Stearoyl-CoA desaturase is a synthetic lethal target in SMAD4-deficient cancers
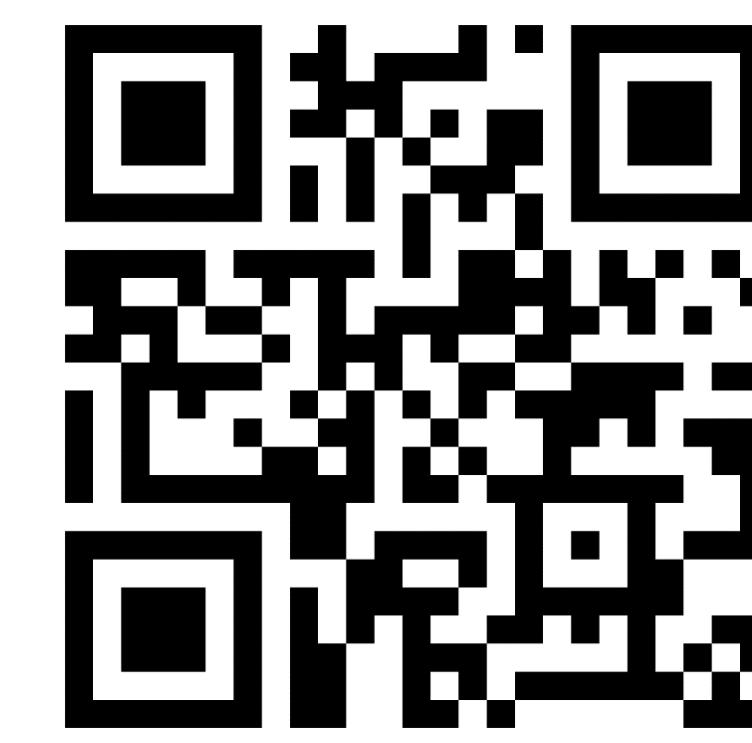
Abstract #558

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Introduction

Mothers against decapentaplegic homolog 4, or SMAD4, is a member of the SMAD family of transcription factors that mediate TGF-β signal transduction. SMAD4 loss of function mutation or deletion is found in about 30% of pancreatic ductal adenocarcinoma (PDAC) and 15% of colorectal adenocarcinoma and esophageal adenocarcinoma patients, and it is associated with their poor prognosis. Over the past two decades, its tumor suppressor role has been elucidated, and the loss of SMAD4 is sufficient to promote tumorigenesis in multiple GEM models. To identify novel therapeutic vulnerabilities for SMAD4-deficient cancers, a CRISPR dropout screening approach was employed in SMAD4 isogenic PDAC models. We identified stearoyl-CoA desaturase, SCD, as a synthetic lethal target in SMAD4-deficient context. SCD is critical for de novo lipid biogenesis and catalyzes the rate-limiting step in the production of monounsaturated fatty acid. Genetic and pharmacologic studies in vitro confirmed this synthetic lethal relationship. Additionally, drug-anchored CRISPR dropout screening and RNA expression profiling demonstrated that accumulation of saturated fatty acid in response to SCD inhibition drives cytotoxicity in SMAD4-deficient cells. Mouse studies with CRISPR-based knockout of SCD and a well-characterized SCD inhibitor (A939572) demonstrated anti-tumor efficacy in SMAD4-mutant xenograft models. However, compared to genetic knockout (KO) of SCD the pharmacological inhibitor was less effective at inhibiting tumor proliferation in vivo. Together, these data identify SCD as a selective vulnerability in SMAD4-mutant cancers.

## SMAD4 deficiency defines patients with unmet medical needs
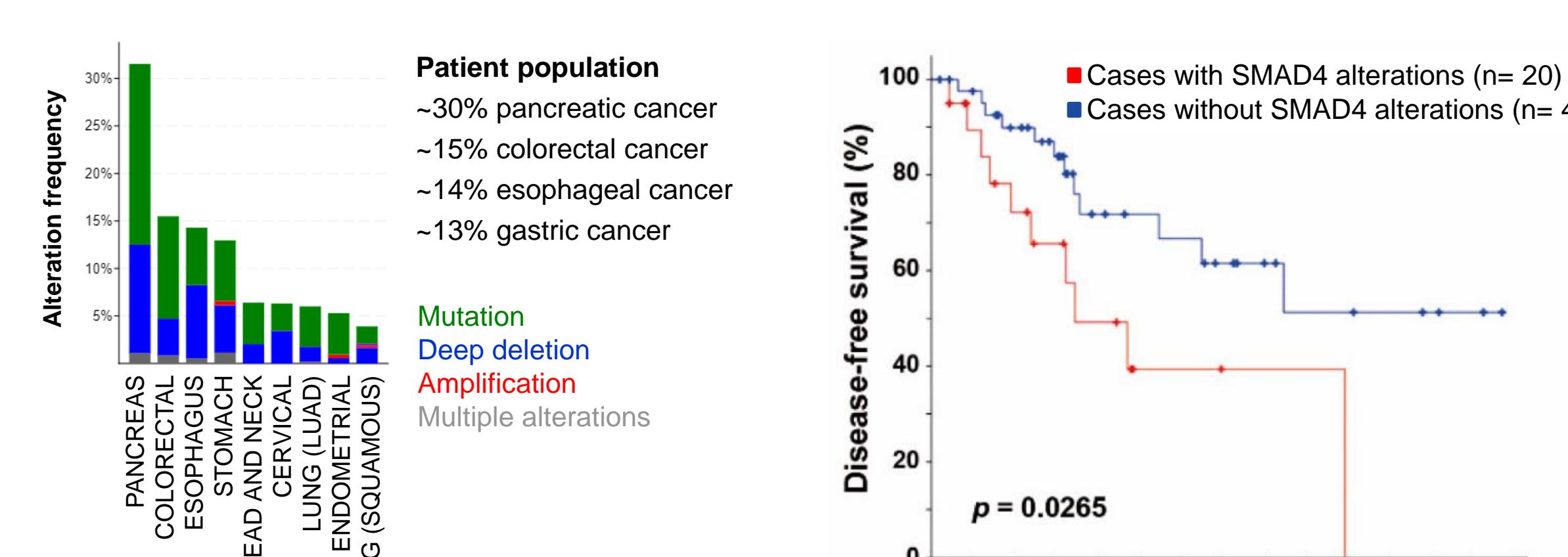
SMAD4/fatty acid biosynthesis synthetic lethality discovered through CRISPR screens


