Supplemental Methods Table 1.
Clinical characteristics of desmoid tissues collected for U133A gene expression analysis

Characteristic	number (%) n=55
Gender	
Male	15 (27%)
Female	40 (73%)
Size	
<=5cm	18 (33%)
5-10cm	18 (33%)
>=10cm	19 (35%)
Age at diagnosis	
<25yo	19 (35%)
25-25yo	33 (60%)
>45yo	15 (27%)
Site	
Abdominal wall	12 (22%)
Chest wall/flank	16 (29%)
Intra-abdominal/retroperitoneal	12 (22%)
Upper extremity	11 (20%)
Lower extremity	14 (25%)
Presentation status	
Primary	38 (69%)
Local recurrence	17 (26%)

Supplemental Methods Table 2. Clinical Characteristics of Samples from Alliance A091105

Patient number	Fresh frozen samples (Immunoblot)	FFPE samples (IHC)	Disease presentation	Site	Multifocal	RECIST progression prior to enrollment
Sorafenib arm						
1	X	X	primary	lower extremity	N	N
2	X	X	recurrent	lower extremity	Y	N
3	X	X	primary	upper extremity	N	Y
4	X	X	primary	lower extremity	unknown	Y
5	X		recurrent	lower extremity	Y	Y
6	X	X	primary	chest wall/flank	N	N
7	X	X	primary	upper extremity	N	N
8	X	X	primary	upper extremity	unknown	Y
9		X	recurrent	intra-abdominal/retroperitoneal	N	N
10		X	primary	lower extremity	N	N
11		X	recurrent	upper extremity	N	N
12		X	primary	upper extremity	N	N
13		X	recurrent	lower extremity	Y	N
14		X	recurrent	chest wall/flank	N	Y
15		X	recurrent	lower extremity	N	Y
16		X	recurrent	lower extremity	N	N
17		X	recurrent	intra-abdominal/retroperitoneal	Y	Y
18		X	primary	chest wall/flank	unknown	Y
19		X	recurrent	chest wall/flank	N	N
20		X	primary	intra-abdominal/retroperitoneal	N	N
Placebo arm						
1	X	X	recurrent	intra-abdominal/retroperitoneal	N	N
2	X	X	primary	chest wall/flank	unknown	Y
3	X		recurrent	head and neck	N	N
4	X	X	recurrent	abdominal wall	Y	N
5	X	X	primary	abdominal wall	N	N
6	X	X	recurrent	chest wall/flank	Y	N
7	X	X	recurrent	intra-abdominal/retroperitoneal	unknown	Y
8	X	X	recurrent	lower extremity	N	N
9		X	recurrent	chest wall/flank	unknown	Y
10		X	recurrent	abdominal wall	N	N
11		X	recurrent	abdominal wall	Y	Y
12		X	recurrent	abdominal wall	unknown	Y
13		X	primary	intra-abdominal/retroperitoneal	N	Y
14		X	primary	upper extremity	N	N
15		X	primary	abdominal wall	unknown	N
16		X	recurrent	upper extremity	Y	Y
17		X	recurrent	upper extremity	N	N
18		X	primary	intra-abdominal/retroperitoneal	N	N
19		X	primary	intra-abdominal/retroperitoneal	unknown	N

Supplemental Methods Table 3. Representativeness of study participants

Cancer type: Desmoid-type fibromatosis

Considerations related to:

Sex	The largest series reporting on desmoid patients include cohorts of approximately 500 patients; studies show rates of diagnosis in female patients is approximately twice that in men.
Age	Approximately half of patients are diagnosed between the ages of 25 and 45 years.
Race and Ethnicity	No large reports have addressed predilection of the disease to cohorts of specific race or ethnicity.
Geography	Desmoid-type fibromatosis is a rare disease diagnosed in only approximately 1500 patients in the United States yearly.
Study Population	Specimens from Alliance A091105 used in this study included a population that was 68% female (concordant with increased incidence of desmoids in women vs. men). 72% of patients were white, 12% were black or African American and race was unknown in 10% of patients. 10% of participants identified as Hispanic. The study was conducted through the Alliance for Clinical Trials in cooperation with ECOG-ACRIN, SWOG, NRG, and NCIC-CTG so that enrollment was open to patients at institutions across the US and Canada.

Supplemental Methods Table 4. shRNA sequences

CTNNB1

shRNA E1: *5'*-GCTTGGAATGAGACTGCTGAT-*3'*

shRNA E2: *5'*-CCTTTAGCTGTATTGTCTGAA-*3'*

HIF1A

shRNA #1 (2294): *5'*-TTAACTTGATCCAAAGCTCTGA-*3'*

shRNA #2 (288): *5'*-TTATCAGAAATGTAAATCATGT-*3'*

ABL1

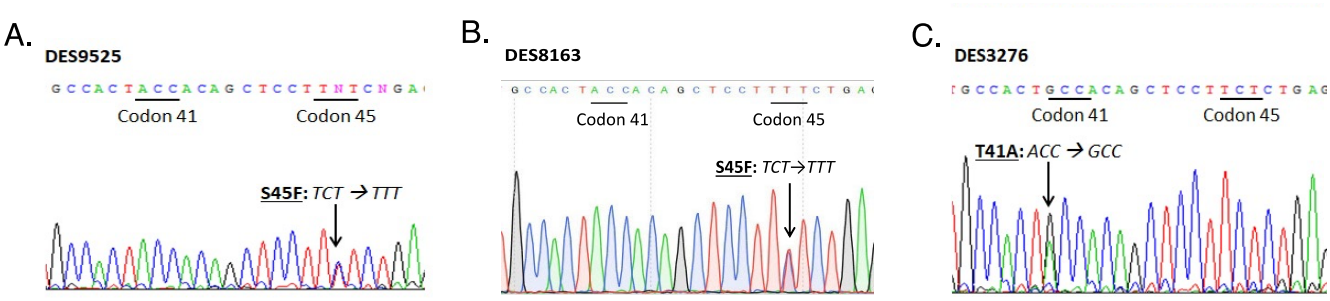
shRNA #1: *5'*-AATGATGATGAACCAACTCGG-*3'*

