Supplemental Figures:

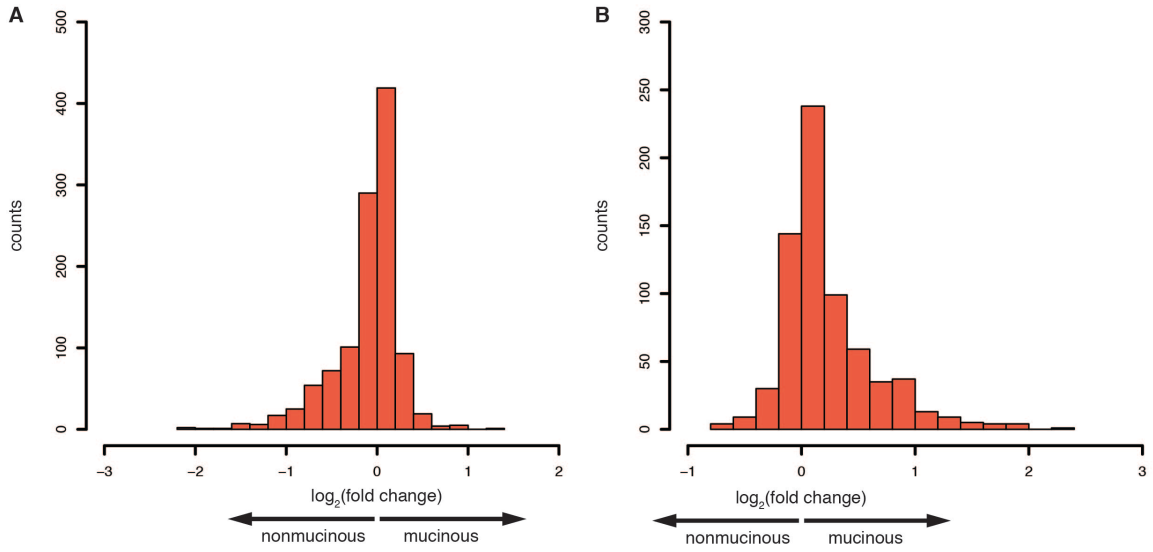

Fig S1. Comparison of global proteolytic activity in mucinous and nonmucinous cysts by MSP-MS. Histograms depicting the number of cleavages enriched in mucinous and nonmucinous cysts at pH 3.5 **(B)** and pH 7.5 **(B)**. Spectral counts of peptide cleavage products were used for quantification of the fold change (mucinous/nonmucinous) and counts are the number of peptide cleavages in each bin.

