Antibodies against the erythroferrone N-terminal domain prevent hepcidin suppression and ameliorate murine thalassemia

Supervisor:Dr roohollah Mirzaee

Presented by:Somayeh Daneshikohan

blood
Regular Article

RED CELLS, IRON, AND ERYTHROPOIESIS

Antibodies against the erythroferrone N-terminal domain prevent hepcidin suppression and ameliorate murine thalassemia

João Arezes,[1,*] Niall Foy,[2,*] Kirsty McHugh,[3] Doris Quinkert,[3] Susan Benard,[4] Anagha Sawant,[5] Joe N. Frost,[1] Andrew E. Armitage,[1] [illegible] Jin Lim,[1] May S. Tam,[5] Edward Lavallie,[5] Debra D. Pittman,[5] Orla Cunningham,[2] Matthew Lambert,[2] [illegible] Drakesmith[1,8,*] [illegible] United Kingdo

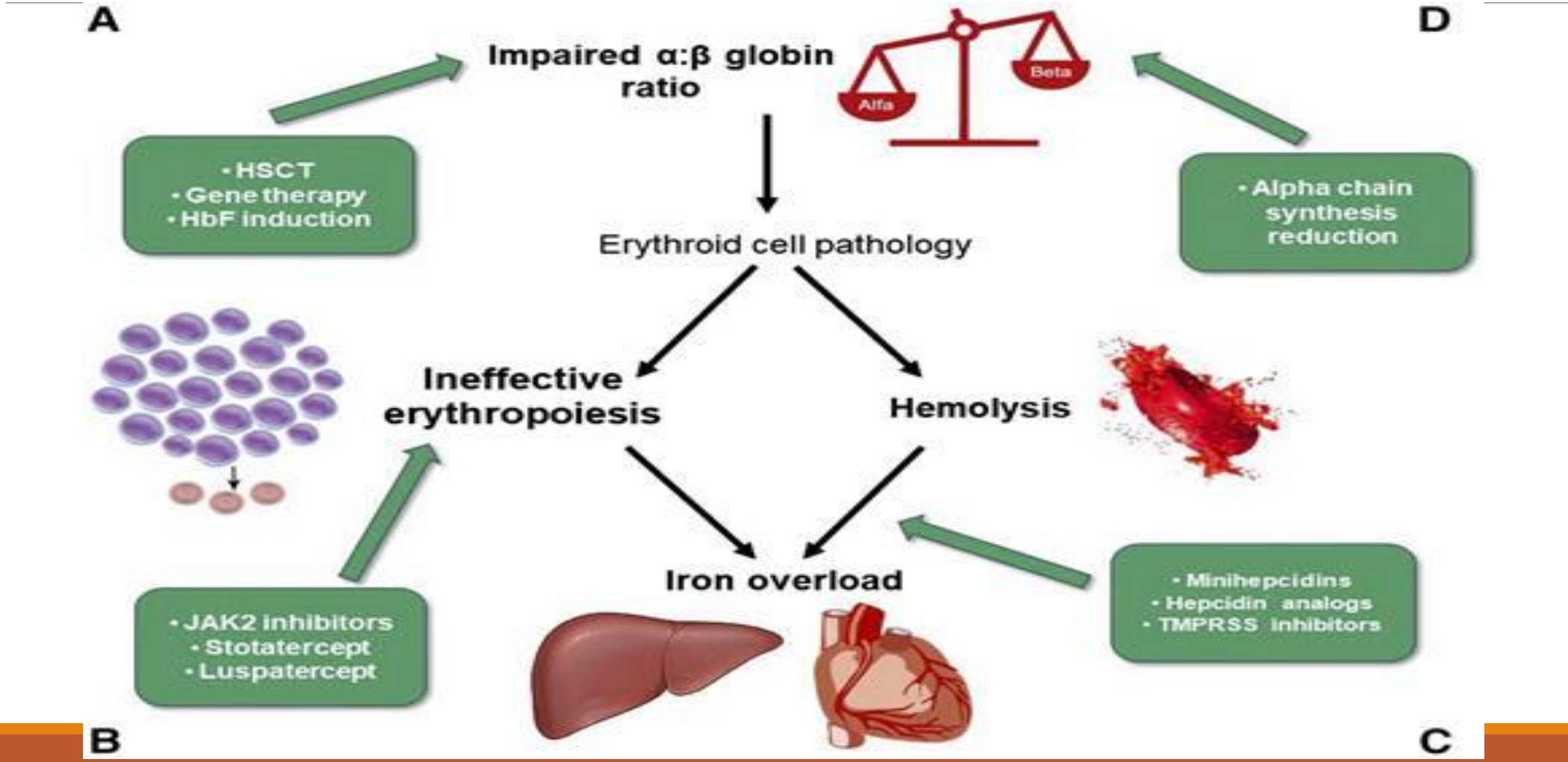
IF: 17.5

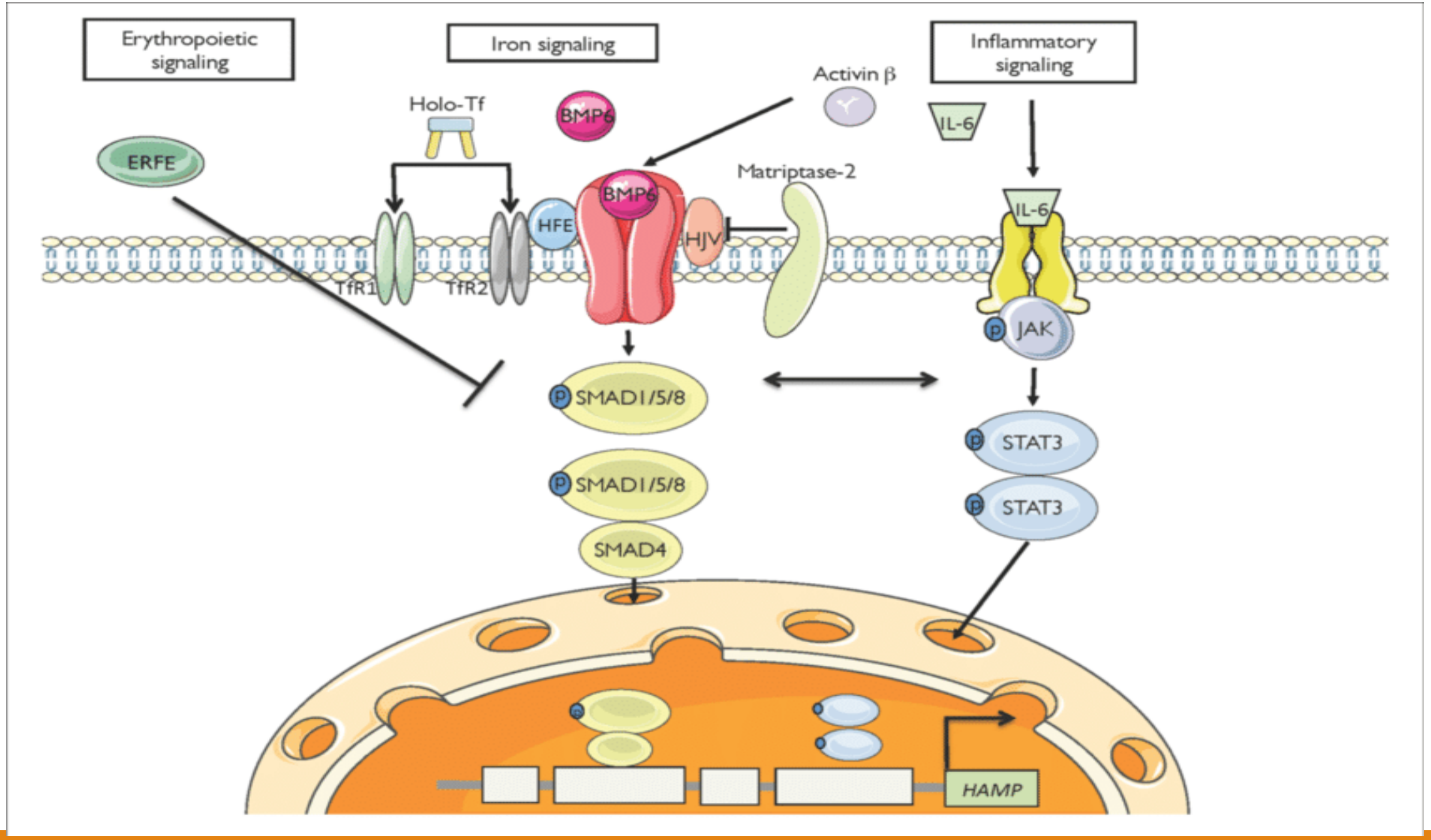
Thalassemia

- 1.5% of the global population (migration)
- prevalent in the Mediterranean, Middle East, Indian, and East and Southeast Asia , Europe and North America
- The imbalance in the α/β-globin chain ratio (ineffective erythropoiesis, chronic hemolytic anemia, and compensatory hemopoietic expansion)
- blood transfusion, splenectomy,iron chelation therapy , hematopoietic stem-cell transplantation (HSCT)

