



P3.228: Adenosine deaminase acting on RNA (ADAR1) mediates Osimertinib resistance in EGFR-mutant NSCLC

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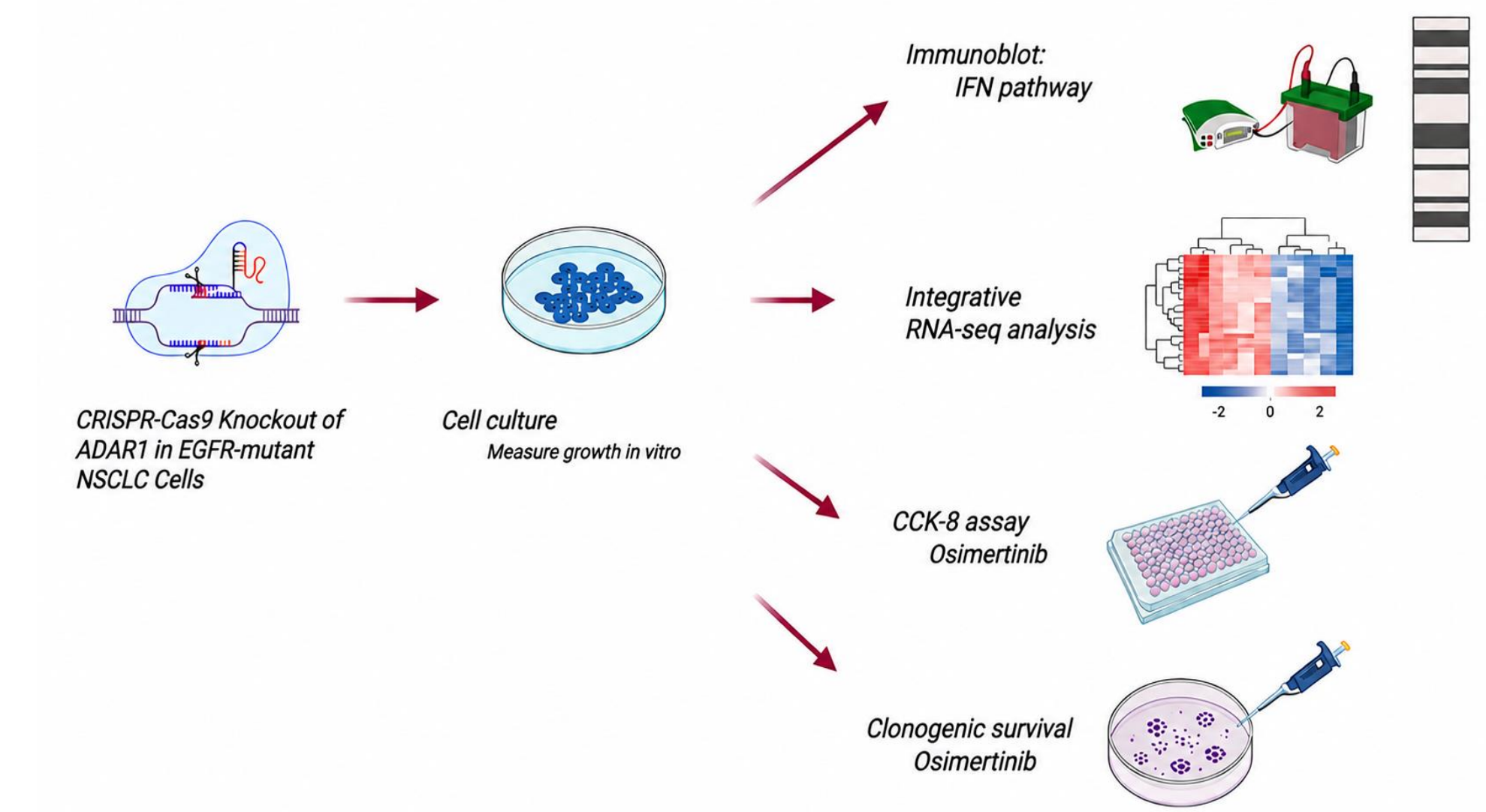
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Background

- Osimertinib achieves >80% response rates in EGFR-mutant NSCLC, yet acquired resistance develops in virtually all patients within 18–21 months.
- ADAR1 is overexpressed in NSCLC, required for tumorigenesis and dampens IFN responses.
- Osimertinib treatment acutely induces IFN signaling
- We hypothesize that osimertinib-induced IFN signaling upregulates ADAR1-p150, suppressing innate immune activation to enable drug tolerance and resistance.

