Supplementary Figure Legends

Supplementary Fig. S1. μCT quantification of cortical and trabecular bone. 2 month-old WT and *mda-9*$^{-/-}$ mice were given intracardiac (I.C.) injection of murine PC cells (RM1-BM-*Luc*, Experiment 1A). Femurs were harvested 10-days post injection and μCT analysis was conducted. N = 6 legs/condition; ANOVA-Tukey $p<0.05$ using SEM. Groups not sharing a letter are statistically significant.

Supplementary Fig. S2. A summary of "death" and "metastatic events" from bone marrow transfusion studies (From Fig. 1E) are tabulated. "±" weakly positive.

Supplementary Fig. S3. A) Blots from antibody-based arrays showing differential expression levels of various chemokines in tumor-bearing serum isolated from WT vs *mda-9*$^{-/-}$ animals. **B)** Bone marrow (BM) cells were isolated from WT and *KO (mda-9*$^{-/-}$) mice and stimulated with different murine tumor cell-derived conditioned media, C2-TRAMP or B6CaP, for 12 hr. Total cellular RNA was extracted, and qPCR was performed to detect mouse CXCL5 mRNA. Data is presented as fold-change relative to the unstimulated wild type (BM^{WT}) group. Different letters in two variables are statistically significant ($p<0.05$). **C)** 3 X 10^4 RM1-BM-*Luc* cells were injected by the intracardiac (I.C.) route into WT and *mda-9*$^{-/-}$ mice. 14-day post-injection, total BM cells were isolated and stained for Macrophage ($CD45^+CD11b^+$), NK cell ($CD45^+NK1.1^+$), Dendritic Cell ($CD45^+CD11b/c^+$) and intracellular CXCL5 expression for identifying the specific cell population(s) that differentially respond to tumor cells. Percentages of CXCL5 expression in different populations is presented. **D)** Bone marrow-derived mesenchymal stromal cells (BM-MSCs) were isolated from WT and *mda-9*$^{-/-}$ mice and stimulated with tumor cell-derived conditioned media for 12 hrs. Total cellular RNA was extracted, and qPCR was performed to detect mouse *CXCL10* and *CCL11* mRNA. Data is presented as fold change relative to the unstimulated wild type ($BM\text{-}MSC^{WT}$) group. *$p<0.05$. "ns": non-significant.

Supplementary Fig. S4. Therapeutic potential of Anti-CXCL5. 1 x 10^4 RM1-BM-*Luc* cells were surgically implanted in the left femur and treated with control or anti-CXCL5 (three doses) within the first three days. **A)** Mice were imaged weekly and luciferase intensity was calculated using a built-in Living Image Software v.2.50. Representative photographs at day-7 and -14 are presented. **B)** Calculated ROI is presented. **C)** Changes of BLI intensity during two weeks of tumor growth of individual mice are graphically presented. Red Circle: Control

Antibody; Blue square: Anti-CXCL5. Solid line from each group is the average value from 7 animals. * Statistically significant.

