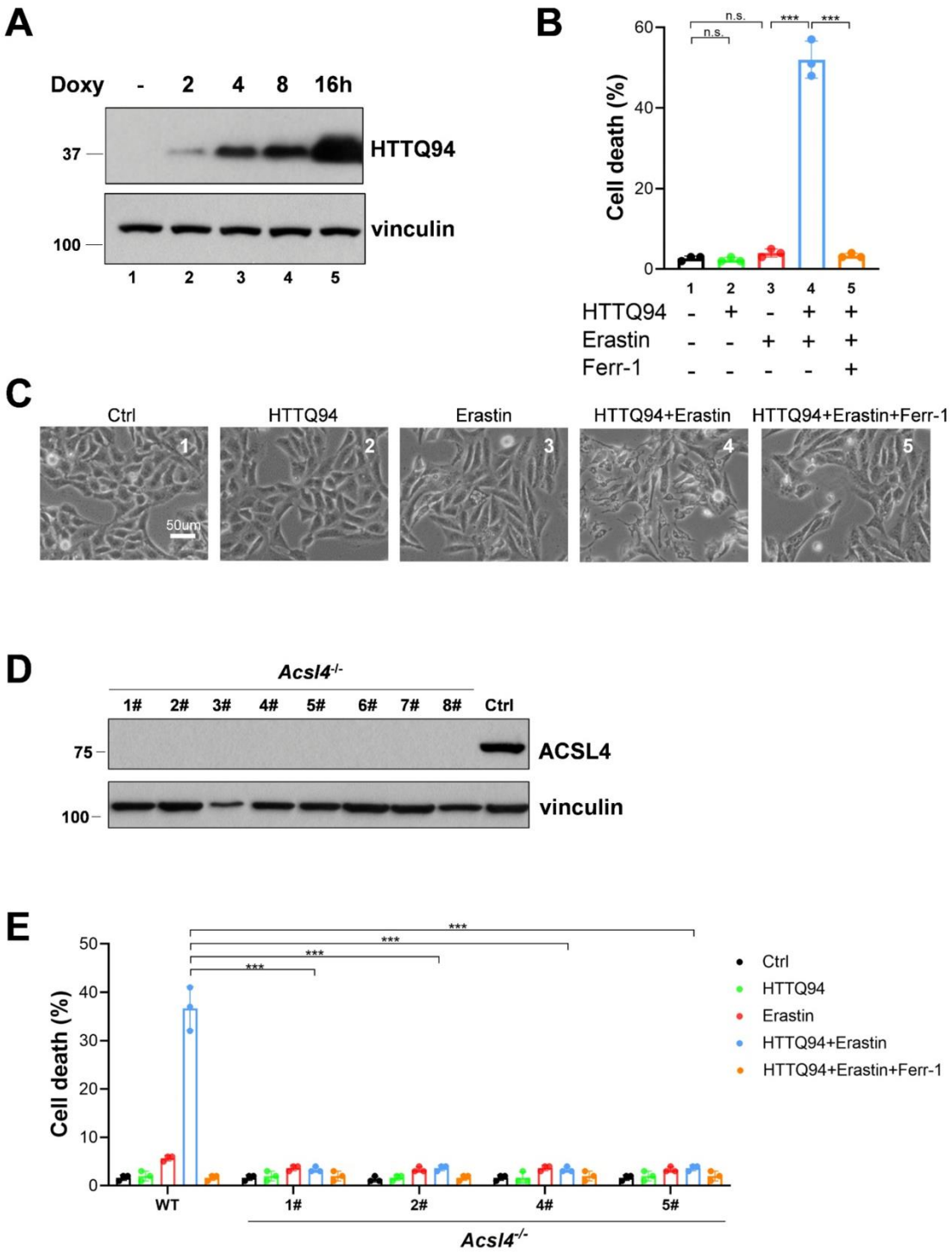
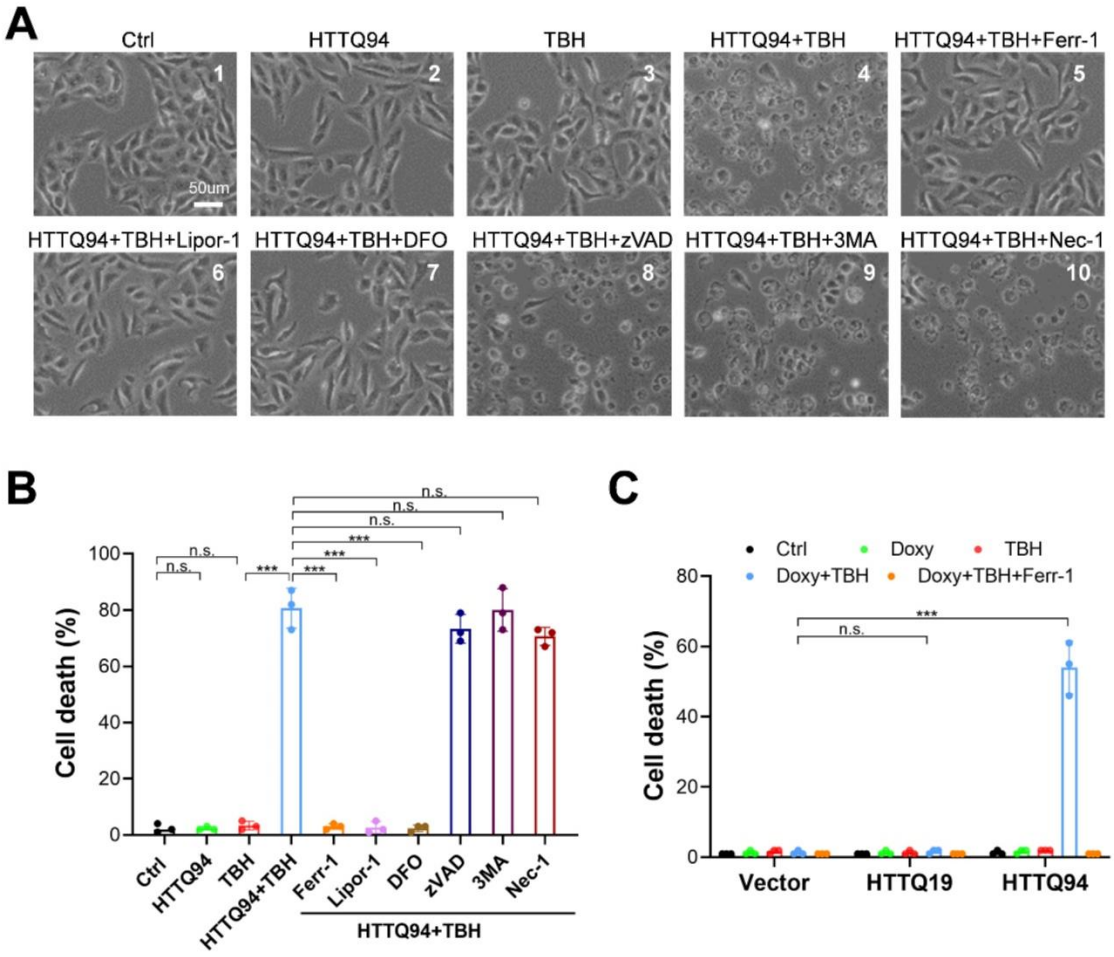
Supplemental Figures and legends

Song et al. Supplemental Figure 1



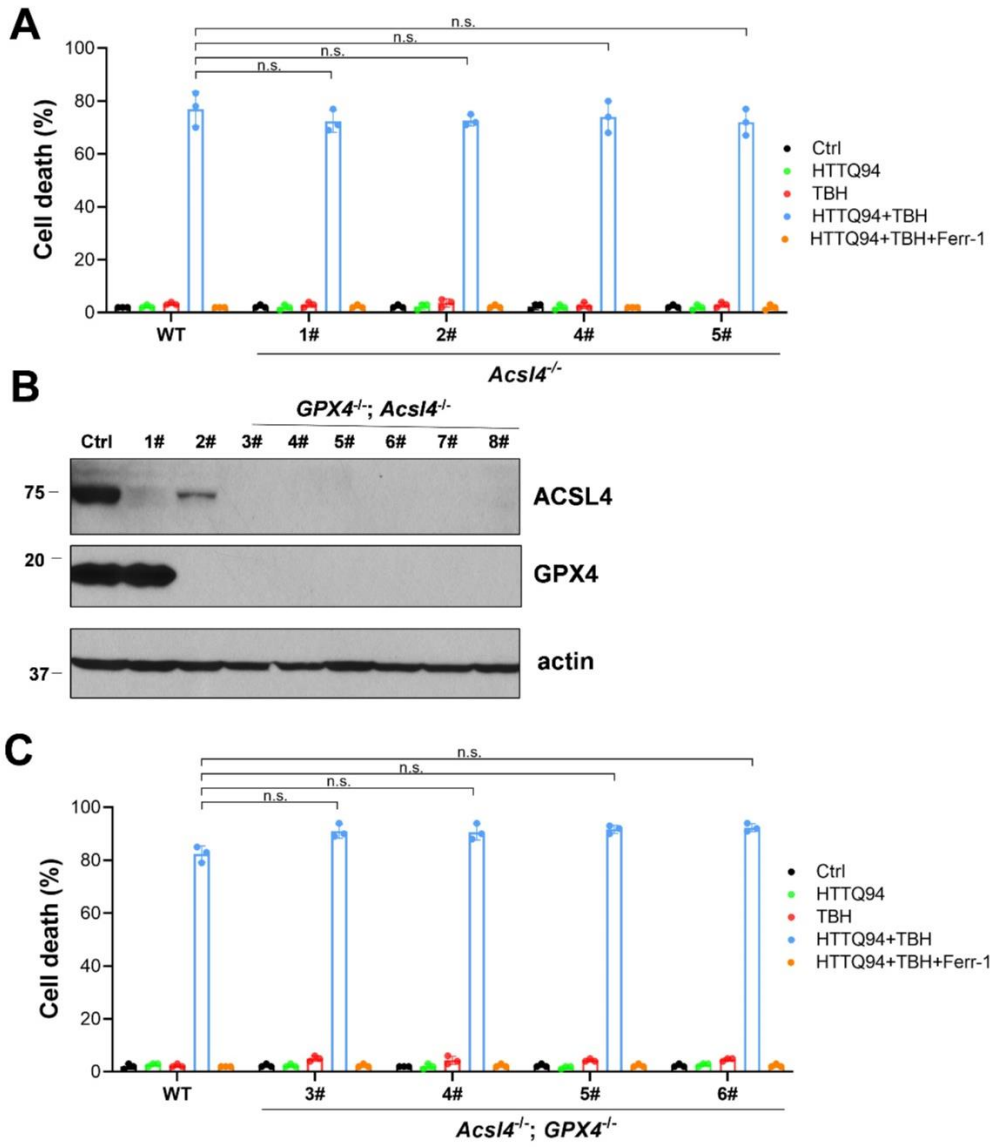
Supplemental Figure 1. The effect of HTTQ94 on ACSL4-dependent ferroptotic responses. *(A)* Western blot analysis of HTTQ94 in HTTQ94 Tet-on SK-N-BE(2)C cells treated with doxycycline (0.5µg/ml; Tet) for 2, 4, 8 and 16h. *(B)* HTTQ94 Tet-on SK-N-BE(2)C cells pre-incubated with doxycycline (0.5µg/ml) for 16h were treated with Erastin (40µM) for 32h with/without Ferr-1 (2µM). *(C)* Representative phase-contrast images of cell death from, related to panel B. *(D)* Western blot analysis for ACSL4 in different *Acsl4* crispr knockout clones from the HTTQ94 Tet-on SK-N-BE(2)C cell line. *(E)* Cell death assay. The HTTQ94 Tet-on SK-N-BE(2)C control crispr and four independent *Acsl4* crispr cell clones pre-incubated with doxycycline (0.5µg/ml) for 16h were treated with erastin (40µM) for 32h with/without Ferr-1 (2µM). Cell death were calculated from three replicates; Data shown in (*B*) and (*E*) are means ± SD. P-values were derived from two-tailed unpaired t-test. (***) P≤0.001, (**) P≤0.01, (*) P≤0.05, (n.s.) P > 0.05.

