

La ferroptosi come driver dell'inflamm-aging nei lungosopravviventi da tumore pediatrico: riprogrammazione macrofagica indotta da ferrochelanti e antiossidanti

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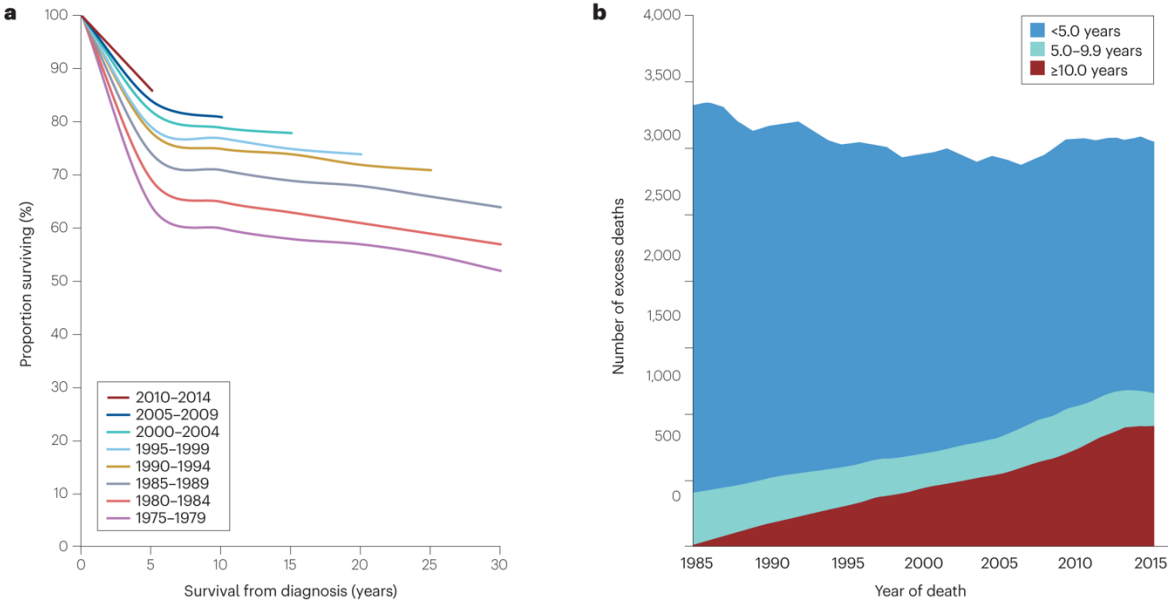
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Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other

Childhood Cancer Survivors (CCS)

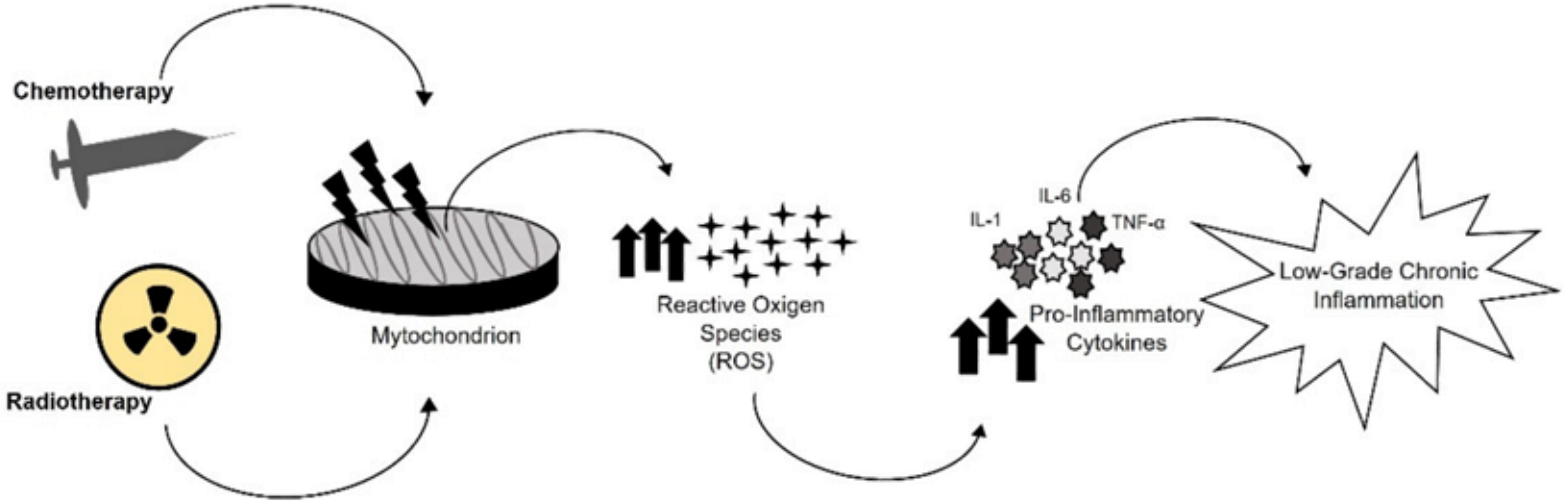
- One of the greatest medical successes over the past five decades is the improvement in survival among children with cancer.
- A total of 80% of children treated for childhood cancer achieve 5-year survival, becoming long-term survivors.



Ehrhardt MJ, Krull KR, Bhakta N, Liu Q, Yasui Y, Robison LL, Hudson MM. Improving quality and quantity of life for childhood cancer survivors globally in the twenty-first century. Nat Rev Clin Oncol. 2023 Oct;20(10):678-696.

Chronic Late effects related to anti-cancer treatments

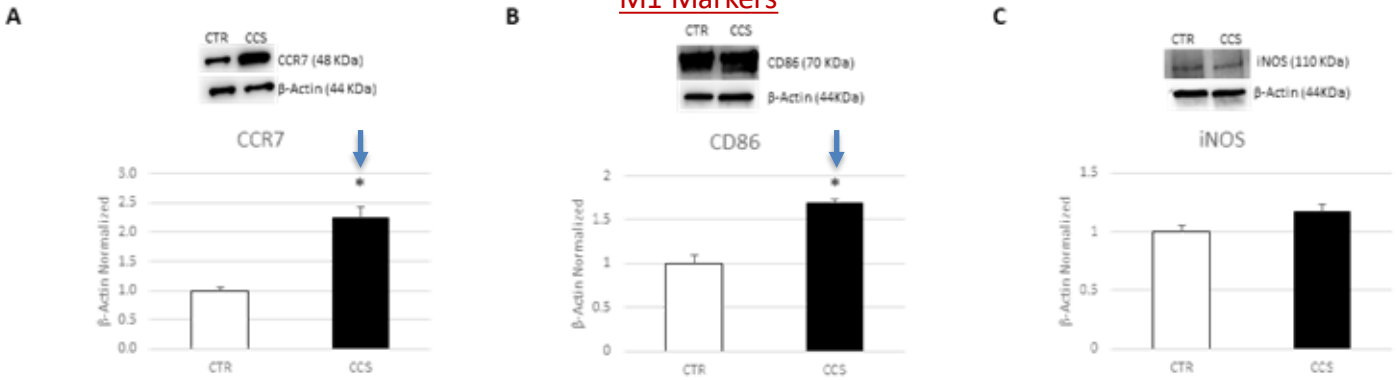
- Chemotherapy and radiotherapy leads to inflammation, the accumulation of senescent cells, and the increasing of reactive oxygen species (ROS) and DNA mutations.
- Mitochondria are the major source of reactive oxygen species (ROS). Due to the proximity of mitochondrial DNA (mtDNA) to sites of ROS generation, mtDNA is prone to accumulating mutations after exposure to chemotherapy and radiation.



Rossi F, Di Paola A, Pota E, Argenziano M, Di Pinto D, Marrapodi MM, Di Leva C, Di Martino M, Tortora C. Biological Aspects of Inflamm-Aging in Childhood Cancer Survivors. Cancers (Basel). 2021 Sep 30;13(19):4933.

Macrophages characterization in CCS

M1 Markers



M2 Markers

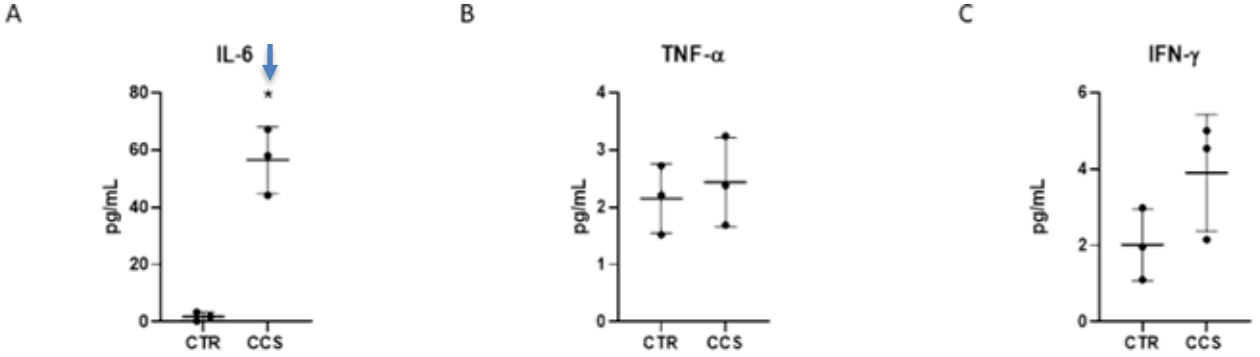


- We revealed an increase of M1 markers protein expression levels (CCR7, CD86 and iNOS) in CCS compared to CTR.
- We also observed a decrease of protein expression levels of M2 phenotype markers (CD206 and pSTAT6) in CCS compared to CTR.

Di Paola A, Marrapodi MM, Pota E, Colucci Cante R, Rana D, Giliberti G, Di Feo G, Ahmed S, Roberti D, Nigro R, Rossi F, Argenziano M. Role of Nutraceuticals in Counteracting Inflammation in In Vitro Macrophages Obtained from Childhood Cancer Survivors. Cancers (Basel). 2024 Feb 8;16(4):714. doi: 10.3390/cancers16040714. PMID: 38398105; PMCID: PMC10886672.

