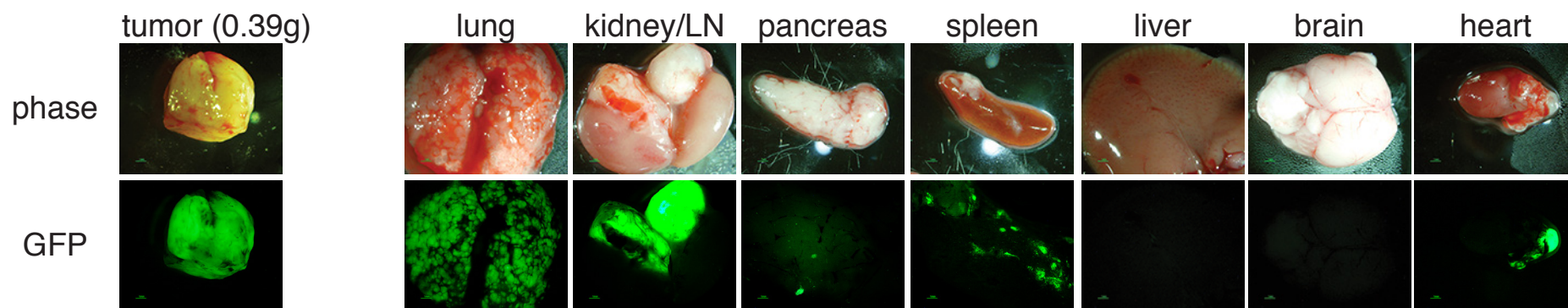


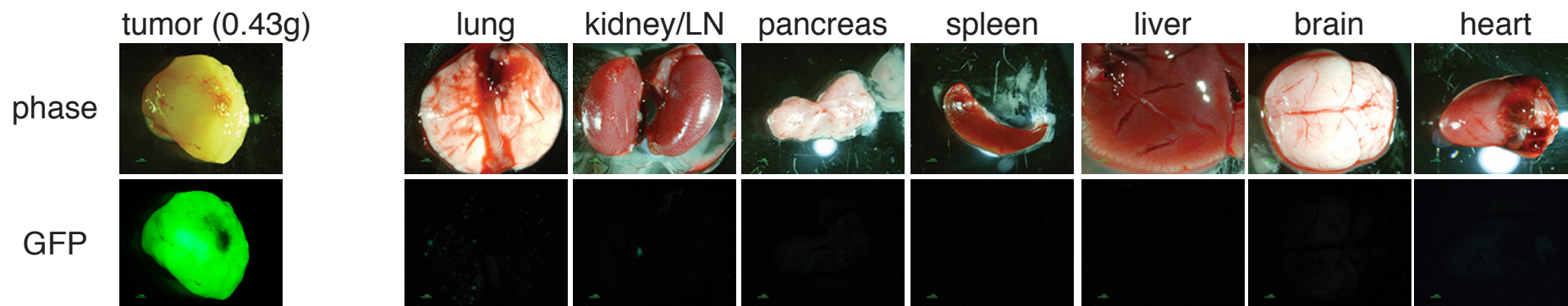
**Figure S1.** DP-implanted PCa cells demonstrate more extensive metastasis than SC-implanted PCa cells. The NOD/SCID mouse (#3793) was implanted with 100,000 PC3-Luc/GFP cells into the DP (**A**) and a separate mouse (#3750) was implanted with 100,000 PC3-Luc/GFP cells SC (**B**). Shown are representative phase and GFP images of the primary tumor for both mice (left) and of the lung, kidney/LN, pancreas, spleen, liver, brain and heart. Note that DP implanted PC3-Luc/GFP cells metastasized to many end organs whereas SC implanted cells did not metastasize in spite of very similar tumor sizes.

