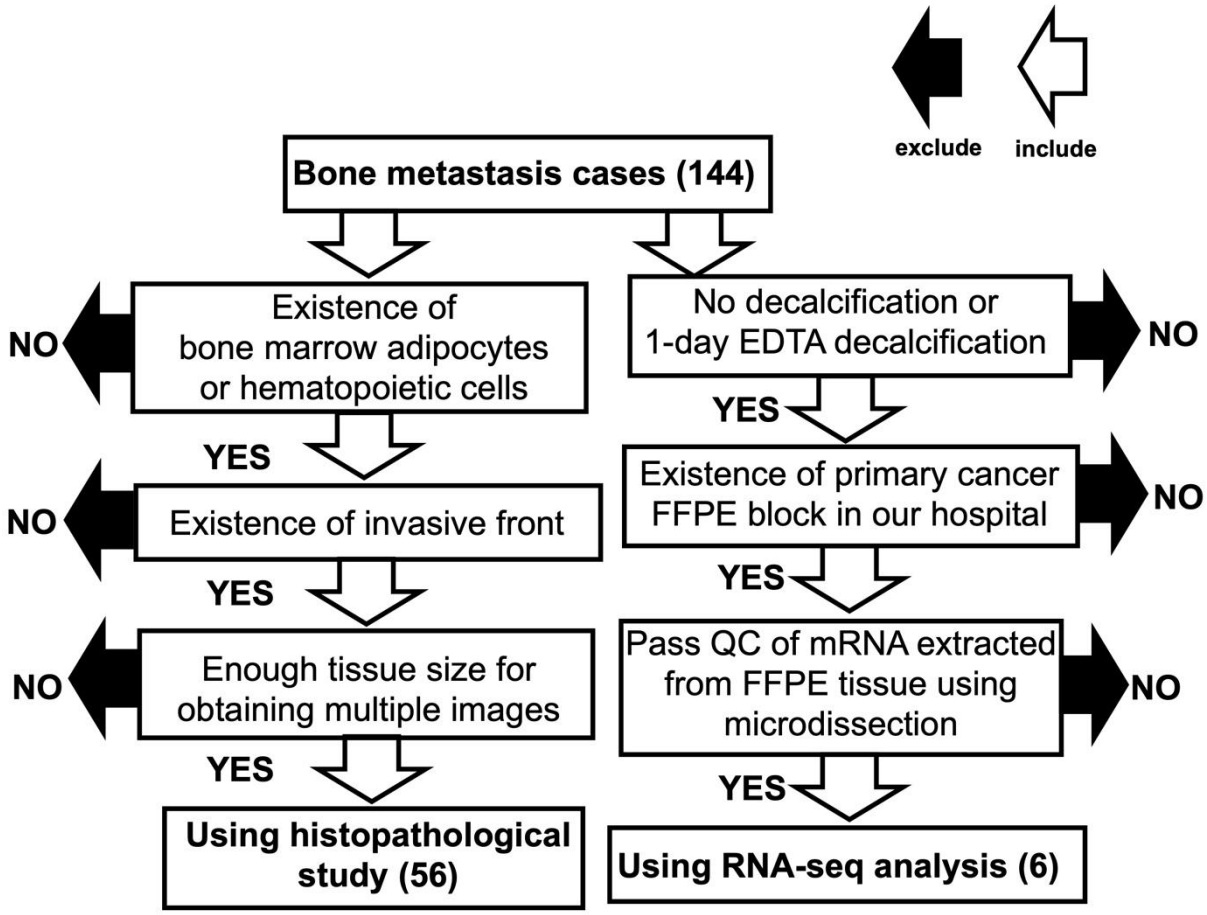
Supporting information

Supplementary Figure 1. Bone metastasis specimen selection procedure.

