A iNFiXion BioScience neurofibromin iNF-07E antibody epitope schematic



## **B** Neurofibromin iNF-07E antibody IHC signal is *NF1*-dependent





## C Neurofibromin iNF-07E antibody is specific to neurofibromin and preferentially binds neurofibromin at 250 kDa



## Supplementary Figure 1: iNFiXion NF1 iNF-07E antibody data

A) iNFiXion BioScience neurofibromin iNF-07E antibody epitope schematic. The iNF-07E neurofibromin antibody binds at amino acids 861-884 compared to the Bethyl neurofibromin antibody, which binds at amino acids 2760-2818. Data provided by iNFiXion BioScience. B) iNFiXion BioScience neurofibromin iNF-07E antibody IHC signal is *NF1*-dependent. Data provided by iNFiXion BioScience. C) iNFiXion BioScience neurofibromin iNF-07E antibody western blot signal is specific to neurofibromin and preferentially binds neurofibromin at 250kDa. Data provided by iNFiXion BioScience.



Dialysis depletes polar and Β transition metabolites 3×10<sup>7</sup> **¬** 2×10<sup>7</sup> -

🕹 💑 👁

1×10<sup>7</sup> -

8×10<sup>7</sup> -

6×10<sup>7</sup> –

4×10<sup>7</sup>

2×10<sup>7</sup> ·

4×10<sup>6</sup> -

3×10<sup>6</sup> –

2×10<sup>6</sup> -

- derived compounds Glutamine **FBS** dFBS Z-Score 2  $-\infty$ 0 -1 Gluta -**O**-**O**-**O**-Gluc D
- FBS dialysis decreases most metabolites but С introduces SnakeSkin<sup>™</sup> Dialysis Tubing-



#### M3/M5 Glutamate D

25

20

15

10

5

NFAIEN

ns

NFAMS





**Biological Replicate** (z-score calculated from mean peak area)

## Supplementary Figure 2: FBS dialysis depletes most measured metabolites A) PCA analysis complete vs. dialyzed FBS (n=3, 3 technical replicates per biological replicate). B) Comparison of selected metabolite peak values between complete and dialyzed FBS (n=3, 3 technical replicates per biological replicate). C) Comparison of relative abundance of all measured and identified metabolites between complete and dialyzed FBS (n=3, 3 technical replicates per biological replicate). D) NF1 deficiency does not impact the ratio of M+3 glutamate to M+5 glutamate (n=1, 3 technical replicates per biological replicate, sig. calculated by Welch t-test)

#### MCF7 Inhibitor Dosage References Α

Drug	Dose Range	Publication	Metabolic Effect	Proliferative / Viability Effect
Tamoxifen	100nM	Yee et al. 2017	NA	~50% reduction in cell viability
Cobimetinib	100nM	Mills et al. 2023	NA	~25% reduction in cell viability
Everolimus	25nM	Lewis-Wambi et al. 2016	NA	50% reduction in cell viability
2DG	5mM	Prehn et al.	Increased intracellular glucose	NA
BPTES	10µM	Jeong et al. 2019	No change in intracellular glutamine, decreased GLS protein expression	No proliferative or viability effect
	10µM	Di et al. 2015	Decreased glutamine uptake, decreased ATP	NA
	20µM	Raftery et al. 2018	Decreased intracellular glutamate	NA
Etomoxir	10-200µM	Patti et al. 2018	Inhibited fatty acid oxidation at $10\mu$ M, Complex I inhibition at $200\mu$ M	Decreased cell proliferation at 200µM
PF-06424439	10µM	Frasor et al. 2018	Decreased triglycerides after treatment (combo DGAT1/2i)	Decreased final confluency
PF-04620110	10µM	Seco et al. 2021	Decreased lipid droplets, altered lipid gene expression	Decreased cell proliferation at 100µM
	10µM	Frasor et al. 2018	Decreased triglycerides after treatment (combo DGAT1/2i)	Decreased final confluency

