

Figure S1. Experimental design of the present study. NF-1, neurofibromin; CRC, colorectal cancer.

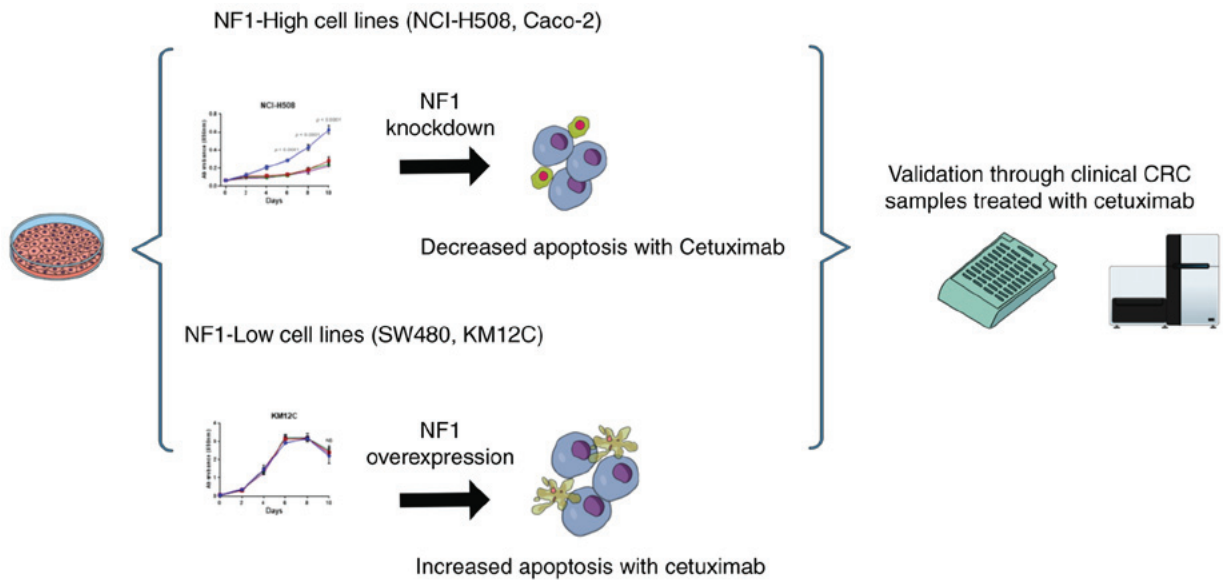


Figure S2. Estimated NF1 expression in the tumor samples. NF1 Fragments Per Kilobase of transcript per Million values according to sample acquisition site, stage at diagnosis, primary tumor site and the line of treatment. dx, diagnosis.