Song et al. Supplemental Figure 2



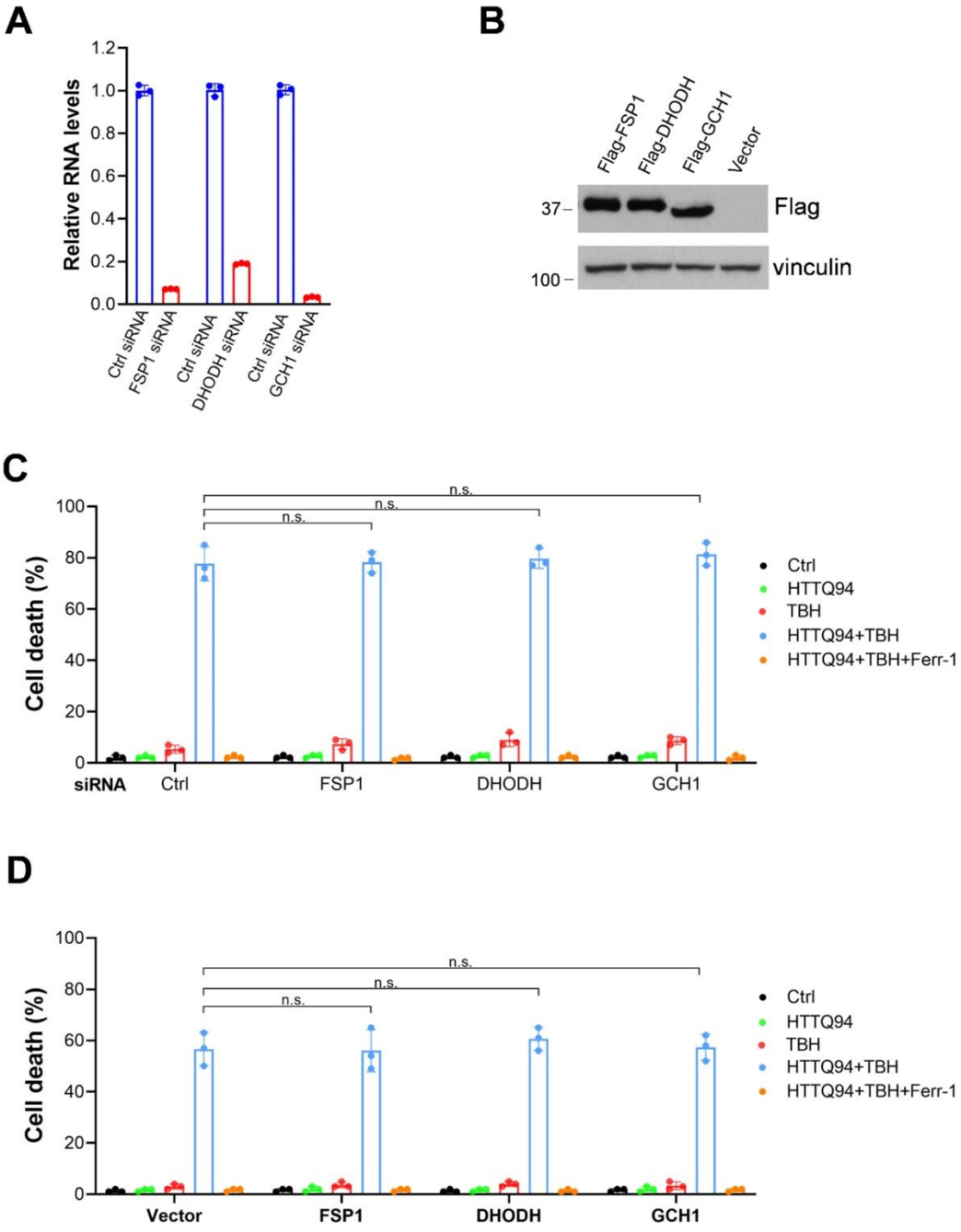
Supplemental Figure 2. HTTQ94 mediated ferroptotic responses upon ROS stress. *(A)* Representative phase-contrast images of cell death from the HTTQ94 tet-on SK-N-BE(2)C cells. HTTQ94 Tet-on SK-N-BE(2)C cells pre-incubated with doxycycline (0.5µg/ml) for 16h were treated with TBH (350µM) for 24h in the presence or absence of the ferroptosis inhibitors (ferrostatin-1, Ferr1, 2µM; liproxstatin-1, Lipor1, 2µM and DFO,100µM), apoptosis inhibitor (Z-VADFMK, zVAD, 10µM), autophagy inhibitor (3-methylademine, 3MA, 2mM) or necroptosis inhibitor (necrostatin-1, Nec1, 10µM). *(B)* Quantification of cell death, related to panel A; *(C)* Cell death assay. Control, HTTQ94 and HTTQ19 fragment Tet-on H1299 cells pre-incubated with doxycycline (0.5µg/ml) for 16h were treated with TBH (50µM) for 24h with/without Ferr-1 (2µM). Cell death were calculated from three replicates; Data shown in (*B*) and (*C*) are means ± SD. P-values were derived from two-tailed unpaired t-test. (***) P≤0.001, (**) P≤0.01, (*) P≤0.05, (n.s.) P > 0.05.

