Supporting Information

Probing the Cytotoxic Signaling Induced by Eupenifeldin in Ovarian Cancer Models

Amanda C. Maldonado[1‡], Monica A. Haughan[2‡], Manead Khin[2], Julia Ekiert[2,] Ziwei Zhang[2], Daniel Lantvit[2], Zeinab Y. Al Subeh[3], Herma C. Pierre[4], Maryna Salkovski[5], Tal Hirschhorn[6], Yu (Tom) Gao[2], Cedric J. Pearce.[7], Brent R. Stockwell[6,8] Leslie N. Aldrich[5], Nicholas H. Oberlies[4], and Joanna E. Burdette[2*]

[1]Chicago Biomedical Consortium, Northwestern University, Evanston, Illinois, United States

[2]Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois, United States

[3]Department of Medicinal Chemistry and Pharmacognosy, Jordan University of Science and Technology, Irbid, Jordan

[4]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27402, United States

[5]Department of Chemistry, College of Liberal Arts and Sciences, University of Illinois at Chicago, Chicago, Illinois, United States

[6]Department of Biological Sciences, Columbia University, New York, NY, 10027, United States

[7]Mycosynthetix Inc., Hillsborough, NC 27278, United States

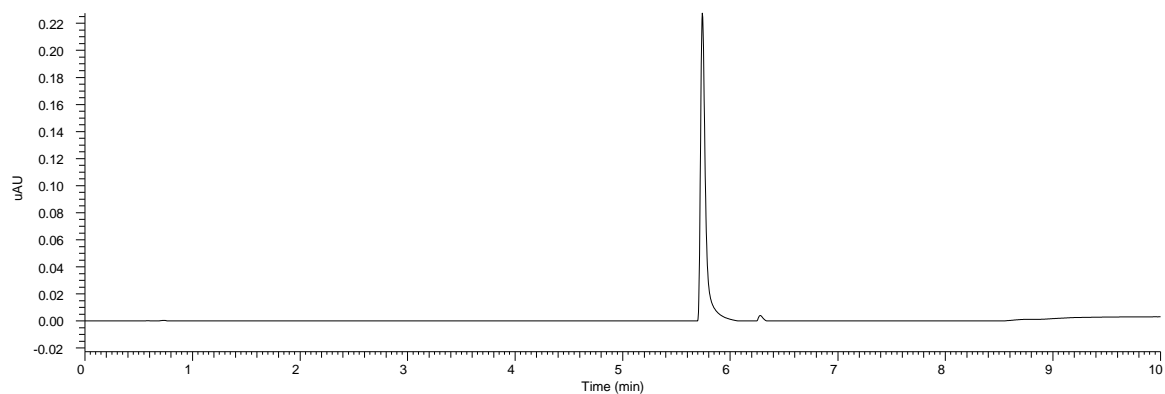[8]Department of Chemistry, Columbia University, New York, NY, 10027, United States

 *Corresponding author
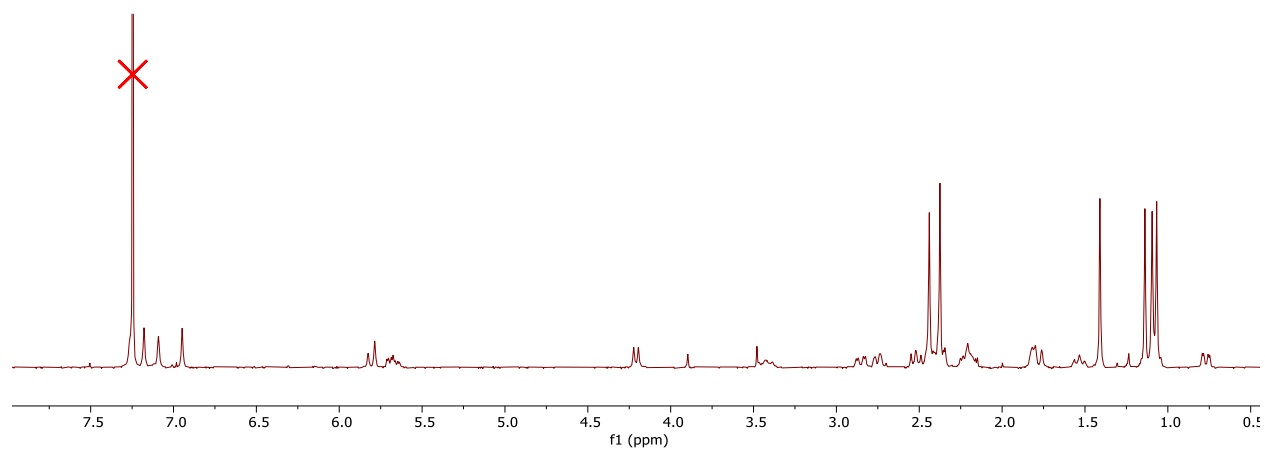
‡Equal contribution