Materials and Methods

- CRISPR/Cas9 ADAR1 KO was generated in H1975 cells (EGFR T790M) and assessed for growth, osimertinib sensitivity (CCK-8), and resistance acquisition.
- Innate immune activation (phospho-PKR, phospho-eIF2 α , MDA5) was assessed by western blot in H1975 WT, KO, and OsiR cells.
- Integrative RNA-seq analysis of GSE193258 and GSE278222 characterized the IFN transcriptional response to acute osimertinib and in resistant cells.



Conclusions

- ADAR1 KO sensitizes H1975 cells to osimertinib and prevents acquired resistance**
- Osimertinib drug tolerance requires ADAR1**
- Osimertinib acutely activates IFN signaling that is suppressed as resistance develops.**
- ADAR1 KO restores IFN signaling after osimertinib treatment**

Future Directions

- Future studies will test whether ADAR1 knockdown reverses resistance in OsiR cells and organoids
- Develop ADAR1 targeted approaches for clinical translation

REFERENCES

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ChatGPT was used to edit the schematic image describing the Methods for the experimental work. AI was NOT used for any other portion of creating or writing this abstract or poster.

Results

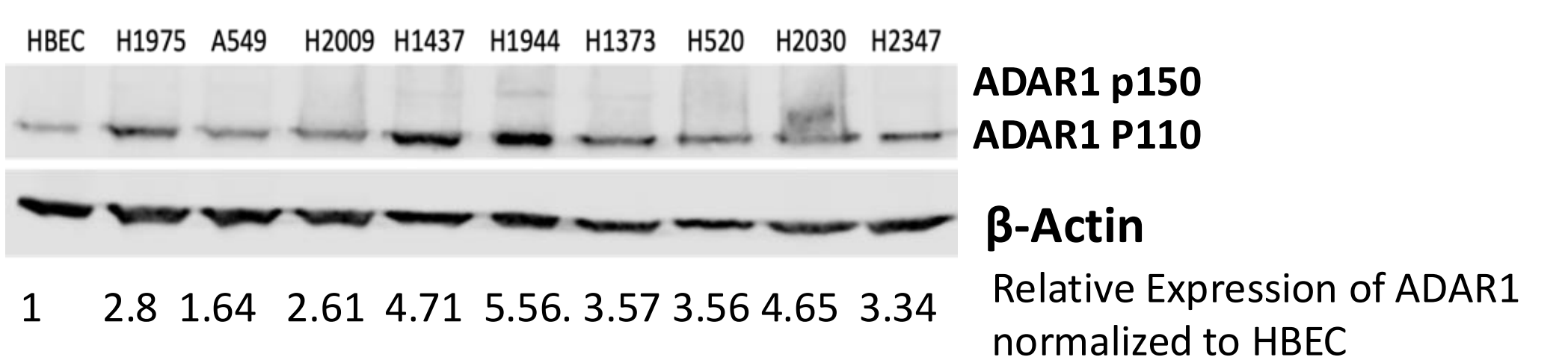


Figure 1. ADAR1 expression in human NSCLC cell lines. A panel of human NSCLC cells were assayed by immunoblot for ADAR1. Human bronchial epithelial cells (HBEC) are noncancerous cells used as a control. ADAR1 levels were normalized to the optical density of ADAR1/β-actin in HBEC.

