Figure S1

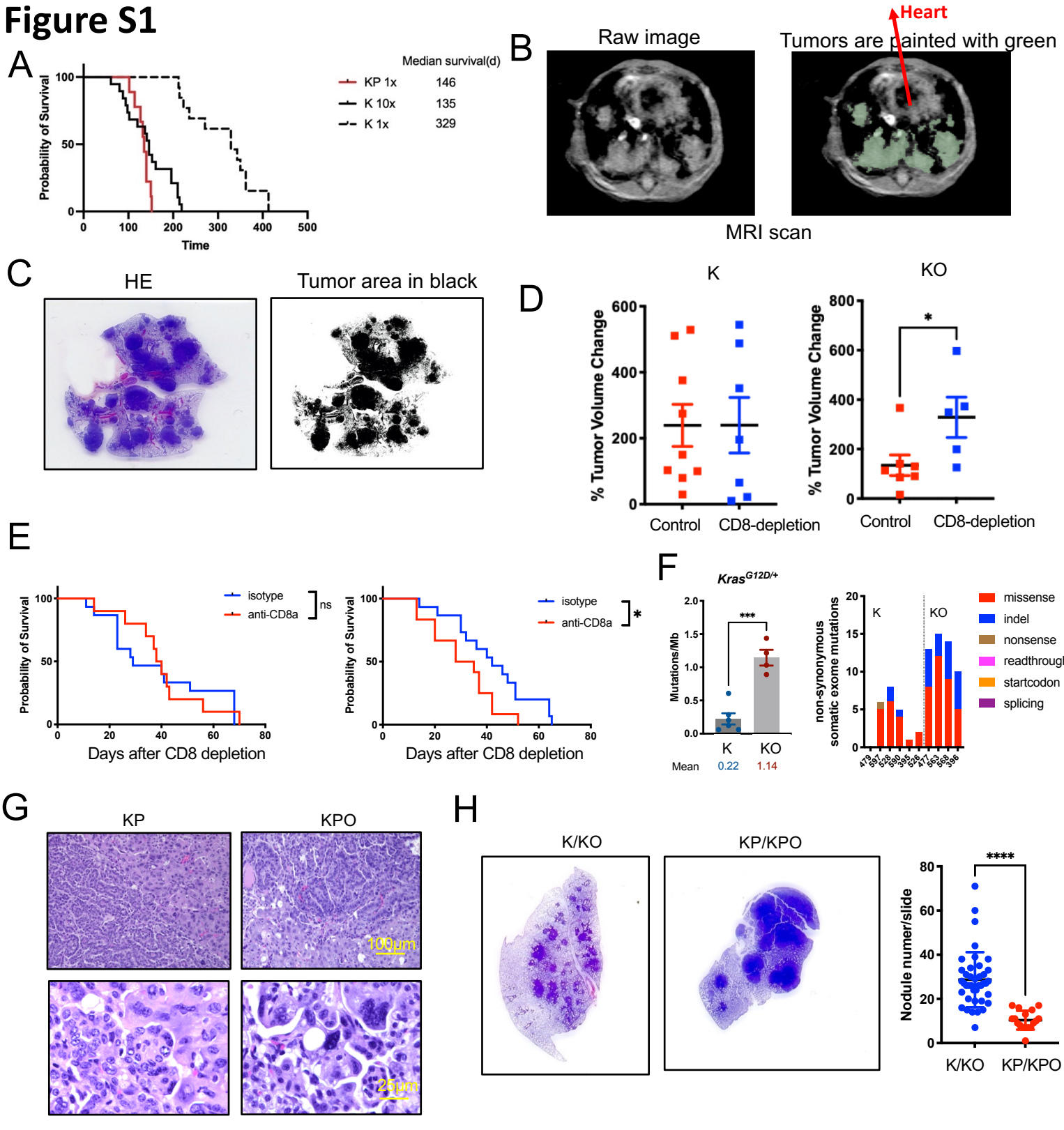
A
Probability of Survival
Time
Median survival(d)
KP 1x 146
K 10x 135
K 1x 329
B
Raw image
Tumors are painted with green
Heart
MRI scan
C
HE
Tumor area in black
D
K
KO
% Tumor Volume Change
Control
CD8-depletion
E
isotype
anti-CD8a
ns
*
Days after CD8 depletion
F
Kras G12D/+
Mutations/Mb
Mean 0.22 1.14
non-synonymous somatic exome mutations
missense
indel
nonsense
readthrough
startcodon
splicing
G
KP
KPO
100μm
25μm
H
K/KO
KP/KPO
Nodule numer/slide
****

Figure S1: *Pole-P286R* induces immunogenicity in *Kras*^{G12D} mice, related to Figures 1 and 2.

(A) Survival of K/KP GEMMs induced with different titers of Adeno-Cre: 1x (2.5x10^7) or 10x (2.5x10^8).

(B) Labelling of lung tumors on an axial slice from MRI scan using 3D Slicer software (green marks tumor areas). All tumor containing slices were marked for volume quantification.

(C) Method for quantifying tumor area from H&E-stained sections using Image J. Original H&E is shown on the left. Example masked tumor area generated by Image J is shown on the right.

(D) Change in tumor volume after 2 weeks of treatment with Cd8 depletion antibody or isotype in K vs KO mice.

(E) Survival of K/KO GEMMs treated with isotype or CD8 depletion antibody after tumor confirmation by MRI.

(F) Total mutations/MB determined by whole exome sequencing of K vs. KO lung tumors(left). Distribution of the type of mutations in K vs KO tumors (right).

(G) Representative H&E of KP and KPO GEMMs.

(H) Scans of H&E-stained tissue sections of p53 WT (Wild Type) and mutant lung tumors (left) and quantification of tumor nodule number in K and KO vs KP and KPO tumors.

