Bailly-Maitre et al. Supplemental Experimental Procedures

Materials

Tunicamycin was purchased from Calbiochem EMB Bioscience. Doxycycline was obtained from Sigma.

Cell culture and cell lines

The tetracycline-inducible HeLa cell lines conditionally overexpressing BI-1 have been described (23). Cells were maintained in DMEM (Gibco) with 10% of Tet-system-approved fetal bovine serum (Clontech) in the presence of 100 µg/ml G418 (Gibco) and 400 µg/ml hygromycin (Invitrogen). To induce expression of BI-1, cells were left untreated (controls) or treated for 24 h with 1 µg/ml doxycycline (Sigma).

XBP-1 Splicing assay

Total RNAs from cultured HeLa cells were prepared using Trizol reagent (Invitrogen). The cDNA was reversely transcribed from 200 ng of total RNA using SuperScript Reverse Transcriptase (Invitrogen). PCR was then conducted using specific primers flanking the splice site 5'-aaa cag agt agc agc tca gac tgc-3' and 5'-tcc ttc tgg gta gac ctc tgg ga-3'. Unspliced Xbp-1 gave a product of 480 bp, and the spliced cDNA was 454 bp.

Bailly-Maitre et al. Supplemental Figure Legends

Figure 1: Adenoviral expression of HA-BI-1 and GFP.

a) HeLa cells were infected with adenoviruses expressing BI-1 (Ad BI-1) and control GFP (Ad GFP) at a MOI of 10 and 50. Cells were harvested at 16 h post-infection. Expression of BI-1 and GFP was assessed by immunoblotting.

b) Expression of HA-BI-1 was measured in livers from Ad GFP and Ad BI-1 inj. animals.

Figure 2: BI-1 overexpression regulates IRE1α signaling in vitro

a) ER stress responses in cells overexpressing BI-1 and control cells were investigated. To induce exogenous BI-1 expression, HeLa cells stably transfected with tet-inducible-BI-1 plasmid were exposed to 1 μg/ml doxycycline for 24 h. Control and BI-1 overexpressing Hela cells were subjected to treatment with tunicamycin (Tm, 3 μg/ml) for different time periods and total proteins were extracted. Expression of XBP-1s, GRP78, ATF-6 and HA-BI-1 was analyzed by immunoblotting. Representative blots are shown from a minimum of three independent determinations.

b) ER stress was induced by Tm (2μg/ml) for 12 h. Cytoplasmic IRE1α-mediated *Xbp1* mRNA splicing was detected by reverse transcription (RT) reaction followed by PCR amplification of spliced and unspliced *Xbp1*. *Gapdh* was used as a housekeeping gene. PCR products were separated in 3% agarose gels.

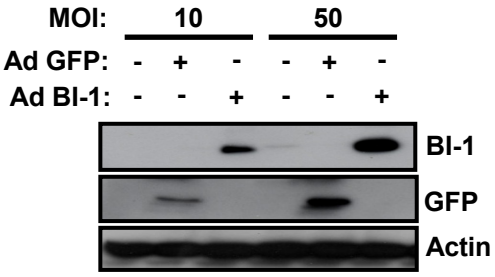
Figure 3: Hepatic BI-1 overexpression in db/db mice

a) Representative Oil-Red O stainings of liver sections from *db/db* mice. Three animals of each treatment group were analyzed.

b) Serum levels of ALT and AST from blood collected from random fed *db/db* mice treated with either Ad GFP or Ad BI-1.

Bailly-Maitre et al. Supplementary Figure 1

a



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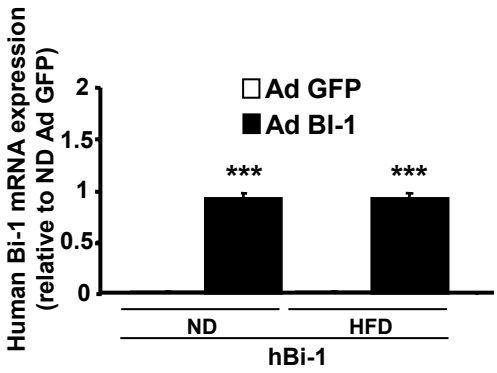
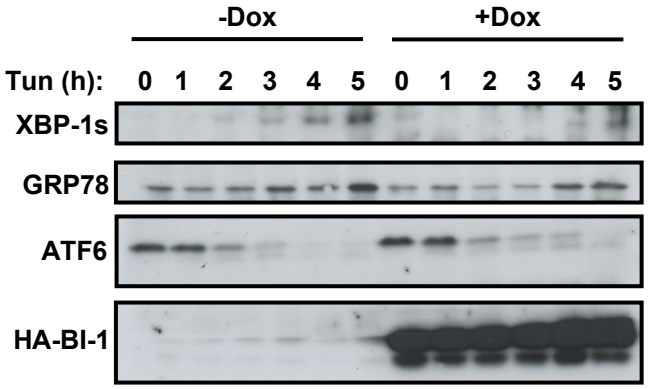


Bailly-Maitre et al. Supplementary Figure 2

a



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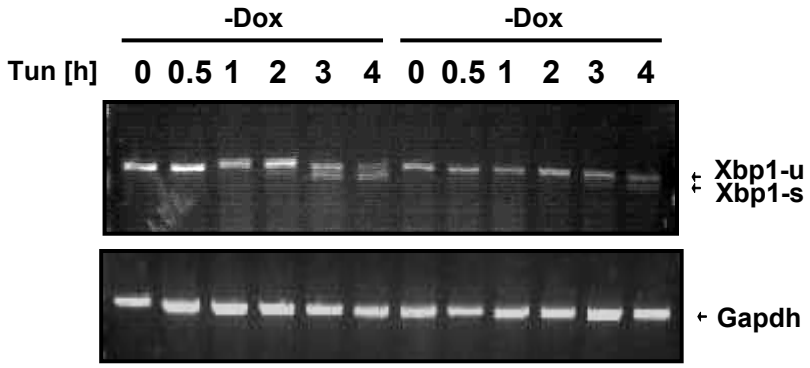
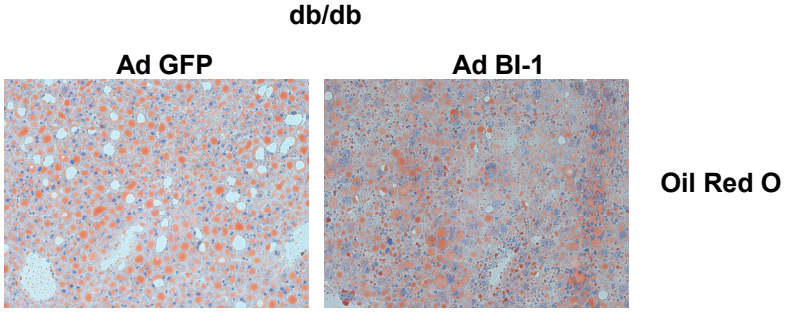


Bailly-Maitre et al. Supplementary Figure 3

a



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