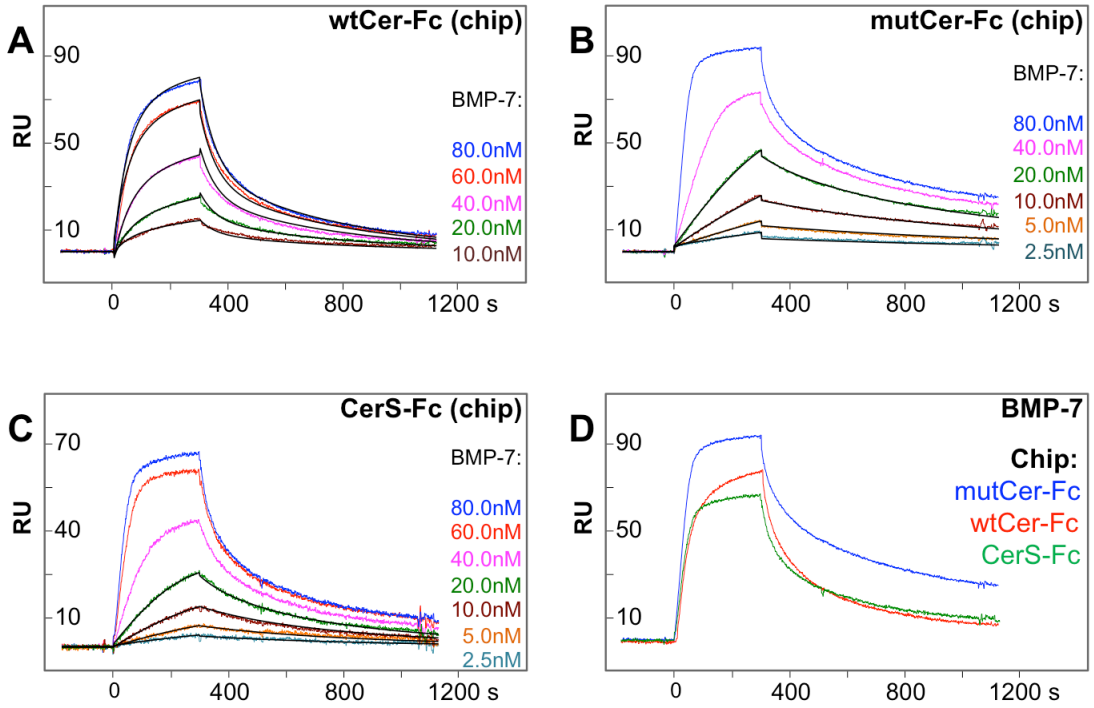
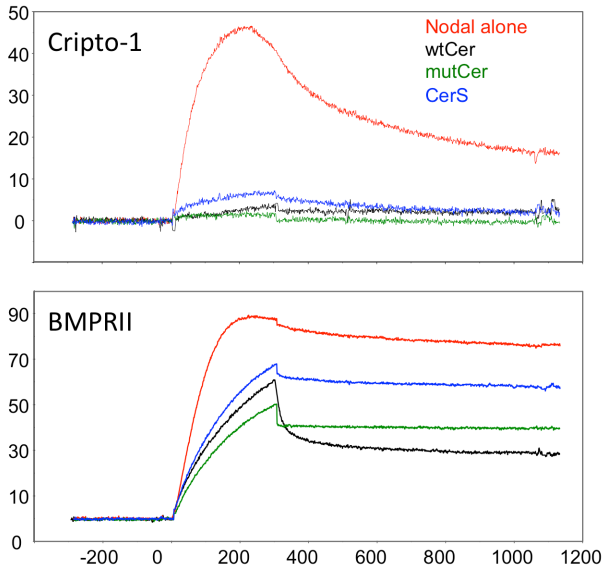
