Altieri P *et al.*, 5-fluorouracil causes endothelial cell senescence: potential protective role of glucagon-like peptide 1.

Supporting Information

Table S1. Primers used in the study

Gene	Accession number	Primer sequence (5′-3′)	Product length
VCAM1	NM_001078.3	Fwd: TCAGATTGGAGACTCAGTCATGT Rev: ACTCCTCACCTTCCCGCTC	109
ICAM1	NM_000201.2	Fwd: GGCCGGCCAGCTTATACAC Rev: AGACACTTGAGCTCGGGCA	166
TYMP	NM_001257989.1	Fwd: CCTGCGGACGGAATCCTATA Rev: TGTGATGAGTGGCAGGCTGT	69
GAPDH	NM_001289746.1	Fwd: TGGTCACCAGGGCTGCTTT Rev: AGCTTCCCGTTCTCAGCCT	151

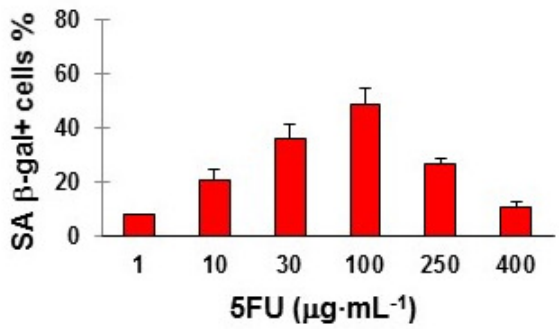

Fig. S1. Senescence of EA.hy926 cells in response to increasing concentrations of 5FU.

EA.hy926 cells were incubated for 4 hours with the indicated concentrations of 5FU. After this treatment, senescence was assessed as percentage of SA β-gal positive cells. No apoptosis, as evaluated by immunocytochemistry using a rabbit monoclonal anti-cleaved caspase-3 antibody (clone 5A1E, Cell Signaling Technologies), was observed. The experiment was repeated 5 times.

