

RBC Alloimmunization in Type 1 Interferon-Dependent and -Independent Lupus Models

June Young Lee¹, Emaan Madany PhD¹, Sumaarg Pandya¹, Michifumi Yamashita MD PhD¹, Caroline Jefferies PhD², and David R. Gibb MD PhD^{1,3}

¹Department of Pathology and Laboratory Medicine, ²Department of Internal Medicine, Division of Rheumatology

³Division of Transfusion Medicine

Cedars Sinai Medical Center, Los Angeles

RBC alloimmunization and type 1 interferons in patients with SLE

Patients with autoimmune diseases, including systemic lupus erythematosus (SLE), have an increased frequency of red blood cell (RBC) alloimmunization. However, underlying mechanisms are poorly understood. Two-thirds of patients with systemic lupus erythematosus (SLE) express a type 1 interferon (IFN α/β) gene signature that is associated with disease severity. A recent report showed that RBCg alloimmunization is enhanced in the pristane-induced lupus mouse model, in which administration of pristane oil induces IFN α/β production and a lupus-like phenotype. However, it is uncertain whether the lupus-like phenotype or IFN α/β directly promotes alloimmunization. While pristane induces autoimmunity by an IFN α/β -dependent manner, MRL/lpr mice contain mutations in the B cell expressed pro-apoptotic gene, Fas, that causes spontaneous autoantibody production and a lupus-like phenotype in the absence of IFN α/β . Thus, we utilized these IFN α/β -dependent (pristane and IFN α/β -independent (MRL/lpr) models to test the hypothesis that IFN α/β -mediated inflammation promotes RBC alloimmunization in lupus.

Objectives:

- 1) Determine the degree to which a lupus-like phenotype promotes RBC alloimmunization in multiple murine models.
- 2) Identify the role of type 1 interferons (IFN α/β) in promoting RBC alloimmunization in:
 - IFN α/β -dependent lupus model (pristane-induced)
 - IFN α/β -independent lupus model (MRL/lpr)

