The lipid peroxidation product 4-hydroxynonenal contributes to oxidative stress-mediated deterioration of the ageing oocyte

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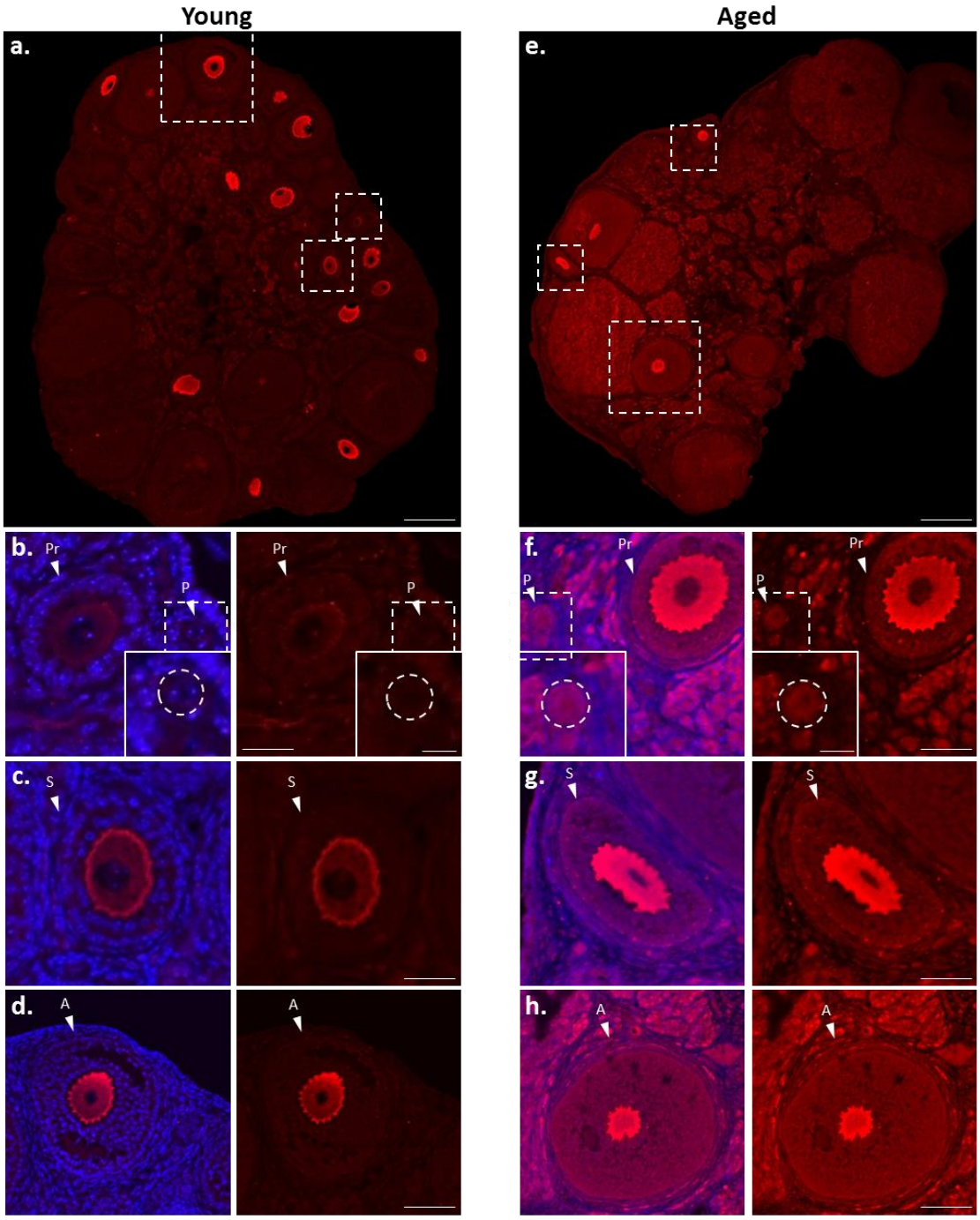

Fig. S1: **4-HNE is a by-product of lipid peroxidation in the mouse ovary.** The production and localisation of 4-HNE between young (4 to 6 weeks) and aged (14 months) mouse ovaries was determined using immunofluorescence**. (a)** 4-HNE expression (red) was observed at basal levels within all cell types within the young ovary (scale bar = 200 µm) including oocytes in **(b)** primordial (P), primary (Pr, scale bar = 20 µm), **(c)** secondary (S, scale bar = 40 µm) and **(d)** antral follicles (A, scale bar = 80 µm). **(e)** 4-HNE generation was also observed in all cell types within the aged ovary (scale bar = 300 µm) including oocytes in **(f)** primordial, primary (scale bar = 20 µm) **(g)** secondary (scale bar = 40 µm) and **(h)** antral follicles (scale bar = 80 µm). Nuclei were counterstained with DAPI (blue). Immunofluorescence assays were performed in both technical and biological triplicate.

