Supplemental figures:

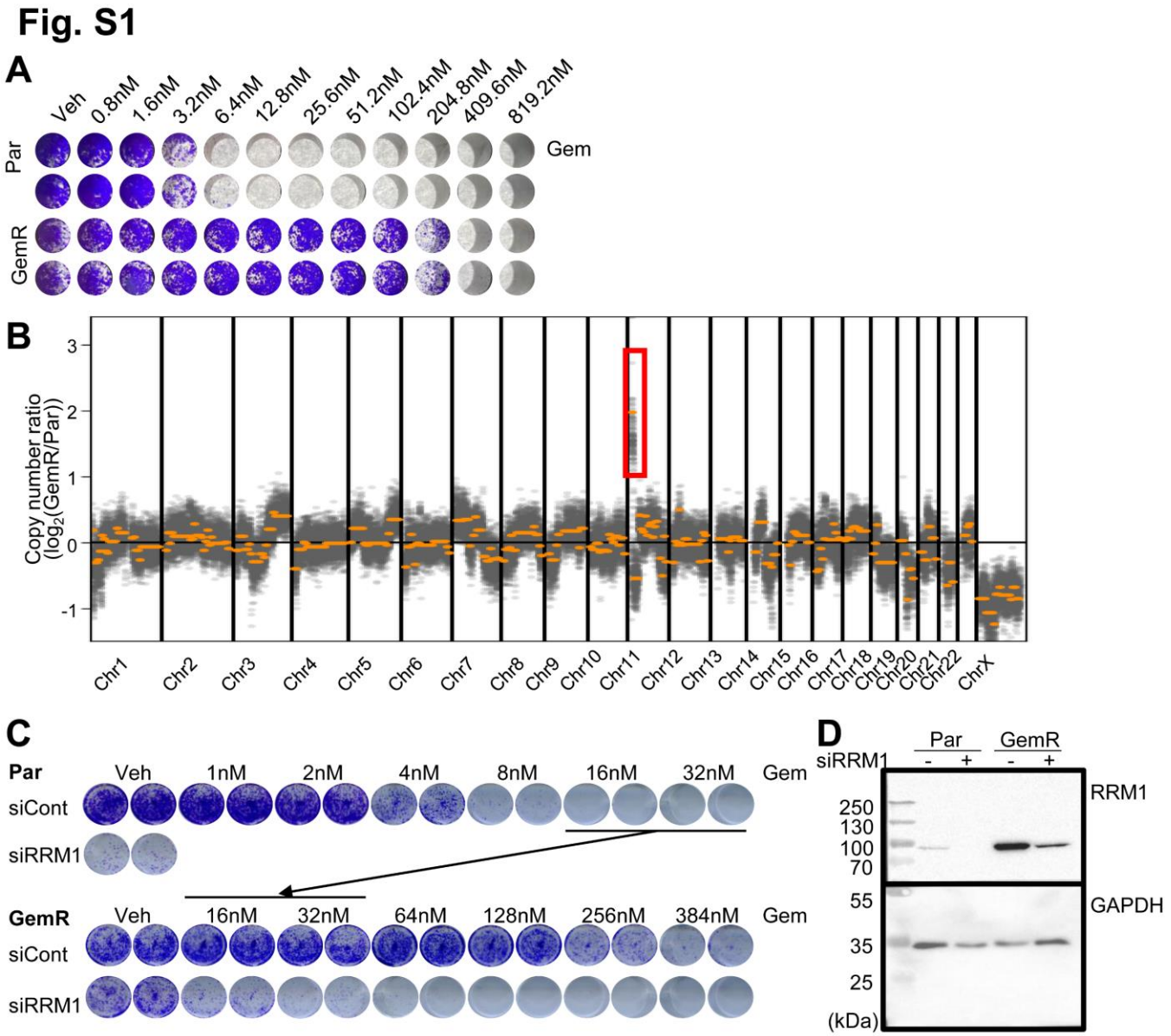

Fig. S1 (A) Crystal violet staining of a 7 days proliferation assay of Par and GemR treated with gemcitabine (Gem). (B) Genome-wide copy number variation analysis of GemR compared to Par. Highlighted in a red box is the identified amplification in chr11. (C) Crystal violet staining of a 7 days proliferation assay upon RRM1 knockdown and treatment with gemcitabine (Gem) in Par and GemR. (D) Full western blot validation of RRM1 knockdown in Par and GemR.

