Electronic Supplementary Material (ESI) for Integrative Biology.

Mapping lung tumor cell drug responses as a function of matrix context and genotype using cell microarrays

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Supplemental Materials and Methods

Analysis of microarrays

Following imaging of entire arrays as described in the Materials and Methods section, we applied the following analytical steps:

1. **Image processing in Fiji (ImageJ).** Array images from each channel (*i.e.*, blue, red, or green) were converted from 32-bit TIFF images to 8-bit TIFF images in Fiji (ImageJ version 1.51a)[1, 2] in order to reduce computational load during analysis. For similar reasons, we applied 2×2 or 4×4 binning in Fiji to reduce image size from >500 megapixels to 29–32 megapixels per channel.
2. **Single-cell analysis in CellProfiler.** Binned 8-bit TIFF images were analyzed in CellProfiler (version 2.1.1)[3] using the following modules: IdentifyPrimaryObjects, IdentifySecondaryObjects, and MeasureObjectIntensity. IdentifyPrimaryObjects was used to identify nuclei for cell counts while IdentifySecondaryObjects and MeasureObjectIntensity were used to quantify immunolabel intensity, namely for assessment of proliferation by BrdU and apoptosis by cleaved caspase-3. Single-cell data from all three modules were output as CSV files by channel for downstream analysis.
3. **Statistical analysis in R.** Before further analysis, we recorded pixel coordinates of dextran-rhodamine markers for each array. Using single-cell CSV files from Step 2 in conjunction with the dextran-rhodamine coordinates and a transfer list defining the rows and columns of each ECM condition, we automatically assigned ECM conditions to the objects identified in CellProfiler. We manually validated the results of this assignment with the associated images and corrected or otherwise discarded data from arrays with poor signal-to-noise ratios or image artifacts. Using each biological replicate (1–2 arrays with 10–20 islands per arrayed condition) as our fundamental statistical unit, we calculated the following measures in R (R

Core Team, 2016, R Foundation for Statistical Computing) for individual ECM conditions by treatment and cell type:

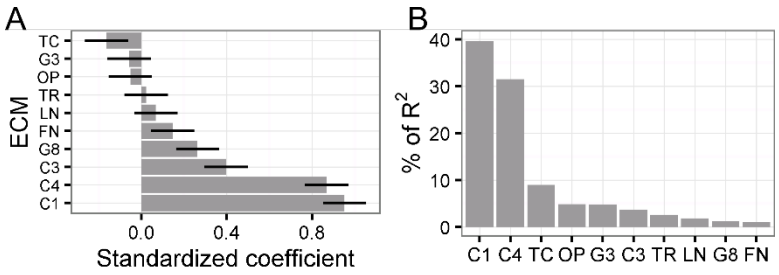
i. **Cells/island.** We calculated cells/island by dividing cell count by the known number of islands per array and ECM condition: $\text{cells/island} = \frac{\text{cell count per array}}{\text{\# of islands per array}}$.

ii. **Percentage of control.** In order to accurately estimate the ECM-specific effect of drug treatment, we first removed the following ECM conditions for which control cells/island was ≤3: C3-OP, FN-G3, G3-TC, G3-TR, G8, LN-G3, OP, TC, TC-OP, TC-TR, and TR. For the remaining ECM conditions, we calculated the percentage of control for each drug treatment: $\%\ \text{of control} = 100\times \frac{\text{cells/island (drug)}}{\text{cells/island (control)}}$. This calculation was performed with respect to controls within each biological replicate before pooling for later statistical analysis.

iii. **Intensity.** To account for drift between biological replicates, we calculated quantile normalized intensity for labels (namely caspase-3 or BrdU) similarly to its application to oligonucleotide microarrays.[4] Specifically, single cell intensity observations per channel were ranked within each biological replicate. We then calculated the mean of observations at equal ranks across biological replicates and assigned that value to all observations of that rank.

