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| 13. SUPPLEMENTARY NOTES                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                  |                          |                                      |                                                          |                                            |
| 14. ABSTRACT<br>During this year, we generated Glipr1 WT (wild-type) and KO (knockout) male mice, surgically castrated them, and collected the VP and epididymal WAT on days 3, 14, and 35 after castration, as outlined in the Statement of Work ([SOW], Aim 1, Tasks 1-3). We isolated RNA and performed microarray analysis to characterize genes affected by castration in WAT obtained 14 days after castration from both mouse genotypes. Analyses of all VP tissue and of WAT collected 3, 14, and 35 days after castration and studies outlined in the SOW, Aim 2, Task 1, are ongoing. Our major findings are 1) Castration induces WAT wet weight (ww) and WAT ww: body weight ratio reductions in mice of both genotypes, but with different kinetics: the reduction is delayed with Glipr1 knockout. Further, testosterone offsets the effect of castration on WAT ww 14 and 35 days after castration in both genotypes. 2) Castration induces morphologic changes in adipocytes: cells become smaller, with larger stromal compartments. Kinetics of the castration-induced WAT ww reduction differs between the two genotypes. 3) Castration induces changes in the WAT genome: we detected genes whose up- or down-regulation was Glipr1-genotype specific. |                  |                          |                                      |                                                          |                                            |
| 15. SUBJECT TERMS<br><br>Prostate cancer                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                  |                          |                                      |                                                          |                                            |
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## 2011 PROGRESS REPORT

### INTRODUCTION

Prostate cancer (PCa) cells are initially sensitive to hormonal manipulation, and androgen-deprivation therapy (ADT) generally reverses androgen receptor (AR)–dependent growth and proliferation. ADT is one of the main treatment modalities in the clinical management of PCa, but ADT is only palliative, and PCa eventually progresses to an androgen-insensitive stage, i.e., castrate-resistant PCa (CRPC), after a median of 12–20 months. Progression to CRPC is a dynamic process that is incompletely understood as yet. Potential mechanisms contributing to the development of CRPC include selective growth of a preexisting hormone-insensitive population of cancer cells as a result of suppression by androgen ablation of the androgen-dependent cell population; activation of oncogenes; inactivation of tumor suppression genes; and interaction between cancer cells and tumor-associated stroma and tumor-associated macrophages. The object of this research project is to investigate the effect of castration on epididymal white adipose tissue (WAT), ventral prostate (VP) tissue, and adipose stromal cells (ASCs) from male *Glipr1*<sup>+/+</sup> wild-type (WT) and *Glipr1*<sup>-/-</sup> knockout (KO) mice. We are testing our hypothesis that the biologic activity of WAT is affected by castration and that although the acute effects of castration (e.g., GLIPR1 induction) may suppress cancer-promoting adipokines, long-term ADT results in monocyte infiltration and the generation of WAT-associated macrophages (WAMs). WAMs, in turn, produce cytokines and promote the growth and survival of growth factor–expressing ASCs, which enter the systemic circulation and promote PCa progression. An important note is that the prostate, an androgen target organ, is significantly affected by castration and also produces cytokines and cytokine receptors that may, in concert with WAT-derived cytokines, contribute to the progression of already established local tumors. We also hypothesize that Glipr1/GLIPR1 protein regulates castration-induced WAMs and ASCs. Our overarching hypothesis is that castration induces alterations in WAT that promote the development of CRPC. During this initial funding period, we have performed the following studies proposed in Specific Aims 1 and 2a. First, we generated a sufficient number of 12-week-old *Glipr1* WT and KO male mice. We then surgically castrated those mice and collected the VP and epididymal WAT on days 3, 14, and 35 after castration. We have also isolated the total RNA from those tissues. Further, we have performed microarray analyses to characterize the genes in the VP and WAT that were affected on day 14 after castration. The isolation and characterization of ASCs is in progress.

### Key Personnel

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### STATEMENT OF WORK

**Aim 1: Identify castration-affected and/or Glipr1-regulated genes in ventral prostate (VP) tissue, epididymal white adipose tissue (WAT), and adipocyte stromal cells (ASCs) using in vivo models.**

1. Generate a sufficient number of *Glipr1* wild-type (WT) and *Glipr1* knockout (KO) 12-week-old male mice (1–6 months).
2. Perform the surgical castration experiment using the *Glipr1* WT and KO male mice, and collect VP, WAT, and ASCs on days 3, 14, and 35 after castration (6–9 months).
3. Isolate RNA and perform microarray analyses to characterize genes affected by castration in VP, WAT, and ASCs in *Glipr1* WT and KO male mice (9–12 months).

