

SUPPLEMENTARY TABLES FOR

Circulating tumor cells exhibit metastatic tropism and reveal brain metastasis drivers

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File includes supplementary tables S1 to S10
(Large Supplementary Table S3, S4, S8 and S10 were uploaded as separate xlsx files)

Table S1. Breast cancer patient information. Clinical information regarding metastatic lesions are reported for patient from whom CTC lines were generated. To ensure genetic stability in CTC line over time in culture, we used the Ovation Target Enrichment System for identification of changes in key driver mutations allele frequency in BRx68

Patient No	BRx07	BRx42	BRx50	BRx68				
Blood Collection	08/15/2012	09/24/2013	04/22/2013	05/08/2013				
Bone	Yes	Yes	Yes	Yes				
Lung	Yes (small nodules <1cm)	No	No	Yes (LNs)				
Ovary	No	Yes	No	No				
Brain	No (MRI brain negative in 2011)	No (developed later. MRI brain positive on 10/31/14)	No (MRI brain negative in 2011)	No (MRI brain negative in 2011)				
Mutation detected in CTC lines (as described in Yu and al., 2014)	BRx07	BRx42	BRx50	BRx68	Mutant allele frequency over time in culture in BRx68			
	TP53	KRAS	BRCA2	TP53	7 months	8 months	12 months	18 months
	PIK3CA	PIK3CA	IKZF1	PIK3CA	0.685	0.818	0.852	0.75
	FGFR2	IGF1R	ESR1	ESR1	0.744	0.707	0.719	0.784
	CDH1			MSN	0.5	0.697	0.5	0.469
APC								
DGKQ								
MAML2								

Table S2. Metastatic potential in breast cancer patient-derived CTC lines. Comparison of organ tropism between parental CTC lines and CTC-derived metastatic variants. Percent of mice exhibiting brain, bone, lung and ovary metastases, 8 months (BRx50) or 5 months (BRx68 and BRx07) after inoculation with parental lines (first generation) and metastatic variants (second or third generation). n = number of mice.

CTC lines (intracardiac injection)	Metastases n (%)			
	Brain	Bone	Lung	Ovary
BRx07				
BRx07 (1st generation) n=18	0 (0%)	3 (17%)	13 (72%)	13 (72%)
BRx07LuM1 (2nd generation) n=6	0 (0%)	2 (33%)	6 (100%)	5 (83%)
BRx07LuM2 (3rd generation) n=5	0 (0%)	0 (0%)	5 (100%)	4 (80%)
Fisher's Exact Test <i>P-value</i> (1st vs 2nd)	1.00	0.57	0.28	1.00
Fisher's Exact Test <i>P-value</i> (1st vs 3rd)	1.00	1.00	0.55	1.00
Fisher's Exact Test <i>P-value</i> (2nd vs 3rd)	1.00	0.45	1.00	1.00
BRx50				
BRx50 (1st generation) n=18	1 (6%)	1 (6%)	0 (0%)	0 (0%)
BRx50BrM1 (2nd generation) n=3	3 (100%)	1 (33%)	0 (0%)	2 (67%)
BRx50BrM2 (3rd generation) n=8	4 (50%)	2 (25%)	0 (0%)	2 (25%)
Fisher's Exact Test <i>P-value</i> (1st vs 2nd)	0.003	0.27	1.00	0.01
Fisher's Exact Test <i>P-value</i> (1st vs 3rd)	0.02	0.22	1.00	0.09
Fisher's Exact Test <i>P-value</i> (2nd vs 3rd)	0.24	1.00	1.00	0.49
BRx68				
BRx68 (1st generation) n=12	0 (0%)	4 (33%)	1 (3%)	5 (42%)
BRx68BoM1 (2nd generation) n=16	2 (12.5%)	10 (62.5%)	0 (0%)	9 (56%)
Fisher's Exact Test <i>P-value</i>	0.49	0.25	0.43	0.7

Table S5. List of differentially expressed genes in bone stromal cells after tumor formation.

