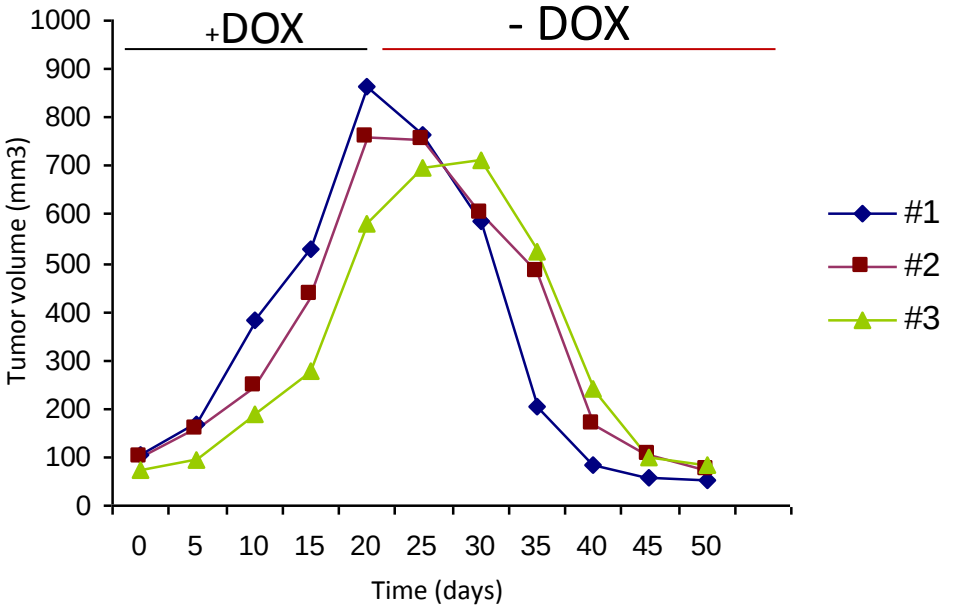
Supplementary figures

A



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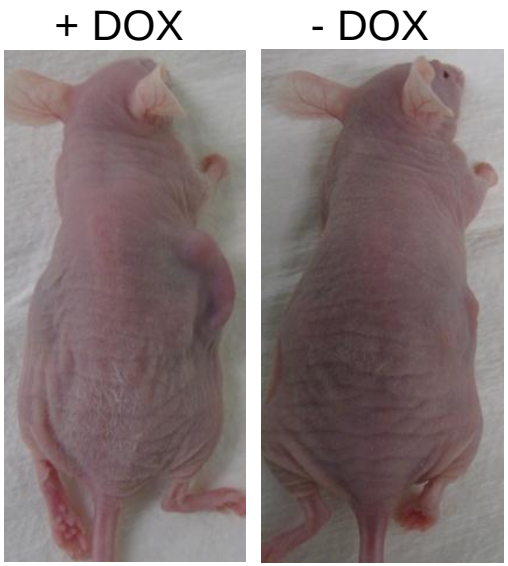


A: Tumor growth and regression of 3 independent iKRAS-derived allografts with or without doxycycline in the drinking water.

B: Representative iKRAS-derived allografts before and after doxycycline witdrawal for 15 days

Figure S1

Figure S2

A: Adhesion-independent colony forming assay of 3 independent iKRAS lines cultured in the presence or absence of 2 μg/ml doxycyline

B: Crystalviolet staining of 3 independent iKRAS lines cultured in adherent tissue culture conditions in the presence or absence of 2 μg/ml doxycyline for 7 days

C: RAF-pulldown assay to determine the amount of GTP-bound (active) RAS in cells cultured in the presence or absence of of 2 μg/ml doxycyline for 48 hours. An aliquot of the lysates utilized in the pulldown assay was independently subjected to western blot with anti β-actin as loading control of the pulldown.

D: Annexin V/7AAD flow cytometry analysis of 3 independent iKRAS lines cultured in adherent tissue culture conditions in the presence or absence of 2 μg/ml doxycyline for 7 days