Supplemental Figures

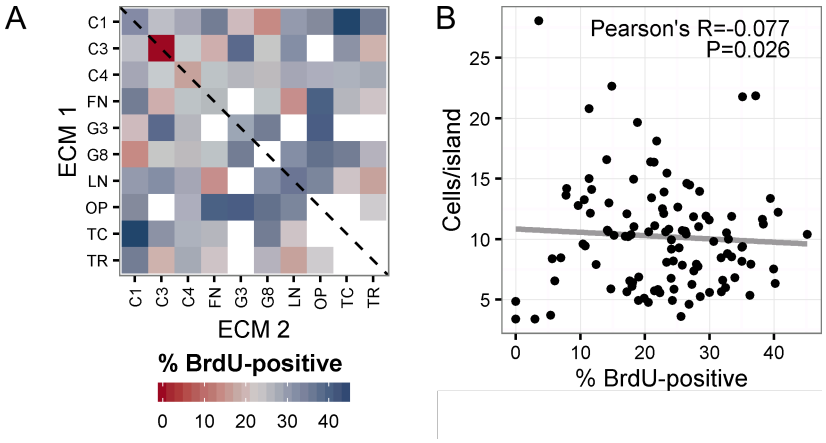
Supplemental Figure S1: Main effects of ECM on A549-WT cells/island



A. Standardized regression coefficients for each ECM protein; larger coefficients were associated with greater cells/island.
B. Relative importance of each ECM protein represented as a percentage of R^2 of the regression in Supplemental Table S1.

Error bars are 95% CI. See also Figure 1 and Supplemental Table S1.

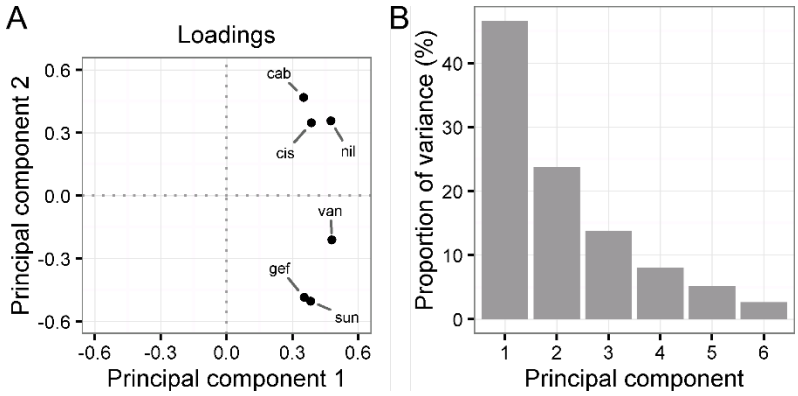
Supplemental Figure S2: Analysis of A549-WT cell proliferation



A. Combinatorial heat map showing A549-WT cell proliferation as a function of all 55 ECM conditions. White boxes indicate conditions removed due to low adhesion in controls.

B. Correlation of A549-WT cells/island with percentage of A549-WT cells positive for BrdU.

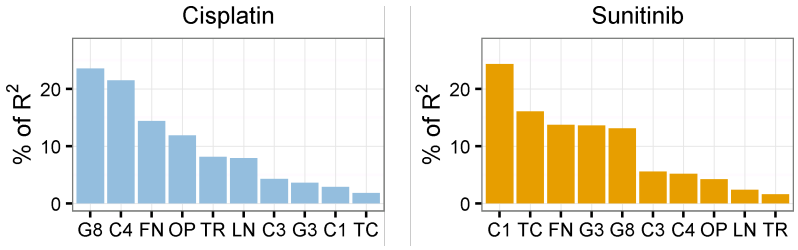
Supplemental Figure S3: PCA of A549-WT drug response



A. Loadings for principal components 1 and 2. Principal component 1 represents overall response to drug. Principal component 2 represents differential response to drugs in Group 1 (cabozantinib, cisplatin, and nilotinib) and Group 2 (vandetanib, gefitinib, and nilotinib).
B. Scree plot showing proportion of variance explained by each principal component. The first and second principal components together explain 70.4% of variance.

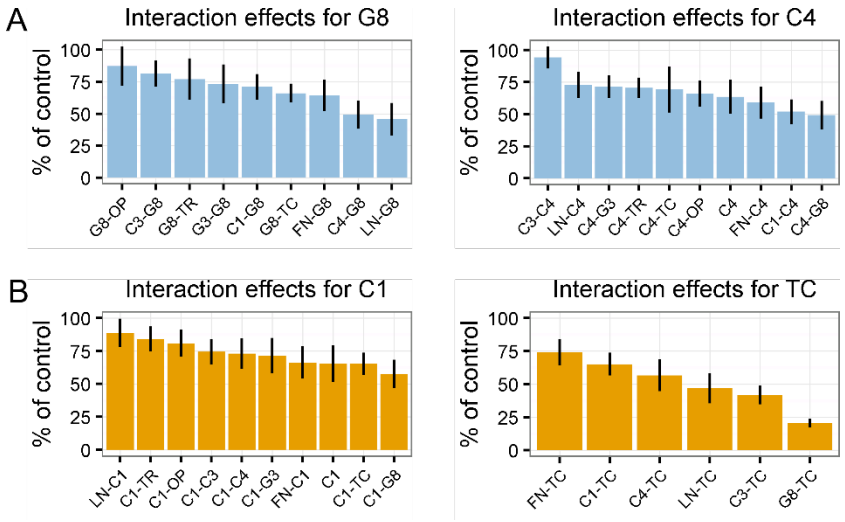
See also Figure 2.

