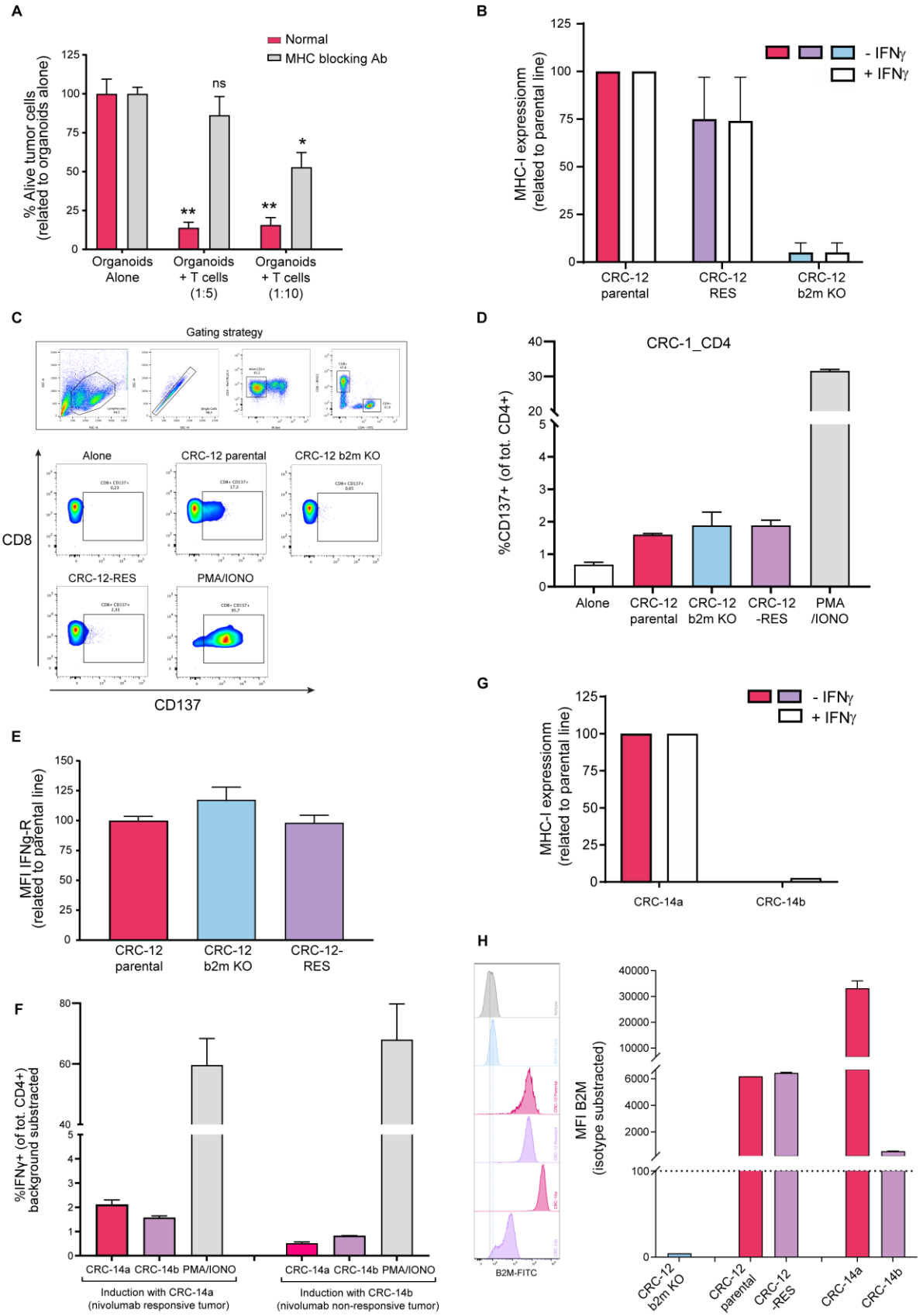
A
Normal
MHC blocking Ab
% Alive tumor cells (related to organoids alone)
ns
**
**
*
Organoids Alone
Organoids + T cells (1:5)
Organoids + T cells (1:10)
B
MHC-I expressionm (related to parental line)
- IFNγ
+ IFNγ
CRC-12 parental
CRC-12 RES
CRC-12 b2m KO
C
Gating strategy
Alone
CRC-12 parental
CRC-12 b2m KO
CRC-12-RES
PMA/IONO
CD8
CD137
D
CRC-1_CD4
%CD137+ (of tot. CD4+)
Alone
CRC-12 parental
CRC-12 b2m KO
CRC-12 -RES
PMA /IONO
E
MFI IFNg-R (related to parental line)
CRC-12 parental
CRC-12 b2m KO
CRC-12-RES
F
%IFNγ+ (of tot. CD4+) background substracted
CRC-14a CRC-14b PMA/IONO
CRC-14a CRC-14b PMA/IONO
Induction with CRC-14a (nivolumab responsive tumor)
Induction with CRC-14b (nivolumab non-responsive tumor)
G
MHC-I expressionm (related to parental line)
- IFNγ
+ IFNγ
CRC-14a
CRC-14b
H
B2M-FITC
MFI B2M (isotype substracted)
CRC-12 b2m KO
CRC-12 parental
CRC-12 -RES
CRC-14a
CRC-14b

Supplementary Figure S5. ***Validation experiments in patient-derived dMMR/MSI tumor organoid-T cell co-culture system. A****, CRC-12 parental organoids viability upon T cell co-culture with luciferase reporter assay. Error bars represent the mean and SEM of at least two biological replicates. Significance was determined by two-tailed Student's t test. ns, not significant; ** P ≤0.01 and *** P≤0.001.* ***B****, Cell surface MHC class I expression in CRC-12, CRC-12-RES and CRC-12-B2M knockout cells as determined by flow cytometry. Organoids were stimulated with 200 ng/mL IFNγ for 24 hr or were left unstimulated. Bar graphs indicate median fluorescence intensity (MFI) of anti-HLA-A, -B, and -C-PE minus MFI of isotype control, normalized to the parental line. Error bars represent SEM of at least two independent experiments.