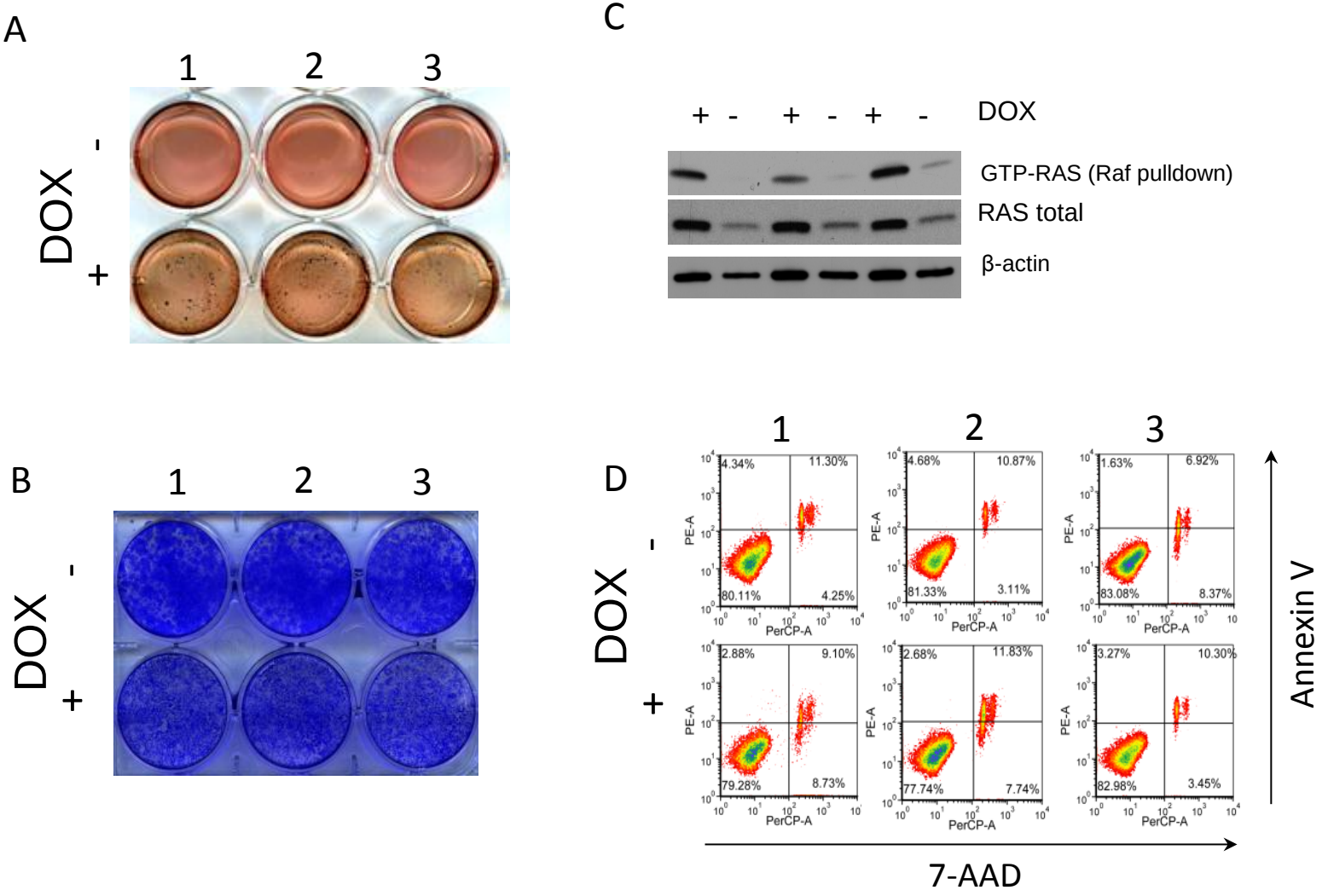
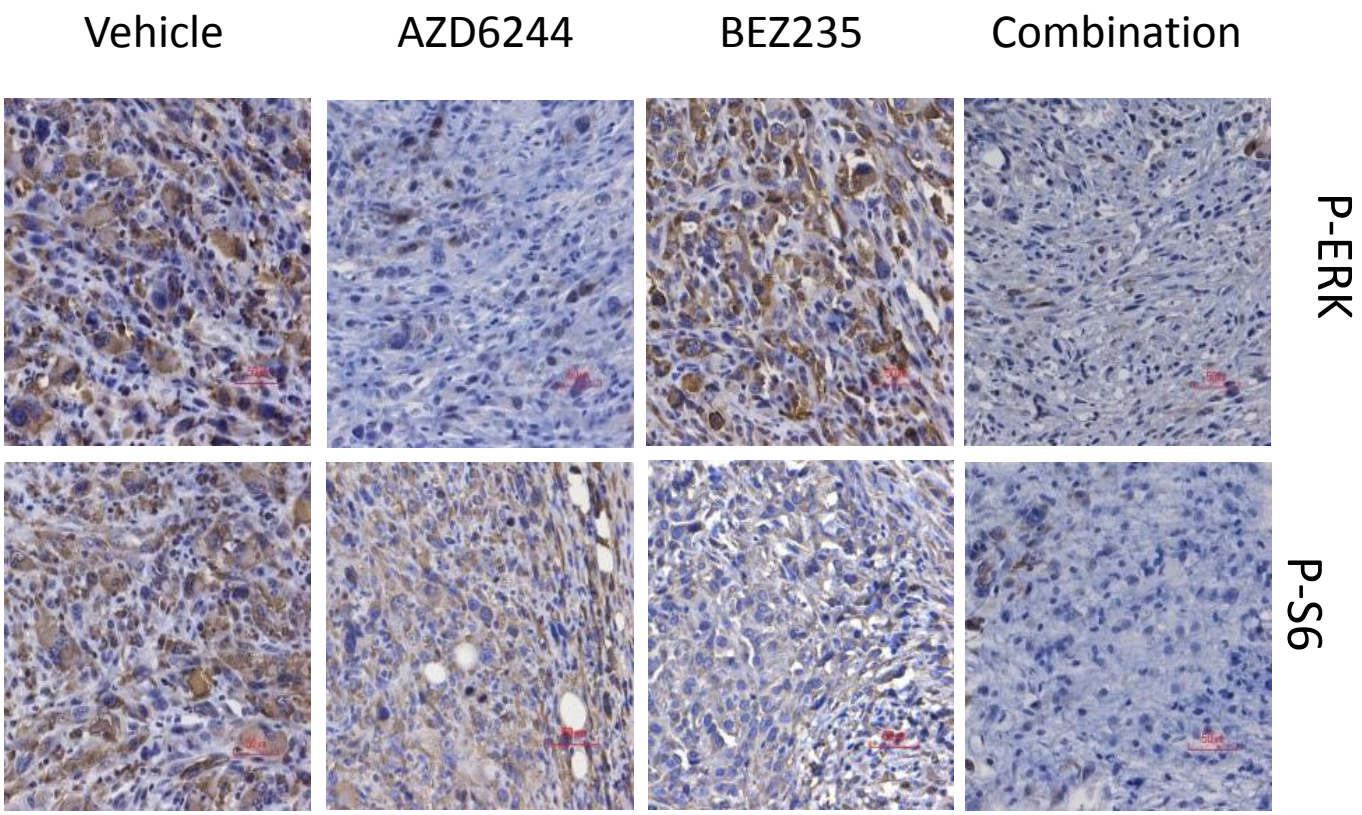
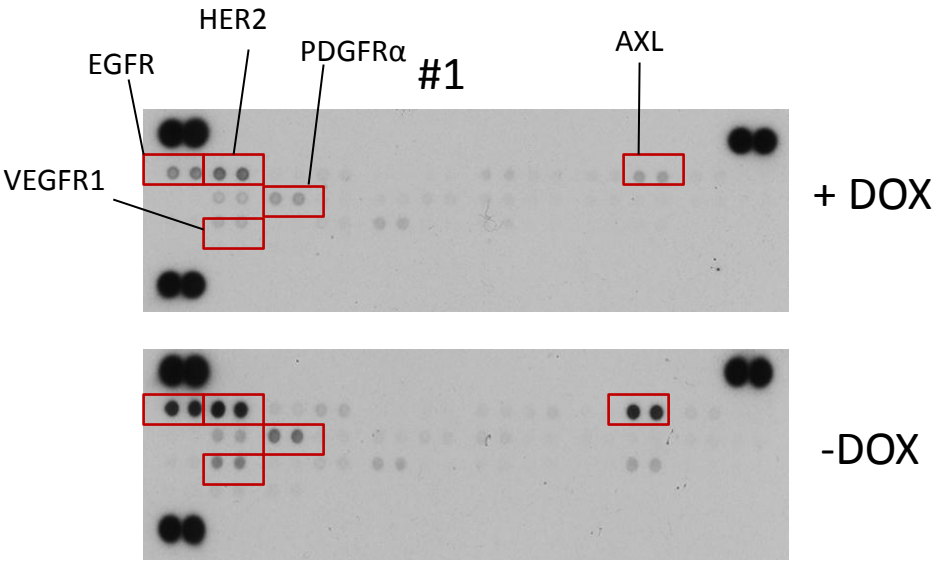

