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Figure S1. Design of 16 cell colour codes. A. Combination of four fluorophores for 16 colour codes. 1 and 0 respectively indicate bright and dim. B. Design of DNA tag transfected to the cells. C. Mean fluorescence intensities of colour-coded cells.



Figure S2. DNA-barcoded hydrogel beads with 16 colour codes. A. Combination of four fluorophores for 16 colour codes. 1 and 0 respectively indicate bright and dim. B. Schematic images of barcoded primers attached to hydrogel beads. C. Example schematic images of colour code staining. The designated fluorophores are attached to beads via branch DNAs hybridising to the sequence of bead colour code. D. Mean fluorescence intensities of colour-coded beads.



Figure S3. Procedures of species-mixing experiment. We divided human cells (HeLa) and mouse cells (NIH/3T3) into the eight sub-pools respectively for a total of 16 sub-pools. We individually stained with different combinations of fluorescence dyes and transfected with corresponding DNA tags via electroporation. We combined all sub-pools of colour-coded cells into a single tube and captured the cells in a microwell array. After cell imaging, we introduced the colour-coded hydrogel beads into the microwells, creating pairs of a colour-coded cell and bead in each microwell. Upon cell lysis, released mRNAs and DNA tags hybridise with barcoded dT primers on hydrogel beads. We imaged the hydrogel beads and proceeded to reverse transcription, PCR, and next-generation sequencing.



Figure S4. Library designs of cDNA and DNA tag for Illumina sequencing. A cDNA library. B. DNA tag library.



Figure S5. HeLa cell response to paclitaxel on dish A. The drug response curve of HeLa cells treated with paclitaxel for 24 h. The red broken line indicates the half-maximal inhibitory concentration (IC50). B-D. Images of cells in bright fields and nuclei stained with Hoechst 33342 of HeLa cells treated with paclitaxel (B. vehicle, C. 5 nM, and D. 50 nM) for 24 h in standard couture dishes. The scale bars on the bottom right are 50 μm. E. Images of nuclei in individual HeLa cells treated with different concentrations of paclitaxel. Cian points are the detected positions of spindle poles. The colour labels on the bottom of the images indicate the number of spindle poles. The images are stratified according to the number of spindle poles. F. Distributions of the number of spindle poles in HeLa cells.