Conformation-specific inhibitory anti-MMP-7 monoclonal antibody sensitizes pancreatic ductal adenocarcinoma cells to chemotherapeutic cell kill

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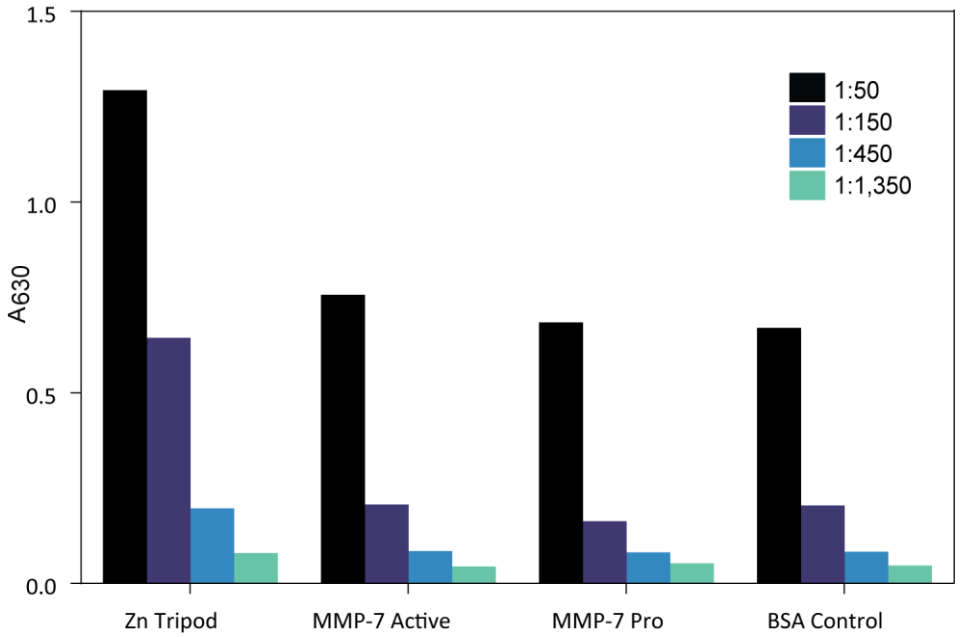

Figure S1. ELISA showing lack of anti MMP-7 antibodies**, in immunization with synthetic Zinc- tripod alone.** Immune responses were examined in serum using ELISA against antigens Zinc-Tripod, MMP-7 active form, MMP-7 zymogen form and BSA control in bleed from mouse immunized with Zinc-Tripod alone. The ELISA did not show presence of antibodies against MMP-7 catalytic or zymogen forms.