Figure S2

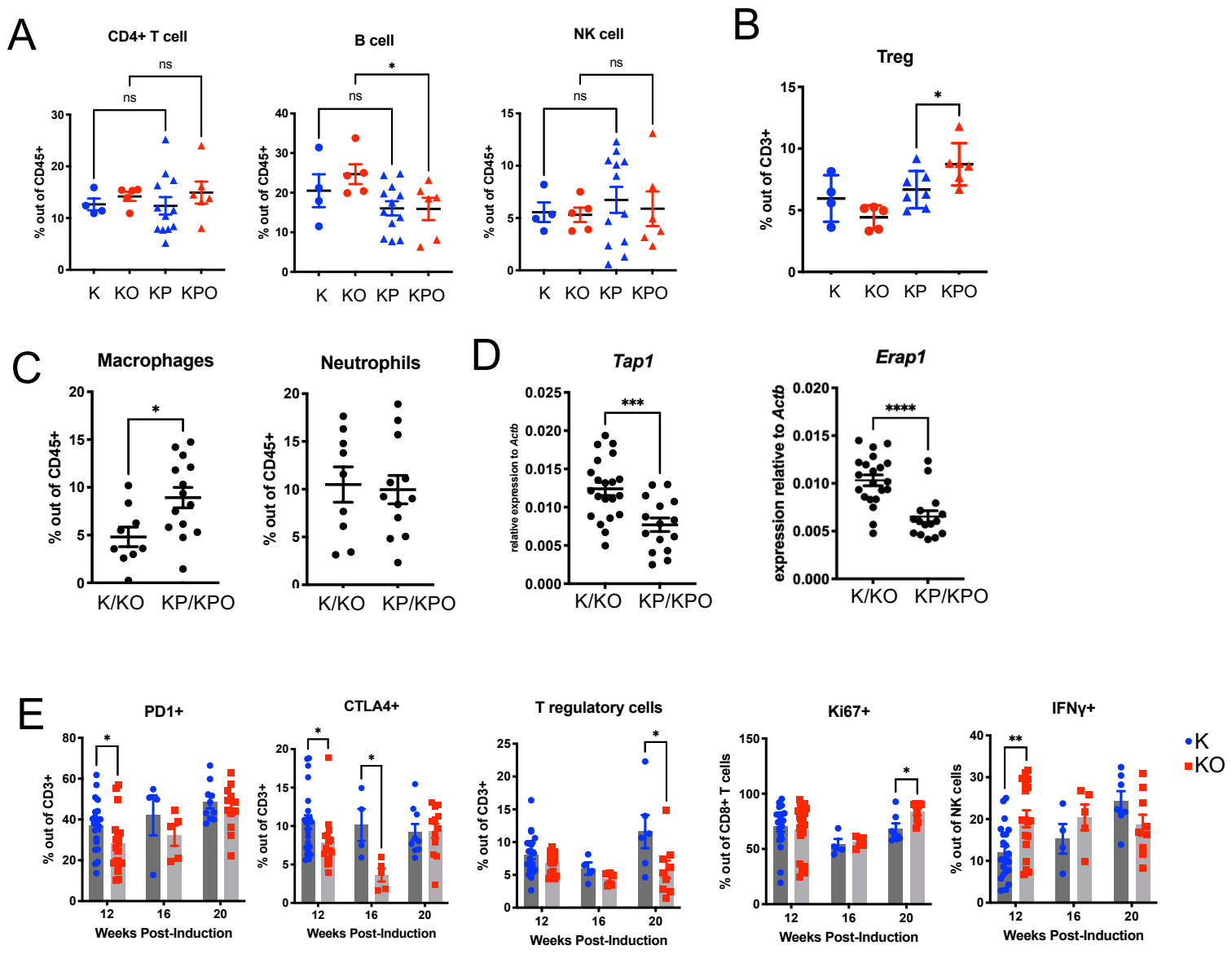
A
CD4+ T cell
B cell
NK cell
% out of CD45+
ns
*
K KO KP KPO
B
Treg
% out of CD3+
C
Macrophages
Neutrophils
K/KO KP/KPO
D
Tap1
Erap1
relative expression to Actb
expression relative to Actb
***
****
E
PD1+
CTLA4+
T regulatory cells
Ki67+
IFNγ+
% out of CD8+ T cells
% out of NK cells
**
Weeks Post-Induction
K
KO

Figure S2: Immune phenotyping of GEMMs, related to Figure 4.

(A) CD4+ T cell, B cell and NK (Natural Killer) cell percentages out of all CD45+ cells in K, KO, KP, and KPO GEMM (Genetically Engineered Mouse Models) tumors.

(B) Quantification of Tregs (CD4+FoxP3+) in KP/KPO tumors.

(C) Quantification of alveolar macrophages (Cd11c+ Cd11b- Cd103-) and neutrophils (Cd11b+ Ly6G+) in K and KO vs KP and KPO tumors.

(D) *Tap1* and *Erap1* expression in tumors were determined by qPCR of RNA extracted from lung tumor tissue.

(E) Expression of checkpoint receptors (PD1, CTLA4), percentage of Tregs, proliferation (Ki67+) of CD8+ T cells, and activation (IFNγ+) of NK cells in the tumor microenvironment determined by flow cytometry in K and KO GEMMs.