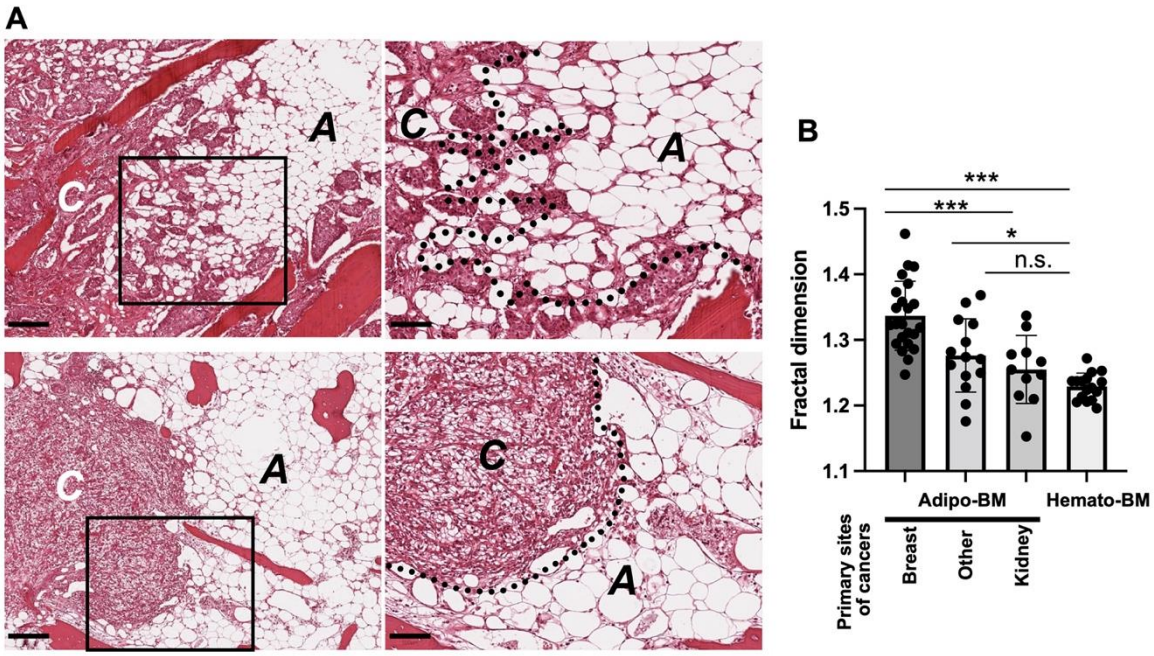
In total, 144 patients with bone metastases were examined; 56 cases were available for histopathological analysis, and six for RNA-seq analysis.

Supplementary Figure 1



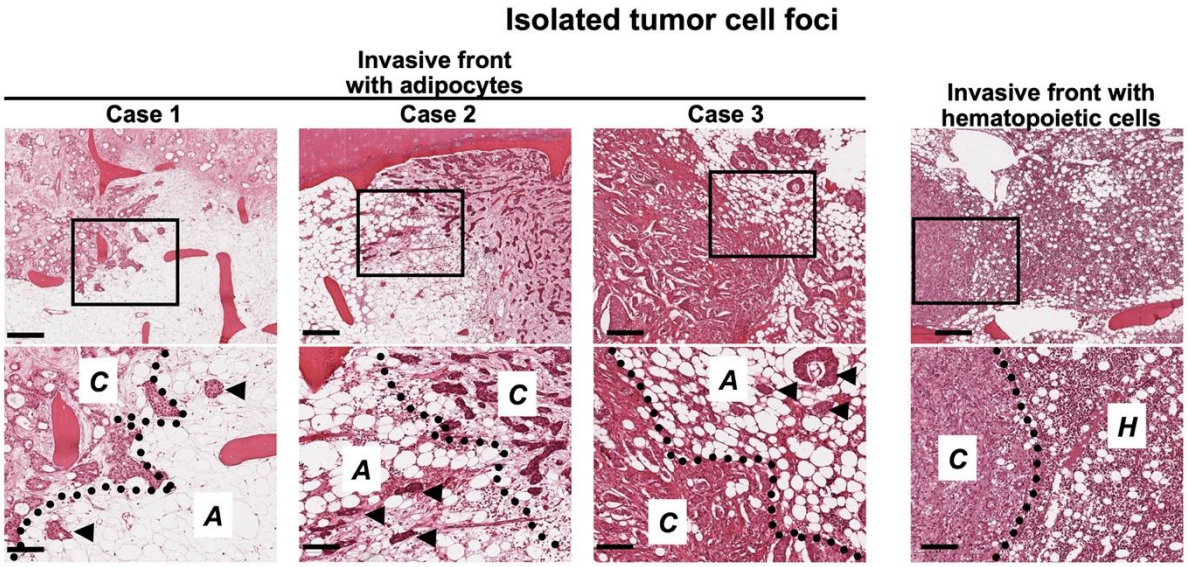
Supplementary Figure 2. Complexity of invasive fronts with different primary sites. **A**. Representative images of invasive fronts with adipo-BM, breast cancer (above), and renal cell carcinoma (below). Scale bar: 100 µm. *A*: Bone marrow adipocytes; *C*: Cancer cells. **B**. Comparison of fractal dimensions between adipo-BM and hemato-BM invasive fronts with different primary sites (One-way ANOVA and Tukey's multiple comparison tests; ***, $P < 0.001$).

Supplementary Figure 2



Supplementary Figure 3. Isolated tumor foci (ICF) at invasive fronts with adipo-BM or hemato-BM. *A*: bone marrow adipocytes; *H*: hematopoietic cells; *C*: cancer cells. Dotted line: invasive front. Arrowhead: ICF. Scale bars: 500 µm (above) and 100 µm (below). Table: frequency of ICF at the adipo-BM and hemato-BM invasive fronts (Fisher's exact test).