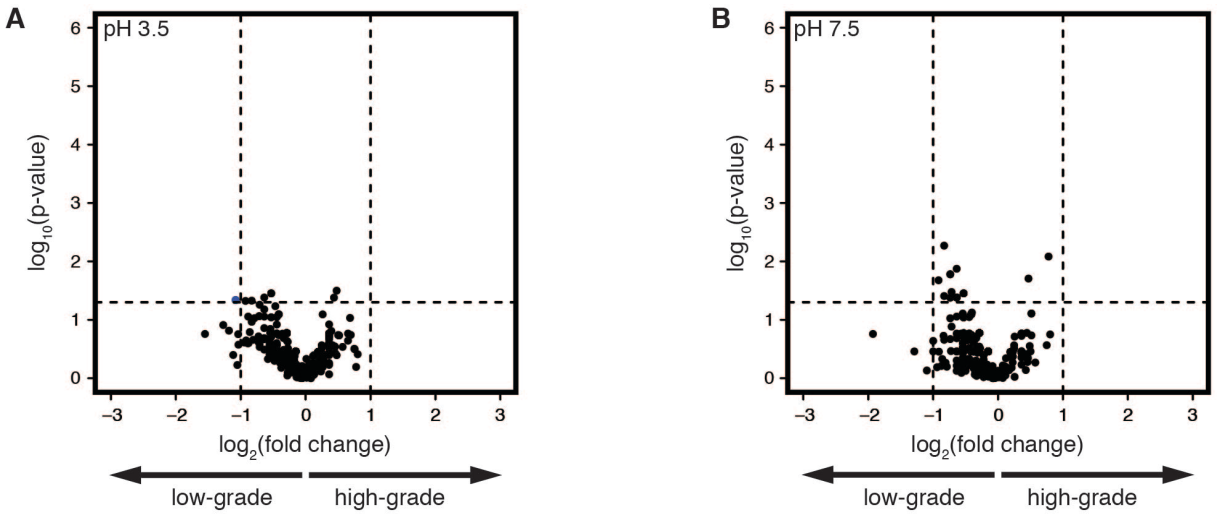

Fig S2. Comparison of global proteolytic activity in mucinous cysts with low- and high-grade dysplasia. Volcano plots displaying the peptide cleavages generated by the mucinous cysts with low-grade dysplasia (n=9) and high-grade dysplasia (n=7) at pH 3.5 **(A)** and pH 7.5 **(B)**. Fold change corresponds to low-grade dysplasia/high-grade dysplasia.

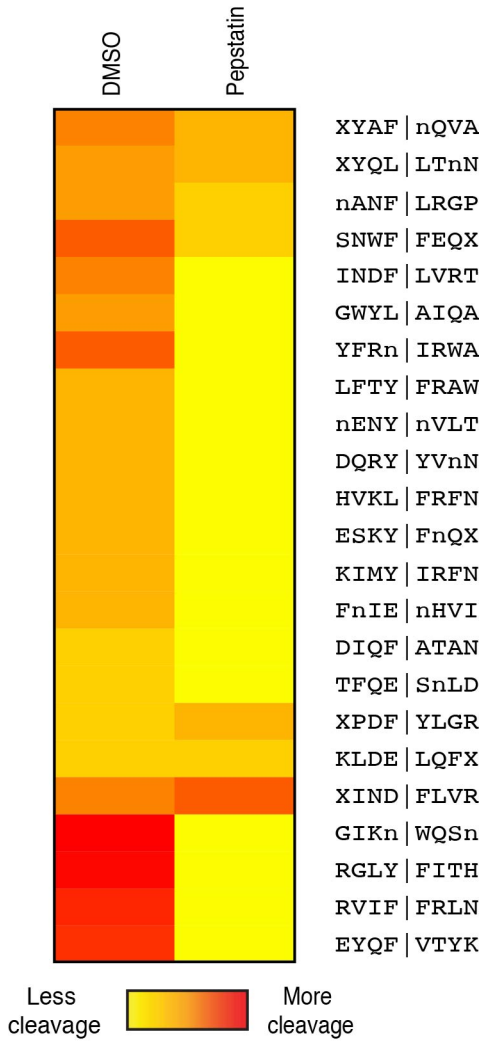

Fig S3. Analysis of pepstatin inhibition on protease activity through MSP-MS. Heatmap displaying cleavage of 28 mucinous-specific substrates following treatment of a mucinous cyst fluid sample with DMSO or pepstatin. Spectral counts were used for relative quantification of peptide cleavage products. Vertical bar (|) indicates the site of cleavage within substrates.

