Heparan sulfate inhibits transforming growth factor β signaling and functions *in cis* and *in trans* to regulate prostate stem/progenitor cell activities

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Running title: *Heparan sulfate regulates prostate stem/progenitor cell functions*

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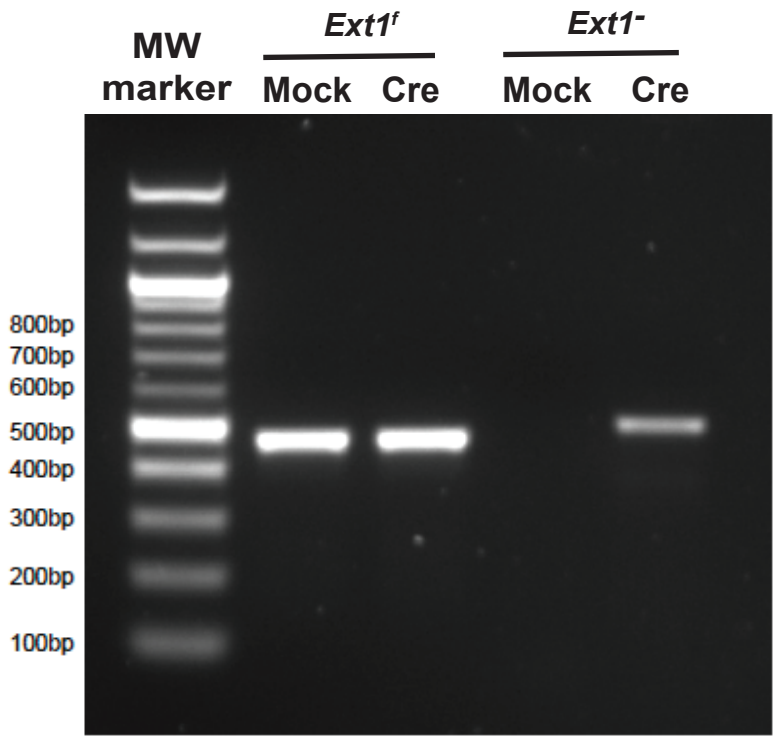


Figure S1. Cre mediated excision of *Ext1* allele. Genomic DNA isolated from primary spheres generated by mock- or Cre- lentivirus transduced Ext1$^{f/f}$ PrECs was subjected to PCR analysis to detect loxP flanked *Ext1* (*Ext1^{f} allele*, 460 bp) and Cre-excised *Ext1* deletion (*Ext1^{-}*, 500 bp) allele. Primary cultures are a mixed population of transduced and un-transduced spheres thus resulting in *Ext1^{floxed}* allele being detected in Cre-transduced PrECs.

