

**Table S1. Effect of limiting energy availability on carcinogenic response in mammary gland, target proteins in mammary carcinomas and plasma lipid profile<sup>a</sup>**

Treatment	Control	DER	WRL	WRH
Cell cycle and apoptosis in mammary carcinomas				
Cyclin D1	929 ± 31 <sup>a</sup>	805 ± 28 <sup>b</sup>	824 ± 19 <sup>ab</sup>	710 ± 31 <sup>b</sup>
p27	1002 ± 41 <sup>a</sup>	1171 ± 41 <sup>ab</sup>	1059 ± 51 <sup>ab</sup>	1192 ± 49 <sup>b</sup>
Bax	212 ± 7 <sup>a</sup>	230 ± 13 <sup>ab</sup>	223 ± 10 <sup>a</sup>	270 ± 14 <sup>b</sup>
Bcl-2	695 ± 26 <sup>ab</sup>	686 ± 41 <sup>ab</sup>	716 ± 35 <sup>a</sup>	590 ± 18 <sup>b</sup>
Bax/Bcl-2	0.31 ± 0.01 <sup>a</sup>	0.34 ± 0.02 <sup>a</sup>	0.31 ± 0.01 <sup>a</sup>	0.46 ± 0.02 <sup>b</sup>
pAkt and SIRT1 in mammary carcinomas				
pAkt <sup>Ser473</sup>	856 ± 55 <sup>a</sup>	495 ± 36 <sup>b</sup>	574 ± 35 <sup>b</sup>	433 ± 28 <sup>b</sup>
SIRT1	443 ± 25 <sup>a</sup>	591 ± 25 <sup>b</sup>	568 ± 28 <sup>b</sup>	527 ± 23 <sup>b</sup>
mTOR-related regulatory nodes pathway in mammary carcinomas				
LKB <sup>Ser428</sup> ratio	0.034 ± 0.001 <sup>a</sup>	0.045 ± 0.001 <sup>b</sup>	0.036 ± 0.001 <sup>a</sup>	0.036 ± 0.001 <sup>a</sup>
AMPK <sup>Thr172</sup> ratio	0.030 ± 0.003	0.039 ± 0.004	0.031 ± 0.003	0.035 ± 0.002
PI3Kp110	547 ± 29 <sup>a</sup>	393 ± 11 <sup>b</sup>	461 ± 25 <sup>ab</sup>	433 ± 23 <sup>b</sup>
Akt <sup>Ser473</sup> ratio	0.34 ± 0.01 <sup>a</sup>	0.18 ± 0.01 <sup>b</sup>	0.22 ± 0.03 <sup>b</sup>	0.22 ± 0.01 <sup>b</sup>
TORC1	479 ± 23	410 ± 21	446 ± 21	439 ± 17
Raptor <sup>Ser792</sup> ratio	0.045 ± 0.001 <sup>a</sup>	0.096 ± 0.003 <sup>b</sup>	0.049 ± 0.01 <sup>a</sup>	0.063 ± 0.012 <sup>a</sup>
PRAS-40 <sup>Thr246</sup> ratio	0.109 ± 0.003 <sup>a</sup>	0.096 ± 0.004 <sup>b</sup>	0.105 ± 0.02 <sup>ab</sup>	0.100 ± 0.003 <sup>ab</sup>
mTOR <sup>Ser2448</sup> ratio	0.30 ± 0.01 <sup>a</sup>	0.25 ± 0.01 <sup>b</sup>	0.28 ± 0.01 <sup>ab</sup>	0.26 ± 0.01 <sup>ab</sup>
P70S6K <sup>Thr389</sup> ratio	0.45 ± 0.02 <sup>a</sup>	0.36 ± 0.02 <sup>b</sup>	0.41 ± 0.01 <sup>ab</sup>	0.40 ± 0.02 <sup>ab</sup>
4EBP1 <sup>Thr37/46</sup> ratio	0.26 ± 0.006 <sup>a</sup>	0.228 ± 0.005 <sup>b</sup>	0.241 ± 0.008 <sup>ab</sup>	0.238 ± 0.02 <sup>ab</sup>
Transcription related factors in mammary carcinomas				
FoxO1 <sup>Thr24</sup> ratio	0.305 ± 0.005 <sup>a</sup>	0.175 ± 0.035 <sup>b</sup>	0.171 ± 0.026 <sup>b</sup>	0.174 ± 0.027 <sup>b</sup>
FoxO3a <sup>Thr32</sup>	66 ± 3 <sup>a</sup>	45 ± 4 <sup>b</sup>	44 ± 6 <sup>b</sup>	42 ± 8 <sup>b</sup>

