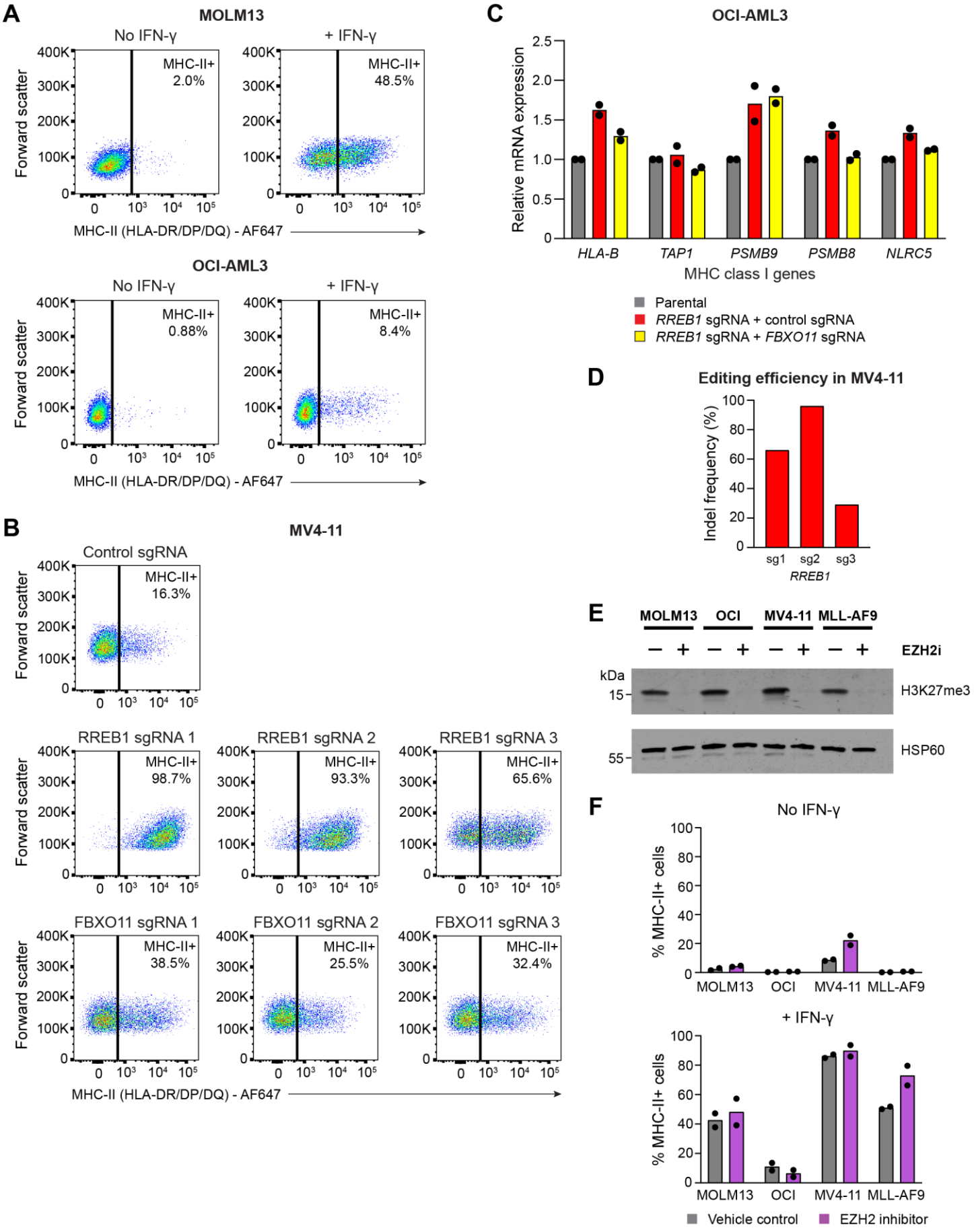
Figure S1. Related to Figure 1. Loss of RREB1 or FBXO11 induces MHC-II upregulation in deficient AML cell lines.



(A) Cell surface MHC-II expression in MOLM13 and OCI-AML3 cells in the presence or absence of IFN-γ 25 ng/mL for 48 hours. Representative data from 3 experiments.

(B) Cell surface MHC-II expression in MV4-11 Cas9 cells transduced with individual sgRNAs targeting the indicated genes or a control sgRNA. Representative data from 3 experiments.

