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Supporting Information for

Heparin is essential for optimal cell signaling by FGF21 and for regulation of βKlotho cellular stability

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Figures S1 to S7

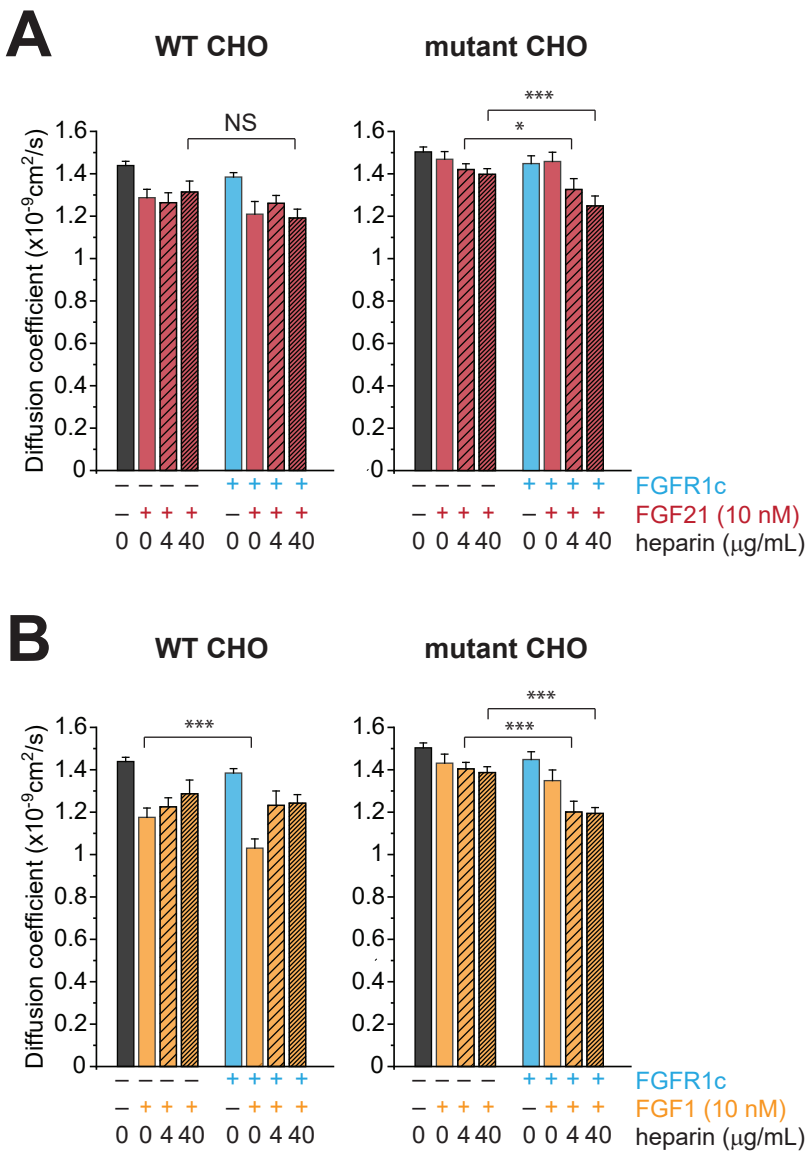

Figure S1. Diffusion coefficient measurements of HaloTag-βKlotho stimulated by FGF21 or FGF1 in WT or HSPG-deficient CHO cells.