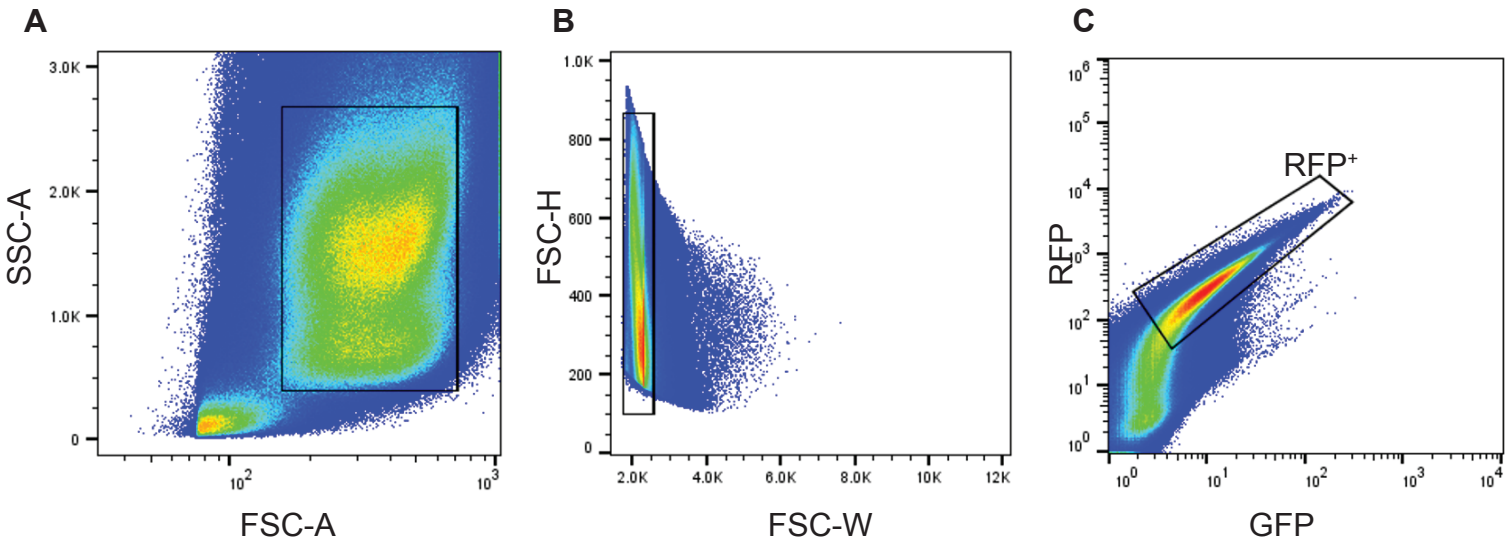


Figure S2. Gating strategy to collect RFP-expressing PrECs from primary spheres. PrECs transduced with control or Cre (with RFP marker) expressing lentivirus. Primary prostate spheres were dissociated and sorted. (**A**) Exclusion of cell debris. (**B**) Doublet discrimination to obtain a single cells suspension. (**C**) Identification of RFP-expressing PrECs.

