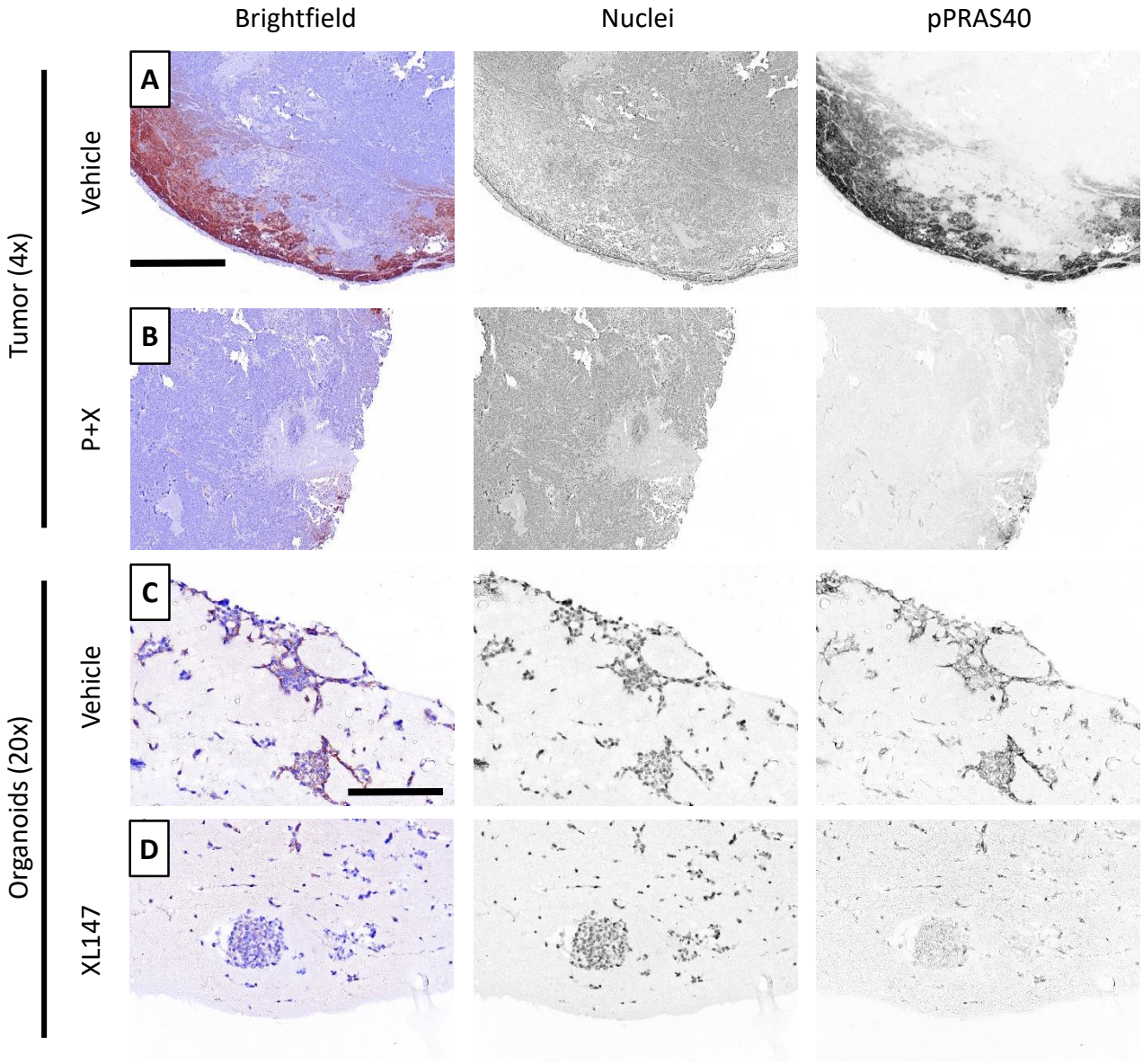
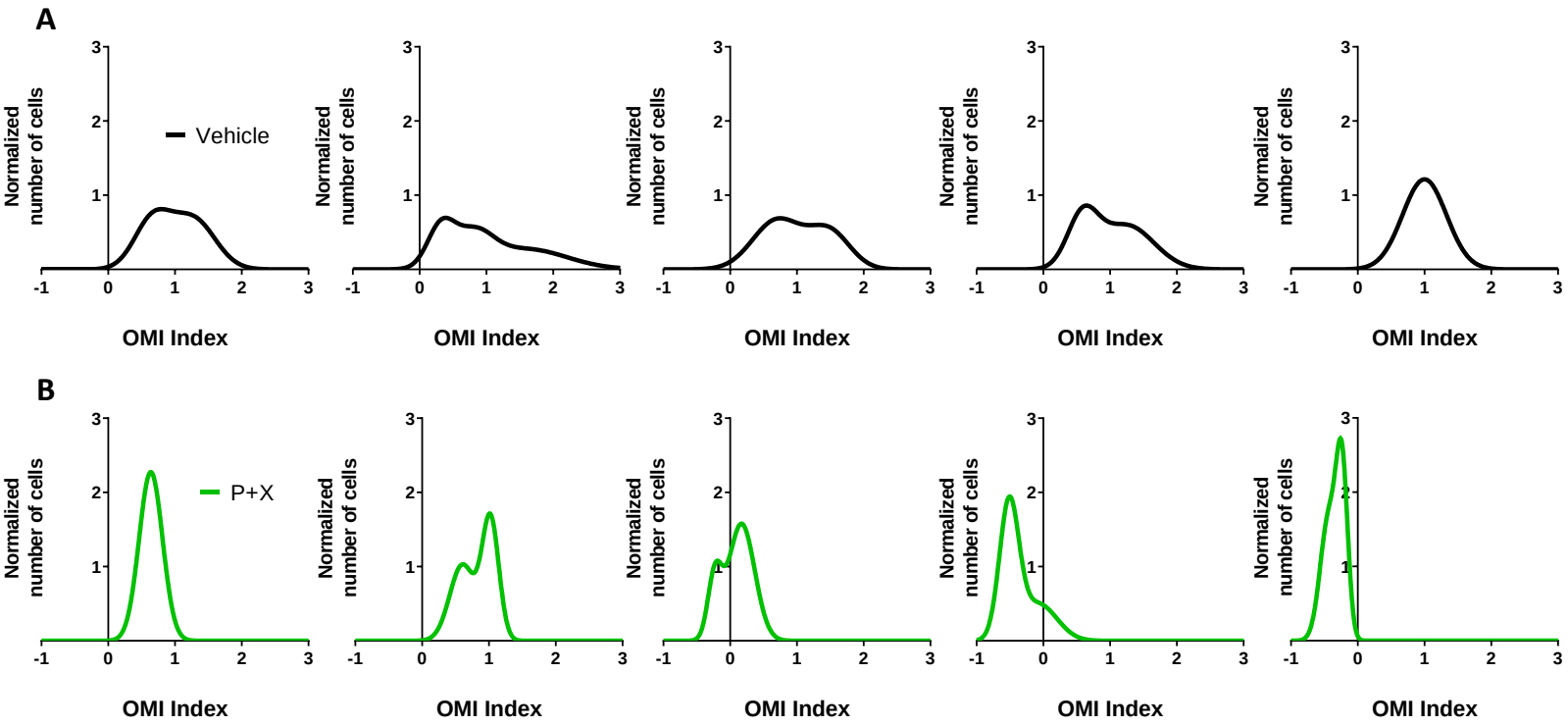
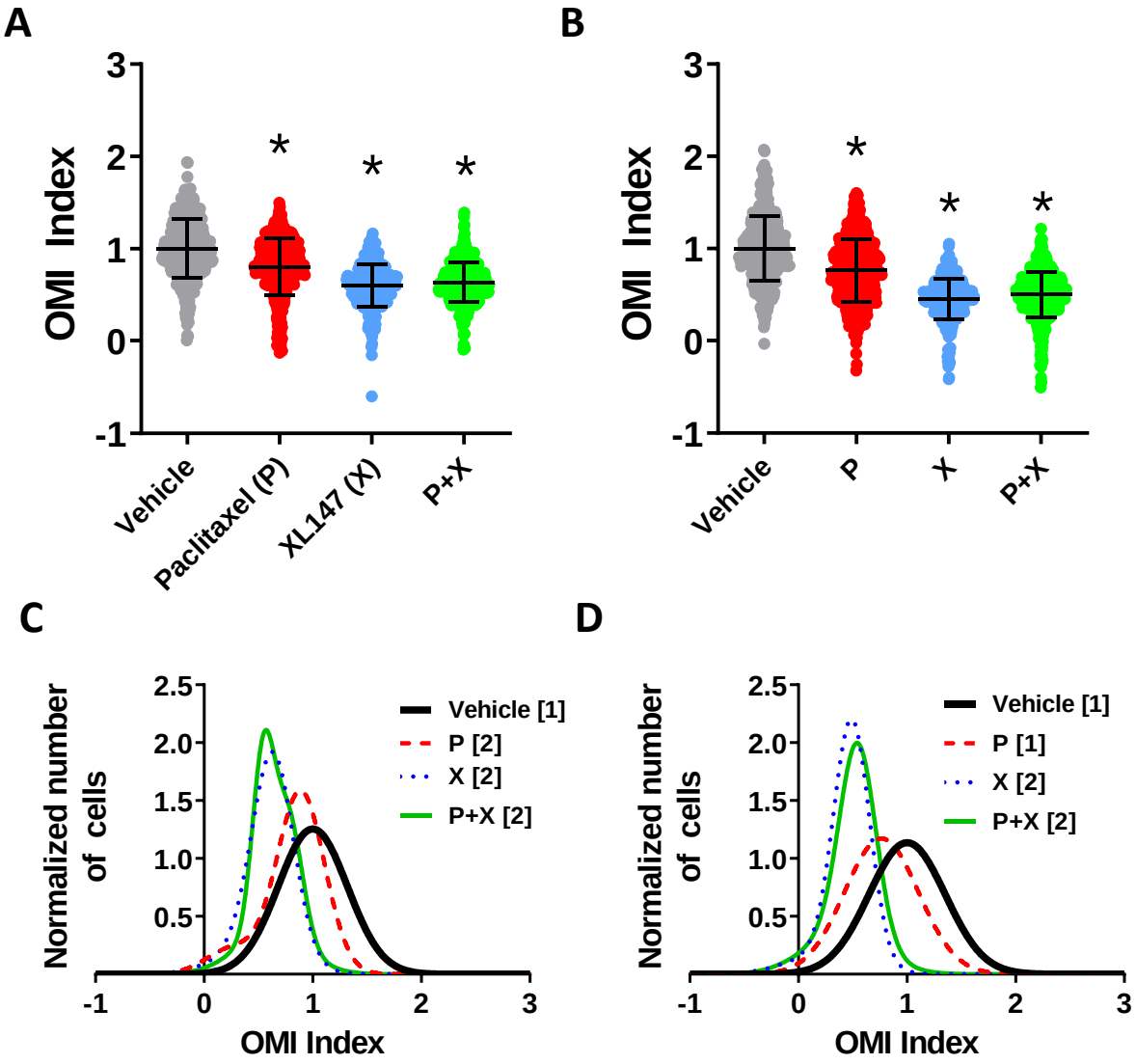
Supplemental info



