Supplementary Information

JAK1 truncating mutations in gynecologic cancer define new role of cancer-associated protein tyrosine kinase aberrations

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sTable 1: Complete list of samples with JAK1 mutations in the TCC project

Type	Total case	NS mutation*	NS mutation rate (%)	Truncation*	Truncation rate (%)		Case	NS mutation	Truncation
Brain	79	1	1.2	0					
Breast	427	10	2.3	1	0.2				
Cervix	49	2	4.1	2	4.1	**Combined GYN**	635	53** (8.3%)	36 (5.7%)
Endometrium	200	24	12	19	9.5				
Gynecologic	1	0		0					
Ovary	235	7	3	1	0.4				
Uterus	148	19	12.8	14***	9.5				
Vagina	1	0		0					
Vulva	1	1	100	0					
Esophagus	44	2	4.5	0					
Gallbladder	2	1	50	0					
HEME-CLL	94	2	21.3	0					
Kidney	243	8	3.3	0					
Large bowel	460	22	4.8	8	1.7	**Combined CRC**	539	23 (4.3%)	9 (1.7%)
Small Intestine	7	0		0					
Rectum-anus	72	1	1.4	1	1.5				
Lung	603	14	2.3	2	0.3				
Pancreas	161	4	2.5	1	0.6				
Prostate	52	1	1.9	0					
Skin	209	10	4.8	1	0.4				
Soft tissue	45	2	4.4	0					
Stomach	55	4	7.3	2	3.6				
Adrenal	1	0							
Ampulla Of Vater	1	0							
Bladder	6	0							
Bone	2	0							
HEME-AML	36	0							
Larynx	24	0							
Lymph Nodes	2	0							
Mandible	3	0							
Maxilla	2	0							
Mesenteric	3	0							
Nose	2	0							
Oral Cavity	30	0							
Penis-Scrotum	1	0							
Peritoneum	9	0							
Pharynx	3	0							
Pleura	3	0							
Renal Pelvis	5	0							
Retroperitoneum	3	0							
Salivary Gland	7	0							
Spleen	2	0							
Testes	1	0							
Thoracic	1	0							
Thyroid	12	0							
Tongue	5	0							
Tonsils	1	0							
Total	3274	134		52					

* Some tumors have more than 1 mutations. The total number of ns mutations detected is 166.