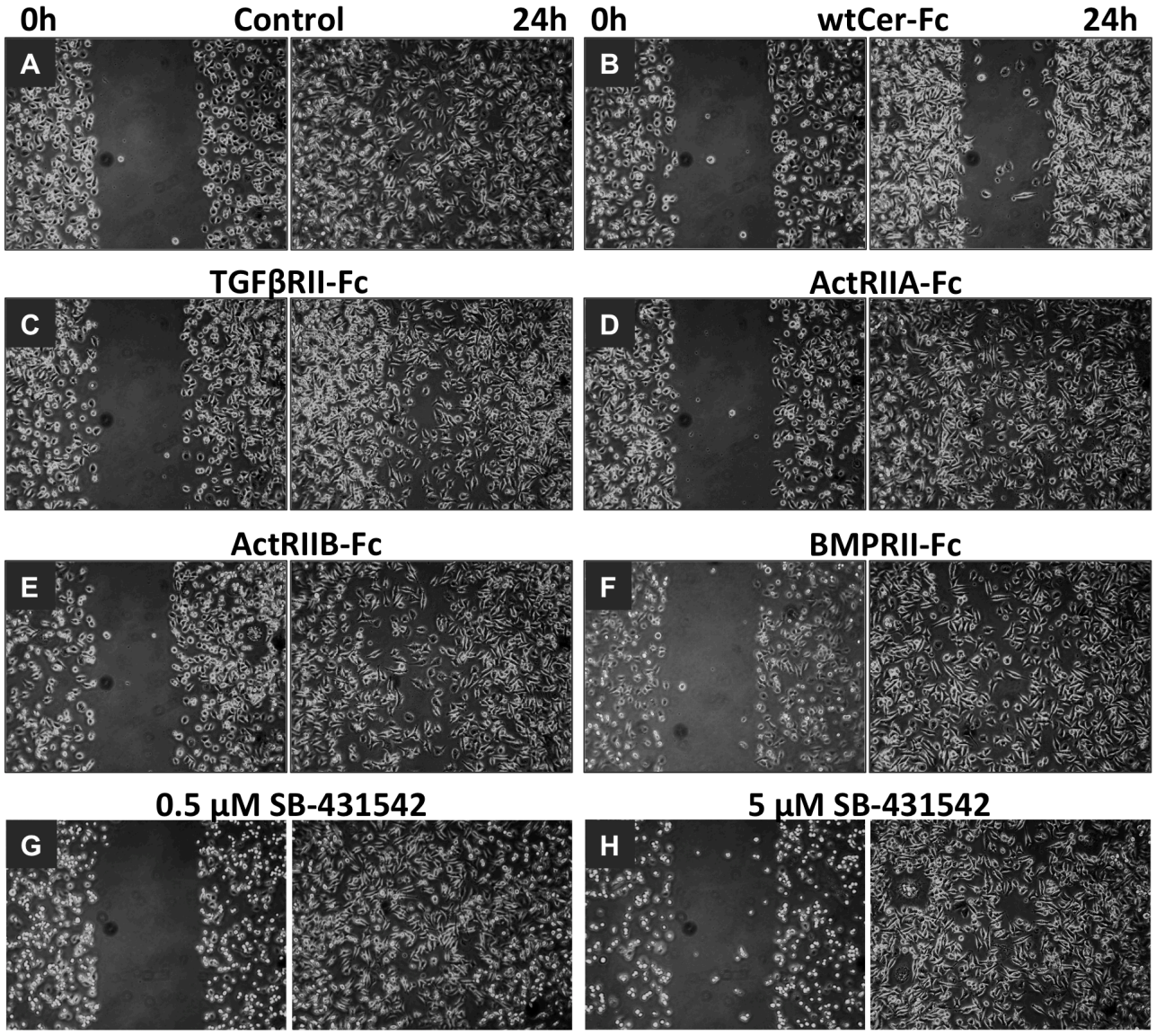