Figure S3

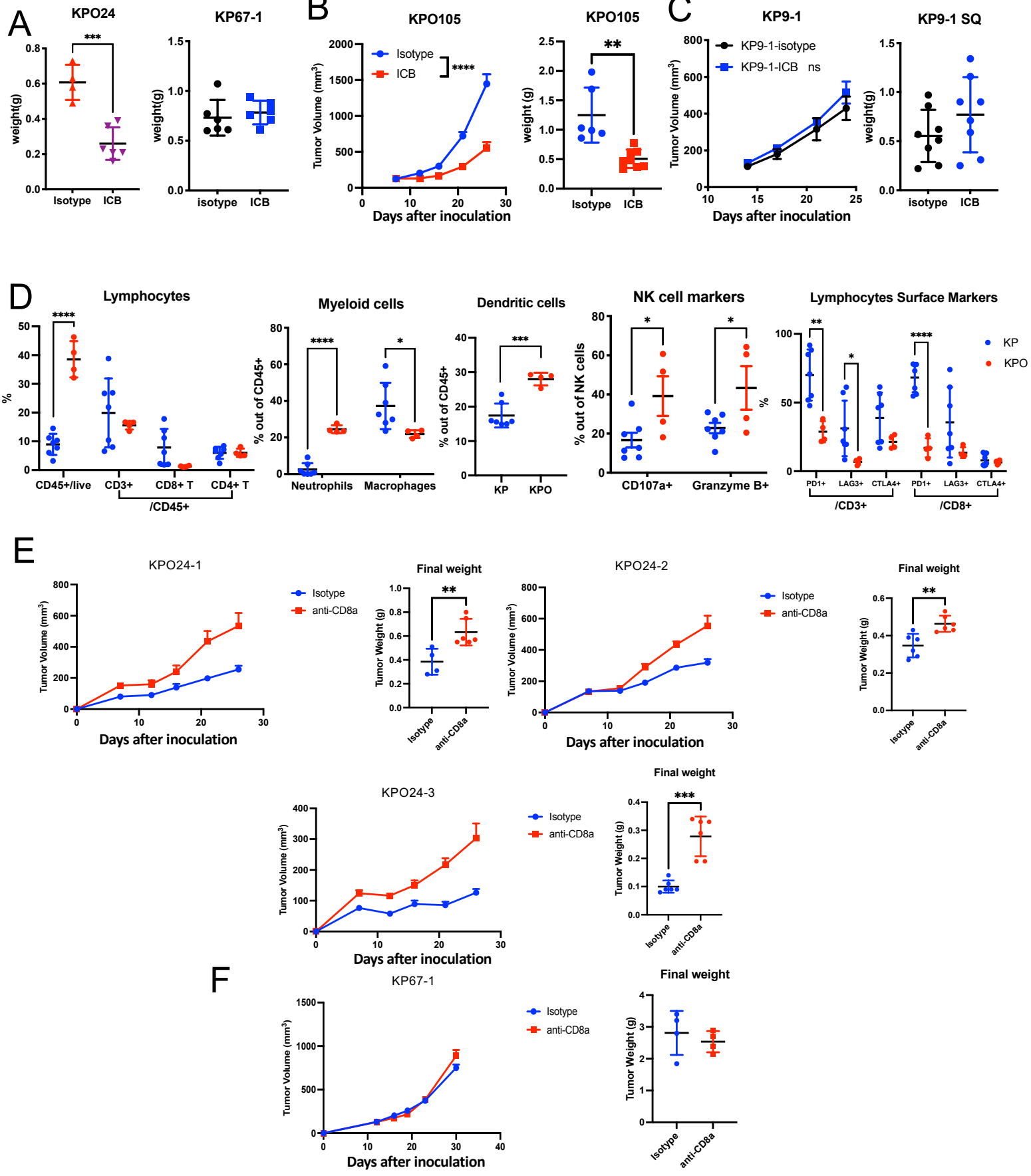
A
KPO24
weight(g)
Isotype
ICB
***
KP67-1
weight(g)
isotype
ICB
B
KPO105
Tumor Volume (mm3)
Isotype
ICB
****
Days after inoculation
KPO105
weight (g)
**
Isotype
ICB
C
KP9-1
Tumor Volume (mm3)
KP9-1-isotype
KP9-1-ICB ns
Days after inoculation
KP9-1 SQ
weight(g)
isotype
ICB
D
Lymphocytes
%
****
CD45+/live
CD3+
CD8+ T
CD4+ T
/CD45+
Myeloid cells
% out of CD45+
****
*
Neutrophils
Macrophages
Dendritic cells
% out of CD45+
***
KP
KPO
NK cell markers
% out of NK cells
*
*
CD107a+
Granzyme B+
Lymphocytes Surface Markers
%
**
*
****
PD1+
LAG3+
CTLA4+
PD1+
LAG3+
CTLA4+
/CD3+
/CD8+
KP
KPO
E
KPO24-1
Tumor Volume (mm3)
Isotype
anti-CD8a
Days after inoculation
Final weight
Tumor Weight (g)
**
Isotype
anti-CD8a
KPO24-2
Tumor Volume (mm3)
Isotype
anti-CD8a
Days after inoculation
Final weight
Tumor Weight (g)
**
Isotype
anti-CD8a
KPO24-3
Tumor Volume (mm3)
Isotype
anti-CD8a
Days after inoculation
Final weight
Tumor Weight (g)
***
Isotype
anti-CD8a
F
KP67-1
Tumor Volume (mm3)
Isotype
anti-CD8a
Days after inoculation
Final weight
Tumor Weight (g)
Isotype
anti-CD8a

Figure S3: Characterization of syngeneic models derived from GEMMs, related to Figure 5.

(A) Weights of final dissected subcutaneous tumors of KPO24 and KP67-1 treated with isotype or ICB (Immune Checkpoint Blockade) from growth curves are main figure 4.

(B) Growth curve of subcutaneous tumors of KPO105 treated with ICB. Weights of final dissected tumors are shown on the right.

(C) Growth curve of subcutaneous tumors of KP9-1 treated with isotype control or ICB (left) and weights of dissected tumors at endpoint are shown on the right.

