# A genetically-encoded sender-receiver system in 3D mammalian cell culture

**Supplementary Information S1.**

This file contains Supplementary Figures S1-S5, annotated DNA sequences of the final constructs used in this study and the computational model of diffusion and repression.

**Contents**

**1. Supplementary figures:**

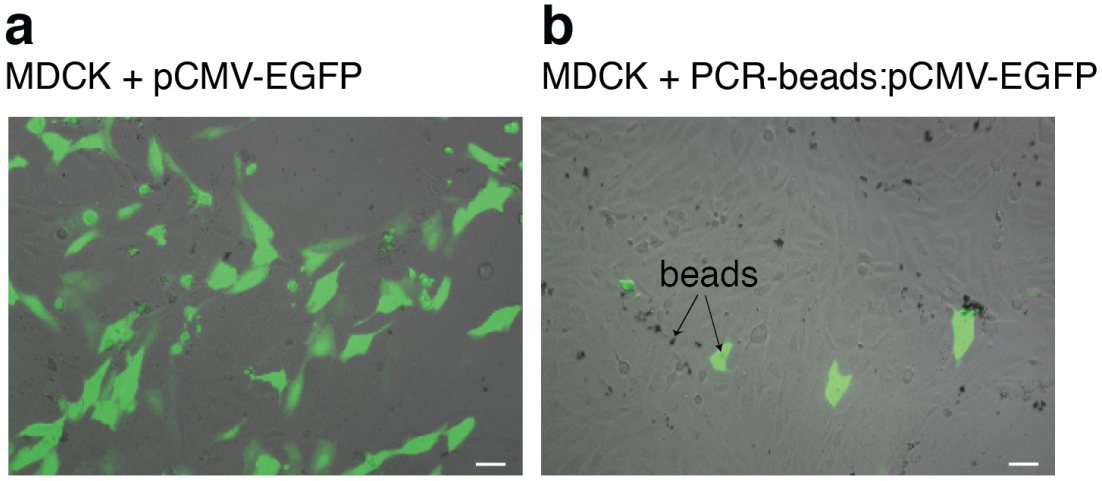


**Figure S1. Comparison of lipofectamine and bead-based localised transfection of MDCK cells in standard 2D cell culture.** Fluorescence microscopy images are of cells transfected with pCMV-EGFP. (a) About ~60% transfection efficiency is achievable with standard lipofectamine plasmid transient transfection. (b) Using a bead-based localized PCR transfection method (*Nat Methods* **2005,** *2* (2), 113-8), less than 1% of MDCK cells are transfected. Green cells are transfected by streptavidin-coated paramagnetic beads, coated with biotinylated PCR DNA. Scale bars, 50 µm.

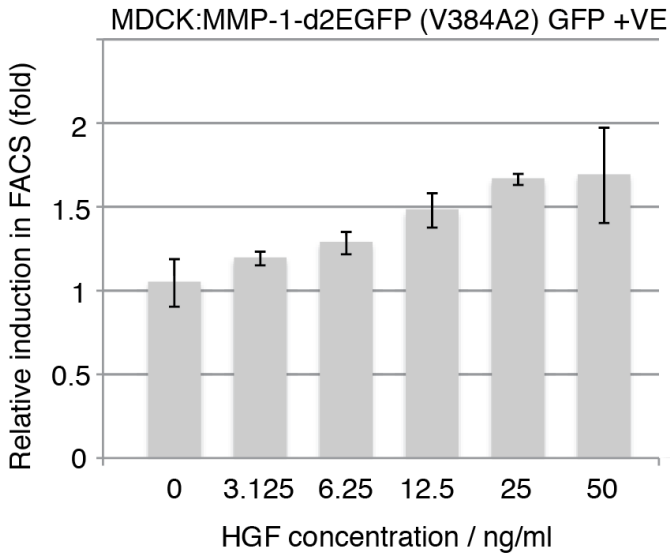


**Figure S2. HGF induction of MDCK:pMMP-1-d2EGFP stable cell line in 2D culture.** Analytical FACS was used to count GFP positive cells in samples treated with different concentrations of HGF for 24 h. The same gate is used in all experiments defined by a FITC/PE-A dotplot, such that there are 0% WT MDCK cells and 99.9% of MMP1-d2EGFP transfected cells (V384A2 candidate) in this gate. The geometric mean of FITC (EGFP) was extracted. To obtain fold changes, each individual triplicate repeat was normalised for the geometric mean of the 0 HGF sample. Error bars: 1 s.d.

