# Mechanisms Associated with Dietary Energy Balance Effects on Tumor Development: Alterations in Growth Factor and Energy Sensing Pathways

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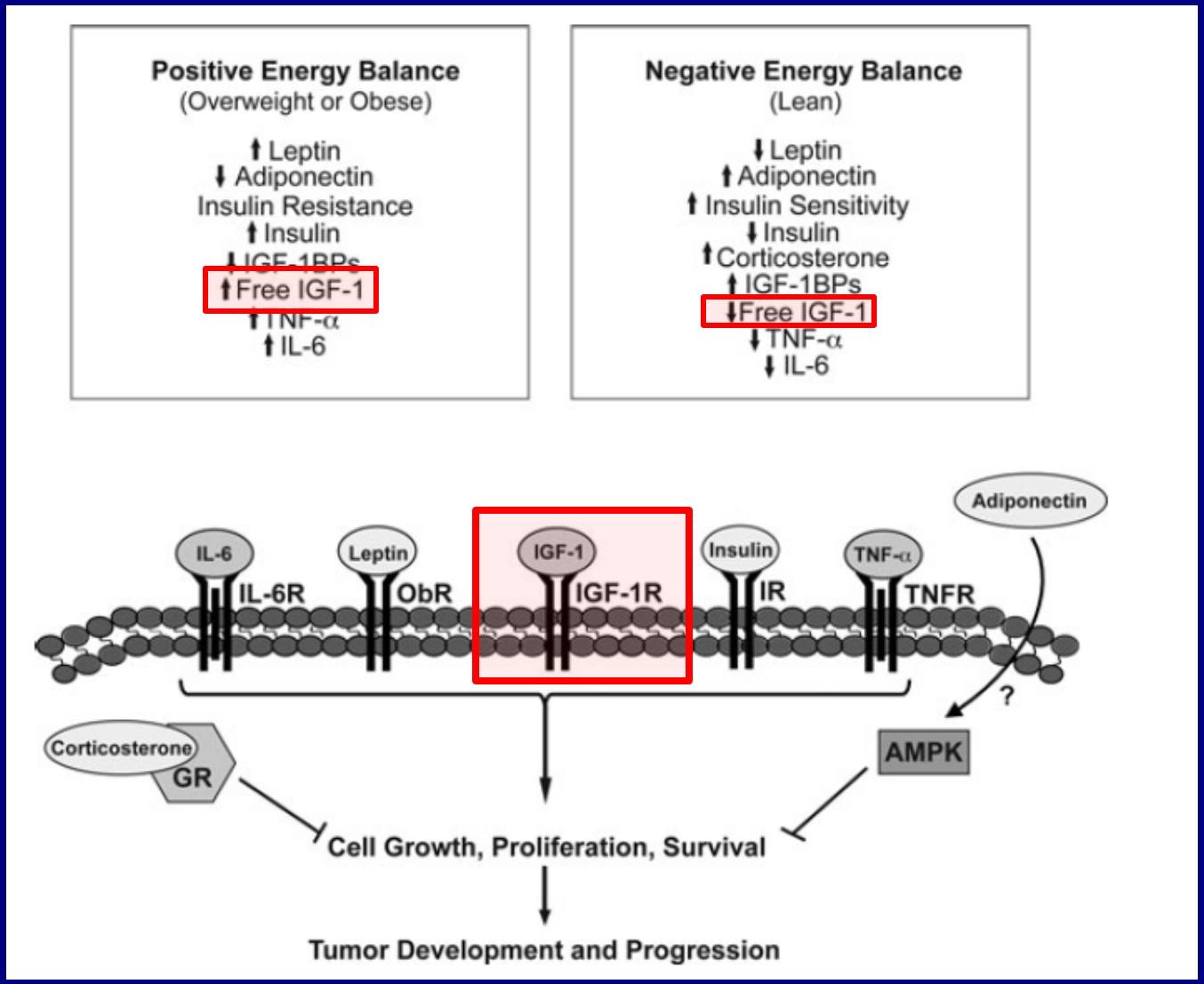
# Presentation Outline

- **Part I**
  - Dietary energy balance modulates skin tumor promotion via altered growth factor signaling (e.g., Akt/mTOR) and proliferation during tumor promotion.
  - Possible role of altered IGF-1R/EGFR crosstalk in dietary energy balance effects on tumor promotion.
  - Targeting mTORC1 as a strategy for inhibition of tumor promotion mimicking the effects of CR.
- **Part II**
  - Dietary energy balance modulates prostate cancer progression in Hi-Myc mouse model
  - Dietary energy balance effects growth factor signaling pathways in prostate
  - Possible role of inflammation and inflammatory signaling in prostate cancer progression in Hi-Myc Mice

# Potential Mechanisms Underlying the Link Between Energy Balance and Cancer




Moore et al, ANYAS, 2011

# Dietary Energy Balance Modulation Alters Steady State Growth Factor Signaling Pathways in Epithelial Tissues of Normal Mice

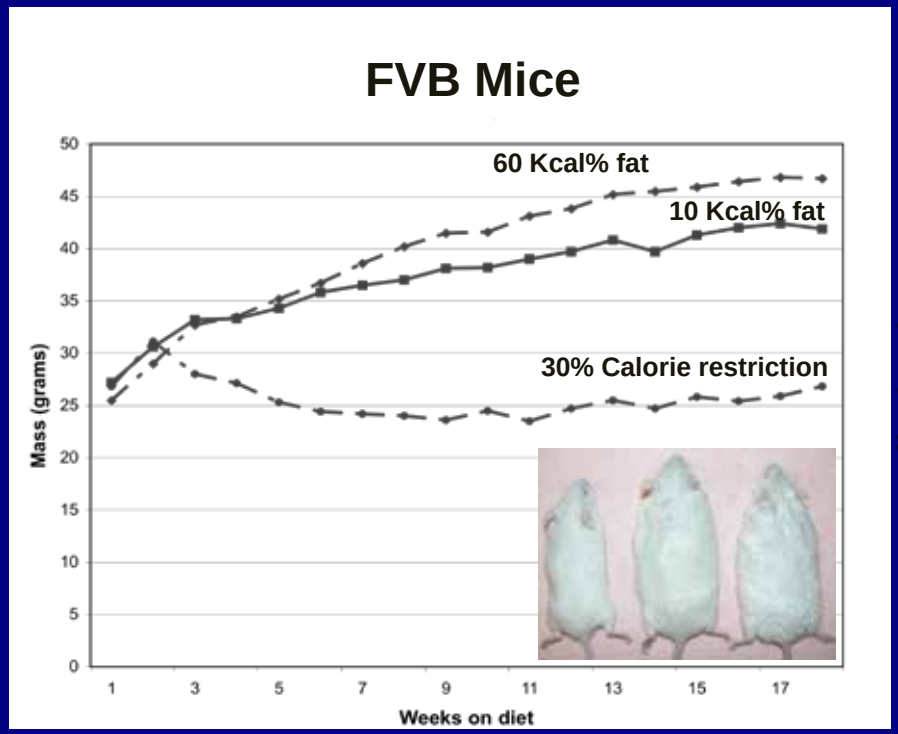
**Epidermis**
**Liver**
**Prostate**

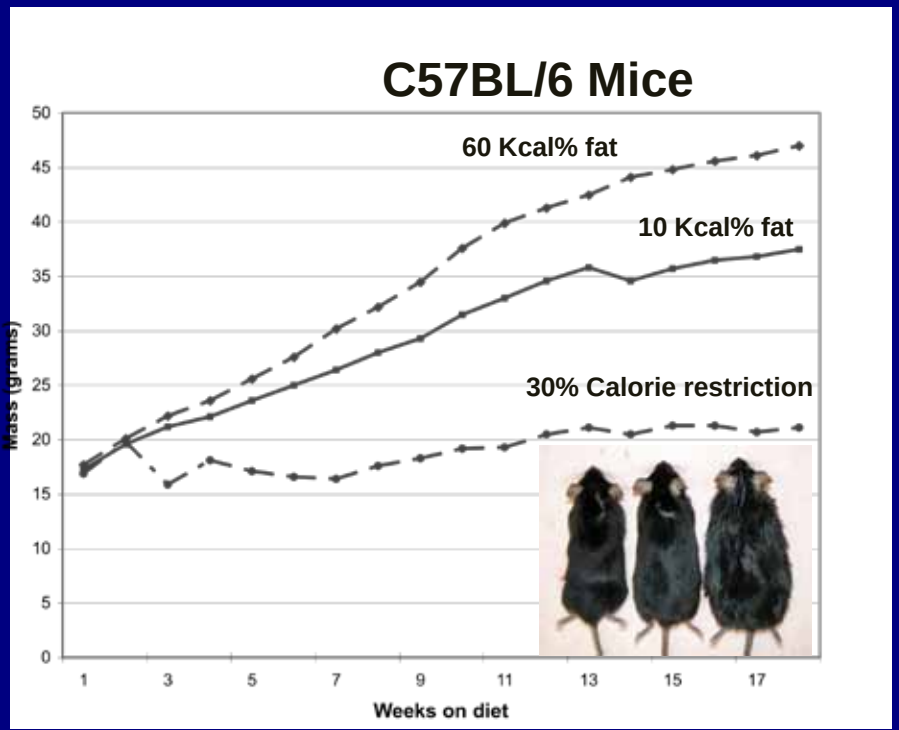
**Caloric Density of Diets Used to Examine the Effect of Dietary Energy Balance on Growth Factor Signaling in Epithelial Tissues**

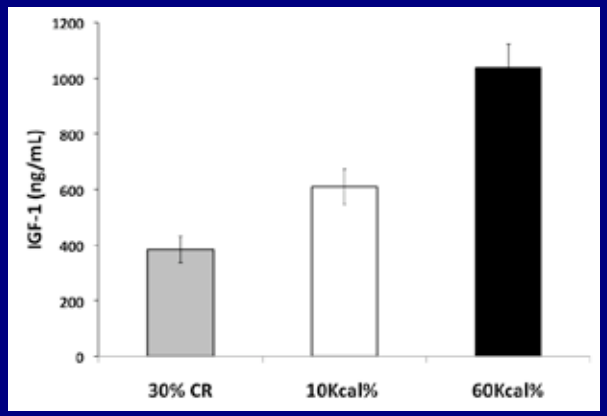
| *Experimental diet* | *Experimental Phenotype* | *Protein (kcal%)* | *Carbohydrate (kcal%)* | *Fat (kcal%)* |
|---|---|---|---|---|
| *30% CR[1]* | Lean | 29 | 57 | 14 |
| *15% CR[2]* | Normal | 24 | 65 | 12 |
| *AIN76A[3]* | Overweight | 20 | 70 | 10 |
| *DIO* | Obese | 20 | 20 | 60 |

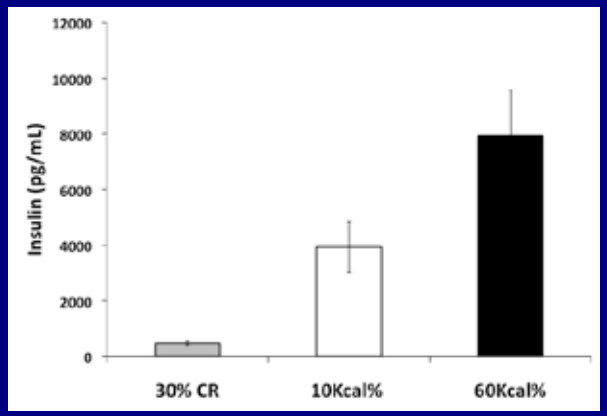
Moore et al, Cancer Prev. Res., 2008

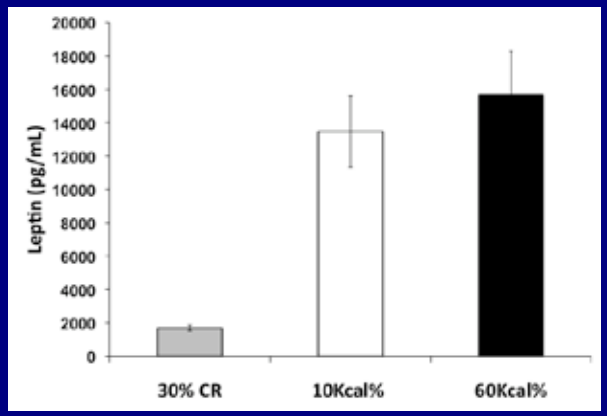
# Effect of Dietary Energy Balance Modulation on Weight Distribution and Serum Hormone Levels in Male Mice