shRNA #2: *5'*-ATGATGATGAACCAACTCGGC-*3'*

Supplemental Methods Table 5. RRIDs for Commercially Available Materials Used in This Study

Antibodies		
β-catenin	Cell Signaling Technology #9562	RRID: AB_331149
HIF1-α	Cell Signaling Technology #36169	RRID: AB_2799095
phospho-AKT (Ser473)	Cell Signaling Technology #9271	RRID: AB_329825
AKT	Cell Signaling Technology #9272	RRID: AB_329827
phospho-PDGFRβ (Y751)	Cell Signaling Technology #3161	RRID: AB_331053
PDGFRβ	Cell Signaling Technology #3169	RRID: AB_2162497
phospho-VEGFR2 (Y1175)	Cell Signaling Technology #2478	RRID: AB_331377
c-Abl	Cell Signaling Technology #2862	RRID: AB_2257757
phospho-c-Abl (Y412)	Millipore #07-788	RRID: AB_11212018
phospho-Crkl	Cell Signaling Technology #3181	RRID: AB_331068
phospho-CrkII	Cell Signaling Technology #3491	RRID: AB_2229920
β-actin	Cell Signaling Technology #8457	RRID: AB_10950489
Vinculin	Abcam #ab91459	RRID: AB_2050446
FosB	Cell Signaling Technology #2251	RRID: AB_2106903
EGR1	R&D Systems MAB2818	RRID: AB_2097028
p21	Millipore OP64	RRID: AB_2335868
Software and Algorithms		
HTSeq		RRID: SCR_005514
DeSeq		RRID: SCR_000154
Bedtools		RRID: SCR_006646
Bowtie2		RRID: SCR_016368
ImageJ		RRID: SCR_003070

Supplemental Methods. Figure 1.



Supplemental Methods Figure 1. Validation of *CTNNB1* mutation in desmoid cell lines by Sanger sequencing. (A) DES9525, (B) DES3276, and (C) DES8163 cells. Mutations in exon 3 of *CTNNB1* resulting in the canonical S45F and T41A alterations in β-catenin are highlighted.

Supplemental Table 1 (see Excel). Gene set enrichment analysis of genes dysregulated in *CTNNB1* knockdowns as compared to scramble controls. Experiment was completed in DES9525 cell line.

Supplemental Table 2 (see Excel). Genes included in HIF1- and angiogenesis-related pathways and used for supervised clustering of desmoid tumors. Pathways in which each gene are included are annotated in the table.

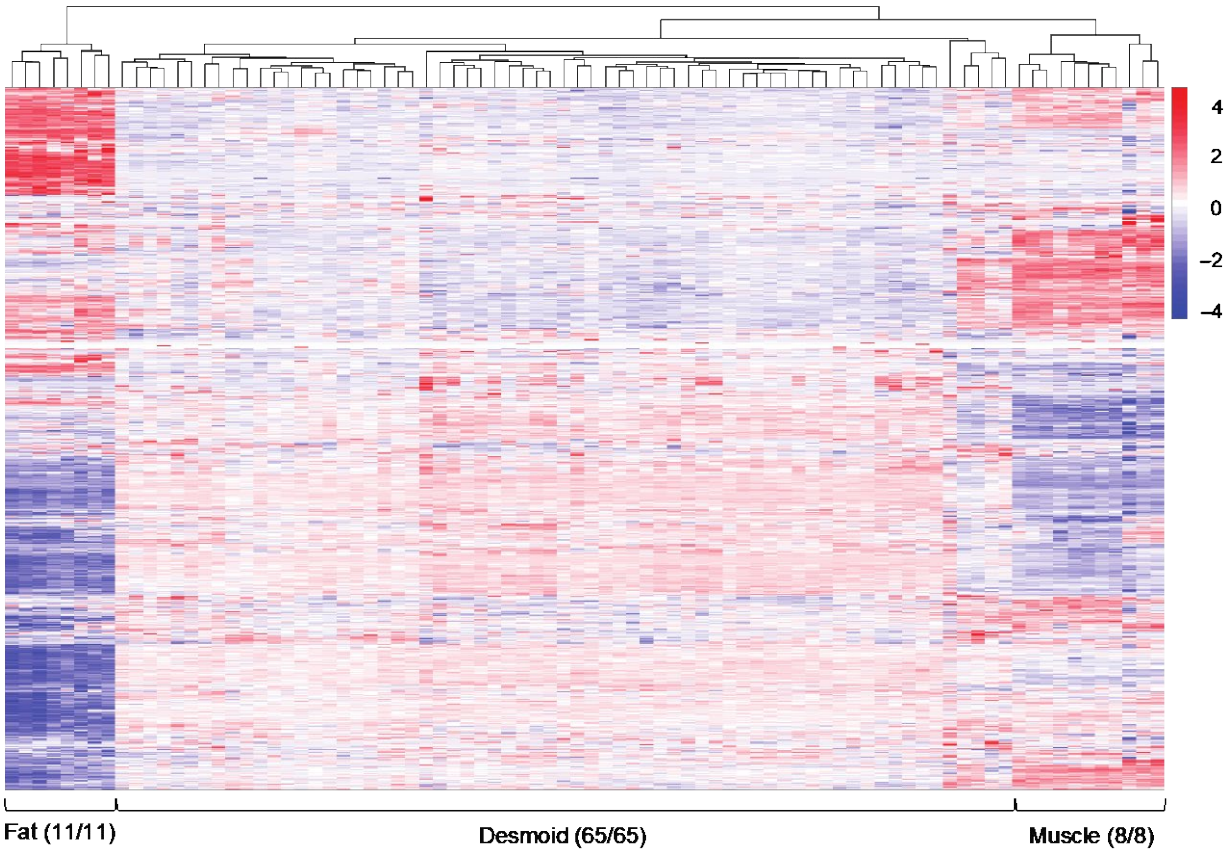
Supplemental Table 3 (see Excel). Genes noted to be altered in DES9525 after *CTNNB1* knockdown as identified by RNA-seq. Fold change is relative to expression in cells treated with scramble control.

