Glutathione peroxidase 4-regulated neutrophil ferroptosis induces systemic autoimmunity

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The linkage between neutrophil death and the development of autoimmunity has not been thoroughly explored. Here, we show that neutrophils from either lupus-prone mice or patients with systemic lupus erythematous (SLE) undergo ferroptosis. Mechanistically, autoantibodies and interferon-α present in the serum induce neutrophil ferroptosis through enhanced binding of the transcriptional repressor CREMα to the glutathione peroxidase 4 (Gpx4, the key ferroptosis regulator) promoter, which leads to suppressed expression of Gpx4 and subsequent elevation of lipid-reactive oxygen species. Moreover, the findings that mice with neutrophil-specific Gpx4 haploinsufficiency recapitulate key clinical features of human SLE, including autoantibodies, neutropenia, skin lesions and proteinuria, and that the treatment with a specific ferroptosis inhibitor significantly ameliorates disease severity in lupus-prone mice reveal the role of neutrophil ferroptosis in lupus pathogenesis. Together, our data demonstrate that neutrophil ferroptosis is an important driver of neutropenia in SLE and heavily contributes to disease manifestations.

SLE is a chronic debilitating autoimmune disease characterized by a global loss of immune tolerance with activation of both innate and adaptive branches of the immune system. Central features include accumulated noncleaved cell debris derived from various forms of cell death, elevated type I interferon (IFN) signaling and increased autoantibody production. Neutrophils are the dominant immune cells in the circulation and contribute to a variety of autoimmune disorders. In patients with SLE, a number of abnormalities in neutrophils have been reported, including impaired phagocytosis, increased aggregation and accelerated cell death. Elevated levels of anti-neutrophil cytoplasmic antibodies in the sera of patients with SLE suggest that neutrophil death together with ineffective clearance of the subsequent debris provides a stable source of autoantigens for disease initiation and propagation. However, the main forms of neutrophil death in SLE and the underlying mechanisms have not been fully characterized.

Ferroptosis is a newly recognized form of programmed cell death that is morphologically, biochemically and genetically distinct from other forms of regulated cell death such as apoptosis, necroptosis and pyroptosis. It is characterized by production of lipid-reactive oxygen species (ROS) and iron overload, leading to caspase- and necrocase-independent cell death. As a key regulator of ferroptosis, phospholipid oxidase glutathione peroxidase 4 (GPX4) can detoxify hydroperoxides in membrane lipids directly, thereby reducing damage to membrane function and preventing the generation of lipid peroxidation-derived reactive products and mitigating ferroptosis. Inhibition or ablation of GPX4 induces ferroptosis in different cell types. Here, we describe how autoantibodies and type I IFNs cooperatively induce neutrophil ferroptosis in lupus through the calcium/calmodulin kinase IV (CaMKIV)/CREMα signaling axis. Importantly, the observations that neutrophil-specific Gpx4 haploinsufficiency in mice gives rise to SLE-like phenotypes and that in vivo inhibition of ferroptosis mitigates disease progression in lupus-prone MRL/lpr mice confirm the immunopathogenic effects of neutrophil ferroptosis. Therefore, our results, reported herein, identify a central cellular defect and provide the missing link between neutropenia and lupus pathogenesis.

Results

Serum factors modulate neutrophil viability in SLE. To substantiate the concept that neutropenia is a common feature in SLE, a total of 126 patients with SLE were included in the analysis (Supplementary Tables 1–3). Compared with healthy controls (HCs), neutrophil counts in patients with SLE were significantly reduced, with 35% of them presenting a neutrophil count below 2×10^9 l^-1. In contrast, this was not observed in patients with other autoimmune diseases (Fig. 1a). Moreover, to exclude the potential interference of treatment, 98 patients with newly diagnosed untreated SLE were selected and, as expected, the neutrophil counts correlated inversely with disease activity as measured by the Systemic Lupus Erythematous Disease Activity Index (SLEDAI) (Fig. 1b). Importantly, the numbers of neutrophils were restored to normal levels after effective treatment with standard-of-care medications (Fig. 1c and Supplementary Table 4).

We next evaluated the viability (live cells were gated as 7AAD^-Annexin V^-) of fresh neutrophils isolated from SLE patient blood and, as expected, they displayed lower cell viability compared with those from HCs; notably, effective treatment helped restore neutrophil viability (Fig. 1d). Collectively, these observations led us to...
Fig. 1 | SLE IgG and IFN-α modulate neutrophil viability. a. The numbers of peripheral neutrophils from either HCs or patients with different rheumatic diseases including SLE, RA, Behcet’s disease (BD) and ankylosing spondylitis (AS) (HC: n = 188; SLE: n = 126; RA: n = 50; BD: n = 50; AS: n = 50). b, The numbers of peripheral neutrophils in SLE correlated negatively with disease activity as measured by the SLEDAI (n = 98). c. The numbers of peripheral neutrophils from either HCs or patients with different rheumatic diseases including SLE, RA, Behcet’s disease (BD) and ankylosing spondylitis (AS) (HC: n = 50; BD: n = 126; RA: n = 98). d, Flow cytometry quantification of cell viability of neutrophils cultured in vitro with 20% serum from either HCs (n = 7) versus HCs. e, The in vitro effect of SLE sera on neutrophil cell viability correlated with the peripheral neutrophil counts in patients with SLE (n = 98) before and after treatments versus HCs. f, The numbers of peripheral neutrophils in patients with SLE correlated negatively with disease activity as measured by the SLEDAI (n = 98) for 16 h. g, Multiplex cytokine array of the inflammatory factors present in sera from patients with SLE versus HCs (SLE = 39, HC = 37). h, Venn diagram showing serum factors specifically increased in SLE. i, Flow cytometry quantification of cell viability of neutrophils (n = 7) cultured in vitro with SLE serum supplemented with blocking antibody targeting IFN-α at different dosages (0.1, 1, 10 μg ml⁻¹) (j), or with HC serum with the addition of IFN-α at different dosages (10⁻³, 10⁻⁴, 10⁻⁵ U ml⁻¹) (j) for 16 h. k, Flow cytometry quantification of cell viability of neutrophils (n = 7) from HCs cultured with HC serum with the addition of SLE IgG at different dosages (1.2, 2.4, 3.6 g l⁻¹) (k), or SLE serum with/without IgG depletion (l), for 16 h. Data are shown as mean ± s.d. Not significant (NS) P > 0.05. Two-tailed unpaired or paired Student’s t-test was applied. Ctrl, control; Tx, treatment.
hypothesize that the sera from patients with active SLE may promote neutrophil death. As expected, neutrophils cultured with SLE sera displayed significantly reduced viability (Fig. 1e and Extended Data Fig. 1a) and this reduction correlated positively with the severity of neutropenia in the patients (Fig. 1f and Supplementary Table 5). Of note, serum from patients with rheumatoid arthritis (RA) did not have the same effect (Extended Data Fig. 1b). To determine key factors in SLE sera responsible for this effect, we compared cytokine profiles in SLE sera with those in other autoimmune diseases and HCs. Four cytokines were specifically increased in SLE, namely IFN-α, chemokine C-X-C motif ligand 11 (CXCL11), interleukin-12p40 (IL-12p40) and IL-23 (Fig. 1g,h and Extended Data Fig. 1c). However, blockade of type I IFN signaling, but not of the other cytokines13, abrogated the enhanced neutrophil death mediated by SLE sera (Fig. 1i and Extended Data Fig. 1d), and the addition of IFN-α to HC serum promoted neutrophil death (Fig. 1j).

