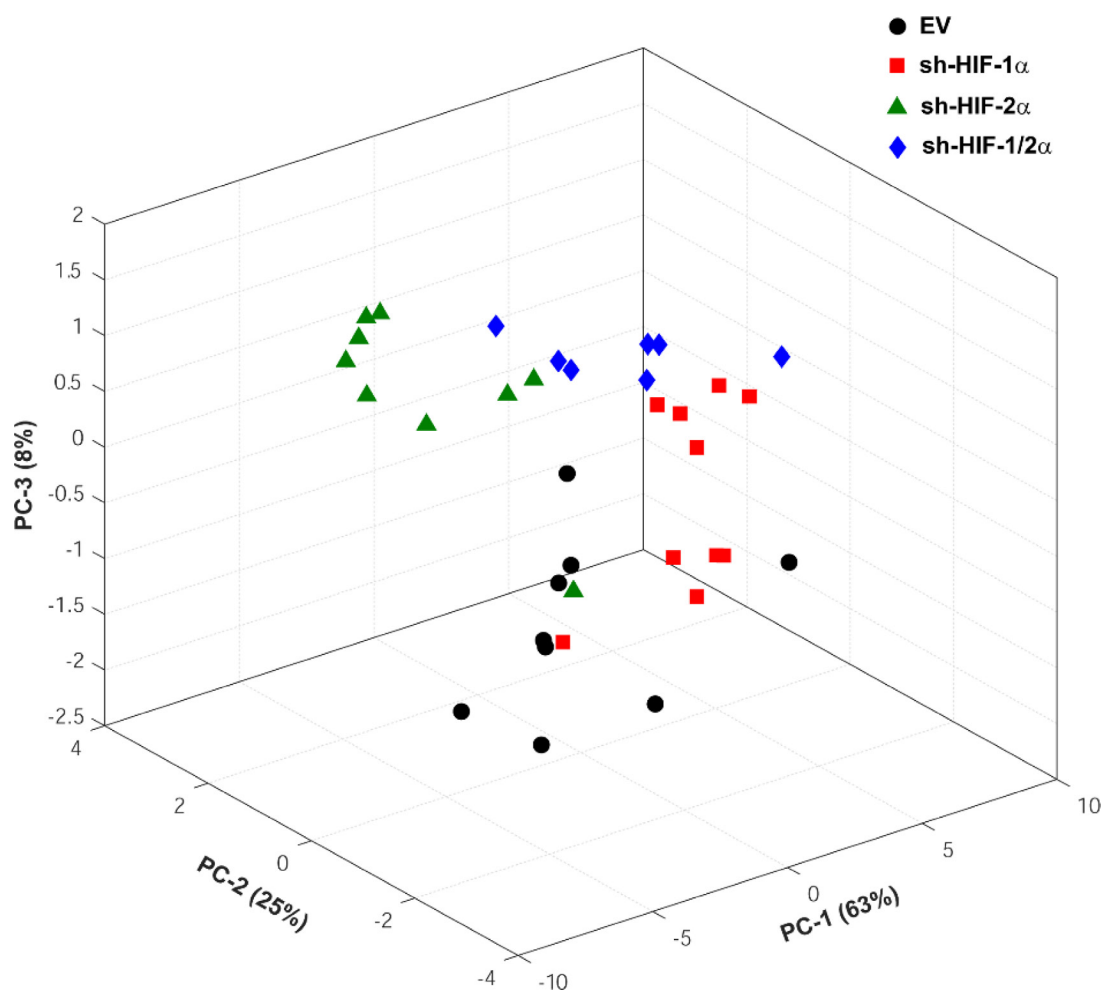


## Metabolic consequences of HIF silencing in a triple negative human breast cancer xenograft

### SUPPLEMENTARY MATERIALS



**Supplementary Figure 1: Multivariate principal component analysis (PCA) of metabolites.** A three dimensional scatter score plot shows clear clustering of 231-EV (black dot), sh-HIF-1 $\alpha$  (red square), sh-HIF-2 $\alpha$  (green triangle), and sh-HIF-1/2 $\alpha$  (blue diamond) tumors.

