

## **Supplementary Tables for**

**FGFR3 alterations in bladder cancer stimulate serine synthesis to induce immune-inert macrophages that suppress T-cell recruitment and activation**

**Supplementary Table 1. Key resources and reagents used in this study.**

<b>Reagents or Resources</b>	<b>Source</b>	<b>Identifier</b>
<b>Antibodies</b>		
Brilliant Violet 421™ anti-mouse CD45 (30-F11)	BioLegend	Cat# 103134, RRID: AB_2562559
Brilliant Violet 650™ anti-mouse CD8α (53-6.7)	BioLegend	Cat# 100741; RRID: AB_11124344
PE anti-mouse CD3 (17A2)	BioLegend	Cat# 100206, RRID: AB_312663
PE/Cyanine7 anti-mouse CD4 (GK1.5)	BioLegend	Cat# 100421, RRID: AB_312706
Alexa Fluor 647 anti-human/mouse Granzyme B (GB11)	BioLegend	Cat# 515406, RRID: AB_2566333
PE/Cyanine7 anti-mouse/human CD11b (M1/70)	BioLegend	Cat# 101215, RRID: AB_3127982
Brilliant Violet 510™ anti-mouse F4/80 (BM8)	BioLegend	Cat# 123135, RRID: AB_2562622
Alexa Fluor 647 anti-mouse CD206 (C068C2)	BioLegend	Cat# 141711, RRID: AB_10900240
PE anti-mouse CXCL9 (MIG-2F5.5)	BioLegend	Cat# 515603, RRID: AB_2245490
Brilliant Violet 605™ anti-mouse I-A/I-E (M5/114.15.2)	BioLegend	Cat# 107639, RRID: AB_2565894
Phospho-AKT1 (Ser473) (SDRNR)	Thermo Fisher	Cat# 12-9715-42, RRID: AB_2637101
PE anti-human HLA-DR	BioLegend	Cat# 422302, RRID: AB_2818986
PE anti-human CXCL9	BD Biosciences	Cat# 566013, RRID: AB_2739458)
Mouse anti-Alpha Tubulin Monoclonal	Proteintech	Cat# 66031-1-Ig, RRID: AB_11042766
Rabbit anti-Phospho-p44/42	Cell Signaling	Cat# 4s370, RRID: AB_2315112

MAPK (Erk1/2) (Thr202/Tyr204)	Technology	
Rabbit anti-Phospho-Erk5 (Thr218/Tyr220)	Cell Signaling Technology	Cat# 3371, RRID: AB_2140424
Rabbit anti-PSAT1	Proteintech	Cat# 10501-1-AP, RRID: AB_2172597
Mouse anti-AKT	Proteintech	Cat# 60203-2-Ig, RRID: AB_10912803
Rabbit anti-Tri-Methyl Lysine Motif [tme-K] (D1L1X)	Cell Signaling Technology	Cat# 14680, RRID: AB_2798568
HRP-labeled Goat Anti- Rabbit IgG (H+L) antibody (for WB)	Servicebio	Cat# GB23303, RRID: AB_2811189
HRP-labeled Goat anti- mouse IgG antibody (for WB)	Servicebio	Cat# GB23301, RRID: AB_2904020
Rabbit anti-F4/80 (D2S9R)	Cell Signaling Technology	Cat# 70076, RRID: AB_2799771
Rabbit anti-CD206/MRC1 (E6T5J)	Cell Signaling Technology	Cat# 24595, RRID: AB_2892682
Goat Anti-Mouse Cxcl9 / mig Polyclonal antibody	R&D Systems	Cat# AF-492-NA, RRID: AB_2086734
Rabbit monoclonal anti- CD8 (D4W2Z)	Cell Signaling Technology	Cat# 98941, RRID: AB_2756376
Rabbit anti-Granzyme B (D6E9W)	Cell Signaling Technology	Cat# 46890, RRID: AB_2799313
Mouse anti-CD68 (PG-M1)	Zhongshan Golden Bridge	Cat# ZM-0464
Mouse anti-HLA-DR	Zhongshan Golden Bridge	Cat# ZM-0136
Rabbit anti-CD8 Antibody	Abcam	Cat# MA5-14548, RRID: AB_10984334

