# SUPPLEMENTARY INFORMATION

## Bioactive poly(2-oxazoline)-based nanomaterials bearing arylalkylamine and benzamide motifs possess intrinsic radical trapping and anti-ferroptosis properties

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# Abbreviations

ROS reactive oxygen species
GPX4 glutathione peroxidase 4
DMT1 Divalent metal transporter 1
ACSL3/4 acyl-CoA synthetase long chain family member 3 and 4
ALOX Arachidonate lipoxygenase
AD Alzheimer's disease
Fer-1 ferrostatin-1
Lip-1 liproxstatin-1
Cy5 cyanine 5
RTA radical trapping antioxidants
BBB blood brain barrier
POx-poly(oxazoline)s
PDCs polymer drug conjugates
PD pharmacodynamics
MeOx-2-methyl-2-oxazoline
PhOx 2-phenyl-2-oxazoline
DPhOx 2-(4-(1,3-dioxolan-2-yl)phenyl)-2-oxazoline
AldOx aldehyde functional deprotected-2-(4-(1,3-dioxolan-2-yl)phenyl)-2-oxazoline
BuOx 2-butyl-2-oxazoline
CCMs core crosslinked micelles
CMC critical micelle concentration
SAR structure activity relationship
$C_{11}$-BODIPY membrane sensor for lipid peroxidation
BODIPY-(488/530) oxidized membrane sensor for lipid peroxidation

<u>Polymer library</u>

M45F4R MeOx45Ferrostatin4 reduced
M45D4 MeOx45Diarylamine4
M45P5 MeOx45PhOx5
M20P40 MeOx20PhOx40
M30P20 MeOx30PhOx20
M40P10 MeOx40PhOx10
M30P15Ald5 MeOx30PhOx15AldOx5
M30P15D4 MeOx30PhOx15Diarylamine5
M36B23 MeOx36BuOx23
Et-CCM ethylenediamine crosslinked core crosslinked micelles
Ph-CCM p-phenylenediamine crosslinked core crosslinked micelles

**Table S1**. Further characterization of DPhOx based copolymers and related nanomaterials.

| Polymer | DP (MeOx) | DP (co-monomer) | Pre-modification | | Đ |
|---|---|---|---|---|---|
| | | | $M_{n,\ NMR}$ (g mol-1) | $M_{n,\ SEC}$ (g mol-1) | |
| **M45F4R** | 45 | 4 | 5606 | 12500 | 1.21 |
| **M45D4** | 45 | 4 | 4861 | 10600 | 1.14 |
| **M45P5** | 45 | 5 | 4598 | 9300 | 1.14 |

**Table S2.** $EC_{50}$ values for bioactive polymers in this study

| Polymer | $EC_{50}$ (mM) |
|---|---|
| **M45P5** | 1.32 |
| **M45D4** | 0.13 |
| **M45F4R** | $0.66 \times 10^{-3}$ |
| **M40P10** | 0.25 |
| **M30P20** | 0.11 |
| **M30P15D5** | 0.052 |
| **M20P40** | 0.03 |
| **Ph-CCM** | 0.008 |
| **Et-CCM** | 0.024 |