**Aim 2: Study the interactions between ASCs isolated from *Glipr1* WT (ASCs-WT) and KO (ASCs-KO) male mice and human prostate cancer cell lines in vitro and in vivo.**

1. Isolate, expand, and prepare a stock of frozen ASCs from *Glipr1* WT and KO male mice (6–12 months).

## **RESEARCH**

### **Materials and Methods**

Animals and treatments. During this first year of the funding period, we initiated studies to satisfy Aim 1. We bred the *Glipr1*<sup>+/+</sup> (*Glipr1* WT) and *Glipr1*<sup>-/-</sup> (*Glipr1* KO) mice [the latter mice were generated as previously described (1)]. Twelve-week-old *Glipr1* WT and KO mice were each randomly allocated to three subgroups of 5–10 mice each. One subgroup underwent sham surgery, and the other two, surgical castration. The sham-operated animals and half of the castrated ones then received a subcutaneously implanted empty pellet (EP) on their back, creating, respectively, control (sham + EP) and castrated + EP (Cx + EP) subgroups. The second half of the castrated animals received implanted testosterone pellets (TP), creating a Cx + TP subgroup. Thus, we established the following experimental groups and subgroups\*:

Group 1, 3 days postoperatively: WT control – 3d; Cx + EP – 3d; and Cx + TP – 3d

Group 2, 14 days postoperatively: WT control – 14d; Cx + EP – 14d; and Cx + TP – 14d

Group 3, 35 days postoperatively: WT control – 35d; Cx + EP – 35d; and Cx + TP – 35d

Group 4, 3 days postoperatively: KO control – 3d; Cx + EP – 3d; and Cx + TP – 3d

Group 5, 14 days postoperatively: KO control – 14d; Cx + EP – 14d; and Cx + TP – 14d

Group 6, 35 days postoperatively: KO control – 35d; Cx + EP – 35d; and Cx + TP – 35d

\*Key: control = sham-operated + empty pellet (EP); Cx + EP = castration + EP; and Cx + TP = castration + testosterone pellet (TP).

Blood and tissue sampling and processing. We euthanized mice on the specified days after the surgical and pellet-implantation procedures. Their body weight and length were recorded. Blood samples were collected from the posterior vena cava, allowed to clot overnight at 4°C, and then centrifuged for 20 minutes at 2,000 *g*. The resulting serum was stored frozen at –80°C.

Further, the epididymal WAT pads were collected from each mouse, as were the anterior, ventral (VP), and dorsolateral lobes of the prostate, an androgen-dependent organ used as a control, and the wet weight (ww) of all those tissues was measured. WAT pads were processed as follows: one half was snap-frozen in liquid nitrogen and stored at –80°C for further analysis of protein expression and RNA extraction. The second half of the WAT was fixed in paraformaldehyde, embedded in paraffin, and cut into 5-μm sections for subsequent histologic and immunohistochemical analyses. The prostate lobes were fixed in paraformaldehyde and embedded in paraffin or were pooled in RNAlater Solution (Ambion/Invitrogen Corporation, Carlsbad, CA) and stored at 4°C. The next day, the solution was removed, and the tissues were stored at –80°C for use in further RNA extraction.

Histologic analysis of WAT. Hemotoxylin and eosin–stained WAT sections were quantitatively evaluated at 20× magnification. The analysis was performed with a Nikon Eclipse 90i image analysis system and the NIS-Elements AR 3.0 software (both from Nikon Instruments, Inc., Melville, NY). Using the manual function of the system, we outlined 100 randomly selected adipocytes from each section and recorded the areas of individual adipocyte profiles. To analyze the size distribution of adipocytes, we used the histogram function in Microsoft Excel.

**Gene-expression analysis.** We isolated total RNA from the frozen WAT and VP specimens by using a standard extraction protocol with Trizol reagent (Invitrogen). Biotin-labeled cRNA samples for hybridization were prepared by using an Illumina TotalPrep RNA amplification kit (Applied Biosystems/Ambion, Austin, TX). Total RNA (500 ng) was used for cDNA synthesis, which was then amplified and biotin labeled. The biotinylated cRNA (1.5  $\mu$ g) was hybridized to the Mouse-6 v.2 Expression BeadChip microarray kit (Illumina, San Diego, CA). Signals were developed by using Amersham fluorolink streptavidin-Cy3 (GE Healthcare Bio-Sciences, Piscataway, NJ). Gene-expression data were collected by using a bead array reader confocal scanner (BeadStation 500GXDW; Illumina). The microarray data were normalized and statistical analysis was performed as previously described (2).

**Isolation of stromal cells from WAT.** WAT pads were collected from 12-week old male *Glipr1* WT and KO mice. The tissue was cut with scissors and digested with collagenase I for up to 60 min at 37°C. The digested tissue was then filtered through a 100- $\mu$ m filter. Next, the filtrate was centrifuged at 150 *g* for 5 min. The pellet was treated with erythrocyte lysis buffer and centrifuged again at 150 *g* for 5 min. The resulting cell pellet was resuspended in high-glucose DMEM with 10% fetal bovine serum, and the suspension was plated onto 10-cm culture plates.

**Statistical analysis.** The statistical significance of the differences between the WAT ww's and the WAT ww:body weight ratios in the various subgroup comparisons was determined by using two-tailed Student's *t* testing. The level of significance was set at  $P < 0.05$ . Data are expressed as means  $\pm$  SE.

## Results

### Aim 1

**Altered response of WAT to castration in *Glipr1* WT and KO mice** (Figure 1). Analysis of epididymal WAT in *Glipr1* WT mice revealed that in the Cx + EP subgroups on days 3, 14, and 35 after castration, the WAT ww's relative to those in the sham + EP control subgroups were  $64 \pm 6\%$  ( $P = 0.037$ ),  $49 \pm 6\%$  ( $P = 0.002$ ), and  $60 \pm 10\%$  ( $P = 0.020$ ), respectively.

