Diabetic Complications Consortium

Application Title: Innovative SDF-1 mRNA delivery to reverse diabetic neurovascular erectile dysfunction.

Principal Investigator: Johanna Hannan, PhD

1. Project Accomplishments:

1.1 Introduction

Diabetes mellitus (DM) is a chronic disease that impacts over 422 million people worldwide and more than 90% of cases are type 2 DM (T2DM). Uncontrolled T2DM leads to vascular dysfunction, neuropathy, and erectile dysfunction (ED). ED is a highly prevalent, often-overlooked chronic health challenge with profound negative impacts on quality of life and well-being. Current ED therapies do not treat the underlying pathological changes to the penile neurovascular architecture that occur with DM. A long-lasting treatment to prevent or reverse the underlying pathology of T2DM ED is sorely needed.

Stromal cell-derived factor-1 (SDF-1) is a widely explored chemokine important for stem cell biology and a promising new therapeutic target to rescue erectile function. SDF-1 can activate stem-cell associated repair pathways to enhance the integrity of penile neurovascular architecture to potentially recover erectile physiology. Recent advances in synthetic mRNA engineering have made mRNA safe, potent, stable, and easy to manufacture. SDF-1 mRNA injected into injured DM penile tissue can translate into multiple SDF-1 proteins, providing sustained delivery over days. We proposed to deliver SDF-1 mRNA in a poly disulfide amine nanoparticle (PDSA-NP) via intracavernosal injection to Zucker Diabetic Fatty (ZDF) obese rats, a preclinical model of T2DM ED.

1.2 Accomplishments

Over the last two years, we have determined the optimal dose if SDF-1 mRNA to be administered via intracavernosal injections and have shown that we were able to improve erectile function in T2DM ZDF obese rats. DiaComp Pilot funding was key in generating preliminary data for an R01 which was awarded in 2022. We are currently working on a second R01 submission for February 2024 to further explore the mechanisms of improved erectile function via SDF-1 and plasmid SDF-1 delivery. Work supported by this funding will be presented as an oral presentation at the upcoming SMSNA Fall meeting in November in San Diego, CA. Additionally, the SDF-1 efficacy data is submitted to the AUA 2024 annual meeting. We are currently working on two manuscripts to be submitted this fall.

2. Specific Aims:

Specific Aim 1. Determine the optimal therapeutic dose of two SDF-1 mRNA candidates for localized penile mRNA delivery.

Results: SDF-1 mRNA was delivered in PDSA-NP via intracavernous penile injection to healthy Sprague-Dawley rats. We qualitatively confirmed via live animal imaging with an In Vivo Imaging System (IVIS) that our prototype PDSA NP was capable of mediating luciferase mRNA (mLuc) translation in penile tissues. We repeated this study with PDSA-NPs carrying incrementing mLuc doses and quantitatively determined the in vivo translation efficiency using tissue homogenate-based luciferase assay. The luciferase activity was elevated when the dose was increased from 10 to 50 μg, but further dose incrementation to 100 μg did not increase the luciferase activity (Figure 1A), presumably indicating that 50 μg was a saturating dose. Next, we treated rats with PDSA-NPs carrying stomal cell-derived factor-1 (SDF-1) mRNA (mSDF-1) at two dose levels (i.e., 10 to 50 μg) and confirmed dose-dependent SDF-1 expression in rat cavernous tissue (Figure 1B). In parallel, we tested the effect of long-term storage of PDSA NPs carrying mLuc at 4°C on their performances in vivo. We found that the particle physicochemical properties changed over time reaching near double the particle size after a 3-week storage. We then treated animals with PDSA-NPs stored for different time periods, including 1 or 3 week(s), and compared with animals treated with freshly prepared particles. As expected from the alteration of particle properties, the ability of PDSA-NPs to mediate luciferase activity was significantly reduced by the long-term storage (Figure 1C).