Supplemental Figure S4: Relative importance of ECM in A549-WT response to cisplatin and sunitinib



Relative importance of each ECM in the cisplatin and sunitinib treatments. See also Figure 3 and Supplemental Table S3.

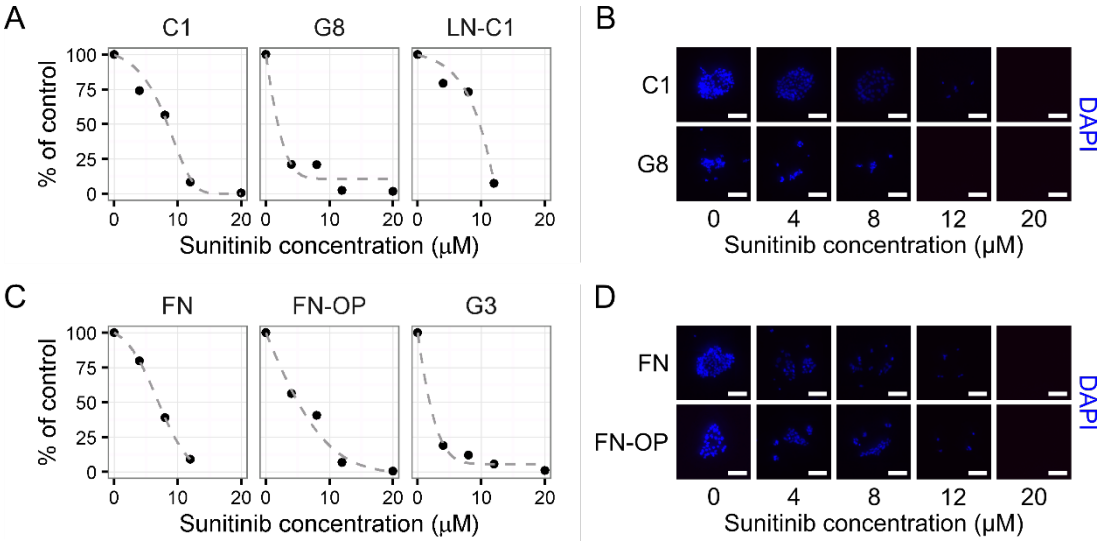
Supplemental Figure S5: Interaction effects for select highly-ranked ECM proteins



A. Interaction effects as a percentage of control for G8- and C4-containing ECM conditions treated with cisplatin (5 μM).
B. Interaction effects as a percentage of control for C1- and TC-containing ECM conditions treated with sunitinib (2 μM).

Error bars are SEM. See also Figure 4.

Supplemental Figure S6: Dose-response curves for select ECM conditions treated with sunitinib



A. Dose-response curves as a percentage of control for cells on C1, G8, and LN-C1 treated with sunitinib at 0, 4, 8, 12, and 20 μM.

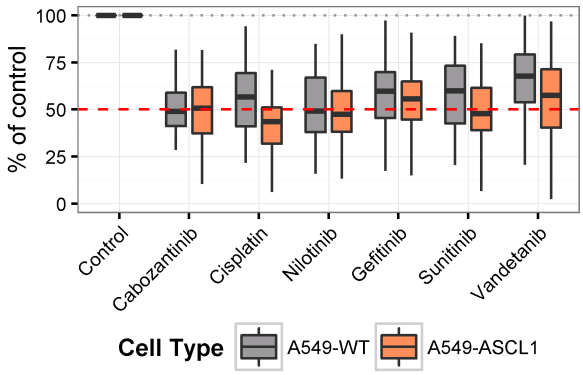
B. Representative micrographs of data in (A) for C1 and G8 labeled for nuclei (DAPI).

