Supplementary Materials for

Chimeric antibody targeting unique epitope on onco-Mucin 16 reduces tumor burden in pancreatic and lung malignancies

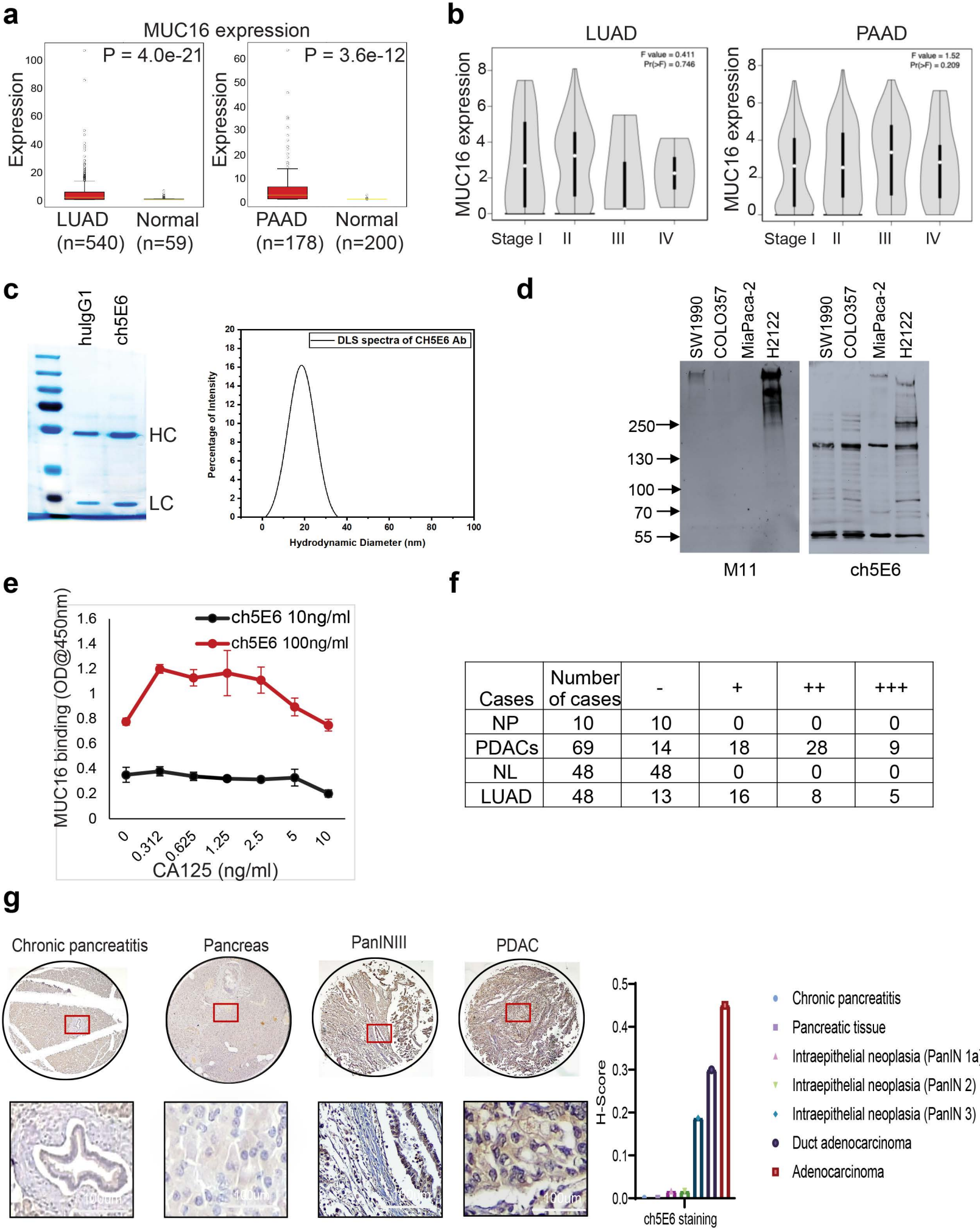
Supplementary Table 1: List of primers used in the study

Primer	Sequence
5E6-VH-F1	AATAGGGAATTCGGAGGTGAAG
5E6-VH-R1	CCTTGGTGCTAGCTGAGGA
5E6-VL-F1	CCTTGGTGCTAGCTGAGGA
5E6-VL-R1	AGCCACCGTACGCTTTAT
VH seq	ATTAAAGAGGAGAAATTAACC A
VL seq	AGGCCTCTAGATTAATGATGAT
pFUSE-CHIg/CLIg seq	TGCTTGCTCAACTCTACGTC

Supplementary Table 2: List of antibodies used in the study

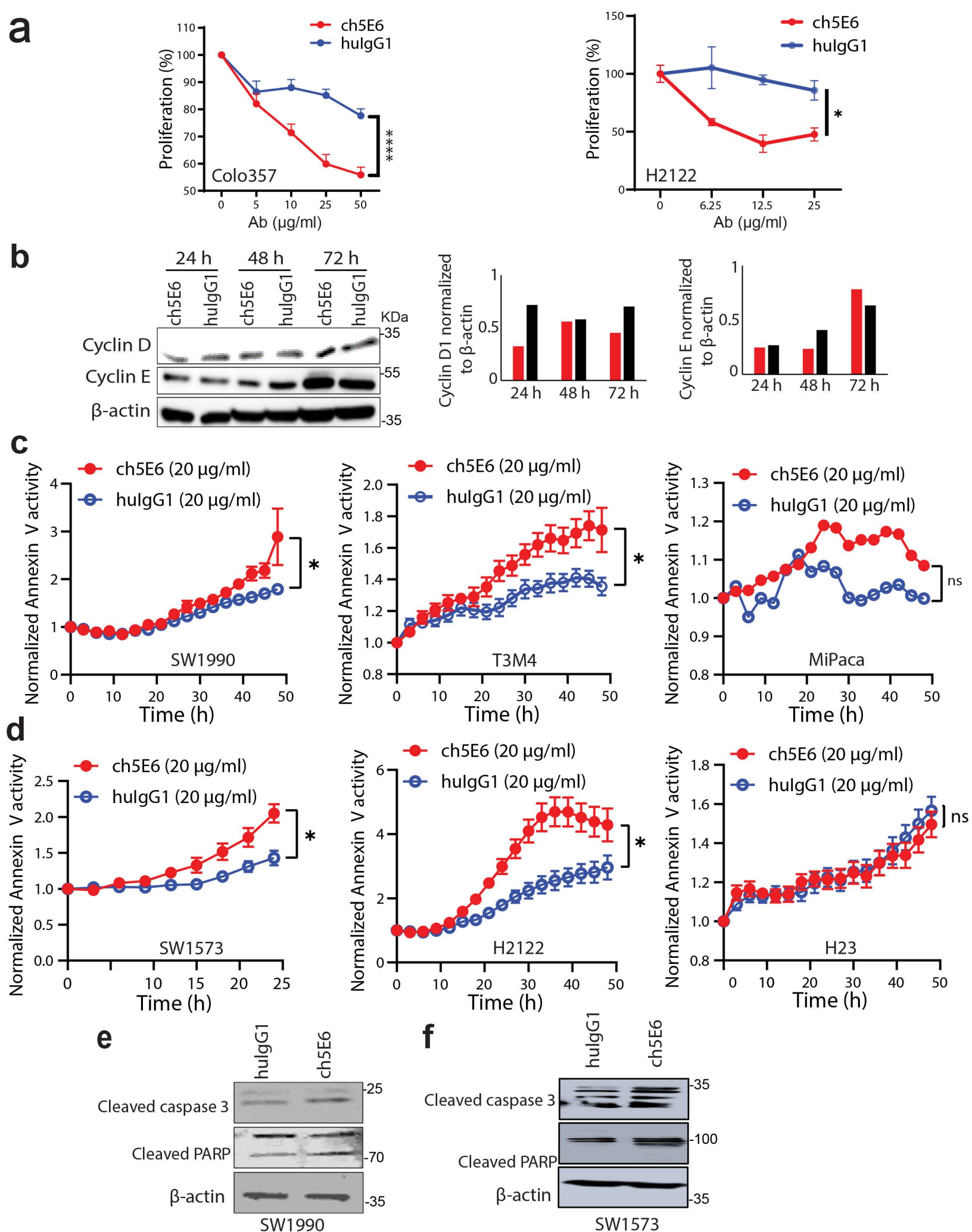
ANTIBODIES	SOURCE	IDENTIFIER/DILUTION
List of primary antibodies used in Immunofluorescence		
E-cadherin	Santa Cruz	Cat# Sc-55510 (Ms) (1:100)
N-cadherin	Cell Signaling Technology	Cat# 13116S (Rb) (1:200)
M11	Agilent Dako	Cat# GA701 (Ms) (1:1000)
Actin Cytoskeleton / Focal Adhesion Staining Kit	Sigma	FAK100 (1:100)
pFAK (Y397)	Life Technologies	Cat # 700255 (Rb) (1:250)
List of secondary antibodies used in Immunofluorescence		
Alexa Fluor 568 goat anti-mouse IgG	Life Technologies	Cat#A11031 (1:300)
Alexa Fluor 568 goat anti-rabbit IgG	Life Technologies	Cat# A11011 (1:300)
Alexa Fluor 488 goat anti-mouse IgG	Life Technologies	Cat# A28175 (1:300)
Alexa Fluor 488 goat anti-rabbit IgG	Life Technologies	Cat# A11008 (1:300)
Alexa Fluor 488 goat anti-human IgG	Life Technologies	Cat# A-11013 (1:300)
List of antibodies used in Western Blotting		
N-cadherin	Cell Signaling Technology	Cat# 13116S (Rb) (1:1000)
E-cadherin	Cell Signaling Technology	Cat# 3195 (Rb) (1:1000)
Vimentin	Cell Signaling Technology	Cat#5741S (Rb) (1:100)

