

Elevated Level of Nerve Growth Factor (NGF) in Serum-Derived Exosomes Predicts Poor Survival in Patients with Breast Cancer Undergoing Neoadjuvant Chemotherapy

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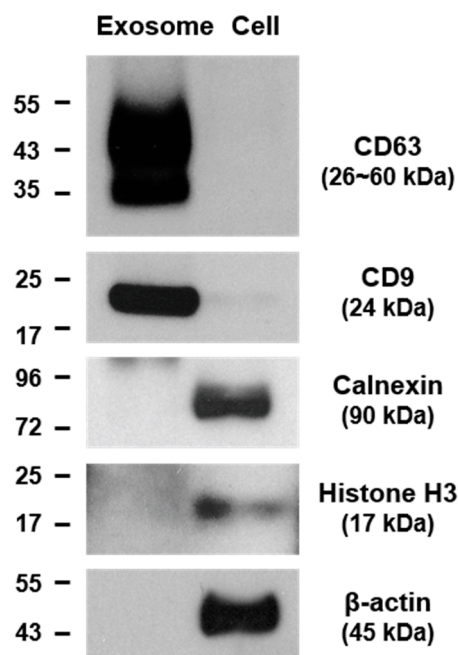


Figure S1. Characterization of exosome preparation by Western blot. Exosomes were isolated as described in Section 2.2. in Materials and Methods. The lysate of MDA-MB-231 cell line was loaded as control. The equal amounts of protein samples (10 μ g protein) were subjected to SDS-PAGE, followed by immunoblot with the indicated antibodies. The presence of exosomal markers (CD63 and CD9), and the absence of non-exosomal markers (Calnexin, Histone H3, and β -actin) demonstrates a pure exosome preparation.

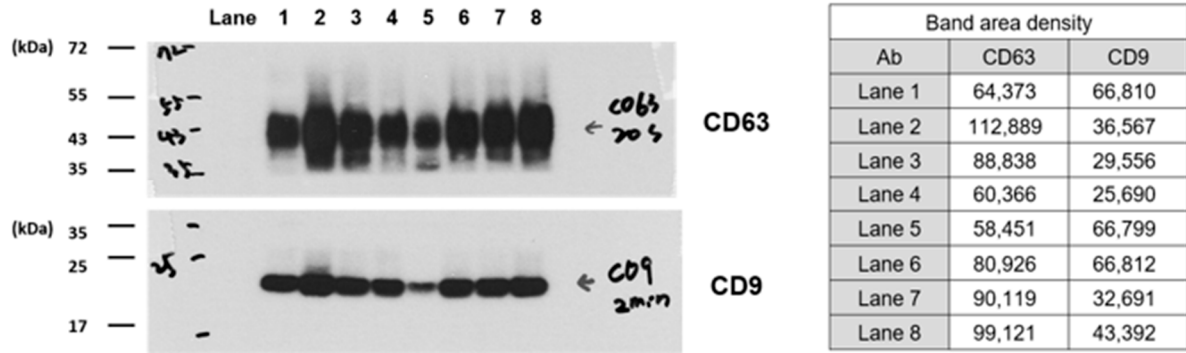


Figure S2. Uncut blots for Figure 1E.

