SUPPLEMENTARY INFORMATION

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. IRP2 and experimental COPD. (**a**) Representative Irp2 immunostaining in the airways of mice (arrows indicate Irp2) in WT mouse lungs exposed to room air (RA) or CS (1–6 months) exposed to RA or CS for 1month or 6 months. *n* = 2 technical replicates. Scale bar 50 μm. (**b**) Co-staining of Irp2 (red), podoplanin (type I-cell), pro-SPC (type II-cell), acetylated alpha-tubulin (ciliated cell), uteroglobin (secretory cell), F4/80 (alveolar macrophage) or cytokeratin-5 (basal cell) or Pro3/Hoechst (nuclei) in mice exposed to RA or CS (6 months) (*n* = 2 biological and *n* =2 technical replicates). Scale bar 50 μm or 10 μm (F4/80 only). (**c-d**) IRP2 immunoblot expression in (**c**) primary human bronchial epithelial cells and (**d**) in the human airway epithelial cell line Beas2B exposed to cigarette smoke extract (20%) for the indicated times (*left*) with confocal analysis of IRP2 staining in Beas2B cells treated with CSE (20%) for 30 minutes (*right*). Scale bar 10 μm. *n* = 3 biological replicates. All data are representative of three independent experiments.

Supplementary Figure 2. *Irp2*$^{-/-}$ mice are protected from CS-induced emphysema independently of Irp1. (**a**) Mean chord lengths (*left*) and weighted mean diameters (*right*) of *WT* (RA, *n* = 5;CS, *n* = 3) or *Irp2*$^{-/-}$ (RA *n* = 6, CS *n* = 6) C57/BL6 mice exposed to room air (RA) or cigarette smoke (CS) for 4 months (*n* = 10 images per mouse). (**b**) Cleaved caspase 3 or Bcl 2 expression levels by immunoblot analysis (WTRA *n* = 5; WTCS *n* = 3; *Irp2*$^{-/-}$ RA, CS *n* = *6*; *n* = 2 technical replicates). (**c**) LC3B (*left*) and Atg 7 (*middle*) immunoblot expression and LC3B immunostaining (*right*) (representative images of *n* = 2 technical and *n* = 4 biological replicates per group) of WT and *Irp2*$^{-/-}$ murine lungs exposed to RA or CS (4 months). (**d**) Irp1 immunoblot (*left*) analysis with densitometry (*right*) in WT or *Irp2*$^{-/-}$ mice exposed to RA or CS for 4 months. (**e**) Total leukocyte counts, total alveolar macrophages, total lymphocytes, total PMN's and (**f**) total BAL protein in WT mice exposed to CS (1 month)*, n* = 3 technical replicates. All data are mean ± s.e.m. **P* < 0.05, ***P* < 0.01 by Two-Way ANOVA followed by Bonferroni correction. $^{\#}P$ < 0.05 by unpaired Student's t-test. $^{\#}P$ < 0.05, $^{\#\#\#}P$ < 0.01 by unpaired Student's *t*-test. n.s., not significant.

Supplementary Figure 3. Irp2 deficient mice and hyperoxia induced lung injury and CLP models. (**a**) Survival to hyperoxia (> 99% O_2) over 0-120 hours in WT (*n* = 10) and *Irp2*$^{-/-}$ (*n* = 17) mice. (**b**) Total BAL leukocytes (*left*), total BAL protein (*right*), (**c**) BAL IL-6 (*left ELISA*) and BAL IL-33 (*right ELISA*) concentrations in WT (control *n* = 4;hyperoxia *n* = 5) and *Irp2*$^{-/-}$ mice (control *n* = 5;hyperoxia *n* = 4) exposed to hyperoxia for 70 hours. (**d**) CLP survival over 0-168 hours in *WT* (*n* = 7) and *Irp2*$^{-/-}$ (*n* = 8) mice. (**e**) Total BAL leukocytes (*left*), total BAL protein (*right*) levels, (**f**) BAL IL-6 (*left ELISA;* WT sham *n* = 2;CLP *n* = 4; *Irp2*$^{-/-}$ sham *n* = 3;CLP *n* = 4;) and BAL IL-33 (*right ELISA;* WT sham *n* = 2;CLP *n* = 4; *Irp2*$^{-/-}$ sham *n* = 3;CLP *n* = 4) concentrations in WT and *Irp2*$^{-/-}$ mice 24 hours after CLP surgery. (**g**) Plasma IL-1β (*left ELISA;* WT sham *n* = 2;CLP *n* = 4; *Irp2*$^{-/-}$ sham *n* = 3;CLP *n* = 3) and plasma IL-18 (*right ELISA;* WT sham *n* = 2;CLP *n* = 4; *Irp2*$^{-/-}$ sham *n* = 3;CLP *n* = 3) levels in WT and *Irp2*$^{-/-}$ mice 24 hours after CLP surgery. All data are mean ± s.e.m. **P* < 0.05. ***P* < 0.01, ****P* < 0.005 by one-way ANOVA.

Supplementary Figure 4. RNA-immunoprecipitation of IRP2-RNA complexes from Beas2B cells treated with and without DFO. (**a**) Workflow of RIP-Seq. (**b**) Immunoblot of

IRP2 immunoprecipitation with IgG, 2 µg or 5 µg IRP2 IgG in Beas2B cells. (**c**) Quality of RNA-Seq library generated. (**d**) To identify RIP-Seq peaks four primary comparisons were made: CTL compared to IgG-CTL (CTL/IgG), DFO compared to IgG-DFO (DFO/IgG), CTL compared to DFO (CTL/DFO) and DFO compared to CTL (DFO/CTL). (**e**) Enrichment of Peak scores of known IRP2 targets, ferritin (FTL) and transferrin receptor 1 (TfR) in the RIP-Seq data set and (**f**) validated by q-PCR. (**g**) A clustering of the annotations (black ticks) between the 4375 genes (x-axis) that had a RIP-Seq peak in any of the defined sets (CTL-specific, DFO-specific or Common) and the GO categories found to be enriched (FDR < 10^{-3}) in these genes (y-axis) based on DAVID analysis. Five clusters, or "communities", of genes and enriched GO categories emerged (denoted by blue: community 1, yellow: community 2, turquoise: community 3, red: community 4 and green: community 5). This data was used to define each of the "community" segments of the Circos plot. All data are mean ± s.e.m. **P* < 0.05, by Two-Way ANOVA followed by Bonferroni correction. $^{\#}P$ < 0.05, $^{\#\#}P$ < 0.001 by unpaired Student's *t*-test. ND, not detected.

Supplementary Figure 5. Within Community 2 we identified three collections of highly related GO categories (*see Fig. 3a*). These collections are composed of functions related to mitochondria, protein localization or other GO terms. Here we show genes within Community 2 that are significantly differentially-expressed (*P* < 0.01 based on unpaired two-sample *t*-test), based on a microarray study of lungs from *Irp2*$^{-/-}$ and WT mice and are also annotated to GO categories within the (**a**) 'Protein localization' collection of categories or (**b**) the 'Other' collection of categories. The expression values shown for each gene (row) are *z*-score normalized for visualization purposes.

