Supplemental Figures

Figure S1

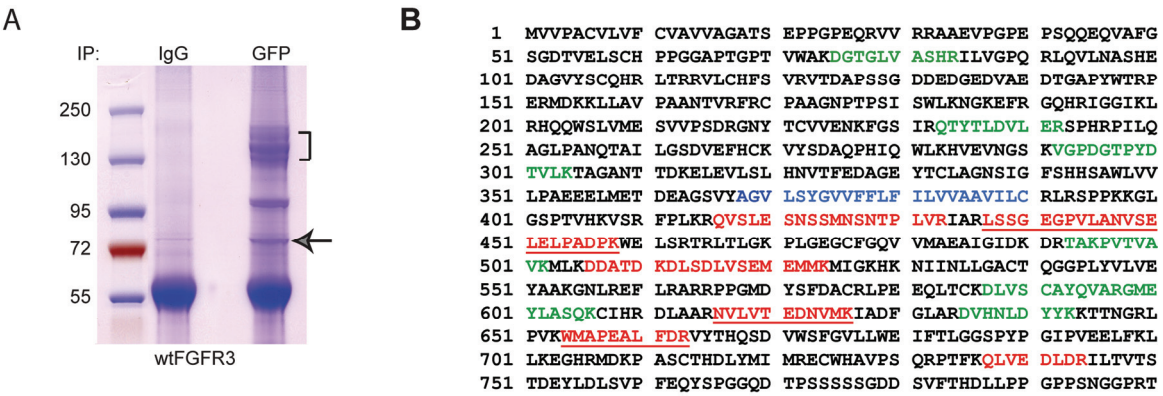

Figure S1. Mass Spectroscopy analysis of intact and cleaved FGFR3.

(A) wtFR3-GFP IP'd with antibodies to GFP (right lane) or control IgG (left lane). Bracket: multiple glycosylation species of full-length FGFR3. Arrow: cleavage fragment subjected to analysis by mass spectroscopy. **(B)** Murine FGFR3 amino acid sequence. Green: sequences identified from intact fragment. Red: sequences identified from cleavage fragment. Underlined red: sequences identified from both samples. Blue: transmembrane domain, for orientation purposes.

Figure S2

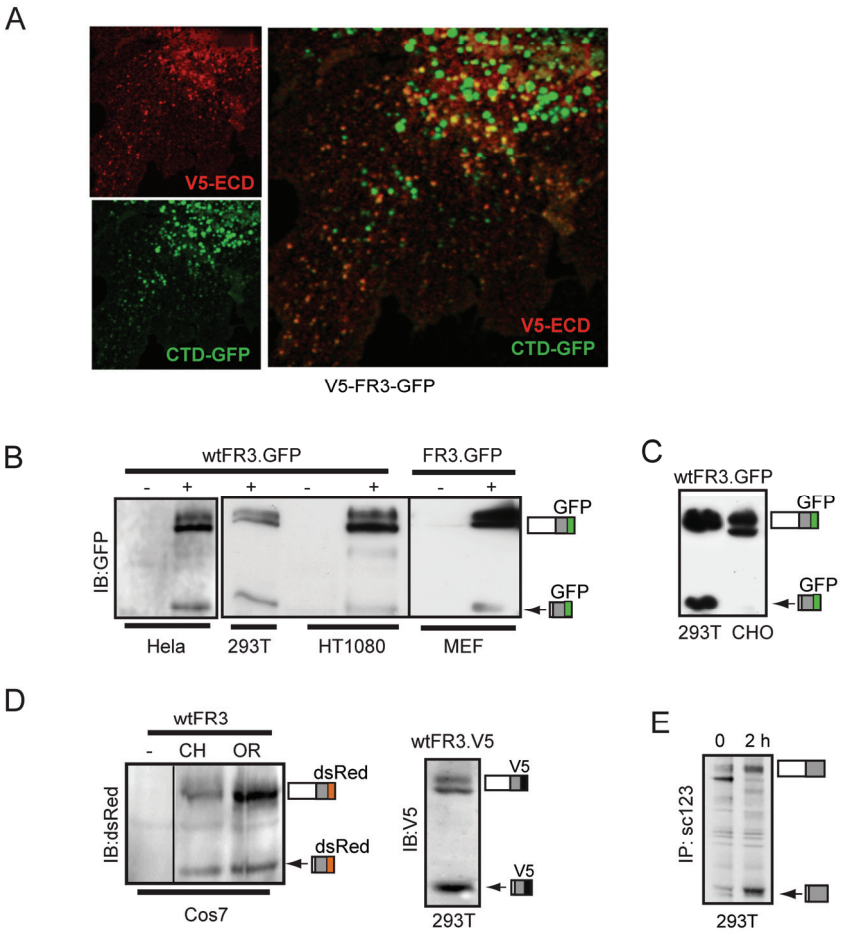

Figure S2. Cleavage of FGFR3 is detected in multiple cell types and is independent of the epitope tag.