CFPAC-1 (SMAD4-null)
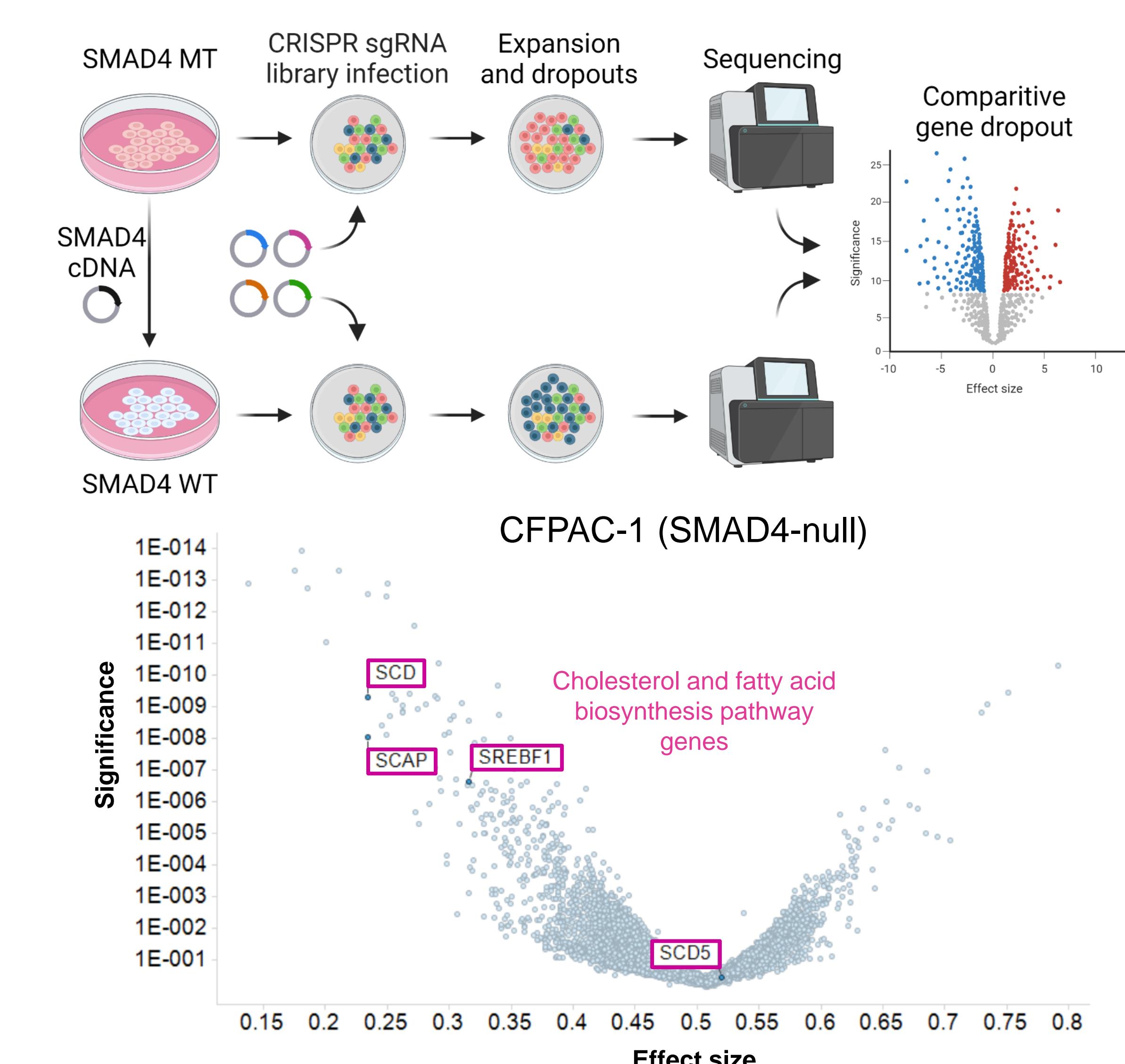
SMAD4/SCD synthetic lethal interaction validates in orthogonal datasets
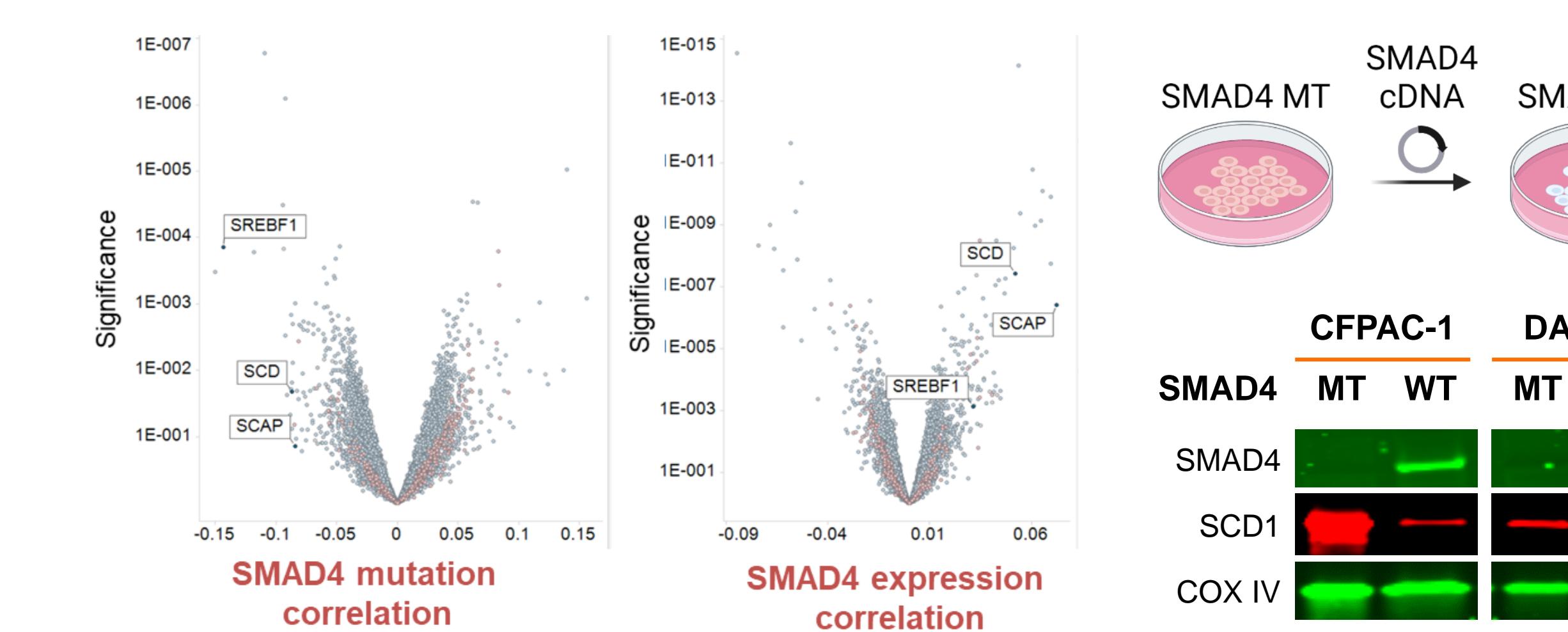
## Knockout of SCD is efficacious in vitro and in SMAD4-deficient pancreatic CDX model

