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(54) Title: COMBINATION THERAPIES INCLUDING PARP1 INHIBITORS

(57) Abstract: Described herein are methods and compositions that include use of one or more PARP1 inhibitors and one or more inhibitors of activated stromal / activated cancer-associated fibroblasts. Such methods and compositions are useful for treating cancer.



COMBINATION THERAPIES INCLUDING PARP1 INHIBITORS**Cross-Reference to Related Applications**

This application claims the benefit of the filing date of U.S. application No.
5 63/296,266, filed on January 4, 2022, the disclosure of which is incorporated by
reference herein.

Government Funding

This invention was made with government support under R01CA218254
awarded by the National Institutes of Health. The government has certain rights in
10 the invention.

Incorporation by Reference of Sequence Listing

A Sequence Listing is provided herewith as an xml file, "2298710.xml"
created on January 3, 2023, 2022 and having a size of 95,728 bytes. The content of
15 the xml file is incorporated by reference herein in its entirety.

Background

The tumor microenvironment (TME), including stromal fibroblasts, can be a
driver of tumorigenesis in many types of tumors. Activation of tumor stromal fibroblasts
to generate cancer-associated fibroblasts (CAFs) can embolden epithelial cancer cells to
20 progress to more malignant stages and affect disease outcomes (Barron and Rowley,
2012; Cunha et al., 2002; Rowley, 1998; Tuxhorn et al., 2001). Cancer-associated
fibroblasts impact the tumor epithelium by modulating many functions through the
secretion of stromal growth factors and inflammatory mediators, by reprogramming
their metabolism to provide nutrients and survival factors, and by remodeling the
25 extracellular matrix (Hanahan and Weinberg, 2011; Kasashima et al., 2020; Sahai et al.,
2020).

However, the master regulators of the acquisition of the CAF phenotype, as well
as the molecular mechanisms whereby the tumor epithelium drives this process, are
currently unknown.

Summary

As described herein, tumor-secreted lactate activates cancer associated
fibroblasts (CAF) through downregulation of p62, a master regulator of CAF.
Lactate reduces the NAD⁺/NADH ratio, which impairs PARP1 activity and inhibits
p62. NAD⁺ supplementation can revert stromal activation. PARP1 inhibitors

(PARP1i) such as Olaparib, mimic lactate and activate CAF *in vitro* and *in vivo*.

Olaparib-induced

stroma is rich in hyaluronan. Co-treatment of Olaparib and PEGPH20, a clinical grade hyaluronidase reverts stroma activation and enhances Olaparib anti-tumor

5 activity.

Combinatorial therapies are described herein that can improve the efficacy of currently available cancer treatments. For example, compositions and methods that include use of PARP1 inhibitors can be combined with drugs that target the stroma to enhance PARP1 inhibitor efficacy or drugs that block or inhibit TNFalpha, IL-6
10 or Janus kinase (JAK).

The PARP1 inhibitors can include olaparib (Lynparza), niraparib (Zejula), rucaparib (Rubraca), talazoparib (Talzenna), veliparib (ABT-888), or a combination thereof.

Examples of agents that can be used to treat or inhibit activated stromal /
15 activated cancer-associated fibroblasts include but are not limited to hyaluronan (HA) synthase inhibitors, hyaluronan build-up inhibitors (e.g., PEGylated Recombinant Human Hyaluronidase, PEGPH20), autophagy inhibitors (e.g., ULK inhibitors such as SBI-0202965), fibroblast activation protein alpha (FAPα) inhibitors, GW4064 (farnesoid X receptor (FXR) agonists, Pirfenidone (PDF)
20 combined with doxorubicin, SMO-inhibitors (e.g., vismodegib, sonidegib), amphiregulin inhibitors, CXCL12 antagonists (e.g., an E5 antagonistic peptide), DDR2 inhibitors (e.g., WRG-28, which is a small molecule inhibitor of DDR2), or a combination thereof.

Examples of agents that can inhibit JAK include but are not limited to
25 lesataurtinib, baricitinib, tofacitinib, upadacitinib, filgonitib, delgocitinib, deucravacitinib, LS104, ON044580, NVP-BBT594, or NVP-CHZ868, or a combination thereof.

Examples of agents that can inhibit IL6 include but are not limited to tocilizumab, sarilumab, siltuximab, olokizumab, elsilimomab, clazakizumab,
30 sirukumab, levimumab, CPSI-2364, ARGX-109, FE301 or FM101, or any combination thereof.

Examples of agents that can inhibit TNFalpha include but are not limited to adalimumab, certolizumab pegol, etanercept, golimumab, or infliximab, or a combination thereof.

Brief Description of the Figures

FIGs. 1A-1L. PCa cells secrete a soluble factor that reduces p62 expression in stromal cells. **FIG. 1A** shows qPCR of *Sqstm1*, *Sdf-1* and *Tgfb β* levels in mPSC-GFP cells mixed with TRAMPC2 cells and cultured for 7 days and western blot.

5 **FIGs. 1B** and **1C** show immunoblot analysis for the indicated proteins (**FIG. 1B**) and qPCR of *SQSTM1* (**FIG. 1C**) in WPMY-1 cells co-cultured with PCa cells during 48 h (n= 3). **FIGs. 1D** and **1E** provide immunoblot analysis for the indicated proteins (**FIG. 1D**) and qPCR of *SQSTM1* (**FIG. 1E**) in WPMY-1 cells incubated 48 hours with conditioned media (CM) from PrEC, RWEP1, PC3, DU145, TRAMPC2

10 and LNCAP cells (n= 3). **FIG. 1F** illustrates *SQSTM1* promoter-driven luciferase in WPMY-1 incubated 48 hours with conditioned media from PrEC, RWEP1, PC3, DU145, TRAMPC2 and LNCAP cells (n= 3). **FIGs. 1G** and **1H** show immunoblot analysis for the indicated proteins (**FIG. 1G**) and qPCR of *SQSTM1* levels (**FIG. 1H**) in WPMY-1 cells incubated 48 h with fractionated CM from PC3 cells (n= 3).

15 **FIG. 1I** shows *SQSTM1* promoter-driven luciferase in WPMY-1 cells incubated 48 hours with fractionated CM from PC3 cells (n= 3). **FIGs. 1J** and **1K** show immunoblot analysis for the indicated proteins (**FIG. 1J**) and qPCR of *SQSTM1* levels (**FIG. 1K**) in WPMY-1 cells incubated 48 hours with normal or boiled CM from PC3 cells (n= 3). **FIG. 1L** *SQSTM1* promoter-driven luciferase in WPMY-1

20 cells incubated 48 hours with normal or boiled CM from PC3 cells (n= 3). Results are shown as mean $\pm$ SEM *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

FIGs. 2A-2L. Lactate is sufficient to downregulate p62. **FIG. 2A** shows lactate secretion was determined by YSI2950 analyzer from PCa cells for 72 hours. Results are normalized to total protein content (n= 3). **FIGs. 2B** and **2C** show

25 immunoblot analysis for the indicated proteins (**FIG. 2B**) and qPCR of *SQSTM1* and *MCT1* levels (**FIG. 2C**) in WPMY-1 cells, transduced with the indicated siRNAs, incubated with CM from PC3 cells for 48 hours (n= 3). **FIGs. 2D** and **2E** show immunoblot analysis for the indicated proteins (**FIG. 2D**) and qPCR of *SQSTM1* and *MCT4* levels (**FIG. 2E**) in WPMY-1 cells incubated with CM from PC3 cells,

30 transduced with the indicated siRNAs, for 48 hours (n= 3). **FIGs. 2F** and **2G** show immunoblot analysis for the indicated proteins (**FIG. 2F**) and qPCR of *SQSTM1* levels (**FIG. 2G**) in WPMY-1 cells, treated with AZD3965, and incubated with CM from PC3 cells for 48 hours (n= 3). **FIGs. 2H** and **2I** show immunoblot analysis for the indicated proteins (**FIG. 2H**) and qPCR of *SQSTM1* levels (**FIG. 2I**) in WPMY-

1 cells incubated with Lactate (24 mM) at different times (n= 3). **FIGs. 2J and 2K**
 provide immunoblot analysis for the indicated proteins (**FIG. 2J**) and qPCR of
SQSTM1 levels (**FIG. 2K**) in WPMY-1 cells incubated with different dose of
 Lactate for 48 hours (n= 3). **FIG. 2L** provides immunoblot analysis for the indicated
 5 proteins in WPMY-1 cells incubated with Lactate 24 mM (pH 6.5) or DMEM at
 different pH for 48 hours (n = 3). Results are shown as mean $\pm$ SEM *p < 0.05,
 p < 0.01, *p < 0.001, ****p < 0.0001.

FIGs. 3A-3M. AP1 elements control p62 downregulation by lactate. **FIG.**
3A illustrates four clusters (C1, C2, C3 and C4) heatmap of ATAC-seq peaks, +/- 2
 10 Kb from TSS in WPMY-1 cells treated or not with lactate for 48 hours. **FIG. 3B**
 illustrates HOMER discovery analysis of TFs enriched promoter regions (1-2 Kb)
 closed in WPMY-1 cells treated with Lactate (Control vs Lactate). **FIG. 3C** shows
 genome browser view at *SQSTM1* promoter. Chromatin accessibility (ATAC-seq)
 and RNA-seq profiles are shown in WPMY-1 cells treated or not with Lactate. **FIG.**
 15 **3D** illustrates AP1-driven luciferase in WPMY-1 cells treated with lactate for 48
 hours (n= 3). **FIG. 3E** shows *SQSTM1* promoter-driven luciferase in WPMY-1 cells,
 transduced with the indicated plasmids, and treated with lactate for 48 hours (n= 3).
FIG. 3F shows immunoblot analysis in sgC and sgAP-1A WPMY-1 cells for the
 indicated proteins. **FIGs. 3G and 3H** show immunoblot analysis for the indicated
 20 proteins (**FIG. 3G**) and qPCR of *SQSTM1* levels (**FIG. 3H**) in sgC and sgAP-1A
 WPMY-1 cells incubated with lactate (n= 3). **FIG. 3I** illustrates ChIP-PCR analysis
 of *SQSTM1* promoter (AP-1A) occupancy of c-JUN, c-FOS, FOSB or JUNB in
 WPMY-1 cells treated or not with lactate (24 mM) for 48 hours (n= 3). **FIGs. 3J**
 and **3K** show immunoblot analysis for the indicated proteins (**FIG. 3J**) and qPCR of
 25 *SQSTM1* levels (**FIG. 3K**) in WPMY-1 cells incubated with lactate and transduced
 with the indicated siRNAs (n= 3). **FIG. 3L and 3M** show immunoblot analysis for
 the indicated proteins (**FIG. 3L**) and qPCR of *SQSTM1* levels in WPMY-1 cells
 incubated with lactate and treated with 10 μ M of SP600125 (n= 3). Results are
 shown as mean $\pm$ SEM *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

30 **FIGs. 4A-4G.** Changes in NAD⁺/NADH levels by lactate are critical for p62
 downregulation. **FIGs. 4A and 4B** show immunoblot analysis for the indicated
 proteins (**FIG. 4E**) and qPCR of *SQSTM1* levels (**FIG. 4F**) in WPMY-1 incubated
 with Lactate or Pyruvate for 48 hours (n= 3). **FIG. 4C** illustrates intracellular NAD
 levels in WPMY-1 cells treated or not with lactate (n= 3). **FIGs. 4D and 4E** show

immunoblot analysis for the indicated proteins and qPCR of *SQSTM1* levels in WPMY-1 cells incubated with Lactate and NAD (150 μ M) for 48 hours (n= 3).

FIGS. 4F and 4G show immunoblot analysis for the indicated proteins and qPCR of *SQSTM1* levels in WPMY-1 cells incubated with Lactate or NR (200 μ M) for 48 hours (n= 3). Results are shown as mean $\pm$ SEM **p< 0.01, ***p < 0.001, ****p < 0.0001.

FIGS. 5A-5T. PARP-1 is critical for the control of p62 levels in stromal cells. **FIG. 5A** shows immunoblot analysis for the indicated proteins in WPMY-1 cells transduced with the indicated siRNAs. **FIGS. 5B and 5C** show immunoblot analysis for the indicated proteins (B) and qPCR of *SQSTM1* levels (**FIG. 5C**) in WPMY-1 cells transduced with the indicated siRNAs. **FIGS. 5D and 5E** show immunoblot analysis for the indicated proteins and qPCR of *PARP-1* (**FIG. 5D**) and *SQSTM1* (**FIG. 5E**) levels in WPMY-1 cells transduced with the indicated siRNAs. **FIGS. 5F and 5G** show immunoblot analysis for the indicated proteins (**FIG. 5F**) and qPCR of *SQSTM1* levels (**FIG. 5G**) in WPMY-1 cells incubated with PJ34 for 24 hours (n= 3). **FIGS. 5H and 5I** show immunoblot analysis for the indicated proteins (**FIG. 5H**) and qPCR of *SQSTM1* levels (**FIG. 5I**) in WPMY-1 cells incubated with Olaparib for 48 hours (n= 3). **FIGS. 5J and 5K** illustrate ChIP-PCR analysis of *SQSTM1* promoter (AP-1A) occupancy of c-FOS (**FIG. 5J**) or c-JUN (**FIG. 5K**) in WPMY-1 cells treated or not with Olaparib (20 μ M) for 48 hours (n= 3). **FIG. 5L** shows immunoblot analysis for the indicated proteins and PARylated protein levels in WPMY-1 cells incubated with lactate for the indicated times. **FIGS. 5M and 5N** illustrate PARylation of c-JUN (**FIG. 5M**) or c-FOS (**FIG. 5N**) in WPMY-1 treated or not with lactate for 48 h (n= 2). **FIGS. 5O and 5P** illustrate PARylation of c-JUN (**FIG. 5O**) or c-FOS (**FIG. 5P**) in WPMY-1 treated or not with lactate and NAD for 48 hours (n= 2). **FIG. 5Q and 5R** illustrate ChIP-PCR analysis of *SQSTM1* promoter (AP-1A) occupancy of c-FOS (**FIG. 5Q**) or c-JUN (**FIG. 5R**) in WPMY-1 cells treated or not with lactate and NAD for 48 hours (n= 3). **FIGS. 5S and 5T** illustrate ChIP-PCR analysis of *SQSTM1* promoter (AP-1A) occupancy of c-FOS (**FIG. 5S**) or c-JUN (**FIG. 5T**) in WPMY-1 cells treated or not with lactate and NR for 48 hours (n= 3). Results are shown as mean $\pm$ SEM *p < 0.05, **p< 0.01, ***p < 0.001, ****p < 0.0001.

FIGS. 6A-6M. AP1 control of the *SQSTM1* promoter in stromal cells is critical for PCa tumor growth and invasion. **FIG. 6A** shows qPCR analysis of

mRNA levels of the indicated genes in WPMY-1 cells incubated with Lactate (24 mM) for 48 hours (n = 3). **FIG. 6B** shows qPCR analysis of mRNA levels of the indicated genes in WPMY-1 cells treated or not with lactate and NAD for 48 hours (n = 3). **FIG. 6C** illustrates GSEA analysis of “LACTATE UP” and “LACTATE DOWN” gene signatures in CAFs vs. NAFs gene set GSE34312. **FIG. 6D** illustrates qPCR analysis of mRNA levels of indicated genes in sgC and sgAP-1A WPMY-1 cells (n= 3). **FIG. 6E-6G** show experimental design (**FIG. 6E**), Migration (**FIG. 6F**), and Invasion (**FIG. 6G**) of PC3 cells co-cultured with sgC or sgAP-1A WPMY-1 cells for 16 h. Representative images and quantification (n= 3). **FIG. 6H** and **6I** show experimental design (**FIG. 6H**) and H&E staining (**FIG. 6I**) of organotypic gels combining PC3 cells with sgC and sgAP-1A WPMY-1 cells. Quantification of PC3 cells invasion and proliferation of experiments shown in (**FIG. 6I**). **FIG. 6J-6M** illustrate subcutaneous xenograft co-implantation in castrated NSG mice (male, 7 weeks old) of PC3 PCa cells with sgC or sgAP-1A WPMY-1 cells (PC3 + sgC WPMY-1 n=8; PC3 + sgAP-1A WPMY-1 n=8). Experimental design (**FIG. 6J**), Tumor weight (**FIG. 6K**), gross images (**FIG. 6L**), IHC for Masson Trichrome, HA and aSMA (**FIG. 6M**), scale bars 50 μ m. Results are shown as mean $\pm$ SEM *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

FIGs. 7A-7T. Olaparib treatment mimics lactate loss of p62 and promotes stromal activation. **FIG. 7A** illustrates enriched pathways obtained by GSEA pre-ranked analysis using DEG in Olaparib-treated WPMY-1 cells. **FIG. 7B** illustrates GSEA analysis for stromal activation and CAF phenotype gene signatures in Olaparib-treated WPMY-1 cells. **FIG. 7C** illustrates GSEA analysis of “Olaparib_UP”, “Olaparib_DN”, “Olaparib_ADT_UP”, “Olaparib_ADT_DN” gene signatures in CAFs vs. NAFs gene set GSE34312. **FIG. 7D** shows qPCR analysis of mRNA levels of indicated genes in Olaparib-treated WPMY-1 cells (n= 3). **FIG. 7E** shows four clusters (C1, C2, C3 and C4) heatmap of ATAC-seq peaks, +/- 2 Kb from TSS, upon Olaparib treatment for 96 hours in basal conditions. **FIG. 7F** shows HOMER discovery analysis of TFs enriched in promoter regions (1-2 Kb) closed upon Olaparib treatment in WPMY-1 cells (DMSO vs Olaparib). **FIG. 7G** shows integrative Genomics Viewer (IGV) of ATAC-seq peaks in *SQSTM1* promoter in Olaparib-treated WPMY-1 cells in basal conditions. **FIGs. 7H-7J** show trans-well migration and invasion assays. Experimental design (**FIG. 7H**), left, trans-well migration and invasion assay of PC3 PCa cultured with Olaparib pre-treated

WPMY-1 cells for 20 hours, scale bar 50 μ m. Right, relative number of migrating and invading cells (**FIGs. 7I-7J**). **FIG. 7K** and **7L** show click-iT EdU incorporation assay. Experimental design (**FIG. 7K**), left, representative images of EdU incorporation in PC3 PCa cells treated with CM from Olaparib-treated WPMY-1 cells for 24 hours. Right, percentage of Edu positive cells (n= 3) (**FIG. 7L**), scale bar 50 μ m. **FIG. 7M** and **7N** show survival assay of GFP-labeled PC3 PCa cells co-cultured with WPMY-1 cells or alone, treated with DMSO or Olaparib, in basal and ADT conditions. Experimental design (**FIG. 7M**), relative GFP positive area after 10 days of culture (n= 6) (**FIG. 7N**). **FIGs. 7O-7T** illustrate subcutaneous xenograft co-implantation in NSG mice (male, 7 weeks old) of PC3 PCa cells with WPMY-1 cells (PC3 + WPMY-1 n= 20). Mouse were treated twice a week with Olaparib 40 mg/Kg for 2 weeks. Experimental design (**FIG. 7O**), tumor weight (g) (**FIG. 7P**), tumor volume (mm³) (**FIG. 7Q**), gross images (**FIG. 7R**), IHC for Masson Trichrome, HA, and aSMA (**FIG. 7S**). Immunofluorescence (IF) for aSMA, p62, and DAPI. scale bars 50 μ m (**FIG. 7T**). Results are shown as mean $\pm$ SEM *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

FIGs. 8A-8N. Stromal activation upon Olaparib treatment impacts prostate epithelial cells. **FIG. 8A** and **8B** illustrate TRAMP⁺ mice treated with Olaparib 40 mg/Kg, 5 days a week for 4 weeks. Experimental design (**FIG. 8A**), IHC for HA, and aSMA (**FIG. 8B**), scale bars 50 μ m. **FIG. 8C** and **8D** show scRNAseq compartment identification. UMAP plot with clustering results color-coded by compartment types (**FIG. 8C**). Proportion cell number of the identified cell compartments (**FIG. 8D**). **FIG. 8E** and **8F** show scRNAseq fibroblast subset. UMAP plot with re-clustering of fibroblast showing the main markers of each cluster (**FIG. 8E**). Feature plot for the expression of indicated genes (**FIG. 8F**). (**FIG. 8G** shows qPCR analysis of mRNA levels of indicated genes in Olaparib-treated WPMY-1 cells (n= 3). **FIG. 8H** shows IHC for SPINK1, scale bars 50 μ m. **FIG. 8I-8L** illustrate scRNAseq epithelial subset. UMAP plot with re-clustering of epithelial cells color-coded by cell type (**FIG. 8I**). Violin plots of specific epithelial cell markers (**FIG. 8J**). Proportion cell number of the identified epithelial cell populations (**FIG. 8K**). Violin plots of indicated gene signatures (**FIG. 8L**). **FIG. 8M** shows IHC for pSMAD2, scale bars 50 μ m. **FIG. 8N** illustrates Cellphone DB analysis showing ligand-receptor interactions between identified epithelial and fibroblast populations.

FIGs. 9A-9G. Stroma activation blockade reduce the tumor supporting effects of Olaparib. **FIG. 9A** shows IF for HABP in WPMY- cells treated with vehicle (DMSO), Olaparib 10 μ M or Olaparib 10 μ M +PEGPH20 2.5 μ g/ml for 96-hour, scale bars 50 μ m. **FIG. 9B** and **9C** illustrate trans-well migration assays. Experimental design (**FIG. 9B**), relative number of migrating PC3 PCa cultured with Olaparib 10 μ M or Olaparib 10 μ M +PEGPH20 2.5 μ g/m pre-treated WPMY-1 cells for 20 hours, basal and ADT conditions (**FIG. 9C**). **FIG. 9D** and **9E** show Click-iT EdU incorporation assay. Experimental design (**FIG. 9D**), left, representative images of EdU incorporation in PC3 PCa cells treated with CM from Olaparib 10 μ M, Olaparib 10 μ M +PEGPH20 2.5 μ g/m or Olaparib 10 μ M +CM incubation with Galunisertib 10 μ M WPMY-1 cells for 24 hours. Right, percentage of Edu positive cells (n= 3) (**FIG. 9E**), scale bar 50 μ m. **FIG. 9F** and **9G** illustrate subcutaneous xenograft co-implantation in NSG mice (male, 7 weeks old) of PC3 PCa cells with WPMY-1 cells (PC3 + WPMY-1 n= 20). Mouse were treated twice a week with Olaparib 40 mg/Kg or Olaparib + PEGPH20 0.0375 mg/Kg for 2 weeks. Experimental design (**FIG. 9F**), tumor volume (mm³) (**FIG. 9G**).

FIGs. 10A-10F. Lactate-driven p62 downregulation is not affected by different degradative mechanisms. **FIG. 10A** shows WPMY-1 cells, incubated or not with lactate for 48 hours, were incubated with 50 ug/ml of cycloheximide (CHX) and protein stability was determined by immunoblotting at indicated time points. P62 levels were normalized to actin (n = 3). **FIG. 10B** and **10C** show immunoblot analysis for indicated proteins in WPMY-1 cells treated or not with lactate and MG132 (**FIG. 10B**) or Bafilomycin A1 (**FIG. 10C**) for 48 hours. **FIG. 10D** shows immunoblot analysis for indicated proteins in WPMY-1 cells, treated or not with lactate for 48 hours, and transduced with the indicated siRNAs. **FIG. 10E** and **10F** show immunoblot analysis for indicated proteins in WPMY-1 cells, treated or not with lactate for 48 hours and a dose-response of the Calpain inhibitor (**FIG. 10E**) or Caspase inhibitor (**FIG. 10F**).

FIG. 11A-11F. Selective downregulation of p62 by PARP1 deletion. **FIG. 11A** and **11B** show qPCR analysis of mRNA levels of the indicated genes in WPMY-1 cells, transduced with the indicated siRNAs. **FIG. 11C** shows qPCR analysis of mRNA levels of the indicated genes and immunoblot analysis for indicated proteins in sgPARP-1 and sgC WPMY-1 cells. **FIG. 11D-11E** illustrate Olaparib induces autophagy. Immunoblot analysis of p62 (**FIG. 11D**) and LC3

(**FIG. 11E**) in WPMY-1 cells treated with Olaparib in the presence of lysosomal inhibitors (PI). **FIG. 11F** shows treatment with an autophagy inhibitor (ULK inhibitor, SBI-0202965) reverts the stroma activation induced by Olaparib as determined by qPCR of the CAF marker, ACTA2.

5 **FIGs. 12A-12D.** Olaparib induces fibroblast activation in different stromal cells. **FIG. 12A-12D** show qPCR analysis of mRNA levels of the indicated genes in (**FIG. 12A**) WPMY-1 cells (**FIG. 12B**) human normal primary fibroblast cells (**FIG. 12C**) mouse normal fibroblast cells (**FIG. 12D**) shows mouse hepatic stellate cells treated with Olaparib.

10 **FIGs. 13A-F.** PARP inhibitors induce stromal activation in different cell lines and *in vivo* models. **FIG. 13A-13D** show immunoblot analysis for P62 protein in primary prostate fibroblasts (**FIG. 13A**), lung primary fibroblasts (**FIG. 13B**), breast primary fibroblasts (**FIG. 13C**), and endometrial primary fibroblasts (**FIG. 13D**), veh-treated or Olaparib-treated, 20 μ M, for 4 days. **FIG. 13E-13F** show
15 GSEA analysis for the indicated stromal activation signatures comparing Talazoparib- (PARP inhibitor) vs veh- treated in mammary gland tumors (GSE125206) (**FIG. 13E**) and Niraparib (PARP inhibitor) vs veh-treated in human ovarian cancer PDXs (GSE163854) (**FIG. 13F**) showing stromal activation in PARP inhibitors treated samples.

20 **FIGs. 14A-14F.** Targeting IL6/JAK/STAT or TNF α signaling decreases stromal activation and rescue the proliferative effect conferred to PC3 prostate tumor epithelial cells. **FIG. 14A-14C** show GSEA analysis for the indicated signatures in Olaparib 20 μ M vs DMSO-treated WPMY-1 cells (**FIG. 14A**), Talazoparib- (PARP inhibitor) vs saline-treated in mammary gland tumors (GSE125206, **FIG. 14B**) and
25 Niraparib- (PARP inhibitor) vs veh-treated in human ovarian cancer PDXs (GSE163854, **FIG. 14C**), showing increased inflammatory related signatures upon PARP inhibitor treatment. **FIG. 14D** shows experimental design. **FIG. 14E** shows qPCR analysis of mRNA levels of indicated genes in WPMY-1 cells treated with DMSO, Olaparib 10 μ M, Lestaurtinib (JAKi) 50nM or Olaparib + Lestaurtinib
30 50nM (n= 3). **FIG. 14F** shows Click-iT EdU incorporation assay, left, representative images of EdU incorporation in PC3 PCa cells treated 24 h with CM from WPMY-1 treated with DMSO, Olaparib 10 μ M, Olaparib 10 μ M + Lestaurtinib 50nM, Olaparib 10 μ M + IL-6 capture antibody (2h), Olaparib 10 μ M +TNF α neutralizing antibody 1 μ g/ml (1 h), Lestaurtinib 50 nM, IL-6 capture antibody (2 h) or TNF α neutralizing

antibody 1 μ g/m (1 h). Right, percentage of Edu positive cells (n= 3) scale bars 50 μ m.

FIGs. 15A-15K. Olaparib induces stromal activation *in vivo* both in healthy and metastatic tissues. **FIG. 15A-15C** shows Olaparib induces stromal activation in tumor-invaded lymph nodes and bones in TRAMP⁺/FVB castrated mice. Experimental design (**FIG. 15A**). IHC for HA and α SMA in tumor-invaded lymph nodes from Olaparib- or Veh-treated mice (n=16) showing increased stromal content upon Olaparib treatment, scale bars 50 μ m **FIG. 15B** shows IHC for HA in bone from Olaparib- or Veh-treated mice (n=3) showing increased HA deposition in the growth plate and stromal areas, scale bars 100 μ m (C). **FIG. 15D-15E** show bone in culture (BICA) assay on femur pieces pre-treated or not with Olaparib 20 μ M for 48 h cocultured with RM1-BoMe3 tumor epithelial cells for 48 h. Experimental design (**FIG. 15D**). Left representative images of RFP⁺ RM1-BoMe3 cells attached to the bone. Right, graph or RFP⁺ positive area showing increased tumor homing of RM1-BoMe3 in Olaparib pre-treated bones (**FIG. 15E**). **FIG. 15F** shows qPCR analysis of mRNA levels of indicated genes in isolated bone marrow cells from Olaparib-40mg/kg or veh-treated mice (n=3). **FIG. 15G** shows qPCR analysis of mRNA levels of indicated genes in cultured primary bone marrow cells treated with Olaparib 10 μ M or DMSO (96 h). **FIG. 15H-15J** illustrate caudal artery injection of RM1-BoMe3 mouse metastasis model showing that Olaparib induces HA deposition and activation of TGF β signaling. Experimental design (**FIG. 15H**). IHC for HA showing increased HA deposition in Olaparib-treated liver metastasis (n=3), scale bars 100 μ m (**FIG. 15I**). IHC for pSMAD2 showing an increased pSMAD2 staining in bone metastasis in Olaparib- treated mice (n=3), scale bars 100 μ m (**FIG. 15J**). **FIG. 15K** shows IHC for HA in pre- and post- Olaparib treatment in a prostate cancer bone metastasis showing increased HA deposition in Olaparib-treated mice, scale bars 100 μ m.

Detailed Description

Mounting evidence identifies the tumor microenvironment (TME), including stromal fibroblasts, as a driver of tumorigenesis in many types of tumors, including prostate cancer (PCa) (Klemm and Joyce, 2015; Nakanishi et al., 2018; Zhang et al., 2013; Zhang et al., 2020). It is well accepted now that the activation of tumor stromal fibroblasts to a state commonly known as cancer-associated fibroblasts (CAFs) can embolden epithelial cancer cells to progress to more malignant stages

and affect disease outcomes (Barron and Rowley, 2012; Cunha et al., 2002; Rowley, 1998; Tuxhorn et al., 2001). CAFs impact the tumor epithelium by modulating many functions through the secretion of stromal growth factors and inflammatory mediators, by reprogramming their metabolism to provide nutrients and survival factors, and by remodeling the extracellular matrix (Hanahan and Weinberg, 2011; Kasashima et al., 2020; Sahai et al., 2020). This fibroblast-epithelium synergistic crosstalk promotes malignancy and is believed to negatively impact cancer treatment by promoting the accumulation of “persisting” or “resistant” tumor cells, which limit therapy efficacy and promote resistance (Bluemn et al., 2017; Linares et al., 2017; Zhang et al., 2020). The renewed interest in the study of CAFs has resulted in the extensive characterization of their cell heterogeneity as well as the establishment of a complex catalogue of the myriad of secreted intermediates that likely impact tumor progression and response to therapy. However, a major gap in the field is the identification of the key master regulators of the acquisition of the CAF phenotype, as well as the molecular mechanisms whereby the tumor epithelium drives this process.

The autophagy substrate and signaling adaptor p62 (encoded by *SQSTM1* gene) is a regulator of CAF biology (Huang et al., 2018; Linares et al., 2017; Valencia et al., 2014). P62 has a dual role in cancer (Duran et al., 2011; Duran et al., 2016; Duran et al., 2008; Moscat et al., 2016; Umemura et al., 2016). Thus, while it plays an important oncogenic function in the tumor epithelium, it is a suppressor of tumor progression by restraining CAF activation (Duran et al., 2016; Goruppi and Dotto, 2013; Linares et al., 2017; Reina-Campos et al., 2018; Valencia et al., 2014). It is well established that p62 is upregulated in the epithelium of many types of tumors, which invariably associates with more aggressive cancer and poorer overall survival (Moscat and Diaz-Meco, 2009; Moscat et al., 2016). However, many tumors display reduced levels of p62 in their stroma, especially in CAFs (Duran et al., 2016; Goruppi and Dotto, 2013; Linares et al., 2017; Valencia et al., 2014). p62 downregulation is a central event in the acquisition of the CAF phenotype and in creating a pro-tumorigenic microenvironment conducive to cancer (Linares et al., 2017; Valencia et al., 2014). From a mechanistic point of view, p62 inactivation in prostate stromal fibroblasts impairs their metabolic detoxification capacity and leads to the accumulation of reactive oxygen species, which in turn results in the release of pro-survival inflammatory cytokines (Valencia et al., 2014). Interestingly, under

nutrient stress conditions, the downregulation of p62 in fibroblasts also promotes tumorigenesis by enabling their own survival and that of tumor epithelial cells (Linares et al., 2017). Specifically, under conditions of glutamine deprivation, a common situation in the TME of highly aggressive cancers, the loss of p62 in
5 stromal fibroblasts reprograms their metabolism to produce and secrete asparagine to maintain protein synthesis in the tumor epithelium (Linares et al., 2017). These studies highlight the pivotal role of p62 as a tumor suppressor in the stroma by activating pro-survival pathways and the cellular adaptation to nutrient deprivation.

Therefore, whereas the genetic inactivation of p62 selectively in the tissue
10 epithelium impairs tumorigenesis, that in the stromal fibroblasts has the opposite effect by impacting several metabolic and inflammatory pathways (Moscato et al., 2016). The fact that whole body genetic inactivation of p62 results in increased tumorigenesis, mimicking the phenotype of mice with p62 deficiency in the fibroblast stromal compartment, strongly suggests that the stromal role of p62 as a
15 tumor suppressor is what determines the global impact of p62 in cancer (Valencia et al., 2014). This indicates that the tumor-promoting signals produced by the loss of p62 in CAFs override the requirement for p62 in the tumor epithelium, which highlights the importance of studying how p62 is lost in the tumor's fibroblast compartment. In this regard, a key pending question is to determine how tumors
20 downregulate p62 in the stroma to promote the CAF phenotype. Identifying this mechanism is of paramount importance because, given the role of p62 in CAF activation and function, this will provide a paradigm for understanding of the master regulators of stromal activation, which will be more amenable to therapeutic intervention than trying to affect the complex downstream effectors unleashed upon
25 stromal activation.

PARP1

The poly (ADP-ribose) polymerase 1 (PARP1) enzyme helps cells repair themselves if they become damaged by serving as a "sensor" for DNA strand breaks. Increased PARP1 expression is sometimes observed in melanomas, breast cancer,
30 lung cancer, and other neoplastic diseases. PARP inhibitors stop PARP from repairing cancer cells.

The human PARP1 gene (gene ID: 142) is located on chromosome 1 (NCBI NC_000001.11 (226360691..226408093, complement)). An amino acid sequence

for human PARP1 is available in the Uniprot database as accession no. P09874 and shown below as SEQ ID NO:1.

	10	20	30	40	50
5	MAESSDKLYR	VEYAKSGRAS	CKKCSESIPK	DSLRLAIMVQ	SPMFDGKVPH
	60	70	80	90	100
	WYHFSDFWKV	GHSIRHPDVE	VDGFSELRWD	DQQKVKKTAE	AGGVTGKGQD
	110	120	130	140	150
	GIGSKAEKTL	GDFAAEYAKS	NRSTCKGCME	KIEKGQVRLS	KKMVDPEKPQ
10	160	170	180	190	200
	LGMIDRWYHP	GCFVKNREEL	GFRPEYSASQ	LKGFSLATE	DKEALKKQLP
	210	220	230	240	250
	GVKSEGRKRG	DEVGDVDEVA	KKKSKKEKDK	DSKLEKALKA	QNDLIWNIKD
	260	270	280	290	300
15	ELKKVCSTND	LKELLIFNKG	QVPSGESAIL	DRVADGMVFG	ALLPCEECSE
	310	320	330	340	350
	QLVFKSDAYY	CTGDVTAWTK	CMVKTQTPNR	KEWVTPKEFR	EISYLKKLKV
	360	370	380	390	400
	KKQDRIFPPE	TSASVAATPP	PSTASAPAAV	NSSASADKPL	SNMKILTGLK
	410	420	430	440	450
20	LSRNKDEVKA	MIEKLGGKLT	GTANKASLCI	STKKEVEKMN	KKMEEVKEAN
	460	470	480	490	500
	IRVVEDFLQ	DVSASTKSLQ	ELFLAHILSP	WGAEVKAEPV	EVVAPRGKSG
	510	520	530	540	550
25	AALSKKSKGQ	VKEEGINKSE	KRMKLTLLKG	AAVDPDSGLE	HSAHVLEKGG
	560	570	580	590	600
	KVFSATLGLV	DIVKGTNSYY	KLQLLEDDKE	NRYWIFRSWG	RVGTVIGSNK
	610	620	630	640	650
	LEQMPSKEDA	IEHFMKLYEE	KTGNAWHSKN	FTKYPKKFYP	LEIDYGQDEE
	660	670	680	690	700
30	AVKKLTVNPG	TKSKLPKPVQ	DLIKMIFDVE	SMKKAMVEYE	IDLQKMPLGK
	710	720	730	740	750
	LSKRQIQAAAY	SILSEVQAV	SQGSSDSQIL	DLSNRFYTLI	PHDFGMKKPP
	760	770	780	790	800
35	LLNNADSVQA	KVEMLDNLLD	IEVAYSLLRG	GSDDSSKDPI	DVNYEKLKTD
	810	820	830	840	850
	IKVVDKRDSEE	AEIIRKYVKN	THATTHNAYD	LEVIDIFKIE	REGECQRYKP
	860	870	880	890	900
	FKQLHNRRL	WHGSRTTNFA	GILSQGLRIA	PPEAPVTGYM	FGKGIYFADM
	910	920	930	940	950
40	VSKSANYCHT	SQGDPIGLIL	LGEVALGNMY	ELKHASHISK	LPKGKHSVKG
	960	970	980	990	1000
	LGKTTDPDSA	NISLDGVDVP	LGTGISSGVN	DTSLLYNEYI	VYDIAQVNLK
	1010				
45	YLLKLKFNFK	TSLW			

A cDNA sequence for human PARP1 is available in the NCBI database as accession no. NM_001618.4 and shown below as SEQ ID NO:2.

	1	AGCAATCTAT	CAGGGAACGG	CGGTGGCCGG	TGCGGCGTGT
	41	TCGGTGGCGG	CTCTGGCCGC	TCAGGCGCCT	GCGGCTGGGT
50	81	GAGCGCACGC	GAGGCGGCGA	GGCGGCAGCG	TGTTTCTAGG
	121	TCGTGGCGTC	GGGCTTCCGG	AGCTTTGGCG	GCAGCTAGGG
	161	GAGGATGGCG	GAGTCTTCGG	ATAAGCTCTA	TCGAGTCGAG
	201	TACGCCAAGA	GCGGGCGCGC	CTCTTGCAAG	AAATGCAGCG
	241	AGAGCATCCC	CAAGGACTCG	CTCCGGATGG	CCATCATGGT
55	281	GCAGTCGCCC	ATGTTTGATG	GAAAAGTCCC	AACTGGGTAC
	321	CACTTCTCCT	GCTTCTGGAA	GGTGGGCCAC	TCCATCCGGC

	361	ACCCTGACGT	TGAGGTGGAT	GGGTTCTCTG	AGCTTCGGTG
	401	GGATGACCAG	CAGAAAGTCA	AGAAGACAGC	GGAAGCTGGA
	441	GGAGTGACAG	GCAAAGGCCA	GGATGGAATT	GGTAGCAAGG
	481	CAGAGAAGAC	TCTGGGTGAC	TTTGCAGCAG	AGTATGCCAA
5	521	GTCCAACAGA	AGTACGTGCA	AGGGGTGTAT	GGAGAAGATA
	561	GAAAAGGGCC	AGGTGCGCCT	GTCCAAGAAG	ATGGTGGACC
	601	CGGAGAAGCC	ACAGCTAGGC	ATGATTGACC	GCTGGTACCA
	641	TCCAGGCTGC	TTTGTCAAGA	ACAGGGAGGA	GCTGGGTTTC
	681	CGGCCCAGT	ACAGTGCAG	TCAGCTCAAG	GGCTTCAGCC
10	721	TCCTTGCTAC	AGAGGATAAA	GAAGCCCTGA	AGAAGCAGCT
	761	CCCAGGAGTC	AAGAGTGAAG	GAAAGAGAAA	AGGCGATGAG
	801	GTGGATGGAG	TGGATGAAGT	GGCGAAGAAG	AAATCTAAAA
	841	AAGAAAAAGA	CAAGGATAGT	AAGCTTGAAA	AAGCCCTAAA
	881	GGCTCAGAAC	GACCTGATCT	GGAACATCAA	GGACGAGCTA
15	921	AAGAAAGTGT	GTTCAACTAA	TGACCTGAAG	GAGCTACTCA
	961	TCTTCAACAA	GCAGCAAGTG	CCTTCTGGGG	AGTCGGCGAT
	1001	CTTGGACCGA	GTAGCTGATG	GCATGGTGTT	CGGTGCCCTC
	1041	CTTCCCTGCG	AGGAATGCTC	GGGTCAGCTG	GTCTTCAAGA
	1081	GCGATGCCTA	TTACTGCACT	GGGGACGTCA	CTGCCTGGAC
20	1121	CAAGTGTATG	GTCAAGACAC	AGACACCCAA	CCGGAAGGAG
	1161	TGGGTAACCC	CAAAGGAATT	CCGAGAAATC	TCTTACCTCA
	1201	AGAAATTGAA	GGTTAAAAAA	CAGGACCGTA	TATTCCCCCC
	1241	AGAAACCAGC	GCCTCCGTGG	CGGCCACGCC	TCCGCCCTCC
	1281	ACAGCCTCGG	CTCCTGCTGC	TGTGAACTCC	TCTGCTTCAG
25	1321	CAGATAAGCC	ATTATCCAAC	ATGAAGATCC	TGACTCTCGG
	1361	GAAGCTGTCC	CGGAACAAGG	ATGAAGTGAA	GGCCATGATT
	1401	GAGAAACTCG	GGGGGAAGTT	GACGGGGACG	GCCAACAAGG
	1441	CTTCCCTGTG	CATCAGCACC	AAAAAGGAGG	TGGAAAAGAT
	1481	GAATAAGAAG	ATGGAGGAAG	TAAAGGAAGC	CAACATCCGA
30	1521	GTTGTGTCTG	AGGACTTCCT	CCAGGACGTC	TCCGCCTCCA
	1561	CCAAGAGCCT	TCAGGAGTTG	TTCTTAGCGC	ACATCTTGTC
	1601	CCCTTGGGGG	GCAGAGGTGA	AGGCAGAGCC	TGTTGAAGTT
	1641	GTGGCCCCAA	GAGGGAAGTC	AGGGGCTGCG	CTCTCCAAAA
	1681	AAAGCAAGGG	CCAGGTCAAG	GAGGAAGGTA	TCAACAAATC
35	1721	TGAAAAGAGA	ATGAAATTAA	CTCTTAAAGG	AGGAGCAGCT
	1761	GTGGATCCTG	ATTCTGGACT	GGAACACTCT	GCGCATGTCC
	1801	TGGAGAAAGG	TGGGAAGGTC	TTCAGTGCCA	CCCTTGGCCT
	1841	GGTGGACATC	GTTAAAGGAA	CCAACCTCCTA	CTACAAGCTG
	1881	CAGCTTCTGG	AGGACGACAA	GGAAAACAGG	TATTGGATAT
40	1921	TCAGGTCCTG	GGGCCGTGTG	GGTACGGTGA	TCGGTAGCAA
	1961	CAAACCTGGAA	CAGATGCCGT	CCAAGGAGGA	TGCCATTGAG
	2001	CACTTCATGA	AATTATATGA	AGAAAAAACC	GGGAACGCTT
	2041	GGCACTCCAA	AAATTTACAG	AAGTATCCCA	AAAAGTTCTA
	2081	CCCCCTGGAG	ATTGACTATG	GCCAGGATGA	AGAGGCAGTG
45	2121	AAGAAGCTGA	CAGTAAATCC	TGGCACCAAG	TCCAAGCTCC
	2161	CCAAGCCAGT	TCAGGACCTC	ATCAAGATGA	TCTTTGATGT
	2201	GGAAAGTATG	AAGAAAGCCA	TGGTGGAGTA	TGAGATCGAC
	2241	CTTCAGAAGA	TGCCCTTGGG	GAAGCTGAGC	AAAAGGCAGA
	2281	TCCAGGCCGC	ATACTCCATC	CTCAGTGAGG	TCCAGCAGGC
50	2321	GGTGTCTCAG	GGCAGCAGCG	ACTCTCAGAT	CCTGGATCTC
	2361	TCAAATCGCT	TTTACACCCT	GATCCCCCAC	GACTTTGGGA

	2401	TGAAGAAGCC	TCCGCTCCTG	AACAATGCAG	ACAGTGTGCA
	2441	GGCCAAGGTG	GAAATGCTTG	ACAACCTGCT	GGACATCGAG
	2481	GTGGCCTACA	GTCTGCTCAG	GGGAGGGTCT	GATGATAGCA
	2521	GCAAGGATCC	CATCGATGTC	AACTATGAGA	AGCTCAAAAC
5	2561	TGACATTAAG	GTGGTTGACA	GAGATTCTGA	AGAAGCCGAG
	2601	ATCATCAGGA	AGTATGTTAA	GAACACTCAT	GCAACCACAC
	2641	ACAATGCGTA	TGACTTGGA	GTCATCGATA	TCTTTAAGAT
	2681	AGAGCGTGAA	GGCGAATGCC	AGCGTTACAA	GCCCTTTAAG
	2721	CAGCTTCATA	ACCGAAGATT	GCTGTGGCAC	GGGTCCAGGA
10	2761	CCACCAACTT	TGCTGGGATC	CTGTCCCAGG	GTCTTCGGAT
	2801	AGCCCCGCCT	GAAGCGCCCG	TGACAGGCTA	CATGTTTGGT
	2841	AAAGGGATCT	ATTTGCTGA	CATGGTCTCC	AAGAGTGCCA
	2881	ACTACTGCCA	TACGTCTCAG	GGAGACCCAA	TAGGCTTAAT
	2921	CCTGTTGGGA	GAAGTTGCCC	TTGGAAACAT	GTATGAACTG
15	2961	AAGCACGCTT	CACATATCAG	CAAGTTACCC	AAGGGCAAGC
	3001	ACAGTGTCAA	AGGTTTGGGC	AAAACCTACC	CTGATCCTTC
	3041	AGCTAACATT	AGTCTGGATG	GTGTAGACGT	TCCTCTTGGG
	3081	ACCGGGATTT	CATCTGGTGT	GAATGACACC	TCTCTACTAT
	3121	ATAACGAGTA	CATTGTCTAT	GATATTGCTC	AGGTAAATCT
20	3161	GAAGTATCTG	CTGAAACTGA	AATTCAATTT	TAAGACCTCC
	3201	CTGTGGTAAT	TGGGAGAGGT	AGCCGAGTCA	CACCCGGTGG
	3241	CTCTGGTATG	AATTCACCCG	AAGCGCTTCT	GCACCAACTC
	3281	ACCTGGCCGC	TAAGTTGCTG	ATGGGTAAGTA	CCTGTACTAA
	3321	ACCACCTCAG	AAAGGATTTT	ACAGAAACGT	GTTAAAGGTT
25	3361	TTCTCTAACT	TCTCAAGTCC	CTTGTTTTGT	GTTGTGTCTG
	3401	TGGGGAGGGG	TTGTTTTGGG	GTTGTTTTTG	TTTTTTCTTG
	3441	CCAGGTAGAT	AAAACCTGACA	TAGAGAAAAG	GCTGGAGAGA
	3481	GATTCTGTTG	CATAGACTAG	TCCTATGGAA	AAAACCAAGC
	3521	TTCGTTAGAA	TGTCTGCCTT	ACTGGTTTCC	CCAGGGAAGG
30	3561	AAAAATACAC	TTCCACCCTT	TTTTCTAAGT	GTTTCGTCTTT
	3601	AGTTTTGATT	TTGGAAAGAT	GTTAAGCATT	TATTTTTAGT
	3641	TAAAAATAAA	AACTAATTTC	ATACTATTTA	GATTTTCTTT
	3681	TTTATCTTGC	ACTTATTGTC	CCCTTTTTAG	TTTTTTTTGT
	3721	TTGCCTCTTG	TGGTGAGGGG	TGTGGGAAGA	CCAAAGGAAG
35	3761	GAACGCTAAC	AATTTCTCAT	ACTTAGAAAC	AAAAAGAGCT
	3801	TTCTTCTCC	AGGAATACTG	AACATGGGAG	CTCTTGAAAT
	3841	ATGTAGTATT	AAAAGTTGCA	TTTGAAATTC	TTGACTTTCT
	3881	TATGGGCACT	TTTGTCTTCC	AAATTAAAAC	TCTACCACAA
	3921	ATATACTTAC	CCAAGGGCTA	ATAGTAATAC	TCGATTAAAA
40	3961	ATGCAGATGC	CTTCTCTA		

Subjects can be effectively treated by the compositions and methods described herein even though those subjects have PARP1 isoforms and variants with somewhat different PARP1 sequences than the examples of PARP1 sequences described herein. Such PARP1 isoforms and variants can have polypeptide or nucleic acid sequences with between 55-100% sequence identity to a reference sequence (e.g., a sequence described herein). For example, PARP1 isoforms and variants can have at least 55% sequence identity, e.g., at least 60%, at least 70%, at

least 80%, at least 90%, at least 95%, at least 96%, at least 97% sequence, at least 98%, at least 99% identity to a reference sequence (e.g., a sequence described herein) over a specified comparison window. Optimal alignment may be ascertained or conducted using the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443-53 (1970).

Hyaluronan Synthase

Hyaluronan or hyaluronic acid is a high molecular weight unbranched polysaccharide synthesized within a variety of organisms, including mammals. Hyaluronan or hyaluronic acid is a constituent of the extracellular matrix. It consists of alternating glucuronic acid and N-acetylglucosamine residues that are linked by beta-1-3 and beta-1-4 glycosidic bonds. Hyaluronic acid is synthesized by membrane-bound synthase at the inner surface of the plasma membrane, and the chains are extruded via ABC-Transporter into the extracellular space. It serves a variety of functions, including space filling, lubrication of joints, and provision of a matrix through which cells can migrate.

For example, a human hyaluronan synthase 1 (HAS1) gene is on chromosome 19 (NCBI NC_000019.10 (51713112..51723991, complement), and HAS1 encodes the following human amino acid sequence (NCBI accession no. Q92839.2; SEQ ID NO:3).

```

20      1 MRQQDAPKPT PAACRCSGLA RRVLTIAFAL LILGLMTWAY
      41 AAGVPLASDR YGLLAFGLYG AFLSAHLVAQ SLFAYLEHRR
      81 VAAAARGPLD AATARSVALT ISAYQEDPAY LRQCLASARA
     121 LLYPRARLRV LMVVDGNRAE DLYMVDMFRE VFADEDPATY
     161 VWDGNYHQPW EPAAAGAVGA GAYREVEAED PGRLAVEALV
25     201 RTRRCVCVAQ RWGGKREVMY TAFKALGDSV DYVQVCDSDT
     241 RLDPMALLEL VRVLDEDPRV GAVGGDVRIL NPLDSWVSFL
     281 SSLRYWVAFN VERACQSYFH CVSCISGPLG LYRNNLLQQF
     321 LEAWYNQKFL GTHCTFGDDR HLTNRMLSMG YATKYTSRSR
     361 CYSETPSSFL RWLSQQTRWS KSYFREWLYN ALWWHRHHAW
30     401 MTYEAVVSGL FPFVFAATVL RLFYAGRPWA LLWVLLCVQG
     441 VALAKAAFAA WLRGCLRMVL LSLYAPLYMC GLLPAKFLAL
     481 VTMNQSGWGT SGRRKLAANY VPLLPLALWA LLLLGGGLVRS
     521 VAHEARADWS GPSRAAEAYH LAAGAGAYVG YWVAMLTLYW
     561 VGVRRLCRRR TGGYRVQV

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A cDNA sequence for an HAS1 polypeptide can have the following human nucleotide sequence (NCBI accession no. NM_001297436.2; SEQ ID NO:4).

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1 ACAGAGAGGC TGGGAGAGCA AGCCGGAGAG AAGAGAGAGC

```

	41	CCGGCCAGAC	CCACTGCGAT	GAGACAGGAC	GCGCCCAAGC
	81	CCACTCCTGC	AGCCTGCCGC	TGCTCCGGCC	TGGCCCGGAG
	121	GGTGCTGACC	ATCGCCTTCG	CCCTGCTCAT	CCTGGGCCTC
	161	ATGACCTGGG	CCTACGCCGC	CGGGGTGCCG	CTGGCCTCCG
5	201	ATCGCTACGG	CCTCCTGGCC	TTCGGCCTCT	ACGGGGCCTT
	241	CCTTTCAGCG	CACCTGGTGG	CGCAGAGCCT	CTTCGCGTAC
	281	CTGGAGCACC	GGCGGGTGGC	GGCGGCGGCG	CGGGGGCCGC
	321	TGGATGCAGC	CACCGCGCGC	AGTGTGGCGC	TGACCATCTC
	361	CGCCTACCAG	GAGGACCCCG	CGTACCTGCG	CCAGTGCCTG
10	401	GCGTCCGCCC	GCGCCCTGCT	GTACCCGCGC	GCGCGGCTGC
	441	GCGTCCTCAT	GGTGGTGGAT	GGCAACCGCG	CCGAGGACCT
	481	CTACATGGTC	GACATGTTCC	GCGAGGTCTT	CGCTGACGAG
	521	GACCCCGCCA	CGTACGTGTG	GGACGGCAAC	TACCACCAGC
	561	CCTGGGAACC	CGCGGCGGCG	GGCGCGGTGG	GCGCCGGAGC
15	601	CTATCGGGAG	GTGGAGGCGG	AGGATCCTGG	GCGGCTGGCA
	641	GTGGAGGCGC	TGGTGAGGAC	TCGCAGGTGC	GTGTGCGTGG
	681	CGCAGCGCTG	GGGCGGCAAG	CGCGAGGTCA	TGTACACAGC
	721	CTTCAAGGCG	CTCGGAGATT	CGGTGGACTA	CGTGCAGGTC
	761	TGTGACTCGG	ACACAAGGTT	GGACCCCATG	GCACTGCTGG
20	801	AGCTCGTGCG	GGTACTGGAC	GAGGACCCCG	GGGTAGGGGC
	841	TGTTGGTGGG	GACGTGCGGA	TCCTTAACCC	TCTGGACTCC
	881	TGGGTCAGCT	TCCTAAGCAG	CCTGCGATAC	TGGGTAGCCT
	921	TCAATGTGGA	GCGGGCTTGT	CAGAGCTACT	TCCACTGTGT
	961	ATCCTGCATC	AGCGGTCCTC	TAGGCCTATA	TAGGAATAAC
25	1001	CTCTTGACAGC	AGTTTCTTGA	GGCCTGGTAC	AACCAGAAGT
	1041	TCCTGGGTAC	CCACTGTACT	TTTGGGGATG	ACCGGCACCT
	1081	CACCAACCGC	ATGCTCAGCA	TGGGTTATGC	TACCAAGTAC
	1121	ACCTCCAGGT	CCCGCTGCTA	CTCAGAGACG	CCCTCGTCCT
	1161	TCCTGCGGTG	GCTGAGCCAG	CAGACACGCT	GGTCCAAGTC
30	1201	GTACTTCCGT	GAGTGGCTGT	ACAACGCGCT	CTGGTGGCAC
	1241	CGGCACCATG	CGTGGATGAC	CTACGAGGCG	GTGGTCTCCG
	1281	GCCTGTTCCC	CTTCTTCGTG	GCGGCCACTG	TGCTGCGTCT
	1321	GTTCTACGCG	GGCCGCCCTT	GGGCGCTGCT	GTGGGTGCTG
	1361	CTGTGCGTGC	AGGGCGTGGC	ACTGGCCAAG	GCGGCCTTCG
35	1401	CGGCCTGGCT	GCGGGGCTGC	CTGCGCATGG	TGCTTCTGTC
	1441	GCTCTACGCG	CCCCTCTACA	TGTGTGGCCT	CCTGCCTGCC
	1481	AAGTTCCCTG	CGCTAGTCAC	CATGAACCAG	AGTGGCTGGG
	1521	GCACCTCGGG	CCGGCGGAAG	CTGGCCGCTA	ACTACGTCCC
	1561	TCTGCTGCCC	CTGGCGCTCT	GGGCGCTGCT	GCTGCTTGGG
40	1601	GGCCTGGTCC	GCAGCGTAGC	ACACGAGGCC	AGGGCCGACT
	1641	GGAGCGGCCC	TTCCCGCGCA	GCCGAGGCCT	ACCACTTGGC
	1681	CGCGGGGGCC	GGCGCCTACG	TGGGCTACTG	GGTGGCCATG
	1721	TTGACGCTGT	ACTGGGTGGG	CGTGCGGAGG	CTTTGCCGGC
	1761	GGCGGACCGG	GGGCTACCGC	GTCCAGGTGT	GAGTCCAGCC
45	1801	ACGCGGATGC	CGCCTCAAGG	GTCTTCAGGG	GAGGCCAGAG
	1841	GAGAGCTGCT	GGGCCCCGAG	CCACGAACCT	GCTGGGTGGT
	1881	TCTCTGGGCC	TCAGTTTCCC	TCCTCTGCAA	AACGAGGGGG
	1921	TCAGCCCAAG	ATTCTTCAGT	CTGGACTATA	TTGGGACTGG
	1961	GACTTCTGGG	TCTCCAGGGA	GGGTATTTAT	TGGTCAGGGT
50	2001	GTGGGATCTG	AGGAGTGGAG	GGAAAGGGTC	CTGCTTTCTC
	2041	CTCGTTCTTA	TTTAATCTCC	ATTTCTACTG	TGTGATCAGG

2081 ATGTAATAAA GAATTTTATT TATTTTC

In another example, a human hyaluronan synthase 2 (HAS2) gene is on chromosome 8 (NCBI NC_000008.11 (121612116..121641440, complement), and

5 HAS2 encodes the following human amino acid sequence (NCBI accession no. NP_005319.1; SEQ ID NO:5).

	1	MHCERFLCIL	RIIGTTLFGV	SLLLGITAAY	IVGYQFIQTD
	41	NYFYSFGLYG	AFLASHLIIQ	SLFAFLEHRK	MKKSLETPIK
	81	LNKTVALCIA	AYQEDPDYLR	KCLQSVKRLT	YPGIKVVMVI
10	121	DGNSEDDLYM	MDIFSEVMGR	DKSATYIWKN	NFHEKGPGET
	161	DESHKESSQH	VTQLVLSNKS	ICIMQKWGGK	REVMYTAfra
	201	LGRSVDYVQV	CSDTMLDPA	SSVEMVKVLE	EDPMVGGVGG
	241	DVQILNKYDS	WISFLSSVRY	WMAFNIERAC	QSYFGCVQCI
	281	SGPLGMYRNS	LLHEFVEDWY	NQEFMGNQCS	FGDDRHLTNR
15	321	VLSLGYATKY	TARSKCLTET	PIEYLRWLNQ	QTRWSKSYFR
	361	EWLYNAMWFH	KHHLWMTYEA	IITGFFPFPL	IATVIQLFYR
	401	GKIWNILLFL	LTVQLVGLIK	SSFASCLRGN	IVMVFMSLYS
	441	VLYMSSLLPA	KMFAIATINK	AGWGTSGRKT	IVVNFIGLIP
	481	VSVWFTILLG	GVIFTIYKES	KRPFSESKQT	VLIVGTLLYA
20	521	CYWVMLLTLY	VVLINKCGRR	KKGQQYDMVL	DV

A cDNA encoding this HAS2 amino acid sequence is shown below as SEQ ID NO:6 (NCBI accession no. NM_005328.3)

	1	AGACCCCTT	AAGTTGGAGG	AGGCAGAAGG	GCAACAACGG
25	41	CGGGGAAGGA	GAAGTCAAGA	CGTCTGGAAA	GAATTACCCA
	81	GTCCTGGCTT	CGAGCAGCCC	ATTGAACCAG	AGACTTGAAA
	121	CAGCCCCAGC	CAAAGACTTT	TCTCCCAATT	CTGCGCTTCC
	161	TGGGTTCCTG	TGAGTCTTCC	ACAGGCTTTT	TTTTTTTTTT
	201	TTTTTTTTTT	TAAGACGAAA	AAGAGATTTT	CTGTTATCGG
30	241	GGGCAGAAAG	ACTGAAGCCC	AAAAAAAAAA	AAAAAAAAAA
	281	AAGAAAAGAA	AAGAAAAAAG	AAAAGTTAAT	TTATTTTTAA
	321	AGCATAATTT	TTTTAAGAAT	TAGACTGAAG	TGCAACGGAA
	361	ACATAAAGAG	AATATTAGTG	AAATTATTTT	TTAAAGTGGG
	401	GAAGAATCAA	ACATTTAAGA	CTCCCCTATC	CTTTTTAAAT
35	441	GTTGTTTTTA	AATTTCTTAT	TTTTTTTGGC	CGGTCGTCTC
	481	AAATTCATCT	GATCTCTTAT	TACCTCAATT	TTGGAAACTG
	521	CCCGCCACCG	ACCCTCCGGG	ACCACACAGA	CAGGCTGAGG
	561	ACGACTTTAT	GACCAAGAGC	TGAACAAGAT	GCATTGTGAG
	601	AGGTTTCTAT	GTATCCTGAG	AATAATTGGA	ACCACACTCT
40	641	TTGGAGTCTC	TCTCCTCCTT	GGAATCACAG	CTGCTTATAT
	681	TGTTGGCTAC	CAGTTTATCC	AAACGGATAA	TTACTATTTT
	721	TCTTTTGGAC	TGTATGGTGC	CTTTTGGCA	TCACACCTCA
	761	TCATCCAAAG	CCTGTTTGCC	TTTTTGGAGC	ACCGAAAAAT
	801	GAAAAAATCC	CTAGAAACCC	CCATAAAGTT	GAACAAAACA
45	841	GTTGCCCTTT	GCATCGCTGC	CTATCAAGAA	GATCCAGACT
	881	ACTTAAGGAA	ATGTTTGCAA	TCTGTGAAAA	GGCTAACCTA
	921	CCCTGGGATT	AAAGTTGTCA	TGGTCATAGA	TGGGAACCTA

	961	GAAGATGACC	TTTACATGAT	GGACATCTTC	AGTGAAGTCA
	1001	TGGGCAGAGA	CAAATCAGCC	ACTTATATCT	GGAAGAACAA
	1041	CTTCCACGAA	AAGGGTCCCG	GTGAGACAGA	TGAGTCACAT
	1081	AAAGAAAGCT	CGCAACACGT	AACGCAATTG	GTCTTGTTCA
5	1121	ACAAAAGTAT	CTGCATCATG	CAAAAATGGG	GTGGAAAAAG
	1161	AGAAGTCATG	TACACAGCCT	TCAGAGCACT	GGGACGAAGT
	1201	GTGGATTATG	TACAGGTTTG	TGATTTCAGAC	ACTATGCTTG
	1241	ACCCAGCCTC	ATCTGTGGAG	ATGGTAAAAG	TTTTAGAAGA
	1281	AGATCCCATG	GTTGGAGGTG	TTGGGGGAGA	TGTCCAGATT
10	1321	TTAAACAAGT	ACGATTCCCTG	GATCTCATTG	CTCAGCAGTG
	1361	TAAGATATTG	GATGGCTTTT	AATATAGAAA	GGGCCTGTCA
	1401	GTCTTATTTT	GGGTGTGTTT	AGTGCATTAG	TGGACCTCTG
	1441	GGAATGTACA	GAAACTCCTT	GTTGCATGAG	TTTGTGGAAG
	1481	ATTGGTACAA	TCAAGAATTT	ATGGGCAACC	AATGTAGCTT
15	1521	TGGTGATGAC	AGGCATCTCA	CGAACCAGGT	GCTGAGCCTG
	1561	GGCTATGCAA	CAAAATACAC	AGCTCGATCT	AAGTGCCTTA
	1601	CTGAAACACC	TATAGAATAT	CTCAGATGGC	TAAACCAGCA
	1641	GACCCGTTGG	AGCAAGTCCT	ACTTCCGAGA	ATGGCTGTAC
	1681	AATGCAATGT	GGTTTCACAA	ACATCACTTG	TGGATGACCT
20	1721	ACGAAGCGAT	TATCACTGGA	TTCTTTCCTT	TCTTCTCAT
	1761	TGCCACAGTA	ATCCAGCTCT	TCTACCGGGG	TAAAATTTGG
	1801	AACATTCTCC	TCTTCTTGTT	AAGTGTCCAG	CTAGTAGGTC
	1841	TCATAAAATC	ATCTTTTGCC	AGCTGCCTTA	GAGGAAATAT
	1881	CGTCATGGTC	TTCATGTCTC	TCTACTCAGT	GTTATACATG
25	1921	TCGAGTTTAC	TTCCCGCCAA	GATGTTTGCA	ATTGCAACAA
	1961	TAAACAAAGC	TGGGTGGGGC	ACATCAGGAA	GGAAAACCAT
	2001	TGTTGTTAAT	TTCATAGGAC	TCATTCCAGT	ATCAGTTTGG
	2041	TTTACAATCC	TCCTGGGTGG	TGTGATTTTC	ACCATTTATA
	2081	AGGAGTCTAA	AAGGCCATTT	TCAGAATCCA	AACAGACAGT
30	2121	TCTAATTGTT	GGAACGTTGC	TCTATGCATG	CTATTGGGTC
	2161	ATGCTTTTGA	CGCTGTATGT	AGTTCTCATC	AATAAGTGTG
	2201	GCAGGCGGAA	GAAGGGACAA	CAATATGACA	TGGTGCTTGA
	2241	TGTATGATCT	TCCATGTTTT	GACGTTTGCA	GTCACACACA
	2281	ACACCTTAGT	TCCTCTAGGG	GCTGTACAGT	ATTGTGGCAT
35	2321	CAGATAATGC	CACCAAAGGA	GACATATCAC	TGCTGCTGGG
	2361	ACTTGAACAA	AGACATTTAT	ATGGGTTTAT	TTTCATTCTG
	2401	CCAAAGTAAA	ACAATACATC	AACAAGAAGA	AACTCAGATT
	2441	TAACCTGTTA	TTTCTATGAA	AATGGGATGA	ATTCTTTGTT
	2481	TATGCACTTT	TTCTTACTG	TGCATCCGCC	TGAAAGTGTT
40	2521	TTGCCCTATA	TACCTCACTA	GCCATGCTTT	ATGTGGGTGA
	2561	TCATGGAAGA	AAAGGATTTT	GGAAACTCAA	GGAAAAGTTC
	2601	TTTCAACCTA	TACAACCTAA	CTTATGGACT	GTTTTGATAG
	2641	ATGATAATTT	TTTTTTTTTA	GGAAGGATTT	TCTTTTTAAC
	2681	TTTACCAAAT	GAAATGCCAA	AGGAAGTTTT	AAAGGCCGTT
45	2721	GGCTGTGCTG	TATTTTGATA	TAATTGTACT	GTGTTTTTAA
	2761	ATTTTGTATG	CCAATCTTAA	AGACAAATTT	TGCATATTCT
	2801	CTATTTTACT	TTTCTGCCAA	AATAAACCTG	TTCTTCCTTT
	2841	TTTAAAATAA	AATAAGTTCT	TAAAAAATTT	ATACTTAAAA
	2881	AATCCTGCCC	AAAATGTGAA	GCTTGGTTGA	CTGATGTTCA
50	2921	TGATAGAAAG	AATAAAATGT	TTCTCTCTCT	CTACCTTTTA
	2961	AAATTGAATA	GTTTATTTCT	GTGAAAGAAG	TATTTAAACT

	3001	TTCAATATTT	TAACTTTTTG	TTTTTATTTT	TTTGTAGAAAA
	3041	GGCCAATATA	CCTATCACAC	TTTGGAAGTA	AAAATACACA
	3081	CTTTCGTGTG	TACCTAAAAA	AAAAATCGTT	GAAAATCAAG
	3121	GCCAAAGGTA	GTGCAATTTT	TTCATTAAGA	TTTAAAAAAA
5	3161	AGGGAATGAT	AGTCTTTGAA	AGAAAACAGT	AGGCATCCAG
	3201	CACTGGACAA	AACATGGGTA	TCAAAGATGA	ATAATCTTTG
	3241	GAGATTCTGG	CAGTGTTTTT	CCAGAACAAG	TCAAGTGGAA
	3281	AGTGGAGAAA	TTATCTGTAT	AATTTTGGAC	ACATACAATG
	3321	CAGTTTATCA	AAGGTTTTGT	TCTGTGGCCT	GAATTTACTG
10	3361	GGTCCTACCT	ACACATTGAA	CATGTTTTGC	CTGTCTTTTT
	3401	TTTTTTTTTT	AACTTGCCAG	TTCACTTTAC	ATGTTAGTAT
	3441	AATGTTTACA	CGGGTGAGTT	GGATAAATTA	TAAAACATAT
	3481	AAATTCAAAA	TTGGCAGATA	GAATCACCAA	GTATCTATCC
	3521	TCTTTTACTT	TCAAATGAGG	AATTTTGTGT	TTCTGAATTA
15	3561	CACAGATCAT	CACTTCCTAT	TTCCTGTTCT	GGACCTGTAT
	3601	AAAAATGTCT	ACACAGTAGA	AGTGACATCA	AGGTTTAATA
	3641	AGTATATCAA	TGATTGGCAC	ATATAAAAAT	TGTTGAACCA
	3681	CATACTCTGA	ACTTGGCTAA	TTTAGTTACT	GCAAGGCCTC
	3721	CATTATCCAG	TTTTATTTTT	TACACGATTG	ACCTTGCCTT
20	3761	GTAGCTGGTG	CTGTGTAGAC	CTGTGTTGAA	AACACAATCG
	3801	GAATATATGA	ATAATTGAAT	AAACAGCATT	ATGGTGAGGC
	3841	AGAGACACAT	GGAGAAGTGT	TAAAAAATAA	ATGGGCTTCC
	3881	TGCCTTTCTG	CCTCTTTTTT	ATGCAGTCAT	CTATGTTACA
	3921	TCTATCCTGC	CTAAGAAAAA	GCTGCACATC	CTACCTTCAG
25	3961	AGTACAAAAA	GGTACATCTG	AGACTCAAGA	CTCTCACTGA
	4001	TTGGAGAGCT	TGTGGAAAAC	AAAACACACC	ATGCCAATAA
	4041	ATGAGATGAA	AACTTGAGTT	TGCCTTTTTA	ACTATTTATG
	4081	TTCTAAGTTA	AGCTTTGATA	ACATTCAAAT	GTCAAATTCT
	4121	CTCATTCTTA	TAAAAAGTTG	AATTAATTGC	CTGTATTTAT
30	4161	TTTAGCAATT	ATTCAATGTA	TTTCCAGTAT	AGGATGTATA
	4201	GTATAATTAA	TTTTTTGTAA	ATAAAATATT	TTTGATAAGA

Subjects can be effectively treated by the compositions and methods described herein even though those subjects have HAS1 or HAS2 isoforms and variants with somewhat different sequences than the examples of HAS sequences described herein. Such HAS isoforms and variants can have polypeptide or nucleic acid sequences with between 55-100% sequence identity to a reference sequence (e.g., a sequence described herein). For example, HAS isoforms and variants can have at least 55% sequence identity, e.g., at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97% sequence, at least 98%, at least 99% identity to a reference sequence (e.g., a sequence described herein) over a specified comparison window. Optimal alignment may be ascertained or conducted using the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443-53 (1970).

Hyaluronidase

Hyaluronidase is an enzyme that breaks down hyaluronic acid. Although it has been used in medical applications for over 60 years, it is thought exacerbate the spread (metastasis) of cancer. Clinical trials of PEGPH20, a clinical grade hyaluronidase, were discontinued after the investigational drug failed to meet the primary endpoint in a phase 3 trial in metastatic pancreas cancer. However, as illustrated herein, PEGPH20 reverts stroma activation and enhances Olaparib anti-tumor activity.

An example of a hyaluronidase sequence is the following hyaluronidase PH20 sequence (Uniprot P38567-1; SEQ ID NO:7).

10		10	20	30	40
	50				
	MGVLKFKHIF	FRSFVKSSGV	SQIVFTFLLI	PCCLTLNFRA	
	PPVIPNPVFL				
		60	70	80	90
15	100				
	WAWNAPSEFC	LGKFDEPLDM	SLFSFIGSPR	INATGQGVTI	
	FYVDRLGYYP				
		110	120	130	140
	150				
20	YIDSITGVTV	NGGIPQKISL	QDHLDKAKKD	ITFYMPVDNL	
	GMAVIDWEEW				
		160	170	180	190
	200				
	RPTWARNWKP	KDVYKNRSIE	LVQQQNVQLS	LTEATEKAKQ	
25	EFEKAGKDFL				
		210	220	230	240
	250				
	VETIKLGKLL	RPNHLWGYYL	FPDCYNHMYK	KPGYNGSCFN	
	VEIKRNDDLS				
30		260	270	280	290
	300				
	WLWNESTALY	PSIYLNTQQS	PVAATLYVRN	RVREAIRVSK	
	IPDAKSPLPV				
		310	320	330	340
35	350				
	FAYTRIVFTD	QVLKFLSQDE	LVYTFGETVA	LGASGIVIWG	
	TLSIMRSMKS				
		360	370	380	390
	400				
40	CLLLDNYMET	ILNPYIINVT	LAAKMCSQVL	CQEQGVCIRK	
	NWNSSDYLHL				
		410	420	430	440
	450				
	NPDNFAIQLE	KGGKFTVRGK	PTLEDLEQFS	EKFYCSCYST	
45	LSCKEKADV				
		460	470	480	490
	500				

DTDAVDVCIA DGVCIDAFK PPMETEEPQI FYNASPSTLS
ATMFIVSILF

LIISVASL

5

A cDNA sequence for such a hyaluronidase is shown below (NCBI L13781.1; SEQ ID NO:8).

