Supporting Information

Tarn *et al*. 10.1073/pnas.0803383105

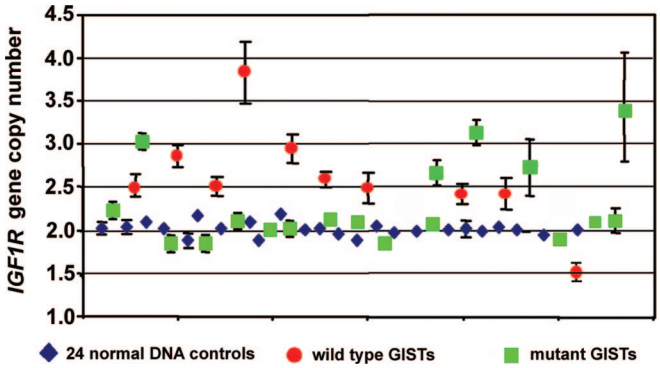

Fig. S1. *IGF1R* gene amplification in gastrointestinal stromal tumor (GIST) biopsies. Genomic quantitative PCR (qPCR) analyses of *IGF1R* gene copy in 24 normal samples (blue symbols), 16 mutant clinical GIST samples, and 2 mutant GIST cell lines (green symbols), and in 10 WT GISTs (red symbols) are shown. qRT-PCR for detecting *IGF1R* gene amplification was carried out using custom-designed primers and Fam-labeled probe, which results in an 80-bp PCR product. Normal blood DNAs were either made into 2-fold serial dilutions and used to generate standard curves for IGF1R or RNase P or used as normal sample controls. Gene copy number was calculated as gene dose according to the linear regression curves derived from standard curves for each gene.

