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TITLE: Investigate the Role of Obesity in Ovarian Cancer Initiation and Progression

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**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

During this funding period we focused on generating the mouse models that will enable achieve the goals of the proposed research. The aim of this project is to identify genes and pathways in ovarian stem cells and in transformed ovarian cells affected by obesity that lead to ovarian cancer initiation and progression.

**15. SUBJECT TERMS**

Obesity, Ovarian Cancer, ovarian stem cells, inflammation, adipose tissue

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## 1. Introduction

The objective of this project is to determine the effects of obesity on ovarian stem cell activity (ovarian stem cells are prone to tumorigenesis) as well as the growth and metastatic potential of transformed ovarian epithelial cells in obese animals. The goal is to identify genes and pathways that lead to ovarian cancer initiation and progression. We also aim to identify secreted factors from adipose tissue that promote ovarian cancer initiation and progression in obesity. Identification of new genes and pathways will set the basis for the development of new therapeutics. Given that SIRT1 activity in adipose tissue protects from obesity-related metabolic dysfunction and inflammation, we will also test the hypothesis that adipose SIRT1 counteracts ovarian cancer initiation and progression and its loss in obese animals contributes to ovarian cancer. As SIRT1 can be pharmacologically activated, combinatorial therapies that target ovarian cancer cells and induce SIRT1 activity in adipose tissue might prove a powerful approach in treating patients.

## 2. Keywords

Obesity
Adipose tissue
Inflammation
SIRT1
Ovarian stem cells
Ovarian cancer

## 3. Accomplishments

**What were the major goals of the project?**

**Major Goals for year 1 (from statement of work)**

***A. Determine the effects of high-fat diet and adipose SIRT1 on the ovarian stem cell niche***

- **Major Task 1**: Determine the effects of high-fat diet on the ovarian stem cell niche
  - **Subtask 1**: Generate mice with EGFP expressing-ovarian stem cells, which will be fed high-fat and chow diets.
  - **Subtask 2**: High-fat and chow diet feeding for 1-5 months.
  - **Subtask 3:** Analyze ovaries (histology) and ovarian stem cell activity (in vitro).
  - **Milestones:**
    - Local IRB/IACUC Approval (for all procedures/subtasks)
    - ACURO Approval (for all procedures/subtasks)
    - Analysis of ovarian stem cell activity in obesity
- **Major Task 2**: Identification of genes and pathways in ovarian stem cells affected by high-fat diet
  - **Subtask 1**: Gene expression analyses of the ovarian stem cells isolated from mice fed high-fat and chow diets for 4, 8, 16, and 20 weeks.
  - **Subtask 2**: Gene expression analyses of adipose tissue and cytokine/adipokine arrays using serum and peritoneal fluid of the mice used in major task 2/ subtask 1.

- **Subtask 3:** Test the effects of the identified secreted factors on primary ovarian stem cells.
- **Milestones:**
  - Identification of targets in ovarian stem cells influenced by obesity.
  - Identification of secreted factors from adipose tissue that affect ovarian stem cells.

- **Major Task 3:** Determine the effects of adipose SIRT1 on ovarian stem cells
  - **Subtask 1**: Generate SIRT1 adipose tissue-specific knockout (FKO) and transgenic (FTg) mice with EGFP-expressing ovarian stem cells.

***B. Determine the effects of high-fat diet and adipose SIRT1 on ovarian cancer***

- **Major Task 4:** Determine the effects of high-fat diet on ovarian cancer initiation.
  - **Subtask 1**: Generate mice with ovarian stem cells null for p53 and Rb.

**What was accomplished under these goals?**

During the first year of funding, we obtained approval from the local IRB/IACUC and the ACURO, both of which were lengthy processes of multiple revisions. Our protocol also had to be reviewed again by the ACURO as the local protocol expired after few months of the first ACURO approval.

Our studies require the generation of cohorts of female mice with certain genotypes. This process took longer than anticipated due to breeding problems. We now have enough female mice of similar ages expressing EGFP in ovarian stem cells that we will feed high fat diet and we will proceed with the analyses. Most of the analyses listed as milestones required the mice, which took us longer than anticipated to generate.

