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**Xingxing Kong**

kongxingxing@fudan.edu.cn

Fudan university

**Jiyang Ding**

Fudan University

**Xianmin Wu**

Inner Mongolia University

**Yanjun Wu**

Fudan University

**Yonghao Feng**

Fudan University Jinshan Hospital

**Jinwei Feng**

Fudan University

**Ying Wang**

Inner Mongolia University

**Xinyi Zhang**

Fudan University

**Shuang Zhang**

Fudan University

**Hui Wang**

Fudan University

**Hongmei Yan**

Fudan University Zhongshan Hospital

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# Adipose myostatin mediates lipid signaling from obese adipose tissue to tumors

Jiyang Ding<sup>1, #</sup>, Xianmin Wu<sup>1, 2, #</sup>, Yanjun Wu<sup>3, #</sup>, Yonghao Feng<sup>4</sup>, Jinwei Feng<sup>1, 5</sup>, Ying Wang<sup>1, 2</sup>, Xinyi Zhang<sup>1, 5</sup>, Shuang Zhang<sup>1</sup>, Hui Wang<sup>1</sup>, Hongmei Yang<sup>6</sup>, Xingxing Kong<sup>1, 2, 3, 7</sup>

<sup>1</sup> State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, Shanghai 200438, China.

<sup>2</sup> Institutes of Biomedical Sciences, School of Life Sciences, Inner Mongolia University, Hohhot 010000, China.

<sup>3</sup> Shanghai Key Laboratory of Metabolic Remodeling and Health, Institute of Metabolism and Integrative Biology, Fudan University, Shanghai, 200438 China.

<sup>4</sup> Department of Endocrinology, Jinshan Hospital, Fudan University, Shanghai, 200438 China.

<sup>5</sup> Human Phenome Institute, Fudan University, Shanghai, 200438 China.

<sup>6</sup> Department of Endocrinology and Metabolism, Zhongshan Hospital, Fudan University, Shanghai, 200438 China.

<sup>7</sup> Department of Endocrinology, Huadong Hospital, Fudan University, Shanghai, 200438 China.

# These authors contributed equally.

## Abstract

Obesity promotes cancer progression, but how excess adiposity is molecularly coupled to tumor growth remains poorly understood. Here, we identify adipose myostatin (MSTN) as a regulator of obesity-associated tumor progression. Adipocyte-specific, but not muscle-specific, deletion of *Mstn* suppressed tumor growth in obese mice, revealing a previously unrecognized role for adipose MSTN in tumor-host communication. Mechanistically, intracellular full-length MSTN activated SMAD2/3-dependent *Scd1* expression in adipocytes, thereby increasing production of the lipokine palmitoleic acid (POA). Circulating POA directly engaged pyruvate kinase M2 (PKM2) in tumor cells, promoting PKM2 dimerization and nuclear accumulation and activating tumor-promoting transcriptional programs. Restoring POA reversed the tumor-suppressive effect of adipocyte *Mstn* deletion *in vivo*. Together, these findings reveal an adipose MSTN-SCD1-POA-PKM2 signaling circuit that links obesity-associated adipose dysfunction to tumor-cell signaling and progression, establishing systemic lipid communication as a mechanism by which excess adiposity promotes cancer.

## Introduction

Increasing evidence indicates that obesity promotes tumor progression not only through systemic metabolic dysfunction but also through active communication between adipose tissue and tumors<sup>1</sup>. As a major endocrine and metabolic organ, adipose tissue releases fatty acids, adipokines and other signaling molecules into the circulation, and obesity profoundly remodels this secretory landscape<sup>2-4</sup>. Adipocyte-derived lipids can serve as metabolic substrates or signaling molecules for tumor cells, highlighting adipose tissue as an important regulator of tumor biology<sup>2,3</sup>. Despite these observations, the adipose-intrinsic mechanisms that determine which lipid signals are produced and how they are transmitted to tumors remain poorly understood.

Myostatin (MSTN, GDF8) is a transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily member best known as a potent negative regulator of skeletal muscle growth and systemic metabolism<sup>5</sup>. This muscle-centric view of MSTN biology is being revisited as pharmacological inhibition of the MSTN pathway emerges as a strategy to improve body composition and preserve lean mass during weight loss. Although MSTN is also expressed in adipose tissue<sup>1,4,5</sup>, its cell-intrinsic functions in adipocytes remain poorly defined. Moreover, MSTN biology may extend beyond its canonical secreted C-terminal ligand, as intracellular or incompletely processed MSTN can retain biological activity<sup>2</sup>. Whether intracellular MSTN regulates adipocyte metabolism and thereby influences signals communicated to distant tissues is unknown.

Among adipose-derived metabolic signals, palmitoleic acid (POA, C16:1n-7), generated by stearoyl-CoA desaturase 1 (SCD1), is an adipose-derived lipid with established metabolic and signaling functions<sup>3,6</sup>. Circulating POA levels are altered in obesity and metabolic dysfunction and have been associated with tumor progression in experimental models and human studies<sup>7-10</sup>. Yet whether POA can directly engage intracellular proteins to alter tumor-cell signaling remains unclear. Pyruvate kinase M2 (PKM2) is a particularly relevant candidate, as its oligomeric state and nuclear localization couple metabolic signals to oncogenic transcriptional programs<sup>11-15</sup>. Whether an adipose-derived lipid such as POA can directly regulate PKM2 to transmit metabolic information from adipose tissue to tumor cells remains unknown.

Here, we investigated whether MSTN exerts tissue-specific functions that regulate metabolic communication beyond skeletal muscle. We found that adipocyte-specific, but not muscle-specific, deletion of *Mstn* suppressed obesity-associated tumor growth, revealing an unexpected adipose function of MSTN in tumor progression. Mechanistically, intracellular full-length MSTN activated a noncanonical SMAD2/3-SCD1 pathway in adipocytes to increase POA biosynthesis. Adipose-derived POA accumulated in tumors and directly engaged PKM2, promoting its dimerization and nuclear translocation and activating transcriptional programs that support tumor progression. Restoration of POA levels rescued tumor growth in adipose *Mstn*-deficient mice, establishing POA as a functional mediator of adipose-tumor communication. Together, these findings expand MSTN biology beyond its canonical muscle-centric function and identify an adipose MSTN-POA-PKM2 axis that links a tissue-specific protein signal into a circulating lipid signal to regulate tumor-cell state.

## Results

### Adipose, but not muscle, MSTN promotes obesity-associated tumor progression

Myostatin (MSTN) is best known as a muscle-derived regulator of skeletal muscle growth, but whether its functions in obesity extend beyond skeletal muscle remains poorly understood. Because global *Mstn* deletion has been reported to suppress tumor growth<sup>16</sup>, we first asked whether muscle-derived MSTN accounts for this effect. Given that skeletal muscle is the predominant source of circulating MSTN *in vivo*<sup>5,17</sup>, we generated skeletal muscle-specific *Mstn* knockout (MKO) mice by crossing *Myf1-Cre* with *Mstn*-flox mice (Extended Data Fig.S1a,b). Under normal chow conditions, MKO mice phenocopied the tumor-suppressive effect observed in global *Mstn* knockout mice<sup>16</sup>, exhibiting reduced MC38 tumor growth compared with control mice (Extended Data Fig.1c,d). Strikingly, this effect was lost under obesity. Following high-fat diet (HFD) feeding, MC38 tumors exhibited comparable growth kinetics in MKO and control mice, with no significant differences in tumor volume, tumor burden, or tumor-free body weight (Extended Data Fig.1e-h). Thus, despite being the predominant source of circulating MSTN, skeletal muscle-derived MSTN is dispensable for tumor progression in the obese state, suggesting that obesity may engage a distinct tissue source or mode of MSTN action.

We therefore investigated whether another MSTN-producing tissue becomes functionally relevant in obesity. Adipose tissue expresses MSTN<sup>1,4,18</sup>, and analysis of human samples revealed increased *MSTN* mRNA and protein abundance in adipose tissue from patients with cancer (Fig.1a,b). We next generated adipocyte-specific *Mstn* knockout (AKO) mice by crossing *Adipoq-Cre* with *Mstn*-flox mice (Fig.1c and Extended Data Fig.2a-c). Unlike muscle-specific deletion, adipocyte-specific *Mstn* deletion had little effect on tumor progression under normal chow conditions. MC38 colorectal and Panc02 pancreatic tumors grew similarly in AKO and control mice (Extended Data Fig.2d-j). In contrast, after HFD feeding, adipocyte *Mstn* deletion markedly restrained tumor progression. In both tumor models, AKO mice displayed significantly reduced tumor growth and improved survival compared with floxed controls (Fig.1d-j). AKO tumors showed reduced Ki67-positive tumor-cell proliferation and CD31-positive vessel density (Fig.1k-p).

This phenotype was maintained in an orthotopic model of pancreatic cancer. In HFD-fed mice, adipocyte *Mstn* deletion suppressed orthotopic Panc02 tumor growth and prolonged survival, while substantially reducing hepatic invasion and metastatic dissemination (Fig.1q-x). These effects occurred without differences in tumor-free body weight. Together, these findings reveal a previously unrecognized tissue-specific function of MSTN in obesity: whereas skeletal muscle-derived MSTN is dispensable for tumor progression in the obese state, adipose-derived MSTN becomes a critical driver of tumor growth and metastatic behavior.

