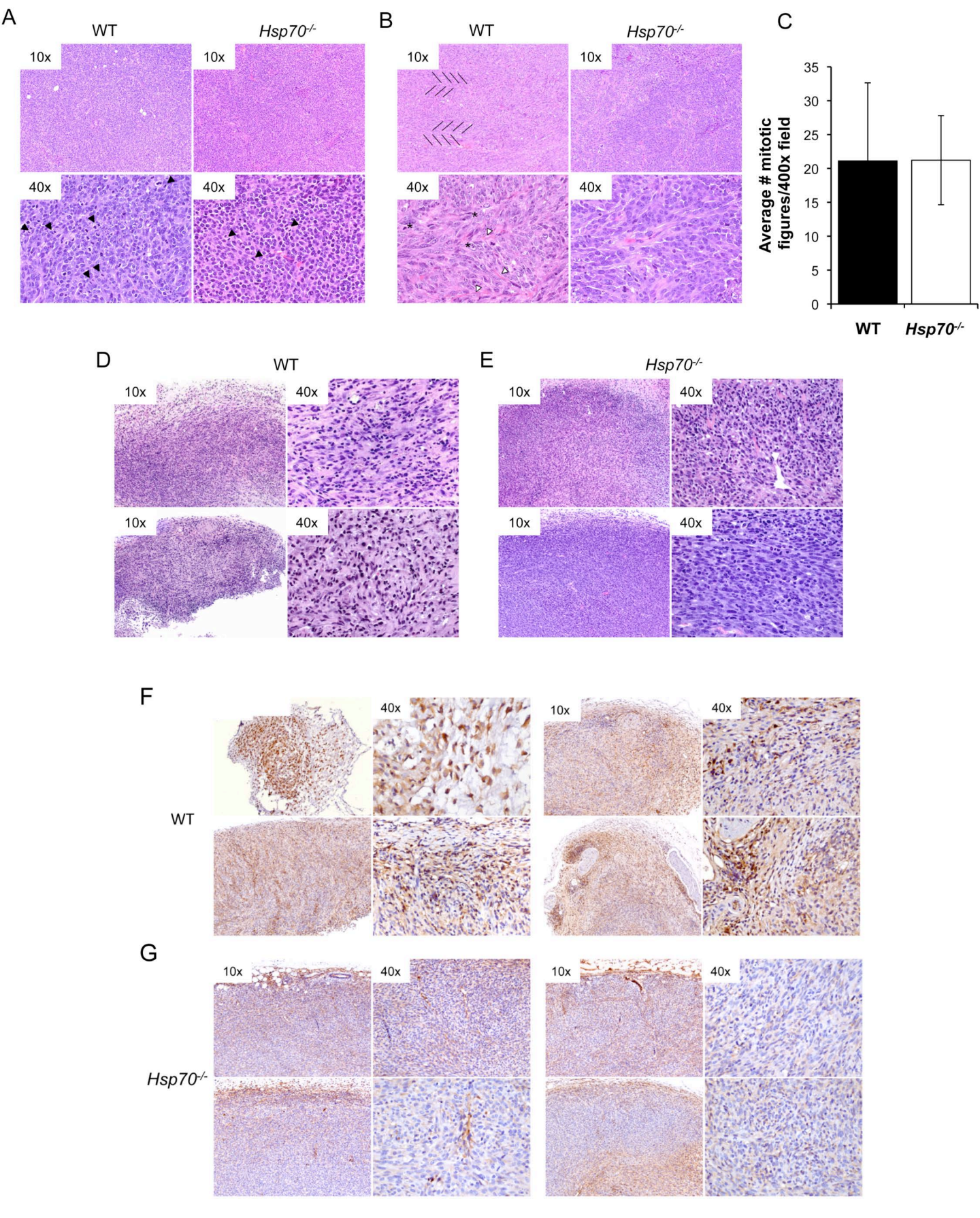