(D) Flow cytometry analysis of KP67-1 vs KPO24 models showing percentage of lymphocytes, macrophages Cd11c+ Cd11b- Cd103-, neutrophils (Cd11b+ Ly6G+), dendritic cells (Ly6C-Ly6G-CD103-CD11b+/ Ly6C-Ly6G-CD103+CD11b-), activation marker for NK cells, and immune checkpoint receptor expression on CD3+ or CD8+ T cells.

(E) *In vivo* tumor growth of single cell clones KPO24-1, KPO24-2 and KPO24-3 implanted subcutaneously and treated with isotype or anti-CD8a depleting antibodies. Weight of dissected tumor was graphed next to the growth curves.

(F) *In vivo* tumor growth of KP67-1 implanted subcutaneously and treated with isotype or anti-CD8a depleting antibody. Weight of dissected tumor graphed on the right.

Figure S4

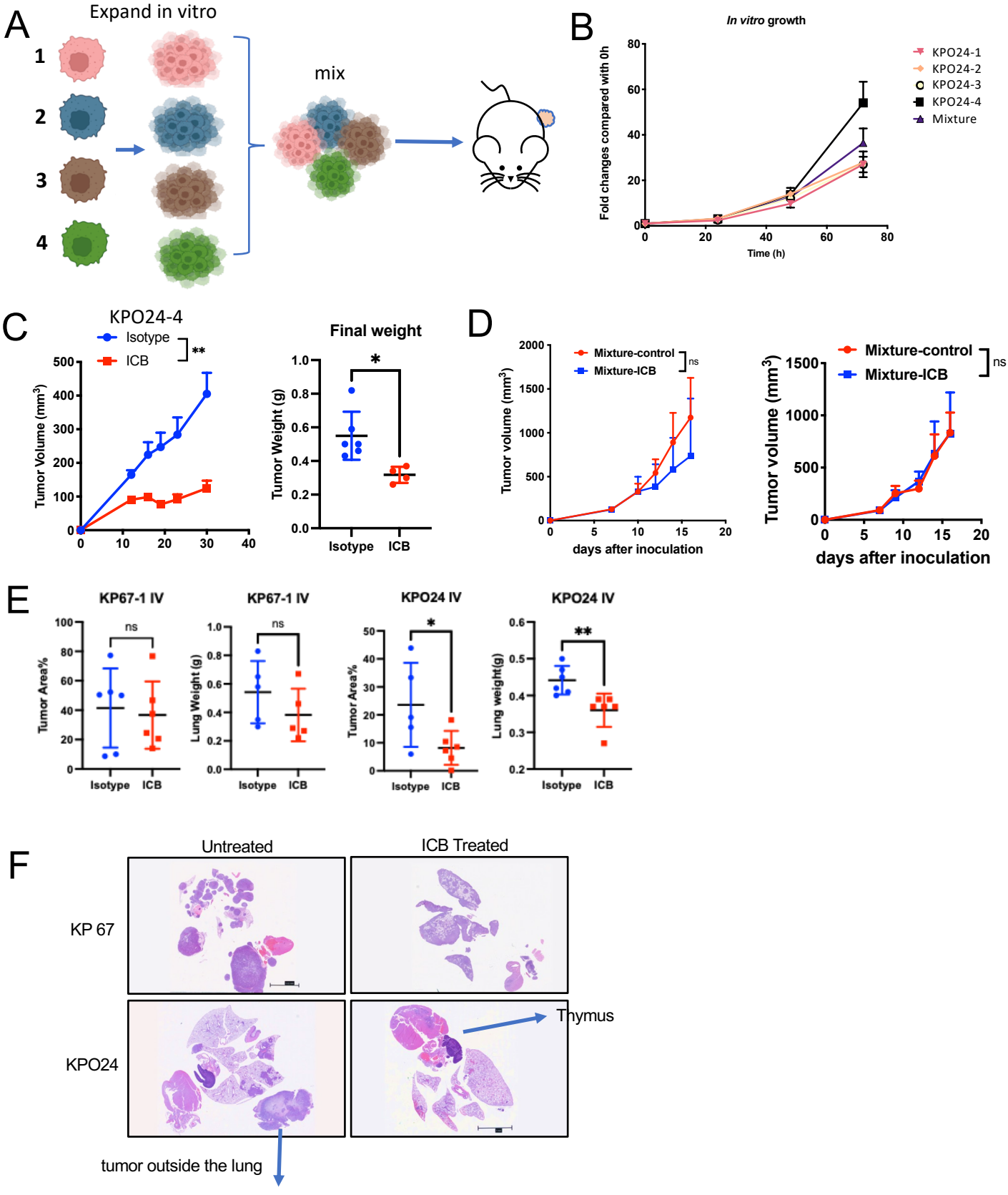
A
Expand in vitro
1
2
3
4
mix
B
In vitro growth
Fold changes compared with 0h
Time (h)
KPO24-1
KPO24-2
KPO24-3
KPO24-4
Mixture
C
KPO24-4
Isotype
ICB
**
Tumor Volume (mm3)
Final weight
*
Tumor Weight (g)
Isotype
ICB
D
Mixture-control
Mixture-ICB
ns
Tumor volume (mm3)
days after inoculation
E
KP67-1 IV
KPO24 IV
Tumor Area%
Lung Weight (g)
Lung weight(g)
ns
*
**
Isotype
ICB
F
Untreated
ICB Treated
KP 67
KPO24
Thymus
tumor outside the lung

Figure S4: Response of KPO clone mixtures or pool to ICB in subcutaneous tissue or lung, related to Figure 5

(A) Schema for mixture experiment. 4 single cell derived models derived from KPO24 were cultured *in vitro* to expand then pooled together right before *in vitro* or *in vivo* experiments. Clone 4 is *Pole* WT.