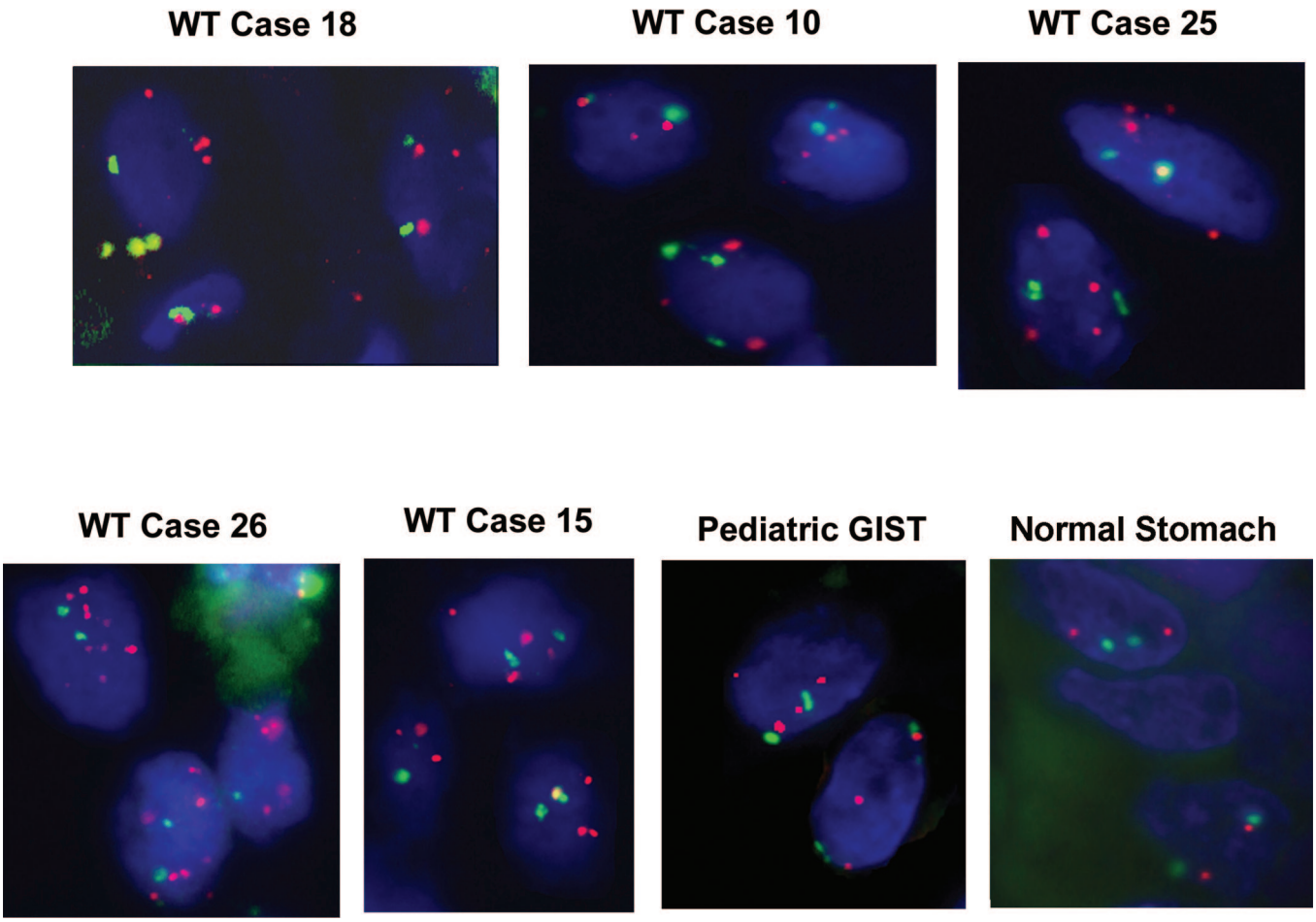

Fig. S2. *IGF1R* gene amplification in GIST biopsies. FISH results of five WT GISTs and a pediatric GIST showing *IGF1R* amplification. Normal stomach shows two *IGF1R* copies. Green, *CEP 15* signals; red, *IGF1R* signals. Five-micrometer paraffin-embedded tumor tissue sections were cohybridized with the CEP15 SpectrumGreen probe and the labeled (SpectrumOrange dUTP) RP11-654A15 BAC probe encompassing the *IGF1R* gene. Slides were counterstained with DAPI, and cells were observed using a fluorescence microscope. Images were captured using a CCD camera operated by a Metasystems ISIS FISH imaging system. Quantification of the *IGF1R* and *CEP 15* signals was performed with hematoxylin and eosin staining, and 200 cells were scanned for each slide. Increased copy number was defined as the presence of three or more signals per nucleus.

