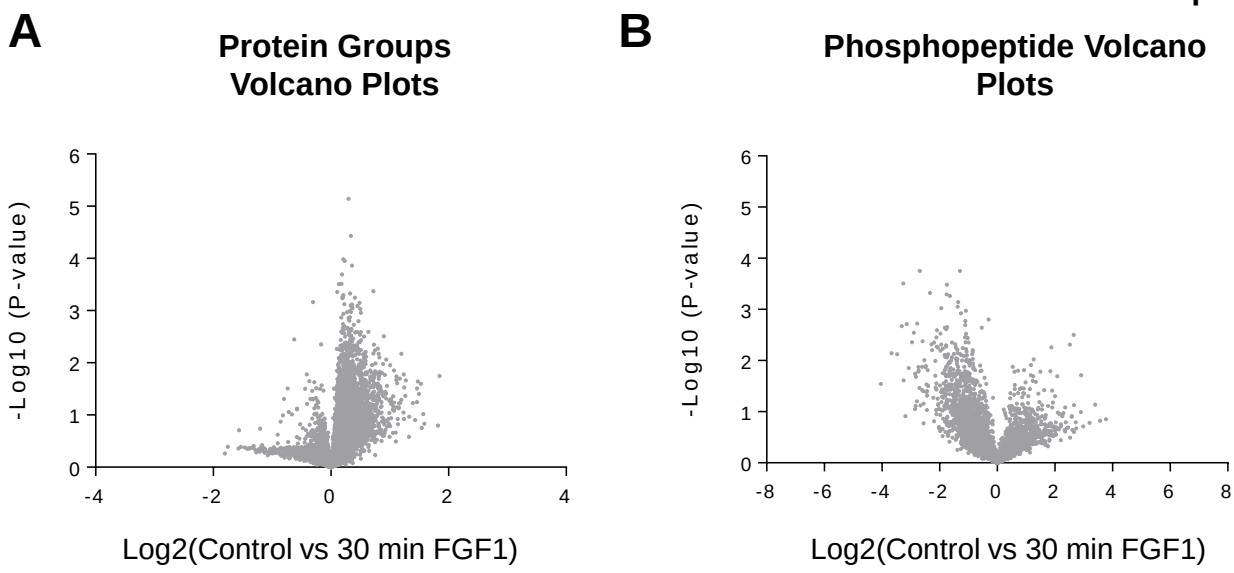


Figure S1. Volcano plots of quantified proteins and phosphopeptides.
Volcano plots of global proteome **(A)** and phosphoproteome **(B)** comparing no FGF treatment with 30 minutes of FGF1 treatment. A two-sided t-test was performed correcting for multiple testing by controlling for FDR at 5%. Protein or peptides with a q-value of 0.05 or less are considered statistically significant. There were no statistically significant changes between two samples in the global and phospho-proteome.