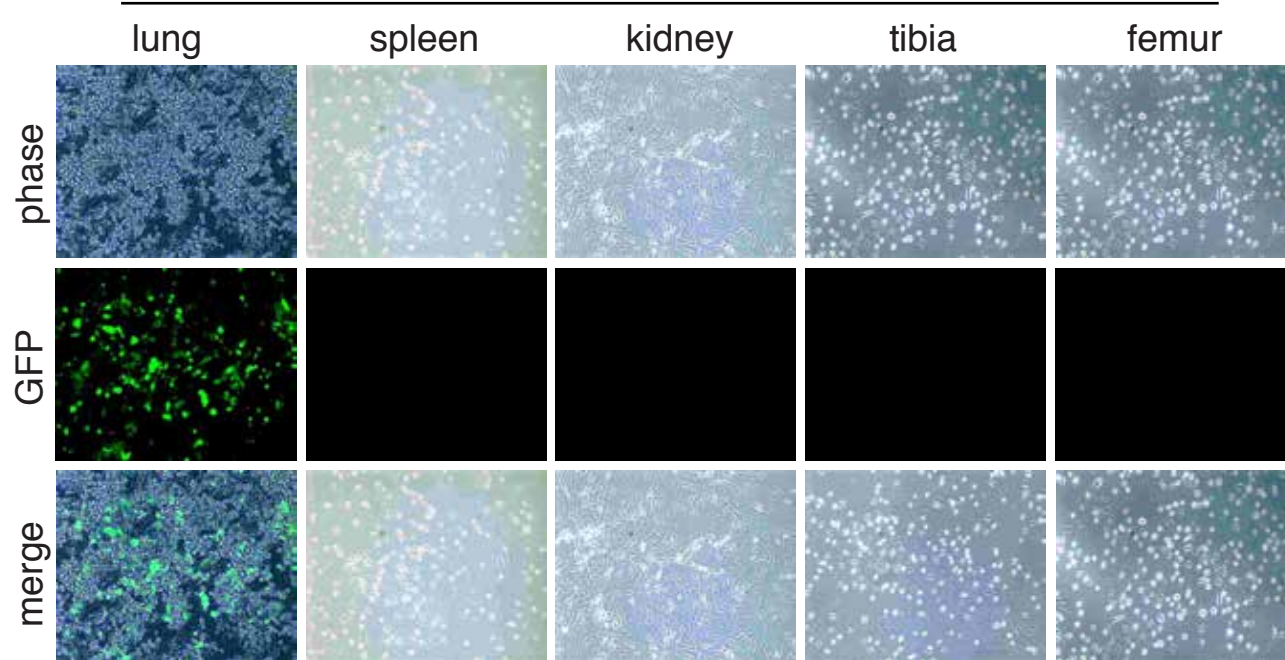
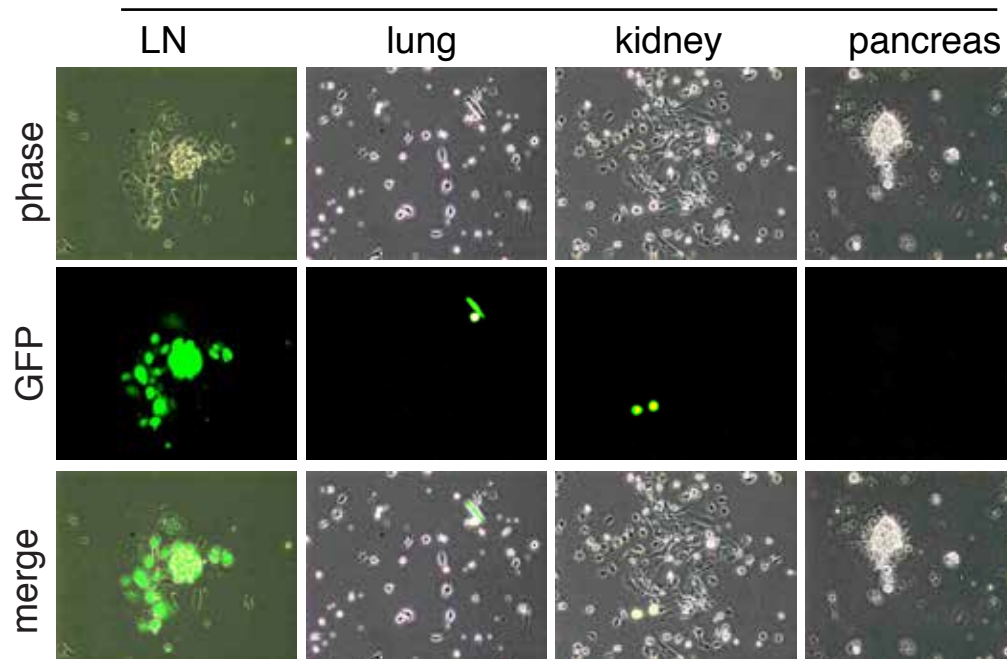
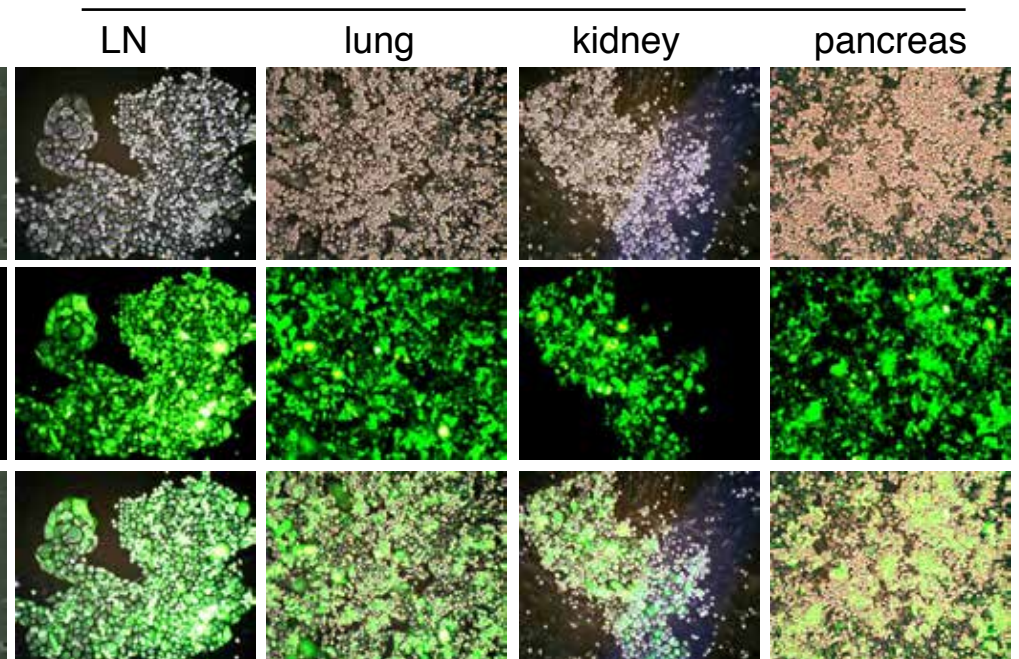
**Figure S2.** AP-implanted PC3 cells metastasize to many organs. (**A**) Shown are representative images of PC3-GFP cells cultured (~2 weeks after plating) from various end organs. The animal was implanted with 1,000,000 PC3-GFP cells SC and sacrificed one month after implantation. Note that PC3-GFP cells metastasized to the lung but not to other organs. The GFP<sup>-</sup> cells on the background are the host cells. (**B**) Images of PC3-GFP cells from end organs cultured for 2 or 10 days. The mouse (#0020) was implanted with 100,000 PC3-GFP cells in the AP. Note abundant PC3-GFP cells that had metastasized to the LN with fewer cells to the lung, kidney, and pancreas (left). However, the few cells that metastasized to these latter organs were live cells as they grew up to form colonies in 10 days (right). Original magnifications, x200.

