Supplemental Material to:

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Biochemical and functional analyses of gp130 mutants unveil JAK1 as a novel therapeutic target in human inflammatory hepatocellular adenoma

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Table S1

n°ID	Gender	age	Diagnosis	IL6ST (gp130) nucleotide change	IL6ST (gp130) amino acid change	CTNNB1 exon 3 Mutation Nc; AA
CHC555T*	F	41	IHCA	c.503_580del	p.Lys168_Asn193del	NM
CHC691T*	F	35	IHCA	c.518_532del	p.Lys173_Asp177del	NM
CHC1342T	F	40	IHCA	c.518_532del	p.Lys173_Asp177del	NM
CHC786T*	M	61	bIHCA	c.551_559del	p.Val184_Ser187delinsAla	c.13+9_399del; p.?
CHC756T	F	48	bIHCA	c.552_566del	p.Asp185_Val189del	c.121A>G; p.Thr41Ala
CHC469T*	F	32	bIHCA	c.557_571del	p.Tyr186_Tyr190del	c.100G>A; p.Gly34Arg
CHC470T*	F	32	bIHCA	c.557_571del	p.Tyr186_Tyr190del	c.100G>A; p.Gly34Arg
CHC528T	M	39	IHCA	c.557_571del	p.Tyr186_Tyr190del	NM
CHC769T	F	34	IHCA	c.557_571del	p.Tyr186_Tyr190del	NM
CHC1121T*	F	25	IHCA	c.557_571del	p.Tyr186_Tyr190del	NM
CHC1020T*	F	40	IHCA	c.557_571del	p.Tyr186_Tyr190del	NM
CHC1350T	F	44	IHCA	c.557_571del	p.Tyr186_Tyr190del	NM
CHC1386T	F	44	IHCA	c.557_571del	p.Tyr186_Tyr190del	NM
CHC821T	F	46	IHCA	c.557_571del	p.Tyr186_Tyr190del	NM
CHC955T	F	38	IHCA	c.556_571delinsA	p.Tyr186_Phe191delinsIle	NM
CHC390T*	M	49	IHCA	c.557_574del	p.Tyr186_Val192delinsPhe	NM
CHC344T*	F	39	IHCA	c.560_571del	p.Ser187_Tyr190del	NM
CHC362T*	F	54	IHCA	c.560_571del	p.Ser187_Tyr190del	NM
CHC382T*	F	40	IHCA	c.560_571del	p.Ser187_Tyr190del	NM
CHC466T*	F	50	IHCA	c.560_571del	p.Ser187_Tyr190del	NM
CHC581T	F	51	IHCA	c.560_571del	p.Ser187_Tyr190del	NM
CHC820T	F	46	bIHCA	c.560_571del	p.Ser187_Tyr190del	c.116C>G; p.Ala39Gly
CHC670T*	F	38	IHCA	c.560_571del	p.Ser187_Tyr190del	NM
CHC823T	F	46	bIHCA	c.560_571del	p.Ser187_Tyr190del	c.116C>G; p.Ala39Gly
CHC975T	F	50	IHCA	c.560_571del	p.Ser187_Tyr190del	NM
CHC751T	F	39	IHCA	c.560_571del	p.Ser187_Tyr190del	NM
CHC1434T	F	38	IHCA	c.560_571del	p.Ser187_Tyr190del	NM
CHC1329T	F	40	IHCA	c.560_571del	p.Ser187_Tyr190del	NM
CHC772T	F	34	IHCA	c.562_573del	p.Thr188_Phe191del	NM
CHC745T	F	42	IHCA	c.562_573del	p.Thr188_Phe191del	NM
CHC858T	F	51	IHCA	c.563_574del	p.Thr188_Val192delinsIle	NM
CHC552T*	F	54	IHCA	c.563_574del	p.Thr188_Val192delinsIle	NM
CHC1232T*	F	36	IHCA	c.563_574del	p.Thr188_Val192delinsIle	NM
CHC594T	F	37	IHCA	c.563_574del	p.Thr188_Val192delinsIle	NM
CHC680T	F	41	IHCA	c.563_577del	p.Thr188_Val192del	NM
CHC364T*	F	35	IHCA	c.565_576del	p.Val189_Val192del	NM
CHC377T*	F	42	IHCA	c.565_576del	p.Val189_Val192del	NM
CHC455T	F	27	IHCA	c.565_576del	p.Val189_Val192del	NM
CHC456T	F	27	IHCA	c.565_576del	p.Val189_Val192del	NM
CHC473T	F	37	IHCA	c.564_575del	p.Val189_Val192del	NM
CHC771T*	F	34	IHCA	c.564_575del	p.Val189_Val192del	NM