** The number of truncating mutations detected in GYN cancer is 78.

***All are endometrial adenocarcinoma except 1 case that medical record is not available

sTable 2: JAK1 mutations in CCLE

Protein Change	Chror	Start	End	Sample	NCBI Build	Strand	Variant Classification	Variant	Refe	Tun	Tun	dbS	Method	Oncomap Assays
	1	65300158	65300158	TF1 HAEMATOPOIETIC AND LYMPHOI	37	+	UTR 3	SNP	C	G	G		Hybrid_(	NA
	1	65300162	65300162	JHUEM7 ENDOMETRIUM	37	+	UTR 3	SNP	A	C	C		Hybrid_(	NA
	1	65300199	65300199	SKMEL2 SKIN	37	+	UTR 3	SNP	G	A	A		Hybrid_(	NA
p.R1113H	1	65301110	65301110	639V URINARY TRACT	37	+	Missense Mutation	SNP	C	T	T		Hybrid_(	NA
p.E1051Q	1	65301888	65301888	HT55 LARGE INTESTINE	37	+	Missense Mutation	SNP	C	G	G		Hybrid_(	NA
p.G990_splice	1	65303789	65303789	MFE319 ENDOMETRIUM	37	+	Splice Site SNP	SNP	T	C	C		Hybrid_(	NA
p.K982T	1	65304170	65304170	EM2 HAEMATOPOIETIC AND LYMPHOI	37	+	Missense Mutation	SNP	T	G	G		Hybrid_(	NA
p.P969S	1	65304210	65304210	SJRH30 SOFT TISSUE	37	+	Missense Mutation	SNP	G	A	A		Hybrid_(	NA
p.E966V	1	65304218	65304218	HPBALL HAEMATOPOIETIC AND LYMP	37	+	Missense Mutation	SNP	T	A	A		Hybrid_(	NA
p.G948R	1	65305286	65305286	SW1116 LARGE INTESTINE	37	+	Missense Mutation	SNP	C	T	T		Hybrid_(	NA
p.I943N	1	65305300	65305300	CW2 LARGE INTESTINE	37	+	Missense Mutation	SNP	A	T	T		Hybrid_(	NA
p.K939E	1	65305313	65305313	CAL51 BREAST	37	+	Missense Mutation	SNP	T	C	C		Hybrid_(	NA
p.V938A	1	65305315	65305315	CCK81 LARGE INTESTINE	37	+	Missense Mutation	SNP	A	G	G		Hybrid_(	NA
p.I928T	1	65305345	65305345	KMS28BM HAEMATOPOIETIC AND LYI	37	+	Missense Mutation	SNP	A	G	G		Hybrid_(	NA
p.K924fs	1	65305357	65305357	RL952 ENDOMETRIUM	37	+	Frame Shift Del	Del	T	-	-		Hybrid_(	NA
p.K908T	1	65305405	65305405	HEC251 ENDOMETRIUM	37	+	Missense Mutation	SNP	T	G	G		Hybrid_(	NA
p.A906V	1	65305411	65305411	SNU1040 LARGE INTESTINE	37	+	Missense Mutation	SNP	G	A	A		Hybrid_(	NA
p.R879C	1	65306942	65306942	MOLM6 HAEMATOPOIETIC AND LYMPI	37	+	Missense Mutation	SNP	G	A	A		Hybrid_(	NA
p.K860fs	1	65306997	65306997	HEC265 ENDOMETRIUM	37	+	Frame Shift Del	Del	T	-	-		Hybrid_(	NA
p.K860fs	1	65306997	65306997	RL952 ENDOMETRIUM	37	+	Frame Shift Del	Del	T	-	-		Hybrid_(	NA
p.K860fs	1	65306997	65306997	MFE296 ENDOMETRIUM	37	+	Frame Shift Del	Del	T	-	-		Hybrid_(	NA
p.K860fs	1	65306997	65306997	JHUEM1 ENDOMETRIUM	37	+	Frame Shift Del	Del	T	-	-		Hybrid_(	NA
p.K860fs	1	65306997	65306997	HEC108 ENDOMETRIUM	37	+	Frame Shift Del	Del	T	-	-		Hybrid_(	NA
p.K860fs	1	65306997	65306997	SNU1 STOMACH	37	+	Frame Shift Del	Del	T	-	-		Hybrid_(	NA
p.K860fs	1	65306997	65306997	IGROV1 OVARY	37	+	Frame Shift Del	Del	T	-	-		Hybrid_(	NA
p.K860fs	1	65306997	65306997	CAL51 BREAST	37	+	Frame Shift Del	Del	T	-	-		Hybrid_(	NA
p.K860fs	1	65306997	65306997	ISHIKAWAHERAKLIO02ER ENDOMETR	37	+	Frame Shift Del	Del	T	-	-		Hybrid_(	NA
p.K860fs	1	65306997	65306997	TOV21G OVARY	37	+	Frame Shift Del	Del	T	-	-		Hybrid_(	NA
p.N833S	1	65307190	65307190	HCC95 LUNG	37	+	Missense Mutation	SNP	T	C	C		Hybrid_(	NA
p.L799P	1	65309754	65309754	EN ENDOMETRIUM	37	+	Missense Mutation	SNP	A	G	G		Hybrid_(	NA
p.E765E	1	65309855	65309855	BICR18 UPPER AERODIGESTIVE TRAC	37	+	Synonymous	SNP	C	T	T		Hybrid_(	NA
p.E751K	1	65310437	65310437	HEC251 ENDOMETRIUM	37	+	Missense Mutation	SNP	C	T	T		Hybrid_(	NA
p.R681W	1	65311270	65311270	LS180 LARGE INTESTINE	37	+	Missense Mutation	SNP	G	A	A		Hybrid_(	NA
p.V658F	1	65312347	65312347	OCIM1 HAEMATOPOIETIC AND LYMPH	37	+	Missense Mutation	SNP	C	A	A		Hybrid_(	NA