These results indicate that type I IFN contributes to neutrophil death in patients with lupus.

The production of autoantibodies is a hallmark of SLE and plays a critical role in disease pathogenesis14. Interestingly, the proportion of autoantibodies, such as anti-dsDNA IgG, in total IgG correlated positively with the severity of neutropenia (Extended Data Fig. 1f,i,g). To investigate whether autoantibodies are involved in neutrophil death, we purified total IgG from either healthy or SLE sera (Extended Data Fig. 1h,i,j), and applied them to healthy neutrophils in the presence of HC serum. As expected, SLE but not healthy IgG reduced the viability of neutrophils in a concentration-dependent manner (Fig. 1k and Extended Data Fig. 1k). Consistently, the depletion of IgG from SLE serum reduced the ability of serum to decrease cell viability of cultured neutrophils and the addition of IFNAR-neutralizing antibody further curtailed this modulating capability (Fig. 1l and Extended Data Fig. 1l,j,k). Taken together,
Neutrophil ferroptosis is prevalent in patients with SLE. NETosis, present in SLE sera 
α-antitrypsin, was defined as cells with a surrounding 
deocondensed chromatin and granular contents to the extracellular 
Neutrophil ferroptosis is prevalent in patients with SLE. NETosis, 
the presence of HC or SLE serum with or without addition of 
induced death in neutrophils cultured with HC serum, two ferroptosis 
and超高量の児童期が示されている(n = 4). We observed only a small 
morphological characteristics of ferroptosis5, including mitochondrial 
and disappearance of mitochondrial cristae (Fig. 2b). Similar 
but were remarkably reduced after effective systemic treatment 
(Fig. 2d). Of note, the increased levels of lipid-ROS were restricted 
to neutrophils and did not extend to lymphocytes or monocytes 
from patients with SLE, and consistently SLE serum induced 
lipid-ROS and reduced cell viability in neutrophils only (Fig. 2e,f 
and Extended Data Fig. 2a,b). In contrast to the finding that RSL-3 
duced death in neutrophils cultured with HC serum, two ferroptosis 
inhibitors, liproxstatin-1 (LPX-1) and the iron chelator 
deferoxamine (DFO), rescued neutrophil death induced by SLE 
serum (Fig. 2d). Of note, low dose of 
these results suggest that SLE IgG and IFN-α present in SLE sera 
induce neutrophil death. 

Neutrophil ferroptosis is prevalent in patients with SLE. NETosis, a unique form of neutrophil death characterized by the release of 
decondensed chromatin and granular contents to the extracellular 
space, has been proposed as an important cause of 
neuropenia in lupus15. To test this, peripheral neutrophils from both HCs and 
patients with SLE were stained with propidium iodide (PI) to 
detect dead cells and DAPI as counterstain, and neutrophil extracellular 
traps (NETs) were defined as cells with a surrounding 
DNA area exceeding 400 μm² (ref. 16). We observed only a small 
portion of dead neutrophils that could be attributed to NETosis 
(Fig. 2a). Therefore, we further looked into neutrophil death by 
electron microscopy, and we found that a significant portion of 
neutrophils isolated from patients with SLE exhibited typical morphological 
characteristics of ferroptosis5, including mitochondrial 
vacuole formation with increased mitochondrial membrane 
density and disappearance of mitochondrial cristae (Fig. 2b). Similar 
morphologic changes were recapitulated in HC neutrophils treated 
with RSL-3, a ferroptosis inducer (Fig. 2c). Consistent with this, 
the levels of lipid-ROS, both an indicator and a strong inducer of 
ferroptosis5, were higher in patients with SLE with active diseases 
and 

Fig. 3 | Neutrophil ferroptosis, the main form of neutrophil death in SLE, is induced by autoantibodies and IFN-α. a. HC neutrophil cell viability when cultured with 20% HC serum supplemented with RSL-3 (10 μM) or dimethylsulfoxide for 16 h; cell viabilities were assessed by flow cytometry (n = 4). b. HC neutrophil cell viability when cultured in 20% HC or SLE serum supplemented with increasing dosages of various reagents, including two ferroptosis inhibitors, LPX-1 (1/100/1,000 nM) or DFO (1/10/100 μM); two necroptosis inhibitors, Nec-1 (1/10/1,000 nM) or NSA (1/10/100 μM); or apoptosis inhibitor Z-VAD (0.1/1/10 μM); two necroptosis inhibitors, LPX-1 (10/100/1,000 μM) or DFO (1/10/100 μM) for 16 h; cell viabilities were assessed by flow cytometry (n = 5). c. HC neutrophils were cultured in the presence of HC or SLE serum with or without addition of Cl-amidine (Cl) (an inhibitor of NETosis, 100 μM) or LPX-1 (1 μM) for 16 h, and NETs were counted in SYTOX Green+ cells by morphology (n = 8). Neutrophils with DNA area greater than 400 μm² were considered to have undergone NETosis. Dot plots show the percentage of NETosis in all dead neutrophils in the indicated group. d. Lipid-ROS production by HC neutrophils when cultured with 20% HC serum supplemented with SLE IgG, or with SLE serum with or without IgG depletion, for 16 h (n = 11). e. Lipid-ROS production by HC neutrophils when stimulated with IFN-α at different concentrations (10-5, 10-4, 10-3 U ml⁻¹) for 16 h (n = 6). f. Lipid-ROS production by HC neutrophils when cultured with SLE serum in the absence or presence of IFNAR blocking antibody at different dosages (0.1, 1, 10 μg ml⁻1) for 16 h (n = 6). Data are shown as mean ± s.d. * or † P < 0.05, ** or 

\[ \text{Lipid-ROS MFI} \]
\[ \text{Ctrl RSL-3 SLE serum} \]
\[ \text{HC serum} \]
\[ \text{SLE serum} \]
\[ \text{LPX-1 DFO Nec-1 NSA Z-VAD} \]
\[ \text{HC serum} \]
\[ \text{SLE serum} \]
\[ \text{Ctrl IgG depletion SLE serum} \]
\[ \text{Ctrl} \]
\[ \text{Anti-IFNAR} \]
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As was expected, the addition of SLE IgG or IFN-α to HC serum increased neutrophil lipid-ROS in a concentration-dependent manner (Fig. 3d,e), whereas IgG depletion or treatment with IFNAR-neutralizing antibodies reduced the capacity of SLE sera to induce lipid-ROS production by neutrophils (Fig. 3d,f and Extended Data Fig. 1e). Although both IFN-α and SLE IgG induced neutrophil ferroptosis, SLE IgG was a more potent inducer. Of note, although there was approximately 20% NETosis in total dead neutrophils induced by IFN-α stimulation alone, the addition of SLE IgG clearly re-programmed the neutrophil death program indicated by a significant decrease of NETosis in the face of slightly enhanced neutrophil death (Extended Data Fig. 3f–i). Taken together, our results suggest
that in the SLE milieu, neutrophil ferroptosis is a dominant form of neutrophil death considering the co-presence of IFN-α and IgG.