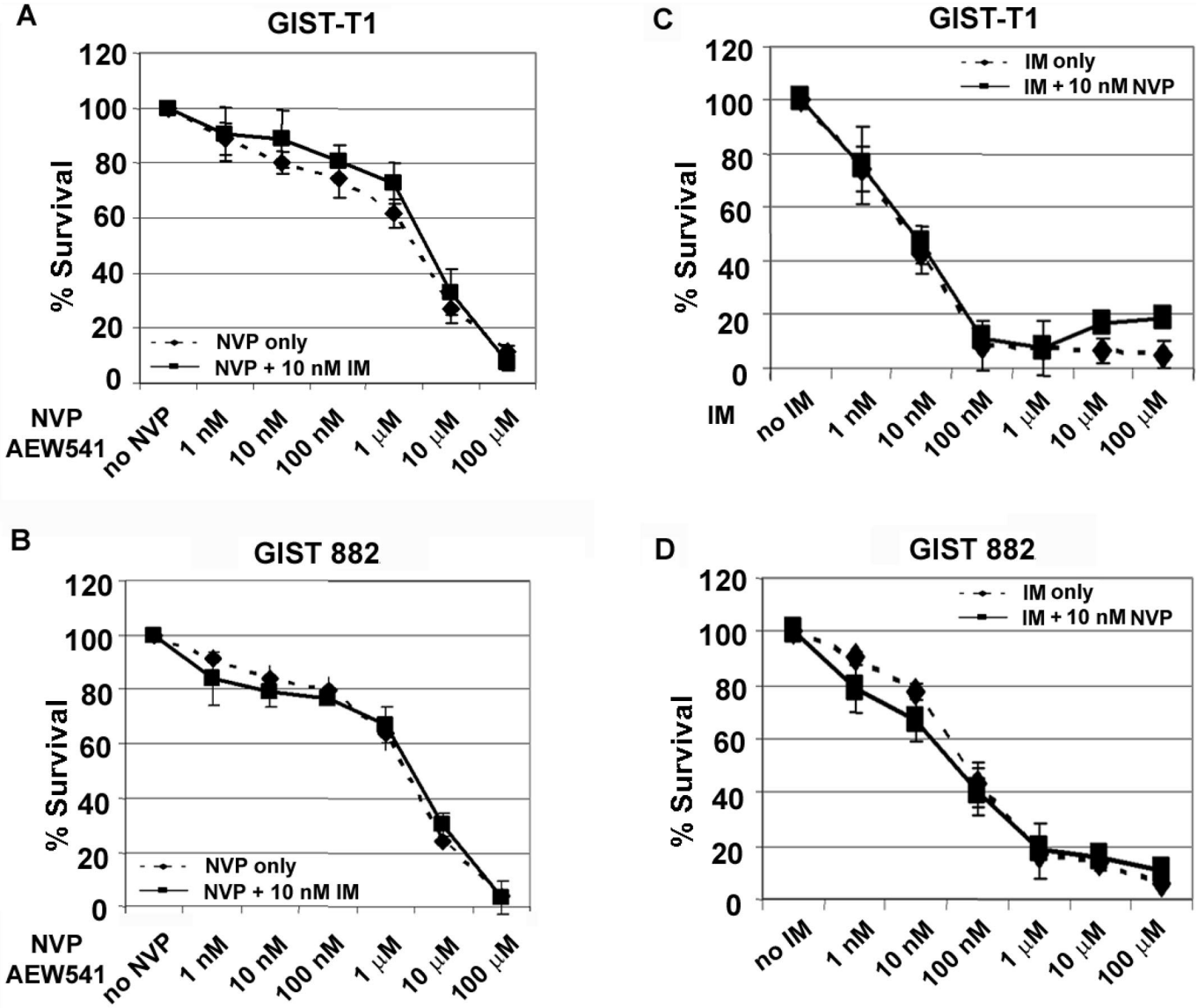

Fig. S3. Cell proliferation/viability assays. (*A* and *B*) GIST-T1 and GIST 882 cells were treated with NVP-AEW541 at the indicated concentrations for 72 h followed by WST-1 assays to measure cell viability. Dotted lines and solid lines represent cells treated with NVP alone or treated with NVP plus 10 nM imatinib, respectively. (*C* and *D*) GIST-T1 and GIST 882 cells were treated with imatinib at the indicated concentrations for 72 h followed by WST-1 assays. Dotted lines and solid lines represent cells treated with imatinib alone or treated with imatinib plus 10 nM NVP-AEW541, respectively.