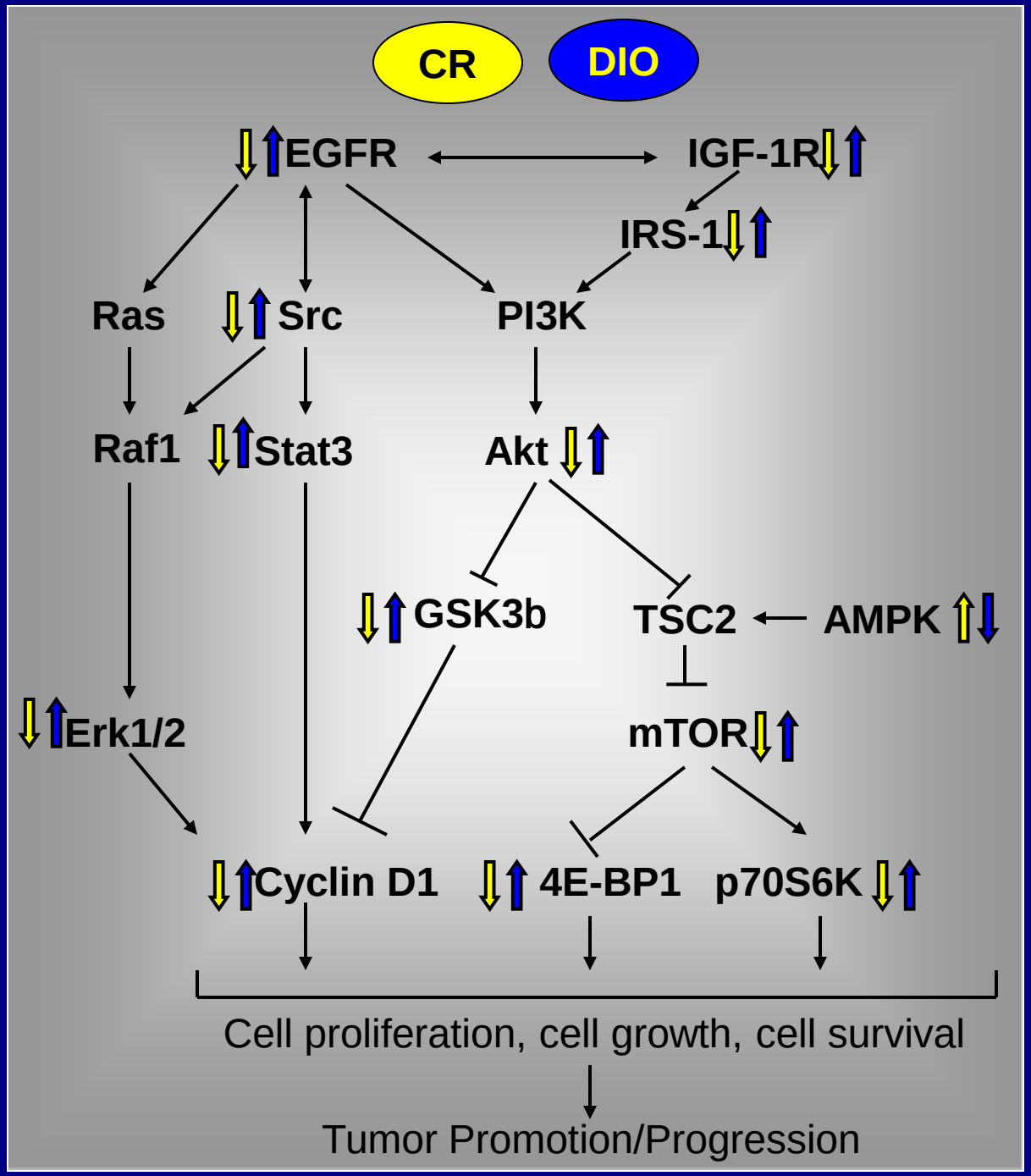



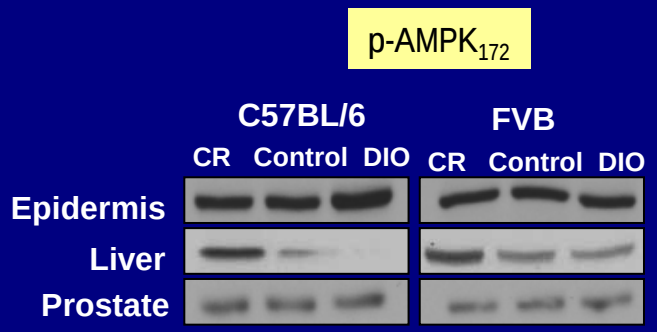




Moore et al, Cancer Prev Res 2008

# Effect of Dietary Energy Balance Modulation on Growth Factor Signaling in Epithelial Tissues of Untreated Mice



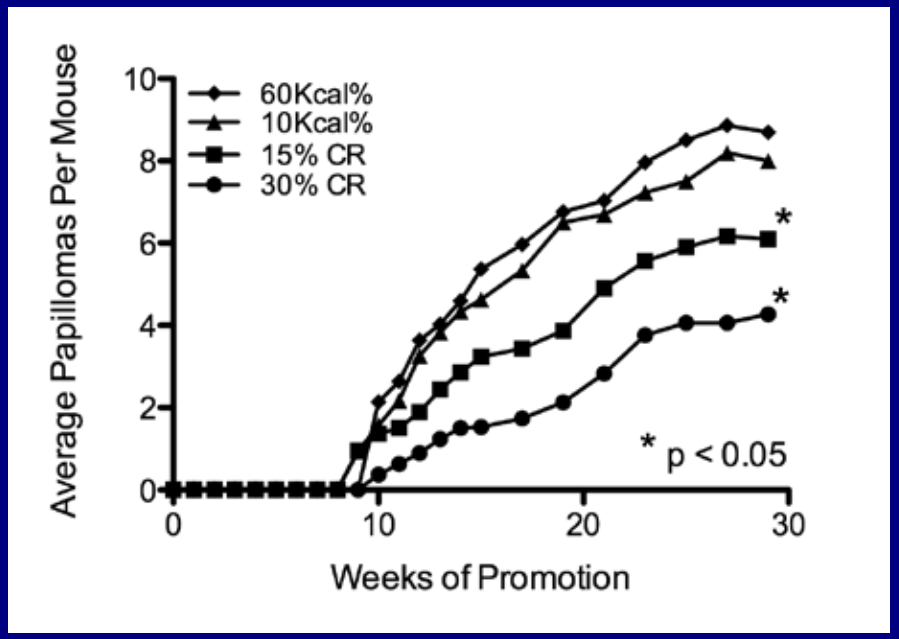



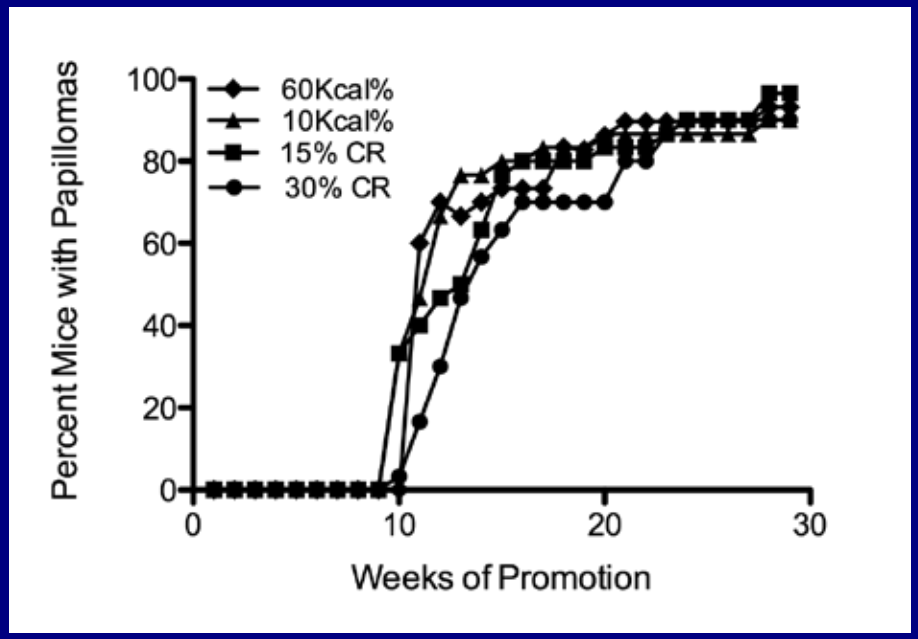

Moore et al, Cancer Prev. Res. 2008

# Dietary Energy Balance Modulation and Two-Stage Skin Carcinogenesis

- Calorie restriction (CR) significantly reduces tumor incidence, multiplicity, and papilloma size during two-stage skin carcinogenesis (Boutwell, 1964; Birt et al, 1991; Birt et al, 1993).
- Inhibitory effects of CR are observed primarily during tumor promotion (Birt et al, 1991).
- Potential mechanisms of inhibition:
  - 40% CR inhibits ERK, leading to decreased proliferation (Liu et al, 2001);
  - Levels of corticosterone may mediate inhibitory effects of 40%CR (Stewart et al, 2005)';
  - Reduced PI3K and Ras signaling following treatment with TPA (Xie et al, 2007);
  - Altered growth factor signaling via reduced circulating IGF-1 (Moore et al, CPR 2008; Cancer Res., 2008)
- Effect of positive energy balance is less clear.

# Dietary Energy Balance Modulation of Skin Tumor Promotion by TPA in ICR Mice




Moore et al, unpublished data

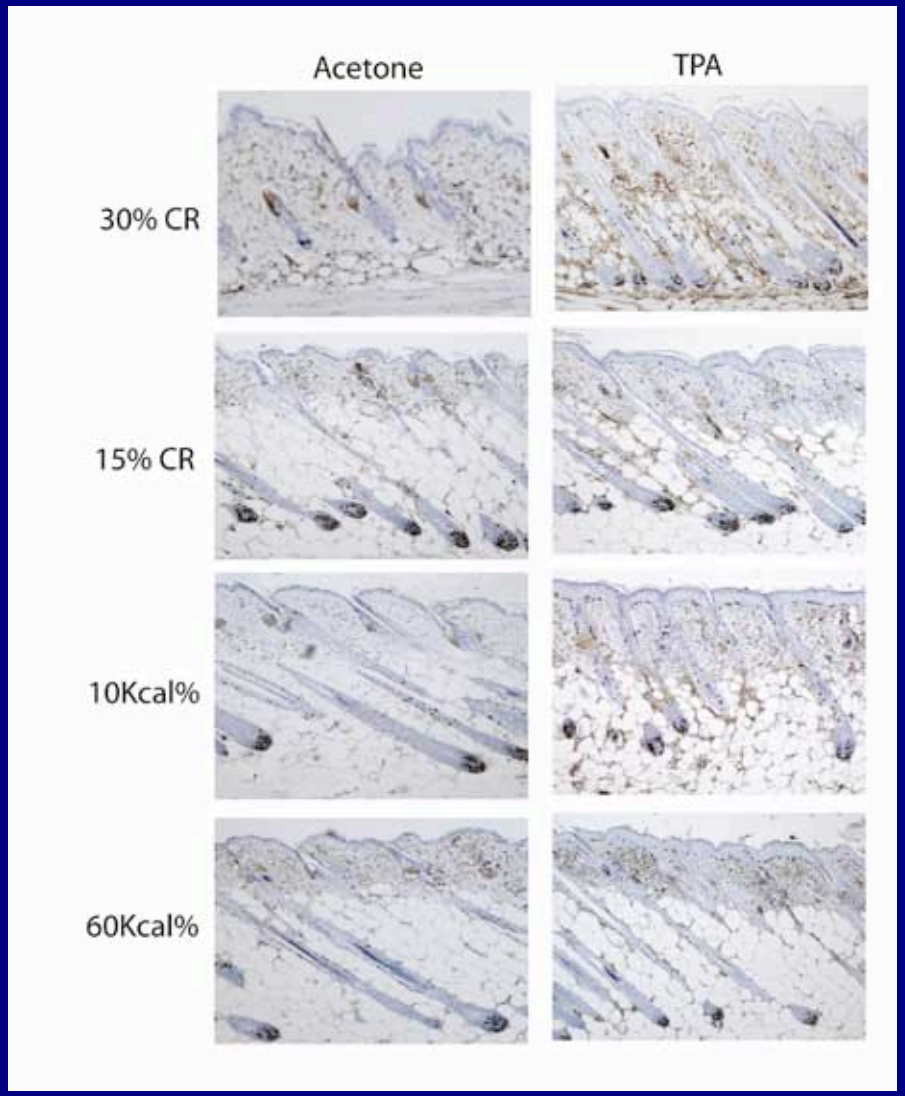
# Dietary Energy Balance Does Not Affect the Rate of Malignant Conversion of Papillomas to SCCs

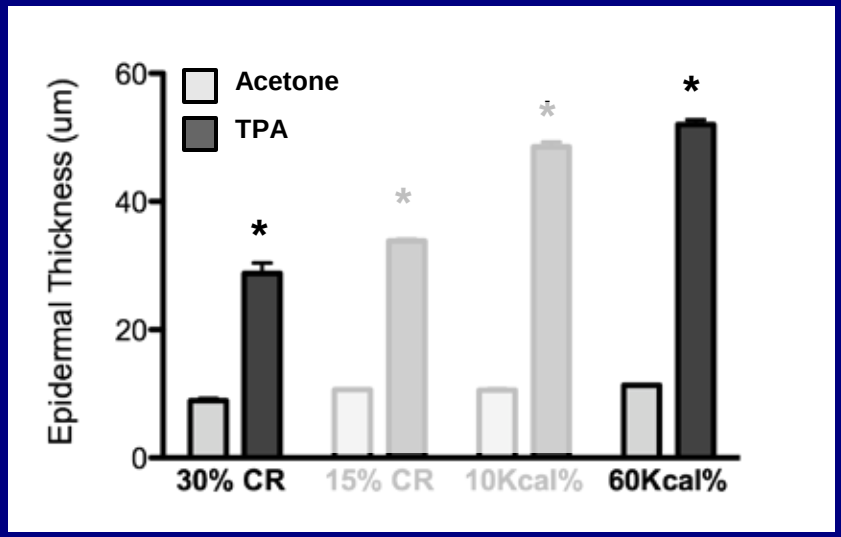
| Experimental Diet | Total Number of Mice | Average Papillomas Per Mouse[1] | Carcinoma Incidence (%) | Carcinomas per Mouse | Conversion Ratio[2] |
|---|---|---|---|---|---|
| 60kcal% fat | 25 | 8.86 | 96.0 | 2.28[a] | 0.26 |
| 10kcal% fat | 27 | 8.20 | 92.3 | 1.58 | 0.20 |
| 15% CR | 29 | 6.17[a] | 69.0 | 1.59 | 0.26 |
| 30% CR | 26 | 4.27[a] | 57.7 | 0.96[a] | 0.23 |

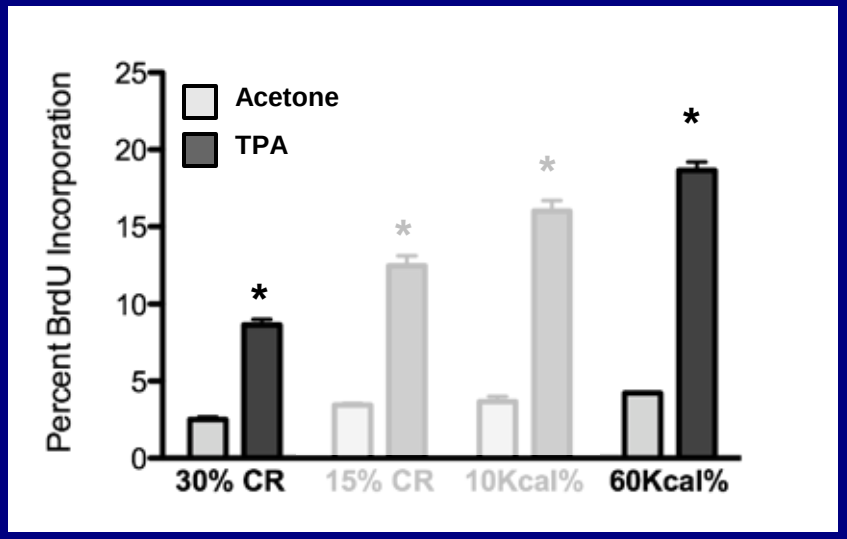
[1]Data taken at 27 weeks of promotion with 3.4 nmol TPA after which the papilloma response had reached a plateau
[2]Ratio of the average number of SCCs at 50 weeks to average number of papillomas at 27 weeks
[a]Significantly different from all groups, Wilcoxon Rank Sum ($p<0.05$)

Moore et al, unpublished data

# Dietary Energy Balance Modulation Alters Epidermal Proliferation Following TPA Treatment



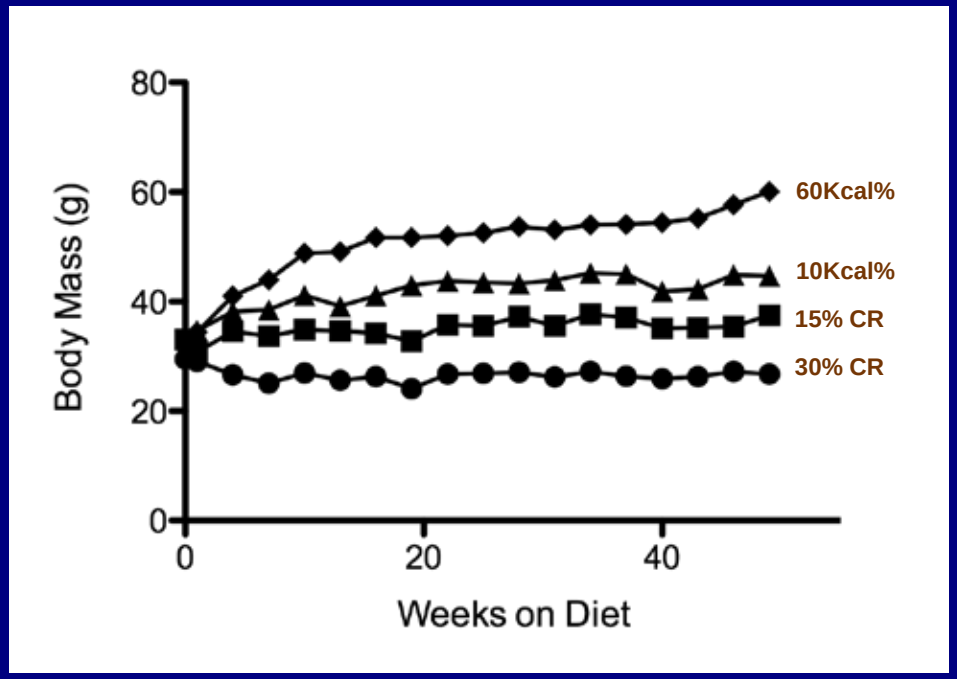



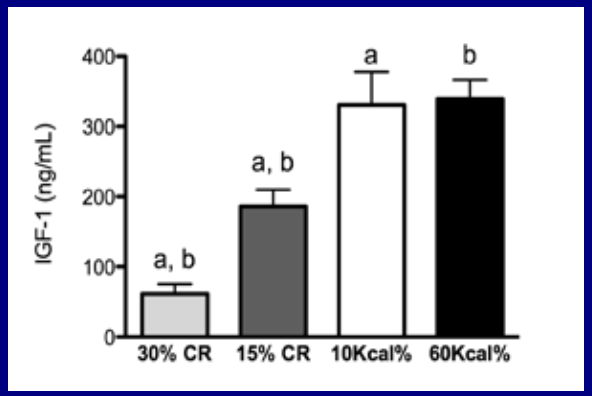



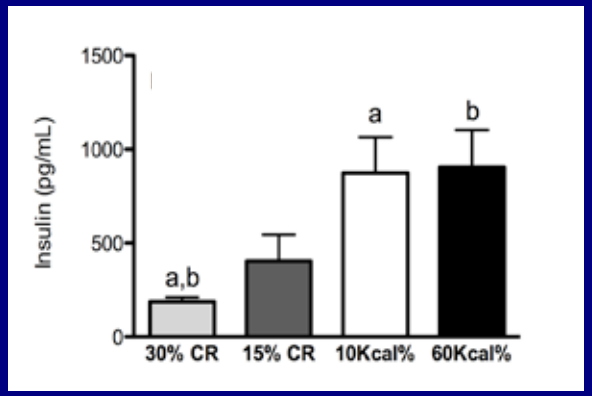

Moore et al, unpublished data

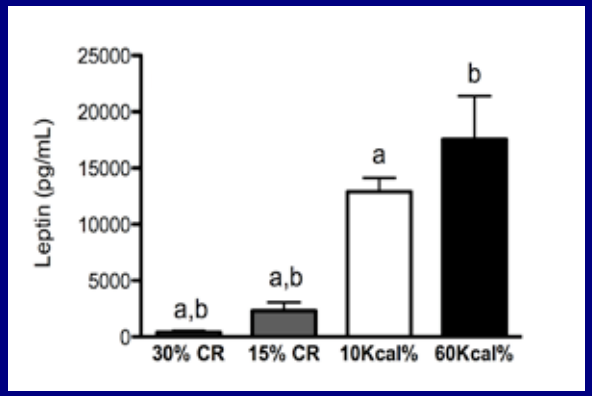
* Significantly different from other groups Wilcoxon Rank Sum p < 0.05