Supplemental Table 4 (see Excel). *CTNNB1* mutations detected in pre- and post-treatment biopsies from patients on ALLIANCE A091105.

Supplemental Table 5 (see Excel). Genes with altered expression in DES9525 cells after treatment with PDGF-BB as compared to untreated cells as determined by RNA-seq.

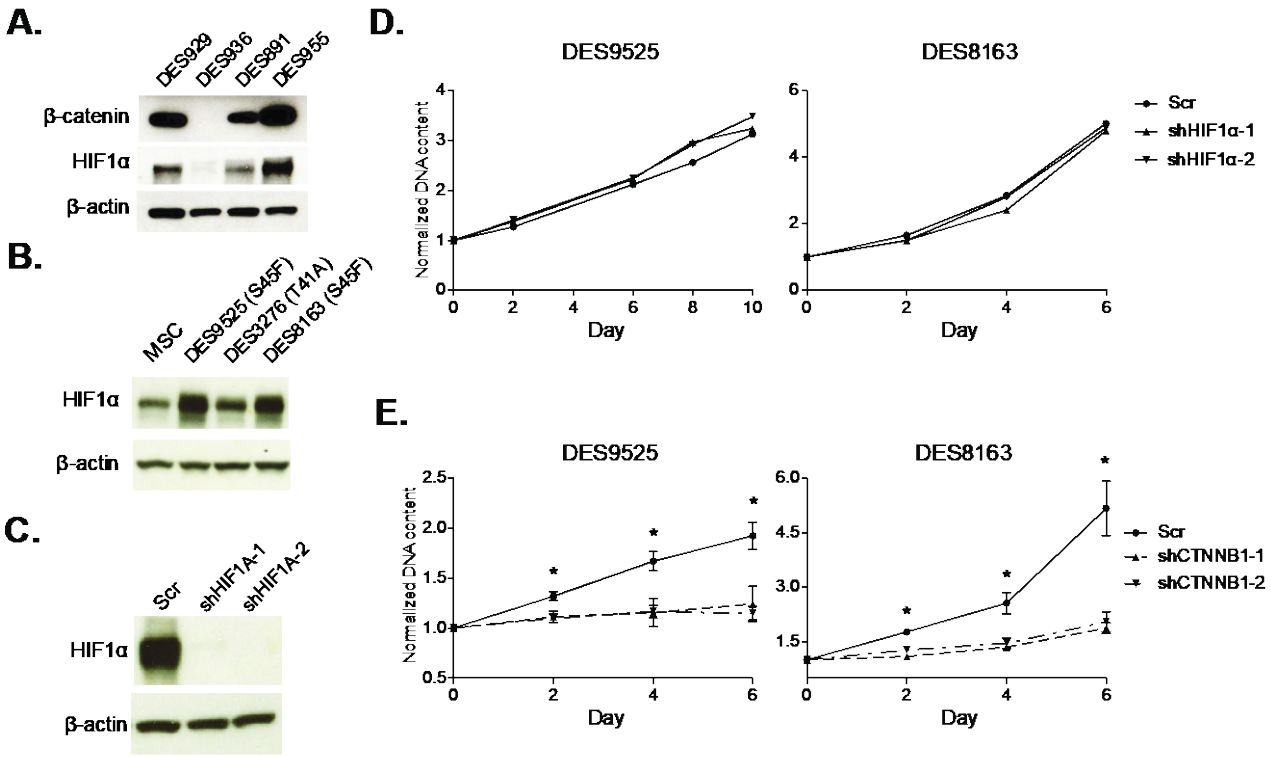
Supplemental Table 6 (see Excel). Gene set enrichment analysis performed on RNA-seq from pre-treatment biopsies taken from patients on the placebo arm of ALLIANCE A091105.

Supplemental Figure 1.



