Supplemental File

TRAF6 is an amplified oncogene bridging the Ras and nuclear factor-κB cascade in human lung cancer

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Supplemental Table 1. Candidate genes within the minimally amplified region on 11p12-13.

Gene	BP Start	BP End	Corrected p-value	$-\log^2$ (p-value)
WT1	32367179	32374525	8.11	-3.02
WIT1	32416050	32418194	8.64	-3.11
CCDC73	32583302	32583772	2.18	-1.13
PRRG4	32808208	32831960	6.15	-2.62
QSER1	32954688	32955419	0.87	0.20
DEPDC7	32993984	33011702	2.19	-1.13
TCP11L1	33018152	33050970	0.09	3.46
HIPK3	33236667	33317648	4.37	-2.13
C11orf41	33521049	33648259	17.00	-4.09
CD59	33683293	33685857	0.17	2.59
FBXO3	33724529	33752604	1.39	-0.47
LMO2	33849728	33850120	3.40	-1.76
NAT10	34083724	34125033	2.92	-1.55
ABTB2	34129110	34138411	6.16	-2.62
CAT	34417068	34450183	3.12	-1.64
ELF5	34456918	34491891	5.59	-2.48
EHF	34620414	34625184	3.99	-1.99
APIP	34860420	34894484	2.67	-1.42
PDHX	34894740	34974092	1.28	-0.35
NA	35183185	35186287	0.50	0.99
CD44	35208209	35210525	0.13	2.99
SLC1A2	35369662	35398088	8.65	-3.11
DKFZP586H2123	35409953	35411017	9.30	-3.22
FJX1	35596630	35598988	5.03	-2.33
TRIM44	35640926	35789178	1.08	-0.11
COMMD9	36252085	36267555	0.10	3.38
FLJ14213	36430384	36430982	0.23	2.10
TRAF6	36467531	36488372	0.01	6.43
RAG1	36546138	36557840	16.43	-4.04
RAG2	36570311	36571274	16.50	-4.04
C11orf74	36572632	36637398	7.65	-2.93

Supplemental Table 2. Lung cancer cell lines.