CHC684T	F	29	IHCA	c.565_576del	p.Val189_Val192del	NM
CHC1131T	F	30	bIHCA	c.564_576delinsA	p.Val189_Val192del	c.121A>G; p.Thr41Ala
CHC1237T	F	41	IHCA	c.565_574delinsT	p.Val189_Val192delinsPhe	NM
CHC536T	F	46	IHCA	c.566_577del	p.Val189_Asn193delinsAsp	c.94G>A; p.Asp32Asn
CHC503T	F	49	IHCA	c.566_580delinsATG	p.Val189_Ile194delinsAspVal	NM
CHC1328T	F	40	IHCA	c.567_578del	p.Tyr190_Asn193del	NM
CHC698T	F	31	IHCA	c.568_579del	p.Tyr190_Asn193del	NM
CHC1331T	F	40	IHCA	c.567_578del	p.Tyr190_Asn193del	NM
CHC973T	F	52	IHCA	c.569_578delinsC	p.Tyr190_Asn193delinsSer	NM
CHC976T	M	63	IHCA	c.570_578del	p.Phe191_Asn193del	NM
CHC1124T*	M	50	bIHCA	c.571_579del	p.Phe191_Asn193del	c.121A>G; p.Thr41Ala
CHC1238T*	F	41	IHCA	c.573_582delinsG	p.Phe191_Ile194delinsLeu	NM
CHC867T*	F	38	IHCA	c.583_588del	p.Glu195_Val196del	NM
CHC481T	F	30	IHCA	c.616_645dup	p.Lys206_Asp215dup	NM
CHC1433T	F	38	IHCA	c.618_641dup	p.Asn213_Phe214ins8	NM
CHC1023T*	M	35	bIHCA	c.646_647ins33	p.Asp215_Pro216ins11	c.121A>G; p.Thr41Ala
CHC1275T	F	40	bIHCA	c.643_645del	p.Asp215del	c.121A>G; p.Thr41Ala
CHC1301T	F	43	IHCA	c.643_645del	p.Asp215del	NM
CHC711T*	F	26	bIHCA	c.643_645del	p.Asp215del	c.121A>G; p.Thr41Ala
CHC754T	M	29	bIHCA	c.647C>A	p.Pro216His	c.69_425del; p.His24_Tyr142del
CHC839T	F	46	IHCA	c.647C>A	p.Pro216His	NM
CHC974T	F	42	bIHCA	c.647C>A	p.Pro216His	c.134C>T; p.Ser45Phe
CHC1123T	F	46	IHCA	c.647C>A	p.Pro216His	NM
CHC979T	M	58	bIHCA	c.646_648del	p.Pro216del	c.97T>C; p.Ser33Pro
CHC1337T	F	46	IHCA	c.1252_1263del	p.Ala418_Phe421del	NM

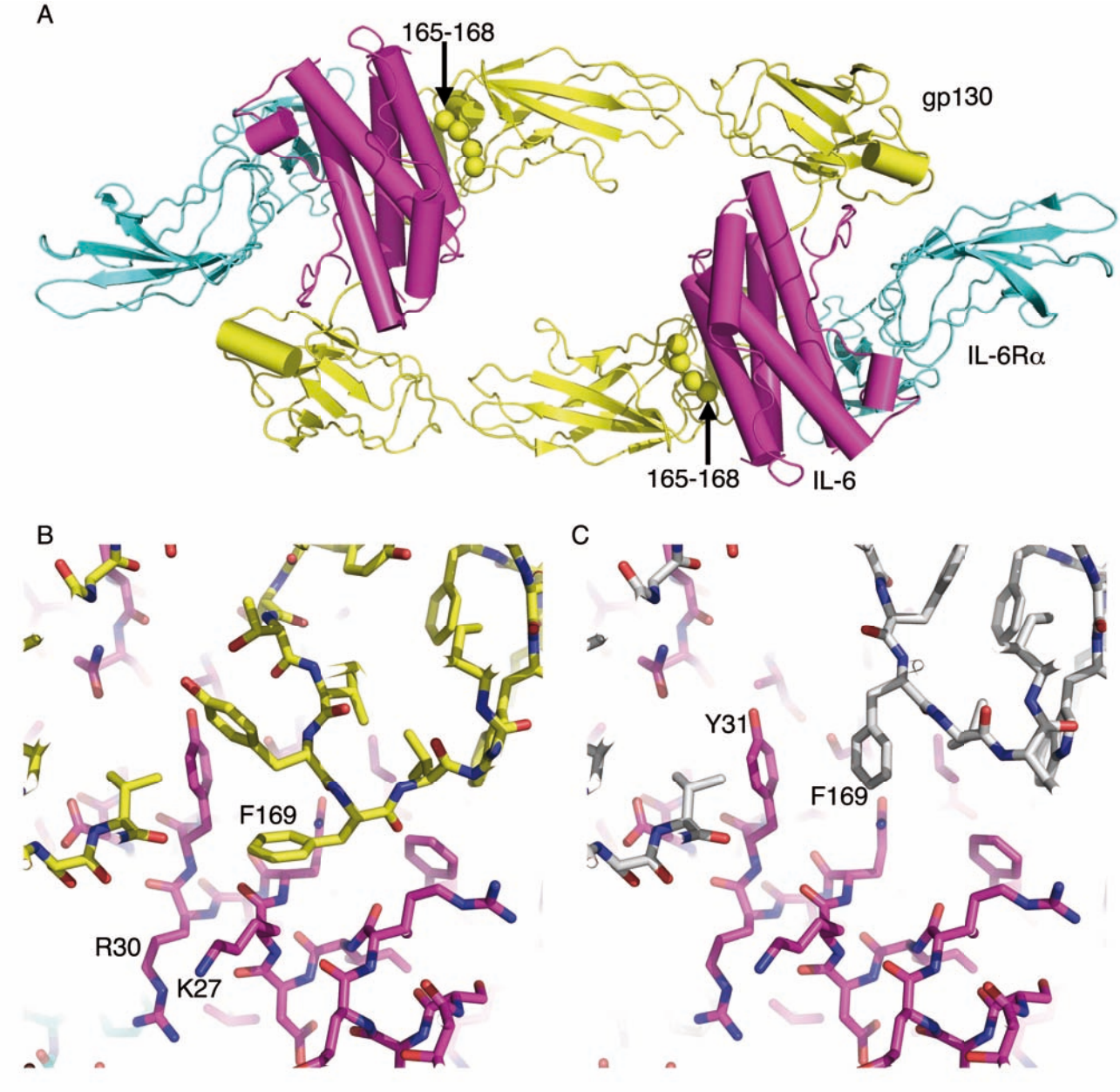
*previously described in Rebouissou et al, Nature 2009