We also have generated mice that are homozygotes for the conditional p53 and Rb alleles, and express the cre recombinase under the LGR5 promoter in order to drive the excision specifically in stem cells. This breeding scheme was particularly demanding, but we generated enough mice to proceed with the experiments described below.

***Wild type mice***

We have now carried out the high fat diet (HFD) experiments in mice in the proestrus phase of the estrus cycle (Fig. 1). It is critical to use mice in this phase because estrus phase mice have ovarian stem cells that are activated for cell division (Fleshken-Nikitin et al., *Nature*, 2013). In the first study, mice were fed a normal chow or HFD for four months prior to analysis. We then counted the number of stem cells by extracting ovaries from Lgr5-EGFP-IRES-creERT2 expressing mice (to mark the stem cells), dissociating cells by 40 min trypsin-EDTA treatment, and counting hi-GFP cells by FACS. This analysis clearly indicated that HFD reduced the stem cell number (compared to total ovarian epithelial cells) by about half (Fig. 2). This reduction might be expected to lead to defects in ovulation in at least some cases.

Next we assessed the activity of the stem cells by an *ex vivo* assay for colony formation. In this assay, "activated" stem cells will give rise to colonies in matrigel. We thus plated 4,000 hi-GFP cells per well and found that HFD mice yielded a two fold increase in colony number compared to chow fed mice (Fig. 3). Moreover, the colonies from HFD mice grew much larger than those

from chow fed mice (Fig. 4). In summary, these results suggest that the HFD may decrease the efficiency of ovulation by reducing the stem cell number. However, such diets might also increase the incidence of ovarian cancer by giving rise to hyper-activated stem cells.

***p53 and Rb deleted mice***

p53 and Rb are frequently lost in ovarian cancers. It was thus important to query whether the effects of the HFD on ovarian stem cell activation were due to a heightened loss of these tumor suppressors or some other reason; i. e. would the effect of the HFD be observed in the absence of p53 and Rb. We therefore bred mice to contain homozygous floxed alleles of TP53 and Rb, as well as the Lgr5-EGFP-IRES-creERT2. Tamoxifen feeding of mice (2 mg/day for five days), unfortunately, gave rise to only partial excision of the floxed cassettes (by RT-PCR assays). To get around this technical problem, we isolated the ovarian stem cells from the doubly floxed and Lgr5-EGFP-IRES-creERT2 bearing mice and excised the cassettes by tamoxifen addition *ex vivo*. After plating 4,000 cells of wild type or knock out cells from mice fed chow or the HFD, we found an increase in colony formation by deleting p53 and Rb, an increase by the HFD, and critically, these two effects were additive (Fig. 5). We conclude that the HFD activates ovarian stem cells by a mechanism other than p53 and Rb loss.

**What opportunities for training and professional development has the project provided?**
Nothing to report

**How were the results disseminated to communities of interest?**
Nothing to report

**What do you plan to do during the next reporting period to accomplish the goals**?
Not applicable

## 4. Impact

**What was the impact on the development of the principal disciplines of the project?**
Our findings show that a high fat diet exerts two effects on ovarian stem cells. First, the number of stem cells is reduced by one half. Second, the remaining stem cells are hyper-activated as determined by increases in number and size of stem cell derived colonies ex vivo.

**What was the impact on other disciplines?**
Nothing to report

**What was the impact on technology transfer?**
Nothing to report

**What was the impact on society beyond science and technology?**
Our findings suggest possible links between diet and reproduction as well as cancer.

## 5. Changes/Problems

**Changes in approach and reasons for change**
Nothing to report

**Actual or anticipated problems or delays and actions or plans taken to resolve them**
The generation of mice took longer than anticipated because not enough mice were produced. Thus, we were unable to pursue aims related to possible interactions between adipose tissue and ovarian cancer. So, the mechanisms responsible for our observed effects of diet on stem cells are unknown.

**Changes that had a significant impact on expenditures**
Nothing to report

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
Nothing to report

**Significant changes in use or care or human subjects**
Nothing to report

**Significant changes in use or care or vertebrate animals**
Nothing to report

**Significant changes in use of biohazards and/or select agents**
Nothing to report

## 6. Products
Nothing to report

## 7. Participants & Other Collaborating Organizations

**What individuals have worked on the project?**

Name: Leonard P. Guarente
Project Role: PI
Nearest person month worked: 0.5 month (summer)
Contribution to the project: Project guidance

Name: Angeliki Chalkiadaki
Project Role: Research scientist
Nearest person month worked: 12 months
Contribution to the project: All the experiments described in the proposal.