(A) Chondrocytes transfected with V5-FR3-GFP were fixed, permeablized and stained for V5 epitope ($Alexa_{568}$). Cells were imaged using confocal microscopy for GFP (green, CTD) and V5 (red, ECD). Co-localized vesicles image yellow. (**B-E**) Arrow: cleaved FR3 CTD. (**B**) Western blots of WCL from Hela, 293T, HT1080 and MEF cells transfected with or without wtFR3-GFP and probed for the GFP epitope. (**C**) Western blot of WCL from CHO cells stably expressing wtFR3-GFP, cultured 8 h in the presence of FGF1 and then probed for the GFP epitope. (**D**) Western blots of Cos7 cells stably expressing wtFR3 fused with mCherry (CH) or mOrange (OR) and probed using dsRed antibody (left); T-Rex 293 cells stably expressing wtFR3-V5 and probed for V5 epitope (right). (E) Pulse-chase analysis of T-Rex 293 cells stably expressing untagged FR3, IP'd using C-terminal FR3 antibodies (sc-123).

Figure S3

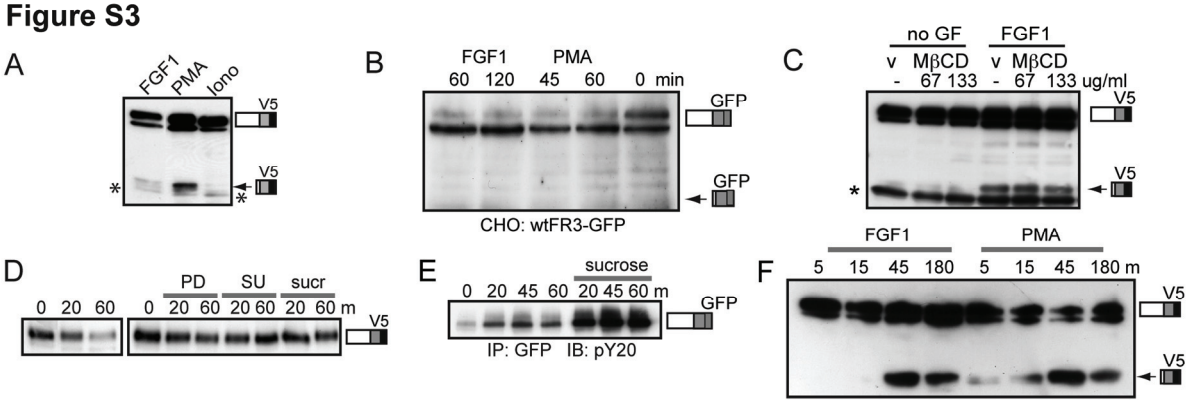

Figure S3. FGF1-induced cleavage of FGFR3 requires both kinase activity and endocytosis whereas PMA-induced cleavage is more rapid. (**A-F**) wtFR3-V5 (wtFR3-GFP in panel B, E) was induced 8h with tet and then serum-starved overnight in the presence of tet. Arrow: cleaved FR3. (**A**) Western blot of WCL from serum-starved cells cultured 30 min with FGF1, 100nM PMA or 100nM ionomycin (Iono), probed for V5 epitope. **(B)** CHO cells stably expressing wtFR3-GFP were serum-starved overnight and then treated with FGF1 or 100ng/ml PMA for the times indicated. Western probed for C-terminal GFP tag. **(C)** Serum-starved cells were pre-treated 1h with MβCD or v (DMSO) prior to addition of vehicle, FGF1 or PMA. Western blot of WCL proved for V5 epitope. Asterisk: non-specific band. **(D)** Kinase inhibitors and sucrose block wtFR3 endocytosis. Serum-starved cells were pre-treated 20 min +/- 1 uM PD173074 (PD), 10 uM Su5402 (SU) or 0.45 M sucrose (sucr) prior to FGF1 addition at 37ºC. Cells were surface biotinylated at the times indicated after which equal ug lysates were P'd with neutravidin gel to assay the fraction of receptor remaining at the PM following FGF1 stimulation. Equal volumes of P'd lysates were subject to western blotting and probed for V5 epitope. (**E**) Sucrose inhibits FGFR3 endocytosis but not its phosphorylation. Serum-starved cells were pretreated for 20 min +/- 0.45 M sucrose prior to the addition of FGF1. Cell lysates were harvested at the times indicated and equal ug lysate was IP'd for GFP epitope. Western blots were probed for phosphotyrosine (pY20). **(F)** Serum-starved cells were treated with FGF1 or 100 ng/ml PMA for the times indicated. Western blot of WCL probed for the V5 epitope.