#### Inhibitor protein target mRNA expression B



С	2DG (HEXi)			BPTES	6 (GLSi)			E		Etomoxi	r (CPT1i)	Bliss
	NF1-EV NF1-45		NF1	EV		NF1-45				NF1-EV	NF1-45	
200	0 19.56 26.96 31.14 0 19.38 37.72 45.43 ±0 ±0.46 ±0.25 ±0.081 ±0 ±0.13 ±0.36 ±0.12	200	$\begin{array}{c} 0 & 9.9 \\ \pm 0 & \pm 0. \end{array}$	4 18.26 59 ± 0.49	0 ± 0	26.78 ± 0.26	35.31 ± 0.3	200	0 ± 0	10.2819.3533.17± 0.2± 0.3± 0.38	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	
کر 100 ا	013.9923.8828.9014.5930.0542.72± 0± 0.067± 0.18± 0.095± 0± 0± 0.15± 0.54± 0.23	โ พี่น 100	$\begin{array}{c} 0 & 5.6 \\ \pm 0 & \pm 0. \end{array}$	514.1641± 0.4	0 ± 0	19.58 ± 0.058	28.36 ± 0.24	<u>کر</u> 100	0 ± 0	7.591329.29± 0.22± 0.21± 0.14	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	50
40H 50	011.5122.627.5012.8828.3440.19± 0± 0.26± 0.29± 0.22± 0± 0.43± 0.49± 0.29	40H1 20	0 9.7 ±0 ±0.	914.6613± 0.37	0 ± 0	15.5 ± 0.26	26.38 ± 0.34	40H 50	0 ± 0	6.259.4124.6± 0.17± 0.18± 0.16	0-0.1617.9341.46 $\pm 0$ $\pm 1.35$ $\pm 0.24$ $\pm 0.57$	0
0	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	0	$\begin{array}{c} 0 & 0 \\ \pm 0 & \pm \end{array}$	$\begin{array}{c} 0 \\ \pm 0 \end{array}$	0 ± 0	0 ± 0	0 ± 0	0	0 ± 0	$\begin{array}{c ccc} 0 & 0 & 0 \\ \pm 0 & \pm 0 & \pm 0 \end{array}$	$\begin{array}{c ccccc} 0 & 0 & 0 & 0 \\ \pm 0 & \pm 0 & \pm 0 & \pm 0 \end{array}$	50
200	0 15.63 35.85 39.2 0 24.75 46.69 50.95 ±0 ±0.21 ±0.31 ±0.29 ±0 ±0 ±0.25 ±0.36 ±0.33	200	$\begin{array}{c} 0 & 8.6 \\ \pm 0 & \pm 0. \end{array}$	7 12.57 33 ± 0.24	0 ± 0	34.59 ± 0.47	41.11 ± 0.32	200	0 ± 0	11.217.1129.54± 0.26± 0.26± 0.33	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	3
(کے 100	0 11.23 32.79 35.31 0 20.02 43.18 47.8 ±0 ±0.19 ±0.3 ±0.21 ±0 ±0.18 ±0.43 ±0.23	(Wu) 100	$\begin{array}{c} 0 & 2.6 \\ \pm 0 & \pm 0. \end{array}$	4 11.17 12 ± 0.3	0 ± 0	30.06 ± 0.15	39.37 ± 0.13		0 ± 0	6.4314.5727.37± 0.42± 0.28± 0.25	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	,
BO So 50	07.5630.5732.1018.0942.1945.81±0±0.39±0.32±0.25±0±0±0.35±0.51±0.35	В ОО ОО 50	$ \begin{array}{ccc} 0 & 0.3 \\ \pm 0 & \pm 0. \end{array} $	9 10.45 36 ± 0.11	0 ± 0	25.34 ± 0.46	32.98 ± 0.59	B O O 50	0 ± 0	3.3110.8323.96± 0.37± 0.25± 0.26	023.3733.8842.61 $\pm 0$ $\pm 0.52$ $\pm 0.66$ $\pm 0.68$	3
0	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	0	$\begin{array}{c c} 0 & 0 \\ \pm 0 & \pm \end{array}$	$\begin{array}{c c} 0 \\ \pm 0 \end{array}$	0 ± 0	0 ±0	0 ± 0	0	0 ± 0	$\begin{array}{c ccc} 0 & 0 & 0 \\ \pm 0 & \pm 0 & \pm 0 \end{array}$	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	
50	0 28.65 40.58 44.41 0 33.01 48.51 55.24 ±0 ±0.24 ±0.18 ±0.19 ±0 ±0.2 ±0.15 ±0.16	50	0 26.0 $\pm 0$	2 34.2 23 ± 0.27	0 ± 0	27.54 ± 0.47	35.83 ± 0.53	50	0 ± 0	21.09 26.19 38.48 ±0.75 ±0.81 ±0.99	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	
มี 25	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	(Wu) 25	$\begin{array}{c} 0 \\ \pm 0 \\ \end{array} \begin{array}{c} 27.8 \\ \pm 0. \end{array}$	8 36.16 21 ± 0.23	0 ± 0	27.88 ± 0.29	36.59 ± 0.35	آل     25	0 ± 0	10.0814.6933.71± 0.79± 0.57± 0.4	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	5
ш Ад 12.5	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Ⅲ 入 Ⅲ 12.5	0 21.3 $\pm 0$ $\pm 0.$	3 30.66 53 ± 0.33	0 ±0	23.95 ± 0.48	33.33 ± 0.45	Ш 12.5	0 ± 0	17.5217.7535.43± 0.4± 0.92± 0.6	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	5
0	$ \begin{vmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ \pm 0 & \pm$	0	$ \begin{array}{c c} 0 & 0 \\ \pm 0 & \pm 0 \end{array} $	$\begin{array}{c c} 0 \\ \pm 0 \end{array}$	0 ±0	0 ±0	0 ± 0	0	0 ± 0	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	
I	0 2 10 20 0 2 10 20		0 5	. 10	0	5	10	L	0	10 100 200	0 10 100 200	<b>_</b>