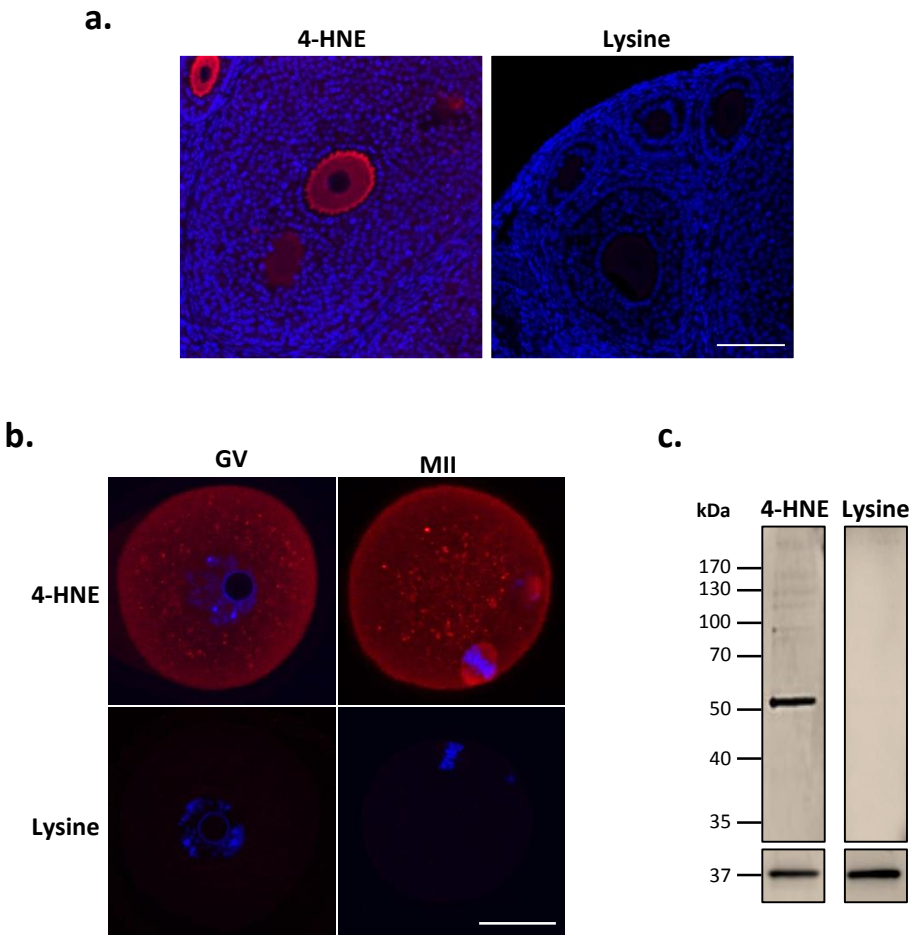

Fig. S2: **4-HNE antibody specificity.** L-Lysine-4-HNE bound anti-4-HNE antibody controls (lysine) revealed no unspecific primary or secondary antibody binding during **(a)** Immunofluorescence (scale bar = 40 µm) or **(b)** immunocytochemistry (scale bar = 20 µm) compared to unbound anti-4-HNE antibody (red). Nuclei were counterstained with DAPI (Immunofluorescence) or Hoechst (immunocytochemistry) (blue). Antibody specificity was also confirmed for immunoblotting, which also revealed no unspecific primary or secondary antibody binding in L-Lysine-4-HNE bound anti-4-HNE antibody controls (lysine). Immunoblots were stripped and re-probed with GAPDH as a loading control.