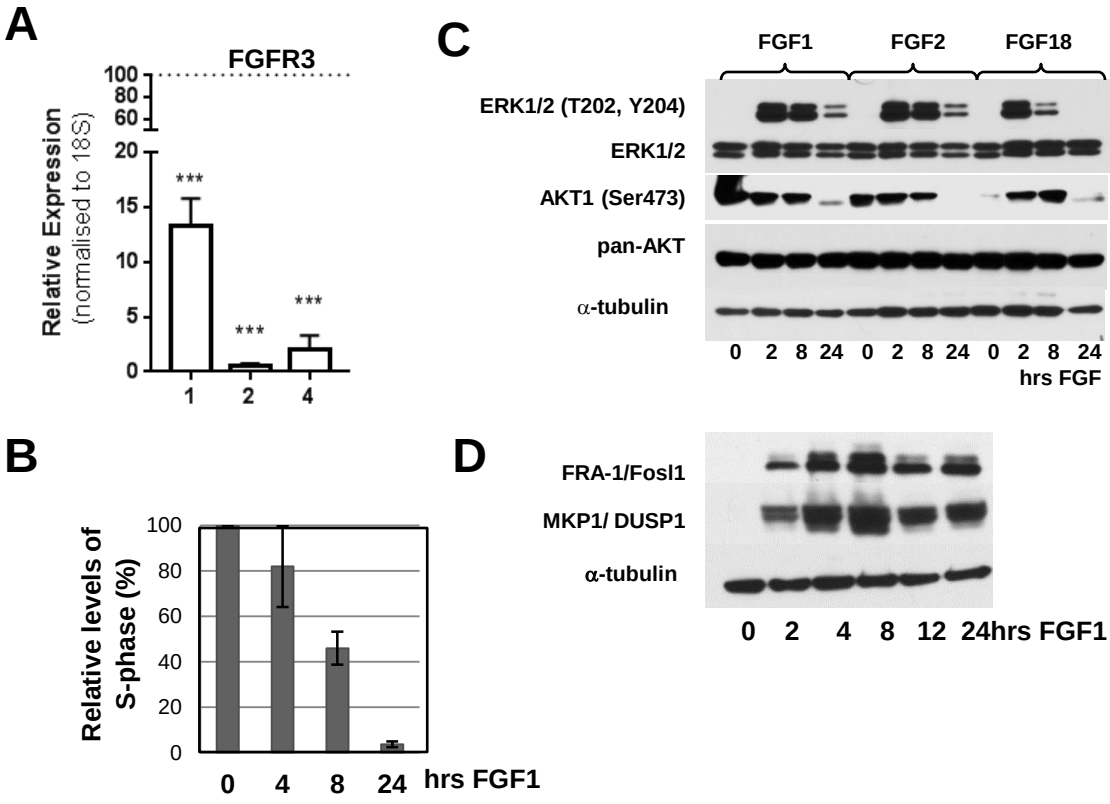


Figure S2. **FGF signaling in RCS cells**. **(A)** Relative levels of FGFR1-4 mRNAs in RCS cells were determined using specific primers. 18S was used as a normalization control, data were plotted relative to FGFR3 expression (100%). Rat Chondrosarcoma (RCS) cells were treated with FGF1, FGF2 and FGF18 for the times indicated, and were analyzed by **(B)** FACScan™ analysis and **(C,D)** Immunoblotting . Numbers on the Y-axis indicate relative percentage of total cells in S-phase. **(C,D)** 10 μg of total protein was used for immunodetection and equal amount of protein loading was confirmed by immunodetection of a-tubulin.

