

## Kallikrein-related peptidase 5 induces miRNA-mediated anti-oncogenic pathways in breast cancer

### Supplementary Material

**Supplementary Table 1:** Enriched miRNA target pathways in KLK5 reconstituted expressing breast cancer cells.

Down-regulated miRNAs				Up-regulated miRNAs			
KEGG Pathway	p-value	# genes	# miRNAs	KEGG Pathway	p-value	# genes	# miRNAs
MAPK signaling	0.00472 3	132	44	Glycosaminoglycan biosynthesis - heparan sulfate	1.15E-13	10	13
Apoptosis	0.04055 8	43	37	TGF-beta signaling pathway	4.83E-06	45	18
p53 signalling	0.04580 7	37	31	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	1.24E-05	36	18
Oocyte meiosis	0.00247 9	65	42	Endocytosis	1.52E-05	90	20
Osteoclast differentiation	0.04580 7	63	42	ECM-receptor interaction	1.52E-05	35	15
Vascular smooth muscle contraction	0.03920 3	58	39	Glioma	8.67E-05	32	17
T cell receptor signaling	0.04986 9	55	39	Axon guidance	0.00018	57	20
Amoebiasis	0.04580 7	50	39	Regulation of actin cytoskeleton	0.00107	93	19
mRNA surveillance	0.00891 3	48	38	Adherens junction	0.0039	38	14
Gastric acid secretion	0.00891 3	43	39	Pathways in cancer	0.0054	135	21
Calcium signalling	0.00793 7	89	46	Focal adhesion	0.0054	85	19
Renal cell carcinoma	0.00703 8	38	38	Wnt signaling pathway	0.0057	66	18
Protein processing in endoplasmic reticulum	0.00049 5	89	43	Neurotrophin signaling pathway	0.0068	57	19
Hedgehog signaling	0.02219 2	32	37	ErbB signaling pathway	0.0068	43	18
Basal cell carcinoma	0.03920 3	31	30	Salivary secretion	0.0068	42	17
Vasopressin-regulated water reabsorption	0.03401 1	29	25	Long-term potentiation	0.0068	35	17
Prion diseases	1.48E-09	19	24	GnRH signaling pathway	0.0384	43	19
D-Glutamine and D-	0.02659	4	5	Ubiquitin mediated	0.0092	59	19

glutamate metabolism	7			proteolysis			
Fatty acid biosynthesis	0.00368 5	1	1	Melanogenesis	0.0190	44	17

**Supplementary Table 2:** Combined (up and downregulated) miRNA target pathways in KLK5 reconstituted expressing breast cancer cell lines.

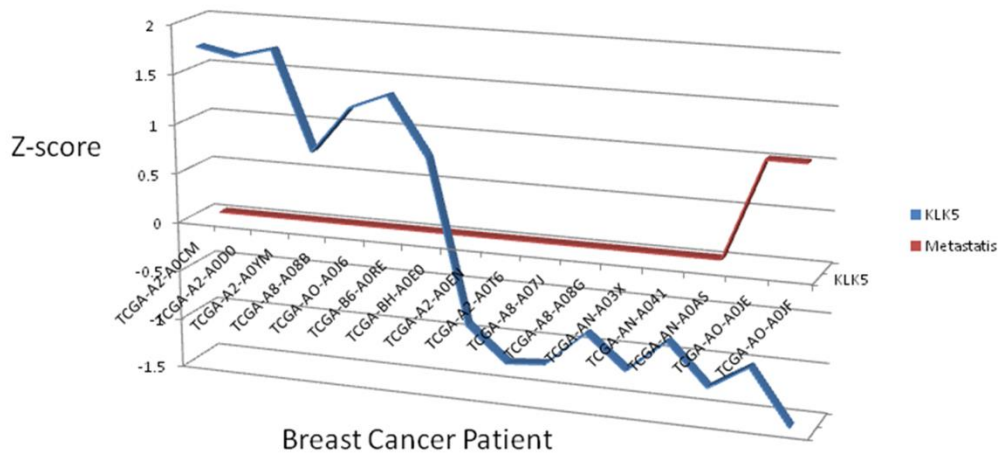
<b>KEGG Pathway</b>	<b>p-value</b>	<b># genes</b>
Pathways in cancer	<0.0001	70
Endocytosis	<0.0001	65
Ubiquitin mediated proteolysis	<0.0001	62
Focal adhesion	<0.0001	63
Wnt signaling pathway	<0.0001	63
Regulation of actin cytoskeleton	<0.0001	64
Neurotrophin signaling pathway	<0.0001	60
Insulin signaling pathway	<0.0001	60
Salivary secretion	<0.001	56
ErbB signaling pathway	<0.001	61
Pancreatic cancer	<0.001	59
Prostate cancer	<0.001	53
Melanogenesis	<0.001	53
Adherens junction	<0.001	56
Long-term potentiation	<0.001	52
Glioma	<0.001	59
TGF-beta signaling pathway	<0.001	57
Gap junction	<0.001	49
mTOR signaling pathway	<0.001	48

