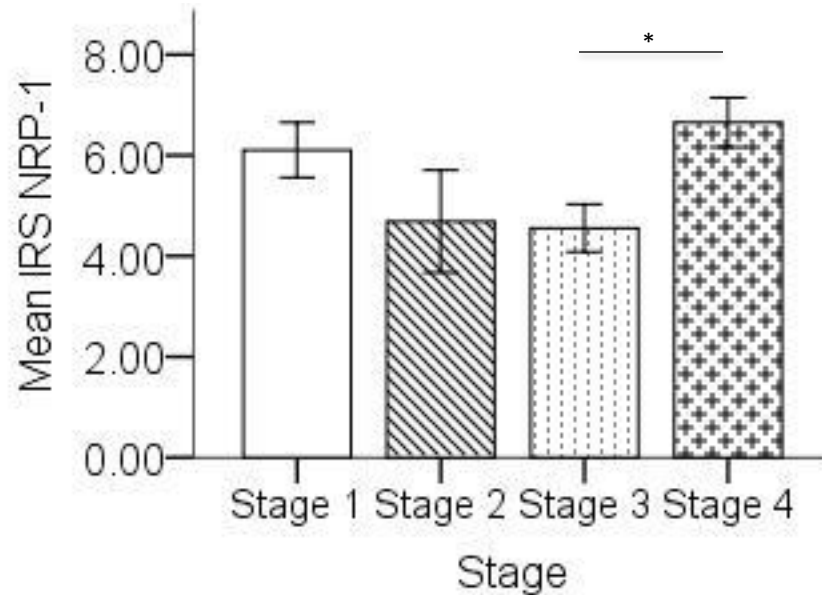
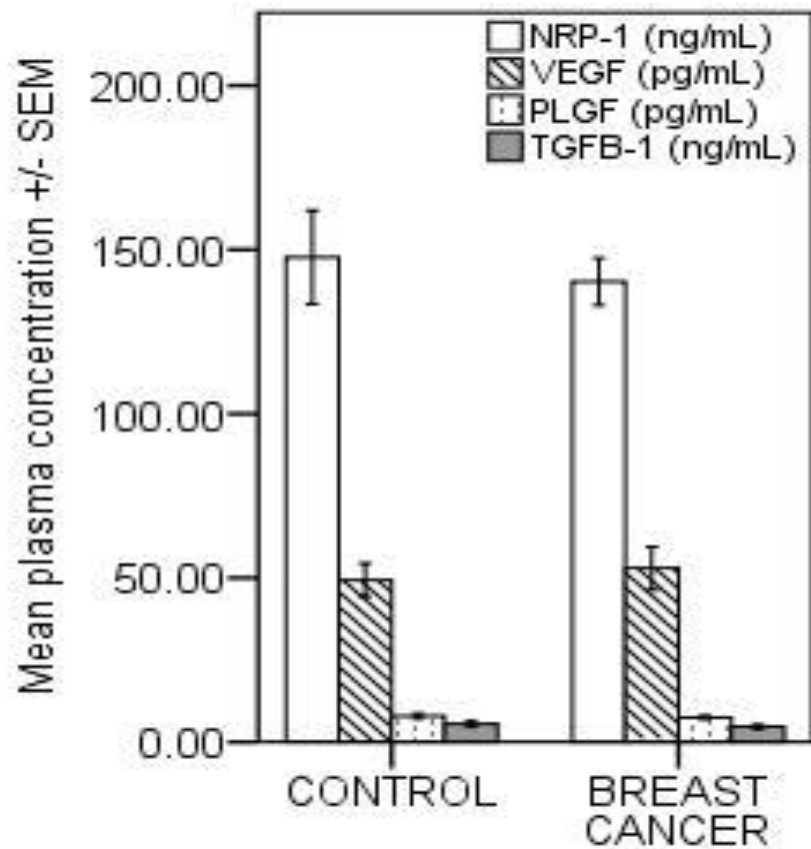


Neuropilin-1 Associated Molecules in the Blood Distinguish Poor Prognosis Breast Cancer: A Cross-Sectional Study

