Figure S-1. Immunofluorescent analysis of DMT1 expression for the cell lines by confocal microscopy. The 2/-IRE line (A-F) or the FLAG/1A/+IRE line (G-L) or the 1A/+IRE line (M-N) were grown in the absence (A-C, G-I and M) or presence (D-F, J-L and N) of doxycycline then reacted with primary antibody for -IRE DMT1 and secondary Alexa-568 anti-rabbit IgG (A, D) or primary antibody for +IRE DMT1 and secondary Alexa-568 anti-rabbit IgG (G, J, M, N) and primary antibody for FLAG® and secondary Alexa-488 anti-mouse IgG (B, E, H, K). Images were collected with settings appropriate for the secondary antibody; pseudo-color was added (A-L) so that the end result was in red (+/-IRE Ab in A, D, G, J), green (FLAG Ab in B, E, H, K) or yellow to orange, indicating colocalization (merge in C, F, I, L). This series of images represents multiple other confocal slices from the same cell preparations and the images resemble those from 6 repetitions of the results.

Figure S-2. Immunoblotting to visualize increased DMT1 expression. Lysates from the FLAG/2/-IRE line (A) and the FLAG/1A/+IRE line (B) were electrophoresed in SDS gels and stained with FLAG® antibody. In each panel, the comparison is between lysates from cells grown with and without doxycycline.

Figure S-3. Dependence on pH of divalent metal ion transport. $^{54}Mn^{2+}$ (A and B) and $^{59}Fe^{2+}$ (C and D) uptake were compared for (A and C) the 2/-IRE line or (B and D) the 1A/+IRE line in each case with (open bars) and without (solid bars) doxycycline. The data shown are representative of 6 separate experiments for Mn and 4, for Fe. Cells often exhibited signs of stress at pH < 5.5 so experiments where the doxycycline treated cells showed minimal indication of the problem were selected for the figure. There was still some toxicity at low pH even for the selected experiments with the 2/-IRE cells and no doxycyline.

Figure S-4. Temperature dependence of divalent metal ion transport. $^{54}Mn^{2+}$ (A and B) and $^{59}Fe^{2+}$ (C and D) uptake were compared for (A and C) the 2/-IRE line or (B and D) the 1A/+IRE line in each case with (open bars) and without (solid bars) doxycycline.

