

Supplementary Materials and Methods

Phospho antibody array

Meta4 organoids were treated with either DMSO or Pyrvinium for 24 hours to perform the antibody array covering 227 antibodies was performed using the ERK signaling phospho antibody microarray according to the manufacturer's manual (Full Moon BioSystems). Briefly, protein extraction from organoid was performed using protein extraction buffer containing a 1% protease inhibitor cocktail (Sigma), 1% phosphatase inhibitor cocktail (Sigma), and lysis beads. Extracted protein solution was purified using a gel matrix column. Concentration of the purified protein was measured with Pierce™ BCA protein assay kit (Thermo Fisher Scientific). Protein samples (60 µg) were treated with biotin/N,N-dimethylformamide solution for 90 min and stop reagent for 30 min. The slides were incubated with blocking solution on a shaker at 60 rpm for 30 min at RT. Next, protein samples were mixed into the coupling solution while the slides were washed ten times with high-grade distilled water. The blocked array slides were incubated with coupling mixture for 2 hours RT, and then the slides were washed with washing solution. Alexa Fluor™ 555 - streptavidin (Thermo Fisher Scientific) was mixed in detection buffer, treated to the coupled array slides for 20 min at RT. The slides were washed with washing solution and scanned using a GenePix 4100A scanner (Axon Instrument), and data were analyzed using GenePix 7.0 (Axon Instrument).

Flow cytometry

To obtain single cells, the Meta4 organoids were washed with ice-cold phosphate-buffered saline (PBS) and incubated with Gentle Cell Dissociation buffer (Stemcell Technologies) for 1 hour at 4°C and TrypLE (Gibco) for 5 min at 37°C. Cells were pelleted at 500×g for 5 min, washed with ice-cold Flow Cytometry Staining Buffer (eBioscience), and centrifuged at 500×g for 5 min. Cells were then incubated with fluorescence-conjugated antibodies diluted

in Flow Cytometry Staining Buffer for 30 min at 4°C, washed and resuspended in 1X Annexin-binding buffer. Pacific Blue™ Annexin V (5 µL) and 1 µL of 500 µM SYTOX™ AADvanced™ Dead Cell Stain solution was then added to each 100 µL of cell suspension. All antibodies used for Flow cytometry were listed in Supplementary Table 2. The cells were analyzed using the 5-Laser LSR II (BD Biosciences) in the Vanderbilt Flow Cytometry Shared Resource.

Immunostaining

To prepare formaldehyde-fixed paraffin-embedded (FFPE) organoids, organoids were fixed in 4% paraformaldehyde (PFA) at room temperature (RT) for 30 min. The fixed organoids were embedded in HistoGel™ (Thermo Fisher Scientific) and washed with PBS for 3 min. The samples were then processed using a standard histological protocol for paraffin embedding. Human tissues were fixed in 10% neutral buffered formalin for 1 week at RT and mouse tumor tissues were fixed in 4% PFA overnight at 4°C. The fixed tissues were processed according to a standard histological. Four to five micrometers of organoid or tissue paraffin sections were de-paraffinized in HistoClear solution (Electron Microscopy Services) and rehydrated through a series of ethanol (100%, 95% and 75%). Antigen retrieval was performed using a target retrieval solution (pH6, Dako) in a pressure cooker for 15 min. For immunofluorescence staining, each section was incubated in serum-free protein block solution (Dako) at RT for 1.5 hours. Primary antibodies were diluted in Antibody Diluent with background reducing components (Dako) and applied to sections. After overnight incubation at 4°C, slides were washed in PBS and secondary antibodies diluted in Antibody Diluent (Dako) were applied to each section. Sections were incubated at RT for 1 hour and Hoechst was applied to the sections for 5 min for nuclei counterstaining. All fluorescence images were acquired using a Zeiss Axio Imager M2 microscope with Axiovision digital imaging system or scanned using the Aperio Versa 200 Fluorescent Slide Scanner (Leica) at

the Vanderbilt Digital Histology Shared Resource. Preparation and overlay of fluorescence images were performed using Adobe Photoshop. For immunohistochemical staining, sections were blocked in Peroxidase Blocking Solution (Vector Laboratories) at RT for 20 min followed by serum-free protein block solution (Dako) at RT for 1.5 hours. Primary antibodies were diluted in Antibody Diluent with background reducing components (Dako) and applied to sections, then incubated overnight at 4°C. Horseradish-peroxidase-conjugated secondary antibodies were applied to sections and incubated for 15 min at RT followed by washes in PBS. ImmPRESS polymer detection reagent and ImmPACT DAB substrate kits (Vector Laboratories) were used for antibody detection. Images were scanned on a SCN400 slide scanner (Leica) at 20x magnification. All primary and secondary antibodies used for immunostaining are listed in Supplementary Table 2.