Supplementary Figure 6. Expression of mitochondrial genes in the (**a**) LGRC and (**b**) ECLIPSE COPD cohorts. Expression data was downloaded from LGRC (GSE47460); selected mitochondrial genes (**Fig. 3a**) were measured in patients with high or low *IRP2* expression. In each heat map, the subjects are ordered (from left to right) based on increasing values of IRP2 gene expression, with a white bar delimiting which individuals were identified as having "low" *IRP2* expression (less than the median across all subjects) or "high" *IRP2* expression (greater than the median across all subjects). Rows are ordered the same as **Fig.3a** and each row is *z*-score normalized for visualization purposes. LGRC; *n* = 121 COPD patients, *n* = 20 non-smokers and *n* = 18 smokers. ECLIPSE; *n* = 136 COPD subjects, *n* = 84 smoker controls, *n* = 6 non-smoker controls. The overall "meta" *P*-values (for the genes in the heat map) were: COPD-subjects: 1.4e–3, Smoker-controls: 0.9564, Non-smoker controls: 0.5303.

Supplementary Figure 7. IRP2 and cell death (**a**) Representative cytochrome c immunostaining of WT and *Irp2*$^{-/-}$ murine airways exposed to RA or CS (6 months), *n* = 2 technical replicates. Scale bar: 100 µm. (**b**) Human airway epithelial cells (Beas2B) treated with siRNA to *IRP2* are protected from CS-induced cell death and mitochondrial reactive oxygen species (mROS) production. *n* =2 technical duplicates of *n* =3 biological replicates. (**c**) Flow cytometry analysis of mROS production using MitoSOX in CSE-treated Beas-2B cells with non-target (NT) siRNA or si*IRP2* siRNA. Data was normalized to Beas2B cells transfected with control non-target (NT) siRNA. *n* =2 technical duplicates of *n* =3 biological replicates. All data are mean ± s.e.m. of three independent experiments **P*< 0.05, by Two-Way ANOVA followed by Bonferroni correction.

Supplementary Figure 8. CS increases the labile iron pool and disrupts Fe-S assembly in human epithelial cells. (**a**) Immunoblot expression of IRP2 in human epithelial Beas2b cells treated with empty vector (CTL) or five shIRP2 clones showing efficiency of IRP2 knockdown in Clone #3 (*left*). Labile iron pool was measured in CSE-treated (20% 24h) Beas2B cells (+/- clone 3 sh*IRP2*) (*right*). Cells were treated with ferric ammonium citrate (Fe^{3+}) (FAC) as a positive control. (**b**) Perls' staining quantification (*n* = 10 images per mouse, *n* = 2 technical replicates) (NC, negative control) in WT and *Irp2*$^{-/-}$ mice exposed to RA or CS (4 months), *n* = 3 per group. (**c**) Frataxin expression by immunoblot or RT-PCR analysis in Bea2B cells treated with 20% CSE for the indicated times. (**d**) Immunoblot analysis of ferritin expression in WT or *Irp2*$^{-/-}$ mice exposed to RA or CS for 4 months, *n* = 3 per group. (**e**) Fold change non-heme iron in cytosolic fractions WT (RA, *n* =12; 1 month CS *n* =5; 4 months CS *n* =3; 6 months CS, *n* = 5) or *Irp2*$^{-/-}$ (RA, *n* = 12; 1 month CS *n* =5; 4 months CS *n* =3; 6 months CS, *n*= 5) mice exposed to RA or CS for 1–6 months. (**f**) Fold change heme iron levels in cytosolic fractions WT (RA, *n* = 8; 1 month CS *n* = 5; 6 months CS, *n* = 5) or *Irp2*$^{-/-}$ (RA, *n* = 8; 1 month CS *n* = 5; 6 months CS, *n* = 5) mice exposed to RA or CS for 1 or 6 months. (**g**) Immunoblot analysis of FBXL5 expression in WT or *Irp2*$^{-/-}$ mice exposed to RA or CS for 1-6 months. *n* =3 per group. (**g**) Fluorescent assay for the detection of 2Fe2S mitochondrial (l*eft*) or cytosolic (*right*) Fe-S clusters. (**h**) Immunoblot analysis of heme oxygenase 1 expression in WT or *Irp2*$^{-/-}$ mice exposed to RA or CS for 4 months, *n* = 3 per group. All data are mean ± s.e.m. **P* <0.05, ****P* <0.005 by one-way or ANOVA followed by Bonferroni correction. $^{\#}P$ <0.05, $^{\#\#}P$ <0.01 by student's unpaired *t*-test.

Supplementary Figure 9. IRP2 regulates the response of ETC Complexes to CS**.** (**a**) Immunoblot analysis of Complexes I-V of the electron transport chain in normalized mitochondrial-enriched fractions generated from the lungs of WT or *Irp2*$^{-/-}$ mice exposed to RA or CS (4 months), *n* =3 per group. (**b**) COX4I2 expression and densitometry of WT or *Irp2*$^{-/-}$ mice exposed to RA or CS (6 months), *n* =3 per group. (**c**) MCC of mice with two knockin mutated alleles of Sco2 (*Sco2*$^{ki/ki}$) after 1 month exposure to RA or CS. (**d**) Infiltrating alveolar macrophages in the BAL of WT and *Sco2*$^{ki/ko}$ mice exposed to RA or CS (1 month). (**e**) Fold change non-heme iron levels and (**f**) heme iron levels in cytosolic fractions of WT and *Sco2*$^{ki/kio}$ mice after 1-month exposure to RA or CS, *n* =3 per group. All data are mean ± s.e.m. **P* <0.05 by one-way ANOVA. $^{\#}P$ <0.05 by unpaired Student's t-test.

Supplementary Figure 10. Mitochondrial iron chelation or a low iron diet alleviates CS-induced bronchitis. (**a**) Percentage weight gain (*left*) and non-heme iron content of serum (*right*) over 6 weeks of WT mice on a control (300 ppm iron), low iron (6 ppm iron) or high iron diet (2% carbonyl iron). (**b**) Non-heme iron content (*left*) (CTL *n* =8; High *n* =13;low *n* =12) and serum non-heme iron (*right*) (CTL *n* =8; High *n* =6;low *n* =7) in mice on a control, low or high iron diet. (**c**) Total macrophage (*left*), lymphocyte (*middle*), PMN (*right*) BAL cell counts, (**d**) total BAL IL-33 (ELISA) (control n= 9;DFP control n= 10; 1 month CS n= 6; 6 weeks CS n= 6; 6 weeks +DFP n= 6; 8 weeks CS *n* = 4; 8 weeks CS +DFP *n* = 5) and (**e**) whole lung IL-6 (ELISA) expression (control *n* = 8;DFP control *n* = 8; 1 month CS *n* = 9; 6 weeks CS *n* = 5; 6 weeks +DFP *n* = 5; 8 weeks CS *n* = 3; 8 weeks CS +DFP *n* = 5) in WT mice exposed to RA or CS (1 month), followed by treatment with DFP for 2 weeks or 4 weeks, *n* =2 technical replicates. Black arrows indicate time points and blue arrows indicate time of DFP addition. All data are mean ± s.e.m. **P* <0.05. ***P* <0.01, ****P* <0.005 by one-way ANOVA.