(**A**) Diffusion coefficients of HaloTag-βKlotho particles in WT and mutant (HSPG deficient) CHO cells with or without exogenous FGFR1c under the following FGF21 conditions: no ligands (40 cells each for WT and mutant cells), FGF21 only (9 and 14 cells, respectively), FGF21 and 4 μg/mL heparin (6 and 24 cells, respectively), FGF21 and 40 μg/mL heparin (5 and 26 cells, respectively), FGFR1c only (34 and 41 cells, respectively), FGFR1c and FGF21 (7 and 26 cells, respectively), FGFR1c, FGF21 and 4 μg/mL heparin (8 and 22 cells, respectively) and FGFR1c, FGF21 and 40 μg/mL heparin (7 and 23 cells, respectively). (**B**) Diffusion coefficients of HaloTag-βKlotho particles in WT and HSPG-deficient CHO cells under the following FGF1 conditions: no ligands (40 cells each for WT and mutant cells), FGF1 only (8 and 24 cells, respectively), FGF1 and 4 μg/mL heparin (6 and 23 cells, respectively), FGF1 and 40 μg/mL heparin (8 and 24 cells, respectively), FGFR1c only (34 and 41 cells, respectively), FGFR1c and FGF21 (17 and 13 cells, respectively), FGFR1c, FGF21 and 4 μg/mL heparin (7 and 19 cells, respectively) and FGFR1c, FGF21 and 40 μg/mL heparin (8 and 24 cells, respectively). Error bars indicate mean ± SE, *P < 0.05, ***P < 0.001 by Student's t test. Note that diffusion coefficients with exogenous FGFR1c are redisplayed from Fig. 1 and 3 for comparison.

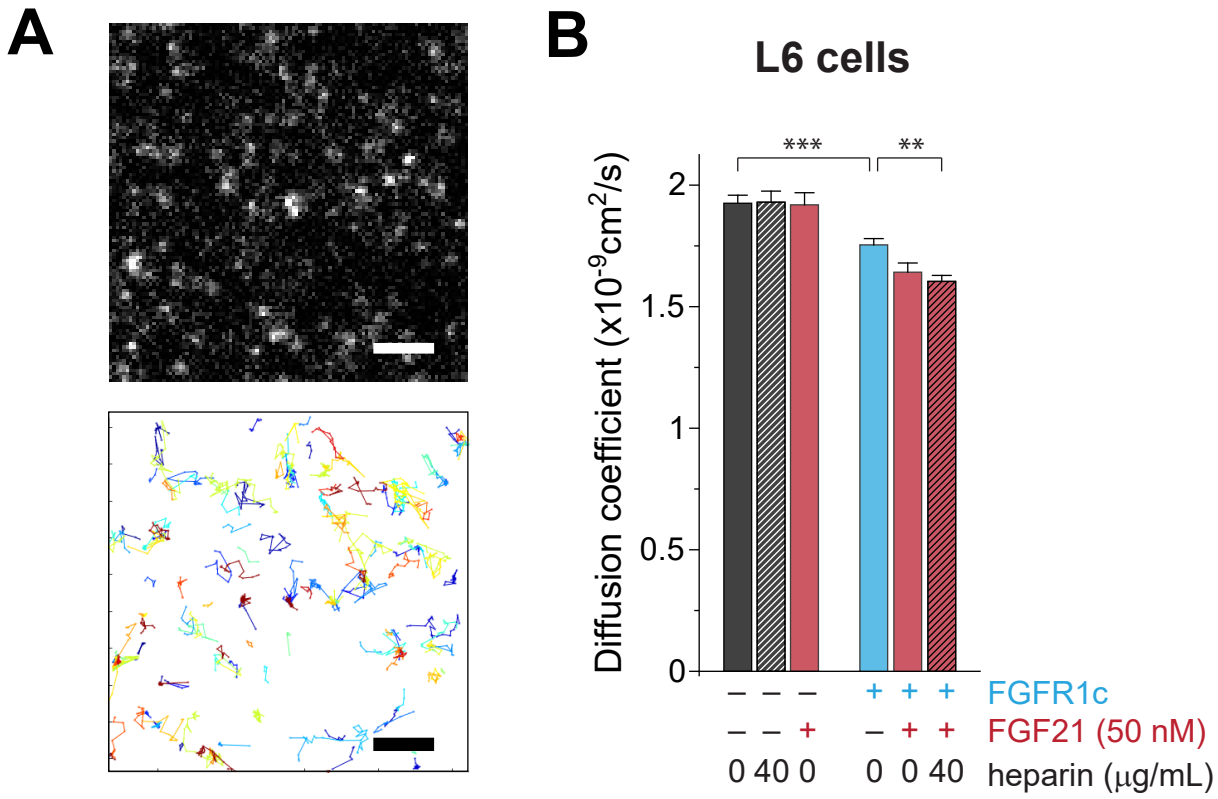

Figure S2. Diffusion coefficient measurements of HaloTag-βKlotho expressed in L6 cells.