# Weight Gain and Selected Serum Protein Profiles of Female ICR Mice in the Two-Stage Skin Carcinogenesis Study



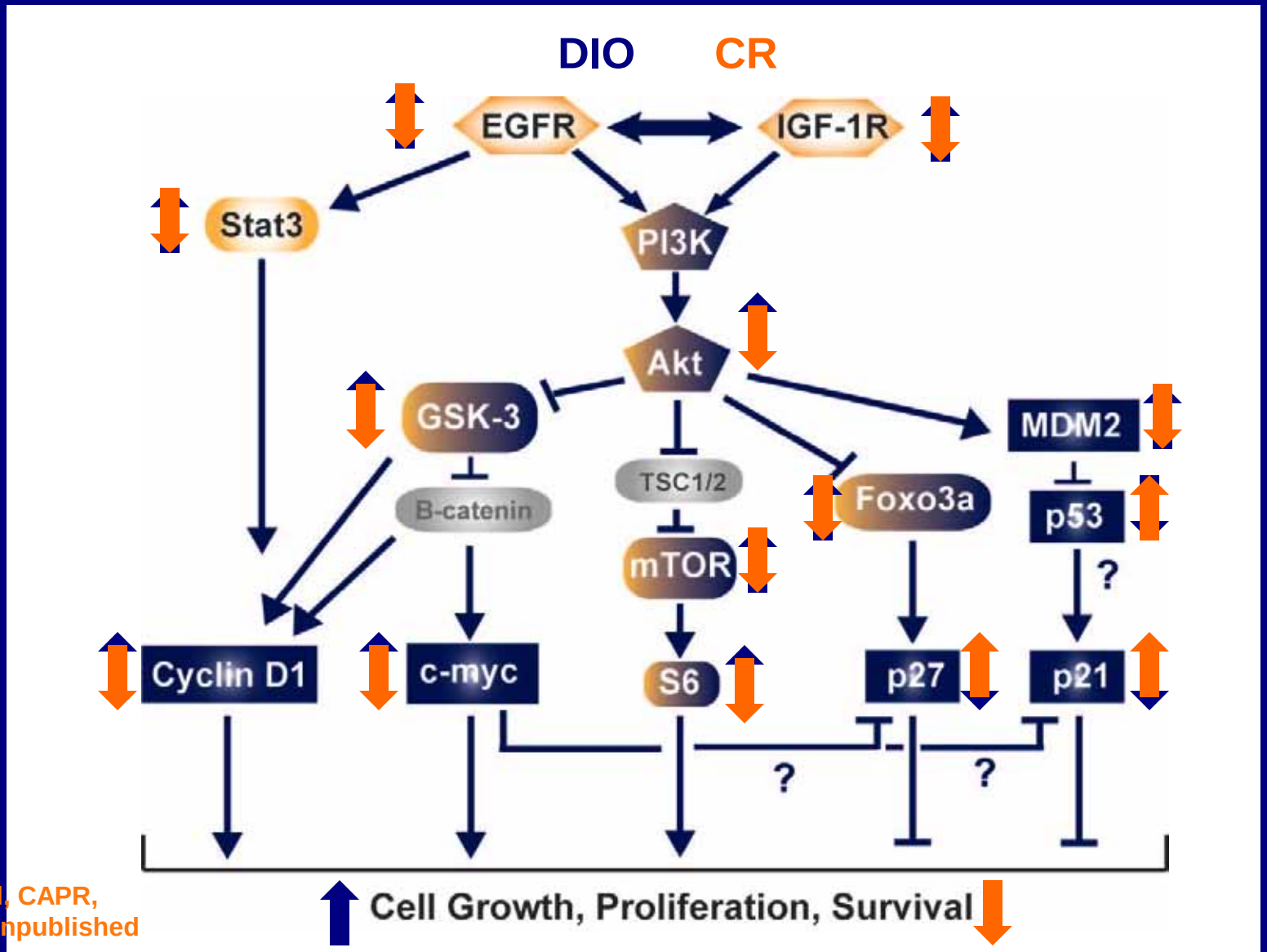




[a,b] Significantly different from values with the same lettering Wilcoxon Rank Sum ( $p < 0.05$)

Moore et al, unpublished data

# Summary of Dietary Energy Balance Effects on Growth Factor Signaling and Cell Cycle Related Proteins in Epidermis During Tumor Promotion



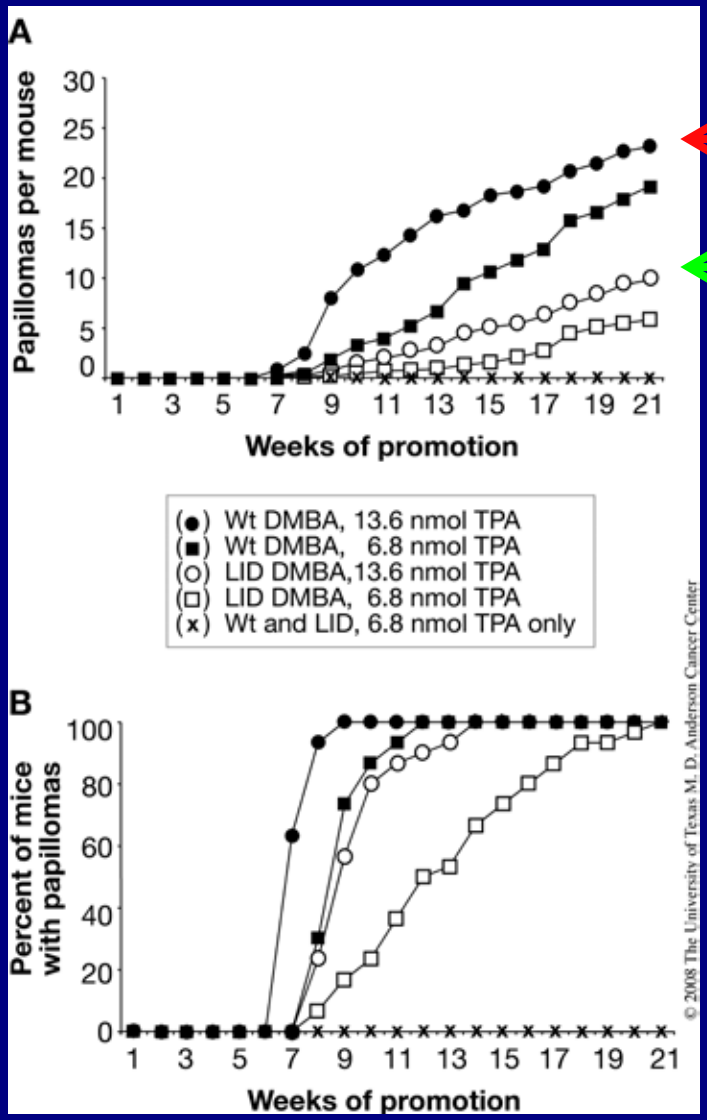

Moore et al, CAPR, 2008 and unpublished data

# Reduced Circulating IGF-1 Inhibits Two-Stage Skin Carcinogenesis

## LID Mice

- Circulating IGF-1 is produced primarily by the liver
- Cre/loxP system was utilized to create a targeted deletion of liver specific IGF-1 production
- Results in a 75% reduction in circulating IGF-1
- Targeted deletion had no effect on post-natal growth

