

Supplementary Tables

Supplementary Table S1: A list of materials and reagents that are recommended for the GeoMx DSP assays that are not supplied by NanoString.

Reagent/Material	Source/Part Number
10% neutral buffered formalin (NBF)	EMS Diasum, 15740-04
100% deionized formamide	VWR, VWRV0606
100% ethanol (EtOH): ACS grade or better	Various
10X citrate buffer pH 6	Sigma-Aldrich, C9999-1000ML
10X phosphate buffered saline pH 7.4 (PBS)	Invitrogen, 00652158
10X TBS with tween 20 (TBS-T)	Cell Signaling Technologies, 9997S
10X tris buffered saline (TBS)	Cell Signaling Technologies, 12498S
16% paraformaldehyde (PFA)	16% stock, 28908
1X phosphate buffered saline pH 7.4 (PBS)	ThermoFisher, 10010031
20X SSC (DNase, RNase free)	Sigma-Aldrich, S6639
BOND Dewax Solution, 1 L	Leica Biosystems, AR9222
BOND Epitope Retrieval 1, 1 L	Leica Biosystems, AR9961
BOND Epitope Retrieval 2, 1 L	Leica Biosystems, AR9640
BOND Wash Solution 10X Concentrate, 1 L	Leica Biosystems, AR9590
CitriSolv	Fisher Scientific, 04-355-121
DEPC-treated water	ThermoFisher, AM9922
Glycine	Sigma-Aldrich, G7126
Proteinase K	Ambion, AM2546
Tris base	Sigma-Aldrich, 10708976001
HybriSlip hybridization covers (22 mm x 40 mm x 0.25 mm)	Grace Bio-Labs, 714022
RNase Away	Thermofisher, 7003PK
Dextran sulfate	Sigma, 67578
BSA	VWR, 97061-420
Deoxyribonucleic acid, single stranded from salmon testes (denatured by heating at 95°C for 10 min prior to use)	Sigma, D7656
Agencourt AMPure XP	Beckman Coulter, A63880
Elution buffer (Tris-HCl 10 mM with 0.05% Tween-20, pH 8.0) *	Teknova, T1485
Bioanalyzer DNA High Sensitivity Kit	Agilent, 5067-4626
Qubit dsDNA HS Assay Kit	Q32854
GFAP antibody (GA-5) Alexa Fluor® 647	Novus Biologicals, NPB2-33184AF647, RRID:AB_2935766
nCounter Master Kit (Maxor FLEX Systems) Reagents and Cartridges	NAA-AKIT-012
BOND Research Detection System (includes 6x 30 mL Open Containers)	Leica Biosystems, DS9455
BOND Titration Kit (includes 50 inserts)	Leica Biosystems, OPT9049
BOND Universal Covertiles	Leica Biosystems, S21.2001
BOND Open Containers 30 mL	Leica Biosystems, OP309700

Supplementary Table S2. A list of NanoString supplied materials and reagents.

