Expanded View Figures

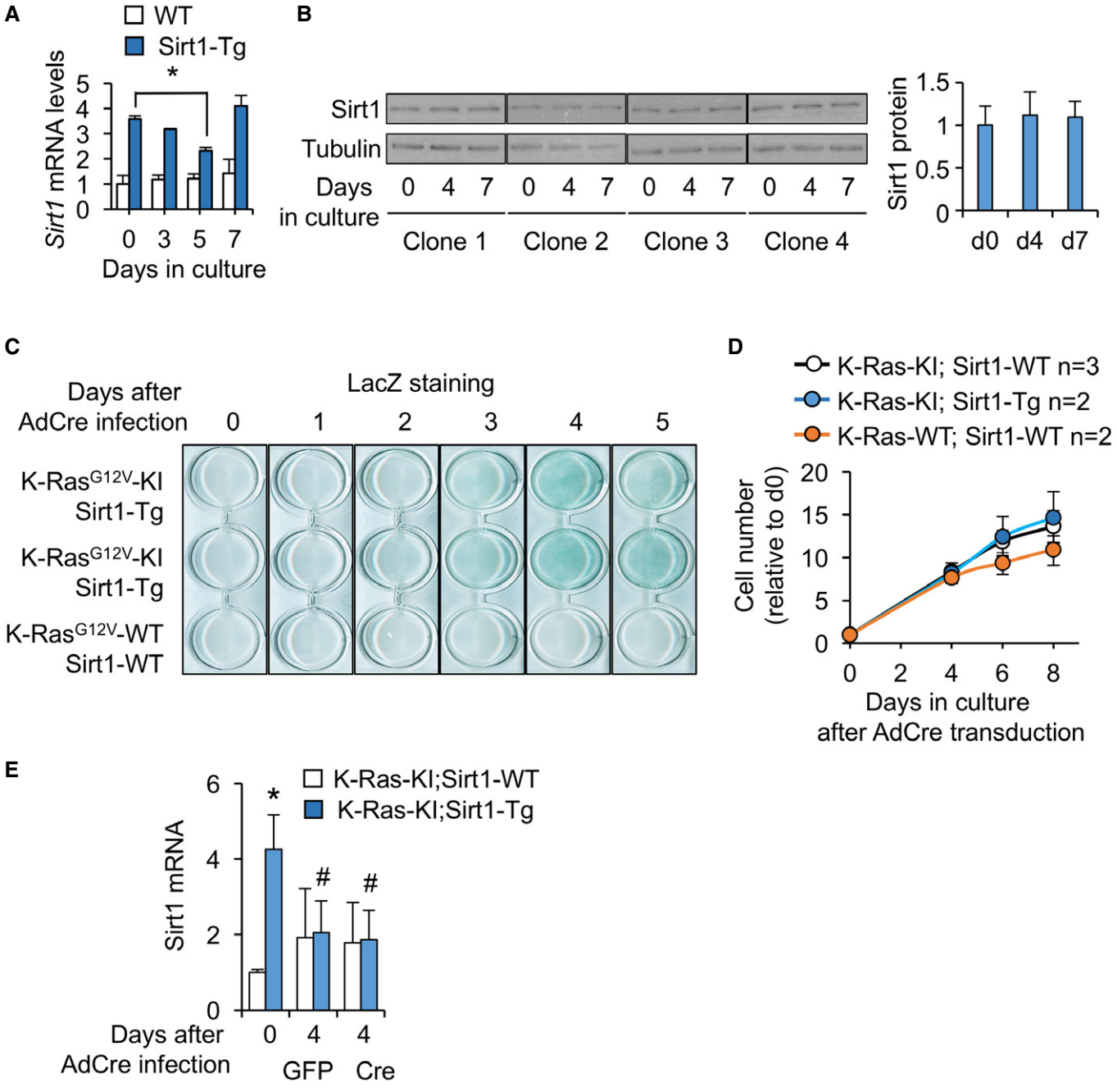


Figure EV1.

Figure EV1. K-Ras activation in Sirt1-WT and Sirt1-Tg MEFs.

A mRNA expression of *Sirt1* from WT or Sirt1-Tg MEFs cultured in 10% FBS for the indicated times. Bars represent the average of at least three independent clones.
B Sirt1 protein expression in four different clones of immortalized MEFs, after culturing them during the indicated time. Quantification of the band intensity is shown in the right panel.
C X-Gal staining of MEFs of the indicated genotypes infected with adeno-Cre (AdCre) at the indicated times after infection, to detect the LacZ reporter placed in the same polycistronic mRNA with the oncogene K-Ras^{G12V}.
D Growth curve with MEFs of the indicated genotypes after Adeno-Cre infection.
E mRNA expression of *Sirt1* from three independent replicates of K-Ras-KI; Sirt1-WT or K-Ras-KI; Sirt1-Tg MEFs 4 days after infection with the indicated adenoviruses.

