Supplementary Materials: Hepatitis B Virus Protein X Induces Degradation of Talin-1

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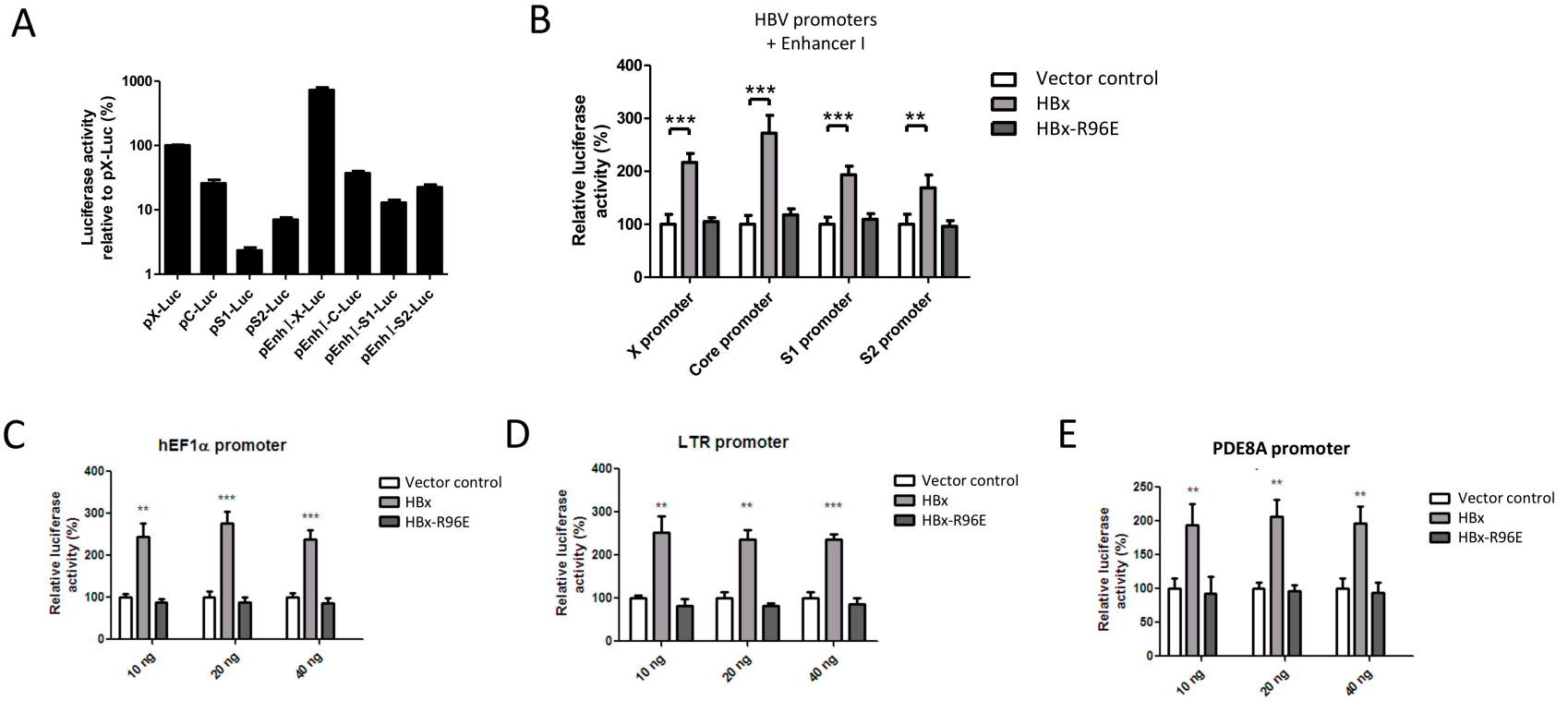