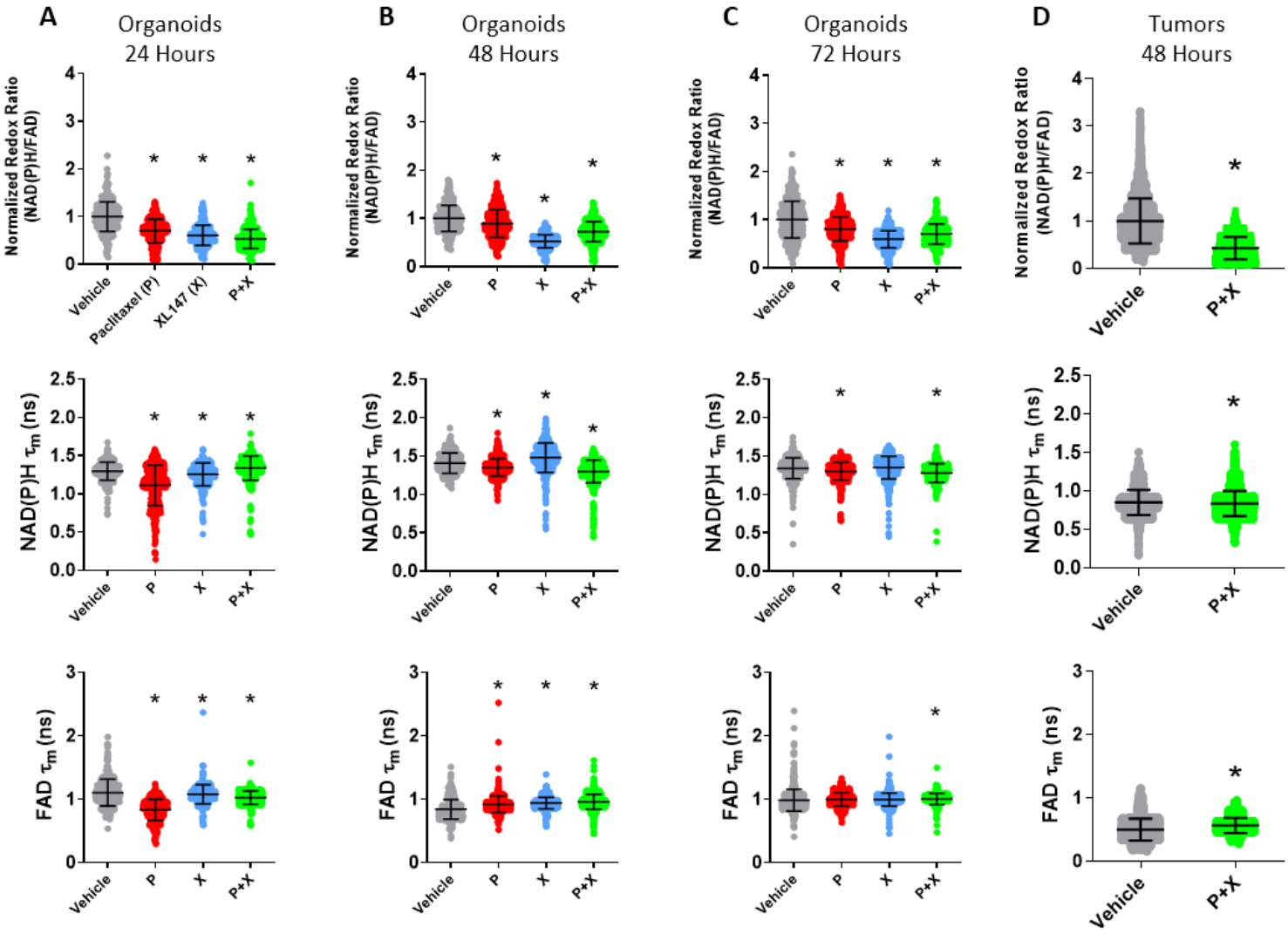
Supplementary Figure S1. Decreased expression of pPRAS40 with treatment in PyVmT tumors and organoids. A-B, Example histology and spectral unmixing of pPRAS40 expression in PyVmT tumors treated with vehicle (**A**) and paclitaxel and XL147 (P+X) for 48 hours (**B**). **C-D**, Examples of pPRAS40 expression in PyVmT organoids treated with vehicle (**C**) and XL147 for 72 hours (**D**). 4x scale bar = 1 mm. 20x scale bar = 200 μm.



