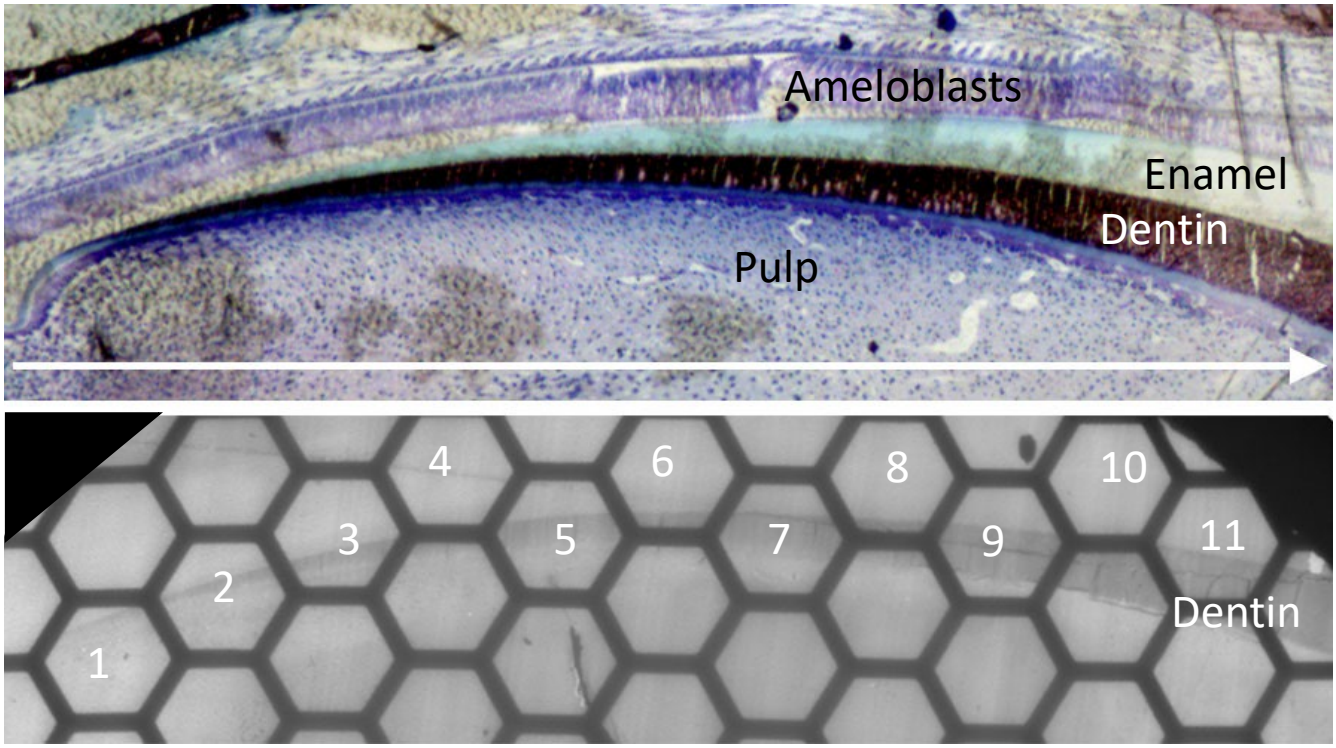
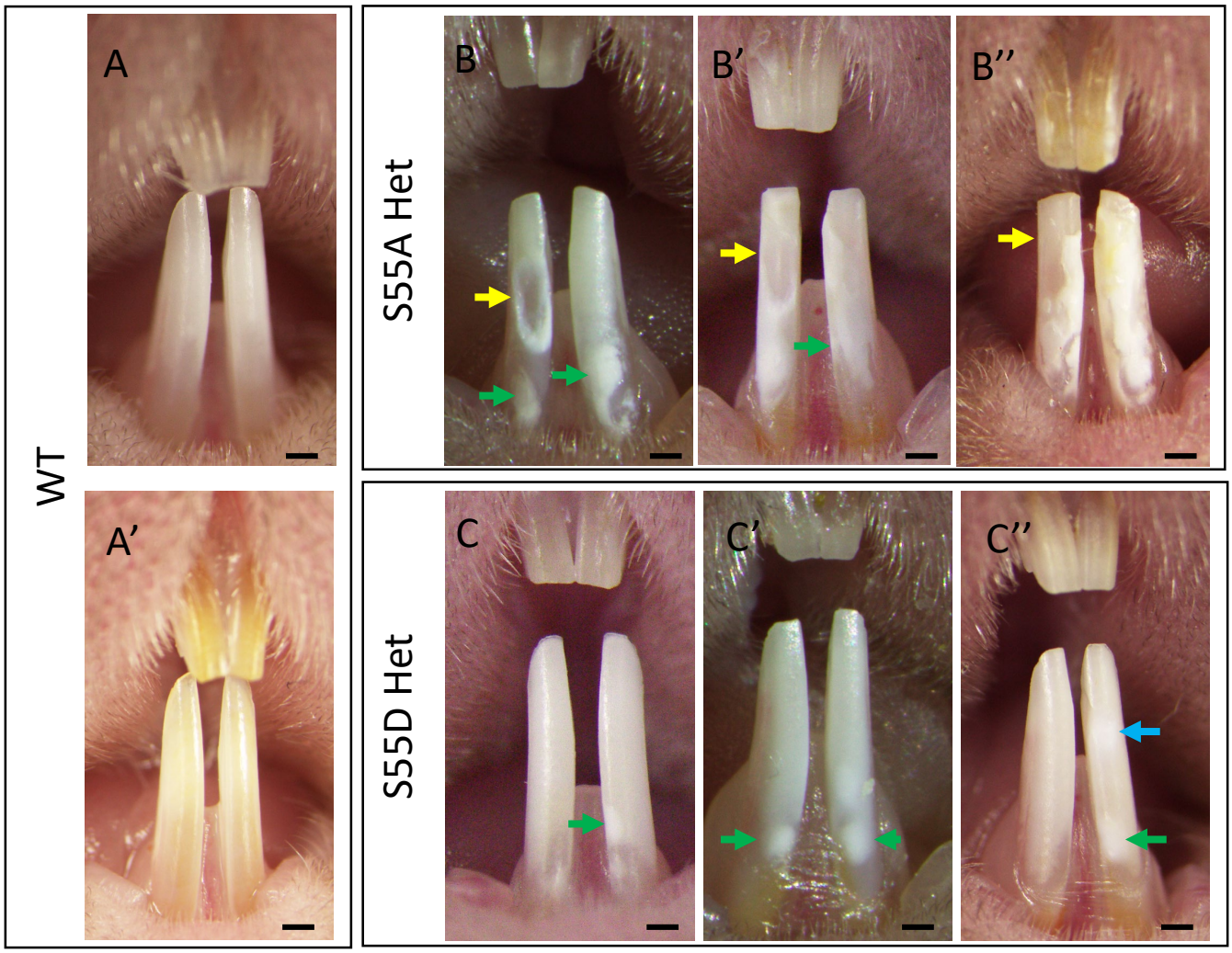


Supplementary FIGURE 1 | Light and TEM micrographs illustrate the method of acquiring serial TEM micrographs from apical portions of constantly growing mouse incisors. Differentiation of ameloblasts progresses in the apical-coronal axis identified by the white arrow. All stages of enamel deposition from pre-secretory to maturation are captured in one single section and sequential progression of enamel secretion can be observed in a series of grid windows (grids) starting from initial enamel deposition of the dentin surface in pre-secretory stage (grid 1) to late-secretory stage (grid 11).