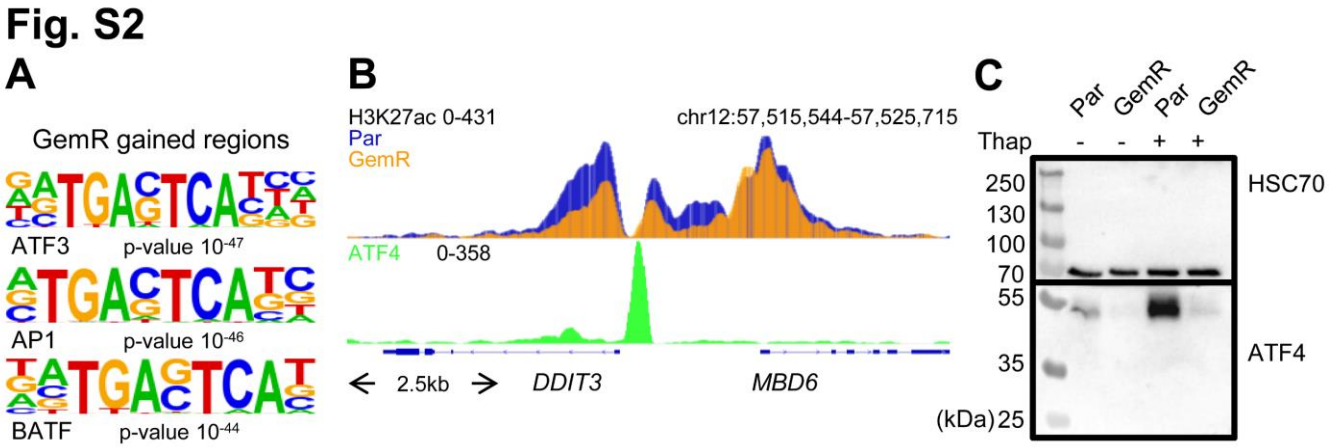

Fig. S2 (A) Top three hits of motif analysis in H3K27ac gained regions in GemR compared to Par. (B) ATF4 and H3K27ac profiles around the TSS of the stress responsive gene *DDIT3*. (C) Full western blot of ATF4 levels upon thapsigargin (Thap) treatment in Par and GemR. The same western blot is shown in Fig. 2E.

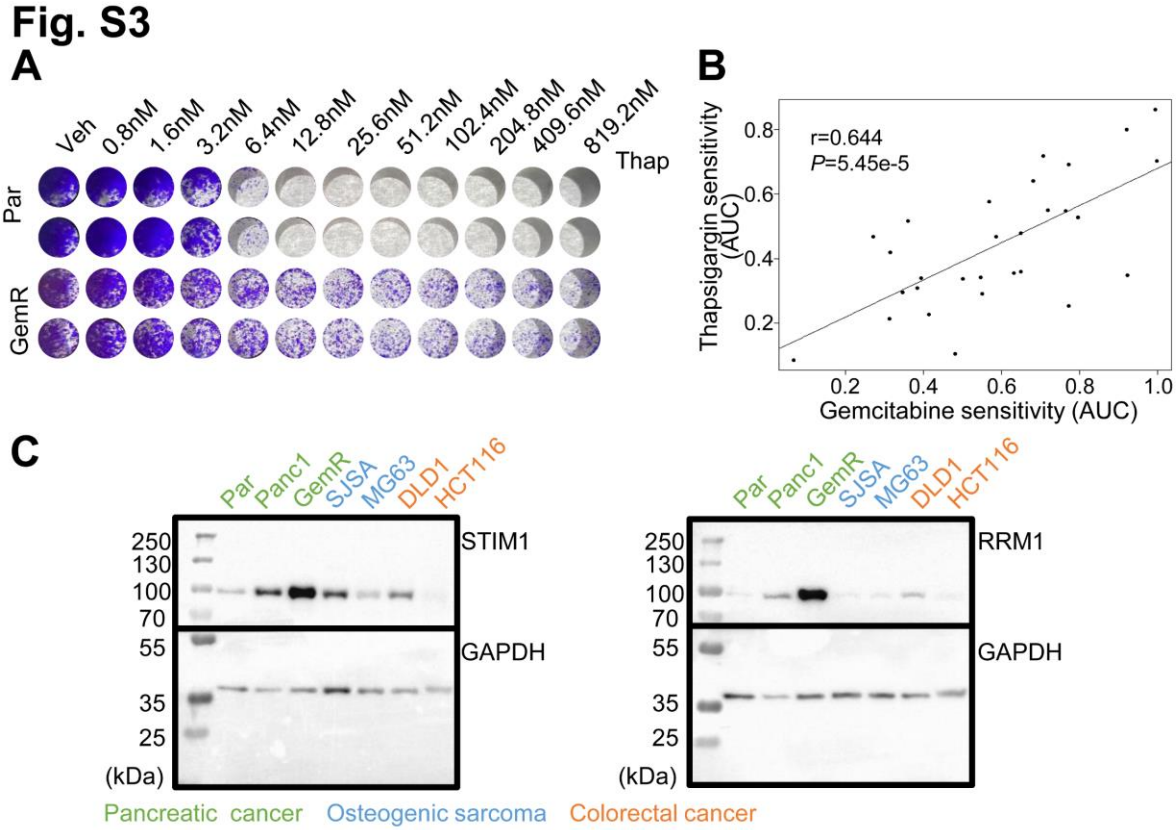

Fig. S3 (A) Crystal violet staining of a 7 days proliferation assay of Par and GemR treated with thapsigargin (Thap). (B) Scatter plot showing the Spearman correlation of gemcitabine and thapsigargin sensitivity in pancreatic cancer cell lines obtained from DepMap. r=0.644, *P*=5.45e-5. (C) Full western blot of STIM1 and RRM1 levels in pancreatic, colorectal and osteosarcoma cell lines.

