Supplemental materials Section
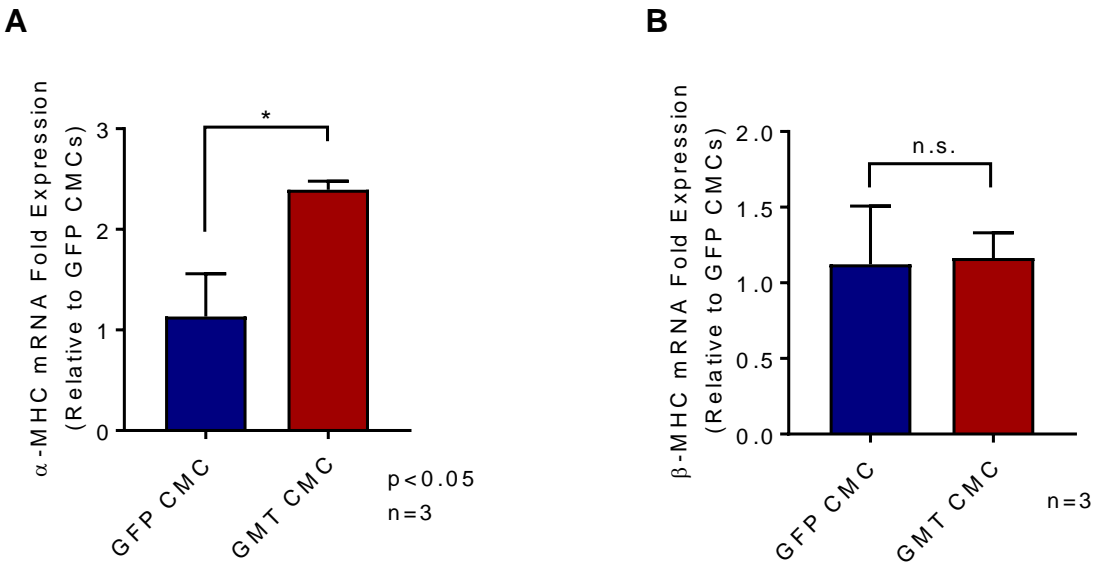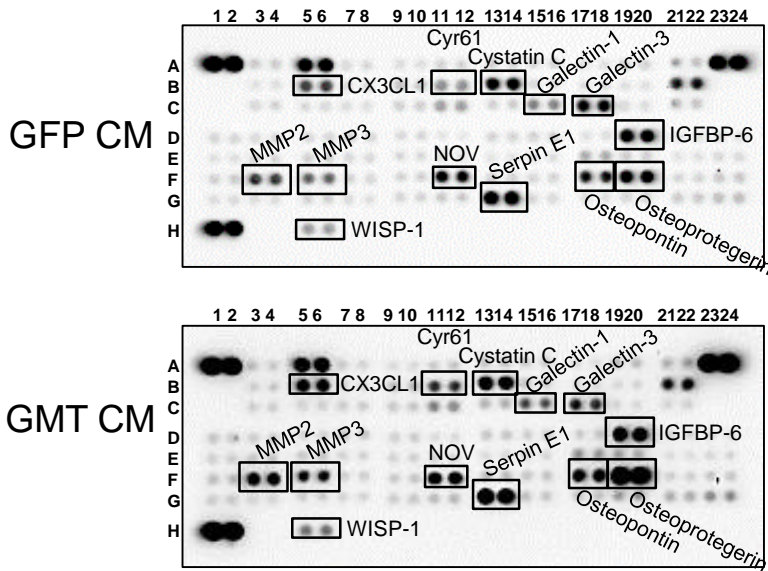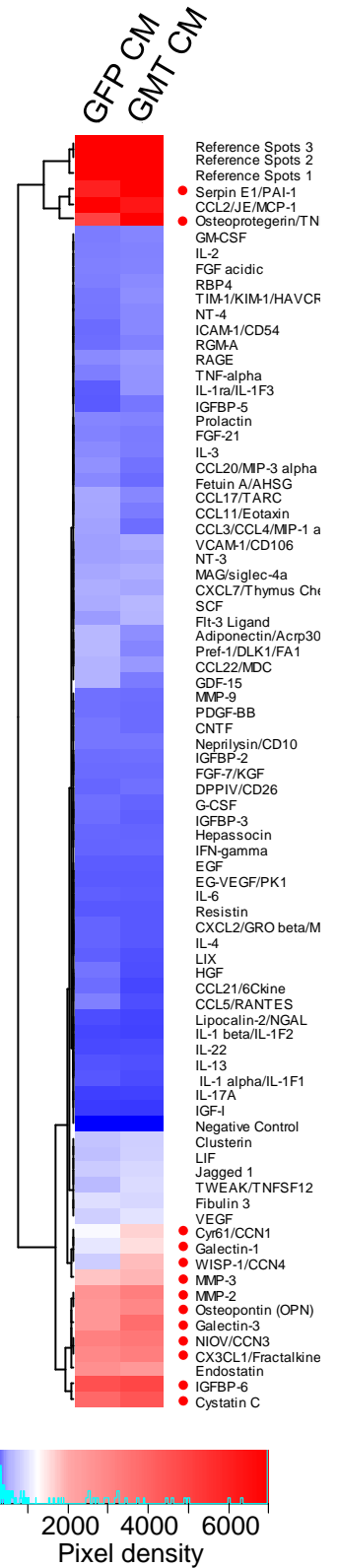
Figure S1