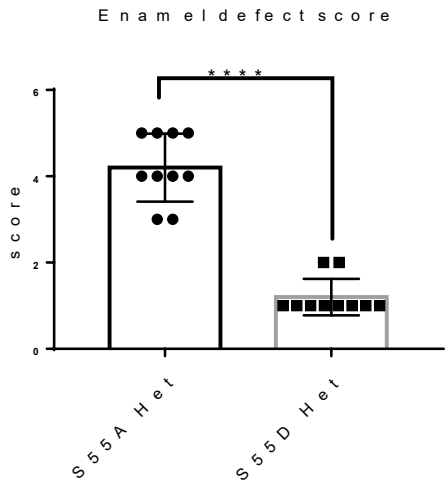


D

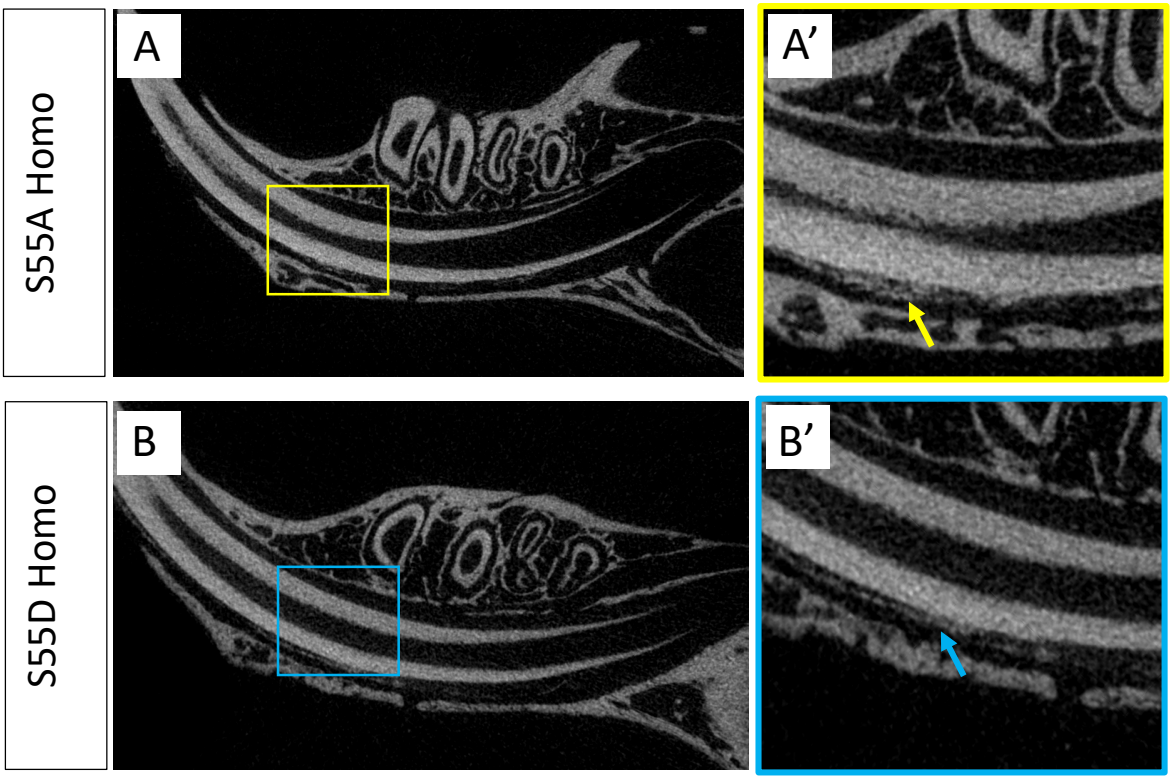
Enamel defects scoring standard

Phenotype	Score
white spots	1
enamel breakage(<1mm)	2
enamel breakage(>1mm)	3
enamel breakage to the tip	4

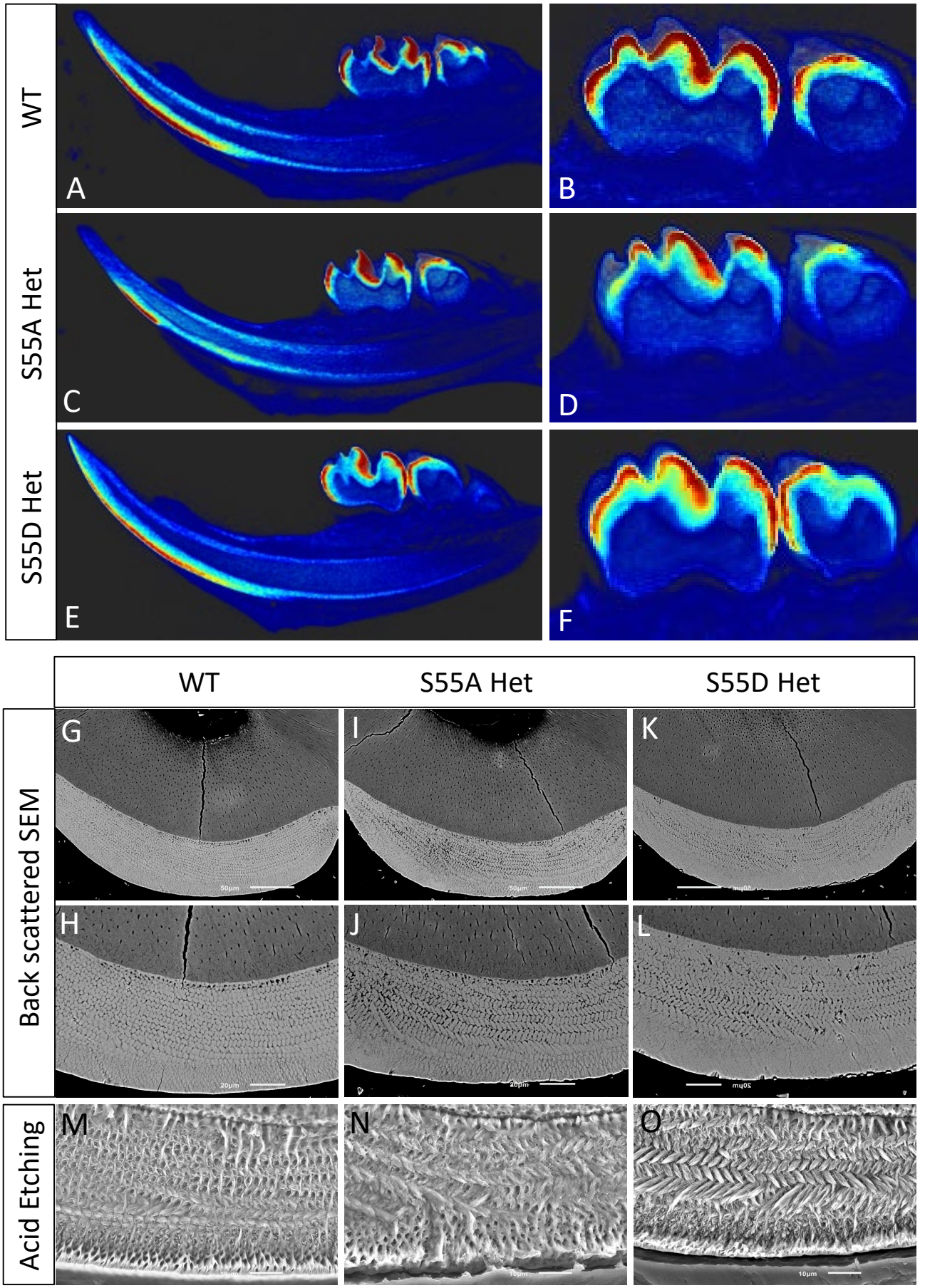
E



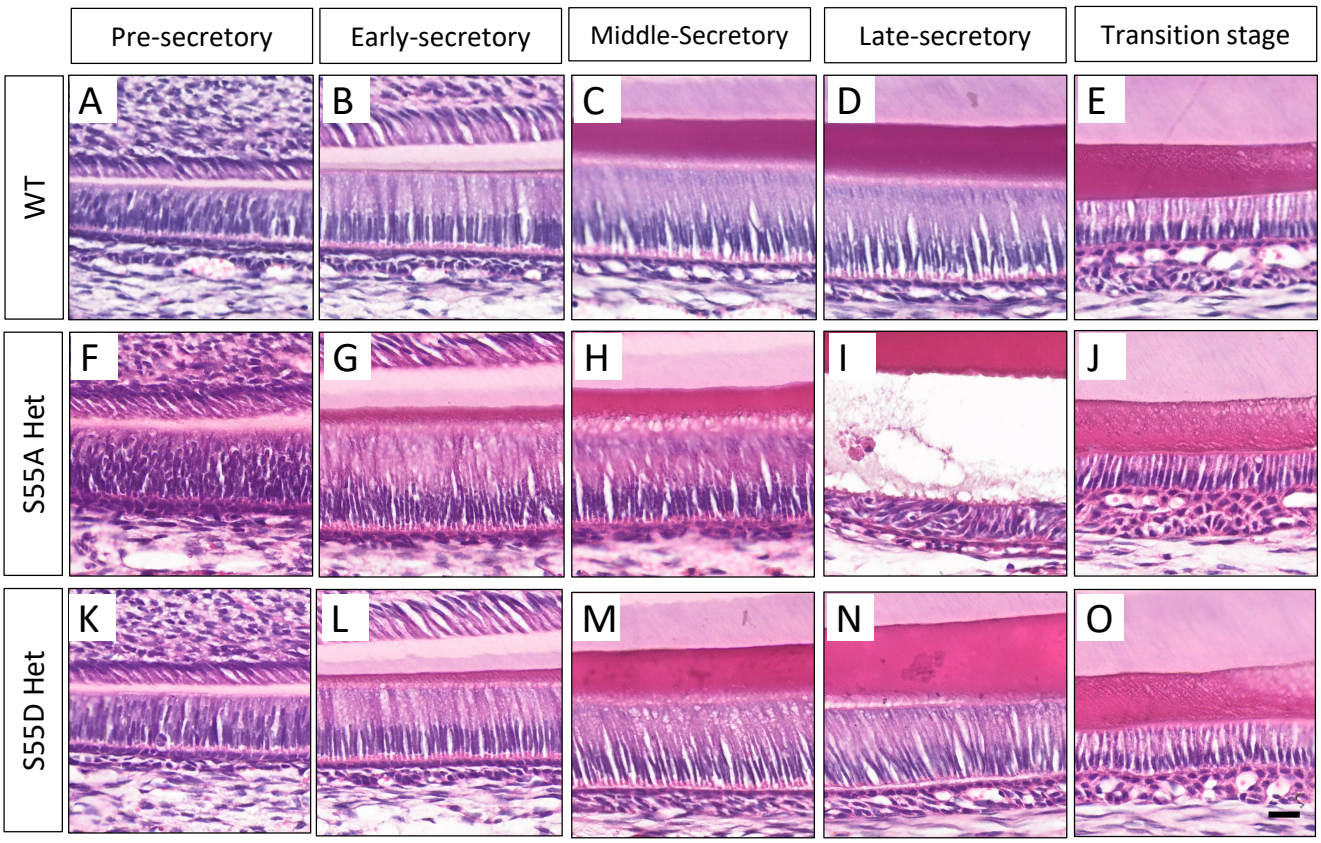
Supplementary FIGURE 2 | The variation of enamel phenotypes in S55A Het and S55D Het. **(A** and **A')** WT incisors. **(B-B")** The