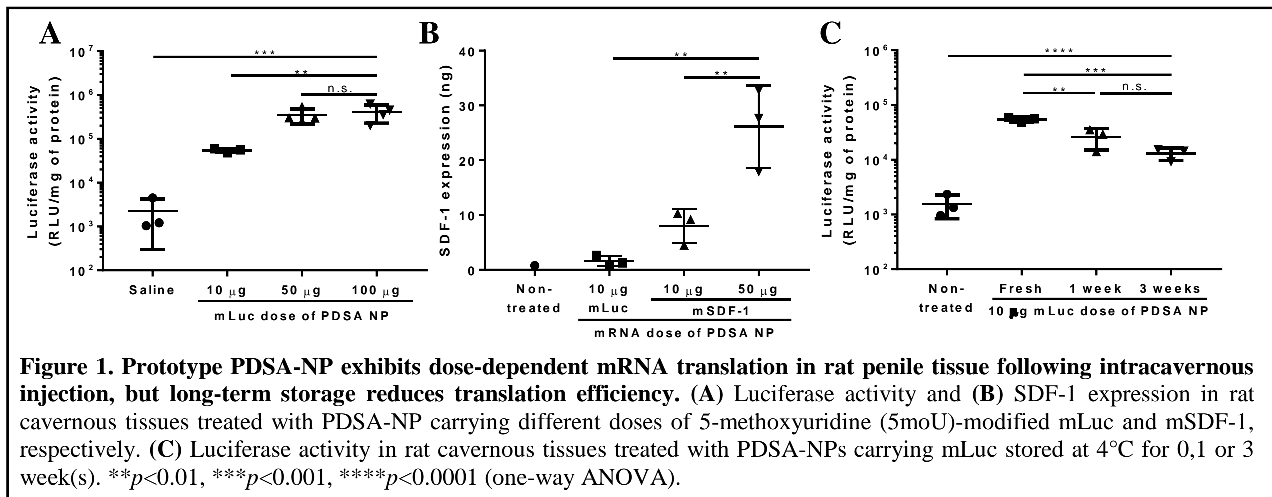

Figure 1. Prototype PDSA-NP exhibits dose-dependent mRNA translation in rat penile tissue following intracavernous injection, but long-term storage reduces translation efficiency. **(A)** Luciferase activity and **(B)** SDF-1 expression in rat cavernous tissues treated with PDSA-NP carrying different doses of 5-methoxyuridine (5moU)-modified mLuc and mSDF-1, respectively. **(C)** Luciferase activity in rat cavernous tissues treated with PDSA-NPs carrying mLuc stored at 4°C for 0,1 or 3 week(s). $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$ (one-way ANOVA).

We also compared the ability of unmodified and chemically modified mSDF-1 to produce SDF-1 protein in vitro, using a commercial transfection agent (i.e., Lipofectamine). We first compared unmodified and N1-methylpseudouridine (N1meΨ)-modified mSDF-1 in primary dermal fibroblast cultures. We found that the SDF-1 levels in the conditioned media from the cell cultures treated with unmodified mSDF-1 were virtually identical to the levels in the media from the normal saline-treated cultures whereas N1meΨ-modified mSDF-1 exhibited dose-dependent SDF-1 production (Figure 2A), suggesting that chemical modification is essential for robust

mRNA translation. We next compared N1meΨ-modified and 5-methoxyuridine (5moU)-modified mSDF-1 in HUVECs cells and found that the levels of SDF-1 expression were comparably robust for two different modifications while exhibiting similar in vitro translation kinetics (Figure 2B). Moving forward, we have used the 5moU-modified SDF-1 for the efficacy experiments.

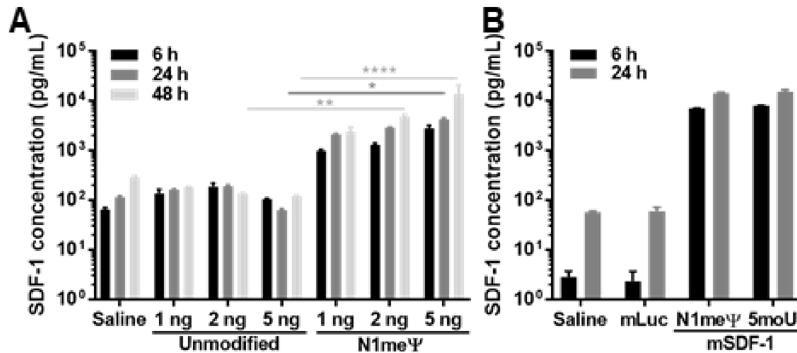

Figure 2. Chemically modified mRNA provided markedly enhanced SDF-1 expression *in vitro*. Relative SDF-1 expression by **(A)** unmodified vs. N1meΨ-modified mSDF-1 in primary dermal fibroblasts and by **(B)** N1meΨ vs. 5moU-modified mSDF-1 in HUVECs $*p < 0.05$, $**p < 0.01$, $****\ p < 0.0001$. (one-way ANOVA).