Song et al. Supplemental Figure 3



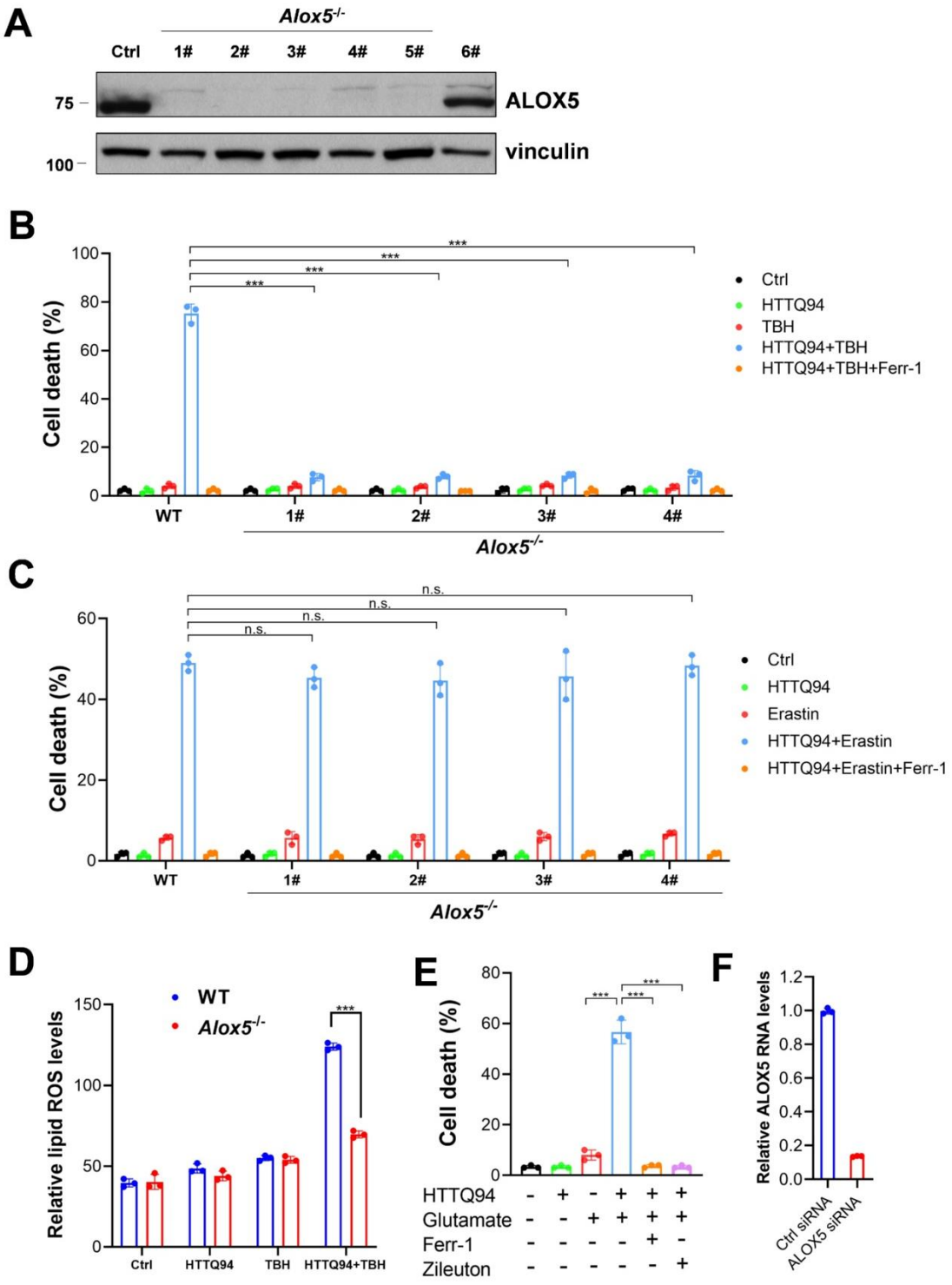
Supplemental Figure 3. HTTQ94 mediated ferroptosis upon ROS stress is independent of GPX4. *(A)* Cell death assay. HTTQ94 Tet-on SK-N-BE(2)C control crispr and four independent *Acsl4* crispr clones pre-incubated with doxycycline (0.5µg/ml) for 16h were treated with TBH (350µM) for 24h with/without Ferr-1 (2µM). *(B)* Western blot analysis of ACSL4 and GPX4 in different *Acsl4/GPX4* double crispr subclones from the HTTQ94 Tet-on SK-N-BE(2)C cell line. *(C)* Cell death assay. HTTQ94 Tet-on SK-N-BE(2)C control crispr and four independent *Acsl4/GPX4* double crispr clones pre-incubated with doxycycline (0.5µg/ml) for 16h were treated with TBH (350µM) for 24h with/without Ferr-1 (2µM). Cell death were calculated from three replicates; Data shown in (*A*) and (*C*) are means ± SD. P-values were derived from two-tailed unpaired t-test. (n.s.) P > 0.05.

Song et al. Supplemental Figure 4



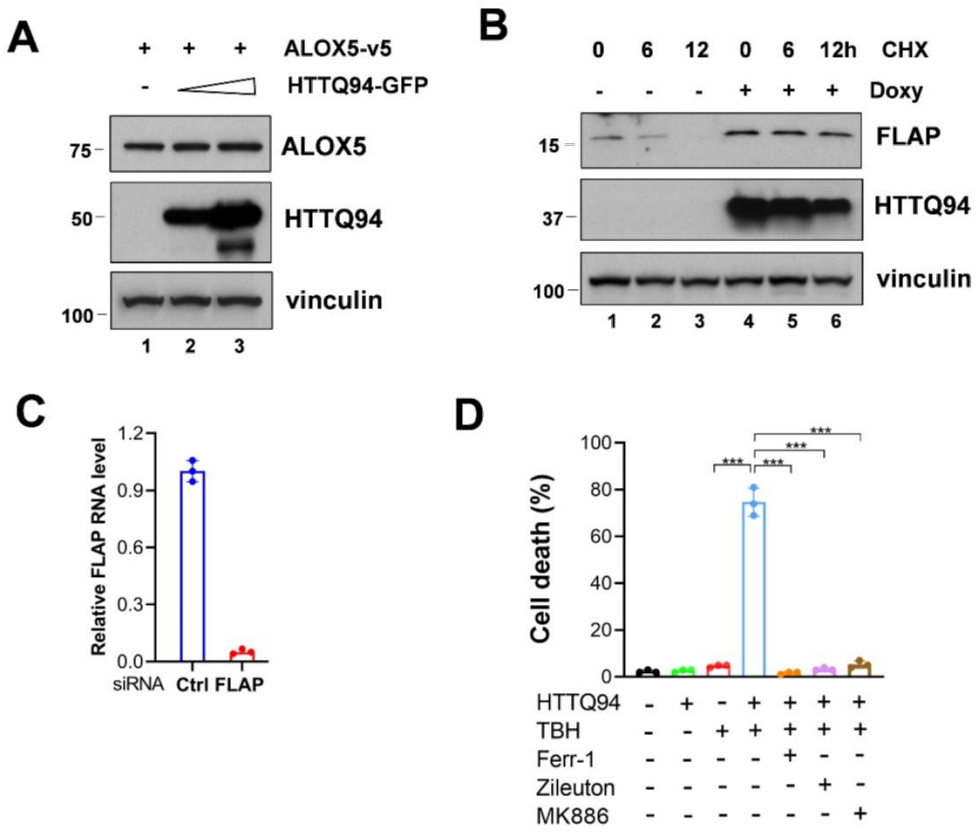
Supplemental Figure 4. HTTQ94 mediated ferroptosis upon ROS stress is independent of FSP1, DHODH and GCH1. *(A)* Q-PCR analysis of the knockdown efficiency of FSP1, GCH1 and DHODH in HTTQ94 Tet-on SK-N-BE(2)C cells transfected with control, FSP1, GCH1 or DHODH siRNA. (*B*) Western blot analysis of FSP1, GCH1 or DHODH in HTTQ94 