**Inhibition of ferroptosis attenuates lupus in mice.** We next examined neutrophil ferroptosis in different lupus-prone mice. Consistent with our finding in patients with SLE, we observed decreased neutrophil viability and increased lipid-ROS production in both MRL/lpr and NZB/W F1 mice (Fig. 4a,b). Furthermore, LPX-1 treatment of MRL/lpr mice at 12 weeks of age efficiently inhibited the production of lipid-ROS in neutrophils, significantly
mitigated disease progression, and reduced the production of autoantibodies and various inflammatory cytokines, as well as increased serum complement component 3 (C3), and limited splenomegaly, lymphadenopathy and severity of lupus nephritis (Fig. 4c–j and Extended Data Fig. 4a–i). Cytoxan (CTX), a drug used to treat people with SLE, was administered as control. Of note, the NETosis inhibitor CI-amidine did not provide equivalent therapeutic values as LPX-1, confirming the distinct role of neutrophil ferroptosis in lupus pathogenesis (Extended Data Fig. 4j–o). Taken together, these results suggest that neutrophil ferroptosis is a main cause of neutropenia in lupus and that targeted therapies and correction of this abnormality yield therapeutic effects.

**SLE IgG and IFN-α suppress GPX4 expression in neutrophils.** We next conducted RNA-sequencing (RNA-seq) analysis and compared neutrophil ferroptosis-related gene expression between HCs and patients with SLE, and identified 21 down-regulated genes and two upregulated genes in SLE neutrophils (Extended Data Fig. 5a and Supplementary Tables 6 and 7). Given that GPX4 has been reported to be a key negative regulator of ferroptotic cell death by removing lipid peroxides, we confirmed that GPX4 expression was significantly decreased in the neutrophils but not other immune cells from both lupus-prone mice and people with SLE (Fig. 5a–e, Extended Data Fig. 6a–e and Supplementary Table 5), although the expression of SLC7A11 was not significantly changed (Extended Data Fig. 6a–e and Supplementary Table 5). Furthermore, when we examined GPX4 expression in neutrophils cultured with different sera, we found, not surprisingly, that GPX4 expression was down-regulated when cultured with SLE serum, especially from patients with active disease and neutropenia, but not HC serum (Fig. 5f–h and Extended Data Fig. 6h,i).
As autoantibodies and type I IFNs were found to be pivotal in inducing neutrophil ferroptosis, we next examined their effect on Gpx4 expression in neutrophils. As expected, we observed that autoantibodies and IFN-α suppressed the expression of Gpx4, whereas addition of anti-IFNAR or IgG depletion reduced the ability of SLE serum to downregulate Gpx4 (Fig. 5i–l). Of note, in healthy donors, neutrophils express significantly lower Gpx4 compared with other immune cells, which may explain why neutrophils are more sensitive to SLE serum-induced ferroptosis considering their much lower threshold of ferroptosis induction (Extended Data Fig. 6f,g). Of note, Gpx4 expression was not affected by NETosis inhibitors (Extended Data Fig. 6j,k). The expression correlation analysis indicated the involvement of FcγR3β in neutrophil ferroptosis by regulating Gpx4 (Extended Data Fig. 7a,b). The high expression of FcγR3β in neutrophils aligned with the finding that the overexpression of FcγR3β significantly enhanced the sensitivity of HL60 cells to SLE IgG-induced Gpx4 reduction, confirming the role of FcγR3β in SLE IgG-mediated neutrophil ferroptosis (Extended Data Fig. 7c–e). Because Toll-like receptor (TLR)-dependent mechanisms have been shown to trigger NETosis3,25, more information is needed to exclude their relevant contribution to neutrophil ferroptosis. Together, our results demonstrate that the autoantibodies and the increased IFN-α in SLE sera downregulate the expression of Gpx4 and lead to neutrophil ferroptosis.

Gpx4fl/fl LysMCre+ mice develop lupus-like disease. To validate the pathogenic role of neutrophil ferroptosis in vivo, we generated a myeloid-cell-specific Gpx4 haploinsufficient mouse (Gpx4fl/fl LysMCre+) to examine the direct contribution of defective Gpx4 expression in neutrophils to the breakdown of immune tolerance. As expected, Gpx4fl/fl LysMCre+ mice showed reduction of Gpx4 expression in neutrophils (Extended Data Fig. 8a,b). Of note, the percentage and absolute cell count of neutrophils in the peripheral blood were significantly decreased in Gpx4fl/fl LysMCre+ mice, which mimicked the neutropenia in patients with SLE (Extended Data Fig. 8c,d). The increased lipid-ROS production and decreased neutrophil viability in these mice phenocopied what we noted in patients with SLE, and also these defects could be rescued by addition of LPX-1 (Extended Data Fig. 8e). Furthermore, we observed lupus-like manifestations, including the production of inflammatory cytokines and autoantibodies, development of skin lesions, splenomegaly, lymphadenopathy, proteinuria and glomerular deposition of IgG, IgM and C1q in Gpx4fl/fl LysMCre+ mice (Fig. 6a–c and Extended Data Fig. 8f,g). Interestingly, Gpx4fl/fl LysMCre+ mice with homozygous Gpx4 reduction exhibited only mild signs of autoimmunity with extremely low neutrophils in vivo, which was consistent with the fact that neutrophils are requisite in lupus pathogenesis (Extended Data Fig. 8h–j). Collectively, the Gpx4fl/fl LysMCre+ mice represent a mouse model to study lupus and the findings herein strongly support the role of neutrophil ferroptosis in the immunopathogenesis in SLE.

SLE IgG and IFN-α promote CREMα binding to the Gpx4 promoter. Next, we aimed to explore the molecular pathway regulating ferroptosis in neutrophils. By nucleotide sequence analysis, we found a conserved CAMP response element (CRE) located at 42~35 base pairs upstream of the human Gpx4 promoter, which was a critical binding site for CAMP response element-binding protein (CREB) and CAMP response element modulator (CREM)26,27. CREMα is a widely expressed transcriptional repressor that has been implicated in the termination of T cell immune responses, and increased CREMα in SLE T cells has been linked to decreased IL-2 and increased IL-17F production28,29. Caki4V has been demonstrated to be involved in the translocation of CREMα to the nucleus and its binding to the CRE sites30. As expected, we observed increased nuclear accumulation of both Caki4V and CREMα in SLE neutrophils (Fig. 7a and Extended Data Fig. 9a). This phenomenon could also be induced in HC neutrophils by culture with SLE serum, or with HC serum supplemented with either SLE IgG or IFN-α (Fig. 7b,c). In contrast, SLE serum with either depletion of IgG or addition of IFNAR-neutralizing antibodies failed to induce nuclear translocation of these two molecules (Fig. 7b,c). These data indicate that autoantibodies and type 1 IFNs induce nuclear translocation of both Caki4V and CREMα in SLE neutrophils.

In agreement with the increased CREMα nuclear accumulation, the enhanced binding of CREMα to the site 42 base pair upstream of Gpx4 promoter was observed in SLE neutrophils (Fig. 7d). In addition, enrichment of CREMα correlated positively with the activation of type 1 IFN signaling (Fig. 7e). Moreover, SLE neutrophils displayed increased binding of nuclear CREMα to the site 42 base pair upstream of the Gpx4 promoter as assessed by DNA pulldown assay (Fig. 7f). Accordingly, the binding of CREMα to the Gpx4 promoter was increased in HC neutrophils cultured with SLE serum, or HC serum supplemented with either SLE IgG or IFN-α, whereas the binding complex was reduced in SLE serum mixed with IFNAR-neutralizing antibodies or depleted of IgG (Extended Data Fig. 9b).