p.C657R	1	65312350	65312350	EN ENDOMETRIUM	37	+	Missense Mutation	SNP	A	G	G		Hybrid_(	NA
p.Y654F	1	65312358	65312358	HUT78 HAEMATOPOIETIC AND LYMPH	37	+	Missense Mutation	SNP	T	A	A		Hybrid_(	NA
p.G610*	1	65313286	65313286	HEC251 ENDOMETRIUM	37	+	Nonsense Mutation	SNP	C	A	A		Hybrid_(	NA
p.T593M	1	65313336	65313336	OC316 OVARY	37	+	Missense Mutation	SNP	G	A	A		Hybrid_(	NA
p.R577Q	1	65316512	65316512	HEC6 ENDOMETRIUM	37	+	Missense Mutation	SNP	C	T	T		Hybrid_(	NA
p.T533M	1	65321242	65321242	MDAPCA2B PROSTATE	37	+	Missense Mutation	SNP	G	A	A		Hybrid_(	NA
p.H525Y	1	65321267	65321267	NCIH1793 LUNG	37	+	Missense Mutation	SNP	G	A	A		Hybrid_(	NA
p.G511D	1	65321308	65321308	HEC151 ENDOMETRIUM	37	+	Missense Mutation	SNP	C	T	T		Hybrid_(	NA
p.C445_splice	1	65325786	65325786	PK45H PANCREAS	37	+	Splice Site SNP	SNP	A	G	G		Hybrid_(	NA
p.P430fs	1	65325832	65325833	LNCAPCLONEFGC PROSTATE	37	+	Frame Shift Ins	Ins	-	G	G		Hybrid_(	NA
p.P430fs	1	65325832	65325833	MFE319 ENDOMETRIUM	37	+	Frame Shift Ins	Ins	-	G	G		Hybrid_(	NA
p.P430fs	1	65325832	65325833	HEC265 ENDOMETRIUM	37	+	Frame Shift Ins	Ins	-	G	G		Hybrid_(	NA
p.P430fs	1	65325833	65325833	HEC1B ENDOMETRIUM	37	+	Frame Shift Del	Del	G	-	-		Hybrid_(	NA
p.A428P	1	65325840	65325840	CW2 LARGE INTESTINE	37	+	Missense Mutation	SNP	C	G	G		Hybrid_(	NA
p.L423V	1	65325855	65325855	NCIH2066 LUNG	37	+	Missense Mutation	SNP	G	C	C		Hybrid_(	NA
p.S383G	1	65330499	65330499	HCC4006 LUNG	37	+	Missense Mutation	SNP	T	C	C		Hybrid_(	NA
p.P370S	1	65330538	65330538	CHL1 SKIN	37	+	Missense Mutation	SNP	G	A	A		Hybrid_(	NA
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p.R343Q	1	65330618	65330618	SW1417 LARGE INTESTINE	37	+	Missense Mutation	SNP	C	T	T		Hybrid_(	NA
p.N339fs	1	65330630	65330630	TOV21G OVARY	37	+	Frame Shift Del	Del	T	-	-		Hybrid_(	NA
p.G282C	1	65332695	65332695	CW2 LARGE INTESTINE	37	+	Missense Mutation	SNP	C	A	A		Hybrid_(	NA
p.D246fs	1	65332803	65332803	SNUC2A LARGE INTESTINE	37	+	Frame Shift Del	Del	C	-	-		Hybrid_(	NA
p.L235V	1	65332836	65332836	KNS60 CENTRAL NERVOUS SYSTEM	37	+	Missense Mutation	SNP	G	C	C		Hybrid_(	NA
p.S228C	1	65332856	65332856	RS5 PLEURA	37	+	Missense Mutation	SNP	G	C	C		Hybrid_(	NA
p.N226S	1	65332862	65332862	SNU1105 CENTRAL NERVOUS SYSTEI	37	+	Missense Mutation	SNP	T	C	C		Hybrid_(	NA
p.N226S	1	65332862	65332862	SQ1 LUNG	37	+	Missense Mutation	SNP	T	C	C		Hybrid_(	NA
p.E223*	1	65332872	65332872	VMRCLCD LUNG	37	+	Nonsense Mutation	SNP	C	A	A		Hybrid_(	NA
p.L166S	1	65335144	65335144	SNU1040 LARGE INTESTINE	37	+	Missense Mutation	SNP	A	G	G		Hybrid_(	NA
p.Q161*	1	65339055	65339055	NCIH1563 LUNG	37	+	Nonsense Mutation	SNP	G	A	A		Hybrid_(	NA
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p.K142fs	1	65339110	65339111	MFE319 ENDOMETRIUM	37	+	Frame Shift Ins	Ins	-	T	T		Hybrid_(	NA
p.K142fs	1	65339111	65339111	LNCAPCLONEFGC PROSTATE	37	+	Frame Shift Del	Del	T	-	-		Hybrid_(	NA
p.K142fs	1	65339111	65339111	HEC151 ENDOMETRIUM	37	+	Frame Shift Del	Del	T	-	-		Hybrid_(	NA
p.K142fs	1	65339111	65339111	22RV1 PROSTATE	37	+	Frame Shift Del	Del	T	-	-		Hybrid_(	NA
p.D82A	1	65344792	65344792	EN ENDOMETRIUM	37	+	Missense Mutation	SNP	T	G	G		Hybrid_(	NA
p.I62V	1	65348981	65348981	NCIH1915 LUNG	37	+	Missense Mutation	SNP	T	C	C		Hybrid_(	NA
	1	65351955	65351955	HGC27 STOMACH	37	+	De novo Start OutOfFram	SNP	G	T	T		Hybrid_(	NA
	1	65351985	65351985	COLO783 SKIN	37	+	UTR 5	SNP	G	A	A		Hybrid_(	NA
	1	65352024	65352024	SNU1040 LARGE INTESTINE	37	+	UTR 5	SNP	G	A	A		Hybrid_(	NA

