Supplemental Information

Supplemental Methods

Microraft Array Fabrication

The 6 monoclonal A375-BAR-mCherry cells were isolated using a microraft array platform. Microraft arrays were fabricated as previously described.[1] Briefly; microraft master molds were prepared by single layer photolithography using 1002F photoresist and a chrome mask with a pattern of 110×110 200 μm square apertures with a 30 μm gap.[2] After fabrication of the master, the microraft array substrate was prepared by casting polydimethylsiloxane (PDMS; Sylgard 184, Dow Corning Corp., Midland MI) onto the mold and curing at 95 ºC for 1 hour. The PDMS well array was then demolded from the master and filled by overlaying a solution of 30% polystyrene in γ-butyrolactone with 3% iron oxide nanoparticles by weight and applying vacuum. The filled microwell array was then lowered into a bath of excess magnetic polystyrene solution and slowly withdrawn at a rate of 25 mm/h to achieve discontinuous dewetting. The dip-coated microwell array was then baked overnight at 95 ºC to remove solvent from the polystyrene and harden the microrafts. The fabricated microraft arrays were mounted to cassettes, oxidized in an air plasma (Harrick Plasma, Ithaca, NY) for 5 min and sterilized with 75% ethanol and air-dried immediately prior to use.

Micropallet Array Fabrication

Micropallet arrays were utilized for the parallel clonogenic screening due to the ability of stable virtual air walls to prevent migration of the cell type employed for extended periods of culture. Micropallet arrays were fabricated as previously described using a composite photoresist of poly(methyl methacrylate-co-methacrylic acid) and 1002F termed PMMA/1002F.[3,4] Briefly, a

35 μm thick layer of photoresist was generated on glass slides by spin coating in two steps: 500 rpm for 10 s followed by 2500 rpm for 30 s. The film was then dried by soft baking at 95 ºC for 1 h. The film was then exposed using a 360 nm long-pass filter for 3 exposures lasting 1 min each with a 1 min gap between each exposure to prevent excessive heating of the photoresist. After exposure the film was cross-linked by baking at 95 ºC for 10 min and developed in 2-methoxypropylacetate for 4 min. After developing the micropallet arrays were oxidized in an air plasma for 5 min prior to silanization. To establish stable virtual air walls, 100 μL of (Heptadecafluoro-1,1,2,2-tetrahydrodecyl)trichlorosilane (Gelest Inc, Morrisville PA) was added to a dish adjacent to micropallet arrays placed in a dry-seal vacuum dessicator (Wheaton, Millville NJ) and a vacuum was applied using an oil-free pump for 2 min. The dessicator was then sealed and incubated for 16 h. After 16 h, vacuum was again applied for 30 minutes to remove excess silane and the treated micropallet arrays were removed. The micropallet arrays were then immediately mounted into cassettes and sterilized with 75% ethanol prior to use.

Supplemental Data

	Clone 1	**Clone 2**	**Clone 3**	**Clone 4**	**Clone 5**	**Clone 6**
Clone 1	1	**1.14E-14**	0.054695	**5.74E-10**	**8.91E-09**	**3.74E-13**
Clone 2	**1.14E-14**	1	**5.49E-06**	**1.69E-29**	**2.05E-29**	**1.88E-22**
Clone 3	0.054695	**5.49E-06**	1	**3.77E-10**	**9.86E-10**	**4.37E-13**
Clone 4	**5.74E-10**	**1.69E-29**	**3.77E-10**	1	0.155183	0.294269
Clone 5	**8.91E-09**	**2.05E-29**	**9.86E-10**	0.155183	1	**0.000509**
Clone 6	**3.74E-13**	**1.88E-22**	**4.37E-13**	0.294269	**0.000509**	1

Table S1. P-values for the two-tailed Wilcoxon rank sum test comparing the median peak reporter activation magnitude between each pair of clonal A375-BAR-mCherry cell lines cultured on polystyrene. Pair-wise comparisons that were found to be significant ($\alpha = 0.01$) are shown in bold.

