**Supplementary Information**

# Hemin-Induced Transient Senescence Via DNA Damage Response: A Neuroprotective Mechanism Against Ferroptosis in Intracerebral Hemorrhage

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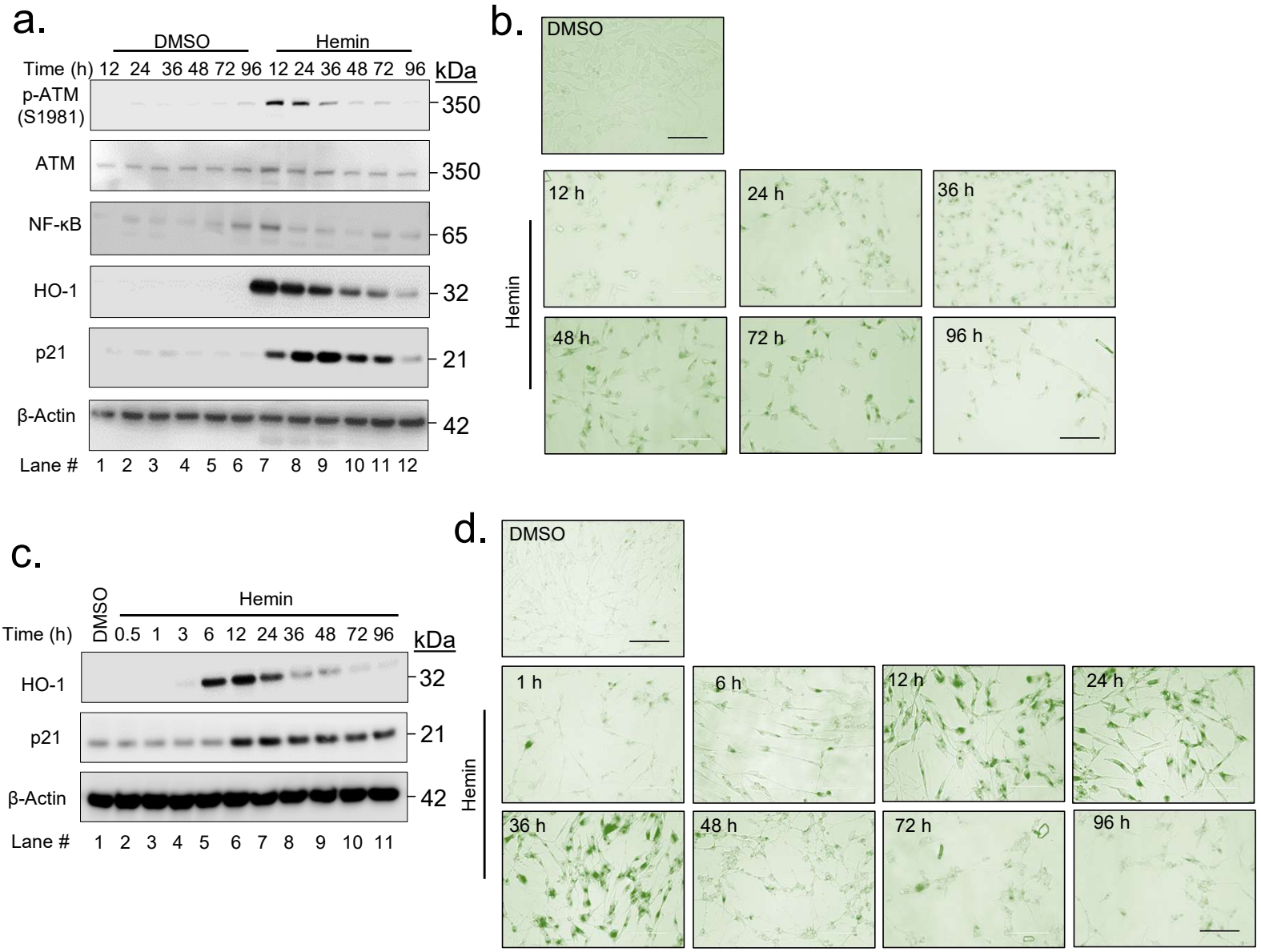
**Supplementary Material** accompanying this paper includes one Table and six Figures.