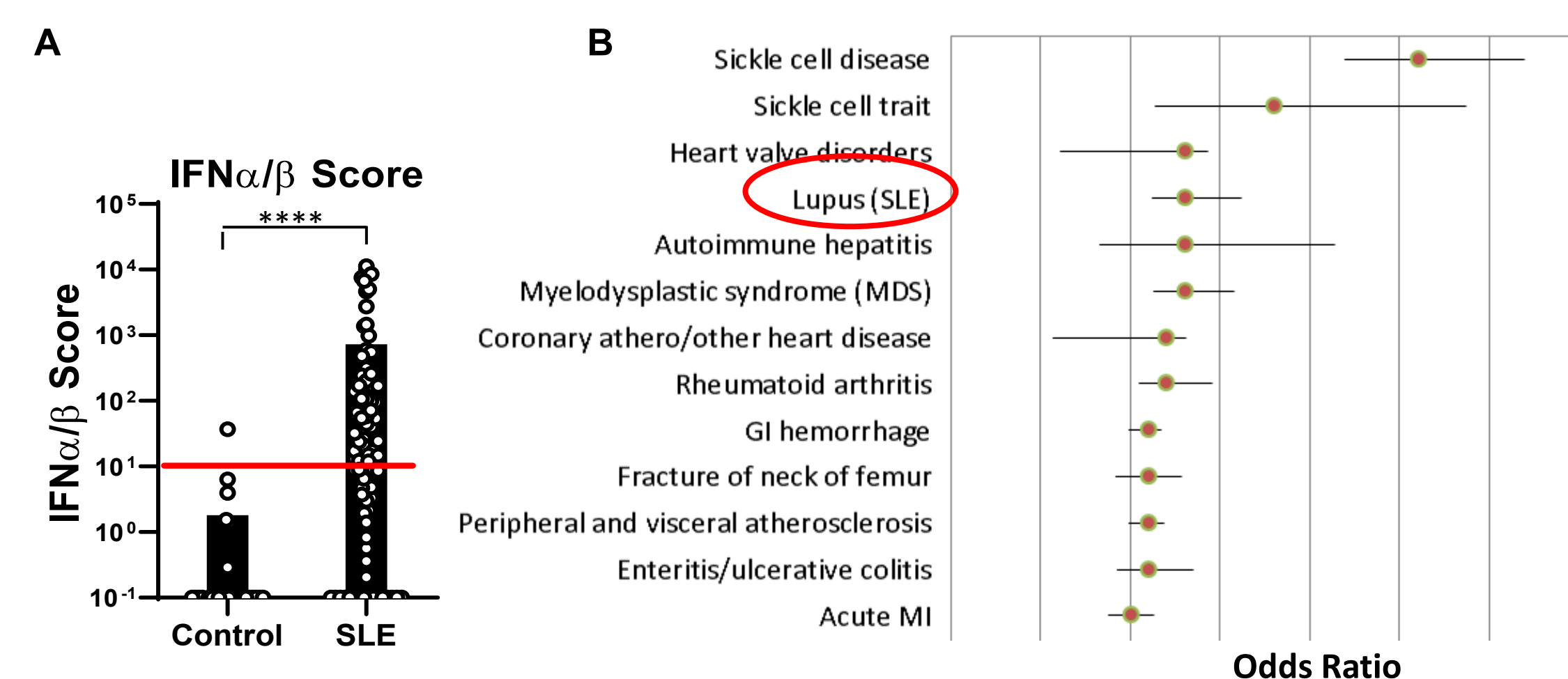


Figure 1. Patients with SLE express an IFN α/β gene signature and have an increased prevalence of RBC alloimmunization. (A) IFN α/β scores, generated from qPCR results of IFN α/β stimulated gene expression, of patients