Supplementary Tables

Supplementary Table 1. Clinicopathological information of gastric cancer patient organoids

Organoids	Gender	Age	Lauren	WHO	T	N	M	Stage	MSI/MSS
hPCO-1	M	50	intestinal	MD	cT2	N0	M0	IA	MSS
hPCO-2	F	81	diffuse	SRCC	cT4a	N3b	M0	IIIC	MSS
hPCO-3	M	51	diffuse	Mixed (PCC90%+PD10%)	T1a	N0	M0	IA	unknown
hPCO-5	F	77	diffuse	Mixed (SRCC70%+PD30%)	T4b	N3a	M0	IIIC	MSS
hPCO-7	F	75	intestinal	PD	T1a	N0	M0	IA	unknown
hPCO-8	F	75	intestinal	PD	T1a	N0	M0	IA	unknown
hPCO-9	F	75	intestinal	PD	T1a	N0	M0	IA	unknown
hPCO-10	F	75	intestinal	PD	T1a	N0	M0	IA	unknown
hPCO-11	F	75	intestinal	PD	T1a	N0	M0	IA	unknown
hPCO-12	F	75	intestinal	PD	T1a	N0	M0	IA	unknown
hPCO-13	M	57	intestinal	MD	T4a	N1	M0	IIIA	MSS
hPCO-14	M	57	intestinal	MD	T4a	N1	M0	IIIA	MSS
hPCO-15	M	57	intestinal	MD	T4a	N1	M0	IIIA	MSS
hPCO-18	F	69	intestinal	MD	T3	N0	M0	IIA	MSI
hPCO-19	M	59	diffuse	SRCC	T4a	N3a	M0	IIIB	MSS
hPCO-25	M	58	intestinal	MD(MC 30%)	T4a	N3a	M0	IIIB	MSI
hPCO-27	M	58	intestinal	WD	T1a	N0	M0	IA	unknown
hPCO-28	M	70	intestinal	MD	T1a	N0	M0	IA	unknown
hPCO-31	M	70	intestinal	MD	T1a	N0	M0	IA	unknown
hPCO-34	M	50	intestinal	MD	T1a	N0	M0	IA	unknown

* MD; Moderate differentiation, PD; poorly differentiation, PCC; poorly cohesive carcinoma, SRCC; signet ring cell carcinoma,

MC; Micropapillary Carcinoma

Supplementary Table 2. Sequences of Primers

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Axin2</i>	ATGGAGTCCCTCCTTACCGCAT	GTTCCACAGGCGTCATCTCCTT
<i>LGR5</i>	AGAGCCTGATACCATCTGCAAAC	TGAAGGTCGTCCACACTGTTGC
<i>ZNRF3</i>	CTCATCGGTTCCACAGGAAGTG	CGTGTAAGGTTGCTTGTCTCCAC
<i>RNF43</i>	CTGGCTATACCAGCATCGGACT	ATGCTGGCGAATGAGGTGGAGT
<i>CDCA4</i>	AGCGACAGTCACTCCTGGACAT	CACACCATCCTGGCTCATTTC
<i>EPHB3</i>	CCTGTGTCAAGATCGAGGAGGT	CTTCAGCGTCTTGATAGCCACG
<i>SP5</i>	TCGTGTGCAACTGGCTCTTCTG	AGGTGATCGCTTCGCATGAAGC
<i>Ccnd</i>	GCAGAAGGAGATTGTGCCATCC	AGGAAGCGGTCCAGGTAGTTCA
<i>Ccnb</i>	AGAGGTGGAACCTTGCTGAGCCT	GCACATCCAGATGTTTCCATCGG
<i>Cdk1</i>	CATGGACCTCAAGAAGTACCTGG	CAAGTCTCTGTGAAGAACTCGCC
<i>Cdkn1a</i>	TCGCTGTCTTGCACTCTGGTGT	CCAATCTGCGCTTGAGTGATAG
<i>Myc</i>	TCGCTGCTGTCTCCGAGTCC	GGTTTGCCTCTTCTCCACAGAC
<i>Pim1</i>	CGCGACATCAAGGACGAGAACA	CGAATCCACTCTGGAGGACTGT
<i>Jun</i>	CAGTCCAGCAATGGGCACATCA	GGAAGCGTGTTCTGGCTATGCA
<i>Fos</i>	GGGAATGGTGAAGACCGTGTC	GCAGCCATCTTATTCCGTTCCC
<i>Bcl2l1</i>	GCCACCTATCTGAATGACCACC	AGGAACCAGCGGTTGAAGCGC
<i>Bcl2</i>	CCTGTGGATGACTGAGTACCTG	AGCCAGGAGAAATCAAACAGAGG
<i>Mcl1</i>	AGTTCATCGAACCATTAGCAGAA	CCTTCTAGGTCTGTACGTGGA
<i>Birc5</i>	CCTACCGAGAACGAGCCTGATT	CCATCTGCTTCTTGACAGTGAGG
<i>P53</i>	ACCGCCGACCTATCCTTACC	TCTTCTGTACGGCGGTCTCTC
<i>Vegf</i>	CTGCTGTAACGATGAAGCCCTG	GCTGTAGGAAGTCTATCTCTCC
<i>Hif1a</i>	CCTGCACTGAATCAAGAGGTTGC	CCATCAGAAGGACTTGCTGGCT
<i>CD133</i>	CTGCGATAGCATCAGACCAAGC	CTTTTGACGAGGCTCTCCAGATC
<i>CD166</i>	AGGAACATGGCGGCTTCAACGA	ACACCACAGTCGCGTTTCCACT
<i>Slfn4</i>	AGGAATGGGTGAAGCGCCAGAT	CAACCAGTGTTCCTTTACGCAGG
<i>Itgam</i>	TACTTCGGGCAGTCTCTGAGTG	ATGGTTGCCTCCAGTCTCAGCA
<i>Nos2</i>	GAGACAGGGAAGTCTGAAGCAC	CCAGCAGTAGTTGCTCCTCTTC
<i>Arg1</i>	CATTGGCTTGCGAGACGTAGAC	GCTGAAGGTCTTCCATCACC