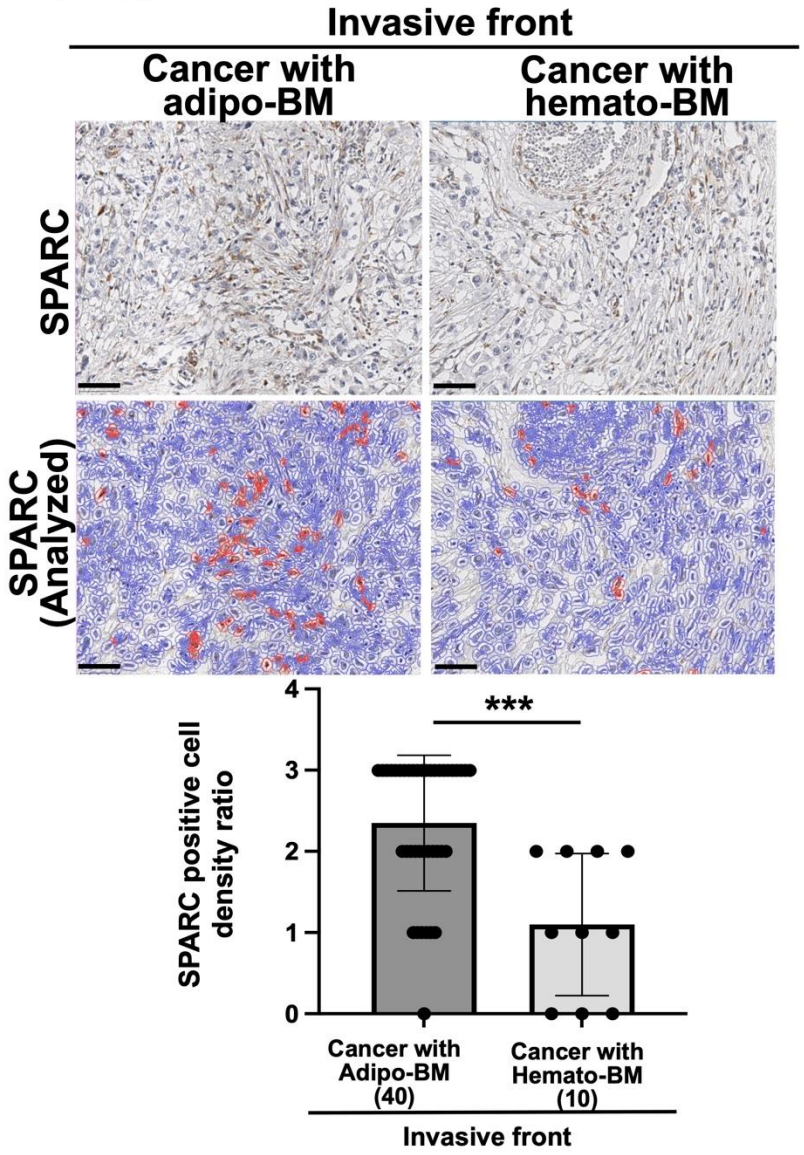
Supplementary Figure 3



Isolated cancer cell foci (ICF)	ICF +	ICF -	p value
Adipo-BM	75.0 (24/32)	25.0 (8/32)	$p < 0.0001$
Hemato-BM	20.0 (4/20)	80.0 (16/20)	

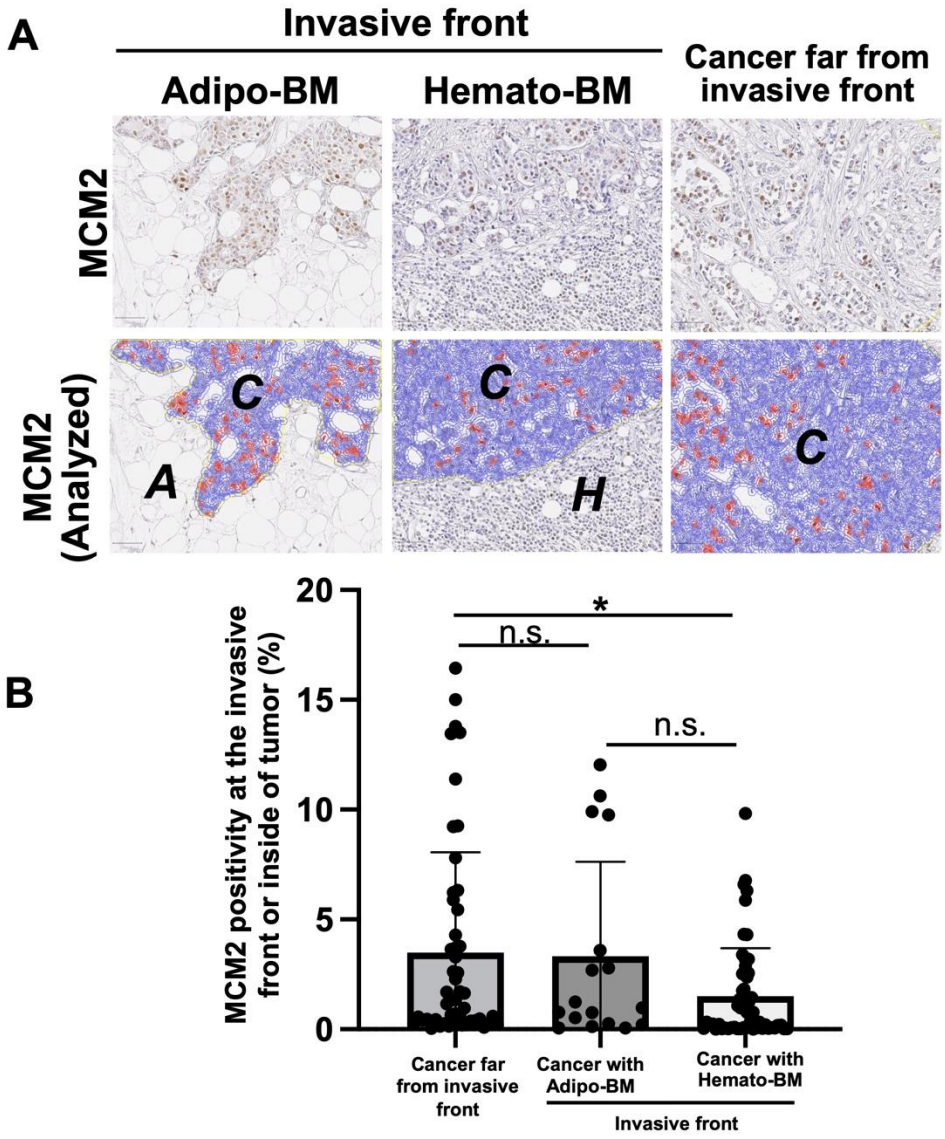
Supplementary Figure 4. Immunohistochemical analysis of SPARC in the adipo-BM or hemato-BM invasive fronts, with representative images. Brown area, positively stained with the anti-SPARC antibody. Scale bar: 100 μm. Bottom, SPARC-positive cell density ratios in the two invasive fronts. The SPARC positivity ratio was set based on the Allred score. 0, 0% positivity; 1, <1% positivity; 2: 1–10% positivity, 3: 10%–33% positivity; 4, 33%–66% positivity; 5, over 67% positivity (zero cases). Unpaired *t*-tests; ***, $P < 0.001$.

