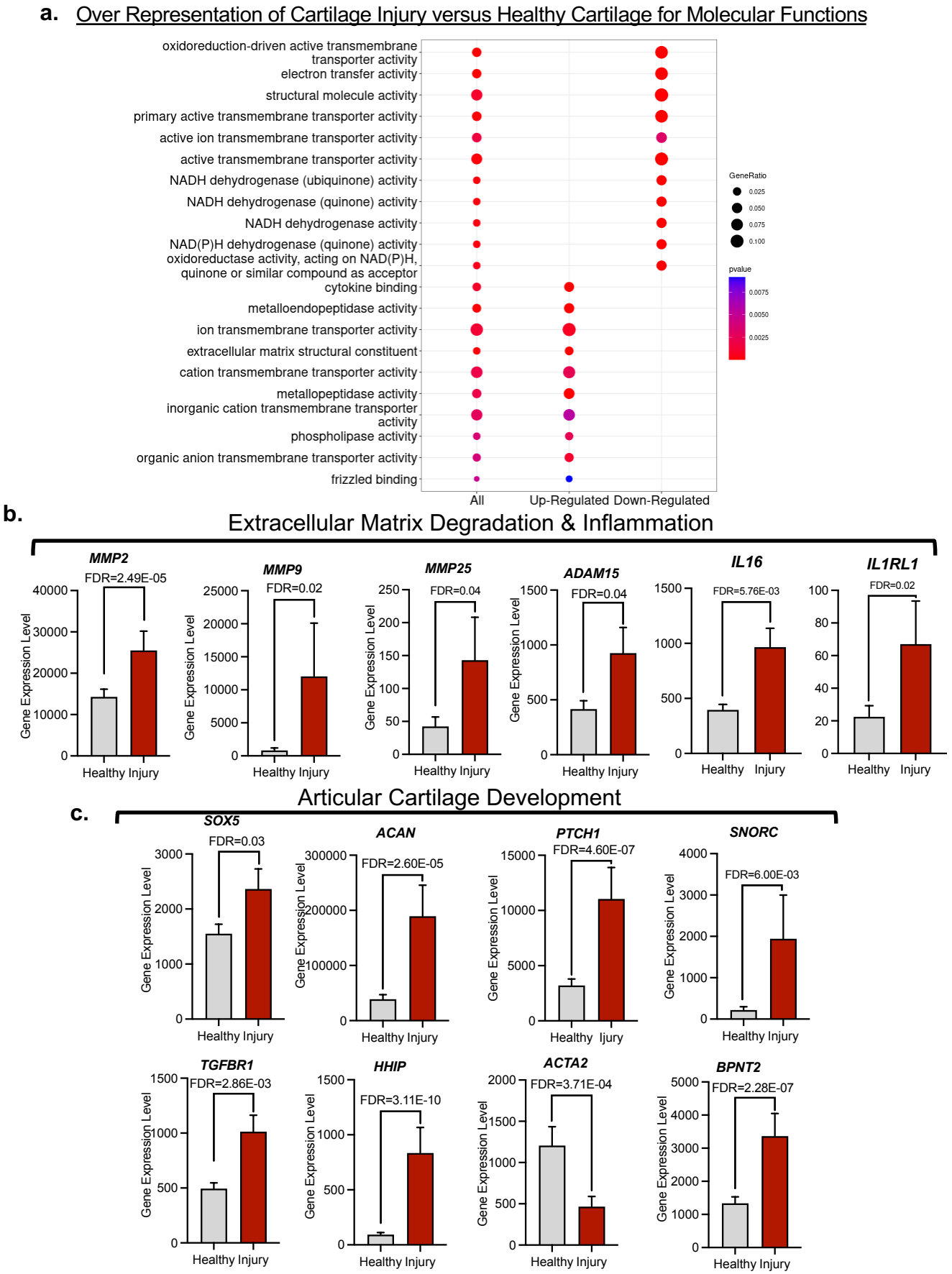
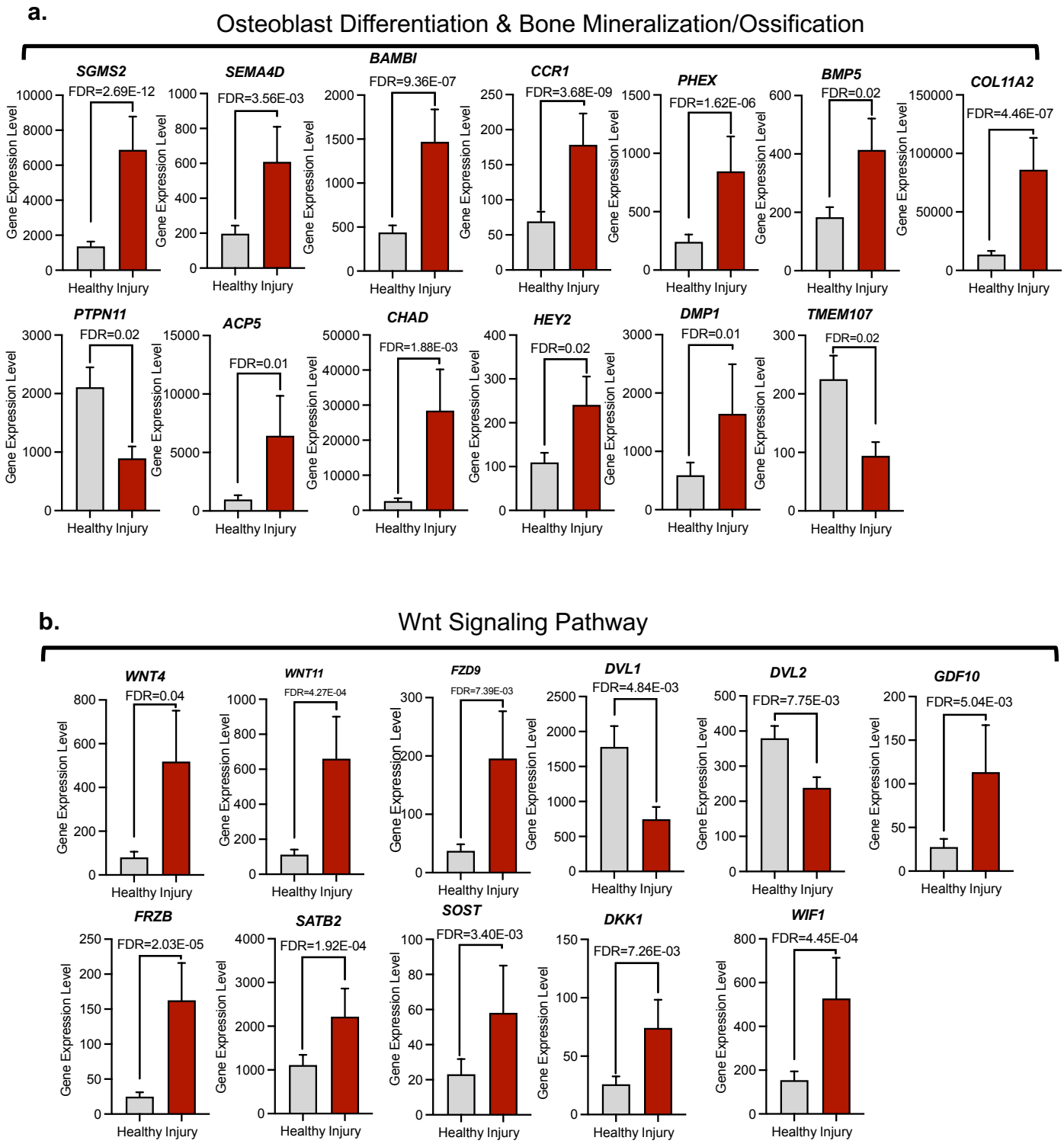
Supplemental Figure 1. Differentially expressed genes related to extracellular matrix degradation, Inflammation, and cartilage development in mini-pig TMJ injury, related to Figure 1.