Data information: Error bars indicate standard error of the mean. For comparison between Sirt1-Tg clones with their corresponding Sirt1-WT controls, statistical significance was assessed using the unpaired, two-tailed Student's *t*-test. $^{*}P < 0.05$. For comparison between Sirt1-Tg clones in different conditions, unpaired two-tailed Student's *t*-test was used. $^{\#}P < 0.05$.
Source data are available online for this figure.

Figure EV2. Regulation of Sirt1 expression by K-Ras in MEFs and in human lung tumor cell lines.

A Western blots of the indicated proteins in WT MEFs treated for 16 h with vehicle (DMSO) or the indicated inhibitors.
B K-Ras-KI MEFs were infected with Adeno-Cre and treated with DMSO (D), MEK inhibitors (Mi) or PI3K inhibitors (Pi) for 16 h, and the indicated proteins were detected by WB.
C Western bots of the indicated proteins from the indicated cell lines, treated with vehicle (DMSO, D), MEK inhibitor (Mi), or PI3K inhibitor (Pi) for 16 h.
D Western blots of the indicated proteins from the cell lines where SIRT1 protein levels were shown to respond to MEK and/or PI3K inhibition in Fig 2E.

Source data are available online for this figure.

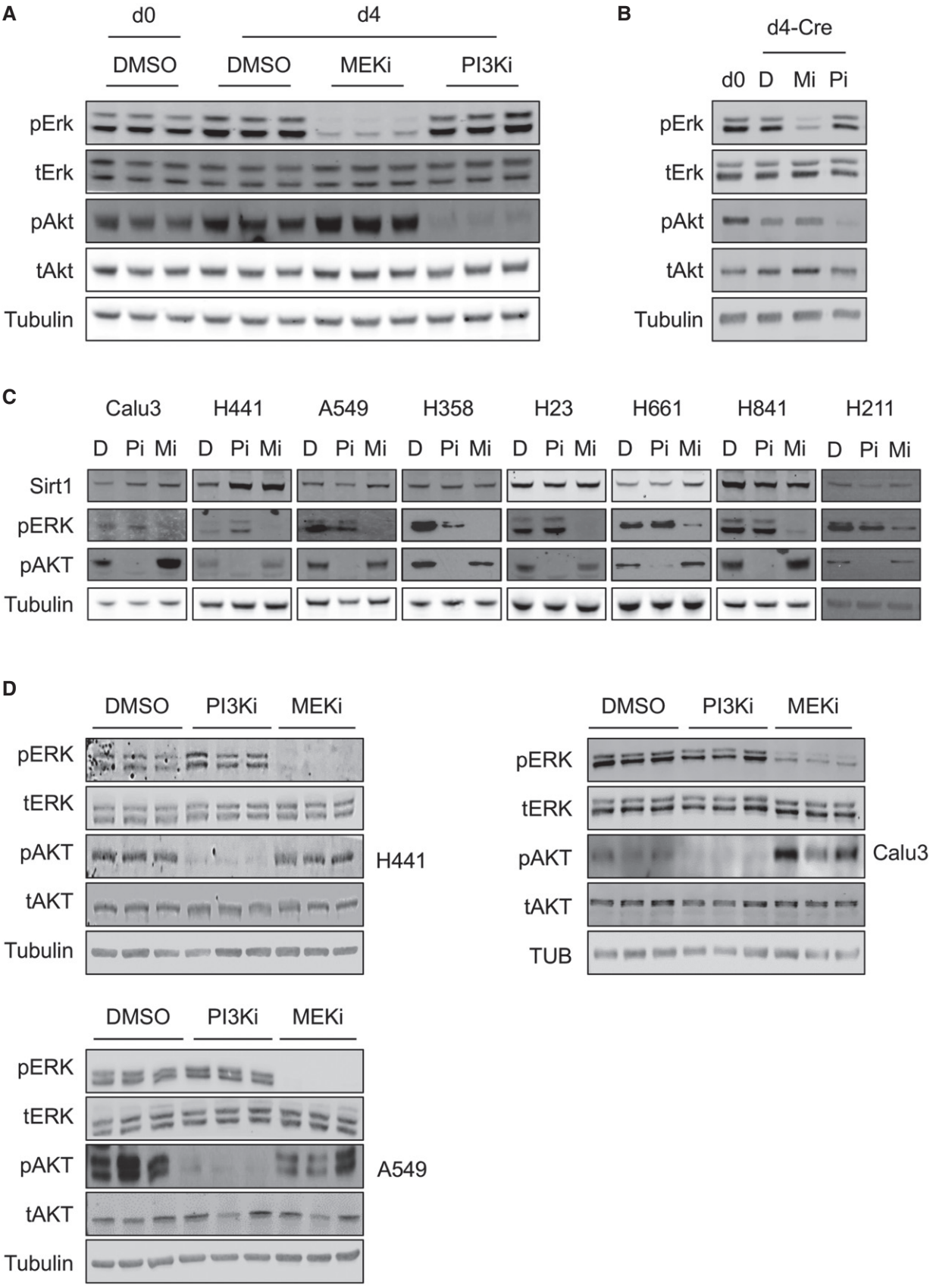
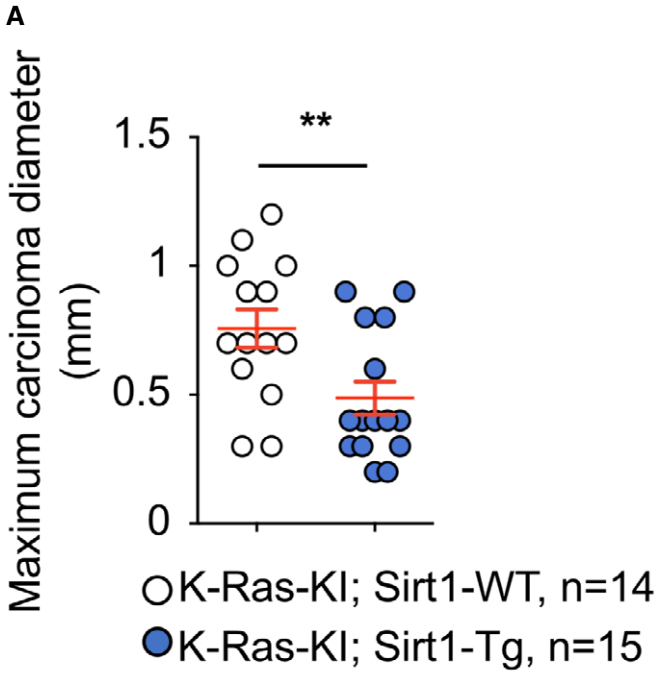

