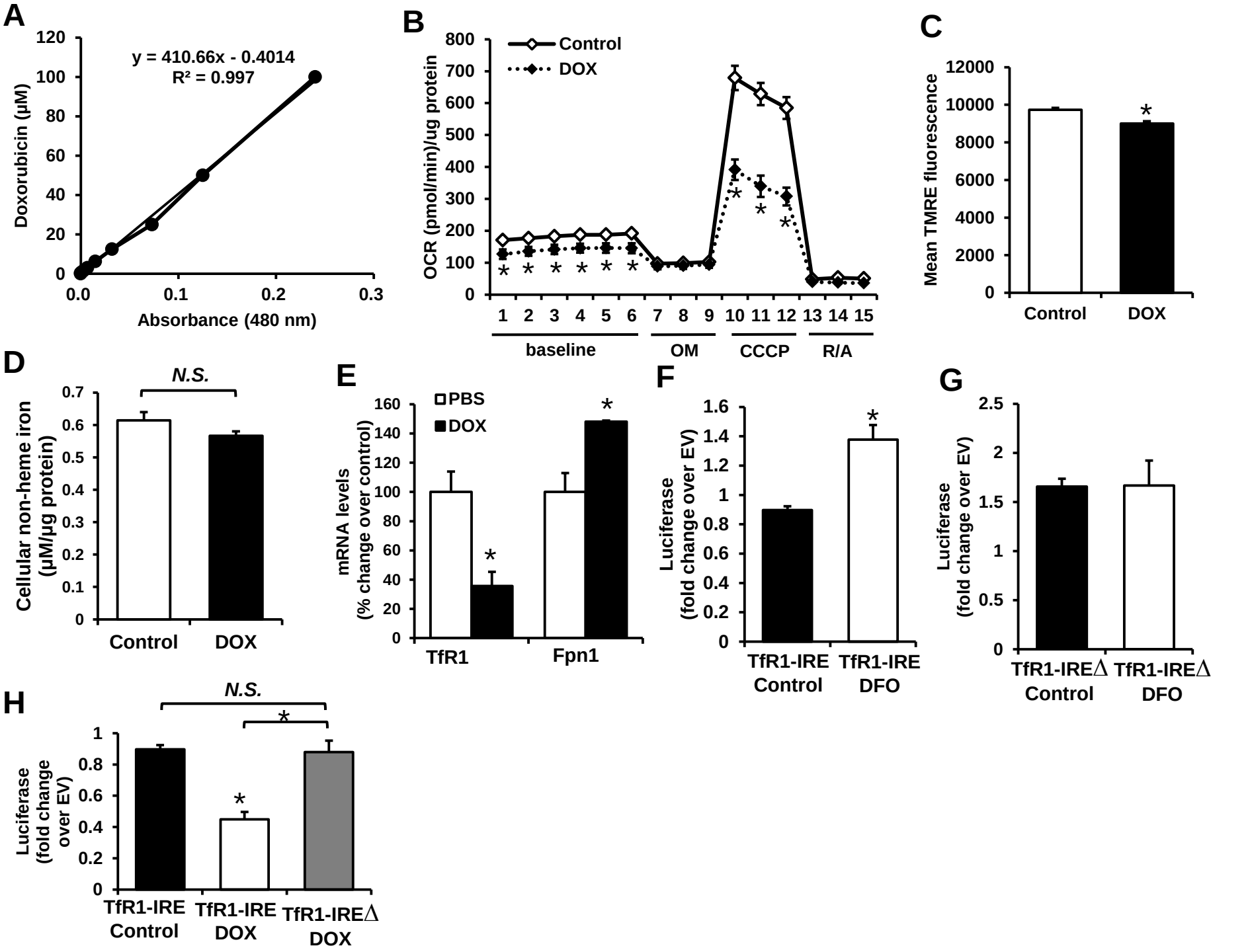
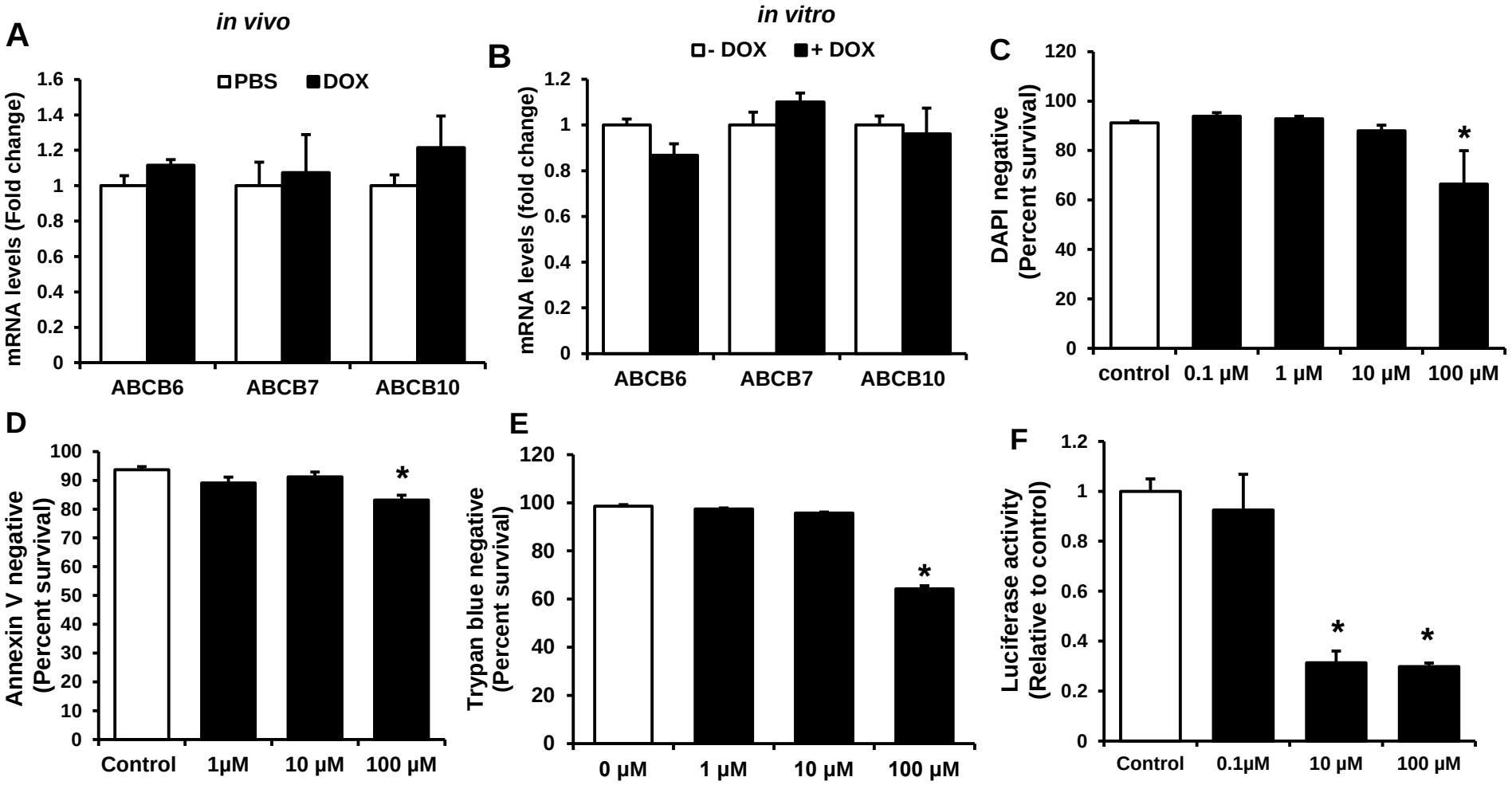

A
y = 410.66x - 0.4014
R² = 0.997
Doxorubicin (μM)
Absorbance (480 nm)
B
Control
DOX
OCR (pmol/min)/ug protein
baseline
OM
CCCP
R/A
C
Mean TMRE fluorescence
Control
DOX
D
N.S.
Cellular non-heme iron (μM/μg protein)
Control
DOX
E
PBS
DOX
mRNA levels (% change over control)
TfR1
Fpn1
F
Luciferase (fold change over EV)
TfR1-IRE Control
TfR1-IRE DFO
G
Luciferase (fold change over EV)
TfR1-IREΔ Control
TfR1-IREΔ DFO
H
N.S.
Luciferase (fold change over EV)
TfR1-IRE Control
TfR1-IRE DOX
TfR1-IREΔ DOX

