SUPPLEMENTARY MATERIAL

Methods

Prior to iron dilution preparations, a stock solution of 10mM $FeSO_4$ and 1mM sodium ascorbate was made in 50ml distilled water by adding 76mg of $FeSO_4$ (Sigma-Aldrich, USA) and 9mg of sodium ascorbate (Sigma-Aldrich, USA). The sodium ascorbate is a vital component of the mixture as this prevents oxidation of iron in solution keeping it in its ferrous (Fe^{2+}) state. A further stock solution of 10mM $FeSO_4$ was prepared without the addition of sodium ascorbate in order to allow oxidation of iron in solution to its oxidised ferric (Fe^{3+}) state. Both of these stock solutions were then filter sterilised and stored at 4^oC. A fresh batch of stock solutions was prepared prior to each set of experiments. All experiments utilising Fe^{3+} were run using the same concentration of iron as the experiments using Fe^{2+}. Methods for PCR, proliferation and other assays were as described in the main manuscript. All Fe^{3+} experiments were run in triplicate on 3 separate occasions, using A549 and QG56 cells; since none of the functional assays were affected by Fe^{2+} in PBECs we restricted the assessment of Fe^{3+} to the cancer lines, where an effect on proliferation had been seen.

Results

The oxidized Fe^{3+} solution resulted in a small rise in ferrozine ($p=0.06$ both lines) and expression of ferritin on PCR in QG56 cells (fold change 1.2) albeit not reaching statistical significance. No change in gene expression was seen for *IREB2* or *TFRC* (all $p>0.05$). On Western blotting a rise in ferritin was seen in both cell lines, similar to that observed with Fe^{2+}(Figure 1, main manuscript and supplementary figure 1), and significantly elevated above untreated cells ($p<0.01$). However, cells did not proliferate significantly in the presence of Fe^{3+} (supplementary figure 2), indeed there was a

reduction in proliferation seen. Apoptosis was elevated in the presence of Fe^{3+}, at a level similar to the incubations with Fe^{2+} (mean 22 and 26% cells positive; A549 and QG56 respectively), albeit not significantly different from untreated cells. Necrosis occurred in 5% of A549 and QG56 cells; again this was no different from untreated cells ($p>0.8$). Migration in the scratch wound assay also showed no significant difference in either cell line ($p>0.4$).