Figure S1. Transcriptional transactivation by hepatitis B virus (HBV) accessory protein X (HBx). (**A**) Basal activity of luciferase reporters under control of the HBV promoters in vectors in the absence and presence of the HBV enhancer I (EnhI) 48 h after transfection in HEK 293 cells. (**B**) Activity of the different HBV promoters in presence enhancer I 48 h after cotransfection with HBx- or HBx-R96E-expressing constructs. (**C**–**E**) Activity of the luciferase reporters under control of the HIV-1 LTR (LTR), the human elongation factor 1α (hEF1α), or the phosphodiesterase 8A (PDE8A) promoters, respectively, 48 h after cotransfection with HBx- or HBx-R96E-expressing constructs in HEK 293 cells. ** $p < 0.01$, *** $p < 0.001$.

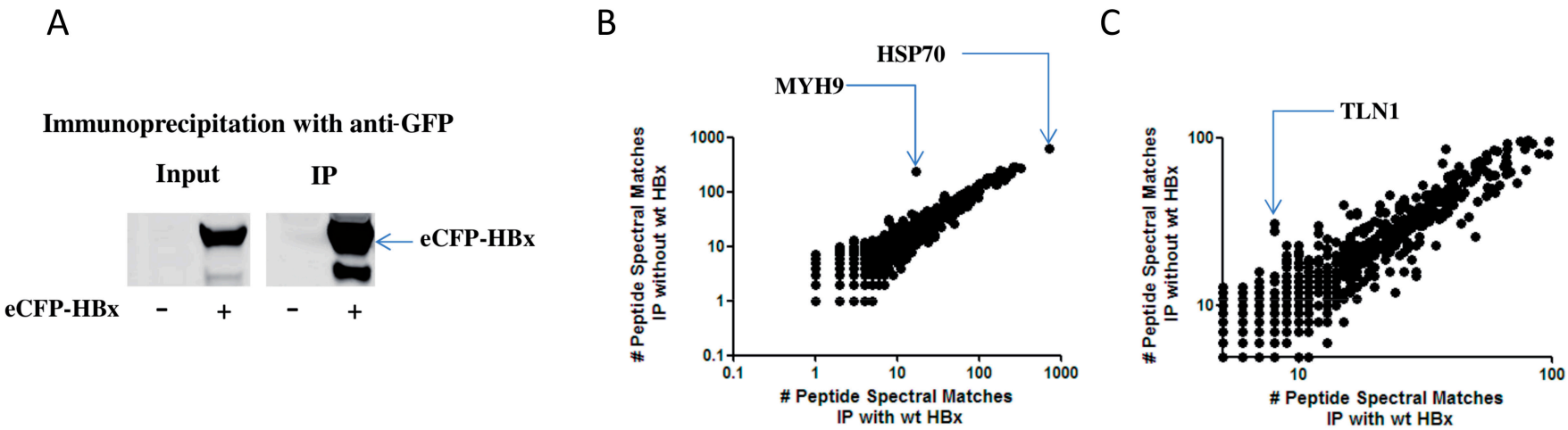

Figure S2. Immunoblot control and peptide spectral matches of HBx-interacting proteins. (**A**) Western blot analysis of eCFP-HBx in HEK 293 cells transfected with an eCFP–HBx expression vector before and after immunoprecipitation (IP) with anti-green fluorescent protein (GFP). (**B**,**C**) The number of peptide spectral matches identified in the immunoprecipitation in the absence of wild-type (wt) HBx is plotted versus the number of peptide spectral matches in the immunoprecipitation in the presence of wild-type HBx based on Proteome Discoverer analysis of mass spectrometry data.