	1	ATTCCATTCC	CTTTCATCTG	TGCTCATACT	TTGCATCAGA
	41	TATTGGGTAA	ACCAAAGTGT	GTAGGAAGAA	ATAAATGTTT
10	81	TCATAGTCAT	TACTCTTTAC	AATGGGAGTG	CTAAAATTCA
	121	AGCACATCTT	TTTCAGAAGC	TTTGTTAAAT	CAAGTGGAGT
	161	ATCCCAGATA	GTTTTACCT	TCCTTCTGAT	TCCATGTTGC
	201	TTGACTCTGA	ATTCAGAGC	ACCTCCTGTT	ATTCCAAATG
	241	TGCCTTTCCT	CTGGGCCTGG	AATGCCCCAA	GTGAATTTTG
15	281	TCTTGGA AAA	TTTGATGAGC	CACTAGATAT	GAGCCTCTTC
	321	TCTTTCATAG	GAAGCCCCCG	AATAAACGCC	ACCGGGCAAG
	361	GAGTTACAAT	ATTTTATGTT	GATAGACTTG	GCTACTATCC
	401	TTACATAGAT	TCAATCACAG	GAGTAACTGT	GAATGGAGGA
	441	ATCCCCCAGA	AGATTTCCTT	ACAAGACCAT	CTGGACAAAG
20	481	CTAAGAAAGA	CATTACATTT	TATATGCCAG	TAGACAATTT
	521	GGGAATGGCT	GTTATTGACT	GGGAAGAATG	GAGACCCACT
	561	TGGGCAAGAA	ACTGGAAACC	TAAAGATGTT	TACAAGAATA
	601	GGTCTATTGA	ATTGGTTCAG	CAACAAAATG	TACAACCTAG
	641	TCTCACAGAG	GCCACTGAGA	AAGCAAAACA	AGAATTTGAA
25	681	AAGGCAGGGA	AGGATTTTCCT	GGTAGAGACT	ATAAAATTGG
	721	GAAAATTACT	TCGGCCAAAT	CACTTGTGGG	GTTATTATCT
	761	TTTTCCGGAT	TGTTACAACC	ATCACTATAA	GAAACCCGGT
	801	TACAATGGAA	GTTGCTTCAA	TGTAGAAATA	AAAAGAAATG
	841	ATGATCTCAG	CTGGTTGTGG	AATGAAAGCA	CTGCTCTTTA
30	881	CCCATCCATT	TATTTGAACA	CTCAGCAGTC	TCCTGTAGCT
	921	GCTACACTCT	ATGTGCGCAA	TCGAGTTCGG	GAAGCCATCA
	961	GAGTTTCCAA	AATACCTGAT	GCAAAAAGTC	CACTTCCGGT
	1001	TTTTGTCATAT	ACCCGCATAG	TTTTTACTGA	TCAAGTTTTG
	1041	AAATTCCTTT	CTCAAGATGA	ACTTGTGTAT	ACATTTGGCG
35	1081	AAACTGTTGC	TCTGGGTGCT	TCTGGAATTG	TAATATGGGG
	1121	AACCCTCAGT	ATAATGCGAA	GTATGAAATC	TTGCTTGCTC
	1161	CTAGACAATT	ACATGGAGAC	TATACTGAAT	CCTTACATAA
	1201	TCAACGTCAC	ACTAGCAGCC	AAAATGTGTA	GCCAAGTGCT
	1241	TTGCCAGGAG	CAAGGAGTGT	GTATAAGGAA	AAACTGGAAT
40	1281	TCAAGTGACT	ATCTTCACCT	CAACCCAGAT	AATTTTGCTA
	1321	TTCAACTTGA	GAAAGGTGGA	AAGTTCACAG	TACGTGGAAA
	1361	ACCGACACTT	GAAGACCTGG	AGCAATTTTC	TGAAAAATTT
	1401	TATTGCAGCT	GTTATAGCAC	CTTGAGTTGT	AAGGAGAAAG
	1441	CTGATGTAAA	AGACACTGAT	GCTGTTGATG	TGTGTATTGC
45	1481	TGATGGTGTC	TGTATAGATG	CTTTTCTAAA	ACCTCCCATG
	1521	GAGACAGAAG	AACCTCAAAT	TTTCTACAAT	GCTTCACCCT
	1561	CCACACTATC	TGCCACAATG	TTCATTGTTA	GTATTTTGTT
	1601	TCTTATCATT	TCTTCTGTAG	CGAGTTTGTA	ATTGCGCAGG
	1641	TTAGCTGAAA	TGAACAATAT	GTCCATCTTA	AAGTGTGCTT
50	1681	CCC			

Subjects can be effectively treated by the compositions and methods described herein even though those subjects have hyaluronidase isoforms and variants with somewhat different sequences than the examples of hyaluronidase sequences described herein. Such hyaluronidase isoforms and variants can have polypeptide or nucleic acid sequences with between 55-100% sequence identity to a reference sequence (e.g., a sequence described herein). For example, hyaluronidase isoforms and variants can have at least 55% sequence identity, e.g., at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97% sequence, at least 98%, at least 99% identity to a reference sequence (e.g., a sequence described herein) over a specified comparison window. Optimal alignment may be ascertained or conducted using the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443-53 (1970).

ULK

Autophagy plays a crucial role in several cellular functions, and its dysregulation is associated with both tumorigenesis and drug resistance. Autophagy is a process of metabolic degradation at the cellular level in which organelles or portions of the cytosol are sequestered by double-membrane autophagosomes and fused with lysosomes for degradation. Unc-51-like kinase 1 (ULK1) is a serine/threonine kinase that participates in the initiation of autophagy. Some studies have indicated that compounds that directly or indirectly target ULK1 could be used for tumor therapy. However, reports of the therapeutic effects of these compounds have come to conflicting conclusions.

ULK1 is a central kinase of the ULK1 complex involved in autophagy initiation, which promotes autophagosome-lysosome fusion. The human ULK1 gene is on chromosome 12 (NC_000012.12 (131894622..131923150)). An example of a human ULK1 amino acid sequence is shown below (Uniprot O75385; SEQ ID NO:9).

	10	20	30	40	50
30	MEPGRGGTET	VGKFEFSRKD	LIGHGAFVAV	FKGRHREKHD	LEVAVKCINK
	60	70	80	90	100
	KNLAQSQTLL	GKEIKILKEL	KHENIVALYD	FQEMANSVYL	VMEYCNGGDL
	110	120	130	140	150
	ADYLHAMRTL	SEDTRLFLQ	QIAGAMRLH	SKGIIHRDLK	PQNILLSNPA
35	160	170	180	190	200
	GRRANPNSIR	VKIADFGFAR	YLQSNMMAAT	LCGSPMYMAP	EVIMSQHYDG
	210	220	230	240	250
	KADLWSIGTI	VYQCLTGKAP	FQASSPQDLR	LFYEKNKTLV	PTIPRETSAP

	260	270	280	290	300
	LRQLLLALLQ	RNHKDRMDFD	EFFHHPFLDA	SPSVRKSPPV	PVPSYPSSGS
	310	320	330	340	350
5	GSSSSSSSTS	HLASPPSLGE	MQQLQKTLAS	PADTAGFLHS	SRDSGGSKDS
	360	370	380	390	400
	SCDTDDFVMV	PAQFPGLVA	EAPSAKPPPD	SLMCSGSSLV	ASAGLESHGR
	410	420	430	440	450
	TPSPSPPCSS	SPSPSGRAGP	FSSSRCGASV	PIPVPTQVQN	YQRIERNLQS
	460	470	480	490	500
10	PTQFQTPRSS	AIRRSBSTSP	LGFARASPS	PAHAEHGGVL	ARKMSLGGGR
	510	520	530	540	550
	PYTSPQVGT	IPERPGWSGT	PSPQGAEMRG	GRSPRPGSSA	PEHSPRTSGL
	560	570	580	590	600
	GCR LH SAPNL	SDLHVVRPKL	PKPPTDPLGA	VFSPPQASPP	QPSHGLQSCR
15	610	620	630	640	650
	NLRGSPKLPD	FLQRNPLPPI	LGSPTKAVPS	FDFPKTPSSQ	NLLALLARQG
	660	670	680	690	700
	VVMTPPRNRT	LPDLSEVGPF	HGQPLGPGLR	PGEDPKGPFG	RSFSTSRITD
	710	720	730	740	750
20	LLLKAAFQTQ	APDPGSTESL	QEKPMETAPS	AGFGGSLHPG	ARAGGTSSPS
	760	770	780	790	800
	PVVFTVGSPP	SGSTPPQGPR	TRMFSAGPTG	SASSSARHLV	PGPCSEAPAP
	810	820	830	840	850
	ELPAPGHGCS	FADPITANLE	GAVTFEAPDL	PEETLMEQEH	TEILRGLRFT
25	860	870	880	890	900
	LLFVQHVLEI	AALKGSASEA	AGGPEYQLQE	SVVADQISLL	SREWGFAEQL
	910	920	930	940	950
	VLYLKVAELL	SSGLQSAIDQ	IRAGKLCLSS	TVKQVVRRLN	ELYKASVVSC
	960	970	980	990	1000
30	QGLSLRLQRF	FLDKQRLDDR	IHSITAERLI	FSHAVQMVQS	AALDEMFOHR
	1010	1020	1030	1040	1050
	EGCVPRYHKA	LLLLEGLQHM	LSDQADIENV	TKCKLCIERR	LSALLTGICA

An example of a human ULK1 cDNA is shown below (NCBI ; SEQ ID NO:10).

35	1	GGATCCGGAT	TCGGATTAGC	AGCCCGGGAA	GAGTGCCGTG
	41	GCACAGGCGC	CGGAGGGAGC	GCGACCCTCG	GACCCCGCCT
	81	GGCCCGCGGG	GCTGGGACCC	GGCCCGGGCC	TGCCCGATGG
	121	GGCGCGCGGC	CCCGGAGATG	CGCCCTCGCC	CGGCCCCGCG
	161	CCCCCGGCCC	CGCGCCCCCG	GCCCGCCCCG	CCCGGCCCCG
40	201	GCCTCCGCCT	GAGTCCCCCG	CGCCTTGCC	CGCCACCCCC
	241	CGCCCGCGC	CCCCGGCCCC	CCTGCGCCAT	GGAGCCCGGC
	281	CGCGGCGGCA	CAGAGACCGT	GGGCAAGTTC	GAGTTCTCCC
	321	GCAAGGACCT	GATCGGCCAC	GGCGCCTTCG	CGGTGGTCTT
	361	CAAGGGCCGC	CACCGCGAGA	AGCACGATTT	GGAGGTCGCC
45	401	GTCAAGTGCA	TTAACAAGAA	GAACCTCGCC	AAGTCTCAGA
	441	CGCTGCTGGG	GAAGGAAATC	AAAATCCTGA	AGGAACTGAA
	481	ACATGAAAAC	ATCGTGGCCC	TGTACGACTT	CCAGGAAATG
	521	GCTAATTCTG	TCTACCTGGT	TATGGAGTAC	TGCAACGGTG
	561	GGGACCTGGC	CGACTACCTG	CACGCCATGC	GCACGCTGAG
50	601	CGAGGACACC	ATCAGGCTCT	TCCTGCAGCA	GATCGCGGGC
	641	GCCATGCGGC	TTCTGCACAG	CAAAGGCATC	ATCCACCGCG
	681	ACCTGAAACC	GCAGAACATC	CTGCTGTCCA	ACCCCGCCGG
	721	CCGCCGCGCC	AACCCCAACA	GCATCCGCGT	CAAGATCGCT

	761	GA	CTTC	GGCT	TC	GC	GC	GGTA	CCT	CC	AG	AGC	AAC	AT	GAT	GG
	801	CG	GC	CAC	ACT	CT	GC	GGCT	CC	CC	AT	GT	TAC	A	TGG	CCCC
	841	GG	TC	AT	CAT	G	TCCC	AG	CA	CT	AC	GAC	GG	AA	GG	CG
	881	TG	GAG	CAT	CG	GC	ACC	AT	CGT	CT	ACC	AG	TGC	CT	GAC	GG
5	921	AG	GC	GC	CCT	CC	AG	GC	CAG	AG	CCCC	CAG	AC	CT	GCG	CCT
	961	GTT	CT	AC	GAG	AA	GA	ACA	AGA	CG	TT	GG	TCCC	CA	CC	AT
	1001	CG	GG	AG	AC	CT	CG	CCCC	CG	GC	GC	CAG	CT	CT	CT	GG
	1041	TAC	TG	CA	ACG	CA	ACC	ACA	AAG	GAC	CG	CA	TGG	ACT	TC	GAT
	1081	GTT	TTTT	TT	CAT	CA	CCC	CT	TTCC	TC	GAT	GCC	AG	CCC	CT	CG
10	1121	AG	GAA	AT	CCC	CA	CCC	CG	TGCC	TGT	GCC	CTCG	TAC	CC	CA	AG
	1161	CG	GGG	TCC	GG	CAG	CAG	CT	CC	AG	CAG	CAG	CT	CC	AC	CT
	1201	CCT	GG	CCT	CC	CC	GC	GT	CCC	TG	GG	CG	AG	AT	GC	AG
	1241	CAG	AAG	AC	CC	TG	GC	CT	CCCC	GG	CT	GAC	ACC	GCT	GG	CT
	1281	TG	CAC	AG	CTC	CC	GG	GACT	CT	GG	TGG	CAG	CA	AG	GA	CT
15	1321	CT	GT	GAC	ACA	GAC	GACT	TCG	TC	AT	GG	TCCC	CG	CG	CAG	TTT
	1361	CC	AG	GT	GAC	TG	GT	GG	CTGA	GG	CG	CCCC	AG	GCC	AA	AC
	1401	CG	CC	AG	AC	AG	CCT	GAT	GTGC	AG	TGG	GAG	CT	CA	CT	GG
	1441	CT	CT	GCG	GGC	TT	GG	AG	AGCC	AC	GG	CCG	GAC	CCC	AT	CT
	1481	TCCC	CAC	CCT	GC	AG	CAG	CTC	CCCC	AG	TCCC	TC	AG	GG	CC	GG
20	1521	CT	GG	CCC	GTT	CT	CC	AG	CAGC	AG	GT	GCG	GCG	CCT	CT	GT
	1561	CAT	CCC	AG	TC	CCC	AC	G	CAGG	TG	CAG	AA	CTA	CC	AG	CG
	1601	GAG	CG	AA	ACC	TG	CAG	T	CACC	CA	CCC	AG	TT	CA	AA	AC
	1641	GG	TCT	CT	GC	CAT	CC	G	CAGG	TC	AG	G	CAG	CA	CC	AG
	1681	GG	GCT	TT	GCA	AG	GG	CC	AGCC	CCT	CG	CCCC	TG	CC	CA	CG
25	1721	GAG	CAT	GG	AG	GCG	TC	C	TGGC	CAG	GA	A	GAT	TCT	CT	GG
	1761	GAG	GCC	GG	CC	CT	AC	G	CCCA	TCT	CCT	CA	AG	TT	G	AA
	1801	CC	CT	GAG	CGG	CC	AG	GCT	GGA	GCG	GG	AC	GCC	CT	CCC	CA
	1841	GG	AG	CT	GAGA	TG	CG	GGG	TGG	CAG	GT	CCC	CT	CG	T	CA
	1881	CCT	CT	G	CACC	CG	AG	C	ACTCT	CCCC	G	C	ACTT	CC	GG	G
30	1921	CT	GCC	GC	CCTG	CAC	AG	C	GC	CCA	AC	C	GT	TG	ACT	TG
	1961	GT	CG	TCC	GCC	CCA	AG	C	TGCC	CAA	AC	CCCC	AC	GG	AC	CCCC
	2001	TG	GG	AG	CTGT	GTT	C	AG	CCCCA	CC	AC	AG	GCCA	GCC	CT	CCCC
	2041	GCC	GT	CCC	CAC	GG	CCT	G	CAGT	CCT	G	CC	GGAA	CCT	G	CG
	2081	TC	AC	CCA	AAGC	TG	CCC	G	ACTT	CCT	G	C	AGCGA	AA	CCCC	CT
35	2121	CCCC	CAT	CCT	GGG	CT	CCCC	AC	CAAG	G	CTG	AC	AG	TG	CC	CT
	2161	TG	ACT	T	CCCG	AAG	AC	CCCC	CA	GCT	CCC	AG	AA	CCT	G	CT
	2201	CT	CCT	AG	CCC	GG	C	AGG	GCGT	GG	T	GAT	GAC	CCCC	CT	GA
	2241	ACC	GG	AC	GCT	GCCC	G	AC	CTC	TC	G	G	AGGTGG	GAC	CT	T
	2281	TG	GT	CAG	CCG	TT	GG	G	CCCTG	GC	CT	G	CGGCC	AG	G	AG
40	2321	CC	CA	AGG	GCC	CCT	TT	G	GCCG	GT	CT	TT	T	AG	C	CG
	2361	T	ACT	G	ACCT	GCT	CCT	T	TAAG	GCG	G	CGTTT	G	GG	A	CA
	2401	CCC	G	G	ACCCG	GG	C	AG	CACGG	AG	AG	CCT	G	GG	A	AG
	2441	AT	GG	AG	ATCG	CA	CC	CT	CAGC	TG	GCT	TT	GGA	GG	G	AG
	2481	ACC	C	AG	GAGC	CC	G	T	GCTGGG	GG	C	ACC	AGCA	GCCC	CT	CCCC
45	2521	GG	TGG	TCT	TC	ACC	G	TGGG	CT	CT	CCCC	C	GAG	CG	G	AG
	2561	CCCC	CCC	CAG	G	GCCC	C	G	CAC	CAG	GAT	G	TT	TC	AG	CG
	2601	CC	ACT	G	GCTC	TG	CC	AG	CTCT	TCT	G	CCCC	GCC	AC	CT	G
	2641	TG	GG	CC	CTGC	AG	C	G	AGGCC	CAG	CCCC	CTGA	GCT	CC	CT	G
	2681	CC	AG	G	ACAG	GCT	G	C	AGCTT	TG	CC	G	ACCC	ATT	G	CT
50	2721	AC	CT	G	GAGGG	GG	CT	G	TGACC	TT	C	G	AGGCC	CC	G	AC
	2761	TG	AG	G	AGACC	CT	C	AT	G	GAG	C	ACAC	GG	AG	AT	C

	2801	CGTGGCCTGC	GCTTCACGCT	GCTGTTCGTG	CAGCACGTCC
	2841	TGGAGATCGC	AGCCCTGAAG	GGCAGCGCCA	GTGAGGCGGC
	2881	GGGGGGCCCT	GAGTACCAGC	TGCAGGAGAG	TGTGGTGGCC
	2921	GACCAGATCA	GCCTGCTGAG	CCGAGAATGG	GGCTTCGCGG
5	2961	AACAGCTGGT	GCTGTACCTG	AAGGTGGCCG	AGCTACTGTC
	3001	CTCCGGCCTG	CAAAGTGCCA	TCGACCAGAT	CCGGGCCGGC
	3041	AAGCTCTGCC	TGTCGTCCAC	TGTGAAGCAG	GTGGTGC GCA
	3081	GGCTGAATGA	GCTGTACAAG	GCCAGCGTGG	TGTCCTGCCA
	3121	GGGCCTGAGC	CTGCGGCTGC	AGCGCTTCTT	CCTGGACAAG
10	3161	CAGCGGCTCC	TGGACCGCAT	TCACAGCATC	ACTGCCGAGA
	3201	GGCTCATCTT	CAGCCACGCT	GTGCAGATGG	TGCAGTCGGC
	3241	TGCCCTGGAC	GAGATGTTCC	AGCACCGTGA	GGGCTGCGTC
	3281	CCACGCTACC	ACAAGGCCCT	GCTGCTCCTG	GAGGGGCTGC
	3321	AGCACATGCT	CTCGGACCAG	GCCGACATCG	AGAACGTCAC
15	3361	CAAGTGCAAG	CTGTGCATTG	AGCGGAGACT	CTCGGCGCTG
	3401	CTGACTGGCA	TCTGTGCCTG	ACCTTTCTGG	CCTGGCTGGG
	3441	CCCCCGTCC	TGCCGAGCCC	TGCAGAGTGG	GCTCTGTGTG
	3481	CTGGCTGGAC	TCCTCGGGAC	AAGCCCATGG	CGCTGATCGC
	3521	TGGTGCTGAG	CCCTGCCCTG	GGCCCCACGG	ACAGTCAGCC
20	3561	TGCCGGCCTC	CCTGCAGCTC	ACGGGGCAGA	ACCAGCACAT
	3601	CTGGAGCCAC	ACAGCTTGGG	GGGTGTCTCC	CATCTTTTAC
	3641	AGGTGGGGAT	CACAGAATTT	CTGCCCCCTC	AGCTGCCTGG
	3681	CTCAGCAGGC	GTGGGTGCCA	CCACCCTCTA	GCCCCAGGGC
	3721	AGCCCCGGAG	GACAGGCAAG	GGCCTGAGAC	CACTGCCGAC
25	3761	TCAAAGCCAA	AGCGAGCTCC	TGCTTAGGGC	AGGTCAGCAG
	3801	GCACTGTGCC	CAGGAAGAGC	CTGCGGCCTC	GGCGTCCCCC
	3841	AGTCTCCAGG	AGCCTCTCCC	TCCGAGATAC	CCACCCAGCT
	3881	TTGTCAATCA	CCCAAGCACT	TTATGCATAT	AGAGACAGAA
	3921	CCTGGACCTC	ACCAGGGACT	GCTGGGCAGC	GATTCCTGGC
30	3961	AGTGGCCTGG	TGTTTGTACA	TACACATATG	CAGACACATG
	4001	CCAGGGCCCC	CCAAGCCCGA	GCACCGGACC	ACGTTGCTGC
	4041	CCAGGTCTGG	ACCTCAGCGG	GAGAACTGGC	TCCGGGGGGA
	4081	GTGGGGCCCT	GCGCTAGAGG	CAGAGGCAGT	TCTTTGTTCA
	4121	AGCGTTCCCT	TGGGGACCGG	CAGCAGAGGC	ACCGTGTTCT
35	4161	CTCAGCCCTG	GATACGTCTT	GTAATCTTTC	ACACTTTATT
	4201	CCTAAAACGT	GTCTTATTTT	TATGCAGCTC	ATTTTTTCTT
	4241	TAAAGGAGAA	AACTTGTAGG	TGTTTAAGAA	TTGGTTTTTG
	4281	GAGGGCGAGG	ACTGGGCCAG	GTTAGAGGCA	GATGGCACAG
	4321	GGGCGTGTGG	CGGGCGGGTG	AGGCTGCTTT	GCACACCTGT
40	4361	GTTGGTGGCT	GTCCCCTGCC	GCCCCTCCCT	GTGGCAGCAG
	4401	CAGGACAGGT	GTGTGCCCAG	CACCCTCCCT	ACCTGGGCCT
	4441	GGAAGCAGAT	GAGGGGAATA	CTTCATGCAA	AGAAAAAAGT
	4481	AACATGTGCA	AAAGCTCCCC	GTCCAGCTTT	GACAGTCAGT
	4521	TTTGATGTCA	GTCCTCGGC	AGGGTAGGCC	TGATGACAGC
45	4561	CCTGTCCCTC	CCTGCCTCCG	CCTTGCCCCA	GGCCACGGAG
	4601	GGCGTCTGCA	GAGAGGCCTG	CCTTCCGGAT	TCCAGGCGGG
	4641	CATGCCCTGC	AAACCCCGCC	TGGGCCTCCC	TTGGTCTGCC
	4681	CAGCCCTCGG	TTAGCCCTGC	CTGAATCAGT	AGATACTTGA
	4721	ACGAGTCCCC	AGTCTGCGGG	AGGCAGTGGT	GGGGCCATGG
50	4761	ACCCATGCGG	GGGGTTCCAG	GGTCACACGC	CACATAACAG
	4801	ACAAAAATAC	ACACACGTGT	GTTTTTCTTT	GCAATACTTG

4841 AAATATTGCC ACTGTGCTTG GACTTAGAAG AAGAAAATCC
 4881 CCGTGACTTC TTCCTCATCA CCTTGATGGC TTTATTCTCA
 4921 CCTTGTGGGG CATGTTTGAA TTTATTGCTT CATGGCCGAC
 4961 TGGAATCCTG AGTCCTGGGA AGCTGGCACT GCGGGGATCT
 5 5001 TGCCCGGTGT CCTGGTCCTC TTGCTTCCGT CGCGGCCGCA
 5041 TGTGCGTGTG TCCAAGCAGG TCCTGGGCGC CTCAACTGCT
 5081 GCCCCTGGTT GAATGTTCTC TTGATAGTGC TGGACCCTTT
 5121 GTCTATTTTA AAGCGAATTT TGTGTGATTT CCTGCCCTTT
 5161 GCGTTATATT GTATAATACC AACGTAAGGA AATAAACCTT
 10 5201 TGGAATTGTT GAAAAAAAAA AAAAAAAAA

Subjects can be effectively treated by the compositions and methods described herein even though those subjects have ULK isoforms and variants with somewhat different sequences than the examples of ULK sequences described herein. Such ULK isoforms and variants can have polypeptide or nucleic acid sequences with between 55-100% sequence identity to a reference sequence (e.g., a sequence described herein). For example, ULK isoforms and variants can have at least 55% sequence identity, e.g., at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97% sequence, at least 98%, at least 99% identity to a reference sequence (e.g., a sequence described herein) over a specified comparison window. Optimal alignment may be ascertained or conducted using the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443-53 (1970).

p62

The p62 protein, also referred to as sequestosome-1, is encoded by the human *SQSTM1* gene, which is found on chromosome 5 (NC_000005.10 (179806393..179838078)). An example of a human p62 amino acid sequence is shown below (Uniprot Q13501-1; SEQ ID NO:11).

	10	20	30	40	50
30	MASLTVKAYL	LGKEDAAREI	RRFSFCCSPE	PEAEAEAAAAG	PGPCERLLSR
	60	70	80	90	100
	VAALFPALRP	GGFQAHYRDE	DGDLVAFSSD	EELTMAMSYV	KDDIFRIYIK
	110	120	130	140	150
	EKKECRRDHR	PPCAQEAPRN	MVHPNVICDG	CNGPVGTRY	KCSVCPDYDL
35	160	170	180	190	200
	CSVCEGKGLH	RGHTKLAFPS	PFGHLSEGFS	HSRWLRVKH	GHFGWPGWEM
	210	220	230	240	250
	GPPGNWSPRP	PRAGEARPGP	TAESASGPSE	DPSVNF LKNV	GESVAAALSP
	260	270	280	290	300
40	LGIEVDIDVE	HGGKRSRLTP	VSPESSTEE	KSSSQPSSCC	SDPSKPGGNV
	310	320	330	340	350
	EGATQSLAEQ	MRKIALESEG	RPEEQMESDN	CSGGDDDWTH	LSSKEVDPST
	360	370	380	390	400

GELQSLQMP E SEGPSSLDPS QEGPTGLKEA ALYPHLPPEA DPR LIESLSQ
 410 420 430 440
 MLSMGFSDEG GWLTRL LQTK NYDIGAALDT IQYSKHPPPL

5 A cDNA encoding a human p62 protein is shown below (SEQ ID NO:12).

	1	GAATTCCCTC	GCCGCTCGCT	ATGGCGTCGC	TCACCGTGAA
	41	GGCCTACCTT	CTGGGCAAGG	AGGACGCGGC	GCGCGAGATT
	81	CGCCGCTTCA	GCTTCTGCTG	CAGCCCCGAG	CCTGAGGCGG
	121	AAGCCGAGGC	TGCGGCGGGT	CCGGGACCCT	GCGAGCGGCT
10	161	GCTGAGCCGG	GTGGCCGCCC	TGTTCCCCGC	GCTGCGGCCT
	201	GGCGGCTTCC	AGGCGCACTA	CCGCGATGAG	GACGGGGACT
	241	TGGTTGCCTT	TTCCAGTGAC	GAGGAATTGA	CAATGGCCAT
	281	GTCCTACGTG	AAGGATGACA	TCTTCCGAAT	CTACATTAAA
	321	GAGAAAAAAG	AGTGCCGGCG	GGACCACCGC	CCACCGTGTG
15	361	CTCAGGAGGC	GCCCCGCAAC	ATGGTGCAAC	CCAATGTGAT
	401	CTGCGATGGC	TGCAATGGGC	CTGTGGTAGG	AACCCGCTAC
	441	AAGTGCAGCG	TCTGCCCAGA	CTACGACTTG	TGTAGCGTCT
	481	GCGAGGGAAA	GGGCTTGAC	CGGGGGCACA	CCAAGCTCGC
	521	ATTCCCCAGC	CCCTTCGGGC	ACCTGTCTGA	GGGCTTCTCG
20	561	CACAGCCGCT	GGCTCCGGAA	GGTGAAACAC	GGACACTTCG
	601	GGTGGCCAGG	ATGGGAAATG	GGTCCACCAG	GAAACTGGAG
	641	CCCACGTCCT	CCTCGTGCAG	GGGAGGCCCG	CCCTGGCCCC
	681	ACGGCAGAAT	CAGCTTCTGG	TCCATCGGAG	GATCCGAGTG
	721	TGAATTTCTT	GAAGAACGTT	GGGGAGAGTG	TGGCAGCTGC
25	761	CCTTAGCCCT	CTGGGCATTG	AAGTTGATAT	CGATGTGGAG
	801	CACGGAGGGA	AAAGAAGCCG	CCTGACCCCC	GTCTCTCCAG
	841	AGAGTTCCAG	CACAGAGGAG	AAGAGCAGCT	CACAGCCAAG
	881	CAGCTGCTGC	TCTGACCCCA	GCAAGCCGGG	TGGGAATGTT
	921	GAGGGCGCCA	CGCAGTCTCT	GGCGGAGCAG	ATGAGGAAGA
30	961	TCGCCTTGGA	GTCCGAGGGG	GCCCCTGAGG	AACAGATGGA
	1001	GTCGGATAAC	TGTTTCAGGAG	GAGATGATGA	CTGGACCCAT
	1041	CTGTCTTCAA	AAGAAGTGGA	CCCGTCTACA	GGTGAAGTCC
	1081	AGTCCCTACA	GATGCCAGAA	TCCGAAGGGC	CAAGCTCTCT
	1121	GGACCCCTCC	CAGGAGGGAC	CCACAGGGCT	GAAGGAAGCT
35	1161	GCCTTGATAC	CACATCTCCC	GCCAGAGGCT	GACCCGCGGC
	1201	TGATTGAGTC	CCTCTCCCAG	ATGCTGTCCA	TGGGCTTCTC
	1241	TGATGAAGGC	GGCTGGCTCA	CCAGGCTCCT	GCAGACCAAG
	1281	AACTATGACA	TCGGAGCGGC	TCTGGACACC	ATCCAGTATT
	1321	CAAAGCATCC	CCCGCCGTTG	TGACCACTTT	TGCCCACCTC
40	1361	TTCTGCGTGC	CCCTCTTCTG	TCTCATAGTT	GTGTTAAGCT
	1401	TGCGTAGAAT	TGGCAGGTCT	CTGTACGGGC	CAGTTTCTCT
	1441	GCCTTCTTCC	AGGATCAGGG	GTTAGGGTGC	AAGAAGCCAT
	1481	TTAGGGCAGC	AAAACAAGTG	ACATGAAGGG	AGGGTCCCTG
	1521	TGTGTGTGTG	TGCTGATGTT	TCCTGGGTGC	CCTGGCTCCT
45	1561	TGCAGCAGGG	CTGGGCCTGC	GAGACCCAAG	GCTCACTGCA
	1601	GCGCGCTCCT	GACCCCTCCC	TGCAGGGGCT	ACGTTAGCAG
	1641	CCCAGCACAT	AGCTTGCCCTA	ATGGCTTTCA	CTTTCTCTTT
	1681	TGTTTTTAAAT	GACTCATAGG	TCCCTGACAT	TTAGTTGATT
	1721	ATTTTCTGCT	ACAGACCTGG	TACACTCTGA	TTTGTAGATAA
50	1761	AGTAAGCCTA	GGTGTGTGTC	GCAGGCAGGC	TGGGGAGGCC
	1801	AGTGTGTGTT	GCCTCCTGCT	GGGACTGAGA	AGGCCACAGA

1841 AGGCGTCCGC AATGTTGGTT TCACTGAGAG CTGCCTCCTG
 1881 GTCTCTTCAC CACTGTAGTT CTCTCATTTC CAAACCATCA
 1921 GCTGCTTTTA AAATAAGATC TCTTTGTAGC CATCCTGTTA
 1961 AATTTGTAAA CAATCTAATT AAATGGCATG CGCAG

5

Subjects can be effectively treated by the compositions and methods described herein even though those subjects have p62 isoforms and variants with somewhat different sequences than the examples of p62 sequences described herein. Such p62 isoforms and variants can have polypeptide or nucleic acid sequences with

10 between 55-100% sequence identity to a reference sequence (e.g., a sequence described herein). For example, p62 isoforms and variants can have at least 55% sequence identity, e.g., at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97% sequence, at least 98%, at least 99% identity to a reference sequence (e.g., a sequence described herein) over a specified comparison

15 window. Optimal alignment may be ascertained or conducted using the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443-53 (1970).

TNF α

An example of a human TNF α amino acid sequence is shown below (SEQ ID NO:71).

20 1 MSTESMIRDV ELAEEALPKK TGGPQGSRRRC LFLSLFSFLI VAGATTLFCL LHFGVIGPQR
 61 EEFPRLSLI SPLAQAVRSS SRTPSDKPVA HVVANPQAEG QLQWLNRRAN ALLANGVELR
 121 DNQLVVPSEG LYLIYSQVLF KGQGCPSTHV LLTHTISRIA VSYQTKVNLL
 SAIKSPCQRE

181 TPEGAEAKPW YEPIYLGGVF QLEKGDRLSA EINRPDYLDF AESGQVYFGI IAL

25 A cDNA encoding a human TNF α protein is shown below (SEQ ID NO:72).

1 AGCAGACGCT CCCTCAGCAA GGACAGCAGA GGACCAGCTA AGAGGGAGAG AAGCAACTAC
 61 AGACCCCCC TGAAAACAAC CCTCAGACGC CACATCCCCT GACAAAGCTGC CAGGCAGGTT
 121 CTCTTCTCT CACATACTGA CCCACGGCTC CACCCTCTCT CCCCTGGAAA
 GGACACCATG

30 181 AGCACTGAAA GCATGATCCG GGACGTGGAG CTGGCCGAGG AGGCGCTCCC
 CAAGAAGACA
 241 GGGGGGCCCC AGGGCTCCAG GCGGTGCTTG TTCCTCAGCC TCTTCTCCTT
 CCTGATCGTG
 301 GCAGGCGCCA CCACGCTCTT CTGCCTGCTG CACTTTGGAG TGATCGGCCC
 CCAGAGGGAA

35 361 GAGTTCCCCA GGGACCTCTC TCTAATCAGC CCTCTGGCCC AGGCAGTCAG
 ATCATCTTCT
 421 CGAACCCCGA GTGACAAGCC TGTAGCCCAT GTTGTAGCAA ACCCTCAAGC
 TGAGGGGCAG

481 CTCCAGTGGC TGAACCGCCG GGCCAATGCC CTCCTGGCCA ATGGCGTGGA
 GCTGAGAGAT
 541 AACCAGCTGG TGGTGCCATC AGAGGGCCTG TACCTCATCT ACTCCCAGGT
 CCTCTTCAAG
 5 601 GGCCAAGGCT GCCCCCTCCAC CCATGTGCTC CTCACCCACA CCATCAGCCG
 CATCGCCGTC
 661 TCCTACCAGA CCAAGGTCAA CCTCCTCTCT GCCATCAAGA GCCCCTGCCA
 GAGGGAGACC
 721 CCAGAGGGGG CTGAGGCCAA GCCCTGGTAT GAGCCCATCT ATCTGGGAGG
 10 GGTCTTCCAG
 781 CTGGAGAAGG GTGACCGACT CAGCGCTGAG ATCAATCGGC CCGACTATCT
 CGACTTTGCC
 841 GAGTCTGGGC AGGTCTACTT TGGGATCATT GCCCTGTGAG GAGGACGAAC
 ATCCAACCTT
 15 901 CCCAAACGCC TCCCCTGCCC CAATCCCTTT ATTACCCCCT CCTTCAGACA
 CCCTCAACCT
 961 CTTCTGGCTC AAAAAGAGAA TTGGGGGCTT AGGGTCGGAA CCCAAGCTTA
 GAACTTTAAG
 1021 CAACAAGACC ACCACTTCGA AACCTGGGAT TCAGGAATGT GTGGCCTGCA
 20 CAGTGAAGTG
 1081 CTGGCAACCA CTAAGAATTC AAACCTGGGGC CTCCAGAACT CACTGGGGCC
 TACAGCTTTG
 1141 ATCCCTGACA TCTGGAATCT GGAGACCAGG GAGCCTTTGG TTCTGGCCAG
 AATGCTGCAG
 25 1201 GACTTGAGAA GACCTCACCT AGAAATTGAC ACAAGTGGAC CTTAGGCCTT
 CCTCTCTCCA
 1261 GATGTTTCCA GACTTCCTTG AGACACGGAG CCCAGCCCTC CCCATGGAGC
 CAGCTCCCTC
 1321 TATTTATGTT TGCACCTGTG ATTATTTATT ATTTATTTAT TATTTATTTA
 30 TTTACAGATG
 1381 AATGIATTTA TTTGGGAGAC CGGGGTATCC TGGGGGACCC AATGTAGGAG
 CTGCCTTGGC
 1441 TCAGACATGT TTTCCGTGAA AACGGAGCTG AACAATAGGC TGTTCCCATG
 TAGCCCCCTG
 35 1501 GCCTCTGTGC CTTCTTTTGA TTATGTTTTT TAAAATATTT ATCTGATTAA
 GTTGTCTAAA
 1561 CAATGCTGAT TTGGTGACCA ACTGTCACTC ATTGCTGAGC CTCTGCTCCC
 CAGGGGAGTT
 1621 GTGTCTGTAA TCGCCCTACT ATTCAGTGGC GAGAAATAAA GTTTGCTTAG AAAAGAAA
 40

Subjects can be effectively treated by the compositions and methods described herein even though those subjects have TNF α isoforms and variants with somewhat different sequences than the examples of TNF α sequences described herein. Such TNF α isoforms and variants can have polypeptide or nucleic acid sequences with between 55-100% sequence identity to a reference sequence (e.g., a sequence described herein). For example, TNF α isoforms and variants can have at least 55% sequence identity, e.g., at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97% sequence, at least 98%, at least 99% identity to a reference sequence (e.g., a sequence described herein) over a specified comparison window. Optimal alignment may be ascertained or conducted using the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443-53 (1970).

IL-6

An example of a human IL-6 amino acid sequence is shown below (SEQ ID NO:73).

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1 MNSFSTSAFG PVAFSLGLLL VLPAAFPAPV PPGEDSKDVA APHRQPLTSS ERIDKQIRYI
61 LDGISALRKE TCNKSNCES SKEALAENNL NLPKMAEKDG CFQSGFNEET CLVKIITGLL
121 EFEVYLEYLQ NRFESSEEQA RAVQMSTKVL IQFLQKKAKN LDAITTPDPT
TNASLLTKLQ
20 181 AQNQWLQDMT THLILRSFKE FLQSSLRALR QM

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A cDNA encoding a human IL-6 protein is shown below (SEQ ID NO:74).

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1 ATTCTGCCCT CGAGCCCACC GGAACGAAA GAGAAGCTCT ATCTCCCCTC CAGGAGCCCA
61 GCTATGAACT CCTTCTCCAC AAGCGCCTTC GGTCCAGTTG CCTTCTCCCT GGGGCTGCTC
121 CTGGTGTGTC CTGCTGCCTT CCCTGCCCCA GTACCCCAG GAGAAGATTC
25 CAAAGATGTA
181 GCCGCCCCAC ACAGACAGCC ACTCACCTCT TCAGAACGAA TTGACAAACA
AATTCGGTAC
241 ATCCTCGACG GCATCTCAGC CCTGAGAAAG GAGACATGTA ACAAGAGTAA
CATGTGTGAA
30 301 AGCAGCAAAG AGGCACTGGC AGAAAACAAC CTGAACCTTC CAAAGATGGC
TGAAAAAGAT
361 GGATGCTTCC AATCTGGATT CAATGAGGAG ACTTGCCTGG TGAAAATCAT
CACTGGTCTT
421 TTGGAGTTTG AGGTATACCT AGAGTACCTC CAGAACAGAT TTGAGAGTAG
35 TGAGGAACAA
481 GCCAGAGCTG TGCAGATGAG TACAAAAGTC CTGATCCAGT TCCTGCAGAA
AAAGGCAAAG

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541 AATCTAGATG CAATAACCAC CCCTGACCCA ACCACAAATG CCAGCCTGCT
 GACGAAGCTG
 601 CAGGCACAGA ACCAGTGGCT GCAGGACATG ACAACTCATC TCATTCTGCG
 CAGCTTTAAG
 5 661 GAGTTCCTGC AGTCCAGCCT GAGGGCTCTT CGGCAAATGT AGCATGGGCA
 CCTCAGATTG
 721 TTGTTGTAA TGGGCATTCC TTCTTCGGT CAGAAACCTG TCCACTGGGC
 ACAGAACTTA
 781 TGTTGTTCTC TATGGAGAAC TAAAAGTATG AGCGTTAGGA CACTATTTTA
 10 ATTATTTTAA
 841 ATTTATTAAT ATTTAAATAT GTGAAGCTGA GTTAATTTAT GTAAGTCATA
 TTTATATTTT
 901 TAAGAAGTAC CACTTGAAAC ATTTTATGTA TTAGTTTTGA AATAATAATG
 GAAAGTGGCT
 15 961 ATGCAGTTTG AATATCCTTT GTTTCAGAGC CAGATCATTT CTGGAAAGT
 GTAGGCTTAC
 1021 CTCAAATAAA TGGCTAACTT ATACATATTT TTAAAGAAAT ATTTATATTG
 TATTTATATA
 1081 ATGTATAAAT GGTTTTTATA CCAATAAATG GCATTTTAAA AAATTCA
 20

Subjects can be effectively treated by the compositions and methods
 described herein even though those subjects have IL-6 isoforms and variants with
 somewhat different sequences than the examples of IL-6 sequences described
 herein. Such IL-6 isoforms and variants can have polypeptide or nucleic acid
 25 sequences with between 55-100% sequence identity to a reference sequence (e.g., a
 sequence described herein). For example, IL-6 isoforms and variants can have at
 least 55% sequence identity, e.g., at least 60%, at least 70%, at least 80%, at least
 90%, at least 95%, at least 96%, at least 97% sequence, at least 98%, at least 99%
 identity to a reference sequence (e.g., a sequence described herein) over a specified
 30 comparison window. Optimal alignment may be ascertained or conducted using the
 homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443-53
 (1970).

JAK

An example of a human JAK amino acid sequence is shown below (SEQ ID
 35 NO:75).

1 MQYLNIEDC NAMAFCAKMR SSKKTEVNLE APEPGVEVIF YLSDREPLRL GSGEYTADEL
 61 CIRAAQACRI SPLCHNLFAL YDENTKLWYA PNRTITVDDK MSLRLHYRMR FYFTNWHGTN

181 DGHDIENECL GMAVLAISHY AMMKMKQLPE LPKDISYKRY IPETLNKSIR
 QRNLLTRMRI
 241 NNVFKDFLKE FNNKTICDSS VSTHDLKVY LATLETCLKH YGAEIFETSM
 LLISSENNEMN
 5 301 WFHSNDGGNV LYYEVMVTGN LGIQWRHKPN VVSVEKEKNK LKRKKLENKH
 KKDEEKNKIR
 361 EEWNNSYFYP EITHIVIKES VVSINKQDNK KMELKLSSHE EALS FVSLVD
 GYFRLTADAH
 421 HYLCTDVAPP LIVHNIQNGC HGPICTEYAI NKLRQEGSEE GMYVLRWSCT
 10 DFDNILMTVT
 481 CFEKSEQVQG AQKQFKNFQI EVQKGRYSLH GSDRSFPSLG DLMShLKKQI
 LRTDNISFML
 541 KRCCQPKPRE ISNLLVATKK AQEWQPVYPM SQLSFDRILK KDLVQGEHLG
 RGTRTHIYSG
 15 601 TLMDYKDDG TSEEKIKVI LKVLDPShRD ISLAFEEAAS MMRQVShKHI
 VYLYGVCVRD
 661 VENIMVEEFV EGGPLDLFMH RKSDVLTTPW KFKVAKQLAS ALSYLEDKDL
 VHGNVCTKNL
 721 LLAREGIDSE CGPFIKLSDP GIPITVLSRQ ECIERIPWIA PECVEDSKNL
 20 SVAADKWSFG
 781 TTLWEICYNG EIPLKDKTLI EKERFYESRC RVPVTPSCKEL ADLMTRCMNY
 DPNQRPFRA
 841 IMRDINKLEE QNPDIVSEKK PATEVDPTHF EKRFLKRIRD LGEGHFGKVE
 LCRYDPEGDN
 25 901 TGEQVAVKSL KPESGGNHIA DLKKEIEILR NLYHENIVKY KGICTEDGGN
 GIKLIMEFLP
 961 SGSLKEYLPK NKNKINLKQQ LKYAVQICKG MDYLGSRQYV HRDLAARNVL
 VESEHQVKIG
 1021 DFGLTKAIET DKEYYTVKDD RDSPVFWYAP ECLMQSKFYI ASDVWSFGVT
 30 LHELLTYCDS
 1081 DSSPMALFLK MIGPTHGQMT VTRLVNTLKE GKRLPCPPNC PDEVYQLMRK
 CWEFQPSNRT
 1141 SFQNLIEGFE ALLK

35 A cDNA encoding a human JAK protein is shown below (SEQ ID NO:76).

1 GCGTCGCTGA GCGCAGGCCG CGGCGGCCGC GGAGTATCCT GGAGCTGCAG ACAGTGCGGG
 61 CCTGCGCCCA GTCCCGGCTG TCCTCGCCGC GACCCCTCCT CAGCCCTGGG CGCGCGCACG
 121 CTGGGGCCCC GCGGGGCTGG CCGCCTAGCG AGCCTGCCGG TCGACCCAG
 CCAGCGCAGC
 40 181 GACGGGGCGC TGCCTGGCCC AGGCGCACAC GGAAGTGC GC TTCTCTGAAG
 TAGCTTTGGA

241 AAGTAGAGAA GAAAATCCAG TTTGCTTCTT GGAGAACACT GGACAGCTGA
 ATAAATGCAG
 301 TATCTAAATA TAAAAGAGGA CTGCAATGCC ATGGCTTTCT GTGCTAAAAAT
 GAGGAGCTCC
 5 361 AAGAAGACTG AGGTGAACCT GGAGGCCCTT GAGCCAGGGG TGGAAGTGAT
 CTTCTATCTG
 421 TCGGACAGGG AGCCCCTCCG GCTGGGCAGT GGAGAGTACA CAGCAGAGGA
 ACTGTGCATC
 481 AGGGCTGCAC AGGCATGCCG TATCTCTCCT CTTTGTACACA ACCTCTTTGC
 10 CCTGTATGAC
 541 GAGAACACCA AGCTCTGGTA TGCTCCAAAT CGCACCATCA CCGTTGATGA
 CAAGATGTCC
 601 CTCCGGCTCC ACTACCGGAT GAGGTTCTAT TTCACCAATT GGCATGGAAC
 CAACGACAAT
 15 661 GAGCAGTCAG TGTGGCGTCA TTCTCCAAAG AAGCAGAAAA ATGGCTACGA
 GAAAAAAAAG
 721 ATTCCAGATG CAACCCCTCT CTTGATGCC AGCTCACTGG AGTATCTGTT
 TGCTCAGGGA
 781 CAGTATGATT TGGTGAAATG CCTGGCTCCT ATTCGAGACC CCAAGACCGA
 20 GCAGGATGGA
 841 CATGATATTG AGAACGAGTG TCTAGGGATG GCTGTCCTGG CCATCTCACA
 CTATGCCATG
 901 ATGAAGAAGA TGCAGTTGCC AGAACTGCCC AAGGACATCA GCTACAAGCG
 ATATATTCCA
 25 961 GAAACATTGA ATAAGTCCAT CAGACAGAGG AACCTTCTCA CCAGGATGCG
 GATAAATAAT
 1021 GTTTTCAAGG ATTTCTTAAA GGAATTTAAC AACAAGACCA TTTGTGACAG
 CAGCGTGTCC
 1081 ACGCATGACC TGAAGGTGAA ATACTTGGCT ACCTTGAAAA CTTTGACAAA
 30 ACATTACGGT
 1141 GCTGAAATAT TTGAGACTTC CATGTTACTG ATTTTCATCAG AAAATGAGAT
 GAATTGGTTT
 1201 CATTCGAATG ACGGTGAAAA CGTTCTCTAC TACGAAGTGA TGGTGACTGG
 GAATCTTGGA
 35 1261 ATCCAGTGGA GGCATAAACC AAATGTTGTT TCTGTTGAAA AGGAAAAAAA
 TAACTGAAG
 1321 CGGAAAAAAC TGGAAAATAA ACACAAGAAG GATGAGGAGA AAAACAAGAT
 CCGGGAAGAG
 1381 TGGAACAATT TTTCTTACTT CCCTGAAATC ACTCACATTG TAATAAAGGA
 40 GTCTGTGGTC

1441 AGCATTAAACA AGCAGGACAA CAAGAAAATG GAACTGAAGC TCTCTTCCCA
CGAGGAGGCC

1501 TTGTCCCTTTG TGTCCCTGGT AGATGGCTAC TTCCGGCTCA CAGCAGATGC
CCATCATTAC

5 1561 CTCTGCACCG ACGTGGCCCC CCCGTTGATC GTCCACAACA TACAGAATGG
CTGTCAATGGT

1621 CCAATCTGTA CAGAATACGC CATCAATAAA TTGCGGCAAG AAGGAAGCGA
GGAGGGGATG

1681 TACGTGCTGA GGTGGAGCTG CACCGACTTT GACAACATCC TCATGACCGT

10 CACCTGCTTT

1741 GAGAAGTCTG AGCAGGTGCA GGTGCCCAG AAGCAGTTCA AGAACTTTCA
GATCGAGGTG

1801 CAGAAGGGCC GCTACAGTCT GCACGGTTCG GACCGCAGCT TCCCCAGCTT
GGGAGACCTC

15 1861 ATGAGCCACC TCAAGAAGCA GATCCTGCGC ACGGATAACA TCAGCTTCAT
GCTAAAACGC

1921 TGCTGCCAGC CCAAGCCCCG AGAAATCTCC AACCTGCTGG TGGCTACTAA
GAAAGCCCAG

1981 GAGTGGCAGC CCGTCTACCC CATGAGCCAG CTGAGTTTCG ATCGGATCCT

20 CAAGAAGGAT

2041 CTGGTGCAGG GCGAGCACCT TGGGAGAGGC ACGAGAACAC ACATCTATTG
TGGGACCCTG

2101 ATGGATTACA AGGATGACGA AGGAACTTCT GAAGAGAAGA AGATAAAAGT
GATCCTCAAA

25 2161 GTCTTAGACC CCAGCCACAG GGATATTTCC CTGGCCTTCT TCGAGGCAGC
CAGCATGATG

2221 AGACAGGTCT CCCACAAACA CATCGTGTAC CTCTATGGCG TCTGTGTCCG
CGACGTGGAG

2281 AATATCATGG TGGAAGAGTT TGTGGAAGGG GGTCTCTGG ATCTCTTCAT

30 GCACCGGAAA

2341 AGCGATGTCC TTACCACACC ATGGAAATTC AAAGTTGCCA AACAGCTGGC
CAGTGCCCTG

2401 AGCTACTTGG AGGATAAAGA CCTGGTCCAT GGAAATGTGT GTAATAAAAA
CCTCCTCCTG

35 2461 GCCCGTGAGG GCATCGACAG TGAGTGTGGC CCATTCATCA AGCTCAGTGA
CCCCGGCATC

2521 CCCATTACGG TGCTGTCTAG GCAAGAATGC ATTGAACGAA TCCCATGGAT
TGCTCCTGAG

2581 TGTGTTGAGG ACTCCAAGAA CCTGAGTGTG GCTGCTGACA AGTGGAGCTT

40 TGGAACCACG

2641 CTCTGGGAAA TCTGCTACAA TGGCGAGATC CCCTTGAAAG ACAAGACGCT
GATTGAGAAA
2701 GAGAGATTCT ATGAAAGCCG GTGCAGGCCA GTGACACCAT CATGTAAGGA
GCTGGCTGAC
5 2761 CTCATGACCC GCTGCATGAA CTATGACCCC AATCAGAGGC CTTTCTTCCG
AGCCATCATG
2821 AGAGACATTA ATAAGCTTGA AGAGCAGAAT CCAGATATTG TTTCAGAAAA
AAAACCAGCA
2881 ACTGAAGTGG ACCCCACACA TTTTGAAAAG CGCTTCCTAA AGAGGATCCG
10 TGACTTGGGA
2941 GAGGGCCACT TTGGGAAGGT TGAGCTCTGC AGGTATGACC CCGAAGGGGA
CAATACAGGG
3001 GAGCAGGTGG CTGTAAATC TCTGAAGCCT GAGAGTGGAG GTAACCACAT
AGCTGATCTG
15 3061 AAAAAGGAAA TCGAGATCTT AAGGAACCTC TATCATGAGA ACATTGTGAA
GTACAAAGGA
3121 ATCTGCACAG AAGACGGAGG AAATGGTATT AAGCTCATCA TGGAATTTCT
GCCTTCGGGA
3181 AGCCTTAAGG AATATCTTCC AAAGAATAAG AACAAAATAA ACCTCAAACA
20 GCAGCTAAAA
3241 TATGCCGTTC AGATTTGTAA GGGGATGGAC TATTTGGGTT CTCGGCAATA
CGTTCACCGG
3301 GACTTGGCAG CAAGAAATGT CCTTGTGAG AGTGAACACC AAGTGAAAT
TGGAGACTTC
25 3361 GGTTTAACCA AAGCAATTGA AACCGATAAG GAGTATTACA CCGTCAAGGA
TGACCGGGAC
3421 AGCCCTGTGT TTTGGTATGC TCCAGAATGT TTAATGCAAT CTAAATTTTA
TATTGCCTCT
3481 GACGTCTGGT CTTTGGAGT CACTCTGCAT GAGCTGCTGA CTTACTGTGA
30 TTCAGATTCT
3541 AGTCCCATGG CTTTGTTCCT GAAAATGATA GGCCCAACCC ATGGCCAGAT
GACAGTCACA
3601 AGACTTGTGA ATACGTTAAA AGAAGGAAAA CGCCTGCCGT GCCCACCTAA
CTGTCCAGAT
35 3661 GAGGTTTATC AACTTATGAG GAAATGCTGG GAATTCCAAC CATCCAATCG
GACAAGCTTT
3721 CAGAACCTTA TTGAAGGATT TGAAGCACTT TTAAAATAAG AAGCATGAAT
AACATTTAAA
3781 TTCCACAGAT TATCAAGTCC TTCTCCTGCA ACAAATGCCC AAGTCATTTT
40 TTAAAAATTT

3841 CTAATGAAAG AAGTTTGTGT TCTGTCCAAA AAGTCACTGA ACTCATACTT
CAGTACATAT
3901 ACATGTATAA GGCACACTGT AGTGCTTAAT ATGTGTAAGG ACTTCCTCTT
TAAATTTGGT
5 3961 ACCAGTAACT TAGTGACACA TAATGACAAC CAAAATATTT GAAAGCACTT
AAGCACTCCT
4021 CCTTGTGGAA AGAATATAACC ACCATTTTCAT CTGGCTAGTT CACCATCACA
ACTGCATTAC
4081 CAAAAGGGGA TTTTGA AAA CGAGGAGTTG ACCAAAATAA TATCTGAAGA
10 TGATTGCTTT
4141 TCCCTGCTGC CAGCTGATCT GAAATGTTTT GCTGGCACAT TAATCATAGA
TAAAGAAAGA
4201 TTGATGGACT TAGCCCTCAA ATTCAGTAT CTATACAGTA CTAGACCATG
CATTCTTAAA
15 4261 ATATTAGATA CCAGGTAGTA TATATTGTTT CTGTACAAAA ATGACTGTAT
TCTCTCACCA
4321 GTAGGACTTA AACTTTGTTT CTCCAGTGGC TTAGCTCCTG TTCCTTTGGG
TGATCACTAG
4381 CACCCATTTT TGAGAAAGCT GGTCTACAT GGGGGGATAG CTGTGGAATA
20 GATAATTTGC
4441 TGATGTTAA TTCTCAAGAA CTAAGCCTGT GCCAGTGCTT TCCTAAGCAG
TATACCTTTA
4501 ATCAGAACTC ATTCCCAGAA CCTGGATGCT ATTACACATG CTTTAAAGAA
ACGTCAATGT
25 4561 ATATCCTTTT ATAACCTCTAC CACTTTGGGG CAAGCTATTC CAGCACTGGT
TTTGAATGCT
4621 GTATGCAACC AGTCTGAATA CCACATACGC TGCCTGTTC TTAGAGGGTT
TCCATACTTA
4681 CCACCGATCT ACAAGGGTTG ATCCCTGTTT TTACCATCAA TCATCACCTT
30 GTGGTGCAAC
4741 ACTTGAAAAGA CCCGGCTAGA GGCATATGG ACTTCAGGAT CCACTAGACA
GTTTTAGTT
4801 TGCTTGAGG TAGCTGGGTA ATCAAAAATG TTTAGTCATT GATTCAATGT
GAACGATTAC
35 4861 GGTCTTTATG ACCAAGAGTC TGAAAATCTT TTTGTTATGC TGTTTAGTAT
TCGTTTGATA
4921 TTGTTACTTT TCACCTGTTG AGCCCAAATT CAGGATTGGT TCAGTGGCAG
CAATGAAGTT
4981 GCCATTTAAA TTTGTTTATA GCCTACATCA CCAAGGTCTC TGTGTCAAAC
40 CTGTGGCCAC
5041 TCTATATGCA CTTTGTTTAC TCTTTATACA AATAAATATA CTAAAGACTT TA

Subjects can be effectively treated by the compositions and methods described herein even though those subjects have JAK isoforms and variants with somewhat different sequences than the examples of JAK sequences described herein. Such JAK isoforms and variants can have polypeptide or nucleic acid sequences with between 55-100% sequence identity to a reference sequence (e.g., a sequence described herein). For example, JAK isoforms and variants can have at least 55% sequence identity, e.g., at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97% sequence, at least 98%, at least 99% identity to a reference sequence (e.g., a sequence described herein) over a specified comparison window. Optimal alignment may be ascertained or conducted using the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443-53 (1970).

Inhibitory Nucleic Acids

The expression of hyaluronan synthase can be inhibited, for example by use of an inhibitory nucleic acid that specifically recognizes a nucleic acid that encodes the hyaluronan synthase.

An inhibitory nucleic acid can have at least one segment that will hybridize to a hyaluronan synthase nucleic acid, ULK1 nucleic acid, TNF α nucleic acid, IL6 nucleic acid or JAK nucleic acid under intracellular or stringent conditions. The inhibitory nucleic acid can reduce expression of a nucleic acid encoding hyaluronan synthase, ULK1, TNF α , IL6 or JAK. A nucleic acid may hybridize to a genomic DNA, a messenger RNA, or a combination thereof. An inhibitory nucleic acid may be incorporated into a plasmid vector or viral DNA. It may be single stranded or double stranded, circular or linear.

An inhibitory nucleic acid is a polymer of ribose nucleotides or deoxyribose nucleotides having more than 13 nucleotides in length. An inhibitory nucleic acid may include naturally occurring nucleotides; synthetic, modified, or pseudo-nucleotides such as phosphorothiolates; as well as nucleotides having a detectable label such as P³², biotin or digoxigenin. An inhibitory nucleic acid can reduce the expression and/or activity of a hyaluronan synthase nucleic acid, ULK1 nucleic acid, TNF α nucleic acid, IL6 nucleic acid or JAK nucleic acid. Such an inhibitory nucleic acid may be completely complementary to a segment of an endogenous hyaluronan synthase nucleic acid (e.g., a hyaluronan synthase RNA) or ULK1 nucleic acid (e.g.,

an ULK1 mRNA) or TNF α nucleic acid (e.g., a TNF α mRNA) or IL-6 nucleic acid (e.g., a IL-6 mRNA) or JAK nucleic acid (e.g., a JAK mRNA). Alternatively, some variability is permitted in the inhibitory nucleic acid sequences relative to hyaluronan synthase, ULK1, TNF α , IL6 or JAK. An inhibitory nucleic acid can

5 hybridize to a hyaluronan synthase nucleic acid or ULK1 nucleic acid or a TNF α nucleic acid or a IL-6 nucleic acid or a JAK nucleic acid under intracellular conditions or under stringent hybridization conditions and is sufficiently complementary to inhibit expression of the endogenous hyaluronan synthase nucleic acid or ULK1 nucleic acid or TNF α nucleic acid or IL-6 nucleic acid or JAK nucleic

10 acid. Intracellular conditions refer to conditions such as temperature, pH and salt concentrations typically found inside a cell, e.g., an animal or mammalian cell. One example of such an animal or mammalian cell is a stromal or epithelial cell. Another example of such an animal or mammalian cell is a cancer cell. Generally, stringent hybridization conditions are selected to be about 5°C lower than the thermal melting

15 point (T_m) for the specific sequence at a defined ionic strength and pH. However, stringent conditions encompass temperatures in the range of about 1°C to about 20°C lower than the thermal melting point of the selected sequence, depending upon the desired degree of stringency as otherwise qualified herein. Inhibitory oligonucleotides can, for example, have 2, 3, 4, or 5 or more stretches of contiguous

20 nucleotides that are precisely complementary to a hyaluronan synthase coding sequence or ULK1 coding sequence or TNF α coding sequence or IL-6 coding sequence or JAK coding sequence, wherein each complementary stretch is separated by one or more contiguous nucleotides that are not complementary to adjacent coding sequences, can inhibit the function of a hyaluronan synthase nucleic acid or

25 ULK1 nucleic acid or TNF α nucleic acid or IL-6 nucleic acid or JAK nucleic acid. In general, each stretch of contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences may be 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an inhibitory nucleic acid hybridized to a sense nucleic acid to estimate the

30 degree of mismatching that will be tolerated for inhibiting expression of a particular target nucleic acid. Inhibitory nucleic acids of the invention include, for example, a short hairpin RNA, a small interfering RNA, a ribozyme or an antisense nucleic acid molecule.

The inhibitory nucleic acid molecule may be single or double stranded (e.g., a small interfering RNA (siRNA)) and may function in an enzyme-dependent manner or by steric blocking. Inhibitory nucleic acid molecules that function in an enzyme-dependent manner include forms dependent on RNase H activity to degrade target mRNA. These include single-stranded DNA, RNA, and phosphorothioate molecules, as well as the double-stranded RNAi/siRNA system that involves target mRNA recognition through sense-antisense strand pairing followed by degradation of the target mRNA by the RNA-induced silencing complex. Steric blocking inhibitory nucleic acids, which are RNase-H independent, interfere with gene expression or other mRNA-dependent cellular processes by binding to a target mRNA and getting in the way of other processes. Steric blocking inhibitory nucleic acids include 2'-O alkyl (usually in chimeras with RNase-H dependent antisense), peptide nucleic acid (PNA), locked nucleic acid (LNA) and morpholino antisense.

Small interfering RNAs, for example, may be used to specifically reduce translation of hyaluronan synthase or ULK1 or TNF α or IL-6 or JAK nucleic acid such that translation of the encoded hyaluronan synthase, ULK1, TNF α , IL-6 or JAK is inhibited. SiRNAs mediate post-transcriptional gene silencing in a sequence-specific manner. See, for example, website at invitrogen.com/site/us/en/home/Products-and-Services/Applications/rnai.html. Once incorporated into an RNA-induced silencing complex, siRNA mediate cleavage of the homologous endogenous mRNA transcript by guiding the complex to the homologous mRNA transcript, which is then cleaved by the complex. The siRNA may be homologous and/or complementary to any region of the hyaluronan synthase transcript or to any region of an ULK1 transcript or to any region of a TNF α transcript or any region of a IL-6 transcript or any region of a JAK transcript. The region of homology may be 30 nucleotides or less in length, e.g., less than 25 nucleotides such as about 21 to 23 nucleotides in length. SiRNA is typically double stranded and may have two-nucleotide 3' overhangs, for example, 3' overhanging UU dinucleotides. Methods for designing siRNAs are known to those skilled in the art. See, for example, Elbashir *et al. Nature* **411**: 494-498 (2001); Harborth *et al. Antisense Nucleic Acid Drug Dev.* **13**: 83-106 (2003).

The pSuppressorNeo vector for expressing hairpin siRNA, commercially available from IMGENEX (San Diego, California), can be used to generate siRNA for inhibiting expression of hyaluronan synthase or ULK1. The construction of the

siRNA expression plasmid involves the selection of the target region of the mRNA, which can be a trial-and-error process. However, Elbashir et al. have provided guidelines that appear to work ~80% of the time. Elbashir, S.M., et al., *Analysis of gene function in somatic mammalian cells using small interfering RNAs*. Methods, 5 2002. **26**(2): p. 199-213. Accordingly, for synthesis of synthetic siRNA, a target region may be selected, e.g., 50 to 100 nucleotides downstream of the start codon. The 5' and 3' untranslated regions and regions close to the start codon should be avoided as these may be richer in regulatory protein binding sites. As siRNA can begin with AA, have 3' UU overhangs for both the sense and antisense siRNA 10 strands, and have an approximate 50 % G/C content. An example of a sequence for a synthetic siRNA is 5'-AA(N19)UU, where N is any nucleotide in the mRNA sequence and the siRNA should be approximately 50% G-C content. The selected sequence(s) can be compared to others in the human genome database to minimize homology to other known coding sequences (e.g., by Blast search, for example, 15 through the NCBI website).

SiRNAs may be chemically synthesized, created by *in vitro* transcription, or expressed from an siRNA expression vector or a PCR expression cassette. See, e.g., website at invitrogen.com/site/us/en/home/Products-and-Services/Applications/rnai.html. When an siRNA is expressed from an expression 20 vector or a PCR expression cassette, the insert encoding the siRNA may be expressed as an RNA transcript that folds into an siRNA hairpin. Thus, the RNA transcript may include a sense siRNA sequence that is linked to its reverse complementary antisense siRNA sequence by a spacer sequence that forms the loop of the hairpin as well as a string of U's at the 3' end. The loop of the hairpin may be 25 of any appropriate lengths, for example, 3 to 30 nucleotides in length, e.g., , 3 to 23 nucleotides in length, and may be of various nucleotide sequences including, AUG, CCC, UUCG, CCACC, CTCGAG, AAGCUU, CCACACC and UUCAAGAGA. SiRNAs also may be produced *in vivo* by cleavage of double-stranded RNA introduced directly or via a transgene or virus. Amplification by an RNA-dependent 30 RNA polymerase may occur in some organisms.

An inhibitory nucleic acid such as a short hairpin RNA siRNA or an antisense oligonucleotide may be prepared using methods such as by expression from an expression vector or expression cassette that includes the sequence of the inhibitory nucleic acid. Alternatively, it may be prepared by chemical synthesis

using naturally occurring nucleotides, modified nucleotides or any combinations thereof. In some embodiments, the inhibitory nucleic acids are made from modified nucleotides or non-phosphodiester bonds, for example, that are designed to increase biological stability of the inhibitory nucleic acid or to increase intracellular stability of the duplex formed between the inhibitory nucleic acid and the target hyaluronan synthase nucleic acid.

An inhibitory nucleic acid may be generated, for example, by expression from an expression vector encoding a complementarity sequence of the hyaluronan synthase nucleic acid, ULK1 nucleic acid, TNF α nucleic acid, IL-6 nucleic acid or JAK nucleic acid. Alternatively, it may be prepared by chemical synthesis using naturally occurring nucleotides, modified nucleotides or any mixture of combination thereof. In some embodiments, the hyaluronan synthase nucleic acids or ULK1 nucleic acids or TNF α nucleic acids or IL-6 nucleic acids or JAK nucleic acids are made from modified nucleotides or non-phosphodiester bonds, for example, that are designed to increase biological stability of the nucleic acids or to increase intracellular stability of the duplex formed between the inhibitory nucleic acids and other (e.g., endogenous) nucleic acids.

For example, the inhibitory hyaluronan synthase nucleic acids or inhibitory ULK1 nucleic acids or inhibitory TNF α nucleic acids or inhibitory IL-6 nucleic acids or inhibitory JAK nucleic acids can be peptide nucleic acids that have peptide bonds rather than phosphodiester bonds.

The inhibitory nucleic acids of the hyaluronan synthase or ULK1 or TNF α or IL-6 or JAK described herein may include modified nucleotides, as well as natural nucleotides such as combinations of ribose and deoxyribose nucleotides.

Naturally occurring nucleotides that can be employed in the hyaluronan synthase nucleic acids or ULK1 nucleic acids or TNF α nucleic acids or IL-6 nucleic acids or JAK nucleic acids can include the ribose or deoxyribose nucleotides adenosine, guanine, cytosine, thymine and uracil.

Examples of modified nucleotides that can be employed in the inhibitory hyaluronan synthase nucleic acids or inhibitory ULK1 nucleic acids or inhibitory TNF α nucleic acids or inhibitory IL-6 nucleic acids or inhibitory JAK nucleic acids include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-

carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxoacetic acid, wybutosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxoacetic acid methylester, uracil-5-oxoacetic acid, 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The inhibitory nucleic acids may be of same length as wild type hyaluronan synthase or as any isoforms or variants of hyaluronan synthase or ULK1 or TNF α or IL-6 or JAK. The inhibitory nucleic acids of the hyaluronan synthase, ULK1, TNF α , IL-6 or JAK described herein can also be longer and include other useful sequences. In some embodiments, the inhibitory nucleic acids of the hyaluronan synthase, ULK1, TNF α , IL-6 or JAK are somewhat shorter. For example, inhibitory nucleic acids of hyaluronan synthase, ULK1, TNF α , IL-6 or JAK can include a segment that has a nucleic acid sequence that can be missing up to 5 nucleotides, or missing up to 10 nucleotides, or missing up to 20 nucleotides, or missing up to 30 nucleotides, or missing up to 50 nucleotides, or missing up to 100 nucleotides from the 5' or 3' end.

The inhibitory nucleic acids can be introduced via one or more vehicles such as via expression vectors (e.g., viral vectors), via virus like particles, via ribonucleoproteins (RNPs), via nanoparticles, via liposomes, or a combination thereof. The vehicles can include components or agents that can target particular cell types, facilitate cell penetration, reduce degradation, or a combination thereof.

Antibodies

Antibodies can be used as inhibitors of hyaluronan synthase, ULK1, TNF α , IL-6 or JAK. Antibodies can be raised against various epitopes of hyaluronan synthase, ULK1, TNF α , IL-6 or JAK. Some antibodies for hyaluronan synthase, ULK1, TNF α , IL-6 or JAK may also be available commercially. However, the antibodies contemplated for treatment pursuant to the methods and compositions

described herein are in one embodiment human or humanized antibodies and are highly specific for their targets.

In one aspect, the present disclosure relates to use of isolated antibodies that bind specifically to hyaluronan synthase, ULK1, TNF α , IL-6 or JAK. Such antibodies may be monoclonal antibodies. Such antibodies may also be humanized or fully human monoclonal antibodies. The antibodies can exhibit one or more desirable functional properties, such as high affinity binding to hyaluronan synthase, ULK1, TNF α , IL-6 or JAK.

Methods and compositions described herein can include antibodies that bind hyaluronan synthase, ULK1, TNF α , IL-6 or JAK, or a combination of antibodies where each antibody type can separately bind hyaluronan synthase, ULK1, TNF α , IL-6 or JAK.

The term "antibody" as referred to herein includes whole antibodies and any antigen binding fragment (i.e., "antigen-binding portion") or single chains thereof. An "antibody" refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, C_{H1}, C_{H2} and C_{H3}. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system.

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., a peptide or domain of hyaluronan

synthase, ULK1, TNF α , IL-6 or JAK). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment
5 consisting of the V_L, V_H, C_L and C_{H1} domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_{H1} domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546), which consists of a V_H domain;
10 and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci.
15 USA 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using available techniques, and the fragments are screened for utility in the same manner as are intact antibodies.

20 An "isolated antibody," as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds hyaluronan synthase, ULK1, TNF α , IL-6 or JAK is substantially free of antibodies that specifically bind antigens other than a hyaluronan synthase, ULK1, TNF α , IL-6 or JAK). An isolated antibody
25 that specifically binds hyaluronan synthase, ULK1, TNF α , IL-6 or JAK may, however, have cross-reactivity to related antigens, such as isoforms or variants of hyaluronan synthase, ULK1, TNF α , IL-6 or JAK from various humans or from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

30 The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

The term "human antibody," as used herein, is intended to include antibodies having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo). However, the term "human antibody," as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "human monoclonal antibody" refers to antibodies displaying a single binding specificity which have variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. In one embodiment, the human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic nonhuman animal, e.g., a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

The term "recombinant human antibody," as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom (described further below), (b) antibodies isolated from a host cell transformed to express the human antibody, e.g., from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial human antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable regions in which the framework and CDR regions are derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the V_L and V_H regions of the recombinant antibodies are sequences that, while

derived from and related to human germline V_L and V_H sequences, may not naturally exist within the human antibody germline repertoire in vivo.

As used herein, "isotype" refers to the antibody class (e.g., IgM or IgG1) that is encoded by the heavy chain constant region genes.

5 The phrases "an antibody recognizing an antigen" and "an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen."

The term "human antibody derivatives" refers to any modified form of the human antibody, e.g., a conjugate of the antibody and another agent or antibody.

10 The term "humanized antibody" is intended to refer to antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Additional framework region modifications may be made within the human framework sequences.

The term "chimeric antibody" is intended to refer to antibodies in which the
15 variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

As used herein, an antibody that "specifically binds to human hyaluronan
20 synthase" or an antibody that "specifically binds to human ULK1" or an antibody that "specifically binds to $TNF\alpha$ " or an antibody that "specifically binds to IL-6" or an antibody that "specifically binds to JAK" is intended to refer to an antibody that binds to human hyaluronan synthase or ULK1 or $TNF\alpha$ or IL-6 or JAK with a K_D of 1×10^{-7} M or less, 5×10^{-8} M or less, 1×10^{-8} M or less, 5×10^{-9} M or less, or between
25 1×10^{-8} M and 1×10^{-10} M or less.

The term " K_{assoc} " or " K_a ," as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term " K_{dis} " or " K_d ," as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term " K_D ," as used herein, is intended to refer to
30 the dissociation constant, which is obtained from the ratio of K_d to K_a (i.e., K_d / K_a) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art. One method for determining the K_D of an antibody is by using surface plasmon resonance, e.g., using a biosensor system such as a Biacore™ system.

The antibodies of the invention are characterized by particular functional features or properties of the antibodies. For example, the antibodies bind specifically to human hyaluronan synthase, ULK1, TNF α , IL-6 or JAK. In one embodiment, an antibody of the invention binds to hyaluronan synthase, ULK1, TNF α , IL-6 or JAK with high affinity, for example with a K_D of 1×10^{-7} M or less. The antibodies can exhibit one or more of the following characteristics:

(a) binds to human hyaluronan synthase, ULK1, TNF α , IL-6 or JAK with a K_D of 1×10^{-7} M or less;

(b) inhibits the function or activity of hyaluronan synthase, ULK1, TNF α , IL-6 or JAK;

(c) inhibits cancer (e.g., metastatic cancer); or

(d) a combination thereof.

Assays to evaluate the binding ability of the antibodies toward hyaluronan synthase, ULK1, TNF α , IL-6 or JAK can be used, including for example, ELISAs, Western blots and RIAs. The binding kinetics (e.g., binding affinity) of the antibodies also can be assessed by standard assays known in the art, such as by Biacore™ analysis.

