

Title	TGR(mREN2)27 rats develop non-alcoholic fatty liver disease- associated portal hypertension responsive to modulations of Janus-kinase 2 and Mas receptor
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University College Cork, Ireland Coláiste na hOllscoile Corcaigh 1 TGR(mREN2)27 rats develop non-alcoholic fatty liver disease-associated portal 2 hypertension responsive to modulations of Janus-kinase 2 and Mas receptor

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1 Supplemental Figure 1:

2 A) Quantification of hepatic Sirius red staining. Quantified hepatic Sirius red stainings represented in Figure 1 were analyzed using 3DHISTECH Ltd. Software. Quantified Sirius red 3 stainings of TGR(mREN2)27 rats were compared to respective WT quantifications in 4 control,BDL (2w, 4w) and CCl₄ (10w) groups. Results are expressed as percentage of positive 5 stained area. B) Quantification of hepatic aSMA staining. Positive aSMA stainings of healthy, 6 injured BDL (2w, 4w) and CCl₄ (10w) intoxicated TGR(mREN2)27 livers were compared with 7 positive aSMA stainings in respective WT livers. Results are shown as percentage of positive 8 stained area. C) Hepatic protein expressions downstream AT1R. Protein expression levels of 9 Jak2, pJak2, Arhgef1 and aSMA were detected by Western blots. Each representative 10 Western blot shows on the left hand side, the expression of hepatic WT proteins and on the 11 right hand side, the expression of hepatic TGR(mREN2)27 proteins. Each hepatic protein 12 expression is illustrated for sham operated, BDL (2w, 4w) and CCl₄ (10w) intoxicated rats. D) 13 Hepatic protein expression of AT1R and Mas. The hepatic protein expression of AT1R and 14 15 Mas were investigated by Western blot. Each Western blot shows hepatic WT protein expressions (left) and hepatic TGR(mREN2)27 protein expressions (right). Protein 16 17 expressions of AT1R and Mas are shown for healthy sham operated, BDL (2w, 4w) and CCl4 (10w) intoxicated livers. E) mRNA expressions of hepatic ACE mRNA without and with AVE 18 injection in TGR(mREN2)27 rats. Livers of TGR(mREN2)27 rats were analyzed for ACE mRNA 19 20 expressions with and without AVE injection. Protein expression levels of healthy control 21 livers and injured livers (BDL, CCl₄) were normalized to healthy control livers without AVE injection. Hepatic mRNA expressions are represented in x-fold change. F) Hepatic AT1R 22 23 mRNA expression with and without AVE injection in TGR(mREN2)27 rats. Hepatic AT1R mRNA expression of TGR(mREN2)27 rats were normalized to control TGR(mREN2)27 values 24 and represented as x-fold change. Bars are illustrated as means ± s.e.m. Statistical analyzes; 25 Mann-Whitney t-test. */** indicates p<0.05/p<0.005 compared to respective WT or 26 TGR(mREN2)27 controls. 27

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Supplemental Figure 1:



D. Hepatic protein expression of AT1R and Mas

A. Quantification of hepatic sirius red staining

B. Quantification of hepatic α SMA staining



C. Hepatic protein expressions downstream AT1R



*/**p<0.05/p<0.005

E. Hepatic ACE mRNA expression without and with AVE injection in TGR(mREN2)27 rats



F. Hepatic AT1R mRNA expression without and with AVE injection in TGR(mREN2)27 rats



WT TGR(mREN2)27