Supplemental Information:

Supplemental Methods:

Gene Expression Analysis:

RNAscope™ was utilized to quantify and localize transcripts of gene expression in 4% paraformaldehyde fixed mandibular condyles. Manufacture's protocols were followed for the RNAscope™ assays using the ACD 2.5 HC Assay-Brown and 2.5HD Duplex Assay kits catalog #322300 and #322430 respectively. The following RNAscope™ probes were used: Mm-*Adamts5* #427621, Mm-*Adamts5*-01 #518711-C2, Mm-*Bglap*-01 #478941, Mm-*Mmp13* #427601, Mm-*Ctsk* #464071, Mm-*Vcantv2* #428311 and Mm-*Gli1* #311001.

Microscopy, Quantification:

Adamts5$^{+/+}$ and *Adamts5*$^{-/-}$ Safranin O/Fast Green images were acquired with a Zeiss Axio.A1 Microscope. ZenBlue™ was used to measure the distance of bone marrow infiltration. To standardize the distance from the hypertrophic chondrocytes to bone marrow (defined by the characteristic dark purple, round cells within the trabecular spaces) the widest portion of the condylar head in the saggital plane was chosen. For each animal the distance was measured and averaged from 3 serial sections.

For RNAscope™ quantification, *Adamts5*$^{+/+}$ and *Adamts5*$^{-/-}$ images were acquired with a Zeiss Axio.A1 Microscope. Pixel intensity of *Bglap, Mmp13* and *Mmp9* was measured by calculating the total number of positive pixels above a standardized threshold. *Bglap, Mmp13* and *Mmp9* positive pixels were normalized to the subchondral bone volume (8 µm depth; *n* = 5/genotype), and *Mmp13* positive pixels were normalized to the hypertrophic and subchondral bone volume (8 µm depth; *n* = 3+/genotype for each age group). Since the *Vcan and Gli1* Riboprobe hybridization signals were relatively weak, the number of positive *Vcan and Gli1* cells were counted and divided by the total number of cells in the transition zone and expressed as percent Vcan and Gli1 cells.

Supplemental Table 1: **Male *Adamts5*$^{+/+}$ and *Adamts5*$^{-/-}$ mice demonstrate more significant alterations in total volume, bone volume/total volume, and trabecular spacing compared to female *Adamts5*$^{+/+}$ and *Adamts5*$^{-/-}$.** Mean values were reported for total volume (TV), bone volume (BV), bone volume/total volume (BV/TV), trabecular thickness (TbTh) and trabecular spacing (TbSp) within each group (*Adamts5*$^{+/+}$ female [+/+ ♀]; *Adamts5*$^{+/+}$ male [+/+ ♂]; *Adamts5*$^{-/-}$ female [-/- ♀]; *Adamts5*$^{-/-}$ male [-/- ♂]; gray boxes). The *P*-values are shown for all of the combination of comparisons with *P*-values (<0.05) shown in bold. The 95% confidence intervals are provided below the *P* value.

Table 1:

	Group ID		+/+	-/-
		Mean	0.2792	0.3292
Total Volume (TV)	+/+	0.2792	-	-
	-/-	0.3292	***P*=0.0053** 95%CI 0.01546 to 0.08456	-
	Group ID		+/+	-/-
		Mean	0.2173	0.2385
Bone Volume (BV)	+/+	0.2173	-	-
	-/-	0.2385	*P*=0.0869 95%CI 0.003168 to 0.04561	-