**Supplementary Table 1: Metabolites identified and quantified in aqueous and lipid phase extracts of tumors derived from EV, sh-HIF-1 $\alpha$ , sh-HIF-2 $\alpha$  shRNA, sh-HIF-1/2 $\alpha$  cells**

Metabolites	Chem Shift	EV		sh-HIF-1 $\alpha$		sh-HIF-2 $\alpha$			sh-HIF-1/2 $\alpha$		
	ppm	Avg	$\pm$ SEM	Avg	$\pm$ SEM	<sup>a</sup> P-value	Avg	$\pm$ SEM	<sup>#b</sup> P-value	Avg	$\pm$ SEM
Amino Acids	BCA	0.99	1.01 $\pm$ 0.10	1.03 $\pm$ 0.111	(0.436)	0.96 $\pm$ 0.068	(0.343)	0.88 $\pm$ 0.119	(0.210)		
	Valine	1.05	0.17 $\pm$ 0.018	0.19 $\pm$ 0.02	(0.212)	0.17 $\pm$ 0.012	(0.458)	0.16 $\pm$ 0.023	(0.348)		
	Alanine	1.48	1.21 $\pm$ 0.082	0.78 $\pm$ 0.09	(0.002)	0.77 $\pm$ 0.056	(0.001)	0.63 $\pm$ 0.086	(0.000)		
	Lysine	1.73	0.84 $\pm$ 0.05	0.76 $\pm$ 0.079	(0.210)	0.84 $\pm$ 0.06	(0.467)	0.73 $\pm$ 0.10	(0.153)		
	Glutamate	2.35	1.84 $\pm$ 0.096	1.29 $\pm$ 0.16	(0.005)	2.15 $\pm$ 0.15	(0.052)	1.16 $\pm$ 0.135	(0.000)		
	Glutamine	2.45	0.53 $\pm$ 0.054	0.40 $\pm$ 0.039	(0.033)	0.63 $\pm$ 0.044	(0.083)	0.44 $\pm$ 0.043	(0.118)		
	Aspartate	2.80	0.16 $\pm$ 0.014	0.07 $\pm$ 0.018	(0.000)	0.15 $\pm$ 0.019	(0.245)	0.05 $\pm$ 0.018	(0.000)		
	Glycine	3.57	0.76 $\pm$ 0.067	0.40 $\pm$ 0.046	(0.001)	0.81 $\pm$ 0.073	(0.317)	0.44 $\pm$ 0.055	(0.002)		
	Tyrosine	6.90	0.04 $\pm$ 0.005	0.04 $\pm$ 0.005	(0.185)	0.03 $\pm$ 0.005	(0.133)	0.03 $\pm$ 0.003	(0.025)		
	Phenylalanine	7.41	0.08 $\pm$ 0.01	0.09 $\pm$ 0.009	(0.253)	0.07 $\pm$ 0.004	(0.239)	0.07 $\pm$ 0.008	(0.284)		
Organic Acids	Histidine	7.69	0.01 $\pm$ 0.004	0.01 $\pm$ 0.004	(0.330)	0.007 $\pm$ 0.002	(0.212)	0.01 $\pm$ 0.004	(0.321)		
	Lactate	1.34	6.87 $\pm$ 0.702	6.23 $\pm$ 0.719	(0.269)	5.56 $\pm$ 0.347	(0.057)	6.10 $\pm$ 0.885	(0.250)		
	Acetate	1.92	0.26 $\pm$ 0.068	0.16 $\pm$ 0.024	(0.079)	0.13 $\pm$ 0.019	(0.038)	0.11 $\pm$ 0.022	(0.042)		
	Fumarate	6.51	0.005 $\pm$ 0.001	0.003 $\pm$ 0.001	(0.222)	0.004 $\pm$ 0.001	(0.308)	0.002 $\pm$ 0.001	(0.052)		
	Formate	8.45	0.06 $\pm$ 0.019	0.056 $\pm$ 0.009	(0.429)	0.042 $\pm$ 0.005	(0.193)	0.042 $\pm$ 0.012	(0.240)		
	Pyruvate	2.40	0.33 $\pm$ 0.034	0.25 $\pm$ 0.037	(0.067)	0.31 $\pm$ 0.016	(0.276)	0.23 $\pm$ 0.024	(0.022)		
	Choline	3.21	0.79 $\pm$ 0.111	0.61 $\pm$ 0.084	(0.108)	0.83 $\pm$ 0.069	(0.358)	0.57 $\pm$ 0.064	(0.073)		
	Cholines	PC	3.22	2.90 $\pm$ 0.195	1.79 $\pm$ 0.237	(0.001)	4.77 $\pm$ 0.51	(0.002)	3.07 $\pm$ 0.325	(0.325)	