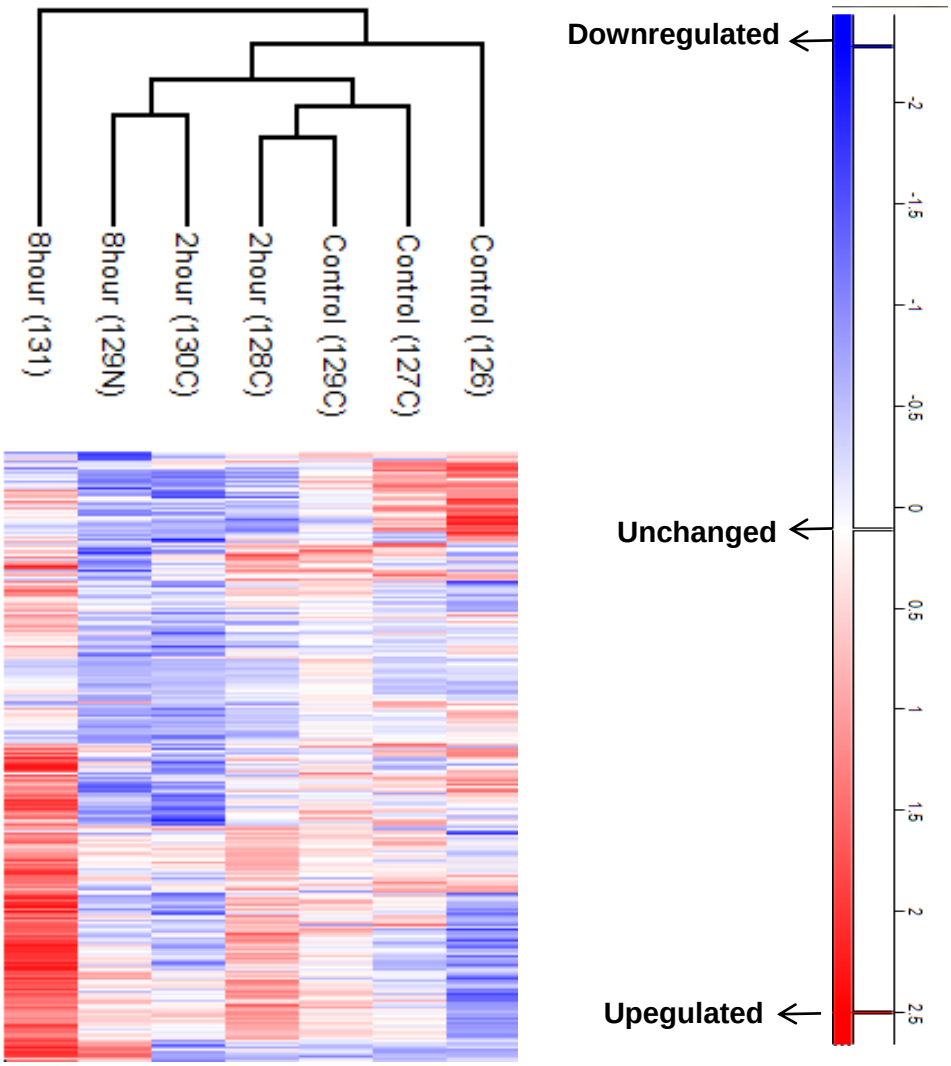
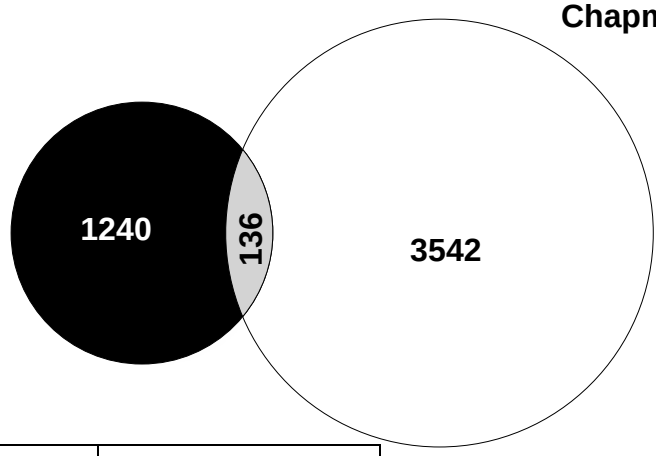


Figure S3. Heatmap of quantified proteins.
Unsupervised hierarchical clustering is performed based on the Z-score (difference from the mean) of log2 protein intensities.

A

B



C

	All Peptide Groups	Phosphopeptide Groups
Unique SCX	77829	1240
Unique SAX	25499	3542
Common SCX + SAX	19893	136
Total	123221	4918

