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SUPPLEMENTARY INFORMATION

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[1]Department of Biomedicine, Cancer Metastasis Lab, University of Basel and University Hospital Basel, Basel, Switzerland. [2]SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland. [3]Gynecologic Cancer Center, University Hospital Basel, Basel, Switzerland. [4]Department of Medical Oncology, University Hospital Basel, Basel, Switzerland. [5]Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland. [6]Breast Center, University of Basel and University Hospital Basel, Basel, Switzerland. *e-mail: Nicola.Aceto@unibas.ch

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Neutrophils Escort Circulating Tumor Cells to Enable Cell Cycle Progression

Barbara Maria Szczerba[1], Francesc Castro-Giner[1,2], Marcus Vetter[3,4], Ilona Krol[1], Sofia Gkountela[1], Julia Landin[4], Manuel C. Scheidmann[1], Cinzia Donato[1], Ramona Scherrer[1], Jochen Singer[5,2], Christian Beisel[5], Christian Kurzeder[3,6], Viola Heinzelmann-Schwarz[3], Christoph Rochlitz[4], Walter Paul Weber[6], Niko Beerenwinkel[2,5] & Nicola Aceto[1,*]

[1] Department of Biomedicine, Cancer Metastasis Lab, University of Basel and University Hospital Basel, 4058 Basel, Switzerland
[2] SIB Swiss Institute of Bioinformatics, 1015 Lausanne, Switzerland
[3] Gynecologic Cancer Center, University Hospital Basel, 4056 Basel, Switzerland
[4] Department of Medical Oncology, University Hospital Basel, 4056 Basel, Switzerland
[5] Department of Biosystems Science and Engineering, ETH Zurich, 4058 Basel, Switzerland
[6] Breast Center, University of Basel and University Hospital Basel, 4056 Basel, Switzerland

* To whom correspondence should be addressed:

Prof. Nicola Aceto
University of Basel and University Hospital Basel
Phone: +41-61-207-0773
Nicola.Aceto@unibas.ch

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Supplementary Table 1. Clinical features of 34 breast cancer patients with detectable circulating tumor cells (CTCs). Table showing the age (years) at the moment of primary diagnosis, age at CTC evaluation and summarizing the primary tumor parameters: % of ER+ cells, % of PR+ cells, % of Ki67+ cells, HER2 status ("+": positive, "-": negative), histologic subtype (IL: invasive lobular; ID: invasive ductal; Inf. ID: inflammatory invasive ductal). NA: not available.

Patient ID	Age at primary diagnosis	Age at CTC evaluation	% of ER+ cells	% of PR+ cells	% of Ki67+ cells	HER2 status	Histologic subtype	Tumor grade
Br2	58	72	1	1	NA	NA	IL	3
Br3	57	57	35	0	40	+	ID	3
Br7	42	42	90	90	25	-	Inf. ID	2
Br9	60	63	70	30	60	-	ID	3
Br11	54	57	0	0	50	-	ID	3
Br13	56	62	100	5	5	-	IL	2
Br14	73	75	100	10	NA	-	ID	2
Br15	38	43	100	75	NA	-	ID	1
Br16	38	49	100	75	NA	-	ID	2
Br17	53	67	1	1	NA	-	ID	2
Br23	63	63	100	0	NA	-	IL	2
Br25	39	46	70	15	NA	-	ID	2
Br26	68	68	NA	NA	NA	NA	NA	NA
Br27	62	64	100	5	20	+	ID	2
Br30	49	51	80	70	25	-	ID	3
Br35	72	78	70	50	15	-	IL	2
Br37	67	67	70	0	20-35	+	ID	3
Br38	36	36	90	50	50	-	ID	2
Br39	47	53	60	30	10	-	ID	3
Br44	47	50	100	80	30	+	ID	2
Br45	45	49	100	80	10	-	ID	1
Br46	49	49	0	0	80	-	ID	3
Br51	54	55	0	0	25-30	-	ID	3
Br52	49	68	100	50	35	-	ID	3
Br53	38	60	1	0	NA	-	ID	NA
Br55	63	63	0	0	15	+	ID	2
Br56	70	74	50	0	35	-	ID	3
Br57	56	56	95	5	NA	-	ID	3
Br61	60	63	60	0	NA	-	ID	2
Br63	58	61	0	0	90	-	ID	3
Br64	41	51	90	2	NA	+	ID	3
Br65	70	70	0	0	20	-	Inf. ID	3
Br67	57	70	90	90	NA	-	ID	1
Br73	58	79	NA	NA	NA	NA	NA	NA