In the Cx + TP WT subgroups, the WAT ww's were  $83 \pm 7\%$ ,  $113 \pm 7\%$ , and  $112 \pm 15\%$  of those of the sham + EP control subgroups on days 3, 14, and 35, respectively. The WAT ww:body weight ratios in the Cx + EP subgroups were  $68 \pm 6\%$  ( $P = 0.030$ ),  $51 \pm 7\%$  ( $P = 0.001$ ), and  $65 \pm 9\%$  ( $P = 0.016$ ) of those of the sham + EP controls on days 3, 14, and 35 after the surgery, respectively, whereas in the Cx + TP subgroups, the ratios were  $81 \pm 5\%$ ,  $94 \pm 4\%$ , and  $96 \pm 11\%$  of those in the controls.

Similar analysis of the WAT from the *Glipr1* KO mice revealed that the ww's in the Cx + EP subgroups were  $93 \pm 7\%$ ,  $67 \pm 5\%$  ( $P < 0.001$ ), and  $48 \pm 4\%$  ( $P < 0.001$ ) of those of the sham + EP controls on days 3, 14, and 35 days after the surgery, respectively.

In the Cx + TP group, the respective WAT ww's were  $87 \pm 6\%$ ,  $138 \pm 15\%$  ( $P = 0.019$ ), and  $128 \pm 7\%$  ( $P = 0.012$ ) of those of the controls. The WAT ww:body weight ratios in the Cx + EP subgroups were  $93 \pm 7\%$ ,  $65 \pm 4\%$  ( $P < 0.001$ ), and  $53 \pm 4\%$  ( $P < 0.001$ ) of those of the sham + EP control subgroups, respectively, on days 3, 14, and 35 after the surgery. In the Cx + TP group, those ratios relative to the control subgroups were  $82 \pm 6\%$  ( $P = 0.040$ ),  $126 \pm 11\%$  ( $P = 0.041$ ), and  $127 \pm 8\%$  ( $P = 0.018$ ), respectively.

Together, these results indicate that in the WAT from *Glipr1* KO mice, the response to castration is delayed relative to that in the WAT from the *Glipr1* WT mice. Further, the differences in WAT ww in the Cx + EP subgroups of the *Glipr1* WT and KO mice, expressed as percentages of that in the sham surgery + EP control subgroups, were statistically significant on days 3 ( $P = 0.008$ ) and 14 ( $P = 0.035$ ) after the surgical procedures (Figure 1). Also, testosterone supplementation after surgical castration (i.e., Cx + TP) offset the effect of castration alone (i.e., Cx + EP) on days 14 and 35 days after castration.

Further, testosterone caused a statistically significant increase in WAT ww's in *Glipr1* KO Cx + TP subgroups on days 14 and 35 after the surgery (Table 1).

We have evaluated changes in the size of adipocytes using hematoxylin and eosin–stained paraffin-embedded sections of the WAT (Figure 2 ) from each group of mice. Castration also induced morphologic changes in adipocytes: the cells become smaller and have a larger stromal compartment.

In summary, surgical castration induces reductions in WAT ww and WAT ww:body weight ratios in both *Glipr1* WT and *Glipr1* KO mice, although with different kinetics; the response is delayed in WAT from the *Glipr1* KO mice. Testosterone treatment offset the effect of castration on WAT ww on days 14 and 35 after the surgery in both genotypes.

Identification of genes in epididymal WAT that are altered by castration. As noted in the Methods, we used an Illumina microarray chip set to detect genes in the WAT whose expression was affected by castration in the *Glipr1* WT and KO Cx + EP and Cx + TP subgroups by postoperative day 14.

We considered only the genes that were up-regulated to at least twice their initial level of expression or down-regulated to half or less of their initial level of expression. We detected 40 up-regulated and 111 down-regulated genes in the WAT from the *Glipr1* WT Cx + EP subgroup and 389 up-regulated and 75 down-regulated genes in the Cx + TP subgroup. In the WAT from the *Glipr1* KO mice, we detected 126 up-regulated and 96 down-regulated genes in the Cx + EP subgroup and 548 up-regulated and 389 down-regulated genes in the Cx + TP subgroup. The most up-regulated and down-regulated genes in the experimental subgroups are listed in Tables 2 and 3.

Next, we analyzed genes that were expressed differently in the WAT from the *Glipr1* KO sham + EP subgroup than they were in that from the *Glipr1* WT sham + EP subgroup. We found 54 genes that were overexpressed and 62 genes that had reduced expression in *Glipr1* KO WAT relative to their expression in *Glipr1* WT WAT (Table 4).

Castration-induced chemokines in the WAT from *Glipr1* WT and KO mice. Further analysis of the gene-expression data we obtained revealed that castration induced an increase in a limited number of chemokines: *Cxcl13* (3.4-fold) and *Ccl5* (2.3-fold) in the *Glipr1* WT Cx + EP subgroup and *Cxcl14* (2.4-fold) in the Cx + TP subgroup. In the *Glipr1* KO mice, we detected increases in *Ccl4* (2.6-fold), *Ccl9* (2.1-fold), and *Ccl3l3* (2.1 fold) in the Cx + EP group and *Cxcl14* (3.3-fold) in the Cx + TP group.