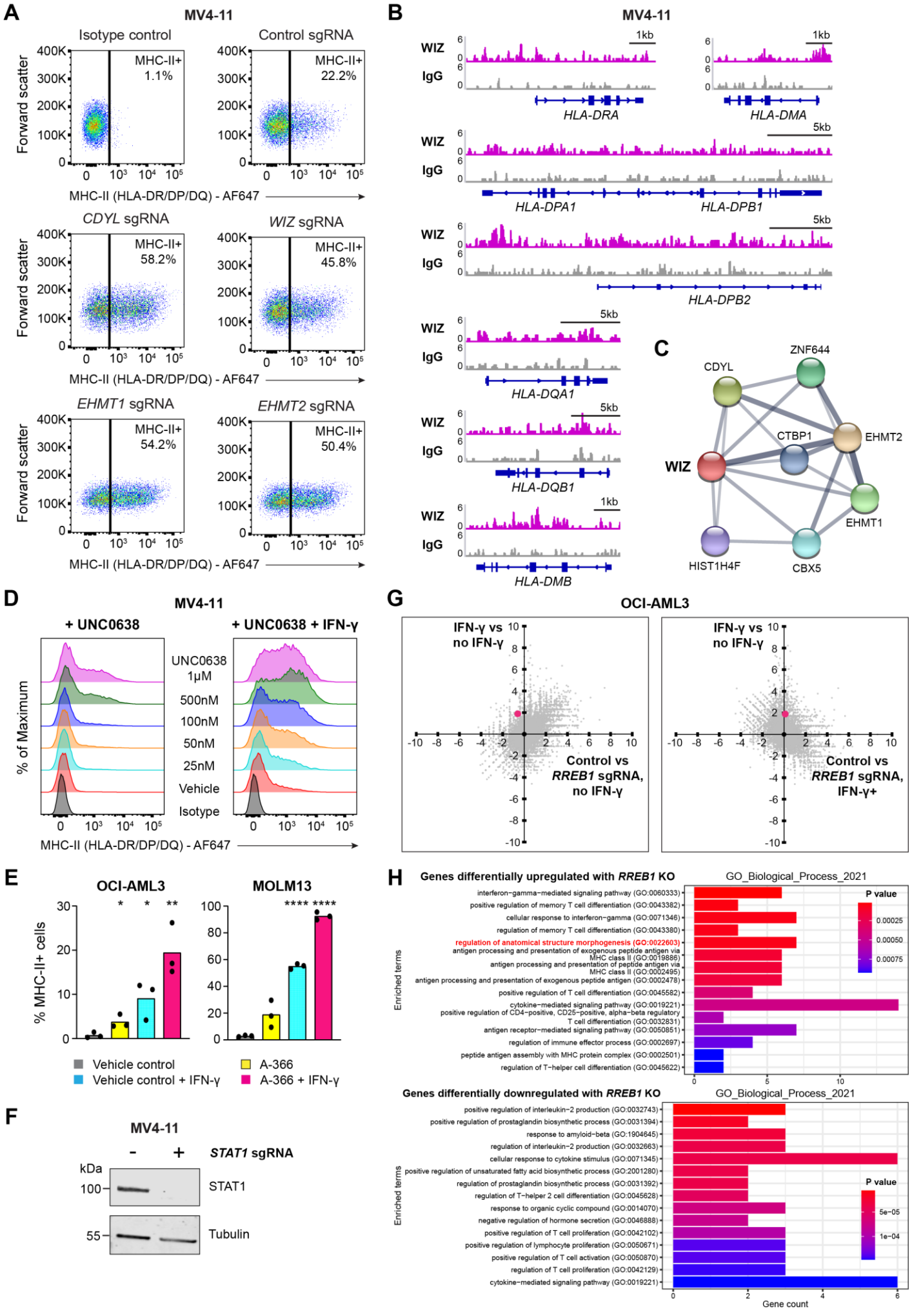
(C) mRNA expression of MHC-I pathway genes in OCI-AML3 cells transduced with sgRNAs targeting the indicated genes. Bars depict mean fold change in expression from 3 independent experiments and points indicate the mean of technical triplicates from individual experiments.

(D) Indel frequency for *RREB1* sgRNA as determined by ICE analysis.

(E) Immunoblot of the indicated cell lines treated with EZH2 inhibitor (EPZ-011989 3μM) or vehicle control for 7 days.

(F) Cell surface MHC-II expression in the indicated cell lines treated with EZH2 inhibitor (EPZ-011989 3μM) for 7 days, in the presence or absence of human IFN-γ 25 ng/mL (MOLM13 and OCI-AML3), human IFN-γ 10 ng/mL (MV4-11) or mouse IFN-γ 10 ng/mL (MLL-AF9) for 48 hours. Bars depict the mean percentage of MHC-II^{+} cells from 3 independent experiments indicated by points.

Figure S2. Related to Figure 2. CtBP complex targeting induces MHC-II expression.