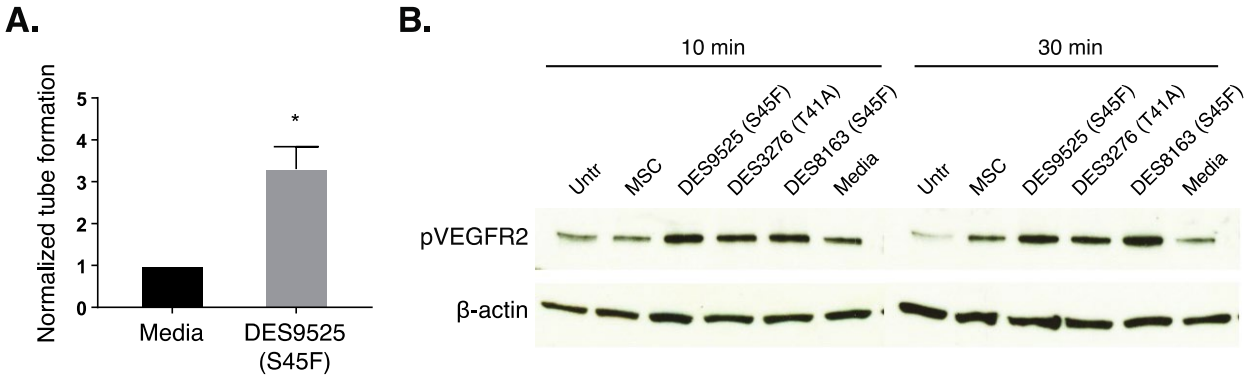
Supplemental Figure 1. Unsupervised clustering based on expression of hypoxia- and VEGF-related genes accurately differentiates desmoids from normal muscle and normal fat.

Supplemental Figure 2.



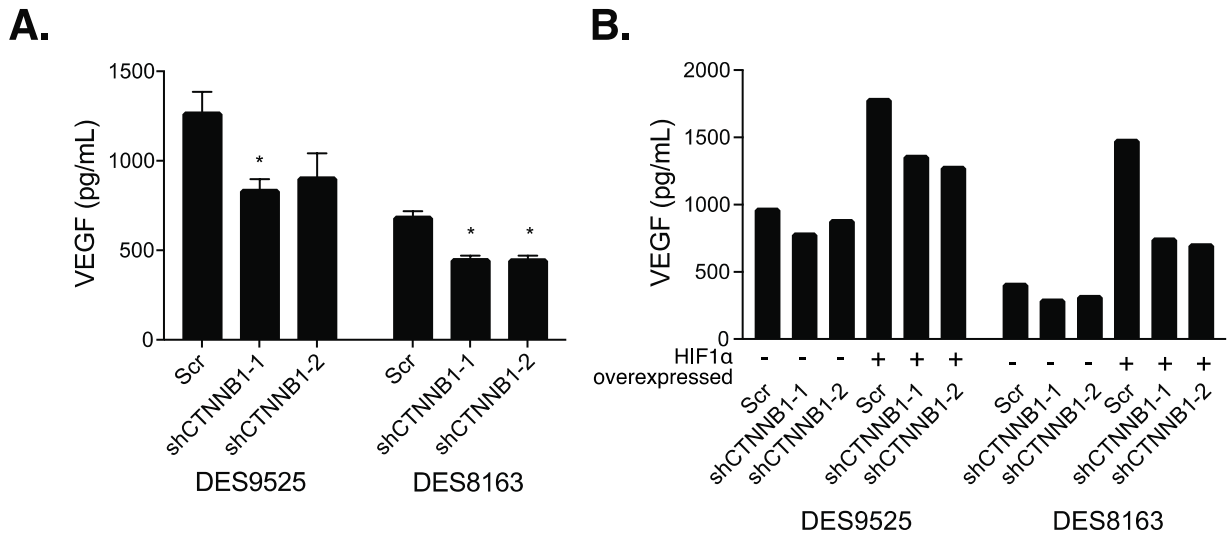
Supplemental Figure 2. β-catenin, but not HIF1α, is required for desmoid cell proliferation. (A-B) Immunoblots for β-catenin and HIF1α in (A) desmoid tumors and (B) desmoid cell lines. (C-D) Effect of *HIF1A* knockdown on (C) HIF1α protein expression in DES9525 cells and (D) proliferation in multiple desmoid cell lines. (E) Effect of *CTNNB1* knockdown on proliferation (*, $p<0.05$).

Supplemental Figure 3.



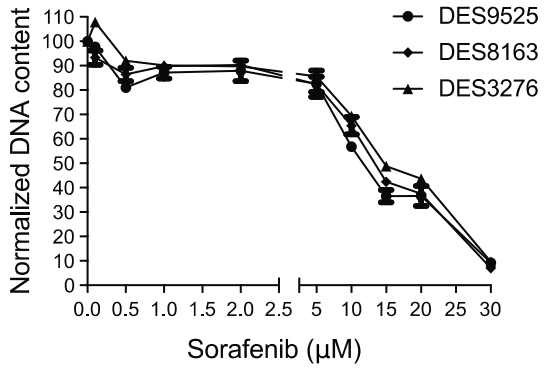
Supplemental Figure 3. VEGFR2 phosphorylation is associated with desmoid cell-induced tube formation in HUVEC cells. (A) Relative rate of tube formation by HUVEC cells co-cultured with DES95252 compared with media alone. Mean of 3 biologic replicates; *, p<0.05. (B) VEGFR2 phosphorylation in HUVEC cell lysates after treatment with conditioned media from mesenchymal stem cells (MSC), desmoid cultures (DES9525, DES3726, and DES8163), or media alone (M Ctrl).

Supplemental Figure 4.



