Figure S6

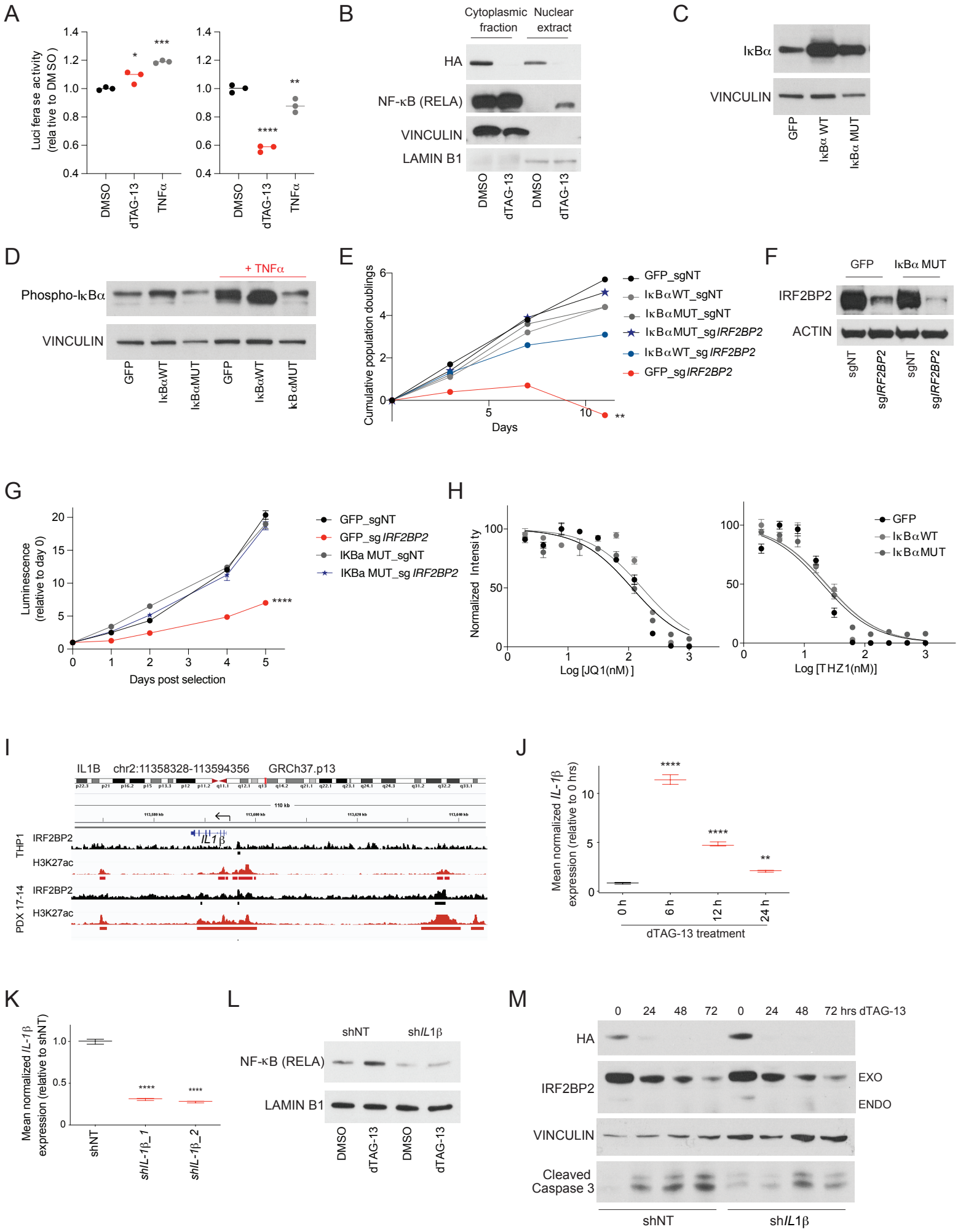
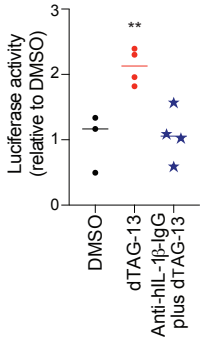
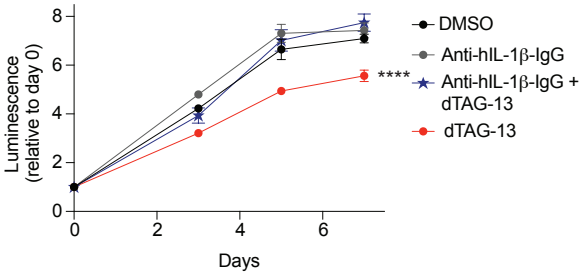