Supplementary Table 2. Circulating tumor cells (CTCs) enumeration and composition in 34 breast cancer patients. Table describing the presence of metastatic disease at the time of CTC enumeration and total numbers and percentages of single CTCs, CTC-clusters without white blood cells (WBCs) and CTC-clusters with WBCs (CTC-WBC-clusters) per 7.5ml of peripheral blood.

Patient ID	Established metastatic disease at CTC evaluation	# of Single CTCs	# CTC-cluster without WBCs	# CTC-WBC-clusters	% of Single CTCs	% CTC-cluster without WBCs	% CTC-WBC-clusters	Total # of CTCs per patient
Br2	yes	10	0	0	100	0	0	10
Br3	yes	3	0	0	100	0	0	3
Br7	yes	100	20	10	77	15	8	130
Br9	yes	2	0	0	100	0	0	2
Br11	yes	9	1	0	90	10	0	10
Br13	yes	1	0	0	100	0	0	1
Br14	yes	1	0	0	100	0	0	1
Br15	yes	4	1	0	80	20	0	5
Br16	yes	100	25	0	80	20	0	125
Br17	yes	24	4	0	86	14	0	28
Br23	yes	100	100	5	49	49	2	205
Br25	yes	5	0	0	100	0	0	5
Br26	yes	4	1	0	80	20	0	5
Br27	yes	2	0	0	100	0	0	2
Br30	yes	10	0	3	77	0	23	13
Br35	yes	3	0	0	100	0	0	3
Br37	yes	4	0	1	80	0	20	5
Br38	no	9	1	0	90	10	0	10
Br39	yes	15	7	1	65	30	4	23
Br44	yes	10	0	1	91	0	9	11
Br45	yes	28	3	0	90	10	0	31
Br46	no	4	0	0	100	0	0	4
Br51	yes	1	0	0	100	0	0	1
Br52	yes	1	0	0	100	0	0	1
Br53	yes	4	2	0	67	33	0	6
Br55	yes	2	0	0	100	0	0	2
Br56	yes	1	0	0	100	0	0	1
Br57	yes	20	1	2	87	4	9	23
Br61	yes	25	10	7	60	24	17	42
Br63	yes	20	0	1	95	0	5	21
Br64	yes	4	0	0	100	0	0	4
Br65	yes	1	0	0	100	0	0	1
Br67	yes	13	0	0	100	0	0	13
Br73	yes	6	4	2	50	33	17	12

Supplementary Table 3. Genes upregulated in CTCs from CTC-neutrophil-clusters obtained from the Balb/c-4T1 mouse model. Table listing the genes upregulated in CTCs from CTC-neutrophil-clusters compared to CTCs alone (*n*=29). Gene ID, fold change, *P* value and *Q* value are shown (edgeR likelihood ratio test for differential expression, with Benjamini-Hochberg adjustment).

