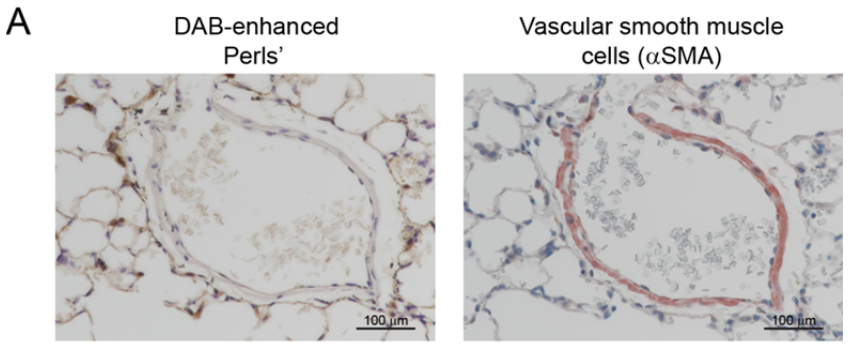


Figure S1

**Figure S1 – Pulmonary vascular smooth muscle cell layers in Slc40a1$^{C326S/C326S}$ mice.** A) DAB-enhanced Perls' staining (left) and immunohistochemistry for alpha smooth muscle actin (αSMA) (right) of consecutive lung sections from Slc40a1$^{C326S/C326S}$ female 36-week old mice.

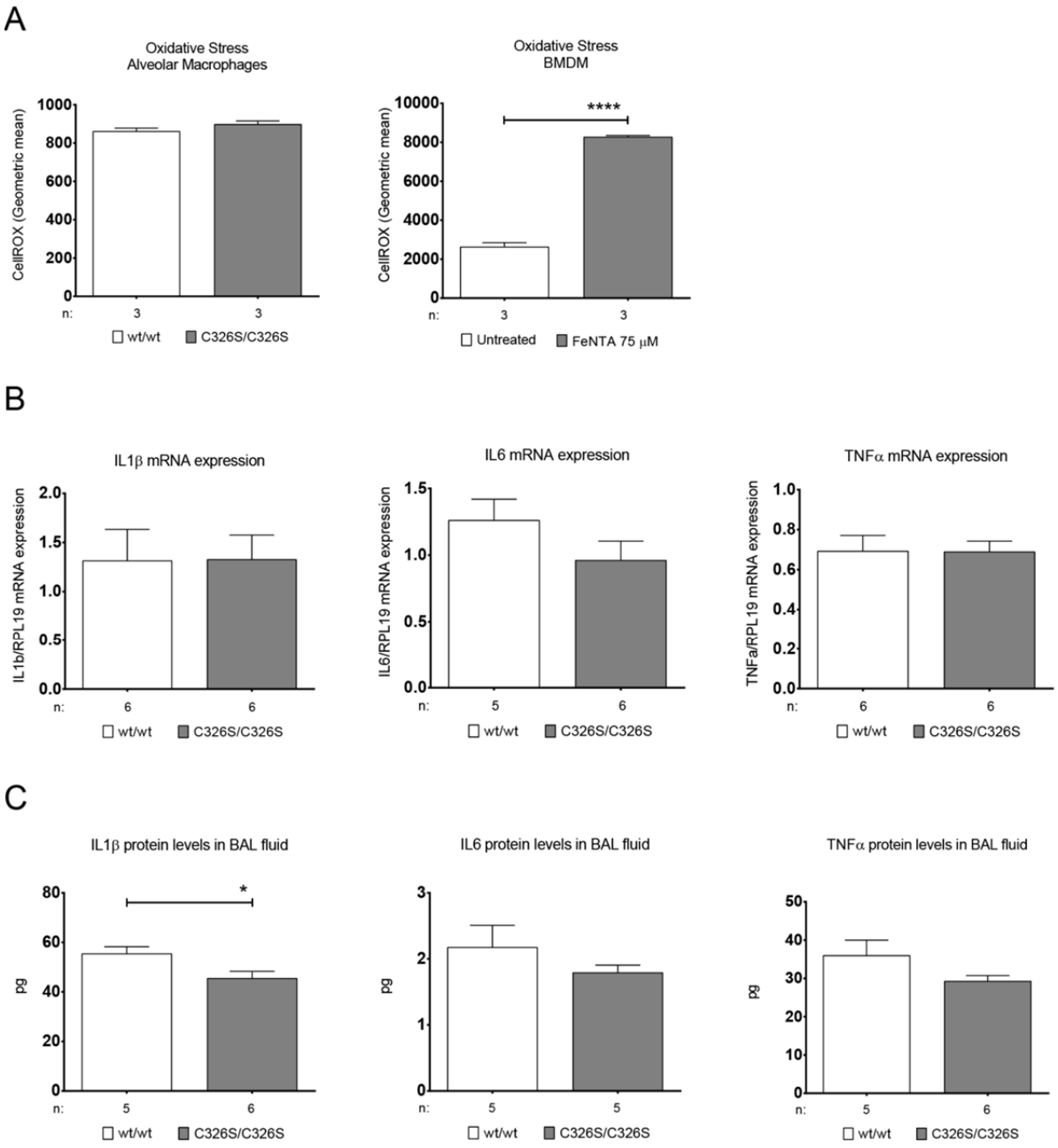


Figure S2

**Figure S2 – Slc40a1$^{C326S}$ mice do not show signs of pulmonary inflammation.** A) FACS analysis of the CellROX oxidative stress tracer of iron treated BMDM or AM isolated from the BAL of 24-week old male mice. B) qRT-PCR analysis of IL1β, IL6 and TNFα mRNA expression in total lung from female 36-week old mice. C) Luminex analysis of IL1β, IL6 and TNFα protein levels in the BAL fluid supernatant of female 36-week old mice. Data are reported as mean ± SEM. Student's t test: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