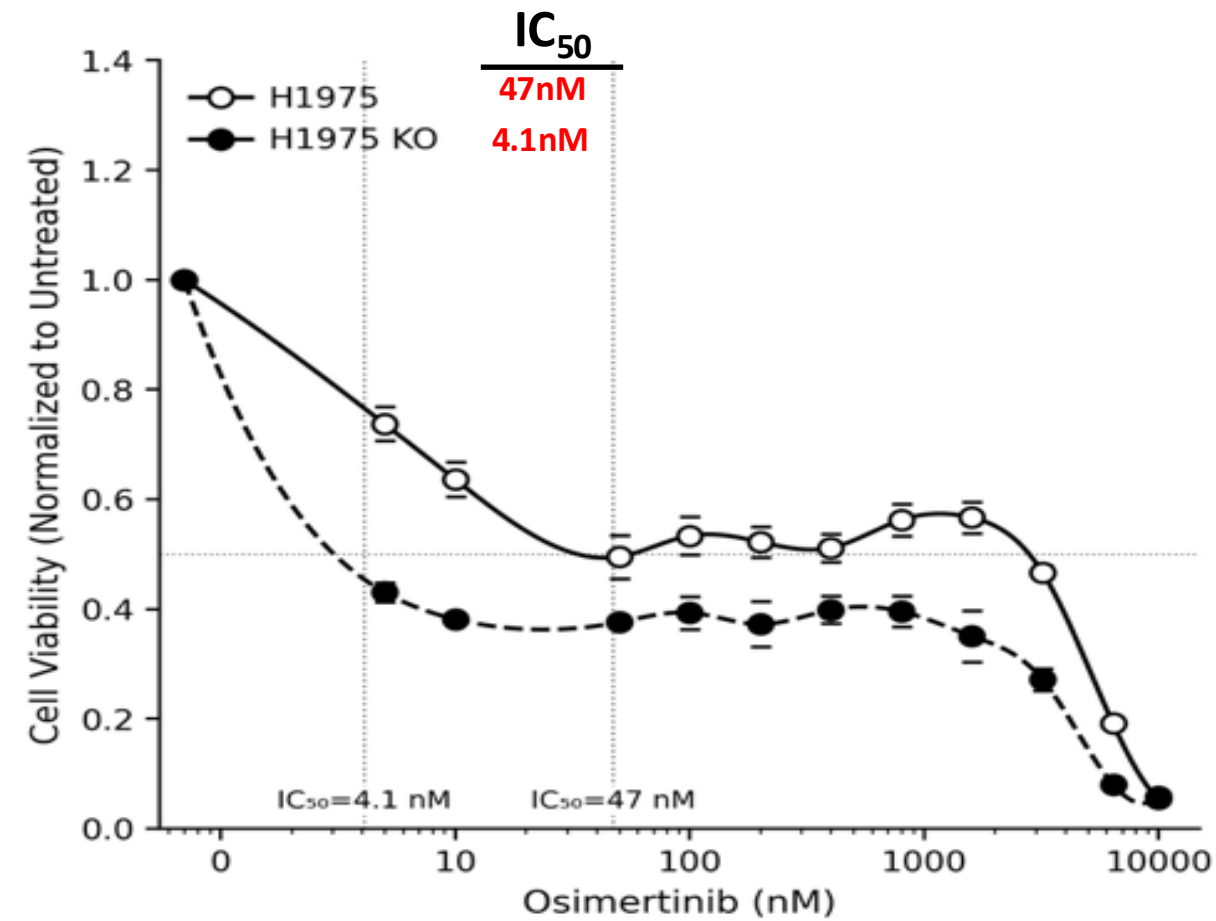


Figure 3. ADAR1 KO dramatically sensitizes H1975 cells to osimertinib. H1975 WT and ADAR1 KO cells were treated with increasing doses of osimertinib and cell viability assessed at 72 hours (triplicate). ADAR1 KO cells show markedly reduced IC50 compared to WT cells.