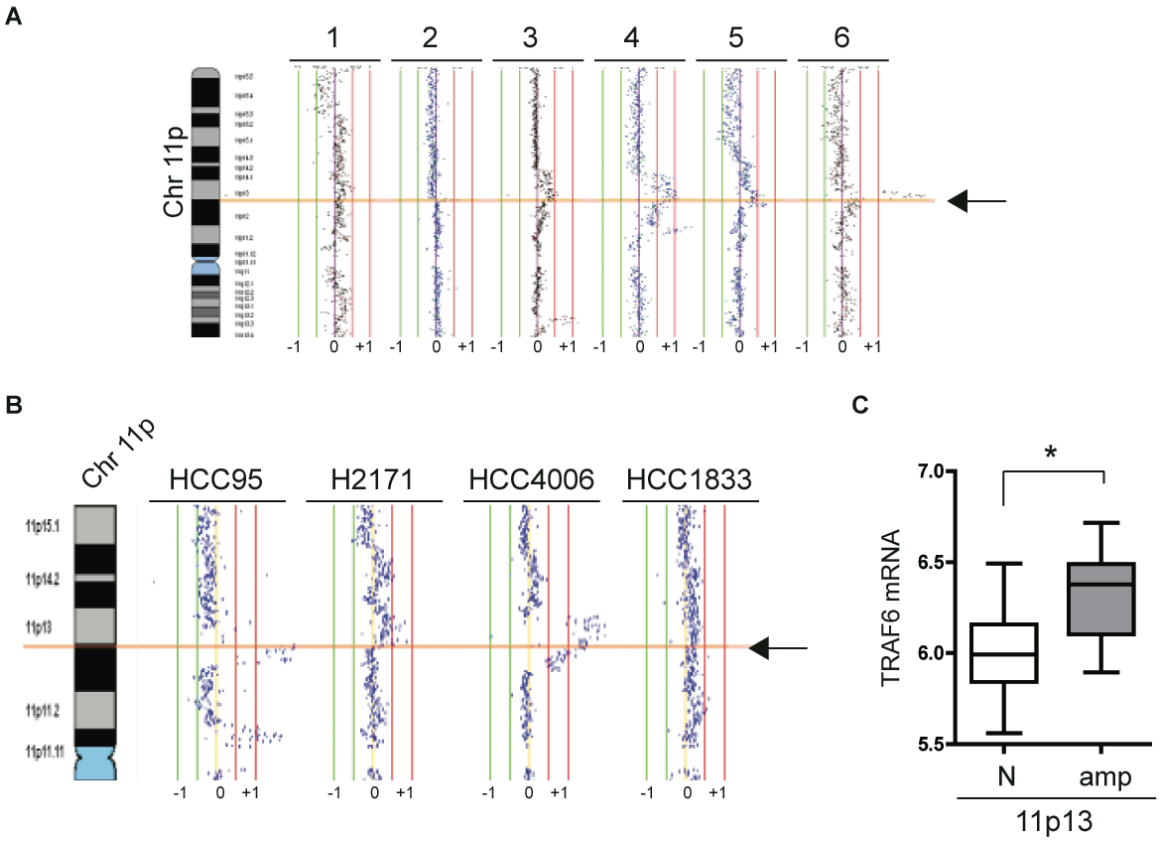
Name	ATCC Number	Classifier	amp 11p13*
A549	CCL-185	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	Yes
Calu-1	HTB-54	Epidermoid Carcinoma	
Calu-3	HTB-55	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
Calu-6	HTB-56	Anaplastic Carcinoma	
EKVX	N/A	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC), NCI-60	
H1155	CRL-5818	Non-Small Cell Lung Cancer (NSCLC), Large Cell Carcinoma (LCC)	
H1184	CRL-5858	Small Cell Lung Cancer (SCLC)	
H1299	CRL-5803	Non-Small Cell Lung Cancer (NSCLC), Large Cell Carcinoma (LCC)	
H1355	CRL-5865	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
H1395	CRL-5868	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
H1437	CRL-5872	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
H146	HTB-173	Small Cell Lung Cancer (SCLC)	
H157	CRL-5802	Non-Small Cell Lung Cancer (NSCLC), Squamous Cell Carcinoma (SqCC)	Yes
H1607	N/A	Small Cell Lung Cancer (SCLC)	
H1648	CRL-5882	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
H1650	CRL-5883	Non-Small Cell Lung Cancer (NSCLC), Bronchioalveolar carcinoma (BAC)	Yes
H1666	CRL-5885	Non-Small Cell Lung Cancer (NSCLC), Bronchioalveolar carcinoma (BAC)	
H1672	CRL-5886	Small Cell Lung Cancer (SCLC)	
H1755	CRL-5892	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
H1770	CRL-5893	Neuroendocrine	
H1781	CRL-5894	Non-Small Cell Lung Cancer (NSCLC), Bronchioalveolar carcinoma (BAC)	
H1819	CRL-5897	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
H187	CRL-5804	Small Cell Lung Cancer (SCLC)	
H1975	CRL-5908	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
H1993	CRL-5909	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
H2009	CRL-5911	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
H2052	CRL-5915	Mesothelioma (ME)	
H2073	CRL-5918	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
H2085	CRL-5921	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
H2087	CRL-5922	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
H2107	CRL-5983	Small Cell Lung Cancer (SCLC)	
H2122	CRL-5985	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
H2126	CCL-256	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
H2141	CRL-5927	Small Cell Lung Cancer (SCLC)	
H2170	CRL-5928	Non-Small Cell Lung Cancer (NSCLC), Squamous Cell Carcinoma (SqCC)	
H2171	CRL-5929	Small Cell Lung Cancer (SCLC)	Yes
H2228	CRL-5935	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
H226	CRL-5826	Non-Small Cell Lung Cancer (NSCLC), Squamous Cell Carcinoma (SqCC)	
H2347	CRL-5942	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
H2882	N/A	Non-Small Cell Lung Cancer (NSCLC)	
H2887	N/A	Non-Small Cell Lung Cancer (NSCLC)	Yes
H289	N/A	Small Cell Lung Cancer (SCLC)	