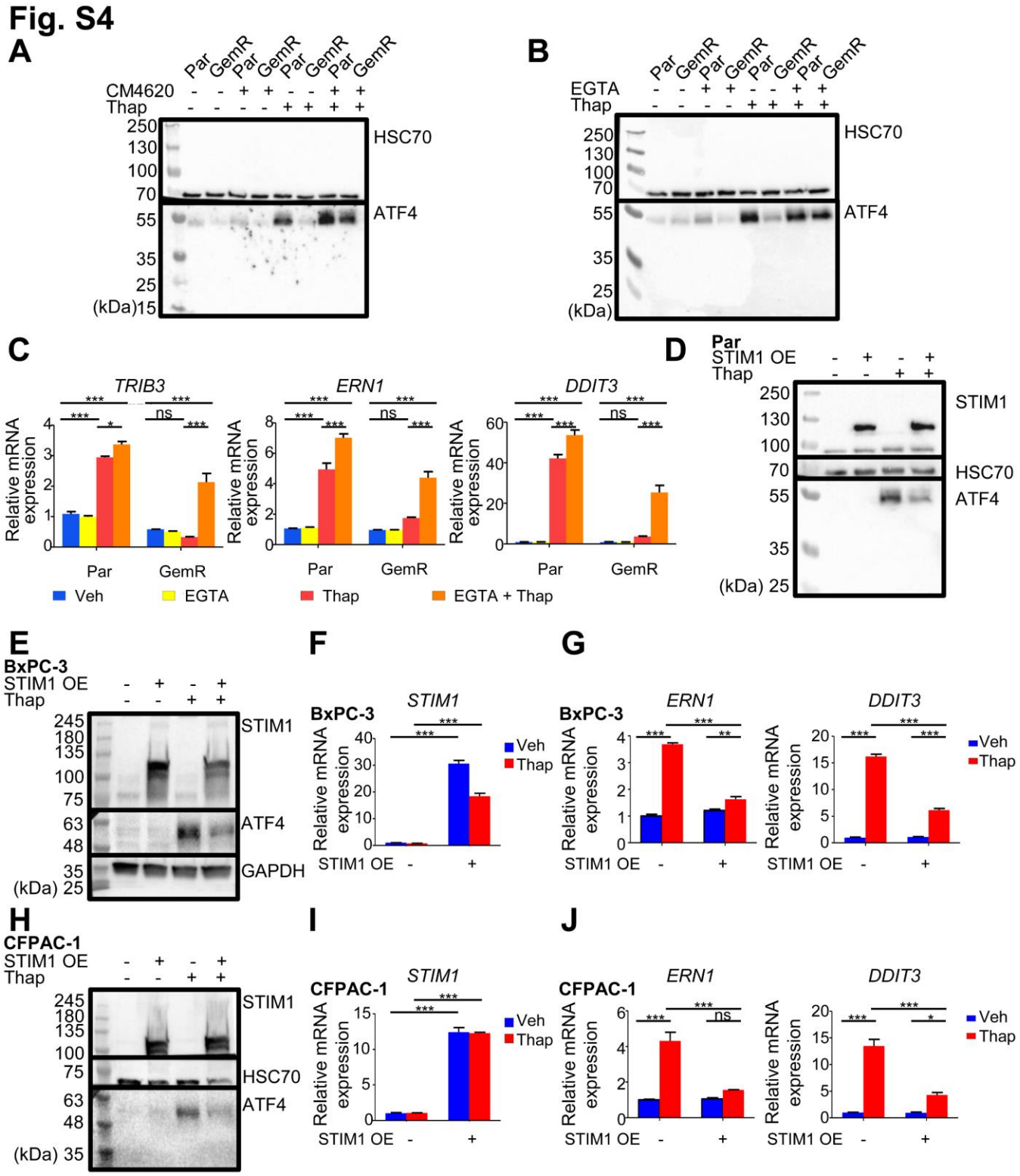

Fig. S4 (A) Full western blot depicting ATF4 levels upon SOCE inhibition by CM4620 and thapsigargin (Thap) treatment in Par and GemR. The same western blot is shown in Fig. 3D. (B) Full western blot depicting ATF4 levels upon EGTA and thapsigargin (Thap) treatments in Par and GemR. (C) Expression of stress responsive genes upon EGTA and thapsigargin (Thap) treatment in Par and GemR. Mean ±SD, *n*=3. (D) Full western blot of STIM1 and ATF4 levels upon STIM1 overexpression and thapsigargin (Thap) treatment in Par. The same western blot is shown in Fig. 3F. (E) Full western blot of STIM1 and ATF4 levels upon STIM1 overexpression and thapsigargin (Thap) treatment in BxPC-3. (F) Validation of *STIM1* overexpression in BxPC-3. Mean ±SD, *n*=3. (G) Expression of stress responsive genes upon STIM1 overexpression and thapsigargin (Thap) treatment in BxPC-3. Mean

±SD, *n*=3. (H) Full western blot of STIM1 and ATF4 levels upon STIM1 overexpression and thapsigargin (Thap) treatment in CFPAC-1. (I) Validation of *STIM1* overexpression in CFPAC-1. Mean ±SD, *n*=3. (J) Expression of stress responsive genes upon STIM1 overexpression and thapsigargin (Thap) treatment in CFPAC-1. Mean ±SD, *n*=3. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ns=not significant.