In summary, surgical castration induced changes in the genome of epididymal WAT. We identified genes that were up-regulated and down-regulated in WAT from the Cx + EP and Cx + TP subgroups of both *Glipr1* WT and KO mice. We also identified the postcastration up-regulation of a limited number of genes coding for cytokines.

Analyses of the RNAs from WAT collected from the experimental subgroups on days 3 and 35 after castration and of the RNAs from VP samples are in progress.

## Aim 2

Isolation of ASCs from the *Glipr1* WT and KO mice and testing conditions for the co-culturing experiments are in progress. We will be initiating the study of the effect of ASCs on growth rate of human prostate cancer cell lines.

## KEY RESEARCH ACCOMPLISHMENTS

1. The experimental groups were established, and biologic materials, including WAT, VP, and serum specimens, were collected as proposed.

2. We found that castration induces reductions of WAT wet weight and WAT wet weight:body weight ratios in *Glipr1* WT and *Glipr1* KO mice with different kinetics; the response is delayed in *Glipr1* KO WAT. Testosterone treatment offsets the effect of castration on WAT wet weight on days 14 and 35 after the surgery in both genotypes.
3. Castration induces morphologic changes in adipocytes: cells become smaller and have a larger stromal compartment. The kinetics in the castration-induced WAT wet weight reduction differs between *Glipr1* WT and KO mice.
4. Castration induces changes in the genome of WAT. We detected genes in which the up- or down-regulation was specific to the *Glipr1* WT or KO genotype.

## REPORTABLE OUTCOMES

1. Floryk D, Kurosaka S, Tanimoto R, Yang G, Goltsov A, Park S, Thompson TC. Castration-induced changes in mouse epididymal white adipose tissue. (Submitted.)
2. Floryk D, Hirayama T, Tanimoto R, Kurosaka S, Yang G, Goltsov A, Thompson TC. Castration-induced changes in *Glipr1* WT and KO mouse epididymal white adipose tissue. (In preparation.)

## CONCLUSIONS

Castration induces reduced WAT wet weights and the WAT wet weight:body weight ratios in *Glipr1* WT and KO mice, with different kinetics; the response is delayed in *Glipr1* KO WAT. Testosterone treatment offsets the effect of castration on WAT wet weight on days 14 and 35 days after surgery in both genotypes. Castration also induces morphologic changes in adipocytes: cells become smaller and have a larger stromal compartment.

Castration induces changes in the genome of epididymal WAT. We identified up-regulated and down-regulated genes in WAT from both castrated and castrated + testosterone-supplemented *Glipr1* WT and *Glipr1* KO mice. Finally, we identified a limited number of genes coding for cytokines that were up-regulated after castration.

## REFERENCES

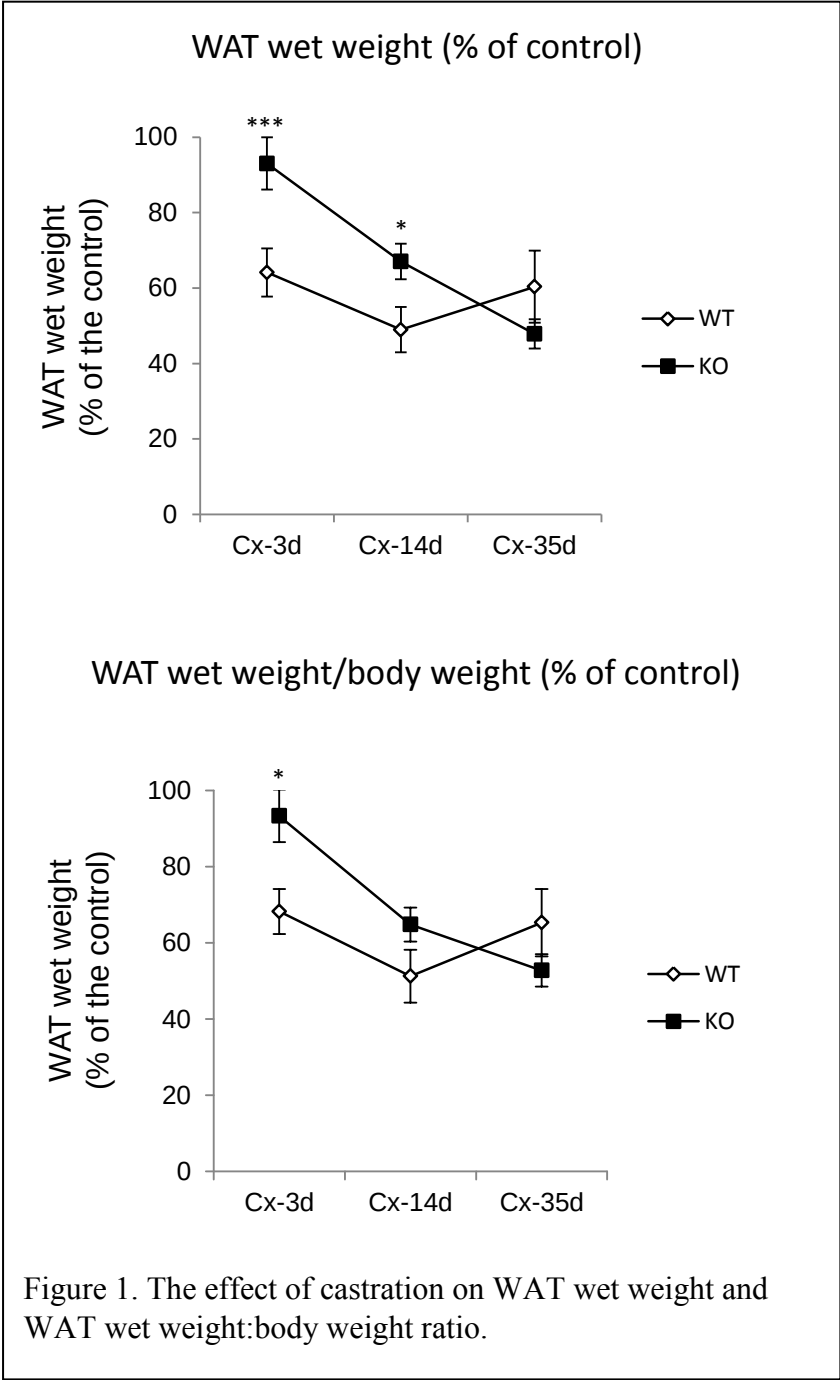
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## **APPENDICES**