(A) Expanded view of HaloTag-βKlotho particles on the surface of an L6 cell imaged by TIRFM (upper panel). A selected frame (100-ms exposure) from a 10-Hz recording is shown. Scale bar, 2 μm. Automated detection and tracking of moving HaloTag-βKlotho particles during a 10-s recording period (lower panel). Single-particle tracking was performed as described in Materials and Methods. (B) Diffusion coefficient of HaloTag-βKlotho particles in L6 cells under the following conditions: no ligands (33 cells), 40 μg/mL heparin only (10 cells), FGF21only (10 cells), FGFR1c only (42 cells), FGFR1c and FGF21 (12 cells) and FGFR1c, FGF21 and 40 μg/mL heparin (15 cells). Error bars indicate mean ± SE, **P < 0.01 and ***P < 0.001 by Student's t test.

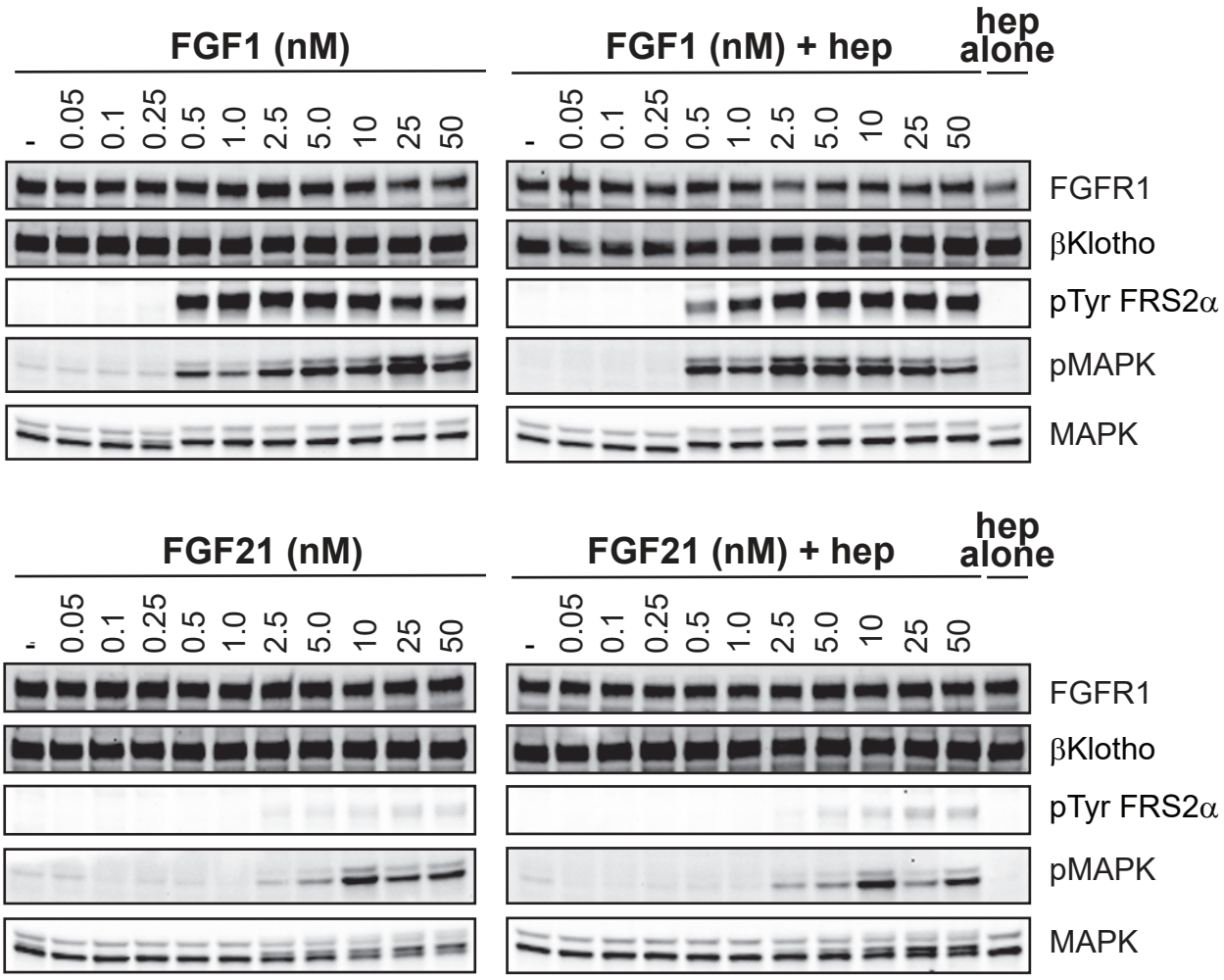

Figure S3. Cell signaling in WT CHO cells stably expressing FGFR1c together with βKlotho induced by different stimuli.

Wild-type CHO cells stably expressing FGFR1c together with βKlotho were left unstimulated or stimulated with either FGF1, FGF1 together with heparin (5 μg/mL), FGF21, or FGF21 together with heparin (5 μg/mL) in increasing concentrations (as indicated) for 10 minutes at 37°C. Cells treated with heparin alone were used as controls. Cell lysates were subjected to SDS/PAGE and analyzed for tyrosine phosphorylation of FRS2α and MAPK activation by immunoblotting with anti-pFRS2α and anti-pMAPK antibodies respectively. Anti-FGFR1c, anti-HA and anti-MAPK antibodies were used as loading controls.