Specific Aim 2. Evaluate the therapeutic efficacy of two SDF-1 mRNA candidates using a rat model of T2DM ED.

Results: ZDF obese rats received a penile injection of 50µL (50µg) mSDF-1. ZDF lean rats received penile injections of 50µL (50µg) mLUC. All injections were done with a tourniquet placed at the base of the penis for five minutes. Both groups received injections every five days for 15 days (total of 4 injections). Ten days after the last injection, erectile function was assessed, and tissues harvested. Lean and obese rats received daily oral trimethoprim sulfate (75mg/kg) to prevent penile infections. Prior to starting treatment, ZDF lean and obese rats grew normally, and obese rats developed T2DM indicated by a non-fasted blood glucose above 400mg/dL (Figure 3A). At day 0 of mSDF-1 administration, ZDF obese rats averaged 435.5g and at day 25 they weighed on average 410.0g, an average loss of about a gram a day (Figure 3B). At day 0 of mLUC administration, ZDF lean rats average weight was 427.0g and after 25 days they weighed 416.1g, an average loss of a little less than half a gram (0.44g) a day. Neither mLUC nor mSDF-1 changed the non-fasted blood glucose of ZDF lean or obese rats normalized to body weight (Figure 3C).

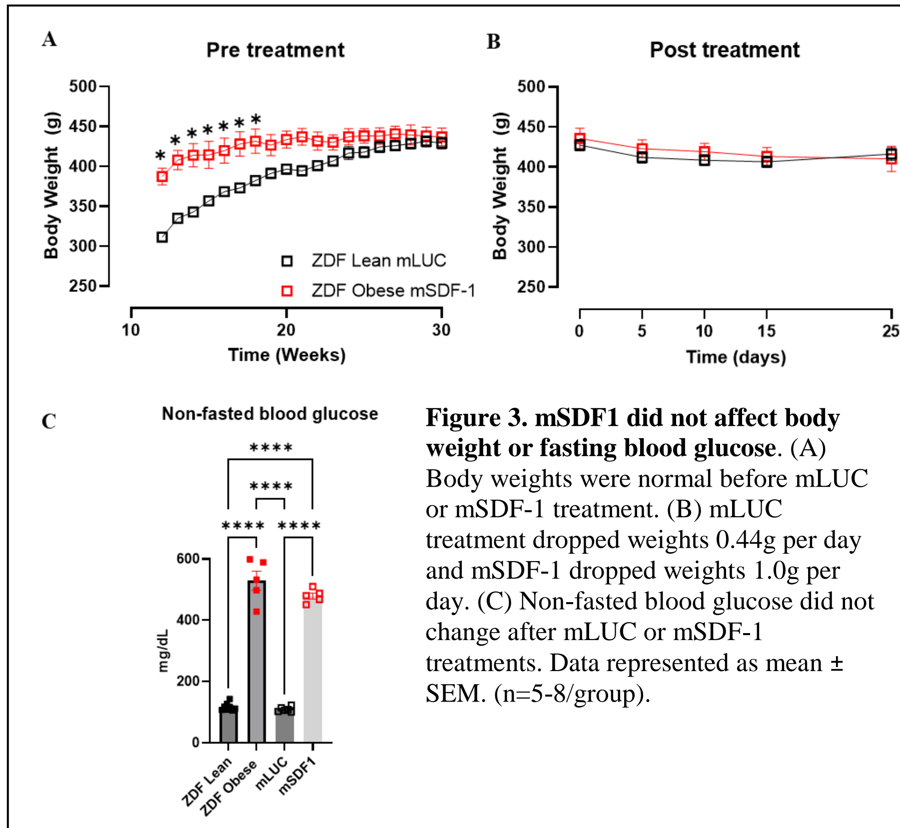

Figure 3. mSDF1 did not affect body weight or fasting blood glucose. (A) Body weights were normal before mLUC or mSDF-1 treatment. (B) mLUC treatment dropped weights 0.44g per day and mSDF-1 dropped weights 1.0g per day. (C) Non-fasted blood glucose did not change after mLUC or mSDF-1 treatments. Data represented as mean ± SEM. (n=5-8/group).