**Not applicable**

SUPPORTING DATA



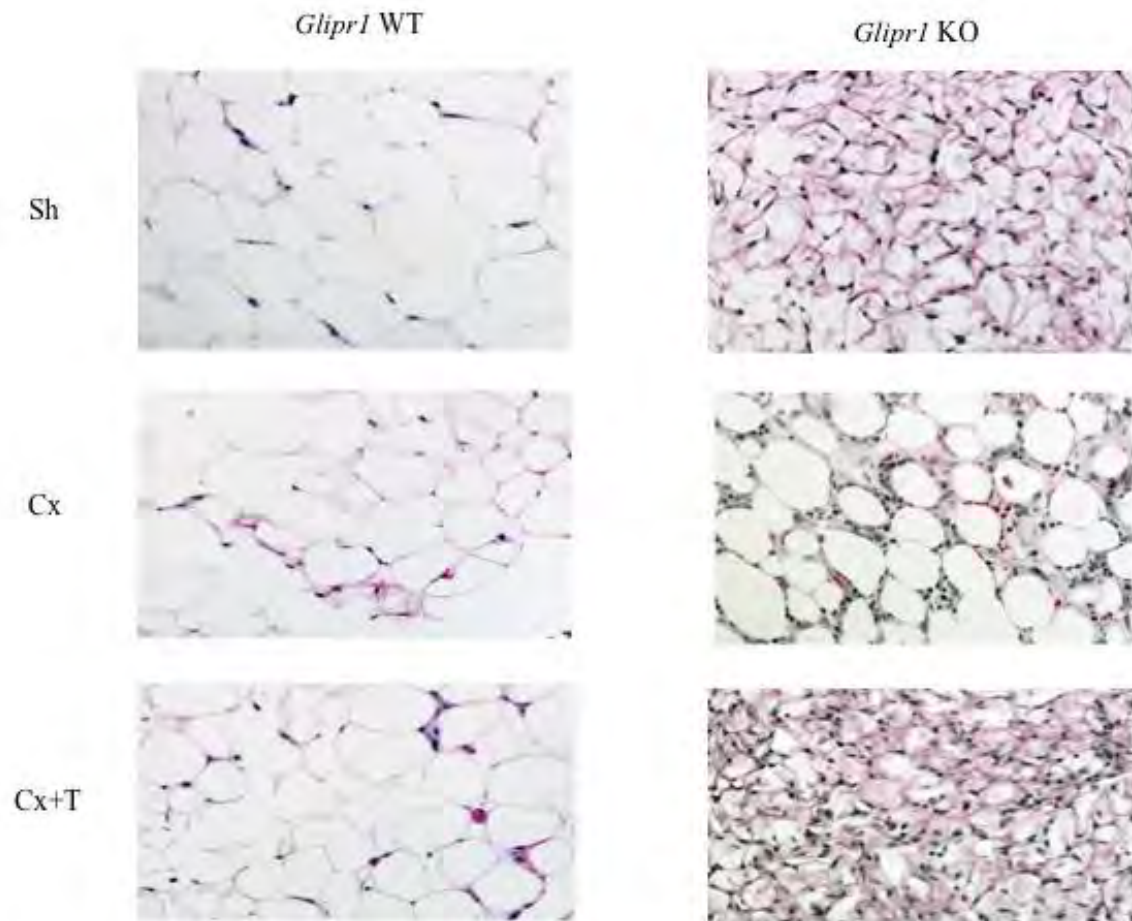


Figure 2. H&E staining of WAT from experimental *Glpr1* WT and *Glpr1* KO mice 3 days after the surgical procedure. Sh, sham-operated group; Cx, castration group; Cx+T, castration + testosterone pellet group.

