Figure S1

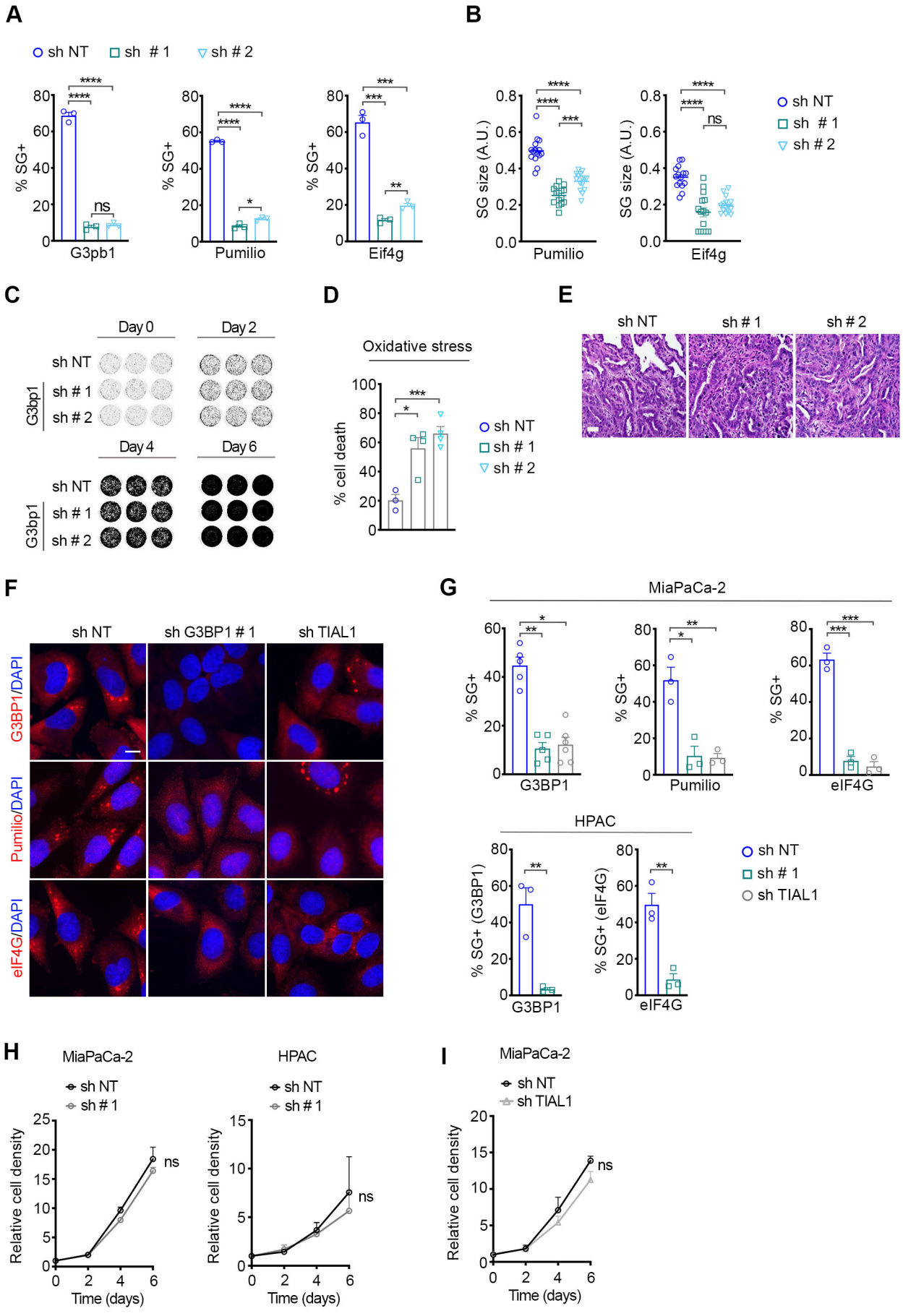

A
sh NT
sh # 1
sh # 2
% SG+
G3pb1
Pumilio
Eif4g
B
SG size (A.U.)
Pumilio
Eif4g
C
Day 0
Day 2
Day 4
Day 6
G3bp1
D
Oxidative stress
% cell death
E
F
sh NT
sh G3BP1 # 1
sh TIAL1
G3BP1/DAPI
Pumilio/DAPI
eIF4G/DAPI
G
MiaPaCa-2
HPAC
% SG+ (G3BP1)
% SG+ (eIF4G)
G3BP1
eIF4G
H
MiaPaCa-2
HPAC
Relative cell density
Time (days)
ns
I
MiaPaCa-2
sh TIAL1

Figure S1. Related to Figure 1.

(A) Quantification of the percent SG positive (+) cells as detected by G3bp1, Pumilio, and Eif4g, in KPC-4662 cells expressing the indicated shRNAs and stressed as in 1A. Data are shown as mean +/- SEM of 3 independent experiments. The individual values for each experiment are also shown. ***p<0.001, ****p<0.0001 by unpaired t-test.

(B) Quantification of the average size of SGs and number of SGs per cell in cells as in 1A. Data from a representative experiment as in 1A are shown. ***p<0.001, ****p<0.0001, ns = non-significant by unpaired t-test.

(C) Representative images of Syto60 relative cell density measurements of KPC-4662 cells induced to express the indicated constructs.

(D) Percent cells undergoing cell death in KPC-4662 cells expressing the indicated constructs and subjected to oxidative stress via SA (300 μM) for 6 hr. Cell death was determined by time-lapse microscopy. Cells undergoing blebbing were determined to be undergoing cell death. Measurements from a representative experiment are shown. Data is shown as mean +/- SEM for 4 non-overlapping FOV imaged at 20x.

(E) Representative H&E staining of sections from orthotopic tumors in Figure 1F-H. Scale bar, 50 μm.

(F) Representative immunostaining of the SG markers G3BP1, Pumilio, and eI4FG and DAPI in MiaPaCa-2 cells subjected to oxidative stress following Dox-induced expression of the indicated shRNAs. Oxidative stress was induced via treatment with 100 μM sodium arsenate (SA) for 1 hr. Scale bar, 10 μm.

(G) Quantification of SG index as detected by G3BP1, Pumilio, and eIF4G, in MiaPaCa-2 and HPAC cells expressing the indicated shRNAs and stressed as in 1A. Data are

shown in arbitrary units (a.u.) for each marker and as mean +/- SEM of 3 independent experiments. The individual values for each experiment are also shown. *p<0.05, **p<0.01, ***p<0.001 by unpaired t-test.