### Adipose MSTN controls tumor progression through a circulating lipokine

The absence of an obesity-associated tumor phenotype in MKO mice, together with the lack of an effect of adipocyte *Mstn* deletion on circulating MSTN concentrations (Extended Data Fig.2k), suggested that adipose MSTN may regulate tumor progression indirectly by altering the metabolic output of adipose tissue. To address this possibility, we established a conditioned-medium system. Primary adipocytes isolated from control and AKO mice were exposed to palmitate (PA), and conditioned media were subsequently applied to Panc02 or

MC38 cells (Fig.2a). Conditioned medium from PA-treated Flox adipocytes markedly enhanced tumor cell proliferation and scratch assay, whereas these tumor-promoting effects were attenuated in conditioned medium derived from AKO adipocytes (Fig.2b-d and Extended Data Fig.3a-c). In contrast, direct exposure of tumor cells to equivalent concentrations of PA did not produce genotype-dependent effects, suggesting that adipose MSTN regulates a secondary adipocyte-derived signal rather than the response to PA itself.

To identify this signal, we performed plasma metabolomic profiling of tumor-bearing control and AKO mice. Principal-component analysis revealed a distinct metabolic signature associated with adipocyte *Mstn* deletion (Fig.2e), with pathway analysis highlighting broad alterations in lipid metabolism (Fig.2f). Among the altered metabolites, palmitoleic acid (POA; C16:1n7) was consistently reduced in AKO mice (Fig.2g,h and Extended Data Fig.3d). Circulating POA was unchanged in MKO mice (Extended Data Fig.3e), paralleling the absence of an obesity-associated tumor phenotype in these animals. Targeted LC-MS/MS independently confirmed reduced circulating POA in both subcutaneous and orthotopic pancreatic tumor models following adipocyte *Mstn* deletion (Fig.2i,j). POA was also reduced in white adipose depots from AKO mice (Fig.2k,l). Thus, adipose MSTN controls the systemic availability of POA, identifying this lipid as a candidate mediator through which adipose tissue communicates with tumors.

### **Intracellular MSTN couples adipose signaling to SCD1-dependent POA biosynthesis**

We next asked how adipose MSTN controls POA production. Transcriptomic profiling of iWAT and eWAT from tumor-bearing control and AKO mice revealed extensive remodeling of adipose gene expression following *Mstn* deletion (Fig.3a,b and Extended Data Fig.4a,b). Among the most strongly suppressed pathways was unsaturated fatty-acid biosynthesis (Fig.3c and Extended Data Fig.4c). *Scd1*, which encodes the rate-limiting desaturase responsible for converting palmitate into palmitoleic acid<sup>19,20</sup>, was downregulated in *Mstn*-deleted adipose tissue (Fig.3d,e and Extended Data Fig.4d-g). In contrast, SCD1 expression was unchanged in skeletal muscle and tumor tissues (Extended Data Fig.4h-m) and was similarly unaffected in MKO mice (Extended Data Fig.4n). Consistent with these *in vivo* findings, ablation of *Mstn* in primary adipocytes markedly reduced SCD1 expression at both the mRNA and protein levels (Fig.3f,g). Conversely SCD1 expression was elevated in adipose tissue from cancer patients and in independent public datasets (Fig.3h,i and Extended Data Fig.4o,p). These findings identify SCD1 as a candidate metabolic effector linking adipose MSTN to POA biosynthesis.

MSTN is generally viewed as a secreted TGF- $\beta$  family ligand whose mature C-terminal dimer activates activin type II receptors and downstream SMAD2/3 signaling. We therefore asked whether canonical extracellular MSTN signaling was required for the regulation of *Scd1*. Cleavage-resistant MSTN variants carrying a BMP1/Tolloid cleavage-site mutation (MSTN<sup>D100A</sup>)<sup>21,22</sup> or combined BMP1/Tolloid and furin cleavage defects (MSTN<sup>Dmut</sup>) remained capable of activating SMAD2/3 and a TGF- $\beta$ -responsive CAGA reporter<sup>23</sup> despite predominantly retaining the full-length form (Fig.3j and Extended Data Fig.5a-e). Moreover, an N-terminal signal-peptide deletion mutant (MSTN <sup>$\Delta$ 25AA</sup>), which remained intracellular and was undetectable in conditioned medium, retained the ability to activate the *Scd1* promoter (Fig.3k). Both wild-type and cleavage-resistant MSTN activated the *Scd1* promoter, whereas conditioned medium from MSTN-expressing cells failed to reproduce this activity (Fig.3l,m). Pharmacological inhibition of ALK2/ALK3 signaling (LDN193189)<sup>24</sup> likewise failed to block

MSTN-dependent *Scd1* activation (Fig.3n). Together, these experiments indicate that extracellular, canonically processed MSTN is neither necessary nor sufficient for this adipocyte response.

We next defined the downstream transcriptional mechanism. Inhibition of SMAD2/3 signaling with TP-HCl reduced *Scd1* promoter activity and SCD1 protein abundance (Fig.3o and Extended Data Fig.5f). Promoter truncation and mutagenesis identified a SMAD-responsive element between –309 and –298 bp of the *Scd1* promoter that was required for MSTN-dependent transactivation (Fig.3p-r and Extended Data Fig.5g,h). Thus, intracellular full-length MSTN engages a SMAD2/3-dependent transcriptional program to increase SCD1 expression and POA biosynthesis in adipocytes.

### **Palmitoleic acid directly engages PKM2 to activate tumor-cell signaling**

We next investigated how POA transmits the adipose-derived signal to tumor cells. Exogenous POA promoted proliferation and migration of both Panc02 and MC38 cells (Fig.4a-d and Extended Data Fig.6a,b), without significantly altering oxygen consumption (Fig.4e and Extended Data Fig.6c), arguing against a primary effect on mitochondrial oxidative metabolism. We therefore considered whether POA acts as a signaling lipid by directly engaging intracellular proteins. Affinity purification using a biotinylated POA probe followed by mass spectrometry identified pyruvate kinase M2 (PKM2) among candidate POA-interacting proteins (Fig.4f-h). The interaction was confirmed by co-immunoprecipitation and was preferential for POA over palmitate (Fig.4i,j).

Structural docking identified a candidate POA-binding pocket in PKM2. Systematic alanine substitution of predicted contact residues and previously reported functional residues<sup>13,14,25-29</sup> showed that R455 and N456 were uniquely required for POA binding (Fig.4k,l). POA increased PKM2 dimerization, providing a potential molecular link between lipid engagement and PKM2 nuclear signaling (Fig.4m). Consistent with this model, POA induced robust nuclear accumulation of PKM2 in Panc02 and MC38 cells, whereas this response was attenuated by mutation of the POA-binding residues (Fig.4n and Extended Data Fig.6f-j). POA did not induce comparable PKM2 nuclear accumulation in non-transformed HEK293T cells (Extended Data Fig.6k), suggesting that this response is preferentially engaged in tumor cells.

We next tested whether PKM2 nuclear signaling was required for the biological activity of POA. TEPP-46, which stabilizes tetrameric PKM2 and limits its nuclear translocation<sup>30,31</sup>, blocked POA-induced PKM2 nuclear accumulation and substantially attenuated POA-induced proliferation and migration in both tumor models (Fig.4n-q and Extended Data Fig.6l-o). Genetic experiments provided complementary evidence: PKM2 depletion suppressed tumor-cell proliferation and migration, whereas re-expression of wild-type PKM2 restored these responses. In contrast, the POA-binding-deficient PKM2 mutant failed to restore POA responsiveness (Fig.4r-t and Extended Data Fig.6p-t). These findings establish PKM2 as a direct molecular sensor of POA through which an adipose-derived lipid signal is translated into tumor-cell signaling.

### **Adipose MSTN-POA signaling rewires tumor-cell transcription and drives tumor progression**

Having identified a direct POA-PKM2 interaction, we asked whether this pathway accounts for the transcriptional effects of adipose MSTN. Tumor cells were either treated with POA or co-cultured with Flox or AKO adipocytes in the presence of palmitate (Extended Data

Fig.7a). Transcriptomic analysis revealed substantial overlap in differentially expressed genes between these conditions (Extended Data Fig.7b). Notably, these shared transcriptional changes were consistent with a nuclear PKM2-driven oncogenic program<sup>15</sup>. The shared genes were enriched for pathways associated with oncogenic signaling, including JAK-STAT, PI3K-AKT and IL-17 signaling, as well as proliferation, cell-cycle progression and survival (Extended Data Fig.7c-f). These transcriptional changes were consistent with a nuclear PKM2-dependent tumor-promoting program, linking the metabolic output of adipose MSTN signaling to tumor-cell state.

We next examined the broader relevance of POA in human cancer. Across published studies encompassing multiple cancer types and biological matrices, increased circulating or tissue-associated POA has been associated with cancer occurrence, disease progression or poorer clinical outcomes (Fig.5a,b). Although these associations do not establish causality, together with our mechanistic findings they support the potential relevance of POA as a tumor-promoting lipid signal.

