Expanded View Figures

Figure EV1. Characterization and generation of RKO PD-L1-eGFP reporter cell line.

A Analysis of *PD-L1* transcript expression (black) in 675 cancer cell lines (*x*-axis) and expression of the house keeping gene TATA-box binding protein (TBP) mRNA (gray). RKO cells show highest PD-L1 transcript expression (red).

B *PD-L1* genomic locus with schematic transcript (ID: ENST00000381577.3) and exon structure. Numbers on top indicate genomic positions on chromosome 9. Arrows indicate primer-annealing sites for genotyping (P1-P6). Sequence of protospacer and PAM for Cas9 targeting in its genomic context (top, stop codon bold). Schematic of the template employed for homology-directed repair (HDR) is shown below the transcript (HA: homology arm, eGFP: enhanced green fluorescent protein, bsr: blasticidin resistance gene, P2A: porcine teschovirus-1 2A).

C Agarose gel of PCR products from genomic DNA of PD-L1-eGFP reporter cells (left). Primer-annealing sites (P) as in shown in (B). Marker band sizes (M) as indicated. Sequence chromatogram (right) of the PCR product with primer combination P3/P4 spanning the genomic insertion site and parts of the HDR (bsr) and the PD-L1 genomic locus (3′-UTR).

D Immunofluorescence microscopy images of reporter cells stained with α-tubulin, eGFP antibodies, and DAPI. Arrowheads indicate PD-L1-eGFP protein localized to plasma membrane (scale bar: 10 μm).

E PD-L1-eGFP reporter expression in cells treated with various amounts of IFN-γ as indicated (KI: knockin RKO reporter cells).

F Half maximal effective concentration (EC_{50}) of IFN-γ in RKO reporter cells.

