Cardiovascular Consequences of Prostanoid I Receptor Deletion in Microsomal Prostaglandin E Synthase-1 Deficient Hyperlipidemic Mice

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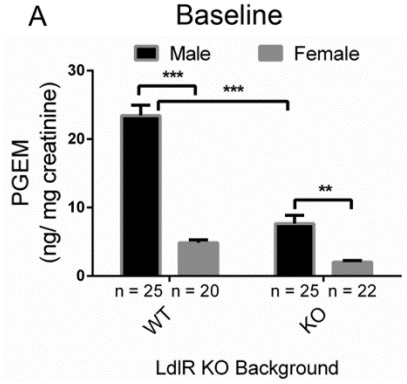
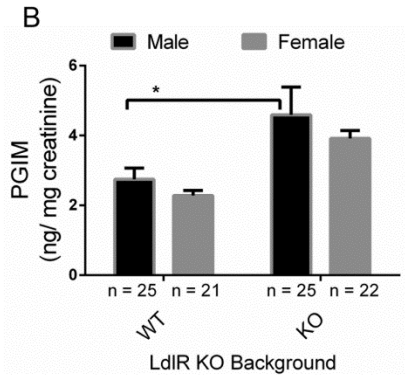
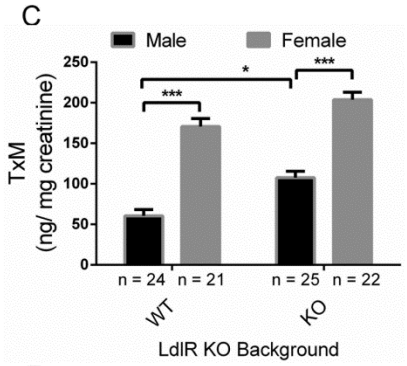
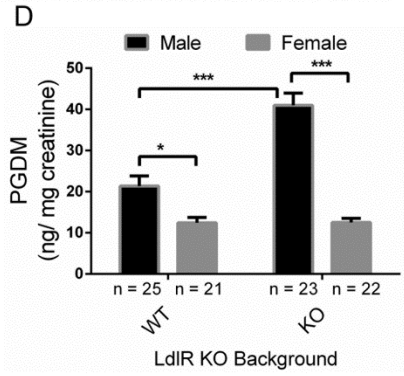
Supplemental Figures



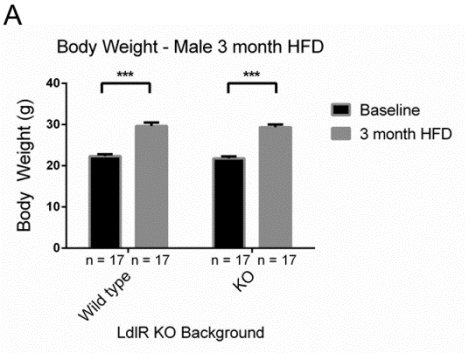






Supplemental Figure 1. Impact of prostacyclin receptor (Ip) and microsomal prostaglandin E synthase 1 (mPges-1) deletion on prostaglandin biosynthesis in mice. Fasting (9am-5 pm) urine samples from LdlR KO (WT) and Ip/mPges-1/ LdlR TKO (KO) mice were collected before feeding a HFD, and prostanoids metabolites were analyzed by liquid chromatography/ mass spectrometry, as described in the Methods. Deletion of Ip and mPges-1 suppressed PGE_2 but increased PGI_2 biosynthesis as reflected in their urinary PGEM (7-hydroxy-5, 11-diketotetranorprostane-1, 16-dioic acid) (A) and PGIM (2, 3-dinor 6-keto $PGF_{1\alpha}$) (B) metabolites, respectively. Urinary 2, 3-dinor TxB_2 (TxM) was also elevated in TKO mutants in both sexes (C). PGDM (11, 15-dioxo-9α-hydroxy-2,3,4,5-tetranorprostan-1,20-dioic acid) levels in male mice were augmented in the TKO mutants (D). PGDM levels in female were not changed. Two-way ANOVA showed a significant effect of genotype and/ or gender on urinary prostanoid levels (PGEM, genotype, $p< 0.0001$, gender, $p< 0.0001$, interaction, $p< 0.0001$; PGIM, genotype, $p= 0.0005$, gender, $p= 0.2363$, interaction, $p= 0.8289$; TxM, genotype, $p< 0.0001$, gender, $p< 0.0001$, interaction, $p= 0.4178$; PGDM, genotype, $p< 0.0001$, gender, $p< 0.0001$, interaction, $p< 0.0001$). Multiple comparison tests (Holm-Sidak) were used to test significant differences between WT (LdlR KO) and KO (Ip/mPges-1/ LdlR TKOs). Data are expressed as means ± SEMs. *$p<0.05$, **$p< 0.01$, ***$p<0.001$; n=20-25 (male) and female (22-25) per genotype.