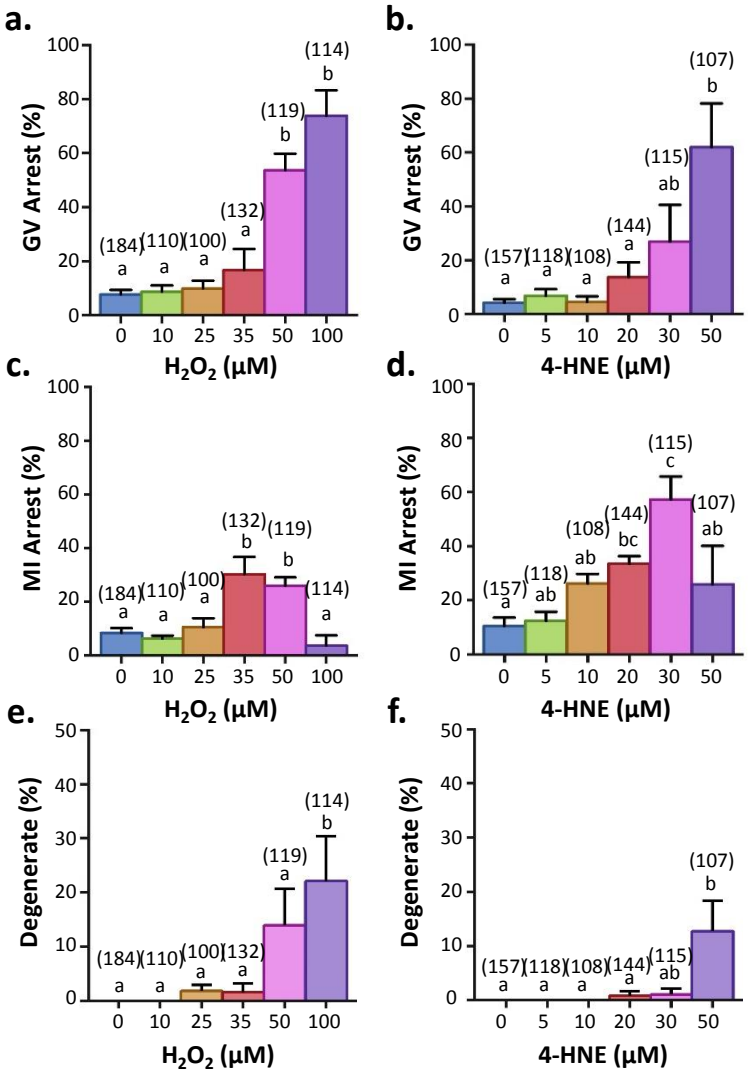

Fig. S3: **Acute exposure to H_2O_2 and 4-HNE at GV stage causes meiotic arrest and degeneration.** Oocytes at GV stage were treated with either H_2O_2 for 1 h or 4-HNE for 2 h prior to IVM for 16 h. For the purpose of this analysis, GV oocytes were identified by the presence of a nuclear envelope and nucleolus, MI oocytes by the absence of the nuclear envelope and nucleolus and degenerative oocytes were identified *via* cytoplasmic fragmentation. **(a)** A dose-depended increase in GV arrest was observed after H_2O_2 (one-way ANOVA; $p \leq 0.0011$) and **(b)** 4-HNE (one-way ANOVA; $p \leq 0.0037$) treatment. **(c)** A dose-dependent increase following a significant decrease was also observed in MI arrest after H_2O_2 (one-way ANOVA; $p \leq 0.0456$) and **(d)** 4-HNE (one-way ANOVA; $p \leq 0.0420$) treatment. **(e)** Furthermore, a dose-depended increase in oocyte degeneration was observed after H_2O_2 (one-way ANOVA; $p \leq 0.0037$) and **(f)** 4-HNE (one-way ANOVA; $p \leq 0.0003$) treatment. Error bars represent SEM. IVM experiments were performed with a minimum of five replicates with each replicate containing between 20-50 oocytes pooled from a minimum of three animals.