Supplemental Figure 1. Differentially expressed genes related to extracellular matrix degradation, inflammation, and cartilage development in mini-pig TMJ injury, related to Figure 1. (a) Dot plot of GO terms of molecular functions in injured vs uninjured mini-pig condyles from bulk RNAseq analysis. **(b-c)** Relative gene expression levels of selected extracellular degradation and inflammation genes (**b**) and cartilage development genes (**c**) determined from GO enrichment analyses.

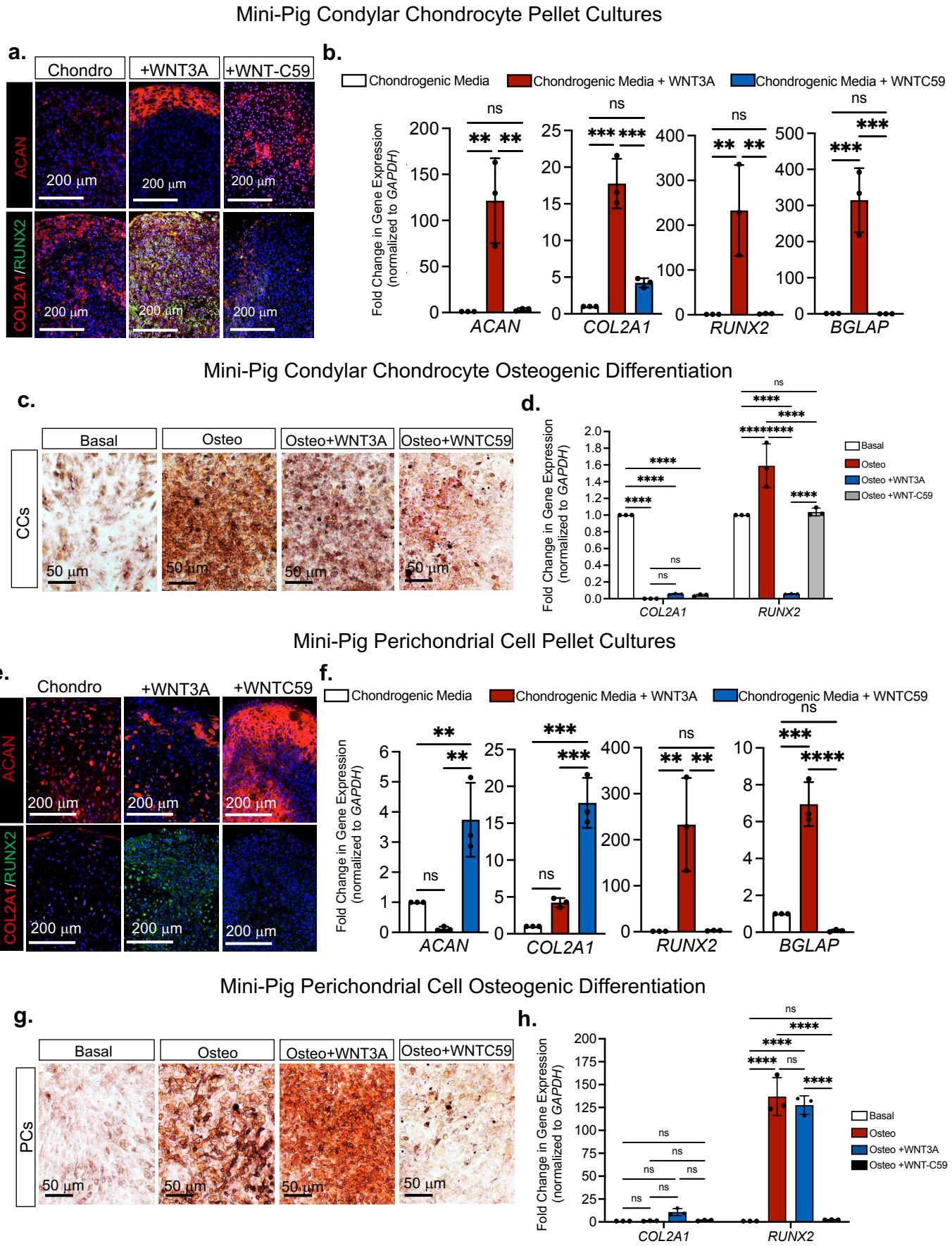
Supplemental Figure 2. Differentially expressed genes associated with osteoblast differentiation, bone mineralization, and Wnt signaling in mini-pig TMJ injury, related to Figure 1



