**Figure S1:** c-cbl but not cbl-b is ubiquitously expressed during early development and the over-expression of a dominant negative Cbl causes phenotypes similar to Fgf8 gain-of-function. (a-n) Whole mount in situ hybridizations with anti-sense riboprobes detecting either c-cbl (a-g) or cbl-b (h-n) mRNAs at indicated stages of development. For gastrulation stage embryos shown orientation is animal pole to the top (a-d, h-k), dorsal to the right (c,d,j,k). For embryos in somitogenesis and beyond (e-g, l-n) anterior is to the left and dorsal to the top. c-cbl mRNA is maternally provided and zygotically expressed ubiquitously at all investigated stages (a-g). cbl-b mRNA is maternally provided, but not zygotically expressed until early somitogenesis stages where it shows low level ubiquitous expression and higher level expression in defined tissues (h-n). (a-o) Cbl-YF over-expression causes phenotypes resembling Fgf8 gain-of-function. Embryos were injected with either cbl-YF (p,v), cbl-YF and XFD (q,w), wild-type c-cbl (r,t) or ffg8 (t,y) mRNAs, fixed at 60% epiboly and stained for gsc (o-t) or gbx2 (u-y) expression by in-situ hybridization. (o,u) Un-injected control embryos. Shown orientation of embryos is animal pole to the front, dorsal the right. Cbl-YF causes an expansion of the dorsal marker gsc (p) and a reduction of the ventral marker gbx2 (w) similar to Fgf8 over-expression (t,y). This effect is counteracted by XFD (q,w). Arrow heads mark expression domain boundaries. Scale bars are 100 μm.
**Figure S2:** Morpholino knock-down of c-Cbl mimics effects of Cbl-YF over-expression. (a) Quantification of *in situ* expression domain analysis. Embryos were injected with indicated morpholinos (MO) or mRNAs and embryos were processed for *in situ* hybridization. The marginal expression domains of *fgf8*, *spry4*, *spry2* and *pea3* were measured. Gray bars show values for un-injected controls. N numbers are given underneath each respective data bar. Error bars denote the standard deviation (s.d.). Statistical significance was analyzed by Anova tests (*Pfgf8 = 1.9e-12; Pspry4 = 4.6e-13; Pspry2 = 9.5e-24; Ppea3 = 6.9e-22*) followed by post-hoc Tukey tests. Asterisks indicate significant differences between control and the respective treatment (see Methods section for details). Injection either c-cbl ATG or splice Morpholinos causes mild phenotypes similar to Cbl-YF over-expression. This effect is strengthened by injection of a mixture of ATG and splice MOs. Cbl-b MOs do not cause any phenotype. Note that the cbl-b MO does not have an influence on the maternally provided cbl-b mRNA levels (d) and can thus be considered as a negative control. (b) C-Cbl MOs cause a delayed sorting of Fgf8 to the late endosome. Cy5-Fgf8 coated beads were implanted into the animal pole of sphere stage embryos. Embryos were mounted and imaged from 10 minutes post implantation (mpi) until 90 mpi in 10 minutes intervals. Quantification of Cy5-Fgf8 co-localization in embryos injected with CFP-Rab7 and a mixture of cbl ATG and splice morpholinos (cbl MO, green) or cbl-YF mRNA (red) and control embryos (blue). Percentage co-localization of Cy5-Fgf8 positive endosomes with Rab7 endosomes (y-axis) is plotted against time (minutes post implantation, mpi, x-axis). n-numbers are given at the right end of respective curves. Error bars denote the standard deviation. Statistical significance was analyzed by Anova tests followed by post-hoc Tukey tests. Post-hoc statistical significance is indicated by solid circles for ctr – cbl-YF, empty circles for ctr – cbl MO, and asterisks for cbl-YF – cbl MO pairs (see Methods section for details). Co-localization values for cbl-MO are in between the values for cbl-YF and ctr. (c) Integrated co-localization values for Fgf8 and Rab7 over the whole time-course for ctr (blue), cbl-YF (red) and cbl MO (green). Statistical significance was analyzed by an Anova test (*P 3.3e-19*) followed by post-hoc Tukey tests (values shown for the respective data pairs). cbl MO causes a significant reduction in Fgf8 co-localization with Rab7, but to a smaller extend than cbl-YF. (d) RT-PCR control for the efficiency of splice MO knock down. Embryos were injected with either c-cbl (left panel) or cbl-b (right panel) splice MOs and mRNA of injected and control embryos was harvested at 60% epiboly. cDNA was generated by reverse transcription and used as template for PCR analysis with primers specific for c-cbl (left panel) or cbl-b (right panel) covering the respective splice sites. c-cbl splice MO efficiently targets the c-cbl mRNA leading to a changed transcript. Note residual wild type mRNA likely due to maternal mRNA contribution. cbl-b MO has no effects, probably due to maternal mRNA contribution and lack of zygotic cbl-b expression.