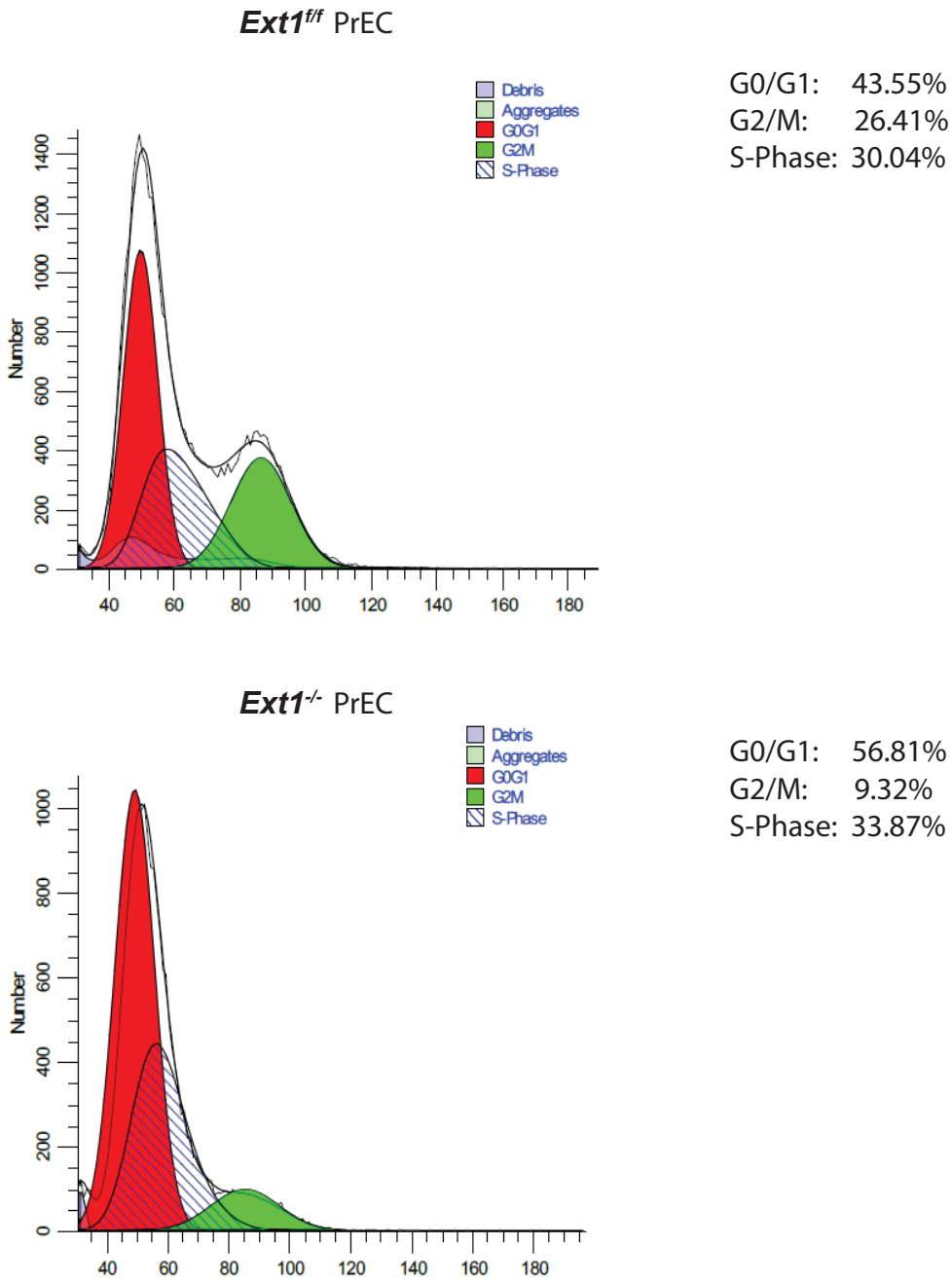


Figure S3. Loss of HS expression arrests cell cycle progression. Primary spheres infected with control or Cre expressing virus were dissociated, sorted and cultured in Matrigel