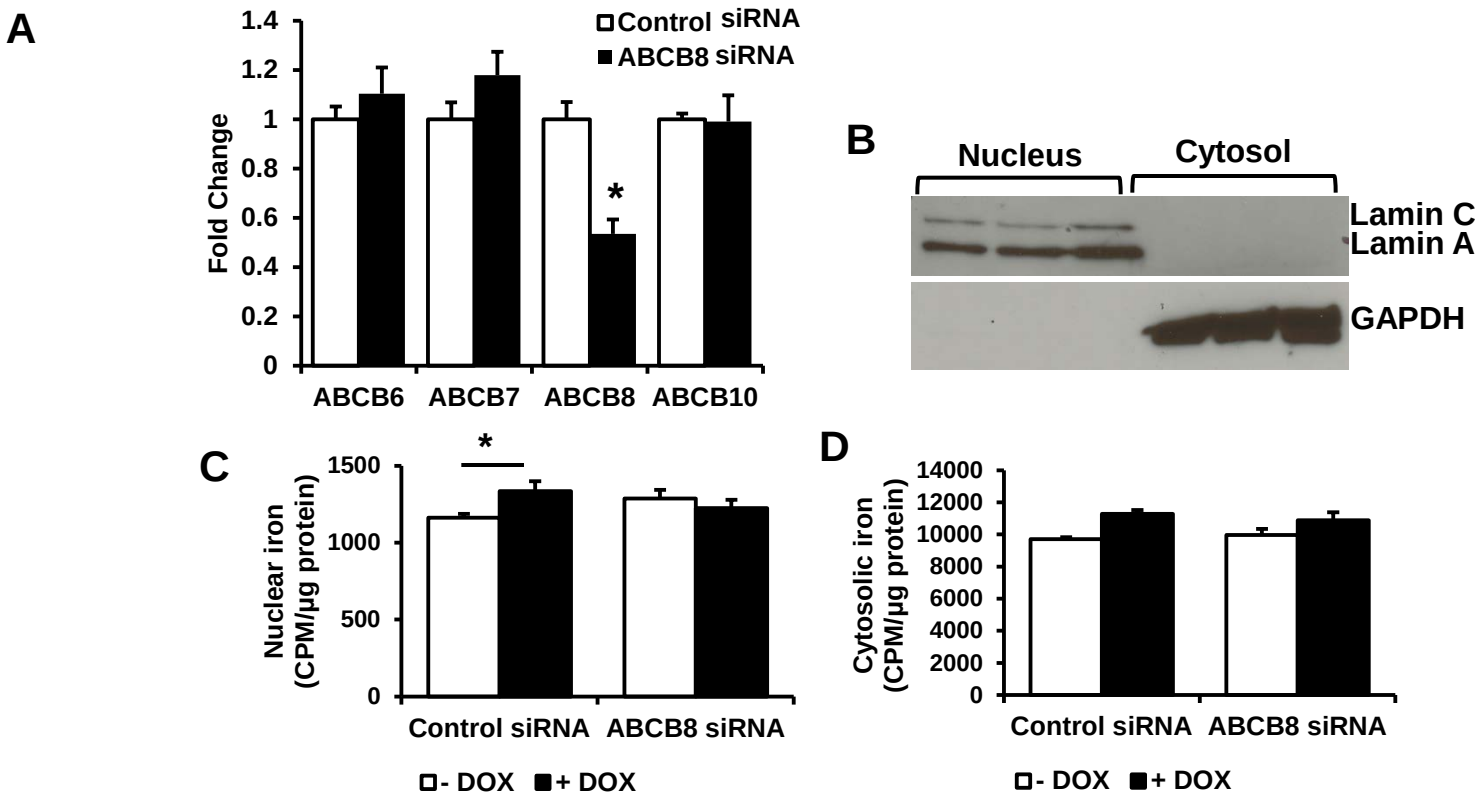
Supplemental Figure 1. **Doxorubicin regulates cellular iron homeostasis**

(**A**) Absorbance (480 nm) was measured in NRCM at increasing concentrations of DOX. (**B**) Analysis of mitochondrial oxygen consumption rate (OCR) in NRCM with DOX or control treatment quantified using Seahorse Bioanalyzer. Respiration rates are measured at baseline, in the presence of oligomycin (OM, inhibitor of ATP synthase), carbonyl cyanide m-chlorophenol (CCCP, dissipation of proton gradient), and rotenone/antimycin A (R/A, respiratory chain inhibitors) and normalized to protein concentration of each sample (n=12). (**C**) Mitochondrial membrane potential assessed by TMRE staining and flow cytometry in NRCM treated with 10μM DOX for 16 hours (n=3). (**D**) Total cellular non-heme iron levels in NRCM treated with 10μM DOX for 16 hours determined by Ferene S assay (n=3). (**E**) mRNA expression of cellular iron regulatory proteins, transferrin receptor 1 (TfR1) involved in iron import, and ferroportin 1 (Fpn1) involved in iron export, in NRCM treated with 10μM DOX for 16 hours (n=6). (**F-H**) Luciferase assay of regulation of either full-length 3'UTR of TfR1 (TfR1-IRE) or 3'UTR of TfR1 with deletion of all five IREs (TfR1-IRE∆) by 200 μM DFO (**F,G**) and 10μM DOX (**H**) in HEK293 cells treated for 16 hours and normalized to renilla luciferase expression and empty vector (EV) expression (n=12). Data are presented as mean±SEM. *$P < 0.05$.

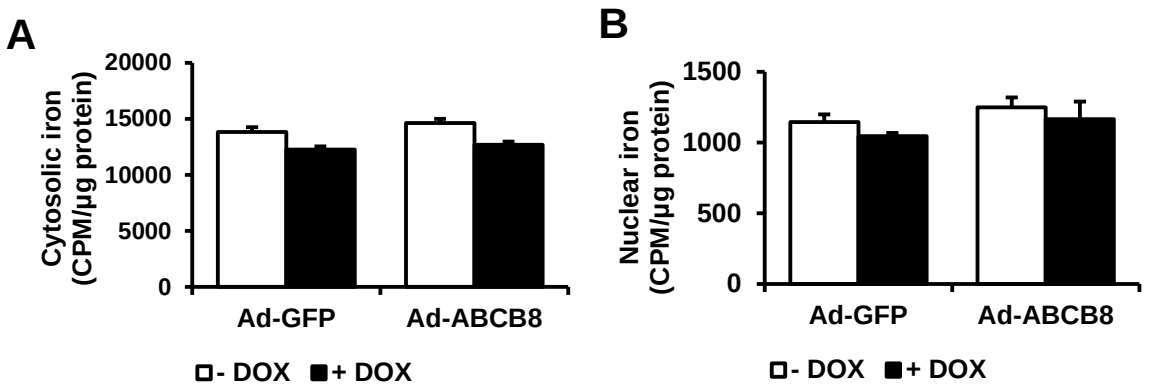


Supplemental Figure 2. **Doxorubicin does not regulate other mitochondrial ABC proteins and DOX regulation of ABCB8 is not through its effects on cell death**

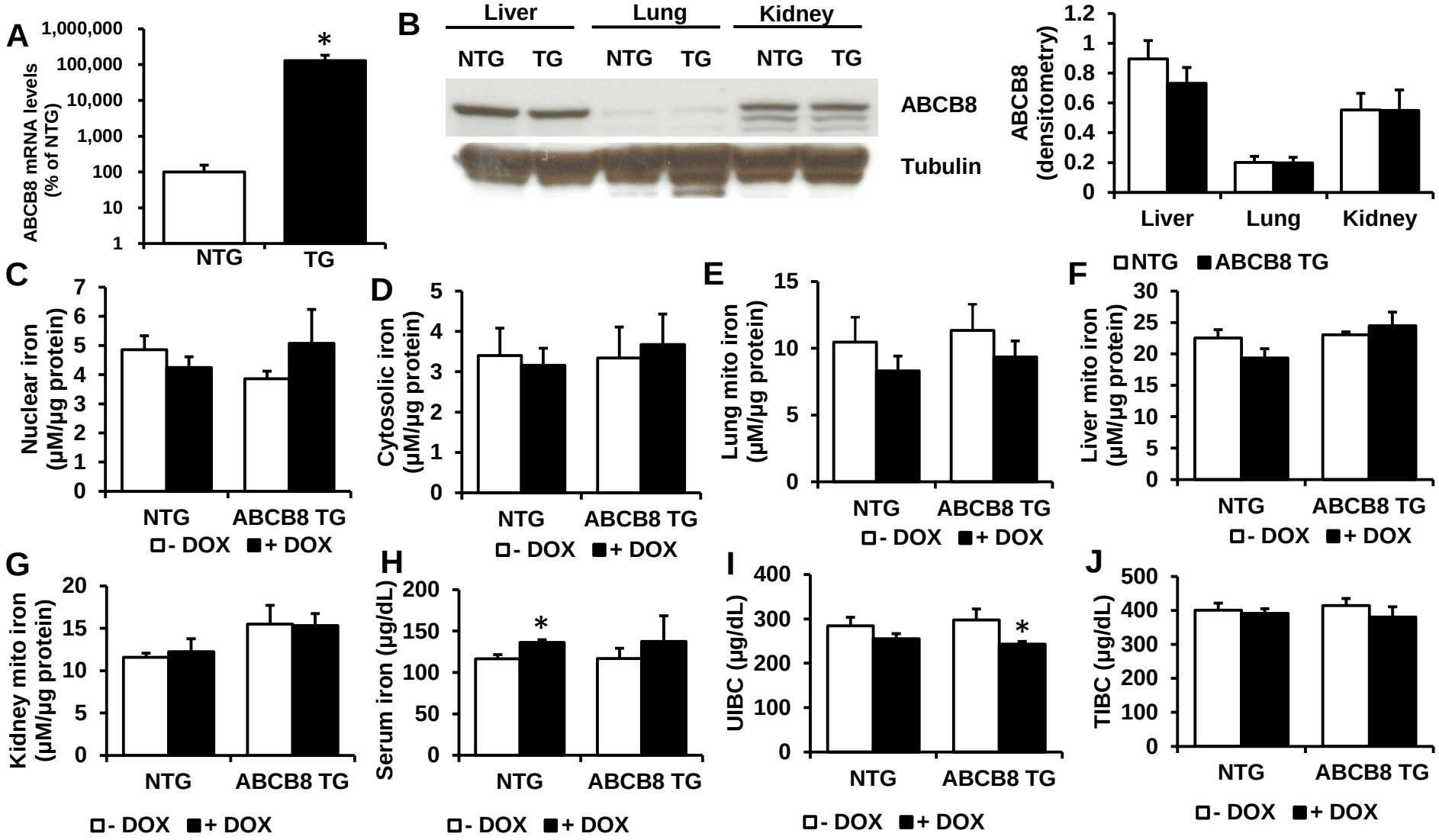
(**A**) mRNA levels of three other mitochondrial ABC transporter genes were measured in mouse heart that had been treated with 6 mg/kg per day injection every third day for a total of four injections (Protocol 2) or saline (PBS) (n=4). Measurements were performed via qRT-PCR, normalized to 18S rRNA expression, and reported as a percentage of measurements obtained in the absence of DOX treatment. (**B**) mRNA levels of mitochondrial ABC proteins in NRCM treated with 10 μM DOX or control vehicle (n=6). (**C-E**) Cell death in HEK293 cells in response to various doses of DOX, as assessed by DAPI staining (n=6) (**C**), Annexin V staining (n=6) (**D**), and Trypan blue staining (n=3) (**E**). (**F**) Luciferase assay of the effects of DOX on the expression of luciferase construct driven by 6 kb of ABCB8 promoter (n=6). DOX at doses that did not affect cell death (i.e., 10 μM) still caused a reduction in the levels of ABCB8. Data are presented as mean±SEM. *$P < 0.05$.