Fibronectin	ABclonal	Cat# A7488 (Rb) (1:1000)
p-P70S6K (T389)	Cell Signaling Technology	Cat# 9205 (Rb) (1:1000)
p-JNK	Cell Signaling Technology	Cat# 9251S (Rb) (1:1000)
pFAK (Y397)	Cell Signaling Technology	Cat#D20B1 (Rb) (1:1000)
P-p44/42 MAPK (Y202/204)	Cell Signaling Technology	Cat#9101S (Rb) (1:1000)
pAkt	Cell Signaling Technology	Cat#D9E (Rb) (1:1000)
Cyclin D	Cell Signaling Technology	Cat#92G2 (Rb) (1:500)
List of antibodies used in Immunohistochemistry		
N-cadherin	Santa Cruz	Cat# Sc-55510 (Ms) (1:100)
M11	Agilent Dako	Cat# GA701 (Ms) (1:100)
Ki67	Cell Signaling Technology	Cat# D385 (Rb) (1:500)
Cleaved caspase 3	Cell Signaling Technology	Cat# D175 (Rb) (1:200)

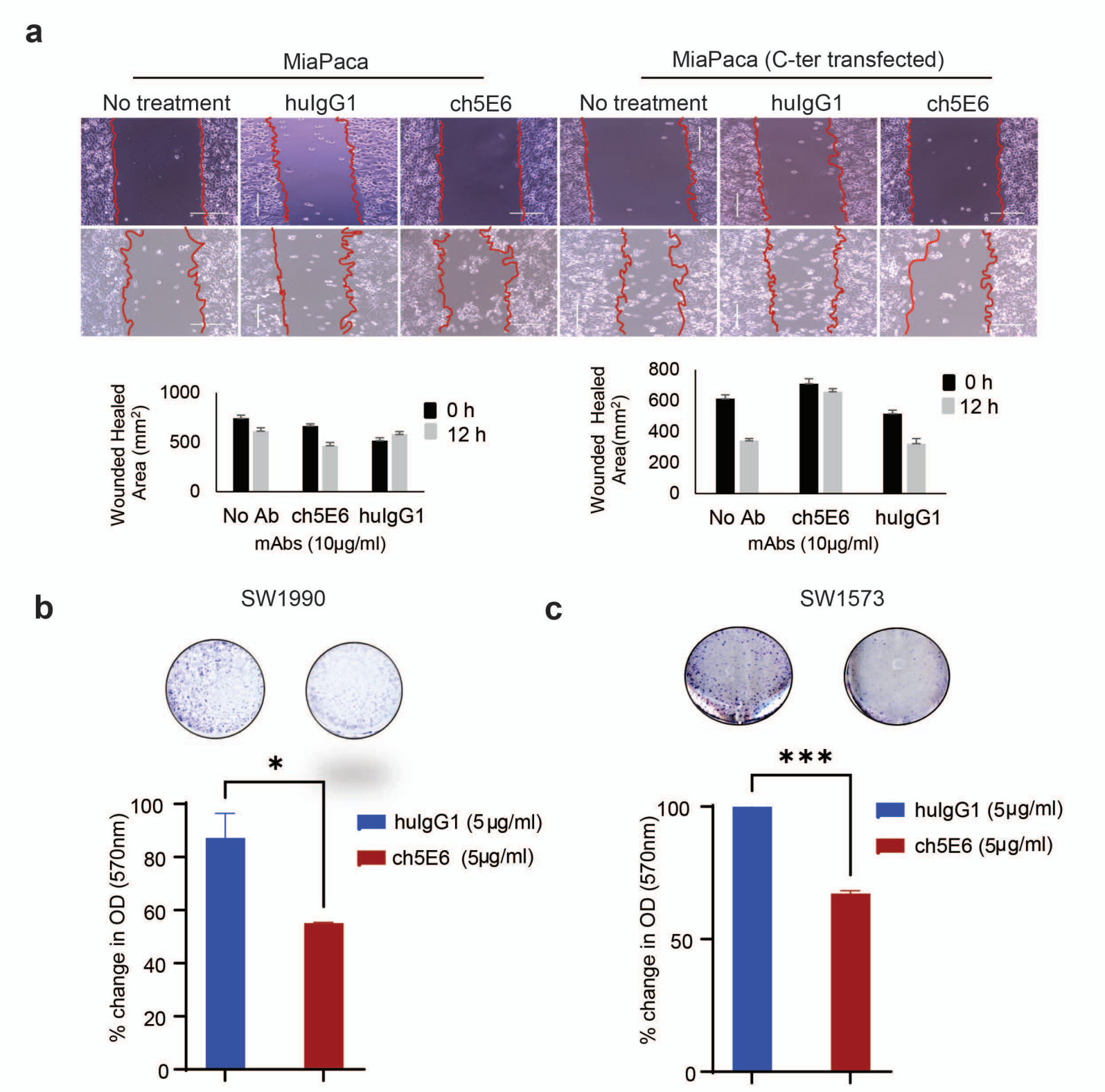


Cases	Number of cases	-	+	++	+++
NP	10	10	0	0	0
PDACs	69	14	18	28	9
NL	48	48	0	0	0
LUAD	48	13	16	8	5