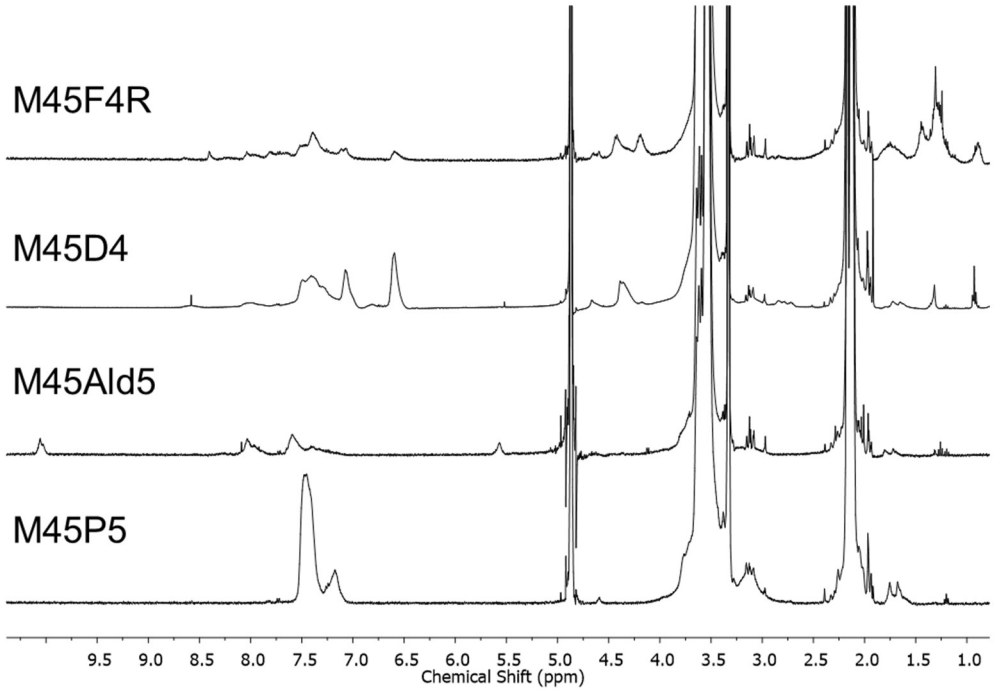


**Figure S1**. $^{1}$H NMR spectra of the PhOx containing polymers at different stages of synthesis.

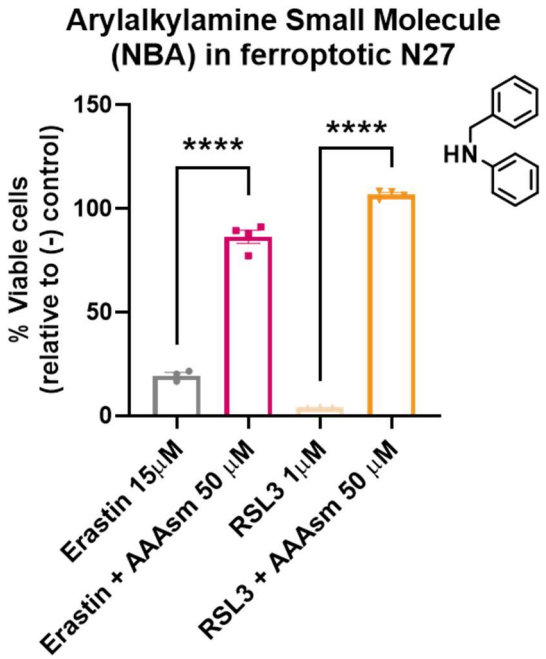


**Figure S2**. N-Benzylaniline (NBA) suppresses ferroptosis at concentration equal to number of polymeric units of arylalkylamine.