A
1
2
3
DOX
-
+
B
1
2
3
DOX
-
+
C
+ - + - + -
DOX
GTP-RAS (Raf pulldown)
RAS total
β-actin
D
1
2
3
DOX
-
+
4.34%
11.30%
80.11%
4.25%
4.68%
10.87%
81.33%
3.11%
1.63%
6.92%
83.08%
8.37%
2.88%
9.10%
79.28%
8.73%
2.68%
11.83%
77.74%
7.74%
3.27%
10.30%
82.98%
3.45%
PE-A
PerCP-A
Annexin V
7-AAD

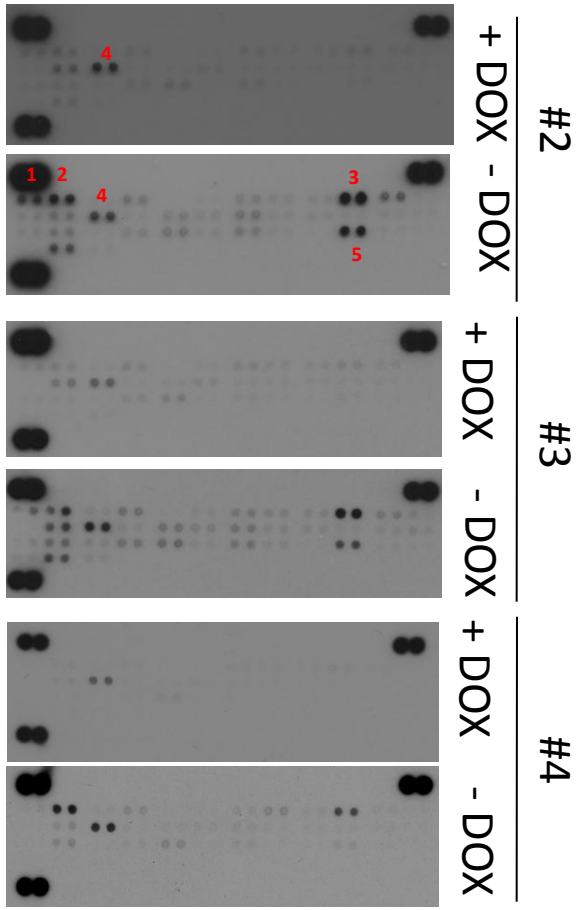
Figure S2



Representative IHC of P-ERK and P-S6 of the tumor study reported in figure 2D

Figure S3





Phospho RTK array of 4 independent iKRAS lines cultured in the presence or absence of 2 µg/ml doxycycline

1: P-EGFR 2: P-HER2 3: P-AXL 4: P-PDGFRA 5: P-EPHA7

Figure S4

Figure S5

A: Anti AXL antibody validation on murine 4T1 cells. lysates were obtained from cells transduced with shRNA for GFP as control or with different shRNA for murine AXL.

B: Anti AXL antibody validation on human PATU8988T cells. lysates were obtained from cells transduced with shRNA for GFP as control or with different shRNA for human AXL.

C: Anti AXL antibody validation for immunohistochemical detection of AXL performed on PATU8988T cells stransduced with shRNA for GFP as control or ShRNA for human AXL.

C: Immunoprecipitation from lysates derived from PATC53 xenograft lysates. Right IP anti P-tyr wb anti P-AXL left IP anti AXL wb anti P-Tyr or anti AXL

D: Detection of phospho-AXL in tumor lysates derived from xenograft of the human pancreatic cancer primary lines PATC53 or PATC66