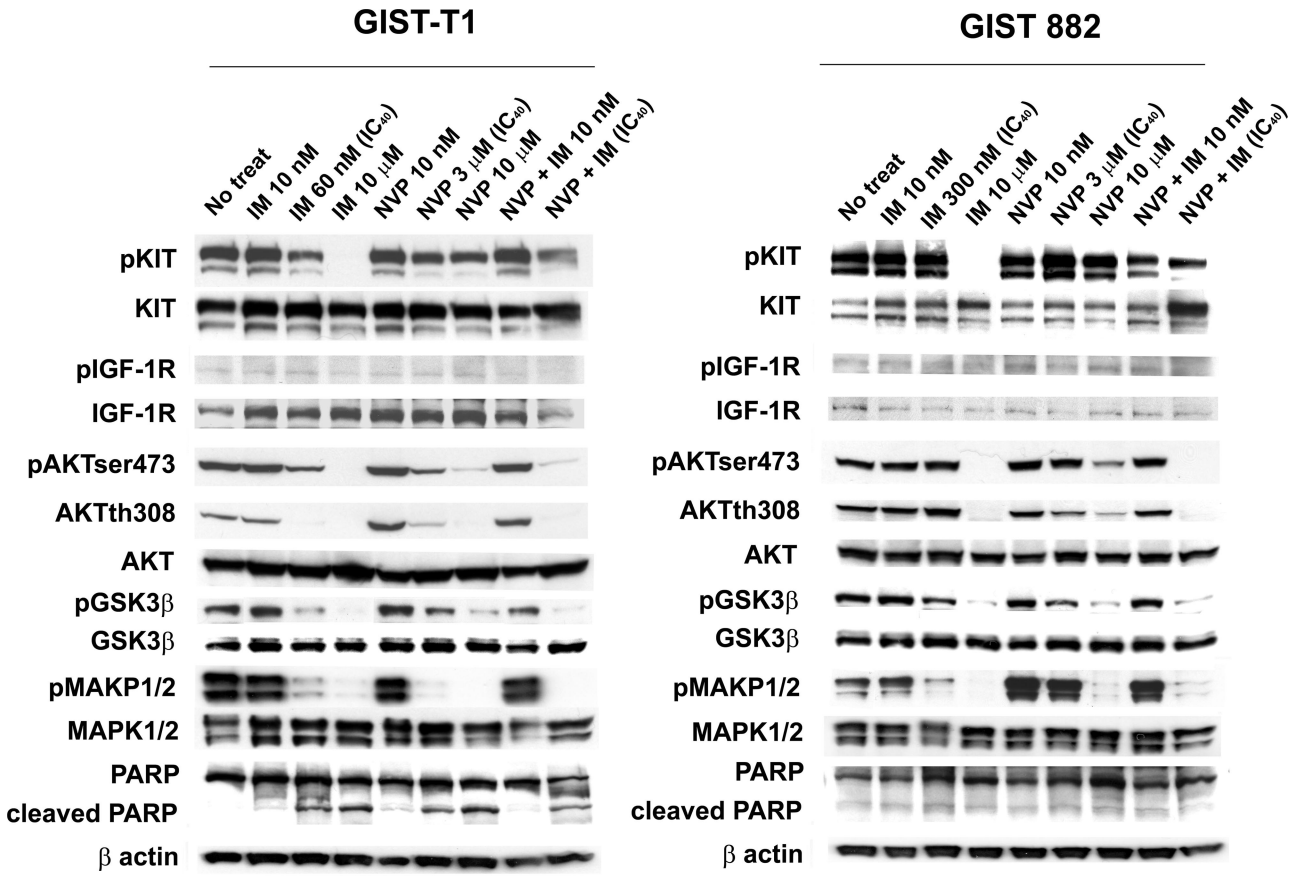

Fig. S4. NVP-AEW541 and imatinib have additive effects on IGF1R and KIT signaling. GIST-T1 cells and GIST 882 cells were treated with 10 nM, IC_{40}, or 10 μM imatinib or NVP-AEW541; with 10 nM each NVP-AEW541 and imatinib; or with NVP-AEW541 and imatinib at IC_{40} concentrations for 6 h. Equal amounts (40 μg) of WCE from each sample were subjected to immunoblotting using specific antibodies as indicated.