	Clone 1	**Clone 2**	**Clone 3**	**Clone 4**	**Clone 5**	**Clone 6**
Clone 1	1	**5.69E-05**	**0.000226**	**1.43E-10**	**3.65E-10**	**5.57E-06**
Clone 2	**5.69E-05**	1	0.789757	**0.006017**	0.041012	0.495437
Clone 3	**0.000226**	0.789757	1	0.057556	0.174166	0.625638
Clone 4	**1.43E-10**	**0.006017**	0.057556	1	0.407026	0.113721
Clone 5	**3.65E-10**	0.041012	0.174166	0.407026	1	0.355528
Clone 6	**5.57E-06**	0.495437	0.625638	0.113721	0.355528	1

Table S2. P-values for the two-tailed Wilcoxon rank sum test comparing the median time to reach peak reporter activation between each pair of clonal A375-BAR-mCherry cell lines cultured on polystyrene. Pair-wise comparisons that were found to be significant ($\alpha = 0.01$) are shown in bold.

	Clone 1	Clone 2	Clone 3	Clone 4	Clone 5	Clone 6
Clone 1	1	0.057693	0.450691	0.038412	0.576431	0.099773
Clone 2	0.057693	1	0.740352	**0.001893**	0.169575	0.971958
Clone 3	0.450691	0.740352	1	0.044307	0.657881	0.787086
Clone 4	0.038412	**0.001893**	0.044307	1	0.010573	**0.002302**
Clone 5	0.576431	0.169575	0.657881	0.010573	1	0.246888
Clone 6	0.099773	0.971958	0.787086	**0.002302**	0.246888	1

Table S3. P-values for the two-tailed Wilcoxon rank sum test comparing the median time for signal to relax to half peak fluorescence between each pair of clonal A375-BAR-mCherry cell lines cultured on polystyrene. Pair-wise comparisons that were found to be significant ($\alpha = 0.01$) are shown in bold.

Clone	1	2	3	4	5	6
PS vs Fbn	0.502104	0.275798	0.06618	**0.00258**	0.358901	0.341907
PS vs Gel	0.205828	0.284172	0.038567	0.793116	0.874622	0.411383
Fbn vs Gel	0.165635	0.048529	**1.83E-05**	**0.005946**	0.464554	0.657918

Table S4. P-values for the two-tailed Wilcoxon rank sum evaluating the null hypothesis that the median time to reach peak fluorescence for each test pair is not significantly different. The test failed to reject the null hypothesis for all but three cases for $\alpha = 0.01$ level. In clones 3 and 4, the median time to reach peak fluorescence for cells cultured on fibronectin was significantly longer than for cell cultured on gelatin and in clone 4 the median time was also significantly longer for cells cultured on fibronectin than for cells cultured on polystyrene.

Clone	1	2	3	4	5	6
PS vs Fbn	0.015467	0.930512	0.067268	0.383456	0.469664	0.013488
PS vs Gel	0.683797	0.099148	0.856897	0.500818	0.55149	0.538264
Fbn vs Gel	0.048942	0.151066	0.043366	0.970127	0.249788	0.073528

Table S5. p-values for the two-tailed Wilcoxon rank sum test to evaluate the null hypothesis that the median times to reach half peak fluorescence are not different for each pair of cell culture substrates tested for each clone. At the $\alpha = 0.01$ level, the test failed to reject the null hypothesis for all comparisons.

Clone	1	2	3	4	5	6
PS vs Fbn	1.42E-08	0.00057	2.55E-09	**0.0412**	9.71E-06	**0.0269**
PS vs Gel	**0.02896**	**0.66139**	8.16E-05	0.0032	0.00011	1.34E-11
Fbn vs Gel	1.72E-05	2.41E-09	1.29E-18	**0.96308**	**0.37721**	4.76E-05

Table S6. p-values for the two-tailed Wilcoxon rank sum test to evaluate the null hypothesis that the median peak activation magnitude of cells cultured on condition is not statistically significantly different. Values that fail to reject the null hypothesis at the $\alpha = 0.01$ level are shown in bold.

Clone	1	2	3	4	5	6
PS	7.10E-09	2.86E-04	1.27E-09	**0.0206**	**1**	**0.987**
Gel	8.59E-06	1.20E-09	6.47E-19	**0.519**	**0.812**	2.38E-05

Table S7. p-values for the single-tailed Wilcoxon rank sum test to evaluate the null hypothesis that the median intensity of the fibronectin distribution is not greater than the median intensities of PS or Gel. At the $\alpha = 0.01$ level, the rank sum test shows a statistically significantly higher median fluorescence intensity for Clones 1-3 when cultured on fibronectin than either of the alternate substrates and a high median fluorescence intensity for Clone 6 when cultured on fibronectin than on gelatin.