H3122	N/A	Non-Small Cell Lung Cancer (NSCLC)	
H322	N/A	Non-Small Cell Lung Cancer (NSCLC), Bronchioalveolar carcinoma (BAC)	
H3255	N/A	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	Yes
H358	CRL-5807	Non-Small Cell Lung Cancer (NSCLC), Bronchioalveolar carcinoma (BAC)	
H378	CRL-5808	Small Cell Lung Cancer (SCLC)	
H441	HTB-174	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
H460	HTB-177	Non-Small Cell Lung Cancer (NSCLC), Large Cell Carcinoma (LCC)	
H520	HTB-182	Non-Small Cell Lung Cancer (NSCLC), Squamous Cell Carcinoma (SqCC)	
H524	CRL-5831	Small Cell Lung Cancer (SCLC)	
H526	CRL-5811	Small Cell Lung Cancer (SCLC)	Yes
H82	HTB-175	Small Cell Lung Cancer (SCLC)	
H820	N/A	Non-Small Cell Lung Cancer (NSCLC), Bronchioalveolar carcinoma (BAC)	
H841	CRL-5845	Small Cell Lung Cancer (SCLC)	
H889	CRL-5817	Small Cell Lung Cancer (SCLC)	
HCC1171	N/A	Non-Small Cell Lung Cancer (NSCLC)	
HCC1195	N/A	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
HCC1359	N/A	Non-Small Cell Lung Cancer (NSCLC), Large Cell Carcinoma (LCC)	
HCC15	N/A	Non-Small Cell Lung Cancer (NSCLC), Squamous Cell Carcinoma (SqCC)	
HCC1719	N/A	Lung Cancer	Yes
HCC1833	N/A	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
HCC193	N/A	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	Yes
HCC2279	N/A	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
HCC2450	N/A	Non-Small Cell Lung Cancer (NSCLC), Squamous Cell Carcinoma (SqCC)	
HCC2935	CRL-2869	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
HCC33	N/A	Small Cell Lung Cancer (SCLC)	
HCC366	N/A	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	Yes
HCC4006	CRL-2871	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	Yes
HCC4011	N/A	Non-Small Cell Lung Cancer (NSCLC)	
HCC4017	N/A	Non-Small Cell Lung Cancer (NSCLC)	
HCC44	N/A	Non-Small Cell Lung Cancer (NSCLC)	
HCC461	N/A	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	Yes
HCC515	N/A	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
HCC78	N/A	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
HCC827	CRL-2868	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC)	
HCC95	N/A	Non-Small Cell Lung Cancer (NSCLC), Squamous Cell Carcinoma (SqCC)	Yes
HOP 62	N/A	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC), NCI-60	
HOP 92	N/A	Non-Small Cell Lung Cancer (NSCLC), Large Cell Carcinoma (LCC), NCI-60	
NCI-H23	CRL-5800	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC), NCI-60	
NCI-H522	CRL-5810	Non-Small Cell Lung Cancer (NSCLC), Adenocarcinoma (AC), NCI-60	Yes
PC3	N/A	Lung Cancer	
PC9	N/A	Lung Cancer	Yes
SK-MES-1	HTB-58	Non-Small Cell Lung Cancer (NSCLC), Squamous Cell Carcinoma (SqCC)	Yes
SW900	HTB-59	Non-Small Cell Lung Cancer (NSCLC), Squamous Cell Carcinoma (SqCC)	Yes