Ensembl gene id	Gene symbol	log2FoldChange	pvalue	padj
ENSMUSG00000021194	Chga	6.101342879	5.40E-08	7.52E-05
ENSMUSG00000074006	Omp	6.169660643	5.83E-07	0.0006210538443
ENSMUSG00000038115	Ano2	4.987103786	1.45E-06	0.001310478896
ENSMUSG000000051111	Sv2c	4.87675386	3.57E-06	0.002939848026
ENSMUSG00000023032	Slc4a8	2.862484226	4.23E-06	0.003196940916
ENSMUSG00000006575	Rundc3a	3.1135593	4.24E-06	0.003196940916
ENSMUSG00000003410	Elavl3	4.918516724	4.74E-06	0.003435269758
ENSMUSG00000027895	Kcnc4	4.383850221	9.82E-06	0.006585568415
ENSMUSG00000053693	Mast1	2.054807541	1.55E-05	0.008793944672
ENSMUSG00000044217	Aqp5	4.328279112	1.53E-05	0.008793944672
ENSMUSG00000032452	Clstn2	4.150347977	1.66E-05	0.009092529073
ENSMUSG00000021803	Cdhr1	4.509507339	1.99E-05	0.01027412167
ENSMUSG000000031026	Trim66	4.435764827	2.06E-05	0.01037096975
ENSMUSG00000039809	Gabbr2	5.820666875	2.58E-05	0.01197580834
ENSMUSG00000000247	Lhx2	4.244331078	2.67E-05	0.01209647359
ENSMUSG000000035355	Kcnh4	3.818751754	3.25E-05	0.0140115914
ENSMUSG00000047787	Flrt1	3.281575546	3.83E-05	0.01576444324
ENSMUSG00000000983	Wfdc18	6.198374563	4.37E-05	0.0172085121
ENSMUSG000000029168	Dpysl5	4.026436021	4.83E-05	0.0175169607
ENSMUSG00000022619	Mapk8ip2	4.979405295	4.84E-05	0.0175169607
ENSMUSG00000058589	Anks1b	3.732938583	4.98E-05	0.01766607684
ENSMUSG000000038453	Scin1	3.480769058	5.37E-05	0.01833262313
ENSMUSG00000078161	BC007180	4.108538751	7.66E-05	0.02476103474
ENSMUSG00000025813	Homer2	2.671286211	8.20E-05	0.02558369884
ENSMUSG000000098220	Gm27031	4.257502883	8.19E-05	0.02558369884
ENSMUSG00000028017	Egf	3.212271515	8.77E-05	0.02618894743
ENSMUSG000000041653	Pnpla3	3.380347479	8.83E-05	0.02618894743
ENSMUSG000000037386	Rims2	5.498030283	8.68E-05	0.02618894743
ENSMUSG00000022490	Ppp1r1a	2.514050138	9.78E-05	0.02673623515
ENSMUSG00000024411	Aqp4	6.101372765	9.37E-05	0.02673623515
ENSMUSG000000045761	Fam179a	4.645715771	0.0001024797009	0.0272399953
ENSMUSG00000029189	Sel1l3	2.696390456	0.0001059895149	0.0274073744
ENSMUSG00000040564	Apoc1	4.452734331	0.0001101433669	0.02808035331
ENSMUSG000000043969	Emx2	4.636481328	0.0001129114868	0.02838626143
ENSMUSG00000022780	Mfi2	3.073603352	0.0001170691427	0.02863606152
ENSMUSG00000025202	Scd3	4.487023799	0.0001161534767	0.02863606152
ENSMUSG000000032890	Rims3	3.132328051	0.0001265906866	0.02975867556
ENSMUSG00000029657	Hsph1	2.865108956	0.0001318870019	0.0302342399
ENSMUSG00000030650	Tmc5	3.891722279	0.0001389301683	0.03119227362
ENSMUSG000000056216	Cebpg	0.7572180985	0.0001458599257	0.03170381171
ENSMUSG00000014725	Adam28	4.231171674	0.0001506285734	0.03170381171
ENSMUSG000000037974	Muc5ac	4.904992428	0.0001477986136	0.03170381171
ENSMUSG000000042672	Dcst1	2.501674037	0.0001534861897	0.03193394851
ENSMUSG00000022949	Clic6	4.083132909	0.0001645088841	0.03345814957
ENSMUSG000000031709	Tbc1d9	1.327260987	0.0001696141269	0.03411317012