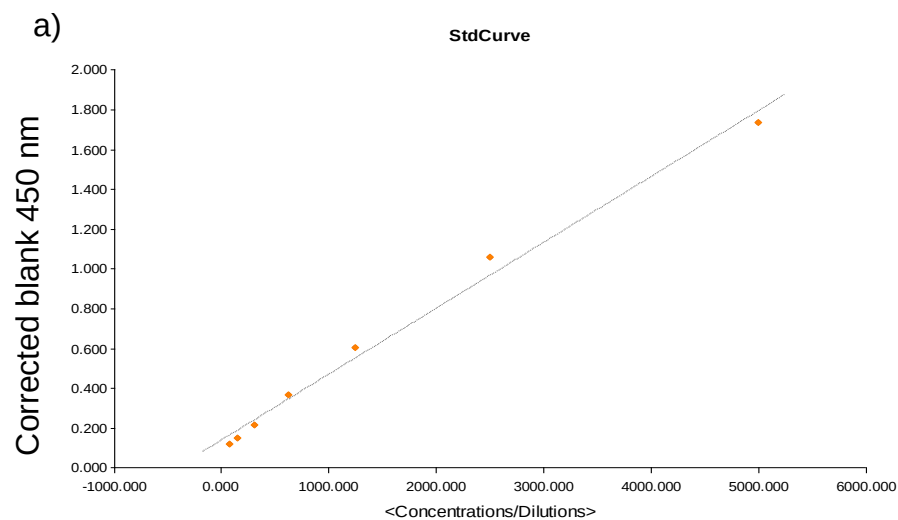
Adviti Naik¹, Noura Al-Zeheimi¹, Charles Saki Bakheit², Marwa Al Riyami³, Adil Al Ajarrah⁴, Mansour S. Al Moundhri⁵, Zamzam Al Habsi⁴, Maysoon Basheer¹, Sirin A. Adham^{1*}



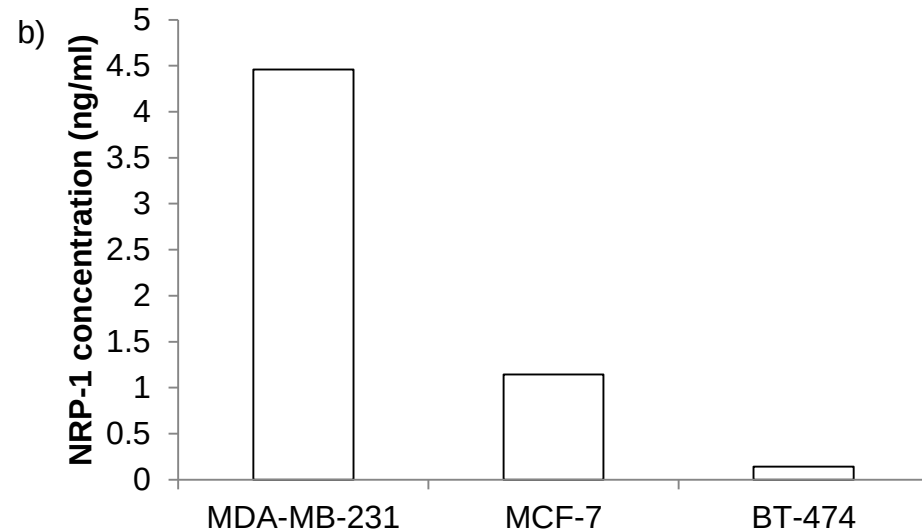
Supplementary Figure S1. Mean IRS score of NRP-1 in breast cancer stages. Graph represents calculated mean immuno-reactive score (IRS) of NRP-1 expression $\pm$ SEM in breast tumour tissue sub grouped according to overall disease stage. Stage 4 tumors were represented with higher levels of NRP-1 compared to stage 3 tumors $p=0.012$.