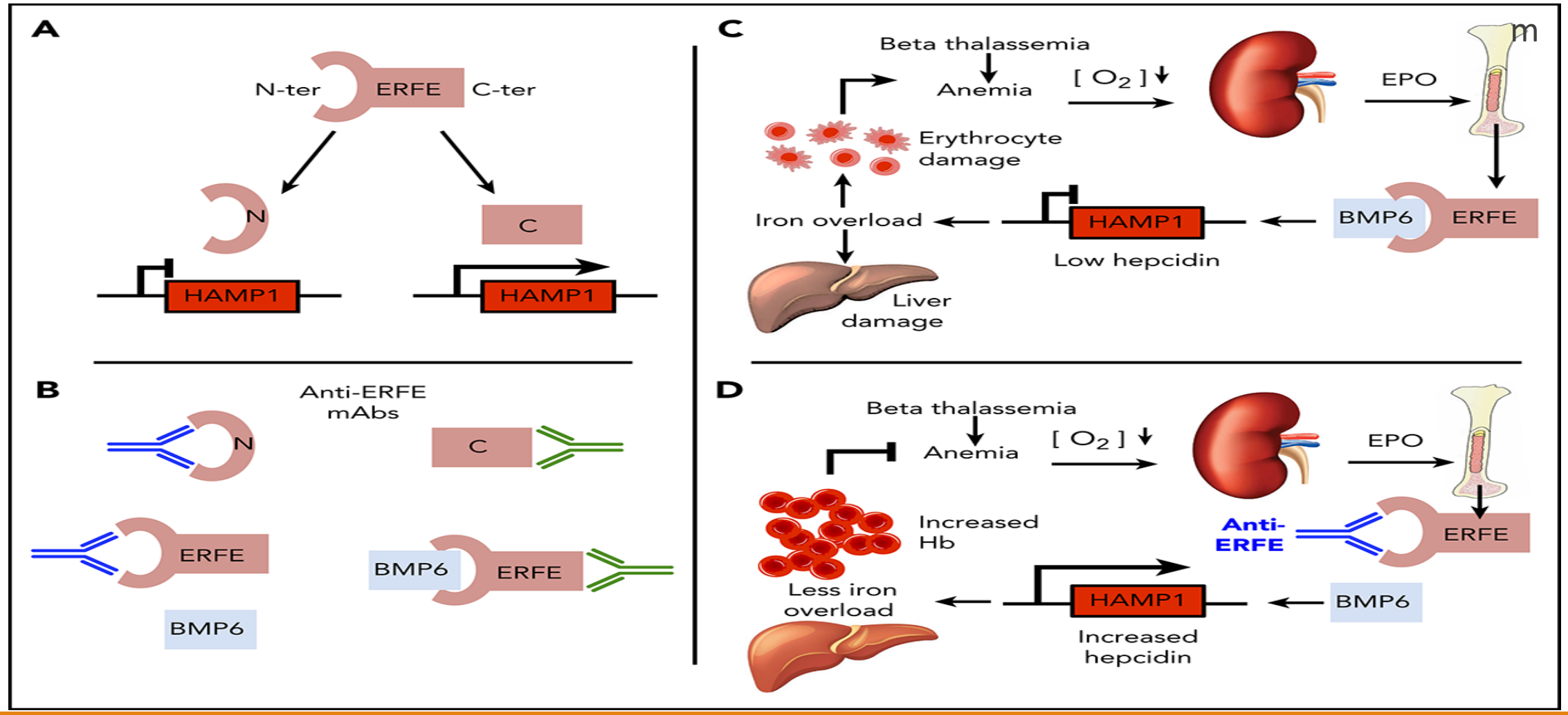
A main cause of morbidity in these patients is iron overload (transfusion & chelators)

Understanding the mechanism underlying iron accumulation may contribute to the design of better therapies to improve the clinical outcome




Erythropoietic signaling
Iron signaling
Inflammatory signaling
Activin β
Holo-Tf
BMP6
IL-6
ERFE
Matriptase-2
BMP6
HFE
HJV
IL-6
TfR1
TfR2
JAK
P
SMAD1/5/8
STAT3
P
SMAD1/5/8
STAT3
SMAD4
HAMP

erythropoiesis requires iron avaibility → suppression of hepcidin

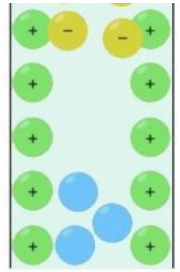


What was examined in this article?

- Do ERFE bind s to BMP2, BMP4, and BMP6 with different affinities?
- isThe N-terminal domain of ERFE sufficient to suppress hepcidin?
- Do Neutralizing anti-ERFE antibodies bind to the N-terminal region of ERFE?
- Do Antibodies binding ERFE N-terminal domain block hepcidin suppression in EPO-treated mice ?
- Do Anti-ERFE antibodies decrease iron levels and alter blood parameters in thalassemic mice?

Protein production(ERFE)

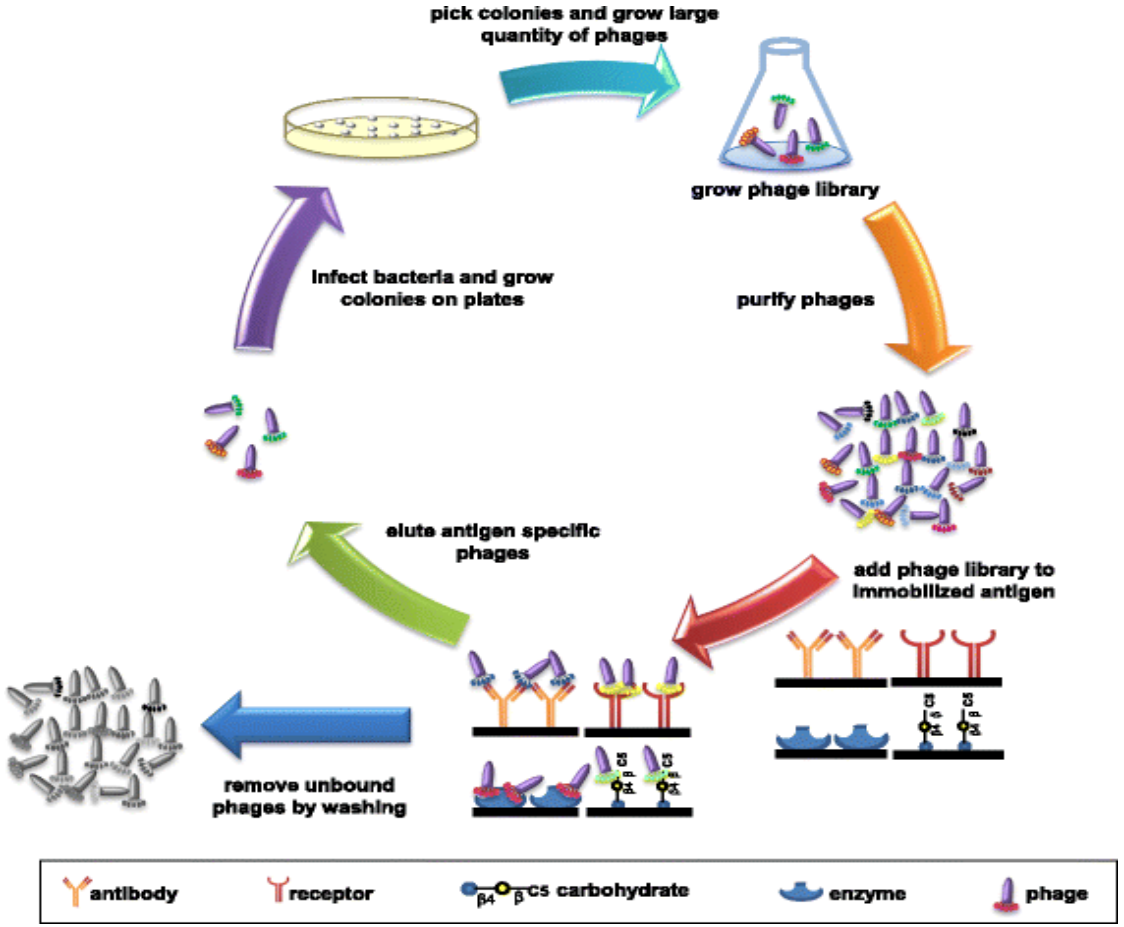
Expi293f cell 36°c → Transfected with vector DNA (human or mouse ERFE) → Shaking incubator at 36°c &8%co2 No trypton feed → Cm collect 120h →  sterile Nalgene bottle →  Sorvall H-6000A ROTOR 6min/1800rpm/4°c → 