Given that each of the subject antibodies can bind to hyaluronan synthase, ULK1, TNF α , IL-6 or JAK the V_L and V_H sequences or the respective antibodies can be "mixed and matched" to create other binding molecules that bind to hyaluronan synthase, ULK1, TNF α , IL-6 or JAK, respectively. The binding properties of such "mixed and matched" antibodies can be tested using the binding assays described above and assessed in assays described in the examples. When V_L and V_H chains are mixed and matched, a V_H sequence from a particular V_H / V_L pairing can be replaced with a structurally similar V_H sequence. Likewise, a V_L sequence from a particular V_H / V_L pairing may be replaced with a structurally similar V_L sequence.

Accordingly, in one aspect, the invention provides an isolated monoclonal antibody, or antigen binding portion thereof comprising:

- (a) a heavy chain variable region comprising an amino acid sequence; and
(b) a light chain variable region comprising an amino acid sequence;

wherein the antibody specifically binds hyaluronan synthase, ULK1, TNF α , IL-6 or JAK.

In some cases, the CDR3 domain, independently from the CDR1 and/or CDR2 domain(s), alone can determine the binding specificity of an antibody for a cognate antigen and that multiple antibodies can predictably be generated having the same binding specificity based on a common CDR3 sequence. See, for example,

5 Klimka et al., British J. of Cancer 83(2):252-260 (2000) (describing the production of a humanized anti-CD30 antibody using only the heavy chain variable domain CDR3 of murine anti-CD30 antibody Ki-4); Beiboer et al., J. Mol. Biol. 296:833-849 (2000) (describing recombinant epithelial glycoprotein-2 (EGP-2) antibodies using only the heavy chain CDR3 sequence of the parental murine MOC-31 anti-

10 EGP-2 antibody); Rader et al., Proc. Natl. Acad. Sci. U.S.A. 95:8910-8915 (1998) (describing a panel of humanized anti-integrin $\alpha_v\beta_3$ antibodies using a heavy and light chain variable CDR3 domain. Hence, in some cases a mixed and matched antibody or a humanized antibody contains a CDR3 antigen binding domain that is specific for hyaluronan synthase, ULK1, TNF α , IL-6 or JAK.

15 **Expression Systems**

Nucleic acid segments encoding one or more inhibitory nucleic acids, proteins (e.g., hyaluronidase), or antibodies can be inserted into or employed with any suitable expression system. Commercially useful and/or therapeutically effective quantities of one or more inhibitory nucleic acids, proteins (e.g., hyaluronidase), or

20 antibodies can also be generated from such expression systems. In some cases, the expression systems can be used to express hyaluronidases. In some cases, the expression systems can be used to express inhibitory nucleic acids that can reduce endogenous hyaluronan synthase levels.

Recombinant expression of nucleic acids is usefully accomplished using a

25 vector, such as a plasmid. The vector can include a promoter operably linked to nucleic acid segment encoding one or more of the hyaluronidase or an inhibitory nucleic acid for hyaluronan synthase nucleic acids, or other proteins (e.g., hyaluronidase), or antibodies.

The vector can also include other elements required for transcription and

30 translation. As used herein, vector refers to any carrier containing exogenous DNA. Thus, vectors are agents that transport the exogenous nucleic acid into a cell without degradation and include a promoter yielding expression of the nucleic acid in the cells into which it is delivered. Vectors include but are not limited to plasmids, viral

nucleic acids, viruses, phage nucleic acids, phages, cosmids, and artificial chromosomes.

5 A variety of prokaryotic and eukaryotic expression vectors suitable for carrying, encoding and/or expressing hyaluronidase, inhibitory nucleic acids, proteins, or antibodies can be used. A variety of prokaryotic and eukaryotic expression vectors suitable for carrying, encoding and/or expressing hyaluronidase, inhibitory nucleic acids, proteins, or antibodies can be employed. Such expression vectors include, for example, pET, pET3d, pCR2.1, pBAD, pUC, and yeast vectors. The vectors can be used, for example, in a variety of *in vivo* and *in vitro* situations.

10 The expression cassette, expression vector, and sequences in the cassette or vector can be heterologous. As used herein, the term "heterologous" when used in reference to an expression cassette, expression vector, regulatory sequence, promoter, or nucleic acid refers to an expression cassette, expression vector, regulatory sequence, or nucleic acid that has been manipulated in some way. For example, a heterologous promoter can be a promoter that is not naturally linked to a nucleic acid of interest, or that has been introduced into cells by cell transformation procedures. A heterologous nucleic acid or promoter also includes a nucleic acid or promoter that is native to an organism but that has been altered in some way (*e.g.*, placed in a different chromosomal location, mutated, added in multiple copies, linked to a non-native promoter or enhancer sequence, *etc.*). Heterologous nucleic acids may comprise sequences that comprise cDNA forms. Heterologous coding regions can be distinguished from endogenous coding regions, for example, when the heterologous coding regions are joined to nucleotide sequences comprising regulatory elements such as promoters that are not found naturally associated with the coding region, or when the heterologous coding regions are associated with portions of a chromosome not found in nature (*e.g.*, genes expressed in loci where the protein encoded by the coding region is not normally expressed). Similarly, heterologous promoters can be promoters that at linked to a coding region to which they are not linked in nature.

30 Viral vectors that can be employed include those relating to lentivirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, polio virus, AIDS virus, neuronal trophic virus, Sindbis and other viruses. Also useful are any viral families which share the properties of these viruses which make them suitable for use as vectors. Retroviral vectors that can be employed include those described in by

Verma, I.M., Retroviral vectors for gene transfer. In Microbiology-1985, American Society for Microbiology, pp. 229-232, Washington, (1985). For example, such retroviral vectors can include Murine Maloney Leukemia virus, MMLV, and other retroviruses that express desirable properties. Typically, viral vectors contain,

5 nonstructural early genes, structural late genes, an RNA polymerase III transcript, inverted terminal repeats necessary for replication and encapsidation, and promoters to control the transcription and replication of the viral genome. When engineered as vectors, viruses typically have one or more of the early genes removed and a gene or gene/promoter cassette is inserted into the viral genome in place of the removed

10 viral nucleic acid.

A variety of regulatory elements can be included in the expression cassettes and/or expression vectors, including promoters, enhancers, translational initiation sequences, transcription termination sequences and other elements. A “promoter” is generally a sequence or sequences of DNA that function when in a relatively fixed

15 location in regard to the transcription start site. For example, the promoter can be upstream of the nucleic acid segment encoding one or more hyaluronidase, inhibitory nucleic acid, protein, or antibody.

A “promoter” contains core elements required for basic interaction of RNA polymerase and transcription factors and can contain upstream elements and

20 response elements. “Enhancer” generally refers to a sequence of DNA that functions at no fixed distance from the transcription start site and can be either 5’ or 3’ to the transcription unit. Furthermore, enhancers can be within an intron as well as within the coding sequence itself. They are usually between 10 and 300 by in length, and they function in cis. Enhancers function to increase transcription from nearby

25 promoters. Enhancers, like promoters, also often contain response elements that mediate the regulation of transcription. Enhancers often determine the regulation of expression.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human or nucleated cells) can also contain sequences for the termination of

30 transcription, which can affect mRNA expression. These regions are transcribed as polyadenylated segments in the untranslated portion of the mRNA encoding tissue factor protein. The 3’ untranslated regions also include transcription termination sites. The transcription unit may also contain a polyadenylation region. One benefit of this region is that it increases the likelihood that the transcribed unit will be

processed and transported like mRNA. The identification and use of polyadenylation signals in expression constructs is well established. Homologous polyadenylation signals may be used in the transgene constructs.

5 The expression of one or more type of hyaluronidase, inhibitory nucleic acid, protein, or antibody from an expression cassette or expression vector can be controlled by any promoter capable of expression in prokaryotic cells or eukaryotic cells. Examples of prokaryotic promoters that can be used include, but are not limited to, SP6, T7, T5, *tac*, *bla*, *trp*, *gal*, *lac*, or maltose promoters. Examples of eukaryotic promoters that can be used include, but are not limited to, constitutive
10 promoters, e.g., viral promoters such as CMV, SV40 and RSV promoters, as well as regulatable promoters, e.g., an inducible or repressible promoter such as the tet promoter, the hsp70 promoter and a synthetic promoter regulated by CRE. Vectors for bacterial expression include pGEX-5X-3, and for eukaryotic expression include pCIneo-CMV.

15 The expression cassette or vector can include nucleic acid sequence encoding a marker product. This marker product is used to determine if a vector or expression cassette encoding one or more hyaluronidase, inhibitory nucleic acid, protein, or antibody have been delivered to the cell and once delivered, is being expressed. Marker genes can include the *E. coli* lacZ gene which encodes β -galactosidase, and
20 green fluorescent protein. In some embodiments the marker can be a selectable marker. When such selectable markers are successfully transferred into a host cell, the transformed host cell can survive if placed under selective pressure. There are two widely used distinct categories of selective regimes. The first category is based on a cell's metabolism and the use of a mutant cell line which lacks the ability to
25 grow independent of a supplemented media. The second category is dominant selection which refers to a selection scheme used in any cell type and does not require the use of a mutant cell line. These schemes typically use a drug to arrest growth of a host cell. Those cells which have a novel gene would express a protein conveying drug resistance and would survive the selection. Examples of such
30 dominant selection use the drugs neomycin (Southern P. and Berg, P., J. Molec. Appl. Genet. 1: 327 (1982)), mycophenolic acid, (Mulligan, R. C. and Berg, P. Science 209: 1422 (1980)) or hygromycin, (Sugden, B. et al., Mol. Cell. Biol. 5: 410-413 (1985)).

Gene transfer can be obtained using direct transfer of genetic material, in but not limited to, plasmids, viral vectors, viral nucleic acids, phage nucleic acids, phages, cosmids, and artificial chromosomes, or via transfer of genetic material in cells or carriers such as cationic liposomes. Such methods are available in the art and readily adaptable for use in the method described herein. Transfer vectors can be any nucleotide construction used to deliver genes into cells (e.g., a plasmid), or as part of a general strategy to deliver genes, e.g., as part of recombinant retrovirus or adenovirus (Ram et al. *Cancer Res.* 53:83-88, (1993)). Appropriate means for transfection, including viral vectors, chemical transfectants, or physico-mechanical methods such as electroporation and direct diffusion of DNA, are described by, for example, Wolff, J. A., et al., *Science*, 247, 1465-1468, (1990); and Wolff, J. A. *Nature*, 352, 815-818, (1991).

For example, the nucleic acid molecules, expression cassette and/or vectors encoding one or more hyaluronidase, inhibitory nucleic acid, protein, or antibody can be introduced to a cell by any method including, but not limited to, calcium-mediated transformation, electroporation, microinjection, lipofection, particle bombardment and the like. The cells can also be expanded in culture and then administered to a subject, e.g. a mammal such as a human. The amount or number of cells administered can vary but amounts in the range of about 10^6 to about 10^9 cells can be used. The cells are generally delivered in a physiological solution such as saline or buffered saline. The cells can also be delivered in a vehicle such as a population of liposomes, exosomes or microvesicles.

In some cases, the transgenic cell can produce exosomes or microvesicles that contain nucleic acid molecules, expression cassettes and/or vectors expressing hyaluronidases, inhibitory nucleic acids, proteins, antibodies, or a combination thereof. In some cases, the transgenic cell can produce exosomes or microvesicles that contain nucleic acid molecules that encode one or more hyaluronidases, inhibitory nucleic acids, proteins, or antibodies to particular tissues. Microvesicles can mediate the secretion of a wide variety of proteins (e.g., hyaluronidase), lipids, mRNAs, and micro RNAs, interact with neighboring cells, and can thereby transmit signals, proteins (e.g., hyaluronidase), lipids, and nucleic acids from cell to cell (see, e.g., Shen et al., *J Biol Chem.* 286(16): 14383–14395 (2011); Hu et al., *Frontiers in Genetics* 3 (April 2012); Pegtel et al., *Proc. Nat'l Acad Sci* 107(14): 6328–6333 (2010); WO/2013/084000; each of which is incorporated herein by reference in its

entirety. Cells producing such microvesicles can be used to provide one or more hyaluronidase, inhibitory nucleic acid, protein, or antibody.

Transgenic vectors or cells with a heterologous expression cassette or expression vector can express one or more hyaluronidase, inhibitory nucleic acid, protein, or antibody. Any of these vectors or cells can be administered to a subject. Exosomes produced by transgenic cells can also be used to administer one or more hyaluronidase, inhibitory nucleic acid, protein, or antibody to the subject.

Compositions

The invention also relates to compositions containing PARP1 inhibitors and activated stromal / activated cancer-associated fibroblast inhibitors or inhibitors of TNF α , IL-6 or JAK. Such inhibitors can be small molecules, antibodies, polypeptides, inhibitory nucleic acids, nucleic acids encoding inhibitory nucleic acids, nucleic acids encoding a polypeptide (e.g., within an expression cassette or expression vector), or a combination thereof. The compositions can be pharmaceutical compositions. In some embodiments, the compositions can include a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" it is meant that a carrier, diluent, excipient, and/or salt is compatible with the other ingredients of the formulation, and not deleterious to the recipient thereof.

The composition can be formulated in any convenient form. In some embodiments, the therapeutic agents of the invention (e.g., small molecules, inhibitory nucleic acids, nucleic acids encoding inhibitory nucleic acids, polypeptides, nucleic acid encoding polypeptides (e.g., within an expression cassette or expression vector), a compound identified by a method described herein, or a combination thereof), are administered in a "therapeutically effective amount." Such a therapeutically effective amount is an amount sufficient to obtain the desired physiological effect, such a reduction of at least one symptom of cancer. For example, chemotherapeutic agents can reduce cell metastasis by 5%, or 10%, or 15%, or 20%, or 25%, or 30%, or 35%, or 40%, or 45%, or 50%, or 55%, or 60%, or 65%, or 70%, or 80%, or 90%, or 95%, or 97%, or 99%, or any numerical percentage between 5% and 100%. Symptoms of cancer can also include tumor cachexia, tumor-induced pain conditions, tumor-induced fatigue, tumor growth, and metastatic spread. Hence, the chemotherapeutic agents may also reduce tumor cachexia, tumor-induced pain conditions, tumor-induced fatigue, tumor growth, or a combination thereof by 5%, or 10%, or 15%, or 20%, or 25%, or 30%, or 35%, or 40%, or 45%,

or 50%, or 55%, or 60%, or 65%, or 70%, or 80%, or 90%, or 95%, or 97%, or 99%, or any numerical percentage between 5% and 100%.

To achieve the desired effect(s), the therapeutic agents may be administered as single or divided dosages. For example, therapeutic agents can be administered in dosages of at least about 0.01 mg/kg to about 500 to 750 mg/kg, of at least about 0.01 mg/kg to about 300 to 500 mg/kg, at least about 0.1 mg/kg to about 100 to 300 mg/kg or at least about 1 mg/kg to about 50 to 100 mg/kg of body weight, although other dosages may provide beneficial results. The amount administered will vary depending on various factors including, but not limited to, the type of small molecules, compounds, polypeptides, antibodies, nucleic acids, or combinations thereof chosen for administration, the disease, the weight, the physical condition, the health, and the age of the mammal. Such factors can be readily determined by the clinician employing animal models or other test systems that are available in the art.

Administration of the therapeutic agents in accordance with the present invention may be in a single dose, in multiple doses, in a continuous or intermittent manner, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of the therapeutic agents and compositions of the invention may be essentially continuous over a preselected period of time or may be in a series of spaced doses. Both local and systemic administration is contemplated.

To prepare the composition, small molecules, compounds, polypeptides, nucleic acids, expression cassettes, and other agents are synthesized or otherwise obtained, purified as necessary or desired. These small molecules, compounds, polypeptides, antibodies, nucleic acids, expression cassettes, and other agents can be suspended in a pharmaceutically acceptable carrier and/or lyophilized or otherwise stabilized. The small molecules, compounds, polypeptides, nucleic acids, expression cassettes, other agents, and combinations thereof can be adjusted to an appropriate concentration, and optionally combined with other agents. The absolute weight of a given small molecule, compound, polypeptide, antibodies, nucleic acid, and/or other agents included in a unit dose can vary widely. For example, about 0.01 to about 2 g, or about 0.1 to about 500 mg, of at least one molecule, compound, polypeptide, nucleic acid, and/or other agent, or a plurality of molecules, compounds, polypeptides, nucleic acids, and/or other agents can be administered. Alternatively, the unit

dosage can vary from about 0.01 g to about 50 g, from about 0.01 g to about 35 g, from about 0.1 g to about 25 g, from about 0.5 g to about 12 g, from about 0.5 g to about 8 g, from about 0.5 g to about 4 g, or from about 0.5 g to about 2 g.

Daily doses of the chemotherapeutic agents of the invention can vary as well.

- 5 Such daily doses can range, for example, from about 0.1 g/day to about 50 g/day, from about 0.1 g/day to about 25 g/day, from about 0.1 g/day to about 12 g/day, from about 0.5 g/day to about 8 g/day, from about 0.5 g/day to about 4 g/day, and from about 0.5 g/day to about 2 g/day.

- 10 It will be appreciated that the amount of therapeutic agent for use in treatment will vary not only with the particular carrier selected but also with the route of administration, the nature of the cancer condition being treated and the age and condition of the patient. Ultimately the attendant health care provider can determine proper dosage. In addition, a pharmaceutical composition can be formulated as a single unit dosage form.

- 15 Thus, one or more suitable unit dosage forms comprising the therapeutic agent(s) can be administered by a variety of routes including parenteral (including subcutaneous, intravenous, intramuscular and intraperitoneal), oral, rectal, dermal, transdermal, intrathoracic, intrapulmonary and intranasal (respiratory) routes. The therapeutic agent(s) may also be formulated for sustained release (for example, 20 using microencapsulation, see WO 94/ 07529, and U.S. Patent No.4,962,091). The formulations may, where appropriate, be conveniently presented in discrete unit dosage forms and may be prepared by any of the methods well known to the pharmaceutical arts. Such methods may include the step of mixing the therapeutic agent with liquid carriers, solid matrices, semi-solid carriers, finely divided solid 25 carriers or combinations thereof, and then, if necessary, introducing or shaping the product into the desired delivery system. For example, the therapeutic agent(s) can be linked to a convenient carrier such as a nanoparticle, albumin, polyalkylene glycol, or be supplied in prodrug form. The therapeutic agent(s), and combinations thereof can be combined with a carrier and/or encapsulated in a vesicle such as a 30 liposome.

The compositions of the invention may be prepared in many forms that include aqueous solutions, suspensions, tablets, hard or soft gelatin capsules, and liposomes and other slow-release formulations, such as shaped polymeric gels.

Administration of inhibitors can also involve parenteral or local administration of the in an aqueous solution or sustained release vehicle.

Thus, while the therapeutic agent(s) and/or other agents can sometimes be administered in an oral dosage form, that oral dosage form can be formulated so as to protect the small molecules, compounds, polypeptides, nucleic acids, expression cassettes, and combinations thereof from degradation or breakdown before the small molecules, compounds, antibodies, polypeptides, inhibitory nucleic acids, nucleic acids encoding such polypeptides, and combinations thereof provide therapeutic utility. For example, in some cases the small molecules, compounds, inhibitory nucleic acids, polypeptides, nucleic acids encoding such polypeptide, antibodies, and/or other agents can be formulated for release into the intestine after passing through the stomach. Such formulations are described, for example, in U.S. Patent No. 6,306,434 and in the references contained therein.

Liquid pharmaceutical compositions may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, dry powders for constitution with water or other suitable vehicle before use. Such liquid pharmaceutical compositions may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives. The pharmaceutical compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Suitable carriers include saline solution, encapsulating agents (e.g., liposomes), and other materials. The chemotherapeutic agent(s) and/or other agents can be formulated in dry form (e.g., in freeze-dried form), in the presence or absence of a carrier. If a carrier is desired, the carrier can be included in the pharmaceutical formulation, or can be separately packaged in a separate container, for addition to the inhibitor that is packaged in dry form, in suspension or in soluble concentrated form in a convenient liquid.

Therapeutic agent(s) and/or other agents can be formulated for parenteral administration (e.g., by injection, for example, bolus injection or continuous infusion) and may be presented in unit dosage form in ampoules, prefilled syringes, small volume infusion containers or multi-dose containers with an added preservative.

The compositions can also contain other ingredients such as chemotherapeutic agents, anti-viral agents, antibacterial agents, antimicrobial agents and/or preservatives. Examples of additional therapeutic agents that may be used include, but are not limited to: alkylating agents, such as nitrogen mustards, alkyl sulfonates, nitrosoureas, ethylenimines, and triazenes; antimetabolites, such as folate antagonists, purine analogues, and pyrimidine analogues; antibiotics, such as anthracyclines, bleomycins, mitomycin, dactinomycin, and plicamycin; enzymes, such as L-asparaginase; farnesyl-protein transferase inhibitors; hormonal agents, such as glucocorticoids, estrogens/antiestrogens, androgens/antiandrogens, progestins, and luteinizing hormone-releasing hormone antagonists, octreotide acetate; microtubule-disruptor agents, such as ecteinascidins or their analogs and derivatives; microtubule-stabilizing agents such as paclitaxel (Taxol®), docetaxel (Taxotere®), and epothilones A-F or their analogs or derivatives; plant-derived products, such as vinca alkaloids, epipodophyllotoxins, taxanes; and topoisomerase inhibitors; prenyl-protein transferase inhibitors; and miscellaneous agents such as, hydroxyurea, procarbazine, mitotane, hexamethylmelamine, platinum coordination complexes such as cisplatin and carboplatin; and other agents used as anti-cancer and cytotoxic agents such as biological response modifiers, growth factors; immune modulators, and monoclonal antibodies. The compositions can also be used in conjunction with radiation therapy.

Treatment

The one or more PARP1 inhibitors and inhibitors of activated stromal / activated cancer-associated fibroblasts, TNF α , IL-6 or JAK are useful for selectively targeting tumors or treating cancers.

“Treatment” or “treating” refers to both therapeutic treatment, and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those prone to have the disorder, or those in whom the disorder is to be prevented.

“Subject” for purposes of treatment refers to any animal classified as a mammal or bird, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. In one embodiment, the subject is human.

As used herein, the term “cancer” includes solid animal tumors as well as hematological malignancies. The terms “tumor cell(s)” and “cancer cell(s)” are used interchangeably herein.

"Solid animal tumors" include cancers of the head and neck, lung, mesothelioma, mediastinum, lung, esophagus, stomach, pancreas, hepatobiliary system, small intestine, colon, colorectal, rectum, anus, kidney, urethra, bladder, prostate, urethra, penis, testis, gynecological organs, ovaries, breast, endocrine system, skin central nervous system; sarcomas of the soft tissue and bone; and melanoma of cutaneous and intraocular origin. In addition, a metastatic cancer at any stage of progression can be treated, such as micrometastatic tumors, megametastatic tumors, and recurrent cancers.

The term "hematological malignancies" includes adult or childhood leukemia and lymphomas, Hodgkin's disease, lymphomas of lymphocytic and cutaneous origin, acute and chronic leukemia, plasma cell neoplasm and cancers associated with AIDS.

The inventive methods and compositions can also be used to treat cancer of the breast, cancer of the lung, cancer of the adrenal cortex, cancer of the cervix, cancer of the endometrium, cancer of the esophagus, cancer of the head and neck, cancer of the liver, cancer of the pancreas, cancer of the prostate, cancer of the thymus, carcinoid tumors, chronic lymphocytic leukemia, Ewing's sarcoma, gestational trophoblastic tumors, hepatoblastoma, multiple myeloma, non-small cell lung cancer, retinoblastoma, or tumors in the ovaries. A cancer at any stage of progression can be treated or detected, such as primary, metastatic, and recurrent cancers. In some cases, metastatic cancers are treated but primary cancers are not treated. Information regarding numerous types of cancer can be found, e.g., from the American Cancer Society (cancer.org), or from, e.g., Wilson et al. (1991) Harrison's Principles of Internal Medicine, 12th Edition, McGraw-Hill, Inc.

In some embodiments, the cancer and/or tumors to be treated are prostate cancers, lung cancers, or breast cancers.

Treatment of, or treating, metastatic cancer can include the reduction in cancer cell migration or the reduction in establishment of at least one metastatic tumor. The treatment also includes alleviation or diminishment of more than one symptom of metastatic cancer such as coughing, shortness of breath, hemoptysis, lymphadenopathy, enlarged liver, nausea, jaundice, bone pain, bone fractures,

headaches, seizures, systemic pain and combinations thereof. The treatment may cure the cancer, e.g., it may prevent metastatic cancer, it may substantially eliminate metastatic tumor formation and growth, and/or it may arrest or inhibit the migration of metastatic cancer cells.

5 Anti-cancer activity can reduce the progression of a variety of cancers (e.g., breast, lung, or prostate cancer) using methods available to one of skill in the art. Anti-cancer activity, for example, can be determined by identifying the lethal dose (LD100) or the 50% effective dose (ED50) or the dose that inhibits growth at 50% (GI50) of an agent of the present invention that prevents the migration of cancer
10 cells. In one aspect, anti-cancer activity is the amount of the agent that reduces 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% or 100% of cancer cell migration, for example, when measured by detecting expression of a cancer cell marker at sites proximal or distal from a primary tumor site, or when assessed using available methods for detecting metastases.

15 In another example, PARP1 inhibitors and one or more inhibitors of activated stromal / activated cancer-associated fibroblasts, TNF α , IL-6 or JAK can be administered to sensitize tumor cells to immune therapies. By administering PARP1 inhibitors and one or more inhibitors of activated stromal / activated cancer-associated fibroblasts, TNF α , IL-6 or JAK, tumor cells can become more sensitive
20 to the immune system and to various immune therapies.

The invention will be further described by the following non-limiting examples.

Example 1

Reduced p62 levels are associated with the induction of the cancer-associated fibroblast (CAF) phenotype, which promotes tumorigenesis *in vitro* and
25 *in vivo* through inflammation and metabolic reprogramming. However, how p62 is downregulated in the stroma fibroblasts by tumor cells to drive CAF activation is an unresolved central issue in the field. As disclosed herein, tumor-secreted lactate downregulate p62 transcriptionally through a mechanism involving reduction of the
30 NAD⁺/NADH ratio, which impaired poly(ADP-Ribose)-polymerase 1 (PARP1) activity. PARP1 inhibition blocked the poly(ADP-ribosyl)ation of the AP1 transcription factors, c-FOS and c-JUN, which is an obligate step for p62 downregulation. Importantly, restoring p62 levels in CAFs by NAD⁺ rendered CAFs less active. PARP1 inhibitors, such as Olaparib, mimicked lactate in the reduction of

stromal p62 levels, as well as the subsequent stromal activation both *in vitro* and *in vivo*, which suggests that therapies utilizing Olaparib would benefit from strategies aimed at inhibiting CAF activity.

The mechanisms whereby cancer cells promote the acquisition of the pro-tumorigenic CAF phenotype was investigated. As disclosed herein, the lactate secreted by tumor cells downregulates p62 at the transcriptional level to induce stromal activation. Lactate caused a reduction in the NAD⁺/NADH ratio that impaired Poly(ADP-ribose)polymerase 1 (PARP1) activity. The results demonstrate that NAD⁺ supplementation restored p62 levels and impaired CAF activation established the functional relevance of the lactate-PARP1 axis for p62 downregulation and the generation of a reactive stroma conducive to malignancy. Furthermore, the fact that, PARP1 inhibitors used in the clinic, such as Olaparib, mimicked the effect of lactate in downregulating p62 and promoting CAF activity, reveals an unanticipated potential weakness of therapies based on PARP1 inhibitors, and suggest that reprogramming the stroma would enhance the anti-cancer effects of Olaparib.

Experimental Model and Subject Details

Mice

Animal handling and experimental procedures conformed to institutional guidelines and were approved by the Sanford-Burnham-Prebys Medical Discovery Institute Institutional Animal Care and Use Committee, and by the Weill Cornell Medicine Institutional Animal Care and Use Committee. For Olaparib treatment experiments, 13 week-old male TRAMP⁺ mice (C57BL/6-Tg(TRAMP)8247Ng/J, stock No: 003135) were purchased from The Jackson Laboratory, (Bar Harbor, ME, USA). TRAMP⁺ mice were generated in a C57BL/6 background and were born and maintained under pathogen-free conditions. All genotyping was done by PCR. Age-matched mice were used for all experiments. For xenograft experiments, 7-week-old male JAX NSG mice (572NCG) were purchased from Charles River Labs (Wilmington, MA, USA). NSG mice were purchased and maintained under pathogen-free conditions. All mice were maintained on food and water ad libitum and were age-matched and co-housed for all experiments. Mice were sacrificed and prostate, tumors or other organs were collected for analysis.

Cell Lines

WPMY-1 (sex: male), mNAF (sex: male), mHSC (sex: male), hNAF (sex: male), PC3 (sex: male), DU145 (sex: male), TRAMPC2 (sex: male), PrEC (sex: male), RWPE1 (sex: male), LNCAP (sex: male), HEK293T (sex: female) and Phoenix-GP (sex: female) cell lines were purchased from ATCC. WPMY-1, mNAF, mHSC, PC3, DU145, TRAMPC2, HEK293T and Phoenix-GP were cultured in Dulbecco's Modified Eagles Medium (DMEM, Corning). LNCAP, PrEC and RWPE1 cells were cultured in Roswell Park Memorial Institute Medium (RPMI, Corning), hNAF cells were cultured in Fibroblast media (FM, ScienceCell Research Laboratories). All base mediums were supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine in an atmosphere of 95% air and 5% CO₂. Androgen Deprivation Therapy conditions, cells were culture in RPMI media without phenol red (GIBCO) supplemented with 10% charcoal stripped FBS (F6765, Sigma), Glutamax and 100 U/mL penicillin 100 and 100 µg/mL streptomycin. Cultures were tested weekly for mycoplasma contamination.

15 Method Details

Xenograft Experiments

For mouse xenografts using PC3 PCa cells and sgC or sgAP-1 WPMY-1 stromal cells. 7 weeks old NSG mice were surgical castrated and let androgen levels drop for 10 days. Cells were trypsinized washed two times in PBS and resuspend in DMEN. 1x10⁶ PC3 and 1x10⁶ sgC or sgAPI-A WPMY-1 for each mouse were resuspended in 100 µl DMEM:Matrigel (1:1) and injected subcutaneously into both flanks of immunocompromised NSG mice (PC3 + sgC WPMY-1 n=4; PC3 + sgAP-1 WPMY-1 n=4). Tumors were allowed to grow for 1 month. Mice were euthanized and tumors were collected and analyzed histologically. For Olaparib treatment in mouse xenografts. PC3 PCa cells and WPMY-1 stromal cells were trypsinized washed two times in PBS and resuspend in DMEN. 1x10⁶ PC3 and 1x10⁶ WPMY-1 for each mouse were resuspended in 100 µl DMEM:Matrigel (1:1) and injected subcutaneously into both flanks of immunocompromised NSG mice. Tumors were allowed to grow for 14 days, and mouse were randomly divided to receive vehicle, n=10, Olaparib (40 mg/kg, 2 days each week, i.p.), n=10 or Olaparib (40 mg/kg, 2 days each week, i.p.) +_PEGPH20 (0.0375 mg/Kg, 2 days each week, retroorbital injection) n=10. Tumors were measured twice a week. Mice were euthanized 14 days after the initiation of the treatment and tumors were collected and analyzed histologically. For Olaparib treatment in mice, 13 weeks-old male TRAMP⁺, n=18,

were randomly distributed to receive vehicle, n=9, or Olaparib (40 mg/kg/, 5 days each week, i.p.), n=9. Mice were euthanized 1 month after the initiation of the treatment. Prostate and other organs were collected and analyzed histologically.

Cell Culture Experiments

5 To simulate the amounts of lactate that accumulates in the tumor microenvironment as consequence of the Warburg effect, WPMY-1 cells were incubated in culture medium with or without lactic acidosis (24 mM of Lactic acid buffered to pH 6.7-6.8 with NaOH) for 48 hours. To avoid fluctuations in extracellular pH during the Lactate treatment, WPMY-1 cells were grown in a bicarbonate-free DMEM with 30 mM of HEPES in absence of CO₂. To knock out *PARP-1* in WPMY-1 cells, single-guide RNA sequences targeting *PARP-1* exon2 were purchased from Synthego and transduced into WPMY-1 cells with recombinant *Streptococcus pyogenes* Cas9 protein (Truecut Cas9 Protein v2, Thermo), using the Neon Transfection System 1 (Invitrogen) following the manufacturer's protocol and single clones were expanded and screened by protein immunoblotting. To perform *API-A* binding site editing in the *SQSTM1* promoter in WPMY-1 cells, single-guide RNA sequences targeting the human API-A binding site (Synthego) was transduced into cells with a Cas9 protein and a single-stranded donor oligonucleotide (ssODN, IDT) using Neon Electroporation System. Single clones were expanded and screened for *API-A* editing by Sanger sequencing. A point mutation to disrupt the API1 enhancer element in the p62 promoter was introduced by Site Directed Mutagenesis (Stratagene). Knockdown of *MCT1*, *MCT4*, *FOS*, *PARP1*, *PARP2*, *CD38*, *ATG5*, and *SIRT1-7* genes in WPMY-1 cells were achieved by siRNA transfection using Lipofectamine RNAiMAX Transfection Reagent (Invitrogen). Transient overexpression was achieved by transfection using X-tremeGENE HP transfection reagent (Roche). Transfected cells were examined 48 hours after transfection. Establishing stably GFP or RFP expressing cells was achieved by lentivirus-mediated transduction. Lentivirus were produced in HEK293T cells using X-tremeGENE HP transfection reagent (Roche). Virus-containing supernatants were collected 48, 72 and 96 hours after transfection, filtered to eliminate cells, and supplemented with 8 µg/ml polybrene. Cells were infected with three rounds of viral supernatants and selected with puromycin (3 µg/ml). For autophagy or proteasome inhibition, WPMY-1 cells were treated for 12 hours with 100 nM bafilomycin A1 or 12 hours with 20 µM of MG132, respectively,

or vehicle (DMSO). For PARP1 inhibition, WPMY-1 cells were treated with PJ34 (2 days) or Olaparib (4 days) at the doses indicated in each experiment. For MCT1 inhibition, WPMY-1 cells were treated for 48 hours with 10 μ M of AZD3965. For cJUN inhibition, WPMY-1 cells were treated for 48 hours with 10 μ M of SP600125.

5 For calpains and caspases inhibition, WPMY-1 cells were treated for 48 hours with 1-20 μ M of Calpeptin (Tocris) or z-VAD-FMK (Tocris), respectively. For protein synthesis inhibition, WPMY-1 cells were treated with 50 μ g/ml of cycloheximide (Sigma). Conditioned media (CM) was generated by collecting supernatant on day 3. CM was transferred to a 15 ml BD Falcon tube and centrifuged at 1300 rpm for 10

10 minutes. The supernatant was sterile filtered using a 22 mm filter (Millex-GV) with a 10 ml syringe barrel. The samples were stored at 20°C for future experiments. Fractionation of the CM was achieved using Amicon Ultra centrifugal filters (3KUltra, Millipore). The supernatant was centrifuged at 4000 rpm for 1 hour. The supernatant fraction that was >3 kDa remained above the filter and that which

15 was <3kDa passed through to the lower chamber. The >3-kDa fraction was resuspended in DMEM to the pre-filtration volume.

Organotypic Cultures

Organotypic cultures were performed as described previously (Valencia et al., 2014). Briefly, gels were composed of one ml of a mixture of 1.75 volumes of

20 Matrigel, 5.25 volumes of collagen type I, 1 volume of 1x DMEM, 1 volume of 10x DMEM, and 1 volume of filtered FBS. The mixture was plated onto 24-well plates coated with diluted collagen type I. Gels were allowed to equilibrate with 1 ml of 1x DMEM overnight at 37°C. 5 x 10⁵ cells PCa cells and prostate stromal cells (50:50) were then seeded on top of the matrix. Gel rafts were placed onto collagen-coated

25 nylon sheets and lifted using a sterile supporting steel mesh to set up a raised air-liquid culture. Normal medium was changed in alternate days and organotypic cultures were allowed to grow for 14 days. Afterwards, organotypic gels were harvested, fixed in 10% neutral buffered formalin, bisected, and embedded in paraffin. H&E-stained sections were analyzed with a Zeiss light microscope

30 supplemented with Axiovision40 software. Quantification of the invasion assays was performed as described previously (Valencia et al., 2014) using ImageProPlus software.

Migration and Invasion Assay

For co-culture migration and invasion assays, 8×10^4 WPMY-1 cells were plated onto the lower chamber of a 24 well plate in DMEN containing 10% FBS (basal conditions) or 10% charcoal stripped FBS (ADT conditions) and vehicle (DMSO) or Olaparib 10 μ M or Olaparib 10 μ M + PEGPH20 2.5 μ g/ml for 48 hours.

- 5 5×10^4 PC3 were seeded in a transwell chamber (Corning Biocoat control inserts) or in a transwell invasion chamber (Corning BioCoat Matrigel Invasion Chambers), both with 8 μ m membrane. PC3 were allowed to migrate or invade for 20 hours at 37°C, 5% CO₂. Cells were fixed in cold methanol and stained with crystal violet.

Proliferation assay

- 10 WPMY-1 cells were cultured in DMEN containing 10% FBS (basal conditions) or 10% charcoal stripped FBS (ADT conditions), then the cells were treated with vehicle (DMSO), Olaparib 10 μ M, Olaparib 10 μ M + PEGPH20 2.5 μ g/ml for 96 hours, refreshing media and stimuli at 48 hours, conditioned media (CM) was generated by collecting supernatant on day 4. PC3 cells were seeded in a
15 black-walled well (Thermo Fisher) and serum starved overnight. CM from WPMY-1 cells was added to PC3 for 24 h. PC3 were exposed to EdU incorporation for the last 2 hours. Proliferating cells were detected using Click-iT EdU Alexa fluor 488 (Thermo Fisher) following the manufacturer's instructions. Pictures were taken using EVOS M5000 Imaging System and EdU positive were measured using Fiji
20 (Schindelin et al., 2012).

Co-culture assays

- Co-culture assays were performed as described previously (Valencia et al., 2014). Briefly, stromal cells were seeded in TC-treated 6-well plates and allowed to attach for 6 hours before PCa cells were seeded on top of Milicell Cell Culture
25 Inserts (Millipore). Inserts were then placed in the pre-seeded 6-well plate. For co-culture experiments of PC3-GFP label with WPMY-1 cells, 500 PC3 and 1000 WPMY-1 were seeded in a 96 well plate in DMEN containing 10% FBS (basal conditions) or 10% charcoal stripped FBS (ADT conditions) and vehicle (DMSO) or Olaparib 5 μ M for 10 days. Media and treatments were refresh every 48 hours.
30 Pictures were taken using EVOS M5000 Imaging System and GFP positive were measured using Fiji (Schindelin et al., 2012).

Isotopic Labeling

WPMY-1 cells were cultured in DMEM (Cat. #5030, Sigma) medium supplemented with 10% FBS, 4 mM glutamine, 25 mM glucose, 30 mM HEPES,

10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin in a humidified cell culture incubator at 37°C with no CO₂. For isotopic tracing, cells were cultured for 48 hours in growth medium, medium was then changed to growth medium containing 10 mM [3-¹³C]lactate (Cat. # CLM-1578, Cambridge Isotopes) or 10 mM [2-²H]lactate (Cat. #693987, Sigma). Cells were cultured in tracer media for 24 hours. All media was adjusted to pH = 6.8.

Gas Chromatography-Mass Spectrometry (GC/MS)

Metabolites were extracted using a modified Bligh and Dyer method and analyzed as previously described in detail (Linares et al., 2017). Briefly, intracellular metabolites were extracted with 0.25 ml -20°C methanol, 0.1 ml 4°C cold water, and 0.25 ml -20°C chloroform. The extracts were vortexed for 10 minutes at 4°C and centrifuged at 16,000×g for 5 minutes at 4°C. The upper aqueous phase was evaporated under vacuum at -4°C, the lower organic phase under airflow. To determine labeling on lactate in cell culture media, 10 µl of medium was extracted with 90 µl of extraction buffer consisting of 8 parts (v/v) methanol and 1 part (v/v) water, centrifuged at 16,000×g for 5 minutes at 4°C, and 60 µl was dried under vacuum. Derivatization for polar metabolites was performed using a Gerstel MPS with 15 µl of 2% (w/v) methoxyamine hydrochloride (Thermo Scientific) in pyridine (incubated for 60 minutes at 45°C) and 15 µl N-tertbutyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) with 1% tert-butyldimethylchlorosilane (Regis Technologies) (incubated further for 30 minutes at 45°C). Polar derivatives were analyzed by GC-MS using a DB-35MSUI column (30 m x 0.25 i.d. x 0.25 µm) installed in an Agilent 7890B gas chromatograph (GC) interfaced with an Agilent 5977B mass spectrometer (MS) operating under electron impact ionization at 70 eV. The MS source was held at 230°C, the quadrupole at 150°C, helium was used as carrier gas and the GC oven was held at 100°C for 1 minute, increased to 300°C at 10°C/minute, and held at 325°C for 3 minutes. Intracellular labeling on metabolites (corrected for natural abundance using in-house software) is depicted as 1-M0. YSI (yellow springs instrument) was used to quantify the concentration of media lactate and GC/MS to determine lactate labeling in cell culture media at 0 hours and after 24 hours of culture.

Measurement of NAD levels

Quantification was carried out using the NAD/NADH quantification colorimetric kit (Cat. No. MAK037, Sigma) according to the manufacturer's protocol. At least three independent measurements were carried out.

Luciferase assay

5 Subconfluent cultures were transfected with Lipofectamine Plus (Invitrogen) with 100 ng of a *SQSTM1*-luciferase reporter gene plasmid and 2 ng of the *Renilla* control reporter pRL-CMV (Promega). After 48 hours, cells were incubated with conditioned medium for 48 hours. The level of promoter activity was evaluated by determining the firefly luciferase activity relative to *Renilla* luciferase activity using
10 the Dual Luciferase Assay System (Promega) according to the manufacturer's instruction.

Immunofluorescence analysis

WPMY-1 cells were seeded in coverslips and cultured in DMEN containing 10% FBS (basal conditions) or 10% charcoal stripped FBS (ADT conditions), then
15 the cells were treated with vehicle (DMSO), Olaparib 10 μ M, Olaparib 10 μ M + PEGPH20 2.5 μ g/ml for 96 hours, refreshing media and stimuli at 48 hours. Cells were fixed in PFA 4% for 20 minutes at room temperature, permeabilize and blocked following abcam's immunofluorescence protocol. Then, cells were incubated overnight with Hyaluronic acid binding protein (HABP) at 4°C. Cells
20 were incubated with Alexa conjugated streptavidin and mounted in slides with mowiol. Pictures were taken by Zeiss LSM 710 NLO Confocal Microscope.

Histological Analysis

Tissues from indicated mice were isolated, fixed in zinc buffered formalin overnight at 4°C, dehydrated, and embedded in paraffin. Sections (5 μ m) were
25 stained with hematoxylin and eosin (H&E). For immunohistochemistry, sections were deparaffinized, rehydrated, and then treated for antigen retrieval. After blocking in Protein Block Serum-Free solutions (DAKO), tissues were incubated with primary antibody overnight at 4°C followed by incubation with biotinylated secondary antibody. Endogenous peroxidase was quenched in 3% H₂O₂ in water for
30 10 minutes at room temperature. Antibodies were visualized with avidin-biotin complex (Vectastain Elite; Vector Laboratories) using diaminobenzidine as the chromogen. Stained sections were analyzed with a Zeiss light microscope supplemented with Zen 3.3 Blue edition software.

Immunoblotting Analysis

Cells for protein analysis were lysed in RIPA buffer (20 mM Tris-HCl, 37 mM NaCl₂, 2 mM EDTA, 1% Triton-X, 10% glycerol, 0.1% SDS, and 0.5% sodium deoxycholate) with phosphatase and protease inhibitors. For immunoprecipitations, cells were lysed in IP lysis buffer (100 mM NaCl, 25 mM Tris, 1% Triton-X, 10% glycerol, with phosphatase and protease inhibitors) and immunoprecipitated with 25 μ l of 50% slurry of protein Glutathione-Sepharose 4B beads (Bioworld). Immunoprecipitates were washed three times with lysis buffer, once with high salt (500 mM NaCl), and once more with lysis buffer. Protein concentration in lysates was determined by using Protein Assay Kit (Bio-Rad). Cell extracts and immunoprecipitated proteins were denatured, subjected to SDS-PAGE, transferred to PVDF membranes (GE Healthcare). After blocking with 5% nonfat dry milk in Tris-buffered saline and 0.1% Tween (TBS-T), the membranes were incubated with the specific antibodies (as listed in Key Resources Table) overnight at 4°C. After 2 hours incubation with the appropriate horseradish peroxidase-conjugated antibodies, the immune complexes were detected by chemiluminescence (Thermo Scientific) or Near-infrared fluorescence (LI-COR).

RNA extraction and Analysis

Total RNA from mouse tissues, cells and cultured organoids was extracted using the TRIZOL reagent (Invitrogen) and the RNeasy Mini Kit (QIAGEN), followed by DNase treatment. After quantification using a Nanodrop 1000 spectrophotometer (Thermo Scientific), RNA was either processed for RNA-seq or reverse-transcribed using random primers and MultiScribe Reverse Transcriptase (Applied Biosystems). Gene expression was analyzed by amplifying 20 ng of the complementary DNA using the CFX96 Real Time PCR Detection System with SYBR Green Master Mix (BioRad) and primers described in Table S1. The amplification parameters were set at 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds (40 cycles total). Gene expression values for each sample were normalized to the 18s RNA.

Chromatin immunoprecipitation Analysis

WPMY-1 cells were fixed by adding directly to the culture medium formaldehyde (HCHO; from a 37% HCHO–10% methanol stock, Calbiochem) to a final concentration of 1%. After, 20 minutes of incubation with 125 mM glycine, cells were washed with ice-cold phosphate-buffered saline (PBS) and lysed with in 50 mM Tris pH 8.0, 10 mM EDTA, 1% SDS and protease inhibitors and incubated

30 minutes at 4 °C. Chromatin was sheared in a COVARIS S220 Focused-ultrasonicator to yield DNA fragment sizes of 200–1000 base pairs, and diluted 10 times in dilution buffer (20 mM Tris pH 8.0, 2 mM EDTA, 1% triton X-100, 150 mM NaCl and protease inhibitors). Immunoprecipitations were carried out overnight
5 at 4°C using the following protein A-antibodies complexes: cFOS, FOSB, cJUN, JUNB.

Immunocomplexes were washed three times with buffer TSEI (20 mM Tris pH 8.0, 2 mM EDTA, 1% triton X-100, 150 mM NaCl, 0.1% SDS and protease inhibitors), three washes with buffer TSEII (20 mM Tris pH 8.0, 2 mM EDTA, 1%
10 triton X-100, 500 mM NaCl, 0.1% SDS and protease inhibitors), one wash with buffer TSEIII (10 mM Tris pH 8.0, 250 mM LiCl, 1 mM EDTA, 1% NP40, 1% Deoxycholate and protease inhibitors) and one wash with TE pH 8.0. Immunocomplexes were extracted in TE containing 1% SDS, and protein–DNA cross-links were reverted by heating at 65°C overnight. DNA was extracted by using
15 a PCR purification kit (Qiagen). One-tenth of the immunoprecipitated DNA was used in each PCR, for which the promoter-specific primers were used.

RNA-seq Preparation and Sequencing

Total RNA was extracted using Quick-RNA MiniPrep kit (Zymo Research). Libraries were prepared from 200 ng of total RNA using the QuantSeq 3' mRNA-
20 Seq Library Prep Kit FWD for Illumina from Lexogen, and optional UMIs (Vienna, Austria). Barcoded libraries were pooled, and single end sequenced (1X75) on the Illumina NextSeq 500 using the High output V2.5 kit (Illumina Inc., San Diego CA).

ATAC-Seq Library Preparation and Sequencing

The cell pellet was resuspended in 50 µl lysis buffer and then spun down 500
25 × g for 10 minutes at 4°C. The nuclei pellet was resuspended into 50 µl transposition reaction mixture containing Tn5 transposase from Nextera DNA Library Prep Kit (Illumina) and incubated at 37°C for 30 minutes. Then the transposase-associated DNA was purified using MinElute PCR purification kit (QIAGEN). To amplify the library, the DNA was first amplified for 5 cycles using indexing primer from
30 Nextera kit and NEBNext High-Fidelity 2X PCR master mix. To reduce the PCR amplification bias, 5 µl of amplified DNA after the first 5 cycles was used to do qPCR of 20 cycles to decide the number of cycles for the second round of PCR. Usually, the maximum cycle of the second round of PCR is 5 cycles. Then the total amplified DNA was size selected to fragments less than 800 bp using SPRI beads.

Quantification of the ATAC-seq library was done with QuBit. The size of the pooled library was examined by TapeStation. Barcoded ATAC-seq libraries were pooled and paired end sequenced (sX75) on the Illumina NextSeq 500 using the High output V2.5 kit (Illumina Inc., San Diego, CA).

5 Prostate tissue digestion and 10x Library Preparation and Sequencing

Prostate lobes dissected and washed with cold PBS. Prostate tissue was gently minced with scissors and digested in Advance DMEN/F12 media (Gibco, Thermo Fisher) with 5 mg/ml Collagenase type II (Sigma) supplemented with Y27632 (Tocris) for 1 hour at 37°C. Cells were passed through a 70 µm strainer and
10 centrifuge 5 minutes, 1000 rpm at 4°C. After that, cells were incubated with TrypLE Express (Gibco, Thermo Fisher) supplemented with Y27632 for 20 minutes in agitation at 37°C to achieve a single cell suspension. TrypLE Express was quenched and washed with Advance DMEN/F12 media and then cells were centrifuged for 5 minutes, 1000 rpm at 4°C. Dead cells were removed by Annexin V (STEMCELL
15 technologies). scRNA-seq libraries were generated using the Chromium Single Cell 30 Reagent Kit v2 (10X Genomics). Cells were loaded onto the 10X Chromium Single Cell Platform (10X Genomics) at a concentration of 2,000 cells per µl (Single Cell 3' library and Gel Bead Kit v.2) as described in the manufacturer's protocol (10x User Guide, Revision B). On average, approximately 8,000 cells were loaded.
20 Generation of gel beads in emulsion (GEMs), barcoding, GEM-RT clean-up, complementary DNA amplification and library construction were all performed as per the manufacturer's protocol. Individual sample quality was checked using a Bioanalyzer TapeStation (Agilent). Qubit was used for library quantification before pooling. The final library pool was sequenced on an Illumina NovaSeq6000
25 instrument using a S1 flow cell.

Bioinformatics Analysis

For RNA-Seq, sequencing Fastq files were uploaded to BaseSpace and processed with RNASeq Alignment App (Illumina) to obtain raw reads counts for each gene. For 3' RNA-Seq, read data was processed with the BlueBee Genomics
30 Platform (BlueBee, San Mateo, CA). GenePattern (<https://genepattern.broadinstitute.org/gp/pages/index.jsf>) was used to collapse gene matrix files (CollapseDataset module) or to assess the statistical significance of differential gene expression (ComparativeMarkerSelection module for microarray data and DESeq2 module for RNA-seq data). Gene Set Enrichment Analysis

(GSEA) was performed using GSEA v4.1.0 software (<http://www.broadinstitute.org/gsea/index.jsp>) using the default parameters, with customized signatures, using the default parameters with customized signatures. Preranked GSEA analysis was performed using default parameters with MSigDB c2.all.v7.0.symbols (C2) and c5.all.v7.0.symbols (C5) collections. Briefly, differential express genes between Olaparib treated cells and vehicle were obtain using DESeq2, genes were sorted by $\log_2FC \geq \pm 0.5$ and $p_{adj} \geq 0.05$. Ranked genes were used as input for GSEA Preranked analysis. For ATACSeq, FASTQ files from ATAC-seq reads were aligned to UCSC mm10 with Bowtie2 (bowtie2 -very-sensitive -x mm10 -1 FILE_merged_R1.fastq -2 FILE_merged_R2.fastq -X 1000 -p 12 | samtools view -u - | samtools sort - > FILE.bam). Peak calling was performed with MACS2 with a threshold of $q < 0.05$. Peaks were annotated with ChipSeeker R package. For motif enrichment analysis in the differential peaks between vehicle and Olaparib-treated cells, p-values were calculated using findMotifsGenome.pl with -size given, -len 6,8,10,12 and -mask program of HOMER v4.10.3. (Heinz et al., 2010) within 1-2 Kb from the TSS. Preranked GSEA analysis was performed using default parameters with MSigDB c2.all.v7.0.symbols (C2) and c5.all.v7.0.symbols (C5) collections. Briefly, differential express genes between Olaparib treated cells and vehicle were obtain using DESeq2, genes were sorted by $\log_2FC \geq \pm 0.5$ and $p_{adj} \geq 0.05$. Ranked genes were used as input for GSEA Preranked analysis. For ATACSeq, FASTQ files from ATAC-seq reads were aligned to UCSC mm10 with Bowtie2 (bowtie2 -very-sensitive -x mm10 -1 FILE_merged_R1.fastq -2 FILE_merged_R2.fastq -X 1000 -p 12 | samtools view -u - | samtools sort - > FILE.bam). Peak calling was performed with MACS2 with a threshold of $q < 0.05$. Peaks were annotated with ChIPSeeker R package. For motif enrichment analysis in the differential peaks between vehicle and Olaparib-treated cells, p-values were calculated using findMotifsGenome.pl with -size given, -len 6,8,10,12 and -mask program of HOMER v4.10.3. (Heinz et al., 2010) within 1-2 Kb from the TSS. For scRNA-seq, raw sequence reads were quality-checked using FastQC software. The Cell Ranger version 2.1.1 software suite from 10X Genomics (<https://support.10xgenomics.com/single-cell-gene-expression/software/downloads/latest>) was used to process, align and summarize unique molecular identifier (UMI) counts against the mouse mm10 assembly reference genome analysis set, obtained from the University of California Santa

Cruz (UCSC). Raw, unfiltered count matrices were imported into R for further processing. Raw UMI count matrices were filtered using the Seurat v 3.0 R package (Butler et al., 2018) to remove: barcodes with very low (empty wells) and very high (probably doublets) total UMI counts; matrices for which a high percentage of UMIs originated from mitochondrial features (more than 20%); and matrices for which fewer than 250 genes were expressed. Clustering was performed as follows: The percentage of mitochondrial features was a source of unwanted variation and was regressed out using the Seurat package. Dimensionality reduction was performed by principal component analysis and UMAP embedding with resolution 0.5. Cluster gene markers and differentially expressed genes between groups in each cluster were detected using the Wilcoxon Rank Sum test. CellPhoneDB analysis was performed using Cellphonedb method statistical analysis command.

Quantification and Statistical Analysis

All the statistical tests were justified for every figure. All samples represent biological replicates. Data are presented as the mean $\pm$ SEM. Statistical analysis was performed using GraphPad Prism 8 or R software environment (<http://www.r-project.org/>). Significant differences between groups were determined using a Student's t-test (two-tailed) when the data met the normal distribution tested by D'Agostino test. If the data did not meet this test, a Mann-Whitney *U*-test was used. Differences between more than 3 groups were determined using one-way ANOVA test (parametric) or Brown-Forsythe and Welch ANOVA tests (nonparametric) followed by Dunnett post hoc test. If the data did not meet this test, a Mann-Whitney test was used. Differences in Kaplan Meier plots were analyzed by Gehan-Breslow-Wilcoxon test. The chi-square test or Fisher's exact test was used to determine the significance of differences between covariates. Logistic regression analysis was employed to estimate univariate and multivariate odds ratio and 95% confidence interval (CI). Values of $p < 0.05$ were considered as significantly different.

Table 1. List of primers used.

Gene symbol	Forward	Reverse
<i>18s</i>	5'-GTAACCCGTTGAACCCCAT-3' (SEQ ID NO:20)	5'-CCATCCAATCGGTAGTAGCG-3' (SEQ ID NO:21)
<i>SQSTM1</i>	5'-AGCGTCTGCGAGGGAAAG - 3'(SEQ ID NO:22)	5'-ACCCGAAGTGTCCGTGTTT-3'(SEQ ID NO:23)

<i>PARP1</i>	5'-TCTGCCTTGCTACCAATTCC-3' (SEQ ID NO:24)	5'-GATGGGTTCTCTGAGCTTCG -3' (SEQ ID NO:25)
<i>PARP2</i>	5'-CAACACGGCTCCAGAAGACT-3' (SEQ ID NO:26)	5'-GCCTTCACAGATTCATCTTGCT -3' (SEQ ID NO:27)
<i>CD38</i>	5'-TGCTGATGACCTCACATGGT-3' (SEQ ID NO:28)	5'-CCATTGAGCATCACATGGAC -3' (SEQ ID NO:29)
<i>MCT1</i>	5'-TGTTCTCTGTACTCTGGCC-3' (SEQ ID NO:30)	5'-GCAGTTTAGTAGCAAGCCCC-3' (SEQ ID NO:31)
<i>MCT4</i>	5'-CTCGCTCATCATGCTGAACC-3' (SEQ ID NO:32)	5'-ACACAGGAAGACAGGGCTAC-3' (SEQ ID NO:33)
<i>ACTA2</i>	5'-CGATAGAACACGGCATCATC-3' (SEQ ID NO:34)	5'-CATCAGGCAGTTCGTAGCTC-3' (SEQ ID NO:35)
<i>TGFB1</i>	5'-CGTGGAGCTGTACCAGAAATAC-3' (SEQ ID NO:36)	5'-CACAACCTCCGGTGACATCAA-3' (SEQ ID NO:37)
<i>HAS1</i>	5'-GACTCCTGGGTCAGCTTCCTAAG-3' (SEQ ID NO:38)	5'-AAACTGCTGCAAGAGGTTATTCCT-3' (SEQ ID NO:39)
<i>HAS3</i>	5'-AGCACCTTCTCGTGCATCATGC-3' (SEQ ID NO:40)	5'-TCCTCCAGGACTCGAAGCATCT-3' (SEQ ID NO:41)
<i>SFRP1</i>	5'-TCAGGGGCTTCTTCTTTG-3' (SEQ ID NO:42)	5'-TCTGAGGCCATCATTGAACA-3' (SEQ ID NO:43)
<i>MMP9</i>	5'-TCTTCCCTGGAGACCTGAGAAC-3' (SEQ ID NO:44)	5'-GACACCAAACCTGGATGACGATG-3' (SEQ ID NO:45)
<i>SIRT1</i>	5'-TGCTGGCCTAATAGAGTGGCA-3' (SEQ ID NO:46)	5'-CTCAGCGCCATGGAAAATGT-3' (SEQ ID NO:47)
<i>SIRT2</i>	5'-CCATCTGTCACTACTTCATGC-3' (SEQ ID NO:48)	5'-AAGTCCTCCTGTTCCAGC-3' (SEQ ID NO:49)
<i>SIRT3</i>	5'-GCTGGACAGAAGAGATGC-3' (SEQ ID NO:50)	5'-GTGGATGTCTCCTATGTTACC-3' (SEQ ID NO:51)
<i>SIRT4</i>	5'-GCGTGTAAGAAGCCGACT-3' (SEQ ID NO:52)	5'-TTCTTCTCCAGGCAGTCAG-3' (SEQ ID NO:53)
<i>SIRT5</i>	5'-CCCAGAACATCGATGAGC-3' (SEQ ID NO:54)	5'-GCCACAACCTCCACAAGAGG-3' (SEQ ID NO:55)
<i>SIRT6</i>	5'-AGGGACAACTGGCAGAGC-3' (SEQ ID NO:56)	5'-TTAGCCACGGTGACAGAGC-3' (SEQ ID NO:57)

<i>SIRT7</i>	5'-GCAGAGCAGACACCATCC-3' (SEQ ID NO:58)	5'-GTTACGATGTAAAGCTTCG-3' (SEQ ID NO:59)
Chip- <i>AP1-A</i>	5'-ATCCCCCTATTACGACAGCG-3' (SEQ ID NO:60)	5'-GGCCTCCCGGAGGTAAACA-3' (SEQ ID NO:61)
Mutagenesis <i>AP1-A</i>	5'CGGTCATGGGACGCTCTGTCACTG CCGGCCAGACCACCTGACC-3' (SEQ ID NO:62)	5'GGTCAGGTGGTCTGGCCGGCAGTG ACAGAGCGTCCCATGACCG-3' (SEQ ID NO:63)
Mutagenesis <i>AP1-B</i>	5'CAGGCGCCTGGGCTGCTCTGTAC GCTTGGCCAGCACCTGTC-3' (SEQ ID NO:64)	5'GACAGGTGCTGGCCAAGCGTGACA GAGCAGCCAGGCGCCTG-3' (SEQ ID NO:65)
Mutagenesis <i>AP1-C</i>	5'CCCAACTGAGGATATTGCTCTGTC ATGGCCAGGCCCAAGCC-3' (SEQ ID NO:66)	5'GGCTTGGGCTGGCCATGACAGAG CAATATCCTCAGTTGGG-3' (SEQ ID NO:67)

Table 2. List of siRNA oligonucleotides and guides.

Oligonucleotides	Source	Identifier
Human <i>PARP1</i> siRNA	Thermo Fisher Cat#	10038
Human <i>MCT1</i> siRNA	Thermo Fisher Cat#	104272
Human <i>MCT4</i> siRNA	Thermo Fisher Cat#	107503
Human <i>FOS</i> siRNA	Thermo Fisher Cat#	115631
Human <i>CD38</i> siRNA	Thermo Fisher Cat#	119605
Human <i>PARP2</i> siRNA	Thermo Fisher Cat#	111561
Human <i>SIRT1</i> siRNA	Thermo Fisher Cat#	136457

Human <i>SIRT2</i> siRNA	Thermo Fisher Cat#	136455
Human <i>SIRT3</i> siRNA	Thermo Fisher Cat#	136460
Human <i>SIRT4</i> siRNA	Thermo Fisher Cat#	136462
Human <i>SIRT5</i> siRNA	Thermo Fisher Cat#	19661
Human <i>SIRT6</i> siRNA	Thermo Fisher Cat#	116148
Human <i>SIRT7</i> siRNA	Thermo Fisher Cat#	116146
gRNA targeting human <i>PARP-1</i> 5'- GGGACTTTTCCATCAAACAT-3' (SEQ ID NO:68)	Synthego	N/A
gRNA targeting <i>AP1-A</i> in human <i>SQSTM1</i> promoter 5'- ATGGGACGCTGACTCACTGC-3' (SEQ ID NO:69)	Synthego	N/A
ssODN for human <i>SQSTM1</i> promoter editing 5'TCCCCAGCCCAGCCTCCAGGTAAGAGTCACTGAGATGGG TGGCAGCAGGGGCCGGGATCCCCCTATTACGACAGCGGT CATGGGACGCCTGCCGGCCAGACCACCTGACCTCCGCGGC GGGAGGAGAGGGCC-3' (SEQ ID NO:70)	IDT	N/A

Results

Prostate cancer cells secrete a soluble factor that reduces p62 expression in stromal fibroblasts

- 5 To investigate how p62 expression is downregulated in the tumor stroma, an *in vitro* cell system was generated in which mouse GFP-labeled prostate stromal cells (mPSC) mixed with TRAMPC2 PCa epithelial cells were incubated. Analysis of GFP-positive stromal cells isolated by FACS sorting showed reduced *Sqstm1* (coding for p62) mRNA levels in stromal cells upon incubation with tumor cells,
- 10 which also correlated with an increase in bona-fide CAF markers, such as *Tgfb1* and *Sdf-1* (**FIG. 1A**). To test whether the downregulation of p62 required cell contact

between tumor and stromal cells, human prostate stromal fibroblasts (WPMY-1) were co-cultured with different PCa cell lines (PC3, TRAMPC2, and DU145) in a double-chamber setting. p62 was also downregulated at the protein and mRNA levels in stromal cells under these conditions (**FIGs. 1B** and **1C**), indicating that a soluble factor secreted by PCa epithelial cells was sufficient to downregulate p62 in stromal cells. In fact, incubation of stromal cells with conditioned media (CM) from PCa cells (PC3, TRAMPC2, DU145 or LNCAP) effectively reduced p62 protein and mRNA levels in stromal cells, without any effect by the CM from normal prostate epithelial cells (PrEC or RWEP1) (**FIGs. 1D** and **1E**). To determine if the reduction in the mRNA amount of *SQSTM1* was due to inhibited transcriptional activity, cells transfected with a luciferase reporter under the control of the *SQSTM1* promoter (Duran et al., 2008) were used. PCa-conditioned media reduced the activity of the luciferase reporter (**FIG. 1F**), strongly suggesting that the downregulation of stromal p62 by PCa epithelial cells is mediated through the repression of the *SQSTM1* promoter in the stroma by a soluble factor secreted by epithelial tumor cells.

To identify this putative tumor-derived soluble factor, conditioned medium from PC3 cells was size-fractionated to separate molecules that were either less or more than 3 kDa in size, and the ability of either fraction to decrease the amount of stromal p62 was tested. Both the unfractionated tumor-conditioned medium and the <3 kDa fraction comparably downregulated p62 at the protein and mRNA levels in stromal fibroblasts (**FIGs. 1G** and **1H**), as well as the activity of the *SQSTM1* promoter-driven luciferase reporter (**FIG. 1I**). However, the >3 kDa fraction was totally inactive in these assays (**FIGs. 1G-1I**). The active soluble factor was heat-stable (**FIGs. 1J-L**), which suggested that it could be a metabolite.

Lactate downregulates p62 in stromal fibroblasts

Since cancer cells secrete large amounts of lactate that accumulates in the tumor microenvironment as a consequence of the Warburg effect (Vander Heiden et al., 2009), it was speculated that lactate could be a good candidate to be the tumor-derived factor responsible for the downregulation of p62 in stroma cells. Consistent with this hypothesis, it was found that the amount of lactate in the media of different PCa cell lines was higher than in the media of normal prostate epithelial cells (**FIG. 2A**). The cellular uptake of lactate is mediated by different monocarboxylate transporters (MCT1, MCT2, MCT3, and MCT4) (Halestrap, 2013). A prediction

would be that blocking these transporters could alleviate the lactate-induced downregulation of p62. Notably, knockdown of *MCT1*, which controls lactate cellular import in WPMY-1 cells resulted in the inhibition of p62 downregulation induced by CM from PC3 cells (**FIGs. 2B** and **2C**). Likewise, p62 downregulation
 5 in WPMY-1 cells by the same CM was abrogated when *MCT4*, which controls lactate export, was knocked-down in PC3 cells (**FIGs. 2D** and **2E**). Furthermore, treatment with the MCT1 inhibitor, AZD3965, impaired p62 downregulation in WPMY-1 cells treated with PC3 CM (**FIGs. 2F** and **2G**).

Together these results demonstrate that secretion of lactate by PCa cells and
 10 its uptake by stromal cells is a step in the stromal downregulation of p62. To demonstrate that lactate is sufficient to downregulate p62 in stromal cells, WPMY-1 cells were incubated with different concentrations of lactate for 24 hours or 48 hours, which resulted in the downregulation of p62 in a time (**FIGs. 2H** and **2I**) and dose-dependent (**FIGs. 2J** and **2K**) manner. The amount of lactate necessary to
 15 downregulate p62 in stromal cells (**FIGs. 2J** and **2K**) was similar to the secreted by PCa cells (**FIG. 2A**), and consistent with reported data from tumors *in vivo* (Walenta et al., 2000). MCTs are proton-coupled symporters, which explains why lactate-mediated downregulation of p62 was dependent on an acidic pH (**FIG. 2L**). However, the simple acidification of the culture media was not sufficient to
 20 downregulate p62 in stromal cells (**FIG. 2L**). These results demonstrated that lactate secreted by epithelial PCa cells impaired p62 expression in stromal cells at the transcriptional level. To rule out the potential contribution of a hypothetical role of p62 protein stability in the response to lactate, cycloheximide-treated cells were incubated with lactate and p62 protein levels analyzed. Lactate does not affect p62
 25 stability (**FIG. 10A**). In agreement with these results, inhibition of the proteasome (**FIG. 10B**), autophagy (**FIGs. 10C** and **10D**), or cysteine proteases (**FIGs. 10E** and **10F**) did not rescue the reduced p62 protein levels in lactate-treated WPMY-1 cells.

AP1 controls p62 downregulation by lactate

To unravel the mechanisms whereby lactate downregulates stromal p62, the
 30 chromatin accessibility landscape of WPMY-1 cells treated or not with lactate by genome-wide ATAC-seq (assay for transposase-accessible chromatin with high-throughput sequencing) was investigated. This analysis revealed a widespread decrease in chromatin accessibility in lactate-treated WPMY-1 cells (**FIGs. 3A**). HOMER (Hypergeometric Optimization of Motif EnRichment) analysis showed a

significant enrichment of AP-1 transcription factors in the closed chromatin regions of lactate-treated cells (**FIG. 3B**). Analysis of the *SQSTM1* promoter demonstrated the presence of three AP-1 regulatory sites that also showed reduced chromatin accessibility under lactate-treated conditions (**FIG. 3C**). Consistently lactate
 5 repressed an AP-1-driven luciferase reporter (**FIG. 3D**), which collectively indicated that the repression of AP-1 sites in the *SQSTM1* promoter could account for lactate effects in stromal p62 expression.

To establish the contribution of the different AP-1 sites in the *SQSTM1* promoter, AP-1A, AP-1B, and AP-1C) were individually mutated and the impact of
 10 these mutations in the *SQSTM1* promoter activity was determined by luciferase assay. Mutation of AP-1A completely inactivates the *SQSTM1* promoter to levels comparable to those produced by lactate in the intact *SQSTM1* promoter, whereas mutation of AP-1B, or AP-1C had no effect (**FIG. 3E**). These results demonstrate that AP-1A is the important enhancer element in the *SQSTM1* promoter and the most
 15 likely target of lactate-induced repressive effects. To test this hypothesis, in a more physiologically relevant system, the AP-1A element in the endogenous *SQSTM1* promoter was selectively deleted using CRISPR/Cas9 editing to generate four independent WPMY-1 cell cultures (sgAP-1A), which resulted in the inhibition of mRNA and protein p62 expression (**FIGS. 3F-H**). These results establish the
 20 relevance of AP-1A for the regulation of p62 expression in stromal cells. In keeping with this notion, chromatin immunoprecipitation (ChIP) analysis demonstrated that the recruitment of c-FOS and c-JUN, but not of FOSB or JUNB, to the AP-1A regulatory site was significantly decreased in stromal fibroblasts stimulated with lactate (**FIG. 3I**). Consistently, the downregulation of c-FOS (**FIGS. 3J and 3K**) or
 25 the inhibition of c-JUN phosphorylation with a pharmacological inhibitor of JNK (**FIGS. 3L and 3M**), mimicked the effect of lactate on p62 expression both at the protein and mRNA levels. Overall, these data indicate that the impaired recruitment of a c-JUN/c-FOS AP-1 transcriptional complex plays a role in p62 downregulation by lactate in the stroma.

30 Reduction in NAD⁺ levels by lactate metabolism mediates p62 downregulation

Lactate can be reversibly converted into pyruvate with the subsequent depletion of NAD⁺ levels in favor of the generation of NADH (Covarrubias et al., 2021). Importantly, increasing the levels of pyruvate in the culture media, which drives this reaction in the opposite direction, clearly impairs the ability of lactate to

downregulate p62 (**FIGs. 4A and 4B**). Therefore, changes in the NAD^+/NADH ratio could be instrumental for the mechanisms of action of lactate on the regulation of p62 levels. Consistent with this hypothesis, NAD^+ levels in stromal fibroblasts treated with lactate are lower than in untreated cells (**FIG. 4C**). Treatment with

5 NAD^+ or with the NAD^+ precursor, nicotinamide riboside (NR), completely rescued the lactate-induced reduction in p62 (**FIGs. 4D-4G**).

PARP-1 inhibition by lactate downregulates p62 in stromal cells

NAD^+ is a cofactor for three types of transcriptional and posttranslational modulators (Covarrubias et al., 2021). These include the sirtuins (SIRT1 through

10 SIRT7), the cyclic ADP ribose synthases (CD38 and CD157), and the poly-ADP ribose transferases (PARP1/2). Interestingly, only the knockdown of *PARP-1* fully mimicked the effect of lactate on p62 expression (**FIGs. 5A-5E, FIGs. 11A and 11B**). Moreover, treating WPMY-1 cells with the PARP-1 inhibitors PJ34 (**FIGs. 5F and 5G**) or Olaparib (**FIGs. 5H and 5I**) also reduced the expression of p62 in a

15 dose-dependent manner. Of note, Olaparib also induced autophagy activation as determined by LC3 lipidation (**FIGs. 11D and 11E**). Since p62 is a substrate of autophagy, Olaparib impaired p62 expression at the mRNA and protein levels. Interestingly, co-treatment with the autophagy inhibitors (ULKi, SBI-0202965) reverted the Olaparib-induced activation of stroma in a dose dependent manner

20 (Figure S2F), which suggest the use of autophagy inhibitors to overcome the stromal activation in response to PARP1 inhibitors.

PARP1, in addition to its well-known role in DNA damage repair, also controls many other biological processes including transcription (Feng et al., 2015). Thus, PARP-1 reportedly enhances the DNA binding and transactivation of many

25 transcription factors (Gibson and Kraus, 2012). Of special relevance for this study, both c-FOS and c-JUN have been previously shown to be poly(ADP-ribosyl)ated by PARP-1, which increased their DNA binding activity (Huang et al., 2009). Consistently, Olaparib impaired the recruitment of c-FOS and c-JUN to the AP-1A binding site in the *SQSTM1* promoter (**FIGs. 5J and 5K**). Taken together, these

30 results suggest that the regulation of PARP-1 activity by changes in the levels of NAD^+ triggered by lactate metabolism in stromal cells is instrumental for p62 downregulation. Therefore, it was posited that lactate could affect the activation of PARP-1 and, consequently, the poly(ADP-ribosyl)ation of c-JUN and c-FOS. Consistently, lactate-treated cells have reduced total poly(ADP-ribosyl)ation levels

(**FIG. 5L**), and immunoprecipitation experiments demonstrated that lactate impaired the poly(ADP-ribosyl)ation of c-JUN and c-FOS (**FIGs. 5M and 5N**). Adding NAD⁺ to lactate-treated cells rescued the poly(ADP-ribosyl)ation of both transcription factors (**FIGs. 5O and 5P**) and, importantly, their recruitment to the AP-1 regulatory site in the *SQSTM1* promoter (**FIGs. 5Q and 5R**). Similar results were obtained by treating these cells with NR (**FIGs. 5S and 5T**). Taken together, these data demonstrate that depletion of NAD⁺ by lactate metabolism and the subsequent impairment of the poly(ADP-ribosyl)ation of c-FOS and c-JUN, underlies the mechanism whereby lactate triggers the downregulation of p62 in stromal fibroblasts.

