Supplementary Table 1: List of mutations identified exclusively in the post-relapse sample, but not the pre-treatment sample, from a melanoma patient who was treated with vemurafinib and relapsed with a time to disease progression of 5 months.

Chr	Start	End	Ref	Alt	Func.refGe	Gene.refGe	ExonicFunc	AAChange.r	genomicSu	1000g2014	snp138	Polyphen2_	Polyphen2_	LRT_pred	MutationTa	MutationAssessor_pred
chrX	1.23E+08	1.23E+08	G	A	exonic	STAG2	nonsynonyr	STAG2:NM_	.	.		D	D	D	D	M
chr15	42439924	42439924	T	G	exonic	PLA2G4F	nonsynonyr	PLA2G4F:N	.	.		D	D	D	D	M
chr5	1.42E+08	1.42E+08	T	G	exonic	ARHGAP26	nonsynonyr	ARHGAP26:	.	.		D	D	D	D	M
chr5	1.54E+08	1.54E+08	T	G	exonic	GALNT10	nonsynonyr	GALNT10:N	.	.		D	D	D	D	H
chr16	720991	720991	A	C	exonic	RHOT2	nonsynonyr	RHOT2:NM	.	.		D	D	D	D	H
chr4	96124082	96124082	T	G	exonic	UNC5C	nonsynonyr	UNC5C:NM	.	.		D	D	D	D	M
chr17	32962000	32962000	T	G	exonic	TMEM132E	nonsynonyr	TMEM132E	.	.		D	D	D	D	M
chr22	20819608	20819608	C	G	exonic	KLHL22	nonsynonyr	KLHL22:NM	.	.		D	D	D	D	M
chr15	81229165	81229165	A	C	exonic	CEMIP	nonsynonyr	CEMIP:NM_	.	.		D	D	D	D	M
chr21	33074197	33074197	T	G	exonic	SCAF4	nonsynonyr	SCAF4:NM_	.	.		D	D	D	D	M
chr2	2.16E+08	2.16E+08	A	G	exonic	ABCA12	nonsynonyr	ABCA12:NM	.	.		D	D	D	D	M
chr6	2685596	2685596	T	A	exonic	MYLK4	nonsynonyr	MYLK4:NM_	.	.		D	D	D	D	H
chr16	27460543	27460543	A	C	exonic	IL21R	nonsynonyr	IL21R:NM_(	.	.		D	D	D	D	M
chr1	22199894	22199894	T	G	exonic	HSPG2	nonsynonyr	HSPG2:NM_	.	.		D	D	D	D	M
chr15	41029893	41029893	G	T	exonic	RMDN3	nonsynonyr	RMDN3:NM	.	.		D	D	D	D	M
chr10	73767576	73767576	G	C	exonic	CHST3	nonsynonyr	CHST3:NM_	.	.		D	D	D	D	M
chr3	58145336	58145336	C	A	exonic	FLNB	nonsynonyr	FLNB:NM_(	.	.		D	D	D	D	M
chr10	72061204	72061204	T	G	exonic	LRRC20	nonsynonyr	LRRC20:NM	.	.		D	D	D	D	M
chrX	1.53E+08	1.53E+08	G	T	exonic	AVPR2	nonsynonyr	AVPR2:NM_	.	.		D	D	D	D	H
chr11	1.19E+08	1.19E+08	A	C	exonic	DPAGT1	nonsynonyr	DPAGT1:NM	.	.		D	D	D	D	M
chr5	1.49E+08	1.49E+08	T	G	exonic	IL17B	nonsynonyr	IL17B:NM_(	.	.		D	D	D	D	M
chr13	37012872	37012872	T	G	exonic	CCNA1	nonsynonyr	CCNA1:NM_	.	.		D	D	D	D	H
chr21	27372378	27372378	T	C	exonic	APP	nonsynonyr	APP:NM_0(	.	.		D	D	D	D	H
chr9	22006176	22006176	T	G	exonic	CDKN2B	nonsynonyr	CDKN2B:NM	.	.		D	D	D	D	M
chr14	23313599	23313599	T	G	exonic	MMP14	nonsynonyr	MMP14:NM	.	.		D	D	D	D	M
chr1	1.1E+08	1.1E+08	A	C	exonic	AMPD2	nonsynonyr	AMPD2:NM	.	.		D	D	D	D	M
chr1	1.51E+08	1.51E+08	C	G	exonic	RFX5	nonsynonyr	RFX5:NM_(	.	.		D	D	D	D	M
chr9	1.04E+08	1.04E+08	C	T	exonic	GRIN3A	nonsynonyr	GRIN3A:NM	.	.		D	D	D	D	M
chr5	1.73E+08	1.73E+08	T	G	exonic	NKX2-5	nonsynonyr	NKX2-5:NM	.	.		D	D	D	D	M
chr9	96060194	96060194	A	C	exonic	WNK2	nonsynonyr	WNK2:NM_	.	.		D	D	D	D	M
chr1	47283669	47283669	C	T	exonic	CYP4B1	nonsynonyr	CYP4B1:NM	.	.		D	D	D	D	M
chr11	66033214	66033214	G	C	exonic	KLC2	nonsynonyr	KLC2:NM_(	.	.		D	D	D	D	M
chr17	31618750	31618750	T	G	exonic	ASIC2	nonsynonyr	ASIC2:NM_	.	.		D	D	D	D	M
chr11	76796018	76796018	A	C	exonic	CAPN5	nonsynonyr	CAPN5:NM_	.	.		D	D	D	D	H
chr16	20651865	20651865	T	C	exonic	ACSM1	nonsynonyr	ACSM1:NM_	.	.		D	D	D	D	M
chr2	1.56E+08	1.56E+08	A	C	exonic	KCNJ3	nonsynonyr	KCNJ3:NM_	.	.		D	D	D	D	M
chr12	58124709	58124709	C	T	exonic	AGAP2	nonsynonyr	AGAP2:NM_	.	.		D	D	D	D	M
chr11	1.26E+08	1.26E+08	A	C	exonic	DCPS	nonsynonyr	DCPS:NM_(	.	.		D	D	D	D	M
chr16	2522806	2522806	A	C	exonic	NTN3	nonsynonyr	NTN3:NM_(	.	.		D	D	D	D	H
chr19	18119220	18119220	T	G	exonic	ARRDC2	nonsynonyr	ARRDC2:NM	.	.		D	D	D	D	H
chr2	85629027	85629027	A	C	exonic	CAPG	nonsynonyr	CAPG:NM_(	.	.		D	D	D	D	M
chr1	2.27E+08	2.27E+08	A	C	exonic	PARP1	nonsynonyr	PARP1:NM_	.	.		D	D	D	D	H
chr1	26515317	26515317	T	G	exonic	CNKSR1	nonsynonyr	CNKSR1:NM	.	.		D	D	D	D	M
chr9	15422971	15422971	T	G	exonic	SNAPC3	nonsynonyr	SNAPC3:NM	.	.		D	D	D	D	M
chr20	43726317	43726317	C	T	exonic	KCNS1	nonsynonyr	KCNS1:NM_	.	.		D	D	D	D	M
chr13	1.11E+08	1.11E+08	G	A	exonic	COL4A2	nonsynonyr	COL4A2:NM	.	.		D	D	D	D	H
chr12	1.33E+08	1.33E+08	A	C	exonic	EP400	nonsynonyr	EP400:NM_	.	.		D	D	D	D	M
chr12	51868963	51868963	T	A	exonic	SLC4A8	nonsynonyr	SLC4A8:NM	.	.		D	D	D	D	M
chr1	1.87E+08	1.87E+08	A	C	exonic	PLA2G4A	nonsynonyr	PLA2G4A:N	.	.		D	D	D	D	M
chr19	13136208	13136208	T	G	exonic	NFIX	nonsynonyr	NFIX:NM_0	.	.		D	D	D	D	M
chr11	1.2E+08	1.2E+08	A	G	exonic	OAF	nonsynonyr	OAF:NM_1	.	.		D	D	D	D	M
chr2	27707975	27707975	A	C	exonic	IFT172	nonsynonyr	IFT172:NM_	.	.		D	D	D	D	M
chr13	52518307	52518307	C	T	exonic	ATP7B	nonsynonyr	ATP7B:NM_	.	.		D	D	D	D	M
chr22	19213138	19213138	C	A	exonic	CLTCL1	nonsynonyr	CLTCL1:NM	.	.		D	D	D	D	M
chr6	38816525	38816525	T	G	exonic	DNAH8	nonsynonyr	DNAH8:NM	.	.		D	D	D	D	M
chrX	1.06E+08	1.06E+08	A	T	exonic	TBC1D8B	nonsynonyr	TBC1D8B:N	.	.		D	D	D	D	M
chr8	85686850	85686850	C	A	exonic	RALYL	nonsynonyr	RALYL:NM_	.	.		D	D	D	D	M