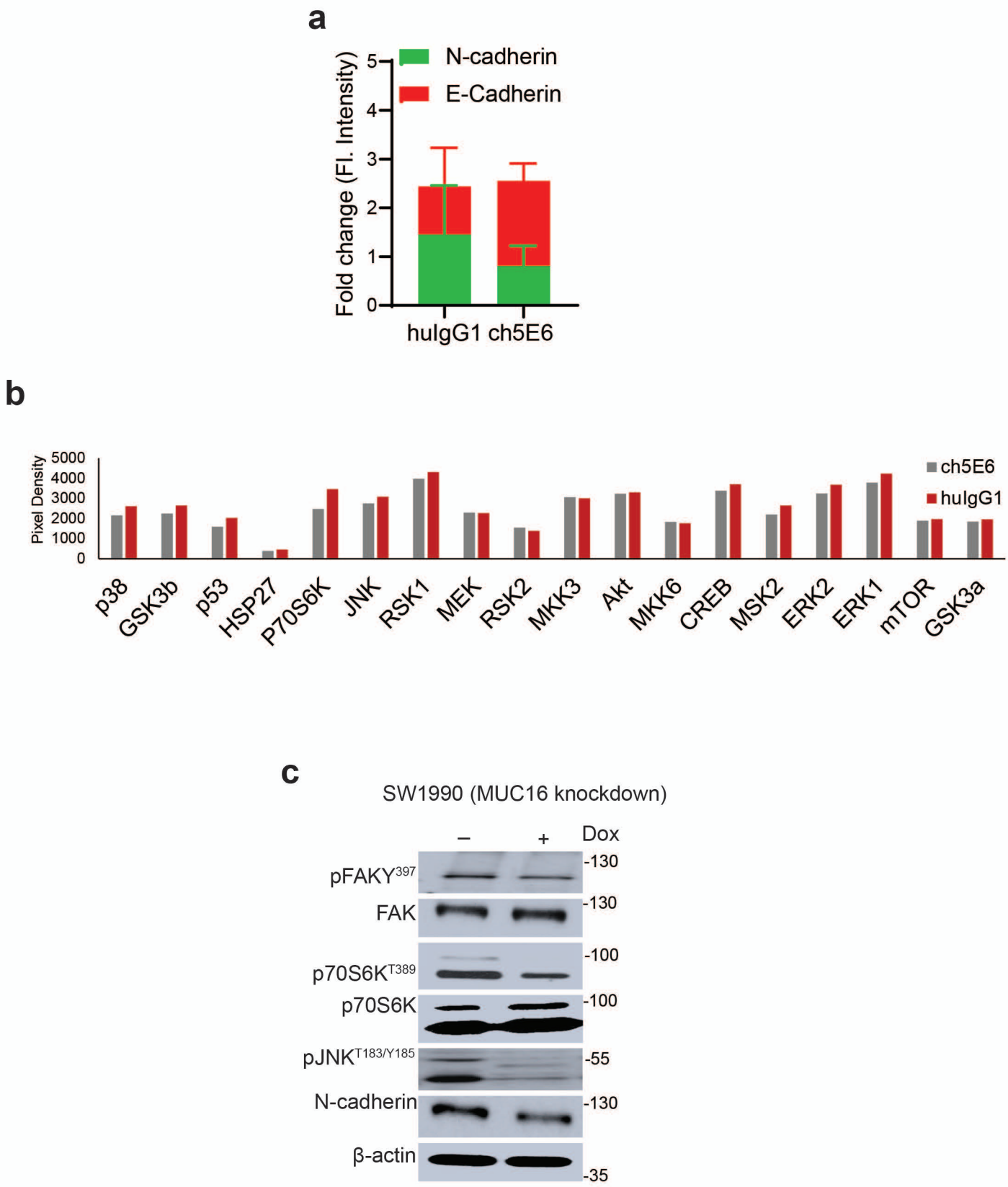
Supplementary Figure 1: A. Transcriptomic expression analysis of MUC16 in PDAC (Pancreatic Ductal Adenocarcinoma) patients (n=178) and adjacent normal (n=200) cases and NSCLC patients (n=540) along with adjacent normal cases (n=59) from GTEx datasets (http://GEPIA.cancer-pku.cn). **B**. Stage-specific expression of MUC16 in both PC and NSCLC patients showing its association with disease aggressiveness. **C.** The characterization of purified ch5E6 on Coomassie-stained gel showing the heavy and light chain bands of chimeric mAb5E6 at 50 and 25 kDa, respectively and Dynamic light scattering (DLS) showing a single peak at hydrodynamic diameter 16nm**. D.** Western blot analysis of cell lysates prepared after the collection of conditioned media showing a specific binding pattern of MUC16 with mAbM11 and ch5E6. Specific bands are indicated by arrow. **E.** Effect on MUC16 binding by ch5E6 in the presence of increasing concentrations of soluble CA125 from 0.3ng/ml to 5ng/ml. **F.** Table showing the percentage of tumors stained by ch5E6 in PC and NSCLC patients. **G.** Immunohistochemical staining of ch5E6 in chronic pancreatitis, PanIN1, PanIN2, PanIN3, and PDAC patients showing specific binding to MUC16 in PDAC patients. Scale bars, 100μm; *, $P < 0.05$; **, $P < 0.005$, ****, $P < 0.0005$.


a
ch5E6
huIgG1
Proliferation (%)
Colo357
****
Ab (μg/ml)
H2122
*
b
24 h
48 h
72 h
KDa
Cyclin D
Cyclin E
β-actin
35
55
35
Cyclin D1 normalized to β-actin
Cyclin E normalized to β-actin
c
ch5E6 (20 μg/ml)
huIgG1 (20 μg/ml)
Normalized Annexin V activity
SW1990
T3M4
MiPaca
ns
Time (h)
d
SW1573
H2122
H23
e
huIgG1
ch5E6
Cleaved caspase 3
Cleaved PARP
β-actin
25
70
35
SW1990
f
35
100
35
SW1573

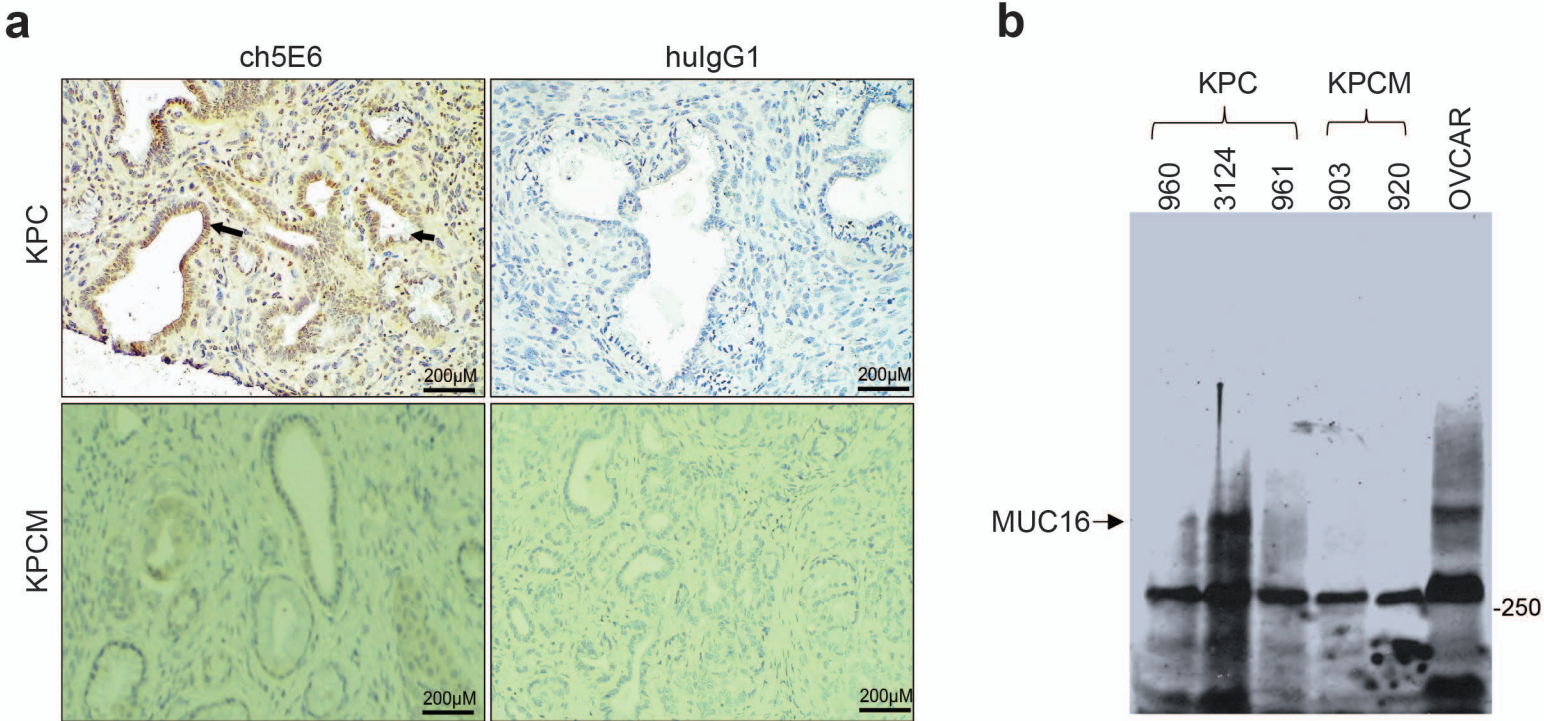
Supplementary Figure 2: A. Real-time MT glo assay showing a specific decrease in cell proliferation by ch5E6 treatment in COLO357 and H2122 cell lines. **B.** Immunoblot analysis of ch5E6 treated lysates for changes in the proliferation markers cyclins D and E at different time points from 24 hours to 72 hours. **C and D.** ch5E6 induced apoptosis in MUC16 expressing PC (SW1990, T3M4) and NSCLC cancer cell lines (SW1573 and H2122) as compared to isotype control mAb huIgG1. MUC16 negative lines MiaPaca-2-2 and H23 were not impacted by treatment. The representative images are shown in the lower panel for each cell line. **E and F.** The apoptosis was validated by increased levels of cleaved caspase 3 in chimeric mAb5E6 treated SW1990 and SW1573 cells. β-actin was used as loading control. Error bars indicate SEM. *, $P < 0.05$; **, $P < 0.005$