Format	Ideal Clonal Yield	Media Consumption per Clone (mL)	Wnt-3a Consumption per clone (µg)
384-well plate	141 ± 12	1.305	0.435
Cell Array	4,047 ± 64	0.006	0.002
Reduction		99.5%	99.5%

Table S8. Comparison of reagent consumption and clonal yield of screening performed using conventional 384 well plates and a 1 in^2 cell array containing 12,100 elements. Expected clonal yield was estimated using the Poisson distribution for a seeding ratio of 1 cell per well or array element; the error shown is the standard deviation of the Poisson distribution for the corresponding mean. Media consumption was calculated for 6 exchanges of 80 µL per well for the 384 well plate and 4 mL per cell array. Wnt-3a consumption was calculated for two 80 µL doses of 1 µg/mL Wnt-3a for the 384 well plate and two 4 mL doses for the cell array.

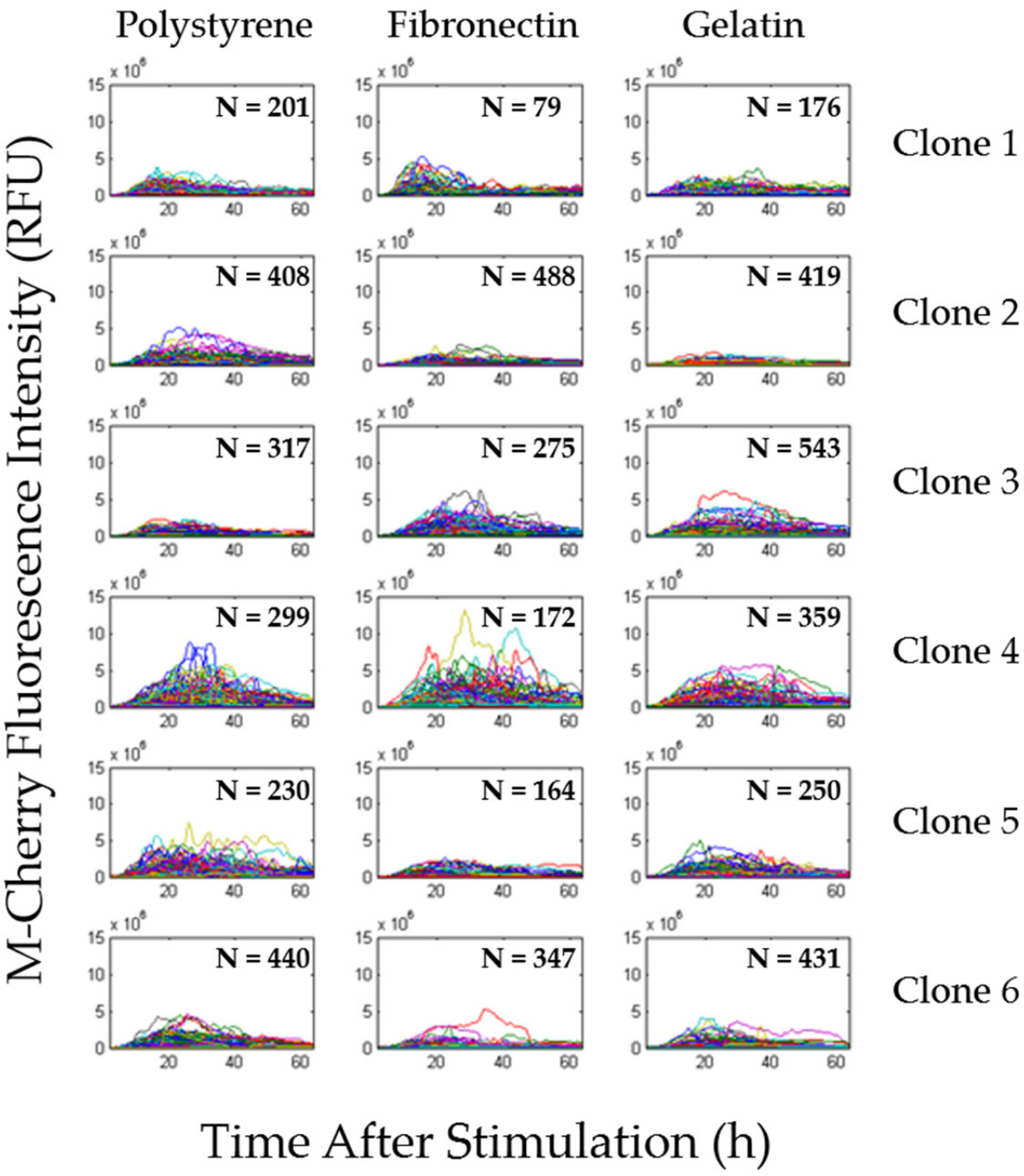

Figure S1. Traces showing the dynamics of mCherry fluorescence after stimulation of 6 clonal A375-BAR-mCherry cell lines with 1 µg/mL recombinant Wnt-3a for 2 h.