(H) Relative cell density of MiaPaCa-2 and HPAC cells expressing the indicated shRNAs measured using a Syto60 stain. Data is mean +/- SD, n=3. ns = non-significant by unpaired t-test

(I) Relative cell density of MiaPaCa-2 cells expressing the indicated shRNAs measured using a Syto60 stain. Data is mean +/- SD, n=3. ns = non-significant by unpaired t-test

Figure S2

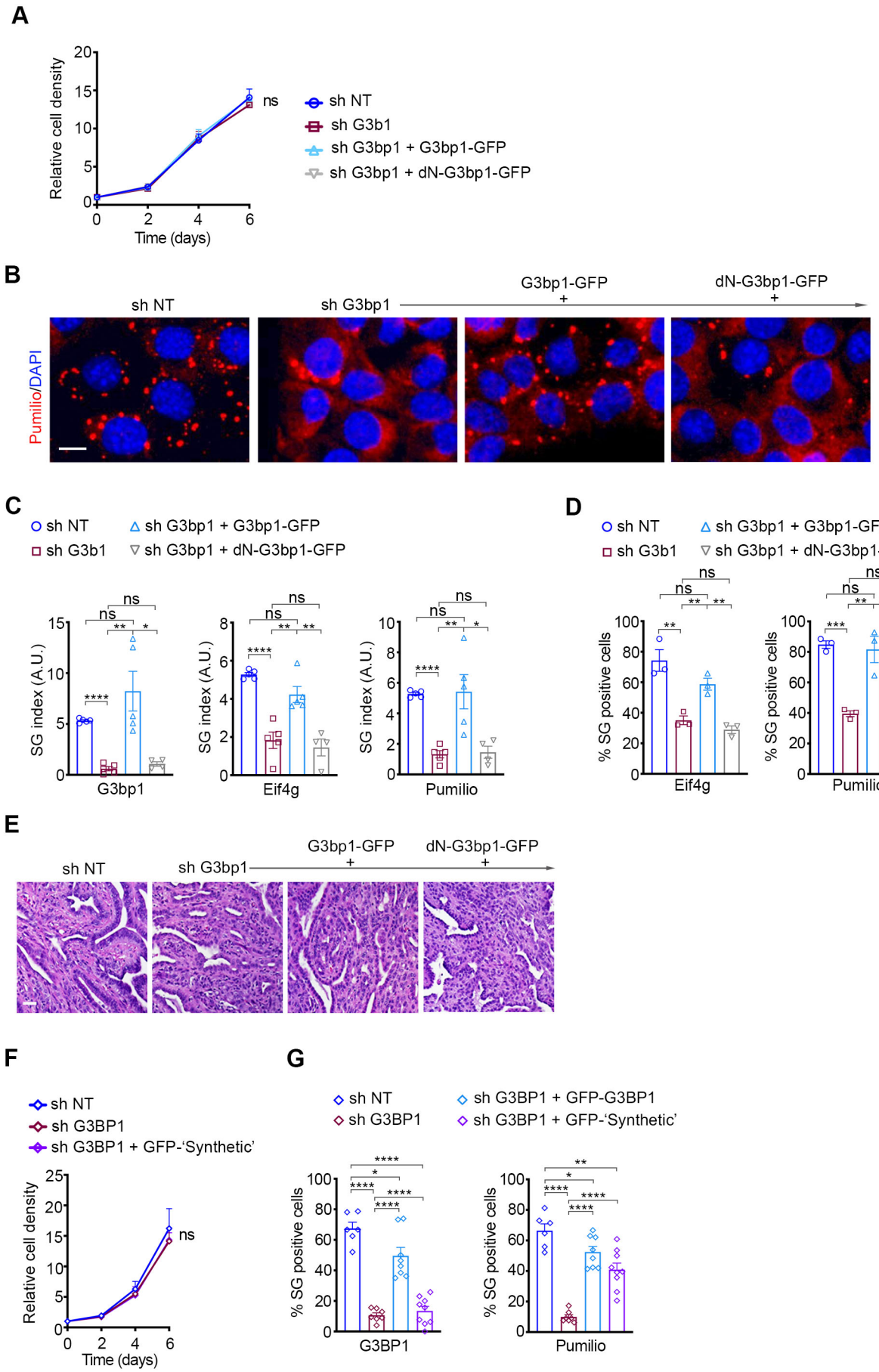

A
Relative cell density
Time (days)
ns
sh NT
sh G3b1
sh G3bp1 + G3bp1-GFP
sh G3bp1 + dN-G3bp1-GFP
B
sh NT
sh G3bp1
G3bp1-GFP
+
dN-G3bp1-GFP
+
Pumilio/DAPI
C
sh NT
sh G3b1
sh G3bp1 + G3bp1-GFP
sh G3bp1 + dN-G3bp1-GFP
SG index (A.U.)
G3bp1
Eif4g
Pumilio
D
% SG positive cells
Eif4g
Pumilio
E
sh NT
sh G3bp1
G3bp1-GFP
+
dN-G3bp1-GFP
+
F
sh NT
sh G3BP1
sh G3BP1 + GFP-'Synthetic'
Relative cell density
Time (days)
ns
G
sh NT
sh G3BP1
sh G3BP1 + GFP-G3BP1
sh G3BP1 + GFP-'Synthetic'
% SG positive cells
G3BP1
Pumilio

Figure S2. Related to Figure 2.

(A) Relative cell density of KPC-4662 cells expressing the indicated shRNAs and constructs measured using a Syto60 stain. Data is mean +/- SD, n=3. ns = non-significant by unpaired t-test

(B) Representative immunostaining of the SG marker Pumilio and DAPI in KPC-4662 expressing the indicated doxycycline-induced shRNAs and constructs. Oxidative stress was induced as in 1A. Scale bar, 10 μm.

(C) Quantification of SG index as detected by G3bp1, Eif4g, and Pumilio in KPC-4662 cells as in 2B. Data are shown in arbitrary units (a.u.) for each marker and as mean +/- SEM of 3 independent experiments. The individual values (circles) for each experiment are also shown. *p<0.05, **p<0.01, ****p<0.0001 by unpaired t-test.

(D) Quantification of the percent SG positive (+) cells as detected by Eif4g and Pumilio, in KPC-4662 cells as in 2B. Data are shown as mean +/- SEM of 3 independent experiments. The individual values (circles) for each experiment are also shown. **p<0.01, ***p<0.01by unpaired t-test.

(E) Representative H&E staining of sections from orthotopic tumors in Figure 2C-D. Scale bar, 50 μm.

(F) Relative cell density of MiaPaCa-2 cells expressing the indicated shRNAs and constructs measured using a Syto60 stain. Data is mean +/- SD, n=3. ns = non-significant by unpaired t-test

(G) Quantification of the percent SG positive (+) cells as detected by G3BP1 and Pumilio, in MiaPaCa-2 cells as in 2F. Measurements from a representative experiment are shown.

Data is shown as mean +/- SEM for 6 non-overlapping FOV imaged at 20x. The individual values for each FOV are also shown. *p<0.05, **p<0.01, ****p<0.0001 by unpaired t-test.