Inflammatory state in macrophages isolated from CCS

Pro-Inflammatory Cytokines



- We revealed an increase of pro-inflammatory cytokines levels (IL-6, TNF-α and IFN-γ) in CCS compared to CTR.

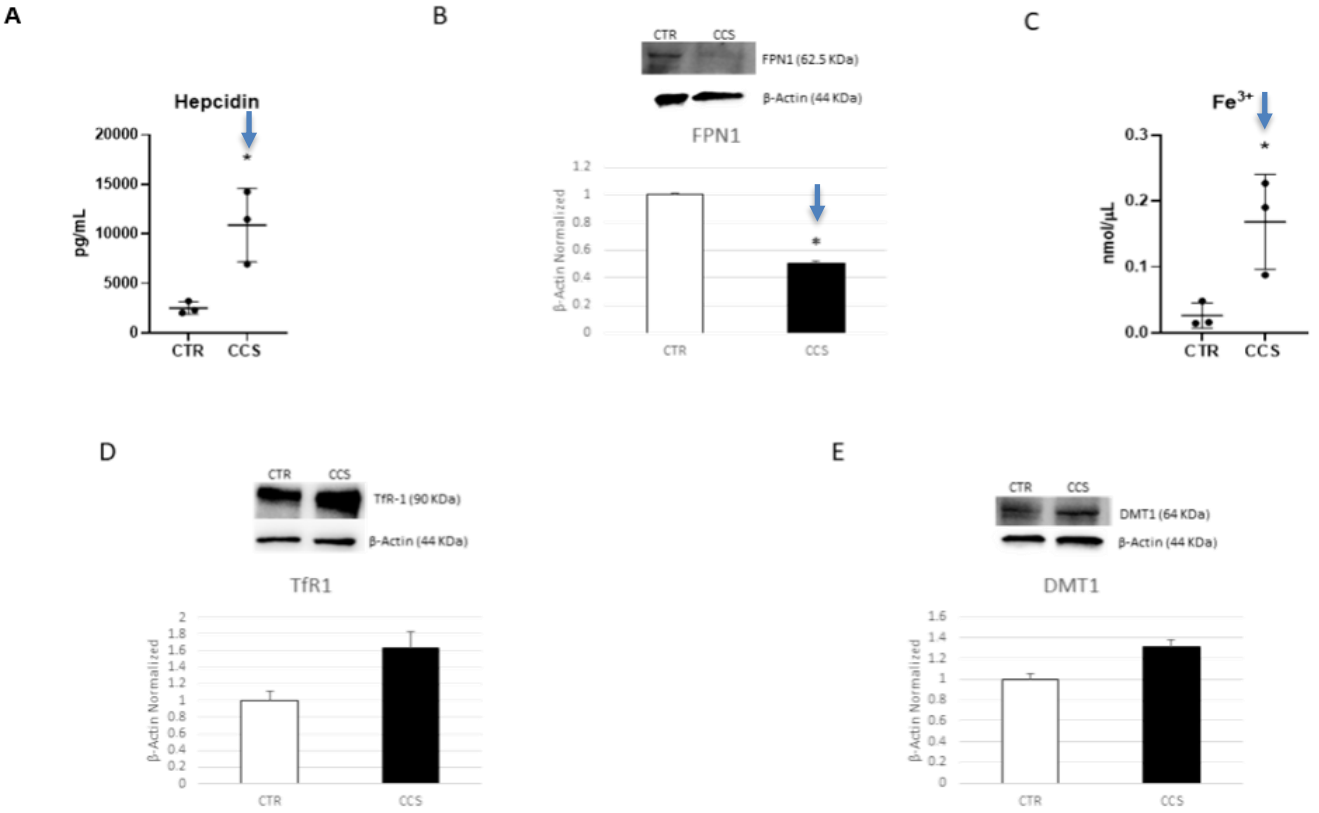
Anti-Inflammatory Cytokines



- We observed a decrease of anti-inflammatory cytokine IL-4 level and any significant variation of the other anti-inflammatory cytokine IL-10 in CCS compared to CTR.

Di Paola A, Marrapodi MM, Pota E, Colucci Cante R, Rana D, Giliberti G, Di Feo G, Ahmed S, Roberti D, Nigro R, Rossi F, Argenziano M. Role of Nutraceuticals in Counteracting Inflammation in In Vitro Macrophages Obtained from Childhood Cancer Survivors. Cancers (Basel). 2024 Feb 8;16(4):714. doi: 10.3390/cancers16040714. PMID: 38398105; PMCID: PMC10886672.

Iron metabolism in macrophages isolated from CCS

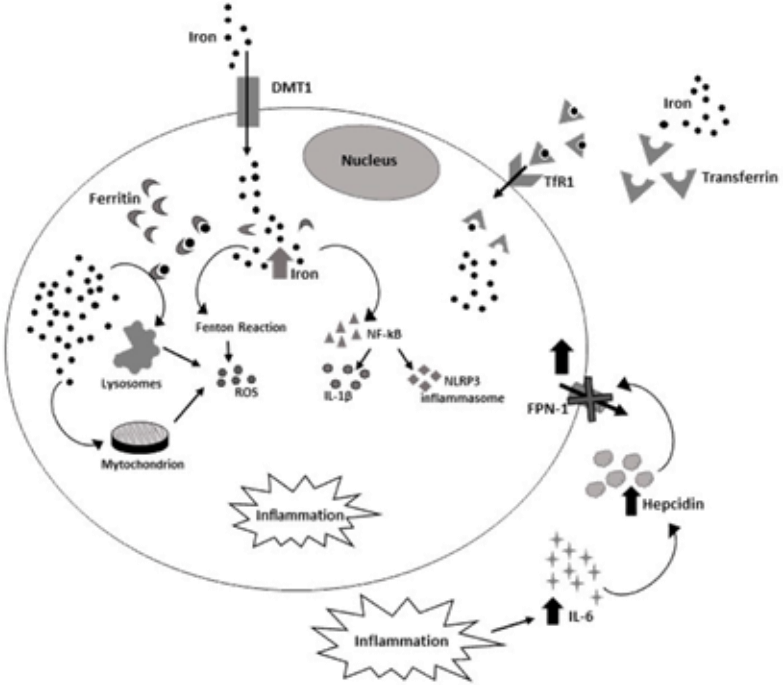


- We observed an impairment of iron metabolism in CCS macrophages, revealing:
- a strong increase of hepcidin levels;
- a marked reduction of FPN1 protein expression levels;
- a trend to increase of TfR1 and DMT1;
- a strong increase of intracellular iron concentration.

Di Paola A, Marrapodi MM, Pota E, Colucci Cante R, Rana D, Giliberti G, Di Feo G, Ahmed S, Roberti D, Nigro R, Rossi F, Argenziano M. Role of Nutraceuticals in Counteracting Inflammation in In Vitro Macrophages Obtained from Childhood Cancer Survivors. Cancers (Basel). 2024 Feb 8;16(4):714. doi: 10.3390/cancers16040714. PMID: 38398105; PMCID: PMC10886672.

Iron and Inflammation

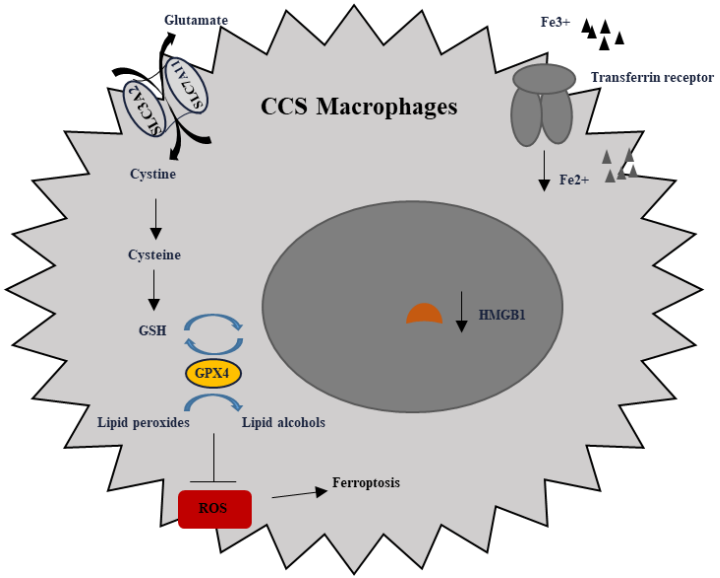
- During inflammation high levels of interleukin-6 cause an increased production of hepcidin which induces a degradation of ferroportin (FPN1), leading to decreased iron efflux that culminates in elevated intracellular iron concentration and consequently iron toxicity in cells and tissues.



Di Paola A, Tortora C, Argenziano M, Marrapodi MM, Rossi F. Emerging Roles of the Iron Chelators in Inflammation. Int J Mol Sci. 2022 Jul 20;23(14):7977.

Ferroptosis and Cell Damage

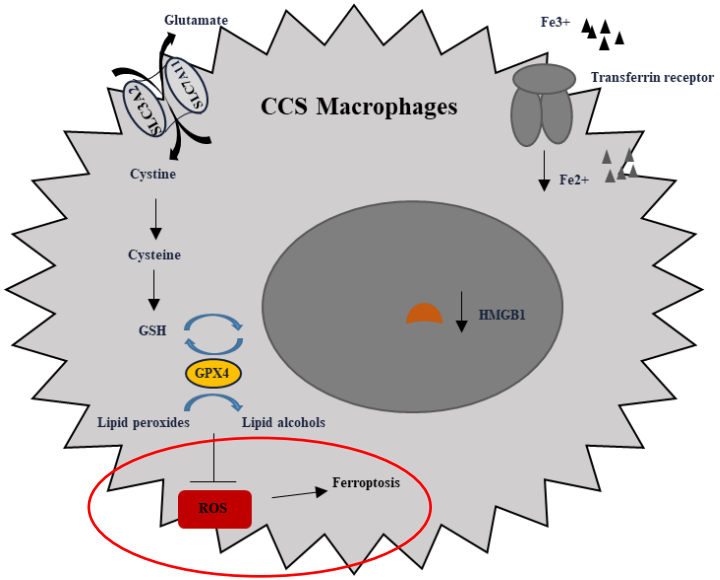
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Ferroptosis and Cell Damage

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- Iron accumulation determines ROS accumulation which induce peroxidation of membrane lipids (polyunsaturated fatty acids, PUFAs), determining the formation of lipid peroxides, altering the fluidity and integrity of the cell membrane, thus leading to the loss of the membrane barrier → cell death.