a
MiaPaca
MiaPaca (C-ter transfected)
No treatment
huIgG1
ch5E6
No treatment
huIgG1
ch5E6
Wounded Healed Area (mm²)
1000
500
0
0 h
12 h
No Ab
ch5E6
huIgG1
mAbs (10µg/ml)
Wounded Healed Area(mm²)
800
600
400
200
0
0 h
12 h
No Ab
ch5E6
huIgG1
mAbs (10µg/ml)
b
SW1990
*
% change in OD (570nm)
100
80
60
40
20
0
huIgG1 (5µg/ml)
ch5E6 (5µg/ml)
c
SW1573
***
% change in OD (570nm)
100
50
0
huIgG1 (5µg/ml)
ch5E6 (5µg/ml)

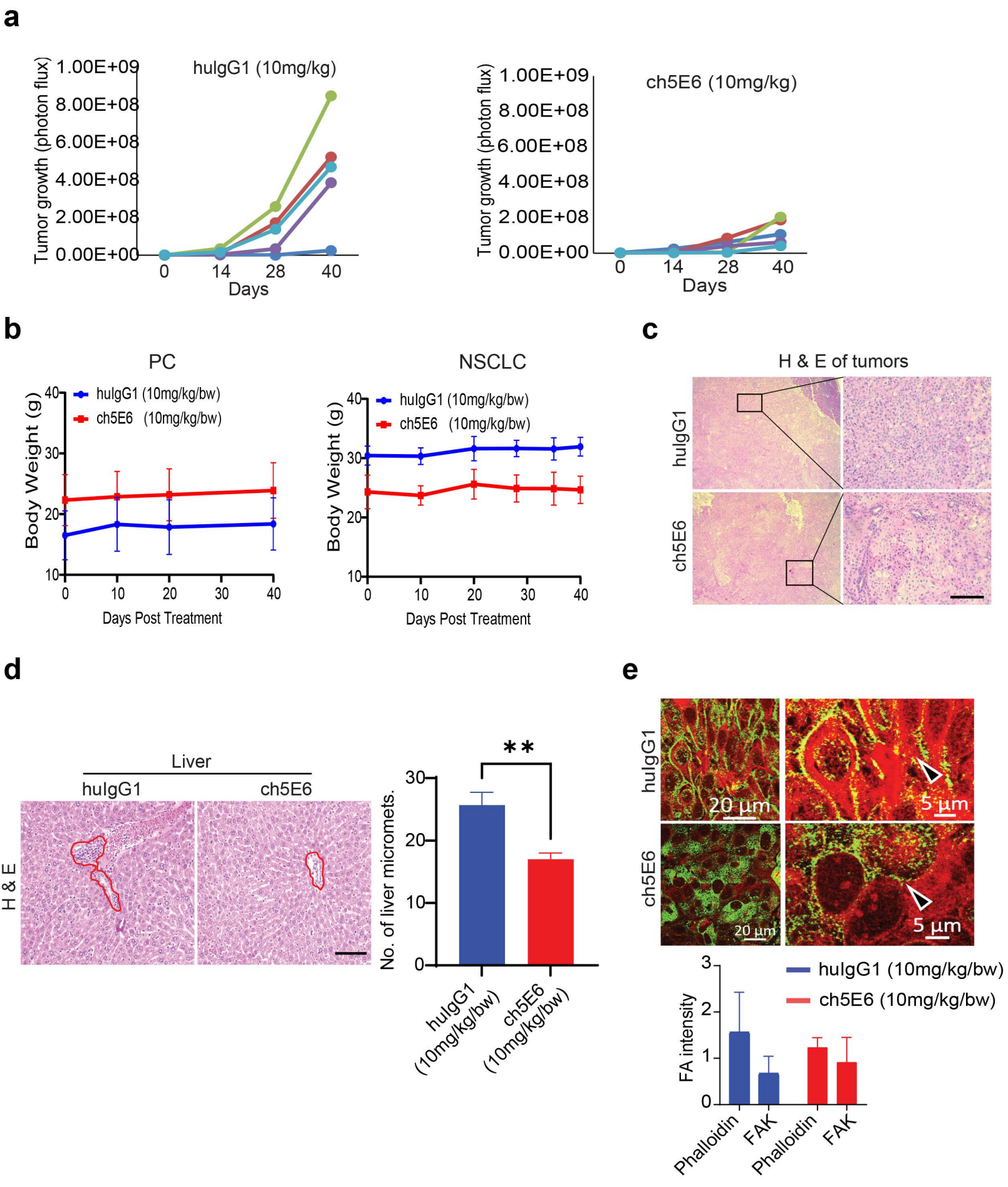
Supplementary Figure 3: A. ch5E6 decreased the migration of MUC16 C-ter transfected MiaPaca-2-2 cells as seen by differences in wound area healed in 12 hours than MiaPaca-2-2 vector control cells. **B.** Impact of ch5E6 on the colony formation ability of SW1990 and SW1573 cells by crystal violet staining. Error bars indicate SEM. *, $P < 0.05$; **, $P < 0.005$


a
N-cadherin
E-Cadherin
Fold change (Fl. Intensity)
0
1
2
3
4
5
huIgG1
ch5E6
b
Pixel Density
0
1000
2000
3000
4000
5000
ch5E6
huIgG1
p38
GSK3b
p53
HSP27
P70S6K
JNK
RSK1
MEK
RSK2
MKK3
Akt
MKK6
CREB
MSK2
ERK2
ERK1
mTOR
GSK3a
c
SW1990 (MUC16 knockdown)
–
+
Dox
pFAKY397
FAK
p70S6KT389
p70S6K
pJNKT183/Y185
N-cadherin
β-actin
-130
-130
-100
-100
-55
-130
-35