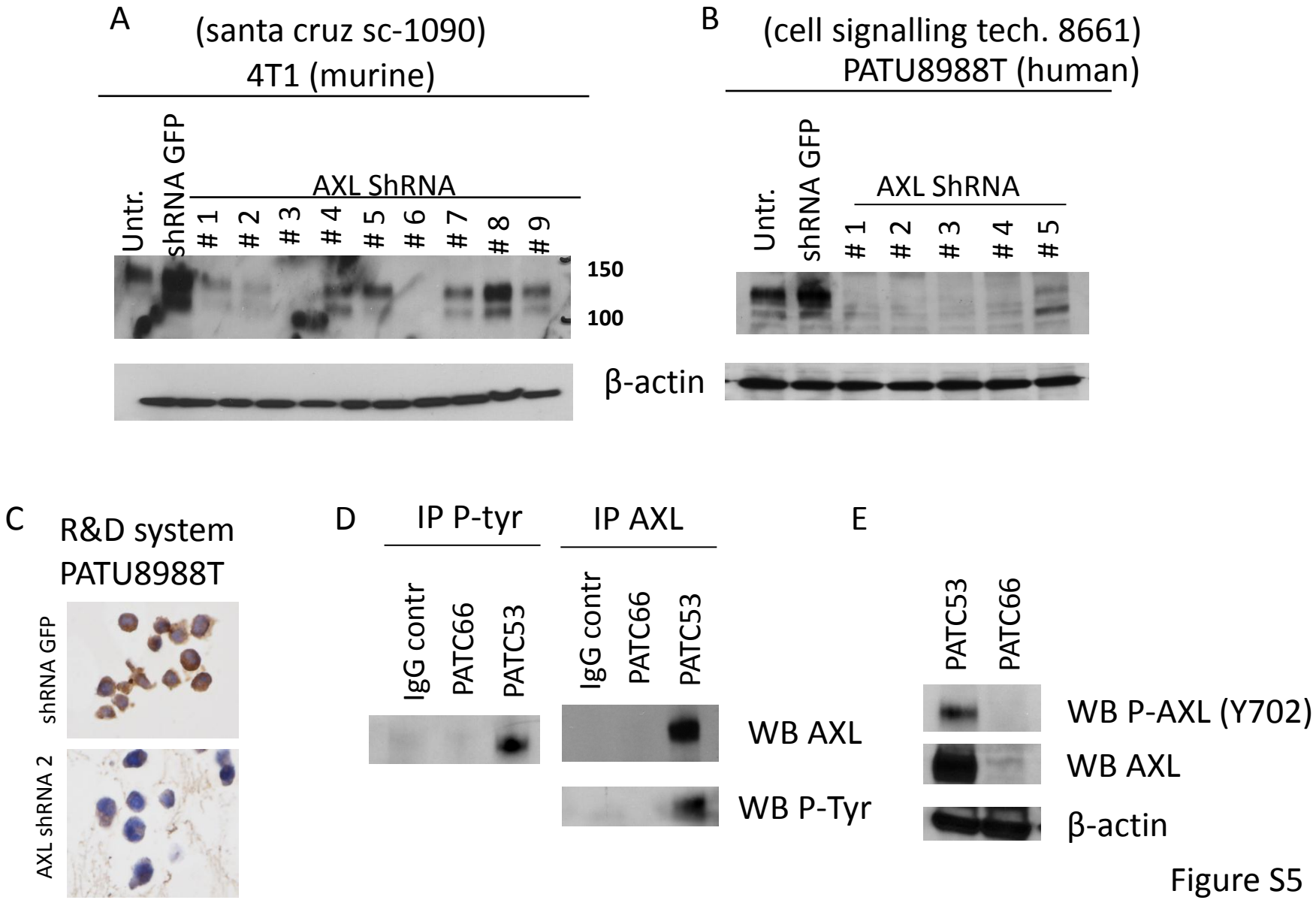

A
(santa cruz sc-1090)
4T1 (murine)
Untr.
shRNA GFP
AXL ShRNA
1
2
3
4
5
6
7
8
9
150
100
β-actin
B
(cell signalling tech. 8661)
PATU8988T (human)
Untr.
shRNA GFP
AXL ShRNA
1
2
3
4
5
C
R&D system
PATU8988T
shRNA GFP
AXL shRNA 2
D
IP P-tyr
IP AXL
IgG contr
PATC66
PATC53
IgG contr
PATC66
PATC53
WB AXL
WB P-Tyr
E
PATC53
PATC66
WB P-AXL (Y702)
WB AXL
β-actin

Figure S5

Figure S6

A: Dose effect of CH5491098 (AXLi) on GAS6-induced AXL phosphorylation on human PATU8988T cells