(B) *In vitro* growth of single cell clones and mixture cultured for 3-days.

(C) Growth curve of subcutaneous tumors of KP24-1 treated with isotype or ICB. Weights of final dissected tumors are shown on the right.

(D) Growth curves of subcutaneous tumors of KP24 clone mixture (1,2,3 and 4) treated with isotype or ICB. Data is from 2 independent experiments.

(E) Mice were intravenously injected with KP67-1 or KPO24 cells and treated with isotype or ICB. Quantification of lung weights and tumor are shown.

(F) Representative H&E images of mouse lungs from (E).

Figure S5

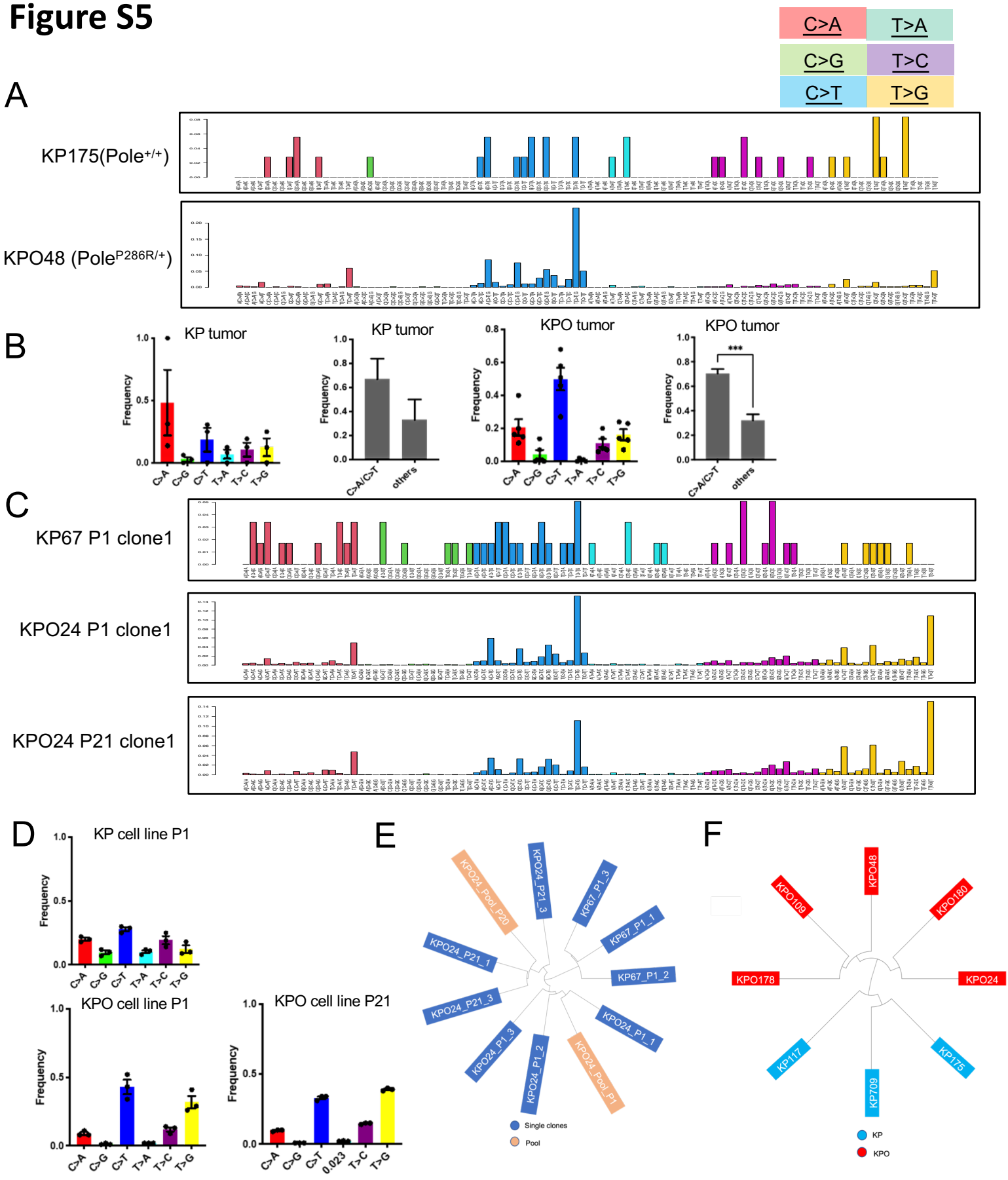
C>A
T>A
C>G
T>C
C>T
T>G
A
KP175(Pole+/+)
KPO48 (PoleP286R/+)
B
KP tumor
KP tumor
KPO tumor
KPO tumor
Frequency
***
C>A
C>G
C>T
T>A
T>C
T>G
C>A/C>T
others
C
KP67 P1 clone1
KPO24 P1 clone1
KPO24 P21 clone1
D
KP cell line P1
KPO cell line P1
KPO cell line P21
0.023
E
KPO24_Pool_P20
KPO24_P21_3
KP67_P1_3
KP67_P1_1
KP67_P1_2
KPO24_P1_1
KPO24_Pool_P1
KPO24_P1_2
KPO24_P1_3
KPO24_P21_3
KPO24_P21_1
Single clones
Pool
F
KPO48
KPO109
KPO180
KPO178
KPO24
KP117
KP175
KP709
KP
KPO

Figure S5: Mutational signatures of KP/KPO tumors and cell lines, related to Figure 5

(A) Representative mutational signature of KP or KPO tumors from GEMMs, KP lines (KP67 P1 clone1) and KPO (KPO24 P1 clone1 and KPO24 P21 clone1) lines.

