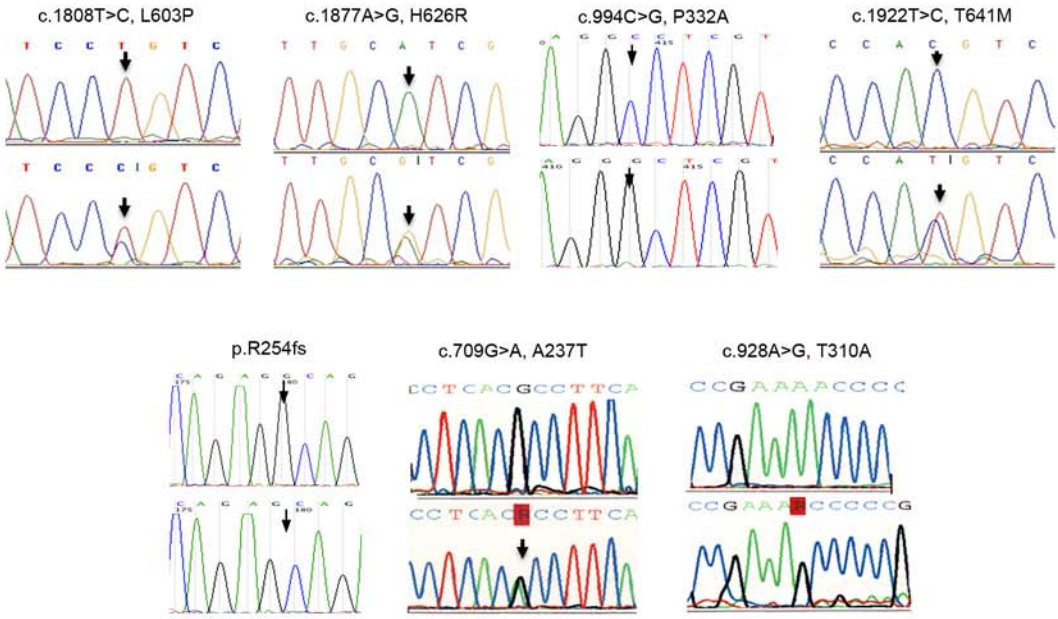
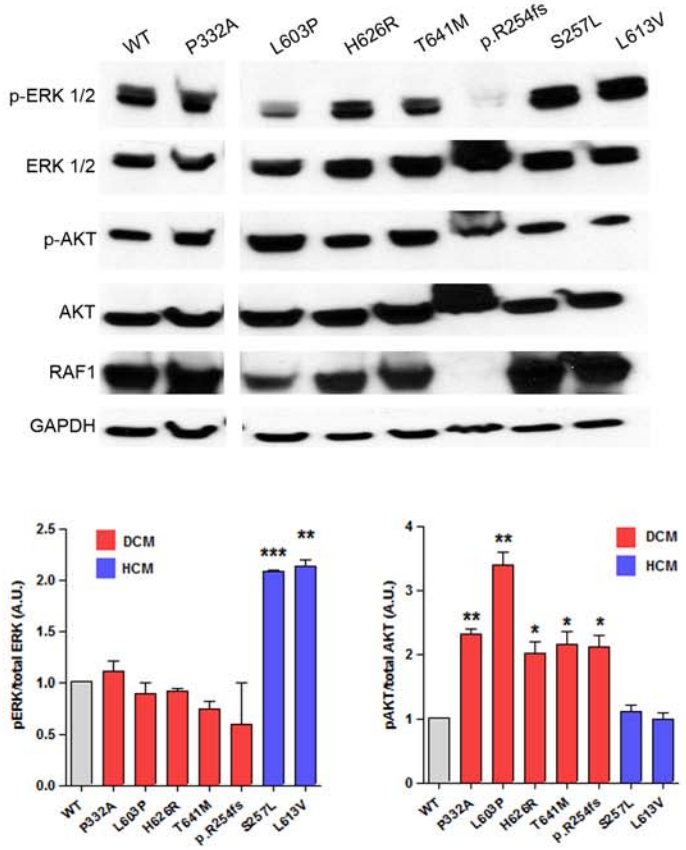
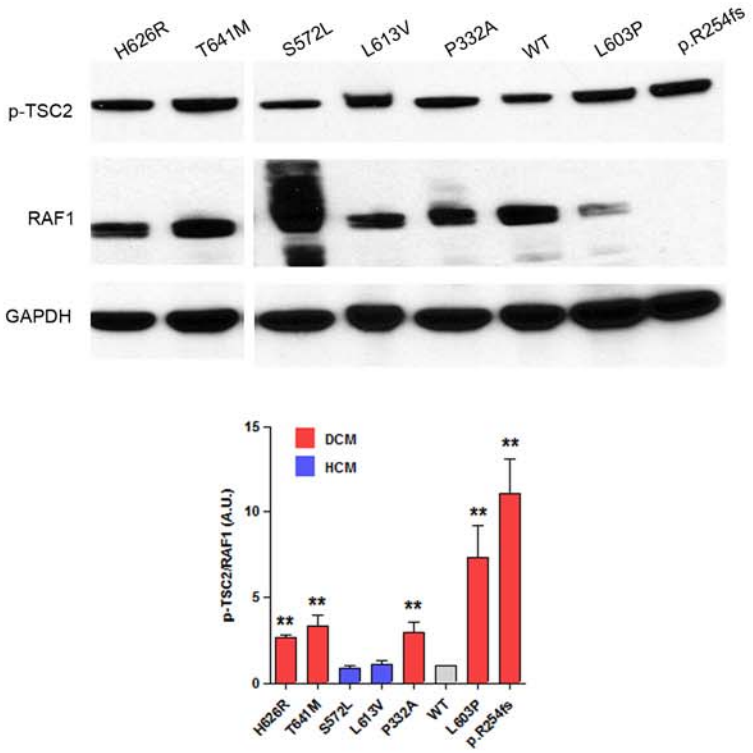