Supplementary Table 3. Reagents and resources

Primary antibodies	Species	Vender	Catalog#
CD44v9 for mouse (1:25000)	Rat	Cosmo Bio Co. LTD.	LKG-M002
Trop2 for mouse (1:500)	Goat	R&D Systems	AF1122
Ki-67 (1:500)	Rat	Abcam	ab16667
UEA1(1:2000)	Conjugtae	Vector	FL-1061
H/K-ATPase (1:10000)	Mouse	Fitzgerald	10R-H100b
CD44v9 for human (1:15000)	Rat	Cosmo Bio Co. LTD	LKG-M001
Trop2 for human (1:500)	Goat	R&D systems	AF650
AQP5 (1:500)	Rabbit	Sigma	HPA065008
GAPDH (1:10000)	mouse	Cell signaling technology	97166S
phospho-STAT3 for IHC (1:2000)	Rabbit	Abclonal	AP1320
Pim1 (1:2000)	Rabbit	Abclonal	A19695
CD25(1:250)	Mouse	Novus Bio	AF2438-sp
CD4 (1:1000)	Rabbit	Abcam	ab183685
CD8 (1:1000)	Mouse	Santacruz	sc-1181
CD19 (1:1000)	Mouse	Santacruz	sc-373897
CD3 (1:2000)	Rabbit	Abcam	ab5690
NK1.1(1:250)	Mouse	Invitrogen	MAI-70100
CD163(1:500)	Rabbit	Proteintech	16646-1-AP
CD68 (1:200)	Mouse	SantaCruz	sc-20060
phospho-STAT3 (Y705) for WB (1:3000)	Rabbit	Cell signaling technology	9145S
phospho-STAT3 (S727) for WB (1:3000)	Rabbit	Cell signaling technology	9143S
total-STAT3 (1:3000)	Rabbit	Cell signaling technology	4904S
phospho-ERK (1:3000)	Rabbit	Cell signaling technology	4370
tERK (1:3000)	Rabbit	Cell signaling technology	4695
Tubulin (1:10000)	Rabbit	Sigma	T5168
Cyclin B1 (1:1000)	Rabbit	Abclonal	A16800
Secondary antibodies		Vender	Catalog#
Donkey anti-Rat IgG (H+L) Highly CrossAdsorbed Secondary Antibody, Alexa Fluor 594		ThermoFisher	A-21209
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488		ThermoFisher	A-21206
Donkey anti-Goat IgG (H+L) CrossAdsorbed Secondary Antibody, Alexa Fluor 647		ThermoFisher	A-21447
Donkey anti-Rat IgG (H+L) CrossAdsorbed Secondary Antibody, DyLight 650		ThermoFisher	SA5-10041
Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 555		ThermoFisher	A-31570
Anti-Mouse IgG (H+L), HRP Conjugate		Promega	W402B
Anti-Rabbit IgG (H+L), HRP Conjugate		Promega	W401B
Streptavidin, Alexa Fluor™ 555 conjugate		Invitrogen	S21381
Fluorescence-conjugated antibodies for FACS		Vender	Catalog#
Alexa Fluor 647 anti-mouse CD133		Biolegend	141216
Fluorescein-conjugated anti-mouse ALCAM/CD166		R&D systems	FAB1172F
Chemicals and Recombinant Proteins		Vender	Catalog#
SuperSignal™ West Femto Maximum Sensitivity Substrate		ThermoFisher	34096
Blotting-Grade Blocker		Bio-Rad	170-6404
Tween20		Sigma	P4916
10xTBS		corning	46-012-CM
Ponceau		Sigma	P7170
10xSDS/Tris/glycine		Bio-Rad	1610772
10xTris/glycine		Bio-Rad	1610771
Dual color protein Ladder		Bio-Rad	1610374