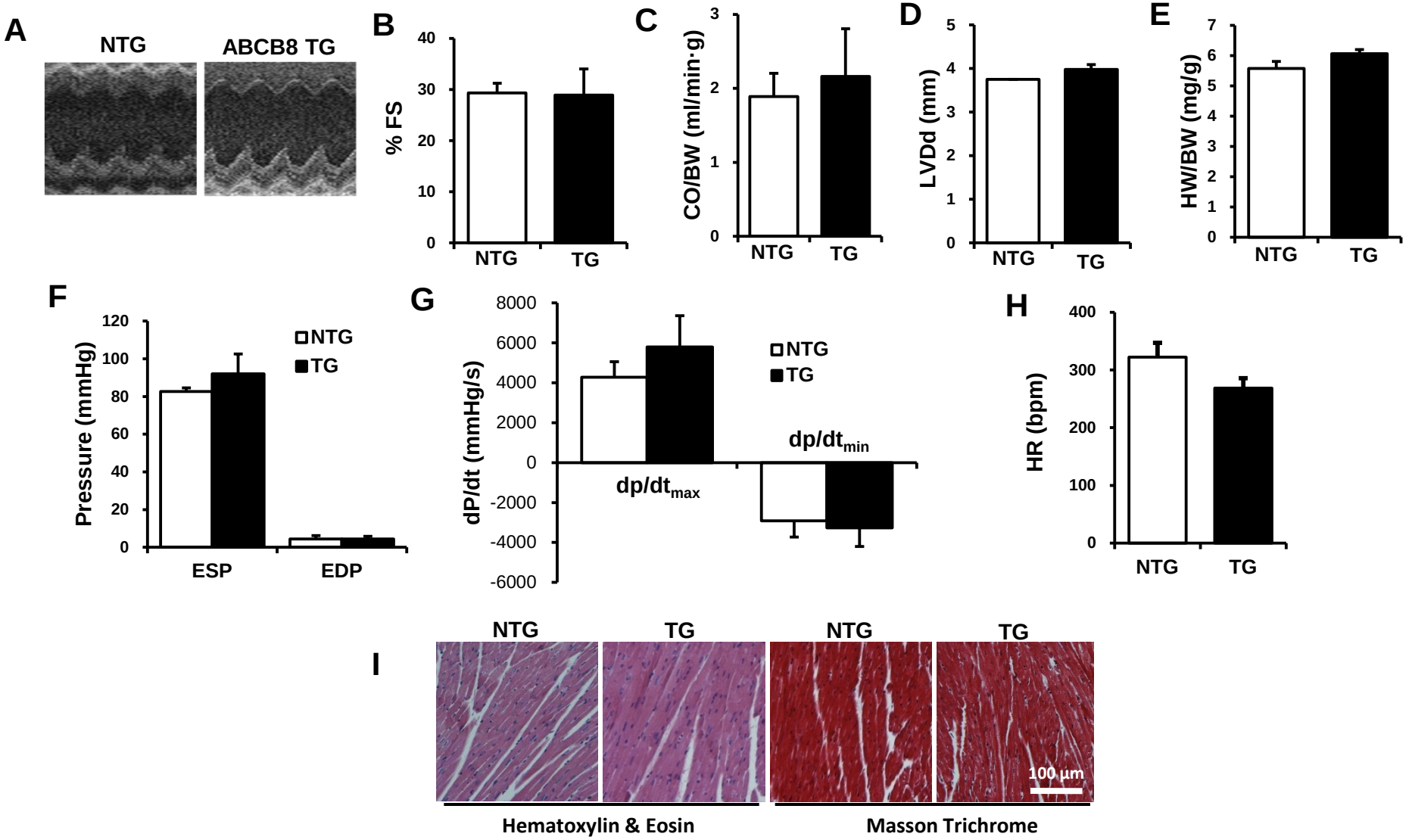
Supplemental Figure 3. **Nuclear and cytosolic iron levels with ABCB8 siRNA with and without DOX in NRCM.**
(A) Levels of other mitochondrial ABC protein in NRCM with and without ABCB8 downregulation. (**B**) Western blot analysis of nuclear and cytosolic fraction purity in NRCM (n=3). (**C, d**) Nuclear (**C**) and cytosolic (**D)** iron with control and ABCB8 siRNA in the presence and absence of DOX (n=3). Data are presented as mean±SEM. *$P < 0.05$.



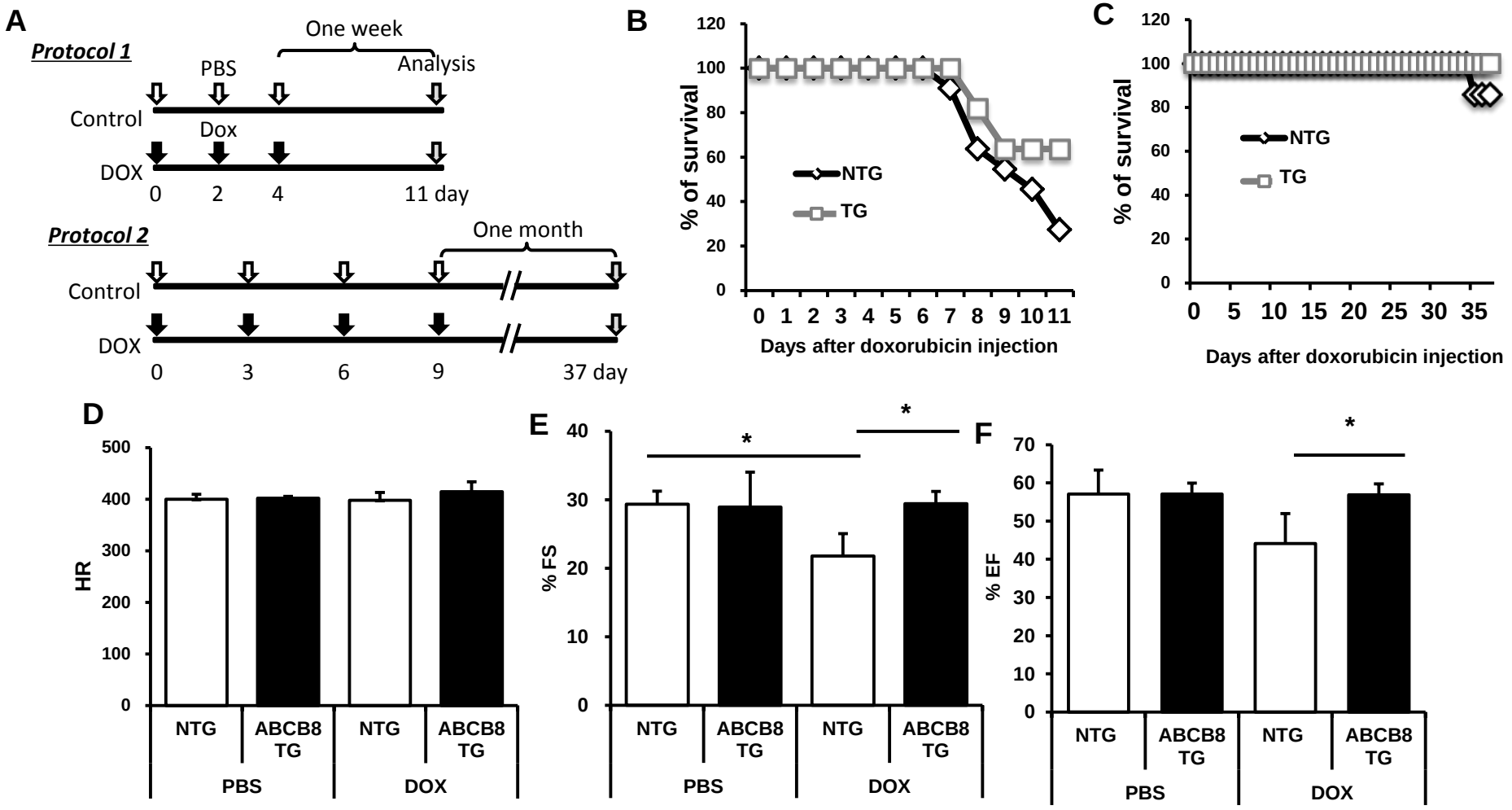
Supplemental Figure 4. **Nuclear and cytosolic iron levels with ABCB8 overexpression with and without DOX in NRCM** (**A**) Nuclear iron and (**B**) cytosolic iron with control and ABCB8 overexpression in the presence and absence of DOX (n=3). Data are presented as mean±SEM. *$P < 0.05$.



