Cathepsin D is essential for the correct collagenolytic activity of macrophages during cholestatic-induced liver fibrosis.

M Fernández-Fernández[1*], P Ruiz-Blázquez[1*], V Pistorio[1,2*], S Núñez[1,3,4], C García-Ruiz[1,3,4,5], JC Fernandez-Checa[1,3,4,5], A Moles[1,3,4,]

[1]Institute of Biomedical Research of Barcelona. Spanish Research Council, Barcelona. [2]University of Naples Federico II, Napoli. [3]Center for the Study of Liver and Gastrointestinal Diseases (CIBERehd), Carlos III National Institute of Health, Madrid. [4]IDIBAPS, Barcelona. [5]Research Center for ALPD, Los Angeles.

* All authors contributed equally to this work.

Correspondence: ana.moles@iibb.csic.es

BACKGROUND: Changes in proteolytic activity are essential to liver fibrosis development. Proteases control not only matrix turnover but also, the activation and repression of growth factors and chemokines influencing disease progression. However, our knowledge about the proteolytic enzymes contributing to liver fibrosis is still very limited.

Thus, the aim of this study was to analyse CtsD cell-specific role during liver fibrosis.

METHODS: Novel macrophage-CtsD knock-out mouse was generated by breeding LysMCre (macrophages) with CtsD floxed mice and validated using WB in macrophages. Fibrosis was established by bile duct ligation in CtsD$^{F/F}$ or CtsD$^{\Delta Mac}$ mice and determined by hydroxyproline and fibrogenic genes by RT-PCR. Collagen degradation and endocytosis was studied using DQ™ Collagen type I and Dextran probes respectively, lysosomal colocalization was determined by IF using LAMP2 and Endo180 and UPAR was analysed by WB in peritoneal macrophages.

RESULTS

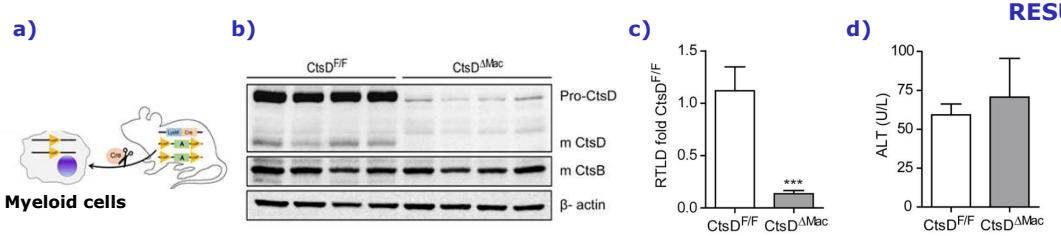
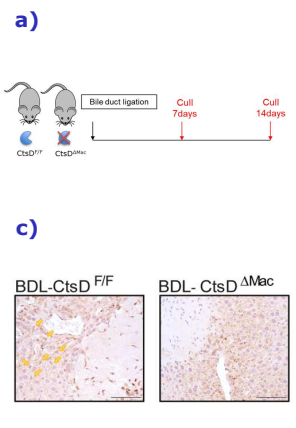


Figure 1: CtsD cell-specific KO mice validation. a) Scheme of the myeloid cell-specific KO mice. **b)** Pro-CtsD, m-CtsD, m-CtsB and β-actin WB, **c)** CtsD gene expression in peritoneal macrophages from CtsD$^{F/F}$ or CtsD$^{\Delta Mac}$ mice. **d)** ALT levels in adult unchallenged CtsD$^{F/F}$ or CtsD$^{\Delta Mac}$ mice.



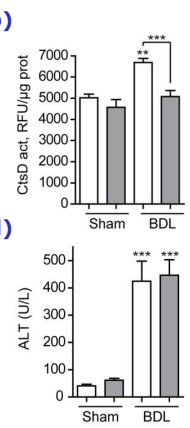


Figure 2: CtsD deletion in macrophages does not affect liver damage after BDL. a) BDL experimental model. **b)** CtsD enzymatic activity, **c)** CtsD immunohistochemistry and **d)** ALT levels of CtsD$^{F/F}$ or CtsD$^{\Delta Mac}$ mice.