73 JAK1 mutations 58 cell lines
72 non-synonymous mutations in 57 cell lines
25 truncating mutations in 19 cell lines
17 frame shift mutation in 12 GYN cancer cell lines
1 splice site mutation in GYN
1 nonsense mutation in GYN
8 missense mutation in GYN cancer
% JAK1 mutations are truncating mutations: 28 of 72 =38.9%
% cell lines with JAK1 truncating mutations are GYN cells: 13/19 =68.4%

Yellow: Truncating mutations in GYN cancer
Orange: Missense mutations in GYN cancer

sTable 3: JAK2 mutations in the TCC project

	Total case*	NS mutation case	NS mutation rate (%)	Truncation case	Truncation rate (%)
Brain	79	1	1.3	0	
Breast	427	32	7.5	0	
Cevix	49	3	6.1	1	2
Endometrium	200	23	11.5	4	2
Ovary	235	15	6.4	1	0.4
Uterus	148	19	12.8	4	2.7
Esophagus	44	1	2.3	0	
HEME-AML	36	1	2.8	0	
HEME-CLL	94	6	6.4	0	
Kidney	243	12	4.9	0	
Large bowel	460	34	7.4	1	0.2
Larynx	24	2	8.3	0	
Liver	30	1	3.3	0	
Lung	603	50	8.3	5	0.8
Oral cavity	30	1	3.3	0	
Pancreas	161	7	4.3	0	
Peritoneum	9	2	22.2	0	
Prostate	52	5	9.6	0	
Rectum-anus	72	4	5.6	1	1.4
Skin	209	11	5.3	0	
Soft tissue	45	3	6.7	0	
Stomach	55	3	5.5	0	
Total	3305	236		17	

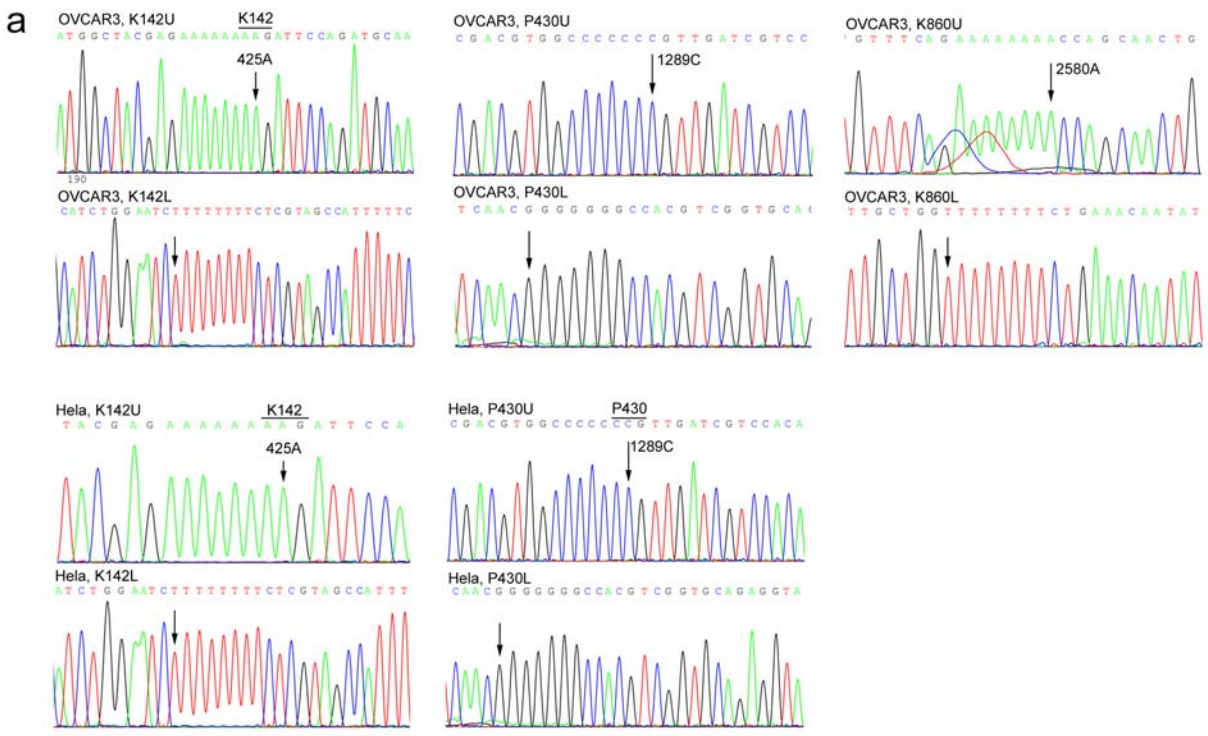
	Case	NS mutation	Truncation
Combined GYN	632	60 (9.4%)	10 (1.6%)