Supplemental Figure 2

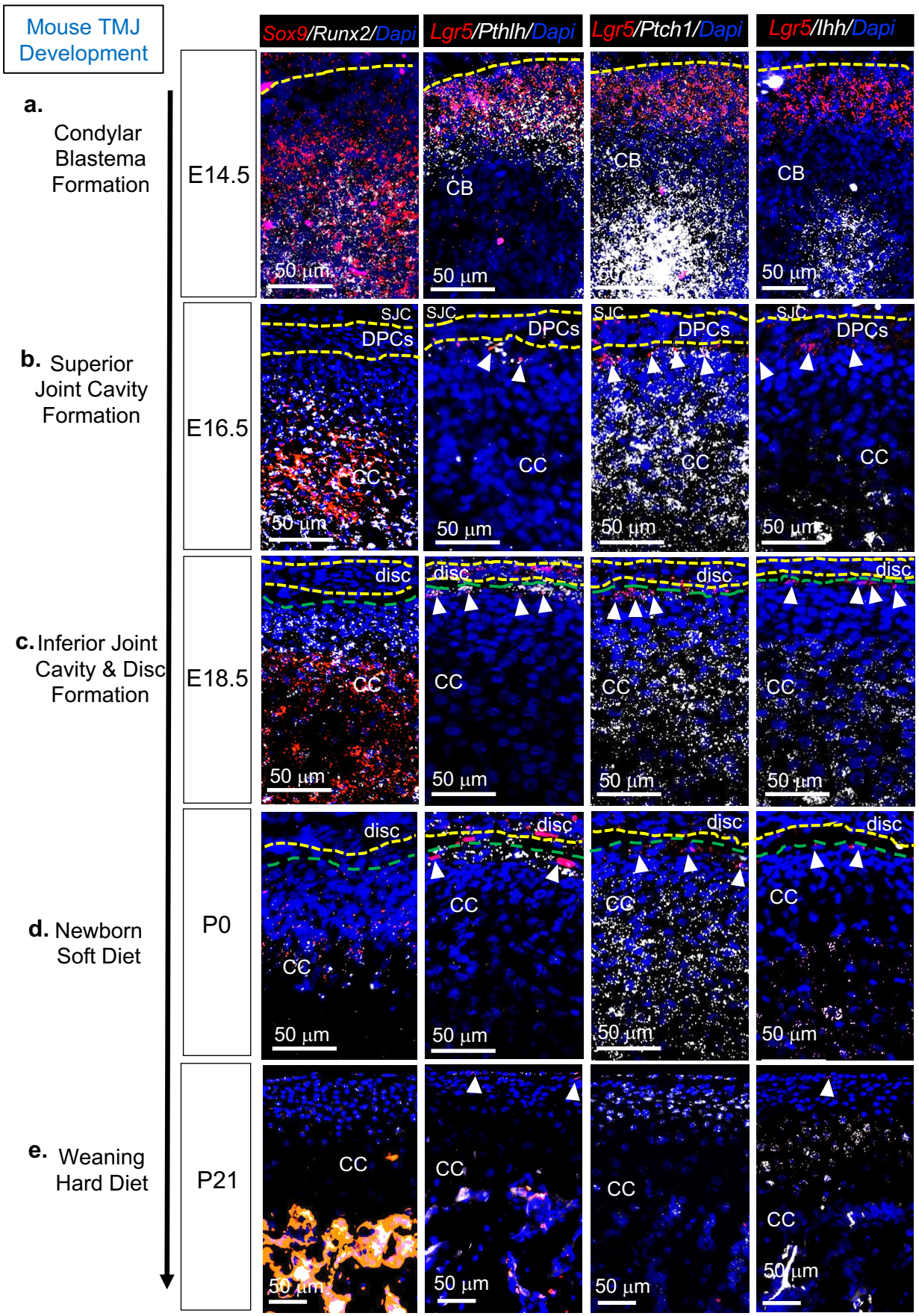
Supplemental Figure 2. Differentially expressed genes associated with osteoblast differentiation, bone mineralization, and Wnt signaling in mini-pig TMJ injury, related to Figure 1. **(a-b)** Relative gene expression levels of selected osteoblast differentiation and bone mineralization/ossification (**a**) and Wnt signaling pathway (**b**) determined from GO enrichment analyses in bulk RNAseq.

Supplemental Figure 3. WNT3a induces loss of chondrocyte identity and osteoblast-like properties in condylar chondrocytes but not in perichondrial cells *in vitro*, related to Figure 1.