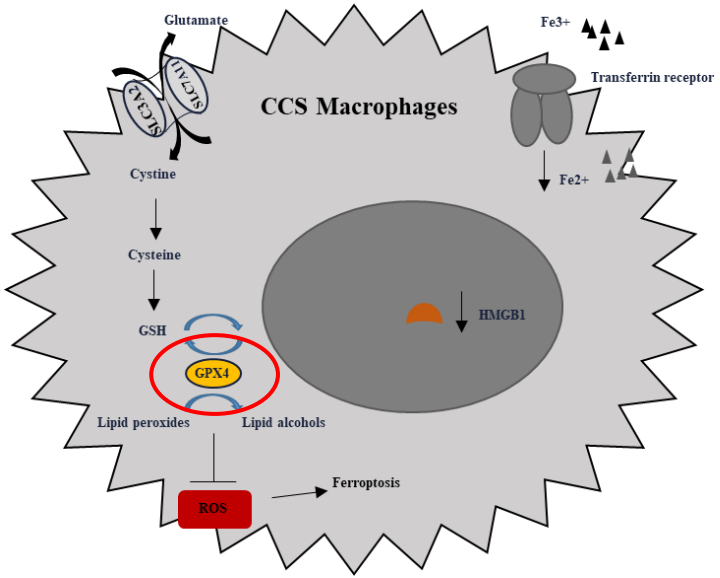


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- Cells have a fine regulated mechanism of defence to inhibit peroxides accumulation. The glutathione peroxidase 4 (GPX4) reduces lipid peroxides to non-toxic lipid alcohols, using reduced glutathione (GSH) as a cofactor. The Xc- system is an antiport that exchanges extracellular glutamate for intracellular cystine, necessary for synthesis of GSH.

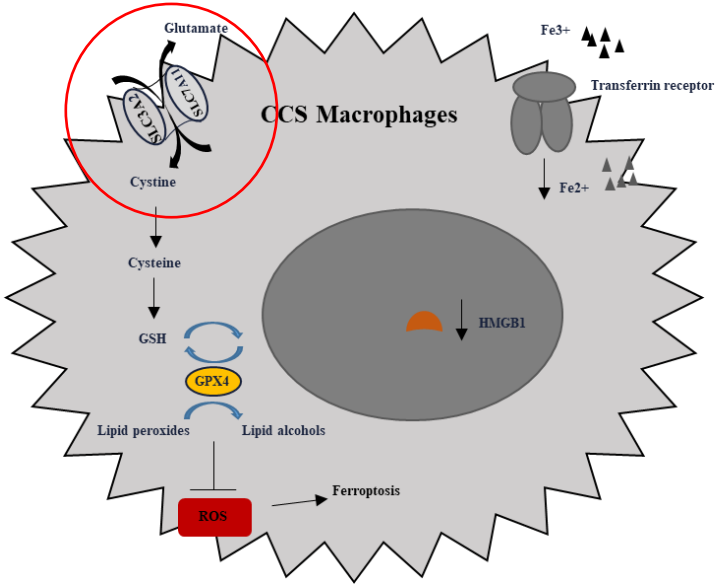


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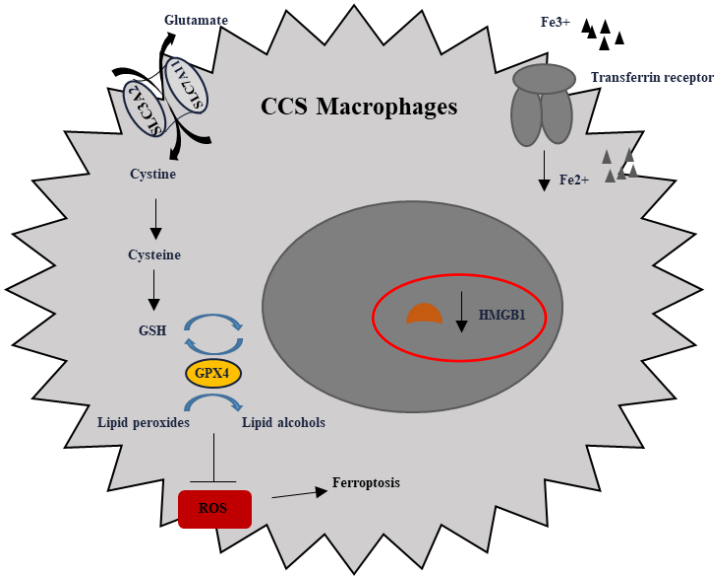
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Ferroptosis and Cell Damage

- When a cell dies, its membrane loses integrity, causing the release of intracellular molecules that are normally not found outside the cell → Damage-Associated Molecular Patterns (DAMPs).
- A key role is played by the acyl-CoA synthetase long-chain family member 4 (ACSL4), an enzyme responsible for the esterification of PUFAs into phospholipids, by adding CoA to the long chain, thus leading to lipoxygenase (ALOX)-dependent lipid peroxidation.



Aim and Experimental Approach

To investigate ferroptosis susceptibility in CCS macrophages and to assess whether nutraceuticals compounds can counteract inflammation by modulating ferroptosis-related markers, inflammatory status and macrophage phenotype.

Experimental model

Peripheral blood-derived macrophages

CCS vs **CTR**

Pharmacological approaches

Ferroptosis inhibition
Ferrostatin-1

Iron chelation
Deferasirox • Eltrombopag

Nutraceuticals treatments
Resveratrol • Tocopherols

Biological readouts

Ferroptosis markers protein expression → Western Blot

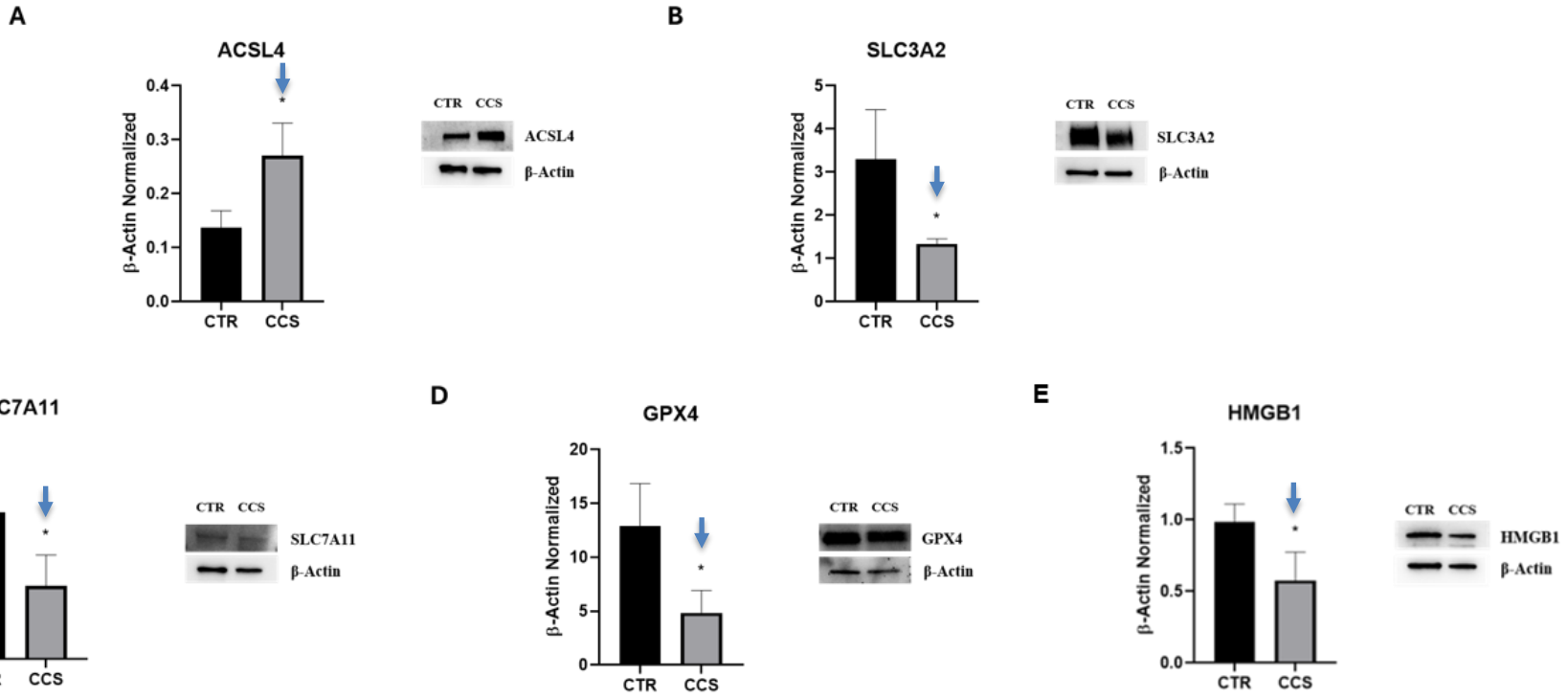
Ferroptosis markers gene expression → Real-Time PCR