B: Dose effect of CH5491098 (AXLi) on GAS6-induced AXL phosphorylation on murine 4T1 cells

C: Dose effect of CH5491098 (AXLi) on PATU8988T (AXL expressing) and DAN-G (AXL non expressing) cells on *in vitro* proliferation

D: Left: real time PCR analysis of AXL expression in PATU8988T and DAN-G cells. Right: western blot analysis of AXL expression in PATU8988T and DAN-G cells

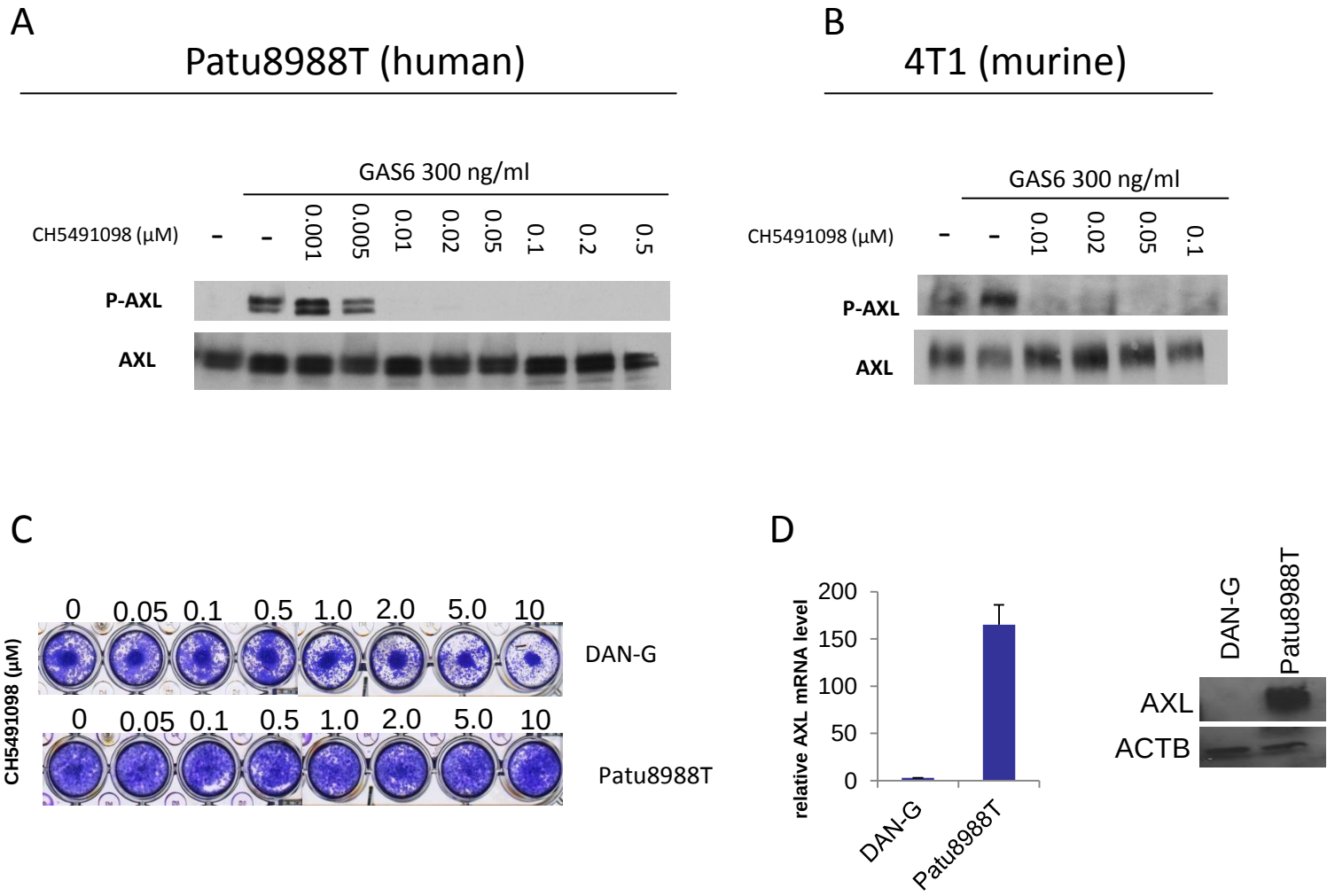
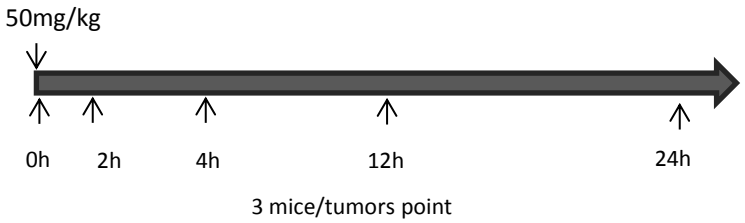