Finally, short interfering RNA (siRNA) Knockdown of CREMα in neutrophil-like HL60 cells significantly abrogated Gpx4 inhibition by SLE serum, autoantibodies or IFN-α; in contrast, overexpression of CREMα decreased Gpx4 expression (Extended Data Fig. 9c–e), supporting that CREMα is a key regulator upstream of Gpx4 in neutrophils. As expected, HL60 cells stimulated with these factors displayed consistent changes in lipid-ROS (Extended Data Fig. 9f).

To validate the CREMα/Caki4V axis in neutrophil ferroptosis in vivo, we examined neutrophils in Camk4−/−/MRL/lpr mice and observed higher levels of Gpx4 and cell viability and lower lipid-ROS (Fig. 7g–i) compared with matched wild-type controls, confirming that Caki4V deficiency promoted Gpx4 expression and reduced neutrophil ferroptosis. Furthermore, we induced lupus-like disease in wild-type, CREM-deficient (Caki4−/−) and Caki4V-deficient (Camk4−/−) mice on the C57BL/6 background by administering pristane and adenosine IFN-α (refs. 31,32).
As expected, the combined effect of autoantibodies and IFN-α significantly decreased neutrophil GPX4 expression and viability and increased the production of lipid-ROS in wild-type but not Crem−/− and Camk4−/− mice (Fig. 7j–m). Together, our findings demonstrate that autoantibodies and IFN-α in SLE promote the nuclear translocation of CREMα and its binding to the Gpx4 promoter in neutrophils, thus suppressing GPX4 expression and resulting in ferroptosis which contributes to disease development (Extended Data Fig. 10).

Discussion
Herein, we verified the key role of GPX4 in neutrophil ferroptosis and identified two pivotal factors, serum autoantibodies and IFN-α, which were responsible for neutropenia in SLE by inducing neutrophil ferroptosis through the activation of the CaMKIV/CREMα axis. The increased nuclear translocation of CaMKIV/CREMα led to the reduction of GPX4 accompanied by increased intracellular lipid-ROS, which finally resulted in neutrophil ferroptosis.
The pathogenic role of neutrophil ferroptosis in SLE was further documented in vivo by the findings that genetic haploinsufficiency of Gpx4 in neutrophils led to the development of lupus-like disease in mice, whereas approaches to reduce neutrophil ferroptosis significantly mitigated disease development in lupus-prone mice. The immune system is poised to recognize and respond to a plethora of dying cells, components of which are potentially immunostimulatory and capable of triggering autoimmunity. Short-lived neutrophils, the most abundant innate immune cells, represent one dominant source of dying cells in the daily burden. In SLE, neutrophils are prone to cell death and the absolute numbers decrease remarkably. Increased levels of neutrophil NETosis have been reported in patients with SLE. However, in our study, by carefully characterizing the pattern of neutrophil death in SLE, we demonstrated that ferroptosis is the major form of neutrophil death, although the contribution of NETosis cannot be excluded. Collectively, our data extend previous understanding of cell ferroptosis, which has been proposed to be associated with ischemic injury and degenerative and neoplastic diseases, and provide a missing link between neutropenia and inflammation in SLE.

Unlike the far better understood programmed cell death processes such as necroptosis and pyroptosis, ferroptosis and its exact role in inflammation and autoimmunity remain largely unknown. Two ways of quenching lipid peroxidation, that is, excessive oxidative modification of polyunsaturated fatty acids and the inhibition of Gpx4, have been shown to induce cellular ferroptosis. Here, serum IgG and IFN-α have been documented to induce neutrophil ferroptosis in patients with SLE by inhibiting Gpx4 expression. However, further efforts are needed to explore additional ferroptosis triggers in SLE, including iron and lipid metabolites and also other potential signaling molecules, such as VDAC2/3, nuclear factor E2-related factor 2 (NRF2), NADPH oxidase (NOX) and p53 (ref. 38). Of note, vitamin E suppresses VDAC2/3, nuclear factor E2-related factor 2 (NRF2), NADPH metabolites and also other potential signaling molecules, such as additional ferroptosis triggers in SLE, including iron and lipid metabolites. However, further efforts are needed to delineate in detail mechanisms whereby neutrophil ferroptosis boosts inflammation in autoimmune diseases and especially in SLE. In addition, ferroptosis of other tissue-resident cells may contribute to the expression of autoimmune diseases. Previously, ferroptosis was demonstrated to account for neuronal cell death in patients with multiple sclerosis and mice immunized to develop encephalomyelitis.

In summary, whereas most current theories concerning the induction of autoimmune diseases implicate the generation of auto-reactive T and B lymphocytes, our findings propose a paradigm which places neutrophil ferroptosis at the center of the pathogenesis of SLE and highlights the importance of targeting Gpx4 transcription and neutrophil ferroptosis in the treatment of lupus.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41590-021-00993-3.

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Methods

Ethics statement. Informed consent was obtained from all human participants. Medical and economic support were provided for participants. All in vivo experiments were performed according to the guidance of the German Animal Welfare Law. Our study was approved by the Institutional Review Board and Animal Care and Use Committee of Peking Union Medical College Hospital (PUMCH) (JS-1196), Beth Israel Deaconess Medical Center's Institutional Review Board (2006P000298) and the Animal Care and Use Committee (O88-2015).

Chemicals, cytokines and antibodies. Chemicals and cytokines used were as follows: recombinant human IFN-γ (1101-01, BPL assay science), R3-SL (3S155, Selleck), LPX-1 (56799, Selleck), DFO (S5742, Selleck), Nec-1 (8037, Selleck), NSA (8251, Selleck), Z-VD-FAK (S7023, Selleck), phosphor-12-myristate 13-acetate (PMA) (P8139, Sigma), GSCK299039 (HY-18950, MedChem Express), APX-15 (HY-120801, MedChem Express), Cl-aminde (506282, Merck), β-ME (M5262, Sigma), propidium iodide (PI; Sigma), Annexin V (APC; BD Pharmingen), T-lymphotropin (T-lymphotropin; J5-1196), BODIPY 581/591 C11 (D3861, Invitrogen), penicillin (Gibco), streptomycin (Gibco), lysing buffer (555899, BD Pharmingen), DAPI (S2130, Solarbio), 7AAD (M6250, Sigma), dimethylsulfoxide (D8371, Solarbio), CTX (S2057, Selleck), β-Glucuronidase (β-Glucuronidase; B3582, Thermo Scientific), Hoechst (62249, Thermo Scientific) and 16% formaldehyde (Gibco), lysing buffer (555899, BD Pharmingen), PI (P304MP, Thermo Scientific), SYTOX Green (S7020, Invitrogen), Hoechst (62249, Thermo Scientific) and 16% formaldehyde (Gibco).

Antibodies used were as follows: anti-Gpx4 (EPNCIR144, ab125066, Abcam; 1:100), anti-CaMKIV (ab3537, Abcam; 1:1,000), anti-β-actin (beta; 1:791, Abcam; 1:1,000), anti-Cit (4.8, ab182451, Abcam; 1:200), anti-neutrophil elastase (ab68672, Abcam; 1:200), anti-CREM (western blot: nbp-21370, 21370-3, PBL Assay Science), anti-CXCL11 (ab9955, Abcam) and centrifuged, following the manufacturer’s instructions. Erythrocytes were collected for analysis. Absolute neutrophil counts were analyzed by flow cytometry. Neutrophils were isolated and cultured in complete medium supplemented with LPX-1 (1 μM) for 16 h and assessed for cell viability and lipid-ROS.