SUPPLEMENTARY TABLE 1. COPD related IRP2 target transcripts

Gene	Encodes for	Protein Function	COPD	$^{\xi}$*IRP2*	*COPD Log2FC (p-value)	$^{\#}$COPD Log2FC (p-value)
Airway Remodeling: Extracellular Matrix						
SERPINE1	Plasminogen activator inhibitor-1 (PAI-1),	Regulator of fibrinolysis at sites of vascular injury	Increased in the Sputum of COPD patients[10]	117.80	-0.01459 (0.82246)	0.68924 (0.36604)
MDM2	nuclear-localized E3 ubiquitin ligase	Targets the tumor suppressor protein p53 for proteasomal degradation.	p53 protein levels are increased in COPD smokers in comparison with non-COPD smokers [11].	117.59	0.02758 (0.04640)	-0.06707 (0.77126)
CCN1	CCN1(cysteine-rich, angiogenic inducer, 61)	Extracellular matrix molecule	Mediates CSE induced IL-8 secretion by lung epithelial cells[12]	95	0.002362 (0.91591)	0.34074 (0.36153)
TGFB1	TGFB1(transforming growth factor, beta-induced, 68kDa) protein	Induced by transforming growth factor-beta (TGF-β) to become a part of the extracellular matrix. Thought to play a role in cell adhesion and cell migration.	While TGFB1 has not been previously associated with the pathogenesis of COPD, TGF-β signaling pathways have been implicated in COPD[13].	42.40	0.01205 (0.28329)	0.28703 (0.26225)
ADAM	ADAM17 (Metallopeptidase domain 17)	Metalloproteinase	ADAM17 may be involved in the mechanism by which CS increases mucin 5AC (MUC5AC), a prevalent airway mucin in the lung[14].	44.65	-0.011018 (0.46769)	0.228256 (0.21523)
Cell cycle						
CDKN1A	p21/Cip1	Cyclin-dependent kinase inhibitor that binds to and inhibits the activity of cyclin-CDK2 or -CDK4 complexes, functioning as a regulator of cell cycle progression at G1.	Increased in COPD patients and has been associated with airway epithelium alterations, including squamous cell metaplasia[15].	60	0.09838 (0.02466)	0.76696 (0.38290)
Stress Responses						
HSPA5	GRP78(glucose-regulated protein, 78kDa)	Member of the HSP family of molecular chaperones required for endoplasmic reticulum integrity and stress-induced autophagy. Plays a central role in regulating the unfolded protein response (UPR)	Up-regulated in smokers compared with nonsmokers and ex-smokers[16].	23.29	-0.00430 (0.62770)	0.05632 (0.73164)
CAV1	CAV-1(caveolin 1)	Scaffolding protein that is the main component of the caveolae plasma membranes found in most cell types.	Cav-1(-/-) mice exhibit higher levels of autophagy and apoptosis in the lung in response to chronic CS exposure *in vivo*[17].	45.5	-0.083373 (6.19E-08)	-0.599497 (0.00066)
Inflammatory						
IL-8	IL-8 (Interleukin 8)	Chemokine produced by macrophages and other cell types	IL-8 secretion has been previously associated with CS exposure[18] and COPD	43	0.19167 (0.01425)	1.78691 (0.14732)

		including epithelial cells, airway smooth muscle cells and endothelial cells. IL-8 is secreted as a result of innate immune receptor signaling and is an important mediator of the immune reaction in the innate immune system response.	patients have significantly higher values of IL-8 when compared to healthy smokers[19].			
IL-11	IL-11 (Interleukin 11)	Stimulate the T-cell-dependent development of immunoglobulin-producing B cells	Altered expression of IL 11 may be involved in the genetic predisposition to COPD[20].	12.4	0.15595 (0.01454)	0.73357 (0.57823)
TNFRSF12A	Tumor necrosis factor receptor superfamily	TNF signaling	Increased TNF signaling in COPD[21,22]	25.6	-0.01204 (0.66970)	0.40211 (0.46526)
IER3	Immediate early response 3	This gene functions in the protection of cells from Fas- or tumor necrosis factor type alpha-induced apoptosis	Increased TNF signaling in COPD[21,22]	63	0.10287 (0.00043)	1.32153 (0.0330)
CCL2	chemokine (C-C motif) ligand 2 also called monocyte chemoattractant protein-1 (MCP-1)	Chemotactic activity for monocytes and basophils but not for neutrophils or eosinophils. It binds to chemokine receptors CCR2 and CCR4	Increased expression in COPD[23]	18	0.12840 (0.00084)	1.13576 (0.24076)
PYCARD (PYD and CARD domain containing)	Apoptosis-associated speck-like protein containing a CARD (ASC) protein	Important adaptor for innate immune receptor signaling via the caspase-1 inflammasome.	Caspase 1 activation has been shown to be central to airway inflammation observed after exposure to CS[24]	25.9	0.04274 (0.00087)	0.16880 (0.46997)
IRAK1	IRAK1 (interleukin-1 receptor-associated kinase 1)	TL4 signaling adaptor	CS-induced MMP-1 is regulated by TLR4 via MyD88/IRAK1[25]	22.65	0.02438 (0.02330)	0.24042 (0.11006)
Other						
ADM	Adrenomedullin	A hypotensive peptide	AM plasma levels are elevated in patients with severe lung disease[26]	20.4	N/D	0.84901 (0.20053)
ANXA2	Annexin A2	Annexin family. Members of this calcium-dependent phospholipid-binding protein family play a role in the regulation of cellular growth and in signal transduction pathways.	Proteomic signature in COPD and lung cancer[27]	208	0.03143 (0.40018)	-0.11488 (0.51776)
THBS1	Thrombospondin 1	Adhesive glycoprotein that mediates cell-to-cell and cell-to-matrix interactions	Differentially expressed in COPD[28]	43	N/D	1.38961 (0.18184)
HMGB1	HMGB1	Nuclear DNA binding protein	BAL levels of HMGB1 were higher in smokers with COPD than in smokers and never-smokers[29]	17.4	-0.01937 (0.04822)	-0.21965 (0.07133)

Definition of abbreviations; COPD = chronic obstructive pulmonary disease;

[§]IRP2. Peak score of RNA-Seq data in each community was analyzed for a fold change of ≥±0.5 in the COPD RNA-Seq and a peak score of >10 in the Rip-Seq.

*COPD. COPD mRNA gene expression from LGRC GSE47460. Log2 Fold change compared in COPD cases compared to smoking controls

[#]COPD. COPD RNA-Seq Log2 Fold change in COPD cases compared to smoking controls

N/D. Not-detected in this data set.

SUPPLEMENTARY TABLE 2. Clinical information of human lung specimens LGRC.