- Liu et. al. *Proc Soc Biol Exp Med*, 2000;Yakar et. al. *PNAS*, 1999

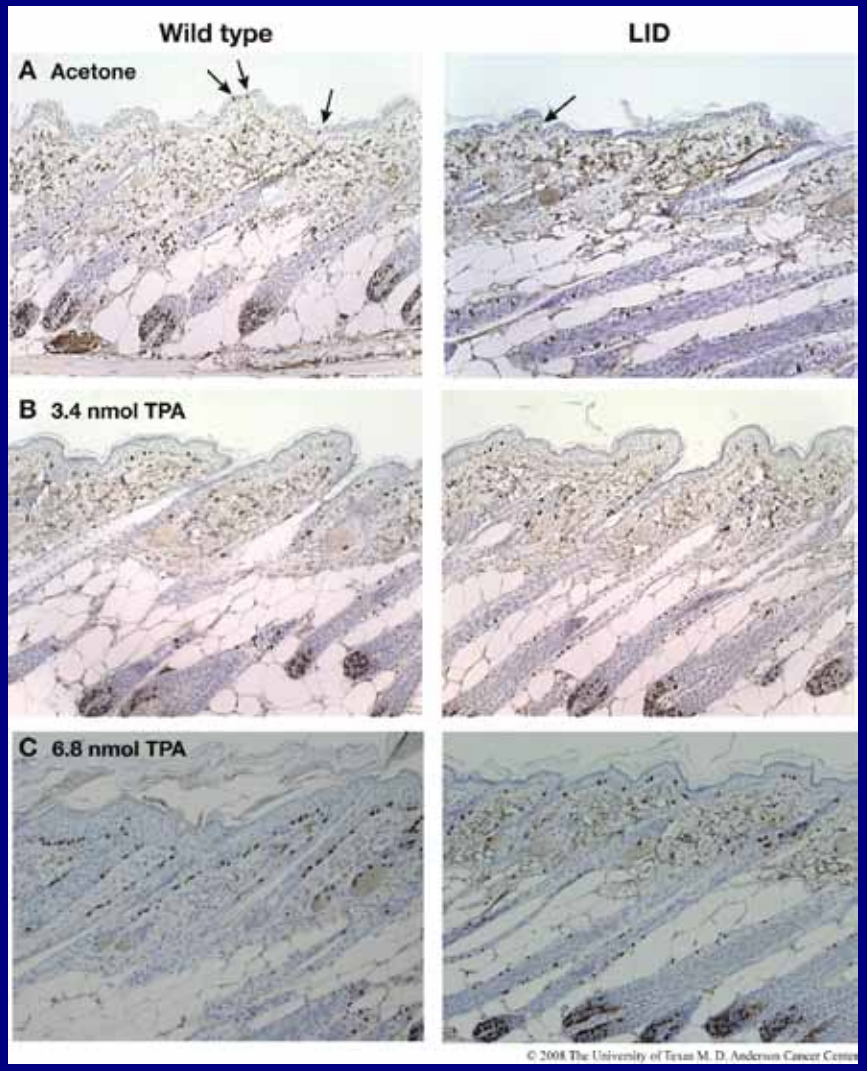


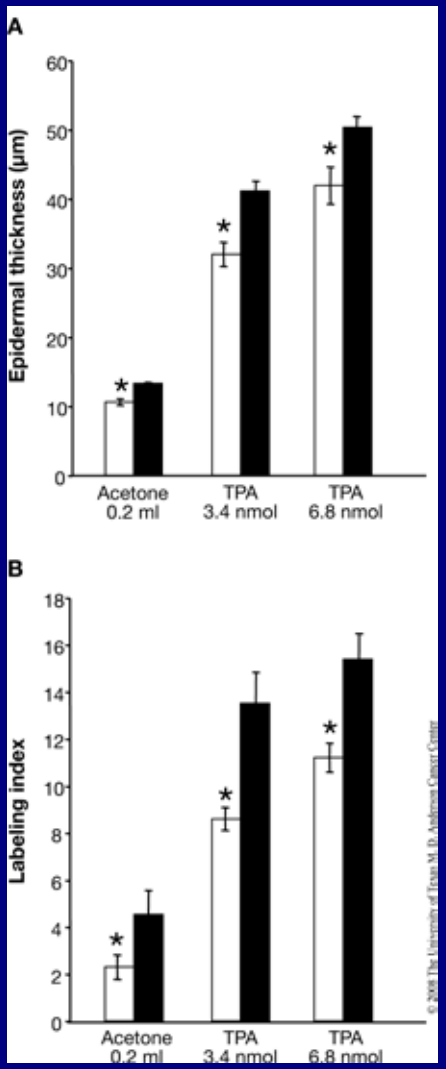

TPA 13.6, Wt

TPA 13.6, LID

Moore et al. *Cancer Res*, 2008

# Reduced Circulating IGF-1 Decreases Epidermal Proliferation Both in the Absence and Presence of TPA Treatment

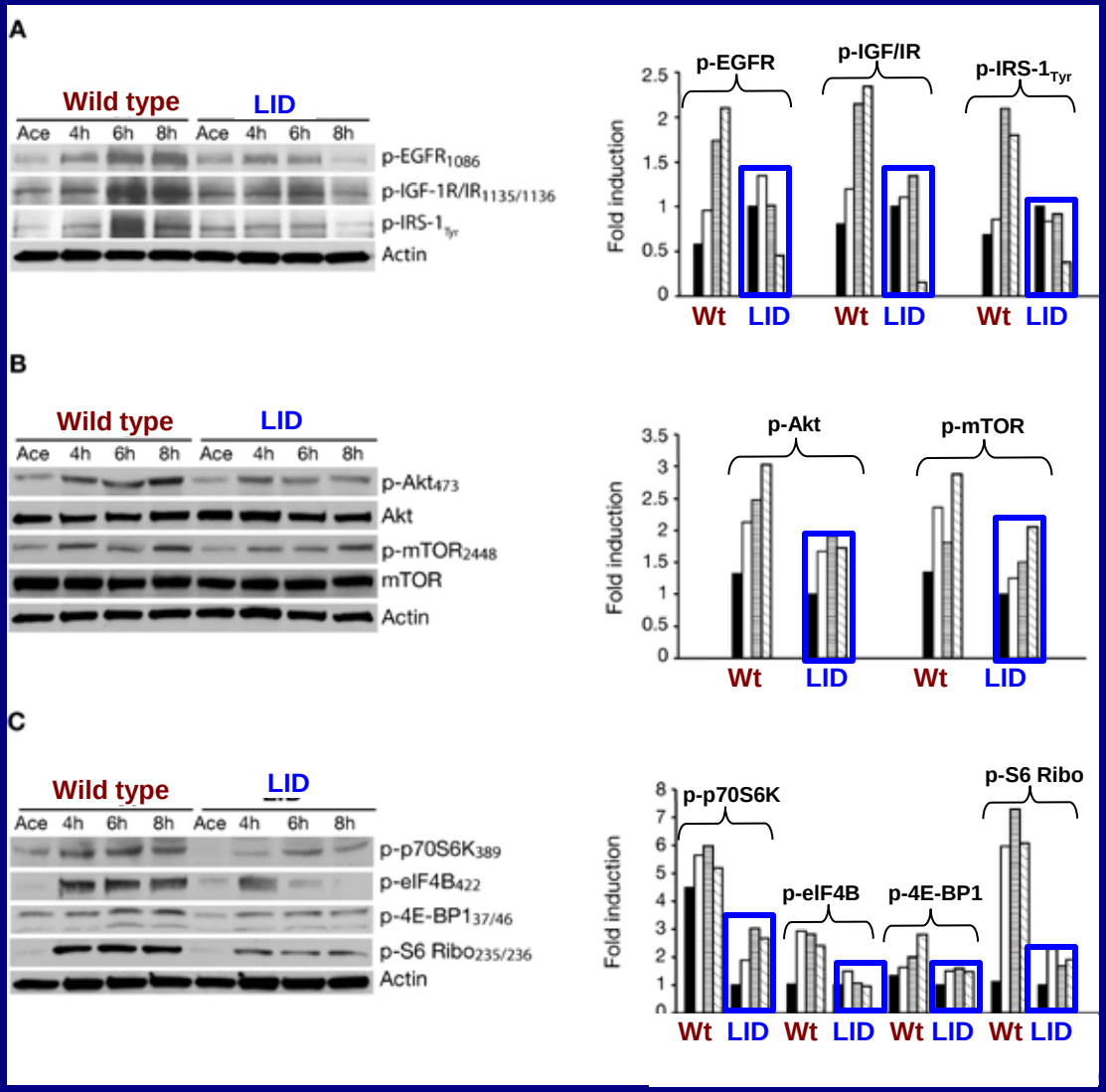



Moore et al. *Cancer Res*, 2008

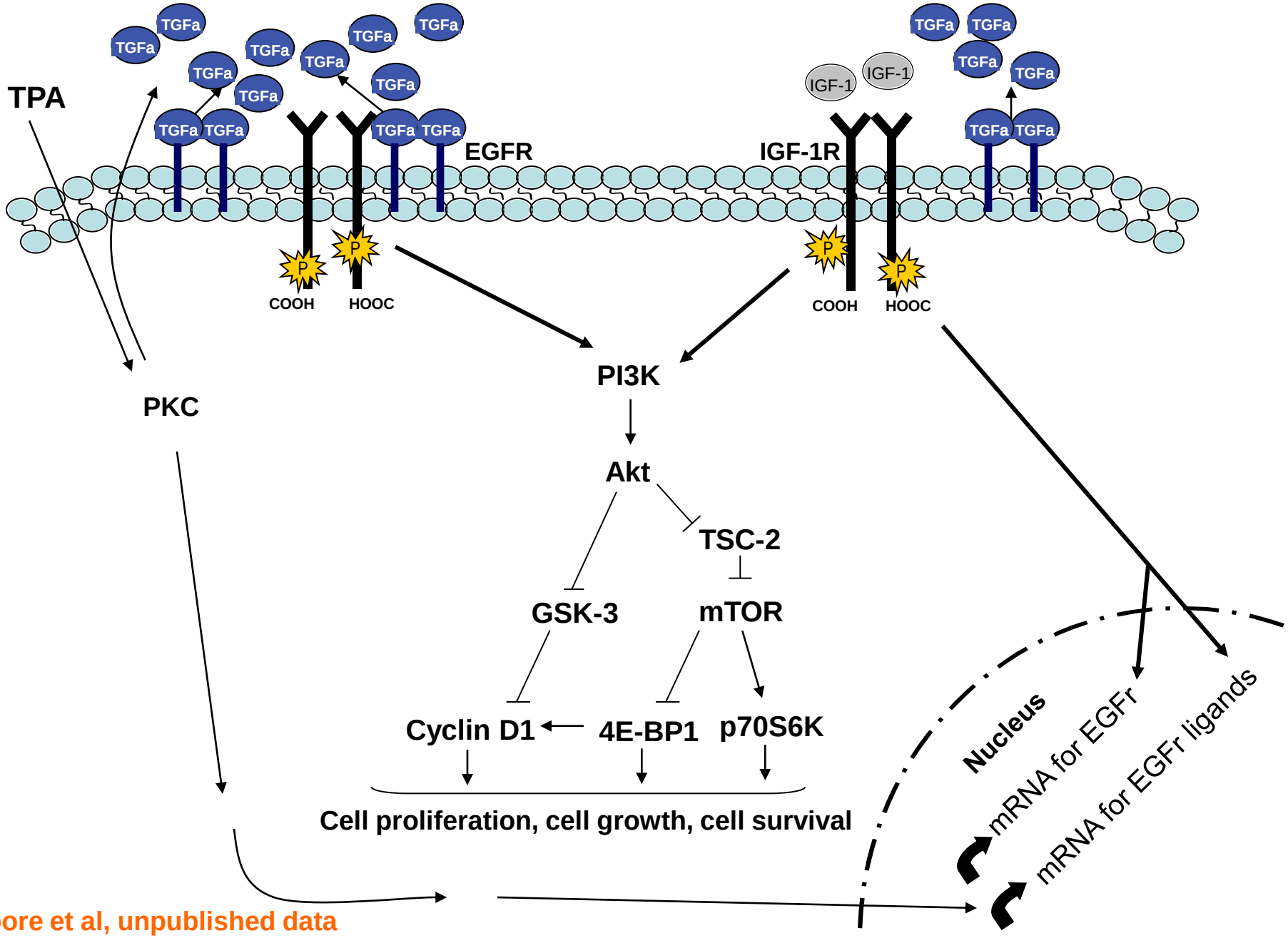
*Significantly different ($p<.05$) from values obtained from wild-type mice

# Reduced Circulating IGF-1 Decreases Activation of EGFR, IGF-1R and Akt/mTOR Signaling Induced by TPA

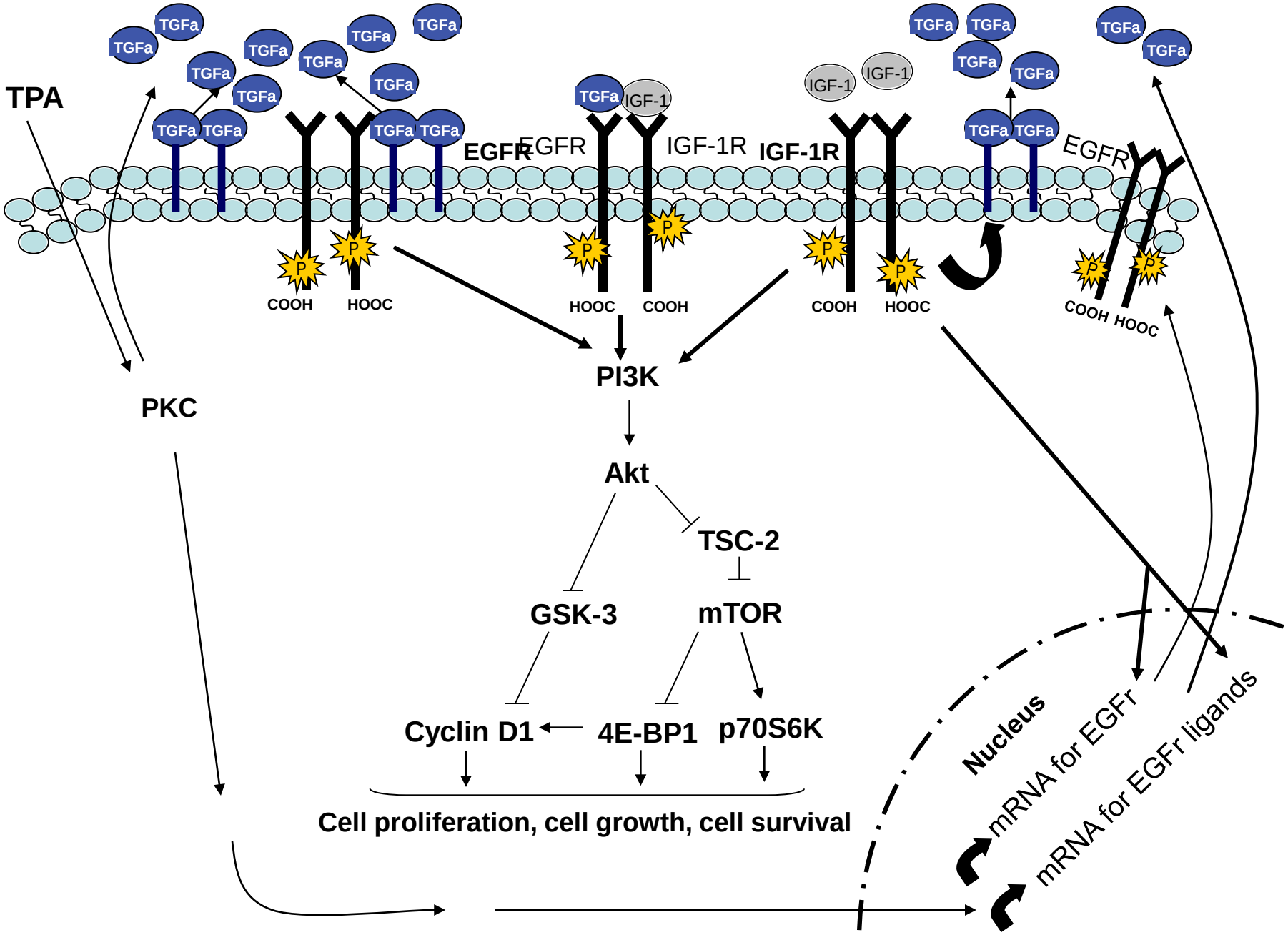



Moore et al. *Cancer Res*, 2008

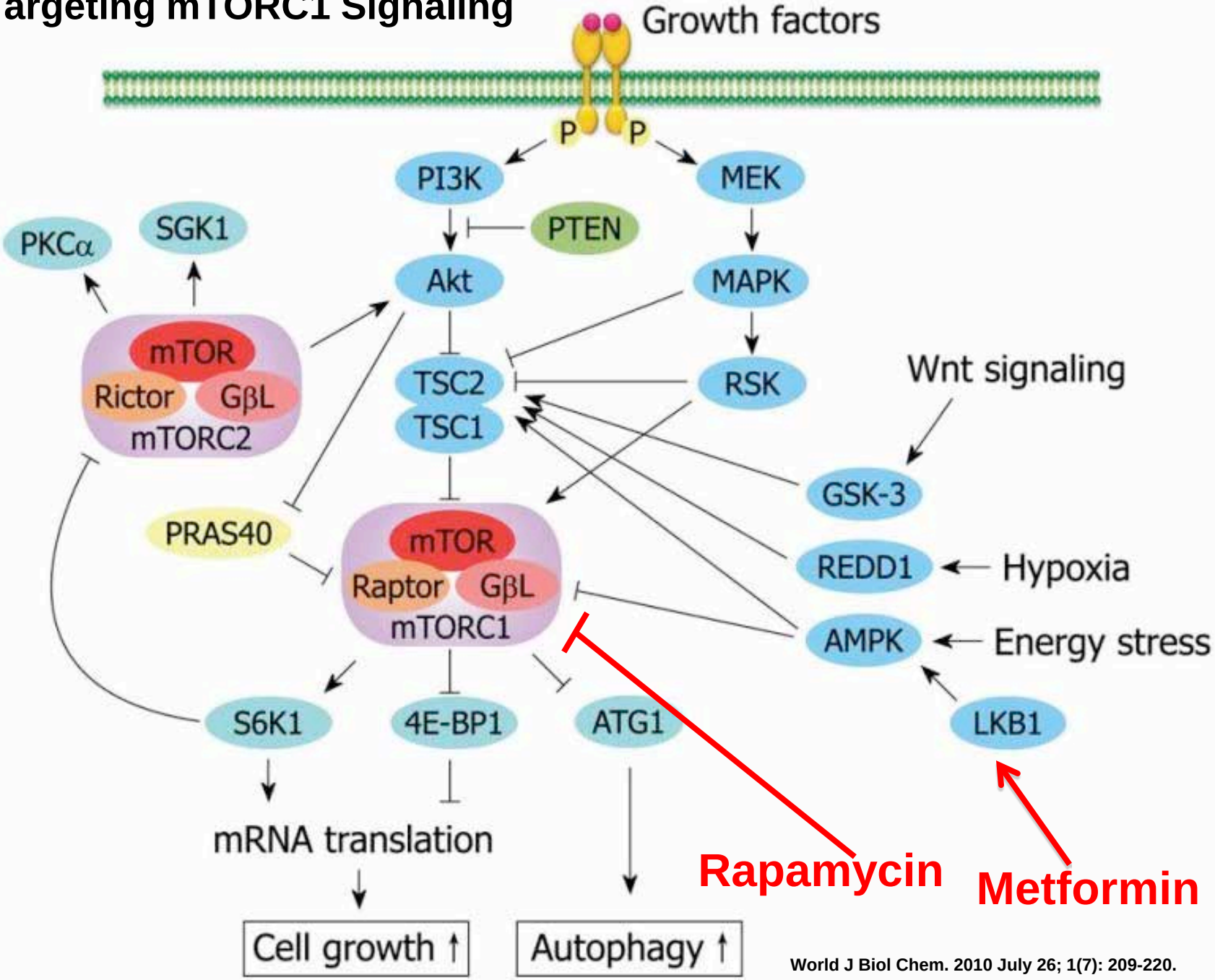
# Mechanisms for IGF-1R/EGFR Cross-Talk




Moore et al, unpublished data

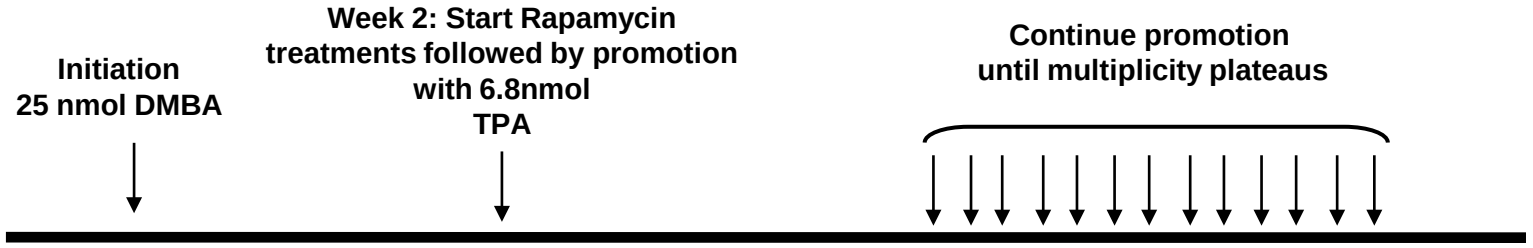

TPA
TGFa
EGFR
EGFR
IGF-1R
IGF-1R
IGF-1
IGF-1
EGFR
P
COOH
HOOC
HOOC
COOH
COOH
HOOC
COOH HOOC
PKC
PI3K
Akt
TSC-2
GSK-3
mTOR
Cyclin D1
4E-BP1
p70S6K
Cell proliferation, cell growth, cell survival
Nucleus
mRNA for EGFr
mRNA for EGFr ligands

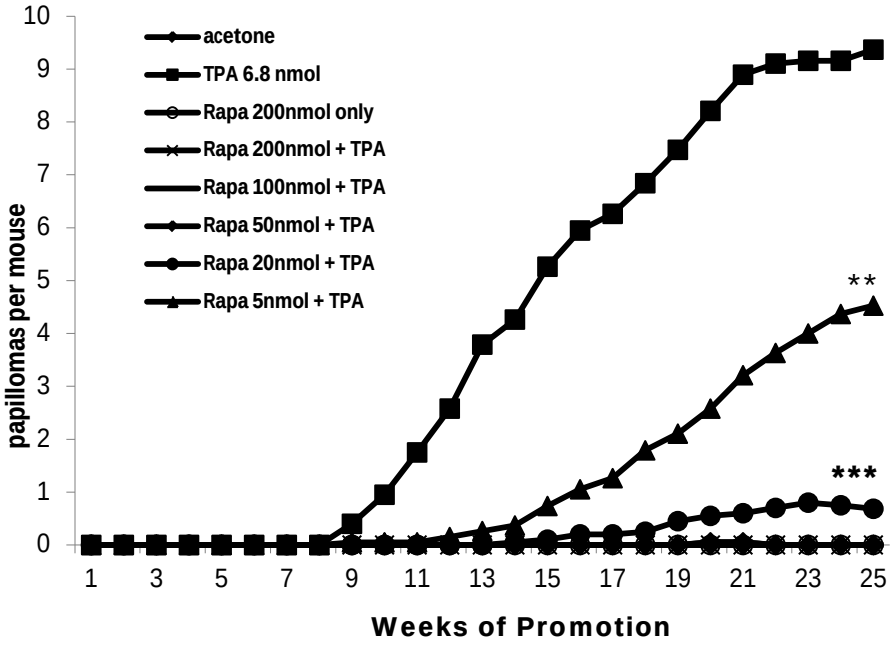
# Targeting mTORC1 Signaling



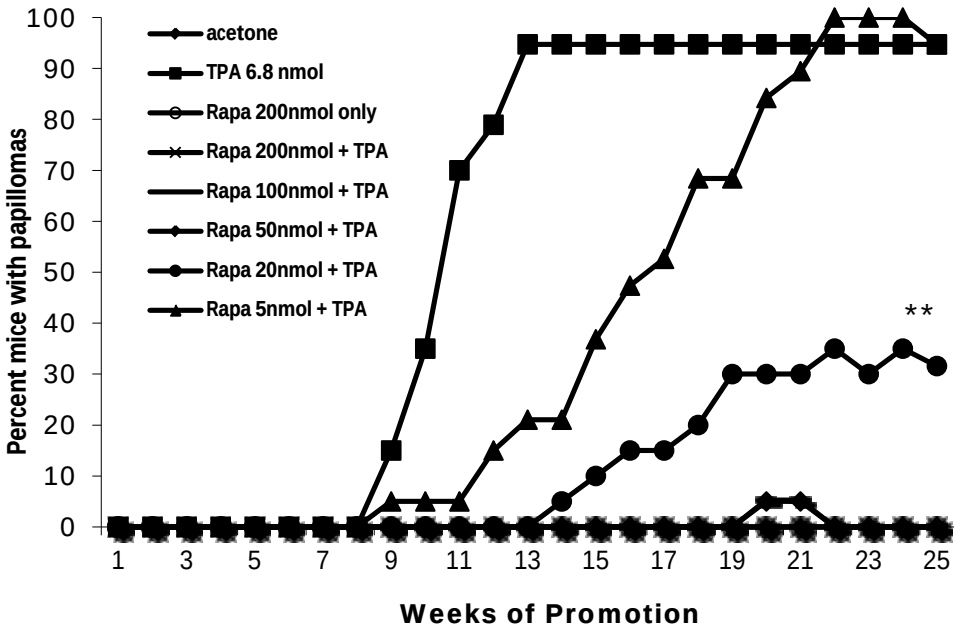

World J Biol Chem. 2010 July 26; 1(7): 209-220.