A
Patu8988T (human)
GAS6 300 ng/ml
CH5491098 (μM)
-
-
0.001
0.005
0.01
0.02
0.05
0.1
0.2
0.5
P-AXL
AXL
B
4T1 (murine)
GAS6 300 ng/ml
CH5491098 (μM)
-
-
0.01
0.02
0.05
0.1
P-AXL
AXL
C
CH5491098 (μM)
0
0.05
0.1
0.5
1.0
2.0
5.0
10
DAN-G
Patu8988T
D
relative AXL mRNA level
200
150
100
50
0
DAN-G
Patu8988T
DAN-G
Patu8988T
AXL
ACTB

Figure S6

Figure S7

A: Study design for evaluation of target engagement
B: Western blot analysis of P-AXL levels in lysates derived from PATC53 xenograft and treated as indicated in A
C: Mean body weight of non tumor bearing nude mice (5 mice/group) treated with CH5491098 daily.
D: Plasma concentrantion of CH5491098. female NCR/nu mice received one dose of 50 mg/kg CH5491098 through either gavage (PO) or intraperitoneal injection (IP), blood was collected at inidicated time points, plasma was derived and subjected to HPLC analysis

A



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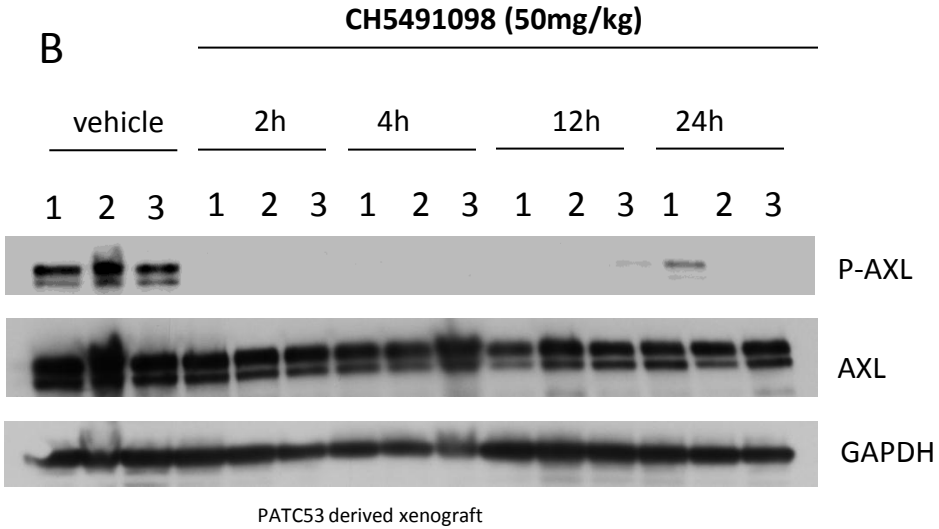
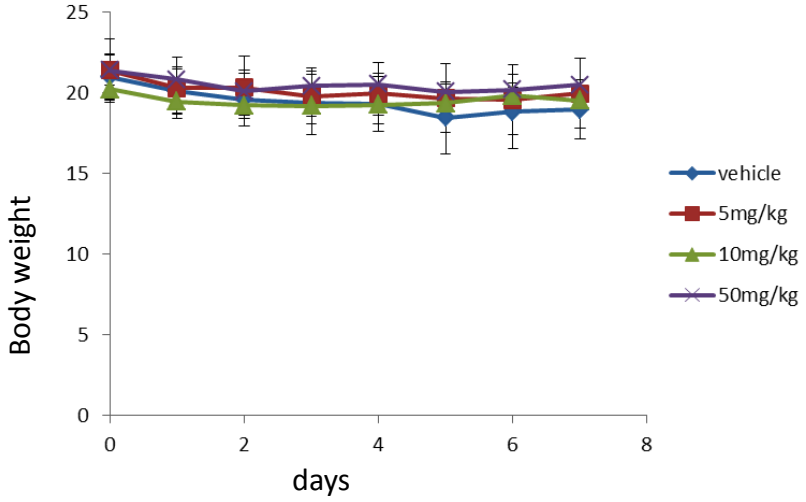