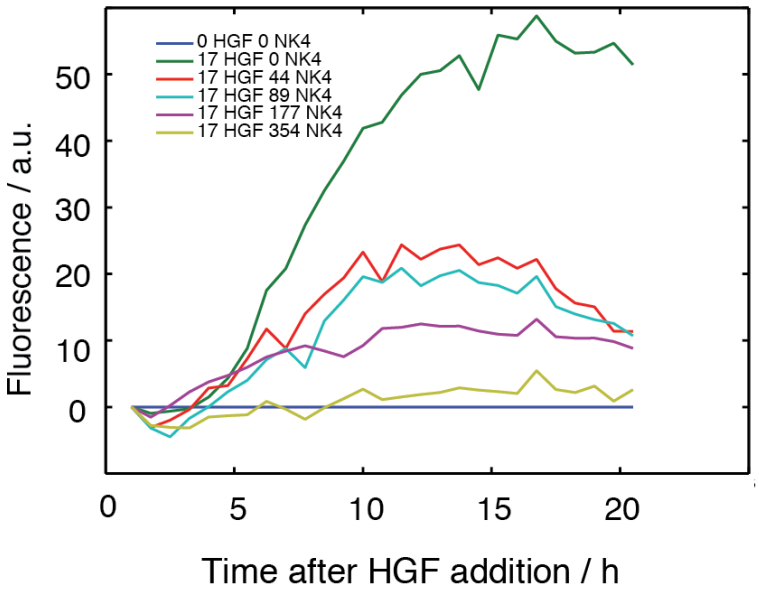


**Figure S3. Time course of repression of HGF by NK4.** Different concentrations of HGF and NK4 were added at time zero, as indicated. Fluorescence was followed by quatitating microscopy images as described in Methods.

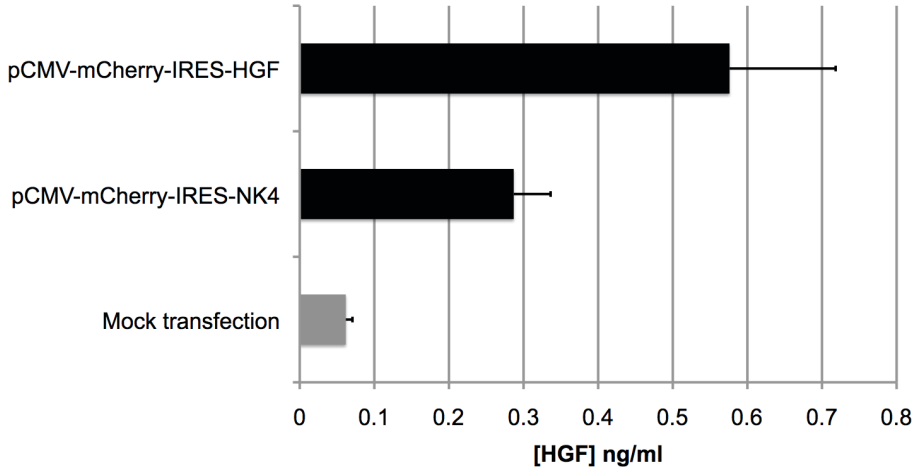


**Figure S4. Secretion of HGF and NK4 from MDCK cells grown in monolayer.** MDCK cells were transfected with plasmids encoding HGF or NK4 secretion constructs. The secreted protein in the medium was measured by ELISA, 24 hrs after transfection, with a Quantikine Human HGF Immunoassay Kit (R&D Systems DHG00). Expression is up to ~0.5 ng/ml (~0.01 nM).