**A** **PC3-Luc/GFP 100k; DP** (animal #3793)



**B** **PC3-Luc/GFP 100k; SC** (animal #3750)



**A**  
**PC3 1x10<sup>6</sup> SC; ~2 wk after plating**  
(animal #0045)**B**  
**PC3 100k AP; 2 d after plating**  
(animal #0020)**PC3 100k AP; 10 d after plating**  
(animal #0020)

## Supplementary Table S1. AP-implanted PC3-GFP cells metastasize to multiple organs

Cell number <sup>a</sup>	Termination <sup>b</sup> (inj. & term. dates)	Metastasis <sup>c</sup>								
		Femur	Tibia	Lung	Kidney	Liver	Pancreas	Brain	Spleen	LN <sup>d</sup>
<b>1,000 cells</b>	<b>0/3</b> (Inj. 09/08/05)									
0012	term: 02/04/06	-	-	-	-	-	-	-	-	-
0013, 0014	term: 03/22/06	-	-	-	-	-	-	-	-	-
<b>10,000 cells</b>	<b>3/3</b> (Inj. 09/08/05)									
0015	term: 01/06/06	-	-	-	±	±	±	-	+	-
0016	term: 01/04/06	-	-	++	±	±	+	-	±	++ <sup>2</sup>
0017	term: 01/02/06	-	-	-	+	-	+	-	±	++ <sup>2</sup>
<b>100,000 cells</b>	<b>3/3</b> (Inj. 09/08/05)									
0018	term: 12/20/05	-	-	+	±	-	++	-	++	++ <sup>2</sup>
0019	term: 12/28/05	-	-	+	+	±	+	-	-	++ <sup>2</sup>
0020	term: 12/21/05	N.D	N.D	+	+	+	+	-	+	++ <sup>2</sup>
<b>1,000,000 cells</b>	<b>3/3</b> (Inj. 09/08/05)									
0021	term: 12/01/05	+	+	+++	+++	++	+++	+	++	+++ <sup>4</sup>
0022	term: 12/08/05	N.D	N.D	++	++	++	+++	+	++	++ <sup>2</sup>
0023	term: 12/08/05	N.D	N.D	++	++	++	+++	+	++	N.D
0805	(inj: 01/26/06) term: 03/28/06	±	±	+++	+++	+++	+++	±	++	+++ <sup>1,3,4</sup>
0807	(inj: 01/26/06) term: 03/26/06	±	±	+++	+++	+++	+++	+	++	+++ <sup>1,3,4</sup>

<sup>a</sup>For AP implantation, cells were first recombined with collagen and then implanted into the AP tubules. Numbers (e.g., 0012) beneath refer to animal tags.