Sartopore 2XL 0.8/0.2 mm filtration → ERFE Pr link to Monofc by G4S linker at the Nterminus → Mono fc-muERFE &Mono fc huERFE purified → MabSelect SuRe/4°C washed extensively with PBS-CMF pH 7.2 and eluted with a decreasing pH gradient → ERFE were exchanged into low sodium chloride pH 8.0 buffer → 

Q Sepharose HP column an increasing sodium chloride gradient → HiLoad superdex 200 column Final pufirication mobile phase containing arginine pH 7.0 → high purity of ERFE PBS-CMF pH 7.2 → furin cleavage sites, at positions 42 and 212

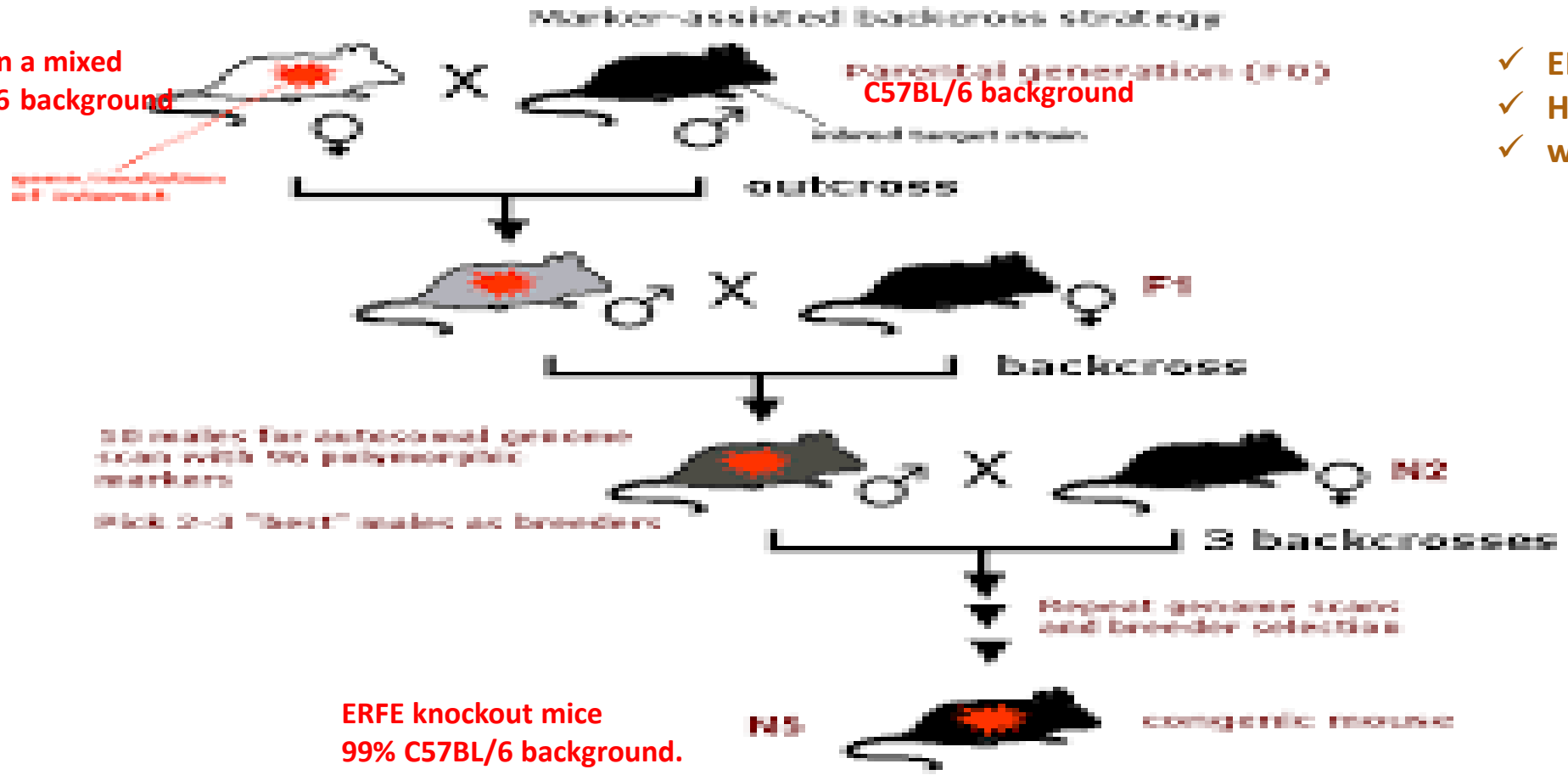
Anti-ERFE

- An antibody scFv phage display library
- Antibodies were screened by using (ELISA)
- Transient HEK293 cells expressing anti-ERFE were cultured
- in FreeStyle 293 medium or Expi293 medium



Animal studies

Erfe+/- mice on a mixed Sv129/C57BL/6 background

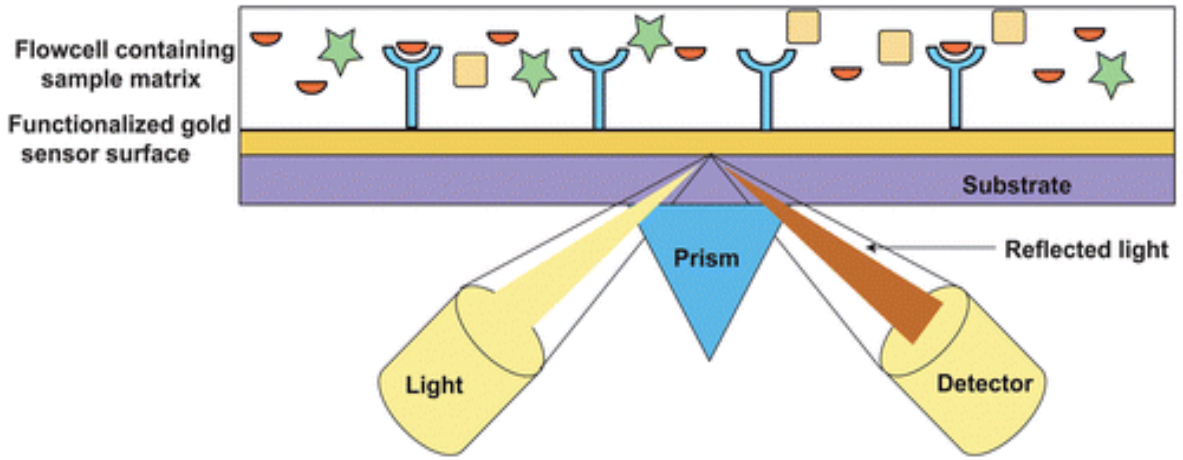


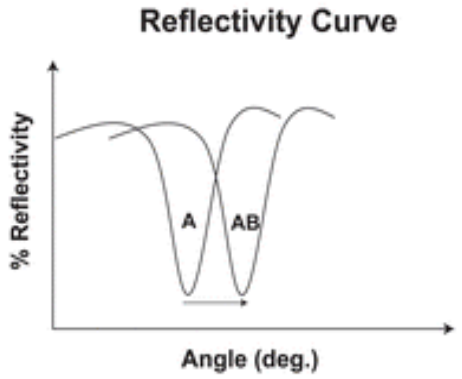
ERFE knockout mice
99% C57BL/6 background.