Supplementary Figure 4



Supplementary Figure 5. Immunohistochemical analysis of MCM2 in adipo-BM or hemato-BM invasive fronts. **A,** Representative images of MCM2-stained invasive fronts or cancer tissue far from the invasive fronts. Brown-stained cancer cells, positively stained with anti-MCM2 antibody. *A*: bone marrow adipocytes; *H*: hematopoietic cells; *C*: cancer cells. Scale bar: 100 μm. **B**, Comparison of the percentage of MCM2 positive cancer cells at different sites. Cancer with Adipo-BM: cancer cells with bone marrow adipocytes within 100 μm of the invasive front; Cancer with Hemato-BM: cancer cells with hematopoietic cells within 100 μm of the invasive front; Cancer far from invasive front: Cancer cells at least 500 μm from invasive front (one-way ANOVA with Tukey's multiple comparison tests; *, $P < 0.05$).

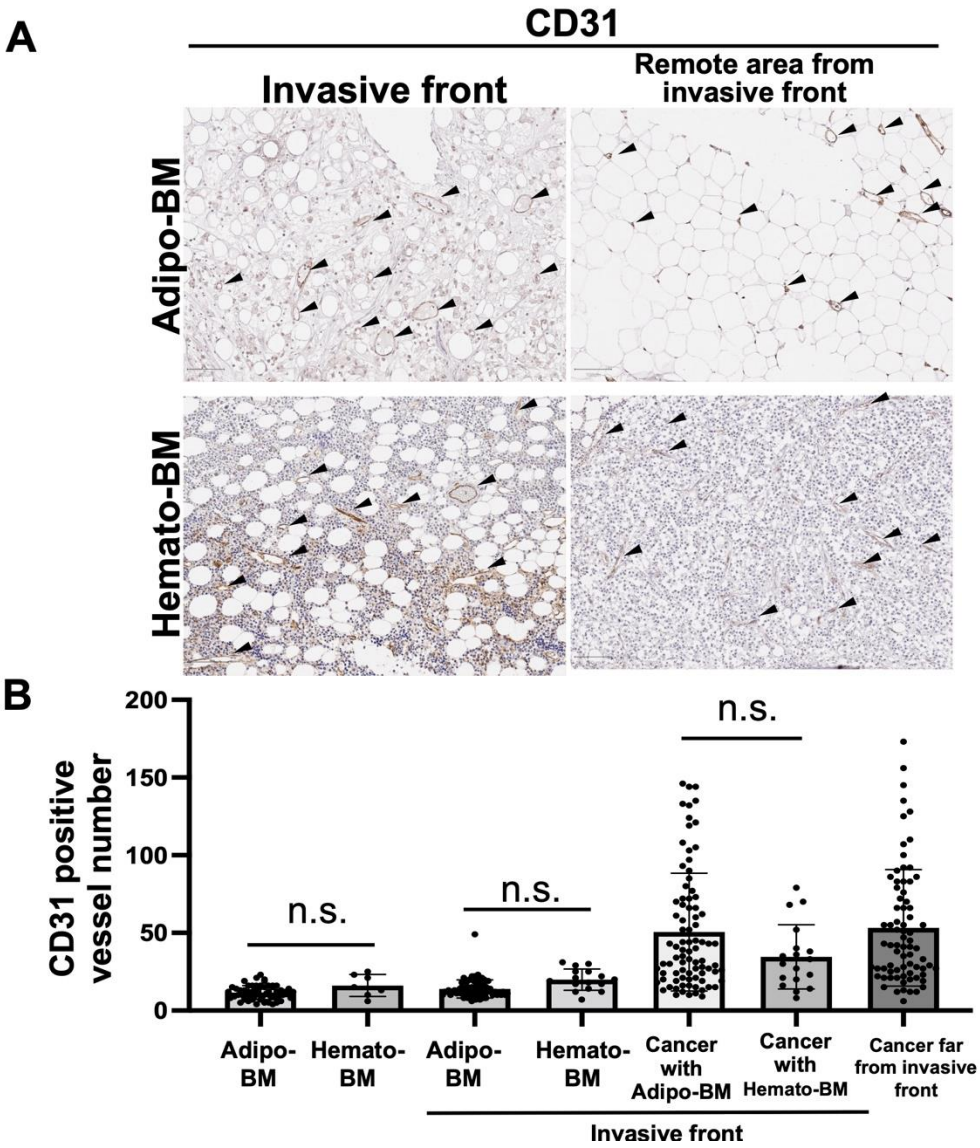
Supplementary Figure 5



Supplementary Figure 6. Immunohistochemical analysis of CD31 in the adipo-BM or haemato-BM invasive fronts, with representative images (**A**). Brown-stained vessels, positively stained with the anti-CD31 antibody (arrowhead). Scale bar: 100 µm. **B**. Comparison of the number of $CD31^+$ vessels at different sites. Adipo-BM: bone