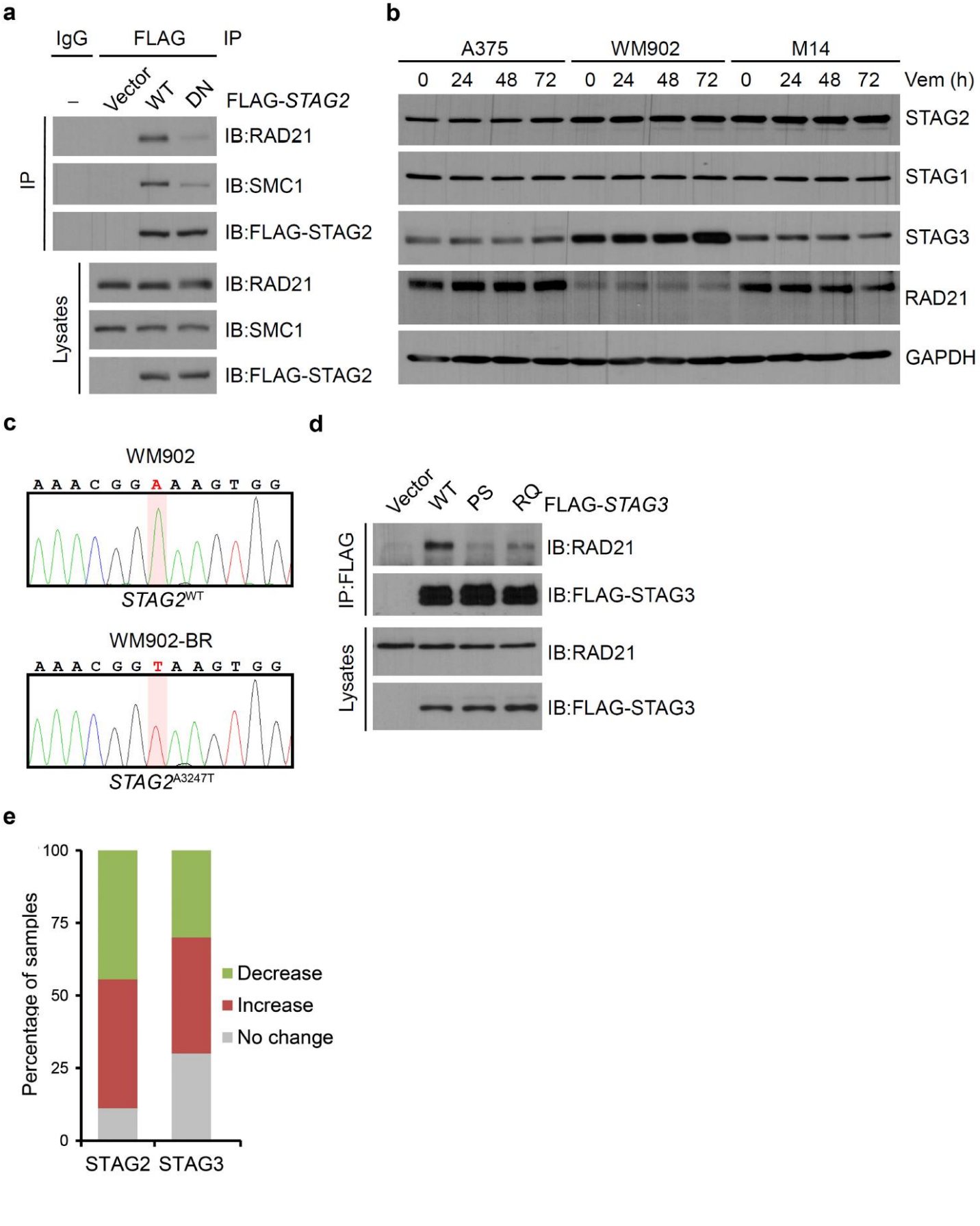
Supplementary Table 2: List of STAG3 mutations found in a study of 45 patients with BRAF Val600-mutant metastatic melanoma who received vemurafenib or dabrafenib monotherapy by Van Allen et al.[4]

Patient #	Early Resistance	STAG3 Mutation
4	yes	Pro272Ser (Pre-treatment)
26	yes	Arg508Gln (Pre-treatment)
40	no	Ala107fs (Post-relapse)
41	no	Ala644Val, Ala1082Val (Post-relapse)
45	no	Gly129Glu (Post-relapse)
46	yes	Glu1064Lys (Pre-treatment)
51	no	Glu683Lys (Post-relapse)
60	yes	Ser1016fs (Post-relapse)
63	no	Asp30Asn, Asp1221Asn (Post-relapse)

Supplementary Table 3: List of antibodies used in this study.