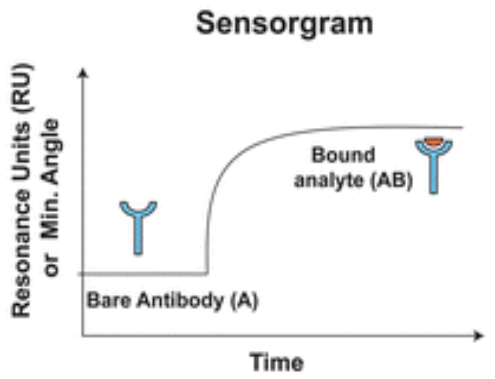
- ✓ ERFE knockout mice
- ✓ Hbb(th3/1) mice
- ✓ wild-type mice

ERFE bind s to BMP2, BMP4, and BMP6 with different affinities

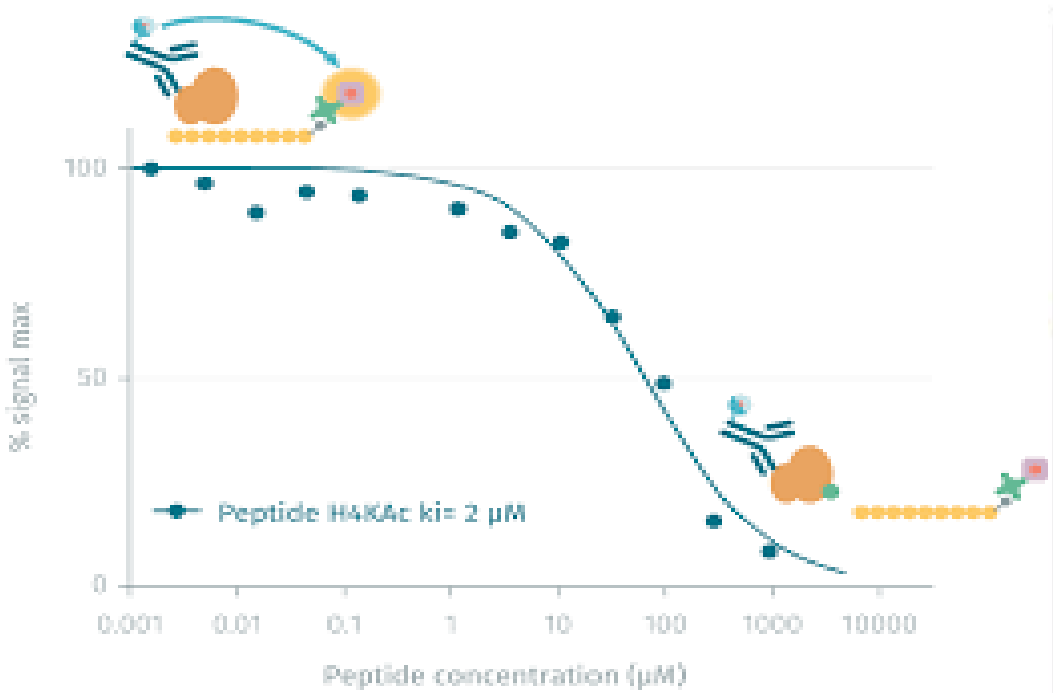
- Biacore T200 instrument (Surface plasmon resonance)
- CM4 Sensor Chip
- Amine Coupling Kit

