Supplementary Data

Antigen	Product	Positive Control	Negative Control	Leukocyte Expression	Dilution
Vimentin (IF)	BD Biosciences, mouse monoclonal IgG1, 550513	PBMCs, PC-3, DU145	T47D, LnCAP, mock	Yes	2:225
N-cadherin (IF, WB)	BD Biosciences (San Jose, CA), mouse monoclonal IgG1, 610920	Sarcoma, rat brain, PC-3	PBMCs, mock	No	4:225
Cytokeratin (pan-CK, IF)	AbD Serotec (Raleigh, NC), mouse monoclonal IgG1, MCA1907HT	T47D, DU145	PBMCs, mock	No	10:225
CD45 (IF)	Invitrogen (Carlsbad, CA), mouse IgG1, HI30, MHCD4500	PBMC	PC-3, DU145, mock	Yes	5:225
CD133 (IF) (PROM1)	Novus Biologics (Littleton, CO), polyclonal rabbit, NB120-16518	CaCo-2 colon cancer cells	PMBCs, mock	Variable	4:225
O-cadherin (IF, WB)	Invitrogen (Carlsbad, CA), mouse IgG1 kappa, 5B2H5, 32-1700	PC-3	PBMCs, mock, T47D	No	5:225
E-cadherin (IF, WB)	BD Biosciences (San Jose, CA), mouse IgG2a, 612131	T47D, PC-3	PMBCs, mock	No	4:225

Supplementary table 1. EMT/stemness antigens assessed in CTCs and control cells. IF=immunofluorescence. WB=western blot.

DEMOGRAPHICS	***N=16***
Median Age (range)	61 (48-81)
Race, Ethnicity	
White, non-hispanic	44 %
Black, non-hispanic	50 %
Asian, non-hispanic	6 %
BASELINE DISEASE HISTORY	
ER/PR positivity	56% / 50%
HER (negative, 1+, 2+)	60% , 27%, 13
Median Baseline Pain, range	0 (0-6)
Median Karnofsky Performance Status (range)	90 (70-90)
Median # of Prior Hormonal Therapies (range)	1 (0-4)
Prior Chemotherapy	81%
SITES OF METASTATIC DISEASE	
Visceral (lung or liver)	75%
Lymph node only	0 %
Lymph Node/, soft tissue, or contralateral breast only	13%
Bone Metastatic:	
Bone metastases with lymph nodes (no visceral metastases)	0 %
Bone metastases without lymph nodes (no visceral metastases)	13 %

Supplementary Table 2. Baseline demographic and clinical characteristics of the women with metastatic BC in this study (n=16). Pain is scored as a linear analog scale (0-10 range).

Subject Number	CTC Count (Cellsearch)[i]	Vimentin (+) CTCs / Total Manual CTC Count[ii]
1	5	4/6
2	4	2/2
3	54	11/11
4	45	6/10
5	626	5/8
6	110	17/21
7	182	5/6
8	17	13/16
9	19	33/34
10	34	12/12
Total	**1127**	**108/126 (86%)**
Subject Number	**CTC Count (Cellsearch)**	**N-Cadherin (+) CTCs/ Total Manual CTC Count**
11	45	13/19
12	12	5/7
13	10	8/8
14	5	7/8
15	12	3/4
16	220	11/13
17	828	81/96
18	26	6/11
19	12	18/22
20	42	15/18
21	485	38/38
Total	**1697**	**205/244 (84%)**
Subject Number	**CTC Count (Cellsearch)**	**CD133 (+) CTCs/ Total Manual CTC Count**
22	16	6/11
23	91	15/21
24	6	0/0
25	36	29/29
26	27	9/9
27	43	10/15
28	2	0/0
29	23	12/14
30	38	23/26
31	30	12/17
32	75	11/11
Total	**387**	**127/153 (83%)**
Subject Number	**CTC Count (Cellsearch)**	**O-Cadherin (+) CTCs/ Total Manual CTC Count**
33	198	23/24
34	180	18/20
35	102	9/14
36	7	15/15
37	55	11/14
38	112	31/33
Total	**654**	**107/120 (89%)**

Supplementary Table 3. Prevalence of EMT and stemness marker expression in individual subjects with metastatic CRPC from this study.