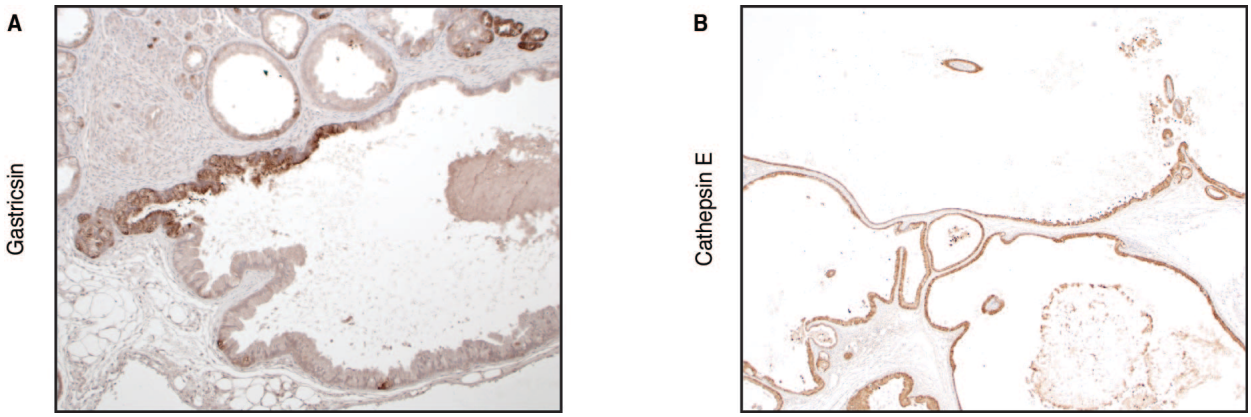

Fig. S4. Immunohistochemical analysis of IPMN genetic mouse model. Immunohistochemical analysis of gastricsin **(A)** and cathepsin E **(B)** in a cystic lesion from a 40-week-old *Ptf1a-Cre; LSL-Kras*^{G12D}*; Brg1*$^{f/f}$ mouse.

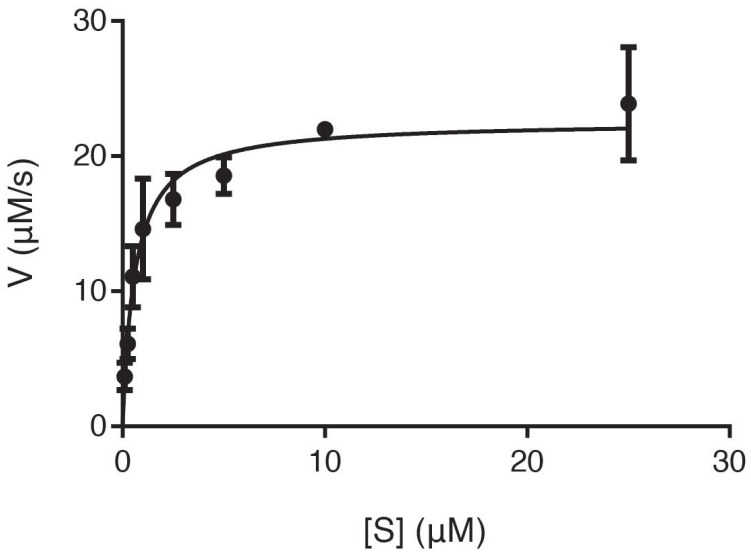

Fig S5. Kinetic analysis of gastricsin selective substrate. Michaelis-Menten analysis of cleavage of DEGW | ALQH substrate by gastricsin. kcat/Km = X, Vmax is Y. Error bars denote SEM from triplicate analysis.

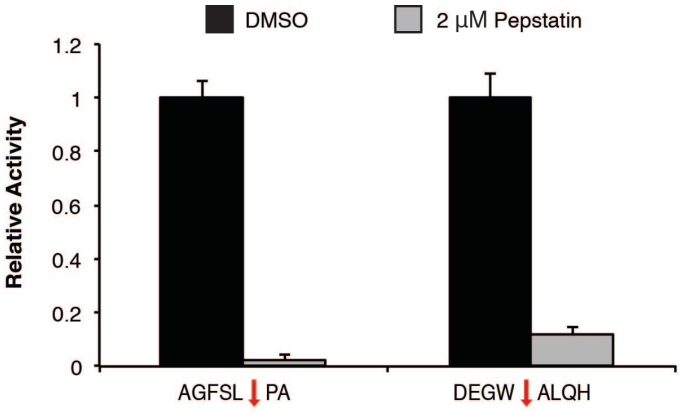

Fig S6. Cleavage of aspartyl protease substrates in mucinous cysts. Pepstatin inhibition of cleavage of fluorescent substrates by a mucinous cyst fluid sample. Activity was normalized relative to DMSO control treatment and error bars denote SEM from triplicate analysis.

