Supplementary Figure 1

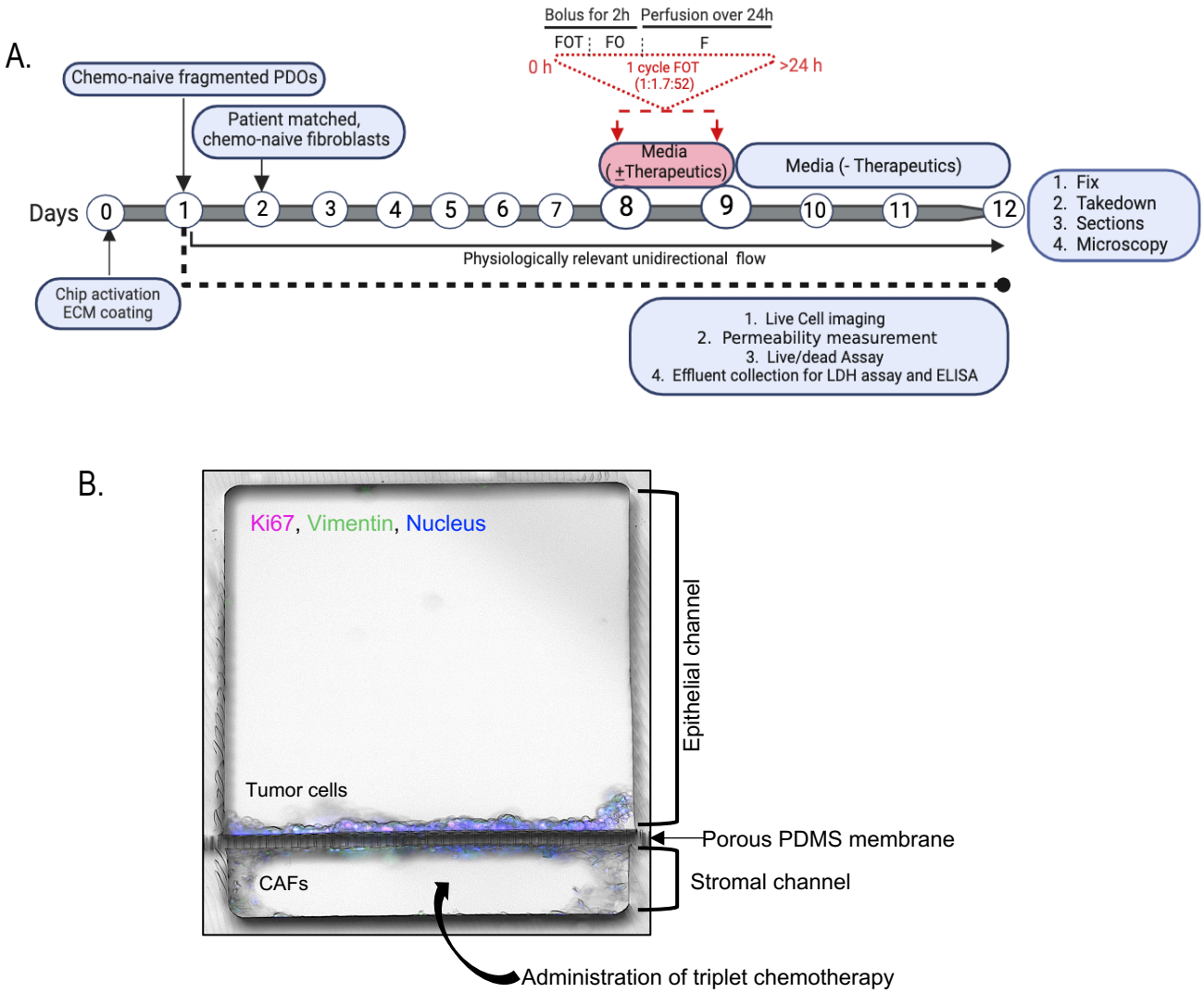


Fig S1. **A.** Detailed schematic representation of the step-by-step procedure involved in the establishment and maintenance of Epithelial-Stromal Interactions in the microfluidic platform. **B.** Low magnification, vertical view of an EAC-chip. Porous PDMS membrane ensures tumor-stroma like communications.