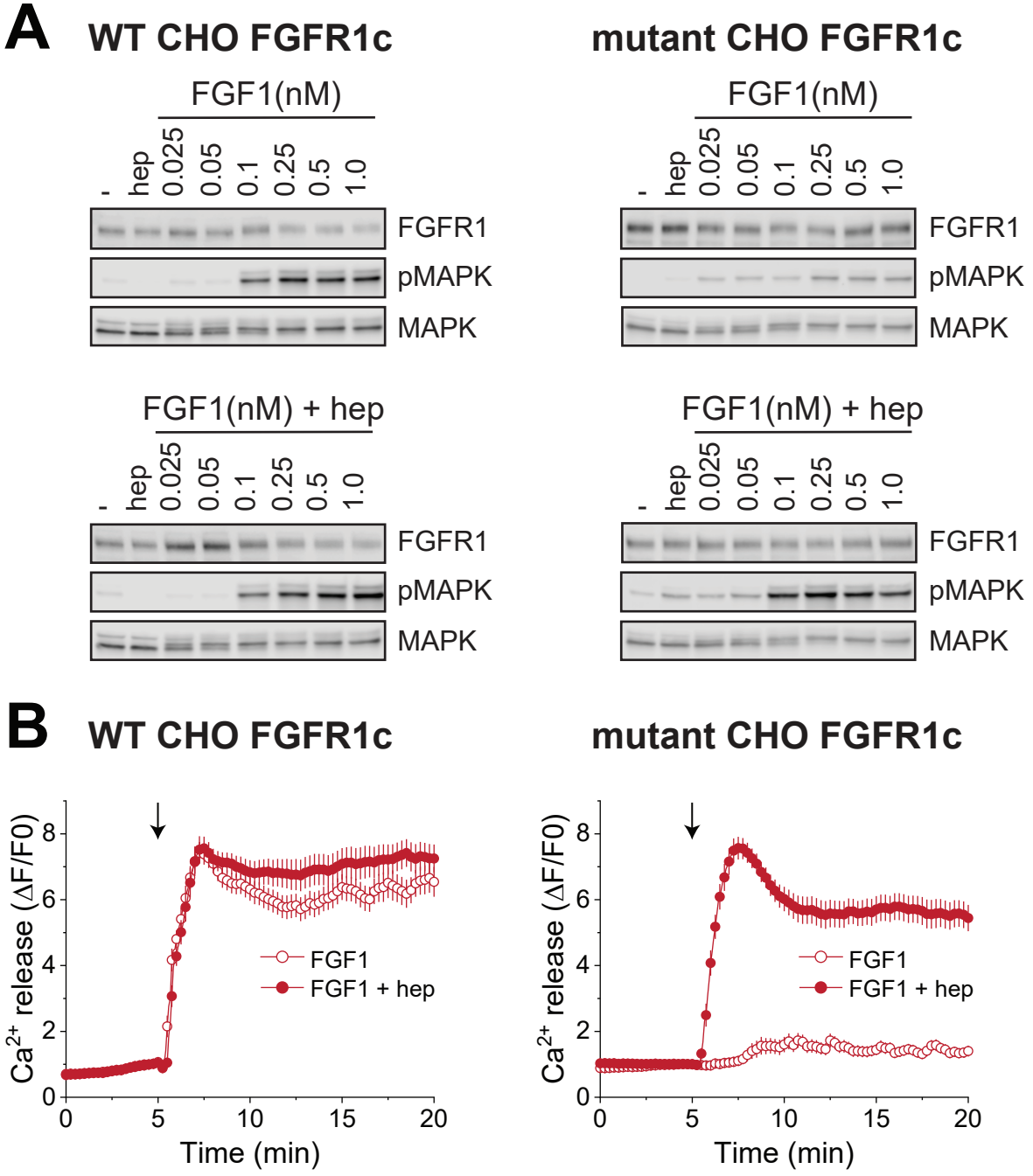

Figure S4. Cell signaling in WT or HSPG-deficient CHO cells stably expressing FGFR1c induced by different stimuli.

(A) WT or HSPG deficient CHO cells stably expressing FGFR1c were left unstimulated or stimulated with increasing concentrations of FGF1 (as indicated) in the absence or presence of heparin (5 µg/mL) for 10 min and 37°C. Cells treated with heparin alone (5 µg/mL) were used as controls. Cell lysates were subjected to SDS-PAGE and analyzed for MAPK activation by immunoblotting with anti-pMAPK. Anti-FGFR and anti-MAPK antibodies were used as controls. **(B)** WT or HSPG-deficient CHO cells stably expressing FGFR1c were loaded with the fluorescent Ca^{2+} indicator Calbryte 520 AM and stimulated with either FGF1 (0.1 nM) alone or FGF1 (0.1 nM) together with heparin (4 µg/mL). The change in fluorescence (ΔF) was normalized to initial fluorescence (F0) and plotted as a function of time. Arrow indicates when ligands were added. Error bars indicate mean ± SE (n = 30 cells for each condition).