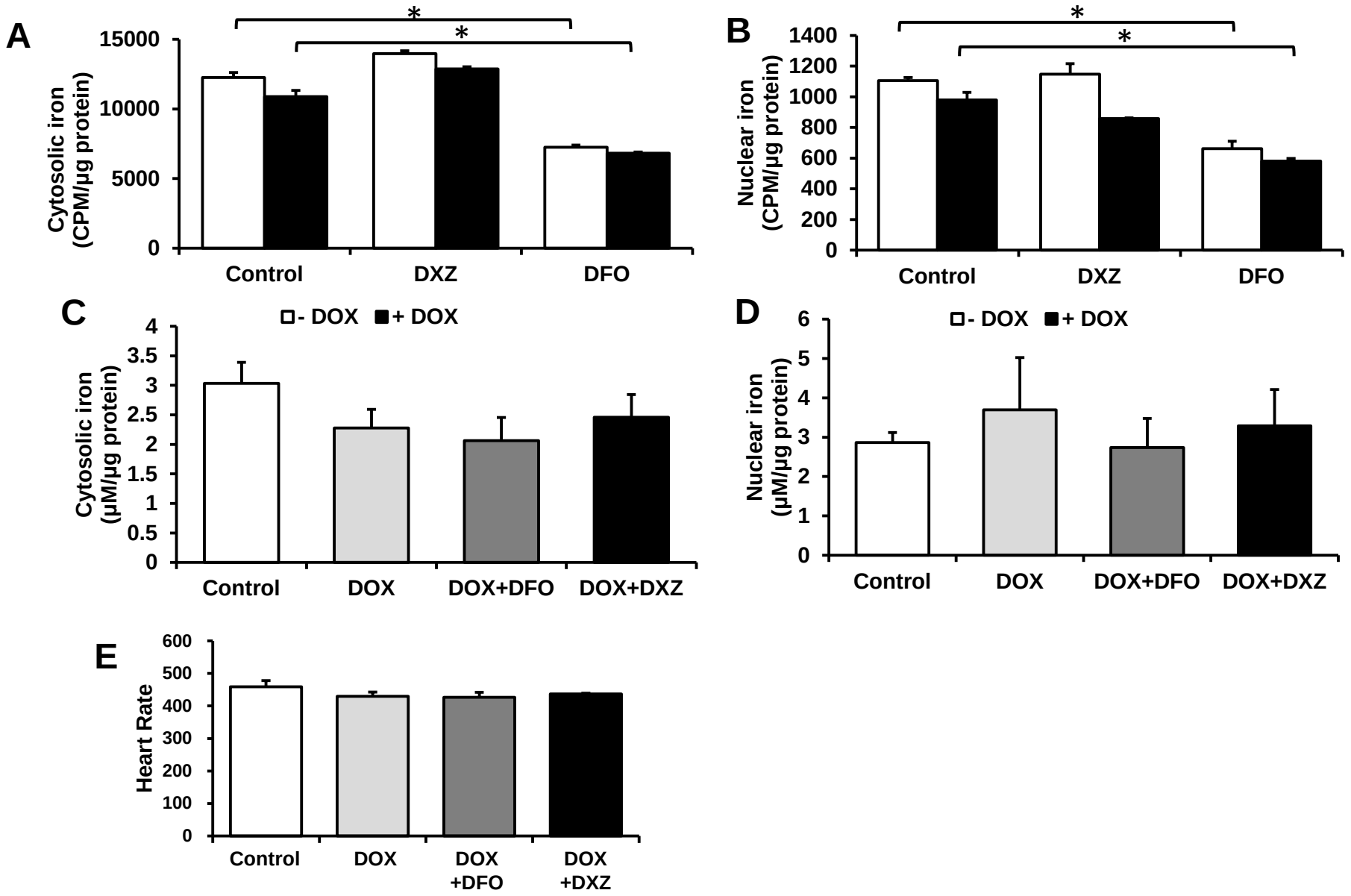
Supplemental Figure 5. **ABCB8 expression and iron parameters in ABCB8 TG mice with and without DOX treatment**
(**A**) mRNA levels of ABCB8 in the hearts of NTG and ABCB8 TG mice (n=3). (**B**) ABCB8 protein levels in the lungs, livers, and kidneys of NTG and ABCB8 TG mice were evaluated via Western blot. Representative image is on the left, densitometry analysis is on the right (n=3). (**C-D**) Nuclear (**C**) and cytosolic (**D**) iron levels in NTG and ABCB8 TG mice with and without DOX treatment (n=4). (**E-G**) Mitochondrial iron levels in the lung (**E**), liver (**F**) and kidney (**G**) of TG and NTG mice with and without DOX (n=3). (**H-J**) Hematologic iron parameters, serum iron (**H**), unsaturated iron binding capacity, UIBC (**I**), and total iron binding capacity, TIBC (**J**) in TG and NTG mice with and without DOX (n=4-5). Data are presented as mean±SEM. *P< 0.05.



Supplemental Figure 6. Cardiac features of ABCB8 TG and NTG mice at baseline in the absence of DOX treatment
M-mode echocardiographic images **(A)** were evaluated for measurements of fractional shortening (FS) (**B**), the ratio of cardiac output to body weight (CO/BW) (**C**), and left-ventricular diastolic diameter (LVDd) (**D**) in the hearts of NTG mice and ABCB8 TG (TG) mice. (**E**) Heart weights (n=5), and (**F-G**) invasive hemodynamic measurements of end systolic pressure (ESP) and end diastolic pressure (EDP) (**F**), dP/dtmax (a marker of systolic function) and dP/dtmin (a marker of diastolic function) (**G**), and heart rate (**H**) were performed in NTG mice and ABCB8 TG mice (n=4). (**I**) H & E-stained and Masson trichrome-stained sections of heart tissue from NTG mice and ABCB8 TG mice were evaluated for histological abnormalities. Data are presented as mean±SEM. *P< 0.05.



Supplemental Figure 7. Survival rates and cardiac features of NTG and ABCB8 TG mice after DOX administration
(**A**) Schematic representation of DOX treatment protocols. Protocol 1 consisted of a 10 mg/kg per day injection administered every other day for a total of three injections, and Protocol 2 used a 6 mg/kg per day injection every third day for a total of four injections. Cardiac function was assessed one week after the last injection for Protocol 1 and one month after the last injection for Protocol 2. (**B,C**) The survival rates of NTG and ABCB8 TG mice were monitored after treatment with 3 intraperitoneal injections of 10 mg/kg DOX (cumulative dose: 30 mg/kg) administered every other day (n=11) (**B**) or 4 intraperitoneal injections of 6 mg/kg DOX (cumulative dose: 24 mg/kg) administered every third day (n=7) (**C**). (**D**) Assessments of heart rate (HR) in NTG and ABCB8 TG mice after DOX treatment according to protocol 2 (n=6-8). (**E,F**) NTG and ABCB8 TG mice were treated with DOX via Protocol 1. One weeks after the final DOX injection (as opposed to one month later for Protocol 2), echocardiographic assessments of fractional shortening (FS) (**E**), and ejection fraction (EF) (**F**) were measured (n=3-5 per group). Data are presented as mean±SEM. *P< 0.05.



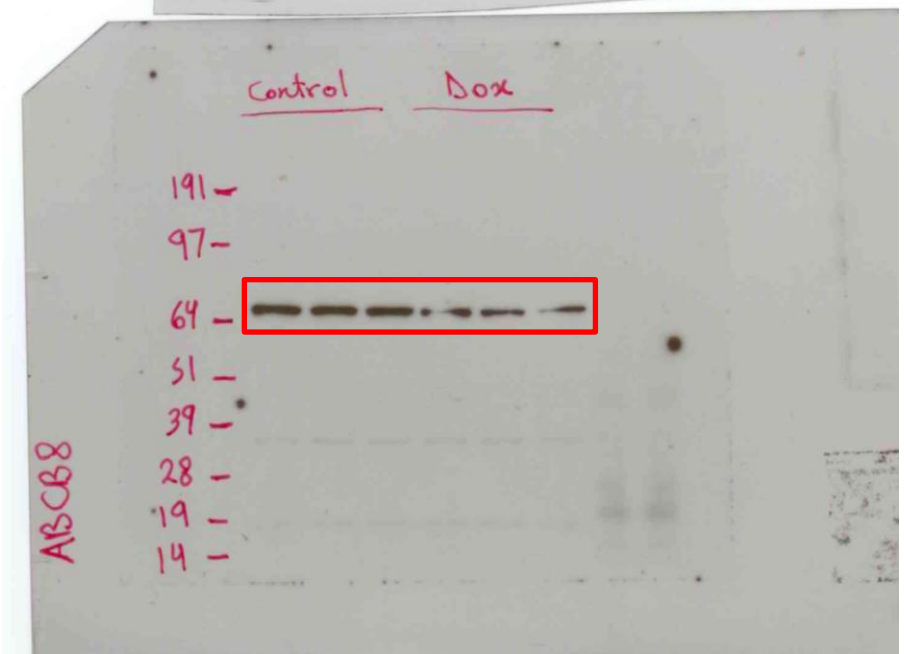
Supplemental Figure 8. Cytosolic and nuclear iron in mice treated with DFO and DXZ

(**A, B**) Cytosolic (**A**) and nuclear (**B**) iron content in NRCM treated with 200µM DFO or 200µM DXZ for 16 hours with and without DOX in NRCM (n=3). (**C,D**) Cytosolic (**D**) and nuclear (**D**) iron levels in the hearts of WT mice treated with DOX, with or without DFO or DXZ (n=3-4). (**E**) Heart rate of control mice and DOX-treated mice that had been co-treated with or without DXZ or DFO. DOX treatment was according to protocol 2 (n=6).