Cell isolation and in vitro culture. To isolate neutrophils and peripheral blood mononuclear cells, blood was layered on Ficoll®-Hypaque density gradients and centrifuged, following the manufacturer’s instructions. Erythrocytes were lysed with Lysis Buffer. Monocytes, B cells and pDCs were purified with CD14 microbeads (130050201, Miltenyi), CD14 microbeads (130050301, Miltenyi) and Plasmacytoid Dendritic Cells Isolation Kit II Human (130097145, Miltenyi), respectively. The preparation contained greater than 98% neutrophils as confirmed by flow cytometry using anti-CD11b and anti-CD11c. Cells were cultured in complete RPMI 1640 basic medium (Gibco) with 100 μM β13-acetate (PMA) (P8139, Sigma), GSK2795039 (HY-18950, MedChem Express), α-ME (10 mg kg−1), β-Glucuronidase (β-Glucuronidase; B3582, Thermo Scientific), Hoechst (62249, Thermo Scientific) and 16% formaldehyde (Gibco), lysing buffer (555899, BD Pharmingen), PI (P304MP, Thermo Scientific), SYTOX Green (S7020, Invitrogen), Hoechst (62249, Thermo Scientific) and 16% formaldehyde (Gibco).
to the manufacturers’ protocols. For kit-isolated IgG, 0.75 M NaHCO3 was used to neutralize the pH of the eluent. IgG was enriched by centrifugal filters (UFC905096, Merck) and resuspended in PBS.

Transmission electron microscopy. Neutrophils freshly isolated or cultured with vehicle (dimethylsulfoxide) or RSL-3 (10 μM) for 4 h were fixed with 2.5% glutaraldehyde in 0.1 M Sorenson’s buffer for 3 h, and then treated with 1% OsO4 in 0.1 M Sorenson’s buffer for 2 h. Enblock staining used 1% tannic acid. After dehydration through an ethanol series, neutrophils were embedded in LX-112 (Ladd Research Industries) and Embed-812 (EMS). The thinly cut sections were stained with 1% uranyl acetate and 0.4% lead citrate and examined under the TEM-1400 Plus electron microscope. Electron micrographs were taken at 5,000–50,000-fold magnification. Eight patients with SLE and six HCs were evaluated for mitochondrial vacuolization by electron microscopy. We randomly collected ten neutrophils per sample under a ¥50,000 lens and counted the number of mitochondria with vacuoles for quantitative analysis. Images were optimized using Photoshop CC 2017 and Illustrator CC 2017 (Adobe).

Flow cytometry. For neutrophil percentage measurements, whole-blood cells of mice were stained with combinations of antibodies including anti-Ly-6G, anti-CD11b and anti-CD45 for 30 min on ice in staining buffer. For detection of intracellular GPX4 expression, cells were stained with anti-ly-6G&ly-6C and anti-GPX4 after fixation and permeabilization with Cytofix/Cytoperm solution anti-CD11b and anti-CD45 for 30 min on ice in staining buffer. For detection of mitochondrial vacuolization by electron microscopy. We randomly collected ten neutrophils per sample under a ¥50,000 lens and counted the number of mitochondria with vacuoles for quantitative analysis. Images were optimized using Photoshop CC 2017 and Illustrator CC 2017 (Adobe).

DNA pulldown. Nuclear lysates were obtained from HC or SLE neutrophils as described above. Biotin-labeled Gpx4 promoter DNA was synthesized by Guangzhou Ribobio (forward: 5ʹ-ATTGGTGAGACTGGTGTTGTT-3ʹ reverse: 5ʹ-CAGAGTGGCAAGACCTC-3ʹ). The biotinylated promoter DNA oligos were annealed and incubated with nuclear lysates at room temperature for 1 h in binding buffer (10 mM Tris, 1 mM KCl, 1% NP-40, 1 mM EDTA, 5% glycerol). Afterward, M-280 streptavidin Dynabeads (10004D, Invitrogen) were added and incubated for 2 h at 4°C with rotation. After three washes with binding buffer, the Gpx4 promoter-binding proteins were eluted by boiling and analyzed by western blot.

RNA isolation and quantitative PCR. Total RNA was extracted with TRizol (10296010, Invitrogen), and converted to complementary DNA using PrimeScript RT Master Mix (RR036A, Takara Bio). Quantitative PCR (qPCR) was carried out with the Applied Biosystems 7500 Fast Real-Time PCR System (CA, USA). The relative expression level was normalized to GAPDH messenger RNA according to the ΔΔCt calculation method. The primers used were as follows: GAPDH Forward: 5ʹ-TCAACGCACATTGTCCAAGCTCA-3ʹ Reverse: 5ʹ-GCTGGTGCCAGGGCTTCTACT-3ʹ Gpx4 Forward: 5ʹ-GAGCAGAACCGAAGTAACACTAC-3ʹ Reverse: 5ʹ-CGGAACGTGTTACAGGGGAA-3ʹ CREM Forward: 5ʹ-CGTCGACCTTCCTTGGCAGC-3ʹ Reverse: 5ʹ-ATGACCATGGAAACAGTTGAACTCCA-3ʹ ISG15 Forward: 5ʹ-TGGAAATTGCGAGCACCTTC-3ʹ Reverse: 5ʹ-TGACCCGTATGCCCTATAGGGGAA-3ʹ

RNA-seq analysis. Total RNA of freshly isolated HC or SLE neutrophils was extracted with TRizol, and subjected to RNA-seq analysis. RNA-seq was performed by the Novogene Experimental Department using the Illumina HiSeq 4000 platform. Raw reads of fastq format were firstly processed through in-house perl scripts and all the downstream analyses were based on the clean data with high quality. An index of the reference genome was built using bowtie2 v.2.2.8 and paired-end clean reads were aligned to the reference genome using HISAT2 v.2.0.4. The mapped reads of each sample were assembled by StringTie (v.1.3.1). Cuffdiff (v.2.1.1) was used to calculate fragments per kilobase million (FPKM) of coding genes in each sample and provided statistical routines for determining differential expression using a model based on the negative binomial distribution. Transcripts with a p-adjust <0.05 were assigned as differentially expressed.

ChIP analysis. ChIP analysis was conducted on neutrophils with anti-CREM (Abclonal) and the assay was performed according to the manufacturer’s instructions (9003, CST). Each sample (4 × 106 cells) was crosslinked with 1% formaldehyde and then subjected to nuclear extraction and chromatin digestion with micrococcal nuclease. Sonication was used for complete lysis of nuclei (10-s pulse and 30-s pause; five cycles). The efficiency of chromatin digestion was determined by nucleic acid electrophoresis. For immunoprecipitation, digested chromatin was incubated with 10 μg of antibodies for 6 h at 4°C with rotation. Afterward, magnetic beads were added to the immunoprecipitation reaction for 2 h at 4°C with rotation. After washing four times, immunoprecipitated chromatin DNA was eluted. Fold enrichment was quantified using qPCR with SYBR Green Realtime PCR Master Mix (QPK-201, TOYOBO) and calculated as a percentage of input chromatin (% input). The primer sequences of Gpx4 promoter were 5ʹ- AACAGTGTCACGCTGGCTT-3ʹ (forward) and 5ʹ-ATGGTGACAGGGCGTCGGTGT-3ʹ (reverse).
Reverse: 5′-UUAACCCUUCGUAGUCUGCTT-3′
siRNA-2
Forward: 5′-GGUGGAACAUCCAGAUVUUTT-3′
Reverse: 5′-AAUUCGUAGUUGCACAACCTT-3′
siRNA-3
Forward: 5′-CCCAAGUUCGAGGUGUUTT-3′
Reverse: 5′-AACACCAUCAGAUCCUGGGTT-3′