(B) Quantification of mutational signatures of KP and KPO GEMM tumors. Quantification and distribution of C>A/C>T substitutions vs other substitutions in KP tumors are shown on the left and Quantification and distribution of C>A/C>T substitutions vs other substitutions in KPO tumors are shown on the right.

(C) Representative mutational signature of KP lines (KP67 P1 clone1) and KPO (KPO24 P1 clone1 and KPO24 P21 clone1) lines.

(D) Quantification of mutational signature of syngeneic KP cell lines at passage 1, KPO cell lines at passage 1 and 21.

(E) Hierarchical clustering determined by somatic mutations in KP single clones (KP67_1, KP67_2 and KP67_3) at passage 1 (P1), KPO24 pool (Passage P1 and P20) and single clones (KPO24_P1_1, KPO24_P1_2 and KPO24_P1_3) and (KPO24_P21_1, KPO24_P21_2 and KPO24_P21_3).

(F) Hierarchical clustering determined by somatic mutations in KP (709, 175, 117) and KPO (48. 178, 24, 180, 109) lung tumors from GEMMs.

Figure S6

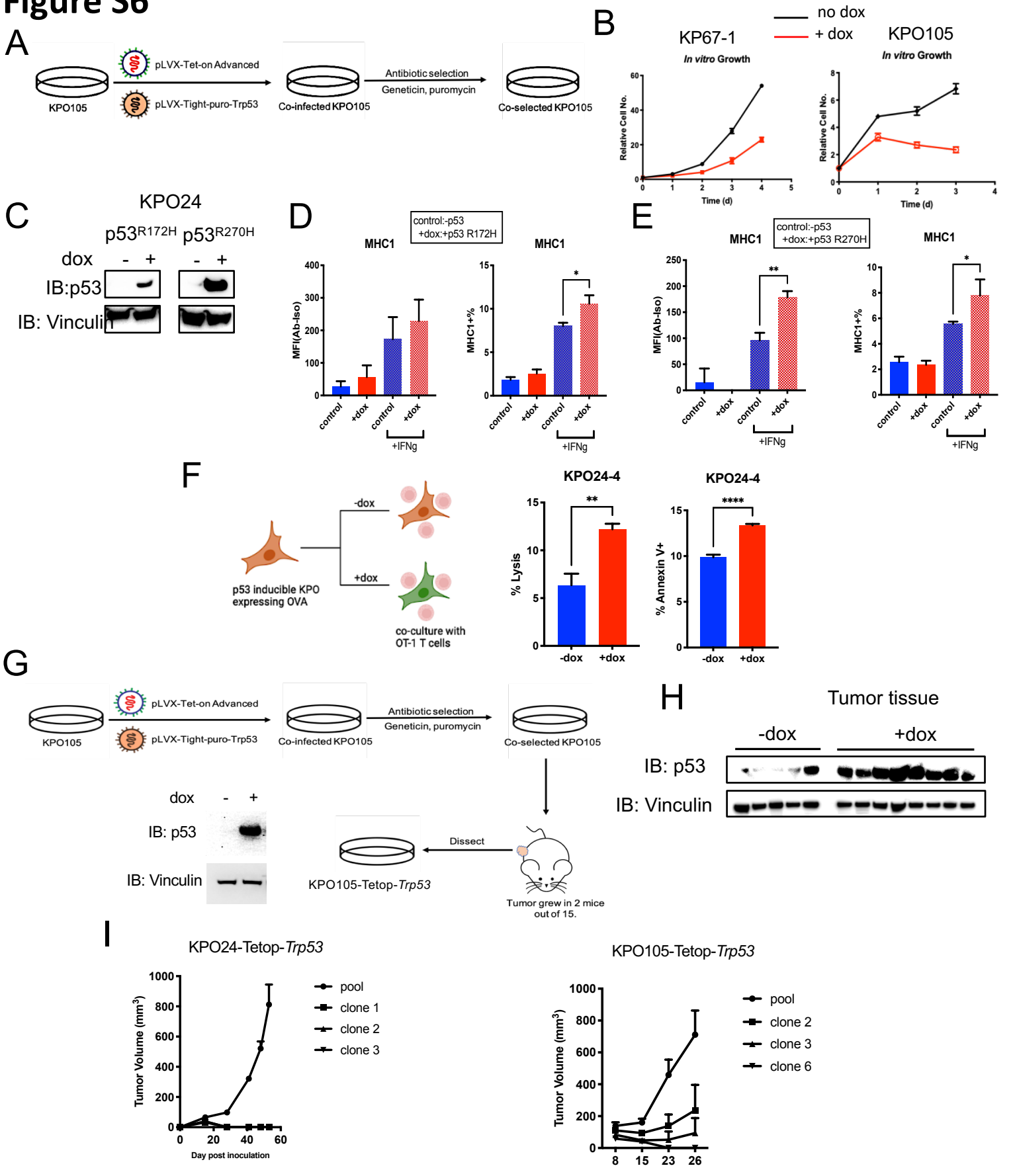
A
KPO105
pLVX-Tet-on Advanced
pLVX-Tight-puro-Trp53
Co-infected KPO105
Antibiotic selection
Geneticin, puromycin
Co-selected KPO105
B
KP67-1
KPO105
no dox
+ dox
In vitro Growth
Relative Cell No.
Time (d)
C
KPO24
p53R172H p53R270H
dox - + - +
IB:p53
IB: Vinculin
D
control:-p53
+dox:+p53 R172H
MHC1
MFI(Ab-Iso)
MHC1+%
control
+dox
+IFNg
E
control:-p53
+dox:+p53 R270H
F
p53 inducible KPO
expressing OVA
-dox
+dox
co-culture with
OT-1 T cells
KPO24-4
% Lysis
% Annexin V+
G
dox - +
IB: p53
IB: Vinculin
Dissect
KPO105-Tetop-Trp53
Tumor grew in 2 mice
out of 15.
H
Tumor tissue
-dox
+dox
IB: p53
IB: Vinculin
I
KPO24-Tetop-Trp53
Tumor Volume (mm3)
Day post inoculation
pool
clone 1
clone 2
clone 3
KPO105-Tetop-Trp53
clone 6

Figure S6: Generation of p53 inducible syngeneic model, related to Figures 6 and 7.