* ***C****, Flow cytometry gating strategies and representative plots of CD8+ T cells tested for tumor reactivity after 2 weeks of co-culture with autologous CRC-12 organoids. T cells were re-stimulated with CRC-12 organoids and evaluated for cell surface staining of CD137. PMA/ionomycin stimulation was included as a positive control.* ***D****, CD137 expression by CD4+ T cells obtained by 2-week co-culture with autologous CRC-12 tumor organoids, on stimulation with CRC-12 organoids. Bars represent the mean and SEM of two biologically independent experiments.* ***E****, Cell surface IFNγ receptor expression of CRC-12, CRC-12-RES and CRC-12-B2M KO organoid lines as determined by flow cytometry. Error bars represent the mean and SEM of at least two biological replicates.* ***F****, Quantification of IFNγ-positive CD4 T cells induced by 2-week co-culture of PBMCs with CRC-14a (responsive) or CRC-14b (non-responsive) organoids derived from the CRC-14 patient. T cells were re-stimulated with CRC-14a (left) or CRC-14b (right) organoids and evaluated for intracellular staining of IFNγ. Background IFNγ-positive cells (in unstimulated condition) were subtracted from the signal. Data represent the mean and SEM of at least 2 independent experiments.* ***G,*** *Cell surface MHC class I expression in CRC-14a and CRC-14b determined by flow cytometry. Organoids were stimulated with 200 ng/mL IFNγ for 24 hr or were left unstimulated. Bar graphs indicate median fluorescence intensity (MFI) of anti-HLA-A, -B, and -C-PE minus MFI of isotype control, normalized to the parental line. Error bars represent SEM of at least two independent experiments. H, Cell surface B2M expression of CRC-12-B2M KO, CRC-12, CRC-12-RES, CRC-14a and, CRC-14b organoid lines as determined by flow cytometry. Error bars represent the mean and SEM of at least two biological replicates.*