Tet-on H1299 cells transfected with either Flag-FSP1, Flag-DHODH, Flag-GCH1 expressing plasmid or an empty vector. (*C*) Cell death assays for SK-N-BE(2)C knockdown cells. HTTQ94 Tet-on SK-N-BE(2)C cells transfected with control (ctrl), FSP1, GCH1 or DHODH siRNA were pre-incubated with doxycycline (0.5μg/ml) for 16h, then treated with TBH (350μM) for additional 24h. (*D*) Cell death assays for the overexpression cells. HTTQ94 Tet-on H1299 cells transfected with either Flag-FSP1, Flag-DHODH, Flag-GCH1 expressing plasmid or an empty vector were pre-incubated with doxycycline (0.5μg/ml) for 16h, then treated with TBH (50μM) for additional 24h. Cell death were calculated from three replicates; Data shown in (*C*) and (*D*) are are means ± SD. P-values were derived from two-tailed unpaired t-test. (n.s.) $P > 0.05$.

Song et al. Supplemental Figure 5



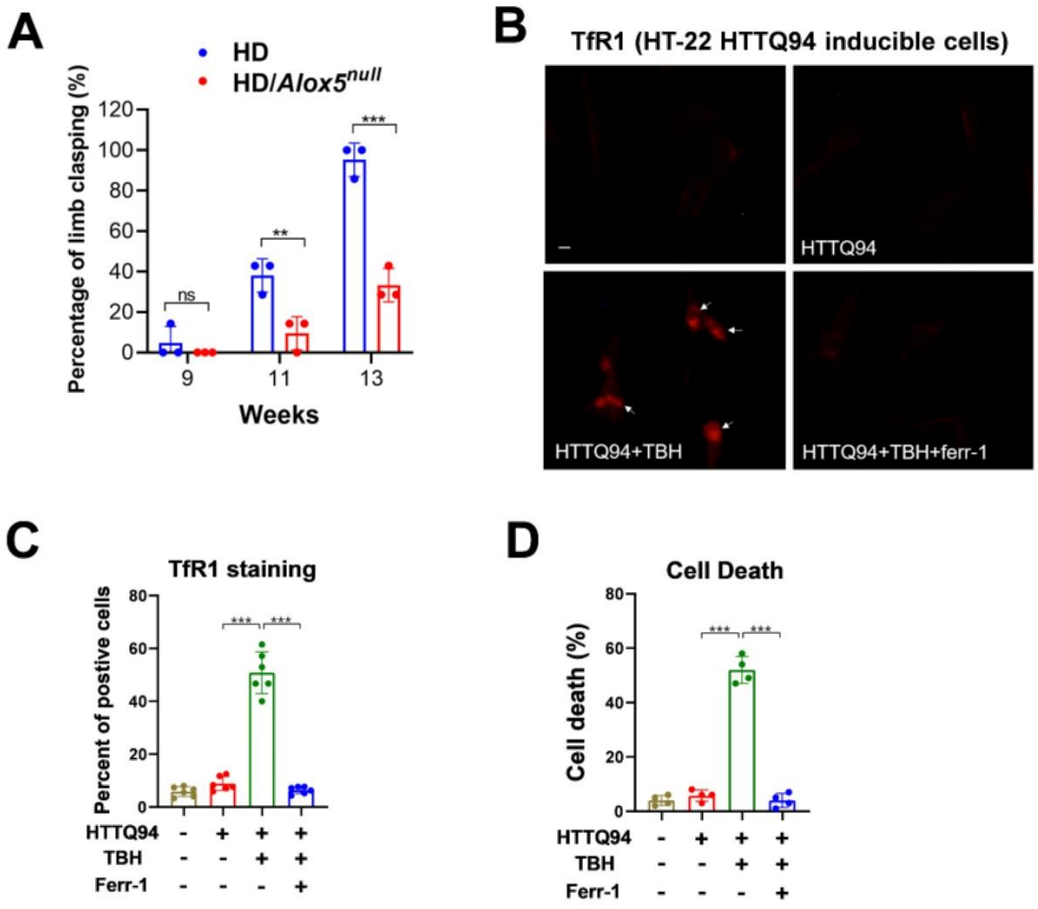
Supplemental Figure 5. Inactivation of ALOX5 abolishes HTTQ94 mediated ferroptotic responses upon ROS-induced stress and glutamate. *(A)* Western blot analysis of ALOX5 in different *Alox5* crispr subclones from the HTTQ94 Tet-on SK-N-BE(2)C cell line. *(B)* Cell death assay. The HTTQ94 Tet-on SK-N-BE(2)C control crispr and four independent *Alox5* crispr cell lines pre-incubated with doxycycline (0.5µg/ml) for 16h were treated with