Table 1. White adipose tissue (WAT) wet weights and wet weight:body weight ratios in *Glipr1* wild-type (*Glipr1* WT) and knockout (*Glipr1* KO) mice after castration and after castration plus testosterone supplementation

| Treatment subgroup                      |                  | Number of days after surgical procedures |                  |         |                  |          |
|-----------------------------------------|------------------|------------------------------------------|------------------|---------|------------------|----------|
| <u><i>Glipr1</i> WT mice</u>            |                  | 3                                        |                  | 14      |                  | 35       |
| WAT wet weight                          | Weight (mg) ± SE | P*                                       | Weight (mg) ± SE | P       | Weight (mg) ± SE | P        |
| Sh + EP                                 | 479.99 ± 66.39   |                                          | 363.45 ± 38.49   |         | 454.31 ± 52.57   |          |
| Cx + EP                                 | 307.84 ± 30.75   | 0.037                                    | 177.97 ± 21.84   | 0.002   | 274.27 ± 43.39   | 0.020    |
| Cx + TP                                 | 396.70 ± 31.90   | 0.273                                    | 412.16 ± 23.65   | 0.267   | 510.44 ± 66.88   | 0.509    |
| <b>WAT wet weight:body weight ratio</b> |                  |                                          |                  |         |                  |          |
|                                         | Ratio            | P                                        | Ratio            | P       | Ratio            | P        |
| Sh + EP                                 | 0.018 ± 0.002    |                                          | 0.014 ± 0.001    |         | 0.016 ± 0.002    |          |
| Cx + EP                                 | 0.012 ± 0.001    | 0.030                                    | 0.007 ± 0.001    | 0.001   | 0.011 ± 0.001    | 0.016    |
| Cx + TP                                 | 0.015 ± 0.001    | 0.151                                    | 0.013 ± 0.001    | 0.508   | 0.016 ± 0.002    | 0.812    |
| <u><i>Glipr1</i> KO mice</u>            |                  |                                          |                  |         |                  |          |
| WAT wet weight                          | Weight (mg) ± SE | P                                        | Weight (mg) ± SE | P       | Weight (mg) ± SE | P        |
| Sh + EP                                 | 290.33 ± 16.55   |                                          | 217.08 ± 10.82   |         | 309.56 ± 20.45   |          |
| Cx + EP                                 | 270.12 ± 19.38   | 0.45                                     | 145.54 ± 10.28   | 0.00006 | 148.04 ± 12.04   | 0.000001 |
| Cx + TP                                 | 253.62 ± 16.76   | 0.13                                     | 298.50 ± 31.59   | 0.0192  | 396.18 ± 22.95   | 0.012    |
| <b>WAT wet weight:body weight ratio</b> |                  |                                          |                  |         |                  |          |
|                                         | Ratio            | P                                        | Ratio            | P       | Ratio            | P        |
| Sh + EP                                 | 0.011 ± 0.001    |                                          | 0.008 ± 0.001    |         | 0.011 ± 0.001    |          |
| Cx + EP                                 | 0.010 ± 0.001    | 0.468                                    | 0.005 ± 0.001    | 0.00006 | 0.006 ± 0.001    | 0.000006 |
| Cx + TP                                 | 0.009 ± 0.001    | 0.040                                    | 0.010 ± 0.001    | 0.041   | 0.013 ± 0.001    | 0.018    |

Abbreviations: SE, standard error; Sh, sham surgery; EP, empty pellet implanted; Cx, castration; TP, testosterone pellet implanted.

\*P value for the comparison with the data for the Sh + EP control group, two-tailed Student's *t* test.

Table 2. Genes with altered expression in the *Glpr1* wild-type (WT) mice after castration

**Genes up-regulated in the castration + EP group**

| Symbol              | Entrez Gene Name                                                               | Fold Change |
|---------------------|--------------------------------------------------------------------------------|-------------|
| <i>LOC100129193</i> | major urinary protein pseudogene                                               | 7.3         |
| <i>IGHG</i>         | immunoglobulin heavy chain (gamma polypeptide)                                 | 5.9         |
| <i>ALDOC</i>        | aldolase C, fructose-bisphosphate                                              | 4.9         |
| <i>IGHA1</i>        | immunoglobulin heavy constant alpha 1                                          | 4.7         |
| <i>SPP1</i>         | secreted phosphoprotein 1                                                      | 4.5         |
| <i>CDH16</i>        | cadherin 16, KSP-cadherin                                                      | 3.7         |
| <i>CXCL13</i>       | chemokine (C-X-C motif) ligand 13                                              | 3.4         |
| <i>ITIH4</i>        | inter-alpha (globulin) inhibitor H4 (plasma Kallikrein-sensitive glycoprotein) | 3.2         |
| <i>CELSR2</i>       | cadherin, EGF LAG seven-pass G-type receptor 2 (flamingo homolog, Drosophila)  | 2.9         |
| <i>ST3GAL6</i>      | ST3 beta-galactoside alpha-2,3-sialyltransferase 6                             | 2.8         |