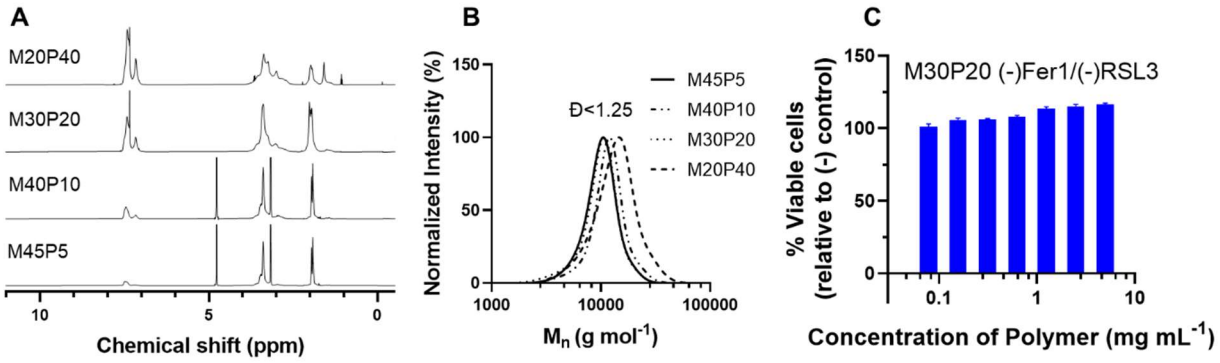


**Figure S3**. Characterization of P(MeOx-grad-PhOx) library. A) H NMR spectra of copolymers with increasing % benzamide content and B) their resulting SEC chromatograms. C) AlamarBlue cell viability assay in N27 cells of M30P20 after 24 hours with up to 5.0 mg $mL^{-1}$ polymer.