(A) Schema for generating stable KP and KPO lines with dox inducible p53 with a two-vector system: pLVX-TetOn Advanced and pLVX-Tight puro Trp53.

(B) *In vitro* growth of p53 inducible KP and KPO lines with doxycycline.

(C) Western blot of KPO line expressing missense mutant p53s (R172H and R270H) on KPO24 cells stably transduced with the two-vector system carrying mutant p53 in panel A.

(D) MHC1 expression KPO24 -TetOp-p53R172H line treated with vehicle or doxycycline determined by flow cytometry

(E) MHC1 expression KPO24 -TetOp-p53R270H line treated with vehicle or doxycycline determined by flow cytometry

(F) T cell specific killing measured by LDH release in a co-culture of KPO24-4 TetOp-p53 (wt) expressing OVA with OT-1 T cells. Apoptosis of tumor cells were measured by Annexin V staining and analyzed by flow cytometry.

(G) Schema for generating p53 inducible KPO lines used in mouse experiments. Dox inducible p53 expressing stable cell line was passaged in mice. Tumor cells from these excised tumors were isolated and expanded for in vivo studies after confirmation of p53.

(H) Western blot for p53 from the subcutaneous tumor of KPO105- TetOp-p53 in main figure 7.

(I) Growth curves for the two different of KPO24 or KPO-105- TetOp-p53 pools or corresponding clones in wildtype mice.

Figure S7

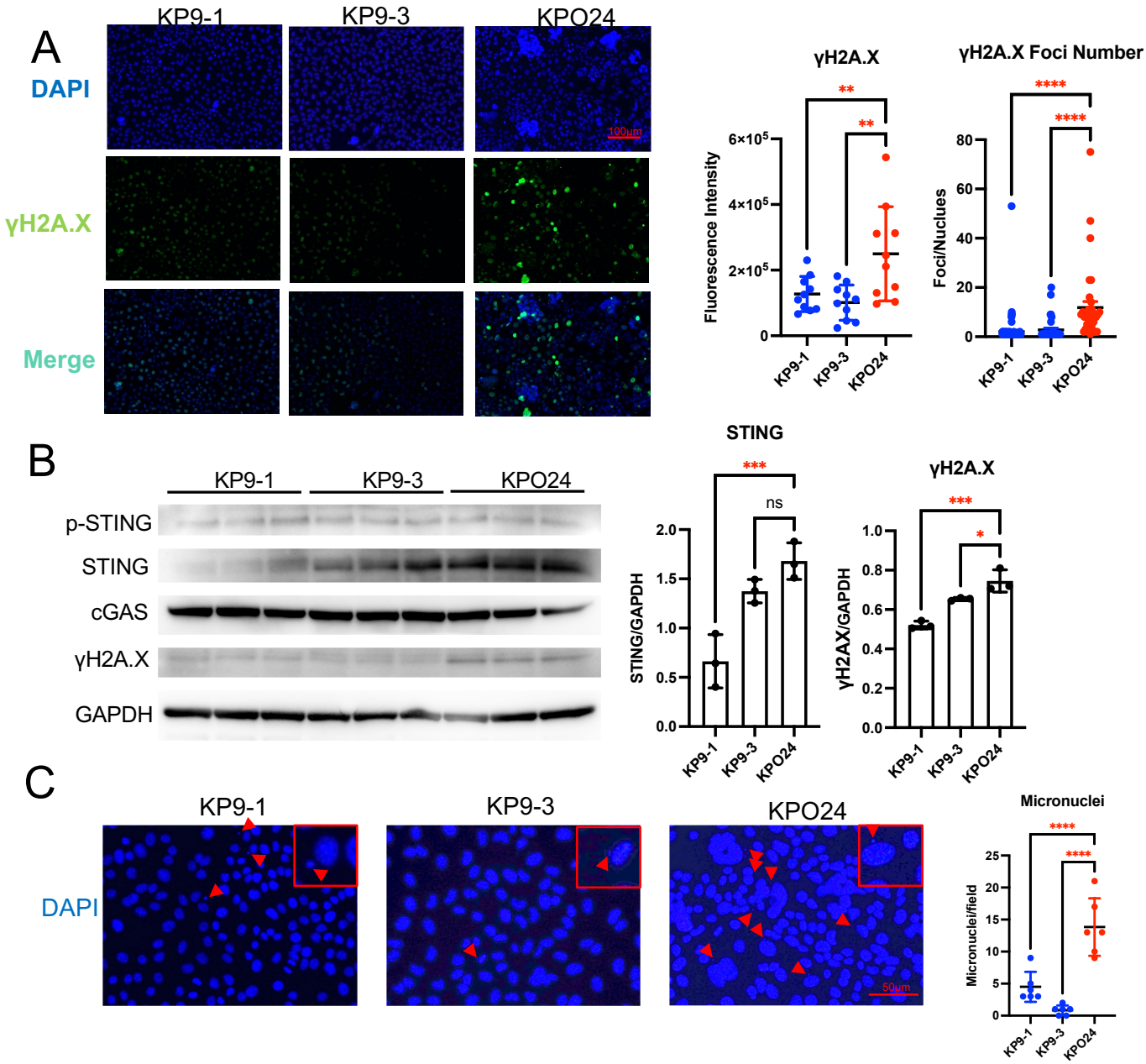
A
KP9-1
KP9-3
KPO24
DAPI
γH2A.X
Merge
100μm
γH2A.X
γH2A.X Foci Number
Fluorescence Intensity
Foci/Nuclues
B
KP9-1
KP9-3
KPO24
p-STING
STING
cGAS
γH2A.X
GAPDH
STING
γH2A.X
STING/GAPDH
γH2AX/GAPDH
ns
C
KP9-1
KP9-3
KPO24
DAPI
50μm
Micronuclei
Micronuclei/field

Figure S7: Analysis of DNA damage and activation of cGAS/STING pathway in KP/KPO lines, related to Figure 8.