**Genes down-regulated in the castration + EP group**

| Symbol         | Entrez Gene Name                              | Fold Change |
|----------------|-----------------------------------------------|-------------|
| <i>PRM1</i>    | protamine 1                                   | -10.9       |
| <i>CUZD1</i>   | CUB and zona pellucida-like domains 1         | -9.5        |
| <i>DEFB129</i> | defensin, beta 129                            | -9.3        |
| <i>SLC38A5</i> | solute carrier family 38, member 5            | -7.7        |
| <i>LY6F</i>    | lymphocyte antigen 6 complex, locus F         | -7.1        |
| <i>RNASE12</i> | ribonuclease, RNase A family, 12 (non-active) | -6.4        |
| <i>HP</i>      | haptoglobin                                   | -6.2        |
| <i>SPINT4</i>  | serine peptidase inhibitor, Kunitz type 4     | -5.9        |
| <i>CES7</i>    | carboxylesterase 7                            | -5.8        |
| <i>CRISP3</i>  | cysteine-rich secretory protein 3             | -5.3        |

**Genes up-regulated in the castration + TP group**

| Symbol          | Entrez Gene Name                                               | Fold Change |
|-----------------|----------------------------------------------------------------|-------------|
| <i>GPX5</i>     | glutathione peroxidase 5 (epididymal androgen-related protein) | 13.6        |
| <i>SPAG11B</i>  | sperm associated antigen 11B                                   | 13.0        |
| <i>SPINT4</i>   | serine peptidase inhibitor, Kunitz type 4                      | 12.7        |
| <i>IDO1</i>     | indoleamine 2,3-dioxygenase 1                                  | 11.5        |
| <i>DEFA-RS1</i> | defensin, alpha, related sequence 1                            | 11.4        |
| <i>PLAC8</i>    | placenta-specific 8                                            | 11.2        |
| <i>LCN5</i>     | lipocalin 5                                                    | 10.6        |
| <i>LY6G5C</i>   | lymphocyte antigen 6 complex, locus G5C                        | 10.2        |
| <i>CRYBA4</i>   | crystallin, beta A4                                            | 9.5         |
| <i>DEFB129</i>  | defensin, beta 129                                             | 9.0         |

Genes **down-regulated** in the castration + TP group

| Symbol          | Entrez Gene Name                                             | Fold Change |
|-----------------|--------------------------------------------------------------|-------------|
| <i>PRM1</i>     | protamine 1                                                  | −4.4        |
| <i>C10ORF10</i> | chromosome 10 open reading frame 10                          | −3.3        |
| <i>MFNG</i>     | MFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase | −3.2        |
| <i>ACAA1B</i>   | acetyl-Coenzyme A acyltransferase 1B                         | −3.1        |
| <i>NNAT</i>     | neuronatin                                                   | −3.1        |
| <i>C3ORF1</i>   | chromosome 3 open reading frame 1                            | −3.0        |
| <i>TMEM45B</i>  | transmembrane protein 45B                                    | −3.0        |
| <i>RETNLA</i>   | resistin like alpha                                          | −3.0        |
| <i>AQP7</i>     | aquaporin 7                                                  | −2.8        |
| <i>PCSK6</i>    | proprotein convertase subtilisin/kexin type 6                | −2.8        |

EP, empty pellet; TP, testosterone pellet.

Table 3. Genes with altered expression in the *Glipr1* knockout (KO) mice after castration

**Genes up-regulated in the castration + EP group**

| Symbol              | Entrez Gene Name                                                | Fold Change |
|---------------------|-----------------------------------------------------------------|-------------|
| <i>MMP12</i>        | matrix metalloproteinase 12 (macrophage elastase)               | 16.9        |
| <i>GPMB</i>         | glycoprotein (transmembrane) nmb                                | 14.7        |
| <i>LOC100129193</i> | major urinary protein pseudogene                                | 12.8        |
| <i>SPP1</i>         | secreted phosphoprotein 1                                       | 9.9         |
| <i>SLC40A1</i>      | solute carrier family 40 (iron-regulated transporter), member 1 | 6.5         |
| <i>VSIG8</i>        | V-set and immunoglobulin domain containing 8                    | 6.3         |
| <i>CLEC4D</i>       | C-type lectin domain family 4, member D                         | 6.3         |
| <i>TREM2</i>        | triggering receptor expressed on myeloid cells 2                | 6.0         |
| <i>CLEC7A</i>       | C-type lectin domain family 7, member A                         | 5.0         |
| <i>LAT2</i>         | linker for activation of T cells family, member 2               | 4.7         |

**Genes down-regulated in the castration + EP group**

| Symbol         | Entrez Gene Name                                                                         | Fold Change |
|----------------|------------------------------------------------------------------------------------------|-------------|
| <i>PRM1</i>    | protamine 1                                                                              | -10.6       |
| <i>HP</i>      | haptoglobin                                                                              | -6.2        |
| <i>TIMP4</i>   | TIMP metalloproteinase inhibitor 4                                                       | -5.1        |
| <i>SNCG</i>    | synuclein, gamma (breast cancer-specific protein 1)                                      | -4.8        |
| <i>SLC6A13</i> | solute carrier family 6 (neurotransmitter transporter, GABA), member 13                  | -4.1        |
| <i>C7</i>      | complement component 7                                                                   | -4.1        |
| <i>HSD11B1</i> | hydroxysteroid (11-beta) dehydrogenase 1                                                 | -4.0        |
| <i>NPR3</i>    | natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C) | -3.9        |
| <i>LRG1</i>    | leucine-rich alpha-2-glycoprotein 1                                                      | -3.3        |
| <i>PTPLB</i>   | protein tyrosine phosphatase-like (proline instead of catalytic arginine), member b      | -3.3        |