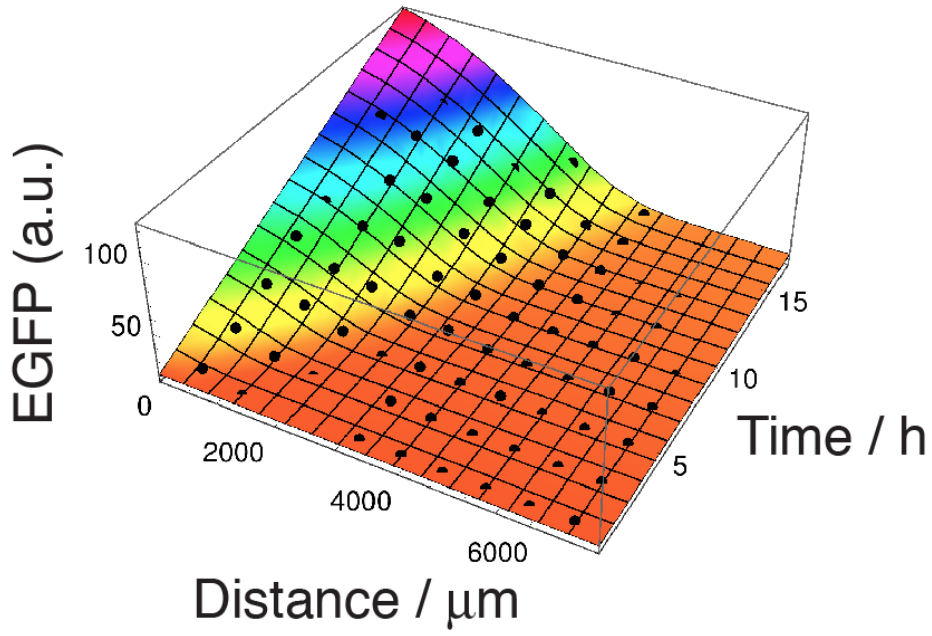


**Figure S5. Quantification of the gradient of HGF-induced GFP.** Black dots are from time-lapse experimental data as in Fig. 6a. The color axis surface profile corresponds to the diffusion profile predicted by Eq. (7), with a diffusion coefficient $D_a = 3.1 \times 10^{-3}$ mm$^2$/min. No delay in response has been considered.

**2. Legends for supporting Quicktime movie files.**

**Supplementary Movie S1.** This movie shows a cyst of the MDCK-MMP-1-d2-EGFP stable cell line, responding to 10 ng/ml HGF over a period of 16 hours, by inducing green fluorescence and tubulating.

**Supplementary Movie S2.** This movie shows a cyst of the MDCK-MMP-1-d2-EGFP stable cell line, locally transfected with pCMV-mCherry-IRES-HGF (red region). Over a period of 16 hours, the neighbouring cells respond to secreted HGF by inducing green fluorescence and tubulating.

**Supplementary Movie S3.** This movie shows another cyst of the MDCK-MMP-1-d2-EGFP stable cell line, locally transfected with pCMV-mCherry-IRES-HGF (red region). Over a period of 16 hours, the neighbouring cells respond to secreted HGF by inducing green fluorescence and tubulating.