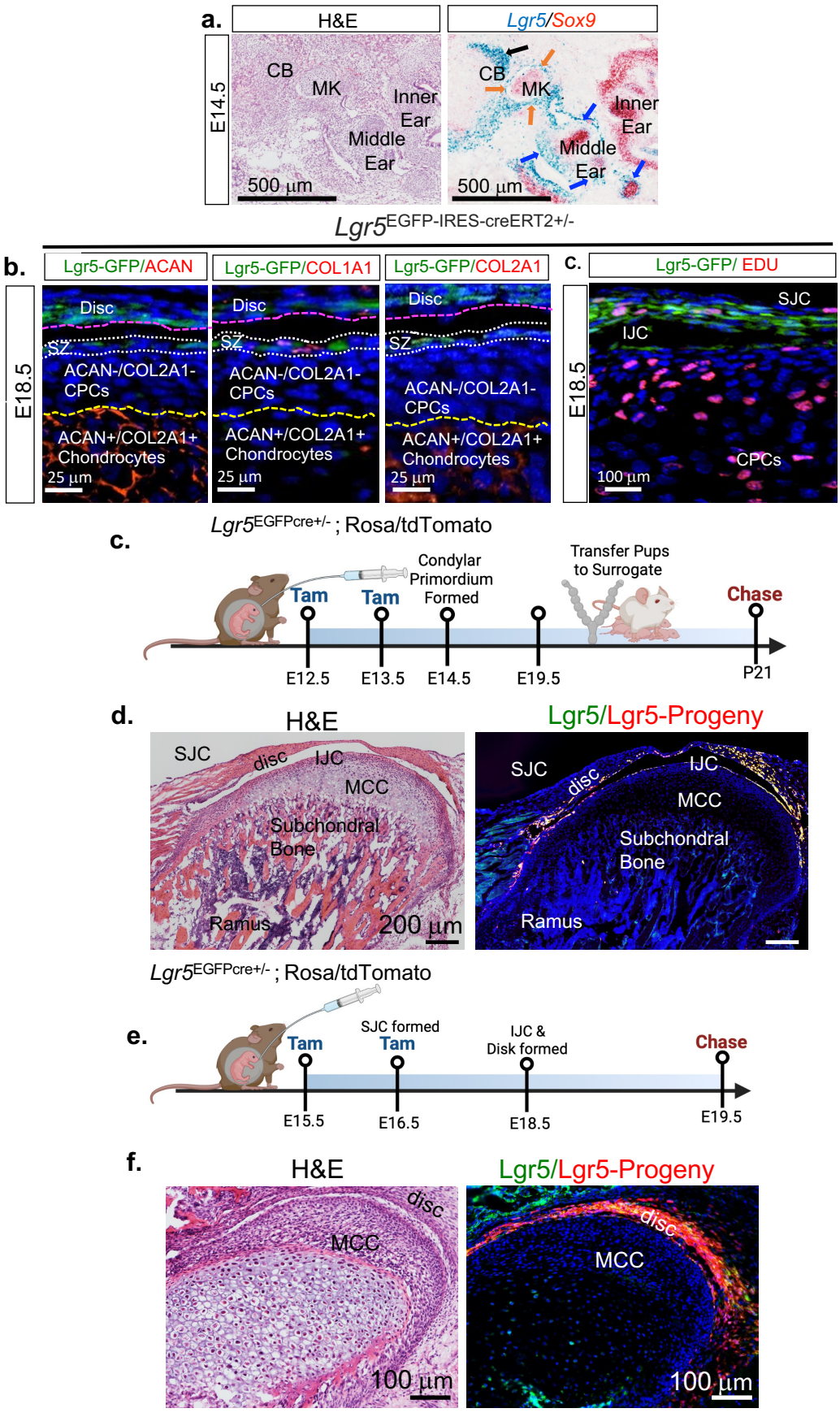
Supplemental Figure 3. WNT3a induces loss of chondrocyte identity and osteoblast-like properties in condylar chondrocytes but not in perichondrial cells *in vitro*, related to Figure 1. (a) Representative immunohistochemistry staining of aggrecan (ACAN), type II collage (COL2A1), and RUNX2 in mini-pig condylar chondrocyte pellet cultures in chondrogenic media (chondro) with WNT3a or WNT-C59. (**b**) qRT-PCR of *ACAN*, *COL2A1*, *RUNX2*, *BGLAP* gene expression in mini-pig condylar chondrocyte pellet cultures. Data presented are mean fold change in gene expression ± SD normalized to GAPDH. **p≤0.01, ***p≤0.001; one-way ANOVA followed by Tukey's post hoc; n=3 pellets. (**c**) Alizarin red staining of mini-pig condylar chondrocytes (CCs) cultured in basal media, osteogenic media, and osteogenic media with WNT3A or WNT-C59. (**d**) qRT-PCR of *COL2A1* and *RUNX2* gene expression in mini-pig condylar chondrocytes. Data presented are mean fold change in gene expression ± SD normalized to GAPDH. ****p≤0.0001; two-way ANOVA followed by Tukey's post hoc; n=3 experiments. (**e**) Representative immunohistochemistry staining of aggrecan (ACAN), type II collage (COL2A1), and RUNX2 in mini-pig perichondrial cell pellet cultures in chondrogenic media (chondro) with WNT3a or WNT-C59. (**f**) qRT-PCR of *ACAN*, *COL2A1*, *RUNX2*, *BGLAP* gene expression in mini-pig perichondrial cell pellet cultures. Data presented are mean fold change in gene expression ± SD normalized to GAPDH. **p≤0.01, ***p≤0.001; one-way ANOVA followed by Tukey's post hoc; n=3 pellets. (**g**) Alizarin red staining of mini-pig perichondrial cells (PCs) cultured in basal media, osteogenic media, and osteogenic media with WNT3A or WNT-C59. (**h**) qRT-PCR of *COL2A1* and *RUNX2* gene expression in mini-pig perichondrial cells. Data presented are mean fold change in gene expression ± SD normalized to GAPDH. ****p≤0.0001; two-way ANOVA followed by Tukey's post hoc; n=3 experiments

Supplemental Figure 4. *Lgr5*-expressing cells are enriched in the outer superficial zone of the perichondrium and differ from chondroprogenitor cells and chondrocytes, related to Figure 2.