marrow adipocytes at least 500 µm from the invasive front; Hemato-BM: hematopoietic cells at least 500 µm from the invasive front; Adipo-BM at the invasive front: bone marrow adipocytes within 100 µm of the invasive front. Hemato-BM at the invasive front: hematopoietic cells within 100 µm of the invasive front. Cancer with adipo-BM: Cancer cells within 100 from the invasive front with bone marrow adipocytes. Cancer with hemato-BM: Cancer cells within

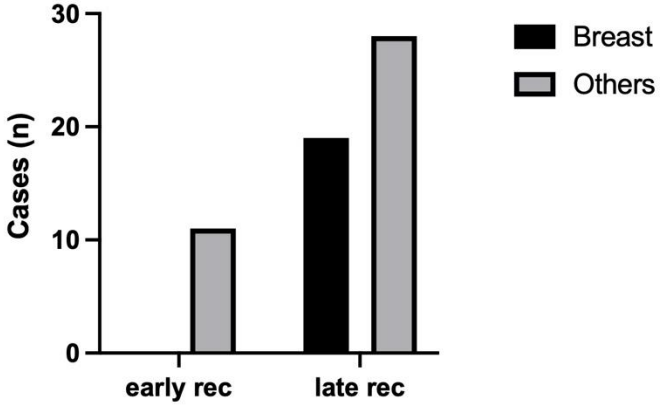
100 μm from the invasive front of hematopoietic cells. Cancer far from the invasive front: Cancer cells at least 500 μm from the invasive front. One-way ANOVA with Tukey's multiple comparison tests; n.s., not significant.

Supplementary Figure 6



Supplementary Figure 7. Duration from diagnosis of the primary site to diagnosis of bone metastasis. Cases with early recurrence (<12 months) and late recurrence (>12 months). Bottom, table of results. Fisher's exact test was used. Statistical significance was set at $P < 0.05$.

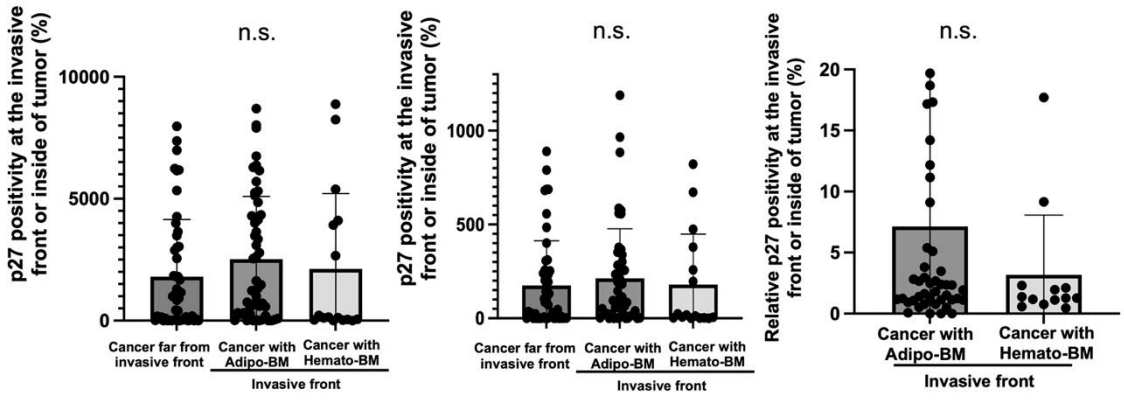
Supplementary figure 7



	Breast	Others	Fisher's exact test
Early recurrence with bone metastasis (<12 m)	0	11	p = 0.0107
Late recurrence with bone metastasis (> 12m)	19	28	