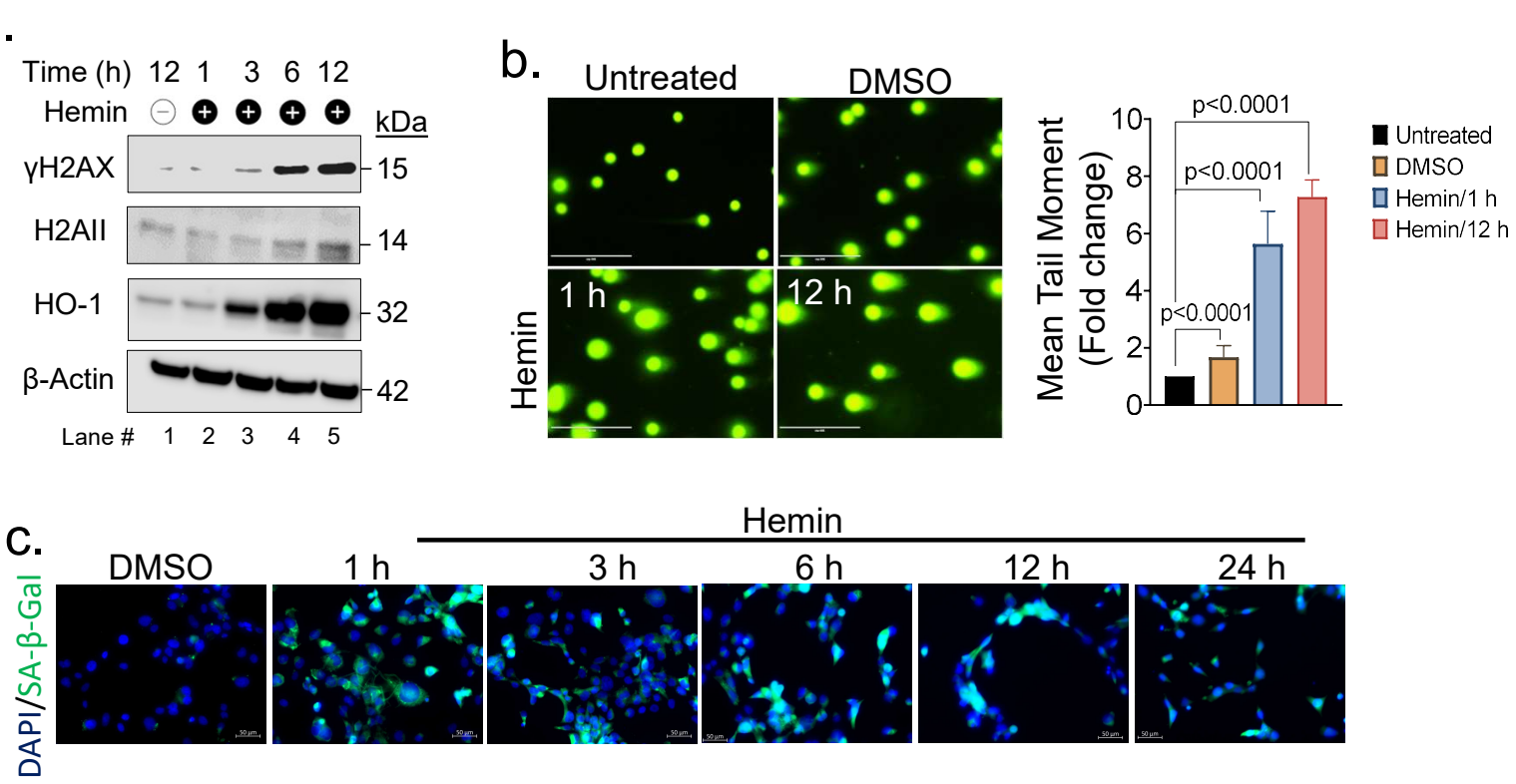
# Supplementary Figure 1




**Supplementary Fig. 1 (Related to Fig. 1): Hemin treatment leads to sequential induction of DDR, senescence, and HO-1 expression at extended time points.**