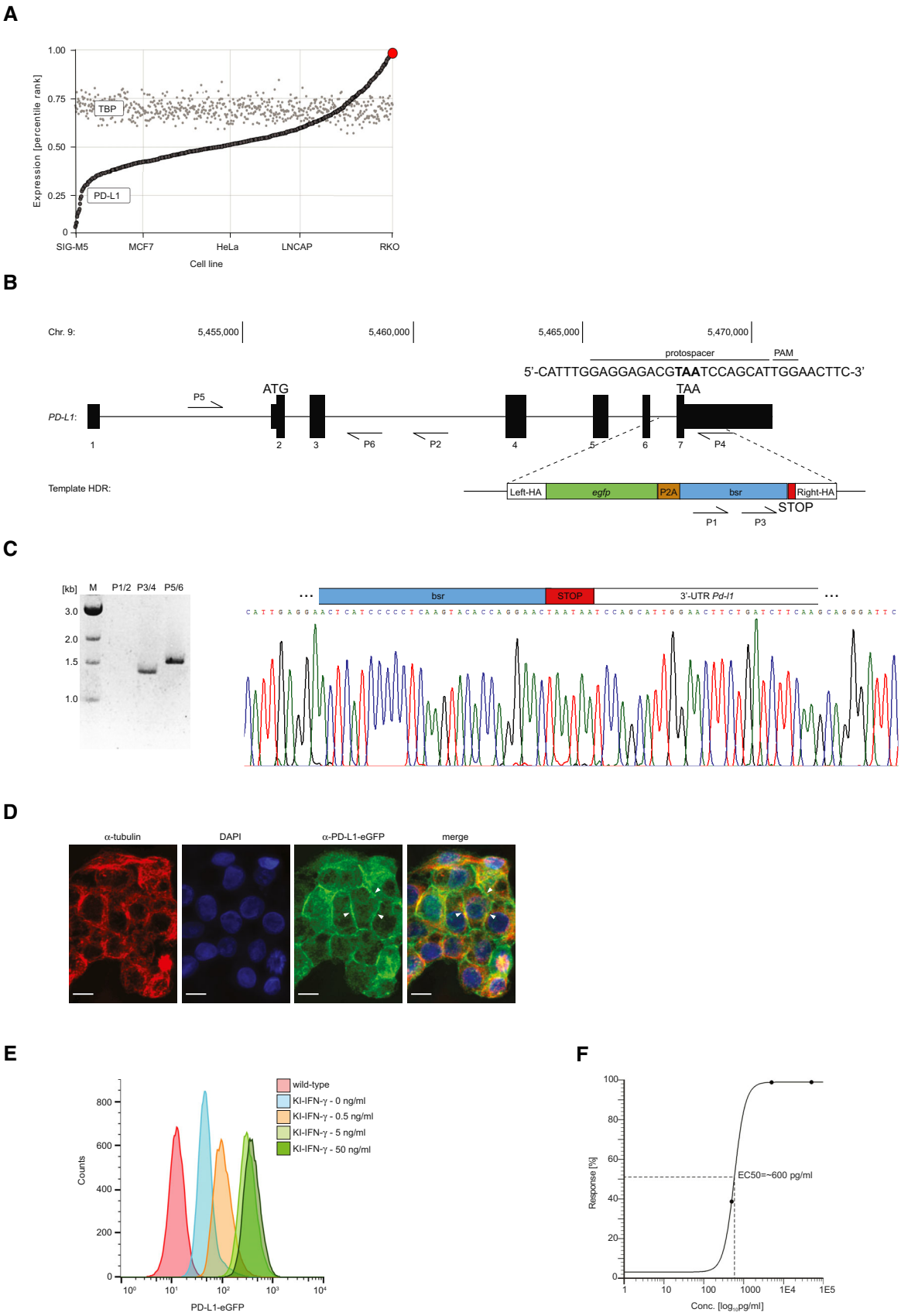
A
1.00
0.75
0.50
0.25
0
Expression [percentile rank]
TBP
PD-L1
SIG-M5
MCF7
HeLa
LNCAP
RKO
Cell line
B
Chr. 9:
5,455,000
5,460,000
5,465,000
5,470,000
protospacer
PAM
5'-CATTTGGAGGAGACGTAATCCAGCATTGGAACTTC-3'
ATG
TAA
P5
PD-L1:
1
2
3
P6
P2
4
5
6
7
P4
Template HDR:
Left-HA
egfp
P2A
bsr
Right-HA
STOP
P1
P3
C
[kb]
M
P1/2
P3/4
P5/6
3.0
2.0
1.5
1.0
bsr
STOP
3'-UTR Pd-l1
D
α-tubulin
DAPI
α-PD-L1-eGFP
merge
E
wild-type
KI-IFN-γ - 0 ng/ml
KI-IFN-γ - 0.5 ng/ml
KI-IFN-γ - 5 ng/ml
KI-IFN-γ - 50 ng/ml
Counts
PD-L1-eGFP
F
Response [%]
EC50=~600 pg/ml
Conc. [log10pg/ml]

Figure EV1.