SMAD4 mutant xenograft

- SCD WT (Cas9 clone)
- SCD WT (Clone 8)
- SCD KO (Clone 7)
- SCD KO (Clone 3)

Cell line: CFPAC-1 (SMAD4-null)
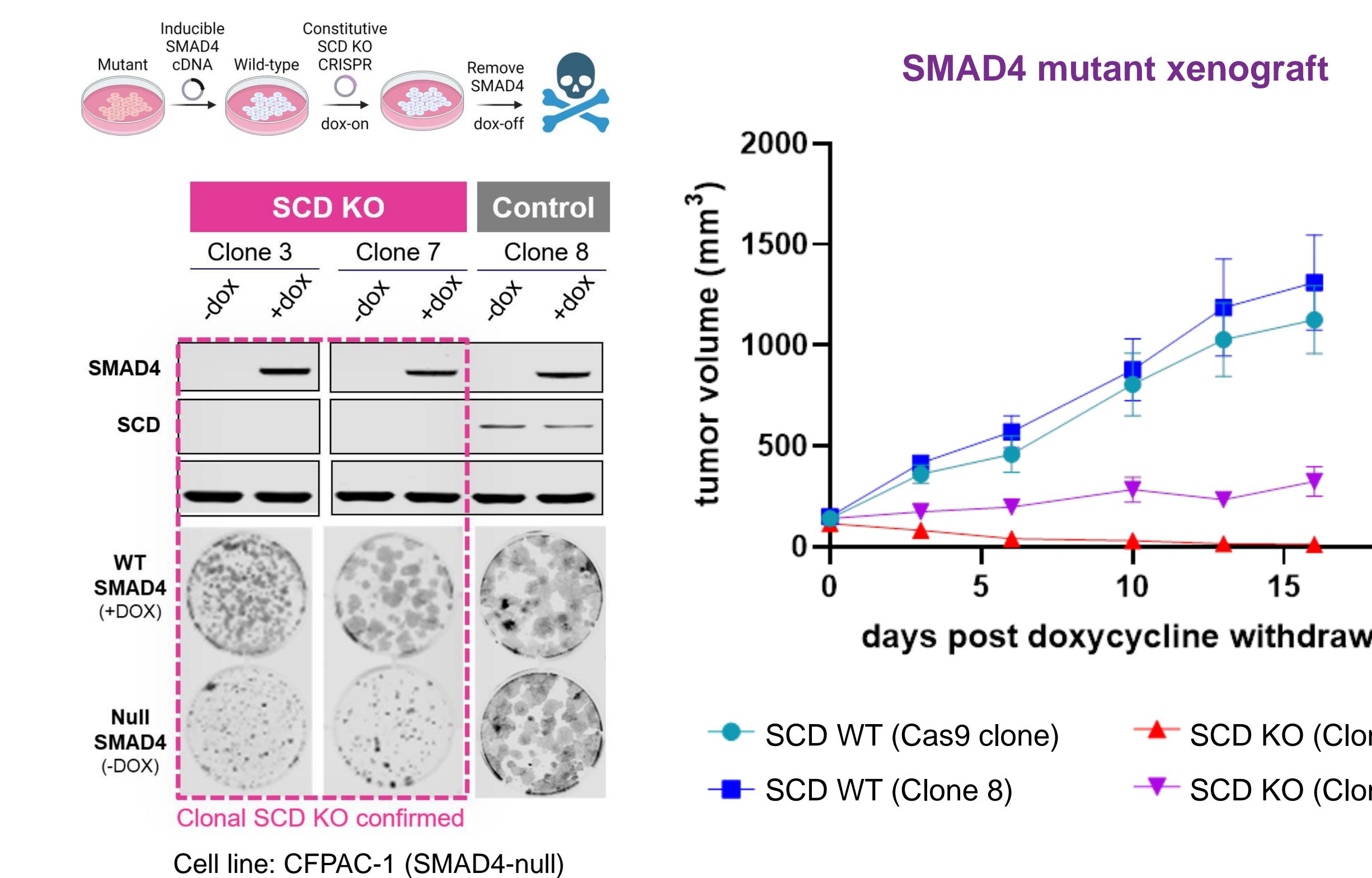
Pharmacological inhibition confirms SMAD4/SCD synthetic lethality