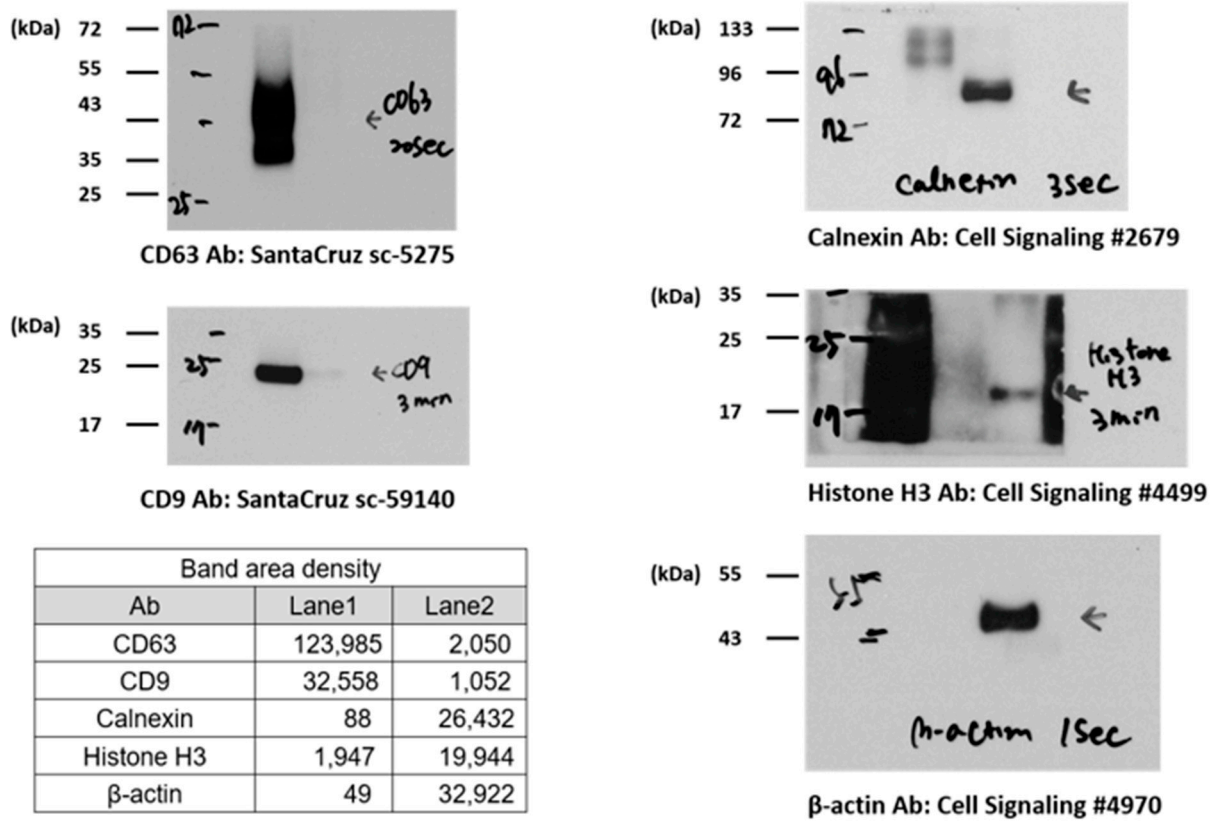


Figure S3. Uncut blots for Figure S1.

Table S1. Full name for each target in the ProcartaPlex immune-related Panel.

