

P3.119 Establishment and Characterization of a Patient-Derived Thoracic Tumor Organoid Library From Diverse Clinical Specimens

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BACKGROUND

- Organoids are stem-cell-derived, self-organizing three-dimensional (3D) culture systems that recapitulate selected cellular, structural, and functional features of their tissue of origin.
- Since the landmark adult stem cell-derived intestinal organoid culture system was reported in 2009, organoids derived from normal tissues and cancers have become widely used. Thoracic tumor organoids (TTOs) enable long-term propagation while preserving key biological features of the original malignancy and are expected to serve as powerful tools for elucidating disease mechanisms.



Figure 1. Representative TTO morphologic evolution during serial culture.

OBJECTIVE

- To define disease- and specimen-specific establishment characteristics, assess how faithfully TTOs recapitulate their corresponding clinical tumors, and clarify their distinct translational research utilities.

METHODS

- We retrospectively reviewed all TTO establishment attempts performed at Keio University Hospital between September 2017 and March 2025.
- Tissue specimens were minced and enzymatically dissociated, whereas fluid specimens were centrifuged to collect cells. Isolated cells or cell clusters were embedded in Matrigel and serially cultured in growth-factor-supplemented medium.
- Successful establishment was defined as stable culture for at least 6 months with sufficient proliferation, together with fulfillment of at least two of the following three criteria:
 - Morphologic features distinct from those of normal organoids.
 - Molecular genetic features consistent with malignancy, as demonstrated by RNA-seq, WES, or other molecular analyses.
 - Growth-factor dependencies distinct from those of normal cells.
- In evaluable cases, organoids were characterized by comparison with matched clinical specimens using histopathology and immunohistochemistry (IHC), whole-exome sequencing (WES), RNA sequencing (RNA-seq), longitudinal clinical correlation, and drug sensitivity testing.

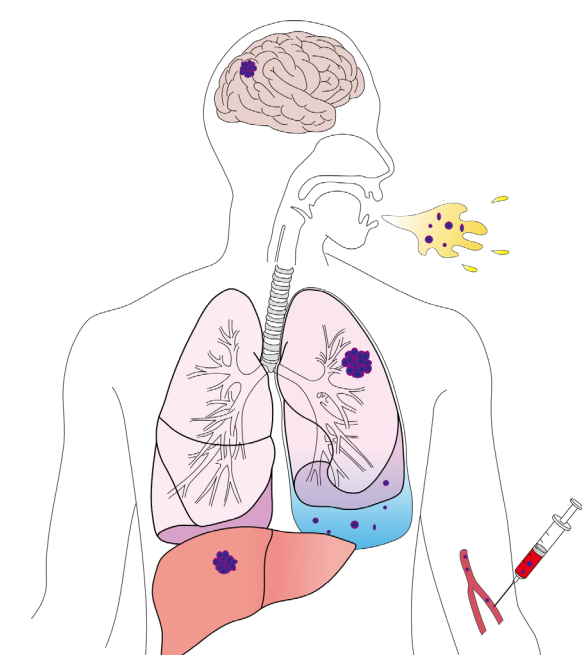


Figure 2. Diverse clinical specimens used for TTO establishment.

DISCUSSION

- The overall establishment rate was 19.7% (261/1,324). A previous study reported a 17% establishment rate using similarly stringent confirmation of tumor-cell propagation, broadly consistent with our findings.
- When analyzed by specimen collection method, establishment rates were higher for fluid specimens, such as pleural effusion, ascites, and pericardial effusion. In surgical and bronchoscopic specimens, contamination by normal airway organoids is often a concern. The higher success rate with fluid specimens may reflect the greater abundance of tumor cells and the lower likelihood of contamination by non-tumor cells at the time of specimen collection.

CONCLUSION

- Across 1,324 establishment attempts, 261 TTO cultures were successfully established (19.7%). Established TTOs recapitulated key features of their source tumors: NSCLC organoids modeled treatment response and acquired resistance, whereas SCLC organoids preserved molecular subtype identity and were efficiently established from bronchoscopic, fluid, and autopsy specimens.