Intracellular ROS levels → ROS assay

Inflammatory cytokines → ELISA

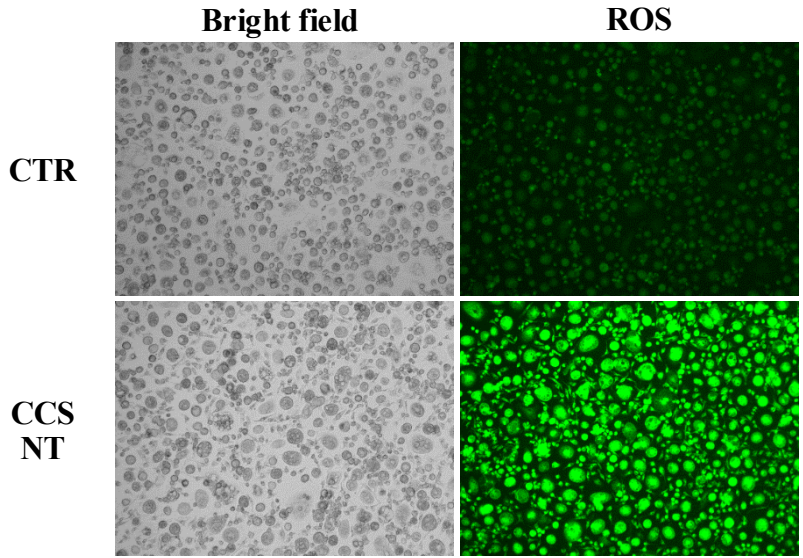
Macrophage phenotype → Western Blot

Ferroptosis markers protein expression levels in CCS macrophages

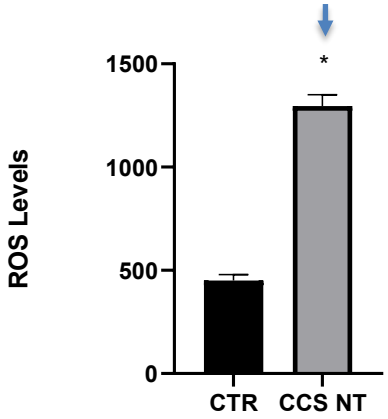


ROS levels in CCS macrophages

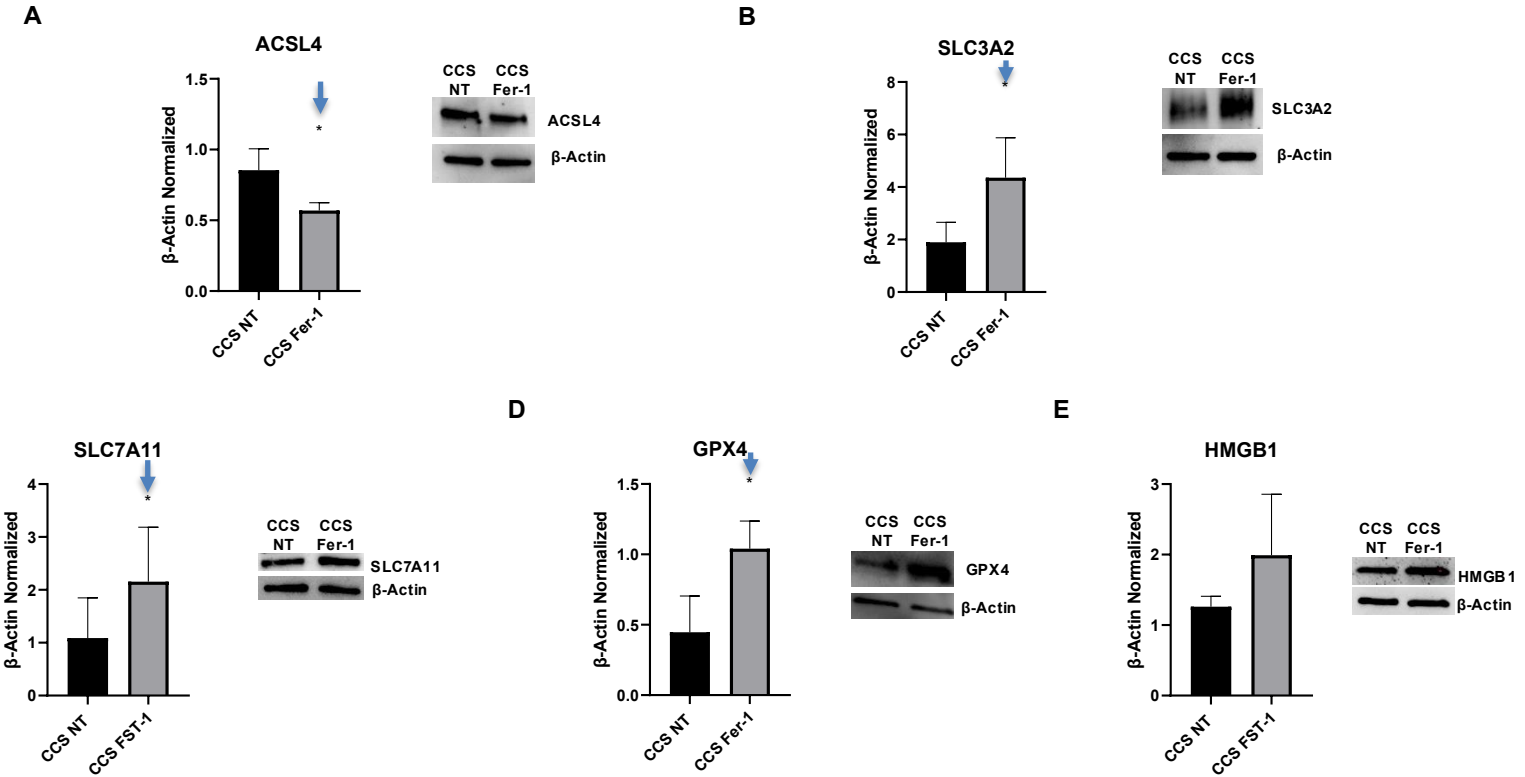
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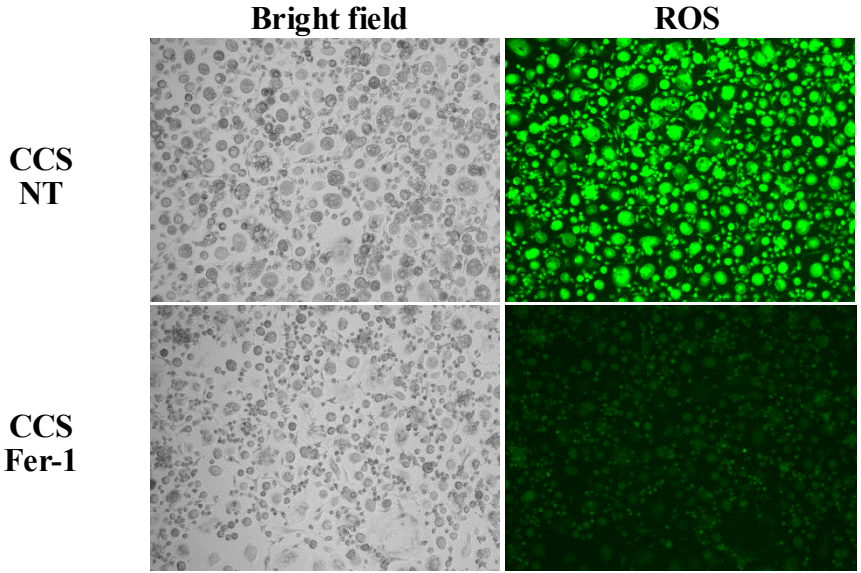


Effects of Ferrostatin-1 (Fer-1) on ferroptosis markers protein expression levels in CCS macrophages

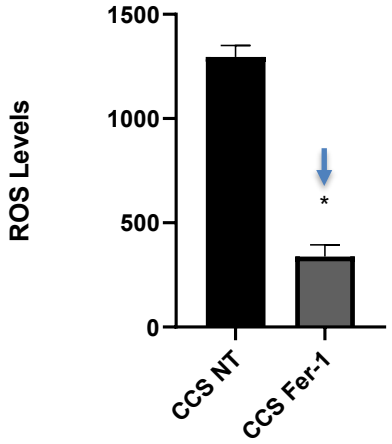


Effect of Fer-1 on ROS levels in CCS macrophages

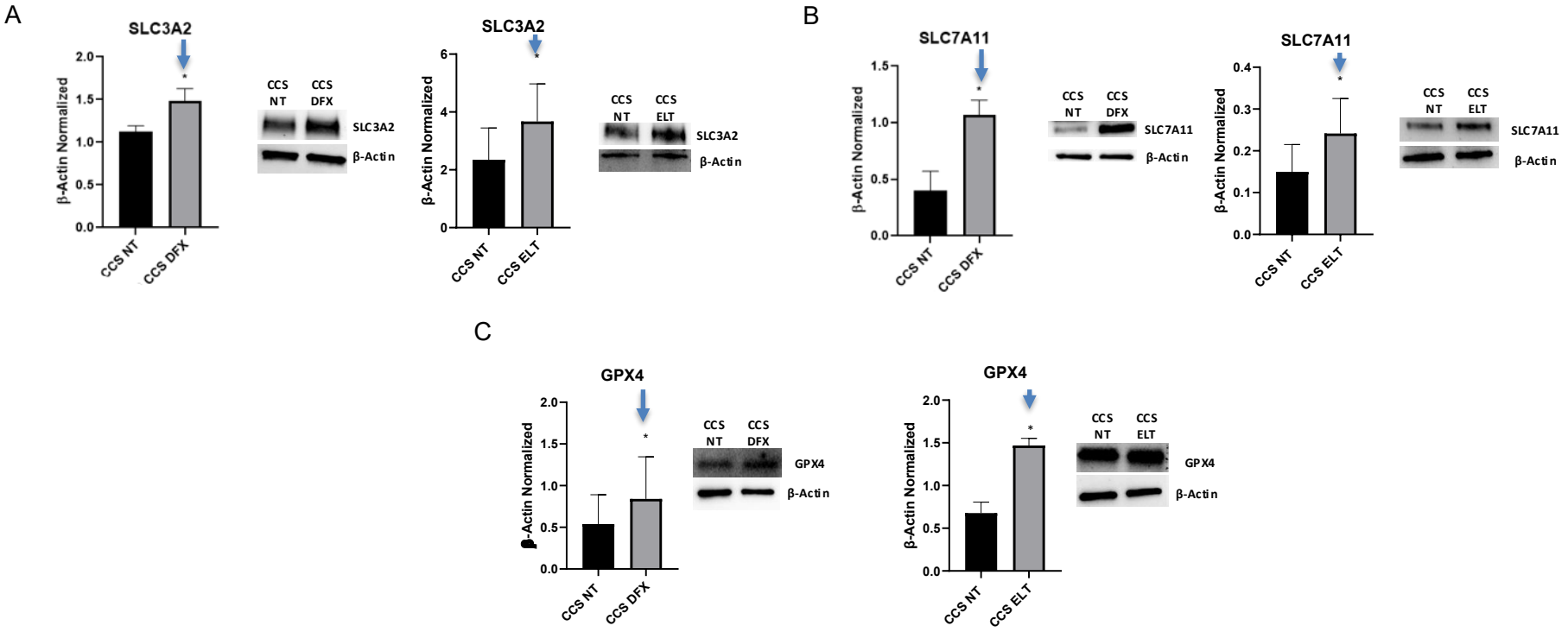
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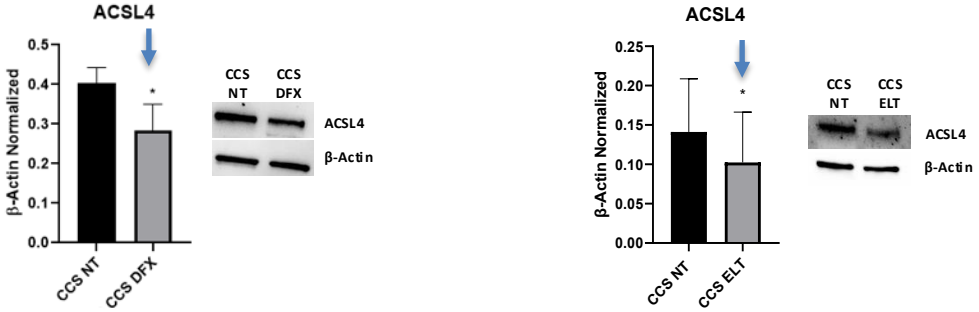