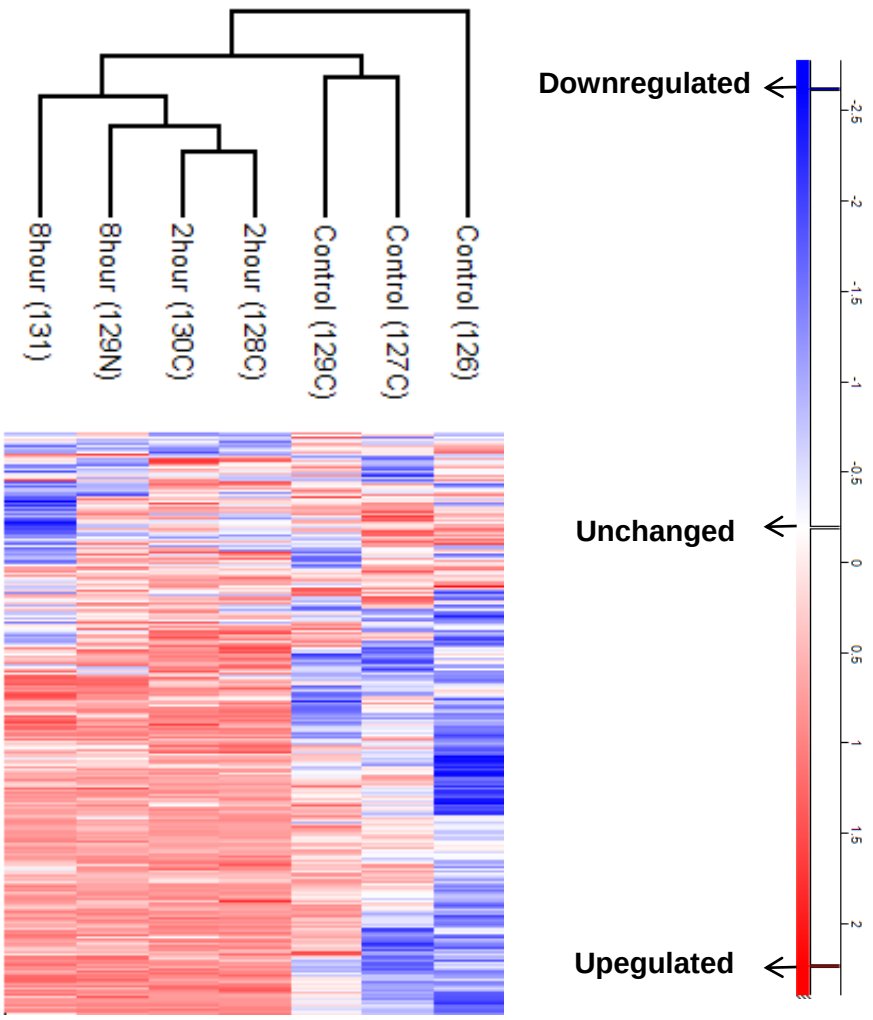
D

	Phosphosites/Peptide			
	Mono	Di	Tri	Tetra
Unique SCX	71%	20%	8%	2%
Unique SAX	72%	18%	7%	3%

Figure S4. Number of peptides and phosphopeptides identified after different TMT clean up schemes.
(A) Peptides identified after either SCX only or SAX clean up of the SCX flow through. **(B)** Phosphopeptides identified after either SCX only or SAX clean up of the SCX flow through. **(C)** Summary of identified peptides and phosphopeptides. **(D)** Distribution of mono, di, tri and tetra phosphorylated peptides.

Note, we capture more peptides with SCX only, but we selectively loose the phosphorylated peptides in the flow through. Using SAX on the SCX flow through we are able to capture over 3000 additional phosphorylated peptides that would have been lost otherwise.