Gene ID	Fold change	*P* value	*Q* value
Col17a1	7.729711543	4.38E-05	0.022963533
Nxt2	7.271228295	1.69E-05	0.014192394
Mecom	7.397491609	3.77E-05	0.022024227
Slfn3	7.077366966	1.04E-04	0.03905821
Adck4	7.427678279	1.30E-04	0.042211886
G630025P09Rik	7.287674752	3.69E-05	0.022024227
Tmem102	7.224902975	3.95E-05	0.022024227
6430548M08Rik	4.592459238	5.28E-05	0.02659683
Osgin2	9.337328143	3.58E-09	0.000045053
Map4k2	4.052985433	3.73E-07	0.001174217
Cerk	6.998938853	7.98E-07	0.002008694
Bcdin3d	7.464061077	9.10E-06	0.009548896
Mcm4	1.966913377	3.12E-05	0.022024227
Mcm6	2.563422692	1.15E-04	0.041240492
Gm19557	3.687694485	8.98E-05	0.038458772
Paqr4	2.968014763	9.96E-07	0.002089226
Ccz1	1.336601221	1.47E-04	0.042232275
Pts	3.629387912	5.73E-05	0.027734751
LOC100861795	1.925876446	1.34E-04	0.042211886
Mad2l1	2.047787406	1.05E-04	0.03905821
Dhx33	2.884231863	1.98E-04	0.049080981
2410016O06Rik	3.79735797	1.43E-04	0.042232275
Tmppe	2.590383285	1.88E-04	0.049080981
Kcnab2	1.503120971	1.60E-04	0.04369843
Dpm2	1.653716335	1.99E-04	0.049080981
Fam188b	7.705872446	8.17E-06	0.009349097
Wdr76	5.585718202	1.46E-04	0.042232275
Ipp	7.760431523	1.23E-05	0.011095049
Kctd18	4.237152031	1.22E-04	0.042211886
LOC100862377	8.145983123	1.52E-08	0.000095428
Cln8	6.156148498	5.46E-08	0.000229294
Zfp768	7.851069906	1.85E-06	0.002905122
Ranbp9	2.990168313	1.53E-04	0.042834776
Zfp606	7.328783228	4.02E-05	0.022024227
Orc6	2.099485226	2.96E-05	0.022024227
Dhx58	7.966691124	1.33E-04	0.042211886
Orc1	4.669427894	3.22E-06	0.004508625
Apitd1	2.609215464	1.75E-04	0.046756691
Smpd2	2.810383775	3.62E-05	0.022024227
Gins2	2.955913697	1.17E-05	0.011095049
Hells	2.268046896	7.26E-05	0.03383987

Supplementary Table 4. Genes downregulated in CTCs from CTC-neutrophil-clusters obtained from the Balb/c-4T1 mouse model. Table listing the genes downregulated in CTCs from CTC-neutrophil-clusters compared to CTCs alone (*n*=29). Gene ID, fold change, *P* value and *Q* value are shown (edgeR likelihood ratio test for differential expression, with Benjamini-Hochberg adjustment).

Gene ID	Fold change	*P* value	*Q* value
9430083A17Rik	-7.011577924	3.55E-05	0.022024227
Cnn2	-1.039965548	1.94E-04	0.049080981
Lima1	-1.928691973	9.52E-05	0.038647688
Fblim1	-1.66298953	6.49E-06	0.00816999
Pdlim5	-1.510923036	1.48E-04	0.042232275
Plekhn1	-1.993109176	7.76E-05	0.03487739
Slc40a1	-7.047808867	1.68E-06	0.002905122
H2−T23	-1.411196227	1.00E-04	0.03905821
6330403M23Rik	-2.306668723	9.16E-05	0.038458772
Lamc3	-6.11620345	1.24E-04	0.042211886

Supplementary Table 5. Genes frequently mutated in CTC-neutrophil donors. Table listing genes mutated in 50% of donors with CTC-neutrophil-clusters. High-impact mutations (as defined by snpEff; see methods section), as well as their predicted effects, are shown across different donors.