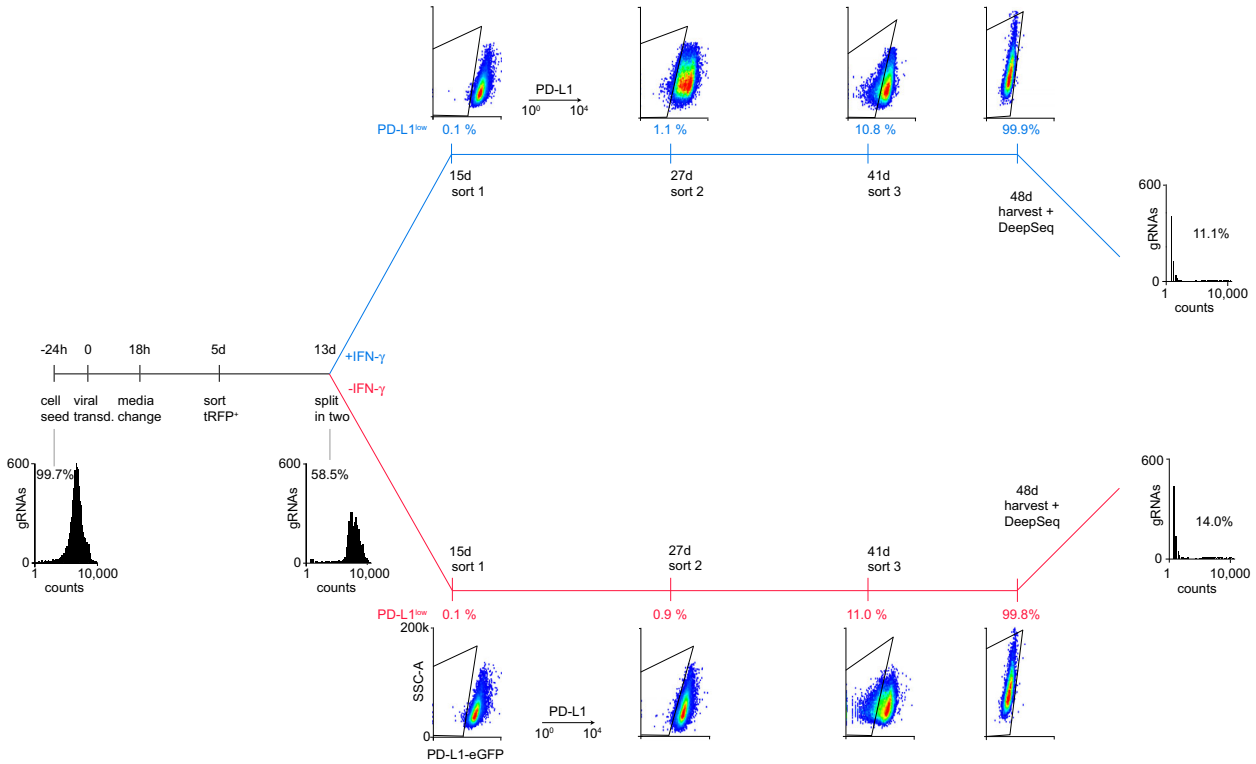

Figure EV2. Schematic of screen for genes regulating *PD-L1*.

Individual steps and time points for the screen with IFN-γ induction (top) and in the absence of IFN-γ (bottom). Sorting by flow cytometry enriches cells with low PD-L1 expression as indicated (percentages of PD-L1$^+$ cells in blue and red). For the time points 24 h, 13 days and 48 days, flow cytometry profiles show decrease in library complexity and enrichment of individual gRNAs (percentages as indicated next to profiles).