N/A, not available; *, amplification includes TRAF6

Supplemental Table 3. Description of TRAF6 and KRAS alterations in the lung cancer cell lines.

	H1395	H520	H460	H2347	HCC95	SK-MES
TRAF6 Amp	No	No	No	No	**Yes**	**Yes**
KRAS Mutation	No	No	**Yes**	**Yes**	No	No
KRAS Signature	No	No	**Yes**	**Yes**	*unknown*	**Yes**

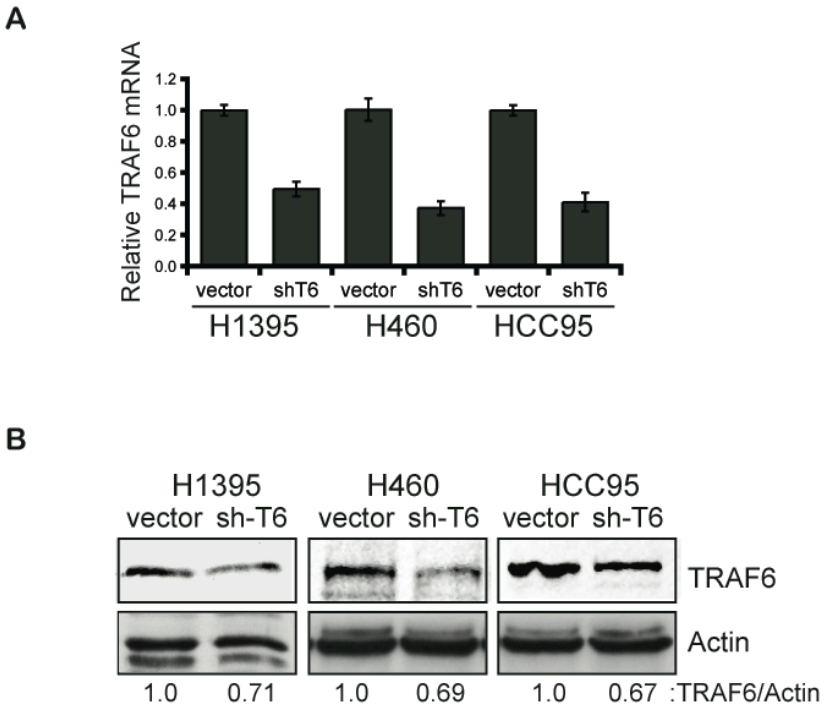
Supplemental Figure 1. TRAF6 mRNA is overexpressed in lung cancer with 11p13 amplifications.



Supplemental Figure 1. ***TRAF6*** **is overexpressed in lung cancer with chr 11p13 amplifications.**

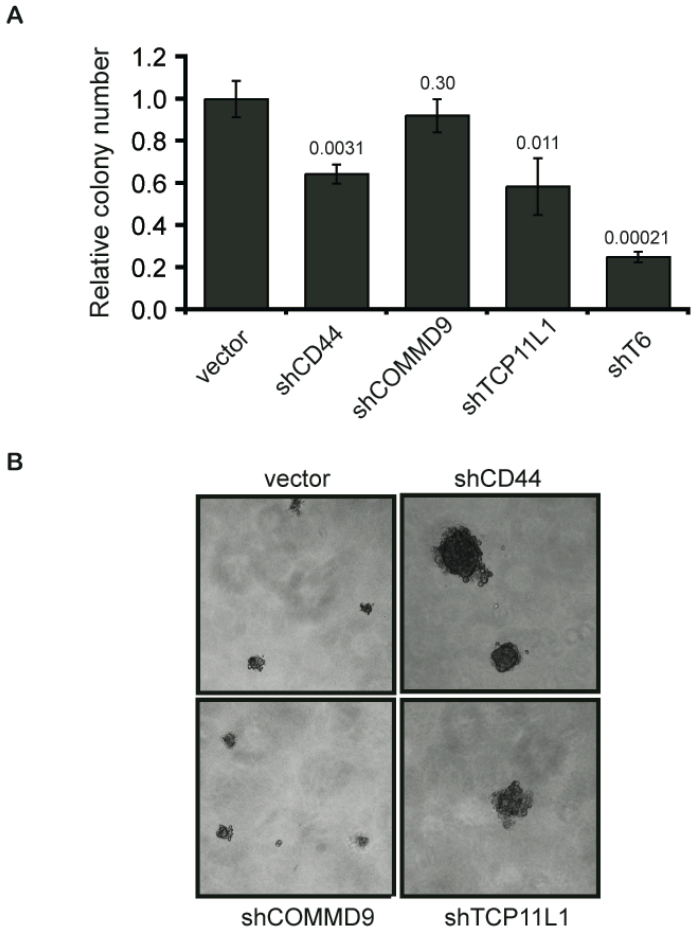
A. The copy number status of chromosome 11p is shown for six representative lung cancer tumors (#1-6). The minimally amplified region spanning chr 11p13 (32,126,542) to chr 11p12 (37,251,933) is indicated with the arrow. Normalized $\log_2$ signal intensity ratios were plotted and visualized by *SIGMA*[2] Vertical lines denote $\log_2$ signal ratios from –1 to +1. Copy number gains are shown to the right of 0 (red). Each black dot represents a single BAC clone. **B.** Alignment of aCGH profiles at chromosome 11p13 from four lung cancer samples. **C.** Expression of *TRAF6* mRNA ($\log_2$) in lung cancer cell lines with (n = 13) and without amplification of the *TRAF6* locus, chr 11p13 (n = 31) ($p = 0.00034$).