C. Dose-response curves as a percentage of control for cells on FN, FN-OP, and G3 treated with sunitinib at 0, 4, 8, 12, and 20 μM.

D. Representative micrographs of data in (C) for FN and FN-OP labeled for nuclei (DAPI).

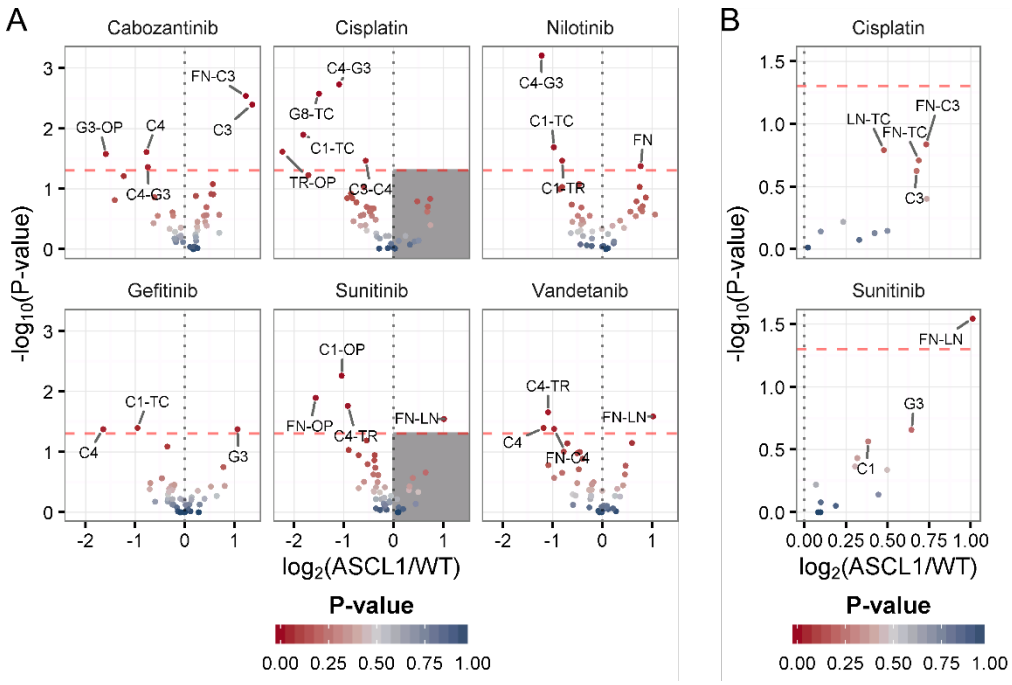
Scale bars are 75 μm.

Supplemental Figure S7: Comparison of overall A549-WT and A549-ASCL1 drug response



Overall percentage of control for A549-WT and A549-ASCL1 cells. See also Figure 6 and Supplemental Figures S8 and S9.

