Hepatic inflammation elicits production of proinflammatory netrin-1 through exclusive activation of translation

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INTRODUCTION

Chronic liver diseases (CLDs) affect more than 1 billion people worldwide. They represent the main etiological conditions for the onset of liver injury, impaired liver function (cirrhosis), and HCC.[1] CLDs share a common factor triggered by different etiologies: hepatic inflammation. Inflammation drives hepatocytes turnover, extracellular matrix accumulation, histological worsening and the long term induction of the tumorigenic factor IL-6, eventually leading to the development of HCC. Hepatic inflammation is a two-edged sword: it helps liver recovery by favoring viral and dead cell clearance, but also exacerbates fibrogenesis, fostering cirrhosis and oncogenesis.

Netrin-1 is a secreted factor well known for preventing cellular apoptosis through its binding to “dependence receptors”. Owing to the causal link between inflammation, cancer in general, and HCC, we hypothesized that hepatic inflammation and netrin-1 may be reciprocal influencers in the liver.

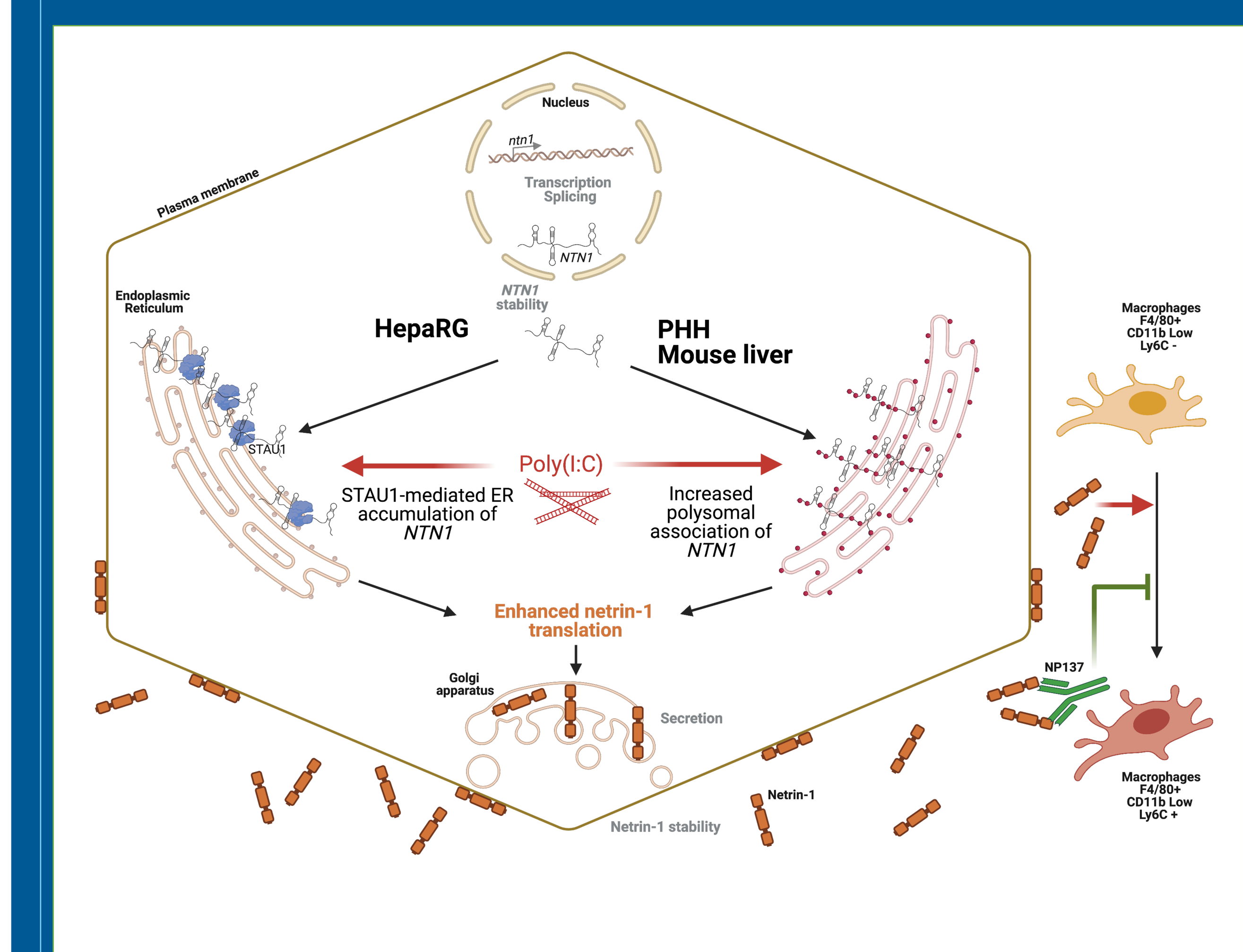
Conflicting data currently depict the implication of netrin-1 in inflammation (exacerbation[2,3] or dampening[4-6]) in different organs and pathologies, also prompting for examination of its functional status in the liver.