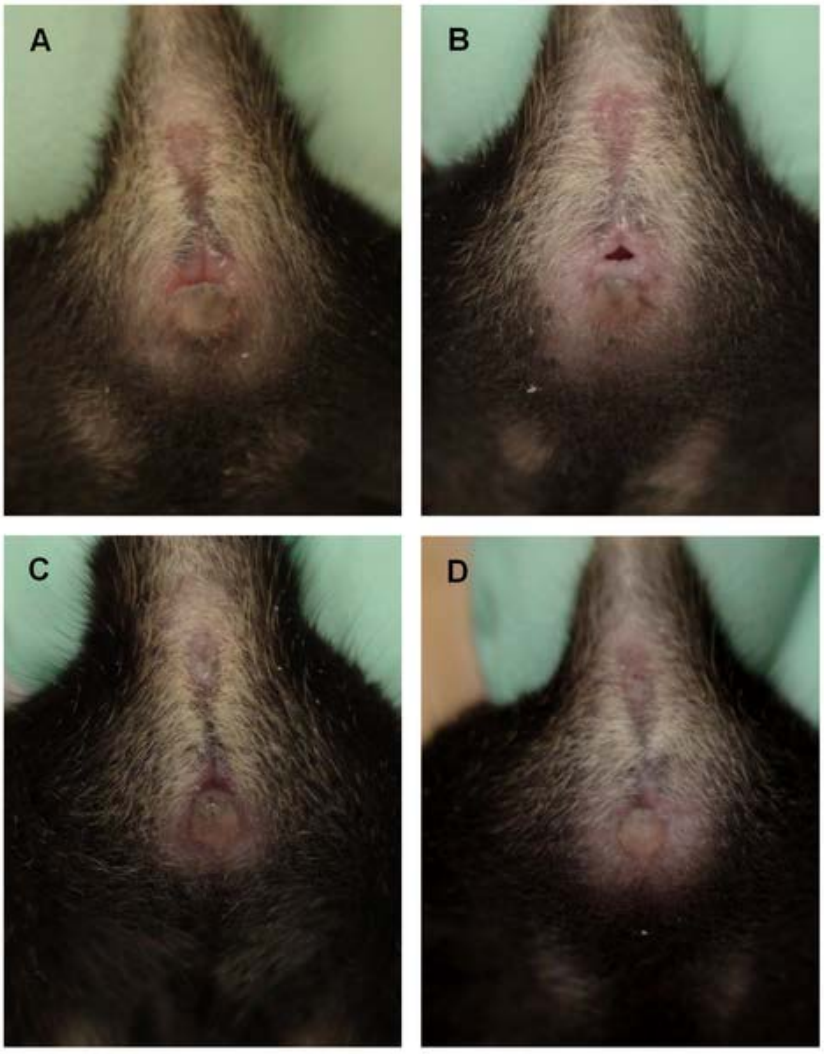
**Has there been a change in the active other support of the PD/PI or senior/key personnel since the last reporting period?**
Nothing to report

**What other organizations were involved as partners?**
Nothing to report

## 8. Special Reporting Requirements
Not applicable

## 9. Appendices
Not applicable

**Figures**




proestrus (**A**),
estrus (**B**),
metestrus (**C**),
diestrus (**D**).
Repeated in total 4-5 days.