20 10 20 10 U U Ζ 2DG (mM)

F DGAT2i (PF-06424439) G DGAT1/2i (PF-04620110, PF-06424439) NF1-45 NF1-EV NF1-EV NF1-45 25.33 27.12 28.13 31.01 38.28 43.43 23.17 24.04 36.33 6.82 10.11 7.6 0 200 200 ± 0.15 ± 0.37 ± 0.35 ± 0.4 ± 0.18 ± 0.21 ± 0.39 ± 0.36 ± 0.34 ± 0 ±0.1 ±0.15 ±0  $\pm 0$ ±0 ± 0.14 (Wu 100 (Mn) 23.23 25.72 21.32 18.29 20.37 29.27 34.24 35.99 7.15 6.44 11.11 0 100 ± 0.14 ± 0.071 ± 0.44 ± 0.21 ± 0.38 ± 0.11 ±0 ± 0.39  $\pm 0.087 \pm 0.13 \pm 0.04$ ± 0.24 ± 0.21 ±0 ±0  $\pm 0$ 40HT 40HT 20.24 22.64 20.57 28.85 16.73 14.68 32.15 6.59 9.04 11.51 35.03 41.31 0 50 50  $\pm 0.26 \pm 0.24 \pm 0.19$ ± 0.66  $\pm 0.62 \pm 0.33$  $\pm 0.16 \pm 0.17 \pm 0.13$ ±0 ± 0.27 ± 0.43 ±0.4 ±0 ±0  $\pm 0$ 0 0 0 0 0 0 ±0 ±0 ±0 ±0 ±0 ±0 ±0  $\pm 0$  $\pm 0$ ± 0 ± 0 ± 0 ±0  $\pm 0$ ±0 26.22 30.68 47.69 22.74 43.15 54.05 16.82 22.53 26.96 36.84 41.43 45.52 200 200 ± 0.15 ± 0.17 ± 0.18 ± 0.37 ± 0.22 ± 0.27 ± 0.14 ± 0 ± 0.21 ±0.3 ± 0.26 ± 0.29 ± 0.38 ± 0 ±0 ±0 <u>ک</u> 100 (Mn) 20.8 23.91 30.6 38.7 51.87 28.08 34.75 39.31 44 21.21 100 ± 0.19 ± 0.092 ± 0.23 ± 0.27 ± 0.11  $\pm 0$   $\pm 0.087$ ± 0.19 ± 0.21 ± 0.17 ±0  $\pm 0.14$   $\pm 0.26$   $\pm 0.14$ ± 0  $\pm 0$ COB COB 22.51 28.01 31.61 39.22 47.01 20.69 26.27 33.22 38.26 16.64 15.09 0 50 50 ± 0.28  $\pm 0.29 \pm 0.35$ ± 0.28 ± 0.35 ± 0.25 ± 0.22  $\pm 0.38$   $\pm 0.19$   $\pm 0.22$ ± 0  $\pm 0.14$ ±0 ± 0.16 ± 0 ±0 ±0 ±0 ±0 ±0 ± 0 ±0 ± 0 ±0 ±0  $\pm 0$  $\pm 0$  $\pm 0$ ±0 ± () 47.79 52.07 42.02 23.41 25.68 27.22 35.23 39.28 43.75 13.73 12.61 14.2 50 50 ± 0.12 ± 0.2  $\pm 0.11 \pm 0.12 \pm 0.15$ ± 0.21 ± 0.22 ± 0.26 ± 0.49 ± 1.26 ±0 ± 0.23 ±0 ± 0.36 ±0  $\pm 0$ (MU) (Mn) 41.42 47.14 51.8 27.04 29.84 32.9 36.48 25.22 15.23 15.92 13.01 25 25  $\pm 0.083 \pm 0.21 \pm 0.052$ ± 0.099 ± 0.2 ± 0.086  $\pm 0.56 \pm 0.82$  $\pm 0.098 \pm 0.16 \pm 0.036$ ± 0.52 ± 0 ± 0 ±0  $\pm 0$ Ш А\_12.5 Ш А\_12.5 41.65 47.66 51.66 29.63 32.34 30.81 36.3 26.51 17.07 18.49 20.12 ± 0.26 ± 0.17 ± 0.2 ± 0.2 ± 0.21 ± 0.16 ± 0.17 ±0.2 ±0.18 ± 0.82 ± 0.68 ±0 ± 0.7 ±0 ± 0 ±0 0 0 0 0 + 0+ 0+ 0**⊥** ∩ + 0+ 0+ 0

