Grade						
1-2	8	25	0.1203	14	19	0.2757
3	5	5		2	8	
Unknown	6	1		5	3	
Metastasis						
M0	19	31	> 0.99999	22	28	> 0.99999
M1	0	0		0	0	

Significant variables (p < 0.05) are bolded and *

Supplemental Table

Table S1 Differentially Expressed Genes in GEO dataset

Table S2 Secretory Factor Set Genes in GeneCard

Table S4 Proteins Binding to NRP-1