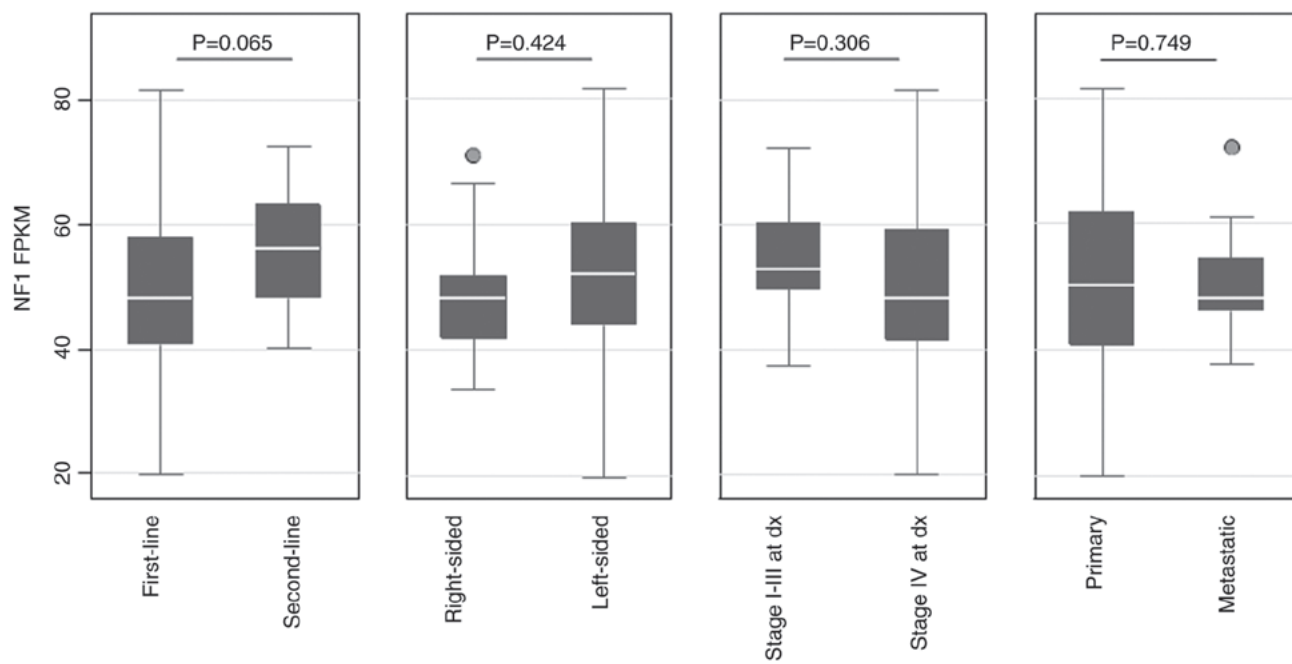


Table SI. Quality of samples submitted for RNA sequencing.

Sample ID	Library ID	Library type	Concentration ( $\mu\text{g}/\mu\text{l}$ )	Concentration (nM)	Size (bp)	Result
18R175	18R175_0011_41338148	TruSeq RNA access	9.26	53.16	268	Pass
18R176	18R176_0020_46008271	TruSeq RNA access	6.63	37.52	272	Pass
18R177	18R177_0021_46800206	TruSeq RNA access	5.77	32.5	273	Pass
18R178	18R178_0022_46750091	TruSeq RNA access	4.36	25.6	262	Pass
18R179	18R179_0023_45349791	TruSeq RNA access	4.46	25.49	269	Pass
18R294	18R294_0001_40759355	TruSeq RNA access	1.3	7.44	268	Pass
18R295	18R295_0003_41338148	TruSeq RNA access	0.79	4.66	262	Fail
18R296	18R296_0008_41897197	TruSeq RNA access	1.1	6.39	264	Pass
18R297	18R297_0009_40759355	TruSeq RNA access	0.45	2.53	273	Fail
18R298	18R298_0010_14863635	TruSeq RNA access	2.47	14.42	263	Pass
18R299	18R299_0025_47345928	TruSeq RNA access	5.51	31.64	268	Pass
18R300	18R300_0027_47642434	TruSeq RNA access	5.7	33.35	263	Pass
18R301	18R301_0002_48218872	TruSeq RNA access	4.72	27.1	268	Pass
18R302	18R302_0004_48434852	TruSeq RNA access	7.17	40.84	270	Pass
18R318	18R318_0005_45349791	TruSeq RNA access	2.42	13.47	276	Pass
18R304	18R304_0006_47345928	TruSeq RNA access	2.25	12.46	278	Pass
18R305	18R305_0007_14863635	TruSeq RNA access	1.56	8.95	269	Pass
18R306	18R306_0012_48434852	TruSeq RNA access	1.41	8.03	270	Pass
18R307	18R307_0013_49456451	TruSeq RNA access	7.79	43.26	277	Pass
18R319	18R319_0014_50587526	TruSeq RNA access	7.12	40.26	272	Pass
18R309	18R309_0015_49456451	TruSeq RNA access	6.7	36.7	281	Pass
18R310	18R310_0016_47642434	TruSeq RNA access	5.12	29.18	270	Pass
18R311	18R311_0018_50587526	TruSeq RNA access	3.87	20.97	284	Pass
18R421	18R421_001_41995161	TruSeq RNA access	3.89	22.24	269	Pass
18R423	18R423_003_40232780	TruSeq RNA access	6.15	35.99	263	Pass
18R426	18R426_006_45932744	TruSeq RNA access	5.89	33.43	271	Pass
18R427	18R427_007_47308099	TruSeq RNA access	7.9	44.86	271	Pass