Supplementary Figure 4: A. Decrease in the fluorescence intensity of N-cadherin by ch5E6 compared to isotype control mAb huIgG1. **B.** The bar graph showing the changes in the expression of various kinases upon treatment with ch5E6 **C.** Immunoblotting analysis of Dox inducible MUC16 knockdown (KD) SW1990 cells showing a decrease in phosphorylated levels of pFAK (Y397), p70S6K, pJNK, and N-cadherin compared to parental cells. Error bars indicate SEM. *, $P < 0.05$; **, $P < 0.005$


a
ch5E6
huIgG1
KPC
KPCM
200μM
200μM
200μM
200μM
b
KPC
KPCM
960
3124
961
903
920
OVCAR
MUC16
-250

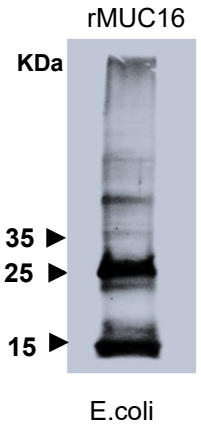
Supplementary Figure 5: A. Immunohistochemical staining of ch5E6 in tumor sections from KPC and KPCM (KPCMUC16 knockout) mice indicating a specific reactivity of ch5E6 to mouse MUC16. **B.** Western blot analysis of pancreatic cell lines derived from KPC (960, 3124, and 961) and KPCM (903,920) mice showing mouse MUC16 recognition by ch5E6.


a
huIgG1 (10mg/kg)
ch5E6 (10mg/kg)
Tumor growth (photon flux)
1.00E+09
8.00E+08
6.00E+08
4.00E+08
2.00E+08
0.00E+00
0
14
28
40
Days
b
PC
NSCLC
huIgG1 (10mg/kg/bw)
ch5E6 (10mg/kg/bw)
Body Weight (g)
Days Post Treatment
c
H & E of tumors
huIgG1
ch5E6
d
Liver
huIgG1
ch5E6
H & E
No. of liver micromets.
**
huIgG1 (10mg/kg/bw)
ch5E6 (10mg/kg/bw)
e
20 μm
5 μm
FA intensity
Phalloidin
FAK

Supplementary Figure 6: A. The line graphs show the change in flux units for each mouse in the treatment group. **C**. The line graph of body weight measured over various time points showing no changes in the treatment group suggesting a safe profile of the therapy. **D.** H&E images showing low proliferative index in ch5E6 treated tumor than isotype control mAb huIgG1 treated one. **E.** H&E images of liver sections showing a significant decrease in the liver mets in ch5E6 treatment group. **F.** Immunofluorescence analysis of FAK100 stained tumors showing a decrease in focal adhesion intensity in ch5E6 treated tumors as measured by phalloidin and vinculin intensity. Error bars indicate SEM. *, $P < 0.05$; **, $P < 0.005$

Supplementary Figure 7 : Full-sized scan of immunoblots in Figure 1 (Fig 1D and Fig 1F)

Figure 1D



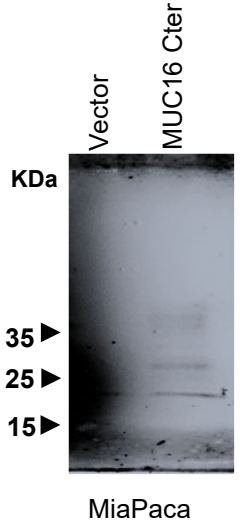
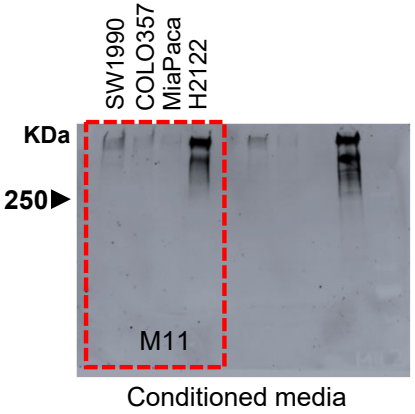
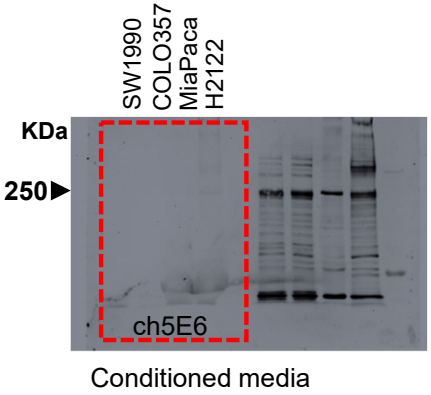
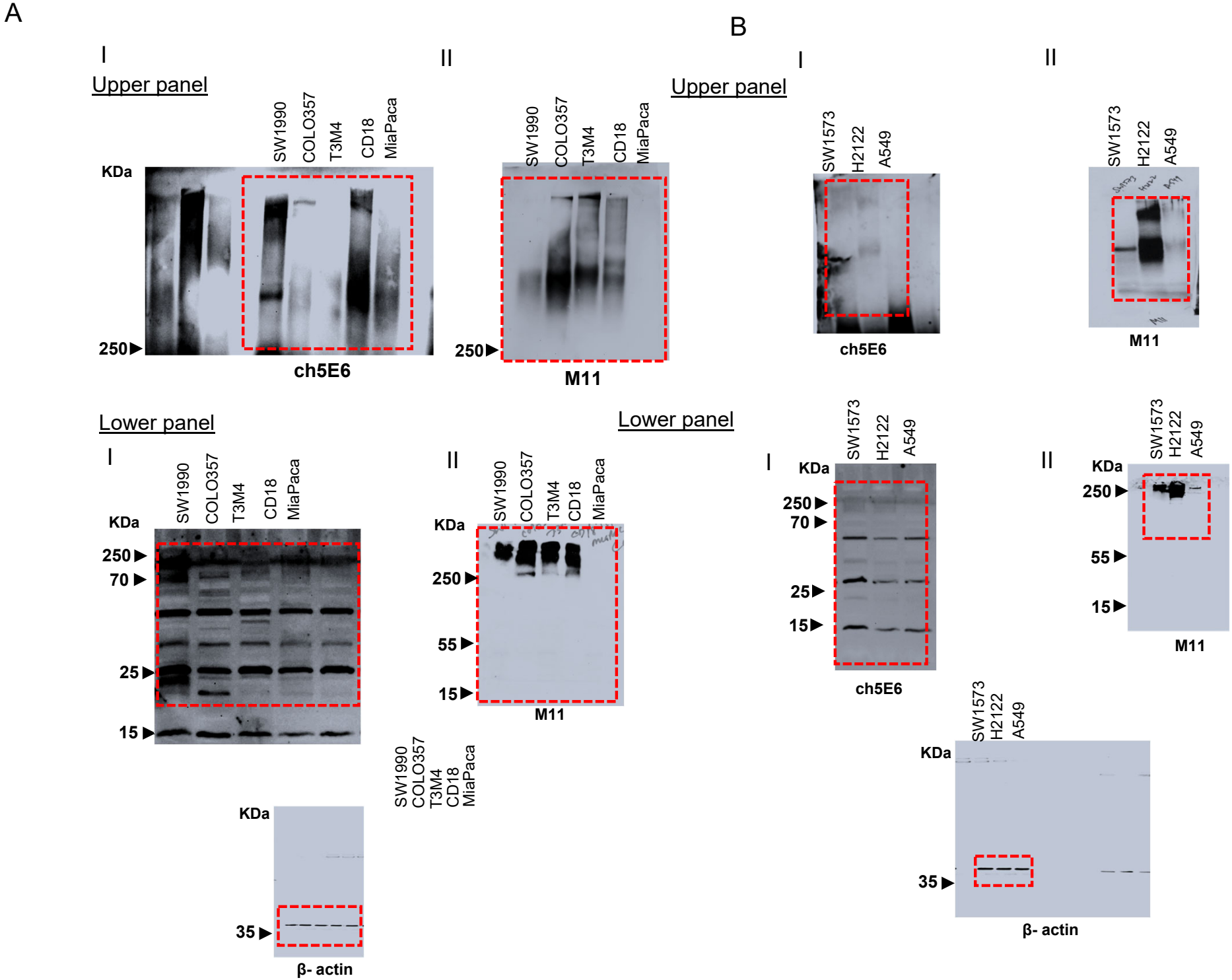