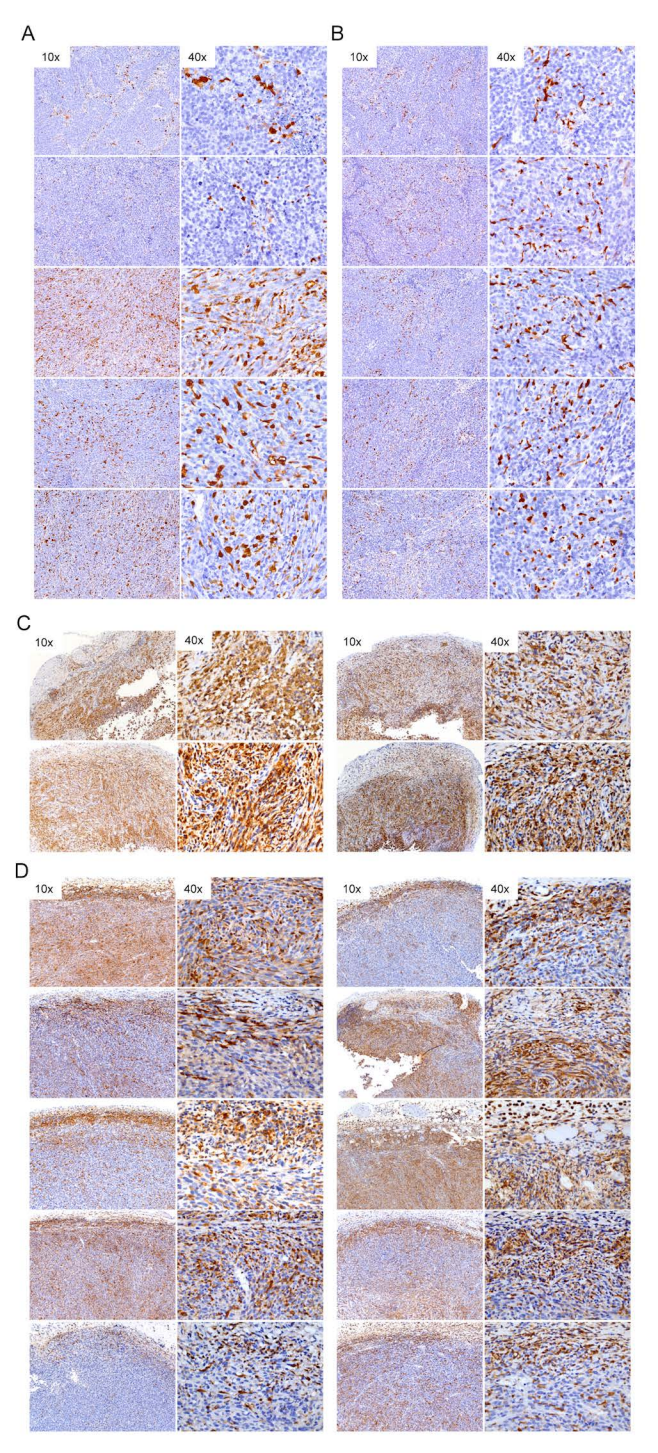