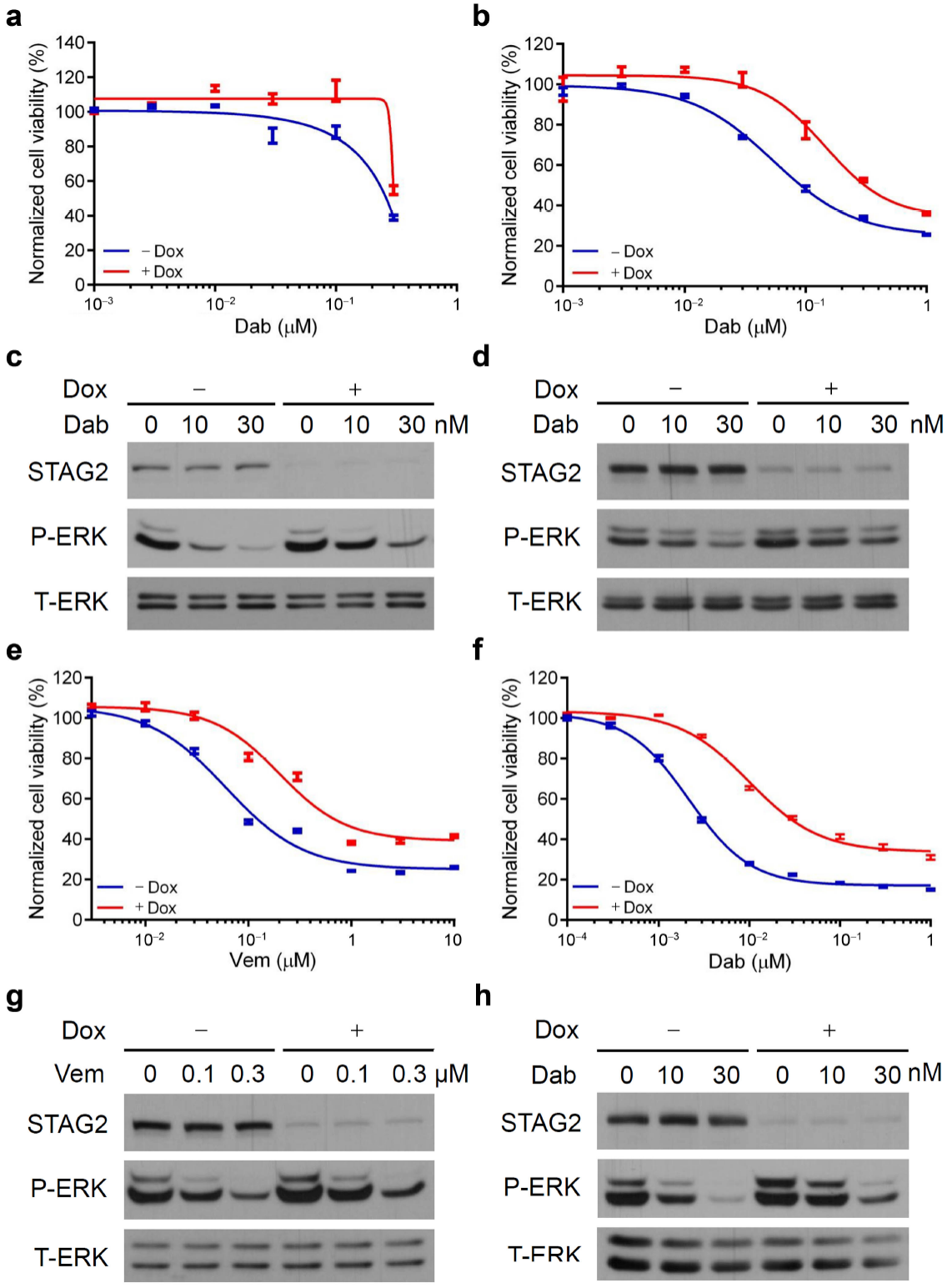
Antigen	Manufacturer	Clone	Catalog #	Technique and dilution
phospho-ERK (Thr202/Tyr204)	Cell signaling Technology		9101	IHC (1:400); WB (1:3000)
ERK	Cell signaling Technology	137F5	4695	WB (1:3000)
MYC	Cell signaling Technology	9B11	2276	WB (1:1000)
phospho-AKT (Thr308)	Cell signaling Technology	C31E5E	2965	WB (1:1000)
AKT	Cell signaling Technology	C67E7	4691	WB (1:1000)
phospho-S6 (Ser240/Ser244)	Cell signaling Technology		2215	WB (1:3000)
S6	Cell signaling Technology	5G10	2217	WB (1:3000)
SMC1	Cell signaling Technology	8E6	6892	WB (1:1000)
EGFR	Cell signaling Technology		2232	WB (1:1000)
GAPDH	Cell signaling Technology	14C10	2118	WB (1:5000)
STAG2	Santa Cruz	J-12	SC-81852	IHC (1:100); WB (1:1000)
STAG3	Abcam		ab185109	IHC (1:200); WB (1:1000)
DUSP4	Abcam		ab72593	WB (1:1000)
DUSP6	Abcam		ab76310	WB (1:1000)
RAD21	Abcam		ab992	WB (1:1000)
FLAG	Sigma	M2	F3165	IP (1:500); WB (1:1000)
pan-Ras	Thermo Secintific		16117	WB (1:1000)
MITF	Thermo Secintific		MS-771-P1	WB (1:200)
STAG1	Novus Biologicals		NB100-298	WB (1:1000)
COT	Biorbyt		orb127540	WB (1:250)
CTCF	Diagenode		C15410210	ChIP (1:500)

Supplementary Figure 1

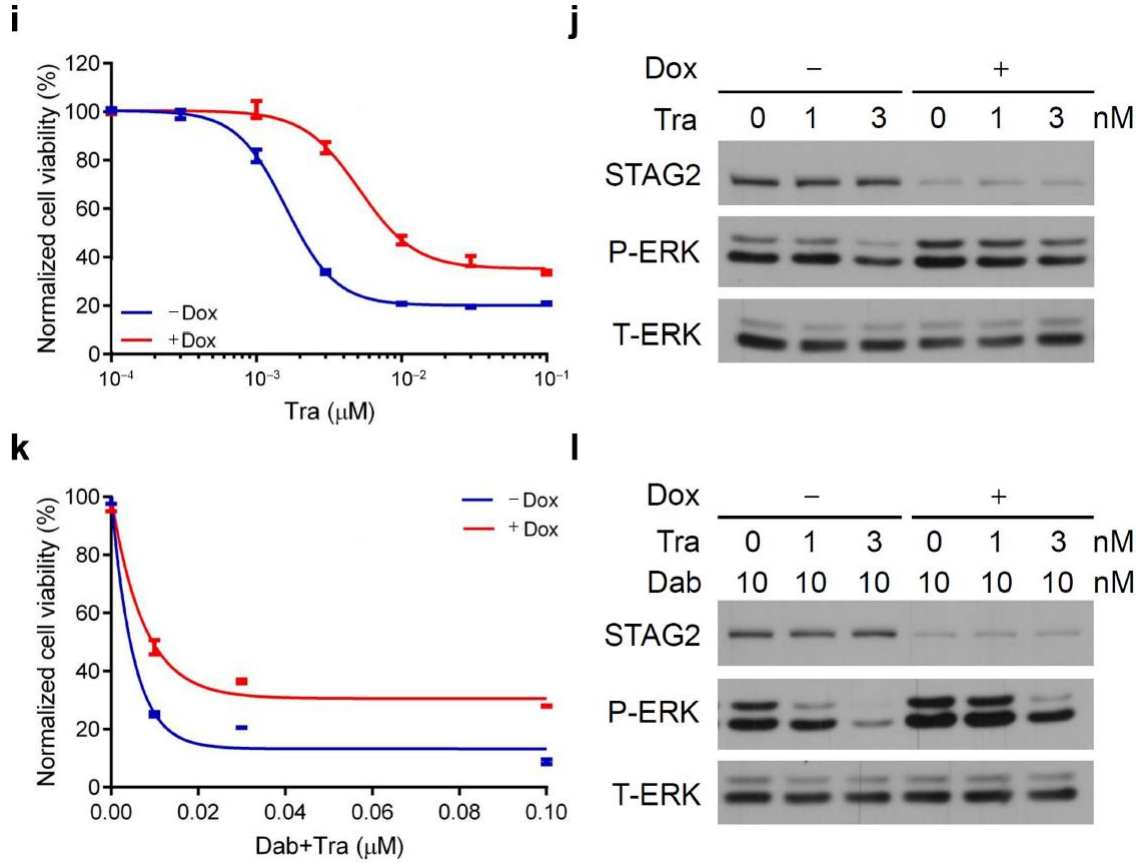


Supplementary Figure 1: Identification and characterization of STAG2 and STAG3 mutations in melanoma. (**a**) HEK293 cells were transfected with FLAG-tagged wild-type STAG2 (WT) or Asp193Asn (DN) mutant. Cell lysates were immunoprecipiated with anti-FLAG antibodies, followed by western blotting. Experiment was performed 3 times. (**b**) A375, WM902 or M14 Cells were incubated in the presence of 0.3 μM vemurafenib. Cell lysates were collected at indicated times and analyzed by western blotting. Experiment was performed 3 times. (**c**) Detection of a *STAG2* mutation in WM902-BR cells by Sanger sequencing. (**d**) HEK293 cells were transfected with FLAG-tagged wild-type STAG3 (WT), Pro272Ser (PS) or Arg508Gln (RQ) mutants. Cell lysates were immunoprecipiated with anti-FLAG antibodies, followed by western blotting. Experiment was performed 3 times. (**e**) Percentages of post-relapse samples from a total of nine patients treated with BRAFi monotherapy or BRAFi and MEKi combination therapy that showed changes of STAG2 or STAG3 expression, compared to their paired pre-treatment samples, in IHC analyses.