Effects of Deferasirox (DFX) and Eltrombopag (ELT) on ferroptosis defense mechanisms markers in CCS macrophages

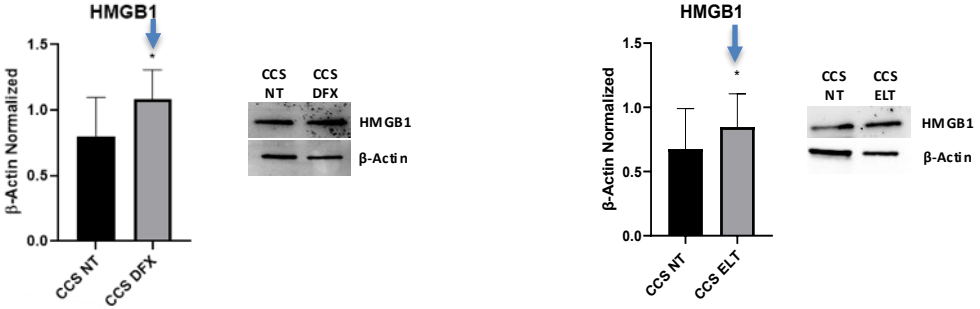


Effects of DFX and ELT on ferroptosis markers protein expression levels in CCS macrophages

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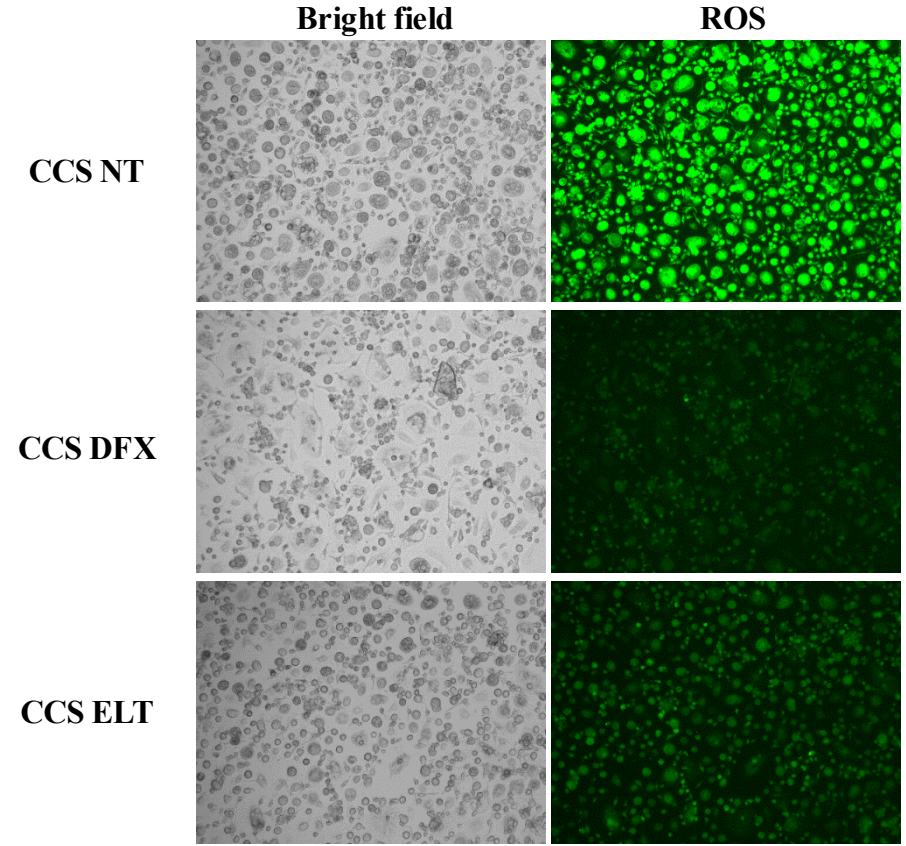


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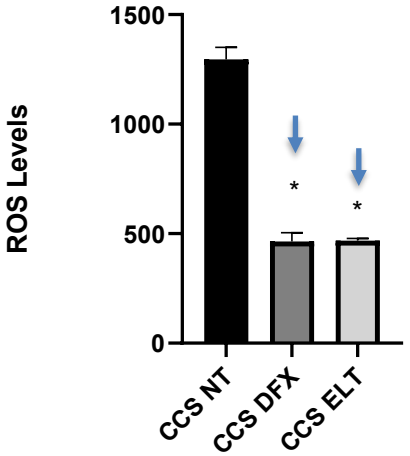


Effect of DFX and ELT on ROS levels in CCS macrophages

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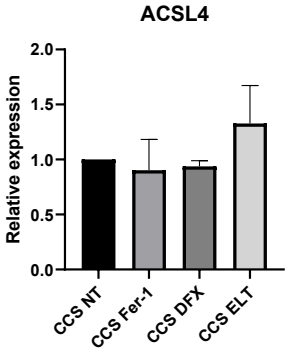


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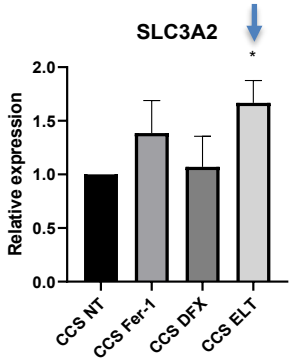


Effects of Fer-1, DFX and ELT on ferroptosis markers gene expression levels in CCS macrophages