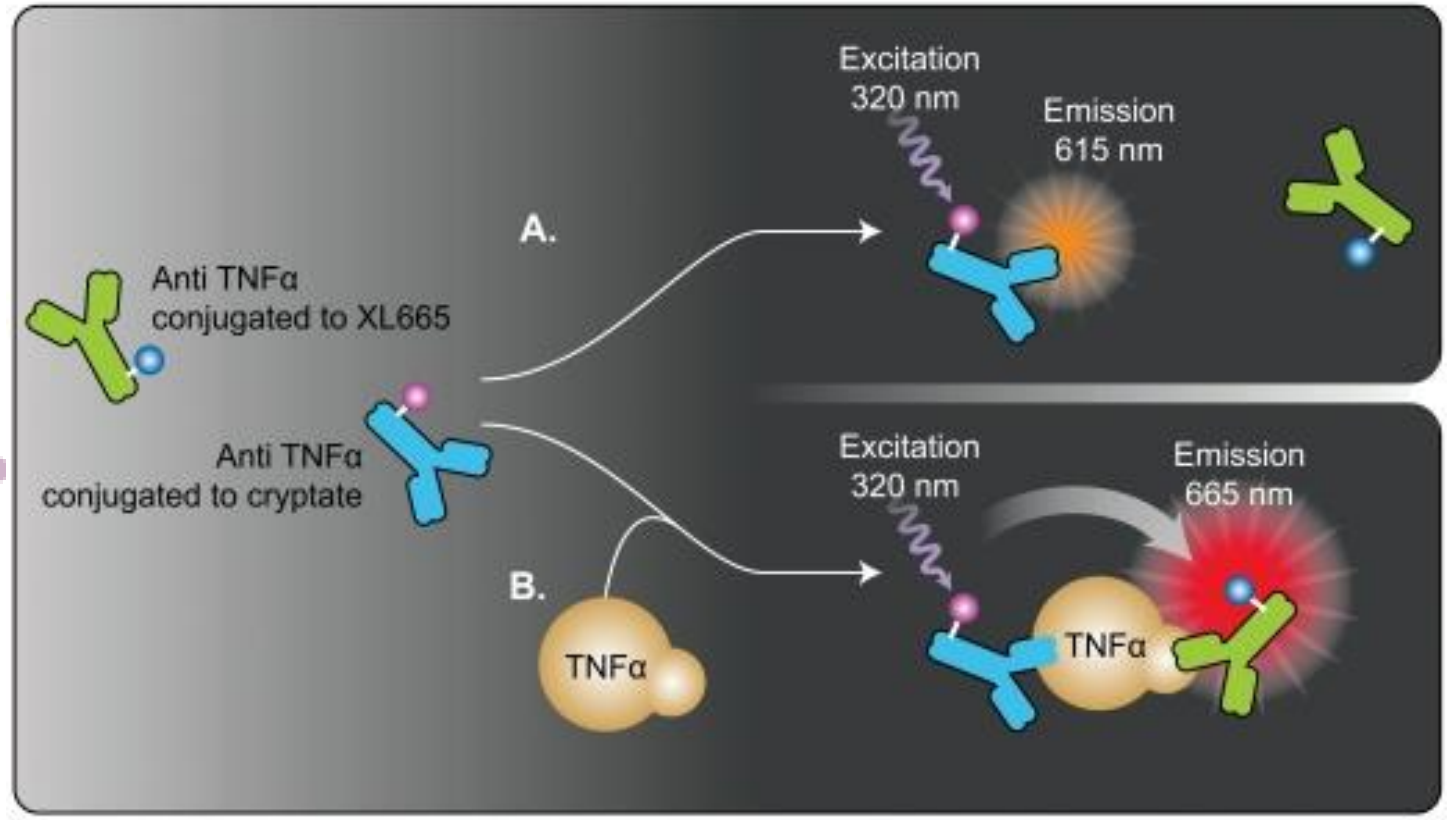






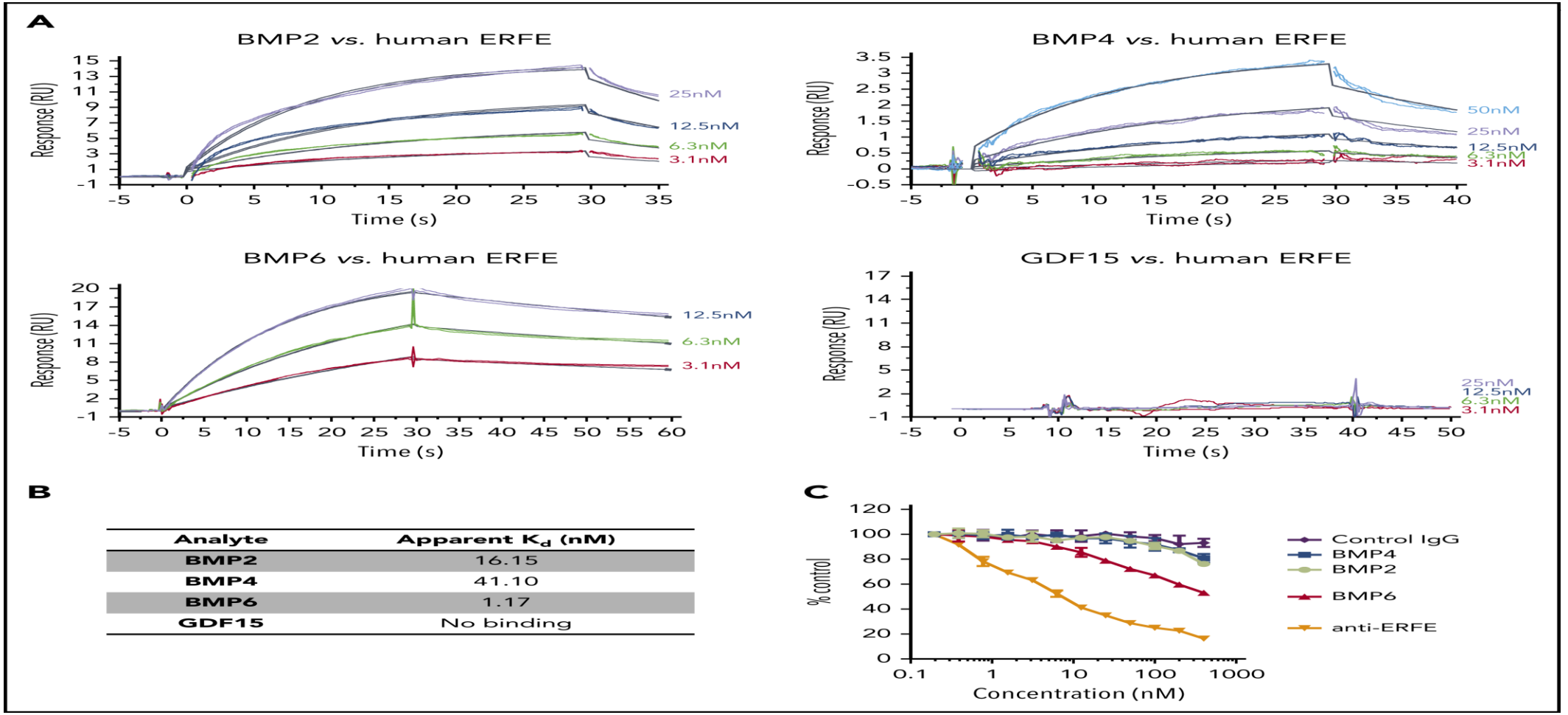
ERFE bind s to BMP2, BMP4, and BMP6 with different affinities





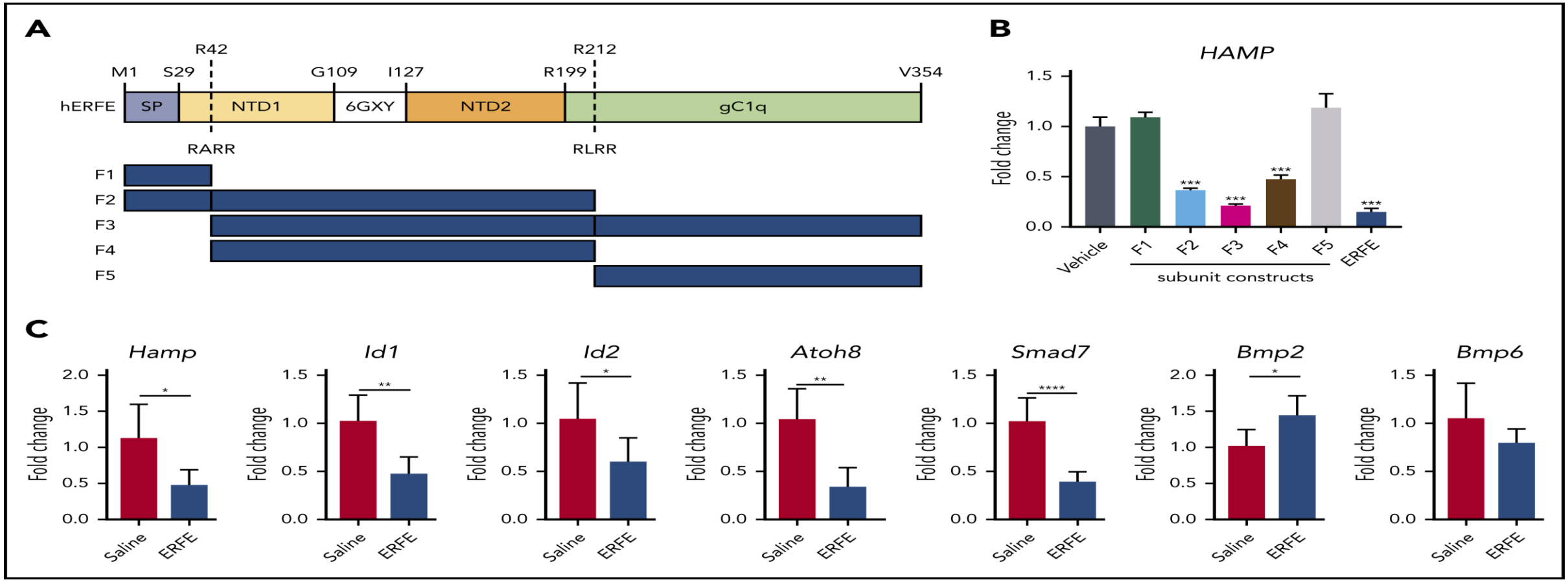
Homogeneous time-resolved fluorescence (HTRF)

ERFE bind s to BMP2, BMP4, and BMP6 with different affinities



Analyte	Apparent K_d (nM)
BMP2	16.15
BMP4	41.10
BMP6	1.17
GDF15	No binding

The N-terminal domain of ERFE is sufficient to suppress hepcidin

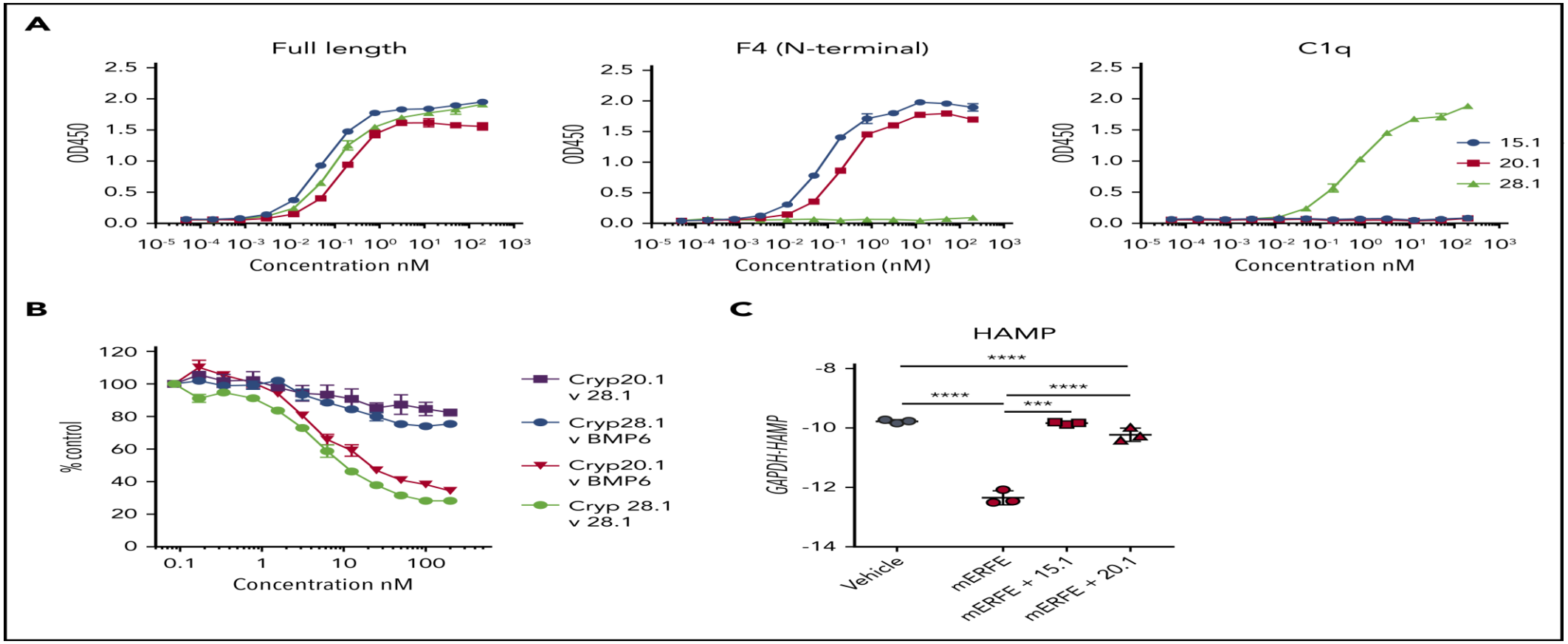


(A) Structure of human ERFE, containing a signal peptide (SP), N-terminal region (NTD) with a collagen-like domain (6 GXY), and a globular C1q domain (Gc1q)