incisors of S55A Het showed severe enamel defects with enamel broke down. The large scale of enamel breakages (yellow arrows) and chalk color change (green arrows) was present. More severely, the defects extended to the incisal tip making the incisor short (B"). **(C-C")** Gross pictures of S55D Het incisors showed enamel phenotypes varying from white spots (green arrows) to small pits (blue arrows). **(D)** The scoring standard of the severity of enamel defects. **(E)** S55A Het showed significantly more severe enamel defects than S55D Het. Scale bars: 500 μm



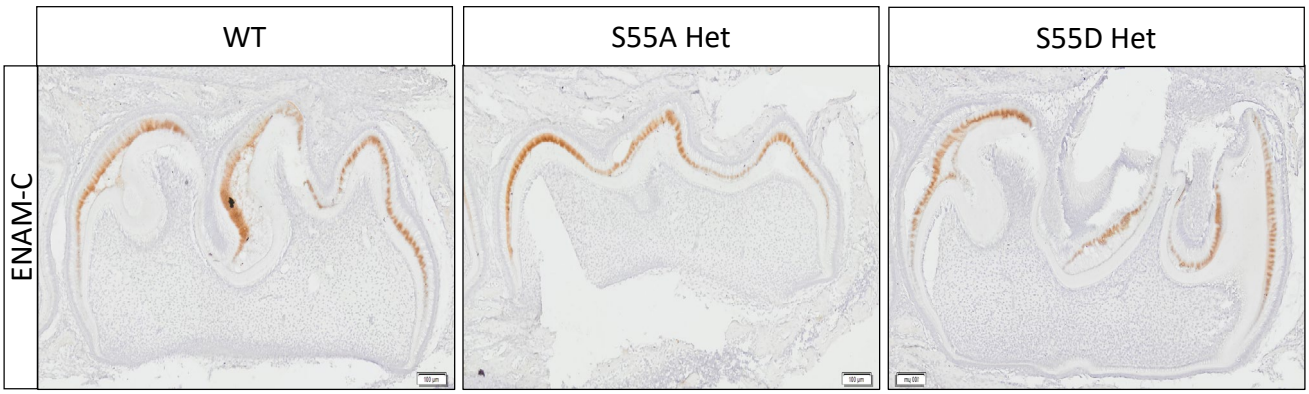
Supplementary FIGURE 3 | No mature enamel was observed in ENAM S55A or S55D Homo incisors above the alveolar bone. However, micro-CT images showed an amorphous enamel matrix with small amounts of radiopaque material between incisor dentin and alveolar bone (yellow and blue arrows in A' and B').