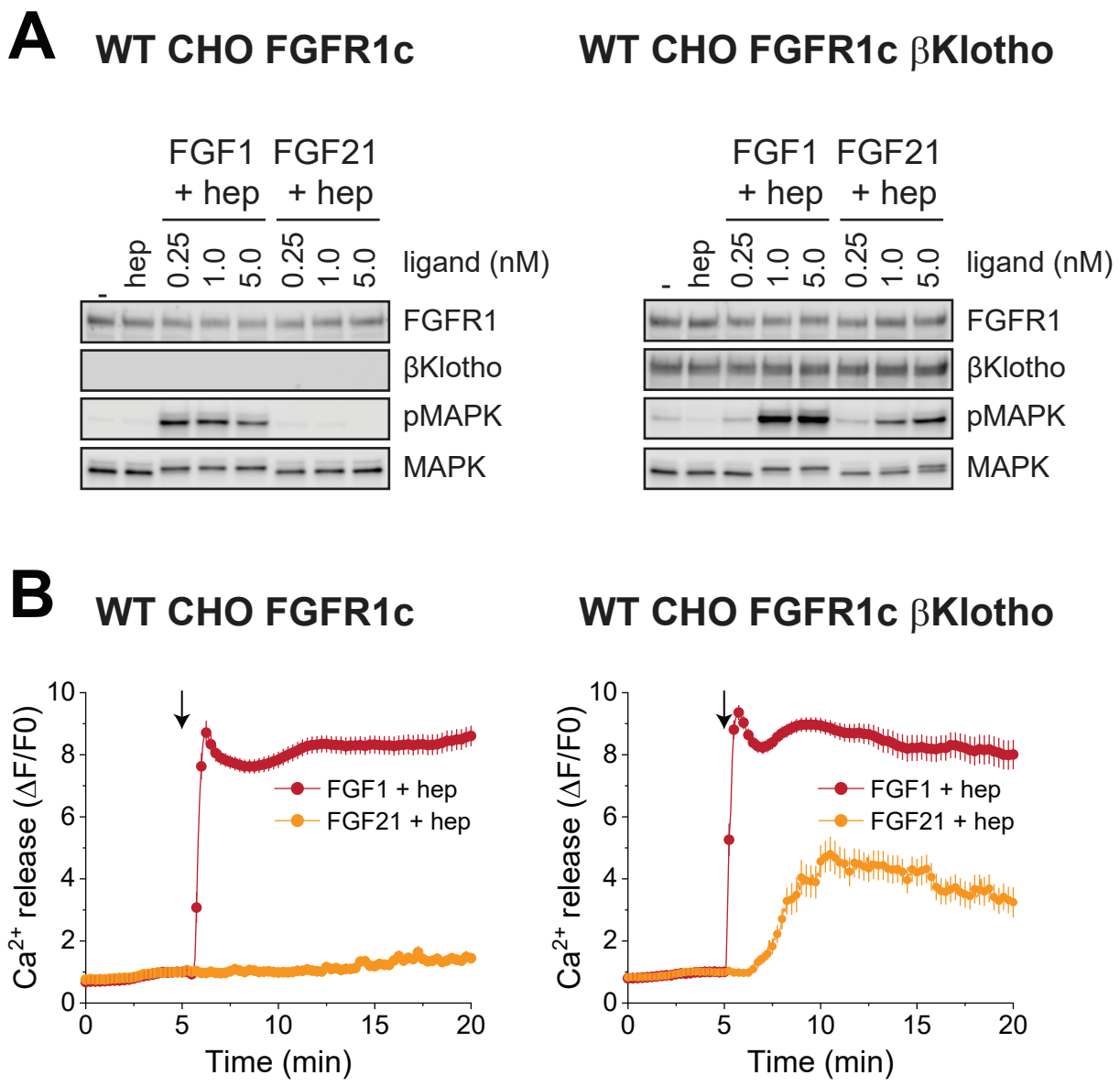

Figure S5. Cell signaling in WT CHO cells stably expressing FGFR1c alone or FGFR1c with βKlotho induced by different stimuli.

(A) WT CHO cells stably expressing FGFR1c alone or FGFR1c together with βKlotho were left unstimulated or stimulated with increasing concentrations of FGF1 or FGF21 (as indicated) in the presence of heparin (5 μg/mL) for 10 min and 37˚C. Cells treated with heparin alone (5 μg/mL) were used as controls. Cell lysates were subjected to SDS-PAGE and analyzed for MAPK activation by immunoblotting with anti-pMAPK. Anti-FGFR and anti-MAPK antibodies were used as controls. **(B)** WT CHO cells stably expressing FGFR1c alone or FGFR1c together with βKlotho were loaded with the fluorescent Ca^{2+} indicator Calbryte 520 AM and stimulated with either FGF1 (5 nM) or FGF21 (5 nM) in the presence of heparin (4 μg/mL). The change in fluorescence (ΔF) was normalized to initial fluorescence (F0) and plotted as a function of time. Arrow indicates when ligands were added. Error bars indicate mean ± SE (n = 30 cells for each condition).

