

Supplementary Material and Methods

Blood Collection and Cotinine Determination

Venous blood was collected after overnight fasting in S-Monovette collection tubes (Sarstedt, Nümbrecht, Germany) and centrifuged at 2000xg for 10 minutes at room temperature. After aliquoting, the serum fraction was immediately frozen at -80°C.

Serum cotinine levels were measured with a solid phase competitive cotinine Enzyme-linked Immunosorbent Assay (ELISA, Abnova, Taipei, China) according to manufacturer's guidelines. The cut-off defining smoker was set to cotinine levels $\geq 0.03\text{nmol/L}$ [57]. Analytical assay sensitivity was 1ng/mL with cross-reactivity for nicotine (<1%), nicotinamide (<1%) and nicotinic acid (<1%).

Measurement of Secreted hCG

Supernatant was collected for all treatments, centrifuged at 1800xg for 10 minutes and stored at -20°C until immunoassay analysis. Human chorionic gonadotropin (hCG) levels were determined using IMMULITE 1000 Systems Immunoassay (Siemens, Munich, Germany). Results were normalized to total protein content in each well.

Supplementary Tables and Figures

Supplementary Table S1. Genes upregulated by maternal obesity in first trimester placental tissue.

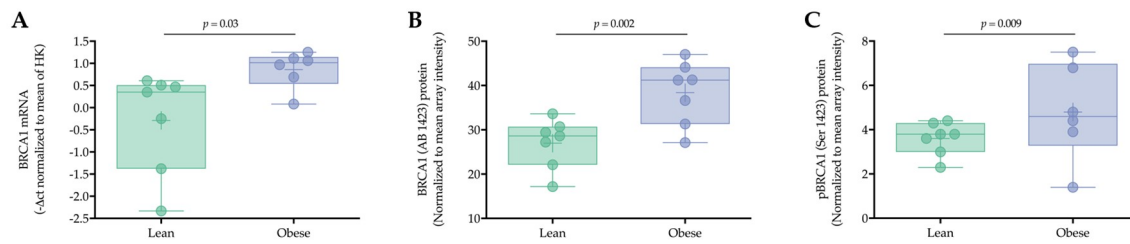
Coefficients					
Dependent Variable	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
<i>ZNF350</i>	-0.367	0.045	-0.452	-8.140	0.015
<i>ERCC5</i>	0.744	0.092	0.945	8.123	0.015
<i>CREBBP</i>	-0.326	0.048	-1.040	-6.804	0.021
<i>SLC35G6</i>	-0.499	0.079	-1.045	-6.359	0.024
<i>LIG4</i>	-0.720	0.122	-0.890	-5.913	0.027
<i>BRCA1</i>	-1.515	0.585	-0.715	-2.590	0.029
<i>POLB</i>	-0.802	0.156	-0.765	-5.127	0.036
<i>FANCL</i>	-0.654	0.143	-0.935	-4.581	0.044
<i>ERCC1</i>	-1.058	0.240	-1.041	-4.418	0.048
<i>PNKP</i>	-0.743	0.173	-1.006	-4.295	0.050
<i>MAX</i>	-0.514	0.128	-1.015	-4.010	0.057
<i>GTF2H1</i>	-0.659	0.175	-1.021	-3.770	0.064
<i>CSNK2A2</i>	-0.596	0.161	-0.962	-3.703	0.066
<i>SUMO1</i>	-0.655	0.181	-0.764	-3.618	0.069
<i>AKT2</i>	-1.046	0.296	-1.013	-3.538	0.071
<i>POLL</i>	-0.866	0.250	-0.959	-3.470	0.074
<i>RAD23B</i>	-1.685	0.520	-0.994	-3.243	0.083
<i>POLR2E</i>	-0.837	0.263	-0.967	-3.185	0.086
<i>RBX1</i>	-1.524	0.499	-0.992	-3.054	0.093

Multivariate linear regression model with BMI as exposure variable, correcting for maternal age.

Supplementary Table S2. Proteins upregulated by maternal obesity in first trimester placental tissue.