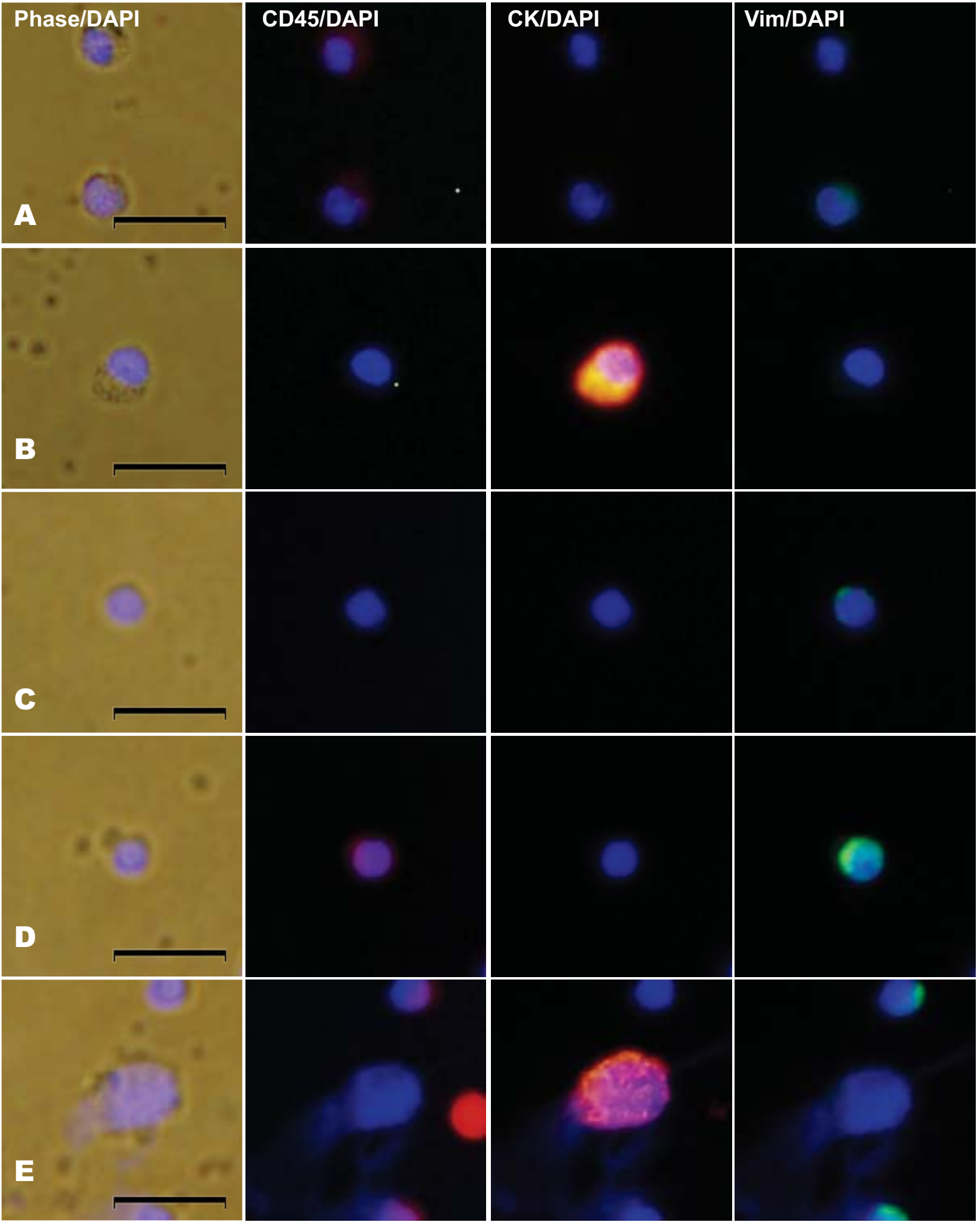
[i] The middle column represents the CTC Count from the FDA-approved Cellsearch® enumeration of CTCs for each subject.

[ii] Right column represents the ratio of vimentin (co-expression of vimentin ranged from 60-100% of cells in a given individual), N-cadherin (Co-expression of N-cadherin ranged from 55-100% of cells in a given individual), CD133 (CD133 co-expression ranged from 55-100% of evaluable cells in a given individual), or O-cadherin (Co-expression of O-cadherin ranged from 64-100% of evaluable cells in a given individual) expressing CTCs among the total number of CTCs that were manually enumerated. A CTC was defined as an intact DAPI positive (nucleated) cell that lacked CD45 expression and expressed cytokeratin.

Subject Number	CTC Count (Cellsearch)	Vimentin (+) CTCs / Total Manual CTC Count
1	21	0/6
2	7	2/2
3	8	4/4
4	21	1/2
5	12	2/2
6	188	21/22
7	324	29/33
8	377	6/23
9	0	0/0
10	3	0/3
Total	**961**	**65/97 (67%)**
Subject Number	**CTC Count (Cellsearch)**	**N-Cadherin (+) CTCs / Total Manual CTC Count**
11	1062	9/13
12	2	0/3
13	147	52/59
14	6	2/5
15	33	15/15
16	2	0/0
Total	**1252**	**78/95 (82%)**

Supplementary Table 4. Expression of vimentin and N-cadherin on CTCs from serial cohorts of 16 women with metastatic progressive breast cancer. The second column is the enumerated CTC count using the approved CellSearch® method, while the third column represents the ratio of EMT marker positive CTCs manually scored compared with the total number of CTCs manually scored from each sample.

