Table S1: Clinical characteristics of melanoma patients (Cohort 1), Related to Figure 1.

	Total Cohort	Immune Checkpoint Blockade	Targeted Therapy	
Number of Patients	389	251	138	
Age at Immunotherapy (Average)	59.6	60.9	57.2	P= 0.019
Gender				
Male	257 (66%)	163 (65%)	94 (68%)	P=0.527
Female	132 (34%)	88 (35%)	44 (32%)	
ECOG Performance Status				P=0.001
0-1	359 (92%)	240 (96%)	119 (86%)	
2+	30 (8%)	11 (4%)	19 (14%)	
Histology				
Melanoma	389(100%)	251 (100%)	138(100%)	
BRAF V600 Mutant	233 (43%)	95 (38%)	138(100%)	P<0.001
Stage at Therapy				
IV	389(100%)	251 (100%)	138(100%)	
Prior Lines of Therapy				
0	295 (76%)	216 (86%)	79 (57%)	P<0.001
1+	94 (24%)	35 (14%)	59 (43%)	
Disease Origin				
Cutaneous	326 (84%)	200 (80%)	126 (91%)	P<0.001
Mucosal	20 (5%)	20 (8%)	0 (0%)	
Ocular	31 (8%)	27 (11%)	4 (3%)	
Unknown	12 (3%)	4 (1%)	8 (6%)	
Therapy				
Dabrafenib	14 (3%)		14 (10%)	
Trametinib	4 (1%)		4 (3%)	
Vemurafenib	36 (9%)		36 (26%)	
Encorafenib	1 (0%)		1 (1%)	
DTIC	1 (0%)		1 (1%)	
Dabrafenib and Trametinib	77 (20%)		77 (56%)	
Vemurafenib and Cobimetinib	3 (1%)		3 (2%)	
Encorafenib and Binimetinib	2 (1%)		2 (1%)	
Nivolumab	38 (10%)	38 (15%)		
Pembrolizumab	96 (25%)	96 (38%)		
Ipilimumab	45 (12%)	45 (18%)		
Ipilimumab and Nivolumab	72 (1%)	72 (29%)		

Abbreviations:
ECOG- Eastern Cooperative Oncology Group

Table S2: Clinical characteristics of NSCLC patients (Cohort 2), Related to Figure 1.

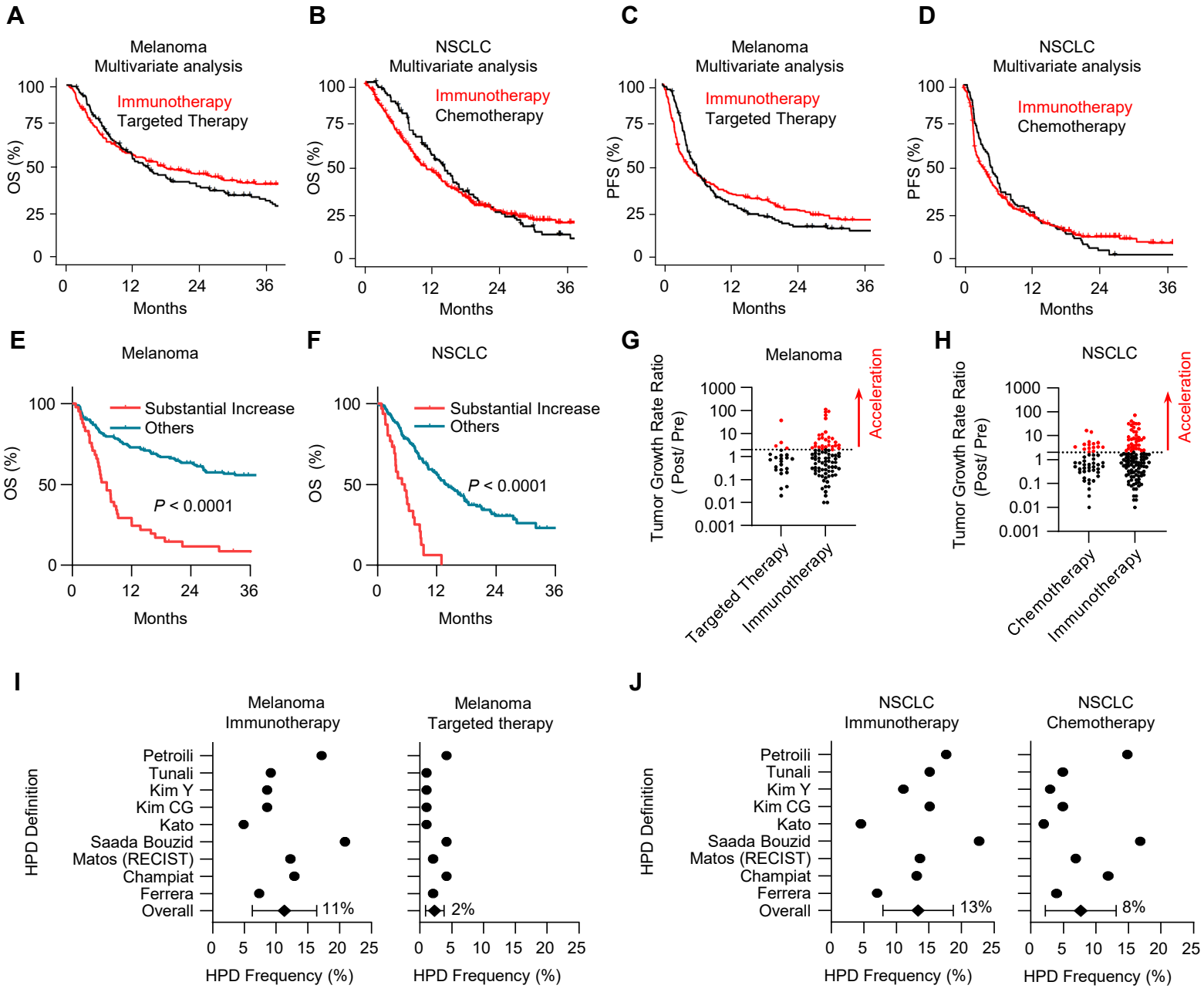
	Total Cohort	Immune Checkpoint Blockade	Chemotherapy	
Number of Patients	375	279	96	
Average Age	66.0	66.0	65.9	P=0.88
Gender				
Male	165 (44%)	109 (39%)	56 (58%)	P=0.001
Female	210 (56%)	170 (61%)	40 (42%)	
ECOG Performance Status				P=0.001
0-1	301 (80%)	213 (76%)	88 (92%)	
2+	74 (20%)	66 (24%)	8 (8%)	
Smoking Status				P=0.159
Never	50 (13%)	37 (13%)	13 (13%)	
Former	279 (75%)	213 (76%)	66 (69%)	
Current	46 (12%)	29 (11%)	17 (18%)	
Stage at Therapy				
IV	375 (100%)	279(100%)	96 (100%)	
Tumor Histology				
Adenocarcinoma	282 (75%)	201 (72%)	81 (85%)	P=0.003
Squamous Cell Carcinoma	66 (18%)	60 (22%)	6 (6%)	
Other	27 (7%)	18 (6%)	9 (9%)	
Prior Lines of Therapy				P<0.001
0	174 (46%)	109 (39%)	65 (68%)	
1	104 (28%)	88 (32%)	16 (17%)	
2+	97 (26%)	82 (29%)	15 (15%)	
Therapy				
Platinum doublet	54 (14%)		54 (56%)	
Gemcitabine	8 (2%)		8 (8%)	
Pemetrexed	12 (3%)		12 (13%)	
Taxane	5 (1%)		5 (5%)	
Other	17 (5%)		17 (18%)	
Atezolizumab	47 (13%)	47 (17%)		
Nivolumab	81 (22%)	81 (29%)		
Pembrolizumab	50 (13%)	50 (18%)		
Carboplatin, pemetrexed, pembrolizumab	82 (22%)	82 (29%)		
Paclitaxel, pemetrexed, pembrolizumab	19 (5%)	19 (7%)		

Abbreviations:
ECOG- Eastern Cooperative Oncology Group

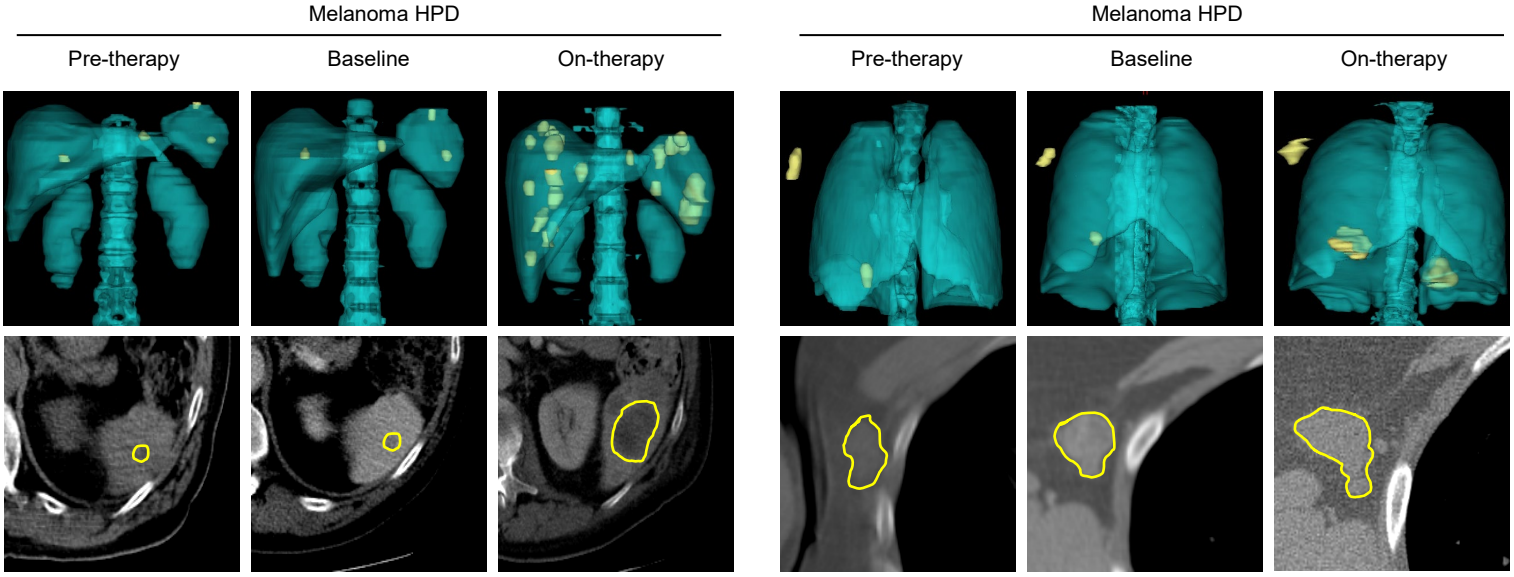
Table S3: HPD definitions, Related to Figure 1.