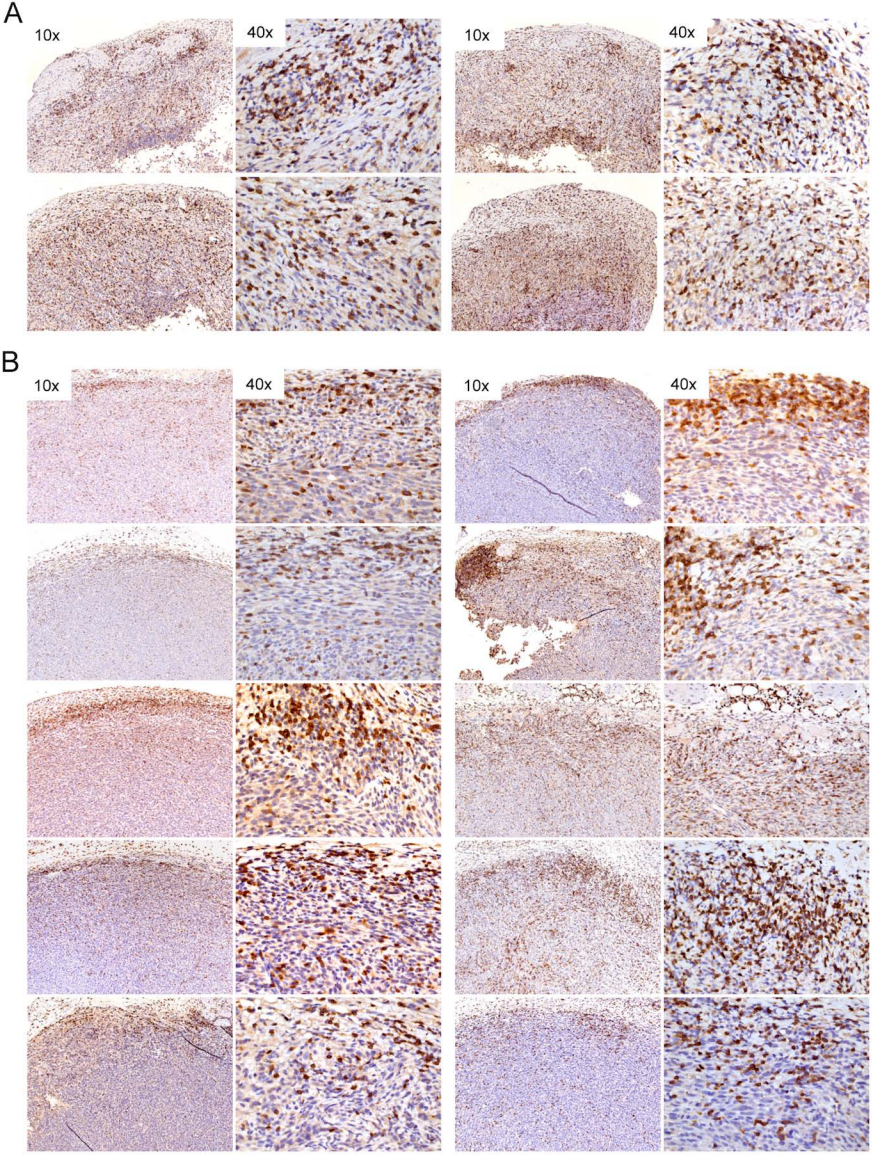
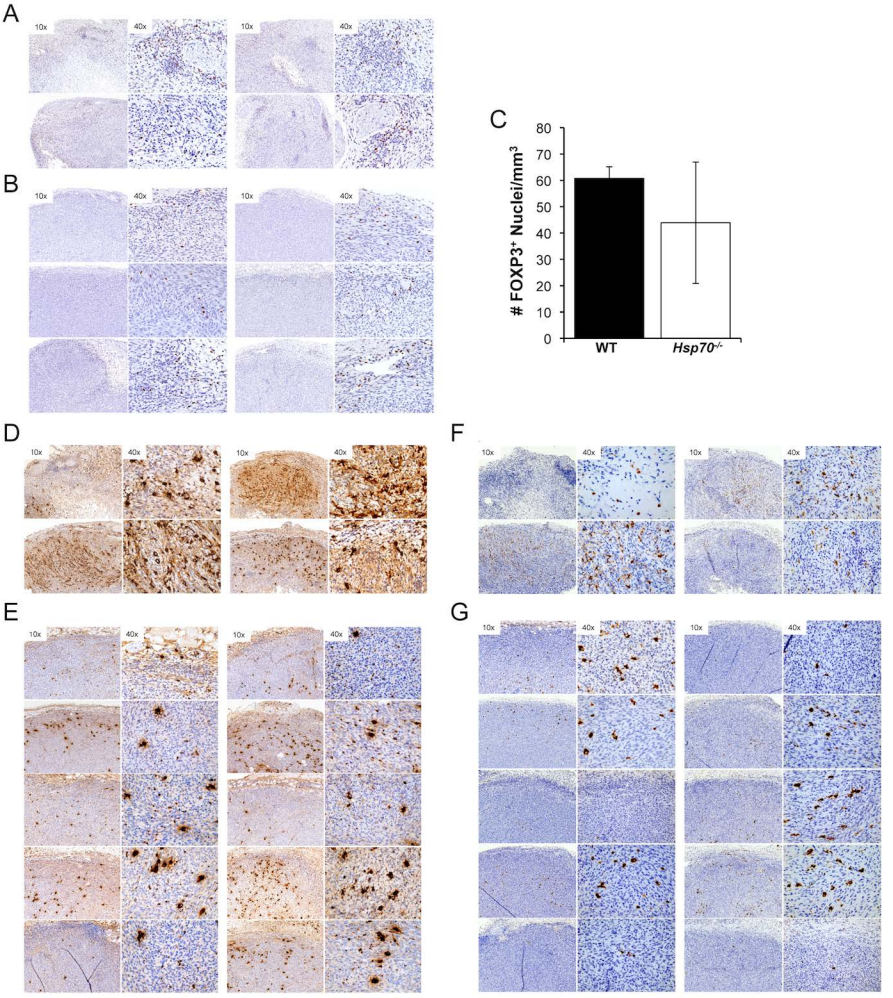