Full data are available in **Hepatology. 2022;00: 1–15. doi:10.1002/hep.32446**

AIM

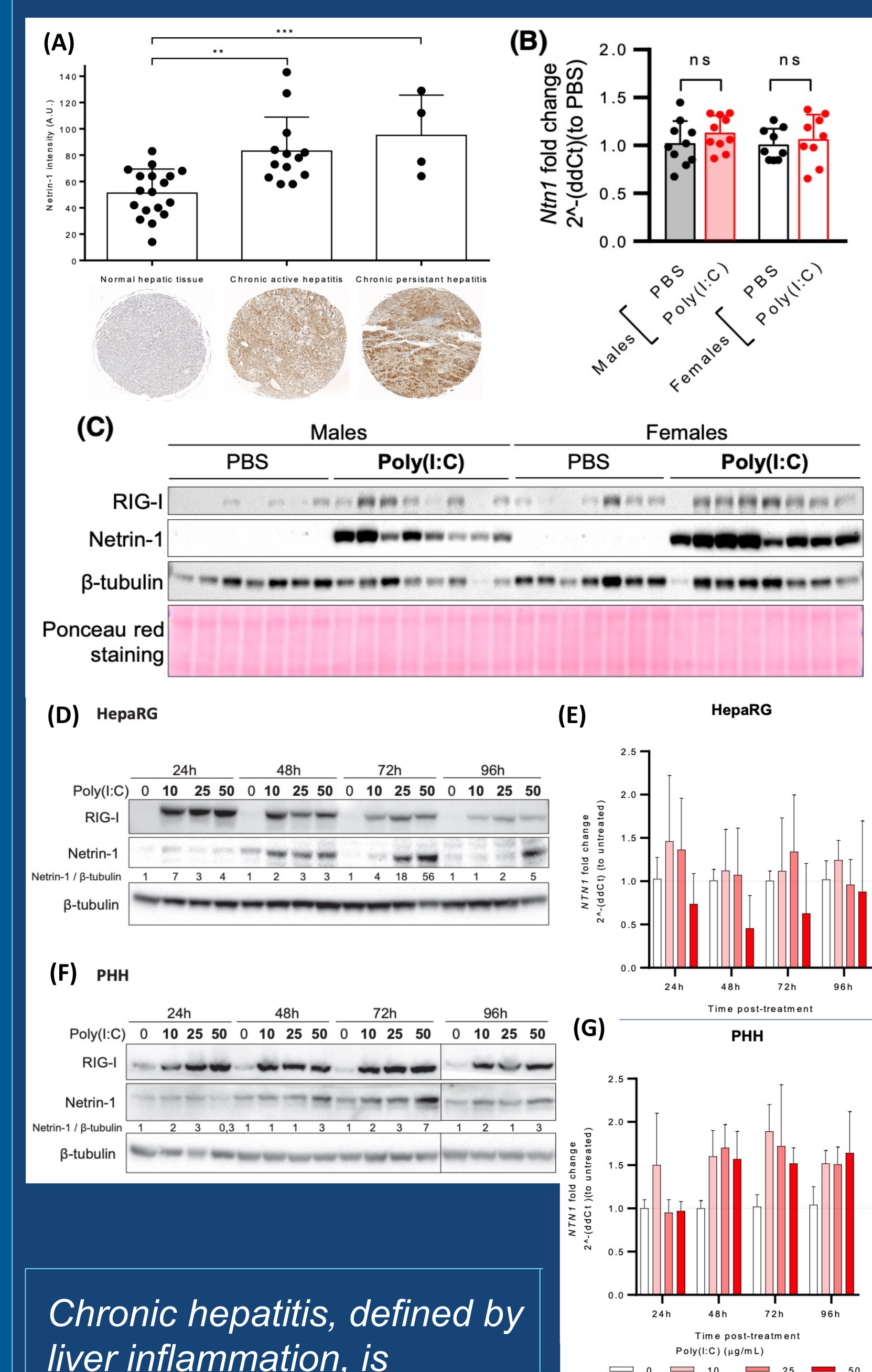
This work aims at testing netrin-1’s inducibility during hepatic inflammation and to decipher the mechanisms leading to its up-regulation in this context, as well at its functional status.