**Supplementary Table 3: miRNA binding sites of selected miRNA target genes.**

miRNA	Gene	RefSeqID	Seed Length	Start	Sequence	End	p-value	SPMS*
miR-1301	COL12A1	NM_080645	8	7468	UUGCAGCU	7461	0.0333	1
miR-1301	COL12A1	NM_004370	8	10960	UUGCAGCU	10953	0.0333	1
miR-183	COL12A1	NM_080645	8	6961	AUGGCACU	6954	0.0333	2
miR-183	COL12A1	NM_080645	8	6869	AUGGCACU	6862	0.0333	2
miR-183	COL12A1	NM_004370	8	10453	AUGGCACU	10446	0.0333	2
miR-183	COL12A1	NM_004370	8	10361	AUGGCACU	10354	0.0333	2
miR-25	COL12A1	NM_080645	8	6817	CAUUGCAC	6810	0.0333	1
miR-25	COL12A1	NM_004370	8	10309	CAUUGCAC	10302	0.0333	1
let-7 a*	COL24A1	NM_152890	8	5678	GAGGUAGU	5671	0.0198	2
miR-183	COL24A1	NM_152890	8	6065	UAUGGCAC	6058	0.0198	2
miR-206	DICER1	NM_030621	9	6681	GGAAUGUAA	6673	0.0163	1
miR-206	DICER1	NM_177438	9	6737	GGAAUGUAA	6729	0.0163	2
miR-19a	DROSHA	NM_001100412	8	5372	AGUUUUGC	5365	0.0142	2
miR-19a	DROSHA	NM_013235	8	5407	AGUUUUGC	5400	0.0142	2
miR-19a	EIF2C1	NM_012199	11	4837	GUUUUGCAUAG	4827	0.0011	2
miR-183	EIF2C2	NM_012154	8	3300	AUGGCACU	3293	0.0143	2
miR-335	EZR	NM_001111077	8	2726	UUUUUCAU	2719	0.0181	1
miR-335	EZR	NM_003379	8	2760	UUUUUCAU	2753	0.0181	1
miR-183	EZR	NM_001111077	8	2317	UAUGGCAC	2310	0.0181	1
miR-183	EZR	NM_003379	8	2351	UAUGGCAC	2344	0.0181	1
miR-181c	ITGB1	NM_033666	8	2801	ACAUUCA	2794	0.0154	2
miR-183	ITGB1	NM_002211	9	3637	UAUGGCACU	3629	0.0048	1
miR-183	ITGB1	NM_033667	9	3532	UAUGGCACU	3524	0.0049	1
miR-183	ITGB1	NM_033668	9	3497	UAUGGCACU	3489	0.0051	1
miR-183	ITGB1	NM_033669	9	3514	UAUGGCACU	3506	0.0049	1
miR-183	ITGB1	NM_133376	9	3552	UAUGGCACU	3544	0.0048	1
miR-19a	ITGB1	NM_033666	8	3024	AGUUUUGC	3017	0.0154	1
miR-19a	ITGB5	NM_002213	7	3203	UGUGCAA	3197	0.0415	1
miR-335	ITGB5	NM_002213	10	3308	UUUUCAUUAU	3299	0.0007	2
miR-335	ITGB5	NM_002213	7	2739	UUUUCAU	2733	0.0415	2
miR-449b	MMP2	NM_001127891	8	2187	AGGCAGUG	2180	0.019	1
miR-449b	MMP2	NM_004530	8	2334	AGGCAGUG	2327	0.019	1
miR-10b*	MMP3	NM_002422	8	1643	ACAGAUUC	1636	0.005	1
miR-520d-3p	MMP3	NM_002422	7	1533	AAAGUGC	1527	0.0199	1
miR-888	MMP3	NM_002422	7	1817	ACUGACA	1811	0.0199	2
miR-519a	MMP3	NM_002422	7	1533	AAAGUGC	1527	0.0199	1
let-7a*	MMP11	NM_005940	7	2111	UGAGGUA	2105	0.0469	1
miR-1301	MMP11	NM_005940	7	2102	UUGCAGC	2096	0.0469	1
let-7a*	MMP13	NM_002427	8	2372	CUAUACAA	2365	0.0193	1