with SLE. (B) Odds ratio of RBC alloimmunization frequency. Figure adapted from Karafin M et al. Br J Haematol 2018. REDS III RBC Alloimmunization Recipient Project.

Results

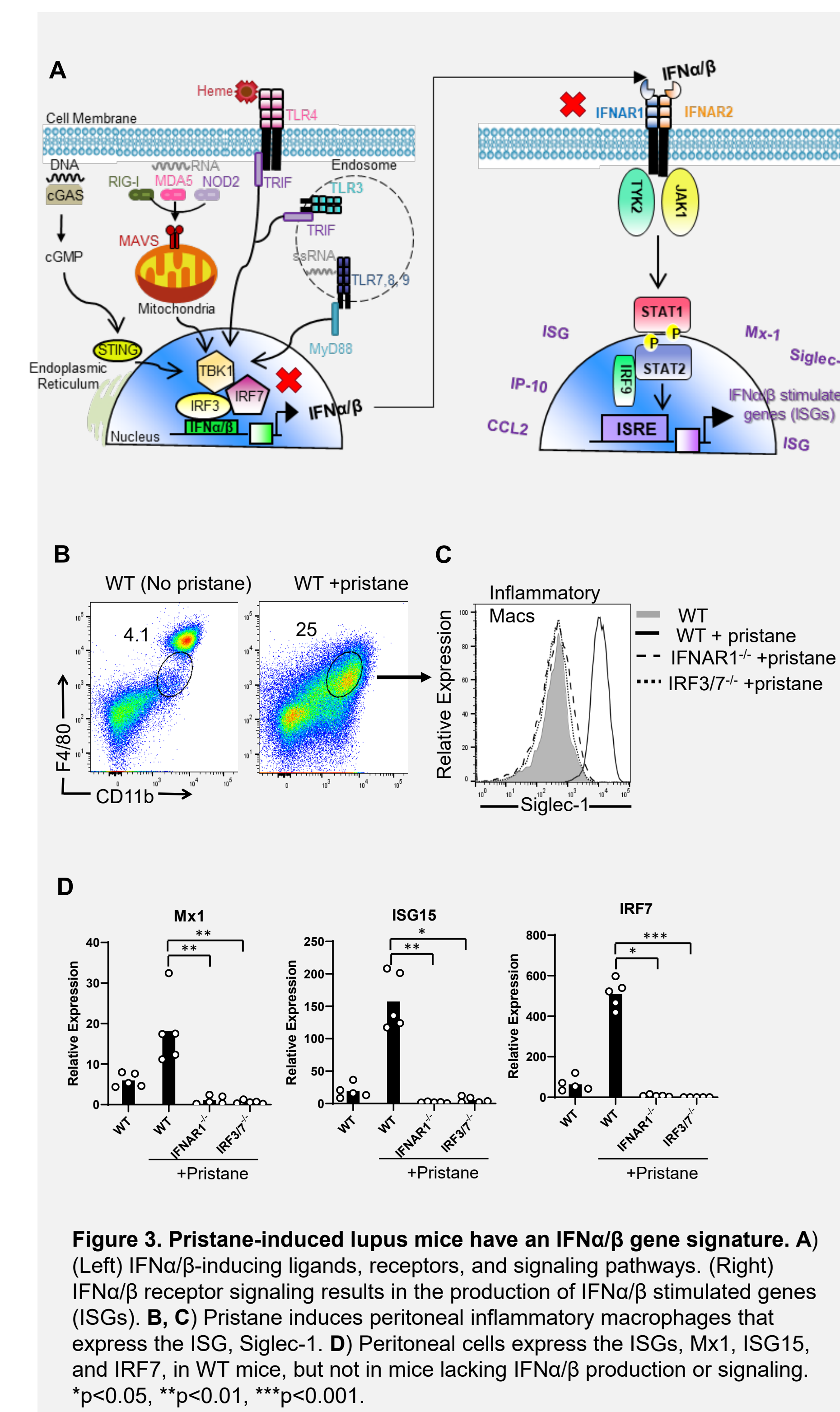
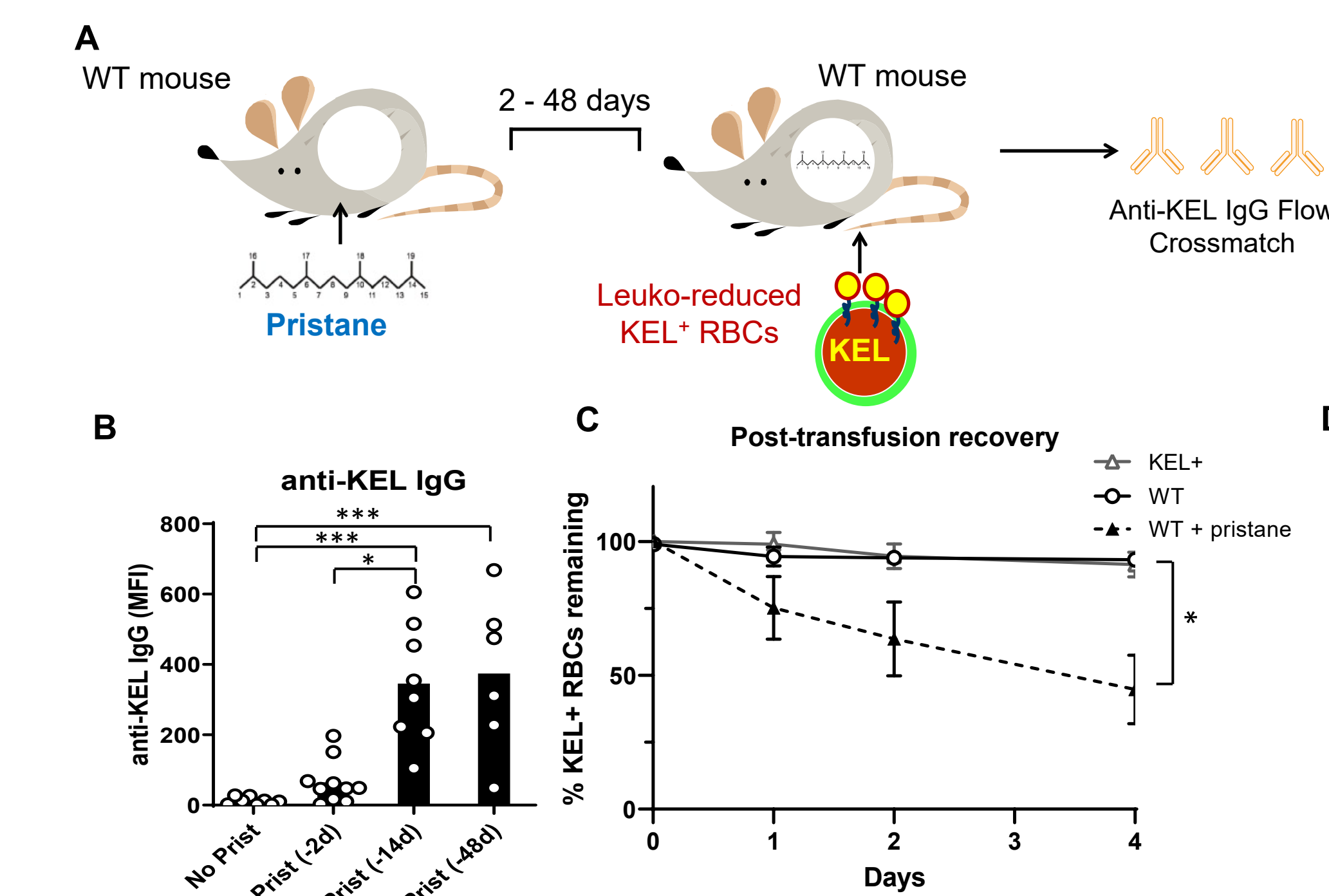