BPTES (mM)

100 10 200 100 200 U IU EX (µM)

Η

200

50

0

200

50

0

50

25

0

ш 212.5

<u>ک</u> 100

COB

(MU)

(آلا کے 100 (آلا

40HT

43.12

0

 $\pm 0$ 

45.98

44.5

 $\pm 0$ 

43.5

43.36

± 0	± 0	ΞU	<u> </u>	± 0	ΞŪ	±Ο	1 O	
0	5	10	20	0	5	10	20	
DGAT	2i (µM	)						

۲	± 0	± 0	±0	±0	± 0	±0	±0	± 0
_	0	5	10	20	0	5	10	20
l	DGAT	1/2i (µ	M)					

## Supplementary Figure 3: Drug synergy data

A) MCF7-specific targeted and metabolic inhibitor dose reference table. B) Mean inhibitor target mRNA expression (n=3) The bar represents the geometric mean, and the error bar boundaries represent the standard deviation. C) Raw Bliss synergy plots describing the synergy between 2DG and 40HT, COB, and EVE. Bliss synergy was calculated and visualized using the SynergyFinder R package (n=2, 8 technical replicates per biological replicate). D) Raw Bliss synergy plots describing the synergy between BPTES and 4OHT, COB, and EVE. Bliss synergy was calculated and visualized using the SynergyFinder R package (n=2, 8 technical replicates per biological replicate). E) Raw Bliss synergy plots describing the synergy between EX and 40HT, COB, and EVE. Bliss synergy was calculated and visualized using the SynergyFinder R package (n=2, 8 technical replicates per biological replicate). F) Raw Bliss synergy plots describing the synergy between DGAT2i and 40HT, COB, and EVE. Bliss synergy was calculated and visualized using the SynergyFinder R package (n=2, 8 technical replicates per biological replicate). G) Raw Bliss synergy plots describing the synergy between DGAT1 and DGAT2 combination treatment and 40HT, COB, and EVE. Bliss synergy was calculated and visualized using the SynergyFinder R package (n=2, 8 technical replicates per biological replicate).

## Raw doublings per day values for Bliss Synergy analysis



Supplementary Figure 4: Raw drug synergy doublings per day data A) Raw doublings per day values for combination inhibitor treatments. The bar represents the geometric mean, and the error bar boundaries represent the standard deviation (n=2, 8 technical replicates per biological replicate).

### Normalized doublings per day values for Bliss Synergy analysis



in doublings per day compared to vehicle

Chan

Ige

**Supplementary Figure 5: Normalized doublings per day values for Bliss Synergy analysis** A) Normalized doublings per day values for combination inhibitor treatments used for Bliss synergy analysis. The symbol represents the geometric mean, and the crossbar boundaries represent the 95% confidence interval (n=2, 8 technical replicates per biological replicate).