C



D

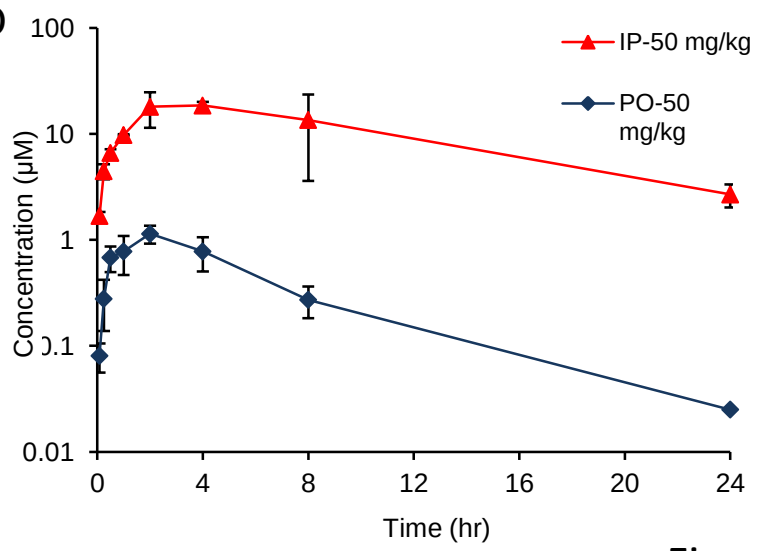


Figure S7

Figure S8

A: Colony forming assay of murine tumor cells derived from the P48-CRE LSL KRAS P53 model tranduced with scramble as control or AXL shRNA.

B: Western blot analysis of AXL in cells utilized in A, in the fourth lane cells transduced with sh RNA for AXL mapping on the 3'UTR were further transduced with AXL encoding lentivirus

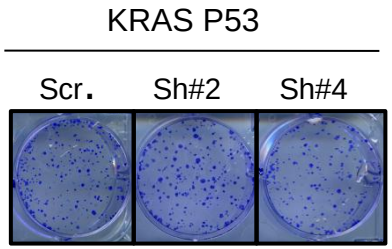
C: Tumor sizes of allograft derived from the P48-CRE LSL KRAS $P53^{fl/+}$ model transduced with the shRNA indicated in B

D: Tumor sizes of allograft derived from the P48-CRE LSL KRAS $P53^{fl/+}$ model treated with vehicle or AXLi 30 mg/kg daily