Figure 3. Pristane-induced lupus mice have an IFN α/β gene signature. (A) (Left) IFN α/β -inducing ligands, receptors, and signaling pathways. (Right) IFN α/β receptor signaling results in the production of IFN α/β stimulated genes (ISGs). (B, C) Pristane induces peritoneal inflammatory macrophages that express the ISG, Siglec-1. (D) Peritoneal cells express the ISGs, Mx1, ISG15, and IRF7, in WT mice, but not in mice lacking IFN α/β production or signaling. *p<0.05, **p<0.01, ***p<0.001.

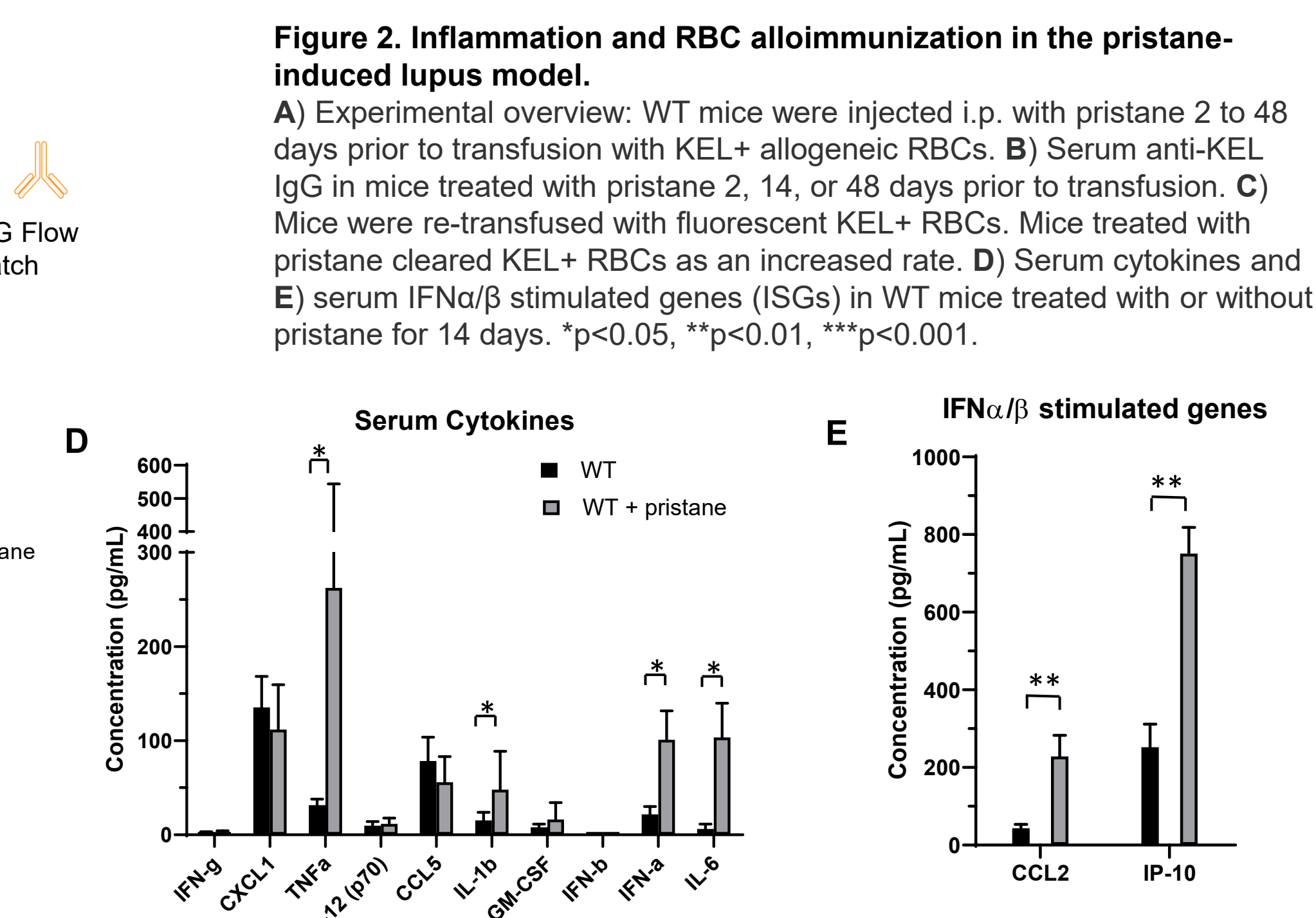


Figure 4. IFN α/β promotes RBC alloimmunization in pristane-induced lupus mice. (A) TNF α and (B) IL-6 production in indicated mice treated with pristane. After KEL+ RBC transfusion, anti-KEL IgG (not IgM) was dependent on IFN α/β production and signaling. *p<0.05.