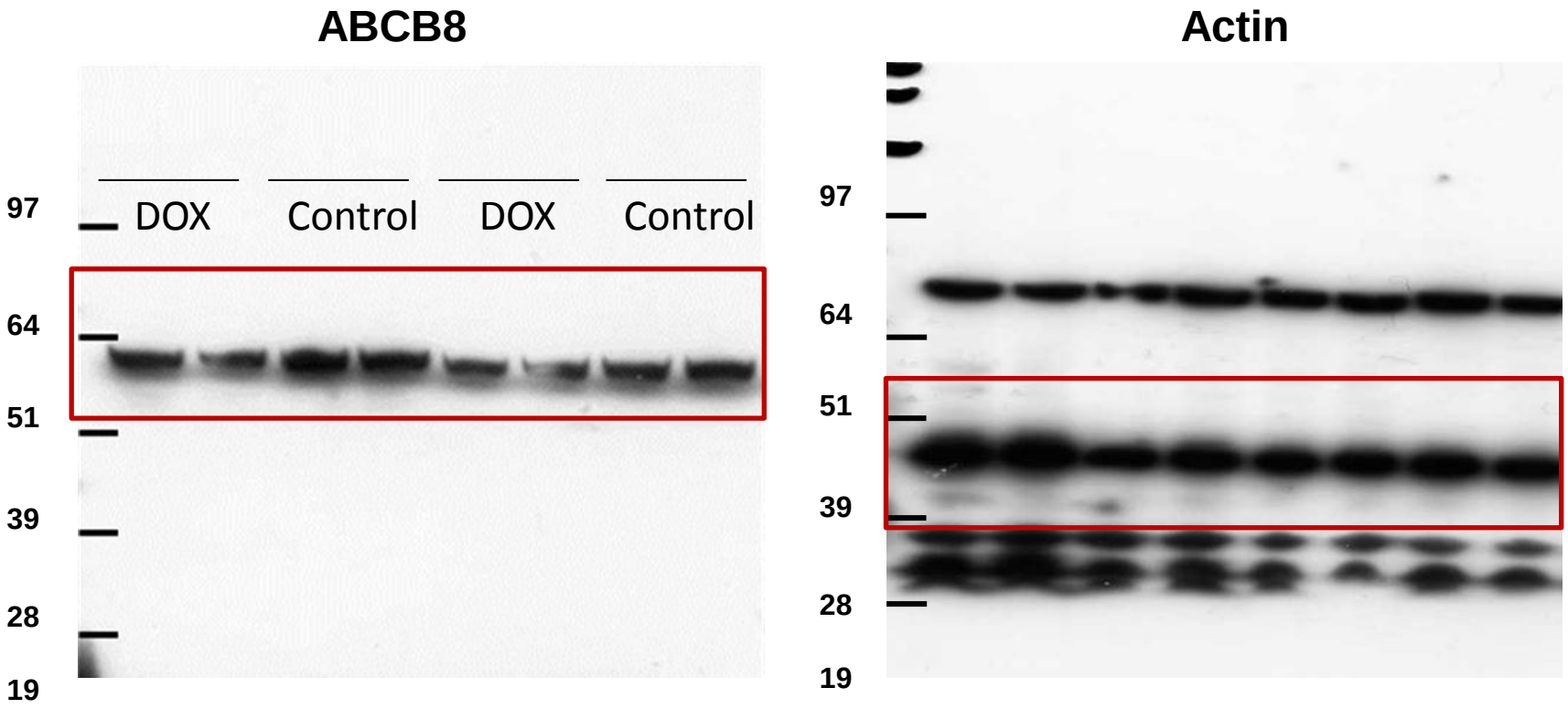
Original Western Blots

Full Unedited Gels for Figure 2A

ABCB8 protein levels in NRCMs
With Dox treatment (20 μM DOX)