Authors	Cancer Type	Definition	DOI
Champiat S, et al.	Multiple Cancer Types	TGR ratio > 2 and PD at 1st assessment	10.1158/1078-0432.CCR-16-1741
Ferrara R, et al.	NSCLC	Delta TGR > 50%	10.1001/jamaoncol.2018.3676
Kim CG, et al.	NSCLC	PD, TTF < 2 months, TGK ratio >2 and TGR ratio >2	10.1093/annonc/mdz123
Kato S, et al.	Multiple Cancer Types	TTF < 2 months, >50% RECIST, >2 fold increase in progression pace	10.1158/1078-0432.CCR-16-3133
Saâda-Bouzid E, et al.	Head and Neck Cancer	TGK ratio > 2	10.1093/annonc/mdx178
Kim Y, et al.	NSCLC	TTF < 2 months, >50% volume increase, TGK >2	10.1016/j.jtho.2019.05.033
Matos I, et al.	Multiple Cancer Types	PD in < 8 weeks, 1.4x increase from baseline or 1.2x increase from baseline with involvement of 2 new organs	10.1158/1078-0432.CCR-19-2226
Tunali I, et al.	NSCLC	TGR ratio >2, TTF < 2 months, AND PD on 1st scan	10.1016/j.lungcan.2019.01.010
Petrioli R, et al.	Multiple Cancer Types	TGR ratio > 2	10.1097/CAD.0000000000000864

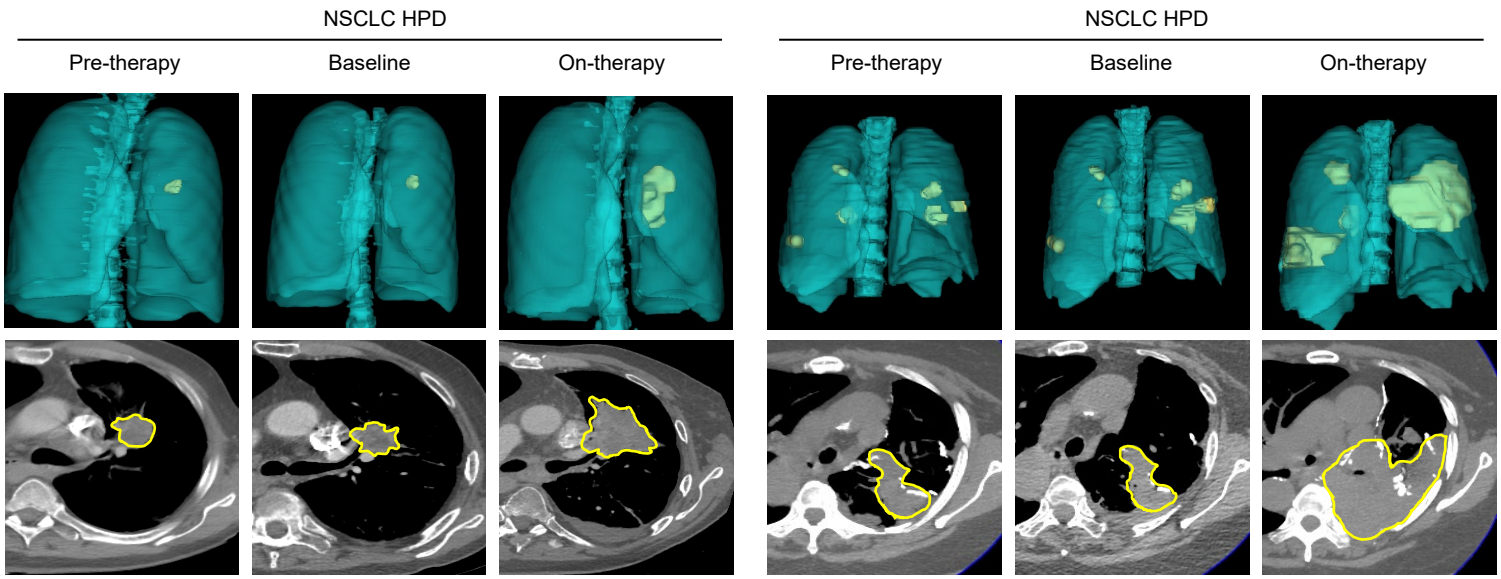
Abbreviations:
HPD- Hyperprogressive Disease
NSCLC- Non-small Cell Lung Cancer
PD- Progressive Disease
RECIST- Response Evaluation Criteria In Solid Tumors
TGK- Tumor Growth Kinetics
TGR- Tumor Growth Rate
TTF- Time to Treatment Failure


A
Melanoma
Multivariate analysis
Immunotherapy
Targeted Therapy
OS (%)
Months
B
NSCLC
Multivariate analysis
Immunotherapy
Chemotherapy
OS (%)
Months
C
Melanoma
Multivariate analysis
Immunotherapy
Targeted Therapy
PFS (%)
Months
D
NSCLC
Multivariate analysis
Immunotherapy
Chemotherapy
PFS (%)
Months
E
Melanoma
Substantial Increase
Others
P < 0.0001
OS (%)
Months
F
NSCLC
Substantial Increase
Others
P < 0.0001
OS (%)
Months
G
Melanoma
Tumor Growth Rate Ratio
(Post/ Pre)
Acceleration
Targeted Therapy
Immunotherapy
H
NSCLC
Tumor Growth Rate Ratio
(Post/ Pre)
Acceleration
Chemotherapy
Immunotherapy
I
Melanoma
Immunotherapy
Melanoma
Targeted therapy
HPD Definition
Petroili
Tunali
Kim Y
Kim CG
Kato
Saada Bouzid
Matos (RECIST)
Champiat
Ferrera
Overall
11%
2%
HPD Frequency (%)
HPD Frequency (%)
J
NSCLC
Immunotherapy
NSCLC
Chemotherapy
HPD Definition
Petroili
Tunali
Kim Y
Kim CG
Kato
Saada Bouzid
Matos (RECIST)
Champiat
Ferrera
Overall
13%
8%
HPD Frequency (%)
HPD Frequency (%)

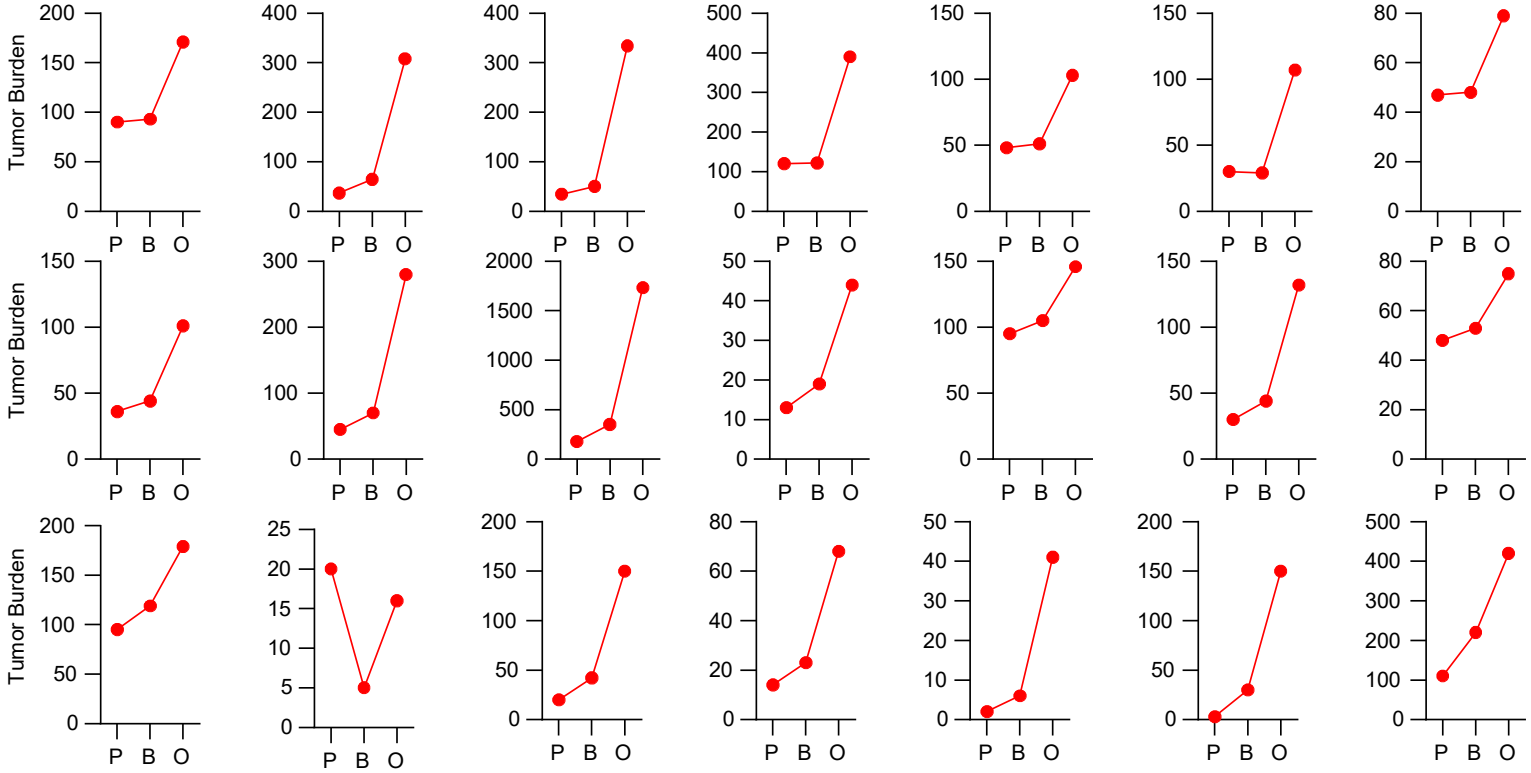
K


Melanoma HPD
Pre-therapy
Baseline
On-therapy
Melanoma HPD
Pre-therapy
Baseline
On-therapy

