Supporting Information

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The Galapagos Chip Platform for High-Throughput Screening of Cell Adhesive Chemical Micropatterns

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2.1. Surface functionalization strategy and characterization

Figure S1: Reaction kinetics of VTMS vapor deposition on the glass substrate. The water contact angle of activated glass substrate increased sharply in the first 2 h of vapor deposition of VTMS,
reaches its maximum after 24 h, and stabilizes after that. Error bars represent SD, n = 3 (number of independent samples).

Figure S2: High-resolution XPS scans of C1s region with PEG-grafted surfaces. Comparison of high-resolution XPS scans of C1s region for VTMS, PEG-thiol-modified VTMS (VTMS-PEG), NbTES, PEG-thiol-modified NbTES, and control surface (glass surface treated with PEG without UV). Raw data are given in blue circles, while fits for single peaks and envelopes are shown in solid green and red lines, respectively.
Table S 1. XPS quantitative analysis of glass substrate at each step of surface modification

| Environment          | Si  | O       | C       | C       | N     | S     | Na/K |
|----------------------|-----|---------|---------|---------|-------|-------|------|
|                      | SiO<sub>2</sub> | SiO<sub>2</sub> + C-O | C-O, C-N | O-C=N | total | S-C  | Total |
| Binding energy (eV)  | 103.0 | 532.9 | 286.7   | 288.5   | 163.4 |       |      |
| Glass (Piranha)      | 21.3 | 41.4   | 2.2     | 2.1     | 0.2   | -     | 2.6  |
| +VTMS<sup>a</sup>    | 22.5 | 42.3   | 2.0     | 1.5     | 0.2   | -     | 1.4  |
| +VTMS-RGD<sup>b</sup>| 20.1 | 38.7   | 3.5     | 3.5     | 3.0   | 0.1   | 2.7  |
| +VTMS-PEG<sup>c</sup>| 21.4 | 47.4   | 14.3    | 0.8     | -     | 0.1   | 1.9  |
| +VTMS-HAVDI<sup>d</sup>| 24.3 | 48.4   | 2.4     | 1.8     | 1.4   | 0.1   | 2.2  |
| +NbTES<sup>e</sup>  | 25.3 | 55.1   | 1.7     | 1.1     | 0.3   | -     | 2.4  |
| +NbTES-RGD           | 21.4 | 48.4   | 5.0     | 3.9     | 3.9   | 0.1   | 2.4  |
| +NbTES-PEG           | 24.6 | 56.5   | 3.7     | 0.9     | 0.3   | -     | 1.2  |

**Note:**

- **a:** +VTMS: Vinyl silane functionalized glass substrate
- **b:** -RGD: CGGGRGDS (RGD) peptide grafted surface.
- **c:** -PEG: PEG thiol (molecular weight 2 kDa) grafted surface.
- **d:** -HAVDI: AcHAVDIGGGC (HAVDI) peptide grafted surface.
- **e:** +NbTES: Norbornene silane functionalized glass substrate
2.3. Fabrication of the Galapagos Chip

![Mask Design](image1.png) 3 µl thiol solution 10 µl thiol solution

**Figure S3**: The resolution of the micropattern is dependent on the thiol solution volume. Thiol solution volume regulates the distance between the substrate and the UV mask, which could regulate the resolution of the patterns. For this study, the obtained resolution was sufficient to transfer pattern design, and the produced patterns were reproducible for a given 3 µl thiol solution volume. Scale bar is 100 µm.

The theoretical resolution as the minimum transferable feature size of our proximity (shadow) printing mode UV-lithographic process due to diffraction effects depends on the distance or proximity gap between the substrate surface to be functionalized and the UV mask, and on the wavelength of the light used. The theoretical resolution is calculated by the given formula: \[^1\] $r = k \sqrt{\lambda * d}$. $k$ is technology parameter ($k = 3/2 = 1.5$), $\lambda$ is wavelength of the light, and $d$ is the proximity gap.

For calculation of the theoretical gap between substrate and mask:

- Volume of the solution stated in $V$, $d$ is the distance of the mask and the substrate, $a$ is the side of the substrate. $V=3\mu l$ and $a=25mm$

\[
V = d * a^2 = 3 \mu l = 3 \text{mm}^3
\]
Theoretical gap is \[ d = \frac{V}{a^2} = \frac{3 \text{mm}^3}{625 \text{mm}^2} = 4.8 \mu\text{m} \]

For calculation of the theoretical resolution:

- We used UV light with a wavelength of \( \lambda = 254 \text{ nm} = 0.254 \mu\text{m} \)
- Calculation of the theoretical resolution is given below. \( k = \frac{3}{2} = 1.5 \), \( \lambda \) is wavelength of the light, and \( d \) is the proximity gap 4.8\( \mu\text{m} \).