GADD153	162 ± 7 <sup>a</sup>	197 ± 6 <sup>b</sup>	190 ± 12 <sup>ab</sup>	197 ± 6 <sup>ab</sup>
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Lipid metabolism in mammary carcinomas

HMGCR	540 ± 21 <sup>a</sup>	473 ± 14 <sup>b</sup>	468 ± 14 <sup>ab</sup>	466 ± 22 <sup>ab</sup>
SREBP1	396 ± 15 <sup>a</sup>	319 ± 19 <sup>b</sup>	288 ± 5 <sup>b</sup>	275 ± 13 <sup>b</sup>
SCD1	224 ± 10	219 ± 9	210 ± 6	216 ± 6
ACC <sup>Ser79</sup> Ratio	1.61 ± 0.05	1.65 ± 0.04	1.61 ± 0.06	1.63 ± 0.06
FASN	4051 ± 193	3387 ± 168	3757 ± 218	3506 ± 250

Plasma lipid profile

Triglyceride (mg/dl)	79 ± 7 <sup>a</sup>	77 ± 6 <sup>a</sup>	61 ± 4 <sup>ab</sup>	53 ± 7 <sup>b</sup>
Cholesterol (mg/dl)	102 ± 4 <sup>a</sup>	94 ± 3 <sup>ab</sup>	84 ± 4 <sup>bc</sup>	78 ± 3 <sup>c</sup>
HDL-Cholesterol (mg/dl)	60 ± 2	58 ± 2	55 ± 3	54 ± 3
LDL-Cholesterol (mg/dl)	26 ± 3 <sup>a</sup>	21 ± 2 <sup>ab</sup>	16 ± 2 <sup>b</sup>	14 ± 1 <sup>b</sup>
HDL-Cholesterol/Cholesterol (%)	60 ± 2 <sup>a</sup>	62 ± 2 <sup>ab</sup>	66 ± 2 <sup>b</sup>	68 ± 2 <sup>b</sup>

<sup>a</sup>Values are means ± SEM for carcinogenic response except incidence and cancer latency (n=30).

Final body weight, cancer multiplicity following square root transformation of cancers counts and plasma lipid profile were analyzed by one-way ANOVA with Bonferroni Post Hoc Test. Cancer incidence was evaluated by Fisher's exact test. Cancer burden was evaluated by the Kruskal Wallis rank test with Dwass-Steel-Chritchlow-Fligner post hoc test. For cancer latency, which was analyzed by survival analysis using the method of Mantel, the values are means and the numbers in the parentheses are the 95% confidence intervals. For target proteins in mammary carcinomas (control, n=8; treatment, n=24), actin-normalized western blot data, which are semi-quantitative estimates of protein expression, were analyzed by Kruskal-Wallis rank test with Dwass-Steel-Chritchlow-Fligner post hoc Test. Ratio, the ratio of phospho-protein (arbitrary units of optical density) to non-phospho-protein (arbitrary units of optical density). Different superscripts (a or c) within the same row are statistically significant among different fatty acids ( $P < 0.05$ ). 4EBP1, eukaryotic translation initiation factor 4E-binding protein 1; ACC, acetyl-CoA carboxylase; Akt, protein kinase B; AMPK, adenosine monophosphate-activated protein kinase;

DER, dietary energy restriction; FASN, fatty acid synthase; FoxO, Forkhead box O; GADD153, growth arrest and DNA damage protein 153; HDL, high-density lipoprotein; HMGCR, 3-hydroxy-3-methyl-glutaryl-CoA reductase; LDL, low-density lipoprotein; LKB1, liver kinase B; mTOR, mammalian target of rapamycin; P70S6K, 70-kDa ribosomal protein S6 kinase; PI3Kp110,

Phosphoinositide 3-kinase p110; Raptor, regulatory associated protein of mTOR; PRAS40, 40-kDa proline-rich protein; SCD1, stearyl-CoA desaturase 1; SIRT1, sirtuin 1; SREBP-1, sterol regulatory element-binding protein 1; TORC1, target of rapamycin complex 1; WRH; wheel running high. WRL, wheel running low.

**Table S2. Summary of orthogonal projections to latent structures-discriminant analysis models<sup>a</sup>**

Model	X	Variations	R2X	R2X(cum)	R2Y(cum)	Q2(cum)
Figure 2	30	Predictive	0.158	0.437	0.893	0.770
		Orthogonal	0.280			
Figure 3	16	Predictive	0.514	0.700	0.856	0.810
		Orthogonal	0.185			

<sup>a</sup>R2X, fraction of X-variation modeled in the component.