A
Luciferase activity (relative to DMSO)
DMSO
dTAG-13
TNFα
B
Cytoplasmic fraction
Nuclear extract
HA
NF-κB (RELA)
VINCULIN
LAMIN B1
C
IκBα
VINCULIN
GFP
IκBα WT
IκBα MUT
D
+ TNFα
Phospho-IκBα
VINCULIN
GFP
IκBαWT
IκBαMUT
kB αMUT
E
Cumulative population doublings
Days
GFP_sgNT
IκBαWT_sgNT
IκBαMUT_sgNT
IκBαMUT_sg IRF2BP2
IκBαWT_sg IRF2BP2
GFP_sg IRF2BP2
F
GFP
IκBα MUT
IRF2BP2
ACTIN
sgNT
sgIRF2BP2
G
Luminescence (relative to day 0)
Days post selection
GFP_sgNT
GFP_sg IRF2BP2
IKBa MUT_sgNT
IKBa MUT_sg IRF2BP2
H
Normalized Intensity
Log [JQ1(nM)]
Log [THZ1(nM)]
GFP
IκBαWT
IκBαMUT
I
IL1B chr2:11358328-113594356 GRCh37.p13
THP1
IRF2BP2
IL1β
H3K27ac
PDX 17-14
IRF2BP2
H3K27ac
J
Mean normalized IL-1β expression (relative to 0 hrs)
0 h
6 h
12 h
24 h
dTAG-13 treatment
K
Mean normalized IL-1β expression (relative to shNT)
shNT
shIL-1β_1
shIL-1β_2
L
shNT
shIL1β
NF-κB (RELA)
LAMIN B1
DMSO
dTAG-13
M
0 24 48 72 0 24 48 72 hrs dTAG-13
HA
IRF2BP2
EXO
ENDO
VINCULIN
Cleaved Caspase 3
shNT
shIL1β

Figure S6

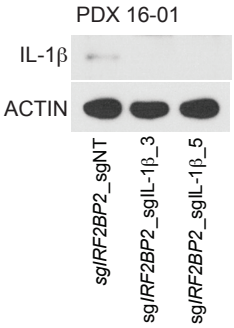
N



O



P



Q

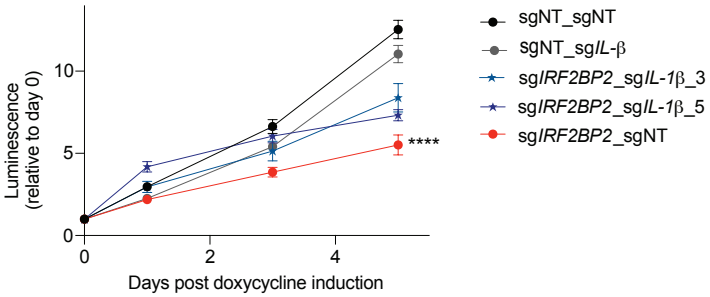


Figure S6. IRF2BP2 controls NF-κB signaling

A, NF-κB reporter assay in MV4-11 cells with degradable IRF2BP2 and NF-κB reporter showing luciferase activity following treatment with dTAG-13 for 24 hours (left panel) and 72 hours (right panel) relative to the luminescence signal of cells treated with DMSO. TNFα was used as a positive CTRL. One-way ANOVA, Dunnett's multiple comparison test, **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

B, Western blot analysis showing NF-κB protein (RELA) in the cytoplasmic fraction and nuclear extracts following degradation of IRF2BP2 after treating PDX 16-01 cells with degradable IRF2BP2 with dTAG-13 for 24 hours. Vinculin indicates the cytoplasmic fraction; lamin B1 is found in nuclear extracts.

C, Western blot analysis for IκBα showing overexpression of GFP control ORF, IκBα WT or IκBα MUT in MV4-11 cells. Vinculin was used as a loading control.