DAN-G isogenics — A939572

CFPAC-1 isogenics — MF-438
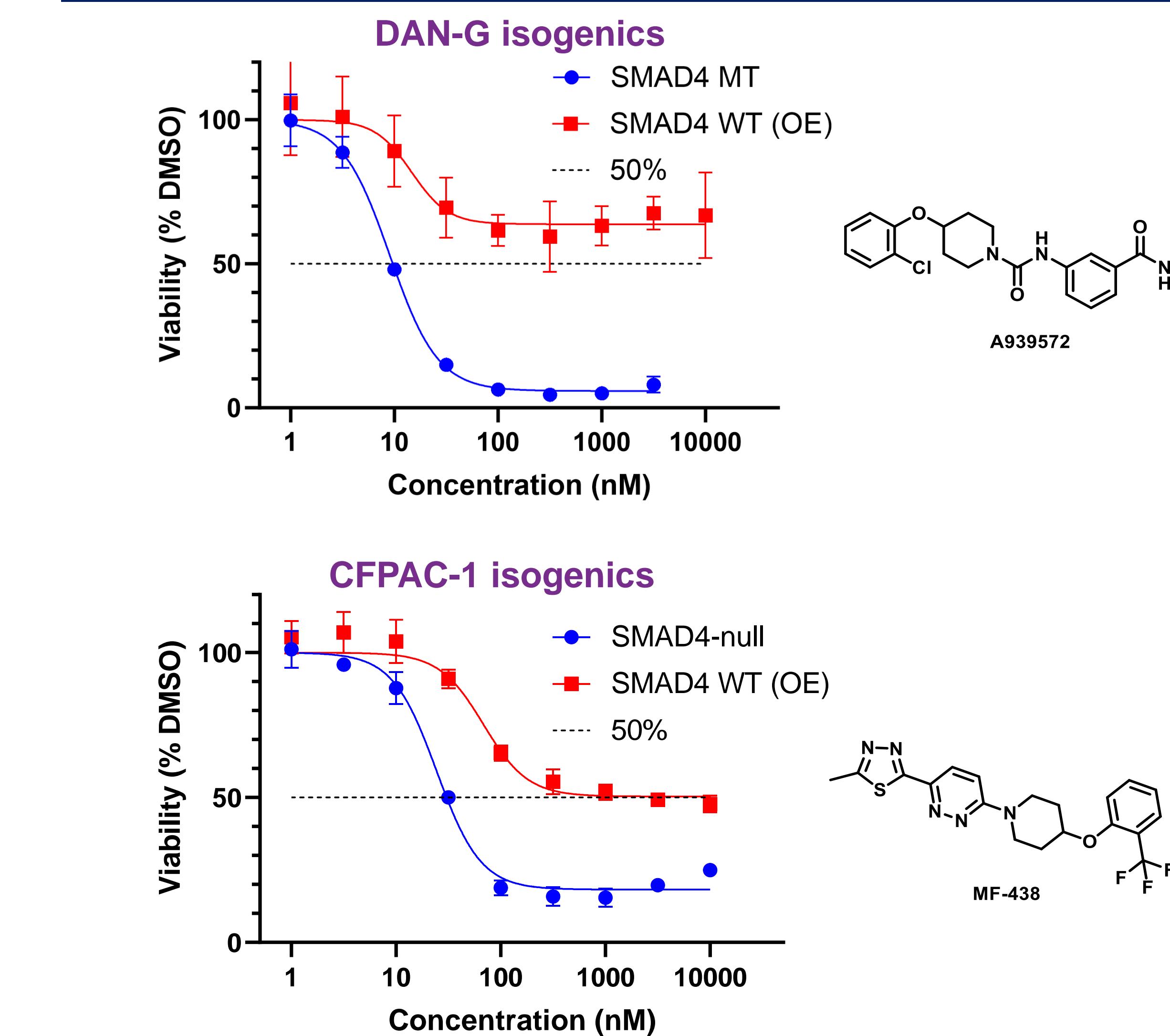
Cell panel profiling confirms synthetic lethal interaction