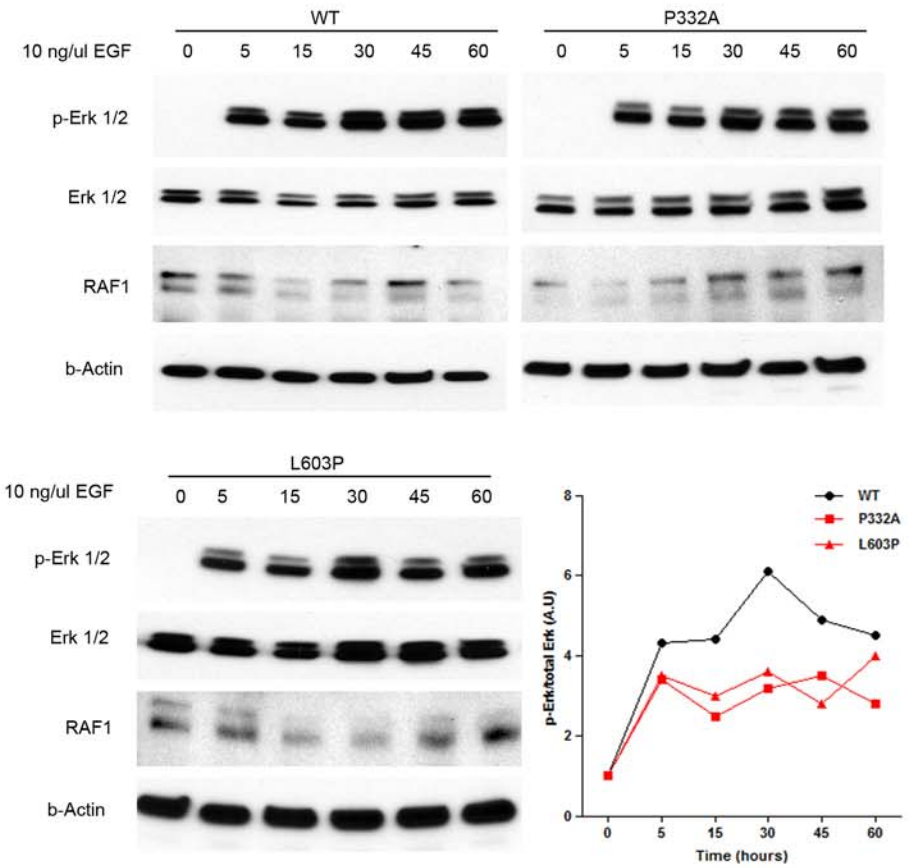
Supplementary Figure 1: DNA sequencing analysis of the DCM patients harboring novel *RAF1* (NM_002880) mutations



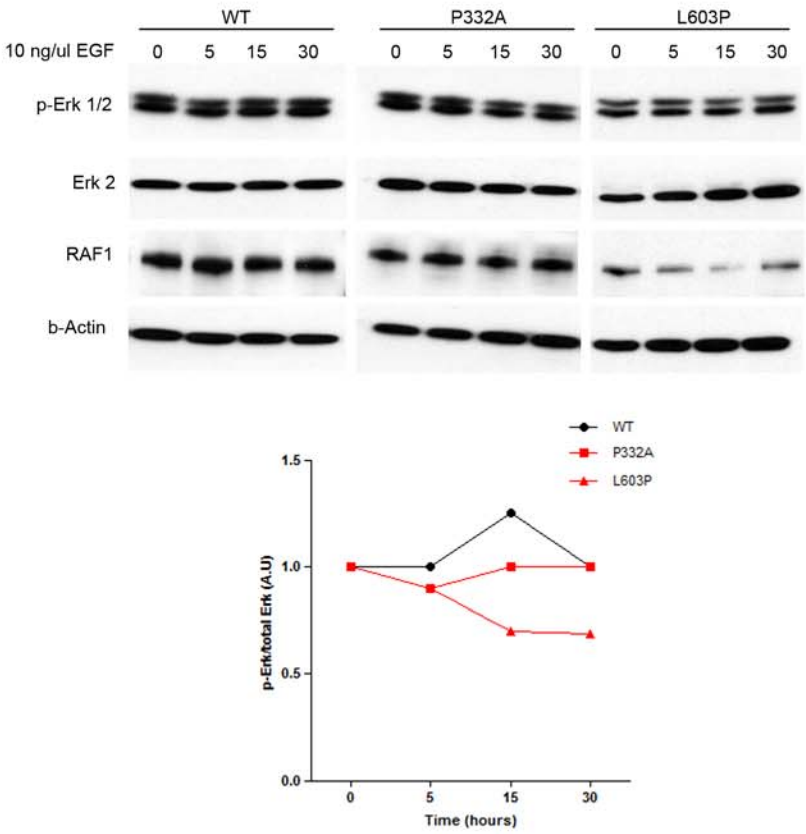
Supplementary Figure 2. DCM-associated mutant RAF1 proteins hyperactivate AKT but not ERK1/2. Representative immunoblot with total lysates from HEK293 cells expressing wild-type (WT), DCM-associated mutant RAF1 proteins (p.Pro332Ala, p.Leu603Pro, p.His626Arg, p.Thr641Met and p.Arg254fs) and HCM-associated mutant RAF1 proteins (p.Ser257Leu and p.Leu613Val) that were stimulated with EGF for 15 minutes. The blot was probed with anti-phospho-ERK1/2, anti-ERK1/2, anti-phospho-AKT, anti-AKT and anti-RAF1 antibodies. Expression levels were normalized to respective total proteins and expressed as Relative Expression compared to the level in the cells overexpressing WT RAF1. GAPDH levels were used as loading control. Data are means ± SD of two independent experiments assayed in duplicates. *p<0.05, **p<0.01, ***p<0.001 vs WT.