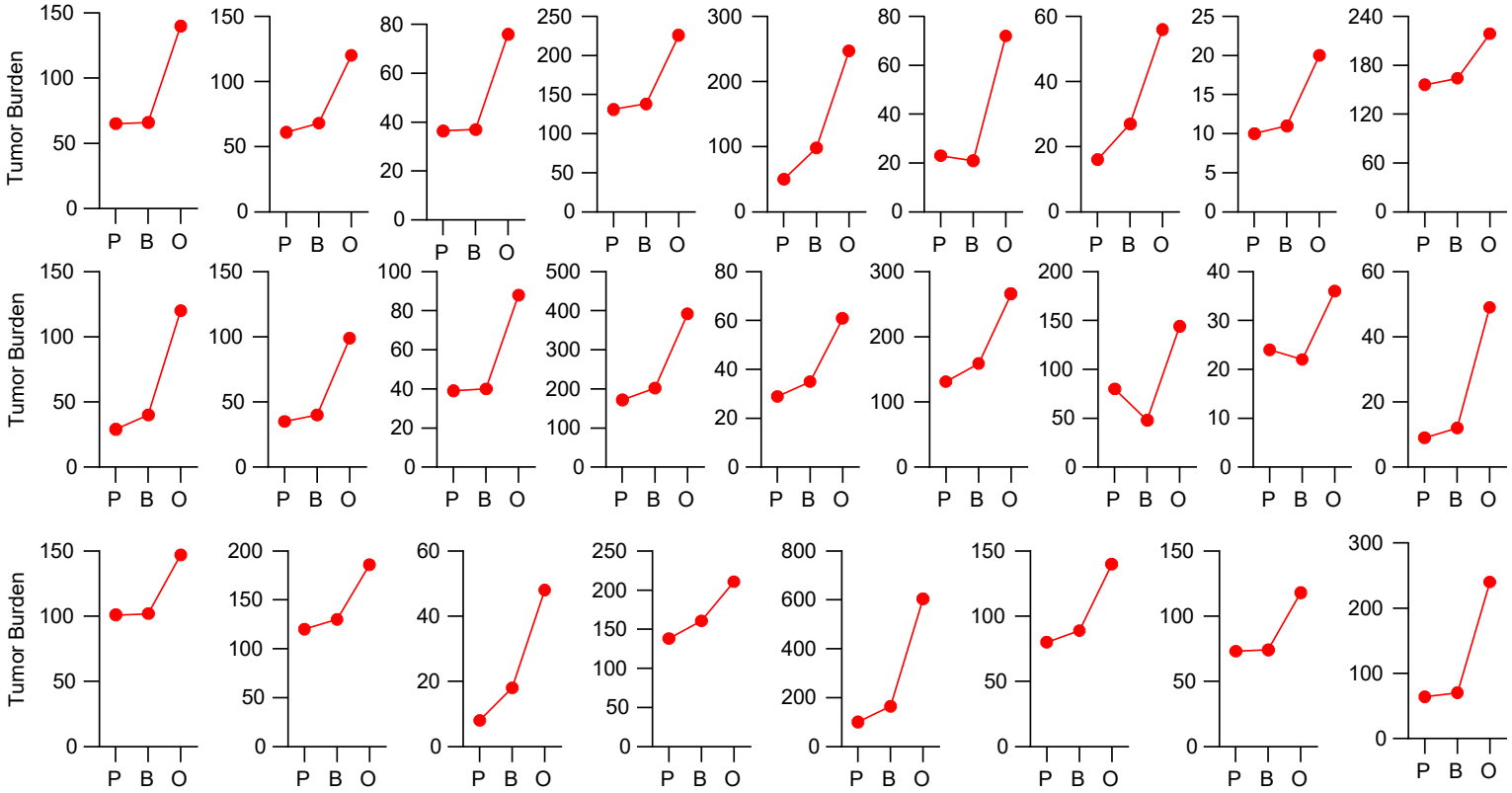
L


NSCLC HPD
Pre-therapy
Baseline
On-therapy
NSCLC HPD
Pre-therapy
Baseline
On-therapy

M



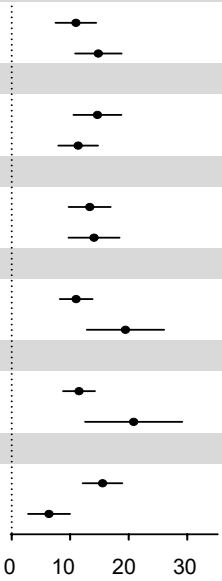
N



O

Melanoma Immunotherapy Cohort

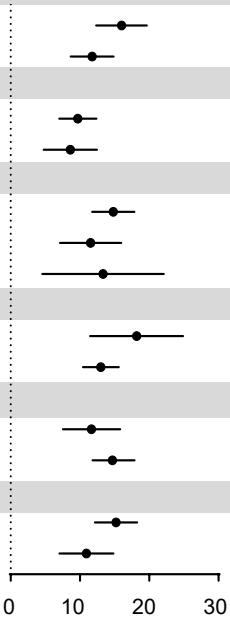
Subgroup	Number of patients evaluated	HPD Frequency (%) (Mean, SD)	P-value
Age			.46445
≥ median	82		
<median	81		
Performance status			.53041
0	75		
1+	88		
BRAF status			.91376
WT	90		
Mutant	64		
Site of Origin			.18311
Cutaneous	127		
Noncutaneous	36		
Line of therapy			.20807
First line	139		
Second line or higher	24		
ICB Therapy			.10609
Single Agent	116		
Dual Agents	47		



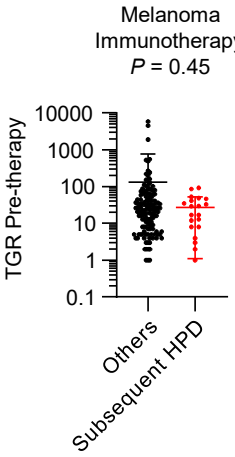
P

NSCLC Immunotherapy Cohort

Subgroup	Number of patients evaluated	HPD Frequency (%) (Mean, SD)	P-value
Age			.38376
< median	100		
≥ median	102		
Performance status			.34836
0-1	158		
2+	44		
Histology			.84312
Adenocarcinoma	135		
Squamous Cell Ca.	52		
Other	15		
Tobacco Use			.28516
Never	33		
Current/Former	169		
Line of therapy			.55734
First line	60		
Second line or higher	142		
Type of therapy			.67080
ICB monotherapy	138		
Chemo + ICB	64		



Q



R

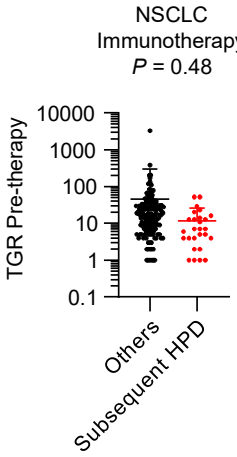


Figure S1: Rapid cancer progression occurs in a subset of patients during immunotherapy. Related to Figure 1.

(A) Multivariable analysis of overall survival (OS) in patients with melanoma (Cohort 1) stratified by type of therapy. RMST at 3 months, HR = 0.900, $P < 0.0001$, Log-rank.

(B) Multivariable analysis of OS in patients with NSCLC (Cohort 2) stratified by type of therapy. RMST at 3 months, HR = 0.942, $P < 0.0001$, Log-rank.

(C) Multivariable analysis of progression-free survival (PFS) in patients with melanoma (Cohort 1) stratified by type of therapy. RMST at 3 months, HR = 0.954, $P = 0.002$, Log-rank.

(D) Multivariable analysis of PFS in patients with NSCLC (Cohort 2) stratified by type of therapy. RMST at 3 months, HR = 0.896, $P = 0.001$, Log-rank.

(E) OS of patients with metastatic melanoma treated with ICB (Cohort 1) stratified by radiographic response, > 50% response n = 41, ≤ 50% response n = 159, Hazard ratio (HR) = 0.1431, $P < 0.0001$ by log-rank test.

(F) OS of patients with metastatic NSCLC treated with ICB (Cohort 2) stratified by stratified by radiographic response, >50% response n = 31; ≤ 50% response n = 181, Hazard ratio (HR) = 0.2678, $P < 0.0001$ by log-rank test.

(G) Tumor growth rate (TGR) of patients with melanoma treated with indicated therapy (Cohort 1). Ratio of TGR following therapy to TGR preceding therapy. Cutoff: More than two-fold increase in TGR (red). targeted therapy n = 96, immunotherapy n = 163. $P = 0.00190$ by Chi squared.

(H) TGR of patients with NSCLC treated with indicated therapy (Cohort 2). Ratio of TGR following therapy to TGR preceding therapy. Cutoff: More than two-fold increase in TGR (red). chemotherapy n = 70, immunotherapy n = 202. $P = 0.04$ by Chi squared.

(I) Frequency of hyperprogression following receipt of immunotherapy (left) or targeted therapy (right) in melanoma patients using indicated criteria from different researchers. paired t test, $P = 0.001$. Data are shown as mean ± s.d.; immunotherapy n = 198, targeted therapy n = 68. Utilizing Champiat et al. definition for HPD: immunotherapy HPD n = 25; targeted therapy n = 4.

(J) Frequency of hyperprogression following receipt of immunotherapy (left) or chemotherapy (right) in NSCLC patients using indicated criteria from different researchers. paired t-test, $P = 0.0001$. Data are shown as mean ± s.d.; immunotherapy n = 161, chemotherapy n = 96. Utilizing Champiat et al. definition for HPD: immunotherapy HPD n = 21; chemotherapy HPD n = 11.

(K-L) Representative cross-sectional and 3D reconstructed computed tomography (CT) images of two patients with metastatic melanoma (**K**) or NSCLC (**L**) with HPD preceding receipt of immunotherapy (left), at baseline preceding immunotherapy (middle), and at first reassessment following immunotherapy (right).

(M-N) Longitudinal tumor burden of individual melanoma (**M**) or NSCLC (**N**) patients with HPD. P, Pre-therapy; B, baseline; O, On-therapy.

(O-P) Proportion of melanoma (**O**) and NSCLC (**P**) patients with hyperprogression stratified by indicated clinicopathologic variables. Chi-squared. Data are shown as mean ± s.d.

(Q-R) Tumor growth rate (TGR) from the period of preceding therapy initiation in patients with melanoma treated with immunotherapy (**Q**) and NSCLC treated with immunotherapy (**R**). Horizontal lines indicate quartiles. Two-tailed t-test.

Table S4: Clinical characteristics of patients with comprehensive sequencing (Cohort 3), Related to Figure 2.

	Total Cohort
Number of Patients	50
Average Age	60.6
Gender	
Male	24 (48%)
Female	26 (52%)
ECOG Performance Status	
0-1	42 (84%)
2+	8 (16%)
Stage at Therapy	
IV	50 (100%)
Tumor Histology	
Melanoma	13 (26%)
NSCLC	5 (10%)
Urothelial Carcinoma	16 (32%)
Sarcoma	7 (14%)
Breast	5 (10%)
Lymphoma	4 (8%)
Therapy	
Atezolizumab	5 (10%)
Ipilimumab	2 (4%)
Nivolumab	8 (16%)
Pembrolizumab	32 (64%)
Combination	3 (6%)