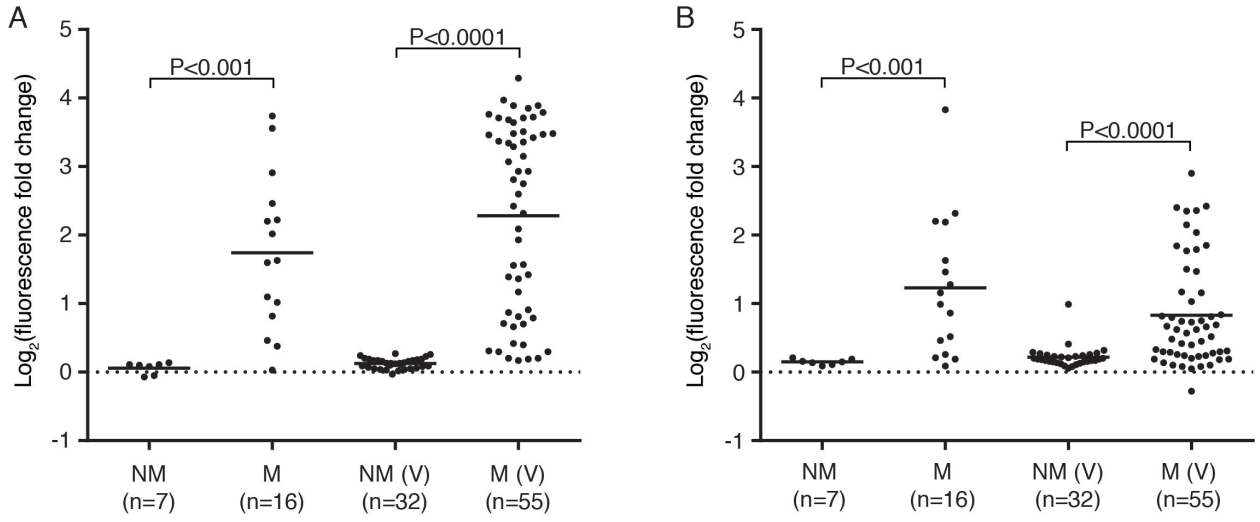

Fig. S7. Quantification of aspartyl protease activity in cyst fluid samples using fluorescent peptide substrates. Gastricsin **(A)** and cathepsin E **(B)** activity in samples analyzed by MSP-MS and fluorescence (n=23) and in samples from validation (V) cohort (n=87) that were just assayed using fluorescent substrates.