(A) Cell surface MHC-II expression in MV4-11 Cas9 cells transduced with a pool of 2 sgRNAs targeting the indicated genes or a control sgRNA. Representative data from 3 experiments.

(B) ChIP-seq plots for WIZ in MV4-11 cells at MHC-II pathway genes.

(C) STRING network analysis of known direct protein interactors with WIZ.

(D) Cell surface MHC-II expression in MV4-11 cells treated with EHMT1/2 inhibitor (UNC0638) at the indicated doses or vehicle control, in the presence or absence of IFN-γ 10 ng/mL for 24 hours. Representative data from 2 experiments.

(E) Cell surface MHC-II expression in MOLM13 and OCI-AML3 cells treated with EHMT1/2 inhibitor (A-366 1μM) or vehicle control, in the presence or absence of IFN-γ 25 ng/mL for 48 hours. Bars depict the mean percentage of MHC-II$^+$ cells from 3 independent experiments indicated by points. Statistical analysis by unpaired t test compared to control cells; p value * < 0.05, ** < 0.01, **** < 0.0001.

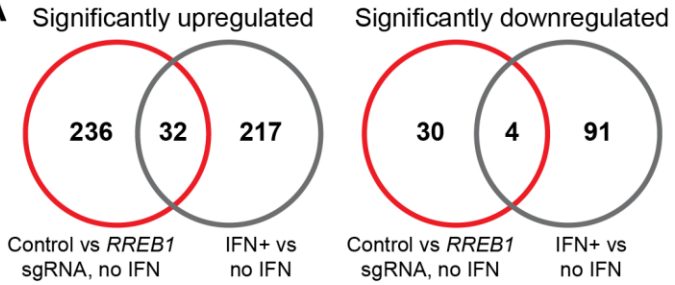
(F) Immunoblot of MV4-11 Cas9 cells transduced with a pool of 2 sgRNAs targeting *STAT1* or a control sgRNA.

(G) RNA-seq correlation plots showing differential gene expression in OCI-AML3 cells in the presence or absence of IFN-γ 25 ng/mL for 48 hours (Y-axis) and OCI-AML3 Cas9 cells transduced with control and *RREB1* sgRNA in the presence or absence of IFN-γ 25 ng/mL for 48 hours (X-axis). HLA-G is highlighted.

(H) Gene set enrichment analysis performed on all differentially upregulated and downregulated genes with *RREB1* KO. For genes differentially upregulated with *RREB1* KO, the term highlighted in red is the only one not associated with MHC-II pathway genes.

Figure S3. Related to Figure 3. Differentially expressed genes with IFN-γ treatment and RREB1 or FBXO11 depletion.

A



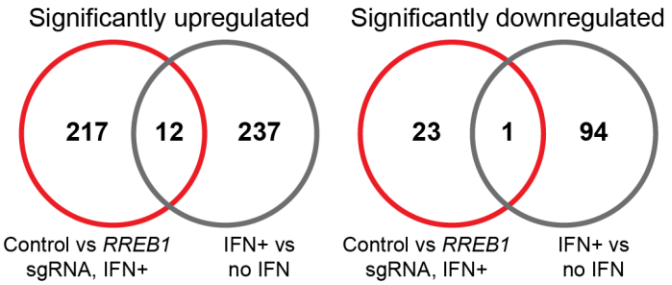
Significantly upregulated in both conditions:

Gene	log_2FC Control vs *RREB1* sgRNA, no IFN	p value Control vs *RREB1* sgRNA, no IFN	log_2FC IFN+ vs no IFN	p value IFN+ vs no IFN
HLA-DPA1	7.439	7.198E-08	4.285	8.301E-03
HLA-DRB1	6.133	1.876E-21	3.384	1.633E-06
HLA-DRA	4.748	6.391E-29	3.780	2.662E-18
AGT	3.903	1.576E-06	3.456	2.477E-05
OTOF	3.329	6.774E-07	2.298	1.912E-03
FAM134B	3.265	4.942E-05	2.584	2.415E-03
KYNU	2.984	1.968E-02	2.841	2.342E-02
CIITA	2.917	3.670E-03	3.108	1.019E-03
PROCR	2.767	1.965E-08	2.579	1.652E-07
MS4A3	2.747	2.082E-13	1.067	3.375E-02
SLC22A23	2.612	1.806E-02	2.688	1.023E-02
NLGN3	2.404	1.435E-02	2.305	1.688E-02
CLU	2.337	1.966E-06	1.820	5.147E-04
KRT81	2.328	2.905E-09	1.501	6.277E-04
IL18BP	2.192	1.432E-02	2.159	1.313E-02
RNF207	2.165	1.475E-02	1.953	2.911E-02
KIF26B	2.105	9.693E-08	1.536	3.143E-04
IL12RB1	2.075	3.164E-06	3.354	1.114E-17
C5orf42	2.008	1.921E-03	3.302	9.331E-10
PRKAR2B	1.894	4.421E-14	1.637	1.575E-10
lnc-BCL2L11-3:1	1.779	2.241E-02	1.785	1.708E-02
LINC00899	1.770	4.766E-05	1.083	3.677E-02
SMARCD3	1.626	9.882E-03	1.750	3.127E-03
SULT1A3	1.545	5.376E-03	1.506	5.647E-03
LINC00599	1.542	5.760E-03	2.830	3.758E-10
SPHK2	1.526	4.010E-02	1.467	4.425E-02
MRAS	1.512	3.545E-04	2.292	4.308E-10
SLAMF8	1.322	1.529E-03	3.006	7.281E-20
APOL4	1.275	3.687E-03	3.744	8.452E-31
FGR	1.227	3.366E-03	1.037	1.746E-02
LINC-PINT:25	1.210	4.243E-02	1.381	1.096E-02
HEG1	1.030	1.059E-02	1.971	6.907E-10