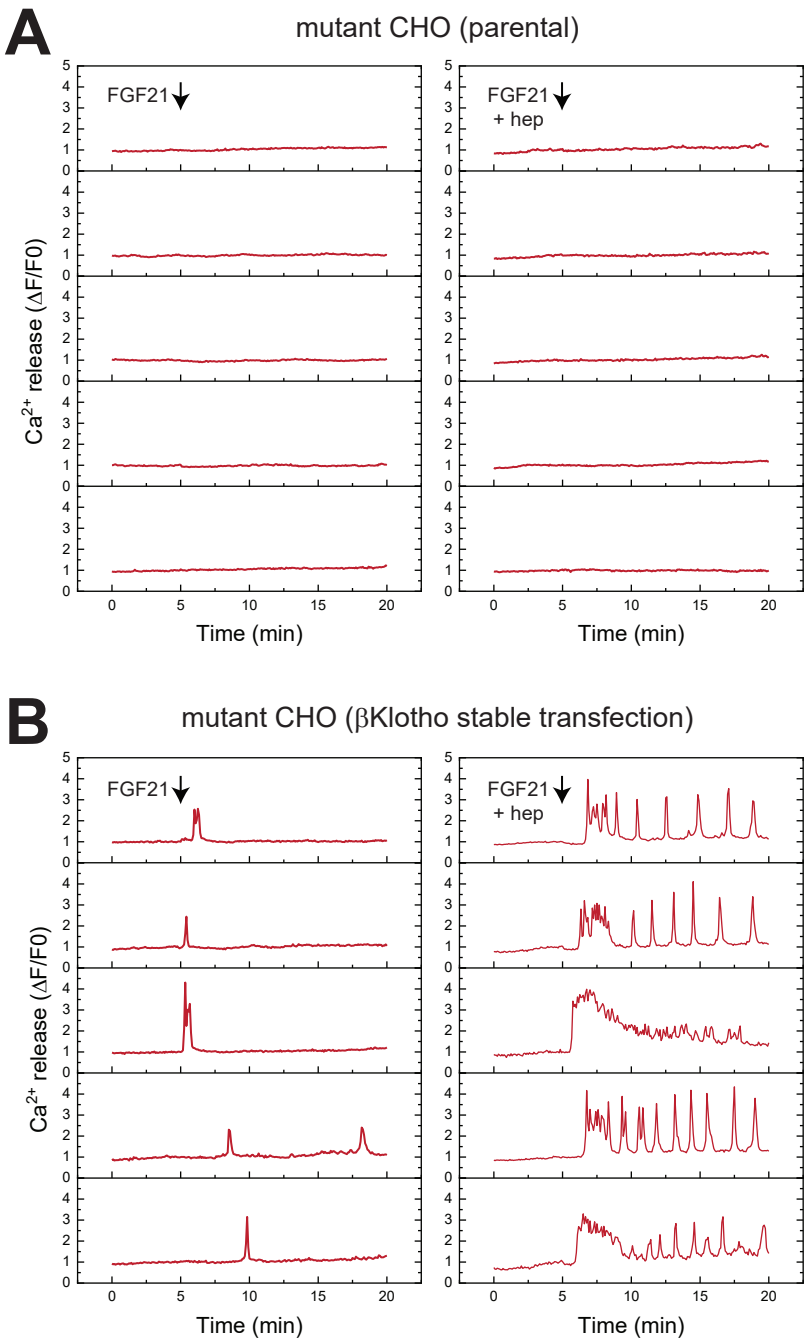

Figure S6. Calcium release measurements of HSPG-deficient CHO cells in response to different stimuli.

(A and **B)** Parental HSPG-deficient CHO cells (**A**) and HSPG-deficient CHO cells stably expressing βKlotho (**B**) were loaded with the fluorescent Ca^{2+} indicator Calbryte 520 AM and stimulated with either FGF21 (50 nM) or FGF21 together with heparin (40 μg/mL). The change in fluorescence (ΔF) was normalized to initial fluorescence (F0) and plotted as a function of time. Individual cell recordings are shown to highlight transient or oscillatory nature of Ca^{2+} release. Arrow indicates when ligands were added.