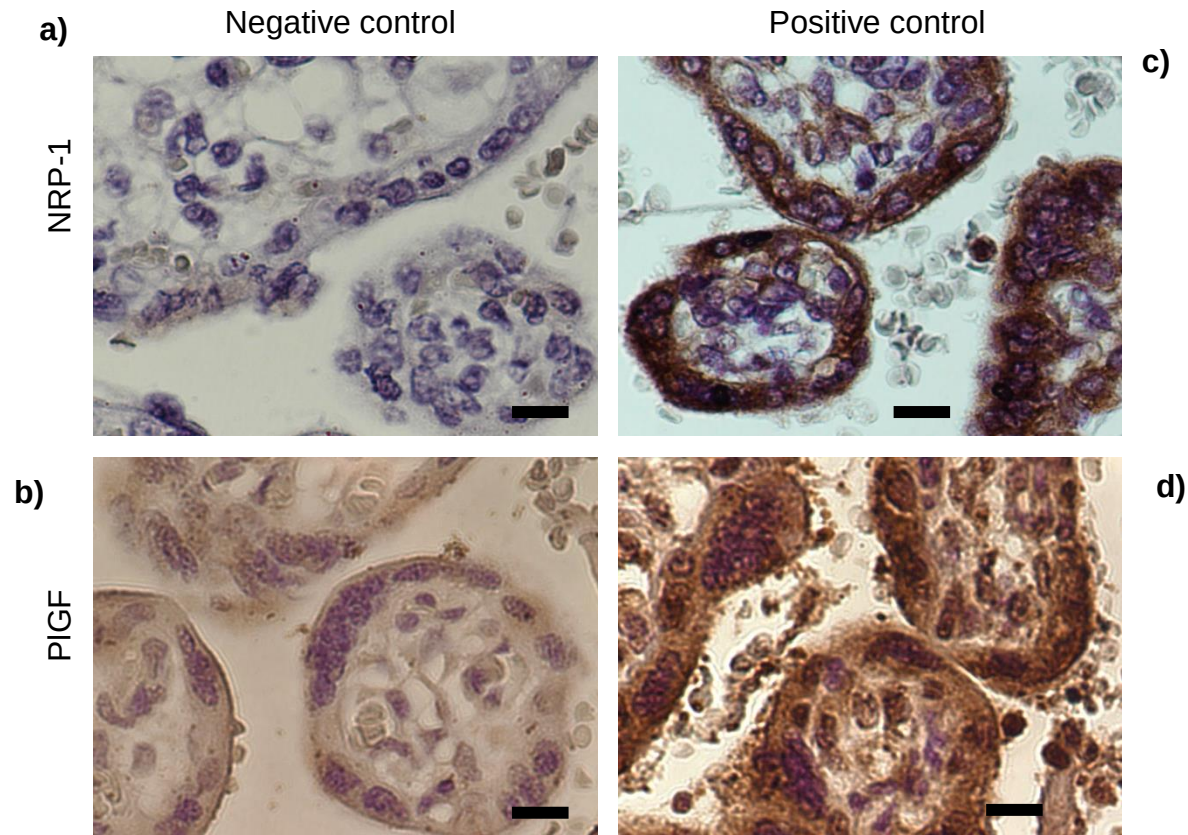
Supplementary Figure S2. Plasma NRP-1, PLGF, VEGF and TGFβ1 concentration in breast cancer and healthy controls. Graph represents a comparison of plasma concentration of NRP-1 (ng/mL), PLGF (pg/mL), VEGF (pg/mL) and TGFβ1 (ng/mL) ($\pm$ SEM) between healthy controls (n=50) and breast cancer patients (n=70) as quantified by ELISA.



	Curve Formula	A	B	R2
StdCurve	$Y=A*X+B$	0.000331	0.138	0.99



Supplementary Figure S3. Validation of ELISA. a) Graph is a representative standard curve generated using recombinant NRP-1/PIGF to determine the specificity and dynamic range of the ELISA. b) NRP-1 concentration, as determined by ELISA, in cell lysates isolated from breast cancer cell lines (MDA-MB-231, MCF-7 and BT-474) with known relative NRP-1 expression confirm the specificity of the ELISA.



Supplementary Figure S4. Immunohistochemistry staining for normal human placental tissue as a positive control for NRP-1. Control placental tissue was simultaneously stained with breast tumour tissue to ensure the specificity of the staining. a) and b) are control negative staining for placental tissue where the primary antibody was not added to the sections serving as a background for staining. c) and d) is full staining with NRP-1 and PLGF respectively, staining shows positive accumulation of both proteins in the placental tissue. Scale bar=50 μ m