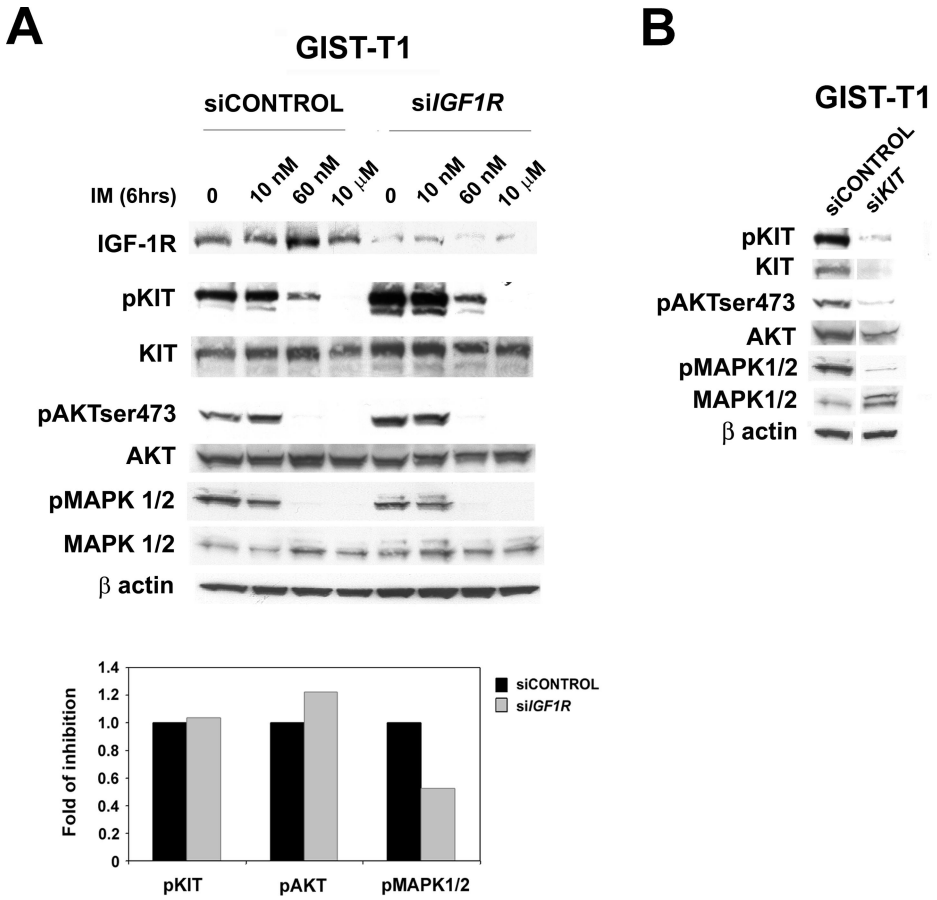

Fig. S5. siRNA knockdown of IGF1R and KIT. (*A* and *B*) GIST-T1 cells were transfected with siRNA-control, siRNA-KIT, or siRNA-IGF1R for 48-h harvest time followed by immunoblotting with the indicated antibodies. Quantification of the inhibition of downstream effectors in siRNA-control vs. siRNA-IGF1R was measured by densitometry and is represented in bar graphs.

Table S1. IGF-1R gene copy numbers and expression levels in GISTs

Case no.	IGF-1R copy no.	IGF-1R copy no. (SNP)	Score IGF1R	Score KIT
WT				
10	2.9	2.4	3	3
15	3	3.3	2	3
18	2.5	3.4	3	3
24	1.5	NA*	2	2
25	2.5	2.9	3	3
26	3.8	2.9	2	2
27	NA	NA	3	3
28	NA	NA	2	3
Pediatric	NA	NA	3	3
Mutant				
1	1.9	2.18	2	3
3	NA	NA	2	3
4	2.0	2.78	1	3
5	3	3.04	1	3
6	2.3	NA	0	3
7	2.1	2.16	1	0
8	2.1	2.6	2	3
9†	3.4	3.04	2	3
11	2.1	NA	2	3
13	2	NA	1	1
14	2.1	NA	1	3
16	2.1	NA	2	3
19	2.7	3.1	1	3
20	3.1	3.26	1	3
22	2.7	2.46	2	3
23	NA	NA	1	2

*NA, not applicable.
†Sample with *PFDGR*α mutation.