		GPC	3.24	3.45 $\pm$ 0.284	1.97 $\pm$ 0.318	(0.002)	2.30 $\pm$ 0.195	(0.002)	1.28 $\pm$ 0.183	(0.000)	
		Total-Choline	3.22	6.81 $\pm$ 0.469	4.16 $\pm$ 0.537	(0.001)	7.54 $\pm$ 0.596	(0.176)	4.67 $\pm$ 0.506	(0.004)	
Creatine		3.04	0.50 $\pm$ 0.044	0.39 $\pm$ 0.032	(0.025)	0.79 $\pm$ 0.069	(0.001)	0.59 $\pm$ 0.064	(0.113)		
Others	Glucose	5.24	0.05 $\pm$ 0.018	0.02 $\pm$ 0.011	(0.102)	0.07 $\pm$ 0.024	(0.233)	0.08 $\pm$ 0.028	(0.153)		
	Glutathione	2.54	0.77 $\pm$ 0.048	0.64 $\pm$ 0.095	(0.133)	0.99 $\pm$ 0.066	(0.009)	0.52 $\pm$ 0.067	(0.004)		
	Taurine	3.43	2.83 $\pm$ 0.17	2.10 $\pm$ 0.197	(0.007)	4.17 $\pm$ 0.326	(0.001)	2.44 $\pm$ 0.359	(0.155)		
	Myo-inositol	4.06	0.79 $\pm$ 0.064	0.27 $\pm$ 0.038	(0.001)	0.51 $\pm$ 0.05	(0.002)	0.33 $\pm$ 0.045	(0.000)		
Nucleotides	Uracil	5.80	0.009 $\pm$ 0.002	0.008 $\pm$ 0.002	(0.966)	0.002 $\pm$ 0.001	(0.033)	0.001 $\pm$ 0.001	(0.015)		
	Adenosine	8.36	0.06 $\pm$ 0.007	0.06 $\pm$ 0.01	(0.395)	0.04 $\pm$ 0.006	(0.025)	0.04 $\pm$ 0.009	(0.151)		
	UDP-N-AcGln+	5.51	0.047 $\pm$ 0.004	0.015 $\pm$ 0.007	(0.001)	0.04 $\pm$ 0.006	(0.183)	0.01 $\pm$ 0.007	(0.000)		
	NADP	9.32	0.03 $\pm$ 0.003	0.02 $\pm$ 0.004	(0.003)	0.02 $\pm$ 0.003	(0.009)	0.01 $\pm$ 0.002	(0.000)		
	ATP/ADP	8.51	0.17 $\pm$ 0.019	0.05 $\pm$ 0.013	(0.000)	0.12 $\pm$ 0.006	(0.015)	0.06 $\pm$ 0.014	(0.000)		
Unidentified	UNK-4.20	4.17	0.97 $\pm$ 0.059	0.67 $\pm$ 0.08	(0.004)	1.48 $\pm$ 0.138	(0.002)	0.95 $\pm$ 0.09	(0.425)		
	UNK-6.15	6.15	0.26 $\pm$ 0.026	0.088 $\pm$ 0.025	(0.000)	0.26 $\pm$ 0.022	(0.497)	0.134 $\pm$ 0.033	(0.003)		
	UNK-6.8	6.80	0.016 $\pm$ 0.001	0.003 $\pm$ 0.001	(0.000)	0.024 $\pm$ 0.002	(0.006)	0.01 $\pm$ 0.002	(0.028)		
	UNK-7.70	7.70	0.001 $\pm$ 0.001	0.04 $\pm$ 0.003	(0.000)	0.025 $\pm$ 0.017	(0.08)	0.04 $\pm$ 0.03	(0.056)		
	UNK-8.27	8.27	0.25 $\pm$ 0.02	0.105 $\pm$ 0.017	(0.000)	0.241 $\pm$ 0.017	(0.385)	0.134 $\pm$ 0.025	(0.002)		

Student *t*-test <sup>a</sup>; EV vs HIF-1 $\alpha$  shRNA, <sup>#b</sup>; EV vs HIF-2 $\alpha$  shRNA, C; EV vs HIF-1 $\alpha$ +2 $\alpha$  shRNA, +UDP-N-Acetyl Glucosamine  
 Values represent Mean  $\pm$  SEM. A one-tailed student t-test was performed on quantified integral peak areas. *P*-values < 0.05 were considered statistically significant. Peak integral areas were normalized to sample weight and dilution factors used during the experimental procedures.