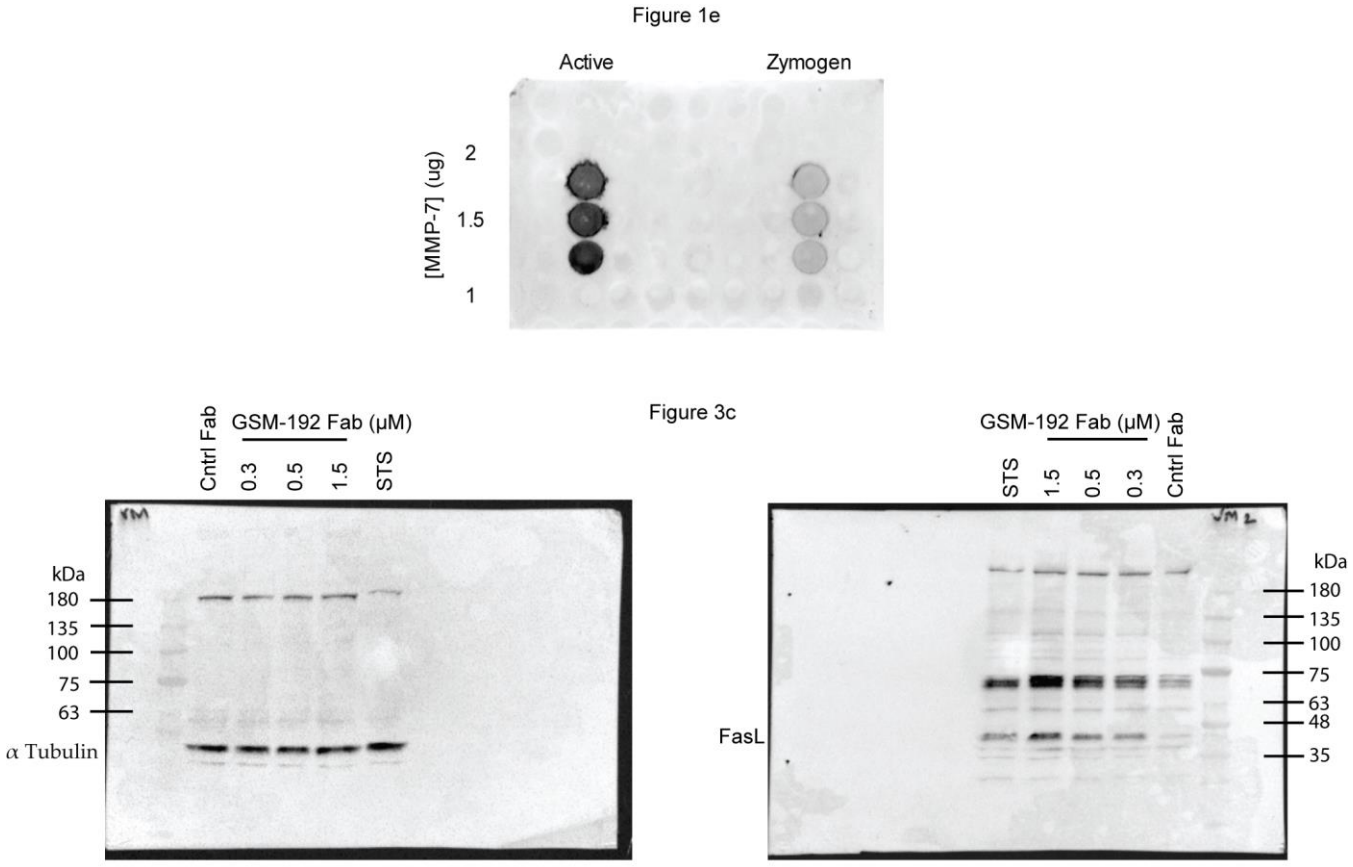

Figure S2. Original unedited western and dot blot images from Figures 1e, 3c.