Supplementary Figure 2

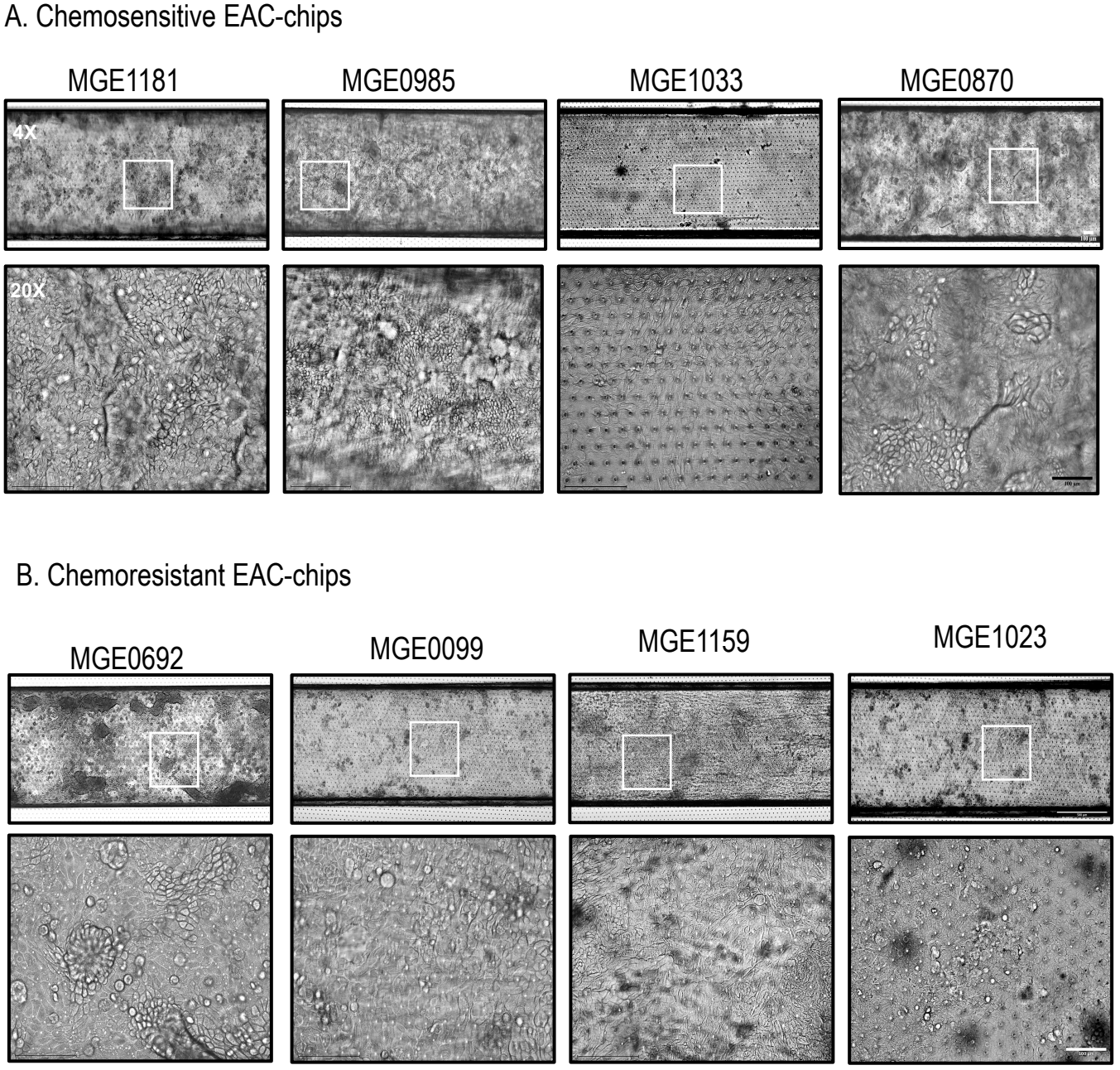


Fig S2.
Bright field horizontal images of all 8 EAC patients derived organ chips exhibiting patient-specific morphological diversity.