(A) Immunofluorescence for γH2A.X and DAPI on KP9-1, KP9-3 and *Kras*$^{G12D/+}$;*p53*$^{-/-}$;*Pole*$^{P286R/+}$ syngeneic cell line KPO24. Intensity for γH2A.X and γH2A.X+ foci per nucleus were quantified.

(B) Western blots for pSTING, STING, cGAS, and γH2A.X on KP9-1, KP9-3 and KPO24. Relative protein intensities for STING and gH2AX were quantified on the right.

(C) DAPI staining for was used to identify micronuclei in KP9-1, KP9-3, and KPO24.

Figure S8

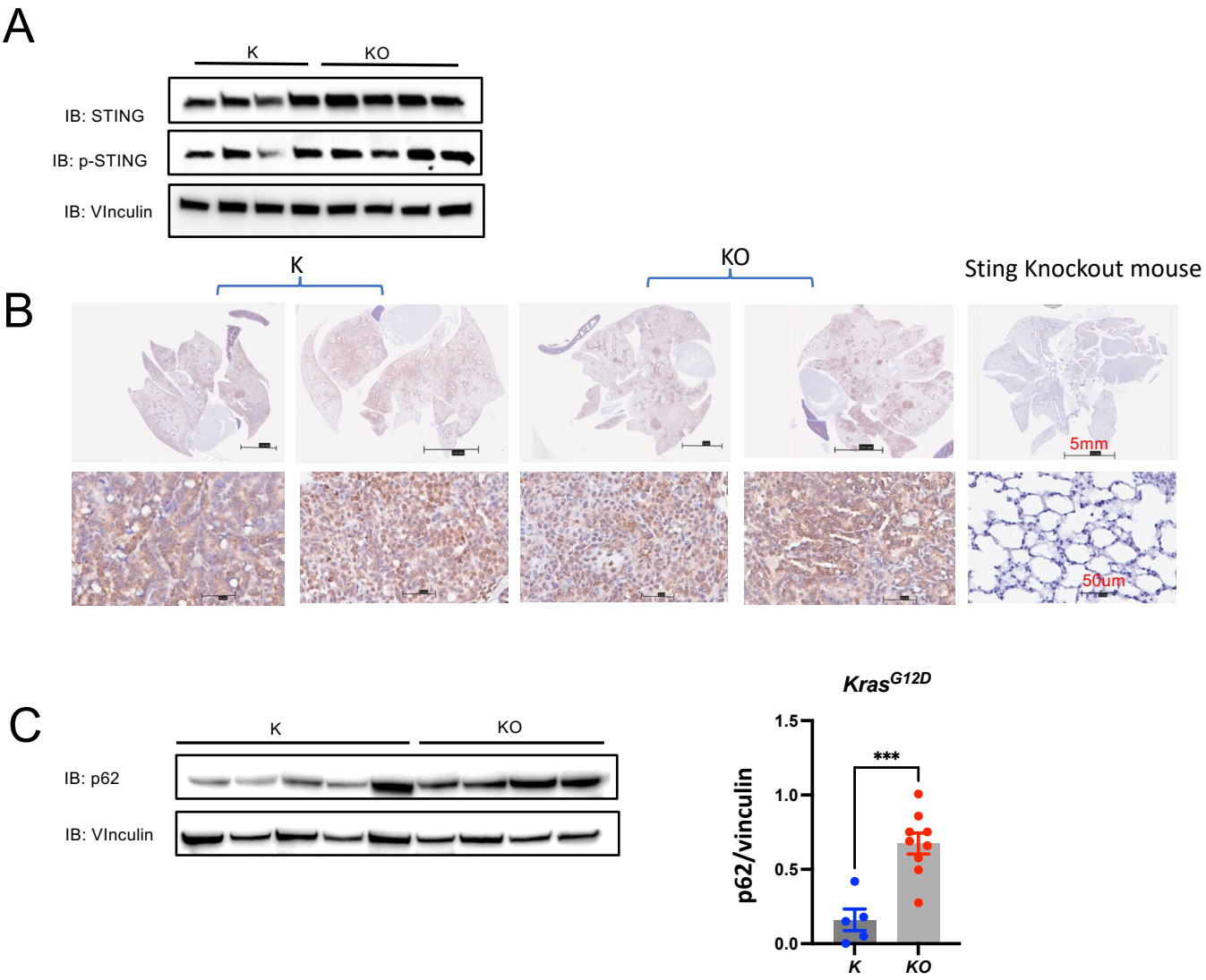
A
K
KO
IB: STING
IB: p-STING
IB: VInculin
B
K
KO
Sting Knockout mouse
5mm
50um
C
K
KO
IB: p62
IB: VInculin
Kras^G12D
p62/vinculin
1.5
1.0
0.5
0.0
***
K
KO

Figure S8: STING expression in K/KO tumors, related to Figure 8.

(A) Western blot for STING and pSTING on lung tissues from K/KO GEMMs.

(B) IHC for STING on lung tissue sections from K/KO GEMMs and STING KO mice. Top- low magnification and bottom- high magnification images.

(C) Western blots for p62 and vinculin on lung tumors from K/KO GEMMs. Relative protein amounts were quantified by image densitometry.