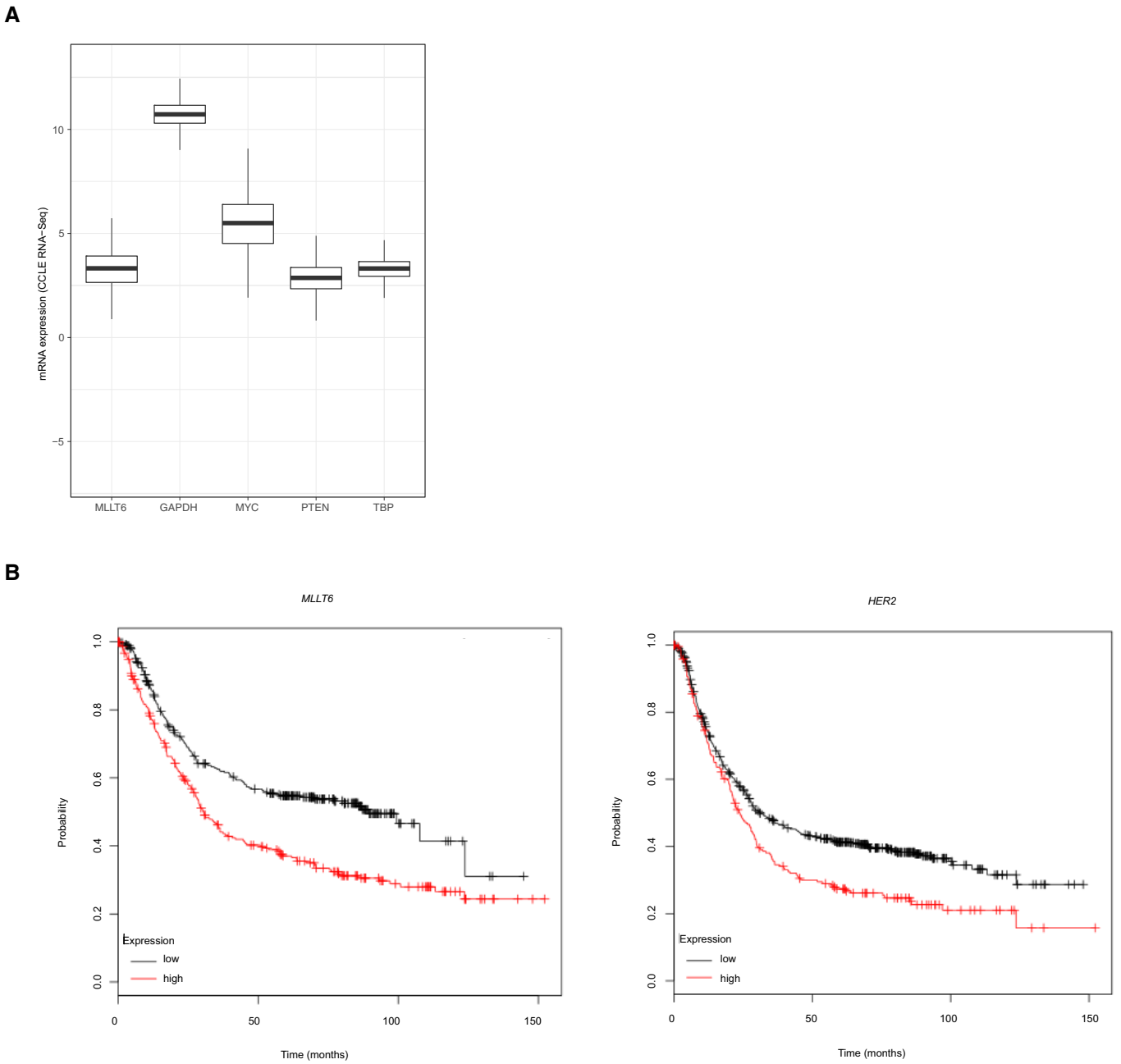

Figure EV3. Expression of *MLLT6* in cancer.

A Distribution of mRNA expression (log scale) of selected genes, across 1,019 cancer cell lines (one measurement per cell line) from the CCLE (Cancer Cell Line Encyclopedia) project. Each box represents an interquartile range (IQR) around the median value indicated by a horizontal line. Whiskers, shown as vertical lines, extend to the minimum and the maximum value not exceeding the 1.5*IQR distance from edges of each box (1st and 3rd quartiles). Outliers are shown as black dots.

B Kaplan–Meier survival plots of patients with gastric cancer with low (black) or high (red) *MLLT6* (left) or *HER2* (right) expression.