(B) Huh7 cells were treated for 24 hours with 1 mg/mL of ERFE subunits F1 to F5, full-length or vehicle, and analyzed for HAMP gene expression

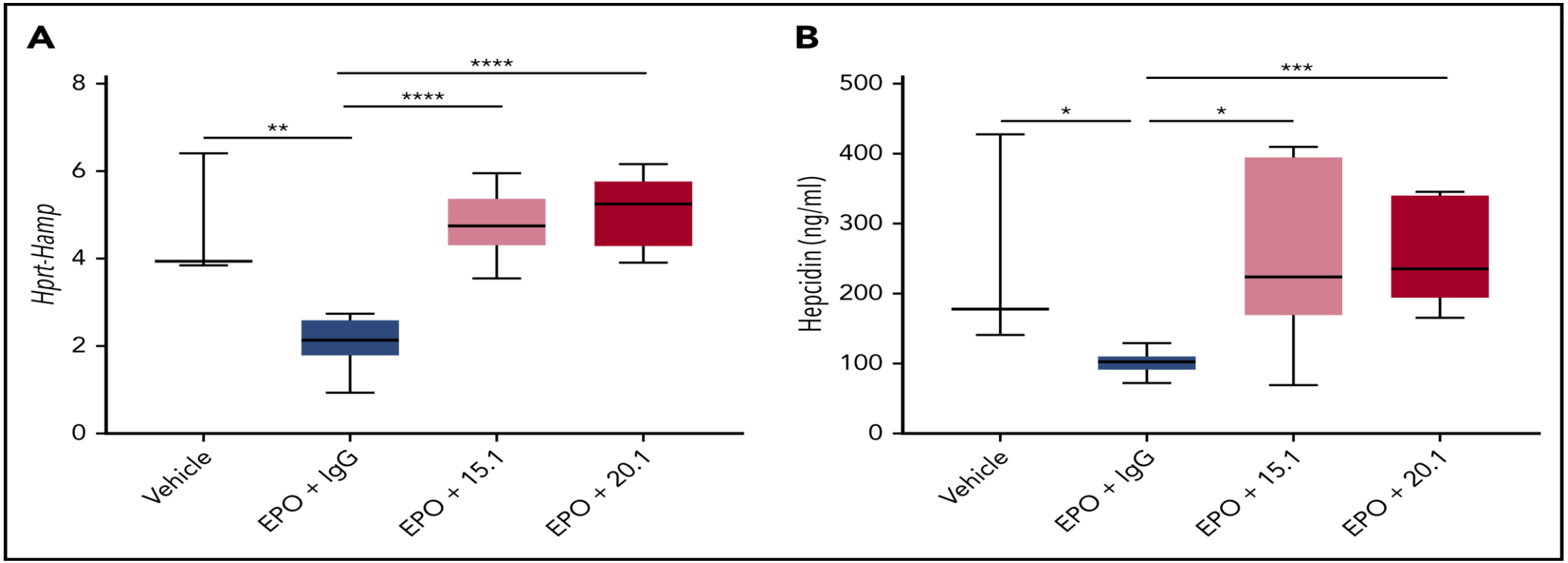
(C)Wild-type mice were treated intraperitoneally with 100 mg of F2 ERFE or saline and analyzed 3 hours after treatment for liver gene expression of 5 BMP target genes (Hamp1, Id1, Id2, Atoh8, Smad7) and Bmp2 and Bmp6

Neutralizing anti-ERFE antibodies bind to the N-terminal region of ERFE



(A) Anti-ERFE antibodies (15.1, 20.1, and 28.1) were assayed at different concentration in ELISA plates
(B) HTRF assay for detection of binding competition using cryptate-labeled anti-ERFE antibodies (20.1 and 28.1) and BMP6 (0.1-200 nM) and unlabeled antibody as positive control.
(C) Huh7 cells were treated for 24 hours with 200 ng/mL of murine ERFE alone or in combination with 10 mg/mL of anti-ERFE antibodies 15.1 and 20.1, and analyzed for HAMP gene expression

Antibodies binding ERFE N-terminal domain block hepcidin suppression in EPO-treated mice

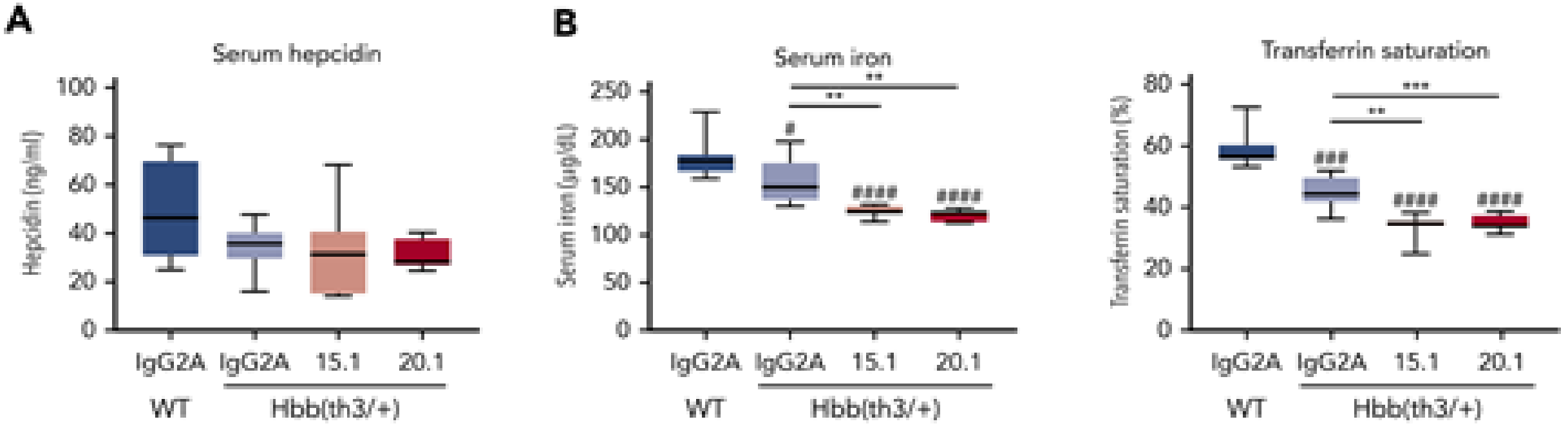


Eight-week-old wild-type malemice were treated intraperitoneally with 200 IU of EPO in combination with intravenous injection of an IgG2A antibody control, anti-ERFE 15.1, or anti-ERFE 20.1
analyzed 18 hours after treatment for assessment of Hamp expression