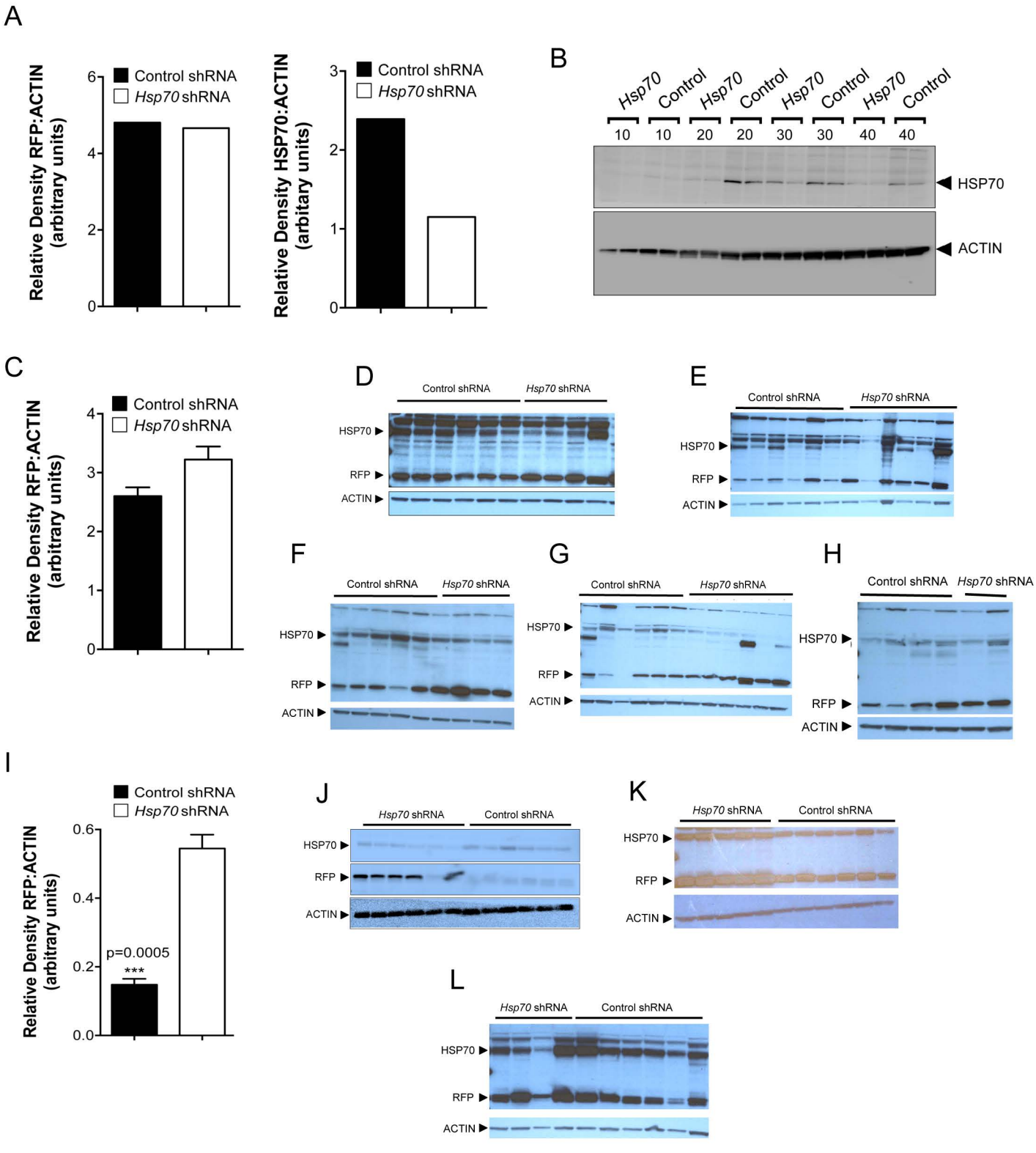