\[
r = k \sqrt{\lambda \cdot d} = 1.5 \cdot \sqrt{(0.254 \cdot 4.8)} = 1.656 \mu\text{m}
\]

Theoretical resolution was found 1.656 \( \mu\text{m} \) for the 3 \( \mu\text{l} \) solution volume for our experimental conditions. Also, we used a non-collimated light source in this study, using a lithographic light source and decreasing thiol solution volume should help to improve the resolution of the pattern.
Figure S4. Surface modification on polymer surfaces and their characterization

A. Examples of UV-induced functionalization of polymer surfaces, polycarbonate (PC), and polyethylene (PE) derivative with RGD peptide. Apparent water contact angle before and after the modification is shown. B. FTIR spectra of non-modified, VTMS- (purple), VTMS-RGD- (purple dashed line), NbTES- (blue line), and NbTES-RGD-modified (dashed blue line) PC surfaces. 1660 cm$^{-1}$ amide signal is visible in RGD-modified surfaces drawn by red dashed line. C Fluorescence image of a fluorescein-labeled PEG patterned NbTES-PE polymer surface. Scale bar is 50 µm. D. The comparison of fluorescent intensity obtained from fluorescein-labeled RGD-modified glass, polycarbonate (PC), and PE polymer surface with VTMS and NbTES. Error bars represent SD, n = 3 (number of independent samples) and significant difference expressed as * (P<0.05).

2.4. hMSC morphology and subcellular features are altered by the underlying RGD micropatterns
Figure S5: hMSC morphology on the Galapagos Chip: RGD/PEG binary micropatterned high-throughput platform. hMSCs cultured for 4 h on the Galapagos Chip platform stained with Cell Painting assay. Different cell morphologies are observed on different micropattern units. Cells labeled with Hoechst 33342 (nuclei, blue), concanavalin A (ER, yellow) and SYTO 14 (nucleoli, yellow), phalloidin (actin, cyan), WGA (Golgi and p-membrane, cyan), MitoTracker Deep Red (mitochondria, red). Scale bar is 50 µm.
Figure S6: Cell viability on the micropatterned surface. HMSCs are cultured on TCP (on the left) and RGD/PEG-patterned surfaces (on the right), and co-stained with calcein-AM and EthD-1. The graphic shows calcein-AM positive cells as a percentage of the total cell number. Scale bar is 100 µm. Error bars represent SD, n = 3 (number of replicas).
2.5. High throughput screening of nuclear localization of YAP

**Figure S7: Cell segmentation and YAP quantification with CellProfiler.** YAP expression and cell morphologies are analyzed by CellProfiler. Representative segmented cells and quantifications are shown. YAP is quantified as YAP ratio, which was obtained by taking the mean pixel intensity in the nucleus and dividing it by the mean pixel intensity of the cytoplasm (\(\text{YAP}_{\text{nuc}}/\text{YAP}_{\text{cyto}}\)).
Figure S8: Machine-learning correlation analysis of YAP expression and subcellular pattern design.  
A. The graphic shows representing mean YAP levels of MSCs cultured on the Galapagos Chip of each individual micro island unit, ranked from highest to lowest. The red line represents the mean YAP level of the non-patterned, uniform RGD. B. The 200 units with the highest and lowest mean YAP levels are chosen as top- and low-hit units, respectively. Error bars represent SD. C. Machine-learning model to predict YAP expression from RGD design parameters. ROC curve showing the prediction performance YAP level by pattern design parameters with XGBoost classification algorithm for training and test datasets. The area under the curve (AUC) indicates the predictive power of the model. D. In the model, FCPN03 and RotSD are some of the most important features to distinguish low- from high-YAP-level-expressing RGD patterns. Scatter plot representing the distribution of the FCPN03 and RotSD for low–hit (blue) and top-hit YAP-expressing cells (yellow) in the whole dataset.
2.6. YAP nuclear localization correlates with cell morphology features

Table S 2. Paxillin and actin textures were measured in the MeasureTexture module of CellProfiler. Descriptions of texture parameters are shown below.

| Texture parameters     | Descriptions                                                                                                                                 |
|------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|
| Correlation            | It measures the linear dependency of intensity values in an image. For an image with large areas of similar intensities, correlation is much higher than for an image with noisier, uncorrelated intensities. It has a value of 1 or -1 for a perfectly positively or negatively correlated image. |
| SumAverage             | The average of the normalized grayscale image in the spatial domain.                                                                                                                                   |
| DifferenceVariance     | The image variation in a normalized co-occurrence matrix.                                                                                                                                             |
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