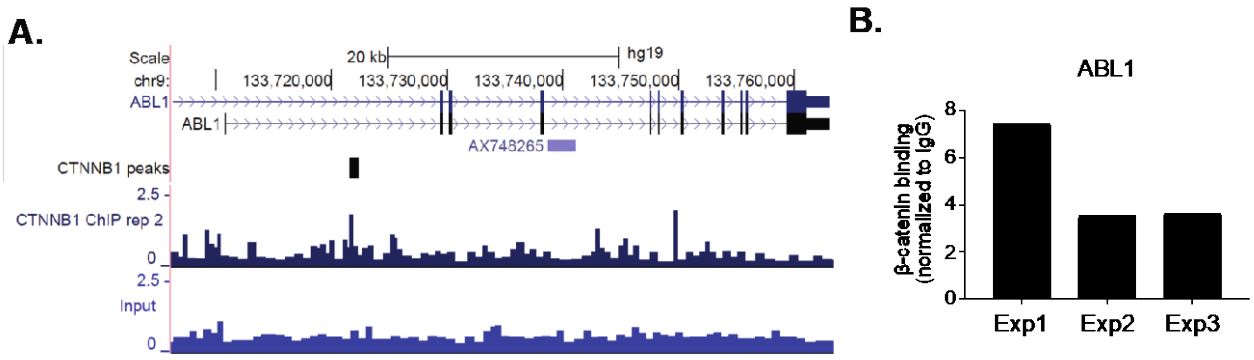
Supplemental Figure 4. (A) *CTNNB1* knockdown reduces and (B) HIF1α overexpression restores VEGF-A secretion by desmoid cells. A shows median of 3 biologic replicates; *, p<0.05. B is a representative experiment. Control for HIF1α overexpression is RFP.

Supplemental Figure 5.



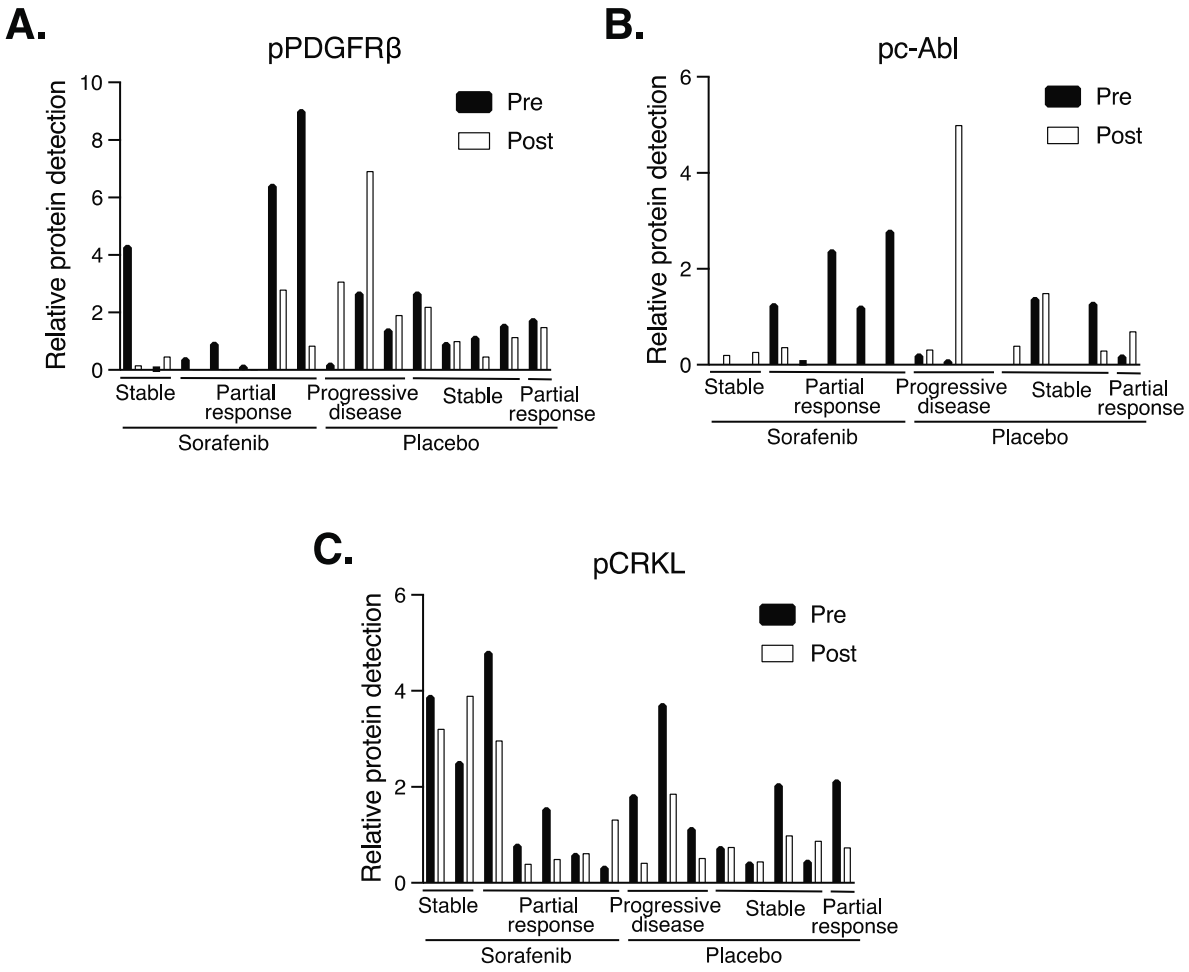
Supplemental Figure 5. Effect of sorafenib on desmoid cell proliferation. Results represent means and standard error of 3 biologic replicates assessed 72 h after addition of drug; data normalized to control (media alone).