Finally, we tested whether restoring POA could reconstitute tumor progression in the absence of adipose MSTN. HFD-fed control and AKO mice bearing orthotopic Panc02 tumors were administered exogenous POA, resulting in a robust increase in circulating POA (Fig.5c,d). POA supplementation accelerated tumor growth in control mice and, importantly, largely restored tumor growth in AKO mice to levels observed in controls (Fig.5e-g). Histological analysis showed corresponding restoration of tumor-cell proliferation and angiogenesis (Fig.5h-j). Thus, restoration of the downstream lipid signal is sufficient to overcome the tumor-suppressive effect of adipocyte *Mstn* deletion *in vivo*, establishing the adipose MSTN-SCD1-POA pathway as a functional mediator of obesity-associated tumor progression.

## Discussion

Obesity promotes cancer progression through complex metabolic and signaling interactions between adipose tissue and tumors, but the molecular signals that connect these compartments remain incompletely understood. Here, we identify adipose MSTN as an upstream regulator of this communication. Adipocyte-intrinsic MSTN promotes SMAD2/3-dependent *Scd1* expression and palmitoleic acid (POA) production, whereas adipose-derived POA engages PKM2 in tumor cells to promote nuclear signaling and tumor progression. This adipose MSTN-SCD1-POA-PKM2 circuit establishes a direct mechanistic link between tissue-specific metabolic regulation and tumor-cell signaling.

Our findings further show that the biological consequences of MSTN depend on its tissue of origin and metabolic context. In obese mice, adipocyte-derived MSTN emerged as the dominant source influencing tumor progression, whereas muscle-specific *Mstn* deletion produced little effect. This distinction is particularly relevant in obesity, where adipocytes undergo profound changes in lipid handling, biosynthetic activity, and secretory function<sup>32-34</sup>. Rather than simply increasing the abundance of metabolic substrates, dysfunctional adipose tissue may actively generate signaling lipids that instruct distant tissues. Our findings place MSTN within this regulatory layer and suggest that established metabolic regulators can acquire distinct inter-organ functions in disease-associated tissue states.

An important conceptual advance is the identification of a noncanonical intracellular function of full-length MSTN in adipocytes. Canonical MSTN signaling is generally

understood as an extracellular process initiated by receptor engagement and SMAD activation<sup>35-37</sup>. In contrast, our data support a model in which intracellular full-length MSTN regulates SMAD2/3-dependent *Scd1* expression, directly coupling MSTN to adipocyte lipid biosynthesis. These findings expand the functional repertoire of MSTN beyond its established role as a secreted ligand and is consistent with emerging evidence that precursor or intracellular pools of TGF $\beta$  superfamily proteins can exert functions distinct from those of their mature extracellular ligands<sup>38</sup>. How intracellular MSTN engages the SMAD2/3 machinery remains an important question and may reveal additional noncanonical functions of TGF $\beta$ -superfamily proteins.

Our study places lipid metabolism at the center of adipose-tumor communication by identifying POA as a metabolic output of adipose MSTN signaling. POA has traditionally been associated with improved insulin sensitivity and metabolic homeostasis<sup>3,39</sup>, but bioactive lipids can exert divergent effects across different cellular and disease settings. For example, sphingolipids such as ceramide and sphingosine-1-phosphate can produce opposing effects on tumor survival and metastasis<sup>40</sup>, while adipose-derived fatty acids can directly participate in tumor-promoting metabolic crosstalk<sup>41</sup>. Recent evidence also suggests that POA can contribute to tumor-promoting lipid remodeling in the tumor-associated adipose microenvironment<sup>42</sup>. Here, POA acts beyond its role as a metabolic substrate: it directly engages tumor-cell PKM2 and promotes its dimerization and nuclear accumulation. The identification of R455 and N456 as critical residues for POA binding provides a molecular link between a circulating lipid signal and the transcriptional state of tumor cells. More broadly, these findings illustrate how lipid-protein interactions can connect systemic metabolic alterations to tumor-cell signaling plasticity.

In summary, these findings support a model in which obesity-associated adipose remodeling generates tumor-promoting signals through tissue-specific MSTN activity. The resulting pathway connects adipose signaling, lipid biosynthesis and tumor-cell transcription within a single inter-organ circuit. This framework suggests that the consequences of targeting MSTN may extend beyond muscle and depend on the tissue compartment in which MSTN is manipulated and the metabolic outputs that are consequently altered. As MSTN-directed therapies enter clinical development for muscle and metabolic disorders, defining these tissue-specific functions will be important for understanding their broader physiological and disease consequences.

## Methods

### Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee. *Mstn*<sup>fl/fl</sup> mice were generated using CRISPR/Cas9 technology targeting exons 2-3 of *Mstn* (ENSMUSG00000026100). Adipose tissue-specific *Mstn* knockout (AKO) mice were generated by crossing *Mstn*<sup>fl/fl</sup> mice with Adiponectin-Cre mice. Muscle-specific *Mstn* knockout (MKO) mice were generated by crossing *Mstn*<sup>fl/fl</sup> mice with Myl1-Cre mice. Cre-negative floxed littermates served as controls (Flox). Mice were maintained on a 12 h light/12 h dark cycle at 23 °C and 50-60% humidity with free access to food and water.

### **Diet intervention**

For HFD experiments, mice were fed a 60% kcal fat diet for 8 weeks before tumor inoculation and remained on the same diet throughout the study. For NCD experiments, mice were maintained on standard chow diet throughout. At the experimental endpoint, BAT, iWAT, eWAT, liver, gastrocnemius, soleus, and tumor tissues were collected.

### **Cell lines, cell culture, and pharmacological treatments**

MC38, Panc02, HEK293T, and C2C12 cells were cultured in high-glucose DMEM (Gibco) supplemented with 10% FBS (Gibco, A5256701) and 1% penicillin-streptomycin (Gibco, 15140122) at 37 °C with 5% CO<sub>2</sub>. HEK293T cells were used for plasmid transfection and luciferase assays. Polyethylenimine (PolySciences, 23966) was used for transfection according to the manufacturer's instructions. Cells were routinely tested for mycoplasma contamination. For pharmacological treatments, HEK293T cells were treated with brefeldin A (YEASEN, 50504ES08) or MG-132 (MCE, HY-13259); Panc02 or MC38 cells were treated with TEPP-46 (MCE) at the indicated concentrations for 24 h. Vehicle-treated cells served as controls.

### **Mouse tumor models**

For subcutaneous tumor models,  $1 \times 10^6$  MC38 or Panc02 cells were resuspended in 100  $\mu$ L PBS and injected subcutaneously into the flank. Tumor dimensions were measured every 3 days using digital calipers, and tumor volume was calculated as  $0.5 \times \text{length} \times \text{width}$ . For orthotopic pancreatic implantation, mice were anesthetized with isoflurane and a small left abdominal incision was made to expose the pancreas.  $5 \times 10^4$  Panc02 cells in 100  $\mu$ L PBS were injected into the pancreatic parenchyma using a fine-gauge syringe. The injection site was checked for leakage, and the abdomen was closed in layers. Mice were monitored until recovery and sacrificed at the indicated endpoint.

### **Survival analysis**

Tumor-bearing mice were monitored daily and euthanized when predefined humane endpoints were reached (body condition score BCS-1). Survival curves were generated using the Kaplan-Meier method and analyzed by log-rank test.

### **Human samples**

Human adipose tissue samples were obtained from patients undergoing surgical treatment at Zhongshan Hospital, Fudan University. Relevant clinical information was collected from medical records. Fresh specimens were snap-frozen in liquid nitrogen and stored at -80 °C. Total protein was extracted for immunoblotting and total RNA for quantitative PCR. All procedures were performed in accordance with the Declaration of Helsinki (2013 revision) and approved by the ethics committee of Zhongshan Hospital, Fudan University. Written informed consent was obtained from each participant.

### **Isolation and differentiation of primary white adipocytes**

Inguinal white adipose tissue was dissected from 6-8-week-old male mice, minced, and digested for 30 min at 37 °C in isolation buffer (PBS containing 4% fatty acid-free BSA and 10 mg/mL collagenase D; Roche). Digested tissue was filtered through a 70  $\mu$ m cell strainer, and the flow-through was centrifuged at 2,000 rpm for 10 min. The supernatant (mature adipocytes) and pellet (stromal vascular fraction, SVF) were collected separately. SVF cells were resuspended and plated for subsequent differentiation.

### **In vivo POA supplementation**

Palmitoleic acid (POA, C16:1n-7) was administered to tumor-bearing mice by oral gavage at 300 mg/kg/daily. Treatment was initiated on day 3 after tumor inoculation and continued until the experimental endpoint. Control mice received an equal volume of vehicle. Body weight and tumor growth were monitored throughout the experiment.

### **Histology**

Tissues were fixed in 4% paraformaldehyde for 24 h, dehydrated through graded ethanol, and embedded in paraffin. H&E staining was performed to evaluate tissue morphology. For immunohistochemistry, paraffin sections were incubated with antibodies against Ki-67 or CD31 (Cell Signaling Technology) overnight at 4 °C, followed by HRP-conjugated secondary antibodies and DAB substrate. Sections were counterstained with hematoxylin. Images were acquired using an Olympus DP80 microscope.

### **Immunofluorescence staining**

Cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, blocked with 5% BSA, and incubated with anti-PKM2 antibody (HUABIO, HA723370) overnight at 4 °C, followed by fluorescent secondary antibodies. Nuclei were stained with DAPI. Images were acquired using a Leica confocal microscope.