Figure 1F





Supplementary Figure 8: Full-sized scan of immunoblots in Figure 2 (Fig 2A and 2B)



Supplementary Figure 9: Full-sized scan of immunoblots in Figure 3A

Figure 3A (SW1990 Panel)

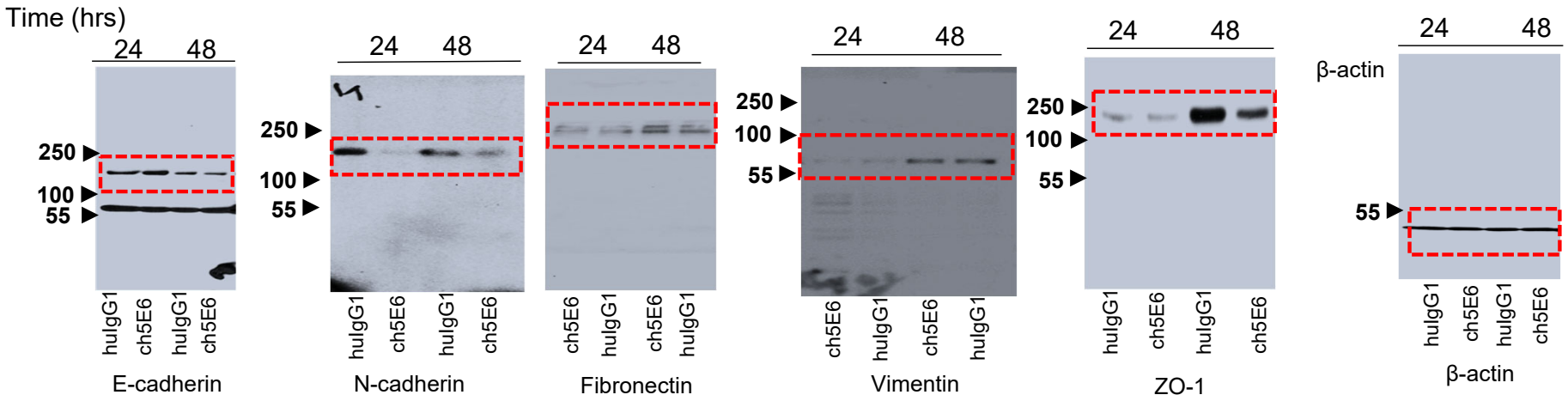
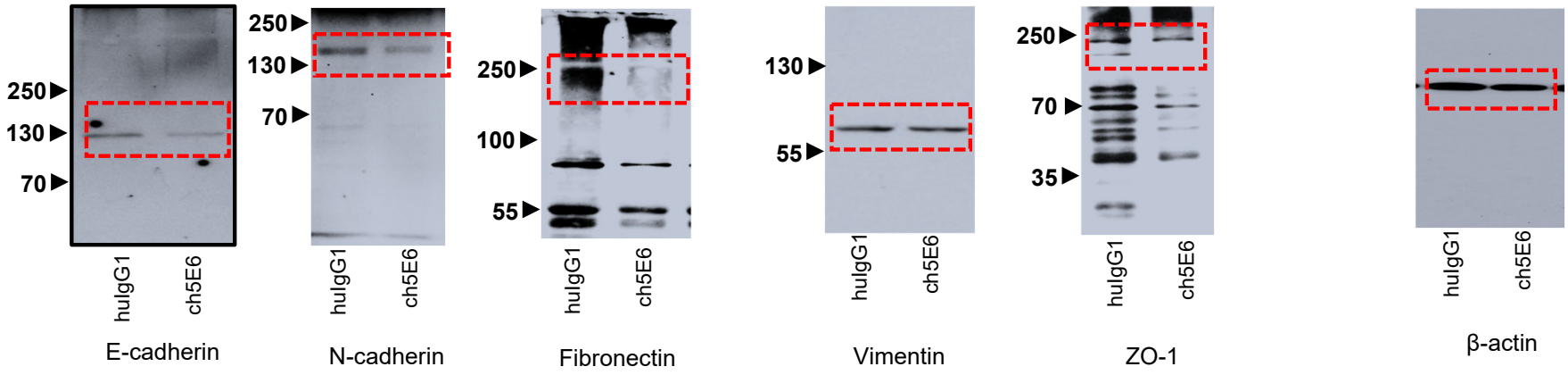
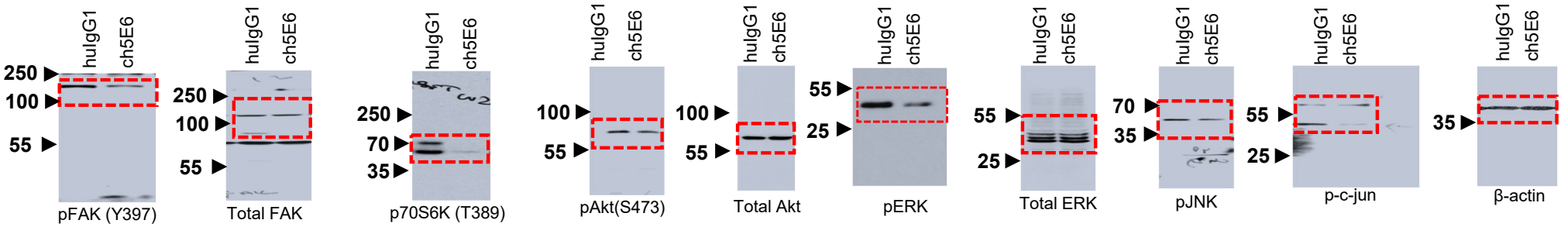


Figure 3A (SW1573 Panel)

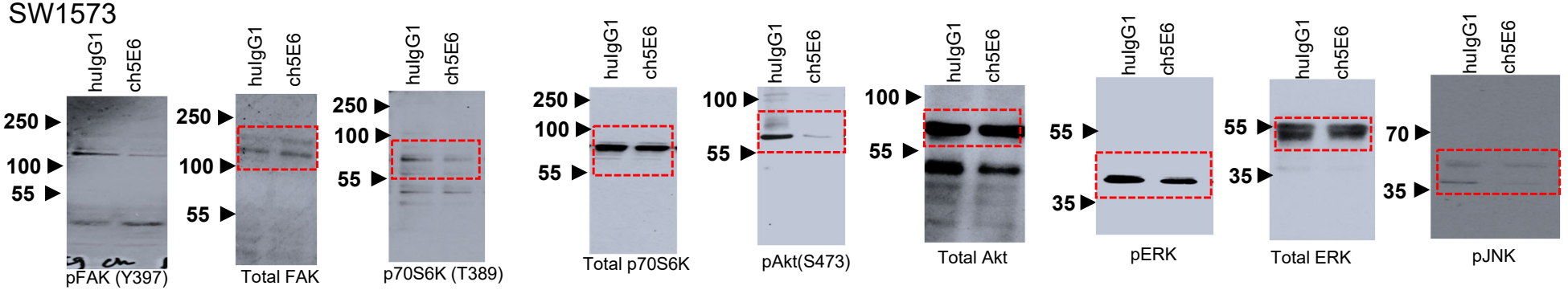


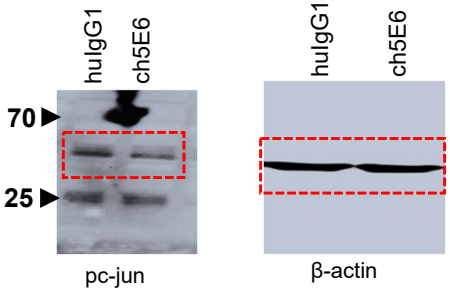
Supplementary Figure 10: Full-sized scan of immunoblots in Figure 3D