Supplementary Figure 2

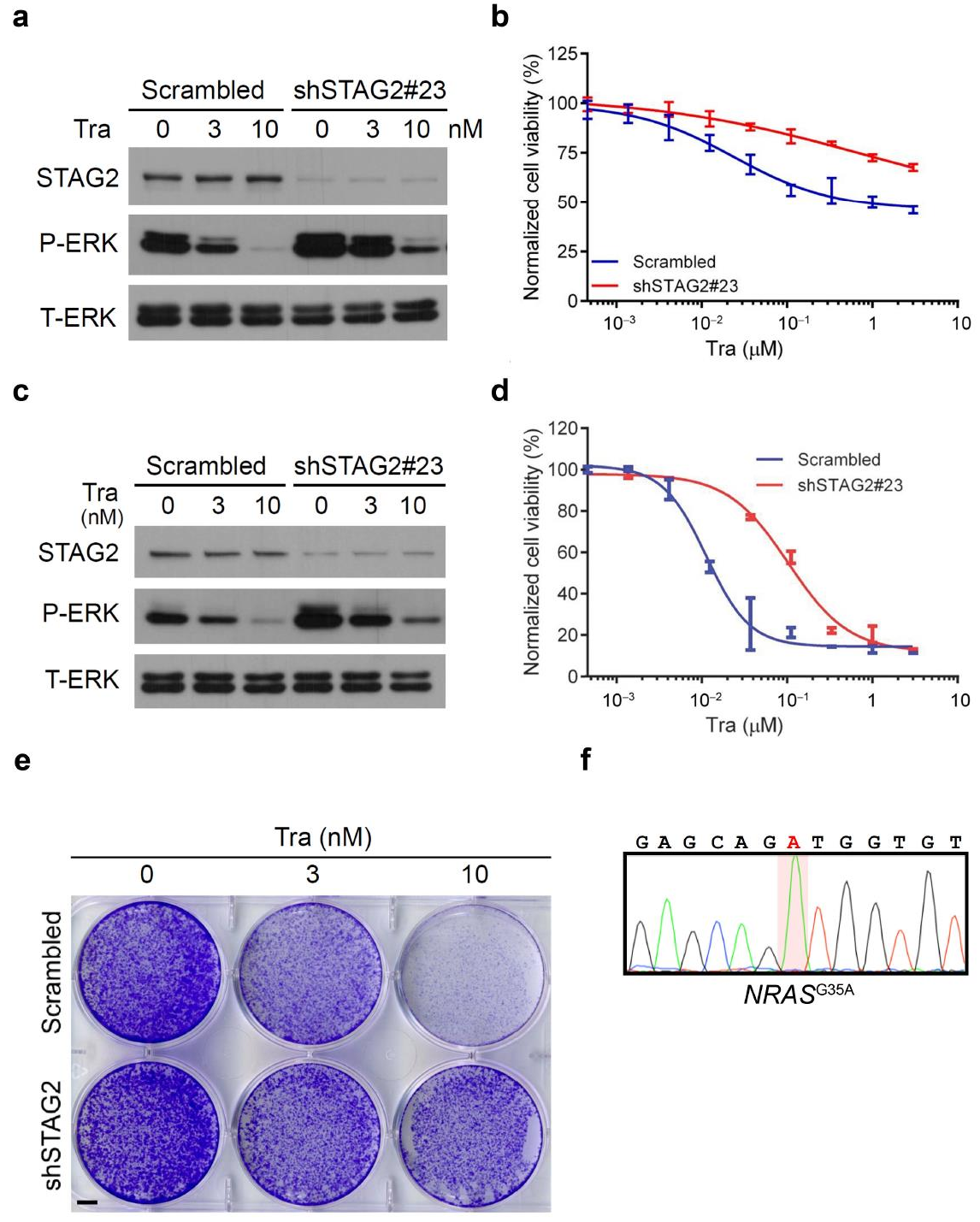


Supplementary Figure 2



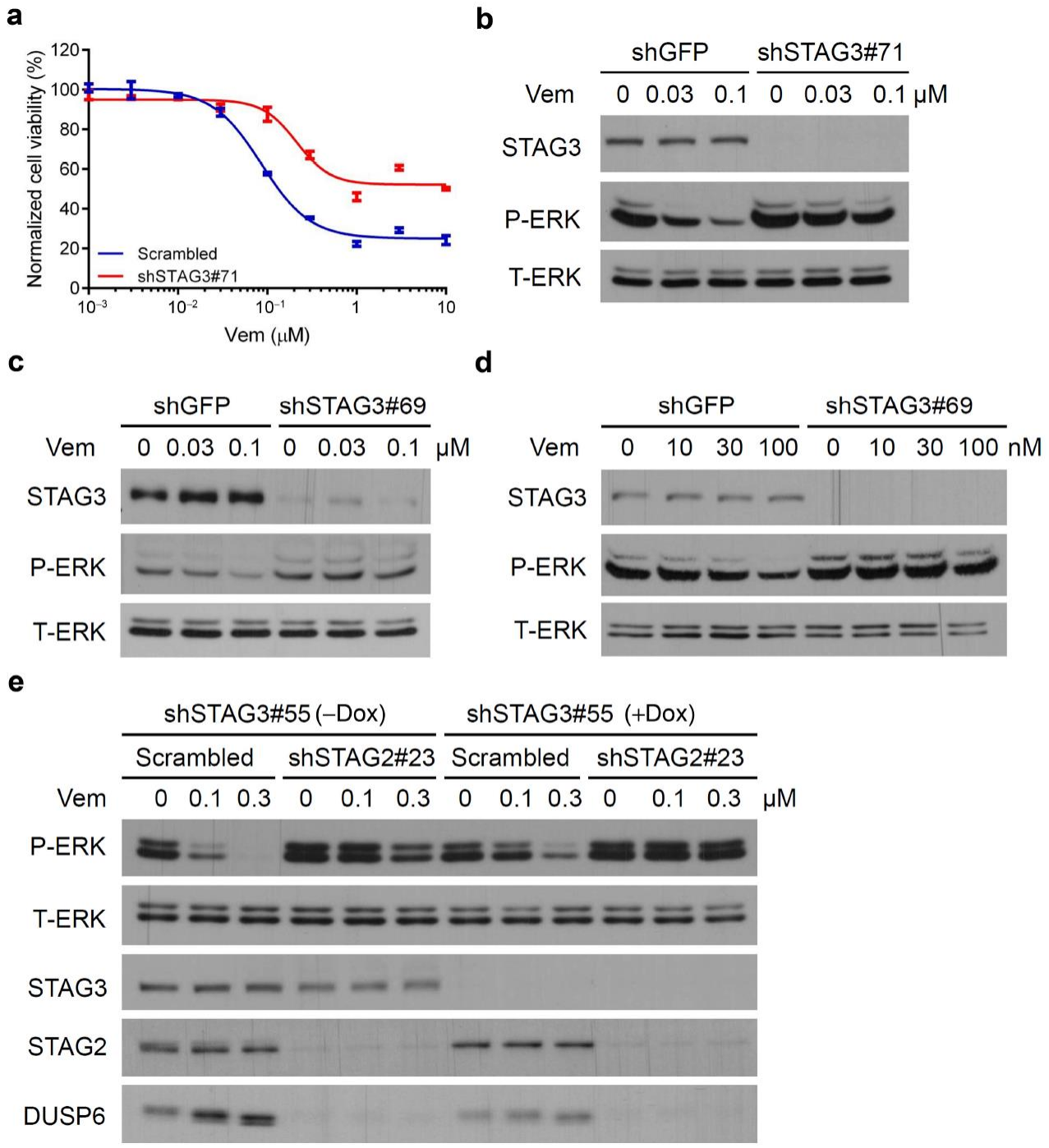
Supplementary Figure 2: Knockdown of STAG2 or STAG3 decreases BRAFi sensitivities in BRAF mutant melanoma cells. (**a**,**b**) Viability of SKMEL28 (a) or M14 (b) cells after treatment with varying concentrations of dabrafenib for 3 d. Experiment was performed 3 times. Data are mean ± s.e.m. (**c**,**d**) SKMEL28 (c) or M14 (d) cells expressing STAG2 inducible shRNA pTRIPZ-shSTAG2#60 were treated with dabrafenib for 2 h. Cell lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times. (**e**,**f**) Viability of A375 cells after treatment with varying concentrations of vemurafenib (e) or dabrafenib (f) for 3 d. Experiment was performed 3 times. Data are mean ± s.e.m. (**g**,**h**) A375 cells cells expressing STAG2 inducible shRNA pTRIPZ-shSTAG2#60 were treated with vemurafenib (g) or or dabrafenib (h) for 2 h. Cell lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times. (**i**) Viability of A375 cells after treatment with varying concentrations of trametinib for 3 d. Experiment was performed 3 times. Data are mean ± s.e.m. (**j**) A375 cells expressing STAG2 inducible shRNA pTRIPZ-shSTAG2#60 were treated with trametinib for 2 h. Cell lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times. (**k**) Viability of A375 cells after treatment of varying concentrations of dabrafenib and trametinib together at a ratio of 10:1 for 3 d. Experiment was performed 3 times. Data are mean ± s.e.m. (**l**) A375 cells expressing STAG2 inducible shRNA pTRIPZ-shSTAG2#60 were treated with dabrafenib and trametinib as indicated for 2 h. Cell lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times.