### **RNA extraction and quantitative real-time PCR**

Total RNA was extracted using TRIzol reagent according to the manufacturer's instructions. 1 µg of RNA was reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher). Quantitative PCR was performed on a QuantStudio 7 Flex system (Thermo Fisher) using SYBR Green PCR Master Mix (Accurate Biotechnology). Rplp0 or Actb (β-actin) was used as the endogenous control.

### **Protein extraction and immunoblotting**

Tissues and cells were lysed in RIPA buffer (Beyotime Biotechnology) supplemented with protease and phosphatase inhibitors (Beyotime Biotechnology). Protein concentration was determined using a BCA Protein Assay Kit (Thermo Fisher). A total of 30 µg protein was loaded onto SDS-PAGE gels, transferred to PVDF membranes (Millipore), and incubated with primary antibodies. Cytoplasmic and nuclear fractions were prepared using a commercial extraction kit (Beyotime Biotechnology). GAPDH served as the cytoplasmic marker and Lamin B1 as the nuclear marker. Primary antibodies: anti-MSTN (R&D Systems, AF788), anti-phospho-SMAD2/3 (CST, 8828S), anti-SMAD2/3 (CST, 8685S), anti-SCD1 (HUABIO, HA723753), anti-β-tubulin (Abclonal, AC008), anti-GAPDH (Abways, AB0037), anti-Lamin B1 (Abclonal, AC057), anti-β-actin (Abclonal, AC026), anti-FLAG (Abclonal, AE005).

### **Plasmids construction**

The full-length coding sequences of mouse Mstn were amplified by PCR using cDNA as the template. The primers used for cloning as follows:

After PCR amplification, the parental methylated plasmids were digested and the products were transformed into competent *E. coli* DH5α cells. Positive colonies were screened by colony PCR, and plasmid DNA was extracted using a plasmid miniprep kit. All constructs were verified by Sanger sequencing before use.

| Primer Name                | Forward                                                       | Reverse                                                        |
|----------------------------|---------------------------------------------------------------|----------------------------------------------------------------|
| Mstn                       | GAACGAATTCGCCACCATGATGCAAAACTG<br>CAAATGTATGTTTATA            | GAACGCGCCGCATGAGCACCCACAGCGG<br>TCTA                           |
| Mstn <sup>Δ25AA</sup>      | GAACGAATTCGCCACCATGAATGAGGGCAGT<br>GAGA                       | GAACGCGCCGCATGAGCACCCACAGCGG<br>TCTA                           |
| Mstn <sup>D100A</sup>      | TACGACGTCCAGAGGGGTGACAGCAGTGATG<br>GC                         | GCCATCACTGCTGTACCCCCTCTGGACGTC<br>GTA                          |
| Mstn <sup>Dmut</sup>       | ACACACCCAAGGACTTTGGGCTTGACTGCGA<br>TG                         | CAAAGTCCTTGGGTGTGTCTGTACACCTTGA<br>CTT                         |
| Scd1                       | GAACGGTACCGGAAGCTCCTGAAGTCTACAG<br>TGGGTGCC,                  | GAACCTCGAGGATGATAGTCAGTTGCTCGC<br>CTCACTTTCCCA                 |
| Scd1- site1                | GAACGGTACCAGAAAAGCGAGAAGAGGAAA<br>AAAAGAAAAGAACT              | GAACCTCGAGGATGATAGTCAGTTGCTCGC<br>CTCACTTTCCCA                 |
| Scd1- site2                | GAACGGTACCAGTGCTGGGATTAAAGGCATG<br>CG                         | GAACCTCGAGGATGATAGTCAGTTGCTCGC<br>CTCACTTTCCCA                 |
| Scd1- site3                | GAACGGTACCAGGCGGTCCCCGGCAGAC                                  | GAACCTCGAGGATGATAGTCAGTTGCTCGC<br>CTCACTTTCCCA                 |
| Scd1- site4                | GAACGGTACCAGAAGCTAGAGGCAGAGGGA<br>ACAGCA                      | GAACCTCGAGGATGATAGTCAGTTGCTCGC<br>CTCACTTTCCCA                 |
| Scd1-site3-mut             | CTCTGCTGCTGCTAGGCGGTCCCCGGCAGAC<br>G                          | GCCTAGCAGCAGCAGAGCCCAATCCTAGCT<br>GCTT                         |
| Pkm2                       | ATGCCGAAGCCACACAGTGAA                                         | TCAAGGTACAGGCACTACACGCATGGTGT<br>GGT                           |
| Pkm2 <sup>S37A</sup>       | TGCCGCTGGACATTGACGCGGCCCATCA<br>C                             | GCGGGCCGTGATGGGGCCGCGTCAATGTC<br>CA                            |
| Pkm2 <sup>Y105A</sup>      | CATCTGATCCCATTCTCGCCGTCCTGTTGCG<br>G                          | AGCCACCGCAACAGGACGGGCGAGAATGG<br>GAT                           |
| Pkm2 <sup>K305A</sup>      | GGGCATTGAGATTCTGCAGAGGCGGTCTTC<br>CTG                         | TCATCTTCTGAGCCAGGAAGACCGCCTCTG<br>CAGGA                        |
| Pkm2 <sup>R399AR400A</sup> | GCTATTCGAGGAACTCGCCGCCCTGGCGCCC<br>A                          | CTGGTAATGGGCGCCAGGGCGGCGAGTTCC<br>TCG                          |
| Pkm2 <sup>K422A</sup>      | GTGCCGTGGAGGCCTCTTCGCATGCTGCAGT<br>G                          | CGATAATGGCCCCACTGCAGCATGCGAAGG<br>AGGCC                        |
| Pkm2 <sup>T432AK433A</sup> | GGCCATTATCGTGCTCGGCCGCTCTGGCAGG<br>A,                         | TGGTGAGCACTCCTGCCAGAGCGGCCGAGC<br>ACGATA                       |
| Pkm2 <sup>R455AN456A</sup> | GGGCTCCTATCATTGCCGTGACTGCAGCGCCC<br>CAGACTG                   | CTGGCGAGCAGTCTGGGGTGATGCAGTCAC<br>GGCAATG                      |
| Pkm2 <sup>G520A</sup>      | GGGTGGCGCCCTGGCTCTGCATTACCAA,<br>scrambled                    | CGCATGGTGTTGGTGAATGCAGAGCCAGG<br>AATTCAAAAACAACAAGATGAAGAGCACC |
| shRNA                      | TTGGTGCTCTTCATCTTGTTGTTTTG                                    | AACTCGAGTTGGTGCTTTCATCTTGTTG                                   |
| shPkm2-1                   | CCGGCAGCAACGCTTGAGTGCTCACTCGAG<br>TGAGCACTACAAGCGTTGCTGTTTTG  | AATTCAAAAACAGCAACGCTTGAGTGCTC<br>ACTCGAGTGAGCACTACAAGCGTTGCTG  |
| shPkm2-2                   | CCGGGACTGGAAACCCTGACTTTATCTCGAG<br>ATAAAGTCAGGGTTTCCAGTCTTTTG | AATTCAAAAAGACTGGAAACCCTGACTTTA<br>TCTCGAGATAAAGTCAGGGTTTCCAGTC |
| shPkm2-3                   | CCGGCATGCAATAGAGACCAGTACTCGAGT<br>AGCTGGTCTCTATTGCATGTTTTG    | AATTCAAAAACATGCAATAGAGACCAGCTA<br>CTCGAGTAGCTGGTCTCTATTGCATG   |

### Luciferase reporter assays

The pGL3-Scd1-promoter (-2000/+100) reporter was constructed by amplifying the corresponding region from C57BL/6J mouse genomic DNA and cloning into pGL3-Basic (Promega). Truncated and site-directed mutant reporters were generated by PCR-based strategies. HEK293T cells were seeded in 24-well plates and co-transfected with the indicated firefly luciferase reporter, pcDNA3.1-MSTN or pcDNA3.1-Δ25AA, and pRL-TK (Renilla control) using ProFection Mammalian Transfection System (Promega). After 48 h, cells were harvested and assayed using the Dual-Luciferase Reporter Assay System (Promega).

### CCK8 and crystal violet assays

For CCK8 assays, cells were seeded in 96-well plates at 3,000 cells per well and treated as indicated. At 12, 24, 48, and 72 h, CCK8 solution (Beyotime Biotechnology) was added, and absorbance at 450 nm was measured after 1 h incubation. For crystal violet assays, cells were fixed, stained with 0.1% crystal violet, washed, imaged, and quantified as indicated.

### **scratch assay**

Cells were seeded into 6-well plates and grown to approximately 80% confluence. A linear wound was created with a sterile pipette tip, and debris was removed by washing with PBS. Cells were cultured in serum-reduced medium. Images were captured at 0, 12, 24, and 48 h using a light microscope, and wound width was quantified using ImageJ (NIH).

### **Metabolomics and metabolite measurements**

Plasma metabolomics was performed by UPLC-MS/MS using a Waters ACQUITY UPLC system coupled with a Waters XEVO TQ-S MS, controlled by MassLynx 4.1. Chromatographic separation was achieved using an ACQUITY BEH C18 column (1.7  $\mu$ m, 100 mm  $\times$  2.1 mm; Waters). The gradient elution used mobile phase A (5 mM ammonium formate in acetonitrile/H<sub>2</sub>O, 95:5 v/v) and mobile phase B (5 mM ammonium formate in acetonitrile/isopropanol, 10:90 v/v) at 0.4 mL/min with the column at 40 °C. Selected lipids were detected using multiple reaction monitoring (MRM) mode. Data integration and quantification were performed using TargetLynx (Waters).