TABLE OF CONTENTS

SUPPLEMENT 1- Eupenifeldin Isolation Purity Data

S1.1 Isolated eupenifeldin HPLC Data

^{1}H NMR Spectrum of Eupenifeldin (400 MHz, CDCl$_3$)


S1.2 Isolated eupenifeldin NMR Data

SUPPLEMENTAL 2 – Additional Hollow Fiber Assay Data

Treatment Group	Mouse ID number	Body Weight (g)					% Change
		Day 0	Day 2	Day 4	Day 6	Day 7	
Vehicle	101	23.5	22.2	22.4	21.8	21.8	7.23
	102	18.3	18.1	18.8	18.3	18.8	2.73
	103	20.9	20.2	20.2	20.1	20.0	4.31
	104	19.1	18.8	19.4	19.0	19.0	0.521
	105	19.1	19.3	19.8	19.6	19.4	1.57
	106	17.8	17.6	18.1	18.0	17.9	0.562
	107	16.9	16.8	16.9	16.6	16.8	0.592
	108	16.9	16.8	17.2	16.9	17.1	1.18
Taxol 2.8mg/kg	109	19.6	19.6	19.2	16.3	16.8	14.3
	110	22.4	22.5	21.2	20	20.4	8.93
	111	19.8	19.5	18.9	16.4	Died	17.2
	112	20.1	19.6	20.5	17.3	17.1	14.9
	113	19.4	19.2	19.3	17.3	Died	10.8
	114	19.7	19.8	19.2	16.9	17.1	13.2
	115	20.0	19.8	19.1	17.4	19.1	4.50
	116	19.9	19.2	18.9	17.4	18.0	9.55
Eupenifeldin 0.5mg/kg	117	17.4	17.7	17.6	16.6	16.1	7.47
	118	17.9	18.4	18.8	17.6	16.5	7.82
	119	16.0	16.7	16.0	14.9	14.1	11.88
	120	21.0	20.8	20.4	20.2	20.4	2.86
	121	17.6	18.1	17.3	16.5	16.1	8.52
	122	18.4	19.3	18.7	18.2	17.7	3.80
	123	19.5	20.3	20.2	19.3	18.8	3.59
	124	18.6	19.2	19.0	18.3	17.6	5.38