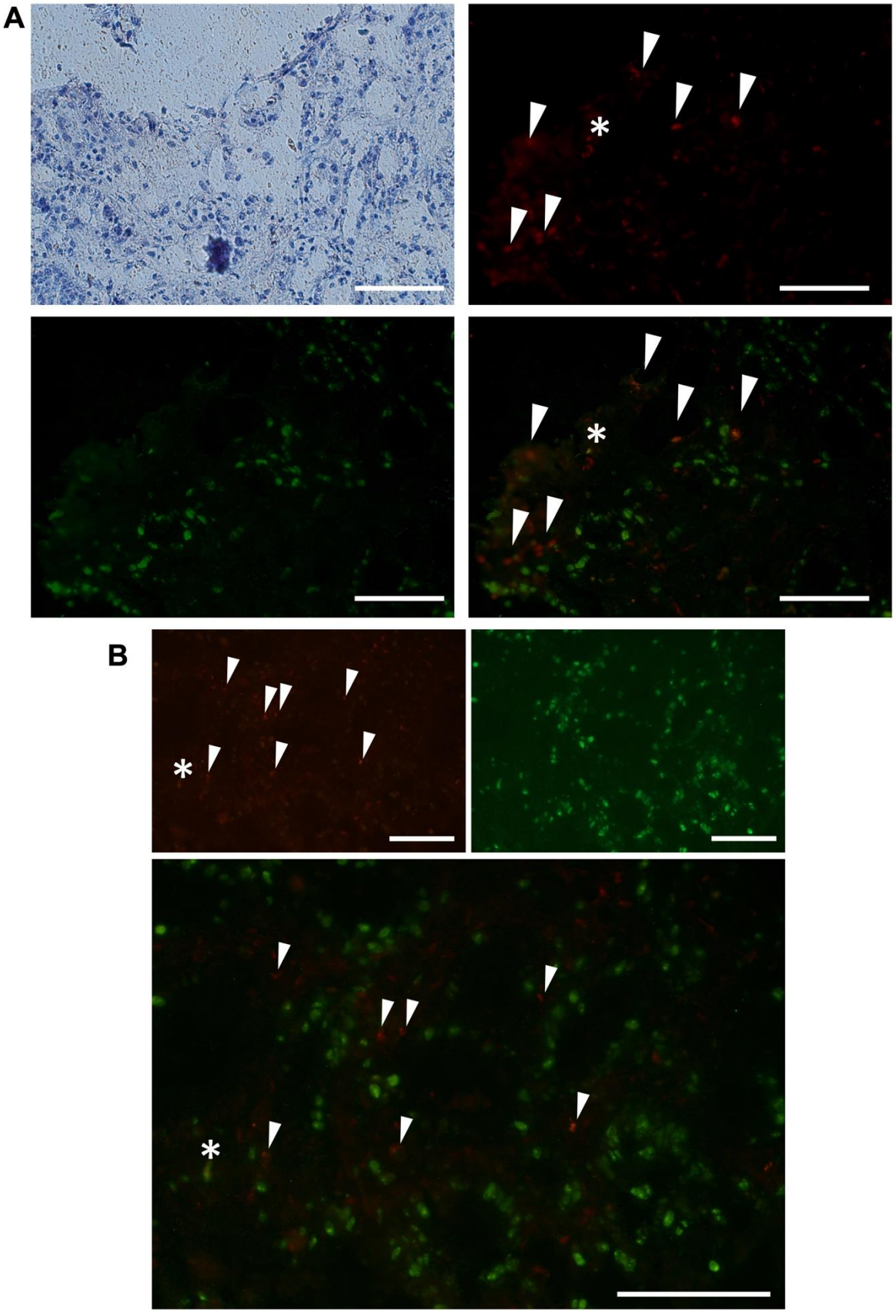
Supplementary Figure 8. Comparison of the percentage of p27-kip positive cancer cells (left) and relative positivity of p27-kip positive cancer cells (middle and right) at different sites. Cancer with Adipo-BM: cancer cells with bone marrow adipocytes within 100 µm of the invasive front; Cancer with Hemato-BM: cancer cells with hematopoietic cells within 100 µm of the invasive front; Cancer far from invasive front: Cancer cells at least 500 µm from the invasive front. One-way ANOVA with Tukey's multiple comparison tests; n.s., not statistically significant.