Supplemental Figure 2. Validation of TRAF6 inhibition by RNAi in lung cancer cells.



Supplemental Figure 2. Validation of TRAF6 inhibition by RNAi in lung cancer cells. Quantitative PCR (TRAF6 and GAPDH mRNA) (**A**) and immunoblotting (TRAF6 and actin protein) (**B**) is shown after inhibition of TRAF6 (shT6) in the indicated cell lines.

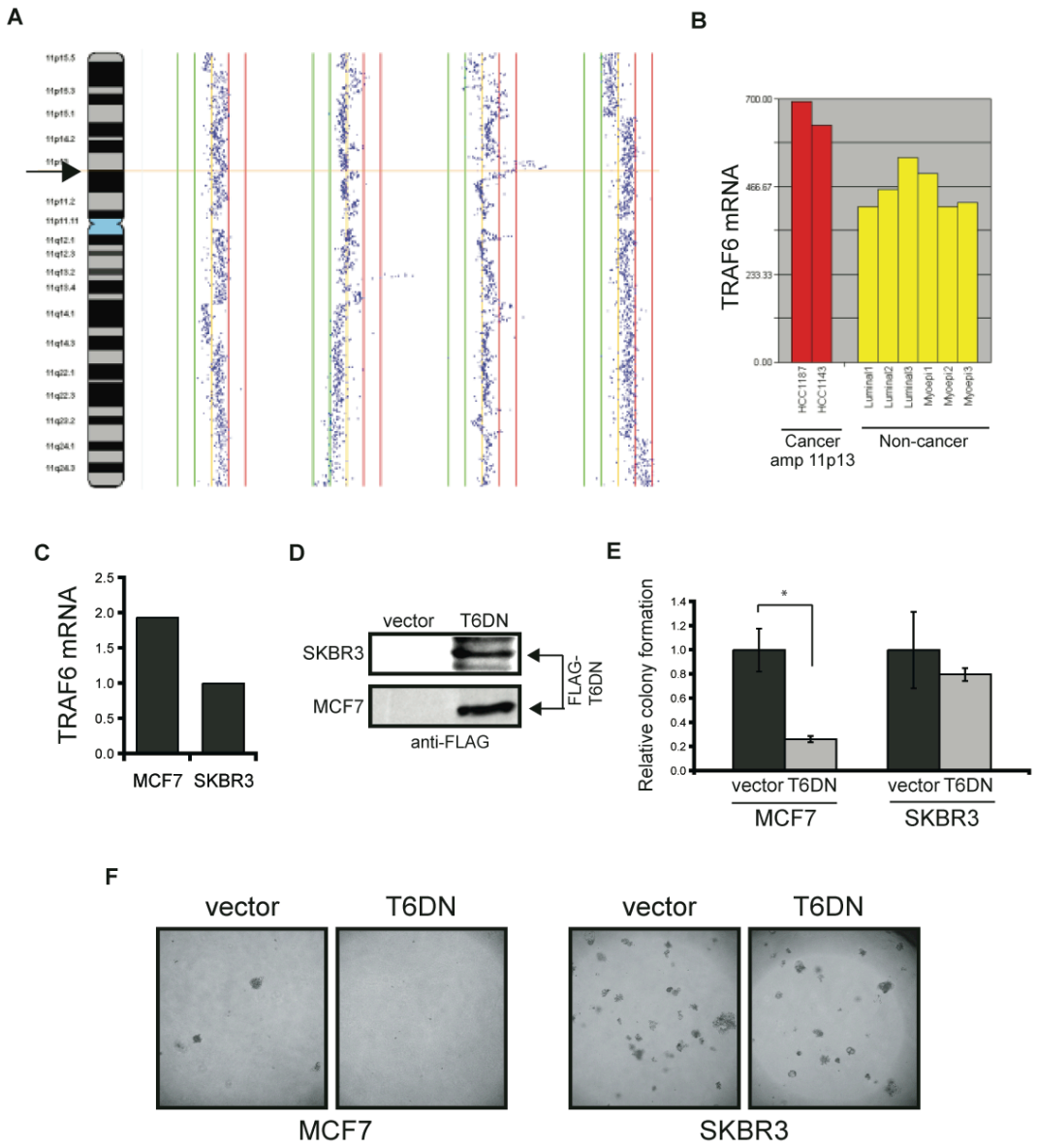
Supplemental Figure 3. Inhibition of candidate genes on chromosome 11p13 in lung cancer cells.