### **Sequence alignment and gene expression analysis**

Total RNA was isolated from adipose tissues and tumor cells using TRIzol. RNA quality was assessed on an Agilent 2100 Bioanalyzer. Libraries were sequenced on the BGISEQ500 platform (BGI-Shenzhen) using 150 bp paired-end reads targeting 30 million reads per sample. Raw reads were trimmed using Trim Galore (v0.6.7), aligned to the mm10 reference genome using STAR (v2.5.2b), and quantified using RSEM (v1.2.28). Differential expression analysis was performed using DESeq2 (v1.42.0). KEGG pathway analysis was conducted on differentially expressed genes. GSEA was performed against KEGG and curated pathway gene sets. For Mendelian randomization analysis, two-sample MR was used to evaluate associations between circulating palmitoleate and pancreatic or colorectal cancer risk, using inverse variance weighted analysis as the primary method with MR-Egger, weighted median, and weighted mode as sensitivity analyses.

### **Pull-down assay and mass spectrometry**

Biotin or POA-biotin was incubated with cell lysates under the indicated conditions. Complexes were captured using streptavidin beads, washed extensively, and analyzed by immunoblotting or LC-MS/MS. Candidate POA-binding proteins were identified by mass spectrometry and ranked by peptide score and confidence.

### **PKM2 docking and mutagenesis**

The PKM2 crystal structure was retrieved from the RCSB PDB database. Protein preprocessing was performed in PyMOL (v2.6.0), including removal of water molecules and phosphate ions. The POA ligand was prepared using OpenBabel (v3.1.1). Molecular docking was performed using AutoDock Vina with the docking center at coordinates (-23.138, -13.977, 7.333) and a grid box size of 15  $\times$  15  $\times$  15. Ligand-receptor interactions were visualized using Discovery Studio 2021 Client, and structural Fig.s were generated in PyMOL.

### **shRNA-mediated knockdown and rescue cell line generation**

Panc02 and MC38 cells with stable PKM2 knockdown were generated using lentiviral shRNAs targeting mouse Pkm2. A non-targeting shRNA was used as control. Lentivirus was produced in HEK293T cells and used to infect Panc02 or MC38 cells in the presence of polybrene. Stable cells were selected with puromycin, and knockdown efficiency was validated by western blotting.

### **Quantification and statistical analysis**

All data are presented as mean  $\pm$  SEM. For two-group comparisons, a two-tailed Student's t test was used. For experiments involving more than two groups, one-way ANOVA followed by Bonferroni post hoc test was applied. Survival analyses were performed using the log-rank (Mantel-Cox) test. Statistical significance was defined as  $p < 0.05$ .

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### **Declaration of interests**

Conflict of interest: The authors have declared that no conflict of interest exists.

### **Author contributions**

All authors had full access to the data in the study and accepted responsibility to submit it for publication. Conceptualization, X.-X.K., H.-M.Y.; methodology, J.-Y.D., and X.-M.W., J.-W.F., Y.-J.W., and Y. W.; formal analysis, J.-Y.D., X.-M.W. Y.-J.W., and J.-W.F.; data curation, X.-Y. Z, and H.-M.Y.; writing - original draft, J.-Y.D., and X.-M.W.; writing - review & editing, X.-X.K.; supervision, X.-X.K., H.W. and S.Z.; project administration, Y.-F.H. and X.-X.K.; funding acquisition, X.-X.K., H.W. and S.Z.

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## Figure Legends

### **Fig. 1 Adipocyte-derived MSTN promotes obesity-associated tumor progression.**

a,b, MSTN mRNA and protein levels in adipose tissue from healthy individuals and patients with cancer,  $n = 6-12$ . c, Mstn mRNA levels in BAT, iWAT, eWAT, and skeletal muscle from Flox and AKO mice,  $n = 5$  mice. d, Schematic of the HFD-fed subcutaneous MC38 and Panc02 tumor models. e-h, Tumor growth and survival in the MC38,  $n = 9-11$  mice and Panc02,  $n = 7-8$  mice. i,j, Gross morphology of excised tumors. k-p, Ki-67 and CD31 staining and quantification in MC38 and Panc02 tumors. q-t, Tumor-free body weight, tumor-to-body-weight ratio, survival, and gross tumor morphology in the orthotopic Panc02 model,  $n = 6-7$ . u-w, Ki-67 and CD31 staining and quantification in orthotopic Panc02 tumors. x, H&E staining and gross morphology of livers from orthotopic tumor-bearing mice; arrows indicate hepatic tumor lesions. Data are presented as mean  $\pm$  SEM. Statistical significance was assessed using two-tailed unpaired Student's t-tests, two-way ANOVA and the log-rank (Mantel-Cox) test. ns, not significant.

### **Fig. 2 Adipocyte MSTN sustains palmitoleate-dependent adipocyte-tumor crosstalk.**

a, Schematic of the conditioned medium experimental design. b,c, Scratch assay in Panc02 cells cultured with indicated CM conditions. d, Proliferation of Panc02 cells cultured with indicated CM conditions,  $n = 6$ . e, PCA of plasma metabolites from Flox and AKO mice,  $n = 4$  mice. f, Metabolite set enrichment analysis of differential plasma metabolites,  $n = 4$  mice. g, Volcano plot of differential plasma metabolites. h, Plasma palmitoleic acid levels,  $n = 4$ . i, C16:1n7 levels in plasma from the Panc02 HFD model,  $n = 7-8$  mice. j, C16:1n7 levels in plasma from the orthotopic pancreatic tumor model,  $n = 5-6$  mice. k, C16:1n7 levels in eWAT from the Panc02 HFD model,  $n = 7-8$  mice. l, C16:1n7 levels in eWAT from the orthotopic model,  $n = 5-6$  mice. Data are mean  $\pm$  SEM. Statistical significance was assessed using two-tailed unpaired Student's t-tests and two-way ANOVA test. ns, not significant.

### **Fig. 3 Intracellular full-length MSTN activates a non-secretory SMAD2/3-SCD1 pathway**

a, PCA of eWAT transcriptomes from orthotopic Panc02 HFD model. b, Heatmap differential genes in AKO eWAT. c, GSEA of unsaturated fatty acid biosynthesis in AKO eWAT. d, e, *Scd1* mRNA and protein levels in iWAT and eWAT from orthotopic Panc02 tumor-bearing mice,  $n = 3-4$  mice. f, g, *Scd1* mRNA and protein levels in isolated mature adipocytes isolated from Flox and AKO mice,  $n = 7$  mice. h, i, *Scd1* mRNA and protein levels in adipose tissue from healthy individuals and patients with cancer,  $n = 6-12$ . j, Design and predicted processing of wild-type MSTN, MSTN $\Delta 25AA$ , MSTN $D100A$ , and MSTN $Dmut$  constructs. k, *Scd1* promoter activity in cells expressing vector, MSTN, or the non-secretory mutant MSTN $\Delta 25AA$ . l, *Scd1* promoter activity in cells expressing vector, MSTN, MSTN $D100A$ , or MSTN $Dmut$ . m, *Scd1* promoter activity in cells treated with the indicated conditioned media. n, *Scd1* promoter activity in the indicated groups in the presence of LDN193189,  $n = 6$ . o, *Scd1* promoter activity in the presence of TP-HCl,  $n = 6$ . p, Predicted SMAD2: SMAD3: SMAD4-binding sites and consensus motifs in the *Scd1* promoter. q, Luciferase activity of serially truncated *Scd1* promoter constructs,  $n = 4$ . r, Luciferase activity of the wild-type and site 3-mutant *Scd1* promoters,  $n = 4$ . Data are mean  $\pm$  SEM. Statistical significance was assessed using two-tailed unpaired Student's t-tests and two-way ANOVA test. ns, not significant.

**Fig. 4 Palmitoleic acid functions as an adipose-derived lipokine mediating obesity-associated tumor progression**

a, Proliferation of Panc02 cells treated with vehicle or POA,  $n = 5$ . b, Colony formation of Panc02 cells treated with vehicle or POA. c, d, Scratch assay in Panc02 cells with indicated conditions. e, Oxygen consumption rate in Panc02 cells treated with vehicle or POA,  $n = 4$ . f, Structure of the biotinylated POA probe. g, Top POA-binding proteins identified by affinity purification-mass spectrometry. h, Associations between candidate protein expression and overall survival. i, POA-biotin pull-down of PKM2. j, Competition of POA-biotin binding to PKM2 by unlabeled PA or POA. k, Predicted POA-binding pocket in PKM2. l, POA-biotin pull-down of wild-type and mutant PKM2. m, PKM2 dimerization in Panc02 and MC38 cells following POA treatment. n, PKM2 localization in Panc02 cells treated with vehicle, POA, or TEPP-46. o, Real-time confluence of Panc02 cells under the indicated treatments,  $n = 5$ . p,q, Scratch assay in Panc02 cells with indicated conditions. r, Proliferation of PKM2-depleted Panc02 cells reconstituted with wild-type or POA-binding-deficient PKM2,  $n = 4$ . s,t, Scratch assay in indicated PKM2-reconstituted cells with indicated conditions. Data are mean  $\pm$  SEM. Statistical significance was assessed using two-tailed unpaired Student's t-tests and two-way ANOVA test. ns, not significant. \* $p < 0.05$ ; \*\* $p < 0.01$ ;

**Fig. 5 POA is associated with adverse cancer outcomes and restores tumor growth in adipocyte-specific Mstn-knockout mice**

a, Summary of published human studies linking POA exposure to cancer occurrence, aggressiveness, and survival. b, Forest plot of reported effect estimates and 95% confidence intervals. c, Schematic of POA supplementation in orthotopic Panc02 AKO mice under HFD. d, Plasma POA levels,  $n = 4-5$  mice. e-g, Tumor weight, tumor / body weight ratio and gross tumor morphology,  $n = 4-5$  mice. h-j, Ki-67 and CD31 staining and quantification. k, Working model. Data are mean  $\pm$  SEM. Statistical significance was assessed using two-tailed unpaired Student's t-tests and two-way ANOVA test. ns, not significant.