Table S2

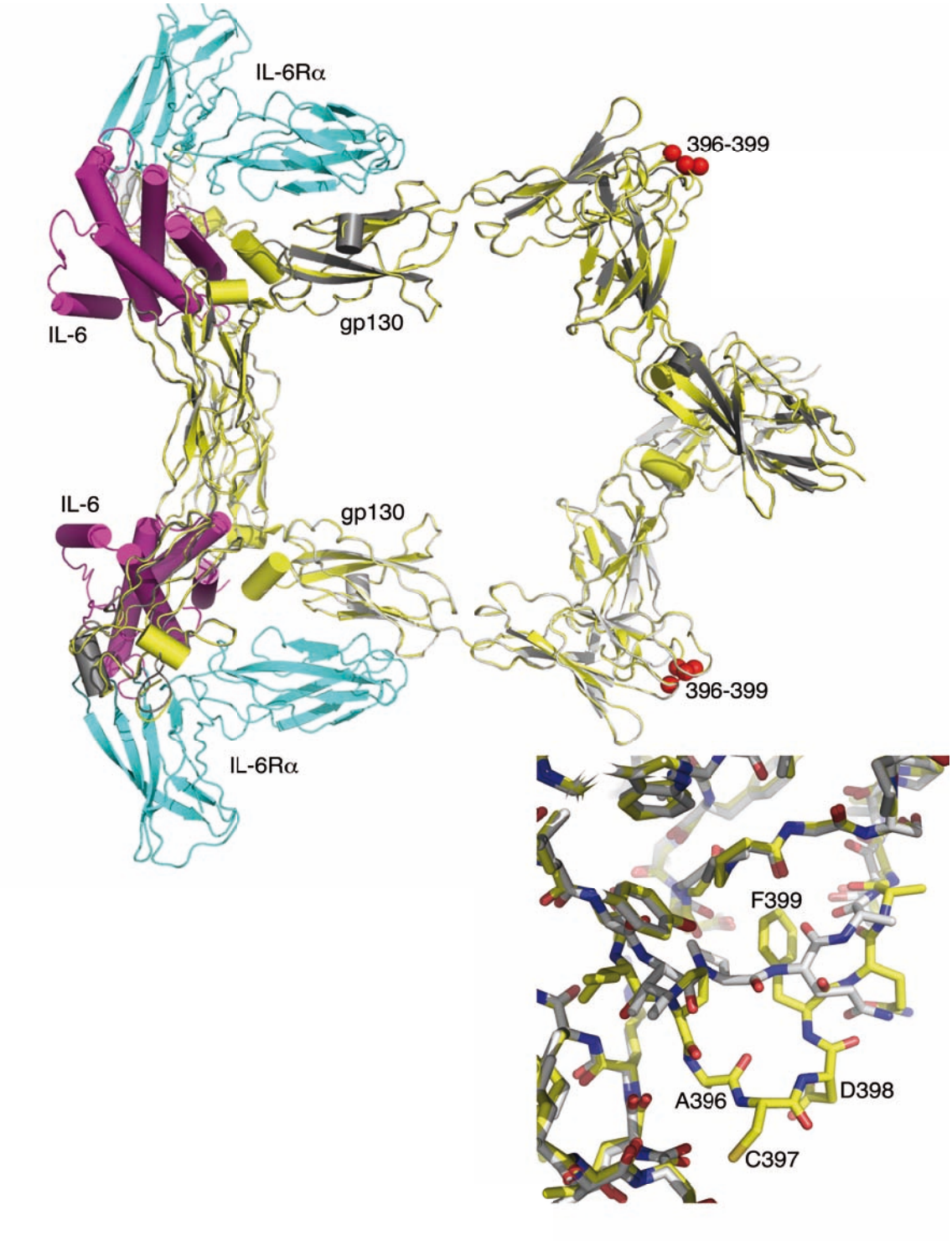
Mutant ID	Localization (cDNA)	Primer sequence (5' To 3')	Primer name
S187	c.559_710del	CCCACCTCATGCACTGTTGATTATTTTGTCAACATTGAAGTCTG	S187 S
		CAGACTTCAATGTTGACAAAATAATCAACAGTGCATGAGGTGGG	S187 AS
Y186	c.556_570 del	CCACCTCATGCACTGTTGATTTTGTCAACATTGAAGTCTG	Y186 S
		CAGACTTCAATGTTGACAAAATCAACAGTGCATGAGGTGG	Y186 AS
V189	c.565_576del	CATGCACTGTTGATTATTCTACTAACATTGAAGTCTGGGTAGAAGC	V189 S
		GCTTCTACCCAGACTTCAATGTTAGTAGAATAATCAACAGTGCATG	V189 AS
K173	c.517_531del	CAAGTTTGCTGATTGCACCCCCACCTCATGCA	K173 S
		TGCATGAGGTGGGGGTGCAATCAGCAAACTTG	K173 AS
P216	c.647C>A	CATCAGATCATATCAATTTTGATCATGTATATAAAGTGAAGCCCAATCC	P216H S
		GGATTGGGCTTCACTTTATATACATGATCAAAATTGATATGATCTGATG	P216H AS
D215	c.643_645del	GGAAGGTTACATCAGATCATATCAATTTTCCTGTATATAAAGTGAAG	D215 S
		CTTCACTTTATATACAGGAAAATTGATATGATCTGATGTAACCTTCC	D215 AS
E195	c.583_588del	TGATTATTCTACTGTGTATTTTGTCAACATTTGGGTAGAAGCAGAGA	E195 S
		TCTCTGCTTCTACCCAAATGTTGACAAAATACACAGTAGAATAATCA	E195 AS