Dependent Variable	Coefficients		Standardized Coefficients	t	Sig.
	Unstandardized Coefficients	Std. Error			
	B		Beta		
RAD52 Ab104	39.565	0.214	1.000	185.074	0.000
HDAC1 Ab421	23.151	2.853	0.951	8.114	0.000
Cyclin E1 Ab77	11.900	1.960	0.916	6.071	0.001
BRCA1 Ab1423	16.374	3.353	0.878	4.883	0.002
Myc Ab358	5.800	1.268	0.849	4.575	0.003
14-3-3 zeta/delta Ab232	5.678	1.249	0.857	4.547	0.003
P90RSK Ab359/363	8.611	2.010	0.850	4.283	0.004
CDC25C Ab216	8.937	2.091	0.830	4.274	0.004
Chk2 Ab68	3.440	0.857	0.834	4.013	0.005
p95 Ab343	2.067	0.516	0.834	4.003	0.005
ATRIP Ab68/72	5.807	1.480	0.816	3.925	0.006
HDAC6 Ab22	2.693	0.699	0.824	3.853	0.006
HDAC3 Ab424	1.517	0.403	0.818	3.761	0.007
HDAC8 Ab39	0.958	0.255	0.817	3.752	0.007
MDM2 Ab166	12.170	3.307	0.811	3.680	0.008
BRCA1 Phospho-Ser1423	2.289	0.636	0.747	3.598	0.009
PP2A Ab307	3.204	0.946	0.786	3.385	0.012
Chk1 Ab286	1.672	0.497	0.770	3.363	0.012
Cyclin D1 Ab286	3.154	0.944	0.780	3.341	0.012
ABL1 Ab204	15.187	4.777	0.768	3.179	0.016
BRCA1 Ab1524	1.405	0.472	0.731	2.976	0.021
Smad2 3 Ab8	0.599	0.214	0.723	2.795	0.027
E2F1 Ab433	3.691	1.324	0.725	2.788	0.027
Cyclin D1 Phospho-Thr286	2.885	1.058	0.717	2.727	0.029
Smad3 Phospho-Ser213	1.043	0.404	0.696	2.585	0.036
TGFBR2 Ab250	1.692	0.664	0.693	2.546	0.038
TOP2A Ab1106	1.169	0.514	0.649	2.272	0.057
p53 Ab20	1.187	0.531	0.639	2.233	0.061
Smad2 3 Phospho-Thr8	0.574	0.257	0.642	2.233	0.061
HDAC1 Phospho-Ser421	1.077	0.487	0.637	2.212	0.063
DNA PK Ab2638	1.230	0.557	0.635	2.209	0.063
PLK1 Ab210	1.510	0.696	0.617	2.169	0.067
Cyclin A1 C-term	2.420	1.211	0.601	1.999	0.086
p53 Ab18	0.527	0.270	0.583	1.951	0.092
Cyclin E2 Ab392	0.610	0.315	0.586	1.934	0.094

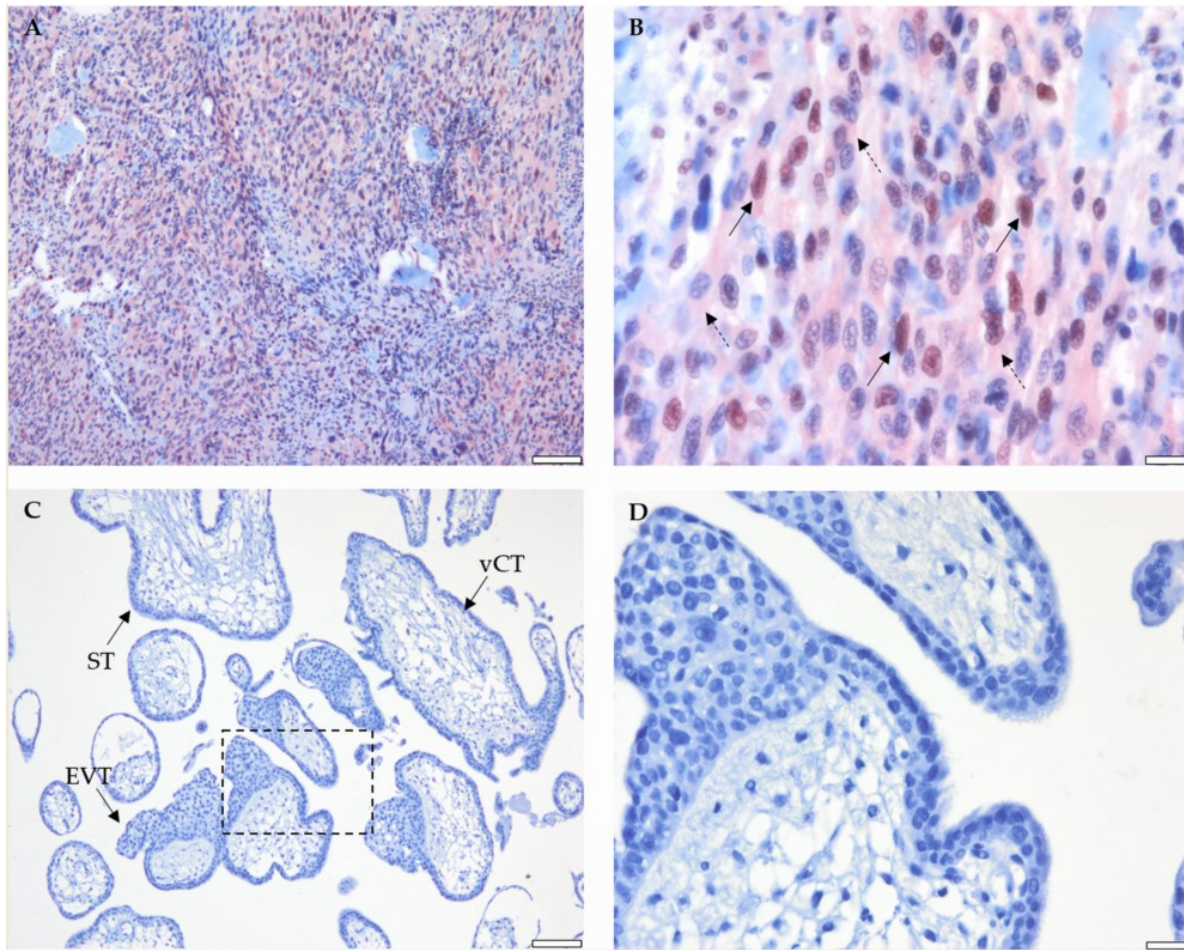
Multivariate linear regression model with BMI as exposure variable, correcting for maternal age.



