PNPLA3 I148M Up-Regulates Hedgehog and Yap Signaling in Human Hepatic Stellate Cells

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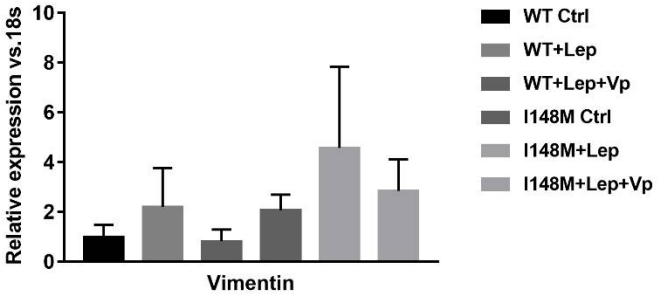
Supporting Materials and Methods:

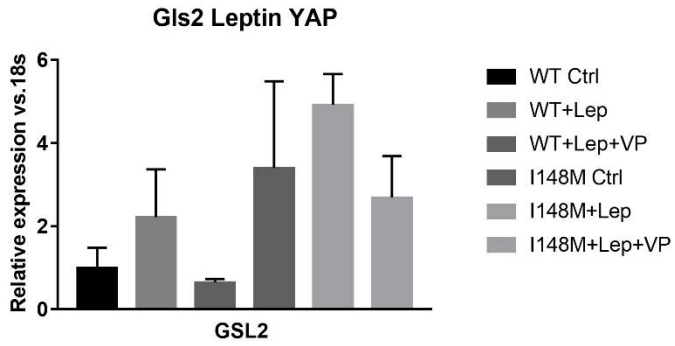
RT-PCR analysis:

RNA was isolated from primary HSCs and LX-2 by NucleoSpin® RNA (MACHEREY-NAGEL GmbH & Co., Germany) according to manufacturer's instructions. One μg of RNA was reverse transcribed using Moloney Murine Leukemia Virus enzyme, 10xbuffer for cDNA synthesis and Deoxynucleotide Mix 10mM (Sigma-Aldrich). Two microliters of diluted cDNA were loaded together with SYBR® Select Master Mix on 96 well-plate to perform RT-PCR analysis (Thermo Fisher Scientific). Forward and reverse primers for each gene of interest were specifically designed and used at 10 pmol/μL (Eurofins Genomics, Germany). Sequences are available upon request.

Supporting Figure and Figure Legend:

Supplementary Fig.1 Bruschi et al.





Supp. Fig.1. Verteporfin inhibits Leptin mediated Hedgehog/Yap target gene expression. LX-2 stably overexpressing cells (n = 3 each PNPLA3 genotype) obtained and cultivated in vitro, as described in Materials & Methods. Where indicated, cells were stimulated with Vp and Leptin (Lep) respectively for 24h and 1h prior the analysis. Expression of Vimentin and GLS2 analyzed by real-time PCR and normalized to 18s.