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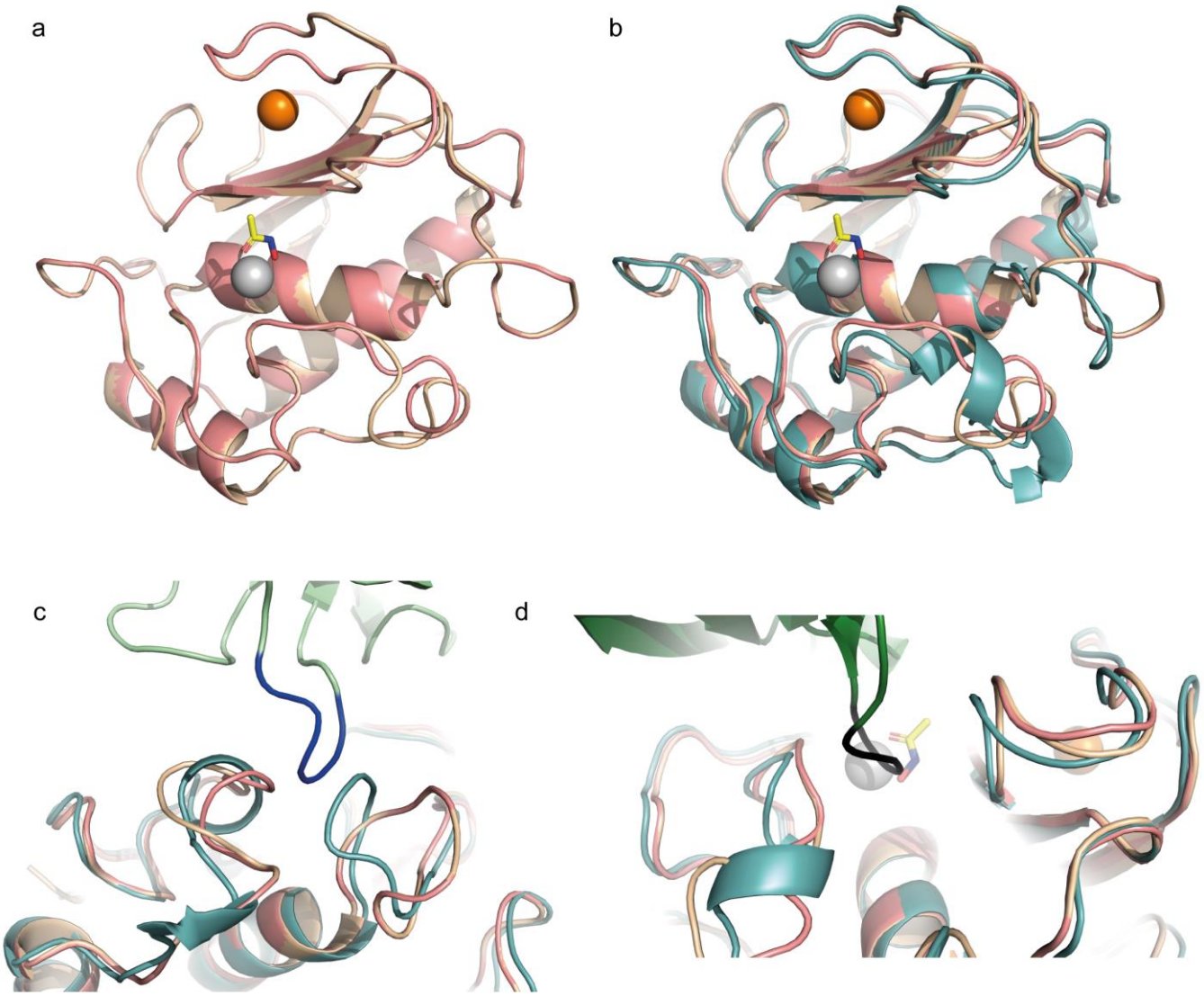