Erectile function was assessed via cavernous nerve mediated electrical stimulation and measurement of intracavernosal pressure (ICP) normalized to mean arterial pressure (MAP). SDF-1 mRNA treatment significantly increased the erectile function of ZDF obese rats. At 6V, ZDF obese rats had a mean ICP/MAP of 0.46, which was less than ZDF lean rats who had an average ICP/MAP of 0.63 (Figure 4A). mSDF-1 increased ICP/MAP, at 6V, ZDF obese rats that received mSDF-1 had an average ICP/MAP of 0.68, which was the highest value of all the groups. Additionally, mSDF-1 improved the maximal ICP of ZDF rats from 49.3mmHG to 73.3mmHG, an increase of 49%. Similarly, mSDF-1 improved the area under the curve (AUC) of ZDF obese rats from 1984.9 mmHg.s to 2888.8 mmHg.s, an increase of 46% (Figure 4B). The ICP/MAP, maximal ICP, or AUC of ZDF lean rats that received mLUC was not different from ZDF lean rats without mLUC (Figure 4C).

Following ICP measurements, we measured the weights of the heart, spleen, kidneys, bladder, testes, or seminal vesicles, and blood was collected for comparative blood cell (CBC) analysis in the two treatment groups. In ZDF obese rats, the bladders and kidneys were larger than the ZDF lean animals due to their diabetic phenotype (Figure 5). Additionally, seminal vesicles were much smaller in the ZDF obese compared to the ZDF lean. Overall, there was no effect of treatment on organ size in either group of rat. CBC analysis showed that the ZDF obese rats that received mSDF-1 had 61% more white blood cells (WBCs) than ZDF lean rats that received mLUC. ZDF obese rats that received mSDF-1 had a three-fold increase in neutrophils and eosinophils, and a five-fold increase in basophils (Table 1). There were no differences in red