A



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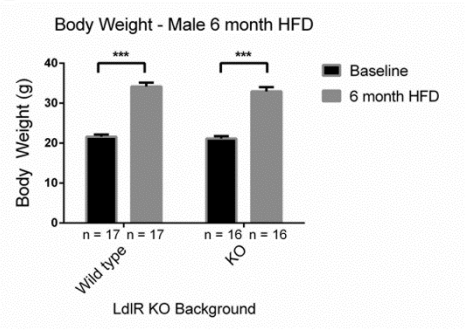

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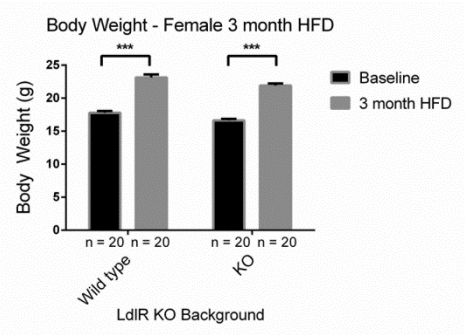

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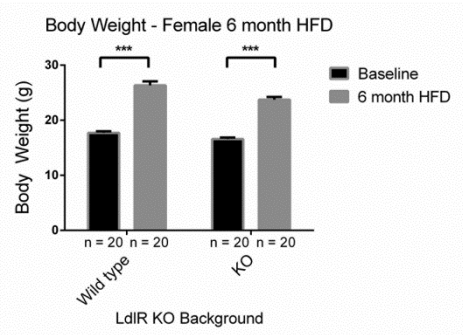
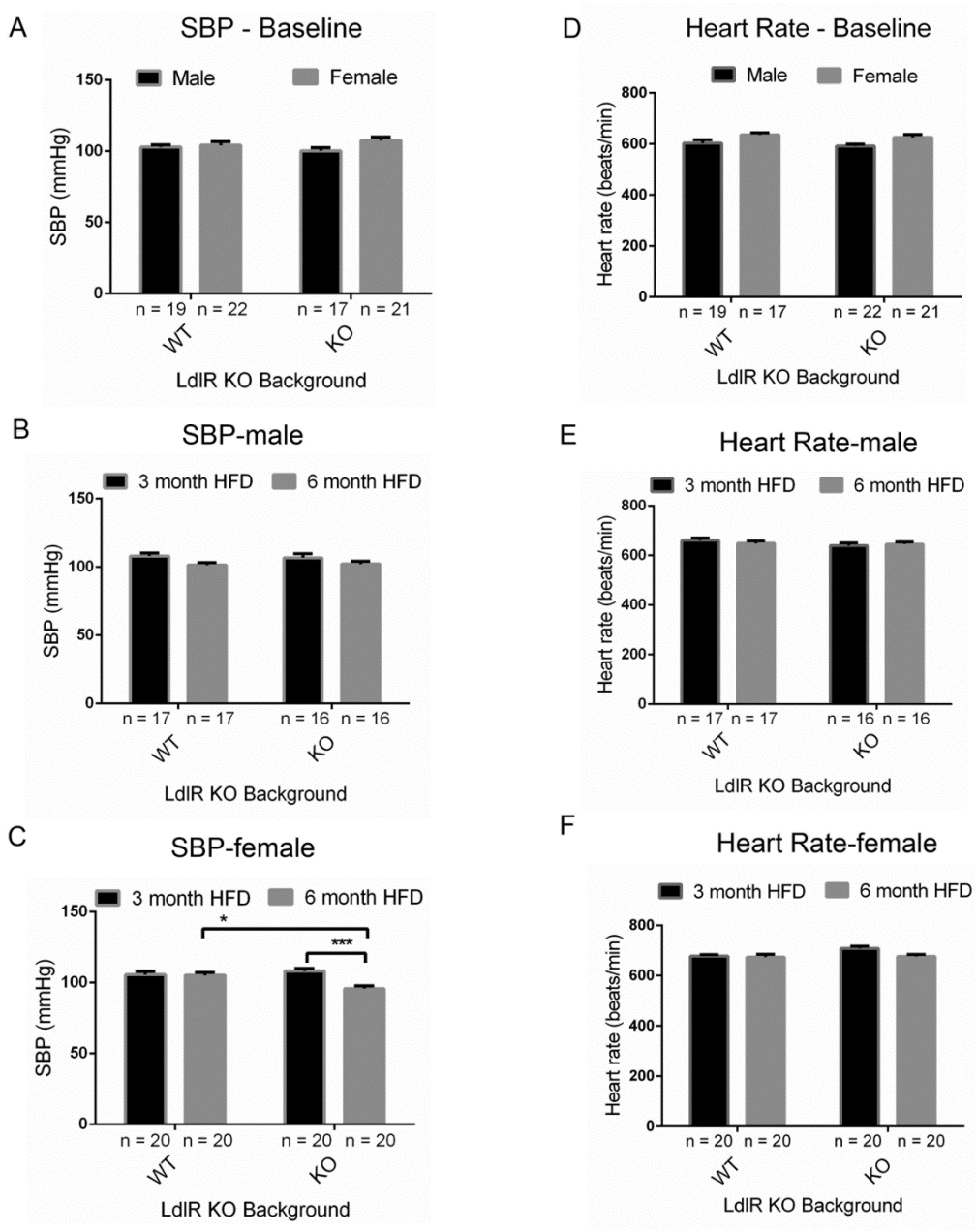