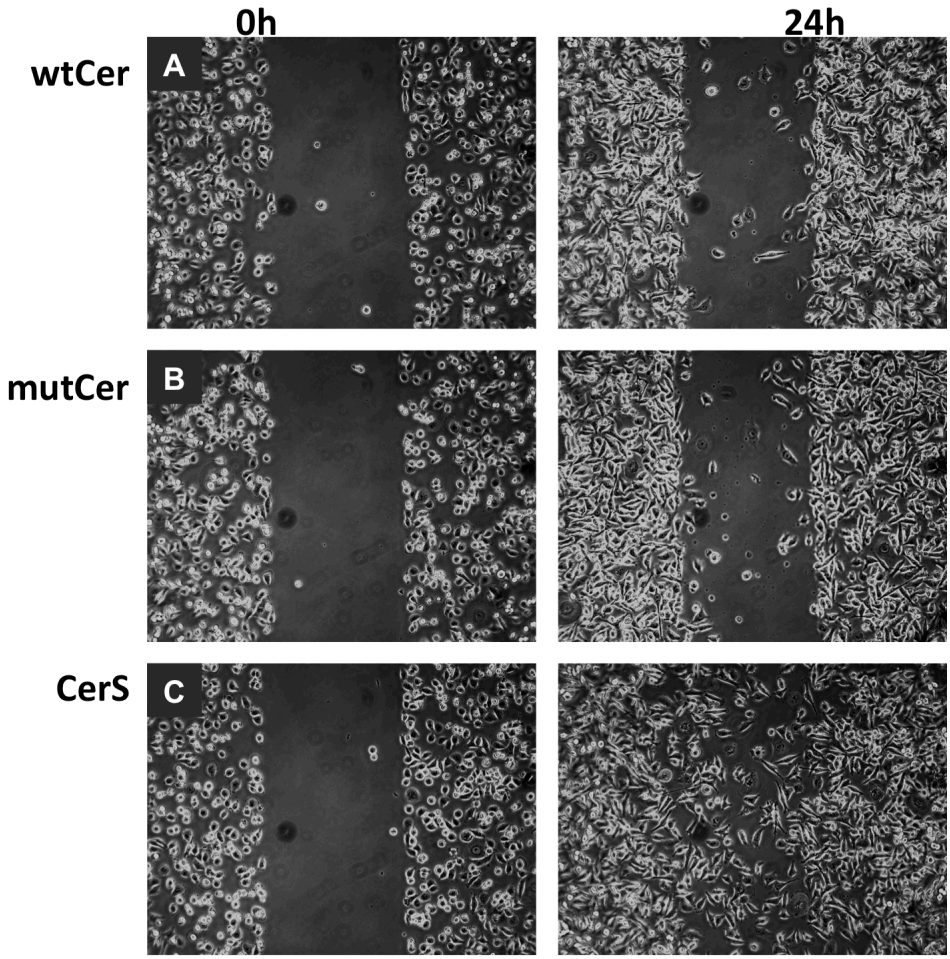
Supplemental Figure S1: Cerberus ligand binding affinities. (A) BMP-7-wtCer-Fc binding. wtCer-Fc was immobilized on the SPR sensor chip and different concentrations of BMP-7 were injected as shown. ***(B)*** BMP-7-mutCer-Fc binding. mutCer-Fc was immobilized and BMP-7 was injected as shown. ***(C)*** BMP-7-CerS-Fc binding. CerS-Fc was immobilized and BMP-7 was injected. Overall, the binding rate constant for BMP-7 is faster than BMP-6, but slower than Activin B. The dissociation rate constant is faster. Data fitting was more problematic for BMP-7, likely due to fast association and fast dissociation rates. Therefore only lower concentrations of BMP-7 were used in evaluation. Fitted curves are superimposed and shown as black lines. ***(D)*** Comparison of BMP-7 binding to wtCer-Fc, mutCer-Fc, CerS-Fc. Equal amounts of wtCer-Fc, mutCer-Fc and CerS-Fc were immobilized on the SPR sensor chip, and 80 nM BMP-7 were injected. wtCer-Fc (red curves), mutCer-Fc (blue curves) and CerS-Fc (green curves) show similar binding profiles, the binding rate of mutCer-Fc is faster.



