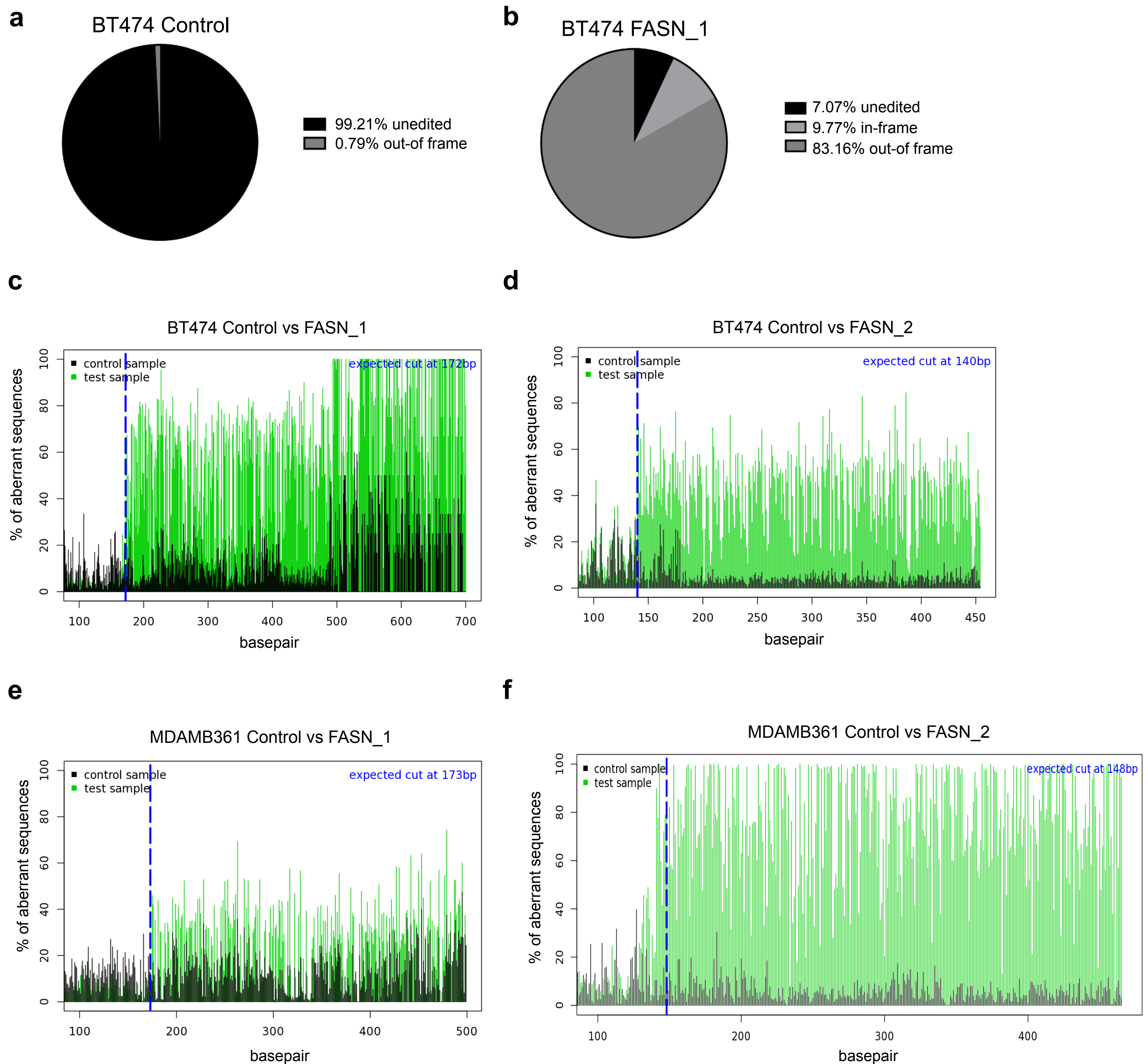
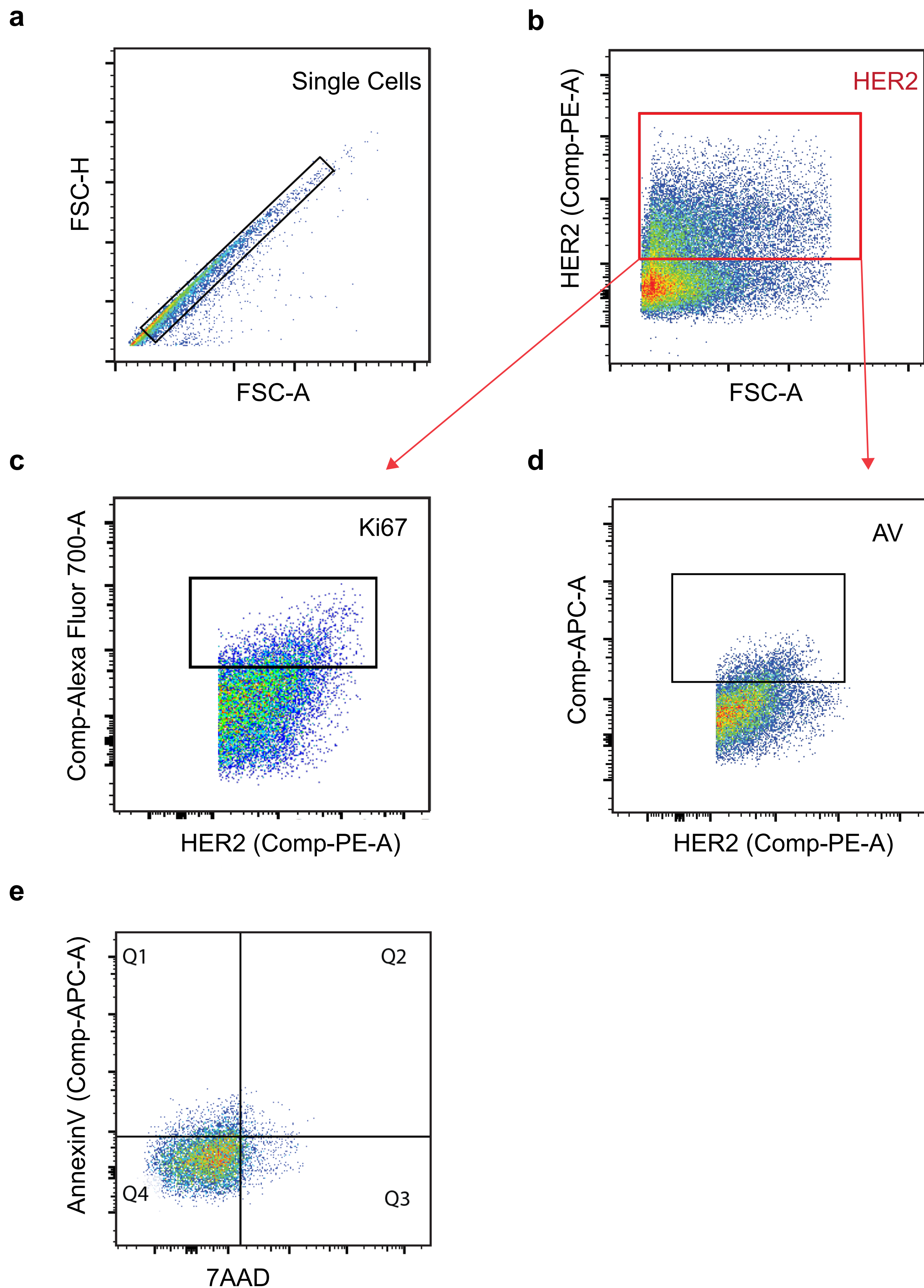


Supplementary Figure 1. CRISPR/Cas9 editing efficiency.
a-b) Next generation amplicon sequencing was performed to assess editing efficiency in BT474 control cells **(a)** and in a clone in which FASN is disrupted by CRISPR/Cas9 (sgFASN_1) **(b)**.
c-f) Sanger sequencing of a PCR amplified region of the FASN gene containg the gRNA sequence in BT474 and MDAMB361 cells (**see Supplementary Table 2**). Data are presented as Tracking of Indels by Decomposition (TIDE) analysis where the blue dotted line indicates the expected cutting site of the sgRNA targeting FASN. The sequences obtained from cells with control and FASN-targeted sgRNAs were compared for each independent guide in each cell line: comparison of BT474 control cells with sgFASN_1 clone (test sample) **(c)**, comparison of BT474 control cells with sgFASN_2 clone (test sample) **(d)**, comparison of MDAMB361 control cells with sgFASN_1 clone (test sample) **(e),** and comparison of MDAMB361 control cells with sgFASN_2 clone (test sample) **(f)**.



Supplementary Figure 2. Flow cytometry gating strategy for brain-tumor derived organotypic slice cultures.
a) Single cells were first identified by forward scatter height (FSC-H) versus forward scatter area (FSC-A) gate.
b) Gating strategy to identify HER2+ BT474 tumor cells.
c,d) Gating strategy to assess Ki67 (proliferation marker) and Annexin V (AV, apoptotic cell marker) staining in HER2+ cells.
e) Gating strategy to identify dead and apoptotic cells using Annexin V and 7-aminoactinomycin (7AAD, viability marker) staining.