Reagent/Material	Source/Item
GeoMx Solid Tumor TME Morphology Kit Human Protein Compatible	Nanostring Technologies, GMX-PRO-MORP-HHST-12
GeoMx Solid Tumor TME Morphology Kit Mouse RNA FFPE Compatible	Nanostring Technologies, GMX-RNA-MORPH-MST-FFPE12
GeoMx Immune Cell Profiling Panel Human Protein Core for nCounter	Nanostring Technologies, GMX-PROCO-NCT-HICP-12
GeoMx Immune Activation Status Panel Human Protein Module for nCounter	Nanostring Technologies, GMX-PROMOD-NCT-HICT(AS)-12
GeoMx Immune Cell Typing Panel Human Protein Module for nCounter	Nanostring Technologies, GMX-PROMOD-NCT-HICT-12
GeoMx IO Drug Target Panel Human Protein Module for nCounter	Nanostring Technologies, GMX-PROMOD-NCT-HIODT-12
GeoMx Human Protein Core for NGS	Nanostring Technologies, GMX-PROCO-NGS-HCORE-12
GeoMx Immune Cell Typing Panel Human Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-HICT-12
GeoMx IO Drug Target Panel Human Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-HIODT-12
GeoMx Immune Activation Status Panel Human Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-HIAS-12
GeoMx Pan-Tumor Panel Human Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-HPT-12
GeoMx Myeloid Panel Human Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-HMY-12
GeoMx MAPK Signaling Panel Human Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-HMAPK-12
GeoMx PI3K/AKT Signaling Panel Human Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-HPI3K-12
GeoMx Neural Cell Typing Panel Human Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-HNCT-12
GeoMx Alzheimer's Pathology Panel Human Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-HADP-12
GeoMx Parkinson's Pathology Panel Human Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-HPDP-12
GeoMx Alzheimer's Pathology Extended Panel Human Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-HADEP-12
GeoMx Cell Death Panel Human Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-HCD-12
GeoMx Glial Cell Subtyping Panel Human Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-HGCS-12
GeoMx Autophagy Panel Human Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-HA-12
GeoMx Mouse Protein Core for NGS	Nanostring Technologies, GMX-PROCO-NGS-MCORE-12
GeoMx Immune Cell Typing Panel Mouse Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-MICT-12
GeoMx IO Drug Target Panel Mouse Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-MIODT-12
GeoMx Immune Activation Status Panel Mouse Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-MIAS-12
GeoMx Pan-Tumor Panel Mouse Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-MPT-12
GeoMx Myeloid Panel Mouse Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-MMY-12
GeoMx MAPK Signaling Panel Mouse Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-MMAPK-12
GeoMx PI3K/AKT Signaling Panel Mouse Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-MPI3K-12
GeoMx Neural Cell Typing Panel Mouse Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-MNCT-12
GeoMx Alzheimer's Pathology Panel Mouse Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-MADP-12
GeoMx Parkinson's Pathology Panel Mouse Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-MPDP-12
GeoMx Alzheimer's Pathology Extended Panel Mouse Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-MADEP-12
GeoMx Cell Death Panel Mouse Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-MCD-12
GeoMx Glial Cell Subtyping Panel Mouse Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-MGCS-12
GeoMx Autophagy Panel Mouse Protein Module for NGS	Nanostring Technologies, GMX-PROMOD-NGS-MA-12
GeoMx Protein Slide Prep Kit for FFPE	Nanostring Technologies, GMX -PREP-PRO-FFPE-12
GeoMx Hyb Code Pack Protein	Nanostring Technologies, GMX-PRO-HYB-96
GeoMx DSP Collection Plate	Nanostring Technologies, GMX-DSP-COLL-PLT-4
GeoMx DSP Instrument Buffer Kit	Nanostring Technologies, GMX-DSP-BUFF-KIT
GeoMx Seq Code Pack	Nanostring Technologies, GMX-NGS-SEQ-AB
GeoMx Human Whole Transcriptome Atlas Human RNA for Illumina Systems	Nanostring Technologies, GMX-RNA-NGS-HuWTA-4
GeoMx RNA Slide Prep Kit for FFPE	Nanostring Technologies, GMX-PREP-RNA-FFPE-12
GeoMx Cancer Transcriptome Atlas Human RNA for Illumina Systems	Nanostring Technologies, GMX-RNA-NGS-CTA-4
GeoMx Mouse Whole Transcriptome Atlas Mouse RNA for Illumina Systems	Nanostring Technologies, GMX-RNA-NGS-MsWTA-4

Supplementary Table S3: A list of Human protein targets by module.