Supplementary Figure 3

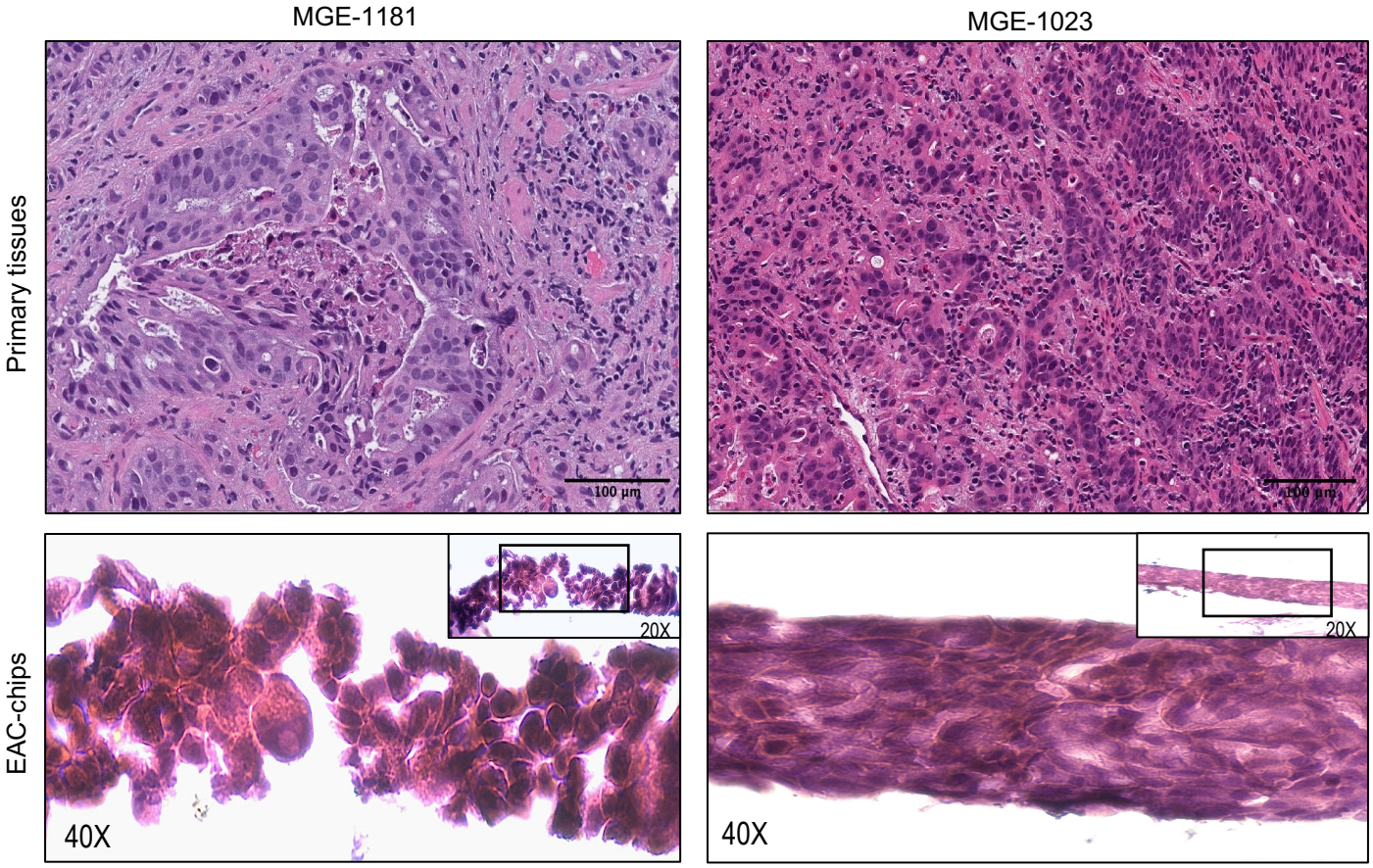
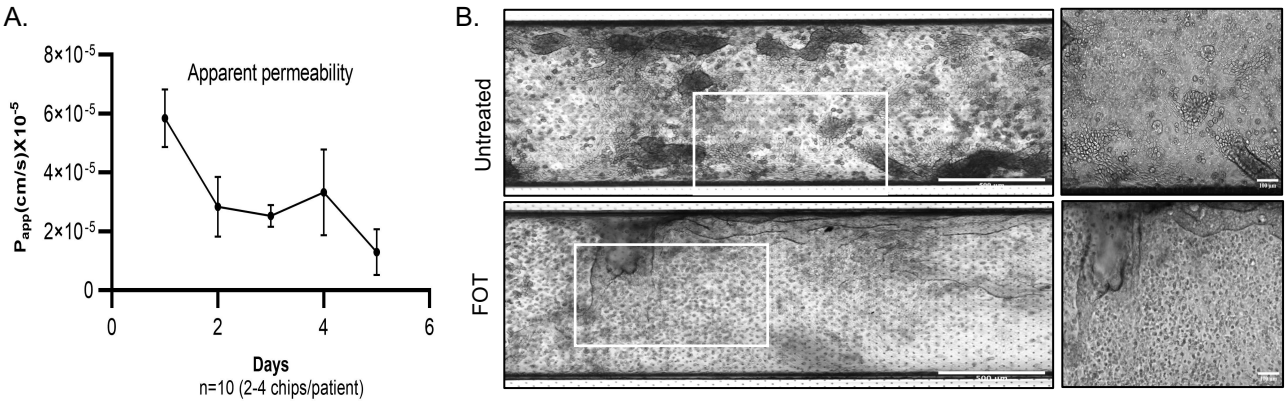