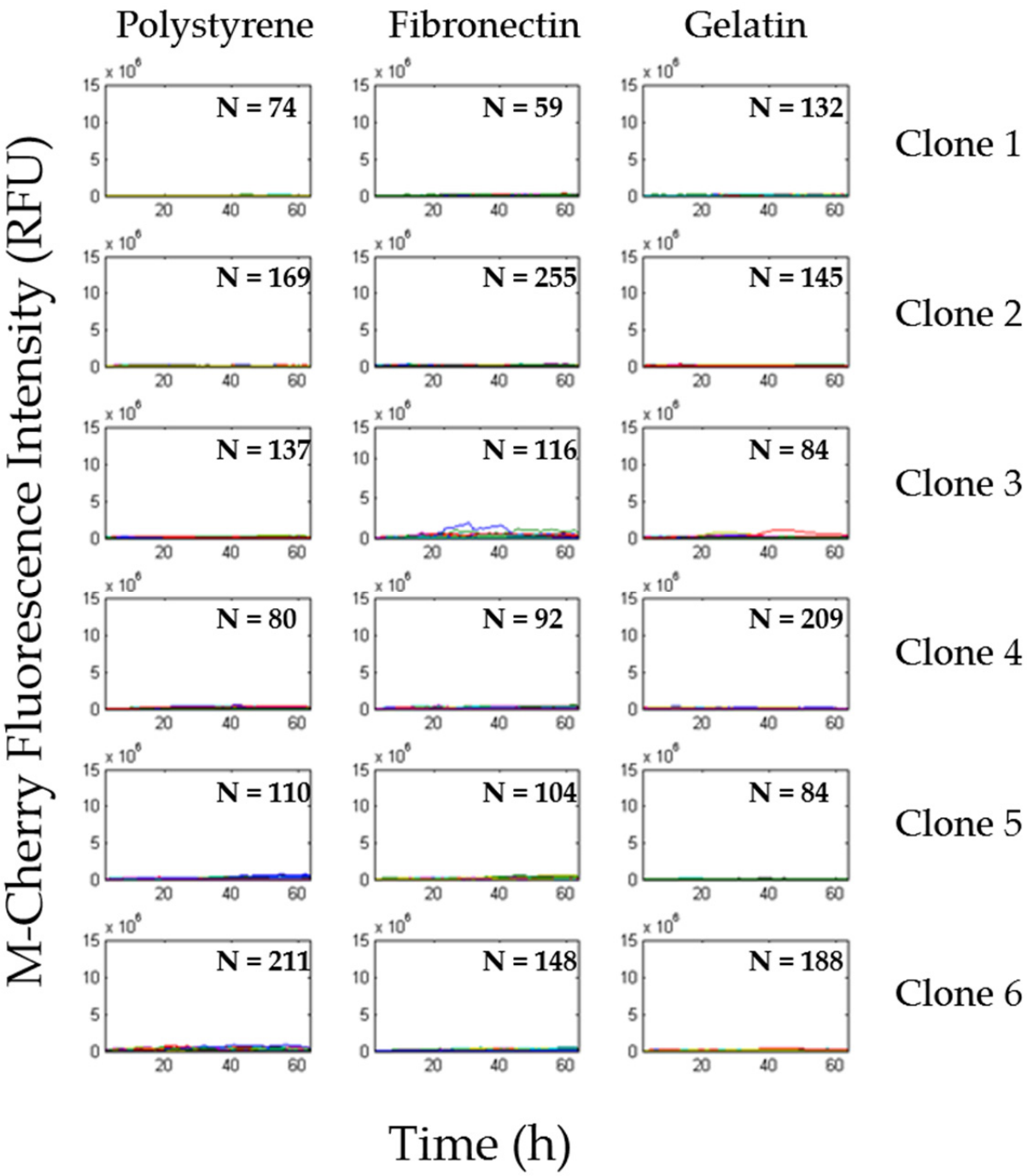

Figure S2. Traces showing measurement of mCherry fluorescence in control A375-BAR-mCherry cells treated with the vehicle for recombinant Wnt-3a (0.1% BSA in DI water stock concentration) for 2 h.