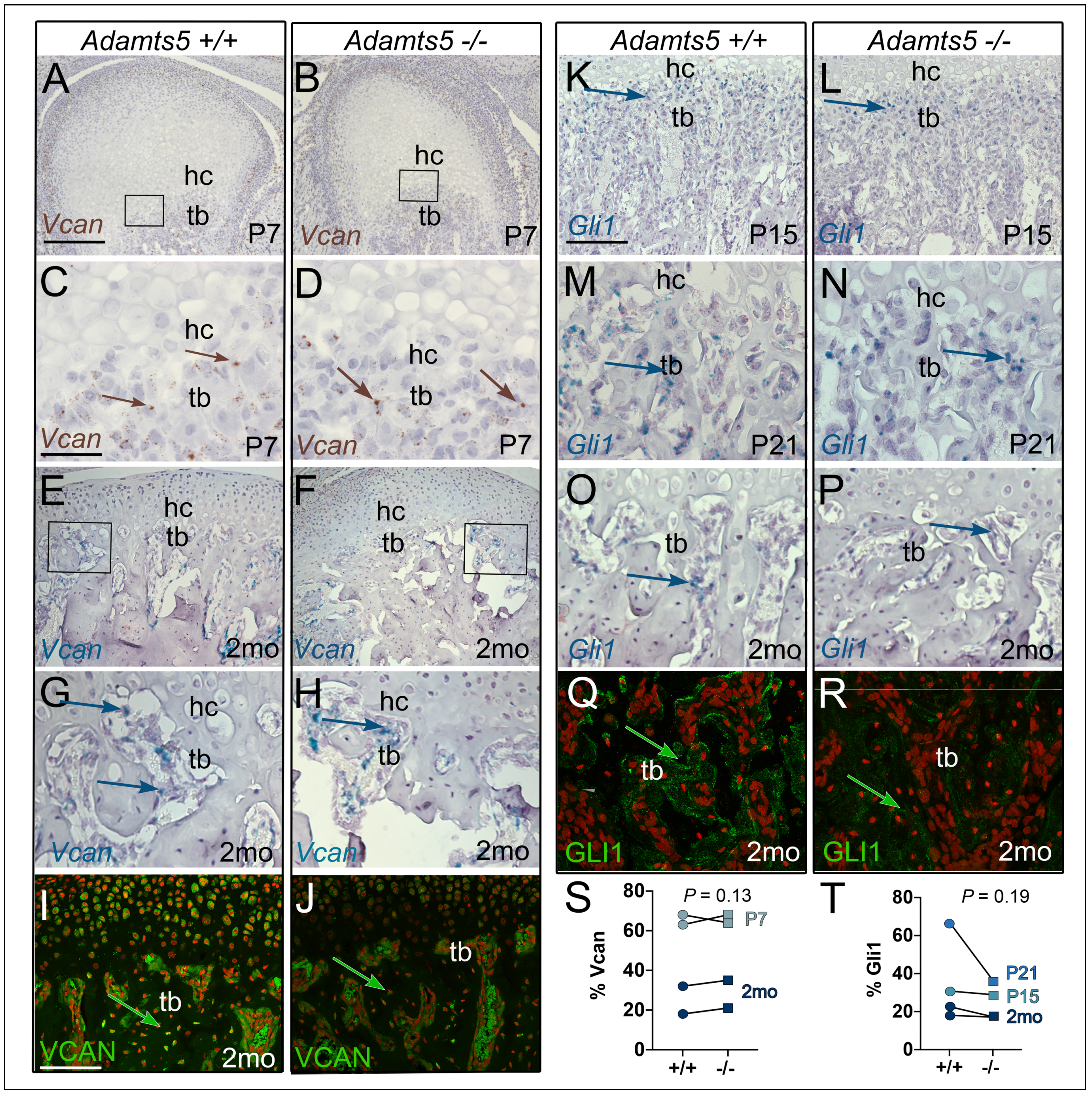
	Group ID		+/+	-/-
		Mean	78.58	69.93
Bone Volume/ Total Volume (BV/TV)	+/+	78.58	-	-
	-/-	69.93	**_P_<0.0001** 95%CI -12.55 to -4.757	-
	Group ID		+/+	-/-
		Mean	0.07162	0.06559
Trabecular Thickness (Tb Th)	+/+	0.07162	-	-
	-/-	0.06559	**_P_=0.0116** 95%CI -0.01066 to -0.001398	-
	Group ID		+/+	-/-
		Mean	0.03876	0.04926
Trabecular Spacing (Tb Sp)	+/+	0.03876	-	-
	-/-	0.04926	**_P_<0.0001** 95%CI 0.006140 to 0.01487	-

	Group ID		+/+♀	+/+ ♂	-/- ♀	-/- ♂
		Mean	0.2886	0.2653	0.3200	0.3409
Total Volume (TV)	+/+ ♀	0.2886	-	-	-	-
	+/+ ♂	0.2653	_P_=0.7266 95%CI 0.08255 to 0.03594	-	-	-

