Sarkar and Zohn, http://www.jcb.org/cgi/content/full/jcb.201105101/DC1

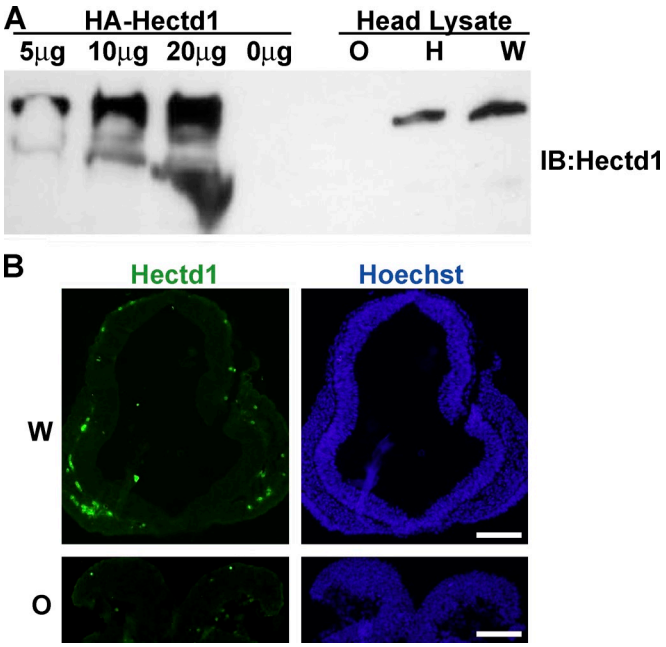

Figure S1. **Specificity of anti-Hectd1 antibody in detection of transfected and endogenous Hectd1.** (A) HEK293T cells were transfected with the indicated amounts of pCMV-HA-Hectd1. Expression of Hectd1 was determined by Western blot analysis using anti-Hectd1 antibody (Biosynthesis, Inc.). Lysates were prepared from *Hectd1*$^{opm/opm}$ (O), *Hectd1*$^{opm/+}$ (H), and *Hectd1*$^{+/+}$ (W) E12.5 embryo heads. (B) Specificity of anti-Hectd1 antibody was confirmed by immunofluorescence analysis on E10.5 wild-type (W) and *Hectd1*^{O} (O) heads where no detectible full-length Hectd1 is synthesized. Bars, 125 mm.

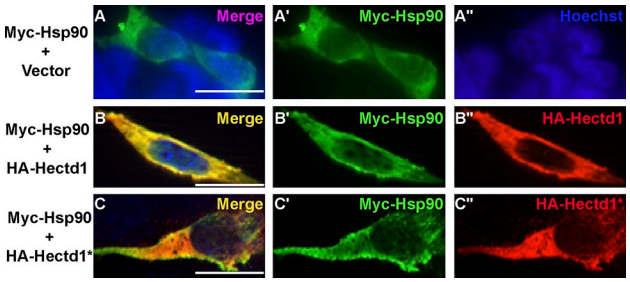

Figure S2. **Colocalization of Hectd1 and Hsp90 in HEK293T cells.** HEK293T cells plated on coverslips were transfected with 1 μg pCMV-Myc-Hsp90 (A) alone, or cotransfected with 20 μg pCMV-HA-Hectd1 (B) or pCMV-HA-Hectd1* (C). Cells were immunostained to detect Myc-Hsp90 (A′-C′) and HA-Hectd1 (A″–C″). Bars, 20 μm

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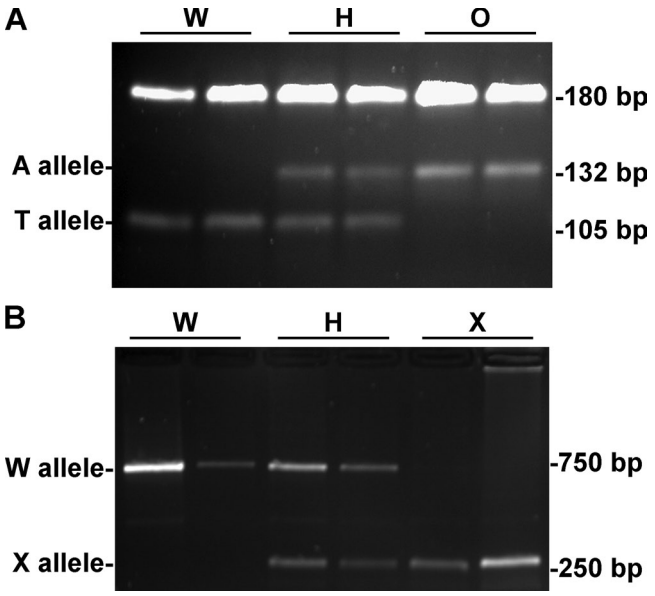

Figure S3. **Genotyping of *Hectd1*^{O} and *Hectd1*^{X} mice.** (A) Tetra-primer ARMS-PCR was performed to distinguish *Hectd1*$^{+/+}$ (W), *Hectd1*$^{opm/+}$ (H), and *Hectd1*$^{opm/opm}$ (O) genotypes. (B) Gap PCR was performed on embryonic yolk sacs or pup tails to distinguish *Hectd1*$^{+/+}$ (W), *Hectd1*$^{X/+}$ (H), and *Hectd1*$^{X/X}$ (X) genotypes.

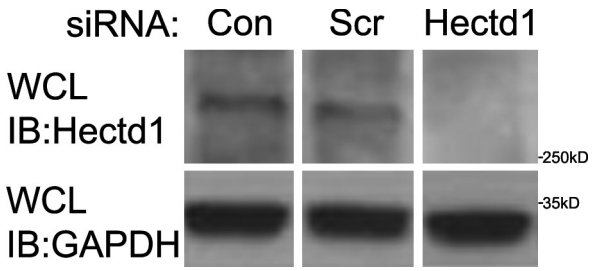

Figure S4. **siRNA targeting Hectd1 results in undetectable protein levels when transfected in HEK293T cells.** HEK293T cells were transfected with control (Con), scrambled (Scr), or siRNA targeting Hectd1. Whole cell lysates (WCL) were subjected to Western blot analyses to detect Hectd1 and GAPDH.