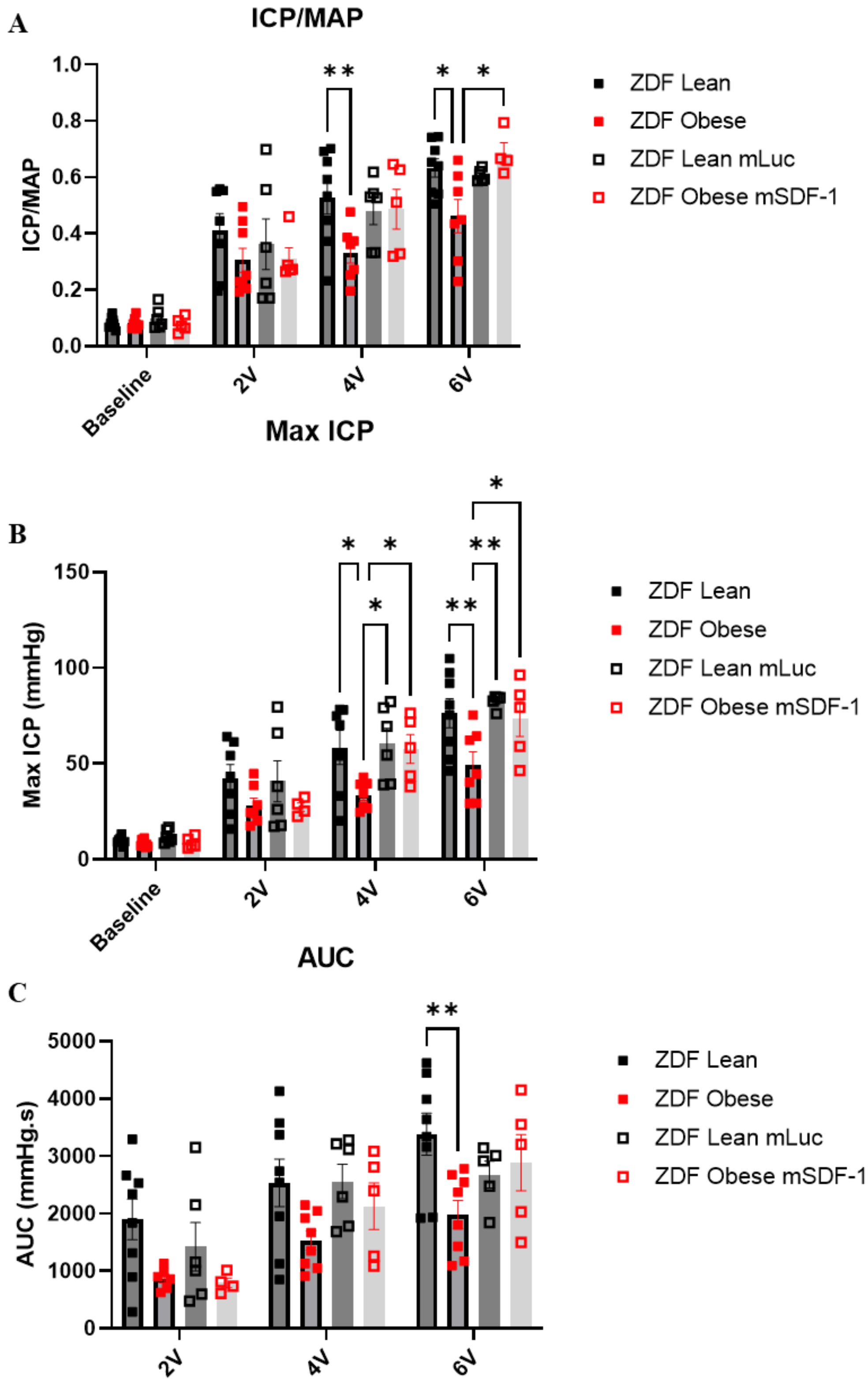

Figure 4. mSDF-1 improved erectile function. (A) mSDF-1 improved the ICP/MAP of ZDF obese rats. (B) mSDF-1 improved the max ICP of ZDF obese rats. (C) The AUC was decreased in ZDF obese rats. Data represented as mean ± SEM. (n=4-8/group).