Supplementary Data: Figures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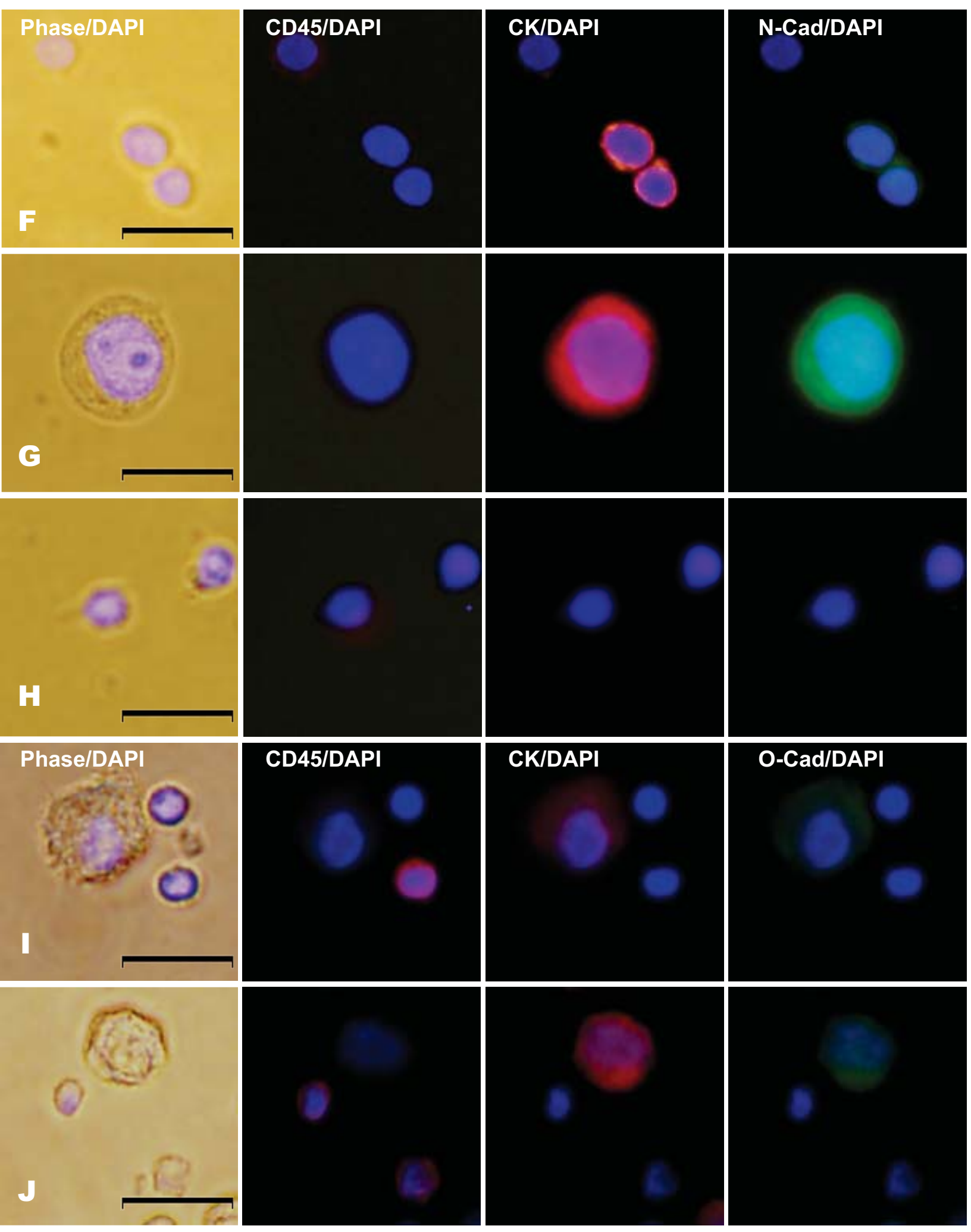
CK/DAPI
Vim/DAPI
A
B
C
D