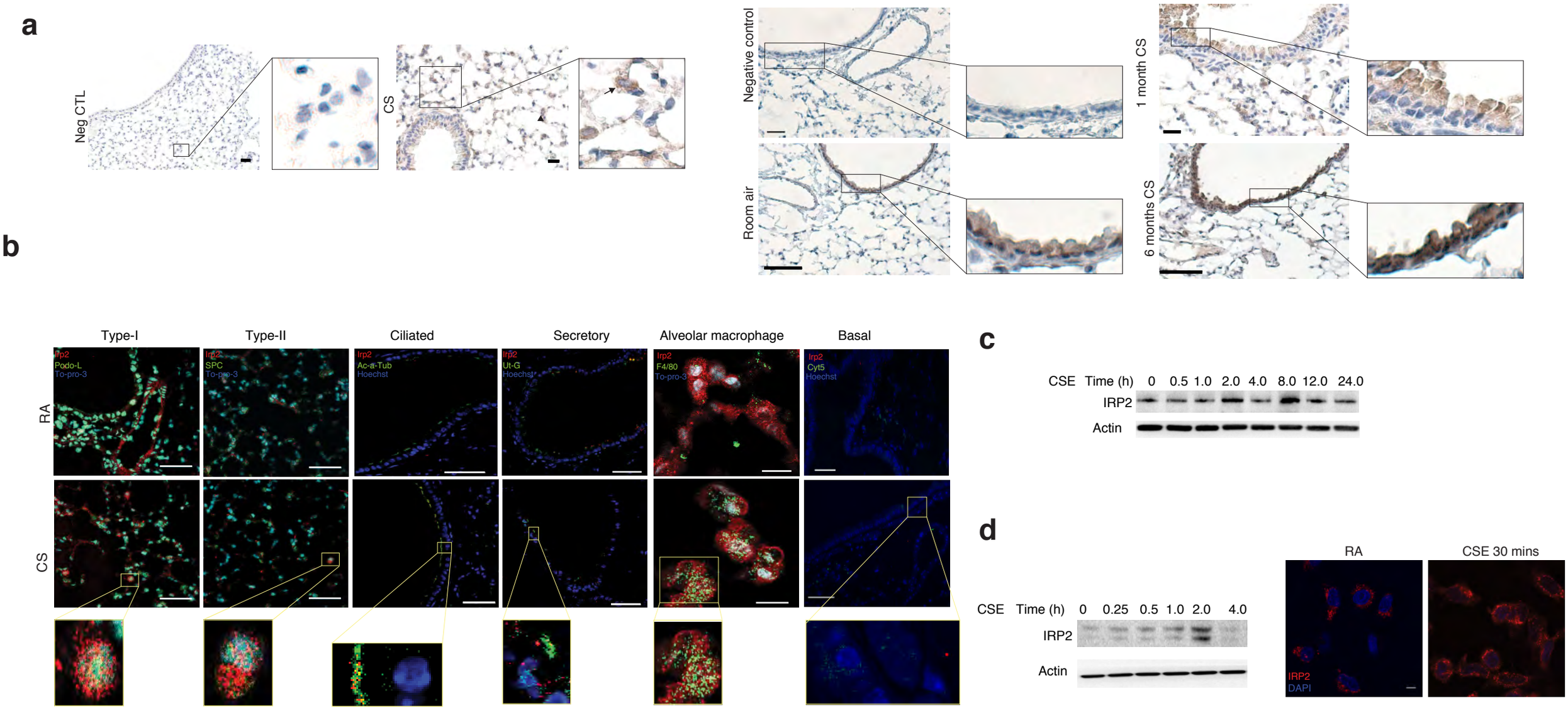
	Never-Smokers (*N* = 5)	COPD GOLD 2 (*N* = 5)
Age, yr.	79 ± 3	70 ± 3
Smoking index at entry, pack-years	0 ± 0	52 ± 4*
Lung function		
FVC, % predicted	99.8 ± 5.2	91.3 ± 2.8
FEV_1, % predicted	102.4 ± 5.7	66.3 ± 1.9*[#]
FEV_1/FVC	0.76 ± 0.02	0.53 ± 0.01*[#]
Emphysema score	0.0 ± 0.0	1.3 ± 0.3

Definition of abbreviations; COPD = chronic obstructive pulmonary disease; GOLD = The Global Initiative for Obstructive Lung Disease; FVC = forced vital capacity; FEV_1 = forced expiratory volume in one second.

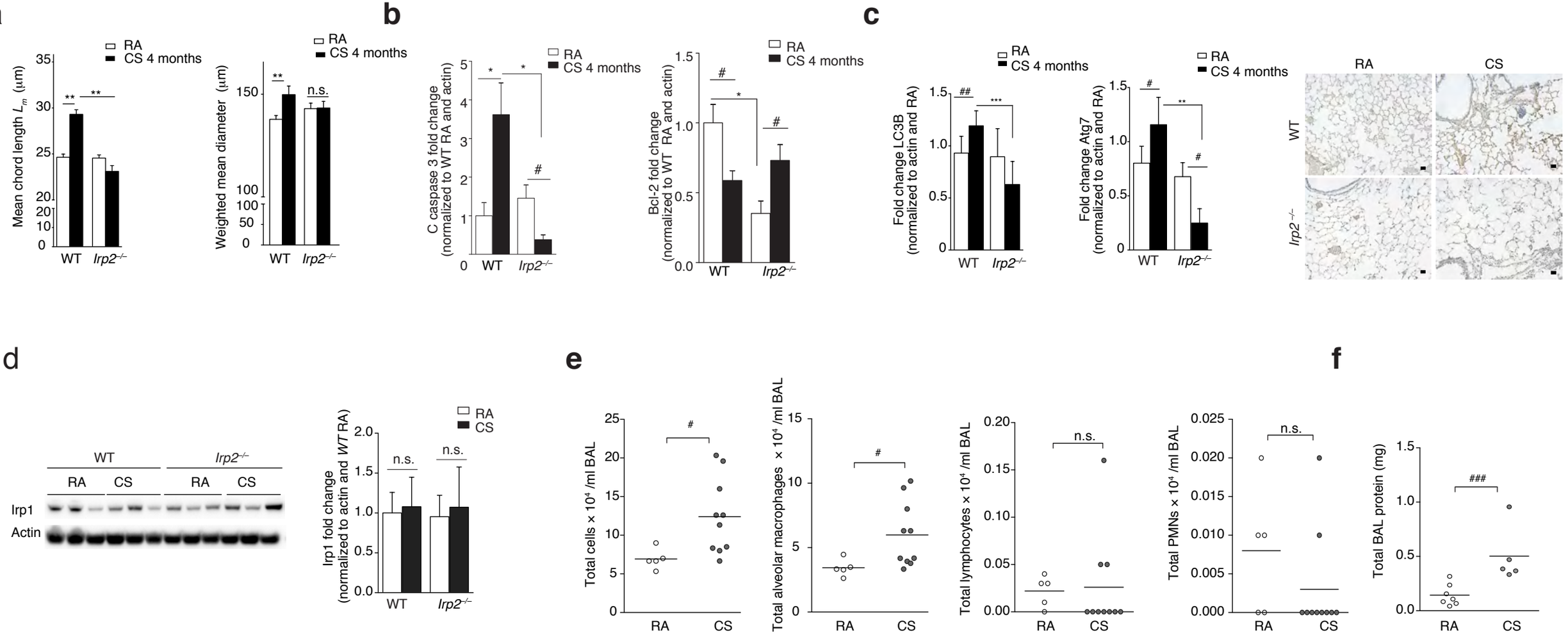
Data expressed as mean ± standard error of the mean (SEM).

* $p < 0.05$, compared with the never-smoker group.