RESULTS | Clinical fidelity and disease-specific utility

Table 1. Establishment efficiency by specimen type and disease group

Specimen source	Non-small cell lung cancer (NSCLC)			Small cell lung cancer	Other	Total
	Adenocarcinoma	Squamous cell carcinoma	NSCLC total			
Surgery	9.1% (15/164)	16.9% (12/71)	11.5% (27/235)	28.6% (4/14)	27.2% (44/162)	18.2% (75/411)
TBB	8.1% (7/86)	2.6% (1/38)	6.5% (8/124)	33.3% (7/21)	0.0% (0/20)	9.1% (15/165)
TBNA	8.7% (4/46)	7.7% (1/13)	8.5% (5/59)	64.0% (16/25)	26.7% (4/15)	25.3% (25/99)
Pleural effusion	30.0% (45/150)	27.8% (5/18)	29.8% (50/168)	64.7% (11/17)	21.9% (14/64)	30.1% (75/249)
Ascites	25.0% (2/8)	—	25.0% (2/8)	—	40.0% (2/5)	30.8% (4/13)
Pericardial effusion	25.0% (1/4)	100.0% (1/1)	40.0% (2/5)	100.0% (1/1)	0.0% (0/1)	42.9% (3/7)
CT-guided biopsy	6.1% (2/33)	7.7% (1/13)	6.5% (3/46)	25.0% (2/8)	0.0% (0/22)	6.6% (5/76)
Sputum	10.5% (2/19)	11.1% (1/9)	10.7% (3/28)	33.3% (4/12)	—	17.5% (7/40)
CTC	0.0% (0/37)	0.0% (0/6)	0.0% (0/43)	24.0% (29/121)	0.0% (0/6)	17.1% (29/170)
Autopsy	0.0% (0/33)	0.0% (0/5)	0.0% (0/38)	61.8% (21/34)	9.1% (1/11)	26.5% (22/83)
CSF	9.1% (1/11)	—	9.1% (1/11)	—	—	9.1% (1/11)
Total	13.4% (79/591)	12.6% (22/174)	13.2% (101/765)	37.5% (95/253)	21.2% (65/306)	19.7% (261/1324)

Values are established/attempted; establishment rate = established/attempted. TBB, transbronchial biopsy; TBNA, transbronchial needle aspiration; CTC, circulating tumor cells; CSF, cerebrospinal fluid.

- High SCLC establishment rates:** SCLC organoids were established from TBNA (64.0%), pleural effusion (64.7%), and autopsy specimens (61.8%).
- Transcriptomic organization:** Library-wide RNA-seq showed broad histology-associated clustering.