Supplemental Figure S2: Cerberus inhibition of Nodal. A) Cerberus inhibition of Nodal-Cripto-1 binding. ***(B)*** Cerberus inhibition of Nodal-BMPRII binding. BMPRII-Fc, or Cripto-1-Fc were immobilized on a sensor chip and 80 nM Nodal pre-incubated with 4 μM Fc free Cerberus (Nodal alone, red; Nodal + wtCer, black; Nodal + mutCer, green; Nodal + CerS, blue) was injected.



Supplemental Figure S3: Inhibition of MDA-MB-231 breast cancer cell migration. MDA-MB-231 breast cancer cells were plated in Ibidi insert dishes and grown to 80% confluence. Inserts were removed to create a gap and medium was exchanged with complete medium containing 2.5 µg/ml Mitomycin C and ***(A)*** 0 mg/ml (control) or 10 mg/ml (approximately 15 nM) ***(B)*** wtCer-Fc, ***(C)*** TGFβRII-Fc, ***(D)*** ActRIIA-Fc, ***(E)*** ActRIIB-Fc, ***(F)*** BMPRII-Fc, ***(G)*** **0.**5 µM SB-431542, and ***(H)*** 5 µM SB-431542. Images were taken after insert removal (0 h) and after 24 h incubation with inhibitors (24 h).



Supplemental Figure S4: Inhibition of MDA-MB-231 breast cancer cell migration with Fc free Cerberus. MDA-MB-231 breast cancer cells were plated in Ibidi insert dishes and grown to 80% confluence. Inserts were removed to create a gap and medium was exchanged with complete medium containing 2.5 μg/ml Mitomycin C and ***(A)*** wtCer, ***(B)*** mutCer, ***(C)*** CerS. Images were taken after insert removal (0 h) and after 24 h incubation with Fc-free Cerberus constructs (24 h).

Rate equations used in biaevaluation for fitting of mass transport limited models

(1) $A + B \leftrightarrow AB$ (1:1 reaction model)

(2) $A_{SOL} \underset{k_t}{\overset{k_t}{\leftrightarrow}} A_{SUR} + B \underset{k_d}{\overset{k_a}{\leftrightarrow}} AB$ (1:1 reaction model with mass transport)

(3) $dA/dt = k_t*(A_{SOL}-A_{SUR}) - (k_a* A_{SUR} *B - k_d* AB)$
$A[t_0] = 0$ (M)

$dB/dt = - (k_a* A_{SUR}*B - k_d*AB)$
$B[t_0]$ = Rmax (Maximum analyte binding capacity)

$dAB/dt = (k_a* A_{SUR}*B - k_d*AB)$
$AB[t_0] = 0$ (M)

A_{SOL} = Bulk concentration analyte (A) in solution (M)
A_{SUR} = Concentration of analyte (A) near the surface (M)
k_a = Association rate constant ($M^{-1}s^{-1}$)
k_d = Dissociation rate constatnt (s^{-1})
k_t = Rate constant for mass transfer (RU $M^{-1}s^{-1}$)
k_m = Mass transport coefficient (s^{-1})
RI = Bulk refractive index contribution (RU)

(4) $k_t = k_m*MW*10^{-9}$

A 1:1 Langmuir interaction model (eq. 1) with a simplified expression for mass transfer limitation (eqs. 2, 3) as implemented in biaevaluation software was used. This model introduces an expression for dA/dt, the rate of change in concentration of analyte A, where A_{SUR} represents the concentration of analyte near the surface that can bind ligand (Cerberus) and A_{SOL} represents the bulk concentration of analyte in solution (Activin B, BMP-7, BMP-6 or Nodal). The model produces a mass transfer rate constant k_t, which is related to the mass transport coefficient k_m (eq. 4).