**Fig. S1 Muscle-specific Mstn deletion suppresses tumor growth under normal-diet but not high-fat-diet conditions.**

a, Generation of muscle-specific *Mstn*-knockout (MKO) mice by crossing Myl1-Cre mice with *Mstn*-floxed mice. Floxed littermates served as controls, and both groups were maintained on a normal diet. b, Relative *Mstn* mRNA expression in the gastrocnemius muscle of Flox and MKO mice,  $n = 7-8$  mice. c,d, MC38 tumor growth curves and tumor / body weight ratio in normal-diet-fed Flox and MKO mice,  $n = 5-6$  mice. e, Schematic of the HFD experimental cohort. Flox and MKO mice were fed an HFD for 8 weeks before subcutaneous inoculation. f, Tumor / body weight ratio at the experimental endpoint,  $n = 8-9$  mice. g,h, MC38 tumor growth and tumor-free body weight in HFD-fed Flox and MKO mice,  $n = 8-9$  mice. Data are presented as mean  $\pm$  SEM. Statistical significance was assessed using two-tailed unpaired Student's t-tests. ns, not significant.

**Fig. S2 Adipocyte-specific Mstn deletion does not alter MC38 or Panc02 tumor growth under normal chow conditions.**

a, Generation of Adipocyte-specific *Mstn*-knockout (AKO) mice by crossing Adiponectin-Cre with *Mstn*-flox mice. b, *Mstn* mRNA expression in isolated mature adipocyte from Flox and AKO mice,  $n = 7$  mice. c, MSTN protein levels in adipose tissue. d, Schematic of the MC38 tumor model under NCD. e, Tumor growth curves in the MC38 NCD model,  $n = 7-9$  mice. f,

Tumor / body weight ratio,  $n = 7-9$  mice. g, Tumor-free body weight,  $n = 7-9$  mice. h, Tumor growth curves in the Panc02 HFD model,  $n = 4-5$  mice. i, Tumor / body weight ratio,  $n = 4-5$  mice. j, Tumor-free body weight,  $n = 4-5$  mice. Data are presented as mean  $\pm$  SEM. Statistical significance was assessed using two-tailed unpaired Student's t-tests. ns, not significant.

**Fig. S3 Adipocyte, but not muscle, MSTN drives palmitoleate-dependent tumor progression.**

a, Proliferation of MC38 cells with indicated CM conditions,  $n = 6$ . b-c, Scratch assay in MC38 cells with indicated CM conditions. d, Heatmap of differential plasma metabolites from Flox and AKO mice. e, C16:1n7 levels in plasma from the MKO HFD model,  $n = 5$  mice. Data are mean  $\pm$  SEM. Statistical significance was assessed using two-tailed unpaired Student's t-tests and two-way ANOVA test. ns, not significant.

**Fig. S4 Adipocyte MSTN selectively sustains adipose SCD1 expression and unsaturated fatty acid biosynthesis in tumor-bearing mice**

a, PCA of iWAT transcriptomes from orthotopic Panc02 HFD model. b, Heatmap of 287 upregulated and 346 downregulated genes in AKO iWAT. c, GSEA of unsaturated fatty acid biosynthesis in AKO iWAT. d,e, *Scd1* mRNA and protein levels in eWAT from the MC38 model,  $n = 10$ . f, g, *Scd1* mRNA and protein levels in eWAT from the Panc02 model,  $n = 5$ . h,i, *Scd1* mRNA and protein levels in MC38 tumors,  $n = 10$ . j,k, *Scd1* mRNA and protein levels in Panc02 tumors,  $n = 5$ . l,m, *Scd1* mRNA in tumors and protein in gastrocnemius muscle and tumors from the orthotopic Panc02 model,  $n = 3-4$ . n, SCD1 protein levels in eWAT and tumors from MKO model. o, Adipose *Scd1* expression in healthy individuals and patients with cancer from GSE131835. p, Adipose *Scd1* expression in control and cachectic patients from GSE20571. Data are mean  $\pm$  SEM. Statistical significance was assessed using two-tailed unpaired Student's t-tests and two-way ANOVA test. ns, not significant.

**Fig. S5 Intracellular MSTN sustains adipose POA biosynthesis through a noncanonical SMAD2/3-SCD1 pathway**

a, Schematic of physiological MSTN processing. b,c, FLAG immunoblotting of MSTN forms in cell lysates and conditioned media. d, Phosphorylated and total SMAD2/3 in cells expressing the indicated MSTN constructs. e, p(CAGA)<sub>12</sub>-Luc reporter activity induced by wild-type or cleavage-site-mutant MSTN,  $n = 3-4$ . f, Immunoblot analysis of p-SMAD2/3, SMAD2/3, and SCD1 in HEK293T cells expressing MSTN or its cleavage-site mutants, with or without TP-HCl or LDN-193189 treatment.,  $n = 3$ . g, MSTN <sup>$\Delta$ 25AA</sup> induced activity of serially truncated *Scd1* promoter constructs,  $n = 5$ . h, MSTN <sup>$\Delta$ 25AA</sup> induced luciferase activity of the wild-type and site 3-mutant *Scd1* promoters,  $n = 5$ . Data are mean  $\pm$  SEM. Statistical significance was assessed using two-tailed unpaired Student's t-tests and two-way ANOVA test. ns, not significant.

**Fig. S6 POA promotes tumor cell proliferation and migration through PKM2-dependent nuclear signaling**

a,b, Scratch assay in indicated PKM2-reconstituted cells with indicated conditions. c, Oxygen consumption rate in MC38 cells treated with vehicle or POA,  $n = 4$ . d,e, POA-biotin pull-down of TKT and ALDOA. f, Cytoplasmic and nuclear PKM2 levels in POA-treated Panc02 cells. g, PKM2 localization in MC38 cells treated with vehicle, POA, or POA plus TEPP-46. h,i, Cytoplasmic and nuclear PKM2 levels in POA-treated MC38 and HEK293T cells. j,k, Nuclear localization of wild-type or POA-binding-deficient PKM2 in indicated PKM2-reconstituted

cells following POA treatment. l, Real-time confluence of MC38 cells under the indicated treatments,  $n = 5$ . m,n, Scratch assay in Panc02 cells with indicated conditions. o, Expression of the indicated tumor-promoting genes in Panc02 cells,  $n = 5$ . p, Validation of PKM2 knockdown in Panc02 and MC38 cells. q, Validation of wild-type and mutant PKM2 re-expression. r, Proliferation of PKM2-depleted MC38 cells reconstituted with wild-type or mutant PKM2,  $n = 4$ . s, t, Scratch assay in indicated PKM2-reconstituted cells with indicated conditions. Data are mean  $\pm$  SEM. Statistical significance was assessed using two-tailed unpaired Student's t-tests and two-way ANOVA test. ns, not significant.

**Fig. S7 POA recapitulates adipocyte MSTN-dependent tumor-promoting transcriptional programs**

a, Schematic of comparing direct treatment or co-culture with adipocytes. b, Overlap between genes upregulated by POA and genes downregulated by co-culture. c, Comparative pathway enrichment analysis of the two gene sets. d, Heatmap of representative shared tumor-promoting genes. e, Expression of *Il6*, *Myc*, *Ccnd1*, *Cdk4*, and *Dnmt1* in Panc02 cells,  $n = 6$ . f, Expression of the indicated genes in Panc02 tumors from Flox and AKO mice,  $n = 6$ . Data are mean  $\pm$  SEM. Statistical significance was assessed using two-tailed unpaired Student's t-tests and two-way ANOVA test. ns, not significant.