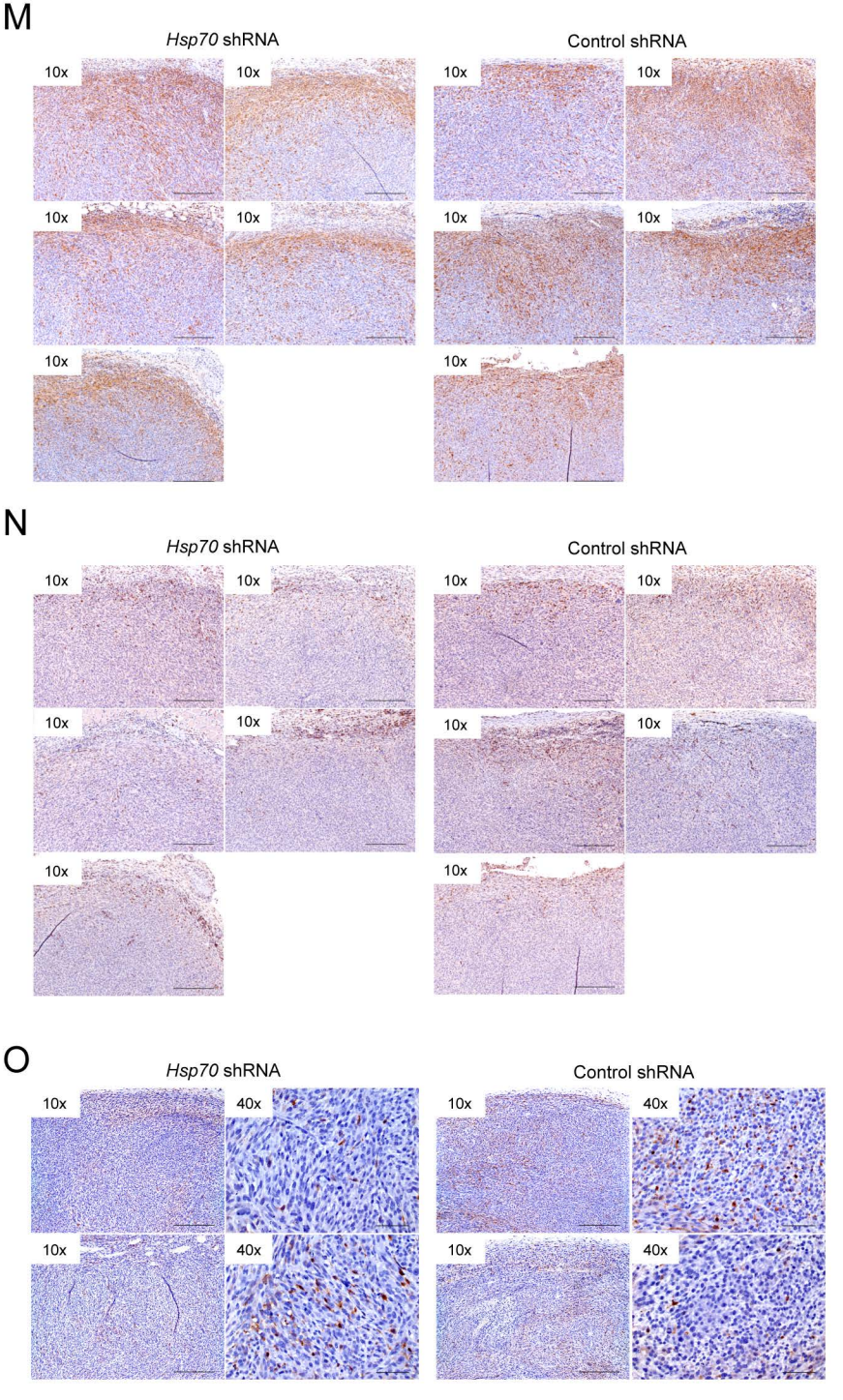