# Rapamycin is a Highly Potent Inhibitor of Skin Tumor Promotion by TPA



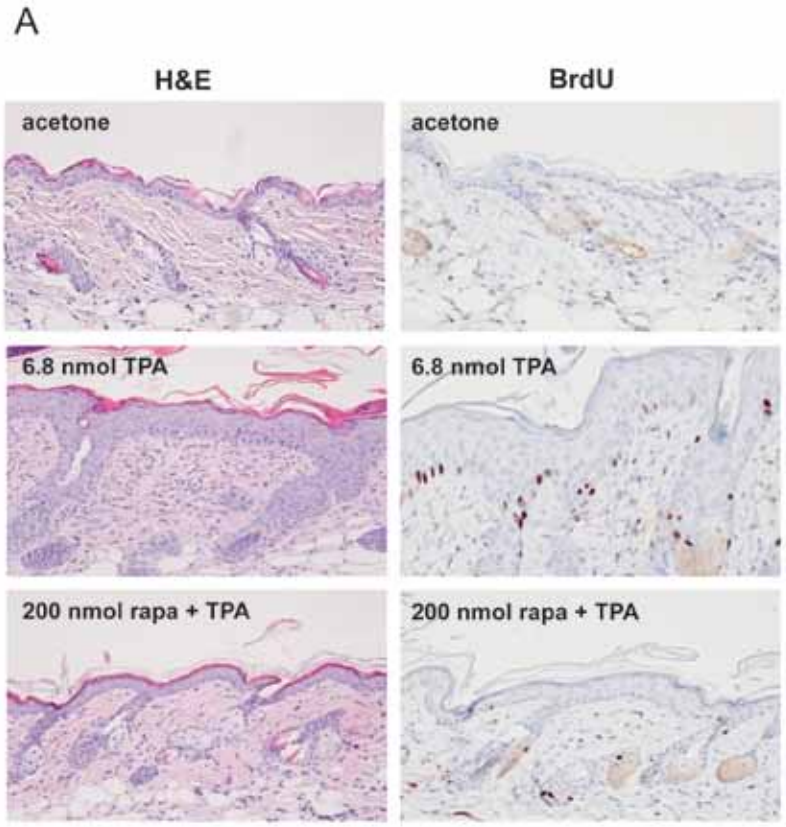

* denotes significance. Differences in the average number of papillomas per mouse at 25 weeks between the TPA control group and cooresponding Rapamycin treated groups for each dose were statistically significant ( P< 0.05, Mann-Whitney *U* Test).

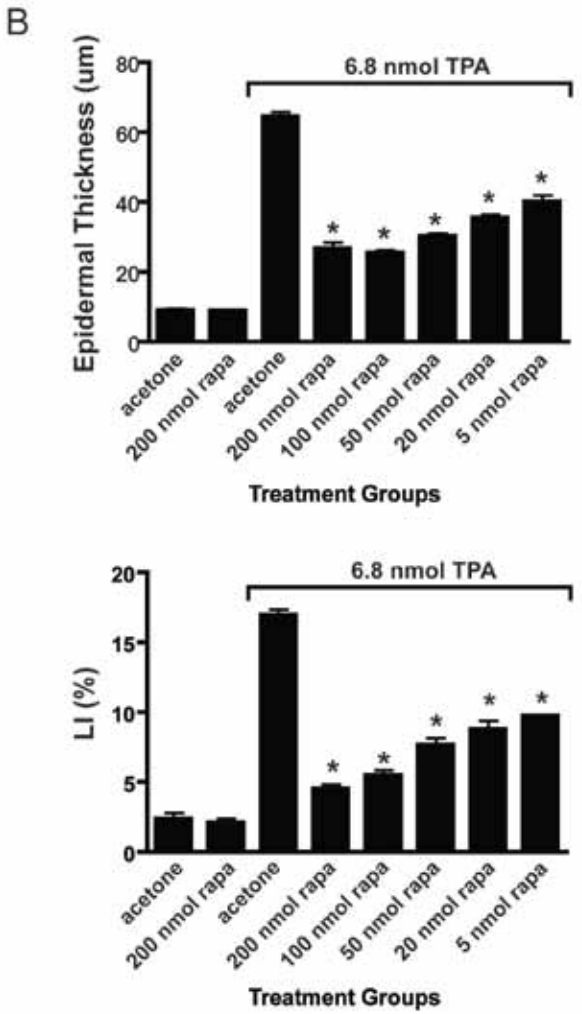



* Significantly different from 6.8nmol TPA ( P< 0.05, Chi-square test).

Checkley et al, CAPR, 2011

# Rapamycin Treatment Inhibits TPA-Induced Epidermal Hyperproliferation

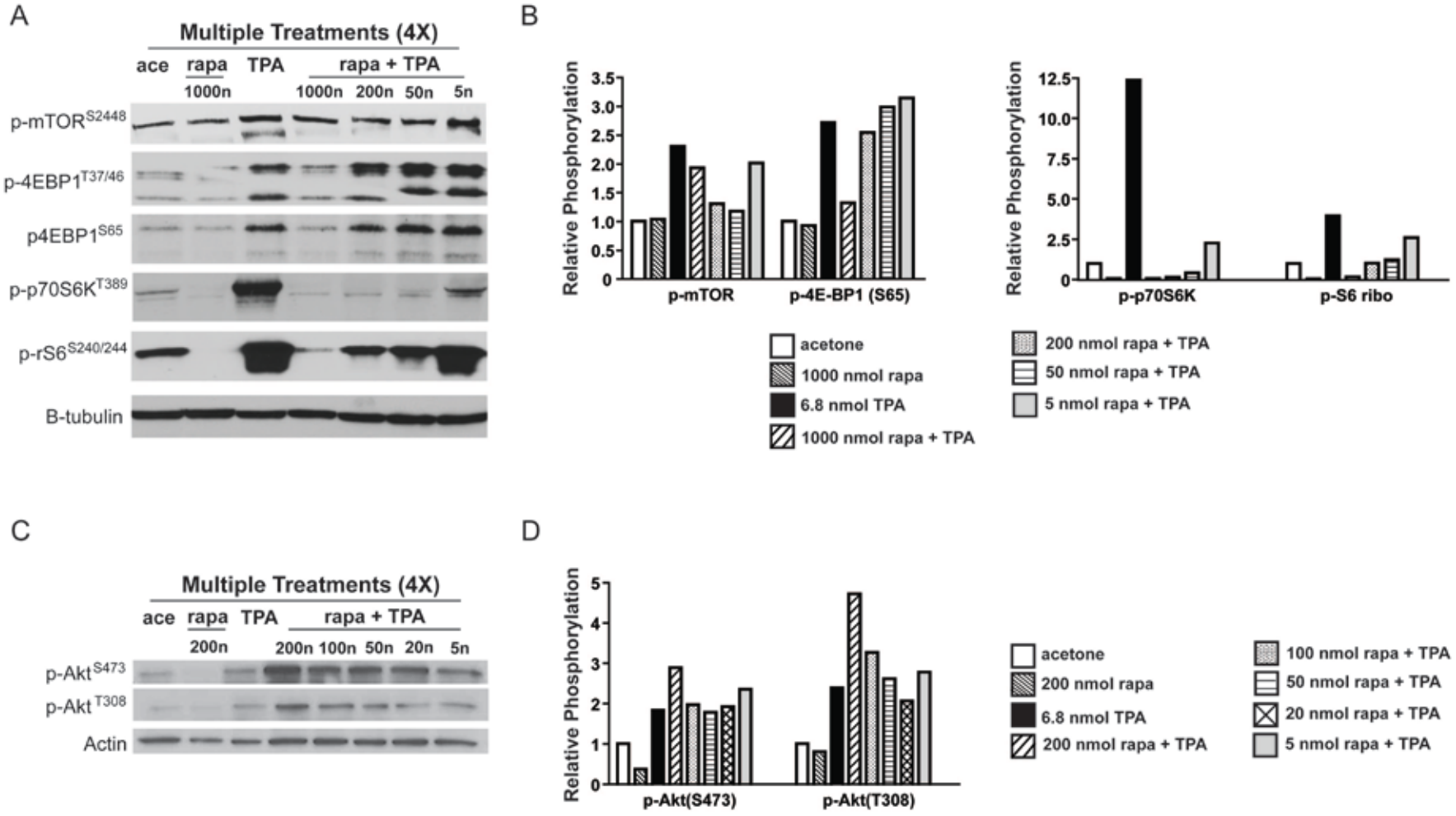



Checkley et al, CAPR, 2011

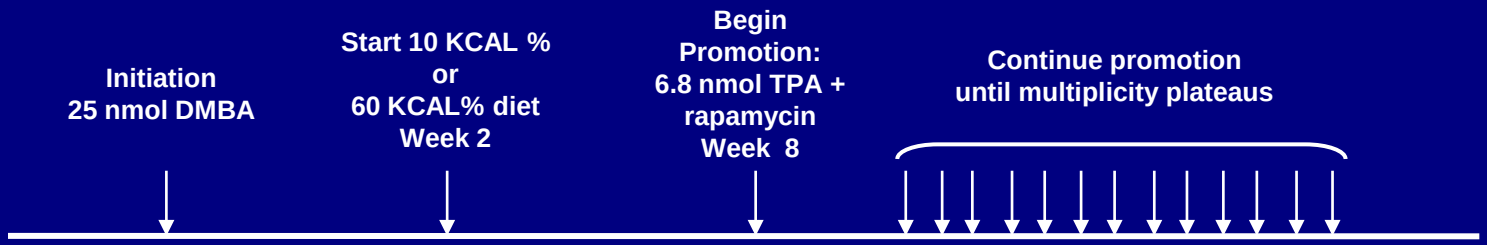
* P < 0.05

# Topical Rapamycin Inhibits TPA-Induced Epidermal mTORC1 Signaling Primarily Through the p70S6K Downstream Signaling Pathway

