Figure S3

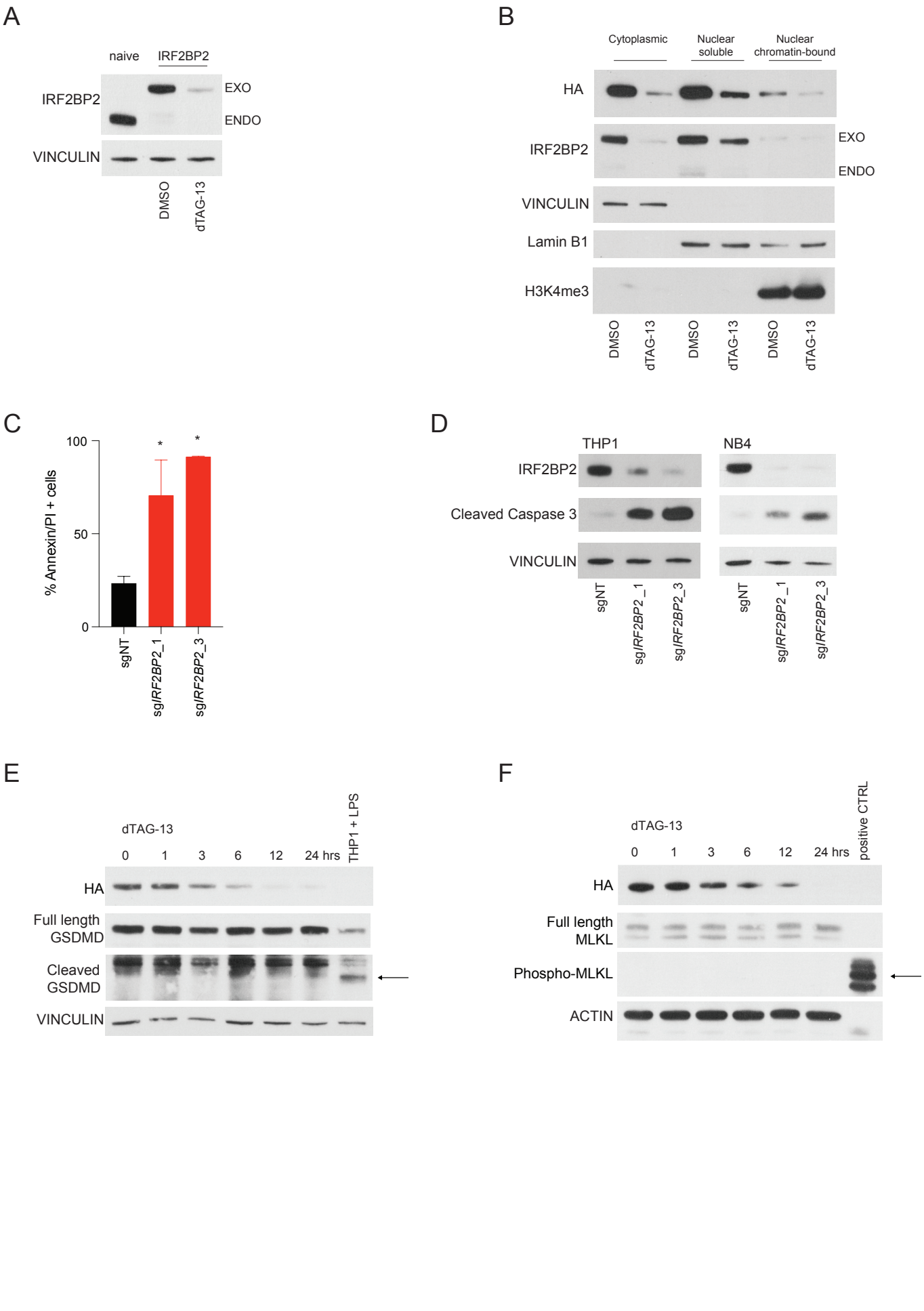
A
naive
IRF2BP2
IRF2BP2
EXO
ENDO
VINCULIN
DMSO
dTAG-13
B
Cytoplasmic
Nuclear soluble
Nuclear chromatin-bound
HA
IRF2BP2
EXO
ENDO
VINCULIN
Lamin B1
H3K4me3
DMSO
dTAG-13
DMSO
dTAG-13
DMSO
dTAG-13
C
% Annexin/PI + cells
100
50
0
*
*
sgNT
sgIRF2BP2_1
sgIRF2BP2_3
D
THP1
NB4
IRF2BP2
Cleaved Caspase 3
VINCULIN
sgNT
sgIRF2BP2_1
sgIRF2BP2_3
sgNT
sgIRF2BP2_1
sgIRF2BP2_3
E
dTAG-13
0
1
3
6
12
24 hrs
THP1 + LPS
HA
Full length GSDMD
Cleaved GSDMD
VINCULIN
F
dTAG-13
0
1
3
6
12
24 hrs
positive CTRL
HA
Full length MLKL
Phospho-MLKL
ACTIN

Figure S3. IRF2BP2 is degraded in all cellular compartments and its perturbation leads to Annexin/PI-positive cells

A, Western blot analysis showing endogenous and exogenous IRF2BP2 expression in naïve MV4-11 cells or MV4-11 cells with a degradable N-terminally tagged $FKBP12^{F36V}$-HA-*IRF2BP2*-fusion and knockout of endogenous *IRF2BP2*, treated with DMSO or dTAG-13 for 24 hours.

B, Western blot analysis showing HA and IRF2BP2 following degradation of IRF2BP2 for 24 hours in the cytoplasmic, nuclear soluble and nuclear chromatin-bound fractions. Vinculin, lamin B1 and H3K4me3 serve as markers of the cellular fractions and loading controls.

C, Quantification of flow-cytometry experiments in MV4-11 cells infected with control guide (sgNT) or hairpins against IRF2BP2 (sg*IRF2BP2*_1 and sg*IRF2BP2*_3) detecting Annexin V/PI + cells. One-way ANOVA, Dunnett's multiple comparison test, * $p < 0.05$.

D, Western blot analysis for cleaved caspase 3 in THP1 (left) and NB4 (right) cells transduced with a non-targeting control CRISPR guide (sgNT) or *IRF2BP2*-targeting sgRNAs. Vinculin was used as a loading control.

E, Western blot analysis for (cleaved) Gasdermin D (GSDMD) in MV4-11 cells over 24 hours with degradation of IRF2BP2. LPS-treated THP1 cells were used as positive control. HA antibody was used to detect IRF2BP2. Vinculin was used as a loading control.

F, Western blot analysis for (phospho)MLKL in MV4-11 cells over 24 hours with degradation of IRF2BP2. Positive control phospho-MLKL lysate was purchased and used following the manufacturers protocol (see methods section).