for 16-20 hrs. Cell cycle analysis of *Ext1*$^{f/f}$ and *Ext1*$^{-/-}$ PrECs was performed and the data was analyzed using ModFit.

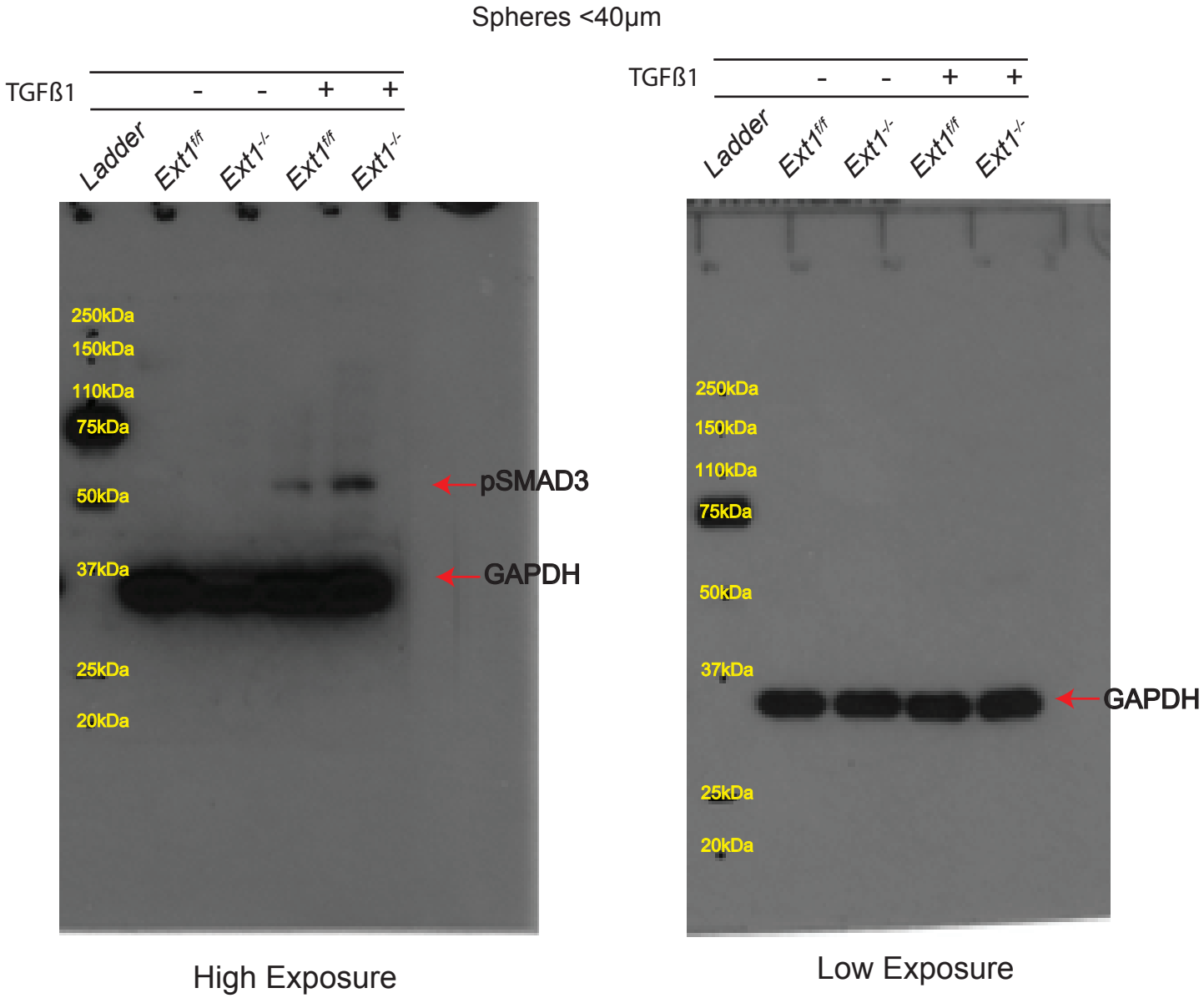
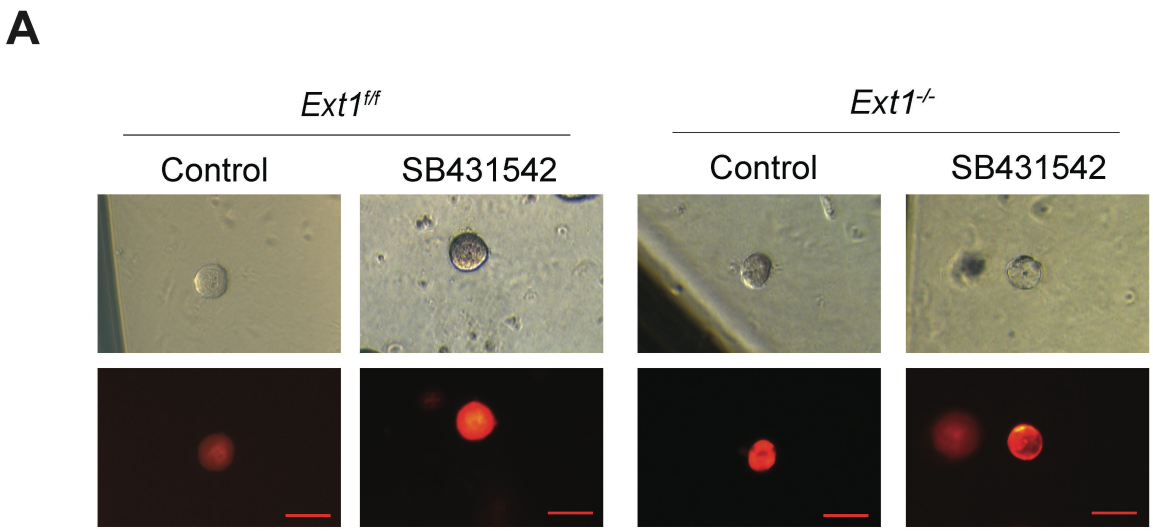