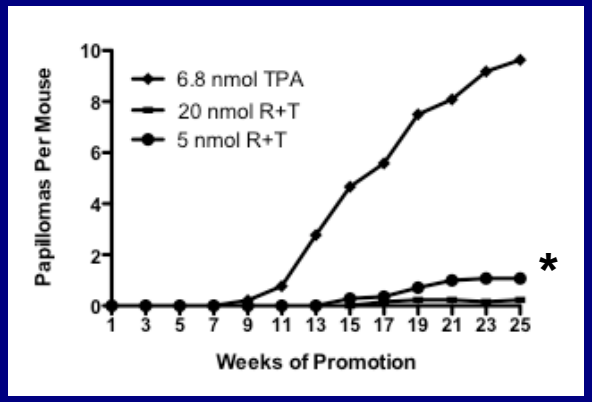


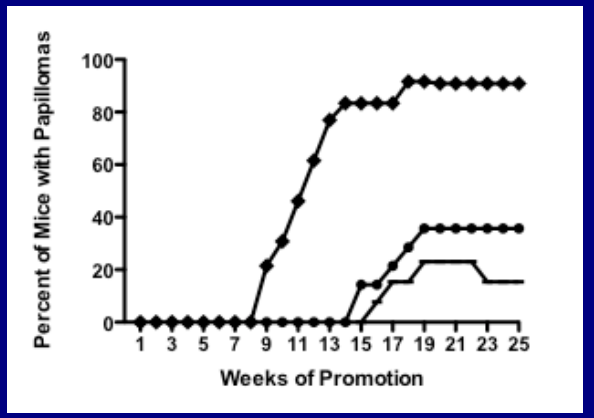

Checkley et al, CAPR, 2011

# Rapamycin Potently Inhibits Skin Tumor Promotion by TPA in Overweight and Obese Mice

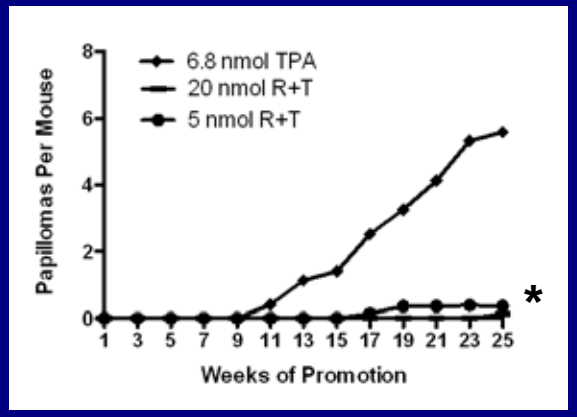


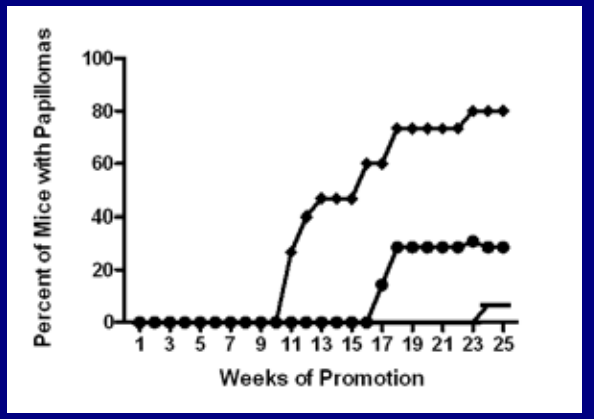

Overweight




Obese

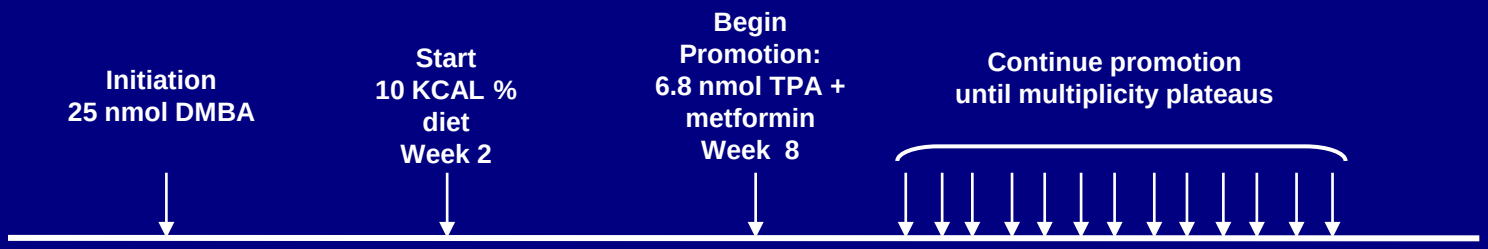


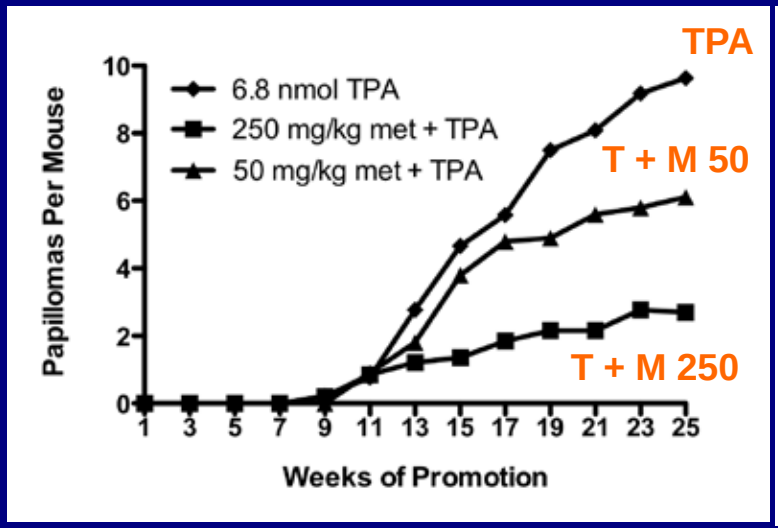



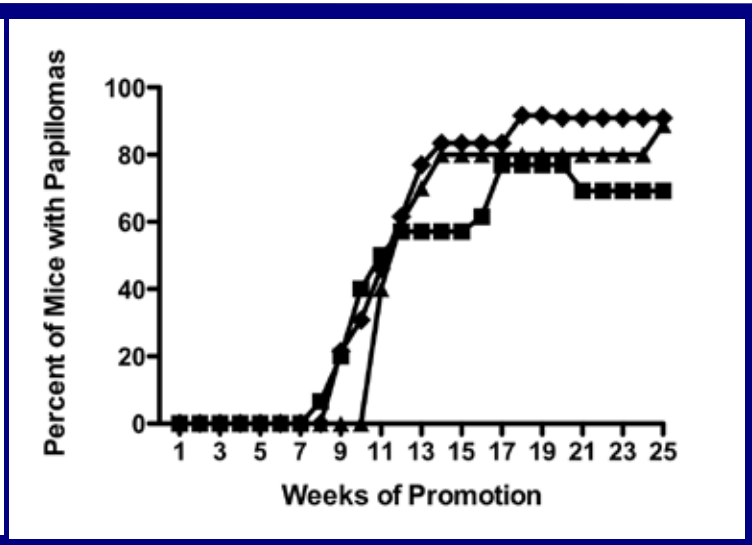

*** Indicates significantly different from 6.8 nmol TPA ($p < .05$)**

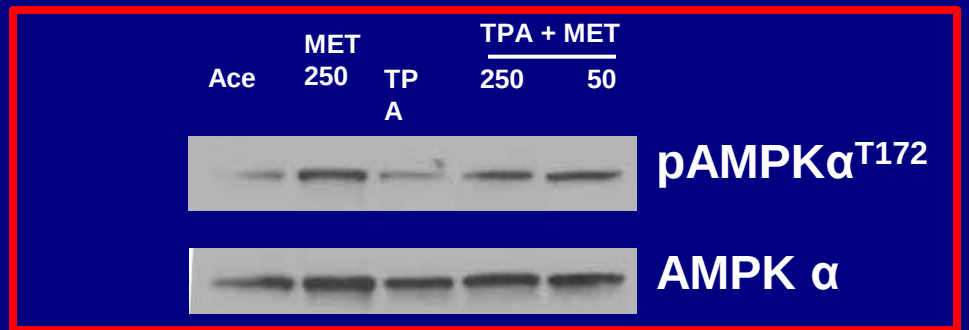
Checkley et al, unpublished

# Metformin Given in the Drinking Water Inhibits Skin Tumor Promotion by TPA in Overweight Mice



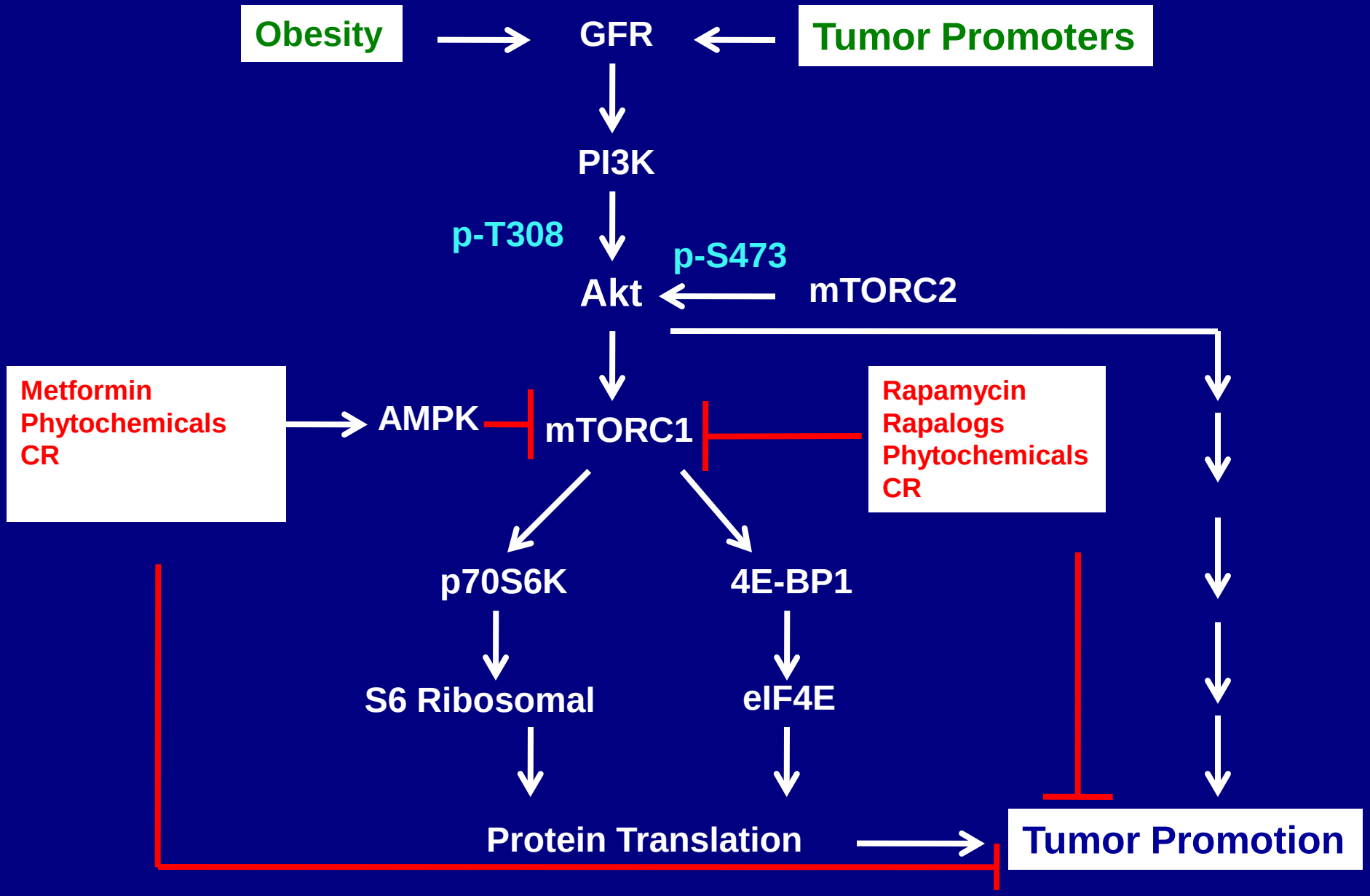




Checkley et al, unpublished

# mTORC1 Signaling Pathway as a Potential Target for Prevention of Obesity Related Cancer Promotion



# Hi-Myc Mouse Model of Prostate Cancer

- Overexpression of c-myc in ARR2PB-myc-PAI transgenic mice resulted in complete penetrance of PIN as early as 2 to 4 weeks of age.
- Progression to locally invasive adenocarcinomas occurs within ages 3 to 6 months for Hi-myc mice and within 10 to 12 months in mice expressing low c-myc levels.
- Ventral Prostate, Dorsolateral Prostate, and Anterior Prostate are the target of c-Myc overexpression.
- Metastases are not present in these transgenic mice.
- This mouse model was the first nonSV40 model to develop advanced adenocarcinoma without NE features.

Elwood-Yen et al, Cancer Cell 4:223-228, 2003

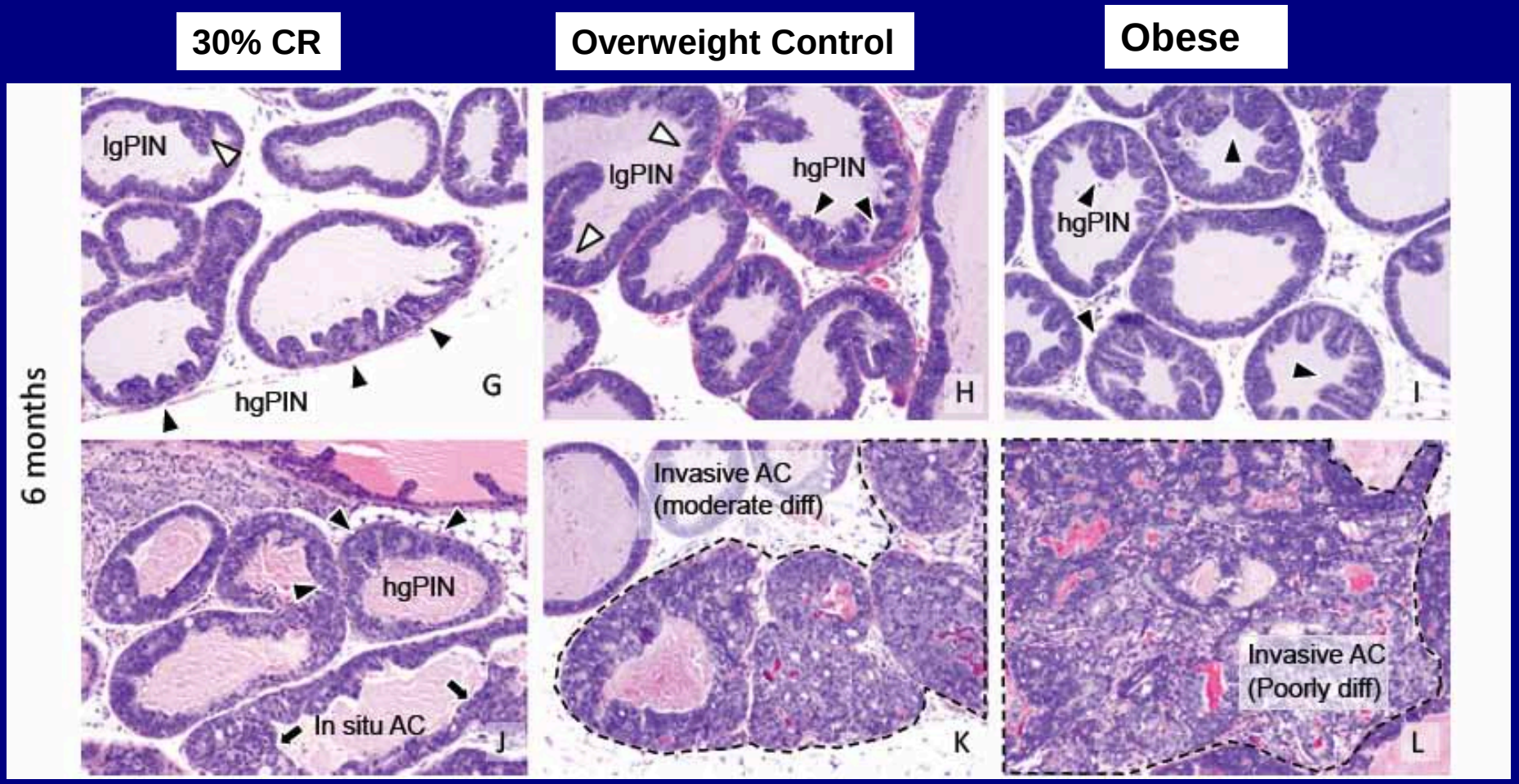
# Effect of Dietary Energy Balance Manipulation on Body Mass, Body Fat Content and Circulating Growth Factors/Adipokines in Hi-Myc Mice.

| | 30% CR | Overweight control | DIO |
|---|---|---|---|
| **Initial mass (g)** | 25.9 ± 0.9 | 24.1 ± 0.96 | 22.3 ± 0.88 |
| **Final mass (g)[b]** | 23.9 ± 1.2 | 40.1 ± 1.2 | 44.9 ± 1.6 |
| **Body fat (%)[b]** | 21.1 ± 0.84 | 38.0 ± 0.87 | 41.2 ± 1.2 |
| **Feed consumption (g/wk)[b]** | 21.2 ± 0.04 | 29.8 ± 0.43 | 28.1 ± 0.8 |
| **IGF-1 (ng/mL)[b]** | 383.7 ± 47.1 | 550 ± 56.8 | 893. ± 90.9 |
| **Insulin (pg/mL)[b]** | 2624.6 ± 235.9 | 3934.2 ± 557.9 | 5682.9 ± 557.5 |
| **Leptin (pg/mL)[c]** | 801.6 ± 293.9 | 10385.9 ± 960.3 | 10392.1 ± 608.9 |
| **Adiponectin (ng/mL)[c]** | 12205.7 ± 898.5 | 9417.3 ± 666.9 | 9654.7 ± 568.2 |
| **Resistin (pg/mL)[b]** | 1516.1 ± 84.7 | 2003.7 ± 213.4 | 2538.5 ± 167.3 |

[a] N=12 mice/group; values are means ± SEM. Serum analyses were performed at study's end.
[b] Value for each diet group differed significantly ($p<0.05$, Wilcoxon rank sum) from that in the other groups for this parameter.
[c] Value differed significantly ($p<0.05$, Wilcoxon rank sum) in the 30% CR group for this parameter relative to the other two groups.

Blando et al, CAPR 2011

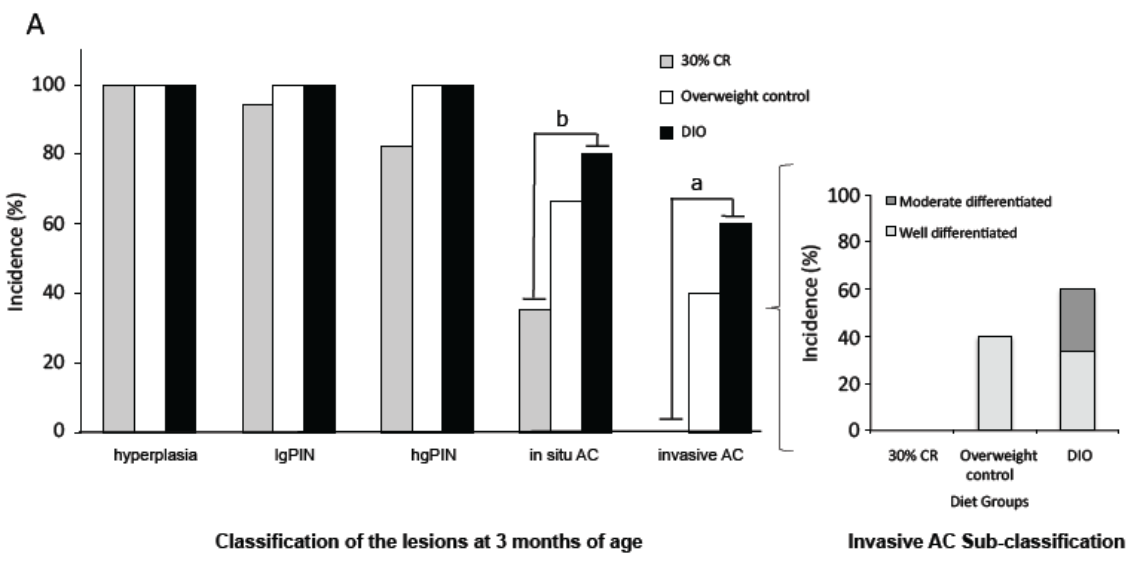
# Characterization of Representative Lesions in Ventral Prostate HI-Myc Mice on the Different Diets at 6 Months