* Not listed tissue types (see sTable 1) do not have non-synonymous JAK2 mutations.

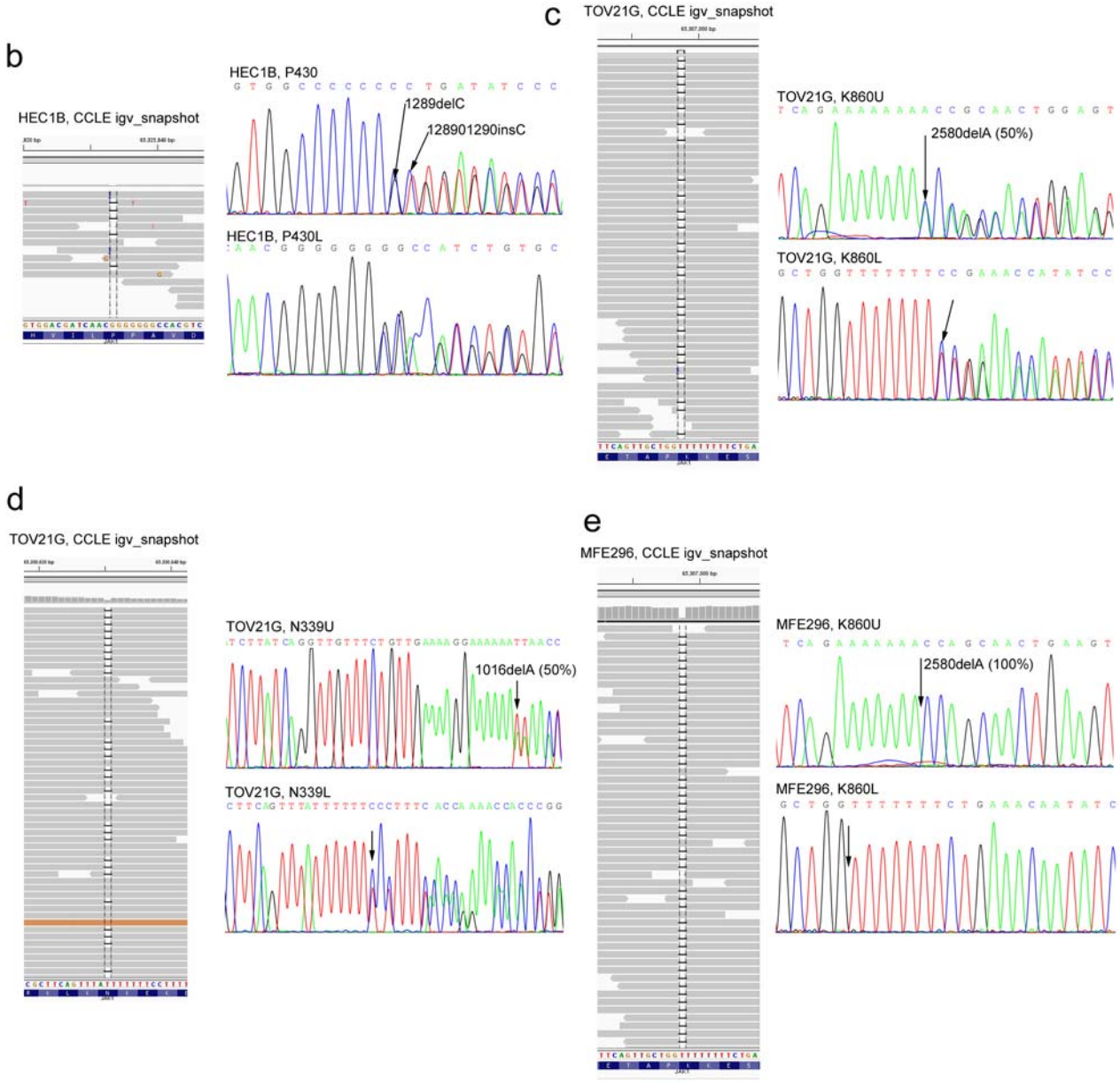
sTable 4: STAT1 mutations in the TCC project