HRP Goat Anti-Rabbit IgG Polymer Detection Kit (for IHC/IF)	Vector Laboratories	Cat# MP-7451; RRID: AB_2631198
HRP Goat Anti-Mouse IgG Polymer Detection Kit (for IHC/IF)	Vector Laboratories	Cat# MP-7444; RRID: AB_2336530
HRP Horse anti-Goat IgG Polymer Detection Kit (for IHC/IF)	Vector Laboratories	Cat# MP-7405; RRID: AB_2336526
<b>Chemicals, peptides, and recombinant proteins</b>		
Collagenase, type I	Servicebio	Cat# GC305013
Collagenase, type II	Servicebio	Cat# GC305014
Collagenase, type IV	Servicebio	Cat# GC305015
0.25% Trypsin-EDTA	Gibco	Cat# R001100
DNase	Servicebio	Cat# 1121MG010
Fixation and Permeabilization Solution	BD Biosciences	Cat# 554722
Perm/Wash Buffer	BD Biosciences	Cat# 554723
Fixable Viability Stain 510	BD Biosciences	Cat# 564406
Fixable Viability Stain 700	BD Biosciences	Cat# 564997
Diaminobenzidine (DAB)	DAKO	Cat# K5007
Hematoxylin	Biosharp	Cat# BL702A
SYBR Premix Ex Taq II	Yeasen	Cat# 11200ES03
Duvelisib	APExBIO	Cat# A1720
Erdafitinib	MCE	Cat# JNJ-42756493
S-Adenosyl-L-methionine chloride dihydrochloride	Sigma-Aldrich	Cat# A7007
L-phenylglycine	Sigma-Aldrich	Cat# 2935-35-5
RIPA Lysis Buffer	CWBIO	Cat# CW2333S
BCA Protein Assay Kit	CWBIO	Cat# CW0014S

Protease inhibitor Cocktail	CWBIO	Cat# CW2200S
Phosphatase Inhibitor Cocktail	CWBIO	Cat# CW2383S
Immobilon Western Chemiluminescent HRP Substrate	Millipore	Cat# WBKLS0500
RBC lysis buffer	CWBIO	Cat# CW0613S
Macrophage colony-stimulating factor	Sino Biological	Cat# 51112-MNAH
<b>Critical commercial assays</b>		
RNA Quick Purification kit	EZbioscience	Cat# B0004DP
PANO Multiplex IHC kit	PANOVUE	Cat# 10203100100
DL-Serine Assay Kit	Sigma-Aldrich	Cat# MAK352
<b>Experimental models: Cell lines</b>		
T24	ATCC	Cat# HTB-4
MB49	Millipore	Cat# SCC148
THP-1	ATCC	Cat# TIB-202
<b>Experimental models: Organisms/strains</b>		
Mouse: C57BL/6J	Hunan Silaikejingda	N/A
Mouse: BALB/c Nude mouse	Beijing Vital River	N/A
Mouse: huPMBC-NOG-dKO humanoid mouse	Beijing Vital River	N/A
<b>Oligonucleotides</b>		
Primers, see Supplementary Table 3	This paper	N/A
<b>Software and algorithms</b>		
Prism 9	GraphPad Software	RRID: SCR_002798, <a href="https://www.graphpad.com/">https://www.graphpad.com/</a>
FlowJo 10.7	Treestar	RRID: SCR_008520, <a href="https://www.flowjo.com/">https://www.flowjo.com/</a>

CytoFLEX 2.5	Beckman Coulter	RRID: SCR_019627, <a href="https://www.beckmancoulter.com">https://www.beckmancoulter.com</a>
GSEA software 4.2.2	Broad Institute	RRID: SCR_003199, <a href="http://www.broadinstitute.org/gsea">http://www.broadinstitute.org/gsea</a>
R 4.0.5	N/A	RRID: SCR_001905, <a href="https://www.r-project.org/">https://www.r-project.org/</a>
Cell Ranger 4.0	10x Genomics	RRID: SCR_017344, <a href="https://www.10xgenomics.com/support">https://www.10xgenomics.com/support</a>
Seurat 4.2.0	Satija Lab	RRID: SCR_016341, <a href="https://satijalab.org/seurat/">https://satijalab.org/seurat/</a>
SeuratWrapper 0.3.0	Satija Lab	RRID:SCR_022555, <a href="https://github.com/satijalab/seurat-wrappers">https://github.com/satijalab/seurat-wrappers</a>
inForm	Akoya	RRID: SCR_019155, <a href="https://www.akoyabio.com/">https://www.akoyabio.com/</a>
Phenochart	Akoya	RRID: SCR_019156, <a href="https://www.akoyabio.com/">https://www.akoyabio.com/</a>