Supplemental Figure 6.



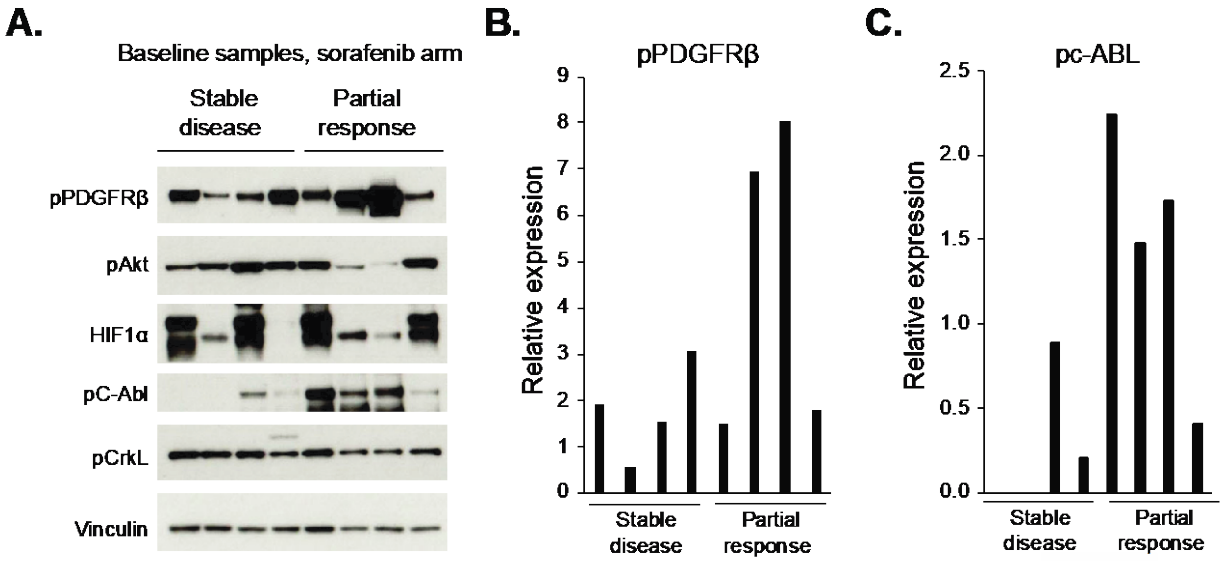
Supplemental Figure 6. β-catenin binds the *ABL1* gene. (A) UCSC genome browser snapshot of normalized ChIP-seq signal profiles for *CTNNB1* with multiple peaks around *ABL1*. (B) Binding of β-catenin to the *ABL1* gene in 3 experiments, normalized to IgG binding.

Supplemental Figure 7.



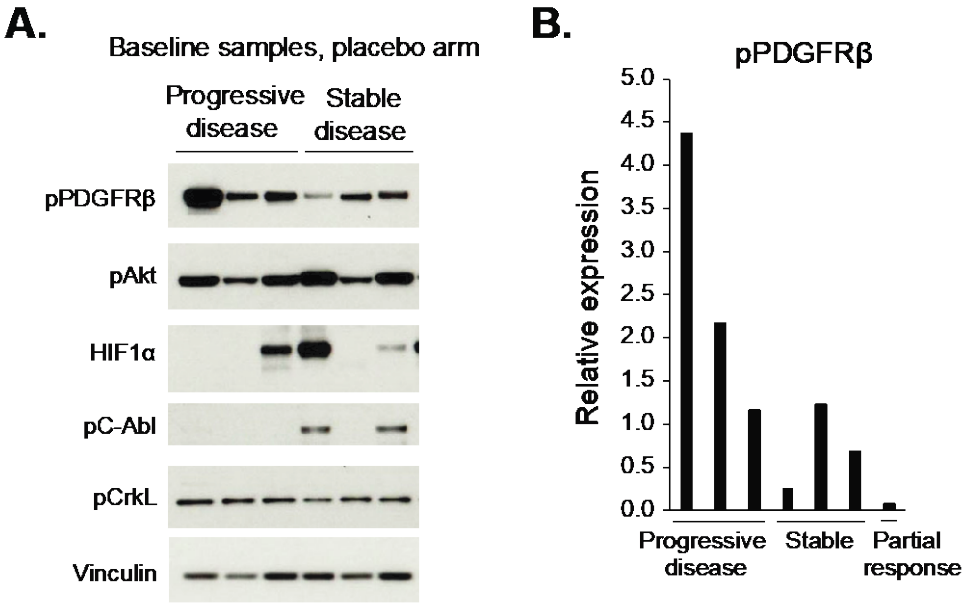
Supplemental Figure 7. Quantitation at baseline (pre) and after (post) treatment of tumor pPDGFRβ, pc-ABL, and pCrkl as assayed by immunoblot and normalized to concurrent vinculin expression.

Supplemental Figure 8.