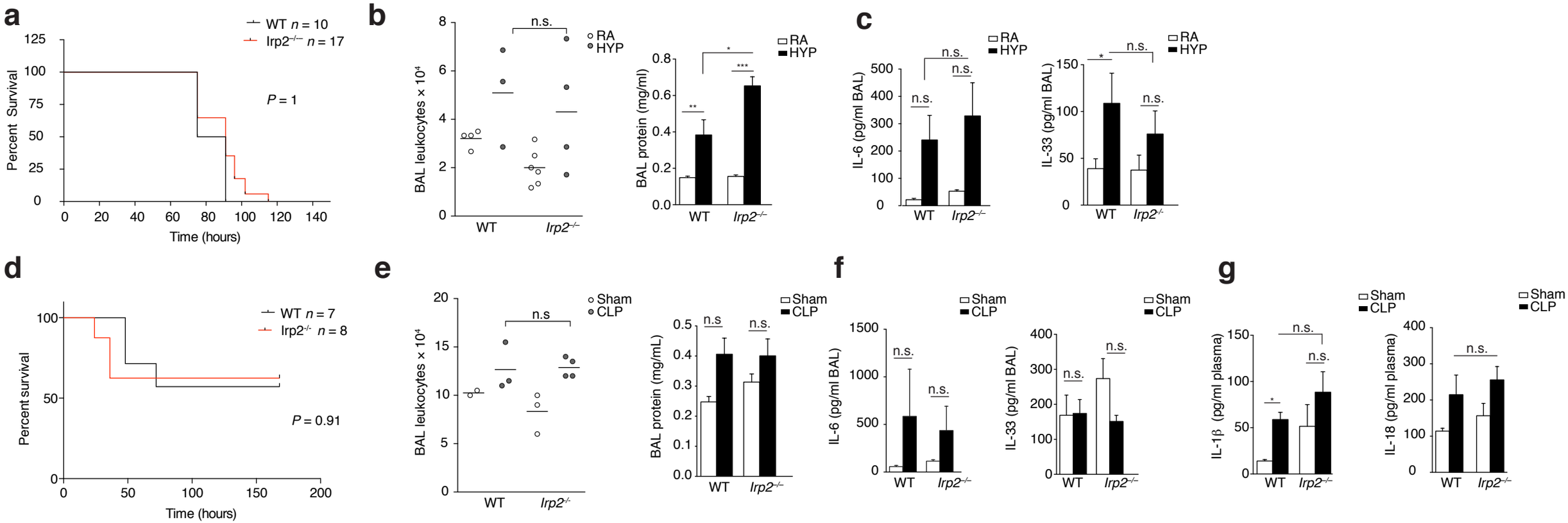
[#] $p < 0.05$, compared with the ever-smoker group.



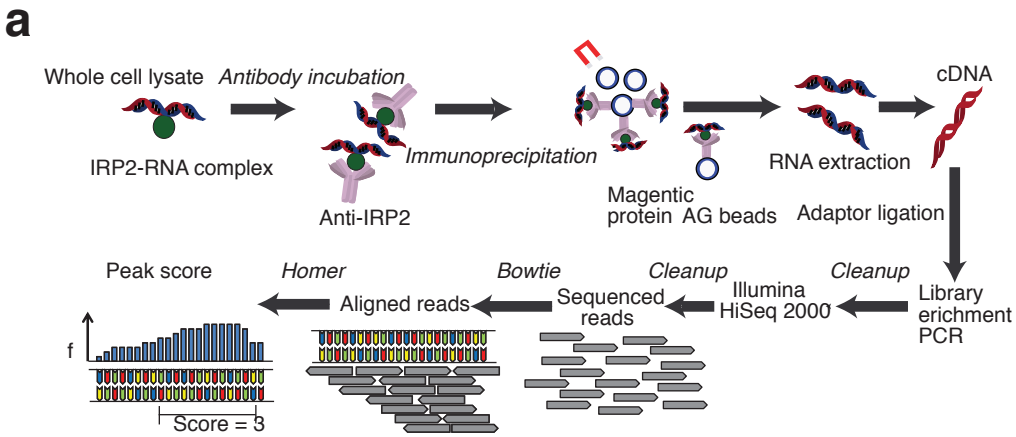
Supplementary Figure. 1

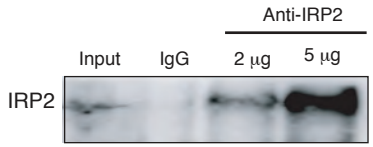


Supplementary Figure.2



Supplementary Figure 3





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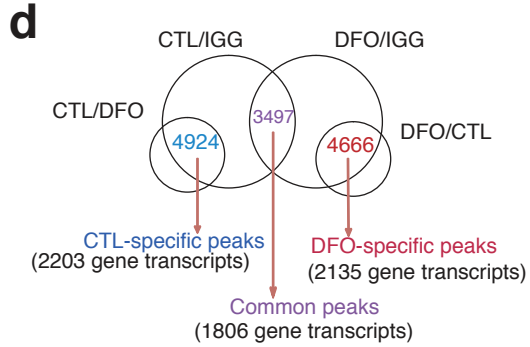
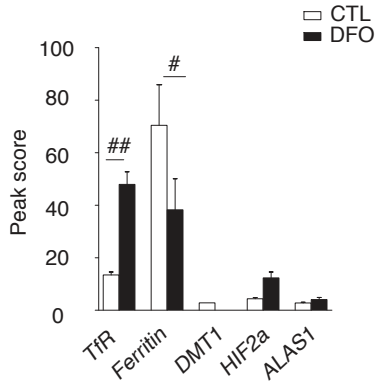
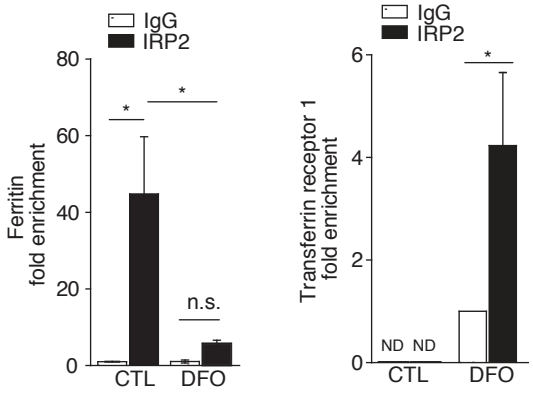
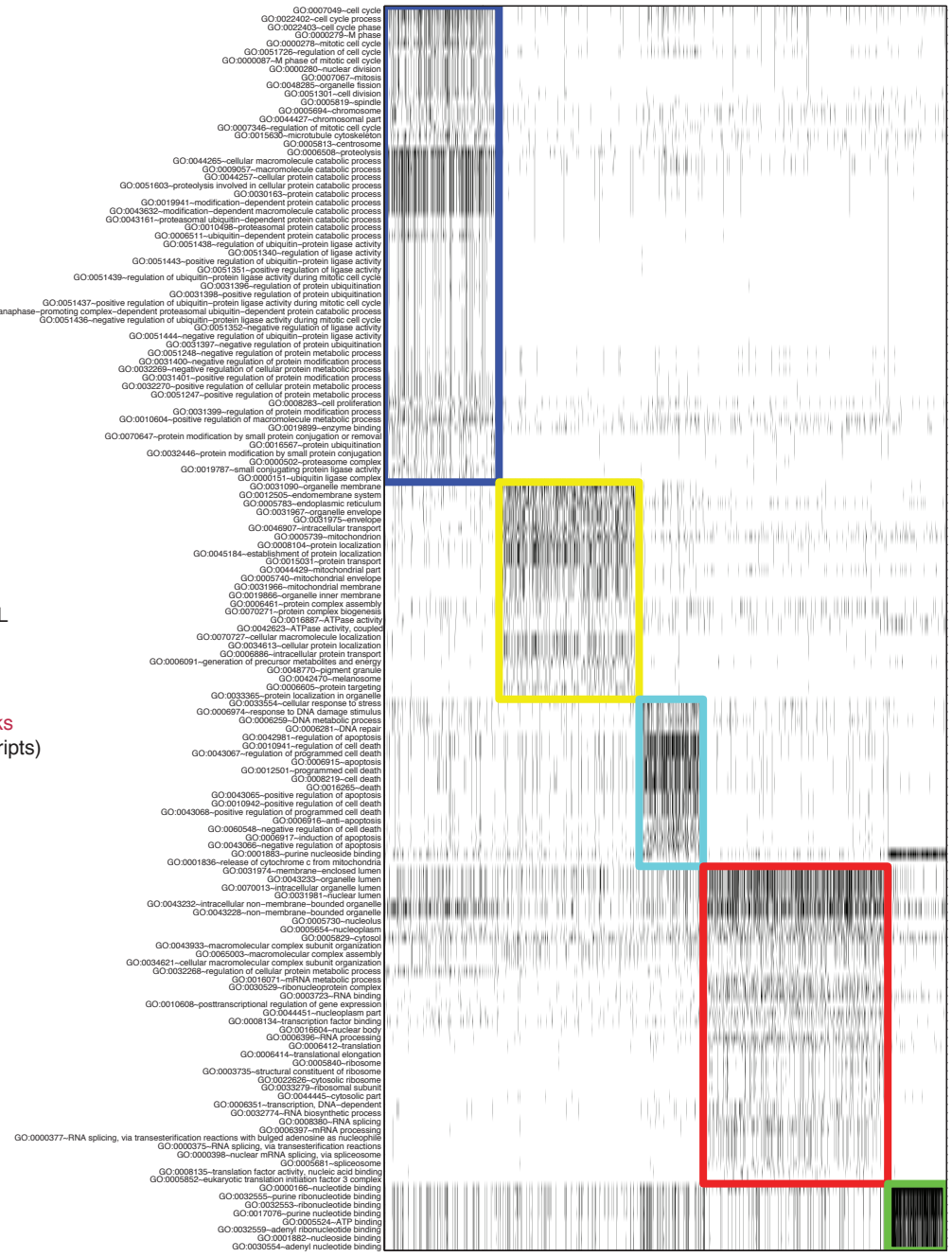
Sample	Bp peak	Library size	Conc (ug/ml)	Qubit nM	qPCR nM
IRP2 CTL	283	183950	30.6	166.3496	232.95
IRP2 DFO	276	179400	35.8	199.5541	308.78
IgG CTL	275	178750	32	179.021	295.9
IgG DFO	272	176800	31.2	176.4706	325.54