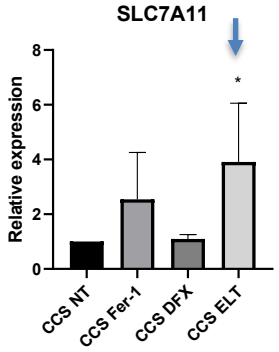
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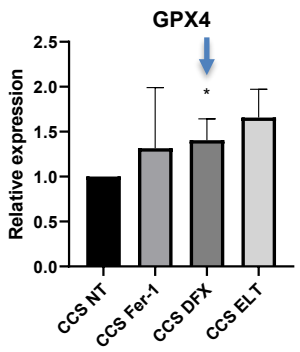
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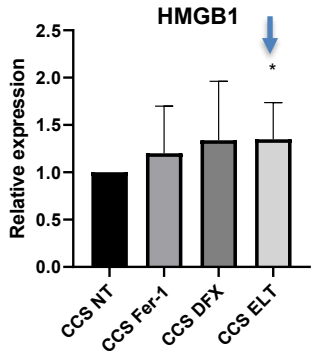
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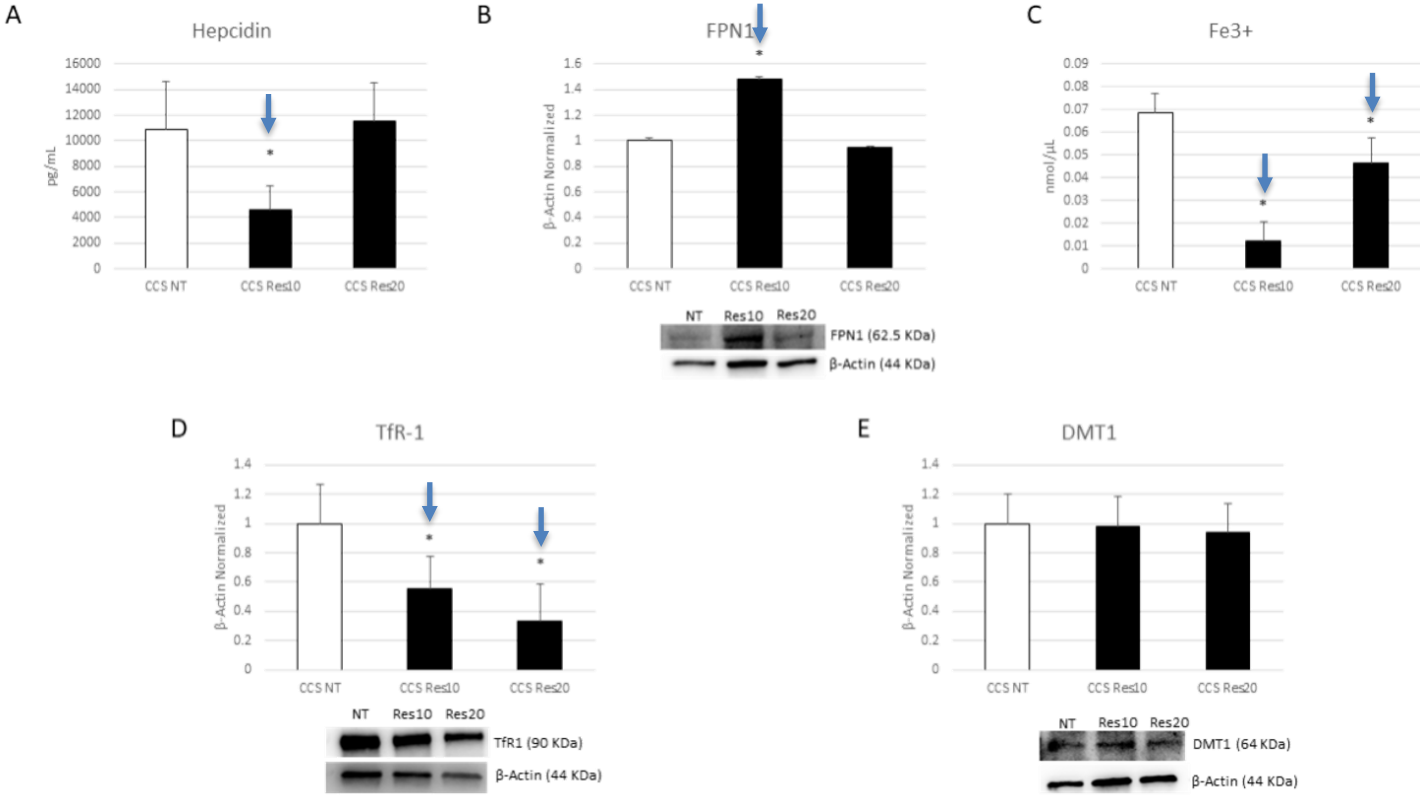
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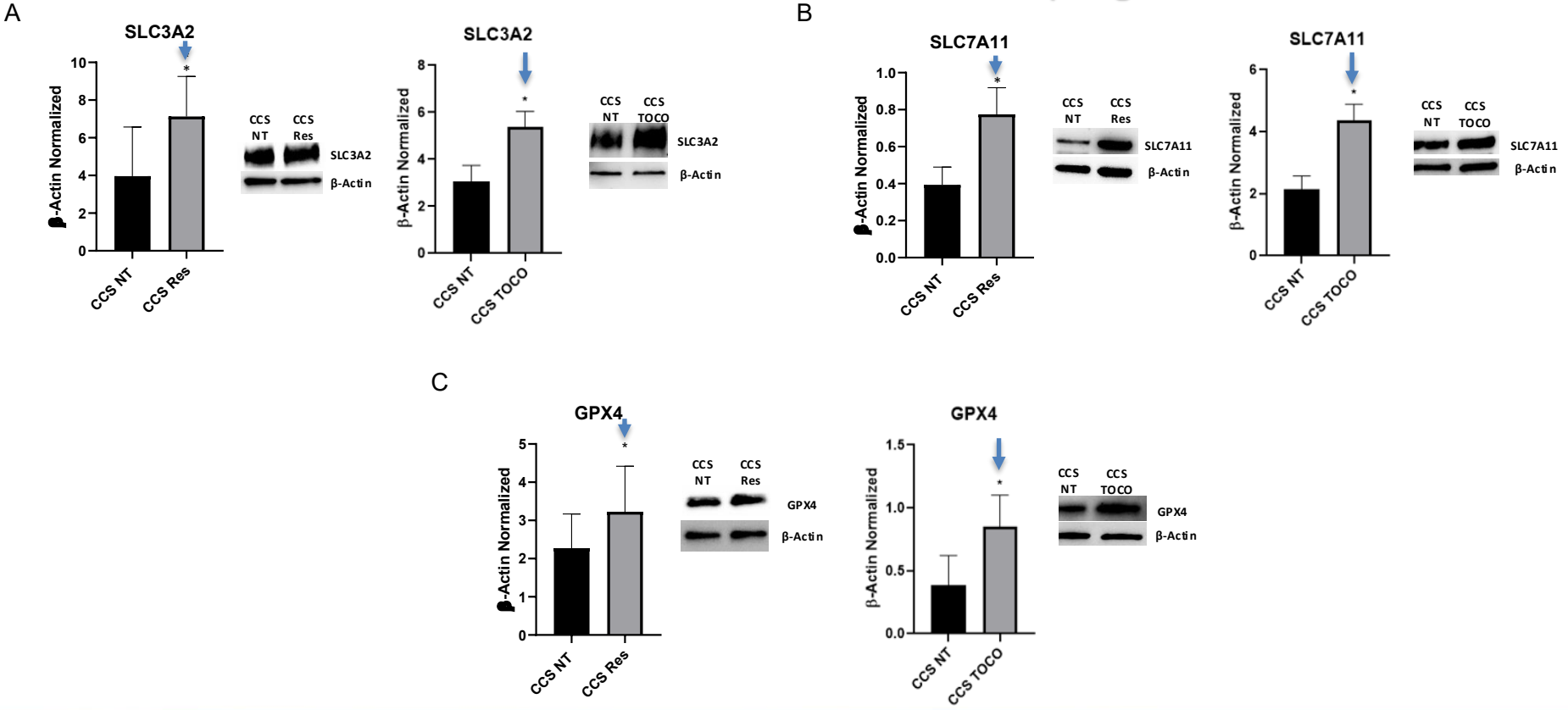


Effects of Res on iron metabolism in CCS macrophage

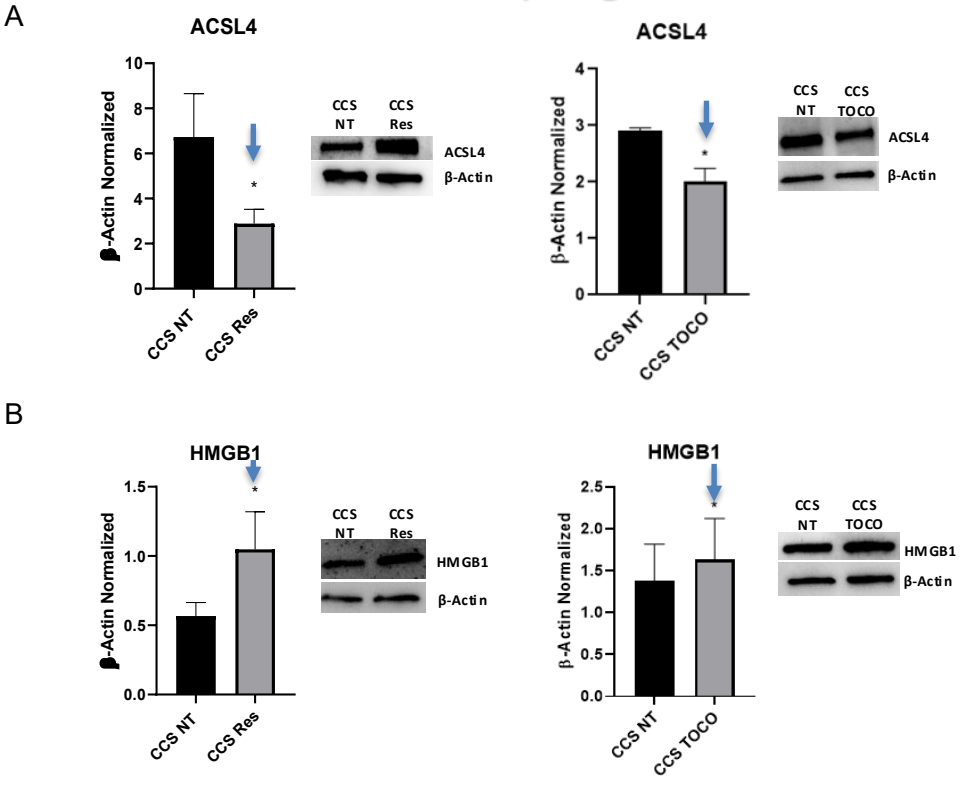


- We demonstrated that Res is able to modulate iron metabolsm in CCS macrophages by:
  - Reducing hepcidin levels;
  - Increasing FPN1 protein expression levels;
  - Reducing TfR1 protein expression levels;
  - Reducing intracellular iron concentration.

Effects of Resveratrol (Res) and Tocopherols (TOCO) on ferroptosis defense mechanisms markers in CCS macrophages

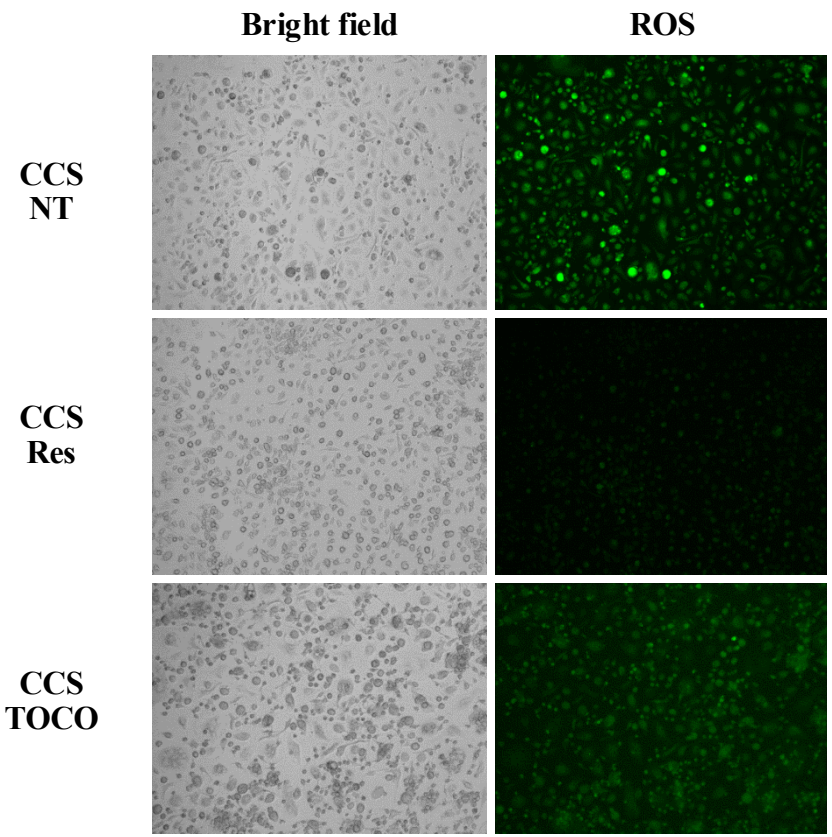


Effects of Res and TOCO on ferroptosis markers protein expression levels in CCS macrophages