V184	c.550_558del, 560T>A	GACACCCCCACCTCATGCACTGCTACTGTGTATTTTGTCA	V184 S
		TGACAAAATACACAGTAGCATGCATGAGGTGGGGGTGTC	V184 AS
A418	c.1252_1263del	CAGCTGTTTTAACTATCCCTCAAGCTACTCACCCTGTAAT	A418 S
		ATTACAGGGTGAGTAGCTTGAGGGATAGTTAAAACAGCTG	A418 AS
Y759F	c.2276A>T	TCGAGCACTGTCCAGTTTTCTACCGTGGTACAC	Y759F S
		GTGTACCACGGTAGAAAACTGGACAGTGCTCGA	Y759F AS
S782A	c.2344T>G	CTTCTCAAGATCCGAGGCTACCCAGCCCTTGTT	S782A S
		AACAAGGGCTGGGTAGCCTCGGATCTTGAGAAG	S782A AS
L786A_L787A	c.2356T>G, 2357T>C,	CCGAGTCTACCCAGCCCGCGGCAGATTCAGAGGAGCGGC	LL/AA S
(LL/AA)	2359T>G, 2360T>C	GCCGCTCCTCTGAATCTGCCGCGGGCTGGGTAGACTCGG	LL/AA S
Flag tag	c.2752ins	GGCGGCTACATGCCTCAGGATTACAAGGATGACGATGACAAGTGAAGGACTAG TAGTTCC	IL6ST Flag S
		GGAACTACTAGTCCTTCACTTGTCATCGTCATCCTTGTAATCCTGAGGCATGTAG CCGCC	IL6ST Flag AS
Myc tag	c.2752ins	AAGGCGGCTACATGCCTCAGGAACAAAAACTTATTTCTGAAGAAGATCTGTGAA GGACTAGTAGTTCCTG	IL6ST Myc S
		CAGGAACTACTAGTCCTTCACAGATCTTCTTCAGAAATAAGTTTTTGTTCCTGAG GCATGTAGCCGCCTT	IL6ST Myc AS