# Figure 1

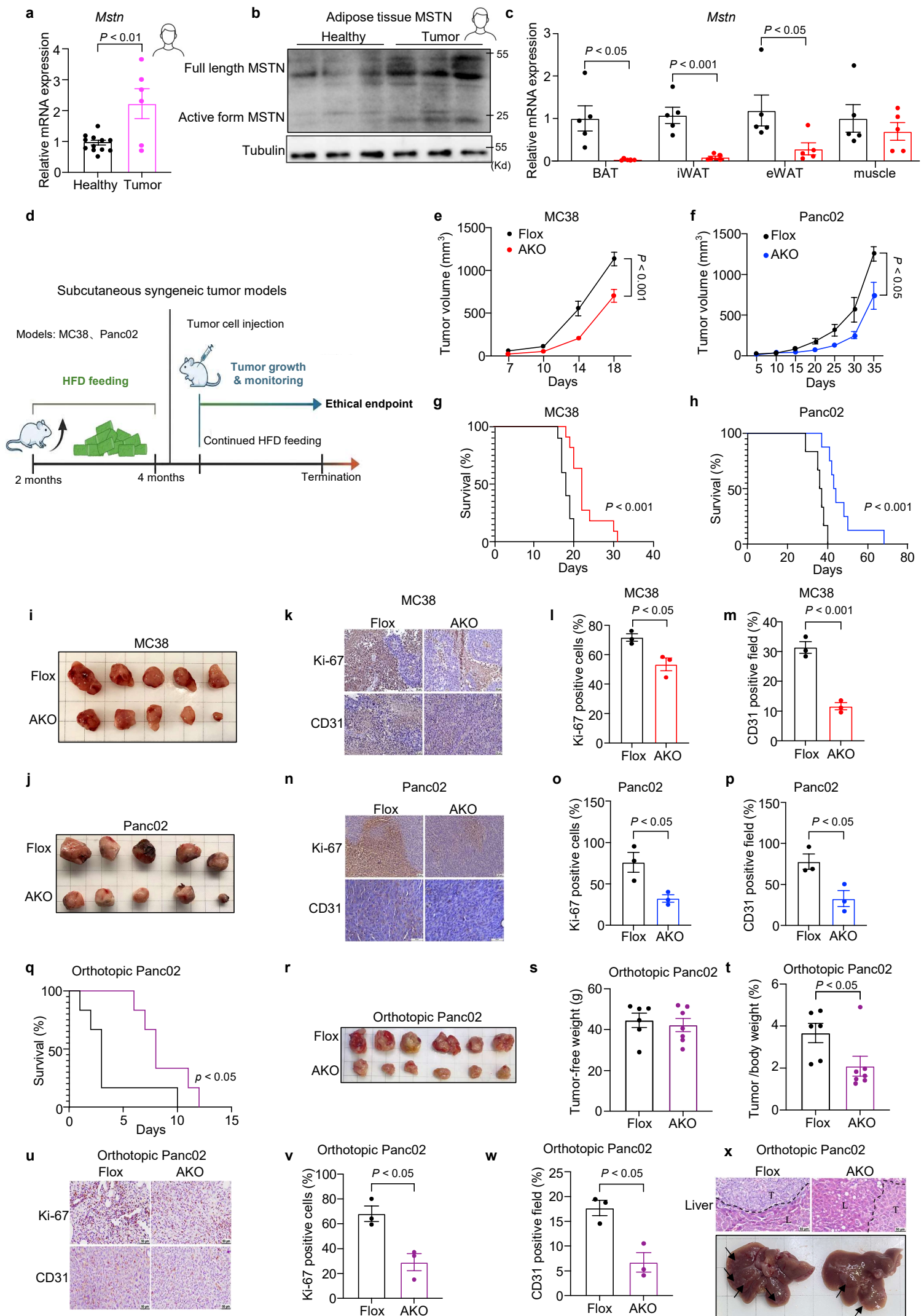


Figure 2

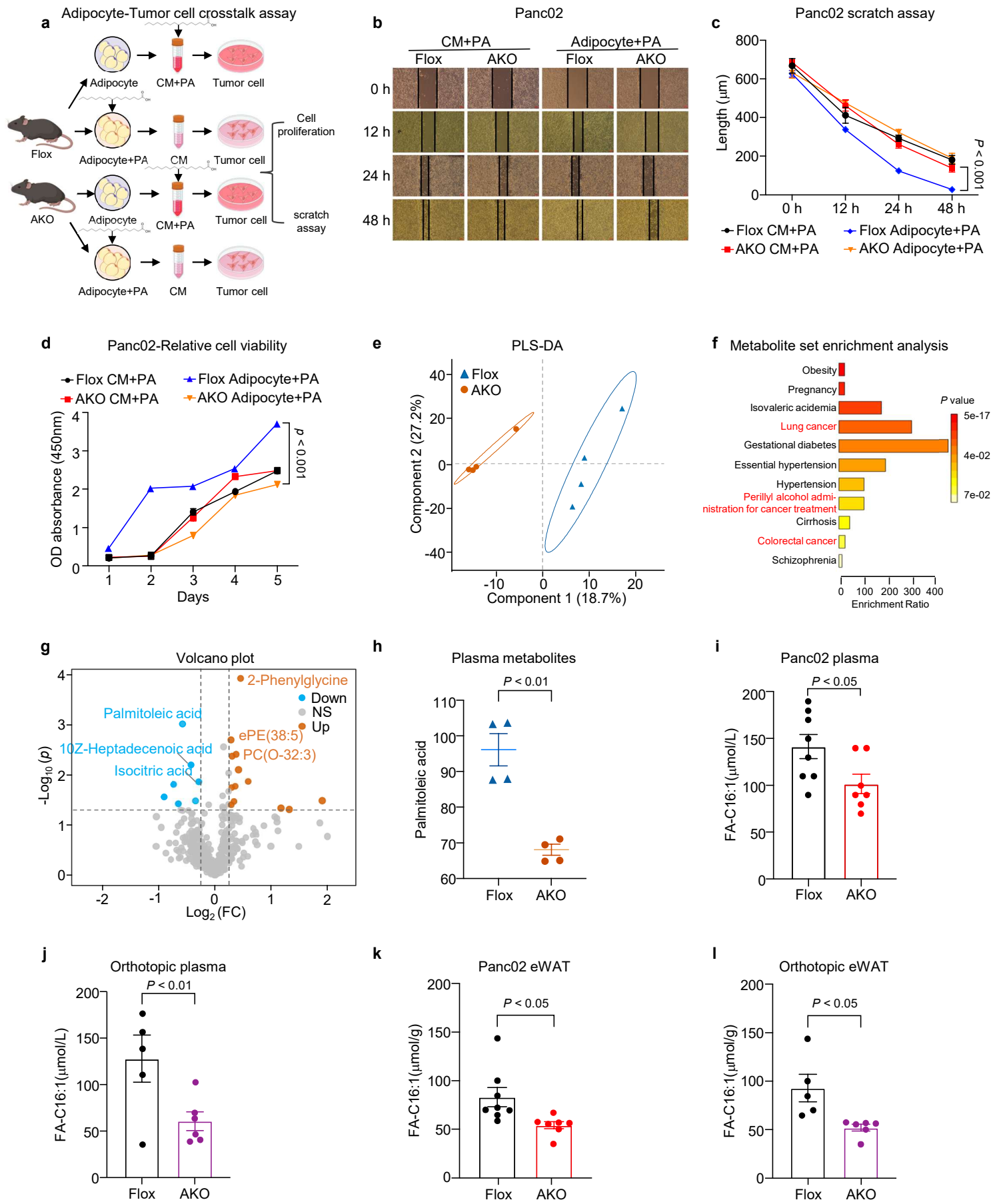


**Figure 3**

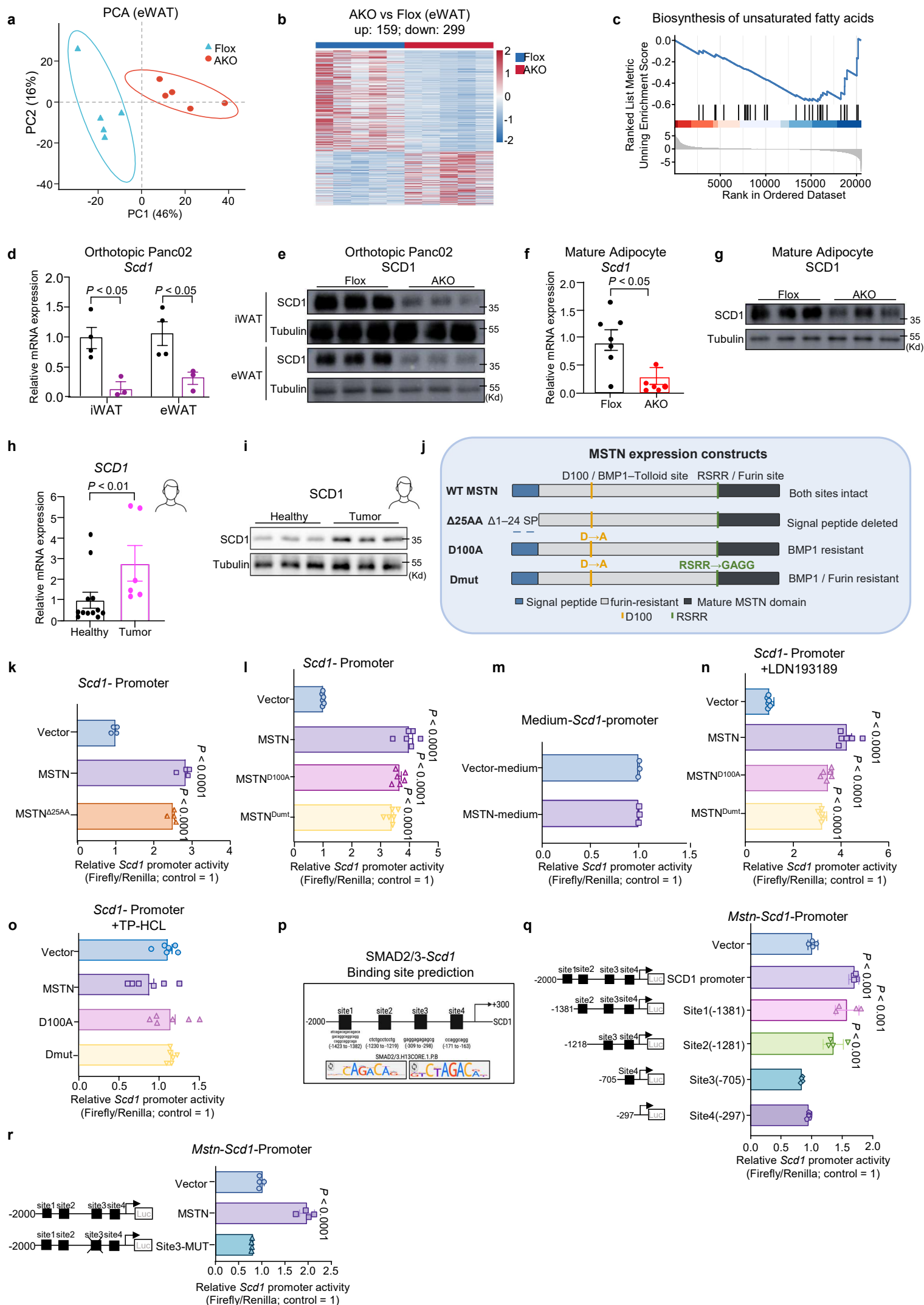


Figure 4