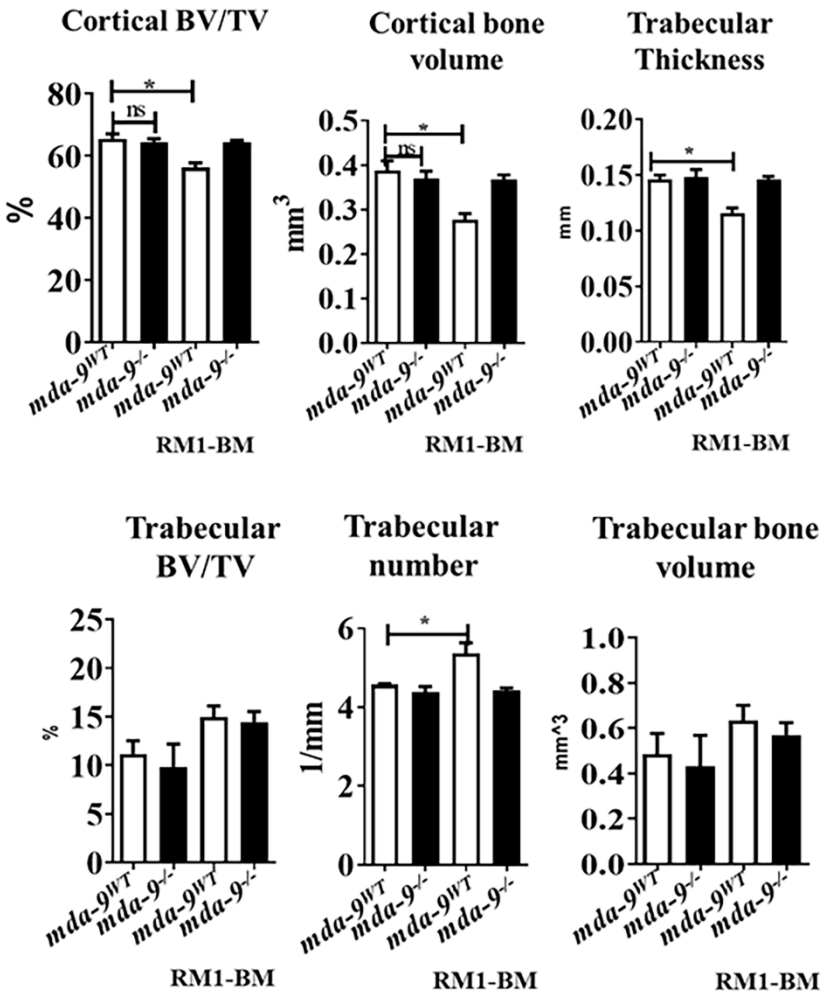
Supplementary Figure S5. A) Expression of MDA-9/Syntenin in PC-3ML and its knockout clones. **B)** HS5 cells were incubated with normalized (equal amount of total protein) tumor cell-derived condition media for 12 hrs. Total RNA was extracted from HS5-cells and analyzed for CXCL5 levels using qPCR. Different letters in two variables are statistically significant ($p<0.05$). **C)** Schematic presentation of the Hippo signaling pathway.

Supplementary Fig. S6. A) Blots from antibody-based arrays showing differential expression levels of various growth factors in tumor cell-derived conditioned media. B) HS5 cells were incubated with GM-CSF or IGFBP4 for 12 hrs. Total RNA was extracted from HS5-cells and analyzed for CXCL5 levels using qPCR. Different letters in two variables are statistically significant ($p<0.05$).

Supplementary Figure S7. A) HS5 cells were incubated with GM-CSF for the indicated time points. CXCL5 secretion was analyzed by ELISA. B) HS5 cells were cultured with indicated PC3-ML conditioned media fractions in the presence or absences of Anti-PDGF-AA for 24 hrs. after initial transfection with a *CXCL5*-Prom. Luciferase activity was measured and presented after normalizing with *Renilla luciferase*. *$p <0.05$. C) PC-3ML cells were treated with commercially available pathway inhibitors and conditioned media were analyzed for PDGF-AA expression. Different letters in two variables are statistically significant ($p< 0.05$).

Supplementary Figure S8. BM-MSCs isolated from WT or KO mice were stimulated with mouse PDGF-AA and *CXCL10* and *CCL11* expression was determined using qPCR. Different letters in two variables are statistically significant ($p<0.05$).