Figure S4. Loss of HS enhances TGFß - Samd2/3 signaling in *Ext1*$^{-/-}$ PrECs. Western blot images for Figure 5E



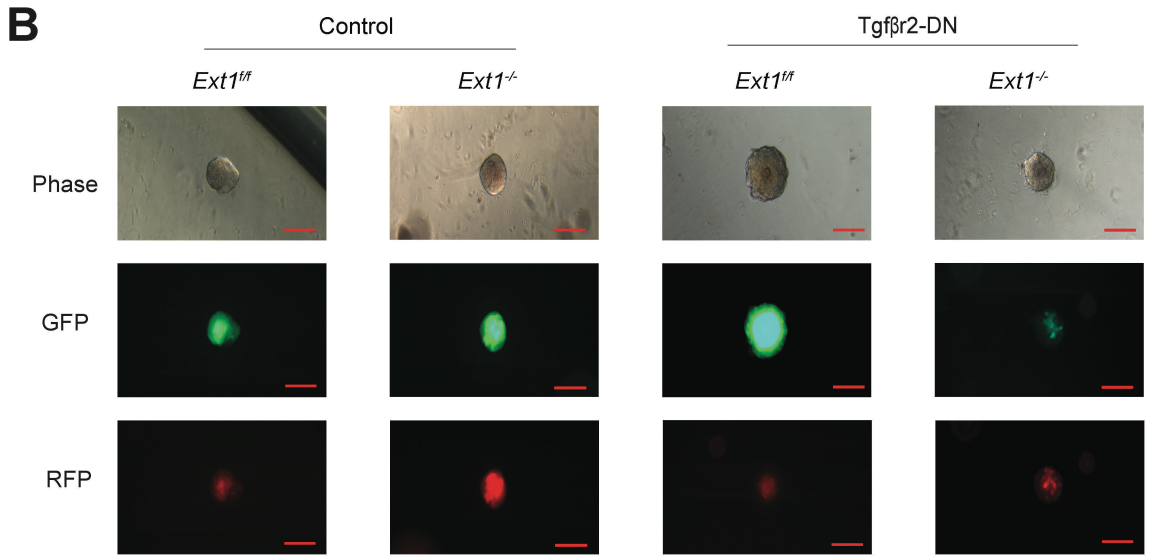


Figure S5. Inhibition of TGFβ signaling restores sphere formation activity of *Ext1*$^{-/-}$ PrSCs. The size of the seconddary spheres formed from primary *Ext1*$^{-/-}$ PrECs was increased either with SB43154 treatment (**A**) or following overexpression of dominant negative form of TGFβR2 (TGFβR2-DN) (**B**). Scale = 100μm. Images shown are representative from three independent experiments.

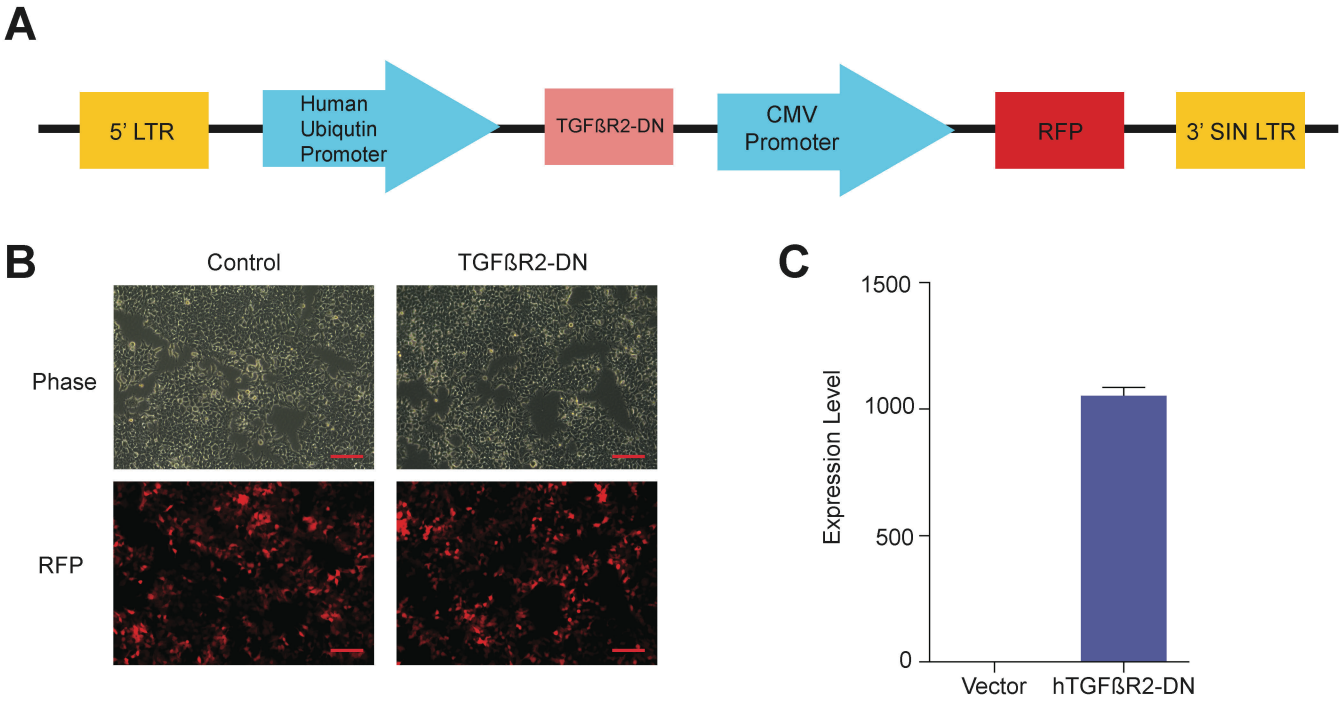


Figure S6. Validation of TGFβR2-DN expression. (**A**) Truncated TGFβR2 (TGFβR2-DN) was cloned into a lentiviral vector as described in the Supplemental Methods. Schematic representation of lentiviral construct expressing TGFβR2-DN (labeled with RFP). (**B**) 293T cells were transduced with control or TGFβR2-DN expressing virus by lentiviral infection. (**C**) The mRNA levels of human TGFβR2 was measured by RT-PCR. Fold Change normalized to Gapdh.