ENSMUSG00000040412	5330417C22Rik	4.238995942	0.000184069661	0.03585784796
ENSMUSG00000047228	BC048546	4.79962377	0.0001896217217	0.03651428495
ENSMUSG00000062444	Ap3b2	3.754786905	0.0001968452381	0.03711557973
ENSMUSG00000029830	Svopl	3.952256412	0.0002038697117	0.03765556787
ENSMUSG00000039092	Sptlc3	6.197728869	0.0002278918423	0.04084227957
ENSMUSG00000028128	F3	2.4120806	0.0002325462034	0.04126783164
ENSMUSG00000037419	Endod1	1.158178019	0.0002380292731	0.0415896479
ENSMUSG00000022871	Fetub	4.497900844	0.0002389549407	0.0415896479
ENSMUSG00000078515	Ddi2	0.4455092089	0.0002440952152	0.04168271217
ENSMUSG00000020396	Nefh	3.744370467	0.0002586323085	0.04341406357
ENSMUSG00000023011	Faim2	3.567432455	0.0002845703017	0.04599113421
ENSMUSG00000042784	Muc1	2.772634653	0.0002969350591	0.04756479207
ENSMUSG00000056880	Gadl1	5.146972635	0.0003082589142	0.04894556671
ENSMUSG00000028699	Tspan1	2.53001592	0.0003151945819	0.04918394076
ENSMUSG00000002190	Clgn	5.409586714	0.0003195314681	0.04943452225
ENSMUSG00000098178	Yam1	-19.70427924	6.50E-19	1.96E-15
ENSMUSG00000064337	mt-Rnr1	-5.972865859	1.96E-10	3.54E-07
ENSMUSG00000064339	mt-Rnr2	-4.614609045	1.37E-05	0.008285677647

Table S6. List of differentially expressed genes in lung stromal cells after tumor formation.

Ensembl gene id	Gene symbol	log2FoldChange	pvalue	padj
ENSMUSG00000053469	Tg	6.737996047	1.67E-11	9.04E-08
ENSMUSG00000022157	Mcpt8	-11.98931127	6.33E-08	0.0001711042441

Table S7. List of differentially expressed genes in brain stromal cells after tumor formation and overlapping with microglia activation signature (Das., et al 2016 (12)).

Ensembl gene id	Gene symbol	log2FoldChange	pvalue	padj
ENSMUSG00000030341	Tnfrsf1a	2.039008872	7.11E-09	5.16E-06
ENSMUSG00000060591	Ifitm2	2.57281611	2.24E-08	1.54E-05
ENSMUSG0000007041	Clic1	2.839077995	5.94E-08	3.66E-05
ENSMUSG00000052837	Junb	1.97263714	6.38E-08	3.75E-05
ENSMUSG00000005823	Gpr108	1.244615898	1.98E-07	0.0001110401322
ENSMUSG00000021281	Tnfaip2	2.718376198	4.31E-07	0.0002047594101
ENSMUSG00000025492	Ifitm3	2.866868511	4.21E-07	0.0002047594101
ENSMUSG00000064215	Ifi27	1.84345901	4.87E-07	0.0002145415641
ENSMUSG00000026547	Tagln2	2.109105472	7.12E-07	0.0002928816872
ENSMUSG00000032000	Birc3	2.572742334	9.95E-07	0.0003960489629
ENSMUSG00000073411	H2-D1	2.527633778	1.06E-06	0.0004070475827
ENSMUSG00000050335	Lgals3	3.393281644	1.10E-06	0.0004107083758
ENSMUSG00000024659	Anxa1	4.240660268	1.76E-06	0.0006036380827
ENSMUSG00000001123	Lgals9	2.499583582	2.43E-06	0.0007880307778
ENSMUSG00000032508	Myd88	1.675145553	2.97E-06	0.0009169863925
ENSMUSG00000024048	Myl12a	1.389170024	4.82E-06	0.001384305619
ENSMUSG00000020806	Rhbdf2	2.26742559	5.04E-06	0.001412565222
ENSMUSG00000046718	Bst2	3.414917182	5.69E-06	0.001560110511
ENSMUSG00000042677	Zc3h12a	2.228688221	6.33E-06	0.001697376062
ENSMUSG00000035778	Ggta1	1.680527217	6.49E-06	0.001697698461
ENSMUSG00000024610	Cd74	3.968603263	7.09E-06	0.00178575374
ENSMUSG00000006498	Ptbp1	1.015709563	8.83E-06	0.002137967909
ENSMUSG00000021822	Plau	2.558993009	1.01E-05	0.002357554545
ENSMUSG00000053175	Bcl3	2.803964397	1.12E-05	0.002559919481
ENSMUSG00000026483	Fam129a	2.141133441	1.39E-05	0.003124703459
ENSMUSG00000032231	Anxa2	2.423230664	1.69E-05	0.00356571194
ENSMUSG00000044786	Zfp36	1.998561642	1.89E-05	0.003801868423
ENSMUSG00000032515	Csmp1	2.007517237	1.93E-05	0.003801868423
ENSMUSG00000056501	Cebpb	2.900627637	1.85E-05	0.003801868423
ENSMUSG00000030342	Cd9	1.854741289	2.06E-05	0.003851681615
ENSMUSG00000016256	Ctsz	2.238187235	2.25E-05	0.004142484691
ENSMUSG00000039899	Fgl2	2.816997691	2.38E-05	0.00418789384
ENSMUSG00000002603	Tgfb1	1.832966068	2.51E-05	0.00429670681
ENSMUSG00000031207	Msn	1.763625687	2.94E-05	0.004710993196
ENSMUSG00000069793	Slfn9	3.32114738	4.49E-05	0.006934115071
ENSMUSG00000042622	Maff	2.667799568	4.62E-05	0.007041083918
ENSMUSG00000030339	Ltbr	1.199116786	6.07E-05	0.008782655951
ENSMUSG00000056737	Capg	2.812724176	6.12E-05	0.008782655951
ENSMUSG00000037820	Tgm2	1.48966277	6.25E-05	0.008863208485
ENSMUSG00000054520	Sh3bp2	1.962214765	6.63E-05	0.00909951932
ENSMUSG00000022175	Lrp10	1.313768879	7.28E-05	0.009558500028
ENSMUSG00000042626	Shc1	1.253440951	9.46E-05	0.01167109623
ENSMUSG00000071637	Cebpd	2.370828678	9.36E-05	0.01167109623