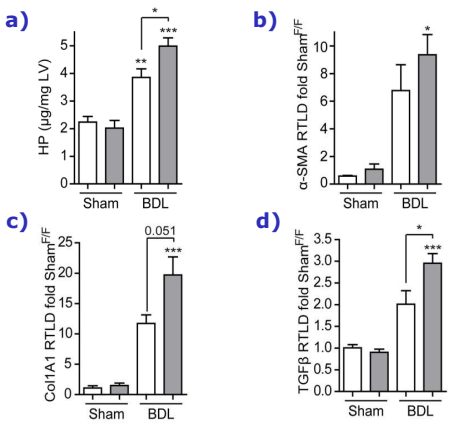


Figure 3: CtsD deletion in macrophages amplifies fibrosis after BDL. a) Hydroxyproline levels and **b)** a-SMA, **b)** Col1A1 and TGFb gene expression in livers from CtsD$^{F/F}$ or CtsD$^{\Delta Mac}$ mice.

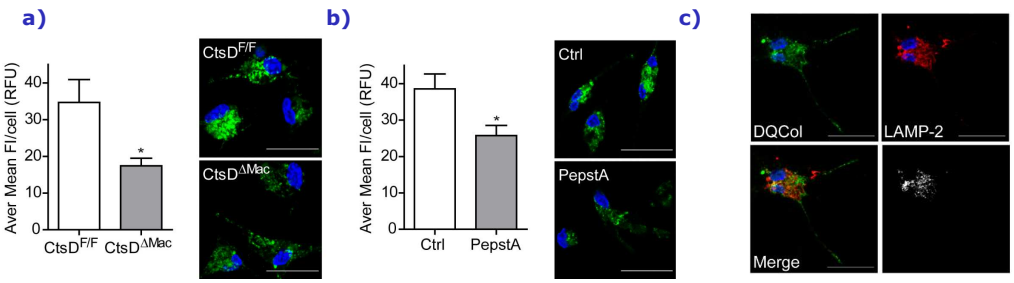


Figure 4: CtsD deficient macrophages present defective collagenolytic activity. a) DQCol degradation in genetically deficient, **b)** pharmacologically inhibited CtsD peritoneal macrophages and **c)** DQCol and LAMP2 colocalization in CtsD$^{F/F}$ peritoneal macrophages.

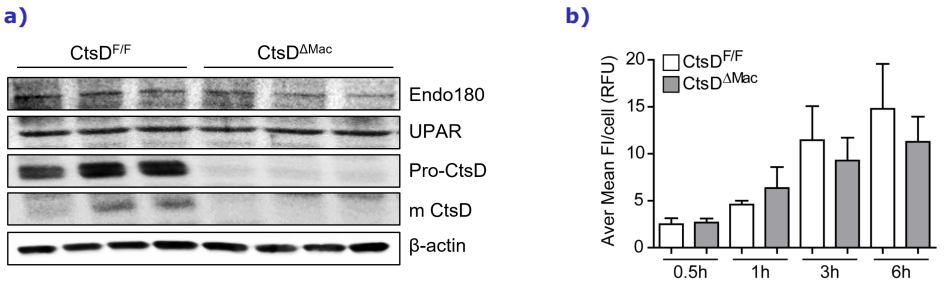


Figure 5: CtsD deficent macrophages present unaffected endocytosis and Endo180/UPAR expression in macrophages. a) Endo180, UPAR (Collagen receptors), Pro-CtsD, mCtsD and b-actin expression and **b)** Dextran internalization time-course assay in CtsD$^{F/F}$ or CtsD$^{\Delta Mac}$ peritoneal macrophages.

CONCLUSION: The correct collagenolytic activity displayed by macrophages is dependent on lysosomal cathepsin D and influences liver fibrosis development.