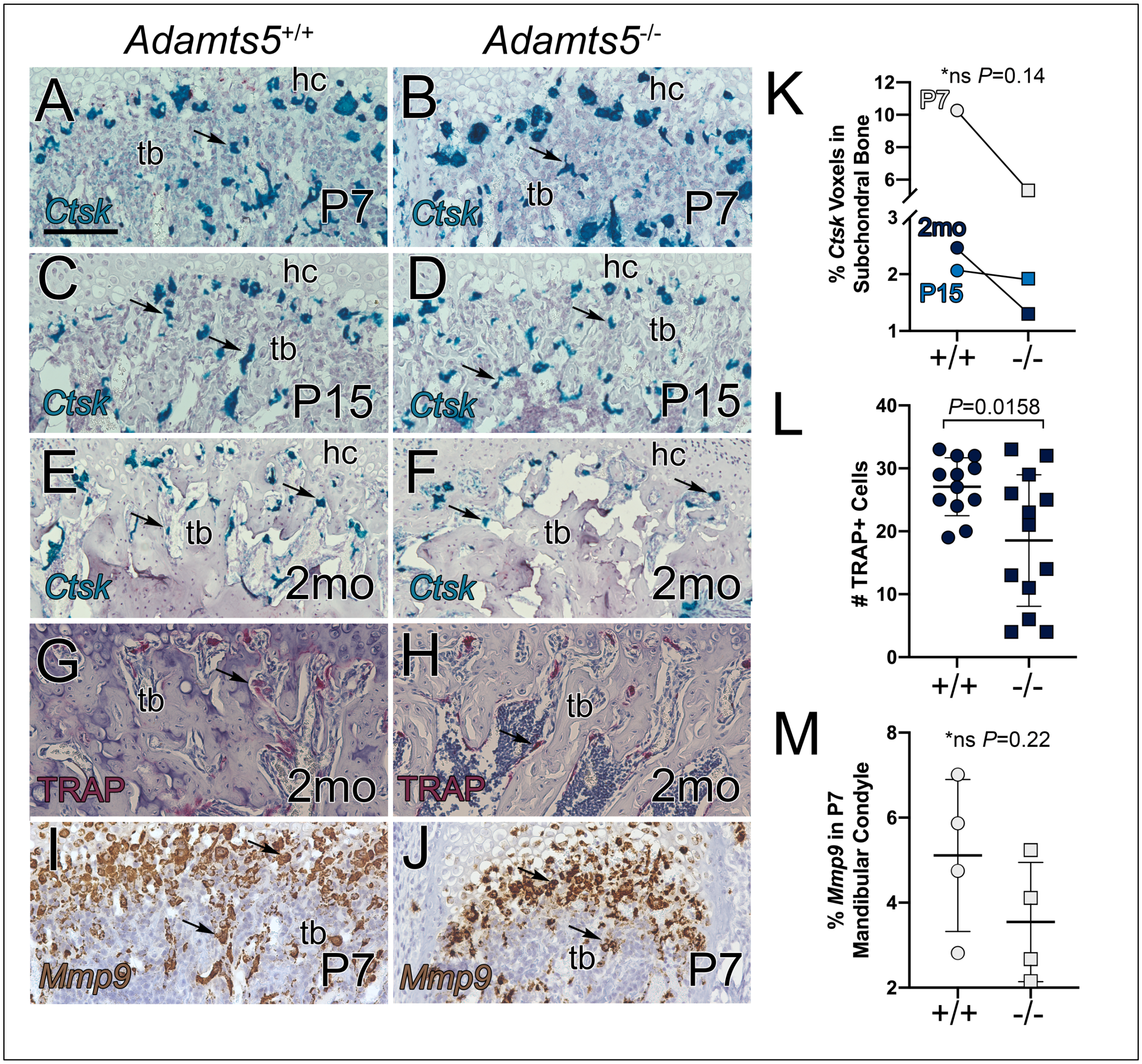
	-/- ♀	0.3200	*P*=0.5221 95%CI 0.09186 to 0.02913	*P*=0.1356 95%CI 0.1204 to 0.01109	-	-
	-/- ♂	0.3409	*P*=0.1601 95%CI 0.1176 to 0.01307	***P*=0.0302** 95%CI -0.1458 to -0.005339	*P*=0.8651 95%CI 0.05038 to 0.09220	-

Bone Volume (BV)	Group ID		+/+♀	+/+ ♂	-/- ♀	-/- ♂
		Mean	0.2273	0.2027	0.2364	0.2408
	+/+ ♀	0.2273	-	-	-	-
	+/+ ♂	0.2027	*P*=0.4267 95%CI 0.06712 to 0.01791	-	-	-
	-/- ♀	0.2364	*P*=0.9442 95%CI 0.05257 to 0.03426	*P*=0.2429 95%CI 0.08095 to 0.01343	-	-
	-/- ♂	0.2408	*P*=0.8528 95%CI 0.05792 to 0.03092	*P*=0.1673 95%CI 0.08622 to 0.0101	*P*=0.9954 95%CI0.04457 to 0.05325	-
Bone Volume/ Total Volume (BV/TV)	Group ID		+/+♀	+/+ ♂	-/- ♀	-/- ♂
		Mean	79.50	77.22	73.82	65.73
	+/+ ♀	79.50	-	-	-	-
	+/+ ♂	77.22	*P*=0.79 95%CI -8.732 to 4.171	-	-	-
	-/- ♀	73.82	*P*=0.11 95%CI -0.9035 to 12.27	*P*=0.59 95%CI -3.757 to 10.56	-	-
	-/- ♂	65.73	***P*<0.001** 95%CI 7.031 to 20.51	***P*<0.001** 95%CI 4.190 to 18.79	***P*=0.03** 95%CI -15.51 to -0.6663	-