E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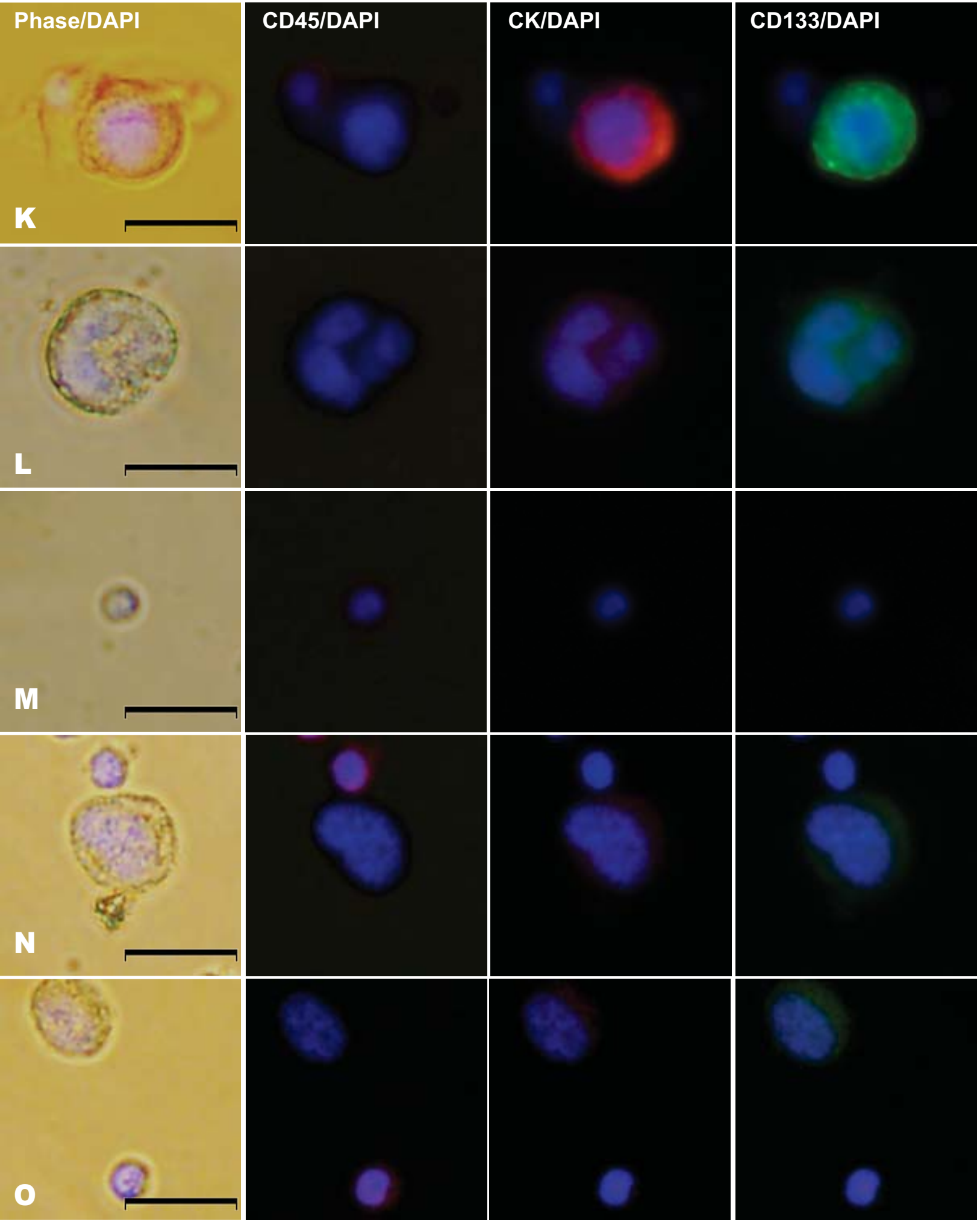
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CK/DAPI