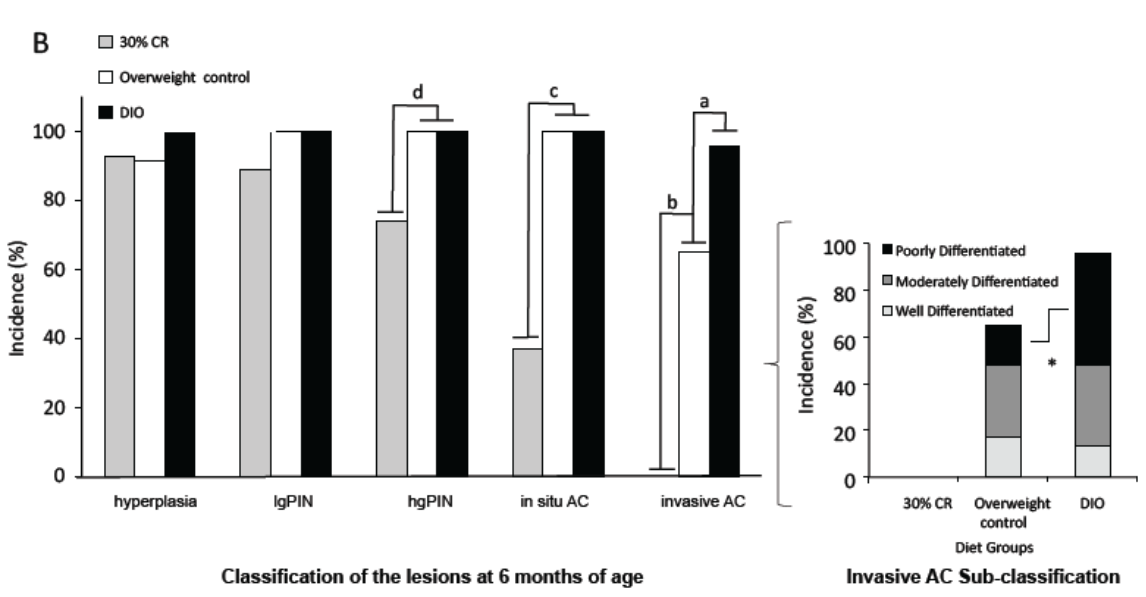

Blando et al, CAPR, 2011

# Dietary Energy Balance Effects Progression of Prostate Cancer in Hi-Myc Mice on At Both 3 and 6 Months

3 Months

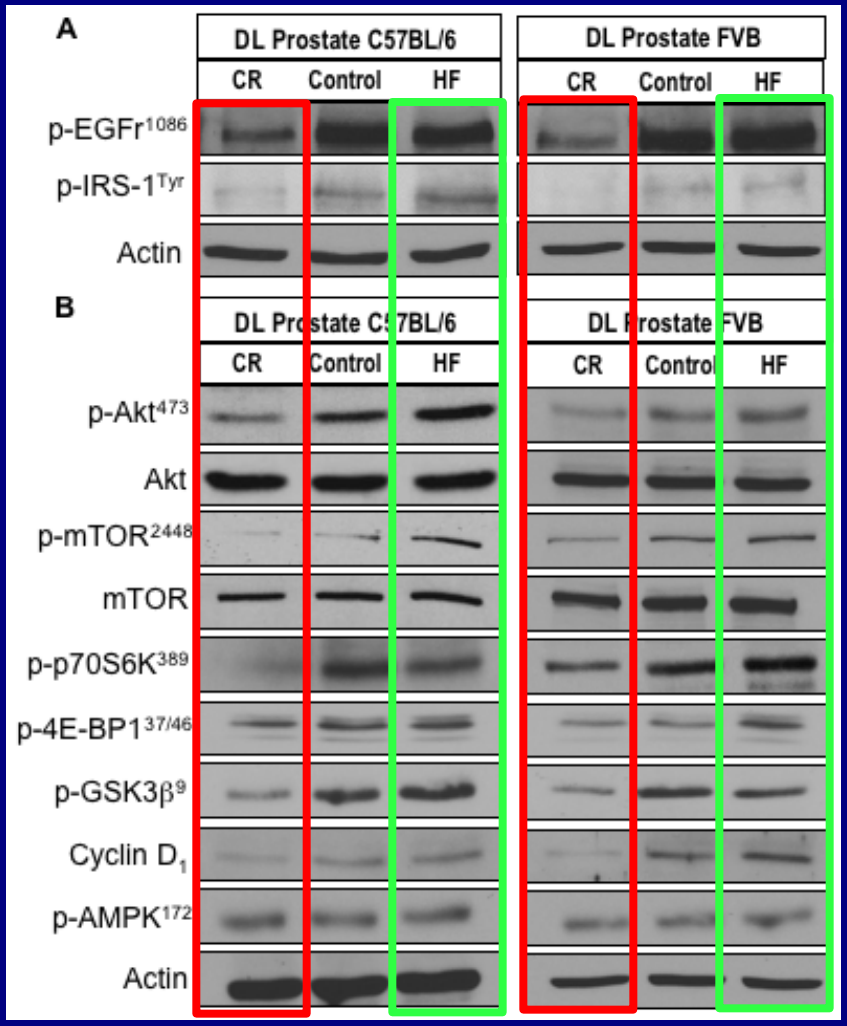



6 Months



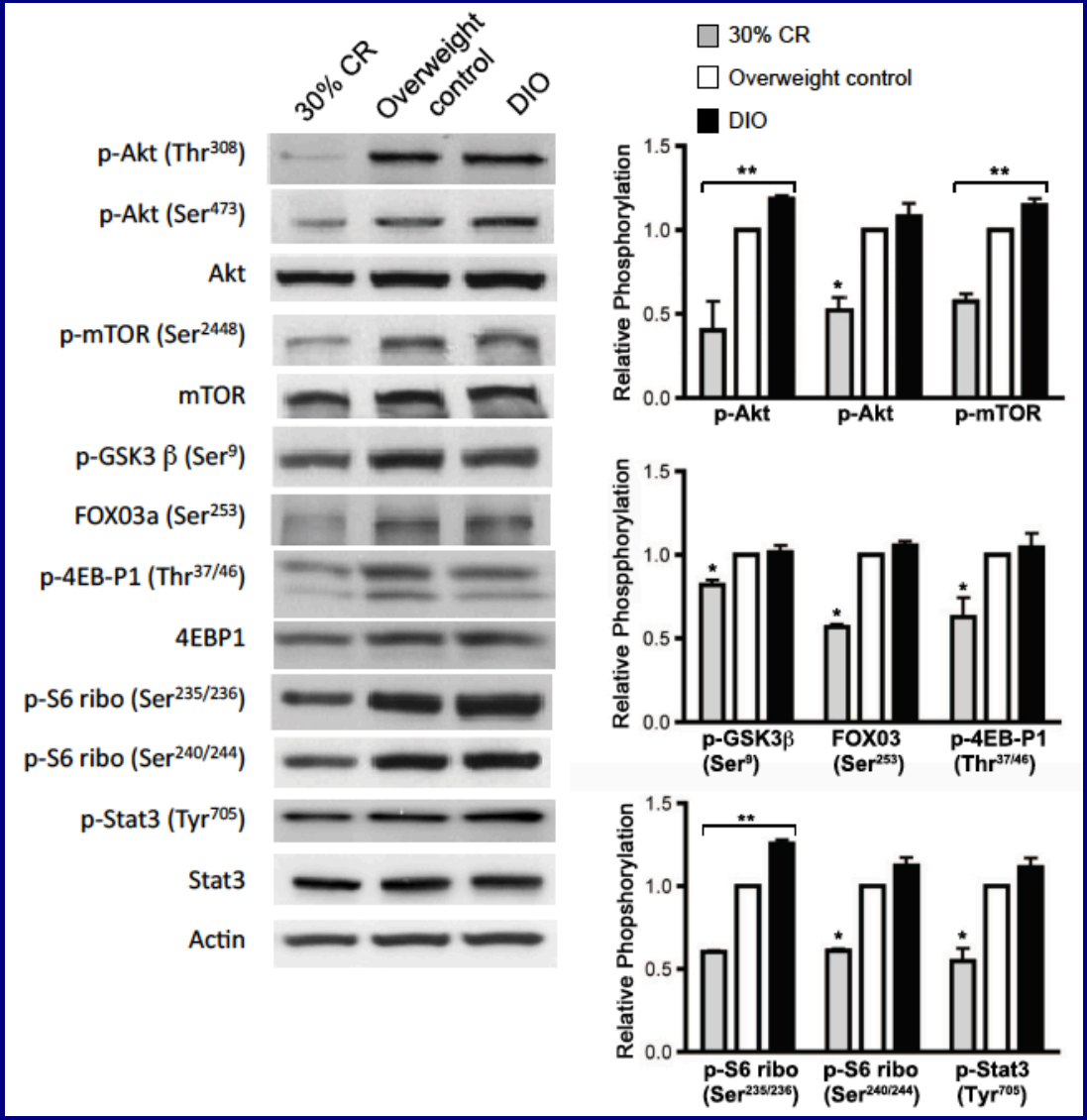

Blando et al, CAPR, 2011

# Dietary Energy Balance Manipulation Modulates Growth Factor Signaling Pathways in Prostate of Male C57BL/6 and FVB/N Mice



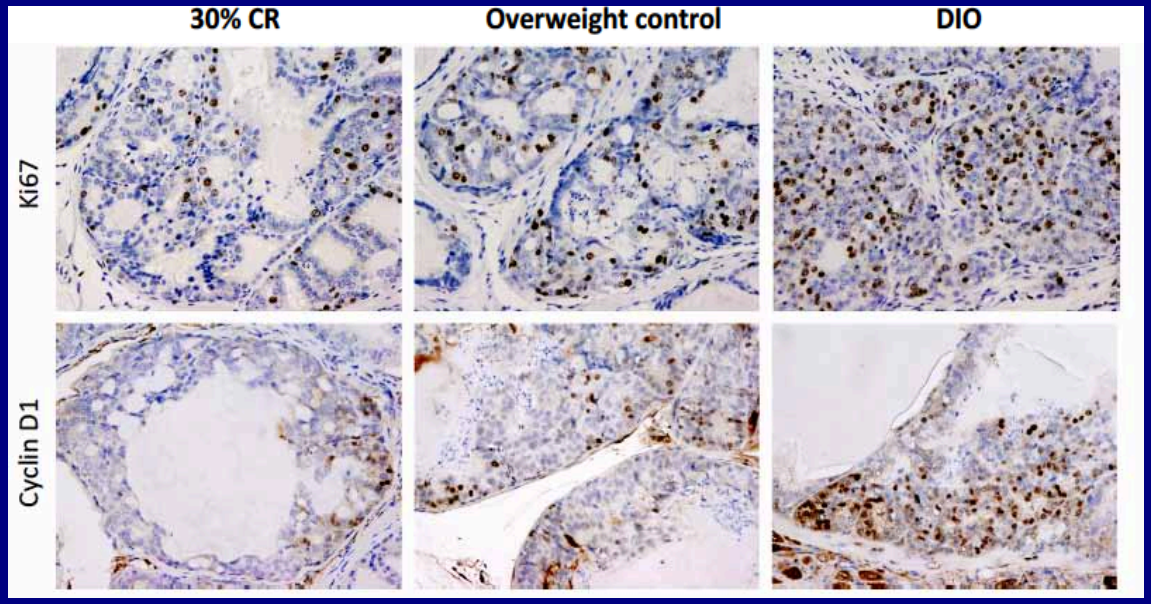

Moore et al, Cancer Prev Res, 2008 and unpublished

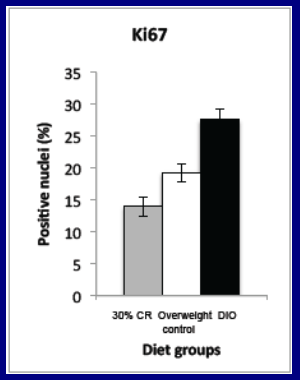
# Dietary Energy Balance Effects Growth Factor Signaling in Ventral Prostate of Hi-Myc Mice

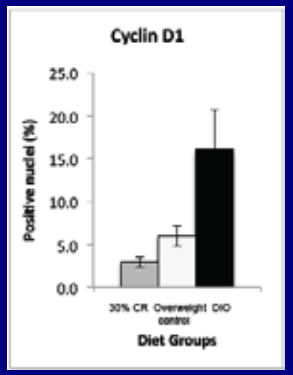


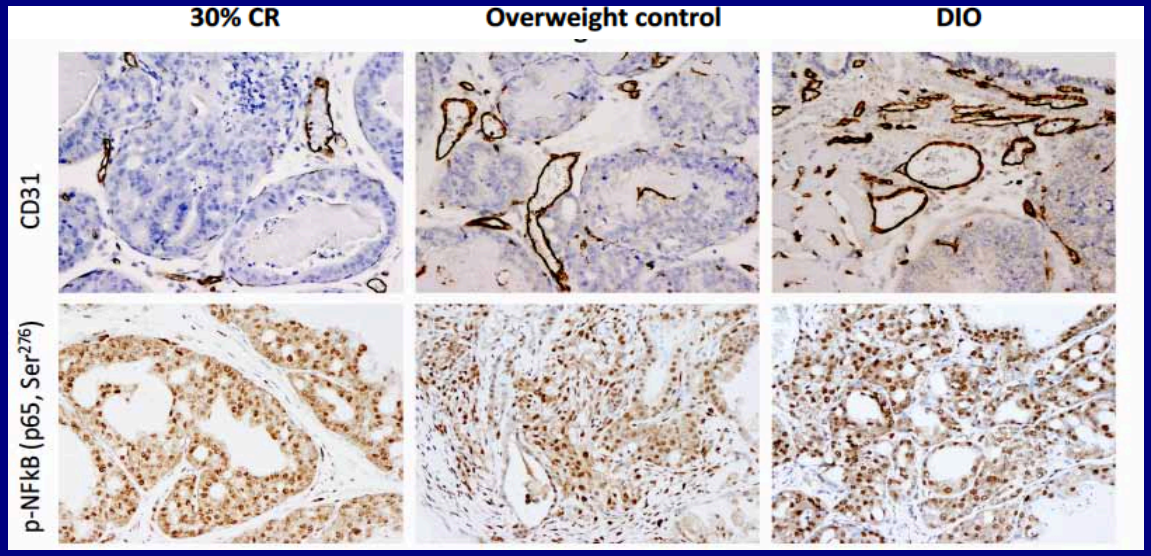

Blando et al, CAPR, 2011

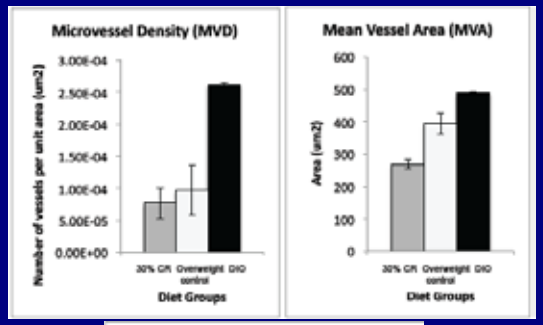
# IHC Staining for KI67, Cyclin D1, CD31 and p-NFkB IN Ventral Prostate of Hi-Myc Mice

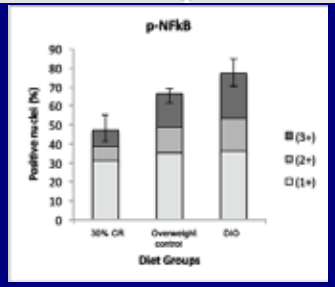


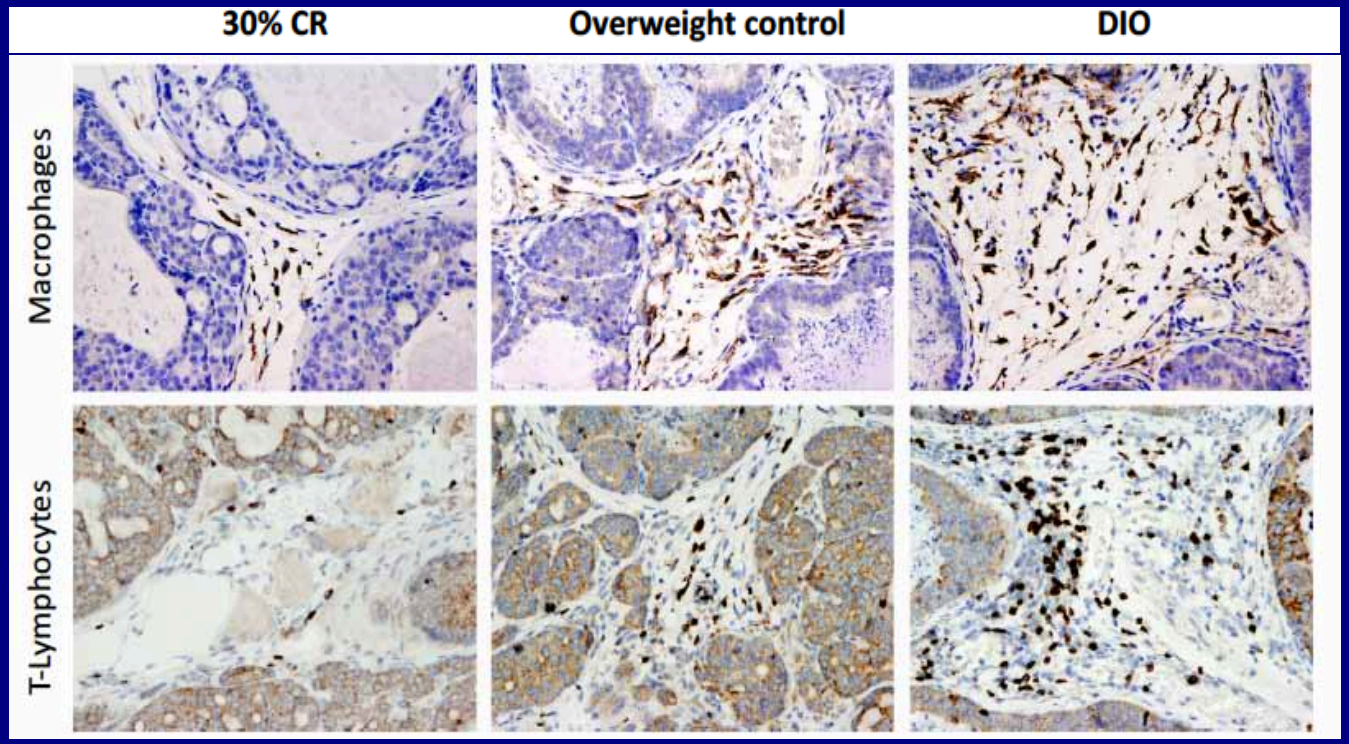



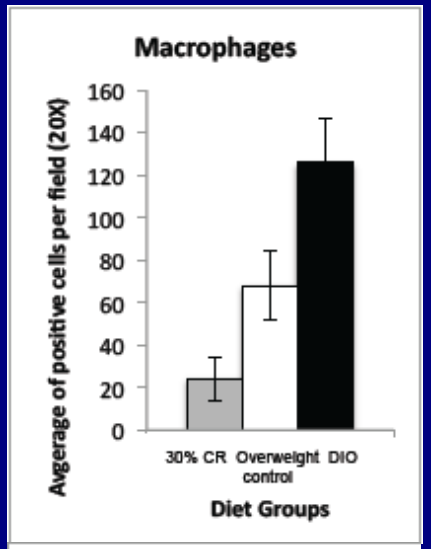



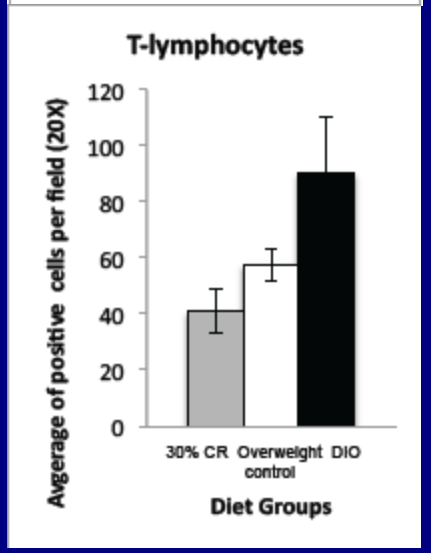



# Dramatic Increases are Seen in Stromal Inflammatory Cell Infiltration in Ventral Prostate of Hi-Myc Mice on an Obesity Inducing Diet