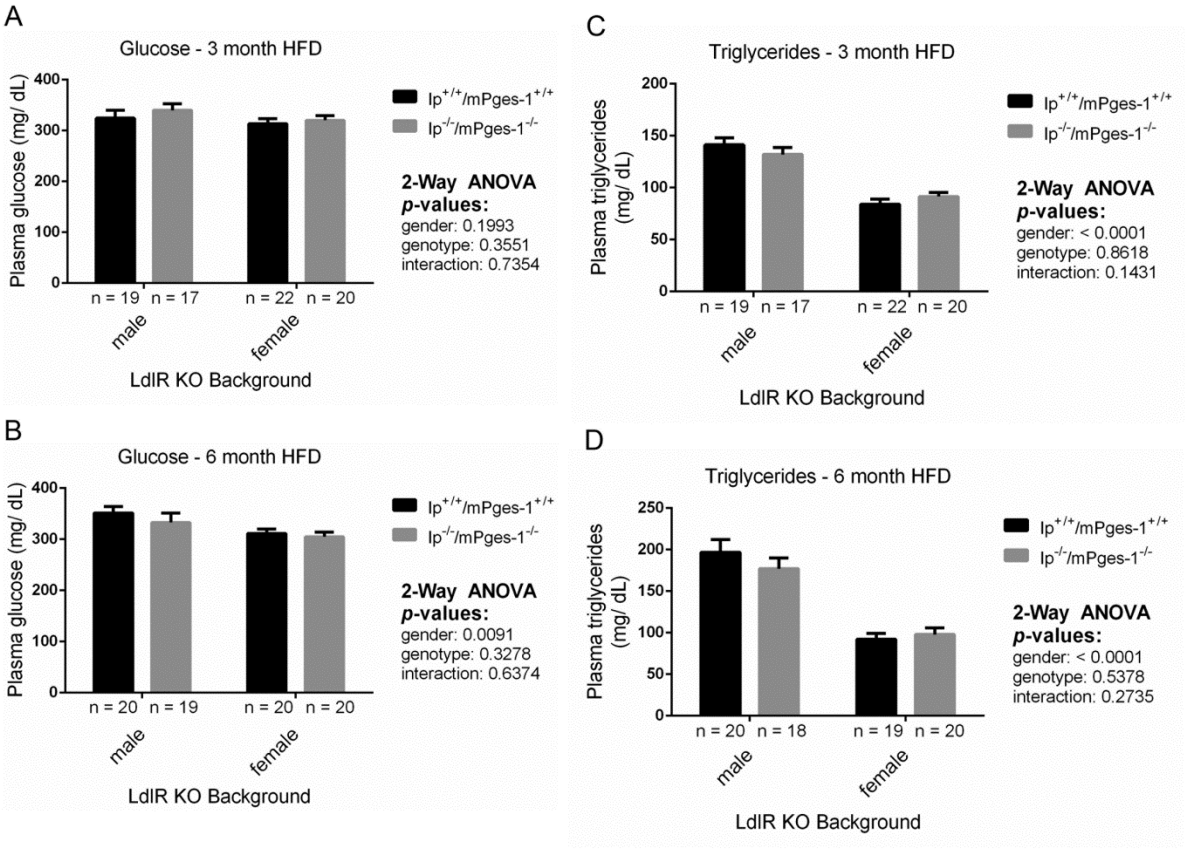
Supplemental Figure 2. Body weight of mice. Both male (A- 3 months, B- 6 months) and female (C- 3 months, D- 6 months) mice were weighed before and after feeding a HFD. Two-