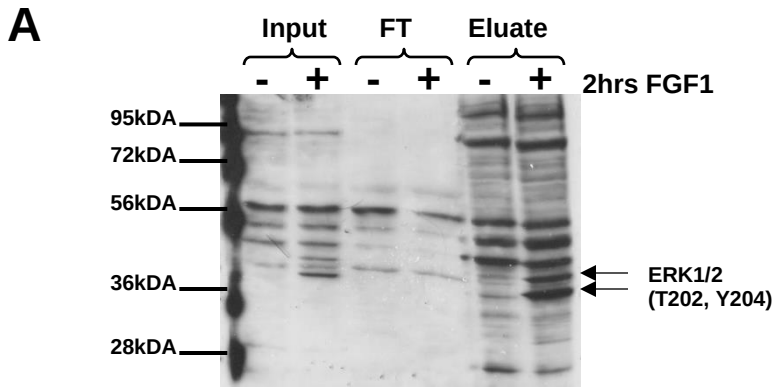
A

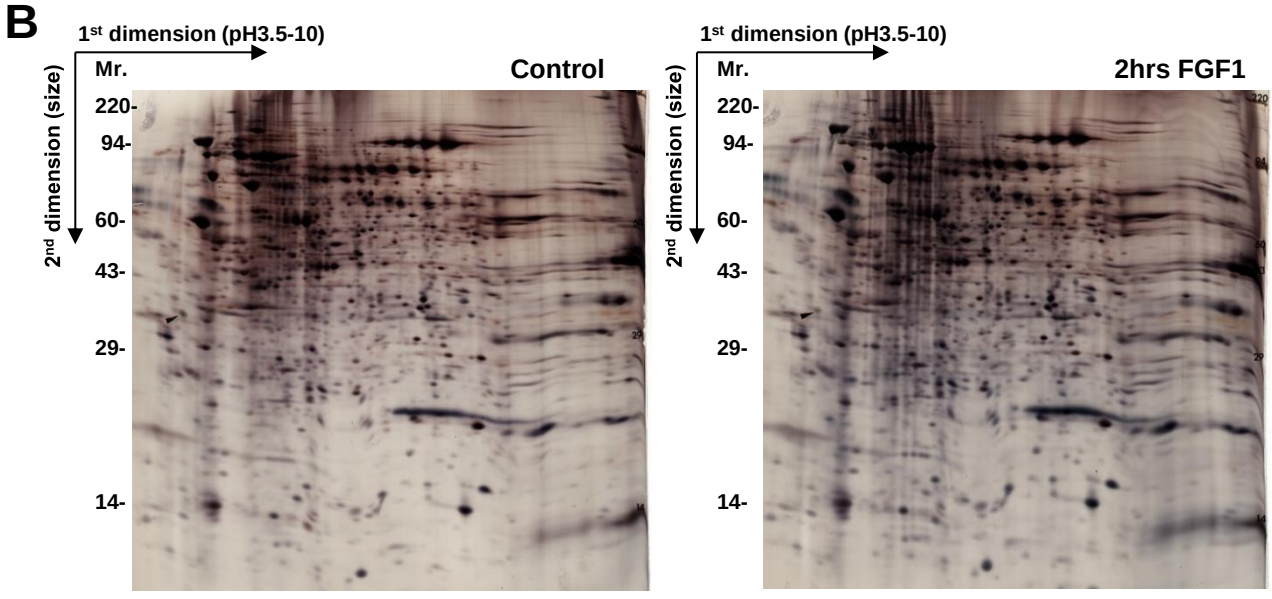


B

Number of phospho-peptides	Ctrl	2hrs FGF	8hrs FGF
3618	+	+	+
1	+	+	
471		+	+
20		+	
1			+

Figure S5. Heatmap of quantified phosphopeptides. **(A)** Unsupervised hierarchical clustering is performed based on the Z-score (difference from the mean) of log2 phosphopeptide intensities. **(B)** Tabular venn diagram of phosphorylated peptides identified showing that the majority of phosphorylated peptides were detected in all samples.





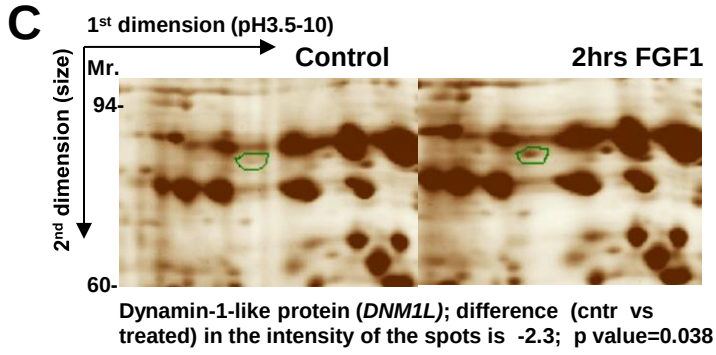


Figure S6. 2D Electrophoresis of total protein lysate of RCS cells.
(A) Analysis of the phosphoprotein enrichment of untreated and FGF1-treated (2hrs) RCS cells. Input, flow through (FT) and eluate fractions were analyzed using a mixture of phospho-pan and ERK1/2 (T202,Y204) antibodies. **(B)** Silver stained 2D gels of FGF1 treated and untreated samples enriched for phosphoproteins (from panel A). **(C)** A fragment of 2D gel (from panel B). An identified protein with the difference in the intensity is circled in green.