SCDi sensitivity (A939572) %GI, pancreatic, gastric, and colorectal

SMAD4 WT
SMAD4 MT/del/CN- or TGFBIIR MT

*** SMAD4 CN loss: KM12
*** SMAD4 WT + TGFBIIR MT: HCT-116 and DLD-1

| Pancreatic, gastric, colorectal	SMAD4 pathway status	
	wild-type	MT/del/CN loss or TGFBIIR MT
Sensitive	2	18
Insensitive	10	6
Total	12	24
% sensitive	16.7%	75.0%
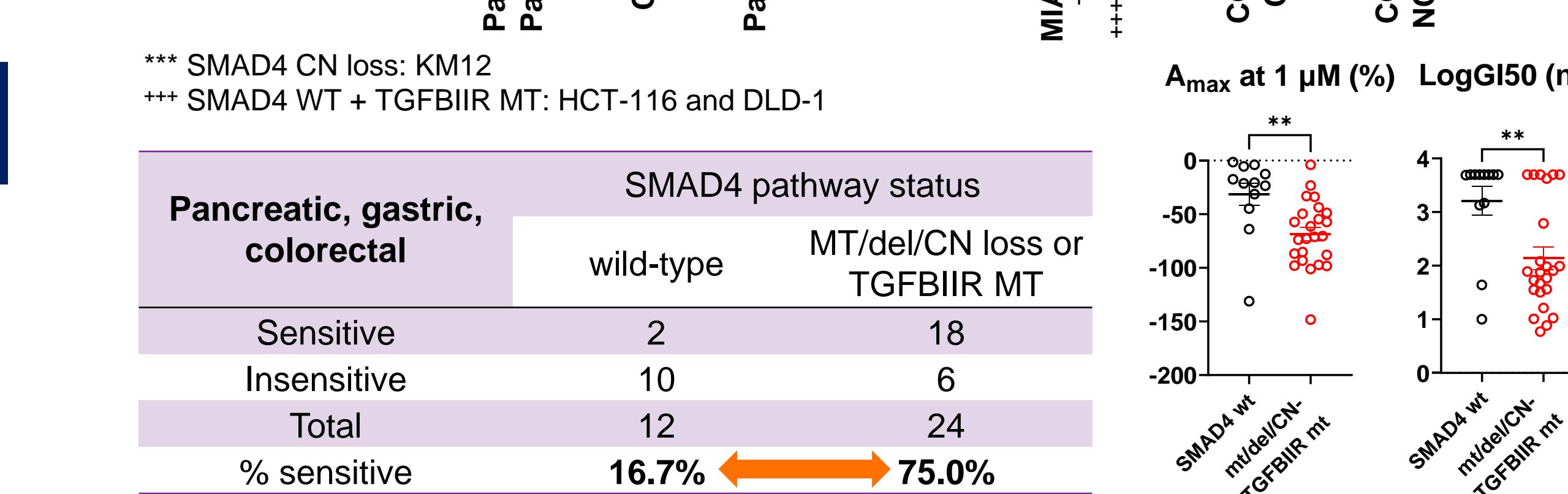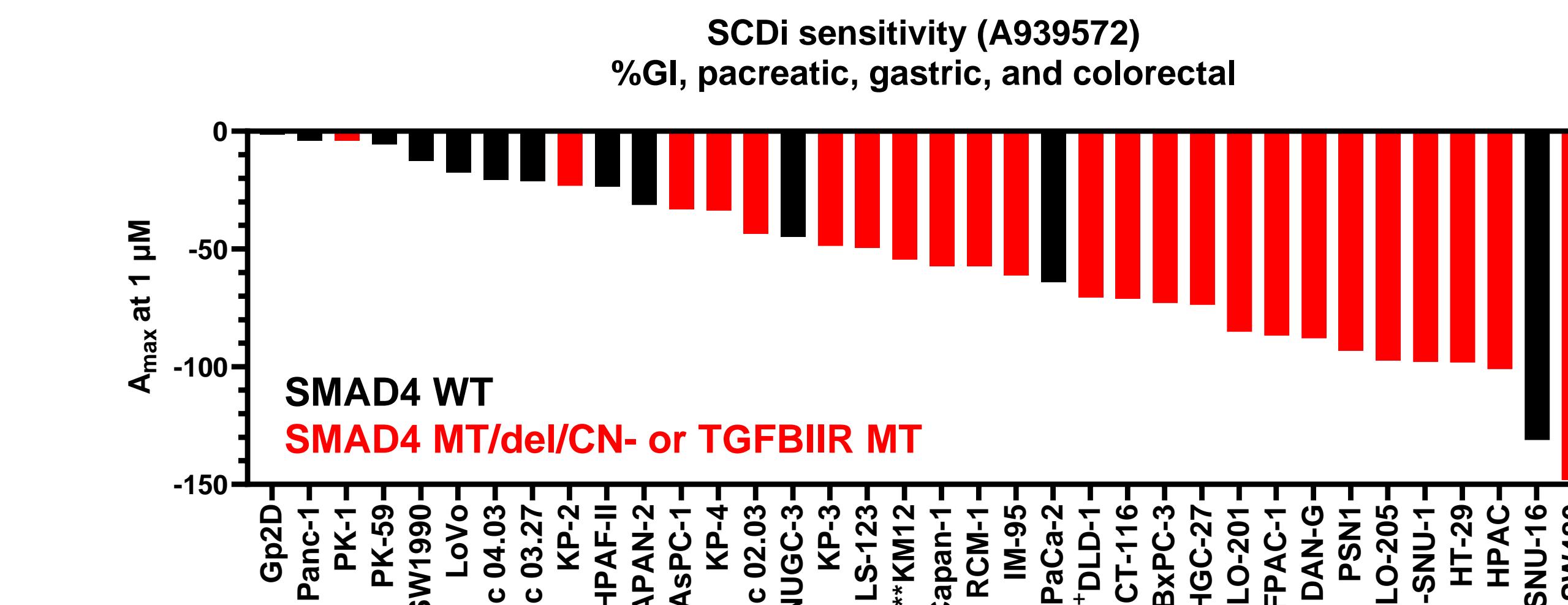
SCDi-anchored CRISPR screen and Nanostring profiling confirms dependency on fatty acid pathway activity

FA genes	Average theta	
FASN	0.83	Enriching genes (Rescuers)
ACACA	0.80	
ACLY	0.72	
LDLR	0.66	
ELOVL6	0.58	
LPIN3	0.55	
SCAP	0.54	
SCD	0.51	
ASCL4	0.49	
ASCL3	0.48	
LPIN2	0.46	
SREBF1	0.45	
GPX4	0.44	
SCD5	0.43	Depleting genes (Sensitizers)
LPIN1	0.38	
FADS2	0.34	