S2.1 Mouse body weights and percent change over the course of treatment in the hollow fiber assay.
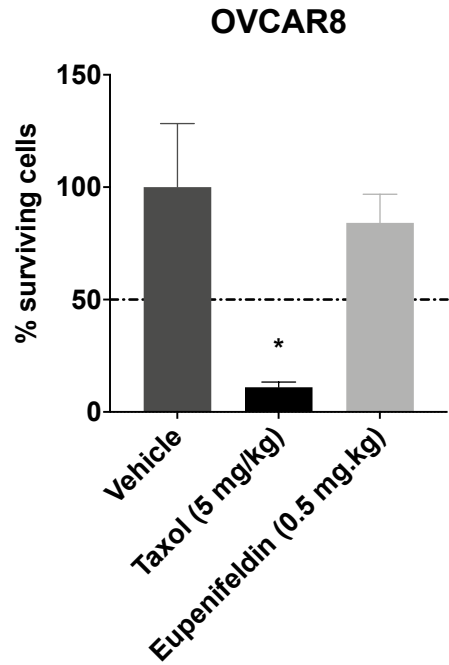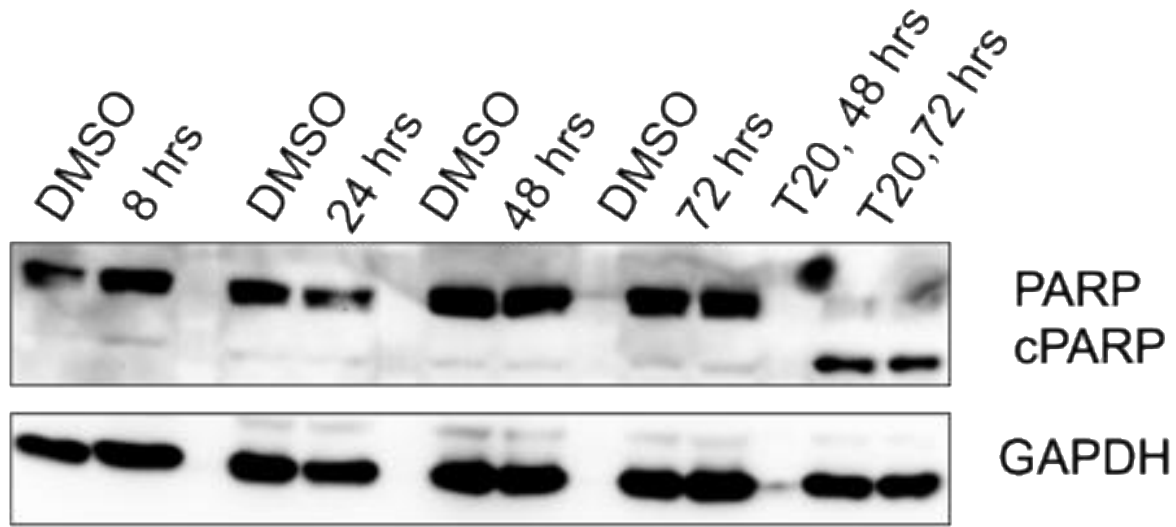
OVCAR8