**Figure 1** – Depicted are mice in the four stages of the estrus cycle.

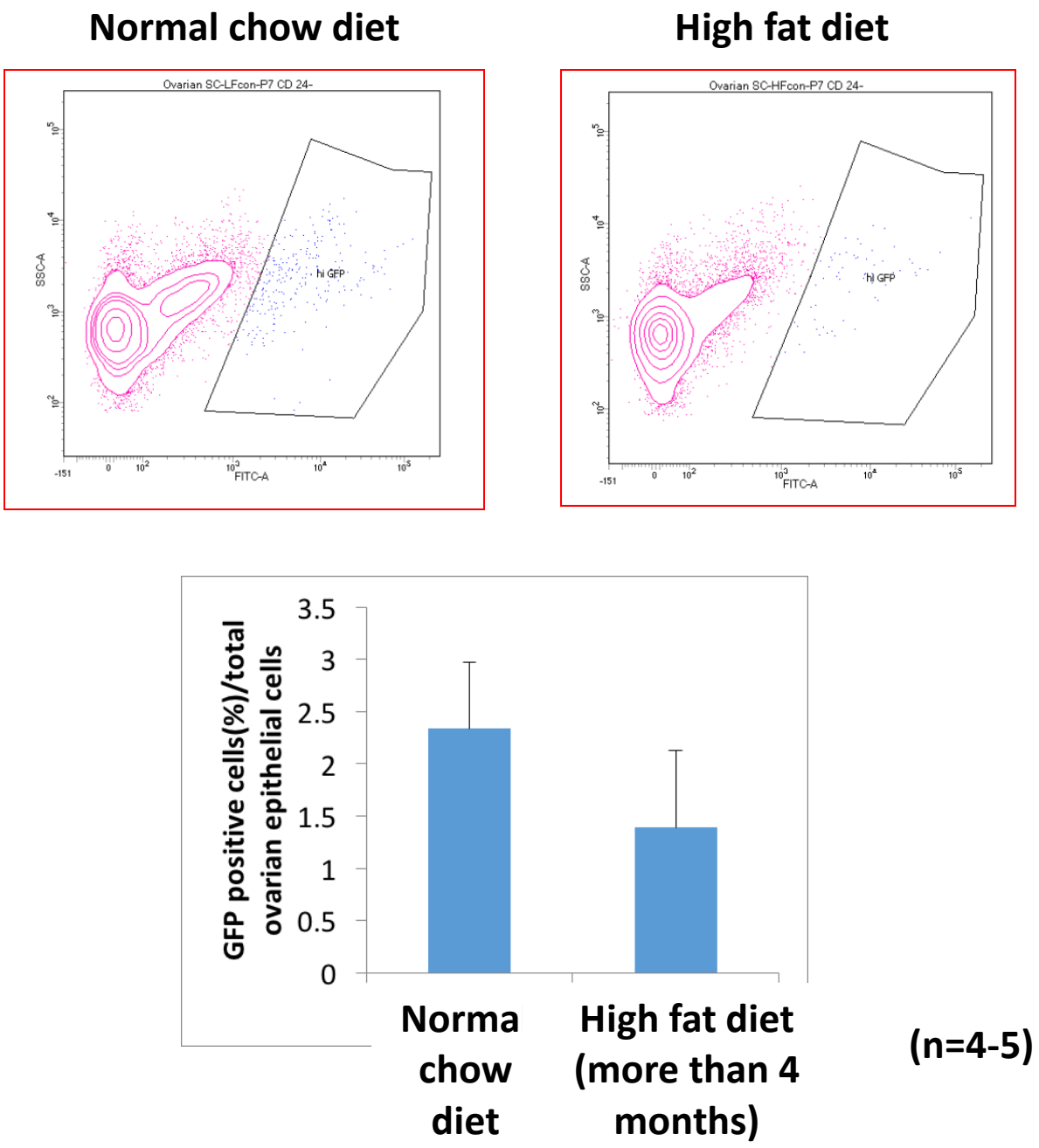



**Figure 2** – FACS sorting of ovarian cells of Lgr5-EGFP-IRES-creERT2 mice for hi-GFP cells shows a decrease in ovarian stem cell number/epithelial cells in HFD animals.