Significantly downregulated in both conditions:

Gene	log_2FC Control vs *RREB1* sgRNA, no IFN	p value Control vs *RREB1* sgRNA, no IFN	log_2FC IFN+ vs no IFN	p value IFN+ vs no IFN
GJA1	-1.926	4.217E-07	-3.047	4.010E-11
MT1H	-1.723	3.343E-19	-1.601	1.055E-16
RN7SK	-1.056	1.532E-06	-1.264	8.438E-09
RGCC	-1.003	1.810E-02	-1.371	6.423E-04

B



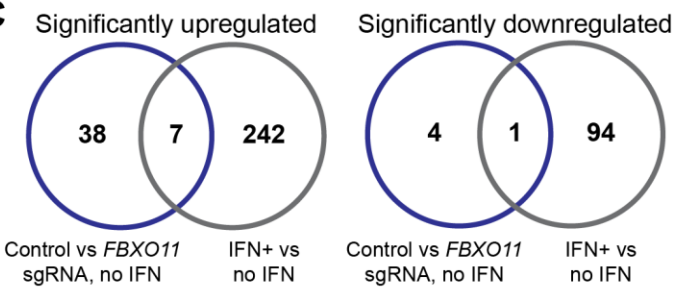
Significantly upregulated in both conditions:

Gene	log_2FC Control vs *RREB1* sgRNA, IFN+	p value Control vs *RREB1* sgRNA, IFN+	log_2FC IFN+ vs no IFN	p value IFN+ vs no IFN
HLA-DPA1	5.020	1.386E-21	4.285	8.301E-03
HLA-DRB1	4.437	3.165E-95	3.384	1.633E-06
HLA-DRA	3.123	1.810E-103	3.780	2.662E-18
CLU	3.006	1.120E-25	1.820	5.147E-04
OTOF	2.823	1.306E-14	2.298	1.912E-03
FAM134B	2.028	8.844E-06	2.584	2.415E-03
AGT	1.833	5.600E-07	3.456	2.477E-05
KRT81	1.824	1.162E-10	1.501	6.277E-04
MS4A3	1.468	7.982E-06	1.067	3.375E-02
PRKAR2B	1.227	9.110E-11	1.637	1.575E-10
PROCR	1.211	1.181E-04	2.579	1.652E-07
KIF26B	1.073	2.031E-03	1.536	3.143E-04

Significantly downregulated in both conditions:

Gene	log_2FC Control vs *RREB1* sgRNA, IFN+	p value Control vs *RREB1* sgRNA, IFN+	log_2FC IFN+ vs no IFN	p value IFN+ vs no IFN
MT1H	-1.167	6.840E-05	-1.601	1.055E-16

C



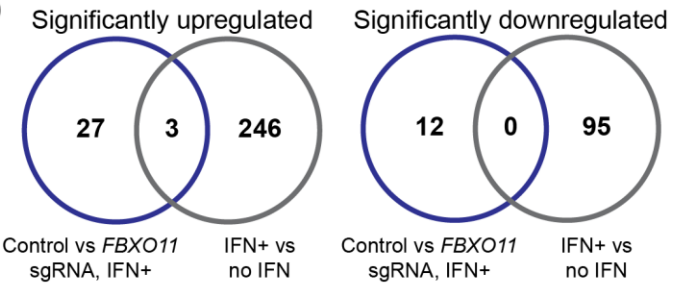
Significantly upregulated in both conditions:

Gene	log_2FC Control vs *FBXO11* sgRNA, no IFN	p value Control vs *FBXO11* sgRNA, no IFN	log_2FC IFN+ vs no IFN	p value IFN+ vs no IFN
MS4A3	2.964	1.452E-14	1.067	3.375E-02
NAV3	2.627	4.710E-06	2.771	2.936E-08
HLA-DRB1	2.459	2.145E-02	3.384	1.633E-06
HLA-DRA	2.122	3.639E-04	3.780	2.662E-18
C5orf42	2.051	1.273E-02	3.302	9.331E-10
GBP4	1.396	1.145E-03	6.605	1.743E-136
GBP5	1.032	2.057E-02	6.608	1.675E-181

Significantly downregulated in both conditions:

Gene	log_2FC Control vs *FBXO11* sgRNA, no IFN	p value Control vs *FBXO11* sgRNA, no IFN	log_2FC IFN+ vs no IFN	p value IFN+ vs no IFN
HES1	-1.491	9.710E-03	-1.461	8.699E-04

D

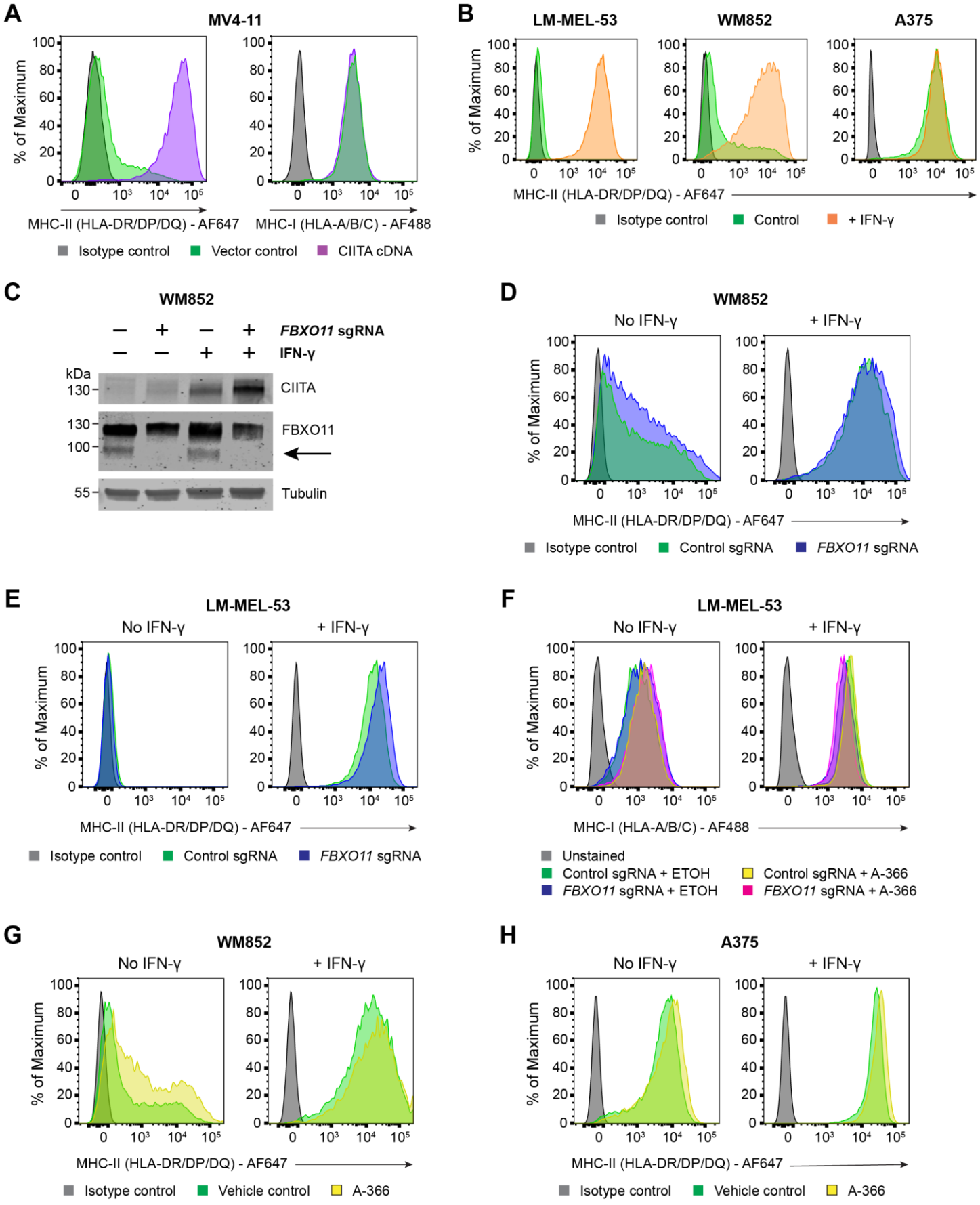


Significantly upregulated in both conditions:

Gene	log_2FC Control vs *FBXO11* sgRNA, IFN+	p value Control vs *FBXO11* sgRNA, IFN+	log_2FC IFN+ vs no IFN	p value IFN+ vs no IFN
HLA-DRB1	2.378	1.520E-23	3.384	1.633E-06
HLA-DRA	2.365	3.090E-56	3.780	2.662E-18
HLA-DPA1	2.003	2.155E-02	4.285	8.301E-03

(A and B) Venn diagrams show the number of significant differentially expressed genes in OCI-AML3 cells in the presence or absence of IFN-γ 25 ng/mL for 48 hours and OCI-AML3 cells transduced with control and *RREB1* sgRNA (A) and with IFN-γ 25 ng/mL for 48 hours (B). Genes that are significantly upregulated or downregulated (defined as fold change > 1.0 and false discovery rate < 0.05) in both conditions are listed, with MHC-II pathway genes highlighted.
(C and D) Venn diagrams show the number of significant differentially expressed genes in OCI-AML3 cells in the presence or absence of IFN-γ 25 ng/mL for 48 hours and OCI-AML3 cells transduced with control and *FBXO11* sgRNA (C) and with IFN-γ 25 ng/mL for 48 hours (D). Genes that are significantly upregulated or downregulated (defined as fold change > 1.0 and false discovery rate < 0.05) in both conditions are listed, with MHC-II pathway genes highlighted.

Figure S4. Related to Figures 3 and 4. CtBP complex and FBXO11 targeting in melanoma cell lines.



(A) Cell surface MHC-II and MHC-I expression in MV4-11 cells transduced with a retroviral vector encoding CIITA cDNA or vector control. Representative data from 3 experiments.

(B) Cell surface MHC-II expression in LM-MEL-53, WM852 and A375 cells, in the presence or absence of IFN-γ 10 ng/mL for 48 hours. Representative data from 2 experiments.

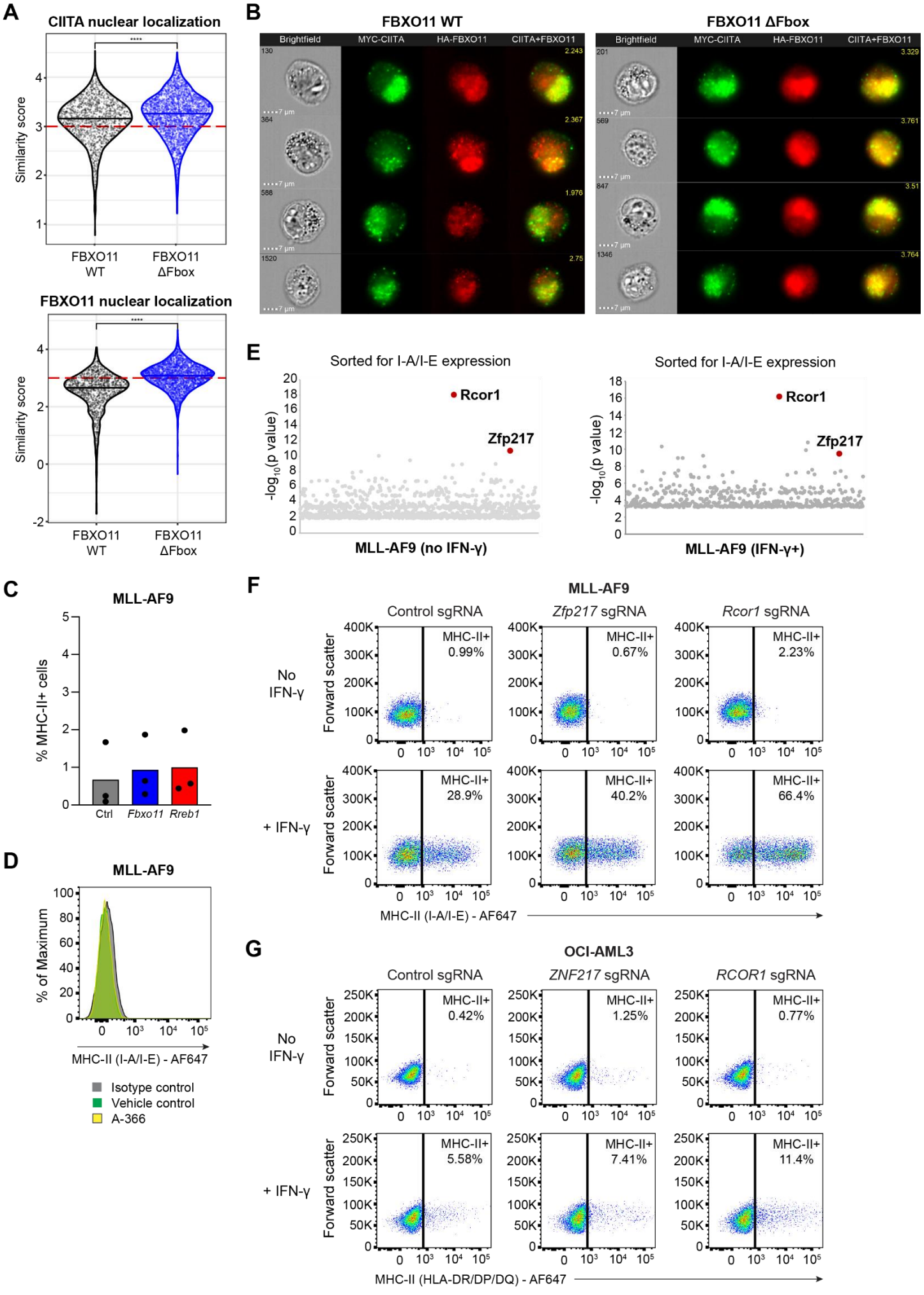
(C and D) Immunoblot (C) and cell surface MHC-II expression (D) in WM852 cells transduced with a retroviral vector encoding CIITA cDNA with or without *FBXO11* sgRNA, in the presence or absence of IFN-γ 10 ng/mL for 48 hours. Representative data from 2 experiments.