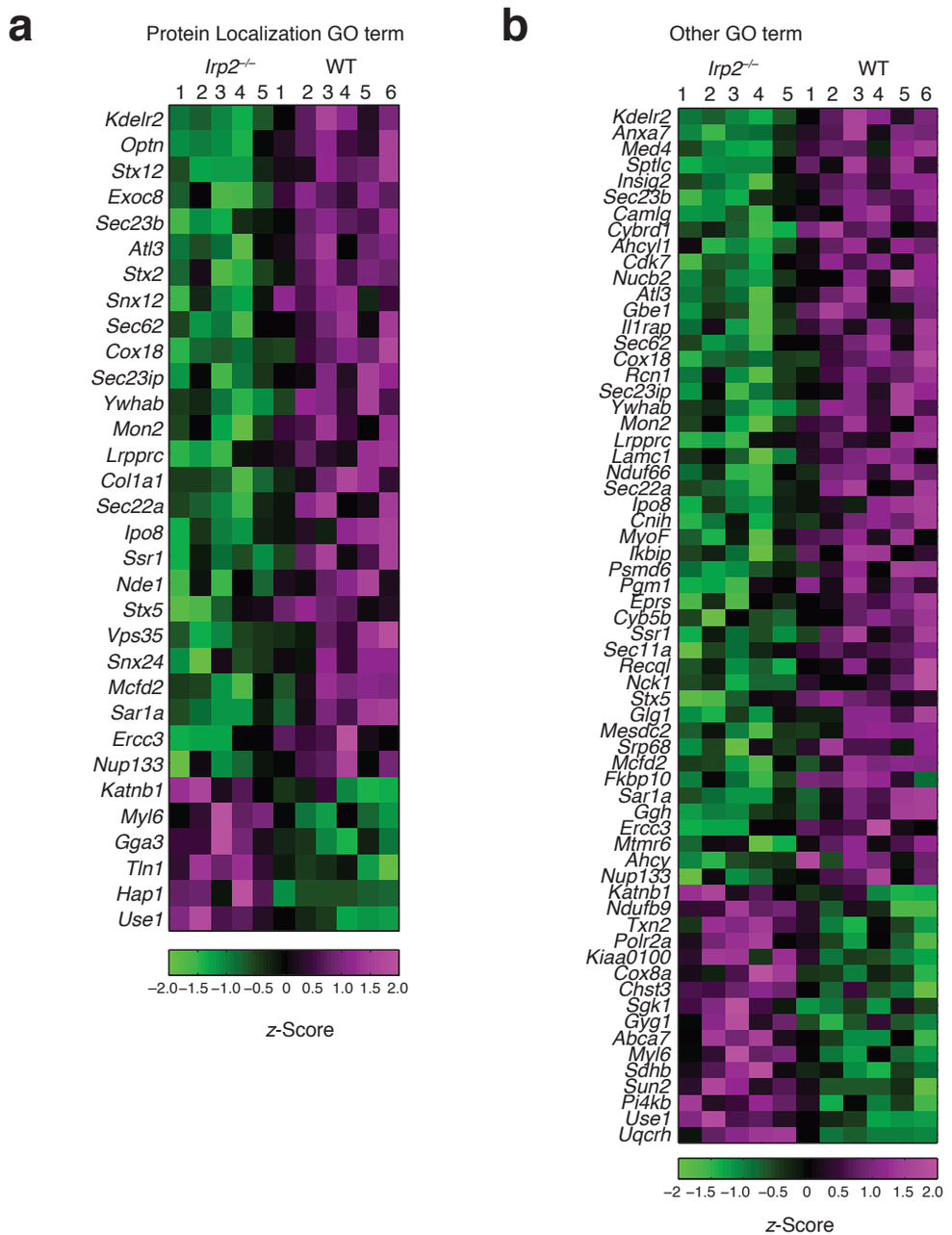




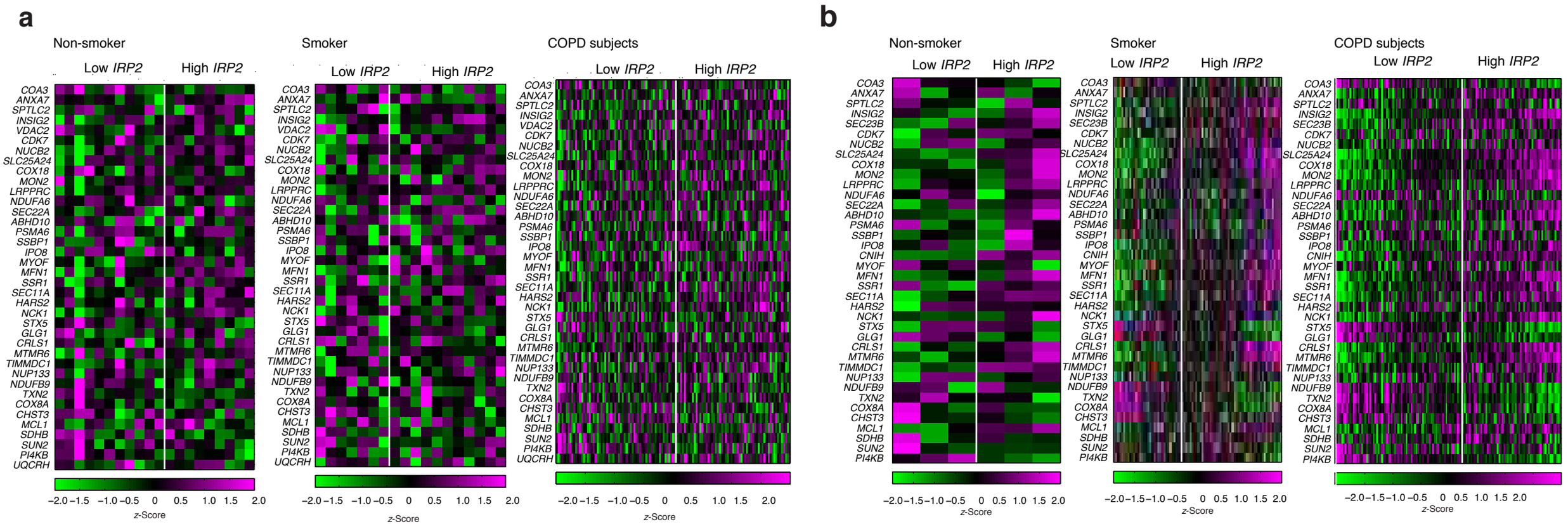




Supplementary Figure.4



Supplementary Figure. 5