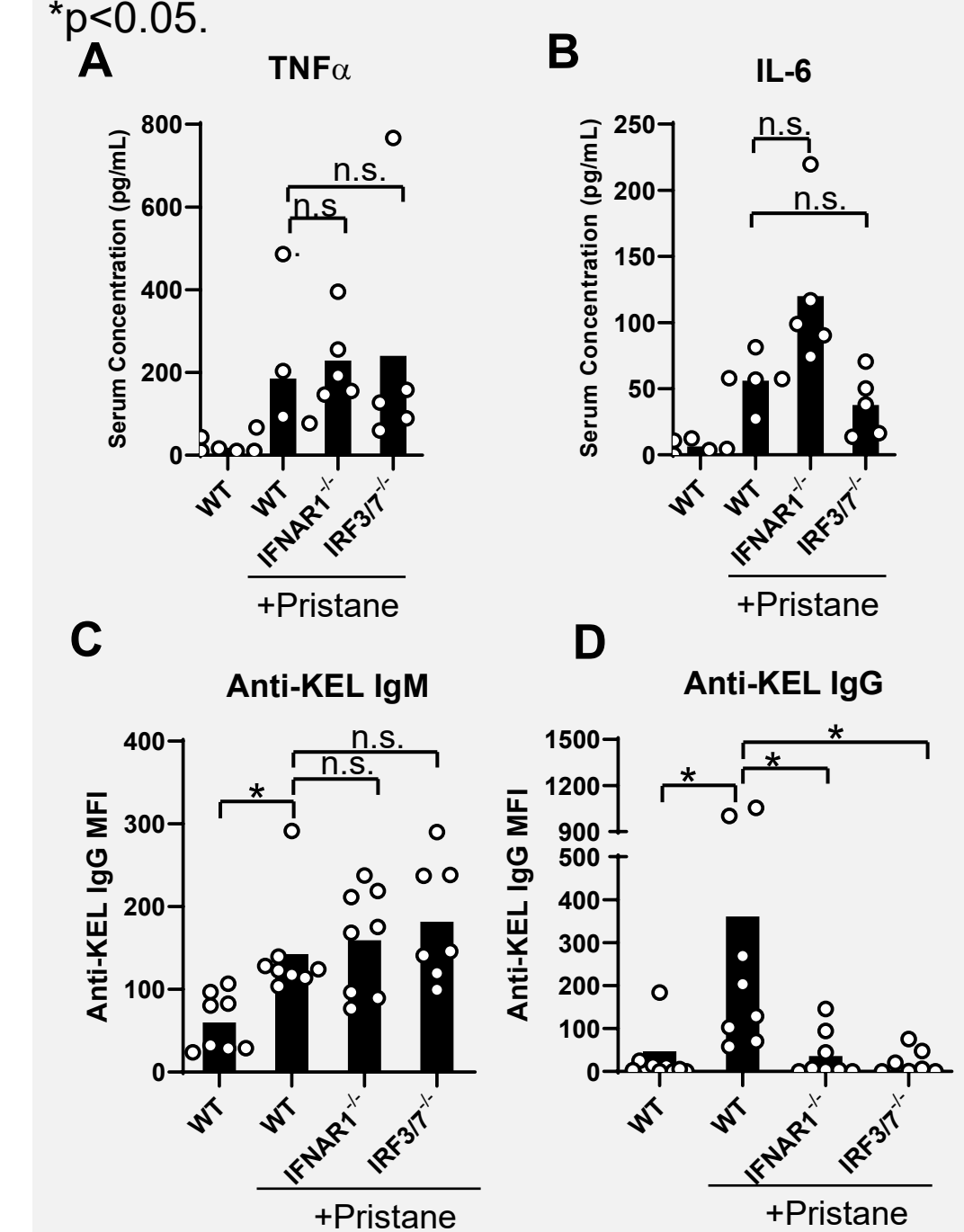


Figure 5. IFN α/β -independent lupus model: MRL/lpr mice. (A) Experimental model of MRL/lpr mice transfused with KEL+ RBCs prior to examination of anti-KEL IgG and KEL RBC clearance. (B) MRL/lpr mice have a mutation in the Fas death receptor expressed by B cells. (C) Atuo-reactive B cells persist in germinal centers, resulting a lupus-like phenotype. Figure from Butt et al. *Immunity*. 2015 May 19;42(5):890-902.