S2.2 Percent surviving OVCAR8 cells from the *in vivo* hollow fiber assay.
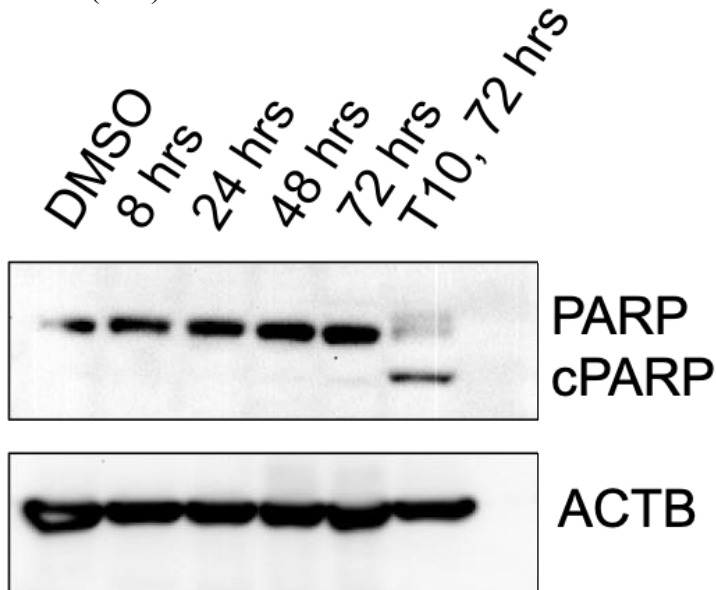
SUPPLEMENTAL 3 Annexin V vs PI
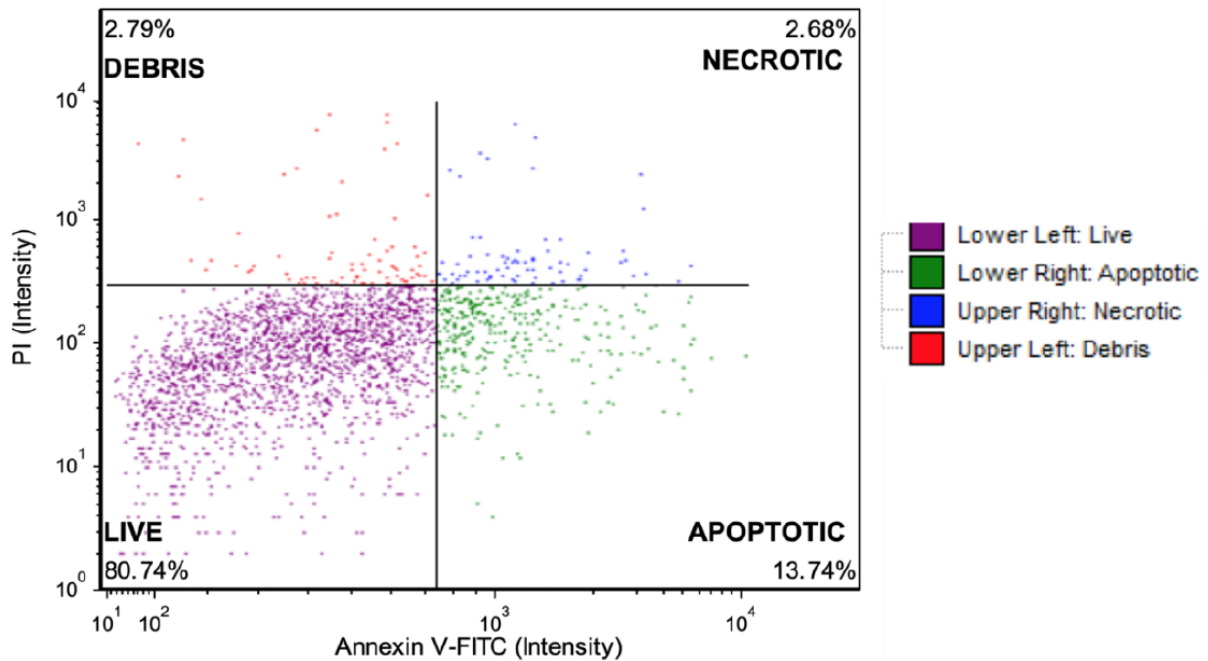
S3.1 Representative plot of DMSO Control.

SUPPLEMENTAL 4- Additional PARP Western Blots

S4.1 Representative western blot of OVCAR8 25nM eupenifeldin treatment compared to 20nM Taxol (T20).


**S4.2** Representative western blot of OVCAR5 25nM eupenifeldin treatment compared to 10nM Taxol (T10).

SUPPLEMENTAL 5 – Proteomic Analysis

Upregulated Proteins	
ASNS	TFRC
CD55	SLC3A2
ATP6V0D1	RAB2A
EIF2B1	FARSB
TMED2	GNAS
ATP2B1	EPHA2
WARS1	EEF1G
AARS1	SEC63
TMOD2	KPNB1
GFPT1	TARS1
PHGDH	UFL1
CANX	KARS1
ANXA5	KNG1
SARS1	CD44

Downregulated Proteins		
POLR2H	SCO1	TCOF1
ETFB	FAM136A	HEXB
FTL	PPIF	UQCRC2
RPL7	PPM1B	ABHD10
RPL11	ATP5MC1	RPL34
TOMM6	AK3	COX5A
COX5B	PHB2	ACO2
FKBP9	PHB	HADHB
RPS29	CTSB	NDUFB10
TUFM	SUCLA2	SDHA
PRDX3	RPL31	NDUFS1
IMMT	ATP5F1E	HIST1H2BO
OGDH	HINT2	RPL37A
PFN2	ATP5F1D	ATP5IF1
RPL10	DLST	ATP5PF
PDHA1	FAU	