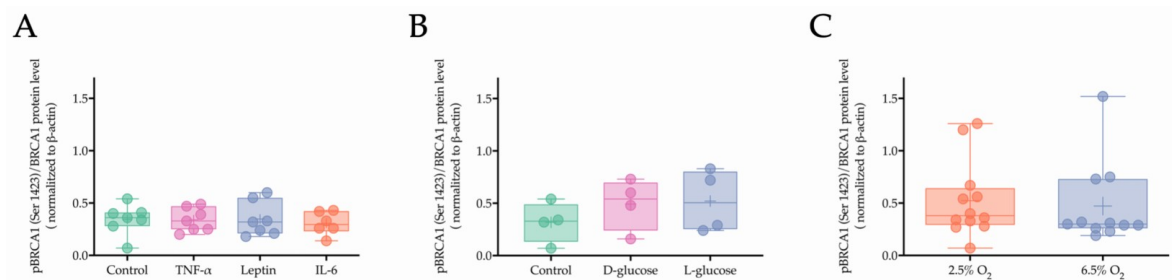
Supplementary Figure S1. Placental BRCA1 is upregulated by maternal obesity. BRCA1 expression and BRCA1 and p(Ser1423)-BRCA1 protein levels were determined by PCR panel (A) and Protein array (B and C), respectively, in first trimester placental tissue (gestational week 7) from lean ($n = 7$) and obese ($n = 6$) women. PCR Panel data are normalized to the mean of two housekeeping genes (HPRT1 and TBP) and Protein array data were normalized on the median intensity value of all antibodies on each array. Results are presented as mean $\pm$ SD and analyzed using a multivariate linear regression model adjusting for maternal age.

Supplementary Table S3. DNA methylation of *BRCA1* gene in lean (gestational week 6⁺⁰-11⁺⁶, *n* = 15) vs. obese (gestational week 5⁺⁰-11⁺², *n* = 15) first trimester placental tissue.