**Supplementary Table 2. Gene set used for GSEA analysis on macrophages in this study.**

Gene sets	Source
GOBP_ANTIGEN_PROCESSING_AND_PRESENTATION_OF_ENDOGENOUS_ANTIGEN	<a href="https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/GOBP_ANTIGEN_PROCESSING_AND_PRESENTATION_OF_ENDOGENOUS_ANTIGEN">https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/GOBP_ANTIGEN_PROCESSING_AND_PRESENTATION_OF_ENDOGENOUS_ANTIGEN</a>
GOBP_LYMPHOCYTE_CHEMOTAXIS	<a href="https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/GOBP_LYMPHOCYTE_CHEMOTAXIS">https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/GOBP_LYMPHOCYTE_CHEMOTAXIS</a>
JAK/STAT Signaling	<a href="https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/KEGG_JAK_STAT_SIGNALING_PATHWAY">https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/KEGG_JAK_STAT_SIGNALING_PATHWAY</a>
JAK/STAT Cascade	<a href="https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/JAK_STAT_CASCADE">https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/JAK_STAT_CASCADE</a>
NFKB Pathway	<a href="https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/BIOCARTA_NFKB_PATHWAY">https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/BIOCARTA_NFKB_PATHWAY</a>
TGFB Pathway	<a href="https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/BIOCARTA_TGFB_PATHWAY">https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/BIOCARTA_TGFB_PATHWAY</a>
GMCSF Pathway	<a href="https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/PID_GMCSF_PATHWAY">https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/PID_GMCSF_PATHWAY</a>
PPARG-KO MACROPHAGE DN	<a href="https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/GSE24292_WT_VS_PPARG_KO_MACROPHAGE_DN">https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/GSE24292_WT_VS_PPARG_KO_MACROPHAGE_DN</a>
TLR4 induced NFKB AND MAPK Activation Signaling	<a href="https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/REACTOME_NFKB_AND_MAP_KINASES_ACTIVATION_MEDIATED_BY_TLR4_SIGNALING_REPERTOIRE">https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/REACTOME_NFKB_AND_MAP_KINASES_ACTIVATION_MEDIATED_BY_TLR4_SIGNALING_REPERTOIRE</a>
TGFB Treated Macrophages UP	<a href="https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/GSE45382_UNTREATED_VS_TGFB_TREATED_MACROPHAGES_UP">https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/GSE45382_UNTREATED_VS_TGFB_TREATED_MACROPHAGES_UP</a>
PI3K/AKT/mTOR Signaling	<a href="https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/HALLMARK_PI3K_AKT_MTOR_SIGNALING">https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/HALLMARK_PI3K_AKT_MTOR_SIGNALING</a>

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PI3K Cascade

[https://www.gsea-  
msigdb.org/gsea/msigdb/cards/REACTOME\\_PI3K\\_  
CASCADE](https://www.gsea-msigdb.org/gsea/msigdb/cards/REACTOME_PI3K_CASCADE)

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**Supplementary Table 3. Primer sequences used in this study.**

Gene		Sequence
<i>Cxcl9</i>	F	TCCTTTTGGGCATCATCTTCC
	R	TTTGTAGTGGATCGTGCCTCG
<i>Cxcl10</i>	F	CCAAGTGCTGCCGTCATTTTC
	R	GGCTCGCAGGGATGATTTCAA
<i>Cxcl11</i>	F	GGCTTCCTTATGTTCAAACAGGG
	R	GCCGTTACTCGGGTAAATTACA
<i>Cd74</i>	F	AGTGCGACGAGAACGGTAAC
	R	CGTTGGGGAACACACACCA
<i>PSAT1</i>	F	TGCCGCACTCAGTGTTGTTAG
	R	GCAATTCCC GCACAAGATTCT
<i>Psat1</i>	F	CTTAGCACCATGGAAGCCAC
	R	TCCAGCTTTCAGGCCAATCA

**Supplementary Table 4. The clinicopathologic characteristics of the SYSMH cohort.**

<b>Variables</b>	<b><i>FGFR3</i>-WT (<i>n</i> = 24)</b>	<b><i>FGFR3</i>-altered (<i>n</i> = 10)</b>
Age (y, median, IQR)	63 (54.75- 70.25)	60.5 (57- 65)
<65	13 (54.2%)	7 (70.0%)
≥65	11 (45.8%)	3 (30.0%)
Gender		
Male	20 (83.3%)	7 (70.0%)
Female	4 (16.7%)	3 (30.0%)
Tumor size (cm)		
<3	10 (41.7%)	6 (60.0%)
≥3	14 (58.3%)	4 (40.0%)
Multifocality		
Unifocal	21 (87.5%)	5 (50.0%)
Multifocal	3 (12.5%)	5 (50.0%)
TNM stage		
I-II	11 (45.8%)	9 (90.0%)
III-IV	13 (54.2%)	1 (10.0%)