Supplemental Figure 3. Inhibition of candidate genes on chromosome 11p13 in lung cancer cells.

Lung cancer cells with amplification of 11p13 (HCC95) were transduced with virus encoding shRNA constructs specific for CD44, COMMD9, TCP11L1, and TRAF6. Transduced cell lines were selected in puromycin and analyzed for colony formation in 0.3% soft agar and confirmation of target gene knockdown by qPCR. **A**. Colony numbers for the indicated transduced cell lines are shown relative to vector-transduced cells (1.0). The corresponding target gene knockdown is shown below. Colony formation of shCOMMD9-expressing cells may be in part explained by the less efficient knockdown of COMMD9. **B**. Representative colonies are shown for the indicated transduced cell lines at 20X magnification. Data is shown from one experiment performed in triplicate.P values are indicated above the histogram. Data are represented as mean ± SEM.

Supplemental Figure 4. Inhibition of TRAF6 suppresses breast cancer lines.



Supplemental Figure 4. Inhibition of TRAF6 suppresses breast cancer lines.
A. Alignment of aCGH profiles at chromosome 11p13 from 4 breast cancer samples. The arrow indicates the location of the *TRAF6* locus. Normalized $\log_2$ signal intensity ratios were plotted and visualized by *SIGMA*[2]. Vertical lines denote $\log_2$ signal ratios from –1 to +1. Copy number decreases are shown to the left of 0 (green). Each black dot represents a single BAC clone. **B.** Expression of *TRAF6* mRNA in breast cancer samples with (n = 2) amplification at band 11p13, as well as normal controls (n = 6). **C.** *TRAF6* mRNA from MCF7 and SKBR3 was measured by qPCR and shown relative to SKBR3 (1.0). As shown, MCF7 cells exhibit higher (two-fold) expression of *TRAF6* mRNA. **D**. MCF7 and SKBR3 were transduced with vector- or TRAF6-dominant negative (FLAG-T6DN)-containing virus. Expression of T6DN was determined by anti-FLAG immunoblotting. **E-F**. Vector- and T6DN-expressing MCF7 and SKBR3 cells were analyzed for colony formation in 0.3% soft agar. Data are represented as mean ± SEM. *, p = 0.024