Fig. S5

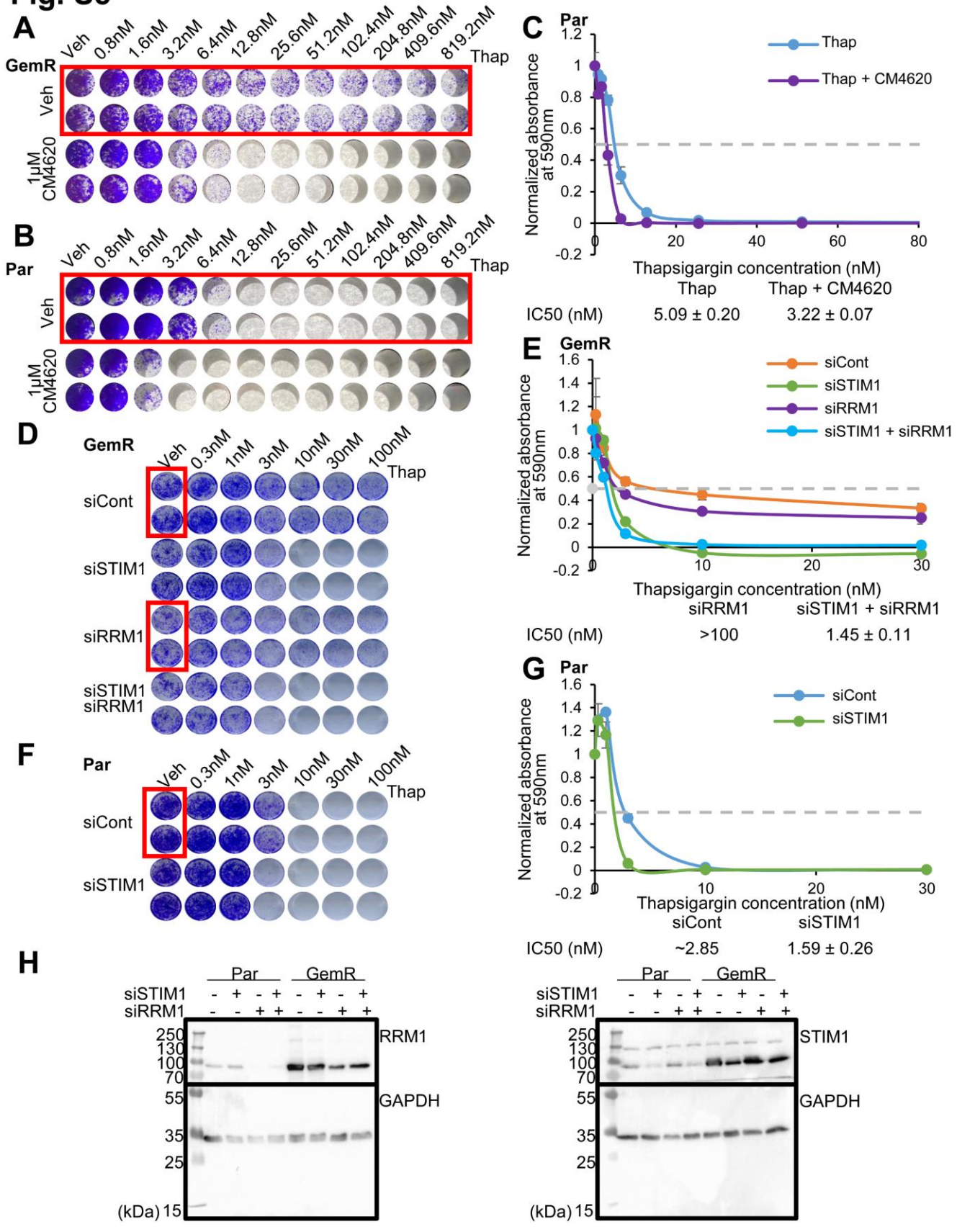
A
GemR
Veh
0.8nM
1.6nM
3.2nM
6.4nM
12.8nM
25.6nM
51.2nM
102.4nM
204.8nM
409.6nM
819.2nM
Thap
Veh
1μM CM4620
B
Par
C
Par
Thap
Thap + CM4620
Normalized absorbance at 590nm
Thapsigargin concentration (nM)
Thap
Thap + CM4620
IC50 (nM)
5.09 ± 0.20
3.22 ± 0.07
D
GemR
0.3nM
1nM
3nM
10nM
30nM
100nM
siCont
siSTIM1
siRRM1
siSTIM1 siRRM1
E
GemR
siCont
siSTIM1
siRRM1
siSTIM1 + siRRM1
Thapsigargin concentration (nM)
siRRM1
siSTIM1 + siRRM1
IC50 (nM)
>100
1.45 ± 0.11
F
Par
G
Par
siCont
siSTIM1
Thapsigargin concentration (nM)
IC50 (nM)
~2.85
1.59 ± 0.26
H
Par
GemR
siSTIM1
siRRM1
250
130
100
70
55
35
25
(kDa) 15
RRM1
GAPDH
STIM1

Fig. S5 (A) Crystal violet staining of a 7 days proliferation assay in GemR upon SOCE inhibition by CM4620 and thapsigargin (Thap) treatment. In a red box are the thapsigargin (Thap) only treated GemR, also shown in Fig. S3A. (B) Crystal violet staining of a 7 days proliferation assay in Par upon CM4620 and thapsigargin (Thap) treatments. In a red box are the thapsigargin (Thap) only treated Par, also shown in Fig. S3A. (C) Proliferation assay of Par treated with CM4620 and thapsigargin (Thap) for 7 days. The absorbance of cell titer blue was normalized to the respective vehicle absorbance. Mean ±SD, *n*=2. IC_{50} values ±SD, *n*=2. The profile of Par treated with thapsigargin (Thap) only was previously depicted in Fig. 3A. (D) Crystal violet staining of a 7 days proliferation assay in GemR upon STIM1 and/or RRM1 knockdown and treatment with thapsigargin (Thap). Highlighted in a red box is the control, vehicle-treated GemR and vehicle-treated RRM1-depleted GemR, which were previously shown in Fig. S1C. (E) Proliferation assay of GemR depleted from STIM1 and/or RRM1 and treated with thapsigargin (Thap) for 7 days. The absorbance of solubilized crystal violet was normalized to the respective vehicle absorbance. Mean ±SD, *n*=2. IC_{50} values ±SD, *n*=2. The profiles of GemR siCont and GemR siSTIM1 were previously depicted in Fig. 3I. (F) Crystal violet staining of a 7 days proliferation assay in Par upon STIM1 depletion and treatment with thapsigargin (Thap). Highlighted in a red box is the control, vehicle-treated Par, which was previously shown in Fig. S1C. (G) Proliferation assay of Par depleted from STIM1 and treated with thapsigargin (Thap) for 7 days. The absorbance of solubilized crystal violet was normalized to the respective vehicle absorbance. Mean ±SD, *n*=2. IC_{50} values ±SD, *n*=2. (H) Full western blot validation of STIM1 and RRM1 knockdown in Par and GemR.