NGS Human Panel	Target	NGS Human Panel	Target
GeoMx Human Immune Activation Status Panel	CD127	GeoMx Human NGS Alzheimer's Extended Pathology Panel	ADAM10
	CD25		BACE1
	CD27		IDE
	CD44		Nephrilysin
	CD45RO		Neurogranin
	CD80		PSEN1
	ICOS		p-Tau (S199)
	PD-1		p-Tau (S396)
GeoMx Human NGS Cell Death Panel	PD-L1	GeoMx Human NGS Alzheimer's Pathology Panel	p-Tau (T214)
	PD-L2		p-Tau (T231)
	BAD		Amyloid Precursor Protein
	BCL6		Amyloid-Beta 1-40
	BCLXL		Amyloid-Beta 1-42
	BIM		APOE
	CD95/Fas		P2RX7
	Cleaved Caspase 9		Phospho-Tau (S404)
GeoMx Human NGS Immune Cell Typing Panel	GZMA	GeoMx Human NGS Glial Cell Subtyping Panel	Phospho-Tdp-43 (S409/S410)
	NF-1 (Neurofibromin-1)		Tau
	p53		Tdp-43
	PARP		Ubiquitin
	CD20		ATG12
	CD3		ATG5
	CD34		BAG3
	CD4		GAB
GeoMx Human NGS MAPK Signaling Panel	CD56	GeoMx Human NGS Neural Cell Typing Panel	HSC70
	CD66b		LAMP2A
	CD8		LC3B
	Fibronectin		P62
	FOXP3		TFEB
	GZMB		VPS35
	4-1BB		C4B
	B7-H3		CD9
GeoMx Human NGS Myeloid Panel	CTLA4	GeoMx Human NGS Parkinson's Pathology Panel	Clec7a
	GITR		CSF1R
	IDO1		Ctsd
	LAG3		Emp1
	OX40L		GPNMB
	STING		Mertk
	Tim-3		S100B
	VISTA		VIM
GeoMx Human NGS Pan-Tumor Panel	BRAF	GeoMx Human NGS Core	GFAP
	EGFR		IBA1
	JNK (phospho T183/Y185)		MAP2
	MEK1 (phospho S217/S221)		Myelin basic protein
	P38 (phospho T180/Y182)		NeuN
	p44/42 MAPK ERK1/2		Neurofilament light
	p44/42 MAPK ERK1/2 (phospho T202/Y204)		Olig2
	P90RSK (phospho T359/S363)		P2ry12
GeoMx Human NGS PI3K/AKT Signaling Panel	pan-RAS	GeoMx Human NGS Core	Synaptophysin
	ARG1		TMEM119
	CD11b		Alpha-synuclein
	CD11c		ApoA-I
	CD14		Calbindin
	CD163		FUS
	CD39		LRRK2
	CD40		Park5
GeoMx Human NGS Cell Death Panel	CD68	GeoMx Human NGS Core	Park7
	HLA-DR		Phospho-Alpha-synuclein (S129)
	Bcl-2		PINK1
	EpCAM		Tyrosine Hydroxylase
	ER-alpha		Beta-2-microglobulin
	Her2		CD31
	MART1		CD45
	NY-ESO-1		GAPDH
GeoMx Human NGS Cell Death Panel	PAN-CK	GeoMx Human NGS Core	Histone H3
	PR		Ki-67
	PTEN		Ms IgG1
	SMA		Ms IgG2a
	AKT (phospho S473)		Rb IgG
	GSK3 (phospho S9)		S6
	GSK3αβ (phospho S21/S9)		
	INPP4B		
MET			
Pan-AKT			
PLCG1			
P-RAS-40 (phospho T246)			
TUBERIN (phospho T1462)			

Supplementary Table S4: A list of Mouse protein targets by module.

NGS Mouse Panel	Target	NGS Mouse Panel	Target
GeoMx Mouse Immune Activation Status Panel	CD127	GeoMx Mouse NGS Alzheimer's Extended Pathology Panel	BACE1
	CD27		IDE
	CD40L		Neprilysin
	CD44		Neurogranin
	CD86		PSEN1
	ICOS		p-Tau (S199)
	PD-1		p-Tau (S396)
	PD-L1		p-Tau (T214)
			p-Tau (T231)
GeoMx Mouse NGS Cell Death Panel	BAD	GeoMx Mouse NGS Alzheimer's Pathology Panel	Amyloid Precursor Protein
	BCLXL		Amyloid-Beta 1-42
	BIM		APOE
	Cleaved Caspase 3 gamma-H2AX		P2RX7
	NF-1 (Neurofibromin-1)		Phospho-Tau (S404)
	p21		Tau
	p53		Tdp-43
	PARP		Ubiquitin
	Perforin		
GeoMx Mouse NGS Immune Cell Typing Panel	BatF3	GeoMx Mouse NGS Autophagy Panel	ATG12
	CD19		ATG5
	CD28		BAG3
	CD3		Beclin-1
	CD34		LC3B
	CD4		P62
	CD8		PLA2G6
	Fibronectin		TFEB
	FOXP3		ULK1
	GZMB		VPS35
GeoMx Mouse NGS IO Drug Target Panel	B7-H3	GeoMx Mouse NGS Glial Cell Subtyping Panel	Aldh111
	CTLA4		CD9
	GITR		CSF1R
	LAG3		Ctsd
	OX40L		GPNMB
	Tim-3		Mertk
	VISTA		MSR1
			S100B
			SPP1
			VIM
GeoMx Mouse NGS MAPK Signaling Panel	BRAF	GeoMx Mouse NGS Neural Cell Typing Panel	GFAP
	EGFR		IBA1
	JNK (phospho T183/Y185)		MAP2
	MEK1		Myelin basic protein
	MEK1 (phospho S217/S221)		NeuN
	P38		Neurofilament light
	p44/42 MAPK ERK1/2		Olig2
p44/42 MAPK ERK1/2 (phospho T202/Y204)	Synaptophysin		
P90RSK (phospho T359/S363)	TMEM119		
	pan-RAS		Alpha-synuclein
GeoMx Mouse NGS Myeloid Panel	CD11b	GeoMx Mouse NGS Parkinson's Pathology Panel	ApoA-I
	CD11c		Calbindin
	CD14		LRRK2
	CD163		Park5
	CD39		Park7
	CD40		Phospho-Alpha-synuclein (S129)
	CD68		PINK1
	F4/80		Tyrosine Hydroxylase
	Ly6G/Ly6C		CD31
	MHC II		CD45
GeoMx Mouse NGS Pan-Tumor Panel	AhR	GeoMx Mouse NGS Core	GAPDH
	AR		GFP
	EpCAM		Histone H3
	ER-alpha		Ki-67
	Her2		Rat IgG2a
	IFNGR		Rat IgG2b
	PAN-CK		Rb IgG
	Pmel17		S6
	SMA		
GeoMx Mouse NGS PI3K/AKT Signaling Panel	AKT (phospho S473)		
	AMPK-alpha_pThr172		
	GSK3αβ (phospho S21/S29)		
	MET		
	Pan-AKT		
	PLCG1		
	P-RAS-40 (phospho T246)		
S6_pS235/236			