A

B

Figure S1. Effect of ectopic GMT expression on myosin cardiac markers.
GMT-transduced CMCs exhibit enhanced expression of (A) α-MHC, but not (B) β-MHC mRNA. Bar graphs denote the arithmetic mean of 3 independent biological replicates ± standard error. Formal tests for normality were performed using the Shapiro-Wilk normality test. P-values were calculated using a two-tailed, unpaired student's t-test.
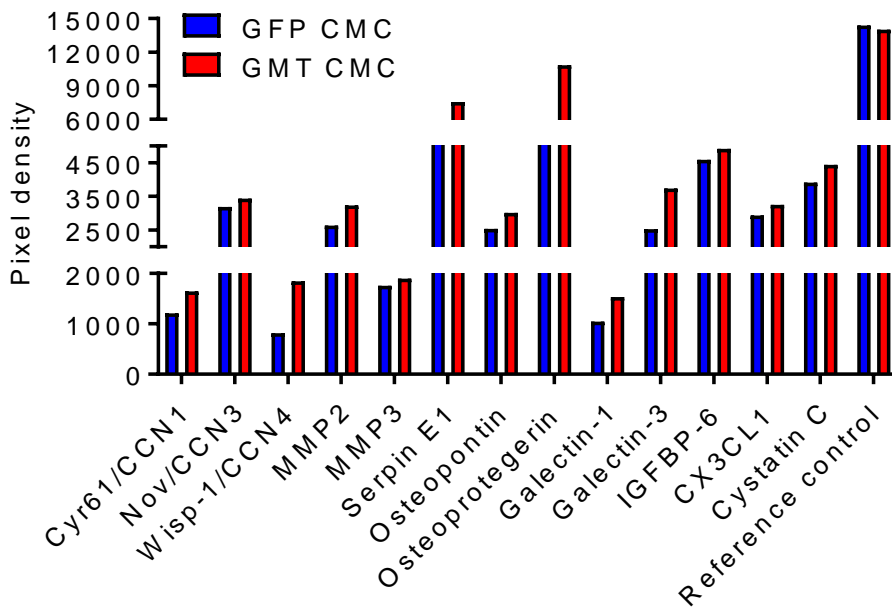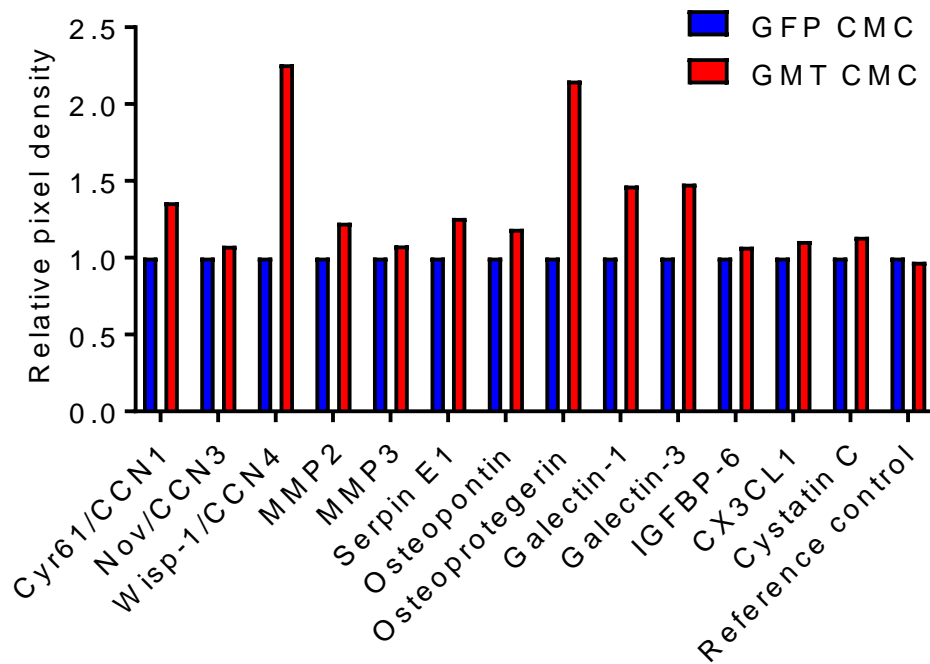
Figure S2