Gene ID	Mutation	Donor ID	Predicted effect (snpEff)
MERTK	c.2466C>T	Br23	structural_interaction_variant
MERTK	c.2173G>A	Br57	structural_interaction_variant
MERTK	c.1816C>T	Br61	structural_interaction_variant
MERTK	c.1994C>T	Br61	structural_interaction_variant
MERTK	c.1995A>G	Br61	structural_interaction_variant
MERTK	c.2007A>G	Br61	structural_interaction_variant
MERTK	c.2031G>A	Br61	structural_interaction_variant
TLE1	c.1908G>A	Br57	structural_interaction_variant
TLE1	c.1787G>A	Br30	structural_interaction_variant
TLE1	c.1551T>C	Br61	structural_interaction_variant
TLE1	c.1509G>C	Br61	structural_interaction_variant
TLR9	c.1219C>T	Br23	stop_gained
TLR9	c.1619C>A	Br30	stop_gained
TLR9	c.1345C>T	Br61	stop_gained
TLR9	c.893G>A	Br61	stop_gained
TRBV6-1	c.340G>T	Br23	stop_gained
TRBV6-1	c.340G>T	Br30	stop_gained
TRBV6-1	c.340G>T	Br57	stop_gained
TTN	c.102513C>G	Br23	stop_gained
TTN	c.97590C>G	Br23	stop_gained
TTN	c.88895-1G>A	Br57	splice_acceptor_variant&intron_variant
TTN	c.83972-1G>A	Br57	splice_acceptor_variant&intron_variant
TTN	c.21067C>T	Br57	stop_gained
TTN	c.20116C>T	Br57	stop_gained
TTN	c.97019G>A	Br61	stop_gained
TTN	c.92096G>A	Br61	stop_gained
TTN	c.70737G>A	Br61	stop_gained
TTN	c.65814G>A	Br61	stop_gained
TTN	c.52315A>T	Br61	stop_gained
TTN	c.47392A>T	Br61	stop_gained
TTN	c.31312C>T	Br61	stop_gained
TTN	c.30361C>T	Br61	stop_gained
ABCA13	c.12412C>T	Br23	stop_gained
ABCA13	c.13285C>T	Br61	stop_gained
ABCC1	c.2530G>A	Br23	structural_interaction_variant
ABCC1	c.2500C>T	Br57	stop_gained
ADGB	c.708G>A	Br23	stop_gained
ADGB	c.1707+1G>A	Br61	splice_donor_variant&intron_variant
AKR1D1	c.513T>C	Br30	structural_interaction_variant
AKR1D1	c.518G>A	Br61	structural_interaction_variant
APOB	c.13188G>A	Br23	stop_gained

APOB	c.2242C>T	Br23	stop_gained&splice_region_variant
APOB	c.693+1G>C	Br57	splice_donor_variant&intron_variant
CENPH	c.595C>T	Br30	stop_gained
CENPH	c.652-1G>A	Br57	splice_acceptor_variant&intron_variant
CERK	c.430G>T	Br57	stop_gained
CERK	c.433C>T	Br61	stop_gained
CLCN1	c.979+1G>T	Br57	splice_donor_variant&intron_variant
CLCN1	c.44G>A	Br61	stop_gained
CSF1R	c.1294C>T	Br23	structural_interaction_variant
CSF1R	c.1665C>T	Br61	structural_interaction_variant
DDB1	c.256G>A	Br23	structural_interaction_variant
DDB1	c.2103T>A	Br61	structural_interaction_variant
DDB1	c.980G>A	Br61	protein_protein_contact
DDX47	c.441A>T	Br57	structural_interaction_variant
DDX47	c.514G>A	Br61	structural_interaction_variant
DECR1	c.271C>T	Br57	structural_interaction_variant
DECR1	c.418-1G>A	Br61	splice_acceptor_variant&intron_variant
DLGAP1	c.2725-1G>A	Br57	splice_acceptor_variant&intron_variant
DLGAP1	c.955C>T	Br61	stop_gained&splice_region_variant
DNAH8	c.8137-2A>T	Br30	splice_acceptor_variant&intron_variant
DNAH8	c.1294-2A>G	Br61	splice_acceptor_variant&intron_variant
DSP	c.754G>A	Br30	structural_interaction_variant
DSP	c.1990C>T	Br61	stop_gained
DUSP5	c.988C>T	Br57	stop_gained
DUSP5	c.633C>T	Br61	structural_interaction_variant
DUSP5	c.792G>A	Br61	structural_interaction_variant
DUSP5	c.799A>G	Br61	structural_interaction_variant
DUSP5	c.801C>T	Br61	structural_interaction_variant
DUSP5	c.807T>G	Br61	structural_interaction_variant
DUSP5	c.903G>T	Br61	structural_interaction_variant
EIF5	c.932A>G	Br57	structural_interaction_variant
EIF5	c.808C>T	Br61	structural_interaction_variant
EPG5	c.2716C>T	Br30	stop_gained&splice_region_variant
EPG5	c.1174C>T	Br61	stop_gained
GTF3C1	c.4540C>T	Br30	stop_gained
GTF3C1	c.2351-1G>A	Br61	splice_acceptor_variant&intron_variant
GUCY1A3	c.1402C>T	Br23	stop_gained
GUCY1A3	c.973G>T	Br61	stop_gained
IDE	c.949T>A	Br57	structural_interaction_variant
IDE	c.1140C>T	Br61	structural_interaction_variant
IFIT1B	c.3G>A	Br57	start_lost&splice_region_variant
IFIT1B	c.414T>A	Br61	stop_gained
IGSF6	c.1A>G	Br23	start_lost
IGSF6	c.201G>A	Br61	stop_gained
IQGAP3	c.2242C>T	Br23	stop_gained
IQGAP3	c.4111C>T	Br61	stop_gained
KDM4A	c.318C>A	Br30	stop_gained
KDM4A	c.98A>G	Br61	structural_interaction_variant