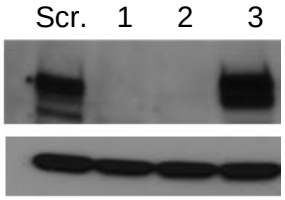
E: Mean ± SD of tumor volumes in C

F: Mean ± SD of tumor volumes in D

A

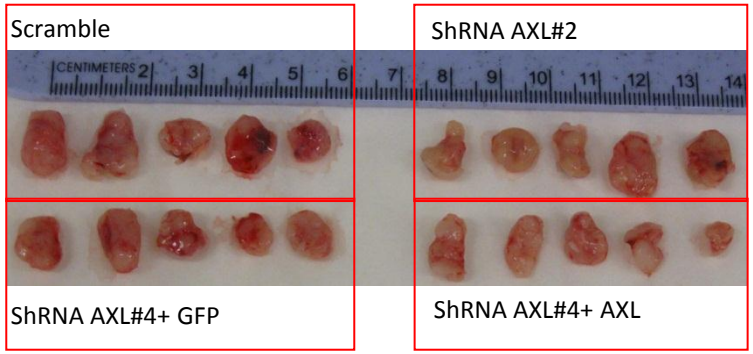


B

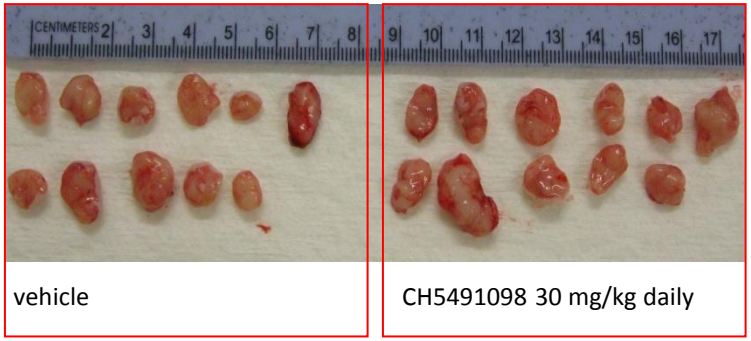


Scr. Scramble
1: ShRNA AXL#2
2 ShRNA AXL#4 3'UTR+GFP
3 : ShRNA AXL4 3'UTR+ AXL

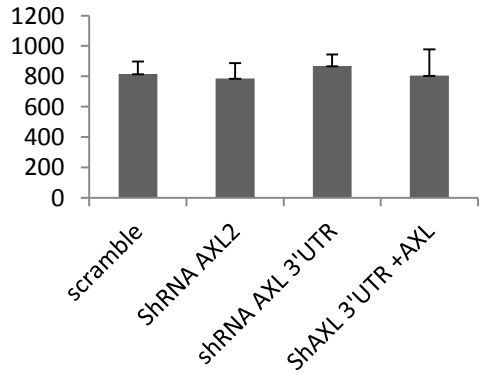
C



D



E



F

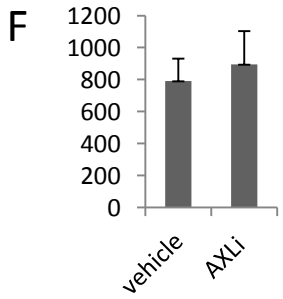
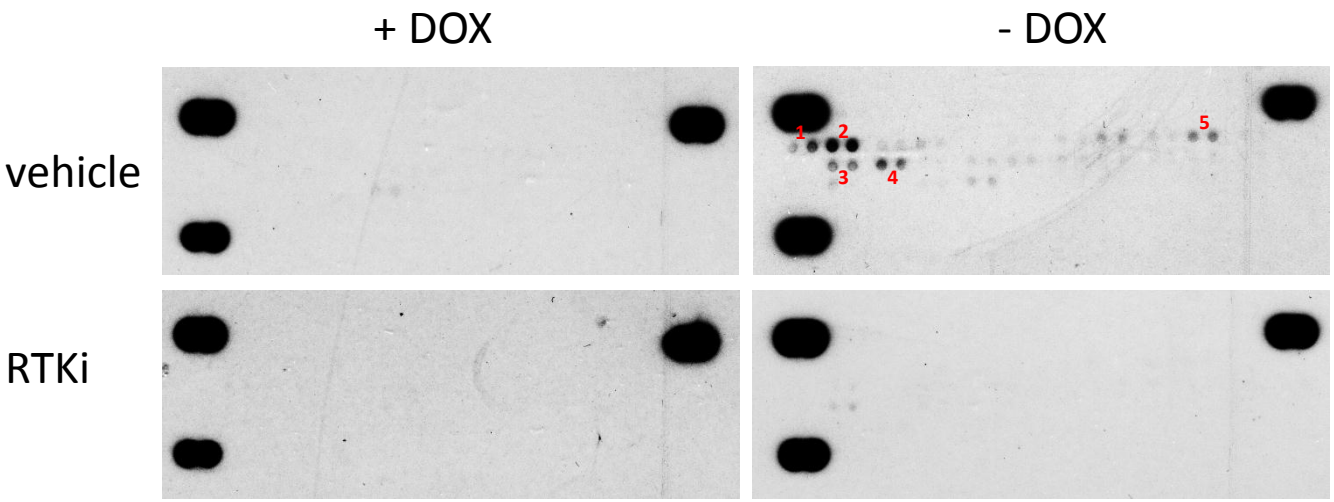


Figure S8



1: EGFR, 2: HER2, 3: MSP-R 4: PDGFRA, 5: AXL

Phospho RTK array of iKRAS lines cultured in the presence or absence of 2 µg/ml doxycycline and treated or not with the combination of RTK inhibitors (RTKi) AXLi (1 µM), lapatinib (1 µM), imatinib (1 µM).

Figure S9

Figure S10

Western blot of Phospho ERK and total ERK of MIA PaCa2 and PANC1 cells transduced with doxycycline inducible shRNA for KRAS and cultured in the presence or absence of doxycycline 2 μg/ml for 72 hours. β-actin serves as loading control