AP-1 is important for CAF activation driven by p62 loss

The loss of p62 drives a CAF phenotype in stromal cells, which promoted tumor progression (Linares et al., 2017; Valencia et al., 2014). Because lactate secretion by PCa cells is necessary and sufficient to downregulate p62 in stromal cells, we next determined whether treating WPMY-1 cells with lactate drives them to a CAF phenotype. Treatment of WPMY-1 cells with lactate increased the expression of bona fide markers of CAF activation such as *ACTA2* and *TGF-β* (**FIG. 6A**), whose expression was reverted by NAD⁺ addition (**FIG. 6B**). Gene set enrichment analysis (GSEA) of genes differentially expressed in lactate-treated WPMY-1 cells showed enrichment in CAF signatures from a human stroma PCa data set (GSE34312) (**FIG. 6C**), validating the human relevance of these observations. Importantly, sgAP-1A fibroblasts in which the *SQSTM1* promoter was endogenously inactivated (**FIG. 3F**) also showed increased expression of CAF markers (**FIG. 6D**). Consistently, experiments in a two-chamber co-culture system demonstrated that sgAP-1A but not sgC fibroblasts promoted the migration and invasion of PC3 cells (**FIGs. 6E-6G**). Similar results were obtained using a previously described 3D organotypic model that recapitulates key aspects of epithelial-stromal crosstalk in the tumor microenvironment (Valencia et al., 2014) (**FIGs. 6H and 6I**). In keeping with the *in vitro* data, subcutaneous xenograft co-implantation of PC3 cells with sgAP-1A in immunodeficient mice (NSG), resulted in increased tumor growth when compared with those co-implanted with sgC fibroblasts (**FIGs. 6J-6L**). Notably, tumors generated with sgAP-1A fibroblasts showed increased markers of stromal activation, such as the augmented deposition of collagen and hyaluronan (HA), and the enhanced expression of αSMA (**FIG.**

6M). Collectively, these results establish that the AP-1-regulatory site in the *SQSTM1* promoter, which is the target of lactate-driven signals and has a pivotal role in the control of endogenous p62 levels in stromal cells, is important for the induction of the CAF phenotype.

5 PARP-1 inhibitors promote a desmoplastic response in stromal fibroblasts through p62

According to the model, lactate triggers the downregulation of p62 in stromal fibroblasts through PARP-1 inhibition. Therefore, it was hypothesized that PARP-1 inhibitors such as Olaparib would be sufficient to induce stromal activation. In
 10 agreement with this hypothesis, GSEA of RNAseq data from WPMY-1 fibroblasts, treated or not with Olaparib under basal or androgen deprivation (ADT) conditions, demonstrated enrichment of signatures corresponding to extracellular matrix remodeling, indicative of CAF activation (**FIG. 7A**), in Olaparib-treated cells, which correlated with well-established gene signatures for stromal activation in several
 15 types of cancers, including PCa (**FIGs. 7B-7C**). Consistently, Olaparib treatment reduced *SQSTM1* mRNA levels and upregulated CAF markers such as *ACTA2*, *SFRP1*, *TGF β* , *MMP9* and the HA synthases in basal and ADT conditions (**FIGs. 7D and 12A**). Similar results were obtained in different human and mouse prostate stromal cell lines and in hepatic stellate cells (**FIGs. 12B-D**), suggesting that the
 20 stromal activation by Olaparib treatment is a general phenotype not linked exclusively to the prostate stroma. Furthermore, ATAC-seq analysis of Olaparib-treated WPMY-1 cells revealed a strong decrease in chromatin accessibility across the genome, like what was observed with lactate treatment (**FIGs. 3A and 7E**). Also, consistent with previous findings in lactate-treated fibroblasts, motif enrichment
 25 analysis using HOMER showed a significant loss of chromatin accessibility in regions with consensus motifs for AP-1 transcription factors (**FIGs. 3B and 7F**). Close inspection of ATAC-seq data demonstrated reduce accessibility of the *SQSTM1* promoter (**FIG. 7G**).

Interestingly, co-culture of PC3 cells with Olaparib-pretreated WPMY-1
 30 cells resulted in increased migration and invasion of PCa cells under basal conditions that was even more pronounced in ADT conditions (**FIGs. 7H-7J**). Moreover, Olaparib-treated WPMY-1 CM led to increased PC3 proliferation (**FIGs. 7K and 7L**). Based on these observations, it was posited that stromal activation in response to Olaparib treatment could hamper its anti-tumor activity. To test this

hypothesis, PC3 cells that were labelled with GFP (PC3^{GFP}) were cultured either with (co-culture) or without (monoculture) WPMY-1 cells in basal or ADT conditions and treated or not with Olaparib for 10 days (**FIG. 7M**). Interestingly, co-culture with WPMY-1 cells rendered PC3^{GFP} cells more resistant to Olaparib as compared to treatment under monoculture conditions (**FIG. 7N**). This effect was even more apparent under ADT conditions (**FIG. 7N**). To investigate the pro-CAF phenotype of Olaparib *in vivo*, PC3 and WPMY-1 cells were co-implanted in NSG mice and then treated either with vehicle or Olaparib (**FIG. 7O**). *In vivo* Olaparib treatment efficiently reduced tumor growth (**FIGs. 7P-7R**), consistent with previous reports (Li et al., 2017; Zhang et al., 2018), but also produced a strong desmoplastic response, characterized by enhanced collagen and HA deposition and increased expression of α SMA and reduced p62 levels (**FIGs. 7S and 7T**).

Single cell analysis of stromal activation and epithelial remodeling in response to Olaparib *in vivo*

To investigate how the treatment with Olaparib triggered the creation of a more activated, protumorigenic microenvironment in PCa, scRNAseq was performed using 10X Genomics Chromium platform on TRAMP+ prostate tumors after treatment with Olaparib for 4 weeks as compared to vehicle controls (**FIG. 8A**). Consistent with the results in the xenograft model (**FIGs. 7O-7T**), *in vivo* treatment of TRAMP⁺ mice induced a strong desmoplastic response in the prostate (**FIG. 8B**). Furthermore, unsupervised clustering identified epithelial cells as well as stromal and immune cells with selective markers for each population (**FIG. 8C**). Consistent with the *in vitro* data of stromal activation, there was an expansion of the stromal and immune populations in response to Olaparib treatment (**FIGs. 8C and 8D**). Further re-clustering of the fibroblast compartment identified six clusters, all enriched in CAF signatures (**FIG. 8E**). Clusters 0 and 2 correspond to the inflammatory CAF subtype (iCAF), whereas clusters 3 and 5 express myofibroblast markers (myCAF). Cluster 3 displayed high levels of two CD44 ligands, *Col1a1* and *Spp1*, while cluster 5 expresses *Acta2*. Interestingly, cluster 1 has features of both iCAF and myCAF and is enriched in growth factors and inflammatory cytokines as well as EMT markers and constitutes the subpopulation with the lowest levels of p62/*Sqstm1*. Cluster 4 is enriched in *Rspo3* and resembled the mesenchymal cell population termed Mes2, previously described in scRNAseq analysis of normal mouse and human prostates (Karthaus et al., 2020). Importantly, differential

expressed gene analysis identified *Has1*, *Spink1* and *Tgfb2* among the most upregulated transcripts in the Olaparib-treated fibroblast population (**FIG. 8F**). *Has1* is a hyaluronan (HA) synthase, one of the three enzymes that synthesize HA, which has been linked to tumor promotion, therapy resistance, and poor outcomes in various tumors, including PCa (Aaltomaa et al., 2002; Lipponen et al., 2001; Toole, 2004; Toole and Slomiany, 2008). Also, recent reports point to a role of HAS-expressing fibroblasts in the promotion of metastatic spreading (Affo et al., 2021). These data are in good agreement with the results in human prostate stromal cells and in *in vivo* mouse models, in which increased deposition of HA upon Olaparib treatment was found. Notably, *Has1* and *Has3* were upregulated in Olaparib-treated WPMY in both basal and ADT conditions (**FIG. 8G**). Of great relevance, SPINK1 (Serine Peptidase Inhibitor, Kazal type 1) expression in PCa has been associated with poor response to ADT, faster progression to the castrate-resistant stage and cancer-associated mortalities (Johnson et al., 2016; Leinonen et al., 2010; Tomlins et al., 2008). Moreover, recent reports showed increased tumor *Spink1* levels in response to AR-targeted therapies (Tiwari et al., 2020) and in prostate stroma after chemotherapy (Chen et al., 2018). The present results show that SPINK1 levels were highly upregulated in human prostate cells under ADT conditions (**FIG. 8G**) and, more importantly, in tumor stroma *in vivo* in Ola-treated mice (**FIG. 8H**). *Tgfb2* is one of the ligands of the TGF β pathway, which has been long recognized to exert opposing roles in cancer progression but that it is an important component of reactive stromas (Lu et al., 2017; Massague, 2008). While TGF β signaling blocks tumorigenesis in primary PCa (Bjerke et al., 2014), it can promote PCa bone metastasis (Fournier et al., 2015) and has been recently associated with resistance to enzalutamide in scRNAseq studies of metastatic CRPC patients (He et al., 2021). Furthermore, anti-TGF β therapies have been proposed to reduce the development and progression of bone metastasis and to enhance the efficacy of immune checkpoint therapy in metastatic CRPC (Jiao et al., 2019). Consistent with enhanced TGF β markers in our scRNAseq data, increased levels of *Tgfb1* were found in both basal and ADT conditions in WPMY cells (**FIG. 8G**). Altogether these results are of great significance for PCa therapy since they identified regulators in the stroma whose therapeutic targeting impairs Ola-induced stromal activation and increases Ola anti-tumor efficacy.

A question that is still to be determined is the precise impact that Olaparib-mediated stromal activation has in the tumor epithelium. To address this question, the scRNAseq data of the PCa tumor epithelial compartment (**FIGs. 8I-8K**) was interrogated. Of potential relevance, Olaparib treatment completely abolished the neuroendocrine cluster 2 (NE1; **FIGs. 8I-8K**), supporting the concept that neuroendocrine tumors display enhanced sensitivity to the treatment with Olaparib (Liu et al., 2019; Zhang et al., 2018). However, Olaparib also expanded several other epithelial clusters: Cluster 0 is a luminal population; cluster 1 is a basal population; clusters 3 and 4 that are luminal but express high levels of EMT markers such as Vimentin (Vim) and Acta 2; and cluster 5 is a new neuroendocrine population that, in contrast to cluster 2, highly expresses the neuronal marker Synaptophysin (Syn), in addition to luminal and EMT markers (**FIGs. 8I and 8J**). Importantly, the Olaparib-treated tumor epithelium showed increased AR, EMT and TGF β signaling, which suggests potential mechanisms of resistance to Olaparib therapy (**FIG. 8L**).

Consistent with enrichment in TGF β signaling signature in Olaparib-treated epithelial cells, there was an increased pSMAD2 staining, an established marker of TGF β activation, in Olaparib-treated prostates (**FIG. 8M**). Moreover, identification of ligand-receptor interactions between the stromal and epithelial populations by CellPhoneDB analysis revealed changes in receptors for HAS1/2 in Olaparib-treated populations (**FIG. 8N**); these included gaining novel CD44-mediated pairs between the stromal populations and the different clusters of tumor cells. Of note, CD44 is a receptor of HA that integrates extracellular matrix cues with growth factor and cytokine signals (Misra et al., 2015; Orian-Rousseau and Sleeman, 2014; Ponta et al., 2003). The data showed that Olaparib increased the HAS1/2-CD44 pairs, but also promoted the interaction of CD44 with other growth-factor receptors, such as FGFR2 or HBEGF (**FIG. 8N**). There was also gain of multiple interactions of the TGF β receptor family and their ligands (**FIG. 8N**) that could account for the increased TGF β signaling and EMT phenotype observed in tumor cells in Olaparib conditions. In this regard, binding of HA induces clustering of CD44 and the complex formation with other receptors (including the receptors for TGF β), which serve to further reinforce the activation of these pathways.

Co-targeting the stroma increases Olaparib anti-tumor activity

Based on the data that Olaparib induces stromal activation, it was hypothesized that strategies targeting the stroma could improve the efficacy of Olaparib as an anti-tumor therapy. Since treatment with Olaparib induces an HA-rich stroma, and HA can be degraded by PEGPH20, a clinical-grade recombinant hyaluronidase (provided by Halozyme Therapeutics), it was tested whether treatment with PEGPH20 could revert Olaparib-induced stroma activation and enhance its anti-tumor activity. As shown in **FIG. 9A**, PEGPH20 efficiently degraded HA deposition in response to Olaparib treatment in WPMY-1 cells, in basal and ADT conditions (**FIG. 9A**). More importantly, PEGPH20 completely blocked the enhanced migration of PC3 cells in response to conditioned media from Olaparib-treated WPMY-1 cells under basal and ADT conditions (**FIGs. 9B and 9C**). Moreover, PEGPH20 also blocked Olaparib-induced proliferation in stromal cells (**FIGs. 9D and 9E**). Likewise, treatment with Galunisertib, a TGF β R inhibitor, completely abolished the stromal proliferation induced by Olaparib (**FIGs. 9D and 9E**). These results indicate that strategies aimed at targeting the stroma could normalize the stromal activation promoted by Olaparib. Consistent with this notion, the *in vivo* data showed that co-treatment with PEGPH20 enhanced Olaparib anti-tumor activity (**FIGs. 9F and 9G**). Collectively, these results demonstrate that combination therapies co-targeting the activated stroma could increase the efficacy of PARP inhibitor therapies such as Olaparib.

Discussion

Despite recent advances in the characterization of the heterogeneity of fibroblast populations in the tumor stroma and the mounting evidence on their role in tumor progression, the molecular mechanisms that control stromal activation by the tumor epithelium remain largely unknown. Here, tumor-secreted lactate was identified as the main signal from the epithelium that activates tumor fibroblasts by downregulating p62. Previous work has shown that p62 is a stromal tumor suppressor whose downregulation in fibroblasts is a key event for CAF activation (Duran et al., 2016; Goruppi and Dotto, 2013; Linares et al., 2017; Valencia et al., 2014). However, the mechanisms whereby p62 is downregulated by the tumor in the stroma were not understood. p62 regulation is subjected to a fine-tune balance between transcription and post-translational mechanisms that control its degradation by autophagy or the proteosome machinery (Moscat and Diaz-Meco, 2009; Moscat et al., 2016). Herein, it was shown that lactate modulates p62 at the transcriptional

level. Thus, although lactate reportedly induces autophagy (Brisson et al., 2016), which can contribute to lactate effects in tumor cells and the stroma, we show here that blocking autophagy or the proteasome failed to prevent p62-downregulation by lactate. At the transcriptional level, p62 has been shown to be regulated by several transcription factors including AP1, NRF2, NF-kB and TFEB under different conditions (Duran et al., 2008; Jain et al., 2010; Ling et al., 2012; Park et al., 2019). The AP-1 binding site in the p62 promoter was identified as the element accounting for lactate-mediated p62 downregulation. Interestingly, these findings are reminiscent of previously published data showing that AP-1 was also the major transcription factor for Ras-induced p62 mRNA expression in the tumor epithelium (Duran et al., 2008), which suggests that AP-1 is a hot spot for the upregulation of p62 by oncogenic transformation in the epithelium and its downregulation in the stroma. Regarding the composition of the AP-1 transcription factors mediating this effect in the stroma, the data demonstrate that c-FOS and c-JUN complexes are important since their recruitment to the p62 promoter was impaired in lactate-treated cells, whereas no changes were observed in the binding of FOSB and JUNB. This is interesting because JUNB has recently been shown to be key in the repression of p62 in keratinocytes (Sukserree et al., 2021), which suggests that different AP-1 proteins play distinct roles in p62 regulation under different cellular contexts.

Lactate has been considered for years as just the end product of glycolysis and as such, a metabolic waste (Ippolito et al., 2019). However more recent evidence has recognized many new roles for lactate including its relevance as a carbon source for cellular metabolism, and potential new functions as a signaling molecule in the TME (Brooks, 2018; Ippolito et al., 2019). Lactate is produced from pyruvate and is exported to the TME, where it can reach high concentrations of up to 40 mM, especially in tumor tissues (Walenta et al., 2000). Most tumor cells can engage in secretion and utilization of lactate depending on the context and extracellular microenvironment (Brooks, 2018). In addition to the shuttling that can take place among different tumor cells, lactate accumulation in the TME has significant effects on the non-malignant compartment, including, as shown here, on the activation of the fibroblasts surrounding the tumor, which results in their acquisition of the CAF phenotype (Bhagat et al., 2019). Therefore, targeting lactate metabolism as a potential therapeutic approach needs to integrate its effects not only on the tumor cell but also in the TME.

Lactate dehydrogenase catalyzes the conversion of lactate into pyruvate. This results in the reduction of NAD⁺ cellular levels, which is an important metabolic and signaling cofactor that impacts multiple functions, often dysregulated in cancer (Chini et al., 2021; Fang et al., 2017). Herein it was shown that low NAD⁺ levels, as
5 consequence of lactate conversion to pyruvate, is central to the ability of lactate to downregulate p62 in fibroblasts and their subsequent conversion into CAF. Our data demonstrating that supplementation with NAD⁺ precursors, such as NR, rescued p62 levels and reverted the CAF phenotype, established that altering the cell NAD⁺/NADH ratio could be a mechanism to modulate stromal activation. In
10 keeping with this notion, downregulation of nicotinamide phosphoribosyl transferase (NAMPT), a rate-limiting enzyme in NAD⁺ synthesis, has been shown to promote renal and lung fibrosis with increased collagen deposition and ECM remodeling (Muraoka et al., 2019). Furthermore, recent evidence demonstrated that stromal nicotinamide N-methyltransferase (NNMT), an enzyme that impairs NAD⁺ biosynthesis by depleting its precursor N-nicotinamide, induces CAF differentiation and cancer progression (Eckert et al., 2019). All these evidences are of potential
15 relevance because they strongly suggest that the stromal activation promoted by NAD⁺ inhibition could limit the efficacy of anti-cancer therapies targeting NAD⁺ biosynthesis, such as NAMPT inhibitors, and could explain their reported limited efficacy *in vivo* (Galli et al., 2020). Supplementation with NR has been studied extensively and its dietary administration is considered safe and effective to increase NAD⁺ levels (Martens et al., 2018). NR supplements are proposed to ameliorate inflammation and metabolic dysfunction in aging, and there is a renewed interest in the potential of NAD-boosting therapies to treat human diseases (Chini et al., 2021;
20 Fang et al., 2017). Based on the present data, an additional benefit of increasing NAD⁺ levels might be to reprogram the stroma back to a less activated phenotype by restoring p62 levels. However, the role of NAD⁺ in cancer is complex and manipulating its levels could be a double-edged sword (Chini et al., 2021; Fang et al., 2017). Thus, increasing the NAD⁺/NADH ratio by administering nicotinamide mononucleotide (NMN), another NAD⁺ precursor, has been shown to have a
25 protumorigenic effect in K-Ras-driven pancreatic cancer (Nacarelli et al., 2019), unveiling the complexity of these therapeutic approaches.

The work herein identified PARP-1 inhibition as the key target of the reduced NAD⁺ levels in the transcriptional downregulation of p62 by lactate.

PARP1 is the major isoform of the PARP enzyme family and, like the other PARPs, its PARylation activity impacts multiple biological processes with a main dual function in DNA damage repair (DDR) and transcriptional regulation (Feng et al., 2015; Gibson and Kraus, 2012). Most of the therapeutic strategies using PARP inhibitors rely on their blockade of DDR and the creation of synthetic lethality in combination with genetic defects in homologous recombination-mediated repair (Farmer et al., 2005; Lord and Ashworth, 2017; McCabe et al., 2006), which are the basis for the treatment of patients with BRCA1- and BRCA2-associated cancers (Mateo et al., 2019). The use of PARP inhibitors has been most successfully exploited in BRCA1/2-deficient breast and ovarian cancers (Sonnenblick et al., 2015), and recently Olaparib has been approved for the treatment of mCRPC in patients with DDR gene mutations (de Bono et al., 2020; Hussain et al., 2020). However, the fact that the percentage of mCRPC patients with these mutations that could benefit from that type of therapy is a minority has led to new efforts for combinatorial strategies by chemically inducing DDR (Li et al., 2017; Zhang et al., 2018). Most of these efforts have been focused on the direct effect of PARP inhibitors on the tumor cell, and much less is known on how the tumor stroma responds to this type of treatment. The present data, showing that lactate inhibits PARP activity, and that PARP-1 knockdown downregulates p62 through the impaired PARylation of AP-1 transcription factors, resulting in CAF activation, might have important therapeutic implications. The results also highlight the increasingly recognized role of PARP1 in transcriptional regulation as a key mechanism of action beyond its role in DDR (Feng et al., 2015; Liu et al., 2019), and identify an unanticipated function for these inhibitors in the activation of the pro-tumorigenic potential of the tumor stroma. These data are consistent with the previously reported protumorigenic phenotype of PARP1 KO mice in the context of the TRAMP⁺ model, indicating the dominant protumorigenic role of the TME in PCa progression under conditions of PARP1 deficiency (Pu et al., 2014). This is an important finding because CAF activation in the TME by PARP inhibitors might limit or even blunt their therapeutic efficacy. Therefore, a better understanding of the fundamental mechanisms controlling the activation of the stromal has the potential to identify vulnerabilities that can improve cancer therapies.

Example 2

Methods

In vivo experiments

For Olaparib treatment in mice, 13 weeks-old male TRAMP⁺/FVB were surgical castrated, after 10 days n=16, were randomly distributed to receive vehicle, n=8, or Olaparib n=8 (5 mg/kg/, 5 days each, i.p.). Mice were euthanized 3 weeks
 5 after the initiation of the treatment. Prostate and other organs were collected and analyzed histologically.

For the metastasis model, caudal artery injection was performed with 500000 RM1-BoMe3 -RFP-Luc prostate cancer epithelial cells in 150 μ l of PBS n=10 in 8-week-old C57Bl6 male mice. After 1-week luciferase was visualized using IVIS to
 10 detect signal in the lower limbs and in less frequently in visceral organs, mice with signal were randomly distributed to receive vehicle, n=5, or Olaparib n=5 (40 mg/kg/, 5 days each, i.p.). After 3 weeks, mice were euthanized and ex-vivo IVIS was performed in lower limbs, vertebrae, and visceral organs to detect metastasis sites. Tissues were collected and analyzed histologically.

Bone in culture

8-week-old C57Bl6 male mice were euthanized, and lower limb bone were collected. Femur epiphyses were dissected and fragmented in 4 pieces by sterilized micro dissecting scissors. Bone pieces were aligned into low-attachment 96-well
 20 plates pre-loaded with 200 μ L DMEM/F12 supplemented with 5% charcoal striped FBS for ADT conditions. Bone pieces were treated with DMSO or Olaparib 20 μ M for 48 h and then 5000 RM1-BoMe3 -RFP-Luc cells were seed on top of the bone pieces. After 48 hours, pictures were taken using EVOS M5000 Imaging System and RFP⁺ positive areas were measured using Fiji.

Stromal-induced activation by PARP inhibitors in other cancers beyond prostate cancer

Olaparib (Ola) downregulated p62 expression, a master regulator of CAFs activation, in stromal cell lines from different tissue origins, including prostate, lung, breast or endometrium (**FIGs. 13A-13D**). These results demonstrate that the stromal activation induced by PARP inhibitors is a general mechanism taking place not only
 30 in the prostate stroma but also in stromal cells from other types of cancer. Consistent with these results, gene set enrichment analysis (GSEA) of RNAseq data of BRCA deficient breast tumors treated with the PARP inhibitor Talazoparib (gene dataset GEO:GSE125206) showed significant enrichment of signatures of stromal activation (**FIG. 13E**). Similar enrichment in signatures of stromal activation was observed in

GSEA of RNAseq data from a PDX model of high grade serous ovarian cancer treated with niraparib (**FIG. 13F**). These results support the notion that co-targeting the stroma enhances the efficacy of PARP inhibitors in other types of cancer beyond prostate cancer, such as breast and ovarian cancer, where this therapy is approved especially for BRCA deficient tumors.

Blocking inflammatory signals reverts stromal activation by PARP inhibitors

To further characterize the molecular mechanisms involved in the stromal activation by PARP inhibitors, RNAseq data of human prostate fibroblasts treated with Ola was analyzed by GSEA. Interestingly, a significant enrichment was found in inflammatory signaling pathways mediated by inflammatory cytokines such as TNF α and IL-6 and the activation of the JAK-STAT cascade (**FIG. 11A**).

Importantly, similar results were found in the analysis of other datasets of two different in vivo models of breast and ovarian tumors, treated with two different PARP inhibitors, Talazoparib and Niraparib (**FIG. 11B**). These results indicate that the activation of these inflammatory pathways is a general signaling mechanism in response to PARP inhibitors, regardless the tumor type. Therefore, it was investigated whether blocking these inflammatory pathways could revert the stromal activation mediated by PARP inhibitors. To do that, fibroblasts were treated with Ola in the presence or absence of a JAK inhibitor, Lestaurtinib (**FIG. 11C**). Of note, incubation with Lestaurtinib and Ola reduces the expression of *HAS3*, *HAS2* and *IL6* induced by Ola (**FIG. 11D**). More importantly, conditioned media from fibroblasts treated with the Lestaurtinib and Ola combination completely blocks the proliferation of cancer cells induced by Ola-treated stromal cells, as determined by Edu (5-ethynyl-2'-deoxyuridine) incorporation (**FIG. 11E**). Similar results were obtained with conditioned media was incubated with anti-IL6 or anti-TNF α antibodies. These results demonstrate that the inflammatory response mediated by TNF α , IL-6 and JAK activation are important events for the stromal activation by PARP inhibitors and that strategies to therapeutically target these signaling cascades could enhance the efficacy of PARP inhibitors. Therefore, the data support the clinical use of combinations of PARP inhibitors and therapies blocking TNF α , IL6 or JAK to improve the clinical response to PARP inhibition in cancer.

PARP inhibitors induce stromal activation in metastatic sites

Since Ola is approved for the treatment of human metastatic castration-resistant prostate cancer (mCRPC), it was investigated whether Ola was able to

induce stromal activation not only in the prostate but also in metastatic sites. Treatment with Ola of castrated TRAMP⁺/FVB F1 mice, which have a high incidence of spontaneous metastasis in lymph nodes, showed increased α SMA expression and higher hyaluronan (HA) deposition in metastatic lymph nodes from Ola-treated mice as compared to those from vehicle-treated mice (**FIGs. 15A and 15B**). Furthermore, analysis of bones from these mice demonstrated higher levels of HA in surrounding bone marrow stromal cells and in the growth plate of Ola-treated mice (**FIG. 15C**). These in vivo mouse data demonstrate that Ola treatment remodels the stromal niche not only in the prostate but also in metastatic sites such as lymph nodes or bone. This remodeling of the metastatic niche could support metastatic growth and predispose these sites to further metastasis seeding. To test this hypothesis, bone-in-culture-arrays (BICA) were used to model bone colonization of cancer cells in *ex vivo* cultures. Mouse fragmented epiphysis and metaphysis of hindlimb bones were treated with Ola in androgen-deprivation conditions (ADT) prior to the seed of luciferase-modified RM1 prostate cancer cells. Of note, Ola significantly increased the seeding of cancer cells onto the bone (**FIGs. 15D and 15E**). In addition, analysis of mouse bone marrow stromal cell cultures isolated from in vivo Ola-treated TRAMP⁺ mice showed enhanced expression of markers of stromal activation such as *Acta2* and of the HA synthase *Has2*, which is important for HA production (**FIG. 15F**). Likewise, *in vitro* Ola treatment of bone marrow stromal cells also showed increased expression of markers of TGF β family (*Tgfb3* and *Tgfb2*) and *Has3* (**FIG. 15G**). These results demonstrate that Ola treatment promotes stromal activation in the bone, which predisposes the bone niche to the seed of metastatic cancer cells. Moreover, deposition of HA was observed in other metastatic sites such as liver after treatment with Ola of mice in an in vivo metastasis model of injection via caudal artery of metastatic cancer cells (**FIG. 15I**). Also, analysis of bone metastasis treated with Ola demonstrated an increased activation of TGF β signaling as shown by enhanced nuclear pSMAD2 in cancer cells (**FIG. 15J**). These observations support clinical studies of combinatorial therapy of PARP inhibitors with TGF β inhibition. To further strengthen the human relevance of these observations, paired biopsies of bone metastasis from a mCRPC patient pre- and post-treatment with Ola were analyzed. Importantly, there was a robust increase in HA deposition in the Ola-treated bone metastasis consistent with our mouse data (**FIG. 15K**).

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All patents and publications referenced or mentioned herein are indicative of the levels of skill of those skilled in the art to which the invention pertains, and each such referenced patent or publication is hereby specifically incorporated by
 15 reference to the same extent as if it had been incorporated by reference in its entirety individually or set forth herein in its entirety. Applicants reserve the right to physically incorporate into this specification any and all materials and information from any such cited patents or publications.

20

The following statements are intended to describe and summarize various embodiments of the invention according to the foregoing description in the specification.

Statements:

- 25 1. A method comprising administering to a subject in need thereof a composition comprising one or more PARP1 inhibitors and one or more inhibitors of activated stromal / activated cancer-associated fibroblasts or one or more inhibitors of TNFalpha, IL6 or JAK.
- 30 2. The method of statement 1, wherein the subject has cancer or is suspected of a having cancer.
3. The method of statement 1 or 2, wherein the subject has primary cancer, metastatic cancer, or recurrent cancer.
4. The method of statement 1, 2 or 3, wherein the subject has cancer of the breast, cancer of the lung, cancer of the adrenal cortex, cancer of the

cervix, cancer of the endometrium, cancer of the esophagus, cancer of the head, cancer of the neck, cancer of the liver, cancer of the pancreas, cancer of the prostate, cancer of the thymus, carcinoid tumors, chronic lymphocytic leukemia, Ewing's sarcoma, gestational trophoblastic tumors, hepatoblastoma, multiple myeloma, non-small cell lung cancer, retinoblastoma, or tumor in the ovaries.

- 5 5. The method of any of statements 1-4, wherein the one or more PARP1 inhibitors are olaparib (Lynparza), niraparib (Zejula), rucaparib (Rubraca), talazoparib (Talzenna), veliparib (ABT-888), or a combination thereof.
- 10 6. The method of any of statements 1-5, wherein the one or more inhibitors of activated stromal / activated cancer-associated fibroblasts include hyaluronan (HA) synthase inhibitors, hyaluronan build-up inhibitors (e.g., PEGylated Recombinant Human Hyaluronidase, PEGPH20),
15 autophagy inhibitors (e.g., ULK inhibitors), fibroblast activation protein alpha (FAP α) inhibitors, GW4064 (farnesoid X receptor (FXR) agonists, Pirfenidone (PDF) combined with doxorubicin, SMO-inhibitors (e.g., vismodegib, sonidegib), amphiregulin inhibitors, CXCL12 antagonists (e.g., an E5 antagonistic peptide), DDR2 inhibitors (e.g., WRG-28,
20 which is a small molecule inhibitor of DDR2), or a combination thereof.
7. The method of any of statements 1-6, wherein the composition comprises Olaparib and PEGPH20.

25 The specific methods and compositions described herein are representative of preferred embodiments and are exemplary and not intended as limitations on the scope of the invention. Other objects, aspects, and embodiments will occur to those skilled in the art upon consideration of this specification and are encompassed within the spirit of the invention as defined by the scope of the claims. It will be
30 readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, or limitation or limitations, which is not

specifically disclosed herein as essential. The methods and processes illustratively described herein suitably may be practiced in differing orders of steps, and the methods and processes are not necessarily restricted to the orders of steps indicated herein or in the claims.

5 As used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to “a nucleic acid” or “a protein” or “a cell” includes a plurality of such nucleic acids, proteins, or cells (for example, a solution or dried preparation of nucleic acids or expression cassettes, a solution of proteins, or a population of
10 cells), and so forth. In this document, the term “or” is used to refer to a nonexclusive or, such that “A or B” includes “A but not B,” “B but not A,” and “A and B,” unless otherwise indicated.

 Under no circumstances may the patent be interpreted to be limited to the specific examples or embodiments or methods specifically disclosed herein. Under
15 no circumstances may the patent be interpreted to be limited by any statement made by any Examiner or any other official or employee of the Patent and Trademark Office unless such statement is specifically and without qualification or reservation expressly adopted in a responsive writing by Applicants.

 The terms and expressions that have been employed are used as terms of
20 description and not of limitation, and there is no intent in the use of such terms and expressions to exclude any equivalent of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention as claimed. Thus, it will be understood that although the present invention has been specifically disclosed by preferred embodiments and
25 optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims and statements of the invention.

 The invention has been described broadly and generically herein. Each of the
30 narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein. In addition, where features or aspects of the invention are described in terms of

Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

WHAT IS CLAIMED IS:

1. A method comprising administering to a subject in need thereof one or more PARP1 inhibitors and one or more inhibitors of activated stromal/activated cancer-associated fibroblasts or one or more modulators of TNFalpha, IL6 or JAK.
5
2. The method of claim 1 wherein the subject is a human.
3. The method of claim 1 or 2, wherein the subject has cancer or is suspected of
10 a having cancer.
4. The method of claim 1, 2 or 3, wherein the subject has primary cancer, metastatic cancer, or recurrent cancer.
- 15 5. The method of any one of claims 1 to 4, wherein the subject has cancer of the breast, cancer of the lung, cancer of the adrenal cortex, cancer of the cervix, cancer of the endometrium, cancer of the esophagus, cancer of the head, cancer of the neck, cancer of the liver, cancer of the pancreas, cancer of the prostate, cancer of the thymus, carcinoid tumors, chronic lymphocytic leukemia, Ewing's sarcoma,
20 gestational trophoblastic tumors, hepatoblastoma, multiple myeloma, non-small cell lung cancer, retinoblastoma, or tumor in the ovaries.
6. The method of any one of claims 1 to 5, wherein the one or more PARP1 inhibitors are olaparib (Lynparza), niraparib (Zejula), rucaparib (Rubraca),
25 talazoparib (Talzenna), veliparib (ABT-888), or a combination thereof.
7. The method of any one of claims 1 to 6, wherein the one or more inhibitors of activated stromal / activated cancer-associated fibroblasts include hyaluronan (HA) synthase inhibitors, hyaluronan build-up inhibitors, autophagy inhibitors,
30 fibroblast activation protein alpha (FAPα) inhibitors, GW4064 (farnesoid X receptor (FXR) agonists, Pirfenidone (PDF) combined with doxorubicin, SMO-inhibitors, amphiregulin inhibitors, CXCL12 antagonists, DDR2 inhibitors, or a combination thereof.

8. The method of claim 7, wherein the DDR2 inhibitor comprises WRG-28.
9. The method of claim 7, wherein the hyaluronan build-up inhibitor comprises PEGylated Recombinant Human Hyaluronidase, PEGPH20.
- 5 10. The method of claim 7, wherein the autophagy inhibitor comprises a ULK inhibitor.
11. The method of claim 7, wherein the SMO inhibitor comprises vismodegib or
10 sonidegib.
12. The method of claim 7, wherein the CXCL12 antagonist comprises an E5 antagonistic peptide.
- 15 13. The method of any one of claims 1 to 6, wherein the Olaparib and PEGPH20 are administered.
14. The method of any one of claims 1 to 13, wherein the one or more PARP1 inhibitors are administered before the one or more inhibitors of activated
20 stromal/activated cancer-associated fibroblasts or one or more modulators of TNFalpha, IL6 or JAK.
15. The method of any one of claims 1 to 13, wherein the one or more PARP1 inhibitors are administered after the one or more inhibitors of activated
25 stromal/activated cancer-associated fibroblasts or one or more modulators of TNFalpha, IL6 or JAK.
16. The method of any one of claims 1 to 13, wherein the one or more PARP1 inhibitors are administered concurrently with the one or more inhibitors of activated
30 stromal/activated cancer-associated fibroblasts or one or more modulators of TNFalpha, IL6 or JAK.
17. The method of any one of claims 1 to 13, wherein a composition comprises the one or more PARP1 inhibitors are administered before the one or more inhibitors

of activated stromal/activated cancer-associated fibroblasts or one or more modulators of TNFalpha, IL6 or JAK.

18. The method of any one of claims 1 to 17, wherein the one or more PARP1
5 inhibitors are injected.

19. The method of any one of claims 1 to 18, wherein the one or more inhibitors
of activated stromal/activated cancer-associated fibroblasts or one or more
modulators of TNFalpha, IL6 or JAK are injected.
10

20. The method of any one of claims 1 to 17, wherein the one or more PARP1
inhibitors are systemically administered.

21. The method of any one of claims 1 to 17 or 20, wherein the one or more
15 inhibitors of activated stromal/activated cancer-associated fibroblasts or one or more
modulators of TNFalpha, IL6 or JAK are systemically administered.

22. The method of any one of claims 1 to 21, wherein the JAK inhibitor
comprises lesataurtinib, baricitinib, tofacitinib, upadacitinib, filgotinib, delgocitinib,
20 deucravacitinib, LS104, ON044580, NVP-BBT594, or NVP-CHZ868.

23. The method of any one of claim 1 to 22, wherein the IL6 inhibitor comprises
an antibody or an antigen binding portion thereof.

24. The method of claim 23, wherein the antibody comprises tocilizumab,
25 sarilumab, siltuximab, olokizumab, elsilimomab, clazakizumab, sirukumab,
levimumab, CPSI-2364.

25. The method of any one of claim 1 to 24, wherein the IL6 inhibitor comprises
30 ARGX-109, FE301 or FM101.

26. The method of any one of claim 1 to 25, wherein the TNFalpha inhibitor
comprises adalimumab, certolizumab pegol, etanercept, golimumab, or infliximab.

27. A composition comprising one or more PARP1 inhibitors and one or more inhibitors of activated stromal/activated cancer-associated fibroblasts or one or more modulators of TNFalpha, IL6 or JAK.

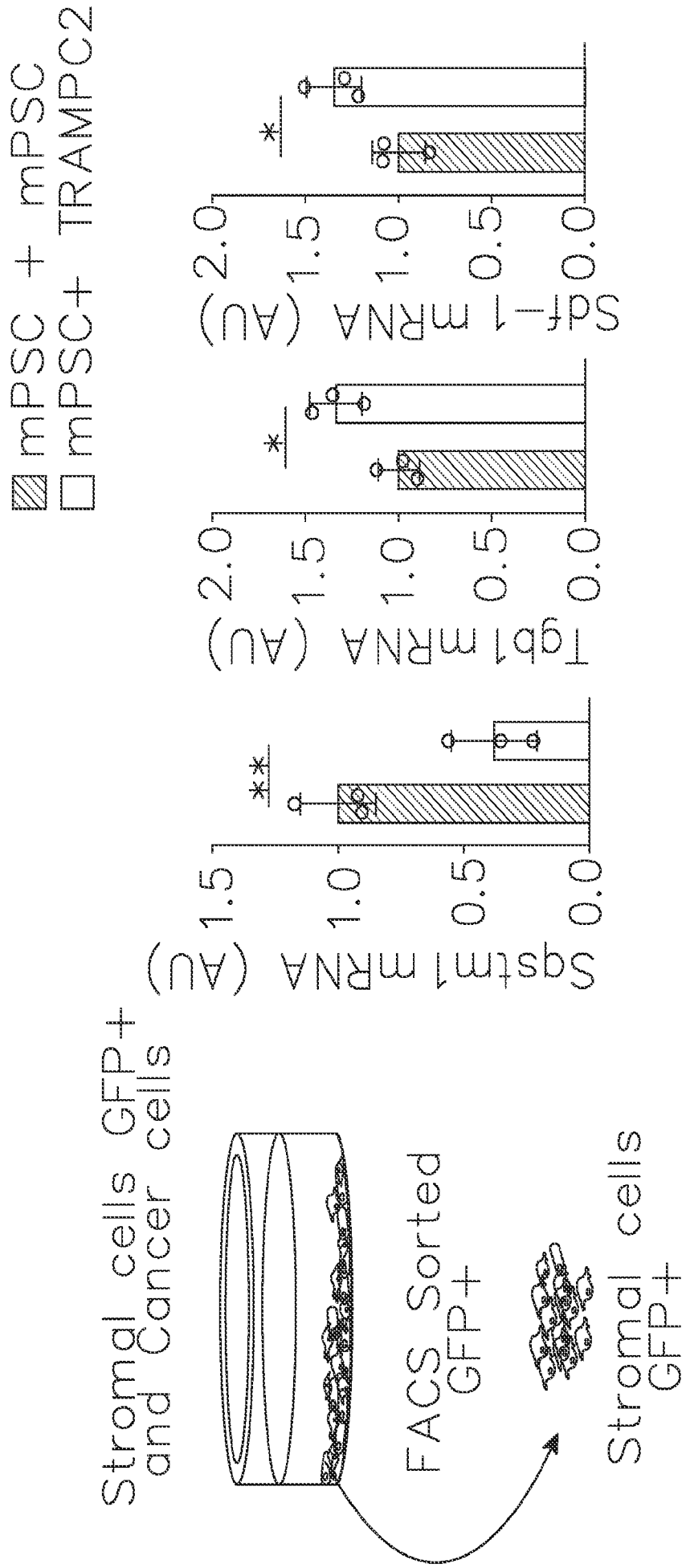


Fig. 1A

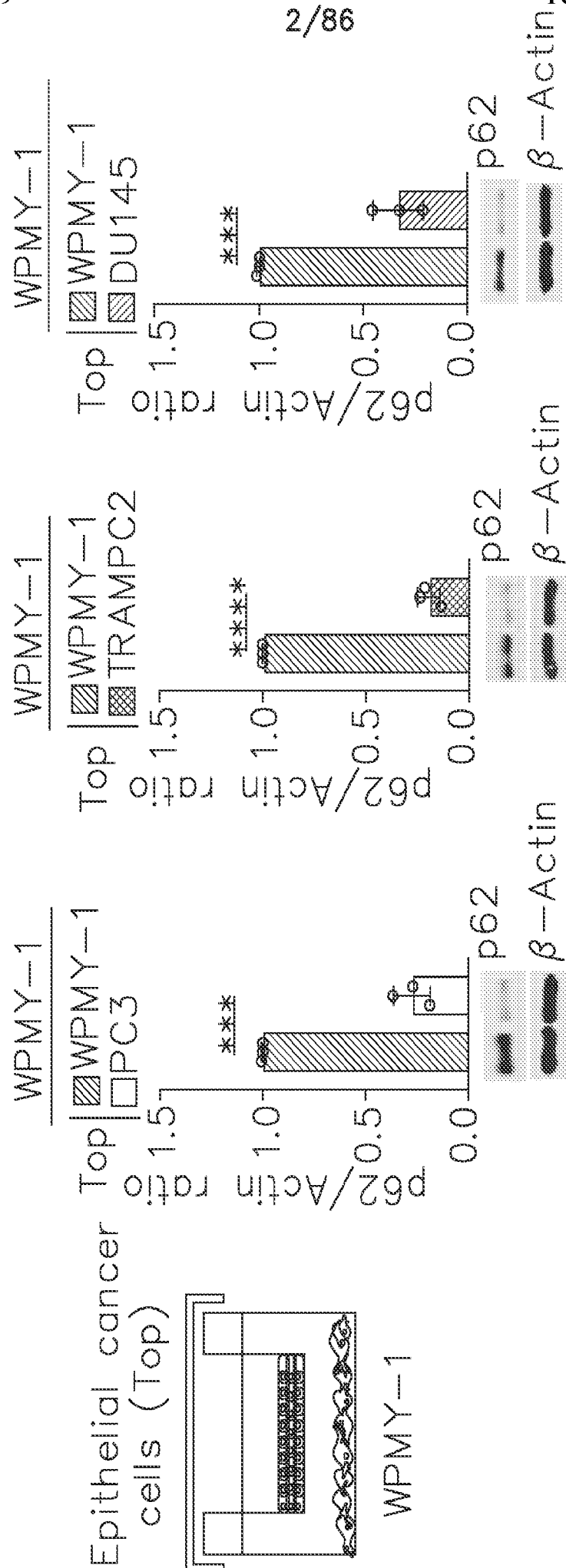


Fig. 1B

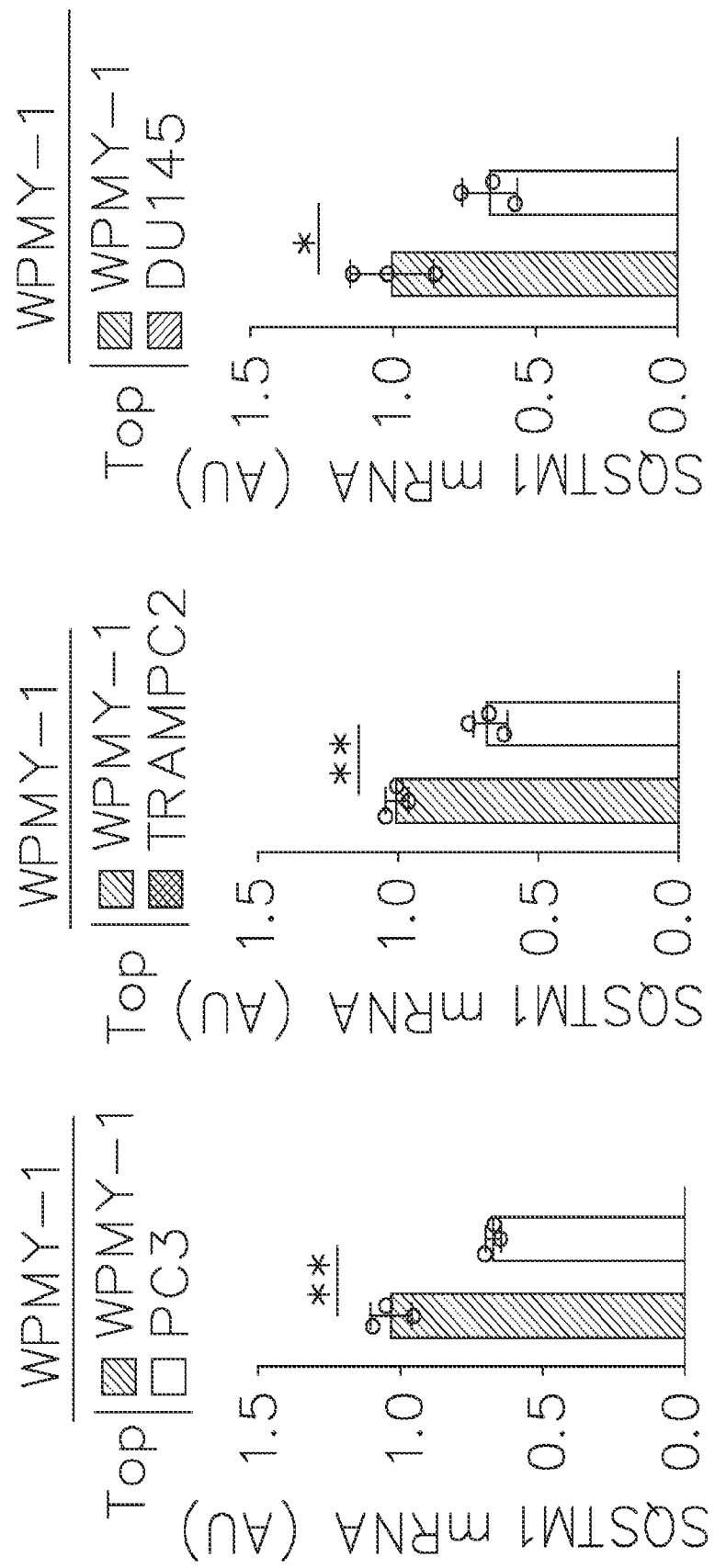
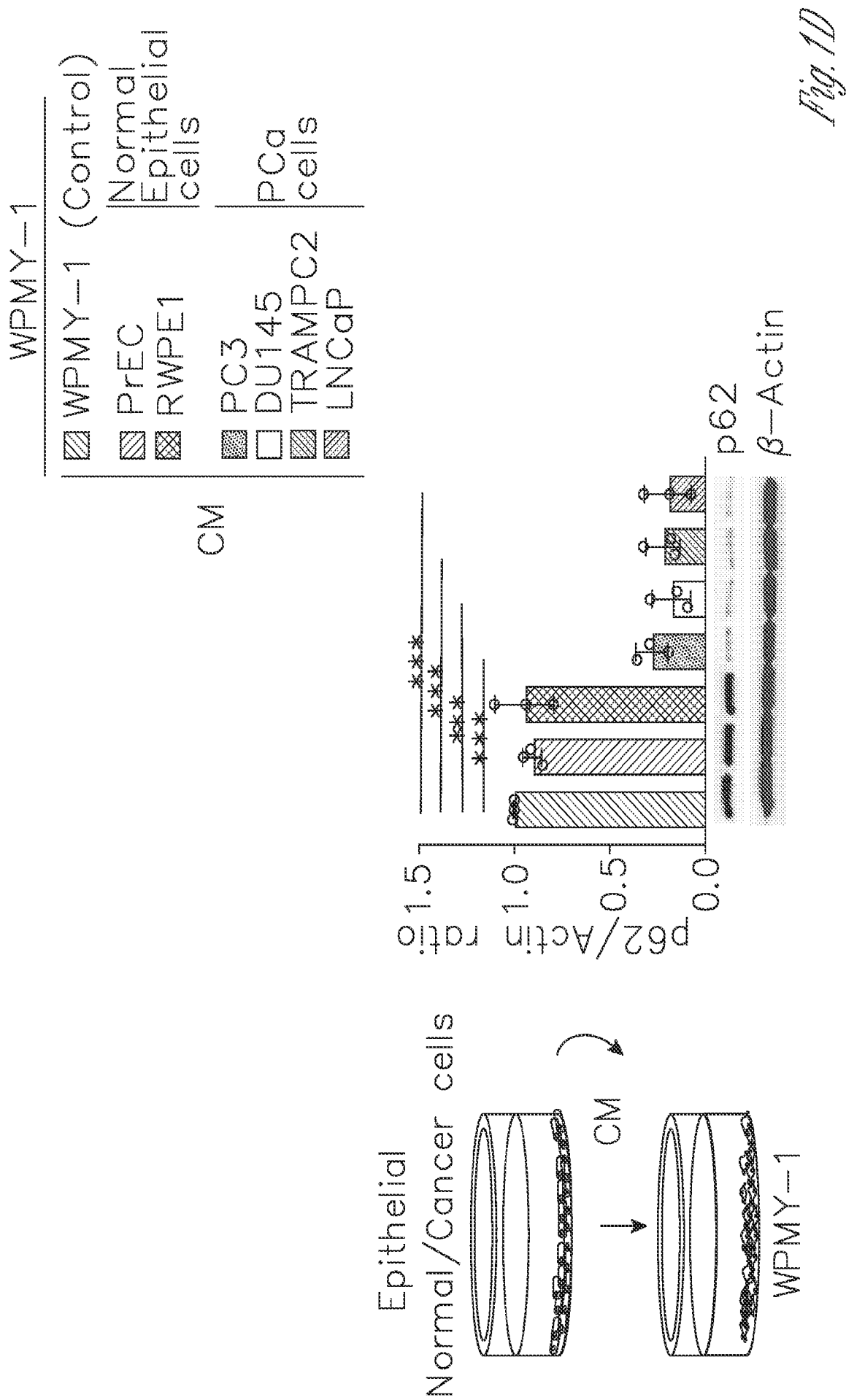
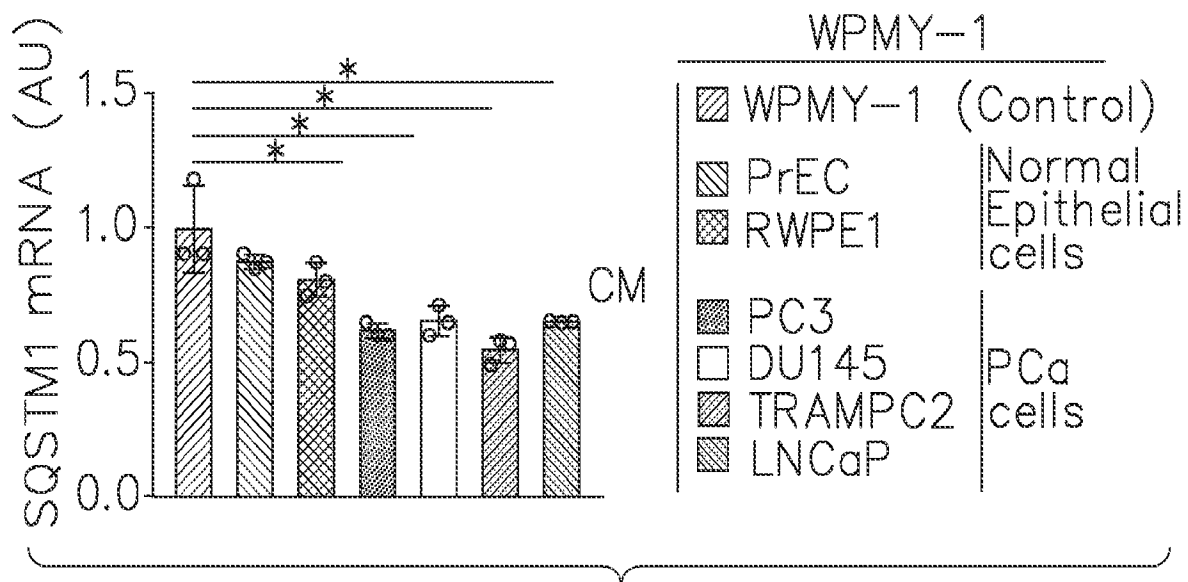
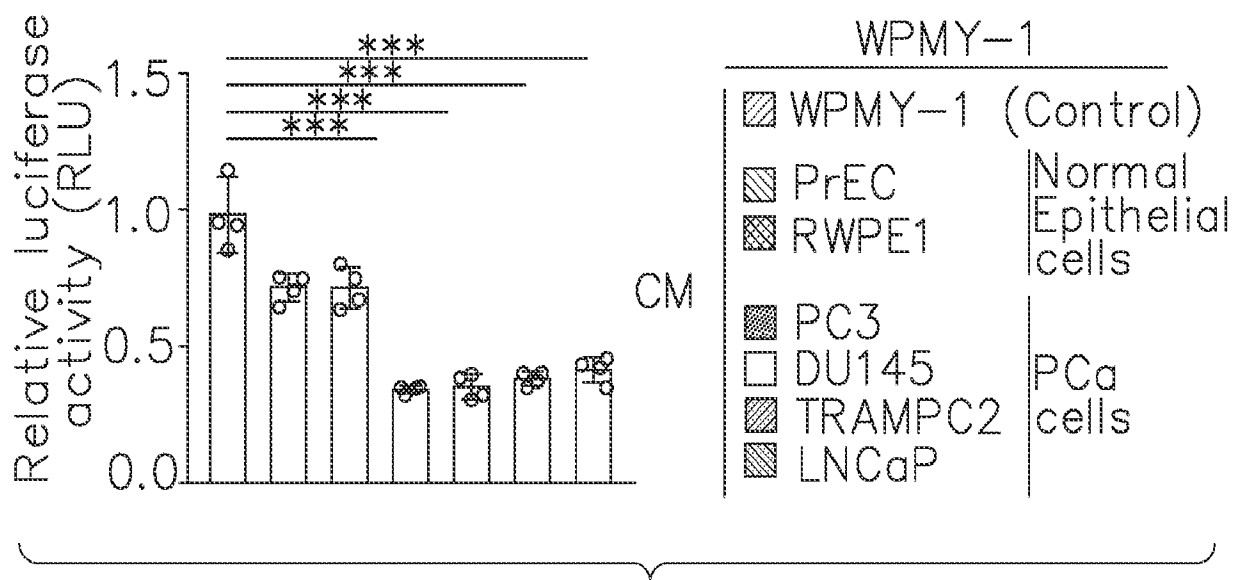
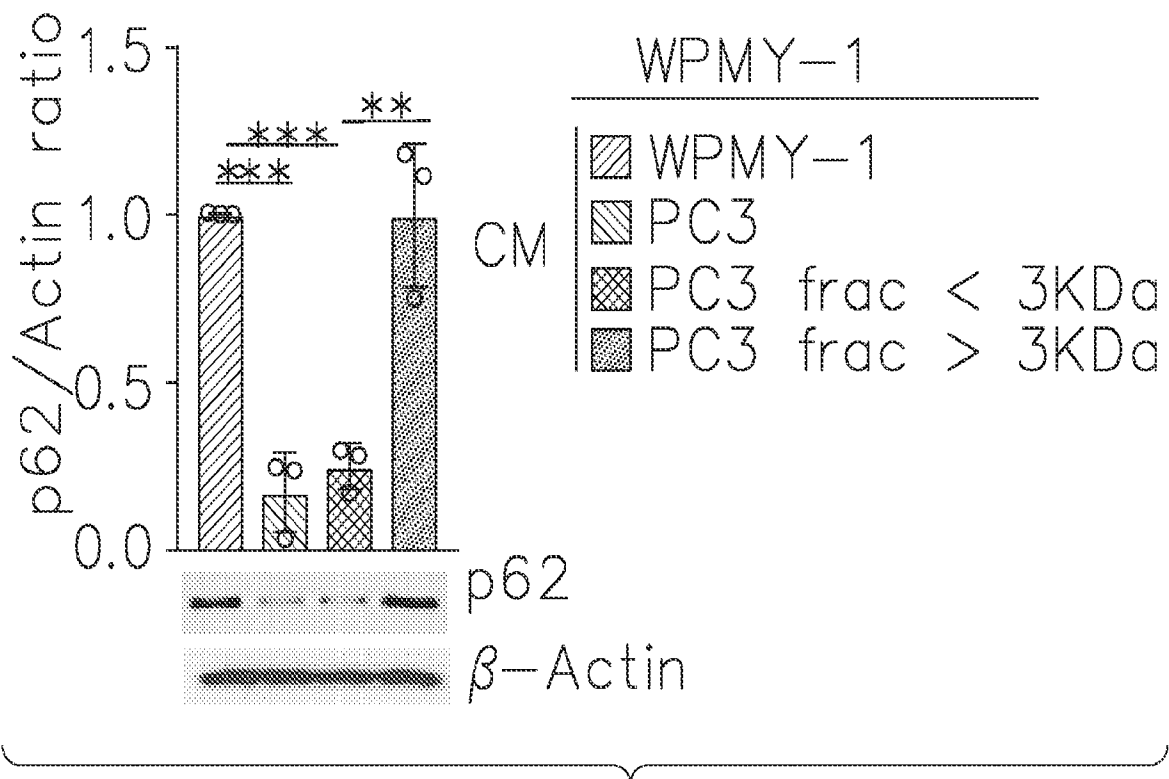
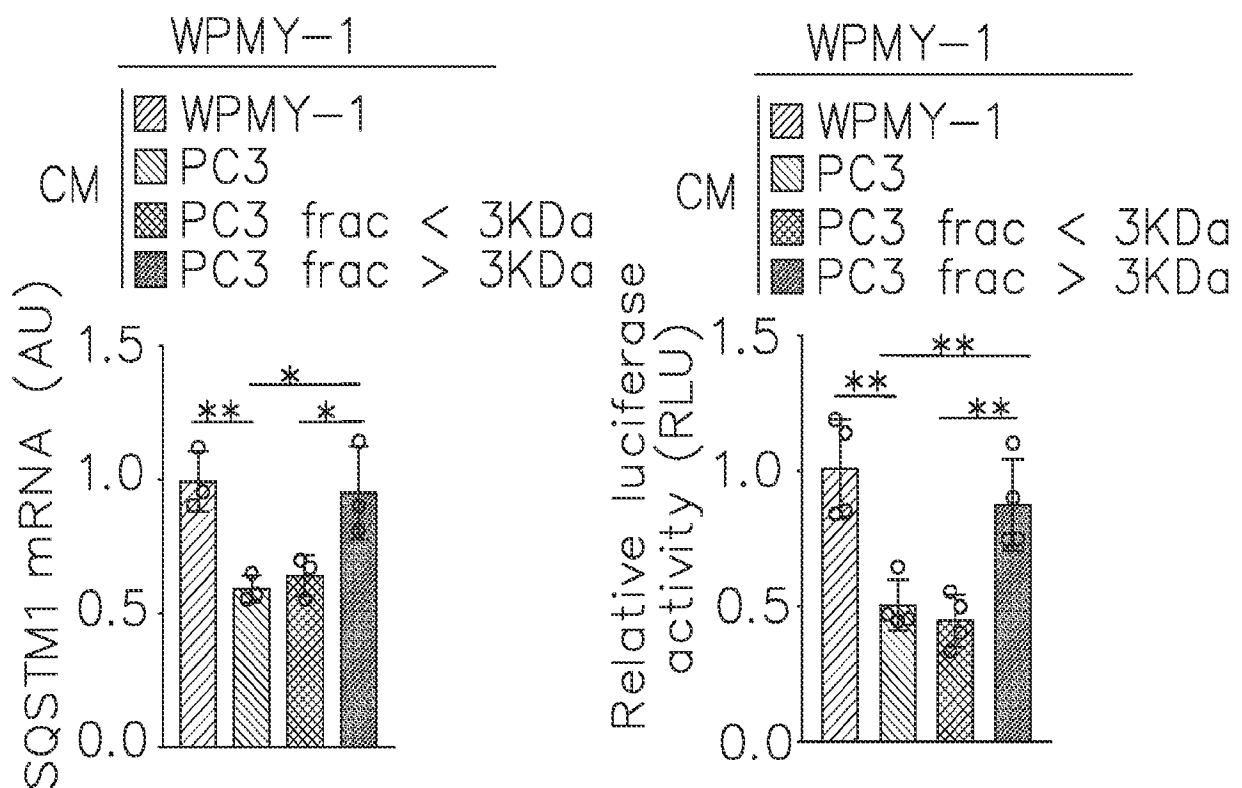


Fig. 1C



*Fig. 1E**Fig. 1F*

*Fig. 1G**Fig. 1H**Fig. 1I*

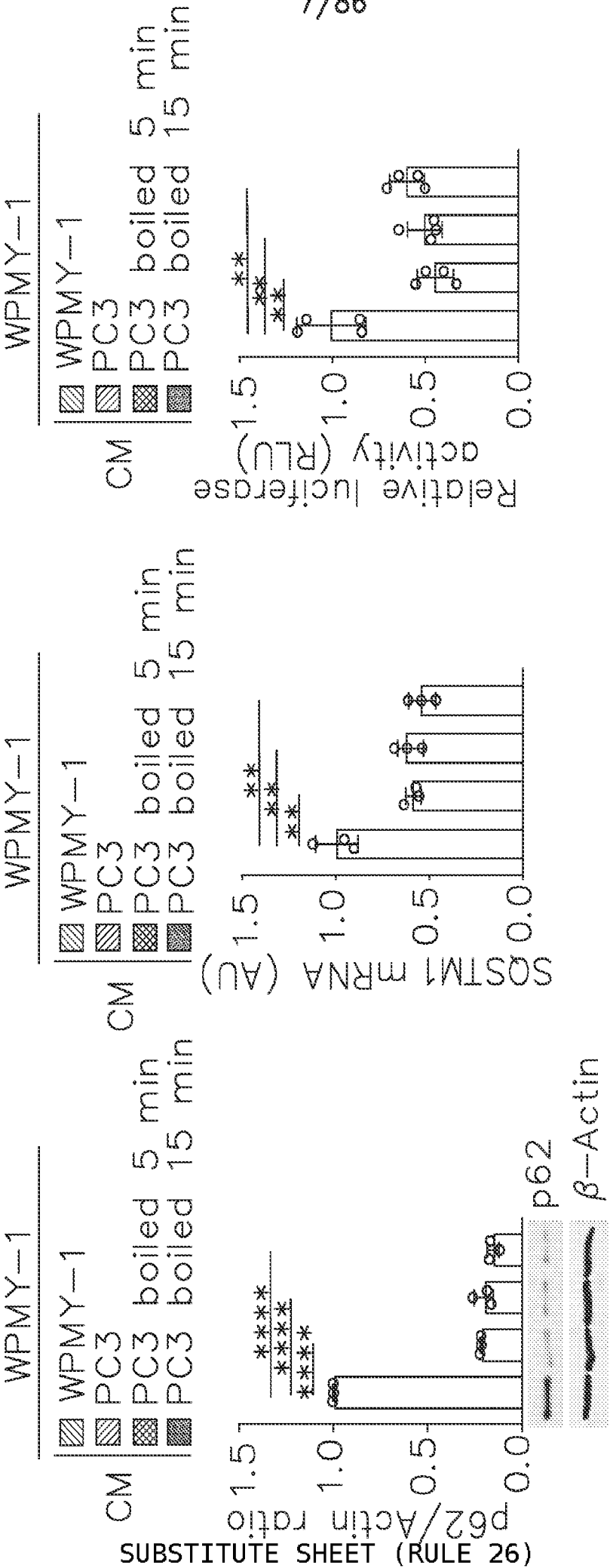
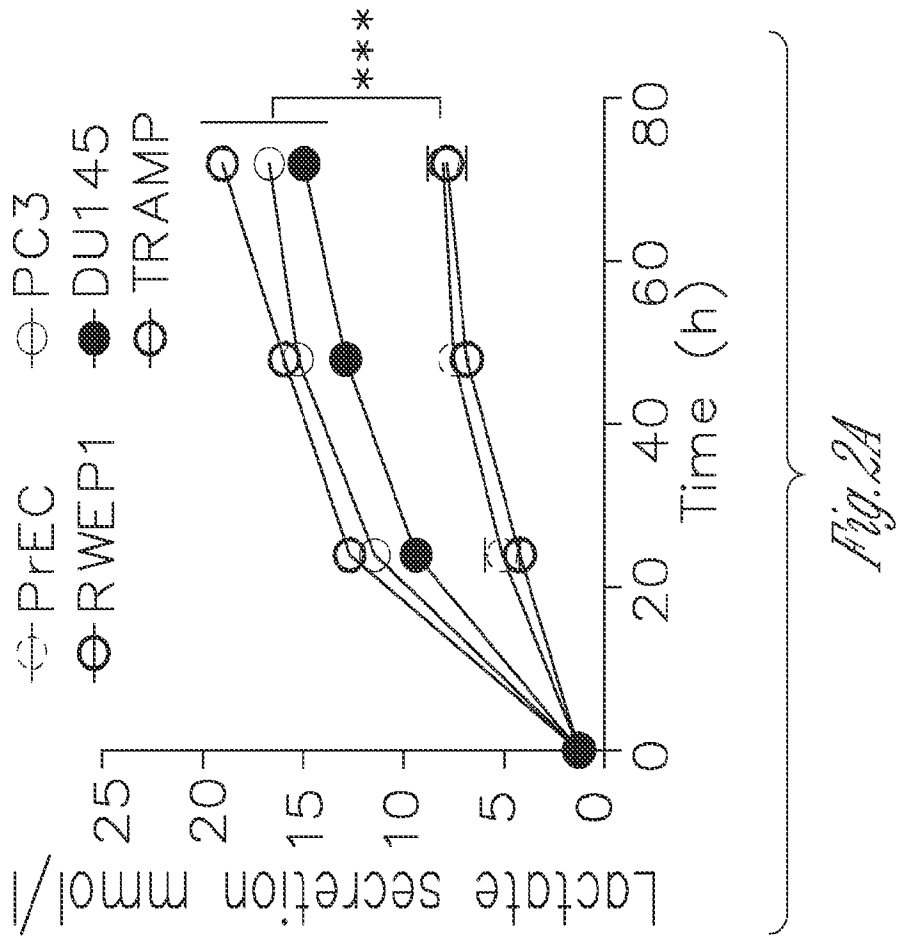
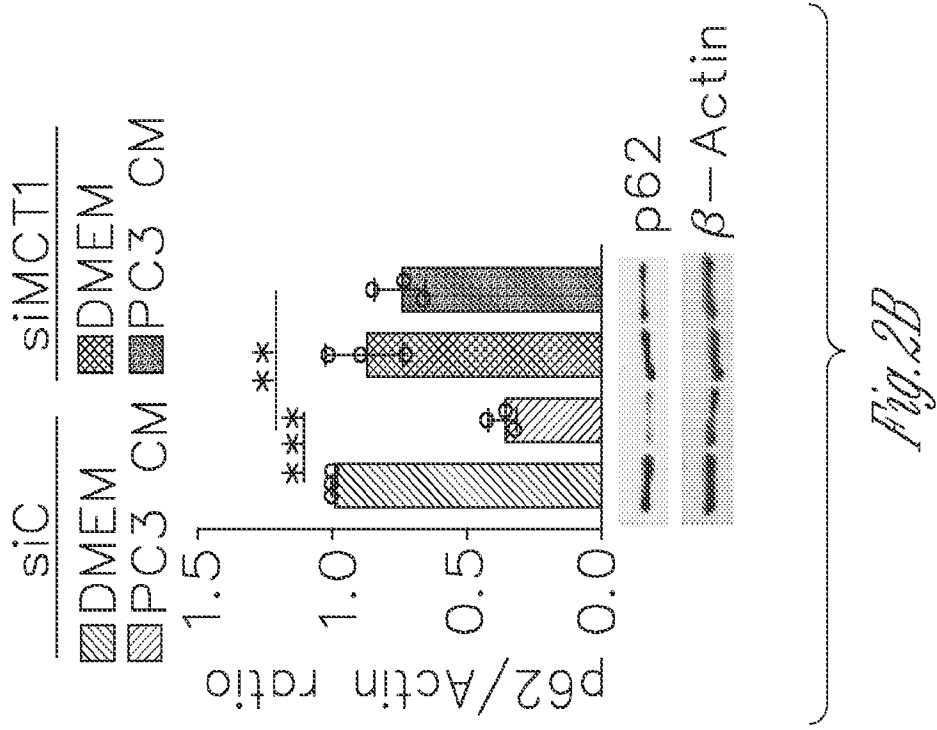
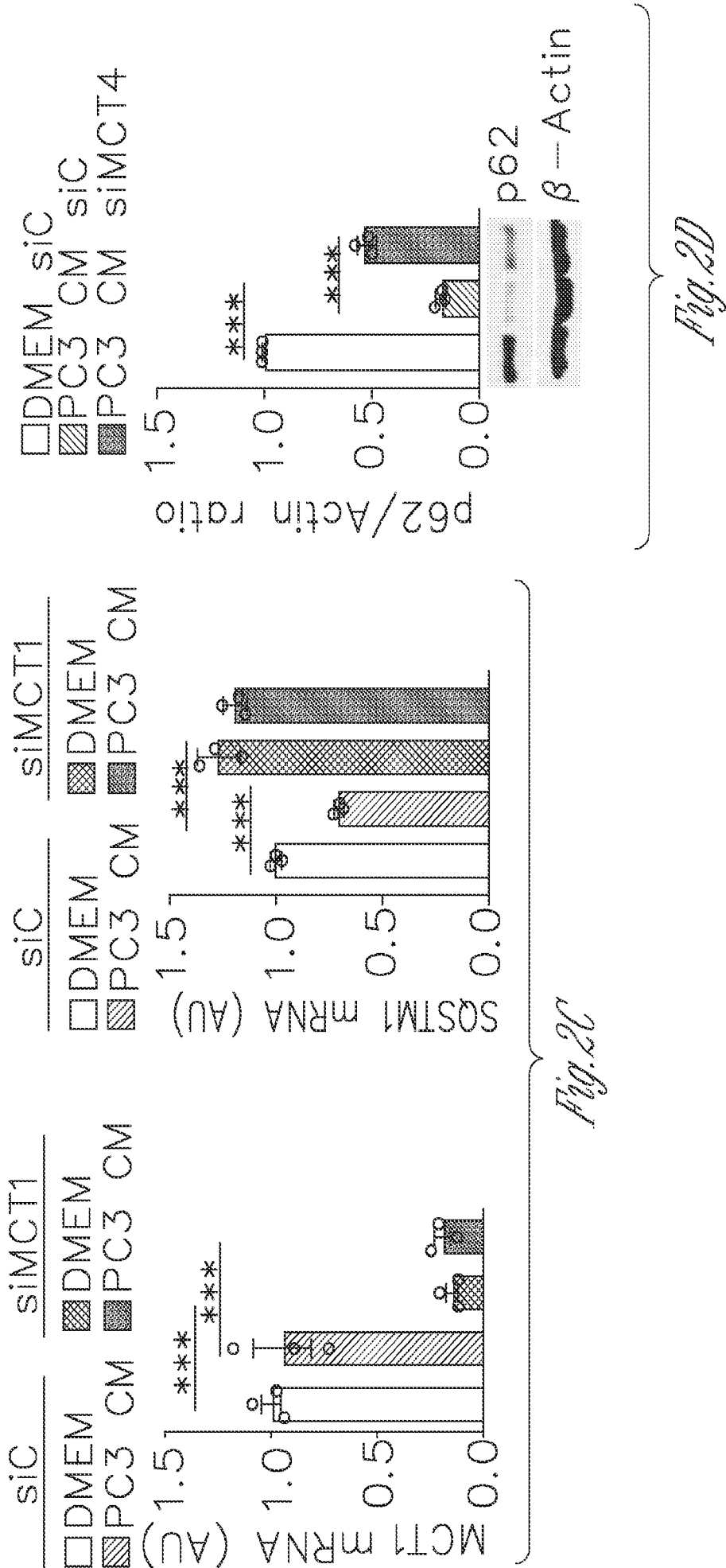