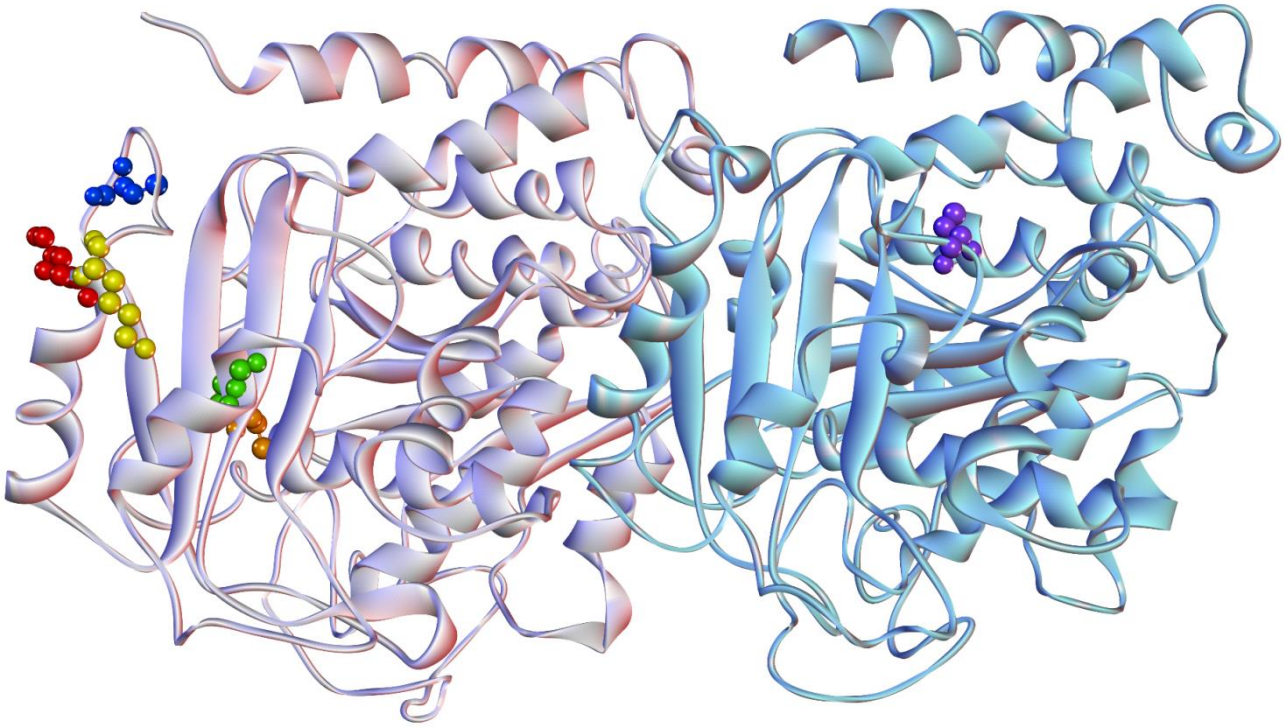

Fig. S4: **α- and β-tubulin dimer homology model with confirmed 4-HNE modifications.** α- tubulin (white) 4-HNE modifications have been identified on Cys295 (green), Lys336 (red), Lys338 (yellow), Cys347 (blue) and Cys376 (orange) as well as on β-tubulin (blue) on Cys303 (purple)[46,62,63].