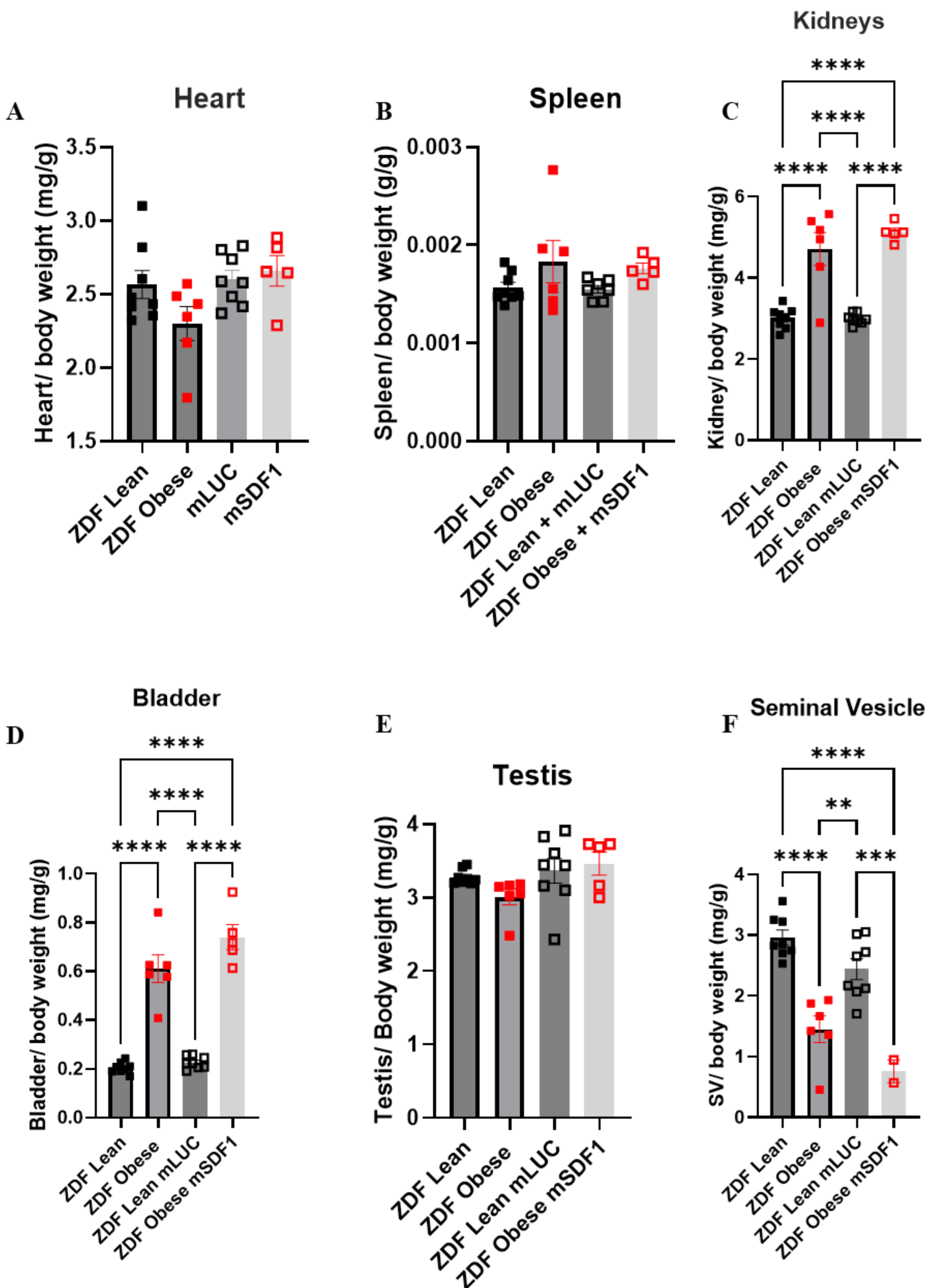

Figure 5. mSDF-1 treatment did not affect organ weights. (A, B, C, D, E, F) mLUC or mSDF-1 did not affect heart, spleen, kidney, bladder, testis, or seminal vesicle weight. Data represented as mean ± SEM. (n=2-8/group).

blood cells (RBCs) or platelets. (Table 1). These findings are likely due to the diabetic phenotype driving increased inflammation and corresponding white blood cells. These data indicate that the treatment appears to be effective and safe in this preclinical T2DM model.

Table 1. ZDF obese rats with mSDF-1 had increased WBCs. Data represented as the mean (n= 5- 7/ group)

Test description	ZDF lean mLuc	ZDF obese mSDF-1	Reference Range	Units	P- value
Leukocytes					
Total WBC	5.9	9.4	1.90 - 16.80	10^3/uL	*
NEU	1.7	4.8	0.35 - 6.30	10^3/uL	***
LYM	4.0	4.1	0.91 - 12.20	10^3/uL	
MONO	0.1	0.4	0.08 - 2.30	10^3/uL	
EOS	0.0	0.1	0.00 - 1.01	10^3/uL	****
BAS	0.0	0.1	0.00 - 0.20	10^3/uL	*
NEU%	27.6	49.9	7.3 - 50.0	%	****
LYM%	68.5	44	40.0 - 88.9	%	****
MONO%	2.8	3.8	2.0 - 18.0	%	
EOS%	0.7	1.2	0.0 - 7.0	%	*
BAS%	0.4	1.1	0.0 - 1.5	%	*
Erythrocytes					
Total RBC	8.2	8.6	5.00 - 9.00	10^3/uL	
HGB	13.8	14.5	11.0 - 17.0	g/dL	
HCT	39.9	42.3	32.0 - 53.0	%	
MCV	48.5	49.5	50.0 - 67.0	fL	
MCH	16.7	16.9	16.0 - 23.0	pg	
MCHC	34.5	34.2	31.0 - 37.0	g/dL	
RDW%	14.1	15.7	11.0 - 16.0	%	**
Platelets					
PLT	991.1	1090.2	250 - 1500	10^3/uL	
MPV	6.7	7.6	4.8 - 7.5	fL	**

Tissues were also collected for molecular and histological analyses. Penile and major pelvic ganglia tissues (MPG) will be stained to assess smooth muscle and collagen content, and nNOS neurons. Additionally, we will assess the expression of SDF-1 receptors, CXCR4. MPG and penile tissues are also in process to undergo NanoString panels for neuroinflammation and fibrosis, respectively. This will give us greater insight into the molecular pathways that are activated in diabetes and how SDF-1 treatment may alter them. These data are key for our upcoming R01 submission and publications.

3. Publications:

None.