Fig S3.

H&E-stained images of a chemosensitive EAC chip (left) and chemoresistant EAC chip (right) sections and corresponding primary tissues.

Supplementary Figure 4



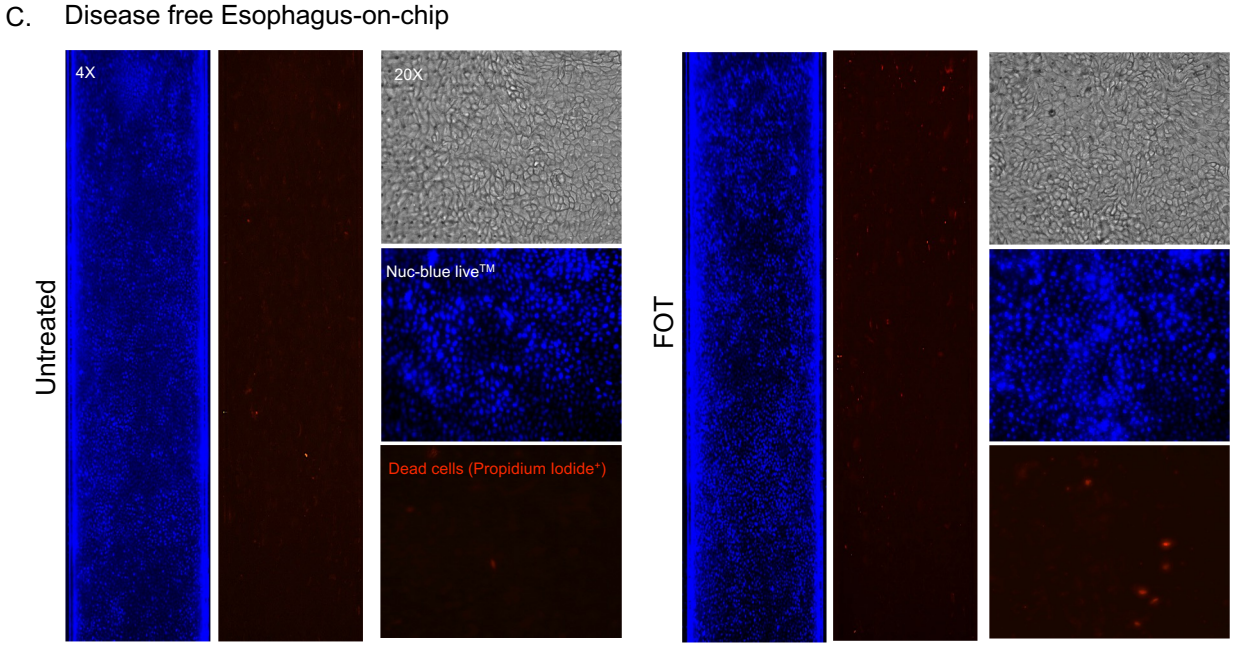
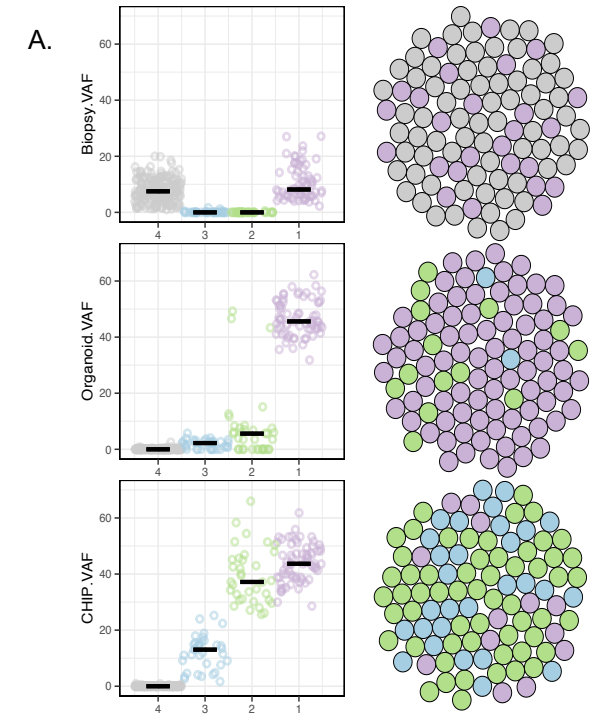