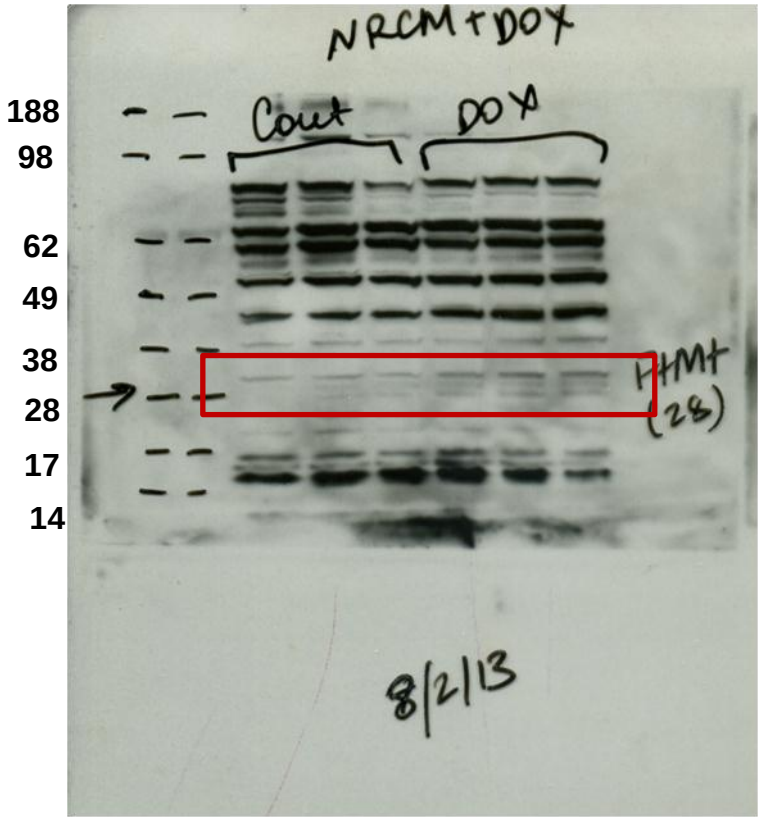


Full Unedited Gels for Figure 2B

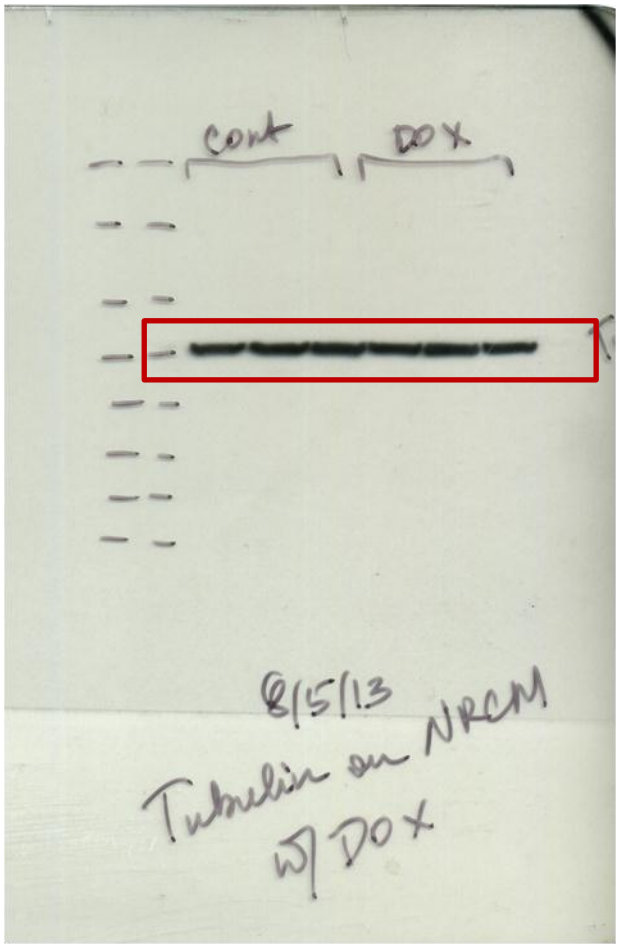


Full Unedited Gels for Figure 2H

Mitochondrial Ferritin

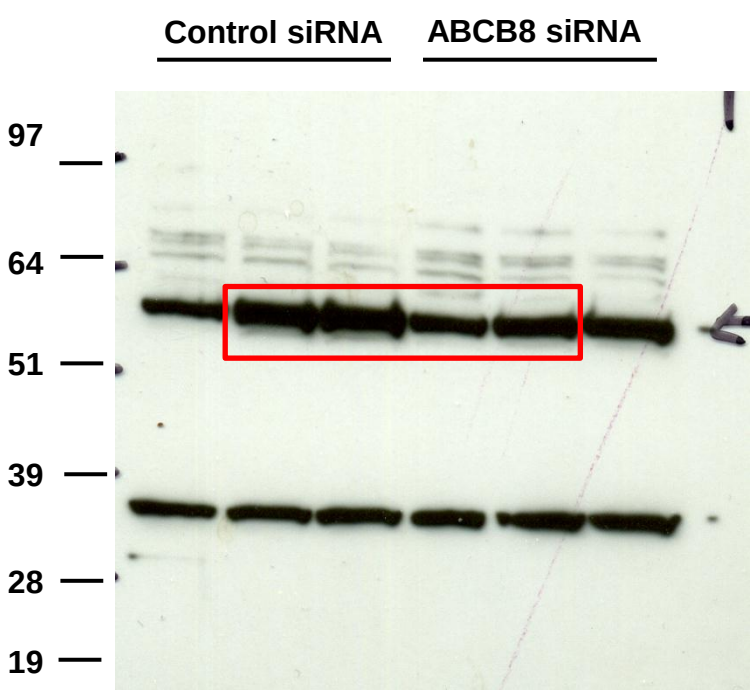


Tubulin

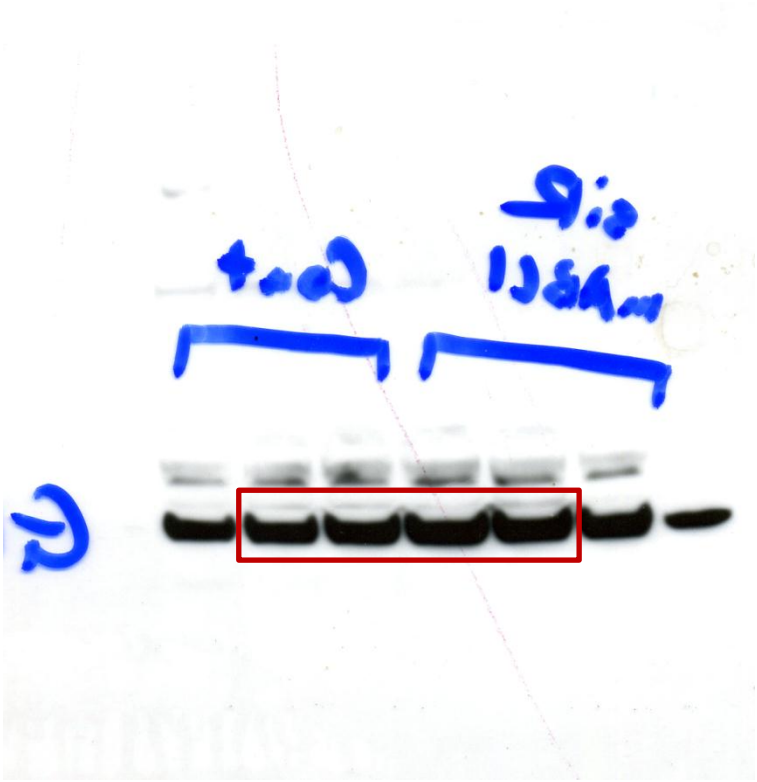


Full Unedited Gels for Figure 3A

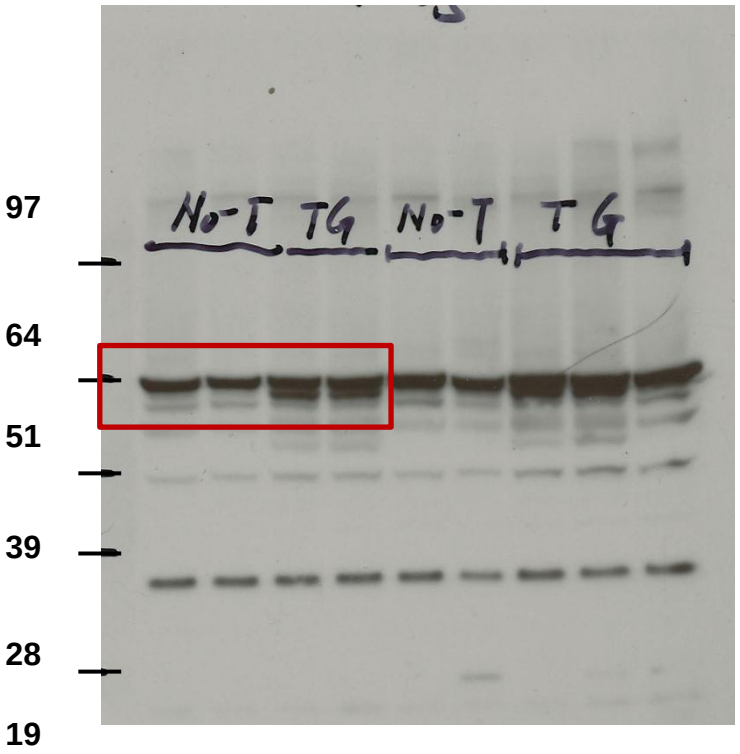
ABCB8