Analyte	Gene Name (HGNC)	Protein Name (Uniprot)	Exosomes (Mean \pm SD)	Serum (Mean \pm SD)
APRIL	TNFSF13	Tumor necrosis factor ligand superfamily member 13	212.1 \pm 224.9	933.8 \pm 727.4
BAFF	TNFSF13B	Tumor necrosis factor ligand superfamily member 13B	5.5 \pm 4.5	0.6 \pm 2.1
BLC	CXCL13	C-X-C motif chemokine 13	13.5 \pm 8	65.1 \pm 36.6
BTLA	BTLA	B- and T-lymphocyte attenuator	151.4 \pm 155	205.5 \pm 138.4
CD137/4-1BB	TNFRSF9	Tumor necrosis factor receptor superfamily member 9	1.1 \pm 3.7	2.7 \pm 6.5
CD152	CTLA4	Cytotoxic T-lymphocyte protein 4	30.6 \pm 46.9	4.9 \pm 8.3
CD27	CD27	CD27 antigen	27.8 \pm 47.9	152.5 \pm 130.8
CD28	CD28	T-cell-specific surface glycoprotein CD28	543.2 \pm 1349.4	21 \pm 95.8
CD30	TNFRSF8	Tumor necrosis factor receptor superfamily member 8	1.7 \pm 2.2	170.2 \pm 137
CD40L	CD40LG	CD40 ligand	1.3 \pm 6.3	20.6 \pm 22.6
CD80	CD80	T-lymphocyte activation antigen CD80	19.9 \pm 20.3	144 \pm 106.7
ENA-78	CXCL5	C-X-C motif chemokine 5	0.7 \pm 1.4	134.3 \pm 112.9
Eotaxin	CCL11	Eotaxin	0.8 \pm 0.7	44.2 \pm 23.7
Eotaxin-2	CCL24	C-C motif chemokine 24	12.5 \pm 9.3	64.9 \pm 31.2
Eotaxin-3	CCL26	C-C motif chemokine 26	28.3 \pm 34.7	0.1 \pm 0.3
FGF-2	FGF2	Fibroblast growth factor 2	100.1 \pm 86.4	-
Fractalkine	CX3CL1	Fractalkine, C-X3-C motif chemokine ligand 1	2.5 \pm 2.8	0.3 \pm 1.2
G-CSF	CSF3	Granulocyte colony-stimulating factor	324.8 \pm 381.1	0.4 \pm 2.5
GITR	TNFRSF18	Tumor necrosis factor receptor superfamily member 18	8.7 \pm 8.4	0.4 \pm 1.8
GM-CSF	CSF2	Granulocyte-macrophage colony-stimulating factor	7.2 \pm 11.4	1.8 \pm 15.1
GRO-alpha	CXCL1	Growth-regulated alpha protein	23.8 \pm 25.8	0.3 \pm 2.9
HGF	HGF	Hepatocyte growth factor	4.3 \pm 4.1	71.4 \pm 63.9
HVEM	TNFRSF14	Tumor necrosis factor receptor superfamily member 14	378.1 \pm 971	0.1 \pm 0.9
IDO	IDO1	Indoleamine 2,3-dioxygenase 1	10.3 \pm 10	11.5 \pm 11.1
IFN-alpha		Interferon alpha	1.5 \pm 1.2	0.1 \pm 0.4
IFN-gamma	IFNG	Interferon gamma	2.2 \pm 2.1	3.2 \pm 3.9
IL-1 alpha	IL1A	Interleukin-1 alpha	7.6 \pm 5.1	0.7 \pm 5.8
IL-1 beta	IL1B	Interleukin-1 beta	7.8 \pm 7.8	4.6 \pm 4.9
IL-10	IL10	Interleukin-10	2.9 \pm 3	-
IL-12p70		Interleukin 12p70	1.2 \pm 0.9	1.4 \pm 0.9
IL-13	IL13	Interleukin-13	0.6 \pm 0.8	0.1 \pm 0.1
IL-15	IL15	Interleukin-15	12.5 \pm 5.1	0.5 \pm 1.4
IL-16	IL16	Pro-interleukin-16	26 \pm 19.2	155.3 \pm 86.5
IL-17A	IL17A	Interleukin-17A	3.2 \pm 4.3	2.2 \pm 6.6
IL-18	IL18	Interleukin-18	4.4 \pm 4.9	7.2 \pm 11.5
IL-2	IL2	Interleukin-2	2.2 \pm 3.3	3.8 \pm 11.4
IL-20	IL20	Interleukin-20	2.2 \pm 10	3.5 \pm 30.2
IL-21	IL21	Interleukin-21	18 \pm 13.2	0.7 \pm 3.7
IL-22	IL22	Interleukin-22	36.8 \pm 24.5	1.1 \pm 5.9
IL-23		Interleukin-23	2968.2 \pm 6362.5	-
IL-27		Interleukin-27	4 \pm 12.2	-
IL-2R		Interleukin-2 receptor	775.4 \pm 464.5	175.1 \pm 657.4
IL-3	IL3	Interleukin-3	20 \pm 25.2	0.5 \pm 3.5
IL-31	IL31	Interleukin-31	66 \pm 77.2	-
IL-4	IL4	Interleukin-4	44.5 \pm 51.7	-
IL-5	IL5	Interleukin-5	15.5 \pm 18.8	-
IL-6	IL6	Interleukin-6	6.7 \pm 7.9	-
IL-7	IL7	Interleukin-7	0.1 \pm 0.2	0.5 \pm 0.8
IL-8	CXCL8	Interleukin-8	5.8 \pm 7.3	0.1 \pm 0.3
IL-9	IL9	Interleukin-9	16.3 \pm 22.1	-
IP-10	CXCL10	C-X-C motif chemokine 10	3.7 \pm 2.1	35.4 \pm 23.1
I-TAC	CXCL11	C-X-C motif chemokine 11	6.9 \pm 15	8.3 \pm 29.7