Supplementary Figure 3



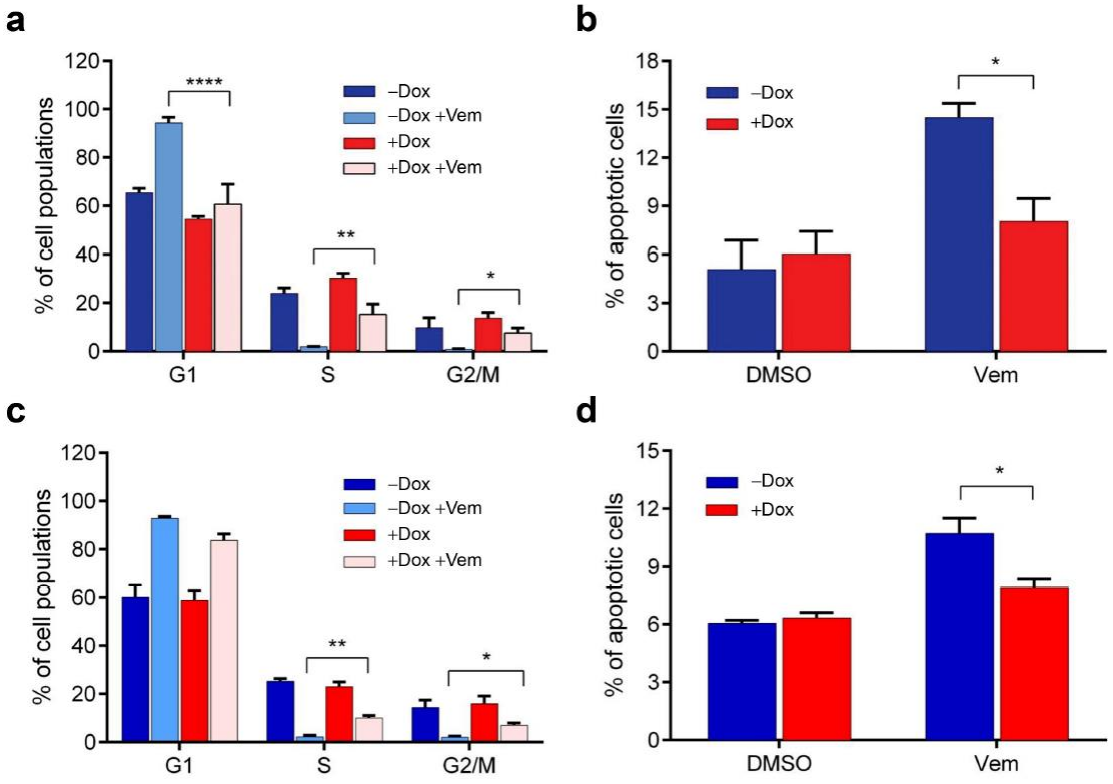
Supplementary Figure 3: Knockdown of STAG2 decreases MEKi sensitivities in NRAS mutant melanoma cells. (**a**) SKMEL103 cells expressing STAG2 shRNA#23 or scrambled control were treated with trametinib for 2 h. Cell lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times. (**b**) Viability of SKMEL103 cells after treatment of varying concentrations of trametinib for 3 d. Experiment was performed 3 times. Data are mean ± s.e.m. (**c**) 501MEL cells expressing STAG2 shRNA#23 or scrambled control were treated with trametinib for 2 h. Cell lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times. (**d**) Viability of 501MEL cells after treatment of varying concentrations of trametinib for 3 d. Experiment was performed 3 times. Data are mean ± s.e.m. (**e**) Viability of 501MEL cells expressing STAG2 shRNA#23 or scrambled control were seeded at $3x10^4$ per well in 6-well plates and treated with trametinib as indicated in clonogenic growth assays. Experiment was performed 3 times. Scale bar: 5 mm. (**f**) Conformation of *NRAS* mutation in 501MEL cells by Sanger sequencing.

Supplementary Figure 4



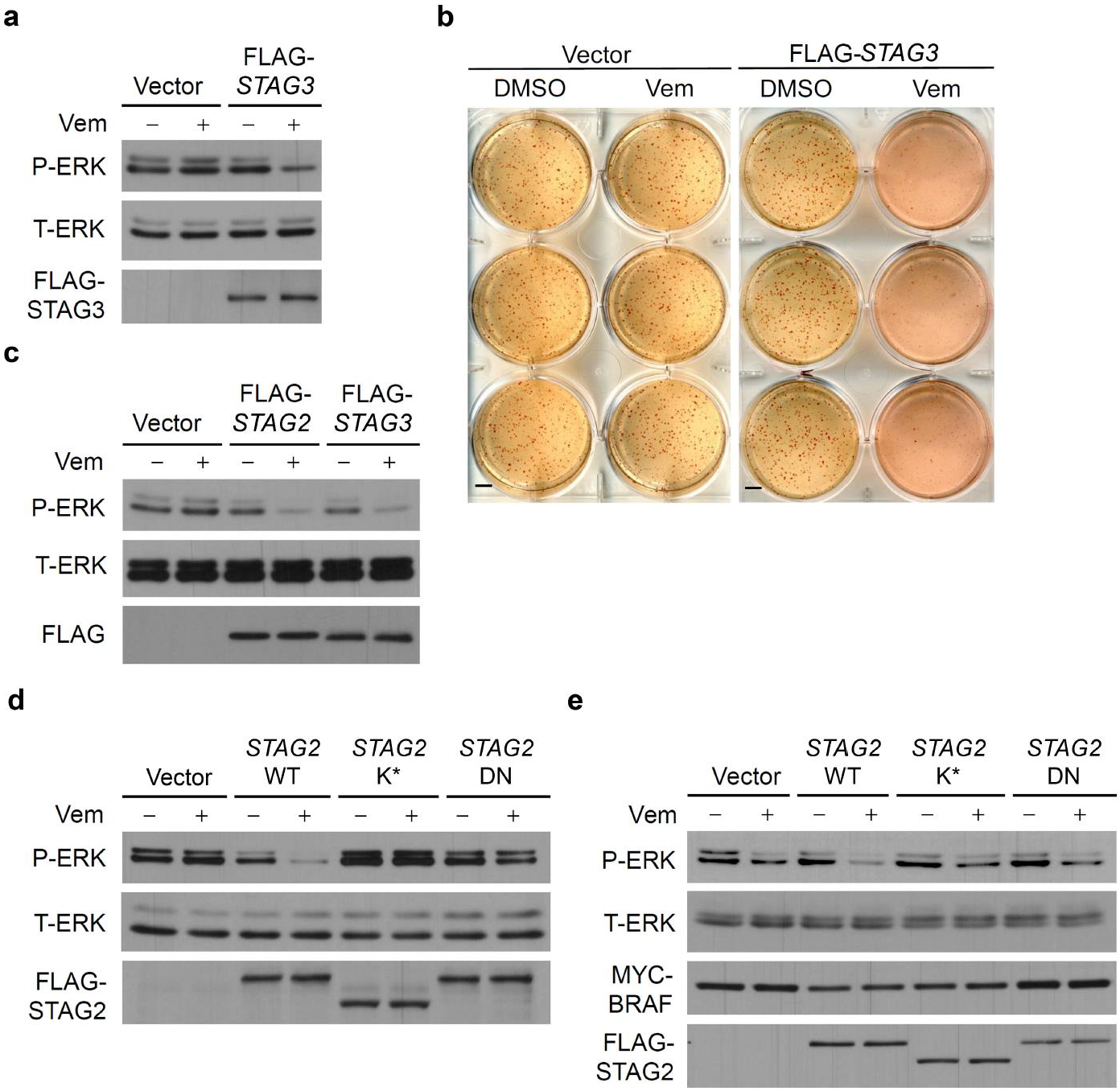
Supplementary Figure 4: Knockdown of STAG3 decreases BRAFi sensitivities in BRAF mutant melanoma cells. (**a**) Viability of M14 cells after treatment of varying concentrations of vemurafenib for 3 d. Experiment was performed 3 times. Data are mean ± s.e.m. (**b**) M14 cells expressing STAG3 shRNA#71 or scrambled control were treated with vemurafenib for 2 h. Cell lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times. (**c**,**d**) A375 (c) or SKMEL28 (d) cells expressing STAG3 shRNA#69 or scrambled control were treated with vemurafenib for 2 h. Cell lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times. (**e**) A375 cells expressing STAG3 inducible shRNA pTRIPZ-shSTAG3#55 were infected with STAG2 shRNA#23 or scrambled control. Cells were cultured in the presence or absence of doxycycline for 5 d. and treated with various concentrations of vemurafenib for 2 h before lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times.