Supplemental Figure S8: ASCL1-specific responses to drug treatment

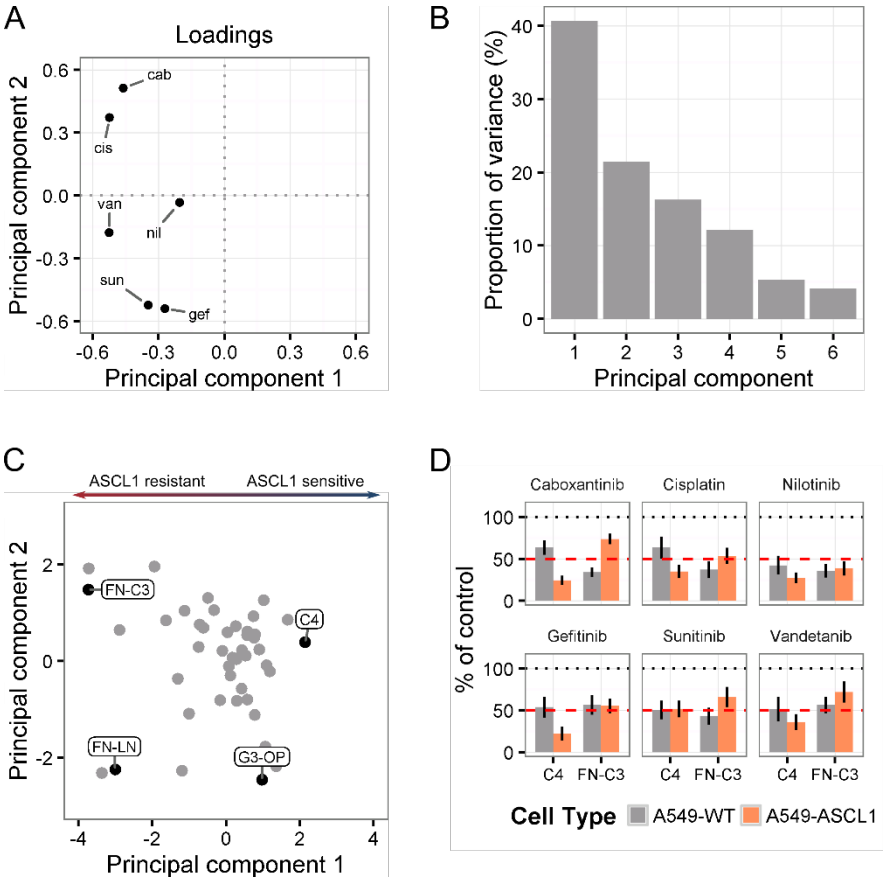


A. Volcano plots showing the ratio of A549-ASCL1 to A549-WT drug response (*x*-axis) against P-values from unpaired Wilcoxon rank sum tests comparing A549-WT and A549-ASCL1 drug response (*y*-axis). Points above the red dashed line are P<0.05; similarly, callouts indicate ECM conditions for which P<0.05. See (B) for detail of areas shaded in gray.

B. Detail of areas shaded in gray in (A) showing ECM conditions treated with cisplatin and sunitinib for which A549-ASCL1 cells were more resistant than A549-WT cells.

See also Figure 6 and Supplemental Figure S9.

Supplemental Figure S9: PCA of WT-normalized A549-ASCL1 drug response as a function of ECM



A. Loadings for principal components 1 and 2. Principal component 1 represents overall WT-normalized response of A549-ASCL1 cells to drug. Principal component 2 represents differential response to drugs in subsets of Group 1 (cabozantinib and cisplatin) and Group 2 (vandetanib and nilotinib).

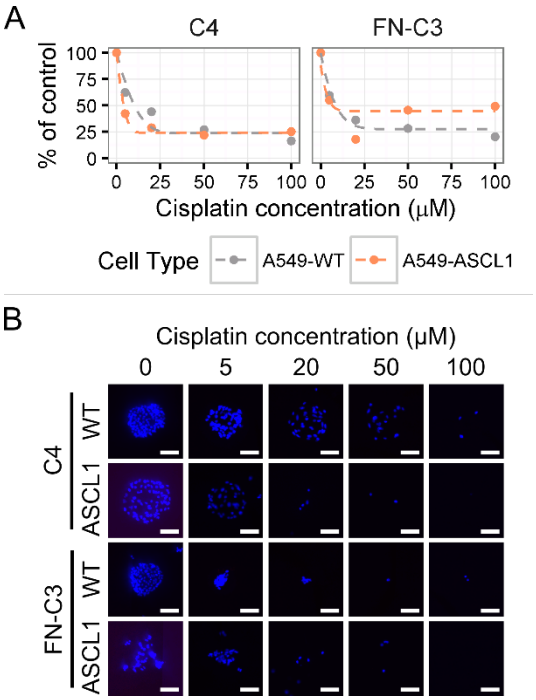
B. Scree plot showing proportion of variance explained by each principal component. Principal components 1 and 2 together explain 62.2% of variance.

C. PCA separates ECM conditions into *ASCL1*-associated sensitivity/resistance.

D. Select ECM conditions identified in (C) validating PCA. Error bars are SEM.

See also Figure 6 and Supplemental Figure S8.

Supplemental Figure S10: Dose-response analysis of C4 and FN-C3



A. Dose-response curves as a percentage of control for A549-WT and A549-ASCL1 cells on C4 and FN-C3 treated with cisplatin at 0, 5, 20, 50, and 100 μM. Dotted lines are 5-parameter logistic fits.

B. Representative micrographs for data in (A) labeled for nuclei (DAPI). Scale bars are 75 μm.

See also Figure 6 and Supplement Figure S8.