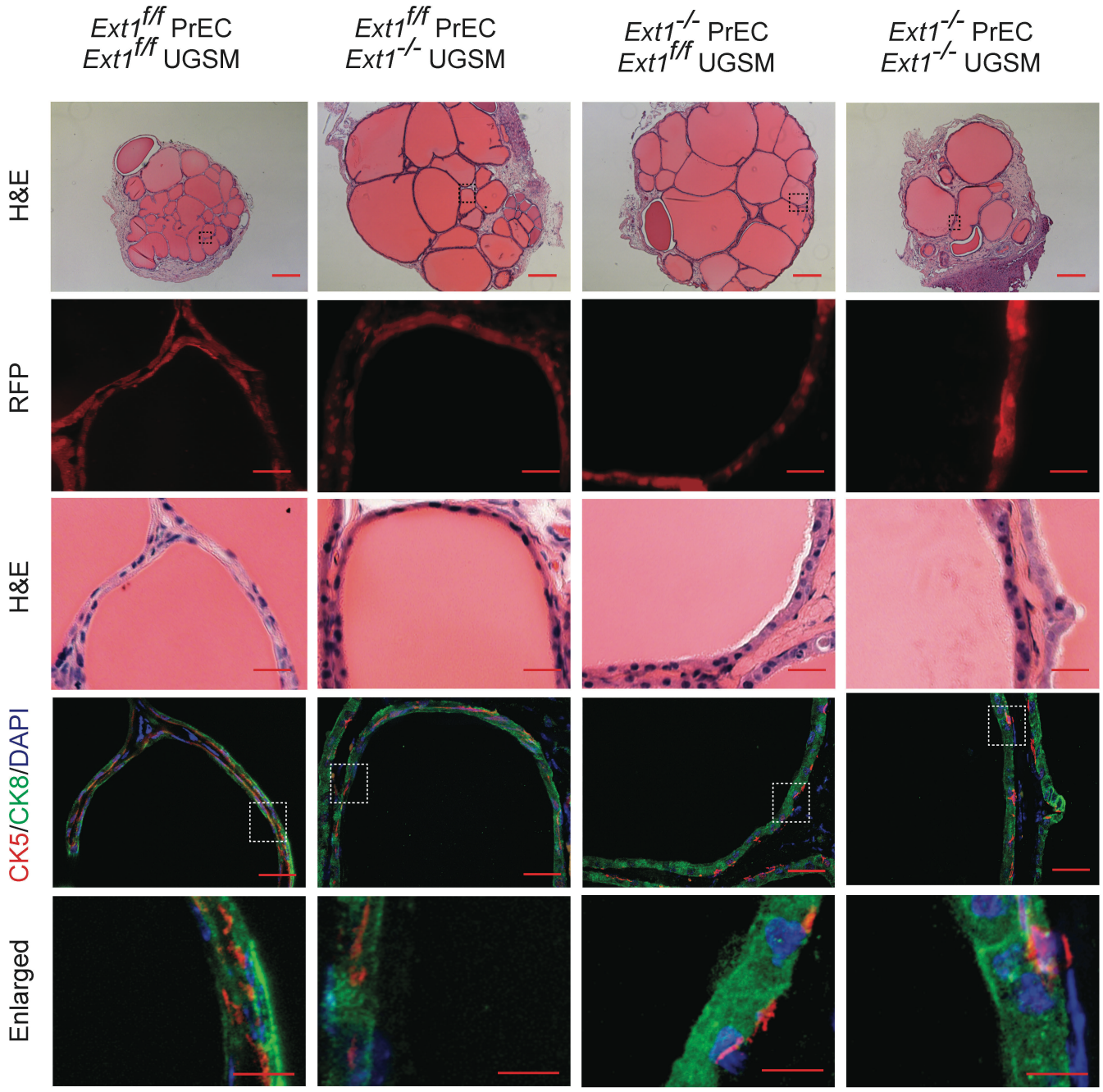


figure S7. Histological and immuno-histochemical analyses of the regenerated grafts. H&E staining of tissue section (scale bar= 200µm). H&E and CK5/CK8 expression of an RFP expressing tubule (Scale bar = 25µm)