A
WT
Hsp70-/-
10x
40x
B
C
Average # mitotic figures/400x field
35
30
25
20
15
10
5
0
WT
Hsp70-/-
D
WT
E
Hsp70-/-
F
WT
G
Hsp70-/-

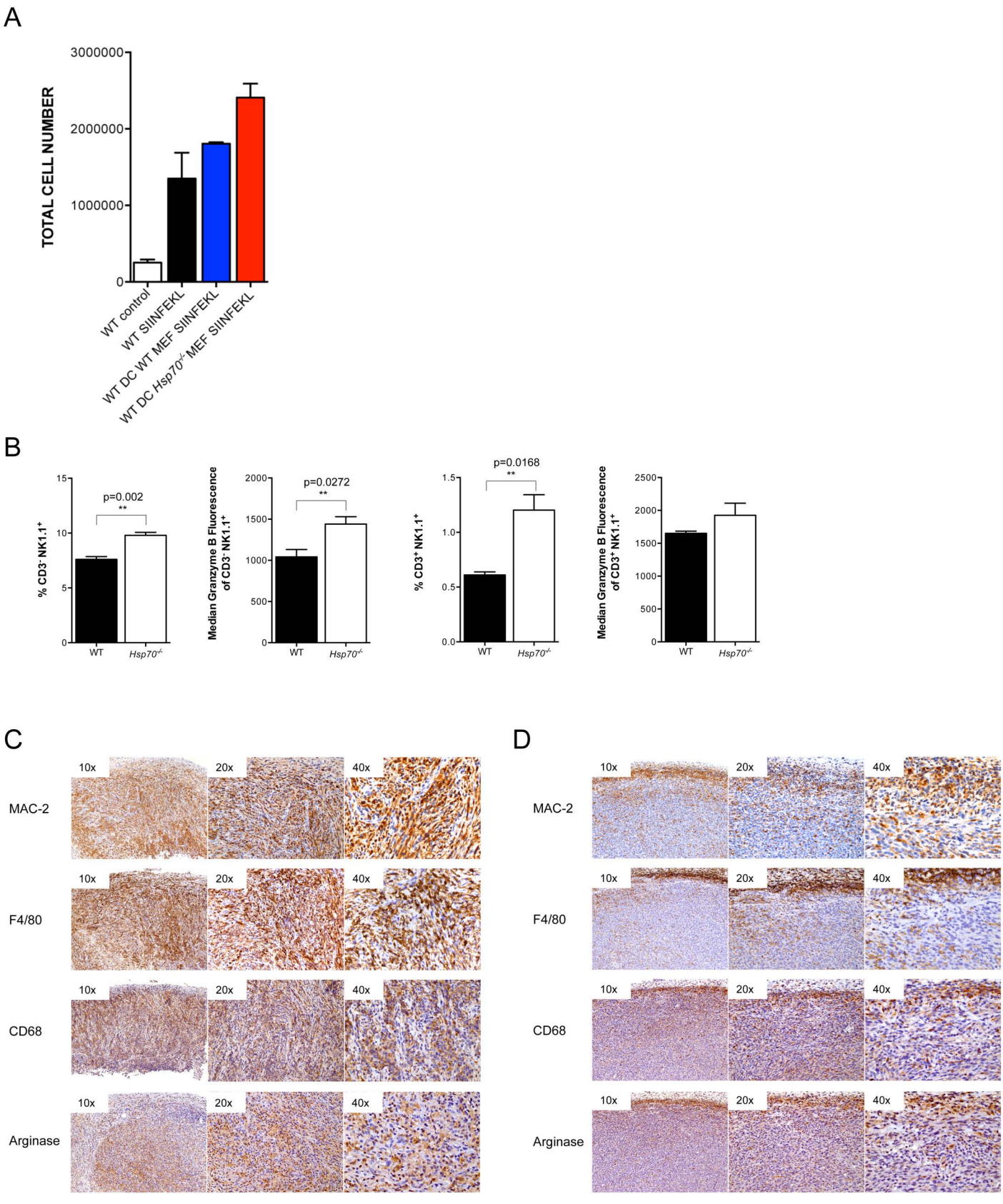

A
10x
40x
B
10x
40x
C
10x
40x
10x
40x
D
10x
40x
10x
40x


A
10x
40x
10x
40x
B
10x
40x
10x
40x


A
10x
40x
10x
40x
B
10x
40x
10x
40x
C
FOXP3+ Nuclei/mm3
80
70
60
50
40
30
20
10
0
WT
Hsp70-/-
D
10x
40x
10x
40x
E
10x
40x
10x
40x
F
10x
40x
10x
40x
G
10x
40x
10x
40x


A
Relative Density RFP:ACTIN (arbitrary units)
Control shRNA
Hsp70 shRNA
Relative Density HSP70:ACTIN (arbitary units)
B
Hsp70
Control
10
20
30
40
HSP70
ACTIN
C
D
E
F
G
H
HSP70
RFP
ACTIN
I
p=0.0005
***
J
K
L


M
Hsp70 shRNA
Control shRNA
10x
N
Hsp70 shRNA
Control shRNA
10x
O
Hsp70 shRNA
Control shRNA
10x
40x


A
TOTAL CELL NUMBER
3000000
2000000
1000000
0
WT control
WT SIINFEKL
WT DC WT MEF SIINFEKL
WT DC Hsp70-/- MEF SIINFEKL
B
% CD3- NK1.1+
p=0.002
**
WT
Hsp70-/-
Median Granzyme B Fluorescence of CD3- NK1.1+
p=0.0272
**
% CD3+ NK1.1+
p=0.0168
**
Median Granzyme B Fluorescence of CD3+ NK1.1+
C
D
MAC-2
F4/80
CD68
Arginase
10x
20x
40x

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Histological analysis of and HSP70 expression in WT and *Hsp70*$^{-/-}$ tumors. Gross histological appearance (hematoxylin and eosin (H&E)) of representative WT and *Hsp70*$^{-/-}$ sarcomas harvested from CD1-*Foxn1*^{nu} **(A)** and **(B)** or C57BL/6J **(D)** and **(E)** hosts. **(A)** and **(B)** show representative images of poorly and moderate to well differentiated tumors respectively of WT and *Hsp70*$^{-/-}$ tumors as indicated. Magnification is shown in the upper left hand corner of each panel. Salient features include mitotic figures (denoted by black arrowheads), karyomegaly (denoted by the asterisks) and matrix deposition (shown by the white arrowheads). Quantification of the average number of mitoses in tumors harvested from CD1-*Foxn1*^{nu} hosts was determined by counting the number of mitotic figures in 10, randomly selected 40x fields and dividing by 10 **(C)**. Panels **(D)** and **(E)** show representative H&E images of two WT and two *Hsp70*$^{-/-}$ tumors harvested from C57BL/6J mice at the magnifications shown. Immunohistochemical staining with an HSP70-specific antibody in 4 WT **(F)** and 4 *Hsp70*$^{-/-}$ **(G)** tumors from C57BL/6J hosts are shown at the magnifications indicated.

Supplementary Figure 2. MAC-2 staining of WT and *Hsp70*$^{-/-}$ tumors. WT and *Hsp70*$^{-/-}$ tumors harvested from CD1-*Foxn1*^{nu} (A) and (B) or C57BL/6J (C) and (D) hosts were prepared for immunohistochemical staining with a MAC-2 antibody to visualize macrophage distribution. Representative images from 5 WT (A) and 5 *Hsp70*$^{-/-}$ (B) tumors from the CD1-*Foxn1*^{nu} hosts and 4 WT (C) and 10 *Hsp70*$^{-/-}$ (D) tumors from the C57BL/6J hosts are shown at the magnifications indicated.

