## **Supplementary Materials**

## Article

## Multiplex Label Free Characterization of Cancer Cell Lines Using Surface Plasmon Resonance Imaging

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KG1a		MCF7		MG-63		NCI-H460			SKBR3					
Marker	ABC	RU (SPRi)	Marker	ABC	RU (SPRi)	Marker	ABC	RU (SPRi)	Marker	ABC	RU (SPRi)	Marker	ABC	RU (SPRi)
CD44	48639	842	CD49f	110012	762	CD44	426853	2329	CD227	89832	997	CD24	191709	1555
CD45	21659	273	ЕрСАМ	98284	887	CD49c	81333	987	CD44	60073	1194	HER2	158838	1300
CD49e	12663	429	CD49b	89604	497	CD49e	64212	654	CD49f	56021	512	ЕрСАМ	123384	1073
CD49d	9994	308	CD24	62994	751	CD227	50185	680	CD56	55119	600	CD227	85931	853
CD166	6090	159	CD49c	55734	600	CD166	19032	438	CD24	27534	495	CD71	35500	366
CD49f	5015	167	HER2	44525	425	CD49f	16082	433	CD71	18187	266	CD49b	20942	243
CD49c	3580	218	EGFR	35005	591	CD49d	12793	496	CD49e	17089	285	CD49c	14023	181
CD71	2822	87	CD44	29102	652	CD105	11200	182	CD49b	16082	169	CD49e	11037	223
CD123	1260	40	CD71	13296	119	HER2	9684	178	EGFR	14629	269	CD166	10486	480
CD49b	1141	78	CD49a	11458	223	CD221	9135	307	CD166	12875	381	EGFR	9325	145
CD61	1039	10	CD166	9564	323	CD49b	7787	134	CD221	9766	271	HER3	6050	299
CD24	1017	28	CD104	9419	196	CD71	7531	111	HER2	8873	267	CD221	4499	159
CD105	1013	71	CD221	5994	194	CD49a	6100	138	CD49a	4731	52	CD324	4206	115
CD33	959	2	CD262	5075	-7	CD56	5928	158	CD49c	4241	82	CD105	3903	142
EGFR	957	24	CD324	4032	138	EGFR	4261	94	HER3	3858	180	CD66b	3386	8
CD261	957	7	HER3	3584	251	CD324	3980	103	CD262	1396	-14	CD49a	3046	3
CD144	924	29	CD261	1052	4	CD262	3183	-26	CD105	1371	35	CD261	3035	-9
CD227	901	18	CD103	948	7	CD113	1895	27	ЕрСАМ	1365	-17	CD140a	2926	-10
EpCAM	860	3	CD49d	691	16	CD104	1754	6	CD113	1095	-8	CD49f	2904	-6
HER2	817	15	CD113	672	1	CD3	1732	-27	CD324	588	13	CD14	2679	-16
CD235a	802	7	CD334	657	25	CD334	1656	18	CD334	588	15	CD20	2548	0
CD56	794	13	CD61	613	16	CD106	1613	-29	CD140a	567	3	CD3	2510	-6
CD262	738	3	CD11c	379	4	CD24	1300	-27	CD123	541	8	CD44	2341	-4
CD140a	736	2	CD235a	348	o	CD103	1300	-15	CD146	478	0	CD25	2319	-5

**Table S1.** responses of all 44 markers obtained with flow cytometry (ABC) and SPRi cytometry (RU SPRi), the data is sorted from high expression (green colors) to low expression (red colors) (as determined by flow cytometry).