18R428	18R428_08_46417008	TruSeq RNA access	6.16	35.5	267	Pass
18R429	18R429_09_11712574	TruSeq RNA access	7.37	42.46	267	Pass
18R430	18R430_010_44458964	TruSeq RNA access	5.05	30.26	257	Pass
18R432	18R432_012_48852948	TruSeq RNA access	9.14	53.25	264	Pass
18R433	18R433_013_49075782	TruSeq RNA access	6.26	35.43	272	Pass
18R434	18R434_014_49432138	TruSeq RNA access	9.8	54.42	277	Pass
18R435	18R435_015_49643363	TruSeq RNA access	7.67	41.97	281	Pass
18R436	18R436_016_29566772	TruSeq RNA access	8.6	49.72	266	Pass
18R437	18R437_018_50181151	TruSeq RNA access	6.06	35.18	265	Pass
18R438	18R438_019_50530171	TruSeq RNA access	7.32	41.57	271	Pass
18R440	18R440_020_50602247	TruSeq RNA access	8.5	49.56	264	Pass
18R442	18R442_021_18517345	TruSeq RNA access	7.05	41.11	264	Pass
18R443	18R443_022_17875226	TruSeq RNA access	7.3	40.42	278	Pass
18R457	18R457_023_51211769	TruSeq RNA access	7.95	42.9	285	Pass
18R458	18R458_025_51283384	TruSeq RNA access	11.64	63.48	282	Pass
18R459	18R459_027_51287920	TruSeq RNA access	10.93	59.82	281	Pass
18R460	18R460_01_51483533	TruSeq RNA access	7.23	40.14	277	Pass
18R461	18R461_02_51508812	TruSeq RNA access	10.62	58.38	280	Pass
18R462	18R462_03_51559733	TruSeq RNA access	10.84	60.87	274	Pass
18R463	18R463_04_45840117	TruSeq RNA access	8.54	47.45	277	Pass
18R464	18R464_05_51589518	TruSeq RNA access	8.29	44.15	289	Pass
18R465	18R465_06_51509435	TruSeq RNA access	5.58	32.13	267	Pass
18R466	18R466_07_39380160	TruSeq RNA access	9.99	54.67	281	Pass
18R467	18R467_08_51775302	TruSeq RNA access	9.93	54.73	279	Pass
18R468	18R468_09_52576072	TruSeq RNA access	12.85	69.37	285	Pass
18R469	18R469_010_52791028	TruSeq RNA access	9.35	53.1	271	Pass
18R470	18R470_011_52842801	TruSeq RNA access	11.36	64.04	273	Pass
18R471	18R471_012_52958148	TruSeq RNA access	8.01	43.69	282	Pass
18R472	18R472_013_45861758	TruSeq RNA access	6.66	36.21	283	Pass
18R473	18R473_014_53010544	TruSeq RNA access	8.72	45.18	297	Pass
18R474	18R474_015_53054056	TruSeq RNA access	9.11	50.4	278	Pass

