Supplementary Methods

Hepcidin standards and research subject serum samples
All hepcidin controls were used as previously described\(^1\). Control donors were non-medicated, healthy on the day of sampling and showed no evidence of anemia during routine monitoring (Hb values of 12.5 g/dL or above: Amgen Occupational Health; consent for research use obtained at time of collection). CKD patient samples were selected based on an estimated GFR (glomerular filtration rate) < 30, not currently on dialysis and dialysis not expected for at least 1 year (supplied by Lake Arrowhead Laboratory Consultants, Lake Arrowhead, CA, USA).

Animal care and housing
All mice were cared for in accordance to the *Guide for the Care and Use of Laboratory Animals, 8\(^{th}\) Edition*. Animals were group-housed at an AAALAC, Intl-accredited facility. All research protocols were approved by the Institutional Animal Care and Use Committee. Animals had *ad libitum* access to pelleted feed and reverse osmosis-purified water. Animals were maintained on a 12:12 hr light: dark cycle and had access to enrichment opportunities. All animals were determined specific pathogen-free.
Hybridoma screening

384 well ELISA plates were prepared by coating 4 µg/ml neutravidin (Thermo Scientific) onto 96 well Costar medium binding ELISA plates (Corning, Lowell, MA) overnight at 4°C. 50 ng/ml biotinylated rhHepc was then loaded onto the neutravidin plates and incubated for 1 hour at room temperature. To differentiate the antibodies by relative binding affinity the panels of antibodies were compared in equilibrium based ELISA binding screens using decreasing amounts of biotinylated hepcidin (4 to 0.16 ng/ml). To establish that antibodies bound to hepcidin in solution, a bridging-ELISA was carried out. An 8:1 molar ratio (antigen excess) of biotin rhHepc:neutravidin-HRP (Thermo Scientific) suspended in PBS tween buffer (PBS +1% BSA + 0.05% tween 20) with 10 mg/ml human gamma globulin (Sigma-Aldrich) was incubated with serially diluted anti-rhHepc antisera overnight at 4 °C in non-binding 384 well plates to form a complex. High binding black 384 well ELISA plates were coated with fibrinogen-streptavidin suspended in 50 mM Tris buffer, pH 8.2 overnight at 4°C. The plates were washed with PBS +1% BSA +0.05% tween 20 and complexes were added. Plates were washed with PBS +0.05% tween20 prior to addition of 20 µl of Femto luminescent substrate (Thermo Scientific). Luminescent RLU were recorded from an M5 plate reader (Molecular Devices).

Polyclonal anti-hepcidin sandwich ELISA

Protein A purified IgG from anti-human hepcidin rabbit antisera (either 4364 described previously\(^2\) or 4366) were coated onto a microtiter plate at a
concentration of 10 µg/ml, incubated with recombinant human hepcidin (rhHepc)³
and sandwiched with biotinylated IgG from the same antiserum used for coating
the plate (10 µg/ml). Detection of biotinylated antibody was carried out with
Streptavidin-conjugated HRP (1 µg/ml).

**Human Hepcidin Detection by ELISA**

Plates were coated with capture antibody 19D12 at 2 µg/ml in PBS and
incubated overnight at 4°C followed by washing and blocking with I-Block™
buffer (Applied Biosystems, Foster City, CA) for 1 hour at room temperature.
Samples were diluted in rabbit serum as needed. Standards, quality control
samples and experimental samples were diluted 1 in 10 dilution in I-block plus
5% BSA and added to the plate for 1.5 hours. After washing, plate was
incubated with detection antibody 23F11-biotin (200 ng/ml) for 1.5 hours. The
plate was subsequently washed and incubated with a 200 ng/ml of streptavidin-
HRP conjugate (Thermo Fisher Scientific, Rockford, IL) for 15 minutes. After
washing the plate, chemiluminescent Femto substrate (Thermo Fisher Scientific)
was added and the signal was read by a SpectraMax 384Plus Luminometer. The
hepcidin assay dynamic range was 0.03-100 ng/ml.
Detection of anti-hepcidin monoclonal antibody sandwiching by Biacore
(detailed method)

Carboxyl groups on the sensor chip surfaces were activated by injecting 60 µl of 0.2 M N-ethyl-N’-(dimethylaminopropyl)carbodiimide (EDC) and 0.05 M N-hydroxysuccinimide (NHS). Specific surfaces were obtained by injecting human anti-human hepcidin antibody 19D12 diluted in 10mM acetate, pH 4.0 at 30 µg/ml onto an EDC and NHS activated sensor chip. Excess reactive groups on the surfaces were deactivated by injecting 60 µl of 1 M ethanolamine. Final immobilized levels were 5,000-6,000 resonance units (RU) for the 19D12 surface. A blank, mock-coupled reference surface was also prepared on the sensor chip. 20nM rhHepc was injected over and bound to the 19D12 surface. Antibodies 23F11, 12B9m, 15E1, 19D12 and 2.7 were tested for sandwiching in turn by injection over the hepcidin/19D12 surface. The surface was regenerated between each antibody by injecting 30 µL 10mM HCl pH2. Addition of mass in relative units (RU) was used to detect binding.