Table S2. FISH results on WT GIST samples

Case no.						
WT GIST 18						
IGF1R signals per nucleus	2	3	4			
% cells	56	40	4			
WT GIST 10						
IGF1R signals per nucleus	2	3	4			
% cells	57	39	4			
WT GIST 25						
IGF1R signals per nucleus	2	3	4	5		
% cells	47	35	15	3		
WT GIST 26						
IGF1R signals per nucleus	2	3	4	5	6	8
% cells	50	18	26	2	2	2
WT GIST 15						
IGF1R signals per nucleus	2	3	4			
% cells	55	36	9			
Pediatric GIST						
IGF1R signals per nucleus	2	3	4	5		
% cells	48	37	11	4		

Table S3. pAKT, pmTOR, pS6 expression levels and clinicopathological features of GISTs

Case no.	Score pAKT	Score pmTOR	Score pS6	Mutational status	Site	Risk	Follow-up	Age/gender
WT								
10	1	2	3	WT	Stomach	High	NED	33/F
15	2	1	2	WT	Stomach	High	LTFU, still alive	86/M
18	2	2	2	WT	Stomach	High	2 mets at dx	53/M
24	1	2	2	WT	Small bowel	High	Alive on IM	51/M
25	2	2	2	WT	Rectum	High	Recurred, NED on IM	48/M
26	1	1	2	WT	Small bowel	High	Mets site	48/M
27	NA*	NA	NA	WT	Multiple sites	High	NA	45/F
28	NA	NA	NA	WT	Small bowel	High	NA	57F
Pediatric Mutant	NA	NA	NA	WT	Stomach	High	NA	12/F
1	2	2	2	9: 74267ins6	Abdominal wall	Int	DOD	43/M
3	3	2	3	11: V559D	Stomach	Very low	NED	56/F
4	1	2	1	11: V560D	1° Stomach, intraabdom.	High	DOD	77/M
5	1	1	1	11: 75684del6	Rectum	Int	NED	63/M
6	0	0	2	11: 75688del3	Small bowel	High	DOD	66/F
7	2	1	2	13: K642E	Small bowel	High	NED	47/M
8	2	3	2	17: Y823D	Stomach	High	LTFU, still alive	81/F
9†	1	1	1	14: N659Y; 18: Y849C	Stomach	High	DOD	71/M
11	2	1	2	9: 74267ins6	Mes. mets	High	DOD	75/F
13	1	1	2	11: V559D	Recurrent	High	Alive on IM	67/F
14	2	2	2	9: 74267ins6	Small bowel	High	DOU	78/M
16	2	2	2	11: delW557_K558	Stomach	Low	NED	71/F
19	3	2	2	11: V559G	Stomach	High	NED	70/F
20	1	0	1	11: 75668del6	Small bowel	Low	NED	66/F
22	1	1	1	11: 75662del24, 75686ins 6	Omentum/ retrop	High	DOD	37/M
23	0	2	2	11: 75686del6	Colon	High	NED	72/F

NED, no evidence of disease; DOD, dead of disease; IM, imatinib mesylate; LTFU, lost of follow-up; DOU, dead of unknown cause.
*NA, not applicable.
†Sample with *PFDGR*α mutation.

Table S4. Primers used for *IGF1R* mutational analysis

Exon	Forward primer	Reverse primer
15	5′- TGAAACTGTTGTAGCGAAGATGAA-3′	5′- CCCAAATTAGCAACCCTCCTGAAA-3′
16	5′- CTGTACCTGCTTTAATTACGG-3′	5′- CTCTCCCTGTGCTGCATTTT-3′
17	5′- GCCGCAGCACCACAGAGACA-3′	5′- TGCAGGGAGATTATAAAGGAAAAG-3′
18	5′- TGATATGCAAACCTCGAAAGAAAT-3′	5′- CTAATGCCAACAAAGTCCTCAAAA-3′
19	5′- TGCTCCAGCGTGTGACTCT-3′	5′- GCTAAAGCTGGCAACGGGTAA-3′
20	5′-TTTCCAAGCTCCTCACAG-3′	5′-GAATGGCTTTAATCTCCTA-3′