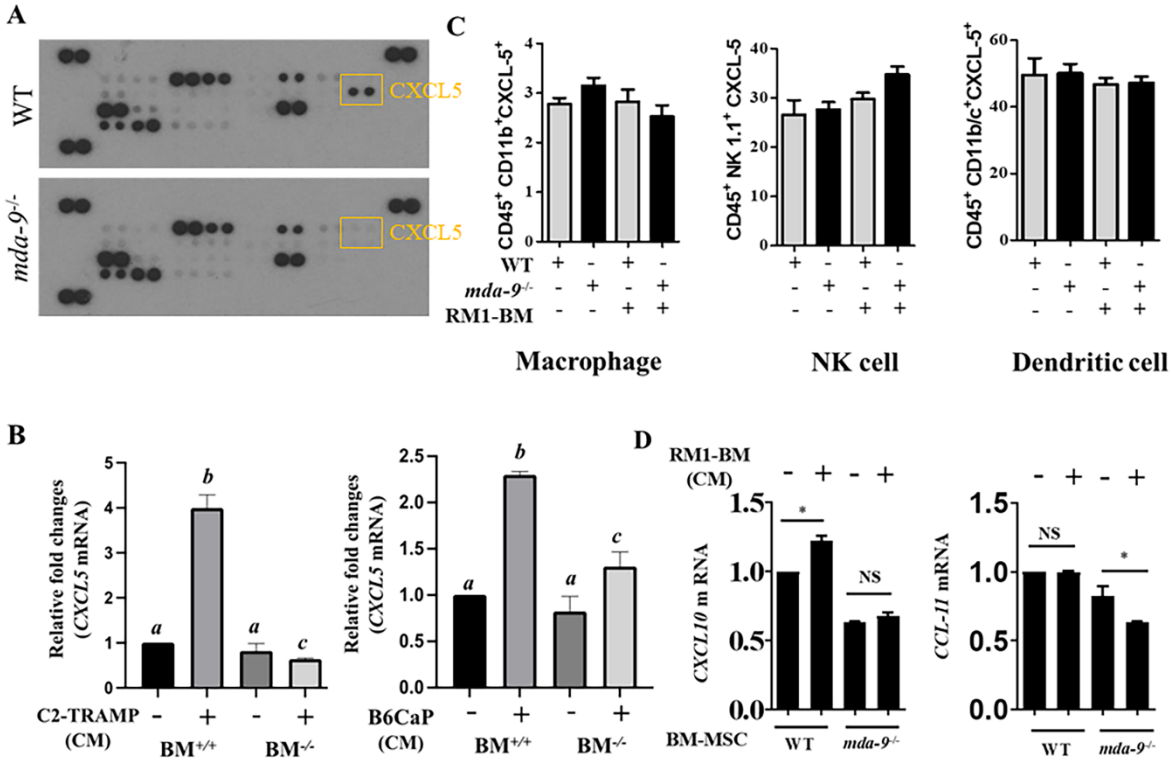
Maji *et al.*, Supplementary Fig. S1



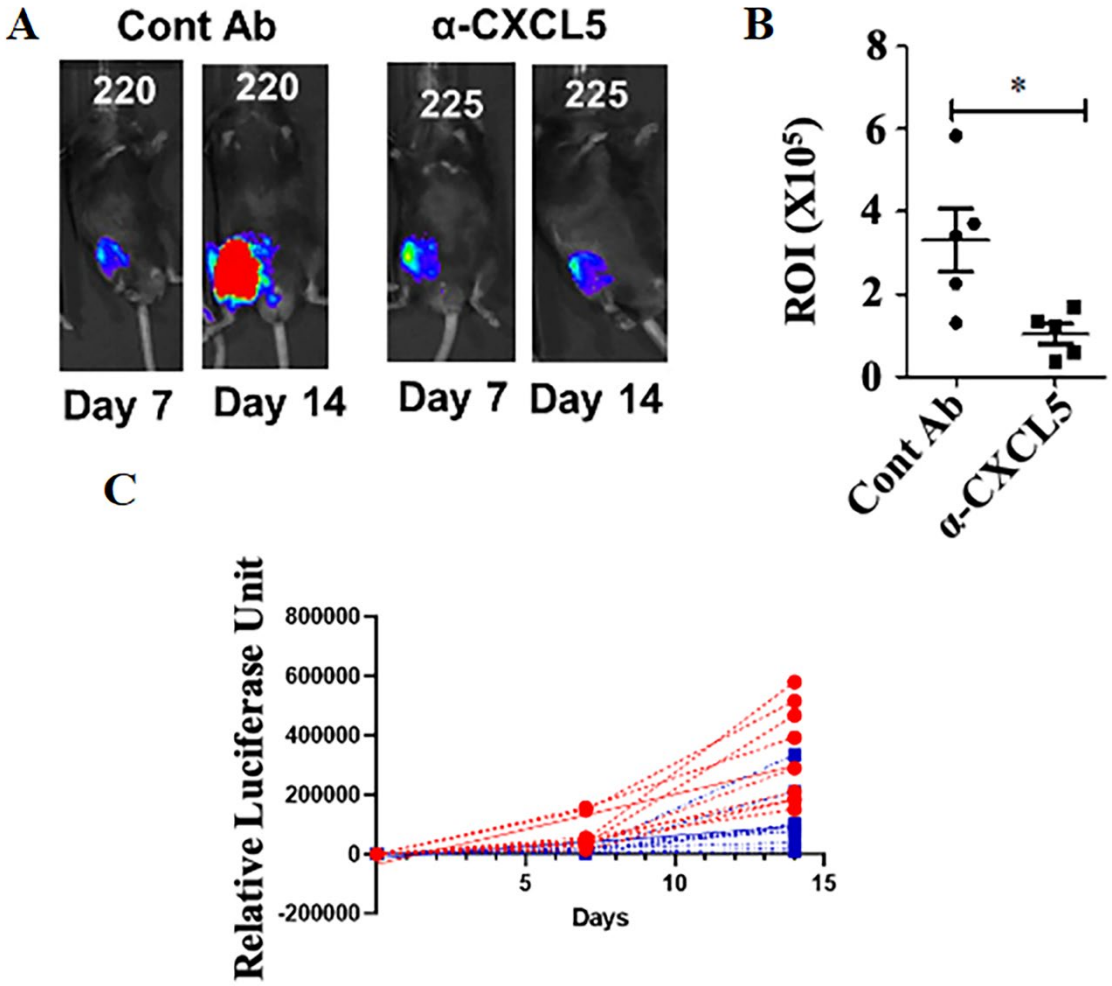
Maji *et al.*, Supplementary Fig. S2

Donor	Recipient	Death by Day 11	Metastatic site(s) (no. of animals examined on Day 12 (BLI)
Sham Transfusion	*mda-9*^{WT}	2	Liver (1/3); Bone (1/3)
BM^{WT}	*mda-9*^{WT}	2	Liver (3/3); Bone (1/3)
Sham Transfusion	*mda-9*$^{-/-}$	0	Liver (±1/5); Bone (0/5)
BM^{WT}	*mda-9*$^{-/-}$	2	Liver (1/3); Bone (2/3)