Supplementary Figure 5



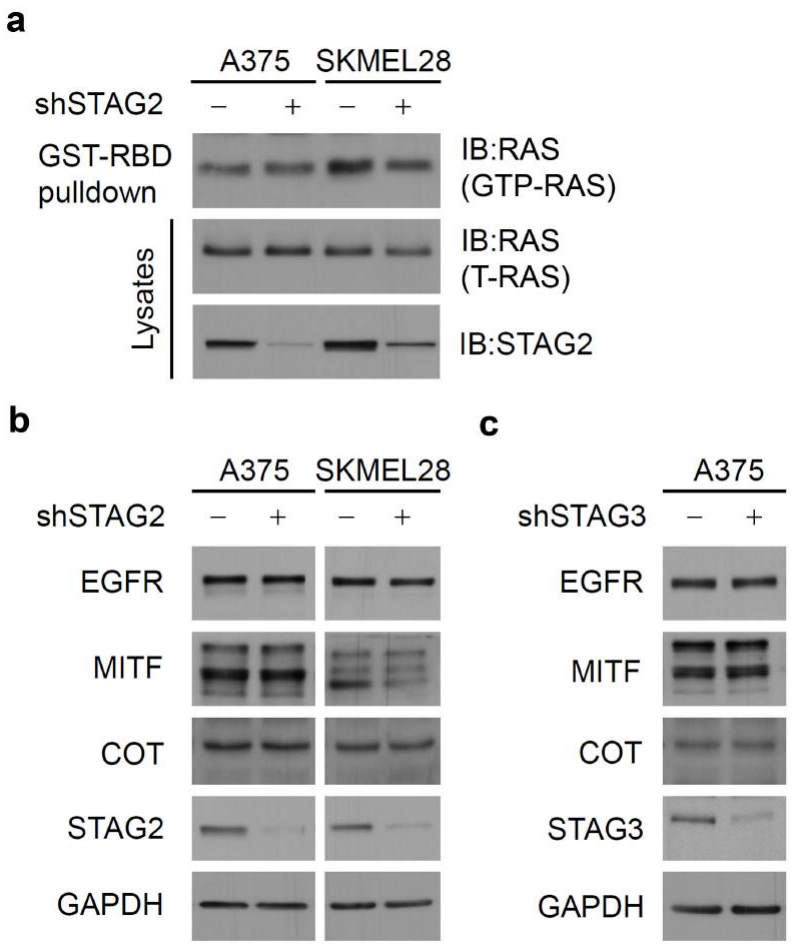
Supplementary Figure 5: Loss of STAG3 impaires the changes in cell cycle progression and reduced the percentages of annexin V-positive apoptotic cells in response to vemurafenib treatment. (**a**,**b**) A375 cells expressing STAG2 inducible shRNA pTRIPZ-shSTAG2#60 were cultured in the presence or absence of doxycycline for 5 d. Cells were treated with or without 1 µM vemurafenib for 72 h before cell cycle (a) and apoptosis (b) analyses were performed. Experiment was performed 3 times. Data are mean ± s.e.m. The *P* values were determined using two tailed Student's *t-test,* * $P < 0.05$; ** $P < 0.01$; **** $P < 0.0001$. The data variance is similar between groups. (**c**,**d**) A375 cells expressing STAG3 inducible shRNA pTRIPZ-shSTAG3#55 were cultured in the presence or absence of doxycycline for 5 d. Cells were treated with or without 1 µM vemurafenib for 72 h before cell cycle (c) and apoptosis (d) analyses were performed. Experiment was performed 3 times. Data are mean ± s.e.m. The *P* values were determined using two-tailed Student's *t-test,* * $P < 0.05$; ** $P < 0.01$. The data variance is similar between groups.