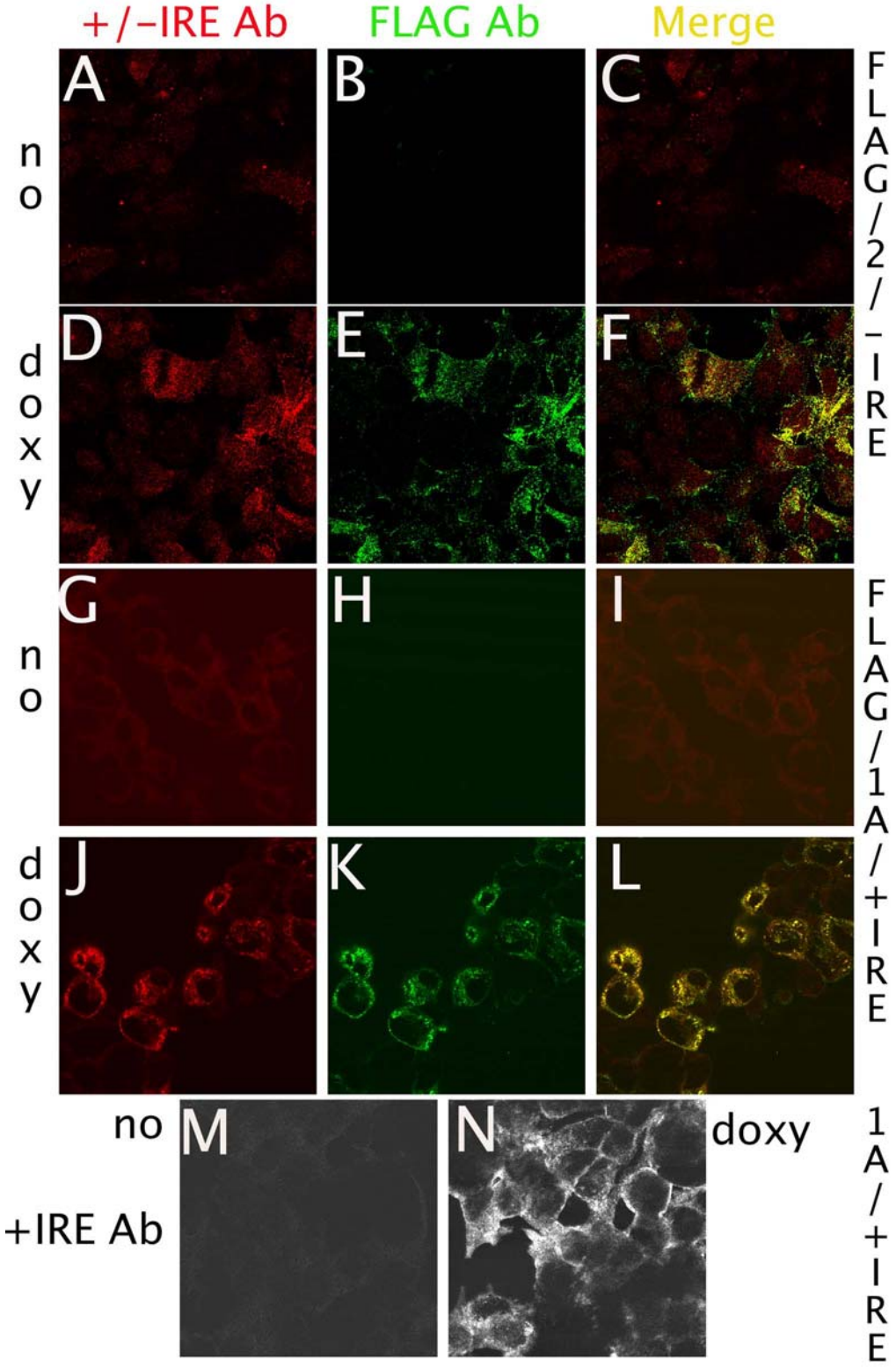

+/−IRE Ab
FLAG Ab
Merge
A
B
C
D
E
F
G
H
I
J
K
L
M
N
no
doxy
FLAG/2/−IRE
FLAG/1A/+IRE
+IRE Ab
1A/+IRE

Figure S-1.

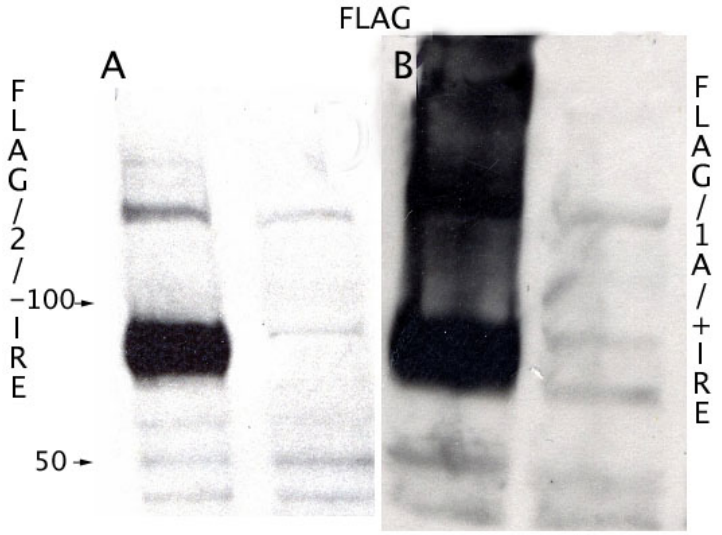


Figure S-2.