NSCLC | Clinical fidelity + functional modeling

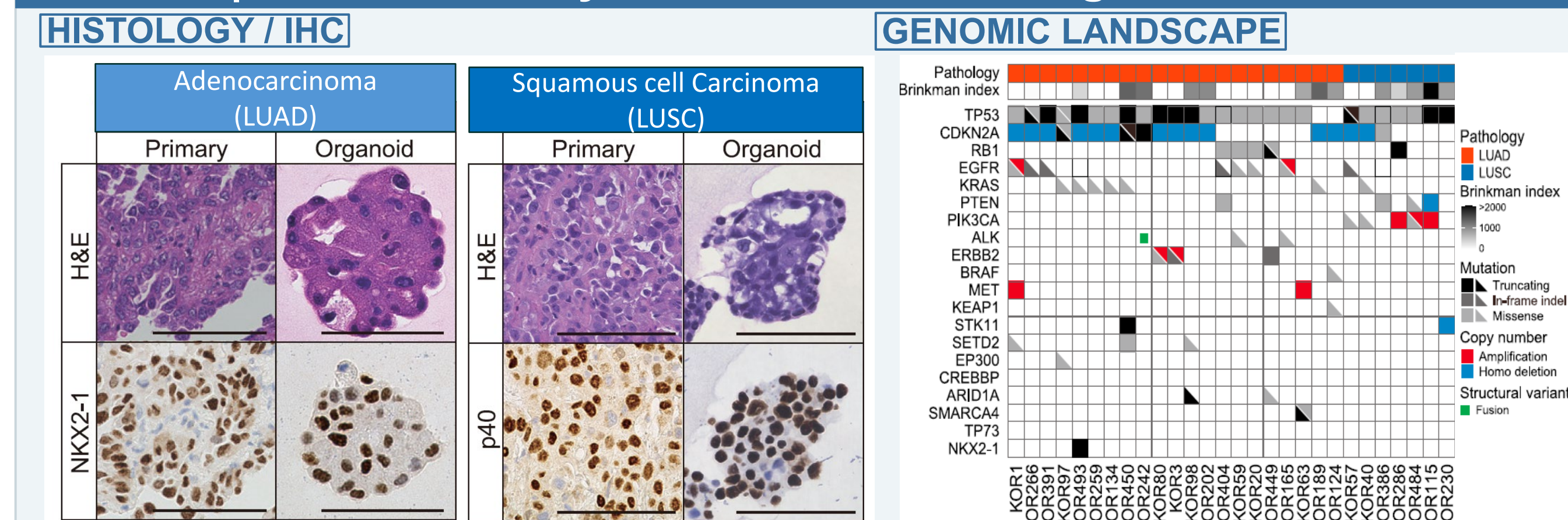


Figure 4. Matched primary tumors and NSCLC organoids preserved morphology and lineage-marker expression.

- Histopathologic fidelity:** Organoids preserved tumor morphology and lineage markers.
- Genomic landscape:** NSCLC organoids harbored major driver alterations, including EGFR and KRAS.

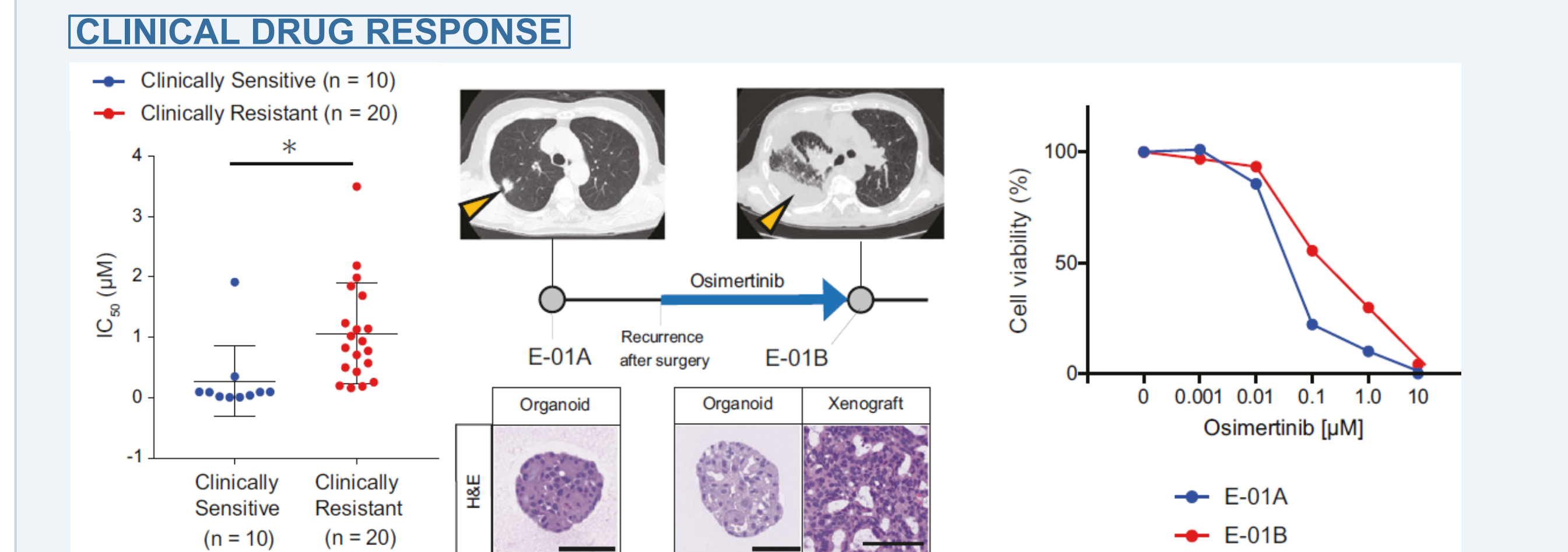


Figure 6. Organoid drug responses reflected clinical sensitivity and recapitulated acquired osimertinib resistance.