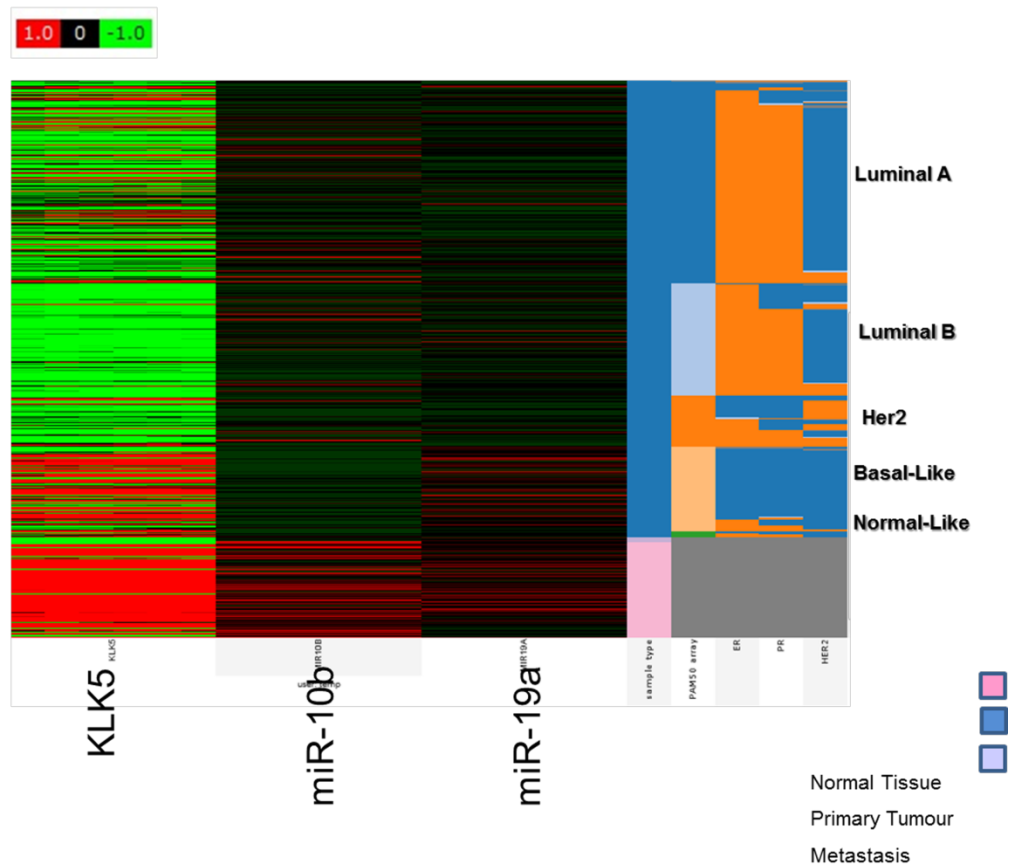
\* Starting Position of miRNA Seed Sequence. EIF2C1 =AGO1, EIF2C2 = AGO2.