Table SI. Continued.

Sample ID	Library ID	Library type	Concentration ( $\mu\text{g}/\mu\text{l}$ )	Concentration (nM)	Size (bp)	Result
18R471	18R471_012_52958148	TruSeq RNA access	8.01	43.69	282	Pass
18R472	18R472_013_45861758	TruSeq RNA access	6.66	36.21	283	Pass
18R473	18R473_014_53010544	TruSeq RNA access	8.72	45.18	297	Pass
18R474	18R474_015_53054056	TruSeq RNA access	9.11	50.4	278	Pass
18R475	18R475_016_53186681	TruSeq RNA access	10.99	58.73	288	Pass
18R476	18R476_018_53202165	TruSeq RNA access	7.94	44.44	275	Pass
18R477	18R477_019_53563916	TruSeq RNA access	8.22	45.83	276	Pass
18R478	18R478_020_53749190	TruSeq RNA access	9.25	52.32	272	Pass
18R479	18R479_021_53839512	TruSeq RNA access	7.17	40.72	271	Pass
18R480	18R480_022_53883324	TruSeq RNA access	8.76	49.37	273	Pass
18R507	18R507_004_42623786	TruSeq RNA access	4.17	25.25	254	Pass
18R508	18R508_011_48483102	TruSeq RNA access	3.68	22.18	255	Pass
18R449	18R449_023_50492510	TruSeq RNA access	4.48	25.42	271	Pass
18R454	18R454_027_51043454	TruSeq RNA access	7.93	44.85	272	Pass
18R455	18R455_01_49432138	TruSeq RNA access	11.76	63.05	287	Pass
18R456	18R456_02_51157593	TruSeq RNA access	5.46	30.75	273	Pass
18R673	18R673_05_49293333	TruSeq RNA access	18.59	100	286	Pass
18R696	18R696_04_51886411	TruSeq RNA access	9.47	51.85	281	Pass
18R700	18R700_08_52405884	TruSeq RNA access	11.3	62.31	279	Pass
18R695	18R695_03_51886411	TruSeq RNA access	17.28	95.29	279	Pass
18R674	18R674_06_49823486	TruSeq RNA access	5.54	31.94	267	Pass
18R699	18R699_07_52405884	TruSeq RNA access	19.72	104.6	290	Pass
18R705	18R705_013_54196465	TruSeq RNA access	15.51	75.49	316	Pass
18R649	18R649_010_37507130	TruSeq RNA access	16.59	93.48	273	Pass
18R646	18R646_007_27383678	TruSeq RNA access	15.24	87.84	267	Pass
18R664	18R664_01_48596185	TruSeq RNA access	13.3	71.81	285	Pass
18R678	18R678_010_50370696	TruSeq RNA access	10.38	58.26	274	Pass
18R682	18R682_014_50881994	TruSeq RNA access	14.7	79.63	284	Pass
18R702	18R702_010_53921194	TruSeq RNA access	22.33	118.89	289	Pass
18R690	18R690_023_51341204	TruSeq RNA access	15.83	83.98	290	Pass
18R653	18R653_014_46669429	TruSeq RNA access	14.18	75.49	289	Pass
18R669	18R669_01_48888682	TruSeq RNA access	11.22	62.77	275	Pass
18R701	18R701_09_53540519	TruSeq RNA access	21.3	114.57	286	Pass
18R675	18R675_07_49823486	TruSeq RNA access	10.38	57.05	280	Pass
18R654	18R654_015_46917825	TruSeq RNA access	14.15	75.31	289	Pass
18R647	18R647_08_33979067	TruSeq RNA access	8.48	47.29	276	Pass
18R694	18R694_02_51857583	TruSeq RNA access	14.37	78.38	282	Pass
18R651	18R651_012_43643024	TruSeq RNA access	17.85	97.7	281	Pass
18R641	18R641_002_17572109	TruSeq RNA access	7.85	43.27	279	Pass
18R663	18R663_027_48580957	TruSeq RNA access	10.59	59.89	272	Pass
18R659	18R659_020_47994630	TruSeq RNA access	9.06	49.78	280	Pass
18R645	18R645_006_21571732	TruSeq RNA access	7.88	44.72	271	Pass
18R661	18R661_021_48205403	TruSeq RNA access	14.27	78.39	280	Pass
18R689	18R689_022_51341204	TruSeq RNA access	8.56	48.76	270	Pass
18R697	18R697_05_52363036	TruSeq RNA access	19.44	106.07	282	Pass
18R642	18R642_003_17843584	TruSeq RNA access	13.62	75.94	276	Pass
18R670	18R670_02_48992947	TruSeq RNA access	13.48	74.05	280	Pass
18R688	18R688_021_51175995	TruSeq RNA access	5.56	30.35	282	Pass
18R677	18R677_09_50204218	TruSeq RNA access	4.66	24.47	293	Pass
18R655	18R655_016_46917825	TruSeq RNA access	5.84	33.39	269	Pass
18R656	18R656_018_47296747	TruSeq RNA access	2.99	17.27	266	Pass
18R665	18R665_02_48596185	TruSeq RNA access	3.43	19.9	265	Pass
18R648	18R648_09_33979067	TruSeq RNA access	2.62	15.17	266	Pass
18R640	18R640_001_17572109	TruSeq RNA access	9.17	49.7	284	Pass
18R683	18R683_015_50977794	TruSeq RNA access	10.12	54.05	288	Pass
18R657	18R657_019_47296747	TruSeq RNA access	5.33	28.99	283	Pass
18R684	18R684_016_50977794	TruSeq RNA access	9.88	52.07	292	Pass
18R671	18R671_03_48992947	TruSeq RNA access	5.75	32.91	269	Pass
18R685	18R685_018_51038902	TruSeq RNA access	1.62	9.59	260	Pass

Table SII. Gene set enrichment analysis for RNA sequencing data from the tumor samples taken from cetuximab-treated patients with metastatic colorectal cancer.