ENSMUSG00000034947	Tmem106a	2.586753764	9.10E-05	0.01167109623
ENSMUSG00000024164	C3	4.417338948	0.001001555992	0.01223980753
ENSMUSG00000029622	Arpc1b	2.26405037	0.001077964748	0.01267173227
ENSMUSG00000071713	Csf2rb	2.694183464	0.000114197902	0.01305133986
ENSMUSG00000003458	Ncstn	0.9039240091	0.0001403490682	0.01455738276
ENSMUSG00000077714	Snord17	3.957020373	0.0001364408886	0.01455738276
ENSMUSG00000025044	Msr1	3.980102061	0.0001708434726	0.01728459822
ENSMUSG00000060802	B2m	2.188011737	0.0001764828938	0.01742662686
ENSMUSG00000031146	Plp2	2.640328954	0.0001788736248	0.01752251707
ENSMUSG00000032690	Oas2	2.899084516	0.0001862010278	0.01795530692
ENSMUSG00000031103	Elf4	2.134146774	0.0002184314689	0.01982426192
ENSMUSG00000031504	Rab20	3.245680158	0.0002169865477	0.01982426192
ENSMUSG00000056569	Mpz	4.794145811	0.0002236141281	0.0200801171
ENSMUSG00000024927	Rela	1.161728014	0.0002452821037	0.02147175182
ENSMUSG00000035678	Tnfsf9	2.495025263	0.0002483461122	0.02158687368
ENSMUSG00000025498	Irf7	3.59459734	0.0002530570127	0.02169085214
ENSMUSG00000002325	Irf9	1.31819847	0.0002611979944	0.02218884948
ENSMUSG00000029304	Spp1	3.007011827	0.0002642599752	0.02218884948
ENSMUSG000000021025	Nfkbia	1.743456459	0.0002810025796	0.0231227656
ENSMUSG00000036461	Elf1	1.537906441	0.0002906035385	0.02355726471
ENSMUSG00000053113	Socs3	2.073017589	0.0003017625677	0.02403003466
ENSMUSG00000069893	9930111J21Ri	2.302459751	0.0003428744865	0.02571362751
ENSMUSG00000037344	Slc12a9	1.007003675	0.0003767462002	0.02784537933
ENSMUSG00000001750	Tcirg1	1.853516925	0.0003801765144	0.02793165903
ENSMUSG000000041515	Irf8	2.527579204	0.0003872043775	0.02811331548
ENSMUSG00000035692	Isg15	3.629665471	0.0003989881442	0.02879947757
ENSMUSG00000025225	Nfkib2	2.066901094	0.0004058713264	0.0289576288
ENSMUSG00000033880	Lgals3bp	2.625863947	0.0004055294193	0.0289576288
ENSMUSG00000024732	Ccdc86	1.339631068	0.0004178814035	0.02947377236
ENSMUSG00000017760	Ctsa	1.48979444	0.000446978337	0.03070944493
ENSMUSG00000010358	Ifi35	2.048470684	0.0004478408886	0.03070944493
ENSMUSG00000070327	Rnf213	1.979368062	0.000451430381	0.03078455908
ENSMUSG00000027642	Rpn2	1.109015071	0.000473520827	0.03175925618
ENSMUSG00000040212	Emp3	2.516926234	0.0004833556062	0.03175925618
ENSMUSG00000028459	Cd72	4.170418301	0.0004801830509	0.03175925618
ENSMUSG00000079111	Kdelr2	1.713849003	0.0005627808393	0.03544083622
ENSMUSG00000068220	Lgals1	2.768181906	0.0005798469293	0.03617543906
ENSMUSG00000034422	Parp14	1.805964973	0.0005887814164	0.03633664511
ENSMUSG00000015839	Nfe2l2	1.698902794	0.0005988428021	0.0364252636
ENSMUSG00000050370	Ch25h	3.439061725	0.0005954429698	0.0364252636
ENSMUSG00000028962	Slc4a2	0.9846151325	0.0006156533514	0.03704770595
ENSMUSG00000040435	Ppp1r15a	1.494796312	0.0006183121952	0.03704770595
ENSMUSG00000027322	Siglec1	3.506800558	0.0006510686348	0.03808597232
ENSMUSG00000005087	Cd44	2.231325846	0.0006879444343	0.03986524955
ENSMUSG00000029605	Oas1b	2.646474318	0.0007359934737	0.0420572567
ENSMUSG00000027580	Helz2	1.737077817	0.000742656296	0.04224242701
ENSMUSG00000026728	Vim	2.122629807	0.0007534359674	0.0426589915
ENSMUSG00000047407	Tgif1	1.770642259	0.0007625222235	0.04297630961