Supplementary Figure 1



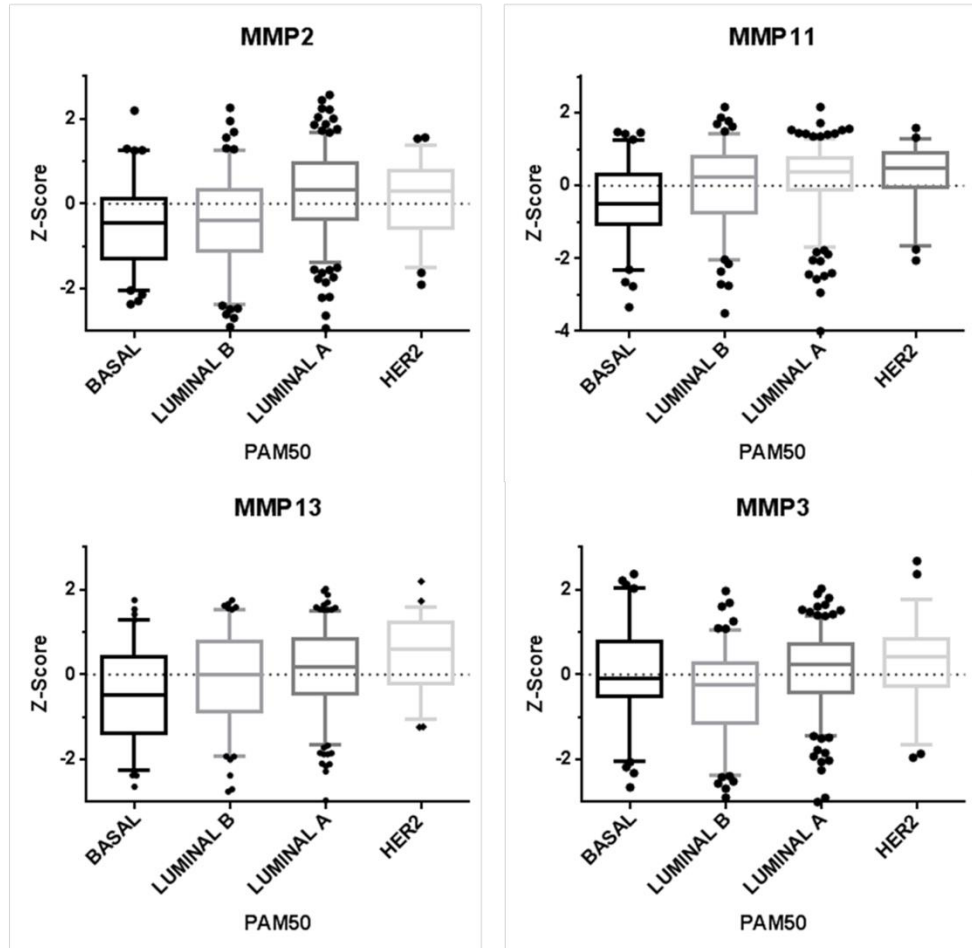
**Supplementary Figure 1:KLK5 expression and metastatic potential.** KLK5 expression plotted as z-score of 746 TCGA breast cancer patients – subset of 16 patients. Metastasis is shown as an absolute value (0 is no metastasis, 1 is metastasis was present as shown by sentinel node biopsy confirmed in the pathology reports). In this subset of patients, metastasis is demonstrated only in two of the lowest z-score KLK5 expressing patients.