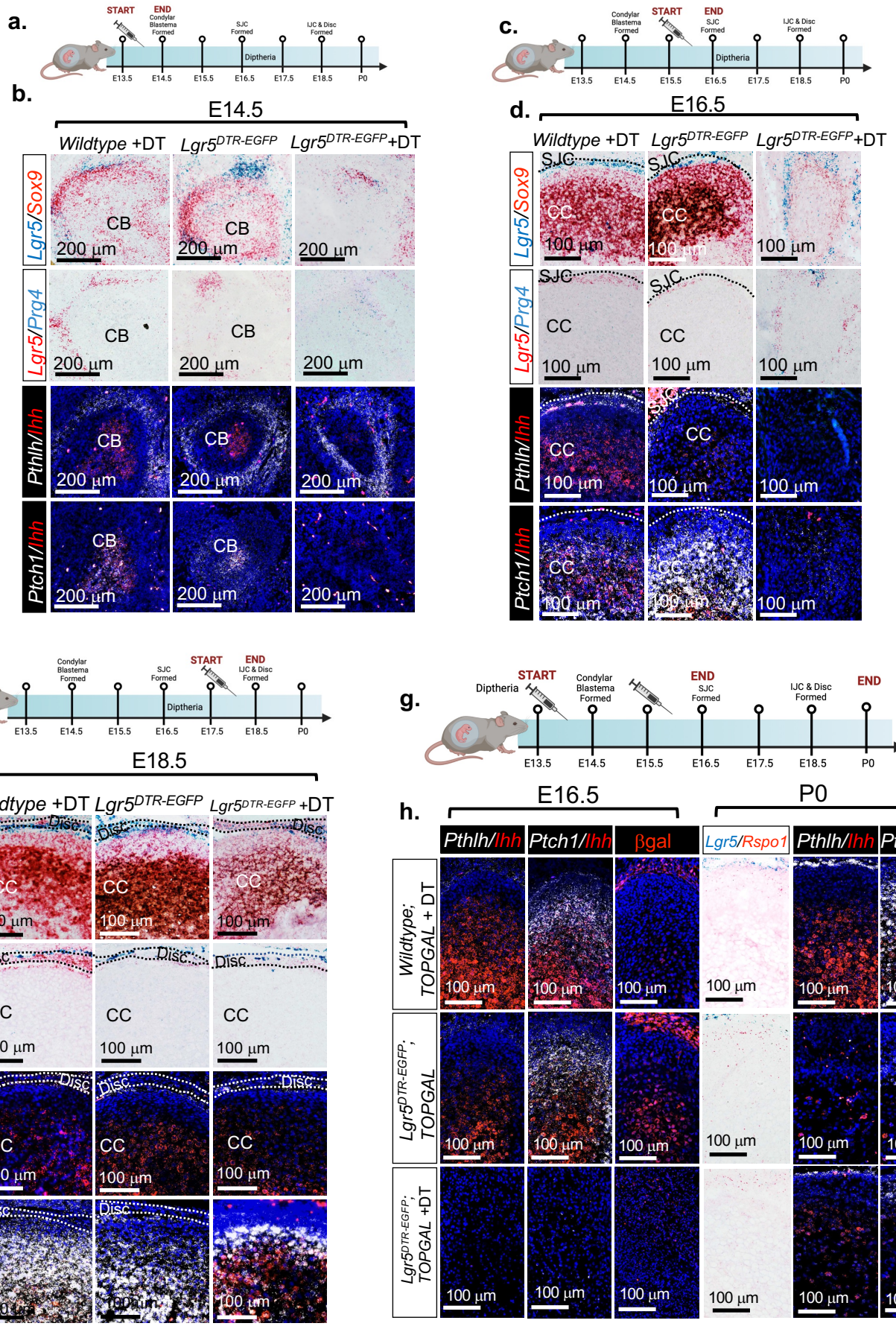
Supplemental Figure 4. *Lgr5*-expressing cells are enriched in the outer superficial zone of the perichondrium and differ from chondroprogenitor cells and chondrocytes, related to Figure 2. **(a-e)** *in situ* hybridization of *Sox9*, *Runx2*, *Lgr5, Pthlh*, *Ptch1*, and *Ihh* in mice during temporomandibular joint morphogenesis at **(a)** E14.5 (condylar blastema formation); **(b)** E16.5 (superior joint cavity formation); **(c)** E18.5 (inferior joint cavity formation); **(d)** P0 (newborn and jaw function); **(e)** P21 (weaning and switch to hard diet). CB=condylar blastema; SJC=superior joint cavity, CC= condylar cartilage, DPC=disc progenitor cells.

Supplemental Figure 5. *Lgr5*-expressing cells are localized to the outer superficial zone of the perichondrium, are not highly proliferative, and supply progeny to disc, perichondrium, periosteum and bone, related to Figures 2-3.