Fig. S6

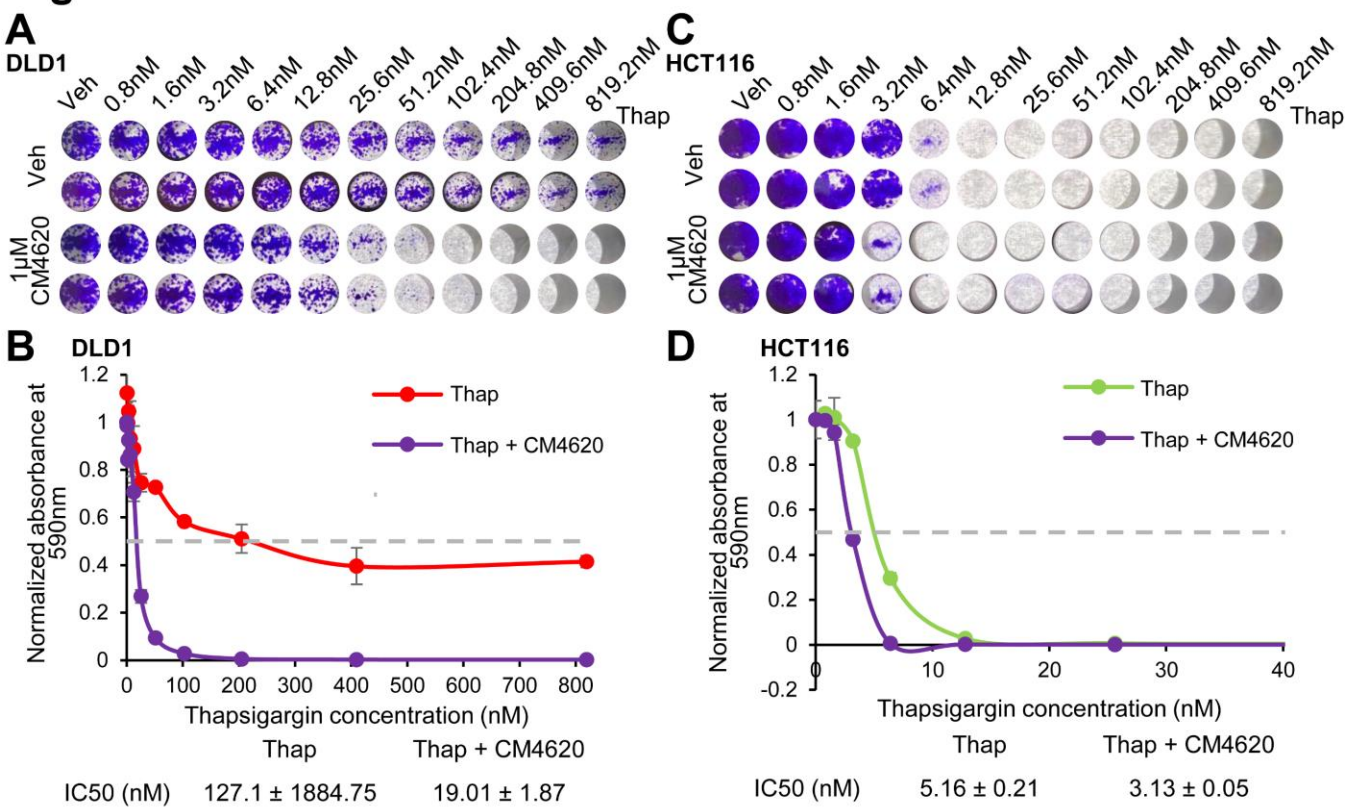

Fig. S6 (A) Crystal violet staining of a 7 days proliferation assay in DLD1 upon SOCE inhibition by CM4620 and thapsigargin (Thap) treatment. (B) Proliferation assay of DLD1 treated with CM4620 and thapsigargin (Thap) for 7 days. The absorbance of cell titer blue was normalized to the respective vehicle absorbance. Mean ±SD, *n*=2. IC_{50} values ±SD, *n*=2. (C) Crystal violet staining of a 7 days proliferation assay in HCT116 upon SOCE inhibition by CM4620 and thapsigargin (Thap) treatment. (D) Proliferation assay of HCT116 treated with CM4620 and thapsigargin (Thap) for 7 days. The absorbance of cell titer blue was normalized to the respective vehicle absorbance. Mean ±SD, *n*=2. IC_{50} values ±SD, *n*=2.