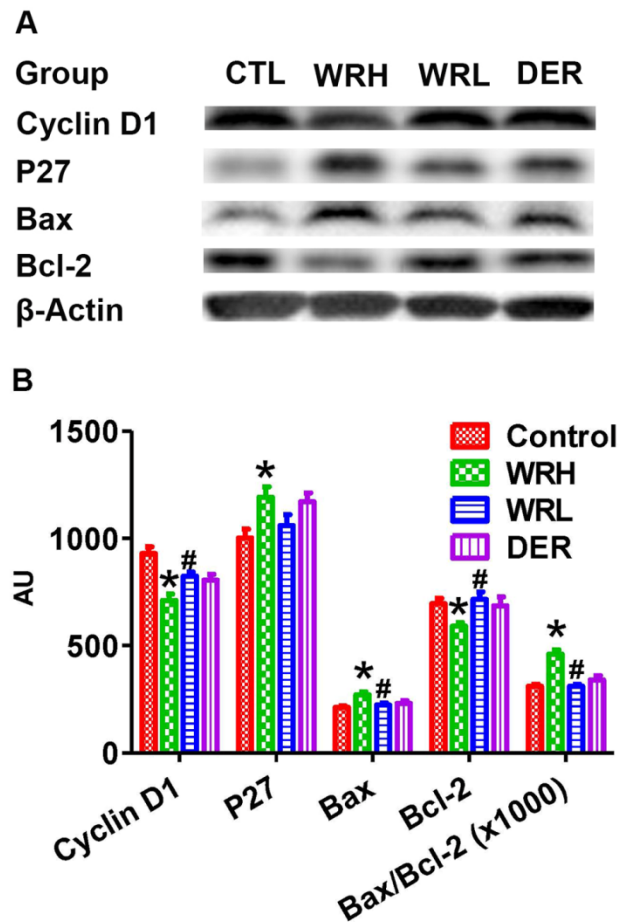
R2X(cum), predictive (X correlated to Y) + orthogonal (X uncorrelated to Y) variation in X that is explained by the model.

R2Y(cum), total sum of variation in Y explained by the model.

Q2(cum): goodness of prediction calculated by full cross validation.

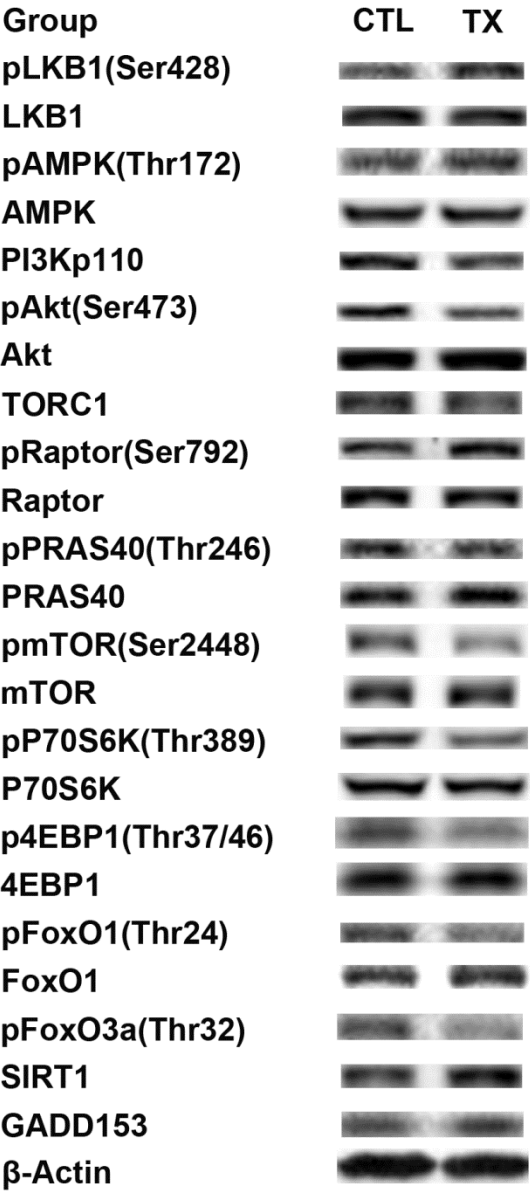
Y variable includes two class, control and limited energy availability

**Figure S1**



**Figure S1.** Effects of limited energy availability via wheel running high (WRH), wheel running low (WRL), or dietary energy restriction (DER) on cell cycle and apoptosis regulators in mammary carcinomas. The images shown are those directly acquired from the ChemiDoc work station that is equipped with a CCD camera having a resolution of 1300 x 1030. (A) A composite image of representative western blots of lysates of carcinomas from rats either control (CTL), WRH, WRL or DER for cell cycle regulators cyclin D1, p27Kip1 and apoptosis regulators Bcl (B cell lymphoma)-associated X (Bax), B cell leukemia oncogene 2 (Bcl-2). Some bands may appear over expressed in this composite photograph of representative bands from the western blot analysis, the actual images evaluated in the ChemiDoc were the linear range based on the threshold and the algorithm used in the Quality one software. (B) Bar graph of cell cycle and apoptosis regulators. AU, arbitrary unit. Compared to control \* or to HWR #,  $P < 0.05$ .