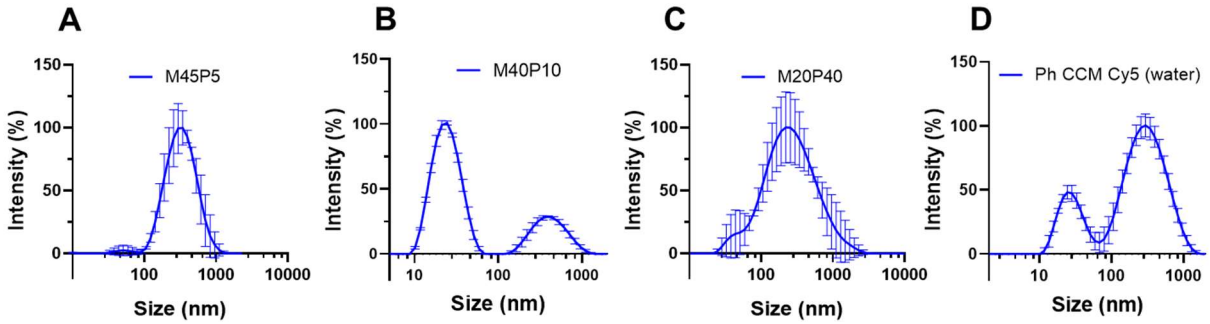


**Figure S4**. DLS study showing normalized % intensity traces of P(MeOx-grad-PhOx) library and Ph-$CCM_{cy5}$.

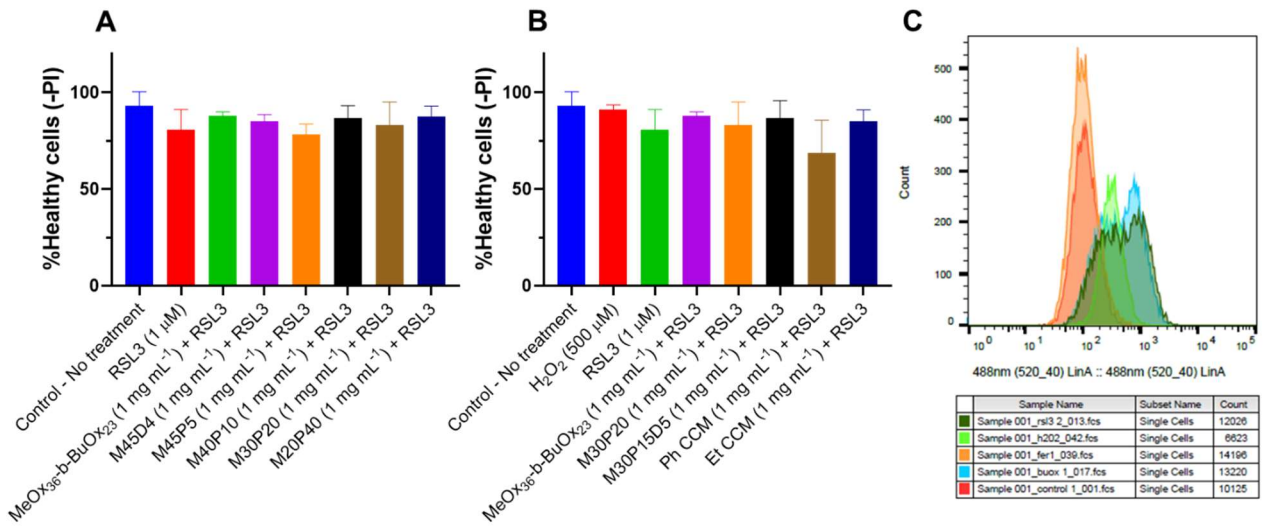


**Figure S5**. % Viable cells determined by PI for treatment conditions used in flow cytometry experiments with $C_{11}$-BODIPY. A) P(MeOx-grad-PhOx) library and M30P15D5. B) CCMs and series. C) Controls for $C_{11}$-BODIPY probe showing average ROS activated BODIPY-(488/530) MFI of cell populations (red-no treatment, light green-$H_2O_2$ 500 µM, blue-RSL3 1 µM, orange-RSL3+Fer-1 1 µM, blue-RSL3+M36B23 1 mg $mL^{-1}$).

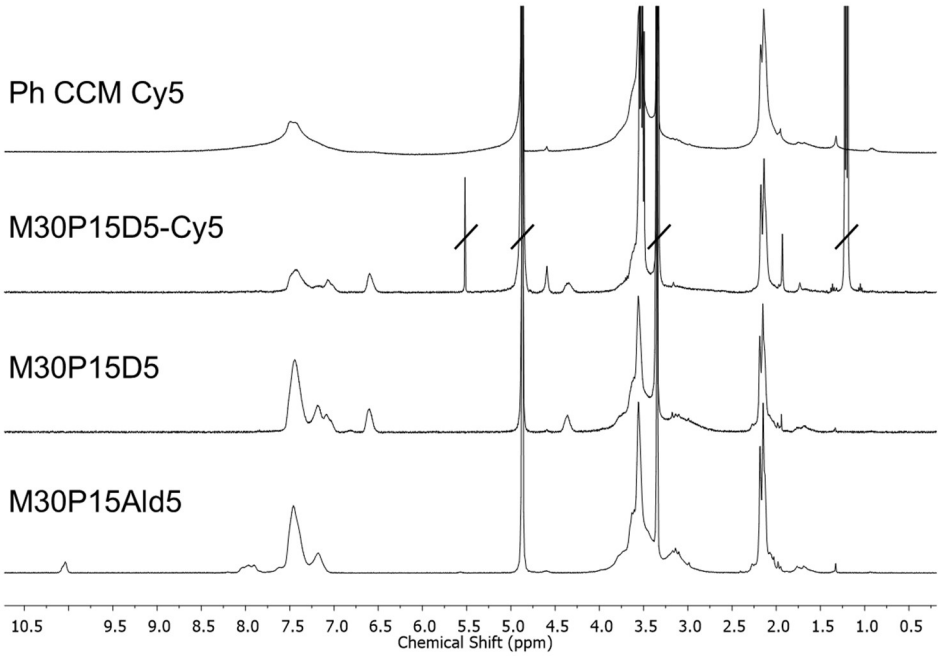


**Figure S6**. $^1$H NMR spectra of linear polymer arm containing AldOx (M30P15Ald5), its modification with Cy5 (M30P15D5-Cy5), aniline (M30P15D5) and then Ph-$CCM_{cy5}$.