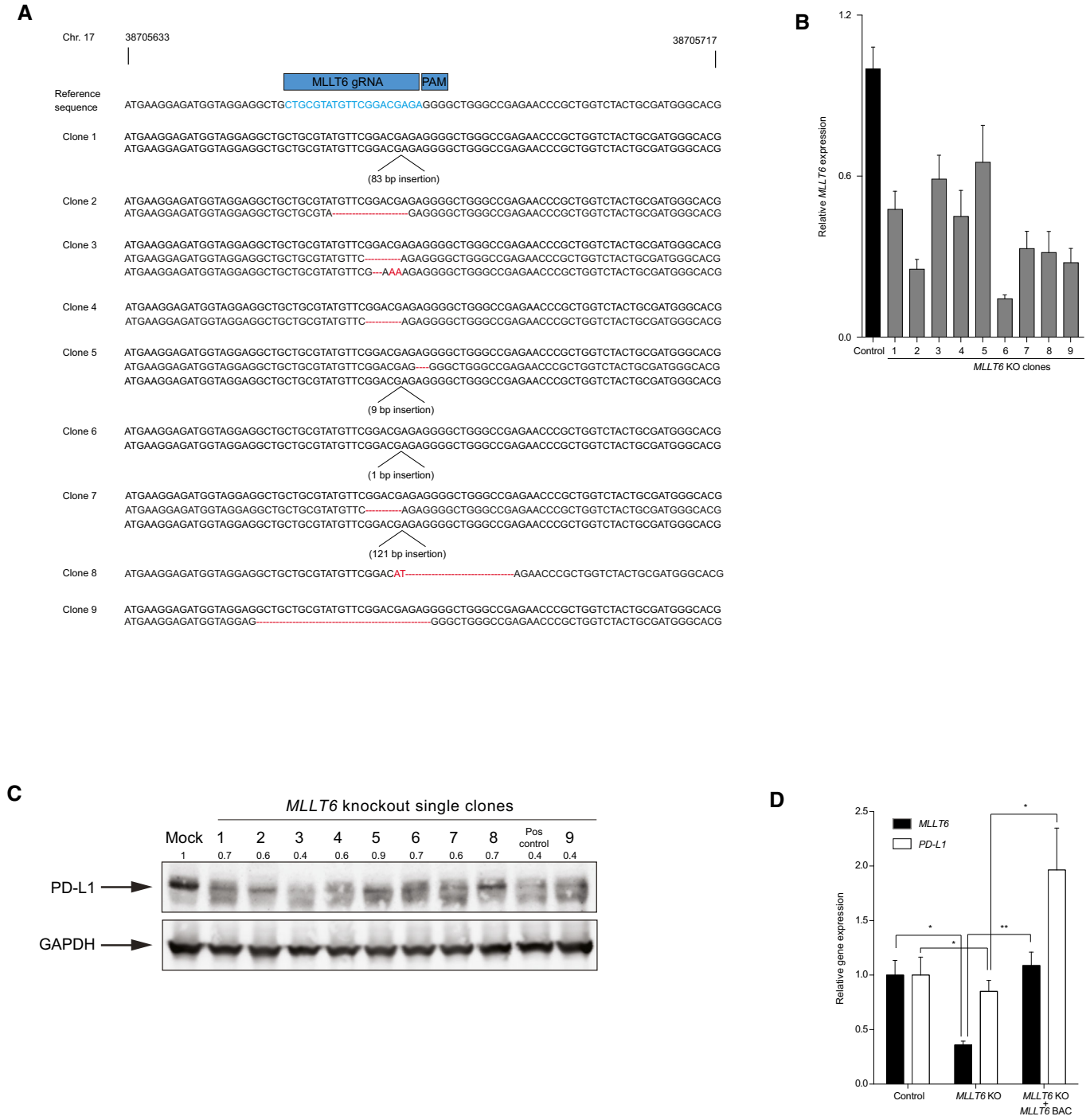

Figure EV4. Characterization of *MLLT6* knockout single clones in RKO cells.

A Genotyping nine RKO *MLLT6* knockout (KO) clones. Sequencing reads from individual clones aligned to reference sequence of *MLLT6* (top row), with sgRNA target site and PAM motif (blue) and insertion-deletion (indel) mutations as indicated (red).

B *MLLT6* transcript levels (± SD) in RKO mock and *MLLT6* KO clones normalized to expression in control cells from four technical replicates.

C Immunoblot showing total cellular protein levels of PD-L1 (top) and GAPDH (bottom) in RKO *MLLT6* KO clones and mock control cells. Numbers indicate relative band intensities of PD-L1 protein normalized to GAPDH.

D *PD-L1* and *MLLT6* transcript levels (± SD) in RKO mock, *MLLT6* KO, and *MLLT6* KO + *MLLT6* BAC normalized to control cells (Student's *t*-test; $*P < 0.05$, $**P < 0.01$; $n = 3$; biological replicates; df = 2).

Source data are available online for this figure.