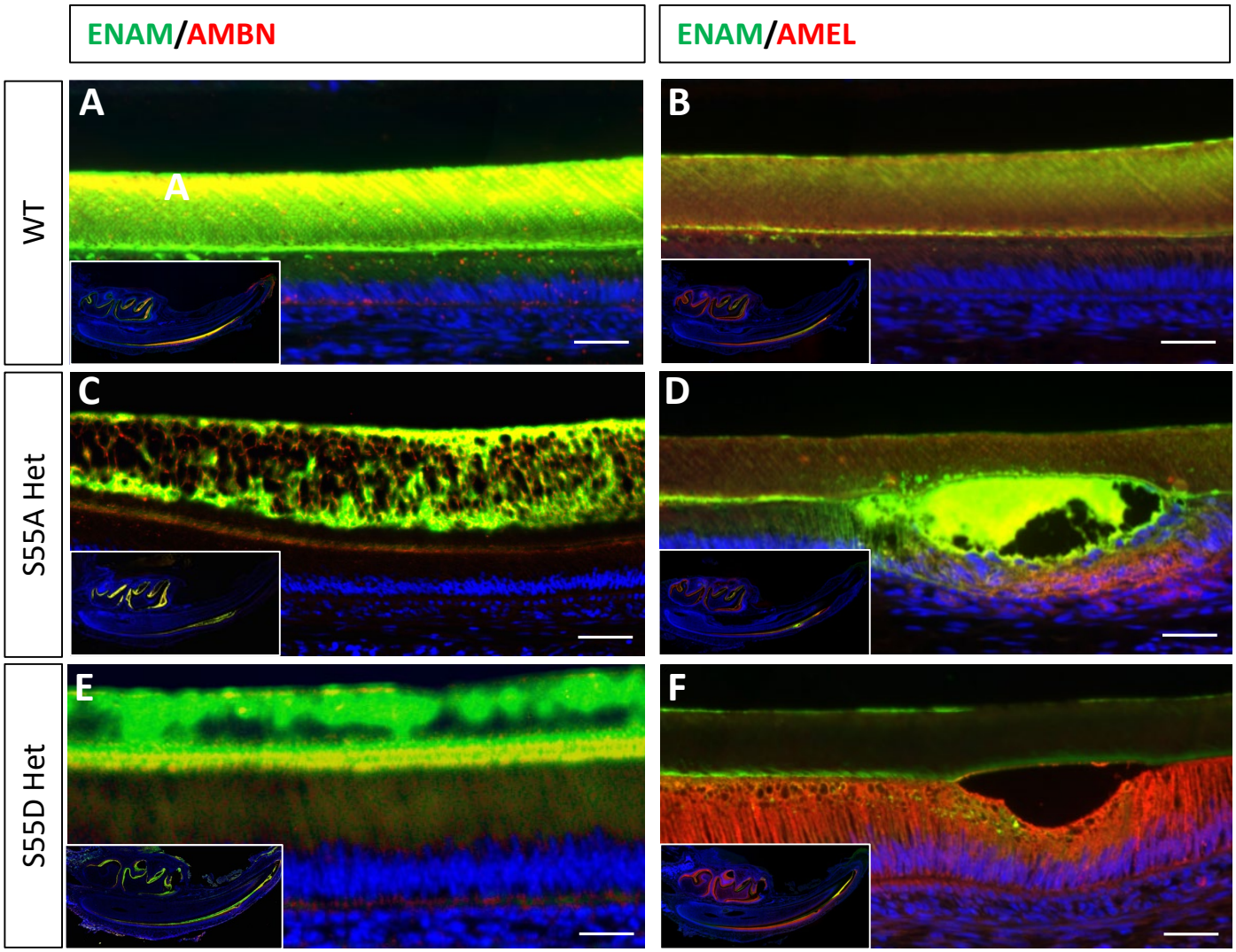
Supplementary FIGURE 4 | Micro-CT and SEM analyses of enamel in 2-week-old WT and ENAM S55A/D mutant mice. **(A-F)** Virtual sagittal sections of lower incisors and molars showed discontinued enamel (red color) in S55A Hets. **(G-L)** Back scattered SEM on the transections of lower incisors showed enamel rods of S55A Hets were aligned more sparsely than WT and S55D Hets. **(M-O)** SEM after acid etching showed disorganized fabric pattern of enamel matrix in S55A Hets compared to WT and S55D Hets. Scale bars: 20 μm in G-L. 10 μm in M-O.