	Total case*	NS mutation	NS mutation rate (%)	Truncation	Truncation rate (%)		Case	NS mutation	Truncation
Brain	79	2	2.5						
Breast	427	8	1.9						
Cevix	49	3	6.1			**Combined GYN**	632	23 (3.6%)	4 (0.6%)
Endometrium	200	8	4.0	1	0.5				
Ovary	235	3	1.3						
Uterus	148	9	6.1	3	2				
Esophagus	44	1	2.3						
HEME-CLL	94	2	2.1						
Kidney	243	4	1.6	1	0.4				
Large bowel	460	11	2.4						
Liver	30	2	6.7						
Lung	603	14	2.3	1	0.2				
Pancreas	161	3	1.9						
Skin	209	9	4.3						
Soft tissue	45	2	4.4						
Thyroid	12	1	8.3						
Total	3039	82		6					

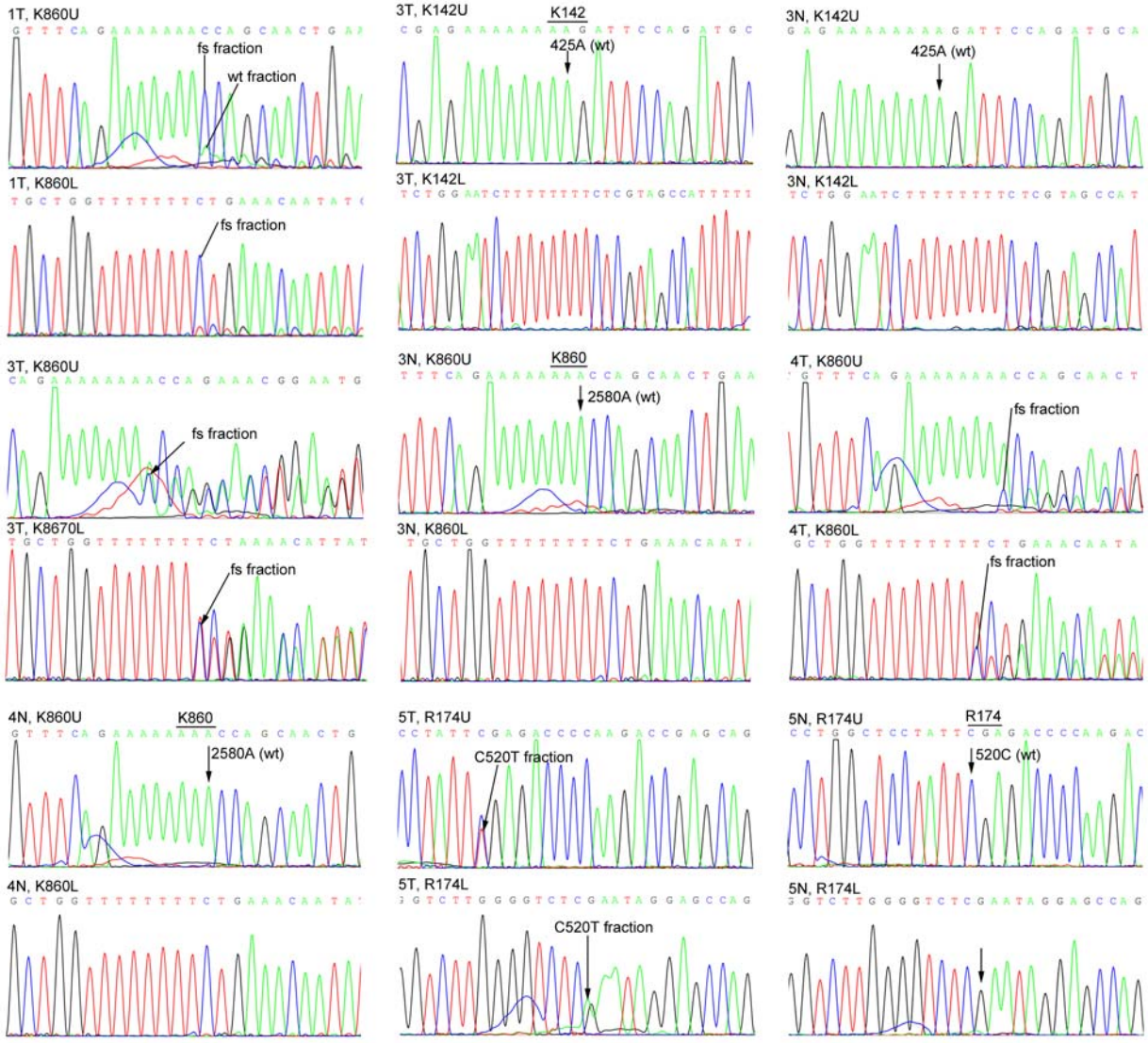
*Not listed tissue types (see sTable 1) do not have non-synonymous STAT1 mutations.