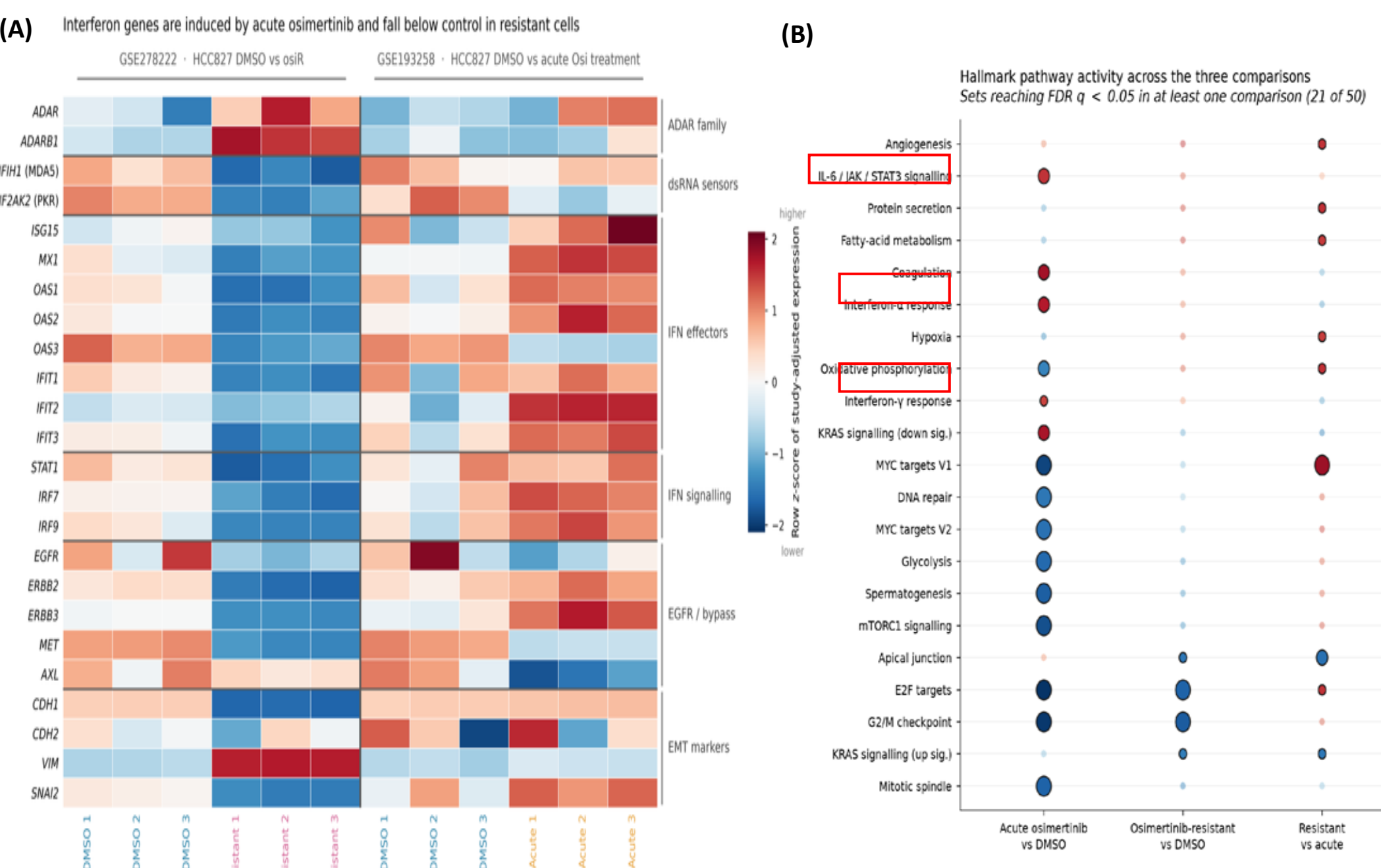


Figure 4. Osimertinib treatment acutely results in enhanced type I IFN response that is markedly suppressed in osimertinib-resistant cells. Publicly available RNA-seq data analysis from two independent studies of HCC827 (EGFR ex19del NSCLC cell line) analyzed together (GSE278222: DMSO, n = 3; osimertinib-resistant, n = 3. GSE193258: DMSO, n = 3; acute osimertinib, n = 3). (A) Heatmap of 24 genes grouped into six functional categories (right-hand labels). (B) Interferon and IL-6/JAK/STAT3 signaling are engaged acutely and not sustained in resistance.

