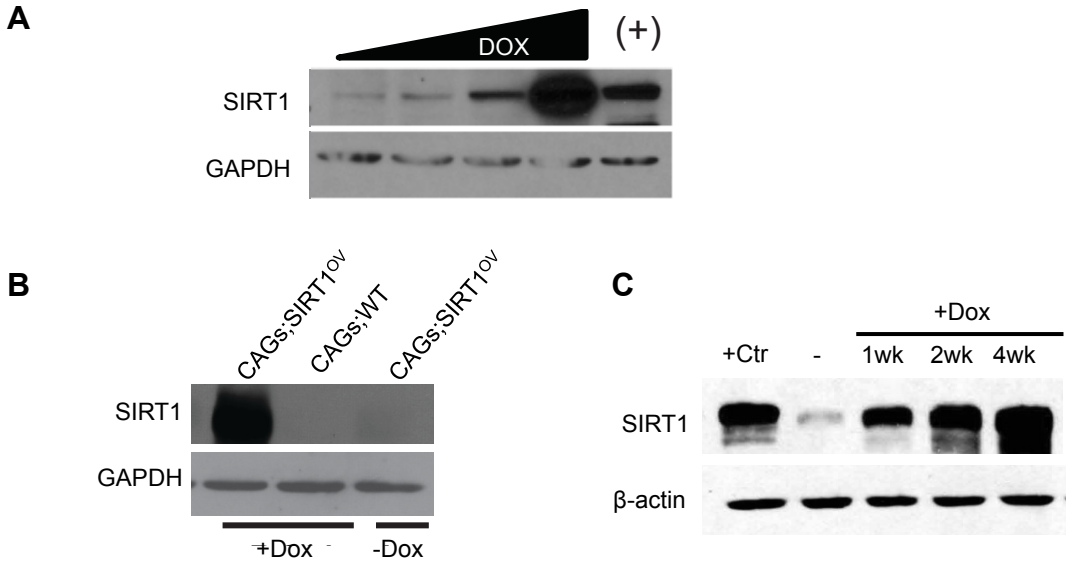
Supplementary Figure 1



Supp. Fig. 1: Generation of Dox-inducible Sirt1 overexpression mice. (A) We generated a DOX-inducible construct containing a human SIRT1 cDNA that is under the regulation of a tetracycline response element (pLP-TRE2-SIRT1). U2OS Tet-ON cells (Clonetech) transfected with the pLP-TRE2-SIRT1 construct were stimulated with 0, 0.01, 0.1 and 1 μg/ml of DOX for 48h to confirm dose-dependent induction of SIRT1. Cells with constitutive overexpression of SIRT1 was used as a positive control (+). (B) Transgenic mice with germline transmission of the pLPTRE2-SIRT1 transgene (TRE-SIRT1^{OV}) were generated by pronuclear injection. Founder mice with germ-line transmission of the transgene were first crossed to the CAGs-rtTA3 driver line to generate mice that were double-transgenic for the TRE-SIRT1^{OV} and CAGs-rtTA3 transgenes (CAGs;SIRT1^{OV}) to test for inducible SIRT1 expression. SIRT1 expression was increased in the kidney cortices of DOX-fed CAGs;SIRT1^{OV} mice compared to DOX-fed, CAGs-positive, pLPTRE2-SIRT1-negative (CAGs;WT) mice and CAGs;SIRT1^{OV} mice without DOX feeding. (C) TRE-SIRT1^{OV} mice were crossed with podocin-rtTA transgenic mice to generate mice with inducible podocyte-specific overexpression of SIRT1 (Pod;SIRT1^{OV}). Western blots confirm the overexpression of SIRT1 in primary podocytes isolated from Pod;SIRT1^{OV} mice with no Dox (-) or with Dox (+Dox) for 1, 2 or 4 weeks. The representative blots of three independent experiments are shown. +Ctr, positive control from the cells with overexpression of SIRT1.