Supplementary Figure 3. CD3 staining of WT and *Hsp70*$^{-/-}$ tumors. WT **(A)** and *Hsp70*$^{-/-}$ **(B)** tumors harvested from C57BL/6J hosts were prepared for immunohistochemical staining with a CD3 antibody to visualize T cell distribution. Representative images from 4 WT **(A)** and 10 *Hsp70*$^{-/-}$ **(B)** tumors are shown at the magnifications indicated.

Supplementary Figure 4. FoxP3, Granzyme B and perforin staining of WT and *Hsp70*$^{-/-}$ tumors. WT **(A)** or *Hsp70*$^{-/-}$ **(B)** tumors harvested from C57BL/6 hosts were prepared for immunohistochemical staining with a

FoxP3 antibody to visualize regulatory T cells. Quantification is shown in **(C)**. WT and *Hsp70*$^{-/-}$ tumors harvested from C57BL/6J hosts were prepared and stained for granzyme B **(D)** and **(E)** or perforin **(F)** and **(G)**. Representative images of 4 WT **(D)** and **(E)** and 10 *Hsp70*$^{-/-}$ **(F)** and **(G)** tumors are shown at the magnifications indicated.

Supplementary Figure 5. *Hsp70* shRNA and control shRNA tumors. WT transformants were transfected with RFP-vectors expressing *Hsp70* or control shRNAs. Following three rounds of sorting (upper 20% of RFP expressing cells was collected each time) cells were stably expressing *Hsp70* or control shRNAs. Immunoblot analysis and densitometric quantification confirmed the equivalent expression of RFP but reduced HSP70 expression in the Hsp70shRNA expressing cell line as compared to the control **(A)**. WT-*Hsp70*-shRNA or WT-control-shRNA tumor cells were subjected to heat shock (42^{o}C for 60 minutes) before recovery for 2 hours. Protein samples (10-40μg as indicated) were loaded in duplicate and immunoblotted for HSP70 (upper panel) and actin control (lower panel) (*Hsp70* or control indicates the cell line used) **(B)**. WT tumors stably expressing *Hsp7*0 or control shRNAs were harvested from C57BL/6J **(D-F)** or CD1-*Foxn1*^{nu} **(G-K)** hosts and immunoblotted for HSP70, RFP and actin as indicated by arrows. Densitometric quantification of RFP expression for tumors harvested from C57BL/6J or CD1-*Foxn1*^{nu} mice is shown **(C)** and **(I)** respectively. WT tumors stably expressing *Hsp70* or control shRNAs were harvested from C57BL/6 hosts **(M)** and **(N)** or CD1-*Fox1*^{nu} hosts **(O)** and stained with MAC-2 **(M)** and **(O)** or CD3 **(N)** antibodies. Representative images from 5 *Hsp70* and 5 control shRNA tumors are shown at the magnifications indicated.

Supplementary Figure 6. The Defective Anti-Tumor Immunity Observed in *Hsp70*$^{-/-}$ Mice Cannot be Attributed to Previously Described Immunomodulatory Activities of HSP70. WT dendritic cells were incubated overnight with irradiated WT or *Hsp70*$^{-/-}$ MEF transformants pre-loaded with SIINFEKL class I MHC OVA peptide. OT-I CD8^{+} transgenic T cells were co-cultured with the DCs for 72h after which T cell number was evaluated **(A)**. WT C57BL/6J hosts were injected bilaterally with WT or *Hsp70*$^{-/-}$ transformants and tumor growth monitored. At the termination of the experiments, spleens were harvested and stained for cell surface CD3 and NK1.1 and intracellular granzyme B. Average % CD3^{-} NK1.1^{+} (NK cells) and CD3^{+} NK1.1^{+} (NKT cells) with the respective median granzyme B fluorescence are shown **(B)**. Average values are calculated from 5

mice of each and p values are generated using t-tests (Prism Software). The tumors generated from WT or *Hsp70*$^{-/-}$ *E1A/Ras* transformants in C57BL/6J mice were harvested at the termination of the experiment and processed for immunostaining with a panel of markers characteristic of tumor associated macrophages. Representative tumors are shown for WT **(C)** and *Hsp70*$^{-/-}$ **(D)** at the magnifications indicated in the upper left corner of each panel.