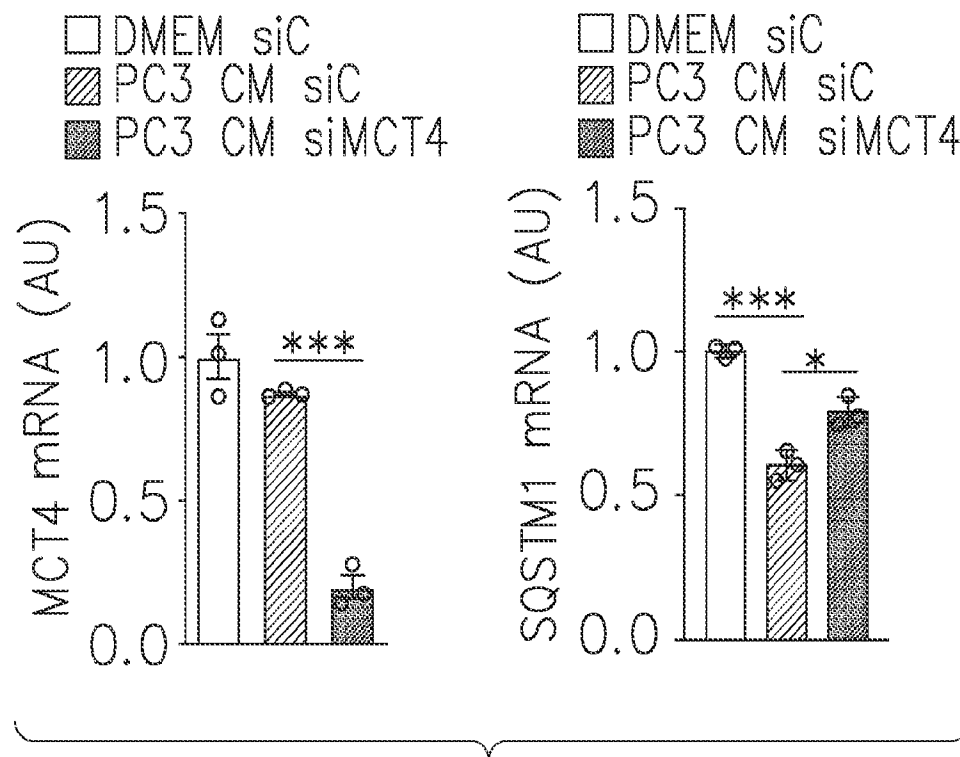
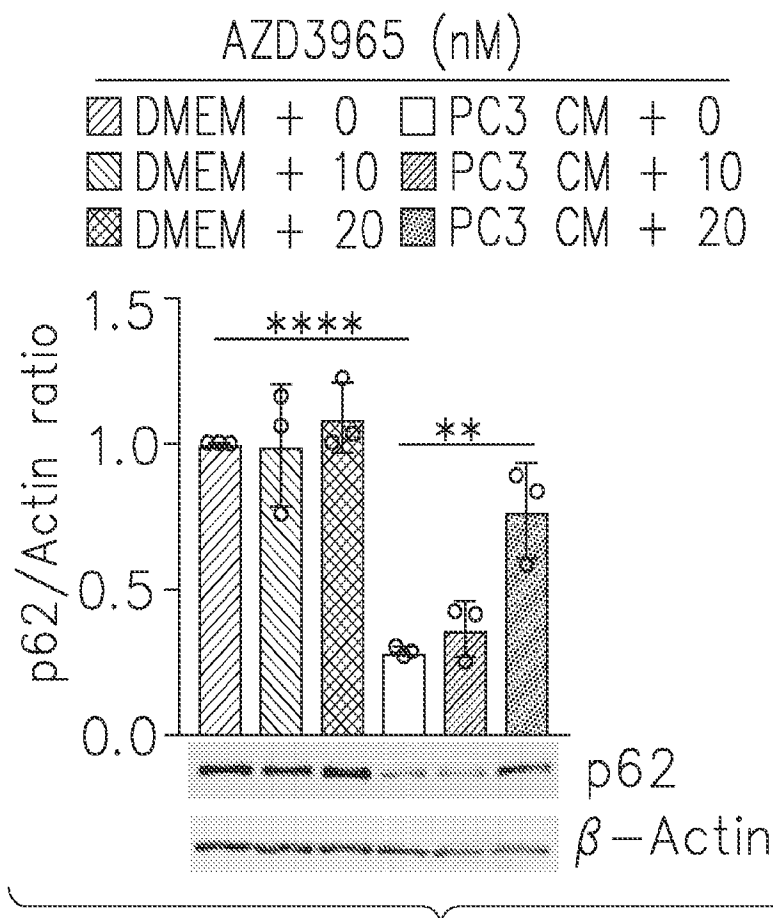
Fig. 1L

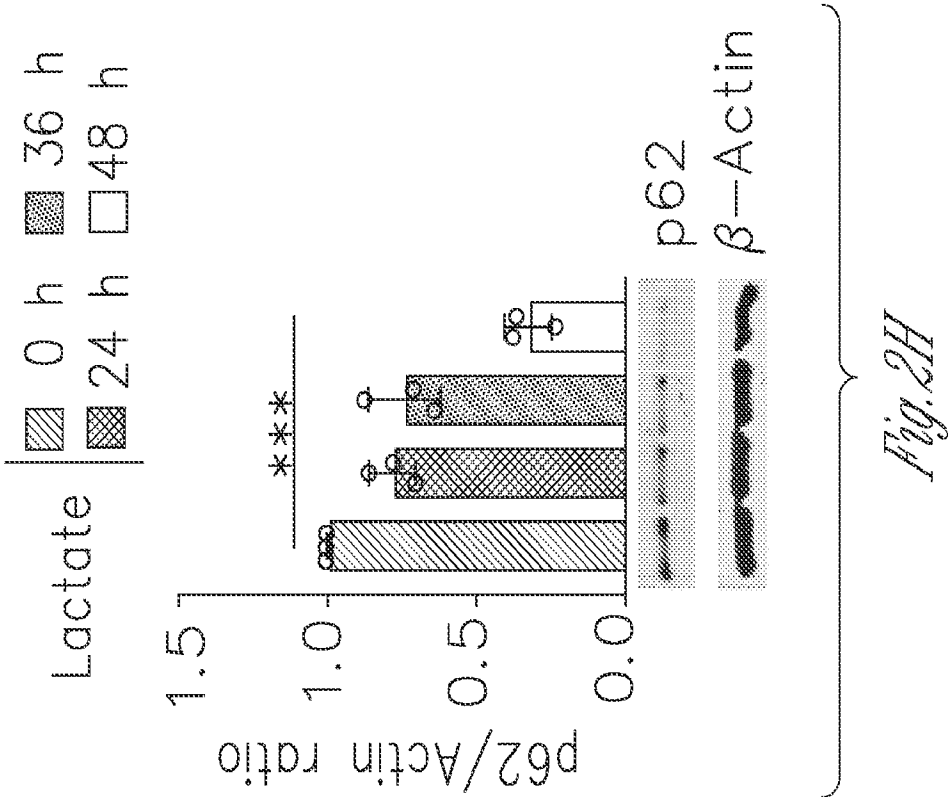
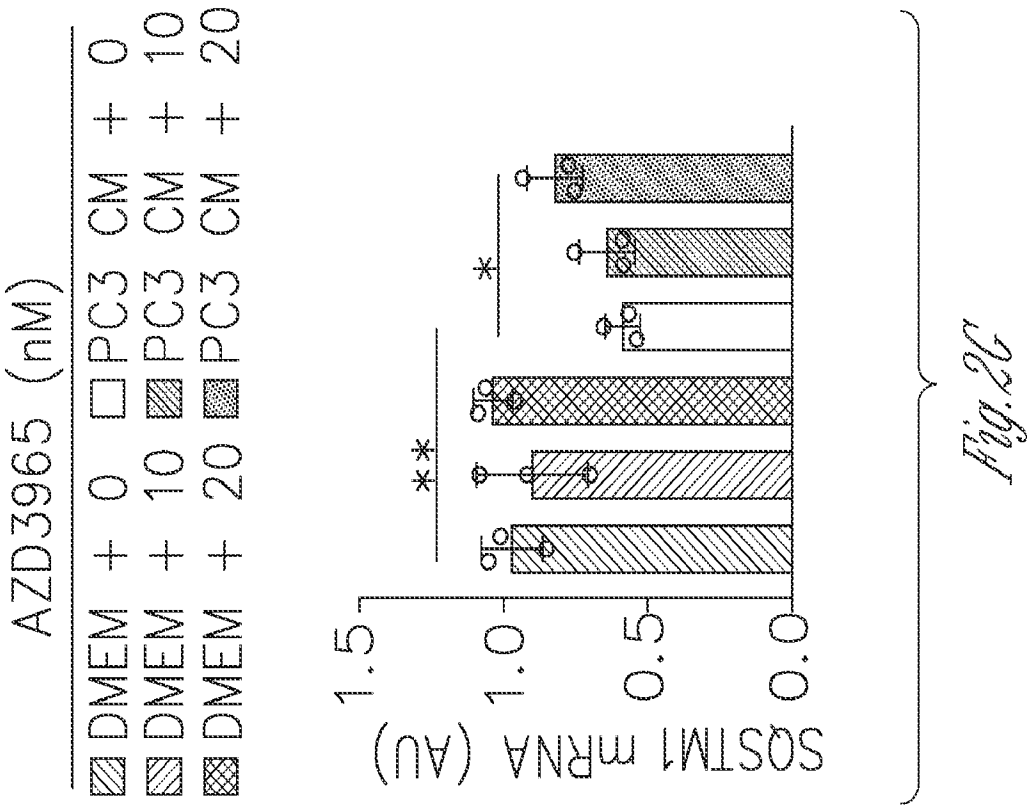
Fig. 1K

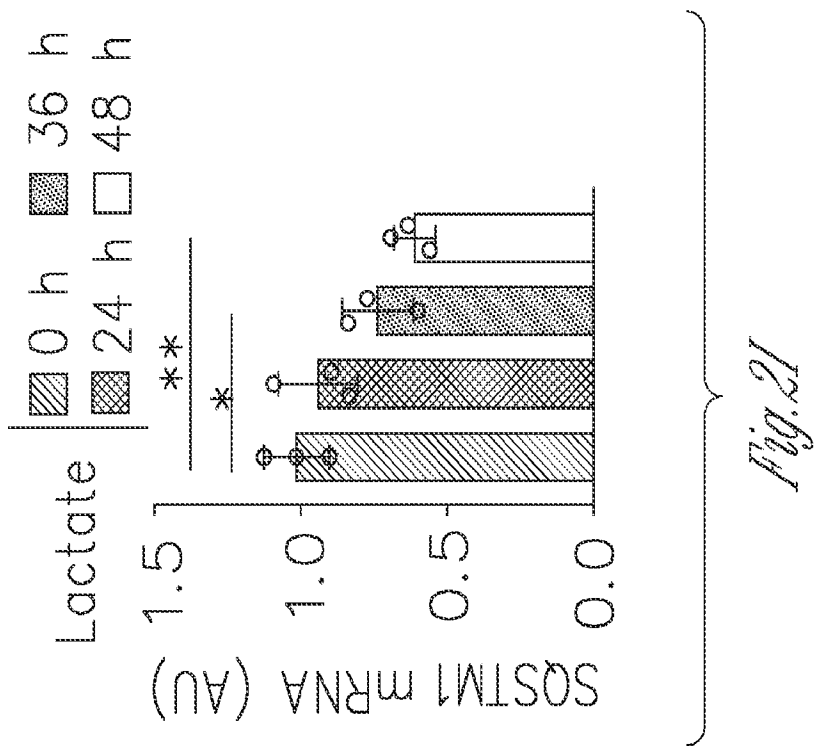
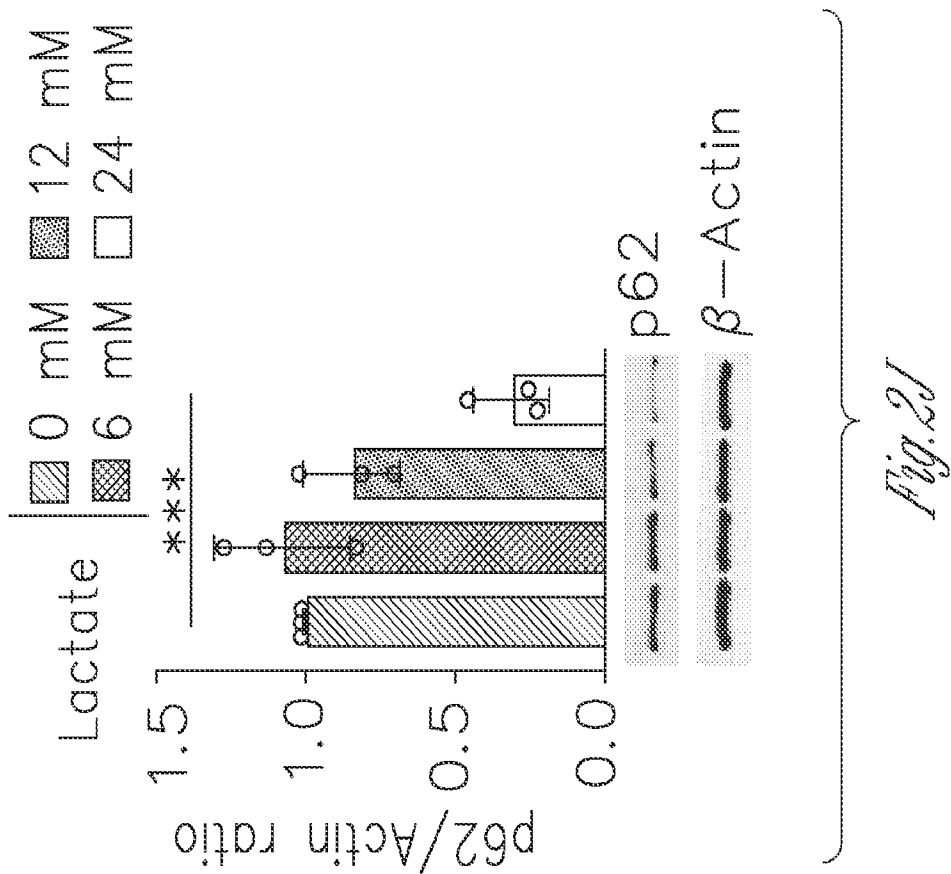
Fig. 1J





*Fig. 2E**Fig. 2F*





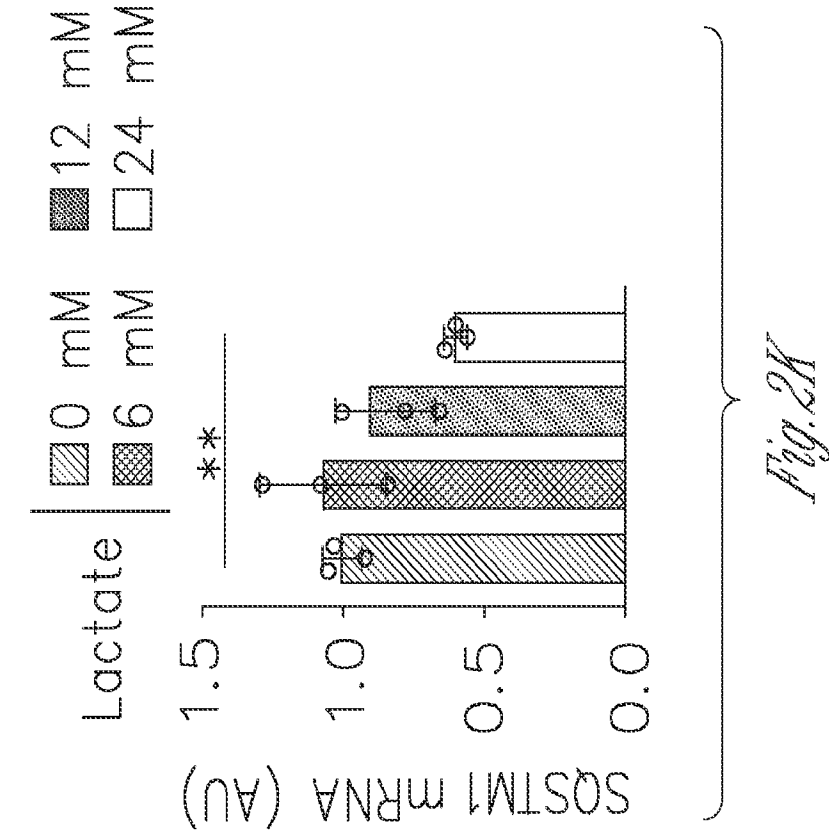


Fig. 2K

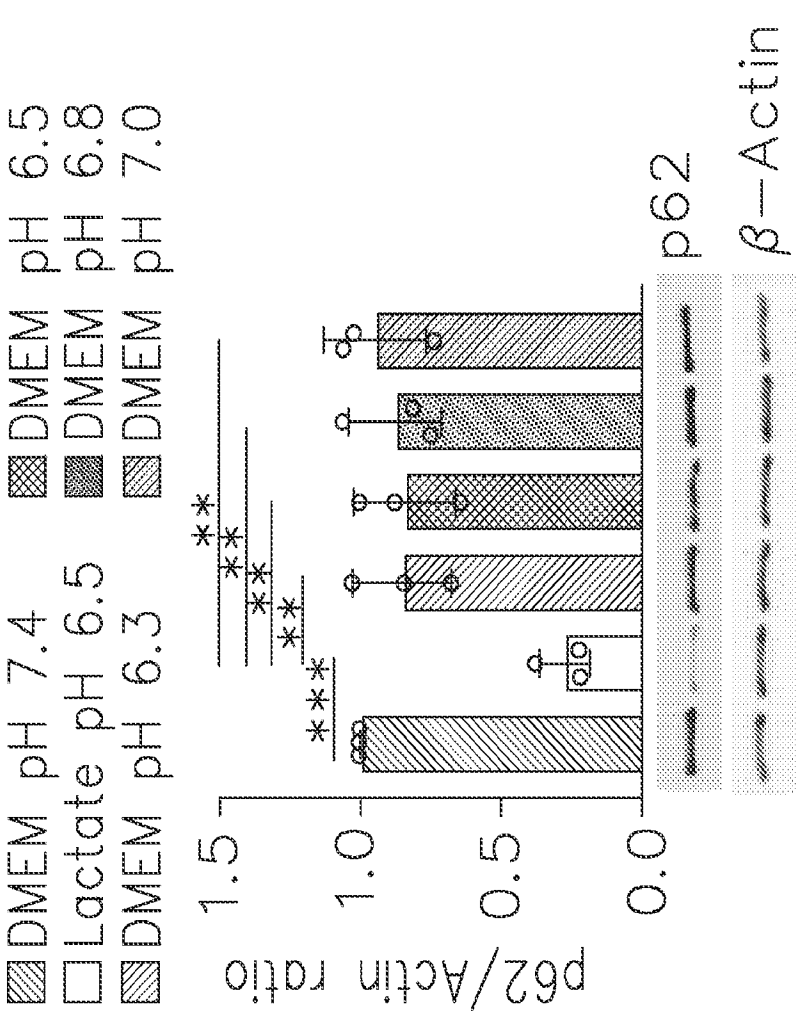


Fig. 2L

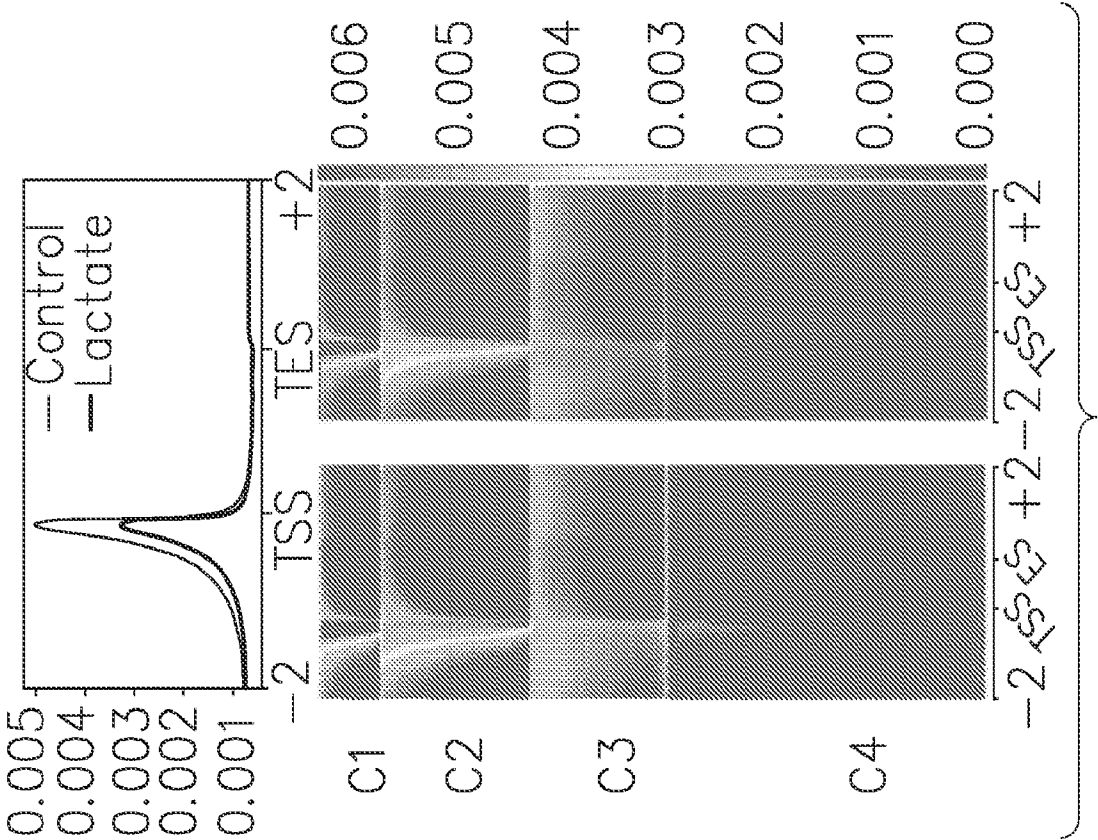


Fig. 3A

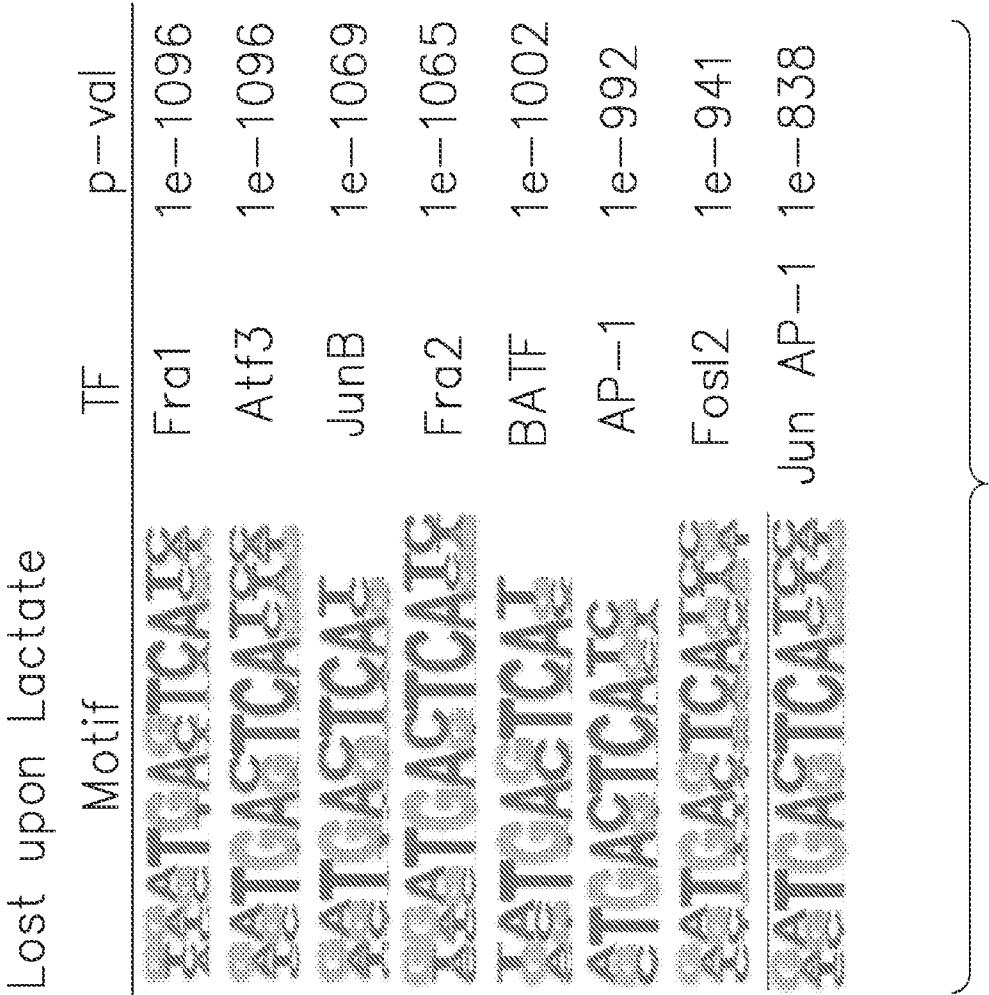


Fig. 3B

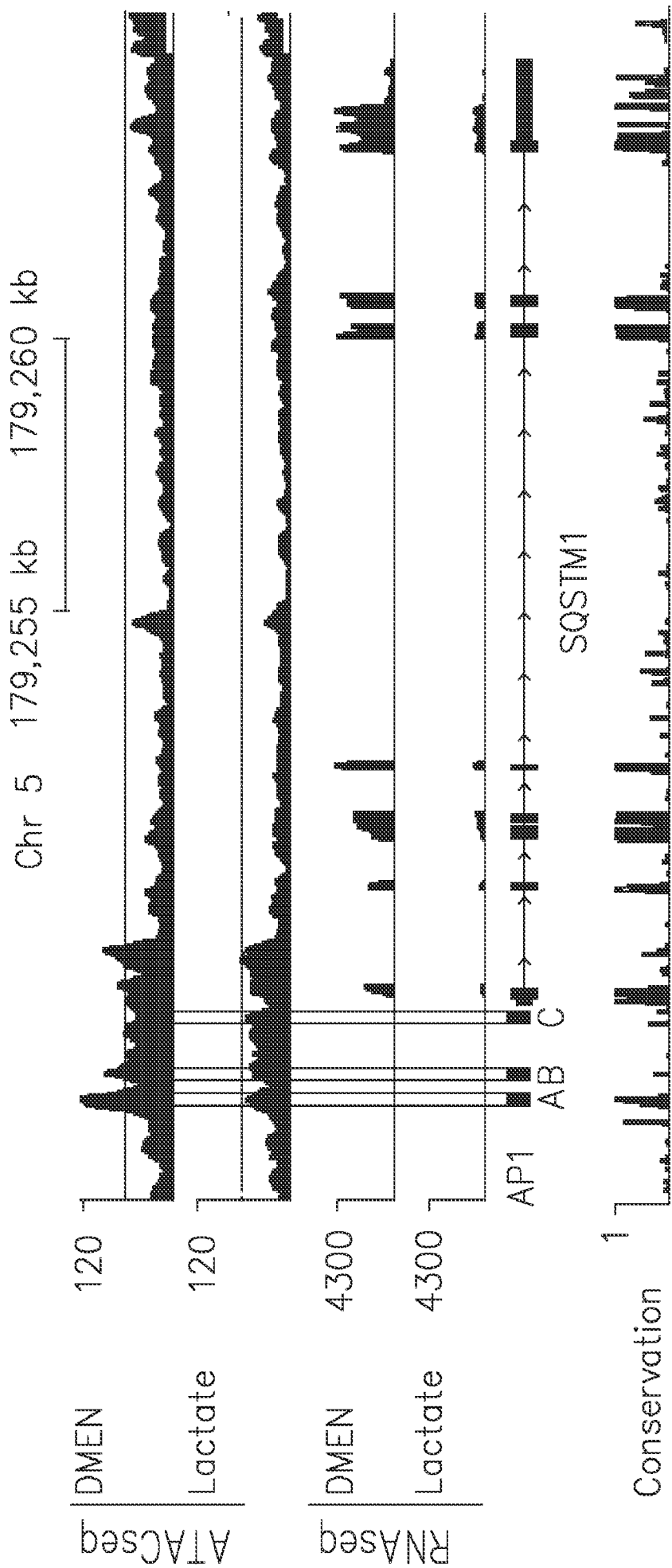
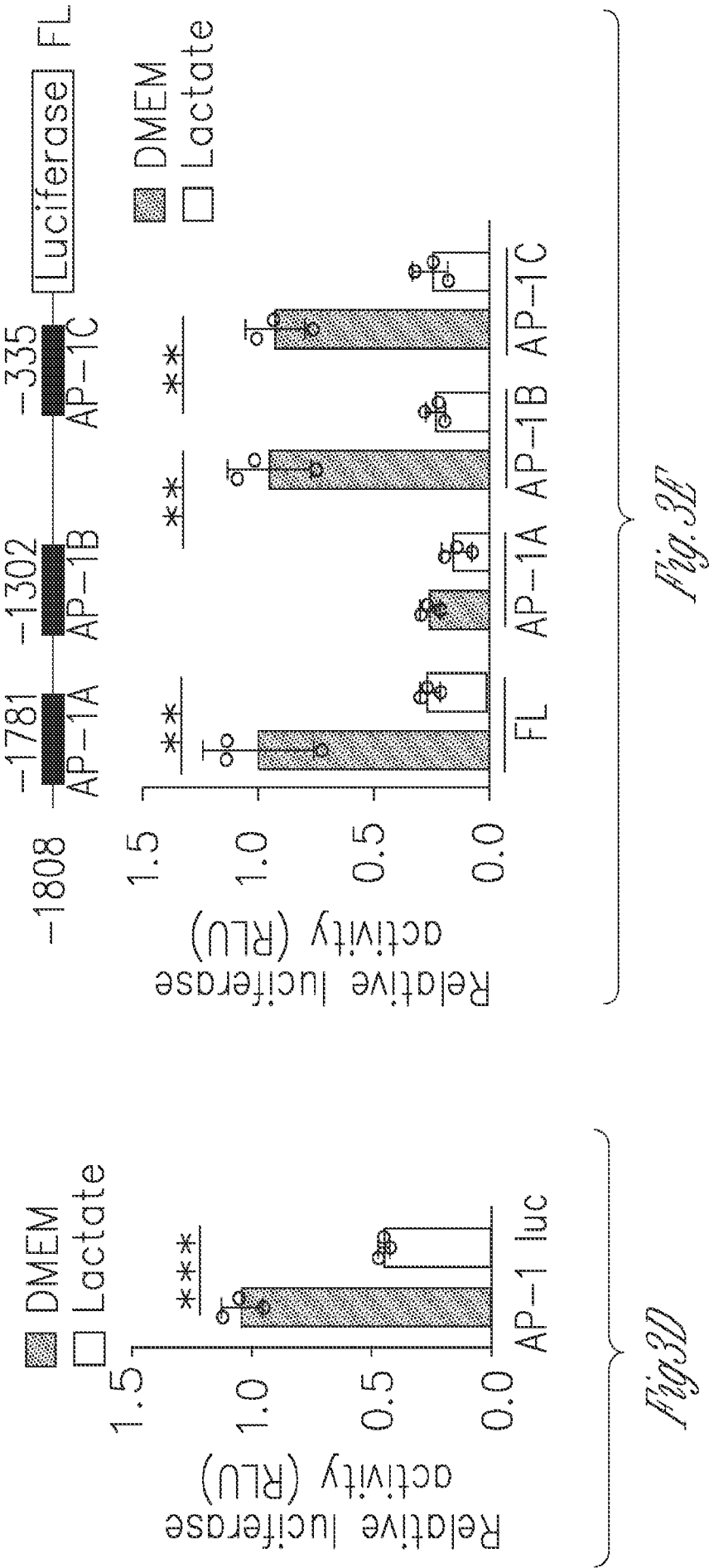
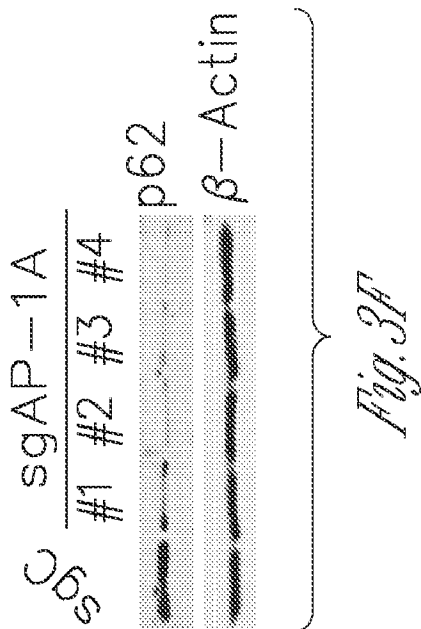
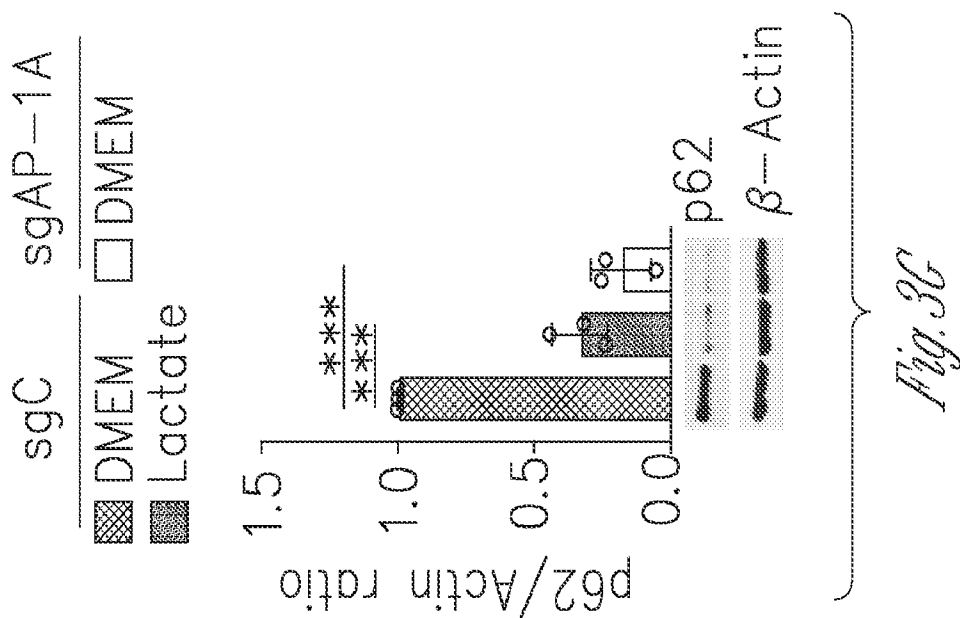
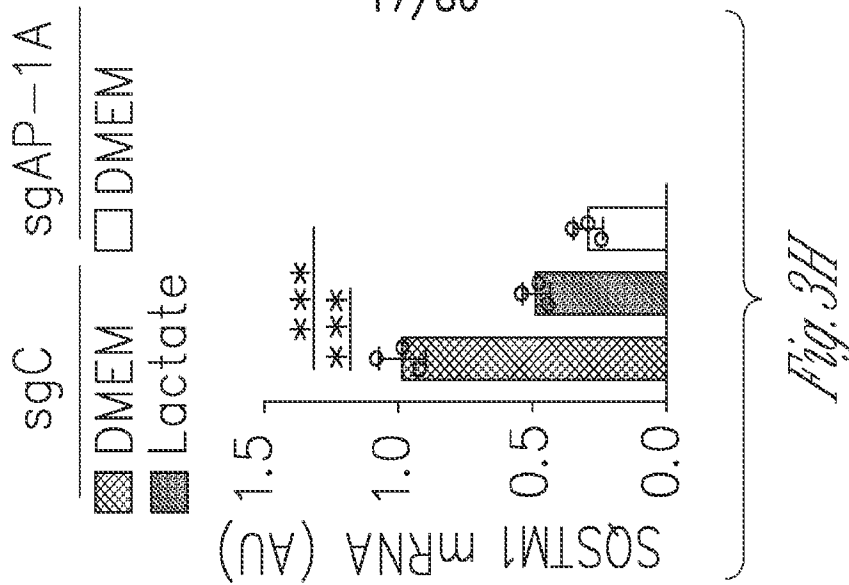
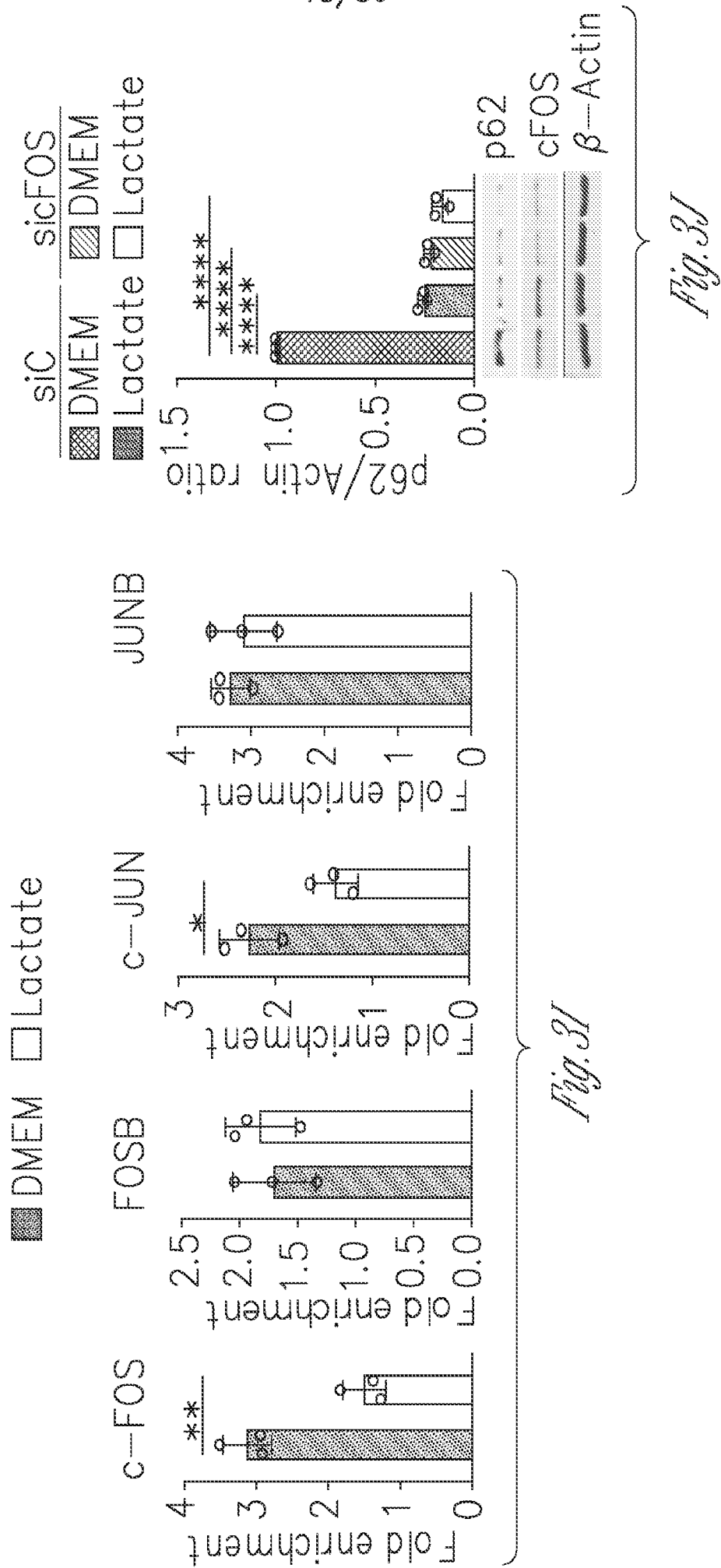
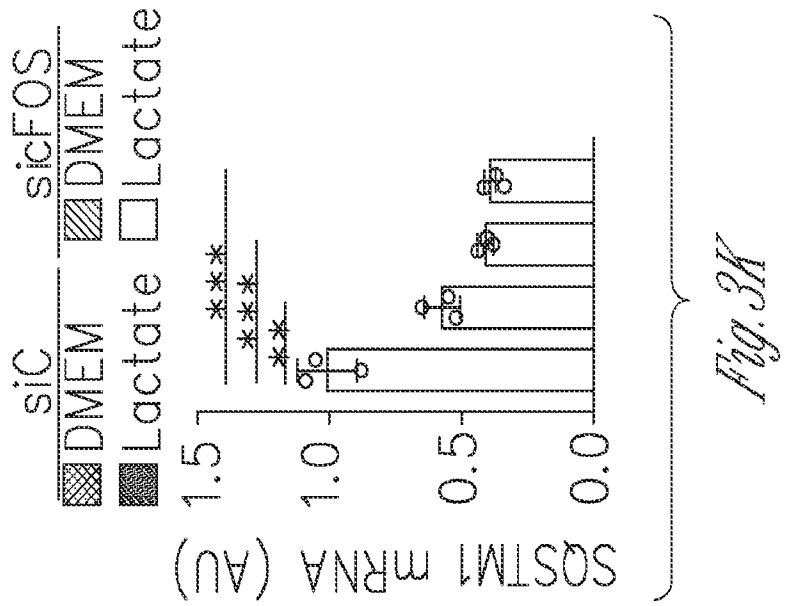
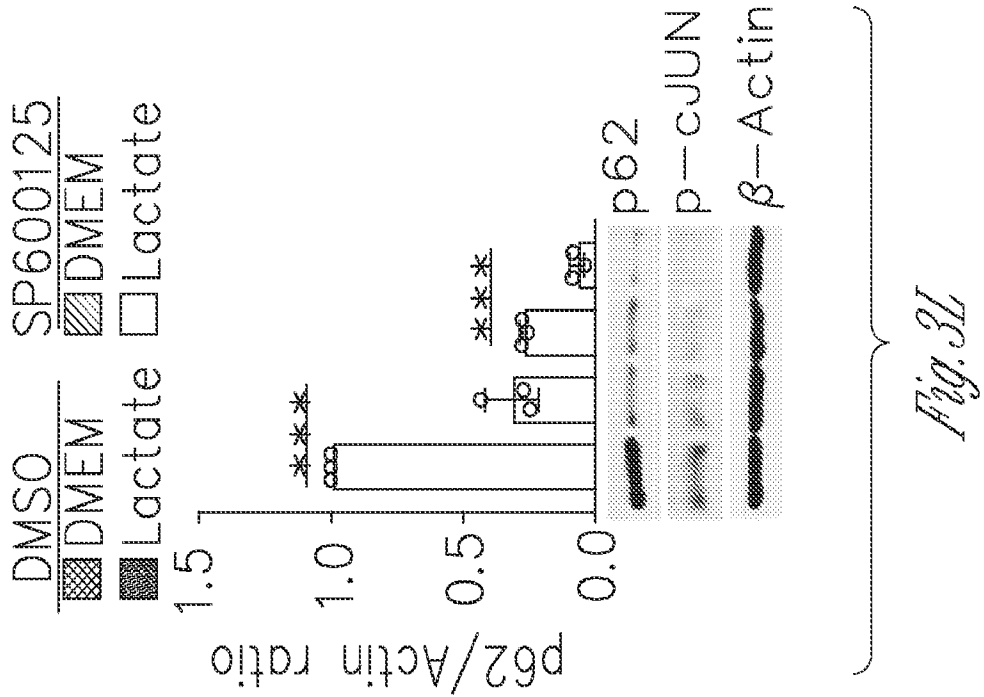
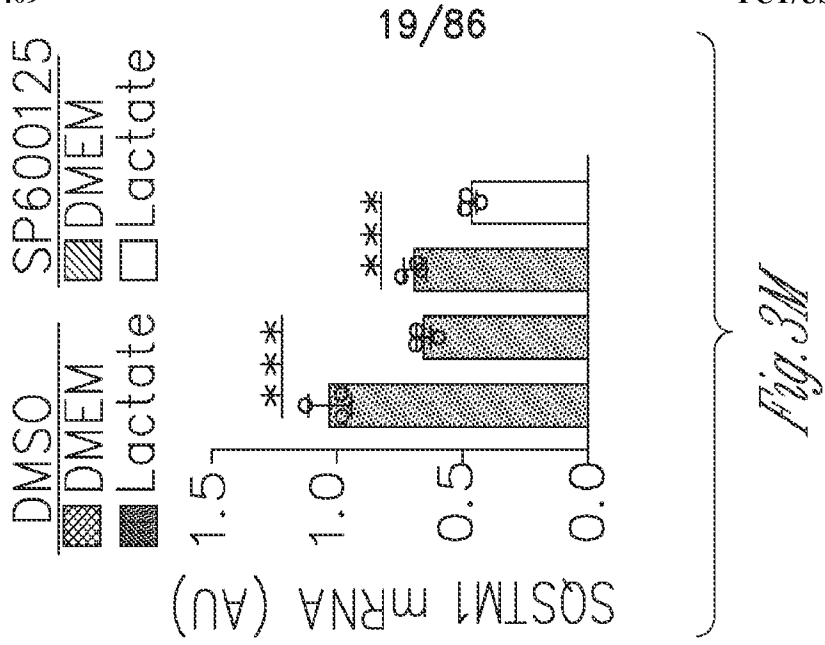


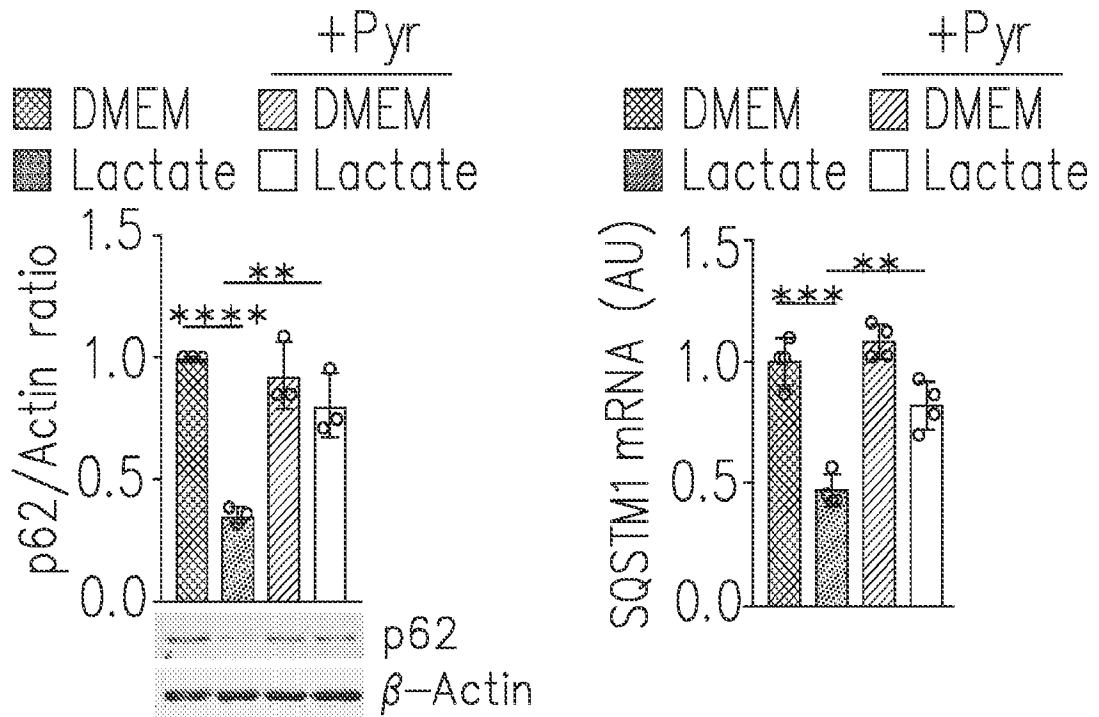
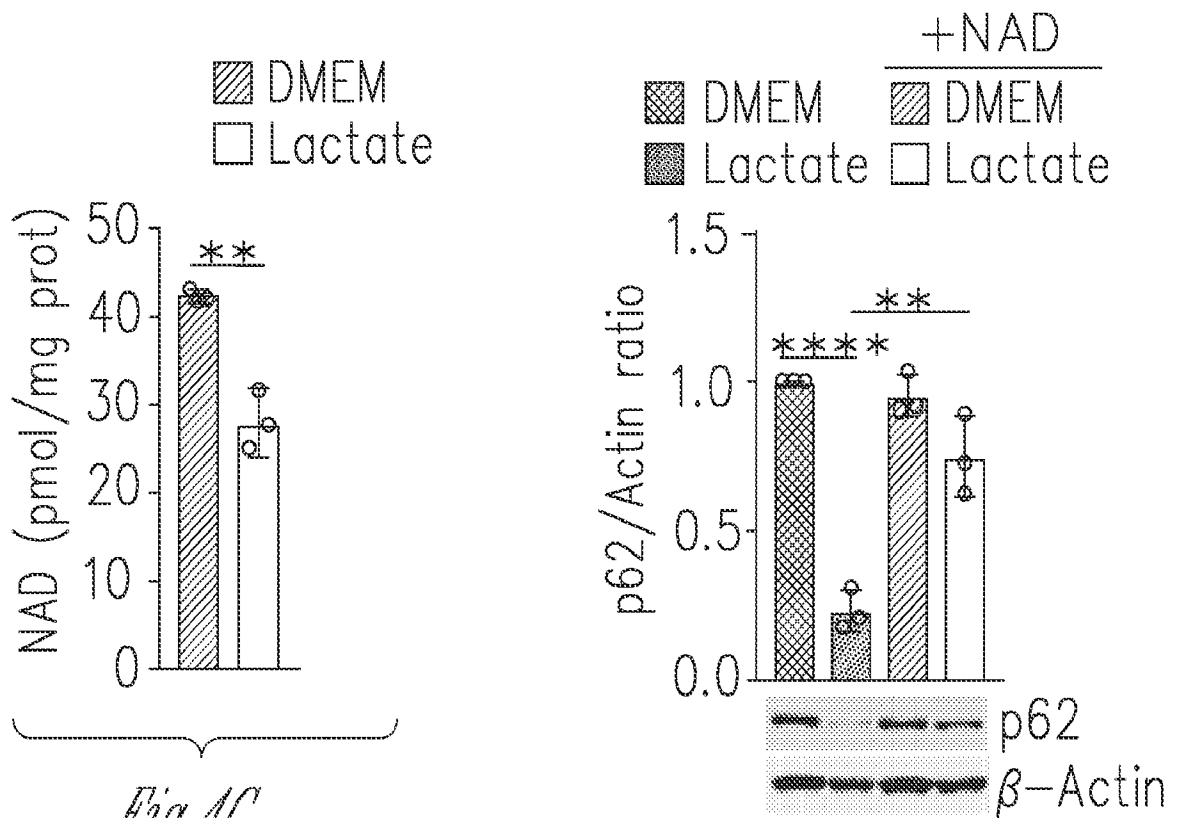
Fig. 3C

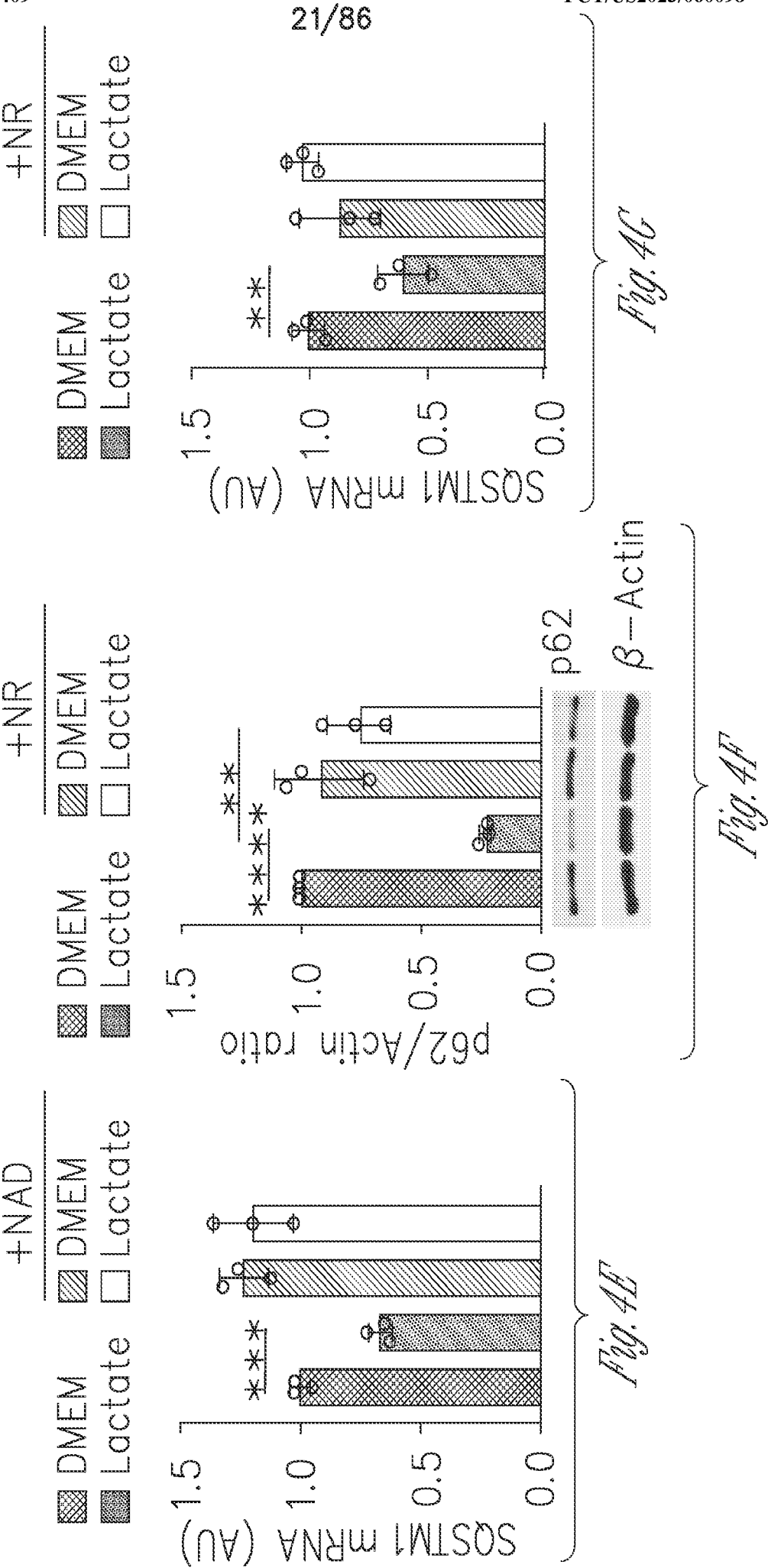


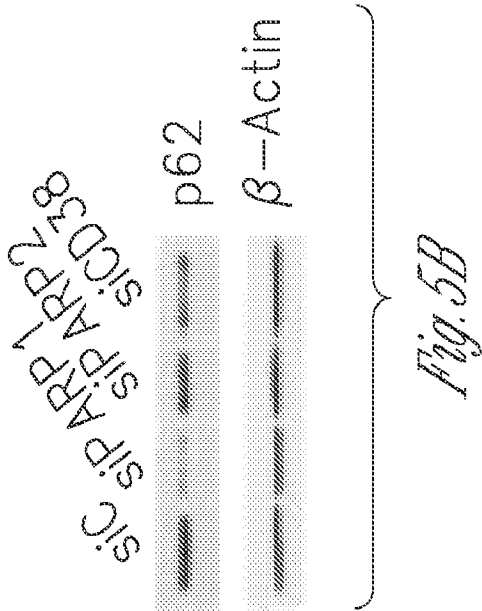
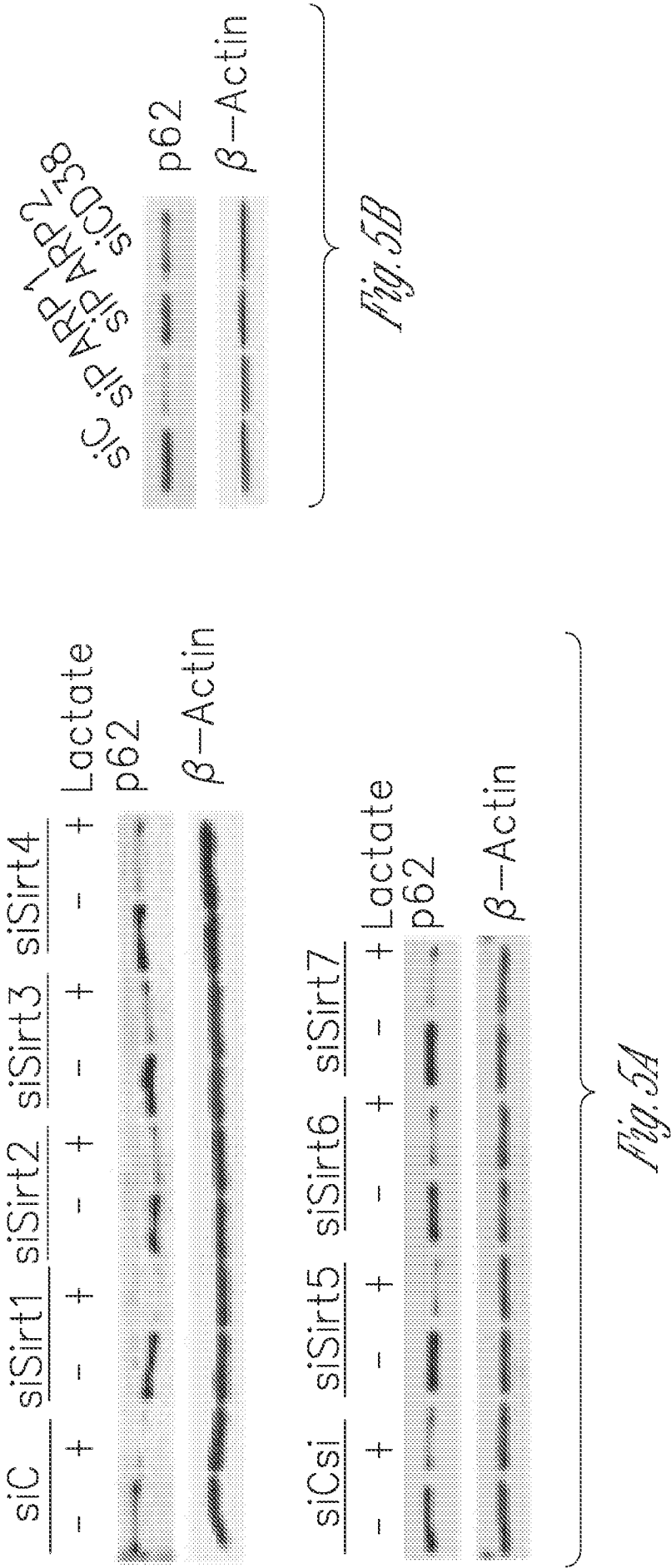






*Fig. 4A**Fig. 4B**Fig. 4C**Fig. 4D*





siC siPARP1

DMEM DMEM

Lactate Lactate

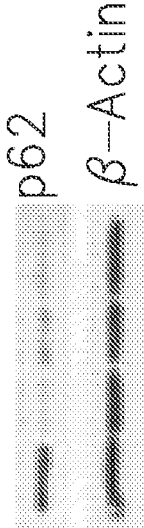
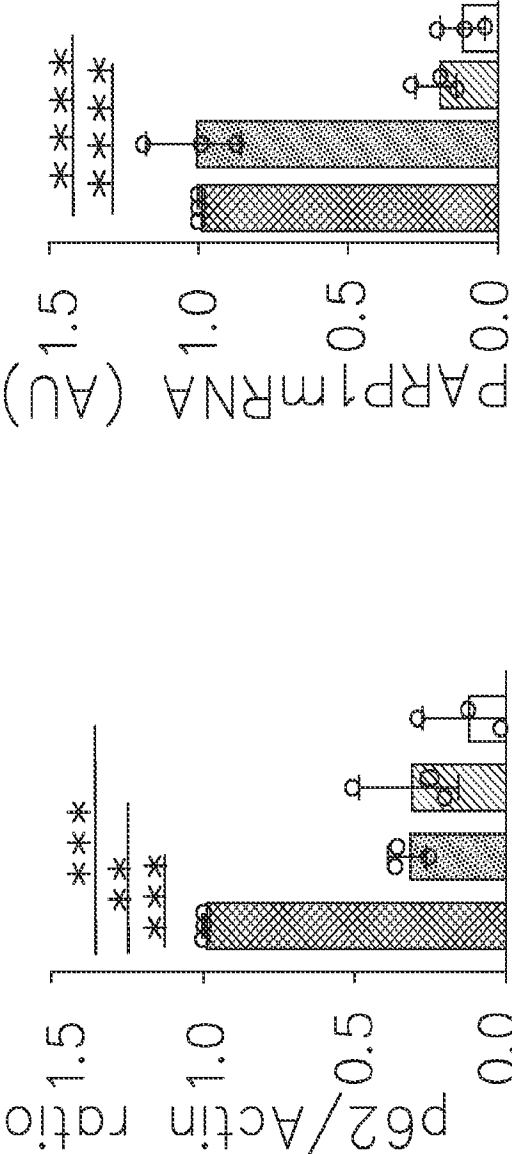


Fig. 5D

siC siPARP2

siPARP1 siCD38

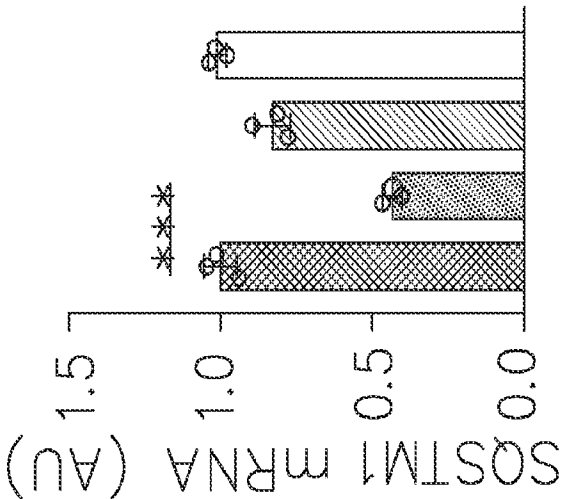
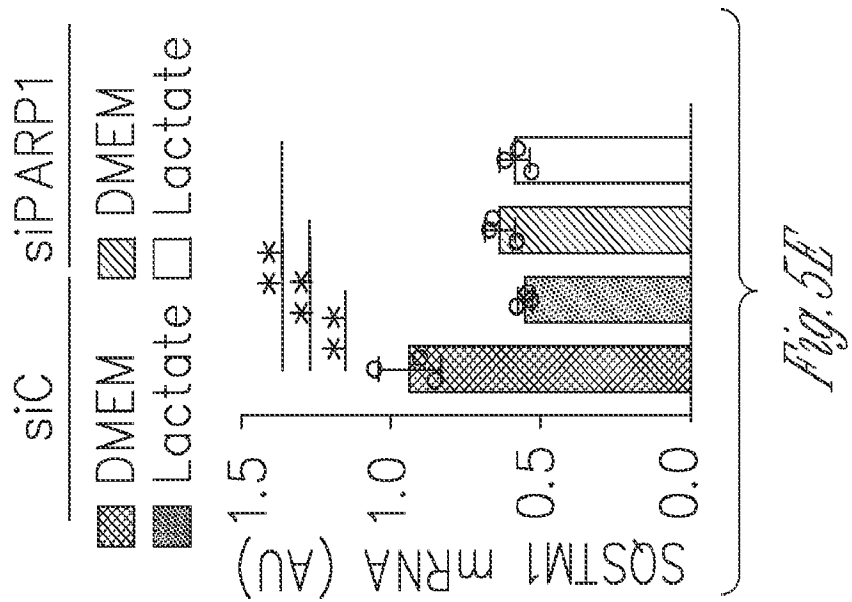
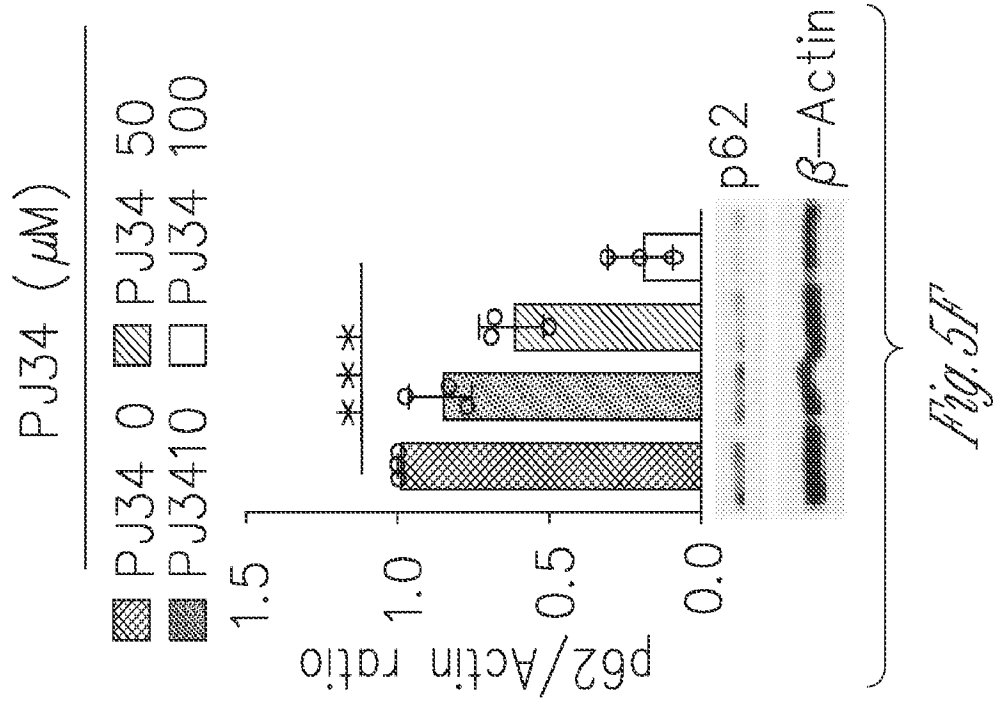
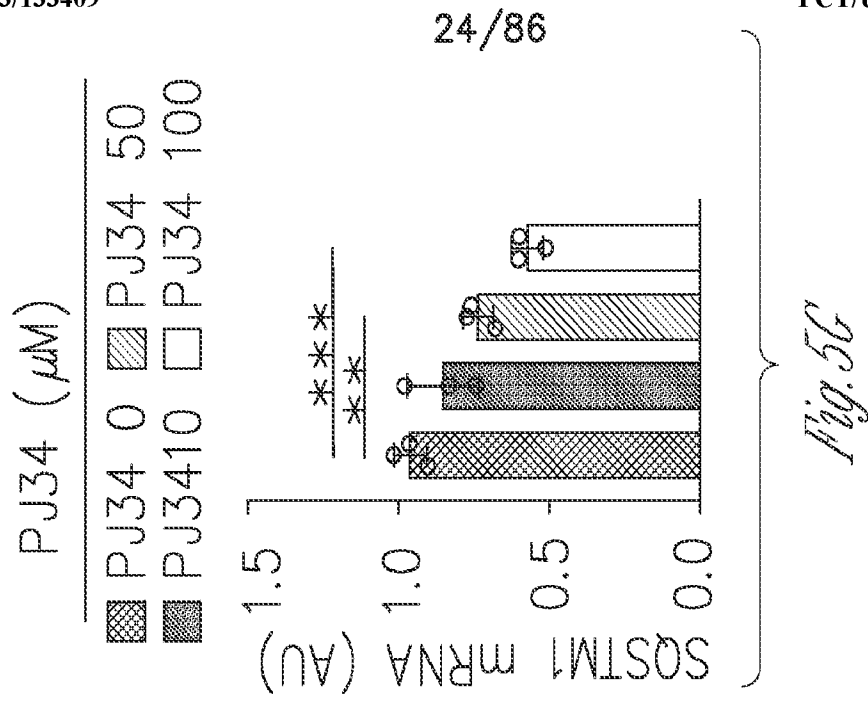
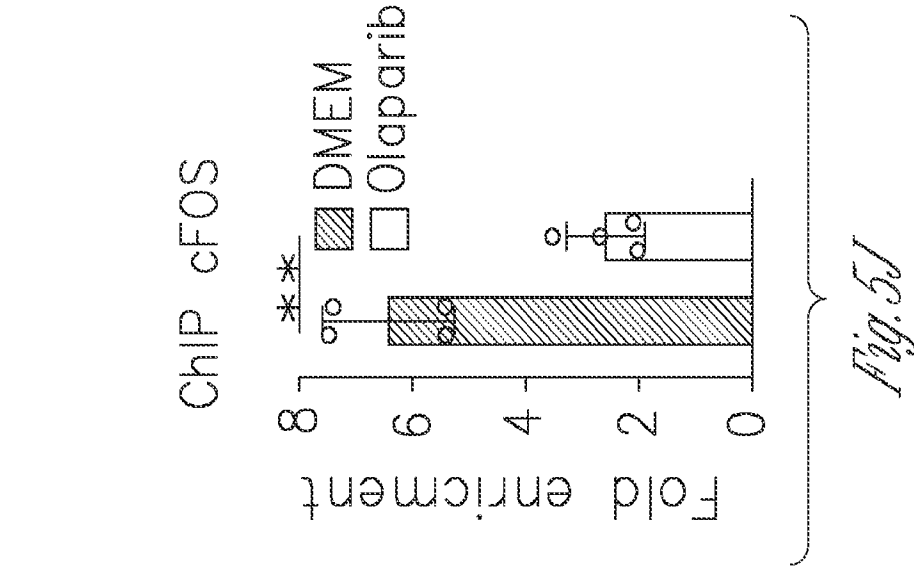
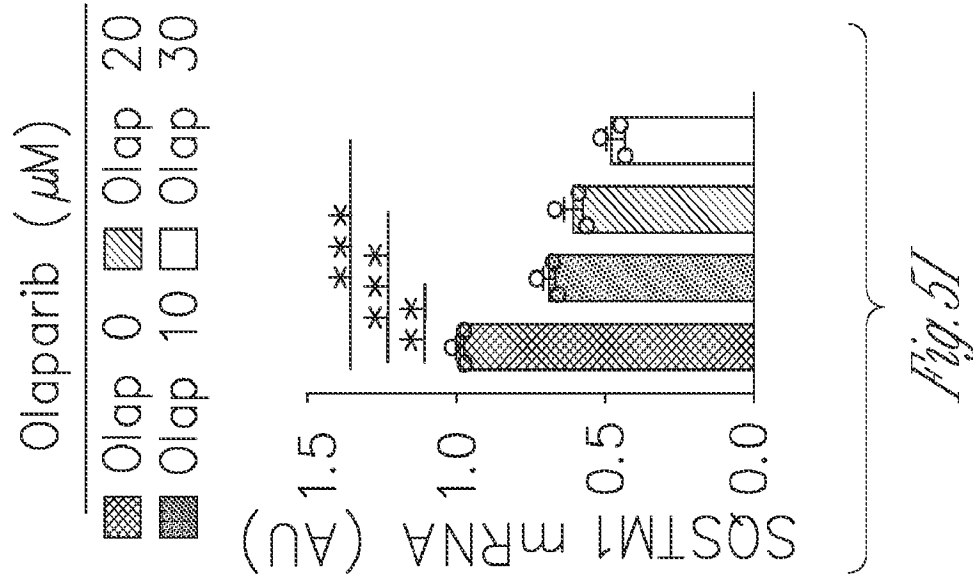
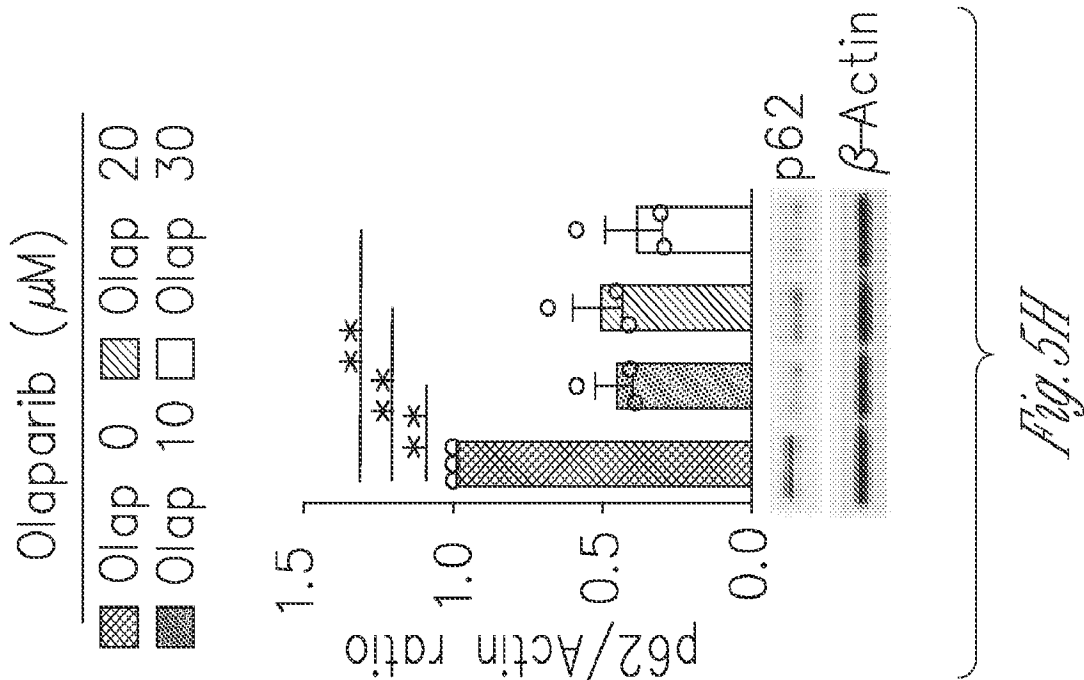
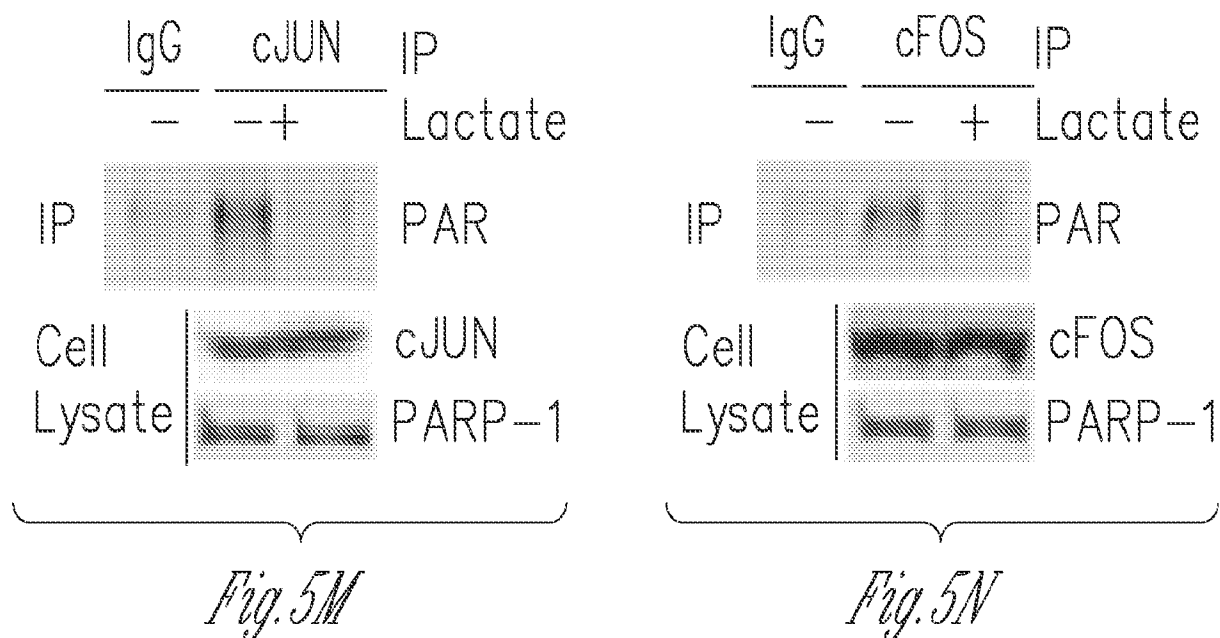
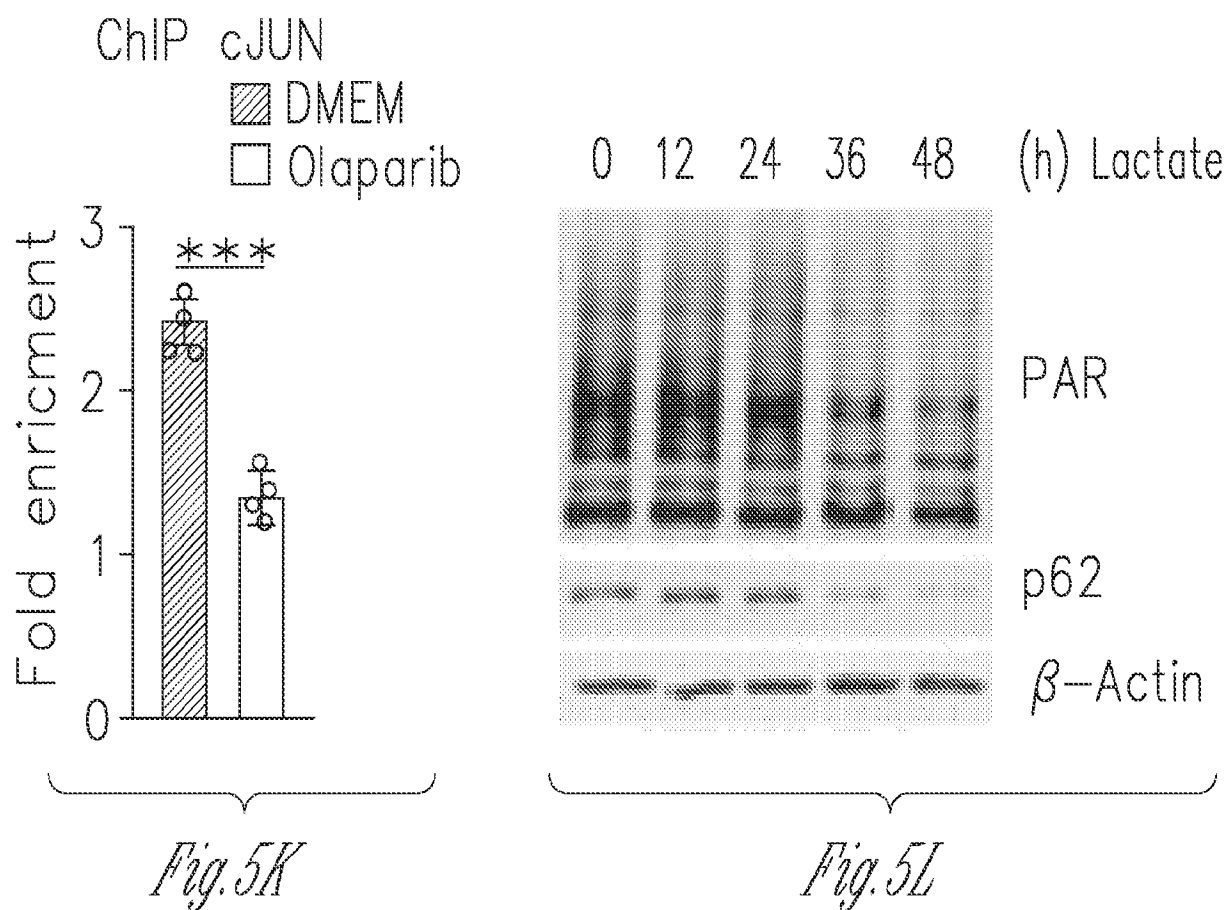
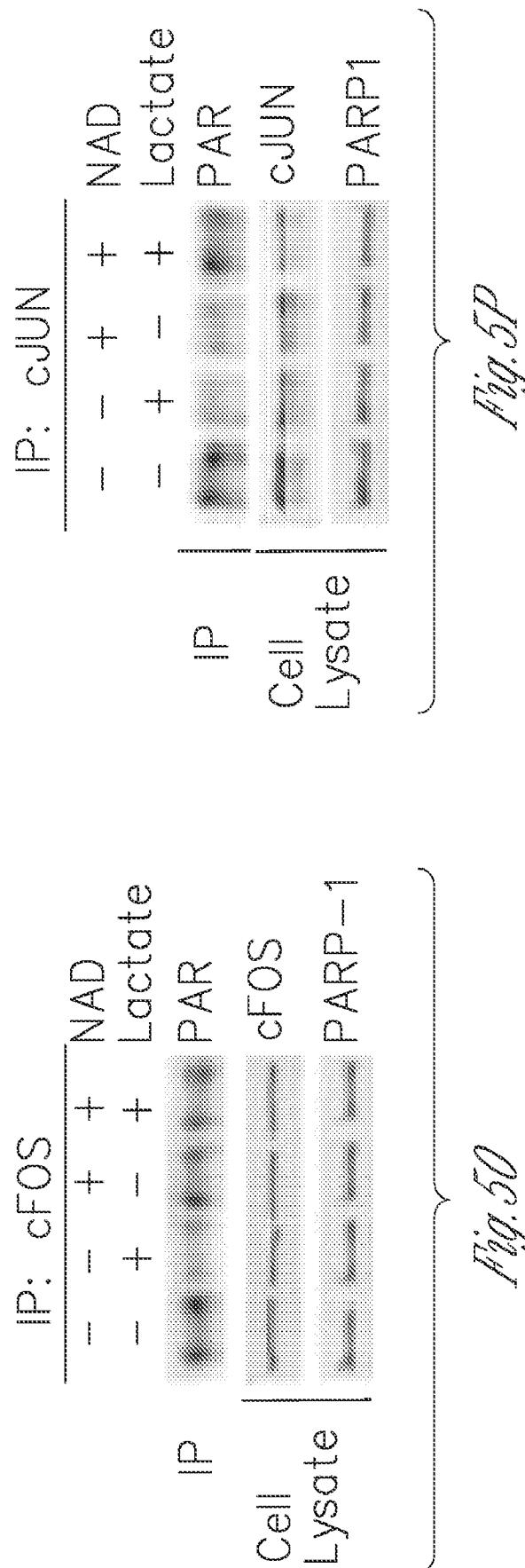