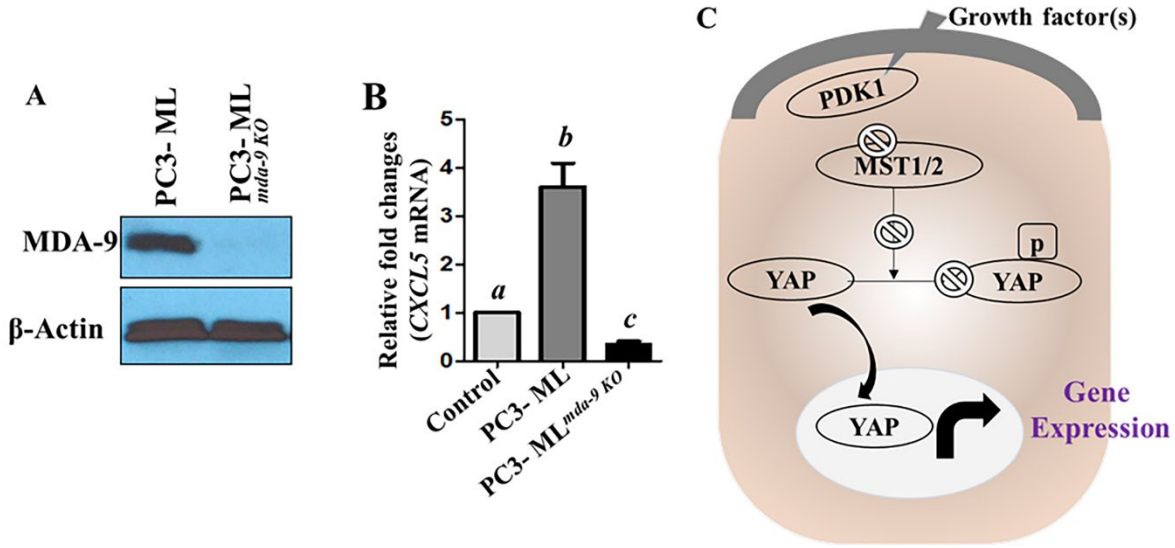
Maji *et al.*, Supplementary Fig. S3



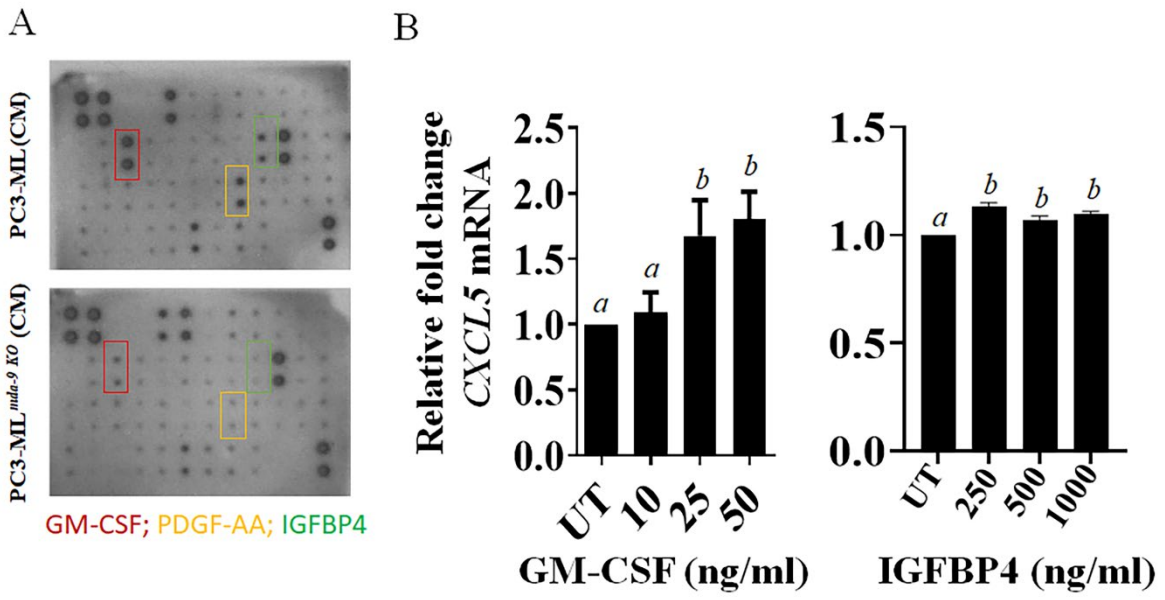
Maji *et al.*, Supplementary Fig. S4



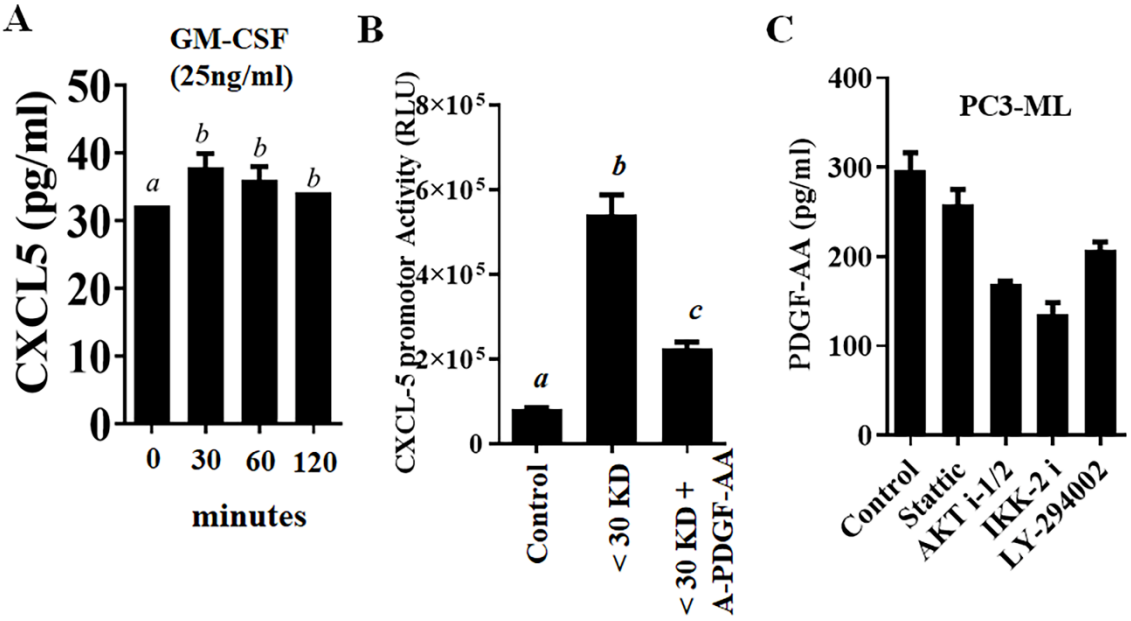
Maji *et al.*, Supplementary Fig. S5