Figure S3

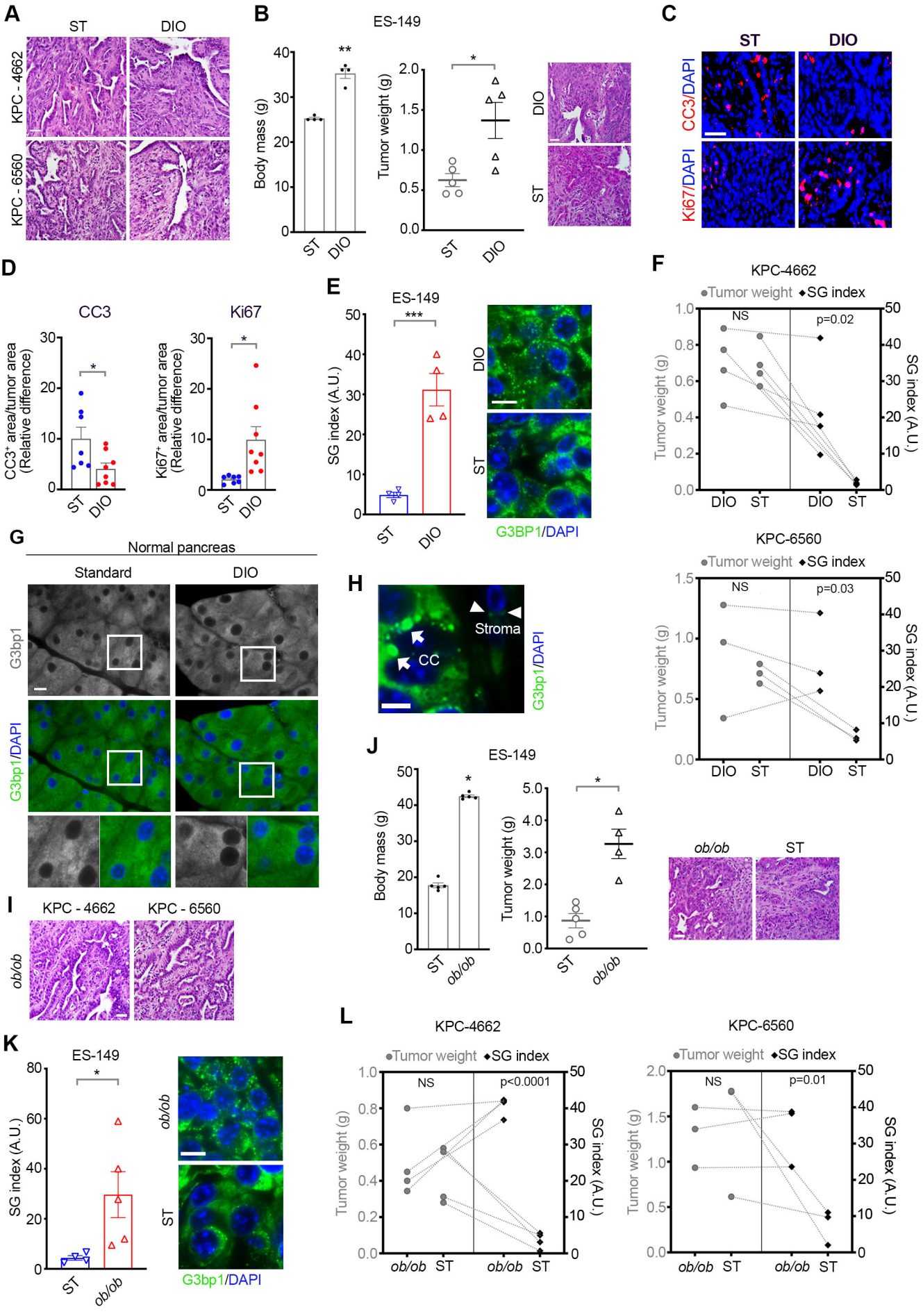

A
ST
DIO
KPC - 4662
KPC - 6560
B
ES-149
Body mass (g)
Tumor weight (g)
ST
DIO
C
CC3/DAPI
Ki67/DAPI
D
CC3
Ki67
CC3+ area/tumor area (Relative difference)
Ki67+ area/tumor area (Relative difference)
E
ES-149
SG index (A.U.)
G3BP1/DAPI
F
KPC-4662
Tumor weight
SG index
NS
p=0.02
Tumor weight (g)
SG index (A.U.)
KPC-6560
p=0.03
G
Normal pancreas
Standard
G3bp1
G3bp1/DAPI
H
Stroma
CC
J
ob/ob
I
K
p<0.0001
p=0.01
L

Figure S3. Related to Figure 3.

(A) Representative H&E staining of sections from orthotopic tumors in Figure 3A-B. Scale bar, 50 μm.

(B) *Left Panel*-Body weight of age-matched mice fed a standard diet (ST) and high-fat diet (DIO) at implantation of ES-149 cells. *Right Panel*- mPDAC tumor growth at experimental endpoint on day 21 post orthotopic implantation of ES-149 cells in mice as in 3A and representative H&E in sections from orthotopic tumors. Data are mean tumor weight at endpoint +/- SEM and individual tumor weights for each mouse. ST n=5, ES-149 DIO n=5, * $p<0.05$, **$p<0.001$ by Mann Whitney t-test.

(C) Representative immunostaining of cleaved caspase 3 (CC3), Ki67, DAPI in sections from KPC-4662 orthotopic tumors in 3B. Scale bar, 50 μm.

(D) Quantification of CC3 or Ki67 positive (+) areas in KPC-4662 tumors in 3B. Data are mean caspase 3 area, or Ki67 area, over total tumor area +/- SEM of individual tumors in mice in each group of A-C. The values for each tumor represent the average of non-overlapping FOV imaged at 20x and covering ~50% of each section. * $p<0.05$ by Mann Whitney t-test.

(E) Quantification of SG index in sections from ES-149 tumors in B as detected by G3bp1 staining and representative images. Data are mean SG index +/- SEM and individual values for each tumor. The SG index for each tumor is the average of 30 non-overlapping FOV imaged at 40x. ST n=4, DIO n=4. *** $p<0.001$ by Mann Whitney t-test. Scale bar, 50 μm.

(F) Assessment of SG index in sections from KPC-4662 and KPC-6560 ST and DIO tumors in 3B that have similar size distribution, as detected by G3bp1. Individual tumor weights and their corresponding SG levels as in 3D are shown.

(G) Representative immunostaining of G3bp1 and DAPI in sections from normal pancreata of ST and DIO mice. Scale bar, 50 μm. Lower panels are 2x-zoom in of boxed regions.