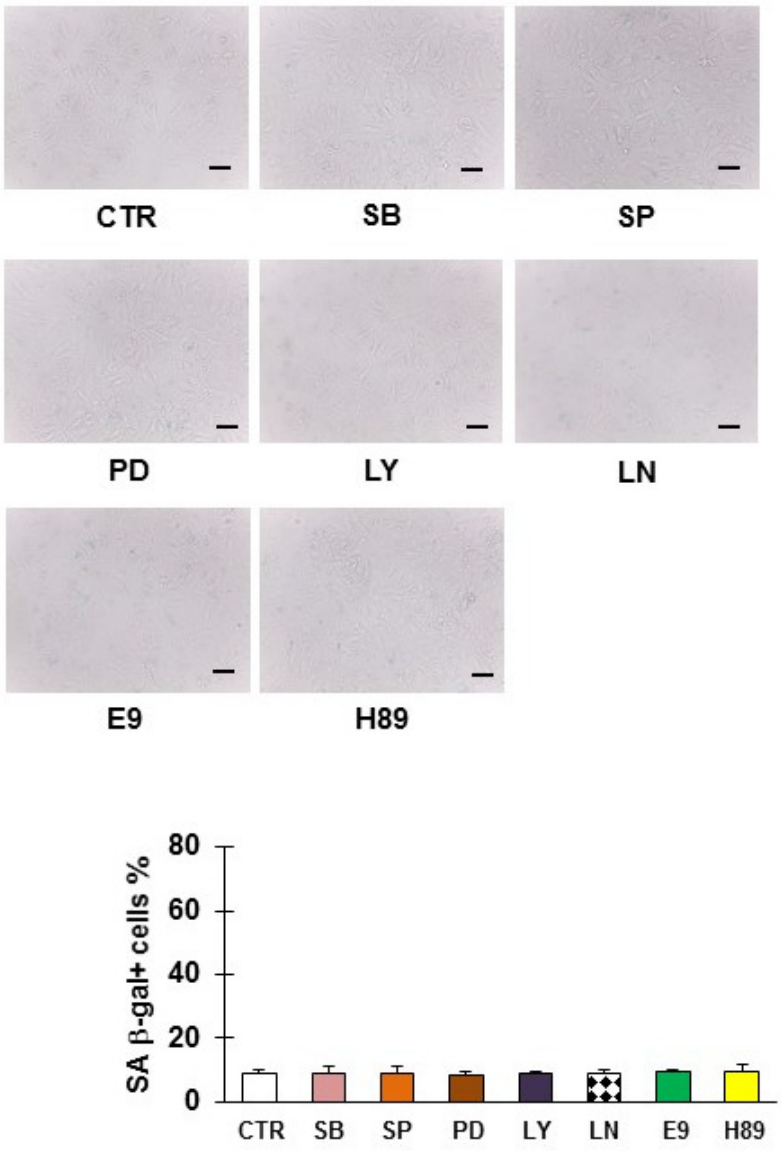

Fig. S2. Effect of the signaling inhibitors used for functional experiments on senescence of EA.hy926 cells.

EA.hy926 cells were treated with the following molecules (see Methods for times and concentrations): SB203580 (inhibitor of p38); SP600125 (inhibitor of JNK); PD98059 (inhibitor of ERK1/2); LY-294002 (inhibitor of phosphatidylinositol-3 kinase); L-NAME (inhibitor of nitric oxide synthase); exendin (9-39) (antagonist of GLP-1 receptor); or H89 (inhibitor of PKA). Senescence was evaluated by staining for SA β-gal and compared to the one found in untreated cells (CTR). The upper part of the Figure shows representative pictures of SA β-gal staining, whilst the percentage of positive cells (mean ± SEM of 5 independent replicates) is presented in the graph in the lower part.

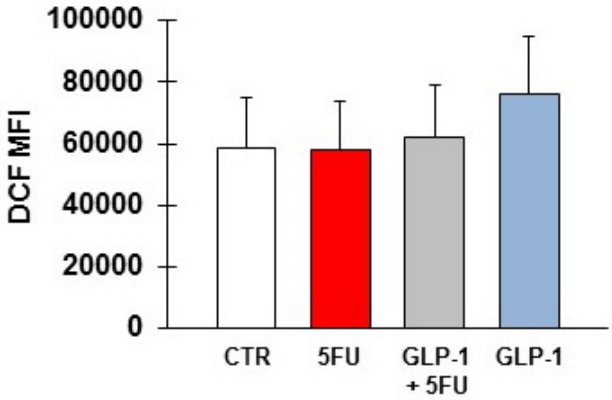

Fig. S3. Oxidative stress in EA.hy926 cells treated with 5FU and/or GLP-1.

Mean fluorescence intensity (MFI) of dichlorofluorescein in EA.hy926 cells after no treatment (CTR) or incubation with 5FU, 5FU preceded by GLP-1, or GLP-1 alone. N = 5.

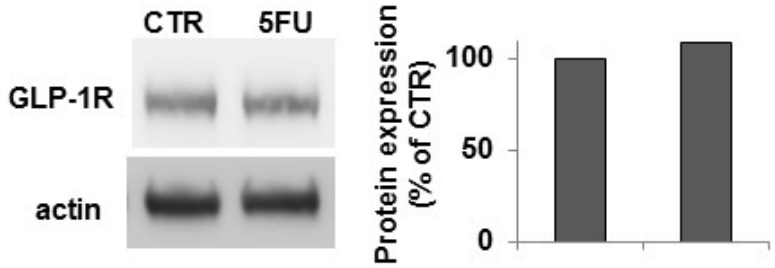

Fig. S4. GLP-1R protein levels in EA.hy926 cells.

Representative western blot and densitometry analysis of the expression of GLP-1R protein in EA.hy926 cells after no treatment (CTR) or exposure to 5FU.