(a) SH-SY5Y cells were treated either with DMSO (lanes 1-6) or hemin (10 μM) (lanes 7-12) and analyzed using western blot for p-ATM, NF-κB, HO-1, and p21 levels. (b) Representative images of cells stained with SA-β-Gal after hemin treatment at indicated time points. (c) Western blot analysis of differentiated SH-SY5Y cells during a time-dependent kinetics study, treated with DMSO (lane 1) or hemin (lanes 2-9) to measure the levels of HO-1 and p21. (d) SA-β-Gal staining of differentiated SH-SY5Y cells in a time-dependent kinetics study after hemin treatment. Scale bar = 20 μm.

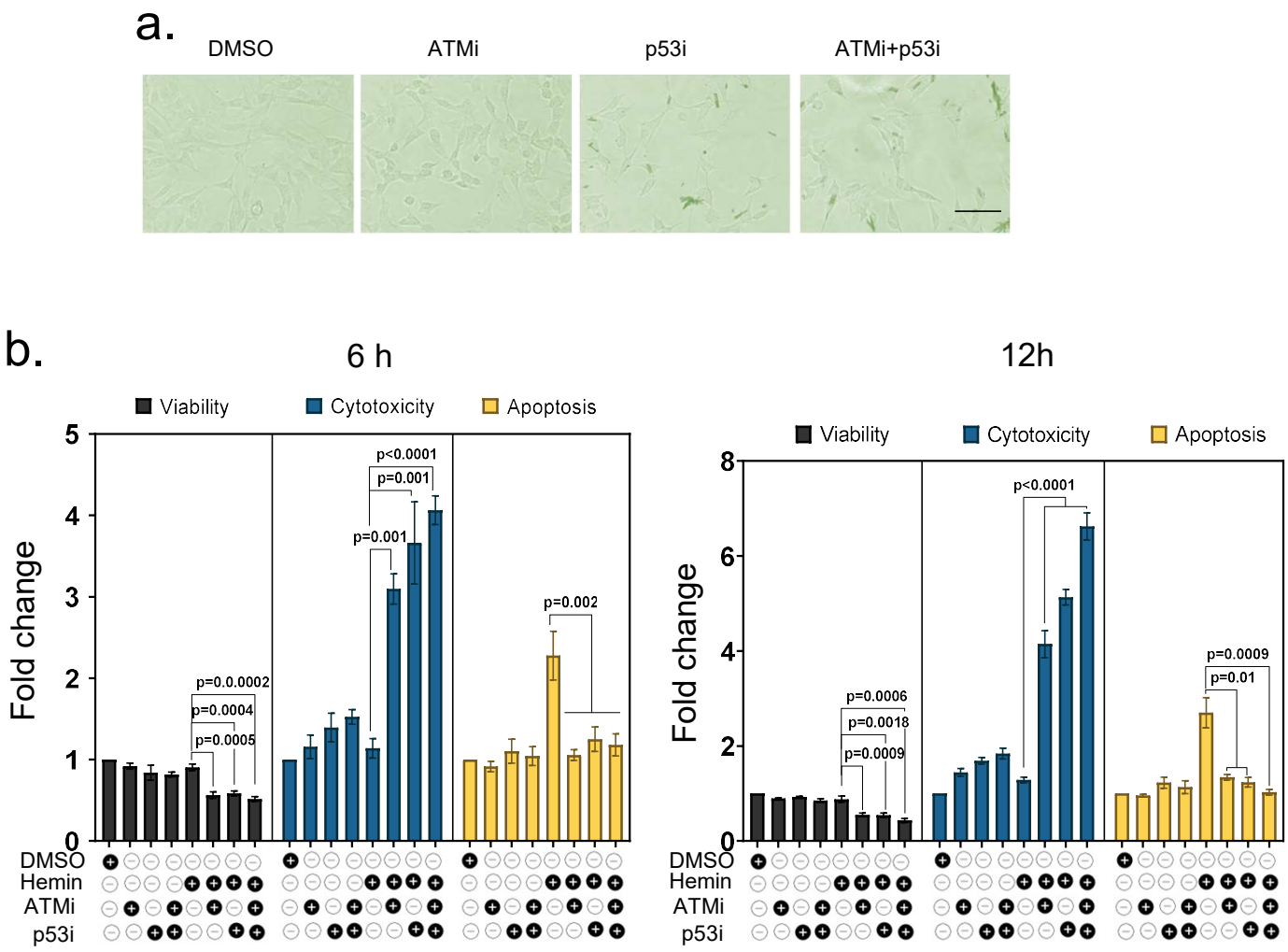
# Supplementary Figure 2




**Supplementary Fig. 2 (Related to Figs. 1 and 2): Hemin-mediated genomic damage, senescence, and HO-1 induction observed in human brain endothelial cells (HBEC-5i).**

(a) Western blot analysis of HBEC-5i cells during a time-dependent kinetics study, treated with hemin (10 μM) (lanes 2-5) or DMSO (lane 1) to measure levels of γH2AX and HO-1. (b) Neutral comet assay of HBEC-5i cells in a time-dependent