(E) Cell surface MHC-II expression in LM-MEL-53 cells transduced with control or *FBXO11* sgRNA, in the presence or absence of IFN-γ 10 ng/mL for 48 hours. Representative data from 2 experiments.

(F) Cell surface MHC-I expression in LM-MEL-53 Cas9 cells transduced with control or *FBXO11* sgRNA and treated with EHMT1/2 inhibitor (A-366 3μM) or vehicle control for 7 days, in the presence or absence of IFN-γ 10 ng/mL for 48 hours. Representative data from 2 experiments.

(G and H) Cell surface MHC-II expression in WM852 (G) and A375 (H) cells treated with EHMT1/2 inhibitor (A-366 3μM) or vehicle control for 7 days, in the presence or absence of IFN-γ 10 ng/mL for 48 hours. Representative data from 2 experiments.

Figure S5. Related to Figures 4 and 5. Regulation of MHC-II expression in melanoma and mouse AML cells.



(A) Summary of CIITA and FBXO11 nuclear localization in LM-MEL-53 cells expressing FBXO11 WT or FBXO11 ΔFbox. High shared localization with DAPI defined by similarity score ≥ 3 (red dotted line), as described in Beum *et al*, 2006; Erie *et al*, 2011; and George *et al*, 2006. Statistical significance was calculated using Dunn's test with Bonferroni correction for multiple comparisons. **** = $p < 0.0001$.

(B) Representative images showing brightfield microscopy (cell number at top left), as well as CIITA and FBXO11 expression (similarity score at top right) in LM-MEL-53 cells expressing FBXO11 WT or FBXO11 ΔFbox.