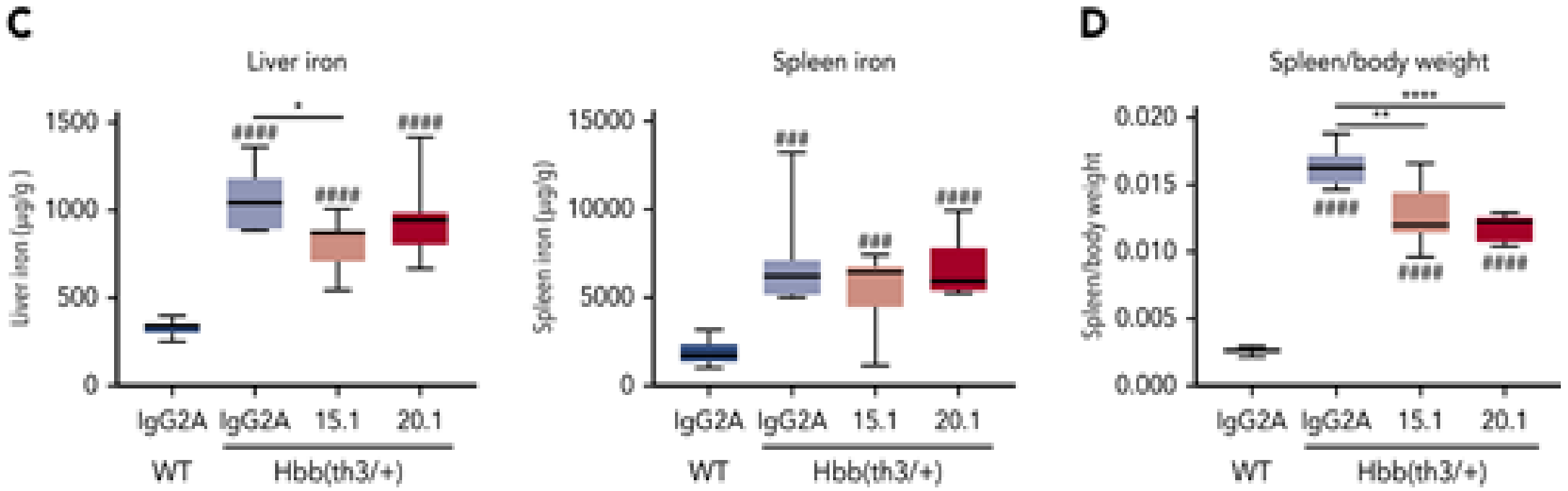
Anti-ERFE antibodies decrease iron levels and alter blood parameters in thalassemic mice

- Four-week-old male Hbb(th3/1)mice were treated intravenously with 5 mg/kg of IgG2A control antibody, anti-ERFE 15.1, or anti-ERFE 20.1, twice a week for 4 weeks
- IgG2A-treated wild-type (WT) mice were used as control for basal levels. After 4 weeks of treatment
- analysis for differences between IgG2A-treated Hbb(th3/1)mice and anti–ERFE-treated mice.
- differences between WT mice and Hbb(th3/1) mice

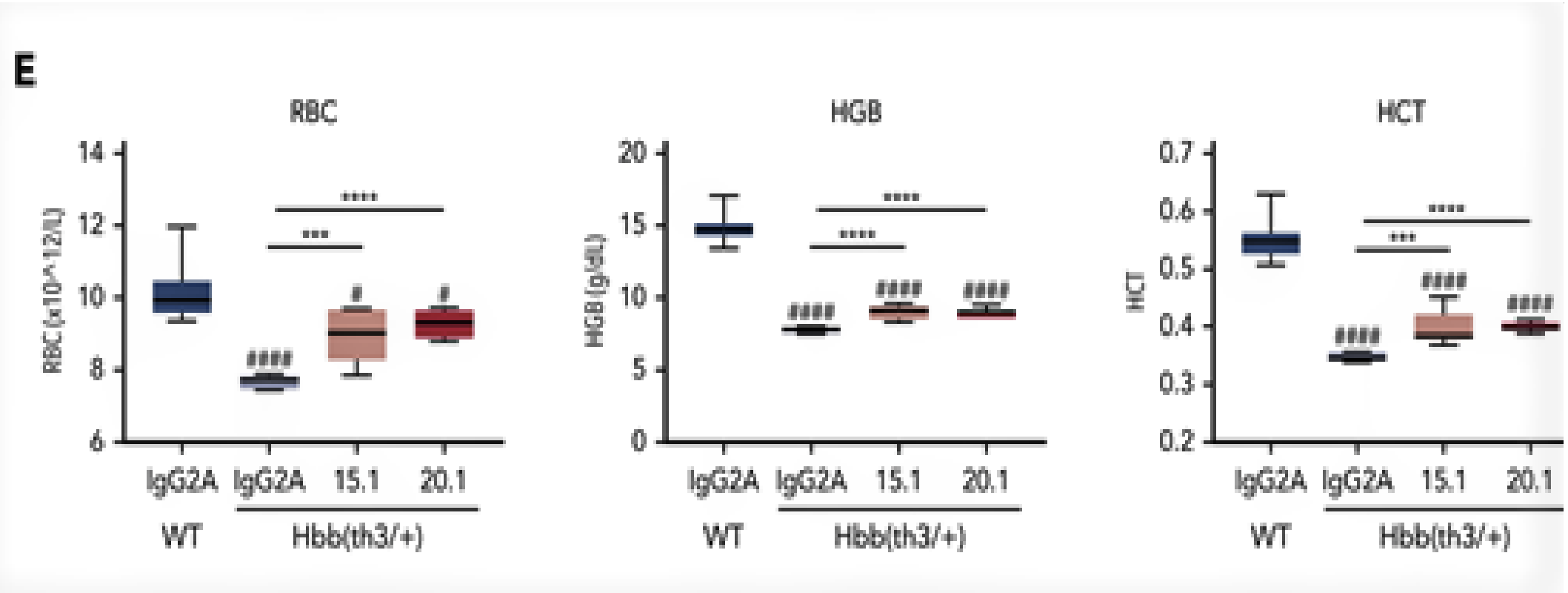
Anti-ERFE antibodies decrease iron levels and alter blood parameters in thalassemic mice



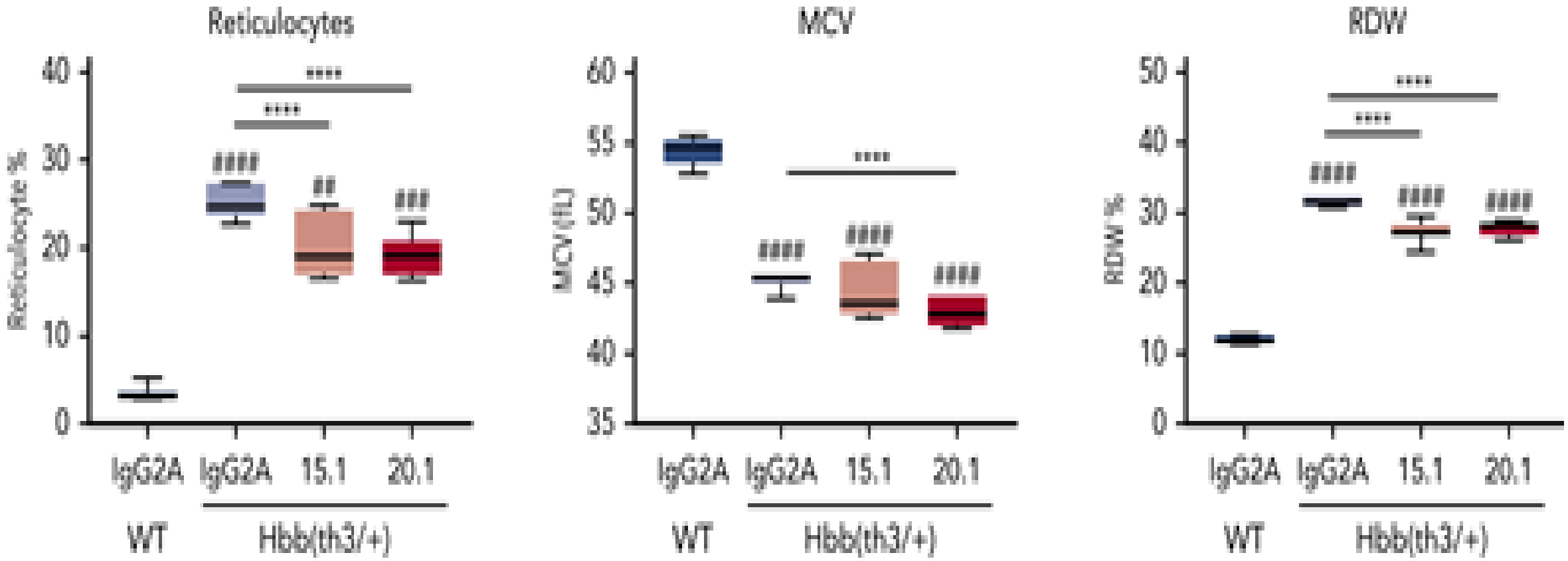
Anti-ERFE antibodies decrease iron levels and alter blood parameters in thalassemic mice



Anti-ERFE antibodies decrease iron levels and alter blood parameters in thalassemic mice



