Supplemental Information

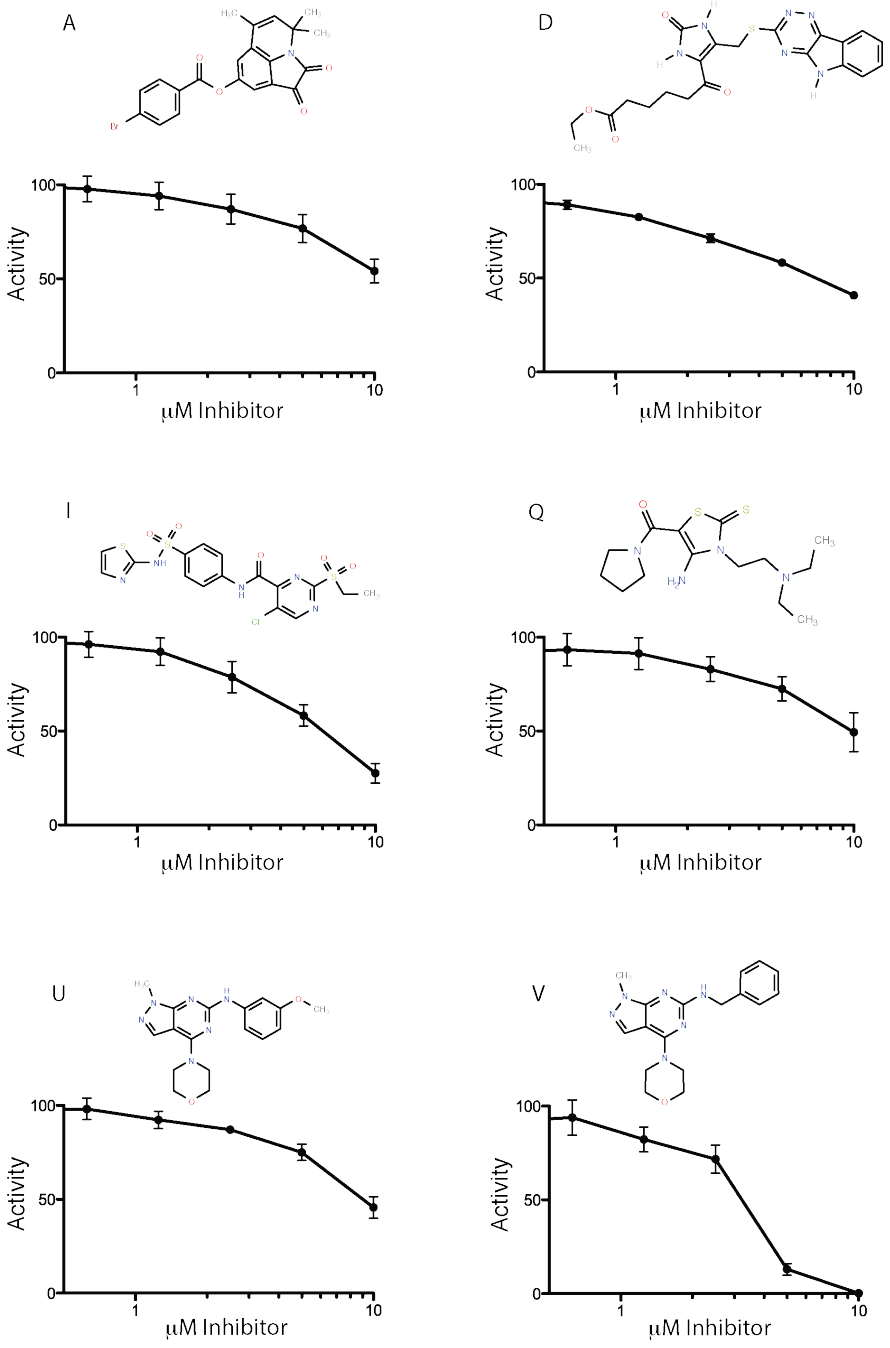

Figure S1. Lead compounds A,D,I,Q, U and V showed positive dose response relationships when used in an AlphaScreen based assay for inhibition of the Flag-PHD2:biotin-4xPXLE peptide interaction. n=3 and error bars represent standard deviation.