kinetics study, treated with or without hemin; DMSO-treated cells were used as the control. DNA integrity is represented as mean comet tail moment fold change. (c) SA-β-Gal staining of HBEC-5i cells in a time-dependent kinetics study, treated with hemin. Scale bar = 50μm. All statistical analyses were performed by two-sided student's t-test, p-values are indicated in the respective graph.

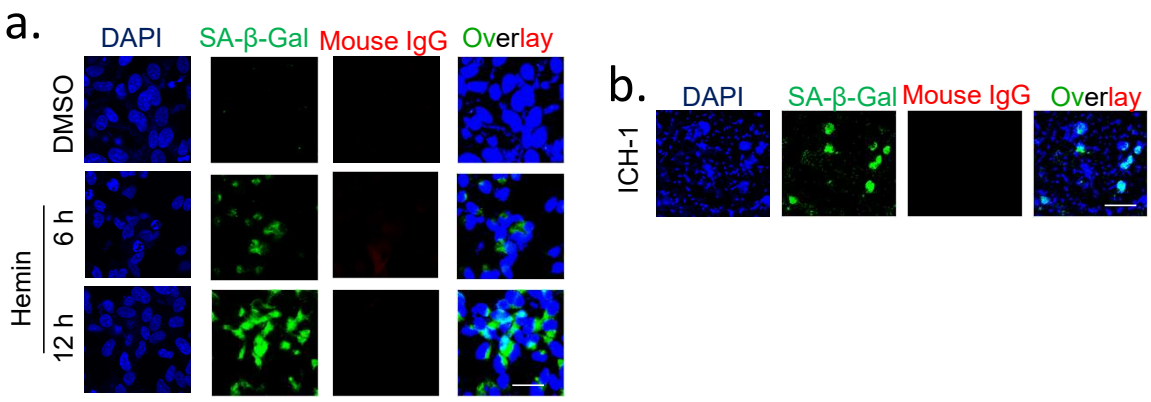
# Supplementary Figure 3




**Supplementary Fig. 3 (Related to Fig. 3): Inhibition of DDR using ATMi or p53i alleviates hemin-induced cell toxicity and death.**

(a) SA-β-Gal staining of SH-SY5Y cells after treatment with pharmacological inhibitors of ATM (KU-55933) and p53 (Pifithrin-α). Scale bar = 20 µm. (b) Viability, cytotoxicity, and apoptosis (caspase-3/7 activity) analysis in cells treated with hemin ± pharmacological inhibitors of ATM and p53 for 6 h and 12 h, DMSO treatment is used as a control. All statistical analyses were performed by two-sided student's t-test, p-values are indicated in the respective graph.