**Genes up-regulated in the castration + TP group**

| Symbol          | Entrez Gene Name                                               | Fold Change |
|-----------------|----------------------------------------------------------------|-------------|
| <i>GPX5</i>     | glutathione peroxidase 5 (epididymal androgen-related protein) | 145.4       |
| <i>LCN5</i>     | lipocalin 5                                                    | 66.3        |
| <i>DEFA-RS1</i> | defensin, alpha, related sequence 1                            | 57.4        |
| <i>IDO1</i>     | indoleamine 2,3-dioxygenase 1                                  | 52.2        |
| <i>CES7</i>     | carboxylesterase 7                                             | 46.4        |
| <i>C4BP</i>     | complement component 4 binding protein                         | 39.2        |
| <i>PI3</i>      | peptidase inhibitor 3, skin-derived                            | 38.0        |
| <i>CRISP3</i>   | cysteine-rich secretory protein 3                              | 28.9        |
| <i>DEFB4A</i>   | defensin, beta 4A                                              | 26.5        |
| <i>LY6G5C</i>   | lymphocyte antigen 6 complex, locus G5C                        | 24.8        |

Genes **down-regulated** in the castration + TP group

| Symbol         | Entrez Gene Name                                    | Fold Change |
|----------------|-----------------------------------------------------|-------------|
| <i>RETNLA</i>  | Resistin-like alpha                                 | -10.7       |
| <i>NNAT</i>    | neuronatin                                          | -10.6       |
| <i>SNCG</i>    | synuclein, gamma (breast cancer-specific protein 1) | -10.0       |
| <i>GSN</i>     | gelsolin                                            | -7.6        |
| <i>ANGPTL4</i> | angiopoietin-like 4                                 | -6.3        |
| <i>ADRB3</i>   | adrenergic, beta-3-, receptor                       | -6.2        |
| <i>MTUS1</i>   | microtubule associated tumor suppressor 1           | -6.2        |
| <i>AQP7</i>    | aquaporin 7                                         | -5.6        |
| <i>GPR81</i>   | G protein-coupled receptor 81                       | -4.8        |
| <i>TIMP4</i>   | TIMP metalloproteinase inhibitor 4                  | -4.8        |

EP, empty pellet; TP, testosterone pellet.



Table 4. Genes with altered expression in the *Glipr1* KO sham-operated subgroup identified during comparison with the *Glipr1* WT sham-operated subgroup

| Symbol                | Entrez Gene Name                                                     | Fold Change |
|-----------------------|----------------------------------------------------------------------|-------------|
| <b>Up-regulated</b>   |                                                                      |             |
| <i>MGST1</i>          | microsomal glutathione S-transferase 1                               | 23.7        |
| <i>ME1</i>            | malic enzyme 1, NADP(+)-dependent, cytosolic                         | 20.6        |
| <i>FCER1G</i>         | Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide | 10.1        |
| <i>MTUS1</i>          | microtubule associated tumor suppressor 1                            | 7.9         |
| <i>NNMT</i>           | nicotinamide N-methyltransferase                                     | 6.6         |
| <i>SNCG</i>           | synuclein, gamma (breast cancer-specific protein 1)                  | 6.2         |
| <i>CAP1</i>           | CAP, adenylate cyclase-associated protein 1 (yeast)                  | 5.7         |
| <i>SUPT16H</i>        | suppressor of Ty 16 homolog (S. cerevisiae)                          | 4.9         |
| <i>CYP2F1</i>         | cytochrome P450, family 2, subfamily F, polypeptide 1                | 4.8         |
| <i>HBD</i>            | hemoglobin, delta                                                    | 4.6         |
| <b>Down-regulated</b> |                                                                      |             |
| <i>SPAG11B</i>        | sperm associated antigen 11B                                         | -12.3       |
| <i>DEFB129</i>        | defensin, beta 129                                                   | -10.6       |
| <i>SPINT4</i>         | serine peptidase inhibitor, Kunitz type 4                            | -10.5       |
| <i>GPX5</i>           | glutathione peroxidase 5 (epididymal androgen-related protein)       | -9.1        |
| <i>RPL29</i>          | ribosomal protein L29                                                | -8.8        |
| <i>CES7</i>           | carboxylesterase 7                                                   | -8.3        |
| <i>NECAP2</i>         | NECAP endocytosis associated 2                                       | -7.2        |
| <i>RNASE12</i>        | ribonuclease, RNase A family, 12 (non-active)                        | -6.5        |
| <i>PRKAG2</i>         | protein kinase, AMP-activated, gamma 2 non-catalytic subunit         | -6.4        |
| <i>RNASE9</i>         | ribonuclease, RNase A family, 9 (non-active)                         | -5.5        |

KO, knockout; WT, wild type.