Figure S4

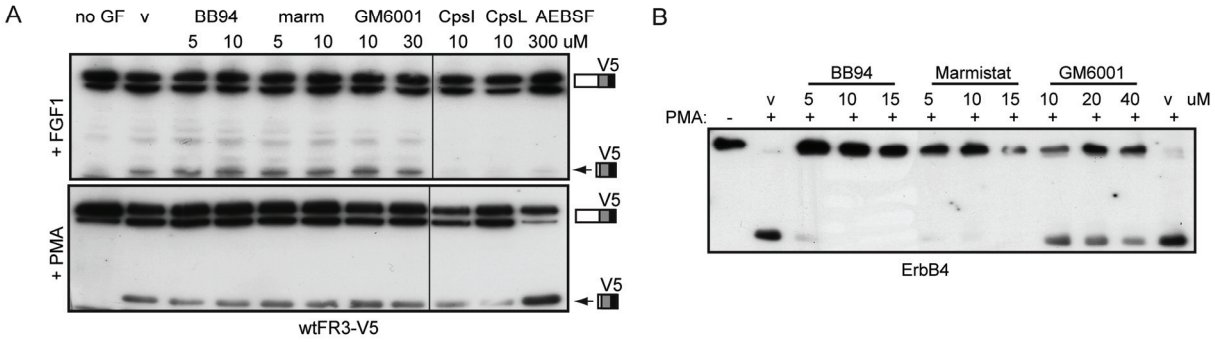

Figure S4. MMP inhibitors block PMA-induced cleavage of ErbB4 but not FGFR3.

(A) Serum-starved T-Rex 293 cells expressing wtFR3-V5 were pretreated 1 h with DMSO (v) or inhibitors prior to stimulation with FGF1 (3 h) or 100ng/ml PMA (45 min). WCL were subject to western blotting and probed for C-terminal V5 tag. v: DMSO BB: BB94; mar: marimastat; CpsI: Cathepsin Inhibitor I: CpsL: Cathepsin Inhibitor L. **(B)** 293 cells transiently transfected with ErbB4 JMa/CYT2, serum-starved overnight, pre-treated 1 h with DMSO or inhibitors before 45 min stimulation with 100nM PMA. Western blot of WCL, probed with antibodies against C-terminal ErbB4.

Figure S5

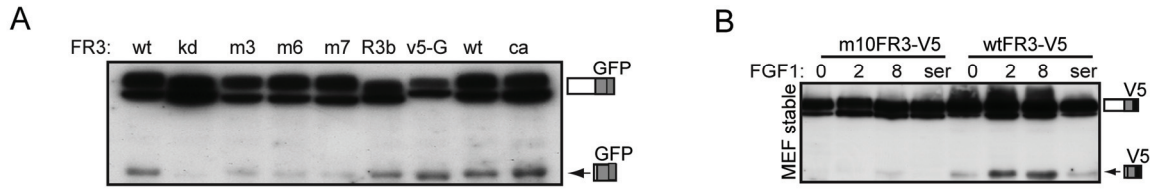

Figure S5. Representative western blots assaying stem mutant cleavage.

(A) Lysates of wt and mutant FR3-GFP expressing T-Rex cells, induced 8 h in the presence of Tet/FGF1 and subject to western blotting. Membranes were probed for GFP epitope. Arrow: cleaved FR3. R3b: FGFR3b; v5-G: FR3(V5)-GFP. **(B)** MEF cell lines stably expressing wt or m10FR3-V5 were serum-starved overnight (or not, serum: ser) and then treated with FGF1 for the hours indicated. Western blot of WCL probed for C-terminal V5 epitope.