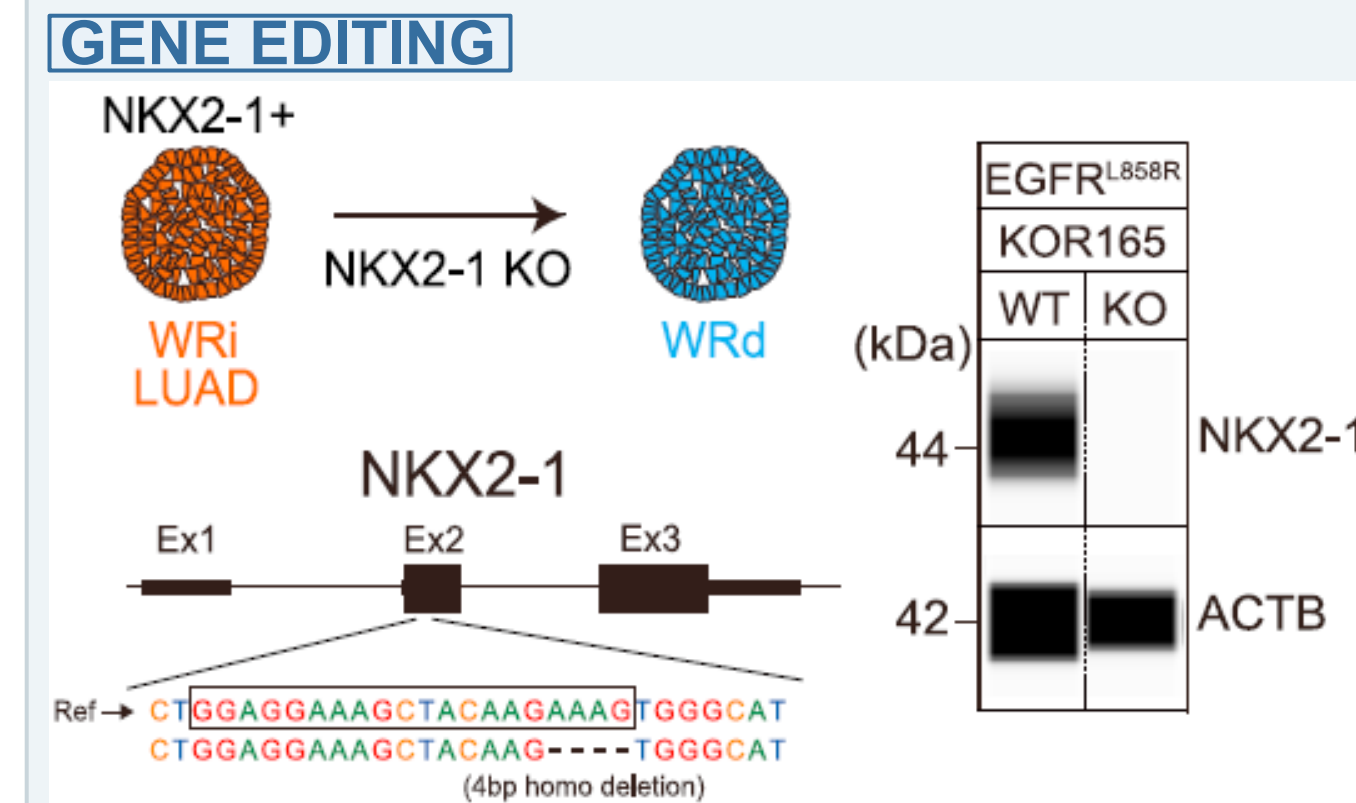


Figure 7. CRISPR-Cas9 editing enabled causal evaluation of lineage regulators such as NKX2-1.