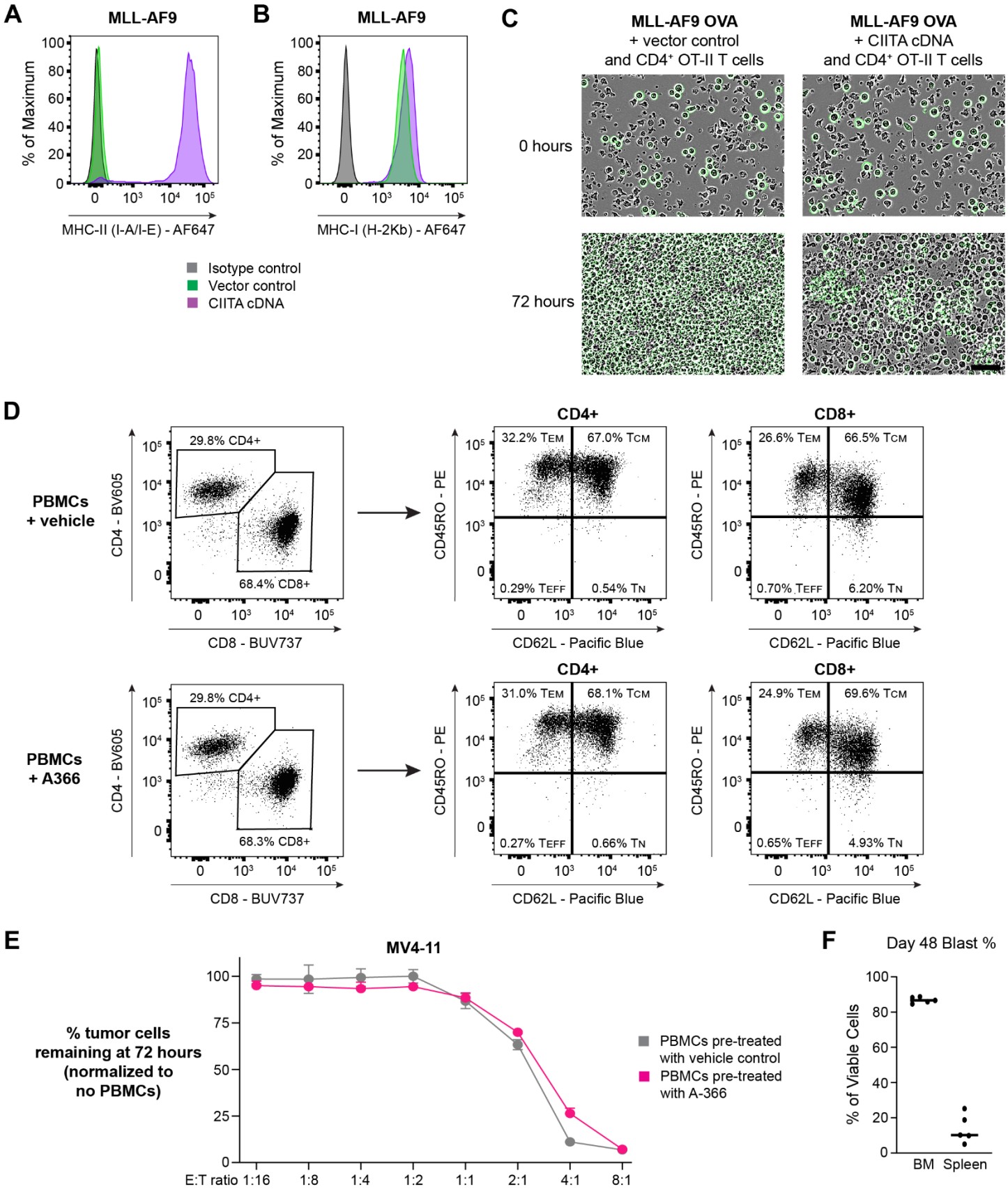
(C) Cell surface MHC-II expression in MLL-AF9 Cas9 cells expressing control (Ctrl), *Fbxo11* or *Rreb1* sgRNA. Bars depict the mean percentage of MHC-II$^+$ cells from 3 independent experiments indicated by points.

(D) Cell surface MHC-II expression in MLL-AF9 cells treated with EHMT1/2 inhibitor (A-366 1μM) or vehicle control for 7 days. Representative data from 3 experiments.

(E) Targeted mouse epigenetic CRISPR screen results. For IFN-γ+ sorts, cells were pulsed with mouse IFN-γ 10 ng/mL for 48 hours prior to each sort. Bubble plots show the top 1,000 enriched genes identified. P values were calculated using the RSA algorithm (König *et al*, 2007).

(F and G) Cell surface MHC-II expression in MLL-AF9 Cas9 (F) and OCI-AML3 Cas9 (G) cells transduced with a pool of 2 sgRNAs targeting the indicated genes or a control sgRNA, in the presence or absence of mouse IFN-γ 10 ng/mL (MLL-AF9) or human IFN-γ 25 ng/mL (OCI-AML3) for 48 hours. Representative data from 3 experiments.

Figure S6. Related to Figure 5. Functional models of AML.



(A and B) Cell surface MHC-II (A) and MHC-I (B) expression in MLL-AF9 cells transduced with a retroviral vector encoding CIITA cDNA or vector control. Representative data from 3 experiments.

(C) Phase contrast microscopy of MLL-AF9 OVA-expressing cells transduced with a retroviral vector encoding CIITA cDNA or vector control, during co-culture with CD4+ OT-II T cells at an E:T ratio of 4:1. GFP-expressing cells are shown with the overlaid green object mask, based on automated detection using the Incucyte SX5 platform. Scale bar, 50µm.

(D) T cell subset analysis in human PBMCs treated with A-366 1μM or vehicle control for 5-7 days. Data is shown from a representative experiment, performed twice.

(E) Percent remaining live MV4-11 cells following 72 hour incubation with human PBMCs at the indicated E:T ratios. PBMCs were pre-treated with A366 1μM or vehicle control for 5-7 days prior to co-culture. Plots show mean ± SEM of technical triplicates from a representative experiment, performed twice.

(F) Percentage of leukemic blasts in bone marrow (BM) and spleen at disease endpoint. Bars represent the mean percentage of leukemic blasts from each mouse indicated by points.