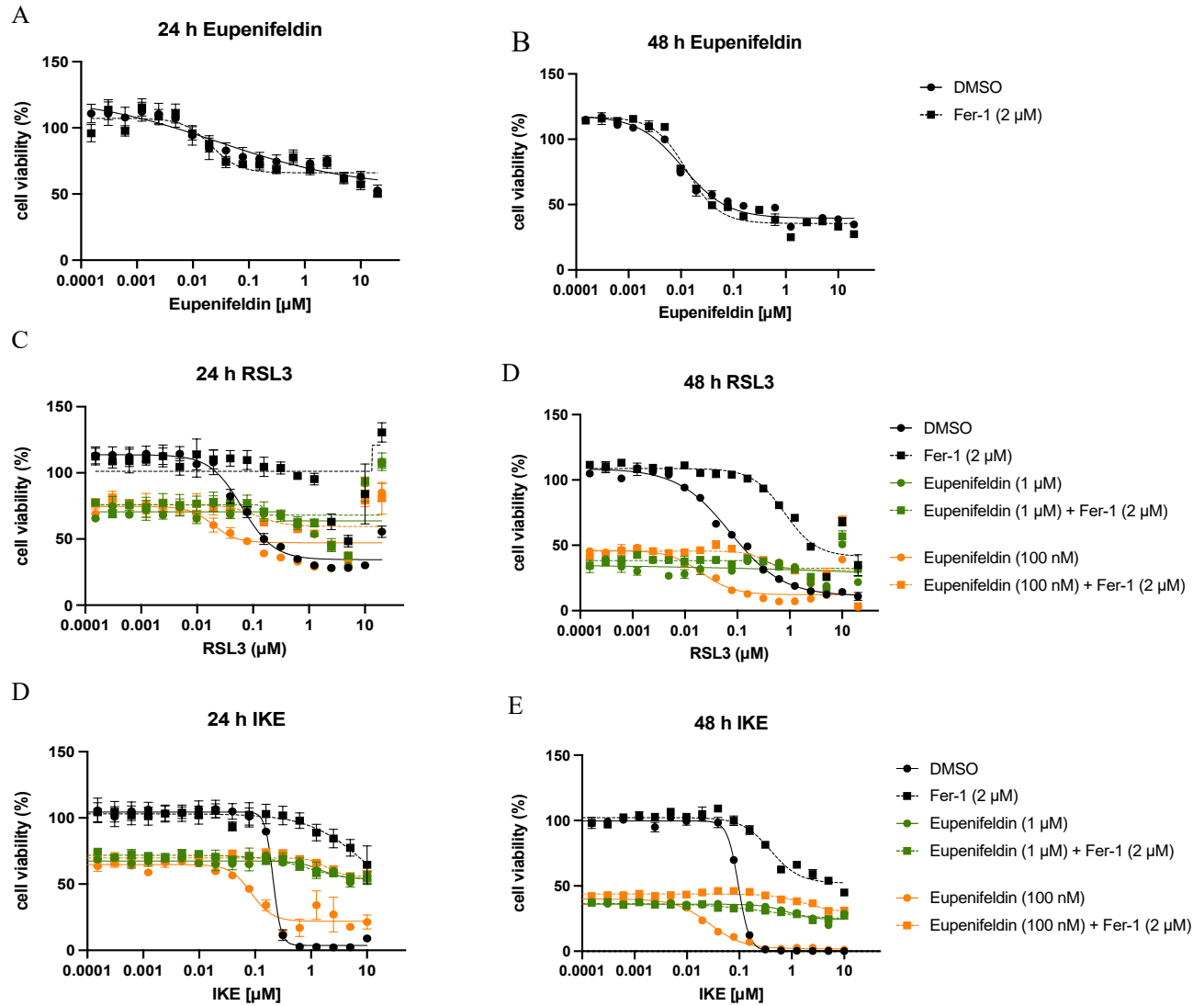**S5.1**- List of All Upregulated and Downregulated Proteins
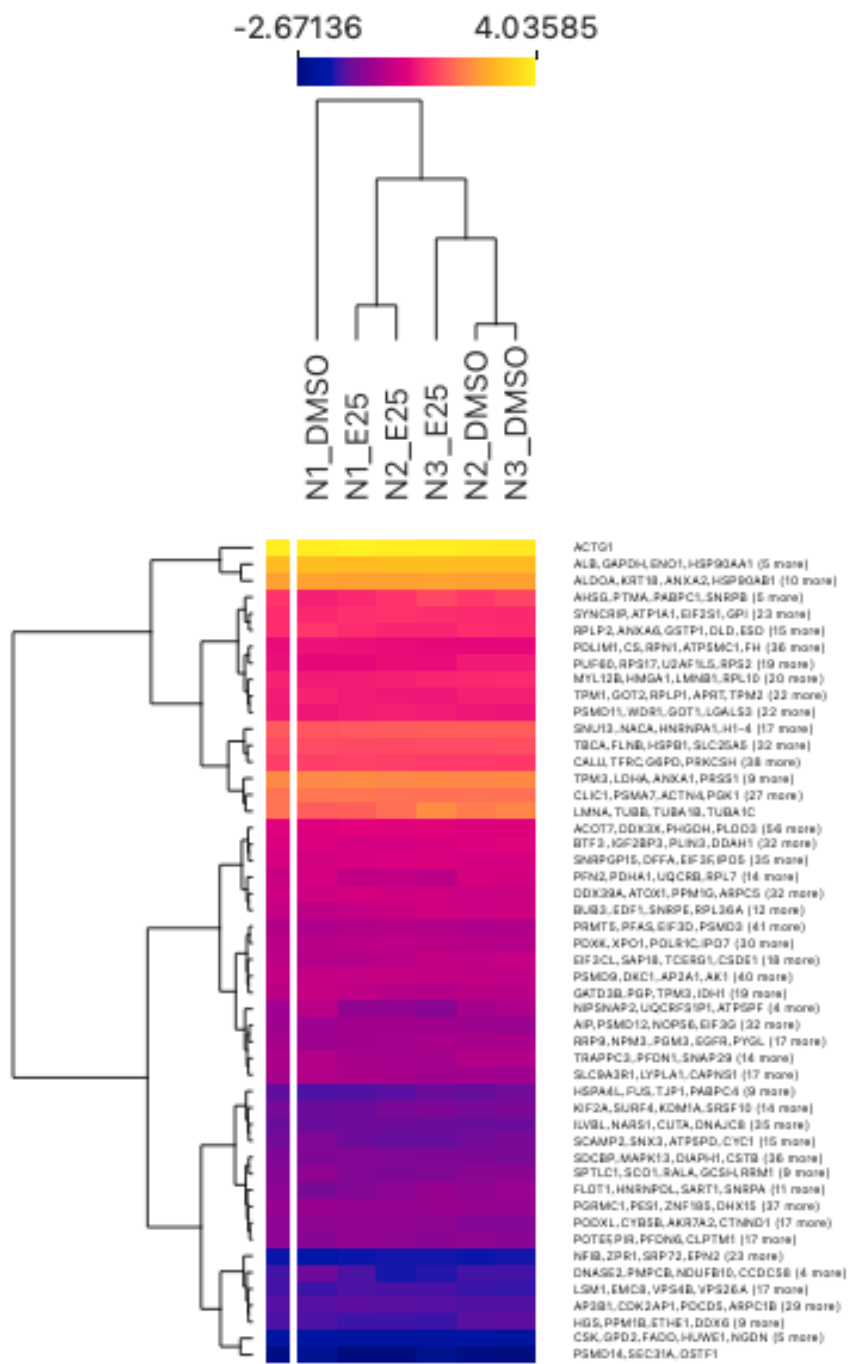
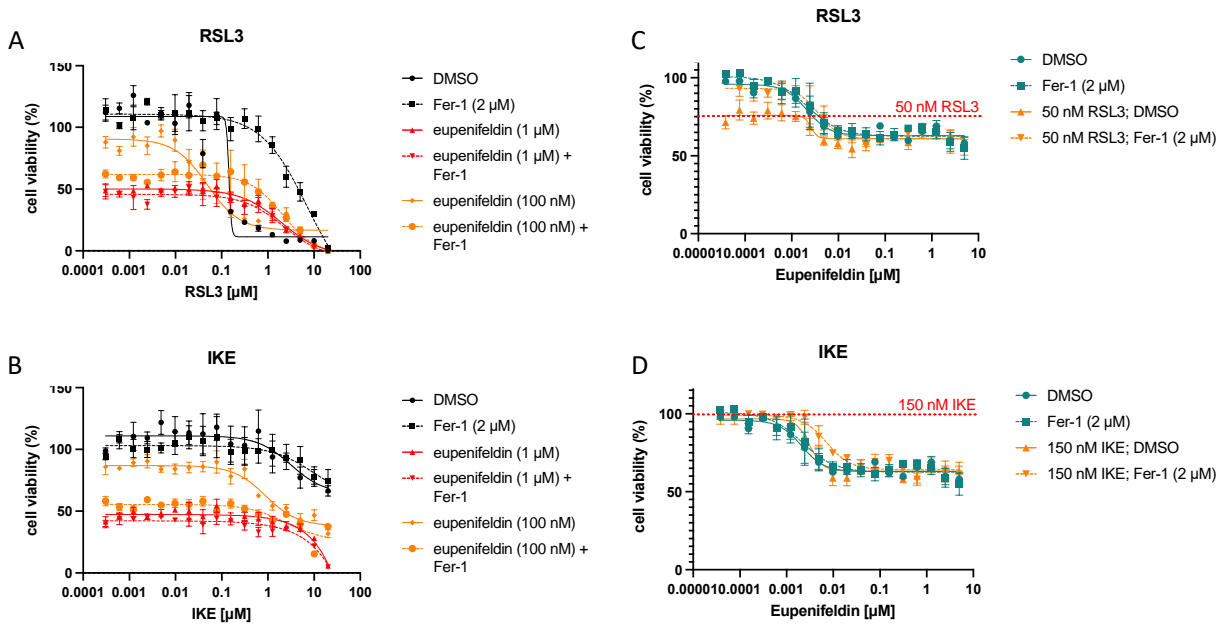**S5.2-** Heatmap of