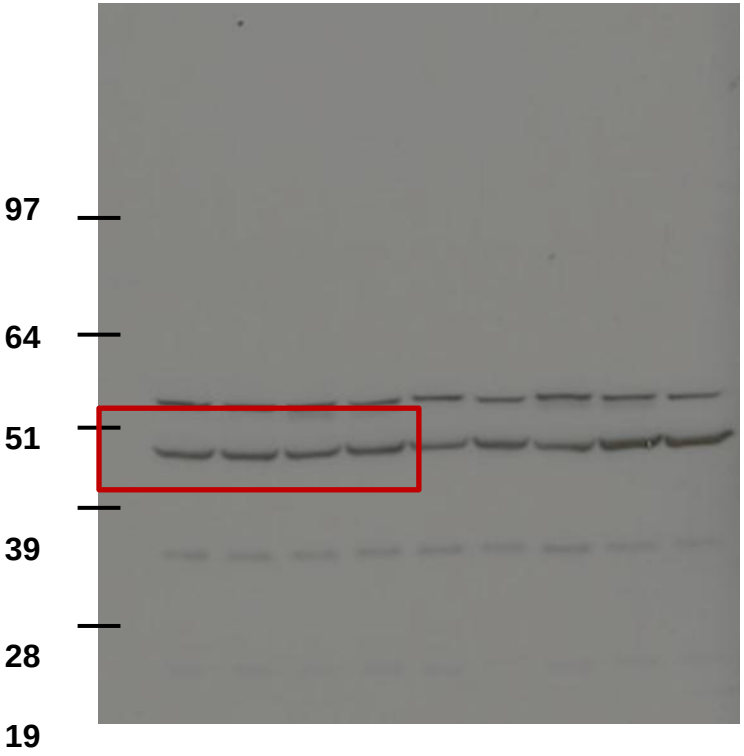


GAPDH



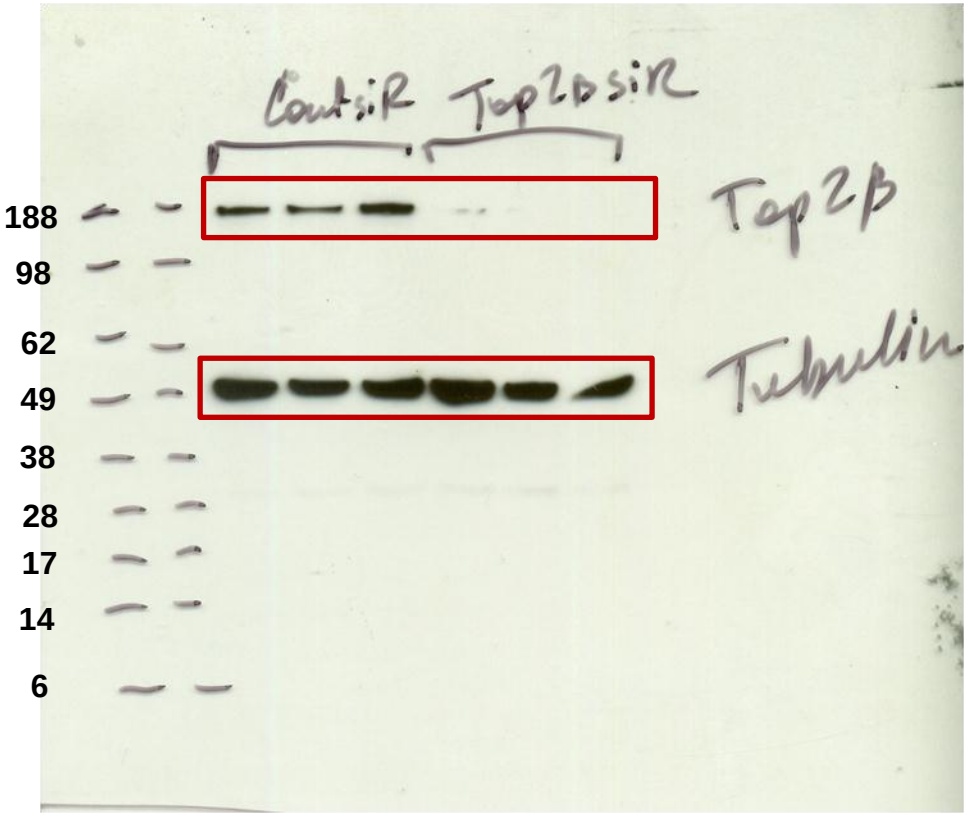
Full Unedited Gels for Figure 5A





Full Unedited Gels for Figure 9A

Topoisomerase 2β and Tubulin



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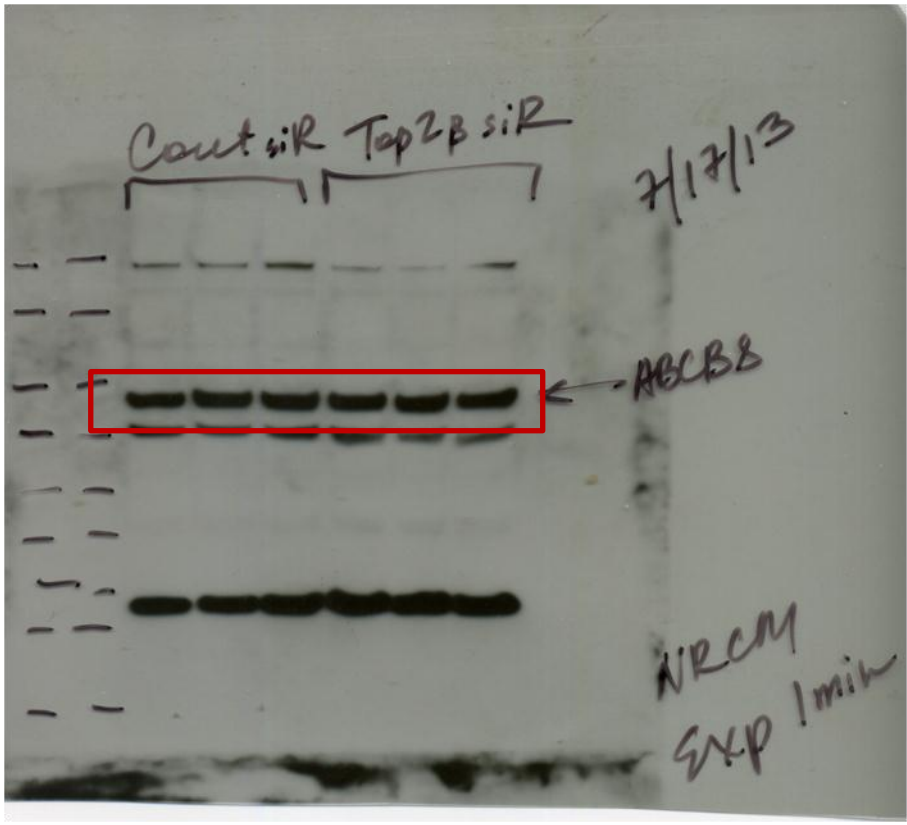
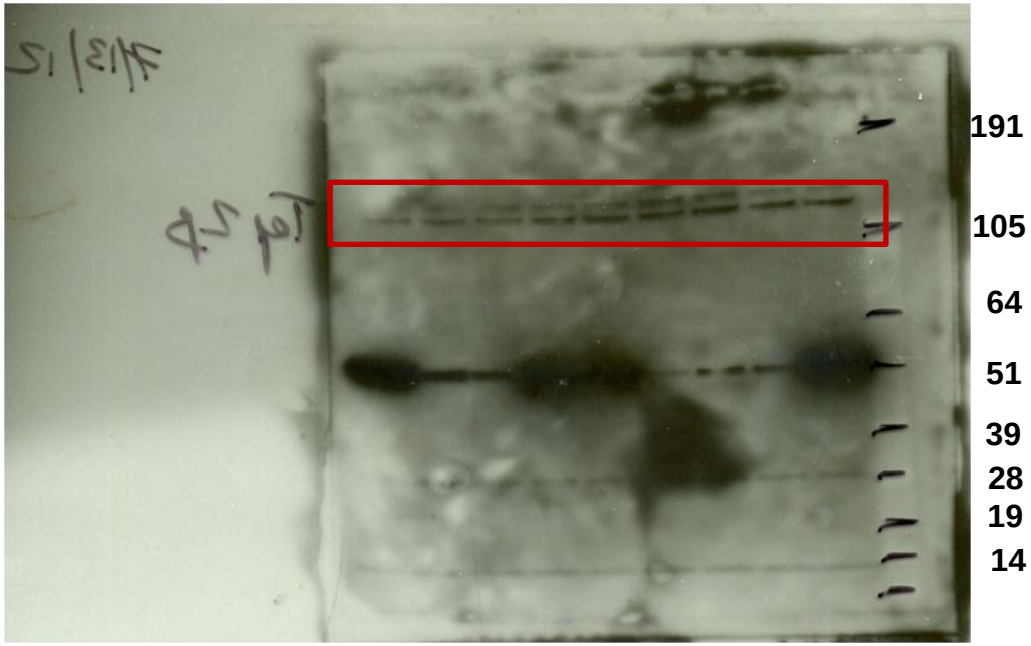
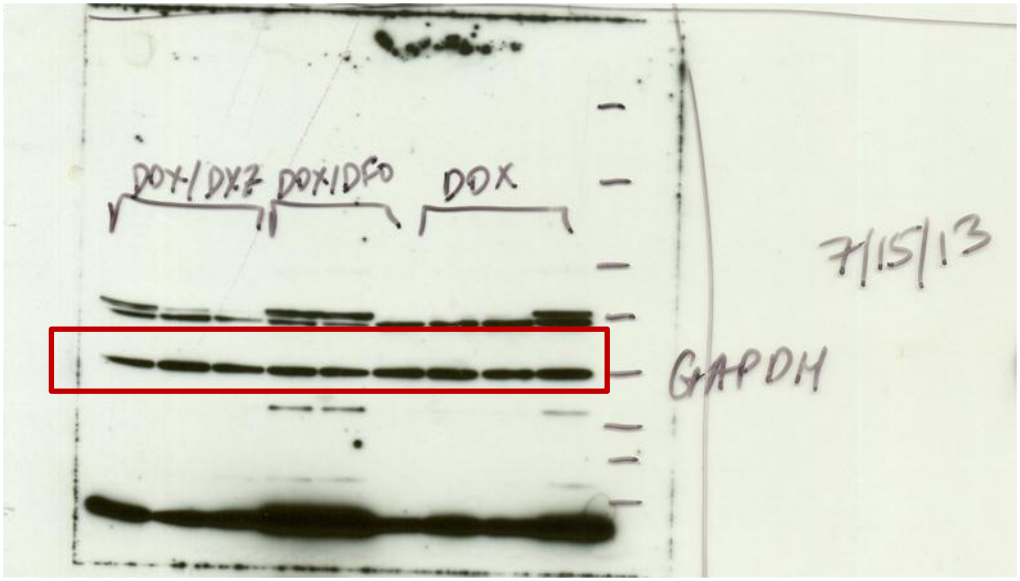