D, Western blot analysis for phospho-IκBα in MV4-11 cells showing overexpression of GFP control ORF, IκBα WT or IκBα MUT after treatment with TNFα for 5 minutes or exposure to the solvent H_2O. Vinculin was used as a loading control.

E, Cumulative population doublings over time assessed by serial cell counting of MV4-11 cells expressing GFP control ORF, IκBα WT or IκBα MUT plus control guide (sgNT) or *IRF2BP2* CRISPR guide (sg*IRF2BP2*). Differential analysis for best-fitted exponential growth curves based on the extra sum of squares (F-test), ** $p < 0.01$.

F, Western blot analysis for IR2BP2 protein in PDX 17-14 cells overexpressing plx317 GFP control ORF (GFP) or plx317 IκBα(S32A/S36A) (IκBαMUT), double-infected with either non-targeting CRISPR guide (sgNT), or sg*IRF2BP2*.

G, Viability assay in PDX 17-14 cells overexpressing plx317 GFP control ORF or plx317 IκBα(S32A/S36A), double-infected with either non-targeting CRISPR guide (sgNT), or an *IRF2BP2* targeting guide (sg*IRF2BP2*). Two-way ANOVA, **** $p < 0.0001$.

H, Viability assay in MV4-11 cells expressing GFP control ORF, IκBα WT or IκBα MUT treated with increasing concentrations of JQ1 (upper graph) or THZ1 (lower graph) for 72 hours.

I, Integrated genomics viewer plots (GRCh37/hg19) showing IRF2BP2 and H3K27ac ChIP-seq RPKM binding signal in the IL-1ß neighborhood region in THP1 cells and PDX 17-14.

J, Quantitative PCR showing mean normalized (to 0 hours) *IL-1ß* expression of at 6, 12 and 24 hours post degradation of IRF2BP2 in MV4-11 cells. One-way ANOVA, Dunnett's multiple comparison test, **** $p < 0.0001$, ** $p < 0.01$.

K, Quantitative PCR showing mean normalized (to shNT) *IL-1ß* expression in MV4-11 cells with degradable IRF2BP2 infected with *IL-1ß* targeting hairpins. One-way ANOVA, Dunnett's multiple comparison test, **** $p < 0.0001$.

L, Western blot analysis for NF-κB (RELA) of nuclear extracts from cellular fractionation in MV4-11 cells with degradable IRF2BP2 and control hairpin or knock-down of *IL-1ß*.

M, Western blot analysis for HA, IRF2BP2, vinculin, and cleaved caspase 3 in MV4-11 cells with degradable IRF2BP2 and non-targeting shRNA (shNT) or shRNA against *IL-1ß* in a time course experiment from 0 to 72 hours after degradation of IRF2BP2.

N, NF-κB reporter assay in MV4-11 cells with degradable IRF2BP2 and NF-κB reporter pre-treated with anti-hIL-1ß-IgG or the solvent H_20, showing luciferase activity following

treatment with dTAG-13 for 6 hours relative to the luminescence signal of cells treated with DMSO. One-way ANOVA, Dunnett's multiple comparison test, ** $p < 0.01$.

O, CellTiter-Glo viability assay in MV4-11 cells with degradable IRF2BP2 in MV4-11 cells upon exposure to DMSO, anti-hIL-1ß-IgG or dTAG-13 (following pre-treatment with anti-hIL-1ß-IgG for 12 hours). Two-way ANOVA, **** $p < 0.0001$.

P, Western blot analysis for IL-1ß in PDX 16-01 cells transduced with an *IRF2BP2*-targeting sgRNA plus a sgRNA targeting *IL-1ß* (*sgIL1β*_3/5) or a non-targeting control sgRNA (sgNT). Actin was used as a loading control.

Q, CellTiter-Glo viability assay in PDX 16-01 double transduced with non-targeting control guides (sgNT_sgNT), non-targeting control guide and *IL-1β*-targeting guide (sgNT_sg*IL-1β*_5), or knockout of *IRF2BP2* (sg*IRF2BP2*_sgNT), or double knockout of *IRF2BP2* and *IL-1β* (sg*IRF2BP2*_sg*IL-1β*_3/5). Two-way ANOVA, **** $p < 0.0001$.