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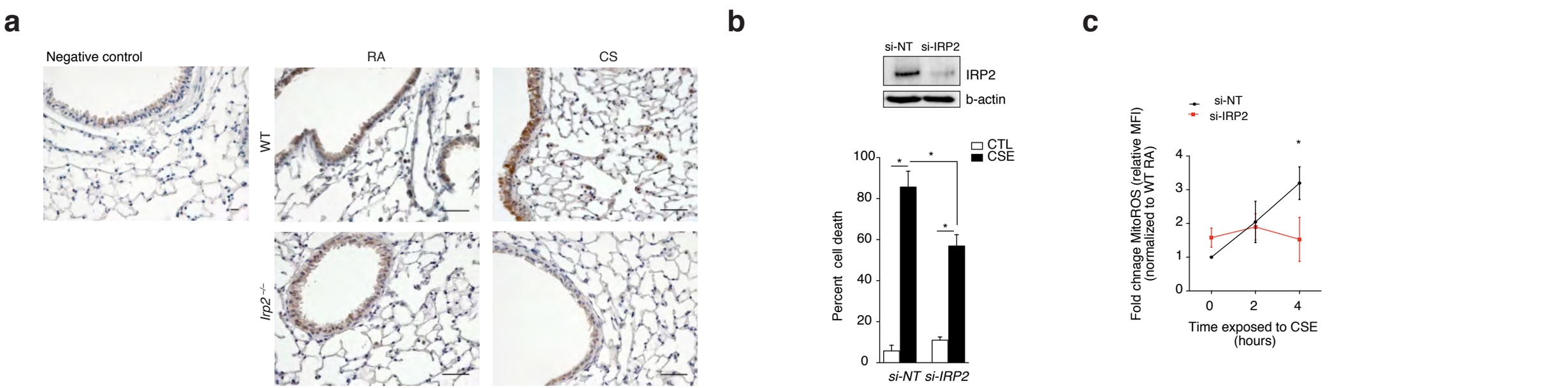
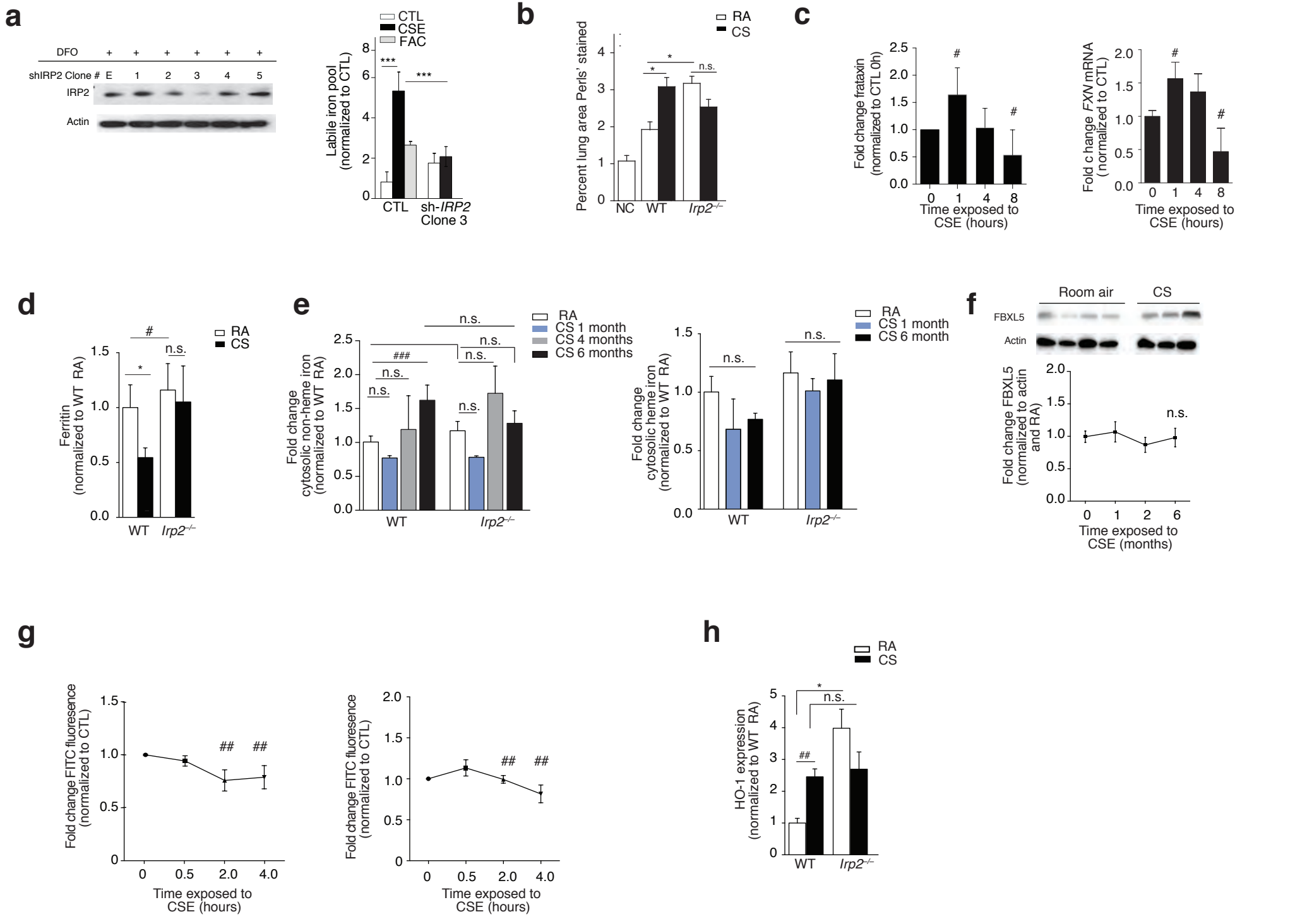
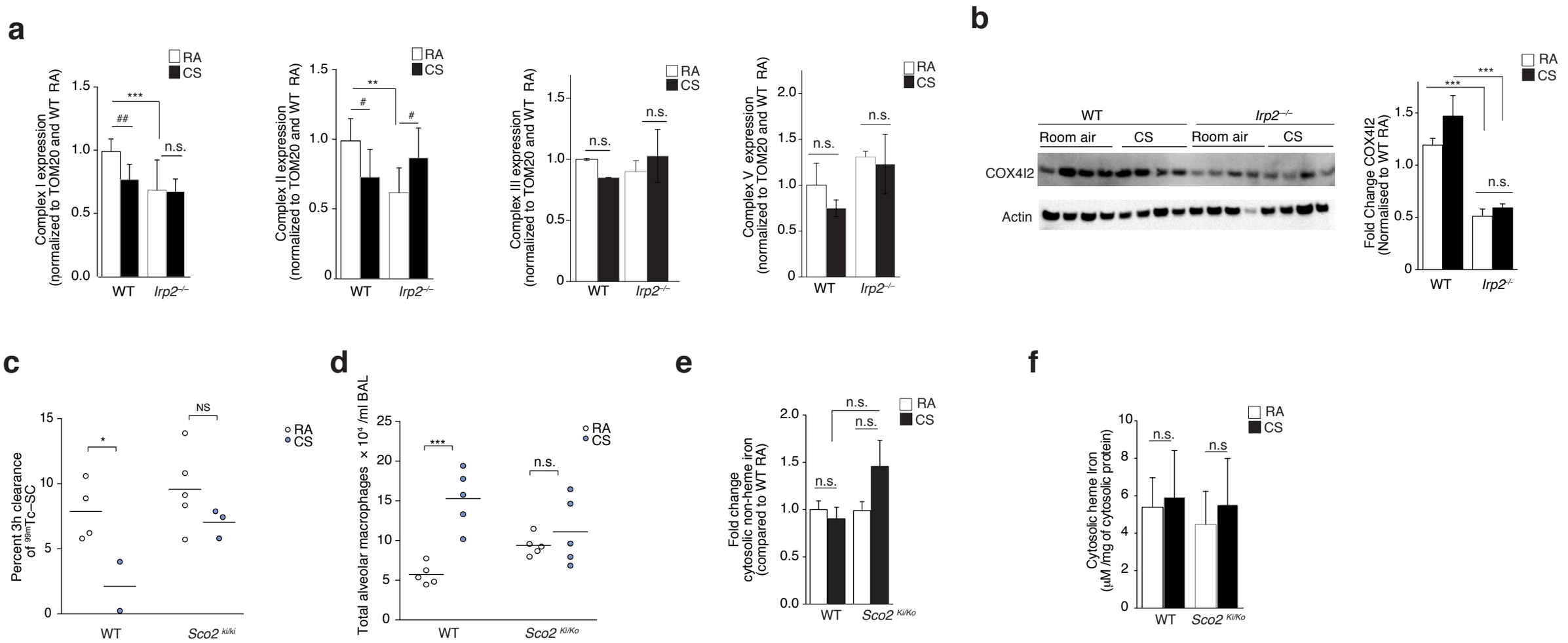