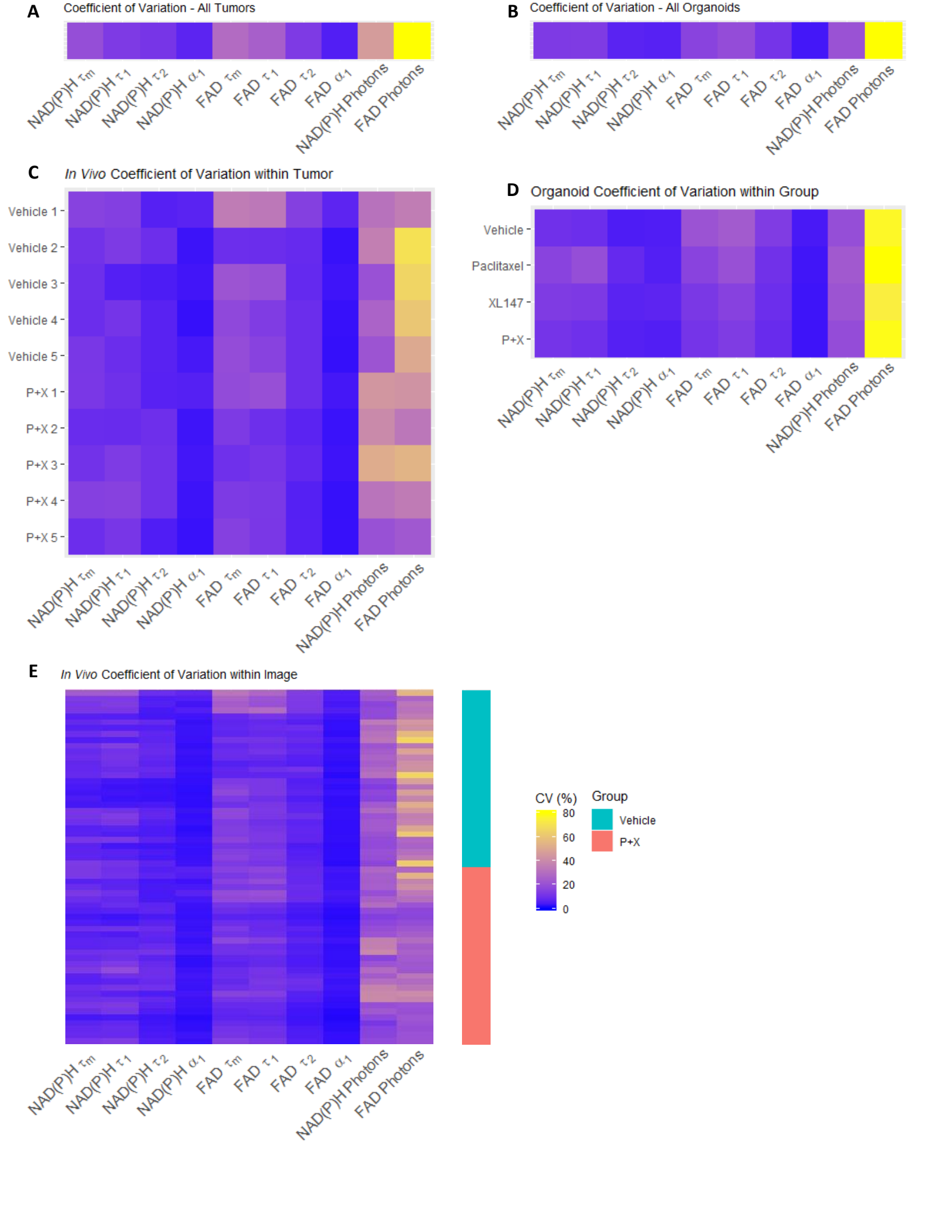
Supplementary Figure S2. OMI index heterogeneity in individual PyVmT tumors. Distributions of drug response at the single-cell level for (**A**) vehicle and (**B**) P+X treated tumors.