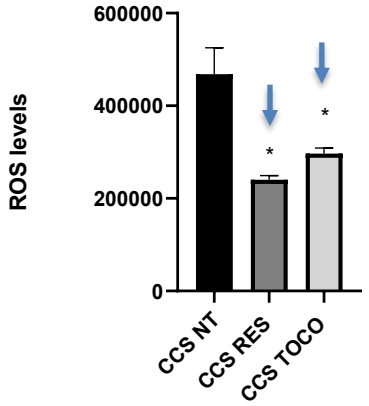


Effect of Res on ROS levels in CCS macrophages

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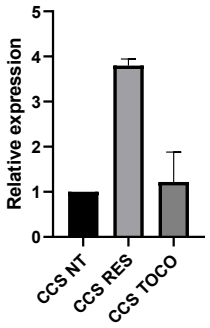


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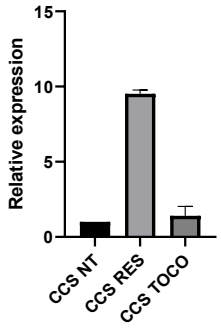


Effects of Res and TOCO on ferroptosis markers gene expression levels in CCS macrophages

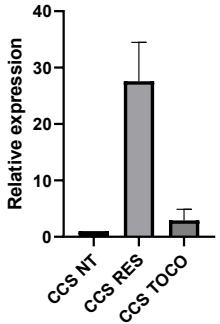
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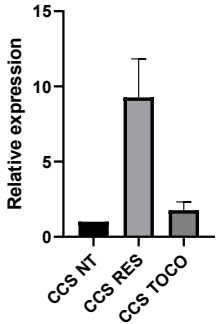
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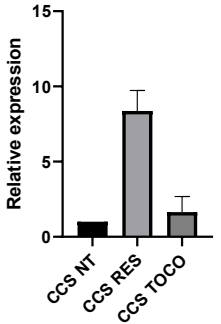
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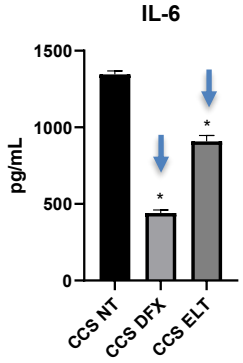


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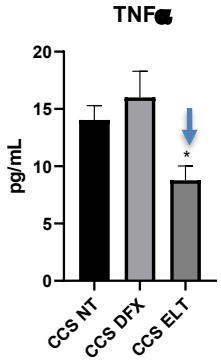


Effects of DFX and ELT on inflammatory state in CCS macrophage

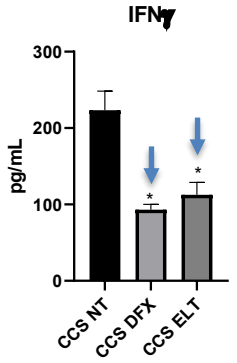
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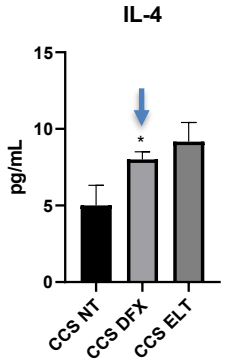
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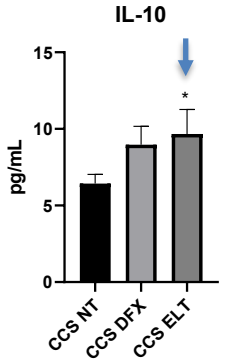
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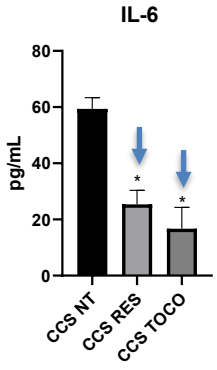


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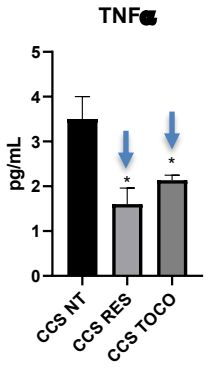


Effects of Res and TOCO on inflammatory state in CCS macrophage

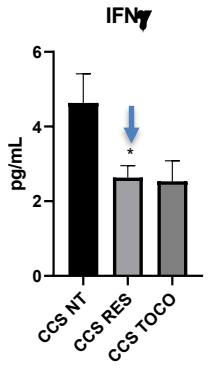
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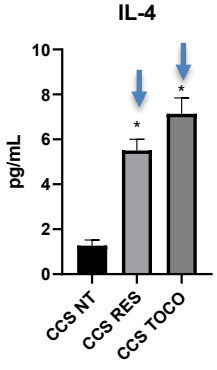
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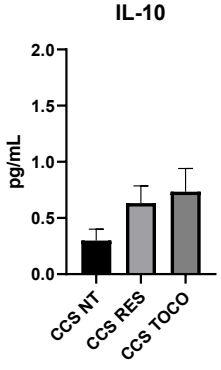
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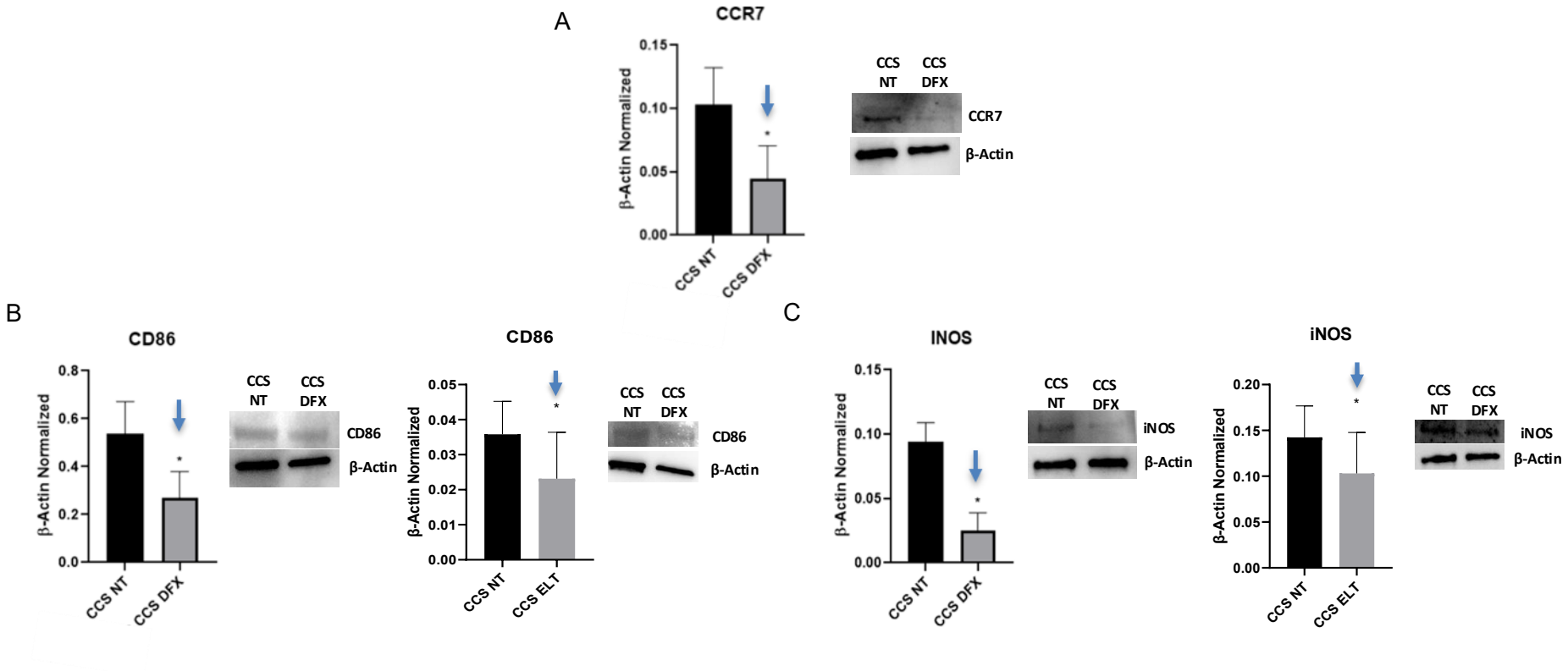


E



Effects of DFX and ELT on macrophage phenotype in CCS

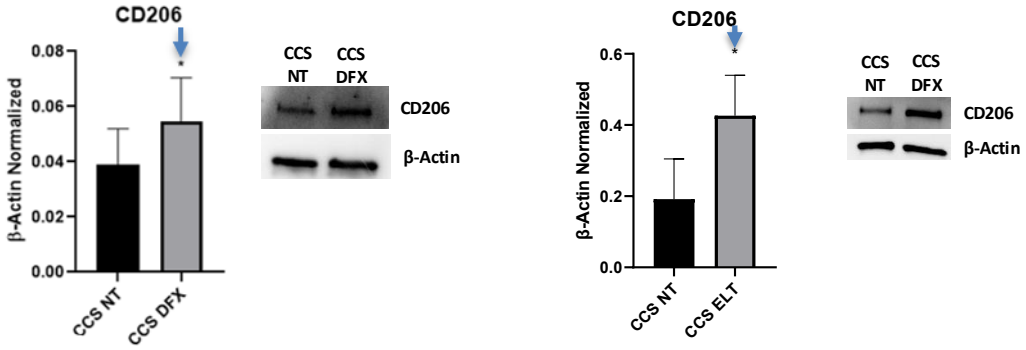
M1 Markers



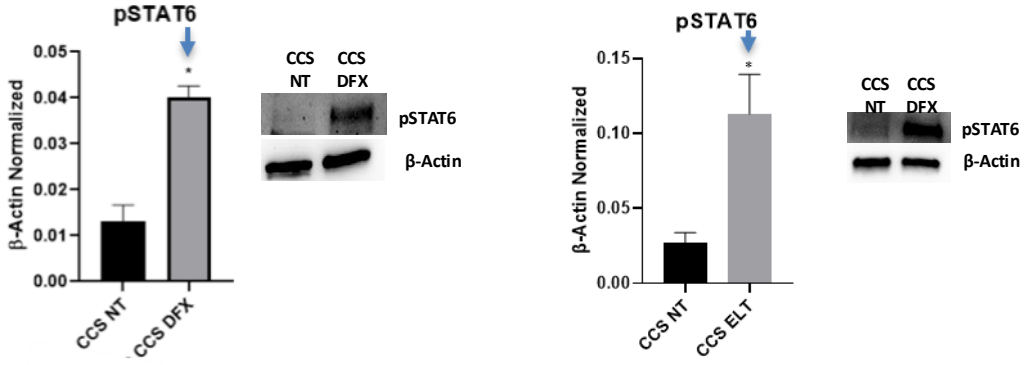
Effects of DFX and ELT on macrophage phenotype in CCS