Figure S3. Superimposition of structures orthologs of MMP-7 orthologs with MMP-3 a closest human paralog. (a) The superimposition of orthologs shows a match in the catalytic cleft region. (b) The structures don't match in comparison between paralogs. hMMP7 – beige, mMMP7 – salmon, MMP3 – teal. (c,d) The catalytic cleft region is important for association with antigen recognizing CDRs CDR1L (blue) and CDR3H(black) of GSM-192.

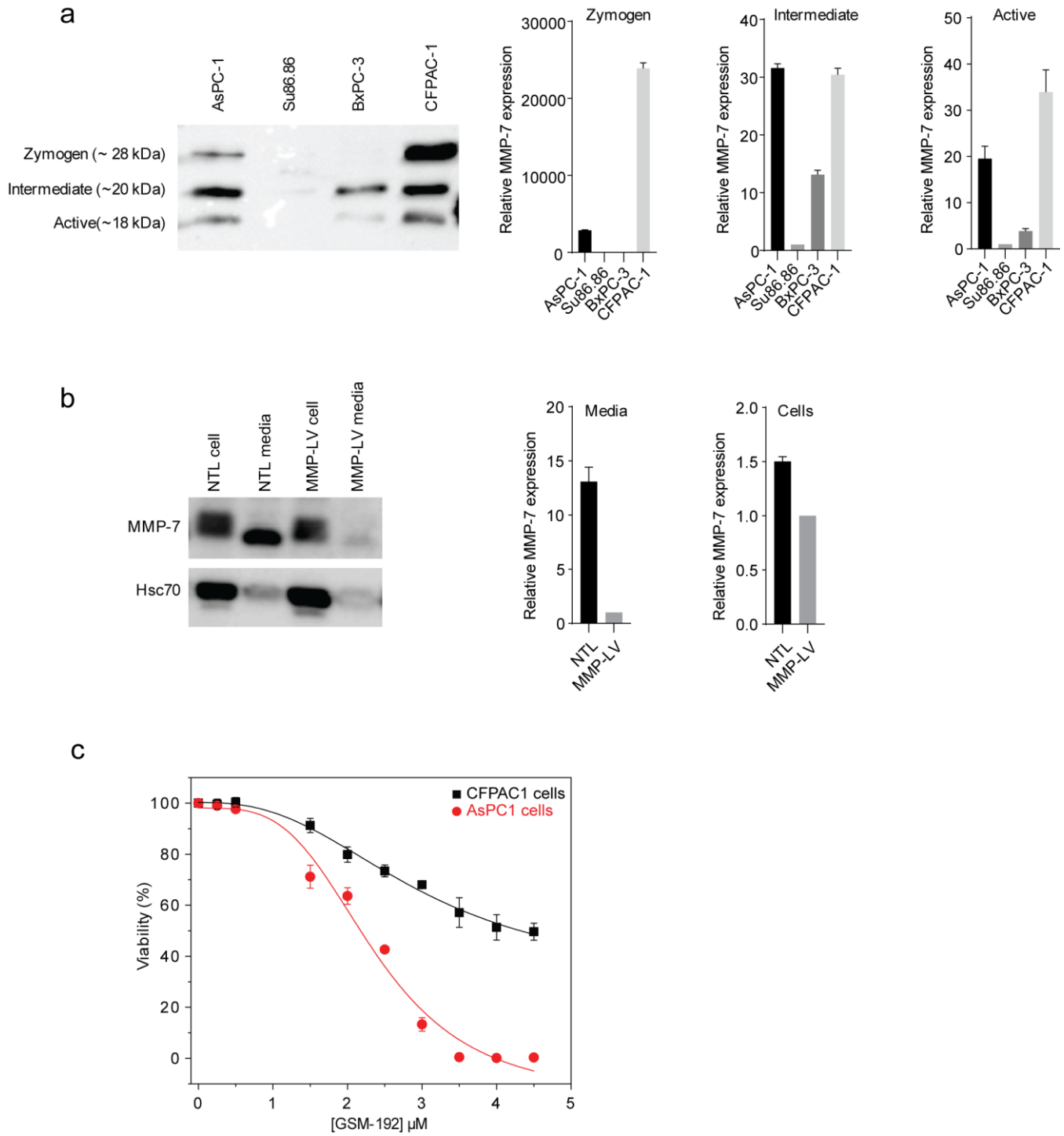

Figure S4. MMP-7 expression and cell survival post GSM-192 treatment. (a) Western blot analysis of cell culture media showing MMP-7 expression in various pancreatic cancer cell lines. Data in the graph represent relative mean densitometry values ± s.e.m. (**b**) Western blot confirming the knockdown efficacy of MMP-7 in lentiviral (LV) silencing. MMP-7 is efficiently knocked down in targeting LV when compared to non-targeting LV samples. Loading control is Hsc70. Data in the graph represent relative mean densitometry values ± s.e.m, cell fraction MMP-7 levels normalized to Hsc70. (**c**) The MTT cell survival assay was used to generate dose response curve-fitting analysis and IC_{50} values for GSM-192 treatment on AsPC-1 and CFPAC-1 pancreatic ductal adenocarcinoma cells were obtained. In this experiment a monoclonal antibody against LOXL-2 (Lysyl Oxidase like 2), was used as an isotype control. Data in the graph represent mean values ± s.e.m,.