Fig S4

A. The EAC Chip also displayed formation of a physiologically relevant tissue barrier (defined as a decrease in apparent permeability) within 5 days. **B.** EAC-Chips were exposed to clinically relevant cytotoxic doses of the same triplet therapy given to the patient (<Cmax, FOT: 10:1:1) for 24 hours starting on day 8 directly to the top epithelial/luminal channel. With chemotherapy delivered *via* the epithelial channel of the EAC-chip failed to accurately replicate the inpatient response, resulting in significant cytotoxicity after one complete cycle on chip of a representative chemo-resistant patient who had poor pathological response (TRG) and stable disease (objective response) after a full cycle of chemotherapy. **C.** Disease free esophagus-on-chips were treated with same clinically relevant cytotoxic doses of FOT-based chemotherapy on day 8. The chemotherapy was administered through the stromal channel of the normal esophagus chips. Live imaging shows an intact epithelial layer, along with some dead cells.

Supplementary Figure 5



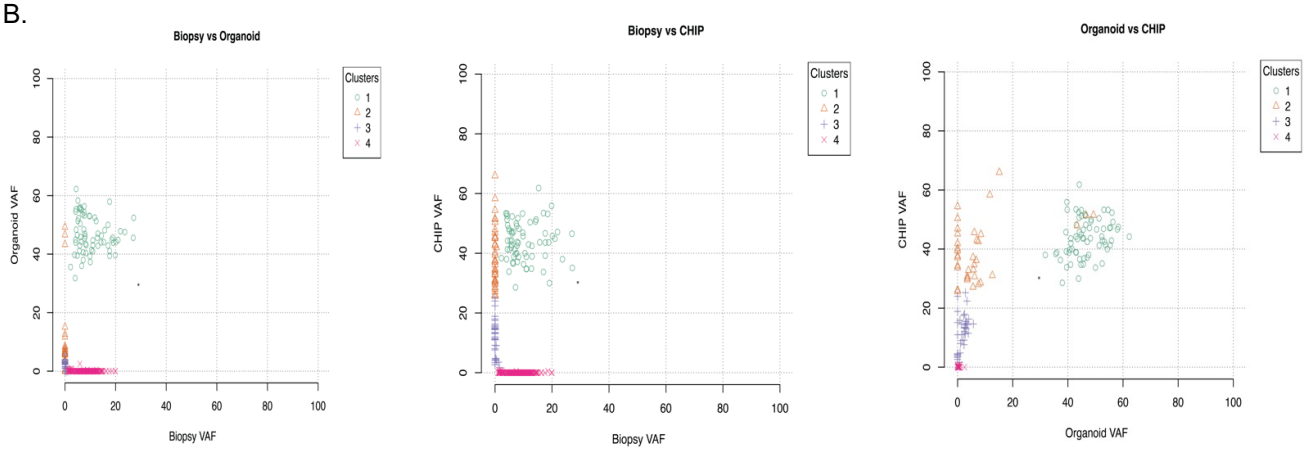
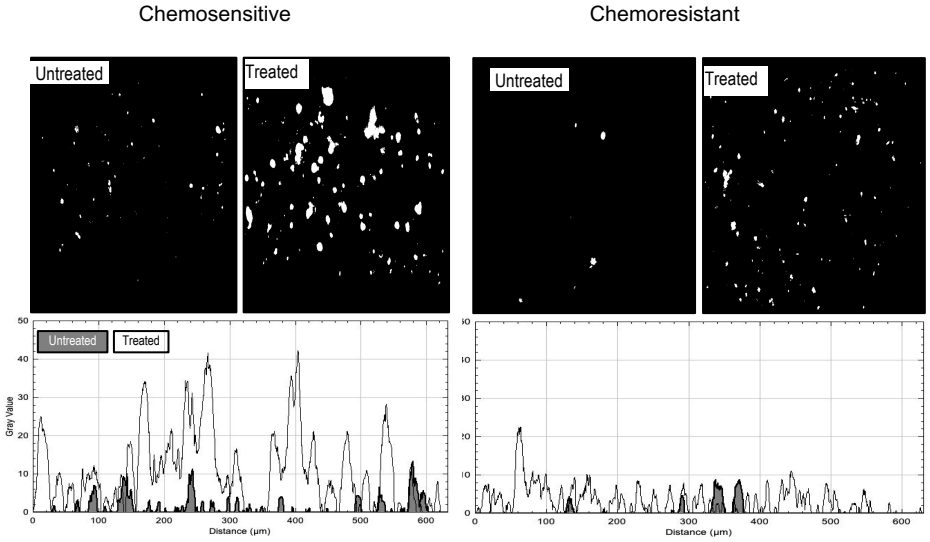