A

GFP CM

GMT CM

C

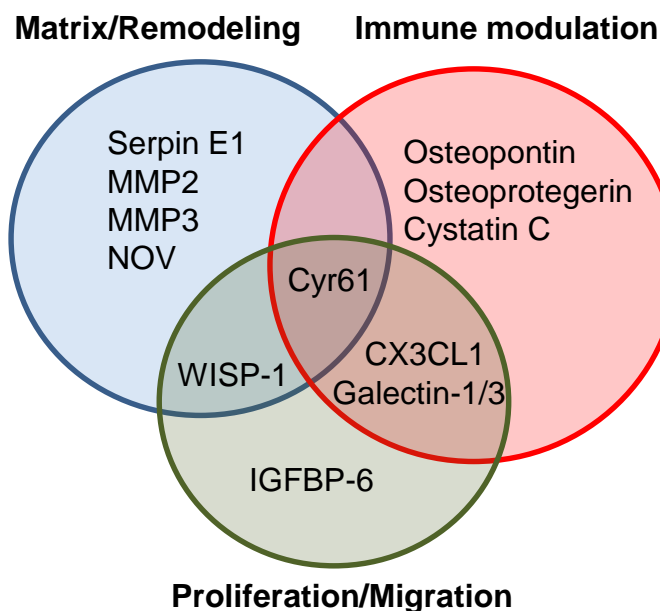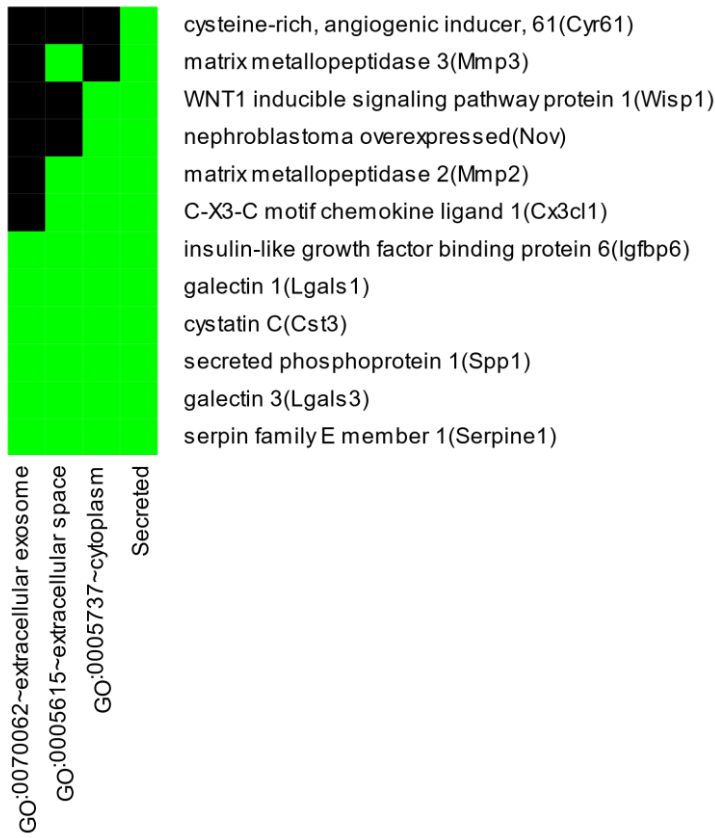

B

D

E

Figure S2. Ectopic GMT expression augments rat CMC cytokine secretion patterns

(A) Representative cytokine array. Conditioned medium (CM), derived from GFP- or GMT-transduced CMCs (n=2 biological replicates for each), was incubated with cytokine array membranes (Proteome Profiler Rat XL Cytokine Array Kit; R&D Systems, Minneapolis, MN) and detected using enhanced chemiluminescence. Black boxes highlight cytokines that are more abundant in CM from GMT CMCs compared to GFP CMCs. (B) Heatmap illustrating post hoc densitometric quantification of resultant cytokine array membranes. Total pixel density is reported. Red dots highlight upregulated cytokines in CM derived from GMT CMCs compared to GFP CMCs. In the figure image, low, medium, and high cytokine expression is indicated using blue, white, and red colors, respectively. Densitometric data graphically presented in bar graphs denoting (C) total pixel density and (D) relative pixel density (relative to GFP