- Functional drug response:** Organoid sensitivity matched clinical response, and paired models recapitulated acquired osimertinib resistance.
- Genetic manipulation:** CRISPR-Cas9-mediated NKX2-1 knockout induced a shift from Wnt-independent to Wnt-dependent growth.

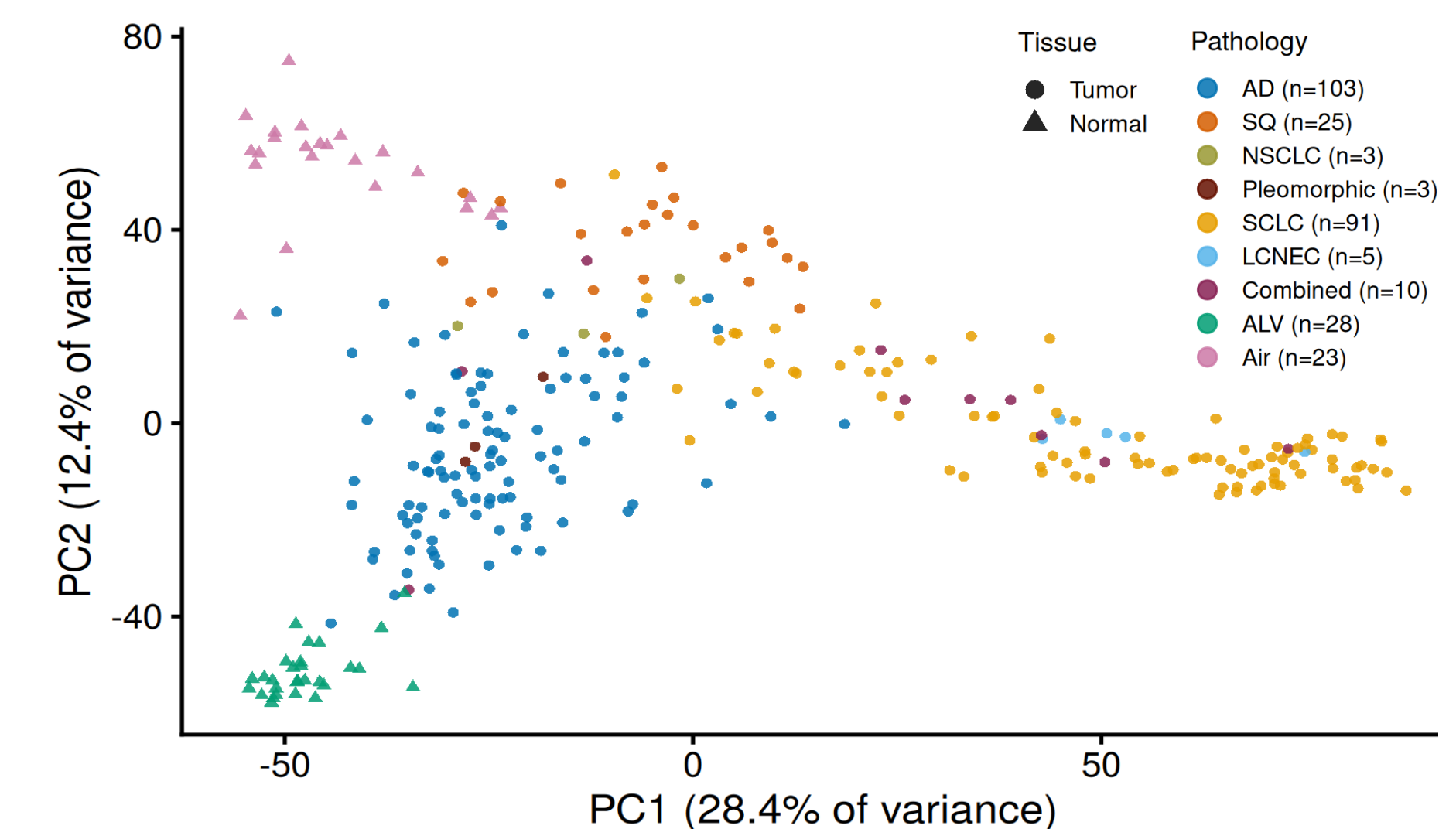


Figure 3. Library-wide RNA-seq showed broad histology-associated clustering across tumor and normal thoracic organoids.