Fig. S7

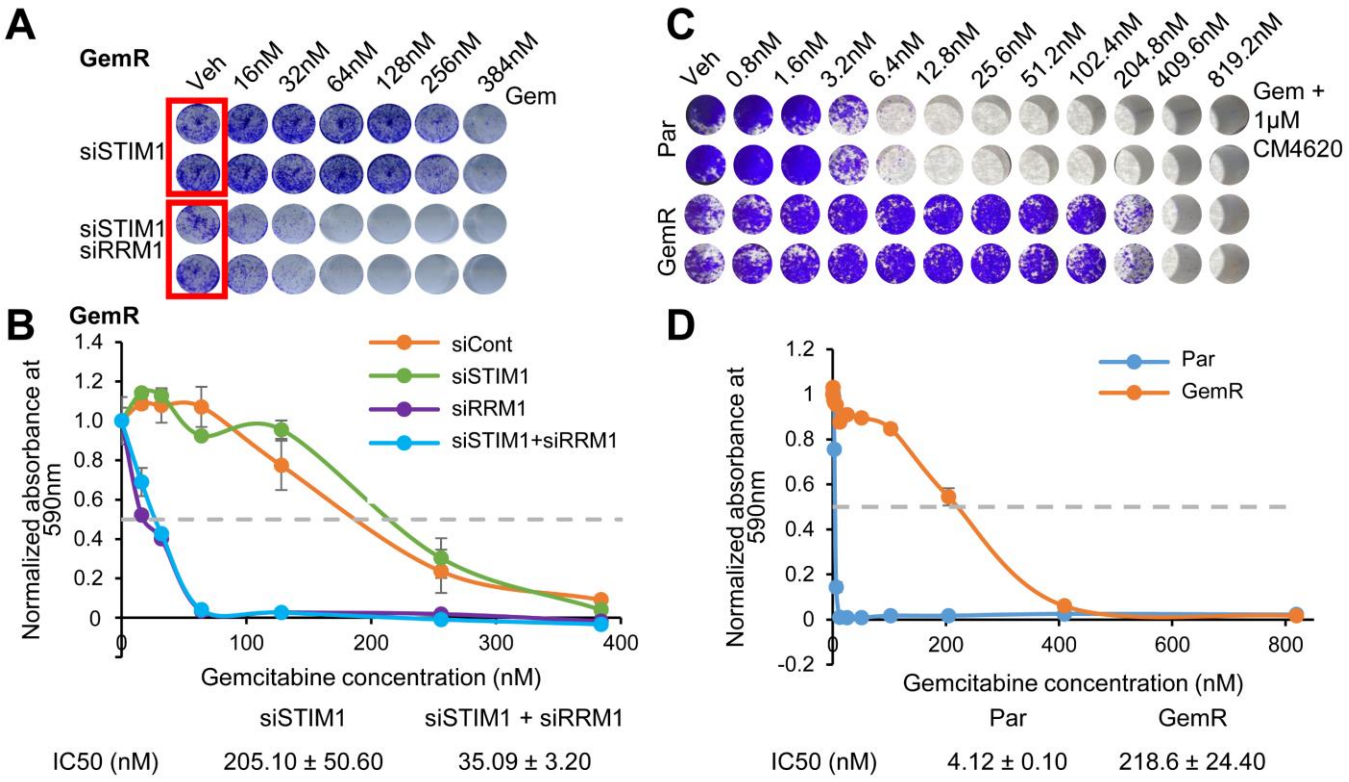

Fig. S7 (A) Crystal violet staining of a 7 days proliferation assay of STIM1 and/or RRM1 depleted GemR treated with gemcitabine (Gem). In red boxes are the vehicle-treated and STIM1 or STIM1 and RRM1 depleted GemR, also shown in Fig. S5D. The crystal violet staining of a 7 days proliferation of control and RRM1-depleted GemR treated with gemcitabine (Gem) can be found in Fig. S1C. (B) Proliferation assay of GemR depleted from STIM1 and/or RRM1 and treated with gemcitabine (Gem) for 7 days. The absorbance of solubilized crystal violet was normalized to the respective vehicle absorbance. Mean ±SD, *n*=2. IC_{50} values ±SD, *n*=2. The profiles of GemR siCont and GemR siRRM1 were previously depicted in Fig. 1F. (C) Crystal violet staining of a 7 days proliferation assay in Par and GemR upon CM4620 and gemcitabine (Gem) treatments. The crystal violet staining of a 7 days proliferation of Par and GemR treated with gemcitabine (Gem) only can be found in Fig. S1A. (D) Proliferation assay of Par and GemR treated with CM4620 and gemcitabine (Gem) for 7 days. The absorbance of cell titer blue was normalized to the respective vehicle absorbance. Mean ±SD, *n*=2. IC_{50} values ±SD, *n*=2. The profiles of Par and GemR treated with gemcitabine (Gem) only can be found in Fig. 1B.