Supplementary Table S5: The 11-cell pellet array (CPA) used in assay development.

	Cell Line	Tissue	Cell Sex	Source, RRID
1	COLO201	Large intestine	Male	(ATCC Cat# CCL-224, RRID:CVCL_F399)
2	DAUDI	Haematopoietic and lymphoid	Male	(ATCC Cat# CCL-213, RRID:CVCL_0008)
3	H596	Lung	Male	(ATCC Cat# HTB178, RRID:CVCL_1571)
4	HDLM2	Haematopoietic and lymphoid	Male	(DSMZ Cat# ACC 17, RRID:CVCL_0009)
5	HEL	Haematopoietic and lymphoid	Male	(ATCC Cat# TIB-180, RRID:CVCL_2481)
6	HS578T	Breast	Female	(ATCC Cat# HTB-126, RRID:CVCL_0332)
7	HUT78	Haematopoietic and lymphoid	Male	(ATCC Cat# TIB161, RRID:CVCL_0337)
8	MALME3M	Skin	Male	(ATCC Cat# HTB-64, RRID:CVCL_1438)
9	OPM2	Haematopoietic and lymphoid	Female	(DSMZ Cat# ACC 50, RRID:CVCL_1625)
10	THP1	Haematopoietic and lymphoid	Male	(ATCC Cat# TIB-202, RRID:CVCL_0006)
11	U118MG	Brain	Male	(ATCC Cat# HTB-15, RRID:CVCL_0633)

Supplementary Table S6: The 45-cell pellet array (CPA) used in assay development.