Fig. 5E

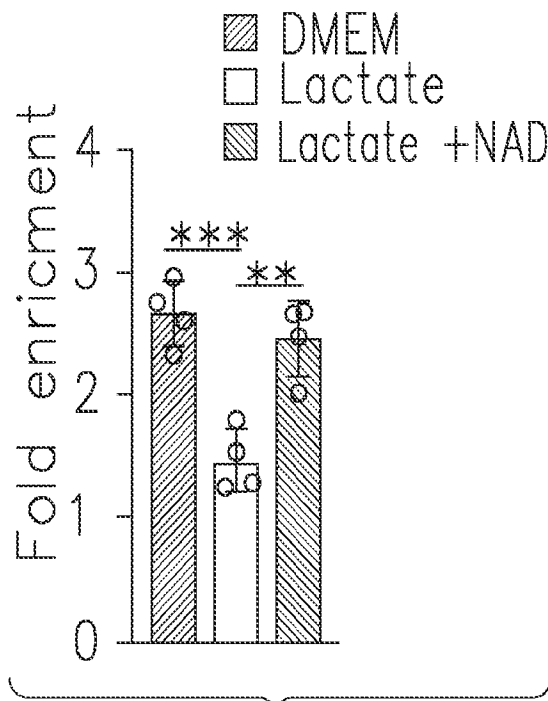




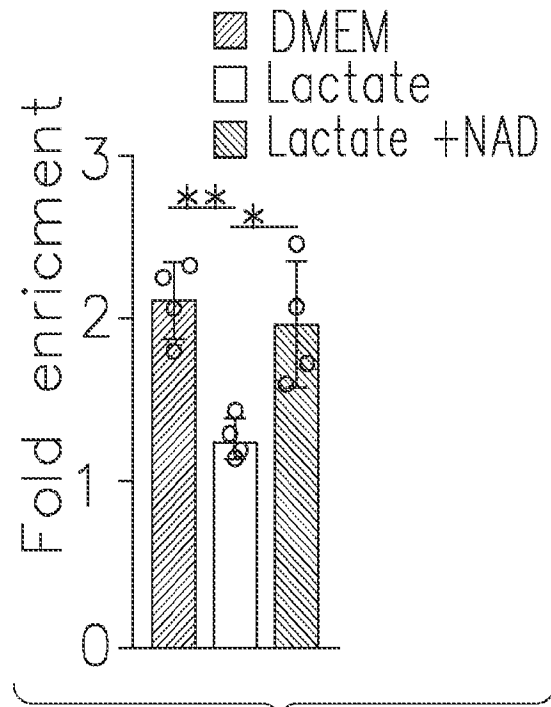




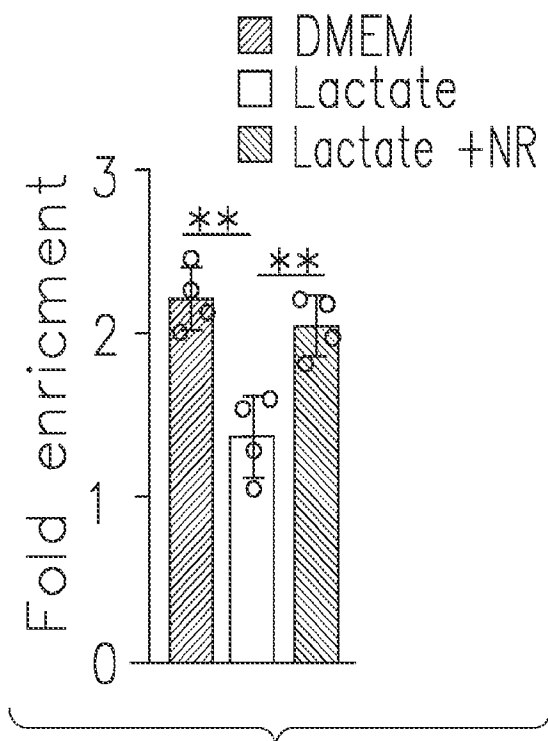
ChIP cFOS

*Fig. 5Q*

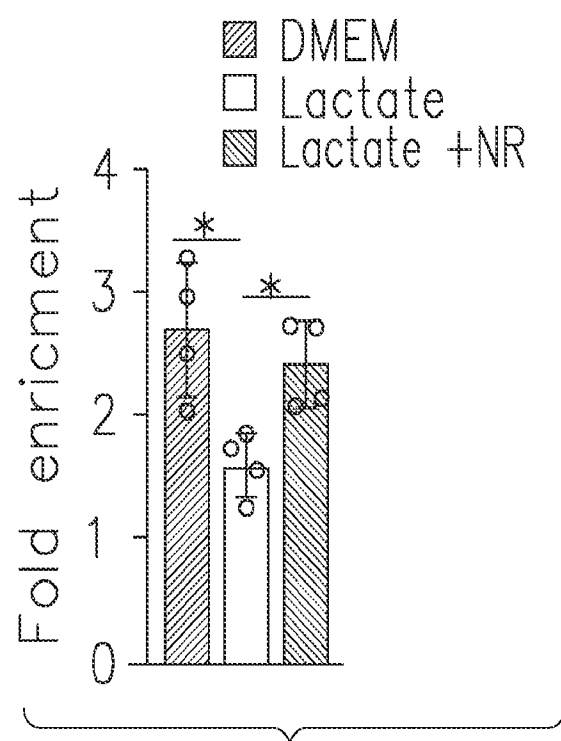
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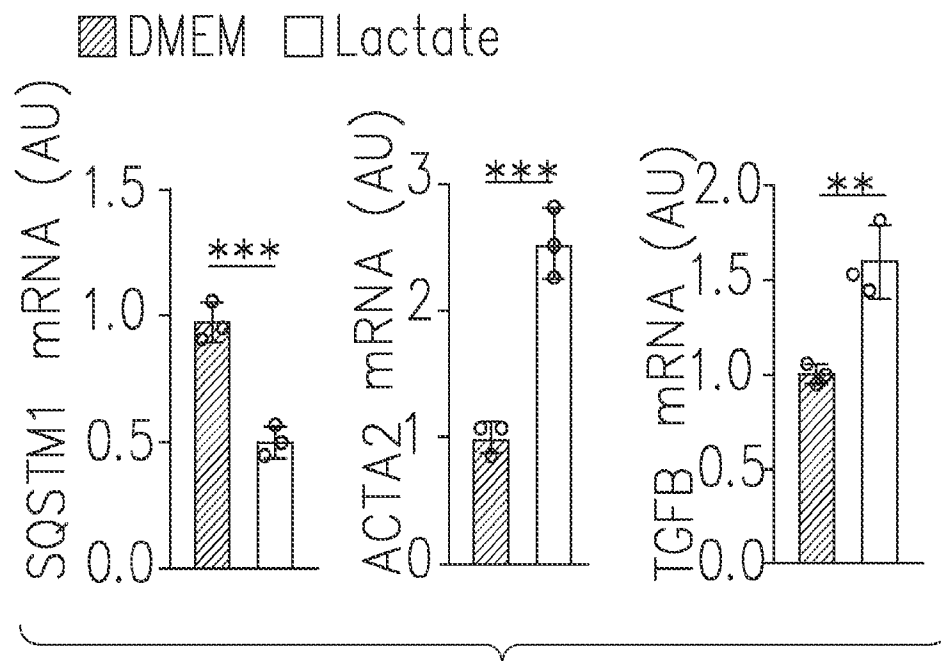
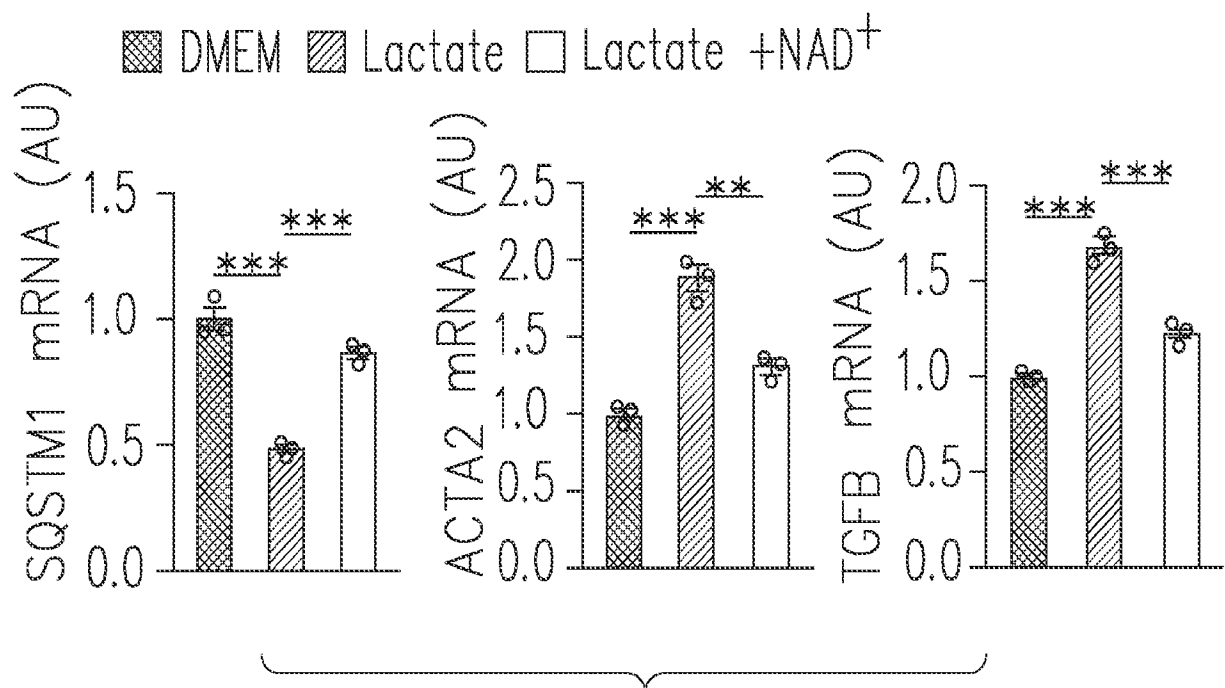
*Fig. 5R*

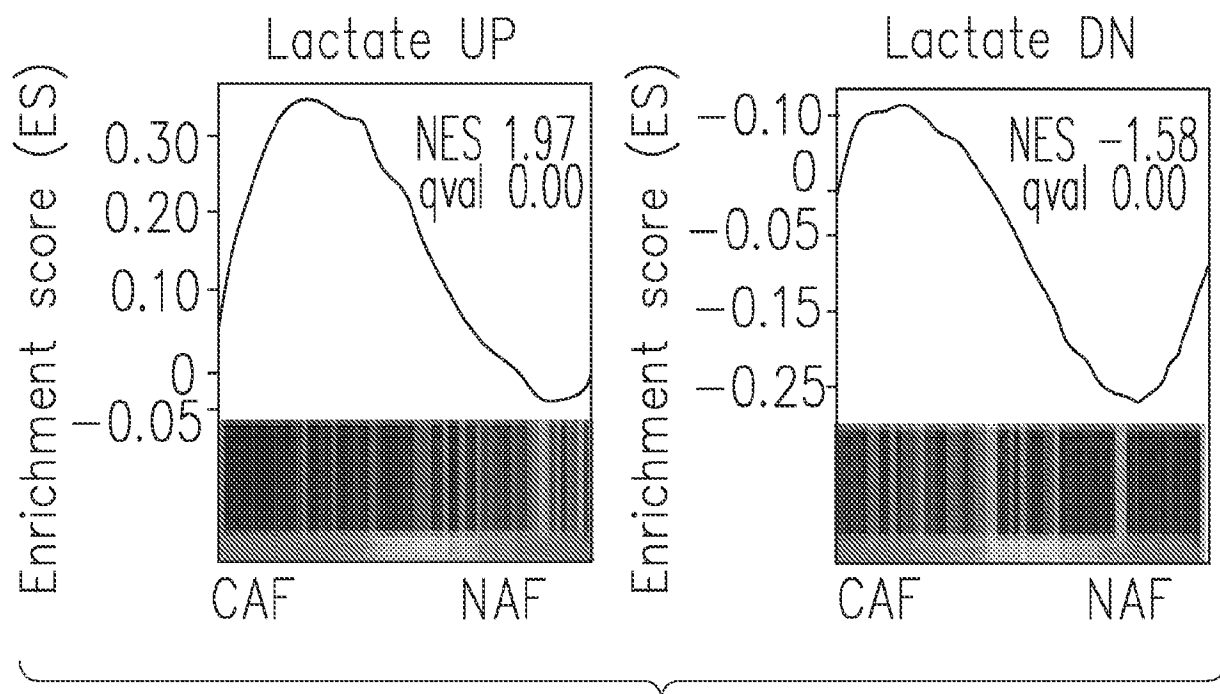
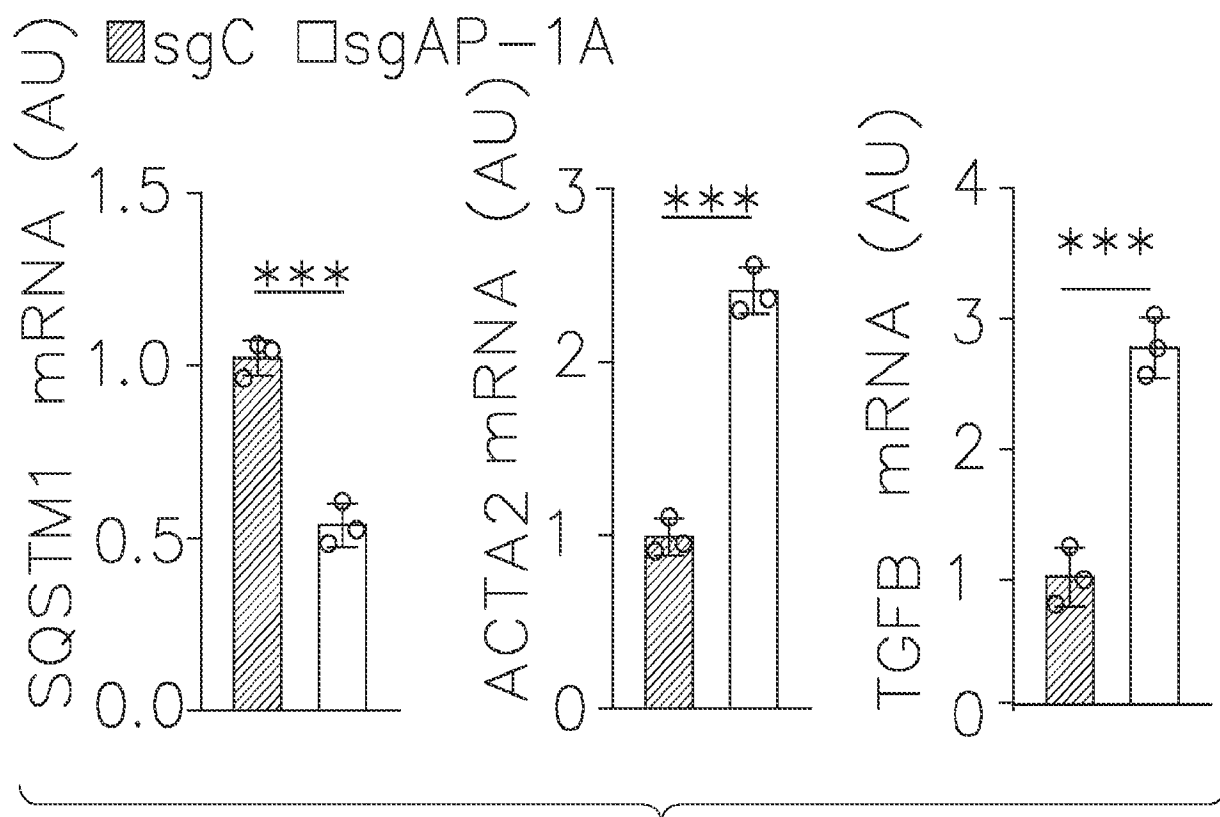
ChIP cFOS

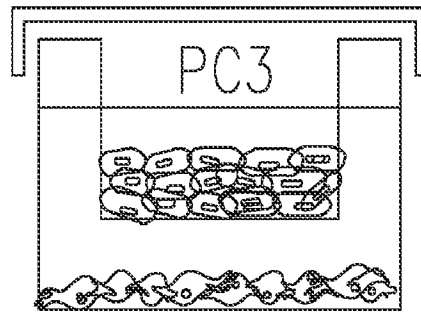
*Fig. 5S*

ChIP cJUN

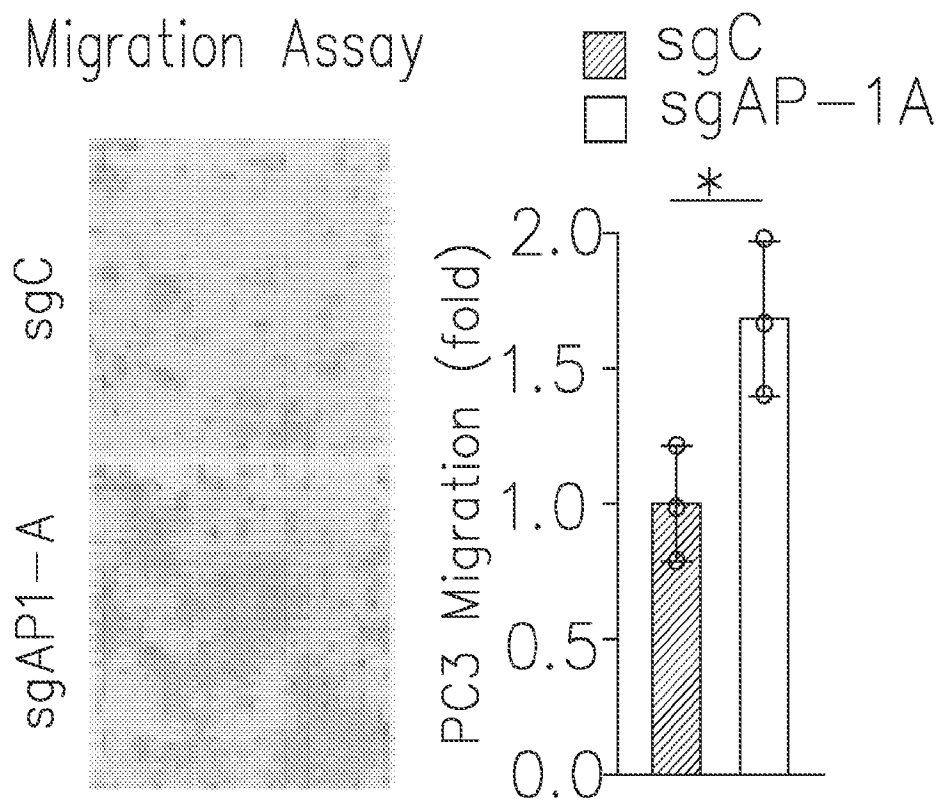
*Fig. 5T*

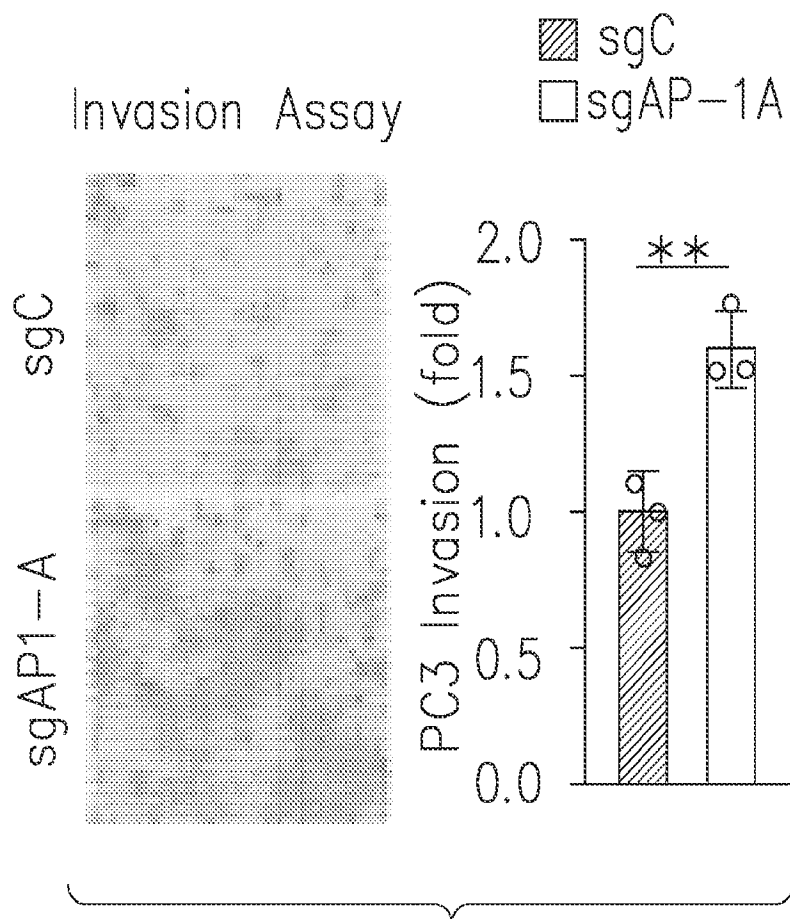
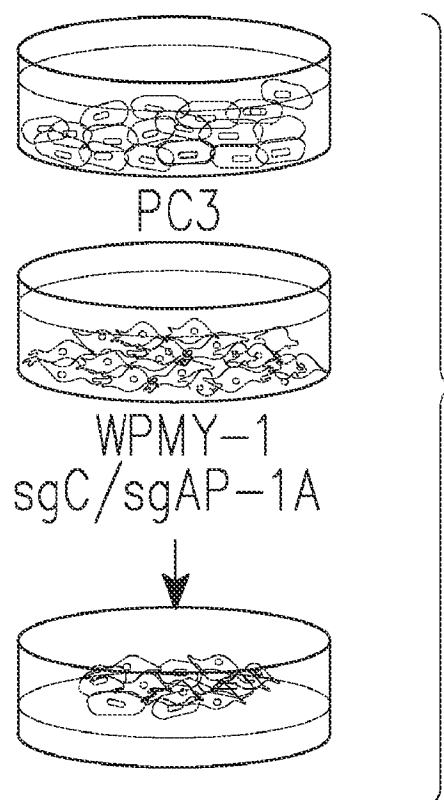
*Fig. 6A**Fig. 6B*

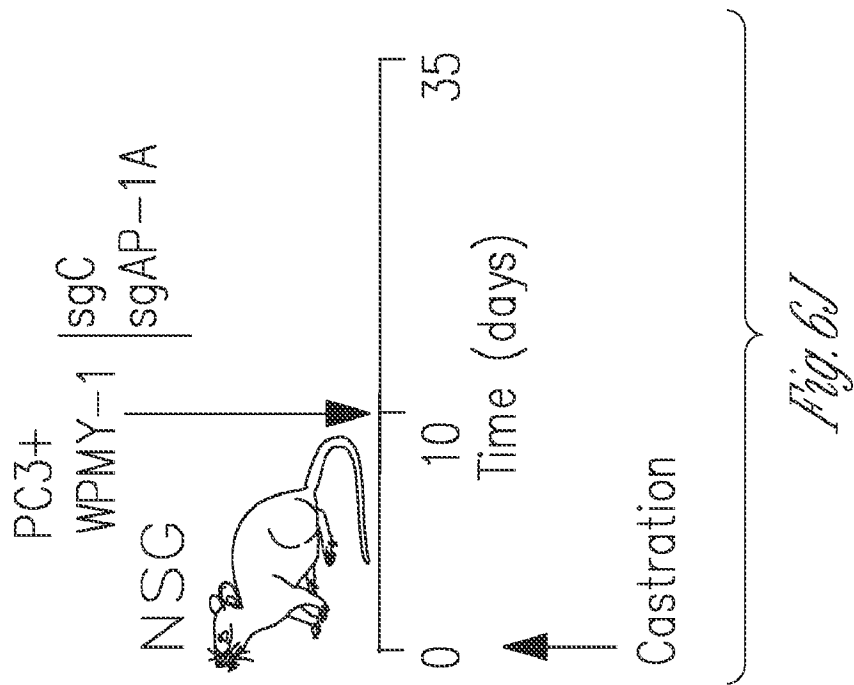
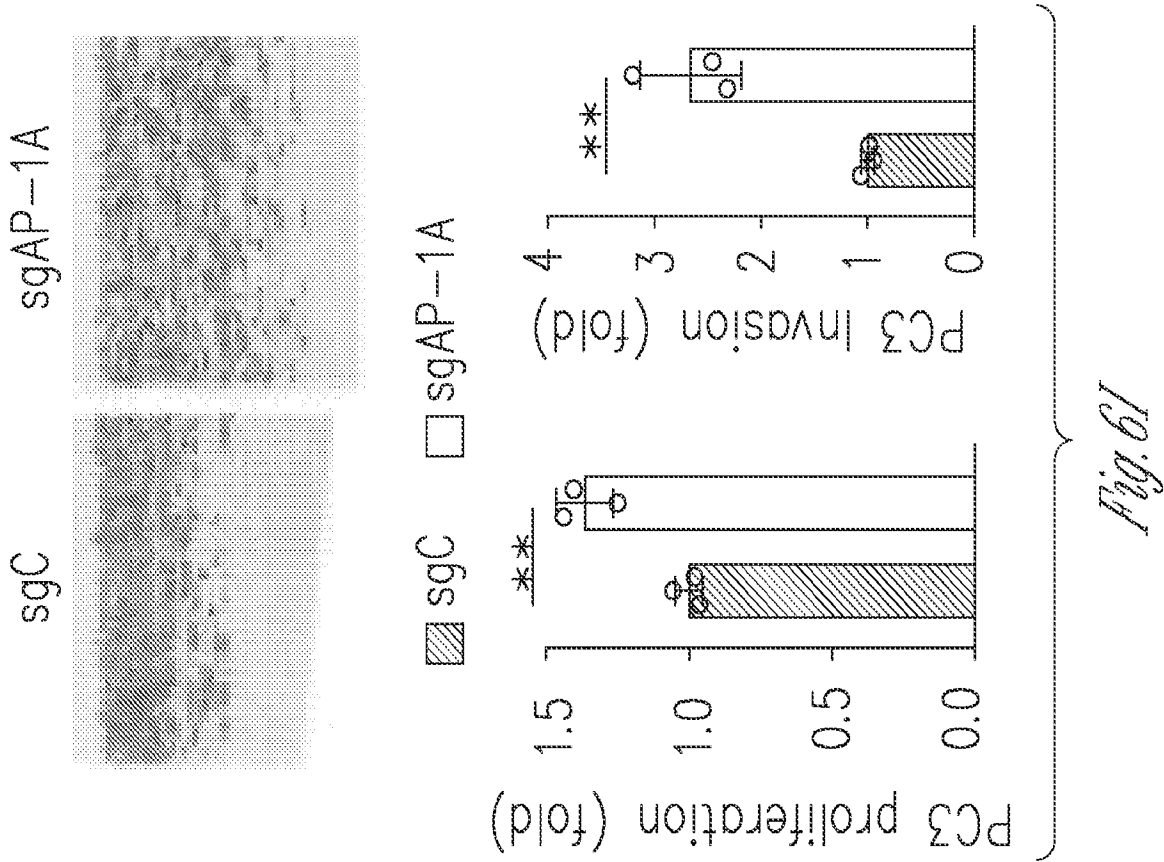
*Fig. 6C**Fig. 6D*

Migration/Invasion
assayWPMY-1
sgC/sgAP-1A*Fig. 6E*

Migration Assay

*Fig. 6F*

*Fig. 6G**Fig. 6H*



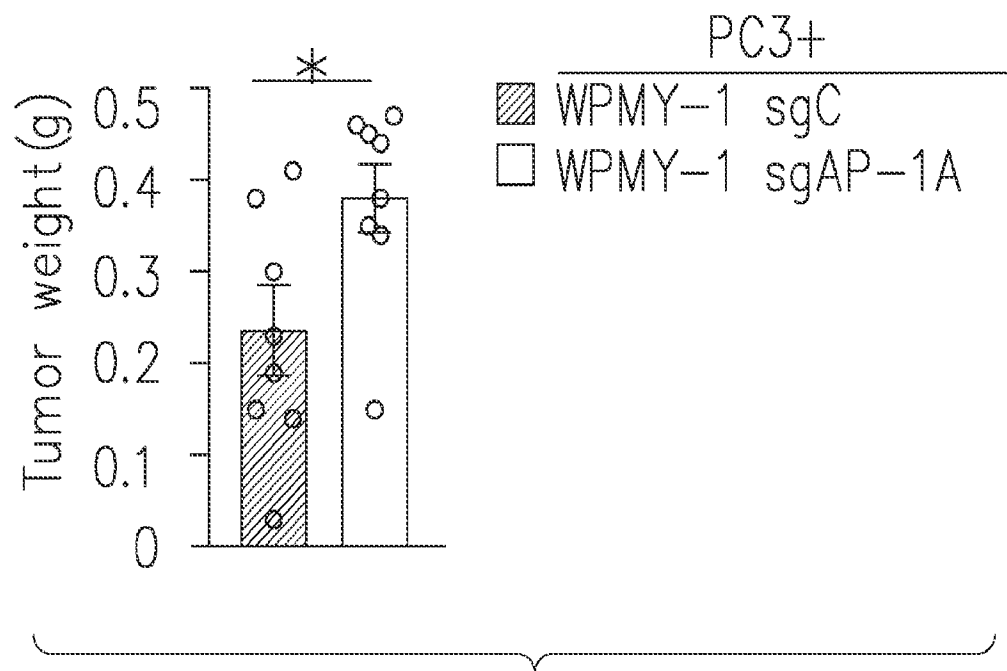


Fig. 6K

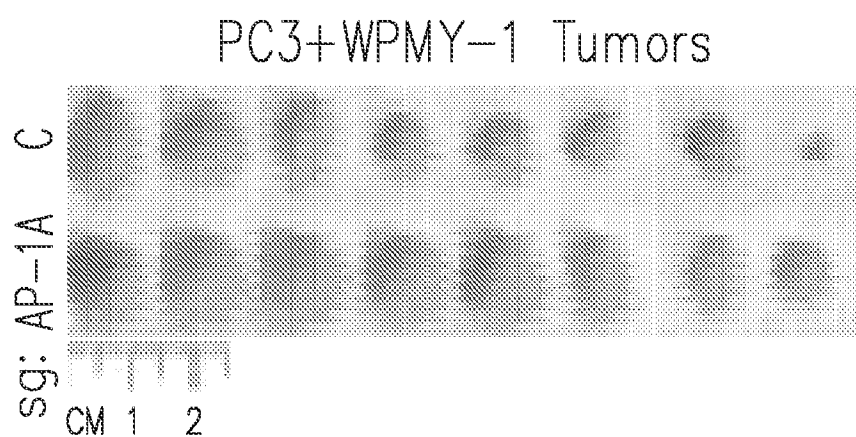


Fig. 6L

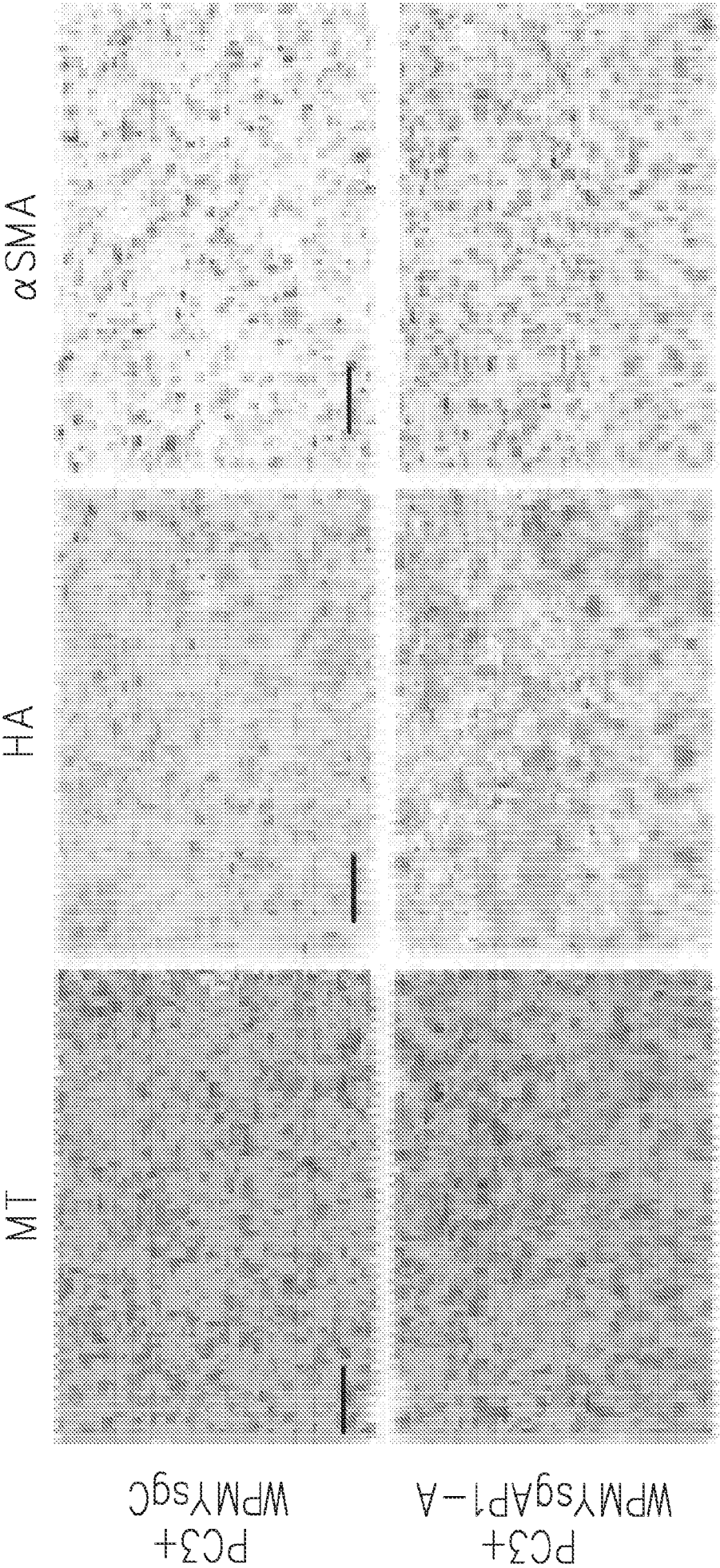


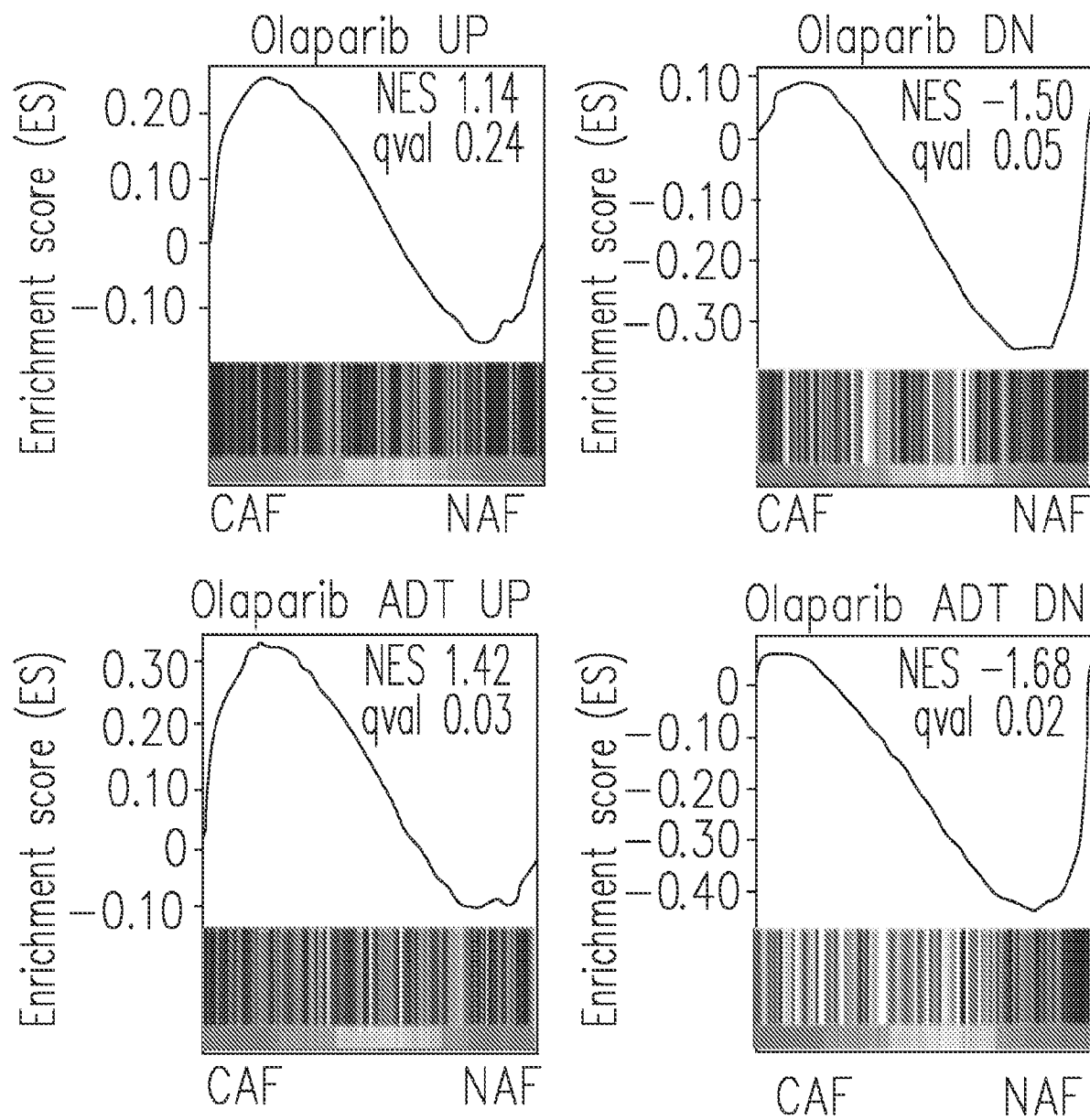
Fig. 6M

Signature		NES	FDR
GO Collagen containing extracellular matrix	Basal	3.00	0.00
	ADT	2.64	0.00
GO Extracellular matrix structural constituent	Basal	2.34	0.02
	ADT	2.15	0.07
GO External encapsulating structure	Basal	3.22	0.00
	ADT	2.75	0.03
GO Connective tissue development	Basal	1.90	0.10
	ADT	NA	NA
GO Cartilage development	Basal	1.57	0.24
	ADT	NA	NA
GO Collagen trimer	Basal	NA	NA
	ADT	1.80	0.19
GO Muscle contraction	Basal	NA	NA
	ADT	1.76	0.21
REACTOME Extracellular matrix organization	Basal	2.02	0.01
	ADT	2.35	0.00
REACTOME Collagen biosynthesis and modifying enzymes	Basal	1.49	0.14
	ADT	1.57	0.12
REACTOME Collagen formation	Basal	1.37	0.22
	ADT	1.46	0.17
REACTOME Assembly of collagen fibrils and other multimeric structures	Basal	NA	NA

Fig. 7A

Olaparib vs Veh			
Signature		NES	FDR
Herrera_CAF (GSE51257)	Basal	1.45	0.082
	ADT	1.42	0.130
Dakhova_Reactive Stroma (GSE9014)	Basal	1.54	0.009
	ADT	1.42	0.062
Tirosh_CAF (GSE103322)	Basal	1.11	NS
	ADT	1.18	0.210
Isella_CAF (GSE56710)	Basal	1.19	0.165
	ADT	1.56	0.002
Loda_Activated Stroma (GSE97284)	Basal	0.97	NS
	ADT	1.29	0.144
Asida_CAF (GSE34312)	Basal	1.35	0.000
	ADT	1.53	0.000
Lee_CAF (GSE132465,GSE132257)	Basal	1.59	0.000
	ADT	1.69	0.001

Fig. 7B

*Fig. 7C*

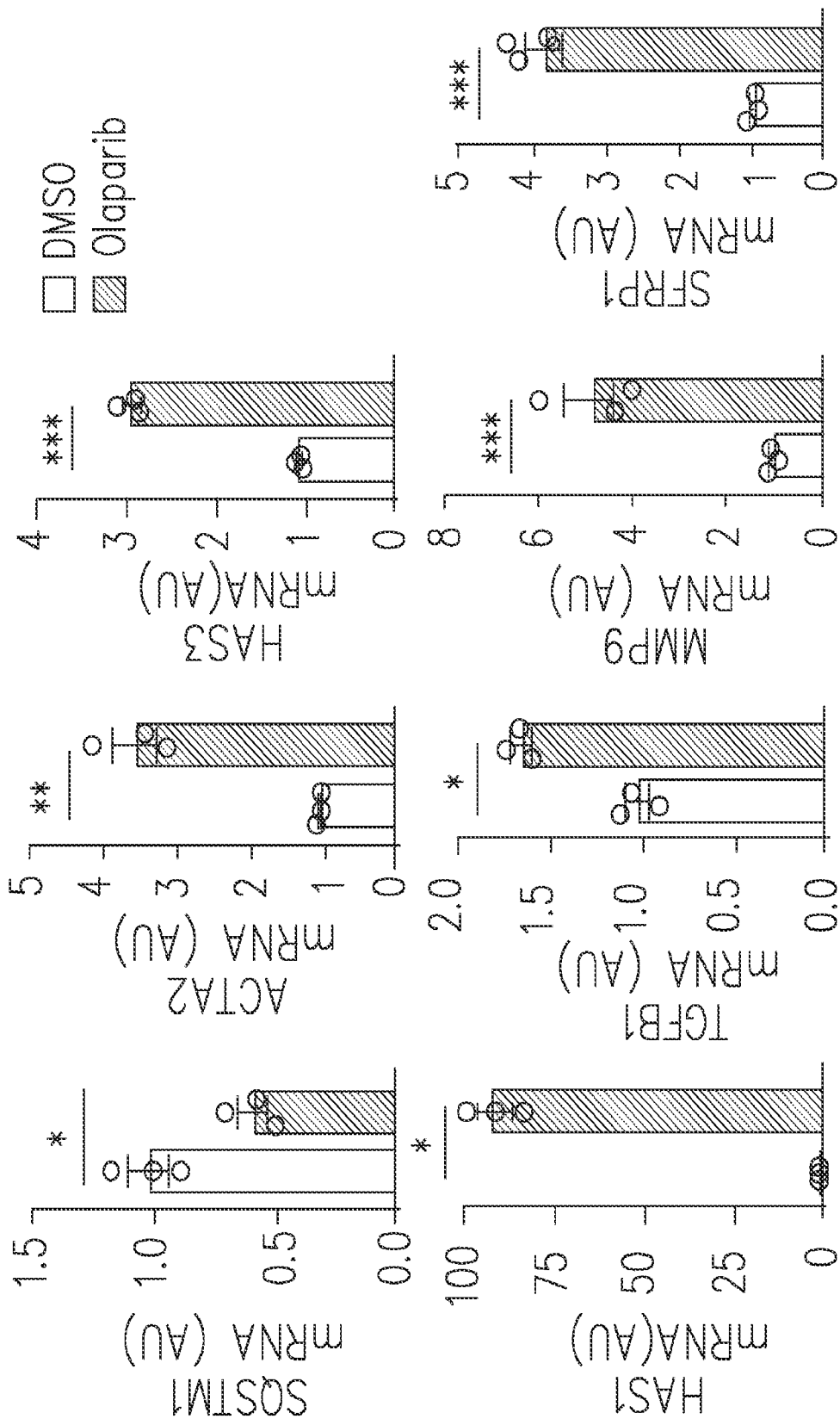


Fig. 7D

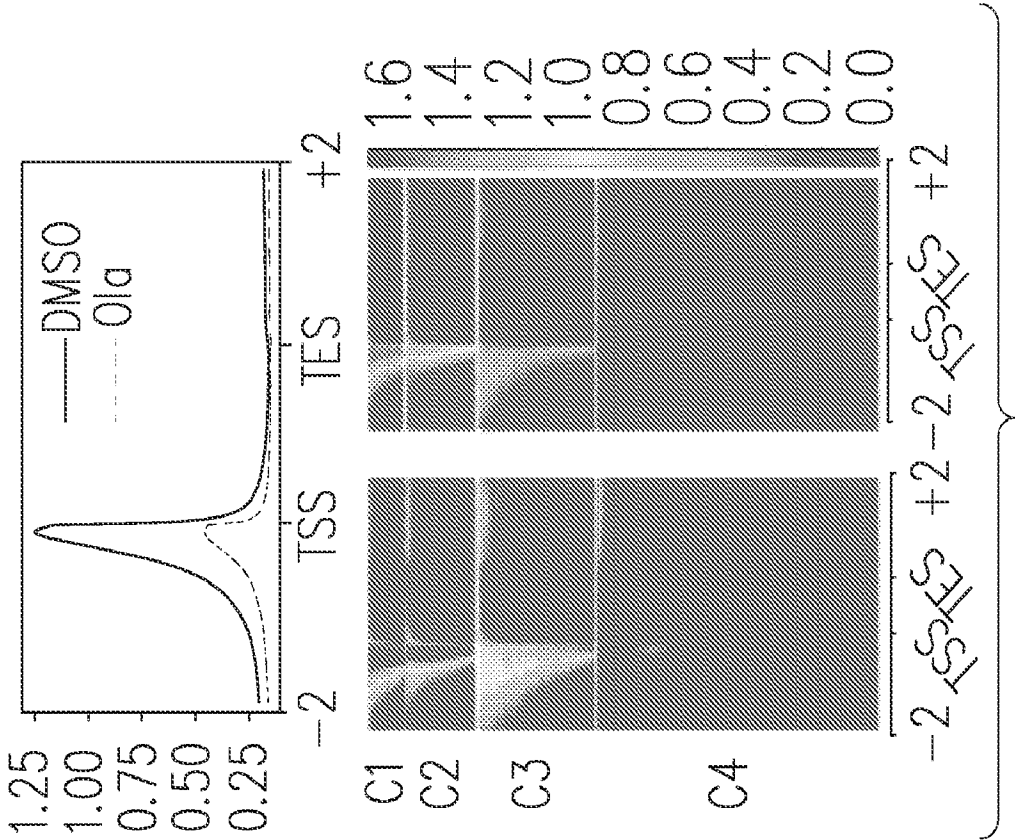


Fig. 7E

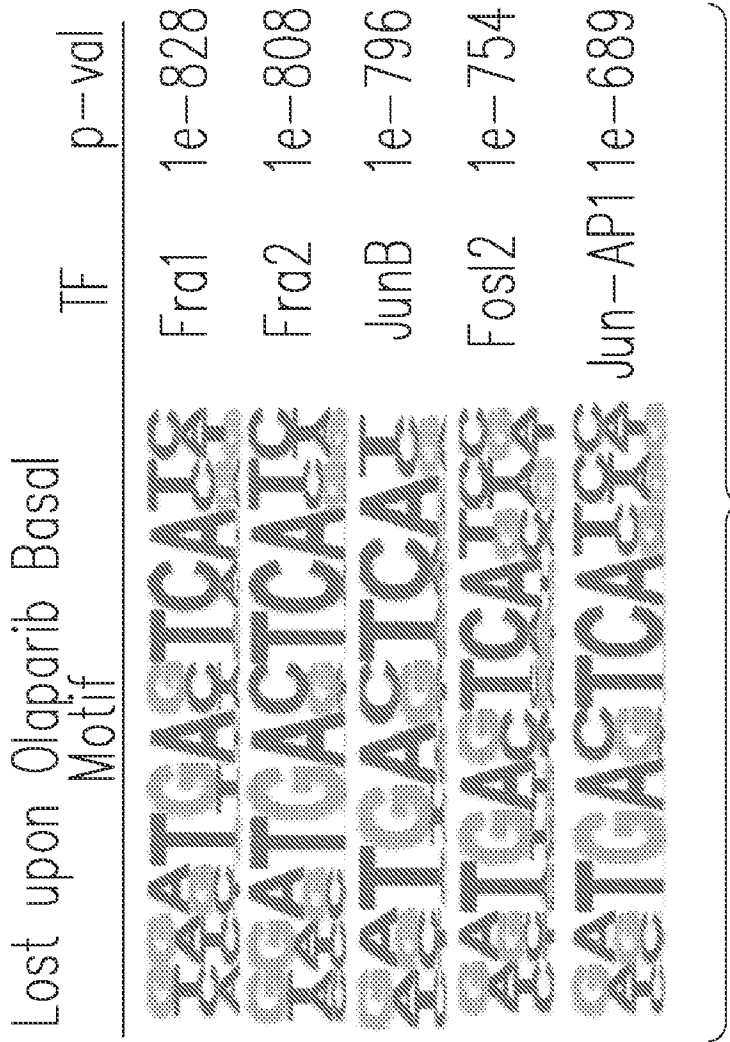
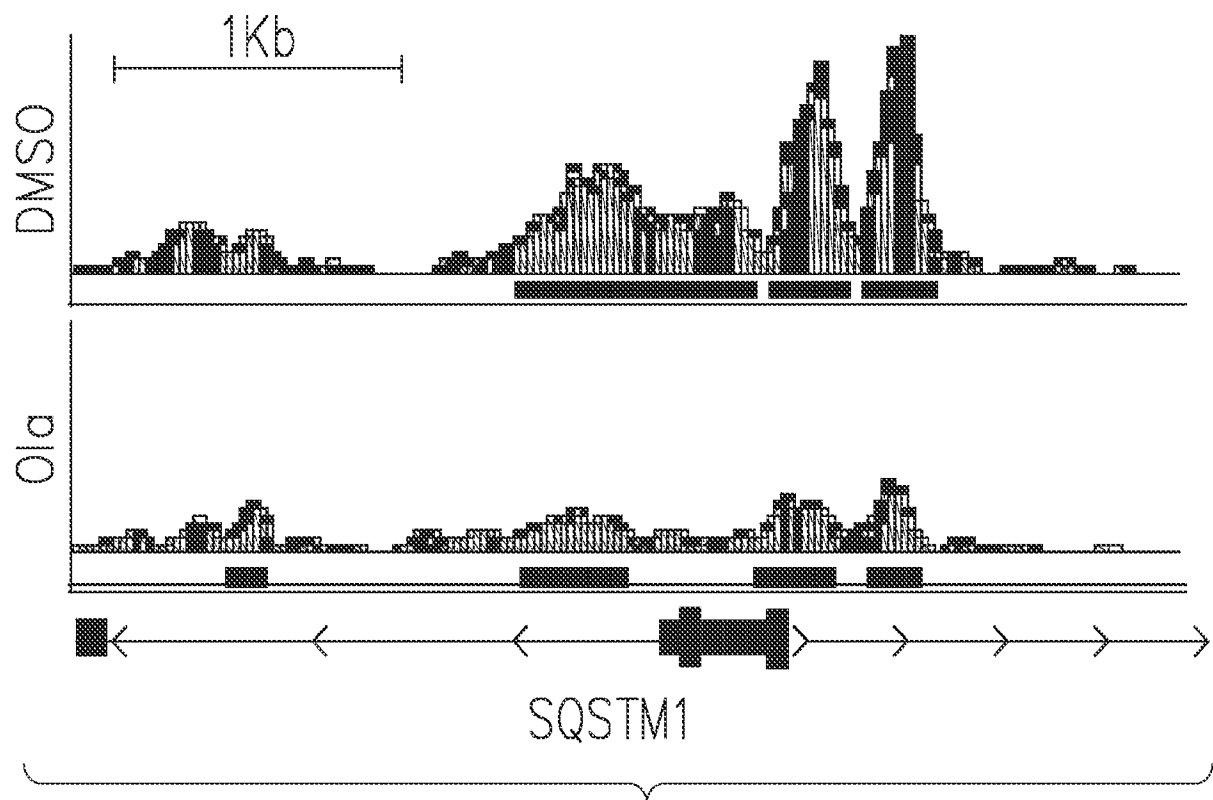
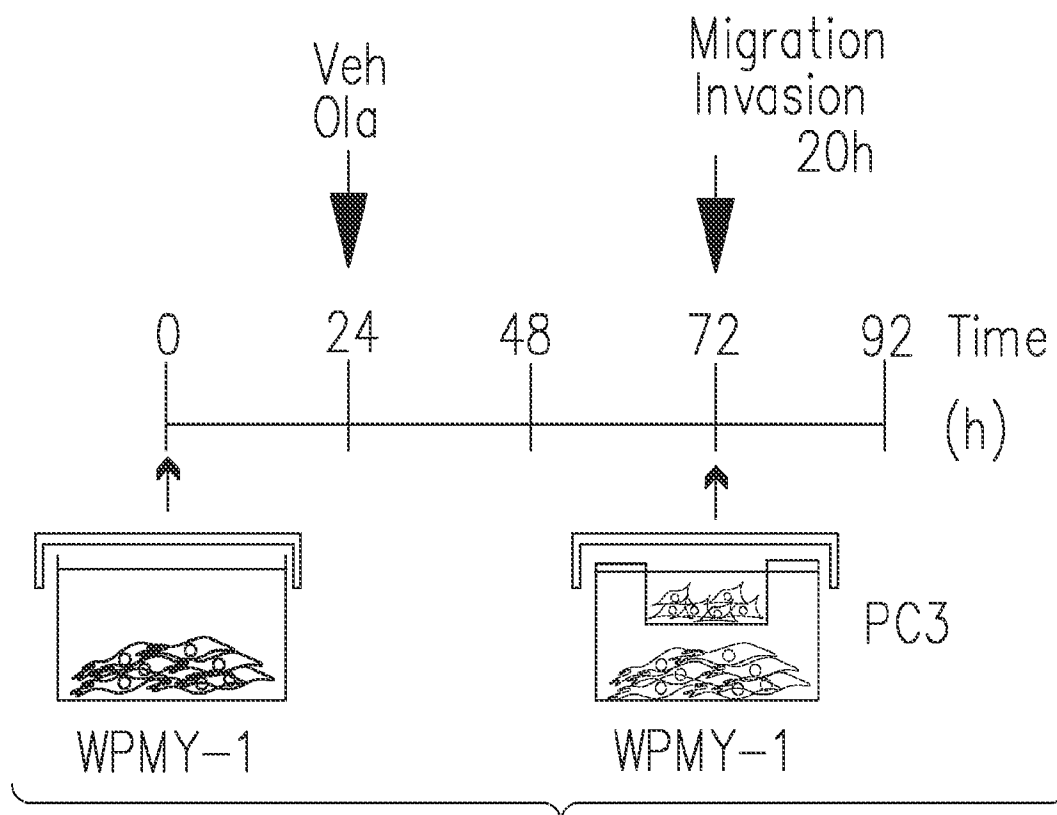
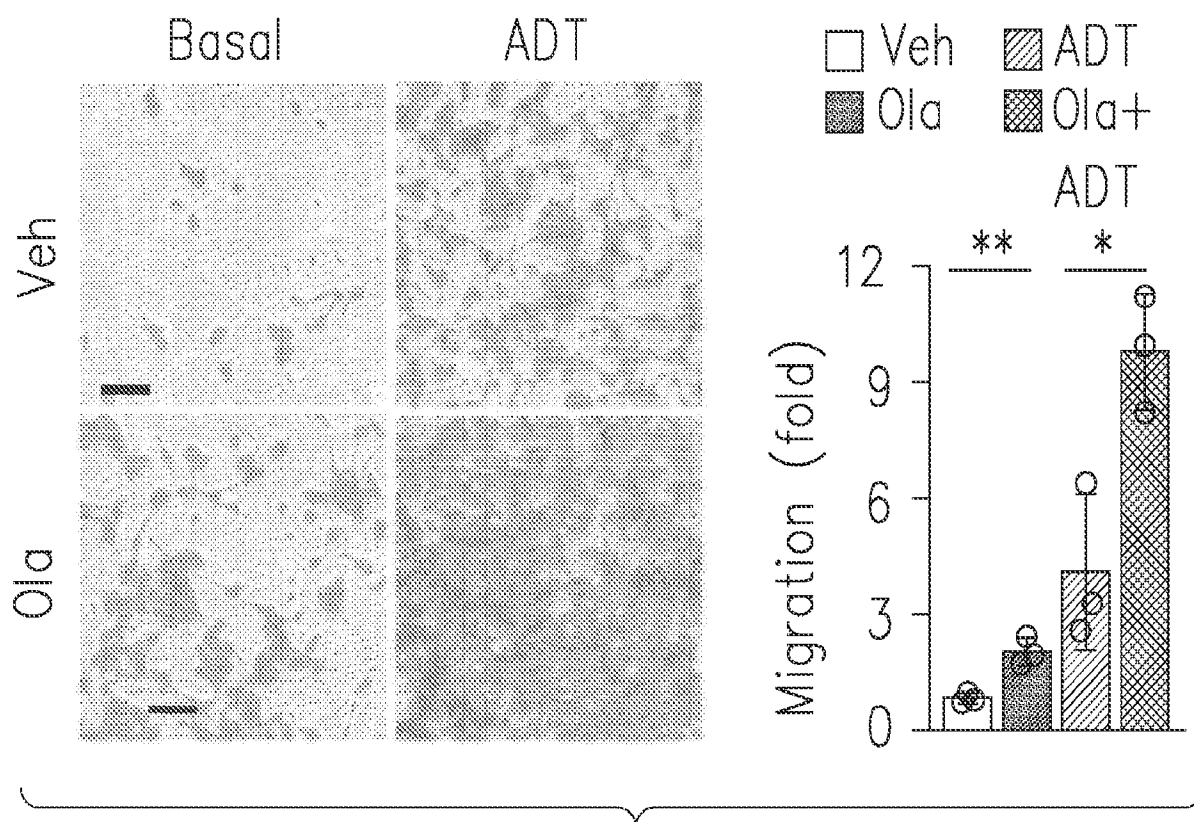
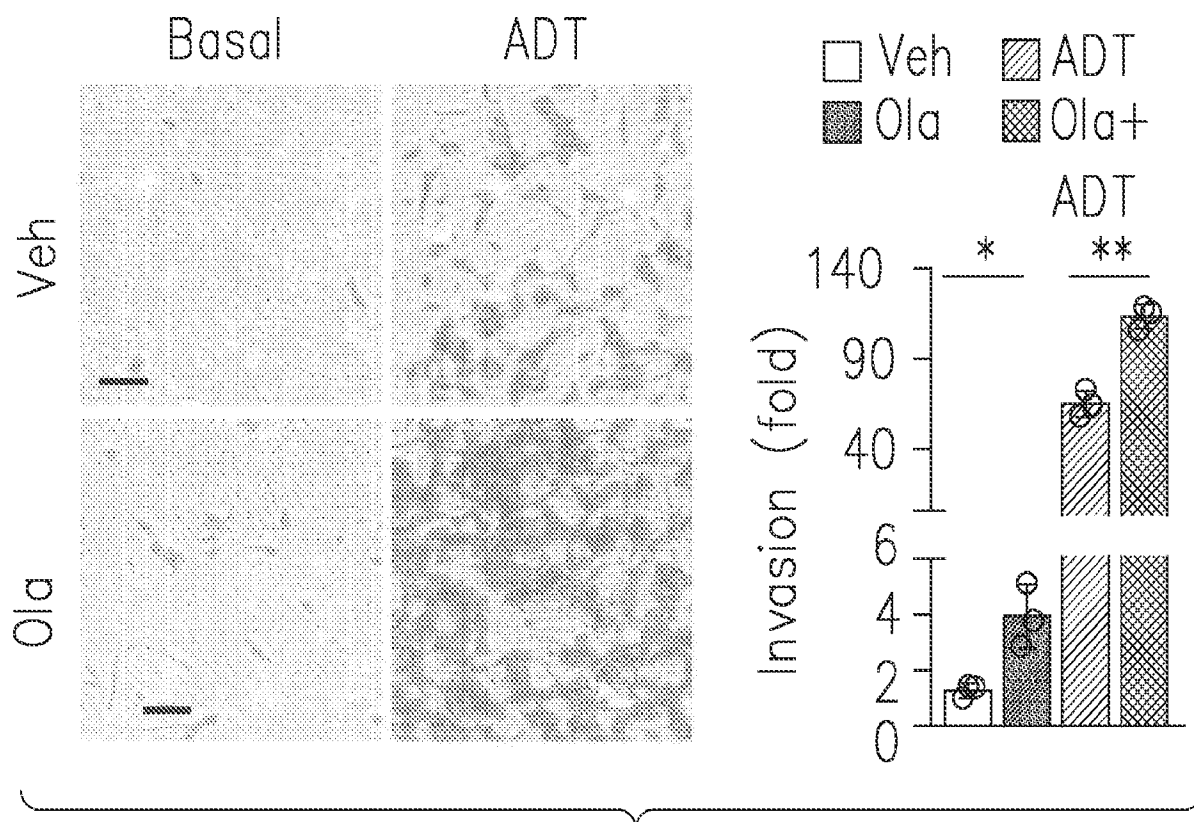
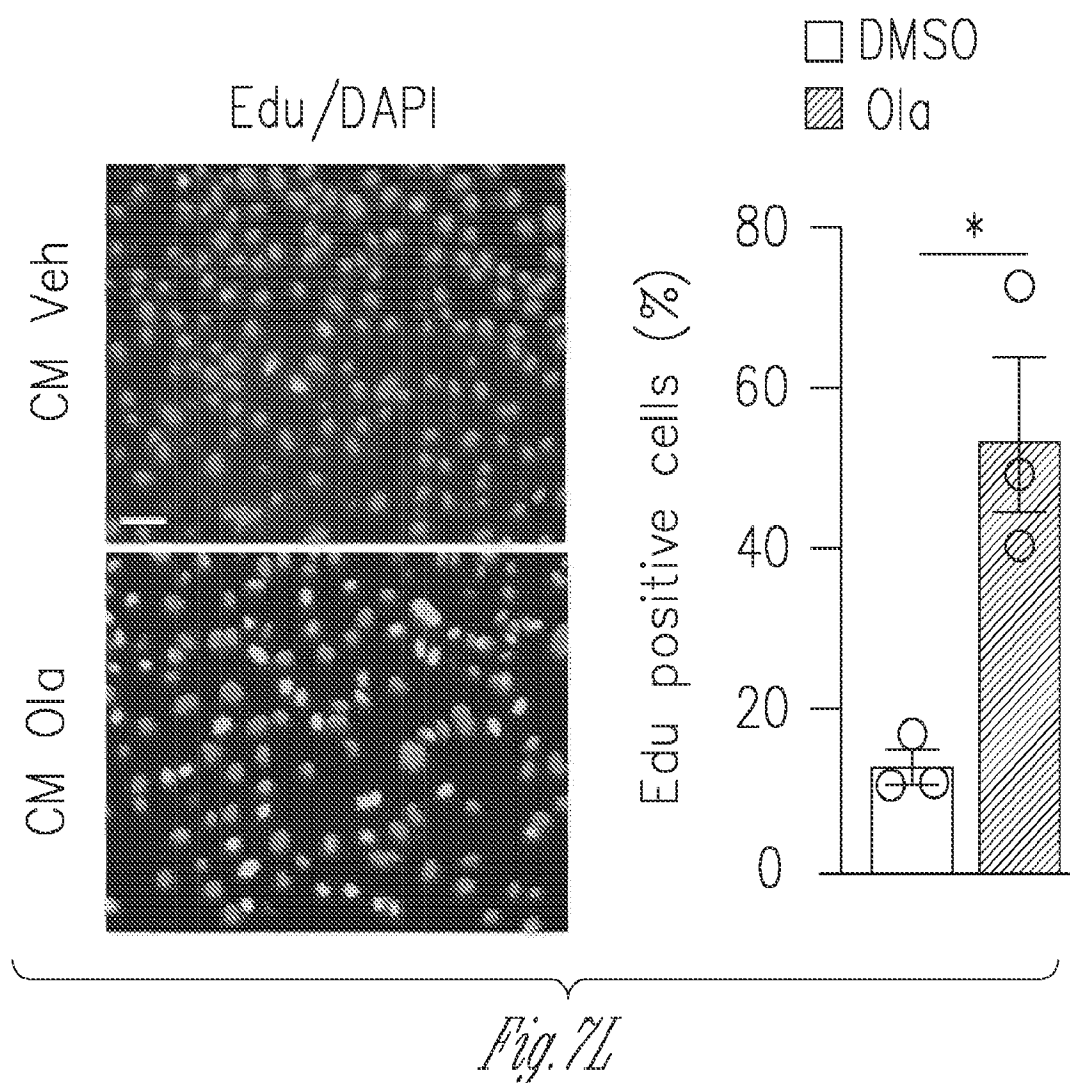
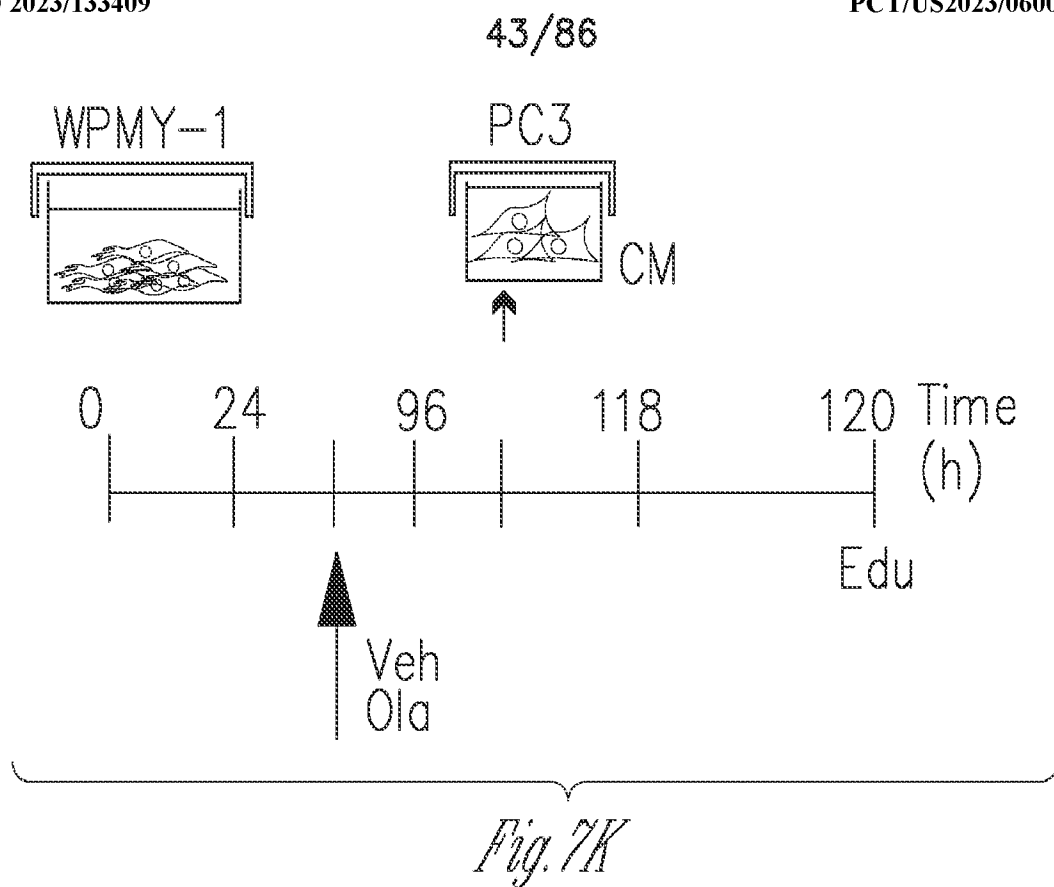
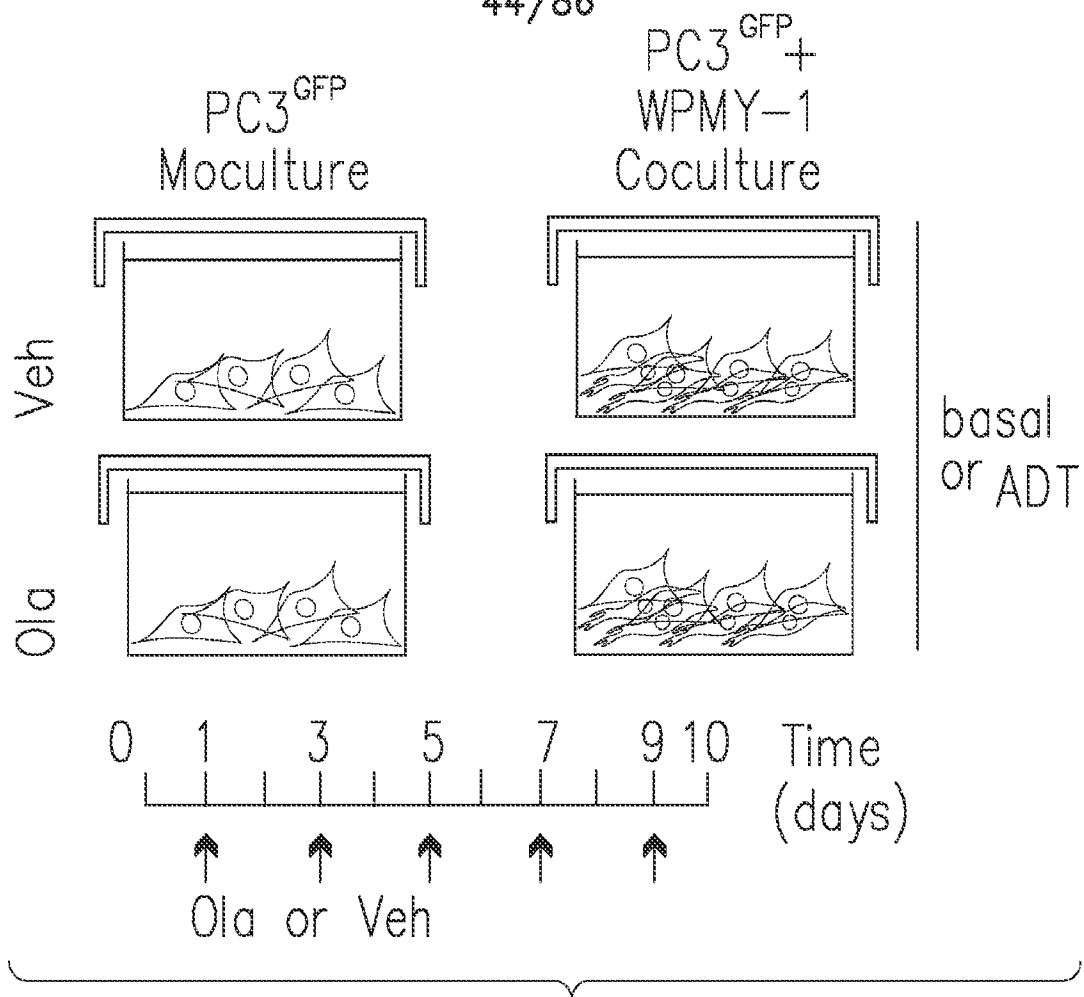
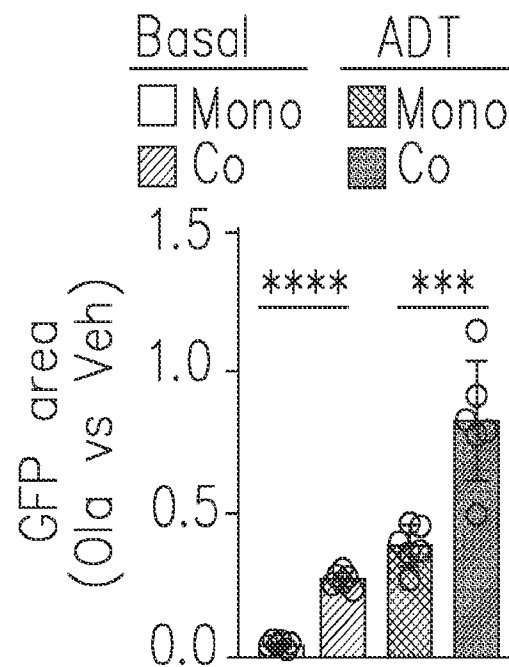