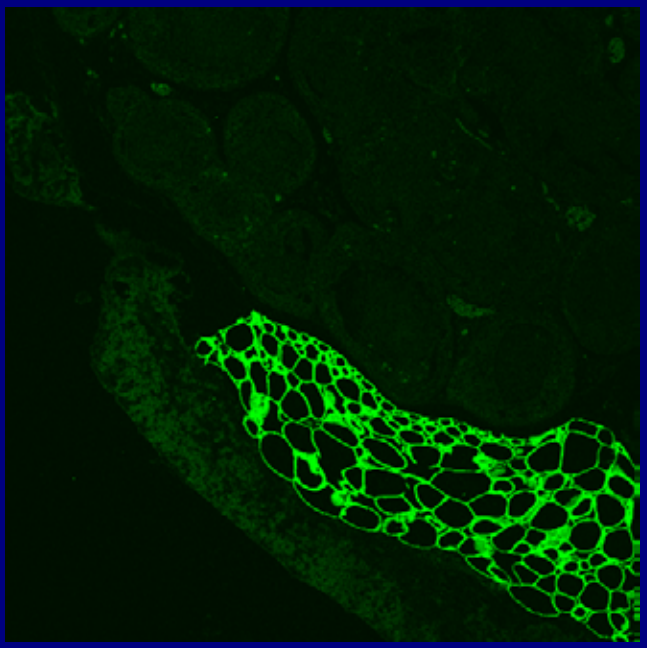


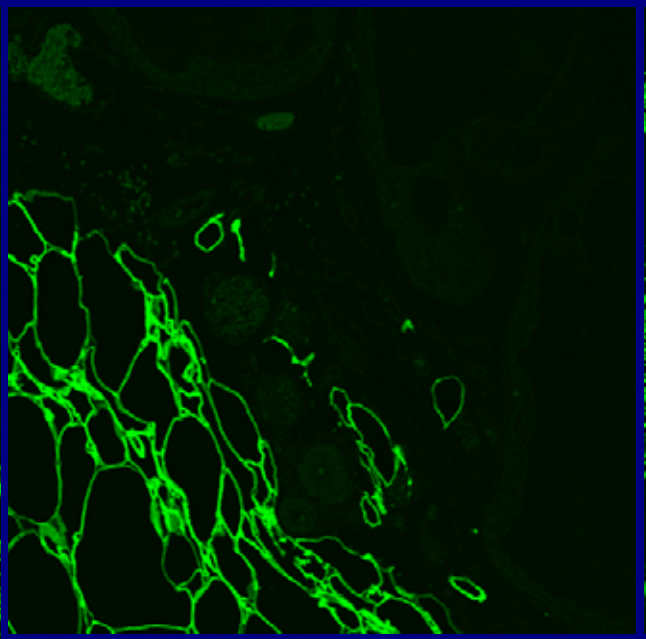



# Perilipin Staining of Adipocytes in Ventral Prostate of Hi-Myc Mice on Different Diets at 6 Months

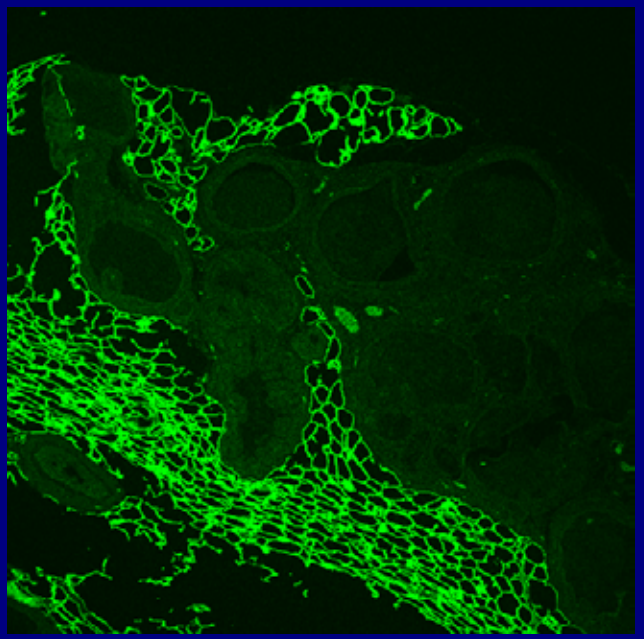
30% CR



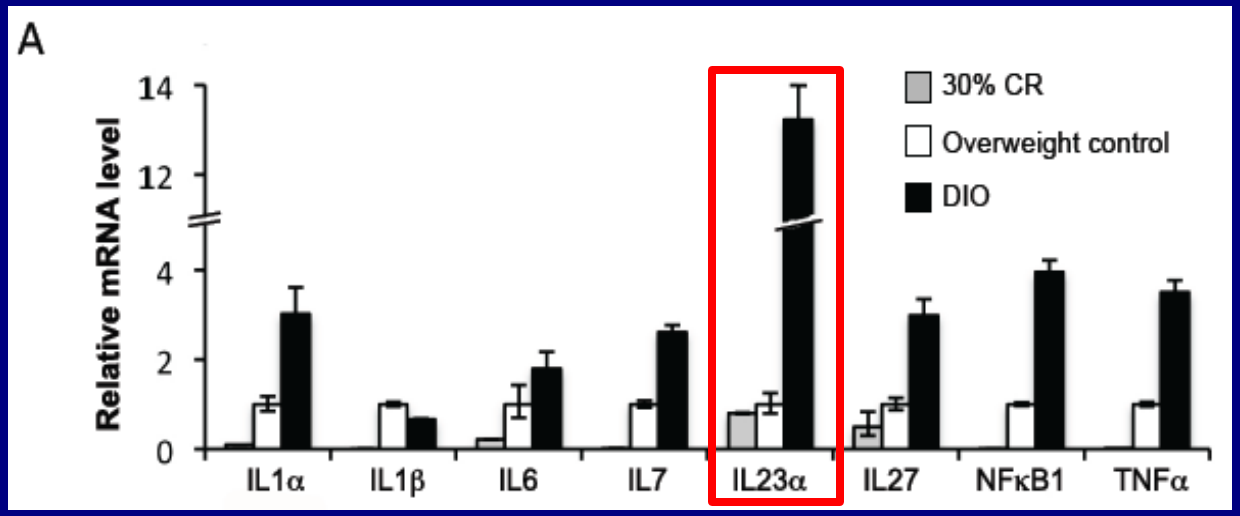
Overweight Control



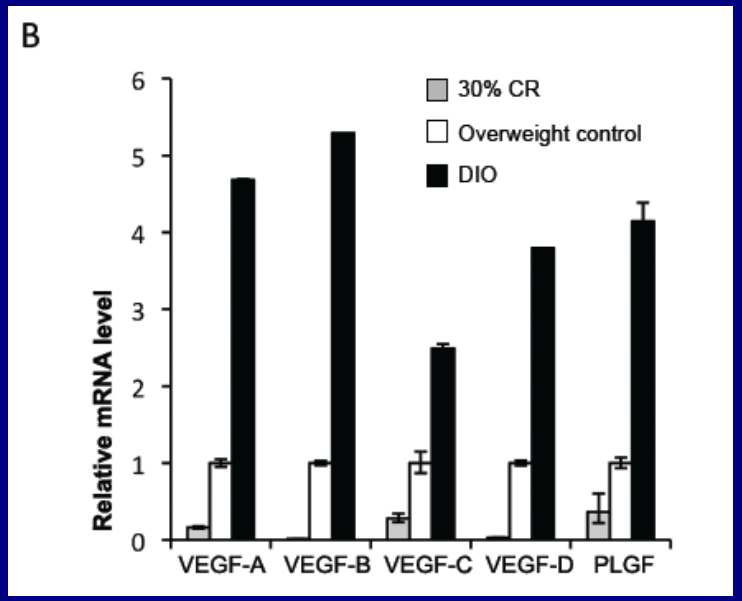
Obese



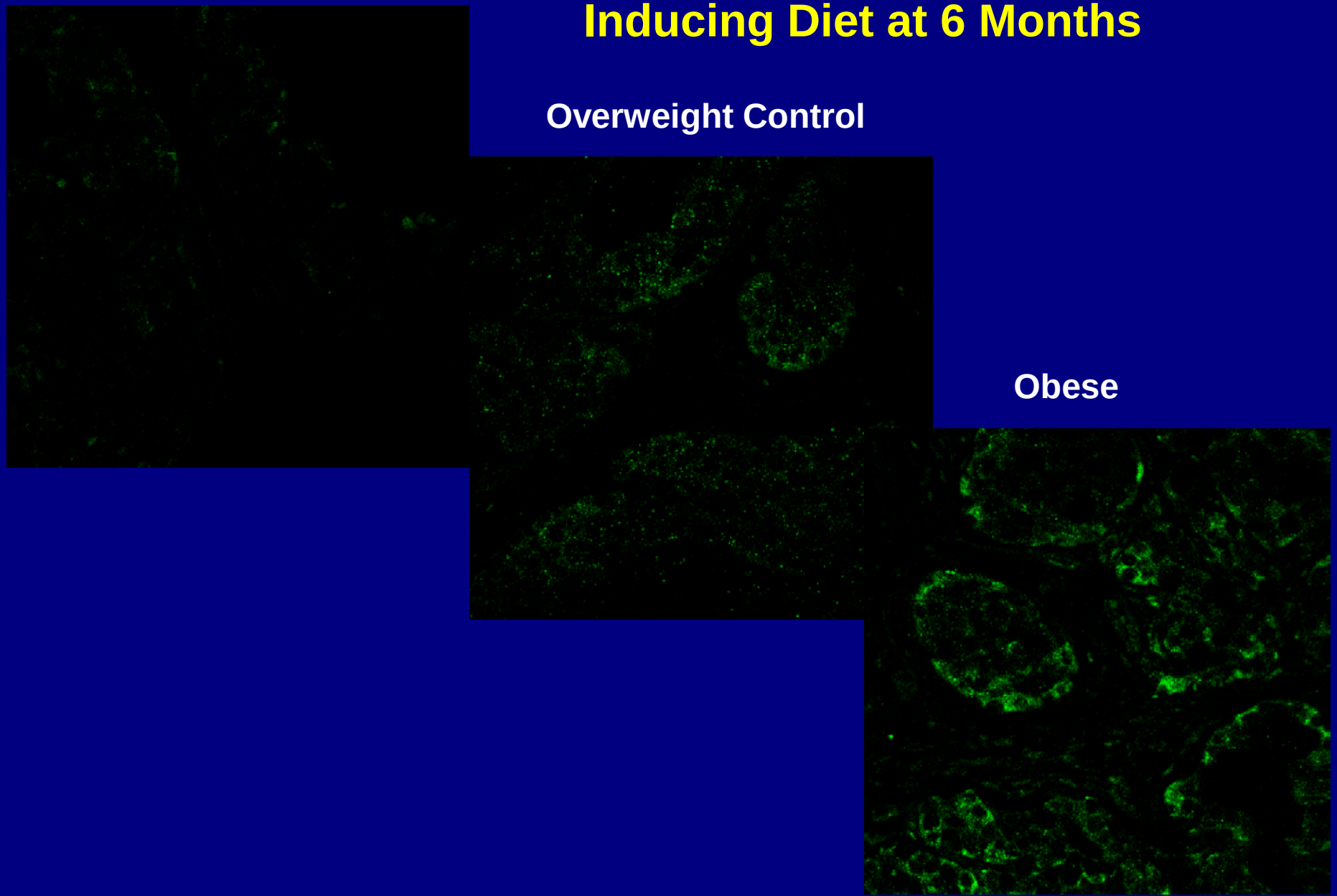
# Dietary Energy Balance Dramatically Effects Expression of Inflammatory Cytokines and VEGF Family Members



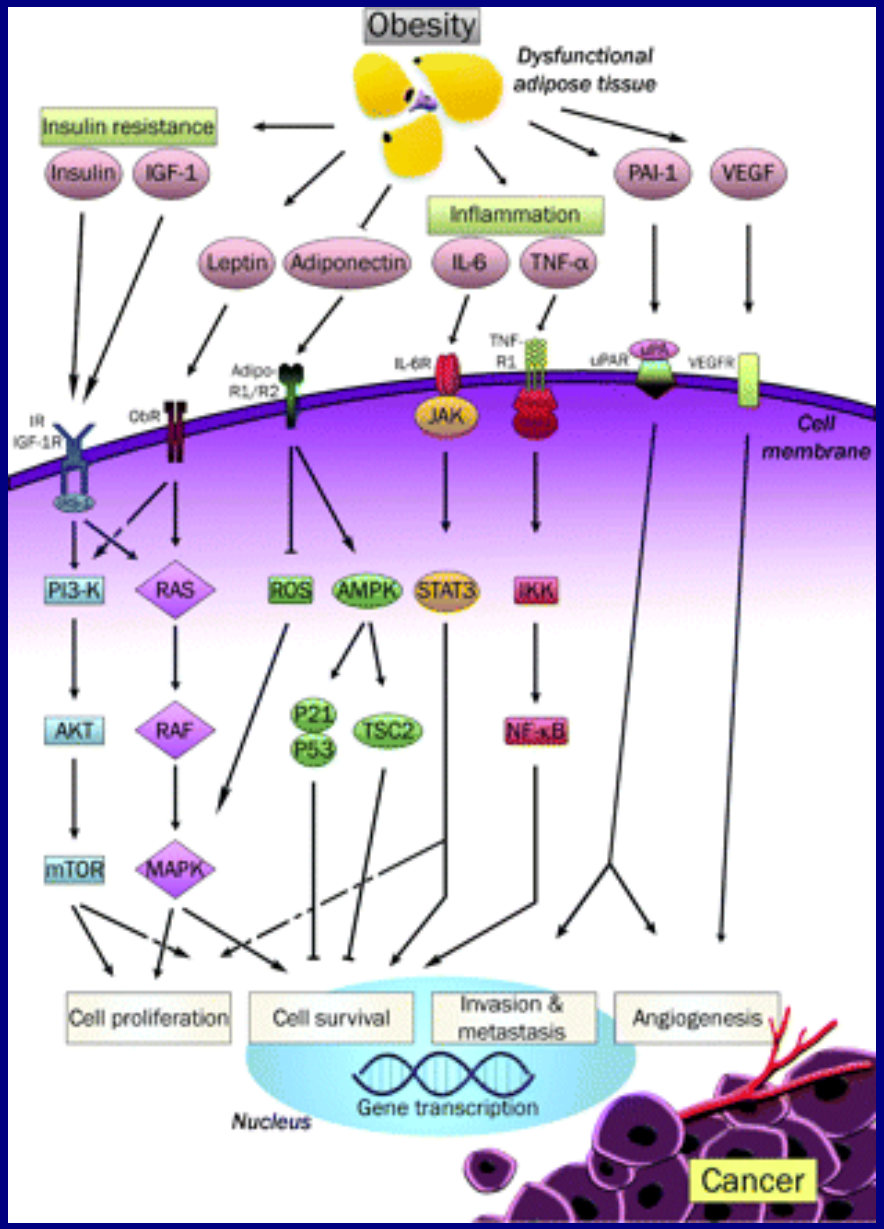

**IL-23 levels are dramatically upreglated**



# The IL-23 Receptor is Highly Upregulated in Ventral Prostate of Hi-Myc Mice on an Obesity Inducing Diet at 6 Months



# Obesity Increases Prostate Cancer Progression Through Multiple Mechanisms




From Kruijsdijk et al, CEBP 2009

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