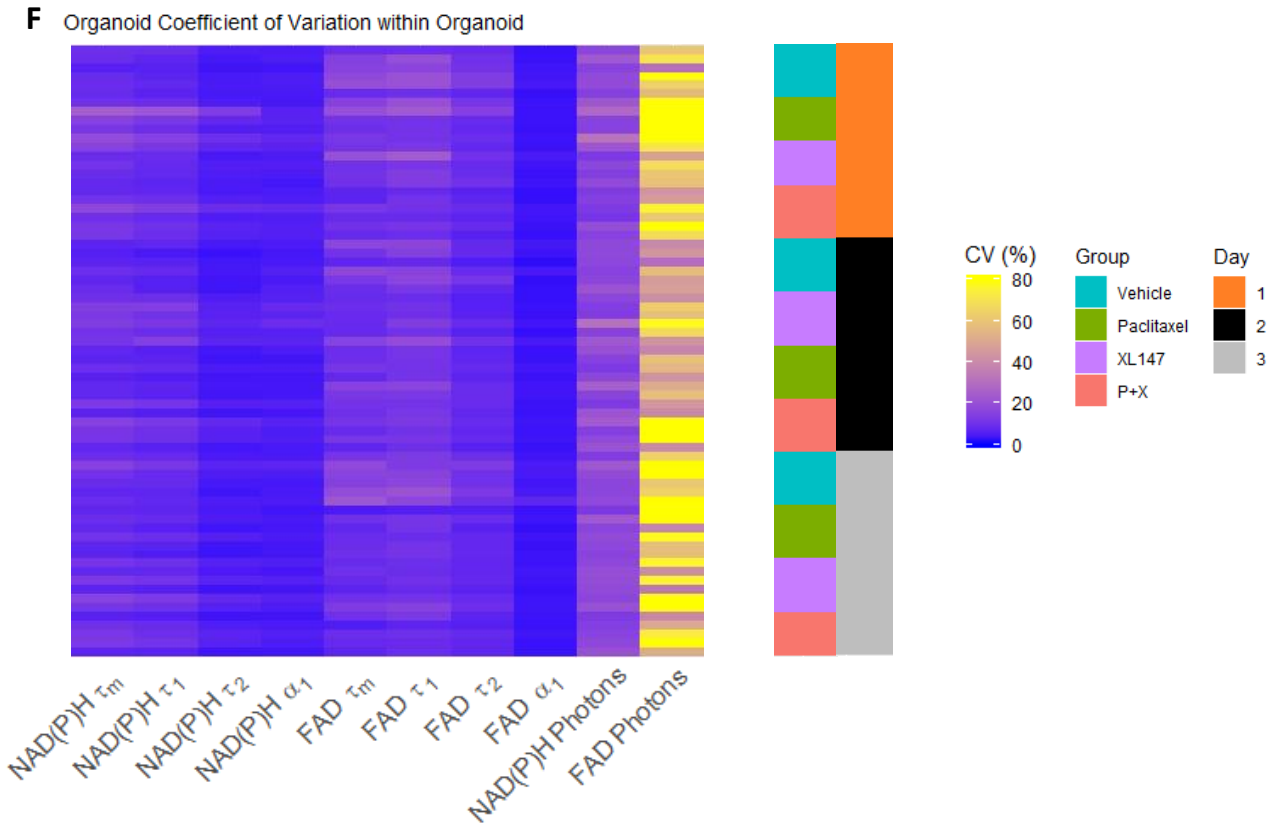


Supplementary Figure S3. OMI of drug response heterogeneity in PyVmT organoids at additional time points. A-B, OMI index quantified at the single cell level at (**A**) 24 hours and (**B**) 48 hours of treatment. N=6 organoids/group. N>290 cells/group. Error bars indicate mean ± SD. * $p<0.0001$ vs. vehicle. **C-D,** Example subpopulation analysis of the OMI index in all organoid cells at (**C**) 24 hours and (**D**) 48 hours of treatment. Brackets in legend indicate number of subpopulations for each group.