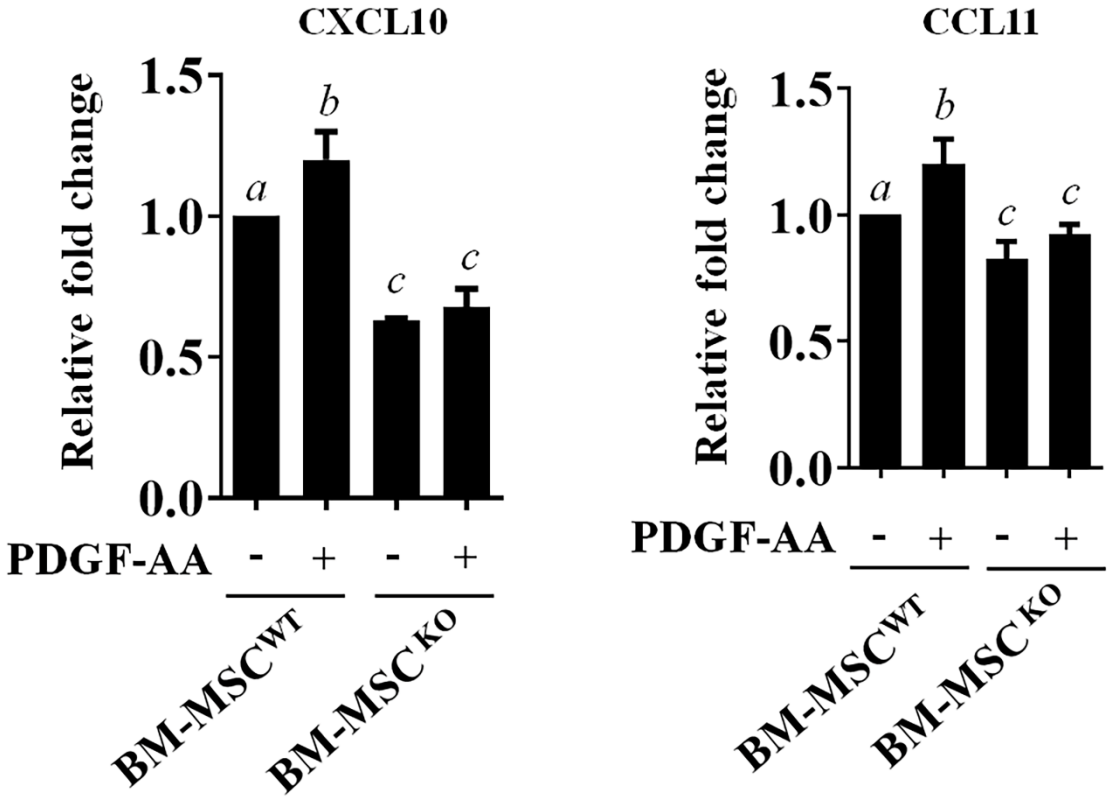
Maji *et al.*, Supplementary Fig. S6



Maji *et al.*, Supplementary Fig. S7



Maji *et al.*, Supplementary Fig. S8



Supplementary Tables

Supplementary Table S1. Proteome Profiler TM Array: Mouse Chemokine Array (ARY020)