SW1990



SW1573





Supplementary Figure 11: Full-sized scan of immunoblots in Figure 3E, 3F and 3G

Figure 3E

FAKi(Y15) treatment

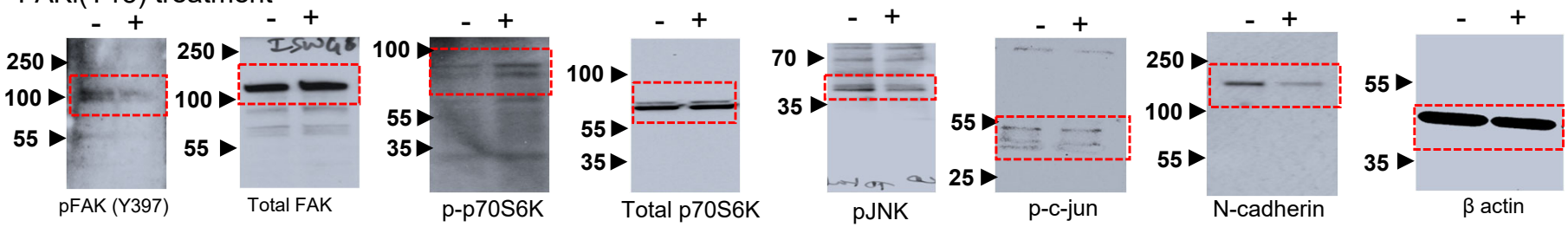


Figure 3F

ERKi (PD98059) treatment

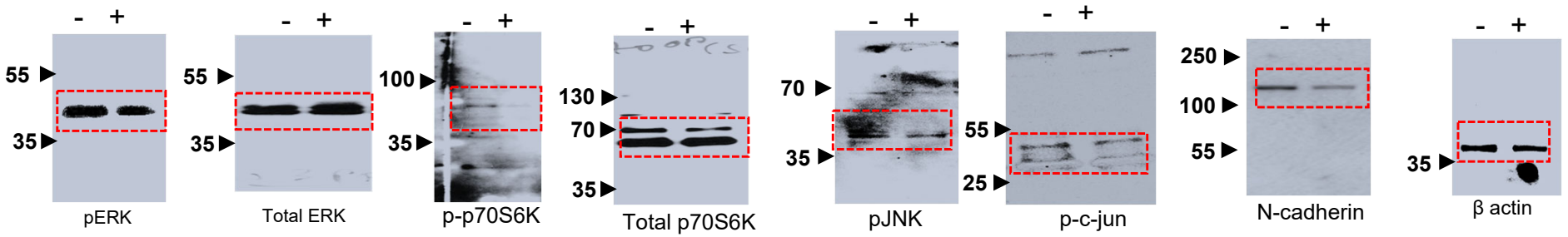
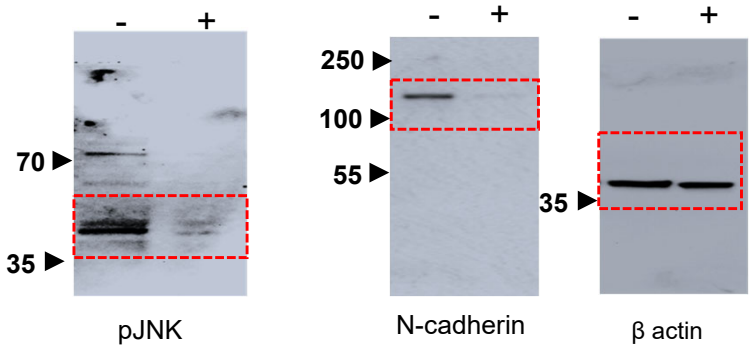


Figure 3G

JNKi (SP60015) treatment



Supplementary Figure 12: Full-sized scan of immunoblots in Figure 6C and D

Figure 6C

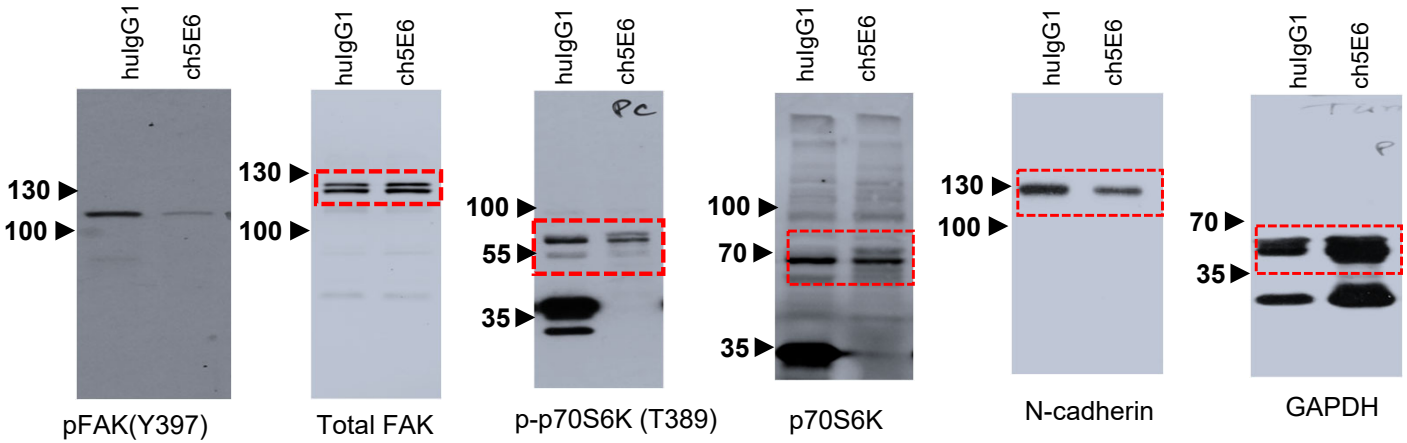
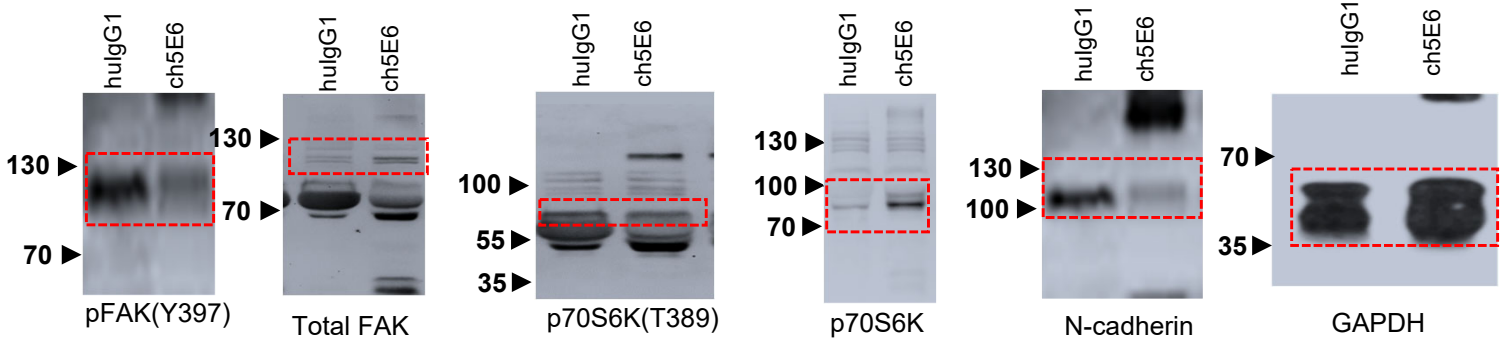
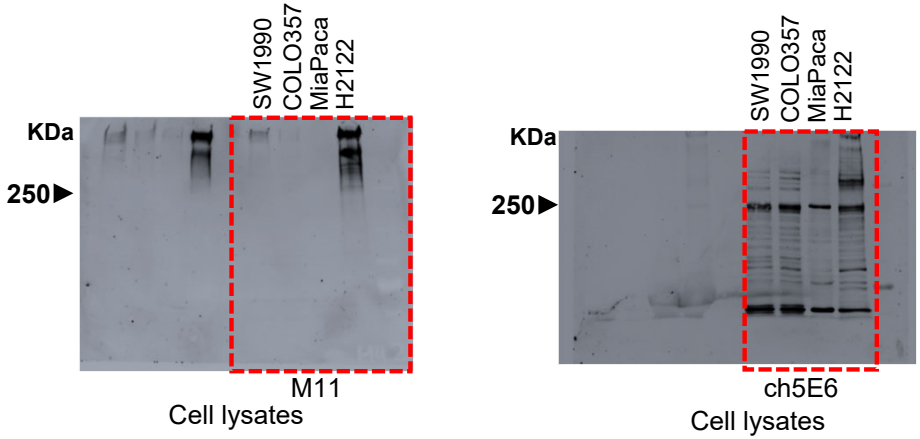