Table S3

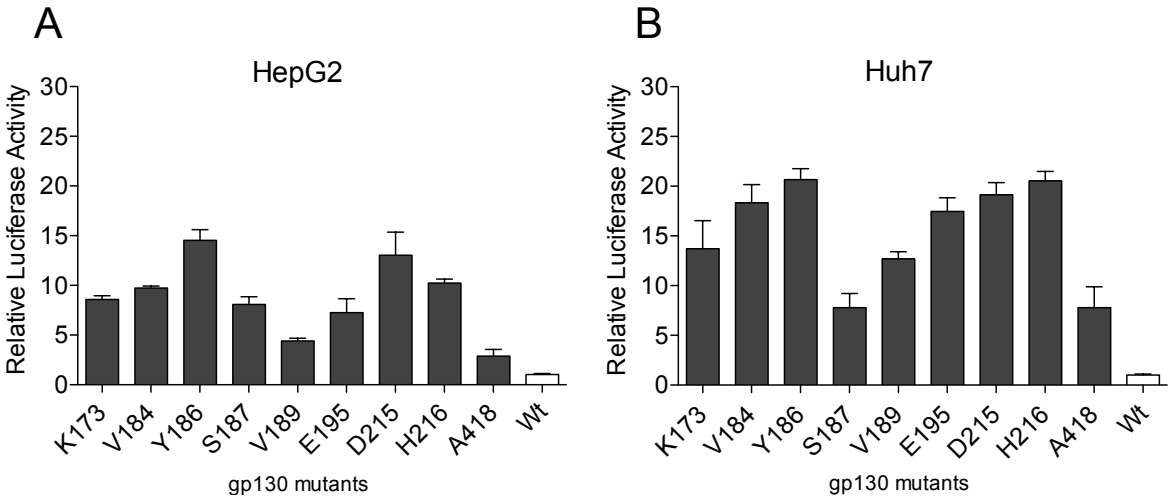
Target gene	si RNA ID	Sequence (5' to 3')	Strand
Human interleukine 6 receptor (hIL6R)	s7316	AGACUACGGUUUGAGCUCATT	sens
		UGAGCUCAAACCGUAGUCUGT	antisens
	s7315	CCACGACUCUGGAAACUAUTT	sens
		AUAGUUUCCAGAGUCGUGGAG	antisens
	s7314	CAACAUGGAUGGUCAAGGATT	sens
		UCCUUGACCAUCCAUGUUGTG	antisens
Human interleukine 6 signal transducer (hIL6ST)	s229006	CACUGUUGAUUAUUCUACUTT	sens
		AGUAGAAUAAUCAACAGUGCA	antisens
	s229007	CUGUUGAUUAUUCUACUGUTT	sens
		ACAGUAGAAUAAUCAACAGTG	antisens
	s229008	UGUUGAUUAUUCUACUGUGTT	sens
		CACAGUAGAAUAAUCAACAGT	antisens
Human Oncostatin M receptor (hOSMR)	s17544	CCAGAUCAGUAGGAUUGAATT	sens
		UUCAAUCCUACUGAUCUGGAT	antisens
	s17542	GUAACUGCAUUCAACUUGATT	sens
		UCAAGUUGAAUGCAGUUACAT	antisens
	s17543	GACUCACAAGAAACCUAUATT	sens
		UAUAGGUUUCUUGUGAGUCTT	antisens
Human Janus kinase 1 (hJAK1)	s7646	CCAUCACCGUUGAUGACAATT	sens
		UUGUCAUCAACGGUGAUGGTG	antisens
	s7647	GUAUUAAGCUCAUCAUGGAtt	sens
		UCCAUGAUGAGCUUAAUACca	antisens
	s7648	GGUGCUGUCUAGGCAAGAAtt	sens
		UUCUUGCCUAGACAGCACCgt	antisens
Human Janus kinase 2 (hJAK2)	s7649	CAAAGAUCCAAGACUAUCAtt	sens
		UGAUAGUCUUGGAUCUUUGct	antisens
	s7650	GGACUGUAUGUACUUCGAUtt	sens
		AUCGAAGUACAUACAGUCCag	antisens
	s7651	CCAGCGGAAUUUAUGCGUAtt	sens
		UACGCAUAAAUUCCGCUGGtg	antisens
Human Tyrosine kinase 2 (hTyk2)	s14535	GAUGCUAUAUUUCCGCAUAtt	sens
		UAUGCGGAAAUAUAGCAUCag	antisens
	s14536	CAUCCACAUUGCACAUAAAtt	sens
		UUUAUGUGCAAUGUGGAUGca	antisens
	s14537	GGAGUAUAAGUUCUACUAUtt	sens
		AUAGUAGAACUUAUACUCCtt	antisens