### 3. pMMP-1-d2EGFP

The pMMP1-d2EGFP plasmid with neomycin resistance, used for the establishment of the MDCK-MMP1-d2EGFP stable cell line, was constructed by PCR and restriction enzyme cloning to give the following sequence, which was verified by Sanger sequencing.

| Key | Position (bp) |
|---|---|
| NheI restriction site (GCTAGC) | 1 – 6 |
| Human MMP-1 promoter (bp -512 to +63) | 7 – 581 |
| BspEI restriction site (TCCGGA) | 582 – 586 |
| d2EGFP CDS (HindIII-d2 PEST sequence) | 587 – 1432 |
| FseI restriction site (GGCCGGCC) | 1447 – 1454 |
| Poly A signal | 1599 – 1649 |
| Kanamycin/Neomycin resistance CDS | 2676 – 3470 |

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### 4. pCMV-HGF-IRES-DsRed

The CMV-HGF-IRES-DsRed plasmid was constructed by standard cloning methods, was verified by DNA sequencing, and contains the following features.

| Key | Position (bp) |
|---|---|
| CMV promoter | 1 – 589 |
| SacI restriction site | 616 – 621 |
| HGF CDS with Kozak sequence | 621 – 2814 |
| XmaI restriction site | 2825-2820 |
| BamHI restriction site | 2819-2824 |
| IRES (internal ribosome entry site) | 2825-3409 |
| BspEI restriction site | 3140-3415 |
| DsRed CDS with Kozak sequence | 3415-4096 |
| NotI restriction site | 4097-4104 |
| Poly A signal | 4249-4299 |

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ATAGCATCAC AAATTTCACA AATAAAGCAT TTTTTTCACT GCATTCTAGT TGTGGTTTGT

NB: the 3 gaps in the middle of the HGF CDS above are where a stop codon can be inserted to convert this into NK4 (481 amino acids).

**5. pCMV-NK4-IRES-DsRed**

The CMV-NK4-IRES-DsRed plasmid was constructed by standard cloning methods, was verified by DNA sequencing, and contains the following features (see Key below). N.B. the fibronectin collagen binding domain coding sequence (CBD; Kitajima T., et al. *Biomaterials* **28:** 1989-1997, 2007) was cloned downstream of NK4. NK4 had similar repressor activity and diffusion profiles in the presence or absence of the CBD fusion (data not shown).

| Key | Position (bp) |
|---|---|
| CMV promoter | 1 – 589 |
| SacI restriction site | 616 – 621 |
| NK4 CDS with Kozak sequence (479 a.a.) | 622 – 2061 |
| FseI restriction site (GGCCGGCC) | 2062 – 2069 |
| CBD | 2070 – 2754 |
| AscI | 2755 – 2762 |
| IRES | 2763 – 3347 |
| BspEI restriction site | 3348 – 3353 |
| DsRed CDS with Kozak sequence | 3354 – 4036 |
| NotI restriction site | 4035 - 4042 |
| Poly A signal | 4187 – 4237 |