Figure S3: A bead implantation assay using Cy5 labeled Fgf8 fully recapitulates Cbl-YF effects on Fgf signaling at the embryonic margin. Cy5-Fgf8 coated beads were implanted into the animal pole of sphere stage embryos injected with mRNA coding for plasma membrane RFP (Ras-RFP, PM; red, a',c') alone (a,b) or together with a dominant negative Fgf receptor (XFD c,d). Embryos were incubated for two hours and analyzed by live confocal microscopy (a,c) followed by in situ hybridization for the detection of spry2 mRNA (b,d). (a,c) Single confocal sections through the animal pole of embryos with the Cy5-Fgf8 bead being visible in the left upper quarter of each panel. (a,c) Cy5 channel showing Cy5-Fgf8 protein on the surface of the bead as well as the Cy5-Fgf8 protein that has penetrated into the tissue. (a',c') Merged image of Cy5 and mRFP channel. Note that Cy5-Fgf8 is almost exclusively localized in intracellular endosomal objects in target cells (a'). In XFD injected embryos Cy5-Fgf8 endocytosis is blocked and the protein stays cell surface associated (c') indicating that Cy5-Fgf8 uptake is strictly dependent on functional Fgf receptors. Scale bars in a,c are 10 µm. (b,d) Cy5-Fgf8 induces Fgf target gene induction in the tissue surrounding the bead (b) while XFD completely blocks this response (d). Scale bars in b,d are 20 µm. (e) Quantification of the target gene induction capabilities of Cy5-Fgf8 (red) and non-labeled Fgf8 (blue) for the expression domain radius of spry4 and spry2 in the tissue surrounding the bead. Numbers below bars give n-number and numbers on top of bars the p-values of indicated populations. The induction radius of spry2 is unchanged for either protein while the induction range of spry4 is reduced by only about 10% for labeled versus unlabeled protein. Thus the labeling procedure has only minimal effects on Fgf8 activity. (f) Implanted embryos were mounted and imaged from 10 minutes post implantation (mpi) until 90 mpi in 10 minutes intervals. Quantification of Cy5-Fgf8 co-localization with CFP-Rab7 in embryos injected with dominant negative Rab5 (red) or control embryos (blue). Percentage co-localization of Cy5-Fgf8 positive endosomes with Rab7 (y-axis) is plotted against time (minutes post implantation, mpi, x-axis). n-numbers are given at the right end of respective curves. Error bars denote the standard deviation. Dominant negative Rab5 causes a delay of lysosomal targeting of Cy5-Fgf8. (g,k) Cbl-YF mediated expansion of Fgf target gene expression is recapitulated in Cy5-Fgf8 bead implantations. Cy5-Fgf8 coated beads were implanted into the animal pole of sphere stage control embryos (g) or embryos injected with mRNA coding for wild-type c-cbl (cbl, h) or cbl-YF (i). Embryos were incubated for two hours and processed for whole mount in situ hybridization. (g-i) Animal view on embryos stained for spry4 mRNA. Cbl-YF causes an expansion of the spry4 expression radius around the bead (i) as compared to control (g) and wild-type c-cbl (cbl, h) injected embryos. Scale bars in g-i are 40 µm. (j) Quantification of expression domain radius of spry4 and spry2 in control embryos (green) and embryos injected with wild-type c-cbl (cbl, blue) or cbl-YF (red) mRNA. Statistical significance was analyzed by an Anova test followed by post-hoc Tukey tests. Results of the post-hoc test are shown for significant differences, only (for spry4 P_{Anova}2.1e-9; spry2 P_{Anova}2e-11). Cbl-YF causes an expansion of the expression domain radius of both spry4 and spry2 as compared to Cbl and control. (k) Fluorescence correlation spectroscopy (FCS) auto-correlation measurements were taken in the extracellular space of live embryos at different distance from a Fgf8-Cy5 coated bead in un-injected control (blue; n=13) or cbl-YF mRNA injected embryos (red; n=13) to determine the extracellular Fgf8 concentration gradient. Data are binned in 20 µm intervals and fit with a radial model 10. Error bars denote standard deviation. Cbl-YF does not influence the Fgf8 gradient.
Figure S4  Cy5-Fgf8 co-localizes with specific endosomal markers within target cells. Cy5-Fgf8 coated beads were implanted into the animal pole of sphere stage embryos. Embryos were mounted and imaged from 10 minutes post implantation (mpi) until 90 mpi in 10 minutes intervals. Shown are representative examples for Cy5-Fgf8 co-localization in embryos injected with CFP-Rab5c (a), YFP-Rab7 (b), mRFP-Lamp1 (c), Cherry-Rab11 (d), Caveolin1-GFP (e), or or plasma membrane RFP (Ras-RFP, PM; f) at 90 mpi. Arrowheads point to endosomes that are positive for both Cy5-Fgf8 and the respective marker. Scale bars are 5 μm.
**Figure S5** Cy5-Fgf8 co-localization with endosomal markers within target cells is independent of the distance to the Fgf8 source. Experimental data shown in Figure 3 was scored in whole confocal sections (all), an area of about 30 μm around the bead (about two cell diameters; close) or the area excluding 30 μm around the bead (far). No significant differences between the different treatments can be detected. Statistical significance was analyzed by Anova tests.
Figure S6 FgfR1-GFP co-localizes with specific endosomal markers within target cells. Embryos were injected with 50pg of FgfR1-GFP mRNA and mRNA coding for tRFP-Rab5c (a), tRFP-Rab7 (b), Cherry-Rab11 (c), Caveolin1-tRFP (d), or plasma membrane RFP (Ras-RFP, PM; e) and were analyzed at sphere stage by confocal microscopy. Arrowheads point to endosomes that are positive for both FgfR1-GFP and the respective marker. Scale bars are 5 μm. (f-i) FgfR1-GFP fusions are constitutively active in zebrafish embryos. Embryos injected with 50pg of FgfR1-GFP mRNA (g,i) and control embryos (f,h) were fixed at 60% epiboly and processed for in situ hybridization. Shown are representative embryos stained for the expression of spry4 (f,g) and spry2 (h,i). FgfR1-GFP injection causes strong ectopic target gene activation. Scale bars are 100 μm.