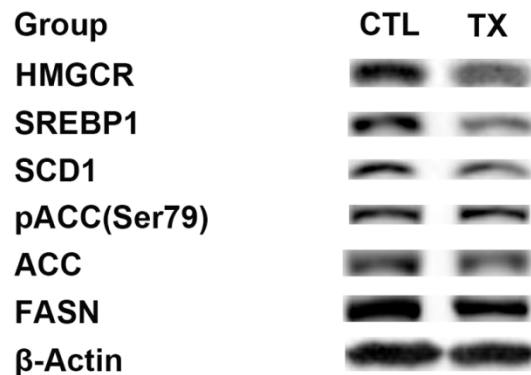
**Figure S2**



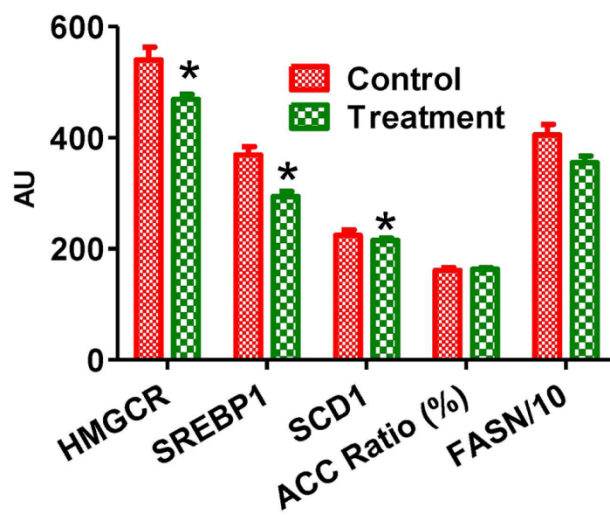
**Figure S2.** Effects of limited energy availability via wheel running high (WRH), wheel running low (WRL), or dietary energy restriction (DER) on cell signaling regulators in mammary carcinomas. The images shown are those directly acquired from the ChemiDoc work station that is equipped with a CCD camera having a resolution of 1300 x 1030. A composite image of representative western blots of lysates of carcinomas from rats either control (CTL) or treatment (TX, including WRH, WRL, and DER). Some bands may appear over expressed in this composite photograph of representative bands from the western blot analysis, the actual images evaluated in the ChemiDoc were the linear range based on the threshold and the algorithm used in the Quality one software. 4EBP1, eukaryotic translation initiation factor 4E-binding protein 1; Akt, protein kinase B; AMPK, adenosine monophosphate-activated protein kinase; FoxO, Forkhead box O; GADD153, growth arrest and DNA damage protein 153; LKB1, liver kinase B; mTOR, mammalian target of rapamycin; P70S6K, 70-kDa ribosomal protein S6 kinase; PI3Kp110, Phosphoinositide 3-kinase p110; Raptor, regulatory associated protein of mTOR; PRAS40, 40-kDa proline-rich protein; SIRT-1, sirtuin 1; TORC1, target of rapamycin complex 1.