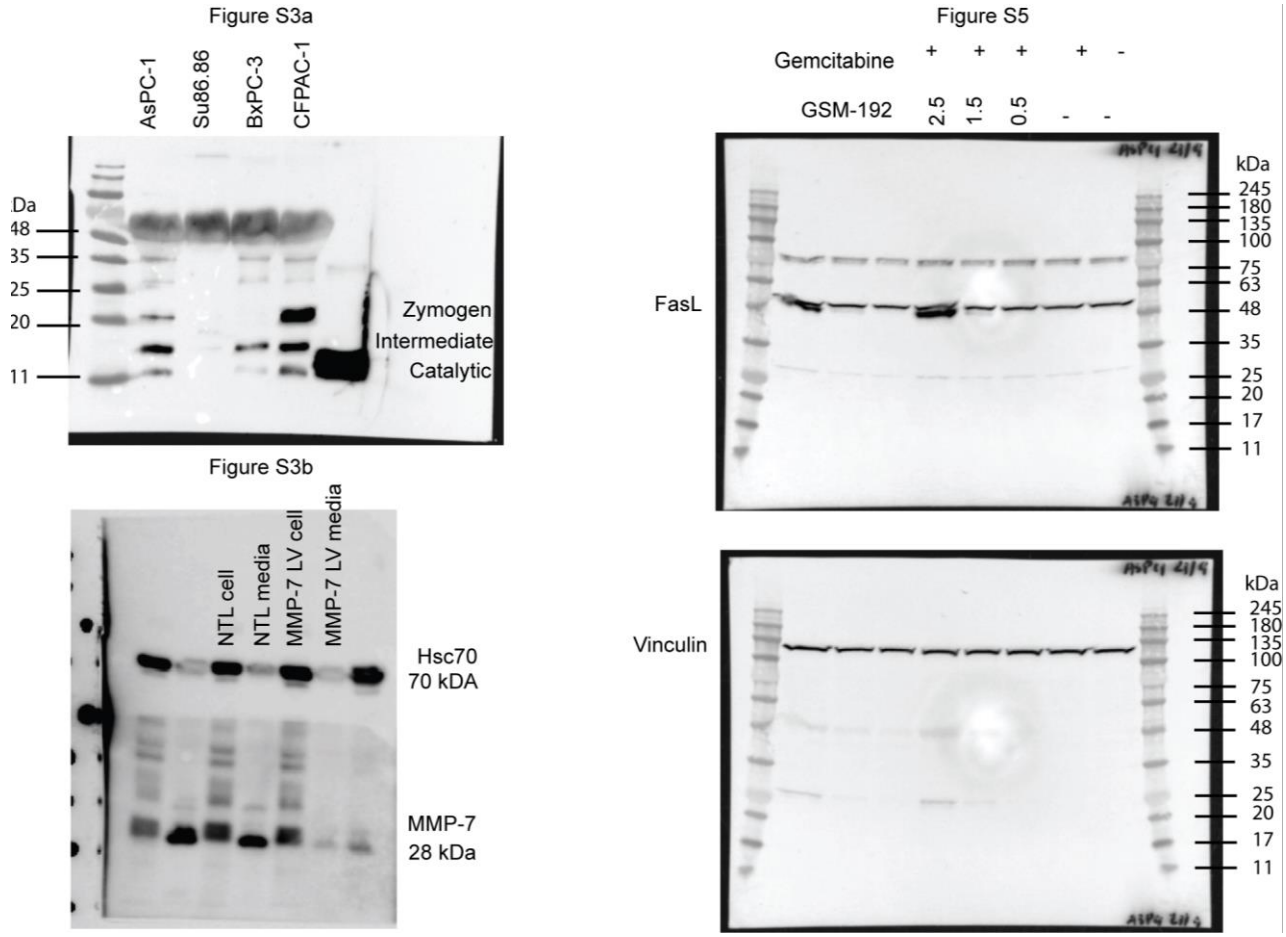

Figure S5. Original unedited western blot images from Figures S3a, S3b, S5.