a
OVCAR3, K142U
K142
425A
OVCAR3, K142L
OVCAR3, P430U
1289C
OVCAR3, P430L
OVCAR3, K860U
2580A
OVCAR3, K860L
Hela, K142U
K142
425A
Hela, K142L
Hela, P430U
P430
1289C
Hela, P430L

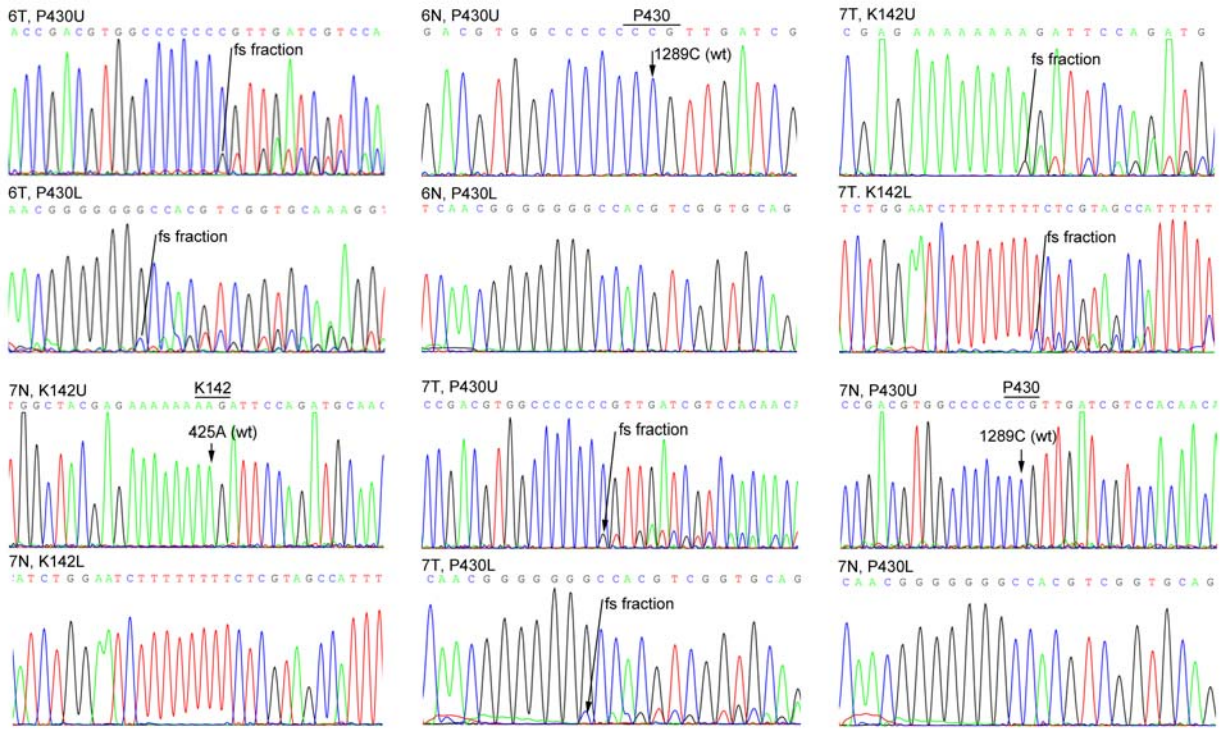
sFig. 1, part a


b
HEC1B, CCLE igv_snapshot
HEC1B, P430
1289delC
128901290insC
HEC1B, P430L
c
TOV21G, CCLE igv_snapshot
TOV21G, K860U
2580delA (50%)
TOV21G, K860L
d
TOV21G, CCLE igv_snapshot
TOV21G, N339U
1016delA (50%)
TOV21G, N339L
e
MFE296, CCLE igv_snapshot
MFE296, K860U
2580delA (100%)
MFE296, K860L

