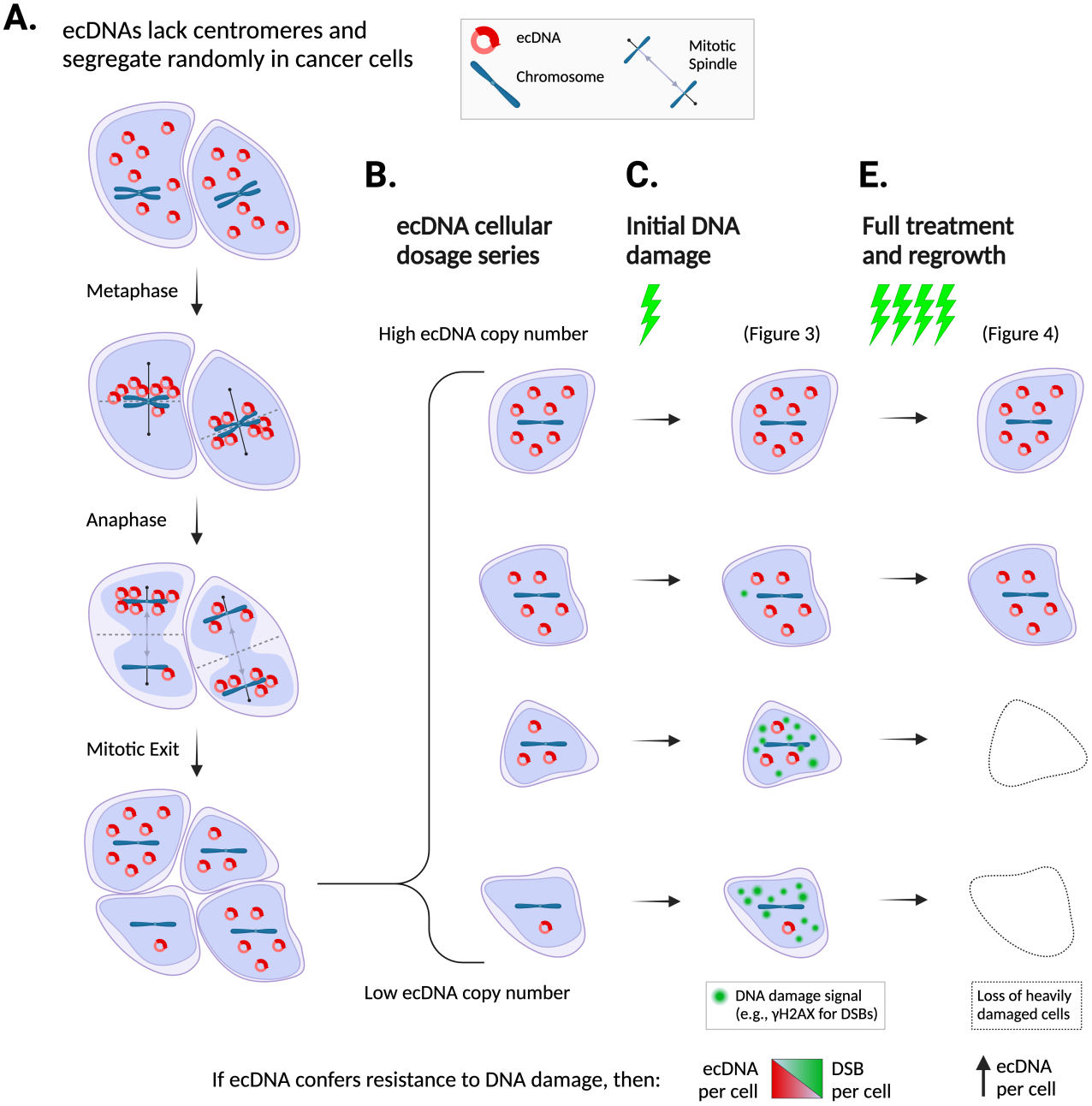
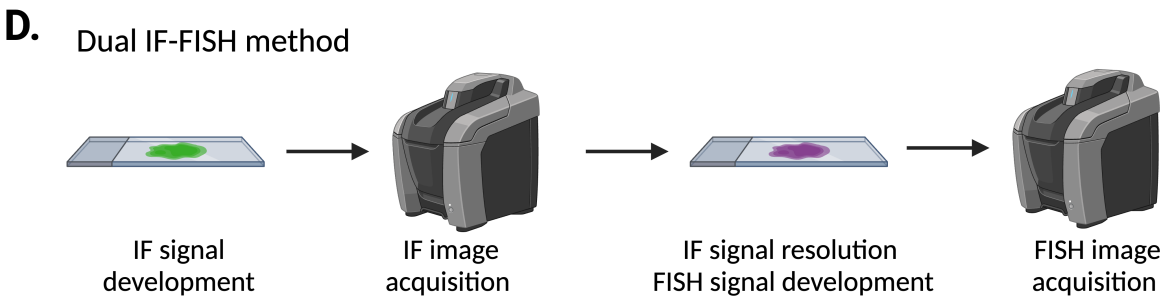
Supplementary Figure S5





Supplementary Figure S5. Method to compare ec*MYC* level with resistance to drug-induced DNA damage. (**A**) Circular ecDNAs lack centromeres and do not attach directly to the mitotic spindle during cell division. This results in random segregation during mitosis, with a binomial distribution of copy numbers in daughter cells (7). If there is a selective advantage for cells with higher ecDNA copy number, e.g., in the case of resistance gene amplifications in the presence of cytotoxic drugs, then repeated rounds of cell divisions and selection will result in tumors with high-level amplifications. (**B**) Here we take advantage of this random asymmetric segregation to generate a natural dosage series of ecDNAs in each xenograft without experimental manipulation. (**C**) Immediately after drug exposure, single-cell measurements of *MYC* FISH for ec*MYC* and γH2AX foci for double-stranded break (DSB) signaling are compared to determine whether DNA damage is induced selectively in cells with lower ec*MYC* levels. (**D**) For the analysis described in **Supplementary Fig. S5C** and presented in **Fig. 3**, formalin-fixed tissue sections are mounted on slides and probed for γH2AX and MYC protein immunofluorescence (IF), with DAPI nuclear staining. The positional coordinates of each IF microscopy field are recorded. The slide is then removed and probed for *MYC* FISH, with re-application of DAPI nuclear staining. The original fields are re-imaged using saved coordinates. DAPI images are used to map nuclei for fine registration of the IF and FISH images. (**E**) Following full cytotoxic regimens and xenograft progression, single-cell measurements of *MYC* FISH determine whether there was selection for higher ec*MYC* copy number. (created with BioRender.com)