Supplemental tables

Supplemental table 1. ELISA data of free IGF-I, total IGF-I, intact IGFBP-3, and GH in *Pappa2* KI and WT mice at 16 weeks of age

	Male			Female		
	Wild-type (N)	**Heterozygous (N)**	**Homozygous (N)**	**Wild-type (N)**	**Heterozygous (N)**	**Homozygous (N)**
Free IGF-I (ng/mL)	2.42 ± 0.17 (13)	0.61 ± 0.08 (14)	0.08 ± 0.03 (21)	1.14 ± 0.20 (17)	0.53 ± 0.08 (15)	0.26 ± 0.10 (16)
Total IGF-I (ng/mL)	31.06 ± 0.66 (13)	35.90 ± 2.12 (14)	49.31 ± 1.66 (21)	31.92 ± 1.38 (17)	38.67 ± 1.59 (15)	58.48 ± 1.82 (16)
% fIGF-I (%)	7.92 ± 0.63 (13)	1.74 ± 0.26 (14)	0.19 ± 0.08 (21)	3.65 ± 0.62 (17)	1.39 ± 0.20 (15)	0.49 ± 0.17 (16)
Intact IGFBP-3 (ng/mL)	406.8 ± 44.9 (13)	617.7 ± 35.2 (14)	1217.6 ± 43.0 (21)	469.4 ± 37.6 (17)	460.0 ± 28.7 (15)	1369.9 ± 83.5 (16)
GH (ng/mL)	5.42 ± 0.50 (13)	5.55 ± 1.22 (14)	6.03 ± 0.54 (21)	4.74 ± 0.22 (17)	5.49 ± 0.54 (15)	10.22 ± 1.10 (16)

Notes: % fIGF-I is the percent ratio of free to total IGF-I value. Values are shown as mean ± SEM.

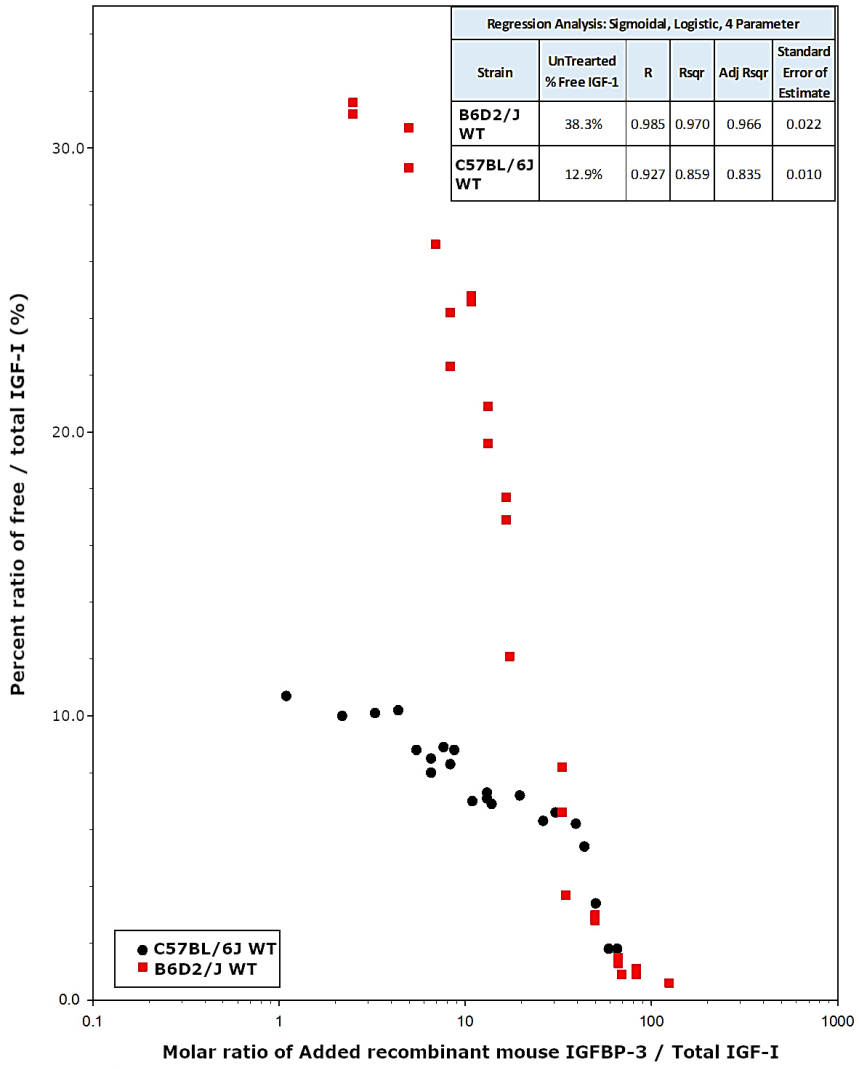
Supplemental table 2. Insulin concentration in *Pappa2* KI and WT mice at 15 weeks of age

	Male			Female		
	Wild-type (N)	**Heterozygous (N)**	**Homozygous (N)**	**Wild-type (N)**	**Heterozygous (N)**	**Homozygous (N)**
Insulin (ng/mL)	0.67 ± 0.10 (6)	0.79 ± 0.28 (6)	1.32 ± 0.25** (5)	0.14 ± 0.04 (6)	0.16 ± 0.06 (6)	0.21 ± 0.02 (3)

Notes: Values are means ± SEM. **, $P < 0.01$ compared with wild-type group by unpaired *t* test.