CM). (E) Venn diagram stratifying secreted cytokines, markedly upregulated in GMT-transduced CMCs, according to canonical biological processes in which they are known to be involved.

Figure S3

Figure S3 (continued)

C

secreted phosphoprotein 1(Spp1)
serpin family E member 1(Serpine1)
WNT1 inducible signaling pathway protein 1(Wisp1)
nephroblastoma overexpressed(Nov)
matrix metallopeptidase 3(Mmp3)
cysteine-rich, angiogenic inducer, 61(Cyr61)
matrix metallopeptidase 2(Mmp2)
galectin 3(Lgals3)
galectin 1(Lgals1)

Extracellular matrix
GO:0005634~nucleus
GO:0007165~signal transduction
Phosphoprotein
GO:0031012~extracellular matrix
GO:0005578~proteinaceous extracellular matrix

D

C-X3-C motif chemokine ligand 1(Cx3cl1)
matrix metallopeptidase 3(Mmp3)
matrix metallopeptidase 2(Mmp2)
nephroblastoma overexpressed(Nov)
WNT1 inducible signaling pathway protein 1(Wisp1)
serpin family E member 1(Serpine1)
insulin-like growth factor binding protein 6(Igfbp6)
cysteine-rich, angiogenic inducer, 61(Cyr61)
secreted phosphoprotein 1(Spp1)
cystatin C(Cst3)
galectin 3(Lgals3)
galectin 1(Lgals1)