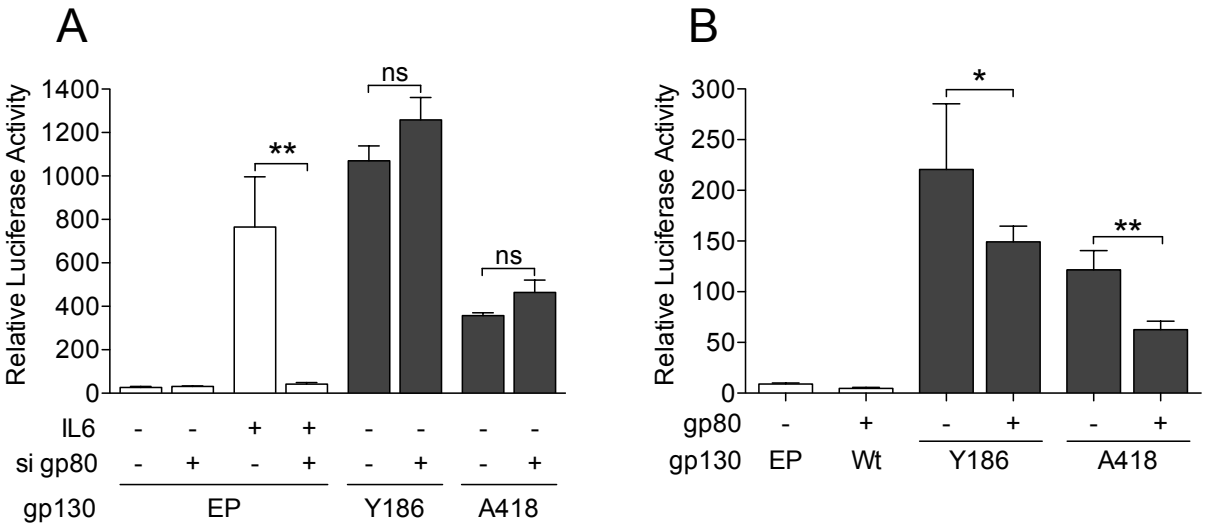
Supplementary Figure S1. Position of the mutations in D2 domain according to the hexameric IL-6/IL-6R/gp130 structure. **A**. Structure (PDB entry 1p9m) of the hexameric IL-6 (both molecules within the hexamer are shown in magenta) bound to IL6-Rα (cyan) in complex with gp130 (yellow). The arrow points to the gp130 residue 168; Cα positions of gp130 residues 165-168 are indicated by yellow spheres. **B.** Close-up view of IL-6 (magenta) interactions with gp130 (yellow). **C.** Close-up view of IL-6 (magenta) interactions with an energy minimized model of gp130Δ165-168 (gray). IL-6 is in the same orientation as shown in panel (B). The numbering of residues corresponds to the *IL6ST* complementary DNA, which has 22 additional amino-terminal residues compared with the polypeptide chain due to the peptide signal.



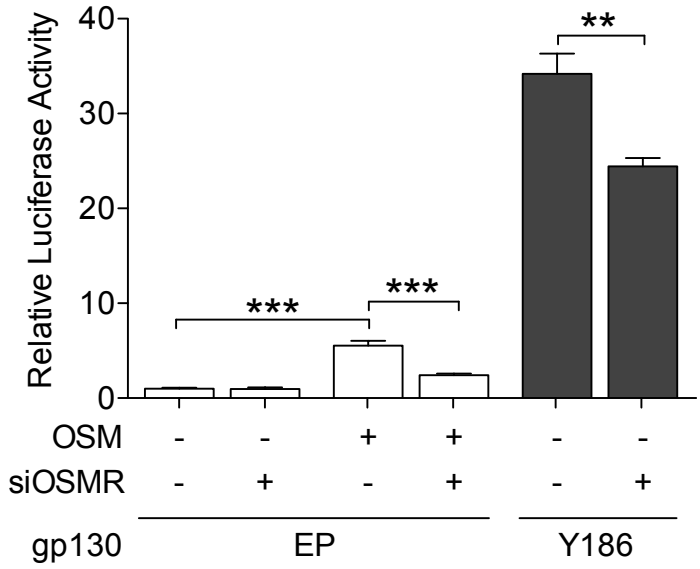
Supplementary Figure S2. Mutations in the structure of the entire extracellular gp130 domain. Superposition of the IL-6/IL6-Rα/gp130 hexamer (PDB entry 1p9m) onto the structure of the entire extracellular gp130 domains (PDB entry 3l5h) and onto our energy-minimized model of gp130 deletion of residues 396-399. IL-6, magenta; IL6-Rα, cyan; gp130, yellow; gp130 396-399 deletion, grey. The gp130 deleted residues 396-399 are indicated by red spheres for their Cα positions in the main Figure and are labeled in the Figure inset. The numbering of residues corresponds to the *IL6ST* complementary DNA, which has 22 additional amino-terminal residues compared with the polypeptide chain due to the peptide signal.