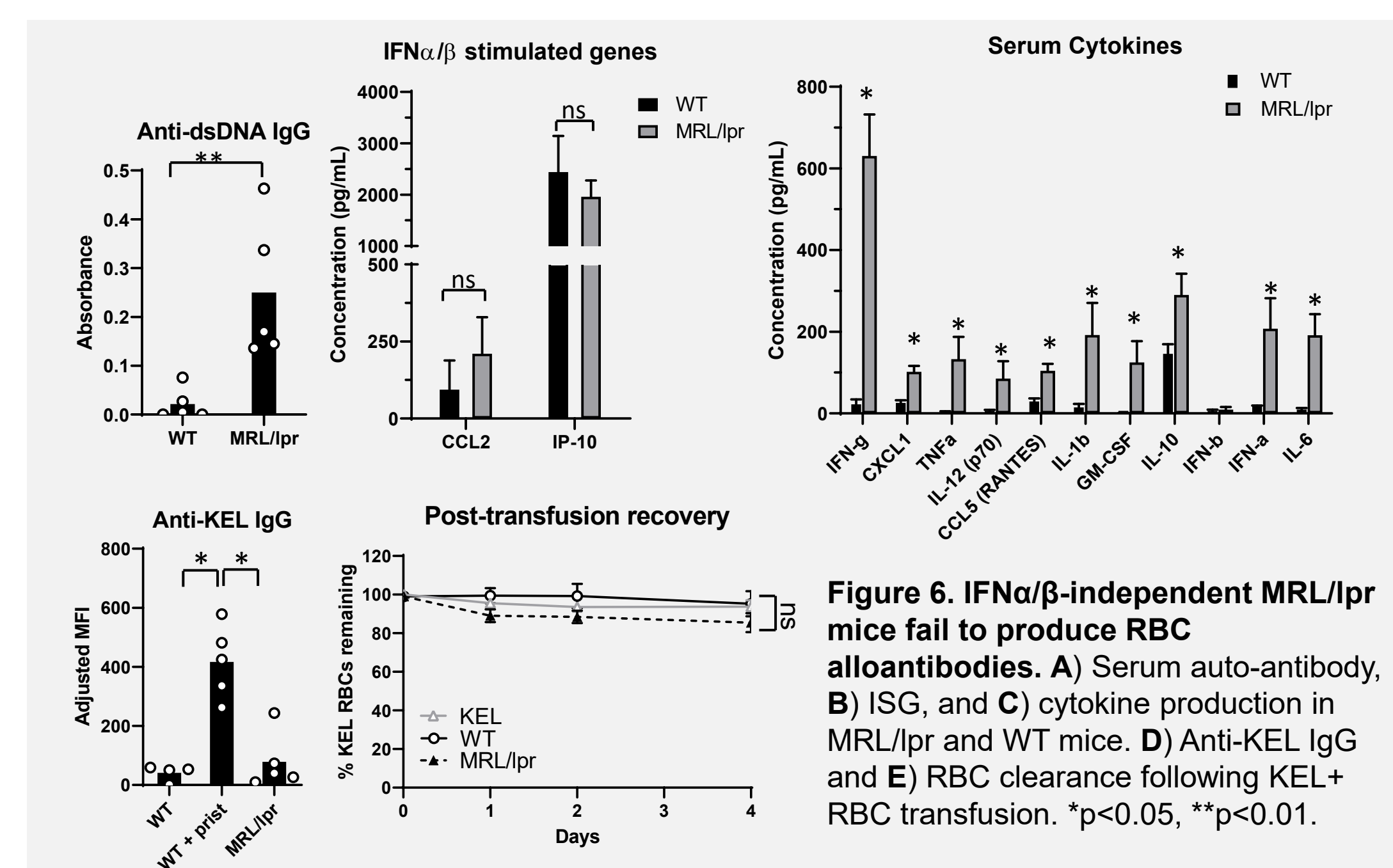


Figure 6. IFN α/β -independent MRL/lpr mice fail to produce RBC autoantibodies. (A) Serum auto-antibody, (B) ISG, and (C) cytokine production in MRL/lpr and WT mice. (D) Anti-KEL IgG and (E) RBC clearance following KEL+ RBC transfusion. *p<0.05, **p<0.01.

Discussion

- Pristane injection induces a lupus-like phenotype, characterized by autoantibody production, renal pathology, and pulmonary hemorrhage. IFN α/β production and signaling are required for pristane-induced lupus pathology
- In the absence of IFN α/β , pristane induces inflammation, including proinflammation cytokine production and expansion of inflammatory neutrophils and macrophages in the peritoneum and spleen.
- However, IFN α/β -independent inflammation was not sufficient to induce RBC alloimmunization, which was IFN α/β -dependent in the pristane model.
- MRL/lpr mice have a lupus phenotype that does NOT require IFN α/β . While autoantibodies and inflammatory cytokines are elevated, ISGs are not increased.
- KEL+ transfusion did not induce anti-KEL IgG or KEL RBC clearance in MRL/lpr mice.

Conclusions

Lupus-associated RBC alloimmunization is induced in IFN α/β -dependent models.

- Transfusion induced alloimmunization in an IFN α/β -dependent lupus model, but not in an IFN α/β -independent model.
- Thus, the lupus-like phenotype of MRL/lpr is not sufficient to induce alloimmunization.
- These results support a prior report indicating that IFN α/β promotes RBC alloimmunization in a lupus mouse model.
- Collectively, these findings warrant investigation of the role of IFN α/β in RBC alloimmunization in patients with SLE and other autoimmune diseases.

Acknowledgements

- Caroline Jefferies, PhD; Jeanne Hendrickson, M.D.
- AABB and National Blood Foundation
- American Society of Hematology
- National Heart, Lung, and Blood Institute

Example document title (Arial 72 pt.)

Department/Division name (Arial 32 pt.)

Faculty/staff/researchers' names & credentials, MD, etc.
Cedars Sinai Medical Center, Los Angeles

Header (Arial 48 pt.)

Text (Arial Regular/Black 32 pt. – resize as needed, extra padding/ spacing built into this text box, can be adjusted by going to Format > Paragraph in the menu)

