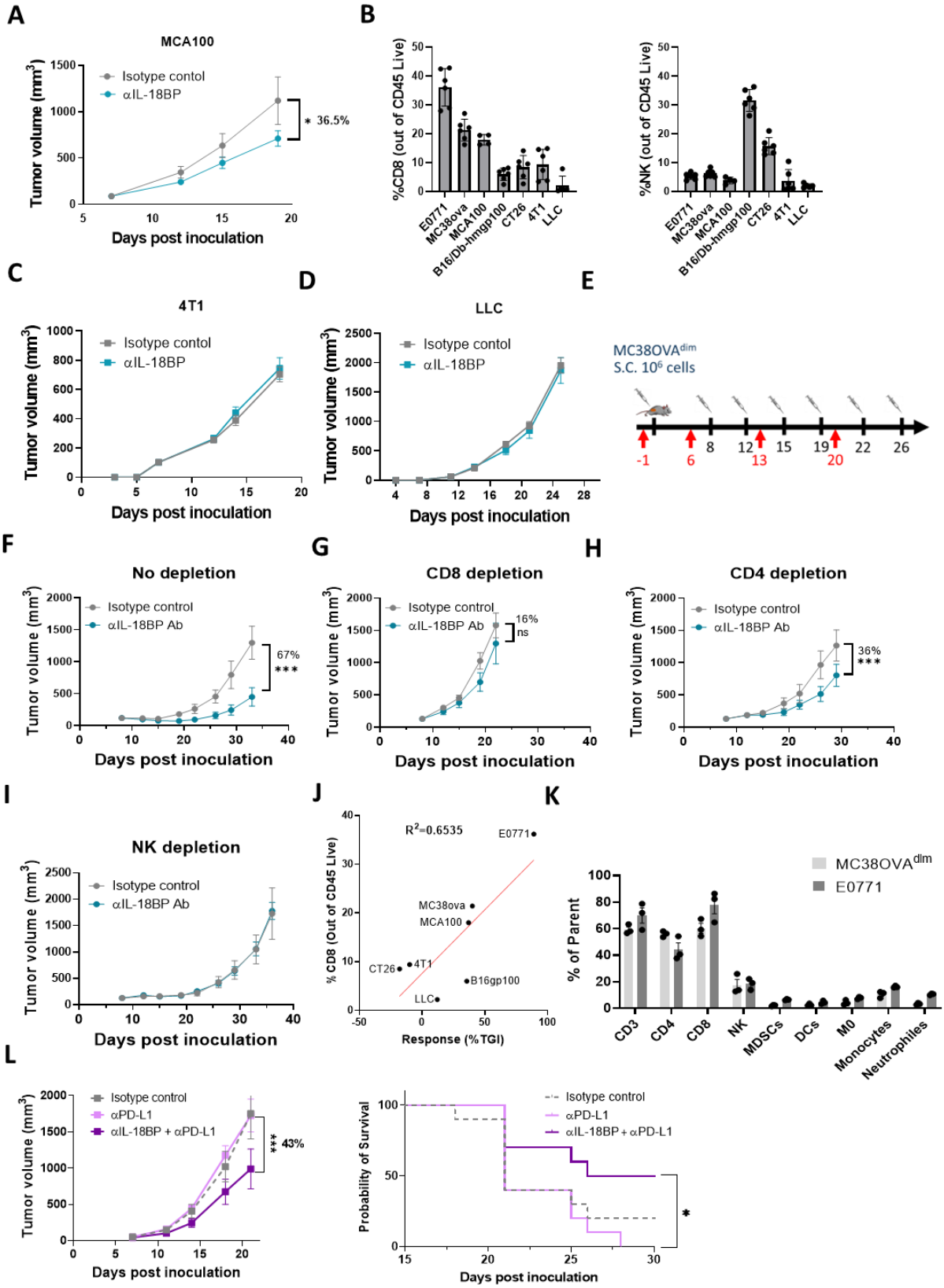
A
MCA100
Isotype contol
αIL-18BP
* 36.5%
Tumor volume (mm3)
Days post inoculation
B
%CD8 (out of CD45 Live)
%NK (out of CD45 Live)
E0771
MC38ova
MCA100
B16/Db-hmgp100
CT26
4T1
LLC
C
4T1
D
LLC
E
MC38OVAdim
S.C. 10^6 cells
-1
6
8
12
13
15
19
20
22
26
F
No depletion
αIL-18BP Ab
67%
***
G
CD8 depletion
16%
ns
H
CD4 depletion
36%
I
NK depletion
J
R2=0.6535
% CD8 (Out of CD45 Live)
Response (%TGI)
K
% of Parent
CD3
CD4
CD8
NK
MDSCs
DCs
M0
Monocytes
Neutrophiles
L
αPD-L1
αIL-18BP + αPD-L1
43%
Probability of Survival

Supplemental Figure S3. Antitumor activity of anti-mouse-IL-18BP Ab in mice studies. (A) MCA100 mouse tumor cells were inoculated in C57Bl/6 mice as described in material and methods. At a tumor volume of 190 mm^3 mice were randomized (n=10 per group) and treated either with anti-IL-18BP Ab or with isotype control (15mg/kg) twice a week for total of 6 treatments. Experiment was performed once. **(B)** E0771, MC38OVA^{dim}, MCA100, B16/Db-hmgp100, CT26, 4T1, and LLC tumors were dissociated by gentleMACS using mouse dissociation kit. Dissociated tumor cells were blocked with a cocktail of anti-CD16, anti-CD32 and anti-CD64 Abs to block nonspecific binding to Fcγ receptors and stained with a target Ab or isotype control cocktail to measure the percentage of $CD8^+$ T and NK cells in each sample. Samples were acquired on a Fortessa X-20 flow cytometer. Bar graphs show the mean ± SD. Each dot represents one mouse. **(C-D)** 4T1 (representative experiment out of 2) **(C)**, and LLC (experiment was performed once) **(D)** mouse tumor cells were inoculated in BALB/c or C57Bl/6 mice as described in material and methods. or on day 4 post inoculation, mice were randomized (n=10 per group) and treated either with anti-IL-18BP Ab or with isotype control (15mg/kg) twice a week for total of 6 treatments. Tumor volumes are represented as the Mean volume ± SEM. Kaplan-Meier. **(E-I)** The