ACACA (ACC): acetyl-CoA carboxylase
FASN: fatty acid synthase

Nanostring Metabolic Pathways panel

Cell line: DAN-G
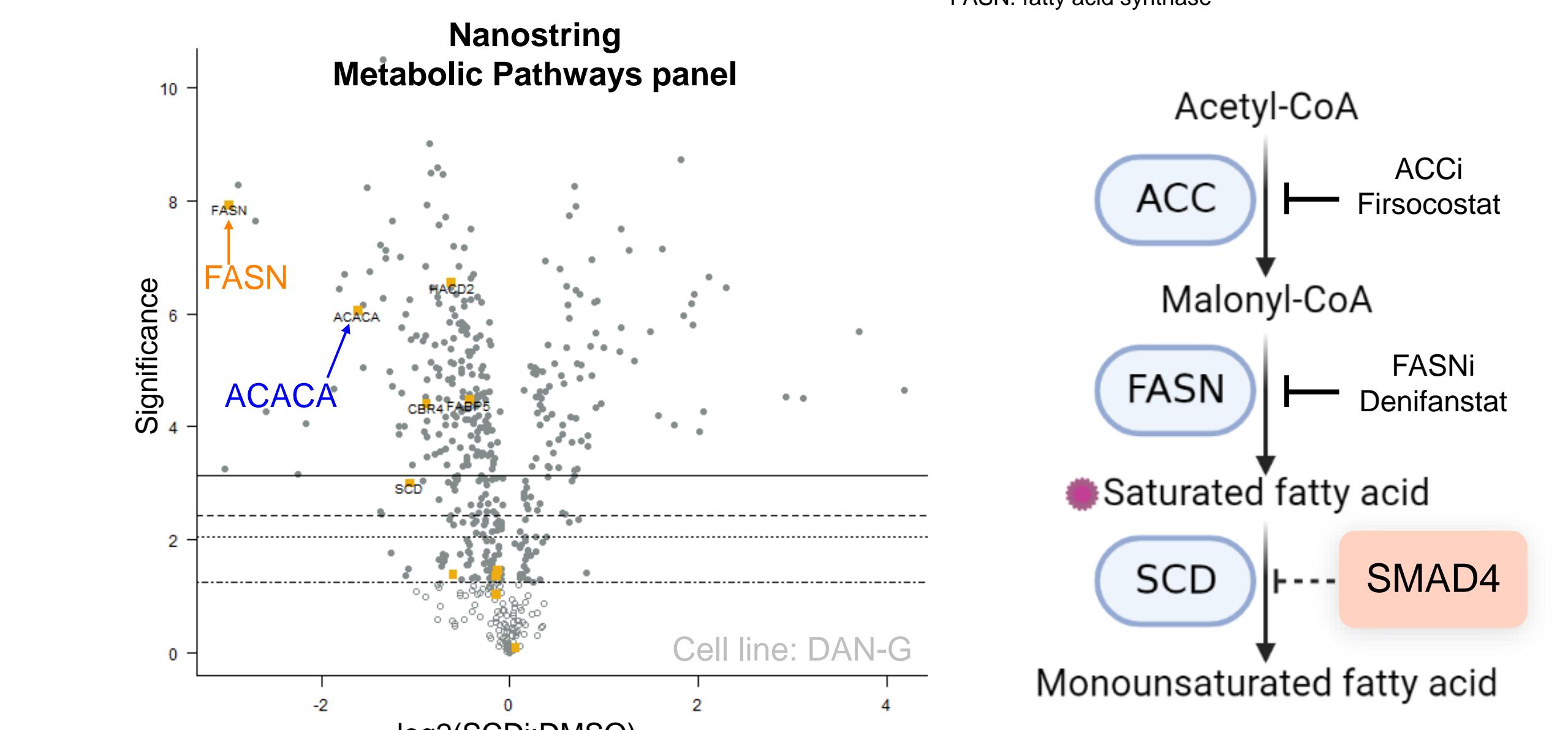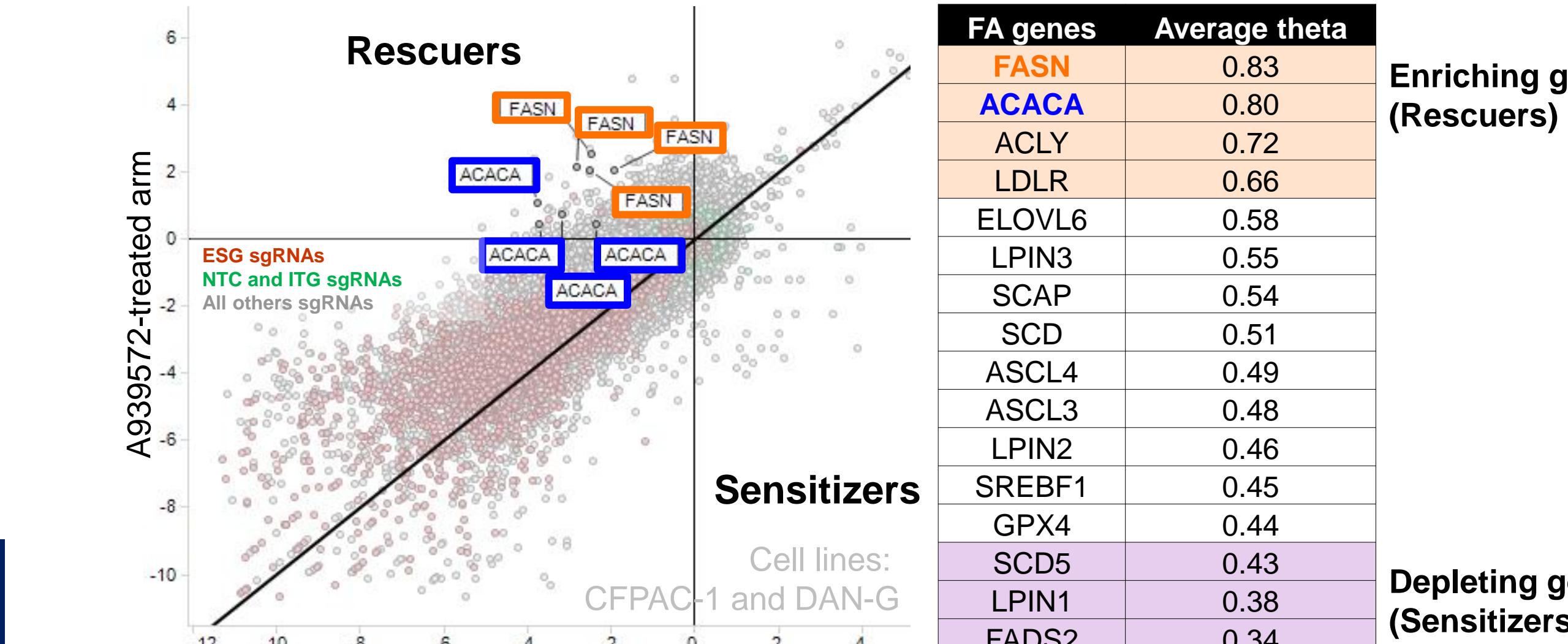
Fatty acid supplementation and inhibition of upstream genes demonstrate cytotoxicity of saturated fatty acid accumulation