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 301 CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA TCGCTATTAC
 361 CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG ACTCACGGGG
 421 ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG TTTTGGCACC AAAATCAACG
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2581 GGCTTTTGCC CCATGGCCGC TCACGAGGAA ATCTGTACCA CAAACGAGGG CGTGATGTAC
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2701 GTGGGAAATG GACGCGGAGA ATGGACATGT ATCGCTTACA GCCAGCTGCG CTAGGGCGCG
2761 CCGCCCCTCT CCCTCCCCCC CCCCTAACGT TACTGGCCGA AGCCGCTTGG AATAAGGCCG
2821 GTGTGCGTTT GTCTATATGT TATTTTCCAC CATATTGCCG TCTTTTGGCA ATGTGAGGGC
2881 CCGGAAACCT GGCCCTGTCT TCTTGACGAG CATTCCTAGG GGTCTTTCCC CTCTCGCCAA
2941 AGGAATGCAA GGTCTGTTGA ATGTCGTGAA GGAAGCAGTT CCTCTGGAAG CTTCTTGAAG
3001 ACAAACAACG TCTGTAGCGA CCCTTTGCAG GCAGCGGAAC CCCCCACCTG GCGACAGGTG
3061 CCTCTGCGGC CAAAAGCCAC GTGTATAAGA TACACCTGCA AAGGCGGCAC AACCCCAGTG
3121 CCACGTTGTG AGTTGGATAG TTGTGGAAAG AGTCAAATGG CTCTCCTCAA GCGTATTCAA
3181 CAAGGGGCTG AAGGATGCCC AGAAGGTACC CCATTGTATG GGATCTGATC TGGGGCCTCG
3241 GTGCACATGC TTTACATGTG TTTAGTCGAG GTTAAAAAAA CGTCTAGGCC CCCCGAACCA
3301 CGGGGACGTG GTTTTCCTTT GAAAAACACG ATGATAATAT GGCCACAtCC GGAACCATGG
3361 CCTCCTCCGA GGACGTCATC AAGGAGTTCA TGCGCTTCAA GGTGCGCATG GAGGGCTCCG
3421 TGAACGGCCA CGAGTTCGAG ATCGAGGGCG AGGGCGAGGG CCGCCCCTAC GAGGGCACCC
3481 AGACCGCCAA GCTGAAGGTG ACCAAGGGCG GCCCCCTGCC CTTCGCCTGG GACATCCTGT
3541 CCCCCCAGTT CCAGTACGGC TCCAAGGTGT ACGTGAAGCA CCCCGCCGAC ATCCCCGACT
3601 ACAAGAAGCT GTCCTTCCCC GAGGGCTTCA AGTGGGAGCG CGTGATGAAC TTCGAGGACG
3661 GCGGCGTGGT GACCGTGACC CAGGACTCCT CCCTGCAGGA CGGCTCCTTC ATCTACAAGG
3721 TGAAGTTCAT CGGCGTGAAC TTCCCCTCCG ACGGCCCCGT AATGCAGAAG AAGACTATGG
3781 GCTGGGAGGC CTCCACCGAG CGCCTGTACC CCCGCGACGG CGTGCTGAAG GGCGAGATCC
3841 ACAAGGCCCT GAAGCTGAAG GACGGCGGCC ACTACCTGGT GGAGTTCAAG TCCATCTACA
3901 TGGCCAAGAA GCCCGTGCAG CTGCCCGGCT ACTACTACGT GGACTCCAAG CTGGACATCA
3961 CCTCCCACAA CGAGGACTAC ACCATCGTGG AGCAGTACGA GCGCGCCGAG GGCCGCCACC
4021 ACCTGTTCCT GTAGgcggcc GCGACTCTAG ATCATAATCA GCCATACCAC ATTTGTAGAG
4081 GTTTTACTTG CTTTAAAAAA CCTCCCACAC CTCCCCCTGA ACCTGAAACA TAAAATGAAT
4141 GCAATTGTTG TTGTTAACTT GTTTATTGCA GCTTATAATG GTTACAAATA AAGCAATAGC
4201 ATCACAAATT TCACAAATAA AGCATTTTTT TCACTGCATT CTAGTTGTGG TTTGTCCAAA

## 6. Calculation of effective diffusion constants

HGF activation and NK4 repression of reporter cysts can be modeled by a set of reaction-diffusion equations:

$$\frac{\partial a(x,t)}{\partial t} = D_a \frac{\partial^2 a(x,t)}{\partial x^2} + J_a(x,t), \quad (1)$$

$$\frac{\partial h(x,t)}{\partial t} = D_h \frac{\partial^2 h(x,t)}{\partial x^2} + J_h(x,t), \quad (2)$$

$$\frac{\partial r(x,t)}{\partial t} = f(a(x,t-\tau_a), h(x,t-\tau_h))), \quad (3)$$

where *a(x, t)*, *h(x, t)* and *r(x, t)* denote the activator, inhibitor and reporter concentrations at time *t* and position *x*, respectively. $J_a(x, t)$ and $J_h(x, t)$ are the source functions that describe both the rate and spatial distribution of activator and inhibitor production, respectively. All physical and chemical processes from the signal recognition, at the cell membrane, up to the reporter protein synthesis can be modeled by an effective regulatory function *f* with delays $\tau s$. Thus, *f* describes the cyst responses at time *t* to a given signaling in the past (the activator at time $t - \tau_a$ and inhibitor concentrations at time $t - \tau_h$).