**Figure S3**

**A**



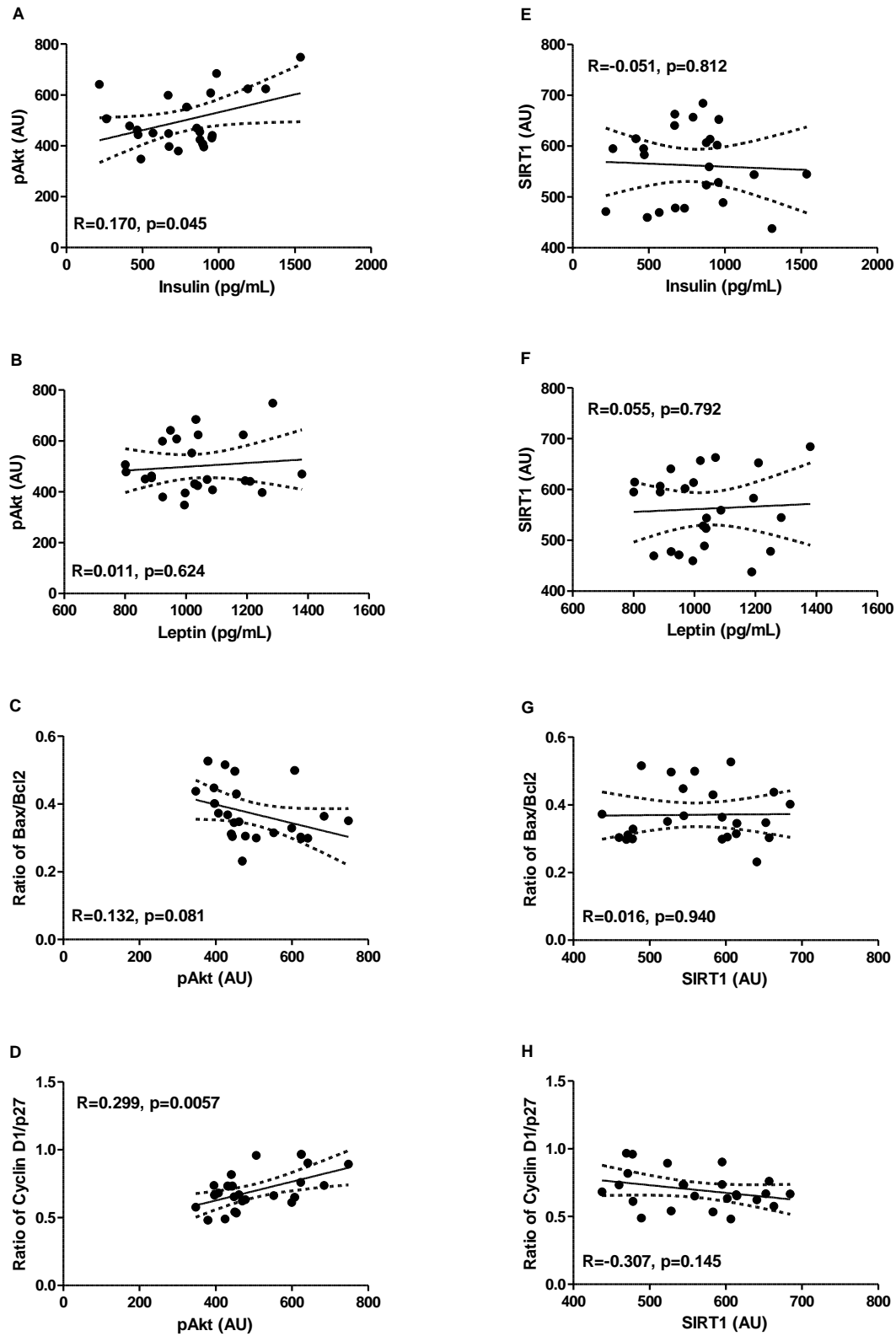
**B**



**Figure S3.** Effects of limited energy availability via wheel running high (WRH), wheel running low (WRL), or dietary energy restriction (DER) on lipid metabolism regulators in mammary carcinomas. The images shown are those directly acquired from the ChemiDoc work station that is equipped with a CCD camera having a resolution of 1300 x 1030. (A) A composite image of representative western blots of lysates of carcinomas from rats either control (CTL) or treatment (TX) for lipid metabolism regulators, 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR), sterol regulatory element-binding protein 1 (SREBP-1), stearoyl-coa desaturase 1 (SCD1), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FASN). Some bands may appear over expressed in this composite photograph of representative bands from the western blot analysis, the actual images evaluated in the ChemDoc were the linear range based on the threshold and the algorithm used in the Quality one software. (B) Bar graph of lipid metabolism regulators.

AU, arbitrary unit. \* Compared to control,  $P < 0.05$ .

Figure S4





**Figure S4.** Regression analyses are shown for levels of pAkt in mammary carcinomas on circulating insulin (panel A), levels of pAkt in mammary carcinomas on ); circulating leptin and (panel B); the Bax/Bcl2 ratio as a reflection of apoptotic potential on pAkt in mammary carcinomas (panel C); the Cyclin D1/p27 ratio in mammary carcinomas on levels of pAkt (panel D); levels of SIRT1 in mammary carcinomas on circulating insulin (panel E); levels of SIRT1 in mammary carcinomas on circulating leptin (panel F); the Bax/Bcl2 ratio in mammary carcinomas on levels of SIRT1 (panel G); the Cyclin D1/p27 ratio in mammary carcinomas on levels of SIRT1 (panel H). Only data from the three LEA intervention groups was included (n=24). AU, arbitrary unit.