SCLC | Clinicopathologic + subtype fidelity

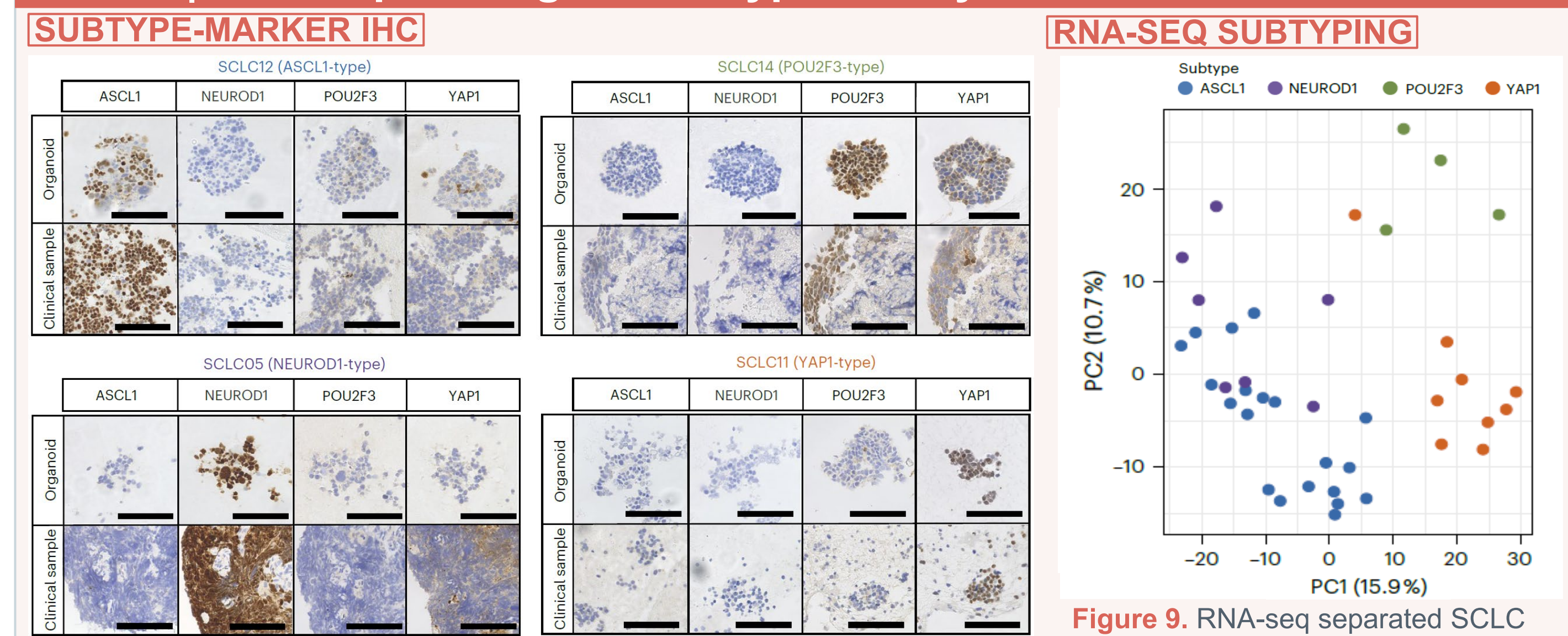


Figure 8. Matched SCLC tumors and organoids preserved ASCL1, NEUROD1, POU2F3, and YAP1 subtype-marker expression.