TBH (350µM) for 24h with/without Ferr-1 (2µM). *(C)* Cell death assay. The HTTQ94 Tet-on SK-N-BE(2)C control crispr and four independent *Alox5* crispr clones pre-incubated with doxycycline (0.5µg/ml) for 16h were treated with Erastin (40µM) for 32h with/without Ferr-1. *(D)* Quantification of lipid ROS levels from three replicates, related to main Figure 4F. *(E)* Cell death assay. The HTTQ94 Tet-on HT-22 cells were treated with doxycycline (0.5µg/ml) and glutamate (10mM) in the presence or absence of Ferr-1(2µM) or Zileuton (10µM) for 20h. *(F)* Q-PCR analysis of knockdown efficiency of ALOX5 in HTTQ94 Tet-on HT-22 cells, related to main Figure 4G. Data shown in (*B*), (*C*), (*D*) and (*E*) are are means ± SD. P-values were derived from two-tailed unpaired t-test. (***) P≤0.001, (**) P≤0.01, (*) P≤0.05, (n.s.) P > 0.05.

Song et al. Supplemental Figure 6



Supplemental Figure 6. The effect of ALOX5 and/or FLAP on HTTQ94-mediated ferroptosis. *(A)* Western blot analysis of V5-ALOX5 and HTTQ94 in HEK293 cells transfected with a V5-ALOX5 expressing plasmid and either an empty vector or indicated mHTT-GFP expressing vector. *(B)* Western Blot analysis for FLAP and HTTQ94 in HTTQ94 Tet-on SK-N-BE(2)C cells pre-incubated with/without doxycycline (0.5μg/ml) for 16h, and then treated with cycloheximide (CHX) at 200μg/ml for