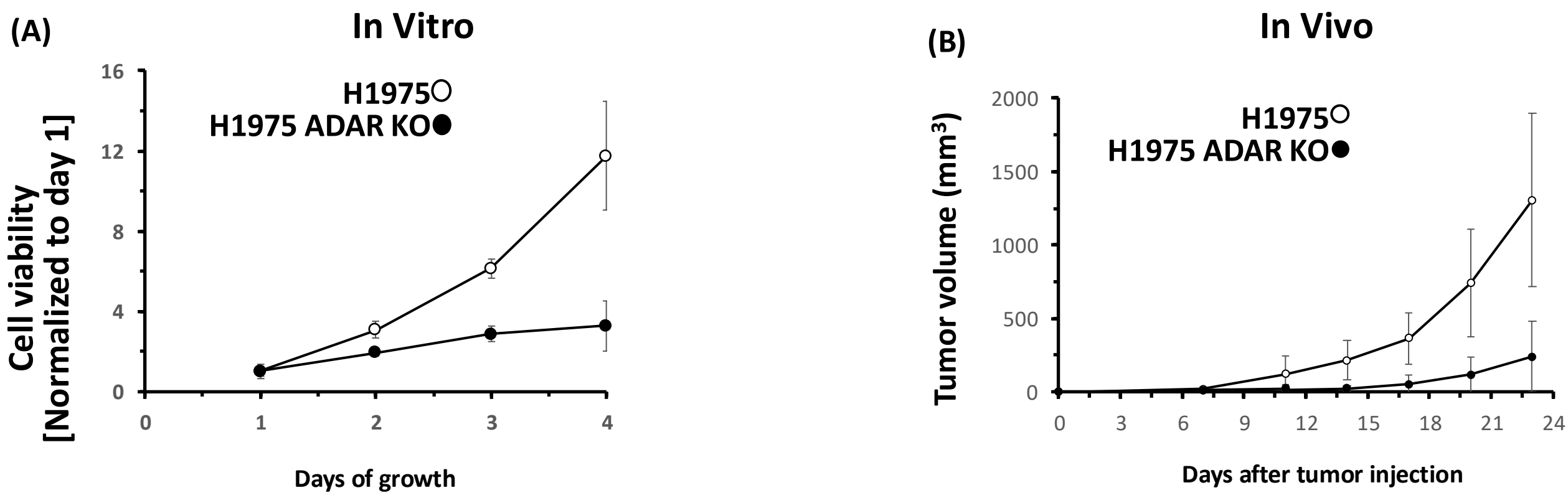


Figure 2. (A) ADAR1 KO reduces baseline proliferation in H1975 cells. Cell viability was analyzed over 96 hours (n=3), (B) **Loss of ADAR1 markedly decreases tumorigenesis in NSCLC.** 1 x 10⁶ ADAR1 WT and ADAR1 KO cells were injected into flanks of mice (n=5 mice).

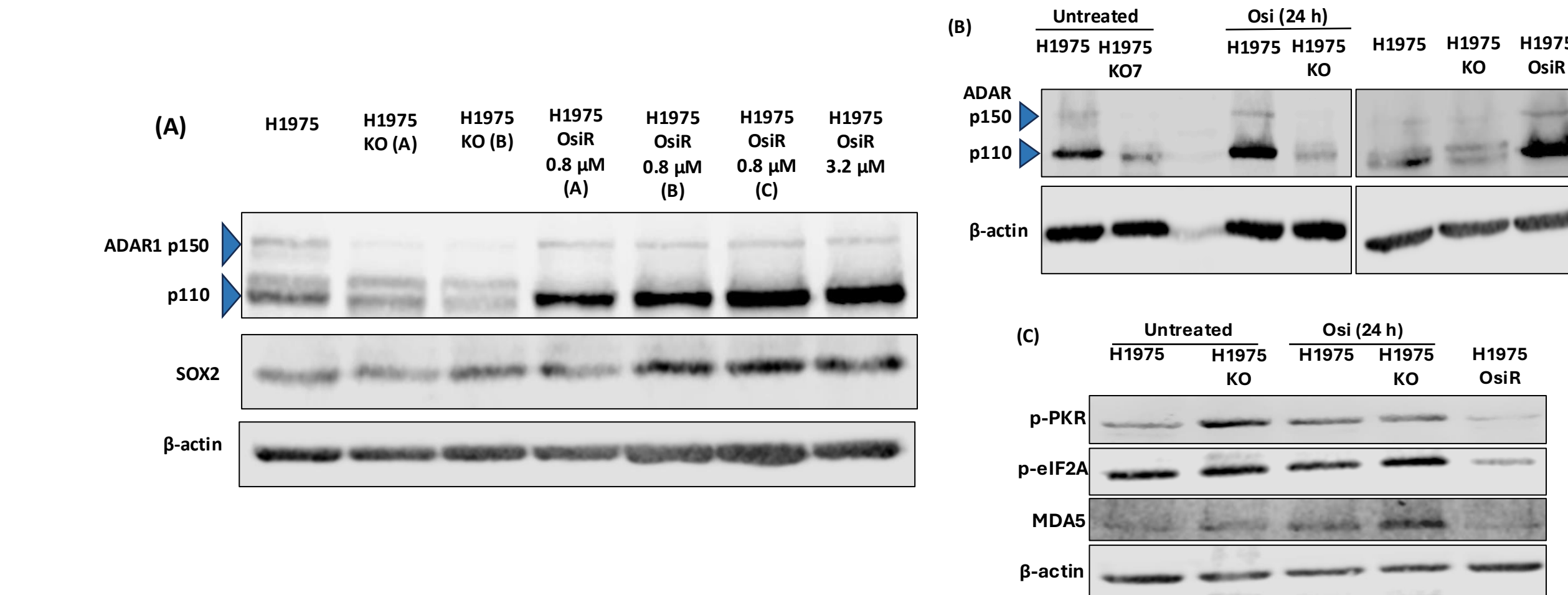


Figure 5. ADAR1 was highly expressed in Osimertinib resistant cells. (A) ADAR1 expression increases as cells develop osimertinib-resistance. **(B)** Both ADAR1-p150 and ADAR1-p110 expression are induced following acute osimertinib treatment (24 h) and remain elevated in osimertinib-resistant cells **(C)** Phospho-PKR, phospho-eIF2 α , and MDA5 are induced following acute osimertinib treatment (24 h) but are suppressed in osimertinib-resistant cells.