LAG-3	LAG3	Lymphocyte activation gene 3 protein	2.9 ± 4.8	22.1 ± 19.5
LIF	LIF	Leukemia inhibitory factor	0.4 ± 0.8	0.1 ± 0.3
MCP-1	CCL2	C-C motif chemokine 2	0.5 ± 1	48.3 ± 26.9
MCP-2	CCL8	C-C motif chemokine 8	0.1 ± 0.2	8.2 ± 4.6
MCP-3	CCL7	C-C motif chemokine 7	19.2 ± 10	3.1 ± 7
M-CSF	CSF1	Macrophage colony-stimulating factor 1	149.6 ± 202.9	0.4 ± 4.5
MDC	CCL22	C-C motif chemokine 22	32.3 ± 38.8	111 ± 51
MIF	MIF	Macrophage migration inhibitory factor	13.9 ± 10.5	45.2 ± 13.5
MIG	CXCL9	C-X-C motif chemokine 9	10.6 ± 13.9	6.2 ± 26.9
MIP-1 alpha	CCL3	C-C motif chemokine 3	0.5 ± 0.9	1.1 ± 2.4
MIP-1 beta	CCL4	C-C motif chemokine 4	0.3 ± 1.4	20.7 ± 25
MIP-3 alpha	CCL20	C-C motif chemokine 20	35 ± 68.3	16 ± 9.7
MMP-1	MMP1	Interstitial collagenase	0.5 ± 0.7	322.7 ± 351.4
NGF beta	NGF	Beta-nerve growth factor	16.8 ± 9.1	0.5 ± 5
PD-1	PDCD1	Programmed cell death protein 1	0.7 ± 1.8	11.3 ± 10.9
PD-L1	CD274	Programmed cell death 1 ligand 1	1 ± 1.1	0.6 ± 0.8
PD-L2	PDCD1LG2	Programmed cell death 1 ligand 2	16.4 ± 15.1	1730.5 ± 719.7
SCF	KITLG	Kit ligand	2.1 ± 2.1	11.2 ± 7.1
SDF-1 alpha	CXCL12	stromal cell-derived factor 1 alpha	160.1 ± 110.5	604.3 ± 157.7
TIM-3	HAVCR2	Hepatitis A virus cellular receptor 2	184.4 ± 270.9	666.1 ± 432.8
TNF alpha	TNF	Tumor necrosis factor	2.1 ± 2.3	2.7 ± 2.5
TNF beta	LTA	Lymphotoxin-alpha	31.1 ± 36	-
TNF-R2	TNFRSF1B	Tumor necrosis factor receptor superfamily member 1B	9.8 ± 8	48.9 ± 20.4
TRAIL	TNFSF10	Tumor necrosis factor ligand superfamily member 10	22.2 ± 16	2.5 ± 7.2
TSLP	TSLP	Thymic stromal lymphopoietin	2.6 ± 2.9	3.2 ± 2.5
TWEAK	TNFSF12	Tumor necrosis factor ligand superfamily member 12	151.9 ± 90.7	433.5 ± 295.1
VEGF-A	VEGFA	Vascular endothelial growth factor A	54.2 ± 40.1	161.3 ± 153.1