Statistics and reproducibility. Each experiment was repeated independently more than three times, and similar results were obtained. All data were analyzed using GraphPad Prism 7 software. Shapiro–Welk test was used for normal distribution verification. For data with a normal distribution and homogeneity of variance, the independent sample t-test was used to compare differences between two groups. In some experiments, a paired Student’s t-test was applied as indicated. For data with non-normal distribution, Mann–Whitney tests were applied. Correlations were calculated using Pearson rank correlation analysis. Sample sizes were determined on the basis of previous experiments using similar methodologies and are detailed in each figure legend. For in vivo studies, mice were randomly assigned to treatment groups. Data in figure legends are presented as mean ± s.d. values or median with interquartile range. P < 0.05 was considered significant.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
All raw source data for all experiments included in this study are provided. RNA sequencing data that support the findings of this study have been deposited with the Gene Expression Omnibus (GEO) repository under accession number GSE153781. Correspondence and requests for materials should be addressed to xzpmch2003@sina.com. Source data are provided with this paper.

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Author contributions
X.Z. and P.L. conceived the project and designed the experiments. P.L., M.I., K.L. and H.L. performed most of the experiments with help from X.X., Y.X. and S.K. Y.Z. and H.L. contributed to discussions. P.L., H.L. and P.E.L. wrote the manuscript. G.C.T. and X.Z. supervised work and acquired funding.

Competing interests
P.E.L. is an employee of AMPLE but has no competing interests with the content of this manuscript. All the authors declare no competing interests.

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Extended Data Fig. 1 | IgG and IFNα but not CXCL11 or IL12/23 p40 present in SLE sera contribute to neutropenia. a–b. Flow cytometry quantification of cell viability of neutrophils (a: n = 3; b: n = 9) in vitro cultured with (a) 5%, 10%, or 20% SLE serum for 6, 16, 24 hours respectively, or with (b) 20% HC, SLE or RA serum respectively for 16 hours. c. Detection of the inflammatory factors in the sera from RA (n = 16), BD (n = 20) and AS (n = 18) patients vs. HCs (n = 19). d–e. Flow cytometry quantification of cell viability and lipid ROS of HC neutrophils (d: n = 7; e: n = 7 for anti-CXCL11 or n = 4 for Ustekinumab) cultured in vitro with 20% SLE serum supplemented with anti-CXCL11 (0.1, 1, 5 µg ml⁻¹) or Ustekinumab (0.1, 1, 10 µg ml⁻¹) for 16 hours. f–g. The proportion of anti-dsDNA in total IgG correlated with SLE neutrophil counts and SLEDAI scores (n = 63). h. Western blot validation of purified IgG from serum and of serum with IgG depletion. i. Flow cytometry quantification of cell viability of neutrophils (n = 5) cultured in vitro with serum in the presence or absence of anti-IFNAR (10 µg ml⁻¹), or IgG depletion, for 16 hours. j–k. Serum IgG was purified or depleted by Protein A/G. (j) Ponceau S staining (upper panel) and western blot (lower panel) detection of purified IgG and of serum with depleted IgG. (k) Flow cytometry quantification of cell viability of neutrophils (n = 4) with HC serum in the presence of HC or SLE IgG at different concentrations (1.2, 2.4, 3.6 g L⁻¹), or SLE serum with/without IgG depletion, for 16 hours. Data are shown as mean ± SD. *p < 0.05, **p < 0.01, ns p > 0.05. Two-tailed paired or unpaired Student’s t-test was applied.
Extended Data Fig. 2 | Ferroptosis is restricted in neutrophils but not other cells in SLE and this could be reverted by addition of Ferroptosis specific inhibitors. a. Flow cytometry quantification of cell viability of HC lymphocytes, monocytes, and neutrophils cultured with 20% HC or SLE serum for 16 hours and the proportion of apoptotic (Annexin V + 7AAD−), necrotic (Annexin V + 7AAD+) and live (Annexin V− 7AAD−) cells in each subset was analyzed (n = 6). b. HC neutrophils were cultured with 20% HC or SLE serum, and lipid-ROS productions at different time points were detected (n = 3). c. Dot plots show cell viability analyzed by lactate dehydrogenase (LDH) release. HC neutrophils (n = 9) were cultured with 20% HC serum supplemented with RSL-3 (10 μM) or SLE serum supplemented with LPX-1 (1μM) for 16 hours before analysis. d-e. Dot plots show flow cytometry quantification of the percentage of apoptotic, necrotic, and live cells. HC neutrophils (n = 5) were cultured with 20% HC or SLE serum in the presence or absence of LPX-1 (1μM) for 16 hours before analysis. f-g. HC B cells (n = 6) were cultured in 20% HC or SLE serum supplemented with LPX-1 for 72 hours, and plasmacytoid dendritic cells (pDC) (n = 3) were cultured for 24 hours, the level of IgG was assessed by ELISA and type I IFNs by flow cytometry individually. h-i. Dot plots show cell viability and lipid-ROS in HC neutrophils (n = 7) cultured with 20% HC or SLE serum supplemented with β-ME (10/50 μM) for 16 hours. Data are shown as mean ± SD. ns p > 0.05. Two-tailed paired Student’s t-test was applied.
Extended Data Fig. 3 | The cooperative effects between IFN-α and SLE IgG on cell death. a-c. HC neutrophils were cultured in the presence of HC or SLE serum with or without the addition of Cl-amidine (Cl) (peptidyl arginine deiminase 4 (PAD4) inhibitor, 100 μM), or LPX-1 (1 μM) for 4 or 16 hours and NETs were assessed in SYTOX Green+ cells based on morphology (n = 6). Neutrophils with DNA area greater than 400 μm² were considered as NETs. Dot plots show the percentage of cells forming NETs in all dead neutrophils from the indicated group. d-e. Representative fluorescent images and related quantification of NETosis. HC neutrophils (n = 6) were stimulated by PMA (50 nM) with or without LPX-1 (1 μM) for 4 hours. f-i. HC neutrophils were cultured with SLE IgG (3.6 g L⁻¹) and/or IFN-α (10⁵ U ml⁻¹) for 4 or 16 hours and cells were stained with SYTOX Green for the detection of NETs. Dot plots show the immunofluorescence microscope quantification of NETosis in total dead neutrophils from the indicated group (4 h: n = 6; 16 h: n = 3). The scale bar represents 50 μm. Data are shown as mean ± SD. ns p > 0.05. Two-tailed paired Student’s t-test was applied.
Extended Data Fig. 4 | The ferroptosis inhibitor ameliorates lupus progression with much better therapeutic effect compared to the NETosis inhibitor.

