



**Supplemental FIGURE 1: Spectral imaging-based and senolytic assay in MEFs.** (A) Representative images of unlabeled MEFs untreated or treated with doxo or  $\gamma$ -IR to induce senescence, acquired with a 488-excitation laser and an emission filter 525/50. Hoechst 33342 was used for nuclear counterstaining. Scale Bar: 50  $\mu$ m. (B) Spectral Imaging analysis of senescent MEFs, untreated or treated with doxo or  $\gamma$ -IR to induce senescence, after excitation at 405nm. Representative images recorded at 414nm, 444nm, 504nm, 514nm, 574nm, 584nm, and 594nm wavelengths are depicted. (C) Mean Fluorescence Intensity of the cytoplasmic and nuclear regions of interest of control and senescent MEFs, acquired at wavelengths comprised between 404nm and 644nm after excitation at 405nm. (D) Representative images and count of H2B-GFP versus H2B-RFP nuclei from H2B-GFP quiescent and H2B-RFP senescent (doxo-treated) MEF cells co-cultures incubated with vehicle (DMSO), navitoclax (Navi), digoxin or fisetin for 48 hours. Data (mean  $\pm$  SD) are represented as Fold Change to DMSO. One representative experiment (N= 3 independent experiments) is shown (\* $p$ < 0.05; \*\* $p$ <0.01, two-tailed Student's t-test compared to senescent DMSO). Scale Bar: 500 nm.