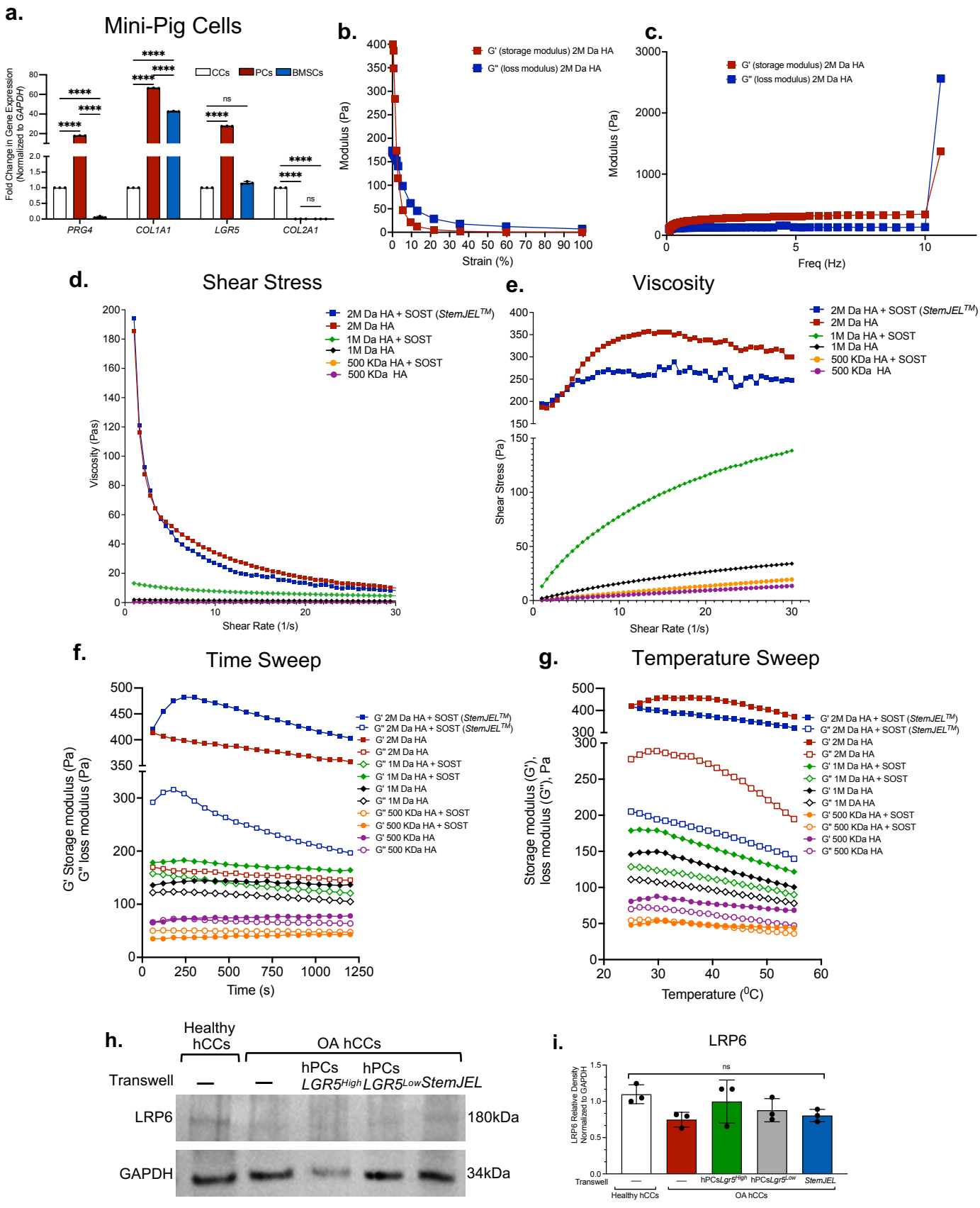
Supplemental Figure 5. *Lgr5*-expressing cells are localized to the outer superficial zone of the perichondrium, are not highly proliferative, and supply progeny to TMJ disc, perichondrium, periosteum and bone, related to Figures 2-3. **(a**) *in situ* hybridization of *Lgr5* (blue) surrounding Meckel's cartilage, condylar blastema and middle ear in the mouse head at E14.5. CB=condylar blastema, MK=Meckel's cartilage. **(b)** Immunohistochemistry of aggrecan (ACAN), type II collagen (COL2A1), and type I collagen (COL1A1) of mandibular condyles from *Lgr5*$^{EFPcre+/-}$ mice at E18.5. SZ=superficial zone, CPCs=chondroprogenitor cells, IJC=inferior joint cavity (IJC), CC= condylar cartilage. **(c)** EDU staining (red) in E18.5 *Lgr5*$^{EFPcre+/-}$ mouse following a 4-hour pulse. SJC=superior joint cavity, IJC=inferior joint cavity. **(c)** Schematic of lineage tracing experiments and tamoxifen-induced recombination in *Lgr5*$^{EGFPcre+/-}$ *; Rosa/tdTomato* mice. Tamoxifen was administered daily at E12.5-E13.5 and Lgr5-progeny were evaluated at P21. To ensure pup survival, a cesarian was performed and pups were transferred to a CD1 surrogate dam. **(d)** H&E and fluorescent images of pups from **a**. SJC=superior joint cavity (SJC), MCC=mandibular condylar cartilage, IJC=inferior joint cavity (IJC). Scale= 200μM. **(c)** Schematic of lineage tracing experiment and tamoxifen-induced recombination in *Lgr5*$^{EGFPcre+/-}$ *; Rosa/tdTomato* mice. **(e)** H&E and fluorescent images of Lgr5-progeny (red) & Lgr5 (green) at E19.5. SJC=superior joint cavity (SJC), MCC=mandibular condylar cartilage, IJC=inferior joint cavity (IJC).

Supplemental Figure 6. Ablation of *Lgr5*-expressing cells disrupts jaw joint morphogenesis, related to Figure 4.



Supplemental Figure 6. Ablation of *Lgr5*-expressing cells disrupts jaw joint morphogenesis, related to Figure 4. **(a)** Experimental timeline of diphtheria toxin administration at E13.5 for 24 hours in *Lgr5*$^{DTR\text{-}EGFP}$ and Wildtype mice. SJC=superior joint cavity; IJC=inferior joint cavity. **(b)** *in situ* hybridization of *Lgr5, SOX9, Prg4, Pthlh, Ptch1, and Ihh* in E14.5 mice in **a**. CB=condylar blastema. **(c)** Experimental timeline of diphtheria toxin administration at E15.5 for 24 hours in *Lgr5*$^{DTR\text{-}EGFP}$ and Wildtype mice. SJC=superior joint cavity; IJC=inferior joint cavity. **(d)** *in situ* hybridization of *Lgr5, SOX9, Prg4, Pthlh, Ptch1, and Ihh* in E16.5 mice in **c**. SJC= superior joint cavity, CC =mandibular condylar cartilage. **(e)** Experimental timeline of diphtheria toxin administration at E17.5 for 24 hours in *Lgr5*$^{DTR\text{-}EGFP}$ and Wildtype mice. SJC=superior joint cavity; IJC=inferior joint cavity. **(f)** *in situ* hybridization of *Lgr5, SOX9, Prg4, Pthlh, Ptch1, and Ihh* in E18.5 mice in E. CC=mandibular condylar cartilage. **(g)** Experimental timeline of diphtheria toxin administration at E13.5 and E15.5 in *Lgr5*$^{DTR\text{-}EGFP}$;Topgal and *Wt*;Topgal mice. Pups were analyzed at E16.5 and P0. **(h)** *in situ* hybridization of *Pthlh, Ihh, Ptch1, Lgr5, Rspo1,* and immunohistochemistry of β-galactosidase (βGgal) in E16.5 and P0 pups from **g**.

Supplemental Figure 7. Isolation of mini-pig TMJ primary cells and rheological properties of *StemJEL*^{TM}, related to Figure 5.