CP#	Cell Line	Tissue Origin	GENE, Accession	Treatment	Cell Sex	Source, RRID
1	HEK293 Tim3	Kidney	TIM3, NM_032782.4		Female	(Crown Bio Cat# C2021)
2	HEK293 4-1BB	Kidney	CD137, NM_001561.5		Female	(Crown Bio Cat# C2012)
3	HEK293 PDL1	Kidney	CD274, NM_014143		Female	(Crown Bio Cat# C2011)
4	HEK293 PDL2	Kidney	PDL2, NM_025239.3	Stable over-expressor in HEK293T (RRID:CVCL_0045)	Female	(Crown Bio Cat# C2025)
5	HEK293 Lag3	Kidney	LAG3, NM_002286.5		Female	(Crown Bio Cat# C2020)
6	HEK293 GITR	Kidney	GITR, NM_009400.2		Female	(Crown Bio Cat# C2011)
7	HEK293 CTLA4	Kidney	CTLA4, NM_005214.4		Female	(Crown Bio Cat# C2015)
8	HEK293 PD1	Kidney	PDCD1, NM_005018		Female	(Crown Bio Cat# C2004)
9	HEK293 ICOS	Kidney	ICOS, NM_012092.3		Female	(Crown Bio Cat# C2016)
10	A431	Skin			Female	(ATCC Cat# CRL1555, RRID:CVCL_0037)
11	CCRF-CEM	Peripheral blood			Female	(ATCC Cat# CCL-119, RRID:CVCL_0207)
12	22rv1	Prostate			Male	(ATCC Cat# CRL2505, RRID:CVCL_1045)
13	H596	Lung			Male	(ATCC Cat# HTB178, RRID:CVCL_1571)
14	HCC78	Lung			Male	(DSMZ Cat# ACC 563, RRID:CVCL_2061)
15	HCT 116	Large intestine			Male	(ATCC Cat# CCL-247, RRID:CVCL_0291)
16	HL-60	Peripheral blood			Female	(ATCC Cat# CCL-240, RRID:CVCL_0002)
17	HUH7	Haematopoietic and Lymphoid			Male	(ECACC Sigma Cat# 01042712, RRID:CVCL_0336)
18	Hut78	Haematopoietic and Lymphoid			Male	(ATCC Cat# TIB161, RRID:CVCL_0337)
19	MDA-MB-468	Breast			Female	(ATCC Cat# HTB-132, RRID:CVCL_0419)
20	K-562	Haematopoietic and Lymphoid			Female	(ATCC Cat# CCL-243, RRID:CVCL_0004)
21	OVCAR8	Ovary			Female	(ATCC Cat# HTB-161, RRID:CVCL_1629)
22	Raji	Haematopoietic and Lymphoid			Male	(ATCC Cat# CCL-86, RRID:CVCL_0511)
23	Ri-1	Haematopoietic and Lymphoid			Female	(DSMZ Cat# ACC 585, RRID:CVCL_1885)
24	SU-DHL-6	B-cell Lymphoma			Male	(ATCC Cat# CRL-2959, RRID:CVCL_2206)
25	RPMI-8226	Haematopoietic and Lymphoid			Male	(ATCC Cat# CCL-155, RRID:CVCL_0014)
26	RAMOS	Haematopoietic and Lymphoid			Male	(ATCC Cat# CRL-1923, RRID:CVCL_0597)
27	SK-BR-3	Breast			Female	(ATCC Cat# HTB-30, RRID:CVCL_0033)
28	SK-MEL-5	Skin			Female	(ATCC Cat# HTB-70, RRID:CVCL_0527)
29	SU-DHL-1	Haematopoietic and Lymphoid			Male	(ATCC Cat# CRI-2955, RRID:CVCL_0538)
30	SU-DHL-4	B-cell Lymphoma			Male	(ATCC Cat# CRI-2957, RRID:CVCL_0539)
31	SK-MEL-2	Skin			Male	(ATCC Cat# HTB-68, RRID:CVCL_0069)
32	WSU-NHL	Haematopoietic and Lymphoid			Female	(DSMZ Cat# ACC 58, RRID:CVCL_1793)
33	THP-1	Peripheral blood			Male	(ATCC Cat# TIB-202, RRID:CVCL_0006)
34	SUP-B15	Haematopoietic and Lymphoid			Male	(ATCC Cat# CRL-1929, RRID:CVCL_0103)
35	U87-MG	Brain			Male	(ATCC Cat# HTB-14, RRID:CVCL_0022)
36	U251-MG	Brain			Male	(ECACC Sigma Cat# 09063001, RRID:CVCL_0021)
37	NB4	Haematopoietic and Lymphoid			Female	(DSMZ Cat# ACC 207, RRID:CVCL_0005)
38	BT-474	Breast			Female	(ATCC Cat# HTB-20, RRID:CVCL_0179)
39	NK-92	Peripheral blood			Male	(DSMZ Cat# ACC-488, RRID:CVCL_2142)
40	SW48	Large intestine			Female	(ATCC Cat# CCL-231, RRID:CVCL_1724)
41	DBTRG-05MG	Brain			Female	(ATCC Cat# CRL-2020, RRID:CVCL_1169)
42	NCI-H2228	Lung			Female	(ATCC Cat# CRI-5935, RRID:CVCL_1543)
43	A431 CA	Skin		Calyculin A (TOCRIS Cat #. 1336, 100 nM, 30 min)	Female	(ATCC Cat# CRL1555, RRID:CVCL_0037)
44	SH-SY5Y CA	Brain			Female	(DSMZ Cat# ACC 209, RRID:CVCL_0019)
45	SKBR3 PI	Breast			Female	(ATCC Cat# HTB-30, RRID:CVCL_0033)