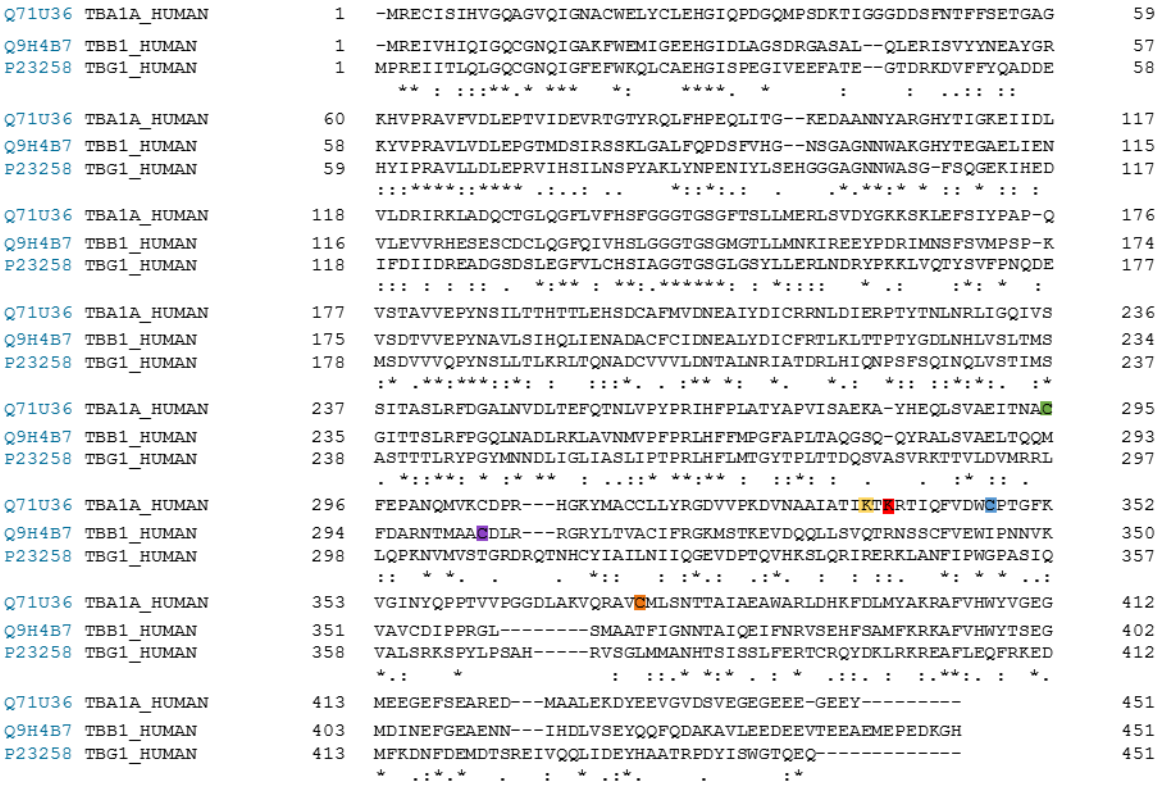

Fig. S5: **Amino acid sequence alignment between α-, β- and γ-tubulin represent a 20% sequence homology.** An asterisk (*) represents residues conserved between three tubulin isoforms, a colon (:) represents conserved substitution of residues between tubulin isoforms and a full stop (.) represents semi-conserved substitutions such as amino acids with similar characteristics. α-tubulin 4-HNE modifications have been identified on Cys295 (green), Lys336 (red), Lys338 (yellow), Cys347 (blue) and Cys376 (orange) as well as on β- tubulin on Cys303 (purple)[46,62,63].

***Table S1*: LC–MS/MS protein identification (ID) of the predominant 55 kDa protein(s) targeted for adduction by 4-HNE.**

Protein ID (gene symbol)	Mascot score	MW [kDa]	No. Peptides
Actin (ACTB)	152.1	41.7	3
Tubulin alpha (TBA3)	81.1	49.9	2
Tubulin beta (TBB4A)	78.1	49.9	2

***Table S2:* Antibodies used for immunocytochemistry (ICC), immunofluorescence (IF), proximity ligation assays (PLA), immunoprecipitation (IP) and immunoblotting (IB).**

Antibody	Species	Concertation	Dilution/ concentration	Source and Cat#
4-HNE	Rabbit polyclonal	1 mg/ ml	1:50 (IF, ICC, PLA) 1:500 (IB)	Cat # HNE11-S, Jomar Diagnostics
4-HNE	Mouse monoclonal	0.1 mg/ ml	1:50 (IB)	Cat # ab48506, Abcam
α-tubulin	Mouse monoclonal	1 mg/ ml	1:400 (ICC, PLA)	Cat # A11126 ThermoFisher
α-tubulin	Rabbit monoclonal	1 mg/ ml	10 µg (IP)	Cat # ab52866, Abcam
α-tubulin	Mouse monoclonal	6 mg/ ml	1:2000 (IB)	Cat # T5168, Sigma-Aldrich
β-tubulin	Rabbit polyclonal	1 mg/ ml	10 µg (IP)	Cat # ab6046, Abcam
β-tubulin	Mouse monoclonal	0.2 mg/ ml	1:100 (ICC, PLA) 1:500 (IB)	Cat# sczsc-5274, Santa Cruz
γ-tubulin	Rabbit polyclonal	0.6 mg/ ml	10 µg (IP)	Cat # ab84355, Abcam
γ-tubulin	Mouse monoclonal	5 mg/ ml	1:1000 (ICC, PLA) 1:5000 (IB)	Cat # T6557, Sigma-Aldrich
CREST	Human monoclonal	1 mg/ ml	1:400 (ICC)	Cat # 90C-CS1058, Fitzgerald
GAPDH	Rabbit polyclonal	5 mg/ ml	1:5000 (IB)	Cat # G9545, Sigma-Aldrich