Supplemental Tables

Supplemental Table S1: Regression against A549-WT cells/island by ECM

	Dependent variable:
	Cells/island
Constant	-0.450*** (-0.725, -0.175)
C1	0.952*** (0.753, 1.151)
C3	0.397*** (0.198, 0.596)
C4	0.868*** (0.669, 1.067)
FN	0.147 (-0.052, 0.346)
G3	-0.057 (-0.257, 0.143)
G8	0.263*** (0.065, 0.461)
LN	0.068 (-0.132, 0.268)
OP	-0.052 (-0.251, 0.147)
TC	-0.164 (-0.363, 0.036)
TR	0.022 (-0.179, 0.222)
Observations	923
R^2	0.230
Adjusted R^2	0.222
Residual Std. Error	0.882 (df = 912)
F Statistic	27.233*** (df = 10; 912)
Note:	*p<0.1; **p<0.05; ***p<0.01
	Values in parentheses are 95% CI

Regression model: $\text{A549-WT control cells/island} \sim \text{C1}+\text{C3}+\text{C4}+\text{FN}+\text{G3}+\text{G8}+\text{LN}+\text{OP}+\text{TC}+\text{TR}$.

Calculated regression coefficients are standardized. See also Figure 1 and Supplemental Figure S1.

Supplemental Table S2: Regression against A549-WT response by drug

	Dependent variable:
	% of control
Control	100.000*** (96.978, 103.022)
Cabozantinib	-49.343*** (-53.658, -45.027)
Cisplatin	-43.287*** (-47.684, -38.890)
Nilotinib	-48.888*** (-53.224, -44.552)
Gefitinib	-40.079*** (-44.454, -35.705)
Sunitinib	-39.172*** (-43.558, -34.787)
Vandetanib	-31.554*** (-35.960, -27.148)
Observations	2,915
R^2	0.199
Adjusted R^2	0.197
Residual Std. Error	32.634 (df = 2908)
F Statistic	120.098*** (df = 6; 2908)
Note:	*p<0.1; **p<0.05; ***p<0.01
	Values in parentheses are 95% CI

Regression model: $\text{A549-WT \% of control} \sim \text{Treatment}$. Calculated regression coefficients are unstandardized. See also Figure 2.

Supplemental Table S3: Regression against A549-WT response by ECM for cisplatin and sunitinib

	Dependent variable:	
	% of control	
	Cisplatin	Sunitinib
Constant	-0.977*** (-1.536, -0.418)	-0.215 (-0.773, 0.343)
C1	0.478** (0.113, 0.843)	0.488*** (0.125, 0.851)
C3	0.557*** (0.182, 0.932)	0.279 (-0.093, 0.652)
C4	0.769*** (0.403, 1.136)	0.280 (-0.080, 0.640)
FN	0.173 (-0.211, 0.556)	0.420** (0.030, 0.809)
G3	0.559** (0.131, 0.988)	-0.257 (-0.684, 0.170)
G8	0.766*** (0.413, 1.120)	-0.177 (-0.522, 0.167)
LN	0.245 (-0.133, 0.624)	0.206 (-0.176, 0.587)
OP	0.669*** (0.300, 1.039)	-0.089 (-0.456, 0.278)
TC	0.443** (0.057, 0.828)	-0.370* (-0.756, 0.017)
TR	0.610*** (0.239, 0.982)	-0.050 (-0.415, 0.316)
Observations	363	369
R^2	0.107	0.114
Adjusted R^2	0.081	0.090
Residual Std. Error	0.959 (df = 352)	0.954 (df = 358)
F Statistic	4.199*** (df = 10; 352)	4.626*** (df = 10; 358)
Note:		*p<0.1; **p<0.05; ***p<0.01
		Values in parentheses are 95% CI

Regression model: $\text{A549-WT control cells/island} \sim \text{C1}+\text{C3}+\text{C4}+\text{FN}+\text{G3}+\text{G8}+\text{LN}+\text{OP}+\text{TC}+\text{TR}$ for cisplatin and sunitinib separately. Regression coefficients are standardized within each model. See also Figure 3 and Supplemental Figure S4.

References

1. C. A. Schneider, W. S. Rasband and K. W. Eliceiri, *Nat Methods*, 2012, **9**, 671-675.
2. J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J. Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak and A. Cardona, *Nat Methods*, 2012, **9**, 676-682.
3. L. Kamentsky, T. R. Jones, A. Fraser, M. A. Bray, D. J. Logan, K. L. Madden, V. Ljosa, C. Rueden, K. W. Eliceiri and A. E. Carpenter, *Bioinformatics*, 2011, **27**, 1179-1180.
4. D. Amaratunga and J. Cabrera, *Journal of the American Statistical Association*, 2001, **96**, 1161-1170.