Figure 6D

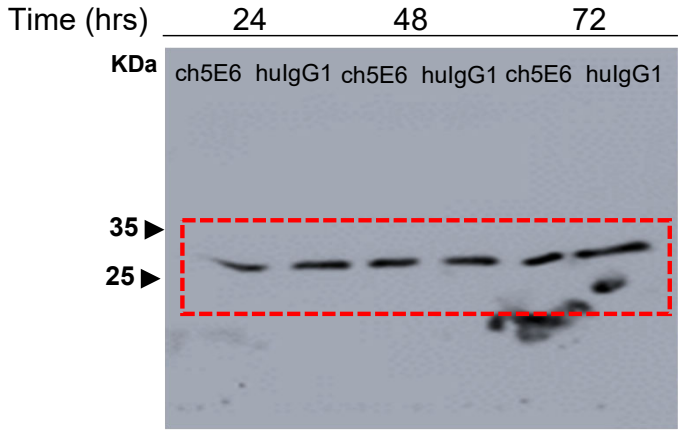


Supplementary Figure 13: Full-sized scan of immunoblots in supplementary figure 1D

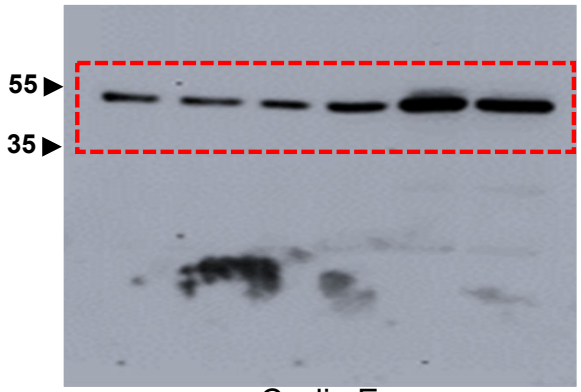


Supplementary Figure 14: Full-sized scan of immunoblots in supplementary figure 2 (S2B, S2E and S2F)

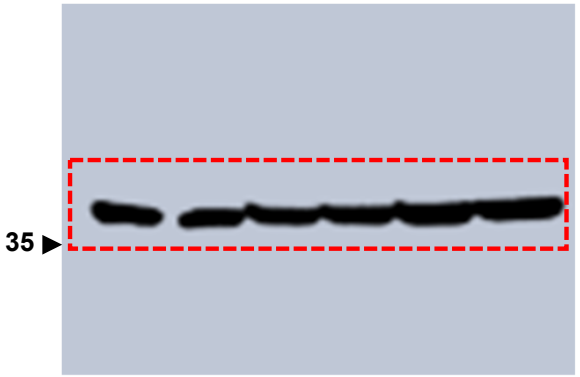
Supplementary Fig 2B



Cyclin D

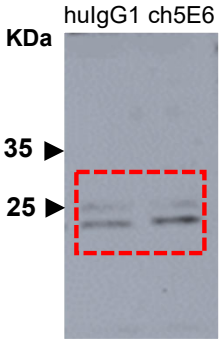


Cyclin E

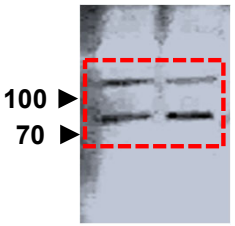


β-actin

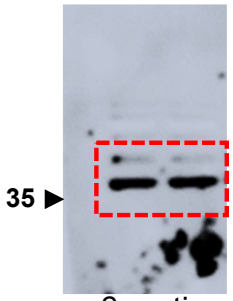
Supplementary Fig 2E



Cleaved Caspase 3

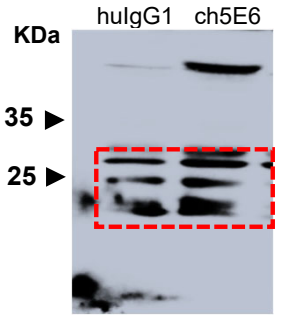


Cleaved PARP

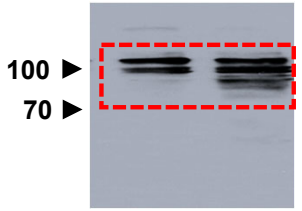


β -actin

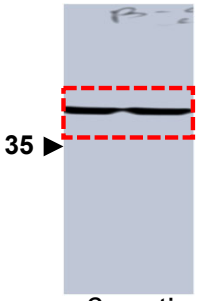
Supplementary Fig 2F

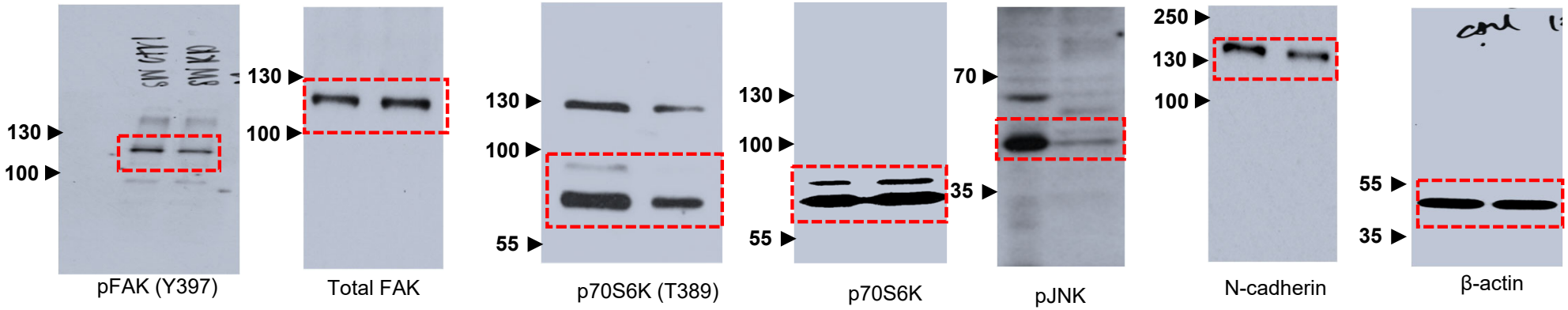


Cleaved Caspase 3



Cleaved PARP



β -actin

Supplementary Figure 15: Full-sized scan of immunoblots in supplementary Figure 4C