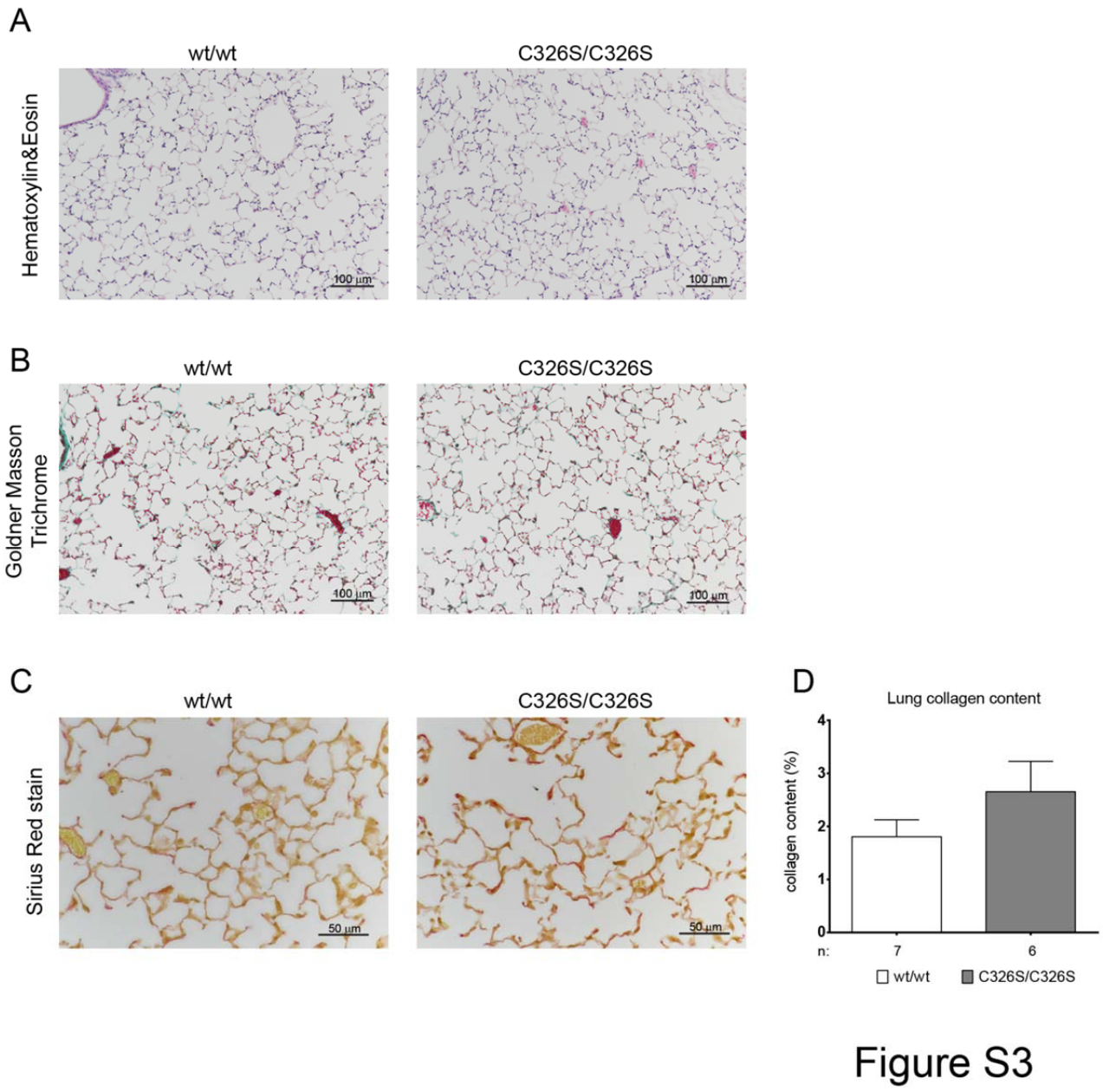


Figure S3

**Figure S3 – The lung structure in Slc40a1$^{C326S/C326S}$ mice is unaltered compared to wild-type mice.** A) Hematoxylin & Eosin stain of lung sections from 36-week old mice. B) Goldner Masson Trichrome stain of lung sections from 36-week old mice. C) Sirius Red stain of lung sections from 36-week old mice. D) Collagen content calculated on Sirius Red stained lung sections from 34-week old mice.