In order to estimate the parameters of the processes governing this system we will estimate the diffusion constant of HGF molecules from the distribution profile of fluorescence data in **Figure 6a**. The gradient of HGF-induced reporter protein *r* obtained in Fig. 6a can be modeled by the equation in 1-dimension:

$$\frac{\partial a}{\partial t} = D_a \frac{\partial^2 a}{\partial x^2} + J_a(x,t), \quad (4)$$

$$\frac{\partial r}{\partial t} = f(a(x,t-\tau_a),0)), \quad (5)$$

As the domain boundaries are far from the source, we can consider the following idealized condition: an unbounded domain where there exists a point source located at $x = 0$ which releases molecules at a constant rate *J*. If the concentration is initially zero everywhere (with the exception of the source position), and the source is switched-on at $t = 0$, then the evolution of the resulting concentration distribution is given by:

$$a(x,t)/J = \frac{x}{4D_a\sqrt{(\pi)}} \int_0^{4D_a t/x^2} \frac{e^{-1/u}}{\sqrt{u}} du. \quad (6)$$

This activator profile and a sigmoidal regulatory function $f(a)$ allow us, by integrating Eq. 5, to determine the theoretical reporter profille $r(x,t)$, which is proportional to:

$$\int_0^t b + \frac{a(x,u)^n}{K_a^n + a(x,u)^n} du. \quad (7)$$

Even when there is not an analytic expression for the theoretical reporter profile, it is possible to find the parameter values of *Da*, *Ka*, *n* and *b* that fit our experimentally observed GFP profile. A numerical fitting procedure determines the following values: $D_a = 3.1 \times 10^{-3}$ mm$^2$/min, $Ka = 0.017$, $n = 2.6$ and $b = 0.02$. **Figure S5** depicts the experimental and theoretical predicted diffusion profile of HGF-induced GFP. Similar analyses are carried out for the other gradient calculations in **Figure 6**.

## 7. Regulatory function calculation - HGF and NK4

### HGF

The HGF dose-response curve *f* can be obtained by considering the expression profile for different amounts of inducer HGF (Main text, Figure 3a). Each individual profile was normalized to the profile at 0 HGF and the maximum value of the relative fluorescence was taken (by fitting a 4th order polynomial; this corresponds to GFP output at approximately 24 h). We then calculated the average of these maximum values over four biological replicates and fitted a sigmoidal function (the black curve in Figure 3b):

$$Va^n/(K_d^n + a^n) + b$$

The variable $a$ corresponds to HGF concentration (ng/ml), the parameter $n$ is the Hill coefficient, $k_d$ is the apparent dissociation constant for activator (equivalent to the effective concentration for 50% activation: $EC_{50}$), $V$ is the difference between the saturation or maximum relative response, $R_{max}$, and the baseline relative response, $b$, in the absence of HGF. Therefore, the maximum GFP fold-induction $R_{max}$ is given by:

$$R_{max} = V + b$$

The fitting procedure leads to the following values: $k_d$ = 17 ng/ml, $n$ = 1.22, $V$ = 3.33 fold, $b$ = 0.75 fold and $R_{max}$ = 4.08 fold.

### NK4

We also quantified the response to the inhibitor NK4 (Main text, Figure 3d). Again, we took the maximum value of the relative fluorescence for each NK4 concentration, and then averaged over the two replicates obtained with 16.7 ng/ml of HGF. Then we fitted the function (the black curve in Figure 3d):

$$\frac{1 + (a/k_a)^n}{(1 + (a/k_a)^n + (h/k_h)^n)}$$

The variable $h$ represents NK4 concentration and $k_h$ is the apparent dissociation constant for NK4 (equivalent to the effective NK4 concentration for 50% inhibition: $IC_{50}$). Using the values of $k_a$ and $n$ obtained above, and $a$ = 16.7 ng/ml, we obtain $k_h$ = 37.6 ng/ml.