Fatty acid supplementation
Palmitic acid (SFA) — Palmitoleic acid (MUFA)
+ vehicle, + 4μM FA, + 20μM FA, + 100μM FA

Co-treatment with upstream inhibitors
Firsocostat (ACCi) — Denifanstat (FASNi)
+ DMSO, + SCDi 4nM, + SCDi 20nM, + SCDi 180nM
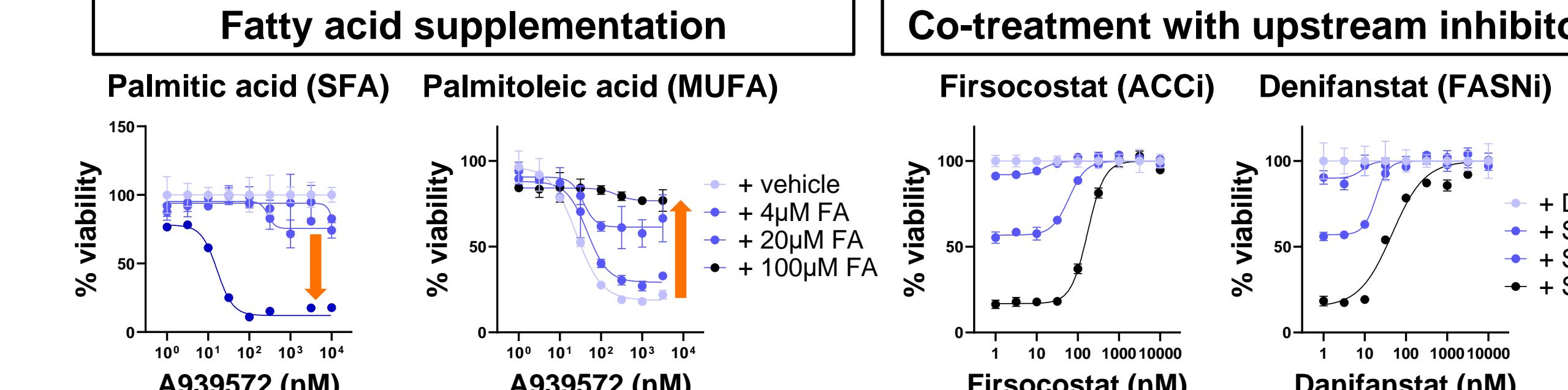
TNG-0770951 demonstrates dose-dependent tumor growth inhibition

BxPC-3 CDX model — A939572

- Vehicle BID
- 30 mpk BID
- 100 mpk BID

~60%TGI
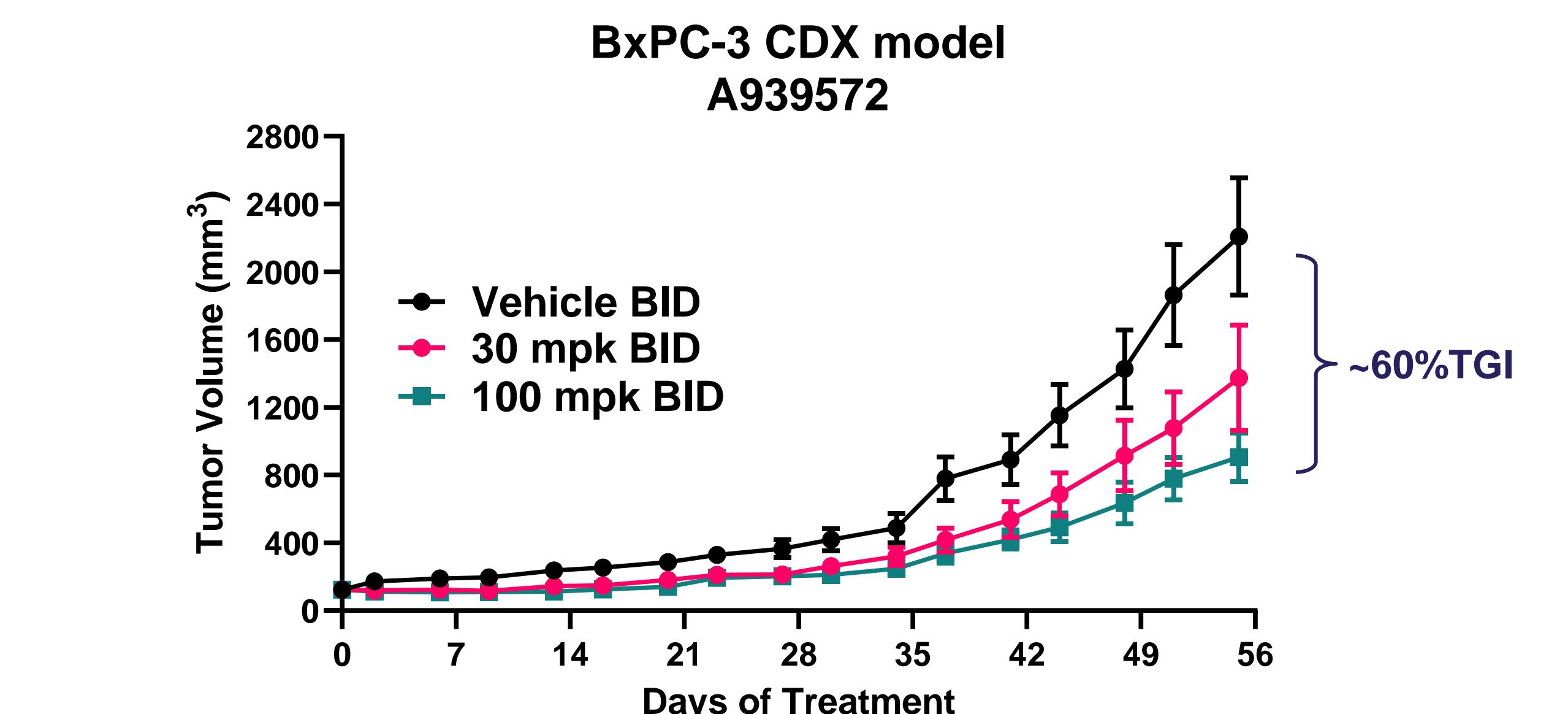
Target engagement and PD modulation were demonstrated in vivo with fatty acid desaturation and compensatory SCD induction