Trabecular Thickness (Tb Th)	Group ID		+/+♀	+/+ ♂	-/- ♀	-/- ♂
		Mean	0.07155	0.07173	0.06757	0.06346
	+/+♀	0.07155	-	-	-	-
	+/+ ♂	0.07173	*P*=0.99 95%CI-0.007955 to 0.00833	-	-	-
	-/- ♀	0.06757	*P*=0.59 95%CI -0.004340 to 0.01229	*P*=0.62 95%CI -0.004875 to 0.0132	-	-
	-/- ♂	0.06346	*P*=0.07 95%CI 0.0004230 to 0.01659	*P*=0.09 95%CI -0.0009429 to 0.01749	*P*=0.65 95%CI -0.01348 to 0.005256	-
Trabecular Spacing (Tb Sp)	Group ID		+/+♀	+/+ ♂	-/- ♀	-/- ♂
		Mean	0.03600	0.04280	0.04507	0.05377
	+/+♀	0.03600	-	-	-	-
	+/+ ♂	0.04280	*P*=0.06 95%CI -0.0001368 to 0.01374	-	-	-
	-/- ♀	0.04507	***P*=0.007** 95%CI -0.01615 to -0.001989	*P*=0.86 95%CI -0.009970 to 0.005427	-	-
	-/- ♂	0.05377	***P*=0.001** 95%CI -0.02502 to -0.01052	***P*=0.003** 95%CI -0.01882 to -0.003119	***P*=0.03** 95%CI 0.0007185 to 0.01668	-



Supplemental Figure 1: Gli1 and Vcan expression are not significantly different in trabecular bone of the *Adamts5* deficient mandibular condyles. *Vcan* expression was evaluated at P7 (A-D) and 2mo (E-J) using RNAscope™ (A-H) and IHC (I, J) *Adamts5* deficient mandibular condyles (n=4) and compared to wildtype (n=4) (S). *Gli1* expression was examined in the developing mandibular condylar transition zone at P15, P21 and 2mo using RNAscope™ (K-P) and IHC (Q, R) in *Adamts5* deficient mandibular condyles (n=4) compared to wildtype (n=4). Quantification of *Vcan* and *Gli1* expression expressed as % positive cells of the total trabecular cells is shown in S (*Vcan*) and T (*Gli1*). The black lines in S and T are drawn from the wildtype to *Adamts5* deficient values in each timepoint pair examined. In graphs circles depict wildtype and squares indicate *Adamts5* deficient values. Each symbol on the graph represents measurements from one condyle. Brown arrows in C, D, blue arrows in G, H -mRNA expression of *Vcan*; green arrows in I, J -Vcan IHC localization. Blue arrows in K-P -mRNA expression of *Gli1*; green arrows in Q, R -Gli1 IHC localization. Bar in A = 250 μm applies to B, F, G; bar in C = 75 μm applies to D, G, H, M-P; bar in I = 50 μm applies to J, Q, R. S-95%CI -4.877 to 19.38; T-95%CI -92.87 to 14.87.



Supplemental Figure 2: ADAMTS5 deficiency reduces osteoclasts at 2 months in the mandibular condylar trabecular bone. *Ctsk* (osteoclast marker) expression was evaluated in the developing mandibular subchondral bone of wildtype (n=3) and *Adamts5* deficient (n=3) mice at P7 (A,B), P15 (C,D), and 2mo (E, F), (blue-green; black arrows). TRAP staining at 2mo is shown in G (wildtype, n=12), H (*Adamts5* deficient (n=13) mandibular condyles (black arrows). Expression of Mmp9 generated by hypertrophic chondrocytes and osteoclasts is shown in I, J (black arrows). Quantification of osteoclasts is shown in graphs: *Ctsk* (K; 95% CI 0.2770 to 1.453), TRAP staining (L; 95% CI -15.33 to -1.759) and *Mmp9* (M; 95% CI -4.345 to 1.136). In graphs circles depict wildtype and squares indicate *Adamts5* deficient values. Each symbol on the graph represents measurements from one condyle. Bar in A = 50 μm applies to B-D; bar in E = 100 μm applies to F-H.