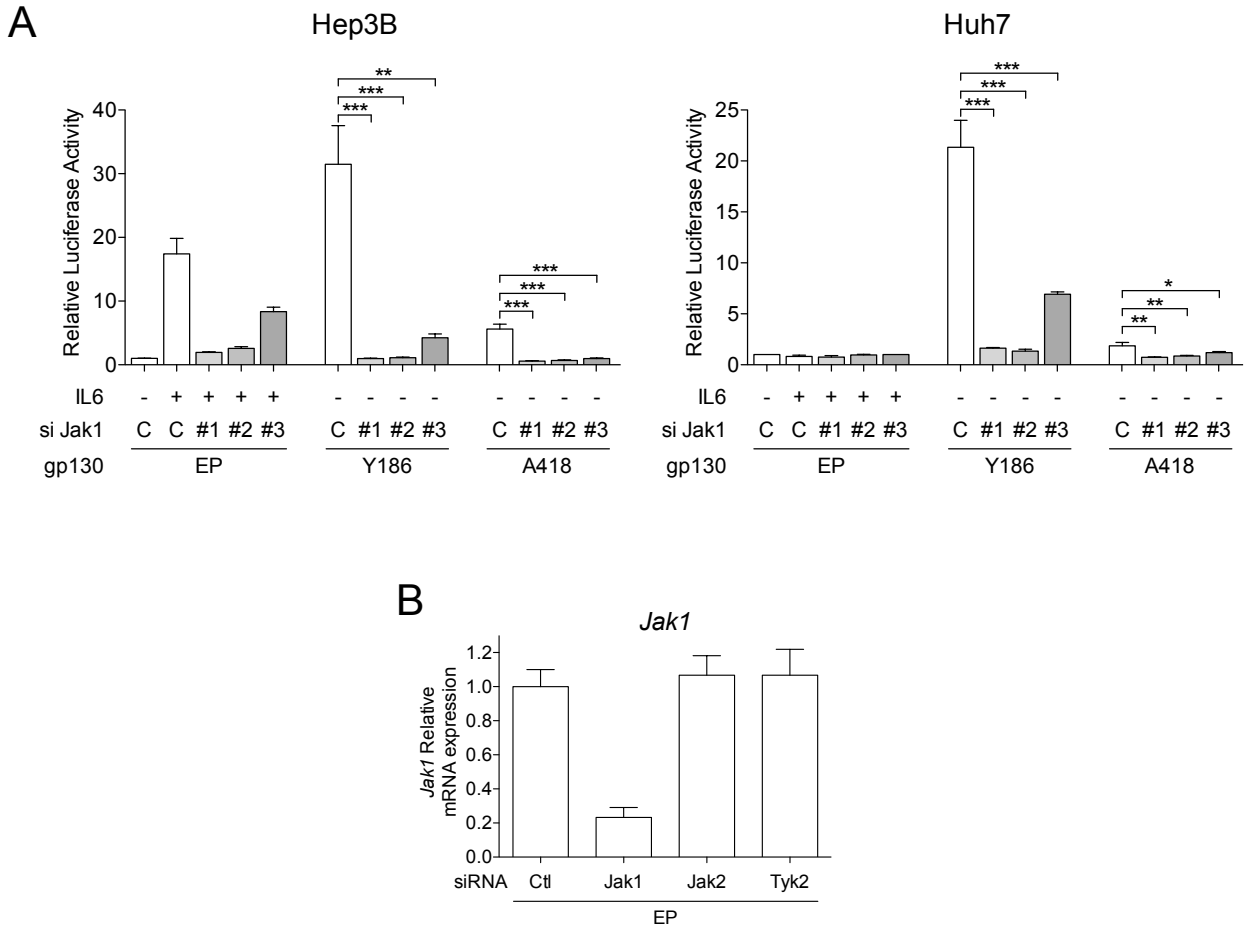
Supplementary Figure S3. Activity of IHCA gp130 mutants in hepatocellular cells. Expression vectors harboring nine different gp130 mutants identified in IHCA or wild type gp130 (Wt) were transfected into HepG2 (**A**) or Huh7 (**B**) cells expressing a STAT3-driven luciferase (Luc) reporter construct. STAT3 activation was measured after 6 hr of serum starvation. Shown is the Luc activity (mean) determined from triplicate co-transfections (± SD) relative to Wt gp130.



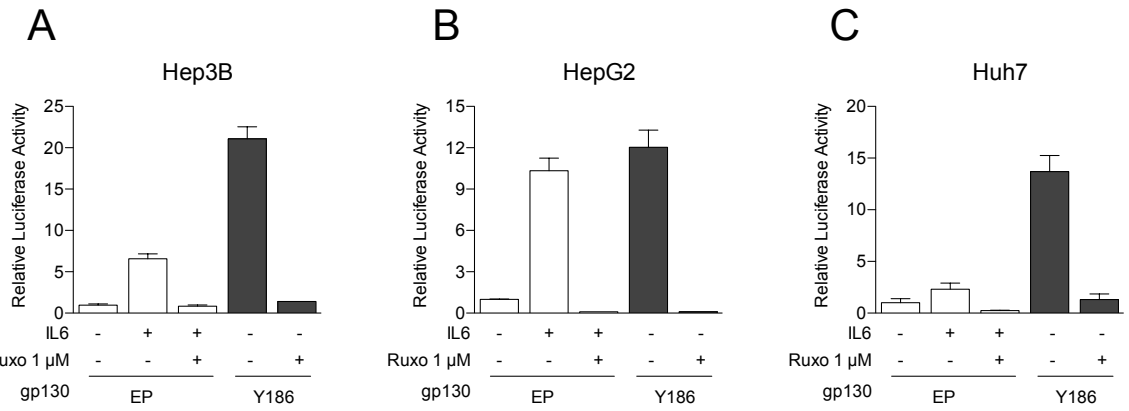
Supplementary Figure S4. Role of gp80 on gp130 mutant activity. **A.** gp130 mutants (Y186 and A418) or empty vector (EP) were co-transfected with gp80 siRNA (+) or control siRNA (-) into Hep3B cells. Results were confirmed with a second gp80 siRNA. **B.** gp130 mutants (Y186 and A418), wild type gp130 (Wt) or control expression vectors (EP) were co-transfected with gp80 (+) or control expression vector (-). Activation of STAT3 is shown by Luc activity (mean) determined from triplicate transfections (± SD) relative to pSIEM-luc alone (EP) with serum starvation. Where indicated, the cells were treated for 3 hr with 100 ng/ml IL6. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, two-tailed Student's *t* test.