Terminal Serum PK — Serum FADI — SCD RNA expression by Nanostring — Tumor FADI
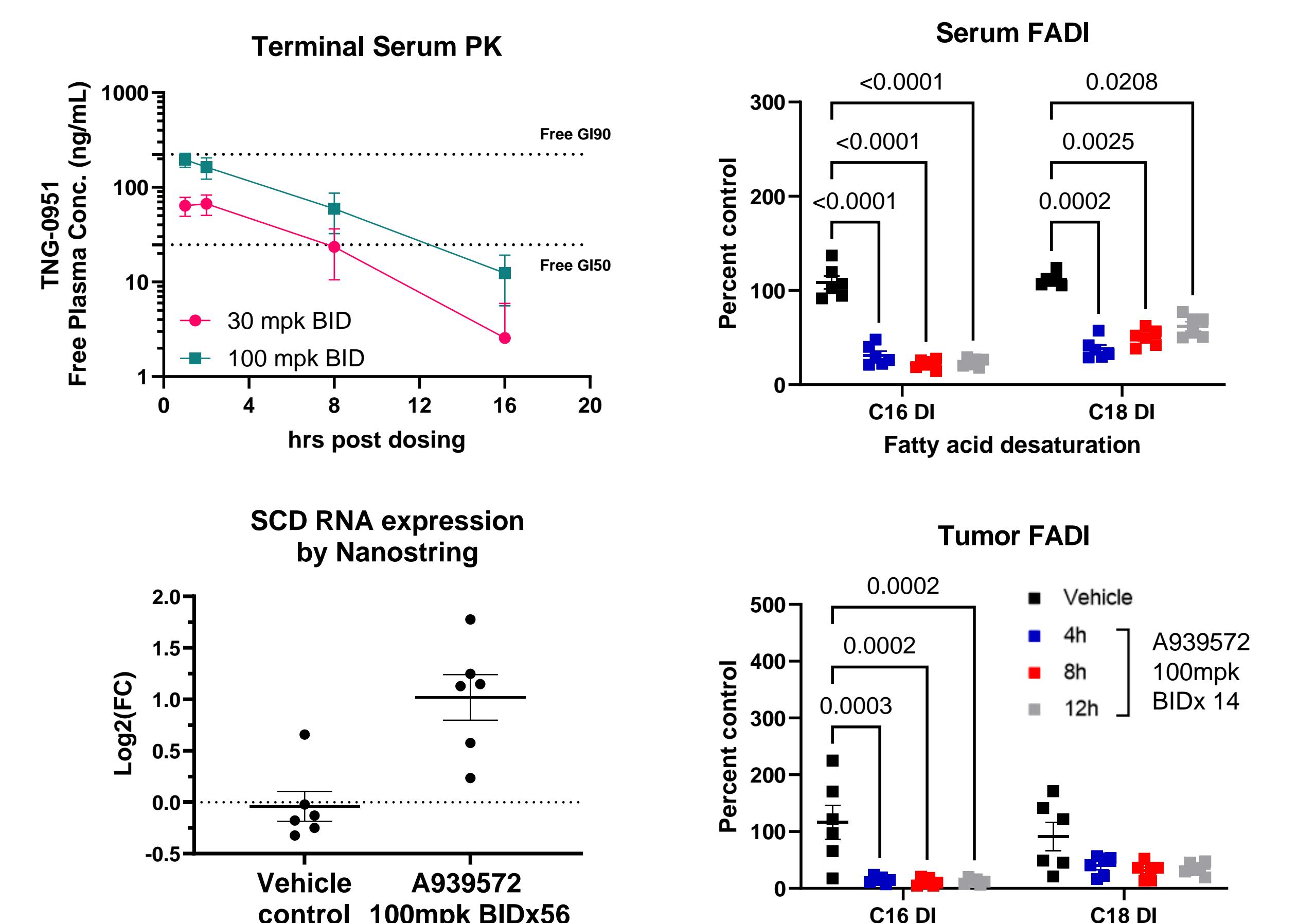
Summary

- SMAD4 is commonly deleted or mutated in large populations of solid tumors of pancreatic, gastric, and colorectal lineages
- Isogenic CRISPR dropout screens identified stearoyl-CoA desaturase as a potential target in SMAD4-deficient cancers
- Genetic and pharmacological studies in vitro validate the synthetic lethal interaction between SMAD4-deficiency and SCD
- Mouse xenograft studies demonstrated on-target efficacy of SCD inhibition
- However, the effect with pharmacological inhibitors was inferior compared to genetic knockout suggesting possible adaptation and resistance as indicated by upregulation of SCD

References

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