GO:0001525~angiogenesis
GO:0034097~response to cytokine
GO:0042060~wound healing
GO:0071347~cellular response to interleukin-1
GO:0007155~cell adhesion
glycosylation site:N-linked (GlcNAc...)
Glycoprotein
disulfide bond
Disulfide bond
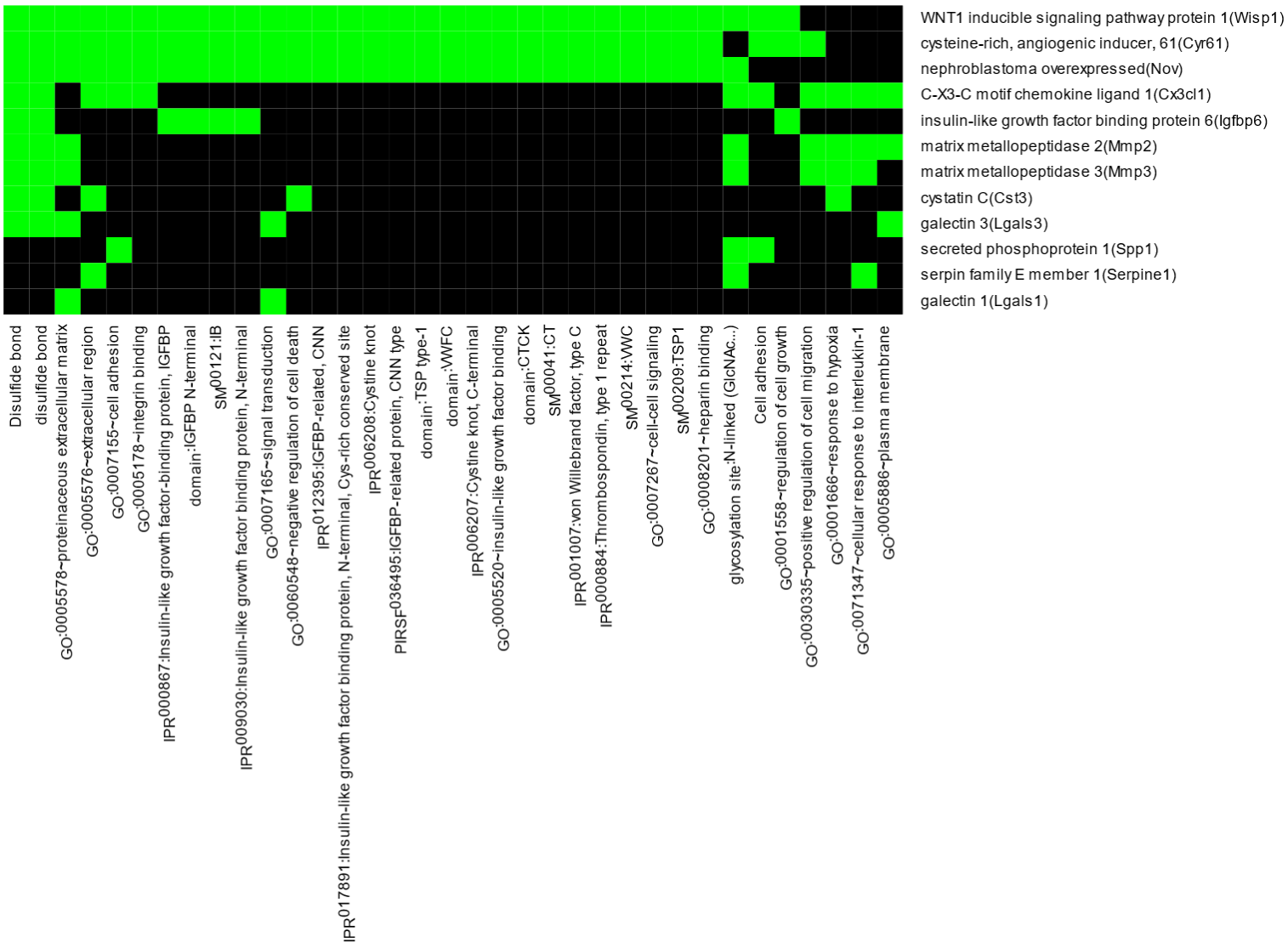Signal
signal peptide
Secreted