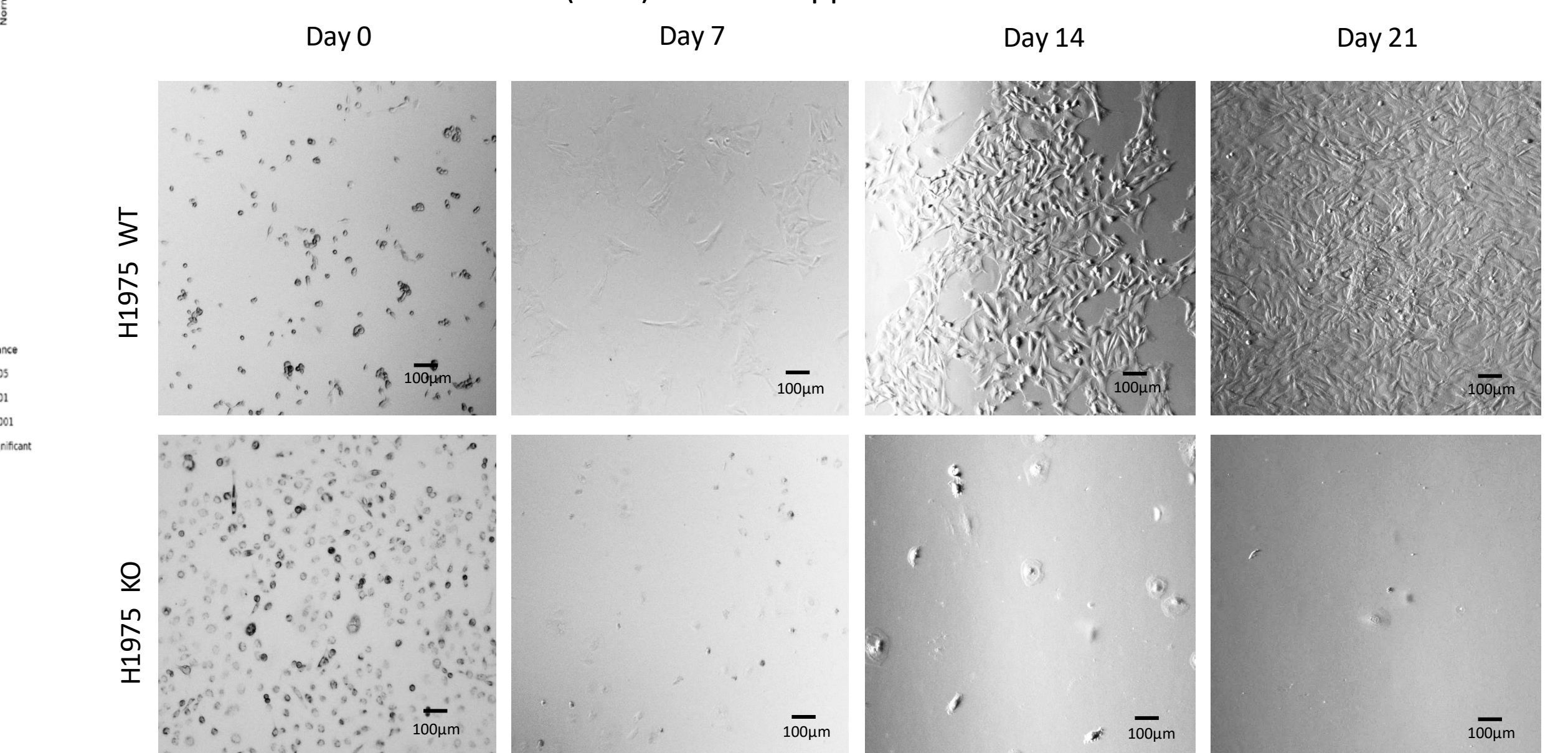


Figure 6. H1975 wild-type and knockout cells seeded at 1x10⁶ cells/plate and treated with osimertinib at 100nM concentration with the media changed every 3 days.