Supplemental figures

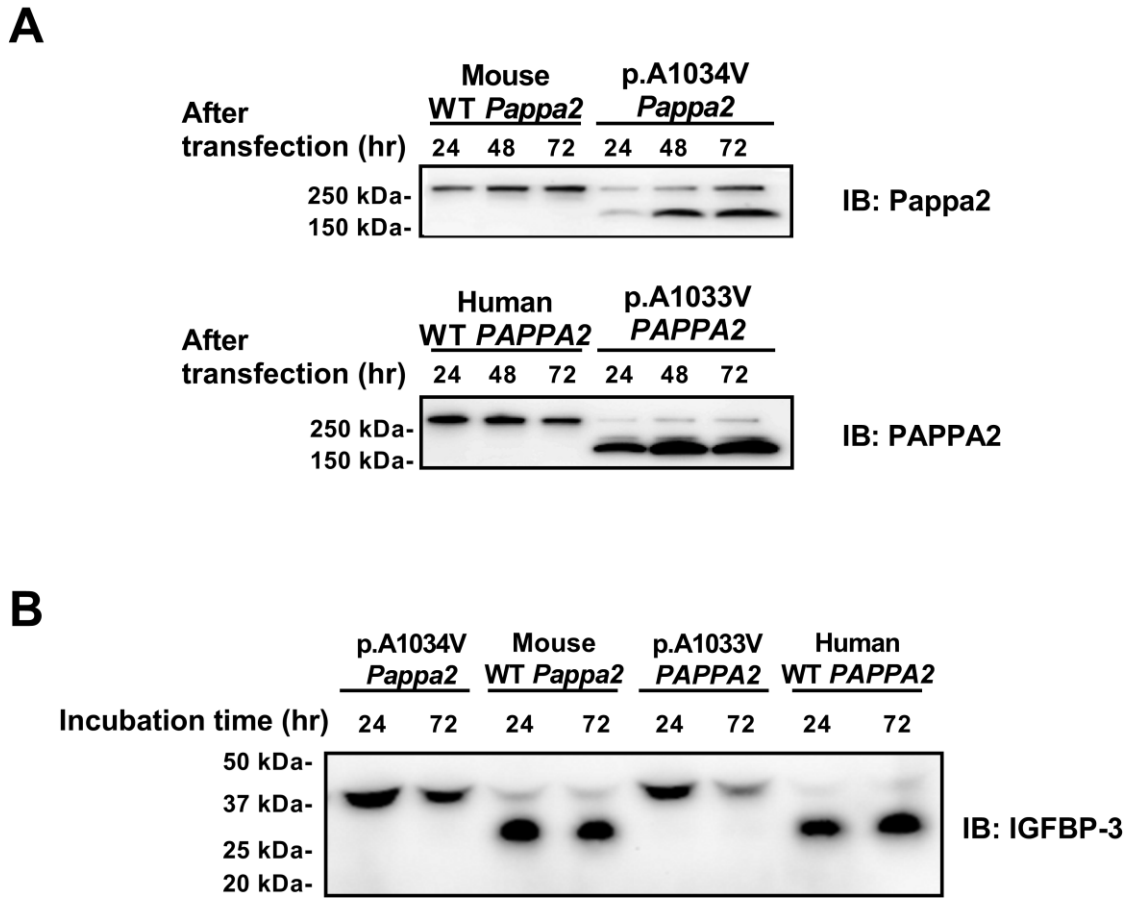
Supplemental figure 1.



Supplemental figure 1. Addition of exogenous recombinant mouse IGFBP-3 decreases free IGF-I in normal mouse serum samples