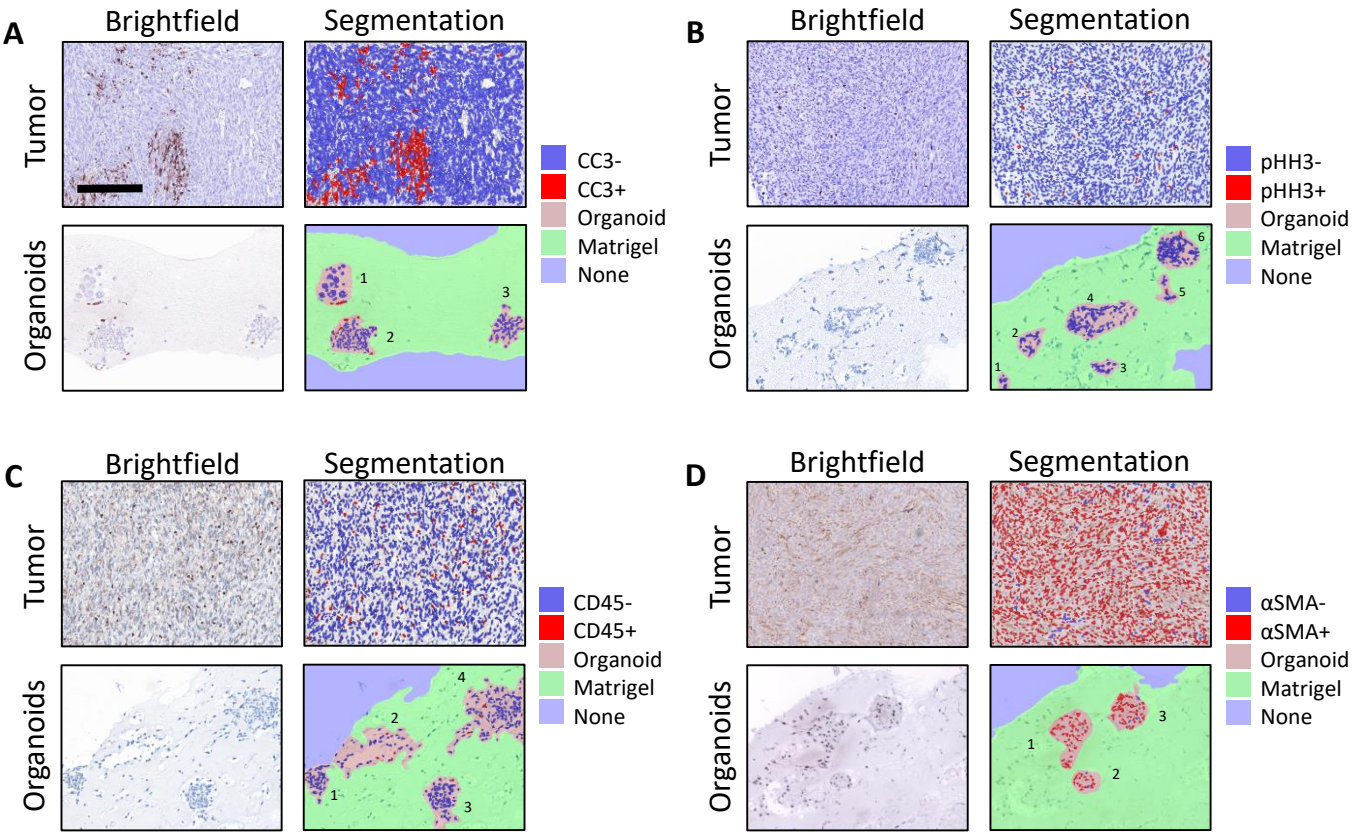


Supplementary Figure S4. OMI endpoints of drug response in single cells. Normalized redox ratio, NAD(P)H τ_m, and FAD τ_m at (A) 24 hours, (B) 48 hours, (C) 72 hours in PyVmT organoids, and at (D) 48 hours in PyVmT tumors. Error bars indicate mean ± SD. * p<0.0001 vs. vehicle. Each dot represents one cell.

