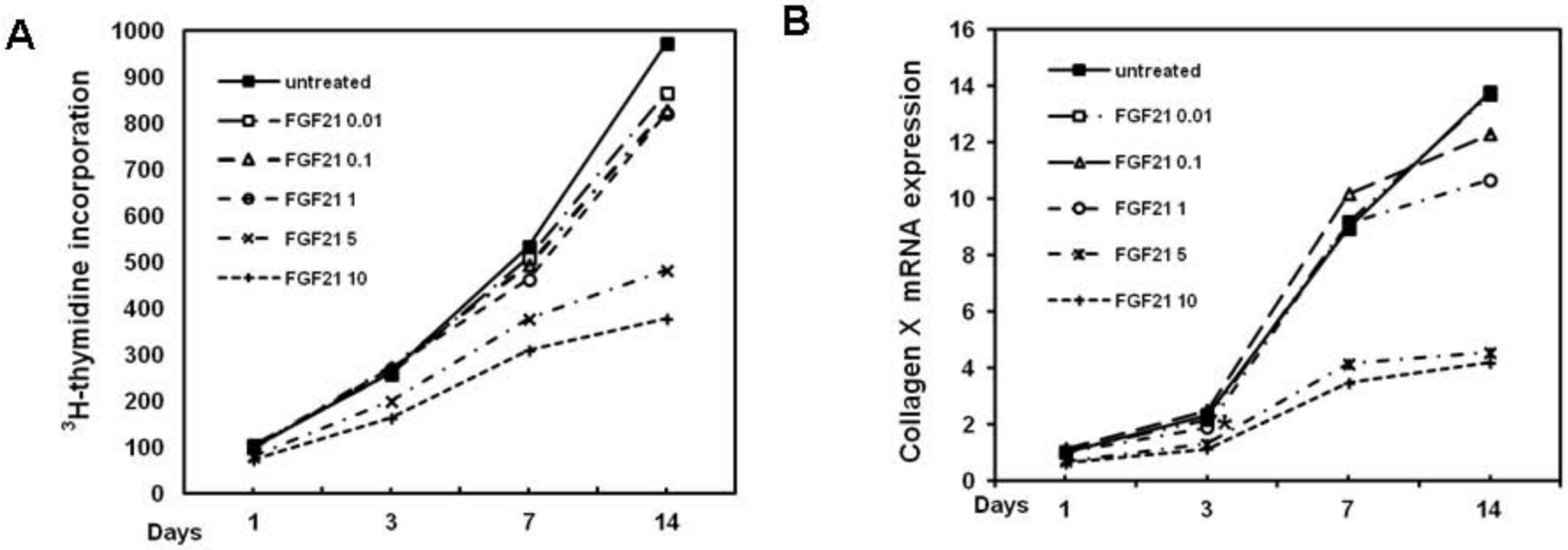
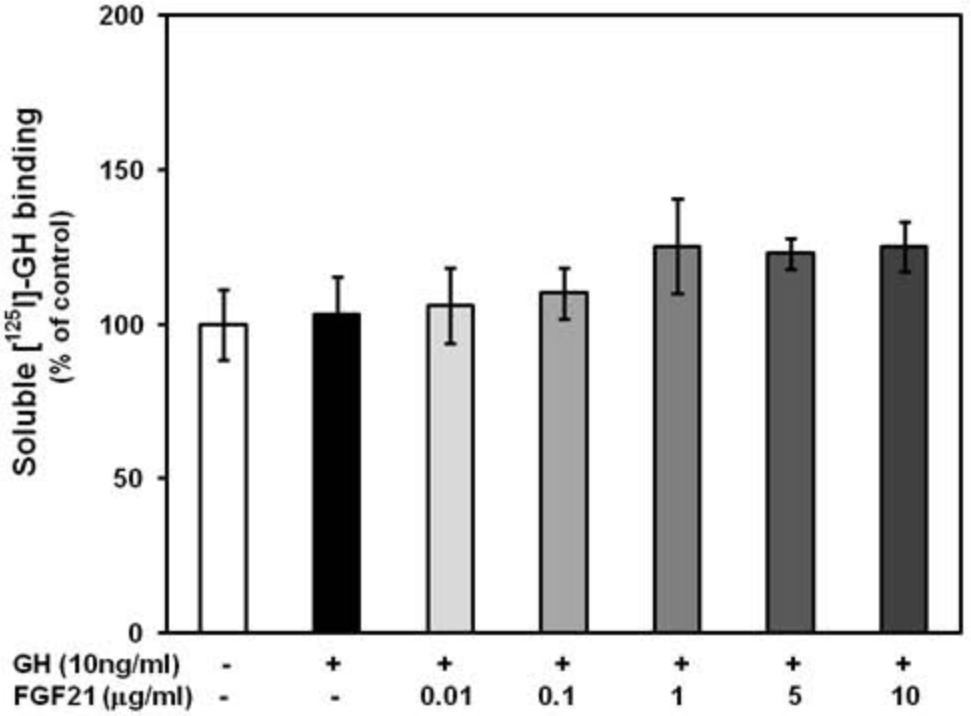