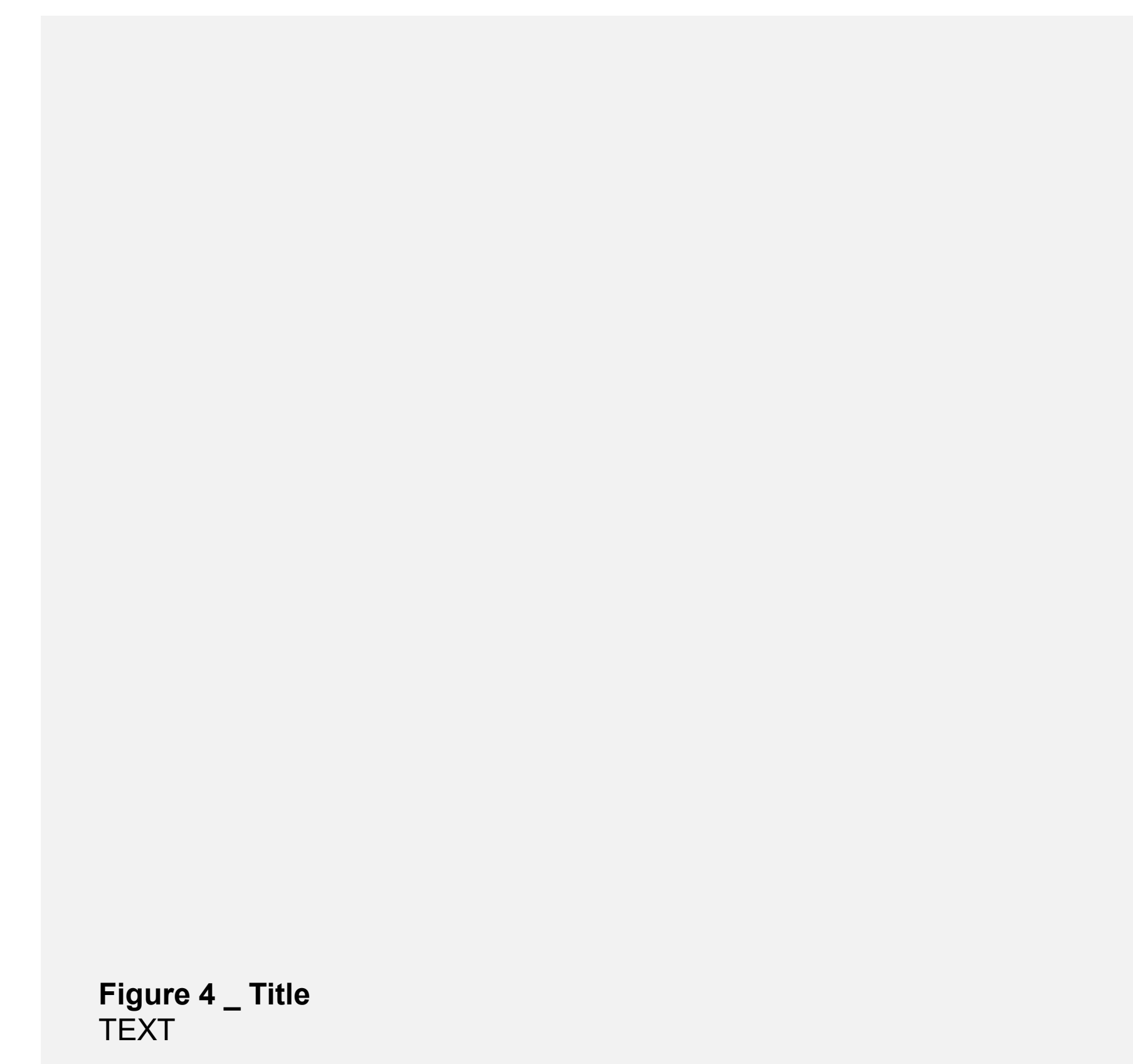
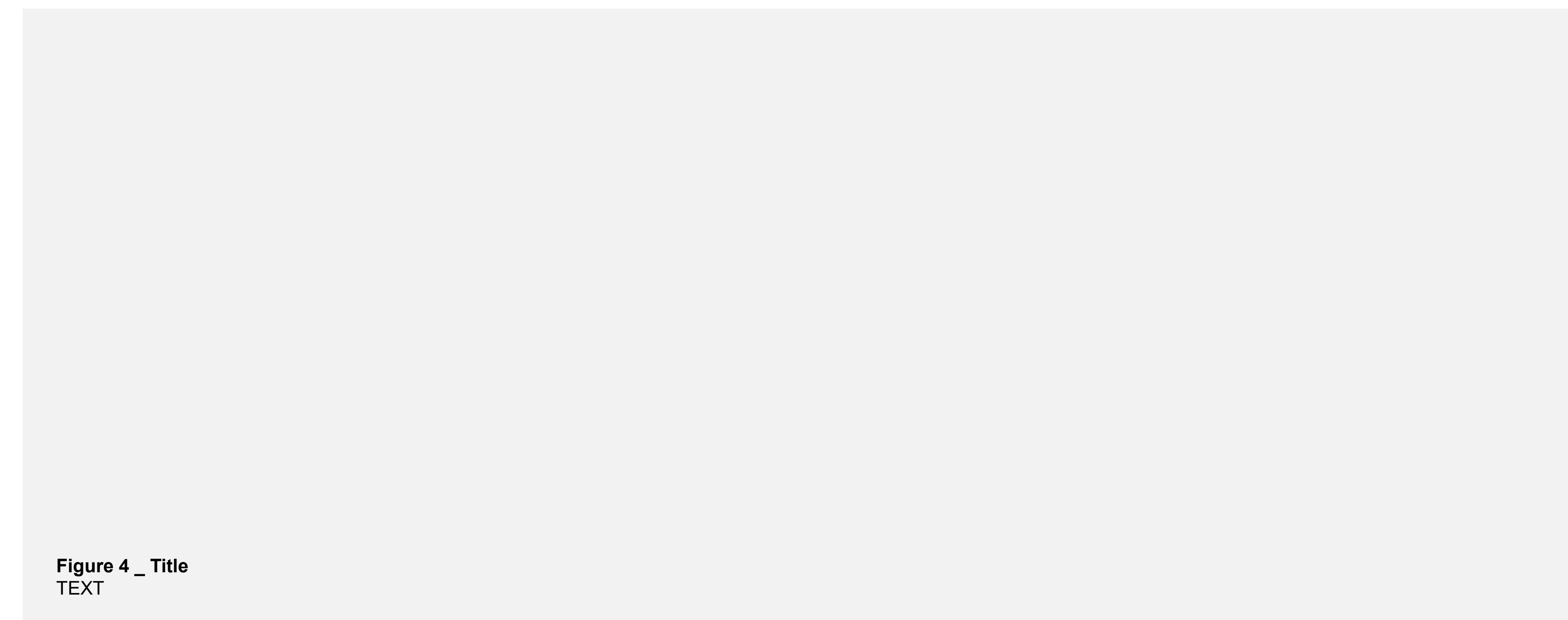
Bold Subheading... This one has 12pt. spacing set before/after the text.