A, Pre-cetuximab vs. post-cetuximab (n=111)						
Kyoto Encyclopedia of Genes and Genomes pathway entry ID	Pathway description	Enrichment Score	NES	P-value	False discovery rate-adjusted P-value	Q-value
hsa01521	EGFR tyrosine kinase inhibitor resistance	-0.2992677	-1.0123052	0.4234973	0.7358541	0.5645651
hsa04014	Ras signaling pathway	-0.2515787	-0.988775	0.4494774	0.737659	0.5659498
hsa04010	MAPK signaling pathway	-0.2265855	-0.9079595	0.7538462	0.9648106	0.740226
B, Within post-cetuximab samples, PD vs. non-PD (n=36)						
Kyoto Encyclopedia of Genes and Genomes pathway entry ID	Pathway description	Enrichment Score	NES	P-value	False discovery rate-adjusted P-value	Q-value
hsa01521	EGFR tyrosine kinase inhibitor resistance	-0.2602811	-0.8329476	0.7782486	1	0.9145312
hsa04014	Ras signaling pathway	-0.2730942	-0.987846	0.4987893	1	0.9145312
hsa04010	MAPK signaling pathway	-0.1952221	-0.7192921	0.9976526	1	0.9145312

ID, identifier; NES, normalized enrichment score; PD, progressive disease.

Table SIII. NF1-truncating mutations detected in the 1,449 study patients with metastatic colorectal cancer.

Study ID	Gene	Variant type	Protein change	Allele frequency
CRC-323	NF1	Frame_Shift_Ins	T32Nfs*6	0.32
CRC-1114	NF1	Frame_Shift_Ins	L2395Ffs*27	0.15
CRC-1114	NF1	Nonsense_Mutation	R1968*	0.13
CRC-1273	NF1	Frame_Shift_Del	F468Lfs*5	0.62
CRC-1273	NF1	Nonsense_Mutation	S1053*	0.35
CRC-1259	NF1	Nonsense_Mutation	Q576*	0.26
CRC-1180	NF1	Nonsense_Mutation	W696*	0.29
CRC-479	NF1	Splice_Site	X464_splice	0.17
CRC-1024	NF1	Nonsense_Mutation	K248*	0.45
CRC-1274	NF1	Nonsense_Mutation	R2450*	0.09
CRC-343	NF1	Frame_Shift_Del	Q1010Kfs*2	0.14
CRC-1275	NF1	Frame_Shift_Del	D1849Mfs*14	0.26
CRC-529	NF1	Splice_Site	X2441_splice	0.08
CRC-535	NF1	Nonsense_Mutation	R1769*	0.26
CRC-305	NF1	Splice_Site	X1371_splice	0.35
CRC-264	NF1	Nonsense_Mutation	R2258*	0.15
CRC-391	NF1	Nonsense_Mutation	R1769*	0.16
CRC-391	NF1	Nonsense_Mutation	E1423*	0.21
CRC-1015	NF1	Nonsense_Mutation	R1241*	0.33
CRC-864	NF1	Splice_Site	X1757_splice	0.62
CRC-942	NF1	Nonsense_Mutation	R1968*	0.13
CRC-1276	NF1	Frame_Shift_Del	S2687Cfs*5	0.66
CRC-1157	NF1	Frame_Shift_Ins	F944Lfs*3	0.18
CRC-1213	NF1	Frame_Shift_Del	Y2285Tfs*5	0.6
CRC-1164	NF1	Nonsense_Mutation	T1770Sfs*3	0.09
CRC-1277	NF1	Splice_Site	X2441_splice	0.18
CRC-1278	NF1	Frame_Shift_Ins	L1147Gfs*49	0.43
CRC-1279	NF1	Frame_Shift_Ins	Y580Lfs*8	0.69
CRC-1280	NF1	Nonsense_Mutation	E1583*	0.45

NF1, neurofibromin.