Figure S2. Comparison between human and mouse PHD2 protein sequences. Amino acid numbering is shown to the right. Amino acids encoded by exon 1 are highlighted with yellow background. Zinc finger is shown in bold.

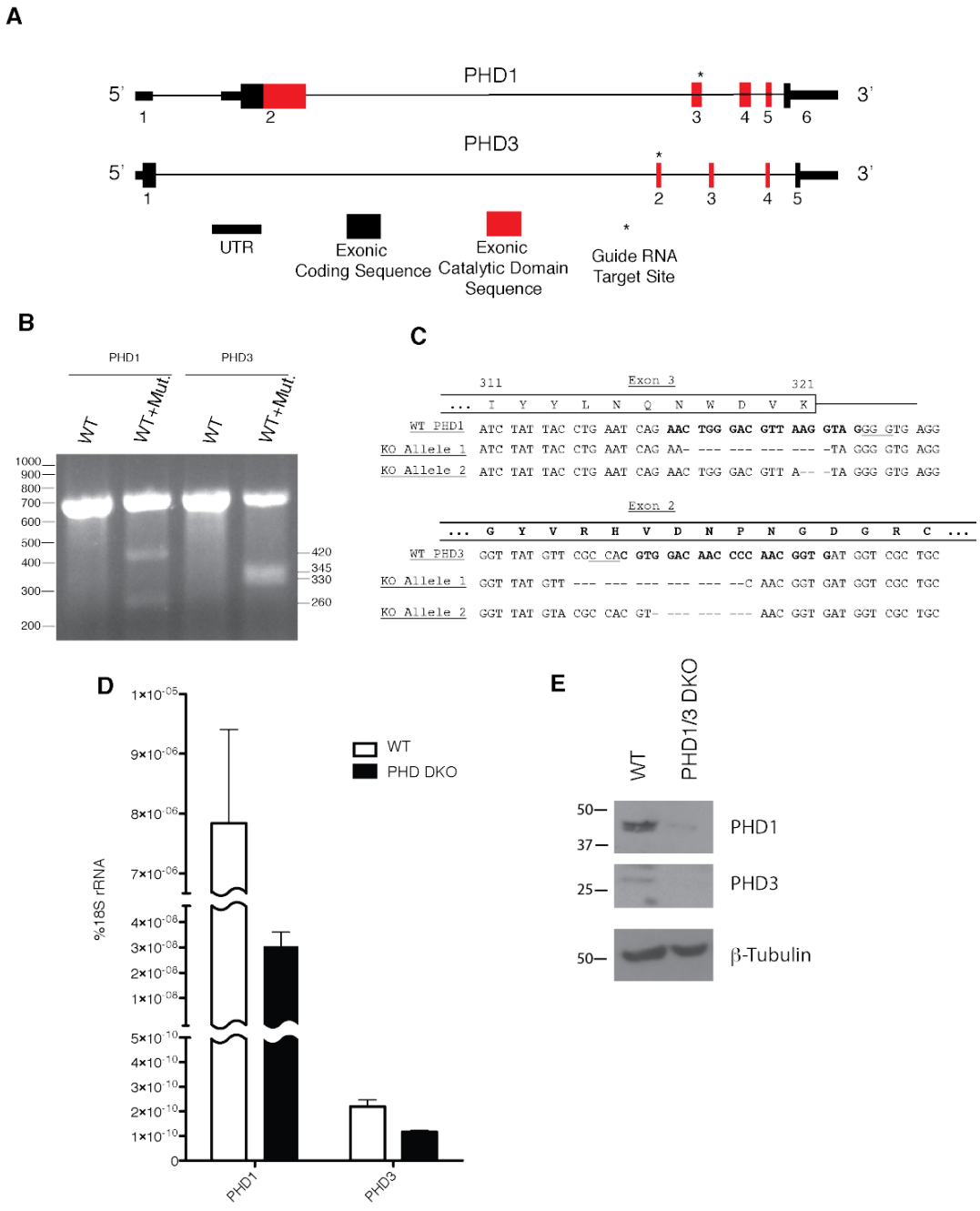

Figure S3. (A) Schematic showing *PHD1* and *PHD3* gene structures. Blocks represent exonic sequences and asterisks (*) indicate sites targeted for Cas9/gRNA-mediated cleavage. (B) Results of Surveyor assay indicating mismatches within PCR product heteroduplexes. (C) Results of deconvoluted Sanger sequencing traces from PCR screening of a *PHD1/3* DKO single cell clone showing deletions present in mutated alleles. (D) Real Time PCR-based assessment of mRNA abundance for *PHD1* and *PHD3* mRNA relative to 18S rRNA in WT Hap1 cells and *PHD1/3* DKO cell line (n=3). (E) Western blot for PHD1 and PHD3 in *PHD1/3* DKO cell line showing loss of expression at both loci.