CONCLUSIONS



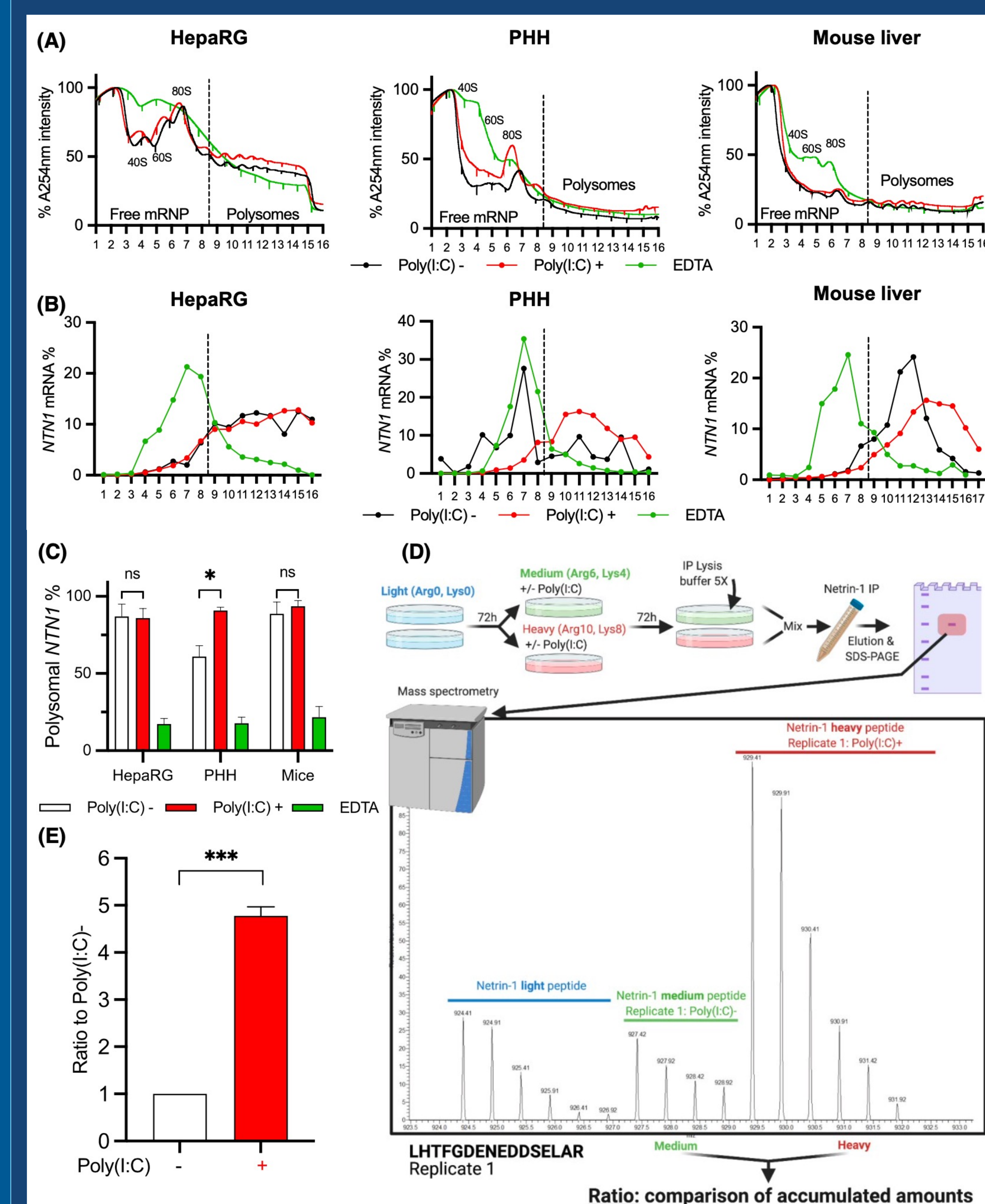
RESULTS

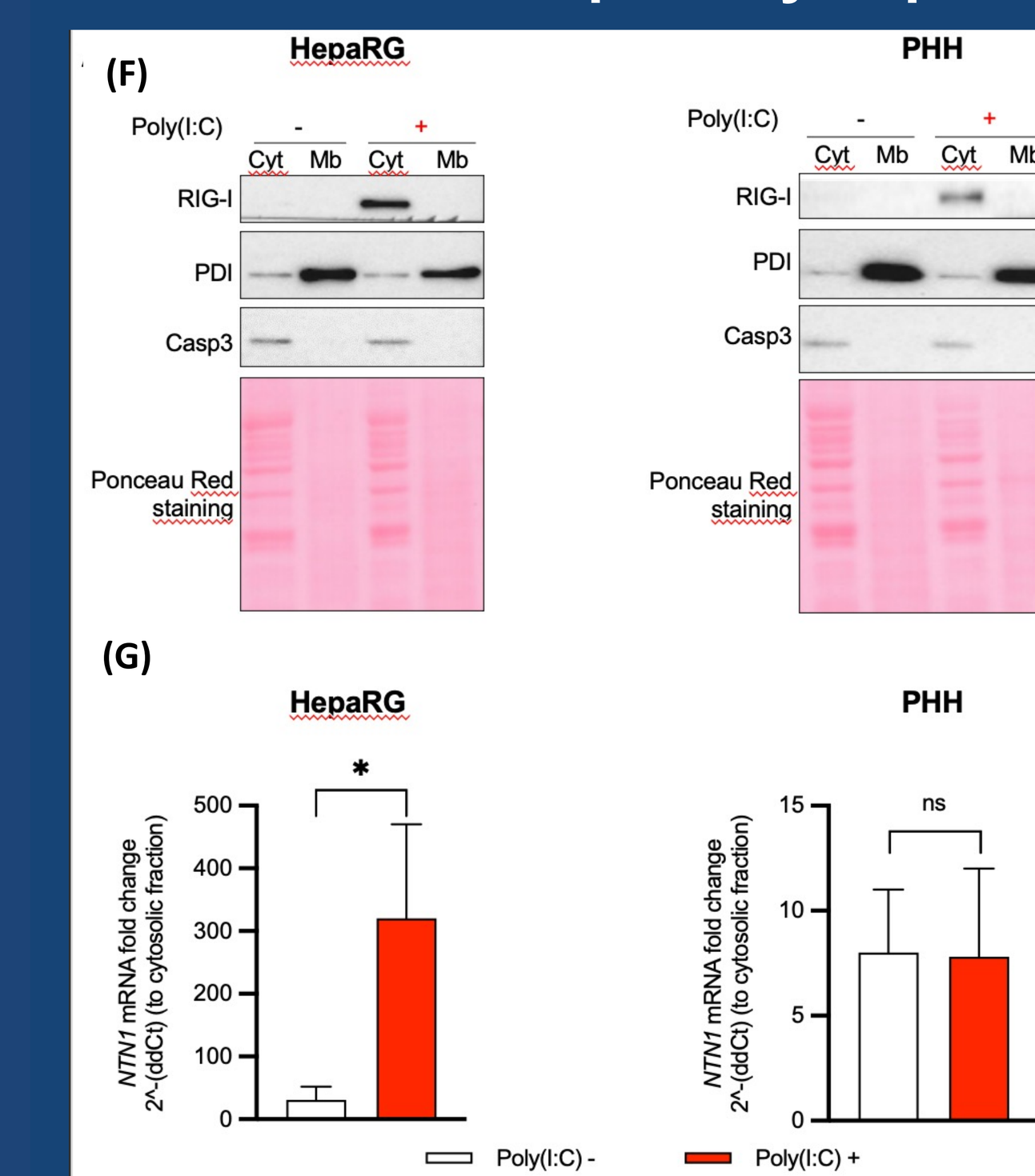
1- Inflammation triggers netrin-1 protein, but not mRNA, up-regulation in clinical samples, *in vitro* and in mice