way ANOVA revealed a significant increase in body weight after 3 or 6 months on a HFD in both sexes (A- male- 3 month HFD, genotype, p= 0.5897, treatment, p< 0.0001, interaction, p= 0.7651; B- male- 6 month HFD, genotype, p= 0.4068, treatment, p< 0.0001, interaction, p= 0.4880; C- female- 3 month HFD, genotype, p= 0.4068, treatment, p< 0.0001, interaction, p= 0.8638; D- female- 6 month HFD, genotype, p= 0.0014, treatment, p< 0.0001, interaction, p= 0.0788). No significant differences were detected between WT and KOs. Data are expressed as means ± SEMs. *p<0.0001; n= 16-17 (male) and 20 (female) per genotype.

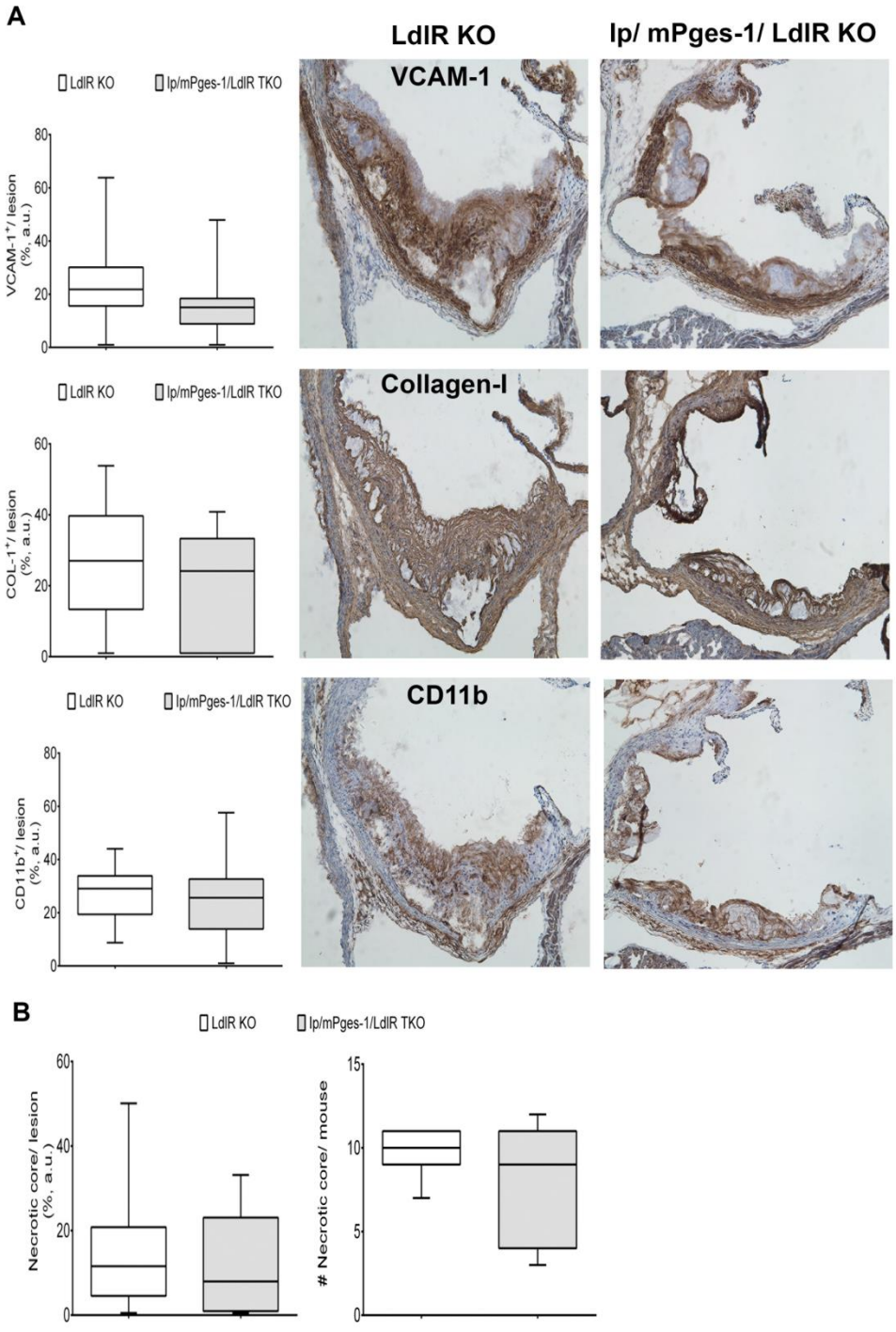


Supplemental Figure 3. Impact of Ip and mPges-1 deletion on systolic blood pressure (SBP) and heart rate of mice on a high fat diet. (A-C) SBP of male and female mice were measured using a tail-cuff system before (A- baseline, male and female) and after feeding a HFD for 3 months or 6 months (B- male, C- female). Two-way ANOVA revealed no significant difference in baseline SBP between WT (LdlR KO) and KO (Ip/mPges-1/ LdlR TKOs) in both sexes

(genotype, p= 0.8491, gender, p= 0.0607, interaction, p= 0.1873) and male mice after 3 or 6 month HFD (genotype, p= 0.9150, treatment, p= 0.0159, interaction, p= 0.6703). SBP of female KOs was significantly reduced at 6 month HFD compared to WTs (genotype, p= 0.0844, treatment, p= 0.0016, interaction, p= 0.0037). Heart rate of mice was also measured simultaneously (D-baseline, male and female, E- male and F- female, 3 or 6 month HFD). No significant difference was detected between WT (LdlR KO) and KO (Ip/mPges-1/ LdlR TKOs) at baseline (genotype, p= 0.3213, gender, p= 0.0023, interaction, p= 0.9508) and in both sexes after HFD feeding (male- genotype, p= 0.1999, treatment, p= 0.7728, interaction, p= 0.3450; female- genotype, p= 0.0511, treatment, p= 0.0414, interaction, p= 0.1069) . Data are expressed as means ± SEMs. *p<0.05, ***p<0.0001; n=17-22 (male) and 16-22 (female) per genotype.