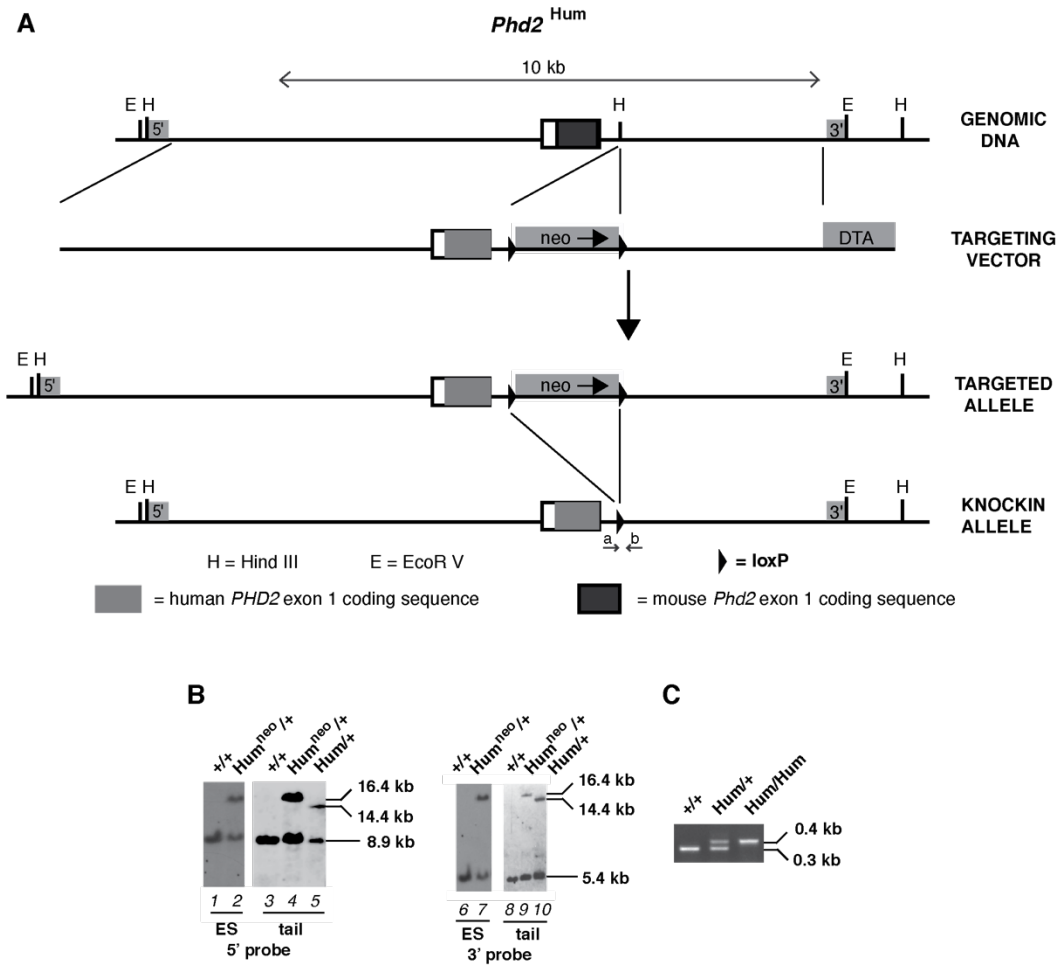

Figure S4. (A) Gene targeting strategy. DTA and neo denote diphtheria toxin A and neomycin cassettes, respectively. Positions of 5' and 3' Southern probes are indicated by 5' and 3', respectively. Positions of CSint1-1 5' and CSint1-1 3' PCR primers are indicated by a and b, respectively. (B) Southern blots employing 5' (left) and 3' (right) probes of Hind III digested ES cell or mouse tail DNA. *Phd2* genotypes are provided at top. The presence of the neomycin cassette is denoted by neo. (C) PCR genotyping of *Phd2* ^{Hum} knock-in mice by using CSint1-1 5' and CSint1-1 3' primers. The 0.3 kb product is derived from the wild type allele, while the 0.4 kb product is derived from the *Phd2* ^{Hum} mutant allele. *Phd2* genotypes are shown at top.