Fig. 7F

*Fig. 7G**Fig. 7H*

*Fig. 7I**Fig. 7J*



*Fig. 7M**Fig. 7N*

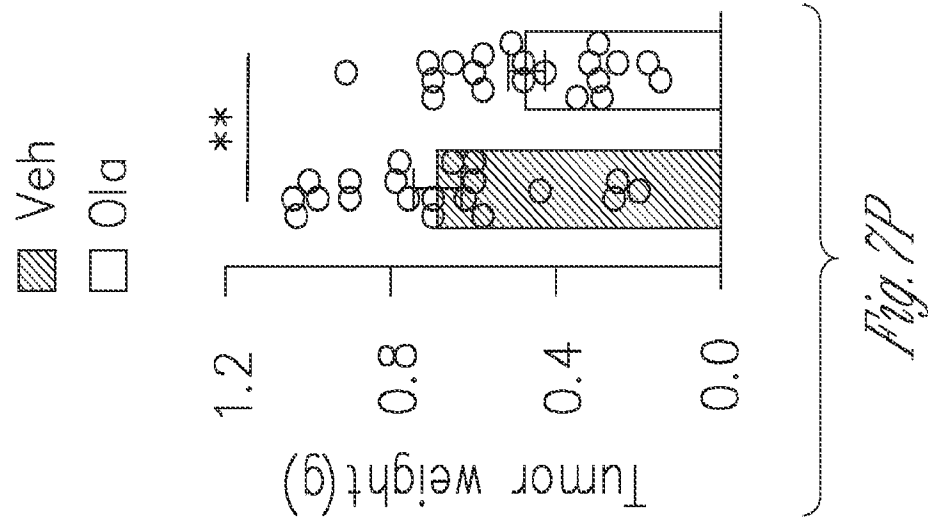


Fig. 7P

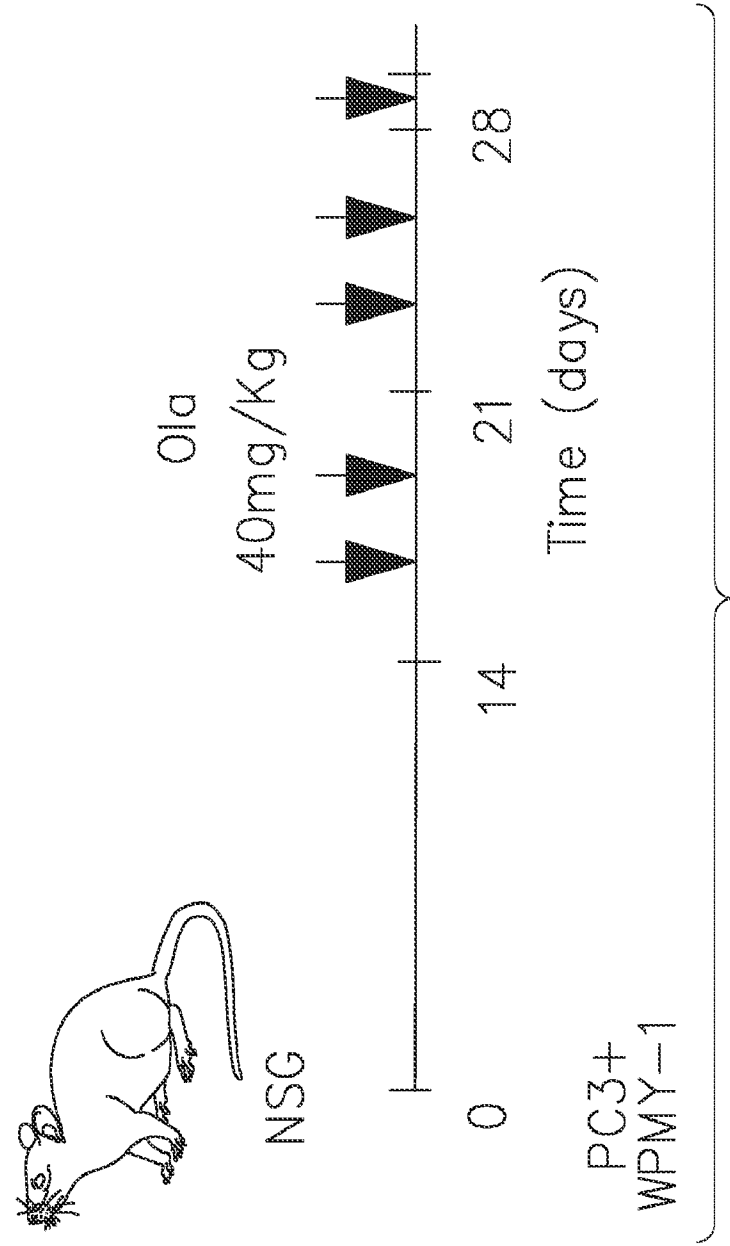


Fig. 7Q

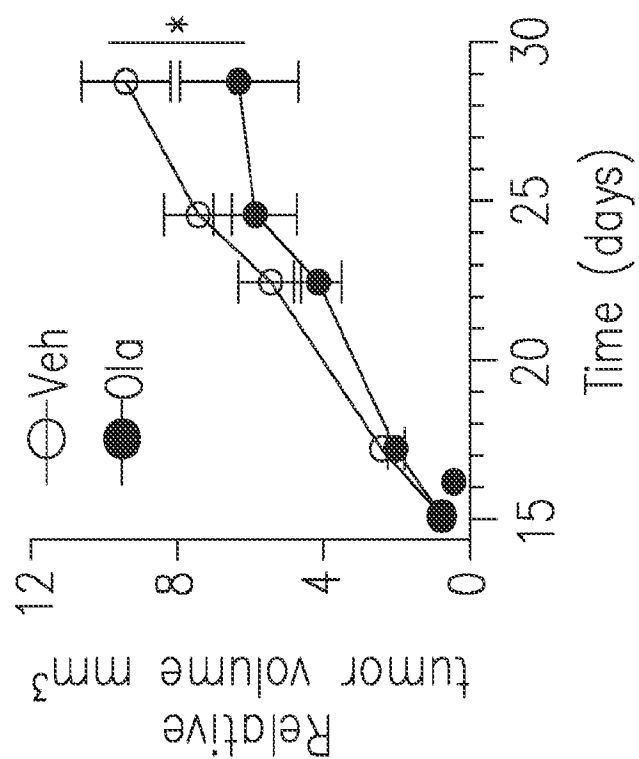
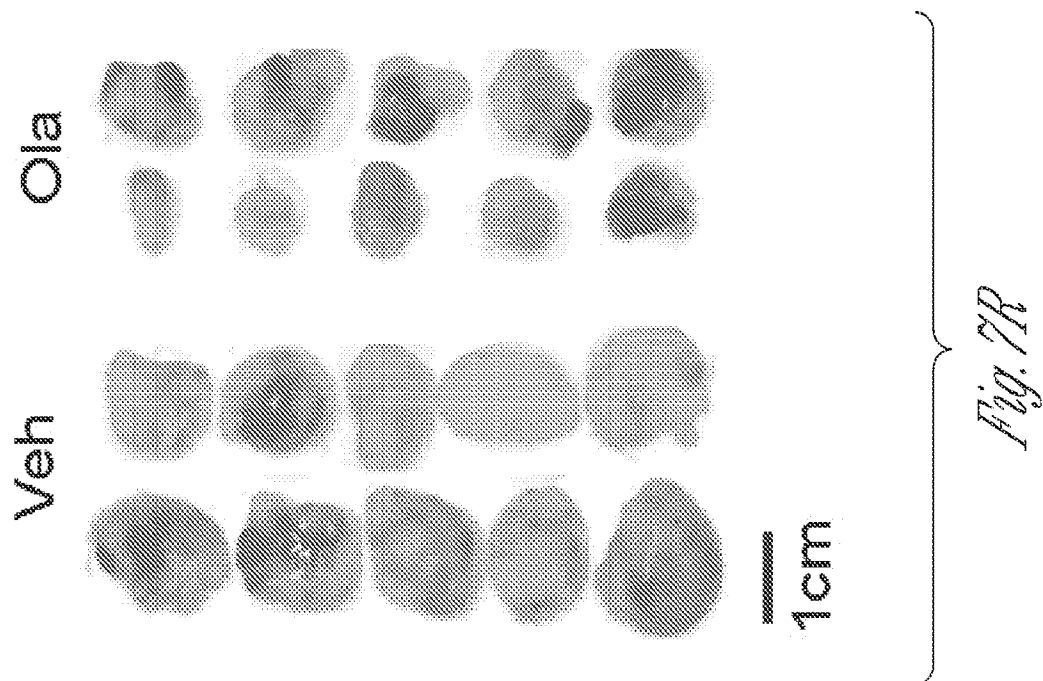


Fig. 7Q

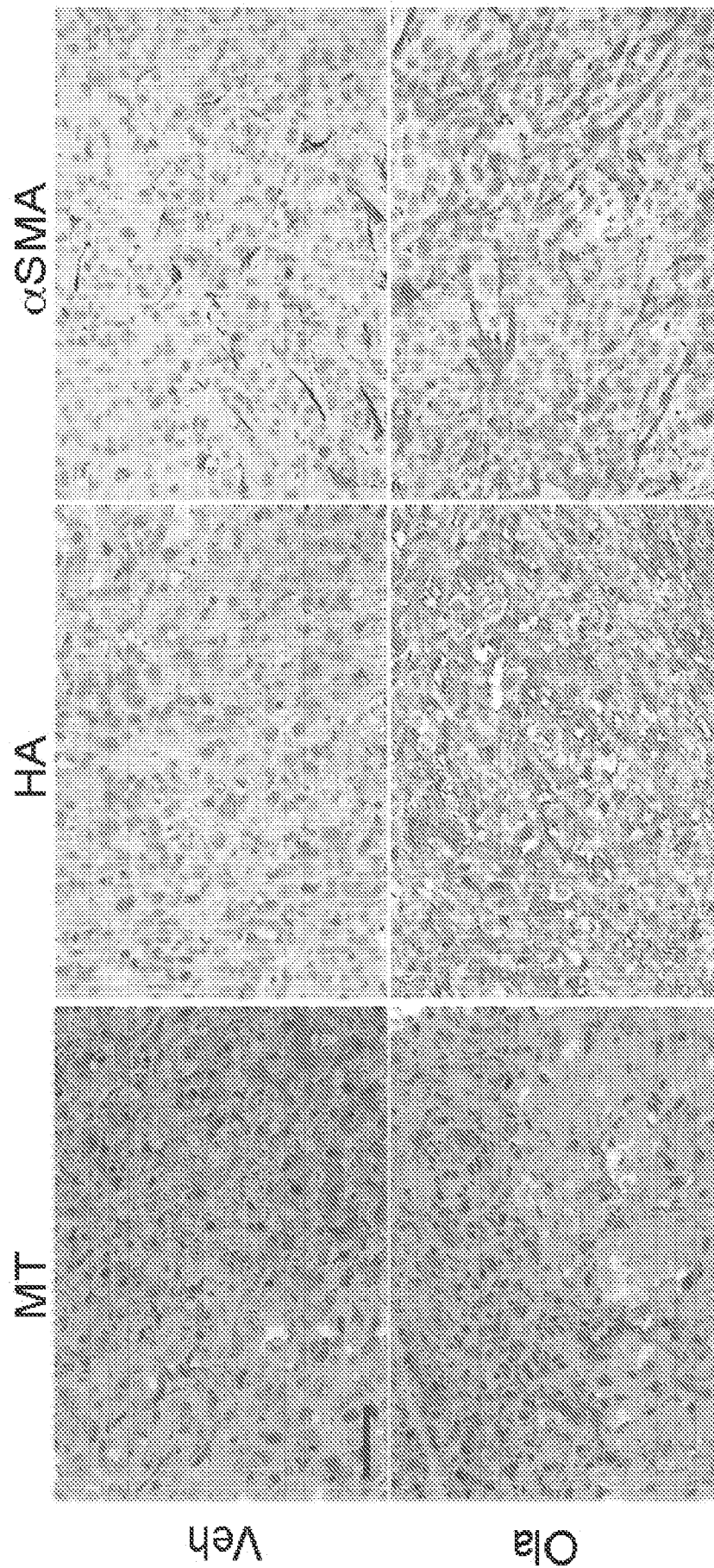
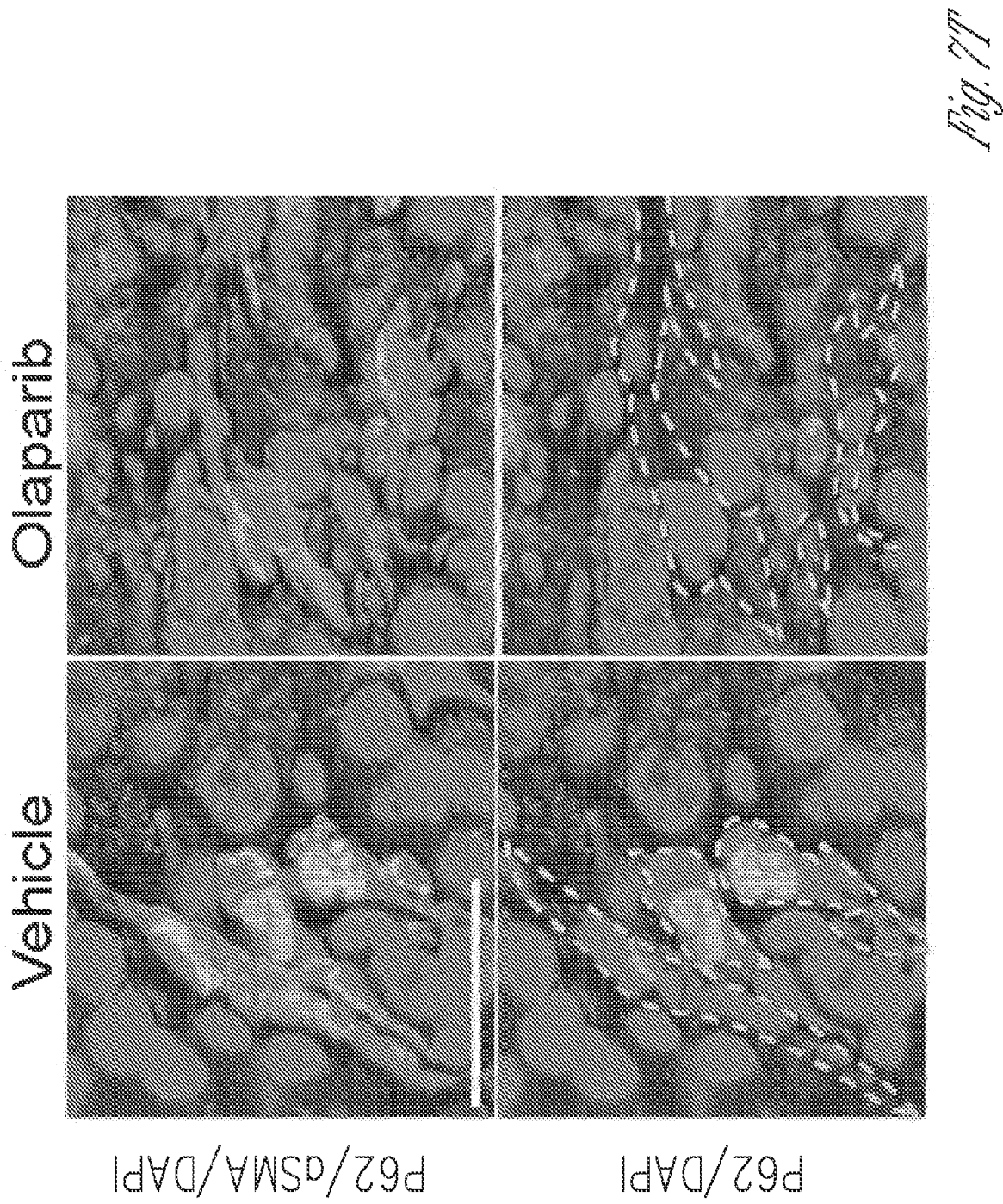
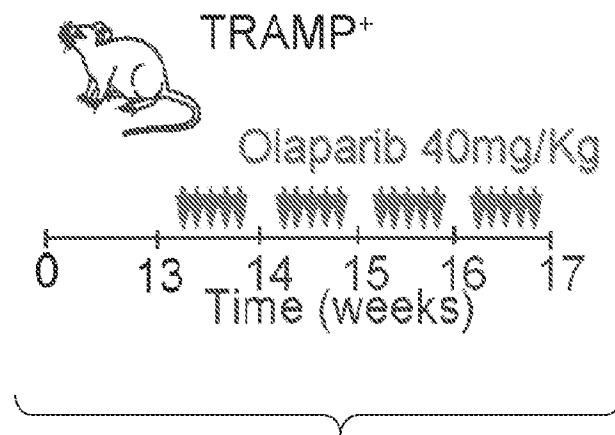
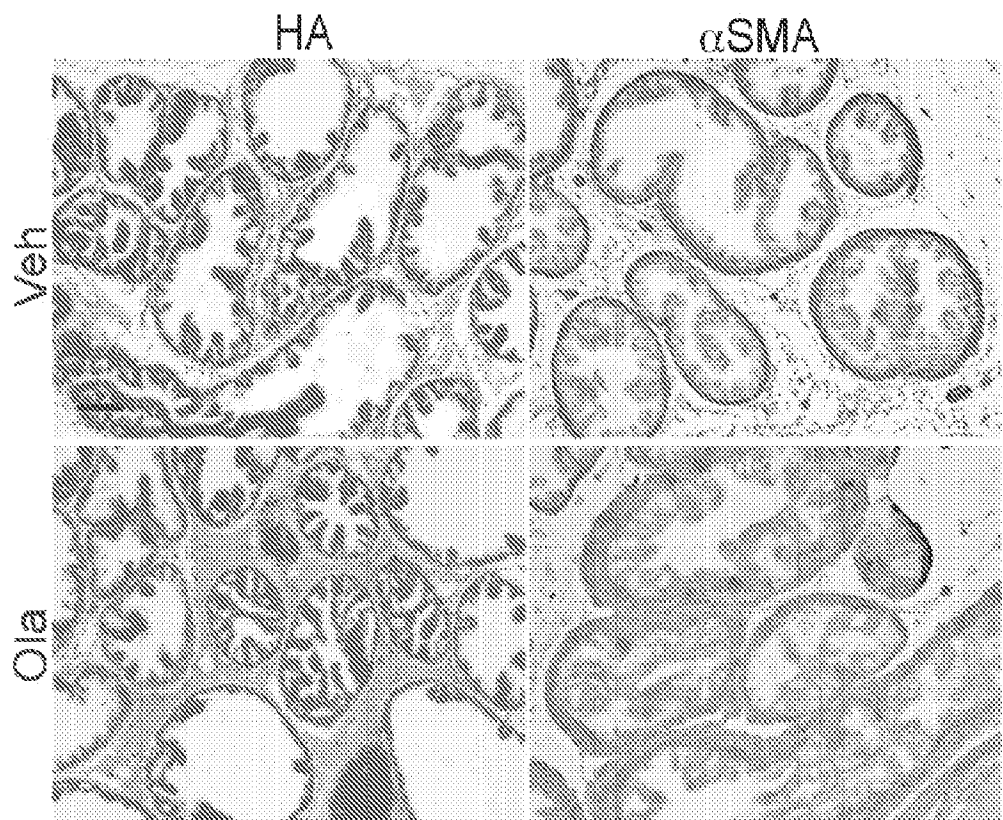


Fig. 2S



*Fig. 8A**Fig. 8B*

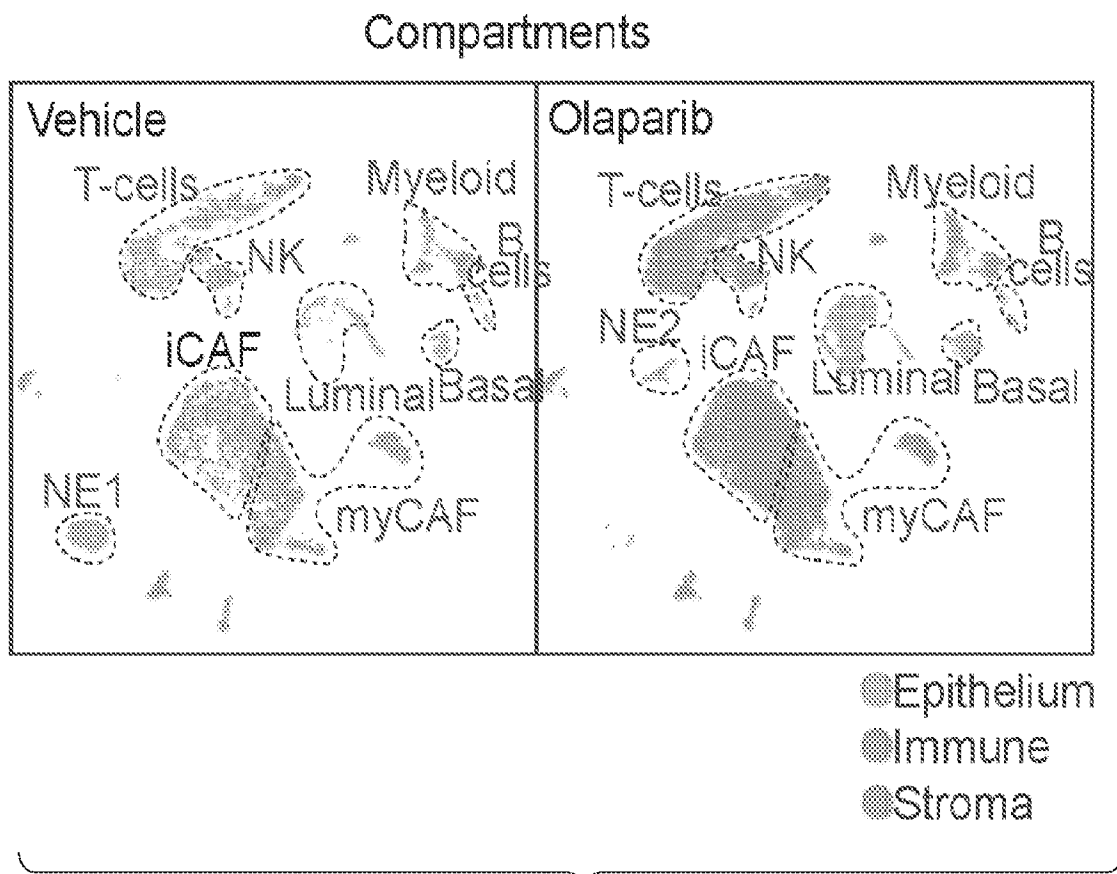


Fig. 8C

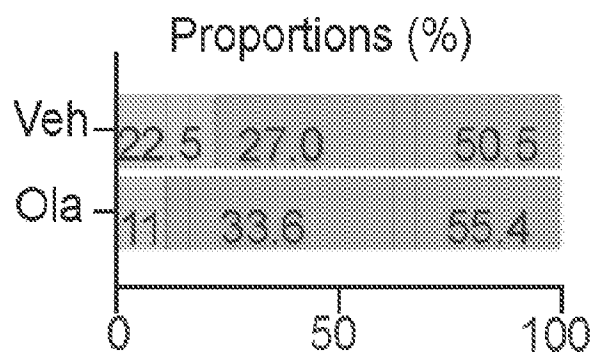


Fig. 8D

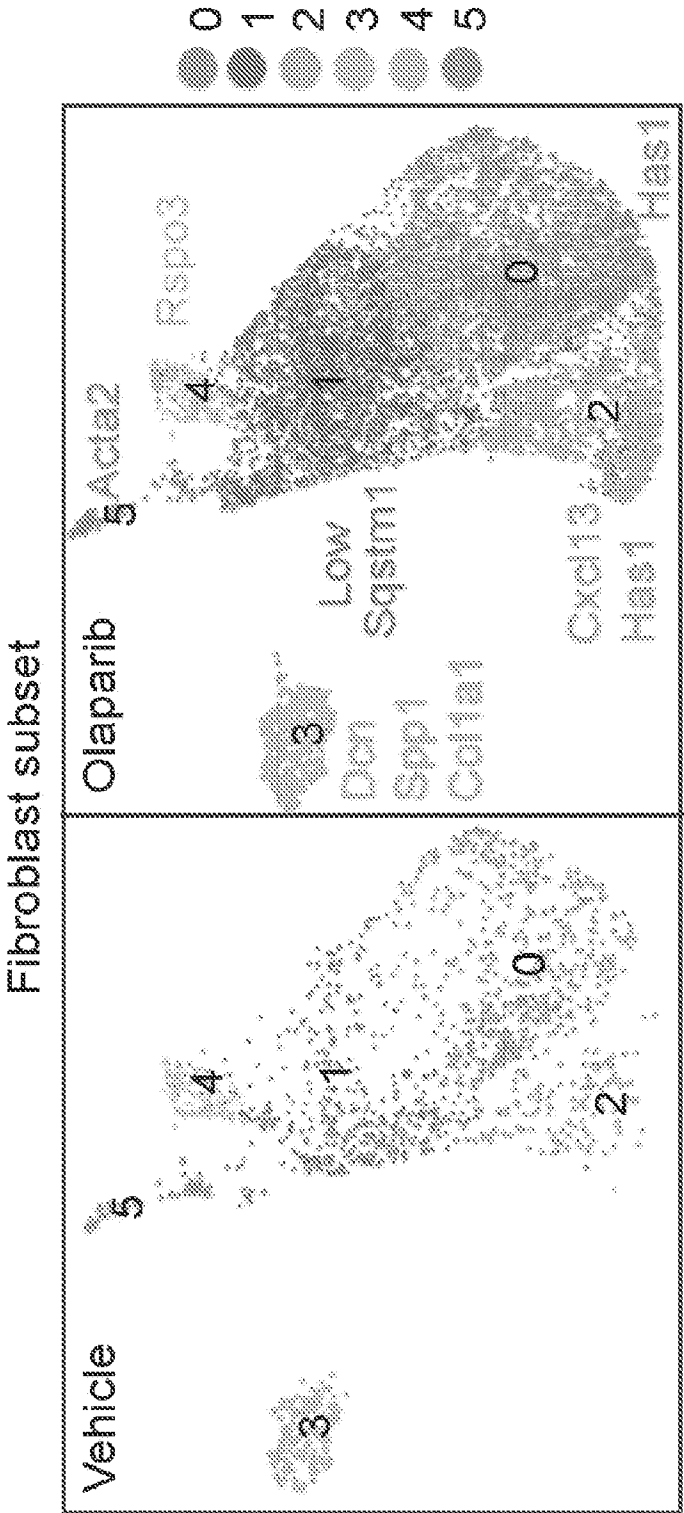


Fig. 8E

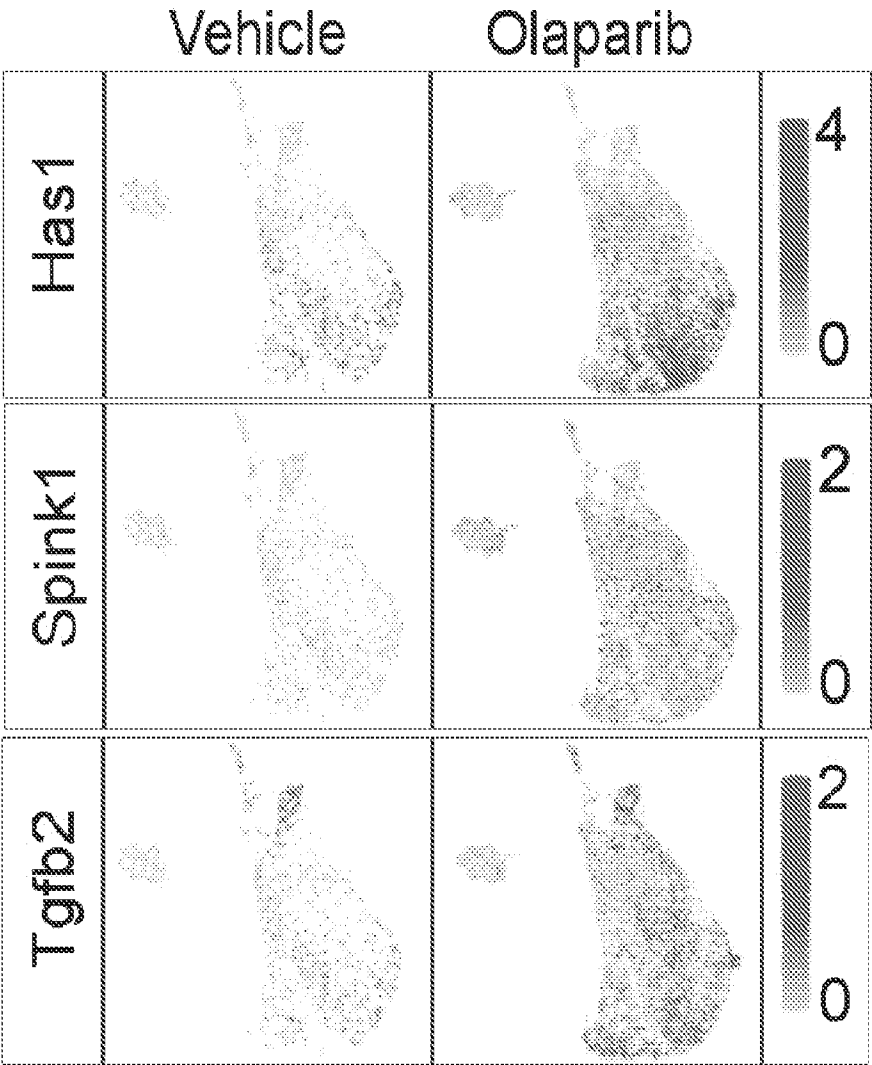


Fig. 8F

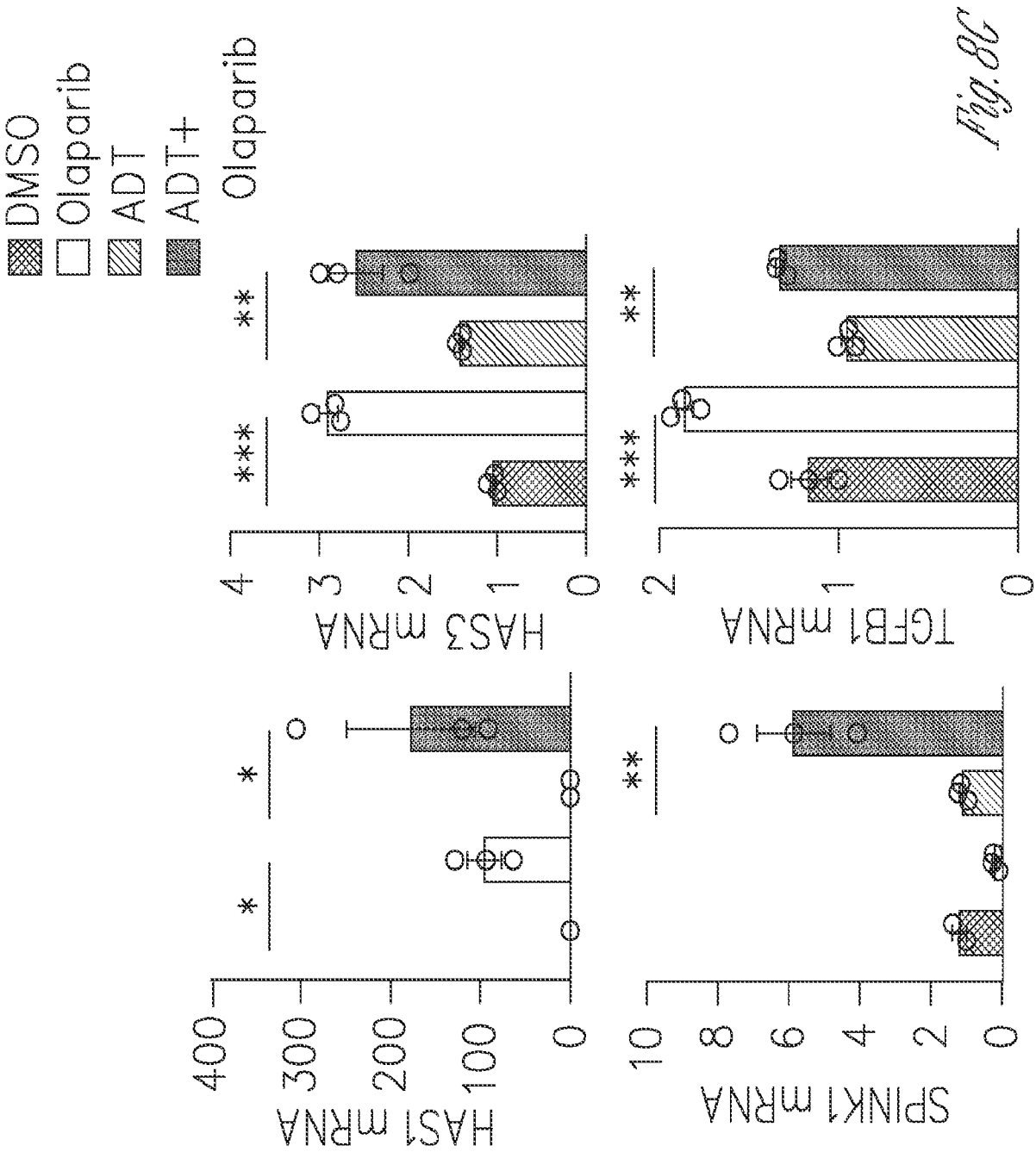


Fig. 8C

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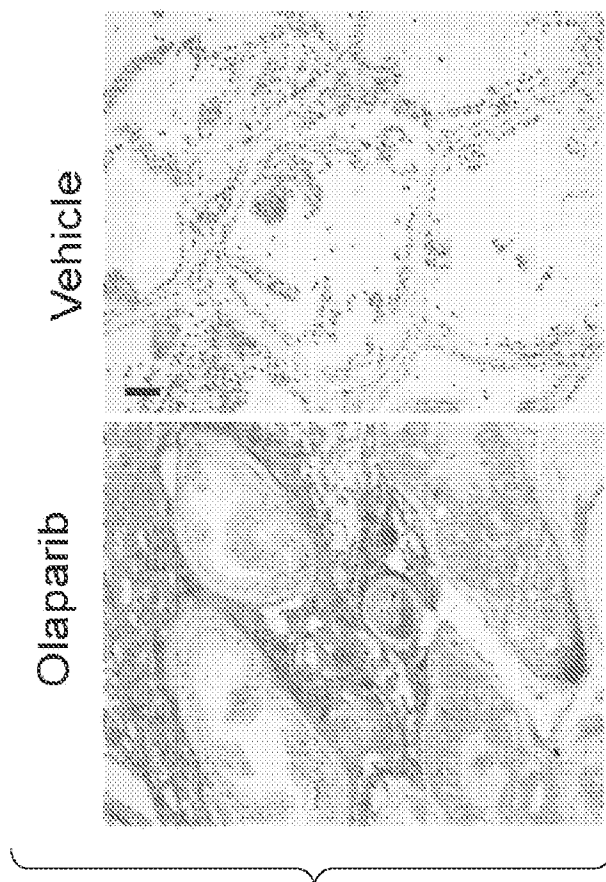


Fig. 8H

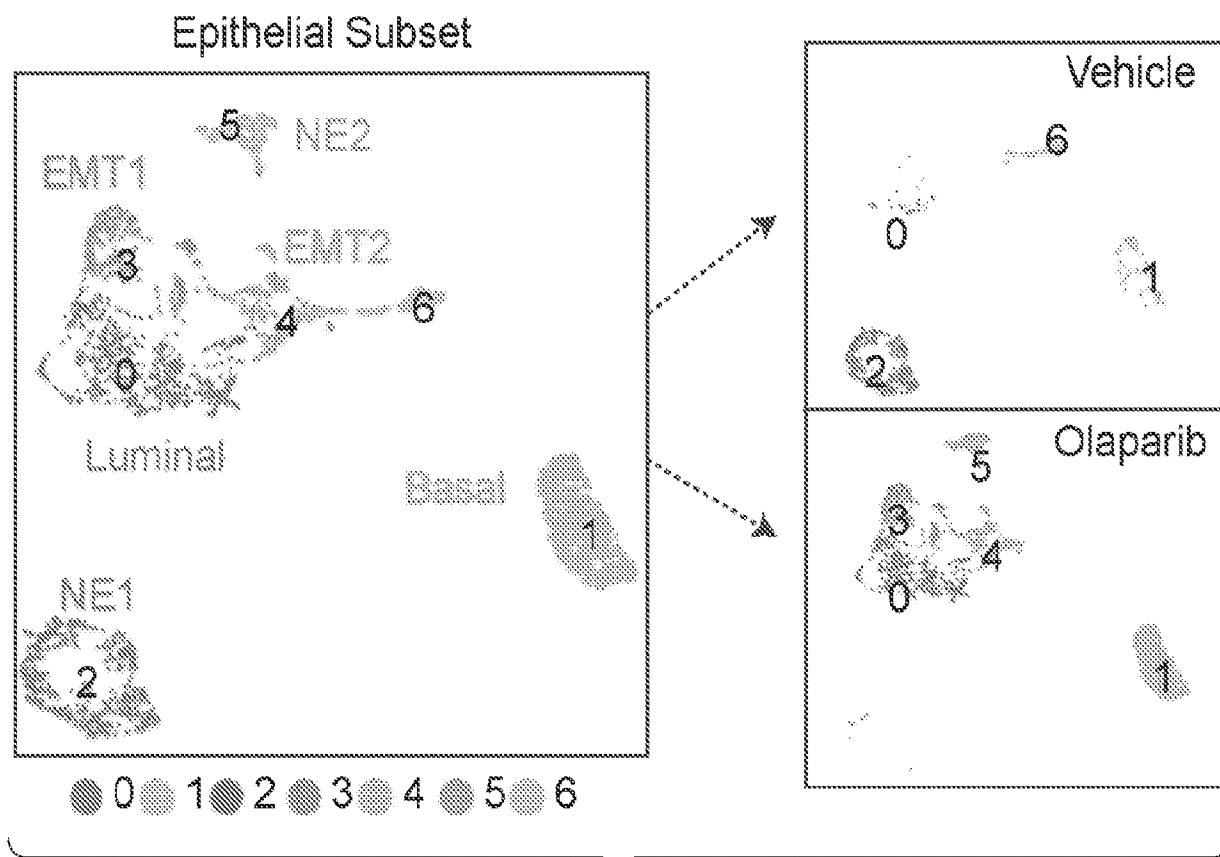


Fig. 8I

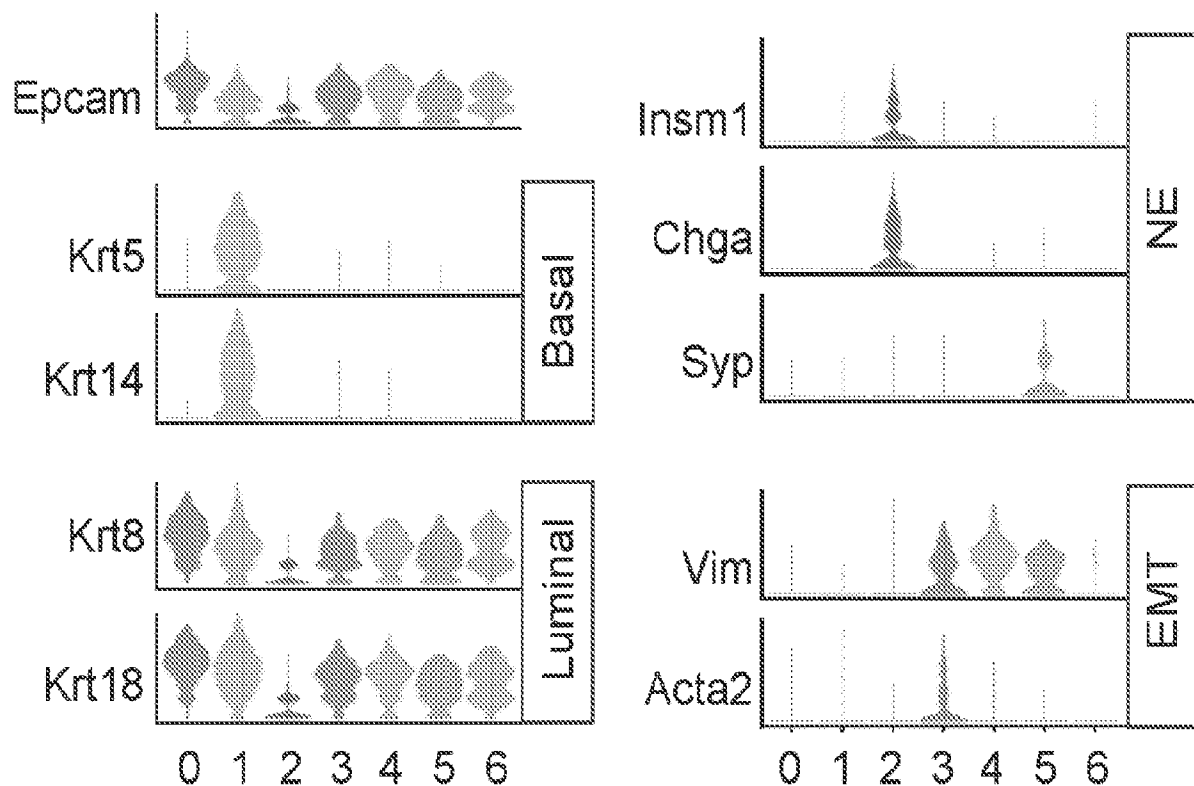


Fig. 8J

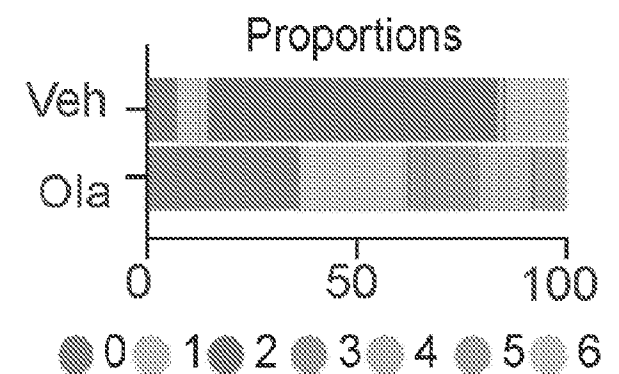
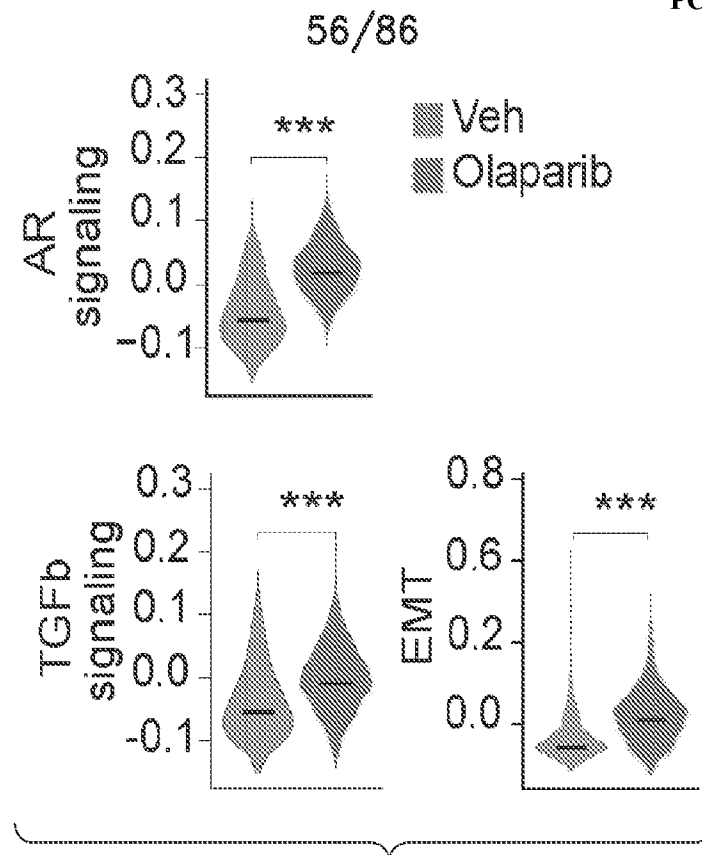
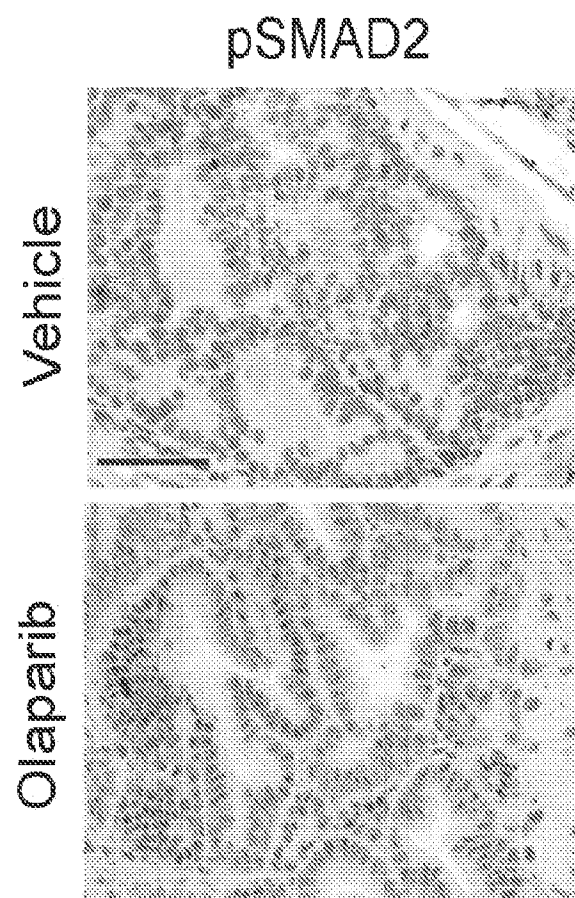


Fig. 8K

*Fig. 8L**Fig. 8M*

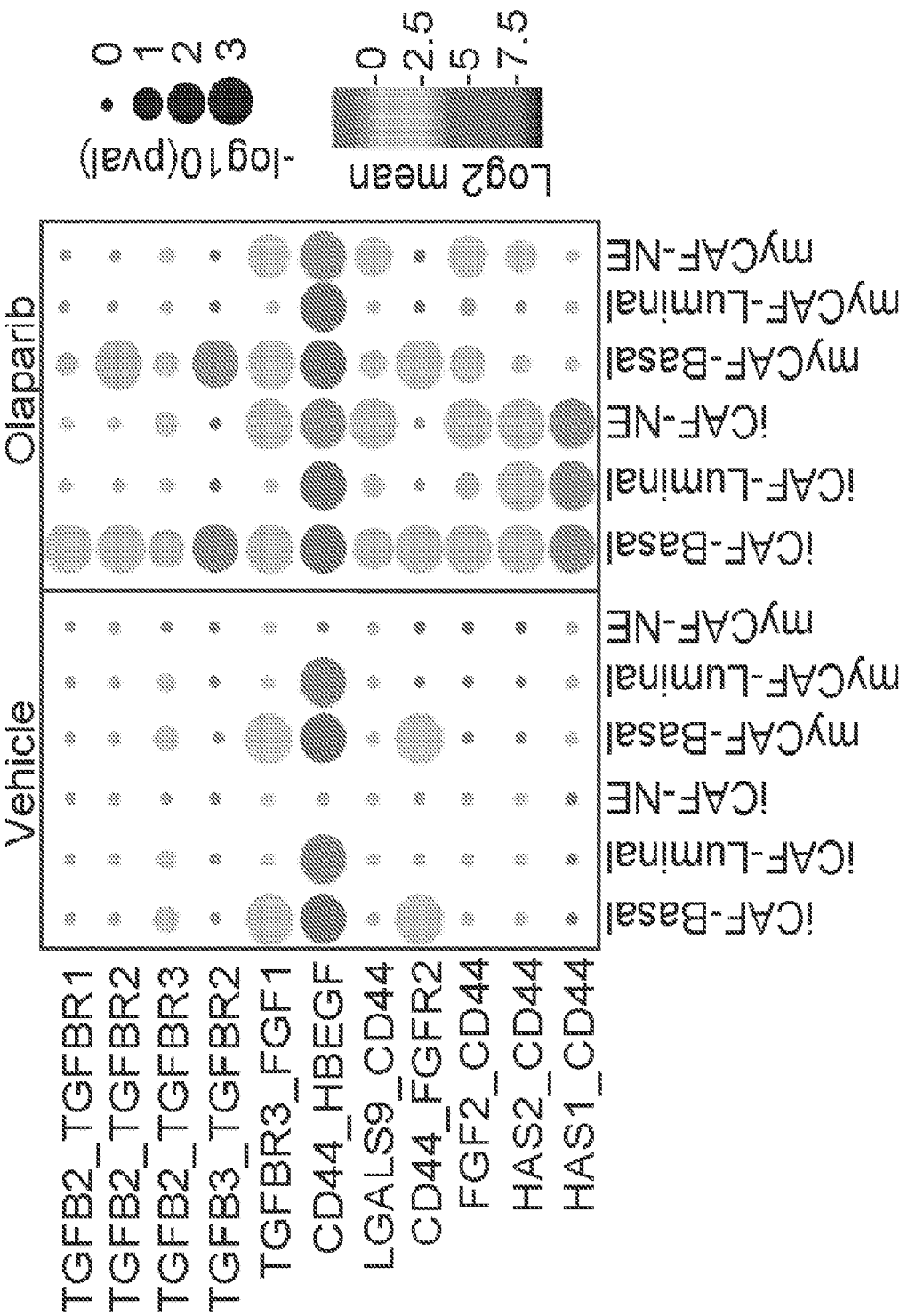


Fig. 8N

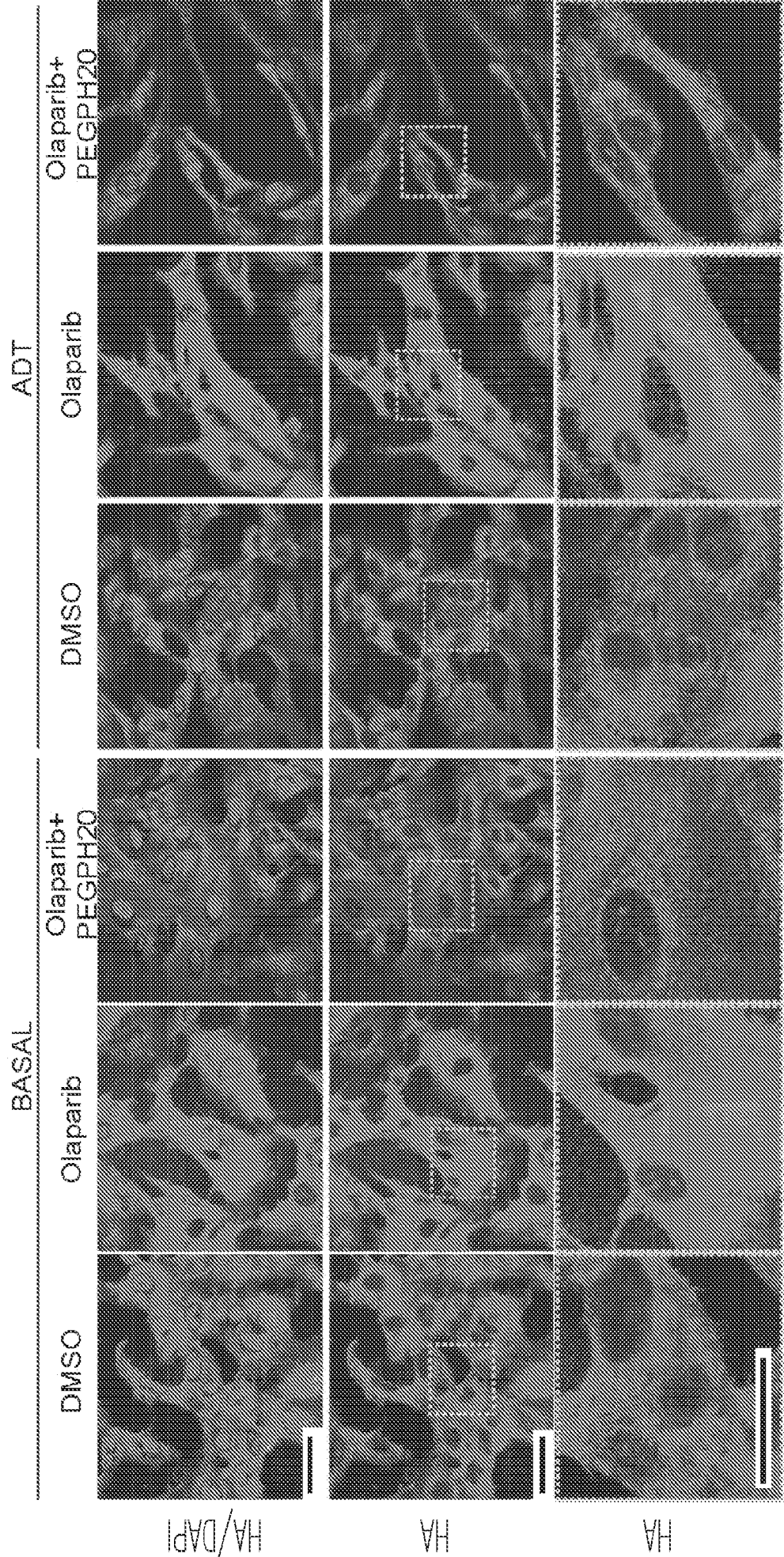


Fig. 9A

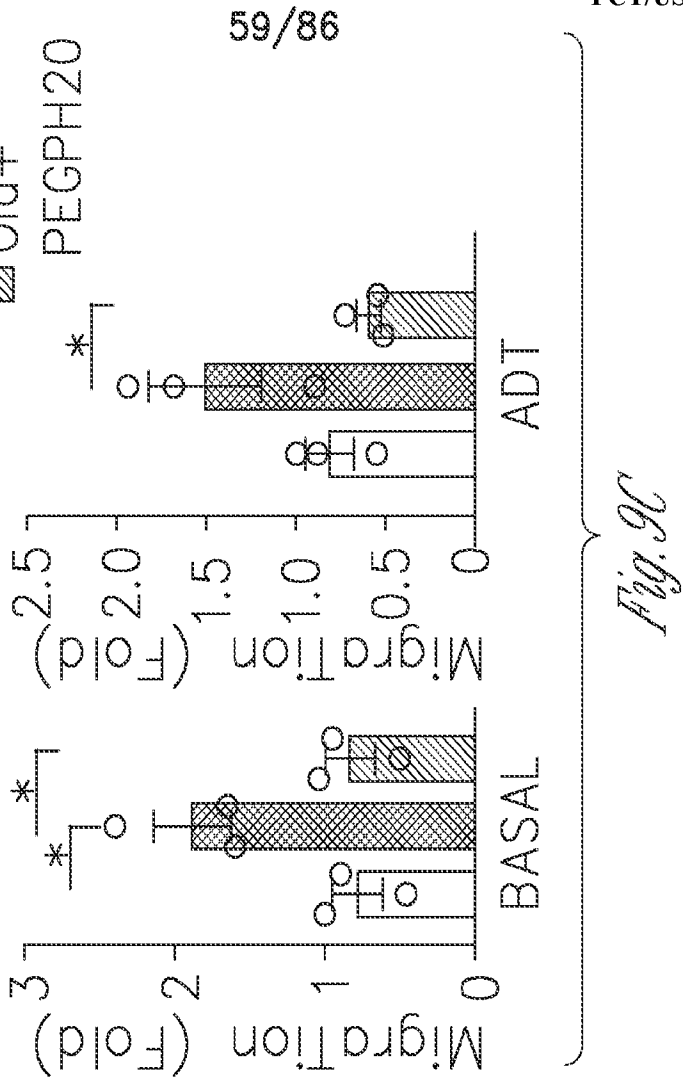


Fig. 9C

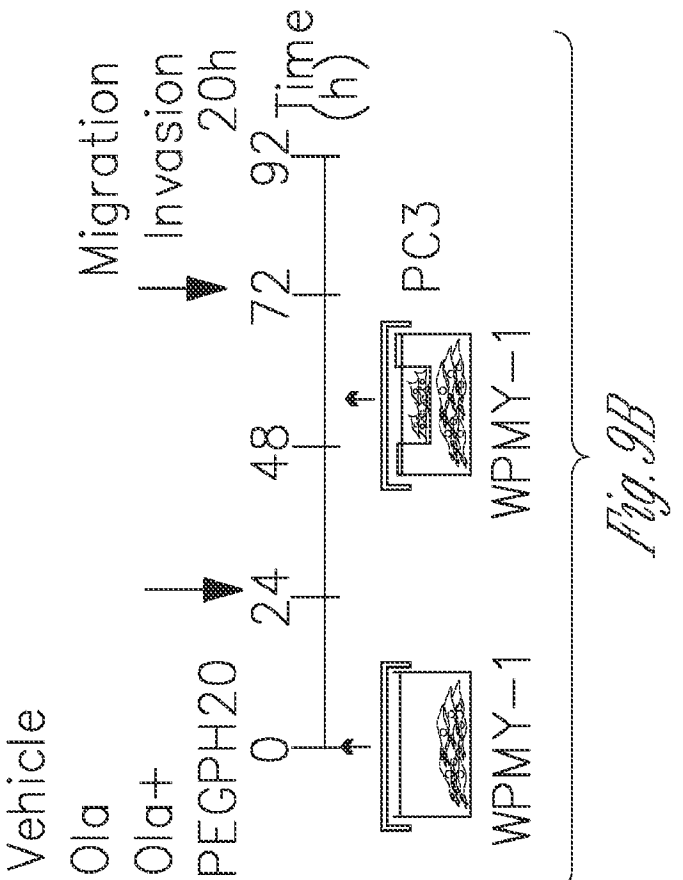
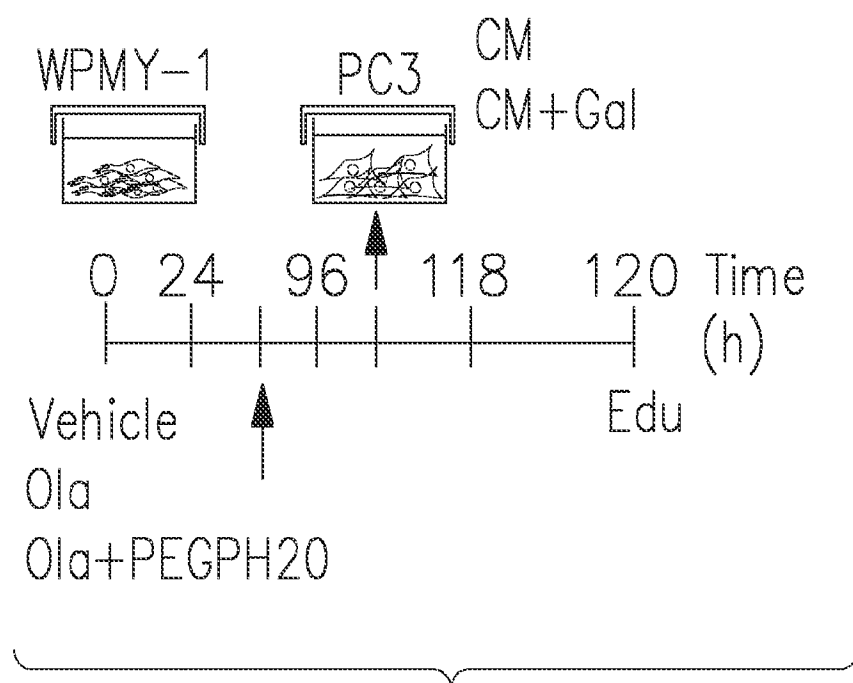
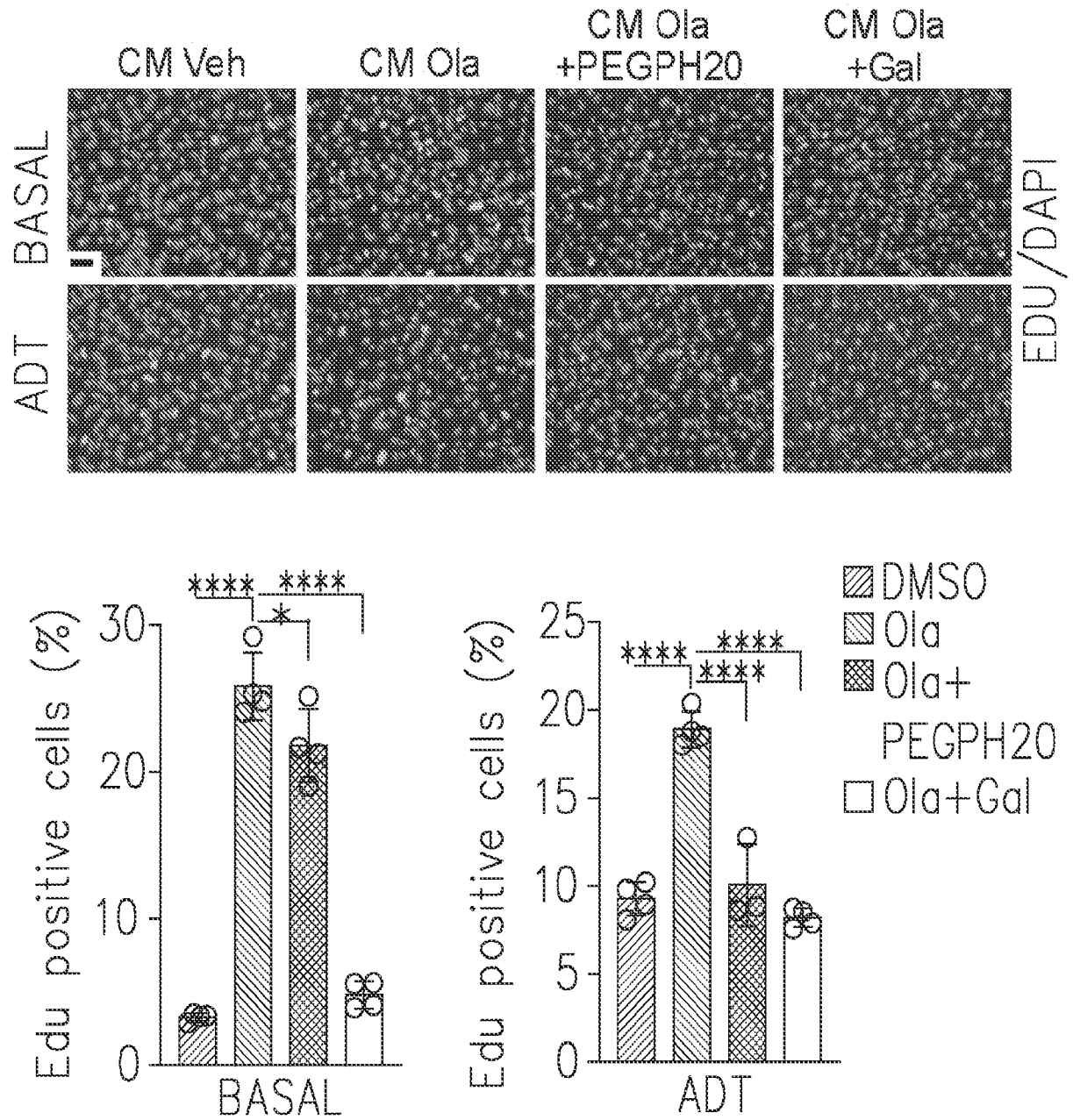


Fig. 9B

*Fig. 9D*

*Fig. 9E*

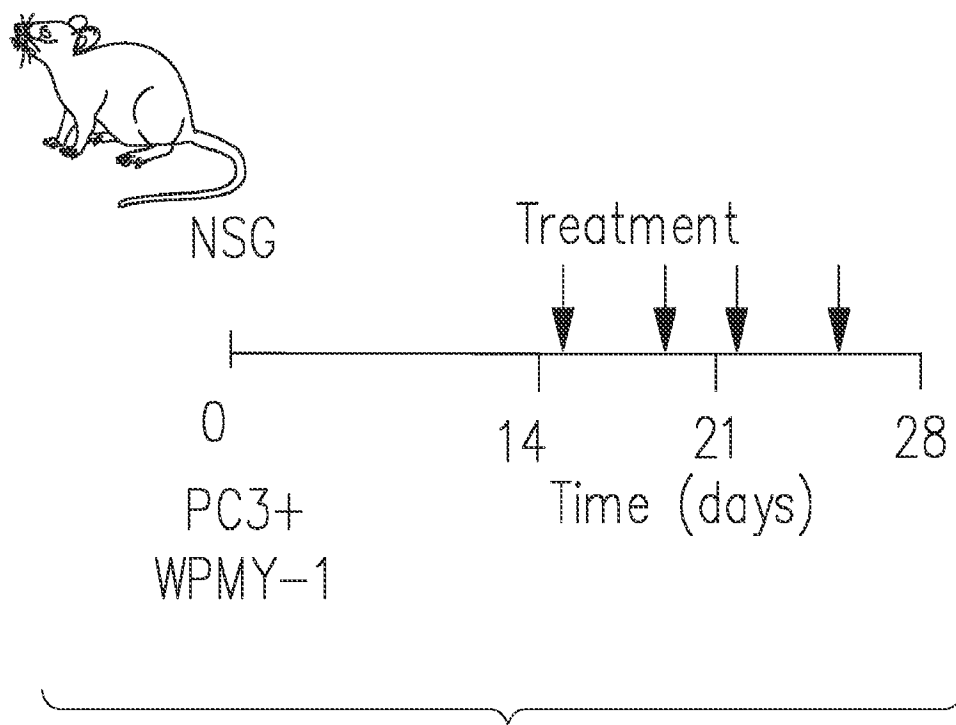


Fig. 9F

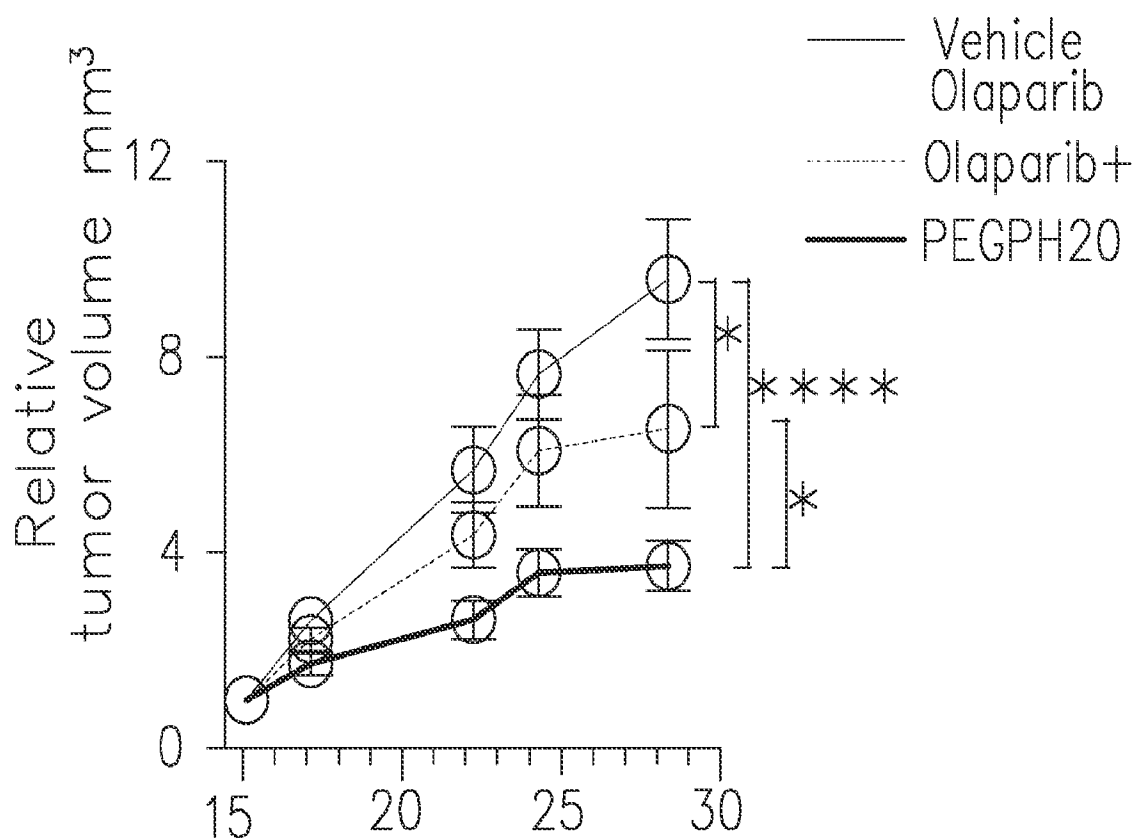


Fig. 9G

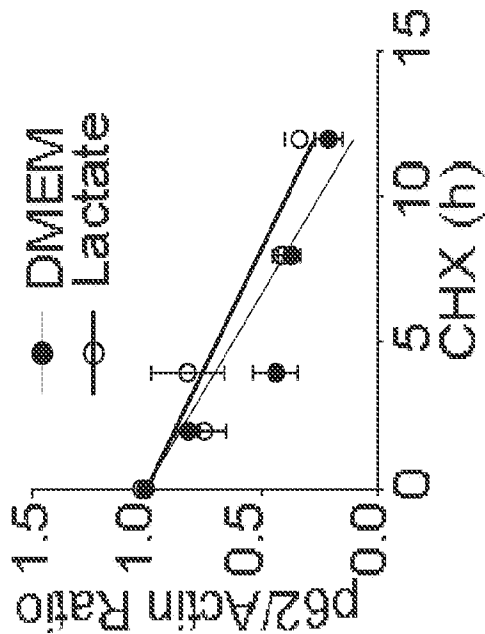


Fig. 10A

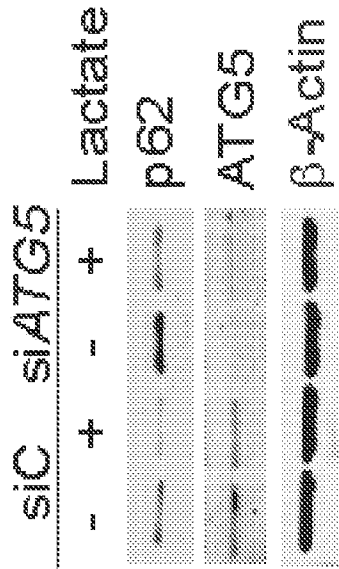


Fig. 10D

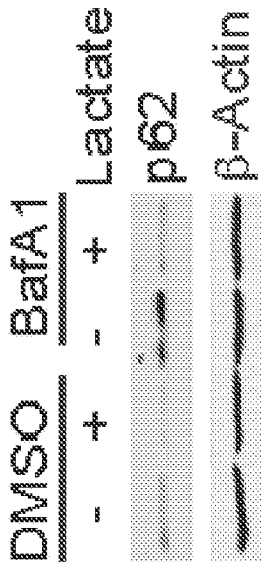


Fig. 10C

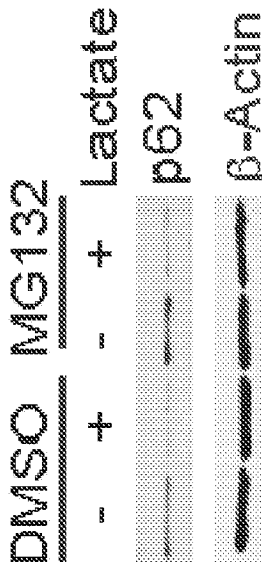
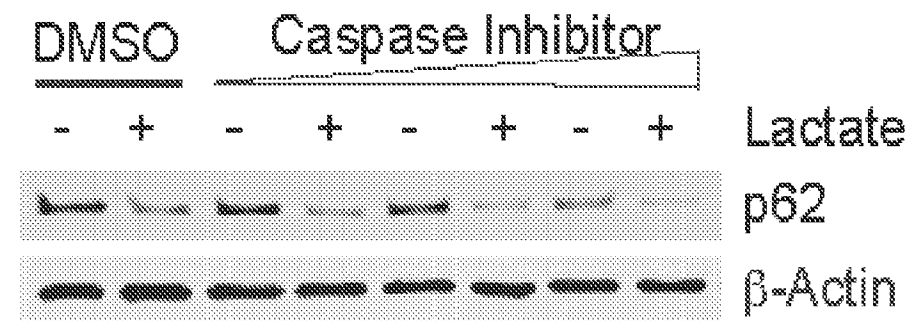