(a–i) MRL/lpr mice (n = 6) were treated with DMSO (0.1 ml 10%), LPX-1 (10 mg/kg) or CTX (20 mg/kg) every other day at week 12 for 6 weeks. DMSO (0.1 ml 10%) was applied to sex-matched MRL/Mpj mice (n = 5) as control. Mice were euthanized at 18 weeks of age for analysis. (a) Flow cytometry quantification of lipid ROS. (b) Representative immunofluorescent images of glomeruli stained with IgG (red), IgM (yellow), C1q (green), and DAPI (blue). (c–e) Flow cytometry quantification of plasma inflammatory factors and (f–i) plasma IgG. (j–o) MRL/lpr mice (DMSO, LPX-1: n = 3; Cl, Cl+LPX-1: n = 4) were treated with DMSO, Cl, LPX-1, or Cl combined with LPX-1 every other day for 3 weeks starting at the age of week 12. Mice were euthanized at 15 weeks of age for analysis. (j–k) Representative images and related quantification of axillary spleens and lymph nodes. (l) Western blot analysis of cit-H3 in circulating neutrophils from mice subjected to the indicated treatment. (m) Dot plots show the ELISA assessment of serum complement 3. (n) Dot plots show the ELISA assessment of serum anti-dsDNA antibodies titers. (o) Dot plots shows the Bicinchoninic acid (BCA) assay of urine proteins. The scale bar represents 50 μm. Data are shown as mean ± SD. ns p > 0.05. Two-tailed unpaired Student’s t-test was applied.
Extended Data Fig. 5 | The expression of cystine transporter SLC7A11 is not different between HC and SLE neutrophils. a, Heatmap visualization of RNA-seq analysis on differentially expressed ferroptosis-related genes (Standardized with GAPDH) in neutrophils between new onset treatment-naïve SLE patients (n = 6) and HCs (n = 6). b, Western blot validation for SLC7A11 antibody. 293 T cells were transfected with Slc7a11 overexpression plasmid and cells without transfection were used as control. c, Western blot assay shows the expression of cystine transporter SLC7A11 in neutrophils from HCs (n = 8) and SLE patients (n = 8). Data are shown as mean ± SD. ns p > 0.05. Two-tailed unpaired Student’s t-test was applied.
Extended Data Fig. 6 | GPX4 reduction was observed in neutrophils but not other immune cells in SLE. a. Flow cytometry quantification of GPX4 expressions in HCs (n = 16) and SLE (n = 12) neutrophils. b. GPX4 expressions in neutrophils from treatment-naïve SLE patients correlated negatively with disease activities as measured by SLEDAI (n = 12). c. Flow cytometry quantification of GPX4 expressions in lymphocytes (including CD4+ T, CD8+ T, and B cells) and monocytes from HCs (n = 11) and SLE patients (n = 9). d-e. Western blot analysis of GPX4 expressions in lymphocytes and monocytes from HCs (n = 11) and SLE patients (n = 10). f-g. Western blot analysis of GPX4 expression in HC neutrophils, monocytes, and lymphocytes (n = 7). h-i. Western blot analysis of GPX4 expression in HC neutrophils, monocytes, and lymphocytes (n = 7) cultured with 20% HC or SLE serum for 30 hours. j-k. Western blot analysis of GPX4 expression in HC neutrophils (n = 3) when cultured with 20% HC serum or SLE serum supplemented with Cl-amidine (Cl, 100 μM), APX-115 (APX) (pan-NADPH oxidase (NOX) inhibitor, 20 μM), and GSK2795039 (GSK) (NOX2 inhibitor, 10 μM). Data are shown as mean ± SD. ns p > 0.05. Two-tailed unpaired Student's t-test was applied.
Extended Data Fig. 7 | FcyR3β is essential for the SLE IgG-mediated GPX4 downregulation in neutrophils. a-b. Expression correlation analysis between different TLRs or FcRs with GPX4 based on RNA-seq data. In SLE neutrophils, (a) TLR signaling pathways are not associated with GPX4 reduction. (b) FcyR3β but not other FcRs’ expression is negatively associated with GPX4 reduction. c. Different Fc receptor expressions in HCs (n = 6) and SLE (n = 6) analyzed by RNA-seq. d. Western blot analysis of FcγR3β expressions in neutrophils, monocytes and lymphocytes from HCs (n = 3). e. GPX4 expressions in HL60 cells after overexpression of FcγR3β (n = 4). Control referred to cells without transfection. Data are presented as mean ± SD or median with interquartile range. ns p > 0.05, one-tailed or two-tailed unpaired Student’s t-test or Mann Whitney test was applied.
Mice with Gpx4 haploinsufficiency in neutrophils developed spontaneous lupus-like disease, while Gpx4^fl/flLysMCre^ mice exhibited mild autoimmunity. a-b. Flow cytometry quantification and western blot analysis of GPX4 in neutrophils (CD45^+CD11b^Ly6G&Ly6C^) and non-neutrophils (including monocytes and lymphocytes) from Gpx4^fl/fl (n = 6) and Gpx4^fl/wtLysMCre^ (n = 9) mice. c-d. Flow cytometry analysis of peripheral neutrophils (CD45^+CD11b^Ly6G&Ly6C^-) and monocytes (CD45^+CD11b^-Ly6G&Ly6C^-) from Gpx4^fl/fl (c: n = 6, d: n = 10) and Gpx4^fl/wtLysMCre^ (c: n = 9, d: n = 13) mice. e. Flow cytometry quantification of lipid-ROS and cell viability in neutrophils (n = 6) from Gpx4^fl/wtLysMCre^ mice cultured in complete RPMI 1640 basic medium in the presence or absence of LPX-1 (1 μM). f. Skin lesions of Gpx4^fl/wtLysMCre^ mice. g. Immunofluorescent images of glomeruli in Gpx4^fl/fl mice and Gpx4^fl/wtLysMCre^ mice. IgG (red), IgM (yellow), C1q (green), and DAPI (blue). h. Dot plots show the proteinuria of Gpx4^fl/fl and Gpx4^fl/wtLysMCre^ mice at 4 months of age assessed by BCA assay. i. ELISA assay shows the levels of serum complement 3 in Gpx4^fl/fl (n = 8) and Gpx4^fl/wtLysMCre^ (n = 8) mice at 6 months of age. j. ELISA assay shows the levels of serum anti-dsDNA antibodies in Gpx4^fl/fl (n = 8) and Gpx4^fl/wtLysMCre^ (n = 8) mice at 6 months of age. The scale bar represents 50 μm. Data are shown as mean ± SD, ns p > 0.05. Two-tailed unpaired or paired Student’s t-test was applied.
Extended Data Fig. 9 | IFNα and SLE IgG enhanced ferroptosis by promoting binding of CREM to the Gpx4 promoter. a. Western blot analysis of CREMα and CaMKIV in cytoplasm and nucleus of neutrophils from HCs and SLE patients. b. Dot plots show the CHIP analysis results on CREMα binding to the promoter of Gpx4 from neutrophils (n=6) with indicated treatment: IFN-α (10^5 U ml⁻¹), anti-IFNAR (10 µg ml⁻¹), SLE IgG (2.4 g L⁻¹) or SLE sera with IgG depletion. c-d. Efficiency of CREMα knockdown by siRNA (n=3) or CREMα over-expression (n=4) in HL-60 cells validated by qPCR (c) and western blot (d). e. Effect of IFN-α or SLE IgG on GPX4 expressions in HL60 cells after knockdown or overexpression of CREMα. f. Efficiency of CREMα knockdown or overexpression on ferroptosis in HL60 cells (n=4), assessed by flow cytometry using BODIPY C11. Data are shown as mean ± SD, ns p > 0.05. Two-tailed unpaired or paired Student’s t-test was applied.
Extended Data Fig. 10 | The hypothetical model for neutrophil ferroptosis in SLE pathogenesis. Autoantibodies and interferon-α present in SLE sera enhance binding of the transcriptional repressor CREMα to Gpx4 promoter, which leads to suppressed expression of GPX4 and subsequent elevation of lipid-ROS. These lead to neutrophil ferroptosis and further promote SLE progression in patients. Moreover, mice with neutrophil-specific Gpx4 haploinsufficiency develop lupus phenotype and inhibition of neutrophil ferroptosis significantly mitigates disease development in lupus-prone mice.
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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
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- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

- BD FACS Aria II (BD, Biosciences, CA, USA) for flow cytometry; TEM-1400 plus electron microscope for Transmission Electron Microscopy; Fluorescence Microscope (ZEISS) for Quantification of NET formation; 7500 Fast Real-Time PCR system (Thermo) for qPCR; A1 HD25/A1R HD25 Nikon confocal laser microscopy (Nikon, Japan) for Immunofluorescence; Illumina Hiseq 4000 platform and StringTie (v1.3.1) for RNA-seq; Western blotting detection system Tanon-5200 (Bio-Tanon, China) for blots and gels; Thermo Scientific Varioskan Flash for ELISA.