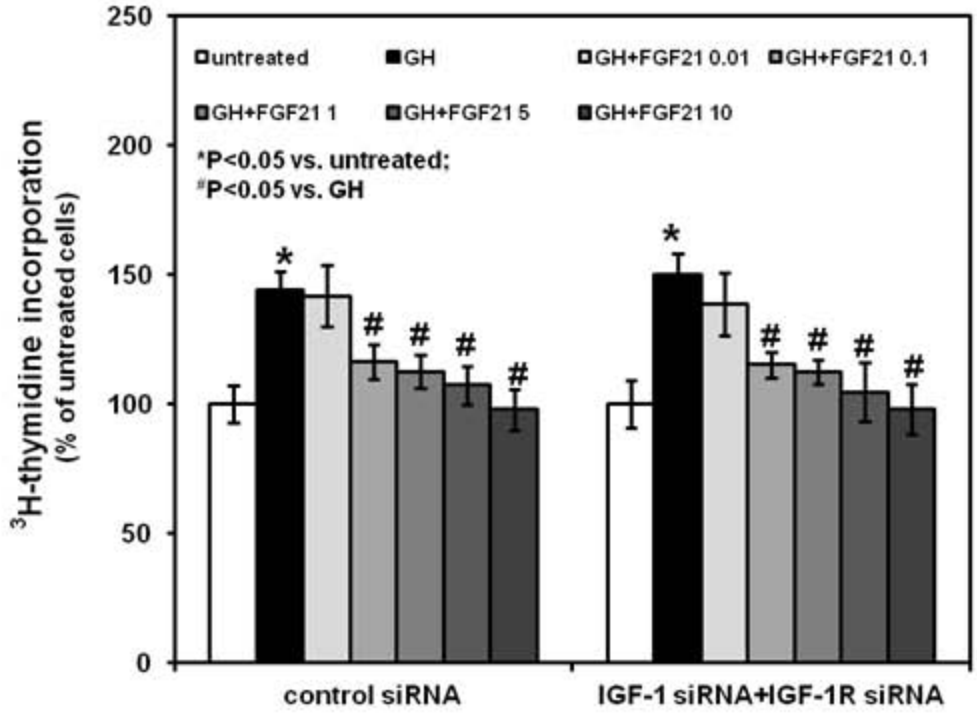