(H) Representative immunostaining of G3bp1 and DAPI in sections from KPC-4662 orthotopic tumors. Cancer cell (CC) SGs are indicated by arrows and Stroma SGs are indicated by triangles. Cancer cells and stroma were identified by staining as positive or negative for CK8 respectively. Scale bar, 10 μm.

(I) Representative H&E staining of sections from KPC-466 and KPC-6560 tumors in Figure 3E-F. Scale bar, 50 μm.

(J) *Left Panel*- Body weight of age-matched of ST and *ob/ob* mice at implantation of ES-149 cells. *Right Panel*- mPDAC tumor growth at experimental endpoint on day 21 post orthotopic implantation of ES-149 cells in mice as in 3E and representative H&E in sections from orthotopic tumors. Data are mean tumor weight at endpoint +/- SEM and individual tumor weights for each mouse. ST n=5, *ob/ob* n=4, * $p<0.05$ by Mann Whitney t-test. Scale bar, 50 μm.

(K) Quantification of SG index in sections from ES-149 ST and *ob/ob* tumors in I as detected by G3bp1 and representative images. Data was derived and shown as in E. * $p<0.05$ by Mann Whitney t-test.

(L) Assessment of SG index in sections from KPC-4662 and KPC-6560 ST and *ob/ob* tumors of similar size distribution in 3B, as detected by G3bp1. Individual tumor weights and their corresponding SG levels as in 3D are shown.

Figure S4

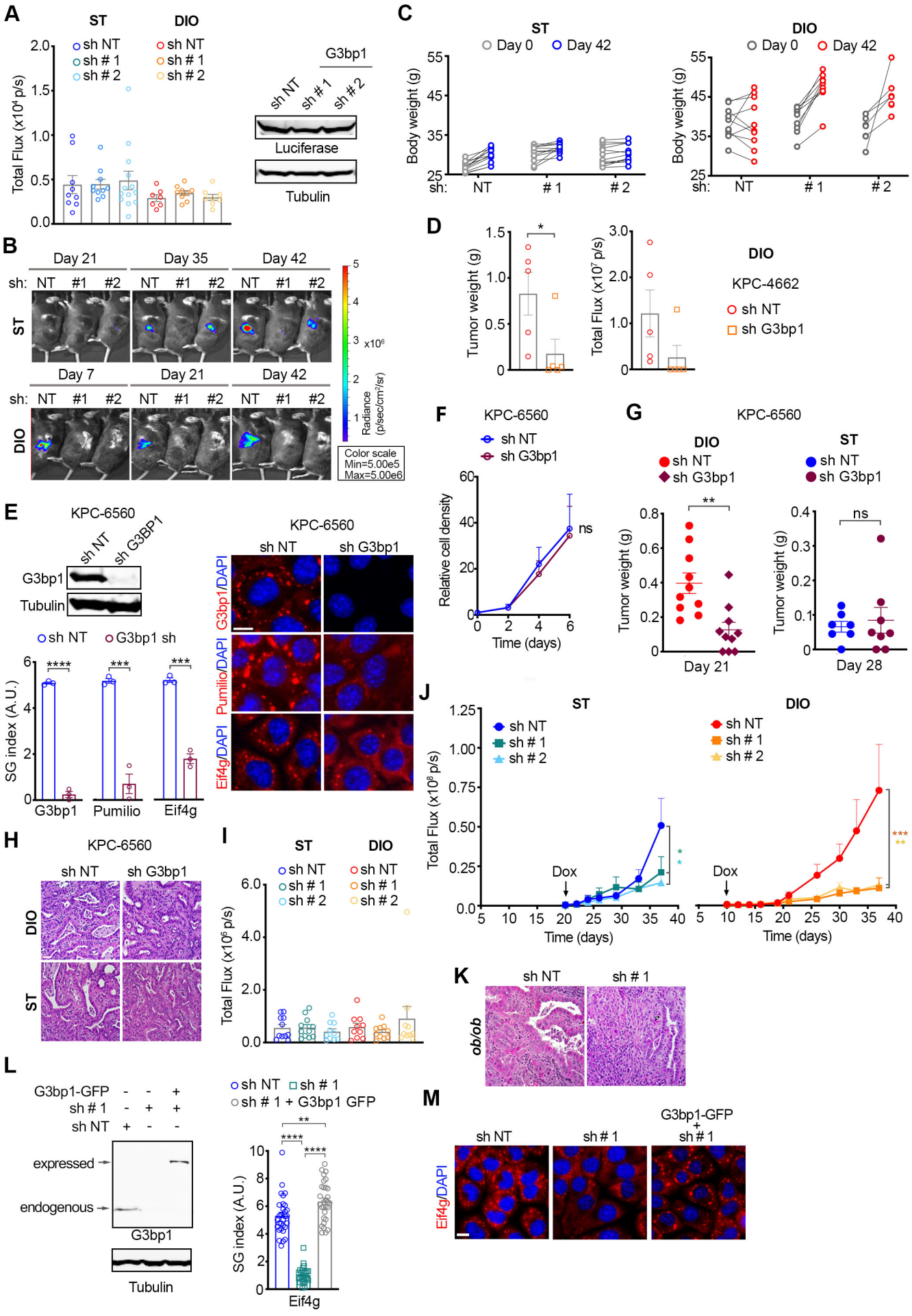

A
ST
DIO
sh NT
sh # 1
sh # 2
Total Flux (x10⁴ p/s)
G3bp1
Luciferase
Tubulin
B
Day 21
Day 35
Day 42
Day 7
sh: NT #1 #2
ST
DIO
Radiance (p/sec/cm²/sr)
x10⁶
Color scale
Min=5.00e5
Max=5.00e6
C
Day 0
Day 42
Body weight (g)
D
Tumor weight (g)
Total Flux (x10⁷ p/s)
KPC-4662
sh G3bp1
E
KPC-6560
sh G3BP1
G3bp1
Tubulin
G3bp1 sh
SG index (A.U.)
Pumilio
Eif4g
G3bp1/DAPI
Pumilio/DAPI
Eif4g/DAPI
F
Relative cell density
Time (days)
ns
G
Day 21
Day 28
H
I
J
Dox
Time (days)
K
ob/ob
L
G3bp1-GFP
expressed
endogenous
sh # 1 + G3bp1 GFP
M
G3bp1-GFP + sh # 1

Figure S4. Related to Figure 4.

(A) Equivalent levels of the indicated luciferase KPC-4662 cells were implanted in the pancreata of ST and DIO mice. Measurements were obtained on day 1 post implantation. Data are mean signal +/- SEM and individual values for each mouse. Western blot showing luciferase expression in KPC-4662 cells.

(B) Representative bioluminescent images of mice in 4B-C. Images were taken at the indicated time points post KPC-4662 orthotopic implantation. The scale indicates radiance expressed as p/sec/cm^2/sr. Each set of images (time) were taken from the same mice.