CD133/DAPI
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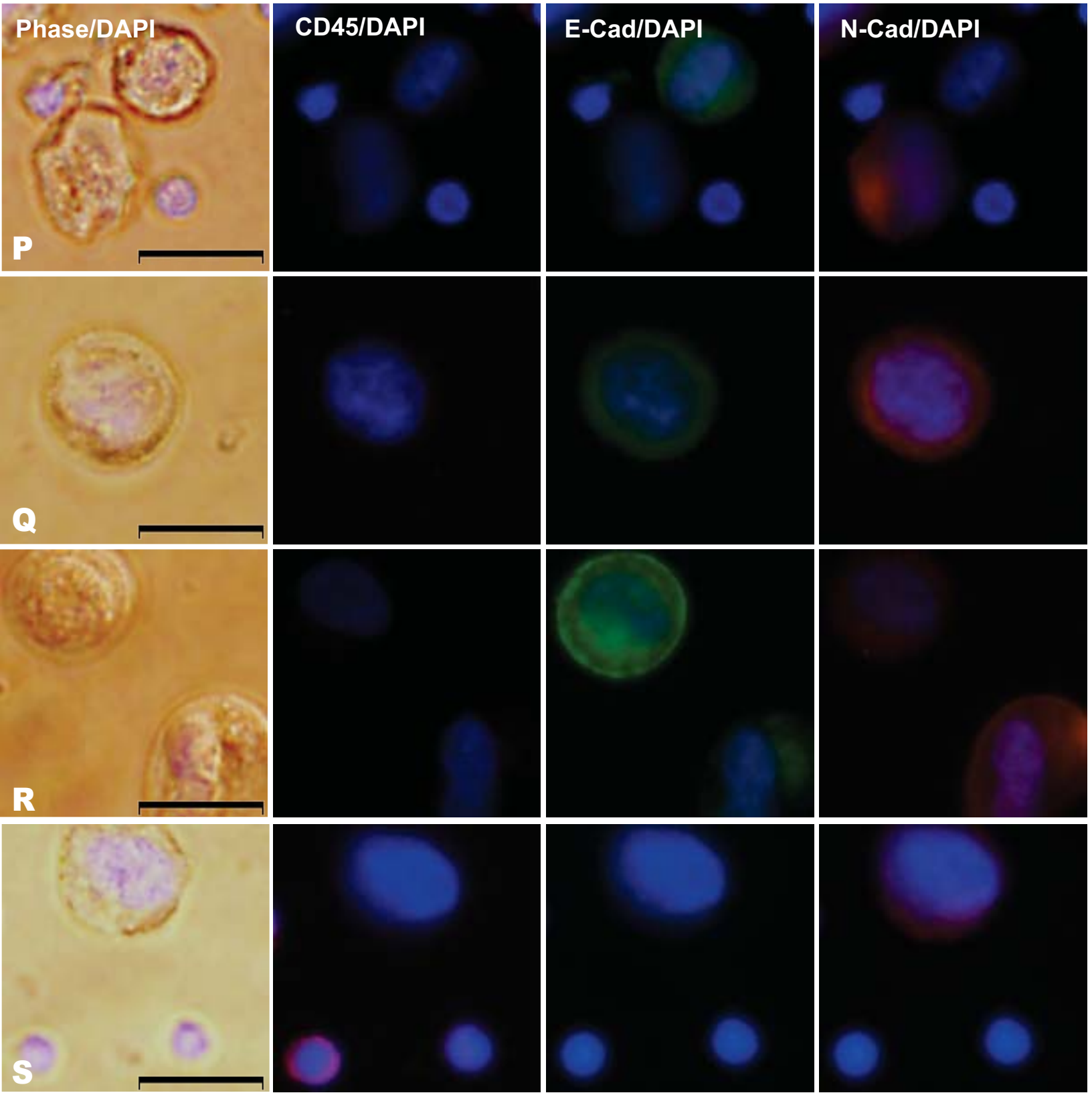
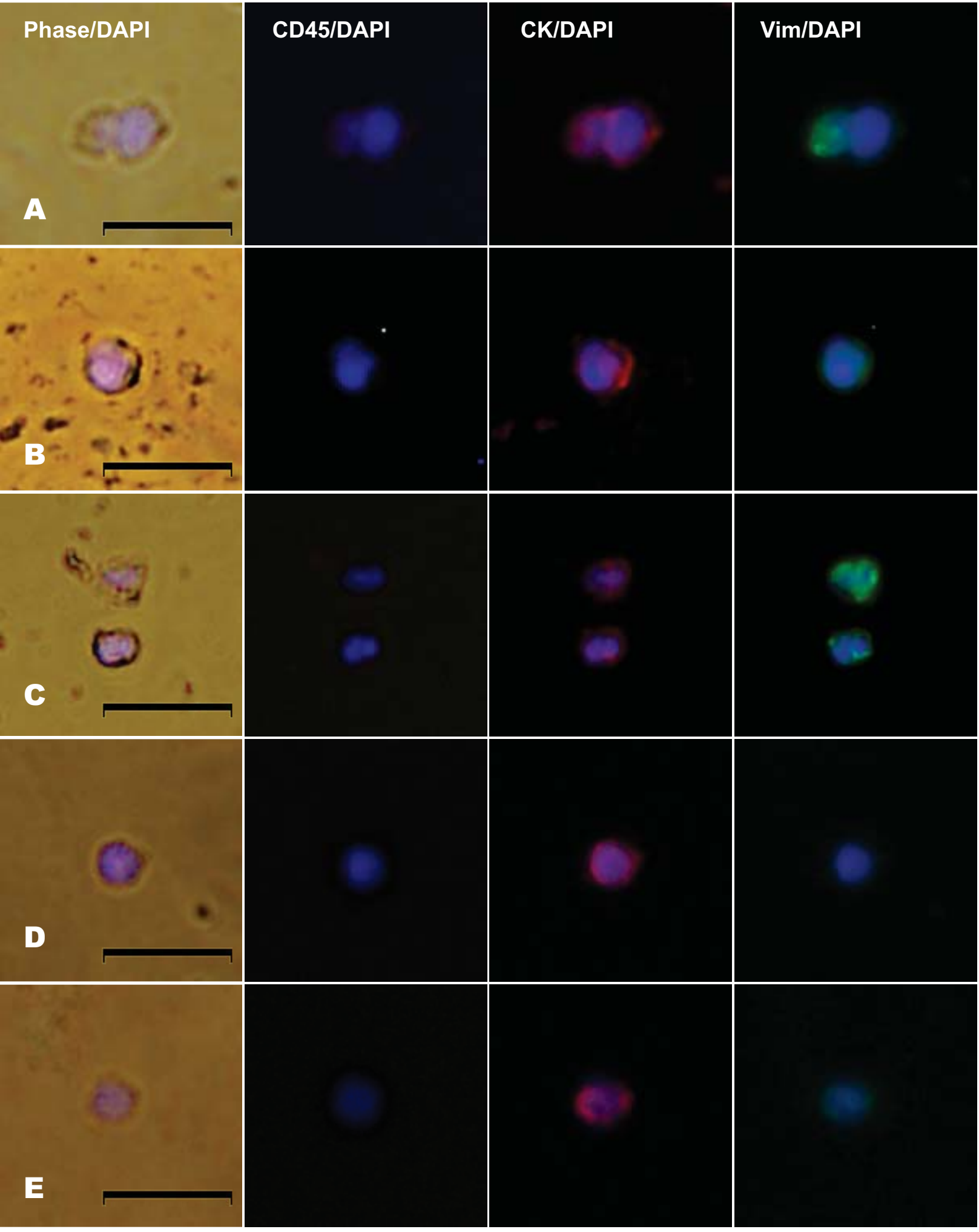