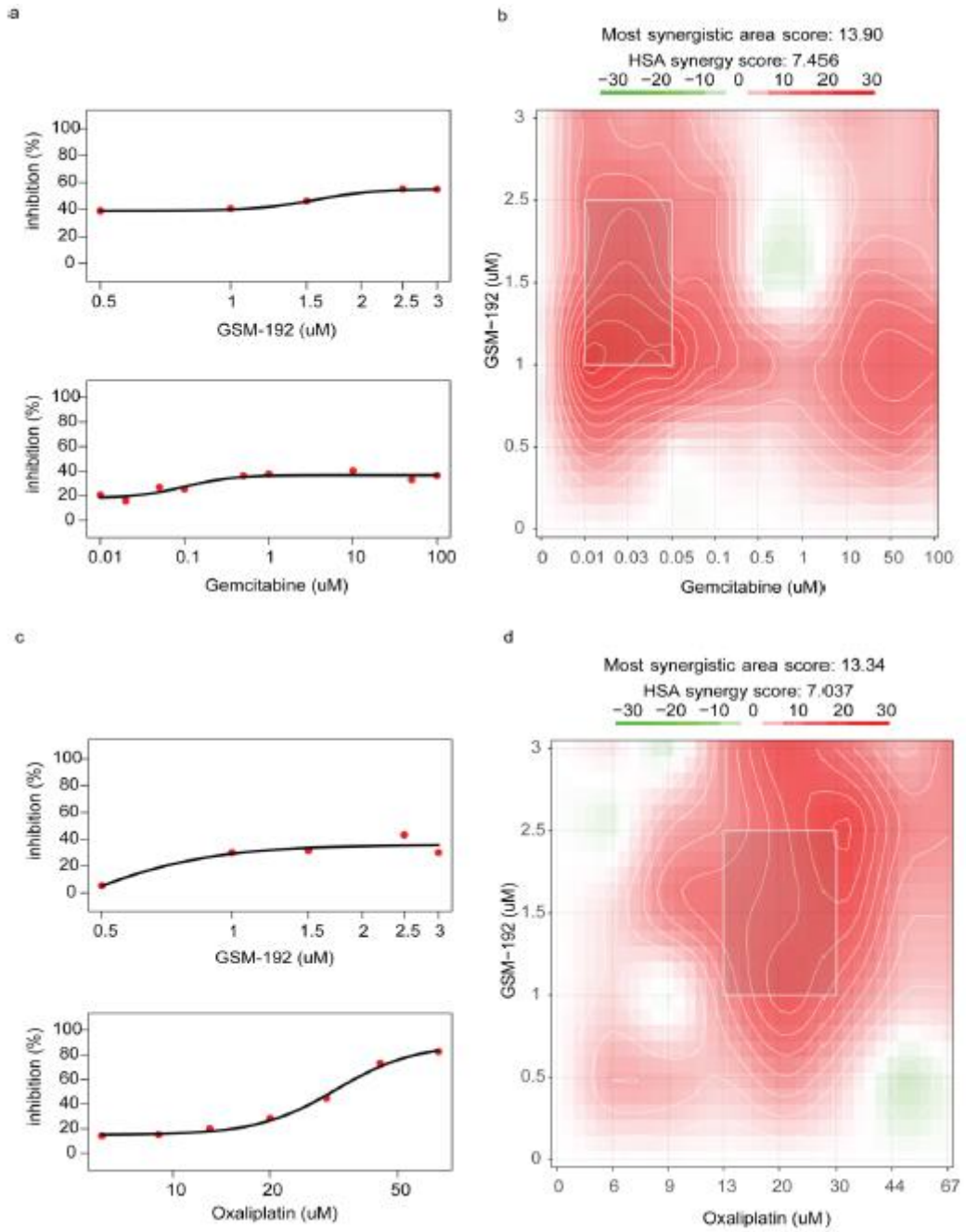

Figure S6. Visualization of synergy scores for drug and GSM-192 combination in AsPC1 cells. SynergyFinder stand-alone web-application for interactive analysis and visualization of multi-drug combination profiling data was used to generate individual dose response curve (**a,c**) and synergy map using highest single agent (HSA) reference model (**b,d**). (**a,b**) correspond to Gemcitabine, GSM-192 combination with a most synergistic areas score of 13.90 signifying a degree of combination synergy. (**d,e**) corresponds to Oxaliplatin, GSM-192 combination with a most synergistic areas score of 13.34 also signifying a degree of combination synergy.