A

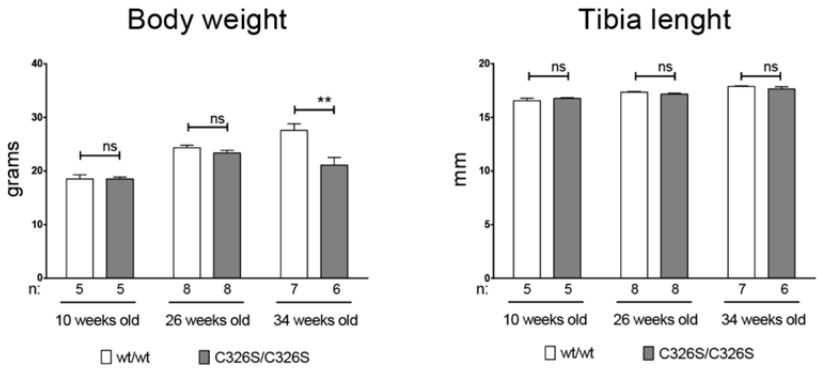


Figure S4

**Figure S4 – Reduced body weight but similar tibia length in 34-week old Slc40a1$^{C326S/C326S}$ mice compared to wild-type mice.** A) Body weight and tibia length of 10-, 26- and 34-week old mice.

A

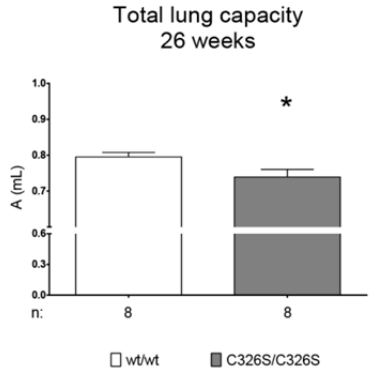


Figure S5

**Figure S5 –** (A) Total lung capacity measured in 26-week old female mice.

Table S1 – Hematological parameters in 36-week old mice

| Parameter | wt/wt | n | C326S/C326S | n | p-value |
|---|---|---|---|---|---|
| Hemoglobin (g/dl) | 16,36 ± 0,06 | 4 | 17,66 ± 0,41 | 4 | * 0,0199 |
| Hematocrit (%) | 53,22 ± 0,53 | 4 | 56,73 ± 1,12 | 4 | * 0,0296 |
| Red blood cells ($10^6$/µL) | 10,60 ± 0,09 | 4 | 11,47 ± 0,05 | 4 | *** 0,0002 |

Data are reported as mean ± SEM

SYBR green qPCR primers

| | Primer Forward | Primer Reverse |
|---|---|---|
| TfR1 | CCCATGACGTTGAATTGAACCT | GTAGTCTCCACGAGCGGAATA |
| DMT1-IRE | AGCTAGGGCATGTGGCACTCT | ATGTTGCCACCGCTGGTATC |
| FPN | TGTCAGCCTGCTGTTTGCAGGA | TCTTGCAGCAACTGTGTCACCG |
| HO1 | AGGCTAAGACCGCCTTCCT | TGTGTTCCTCTGTCAGCATCA |
| RPL19 | AGGCATATGGGCATAGGGAAGAG | TTGACCTTCAGGTACAGGCTGTG |
| TNFα | TGCCTATGTCTCAGCCTCTTC | GAGGCCATTTGGGAACTTCT |
| IL1β | GCAACTGTTCCTGAACTCAACT | ATCTTTTGGGGTCCGTCAACT |
| IL6 | GCTACCAAACTGGATATAATCAGGA | CCAGGTAGCTATGGTACTCCAGAA |

Antibodies used for Western Blotting (WB),immunohistochemistry (IHC) and immunocytochemistry (ICC)

| Antigen | Host | WB dilution | IHC dilution | ICC dilution | Reference/Supplier |
|---|---|---|---|---|---|
| FPN | rabbit | 1:500 | / | 1:200 | MTP11-A / Alphadiagnostics |
| DMT1 | rabbit | / | 1:100 | / | (Galy et al., 2008) |
| FtL | goat | 1:500 | / | / | sc-14420 / SCBT |
| TfR1 | mouse | 1:1000 | / | / | 136800 / Invitrogen |
| β-actin | mouse | 1:5000 | / | / | A1978 / Sigma-Aldrich |
| β-tubulin IV | mouse | / | 1:500 | / | MU178-UC / Biogenex |
| proSP-C | rabbit | / | 1:5000 | / | ab28744-50 / Abcam |
| αSMA | rabbit | / | 1:300 | / | ab5694 / Abcam |

Antibodies used for Flow cytometry

| Antigen | Dilution | Reference/Supplier |
|---|---|---|
| CD45.2 | 1/200 | 109820 / Biolegend |
| CD11c | 1/200 | 550261 / BD Pharmingen |
| SiglecF | 1/200 | 552126 / BD Pharmingen |

## Supplementary references:

Galy, B., Ferring-Appel, D., Kaden, S., Grone, H.J., and Hentze, M.W. (2008). Iron regulatory proteins are essential for intestinal function and control key iron absorption molecules in the duodenum. Cell Metab *7*, 79-85.