Supplementary Figure 8



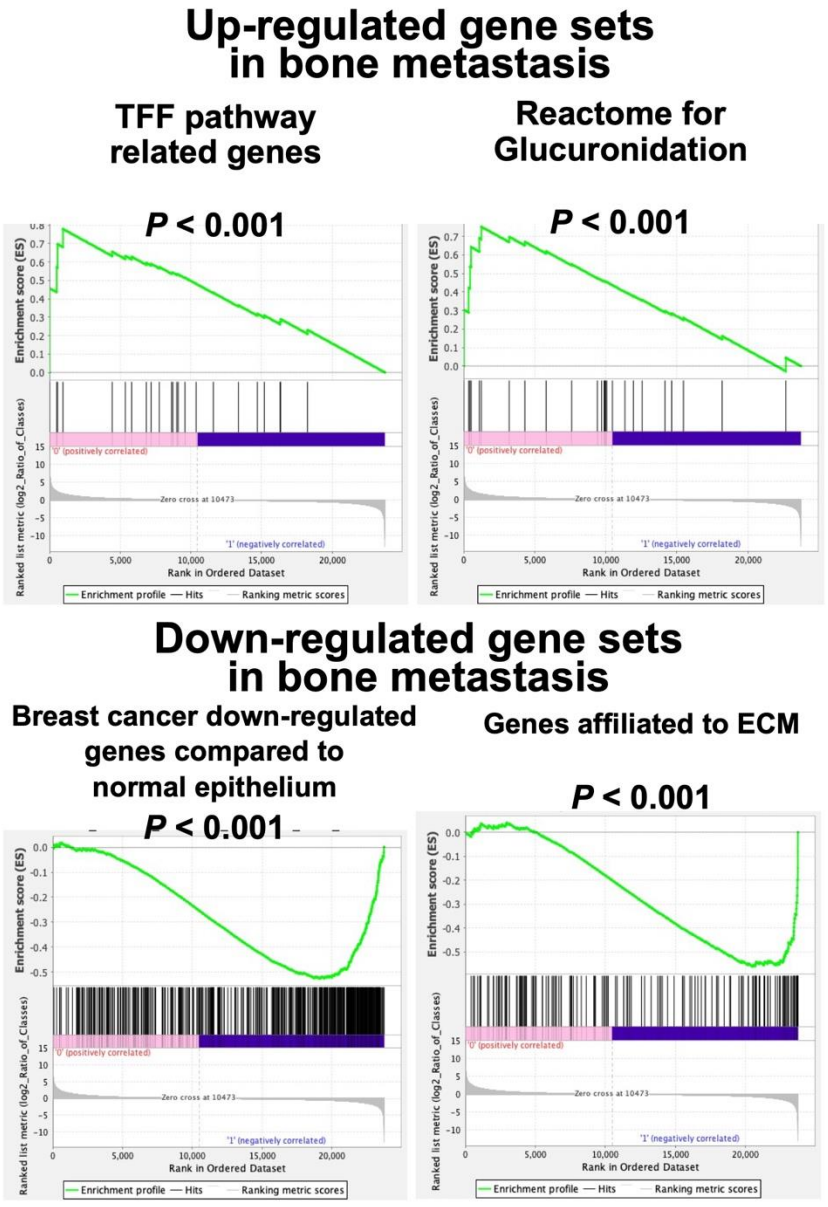
Supplementary Figure 9. Immunofluorescence staining of Ki67 (green) and p21 (red) in the bone metastasis tissue. A and B showed two representative images of different metastatic lesions in the same case. A, Upper left image shows Hematoxylin staining of the serial section of immunofluorescent stained section. Upper right image shows immunofluorescent staining of p21(red). Lower left image shows immunofluorescent staining of Ki67 (green). Lower right image shows the merged image of p21 and Ki67. B, Upper left image shows immunofluorescent staining of p21 (red). Upper right image shows immunofluorescent staining of Ki67 (green). Lower image shows the merged image of p21 and Ki67. Arrowhead: p21 positive cancer cells. Asterisk: p21 and Ki67 positive cancer cells. Scale bar: 100 μm.