*Chronic hepatitis, defined by liver inflammation, is correlated with netrin-1 up-regulation along disease progression **(A)**. Inflammation induction in mice with dsRNA (Poly(I:C)) injection leads to netrin-1 up-regulation at the protein level only **(B-C)**. Indeed, no netrin-1 transcript increase was observed by RT-qPCR. The netrin-1 protein up-regulation in absence of significant netrin-1 transcript induction was confirmed in HepaRG cells **(D-E)** and PHH **(F-G)***

2- Netrin-1 up-regulation is translational and differs in dedifferentiated cells versus primary hepatocytes

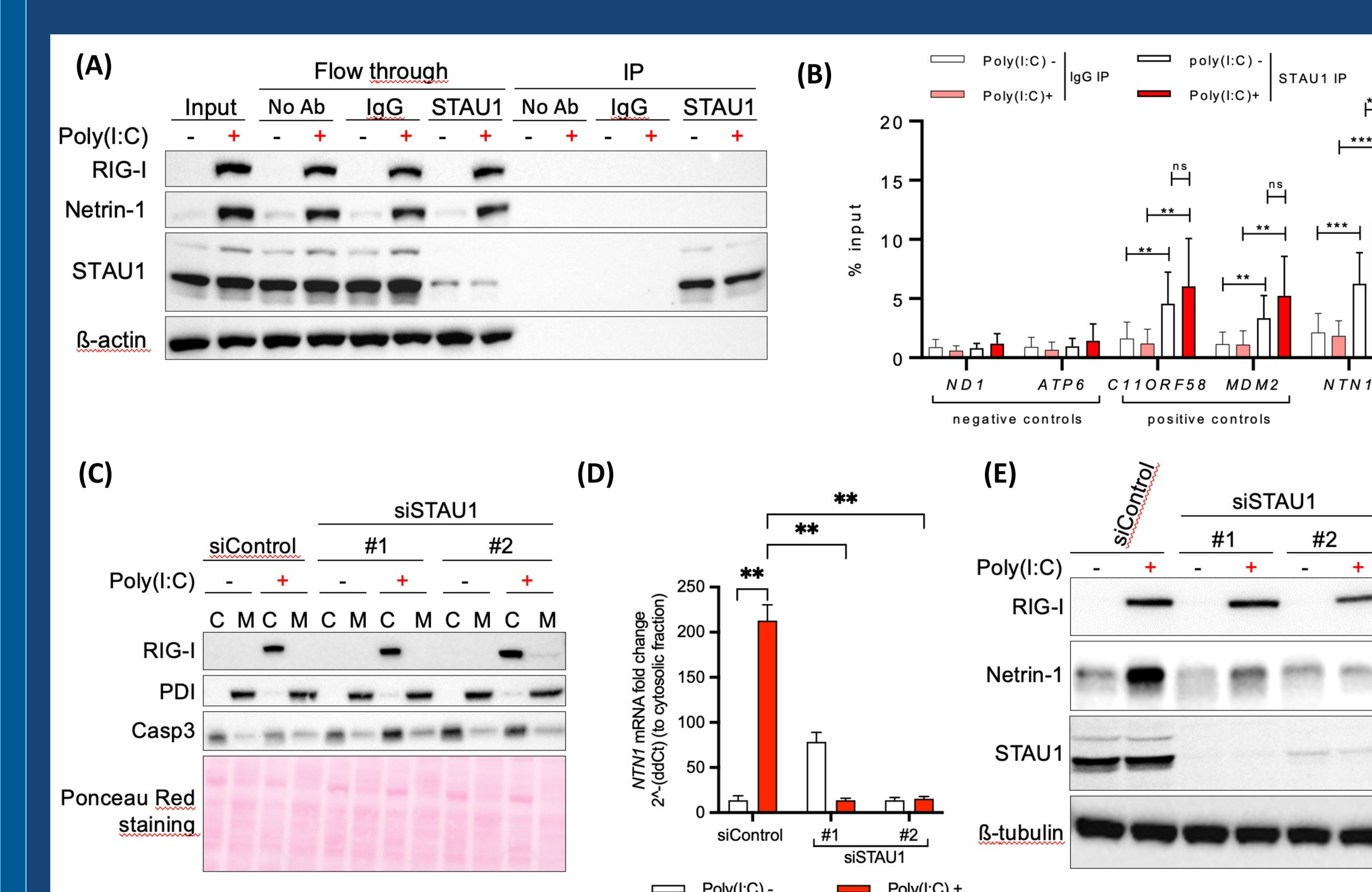




*Netrin-1 being a secreted protein, its translation should occur at the ER-membrane. Sequential fractionation (controlled with cytosolic (RIG-I and Casp3) or membrane (PDI) markers, showed that upon inflammation, the netrin-1 transcript is enriched at the ER-membrane in HepaRG, but not in PHH **(F-G)**.*

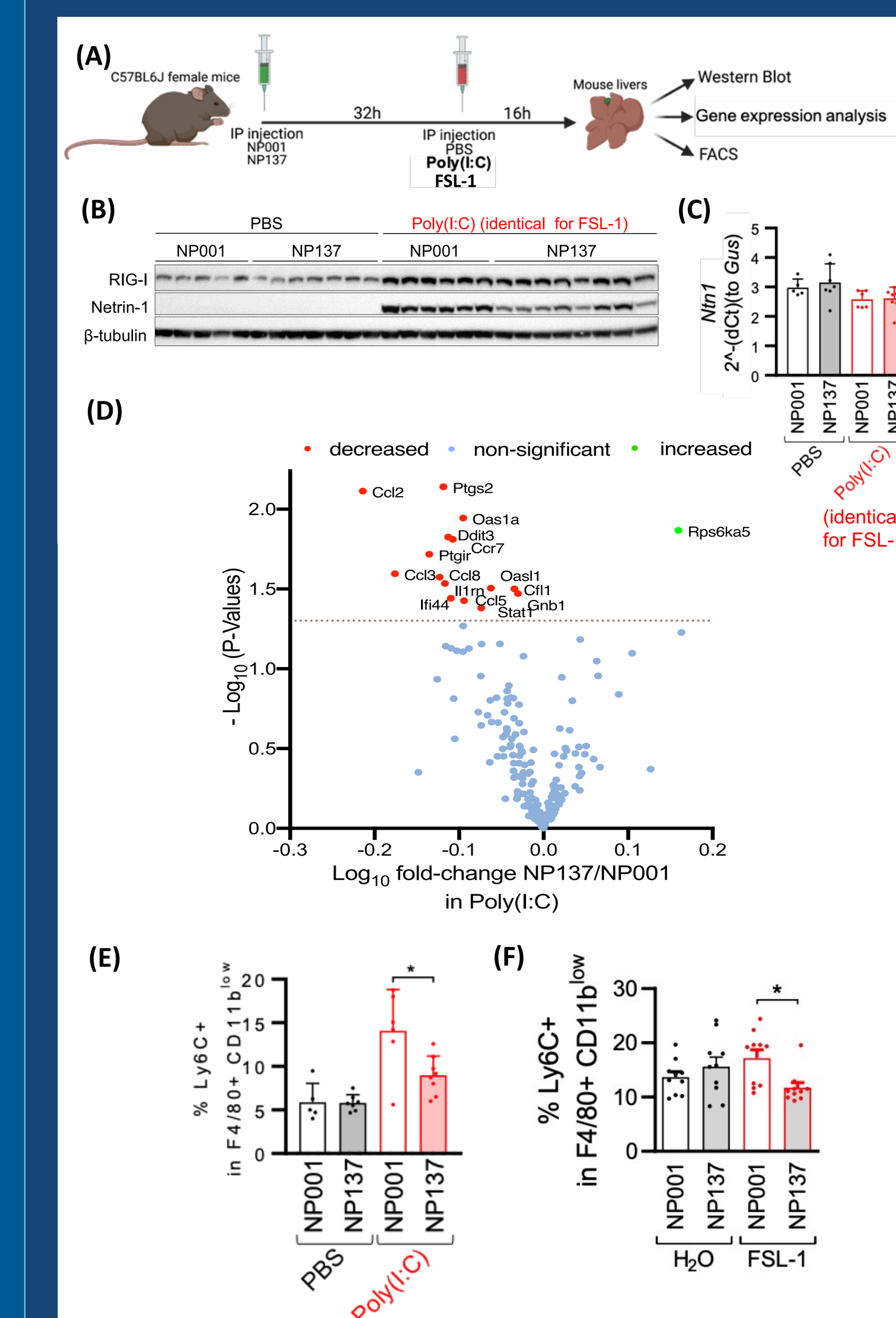
*Netrin-1 upregulation unfolds through accumulation of its transcript in the polysomal fractions**(A-C)** in PHH, as shown here by polysomal fractionation. In mice livers, the netrin-1 transcript is enriched in the heavy fractions (13-17) and can explain the induction of netrin-1 **(B)**. Translational activation of netrin-1 occurs in HepaRG cells (4.7 more netrin-1 residues incorporated during inflammation **(D-E)** despite the absence of netrin-1 transcript accumulation in the polysomal compartment.*