Fig S5

A-B. Representative figure showcasing clonal evolution of somatic variants detected in diagnostic biopsy tissue, organoids, and organ-chip belonging to the same patient. The figure shows that the tumor persisted throughout the models, with a few clones disappearing and additional mutations accumulating, resulting in new subclones. During the diagnostic biopsy, some subclones (purple) comprised nearly one-third of the tumor cells. When the organoid was established and subjected to whole exome sequencing (WES) at passage 6, this led to a shrinkage in tumor content and the complete elimination of some subclones (grey) or the rise (or growth advantage) of a new set of clones (green). Meanwhile, additional subclones (green and sky) might have appeared or were present at very low levels, undetectable by the performed sequencing. Subclones that have significant growth advantages remained conserved from primary tissue to EAC-chip.

Supplementary Figure 6

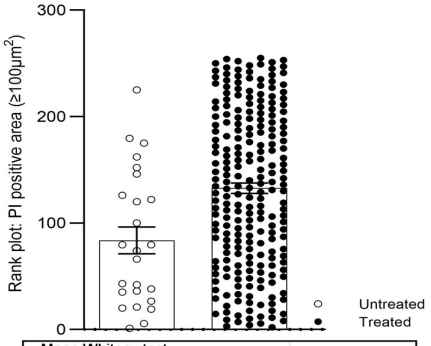
A.



B.

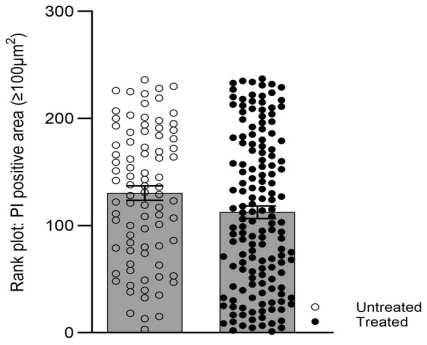
Mann Whitney rank plots for PI positive affected area (>50µm²) comprising dead cells

i) Chemosensitive



Mann Whitney test	
P value	0.0013
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	2094 , 30547
Mann-Whitney U	1769

ii) Chemoresistant



Mann Whitney test	
P value	0.0534
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	11214 , 16989
Mann-Whitney U	5513

Fig S6.

A. Representative PI- positive (dead cells) image profile of chemosensitive and chemoresistant microtissues grown on a microfluidic co-culture model. Gray value in the Y axis. Distance in microns in the X axis. **B.** Rank plots demonstrate areas positive for PI in i) Chemosensitive & ii) Chemoresistant cohort.