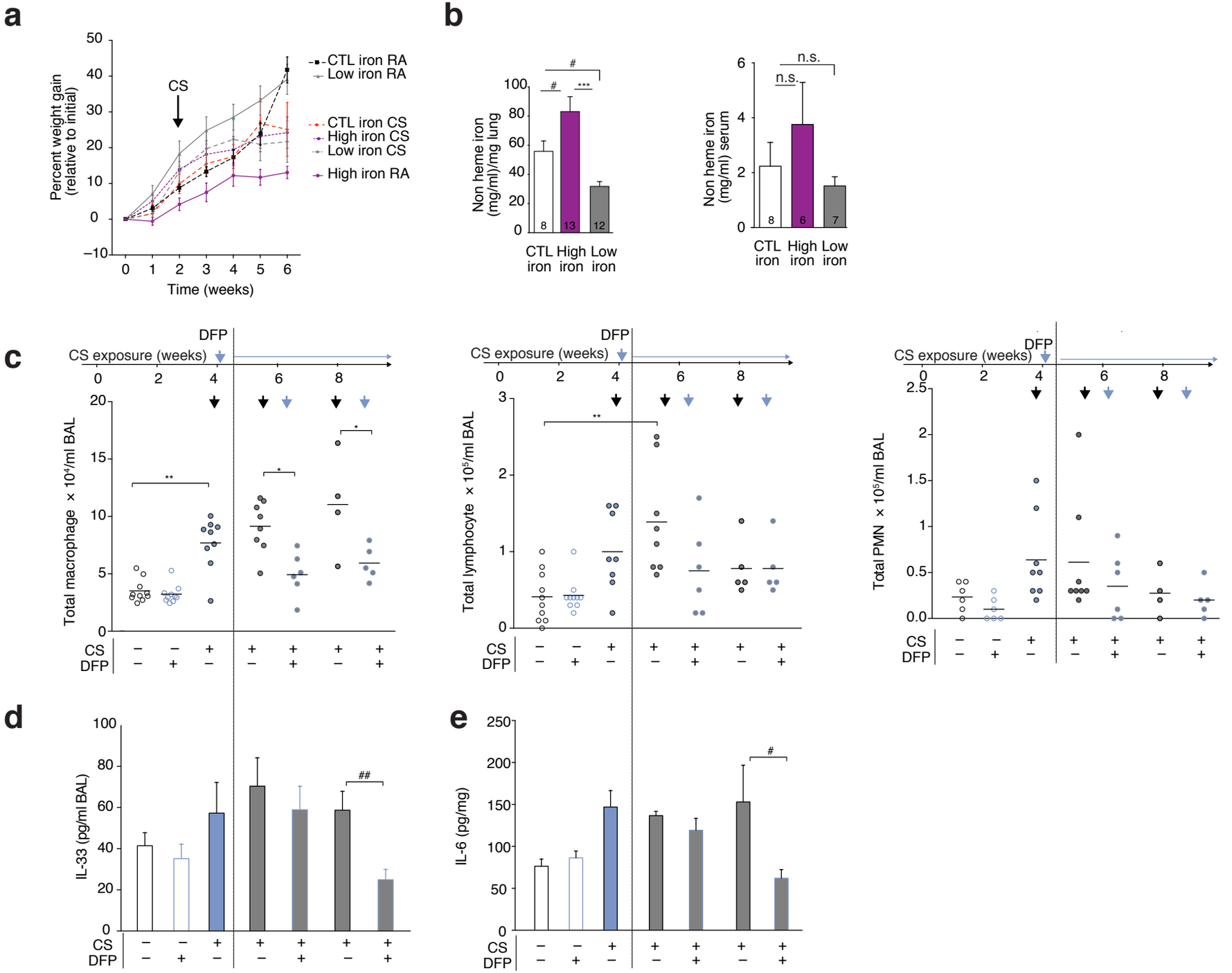
Supplementary Figure 7



Supplementary Figure.8



Supplementary Figure.9



Supplementary Fig.10