P values (FGF21-treated vs. untreated, at the indicated time points)	^{3}H-thymidine incorporation				Collagen X expression			
	1 day	3 days	7 days	14 days	1 day	3 days	7 days	14 days
FGF21 5 μg/ml	<0.05	<0.05	<0.01	<0.01		<0.05	<0.05	<0.01
FGF21 10 μg/ml	<0.05	<0.01	<0.01	<0.01	<0.05	<0.05	<0.05	<0.01

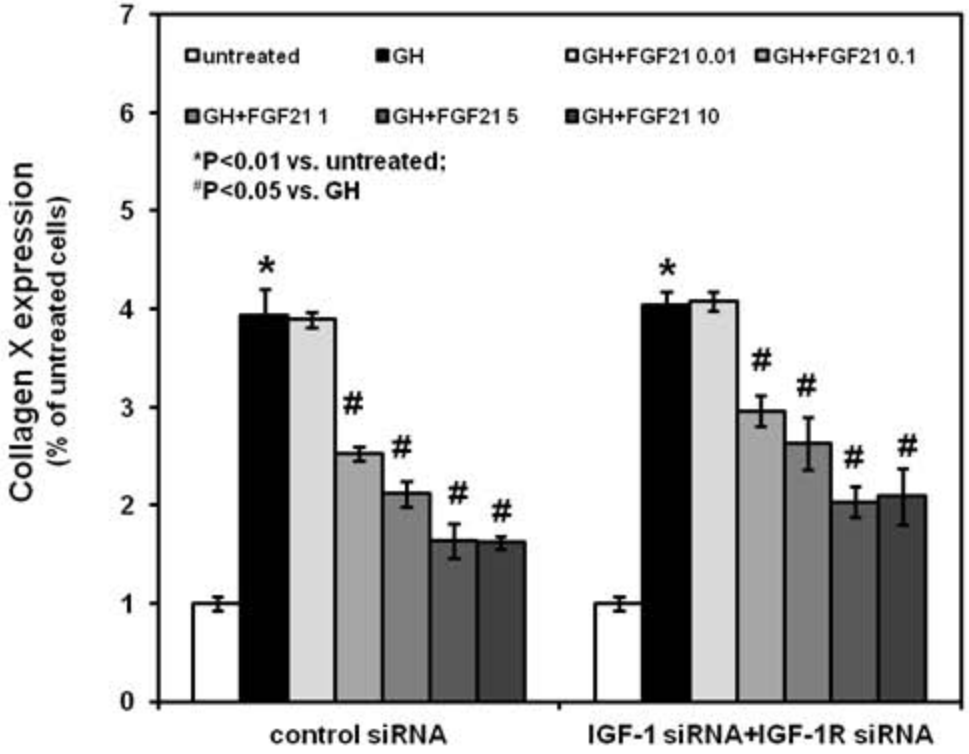
SUPPLEMENTAL FIGURE 1



SUPPLEMENTAL FIGURE 2



SUPPLEMENTAL FIGURE 3



SUPPLEMENTAL FIGURE 4

Supplemental figure 1: Time-dependent and concentration-dependent effects of GH and FGF21 on total ^{3}H-thymidine incorporation and collagen X mRNA expression in chondrocytes. Chondrocytes were washed with fresh serum-free DMEM, seeded in 24-well plate, cultured for 24 hours in the absence or presence of rmGH (10 ng/ml), without or with graded concentrations of rhFGF21 (0.01-10μg/ml). A: At the indicated time points, 2.5μCi/well of ^{3}H-thymidine (Amersham) was added to the culture medium for an additional 3 h. Chondrocytes were released by trypsin and collected onto glass fiber filters. Incorporation of ^{3}H-thymidine was measured by liquid scintillation counting. Results are expressed as % of control (or untreated) and represent mean values obtained from three independent experiments. B: Collagen X mRNA expression was determined by real time PCR. Total RNA was extracted from chondrocytes at the indicated time points and then processed as described in Materials and Methods. The relative expression levels of mRNA were normalized by β-actin in the same samples. Results were expressed as fold change compared to control (or untreated) chondrocytes (mean ±S.E). *P<0.05, **P<0.01 vs. untreated cells at the same time point.

Supplemental figure 2: Effect of rhFGF21 on soluble GH binding activity.

Soluble GH binding was evaluated as described in Materials and Methods. $^{[125I]}$GH-binding activity was measured in conditioned medium of chondrocytes cultured without or with graded concentrations of rhFGF21. Results are expressed as % of control and represent mean values obtained from three independent experiments.

Supplemental figure 3: Effects of FGF21 and GH on ^{3}H-thymidine incorporation in chondrocytes transfected with both IGF-1 siRNAs and IGF-1R siRNAs.

After being previously cultured without or with graded concentrations of FGF21, IGF-1 siRNA + IGF-1R siRNA-transfected chondrocytes were cultured for 24 hours in the absence or presence of rmGH (10 ng/ml), At the end of culture period, 2.5μCi/well of 3H-thymidine (Amersham) was added to the culture medium for an additional 3 h. Incorporation of 3H-thymidine was measured by liquid scintillation counting. Results are expressed as % of control and represent mean values obtained from three independent experiments.

Supplemental figure 4: Effects of FGF21 and GH on Collagen X mRNA expression in chondrocytes transfected with both IGF-1 siRNAs and IGF-1R siRNAs.

Collagen X mRNA expression was measured by real time PCR. Total RNA was extracted from IGF-1 siRNA + IGF-1R siRNA-transfected chondrocytes and then processed as described in

Materials and Methods. The relative expression levels of mRNA were normalized by β-actin in the same samples. Results were expressed as fold change compared to control chondrocytes (mean ±S.E).