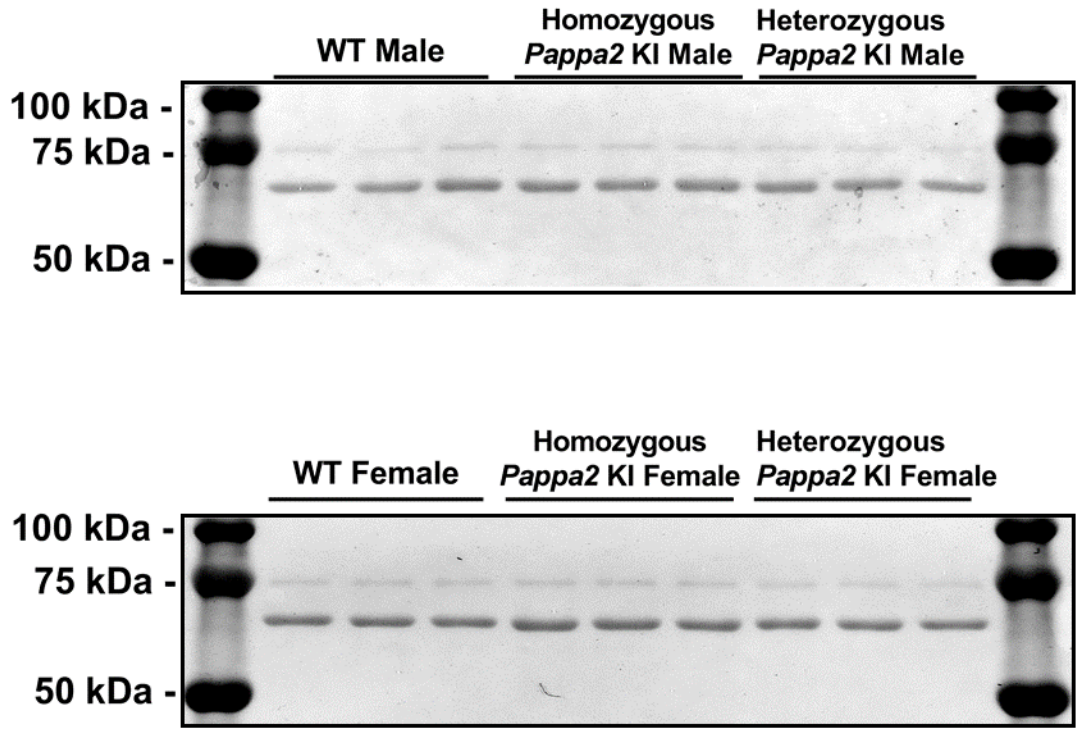
Pooled aliquots of normal mouse serum samples from B6D2/J (background of *Pappa2* KI mice) and from C57BL/6J strains were exposed to increasing concentrations of mouse recombinant IGFBP-3 (#775-B3-025; R&D systems, MN, USA) for 1-2 hour at 37 ˚C prior to analysis. Total and free IGF-I levels were measured by ELISA (AL-137 and AL-136), in replicates of 3 up to 10, and the percent ratio of free IGF-I to total IGF-I was calculated. The coefficient of variation (CV) of replicate determinations was < 11% for mouse serum specimens with free IGF-I concentrations. The limit of detection (LOD) in free IGF-I ELISA kit (AL-136) was < 0.2 ng/mL. The values under LOD was equivalent to 0.6 % of free to total IGF-I ratio after calculation. Red and black dots represent B6D2/J WT and C57BL/6J WT, respectively. R^2 was 0.966 and 0.835 in B6D2/J WT and C57BL/6J WT, respectively.

Supplemental figure 2.



Supplemental figure 2. Recombinant mouse Pappa2 and human PAPPA2 expression and function.
(A) PAPPA2 immunoblots of conditioned mediums (CM) of HEK293 cells transfected with human or mouse PAPPA2 plasmids. Each plasmid was transfected to HEK293 cells as previously described (11). CMs were collected at 24, 48, and 72 hr after transfection. Forty µL of CM was solubilized in SDS sample buffer with 5% β-mercaptoethanol prior to incubation at 95 ˚C for 5 min. The samples were resolved on 7% SDS-polyacrylamide gels and electroblotted onto a nitrocellulose membrane. The membrane was blocked with 3% w/v bovine serum albumin (BSA) and 1X TBS. Primary antibody for immunoblot analysis were a rabbit anti-human PAPPA2 polyclonal antibody (#ab117743; Abcam, Cambridge, MA, USA; RRID: AB_2050158). The mutated Pappa2 and PAPPA2 were secreted in CM from transfected HEK293 cells. (B) IGFBP-3 immunoblots of HEK293 CM combined with human fibroblast CM (known to express and secrete IGFBP-3). CM (100 µL) of HEK293 cells transfected with human and mouse PAPPA2 plasmids was incubated (37 ˚C) with human fibroblast CM (100 µL) containing IGFBP-3. Forty µL of combined CM was resolved on 11% SDS-polyacrylamide gels and electroblotted onto a nitrocellulose membrane. The membrane was blocked with 3% w/v BSA and 1X TBS. Primary antibody for immunoblot analysis were a mouse anti-human IGFBP-3 monoclonal antibody (sc-374365). Molecular weight of intact IGFBP-3 is approximately 40 kDa. The WT Pappa2 and PAPPA2 generated the cleaved IGFBP-3 (~26 kDa), but the mutated proteins could not cleave intact IGFBP-3, even after 72 hr of incubation time.

Supplemental figure 3.



Supplemental figure 3. The total protein staining of membranes used for mouse IGFALS immunoblot analysis.

Total proteins in the membranes used for mouse IGFALS immunoblotting (see Figure 3B), were stained using Pierce™ Reversible Protein Stain Kit.