M2 Markers

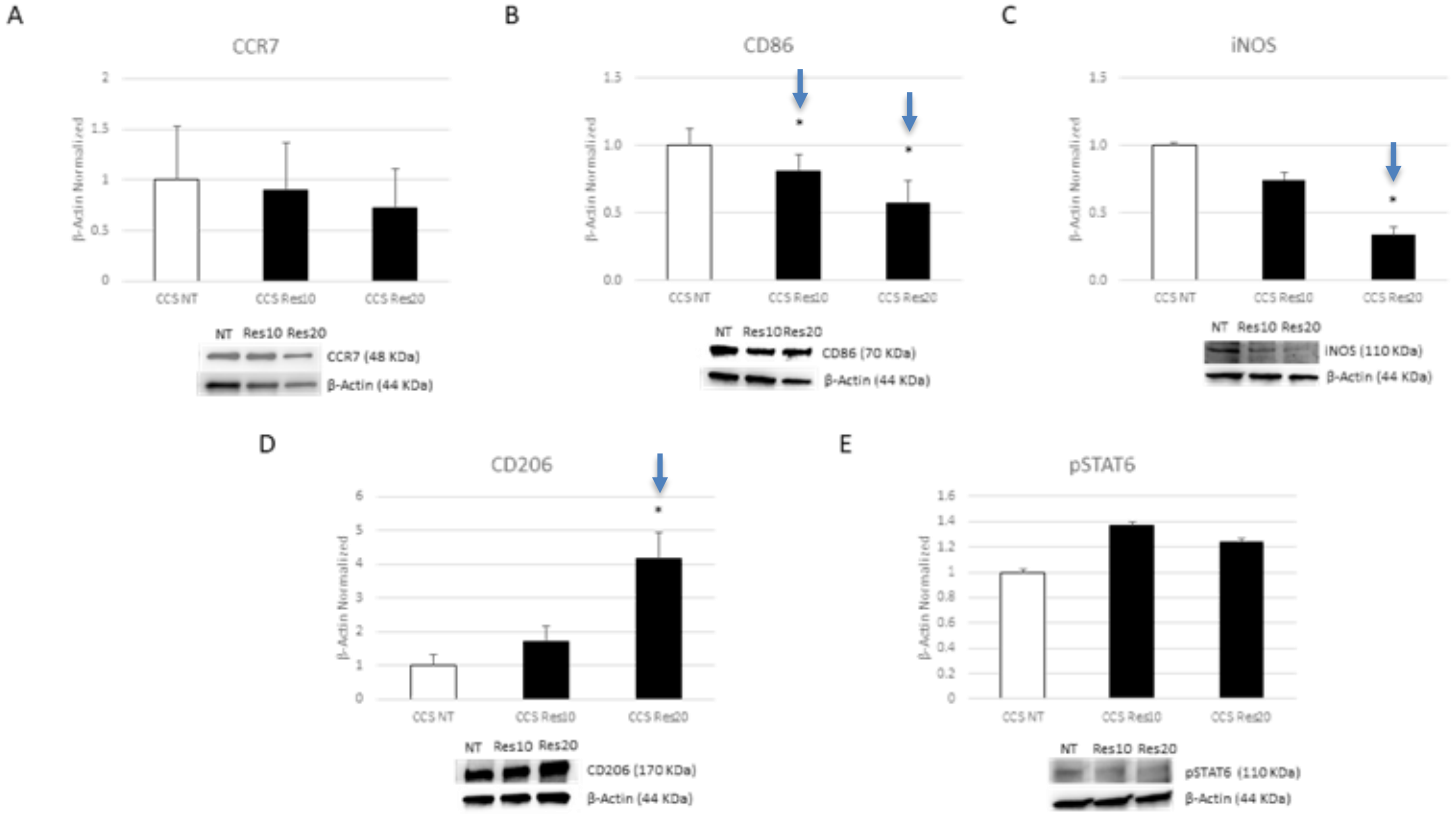
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B

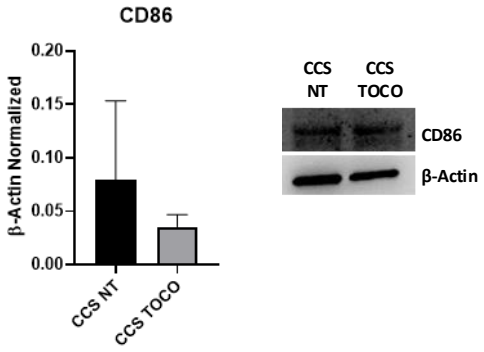


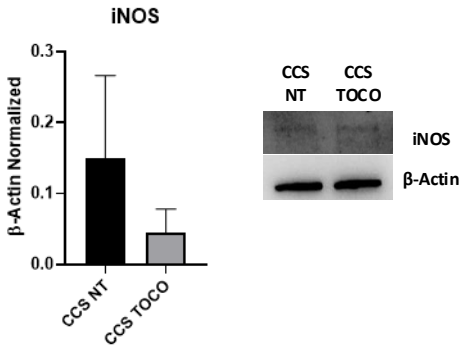
Effects of Resveratrol on macrophage phenotype in CCS

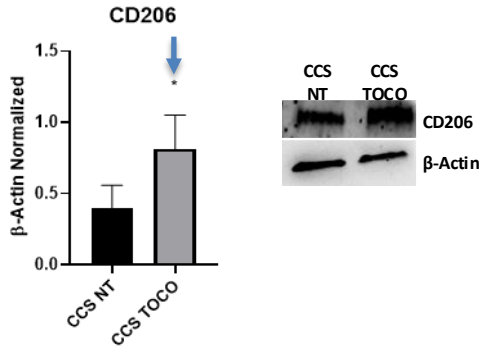


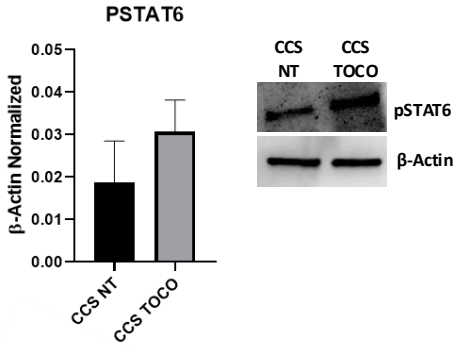
- We revealed that Res is able to reduce M1 protein expression levels markers (particularly CD86 and iNOS) in CCS.
- We revealed that Res is also able to increase M2 phenotype markers (CD206 and pSTAT6) in CCS.

Effects of TOCO on macrophage phenotype in CCS









Conclusions

- We demonstrated for the first time ferroptosis susceptibility in CCS macrophages.
- We suggest that targeting ferroptosis marker could be a novel therapeutic target to counteract inflammation in CCS patients.
- We highlight for the first time the capability of nutraceuticals to exert their anti-inflammatory properties by modulating ferroptosis in CCS, counteracting the low-grade chronic inflammatory state responsible for the inflamm-aging.
- We demonstrated that nutraceuticals induced macrophage switch towards the anti-inflammatory phenotype M2.

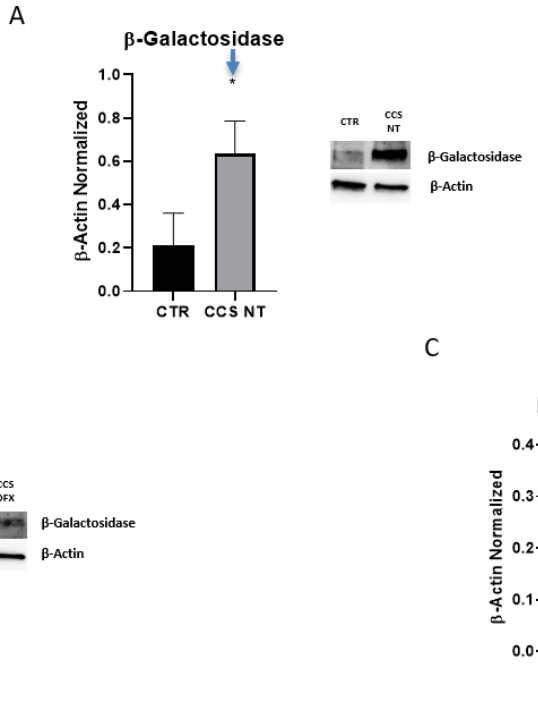
Thank you for your attention!

Thank you for your attention!

Thank you for your attention!

Ongoing investigations - Cellular Senescence

- To further investigate cellular senescence in CCS macrophages.



- Further investigations: Senescence-related markers (p16, p21) and γH2AX (DNA damage-associated markers).

Ongoing investigations – Lipid Peroxidation

- To assess lipid peroxidation in CCS macrophages using the C11-BODIPY assay.

CCS patients (n=50)	
Median age, years (mean ± SD)	14 ± 4
Sex (Female/Male)	26/24
Sideremia (μg/dl)	84.3 ± 22.9
Ferritin (ng/mL)	56 ± 35
Transferrin (mg/dl)	251.8 ± 35.7
C-Reactive Protein (mg/L)	0.17 ± 0.20
Erythrocyte sedimentation rate (mm/h)	8.2 ± 5.2
Hemoglobin (g/dl)	13.8 ± 1.2
Mean Corpuscular Volume fl	84.3 ± 4.6
Transferrin Saturation Index (%)	24.6 ± 5.3