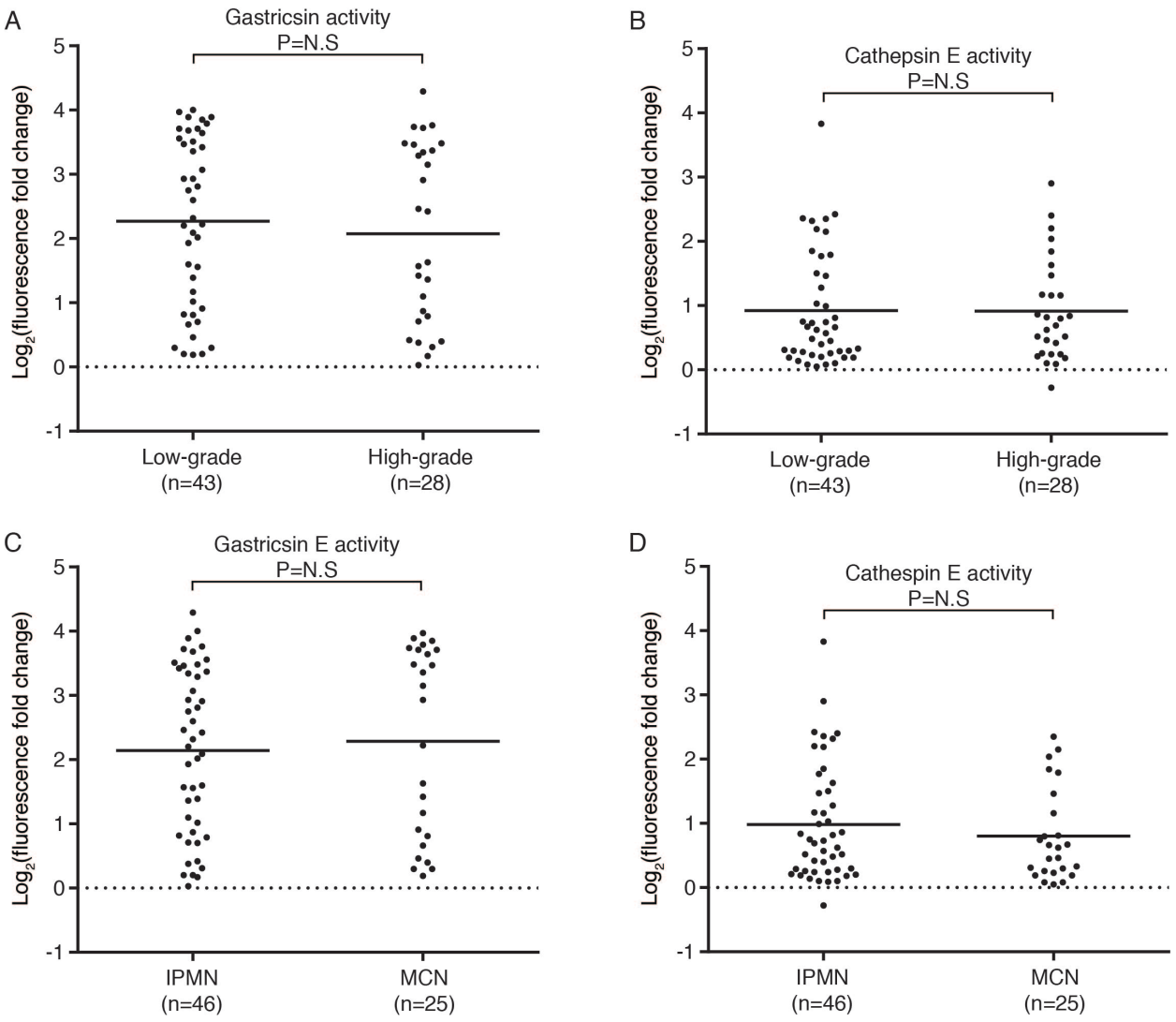

Fig. S8. Analysis of gastricsin and cathepsin E activity in 71 mucinous cyst fluid samples. Gastricsin and cathepsin E activity in mucinous cysts with low- and high-grade dysplasia **(A, B)** or IPMNs and MCNs **(C, D)**.

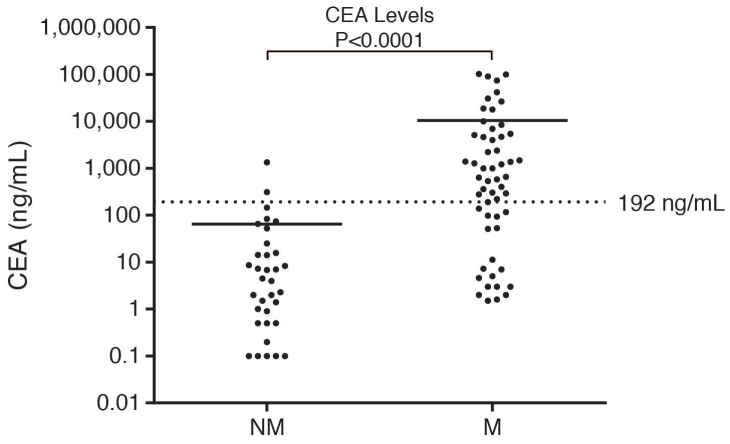

Fig. S9. CEA levels in mucinous and nonmucinous cysts.

Dashed line indicates the standard clinical cutoff of 192 ng/mL.