Supplementary Figure 1

A

Isotype control

Vimentin

AMF-17B

EC-40

CS

V-9

B

Control-TAMRA

CHP-TAMRA

LM7

C

rhVim (ng)

75 kDa

50 kDa

37 kDa

D

IgG IP Control

12-1P

84-1P

Lysate

Markers

75 kDa

50 kDa

37 kDa

E

OS25

SAOS-2

HEK-293T

Vimentin (84-1)

β-actin

F

DRAQ5

84-1

CD45

Merge

DRAQ5

84-1

CD11b

Merge

DRAQ5

84-1

CD3e

Merge

DRAQ5

84-1

Isotype

Merge

G

Cell-surface vimentin (CSV) evaluation in primary osteosarcoma cell lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Disease Variant</th>
<th>Metastasis</th>
<th>CSV</th>
</tr>
</thead>
<tbody>
<tr>
<td>OST-DL-390</td>
<td>Osteosarcoma</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>OST-DL-391</td>
<td>Osteosarcoma</td>
<td>Yes to lung</td>
<td>+</td>
</tr>
<tr>
<td>OST-DL-393</td>
<td>Osteosarcoma</td>
<td>Yes to lung</td>
<td>+</td>
</tr>
<tr>
<td>OST-DL-396A</td>
<td>Osteosarcoma</td>
<td>Yes to brain</td>
<td>++</td>
</tr>
<tr>
<td>OST-DL-399</td>
<td>Osteosarcoma</td>
<td>No</td>
<td>-</td>
</tr>
</tbody>
</table>

LM7 Spiking assay: Sensitivity determination

<table>
<thead>
<tr>
<th>Cells spiked</th>
<th>Average Recovery</th>
<th>Median [range]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>--</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>63%</td>
<td>[1; 2]</td>
</tr>
<tr>
<td>5</td>
<td>70%</td>
<td>[3; 4]</td>
</tr>
<tr>
<td>10</td>
<td>75%</td>
<td>[7; 8]</td>
</tr>
</tbody>
</table>

LM7 Spiking assay: Specificity determination

<table>
<thead>
<tr>
<th>Cells spiked</th>
<th>PBMC Cells</th>
<th>Average Recovery</th>
<th>Background (% specificity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10 x 10^6</td>
<td>--</td>
<td>0 (100%)</td>
</tr>
<tr>
<td>5</td>
<td>10 x 10^6</td>
<td>60%</td>
<td>0 (100%)</td>
</tr>
<tr>
<td>5</td>
<td>20 x 10^6</td>
<td>80%</td>
<td>1 (99.9%)</td>
</tr>
<tr>
<td>5</td>
<td>25 x 10^6</td>
<td>80%</td>
<td>1 (99.9%)</td>
</tr>
</tbody>
</table>

MFI: Mean fluorescence intensity
(-) No MFI change, (+) < 2-fold MFI increase, (++) > 2-fold MFI increase

I

Cell Count

Spiked cells

Recovered cells