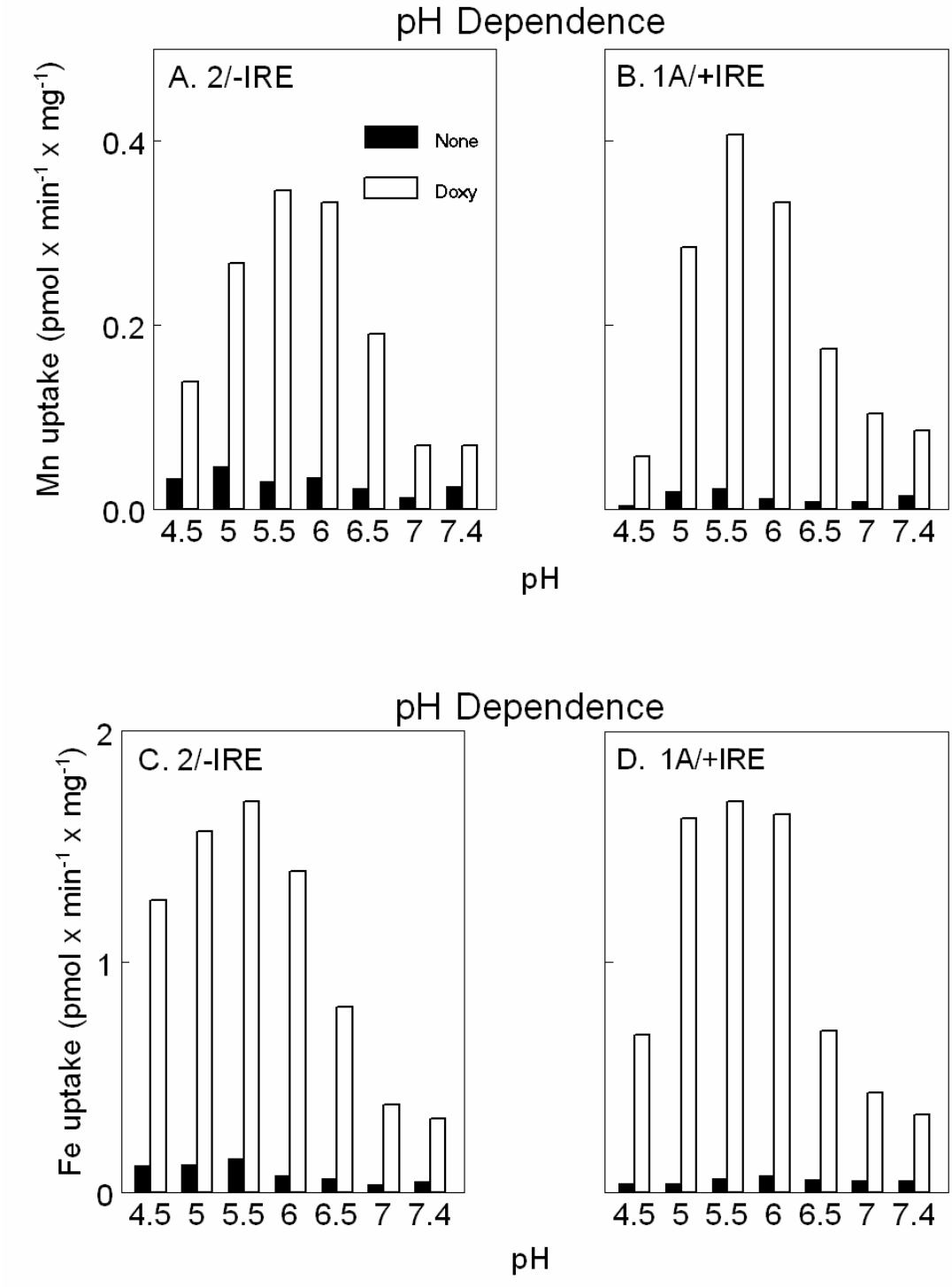
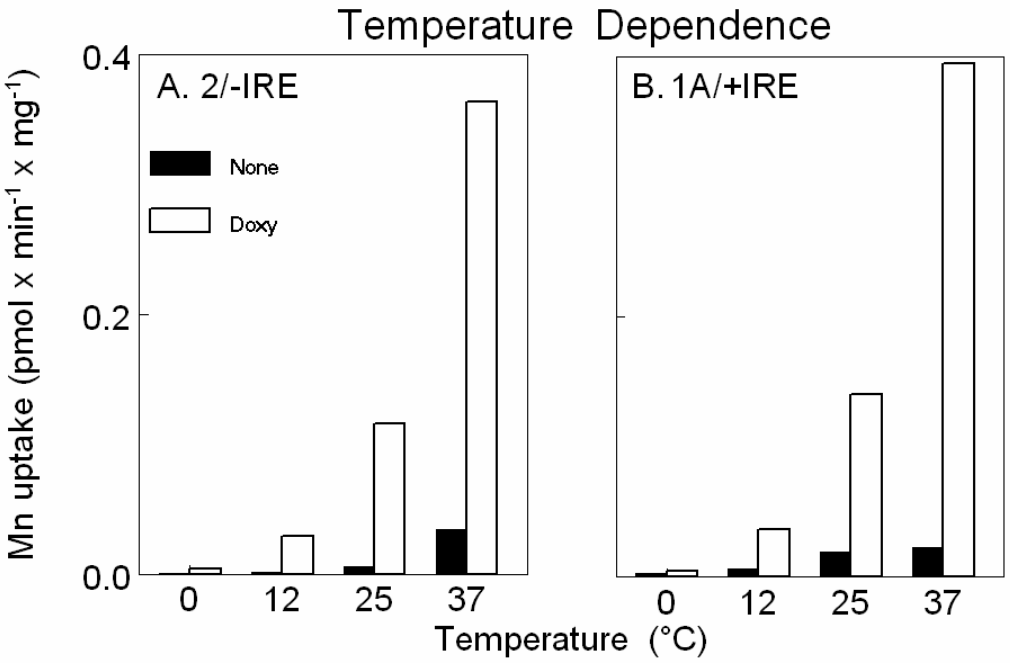