Abbreviations:
ECOG- Eastern Cooperative Oncology Group

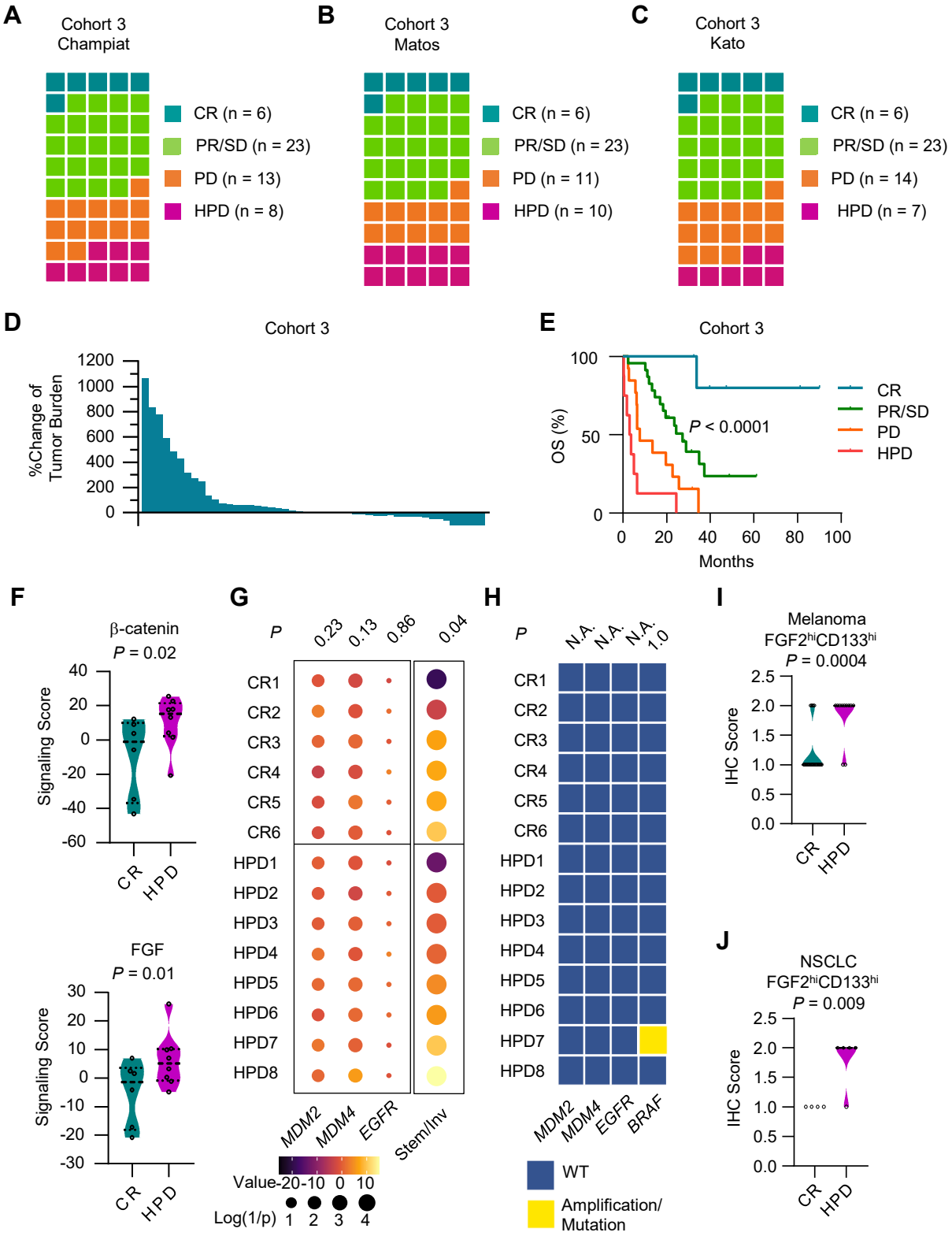

A
Cohort 3
Champiat
CR (n = 6)
PR/SD (n = 23)
PD (n = 13)
HPD (n = 8)
B
Cohort 3
Matos
CR (n = 6)
PR/SD (n = 23)
PD (n = 11)
HPD (n = 10)
C
Cohort 3
Kato
CR (n = 6)
PR/SD (n = 23)
PD (n = 14)
HPD (n = 7)
D
Cohort 3
%Change of Tumor Burden
1200
1000
800
600
400
200
0
E
Cohort 3
OS (%)
100
50
0
P < 0.0001
0
20
40
60
80
100
Months
CR
PR/SD
PD
HPD
F
β-catenin
P = 0.02
Signaling Score
40
20
0
-20
-40
-60
CR
HPD
FGF
P = 0.01
Signaling Score
30
20
10
0
-10
-20
-30
CR
HPD
G
P
0.23
0.13
0.86
0.04
CR1
CR2
CR3
CR4
CR5
CR6
HPD1
HPD2
HPD3
HPD4
HPD5
HPD6
HPD7
HPD8
MDM2
MDM4
EGFR
Stem/Inv
Value -20 -10 0 10
Log(1/p) 1 2 3 4
H
P
N.A.
N.A.
N.A.
1.0
CR1
CR2
CR3
CR4
CR5
CR6
HPD1
HPD2
HPD3
HPD4
HPD5
HPD6
HPD7
HPD8
MDM2
MDM4
EGFR
BRAF
WT
Amplification/
Mutation
I
Melanoma
FGF2hiCD133hi
P = 0.0004
IHC Score
2.5
2.0
1.5
1.0
0.5
0.0
CR
HPD
J
NSCLC
FGF2hiCD133hi
P = 0.009
IHC Score
2.5
2.0
1.5
1.0
0.5
0.0
CR
HPD

Figure S2: Immunogenic and oncogenic pathways correlate in patients with HPD. Related to Figure 2.

(A-C) Best radiographic responses in Cohort 3 based on indicated definitions depicted in a tile diagram. 1 tile represents 1 patient. CR, complete response; PR/SD, partial response/stable disease; PD, progressive disease; HPD, hyperprogressive disease. HPD definition criteria were based on the reports by Drs. Champiat, Matos, or Kato.

(D) Waterfall plot showing change of tumoral burden from initiation of therapy to first surveillance imaging (Cohort 3), Data are shown as percentage change. n = 50.

(E) Overall survival in metastatic patients in Cohort 3 stratified by best radiographic response. HPD definition criteria were based on the report by Dr. Champiat. Log-rank test.

(F) Violin plots displaying β-catenin and FGF signaling scores in Cohort 3 patients stratified by response to immunotherapy. CR n = 6, HPD n = 8. P-values were generated from multivariate mixed effect linear models controlling for biopsy site (fixed effect) and disease type (random effect).

(G) Heat map showing expression levels for *MDM2*, *MDM4*, *EGFR* and stemness and invasiveness gene signature in HPD and CR patients. P-values were generated from multivariate mixed effect linear models controlling for biopsy site (fixed effect) and disease type (random effect).

(H) Tile diagram showed the genetic amplification or mutation of the indicated genes in Cohort 3 patients. Fisher's exact test.

(I-J) IHC scores of $FGF2^{hi}CD133^{hi}$ tumor cells in melanoma (**I**) or NSCLC (**J**) patients with CR or HPD. Chi-square test.

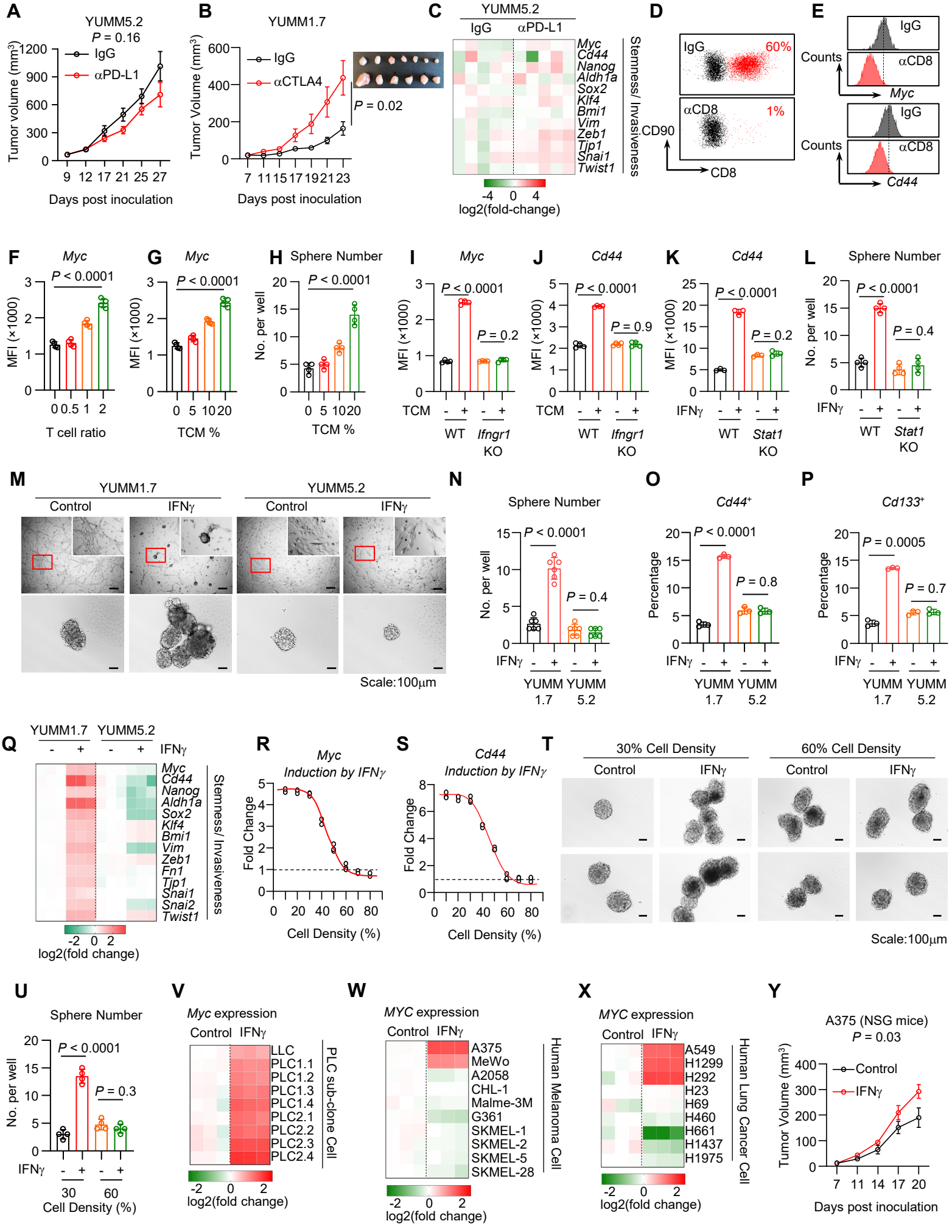

A
YUMM5.2
P = 0.16
IgG
αPD-L1
Tumor volume (mm3)
Days post inoculation
B
YUMM1.7
IgG
αCTLA4
P = 0.02
Tumor Volume (mm3)
Days post inoculation
C
YUMM5.2
IgG
αPD-L1
Myc
Cd44
Nanog
Aldh1a
Sox2
Klf4
Bmi1
Vim
Zeb1
Tjp1
Snai1
Twist1
Stemness/ Invasiveness
log2(fold-change)
D
IgG
60%
αCD8
1%
CD90
CD8
E
IgG
αCD8
Counts
Myc
Cd44
F
Myc
P < 0.0001
MFI (×1000)
T cell ratio
G
Myc
P < 0.0001
MFI (×1000)
TCM %
H
Sphere Number
P < 0.0001
No. per well
TCM %
I
Myc
P < 0.0001
P = 0.2
MFI (×1000)
TCM
WT
Ifngr1 KO
J
Cd44
P < 0.0001
P = 0.9
MFI (×1000)
TCM
WT
Ifngr1 KO
K
Cd44
P < 0.0001
P = 0.2
MFI (×1000)
IFNγ
WT
Stat1 KO
L
Sphere Number
P < 0.0001
P = 0.4
No. per well
IFNγ
WT
Stat1 KO
M
YUMM1.7
YUMM5.2
Control
IFNγ
Scale:100μm
N
Sphere Number
P < 0.0001
P = 0.4
No. per well
IFNγ
YUMM 1.7
YUMM 5.2
O
Cd44+
P < 0.0001
P = 0.8
Percentage
IFNγ
YUMM 1.7
YUMM 5.2
P
Cd133+
P = 0.0005
P = 0.7
Percentage
IFNγ
YUMM 1.7
YUMM 5.2
Q
YUMM1.7
YUMM5.2
IFNγ
Myc
Cd44
Nanog
Aldh1a
Sox2
Klf4
Bmi1
Vim
Zeb1
Fn1
Tjp1
Snai1
Snai2
Twist1
Stemness/ Invasiveness
log2(fold change)
R
Myc
Induction by IFNγ
Fold Change
Cell Density (%)
S
Cd44
Induction by IFNγ
Fold Change
Cell Density (%)
T
30% Cell Density
60% Cell Density
Control
IFNγ
Scale:100μm
U
Sphere Number
P < 0.0001
P = 0.3
No. per well
IFNγ
30
60
Cell Density (%)
V
Myc expression
Control
IFNγ
LLC
PLC1.1
PLC1.2
PLC1.3
PLC1.4
PLC2.1
PLC2.2
PLC2.3
PLC2.4
PLC sub-clone Cell
log2(fold change)
W
MYC expression
Control
IFNγ
A375
MeWo
A2058
CHL-1
Malme-3M
G361
SKMEL-1
SKMEL-2
SKMEL-5
SKMEL-28
Human Melanoma Cell
log2(fold change)
X
MYC expression
Control
IFNγ
A549
H1299
H292
H23
H69
H460
H661
H1437
H1975
Human Lung Cancer Cell
log2(fold change)
Y
A375 (NSG mice)
P = 0.03
Control
IFNγ
Tumor Volume (mm3)
Days post inoculation

Figure S3: $CD8^+$ T cells drive cancer hyperprogression via IFNγ. Related to Figure 3.