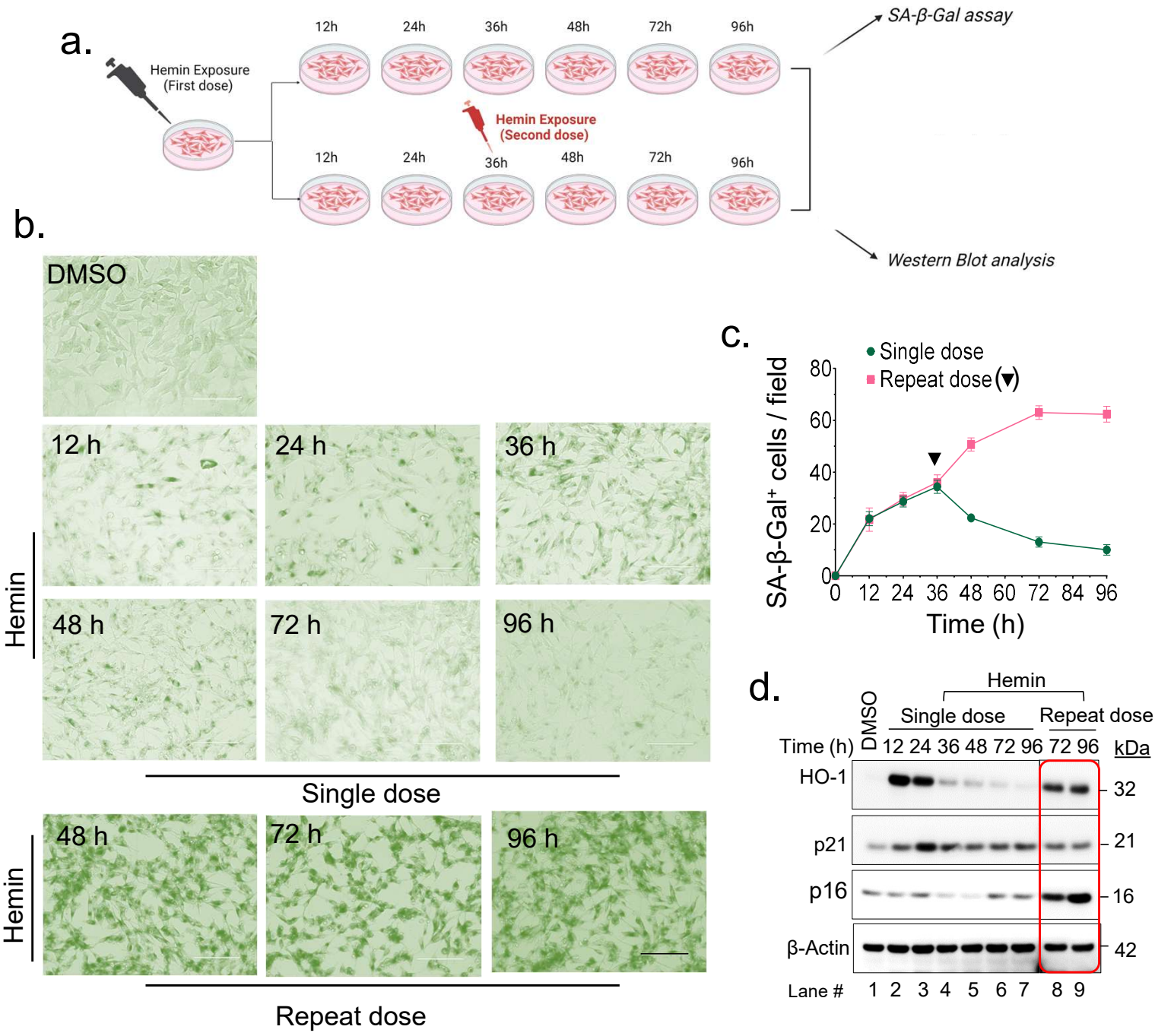
# Supplementary Figure 4




**Supplementary Fig. 4 (Related to Figs. 5 and 6): Validation of antibody specificity.**

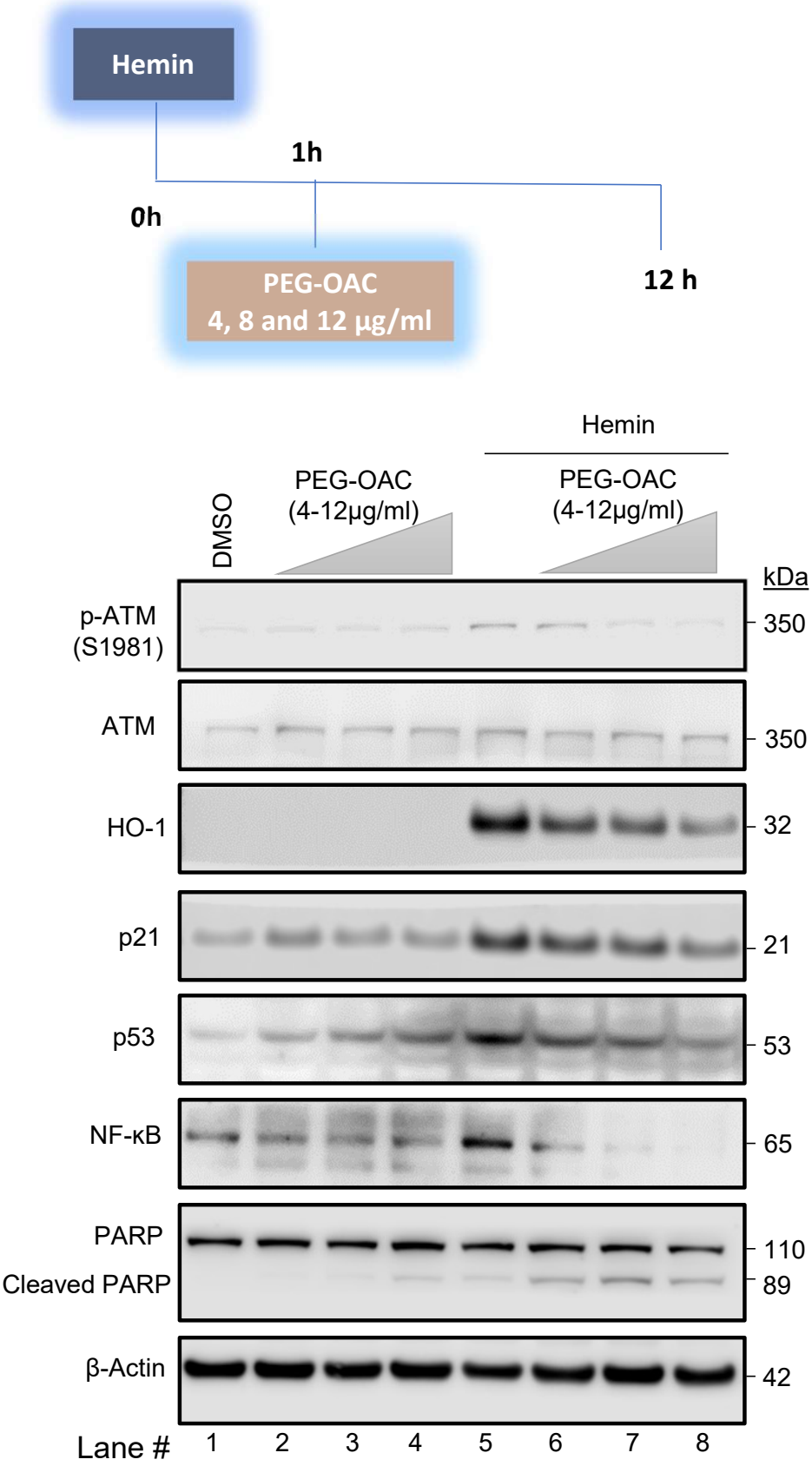
(a) IF co-staining was performed using SA-β-Gal and mouse IgG instead of primary antibody against HO-1 followed by anti-mouse-Alexa Fluor 647 at a 1/1,000 dilution. Nuclear DNA was stained with DAPI in SH-SY5Y cells that were exposed with or without hemin for 6 h and 12 h. Scale bar = 20 µm. (b) In a similar control experiment using ICH patient tissue, IF co-staining was performed using SA-β-Gal and mouse IgG instead of HO-1 primary antibody, counterstained with DAPI. Scale bar = 20 µm.