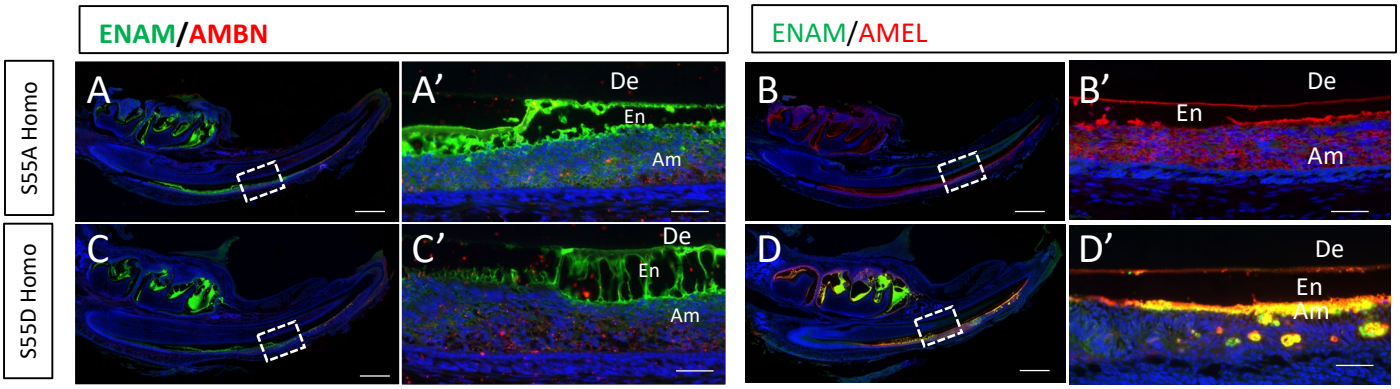
Supplementary FIGURE 5 | Morphological changes of ameloblasts from pre-secretory to transition stages. (A-E) The ameloblasts of WT incisors showed the gradual morphological transition from the pre-secretory stage to the transition stage. At the pre-secretory stage, ameloblasts started to elongate and become polarized. At the secretory lined up and secreted uniform enamel matrix. At the transition stage, ameloblasts lostage, ameloblasts st Tomes' process and shortened in height. (**F-J)** The ameloblasts of S55A Het incisors started showing evident disorganization at the mid-secretory stage, and peeled off from the enamel matrix at late-secretory stage, giving rise to bubble-like structures. (**K-O)** S55D Hets showed regular ameloblasts alignment, similar to WT. Scale bars: 20 um.