(A) YUMM5.2 tumor bearing C57BL/6 mice were treated with control (IgG) or PD-L1 antibody. Tumor growth curves were plotted. Data are shown as mean ± s.d., n = 7 (IgG) or 5 (αPD-L1). Two-tailed t-test.

(B) YUMM1.7 tumor bearing C57BL/6 mice were treated with control (IgG) or CTLA4 antibody. Tumor growth curves were plotted. Data are shown as mean ± s.d., n = 7 (IgG) or 6 (αCTLA4). Two-tailed t-test.

(C) YUMM5.2 tumor bearing C57BL/6 mice were treated with control (IgG) or PD-L1 antibody. On 16^{th} day after tumor inoculation, the indicated gene expression in tumors was determined by qPCR. n = 5 tumors.

(D-E) YUMM1.7 tumor bearing C57BL/6 mice were treated with control (IgG) or CD8 antibody. Tumor infiltrating $CD8^+$ T cells (**D**) and *Myc* (upper) or *Cd44* (lower) expression in $CD45^-$negative tumor cells (**E**) were determined by FACS.

(F-G) YUMM1.7 cells were cultured with indicated ratio of T cells (**F**) or proportion of T cell conditioned medium (TCM) (**G**) for 48 hours. *Myc* expression (MFI) was determined by FACS analysis in $CD45^-CD90^-$ tumor cells. Data are shown as mean ± s.d., n = 4. One-way ANOVA test.

(H) YUMM1.7 cells were cultured with indicated proportion of T cell conditioned medium (TCM) for 48 hours and transferred into 3D-sphere cultures. Tumor spheres were recorded 7 days after 3D-sphere culture. Data are shown as mean ± s.d., n = 4. One-way ANOVA test.

(I-J) WT or *Ifngr1* KO YUMM1.7 cells were cultured with 20% TCM for 48 hours. Tumor intracellular *Myc* (**I**) and surface *Cd44* (**J**) expression was determined by FACS. Data are shown as mean ± s.d., n = 4. Two-tailed t-test.

(K-L) WT or *Stat1* KO YUMM1.7 cells were treated with IFNγ. Surface expression of *Cd44* was determined by FACS 48 hours after treatment (**K**). Tumor spheres were counted on day 7 after 3D-sphere culture (**L**). Data are mean ± s.d., n = 3 (**K**), n = 4 (**L**). Two-tailed t-test.

(M-N) YUMM1.7 and YUMM5.2 cells were treated with IFNγ. Tumor cell morphology in 2D cell culture (upper) or 3D cell culture (lower) was captured after 48 hours treatment (**M**). Tumor spheres were quantified on day 7. Data are shown as mean ± s.d., n = 6. Two-tailed t-test (**N**).

(O-P) YUMM1.7 and YUMM5.2 cells were treated with IFNγ for 48 hours, surface expression of *Cd44* (**O**) or *Cd133* (**P**) were determined by FACS. Data are shown as mean ± s.d., n = 3. Two-tailed t-test.

(Q) YUMM1.7 and YUMM5.2 cells were treated with IFNγ for 48 hours. The indicated gene expression was determined by qPCR. n = 3.

(R-S) Confluent YUMM1.7 cells were trypsinized, counted, seeded at indicated density, and treated with IFNγ for 36 hours. RNA levels of *Myc* (**R**) and *Cd44* (**S**) were determined by qPCR. n =3.

(T-U) YUMM1.7 cells were seeded at 30% or 60% density and treated with IFNγ for 48 hours, followed by 3D-sphere culture. Tumor spheres representative images (**T**) or quantitation (**U**) at day 7 are shown. Data are mean ± s.d., n = 4, Two-tailed t-test.

(V) LLC or PLC cells were treated with IFNγ for 24 hours. *Myc* expression was determined by real-time qRT-PCR. Data are shown as mean ± s.d., n = 3. P value by two-tailed t-test.

(W-X) Human melanoma cell lines (**W**) and human lung cancer cell lines (**X**) were treated with IFNγ for 48 hours. *MYC* expression was determined by qPCR. n = 3.

(Y) A375 tumor-bearing NSG mice were treated with recombinant human IFNγ. Tumor growth curves were plotted. n = 5 (control). n = 6 (IFNγ). Data are shown as mean ± s.d., n = 5. Two-tailed t-test.

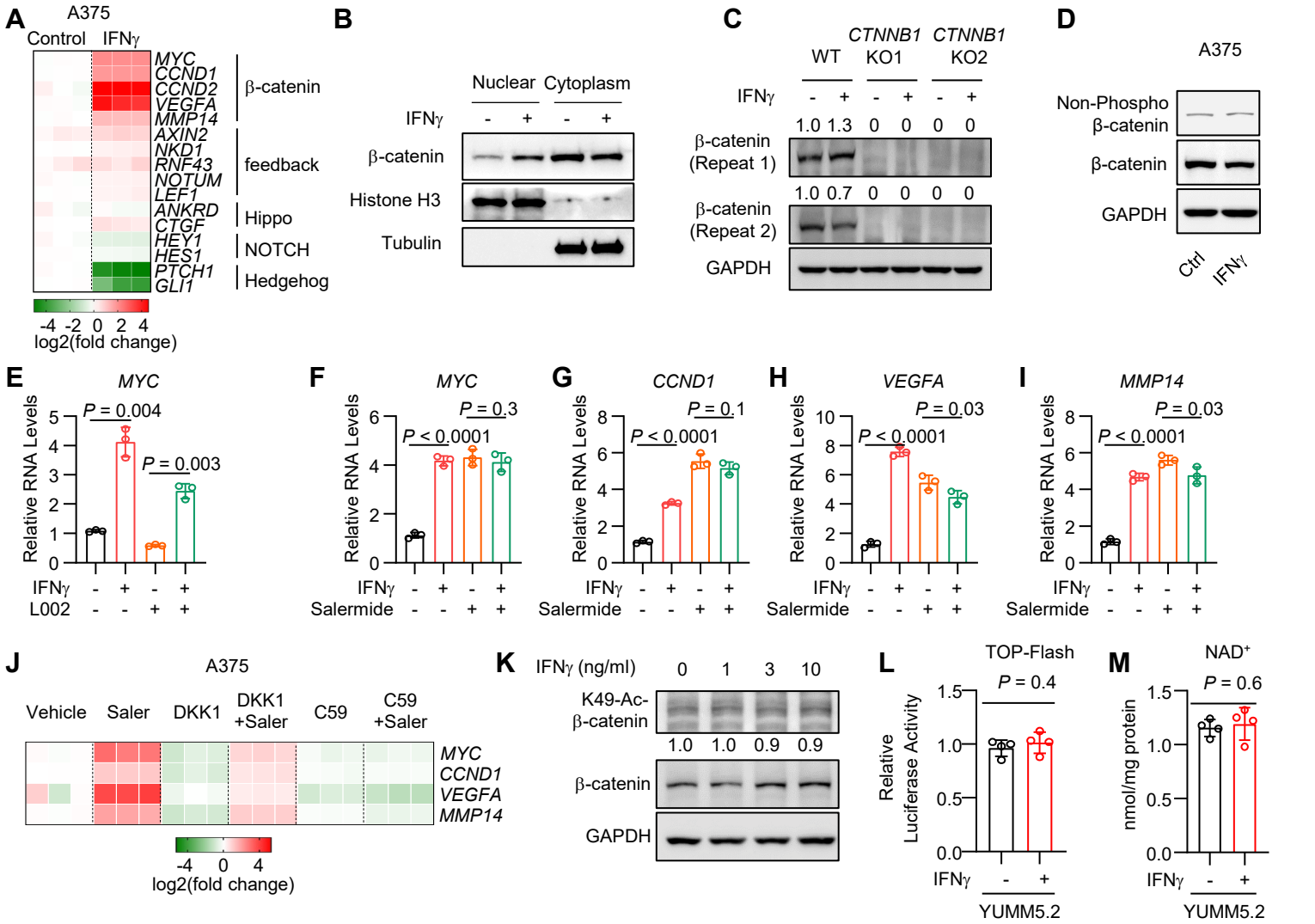


Figure S4: IFNγ reduces NAD⁺ to activate β-catenin acetylation. Related to Figure 4.

(A-B) A375 cells were treated with IFNγ for 36 hours. Indicated transcripts were determined by qRT-PCR. n = 3 (**A**). Indicated proteins were determined in the nuclear or cytoplasmic fractions, respectively. 1 of 2 blots shown (**B**).

(C) Wild type (WT) and *CTNNB1* KO A375 cells were treated with IFNγ for 24 hours. Protein levels of β-catenin (2 repeats) and GAPDH were determined by Western blotting. 1 of 2 Western blots shown.

(D) A375 cells were treated with IFNγ for 24 hours, total or non-phosphorylated β-catenin proteins were determined by Western blot. 1 of 2 Western blots shown.

(E-I). A375 cells were treated with IFNγ, in the presence of L002 (**E**) and Salermide (**F-I**), for 24 hours. β-catenin signaling gene transcripts were determined by qRT-PCR. Data are shown as mean ± s.d., n = 3. Two-tailed t-test.

(J) A375 cells were treated with Salermide (Saler) and DKK1 or Wnt-C59 (C59) for 24 hours. The indicated gene expression was determined by qPCR. n = 3.

(K) A375 cells were treated with IFNγ for 24 hours. Acetylated-β-catenin (K49) was determined by Western blotting. Quantification is indicated. 1 of 3 Western blots shown.

(L) TOP-Flash carrying YUMM5.2 cells were treated with IFNγ for 48 hours. Relative luciferase activity was determined. Data are shown as mean ± s.d., n = 4. Two-tailed t-test.

(M) YUMM5.2 cells were treated with IFNγ for 24 hours. Intracellular NAD⁺ levels were determined by kit. Data are shown as mean ± s.d., n = 4. Two-tailed t-test.