3- Staufen-1 mediates netrin-1 induction through direct interaction with the *NTN1* transcript



*Staufen-1 is a RNA-binding protein specialized in transcripts localisation. A CLIP (UV crosslinking) experiment shows here a direct interaction between Staufen-1 and the netrin-1 transcript **(A-B)**. HepaRG transfection with Staufen-1-siRNA reverses the described phenotype, impairing netrin-1 transcript accumulation at the ER membrane **(C-D)**, and netrin-1 protein synthesis upon inflammation **(E)**.*

4- A phase 2 trial-validated antibody enabling netrin-1 capture unravels its contribution to hepatic inflammation in mice



*Before inflammation induction with poly(I:C) or FSL-1 (bacterial compound), mice were injected with NP137, a phase 2 trial validated anti-netrin-1 antibody **(A)**. Inflammation leads here again to netrin-1 up-regulation at the protein level only with both TLR-ligands **(B-C)**. The analysis of 248 pro-inflammatory genes reveals an effect of the anti-netrin-1 antibody on canonical chemokines (Ccl2 (-1,63 fold), Ccl3 (-1,51 fold), Ccl5 (-1,64 fold) and Ccl8 (-1,33 fold)) and other factors related to the Interferon system in poly(I:C)-treated mice **(D)**. A FACS analysis on liver immune cells revealed a anti-inflammatory effect of the NP137 Ig by downregulating pro-inflammatory macrophages in poly(I:C) **(E)** or FSL-1 **(F)** treated mice, indicating a broader role for netrin-1 than in viral-induced inflammation.*

METHOD

A panel of cell biology and biochemistry approaches (reverse transcription quantitative polymerase chain reaction, reporter assays, run-on, polysome fractionation, cross linking immunoprecipitation, filter binding assay, subcellular fractionation, western blotting, immunoprecipitation, stable isotope labeling by amino acids in cell culture (SILAC)) was used on in vitro–grown primary hepatocytes, human liver cell lines (including HepaRG), mouse samples and clinical samples.

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