Supplementary Figure 9



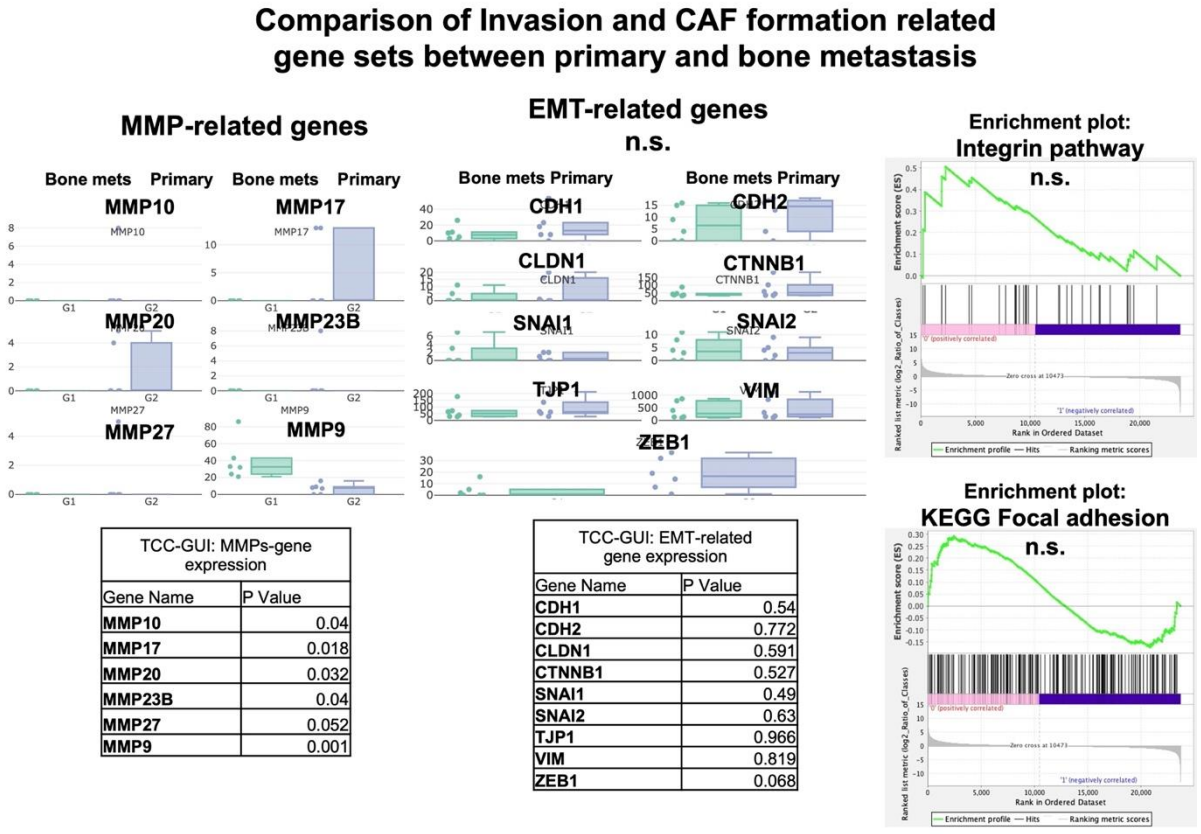
Supplementary Figure 10. Gene set enrichment analysis (GSEA) of upregulated (red bar) or downregulated (blue bar) gene sets in bone metastasis compared to primary cancer. The Y-axis represents enrichment score (ES) and on the X-axis are genes (vertical black lines) represented in gene sets. The green line connects points of ES and genes. Significance threshold set at false discovery rate (FDR) < 0.05.

Supplementary Figure 10



Supplementary Figure 11. (Left) MMP- and EMT-related gene expression in bone metastasis and primary cancer, analyzed using a graphical user interface in the R Bioconducter package (TCG-GUI). (Right) Gene set enrichment analysis (GSEA) of integrin pathways and KEGG focal adhesion-related gene sets. The Y-axis represents enrichment score (ES) and on the X-axis are genes (vertical black lines) represented in gene sets. The green line connects points of ES and genes. Significance threshold set at false discovery rate (FDR) < 0.05.

Supplementary Figure 11



TCC-GUI: MMPs-gene expression	
Gene Name	P Value
MMP10	0.04
MMP17	0.018
MMP20	0.032
MMP23B	0.04
MMP27	0.052
MMP9	0.001

TCC-GUI: EMT-related gene expression	
Gene Name	P Value
CDH1	0.54
CDH2	0.772
CLDN1	0.591
CTNNB1	0.527
SNAI1	0.49
SNAI2	0.63
TJP1	0.966
VIM	0.819
ZEB1	0.068

Supplementary table 1

Duration from the date of primary site's surgery to the date of bone metastasis

Primary site	n	Duration (mean, month)	Duration (range, month)
Breast	19	82.7894737	(14-266)
Lung	7	57.1428571	(2-205)
Prostate	2	26	(17-35)
Kidney	13	45.4285714	(2-180)
Uterus	4	27	(11-48)
hepato	2	25	(24-26)
stomach	2	5.5	(5-6)
Colorectum	2	21	(6-36)
Thyroid	3	20.3333333	(2-47)
Biliary tract	1	14	14
Bladder	1	14	14
Esophagus	1	12	12
Ovary	1	23	23
Total	58	50.50877	(2-266)

Supplementary Table S2: Detailed information of RNA-seq performed bone metastasis cases. From left to right: case numbers, primary sites, MRI findings of bone marrow at the sampling sites, age, height (cm) and weight (kg) were provided in the table.

Case No	Primary site	MRI finding of bone marrow with metastasis	Age	Height (cm)	Weight (kg)
1	Breast	Yellow marrow	59	147.7	51.7
2	Lung	Yellow marrow	60	163	60.4
3	Breast	Yellow marrow	32	153.3	67.7
4	Kidney	Yellow marrow	63	156.3	50
5	Kidney	Yellow marrow	55	175.2	67.6
6	Prostate	Yellow marrow	64	182.4	56.9