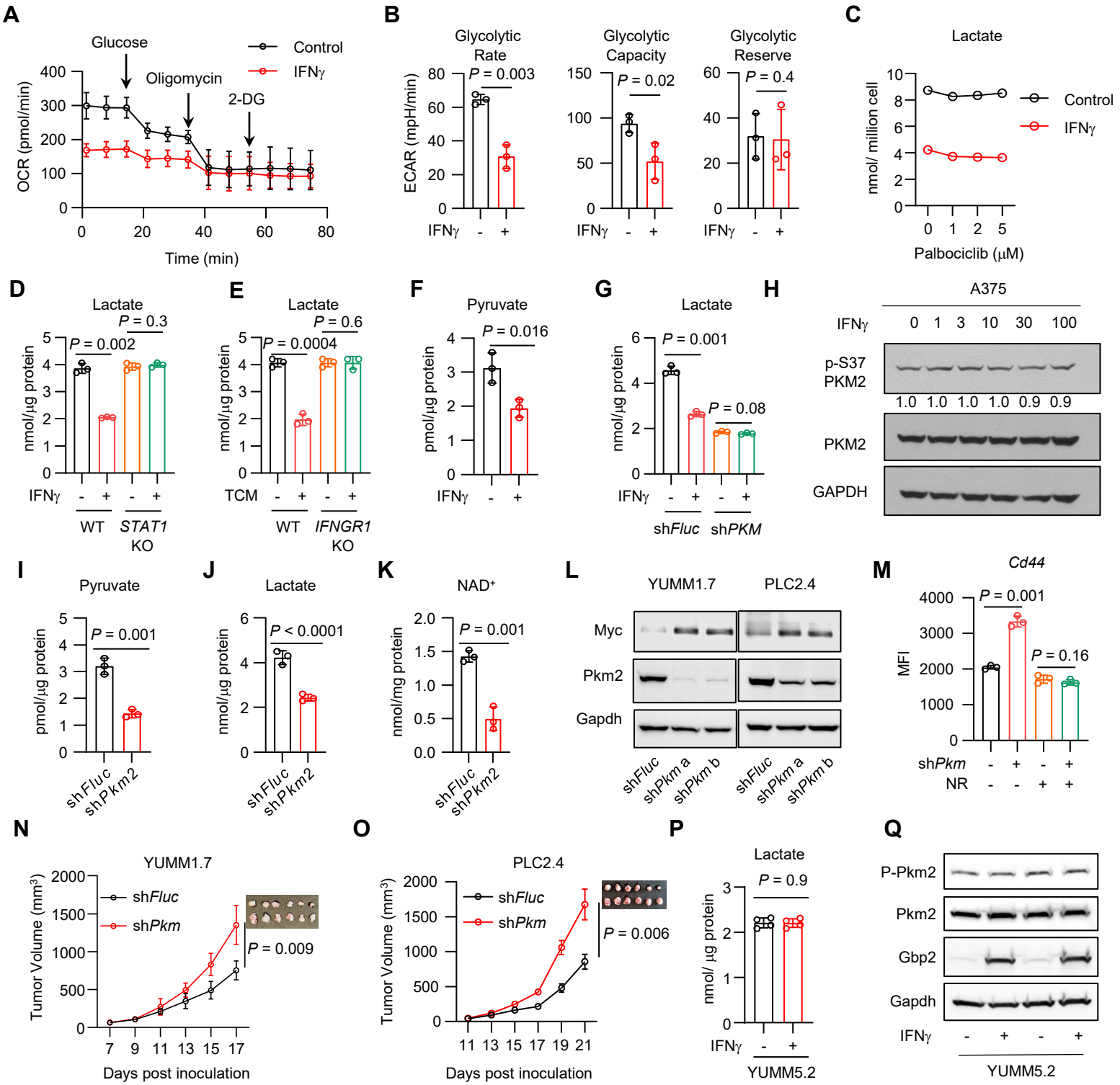

A
Glucose
Oligomycin
2-DG
Control
IFNγ
OCR (pmol/min)
Time (min)
B
Glycolytic Rate
Glycolytic Capacity
Glycolytic Reserve
ECAR (mpH/min)
P = 0.003
P = 0.02
P = 0.4
IFNγ
C
Lactate
nmol/ million cell
Palbociclib (μM)
D
Lactate
P = 0.002
P = 0.3
nmol/μg protein
WT
STAT1 KO
E
Lactate
P = 0.0004
P = 0.6
TCM
IFNGR1 KO
F
Pyruvate
P = 0.016
pmol/μg protein
G
Lactate
P = 0.001
P = 0.08
shFluc
shPKM
H
A375
p-S37 PKM2
PKM2
GAPDH
1.0 1.0 1.0 1.0 0.9 0.9
I
Pyruvate
P = 0.001
shFluc
shPkm2
J
Lactate
P < 0.0001
K
NAD+
P = 0.001
nmol/mg protein
L
YUMM1.7
PLC2.4
Myc
Pkm2
Gapdh
shPkm a
shPkm b
M
Cd44
P = 0.001
P = 0.16
MFI
shPkm
NR
N
YUMM1.7
Tumor Volume (mm3)
shPkm
P = 0.009
Days post inoculation
O
PLC2.4
P = 0.006
P
Lactate
P = 0.9
YUMM5.2
Q
P-Pkm2
Pkm2
Gbp2
Gapdh
YUMM5.2

Figure S5: IFNγ regulates PKM2 phosphorylation to alter NAD^+/ β-catenin signaling. Related to Figure 5.

(A) A375 cells were treated with IFNγ for 24 hours. Seahorse analysis showing the oxygen consumption rate (OCR) in control cells and IFNγ-treated cells in the presence of glucose, oligomycin or 2-DG. Data are shown as mean ± s.d., n = 3.

(B) Seahorse analysis showing glycolytic rate, glycolytic capacity, and glycolytic reserve in control cells or IFNγ-treated A375 cells. Data are shown as mean ± s.d., n = 3, P value by two-tailed t-test.

(C) A375 cells were treated with IFNγ and Palbociclib for 24 hours. Lactate production was determined and normalized with cell numbers. n = 3.

(D) WT or *STAT1* A375 KO cells were treated with IFNγ for 48 hours. Lactate production was determined by quantitation kit. Data are shown as mean ± s.d., n = 3, P value by two-tailed t-test.

(E) WT or *IFNGR1* KO A375 cells were treated with T cell conditioned medium (TCM) for 48 hours. Lactate production quantified by kit. Data are shown as mean ± s.d., n = 3. Two-tailed t-test.

(F) Pyruvate levels were detected in A375 cells treated with medium or IFNγ. Data are shown as mean ± s.d., n = 3. Two-tailed t-test.

(G) Control (sh*Fluc*) or *PKM2* knock down (sh*PKM2*) A375 cells were treated with IFNγ for 24 hours. Lactate production quantified by kit. Data are shown as mean ± s.d., n = 3. Two-tailed t-test.

(H) A375 cells were treated with IFNγ. Phosphorylated (S37) and total protein levels of PKM2 were detected by Western blot. Quantification is indicated. 1 of 2 Western blots shown.

(I-K) Pyruvate (**I**), Lactate (**J**), and NAD^+ (**K**) levels in YUMM1.7 sh*Fluc* and sh*Pkm2* cells were quantified by kits. Data are shown as mean ± s.d., n = 3. Two-tailed t-test.

(L) Myc and Pkm2 proteins were detected by Western blot in sh*Fluc* and sh*Pkm2* YUMM1.7 cells, and PLC2.4 cells. 1 of 2 Western blots shown.

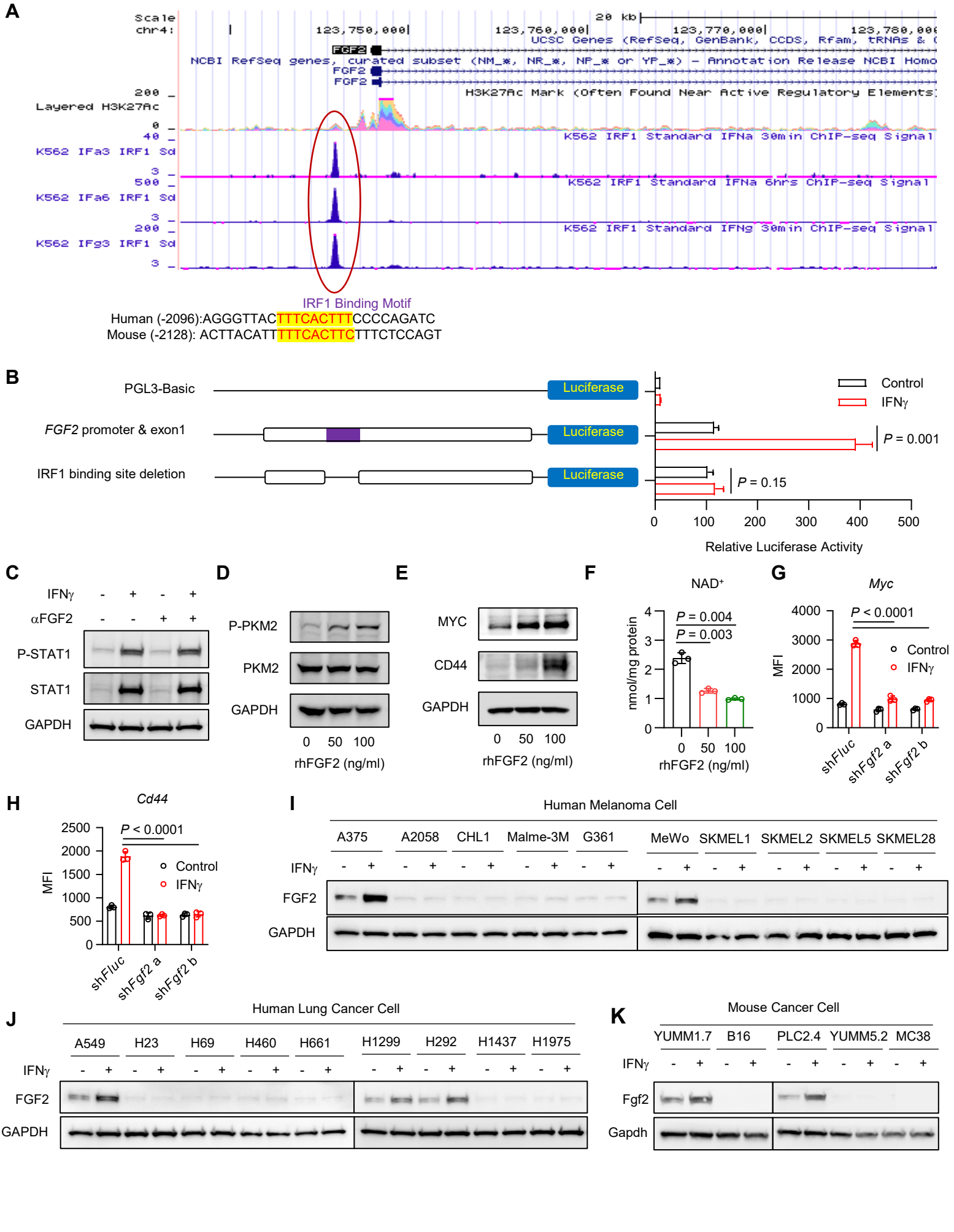
(M) sh*Fluc* or sh*Pkm2* YUMM1.7 cells were treated with nicotinamide riboside (NR) for 48 hours. Surface expression of *Cd44* was determined by FACS. Data are shown as mean ± s.d., n = 3. Two-tailed t-test.