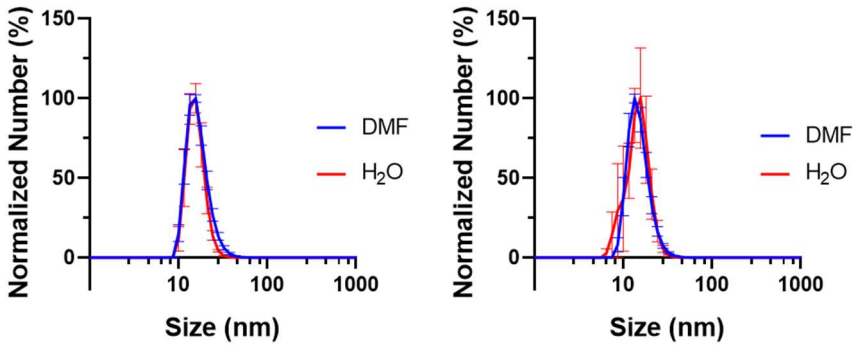


**Figure S7**. DLS of CCMs in different solvents (left: Ph-CCM, right: Et-CCM). M30P20 "arm" was attempted in DMF but did not self-assembly in this solvent. Concentration used for all samples – 1 mg $mL^{-1}$.

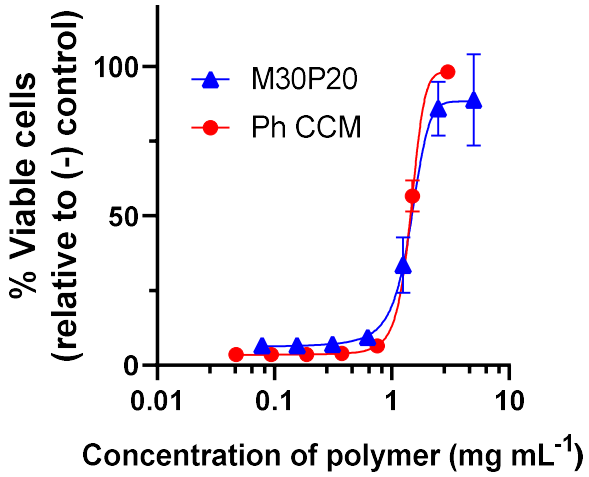


**Figure S8**. Dose-response curves of M30P20 and Ph-CCM plotted against mg $mL^{-1}$ highlighting comparison of relatively similar % benzamide content and response to ferroptosis and additionally the lack of effect of CMC on ferroptosis suppression.

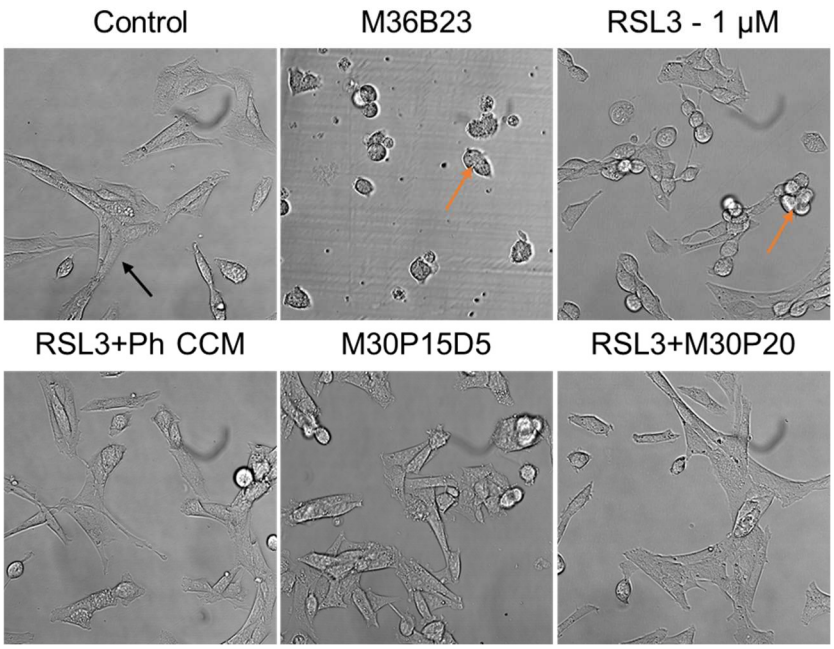


**Figure S9**. Bright field images from $C_{11}$-BODIPY confocal experiment highlighting cell appearance (black arrows: healthy cells with neurite outgrowth, orange: unhealthy constricted cells). RSL3 – 1 μM, polymer – 1 mg $mL^{-1}$.