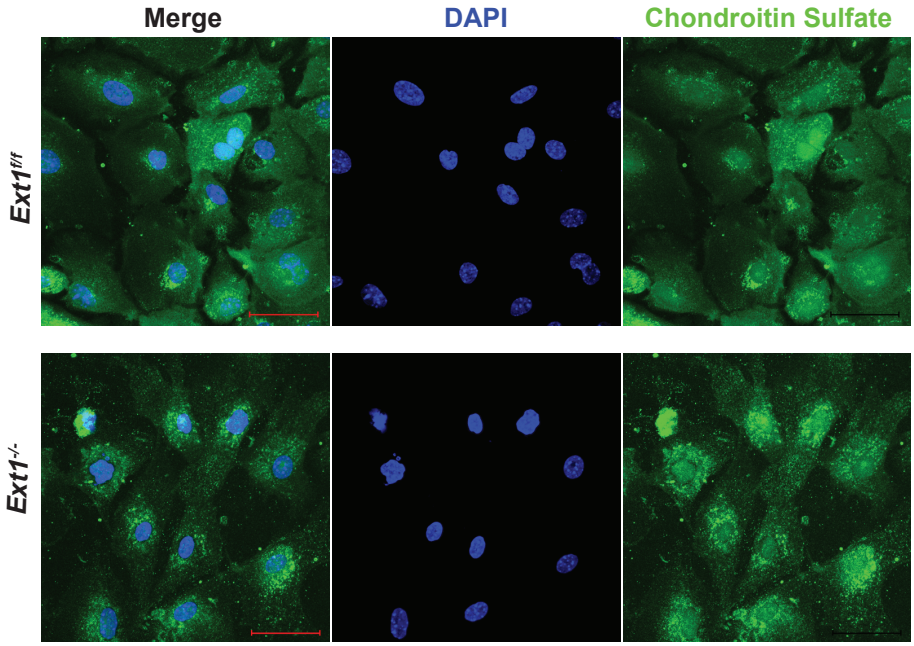


Figure S8. Chondroitin sulfate expression on PrECs. Immunofluorescence of *Ext1*$^{f/f}$ and *Ext1*$^{f/f}$ PrECs from primary prostate spheres were dissociated, cultured as a monolayer and stained for CS epitopes using CS-56 antibody. Scale = 50μm.

Table SI

Primers for mouse genotyping.

Primer	Sequence (5'-3')	Amplicon Size	Comments
Burn 51	GGAGTGTGGATGAGTTGAAG	460bp : floxed allele 389bp : WT allele	Distinguishes between WT and floxed *Ext1* allele
Burn 52	CAACACTTTCAGCTCCAGTC		
Burn 51	GGAGTGTGGATGAGTTGAAG	500bp	Detects *Ext1* deletion band
Burn 29	GAGAACAGGTACCCATGTTC		
P021	CTGAAGAATGGGACAGGCATTG	393bp	Detects the presence of Pb-Cre
C031	CATCACTCGTTGCATCGACC		

Table SII

Primers for amplification of truncated TGFβR2 from pCMV5 HA-TBRII (delta Cyt) plasmid.

Primer	Sequence (5'-3')	Comments
Forward Primer	AAATCTAGAGCCACCATGGGTCGGGGGCTGCTC	TCTAGA - XbaI restriction site GCCACC - Kozak Sequence
Reverse Primer	AAAAAGAATTCTCATGAACTCAGCTTCTGCTGCCG	GAATTC – EcoRI restriction site

Table SIII

Primers for mouse qRT-PCR.

Primer	Sequence (5'-3')	Amplicon Size
Ext1 Forward	TGGAGTCCTGCTTCGATTTC	72bp
Ext1 Reverse	CTTTCTGCTGCGGGTACAC	
p63 Forward	ACAATGCCCAGACTCAATTT	88bp
p63 Reverse	GAGGAGCCGTTCTGAATCTG	
CK5 Forward	GAGATCGCCACCTACAGGAA	117bp
CK5 Reverse	TCCTCCGTAGCCAGAAGAGA	
CK8 Forward	GACATCGAGATCACCACCTA	125bp
CK8 Reverse	GATGAACTCAGTCCTCCTGA	
CDK2 Forward	CTCATCAAGAGCTATCTGTTCC	105bp
CDK2 Reverse	TGCATTGATAAGCAGGTTCT	
CDK4 Forward	GCAGTCTACATACGCAACA	139bp
CDK4 Reverse	AGGCAATCCAATGAGATCAA	
CDK6 Forward	GACTTGACCACTTACTTGGATA	118bp
CDK6 Reverse	GCACTACTCTGTGAGAATGAA	
CyclinA2 Forward	GTCCTTGCTTTTGACTTGGC	139bp
CyclinA2 Reverse	ACGGGTCAGCATCTATCAAAC	
CyclinB1 Forward	AGCGAAGAGCTACAGGCAAG	113bp
CyclinB1 Reverse	TCACACACAGGCACCTTCTC	
CyclinD1 Forward	GCCCTCCGTATCTTACTTCAAG	145bp