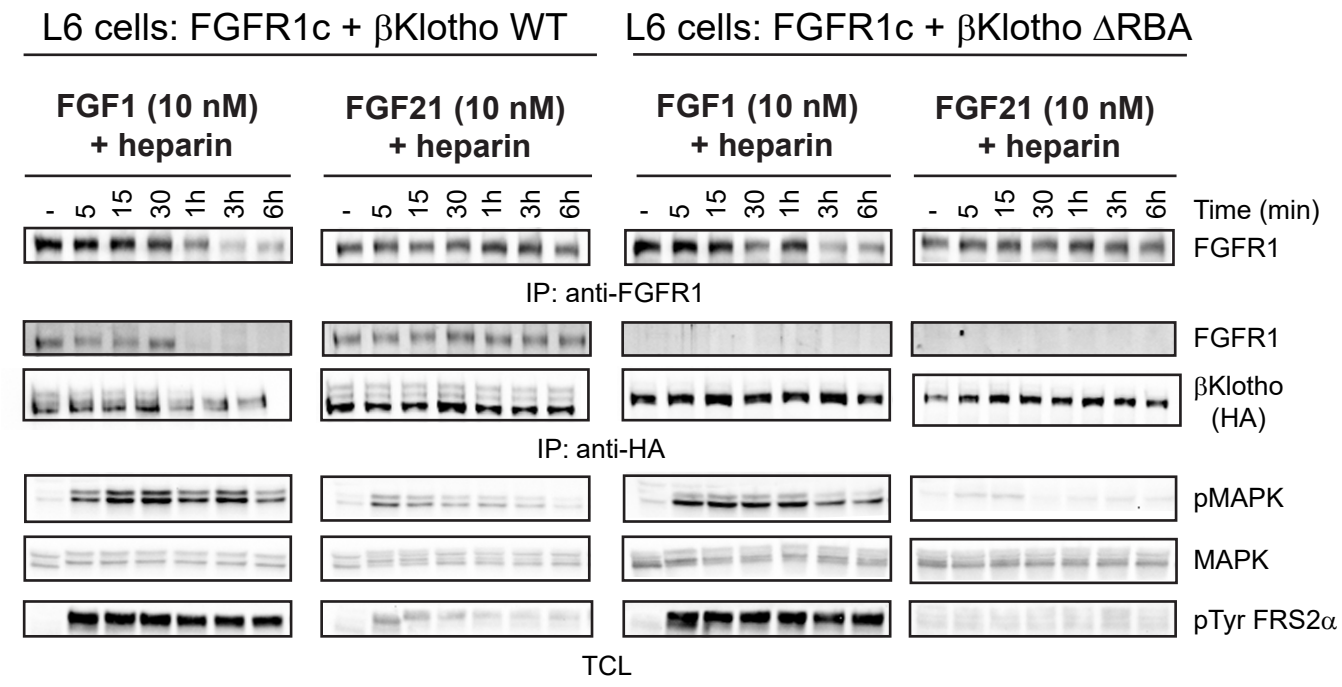

Figure S7. Cell signaling in L6 cells stably expressing FGFR1c together with βKlotho induced by different stimuli.

L6 cells stably expressing FGFR1c together with either βKlotho WT or β*Klotho* ΔRBA (both with an HA tag) were left unstimulated or stimulated with FGF1 together with heparin or FGF21 together with heparin for the indicated time points at 37°C. Cell lysates were subjected to immunoprecipitation (IP) with either anti-FGFR1 antibodies or anti-HA antibodies followed by immunoblotting with anti-FGFR antibodies or anti-HA antibodies. Cell lysates were also analyzed for MAPK activation and tyrosine phosphorylation of FRS2α by immunoblotting with antibodies for pMAPK and pFRS2α respectively. Anti-MAPK antibodies were used as loading control. TCL, total cell lysate.