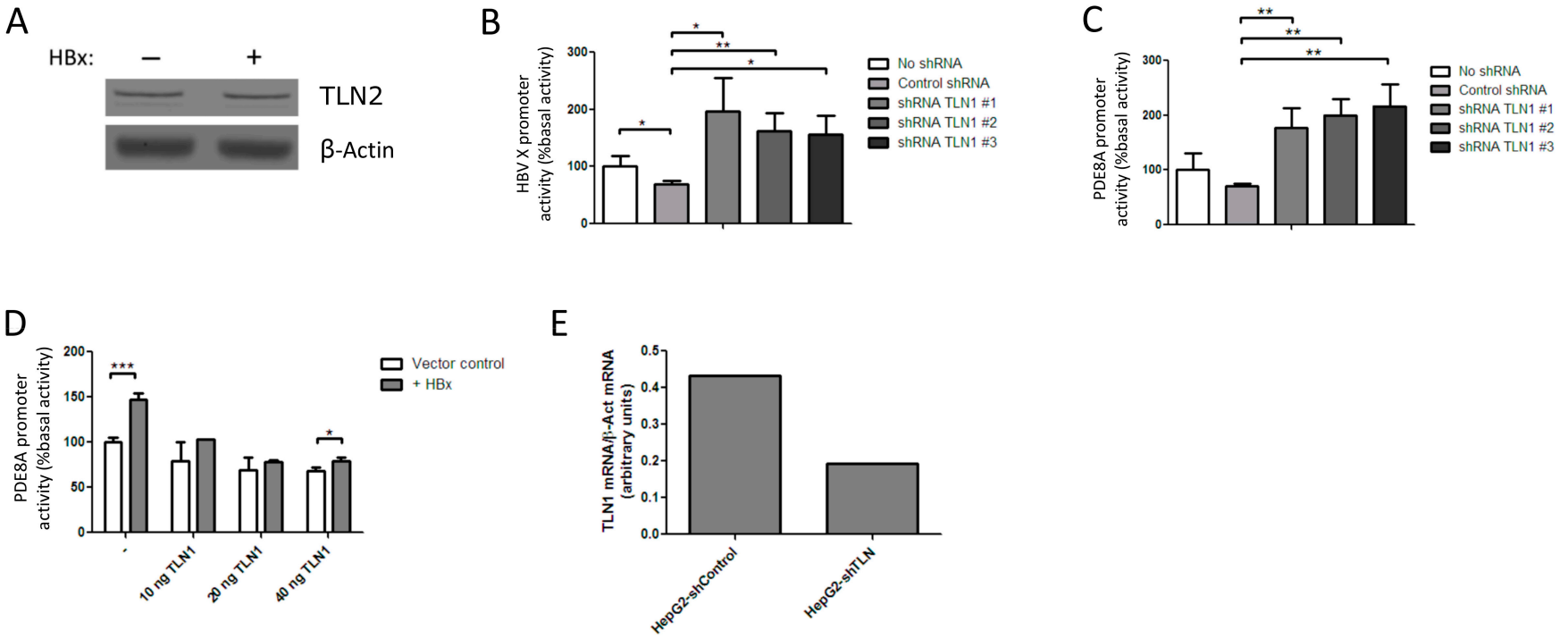

Figure S3. Talin-1 (TLN1) suppresses transcription and is specifically degraded in the presence of HBx. (**A**) Western blot analysis of TLN2, showing TLN2 levels were not affected by HBx expression; (**B**,**C**) HEK 293 cells were transfected with luciferase reporters under control of the HBV X- and human PDE8A promoters, and cotransfected with the plasmids expressing short hairpin RNAs (shRNAs) against TLN1, showing that TLN1 knockdown efficiently transactivates transcription. (**D**) Cotransfection of a vector expressing a biologically active GFP-TLN1 fusion protein with a luciferase reporter under control of the PDE8A promoter prevented transactivation by HBx in a dose-dependent manner. (**E**) TLN1 mRNA was reduced by 55% in HepG2 cells 48 h after transduction with the lentivirus expressing shRNA against TLN1 as measured by the ratio of TLN1 mRNA/β-actin (Act) mRNA. * $p < 0.05$, ** $p < 001$, *** $p < 0.001$.

Table S1. Protein groups identified in the immunoprecipitation experiments based on mass spectrometry data analysed by MaxQuant.

Table S2. Protein groups identified in the immunoprecipitation experiments based on mass spectrometry data analysed by Proteome Discoverer.