CyclinD1 Reverse	GCGGTCCAGGTAGTTCATG	
p15 Forward	CTTTGTGTACCGCTGGGAAC	104bp
p15 Reverse	TTAGCTCTGCTCTTGGGATTG	
p16 Forward	GTGTGCATGACGTGCGGG	146bp
p16 Reverse	GCAGTTCGAATCTGCACCGTAG	
p19 Forward	GCTCTGGCTTTCGTGAACATG	137bp
p19 Reverse	TCGAATCTGCACCGTAGTTGAG	
p21 Forward	TTGCACTCTGGTGTCTGAGC	112bp
p21 Reverse	TCTGCGCTTGGAGTGATAGA	
p27 Forward	GTGGACCAAATGCCTGACTC	122bp
p27 Reverse	TCTGTTCTGTTGGCCCTTTT	
TGFβR1 Forward	TAGCAGCAGACAACAAAGAC	115bp
TGFβR1 Reverse	CCTTCCACAGTAACAGTGTATC	
TGFβR2 Forward	CCAAGTCGGATGTGGAAATGG	103bp
TGFβR2 Reverse	GCCATGACATCACTGTTAAA	
TGFβR3 Forward	GGGAGGTTCACATCCTAAAC	81bp
TGFβR3 Reverse	GGTTCAGATGCAGGGTAAC	
TGFβ1 Forward	GAAGCGGACTACTATGCTAAA	94bp
TGFβ1 Reverse	TACTGTGTGAGATGTCTTTGG	
TGFβ2 Forward	GAGGGATCTTGGATGGAAATG	112bp
TGFβ2 Reverse	GAGGACTTTGGTGTGTTGAG	
TGFβ3 forward	GCATCCACTGTCCATGTCAC	109bp
TGFβ3 Reverse	CCATGGTCATCTTCATTGTCC	
Id1 Forward	AGAACCGCAAAGTGAGCAAG	66bp
Id1 Reverse	GCTGCAGGTCCCTGATGTAG	
Id2 Forward	GCAAAGTACTCTGTGGCTAAA	131bp
Id2 Reverse	CCTGGTGAAATGGCTGATAA	
Id3 Forward	CTGCTACGAGGCGGTGTG	175bp
Id3 Reverse	CACCTGGCTAAGCTGAGTGC	
Col1a1 Forward	GCCAAGAAGACATCCCTGAAG	104bp
Col1a1 Reverse	ATTGTGGCAGATACAGATCAA	
ZFP36L1 Forward	GGGTAACAAGATGCTCAACTA	140bp
ZFP36L1 Reverse	GGTTCTGATGGAACTTGGAGC	
4E-BP1 Forward	GGTCACTAGCCCTACCAG	112bp
4E-BP1 Reverse	GTCCATCTCAAATTGTGACTCT	
E2F1 Forward	CAACTGCTTTCGGAGGACT	149bp
E2F1 Reverse	GTCTCTGAAGAATCCACAGCTT	

Table SIV

Antibodies for Immunohistochemistry and Western Blot

Antibody	Company, Catalog Number	Usage, Dilution
CK5	Covance #PRB-160P	IHC: 1:1000
CK8	Covance #MMS-162P	IHC: 1:2000, WB: 1:1000
p63	Santa Cruz, 4A4	IHC: 1:100, WB: 1:1000
RFP	Rockland, #600-401-379	IHC: 1:1000
Ki67	Novus, #NB500-170	IHC: 1:100
Cleaved Caspase 3	Cell Signaling, #9661	IHC: 1:500

pSmad3	NBP1-77836SS	WB: 1:1000
pSmad2	CST, #3104	ICC: 1:200
Smad2	CST, #3122	ICC: 1:200
GAPDH	Cell Signaling, #2118	WB: 1:5000
Actin	Sigma-Aldrich, A2228	WB: 1:1,000,000
Syndecan-1	Santa Cruz, H-174	WB: 1:1,000
pSmad1	Cell Signaling, #9511	WB: 1:1,000