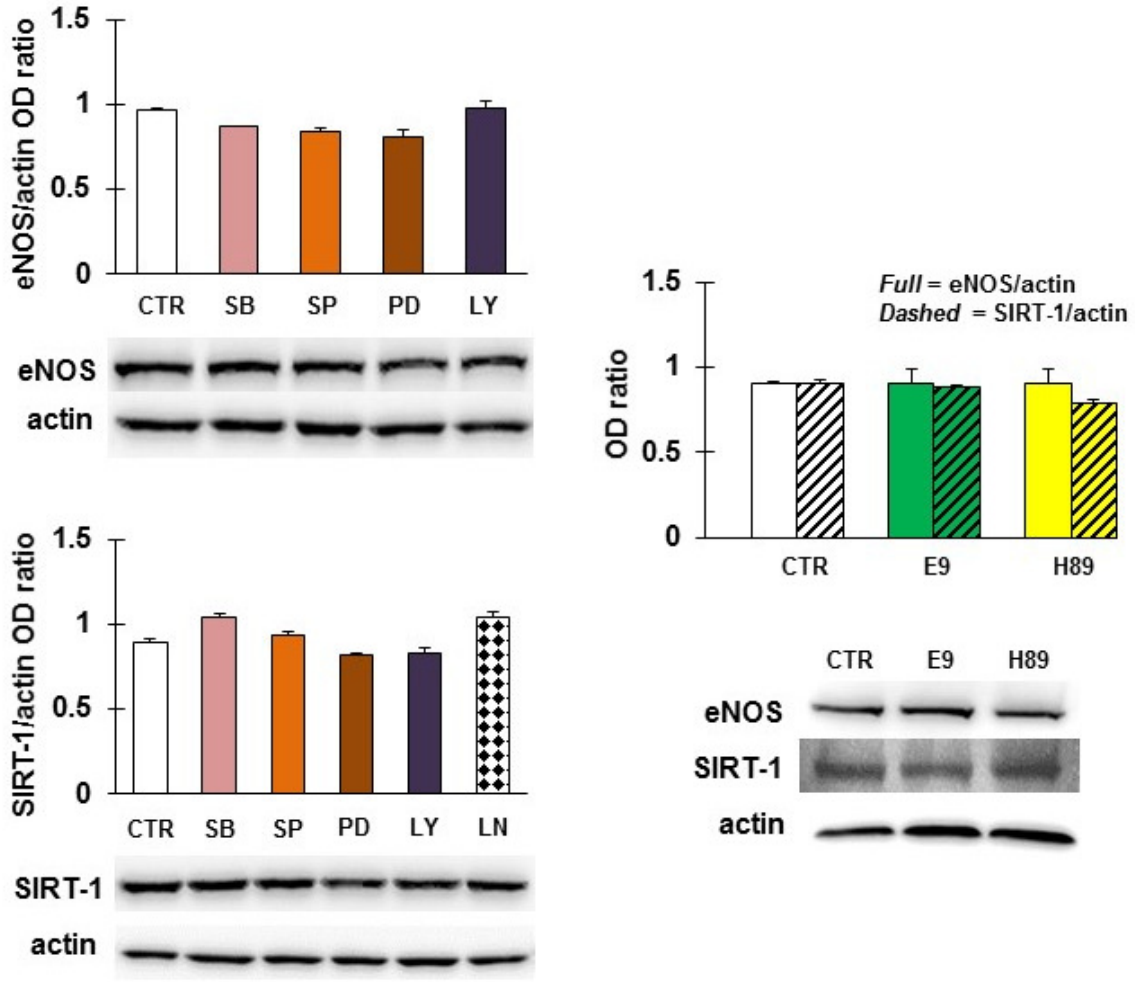

Fig. S5. Effect of the signaling inhibitors used for functional experiments on eNOS and SIRT-1 expression by EA.hy926 cells.

Densitometry analysis and representative western blots for eNOS and SIRT-1 in EA.hy926 after no treatment (CTR) or incubation with the following molecules (see Methods for times and concentrations): SB203580 (inhibitor of p38); SP600125 (inhibitor of JNK); PD98059 (inhibitor of ERK1/2); LY-294002 (inhibitor of phosphatidylinositol-3 kinase); L-NAME (inhibitor of nitric oxide synthase); exendin (9-39) (antagonist of GLP-1 receptor); or H89 (inhibitor of PKA). The optical density of the eNOS and SIRT-1 bands was normalised for the one of actin. Data are mean ± SEM of 5 independent experiments.