activity of IL-18BP blockade depends on $CD8^+$ T cells and on NK cells in MC38OVA^{dim} tumor model. Representative experiment out of 2 is shown. **(E)** MC38OVA^{dim} engrafted mice were assigned into 4 groups (n=8-10) and were injected with anti-CD4 (GK1.5, 300ug), anti-CD8 (Lyt 3.2, 100ug), anti-NK1.1 (PK136, 50ug) or isotype control (100ug) prior to tumor inoculation (day -1), and on days 6, 13 and 20 (red arrows). At tumor volume of 120 mm^3 mice were randomized and treated either with anti-IL-18BP Ab or with isotype control (10mg/kg) twice a week for a total of 6 treatments. Tumor volumes are represented as the Mean volume + SEM. Tumor growth was examined in non-depleted control mice **(F)**, $CD8^+$ cells depleted mice **(G)**, $CD4^+$ cells depleted mice **(H)**, and NK cells depleted mice **(I)**. **(J)** The percent of $CD8^+$ T cells infiltrating the tumor models E0771, MC38OVA^{dim}, MCA100, B16/Db-hmgp100, CT26, 4T1, and LLC was correlated to the tumor growth inhibition (TGI) induced by IL-18BP blockade in each model. **(K)** Immune cell populations were isolated from mouse tumors and stained for IL-18Rα expression by flow cytometry. Bar graphs show the mean ± SEM. Each dot represents one mouse. **(L)** Anti-mouse IL-18BP Ab (15mg/kg) synergized with anti PD-L1 Ab (5mg/kg) to inhibit tumor growth in CT26 tumor model. CT26 tumor cells were inoculated in BALB/c mice as described in material and methods. On day 4 post inoculation, mice were randomized (n=10 per group) and treated either

with anti-IL-18BP Ab, isotype control (15mg/kg), anti-PD-L1 Ab (5mg/kg) or with both agents twice a week for a total of 6 treatments. Representative experiment out of 4 is shown. Tumor volumes are represented as the Mean volume ± SEM. Kaplan-Meier survival curves for each group are shown. $P<0.05$*, $P<0.001$*** by two-way ANOVA with repeated measures and Log Rank Mantel–Cox test for survival analysis