Figure S3. Gene ontology analyses of upregulated cytokines suggest GMT CMCs may exhibit augmented anti-fibrogenic paracrine signaling potency

(A-D) Functional annotation clustering using GO terms associated with those key soluble cytokines identified to be upregulated in condition medium sourced from GMT-transduced CMCs, relative to GFP-transduced controls. Green blocks indicate that a corresponding gene-term association has been positively reported. Black blocks indicate that a corresponding gene-term association has not been reported.

Table S1: Restriction cloning materials

Cloning primer (direction)	Restriction Enzyme	Primer Sequence (5´→3´)	Template	Amplicon (base pairs)
GATA4 (forward)	AgeI	TGC AAT ACC GGT ACC ATG TAC CAA AGC CTG G	pLenti6-3xFLAG-GATA4-V5[a]	1351 bp
GATA4 (reverse)	BsrGI	TAA CGT TGT ACA TCG CGG TGA TTA TGT CC		
MEF2C (forward)	XbaI	TGC AAT TCT AGA ACC ATG GGG AGA AAA AAG ATT CAG	pLenti6-3xFLAG-MEF2C-V5[a]	1427 bp
MEF2C (reverse)	BamHI	TAA CGT GGA TCC CTT TGT TGC CCA TCC TTC AGA AAG TCG		
TBX5 (forward)	BmtI	TGC AAT GCT AGC ATG GCC GAC GCA GAC G	pLenti6-3xFLAG-TBX5-V5[a]	1581 bp
TBX5 (reverse)	SalI	TAA CGT GTC GAC CTA GCT ATT GTC GCT CC		
Puro (forward)	PacI	TGC AAT TTA ATT AAA CTT TGG CCG CGG CTC G	pLK0.1-Puro[b]	1231 bp
Puro (reverse)	PacI	TAA CGT TTA ATT AAG AAC GTT ATT TGC GCT CCT TTC GGT CGG		

[a] Al-Maqtari T *et al*. (2017) Transcription factor-induced activation of cardiac gene expression in human c-kit+ cardiac progenitor cells. PLoS ONE 12(3): e0174242
[b] Sigma-Aldrich

Table S2: Antibodies employed

Antibody	Host	Conjugation	Supplier	Catalog #	Dilution	Exposure Time (minutes:seconds)	Predicted Molecular Weight (kDa)
Anti-GATA4 (D3A3M)	Rabbit mAb	Unconjugated	Cell Signaling Technology	36966	1:2000	1:30	55
Anti-MEF2C (D80C1)	Rabbit mAb	Unconjugated	Cell Signaling Technology	5030	1:1000	30:00	55
Anti-TBX5	Rabbit pAb	Unconjugated	Invitrogen	PA5-29845	1:2000	20:00	55
Anti-Col1A1	Rabbit pAb	Unconjugated	Cell Signaling Technology	84336	1:1000	4:00	250
Anti-Col19A1	Rabbit pAb	Unconjugated	ThermoFisher Scientific	PA5-23977	1:1000	15:00	130
Anti-α-Smooth Muscle Actin (D4K9N) XP	Rabbit mAb	Unconjugated	Cell Signaling Technology	19245	1:1000	0:20	55
Anti-β-Actin	Mouse mAb	Unconjugated	Invitrogen	AM4302	1:5000	0:25	55
Anti-rabbit IgG (secondary antibody)	Goat pAb	HRP-Linked	Cell Signaling Technology	7074	1:5000	N/A	N/A
Anti-mouse IgG (secondary antibody)	Horse pAb	HRP-Linked	Cell Signaling Technology	7076	1:5000	N/A	N/A