# Supplementary Figure 5



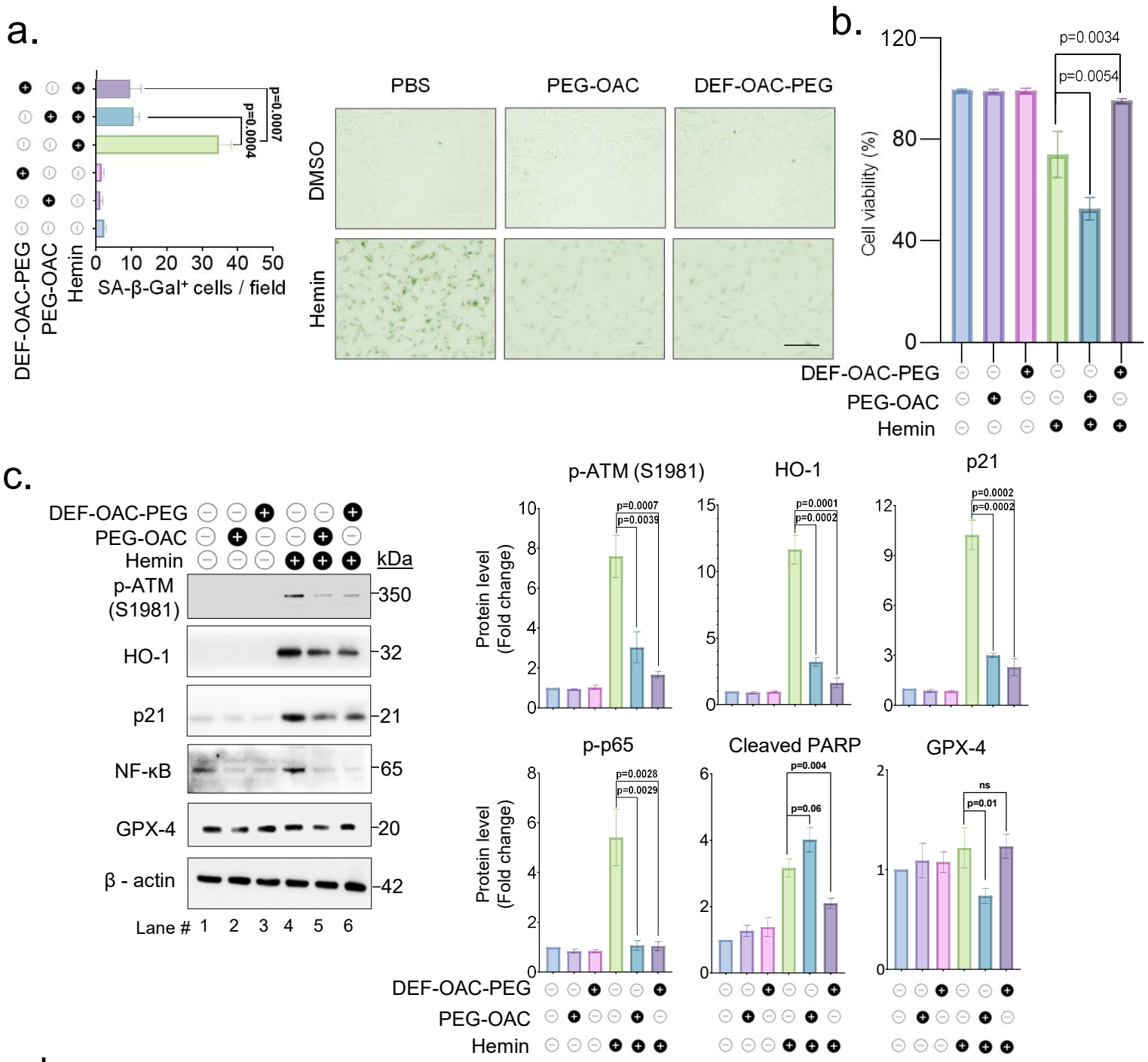

**Supplementary Fig. 5 (Related to Fig. 8):** (a) A schematic diagram illustrating the treatment plan of SH-SY5Y cells with hemin, followed by subsequent experiments. (b) SA-β-Gal staining of SH-SY5Y cells treated with or without hemin (10 μM) at the indicated time points. (c) Quantitation of the SA-β-Gal positive cells per field, arrow indicating the second dose hemin treatment time point. Scale bar = 20 μm. (d) Western blot analysis was performed to evaluate the expression of HO-1, p21, and p16 proteins at indicated time points after hemin treatment. The data was derived from three independent experiments.

# Supplementary Figure 5




**Supplementary Fig. 6: (Related to Fig. 9). PEG-OAC prevents DNA damage and senescence in a dose-dependent manner.** Western blot analysis was conducted with or without PEG-OAC and DEF-OAC-PEG to examine the expression of p-ATM (S1981), HO-1, p21, p53, NF-κB (p65), and cleaved PARP proteins 12h after hemin treatment.

# Supplementary Figure 6



**Supplementary Fig.7 (Related to Fig. 9):**

SH-SY5Y cells were exposed to PEG-OACC and DEF-OAC-PEG nanoparticles 1 h after hemin (10 μM) treatment. (a) Percentage of SA-β-Gal positive cells in experimental groups, along with representative images of SA-β-Gal staining in differentiated NPSCs cells treated with or without hemin and nanoparticles (PEG-OAC and DEF-OAC-PEG) for 24 h. Scale bar = 20 μm. (b) Viability assay of SH-SY5Y cells treated with hemin and nanoparticles (PEG-OAC and DEF-OAC-PEG) for 24 h. (c) Western blot analysis to assess the expression