Chemokine Array		
Coordinate	**Analyte/Control**	**Alternate Nomenclature**
A1, A2, A19, A20	Reference Spots N/A	RS
B3, B4	6Ckine	CCL21, SLC, Exodus-2
B5, B6	BLC	CXCL13, BCA-1
B7, B8	C10	CCL6, MRP-1
B9, B10	Complement Component C5/C5a	C5/C5a
B11, B12	CCL28	MEC
B13, B14	Chemerin	RARRES2
B15, B16	CTACK	CCL27, ALP, ILC, ESkine
B17, B18	CXCL16	SRPSOX
C3, C4	Eotaxin	CCL11
C5, C6	Fractalkine	CX3CL1
C7, C8	IL-16	____
C9, C10	IP-10	CXCL10, CRG-2, C7
C11, C12	I-TAC	CXCL11, H174, SCYB9B
C13, C14	JE	CCL2, MCP-1
C15, C16	KC	CXCL1
C17, C18	LIX	GCP-2, ENA-78
D3, D4	MCP-2	CCL8, HC14
D5, D6	MCP-5	CCL12
D7, D8	MDC	CCL22, ABCD-1

D9, D10	MIG	CXCL9, CRG-10, CMK
D11, D12	MIP-1α/β (pan)	CCL3/CCL4
D13, D14	MIP-1γ	CCL9/10, CCF18, MRP-2
D15, D16	MIP-2	CXCL2, GROβ, GRO2, CINC-3
D17, D18	RANTES	CCL5, SISd
E3, E4	SDF-1	CXCL12, PBSF
E5, E6	Complement Factor D (Sample Control)*	Adipsin, DF, Adn
E7, E8	gp130 (Sample Control)*	IL-6ST, CD130
E9, E10	HSP60 (Sample Control)*	Hspd1
E11, E12	Negative Control N/A	Control (-)
F1, F2	Reference Spots	RS

Supplementary Table S2: Human Growth Factor Antibody Array C1(AAH-GF-1-4)

Each antibody is spotted in duplicate vertically	A	B	C	D	E	F	G	H	I	J	K	L
1	POS	POS	NEG	NEG	AR	bFGF	beta-NGF	EGF	EGFR	FGF-4	FGF-6	FGF-7 (KGF)
2												
3	GCSF	GDNF	GM-CSF	HB-EGF	HGF	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4	IGFBP-6	IGF-1	IGF-1 R
4												
5	IGF-2	M-CSF	M-CSF R	NT-3	NT-4	PDGF R alpha	PDGF R beta	PDGF-AA	PDGF-AB	PDGF-BB	PLGF	SCF
6												
7	SCF R (CD117)	TGF alpha	TGF beta 1	TGF beta 2	TGF beta 3	VEGF-A	VEGFR2	VEGFR3	VEGF-D	BLANK	BLANK	POS
8												

Supplementary Table S3. Clinico-pathological information for patients sample (paraffin section)

Assigned ID	Site	Days From Dx to Met	Days From Dx to Censor	Days From Met to Censor	Dead (1=yes, 0=no)	Treatment	Response
1	right femoral head	Unknown	Unknown	240	0	Radiation to prostate prior to metastasis. No other records available	progression
2	right proximal femur	63	539	476	0	Docetaxel x 6 cycles, palliative radiation to the hip, leuprolide, denosumab	stable
3	right femoral head	39994	40167	173	0	Radiation to the spine for metastasis seen on imaging, leuprolide	unknown
4	right proximal femur	0	13	13	0	Unknown	unknown
5	left femur	0	281	281	1	Bicalutamide, leuprolide, palliative radiation of spine	progression
6	left femoral head	2382	2652	270	0	Leuprolide, Triptorelin, bicalutamide, enzulatamide	progression
7	left femur	40644	40687	43	0	brachytherapy, bicalutamide, leuprolide, zoledronic acid, denosumab, radiotherapy, abiraterone, prednisone, enzalutamide	unknown
8	right femur	1690	1958	268	0	Unknown	unknown
9	left proximal femur	0	273	273	0	Bicalutamide, enzalutamide, docetaxel, ramucirumab	progression