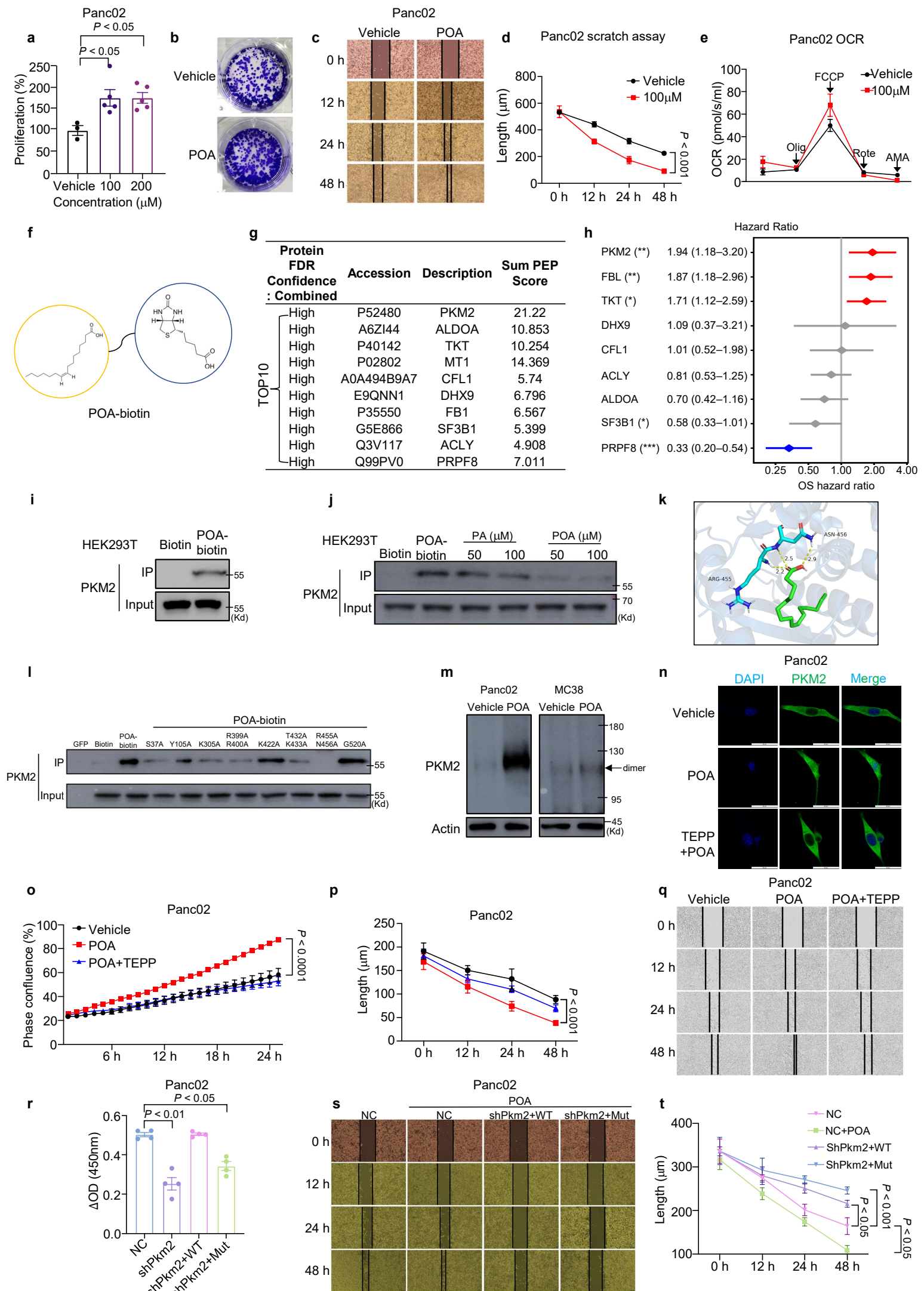
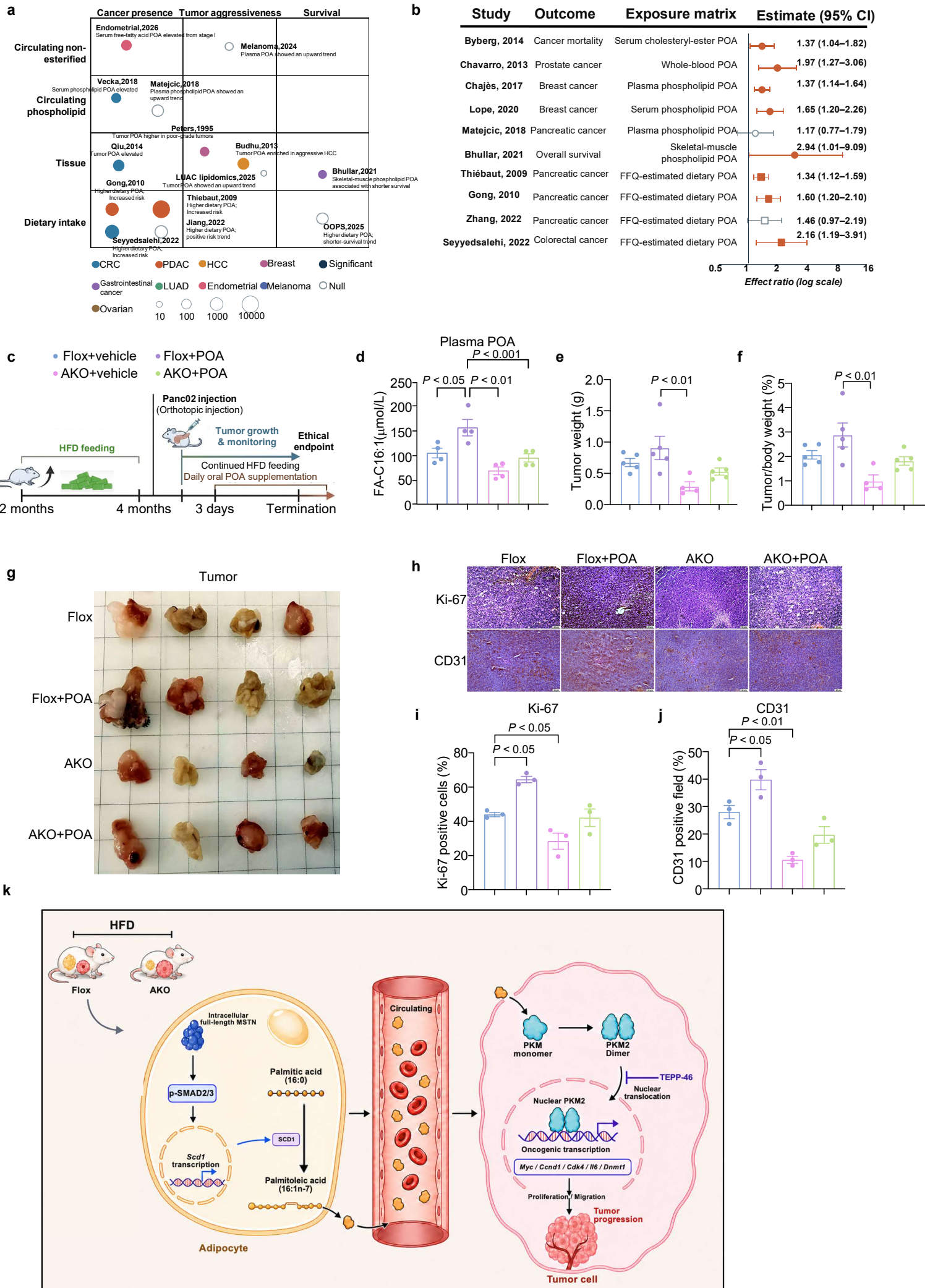


Figure 5



## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementalfigures.pdf](#)