Full Unedited Gels for Figure 9D

Top 2β

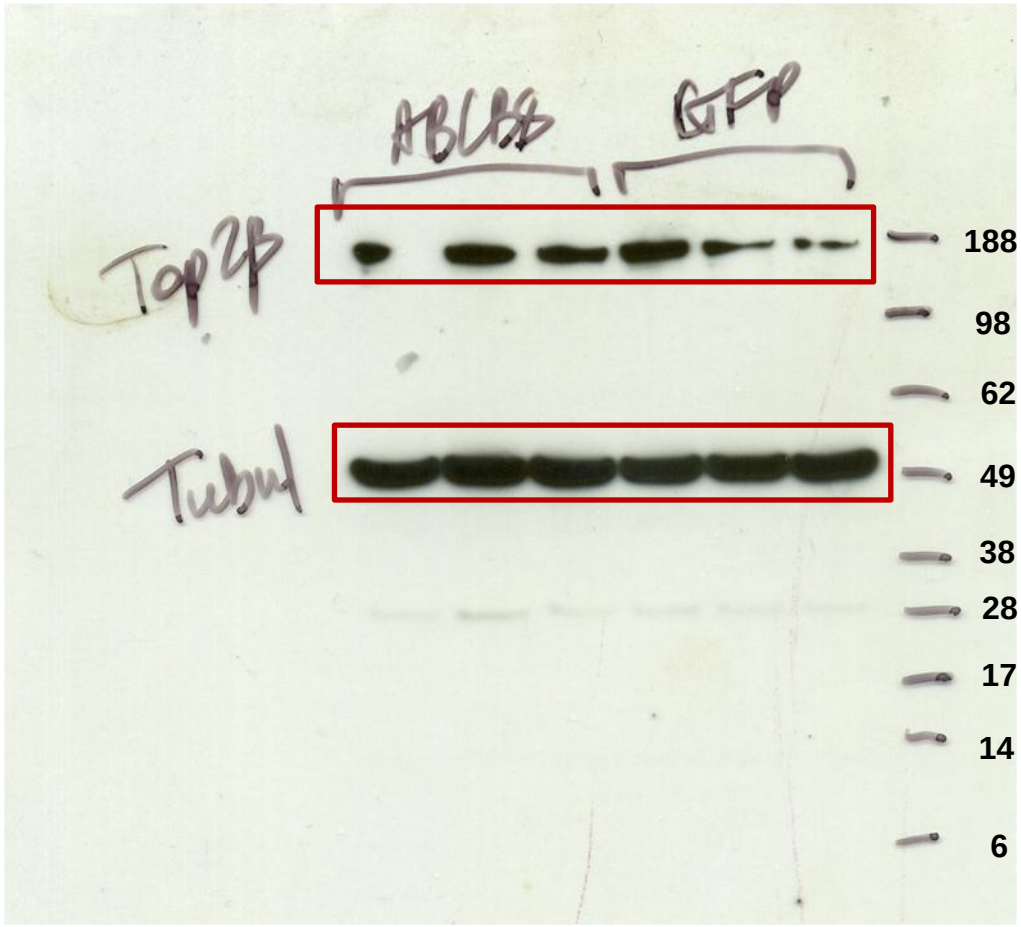


GAPDH



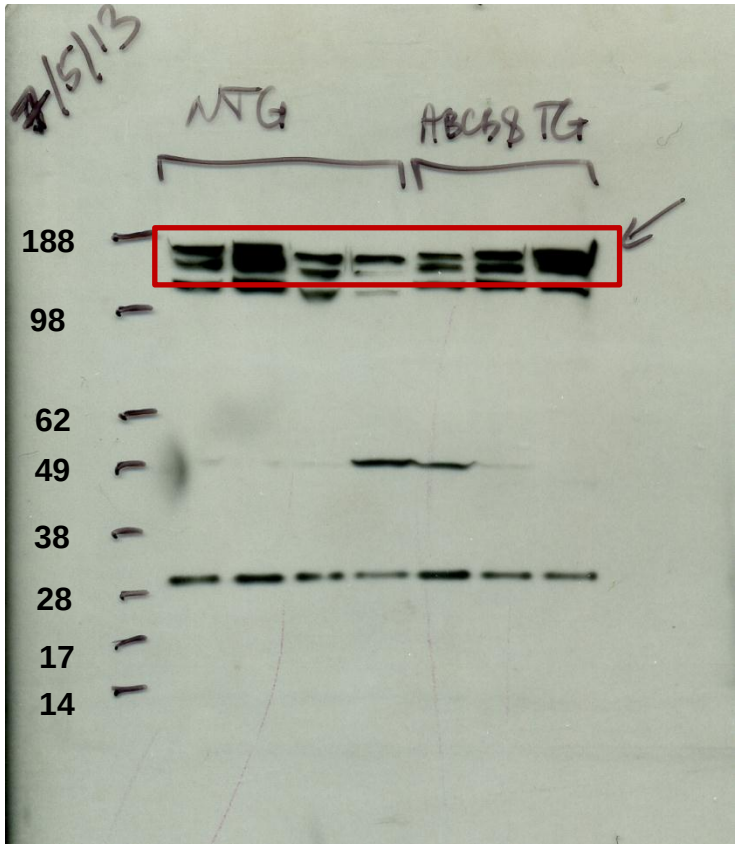
Full Unedited Gels for Figure 9E

Topoisomerase 2β and Tubulin

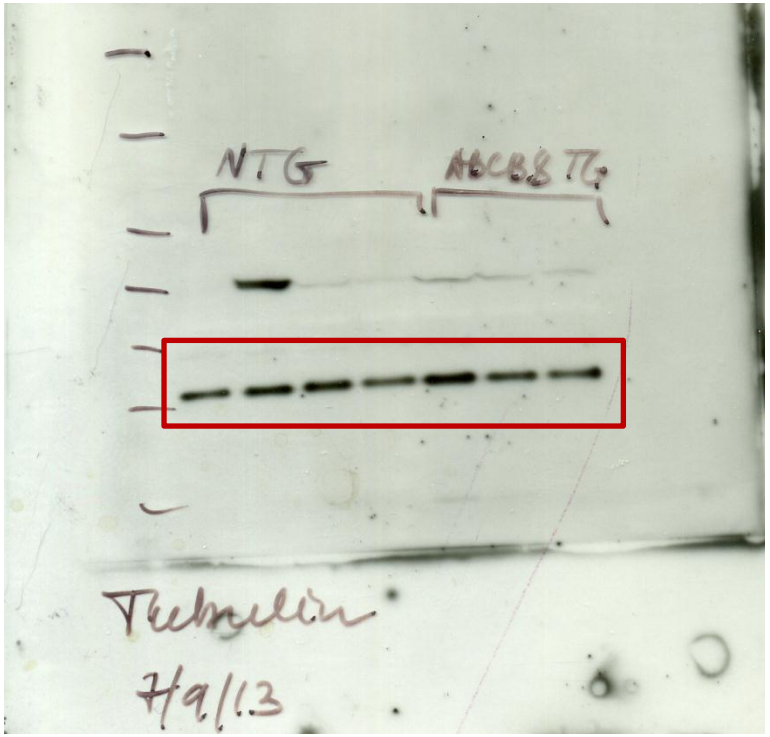


Full Unedited Gels for Figure 9F

Topoisomerase 2β



Tubulin



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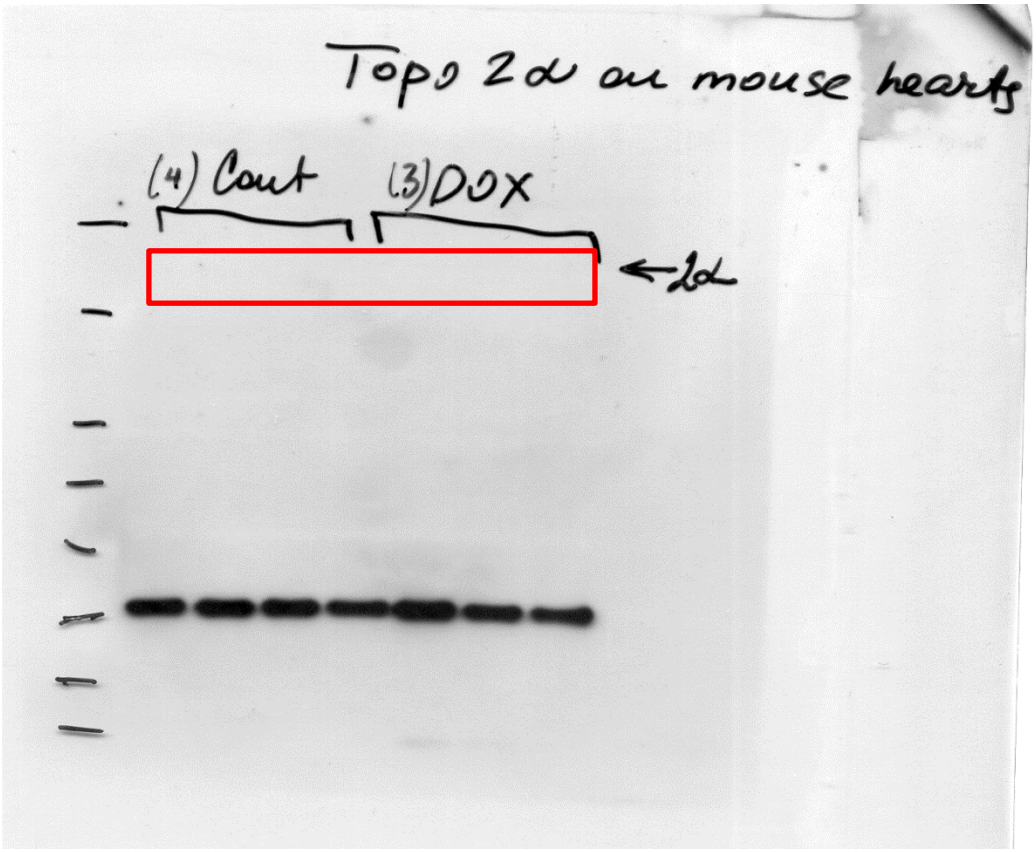
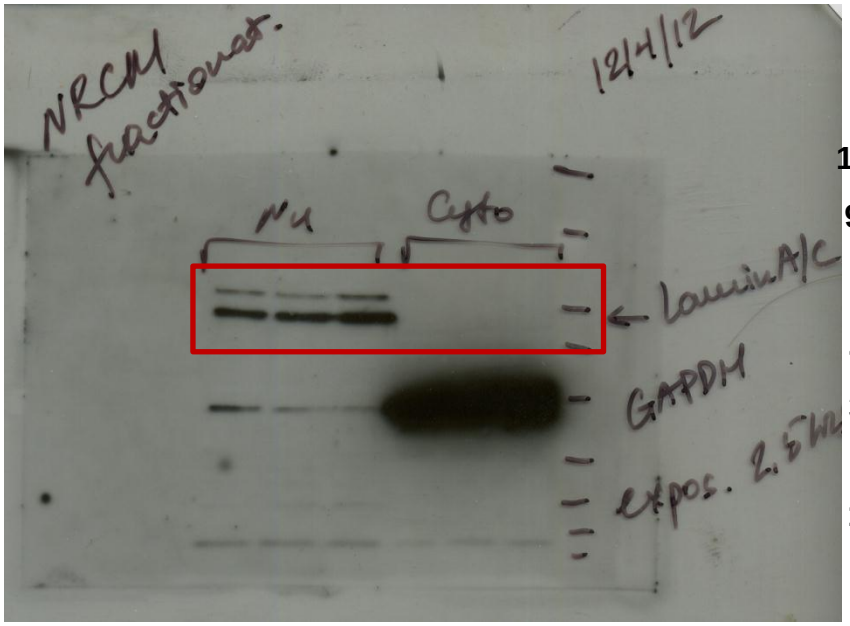


Full Unedited Gels for Supplemental Figure 3A

Lamin A/C



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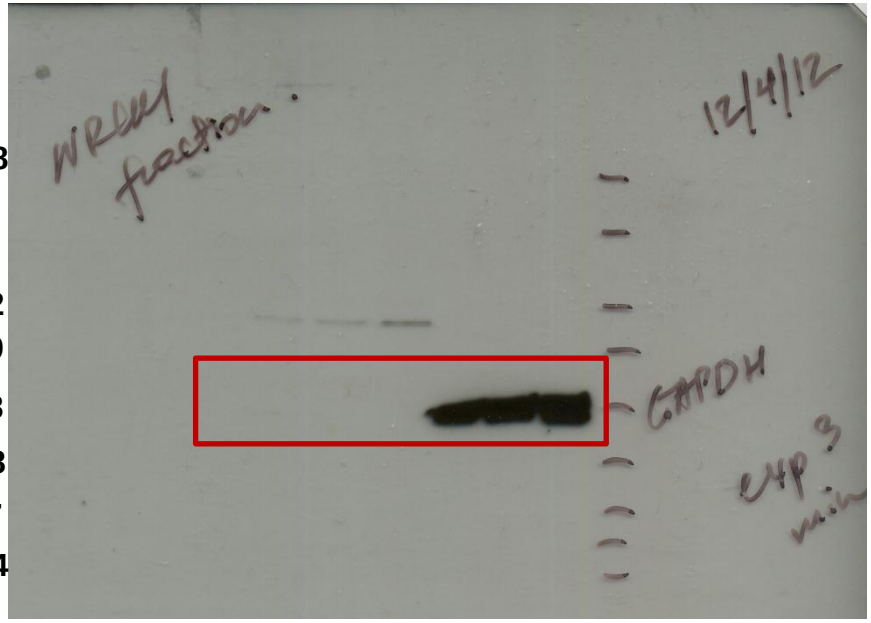
GAPDH



Full Unedited Gels for Figure S5B