ENSMUSG0000009687	Fxyd5	2.292554446 .0008059062446	0.04421022567
ENSMUSG00000037321	Tap1	2.895699509 .0008558003693	0.04632957877
ENSMUSG00000003541	Ier3	2.484449459 .0008805598133	0.04705086483
ENSMUSG00000095115	Itprip12	1.375294008 .0009090068276	0.04794816784
ENSMUSG00000086513	9130208D14R	2.325323112 .0009496017873	0.04843361513
ENSMUSG00000027514	Zbp1	3.876672081 .0009495778691	0.04843361513

Table S9. Characterization of brain metastatic lesions from cancer patients.

BrM (Brain metastasis) Sample ID	Cancer Type	Staining (IHC+IF)					
		Cytokeratin	SEMA4D	c-MYC	Infiltrated Iba1+ cells	% of Ki67 positive cells	% apoptotic positive cells
BrM-3	Breast cancer	+	-	++	yes	24	2
BrM-4	Colon cancer	+	-	++	yes	60	6
BrM-5	Colon cancer	-	+	-	no	59	54
BrM-6	Lung cancer	+	-	+	yes	15	23
BrM-7	Breast cancer	+	-	++	yes	7	2
BrM-8	Lung cancer	+	++	-	yes	14	52
BrM-9	Gastroesophageal junction cancer	+	-	+++	yes	6	9
BrM10	Prostate cancer	+	++	++	yes	29	6
BrM11	Breast cancer	+	+	++	N/A	18	13
BrM12	Breast cancer	+	+	++	yes	8	12
BrM14	Lung cancer	+	+	-	yes	8	45
BrM15	Colon cancer	+	+++	++	yes	4	12

11 out of 12 7 out of 12 9 out of 12 10 out of 11