Supplementary Figure 3: DCM-associated RAF1 mutants activate AKT/mTOR pathway as indicated by tuberin phosphorylation. Representative immunoblot with total lysates from HEK293 cells expressing wild-type (WT), DCM-associated mutant RAF1 proteins (p.Pro332Ala, p.Leu603Pro, p.His626Arg, p.Thr641Met and p.Arg254fs) and HCM-associated mutant RAF1 proteins (p.Ser257Leu and p.Leu613Val) that were stimulated with EGF for 15 minutes. The blot was probed with anti-phospho-TSC2, anti-RAF1 and anti-GAPDH antibodies. Expression levels were normalized to RAF1 and expressed as Relative Expression compared to level in the WT cells. GAPDH levels were used as loading control. Data are means ± SD of two independent experiments assayed in duplicates ***p*<0.01 vs WT.



Supplementary Figure 4. DCM-associated RAF1 mutants show impaired Erk1/2 activation in *Raf1* $^{-/-}$ MEFs. Immunoblot with total lysates from mouse embryonic fibroblasts (MEFs) from Raf1 knockout mice (*Raf1* $^{-/-}$) expressing wild-type (WT) and two representative DCM mutants (p.Pro332Ala and p.Leu603Pro) that were stimulated with EGF at 0, 5, 15, 30, 45 and 60 minutes and probed with anti-phospho-Erk1/2, anti-Erk1/2 and anti-RAF1 antibodies. Expression levels were normalized to respective total proteins and expressed as Relative Expression compared tolevel in the WT cells with indicated time points. Beta-Actin levels were used as loading control.



Supplementary Figure 5: DCM-associated RAF1 mutants fail to activate Erk1/2 in *Braf* $^{-/-}$ MEFs. Immunoblot with total lysates from mouse embryonic fibroblasts (MEFs) from Braf knockout mice (*Braf* $^{-/-}$) expressing wild-type (WT) and two representative DCM mutants (p.Pro332Ala and p.Leu603Pro) that were stimulated with EGF at 0, 5, 15, and 30 minutes and probed with anti-phospho-Erk1/2, anti-Erk 2 and anti-RAF1 antibodies. Expression levels were normalized to respective total proteins and expressed as Relative Expression compared to level in the WT cells with indicated time points. Beta-Actin levels were used as loading control.

Supplementary Table 1. *Titin (*NM_001267550.1) polymorphisms observed in *RAF1* positive patients

Exon	Nucleotide Change	AA Change	Pathogenicity	P1	P2A	P2B	P3	P4	P5	P6	P7	P8
7	c.982C>T	p.Arg328Cys	None known	CC	CT	CT	CC	CC	CC	CT	CT	CT
309	c.64208C>T	p.Thr21403Ile	None known	CC	TT	CT	CC	TT	CC	CT	CT	CT

Supplementary Table 2: Bioinformatic analysis of the effects of *RAF1* mutations

Exon	Nucleotide Change	Amino Acid Change	***PolyPhen-2***	***pMUT***	***SIFT***
5	c.709G>A	p.Ala237Thr	Benign	Neutral	Tolerated
9	c.928A>G	p.Thr310Ala	Benign	Neutral	Tolerated
10	c.994C>G	p.Pro332Ala	Benign	**Pathological**	Tolerated
17	c.1808T>C	p.Leu603Pro	**Probably damaging**	**Pathological**	Affects protein
17	c.1877A>G	p.His626Arg	**Probably damaging**	**Pathological**	Tolerated
17	c.1922C>T	p.Thr641Met	**Probably damaging**	**Pathological**	Affects protein