Supplementary Figure 7

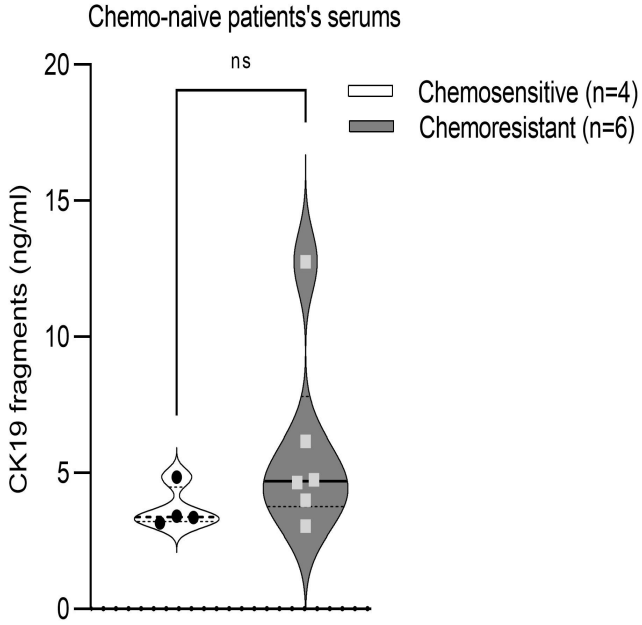


Fig S7.
Serum was isolated from chemo-naïve patients (n=10) at the time of diagnostic biopsy. A serum-based tumor marker, 40 KDa fragment of cytokeratin 19 (CK19) was measured by ELISA. Organoids and matched fibroblasts derived from Chemosensitive (n=3) and Chemoresistant (n=3) patients from this cohort were also used to establish EAC-chip. The median and the upper and lower quartiles are shown. Significance ($p < 0.05$) was determined using an unpaired Student's t-test with Welch's correction.

Supplementary Table 1

	Ratio, Cycles	Chemotherapeutics	Day1 Dose; Time, IV	Mean Cmax in patient with solid tumor (ng/ml)	≈ Mean Cmax (μM)
FLOT *	52:1.7:1 15 days, 4 cycles	Docetaxel (T)	50 mg/m^2, 1h,	- #	
		Oxaliplatin (O)	85 mg/m^2, >2h	2740	6.9
		5-FU (F)	2600 mg/m^2, >24h,	1189 ± 412	9.1
DCF	1:1:10 21 days, 3 cycles	Docetaxel (D)	75 mg/m^2, 1h	2706 ± 715.1	3.3
		Cisplatin (C)	75 mg/m^2, >24h	1180 ± 172	3.9
		5FU (F)	750 mg/m^2,**1-5 days**	265.2 ± 60.8	2.1

* L (Leucovorin) is not a cytotoxic agent

Table S1. Taxane-based neoadjuvant combination chemotherapeutic regimen followed for the treatment of locally advanced, resectable EAC patients. # Data available only for 75 mg/m^2 Docetaxel for 1 h; Mean Cmax ≈ 5.14 μM or 4.15 mg/L. DCF regimen (1:1:10) is almost similar but patient received higher dose of taxane and lower dose of platinum-based drugs.

Supplementary Table 2

Chemotherapeutics	A. Static culture (96 well)	B. Static culture (96 well)	B. Static culture (96 well)	C. In chip (effective stroma inclusive tumor region:18mm^2)
		≈Cmax	1:1.7:52	1:1.7:52
Docetaxel (T)	0-1uM for 72h	3μM, 1h	3.5 nM,1h	3.5 nM, 1h
Oxaliplatin (O)	0-1uM for 72h	6.9 μM , >2h	6 nM, >2h	6 nM, >2h
5-FU (F)	0-10uM for 72h	9.1 μM, , >24h,	182 nM, >24h,	182 nM, >24h

Table S2. Regimen and pharmacokinetic interactions of neoadjuvant chemotherapeutics used for static A. 3D-Matrigel and B. fragmented, dispersed organoids culture as well as C. stroma inclusive EAC chips.

Supplementary Table 3

		Patient #	Location of tumor	Grade	Pathological stage	Pathological response	Objective response
Chemosensitive (SENS)	1	MGE-1199	EGJ	Well & moderate	ypT1N1	TRG 1B	PR
	2	MGE-0985*	Distal Esophagus	GX	ypT3N0	TRG 1A	PR
	3	MGE-1033*	EGJ	Well	ypT2N0	TRG 1B	PR
	4	MGE-0951	EGJ	Poor	ypT2N1	TRG 1B	CR
Chemoresistant (RES)	1	MGE-0099*	EGJ	Moderate	ypT3N2	TRG 2	SD
	2	MGE-1159*	EGJ	Poor	ypT3N3M1	TRG 3	SD
	3	MGE-1023*	EGJ	Poor	ypT3N0	TRG 2	SD
	4	MGE-0975	EGJ	Moderate	ypT3N1	TRG 3	PR
	5	MGE-1076	EGJ	Moderate	NA	TRG3	PR
	6	MGE-1064	EGJ	Poor	ypT2N3	TRG3	PR

Table S3. A cohort of patients with locally advanced, resectable EAC whose sera was collected before undergoing NACT.