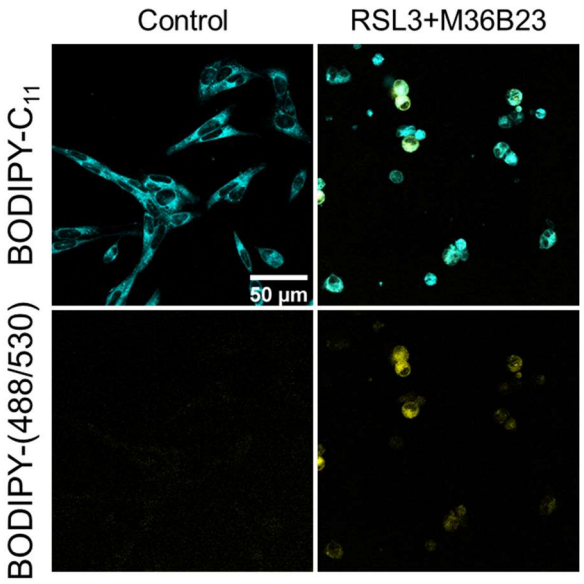


**Figure S10**. Fluorescent images from $C_{11}$-BODIPY experiment showing the M36B23 control producing ROS-activated BODIPY-(488/530) from membrane lipid peroxidation. RSL3 – 1 μM, polymer – 1 mg $mL^{-1}$.