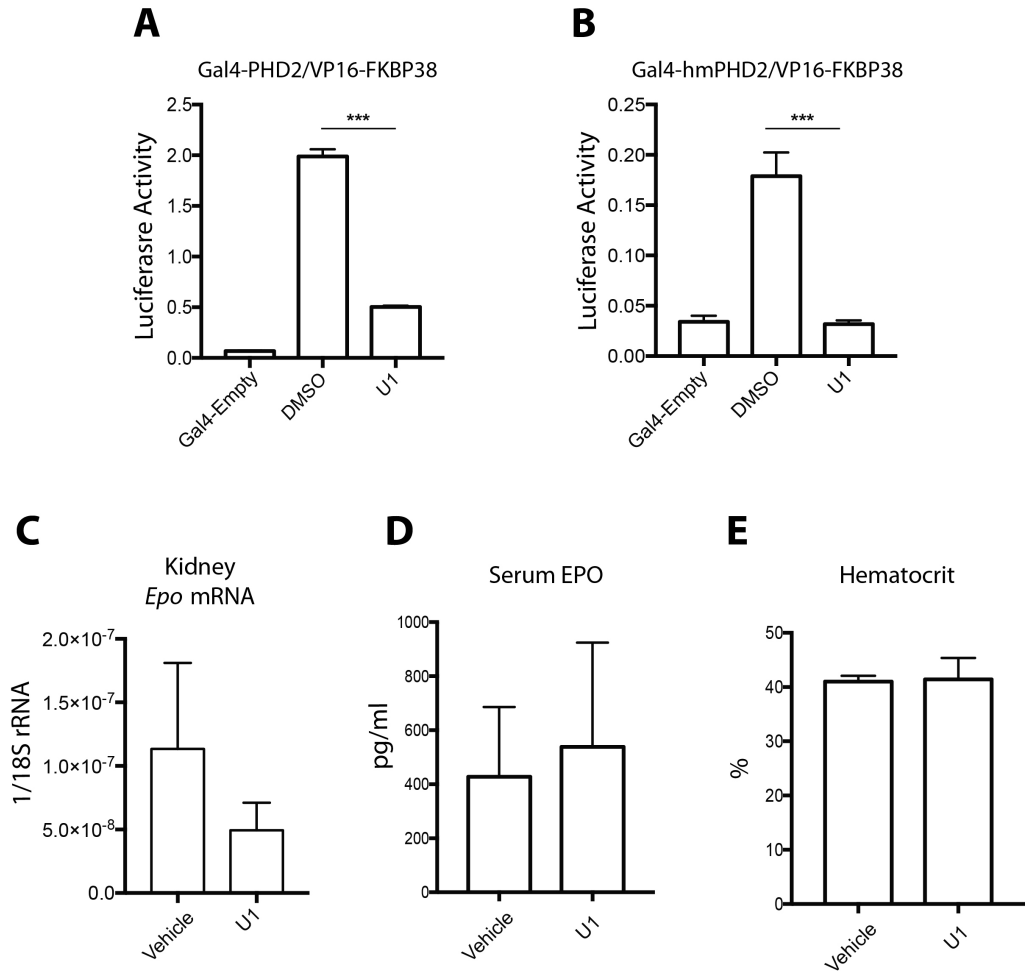

Figure S5. (A-B) Compound U1 (10 μM) inhibits the interaction of both (A) human PHD2 and (B) humanized mPhd2 with the PXLE-containing protein FKBP38 in a mammalian two-hybrid assay. Error bars represent SD. n=3 per group. *** = $P < 0.001$ (C-E) C57BL/6 mice were subjected to 4 days of twice-daily injection with compound U1 at a dose of 20 mg/kg/day as compared to vehicle control. (C) Renal *Epo* mRNA, (D) serum Epo, and (E) hematocrit were measured. Error bars represent SD. n=4 per group. No significant differences were seen between U1 and vehicle injected mice for any of these parameters.