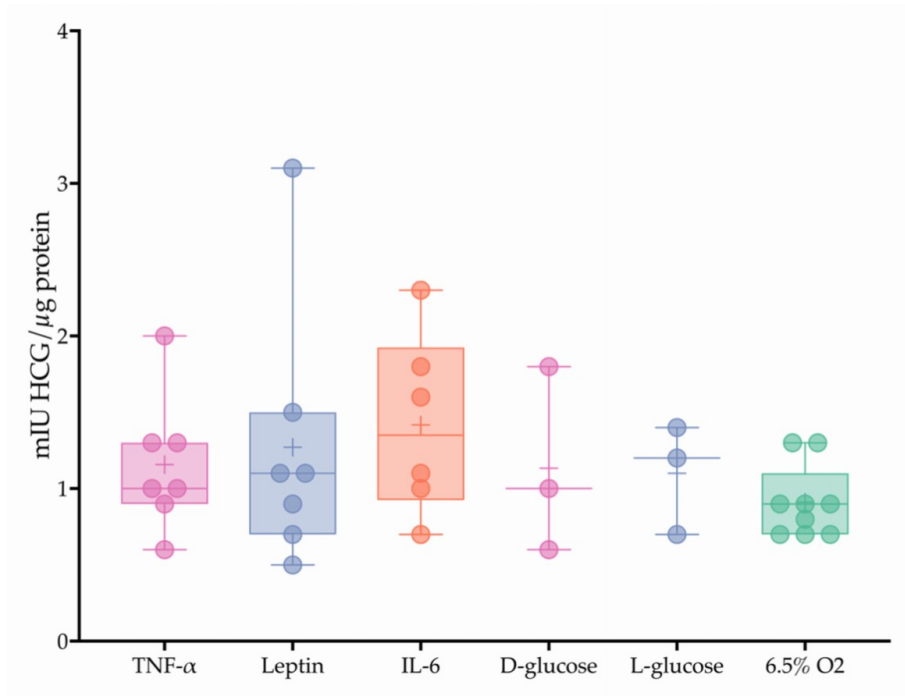
	Lean			Obese				Lean			Obese		
	Mean	SD	n	Mean	SD	n		Mean	SD	n	Mean	SD	n
cg06706355	0.401	0.043	15	0.4	0.025	15	cg16550131	0.489	0.044	15	0.472	0.027	15
cg20334877	0.679	0.034	15	0.695	0.033	15	cg05758696	0.801	0.018	15	0.81	0.019	15
cg02697717	0.439	0.029	15	0.456	0.025	15	cg17526501	0.904	0.019	15	0.918	0.01	15
cg02190078	0.493	0.029	15	0.5	0.029	15	cg09648631	0.878	0.027	15	0.867	0.021	15
cg16490202	0.09	0.009	15	0.082	0.007	15	cg07054526	0.896	0.02	15	0.901	0.017	15
cg05514735	0.179	0.026	15	0.174	0.033	15	cg14048487	0.748	0.037	15	0.742	0.034	15
cg25676905	0.112	0.043	15	0.106	0.022	15	cg19531713	0.1	0.01	15	0.098	0.011	15
cg11808250	0.034	0.007	15	0.033	0.006	15	cg19088651	0.041	0.012	15	0.037	0.008	15
cg09575269	0.041	0.006	15	0.038	0.004	15	cg08386886	0.187	0.018	15	0.184	0.016	15
cg00902415	0.057	0.009	15	0.057	0.007	15	cg08993267	0.044	0.006	15	0.041	0.005	15
cg14995433	0.055	0.006	15	0.055	0.006	15	cg24806953	0.035	0.007	15	0.029	0.008	15
cg18208654	0.035	0.012	15	0.036	0.005	15	cg20187250	0.03	0.005	15	0.029	0.005	15
cg16450103	0.043	0.006	15	0.04	0.006	15	cg15419295	0.046	0.008	15	0.043	0.013	15
cg18831773	0.558	0.018	15	0.559	0.015	15	cg16963062	0.029	0.008	15	0.027	0.006	15
cg08041014	0.481	0.036	15	0.485	0.034	15	cg16630982	0.028	0.007	15	0.028	0.006	15
cg01178231	0.88	0.027	15	0.895	0.017	15	cg21253966	0.034	0.011	15	0.036	0.008	15
cg24818472	0.919	0.019	15	0.912	0.015	15	cg04110421	0.04	0.013	15	0.036	0.006	15
cg20071259	0.885	0.02	15	0.891	0.013	15	cg04658354	0.033	0.006	15	0.029	0.004	15
cg17091024	0.737	0.027	15	0.743	0.026	15	cg17301289	0.042	0.009	15	0.041	0.006	15
cg22325646	0.21	0.027	15	0.213	0.039	15	cg09441966	0.037	0.009	15	0.035	0.011	15
cg25504443	0.127	0.025	15	0.12	0.027	15	cg26891576	0.197	0.031	15	0.182	0.026	15
cg16879222	0.033	0.004	15	0.03	0.004	15	cg20760063	0.049	0.005	15	0.05	0.005	15
cg16246366	0.041	0.006	15	0.042	0.007	15	cg10125569	0.04	0.005	15	0.035	0.005	15
cg17225407	0.157	0.071	15	0.147	0.066	15	cg01587050	0.032	0.006	15	0.028	0.004	15
cg12943674	0.074	0.008	15	0.074	0.005	15	cg10893007	0.031	0.006	15	0.029	0.003	15
cg26524541	0.077	0.007	15	0.076	0.007	15	cg12182452	0.038	0.008	15	0.038	0.007	15
cg10162691	0.05	0.012	15	0.05	0.009	15	cg09831010	0.04	0.005	15	0.037	0.006	15
cg05270634	0.048	0.008	15	0.046	0.009	15	cg25067162	0.073	0.021	15	0.074	0.017	15
cg07591186	0.553	0.052	15	0.558	0.033	15	cg26276233	0.115	0.039	15	0.112	0.028	15
cg08732879	0.42	0.05	15	0.409	0.025	15	cg06001716	0.095	0.059	15	0.087	0.022	15
cg18068798	0.657	0.038	15	0.663	0.028	15	cg02286533	0.113	0.055	15	0.112	0.036	15
cg25061307	0.73	0.05	15	0.726	0.033	15	cg14947218	0.126	0.057	15	0.126	0.037	15
cg14648161	0.387	0.032	15	0.378	0.037	15	cg16006004	0.095	0.051	15	0.095	0.033	15
cg09967497	0.415	0.059	15	0.409	0.04	15	cg18372208	0.146	0.053	15	0.148	0.04	15
cg21983245	0.82	0.028	15	0.832	0.027	15	cg25288140	0.19	0.076	15	0.197	0.059	15
cg05530195	0.446	0.047	15	0.439	0.037	15	cg15065591	0.178	0.079	15	0.181	0.058	15
cg23693293	0.538	0.055	15	0.529	0.044	15	cg11529738	0.144	0.05	15	0.152	0.034	15
cg01879757	0.683	0.09	15	0.709	0.078	15	cg24900425	0.138	0.057	15	0.139	0.041	15
cg16029534	0.876	0.015	15	0.875	0.019	15	cg06973652	0.102	0.035	15	0.106	0.028	15
cg16919093	0.901	0.034	15	0.914	0.019	15	cg20185525	0.42	0.018	15	0.424	0.024	15
cg18830083	0.728	0.045	15	0.74	0.04	15	cg26879546	0.487	0.071	15	0.494	0.083	15
cg25031275	0.68	0.033	15	0.687	0.022	15	cg10200559	0.612	0.04	15	0.615	0.039	15
cg23084950	0.856	0.031	15	0.863	0.015	15	cg14687471	0.858	0.023	15	0.862	0.02	15