Supplementary Figure 6



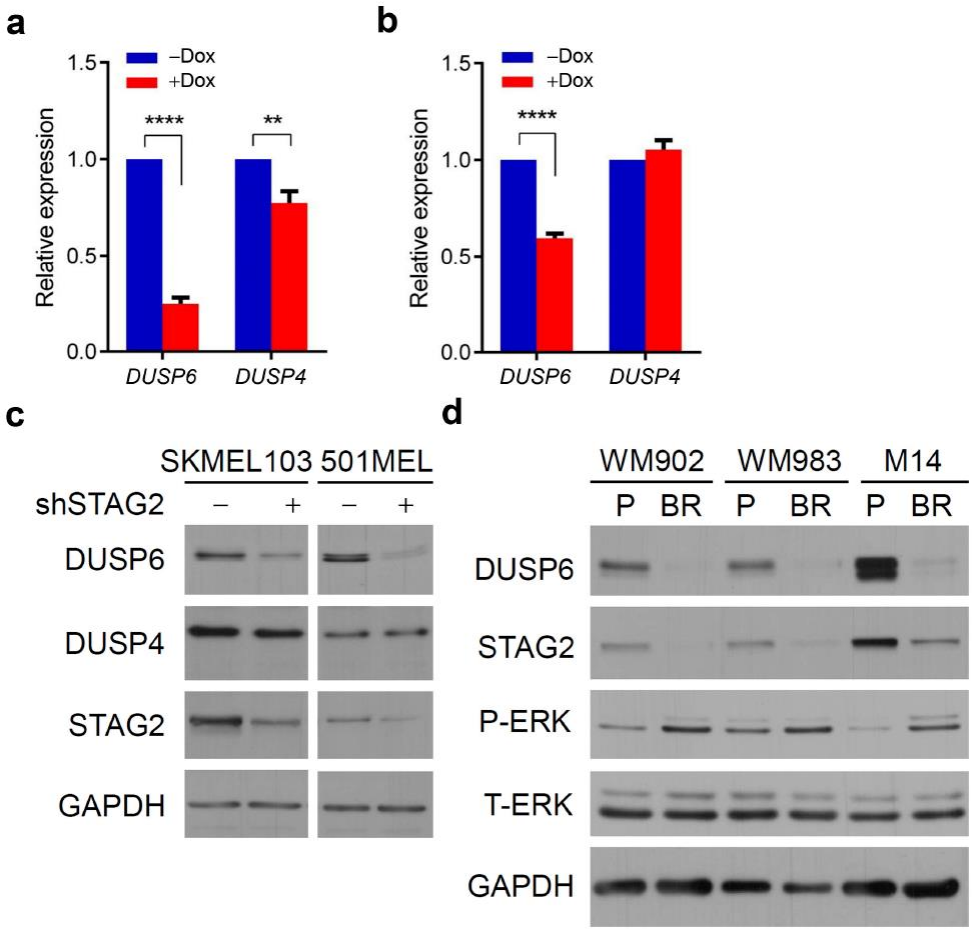
Supplementary Figure 6: Ectopic expression of STAG2 or STAG3 incrases sensitivities to BRAFi in melanoma cells. (**a**) WM902-BR cells stably expressing FLAG-tagged wild-type STAG3 or control vector were treated with 3 μM vemurafenib for 2 h. Cell lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times. (**b**) WM902-BR stable expressing of FLAG-tagged wild-type STAG3 or control vector were used in soft agar assays in the presence or absence of 3 μM vemurafenib. Experiment was performed 3 times. Scale bar: 5 mm (**c**) WM983-BR cells stably expressing FLAG-tagged wild-type STAG2, STAG3 or vector control were treated with 1 μM vemurafenib for 2 h. Cell lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times. (**d**) LOX-IVMI cells stably expressing FLAG-tagged wild-type STAG2 (WT), Lys1083* (K*) or Asp193Asn (DN) mutants were treated with 3 μM vemurafenib for 2 h. Cell lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times. (**e**) HEK293 cells were transfected with MYC-tagged BRAF Val600Glu together with FLAG-tagged wild-type STAG2 (WT), Lys1083* (K*) or Asp193Asn (DN) mutants. Cells were treated with 10 μM vemurafenib for 2 h. Cell lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times. (**f**) HEK293 cells were transfected with MYC-tagged BRAF Val600Glu together with FLAG-tagged wild-type STAG3 (WT), Pro272Ser (PS) or Arg508Gln (RQ) mutants. Cells were treated with 10 μM vemurafenib for 2 h. Cell lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times. (**g**) M14 cells stably expressing FLAG-tagged wild-type STAG2, STAG3 or control vector were treated with 30 nM vemurafenib for 2 h. Cell lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times.

Supplementary Figure 7



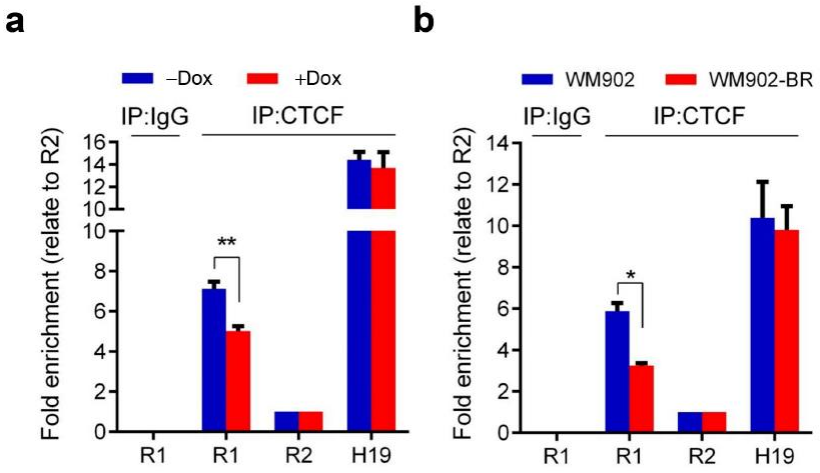
Supplementary Figure 7: Loss of STAG2 does not affect Ras activity or expression of EGFR, MITF or COT in melanoma cells. (**a**) A375 and SKMEL28 cells were stably infected with lentivirus expressing STAG2 shRNA#23 or scrambled control. RAS activation was assessed by pull-down assays with GST-RAF1-RAS binding domain (RBD), followed by western blotting with indicated antibodies. Experiment was performed 3 times. (**b**) A375 or SKMEL28 cells expressing inducible STAG2 shRNA pTRIPZ-shSTAG2#60 were cultured in the presence or absence of doxycycline for 5 d before lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times. (**c**) A375 cells expressing STAG3 shRNA#96 or scrambled control were used for western blotting with indicated antibodies. Experiment was performed 3 times.

Supplementary Figure 8



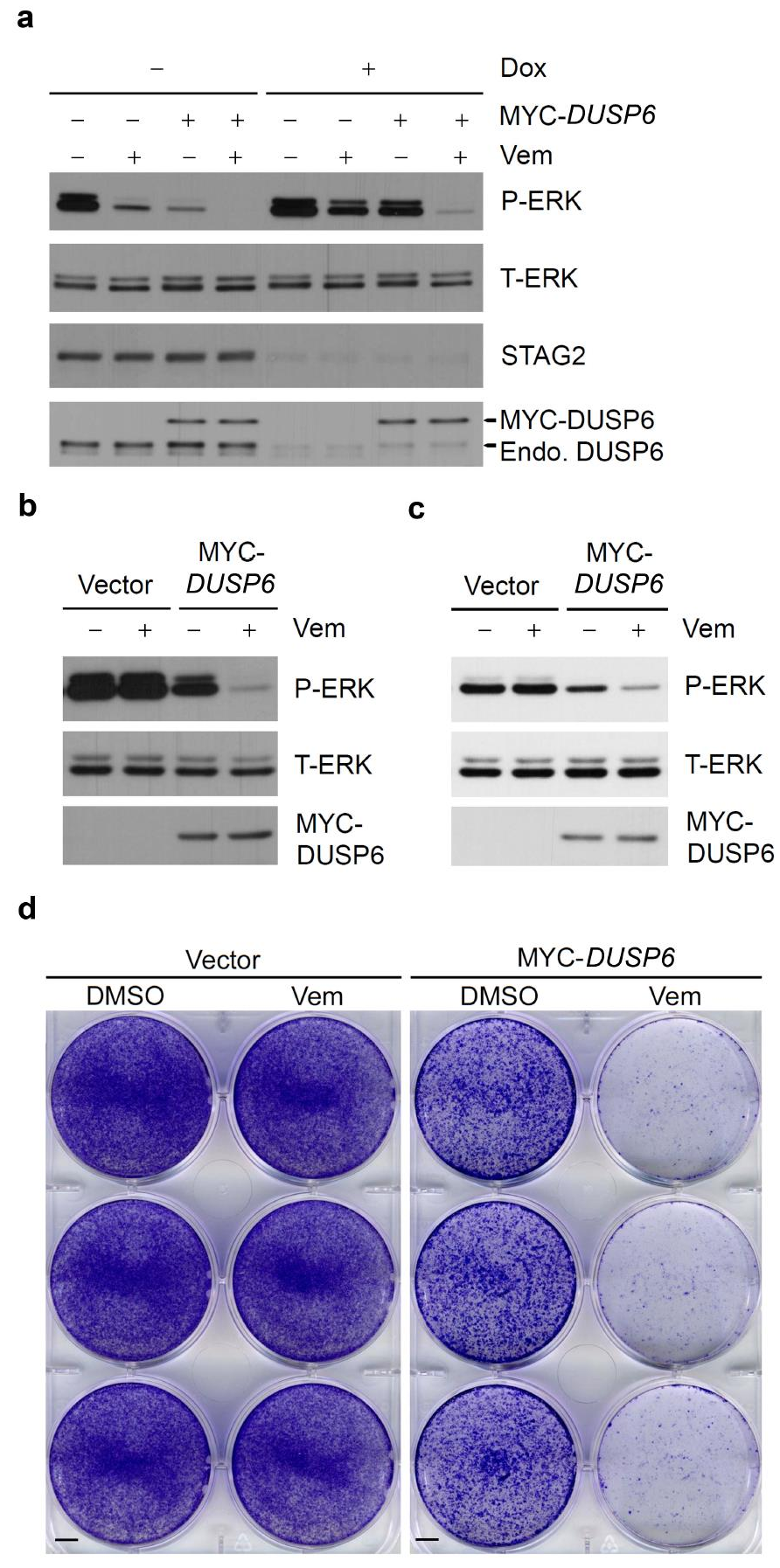
Supplementary Figure 8: STAG2 regulates expression of DUSP6 in melanoma cells. (**a**,**b**) Total RNA from A375 (a) and M14 (b) cells expressing STAG2 inducible shRNA pTRIPZ-shSTAG2#60 were isolated, reverse transcribed, and expression levels of *DUSP4* and *DUSP6* were analyzed by qPCR. Levels of mRNA were calculated relative to the absence of doxycycline control, and housekeeping *GAPDH* gene was used as the reference. *n* = 3 biological replicates. Data are mean ± s.e.m. The *P* values were determined using two-tailed Student's *t-test, ** P < 0.01,;**** P < 0.0001*. The data variance is similar between groups. (**c**) Lysates from SKMEL103 or 501MEL cells expressing STAG2 shRNA#23 or scrambled control were used for western blotting with indicated antibodies. Experiment was performed 3 times. (**d**) Expression of DUSP6 protein in BRAFi-resistant cell lines (BR) and their parental BRAFi-sensitive counterparts (P). Lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times.