Fig. 10B

*Fig. 10E**Fig. 10F*

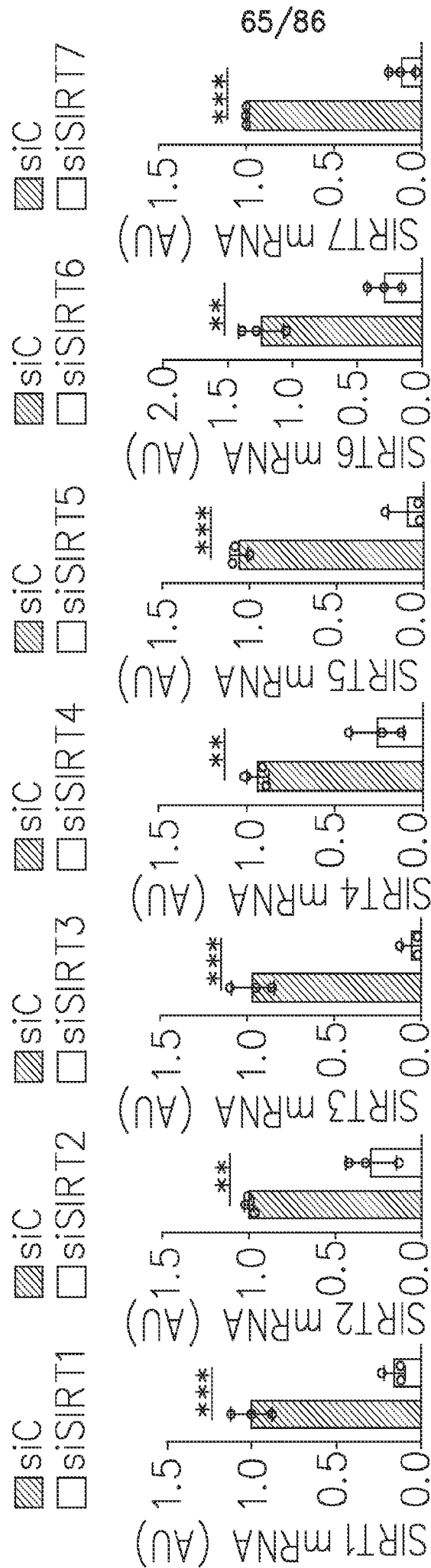
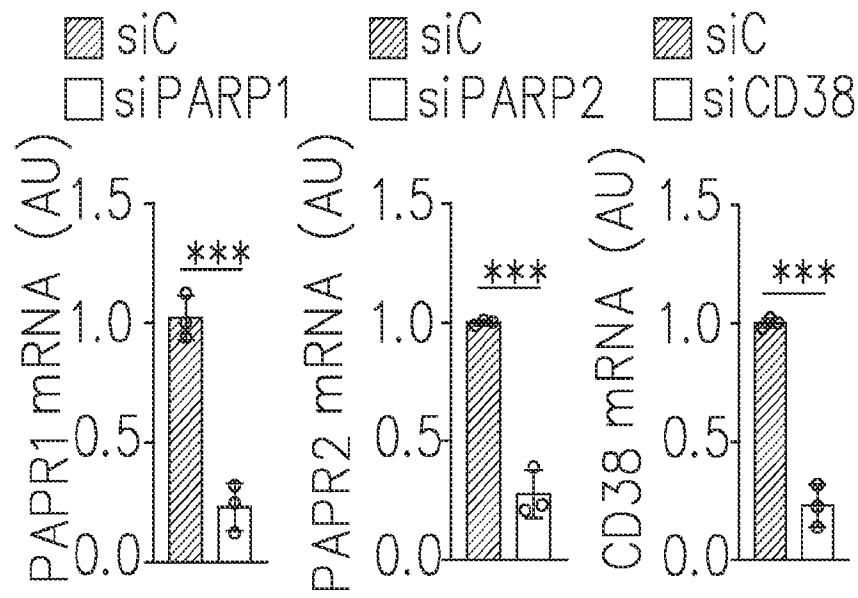
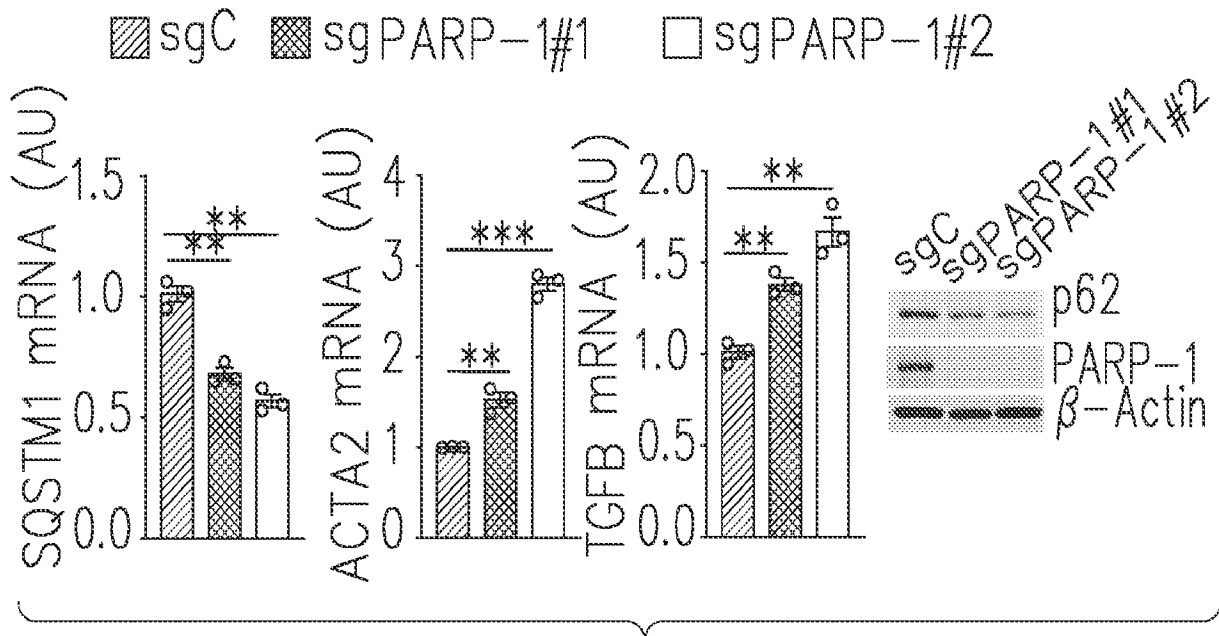


Fig. 11A

*Fig. 11B**Fig. 11C*

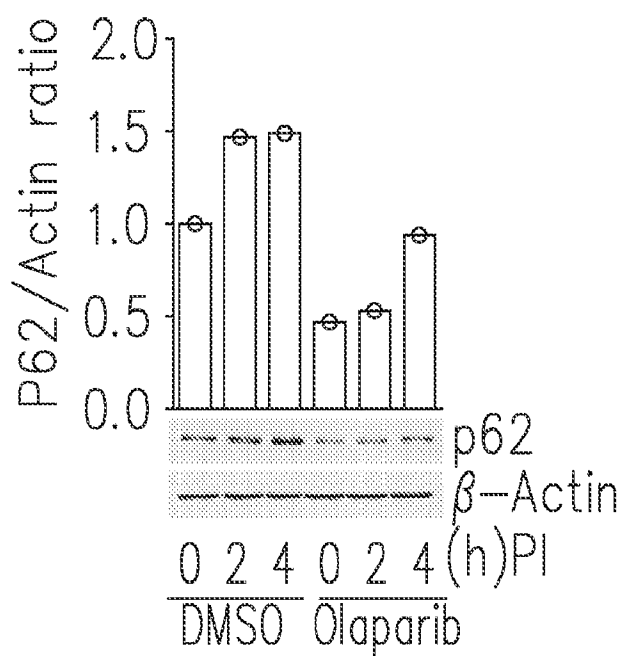


Fig. 11D

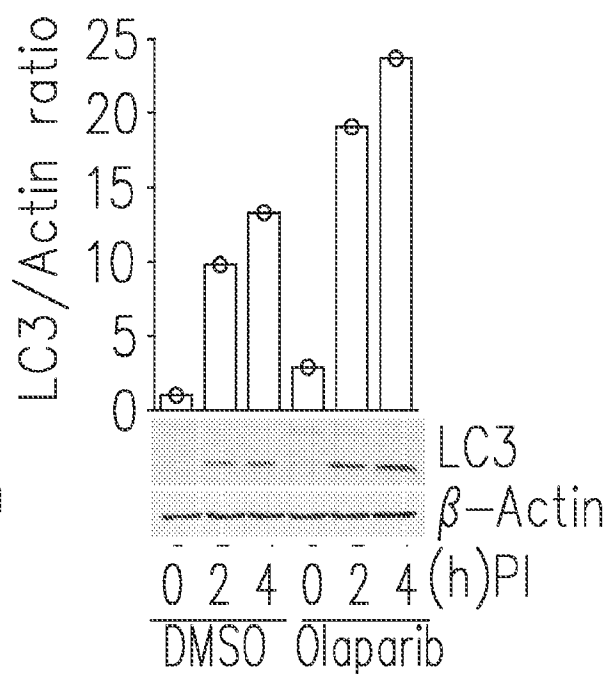


Fig. 11E

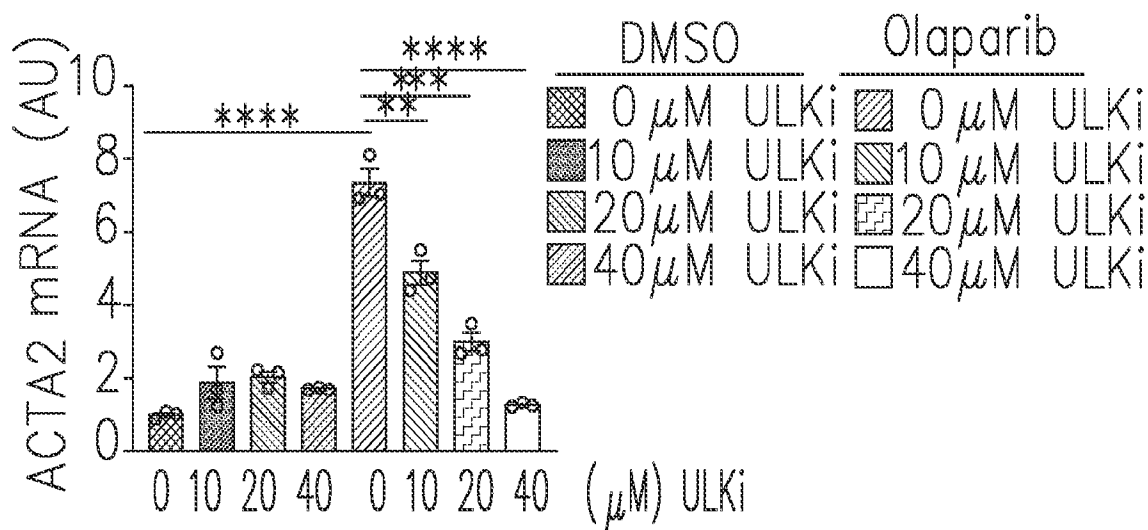
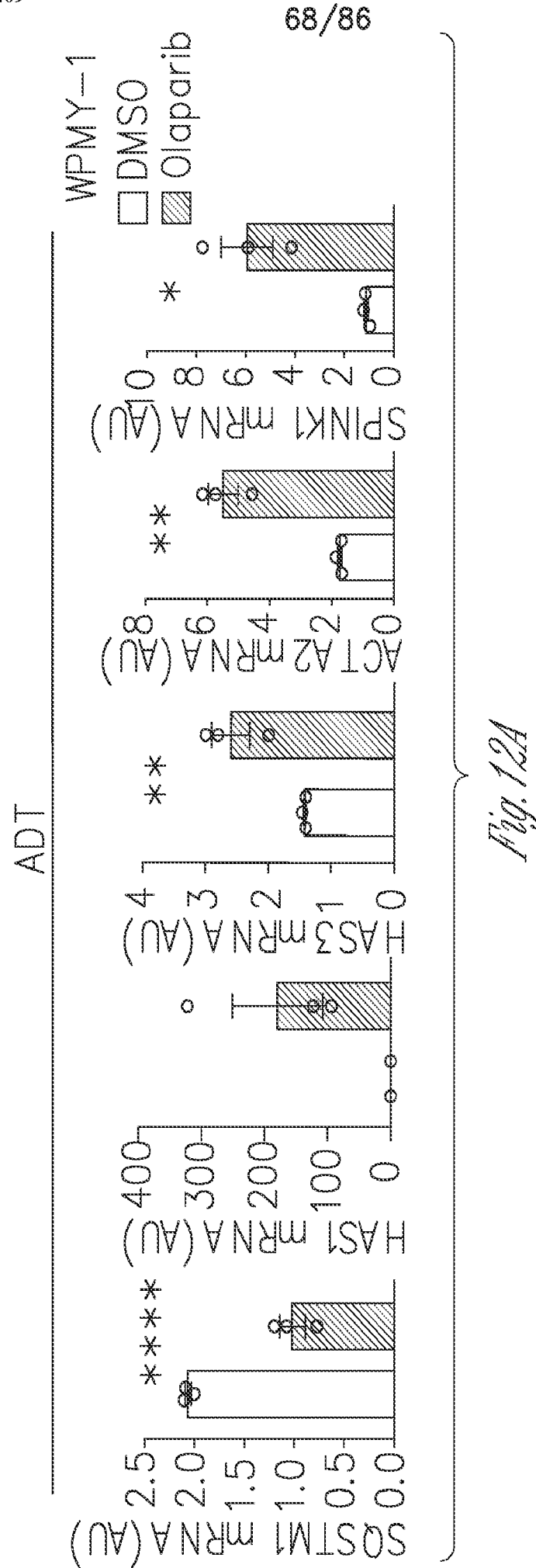


Fig. 11F



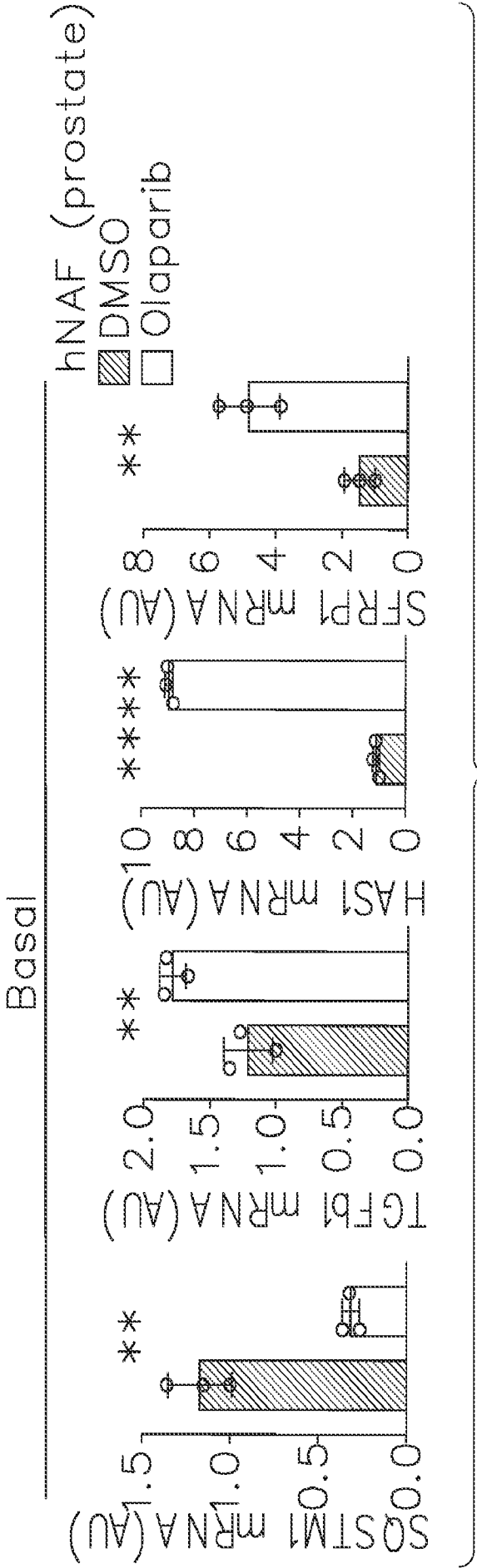


Fig. 12B

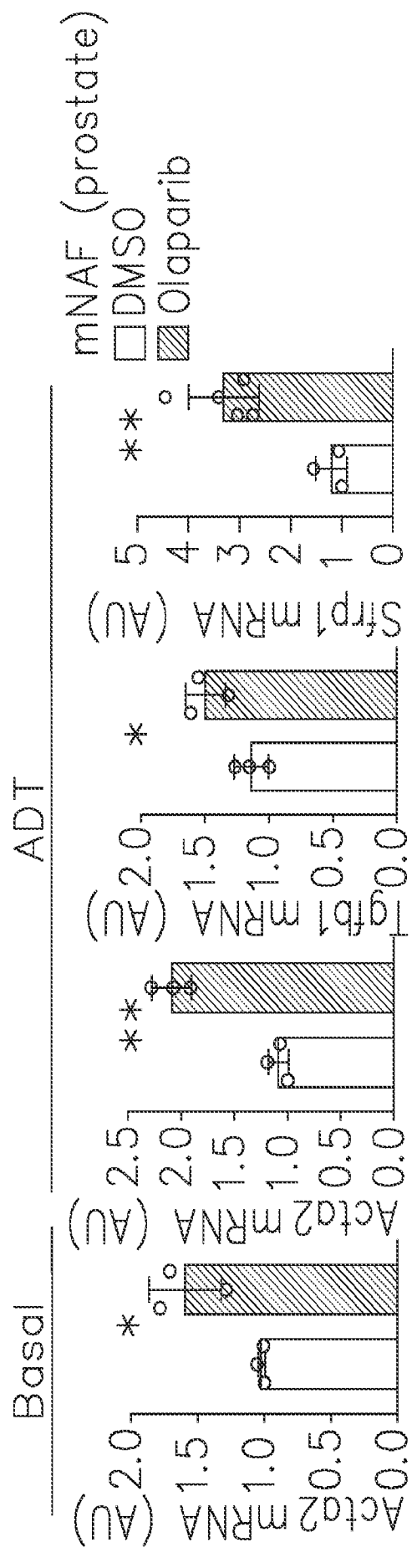
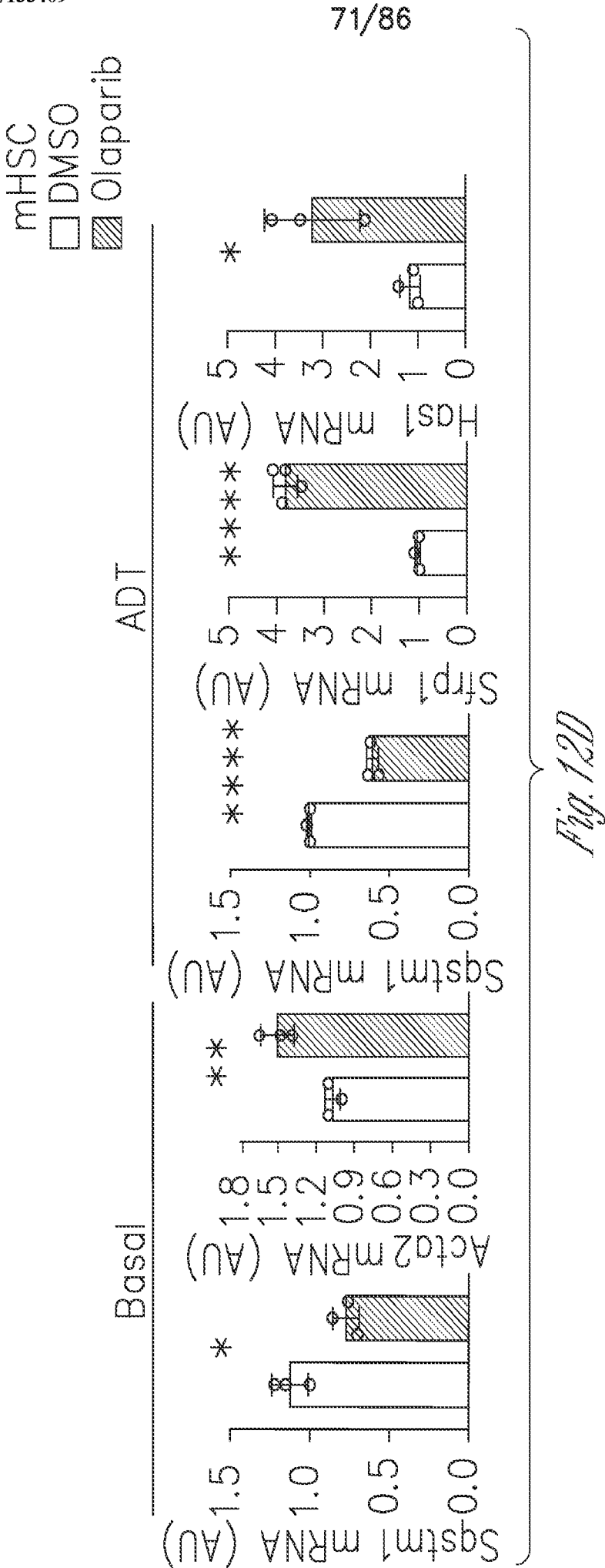
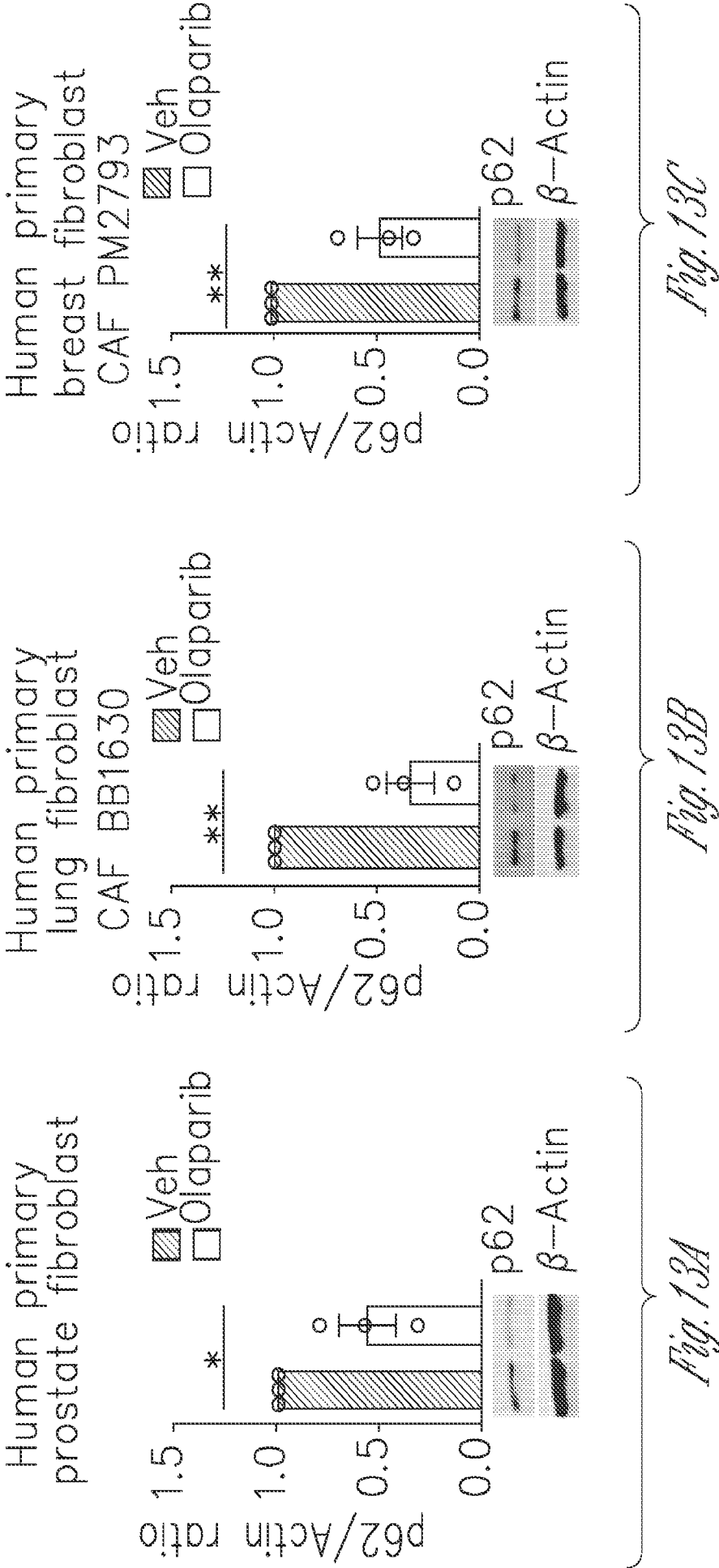


Fig. 12C





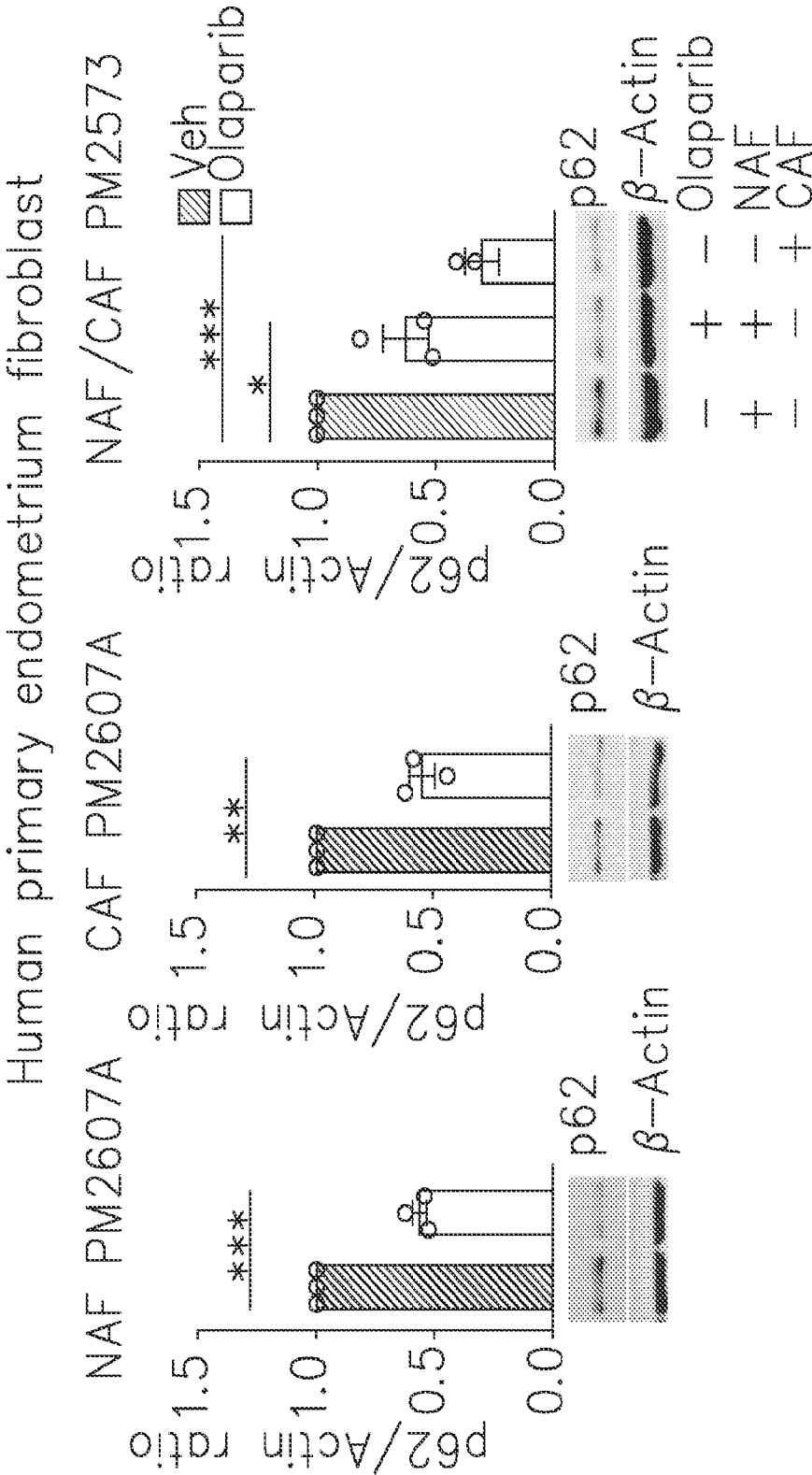
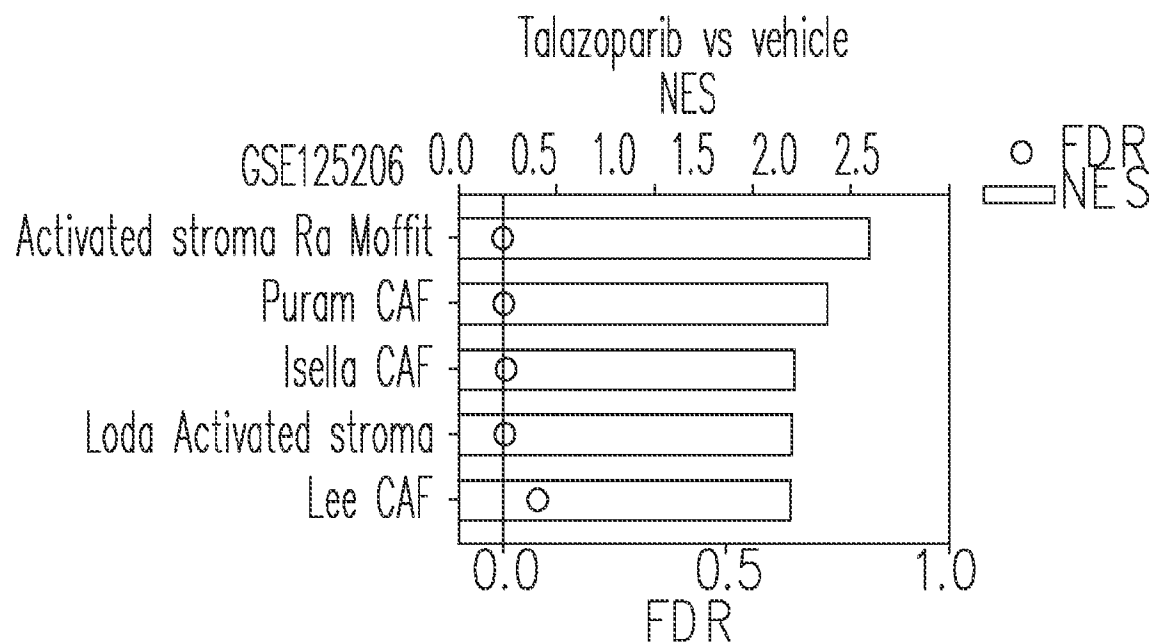
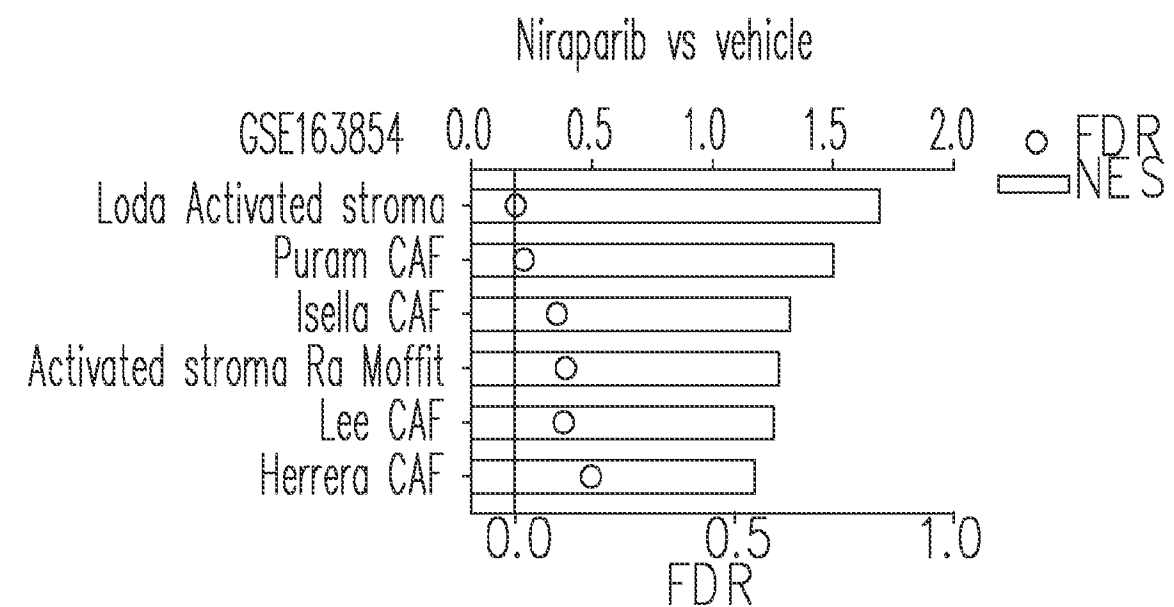
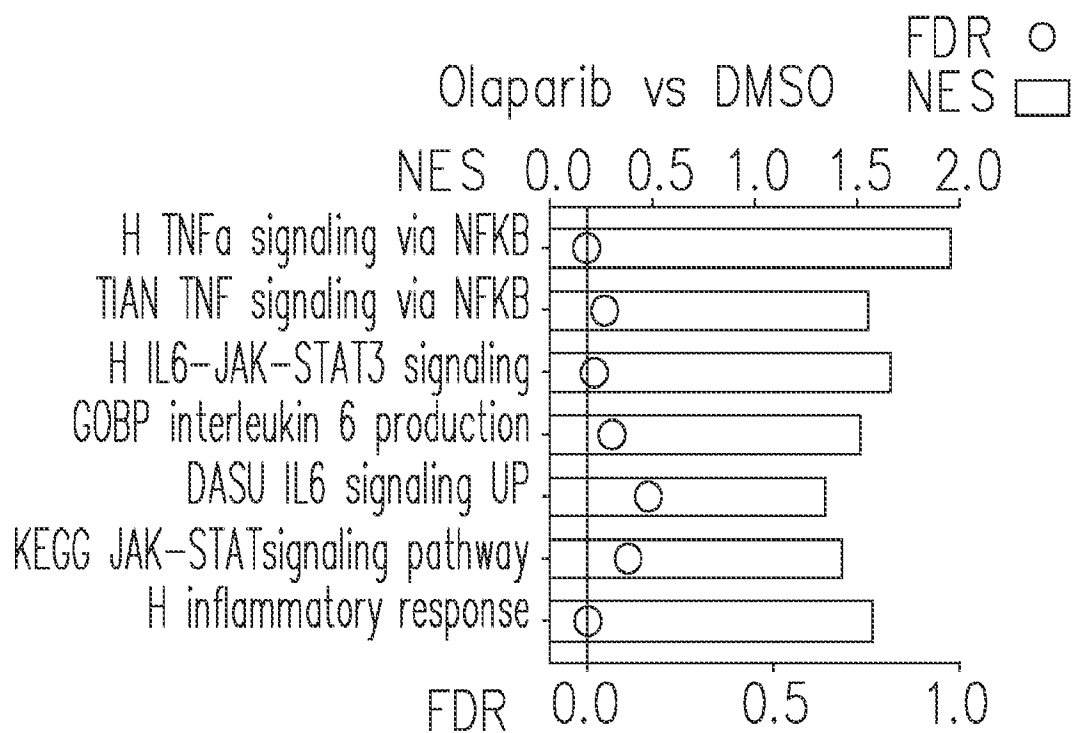


Fig. 13D

*Fig. 13E**Fig. 13F*

*Fig. 14A*

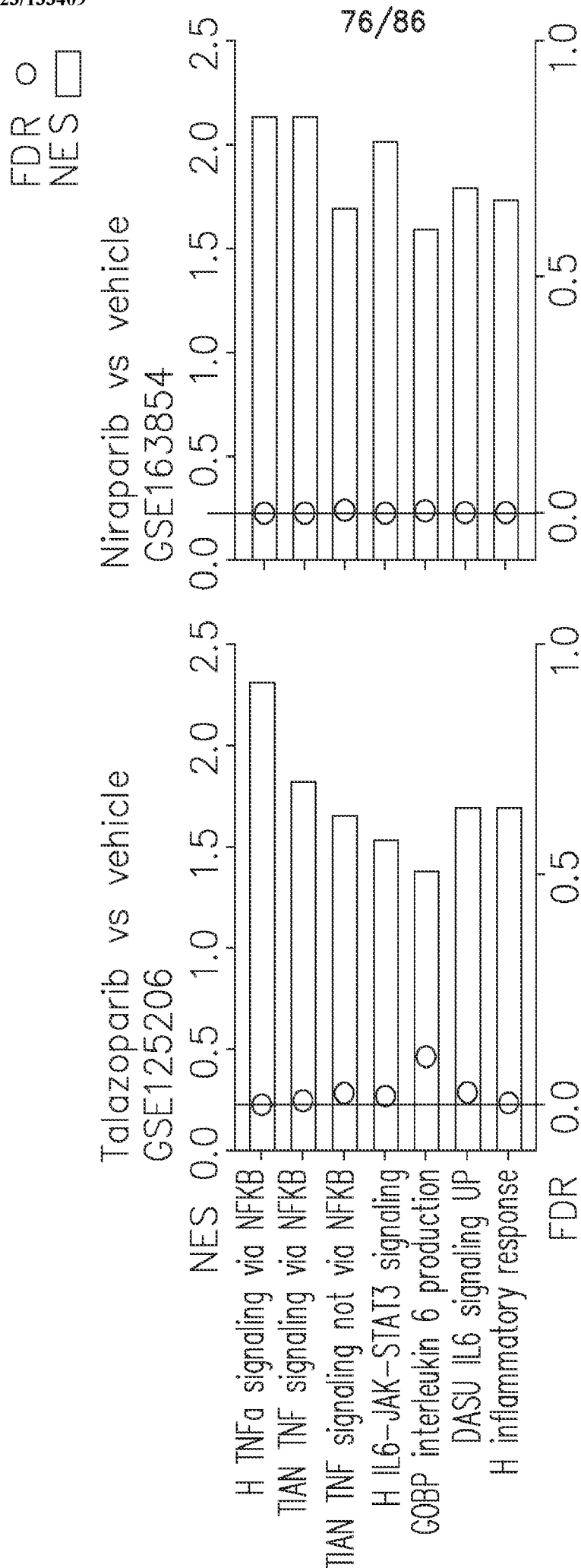
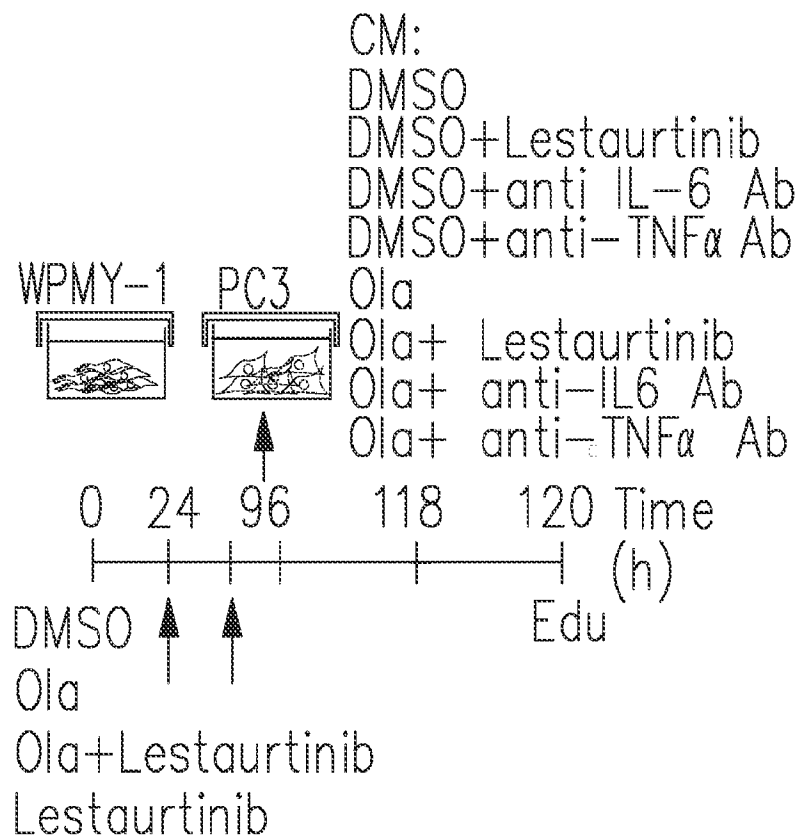


Fig. 14B

*Fig. 14C*

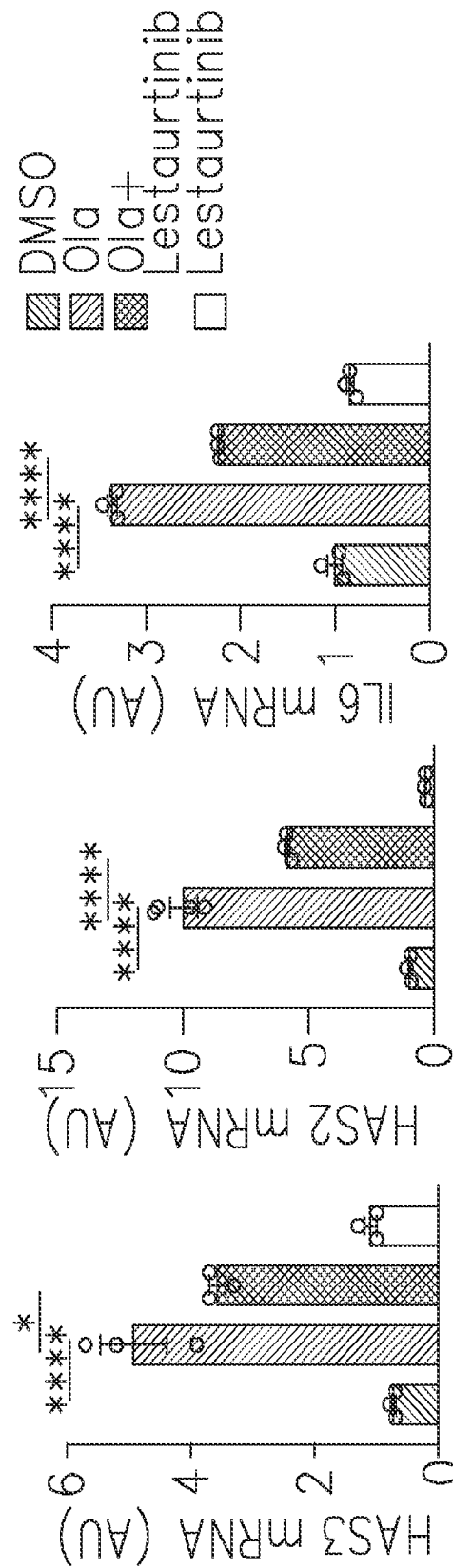


Fig. 14D

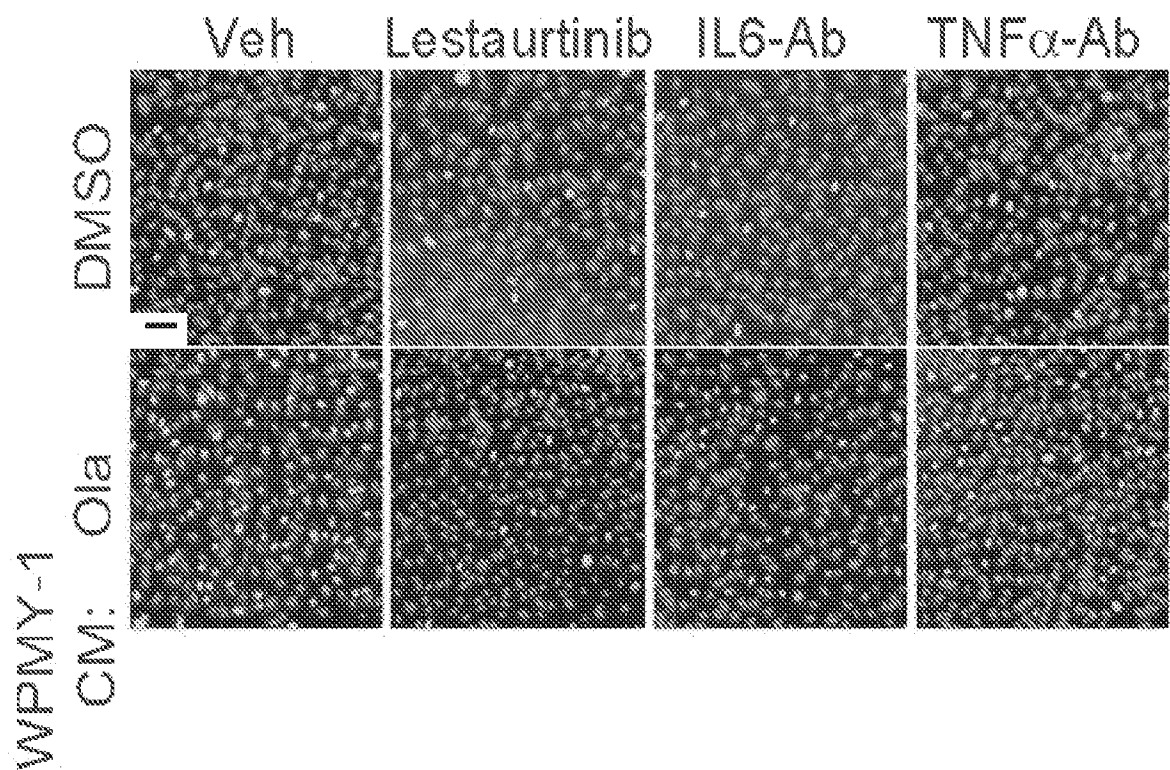


Fig. 14E

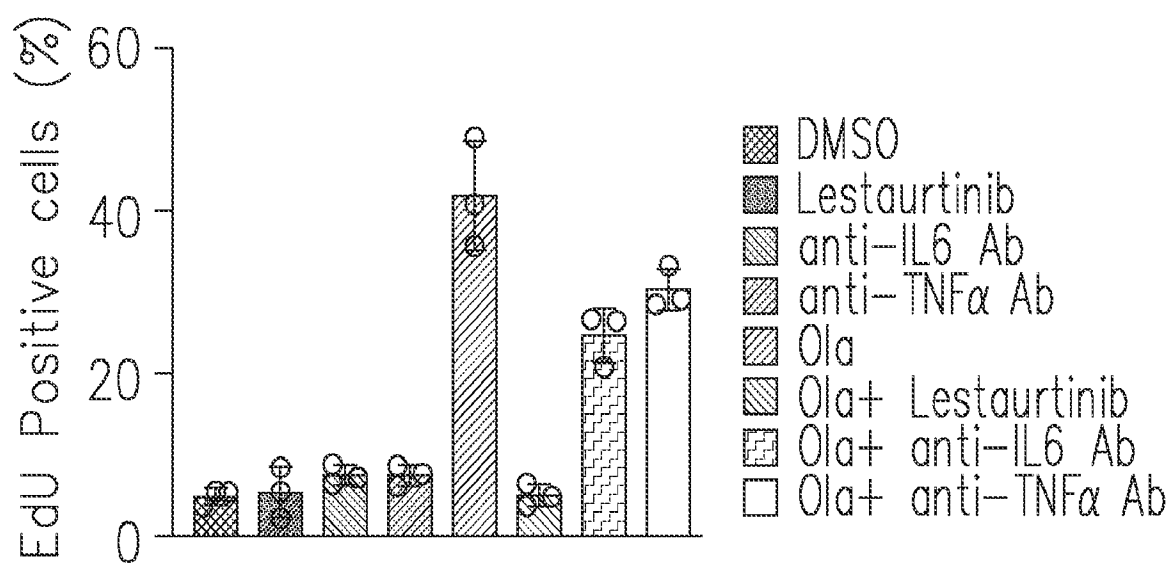
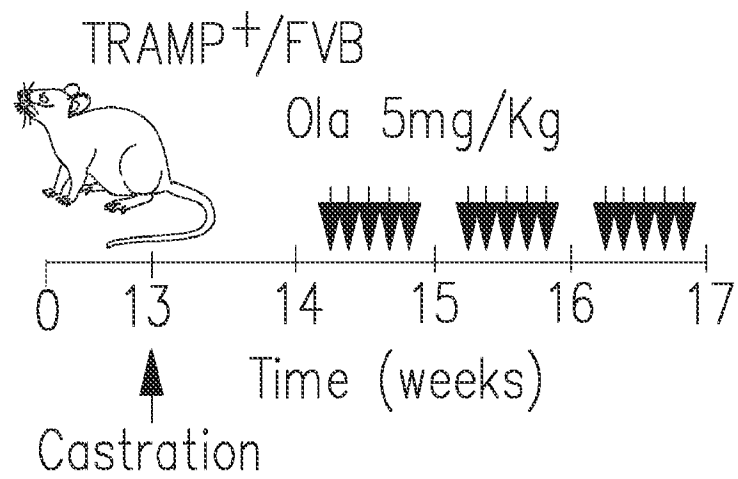
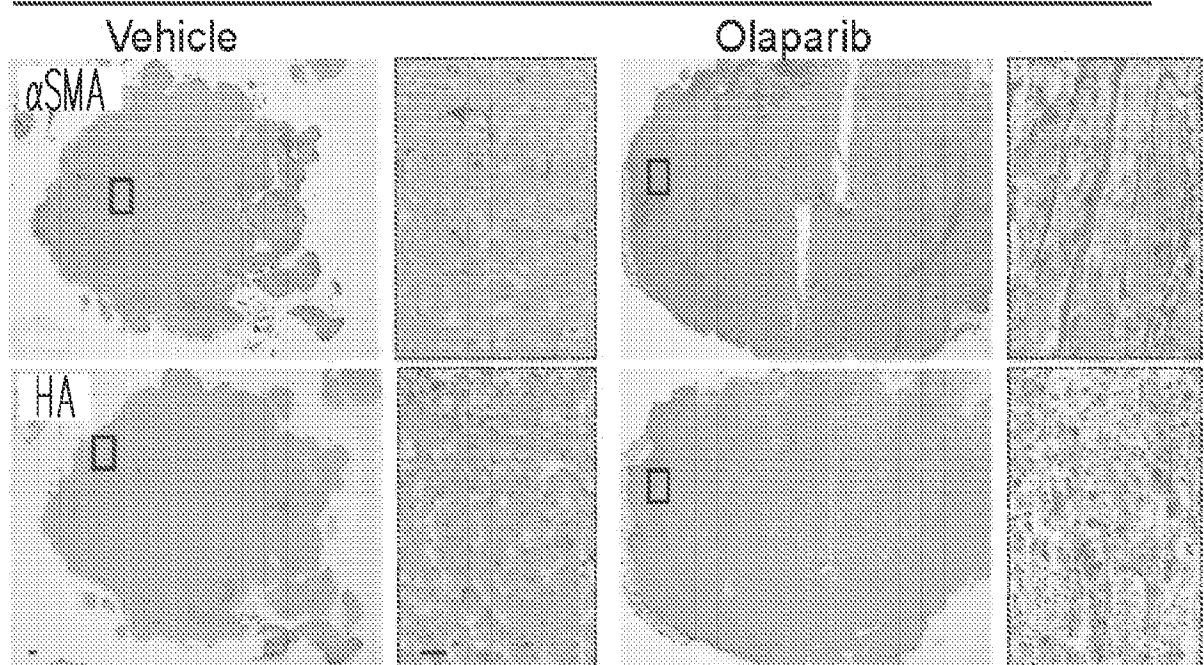
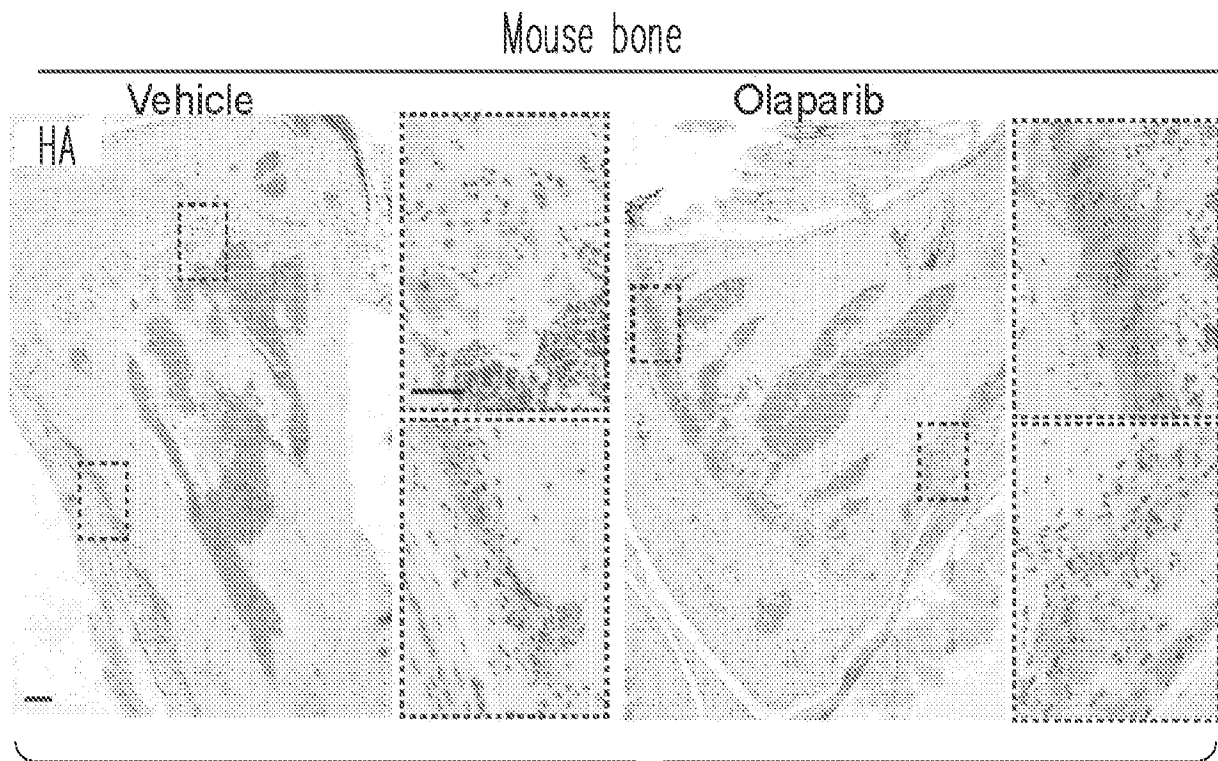
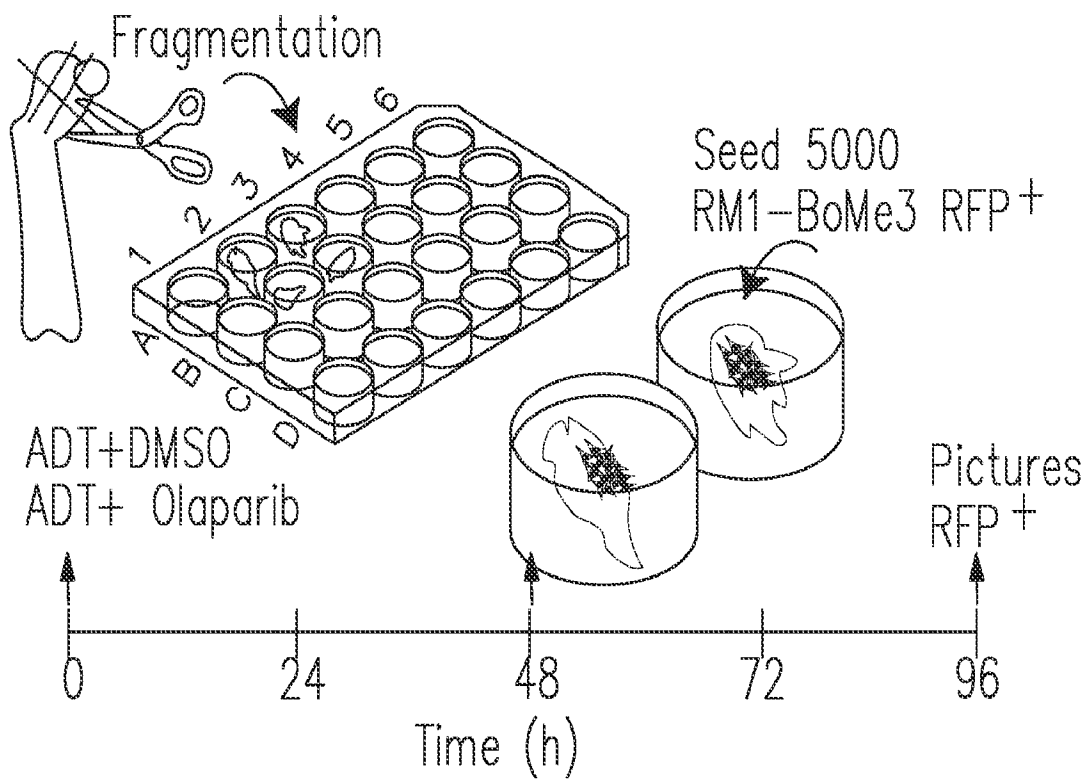


Fig. 14F

*Fig. 15A*

Mouse LN-met

*Fig. 15B*

*Fig. 15C**Fig. 15D*

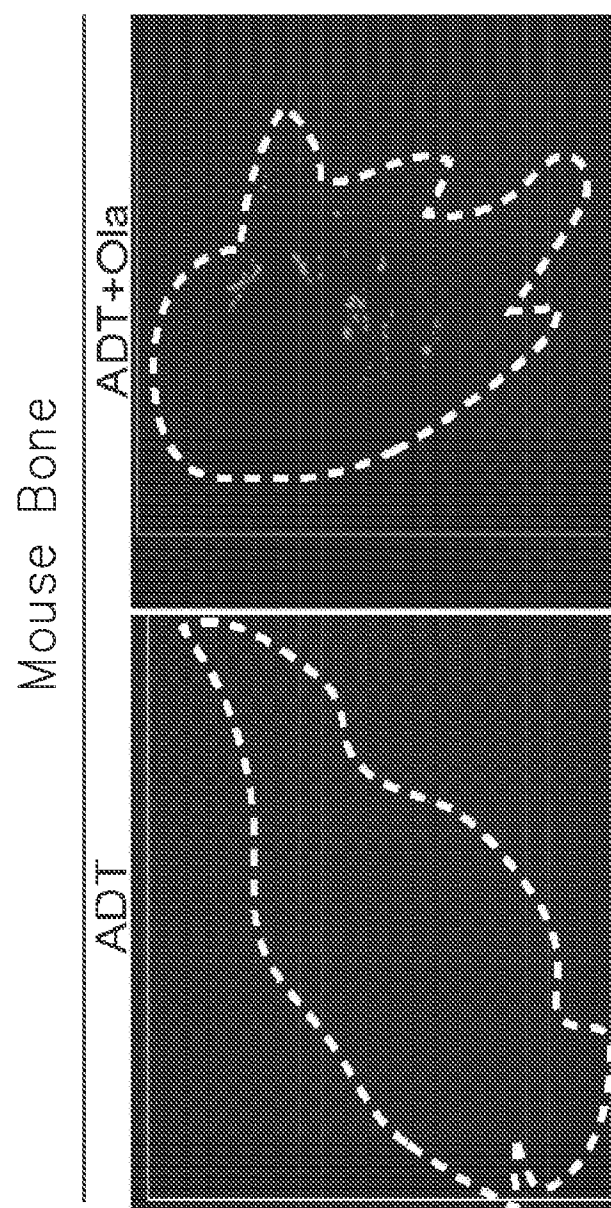
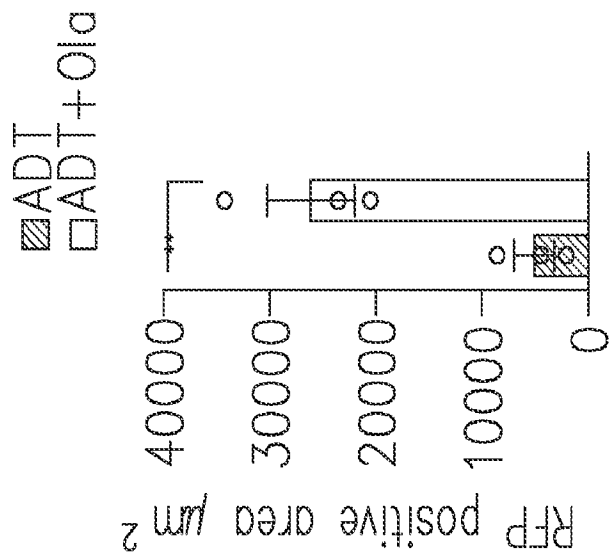
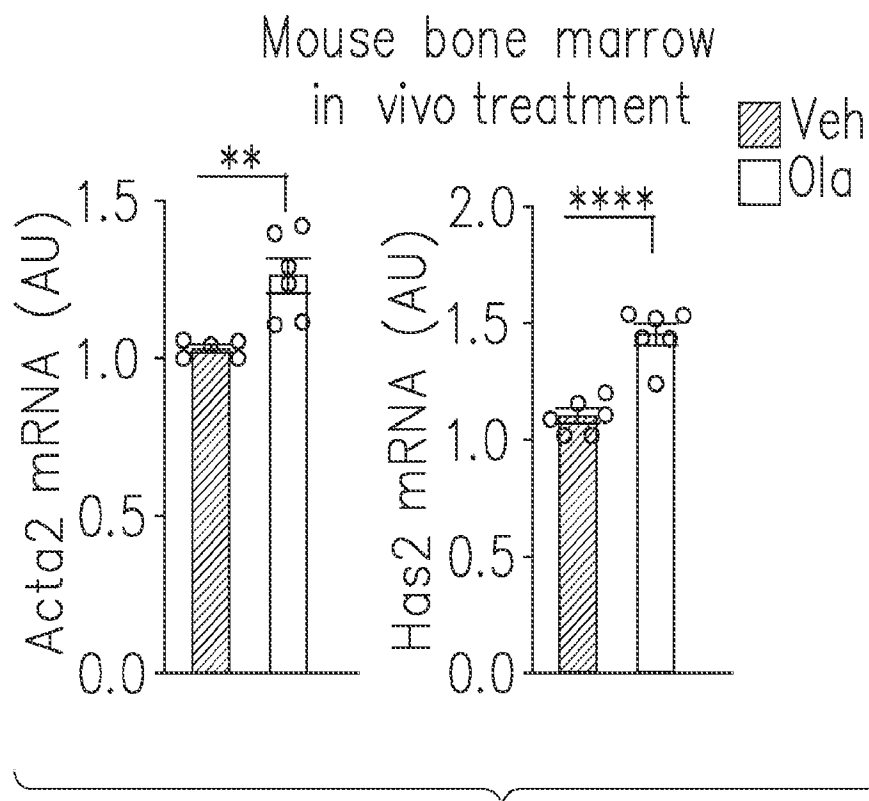
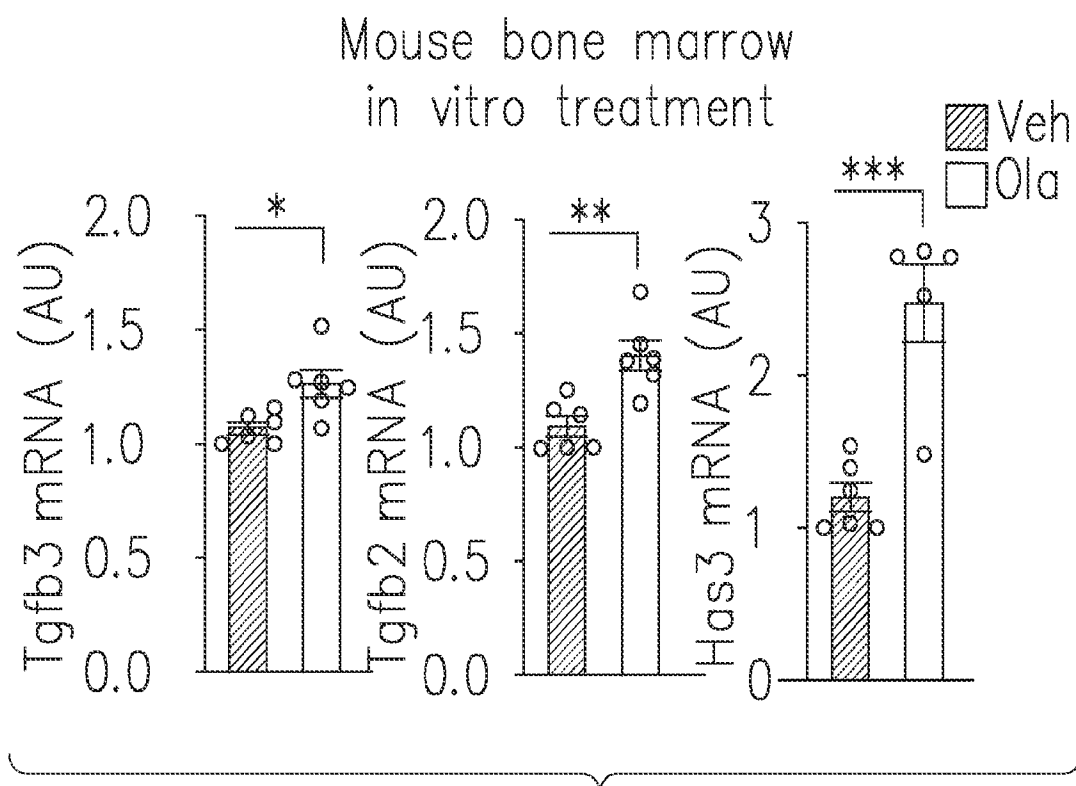
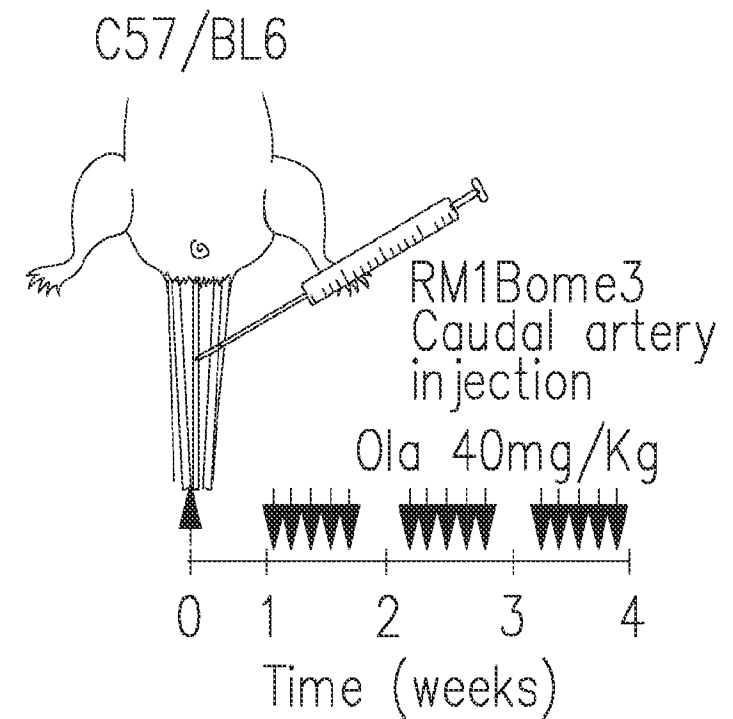
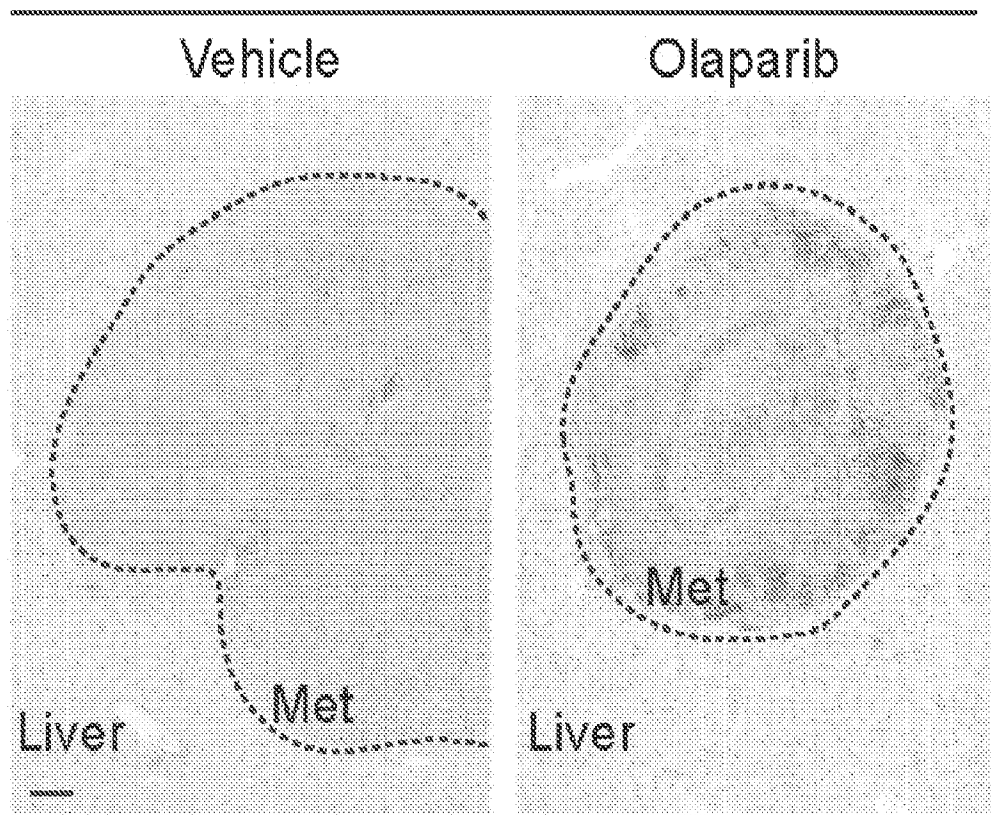


Fig. 15E

*Fig. 15F**Fig. 15G*

*Fig. 15H*

Mouse Liver—met

*Fig. 15I*

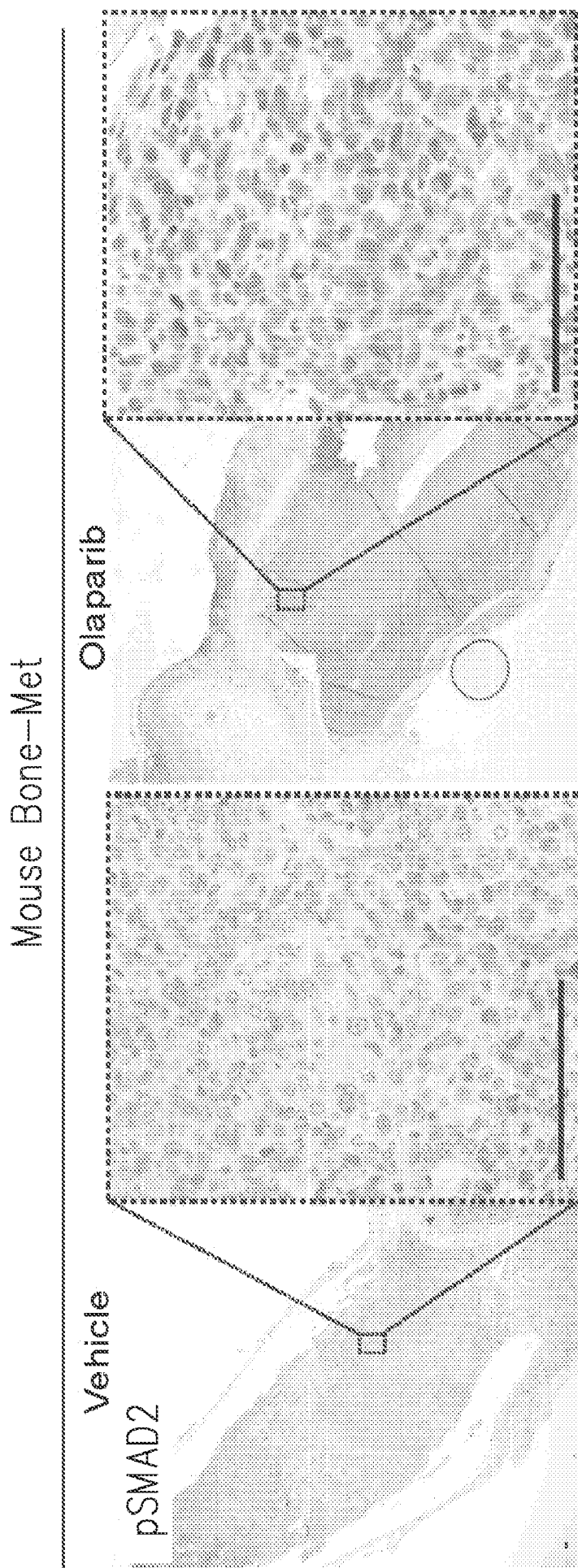


Fig. 15J

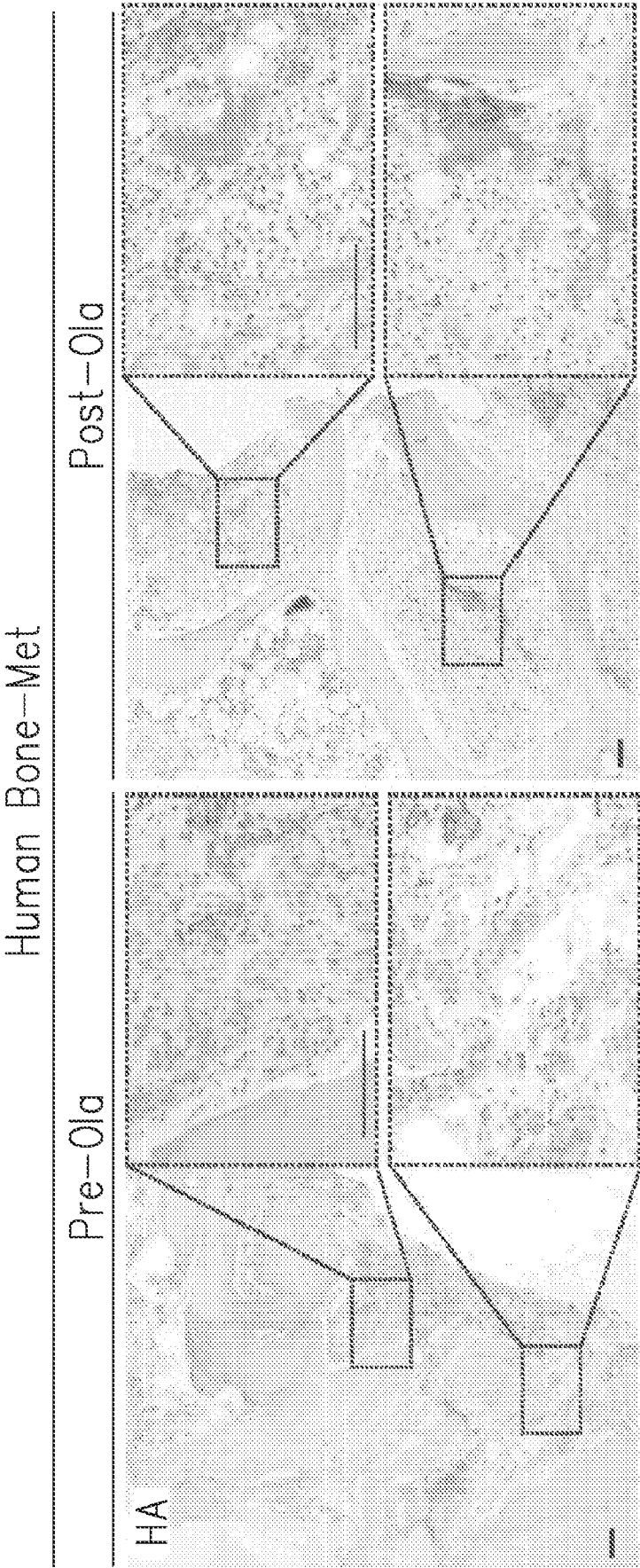


Fig. 15K