ReBlot PluReBlot Plus Strong Antibody Stripping Solution, 10x	Merck	2504
4% paraformaldehyde solution in PBS	Fisher Scientific	AAJ19943K2
Histoclear	National Diagnostics	HS-200
Hoechst, 33242	Invitrogen	62249
Dimethyl sulfoxide (DMSO)	Sigma	D2650
IntestiCult™ Organoid Growth Medium (Mouse)	STEMCELL technologies	06005
IntestiCult™ Organoid Growth Medium (Human)	STEMCELL technologies	06010
Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix	Corning	356231
Cultrex® Reduced Growth Factor Basement Membrane Extract, Type R1	R&D systems	3433-005-R1
Y-27632	MedChemExpress	HY-10583
MycoZap Plus PR	Lonza	VZA-2021
Penicillin/streptomycin	Gibco	15140122
10xPBS	Corning	21-040-CV
ProLong Gold Antifade Mountant	ThermoFisher	P36934
Trizol	Ambionlife technologies	15596026
Pyruvium pamoate	MedChemExpress	
HistoGel™	ThermoFisher	HG-4000-012
Target retrieval solution (pH 6)	Dako	S1699
Protein Block, Serum-Free	Dako	X0909
Antibody Diluent, Background Reducing	Dako	S3022
Antibody Diluent	Dako	S0809
BLOXALL® Endogenous Blocking Solution, Peroxidase and Alkaline Phosphatase	Vector Laboratories	SP-6000
ImmPRESS® HRP Horse Anti-Rabbit IgG Polymer Detection Kit, Peroxidase	Vector Laboratories	MP-7401
ImmPACT® DAB Substrate, Peroxidase (HRP)	Vector Laboratories	SK-4105
Advanced DMEM/F12	Gibco	12634-010
Fetal Bovine Serum (FBS)	PEAK Serum	PS-FB1
Trametinib	MedChemExpress	
CalceinAM	Invitrogen	C1430
EthD-1	Invitrogen	E1169
SSTC	MedChemExpress	
RIPA buffer	Sigma	R0278
4x Laemmli Sample Buffer	Bio-Rad	1610747
eBioscience™ Flow Cytometry Staining Buffer	Invitrogen	00-4222-26
GoScript™ Reverse Transcriptase (Mix+Random Primers)	Promega	A2801
Xylene	Supelco	108297
Tamoxifen	Sigma	T2859
Corn oil	Sigma	C8267
Commercial Assay kits	Vender	Catalog#
iTaq Universal SYBR Green Supermix	Bio-Rad	1725121
iScript™ gDNA Clear cDNA Synthesis Kit	Bio-Rad	1725035
Antibody array assay kit	Full moon bio	KAS02
Erk-phospho antibody array kit	Full moon bio	PEK208
PIPseq™ T2 3' Single Cell RNA Kit v4.0	FluentBio	
Pierce™ BCA Protein Assay Kit	Thermo Scientific	23227
RNeasy FFPE Kit	Qiagen	73504
Software and Algorithms	Vender	Catalog#
Amersham™ Imager 600	GE Healthcare Life Sciences	
EVOS M7000	ThermoFisher	2.0.2094.0
GraphPad Prism	GraphPad Software	Version 9.0.2
AxioVision SE64	Zeiss	versuin 4.9.1
PartekFlow	Partek	
Biorender		
FlowJo	BD Biosciences	Version 10.7.2
CFX Maestro	Bio-Rad	
StepOnePlus software version 2.3	Applied Biosystems	