<sup>b</sup>Animals were terminated (term.) when they became moribund or tumor burden became overwhelming. The injection (Inj.) dates were indicated. Ratios indicate the number of animals bearing tumors / the total number of animals injected. Note that 1,000 PC3-GFP cells implanted at the AP did not develop any tumors when harvested ~5 months after implantation.

<sup>c</sup>Various end organs harvested when animals were terminated were subjected to tissue disaggregation to make single-cell suspensions. The levels of metastasis were semi-quantified by two individuals under a fluorescence microscope to enumerate the numbers of GFP<sup>+</sup> cells. -, no GFP<sup>+</sup> cells; ±, 1-10 GFP<sup>+</sup> cells; +, dozens of GFP<sup>+</sup> cells; ++, hundreds of GFP<sup>+</sup> cells; +++, thousands or tens of thousands of GFP<sup>+</sup> cells. N.D, not determined.

<sup>d</sup>GFP<sup>+</sup> lymph nodes examined. 1, renal, caudal, and sciatic LN; 2, renal LN; 3, mesenteric and inguinal LN; 4, pyloric LN.

**Table S2. Time course of DP-injected PC3-GFP cell metastasis**

Time <sup>a</sup> (after injection)	Termination time <sup>b</sup> (Inj. & term. dates)	Metastasis <sup>c</sup>								
		Femur	Tibia	Lung	Kidney	Liver	Pancreas	Brain	Spleen	LN <sup>d</sup>
<b>PC3-GFP 1,500,000 (DP) Inj:05/19/06</b>										
<b>1 week</b>										
0686	term:05/26/06	-	-	-	-	-	-	-	-	-
0679	term:05/26/06	-	-	-	-	-	-	-	-	-
0678	term:05/26/06	-	-	-	-	-	-	-	-	-
<b>2 weeks</b>										
0682	term:06/02/06	-	-	±	-	-	±	-	-	-
0683	term:06/02/06	-	-	±	-	-	±	-	-	-
0684	term:06/02/06	-	-	±	-	-	±	-	-	-
0685	term:06/02/06	-	-	-	-	-	±	-	-	-
<b>3 weeks</b>										
0024	term:06/09/06	-	-	±	+	+	++	-	++	+
0025	term:06/09/06	-	-	±	-	-	-	-	-	-
0026	term:06/09/06	-	-	+	-	+	±	-	-	+
0027	term:06/09/06	-	-	±	-	-	-	-	-	-
<b>4 weeks</b>										
0028	term:06/16/06	-	-	+	-	-	+	-	-	++
0029	term:06/16/06	-	-	+	+	+	++	-	+	++
0030	term:06/16/06	-	-	+	+	+	+	-	+	+
0031	term:06/16/06	-	-	+	+	-	+	-	+	-
<b>5 weeks</b>										
0032	term:06/23/06	-	-	++	++	±	++	-	+	+++
0033	term:06/23/06	-	-	++	++	±	++	-	+	++
<b>6 weeks</b>										
0034	term:06/30/06	-	-	+++	+++	+	++	-	+	+++
0035	term:06/30/06	-	-	++	++	±	+	-	+	++
0036	term:06/30/06	-	-	++	++	+	++	-	+	++
0037	term:06/30/06	-	-	++	++	±	++	-	+	++
0038	term:06/30/06	-	-	+++	+++	+	++	-	+	+++
<b>7 weeks</b>										
0039	term:07/07/06	±	-	+++	+++	+	++	±	+	+++
0040	term:07/07/06	±	-	+++	+++	+	++	±	+	+++
0041	term:07/07/06	-	-	+++	+++	+	++	+	+	+++
0042	term:07/07/06	-	-	+++	++	+	++	-	++	++
0043	term:07/07/06	-	-	+++	++	±	+	-	+	++

<sup>a-d</sup>PC3 cell metastasis to the lung and pancreas was observed first in the second week after DP implantations, after which time increasing metastasis to many other organs was noted. By 7 weeks, the lung, LN, and kidney showed highest levels of metastasis. Numbers (e.g., 0686) represent animal tags.