pH Dependence
A. 2/-IRE
B. 1A/+IRE
None
Doxy
Mn uptake (pmol x min-1 x mg-1)
0.0
0.2
0.4
4.5 5 5.5 6 6.5 7 7.4
pH
pH Dependence
C. 2/-IRE
D. 1A/+IRE
Fe uptake (pmol x min-1 x mg-1)
0
1
2
4.5 5 5.5 6 6.5 7 7.4
pH

Figure S-3.


Temperature Dependence
A. 2/-IRE
B. 1A/+IRE
None
Doxy
Mn uptake (pmol x min-1 x mg-1)
0.4
0.2
0.0
0
12
25
37
Temperature (°C)

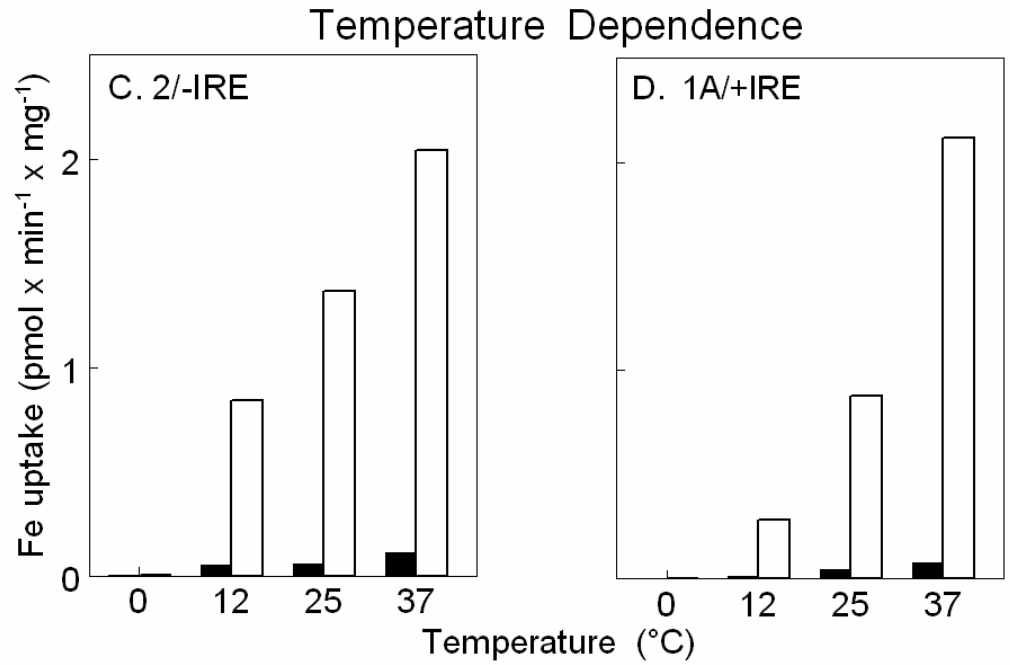

Temperature Dependence
C. 2/-IRE
D. 1A/+IRE
Fe uptake (pmol x min-1 x mg-1)
2
1
0
0
12
25
37
Temperature (°C)

Figure S-4.