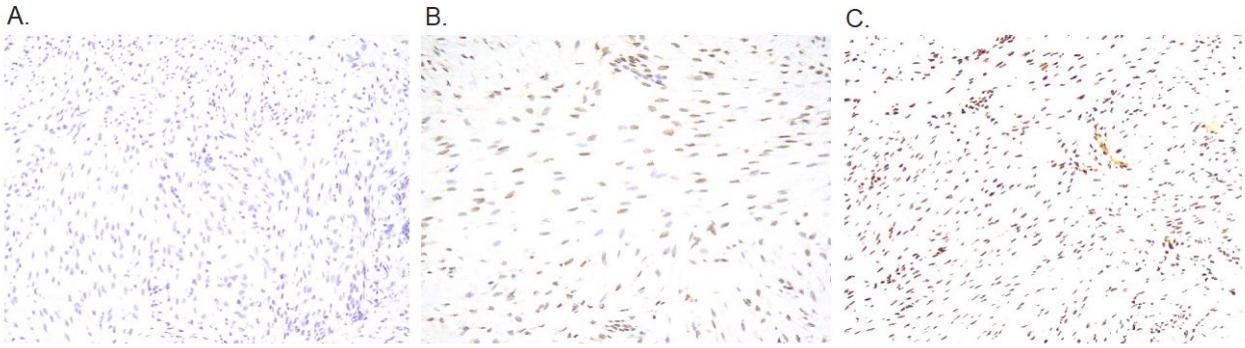
Supplemental Figure 8. Baseline activation of PDGFRβ and c-Abl are non-significantly higher in sorafenib responders compared with patients with stable disease. (A) Immunoblot analysis of baseline biopsies from patients on the sorafenib arm of Alliance AO91105 stratified by best response. (B, C) Quantitation of (B) pPDGFRβ and (C) pc-ABL levels as assayed by immunoblot and normalized to concurrent vinculin expression.

Supplemental Figure 9.



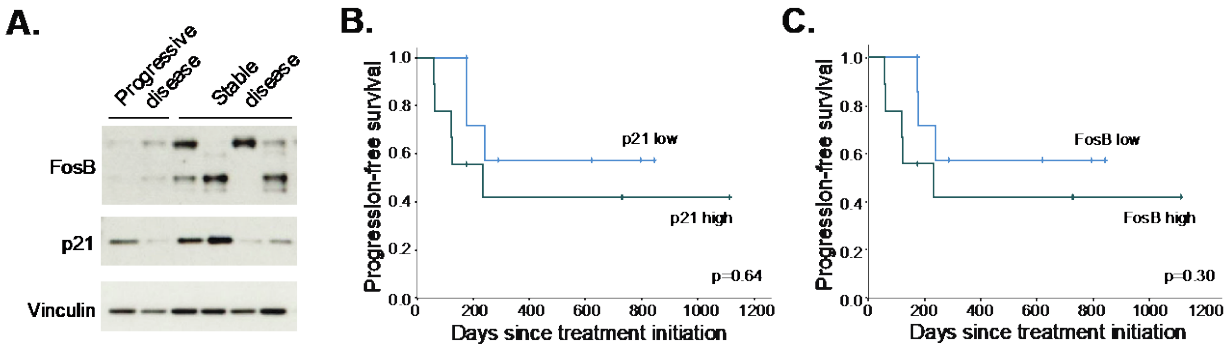
Supplemental Figure 9. Baseline activation of PDGFRβ is non-significantly higher in patients whose disease progressed compared with those with stable disease. (A) Immunoblot analysis of baseline biopsies from patients on the placebo arm of Alliance AO91105 stratified by best response. (B) Quantitation of p-PDGFRβ levels as assayed by immunoblot and normalized to concurrent vinculin expression.

Supplemental Figure 10.



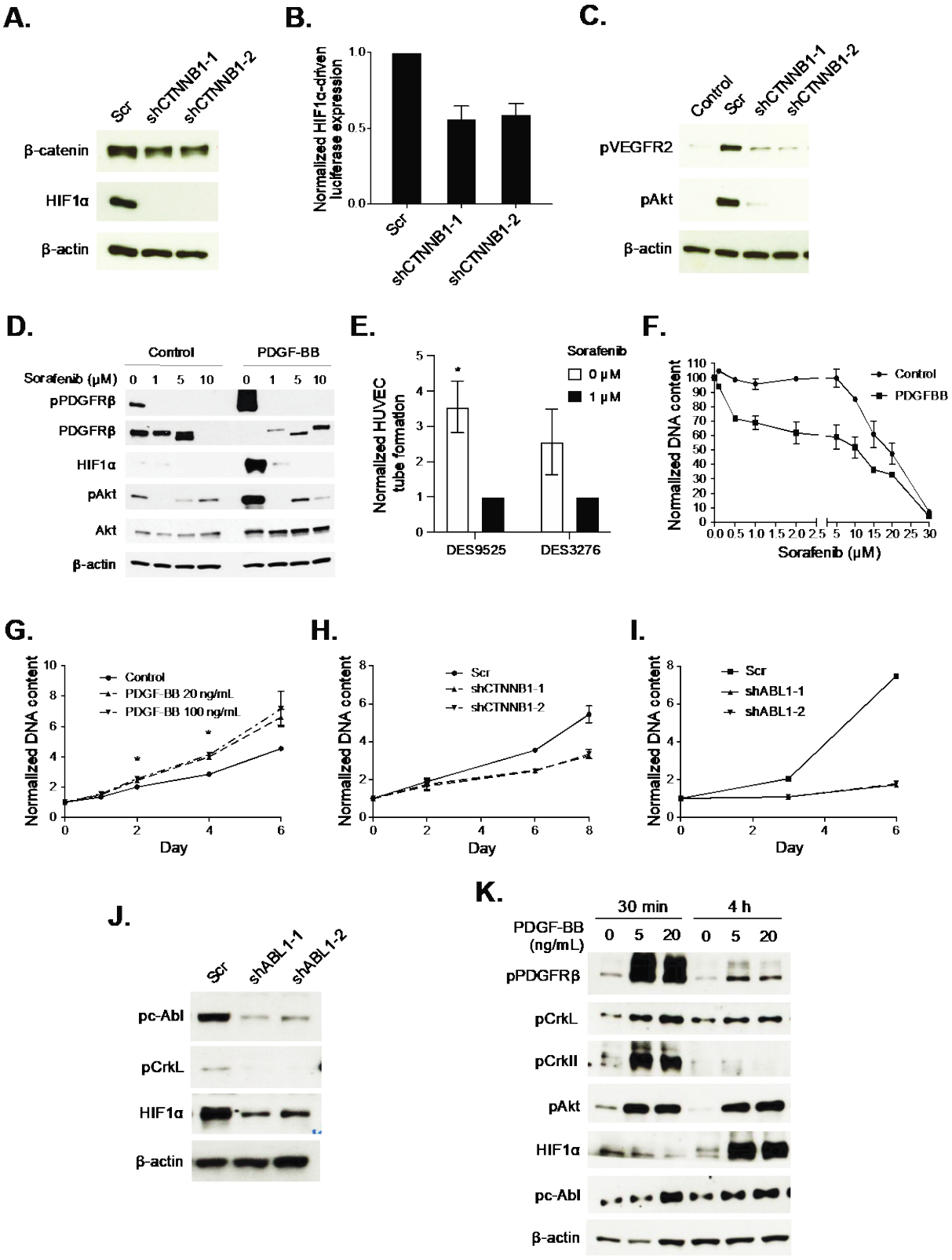
Supplemental Figure 10. Immunohistochemical staining of desmoid cells for nuclear *EGR1*. Representative sections show cells with (A) 0%, (B) ~50%, and (C) >90% staining.