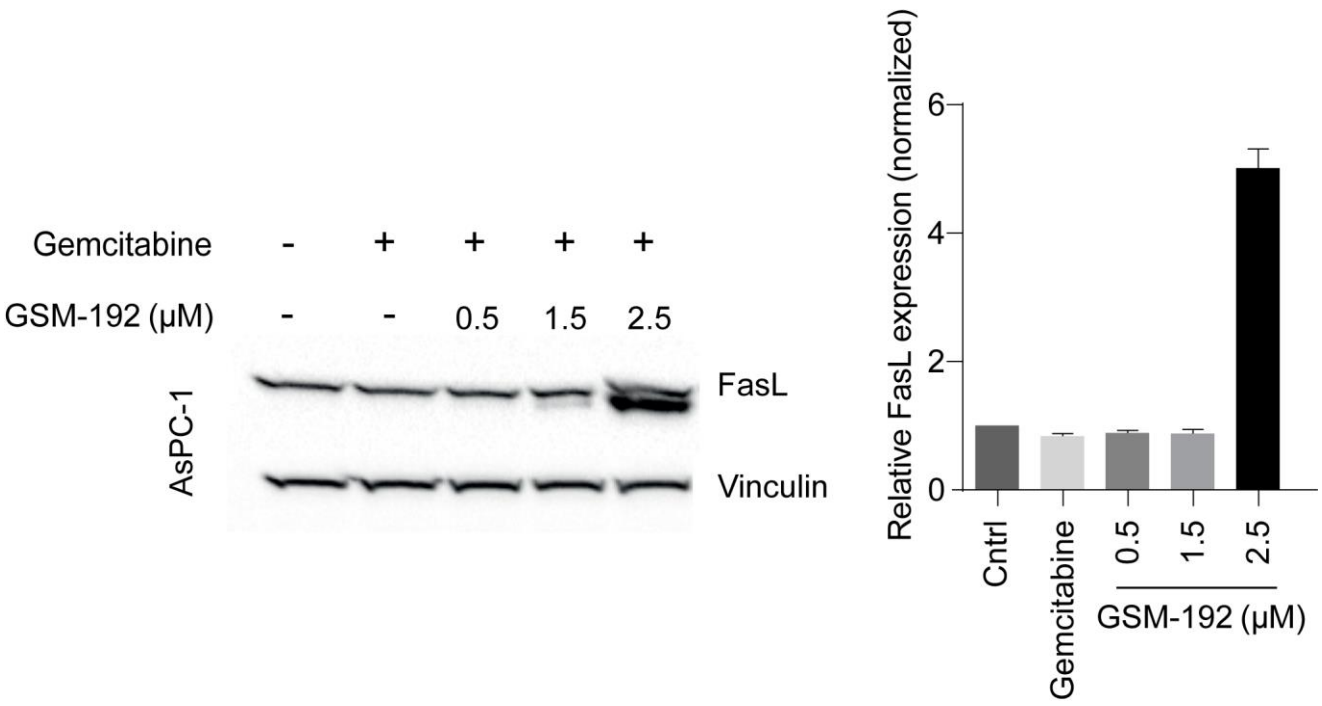

Figure S7. GSM-192 treatment raises FasL levels and synergizes with chemotherapeutic drug gemcitabine for enhanced cell kill. Western blot showing FasL levels in AsPC-1 cells. A concentration dependent increase in FasL levels in GSM-192 treated group enhances extrinsic pathway mediated cell kill in 200μm gemcitabine treated AsPC-1 cells. Gemcitabine treatment alone did not result in noticeable increase in FasL levels. Shown are representative results from 2 independent experiments. Data in the graph represent vinculin normalized relative mean densitometry values, which were further, normalized to the untreated control as baseline ± s.e.m.

Table S1. Data collection and crystallographic refinement statistics of GSM-192 Fab.

Data collection	
Resolution range (Å)	50.0–2.30 (2.34–2.30) [a]
Space group	$P3_121$
Unit cell dimensions	
a, *b*, *c* (Å)	88.44, 88.44, 119.34
g (°)	120
Number of molecules in the asymmetric unit	1
Number of reflections measured	870,771
Number of unique reflections	24,579(1,203) [a]
R_{sym}	0.085 (0.58) [a]
Completeness (%)	100.0 (100.0) [a]
Redundancy	12.0 (11.9) [a]
<I>/<s(I) >	33.0 (5.2)[a]
Refinement statistics	
Resolution (**Å**)	38.3–2.30
Rwork (%)	23.22
Rfree (%)	27.85

B-factor (Å²)		
Protein		37.7
RMSD from ideal geometry		
rmsd bond length (Å)	0.009	
rmsd bond angles (°)		1.2
Ramachandran plot (%)		
Most favored	85.8	
Additional favored		13.3
Generously allowed	0.0	
Disallowed regions		0.9

[a] Values in parentheses correspond to the highest-resolution shell.

Data S1: GSM-192 sequencing.

Immunoglobulin V region genes were cloned and sequenced after amplification by PCR and following sequence was obtained-

GSM-192 Light Chain Sequence

D I V T Q S P A S L A V S L G Q R A T I S C R A S E S F D S Y G N T F V H W Y Q Q K P G Q P P K L L I Y L V S N L E S G V P A G F R G R G S R T D F T L T I D P V E A D D A A T Y Y C Q Q N N E D P Y T F G G G T K L E I K R A

GSM-192 Heavy Chain Sequence

E V Q L Q Q S G P E L V K P G A S V K I P C K A S G Y T F T D Y N M D W M K Q S H G K S L E W I G H I N P N N G G T F Y N Q K F K D K A T F I V D K S S N T A Y M E L R S L T S E D T A V Y F C A R G G G L R R G P F A Y W G Q G T L V T V S