Fig. S8

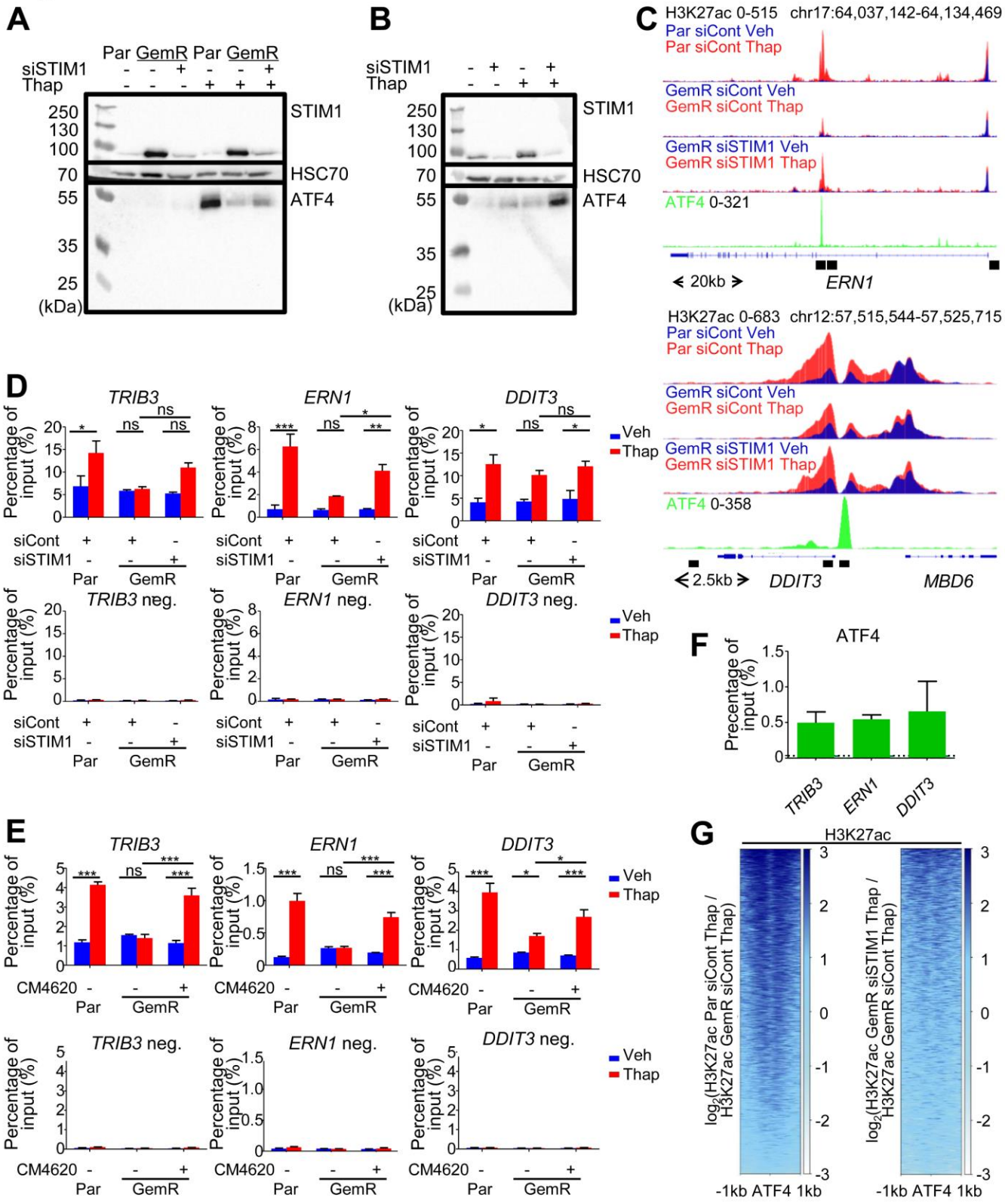

Fig. S8 (A) Full western blot of ATF4 and STIM1 levels upon STIM1 knockdown and thapsigargin (Thap) treatment in Par and GemR. The same western blot is shown in Fig. 4D. (B) Full western blot of ATF4 and STIM1 levels in Panc1 upon thapsigargin (Thap) treatment and STIM1 depletion. The same western blot is shown in Fig. 4F. (C) ATF4 profile of Par treated with thapsigargin (Thap) and H3K27ac profiles of Par, GemR and STIM1-depleted GemR upon thapsigargin (Thap) treatment around the TSS of DN-reversed genes. The regions used for ChIP qPCR are indicated by black boxes.

(D) ChIP qPCR of positive and negative H3K27ac sites around the TSS of *TRIB3*, *ERN1* and *DDIT3* in Par and GemR upon STIM1 knockdown and thapsigargin (Thap) treatment. Each condition is depicted as a percentage of its corresponding input. Mean ±SD, *n*=3. (E) ChIP qPCR of positive and negative H3K27ac sites around the TSS of *TRIB3*, *ERN1* and *DDIT3* in Par and GemR upon SOCE inhibition by CM4620 and thapsigargin (Thap) treatment. Each condition is depicted as a percentage of its corresponding input. Mean ±SD, *n*=3. (F) ChIP qPCR of positive ATF4 sites around the TSS of *TRIB3*, *ERN1* and *DDIT3* in Par treated with thapsigargin (Thap). The average ATF4 signal on the negative sites around the TSS of *TRIB3*, *ERN1* and *DDIT3* is shown as a light green, dark green and black dotted line, respectively. Each condition is depicted as a percentage of its corresponding input. Mean ±SD, *n*=2. (G) Bigwig compare of Par (left) and STIM1-depleted GemR (right) upon thapsigargin (Thap) treatment both compared to GemR treated with thapsigargin (Thap) on ATF4 summits of the overlapping regions identified in Fig. 4I. **P*≤0.05, ***P*≤0.01, ****P*≤0.001, ns=not significant.