- Subtype fidelity:** SCLC organoids preserved matched ASCL1, NEUROD1, POU2F3, and YAP1 IHC patterns and RNA-seq-defined subtype identity.

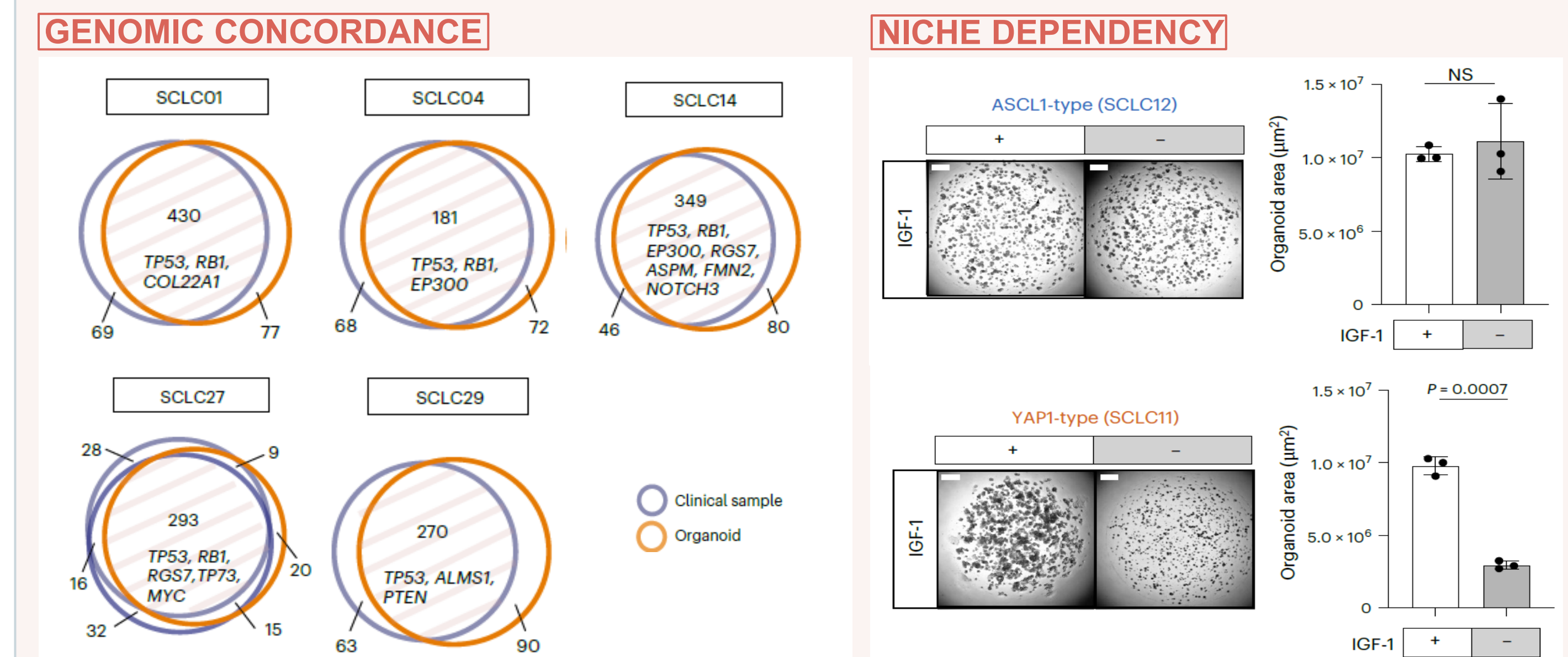


Figure 10. Venn diagrams of matched clinical specimen-organoid pairs showed shared driver alterations by whole-exome sequencing.

- Genomic concordance:** Matched clinical specimens and organoids shared driver alterations by whole-exome sequencing.
- Functional niche modeling:** Niche-factor perturbation revealed subtype-specific growth requirements, including IGF-1 dependence in non-neuroendocrine SCLC organoids.

Figure 11. Niche-factor perturbation identified subtype-specific growth requirements in SCLC organoids.