(C) Body weight of ST and DIO mice on day 0 and 42 post orthotopic implantation of the luciferase KPC-4662 cell lines expressing the indicated shRNAs as in 4A.

(D) mPDAC tumor weight and bioluminescence measurements on day 33 post orthotopic implantation of KPC-4662 cells in DIO mice induced to express the indicated shRNAs as in 1F. Data are mean +/- SEM of individual tumors in each group; sh NT n=5, sh # 1 G3bp1 n=5. *p<0.05 by Mann Whitney t-test.

(E) Western Blot of lysates from KPC-6560 cells expressing the indicated shRNAs. Quantification of SG index as detected by G3bp1, Pumilio, and Eif4g, in KPC-6560 cells expressing the indicated shRNAs and stressed as in 1C. Representative immunostaining of the SG markers G3bp1, Pumilio, and Eif4g and DAPI in KPC-6560 expressing the indicated shRNAs and stressed as in 1C. Scale bar, 10 μm.Data are mean +/- SEM from 3 independent experiments. ***p<0.001, ****p<0.0001 by unpaired t-test.

(F) Relative cell density of KPC-6560 cells expressing the indicated shRNAs measured using a Syto60 stain. Data is mean +/- SD, n=3. ns = non-significant by unpaired t-test.

(G) mPDAC tumor weight at experimental endpoint on day 28 (ST) and 21 (DIO) post orthotopic implantation in immunocompetent mice of KPC-6560 cells induced to express the indicated shRNAs as in 1F. Data are presented as mean +/- SEM and individual tumor weights for each mouse are also shown. ST (sh NT n=7, sh # 3 G3bp1 n=8), DIO (sh NT n=10, sh # 3 G3bp1 n=10). ** $p<0.01$, ns = non-significant by Mann Whitney t-test.

(H) Representative H&E staining of sections from orthotopic tumors in G. Scale bar, 50 μm.

(I) Equivalent bioluminescent signal of indicated KPC-4662 luciferase tumors in ST and DIO mice on day of Dox administration. Data are mean signal +/- SEM and individual values for each mouse.

(J) Quantification of bioluminescent signal over time post Dox administration in ST and DIO mice as in I. Data are mean signal +/- SEM of individual tumors in each group; ST (sh NT n=11, sh # 1 G3bp1 n=11, sh # 2 G3bp1 n=10), DIO (sh NT n=10, sh # 1 G3bp1 n=10, sh # 2 G3bp1 n=10). *$p<0.05$, **$p<0.01$, ***$p<0.001$ by two-way ANOVA.

(K) Representative H&E staining of sections from KPC-6560 orthotopic tumors in 4D. Scale bar, 50 μm.

(L) *Left Panel*-WB of lysates of KPC-4662 cells expressing the indicated Dox-inducible shRNA and shRNA-resistant G3bp1 constructs. *Right Panel*-Quantification of SG index as detected by Eif4g in KPC-4662 cells expressing the indicated constructs and stressed as in 1A. Measurements as in 1C are shown. **$p<0.01$, ****$p<0.0001$ by unpaired t-test.

(M) Representative immunostaining of Eif4g and DAPI in KPC-4662 cells expressing the indicated constructs and stressed as in 1A. Scale bar, 10 μm.