Fig. S9

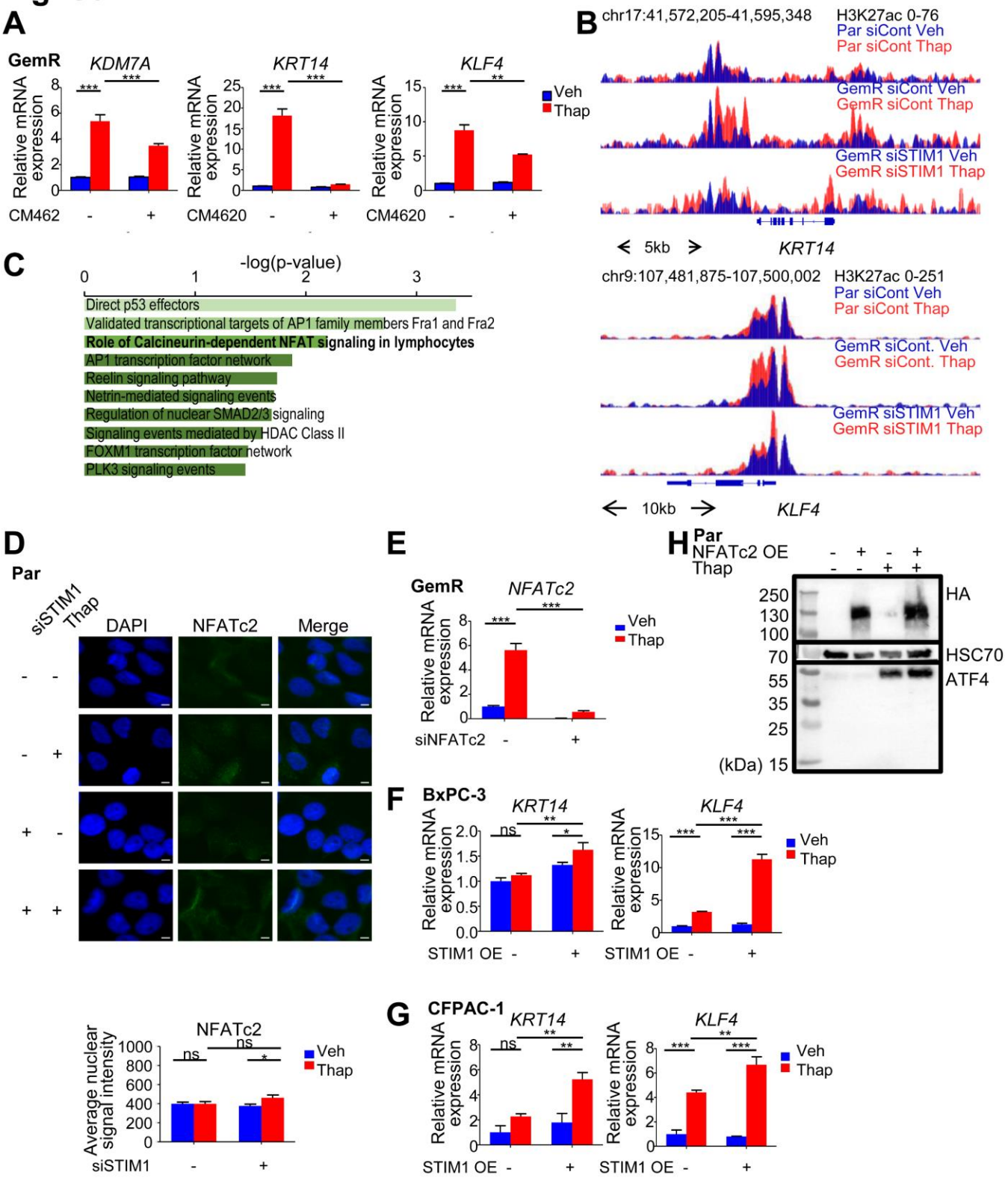

Fig. S9 (A) Gene expression of UP-reversed genes upon thapsigargin (Thap) treatment and SOCE inhibition by CM4620 in GemR. Mean ±SD, *n*=3. (B) H3K27ac profiles around the TSS of *KRT14* and *KLF4* in Par, GemR and STIM1-depleted GemR upon thapsigargin (Thap) treatment. (C) NCI-2016 signature of UP-reversed genes from EnrichR showing an enrichment for Calcineurin-dependent NFAT signaling. (D) NFATc2 immunofluorescence and average nuclear signal intensity in Par. Scale=5µm. Mean ±SEM, *n*=47 (Par siCont Veh), 34 (Par siCont Thap), 34 (Par si*STIM1* Veh), 41 (Par si*STIM1* Thap). (E) NFATc2 knockdown validation. Mean ±SD, *n*=2. (F) Expression of UP-reversed genes upon

STIM1 overexpression and thapsigargin (Thap) treatment in BxPC-3. Mean ±SD, *n*=3. (G) Expression of UP-reversed genes upon STIM1 overexpression and thapsigargin (Thap) treatment in CFPAC-1. Mean ±SD, *n*=3. (H) Full western blot of HA and ATF4 levels upon NFATc2 overexpression and thapsigargin (Thap) treatment in Par. **P*≤0.05, ***P*≤0.01, ****P*≤0.001, ns=not significant.

Fig. S10
A

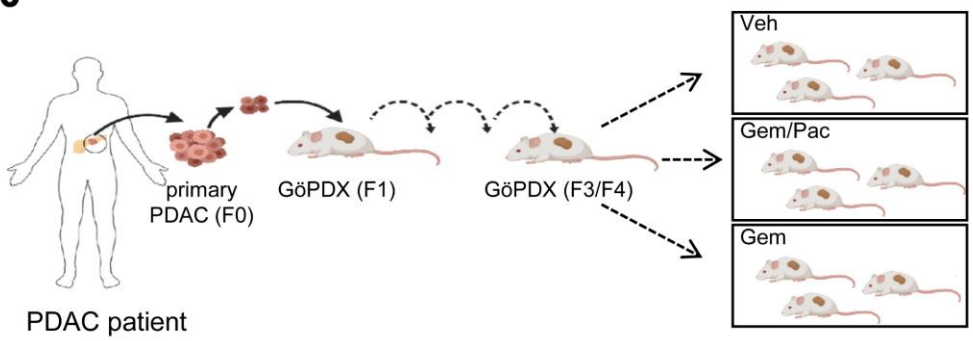

Fig. S10 (A) Scheme depicting the generation of PDXs and the treatment schedule of mice with vehicle (Veh), gemcitabine (Gem) or a combination of gemcitabine and nab-paclitaxel (Gem/Pac).