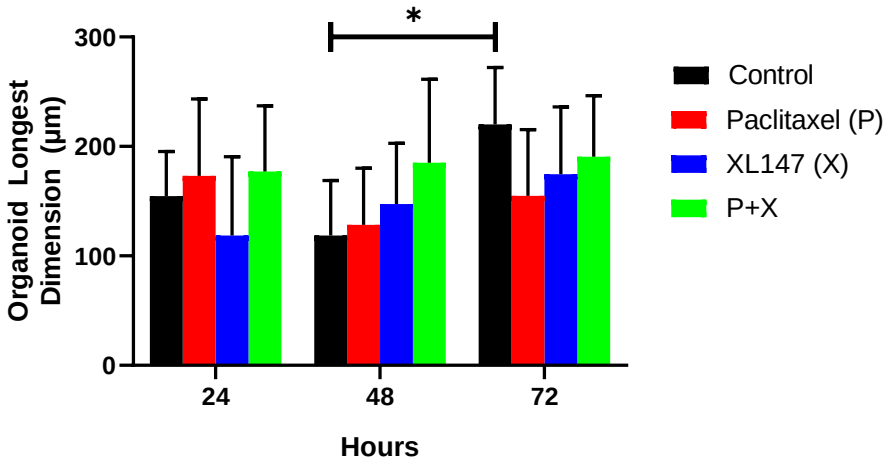

A
Coefficient of Variation - All Tumors
B
Coefficient of Variation - All Organoids
C
In Vivo Coefficient of Variation within Tumor
Vehicle 1
Vehicle 2
Vehicle 3
Vehicle 4
Vehicle 5
P+X 1
P+X 2
P+X 3
P+X 4
P+X 5
D
Organoid Coefficient of Variation within Group
Vehicle
Paclitaxel
XL147
P+X
E
In Vivo Coefficient of Variation within Image
NAD(P)H τm
NAD(P)H τ1
NAD(P)H τ2
NAD(P)H α1
FAD τm
FAD τ1
FAD τ2
FAD α1
NAD(P)H Photons
FAD Photons
CV (%)
80
60
40
20
0
Group
Vehicle
P+X



Supplementary Figure S5. Coefficients of variation of OMI variables across individual cells. A-B, Coefficients of variation (standard deviation divided by the mean, CV) of each OMI variable across all cells imaged (**A**) *in vivo* and (**B**) in tumor-derived organoids. **C,** CV of each OMI variable within each tumor *in vivo.* Each row represents a tumor. **D,** CVs of each OMI variable within each treatment group in organoids. Each row represents a treatment group. **E,** CVs of each OMI variable within each field of view (FOV) imaged *in vivo.* Each row represents a FOV. **F,** CVs of each OMI variable within each organoid. Each row represents an organoid.



Supplementary Figure S6. Automatic cell segmentation and quantification in PyVmT tumors and organoids with histology. Examples images of (**A**) CC3, (**B**) pHH3, (**C**) CD45, and (**D**) αSMA expression in 20x histology images of PyVmT tumor and organoids. Scale bar = 200 μm.



Supplementary Figure S7. Effect of treatment on organoid size. The length of the longest dimension in PyVmT organoids imaged with OMI. Error bars indicate mean ± SD. * $p<0.0005$ using a Wilcoxon rank-sum test. N>5 organoids per group and time point.