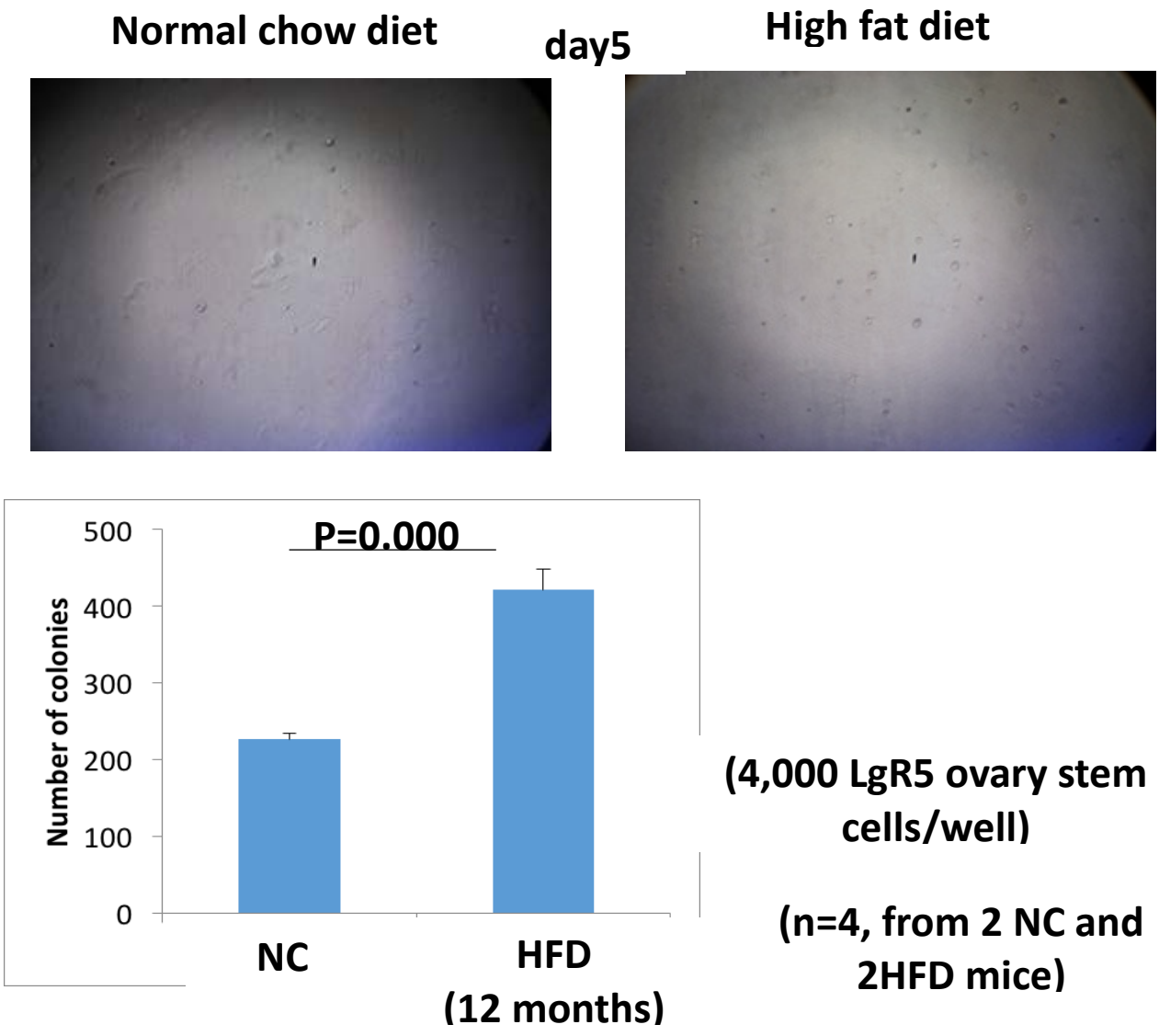



**Figure 3** – Ex vivo colony formation of ovarian hi-GFP cells (stem cells) is increased by HFD.

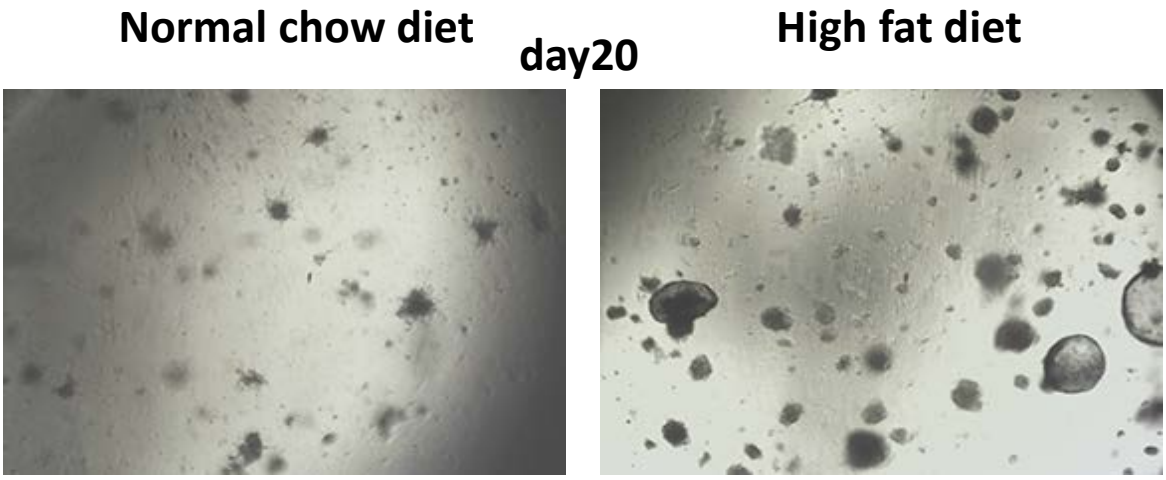



**Figure 4** – Colonies from ovarian stem cells of chow or HFD mice after 20 days. HFD gives rise to larger colonies.