KIF13A	c.3609-1G>A	Br30	splice_acceptor_variant&intron_variant
KIF13A	c.186G>A	Br61	stop_gained
KIF6	c.252-1G>C	Br23	splice_acceptor_variant&intron_variant
KIF6	c.766C>T	Br61	stop_gained
LRP1	c.8308+1G>A	Br44	splice_donor_variant&intron_variant
LRP1	c.2623G>T	Br57	stop_gained
MCF2L2	c.1959G>A	Br30	stop_gained
MCF2L2	c.878+1G>A	Br61	splice_donor_variant&intron_variant
MDC1	c.5681A>G	Br57	structural_interaction_variant
MDC1	c.2422C>T	Br61	stop_gained
MGA	c.950C>G	Br23	stop_gained
MGA	c.4097C>G	Br61	stop_gained
MUC16	c.21001C>T	Br57	stop_gained
MUC16	c.42031C>T	Br61	stop_gained
NOS2	c.1373C>T	Br44	structural_interaction_variant
NOS2	c.938G>A	Br61	structural_interaction_variant
NOTCH2	c.6967C>T	Br57	stop_gained
NOTCH2	c.5293C>T	Br57	stop_gained
NOTCH2	c.4688G>A	Br57	structural_interaction_variant
NOTCH2	c.966T>A	Br61	stop_gained
NRXN1	c.3979A>T	Br57	stop_gained
NRXN1	c.2467+1G>A	Br61	splice_donor_variant&intron_variant
NUP133	c.2295G>A	Br57	stop_gained
NUP133	c.2632C>T	Br61	stop_gained
NUP133	c.1312C>A	Br61	structural_interaction_variant
NUP214	c.873G>A	Br23	structural_interaction_variant
NUP214	c.1026A>G	Br61	structural_interaction_variant
NUP214	c.1051T>C	Br61	structural_interaction_variant
NUP214	c.1092A>T	Br61	structural_interaction_variant
NUP214	c.1097T>C	Br61	structural_interaction_variant
NUP214	c.1099G>A	Br61	structural_interaction_variant
NUP214	c.1104C>T	Br61	structural_interaction_variant
NUP214	c.1114C>G	Br61	structural_interaction_variant
OR10K1	c.7C>T	Br57	stop_gained
OR10K1	c.904C>T	Br61	stop_gained
PGM1	c.1247G>A	Br23	stop_gained
PGM1	c.919G>T	Br57	stop_gained
PIR	c.570G>A	Br57	stop_gained
PIR	c.334C>T	Br57	structural_interaction_variant
PIR	c.838A>G	Br61	structural_interaction_variant
PIR	c.711C>T	Br61	structural_interaction_variant
PPIH	c.275G>A	Br30	structural_interaction_variant
PPIH	c.252T>C	Br61	structural_interaction_variant
PRRC2C	c.6651+2T>C	Br57	splice_donor_variant&intron_variant
PRRC2C	c.7318C>T	Br61	stop_gained
PTPRB	c.4544G>A	Br23	stop_gained
PTPRB	c.5881C>T	Br61	stop_gained
RAB9B	c.375G>A	Br30	structural_interaction_variant