Avolius auctua nonsulibus, mo et; hae tursu st? At abem in tastem prebus, C. Epes, qua aticatquem. Qui iam tam ia consum inime inatrum enam nonsilibul uniusquo erumus, maximus, publicio moermius.

Objectives

- Bullet Point Text (Arial Regular/Gray 32 pt.)
- Catu que aurbi te inc re esta mo addum aus aut factus, nonte consum nonsula buscit;
- Ex nox sentis. Se ac vidiente ficatae con vasdacciorum a elut verem se, consum detiam.
- Avolius auctua nonsulibus, mo et; hae tursu st?
- **Figure 4 _ Title**
Text in tastem prebus, C. Epes, qua aticatquem.
- Qui iam tam ia consum inime inatrum enam nonsilibul uniusquo erumus, maximus, publicio moermius.

Header (Arial 48 pt.)



Materials and Methods (Arial 48 pt.)

Text (Arial Regular/Black 32 pt. – resize as needed, extra padding/spacing built into this text box, can be adjusted by going to Format > Paragraph in the menu)

- Catu que aurbi te inc re esta mo addum aus aut factus, nonte consum nonsula buscit;
- Ex nox sentis. Se ac vidiente ficatae con vasdacciorum a elut verem se, consum detiam.
- Avolius auctua nonsulibus, mo et; hae tursu st?
- At abem in tastem prebus, C. Epes, qua aticatquem.
- Qui iam tam ia consum inime inatrum enam nonsilibul uniusquo erumus, maximus, publicio moermius.

Aus aut factus, nonte consum nonsula buscit; ex nox sentis. Se ac vidiente ficatae con vasdacciorum a elut verem se, consum detiam.

Conclusions

Text (Arial Regular/Black 32 pt. – resize as needed, extra padding/spacing built into this text box, can be adjusted by going to Format > Paragraph in the menu)

- Catu que aurbi te inc re esta mo addum aus aut factus, nonte consum nonsula buscit;
- Ex nox sentis. Se ac vidiente ficatae con vasdacciorum a elut verem se, consum detiam.
- Avolius auctua nonsulibus, mo et; hae tursu st?
- At abem in tastem prebus, C. Epes, qua aticatquem.
- Qui iam tam ia consum inime inatrum enam nonsilibul uniusquo erumus, maximus, publicio moermius.

Conclusions

Text (Arial Regular/Black 24 pt. – resize as needed, extra padding/spacing built into this text box, can be adjusted by going to Format > Paragraph in the menu)

- Catu que aurbi te inc re esta mo addum aus aut factus, nonte consum nonsula buscit;
- Ex nox sentis. Se ac vidiente ficatae con vasdacciorum a elut verem se, consum detiam.
- Avolius auctua nonsulibus, mo et; hae tursu st?

Acknowledgements

Text (Arial Regular/Black 24 pt. – resize as needed, extra padding/spacing built into this text box, can be adjusted by going to Format > Paragraph in the menu)

- Catu que aurbi te inc re esta mo addum aus aut factus, nonte consum nonsula buscit;
- Ex nox sentis. Se ac vidiente ficatae con vasdacciorum a elut verem se, consum detiam.
- Avolius auctua nonsulibus, mo et; hae tursu st?