Figure S5

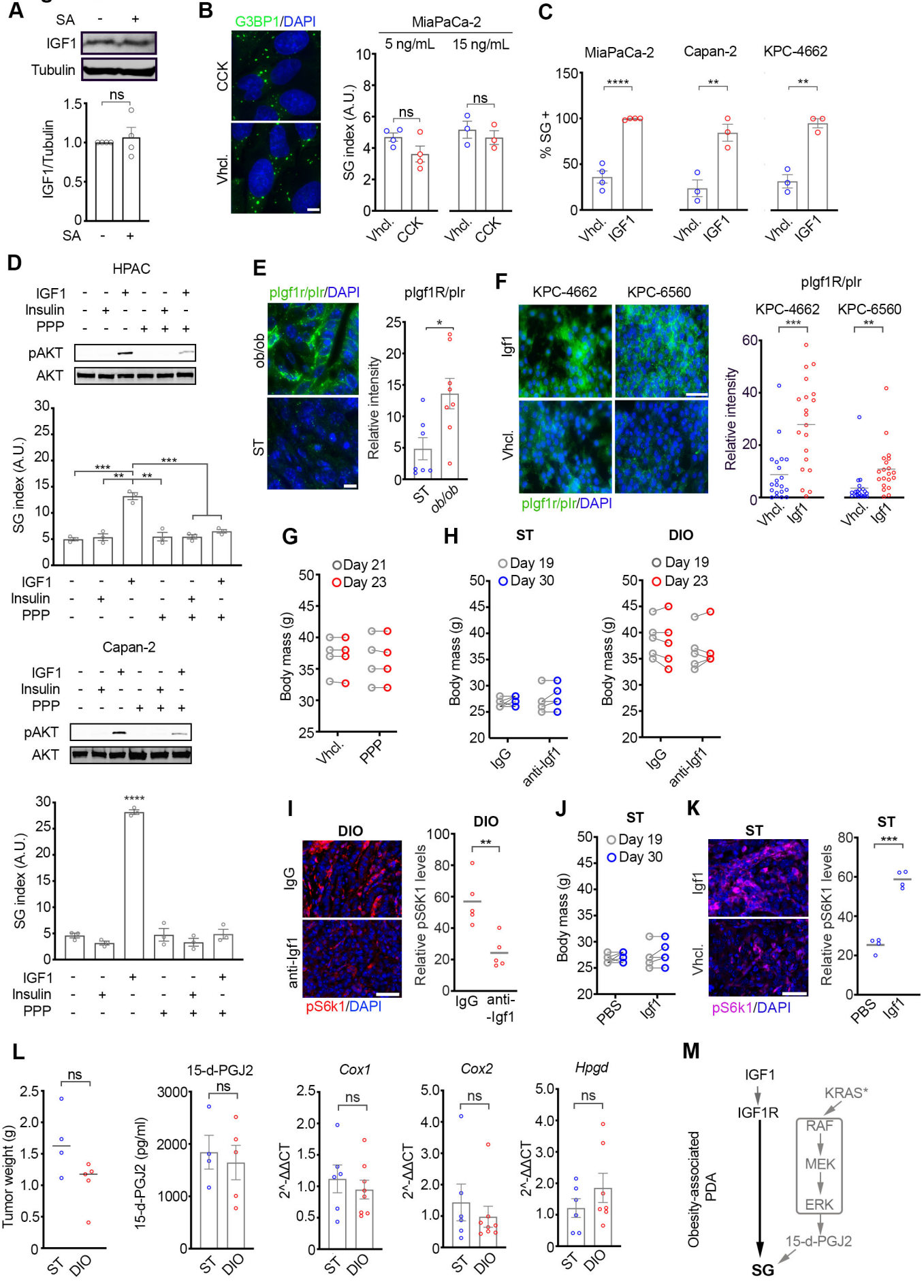

A
SA - +
IGF1
Tubulin
IGF1/Tubulin
ns
SA - +
B
G3BP1/DAPI
CCK
Vhcl.
MiaPaCa-2
5 ng/mL
15 ng/mL
SG index (A.U.)
ns
Vhcl. CCK Vhcl. CCK
C
MiaPaCa-2
Capan-2
KPC-4662
% SG +
****
**
**
Vhcl. IGF1
D
HPAC
IGF1
Insulin
PPP
pAKT
AKT
SG index (A.U.)
Capan-2
E
pIgf1r/pIr/DAPI
ob/ob
ST
pIgf1R/pIr
Relative intensity
ST ob/ob
F
KPC-4662
KPC-6560
Igf1
Vhcl.
pIgf1r/pIr/DAPI
pIgf1R/pIr
Relative intensity
Vhcl. Igf1
G
Day 21
Day 23
Body mass (g)
Vhcl. PPP
H
ST
DIO
Day 19
Day 30
Day 23
IgG anti-Igf1
I
DIO
IgG
anti-Igf1
pS6k1/DAPI
Relative pS6K1 levels
IgG anti--Igf1
J
ST
PBS Igf1
K
ST
pS6k1/DAPI
L
Tumor weight (g)
15-d-PGJ2
15-d-PGJ2 (pg/ml)
Cox1
Cox2
Hpgd
2^-ΔΔCT
ST DIO
M
IGF1
IGF1R
KRAS*
RAF
MEK
ERK
15-d-PGJ2
SG
Obesity-associated PDA

Figure S5. Related to Figure 5.

(A) Western blotting of lysates of MiaPaCa-2 cells treated with vehicle or SA as in 5A. Graph shows quantification of IGF1 levels normalized to tubulin and data are mean +/- SEM, n=4, and individual values for each experiment. ns, non-statistically significant by one sample t-test.

(B) Representative immunostaining of G3BP1 and DAPI and quantification of SG index in MiaPaCa-2 cells pretreated with CCK (5 ng/ml or 15 ng/ml, 2 hr) accordingly, and stressed as in 5A. Data are mean SG index +/- SEM, n=3, and individual values for each experiment. ns, non - significant by unpaired t-test. Scale bar, 10 μm.

(C) Quantification of the percent SG positive (+) cells as detected by G3BP1 in cells as in 5B. Data is shown as mean +/- SEM of 3 independent experiments. The individual values for each experiment are also shown. **p<0.01, ****p<0.0001 by unpaired t-test.

(D) WB of lysates from HPAC and Capan-2 cells treated with PPP or vehicle in the presence or absence of IGF1 and Insulin as in 5E. Quantification of SG index as detected by G3BP1 in HPAC and Capan-2 cells treated as indicated. Data are mean SG index +/- SEM, n = 3. *p<0.05, **p<0.01, ****p<0.0001 by unpaired t-test.

(E) Representative immunostaining of pIgf1r/pIr and DAPI in tissue sections from KPC-4662 tumors in ST and *ob/ob* mice as in 3B. Scale bar, 10 μm. Graph shows mean relative immunofluorescence intensity +/- SEM and values for each tumor. The value for each tumor is the average of non-overlapping FOV covering ~50% of each section imaged at 20x. (ST, n=7; DIO, n=8) * p<0.05 by Mann Whitney t-test.

(F) Representative immunostaining and quantification of pIgf1r and DAPI in KPC-4662 and KPC-6560 cells treated with Igf1 or vehicle as in in 5A. Scale bar, 50 μm. Graph

shows mean relative immunofluorescence intensity and individual values from 20 fields of view at 40x from a representative experiment. *** $p<0.001$ by unpaired t-test. Scale bar, 50 μm

(G) Body weight of DIO mice implanted with KPC-4662 cells prior to and post treatment with vehicle or PPP as in 5G.

(H) Body weight of ST and DIO mice implanted with KPC-4662 cells prior to and post treatment with IgG or anti-Igf1 as in 5H.

(I) Representative immunostaining and quantification of pS6k1 and DAPI in sections from DIO KPC-4662 tumors treated with anti-Igf1 or IgG as in in 5H. Scale bar, 50 μm. Graph shows relative immunofluorescence intensity +/- SEM and values for each tumor. The value for each tumor is the average of non-overlapping FOV covering ~50% of each section imaged at 20x. (ST, n=5; DIO, n=5). ** $p<0.01$ by Mann Whitney t-test.

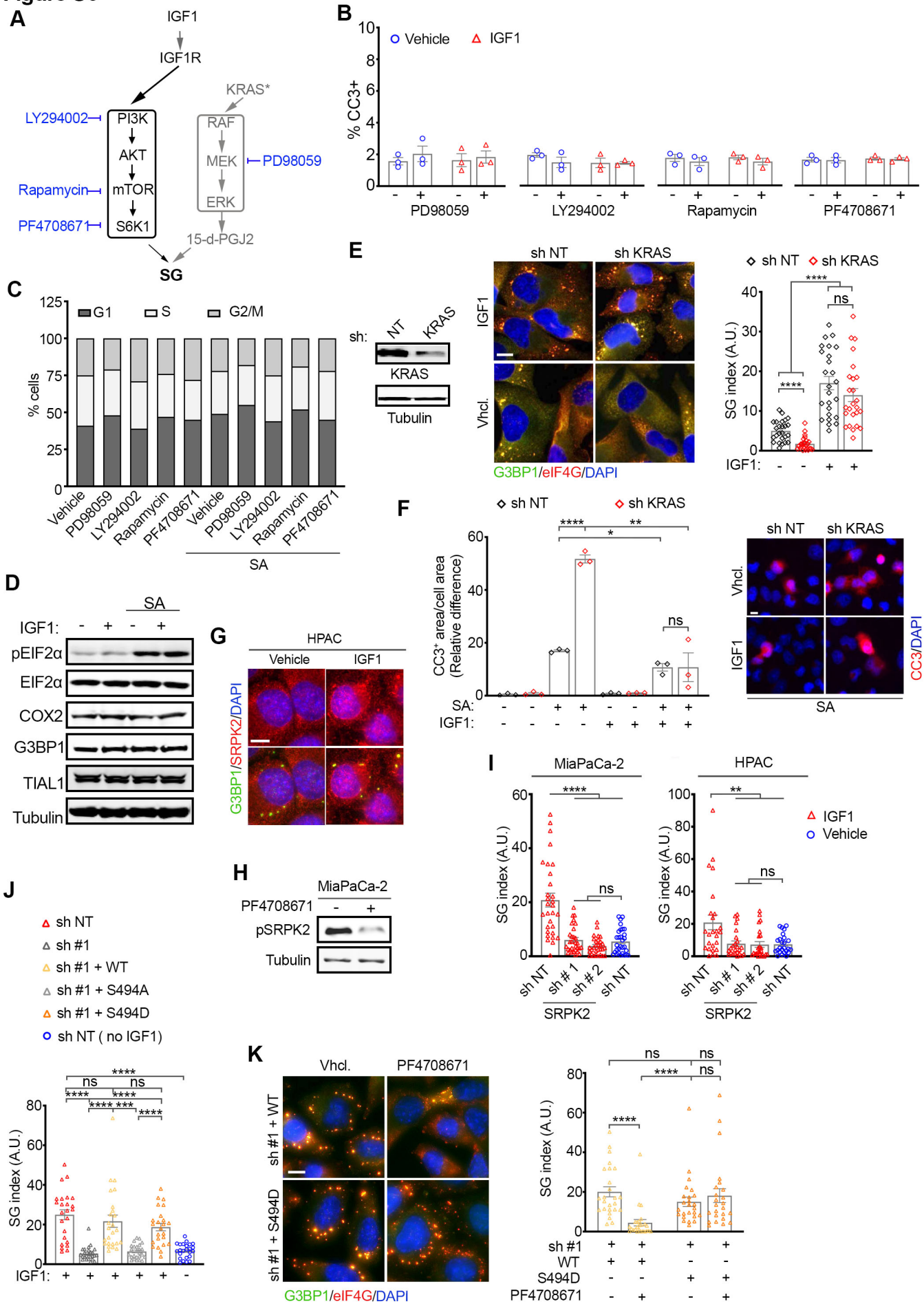
(J) Body weight of ST mice implanted with KPC-4662 cells prior to and post treatment with vehicle or Igf1 as in 5J. (ST, n=4; DIO, n=4).