## Supplementary Figure 2



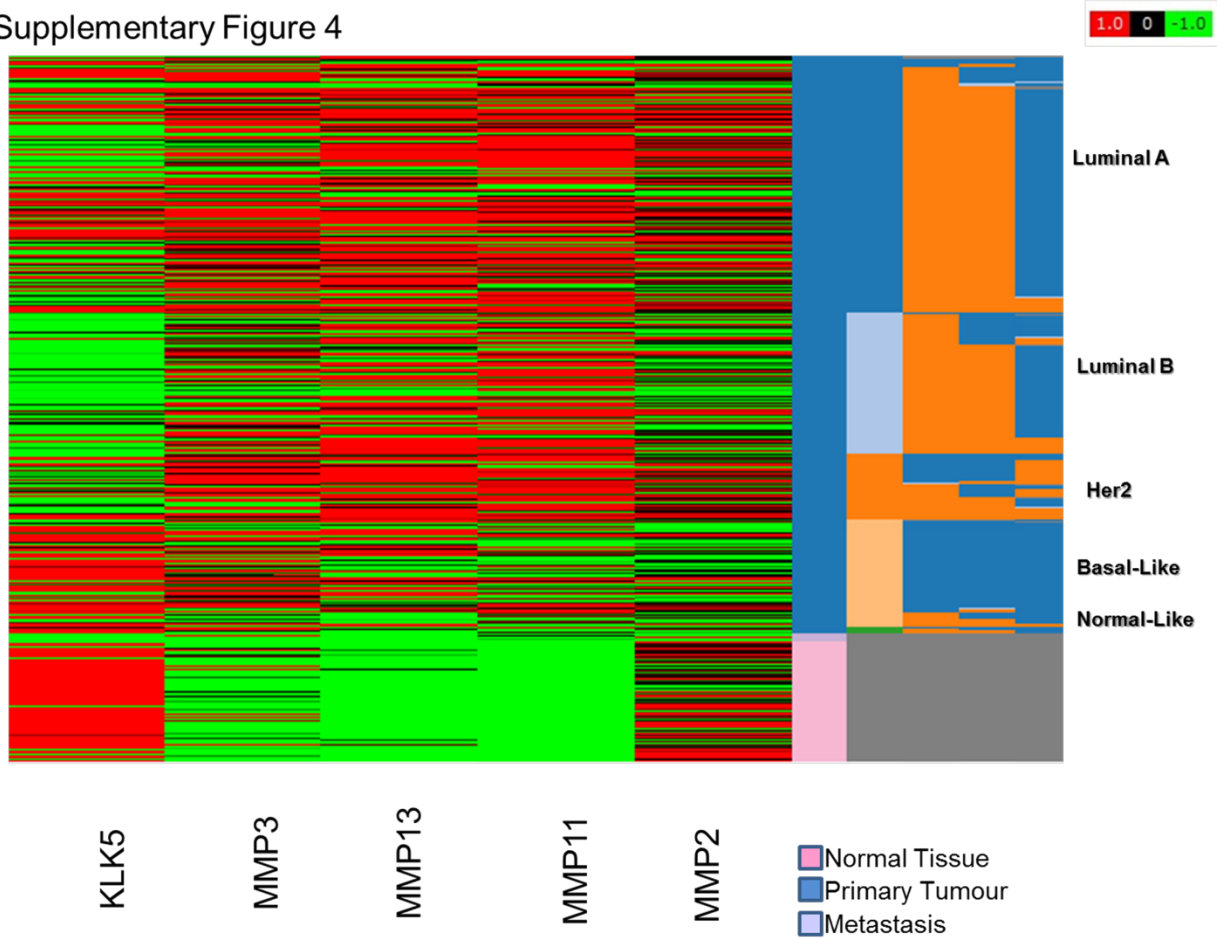
**Supplementary Figure 2:** IlluminaHiSeq data visualized using the Cancer Genomics Browser, n=1106 patients. Red indicates gene expression of +1 Z-score, green indicates -1 Z-score gene expression, and black indicates 0 Z-score<sup>[68]</sup>, [67].

### Supplementary Figure 3



**Supplementary Figure 3: KLK regulated MMP mRNA expression in invasive breast cancer subtypes.** Expression of MMP13, MMP2 and MMP11 are significantly reduced in basal-like human breast cancers as compared to luminal B breast cancer subtypes. MMP3 mRNA expression was increased in basal-like as compared to luminal B human breast cancers. Data plotted as Box and Whiskers Plot (5-95% with outliers plotted), data analyzed from TCGA <sup>[68]</sup>. 81 basal-like, 129 luminal B, 232 luminal A, and 58 Her-2 Breast cancers.

Supplementary Figure 4



**Supplementary Figure 4: Microarray visualization of MMP mRNA expression in invasive breast cancer subtypes.** IlluminaHiSeq data visualized using the Cancer Genomics Browser, n=1106 patients. Red indicates gene expression of +1 Z-score, Green indicates -1 Z-score gene expression, and black indicates 0 Z-score [67].