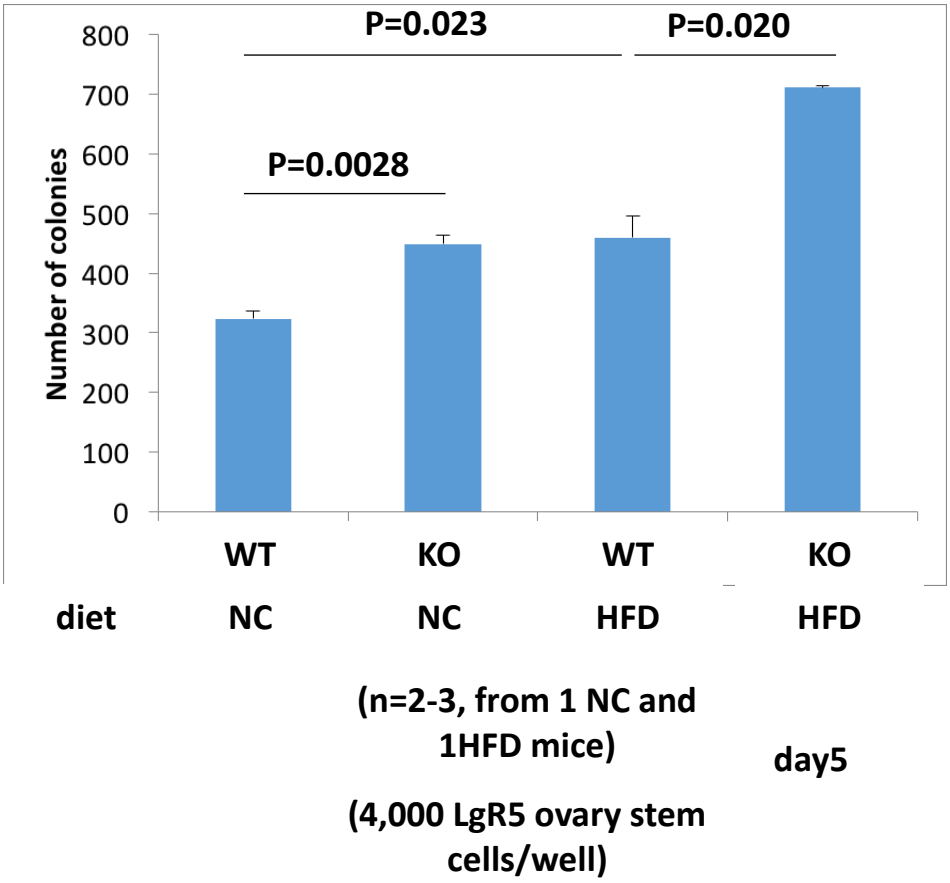



**Figure 5** – Colony formation from wild type (WT) or p53 and Rb deleted (KO) mice fed the normal chow (NC) or hi-fat (HFD) diets. Both deletion of the tumor suppressors and HFD increase colony number additively.