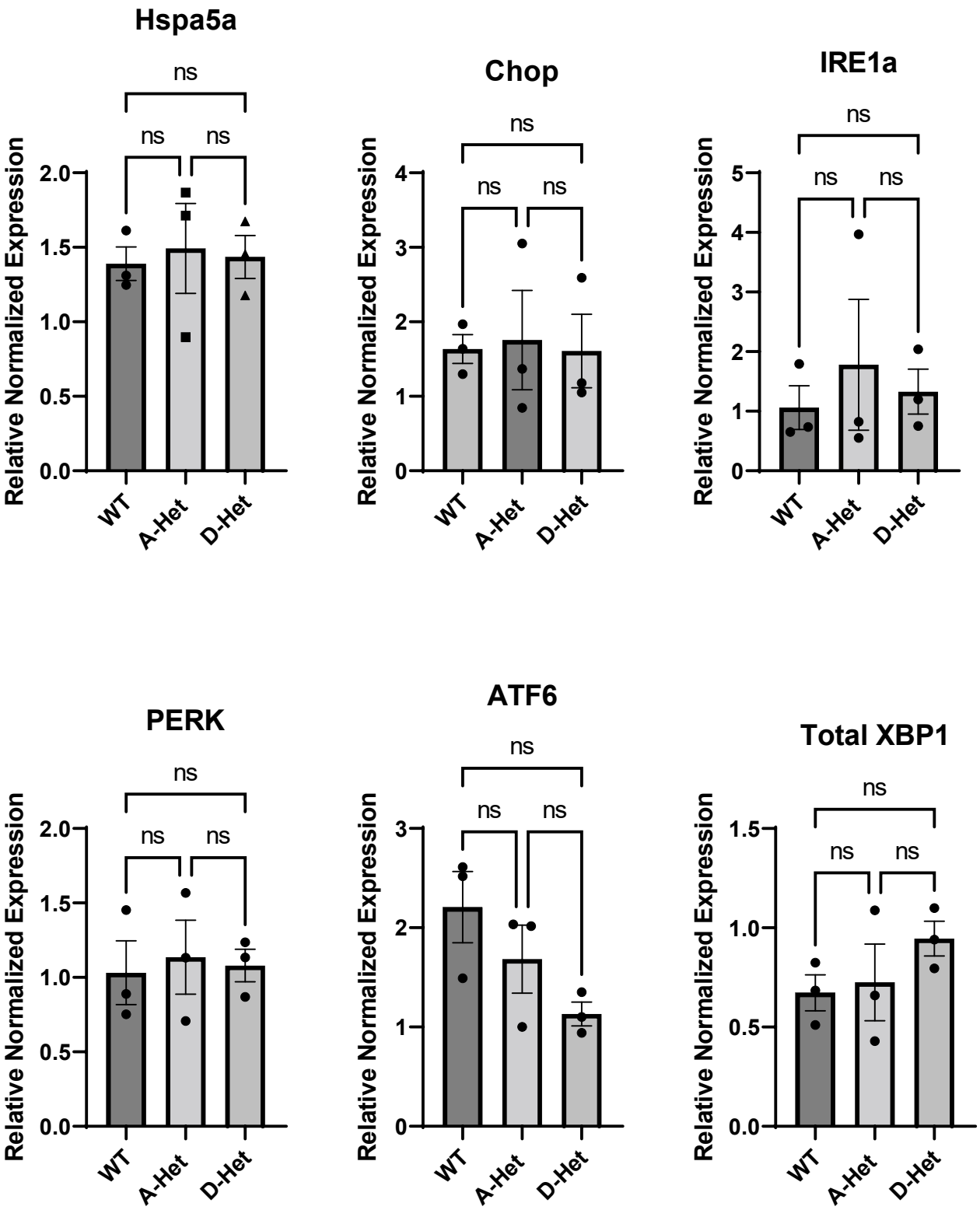
Supplementary FIGURE 6 | No differences were observed in the expression pattern of ENAM-C terminus between WT, S55A Hets, and S55D Hets. Scale bars: 100 um.



Supplementary FIGURE 7| Higher magnification of the distribution and colocalization pattern of ENAM with AMBN or AMEL shown in Fig. 11. **(A, B)** The distribution and colocalization pattern of ENAM with AMBN and AMEL in WT mice. **(C, D)** In S55A Het mice, ENAM was accumulated in the amorphous matrix in the vacuolated spaces formed by detached ameloblasts. AMBN and AMEL showed different distribution patterns from WT. **(E, F)** In S55D Het mice, ENAM and AMBN showed a similar distribution pattern with WT. However, AMEL appeared to be less secreted into the matrix and more retained in ameloblasts. Scale bars: 50 μm.



Supplementary FIGURE 8 | The distribution and colocalization pattern of ENAM with AMBN and AMEL in S55A and S55D Homo mice. Scale bars: 500 μm in A-D; 50 μm in A'-D'.



Supplementary FIGURE 9| Real-time PCR analysis of ER-stress markers in secretory ameloblasts of WT, S55A Het and S55D Het mice. No significant difference was identified in the expression levels of *Hspa5a, Chop, Ire1a, Perk, Atf6* and *Xbp1* between WT and S55A/D mutant mice.