Supplementary Figure 1

Supplementary Figure 1. Mixtures of control cells were assayed in parallel with the patient samples. Scale bars represent 20 μm and were added from a cell image taken from identical magnification and resolution. (A) Parallel control for Fig. 1A: cells that express CD45, lack CK expression, and have variable vimentin expression. (B) Parallel control for Fig. 1A: cell that lacks CD45 expression, expresses CK and has low expression of vimentin. (C) Parallel control for Fig. 1B and 1C (same patient sample, same day of assay): cell that lacks CD45 and cytokeratin expression and expresses vimentin. (D) Parallel control for Fig. 1B and 1C: cell that expresses CD45 and vimentin but lacks CK expression. (E) Parallel control for Fig. 1B and 1C: cell that lacks CD45 expression, expresses CK, and lacks vimentin expression. Note reciprocal expression in nearby cells. (F) Parallel control for Fig. 1D: cell that expresses CD45 and lacks CK and N-cadherin expression. Nearby, two cells that lack CD45 expression and express CK and N-cadherin. (G) Parallel control for Fig. 1E: cell that lacks expression of CD45 and expresses CK and N-cadherin. (H) Parallel control for Fig. 1E: cell that expresses CD45 and lacks CK and N-cadherin expression. (I) Parallel control for Fig. 1F: one large cell that lacks expression of CD45 and expresses CK and O-cadherin. Note nearby cell with CD45 expression and another nearby cell with no expression of CD45, CK, or O-cadherin. (J) Parallel control for Fig. 1G: two cells with CD45 expression and one cell lacking CD45 and expressing CK and O-cadherin.