Supplemental Figure 11



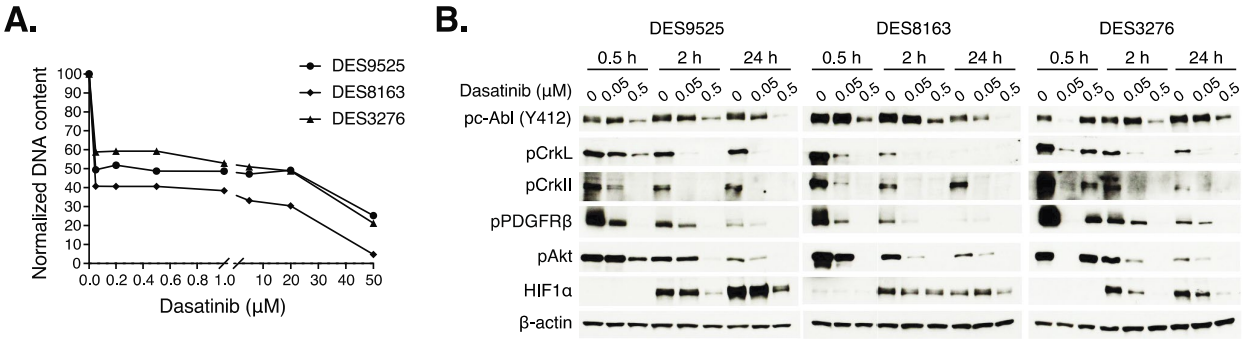
Supplemental Figure 11. Markers of TERT-associated senescence do not correlate with progression risk**.** (A) Expression of senescence markers in baseline biopsies from patients on the placebo arm of A091105 analyzed by immunoblot. Best response as assayed by RECIST criteria is noted. (B-C) Progression-free survival stratified by expression of (B) p21 or (C) FOSB determined by IHC in FFPE samples from a larger cohort (n=17). Low expression, focal/negative or <5%, respectively for p21 and FOSB; high, patchy/positive or ≥5%, respectively.

Supplemental Figure 12.



Supplemental Figure 12. Regulation of PDGFRβ/β-catenin/c-Abl/HIF1α signaling in DES3726 and DES3726T cells, which carry a *CTNNB1* T41A mutation, is similar to that in cells with S45F mutations. Results show data collected in representative experiments. (A-C) Effect of *CTNNB1* knockdown on (A) β-catenin and HIF1α expression, (B) HIF1 α transcriptional activity as assayed by luciferase reporter assay, and (C) VEGFR2 and Akt phosphorylation in HUVEC cells treated with desmoid-conditioned media. (D) Effect of PDGF-BB treatment on PDGFRβ and Akt phosphorylation and HIF1α expression. (E) Effect of sorafenib on desmoid co-culture-induced HUVEC tube formation. (F-G) Effect of PDGF-BB on (F) concentrations of sorafenib needed to inhibit proliferation and (G) proliferation as assessed by DNA content of desmoid cultures. (H-I) Effect of knockdown of (H) *CTNNB1* or (I) *ABL1* on proliferation. (J) Effect of *ABL1* knockdown on Crkl activation and HIF1α expression as assessed by immunoblot. (K) Effect of PDGF-BB on c-ABL activity as assessed by phosphorylation of c-ABL, CrkL and CrkII on immunoblot.

Supplemental Figure 13.



Supplemental Figure 13. Dual PDGFRβ/Src inhibitor dasatinib inhibits, at nanomolar concentrations (A) proliferation of desmoid cells as assessed by DNA content of cultures and (B) c-ABL activity assessed by immunoblot of pc-ABL, pCrkL, and pCrkII.