Supplementary Figure 9



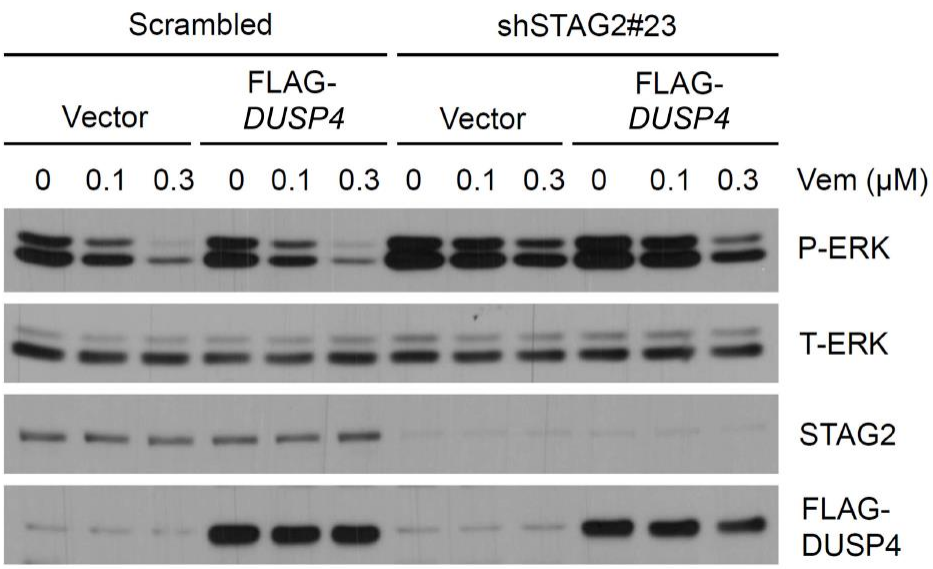
Supplementary Figure 9: STAG2 regulates the binding of CTCF to the *DUSP6* locus. (**a**) M14 cells expressing inducible STAG2 shRNA pTRIPZ-shSTAG2#60 were cultured in the presence or absence of doxycycline for 5 d before ChIP-qPCR assays were performed. Chromatins were immuoprecipitated using CTCF antibody or rabbit IgG. (**b**) Chromatins of WM902 and WM902-BR were immuoprecipitated using CTCF antibody or rabbit IgG. IP-ed chromatins were examined using qPCR with primers for R1 and R2 regions of *DUSP6* and *H19*. Results are expressed as fold enrichment relative to the non-specific region (R2). $n = 3$ biological replicates. Data are mean ± s.e.m. The *P* values were determined using two-tailed Student's *t*-test. $* P < 0.05$; $** P < 0.01$. The data variance is similar between groups.

Supplementary Figure 10



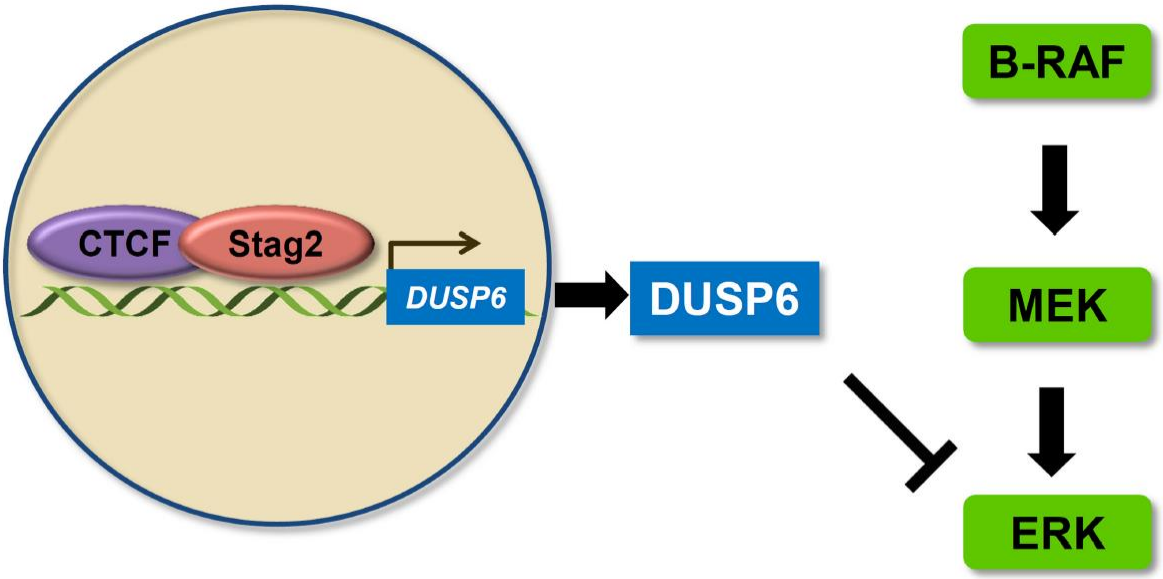
Supplementary Figure 10

e



Supplementary Figure 10: STAG2 regulates ERK activity through controlling expression of DUSP6. (**a**) M14 cells expressing STAG2 inducible shRNA pTRIPZ-shSTAG2#60 were infected with lentivirus expressing MYC-DUSP6 or control vector. Cells were cultured in the presence or absence of doxycycline for 5 d and treated with 0.3 μM vemurafenib for 2 h before lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times. (**b**,**c**) WM902-BR cells (b) and WM983-BR (c) expressing MYC-tagged DUSP6 or vector control were treated with 10 μM vemurafenib for 2 h. Cell lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times. (**d**) WM902-BR cells expressing MYC-tagged DUSP6 or vector control were seeded at $3x10^4$ per well in 6-well plates and treated with in the presence or absence of 1 μM vemurafinib as indicated in clonogenic growth assays. Experiment was performed 3 times. Scale bar: 5 mm (**e**) A375 cells expressing STAG2 shRNA#23 or scrambled control were infected with lentivirus expressing Flag-DUSP4 or control vector. Cells were treated with vemurafenib for 2 h before lysates were used for western blotting with indicated antibodies. Experiment was performed 3 times..

Supplementary Figure 11



Supplementary Figure 11: Schematic model for regulation of BRAF-MEK-ERK signaling pathway by STAG2.