10	right proximal femur	0	450	450	0	Docetaxel, leuprolide, radiation to femur	stable
11	left femoral head	2556	3216	660	0	Unknown	stable
12	right femur	768	870	102	0	Abiraterone, prednisone, enzalutamide, radiation	progression
13	T10	0	310	310	0	Radiation to C and T spine, bicalutamide, docetaxel 6 cycles, and leuprolide	stable
14	T11	0	318	318	1	Palliative radiation to pelvis and sacrum, docetaxel, androgen deprivation therapy	progression
15	T2	3147	4418	1271	0	Radical prostatectomy and lymph node sampling, docetaxel, abiraterone, prednisone, leuprolide, triptorelin, zoledronic acid	stable
16	L5	61	540	479	1	Bicalutamide, leuprolide, enzalutamide, radiation to lumbosacral spine	progression
17	T spine, NOS	0	686	686	0	Radiation to thoracic spine following fusion and laminectomies, androgen deprivation therapy, abiraterone, docetaxel	progression
18	L3	33	69	36	1	Radiation to cervical and thoracic spine and leuprolide	progression
19	L4	39	115	76	1	Radiation to lumbar spine, leuprolide, docetaxel	progression
20	T spine, NOS	0	726	726	0	Bicalutamide, leuprolide	progression
21	T spine, NOS	0	757	757	0	Resection of mass, bicalutamide, abiraterone, prednisone, leuprolide	progression
22	T11	1105	1192	87	0	Unknown	progression
23	T2	0	2233	2233	0	Radiation to C and T spine, bicalutamide, ketoconazole,prednisone, docetaxel	stable
24	T2	3500	3625	125	0	Prostatectomy and radiation to T spine metastasis 9 years	unknown

						after original diagnosis. Received chemotherapy at initial diagnosis. Not known if patient received prior radiation.	
25	C spine, NOS	0	1401	1401	0	Androgen deprivation therapy, docetaxel, bicalutamide	stable

Supplementary Table S4: Sample details for Serum sample (Obtained from Eastern Virginia Medical School, Norfolk, VA)

Sample ID	Clinical Stage T	Clinical Gleason Score	Pathological Stage T	Path Gleason Score
1	cT0	0		
2	cT0	0		
3	cT0	0		
4	T2c	7	pT3b	7
5	T2B	6	pT3c	7
6	cT0	0		
7	cT0	0		
8	T2a	7	pT3b	7
9	T2b	5	pT3b	5
10	T2c	7	pT3b	7
11	cT0	0		
12	cT0	0		
13	T2b	9	pT3b	8
14	T1c	8	pT3b	7
15	cT0	0		
16	T2b	7	pT3b	7
17	T1c	7	pT3b	7
18	cT0	0		
19	T1c	7	pT3b	7
20	cT0	0		
21	T2a	6	pT3b	6

22	T1c	6	pT3b	7
23	Benign	0		
24	Atrophy	0		
25	Benign	0		
26	Atrophy	0		
27	Benign	0		
28	Atrophy	0		
29	Atrophy	0		
30	Atrophy	0		
31	T1c	6	pT3b	7
32	T2a	8	pT3b	8
33	T2a	7	pT3b	7
34	Benign	0		
35	Benign	0		
36	T1c	6	pT3b	7
37	T2a	8	pT3b	8
38	T1c	7	pT3b	7
39	T1c	9	pT3b	9
40	T2b	7	pT3b	7

Sample ID	Clinical Stage T	Clinical Gleason Score	Pathological Stage T	Path Gleason Score	Bone Metastasis
41	T1c	7	pT2c	7	CT Scan
42	T1c	6	pT2c	7	Axumin Scan
43	T1c	7	pT3b	7	Bone Scan
44	T1c	7	pT3a	7	HORMONE REFRACTORY
45	T1c	7	pT2c	7	Metastatic Disease
46	T1c	9	pT3b	7	CT Scan
47	T1c	6	pT2c	7	Bone Scan
48	T1c	8	pT2	7	HORMONE REFRACTORY
49	T1c	6	pT2b	7	Bone Scan
50	T2b	7	pT3a	7	Bone Scan
51	T2a	6	pT4	7	CT Scan
52	T1a	6	pT2c	7	X-Ray
53	T1c	6	pT3a	7	HORMONE REFRACTORY
54	T1c	6	pT3a	7	Axumin Scan
55	T2c	7	pT3b	7	Bone Scan
56	T2a	6	pT2c	7	Bone Scan
57	T1b	7	pT3b	7	Bone Scan
58	T2b	7	pT3a	7	MRI
59	T2a	7	pT3a	7	Bone Scan
60	T1c	6	pT2c	7	PET/CT Scan