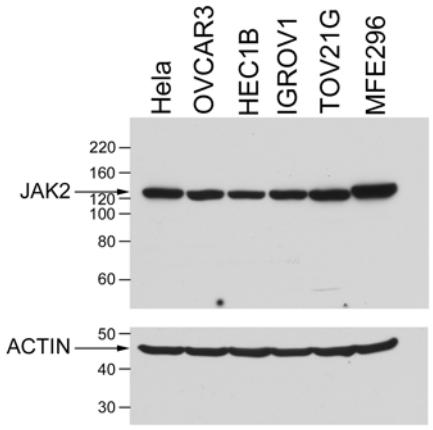
sFig.1, part b


1T, K860U
fs fraction
wt fraction
3T, K142U
K142
425A (wt)
3N, K142U
425A (wt)
1T, K860L
fs fraction
3T, K142L
3N, K142L
3T, K860U
fs fraction
3N, K860U
K860
2580A (wt)
4T, K860U
fs fraction
3T, K8670L
fs fraction
3N, K860L
4T, K860L
fs fraction
4N, K860U
K860
2580A (wt)
5T, R174U
C520T fraction
5N, R174U
R174
520C (wt)
4N, K860L
5T, R174L
C520T fraction
5N, R174L

sFig. 2, part a


6T, P430U
fs fraction
6N, P430U
P430
1289C (wt)
7T, K142U
fs fraction
6T, P430L
fs fraction
6N, P430L
7T, K142L
fs fraction
7N, K142U
K142
425A (wt)
7T, P430U
fs fraction
7N, P430U
P430
1289C (wt)
7N, K142L
7T, P430L
fs fraction
7N, P430L

sFig. 2, part b


Hela
OVCAR3
HEC1B
IGROV1
TOV21G
MFE296
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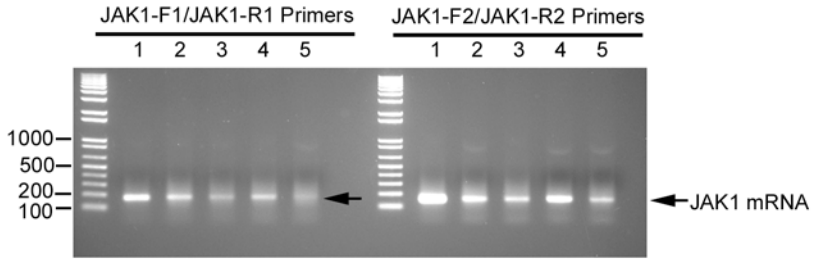
160
JAK2
120
100
80
60
50
ACTIN
40
30

sFig. 3



1. HeLa, 2. TOV21G, 3. HEC1B, 4. IGROV1, 5. MFE296

sFig. 4

Supplementary figure legends

sFigure 1. Sanger sequencing JAK1 in cancer cell lines. (a) Wildtype JAK1 in OVCAR3 and Hela cells. (b) P430 site in HEC1B cells. (c-d) K860 and N339 sites in TOV21G cells. (e) K860 site in MFE296 cells. Genomic DNA was isolated from these cells and coding regions across amino acid K142, N339, P430, or K860 were amplified by PCR and then sequenced. The DNA sequencing spectra in both strands are shown in each panel. Also shown are snapshots of sequence alignment of CCLE exome sequencing data of HEC1B, TOV21G, and MFE296 cells across N339fs, P430fs or K860fs sites.

sFigure 2. DNA sequencing data of tumor and matched normal tissues. The DNA sequencing spectra across mutation sites in tumors and the corresponding regions in normal tissues are shown in both strands.

sFigure 3. Analysis of JAK2 protein in GYN cancer cell lines. Cell lysates from indicated cell lines were analyzed by immunoblotting with indicated antibodies.

sFigure 4. RT-PCR analysis of JAK1 transcripts in GYN cancer cell lines. RT-PCR was performed with ~800 ng of total RNA from each cell line and two different primer pairs. The RT-PCR products were analyzed on a 1.2% agarose gel.