A
d0
d4
DMSO
DMSO
MEKi
PI3Ki
pErk
tErk
pAkt
tAkt
Tubulin
B
d4-Cre
d0 D Mi Pi
pErk
tErk
pAkt
tAkt
Tubulin
C
Calu3
H441
A549
H358
H23
H661
H841
H211
D Pi Mi
Sirt1
pERK
pAKT
Tubulin
D
DMSO
PI3Ki
MEKi
pERK
tERK
pAKT
tAKT
Tubulin
H441
DMSO
PI3Ki
MEKi
pERK
tERK
pAKT
tAKT
TUB
Calu3
DMSO
PI3Ki
MEKi
pERK
tERK
pAKT
tAKT
Tubulin
A549

Figure EV2.



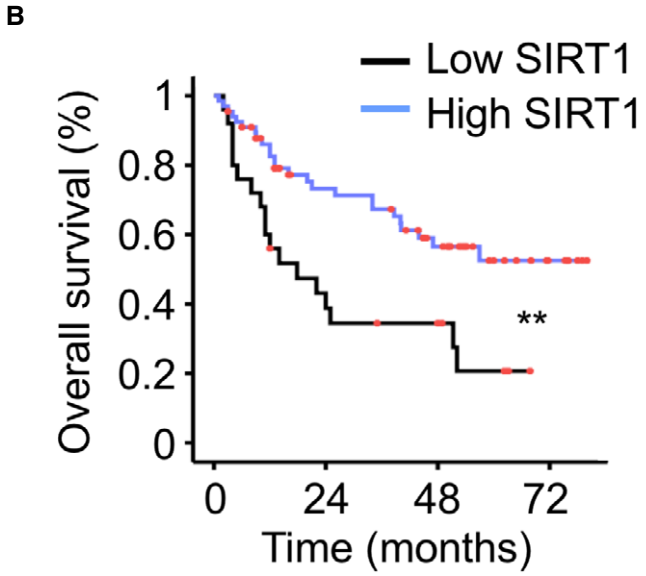


Figure EV3. Sirt1 effects in mouse and human lung carcinoma.

A Quantification of the maximum carcinoma diameter per mouse at the time of death by histopathological analysis. Red lines represent the means and the standard errors of the mean.

B Kaplan–Meier curve with 105 human lung tumor patients indicating that high expression of cytoplasmic SIRT1 is associated with longer overall survival (**P = 0.003).

Data information: Statistical significance was assessed using the two-tailed Student's t-test (A) or the log-rank test (B). **$P < 0.01$.

Figure EV4. Pneumocyte isolation and characterization.