(N-O) Tumor growth curves for sh*Fluc* and sh*Pkm2* YUMM1.7 (**N**) or PLC2.4 (**O**) cells. Data are shown as mean ± s.d., n = 6. Two-tailed t-test.

(P-Q) YUMM5.2 cells were treated with IFNγ for 24 hours. Lactate production (**P**) and Pkm2 phosphorylation (**Q**) was determined by kit and Western blot, respectively. IFNγ-responsive Gbp2 protein was utilized as a positive control. Data are shown as mean ± s.d., n = 4. Two-tailed t-test.

Table S5: FGF/ FGFR family members expression upon IFNγ treatment (RNAseq datasets from GSE99299), Related to Figure 6.

	A375 (RPKM)					B16 (RPKM)			
GENE ID	Ctrl		IFNγ		Gene ID	Ctrl		IFNγ	
FGF1	16	13	22	23	Fgf1	32	42	24	25
FGF2	418	370	1170	1171	Fgf2	0	0	1	0
FGF3	0	0	0	0	Fgf3	0	0	0	0
FGF4	0	0	0	0	Fgf4	0	0	0	0
FGF5	95	110	38	38	Fgf5	0	0	0	0
FGF6	0	0	0	0	Fgf6	0	0	0	0
FGF7	3	0	0	0	Fgf7	2	2	3	4
FGF8	0	0	0	0	Fgf8	3	1	0	0
FGF9	0	0	1	0	Fgf9	2	0	1	0
FGF10	0	0	0	0	Fgf10	0	0	0	0
FGF11	12	24	15	22	Fgf11	0	0	0	0
FGF12	36	40	37	30	Fgf12	0	0	0	0
FGF13	73	74	76	40	Fgf13	0	0	0	0
FGF14	0	1	0	1	Fgf14	0	0	0	0
FGF16	0	0	0	0	Fgf15	0	0	0	0
FGF17	0	0	0	0	Fgf16	0	0	0	0
FGF18	1	1	2	1	Fgf17	0	0	0	0
FGF19	2	2	1	2	Fgf18	0	1	0	1
FGF20	1	2	1	1	Fgf20	0	0	0	0
FGF21	3	1	9	3	Fgf21	0	0	0	0
FGF22	1	0	1	1	Fgf22	0	0	0	0
FGF23	0	0	0	0	Fgf23	0	0	0	0
FGFR1	381	462	415	413	Fgfr1	3	0	0	0
FGFR2	4	1	2	0	Fgfr2	0	3	0	1
FGFR3	25	22	9	9	Fgfr3	24	37	31	37
FGFR4	49	63	24	22	Fgfr4	0	1	0	0


A
Scale
chr4:
123,750,000
123,760,000
123,770,000
20 kb
UCSC Genes (RefSeq, GenBank, CCDS, Rfam, tRNAs &
FGF2
NCBI RefSeq genes, curated subset (NM_*, NR_*, NP_* or YP_*) - Annotation Release NCBI Homo
FGF2
FGF2
H3K27Ac Mark (Often Found Near Active Regulatory Elements)
200
Layered H3K27Ac
0
40
K562 IFa3 IRF1 Sd
K562 IRF1 Standard IFNa 30min ChIP-seq Signal
3
500
K562 IFa6 IRF1 Sd
K562 IRF1 Standard IFNa 6hrs ChIP-seq Signal
3
200
K562 IFg3 IRF1 Sd
K562 IRF1 Standard IFNg 30min ChIP-seq Signal
3
IRF1 Binding Motif
Human (-2096):AGGGTTACTTTCACTTTCCCCAGATC
Mouse (-2128): ACTTACATTTTTCACTTCTTTCTCCAGT
B
PGL3-Basic
Luciferase
FGF2 promoter & exon1
Luciferase
IRF1 binding site deletion
Luciferase
Control
IFNγ
P = 0.001
P = 0.15
0
100
200
300
400
500
Relative Luciferase Activity
C
IFNγ - + - +
αFGF2 - - + +
P-STAT1
STAT1
GAPDH
D
P-PKM2
PKM2
GAPDH
0 50 100
rhFGF2 (ng/ml)
E
MYC
CD44
GAPDH
0 50 100
rhFGF2 (ng/ml)
F
NAD+
P = 0.004
P = 0.003
nmol/mg protein
0
1
2
3
4
0 50 100
rhFGF2 (ng/ml)
G
Myc
P < 0.0001
MFI
0
1000
2000
3000
4000
Control
IFNγ
shFluc
shFgf2 a
shFgf2 b
H
Cd44
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MFI
0
500
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1500
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2500
Control
IFNγ
shFluc
shFgf2 a
shFgf2 b
I
Human Melanoma Cell
A375
A2058
CHL1
Malme-3M
G361
MeWo
SKMEL1
SKMEL2
SKMEL5
SKMEL28
IFNγ - + - + - + - + - + - + - + - + - + - +
FGF2
GAPDH
J
Human Lung Cancer Cell
A549
H23
H69
H460
H661
H1299
H292
H1437
H1975
IFNγ - + - + - + - + - + - + - + - + - +
FGF2
GAPDH
K
Mouse Cancer Cell
YUMM1.7
B16
PLC2.4
YUMM5.2
MC38
IFNγ - + - + - + - + - +
Fgf2
Gapdh

Figure S6: IFNγ induces FGF2 to control PKM2/ NAD^+/ β-catenin signaling. Related to Figure 6.

(A) IRF1 ChIP-seq datasets in K562 cells were obtained from Encode at UCSC. IRF1 binding motif was found in the *FGF2* promoter in human and mouse.

(B) *FGF2* promoter and exon1 were inserted into PGL3-basic plasmid to generate a *FGF2* promoter reporter. IRF1 binding motif was deleted to generate a mutant reporter. A375 cells carrying *FGF2* promoter reporter or mutant reporter were treated with IFNγ for 24 hours. Luciferase activity was determined by dual luciferase activity assay. Data are shown as mean ± s.d., n = 3. Two-tailed t-test.

(C) A375 cells were treated with IFNγ, in the presence or absence of FGF2 neutralizing antibody (αFGF2). Phosphorylated (Y701) and total protein levels of STAT1 were determined at 24 hours by Western blot.

(D-F) A375 cells were treated with recombinant human FGF2. Phosphorylated PKM2 (**D**), MYC, and CD44 (**E**) were determined by Western blotting. 1 of 2 Western blots shown (**D, E**). NAD^+ levels were quantified by kit (**F**). Data are shown as mean ± s.d., n = 3. Two-tailed t-test (**F**).

(G-H) sh*Fluc* or sh*Fgf2* YUMM1.7 cells were treated with IFNγ for 48 hours. *Myc* (**G**) *or Cd44* (**H**) expression were determined by FACS. Data are shown as mean ± s.d., n = 3. Two-tailed t-test.

(I-K) Human melanoma cells (**I**), human lung cancer cells (**J**), and mouse cancer cells (**K**) were treated with IFNγ for 24 hours. FGF2 protein expression was determined by Western blot.

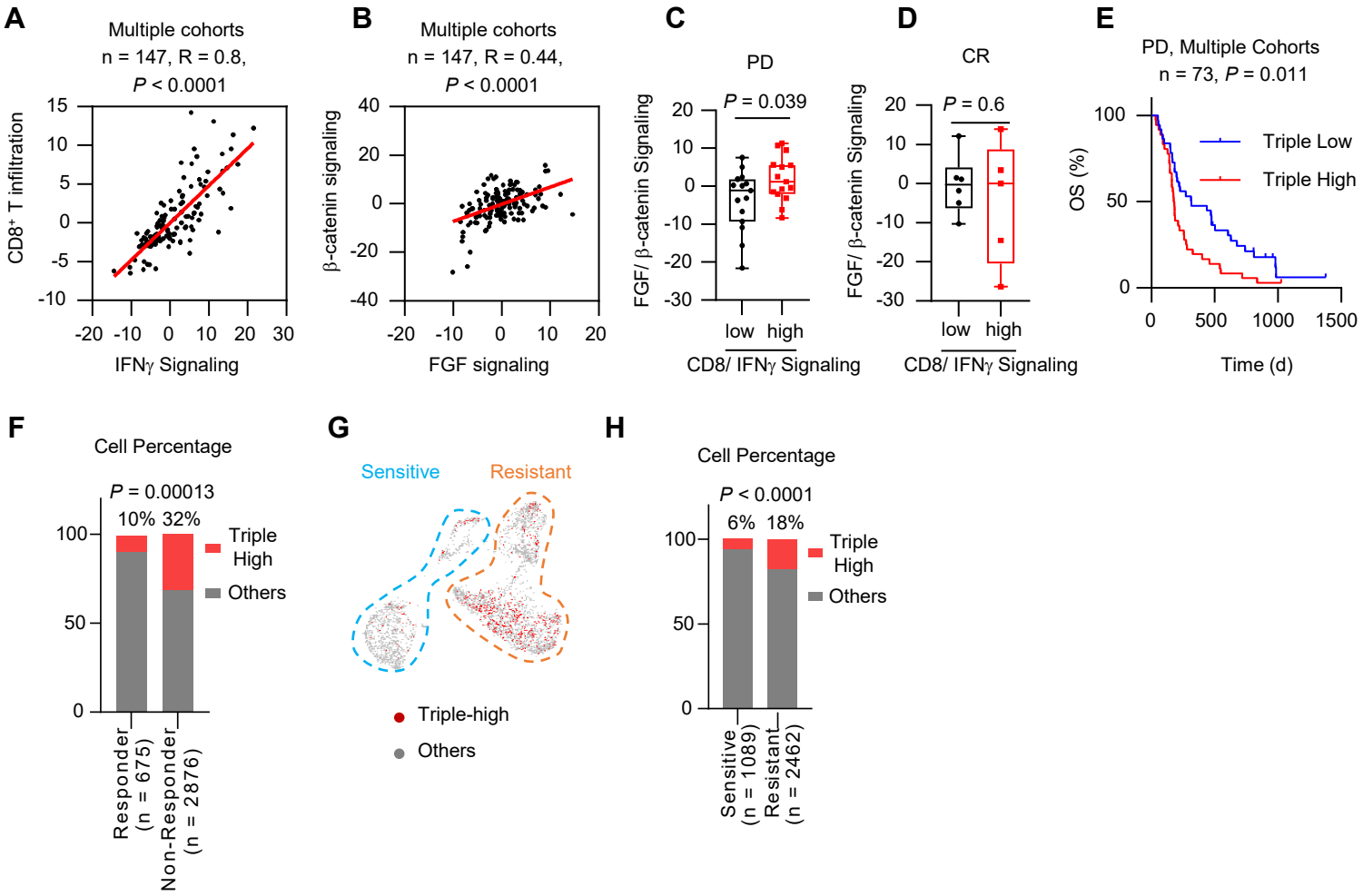

Figure S7: Oncometabolic reprogramming drives cancer hyperprogression during immunotherapy. Related to Figure 6.

(A-B) Correlation between CD8⁺ T cell infiltration and IFNγ signaling (**A**); and FGF signaling and β-catenin signaling (**B**) in 4 public cohorts combined. R and P values were determined by liner regression.