(K) Representative immunostaining and quantification of pS6k1 and DAPI in sections from ST KPC-4662 tumors treated with Igf1 or vehicle as in 5J. Scale bar, 50 μm. Data is shown as in I (ST, n=4; DIO, n=4). *** $p<0.001$ by Mann Whitney t-test.

(L) Similar size distribution of ST and DIO tumors utilized to assess 15-d-PGJ2, *Cox1, Cox2, and Hpgd* levels. 15-d-PGJ2 levels in KPC-4662 tumors from ST and DIO mice were determined by ELISA of the interstitial fluid of the respective tumors (ST, n=4; DIO, n=5). *Cox1, Cox2, and Hpgd* levels were determined by qRT-PCR. Graph shows mean +/- SEM and values for each tumor (ST, n=6; DIO, n=7). ns, non - significant by unpaired t-test.

(M) Proposed model of pathways regulating SG formation in obesity-associated PDAC

Figure S6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IGF1R
KRAS*
LY294002
PI3K
AKT
mTOR
S6K1
Rapamycin
PF4708671
RAF
MEK
ERK
PD98059
15-d-PGJ2
SG
B
Vehicle
IGF1
% CC3+
PD98059
LY294002
Rapamycin
PF4708671
C
G1
S
G2/M
% cells
Vehicle
PD98059
LY294002
Rapamycin
PF4708671
SA
D
SA
IGF1:
pEIF2α
EIF2α
COX2
G3BP1
TIAL1
Tubulin
E
sh NT
sh KRAS
sh:
KRAS
Tubulin
IGF1
Vhcl.
G3BP1/eIF4G/DAPI
SG index (A.U.)
IGF1:
F
CC3+ area/cell area (Relative difference)
SA:
IGF1:
Vhcl.
IGF1
CC3/DAPI
G
HPAC
Vehicle
IGF1
G3BP1/SRPK2/DAPI
H
MiaPaCa-2
PF4708671
pSRPK2
Tubulin
I
MiaPaCa-2
HPAC
SG index (A.U.)
IGF1
Vehicle
sh NT
sh #1
sh #2
SRPK2
J
sh NT
sh #1
sh #1 + WT
sh #1 + S494A
sh #1 + S494D
sh NT (no IGF1)
SG index (A.U.)
IGF1:
K
Vhcl.
PF4708671
sh #1 + WT
sh #1 + S494D
G3BP1/eIF4G/DAPI
SG index (A.U.)
sh #1
WT
S494D
PF4708671

Figure S6. Related to Figure 6.

(A) Schematic of inhibitors and their targets downstream of IGF1 and RAS.

(B) Quantification of percent CC3+ of MiaPaCa-2 cells treated as in 6B. Data are mean +/- SEM from 3 independent experiments and individual values for each experiment.

(C) Cell cycle distribution of MiaPaCa-2 cells treated as in 6B. Data from a representative experiment are shown.

(D) Western Blots of lysates of MiaPaCa-2 cells treated with vehicle or IGF1 and subjected to oxidative stress via SA (100 μM, 1 hr) or vehicle control.

(E) Western Blots of lysates of MiaPaCa-2 expressing the indicated shRNAs. Representative images and quantification of SG index as detected by G3BP1 in MiaPaCa-2 cells expressing the indicated shRNAs. Data from a representative experiment are shown as in 1C. The individual values are also shown. ****p<0.0001, ns = non-significant by unpaired t-test.

(F) Quantification of cell death in MiaPaCa-2 cells expressing the indicated shRNA and treated with vehicle or 500 μM SA for 5 hr as determined by CC3 staining. Representative images are shown. Scale bar, 10 μm. Relative cell death was determined by immunostaining for CC3 as in 4F. Data are mean +/- SEM from 3 independent experiments and individual values for each experiment. *p<0.05, **p<0.01, ****p<0.0001, ns = non-significant by unpaired t-test.

(G) Representative immunostaining of SRPK2, G3BP1, and DAPI in HPAC cells treated as in 6A. Scale bar, 10 μm.

(H) Western Blots of lysates of MiaPaCa-2 cells treated with vehicle or PF4708671.