Supplemental Figure 7. Isolation of mini-pig TMJ primary cells and rheological properties of *StemJEL™*, related to Figure 5. **(a)** qRT-PCR of mini-pig condylar cartilage cells (CCs), mini-pig perichondrial cells (PCs), and mini-pig bone marrow stromal stem cells (BMSCs). Data presented are mean fold change in gene expression ± SD normalized to GAPDH. ****p≤0.0001; two-way ANOVA followed by Tukey's post hoc; n=3 experiments. (**b,c**) Storage (G') and loss (G'') moduli of 2 M Da HA was measured under strain (**b**) and frequency (**c**) sweep tests to determine the linear viscoelastic limit, where 1% strain and 10 Hz were selected. Shear stress **(d)** and viscosity (**e**) was measured as a function of shear rate. Storage (G') and loss moduli (G'') of hydrogels was measured using time (**f**) and temperature (**g**) sweep tests. (**h**) Western blot of LRP6 hCCs cultured**. (i)** Quantification of western blot of LRP5 in hCCs in **h**. Data presented are mean density ± SD normalized to GAPDH; one-way ANOVA followed by Tukey's post hoc; n=3 experiments.

Supplemental Tables

Supplemental Table 1: Gene Ontology, related to Figure 1.

Biological Process/Molecular Function	Gene Ontology
ATP synthesis coupled electron transport	0042773
aerobic electron transport chain	0019646
mitochondrial ATP synthesis coupled electron transport	0042775
oxidative phosphorylation	0006119
respiratory electron transport chain	0022904
electron transport chain	0022900
aerobic respiration	0009060
mitochondrial electron transport, NADH to ubiquinone	0006120
cellular respiration	0045333
ATP metabolic process	0046034
oxidoreduction-driven active transmembrane transporter activity	0015453
electron transfer activity	0009055
cartilage development	0051216
ossification	0001503
biomineral tissue development	0031214
biomineralization	0110148
regulation of ossification	0030278
regulation of biomineral tissue development	0070167
regulation of biomineralization	0110149
bone mineralization	0030282
frizzled binding	0005109

Supplemental Table 2: Mice & PCR Primers for Genotyping, related to STAR Methods.

Mouse	Source	Direction	Sequence
Prg4^{tm1Mawa}*/J*	Jackson Laboratory 025737	Forward (Common)	GGAAGGAGGGACAACACTGA
		Reverse (Wildtype)	TTTGTTGCAGTAGTCTCTTTCG
		Reverse (Mutant)	CCATGCTCCCCACTTTGCGT
Tg (TCF/Lef1-lacZ)34Efu/J	Jackson Laboratory 004623	Forward (Transgene)	ATCCTCTGCATGGTCAGGTC
		Reverse (Transgene)	CGTGGCCTGATTCATTCC
		Forward (IPC)	CAAATGTTGCTTGTCTGGTG
		Reverse (IPC)	GTCAGTCGAGTGCACAGTTT
Gt(ROSA)$^{26Sortm9(CAG-tdTomato)Hze}$/J	Jackson Laboratory 007909	Forward (Wildtype)	AAGGGAGCTGCAGTGGAGTA
		Reverse (Wildtype)	CCGAAAATCTGTGGGAAGTC
		Forward (Mutant)	GGCATTAAAGCAGCGTATCC
		Reverse (Mutant)	CTGTTCCTGTACGGCATGG
Lgr5$^{DTR-EGFP}$	Genentech	Forward	AAGTTCATCTGCACCACCG
		Reverse	TCCTTGAAGAAGATGGTGCG
Lgr5$^{tm1(cre/ERT2)Cle}$*/J*	Jackson Laboratory 008874	Forward	CCGGGCTGCCACGACCAA
		Reverse	GGCGCGGCAACACCATTTTT
CD1-IGS	Charles River 022		

Supplemental Table 3: Primary antibodies used for immunohistochemistry, related to STAR Methods.

Primary Antibody	Source/Catalogue #	Dilution	Species
Mouse anti-Aggrecan	ThermoFisher MA3-16888	1:100	Mouse, Pig
Rabbit anti-βcatenin	Abcam ab6302	1:100	Mouse, Pig
Rabbit anti-βgalactosidase	MBL PM049	1:100	Mouse
Rabbit anti-Collagen I	Abcam ab34710	1:100	Mouse
Mouse anti-Collagen I	Invitrogen ma1-26771	1:100	Pig, Human
Mouse anti-Collagen IIA	Millipore MAB8887	1:100	Mouse, Pig, Human
Rabbit anti-Osteocalcin	EMD Millipore ab10911	1:100	Mouse, Pig, Human
Goat anti-Periostin	R&D Systems, af2955	1:100	Mouse
Rabbit anti-Runx2	Abcam ab23981	1:100	Mouse, Pig

Supplemental Table 4: Secondary antibodies used for immunohistochemistry & western blot, related to STAR Methods.

Secondary Antibody	Source	Dilution
Goat anti-mouse HRP	Invitrogen G21040	1:5000
Goat anti-rabbit HRP	Invitrogen G21234	1:5000
Goat anti-rabbit Alexa Fluor 546	Invitrogen A11010	1:2000
Goat anti-rabbit Alexa Fluor 488	Invitrogen A11008	1:1000
Goat anti-mouse Alexa Fluor 546	Invitrogen A11003	1:2000
Goat anti-mouse Alexa Fluor 488	Invitrogen A11001	1:1000
Donkey anti- rabbit Alexa Fluor 647	Invitrogen A32795	1:2000

Supplemental Table 5: qRT-PCR Primers, related to STAR Methods.

