Article

The Cysteine Protease Legumain Is Upregulated by Vitamin D and Is a Regulator of Vitamin D Metabolism in Mice

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Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1.

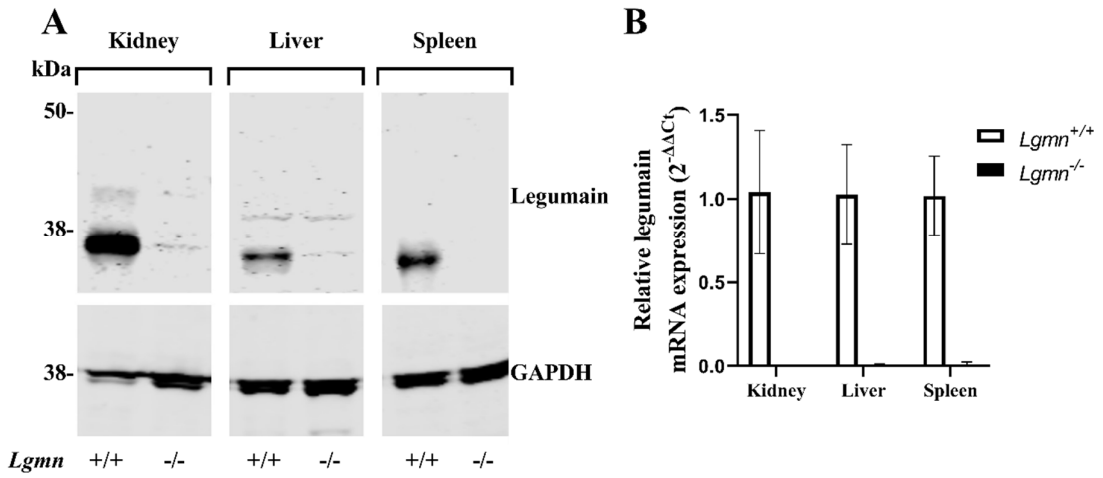

Figure S1. Validation of legumain deficiency in Lgmn$^{-/-}$ mice. Tissues from wild type (Lgmn$^{+/+}$) and legumain deficient (Lgmn$^{-/-}$) mice were harvested. **A.** One representative immunoblot of legumain and GAPDH (housekeeping control) in kidney, liver and spleen (n=3). **B.** Legumain mRNA expression relative to GAPDH (2-ΔΔCT; n=3). Data represent mean ± SEM. Numbers (n) represent individual biological replicates.

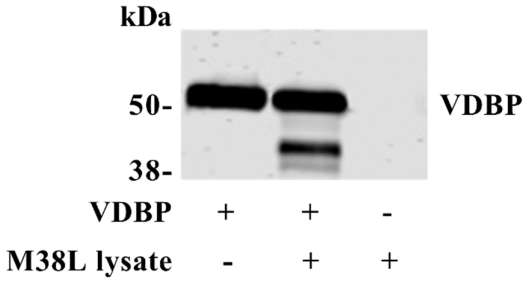

Figure S2. VDBP is cleaved after incubation with lysate from legumain over-expressing HEK293 cells. Purified VDBP (55 kDa) from human plasma (1.9 μM) was incubated in legumain assay buffer (pH 5.8) at 37 °C with or without lysate from legumain over-expressing HEK293 (M38L) cells for 5 hours (n=1).

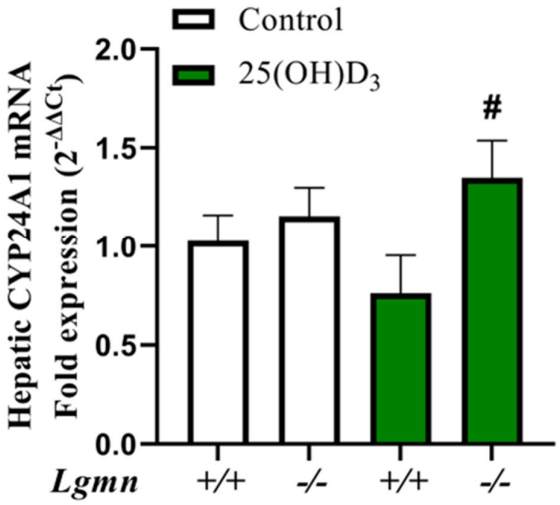

Figure S3. Hepatic CYP24A1 mRNA expression is increased in *Lgmn*$^{-/-}$ mice treated with 25(OH)D$_3$. Wild type (*Lgmn*$^{+/+}$) and legumain deficient (*Lgmn*$^{-/-}$) mice were treated subcutaneously with 50 μg/kg 25(OH)D3 (n=6-7) or an equal volume vehicle (n=7, control) every two to three days (four times in total) for seven days. Tissues were harvested 24 hours after the final injection. Hepatic CYP24A1 mRNA expression relative to the geometric mean of CT values of four housekeeping controls ($2^{-\Delta\Delta CT}$, n=5). Two-way ANOVA. #$p<0.05$ vs different genotype, same treatment. Numbers (n) represent individual biological replicates.

Table S1. Primer sequences used for qPCR.

Human primer sequences		
GAPDH	Forward	GTCTCCTCTGACTTCAACAGCG
	Reverse	ACCACCCTGTTGCTGTAGCCAA
LGMN	Forward	GCAGGTTCAAATGGCTGGTAT
	Reverse	GGAGTGGGATTGTCTTCAGAGT
Mouse primer sequences		
18s	Forward	GGGTCGGGAGTGGGTAATTT
	Reverse	GGGAGCCTGAGAAACGGC
β-actin	Forward	GATATCGCTGCGCTGGTCGTC
	Reverse	ACGCAGCTCATTGTAGAAGGTGTGG
Cyp24a1	Forward	CTGCCCCATTGACAAAAGGC
	Reverse	CTCACCGTCGGTCATCAGC
Cyp27b1	Forward	TCCTGGCTGAACTCTTCTGC
	Reverse	CCAGACCATATTGGCCCGTA
Gapdh	Forward	CATCACTGCCACCCAGAAGACTG
	Reverse	ATGCCAGTGAGCTTCCCGTTCAG
Lgmn	Forward	TGGACGATCCCGAGGATGG
	Reverse	GTGGATGATCTGGTAGGCGT
Rplp0	Forward	GCTTCGTGTTCACCAAGGAGGA
	Reverse	GTCCTAGACCAGTGTTCTGAGC
Vdbp	Forward	ACACCCAACACCTCTCCGGCA
	Reverse	CCAAGCTAGTGCACGGGCCC