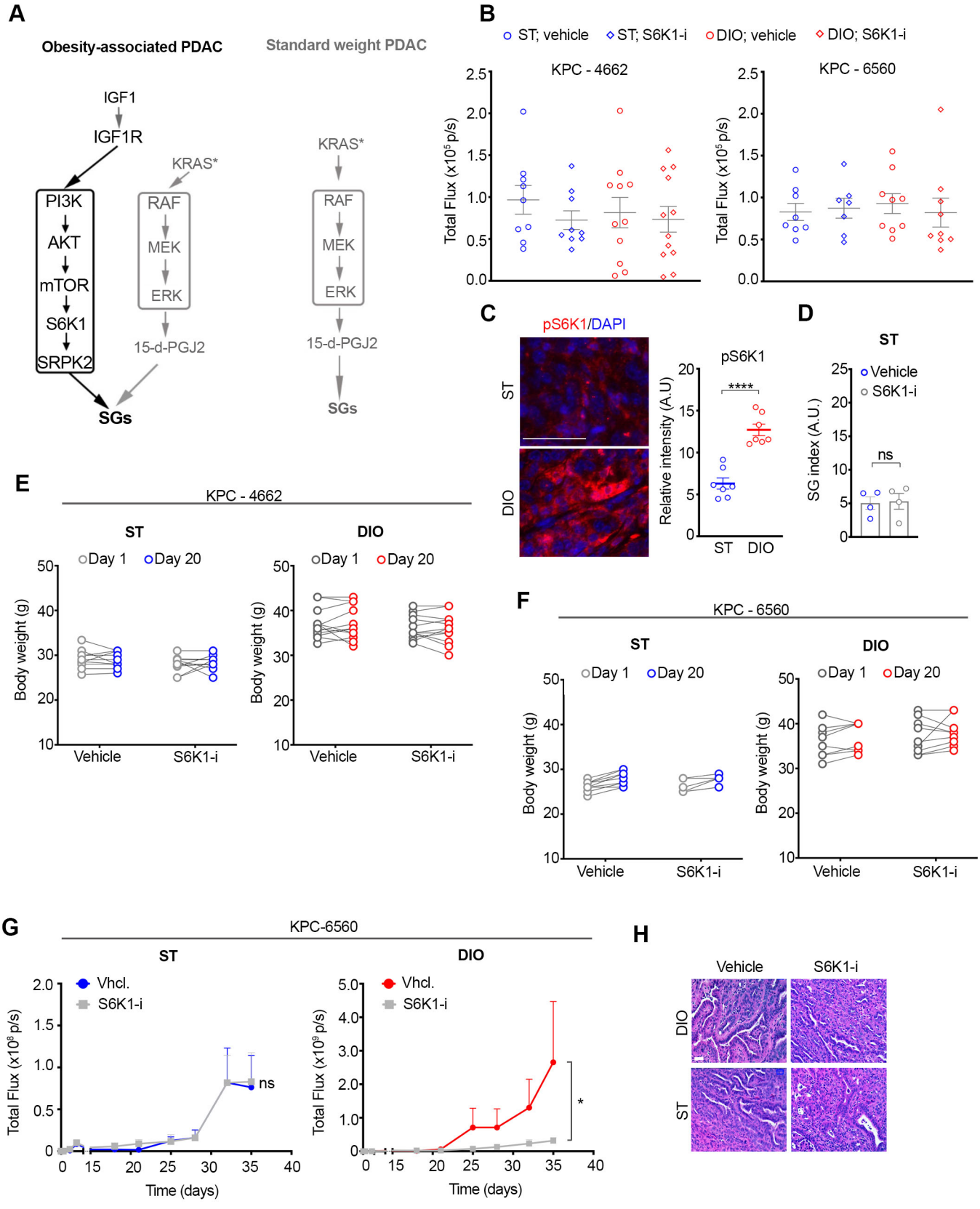
(I) Quantification of SG index as detected by G3BP1 in MiaPaCa-2 cells as in in 6J. Data from a representative experiment are shown in arbitrary units (a.u.) for and as mean +/- SEM for 30 non-overlapping fields of view (FOV) imaged at 20x. The individual values for each FOV are also shown**p<0.01, ****p<0.0001, ns = non-significant by unpaired t-test.

(J) Quantification of SG index as detected by G3BP1 in MiaPaCa-2 expressing the indicated shRNA and constructs. Data is from a representative experiment as in I.

(K) Representative immunostaining of G3BP1, eIF4G, and DAPI and quantification of SG index MiaPaCa-2 expressing the indicated shRNA and constructs and treated with vehicle or PF4708671. Data is from a representative experiment as in I.

(J-K) Mean and individual values are also shown. ****p<0.0001, ns = non - significant by unpaired t-test.

Figure S7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Obesity-associated PDAC
Standard weight PDAC
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IGF1R
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PI3K
AKT
mTOR
S6K1
SRPK2
RAF
MEK
ERK
15-d-PGJ2
SGs
KRAS*
RAF
MEK
ERK
15-d-PGJ2
SGs
B
ST; vehicle
ST; S6K1-i
DIO; vehicle
DIO; S6K1-i
KPC - 4662
KPC - 6560
Total Flux (x10^5 p/s)
C
pS6K1/DAPI
ST
DIO
pS6K1
Relative intensity (A.U)
****
ST
DIO
D
ST
Vehicle
S6K1-i
SG index (A.U.)
ns
E
KPC - 4662
ST
DIO
Day 1
Day 20
Body weight (g)
Vehicle
S6K1-i
F
KPC - 6560
ST
DIO
Day 1
Day 20
Body weight (g)
Vehicle
S6K1-i
G
KPC-6560
ST
DIO
Vhcl.
S6K1-i
Total Flux (x10^8 p/s)
Total Flux (x10^9 p/s)
ns
*
Time (days)
H
Vehicle
S6K1-i
DIO
ST

Figure S7. Related to Figure 7.

(A) Proposed model for pathways regulating SG formation in obesity-associated and standard weight PDAC.

(B) Equivalent numbers of the indicated luciferase KPC-4662 and KPC-6560 cells were implanted in the pancreata of ST and DIO mice. Measurements were obtained on day 1 post implantation. Data are mean signal +/- SEM and individual values for each mouse.

(C) Representative immunostaining of pS6K1 and DAPI in tissue sections from KPC-4662 tumors in DIO mice as in 3B. Graph shows quantification of relative pS6K1 levels as mean intensity +/- SEM and values for each tumor. The value for each tumor is the average of non-overlapping FOV covering ~50% of each section imaged at 10x. *** $p<0.001$ by Mann Whitney t-test. Scale bar, 50 μm.

(D) Quantification of SG index in KPC-4662 tumors sections from ST mice treated with PF4708671 as in 7C. Data are mean SG index +/- SEM, and individual values for each tumor. The SG index for each tumor section is the average of 30 non-overlapping FOV imaged at 40x. ** $p<0.01$ by Mann Whitney t-test.

(E) Body weight of ST and DIO mice on day 0 and 20 post orthotopic implantation of the luciferase KPC-4662 cell line and treated with vehicle control or PF4708671.

(F) Body weight of ST and DIO mice on day 0 and 20 post orthotopic implantation of the luciferase KPC-6560 cell line and treated with vehicle control or PF4708671.

(G) Quantification of tumor bioluminescent signal over time post orthotopic implantation of KPC-6560 cells in ST and DIO mice treated with vehicle or PF4708671 as in 7A. Data are mean signal +/- SEM of individual tumors in each group; ST (vehicle n=8, PF4708671 n=7), DIO (vehicle n=9, PF4708671 n=9). *$p<0.05$ by two-way ANOVA.

(H) Representative immunostaining of H&E in sections from orthotopic tumors in G. Scale bar, 50 μm.