log transformed spectral intensities of all differentially expressed proteins
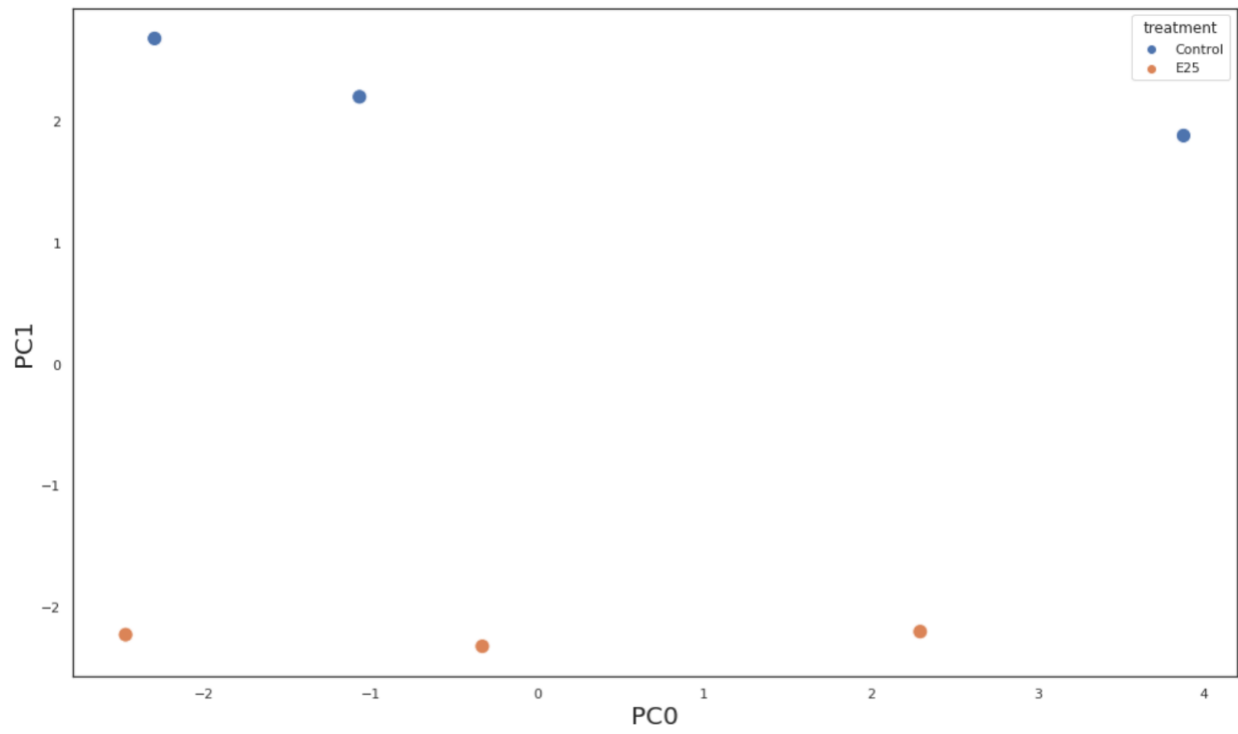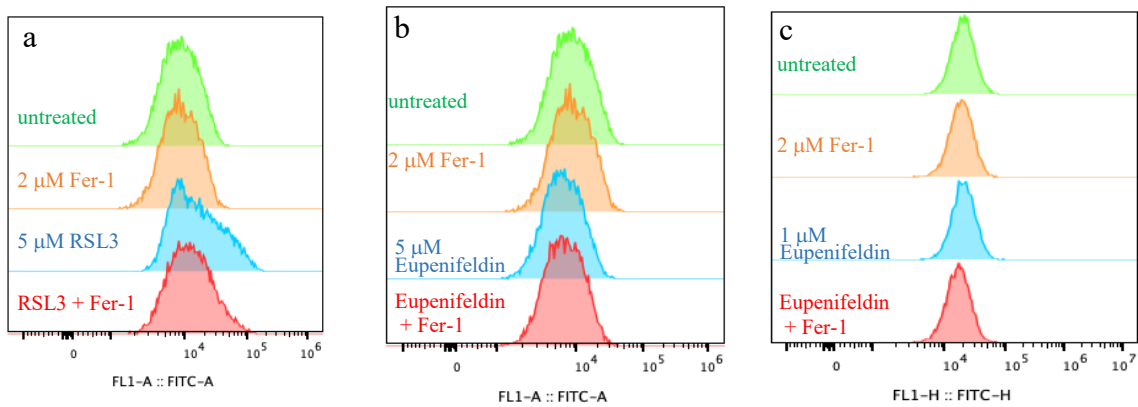
S5.3 Principle Component Analysis Plot

S6.1 HT-1080 cells treated with (A,B) a ferroptosis inducer in a 2-fold dilution series, in the absence or presence of 1 µM or 100 nM Eupenifeldin and (C-E) in combination with ferrostatin-1

S6.2 OVCAR3 potentiation by eupenifeldin for RSL3- or IKE-mediated ferroptosis

S6.3 Lipid Peroxidation Measurements in OVCAR3. (A) 3hr RSL Treatments (B) 3hr Eupenifeldin Treatments (C) 6hr Eupenifeldin Treatments