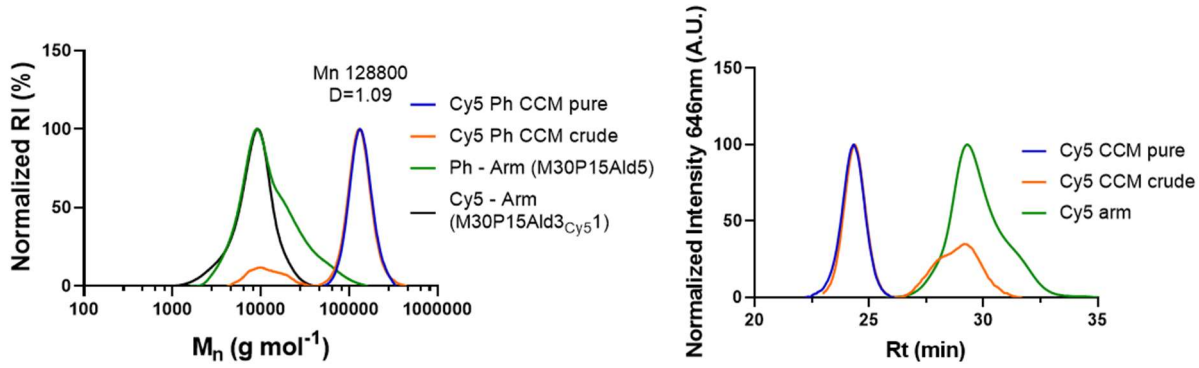


**Figure S11**. Ph-CCM$_{Cy5}$ characterization by SEC chromatography (Normalized UV absorbance at 646 nm).

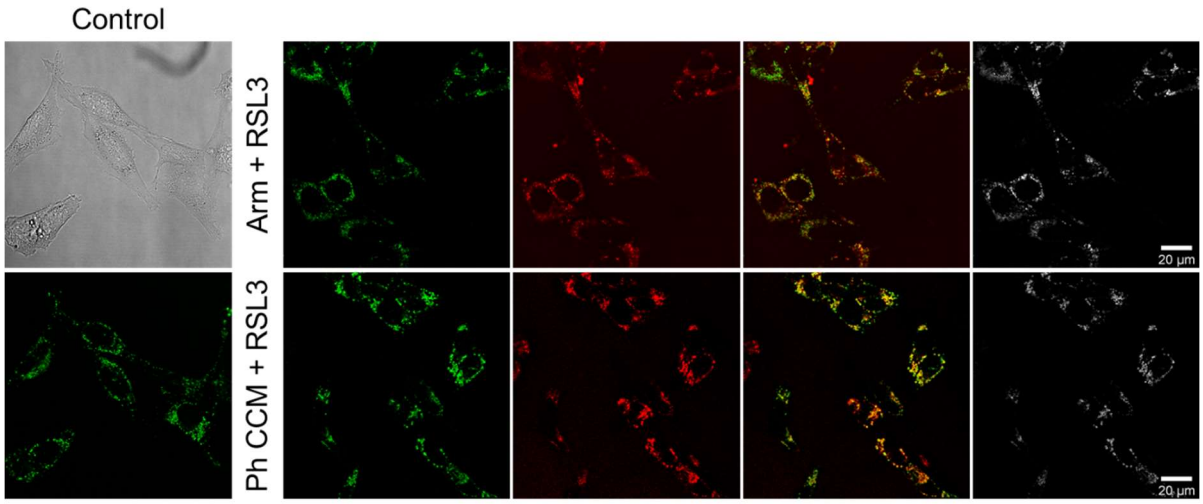


**Figure S12**. Lysosomal colocalization experiment in RSL3 treated cells (RSL3 at 1 µM for 4 hours as cotreatment). Arm-M30P15D5$_{cy5}$, CCM-Ph-CCM$_{cy5}$. Green channel- lysotrackerGreen, red channel- polymer Cy5, grey channel-colocalization using "AND" function in FIJI image calculator. Scale bar 20 µm.

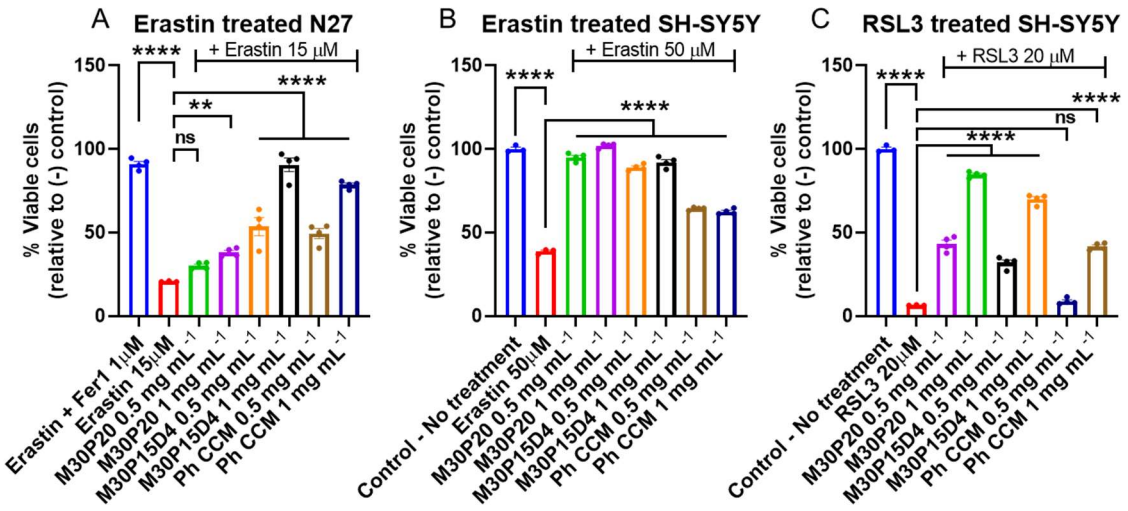


**Figure S13.** Bioactive polymers attenuate alternate models of ferroptosis in N27 and SH-SY5Y.