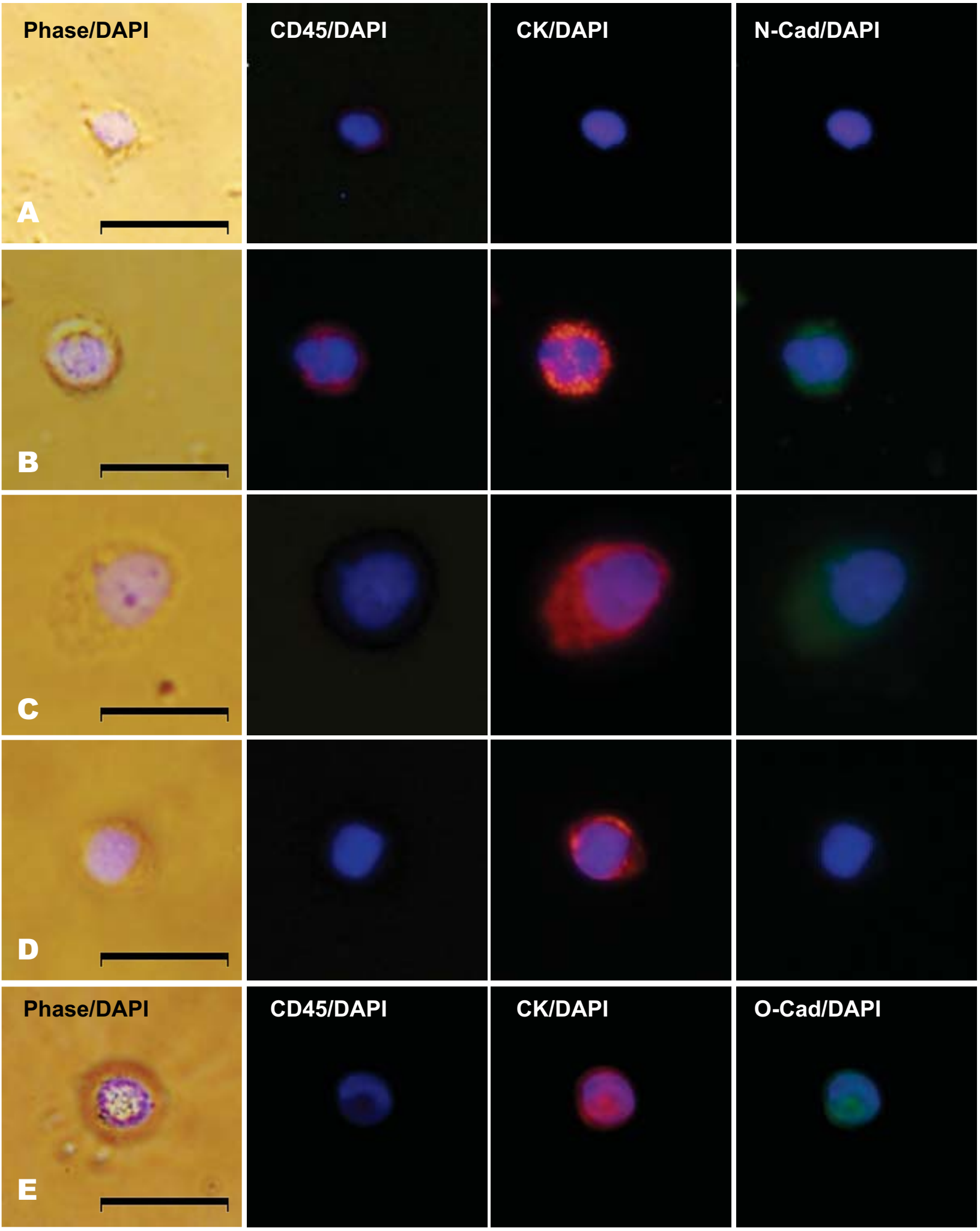
(K) Parallel control for Fig. 3A: cell that expresses CD45 and lacks CK and CD133 expression. Nearby, a cell that lacks CD45 expression and expresses CK and CD133. (L) Parallel control for Fig. 3B: cell that lacks CD45 expression and expresses CK and CD133. (M). Parallel control for Fig. 3B: cell that expresses CD45 and lacks CK and CD133 expression. (N) Parallel control for Fig. 3C: cell that expresses CD45 and lacks CK and CD133 expression. A nearby cell lacks CD45 expression and expresses CK and CD133. (O) Parallel control for Fig. 3D: cell that expresses CD45 and lacks CK and CD133 expression. A nearby cell lacks CD45 expression and expresses CK and CD133.

(P) Parallel control for Fig. 4A: one cell with only E-cadherin expression and another cell with only N-cadherin expression. (Q) Parallel control for Fig. 4B and 4C (same patient sample, same day of assay): cell with expression for both E-cadherin and N-cadherin. (R) Parallel control for Fig. 4D: one cell with strong E-cadherin expression and weak N-cadherin expression and a second cell with weak E-cadherin expression and strong N-cadherin expression. (S) Parallel control for Fig. 4E: one cell with only N-cadherin expression and two cells with only CD45 expression.