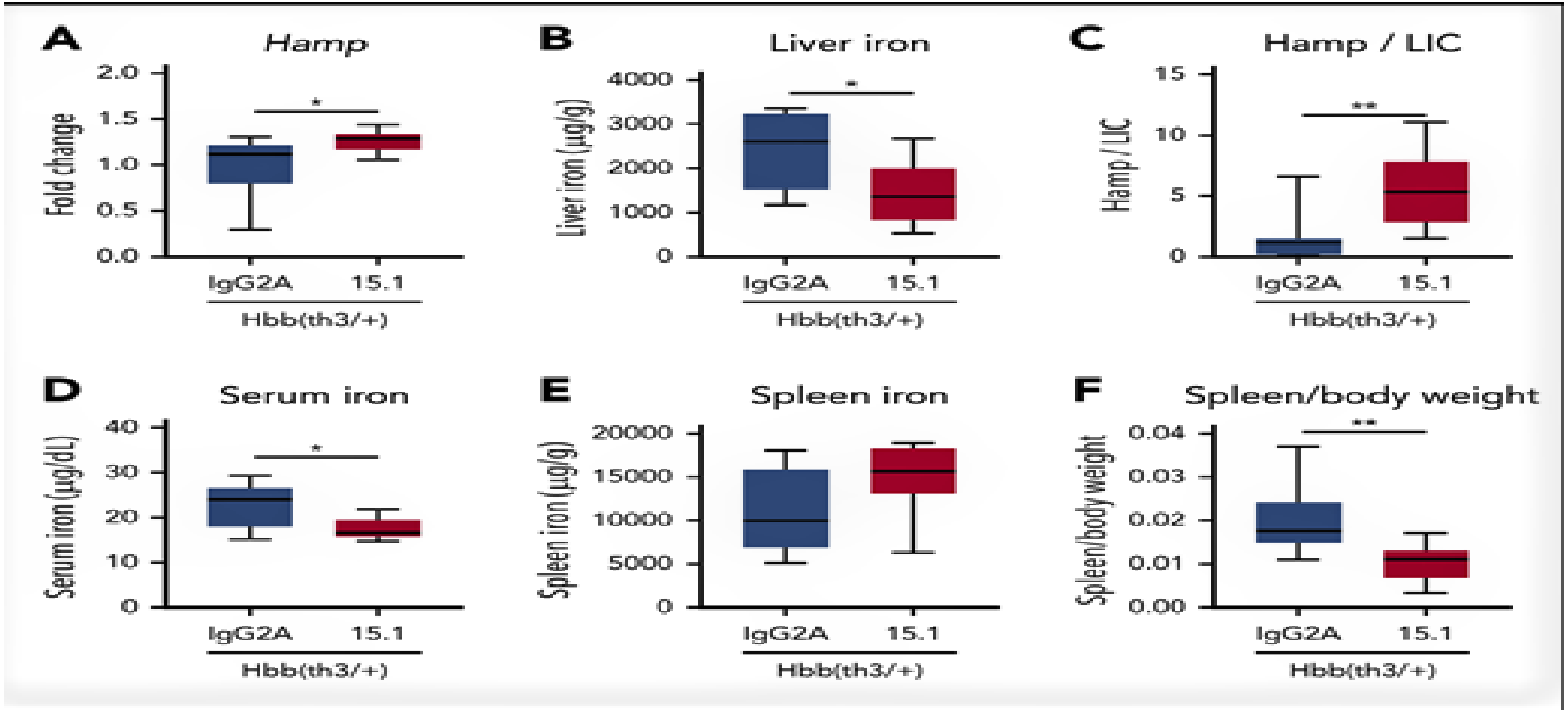
Anti-ERFE antibodies decrease iron levels and alter blood parameters in thalassemic mice



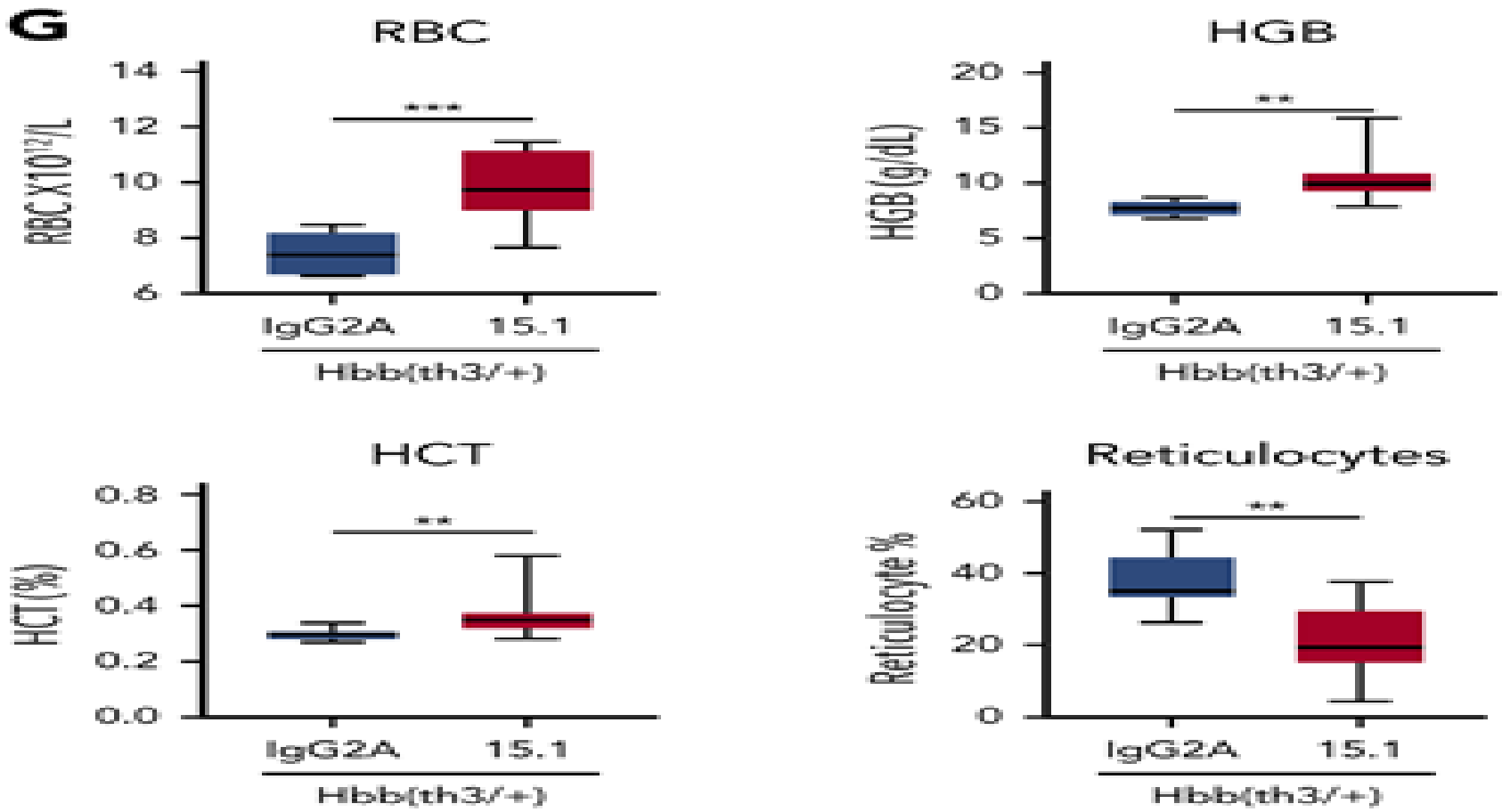
Antibodies targeting the N-terminal domain of ERFE increase hepcidin expression and ameliorate anemia in thalassemic mice

- To further assess the effects of an anti-ERFE antibody, we increased the dosing period to 8 weeks
- Four-week-old male Hbb(th3/1) mice were treated intravenously with 5 mg/kg of IgG2A control antibody, or anti-ERFE 15.1 twice a week for 8 weeks
- After 8 weeks of treatment mice were killed for analysis

Antibodies targeting the N-terminal domain of ERFE increase hepcidin expression and ameliorate anemia in thalassemic mice



Antibodies targeting the N-terminal domain of ERFE increase hepcidin expression and ameliorate anemia in thalassemic mice



conclusion

- an erythroid regulator that increases iron availability in response to erythropoietic

- by identifying the active region of ERFE and the binding affinity to different BMPs, we increased our understanding of ERFE mechanism of action. We describe the development of ERFE N terminus–targeted antibodies that neutralize ERFE-mediated hepcidin suppression and ameliorate the iron-loaded phenotype in a mouse model of bthalassemia, indicating their potential therapeutic utility to treat this disease.

سپاس از توجه شما