Supplemental Figure 4. Impact of Ip and mPges-1 deletion on fasting plasma glucose and triglyceride levels of mice on a high fat diet. Fasting plasma glucose and triglyceride levels were measured using commercial test kit from Stanbio laboratory following manufacturer's instructions. Two-way ANOVA revealed no significant effect of genotype on plasma glucose and triglyceride levels in both male and female mice after feeding a HFD for 3 or 6 months (glucose- 3 month HFD, genotype, p= 0.3551, gender, p= 0.1993, interaction, p= 0.7354; glucose- 6 month HFD, genotype, p= 0.3278, gender, p= 0.0091, interaction, p= 0.6374; triglycerides- 3 month HFD, genotype, p= 0.8618, gender, p< 0.0001, interaction, p= 0.1431; triglycerides- 6 month HFD, genotype, p= 0.5378, gender, p< 0.0001, interaction, p= 0.2735).



Supplemental Figure 5. Morphometric consequences of prostacyclin receptor (Ip) and microsomal prostaglandin E synthase 1 (mPges-1) deletion on lesion development. A. Quantification of immunohistochemical staining of vascular cell adhesion molecule-1 (VCAM-

1), type-I collagen and CD11b in aortic roots of male LdlR KO and TKOs after feeding a HFD for 3 months is shown in parallel with their representative aortic root sections. B. Necrotic core area in lesions was analyzed as per lesion and per mouse are shown. A parametric t-test (2-tailed) revealed no significant effect of genotype on morphological staining for VCAM-1, type-I collagen and CD11b and lesional necrotic core areas. Data are expressed as means ± SEMs. n=7 per genotype (21 lesions).