Gene	Direction	Sequence
Ss GAPDH	Forward	ATCCTGGGCTACACTGAGGAC
	Reverse	AAGTGGTCGTTGAGGGCAATG
Ss COL2a1	Forward	GAGAGGTCTTCCTGGCAAAG
	Reverse	AAGTCCCTGGAAGCCAGAT
Ss LGR5	Forward	CCTTGGCCCTGAACAAAATA
	Reverse	ATTTCTTTCCCAGGGAGTGG
Ss WNT3A	Forward	AGTACTCATCCCTGGGGACA
	Reverse	GGAACTGGTGTTGGCACTCT
Ss PRG4	Forward	ACTGTGGAGAGGACTTCCGA
	Reverse	ACTTCTCACCTTTAGTCACCATT
Ss ACAN	Forward	CCCACCTTTCTCCCTTCTATTC
	Reverse	TCTGGAAACCACTGCTTCTATT
Ss RUNX2	Forward	GAGAGAGAGAGACAGAGAGAAAGA
	Reverse	GGAAAGACACAGAGGAGGTAAAG
Ss SOST	Forward	AGCCAACACTTCTAGCACTTA
	Reverse	ACCTCTCTGTGCTTCCTATCT
Ss BGLAP	Forward	ATGAAGGAAGAGGAGCAAAGAG
	Reverse	ACCACTAGGCTTTGCATCTG
Ss AXIN2	Forward	GAG GGA GGA ATG CGT GGA TA
	Reverse	GGT TTC AGC TGC TTG GAG AC
Ss LEF1	Forward	GGA AAG TGA CTT AGG CGA CA
	Reverse	CTT TCC GTC ATC AGG GTG TT
Ss MMP13	Forward	GGA CCC AGG TGA AGC ATT TA
	Reverse	CCT GCT TCA GTC AAC TAT CTT T
Ss ADAMTS4	Forward	CTC CTG GCT GAA TGG CTT ATA G
	Reverse	CTC TCT CTC CCG TGT CTA TGT
Ss ADAMTS5	Forward	CTT GGG ACC ACC TCA ACA CC
	Reverse	GGG TCA TTG GTA AGG GTC AG
Ss COMP	Forward	ACA GCG ACC AAG ACA AGT AAG

	Reverse	GAG AGA CAA TGA GAC CTC AGA AAG
Hs GAPDH	Forward	CATGAGAAGTATGACAACAGCCT
	Reverse	AGTCCTTCCACGATACCAAAGT
Hs LGR5	Forward	AGTTACGTCTTGCGGGAAAC
	Reverse	AGCTTCTGTGGGTACGTGTC
Hs BGLAP	Forward	TCACACTCCTCGCCCTATTG
	Reverse	CTCTTCACTACCTCGCTGCC
Hs COL2a1	Forward	GCTCCTGCCGTTTCGCTG
	Reverse	ATTATACCTCTGCCCATCCTGC
Hs ACAN	Forward	CTTCCGCTGGTCAGATGGAC
	Reverse	CGTTTGTAGGTGGTGGCTGT
Hs ADAMTS-5	Forward	CAAAGGCCTTCTGCGTTTAAG
	Reverse	CAGTGCTGAATCCTCCAGTTA
Hs ADAMTS-4	Forward	GCC CGC TTC ATA ACT GA
	Reverse	CAA TGG AGC CTC TGG TTT GTC
Hs RUNX2	Forward	AGT AAG AAG AGC CAG GCA GGT
	Reverse	TGG CTG GAT AGT GCA TTC GT
Mm Gapdh	Forward	GTGGAGATTGTTGCCATCAACGA
	Reverse	CCCATTCTCGGCCTTGACTGT
Mm Lgr5	Forward	TCTTCTAGGAAGCAGAGGCG
	Reverse	CAACCTCAGCGTCTTCACCT
Mm Bglap	Forward	ACACCATGAGGACCATCTTTC
	Reverse	GAGACCTTCAGGAGGGTAGTT A
Mm Col2a1	Forward	GCAAGATGAGGGCTTCCATA
	Reverse	CTACGGTGTCAGGGCCAG
Mm Dkk3	Forward	GTGATTGACAGGTGGTGTAGAG
	Reverse	CAGGCAACAGAGGACAGAAA
Mm Sost	Forward	GAAAGACCTGGGACTGGTTATG
	Reverse	TCAGGGTCAGAAACCCTATCT
Mm Prg4	Forward	GAAAATACTTCCCGTCTGCTTGT
	Reverse	ACTCCATGTAGTGCTGACAGTTA
Mm Wnt3a	Forward	CACCACCGTCAGCAACAGCC
	Reverse	AGGAGCGTGTCACTGCGAAAG
Mm Comp	Forward	GCG TAA CTC GGA CAG TGA TAA G
	Reverse	GGC ATC CAG CTC TGT ATC TTT C
Mm Lrp4	Forward	GGCAAAAAGCAGGAACTTGT
	Reverse	TCTACCCAGTGGCCAGAACT
Mm Lrp5	Forward	CTTCCACACACCATGCGAGG
	Reverse	GTCAGACAGGTCATGTACTC
Mm Lrp6	Forward	CTGGATTATTGTCCCCGGAT
	Reverse	GTGTGTATTCCAGTCAGTCC

Supplemental Table 6, related to STAR Methods: Primary antibodies used for western blot

Primary Antibody	Source/Catalogue #	Dilution
Rabbit anti-total βcatenin	Invitrogen 71-2700	1:4000
Rabbit anti-non-phospho (active)-βcatenin	Cell Signaling 8814	1:1000
Rabbit anti-LRP5	Proteintech 24899-1-AP	1:1000
Rabbit anti-LRP6	Cell Signaling 3395	1:1000
Mouse anti-CD44	Proteintech 60224-1	1:5000
Mouse anti-GAPH	Invitrogen MA116757	1:2000

Supplemental Table S7: *in situ* Hybridization Probes

Gene Probe	Source/Catalogue #	Channel
Mm Lgr5	ACD/312171	C1
Mm Col10a1	ACD/426181	C1
Mm Ihh	ACD/413091	C1
Mm Sost	ACD/410031	C1
Mm Prg4	ACD/437661	C1
Mm Lgr5	ACD/312171	C2
Mm Sox9	ACD/401051	C2
Mm Ptch1	ACD/402811	C2
Mm Lgr6	ACD/404961	C2
Mm Col2a1	ACD/407221	C2
Mm Rspo1	ACD/479591	C2
Mm Runx2	ACD/414021	C3
Mm Pthlh	ACD/456521	C3
Mm Dkk3	ACD/400931	C3