of p-ATM (S1981), HO-1, p21, NF-κB (p65), cleaved PARP, and GPX-4 proteins at indicated time points, the data presented is derived from three independent experiments. (d) Representative image of Lipid peroxidation assay using Click-iT™ Lipid Peroxidation Imaging Kit after 24 h treatment with hemin and nanoparticles (PEG-OAC and DEF-OAC-PEG) Scale bar = 20 μm. All statistical analyses were performed by two-sided student's t-test, p-values are indicated in the respective graph.

| Sample ID | Age (years) | Sex | Clinic Diagnosis | Time from ICH to sample collection (Days) | Type of Surgery |
|---|---|---|---|---|---|
| Control 1 | 57 | Female | Unknown | NA | non-neurological postmortem tissue. |
| Control 2 | 43 | Female | Unknown | NA | non-neurological postmortem tissue. |
| Control 3 | 63 | Male | Encephalopathy, broncho-pneumonia and pleuritis, acute renal dysfunction | NA | non-neurological postmortem tissue. |
| ICH 1 | 70 | Male | Intraparenchymal hematoma | ~1 | Left frontal occipital craniotomy and resection of intraventricular hemorrhage with neuronavigation |
| ICH 2 | 30 | Female | Residual vascular malformation with hemorrhage | ~22 | Left frontal craniotomy and microsurgical resection of cavernous vascular malformation with intraoperative MRI and neuronavigation |
| ICH 3 | 64 | Male | Intracerebral hemorrhage with acute hypoxic/ ischemic injury | ~3 | Right-sided craniotomy and microscopic resection of ICH with neuronavigation |

**Supplementary Table 1:** Demographics and clinical diagnosis of ICH patients and non-neurological control brain tissues from the Binghamton Biospecimen Archive.