Data analysis

- GraphPad Prism (version, 7.0A) for all statistical analysis; FlowJo (version 10.4, Tree Star); LEGENDplex™ Data Analysis Software (version 8.0); Cuffdiff (v2.1.1); Applied Biosystems 7500 Real-Time PCR Software (v2.3); ImageJ (version 1.50g, NIH); Photoshop CC 2017 (Adobe); Illustrator CC 2017 (Adobe);

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Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All raw source data for all experiments included in this study are provided. RNA sequencing data that support the finding of this study have been deposited with the Gene expression Omnibus (GEO) repository under accession number GSE153781. In-house perl scripts were used to analyze RNA-seq data, which are patented.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- [ ] Life sciences
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No statistical methods were used to determine the sample size. Sample sizes were chosen to provide sufficient number of human or mice in each group for informative results and statistical testing, accounting for individual variabilities. |
| Data exclusions | No data were excluded in our study. |
| Replication | We have repeated each experiment at least three time to ensure consistent results. All repeats performed showed similar trends. |
| Randomization | Participant samples and experimental mice were allocated into experimental groups at random. For immunofluorescence, transmission electron microscopy and kidney pathology analysis, the areas were randomly selected. |
| Blinding | Blinding was not relevant to our study, for the main group of the study population was women of childbearing age |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Antibodies |
|---------------------------------|-----------|
| Involved in the study           | n/a       |
| Antigens                        | Antibodies |
| Eukaryotic cell lines           | X         |
| Palaeontology and archaeology   | X         |
| Animals and other organisms     | X         |
| Human research participants     | X         |
| Clinical data                   | X         |
| Dual use research of concern    | X         |

| Methods                         | n/a       |
| Involved in the study           | Antibodies |
| ChiP-seq                        | X         |
| Flow cytometry                  | X         |
| MRI-based neuroimaging          | X         |

Antibodies

- Anti-glutathione peroxidase 4 (GPX4) antibody (EPNCIR144, ab125066, Abcam; 1:1000), anti-CaMKIV antibody (ab3557, Abcam; 1:1000), anti-histone 3 antibody (ab1791, Abcam; 1:1000), anti-CIq antibody (4.8, ab182451, Abcam; 1:200), anti-neutrophil elastase antibody (ab68672, Abcam; 1:100), anti-CREM antibody (WB: nbp2-16009, NOVUS; 1:1000), anti-FcγR3b antibody (MMHAR-3, 21370-3, pbl assay science), anti-CXCL11 antibody (ab9955, Abcam), and anti-human IL-12/23 (ustekinumab) (HY-P9909, Medchem Express).

Validation

All antibodies were obtained commercially validated by the respective company. All antibodies had validation statement provided on the website of the manufacturer. Related technical data sheets can be obtained from the manufacturer's website using the catalog number provided above.
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)  
HL-60: KG141, Keygen  
293T: CL-0005, Procell

Authentication  
The HL-60 cell line was purchased from Library of Chinese Academy of Sciences cells; The 293T cell line was purchased from Procell Corporation. Cell line was authenticated prior to receipt by the commercial vendor using the standard method.

Mycoplasma contamination  
Cell line tested negative for mycoplasma contamination

Commonly misidentified lines  
(See ICLAC register)  
The study didn’t involve misidentified lines

Animals and other organisms

Policy information about studies involving animals, ARRIVE guidelines recommended for reporting animal research

Laboratory animals  
Female MRL/Mpj and MRL/Mpj-Faslpr (MRL/lpr) mice were used at an age of 12-18 weeks  
Female Crem-/- CS7BL/6 and Camk4-/- CS7BL/6 mice were used at an age of 8-20 weeks  
Female Camk4-/- MRL/lpr mice were used at an age of 18 weeks  
Female NZB/W F1 mice were used at an age of 8-20 weeks  
Female and male C57/B6-Gpx4fl/fl (000486, Jackson) and C57/B6-LysMcre (000485, Jackson) mice  
The GPX4fl/fl mice were bred to the LysMcre mice to generate female and male GPX4fl/wtLysMcre+ and GPX4fl/flLysMcre+ versions of these strains. These mice were used at an age of 12-52 weeks

Mice were housed with a dark/light cycle of 12 hours, a temperature of 20-24°C, and a humidity of 45-60%.

Wild animals  
The study didn’t involve wild animals.

Field-collected samples  
The study didn’t involve samples collected from the field.

Ethics oversight  
All in vivo experiments were performed according to the guidance of the German Animal Welfare Law. Our study was approved by the Institutional Animal Care and Use Committee of Peking Union Medical College Hospital (PUMCH) (JS-1196), Beth Israel Deaconess Medical Center’s Institutional Animal Care and Use Committee (IACUC-2015).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics  
The characteristics of patients involved in the study were shown in supplementary table 1-5, 7.

Recruitment  
Patients were prospectively recruited in our rheumatology outpatient department fulfilling the diagnosis of SLE. Treated patients involved in the study all received standard medical care to exclude the treatment bias. And sample selection for study inclusion was based on the diagnosis of SLE according to the criteria established by the American College of Rheumatology and therefore no potential self-selection bias is present and hence there is no impact on the results.

Ethics oversight  
Informed consent was obtained from all human participants. Our study was approved by the Institutional Review Board of Peking Union Medical College Hospital (PUMCH) (JS-1196) and Beth Israel Deaconess Medical Center’s Institutional Review Board (2006P000298).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots  
Confirm that:  
✓ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).  
✓ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a “group” is an analysis of identical markers).  
✓ All plots are contour plots with outliers or pseudocolor plots.  
✓ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology  
Sample preparation  
Freshly isolated or cultured neutrophils, lymphocytes and monocytes in different experimental conditions were prepared for flow cytometry analysis.
| Instrument          | BD FACS Aria II (BD Biosciences, CA, USA) |
|---------------------|------------------------------------------|
| Software            | FlowJo (version 10.4, Tree Star)         |
| Cell population abundance | The preparation contained greater than 98% neutrophils as confirmed by flow cytometry using CD16 (Biosciences) and CD11b (Biolegend) antibodies. |
| Gating strategy     | According to the parameters of FSC/SSC, cell debris, lymphocyte population, monocyte population, and neutrophil population were distinguished. The neutrophil population had the largest FSC/SSC parameters. Then live cells were identified as 7AAD (-)/Annexin V (-) group. Monocytes and neutrophils were identified as CD45(+)CD11b(+)Ly6G&Ly6C(-) and CD45(+)CD11b(+)Ly6G&Ly6C(+) respectively in mice. |

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.