A Strategy for pneumocyte isolation: dissociated lungs were analyzed by cytometry to discard aggregates (first panel); stained with DAPI to exclude dead cells (second panel); and further analyzed by size to discard debris (third panel). Finally, single, alive cells were analyzed for their expression of the lymphocytic marker CD45 or the endothelial marker CD31, and double negatives for CD31/CD45 were considered pneumocytes.

B, C Representative immunohistochemistry stainings detecting Katushka-positive cells after only 4 weeks of 4-OH tamoxifen activation (B) or 4 weeks of 4-OH tamoxifen pulse + 2 weeks with no tamoxifen treatment chase (C). Size bars represent 200 μm. Arrows indicate Katushka-positive cells.

D, E KEGG pathway clustering of differentially expressed genes between the K-Ras-KI; Sirt1-WT and K-Ras-KI; Sirt1-Tg pneumocytes isolated by FACS sorting in the pulse phase (D) or in the pulse + chase phase (E). Red columns represent cancer-related pathways.

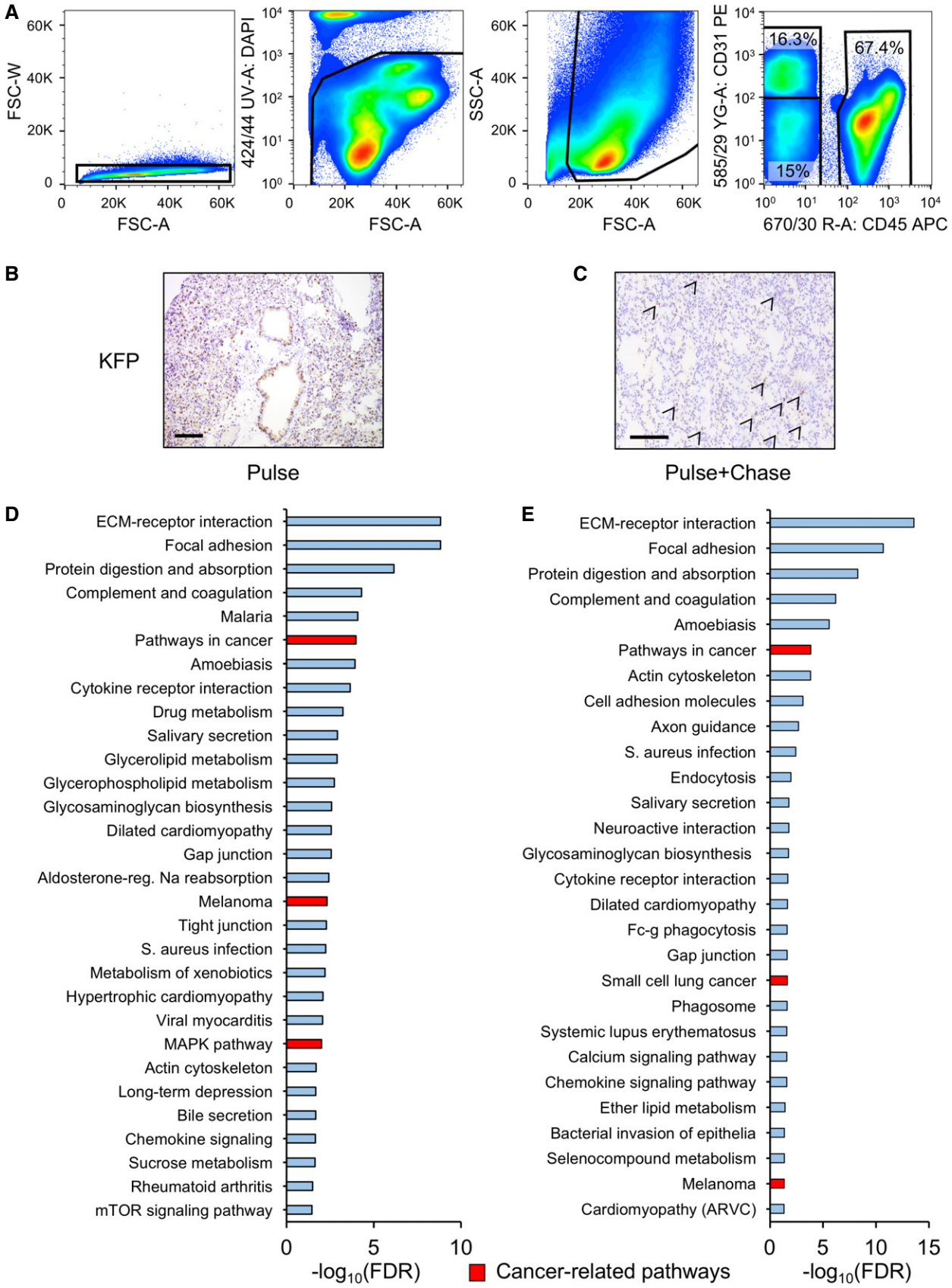


Figure EV4.