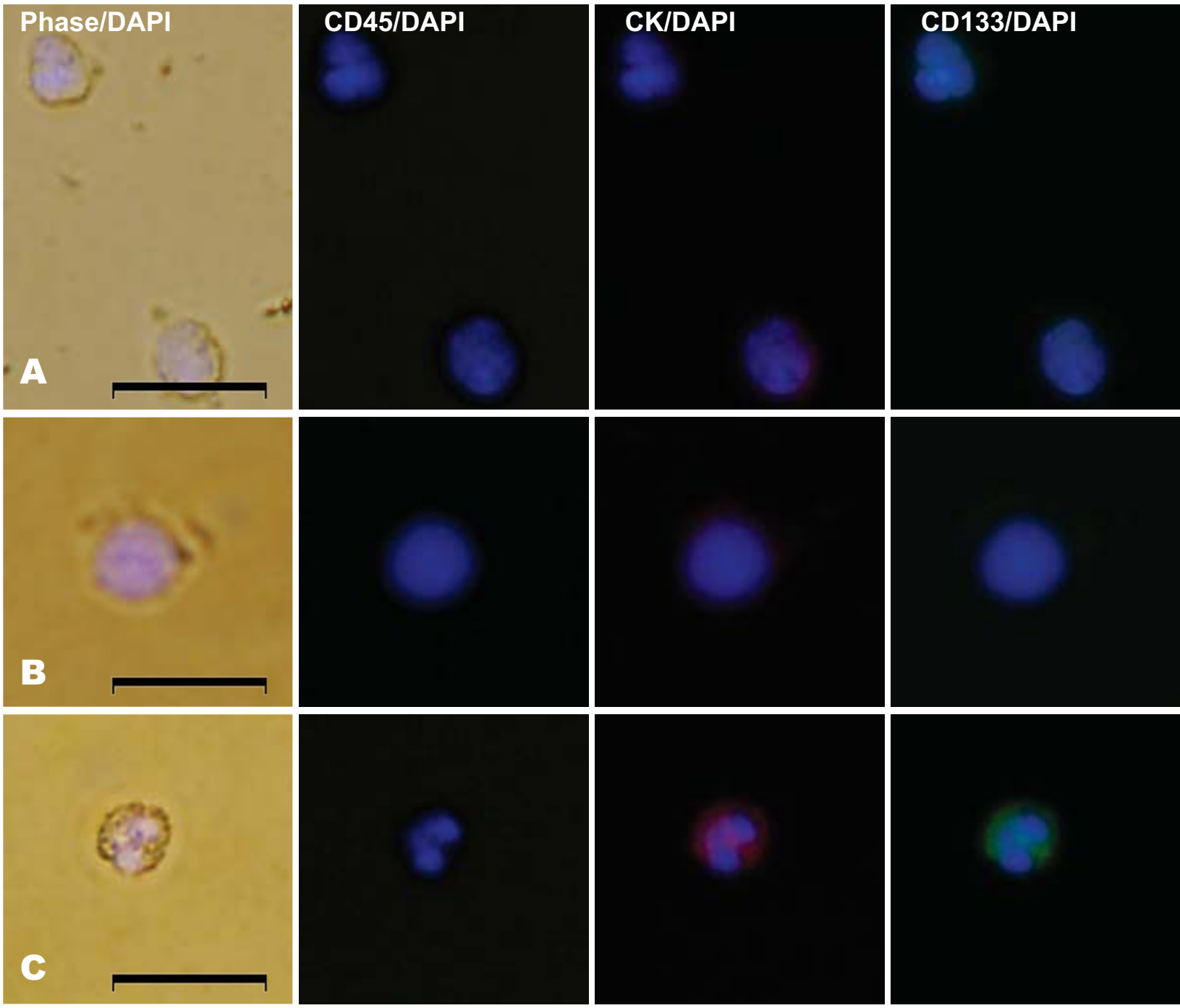
Supplementary Figure 2

Supplementary Figure 2. Additional examples of CTCs based on vimentin expression. Vimentin expression in CTCs from men with progressive metastatic CRPC, (A-C) or women with progressive metastatic breast cancer (D,E). Columns indicate phase/DAPI, CD45/DAPI, CK/DAPI, and vimentin/DAPI expression as indicated. (A) Two CTCs, one with (left) and one without (right) vimentin expression. (B,C) Further examples of vimentin positive CTCs. (D) A CTC from a woman with mBC that lacks vimentin expression. (E) A CTC from a woman with mBC that expresses vimentin.



Supplementary Figure 3

Supplementary Figure 3. Additional examples of CTCs based on N-cadherin or O-cadherin expression. CTCs from men with progressive metastatic CRPC (A, B, E) and women with progressive mBC (C,D). Scale bars represent 20 μm and were added from a cell image taken from identical magnification and resolution. Columns indicate phase/DAPI, CD45/DAPI, CK/DAPI, and N-cadherin/DAPI or O-cadherin expression as indicated. (A) A patient leukocyte with negative N-cadherin expression. (B) A cell of unknown character that expresses CD45, N-cadherin, and cytokeratin (triple positive cell). (C) A CTC from a woman with mBC that expresses N-cadherin. (D) A CTC from a woman with mBC that lacks N-cadherin expression. (E) A CTC from a man with CPRC with CK and O-cadherin expression and no CD45 expression.



Supplementary Figure 4

Supplementary Figure 4. Additional CTCs based on CD133 expression. CD133 expression on CTCs from men with progressive metastatic CRPC, (A-C). Scale bars represent 20 μm and were added from a cell image taken from identical magnification and resolution. Columns indicate phase/DAPI, CD45/DAPI, CK/DAPI, and N-cadherin/DAPI expression as indicated. (A) Two cells with variable CK and CD133 expression. (B) A CTC that lacks CD133 expression. (C) A CTC with strong CD133 expression. Because of its nuclear morphology this cell resembled a leukocyte, but given the criteria defined above (CD45 negative and CK positive) it was scored as a CTC.