(C-D) Correlation between immunogenic and oncogenic signatures in PD (**C**) and CR (**D**) patients. Patients were divided into low and high CD8/ IFNγ signaling groups. The FGF/ β-catenin signaling levels were plotted. Two-tailed t-test.

(E) Overall survival of patients with PD on immunotherapy from multiple datasets stratified by median IFNγ/ FGF/ β-catenin gene signature scores (triple high vs triple low). HR = 0.55. Log-rank test.

(F) Percentages of tumor cells expressing triple high (IFNγ/ FGF/ β-catenin) gene signature in non-responders and responders to PD-1 therapy are shown. Chi-square test.

(G-H) Tumor cells have different sensitivities to anti-PD-1 therapy. Tumor cells expressing triple high (IFNγ/ FGF/ β-catenin) gene signatures are shown in red (**G**). Percentages of tumor cells expressing triple high (IFNγ/ FGF/ β-catenin) gene signatures in ICB-resistant or -sensitive tumor cells are shown (**H**). Chi-square test.

Table S6: Target sequences for gene knock out or knock down. Related to STAR Methods.

ID	Target sequence
Ms Ifngr1 KO1	ATTAGAACATTCGTCGGTAC
Ms Ifngr1 KO2	CTTGAACCCTGTCGTATGCT
Hm IFNGR1 KO1	GGTACTCCCAATATACGATA
Hm IFNGR1 KO2	GGTCCCTGTTTTTACCGTAG
Ms Stat1 KO1	GGTCGCAAACGAGACATCAT
Ms Stat1 KO2	CCAGTACAGCCGCTTTTCTC
Hm STAT1 KO1	GAGGTCATGAAAACGGATGG
Hm STAT1 KO2	ATTGATCATCCAGCTGTGAC
Hm CTNNB1 KO1	CTAACAGCCGCTTTTCTGTC
Hm CTNNB1 KO2	CAACAGTCTTACCTGGACTC
Hm CTNNB1 KO3	AGTCCTGTATGAGTGGGAAC
shFluc	CGCTGAGTACTTCGAAATGTC
Hm shPKM2 1	AACGCTTGTAGAACTCACTCT
Hm shPKM2 2	AAGAAGATCAACGCCTCACTG
Hm shPKM2 3	GAACTCACTCTGGGCTGTAAC
Hm shPKM2 4	CAACGCTTGTAGAACTCACTC
Ms shPkm2 1	AACGCTTGTAGTGCTCACTCT
Ms shPkm2 2	AACATGCAATAGAGACCAGCT
Ms shPkm2 3	GACTGGAAACCCTGACTTTAT
Ms shFgf2 1	AAGAGAGAGGAGTTGTGTCTA
Ms shFgf2 2	AACGAACTGGGCAGTATAAAC

Table S7: Primers for molecular cloning, Related to STAR Methods.

ID	Primer sequence
FGF2 promoter SacI F	ATATGAGCTCGATGTTGAGCCCCTTGTCATGTG
FGF2 promoter MluI R	ATATACGCGTCGTTTTTGCAGTACAGCCGCTT
Ms FGF2 OE SalI F	ATATGTCGACCATGGCTGCCAGCGGCATCAC
Ms FGF2 OE NotI R	ATATGCGGCCGCTCAGCTCTTAGCAGACATTGGAAGAAACAGTATGGC
CTNNB1 K345R F	GCAGAGTGCTGAGGGTGCTATCTGTCTGCTCTAG
CTNNB1 K345R R	ATAGCACCCTCAGCACTCTGCTTGTGGTCCACAG

Table S8: Primers for qPCR, Related to STAR Methods.

ID	Primer Sequence
Hm ACTB QF	GAGCACAGAGCCTCGCCTTT
Hm ACTB QR	ACATGCCGGAGCCGTTGTC
Hm GBP1 QF	AGCCCTACAACTTCGGAACAG
Hm GBP1 QR	TCTGGATTCGCCATCAGTCG
Hm MYC QF	TACAACACCCGAGCAAGGAC
Hm MYC QR	TTCTCCTCCTCGTCGCAGTA
Hm CCND1 QF	GATGCCAACCTCCTCAACGA
Hm CCND1 QR	GGAAGCGGTCCAGGTAGTTC
Hm VEGFA QF	CTTGCCTTGCTGCTCTACC
Hm VEGFA QR	CACACAGGATGGCTTGAAG
Hm MMP14 QF	AGTTCAGTGCCTACCGAAGAC
Hm MMP14 QR	TGTGTGTGGGTACGTAGGTC
Hm PDL1 QF	ACCTGGCTGCACTAATTGTCTA
Hm PDL1 QR	GGTGACTGGATCCACAACCAA
Hm CD47 QF	GCCTATATCCTCGCTGTGGTT
Hm CD47 QR	TTTGAATGCATTAAGGGGTTCCT
Hm FGF2 QF	GCTGTACTGCAAAAACGGGG
Hm FGF2 QR	AGCCAGGTAACGGTTAGCAC
Hm CD44 QF	AAAACTGCAGCCAACTTCCG
Hm CD44 QR	GAATACACCTGCAAAGCGGC
Hm AXIN2 QF	CAAACTTTCGCCAACCGTGGTTG
Hm AXIN2 QR	GGTGCAAAGACATAGCCAGAACC
Hm CCND2 QF	GAGAAGCTGTCTCTGATCCGCA
Hm CCND2 QR	CTTCCAGTTGCGATCATCGACG
Hm NKD1 QF	GAAGATGGAGAGAGTGAGCGAAC
Hm NKD1 QR	GTCATACAGGGTGAAGGTCCAC
Hm RNF43 QF	GGTTACATCAGCATCGGACTTGC
Hm RNF43 QR	ATGCTGGCGAATGAGGTGGAGT
Hm NOTUM QF	CTACTGGTGGAACGCAAACATGG
Hm NOTUM QR	CGCACCACCTCCTGGATGATG
Hm LEF1 QF	CTACCCATCCTCACTGTCAGTC
Hm LEF1 QR	GGATGTTCCTGTTTGACCTGAGG
Hm ANKRD1 QF	CCTGTGGATGTGCCTACGTT
Hm ANKRD1 QR	ACAGGCGATAAGATGCTCCG
Hm CTGF QF	GAGCAGCTGCAAGTACCAGT
Hm CTGF QR	GTCTTCCAGTCGGTAAGCCG
Hm HEY1 QF	GGCTGGTACCCAGTGCTTTT
Hm HEY1 QR	CCCGAAATCCCAAACTCCGA
Hm HES1 QF	GCCAGTGTCAACACGACACC
Hm HES1 QR	CCTCGTTCATGCACTCGCTG
Hm PTCH1 QF	TCGCTCTGGAGCAGATTTCC
Hm PTCH1 QR	TCTCGAGGTTCGCTGCTTTT
Hm GLI1 QF	CGGCACCCCTTCTCTTGCT
Hm GLI1 QR	CATCGAGTTGAACATGGCGTC
Ms Actb QF	CACTGTCGAGTCGCGTCCA
Ms Actb QR	GACCCATTCCCACCATCACA

Ms Myc QF	GTACCTCGTCCGATTCCACG
Ms Myc QR	GCACCTCTTGAGGACCAGTG
Ms Ccnd1 QF	TCAAGTGTGACCCGGACTG
Ms Ccnd1 QR	CACTACTTGGTGGCTCCCG
Ms Fgf2 QF	AAGCGGCTCTACTGCAAGAA
Ms Fgf2 QR	ACACTTAGAAGCCAGCAGCC
Ms Cd44 QF	TGAGACCTGCAGGTATGGGT
Ms Cd44 QR	GCTGAAGCATTGAAGCAATA
Ms Cdh1 QF	GGTCATCAGTGTGCTCACCTCT
Ms Cdh1 QR	GCTGTTGTGCTCAAGCCTTCAC
Ms Nanog QF	GAACGCCTCATCAATGCCTGCA
Ms Nanog QR	GAATCAGGGCTGCCTTGAAGAG
Ms Aldh1a1 QF	GGAATACCGTGGTTGTCAAGCC
Ms Aldh1a1 QR	CCAGGGACAATGTTTACCACGC
Ms Sox2 QF	AACGGCAGCTACAGCATGATGC
Ms Sox2 QR	CGAGCTGGTCATGGAGTTGTAC
Ms Klf4 QF	CTATGCAGGCTGTGGCAAAACC
Ms Klf4 QR	TTGCGGTAGTGCCTGGTCAGTT
Ms Bmi1 QF	ACTACACGCTAATGGACATTGCC
Ms Bmi1 QR	CTCTCCAGCATTCGTCAGTCCA
Ms Vim QF	CGGAAAGTGGAATCCTTGCAGG
Ms Vim QR	AGCAGTGAGGTCAGGCTTGGAA
Ms Zeb1 QF	ATTCAGCTACTGTGAGCCCTGC
Ms Zeb1 QR	CATTCTGGTCCTCCACAGTGGA
Ms Fn1 QF	CCCTATCTCTGATACCGTTGTCC
Ms Fn1 QR	TGCCGCAACTACTGTGATTCGG
Ms Tjp1 QF	GTTGGTACGGTGCCCTGAAAGA
Ms Tjp1 QR	GCTGACAGGTAGGACAGACGAT
Ms Snai1 QF	TGTCTGCACGACCTGTGGAAAG
Ms Snai1 QR	CTTCACATCCGAGTGGGTTTGG
Ms Snai2 QF	TCTGTGGCAAGGCTTTCTCCAG
Ms Snai2 QR	TGCAGATGTGCCCTCAGGTTTG
Ms Twist1 QF	GATTCAGACCCTCAAACTGGCG
Ms Twist1 QR	AGACGGAGAAGGCGTAGCTGAG
Ms Pcna QF	CAAGTGGAGAGCTTGGCAATGG
Ms Pcna QR	GCAAACGTTAGGTGAACAGGCTC
Ms Mki67 QF	GAGGAGAAACGCCAACCAAGAG
Ms Mki67 QR	TTTGTCCTCGGTGGCGTTATCC
Ms Cdk1 QF	CATGGACCTCAAGAAGTACCTGG
Ms Cdk1 QR	CAAGTCTCTGTGAAGAACTCGCC
Ms Cdk2 QF	TCATGGATGCCTCTGCTCTCAC
Ms Cdk2 QR	TGAAGGACACGGTGAGAATGGC
Ms Cdk4 QF	CATACCTGGACAAAGCACCTCC
Ms Cdk4 QR	GAATGTTCTCTGGCTTCAGGTCC
Ms Cdk6 QF	ACCTCTGGAGTGTCGGTTGCAT
Ms Cdk6 QR	TTCCTCTCCTGGGAGTCCAATG
Ms Cdk7 QF	TGAGAATGGAGTTCTGAAACTGGC
Ms Cdk7 QR	CCACACCATACATCCTAGCTCC
Ms Ccna2 QF	TTGTAGGCACGGCTGCTATGCT
Ms Ccna2 QR	GGTGCTCCATTCTCAGAACCTG

Ms Ccnb1 QF	AGAGGTGGAACTTGCTGAGCCT
Ms Ccnb1 QR	GCACATCCAGATGTTTCCATCGG
Ms Ccne1 QF	AAGCCCTCTGACCATTGTGTCC
Ms Ccne1 QR	CTAAGCAGCCAACATCCAGGAC