Supplementary Figure S2. Positive and negative controls for BRCA1 immunohistochemistry of placental tissue. An ovarian cancer specimen was used as positive control (A and B), showing BRCA1 nuclear (B, arrows) and cytosolic (B, dotted arrows). IgG isotype negative control at the same primary antibody concentration showed no staining (C and D). Scale bar: 100 μ m (A and C) or 20 μ m (B and D). Dotted frame in C indicates the field shown with a higher magnification in D.



Supplementary Figure S3. $p^{(\text{Ser1423})}$ -BRCA1/BRCA1 ratio is not regulated by short term exposure to obesity-associated inflammation, hyperglycemia or oxygen tension in early pregnancy. First trimester placental chorionic villous explants from different placental tissues ($n = 4-11$) were cultured at 2.5% O_2 with (A) TNF- α (50ng/mL), leptin (100ng/mL) and IL-6 (100ng/mL) or (B) D-glucose (25nM) and L-glucose (25nM, osmotic control) for 48 hours in triplicates. Explants ($n = 10-12$) were also cultured at 6.5% O_2 (C). $p^{(\text{Ser1423})}$ -BRCA1/BRCA1 protein ratio was calculated based on densitometric analysis from immunoblotting. Results are presented as mean $\pm$ SD. Statistical analysis included Mann Whitney test or Friedman's test followed by Dunn's post hoc analysis.



Supplementary Figure S4. Human chorionic gonadotropin (hCG) levels in culture supernatants of placental chorionic villi explants remained stable after exposure to TNF- α , leptin, IL-6, D-glucose, L-glucose at 2.5% oxygen. Increasing the oxygen tension to 6.5% did not change secreted hCG. Data is shown as mIU hCG normalized on total protein content in the respective well. Results are expressed as mean $\pm$ SD. Statistical analysis was performed using One-way ANOVA.