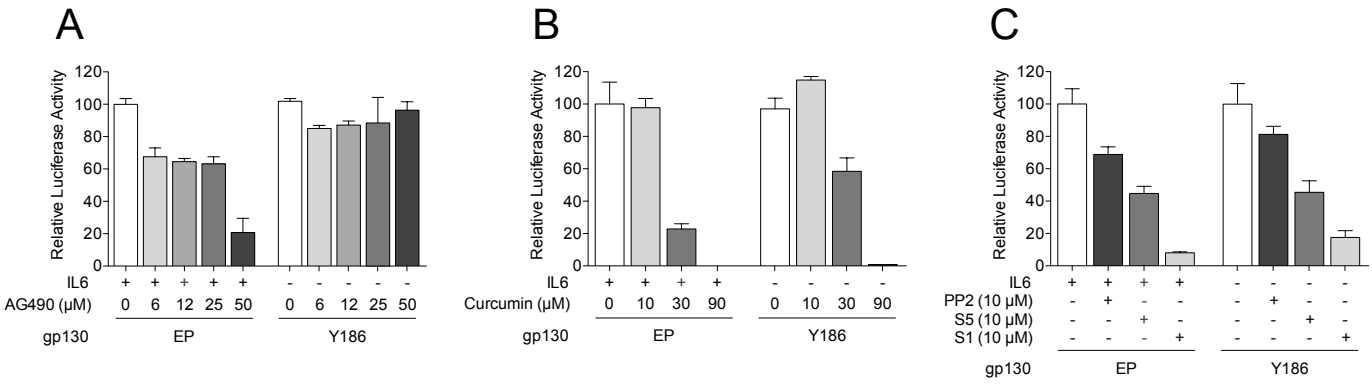
Supplementary Figure S5. Role of OSMR on gp130 mutant activity in Huh7 hepatocellular cells. Knockdown of OSMR in Huh7 cells impairs STAT3 activity induced by the Y186 active gp130 mutant or OSM treatment (100 ng/ml). The data show STAT3-luciferase activity (Luc) relative to cells transfected with the control pSIEM-Luc reporter alone (EP) ± SD (transfections in triplicate). **, $p < 0.01$; ***, $p < 0.001$, two-tailed Student's *t* test.



Supplementary Figure S6. Role of JAK1 on gp130 mutants activation in two additional cell lines. **A.** Hep3B (*left*) or Huh7 (*right*) cells were co-transfected with gp130 mutants (Y186 and A418) or control expression vector (EP) and with three different JAK1 siRNAs or control siRNA (C, white bars). Activation of STAT3 is shown by STAT3-Luc activity (mean) determined from triplicate transfections (± SD) relative to control-Luc (pSIEM-Luc, EP) following serum starvation. **B.** Knockdown efficiency by JAK1 siRNA and specificity of Jak2 and Tyk2 siRNA. Hep3B cells were co-transfected with empty vector (EP) and either control (Ctl), Jak1, Jak2 or Tyk2 siRNA. Shown is the endogenous Jak1 mRNA expression determined from triplicate transfections (± SD) relative to EP with serum starvation. Remaining Jak1 expression in Jak1 siRNA transfected cells was <25% of control. Where indicated, cells were treated for 3 hr with 100 ng/ml IL6. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, two-tailed Student's *t* test.



Supplementary Figure S7. Effect of Ruxolitinib on gp130 mutant activation in hepatocellular cell lines. Hep3B (**A**), HepG2 (**B**) or Huh7 (**C**) cells expressing gp130 mutant (Y186) or control expression vector (EP) were treated with Ruxolitinib (Ruxo; 1 µM, 16 hr) and IL6 (100 ng/ml, 3 hr). Activation of the STAT3 Luc reporter was determined from triplicate transfections (± SD) relative to Hep3B cells transfected with the control pSIEM-Luc reporter (EP) following serum starvation.



Supplementary Figure S8. Effect of different JAK/STAT inhibitors on gp130 mutant activity. Hep3B cells transfected with gp130 mutant (Y186) or control plasmid treated with 100 ng/ml of IL6 (EP) were exposed to increasing concentrations of AG490 for 16 hr (**A**), or to increasing concentrations of Curcumin for 9 hr (**B**). The data shows STAT3 Luc reporter activity relative to transfected cells not treated with the inhibitor. **C.** Hep3B cells transfected with gp130 mutant (Y186) or control plasmid treated with 100 ng/ml of IL6 (EP) were treated with 10μM of Src inhibitor 1 (S1), Src inhibitor 5 (S5) or PP2 for 10 hr. The graph plots STAT3 Luc activity relative to the transfected cells not treated with the inhibitor. All results are from triplicate transfections (± SD) relative to cells transfected with control luciferase reporter (pSIEM-luc, EP) with serum starvation.