Supplementary Table 1. Candidate reference genes primer sequences

	Gene	Primer Sequences	Accession No.	T _m (°C)
1	Ribosomal protein L13a (RPL13A), transcript variant 1	Fp: GAGGCCCTACCACTTCC Rp: AACACCTTGAGACGGTCCAG	NM_012423.3	65.7
2	Beta-2-microglobulin (B2M)	Fp: TAGGAGGGCTGGCAACTTAG Rp: CTTATGCACGCTTAACCTTAACAA	NM_004048.2	55.4
3	Actin, beta (ACTB)	Fp: CCAACCGCGAGAAGATGA Rp: CCAGAGGCGTACAGGGATAG	NM_001101.3	58.5
4	Pumilio RNA-binding family member 1 (PUM1), transcript variant 1	Fp: AGTGGGGGACTAGGCGTTAG Rp: GTTTTCATCACTGTCTGCATCC	NM_001020658.1	46.3
5	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), transcript variant 1	Fp: CCTGGCCAAGGTCATCCATG Rp: GGAAGGCCATGCCAGTGAGC	NM_002046.5	60.4
6	Hypoxanthine phosphoribosyltransferase 1 (HPRT1)	Fp: TTCTTTGCTGACCTGCTGG Rp: TCCCCTGTTGACTGGTCAT	NM_000194.2	63.5
7	Tubulin, beta 2A class IIa (TUBB2A)	Fp: CTGGCACCATGGACTCTG Rp: TCGGCTCCCTCTGTGTAG	NM_001069.2	64.4
8	Peptidylprolyl isomerase A (cyclophilin A) (PPIA), transcript variant 1	Fp: AGACAAGGTCCCAAAGAC Rp: ACCACCCTGACACATAAA	NM_021130.4	61.4
9	Glucuronidase, beta (GUSB), transcript variant 1	Fp: GAAAATACGTGGTTGGAGAGCTCATT Rp: CCGAGTGAAGATCCCCTTTTAA	NM_000181.3	59.4
10	Non-erythropoietic porphobilinogen deaminase(hydroxymethylbilane synthase (PBGD)	Fp: GGCTGCAACGGCGGAA Rp: CCTGTGGTGGACATAGCAATGATT	X04808.1	56.6

Supplementary Table 2. Target genes primer sequences

	Gene	Primer Sequences	Accession No.
1	Placental growth factor (PIGF)	Fp: GGCCAATGTCACCATGCA Rp: GAACGTCAGCTCCACGTAGGA	NM_002632
2	Semaphorin 3A (SEMA3A)	Fp: TTCAGCAATGGAGCTTTCCA Rp: CAACCCCAGCCGTTGAAC	NM_006080.2 NM_002632
3	Semaphorin 4A (SEMA4A)	Fp: TTCTTTGAGAGGCTCCACACATC Rp: CCGCCCACGTCATTCTTG	NM_022367.3
4	Plexin A1 (PLXNA1)	Fp: TCGATGGCAAGTCCGAGTACTT Rp: CGAAGGGATCTTGAGCTGTGA	NM_032242.3
5	Vascular endothelial growth factor receptor 1 (VEGFR1)	Fp: AAAGGCACCCAGCACATCAT Rp: TTCCCCCCTGCATTGGA	NM_002019.4
6	Vascular endothelial growth factor receptor 2 (VEGFR2)	Fp: CCTCCCCCGCATCACAT Rp: GCTCGTTGGCGCACTCTT	NM_002253.2
7	Vascular endothelial growth factor receptor 3 (VEGFR3)	Fp: CAACGGCATCCAGCGATT Rp: AAGGGATTTTCATGCACAATGA	NM_182925.4
8	E-cadherin	Fp: ACAGCCCCGCCTTATGATT Rp: TGAAGGAAGCGGTTCCGA	NM_004360.4
9	N-cadherin	Fp: TGGGAATCCGACGAATGG Rp: GCAGATCGGACCGGATACTG	NM_001792.4
10	Snail family transcriptional repressor 1 (SNAI1)	Fp: CCCCAATCGGAAGCCTAACT Rp: GCTGGAAGGTAACTCTGGATTAGA	NM_005985.3
11	Snail family transcriptional repressor 2 (SNAI2/SLUG)	Fp: CTGCGGCAAGGCGTTT Rp: CGTGTGAGTTCTAATGTGTCCTTGA	NM_003068.4
12	Vimentin (VIM)	Fp: AATGACCGCTTCGCCAACT Rp: ATCTTATTCTGCTGCTCCAGGAA	NM_003380.3
13	Neuropillin-1 (NRP-1)	Fp: CCAGGTCGAATCCGATCCT Rp: CGCTGTCGGTGTA AAAAACCA	NM_003873.5
14	Zinc finger E-box binding homeobox 1 (ZEB1)	Fp: GCCAATAAGCAAACGATTCTG Rp: TTTGGCTGGATCACTTTCAAG	NM_001128128.2
15	Vascular endothelial growth factor A (VEGF)	Fp: ATGACGAGGGCCTGGAGTGTG Rp: CCTATGTGCTGGCCTTGGTGAG	NM_001171623.1
16	Transforming growth factor beta-1 (TGFB1)	Fp: AAGGACCTCGGCTGGAAGTG Rp: CCGGGTTATGCTGGTTGTA	NM_000660.6
17	Hepatocyte growth factor (HGF)	Fp: CTCACACCCGCTGGGAGTAC Rp: TCCTTGACCTTGATGCATTC	NM_000601.5