the indicated times. *(C)* Q-PCR analysis of FLAP knockdown efficiency in HTTQ94 Tet-on SK-N-BE(2)C cells transfected with control siRNA or FLAP specific siRNA. *(D)* Cell death assay. HTTQ94 Tet-on SK-N-BE(2)C cells pre-incubated with doxycycline (0.5μg/ml) for 16h were treated with TBH (400μM) for 24h in the presence or absence of Ferr-1 (2μM), Zileuton (10μM) or MK886 (10μM) for 24h. Data shown in (*D*) are means ± SD. P-values were derived from two-tailed unpaired t-test. (***) P≤0.001.

Song et al. Supplemental Figure 7



Supplemental Figure 7. **TfR1 staining is able to specifically recognize ferroptotic cells induced by HTTQ94 and ROS stress.** *(A)* Limb clasping analysis in HD and HD/*Alox5*^{null} mice (9-, 11- and 13-week-old). p<0.01, n=7. *(B-D)*, TfR1 staining in HT-22 HTTQ94 inducible cells upon TBH treatment. HT-22 HTTQ94 inducible cells were treated 1μg/ml doxycycline O/N to induce HTTQ94 expression, followed by treatment with 30μM TBH for 6h, and then cells were stained with TfR1 antibody. *(B)* Representative images of TfR1 staining. *(C)* Quantification of TfR1 staining. *(D)* Cell death assay, related to panel B. Data shown in (A) are means ± SD. Data shown in (*C*) and (*D*) are means ± SEM. P-values were derived from two-tailed unpaired t-test. (***) P≤0.001, (**) P≤0.01, (*) P≤0.05, (n.s.) P > 0.05.