RAB9B	c.451T>C	Br61	structural_interaction_variant
RAB9B	c.360A>G	Br61	structural_interaction_variant
RAB9B	c.312A>G	Br61	structural_interaction_variant
RAB9B	c.306G>A	Br61	structural_interaction_variant
RAB9B	c.291T>C	Br61	structural_interaction_variant
RAB9B	c.255G>A	Br61	structural_interaction_variant
RAB9B	c.253T>C	Br61	structural_interaction_variant
RAB9B	c.252C>G	Br61	structural_interaction_variant
RAB9B	c.192A>G	Br61	structural_interaction_variant
RAB9B	c.168A>G	Br61	structural_interaction_variant
RAB9B	c.33T>C	Br61	structural_interaction_variant
RAB9B	c.18G>T	Br61	structural_interaction_variant
RELN	c.9370-2A>G	Br57	splice_acceptor_variant&intron_variant
RELN	c.7606G>T	Br57	stop_gained
RELN	c.4696C>T	Br61	stop_gained
RNASEL	c.1322T>C	Br30	structural_interaction_variant
RNASEL	c.1527G>A	Br61	structural_interaction_variant
SEC24D	c.2790G>A	Br30	stop_gained
SEC24D	c.1708-2A>T	Br61	splice_acceptor_variant&intron_variant
SGSM1	c.1786C>T	Br23	stop_gained
SGSM1	c.2227G>T	Br30	stop_gained
SGSM1	c.3385C>T	Br30	stop_gained
SPTAN1	c.742C>T	Br23	stop_gained
SPTAN1	c.4342C>T	Br61	stop_gained&splice_region_variant
SPTB	c.4033C>T	Br23	stop_gained
SPTB	c.1972C>T	Br57	stop_gained
TAS2R43	c.900G>A	Br30	stop_gained
TRIM42	c.43C>T	Br57	stop_gained
TRIM42	c.1189C>T	Br61	stop_gained
UNC80	c.7388+2T>C	Br44	splice_donor_variant&intron_variant
UNC80	c.6268-1G>T	Br61	splice_acceptor_variant&intron_variant
UROD	c.504T>C	Br23	structural_interaction_variant
UROD	c.324C>T	Br61	structural_interaction_variant
USP17L17	c.2T>G	Br30	start_lost
USP17L17	c.21C>G	Br61	stop_gained
USP34	c.7735C>T	Br30	stop_gained
USP34	c.1651C>T	Br61	stop_gained
USP34	c.1441C>T	Br61	stop_gained
VPS33A	c.1033C>T	Br30	stop_gained
VPS33A	c.685T>C	Br61	structural_interaction_variant
VPS33A	c.678T>C	Br61	structural_interaction_variant
VPS33A	c.669T>G	Br61	structural_interaction_variant
VPS33A	c.549A>G	Br61	structural_interaction_variant
WDR89	c.298C>T	Br23	stop_gained
WDR89	c.190C>T	Br23	stop_gained
WDR89	c.298C>T	Br57	stop_gained
XRCC5	c.888C>T	Br23	protein_protein_contact
XRCC5	c.1308A>C	Br61	protein_protein_contact

XRCC5	c.1311C>G	Br61	protein_protein_contact
XRCC5	c.1311C>G	Br61	protein_protein_contact
ZNF202	c.604C>T	Br57	stop_gained
ZNF202	c.1492A>T	Br61	stop_gained
ZNF274	c.1426C>T	Br23	stop_gained
ZNF274	c.1863C>A	Br61	stop_gained
ZNF506	c.1093G>T	Br30	stop_gained
ZNF506	c.641C>G	Br61	stop_gained
ZNF585B	c.1681C>T	Br57	stop_gained
ZNF585B	c.1A>G	Br61	start_lost