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CD334	732	14	CD227	339	-6	HER3	1251	-24	CD117	466	-1	CD262	2281	-6
CD103	725	15	CD14	268	-1	CD144	1187	-6	CD235a	435	-1	CD103	2237	3
HER3	715	25	CD49e	265	11	CD117	1095	-21	CD144	422	7	CD49d	2226	5
CD146	715	15	CD144	265	4	CD11c	1036	-24	CD66b	410	26	CD61	2210	6
CD11c	713	-4	CD20	247	11	CD261	1036	-30	CD33	408	-6	CD106	2210	-5
CD309	706	25	CD106	245	o	CD309	1015	-17	CD61	397	-5	CD104	2194	2
CD117	704	4	CD3	239	9	CD235a	972	-18	CD106	389	-1	CD8a	2188	-16
CD113	691	26	CD117	239	-2	CD14	801	-27	CD19	376	2	CD45	2134	-1
CD106	685	10	CD8a	235	-3	CD8a	790	-28	CD49d	374	5	CD113	2128	89
CD49a	679	8	CD33	229	-3	CD66b	614	-10	CD104	350	-6	CD334	2047	13
CD25	679	7	CD19	227	0	CD45	551	-21	CD261	331	9	CD56	1960	35
CD66b	634	-3	CD25	227	5	ЕрСАМ	487	-27	CD20	308	13	CD11c	1895	-5
CD221	630	-17	CD309	227	6	CD146	487	-21	CD103	284	3	CD19	1862	-7
CD20	630	11	CD56	199	0	CD123	455	-17	CD25	280	6	CD33	1857	-14
CD104	617	27	CD45	178	-4	CD25	434	-17	CD14	272	-4	CD123	1732	5
CD324	600	18	CD140a	174	-6	CD140a	434	-35	CD45	270	0	CD144	1683	-7
CD8a	529	4	CD146	170	-5	CD20	413	-15	CD11c	261	-5	CD117	1678	-10
CD14	520	6	CD123	166	10	CD33	387	-10	CD8a	231	-12	CD309	1651	3
CD19	512	4	CD105	156	10	CD19	355	-27	CD3	223	3	CD235a	1319	-9
CD3	468	14	CD66b	137	7	CD61	334	-21	CD309	193	-6	CD146	779	-5
Correlation	0.7	7856	Correlation	0.8	266	Correlation	0.8	944	Correlation	0.9	9102	Correlation	0.6	5709
Combined correlation	0.7578													
Unstained (flow cytometer)/anti HSA (SPRi)	153	19	Unstained	209	8	Unstained	1911	-13	Unstained	131	-20	Unstained	1373	1
IgG PE control	1280	4	IgG PE control	397	-2	IgG PE control	2453	-26	IgG PE control	424	-6	IgG PE control	2225	-6

Roi nr	Amount of Cells	RU	RU/Cell		
2	22	120	5		
3	18	176	10		
4	25	194	8		
5	23	395	17		
6	17	202	17		
7	18	199	11		
8	16	319	20		
9	15	246	16		
10	18	328	18		
11	18	331	18		
12	16	326	20		
13	12	66	5		
14	18	143	8		
15	25	326	13		
16	15	113	8		
17	24	240	10		
18	29	375	13		
19	28	626	22		
20	33	625	19		
21	38	579	15		
22	40	827	21		
23	37	821	22		
24	20	609	30		
25	10	-28	-3		
26	10	-56	-6		
27	23	256	11		
28	14	42	3		
29	18	136	8		
30	24	341	14		
31	11	-34	-3		
32	21	230	11		
33	19	307	16		
34	8	80	10		
35	15	288	19		
36	28	487	17		
37	21	245	12		
38	34	594	17		
39	27	316	12		
40	28	458	16		
41	23	192	8		
42	36	670	19		

 Table S2. Variation of cell binding seen on a cover coupled sensor.

12	25	FOF	14
43	35	505	14
44	25	201	8
45	10	67	7
46	14	133	10
47	21	497	24
48	25	683	27
st. dev	8	221	19
average	22	311	16
CV (%)	37	71	121

Table 3. Reproducibility values of 3 different cancer cell lines.

Experiment	EpCAM on	EpCAM on	EpCAM on			
	MFC7 Response in	SKDK3 Response in RU	Response in RU			
	RU	Response in Re	Kesponse in Ke			
1	1213	704	1898			
2	1382	782	2242			
3	1543	973	1860			
4	1363	884	1938			
5	1506	678	1886			
6	1537	807	2152			
7	1359	1061	1522			
8	1946	910	1480			
9	1167	1043	1649			
10	1758	1269	1359			
11	1733	925	1808			
12	1403	1168	2299			
13	1731	1589	2192			
14	1607	1292	1645			
15	1767	1166	1674			
16	1787	1406	1622			
17	1531	862	1522			
18	1548	1040	1378			
19	1698	907	1561			
20	2249	1041	2282			
21	1759	766	1544			
22	2864	730	1658			
23	1710	981	1349			
24	1489	1297	1280			
25	1699	1160	1221			
26	1586	1083	1519			
27	2252	1355	1572			
28	2264	1233	1732			

29	1636	1157	1345
30	1751	989	1219
Average	1695	1042	1680
Mean	1667	1041	1634
St. dev.	348	225	317
CV	21	22	19
Combined CV		21	

Supplemental expressing the EpCAM cell surface maker in different amounts.



Figure S1. Cross-sectional schematic of the used SPRi apparatus.



Figure S2. Graph plotting the RU/cell responses of all the markers and repeat experiments performed.



**Figure 3.** Sensorgram showing HS578T cells expressing a very high amount of CD44, despite the fact that the CD44 antibodies were immobilized with the lowest concentration of all the used ligands.



**Figure S4.** Overlay plot showing different amounts of MCF7 cells being analyzed for EGFR expression. As the cell concentration decreases approximately by half, so does the SPRi response.