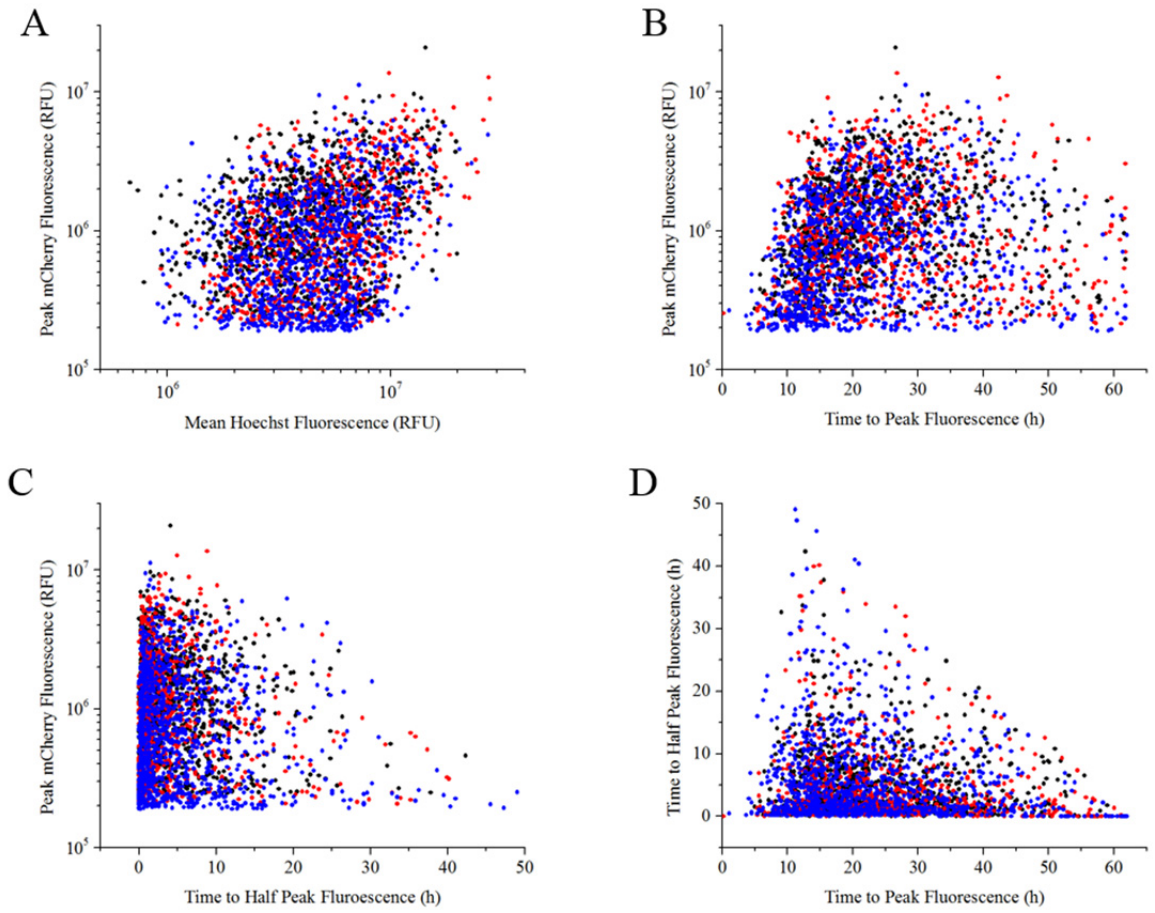

Figure S3. Comparisons between measured parameters from single-cell tracking of A375-BAR-mCherry cells cultured on polystyrene (black), fibronectin (red) and gelatin (black) after treatment with Wnt3a. (A) Peak mCherry fluorescence achieved versus the mean Hoechst fluorescence over the 62 h time-course ($r^2 = 0.09$) (B) Peak mCherry fluorescence versus the time required to reach peak fluorescence ($r^2 = 0.15$) (C) Peak mCherry fluorescence achieved versus the time required for signal to decay to half peak fluorescence ($r^2 = 0.01$) (D) The time required for signal to decay to half peak fluorescence versus the time required for cells to reach peak fluorescence ($r^2 = 0.006$).